Core-box Modelling

Theoretical Contributions and Applications
to Glucose Homeostasis Related Systems

Gunnar Cedersund
Figure on the front: The grey box represents a grey-box model which is built on all literature and in vitro characterisations of the parts. The red sphere represents an identifiable core model which is built on the in vivo data from the intact system. They are put inside each other to represent a core-box model which includes both the two models, and a translation between them.
Love that is not science is not love, just like science that is not love is not absolute science

M. Thomsen (1890-1981)
Abstract

The study of biological systems through a combination of in vitro characterisations, quantitative in vivo experiments, and mathematical modelling, is receiving an increasingly growing interest through the field system biology. Systems biology uses methods from many different research fields, but the existing methods need to be extended and adapted to fit the new situations. This dissertation presents new methods for various modelling situations, and the developments have been driven by examples related to glucose homeostasis.

The modelling methods are contained in a new modelling framework denoted core-box modelling. Core-box modelling attempts to combine the strengths of mechanistic grey-box models (e.g., to describe detailed processes) with the strengths of minimal models (e.g., identifiability and hypothesis testing). The main new steps in the modelling process are: identifiability analysis, model reduction, system identification and a translation between different versions of the same model. In all these sub-disciplines, a review is given and new methods are developed; these contributions are of course valid in any framework. Methods are given for detection and handling of both structural and practical unidentifiability in individual rate expressions. This also yields in vivo expressions for the reaction rates. Special methods are derived for systems with oscillations. First, two methods for identification of the interactions generating the oscillations in a model are given. Then, two methods are presented for parameter estimation of a system close to a Hopf bifurcation. The first method reduces the dimension of the parameters appearing in the differential equations, and the second method eliminates the parameters describing the initial state at time zero. Finally, it is shown how the results can be back-translated to a core-box model; a model with all the details of the original grey-box model, but with quality tags from the core model to its possible predictions.

The new methodologies are applied to the development of three models. The first is a core-box model for insulin signalling in fat cells. The core model is obtained using hypothesis testing, which shows that the internalisation is necessary to generate the observed dynamics, and that the internalisation is of the same time-scale as an observed overshoot in the data. The core-box model is fully integrated, which means that the core and grey-box model may be considered as two aspects of the same model, with the only difference being the degree of ‘zooming’. The second model describes oscillations in yeast glycolysis. Many of the developed methods are applied, and their performances are demonstrated. This leads, e.g., to a refined prediction of the biochemical mechanisms generating the oscillations. The third model describes muscle metabolism under anaerobic exercise. The developed model is used to solve a 25 year old contradiction between data and current biochemical understandings regarding the control of glycolysis following anaerobic contraction. Finally, an existing model of neutrophil metabolism is analysed. The main conclusion is that the model’s apparent lack of robustness lies on the structural level and not on the parametric level. All these contributions show, in various ways, how the combined usage of models, biochemical characterisations, and quantitative in vivo data can work together in a fruitful way; a way which surpasses the abilities of either of these research fields working in isolation.
Preface

This dissertation contains contributions of many different types and I have, of course, been involved in the developments of all these contributions. However, the work has typically been done in collaboration with others, and in some projects I have not even done the majority of the work. Further, many parts of the presented material have already been published, and several other parts are at various stages in the process towards publishing. Now follows an attempt to account for these aspects of the dissertation.

The dissertation is divided in two parts: one for theoretical contributions, and one with applications to specific systems related to glucose homeostasis. The first part of the dissertation is divided in the same order as the presented modelling framework: core-box modelling. This framework is a contribution in itself, even though the contributions in the different sub-results are more important. The idea for this framework has primarily been developed by me, and it was first presented in


A more mature presentation will be available in the following book chapter


These two are the only publications that at this point are relevant to the introductory Chapters 2 and 3. The results regarding structural identifiability in Chapters 4.2 and 10.2.1 is under revision in a slightly different form as


and the results have been developed primarily together with Milena Anguelova and Bernt Wennberg. Milena has made most of the algebraic calculations, and Bernt has laid much of the foundations for the general treatment of the problem. The material regarding algebraic estimation of in vivo kinetics, in Chapters 4.3 and 10.2.2, is the result of a work that has been going on for several years. Dan Fraenkel and Sune Dano have done the experimental work, and I have contributed with most of the theory in the here presented solution. There has been several persons involved in other (abandoned) attempts to solve the problem. Some of these people are: Rolf Wasén, Jan Aslund and Mats Jirstrand.

The methods developed for reduction to an oscillating core model in Chapter 5.2 and its application to the Hynne model in Chapter 10.3.1 is published as

and I have not been doing the main work in neither the method developments nor in the specific application. The further reductions to an identifiable core model and the following back-translations have been carried out together with Henning Schmidt, Bengt Söderberg and Mikael Sunnäker. The two latter are Master Students and have been the main developers of the MATLAB software. I have come with several of the original ideas, and have done most of the supervision of the work related to identifiability and back-translation. The other aspects have been done by the others.

A published version of Chapter 6.2 appears in


and a published version of Chapter 6.3 appears in


Both these last contributions were presented as Chapter 4 and 5 in my Licentiate thesis


and an early version of the basic ideas were presented in


The material has been developed almost exclusively by me, even though Carsten Knudsen has helped with some technical issues, and Lars Nielsen has helped with some valuable ideas regarding the presentation of the material in the Licentiate thesis. The work in Chapter 9 is in the process of publishing through the two articles


A previous version of some of the results was also published in my Licentiate thesis (mentioned above), and some related material was presented as a poster and talk at the ICSB2005 in Boston.

Master students Jenny Andersson and Erik Ulfhielm has helped with many technical details regarding modelling of the big model and on the first two steps, respectively. Peter Ströalfors has been the biological expert and has made sure that suitable experiments have been carried out. Jacob, Henrik and Mats have helped out with the modelling and identification, although I have done the main job.

The publications regarding Chapter 10 have already been mentioned in relation to the corresponding theoretical contribution above. The work presented in Chapter 11 is a collaboration between me, the automatic control group in Linköping, and a group at medical biochemistry in Copenhagen. In Copenhagen Sune Danø and Mads Madsen have been the main developers of the model structure, of the biochemistry behind the reactions and of the handling of the variable pH. Björn Quistorff has helped with the data collection, and has also served as the biological expert on muscle cells. In Linköping Tibor Maksai has carried out much of the calculations, supervised by Jacob Roll and Henrik Tidefelt. I have been trying to mediate between all these different inputs, and have also done most of the final work and analysis. The results will be submitted as


The work presented in Chapter 12 regarding different types of sensitivity in a model for neutrophil metabolism will be submitted as

E. Jacobsen and G. Cedersund. Structural analysis as sensitivity measure in a model for activated neutrophils. *In prep.*, 2006

and an earlier version of the work is already published in


Elling has done the analysis using structural techniques, and I have done the analysis using parametric sensitivity. It could finally be mentioned that projects have been done, published and unpublished, during my Ph.D. time, that are not included in the dissertation, and that are therefore not mentioned here.
Acknowledgements

During the work that has led up to this dissertation there are also many people that have contributed in other ways, ways that are not seen in a publication list. I will now try to mention some of the most important such contributions, even though there will inevitably be people that are unmentioned.

First of all I want to thank my supervisor Mats Jirstrand. He has been my supervisor during my last 1.5 years, i.e., since I joined his group in Gothenburg, and it has been an important time for my own developments as a scientist. One of the main reasons for this is that I have been given the possibility to devote an almost undivided attention to my scientific projects, even though Mats positive attitude, and quick responses to questions have also been much appreciated. Furthermore, Mats has also read and commented on most of the material in this dissertation, something for which I am also grateful. The work has been made possible economically through funding from the EU commission project BioSim (contract nr. LSHB-CT-2004-005137), and through funding from the Swedish Foundation for Strategic Research (project nr. A3 00:89).

I also want to thank my two previous supervisors, Lars Nielsen and Carsten Knudsen. Carsten was my supervisor during my M.Sc. project, and he was also instrumental for the early developments of my first projects as a Ph.D. student. Lars was my supervisor during the Ph.D. time up to my licentiate thesis, and I especially remember the discussions during the final structurings of that thesis as both joyful and rewarding.

Likewise I want to thank Bo Egardt for allowing me to be associated to his group, and for providing a helping hand in the general developments in my Ph.D. studies (including the commenting of substantial parts of the text).

A person that has been with me as a sort of co-supervisor during my whole Ph.D. studies is Peter Strålfors. Your helpful and positive ways and your high-quality scientific inputs have been much appreciated, and I am looking very much forward to working even closer together in the future. Likewise I want to thank the Danish group consisting of Sune Danø (who has also helped with valuable biochemical inputs on parts of the texts in this dissertation), Preben Graee Sørensen, Bjørn Quistorff and Mads Madsen. To be able to work and discuss with you have been one of the corner stones in my entire Ph.D. time, and the Ph.D. school that was arranged by Sune and Preben in 2000 was one of the key events that convinced me to start working in this field.

Another person that has made similar key inputs during my very early developments as a scientist is Rolf Wasén. He was the one that first awoken my interest in the combination of mathematics and biology, and our interactions over the years have always inspired me to keep remembering the big picture. This is definitely true also for my old class mate Gustaf Ullman. Our frequent and long-term interactions have been both inspiring and important to help me remember what it is I am really working towards.

There are several people that have kept me company during this 5 year long scientific journey, and who have also provided much valuable general scientific inputs. Some of the most important such research colleagues are: Mattias Krysander, Robert Blomgran, Henning Schmidt, Jacob Roll, Erik Frisk, and Dan Luciani. Other people that have given me balance in my life are my friends in Bunten;
my yoga friends: Anna Mattsson, Eyal Kropnick, and Erika Bergstedt; my many friends from music and dancing; and my family: Mamma, Pappa and Ollus.

Finally, it is my intention that this dissertation should bring science some important steps closer towards an understanding of life, in particular regarding the relationship between life and biochemistry. However, it is my strong conviction that our understanding of life is instrumental in the creation of our general world-views, i.e., the world-views that guides us in our everyday lives, and the world-views that helps us to decide how to treat each other and how to form our societies. Since I believe that it is on the world-view level that most of our bigger problems lie, it is my hope that this dissertation will contribute to the achievement also of these larger goals. I therefore end by dedicating this dissertation to the continued Growth and Joy of Humanity, and to all people that are taking a conscious part in that development.

Gothenburg, September 2006

Gunnar Cedersund
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Introduction

This dissertation falls within the field *systems biology*, a new and exciting research field that might come to drastically change the traditional approaches to biology – the science of life itself. However, even though systems biology as a term is only widespread since less than a decade, similar efforts and the basic building blocks have been around for much longer than that. In this chapter we will try to put systems biology into a historical perspective that enlightens this, and which also puts these recent trends into the bigger picture of the general developments within science as a whole. We will also take a little closer look at what the basic tools within systems biology are, and especially at those that are in main focus in this dissertation: mathematical models. After these basic concepts have been introduced the main objectives of the work are stated. The chapter ends with an outline of the main contributions. It is the intention that this chapter, except for Section 1.4, should be readable to a wider audience than only those involved in the field, and that all readers willing to give it a serious try should at least get a feeling of what this dissertation is about.

1.1 Perspectives of systems biology

First we consider the developments of systems biology in the larger context of the history of science as a whole. We will see that its emergence is paralleled by similar developments in other research fields, and that it is part of the reversal of a trend that has been going on since the start of modern science. We will then take a closer look at the central approach within systems biology - to consider nature as a system of systems - and finally introduce some of its most important sub-disciplines.
1.1.1 Systems biology in the history of science

The urge to understand oneself and the world one inhabits seems to be at the heart of what it is to be a human being. There are very early evidences of religious rituals for the burial of the dead, which point to the desire to understand the eternal questions of life and death. Similarly there are early evidences of predictions of astronomical phenomena, which point to the desire to understand the physical laws governing the universe. Finally there are early evidences that natural occurrences of the Fibonacci numbers and the golden mean have been discovered, which points to the desire to understand nature through the usage of mathematics.

Evidence of all these things are found, e.g., in the old Greek civilisation. Some of the greatest thinkers in that age are Plato, Socrates, and Euclid, and it is noteworthy that these thinkers considered all aspects of science. They made no clear distinction between what we call religious belief, mathematics, social sciences, and rhetoric, but considered all these subjects as interconnected and part of the same general search for truth. Further, they believed that all these subjects should ideally be part of the same general education.

This ideal was one of the revived concepts during the Renaissance, an era when many of the ideals from the Greek civilisation were readopted. *Uomo Universale* was the name of a man who impersonated this ideal, and he was expected to be well-educated in every cultural form from art to arithmetics, and from physics to philosophy. Leonardo da Vinci is referred to as such a man. In the early Renaissance it was also believed that everything in nature was interconnected as a whole, and that the same symmetries in nature are to be found at all levels of complexity, and in all aspects of life. Therefore, when Kepler designed his laws for the elliptic motions of the planets, he based these on the assumptions that the planets where interconnected as a whole, and that this whole could be interpreted as another manifestation of the same symmetries as those found in the musical scale. Likewise, when Galilei and Newton wrote about their scientific inventions they did that in a continuous text where their religious beliefs appeared side-by-side with the scientific methodologies [6].

Nevertheless, the Renaissance was the era when the modern scientific methods were born, where the mathematics became refined enough to make precise and convincing predictions, and where the first major conflicts between science and religion appeared. For instance, even though Galilei was teaching the grand dutchess Christina about everything from physics to biblical interpretations, and claimed that there is no conflict between the two, his works were condemned by the church, and he was put in house arrest for the last period of his life [6, 45].

Division and specialisation

Because of these conflicts it is maybe not surprising that the developments of the scientific method was followed by a specialisation within science. The scientific questions were narrowed down, and separated from each other. The scientific and objective method so successfully applied in the field of physics was held against the vague and 'unscientific' statements within philosophy. Where previously these two approaches were considered as complementary ways to understand different
aspects of the same eternal truths, which should ideally be used by all scientists, they were now beginning to be considered as opposing methodologies, pointing in different directions. In this way the previously all-encompassing field philosophy was broken down into a multitude of sub-disciplines. First came physics and the other major disciplines like chemistry and biology, but eventually more and more narrow subjects have been invented: organic biochemistry, abstract algebra, high energy particle physics, etc. In this process philosophy, and especially its devotion to the bigger questions, has become more and more marginalised and outside the prime concerns of science. This trend had its first major climax during the Age of Enlightenment, in the end of the 18th century, which was then followed by a trend of a more intuitive and emotional character during Romantic Age in the 19th century.

So far, however, it had mainly been physics and later technology that had been showing the most convincing successes, and it was mainly the findings of physics that had collided with the teachings of the church. Physics was therefore held up as the ideal science, and the methodologies within physics were sought to be applied within all other sciences as well. However, in the second half of the 19th century a new collision between science and church occurred; this time due to the developments within biology. Charles Darwin's two publications [33] and [34], and the following outbreak of discussions between and within the religious and the scientific communities, clearly show what an impact scientific results within biology may have, and how threatening it might feel when the believes about life itself are questioned [6].

The Darwinistic view of an eco-system has many similarities to the Newtonian view of matter. Both picture their system as consisting of isolated entities (atoms or species) which sometimes interact. Further, both picture these interactions as completely local processes, occurring only between the entities that interact. Finally, both picture that the interactions are done by the isolated entities without taking the systems perspective into account. It should be noted that this radically egoistic view has changed slightly within this framework, since it has been noticed, e.g., that collaboration also is an important factor in evolution. Nevertheless, this Newtonian view of an ecosystem has been the prevailing view during the 20th century.

Likewise, an almost exclusively reductionistic approach has been the predominant view in cell biology and biochemistry; the main study object has been the part, often studied isolated from the whole. Biochemical interactions have been studied one by one, by isolating enzymes from the cells and studying them in test tubes (in vitro). Such studies were at their height in the 60s and 70s, when the mechanistic actions of a vast number of enzymes were characterised in great detail. Thereafter, the characterisation of the human DNA was considered as the riddle that would solve everything. That lead to an enormous effort into its characterisation, which was coordinated through the HUGO project. This project was completed in 2003 [27]. The time after this is referred to as the post-genomic era, and while it should be acknowledged that many things are experimentally possible because of this characterisation, it is clear that an understanding of the complete workings of the cell is still far from obtained. One could therefore say that the 20th century biology have to a great extent been devoted to the characterisations of the
individual components in the cells, that this process has in many ways reached a partial completion, and that this has now led to an increased interest for considering the workings of the intact systems. This increased interest is further fueled by increased experimental possibilities that suddenly allows for high-throughput quantitative measurements of intact cells and organisms. All these developments have been important factors for the subsequent emergence of systems biology.

Finally, the same atomistic and reductionistic views have been predominant also in many other research fields during the 20th century. In cultural history the main view have been to consider each culture as an isolated entity, which changes internally and occasionally interacts with other similar entities [140]. The main study object in religion and philosophy has also been on the isolated parts. Individual texts and sentences have been analysed in great detail, and the big picture has often been neglected. And just like the main objectives in biology have been to characterise and systematise the individual biochemical components, e.g., in systematic biology, systematic religion and philosophy have been the prevailing fields for building new theories. It is therefore interesting to note that similar changes towards a systems approach are gaining interest also in these fields.

Precursors to systems biology

The first field that reached the above mentioned exhaustion of the atomistic view is physics. In the end of the 19th century, physics was believed to be a 'dead' subject. All natural laws had been discovered, and it only remained to determine some natural constants to a greater certainty, it was believed. As we all know, this turned out to be completely wrong. The first half of the 20th century was devoted to the developments of a fundamentally different world-view, primarily through the paradigm shifts in mathematics and physics. One important contribution came from Gödel [47], who showed that a scientific theory can never prove its own consistency, i.e., that the consistency has to be verified by something outside the system. Some even bigger changes, however, were due to developments of relativity theory and quantum physics. These showed that the Newtonian concepts of a linear and independent time, and of a world of separated particles, had to be abandoned for an interconnected world, which also includes time [36, 98]. Physics is therefore the first discipline that has changed from a paradigm consisting of isolated particles that sometimes interact, to a paradigm where all the components are inseparable from the system.

The 20th century is, to a great extent, also the century of the big technological revolution. It is therefore interesting that some rather early precursors to systems biology are evident in technological societies. One such example is the field of cybernetics, initiated by Norbert Wiener in the first decades after World War II [134]. This field had as the intention to come up with a theory that encompasses, e.g., technology, biology and psychology. Even though many of the biggest interdisciplinary promises have still remained unfulfilled, these developments were conceptually important, and they are often ascribed to the field systems theory. Some decades later came other important systems theoreticians like Robert Rosen [107] and Mihajlo Mesarovic [87]. These scientists were more purely devoted to biological systems, and they are important forefathers to the concepts within sys-
tems biology. They both developed comprehensive theoretical frameworks for a theoretical treatment of biological systems. It is noteworthy that these frameworks include both the interactions between the parts and their impact on the whole, and the ability of the whole to affect the parts. However, these early precursors within systems theory usually stayed at a theoretical level, and were therefore rather unnoticed in the biological societies.

Some similar works, but later and therefore more simulation based, were developed by theoretical physicists. Their models were typically not constructed out of the mechanistic understanding of a specific system, but instead built on more abstract ideas and on analogical images of the general properties shared by many biological system. These models were therefore not intended to make precise predictions, but instead to come with general statements of biological processes, e.g., regarding self-organisation and evolution. Examples of such works include those of Stuart Kauffman in the 90s [66], and the Hopfield neural networks [59].

There have, on the other hand, also appeared models closer to the mechanistic details in a specific biochemical system. As mentioned above, the 60s and 70s were much devoted to characterisations of individual enzymes. The action of an enzyme is actually a little mini-system in itself, and in the characterisations of these single enzyme systems mathematical modelling was an essential and natural ingredient, acknowledged by both biologists and biochemists. These models, however, described non-living systems of a rather limited complexity. During this time some simple models describing complete systems in intact cells, e.g., glycolysis were also developed [12, 48, 49]. However, these latter systems lacked the experimental data to make them useful for real predictions, and they therefore too had a little impact in the biological societies.

The emergence of systems biology

Gradually, these simple models for the intact systems grew in comprehension and mechanistic realism. Likewise, the experimental possibilities to measure many of these details on the intact systems (in vivo) became more developed. This has allowed for increased possibilities both to characterise the models quantitatively, and to validate or reject the various model predictions beyond course qualitative assessments. These possibilities have been available for technical systems for several decades, and have fueled the theoretical developments of systems theory. One important sub-discipline is system identification, which is devoted to the development and evaluation of quantitative models, and scientists from this field have gradually entered the biological modelling societies as well. All these things together have allowed for modelling to come with non-trivial contributions to specific and important biological questions. This increased predictive power of the models has of course increased the interest for modelling in biological societies, and has led to the emergence of systems biology.

Another factor to this is the completion and exhaustion of some of the naive reductionistic approaches to biology (mentioned above) which have further increased the willingness among the biologist to adopt a systems based approach. Further, the drastically increased amounts of available data have also forced biologists to seek computer aided methods for data interpretation. This latter need first led to
the emergence of the field bioinformatics. Bioinformatics also included some modelling but, since the subsequent emergence of systems biology, the mechanistically based modelling has mostly been ascribed to this field.

It is interesting to note that these changes are paralleled by many similar changes in other scientific disciplines. In cultural history the concept of isolated cultures is now replaced by the concept of a continuously developing whole [140]. Likewise, systems philosophy and process thought are rapidly growing fields within philosophy and religion that considers the universe as a developing whole. These theories also include the latest developments within physics and biology, and are therefore no longer leaving the bigger questions aside [6]. On a higher biological scale it is also becoming more acknowledged that the study of ecosystems must be done also on the systems level, and that an eco-system can be considered as a kind of living organism [57]. It is also apparent that all these disciplines are more and more acknowledging their interdependence, and collaborations over the classical borders between scientific disciplines is becoming more and more encouraged. Compared to the trends and views that have been predominant, more or less since the birth of modern science in the 16th century, these new trends within science are thus of a fundamentally new character.

To conclude this historical perspective of the emergence of systems biology, we can therefore say that it is part of a large trend that is occurring in many other sciences. This trend is to leave the approaches that are satisfied with a systematic characterisation of the individual parts, which are believed to be possible to study independently of each other, to an approach that considers the study of the system as the prime objective, and acknowledges that the parts are intrinsically interconnected with each other and with the whole. This has also implied an increased integration of scientific disciplines, and it is thus in many ways the reversal of a trend that started several centuries ago. Finally, it is interesting to note that both the original trend and the current reversal have been initiated by the developments within physics.

1.1.2 Understanding nature as a system of systems

We have now seen how the emergence of systems biology can be understood as a natural outcome of the general developments within science. We have also seen that two of the fundamental features of systems biology are that it is the system perspective that is in focus, and that it consists of an interaction between many different sub-disciplines. We will now enlarge on the first of these features, and leave the exploration of the sub-disciplines for the next sub-section.

What the systems perspective is in a study depends on the object of study. In this dissertation the object of study is glucose homeostasis, and let us therefore take this as a first example. Glucose homeostasis is the regulation of the sugar level in a system, and the ultimate goal is to understand this regulation in the human body. Further, this regulation is malfunctioning in the rapidly growing decease Diabetes Mellitus, and it is therefore also interesting to understand how the decease makes the regulation malfunction.

A traditional biochemical approach would be to search for individual components that are involved in the homeostasis, and see if it is one of these that mal-
functions in the disease. An individual component could, e.g., be an enzyme, and the importance of the enzyme could be examined by knocking out the gene that codes for the enzyme, or by up- or down-regulating the amount that is produced in the cell. However, the problem with this approach in the case of Diabetes is that the disease occurs in many places at once, and that it is therefore more a malfunctioning on the systems level, than a malfunctioning of an individual enzyme.

A systems biology view of the same system would consider each enzyme as

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**Figure 1.1** The different layers of organisation in a human body.
contained in a network of similar enzymes, and where their interrelation is the
primary study object. The first level of organisation is a small group of enzymes
forming a module with one or several specific functions. A cell’s contribution to the
glucose homeostasis is then considered as the interactions of many such modules
(not all of them enzymatic). Further, the individual cell’s contributions to glucose
homeostasis is considered as a part of the action of many similar cells. Likewise
these groups of cells are grouped together in even larger groups, eventually forming
organs, which interact on the highest system level: the body. These different layers
of interaction are depicted in Figure 1.1.

A systems biology study would then ideally take place in a combined bottom-up
and top-down manner to this system of systems. The bottom-up approach would
start by understanding the smallest functional units. It would then consider the
next higher level of organisation, where each previous object is now a single unit,
and in this way work its way to larger and larger systems. A top-down approach
would instead start at the highest level, the body, and get an understanding at this
level of complexity. Once a model for this level is obtained, the relevant subsystems
are then expanded to a higher level of detail. In this way the top-down approach
works itself to gradually more and more complex descriptions of the whole. The
goal is then, of course, that the top-down and bottom-up approaches should meet
at some point in the middle. In any case, the fundamental aspect of a systems
approach is that one does not consider a unit (independently of which complexity
level that unit is) as a single entity, but as something which is made up as a system
of parts, and which is a part of a larger system.

1.1.3 Sub-disciplines to systems biology

As mentioned above, one of the central features in systems biology is that it is
done by a collaboration between scientists from different disciplines. Which these
are is of course dependent on the specific problem. In the modelling situations
encountered in this dissertation it is my experience that it is best if at least the
three competences depicted in Figure 1.2 are present. Let us now shortly describe
these three competences, as well as some other possible sub-disciplines in a systems
biology project.

Biology

Since the primary study object in systems biology is a biological system, biology is
of course an essential sub-discipline to systems biology (in a way one could even say
that systems biology is a sub-discipline to biology). Biologists are also those that
have the most solid knowledge about the specific system in question, a system which
they have often studied for many years. Further, it is usually the biology group
that performs the experiments since they typically are the ones with access to the
cells or organisms of study, and have access to the best experimental equipments.
However, the general training of a biologist includes no mathematical modelling,
and the handling of such aspects is therefore primarily left to the collaborators.
1.1 Perspectives of systems biology

Figure 1.2 The three major competences needed in a systems biology project.

Biochemistry and theoretical physics

To translate the knowledge about the biological system to a mathematical model is another essential part of a systems biology project. This is often beneficially done by biochemists since they both have the necessary training to speak to the biologists, and to formulate the available knowledge mathematically. Further, such a formulation often requires knowledge about enzyme kinetics, and rate expressions, and this is part of the main training in biochemistry. Finally, if the model is concerned with other types of biological systems, like those dealing with biophysical processes, electropotential changes etc., it is often equally beneficial if a physicist does the model formulation.

Systems theory

When both the model and the experimental data are available, they need to be compared and evaluated with respect to each other. If the comparison is done on an exclusively qualitative bases, e.g., by inspecting if both the model and data oscillates and does so with a similar frequency, or if a given signal goes up or down upon a certain stimuli, this requires no special training, and can be done, e.g., by the people doing the modelling. However, if the full benefit of modelling is to be obtained a quantitative comparison should be done. A quantitative comparison means, e.g., that the exact shape and absolute values of the model simulations and experimental data are compared. Further, a model is most useful if it is determined which parts of the model that are well-determined by the data from the intact system, and which parts of the model that are just based on the characterisations
done on the individual parts. Such analyses are non-trivial and requires special
competences typically not included in the standard training of neither biochemists
nor physicists. It is, however, a part of the background for a systems theoretician,
and the task of evaluating the relation between the data and the model is therefore
often beneficially assigned to scientists with a background in systems theory. It
should also be added that the primary view point of systems biology taken in this
dissertation is that of a systems theoretician.

Other sub-disciplines

There are also several other sub-disciplines that make significant contributions to
the systems biology developments. One such is computer science, since it is en-
abling the necessary software for the modelling. Computer scientists are also much
involved in one of the research fields preceding systems biology: bioinformatics.
Bioinformatics also involves some modelling, but it is more involved with the hand-
ling of the large data sets generated by the new experimental techniques, i.e., to
data mining. Bioinformatics is also a field which receives inputs from several other
sub-disciplines, and some of these are also bringing contributions to the systems
biology community. Such disciplines include statistics, informatics, visualisation
and image analysis. However, at least with respect to the aspects studied in this
dissertation, those contributions are minor.

1.2 Understanding nature with the aid of mathematical modelling

This dissertation takes the perspective of a systems theoretician, and does therefore
deal extensively with the modelling aspects of systems biology, and especially with
the comparison between the model and the data. The kind of models that are
developed in systems biology applications, however, are not models developed for
technical systems. We will now give a short introduction to some of the basic
differences between these modelling traditions, and then turn to the modelling
framework that will be developed during the rest of the dissertation.

1.2.1 The concept of a 'true' model

A model for a system is not the same as the system itself, but an abstraction.
Such an abstraction can be useful in many circumstances, e.g., to study certain
aspects of the real system, or to make a prediction of what the real system
would do. In relation to this it is often assumed that the real system works like
a model. Even though this might not be exactly like the model that is found in
the developments, it is often assumed that there is a model that behaves exactly
like the system. One then refers to this particular model, as the 'true' model for
the system. This assumption is primarily done for practical purposes, since it,
e.g., allows for a facilitated interpretation of the agreement between the model
predictions and the data.
However, one could also argue that the components and interactions in a ‘true’ model correspond exactly to the components and interactions in the real system. Such a standpoint is referred to as realism, and it is not taken in this dissertation. The opposite standpoint to this is to treat models as purely predictive entities, and where the relation between the components and interactions in the model, and the components and interactions in the real system is completely arbitrary and irrelevant. This is a more pragmatic standpoint, and it is often sufficient for technical applications, where a model is primarily used to predict the response of a system to a certain stimuli. For biological applications, however, the relation between the components and interactions in the model and the real system is of interest. This is the case since the modelling is often used as a tool to draw conclusions about what the components and interactions in the real system are, and the accuracy of the model predictions are mostly seen as a way to indirectly assess these things. With such a goal it is important what the relations between the inner workings of the model and the system are, and the inner workings of a model (formalised in the model structure), is therefore developed to mimic certain features of the real system. This, however, does not mean that there is an exact match between the model and the real system, but only that there are correlations. Such a standpoint falls somewhere between the realist and the pragmatic view, and it is referred to as critical realism. This is the standpoint taken in this dissertation.

### 1.2.2 Different kinds of models

When formulating a model there are many choices to be made. There are, e.g., many types of equations to choose between: stochastic differential equations, static model, delay equations, etc. Apart from these choices there is also the choice of what the model should describe, and to which end it is supposed to be used. These leads to different kinds of choices, that we will now consider. More technical details regarding different equation types are given in Chapter 2.

#### Graphical models

A graphical model is a model type that is often created by biologists. It is not a mathematical model, but just a graphical representation of what the components are, and some course information about the interactions in the system. Such a graph will in this dissertation be referred to as an interaction-graph, and an example is given in Figure 1.3. In the modelling process outlined in the next chapter, this will be the first step in the modelling development.

#### Dynamical and static models

A graphical model does only show the basic interactions, but does not give any details to the strengths of the interactions. To quantify this it is easiest to translate the graph into a mathematical model. Further, the graphical model does not give any information to when the interactions occur, and how they change over time. It does also not give any information about how the different components included
in the model change over time. If time is an explicit factor in the model we speak about time-dependent models. Furthermore, if time is involved in the relations between the variables in the model we speak about dynamic models. Note that we may have a situation where there is, e.g., a time-dependent input, but where the other variables in the model are given instantaneously by this input. Such a model would be time-dependent but not dynamic. In this dissertation we will typically deal with dynamical models, since it is the time-evolution within the system that will be our primary concern. However, some technical aspects are simplified if time can be disregarded, and we will therefore also sometimes use models with purely static relations, e.g., when examining the rate of a single reaction.

**White-, black-, and grey-box models**

It was mentioned above that models in technical applications are primarily used to predict the output of a system, given a certain input. That means that the model does not have to mimic what happens in-between the input and the output. If this is the case, the model can be thought of as a black box, that in some ‘magic’ way returns the correct answer, but where the inner workings are unknown. This black-box is then adjusted to the available data to make as good predictions as possible. Such a model type is referred to as a black-box model (see Fig. 1.4). The opposite to this is a white-box model, where the inner workings of the model are completely specified by the previous knowledge of the parts. A white-box model is not at all adjusted to the available data for the whole system. For small systems, that are described by primarily physical processes, this is often sufficient to get
1.2 Understanding nature with the aid of mathematical modelling

![Figure 1.4 A black-box, a grey-box and a white-box model. The name black-box model is chosen because the internal workings of the model are unrelated to the physical processes, but just thought of as a 'magic' box that returns the right output for each input. The star depicts the physical processes going on inside the system. As can be seen, these are clearly seen in both a white- and grey-box model.]

Figure 1.4 A black-box, a grey-box and a white-box model. The name black-box model is chosen because the internal workings of the model are unrelated to the physical processes, but just thought of as a 'magic' box that returns the right output for each input. The star depicts the physical processes going on inside the system. As can be seen, these are clearly seen in both a white- and grey-box model.

While models in technical applications primarily are used for predictions, models in other research fields may have completely different purposes. A model might, e.g., be used to collect and structure the current knowledge about the system. It might also be used to reason about the system, but without comparing the output of the model as a whole, with corresponding data. Such a knowledge storage function of a model is often the case, e.g., for the big biochemical graphical models available...
for large parts of the metabolism in a cell. If a model is used for prediction, it is referred to as a predictive model, and if it is used to describe the system, and to store the knowledge about the system, it is referred to as a descriptive model. It is one of the main objectives of this dissertation to achieve a combination of these two models, i.e., a model that allows for predictions where possible, but that stores also such information that is not sufficient for accurate predictions.

Minimal and full-scale models

Finally, there is an important difference between minimal and full-scale modelling. A minimal model includes only so much complexity as to fulfil the given purpose, and to give the required predictions. If it should be discovered that there are parts of a model that are not necessary to fulfil the purpose with the model, and that are not necessary to obtain the given predictions, these parts would be eliminated in a minimal model. A minimal model is therefore as small as possible, and this usually means that important information is obtained during the model developments. It is, e.g., concluded that all the interactions in the minimal model are crucial for the observed behaviour, i.e., one can draw information about the importance of different interactions. Further, it is also concluded that some interactions are redundant in the generation of certain behaviours. Redundancy is a common feature in biological systems, and one of the reasons for this is that redundancy leads to increased robustness; if one mechanism breaks down, other mechanisms can take over. This is another way in which a minimal model might give an increased understanding for the biological system.

A minimal model is usually built up in an iterative process, where gradually more and more complex models are tested. A full-scale model, on the other hand, is usually constructed by putting all the knowledge there is about the system together in a model structure, and only thereafter are the model parameters adjusted to achieve the desired predictive powers. This means that the predictive powers of a full-scale model are the same as that of the minimal model, but that the descriptive ability is much higher. However, a full-scale model that is developed as above does not include any of the important information about which aspects that are crucial for the observed behaviour, and which aspects are superfluous. Further, a minimal model can often be analysed to get even more precise statements regarding the accuracy of its various predictions. Such quality tags are much harder to achieve for a full-scale model. In this dissertation we will develop a combination between these two model types. It will have the descriptive ability of the full-scale model, and the detailed information about the parts and the quality of the various predictions of the minimal model.

1.2.3 The modelling process

We have already touched upon the modelling process in relation to the minimal and full-scale model, but we will now expand a little bit more on the subject. The basic sequence in the type of modelling pursued in this dissertation is illustrated in Figure 1.5. On the left the input is given. This consists of two parts: experimental data, and prior knowledge about the system. The prior knowledge might, e.g., be
1.2 Understanding nature with the aid of mathematical modelling

available in the form of a graphical model for the system, i.e., a set of hypotheses for which components and interactions that are present in a system. These assumptions are then put together in a model structure, and the resulting model is adjusted to the given estimation data. If a satisfactory agreement between the model and the estimation data is obtained, the grey-box model is then compared with another data set, the validation data. Depending on whether this agreement is satisfactory or not one of the following two conclusions are made:

- If the given model shows an unacceptable agreement with the given data this means that some of the hypothesis are wrong, or that one or several hypotheses is missing. This is an important information, which is often hard to conclude without a modelling approach.

- If the given model shows an acceptable agreement with the qualitatively different validation data, this is an indication that the model has captured some of the properties of the system, and that it has a predictive power. The model can then be used in various circumstances, e.g., to replace some initial animal tests when developing a drug.

This means that both possible outcomes imply a step forward in the developments, even though it is of different kinds depending on the result of the validation test. Finally, it is important to notice that even though a model is validated with respect to qualitatively different data sets than the ones used for the estimation, this is not a guarantee that all aspects of the model have been validated. This is especially true for a full-scale model, which typically includes much redundancy compared to the available measurements. To achieve information about which parts of a model

Figure 1.5 The basic cycle in a modelling process.
that have been validated, and to what extent, without having to resort to purely minimal models, is one of the key contributions of this dissertation.

1.3 Objectives

We have now introduced the basics of systems biology, and introduced the most important components to mathematical modelling; we are therefore ready to introduce the objectives and contributions of this particular dissertation. The dissertation divides in two parts. The first part deals with theoretical developments and new methodologies, and the other part deals with concrete modelling work with the main objective to increase the understanding of specific biological systems.

Theoretical contributions

As we learnt in the previous section, two of the most relevant modelling traditions to systems biology are full-scale grey-box modelling, and minimal modelling using hypothesis testing. These two types of models have complementing strengths and weaknesses. The strengths of the minimal model is that one can draw many conclusions during the development phases, and that the predictions are associated with a quality tag. The strengths of a full-scale grey-box model is that all the detailed knowledge about the mechanisms in the system are included, but as we learnt above, this often comes at the price of a limited knowledge of how accurate the various parts have been determined. The main objective with the first part of the dissertation is to find a way to combine the strengths of these two modelling types.

More specifically, the main objectives are

**Figure 1.6** A core-box model is a combination of an identifiable core model, which has the strengths of a minimal model, and a full-scale grey-box model, which has the strength of detailed predictions and more information about the parts.
1.3 Objectives

- To specify a modelling framework that allows for the development of a grey-box model containing a well-characterised core. This combination will be referred to as a core-box model (see Figure 1.6). The big model should contain all the detailed information about the mechanisms, and the core should contain those parts of the model that are based firmly in the data from the intact system. Further, a translation between the two aspects of the model should be established, and the advantage of the new methodology should be illustrated on an example.

- To identify the most important sub-steps in this modelling framework; to present a review of existing methods for these steps; and to present new methods especially developed to fit into this framework.

- To deal both with the problems of unidentifiability in single rate expressions, which are common in many metabolic models, and with problems of unidentifiability in large mass-action kinetic based models, common in signalling networks.

- To present improved methods in the case of oscillating models, since this is a common feature in many biological systems.

Applications to glucose homeostasis related systems

The developments in the first part of the thesis should both be motivated by and applied to concrete modelling situations on systems related to glucose homeostasis. The strengths of the new methods should be demonstrated, and the strengths of the combined core-box model compared to the corresponding grey-box and core models should be exemplified in illustrative examples. On the other hand, it is not always the case that a complete core-box model needs to be developed, and the chosen model type should be chosen as the one most appropriate for the given biological question.

The following five biological questions should be answered by the modelling:

- When insulin comes to a human fat cell, it binds to an insulin receptor which then sends this signal to the rest of the cell. However, at the same time the receptor leaves the cell membrane to the cytosol, a process that can only be indirectly observed in the available data. What are the main conclusions that can be obtained from the given data with respect to the mechanisms involved in the transport of the receptors to and from the cell membrane?

- Glycolysis is part of the main conversion from sugar to energy, and it has been extensively studied. However, most of its reactions have only been characterised outside the cell (in vitro). What are the relations between the rate expressions and parameters obtained in such studies, compared to what can be indirectly deduced from observations in intact cells (in vivo)?

- Glycolysis is often in a state of oscillations, i.e., a constant movement from high values to low and back again. What are the main interactions in the system leading to this behaviour in the case of yeast glycolysis?
From observations of the metabolism in muscle, it has often been concluded that there seems to be an unknown control of the flux through glycolysis after a muscle cell has done work in the case of no oxygen. Can this conclusion really be drawn from the available data?

Metabolism in activated neutrophils shows a clearly oscillating behaviour. It, however, seems to be difficult to describe this behaviour in few states. What are the reasons for this?

1.4 Outline and contributions

We will now see how the fulfilment of the given objectives relates to the structure of the dissertation. In the process we will also shortly explain what the main contributions are in each chapter. We again divide the material in the same two parts: theoretical contributions and applications to glucose homeostasis related systems.

Theoretical contributions

The core-box modelling framework is closely related to grey-box modelling, and the basic methodologies in this framework are reviewed in Chapter 2. The shortcomings of most grey-box models appearing in the literature today is that they do not provide a quality tag to the various model predictions. The reasons for this is explained in Chapter 3, where the new core-box modelling framework is proposed as a way to solve the problem. A core-box model combines a minimal core model, with established identifiability, with the detailed descriptions available in the grey-box model. The basic new steps in the framework are identifiability and model reduction to an identifiable core model, system identification of the core model, and back-translation of the results to the original grey-box model. Methods for all these steps are then reviewed and developed in the remaining chapters of Part I.

Chapter 4 deals with structural and practical identifiability. Improved methods for unidentifiability in single rate expressions are presented. Structural unidentifiability may occur when all variables from a conserved moiety appear in the same rate expression, e.g., in dehydrogenase reactions. The reasons for this is examined and improved methods for handling of the problem are given, e.g., using linear algebra. Two new methods for handling practical unidentifiability are developed and evaluated. The proposed method is a special case of the core-box modelling problem, that is especially developed to compare in vitro with in vivo characterisations. Chapter 5 shows how these results can be used to help in the reduction towards an identifiable core model. We present a general methodology, with a MATLAB implementation, to obtain practically identifiable models in the case of measurements of all variables. For oscillating models this may then be combined with the reduction to an oscillating core, for which two specialised reduction methods are presented.

Two specialised methods for system identification of oscillating models are also presented. This is done in Chapter 6. The first method is applicable if the input signal corresponding to a Hopf bifurcation is experimentally determined, and shows
1.4 Outline and contributions

a reformulation of this information into a standard constrained optimization problem. Two methods for solving the new problem are presented. The second method is applicable if the system is operating close to the Hopf bifurcation. The parameters describing the initial state are reformulated in minimal degrees of freedom, and solved for in a separate sub-problem for each step in the optimization of the other parameters. It is shown how the sub-problem can be solved in a straightforward manner without integration of the differential equations, and without problems with local minima. This is possible because a combination of a center manifold and normal form reduction is used, which reveals the special structure of the Hopf bifurcation. The advantage of the methods are exemplified on the Brusselator.

Finally, Chapter 7 treats the problem of back-translating the results for the core model to the core-box model, which is then ready to be analysed. Methods are given both for the forward translation $\Phi$ and for the backward translation $\Psi$ for two general models describing the same system. Improved methods are given for structural identifiability, and for the case of structural unidentifiability in single rate expressions caused by conserved moieties analytical translations are provided. Analytical translations are also provided for two common reduction techniques: sensitivity analysis based methods, and variable lumping. Back-translations of both these two types of reductions fulfil a memory constraint, which means that the resulting core-box model will be fully integrated. This means that one may treat the core and the grey-box models as two versions of the same model, with the only difference being the degree of 'zooming'. Finally, the advantages of the core-box model compared to both the grey-box and core model is demonstrated on a simulation based example.

Applications to glucose homeostasis related systems

The second part of the dissertation starts with a review of the whole-body glucose homeostasis; the remaining chapters then present works that are improving the current understanding of some of the most important sub-systems to this homeostasis.

Chapter 9 develops a model for insulin signalling in human fat cells. It is a three-layer core-box model where the middle layer is obtained using hypothesis testing. These tests reveal that a significant pool of internalised free insulin receptors, and recycling of this pool to the membrane are necessary to accurately describe the given data. A three-state minimal model is obtained by model reduction, and a grey-box model is obtained by the addition of known mechanisms in the system. Translations between all these models are established, and the above mentioned memory constraint is fulfilled.

Several of the methods presented in Part I are especially designed for the complexities of the Hynne model for yeast glycolysis. These methods are applied to that model in Chapter 10. The dehydrogenase expressions are replaced by structurally identifiable expressions, and the problems with practical unidentifiability in the phosphoglucoisomerase reaction are handled using the methods in Chapter 4. Finally, the oscillating core of the model is established with a quality tag, and the new MATLAB implementation reduces a third of the parameters in the model.

Even though muscle metabolism accounts for a majority of the energy consump-
tion in the body, it has long been believed that there is a fundamental problem with the understanding of the control of anaerobic glycolysis following sub-maximal contraction. This 25 year old problem is solved in Chapter 11 through a modelling approach. It is shown that the previous assumption of equilibrium in the creatine kinase reaction leads to erroneous results. If this unnecessary assumption is removed, ADP and AMP might do a jump sufficient to cause the observed off-switch of the glycolytic flux. This novel interpretation has the important conceptual implication that ADP and AMP may serve as flux indicators for the given experimental conditions.

Finally, in Chapter 12 the robustness of a model for the oscillatory metabolism in neutrophils is examined. It is shown that the apparent lack of robustness lie on the structural level, and not on the parametric level. This low robustness explains the experienced problems with reductions, and it also shows that a parametric robustness analysis should be interpreted with great care, if it is not complemented with other types of analyses.

All these contributions give new insights into various systems related to glucose homeostasis. The different chapters also show, in various ways, that a systems biology approach – integrating mathematical modelling, quantitative in vivo data, with biochemical characterisation – surpasses the strengths of any of its sub-disciplines working in isolation.
Part I

THEORETICAL CONTRIBUTIONS
Grey-box Modelling

We will now review the most important components that make up the modelling framework that our developments start off from: grey-box modelling. The term grey-box model was shortly introduced in Chapter 1, and we will now more specifically introduce the framework when implemented by nonlinear ordinary differential equations (ODEs). ODEs are introduced in Section 2.1. A grey-box model uses a model structure which is implied by the physical components and processes in the system. How these parts are formulated mathematically for our type of systems is reviewed in Section 2.2. In Section 2.3 and 2.4 we describe how these parts are quantified and put together to form a white-box model. The final tuning and analysis of the grey-box model is described in Section 2.5.

2.1 Nonlinear ordinary differential equations

We will now introduce the basic notations for a system of nonlinear ordinary differential equations (ODEs). ODEs are a common type of equations used to describe time-varying processes in as widespread areas as biology, physics, chemistry, engineering and economics. There are, on the other hand, alternative choices, and we therefore start by shortly describing some of the details regarding this choice. The term 'differential' means that there are derivatives appearing in the equations, and the term 'ordinary' means that there only appears derivatives with respect to time. The equations therefore describe how the state of the system evolve in time. The state of the system is described by a finite number of state variables, and there is one equation for each state variable. The term 'nonlinear' is mostly a historical artifact, and could just as well be left out [123].

Some alternative, and more general, choices would have been partial differ-
ential equations (PDEs) [42], or differential delay equations (DDEs) [35]. Both these descriptions can be arbitrarily well approximated by an ODE by increasing the number of states. PDEs and DDEs are therefore sometimes referred to as infinite dimensional ODEs. In this dissertation we do not need such general descriptions. Another more general equation type would have been the differential algebraic equations (DAEs) [72], but even though we will encounter some algebraic constraints, rewritings to ODEs will always be possible. There will thus not be any need for DAEs either in this dissertation. Some alternative, and more course, description choices would have been linear ODEs and time-discrete predictor models [77]. These are inappropriate because they do not reflect the knowledge about the physical processes in the systems. Yet another choice of equation type would be stochastic models, e.g., based on the Master Equation [13]. Such models are actually more correct versions of the ODEs, but for the systems in this dissertation we are always dealing with so many particles that the error associated with the ODEs can be neglected. The choice of nonlinear ODEs is therefore the most natural choice. Some additional reasons for the choice are related to the fact that there is a strong tradition within the systems biology community to use ODEs, and that there therefore exists many softwares and a well-developed theory for their handling.

The state vector in an ODE is assumed to fully describe the system at a given time-point, \( t \). Let this vector be denoted by \( x(t) \), and let the dimension of \( x(t) \) be denoted by \( n \). This is henceforth written as \( x(t) \in \mathbb{R}^n \), where \( \mathbb{R} \) denotes the set of all real numbers. Except for special cases, the explicit time-dependence will be dropped, and \( x = x(t) \). There might exist input signals to the system that are affecting the system, but which themselves are not affected by the system. Such signals are referred to as control signals, and they are denoted \( u \). The vector \( u \) is also a function of time, and its value will often be both known and possible to control in the experiment. Assume that the other inputs to the system (typically noise and disturbances) are constant, and included in the parameter vector \( p_x \). Let the time-derivative of \( x \) be denoted \( \dot{x} \), and let its relation to the states, parameters and inputs be governed by a nonlinear, smooth, function \( f \). With these notations the system of differential equations is given by

\[
\dot{x} = f(x, p_x, u) \tag{2.1}
\]

Sometimes it will be beneficial to consider the \( u \) and \( p_x \) vector together. Let this pair be denoted \( \mu \)

\[
\mu = (p_x, u)
\]

Note that \( \mu \) is a time-varying vector, and that all the time-dependence lies in \( u \). In an experiment there are certain measurement possibilities, using various sensors. Let the measured signals be given by the time-varying vector \( y \). Assume that the sensor values are a function of \( \mu \), \( x \), and perhaps some additional parameters \( p_y \). Let this functional relationship be described by \( h \)

\[
y = h(x, \mu, p_y) \tag{2.2}
\]

The time when the simulation starts can be given or chosen as part of the experiment design. Let this start time be denoted \( t_0 \) and let the state vector at this time
be denoted \( x(t_0) \). Let \( x_0 \) be the parameter vector giving the start values

\[
x(t_0) = x_0
\]  

(2.3)

In most of the thesis we will have chosen the start time so that it is zero, i.e., \( t_0 = 0 \). In those cases \( x(0) = x_0 \).

Equation (2.1), (2.2), and (2.3) fully specify the system, and we now write these equations together for future reference. To fully specify which vectors are dependent on time, and which are not, the time-dependence is here explicit

\[
\dot{x}(t) = f(x(t), \mu(t)) = f(x(t), p_x, u(t)) \quad (2.4a)
\]

\[
y(t) = h(x(t), p_x, u(t), p_y) \quad (2.4b)
\]

\[
x(t_0) = x_0 \quad (2.4c)
\]

Notice that equation (2.4) allows for a unique predicted (simulated) value for each parameter set \( (p_x, p_y, x_0) \). Collect these parameters in a parameter vector \( p = (p_x, p_y, x_0) \)

To explicitly specify that a given \( y(t) \) is simulated, and dependent on a parameter \( p \), it is denoted \( \tilde{y}(t|p) \). Generally, a mapping from a parameter vector \( p \) to a predicted output \( \tilde{y}(t|p) \) is referred to as a model structure, and it is denoted by \( M \).

\[
M : p \rightarrow \tilde{y}(t|p)
\]

An equation system of the form (2.4) describes the most general model structure when modelled by ODEs. A common objective in this dissertation will be to use experimental data to choose, or estimate, a specific parameter, denoted \( \tilde{p} \), and so obtain an estimated model \( M(\tilde{p}) \). It is thus clear that the formation of a model structure is a central step in a modelling procedure. We will now see how this is typically done for our type of models.

### 2.2 Describing the parts

We will now turn our attention to how the building blocks of the models are formulated. In this thesis the variables of the models will typically correspond to concentrations, and the processes that affect these concentrations will typically be reactions and transport processes.

A chemical reaction converts reactants into products. The rate at which the conversion occurs might also be affected by some modifiers, which are species not themselves affected by the reaction. Consider a reaction occurring in a single compartment, with constant volume, temperature, and pH. Let its substrates be denoted \( S_1, \ldots, S_i \), and let the products be denoted \( P_1, \ldots, P_j \). The stoichiometric coefficients specifies the relative numbers of reactants and products that are involved in a reaction. Let these coefficients be denoted \( \nu_1, \ldots, \nu_{i+j} \). The reaction can then be written

\[
\nu_1 S_1 + \cdots + \nu_i S_i \rightleftharpoons \nu_{i+1} P_1 + \cdots + \nu_{i+j} P_j
\]  

(2.5)
Let the rate at which this reaction occurs be denoted \( v \), and let the kinetic parameters for this reaction be collected in a vector \( k \). Further, let the concentration of a substance \( A \) be denoted \([A]\). Assume that the rate is dependent on the concentration of the substrates and products, and on the concentration of some modifiers denoted \( M_1, \ldots, M_l \).

\[
v = v([S_1], \ldots, [S_i], [P_1], \ldots, [P_j], [M_1], \ldots, [M_l], k)
\]  

(2.6)

We will now consider two common rate expressions of this form.

**Example 2.1** The most basic example of a reaction rate is probably the one based on mass action kinetics. It can be derived from some reasonable physical assumptions [68]. The rate in each direction is modelled as being proportional to the product of the substrate concentrations. For a reversible reaction with substrates \( A \) and \( B \), and products \( C \) and \( D \)

\[
A + B \xrightleftharpoons[k_b\downarrow]{k_f\downarrow} C + D
\]

the rate expression is simply

\[
v = k_f[A][B] - k_b[C][D]
\]

(2.8)

The parameters \( k_f \) and \( k_b \) are the rate constants for the forward and backward reaction. They were implicitly introduced already by their appearance above and below the reaction arrow in (2.7).

**Example 2.2** Another common rate expression is the irreversible Michaelis-Menten rate expression with one substrate and one product. The most important difference between this reaction rate and the mass action rate is that the Michaelis-Menten version has a saturation. This means that the rate will never exceed a certain maximum velocity. This rate is given by a parameter denoted \( V_{\text{max}} \). There is one more kinetic parameter, denoted \( K_M \). It is interpreted as the substrate concentration for which the rate obtains half of its maximum velocity.

\[
v = \frac{V_{\text{max}}[S]}{K_M + [S]}
\]

(2.9)

This, and many other Michaelis-Menten like rate expressions, can be derived from a system of elementary reactions all described by simple mass-action kinetics, plus some assumptions about the relations between their kinetic parameters. This will be described in more detail in Chapter 5.

The principles behind (2.6) can be used to describe a model’s parts also under more general circumstances. For reactions occurring in multiple compartments and for transport processes the same expressions apply directly. The compartment that a concentration refers to is then denoted by an index, which means that \([A]_j\) denotes
the concentration of A in compartment j. If the compartments have different volumes, however, care must be taken when combining concentrations in a single rate expression. One way is to refer all concentrations to the same standard volume, and then account for the volume differences when combining the rate expressions into a model structure (see eq. (2.11) below). The kinetic constants are typically functions of pH and temperature, which means that these functional relations have to be treated in cases where the temperature and pH are not constant. An example of a system where pH is not constant is given in Chapter 11. Finally, even though the mass action and Michaelis-Menten expressions were originally derived for biochemical reactions, the same expressions are often used to describe more general transports, reactions and other processes on other scales, e.g., occurring on the whole-body level.

2.3 Collecting and selecting a model structure

We will now turn to the problem of collecting descriptions of individual processes (reactions, transports, etc.) into a complete model structure of the form (2.4). This process often involve the creation of an interaction graph. This interaction graph is then often converted to a stoichiometry matrix, which can be used to form a model structure of the form (2.4) through a simple matrix multiplication.

An interaction graph

A simple way to declare which variables and interactions that should be included in a model is to form an interaction graph. Each node in such a graph represents either a state $x_i$ directly, or a complex entity referred to as an auxiliary. Each edge in the graph corresponds to an interaction, which typically is a reaction of the form (2.6). To indicate modifications, like those from the $M_i$s in (2.6), one could also add uni-directional arcs going from a node to an edge. An example of an interaction graph is given in Figure 2.1, and it contains five nodes (A,B,C,D,E), three edges ($v_1,v_2,v_3$) and one uni-directional arc (B's inhibition of $v_3$). This system is described in more detail in Example 2.3 below. Since this representation has abstracted all other information, and since it is easy to visualise, it is good when defining the scope of the model. Two important types of decisions when defining the scope of a model are: i) decisions regarding the scope of the model, i.e., the decision of which aspects not to include, and ii) decisions regarding the complexity level of the different parts. One of the main difficulties with excluding parts of a system is due to the many feedbacks and interactions that seem to be present in virtually all biological systems. It is therefore difficult to find truly isolated sub-systems in biology and one typically has to be satisfied if a sub-system can be considered as isolated to a good approximation, at least at the time-scale of interest. Here it is often worthwhile to consider if there are experimental techniques to eliminate different interactions. In this way one can sometimes experimentally create an isolated subsystem. The level of detail at which to describe the processes often has a direct influence on the number of parameters and variables that will be included in a model. In this dissertation we will, e.g., see the process denoted glycolysis
modelled on very different levels of detail. In Chapter 10 we will encounter the Hynne model [62] for yeast glycolysis. The interaction graph for this model is shown in Figure 2.2 and the model uses 20 states and 25 reactions to describe glycolysis. In Chapter 11 we will develop another model for glycolysis in muscle, and that model only contains 6 states and 2 reactions. Both are biochemically formulated models, that serves their particular purpose well.

Another important benefit with the construction of an interaction graph is that biologists and biochemists are already drawing such graphs when studying a system, even if they have no intention of building a mathematical model of the system. It is therefore a convenient way of exchanging information with such collaborators. For most well-studied systems one can therefore find proposals of such graphs, which can be used as starting points for model developments. For many of the well-characterised metabolic systems one is even taught these interaction graphs in basic biochemistry courses.
2.3 Collecting and selecting a model structure

Figure 2.2 Example of an interaction graph showing only the included variables (here metabolites), and their interactions (here reactions and in- and out-flows). This interaction graph corresponds to a model of yeast glycolysis [62], and it is created in the software PathwayLab. When an interaction graph is modelled in such a software each arrow is ‘clickable’, and if all reactions are filled in of the form (2.6), the generation of a an ODE model (2.4a) will be done automatically.
Because there are all these benefits with constructing a model structure in the form of an interaction graph, there are several softwares that allow the user to draw the interaction graph graphically, and to keep that representation also when including details about the individual reactions. Two examples of such softwares are CellDesigner [25] and PathwayLab [96]. The latter of these were used to construct Figures 2.1 and 2.2. Another common alternative for obtaining the model structure (2.4) from an interaction graph, is to use the stoichiometry matrix.

The stoichiometry matrix

We will now see how the stoichiometry indexes \( \nu_i \) can be used to form a model structure from an interaction graph and a set of corresponding rate expressions. Consider an interaction graph of the type described above, and assume that it only consists of reactions with known stoichiometric coefficients. Assume further that each variable \( x_i \) corresponds to the concentration of a substance, and that all reactions occur in a single compartment. Let \( \nu_{ij} \) denote the stoichiometric coefficient for substance \( i \) in reaction \( j \), and distinguish between substrate and product coefficients by having a negative sign in front of the latter. Collect these indexes in a matrix \( N \)

\[
N = \begin{pmatrix}
\nu_{11} & \nu_{12} & \cdots \\
\nu_{21} & \nu_{22} & \vdots \\
\vdots & \vdots & \ddots
\end{pmatrix}
\]

Collect the reaction rates \( \nu_i \) in a vector \( \nu \)

\[
\nu = (\nu_1, \nu_2, \ldots)^T
\]

Then the differential equations corresponding to this interaction graph are found by the following simple matrix multiplication

\[
\dot{x} = N\nu \quad (2.10)
\]

There are a number of situations in which it is not as straightforward as in eq. (2.10) to construct the model structure out of an interaction graph. This happens, e.g., if the model contains states that are not easily interpretable as concentrations (e.g., temperature or pH), if there are processes that are not easily interpretable as reactions (e.g., cell growth), or if there appear compartments with different volumes. In this dissertation we will encounter two such situations: variable pH and multi-compartment systems.

The only system with variable pH is encountered in Chapter 11, and we refer to that chapter for an explanation of how to treat that problem. However, we will encounter several cases where there are variables corresponding to concentrations situated in different compartments with different volumes. In all situations, however, the different volumes will be constant in time, and this allows for a simpler approach than the general one, with time-varying volumes (see, e.g., [46] for a general treatment).

There are many ways to generalise eq. (2.10) to the case with several compartments with constant volumes; here we propose a notationally convenient way.
First, each reaction rate is calculated in terms of a standard volume. Let this standard volume be denoted $V_{\text{vol},r}$. Similarly, denote the volume in which $x_i$ resides by $V_{\text{vol},i}$. Construct the following $n \times n$ matrix $V_{\text{vol}}$

$$V_{\text{vol}} = \begin{pmatrix}
V_{\text{vol},1}/V_{\text{vol},r} & 0 & \ldots & 0 \\
0 & V_{\text{vol},2}/V_{\text{vol},r} & \vdots & 0 \\
\vdots & 0 & \ddots & 0 \\
0 & 0 & \ldots & V_{\text{vol},n}/V_{\text{vol},r}
\end{pmatrix}$$

The generalisation of (2.10) is then given by [62]

$$V_{\text{vol}} \dot{x} = Nv$$

Formulating a reaction network in the form (2.10) has many advantages. There are, e.g., many properties that can be obtained from the $N$ matrix alone. One can for instance detect the presence of conserved moieties by comparing the rank of $N$, $\text{rank}(N)$, with the dimension of $x$, $\text{dim}(x)$. If $\text{rank}(N)$ is less than $\text{dim}(x)$ that is evidence that there are conservation laws in the system. These laws can also be deduced by analysis of this matrix, which is done, e.g., by the command $\text{SBreduce}\_\text{model}$ in the Systems Biology Toolbox for MATLAB [111]. Let us now consider a small example that contains all the grey-box modelling steps considered so far.

**Example 2.3** Consider the simple system depicted in Figure 2.1. This system consists of five metabolites $A$, $B$, $C$, $D$, and $E$. $A$ is situated in a compartment with volume $V_1$ and the other species in a compartment with volume $V_2$. There are three reactions in the system: $v_1$, $v_2$ and $v_3$. One possible choice of interaction graph when modelling this system is Figure 2.1 itself; the metabolites are the nodes and the reactions are the edges (note that we also have a modifying arc from $B$ to $v_3$). However, since there is no feedback from the system $\{D,E\}$ to the system $\{A,B,C\}$, the latter sub-system can be considered in isolation. If possible, it is usually advantageous to do this kind of model restrictions (see also the discussion about model scope definition above). Assume that the two corresponding reactions have the following stoichiometry

$$v_1: \quad A \underset{\rightarrow}{\overset{\leftarrow}{\rightarrow}} B$$
$$v_2: \quad B \underset{\rightarrow}{\overset{\leftarrow}{\rightarrow}} 2C$$

which is equivalent to the following stoichiometric matrix

$$N = \begin{pmatrix}
-1 & 0 \\
1 & -1 \\
0 & 2
\end{pmatrix}$$

For this matrix we have $\text{dim}(x) > \text{rank}(N)$, which means that there exists conserved moieties in the system. Since there are volume differences in the system it is easiest
to handle this after the differential equations have been obtained. Assume that
the reaction rates are formulated in terms of the compartment with volume \( V_2 \).
Equation (2.11) then gives the following differential equations

\[
\frac{d}{dt}[A] = -\frac{V_2}{V_1} v_1
\]

\[
\frac{d}{dt}[B] = v_1 - v_2
\]

\[
\frac{d}{dt}[C] = 2v_2
\]

It is now easy to see that

\[
\frac{d}{dt}([A]V_1/V_2 + [B] + 1/2[C]) = 0
\]

This means that the expression in the bracket is constant over time, and thus a
conserved moiety. Let \( m \) denote the constant value of this moiety.

\[
[A]V_1/V_2 + [B] + 1/2[C] = m
\] (2.12)

Utilising the conserved moiety the model can be formulated by only two differential
equations, e.g., for \( A \) and \( B \). The concentration for \( C \) is then calculated using
eq. (2.12). Assume that the reaction rates for \( v_1 \) and \( v_2 \) are described by simple
reaction kinetics, with \( k_1 \) and \( k_{-1} \) denoting the forward and backward rate constant
for reaction \( i \), respectively. This gives the following ODEs

\[
\frac{d}{dt}[A] = -\frac{V_2}{V_1} (k_1[A] - k_{-1}[B])
\] (2.13a)

\[
\frac{d}{dt}[B] = (k_1[A] - k_{-1}[B]) - (k_2[B] - k_{-2}[C])
\] (2.13b)

\[
[C] = 2(m - [A] * V_1/V_2 - [B])
\]

By identifying \( x \) with ([A],[B]), and \( p_x \) with \( \{V_1,V_2,k_1,k_{-1},k_2,k_{-2},m\} \) eq. (2.13)
is found to be of the form (2.4a). We have thus obtained the non-trivial part of a
model structure for this system. The two remaining equations in (2.4) are easily
obtained from knowledge about how the measurements relate to the state variables,
and of how the initial values are chosen.

### 2.4 Quantifying the parts

Before the model can be used, e.g., in a simulation, one needs parameter values for
the kinetic expressions (2.6), and initial values for the variables (2.4c). One way
to obtain this is to estimate the parameters, by optimizing the agreement between
the model output and the data (see below). An important preparation to this is to
get as many rough estimates as possible. This is often acquired by analysis of the
parts separately, in \textit{in vitro} experiments, or by a search in the literature for similar
model components which have been used under similar circumstances.
In vitro measurements

The Latin phrase *in vitro* means 'in glass', and an *in vitro* experiment means an experiment where a part is studied out of its original context. A common example is an *in vitro* study of an enzyme, which means a study that has been performed on the enzyme outside its normal cellular environment, for instance in a test tube.

*In vitro* experiments are important, e.g., because they allow for measurements of more things than is possible in an intact cell. When studying an enzyme in isolation it is also possible to vary its substrates, co-factors, and possible allosteric regulators, much more than what is feasible in an intact cell; one can then also vary these things independently of each other. In this way it is, e.g., possible to get a mechanistic understanding of what the elementary steps in the enzymatic conversion are. One may also find evidence of approximate relations (like saturations and quasi-steady state equilibria etc) and by combining such findings with the elementary reactions, one can derive Michaelis-Menten like expressions for the enzymatic process.

These expressions are typically similar to that in equation (2.9), but much more complicated. As example we give the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) reaction in the Hynne model [62] which is described by a reversible Michaelis-Menten expression with two non-competing substrate-product couples

\[
V_{\text{GAPDH}} = \frac{V_{\text{max}} ([\text{GAP}][\text{NAD}^+] - [\text{BPG}][\text{NADH}])}{K_{8\text{GAP}}K_{8\text{NAD}}(1 + \frac{[\text{GAP}]}{K_{8\text{GAP}}} + \frac{[\text{BPG}]}{K_{8\text{BPG}}})(1 + \frac{[\text{NAD}^+]}{K_{8\text{NAD}}} + \frac{[\text{NADH}]}{K_{8\text{NADH}}})}
\]  

(2.14)

Although an expression of this kind contains fewer parameters than the underlying network of elementary reactions, there are still many unknown parameters. Their values are then estimated using measurements of the variables representing metabolite concentrations in the rate expressions. In Chapters 4.3 and 5.3 we present new methods for how this can be done in practise. Note, however, that the process of choosing an appropriate rate expression for the reaction, and obtaining feasible parameter values, are often intertwined in each other, i.e., they are often carried out simultaneously. Note also that some parts cannot be treated in *in vitro* experiments. One such example is the glucose transporter [105], which only functions in the membrane between intact cells and their environment.

Note finally that the term *in vitro*, when referred to on the whole-body level, means an experiment of an organ or cell type that has been isolated from its normal environment, i.e., the body.

2.5 Tuning and analysing the grey-box model

According to the so-called 'biochemical promise' [130] it should be enough to take all the steps that has been described up until now, i.e., to characterise and quantify the different parts (for instance by finding *in vitro* estimates of the type (2.14)) and how they are related (for instance by an interaction graph of the type in Figure 2.2), to be able to understand the whole system. A quantitative model can then be put together, and in principle be simulated as a replica of the whole system. This promise was tested by Teusink *et al.* in an article entitled 'Can yeast glycolysis be
understood in terms of *in vitro* kinetics of the constituent enzymes? Testing bioc-chemistry’ [130]. Almost all enzymes in glycolysis were then characterised through *in vitro* experiments performed at the same well-defined state. The remaining few characterisations were taken from similar studies in the literature, and all this was collected into a large system of nonlinear differential equations. However, when the behaviour of this model was compared with corresponding *in vivo* measurements, significant discrepancies were found.

**In vivo** measurements

The Latin phrase *in vivo* means ‘in life’, and in biology it is used in a corresponding way to the phrase *in vitro*, mentioned above. That means that an *in vivo* experiment measures things relating to an enzyme while still in its natural cellular environment, or alternatively things related to the response of a cell or an organ while still contained in the body.

There are of course many advantages with *in vivo* experiments when desiring to understand living processes. This follows since there are many things that change when, e.g., an enzyme is taken out of its normal cellular environment. Examples of important regulating factors for the activity of an enzyme are pH, temperature, and allosteric regulations. An allosteric regulation of an enzyme is an interaction with a molecule at another site than the active site to which the substrate binds. Since one typically does not know all the allosteric regulations of an enzyme, and especially not the cellular concentrations of the corresponding regulators, it is almost impossible to keep these allosteric regulations intact in an *in vitro* experiment. Apart from these unknown effects there is also the important overall regulation of the system (body/organ/cell) on the parts (organs/cells/enzymes). Whether this central feature of all living systems occurs purely through known regulatory mechanisms such as transcription, or whether there are other mechanisms not yet a part of our scientific paradigm, is irrelevant, they will all be included in a ‘perfect’ *in vivo* experiment. These are the major strengths of *in vivo* measurements.

A major drawback of *in vivo* experiments is that it is much more difficult to measure things. It is also impossible to control most of the concentrations that appear, and in this way it is difficult to examine, e.g., saturation effects. The difficulty in perturbing an intact cell also comes from the fact that the different substances need to penetrate the cellular membrane, and this is often difficult to do in a desired time-frame. All these things leads to the fact that *in vivo* measurements usually are much less informative, i.e., that they contain less information about the parameters, than *in vitro* experiments [77]. On the other hand, one could also argue that things that are not excited in an *in vivo* experiment should not be part of the model structure. This, and similar discussions, will be returned to in many places in this dissertation, e.g., in Chapters 3 and 4.

It should finally be mentioned that some types of *in vivo* measurements are more ‘perfect’ than others. This has to do with the method by which the measured signals have been obtained. One can here distinguish between invasive and non-invasive techniques. Invasive methods are done at the cost of destroying the cells, while non-invasive methods are made on intact cells. One common invasive technique is Western blotting (see Chapter 9), and two common non-invasive methods are
auto-fluorescence after exposure to ordinary light, and nuclear magnetic resonance (NMR) (see Chapters 10 and 11, respectively).

### 2.5 Tuning and analysing the grey-box model

#### 2.5.1 Tuning the system behaviour

The major conclusion from the Teusink study [130] was that it is not sufficient to just put together the *in vitro* characterisations from the parts of such a complex system as glycolysis, to obtain a model that agrees with the *in vivo* observations. When facing a disagreement between a white-box model output and corresponding *in vivo* experiments there are two standard options to consider:

- The model structure is wrong
- Some of the given parameter values are wrong

(There are more corresponding explanations in the case of a grey-box model.) In the first of these cases one must change things in the analytical expressions in (2.4a) and/or (2.4b). This may correspond to a change of the kinetic expression for some of the reactions, include some more (or less) reactions in the interaction graph, or add some more (or less) nodes. If one can show that such a modification is necessary it is said that the old model structure is rejected, and this is an important type of understanding that can come from modelling (in some modelling frameworks, one even says that this is the *only* type of understanding that can come from modelling, see Chapter 3.4). However, in order to come to this conclusion one must first test the other option, i.e., whether it is enough to change some of the model’s parameters.

This was tested in a follow-up study to the Teusink article [130] by Hynne, Danø and Sørensen [62]. In this work much of the data obtained in the work by Teusink were re-used and some new data were collected. The new data were collected at a well-defined operating point situated close to a supercritical Hopf bifurcation [32]. The most important difference between [130] and [62], however, is that Hynne et al. assumed that there might have been uncertainties in the *in vitro* estimations. That assumption means that one should examine all parameter combinations that lie within the assumed uncertainties, and see whether some of them can explain the collected *in vivo* data. In [62] this search was done using a method denoted ‘the direct method’, and it combines a search using steady state flux analysis leading to a convex search space in terms of the net velocities, and by using special features that can be utilised only in the vicinity of a Hopf bifurcation.

A more common search method is to form a cost function, denoted $V_N$, which gives a value for each parameter $p$ and time-series, denoted $Z^N$. The capital ‘N’ in the symbol for the cost function and the time-series denotes the number of time-points at which experiment samples were collected. One way to form this cost function is to sum the squares of differences between all the measurements $y_j(t) \in Z^N$ and simulated outputs $\hat{y}_j(t|p)$. For an experiment with $n_y$ measurement signals this becomes

$$V_N(p, Z^N) = \sum_{i=1}^{N} \sum_{j=1}^{n_y} (y_j(t_i) - \hat{y}_j(t_i|p))^2$$
When using flexible model structures, like artificial neural networks, this conclusion is that the present model structure can mimic the data used for model fitting. A minimization is formalised by the operator min and the arg operator returns the argument (here: parameter) that solved the minimization problem. With this minimization the estimated parameter, denoted \( \hat{p} \), is determined by

\[
\hat{p} = \arg \min_{p} V_{N}(p, Z^{N})
\]

and the corresponding model is given by \( M(\hat{p}) \). In Chapter 6.1 we also review different global and local search strategies for solving the minimization problem.

Validating the model

A common argument for why one should not fit a model’s parameters to make the model agree with available in vivo data, is that it makes it impossible to validate the model; if the model has been fitted to agree with some data it is almost obvious that it will agree with the data, and not anything to draw conclusions from. It is also true that the only conclusion that can be drawn from an agreement with data used for model fitting is that the present model structure can mimic the data. When using flexible model structures, like artificial neural networks, this conclusion is almost completely uninteresting, since such model structures can fit almost any data [77].

However, when using model structures that are built on biological and mechanistic assumptions, an agreement with estimation data may be of interest in itself. This is the case for instance if the general belief is that the in vivo observations only can be explained if some new, and as yet unknown, biochemical mechanism is
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included. One would than have shown that this belief is wrong. We will see such an example in Chapter 11. One should, on the other hand, also remember that if one does not allow for variation of the parameters of the model and it does not agree with the available \textit{in vivo} data, as in the case of the work by Teusink [130], it is not possible to draw the conclusion that the model structure cannot agree with the data.

It should, finally, be added, that the comment about the doubtfulness one should have when comparing a model to the same data as it has been fitted against is valid. For this reason a part of the available data, $Z^N$, is often saved for the model validation. This validation data should ideally contain some qualitatively different features than the estimation data, in order to make the validation even more reliable. The agreement between the Hynne model and such independent and qualitatively different validation data is depicted in Figure 2.3. The agreement is quite good and this is a quality mark of the model as a whole. However, as we will argue in the next chapter, there are still many unanswered questions regarding model quality even after such an agreement is obtained.

2.5.2 Analysing and using the model

Once a model has been satisfactorily validated it is ready to be used. There are many ways in which a model can be used; here we will mention two analysis methods: metabolic control analysis and bifurcation analysis, and one type of application: biosimulation of drug action.

Metabolic control analysis

The simplest way of testing a model is by simply performing simulations with the model. However, performing a full evaluation of the properties of a model using only simulations is not feasible. Therefore more systematic analysis methods are used. One such method is metabolic control analysis (MCA). MCA is a type of parametric sensitivity analysis that has been developed for metabolic systems. This analysis was originally developed for simple unbranched metabolic pathways at steady state [54], but has since then been extended to transient systems [40, 86] and branched pathways [43, 133]. Consider a global steady state property $X$, a reaction rate $v_i$ and a parameter $p$. An example of a global steady state property can be the steady state flux through a metabolic pathway. Assume that $p$ is only present in the reaction rate $v_i$. The three basic components in classical MCA to study the interrelation between these quantities are then the control coefficient, $C^X_i$, the elasticity coefficient $\varepsilon^i_p$, and the response coefficient $R^X_p$. The control coefficient measures the change of $X$ with respect to the change in reaction rate $v_i$, the elasticity coefficient measures the change of the reaction rate after a change in the parameter $p$, and the response coefficient measures the change in $X$ after a change in $p$

$$C^X_i := \frac{\partial X/\partial v_i}{X/v_i} = \frac{\partial \ln X}{\partial \ln v_i} \quad \varepsilon^i_p := \frac{\partial \ln v_i}{\partial \ln p} \quad R^X_p := \frac{\partial \ln X}{\partial \ln p} \quad (2.16)$$
Their interrelation is given by the following following *combined response theorem* [101]:

\[ R_p^X = C_i^X \varepsilon_p^i \]  

(2.17)

This relation is exactly fulfilled for steady state calculations, and then one can also derive summation theorems for the different components. There are a number of studies which report generalisations of (2.16) and (2.17) to oscillating systems [9, 31, 99–101, 128]. The most common choice of variable, \( X \), to study have been the amplitude and frequency of the oscillations [99, 128]. However, there are also some studies that have considered other choices of \( X \), like mean value, shape, and phase [101], and stability [100]. The recent article [31], has adapted the theory to systems operating in the vicinity of a Hopf bifurcation and they also show a relation between the response coefficient for the stability and for the amplitude. They also give a nice derivation of summation theorems for a wide class of response coefficients. If one only considers all velocity parameters, i.e., such parameters that scale linearly with the reaction rates, they showed that the following summation rules apply

\[
\sum_i C_i^X = 1 \quad \text{if} \quad X(\alpha t) = \alpha X(t) \quad \forall \alpha \in \mathbb{R}
\]

\[
\sum_i C_i^X = 0 \quad \text{if} \quad X(\alpha t) = X(t) \quad \forall \alpha \in \mathbb{R}
\]

In particular this means that the frequency coefficients sums up to 1, and that the shape, mean value, amplitude and phase coefficients sums up to zero. For the velocity parameters the elasticity coefficients are one, the response and control coefficients are equal, and (2.17) is obviously fulfilled. However, for the other parameters (2.17) is not fulfilled for oscillating systems, not even for the mean values [101].

**Bifurcation analysis**

In this dissertation we will encounter several models that oscillate (see, e.g., Chapters 10 and 12). In all these cases the oscillations appear through a *Hopf bifurcation*. The word bifurcation means ‘branch’, and for dynamical systems it is a branching between two qualitatively different regions in the state-parameter space. For a supercritical Hopf bifurcation it is a branching between an oscillating region and a steady state region.

Bifurcations are originally only defined for autonomous systems, i.e., for systems with constant input signals \( u \). We will simply adopt all the notions from this theory [135] to the more general model structure (2.4a) by considering each value of \( u(t) \) as belonging to a separate system, with a separate set of bifurcations. Another common choice for the generalisation to non-autonomous systems is to introduce a new variable \( s \) with the associated differential equation

\[ \dot{s} = 1 \]  

(2.18)

and by replacing \( u(t) \) by \( u(s) \). This approach was not chosen because it results in an unstable system with unbounded solution.
A bifurcation is often displayed by a bifurcation diagram. A bifurcation diagram for a Hopf bifurcation is shown in Figure 2.4. The diagram corresponds to the following complex differential equation

\[ \dot{z} = (i\omega_0 + \mu\sigma_1)z + g_3|z|^2 \]  

(2.19)

where \( z, g_3, \sigma_1 \in \mathbb{C} \) and \( \omega_0, \mu \in \mathbb{R} \). The upper plot corresponds to the supercritical version, i.e., to \( \text{Re}(g_3) < 0 \). When this condition is fulfilled one can show that the system is stable for all \( \mu < 0 \). This is indicated in the figure by the fact that the fix point, which corresponds to the line, is solid. That the system is stable means that independently of which initial values \( z(0) \) that are chosen for the simulation, the system will asymptotically approach the point \( z = 0 \) as time increases. This is true also at the bifurcation point \( \mu = 0 \), but for \( \mu > 0 \) the system will instead converge to a stable oscillatory regime. The amplitude of the oscillations is depicted by the solid line. Note that there is now an unstable fix point at \( z = 0 \). There is another type of Hopf bifurcation denoted sub-critical. It corresponds to \( \text{Re}(g_3) > 0 \), and then one can show that there is a stable fix point at \( z = 0 \) for all \( \mu < 0 \). However, in the sub-critical case there is an unstable oscillatory behaviour co-existing with the stable steady state. That means that all initial values outside the oscillations will lead to a diverging system, while all initial values inside the oscillations lead to a stable system. Again the bifurcation occurs at \( \mu = 0 \), but now the system is unstable for all initial values, when \( \mu > 0 \).

There are many special features occurring in the vicinity of a bifurcation. Most of them stem from the fact that an arbitrary system undergoing a local bifurcation can have its asymptotic behaviour (the dynamics of the center manifold, see Chapter 6.3) exactly described by a normal form equation. The normal form equation for the Hopf bifurcation is given in (2.19). Let the Hopf bifurcation point in the full state-parameter space be denoted \( (x_b, \mu_b) \). The theory then also ensures the existence of a nonlinear function \( \tilde{h} \) that can translate the 2-dimensional normal form vector \( z \) to the original \( n \)-dimensional state vector \( x \) by

\[ x = x_b + z + \tilde{h}(z, p_\mu) \]  

(2.20)

Note that this is possible for all finite \( n \), i.e., and that the simplification to the two-dimensional formulation (2.19) therefore can be significant. We will make use of this powerful normal form representation when developing special methods for system identification around a Hopf bifurcation in Chapters 6.2 and 6.3. An example of how the transformation (2.20) is calculated is given in the appendix.

**Biosimulation of drug action**

A modelling framework similar to the grey-box modelling framework presented in this chapter is denoted biosimulation. Biosimulation is a commonly used term in the field of drug development, i.e., in the pharmaceutical industry. Here the main objective is often to develop models that can be used in computer simulated drug testings, often referred to as in silico experiments. One way to do this is by performing a sensitivity analysis on the model, which is often done by means of MCA. Another way to do this is by introducing a new input signal, \( u_{\text{drug}} \), which
Figure 2.4 Bifurcation diagram for the Hopf bifurcation. The Hopf bifurcation occurs at the point $\mu = 0$. For lower values of $\mu$, there are only steady states, and for higher values only oscillations.

corresponds to the amount of the drug. One can then simulate different test cases, e.g., different doses, durations, and shapes of this input signal, and in this way compare, e.g., different medication strategies. One can also use biosimulation to pin-point potential drug targets. Since it today is extremely expensive to develop a drug, and since the vast majority of the experiments performed on test animals are more or less of a trial-and-error character, there is a big potential for modelling to enter in this field. The potential for cost reductions and ethical improvements, through the reduction and replacement of animal experiments, are enormous. Furthermore, the usage of modelling also helps to give a better understanding of how a drug is actually working. This might also be beneficial for the development of more individualised drugs.

Summary

In this chapter we have introduced some of the most important components in grey-box modelling. Grey-box modelling distinguishes itself from black-box modelling since its model structure is based on biological and mechanistic assumptions. These assumptions are often translated to an interaction graph, where the nodes corre-
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Table 2.1 The major steps in a grey-box modelling development.

spond to variables (typically concentrations) and the edges to interactions (typically reactions or transports). The edges are then characterised, often through in vitro experiments, giving the kinetic expressions and in vitro estimates of the kinetic parameters. If one only uses literature values from such in vitro characterisations, the process is often referred to as white-box modelling. However, we have seen that a final adjustment to available in vivo data is sometimes necessary to get a model that agrees with in vivo observations. A grey-box model is distinguished from a white-box model if such adjustments are allowed. We have seen that these parameters adjustments does not lead to any problems with interpretation of agreement with data, if such comparisons are done using separate and qualitatively different validation data. A validated model can start to be used for analysis and in silico experimentation, e.g., for biosimulation of drug actions. We summarise all these notions in Table 2.1.

It should finally be emphasised that we have here only mentioned some of the most important ingredients in a grey-box modelling procedure, and that the order in which they should be applied is much case-to-case dependent, and cannot be generalised. Modelling is truly a creative process, which usually involves intuition, re-iteration through the same steps several times, and the ability to see the same problems from many different angles. In Part II of this dissertation we will see several examples of this.
Core-box Modelling

In the previous chapter we introduced the basic methodologies used in grey-box modelling, as it typically is performed in the systems biology community today. Although we stress that modelling is an iterative process, which cannot be described by a linear chain of events, the following three steps could be discerned:

1. **Selection of variables and interactions**: This step mainly consists of a review of qualitative experiments seeking to determine which players and interactions to include in the model. The result of such an analysis is often displayed as an interaction graph (see e.g. Figure 2.2).

2. **Quantification of the parts**: Before a model can be simulated the interactions need to be characterised by analytical expressions. The parameters appearing in these expressions also need to be estimated, or at least chosen. This kind of analysis is often done by studying the individual processes separately, in *in vitro* experiments. We referred to the resulting model as a white-box model, and we exemplified it with the Teusink model for yeast glycolysis [130].

3. **Quantification of the whole model**: Typically biological models are so complex that adjustments are needed to make the model agree with the given *in vivo* data. To avoid over-fitting and other problems with interpretation of the result, one seeks to divide the available *in vivo* data in an estimation part, and a qualitatively different validation part. We referred to the resulting model as a grey-box model, and we exemplified it with the Hynne model, which also describes yeast glycolysis [62].

When the final model has been validated, it is said to be ready to be used. Usage of a model can, e.g., include bifurcation analysis to examine and visualise the
possible qualitative behaviours of the model, sensitivity analysis or model reduction to find out which components are crucial for the observed behaviours, and \textit{in silico} experiments to, e.g., examine the effect of potential drug strategies.

However, in this chapter we will argue that such analysis should ideally be postponed until the model has been developed further. We will start by arguing that there are a number of important questions that cannot be answered with grey-box models as they are described in Chapter 2. This is done in Section 3.1. In Section 3.2 we will then propose an alternative modelling strategy, \textit{core-box modelling}, that will answer these questions. The different steps in this procedure will then be described in more detail, first through a short introduction in this chapter and then in the remaining chapters of Part I. The different parts are \textit{Model reduction to an identifiable core model} (Section 3.3, and Chapters 4 and 5), \textit{System identification of the core model} (Section 3.5 and Chapter 6) and \textit{Back-translation to the core-box model} (Section 3.6 and Chapter 7). The relations between the steps are outlined in Figure 3.1.

3.1 Remaining questions in classical grey-box modelling

Within the systems biology community there exists quite a number of large scale models that have been successfully developed according to the principles and criteria described above, and in the previous chapter. The strengths of these models are that they are based on the mechanistic understanding of what actually goes on in the system, and that they agree with the available \textit{in vivo} data. If they, like the Hynne model, also agree with \textit{in vivo} data that is qualitatively different from the estimation data, this is an additional strength of the model. However, there are a number of natural follow-up questions that still cannot be answered. The most important of these are of the following character:

- What does it mean that the model has an agreement like the one in Figure 2.3, can we trust the model?
- Are there parts or aspects of the model that have been more validated, i.e., that are more trustworthy, than other parts?
- Are there parts or aspects of the model that still are completely unvalidated?

What we are warranting are quality tags to the different parts of the model. Such quality tags would be a great advantage to have when subsequently analysing different features of the model. Assume for instance that some \textit{in silico} experiments have resulted in the prediction that a particular enzyme in a reaction network is very crucial for a specific target feature of the model. There are two fundamentally different cases for how much faith can be put into this prediction. First, this particular prediction from the model can be firmly based in both the \textit{in vivo} and the \textit{in vitro} data. Second, this particular prediction could belong to a part of the model that is rather unrelated to the available \textit{in vivo} data, or to a part of the model whose effect can equally well be accounted for by another part of the model.
In the first case, this prediction is a strong argument to study this enzyme further, and in the second case the prediction can be taken much more lightly. This is one example of how important the above questions can be for the applicability of the model. We will now present an extension of the classical grey-box modelling framework that will seek to remedy these shortcomings.

### 3.2 The core-box modelling framework

The reason why it is not possible to say that a specific part of, e.g., the Hynne model is more reliable than another is that the model is highly over-parametrized with respect to the available data. This over-parametrization means that there are infinitely many parameter combinations that give virtually the same agreement with the existing data. The first step in the analysis is therefore to detect all such over-parametrizations. These over-parametrized parts of the model are then replaced by simpler expressions, using different kinds of model reduction techniques. These steps together are entitled *Model reduction to an identifiable core model*, and they are represented by step 3 in Figure 3.1. We refer to the resulting model as a *core model*. Note that an alternative way to obtain the core model is to formulate it directly from the same information that was used to obtain the grey-box model using the minimal modelling approach in hypothesis testing. This is step 3’ in Figure 3.1, and it is described in Section 3.4.

Once the core model has been formulated, methods from system identification can be applied to estimate the parameters from the *in vivo* data. This step is entitled *System identification of the core model*, and it is depicted as step 4 in Figure 3.1. The system identification framework provides methods that give an uncertainty of the estimated parameters. This uncertainty is then a combination of the original uncertainties from the *in vitro* estimations of the parameters, with the uncertainty that remains after the additional information from the estimation to the *in vivo* data. This model has therefore acquired such quality tags that were warranted above. However, all the parts and processes in the core model have corresponding parts in the original grey-box model. The estimated features and the quality tags, can therefore be back-translated to the grey-box model, which is the next step. This step is referred to as *Back-translation to the core-box model* and it is depicted by step 5 in Figure 3.1.

The advantage of the core-box model compared to the core model is that the core-box model contains all the detailed mechanistic information of the actual processes, which means that it can, e.g., simulate much more detailed *in silico* experiments.
Figure 3.1 This is a schematic drawing of the proposed modelling approach, leading from in vitro biochemistry to an in vivo understanding. In step 1 in vitro experiments are carried out to determine the mechanisms of the involved reactions, and to determine estimates of the corresponding kinetic parameters and concentrations. In step 2 this information is collected into a mechanistic white-box model, which might also be adjusted to agree with the observed in vivo behaviour (grey-box modelling). In step 3 this model is simplified (step 3b) to a smaller model that is identifiable (step 3a) with respect to the in vivo data, and has the same basic features as the original model. This is the core model, and an alternative way is to formulate it directly from the same information that was used to obtain the grey-box model, using the minimal modelling approach in hypothesis testing (step 3'). In step 4 the core model is estimated by one part of the in vivo data (step 4a), and validated by another part (step 4b). This step also includes a determination of quality tags for the different parts of the model. In step 5 the well-determined parts of the core model are translated back into the full-scale model, and the result is a grey-box model with an identified core (a core-box model). It is such a model that we propose is the best candidate for obtaining an in vivo understanding of the cellular processes.
The advantage of the core-box model compared to the grey-box model is that the core-box model has acquired quality tags to its predictions. Further, if the core model is developed using hypothesis testings, the advantages from that process are available as well. In many cases the core-box model should therefore be the most advantageous model to use, and the final analysis step is therefore depicted from the core-box model, in Figure 3.1. It should be stressed that each of these steps, including the final back-translation, are individual sub-disciplines that requires much individual study. Let us therefore view them one by one, first shortly in the rest of this chapter, and then in more detail in the remaining chapters of this theoretical part of the dissertation.

3.3 Model reduction to an identifiable core model

Model reduction involves the identification and elimination of such parts of a model that are unrelated to some specific features of a model. Which those features are might vary from situation to situation. In the core-box modelling framework, the feature in focus is identifiability (and agreement) with respect to the available data, and we therefore introduce this concept first. Then we continue with a short review of how reduction can be performed. In the next section we review the alternative: step 3’ in Figure 3.1.

Identifiability analysis

Consider a parameter $p_j$ in a model for which a particular set of data $Z^N$ has been collected. Loosely speaking this parameter is identifiable with respect to the data if the data set contains sufficient information to uniquely determine the parameter. It is common to distinguish between two levels of identifiability: structural and practical [37].

**Structural identifiability:** If $p_j$ is structurally identifiable it can in principle be estimated from the type of data that is present in $Z^N$, if the data were ‘perfect’. This means that one considers the type of measurement signals ($h$ in (2.4b)), the structure of the dynamic equations ($f$ in (2.4a)), and the way the input signals $u$ enters these equations. What one does not take into account is the practical limitations in the data, i.e., the noise level, the type of excitation of the system, the actual number of samples and sample distance etc. One does also not take into account the practical limitations associated with the optimization step. Structural identifiability is therefore a necessary but not sufficient requirement for practical identifiability.

**Practical identifiability:** If a parameter is practically identifiable it can really be estimated from the actual data. Estimation from a real data set is always associated with an uncertainty, and it is therefore also required that this uncertainty is ‘reasonably low’.

Both these concepts will be treated more formally in Chapter 4, where we will also present methods to automatically analyse and quantify, e.g., what a 'reason-
ably low’ uncertainty is. For now we will be content by an illustration of the concepts on a small example. Note that it is the same example that will be used throughout the whole chapter.

**Example 3.1** The system we will study consists of one substance, A, with concentration \( x = [A] \). There are two types of interactions that effect the concentration negatively: degradation and diffusion. Both these processes are assumed to be irreversible, and to follow simple mass action kinetics with rate constants \( p_1 \) and \( p_2 \), respectively. Further, there is a synthesis of A, which increases its concentration. This synthesis is assumed to be independent of \( x \), and its rate is described by the constant parameter \( p_3 \). Finally, it is possible to measure \( x \), and the measurement noise is denoted \( d \). The system is thus described as a control system (2.4) by the following equations

\[
\begin{align*}
\dot{x} &= -p_1 x - p_2 x + p_3 \quad (3.1a) \\
y &= x + d \quad (3.1b) \\
x(0) &= x_0 \quad (3.1c)
\end{align*}
\]

Assume that *in vitro* experiments have resulted in estimates \( p_1 = 3 \pm 0.2 \) and \( p_3 = 0.02 \pm 0.01 \), but that there is no estimate for \( p_2 \). Assume further that there

![Graph](image-url)

**Figure 3.2** The data from Example 3.1. Note that two of the lines are overlapping.
is one experimental time-series, shown in Figure 3.2, which has been generated by simulations using the parameters

\[(p_1, p_2, p_3, x_0) = (2.8, 1.7, 0.015, 20)\]

Hence, these parameter values are considered as the 'real' parameter values for the system. The solid line in the figure shows the corresponding simulations.

This model illustrates the problems with structural and practical identifiability. The reason why the model is structurally unidentifiable is because the two decay terms in (3.1a) are described in the same way, and because they enter the differential equations in a structurally identical position. Therefore one cannot distinguish the decay caused by the degradation from the decay caused by the diffusion, which means that \(p_1\) and \(p_2\) are unidentifiable. This is true irrespectively of the noise or degree of excitation in the system, which means that they are structurally unidentifiable.

This problem with structural identifiability causes \(p_1\) and \(p_2\) to be practically unidentifiable. However, there are problems with practical identifiability also for the \(p_3\) parameter, even though it is structurally identifiable. This problem occurs because the \(p_3\) term in (3.1a) is much smaller than the two others, because the time-series does not really extend to the stationary part, and because there is a quite significant noise-level. In Chapter 4 we will see how both these results can be derived automatically, and in Chapter 7 we will see how large the uncertainty in \(p_3\) would be if it was to be estimated.

Model reduction

Model reduction is the art of simplifying a model while retaining its most desired features, at least to a given accuracy. In the core-box modelling framework it is assumed that the original model (the grey-box model) is already mimicking the data quite well, and this agreement, together with the parts of the model that are sought to be estimated, are the main features that should be preserved in the reduction. The main objective with the reduction is to make the model identifiable with respect to the available data. Sometimes this is not possible, and then one should at least make the parts one seeks to identify identifiable, and devise a plan for how the remaining uncertainties should be handled (for instance replacing some parameters by fixed numbers). There are many different types of methods for model reduction, and there are many types of complexities that can be reduced.

We will now shortly mention three levels where a reduction in complexity might be obtained. A more complete review, with a higher focus on the existing methods, is given in Chapter 5.

Reduction of rate expressions: In some type of models the rates \(v = v(x, k)\) are highly complex, and involve many parameters and states. This is especially true for many metabolic models (e.g. the Teusink and the Hynne model) which typically involve many enzymatic reactions. The complexity of the enzymatic reaction kinetics is due to the fact that they are composed of a series of elementary reactions that can be described by simple mass action kinetics. These reaction steps can, using some assumptions, be lumped into one single reaction to the price of a much more
complex rate expression. The many parameters appearing in these expressions are, as we will show in later chapters, typically not practically identifiable. This comes from the fact that the actual variability of the corresponding reaction rate is, in practice, governed by a simpler relation between the variables, with fewer degrees of freedom. One type of reduction is therefore to simplify the complicated rate expressions into ones describing such simpler relations. In Chapters 4 and 5 we will develop new such reduction methods, devoted to both structural and practical identifiability.

Elimination and merging of terms: Another level of complexity, where simplifications might be possible, appears at the relation between the terms (that might have been simplified). Then one should compare both the absolute values and the relative variations of the terms. Consider for example the sum of two terms where one term is constantly much smaller than the other. Then one may probably remove the smaller term without changing the dynamics to any great extent. On the other hand, if the absolute values of the terms are comparable, one might still replace a term by a constant (parameter), if the variation of the term is small. Another thing that may be done on this level is to replace several terms by a single term, approximating their joint effect. These kind of term-based simplifications can be denoted numero-symbolic model reduction, and are used extensively by, e.g., the software AnalogInsydes [2]. In the usage of such a software one needs to take precautions not to loose the biochemical interpretability of the model structure.

Reduction and lumping of states: A final thing that can be reduced is the number of differential equations. This directly corresponds to a reduction in the number of states. Such a reduction can be obtained by removing the dynamics of a state, and thus making it a parameter, or by finding new states, obtained by a coordinate transformation. Within the systems and control community there is developed a method that finds a new reduced state vector, which is optimal at preserving the input-output relationships of the model. This method is called balanced truncation [51], and its major drawback for biochemical modelling is that the reduced states are no longer corresponding to physical entities. A method that instead combines the original states into physically interpretable pools is called lumping. Lumping is one of the most used methods within the field of classical biochemical model reduction [94], where it is often done based on intuition.

Lumping of variables might, as described above, lead to simplifications in terms of the number of states and reactions, but to a higher complexity of the kinetics describing the remaining rate expressions. These expressions can then be simplified using the first set of methods, giving new terms that can be compared with the second set of methods and so on. This means that model reduction, just as modelling in general, is not a linear sequence of steps to be taken, but a complex art that requires a re-iteration of previous steps and decisions, as well as the input of biochemical intuition. Just as is the case with modelling in general it is also so that model reduction is always done with a purpose. No single method is superior for all types of complexities, and for all objectives.
Example 3.2  In example 3.1 we saw that the two parameters $p_1$ and $p_2$ are structurally unidentifiable (which means that they can never be estimated, no matter how good the data is), and that $p_3$ is practically unidentifiable (because it cannot be estimated from the present data). The first of these problems can be solved by the following re-parametrization

$$p'_1 = p_1 + p_2$$

which corresponds to a merging of terms

$$p'_1x = p_1x + p_2x$$  \hspace{1cm} (3.2)$$

When parameters are structurally unidentifiable also in the case when all states can be measured, i.e., when $y = x$, one can often perform this kind of symbolic replacements. This is because it is then a question of a pure over-parametrization, and the simplified model is therefore obtained without any approximation in terms of output predictions; the only thing sacrificed is the detailed description of what actually goes on. For the $p_3$ parameter, however, approximations are necessary. Since $p_3$ is small compared to $p'_1x$ during the whole measured process, it can be set to zero as a good approximation (see Figure 3.2). On the other hand, one could argue that it is better to include a small term to acknowledge the fact that there is a small synthesis, but that it cannot be estimated from the data. If $p_3$ is replaced by the constant number 0.02, which is the number estimated in the in vitro experiments, the following reduced model structure is obtained

$$x = p'_1x + 0.02$$  \hspace{1cm} (3.3a)$$

$$y = x$$  \hspace{1cm} (3.3b)$$

$$x(0) = x_0$$  \hspace{1cm} (3.3c)$$

The reduced model is both structurally and practically identifiable from the given data, and this has been obtained without sacrificing the agreement with the data.

3.4 Minimal modelling through hypothesis testing

An alternative way to obtain a core model is to formulate it directly. One then combines the component wise information that was used to form the grey-box model structure, with the limitations implied by the available in vivo data, already at the model formulation step. This alternative way of developing the core model (depicted as step 3' in Figure 3.1) is sometimes an attractive alternative, especially if there does not exist a previously established grey-box model that gives a good agreement with the data. We will see several application examples where this approach is used in Part II. On the other hand this dissertation does not contain any new contributions to this approach, which is denoted statistical testing, and there will therefore be no more detailed review than the one provided here. For more details regarding a statistical approach to modelling of nonlinear differential equations we refer to [113].
Rejection of a null hypothesis

The central step in the statistical testing framework is the rejection of a null hypothesis, $H_0$. A null hypothesis can be formulated in many different ways. In this dissertation we will typically encounter one of the following two null hypotheses:

- That a given model is equivalent to the 'true' model
- That two model structures $M_1$ and $M_2$ explain the data equally good

To determine whether $H_0$ can be rejected or not a test function, $T$, is formed. A test function is a mapping from the available data, $Z^N$, to a scalar value

$$T : Z^N \rightarrow \mathbb{R}$$

Given the assumption that $H_0$ is true this function will have a given probability density function (PDF) to result in a given number

$$f(r|H_0) := \text{the probability density that } T = r \text{ if } H_0 \text{ is true}$$

The probability density function is normalised which means that the integral over the whole real axis is equal to one

$$\int_{-\infty}^{\infty} f(r|H_0) \, dr = 1$$

It will often be interesting to know what the integral is up to a given value, $r$. This is the cumulative distribution function (CDF) $F$

$$F(r|H_0) := \int_{-\infty}^{r} f(r|H_0) \, dr$$

After the choice of test function has been made, the next step is to determine the corresponding distributions. This is often a non-trivial task, and analytical results exist only for ideal situations (see below). Since $f$ is a non-negative function $F$ is a monotonously growing function. That means that the higher value that is returned by $T$, the more unlikely it is that $H_0$ is true (although it, in principle, could generate any value). The next step in the testing procedure is therefore to decide on a limit for how large possibility one accepts for rejecting $H_0$ by mistake. This limit corresponds to a threshold $\delta_\alpha$, which in turn corresponds to a significance level $\alpha$. The significance level is the probability that $H_0$ is rejected while being true

$$\alpha := P(T > \delta|H_0)$$

which means that the null hypothesis will always have been rejected with a certainty of at least $100(1 - \alpha)\%$. If $H_0$ is rejected while being true, it is said that a Type I error is made. A Type II error is the opposite situation: a failure to reject $H_0$ despite its being false. The ability to reject a false model is referred to as the power of the test.
The $\chi^2$ Distribution

The most central distribution we will encounter during tests is the $\chi^2$ distribution. The $\chi^2$ distribution is closely related to the normal distribution. A normal distribution is completely defined by its expectation value, $\mu$, and its variance $\sigma^2$. The normal distribution has the following PDF

$$f(x) = \frac{1}{\sqrt{2\pi\sigma^2}} \exp\left(-\frac{(x-\mu)^2}{2\sigma^2}\right)$$

where $x \in \mathbb{R}$. This is the distribution typically assumed for the measurement noise.

Let the normal distribution be denoted $N(\mu, \sigma)$. The normal distribution is one of the most commonly occurring distributions. One reason for this is given by the central limit theorem, which states that, under certain conditions, the distribution of a sum of a large number of independent variables is approximately normal. There are also several other common distributions (e.g., the Poisson and the Binomial distributions) which approach the normal distribution asymptotically in various limits.

The $\chi^2$ distribution is the distribution for a sum of squares of independent normally distributed $N(0,1)$ variables.

$$Y = \sum_{i=1}^{df} X_i^2 \quad \text{where} \quad X_i \in N(0,1)$$
The new random variable $Y$ is $\chi^2$-distributed

$$Y \in \chi^2_{\text{df}}$$

where df denotes the degrees of freedom of the distribution, which is the same as the number of terms in (3.5). The PDF and CDF for $\chi^2_{\text{df}}$ is given by

$$f(x; \text{df}) = \frac{(1/2)^{\text{df}/2}}{\Gamma(\text{df}/2)} x^{\text{df}/2-1} e^{-x/2}$$

(3.6a)

$$F(x; \text{df}) = \frac{\Gamma(\text{df}/2, x/2)}{\Gamma(\text{df}/2)}$$

(3.6b)

where $\Gamma(a)$ and $\Gamma(a, b)$ is the Gamma and the incomplete Gamma function, respectively. The PDF for df = 101 is plotted in Figure 3.4.

![Figure 3.4](image)

**Figure 3.4** The CDF of a $\chi^2_{101}$ distribution (eq (3.6)). The dashed line is simulated using 4000 bootstraps, and the vertical line corresponds to the threshold for model rejection $\delta_{0.05}$. See Example 3.3 for more explanations.

The method of maximum likelihood

The likelihood function is one of the most central notions in the statistical framework reviewed here. We will now introduce this notion, and see how it can be used to obtain an estimated model. We will then show how such an estimated model
can be subject to a $\chi^2$ test, and how the likelihood function can be used to do a comparative test between two model structures.

A likelihood function is associating a probability (i.e. a likelihood) to the combination of an observation, $Z^N$, and a stochastic model, $M(p)$, which is somehow determined by the parameter $p$. It is thus identical to a PDF and, indeed, if a PDF is available for the same data and model it can be used as a likelihood function. However, the likelihood function is only considered as function of the parameter, and it thus gives a measure of how probable a given observation is for different model parameters. Note that this is the opposite of a classical PDF that gives a measure of how probable different observations are, for a given parameter. Let the likelihood function be denoted $L$

$L : p \rightarrow L(p) =$ a measure of how likely a given observation is, as a function of $p$

Note that the likelihood function, again unlike the PDF, is not necessarily normalised over its definition space. In fact, the likelihood function is actually a class of functions, which are undetermined up to an arbitrary positive scalar. That means that if $L(p)$ is a likelihood function, $aL(p)$ will be an equivalent likelihood function, for any $a > 0$. Let us now turn to the problem of determining the likelihood function for a model structure like (2.4).

The original model structure (2.4) is deterministic. Hence, we first need to specify how to make it a stochastic model suitable for the likelihood framework. In this dissertation it is assumed that the noise enters the equations exclusively through the measurements. Furthermore, it is assumed that the measurement noise is white, normally distributed, and that the random variables for the different time-points and equations are independent. Let the noise at time $t$ and for measurement equation $i$ be denoted $d_i(t)$, and let $d(t)$ denote the corresponding time-dependent vector. Assume finally that the mean of all the noise variables is zero, and that the variance is given by $\sigma^2_i(t)$

$$d_i(t) \in N(0, \sigma_i(t))$$

It will typically be assumed that the given data set, $Z^N$, has been generated by the given model structure $M$ for a 'true' parameter value, $p^0$. Let this model be denoted $M_0$, and let the corresponding 'true' state vectors be denoted $x^0$. The true model structure, corresponding to (2.4) but with a random output, $y$, is then given by

$$\begin{align*}
\dot{x}^0(t) &= f(x^0(t), u^0(t)) = f(x^0(t), p^0_u, u(t)) \quad (3.7a) \\
y(t) &= y(x^0(t), p^0_y, u(t), p^0_y) + d(t) \quad (3.7b) \\
x^0(t_0) &= x^0_0 \quad (3.7c)
\end{align*}$$

To distinguish the simulated data, which is a function of $t$ and $p$, from the measured data, which is only a function of $t$, the former is denoted by $\hat{y}(t|p)$ and the latter by $y(t)$. Note that each $y_i(t)$ appearing in $Z^N$ is to be considered as a specific realisation of a random variable, which contains both a deterministic and a stochastic part. Note also that the residuals, $\epsilon_i(t|p)$, which are defined by the difference between the measured and simulated outputs

$$\epsilon_i(t|p) := y_i(t) - \hat{y}_i(t|p)$$
are purely normally distributed (no deterministic part) at the true parameter values

\[ \varepsilon_i(t|\theta^0) = d_i(t) \in N(0, \sigma_i(t)) \]  

(3.8)

Since the noise is assumed to be independent at each measurement, the corresponding PDF for a whole time-series will be given by the product of the individual PDFs. Since a PDF is also a valid likelihood function we can form a likelihood function in the same way. Using equation (3.4) to obtain the individual PDFs we get the following expression for the complete likelihood function

\[ L(\theta^0) = \prod_{t=1}^{N} \prod_{i=1}^{n_y} \frac{1}{\sqrt{2\pi\sigma_i^2(t)}} \exp \left( \frac{\left( y_i(t) - \hat{y}_i(t|\theta) \right)^2}{2\sigma_i^2(t)} \right) \]

Note that if a parameter \( \theta \) is chosen that is different from the true parameter \( \theta^0 \), this will (under the expectation value operator \( E \)) make the given observation less likely. That means that one way to estimate parameters is to find the parameter values that maximise the likelihood function. This is called the method of maximum likelihood (ML), and if the corresponding estimated parameter is denoted \( \hat{\theta}^{ML} \) we have

\[ \hat{\theta}^{ML} = \arg \max_{\theta} L(\theta) \]  

(3.9)

The concept 'method of maximum likelihood' was first introduced by Fisher in 1922 [44]. We will now use the obtained framework to derive a validation test for an estimated model.

The \( \chi^2 \) test for a single model

Since the logarithm is a strictly increasing function of its argument the logarithm of a function preserves the location of the maximum. Hence, we may consider the log-likelihood function, \( l \), instead

\[ l(\theta) := \log L(\theta) = \sum_{t=1}^{N} \sum_{i=1}^{n_y} \left\{ -0.5 \log(2\pi) - \log(\sigma_i(t)) - \frac{(y_i(t) - \hat{y}_i(t|\theta))^2}{2\sigma_i^2(t)} \right\} \]  

(3.10)

Since the variance of the noise is assumed to be a property that is independent of \( \theta \), the first two terms in (3.10) do not effect the location of the maximum. Hence, we arrive at the cost function that is usually considered during ordinary least squares estimation, after a change in sign and removal of a scalar multiplicative factor

\[ \hat{\theta}^{LS} = \arg \min_{\theta} V_N(\theta, Z^N) := \arg \min_{\theta} \sum_{t=1}^{N} \sum_{i=1}^{n_y} \frac{(y_i(t) - \hat{y}_i(t|\theta))^2}{\sigma_i^2(t)} \]  

(3.11)

Note that the corresponding least squares estimation \( \hat{\theta}^{LS} \) is obtained through a minimisation, while the maximum likelihood estimate is obtained through a maximisation. Note also that the least squares estimation framework considers \( \sigma_i(t) \)
as the weight for the corresponding residual, and that this can be chosen more freely, since it is not linked to an the interpretation of the noise variance. On the other hand, if the maximum likelihood interpretation of $\sigma_i(t)$ is chosen there is a possibility for validating the estimation using a $\chi^2$ test. This test is understood by noting that if $p = p^0$ each term in the summation (3.11) be the square of a normally distributed random variable with zero mean and variance one

$$
\frac{(y_i(t) - \bar{y}_i(t|p^0))}{\sigma_i(t)} \in N(0, 1)
$$

(3.12)

Since the $\chi^2$ distribution is defined as the distribution for such a sum the function $V_N(\hat{\beta}, Z^N)$ will be a $\chi^2$ distributed with $N \times n_y$ degrees of freedom, if the hypothesis $M_0 = M(\hat{\beta})$ is true. Let this hypothesis be denoted $H_0$, i.e., let it be a null hypothesis. We have thus obtained both a test quantity for this null hypothesis ($V_N(\hat{\beta}, Z^N)$), and its distribution ($\chi^2_{N \times n_y}$). After choosing a significance level $\alpha$ it is therefore possible to make a hypothesis test of an obtained estimation by simply using the obtained error function and a table with $\chi^2$ values. If the error function has a value above a threshold corresponding to $\alpha < 0.01$ it is 99% certain that a better model can be found given the current data.

Here it should be noted that the above reasonings are only valid if the test is made upon a different data set than the data set used for the estimation. If the same data set is used for both the test and the estimation, the distribution for the test will have fewer degrees of freedom. This comes from the fact that each identifiable parameter that is estimated implies a constraint on the residuals. The resulting distribution will still be a $\chi^2$ distribution, but the number of degrees of freedom will be reduced by one for each identifiable parameter that has been estimated to fit the particular data set used for the test.

### The likelihood ratio test

The second type of null hypothesis that was mentioned in the beginning of this section assumes that two models are explaining the given data equally well (or that they are identical). Just as for the above $\chi^2$ test we assume that the given models have resulted in concrete models obtained through a maximum likelihood estimation. Let the log-likelihood functions corresponding to models $M_1(p_1)$ and $M_2(p_2)$ be denoted $l_1$ and $l_2$, respectively. The most used test function for the given situation is the following likelihood ratio test [90]

$$
T = 2(l_2 - l_1)
$$

(3.13)

Associated to this test is something denoted standard conditions. Standard conditions are fulfilled if all parameters in both model structures can take any values, and if $M_1$ and $M_2$ are nested. The latter condition means that the model structure $M_1$ can be obtained from $M_2$ by constraining some of its parameters. Constraints can include, e.g., linear combinations, but typically it just means that some parameters are put to zero [26]. Let the difference in the number of parameters for $M_1$ and $M_2$ be denoted by $\Delta df$

$$
\Delta df := \dim(p_2) - \dim(p_1)
$$
Under standard conditions the test statistics (3.13) has a \( \chi^2_{\text{df}} \) distribution. However, standard conditions are never truly fulfilled, and this leads to a number of alterations away from the distribution. For most biochemical models all parameters are restricted to positive values, and this leads to asymptotic distributions consisting of mixtures of \( \chi^2 \) distributions [118]. However, for data sets of limited size these mixtures will also be subject to alterations. Another common violation of the standard conditions for the test statistics (3.13) is that the model structures are non-nested. For this case there exist analytical asymptotical results, but none for the realistic case of limited data sets. For the general situation it is possible to estimate the given distribution through bootstrap procedures [52, 90, 132]. In a bootstrap procedure many new data sets (bootstraps) are generated by simulation using different noise realisations. Usually these data sets are generated so as to mimic the characteristics of the noise in the real data set, and to correspond to the null hypothesis. These bootstrap data are then used to estimate new models, giving new test function values. The obtained function values are then used as an approximation of the real test statistics distribution. Finally, there is one common alternative comparison method, which is referred to as the Akaike Information Criterion (AIC). This method is used and shortly reviewed in Chapter 9.

**Minimal modelling using hypothesis testing**

We have described how a model can be rejected based on the agreement between model output and measurement data (using the \( \chi^2 \) test), and of how one can decide if an alternative model significantly improves the agreement between model and data (using the likelihood ratio test). Now, let us see how these two types of decisions can be used to obtain a minimal model, through an iterative modelling procedure. This approach starts with the same information that was used to form the grey-box model, and it is depicted as step 3’ in Figure 3.1.

The suggested procedure contains the following major steps:

1. Find a model structure \( M_1 \) that is as simple as possible
2. Compute the best agreement \( M_1(p^{ML}) \)
3. Evaluate the obtained model using, e.g., the \( \chi^2 \) test above
   - if not accepted, refine the model by incorporating more information, denote the new model structure \( M_1 \) and go back to 2
4. If the model passes the test, compare it with a more complicated model structure, \( M_2 \), using for instance the likelihood ratio test above
   - If \( M_2 \) is significantly better, set \( M_1 = M_2 \), and go back to step 4
   - If \( M_2 \) is not significantly better, but if there are other possible complicated candidates, \( M'_2 \), set \( M_2 = M'_2 \) and go back to step 4
5. If \( M_2 \) is not significantly better, and all reasonable candidates have been tested the final minimal model is obtained
As usual we would like to start by stressing that all modelling schemes are just outlines of the most important components, and a suggestion for their most logical sequence of application. In practise modelling is carried out in a non-algorithmic way, and one usually needs to go back and re-iterate previous steps and use biochemical intuition to reject or come up with new hypotheses. Such creative processes can never be completely formalised. On the other hand, if it is possible to present a modelling work using the above decision points it is a convincing way to do it. In this dissertation we will see two real modelling examples where these statistical tests are used (Chapters 9 and 11), and there are several examples of convincing work in the systems biology literature where this has been done (see e.g. [126]). Let us now finally see how this procedure can be used in the small example introduced above.

**Example 3.3**  Consider system (3.1a) in Example 3.1. From looking at the data, and from comparing the in vitro estimations of $p_1$ and $p_3$ it is clear that the processes occurring above all implies a reduction of $[A]$. Let us therefore describe this reduction in the simplest possible way, through a constant degradation rate $p_1$. Let this simplest possible model structure be denoted $M_1$, which is given by the following differential equation

$$\dot{x} = -p_1$$

$$x(0) = x_0$$

Step 2 consists of a minimisation using ML. Since this just means fitting a straight line to data, it can be done in many ways. The agreement is is not very convincing, but on the other hand not totally wrong. Step 3 consists of an evaluation of the model using the $\chi^2$ test. We have 102 samples, and therefore we should compare with the $\chi^2_{100}$ distribution. The standard deviation is estimated from the data to be 3, and this gives a $\mathcal{T}$ value of 133. Using the $\chi^2_{100}$ distribution plotted in Figure 3.4 we see that $\delta_{0.05}$ is at 125. This means that the straight-line model can be rejected with a statistical significance of at least 95%, which confirms that the model structure is too bad to be acceptable. Next step is to find a more complicated model structure. Our biochemical knowledge tells us that many decay processes are governed by a term proportional to the concentration. We thus choose a new $M_1$ as

$$\dot{x} = -p_1 x$$

$$x(0) = x_0$$

This model structure gets a $\mathcal{T}$ value of 98. Since we use the same data, the expected distribution is the same. Therefore since $98 < 125$ we cannot reject this $H_0$ (saying that the model structure is true). A comparison with an even more complex model structure could be done, e.g., by replacing $-p_1 x$ for $-p_1 x - p_2 x$, or by adding a small $+p_3$ term. Both these choices of $M_2$ give an agreement that is non-distinguishable from the $M_1$ agreement (see Figure 3.2), which means that we cannot reject $M_1$ either by the $\chi^2$ test or by a likelihood ratio test. In the next section we will see how the same model structure can be validated using an
analysis of the residuals. Note that the hypothesis testing resulted in the same core model as the reduction step from the grey-box model (if the version without the 0.02 term was chosen). This illustrates the fact that although the methods used in Steps 2 + 3 and 3’, in Figure 3.1, are quite different, there does not have to be a difference in the resulting core models.

### 3.5 System identification of the core model

The next step in the core-box modelling framework is the system identification step. This step has similarities to the model tuning step, which is done for grey-box models (see Chapter 2.5.1), but there are some significant differences. The main reason for these is that the model structure is now identifiable. We have now been introduced to this concept, seen two ways how core models can be obtained, and we will now see how the advantage of identifiability can be used. We start by a short review of how the estimation is done.

#### The identification step

The first step in the identification is the choice of cost function, or selection criterion. Usually this is formalised as the minimisation or maximisation of a single valued function, with or without additional constraints. This optimisation problem is then solved using some local or global optimization method.

**Choice of cost function:** In the previous section we saw how the method of maximum likelihood leads to a cost function that should be maximised (eq. (3.9)). We also saw how this choice was the same as the least squares selection criterion (eq. 3.11), with a particular choice of weights on the different samples. Apart from the choice of weights there are also many other choices to make within the least squares framework. One of these is how to treat other types of data than pure time-series, such as prior knowledge of parameter values or knowledge about the phases between oscillating variables without having access to the corresponding time courses. This can, e.g., be done by adding extra terms to the original cost function [77]. If these terms are somehow proportional to the deviation from the extra data, then the optimization can proceed as usual. Other options to treat such data is to formulate the information as constraints on the optimization problem. Two new methods that utilise the latter solution are presented in Chapter 6. Another choice that can be done regards the calculation of \( \hat{y}(t|p) \). The most common option is to simulate the model from the current \( x_0 \) value, and calculate \( \hat{y}(t|p) \) at the time points where measurements data are available through a single simulation. It might sometimes be advantageous to restart the simulation, e.g., if the simulation has diverged too far away from the measured output. An appreciated method that does this is denoted multiple shooting [10, 11], but incorporation of measurement data when computing the prediction \( \hat{y}(t|p) \) is also the basic principle of most prediction error based estimation methods [77]. The multiple shooting method is especially advantageous if all states can be measured. However, if all states can be measured many new options appear. One approach is to estimate some or all of the derivatives and states directly from the time-series, using some kind of interpolation scheme, and
then compare \( \hat{y} \) and \( f(y, p_x, u) \) directly \([131], [46] \). It should finally be said that one could also consider to form more simple criteria for comparison, e.g., only considering the steady state values and the basic oscillatory properties. This is used, e.g., in the 'direct method' that was used to optimize the Hynne model \([62] \). Simple algebraic cost functions have the advantage that they are faster to evaluate, which argues for usage during early screenings of the parameter space.

**Local optimization methods:** Once the cost function has been chosen, the optimization can be performed. If it is possible it is usually advantageous to use a local method. Many local methods are gradient-based, which means that they are using the slope of the cost function to determine in which direction to search. This can be compared to a ball that is rolling down a mountain side, namely it is always going in the direction where it goes quickest downwards. The gradient can be obtained through numerical perturbation or through calculation of the sensitivity equations \([109] \). The Newton method is one of the most basic of these methods. It has the advantage of quadratic convergence (which is very fast) if the starting guess is sufficiently close to the optimum. If the starting guess is close but not that close, it is probably better to use a modification of the Newton method. The Levenberg-Marquardt method and other Gauss-Newton methods are common choices \([92] \). If the starting guess is even further away, and if the cost function is not particularly smooth, it might be better to use a method that does not involve the calculation of the gradient. A common such method, still is local, is the simplex method by Nelder and Mead. This method is the default choice for the function `fminsearch` in MATLAB.

**Global optimization methods:** For parameter estimation in ODEs, the standard least squares cost function is often experienced to be a difficult function to optimize, due to its many local minima. Therefore one often resorts to global optimization methods. Global optimizations are attempting to search the entire parameter space. Since it is impossible to do a full search there are many methods that use different approximations of such a search. Many of these methods are updating a big cluster of many guesses, and even though random variations are typically included, most effort is devoted to search in the vicinity of the currently best estimates. There are also global methods that are completely deterministic. It should be noted that even though there are many that claim that local optimization methods are never applicable to full-scale biology problems (see e.g. \([89] \)), there are also cases where counter-proof has been presented, and in such cases the local optimization approach is often vastly superior (see e.g. \([46] \)). The reason for this is that local methods have a much higher rate of convergence, if one is just sufficiently close to a valid local optimum. This is the reason why hybrid methods are developed, i.e., methods where the initial search is done by a global method, and where the final convergence process is done by a local method \([106] \).

**Determination of quality tags**

Independently of how the cost function has been formulated, and of which optimization methods that have been used, it is central in this step of the core-box...
modelling framework to obtain quality tags to the estimated model. These quality tags can be determined in several ways, but some of the most central concepts when doing so are confidence intervals, cross-validation, and residual tests.

**Confidence intervals:** Even if all free parameters in a core model are supposed to be practically identifiable they will always be determined with an uncertainty. These uncertainties can be quantified in different ways. A common way to perform such an analyses is to consider the parameter regions that are giving 'almost optimal' cost function values. Using statistical assumptions of the noise in the data, one can then translate such regions to statements like: ‘$p_1 = a \pm b$ with a probability of 95%’. One may then vary all parameters within the determined regions, and see how the features of the models changes. In this way one can use the parametric uncertainties, to also draw conclusions about which features of the models that have been estimated to which degree of accuracy.

**Cross-validation and residual tests:** Apart from such parameter based uncertainty measures, there are also more qualitative validation tests that can be done on the model as a whole. In Chapter 2.5 we discussed the necessity of cross-validation with qualitatively different validation data. The features that are tested with such data are, of course, determined with a high certainty. There are, however, typically many properties of a model that are not tested by such data. If an estimated model is accurately describing all aspects of the real system, the only thing that is left in the residuals will be the measurement noise. Therefore one can study different features of the residuals to see whether they have the assumed properties of the noise (typically that it is white and Gaussian). The $\chi^2$ test mentioned in the previous section can actually be considered as such a test. Such residual tests, however, typically only give a validation of the model as a whole, or at least only of the prediction quality of the states that can be measured. The quality of unmeasured features of the model are hard to draw conclusions about using these latter techniques.

Let us now again return to the our little example system, and then move on to the last step: 'Back-translation to the core-box model'.

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**Example 3.4** We are now faced with the problem of estimating the model structure (3.3) with respect to the data in Figure 3.2. Since this problem has already been treated in the previous section, we will instead consider one of the validity tests mentioned above. A $\chi^2$ test gives a $T$ value of 15 which is way below the 95% rejection border at $T = 125$. The residuals are plotted in Figure 3.5, and as can be seen there are no clear trends in the residual. Finally, the $p_1^1$ parameter can be estimated to $p_1^1 = 4.48 \pm 0.3$ where we leave the details of the calculation for later chapters, when the necessary theory has been introduced.
3.6 Back-translation to the core-box model

Assume now that a core model has been estimated, and that the above mentioned quality tags have been determined for the interesting features in the model. One has then a model that answers the questions: 'Which parts/features of the model can we rely on the most, given the current agreement with the data?', 'Are there parts of the model that still are completely unvalidated?' Since it was these questions that were leading us to the development of the core-box modelling framework, we have now come a long way in fulfilling the objectives. There are, however, still some things that could be done, before the maximal information has been included in the model. The identified core model is a minimal model, and it has thus eliminated much of the detailed information contained in the grey-box model. It might, e.g., be the case that an uncertainty relates to a lumped phenomenon, while one is really interested in the uncertainties in the details. It might also be the that one is interested in simulating the response of a particular enzyme, that is lumped together with many others in the core model. To achieve a model that contains all the available knowledge, it therefore seems necessary to translate the results for the core model back to the grey-box model. We divide this in two steps: Back-translation of the estimated features and Back-translation of the quality tags, and finally also mention something about the improved analyses that can be done on the final core-box model.

Figure 3.5 Residual plot for the estimated core model.
Back-translation of the estimated features: Each part in the core model will generally correspond to a module in the grey-box model. A module may consist of one or several parts, and might, e.g., consist of several states that are highly correlated in the grey-box model, and therefore lumped together in the core model. The first step is to translate the behaviours of the parts in the core model back to the corresponding modules in the grey-box model. How this is done will depend very much on the way the reduction has been done. It is therefore best to treat the problem of back-translation already at the reduction step. It might also be so that the sole reason for the modelling is to estimate a certain feature, e.g., the relative effect of two components, and then it is prepared for already during the modelling phase which parts that are involved in the back-translation. For now we will be satisfied with a division in three types of back-translations: calculation of an analytic translation formula, numerical optimization of a single module, and numerical optimization of the entire model. The back-translation is quite straightforward in the first two cases, if the optimization does not involve simulation. If the optimization implies numerical simulation of a cost function, or if one has the last case, the back-translation will be quite tedious, and maybe even comparable to the original estimation problem.

Back-translation of the quality tags: Once the basic behaviours of the core model have been back-translated, one can turn to the problem of back-translating the uncertainties. Also here it is best to treat this problem already during the reduction step. The back-translated uncertainties are connected to the estimations from the \textit{in vivo} data, and are thus of a qualitatively different character than those obtained during the \textit{in vitro} estimations. This means that they should be accounted for separately. As we see in the example below, also the back-translations of the uncertainties may sometimes be made analytically, and just as for the back-translation of the features, it is highly advantageous if it can otherwise be done on parts of the model separately. This is possible, e.g., in the case of lumping and reduction of individual rate expressions (see Chapter 7).

Analysis of the final core-box model: Once the core-box model has been obtained one has full possibilities for doing any \textit{in silico} experiments or other analyses one wants. There are many detailed experiments that are not possible on the preceding core model, simply because the necessary details are not included. This is an important reason why the core-box model is advantageous compared to the core model. Similarly there are many advantages compared to the original grey-box model. Now it is, e.g., possible to judge whether the predicted results are predicted with a high certainty, or just due to some arbitrary choice on an invariant manifold. We will now see an example that clearly reveals the advantage of having such quality tags.

Example 3.5 The reduction to the core model involved two steps: lumping of the two terms describing the elimination, and removal of the term describing the synthesis. We now want to reverse these two reductions, back-translate the obtained \textit{in vivo} estimations and quality tags, and illustrate the advantage of the obtained core-box model.
The elimination of the $p_3$ term was made since it did not affect the model behaviour to any visible extent (see Figure 3.2). This conclusion was basically drawn from the in vitro estimated values. Since this parameter is not affecting the model behaviour the reversal simply means to add it back again; no additional modifications are necessary. Also the back-translation of the merging of the two elimination terms is quite straightforward. This is because we have an analytical expression for how the reduction was done (eq. (3.2)). That means that we have the case i) above, and the reversal is simply done by choosing any values for $p_1$ and $p_2$ that fulfills $p_1' = p_1 + p_2$, and where $p_1$ lies within its uncertainty range from the in vitro calculations.

When back-translating the uncertainties to $p_1$ and $p_2$ we can also use eq. (3.2). It in itself says that it is the sum of the two parameters that has been determined to have the value $4.48 \pm 0.3$. This interpretation is not always that easy to do, and it is step one in the back-translation. Step 2 is to merge the core model uncertainties with the grey-box model uncertainties. The grey-box model uncertainties only stem from the quantification of the parts, which typically consists of in vitro estimations. In the example we have assumed that $p_1$ has been determined to $p_1 = 3 \pm 0.2$, but that there are no estimates of $p_2$. There are no in vivo estimates for these parameters, but using equation (3.2) and the uncertainties in $p_1'$ (from the in vivo data), and the uncertainties in $p_1$ we must have that $p_2 \in (0.98, 1.98)$. That information is new, and is only possible because the in vivo uncertainties have been combined with the in vitro uncertainties. The uncertainties in $p_1$ and $p_3$ have not been improved by the in vivo estimations.

Assume now that we have a potential drug that may reduce $p_2$ by 50%. Let us see how the three available models (the grey-box, the core and the core-box model) relate to that information, and what they can do to analyse the possible effects of such a drug. The grey-box model does not have any uncertainties related to the various parts of the model. It can thus make a prediction of the effect, but there is no way to know how to relate to that prediction, i.e., to know whether it is trustworthy or not. The core model has determined uncertainties, but not to anything relating to $p_2$, since $p_2$ is not a part of that model’s formulation. In order to use that model one would have to know how to translate the 50% reduction in $p_2$ into a reduction in $p_1'$. That translation has been characterised in the core-box model. Using that model one can therefore say that the proposed drug would change the characteristic decay time of the whole system (which is given by $p_1'$) with between 10% and 24%. This little analysis completes our example, that has followed us throughout the whole chapter. In Chapter 7 we will return to it again and do all the back-translation calculations using the full formalism.

Summary

This chapter has outlined a new modelling methodology, core-box modelling, which seeks to answer some important questions that a classical grey-box modelling approach is unable to answer. These questions are of the type: 'Which parts/features of a model can we rely on the most, given the current agreement with the data?', 'Are there parts of a model that still are completely unvalidated?'. The reason
why these questions can not be answered from a classical grey-box model, is that such models are typically highly over-parametrized with respect to the available data. A first step is therefore to characterise these over-parametrizations, using methods from identifiability analysis, and then to simplify these parts so that only the identifiable core of the model remains. Such a minimal model can also be directly obtained from the same information that was used to construct the grey-box model, using methods from hypothesis testing. Once the core model is obtained it is identified with respect to the available data. Since the model is identifiable, we can use the methods of system identification to obtain quality tags to the different model properties. The well-determined properties, and the quality tags, are then finally back-translated to the grey-box model. The resulting model is a combination of all the knowledge about the details that was put into the white-box model, with the information about which features in the resulting model that are based on the \textit{in vivo} data. The resulting model, which we refer to as a core-box model, should therefore be advantageous for analysis, both compared to the minimal core model and compared to the original grey-box model.

We have already presented a short description of all the involved steps. Now we will look at each of them more closely. Each step will be treated in a separate chapter, and for each step we will give both a more extensive review, and present some new results.
Step 3 in the core-box modelling approach is the reduction of the physically formulated grey-box model to an identifiable core model. This step consists of two major components: identifiability analysis and model reduction. If the source of unidentifiability is localised to single rate expressions, the reduction can often be done based on the identifiability analysis alone. We therefore treat both identifiability analysis and such single reaction rate simplifications in this chapter, but leave the general problem of reducing a complete model structure to Chapter 5. It should also be mentioned that there are situations in which the methods in this chapter are sufficient to obtain an identifiable core model for the whole system. This is the case if all variables can be measured and the stoichiometric matrix is of full rank [5]. An identifiable estimation problem is also obtained if all variables in a rate expression and the rate itself can be measured, because one can then form an estimation problem for that reaction rate alone. We start the chapter by a review of general methods and theory for identifiability in Section 4.1. Thereafter, Sections 4.2 and 4.3 presents new methods for handling of structural and practical unidentifiability, respectively. Finally, in Chapter 10.2 the new methods are applied to identifiability problems in the Hynne model.

4.1 General theory and review of methods

Identifiable is treated in many different frameworks. There is unfortunately not a one-to-one correspondence between the concepts in the different frameworks, and it is even so that the answers may differ depending on which kind of identifiability one refers to, and which methods are used to answer it. The general idea, however, is always the same and refers to the possibility for unique estimation of a parameter
from data. If the data leads to a unique value for a parameter, this parameter is said to be identifiable. In the previous chapter we introduced the following differential equation (see Example 3.1)

\[
\begin{align*}
\dot{x} &= -(p_1 + p_2)x + p_3 \\
y &= x + d
\end{align*}
\]

to exemplify the concept. In this example there are two types of unidentifiability present. The first is due to the fact that \(p_1\) and \(p_2\) are indistinguishable, and that only their sum, \(p'_0\), can be identified. This is true for all data sets independently of the signal-to-noise ratio, and this means that \(p_1\) and \(p_2\) are structurally unidentifiable. Structural unidentifiability implies practical unidentifiability. Practical identifiability is obtained if a parameter can be identified from a specific given data set. For the data set in Example 3.1, the parameter \(p_3\) is not practically identifiable. This was concluded from the fact that the \(p_3\) term is much smaller than the \(p'_0x\) term. We will now see how these kind of assessments may be obtained in a more formal setting, and with automatic methods.

**Structural identifiability**

The term structural identifiability is used with different meanings in different frameworks. Here we take the interpretation held in, e.g., [37] which means that a parameter is structurally identifiable if the measurement signals \(y = g(x, \mu, p_y)\) in eq. (2.2) are sufficient for a unique estimation under perfect experimental conditions. The term 'perfect experimental conditions' means that there are no problems with the measurement noise or with the excitation of the system. It is also assumed that there are no computational problems with the estimation, e.g., due to local minima. Other meanings of the term structural identifiability are, e.g., assuming that all states can be directly measured [4], or that the identifiability properties are given by the structure of the interaction-graph [3, 76]. These two variations of the concept are thus weaker statements, and identifiability of those kinds thus follow directly from the kind of structural identifiability that we consider here.

A more formal definition of identifiability is now sketched in terms of the differential algebra framework. A more formal treatment is found in [93, 115]. A central concept in the differential algebra framework is the Lie derivative. It describes the gradient of a scalar function in a certain direction, given by a vector of functions. However, in this dissertation we will only use it in relation to equation (2.4), and we can thus introduce it more specifically. The Lie derivative of an output \(h_i\) is here defined as

\[
\mathcal{L} := \frac{\partial}{\partial t} + \sum_{i=1}^{N} f_i(x) \frac{\partial}{\partial x_i} + \sum_{j \in \mathbb{N}} \sum_{u} u^{(j+1)} \frac{\partial}{\partial u^{(j)}}
\]  \hspace{1cm} (4.1)

Let us now understand these terms one by one. The first operator is simply a derivation with respect to time. Note that this is a partial derivative, which means that it only considers explicit appearances of time (which are rare in this dissertation). The second sum is the time-derivative of an output function in all aspects
that involve the states. This follows from the classical derivation rule of a composite function, and from the fact that $\dot{x}_i = f_i(x)$. The final term is the formal time-derivative with respect to the inputs (remember that $u^{(j)}$ is the $j$th time-derivative of $u$). Since all explicit and implicit time-dependencies in the output function $h(x, u, p_x, p_y)$ are included, the Lie-derivative (4.1) is in this specific setting equal to the total (non-partial) derivative with respect to time of the output function.

The Lie derivative of a scalar function is a scalar, and the Lie derivative of the $n_y$-dimensional column vector $h$ in eq. (2.2) is an $n_y$-dimensional column vector. Now consider multiple applications of the Lie differentiation operator. The subsequent application of $i$ Lie differentiations is denoted

$$L^i := L \cdot L \cdots L \quad i \in \mathbb{N}$$

Note that $L^0$ is the unit operator which leaves a function intact. Consider a vector containing all the outputs, $h$, and their derivatives $L^i h$. This vector describes all the possible dynamics in the output of a system. It is interesting to know how this vector depends on the states, $x$, and on the parameters $p$. This is given by the derivative of the full output vector with respect to the corresponding $x_i$ or $p_j$. If two such derivative vectors are identical this indicates that the corresponding states or parameters have an identical effect on the outputs. This, in turn, indicates that they are unidentifiable. Likewise, if one such derivative vector is equal to a linear combination of other derivative vectors, the involved parameters and states are unidentifiable. These observations mean that if the following matrix

$$O = \left( \begin{array}{cccc}
\frac{dc^0 h}{dx_1} & \cdots & \frac{dc^0 h}{dx_n} & \cdots \\
\frac{dc^0 h}{dp_1} & \cdots & \frac{dc^0 h}{dp_1} & \cdots \\
\vdots & \ddots & \vdots & \vdots \\
\frac{dc^{n+r} h}{dx_1} & \cdots & \frac{dc^{n+r} h}{dx_n} & \cdots \\
\frac{dc^{n+r} h}{dp_1} & \cdots & \frac{dc^{n+r} h}{dp_1} & \cdots \\
\frac{dc^{n+r} h}{dp_r} & \cdots & \frac{dc^{n+r} h}{dp_r} & \cdots 
\end{array} \right)$$

(4.2)

is of less than full rank, there are unidentifiable parameters or states in the system. To test the rank of this matrix is the classical observability test in structural identifiability analysis. Note that the elements in the matrix are functions, i.e., symbolic expressions. The advantage of this is that the result does not depend on the choice of operating point. The disadvantage of this is that the rank calculation is difficult and time-consuming to perform. Note, however, that it is sufficient to include only the first $n + r$ derivatives (proved in [115]).

Apart from obtaining the identifiability of the complete system, it is possible to use the matrix $O$ to determine which individual parameters and states that are identifiable. This is done by removal of a column, and by a check if the rank of the resulting reduced matrix is different from the original rank. If the rank is different this is an indication that a unique contribution to the space of outputs has been

\footnote{The classical convention is to refer to a state that can be uniquely estimated from the outputs as observable. However, since this presentation is only giving an introduction to the basic concepts, we have chosen to refer to both states and parameters as identifiable.}
removed, and that the corresponding state or parameter is identifiable. Finally, the transcendence degree of the system denotes the minimal number of columns that need to be removed before a matrix of full rank is obtained. That means that the transcendence degree denotes the degree of over-parametrization in the system, and that the transcendence degree is zero for an identifiable system. Note that it is possible to have a situation where all parameters are unidentifiable even though the transcendence degree is equal to one. This is the case, e.g., for rational expressions where one parameter appear in front of each individual term both in the numerator and in the denominator. We sum up all these observations in a theorem.

**Theorem 4.1 Criteria for Structural Identifiability**

a) A system is fully identifiable if and only if the matrix $O$ is of full rank, i.e., iff
\[
\text{rank}(O) = n + r
\]  

b) Even if $O$ is not of full rank there might exist identifiable states or parameters. A parameter is identifiable if the rank of the matrix obtained by removing the corresponding column in $O$ is one less than the rank of the original $O$.

As mentioned above, the calculation of the rank tests in Theorem 4.1 are to be calculated over a function space. This is often difficult to perform in a feasible time for realistically sized systems. To overcome these computational complexities Sedoglavic developed an algorithm that performs the calculations slightly differently [115]. Instead of working in a function space, all functions are replaced by Taylor expansions, which allows for corresponding series calculations instead. Further, the calculations are performed modular a large prime number, which also increases the efficiency. The resulting algorithm, which is available in a MAPLE implementation [116], calculates the transcendence degree and a list of identifiable parameters for a system of the form (2.4) in polynomial time. The total time for these calculations is feasible also for large-scale models like, e.g., the Hynne model with 20 states and 60 parameters. The price for the modifications is that the result is only probabilistic for the non-identifiable parameters. However, the probability is very high (often >99.9%) and the result can therefore usually be trusted. Due to all these advantages the algorithm by Sedoglavic has been used in this dissertation whenever the structural identifiability has been calculated. However, the algorithm says nothing about the origin of the unidentifiability, how a reparametrization to identifiable parameters may be chosen, or how a translation between the original and the new parameters may be obtained. Such results are given in Section 4.2.

**Practical identifiability**

**Global simulation based methods**

A parameter is practically identifiable if it really can be estimated from a given data set. This means that the uncertainty associated with the estimation should be acceptably small. The uncertainty in the estimation of a parameter has several sources. One such source is the process of the estimation itself. It is, e.g., often not possible to find the global minimum, since there are too many local minima in the
objective function. The exact location of the global minimum is also dependent
on the exact formulation of the cost function, which often can be formulated in
several ways but still give an acceptable result. One is therefore more interested in
the region of acceptable parameter values for any cost function. This region may
be determined by forming several well-functioning cost functions, and by starting
the estimation from many different initial guesses. This gives a global estimate of
how big the uncertainties are due to the actual estimation.

Another source of uncertainty in an estimated parameter value is due to the
fact that the exact value of the cost function is dependent on the specific noise
realisation. Thus, even if an identical experiment is performed, the noise will be
different, and the exact location of the minimum will change. Furthermore, this
variation increases with the level of the noise. There is thus a relation between
the noise level and the uncertainty in the estimation. One way to determine this
relation is bootstrapping [77]. A bootstrap is a new time-series that has been
simulated with the intention of being identical to the original time-series in the
deterministic part, but with a new noise realisation in the stochastic part [90]. By
doing new estimations on a large number of bootstraps a statistical variation in the
estimation uncertainty is obtained. This variation is a measure of the uncertainty
due to the noise. Bootstrapping is a computationally expensive method since many
estimations must be done. If the estimation itself is on the border of what is
computationally possible, this approach is of course not applicable. Furthermore,
if the bootstraps are based on an experimental data set, the true variation between
different noise realisation is not known, and this leads to an error in the estimation
of the uncertainty. There are, on the other hand, situations where the variation
in the data series due to different noise realisations is known, e.g., when also the
original data series is simulated. If this is the case, and if an individual estimation
is fast, bootstrapping is a sound and comprehensive method for estimation of the
parametric uncertainty due to the noise.

Local sensitivity based methods

If bootstrapping is not applicable other methods have to be used. Typically one
then resorts to local methods based on some kind of sensitivity analysis. The
derivative of the output with respect to the parameters is formally written as
\[ \frac{\partial y(t,p)}{\partial p}, \]
which we will also sometimes refer to as the sensitivity matrices \( S_y^p(t) \)

\[
S^y_p(t) := \frac{\partial y(t)}{\partial p} = \begin{pmatrix}
\frac{\partial y_1(t)}{\partial p_1} & \ldots & \frac{\partial y_1(t)}{\partial p_r} \\
\vdots & \ddots & \vdots \\
\frac{\partial y_n(t)}{\partial p_1} & \ldots & \frac{\partial y_n(t)}{\partial p_r}
\end{pmatrix}
\] (4.4)

They may be calculated either by a new simulation and numerical perturbation or
by solving the following sensitivity equations [109]

\[
\begin{align*}
\frac{dS^x_p}{dt} &= \frac{\partial f}{\partial x} S^x_p + \frac{\partial f}{\partial p} S^p_x \\
S^y_p &= \frac{\partial h}{\partial x} S^x_p + \frac{\partial h}{\partial p} S^p_x
\end{align*}
\] (4.5)
Note that this is a matrix notation of a set of nonlinear ordinary differential equations. Note also that each partial derivative is evaluated at the state $x$, which was given by the original equations (2.4). In practice, however, the equations are usually solved together, and in this dissertation we have used the implementation available in the Systems Biology Toolbox for MATLAB [111].

The output sensitivities may be combined to also account for all co-dependencies between the parameters. A common way to do this is to form the Fisher Information Matrix (FIM) which is defined by the following expression [77]

$$
FIM := \sum_{i=1}^{N} \left( \frac{\partial y(t_i)}{\partial p} \right)^T \Sigma(t_i)^{-1} \left( \frac{\partial y(t_i)}{\partial p} \right)
$$

(4.6)

where $\Sigma(t)$ is the diagonal matrix containing the variance of the measurement noise at time $t_i$. This formulation of FIM is consistent with the same choice of weighting (dividing the residuals by the variance of the measurement noise) for the original cost function, and the $\Sigma(t)$ factor in (4.6) should be removed to be consistent with an ordinary (unweighted) least squares cost function.

It may be shown that the asymptotic distribution of the uncertainty of parameters estimated by a generalised least squares cost function is asymptotically normal as $N$ goes to infinity [77]. Further, the mean value of the normal distribution is zero (i.e., the estimation is unbiased) and the covariance of the noise may be estimated by [113]

$$
\text{Cov}(\hat{p}) = FIM^{-1}
$$

(4.7)

where FIM is given by equation (4.6) with the $\Sigma(t_i)^{-1}$ factor included. If the unweighted cost function is used, and if the noise is assumed to be the same for all samples and time points, equation (4.7) becomes [113]

$$
\text{Cov}(\hat{p}) = \sigma^2 \left[ \sum_{i=1}^{N} \left( \frac{\partial y(t_i)}{\partial p} \right)^T \left( \frac{\partial y(t_i)}{\partial p} \right) \right]^{-1}
$$

(4.8)

where $\sigma^2$ is the true variance of the measurement noise. This variance may be estimated by $\tilde{\sigma}^2$ according to [77]

$$
\tilde{\sigma}^2 = \frac{1}{N n_y - r} \sum_{i=1}^{n_y} \sum_{j=1}^{N} (\varepsilon_i(t_j))^2
$$

(4.9)

where as usual $N$ is the number of time points, $n_y$ is the number of signals measured at each time point, and $r$ is the number of (identifiable) parameters.

It may sometimes be interesting to convert the covariance matrix to numerical uncertainties and confidence regions associated with a certain significance level $\alpha$. In the case of an unweighted cost function of a single output signal, the confidence ellipsoids are given by [113]

$$
\{ p \in \Omega : (p - \hat{p})^T FIM (p - \hat{p}) \leq r \sigma^2 F_{\alpha, N-r} \}
$$

(4.10)
where FIM is to be calculated without the $\Sigma(t_i)^{-1}$ (i.e., equal to the sum inside the square brackets in equation (4.8)), and F is the F-distribution. One may also calculate the uncertainties of an individual parameter according to the following formula [113]

$$
\left( \hat{p} - t_{N-r}^{\alpha/2} \hat{\sigma} \sqrt{d_{ii}}, \hat{p} + t_{N-r}^{\alpha/2} \hat{\sigma} \sqrt{d_{ii}} \right)
$$

(4.11)

where $d_{ii}$ is the i:th diagonal term in the inverse of the unweighted FIM matrix, $([FIM^{-1}])$ and where $t_{N-r}^{\alpha/2}$ is the Student t-distribution with $N - r$ degrees of freedom and confidence level $\alpha$. This means that the true parameters are to be found in the given regions to a probability of 100(1-\(\alpha\))%, and that the cumulative distribution function (CDF) of the given t-distribution at $t_{N-r}^{\alpha/2}$ is $\alpha/2$. It should finally be pointed out that the derivation of all these statistical measures are based on a number of assumptions and a linearisation of the real nonlinear regression problem. There are cases where such local approximations of the confidence regions give very misleading results [38]. A more globally valid region is determined by the level curves in the cost function directly

$$
\{ p : V(p) \leq (1 + \delta)E(\hat{p}) \} \quad \text{where} \quad 0 < \delta \in \mathbb{R}
$$

(4.12)

However, it is hard to determine a statistical uncertainty associated with such a region (even though asymptotic results exists [85]), and such regions are numerically much harder to compute [37]). In any case, the uncertainty region associated with the estimated parameters $\hat{p}$ will henceforth be denoted $\Delta_{\hat{p}}$.

![Figure 4.1 Example of a confidence ellipsoid. The ellipsoid is given by equation (4.10) and the two semi-axes are given by equation (4.43). The longest directions correspond to the most uncertain parameter combinations.](image)

**Identifiability of a single rate expression**

In this chapter we will introduce new methods for handling of unidentifiability located in single rate expressions. When doing so we will often consider the problem of estimating parameters in a single rate expression alone. This is a slightly
different estimation problem than that of estimating parameters in a differential equation like (2.4). That also means that the concepts regarding identifiability will be slightly modified, and now follows a short overview of these modifications.

The differential equations (2.1) are replaced by the equation for the reaction rate, i.e., something of the general form (see Chapter 2.2).

\[ v = v([S_1], \ldots, [S_i], [P_1], \ldots, [P_j], [M_1], \ldots, [M_l], k) = v(x, k) \quad (4.13) \]

It is further assumed that both the flux \( v \), and all substrates \([S]\), products \([P]\), and modifiers \([M]\), can be measured. This means that the parameters can be estimated in this specific reaction alone, without taking the full model into account. This reduces the number of parameters significantly. Further, a cost function may therefore be formed without integration but only using algebraic calculations. The most straightforward cost function is the sum of squares of the residuals, where the residuals are given by the difference between the measured rate, \( y_v(t) \), and the calculated reaction rate \( \tilde{y}_v(t|k) \).

\[ V_N(k) = \frac{1}{2} \sum_{t=1}^{N} (y_v(t) - \tilde{y}_v(t|k))^2 \quad (4.14) \]

The calculated reaction rate is most easily given by (4.13) where the concentrations are replaced by the corresponding measurements. The estimated rate parameters \( \hat{k} \) are as usual given by a minimization of the cost function (cf. (2.15))

\[ \hat{k} = \arg \min_k V_N(k) \quad (4.15) \]

Note that both the cost function (4.14) and the selection criterion (4.15) are identical to the previous notation, except that \( p \) have been replaced by \( k \). The notation \( k \) will be used in the sequel when we want to stress that the only parameters that should be estimated are those contained in a single reaction rate. However, in other cases we will simply let \( p \) denote the parameters to be estimated. In any case, \( p \) and \( k \) are vectors of the same length, \( r \).

Since the cost function and the estimation criterion are identical to the original situation the practical identifiability analysis, which is mostly based on a sensitivity analysis of the cost function, is more or less preserved. The Hessian matrix of the cost function may still be calculated using numerical perturbation and an eigenvector analysis may still be used to approximate the shape of the confidence region through calculation of the semi-axes (see eq. (4.43) below). However, it should be kept in mind that the cost function formed by (4.14) assumes that there is only noise in the measurements for the rate. This assumption is not true since the noise would typically be expected to appear also in the measurements of the concentrations. The covariance matrix, and the statistical interpretation of the uncertainty of the parameters might therefore be more or less inaccurate.

Even though the interpretation of the Hessian may be done as before, the sensitivity equations (4.5) cannot be used to approximate the Hessian via the FIM matrix, since these equations assume underlying differential equations. Instead a
4.1 General theory and review of methods

simpler alternative is used. It is motivated by the following calculations

\[
\frac{\partial V(k)}{\partial k_i} = \frac{\partial}{\partial k_i} \left( \frac{1}{2} \sum_{l=1}^{N} \varepsilon(t_l|\hat{k})^2 \right) = \sum_{l=1}^{N} \varepsilon(t_l|\hat{k}) \frac{\partial \varepsilon(t_l|\hat{k})}{\partial k_i} = -\sum_{l=1}^{N} \varepsilon(t_l|\hat{k}) \frac{\partial \hat{y}_v(t_l|\hat{k})}{\partial k_i} \nonumber
\]

\[
\frac{\partial^2 V(k)}{\partial k_i \partial k_j} = -\sum_{l=1}^{N} \left( \frac{\partial \hat{y}_v(t_l|\hat{k})}{\partial k_j} \frac{\partial \hat{y}_v(t_l|\hat{k})}{\partial k_i} + \frac{\partial^2 \hat{y}_v(t_l|\hat{k})}{\partial k_i \partial k_j} \varepsilon(t_l|\hat{k}) \right) \approx \sum_{l=1}^{N} \left( \frac{\partial \hat{y}_v(t_l|\hat{k})}{\partial k_j} \frac{\partial \hat{y}_v(t_l|\hat{k})}{\partial k_i} \right) \quad (4.16)
\]

The last approximate equality is exact in the limit of infinitely many data points (or under the expectation operator), if the residuals \( \varepsilon(t|\hat{p}) \) are white noise with zero mean. Further, the derivatives \( \frac{\partial \hat{y}_v(t|\hat{p})}{\partial p_i} \) may be calculated symbolically which means that no numerical perturbation is necessary. It is therefore clear that the same assumptions as were used in the motivation for the sensitivity equations (4.5) lead to the even simpler approximation (4.16), in the case of an algebraic cost function. The analog of the unweighted FIM matrix in the algebraic case is equal to the last expression in eq. (4.16). We have now accounted for the most important changes in calculation of the practical identifiability when estimating parameters in a single rate expression.

For structural identifiability there are also straightforward translations available. A parameter in a single rate expression is said to be structurally identifiable if it can be estimated from a perfect data set. We refer to the transcendence degree of the rate expression as the minimal number of parameters that needs to be removed before a structurally identifiable rate expression is obtained. Finally, when calculating the transcendence degree and structural identifiability of an individual rate expression, Sedoglavic’ algorithm may still be used. This can, e.g., be done by constructing a small system of differential equations that contains the relevant reaction rate, that contains the relevant constraints on the state variables which all are assumed to be measured, and where the reaction rate (but not necessarily its parameters) is structurally identifiable.

**System identification of non-identifiable models**

We will now shortly consider different approaches that can be followed when performing system identification of a model that is not identifiable.

Sometimes it is not necessary to take the identifiability issues into account at all. This is the case, e.g., if one is only interested in how good agreement a given model structure can achieve with a given data set. The parameter estimation will ensure that an optimal agreement is obtained, even though the unidentifiable parameters receive arbitrary values (on the invariant manifolds). In this dissertation we encounter a situation where the identifiability is not necessary to establish in Chapter 11.

To optimize a model that is not identifiable might, however, still lead to numerical problems. This might especially happen if boundaries for the unidentifiable parameters are unavailable, since the unidentifiable parameters may then achieve
extremely big or extremely small values. To overcome such problems it is common to replace unidentifiable parameters by reasonable guesses, until a practically identifiable system is obtained. This will make the estimated values of the remaining parameters conditioned on the values that have been chosen for the fixed parameters. It is often argued that all parameter estimations are conditioned on assumptions of the other parameters in the system, and that this is thus unavoidable. The effect of guessing wrong values for the omitted parameters may also be examined using various sensitivity analyses techniques. This method is always possible to follow, and the arguments for it are valid. However, there are situations where better alternatives are possible.

This is, e.g., the case if many parameters have to be set constant in a certain part of the model. The remaining parameters are then conditioned on many other parameters, and this makes a sensitivity analysis cumbersome. Further, the interpretation of what has really been estimated from the data is probably not easily obtained from the obtained parameter value. Say for instance that the data contains information about the time constant describing the overall transport from A to B, but not any information about the detailed processes. If the original model describes all the detailed processes in between, these parameters will of course not be identifiable from the given data. An identifiable model is obtained by replacing all parameters but one with a constant value, which allows for unique estimation of the single remaining parameter. However, even though good guesses might be available for the parameters that were not estimated, it will not be possible to obtain the information that the time constant for the system could be estimated from the data. To obtain such information, a model simplification is necessary. This can be done based on the identifiability analysis, which might also allow for a back-translation leading to a full core-box model. How this can be done in the case of estimation of a single rate expression is now presented: in Section 4.2 for structural unidentifiability, and in Section 4.3 for practical unidentifiability.

4.2 Structural non-identifiability in a single reaction caused by conserved moieties

This section shows how structural non-identifiability may be caused by conserved moieties. The phenomenon is easily understood by studying a small example, and this is done in Section 4.2.1. A general approach to detect and solve this particular source of structural non-identifiability is given in Section 4.2.2 and in Section 4.2.3 the general approach is applied to two more comprehensive examples. In Chapter 7 we will see how estimates of the new identifiable parameters can be back-translated to the original ones, for this particular kind of transformations, and in Chapter 10 we will solve the problem for the Hynne model.

4.2.1 A small example

Consider a reaction with one substrate and one product. Let the concentration of the substrate and product be denoted $x_1$ and $x_2$, respectively. Assume that the
sum of the two concentrations is constant in time, i.e., that

\[ x_1 + x_2 = p \]  

(4.17)

where \( p \) is a constant parameter. The substrate and product then form a conserved moiety. Let the forward rate velocity for this reaction be denoted \( v_f \). According to the classical book on biochemistry [117] a kinetic expression for the forward rate, \( v_f \), in such a system is

\[ v_f = \frac{V_{\text{max}}x_1}{K_S(1 + \frac{x_2}{K_P}) + x_1} \]  

(4.18)

where \( V_{\text{max}}, K_S \) and \( K_P \) are kinetic parameters. Insert (4.17) into (4.18) and find

\[ v_f = \frac{V_{\text{max}}x_1}{K_S(1 + \frac{p - x_1}{K_P}) + x_1} = \frac{x_1}{\tilde{c}_1' + \tilde{c}_2'x_1} \]  

(4.19)

where \( \tilde{c}_1' \) and \( \tilde{c}_2' \) are defined according to

\[ \tilde{c}_1' = \frac{K_S(1 + \frac{p}{K_P})}{V_{\text{max}}} \]

\[ \tilde{c}_2' = \frac{(1 - \frac{K_S}{K_P})}{V_{\text{max}}} \]

From these calculations it is clear that the expression (4.18) suggested in [117] with the three parameters \( K_S, K_P \) and \( V_{\text{max}} \), can in fact be described by 2 parameters, when the assumption (4.17) has been added. Note that (4.18) is not over-parametrized if \( x_1 \) and \( x_2 \) can be varied independently. From this example we have thus seen how the occurrence of all variables from a conserved moiety in the same rate equation may lead to structural unidentifiability. Now we will treat this problem in a more general setting.

### 4.2.2 A general approach to detect and solve the problem

We will treat the problem in a more general form by performing a number of transformations. These are outlined in Figure 4.2, where the original formulation is represented by the leftmost box and the final system with identifiable parameters is represented by the lower rightmost box. We start by rewriting the kinetic expression as a rational expression (i.e. as a fraction of two polynomials) and by understanding some general features about the identifiability aspects of this formulation. This is step 1 in the figure. After that a way to detect the above problem with identifiability due to conserved moieties is introduced, and this is performed together with step 2 in the figure. Should an unidentifiability be detected during step 2 we show how identifiable parameters can be found. This is step 3 in the figure. Finally the identifiable parameters are back-translated to the original identifiable parameters, and this is represented by step 4 and 5 in Figure 4.2. It should also be added that even though we here only consider rational expressions, these results can sometimes be used to analyse, e.g., Hill type expressions as well, as is shown in the second example in Section 4.2.3.
Identifiability of the polynomial formulation

Consider a single reaction rate \( v \). Let \( v \) depend on \( n \) concentrations \( x \) and on \( r \) kinetic parameters \( k \)

\[
v = v(x, k)
\]  

(4.20)

Assume that \( v \) can be reformulated as a rational expression of two polynomials in \( x \). Let the polynomial in the numerator and denominator be denoted \( P \) and \( Q \), respectively. Let the coefficients to the \( i \)th term of \( P \) and \( Q \) be denoted \( a_i \) and \( b_i \), respectively. Collect the coefficients in a vector \( c \)

\[
c = (a_1, a_2, \ldots, b_1, b_2, \ldots)
\]  

(4.21)

Let the list of exponents to the \( i \)th term of \( P \) and \( Q \) be denoted by \( p_i \) and \( q_i \), respectively. These two symbols are members of index sets which means that for instance

\[
p_j = (1, 0, 0, 3) \quad \Rightarrow \quad x^{p_j} = x_1 x_4^3
\]  

(4.22)

Members of index sets are denoted as bold symbols in this thesis. With this notation \( v \) is rewritten as

\[
v(x, k) \Rightarrow v(x, c) = \frac{P(x, c)}{Q(x, c)} = \frac{a_1 x^{p_1} + a_2 x^{p_2} + \ldots}{b_1 x^{q_1} + b_2 x^{q_2} + \ldots}
\]  

(4.23)

The reformulation to this form is step 1 in Figure 4.2. Let the number of terms in this expression be denoted \( n_t \). The number \( n_t \) is also the dimension of \( c \). In
4.2 Handling structural unidentifiability due to conserved moieties

the rational expression (4.23) it is not the original kinetic parameters \( k \) but the coefficients \( c \) that need to be identified. Now follows an important observation regarding the identifiability of these coefficients.

Lemma 4.1 Assume that all variables \( x \) in (4.23) can be measured, and that there is no restriction to how these may vary in relation to each other. Then the coefficients of (4.23) are structurally identifiable apart from an unknown multiplicative scalar.

Proof \( (\Rightarrow) \) Assume that two sets of coefficients \( c^1 \) and \( c^2 \) are related by a single multiplicative scalar, \( \lambda \)

\[
c^2 = \lambda c^1
\]

Then \( v(x, c^1) = v(x, c^2) \) for all \( x \) since both the numerator and denominator have been multiplied by the same factor, which can hence be eliminated.

\( (\Leftarrow) \) Assume that the possibility for a multiplicative factor has been removed, e.g., by fixing the leading coefficient, \( a_1 \), to 1. That gives coefficient vectors \( c^{1'} \) and \( c^{2'} \). Assume finally that \( v(x, c^{1'}) = v(x, c^{2'}) \) for all \( x \). Since the rates are equal for all \( x \), the functions \( v : x \mapsto v(x, c) \) must be equal. Since there is no longer a possibility to obtain identical functions by multiplication of both the numerator and denominator with the same multiplicative scalar, and since each coefficient represents a qualitatively different contribution to the function, the coefficients must be the same. This means that the coefficients are structurally identifiable if the multiplicative factor has been removed.

Remark: This proof is a bit ‘hand-waving’ because we have not developed the necessary framework to do a formal proof. Note also that there is a fundamental difference between identifiability in polynomials and in fractions of polynomials, i.e., in rational expressions. In rational expressions the fact that two expressions give identical outputs for all \( x \), does not always imply that the coefficients are equal, but only in the generic case. Exceptions occur if there are common denominators in the numerator and denominator. However, structural identifiability only deals with the best case scenario, and this problem is therefore not effecting the result in Lemma 4.1. Further, the manifolds in which this problem occurs are of a lower dimension than the full parameter space, and are therefore never run into practical applications.

Remember that \( n_t \) denotes the number of terms in (4.23) and that \( r \) denotes the number of kinetic parameters. Lemma 4.1 then gives us the following easy detection test for non-identifiability of \( k \)

- If \( r > (n_t - 1) \) the kinetic parameters \( k \) are structurally unidentifiable
- If \( r \leq (n_t - 1) \) nothing can be said from this test

Note that \( r < (n_t - 1) \) does occur for some rate expressions, as for instance for the first example in Section 4.2.3. Such expressions could have contained more unknown parameters (for instance, chosen as the coefficients of the polynomial formulation), and still be structurally identifiable. Let us now view the small example of Section 4.2.1 in the light of these results.
Example 4.1 Consider again the small rate expression in equation (4.18). Here the kinetic parameters are \( k = (V_{\text{max}}, K_S, K_P) \) and the polynomial formulation of (4.18) is

\[
v_f(x, c) = \frac{a_1 x_1}{b_1 + b_2 x_1 + b_3 x_2}
\]

where the coefficients are \( c = (a_1, b_1, b_2, b_3) \). Here \( n_t = 4, r = 3 \) and we do not detect any unidentifiability in this expression with the above test. This is good because the kinetic expression (4.18) is structurally identifiable if no assumptions have been made on the variability of the time-varying measurements \( x_1, x_2 \) and \( v_f \). A way to eliminate one coefficient in (4.24) and obtain an identifiable polynomial formulation \( v(x, c') \) is to divide both the numerator and the denominator with \( a_1 \)

\[
v_f(x, c') = \frac{x_1}{b_1' + b_2' x_1 + b_3' x_2}
\]

where \( (b_1', b_2', b_3') = (b_1/a_1, b_2/a_1, b_3/a_1) \)

Detecting the problem

To detect the identifiability problem due to conserved moieties we need to do yet another reformulation: step 2 in Figure 4.2.

Assume that some of the variables in \( x \) make up a conserved moiety. A conserved moiety is an affine relation between the metabolite concentrations that are assumed to be fulfilled for all data-points. Without loss of generality this can be formulated as a constant minus a linear sum of the variables \( x_1, \ldots, x_{n-1} \) which form \( x_n \). Let the real linear coefficients in this sum be denoted \( \alpha_1, \ldots, \alpha_{n-1} \), and let the total concentration of the conserved moiety be denoted \( m \)

\[
x_n = m - \sum_{i=1}^{n-1} \alpha_1 x_i
\]

Insert (4.25) into (4.23) and formulate the result as a new quotient between two polynomials \( P \) and \( Q \). Let the remaining \( n - 1 \) variables, the new coefficients, and the new exponents be denoted as before but with a tilde above. With this notation and with the addition of the knowledge (4.25) the new \( v \), which we denote \( \tilde{v} \), becomes

\[
v(x, c) \overset{(4.25)}{\Rightarrow} \tilde{v}(\tilde{x}, \tilde{c}) = \frac{\tilde{P}(\tilde{x}, \tilde{c})}{\tilde{Q}(\tilde{x}, \tilde{c})} = \frac{\tilde{a}_1 \tilde{x}^{\tilde{p}_1} + \tilde{a}_2 \tilde{x}^{\tilde{p}_2} + \ldots}{\tilde{b}_1 \tilde{x}^{\tilde{q}_1} + \tilde{b}_2 \tilde{x}^{\tilde{q}_2} + \ldots}
\]

The relation between \( c \) and \( \tilde{c} \) is linear. Let the matrix describing the mapping be denoted \( C \)

\[
\tilde{c} = C c
\]

Note that the mapping from \( v \) to \( \tilde{v} \) would not be linear if \( c \) was replaced by \( c' \) or if \( \tilde{c} \) was replaced by a corresponding identifiable coefficient vector \( \tilde{c}' \) (remember
that a little prime $'$ means that a parameter has been eliminated to obtain identifiable parameters, see Figure 4.2). To reveal and use this linear feature of the transformation of $v$ is the reason why the mapping is performed between $c$ and $\bar{c}$, and not between, e.g., $c'$ and $\bar{c}'$. The rank of $C$ describes how many independent coefficients that are formed from this mapping. This observation together with Lemma 4.1 implies the following test for whether the conserved moiety leads to problems with identifiability.

**Theorem 4.2** Assume that the original formulation $v(x, k)$ in (4.20) is structurally identifiable if all variables $x$ can be measured, and when they are assumed independent. Then a conserved moiety as described in (4.25) leads to structural unidentifiability, given the same measurement possibilities, if

$$\text{rank}(C) - 1 < r$$

Here $C$ is defined by equation (4.27) and $r$ is the number of kinetic parameters in the original expression (4.20).

Note that this is only a one-way statement; there might be problems with unidentifiability due to the presence of all variables from a conserved moiety even if $(\text{rank}(C) - 1) \geq r$. On the other hand, for all models that have been tested, and where the appropriate kinetic expressions can be rewritten as a rational expression, the above method has been sufficient to detect and understand the problem. It should also be added that there are examples from the literature where all variables from a conserved moiety appear in the same rate equation and where there are no problems with identifiability [5].

**Finding the identifiable parameters**

If the rank-test shows that the introduction of a conserved moiety has made a previously identifiable kinetic expression unidentifiable, the same transformation (4.27) can be used to obtain identifiable parameters, $\bar{c}'$. This process is depicted by step 3 in Figure 4.2. If the row-rank of $C$ is full, $\bar{c}$ is transformed into $\bar{c}'$ by the same single-coefficient-elimination techniques that was used to transform $c$ into $c'$ (see, e.g., Example 4.1). If the rank is not full, rows should be taken out from $C$ to obtain a sub-matrix that has full rank equal to $\text{rank}(C)$, before eliminating the final coefficient. The remaining parameters, $\bar{c}'$, are structurally identifiable, and the coefficients whose rows were eliminated can be calculated from $\bar{c}'$. An example where $C$ is of less than full rank is given in Section 4.2.3. Finally, we denote the identifiable rate expression $\bar{v}'$, i.e.

$$\bar{v}'(\bar{x}, \bar{c}')$$

(4.28)

is the identifiable expression with the identifiable parameters $\bar{c}'$.

**Example 4.2** Let us again consider the kinetic expression in (4.18). In Example 4.1 (4.18) was reformulated to (4.24). Solve for $x_2$ in (4.17), and insert the
result into (4.24). Rewrite the obtained expression to the form (4.26); this gives
\[ \tilde{v}_t(\tilde{x}, \tilde{c}) = \frac{\tilde{a}_1 x_1}{b_1 + b_2 x_1} \] (4.29)

The transformation between \( c \) and \( \tilde{c} \) is given by the linear matrix \( C \) according to
\[
\begin{pmatrix}
\tilde{a}_1 \\
\tilde{b}_1 \\
\tilde{b}_2
\end{pmatrix} = \tilde{c} = Cc = \begin{pmatrix}
1 & 0 & 0 & 0 \\
0 & 1 & 0 & m \\
0 & 0 & 1 & -1
\end{pmatrix} \begin{pmatrix}
a_1 \\
b_1 \\
b_2 \\
b_3
\end{pmatrix}
\]

The rank of \( C \) is three. Since the original expression (4.18) is identifiable, and since \( 2 = (\text{rank}(C) - 1) < r = 3 \), Theorem 4.2 shows that the introduced conserved moiety (4.17) has caused (4.18) to become unidentifiable. Since \( C \) has full row-rank, a simple elimination is sufficient to obtain \( v(\tilde{x}, \tilde{c}') \). One can for instance divide both the numerator and the denominator of (4.29) with \( \tilde{b}_2 \); this gives
\[ v(\tilde{x}, \tilde{c}') = \frac{\tilde{a}_1 x_1}{\tilde{b}_1' + x_1} \quad \text{and} \quad \tilde{c}' = (\tilde{a}_1', \tilde{b}_1') = (\tilde{a}_1 / \tilde{b}_2, \tilde{b}_1 / \tilde{b}_2) \] (4.30)

Note that this choice leads to the classical Michael-Menten expression, but that we in Section 4.2.1 chose to eliminate the coefficient in the numerator. The choice between these two forms is arbitrary from a mathematical point of view, but the classical Michael-Menten expression is superior in terms of interpretation of the meaning of the parameters.

### 4.2.3 Application to two bigger kinetic expressions

We will now consider two significantly more complex rate expressions than that in equation (4.18). The first is taken from a model by Teusink [130] and the second from a model by Rizzi [104]. In the examples we will also show how some simple rewritings can make the calculations simpler than in the general case, and how some original parameters may be kept invariant in the transformations. The second example will also show how the results can be applied to a wider class than that of rational expressions.

#### The \( v_{ADH} \) expression in the Teusink model

This first example is taken from a model by Teusink [130] and describes the Alcohol Dehydrogenase (ADH) reaction which converts Acetaldehyde (ACA) to ethanol (EtOH) with co-factors NADH and NAD\(^+\). Let \( x \) be the vector of concentrations
\[ x = ([\text{EtOH}], [\text{NAD}^+], [\text{ACA}], [\text{NADH}])^T \]

If the 10 kinetic parameters are denoted
\[ k = (k_1, k_2, k_3, k_4, k_5, k_6, k_7, k_8, k_9, k_{10})^T \]
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the expression is given by

\[
 V_{ADH} = \frac{k_1 \frac{1}{k_2 k_3} x_2 x_1 - k_1 \frac{1}{k_2 k_3 k_4} x_3 x_4}{1 + \frac{1}{k_3} x_2 + \frac{k_5}{k_4 k_5} x_3 + \frac{1}{k_6} x_4 + \frac{k_5}{k_4 k_6} x_1 + \frac{k_5}{k_4 k_7} x_2 x_3 + \frac{1}{k_7 k_8} x_3 x_4 + \frac{1}{k_3 k_7} x_2 x_1 + \frac{k_8}{k_3 k_5 k_6} x_4 x_1 + \frac{k_3 k_4}{k_3 k_5 k_7} x_2 x_3 x_1 + \frac{1}{k_10 k_7 k_8} x_3 x_4 x_1}
\]

Sedoglavic' algorithm (see Section 4.1) shows that all kinetic parameters \( k \) are structurally identifiable if all variables \((x_1, \ldots, x_4)\) and the flow \( v_{ADH} \) can be measured, and if the variables are governed by linearly independent differential equations.

The algorithm also shows that the additional assumption \( x_2 + x_4 = m \), where \( m \) is a constant parameter, makes the expression unidentifiable. The nicotinamides \( x_2 \) and \( x_4 \) do make up a conserved moiety in the Teusink model, and the Teusink model is thus an example of a real published model that contains the reported problem. (There are two other rates that also display this problem, GAPDH and G3PDH [5].) Sedoglavic' algorithm gives the unidentifiability with a high probability of success. It similarly decides that all parameters except \( k_4 \) are unidentifiable with a high probability of success. It calculates the transcendence degree to be one for this expression. We will now see how the new method proves the probabilistic prediction by Sedoglavic' algorithm, and gives a suggestion for how identifiable parameters may be chosen.

Transform the \( v_{ADH} \) expression to the form (4.24). This gives a 13-dimensional \( \mathbf{c} \) vector, and the following rate expression

\[
 v_{ADH} = \frac{a_1 x_2 x_1 + a_2 x_3 x_4}{(b_1 + b_2 x_2 + b_3 x_3 + b_4 x_4 + b_5 x_1 + b_6 x_2 x_3 b_7 x_2 x_1 + b_8 x_3 x_4 + b_9 x_4 x_1 + b_{10} x_2 x_3 x_1 + b_{11} x_3 x_4 x_1 + b_{12} x_3 x_4 x_1 + b_{13} x_3 x_4 x_1)}
\]

Since \( r = 10 \) the first test in Section 4.2.2 does not say anything about the identifiability of \( v_{ADH} \) before the conserved moiety is introduced. Transform the obtained system to the form (4.26). This gives

\[
 \bar{v}_{ADH} = \frac{\bar{a}_1 x_1 + \bar{a}_2 x_3 x_4 + \bar{a}_3 x_4 x_1}{b_1 + b_2 x_3 + b_3 x_4 + b_4 x_1 + b_5 x_3 x_4 + b_6 x_3 x_1 + b_7 x_4 x_1 + b_8 x_3 x_4 x_1}
\]
with the following $C$ matrix transformation

$$
\begin{bmatrix}
\tilde{a}_1 \\
\tilde{a}_2 \\
\tilde{a}_3 \\
b_1 \\
b_2 \\
b_3 \\
b_4 \\
b_5 \\
b_6 \\
b_7 \\
b_8
\end{bmatrix}
= \begin{bmatrix}
m & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
-1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 1 & m & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 1 & 0 & m & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & -1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 1 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 1 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0
\end{bmatrix}
\begin{bmatrix}
a_1 \\
a_2 \\
a_3 \\
b_1 \\
b_2 \\
b_3 \\
b_4 \\
b_5 \\
b_6 \\
b_7 \\
b_8
\end{bmatrix}
$$

(4.31)

The $C$ matrix has rank $\text{rank}(C) = 10$, i.e., one less than its number of rows. Furthermore, $9 = (\text{rank}(C) - 1) < r = 10$, which means that Theorem 4.2 verifies Sedoglavic probabilistic prediction that the parameters in $v_{ADH}$ become unidentifiable when the conserved moiety $x_2 + x_4 = m$ is present (note that Sedoglavic result that the parameters in $v_{ADH}$ are identifiable before the conserved moiety is added, is given as an exact result without probability). To obtain identifiable parameters one could, e.g., replace $\tilde{a}_1$ with $-m\tilde{a}_3$ and divide the resulting numerator and denominator with $\tilde{a}_3$. The first replacement is motivated by the fact that $C$ without its third row has full row-rank, and the second is the same kind of single-coefficient-elimination as was performed in Example 4.1. This gives

$$
v'_{ADH} = \frac{-mx_1 + \tilde{a}_1'x_3x_4 + x_4x_1}{b_1' + b_2'x_3 + b_3'x_4 + b_4'x_3x_4 + b_5'x_3x_1 + b_6'x_3x_1 + b_7'x_4x_1 + b_8'x_3x_4x_1}
$$

(4.32)

where the identifiable parameters $\tilde{c}'$ are given by

$$
\tilde{c}' = (\tilde{a}_1', \tilde{b}_1', \tilde{b}_2', \tilde{b}_3', \tilde{b}_4', \tilde{b}_5', \tilde{b}_6', \tilde{b}_7', \tilde{b}_8') = (\tilde{a}_2, \tilde{b}_1, \tilde{b}_3, \tilde{b}_4, \tilde{b}_5, \tilde{b}_6, \tilde{b}_7, \tilde{b}_8) / \tilde{a}_3
$$
or expressed in the original parameters

\[
\begin{align*}
\tilde{a}_1' &= \frac{1}{k_4} \\
\tilde{b}_1' &= - \left( \frac{1}{1 + \frac{m}{k_5}} \right) \frac{k_2 k_3}{k_1} \\
\tilde{b}_2' &= - \left( \frac{k_5}{k_6 k_7} + \frac{mk_5}{k_3 k_6 k_7} \right) \frac{k_2 k_3}{k_1} \\
\tilde{b}_3' &= - \left( \frac{1}{k_6} - \frac{1}{k_3} \right) \frac{k_2 k_3}{k_1} \\
\tilde{b}_4' &= - \left( \frac{k_8 + m}{k_3 k_2} \right) \frac{k_2 k_3}{k_1} \\
\tilde{b}_5' &= - \left( \frac{1}{k_6 k_7} - \frac{k_5}{k_3 k_6 k_7} \right) \frac{k_2 k_3}{k_1} \\
\tilde{b}_6' &= - \frac{m}{k_9 k_1} \\
\tilde{b}_7' &= - \left( \frac{k_8}{k_3 k_6 k_2} - \frac{1}{k_3 k_2} \right) \frac{k_2 k_3}{k_1} \\
\tilde{b}_8' &= - \left( \frac{1}{k_9 k_1} - \frac{1}{k_3 k_9 k_2} \right) \frac{k_2 k_3}{k_1}
\end{align*}
\]

Since the nonlinear part of the back-translation (step 5 in Figure 4.2) is made up of a quite large equation system, the result is too complex to be interesting. A geometrical interpretation is given in [5].

When performing this analysis no usage was made of the fact that we already knew that \( k_4 \) was identifiable. One could have simplified things by dividing the original kinetic expression \( v(x, k) \) with \( k_2 k_3 \). This results in an expression where all unidentifiable parameters are contained in the denominator. One can then rewrite this as a polynomial, substitute the conserved moiety, and simply identify the coefficients. The result is an expression that is structurally identical to (4.32).

The \( v_{RES,2} \) expression in the Rizzi model

The Rizzi model is also developed for yeast glycolysis. It describes growing cells, and the assumption of conserved nicotinamides nucleotides is introduced explicitly

\[
x_2 = m - x_1
\]

where \( x_1 \) denotes [NAD\(^+\)] and \( x_2 \) [NADH] in the cytosol, and where \( m \) is a constant parameter. The complete expression for \( v_{RES,2} \) is quite comprehensive, but Sedoglavic’s algorithm shows that the only unidentifiable parameters are

\[
(\tau, K_{NAD}, K_{NADH}, K'_{NAD}, K'_{NADH}, L)
\]
These parameters appear exclusively in the following part of the complete expression

\[ v_{RES,2}(x, k) = \frac{c_1 + c_2 x_1 + c_3 x_2)^n - 1 + c_4 (1 + c_5 x_1 + c_6 x_2)^n - 1}{(1 + c_2 x_1 + c_3 x_2)^n + c_7 (1 + c_5 x_1 + c_6 x_2)^n} \]  
(4.37)

Since the same coefficients appear in both the numerator and denominator, they have simply been denoted \( c_i \) in rising order as they appear from left to right. There are 7 coefficients but only 6 original kinetic parameters, and we can thus not draw any conclusions about the identifiability of the kinetic parameters. Sedoglavic’ algorithm, however, shows that they are identifiable.

Step 2 in Figure 4.2 can be modified in the same way as step 1. Insert (4.33) in (4.37) and lump the old coefficients together in a new coefficient vector \( \tilde{c} \). This gives

\[ \tilde{v}_{RES,2}(\tilde{x}, \tilde{c}) = \frac{\tilde{c}_1 (\tilde{c}_2 + \tilde{c}_3 x_1)^n - 1 + \tilde{c}_4 (\tilde{c}_5 + \tilde{c}_6 x_1)^n - 1}{(\tilde{c}_2 + \tilde{c}_3 x_1)^n + \tilde{c}_7 (\tilde{c}_5 + \tilde{c}_6 x_1)^n} \]  
(4.38)

There is no simple rank test to perform at this point, but Sedoglavic’ prediction can be verified by simplifying the expression further. This corresponds to step 3 in Figure 4.2. Extract a coefficient out of all bracket terms so that the constant term starting each bracket expression become 1; this gives

\[ \tilde{v}_{RES,2}(\tilde{x}, \tilde{c}) = \frac{\tilde{c}_1 c_2^{n - 1} (1 + \tilde{c}_4 x_1)^n - 1 + \tilde{c}_4 c_5^{n - 1} (1 + \tilde{c}_6 x_1)^n - 1}{c_2 (1 + \tilde{c}_4 x_1)^n + c_7 c_5 (1 + \tilde{c}_6 x_1)^n} \]  
(4.39)

divide both the numerator and denominator by \( \tilde{c}_2^n \)

\[ \tilde{v}_{RES,2}(\tilde{x}, \tilde{c}) = \frac{\tilde{c}_1 (1 + \tilde{\tilde{c}}_4 x_1)^n - 1 + \tilde{c}_4 c_5^{n - 1} (1 + \tilde{\tilde{c}}_6 x_1)^n - 1}{(1 + \tilde{\tilde{c}}_4 x_1)^n + c_7 c_5 (1 + \tilde{\tilde{c}}_6 x_1)^n} \]  
(4.40)
Lump the coefficients together and denote them $\tilde{c}'_i$ in increasing order as they appear from left to right

$$
\tilde{v}'_{\text{RES,2}}(\tilde{x}, \tilde{c}') = \frac{\tilde{c}'_1 (1 + \tilde{c}'_2 x_1)^n - 1 + \tilde{c}'_3 (1 + \tilde{c}'_4 x_1)^n - 1}{(1 + \tilde{c}'_2 x_1)^n + \tilde{c}'_5 (1 + \tilde{c}'_4 x_1)^n}
$$

That means that there is a formulation that is equivalent to ((4.33) + (4.35)) but that contains only 5 parameters. This verifies Sedoglavic’s probabilistic prediction that ((4.33) + (4.35)) is unidentifiable. The identifiable parameters are

$$
\tilde{c}' = (\tilde{c}'_1, \tilde{c}'_2, \tilde{c}'_3, \tilde{c}'_4, \tilde{c}'_5)
$$

A geometrical interpretation of the back-translation to $k$ (corresponding to step 4 and 5 in Figure 4.2) is given in [5].

**Summary of Section 4.2**

We have reported a new source of structural identifiability caused by the appearance of all variables from a conserved moiety in the same rate expression. A simple expression with one substrate and one product was used to get a quick understanding of the problem. A general framework for detecting the problem was developed for kinetic expressions that can be reformulated into rational expressions, i.e., as a fraction between two polynomials. The test is only sufficient, and does not guarantee that the problem is detected. However, it has detected all examples where it has been tested. If the test detects an unidentifiability, new identifiable parameters, $\tilde{c}'$, are obtained by picking out rows that eliminates left null-space of $C$, and by eliminating a final coefficient. The identifiable parameters $\tilde{c}'$ are expressed as functions of the original kinetic parameters, $k$. The mapping $c \rightarrow \tilde{c}'$ is linear, and this structure is used both when finding the identifiable parameters, and when back-translating the result (the back-translation is shown in Chapter 7). The general method was successfully applied to two comprehensive examples, and it was also showed how the basic steps of Figure 4.2 could be adapted to non-rational expressions. In Chapter 10.2.1 we will see another example of a published grey-box model that is structurally unidentifiable due to its conserved moieties.

### 4.3 Estimation of practically unidentifiable rate expressions

We now turn to the more general problem of handling practical unidentifiability in single rate expressions. We assume that measurements are available for the rate, $v$, and for all the time-varying entities in the rate expression, $x$. We also assume that in vitro estimates, $p^{\text{ivt}}$, of the kinetic parameters are available. If the rate expression was practically identifiable with respect to the given data, one could estimate the parameters from the in vivo data, and thus obtain corresponding in vivo estimations of the same parameters. The two estimates could then be compared and the differences could be analysed. There are few documentations of
such comparisons even though they are important; not only because of the frequent usage of *in vitro* estimated parameter values in modelling, but also because such comparisons drive to the heart of the philosophical question of whether there are any unbridgeable differences between an *in vitro* and an *in vivo* environment.

However, the problem with obtaining such comparisons is that most present *in vitro* derived rate expressions are unidentifiable with respect to the available *in vivo* data. Therefore, a direct identification of all the original kinetic parameters lead to results that typically are highly unrealistic, and that in any case are meaningless to compare with. In this section we will present two types of methods that are meant to overcome this problem. The first approach extracts information from the covariance matrix $\text{Cov}(\vec{p})$ and uses this analysis to identify an expression with identifiable rate expressions, including a back-translation to the original parameters. The second approach achieves an identifiable rate expression through hypothesis testing and minimal modelling, and a simulation approach yields a mapping from the original to the reduced parameters. Both these approaches are exemplified on two small examples in this section, and in Chapter 10.2.2 the second method is applied to a larger expression, for which there also exists real experimental data.

### 4.3.1 Reduction of the unidentifiable directions

**A non-analytical model structure**

Our first approach is based on an analysis of the Fisher Information Matrix (FIM). Remember that $\text{Cov}(\vec{p}) = \text{FIM}^{-1}$, and that these matrices give a measure of how well-determined each parameter is based on the available data (see Section 4.1). There are expressions that calculate the uncertainty in an arbitrary direction based on these matrices [113], but for the present situation it is sufficient to know the most uncertain directions. These are given by the semi-axes to the FIM matrix. A semi-axes is given by the eigenvectors and the eigenvalues to a symmetric and positive definite matrix (which FIM is). Let the $i$:th eigenvalue to FIM be denoted $\lambda_i$, and let the corresponding eigenvector be denoted $u_{F,i}$. The equation for the corresponding semi-axes, denoted $u_{s,i}$, is then given by

$$ u_{s,i} = \frac{1}{\sqrt{\lambda_i}} u_{F,i} \quad (4.43) $$

These semi-axes span the confidence ellipsoids determined by equation (4.10), see Figure 4.1. This means that a long semi-axes correspond to an uncertain parameter direction. As can be seen in equation (4.43), the length of the semi-axes is given by the size of the eigenvalues, and smaller eigenvalues correspond to longer semi-axes. Therefore, an eigenvalue that is equal or very close to zero corresponds to a very high parameter uncertainty and should therefore be removed to achieve an identifiable rate expression. The other directions, on the other hand, should be kept since we want the reduced expression to describe the same relationships as the original expression under the given experimental conditions.

An intuitive reparametrization of $\vec{v}$ in identifiable parameters is therefore obtained by choosing the reduced parameters, $\vec{p}'$, as the coefficients in the space spanned by the eigenvectors corresponding to shorter semi-axes. Assume that the
semi-axes are ordered by length and in increasing order. Assume that there are \( r' \) practically identifiable parameters. Finally, let the identifiable model structure be denoted \( v' \). With these notations, this intuitive choice of identifiable model structure based on the analysis of the FIM matrix is given by

\[
v'(p', x) = v(p(p'), x) \\
p(p') = \tilde{p} + p_{1}u_{F,1} + p_{2}u_{F,2} + \cdots + p_{r'}u_{F,r'}
\]

(4.44a)

(4.44b)

This reparametrization means that \( v(bp) \) is equal to \( v(0) \), i.e., that we have estimated all the identifiable parameters \( \tilde{p} \) to zero. Note, however, that we do not have an uncertainty of \( bp \), since those parameters give a singular FIM matrix (see equation (4.11)). Uncertainties might, on the other hand, be calculated for \( \tilde{p}' \), and this is one of the main reasons for obtaining a reduced identifiable core model (see Chapter 3). In order to compare the estimated confidence regions with the original \textit{in vitro} estimations, we need to obtain a translation between the space of \( p \) and the space of \( p' \). We denote such translations \( \phi \) (from \( p \) to \( p' \)) and \( \Psi \) (from \( p' \) to \( p \)). The determination and analysis of these translations is the topic of Chapter 7. However, since we need them also in the present section, this section also serves as an introduction to the concepts and notations of Chapter 7. The parameters \( p \) and \( p' \) may generally be compared in any space, but for this specific reparametrization it is easiest to compare them in the space of the original parameters, i.e., to determine the back-translation \( \Psi \).

The back-translation \( \Psi \) has the unusual property that it is a mapping from a lower-dimensional space to a higher-dimensional space. To make the mapping unique it is therefore necessary to choose where on the \( \phi \) invariant manifold one should be mapped. We formalise this choice by the addition of design variables \( \eta \). A choice that would go in line with the original reparametrization in the present case would be to let the design variables be the coefficients of the singular eigenvectors.

\[
\Psi := (p', \eta) \rightarrow p = \tilde{p} + p_{1}u_{F,1} + \cdots + p_{r'}u_{F,r'} + \eta_{1}u_{F,r'+1} + \cdots + \eta_{r-r'}u_{F,r} 
\]

(4.45)

By definition, there is no information about the choice of the design variables in the \textit{in vivo} data, as seen from a local perspective. If one therefore seeks to compare the back-translated \textit{in vivo} values with the original \textit{in vitro} estimated values one should optimize the agreement over \( \eta \). However, typically the local view of independence of \( \eta \) is not valid globally, and one should therefore restrict the optimization over \( \eta \) to a region \( \mathcal{N} \subset \mathbb{R}^{r-r'} \) where the local transformations are valid. If one desires to also take the uncertainties into account (which would give a more accurate comparison) one should also optimize over the confidence regions of \( p_{\text{ivt}} \) and \( p' \). The shortest distance is the statistically significant difference between the \textit{in vivo} and \textit{in vitro} estimated values. This difference is thus formally written as

\[
\min_{p, p', \eta} \| p - \Psi(p', \eta) \| \quad \text{where } p \in \Delta_{p_{\text{ivt}}}, \ p' \in \Delta_{\tilde{p}'}, \ \eta \in \mathcal{N}
\]

(4.46)

Recall that \( \Delta_{p_{\text{ivt}}} \) and \( \Delta_{\tilde{p}'} \) denotes the confidence region corresponding to \( p_{\text{ivt}} \) and \( \tilde{p}' \), respectively. It should finally be said that in Chapter 7 we will not focus on a comparison between the information obtained from the \textit{in vivo} and \textit{in vitro} data, but instead on the merging of the two sources of information. Then the design parameters \( \eta \) will be used in a different way.
Figure 4.3 The mappings $\phi$ and $\Psi$ between the original parameter space $\Omega$ and the reduced parameter space $\phi(\Omega)$. The forward translation is determined during the reduction, or by the combination of generating data, $Z^N(p)$, and estimating $\bar{p}'(p)$ based on this data. The back-translation is made unique with the aid of a design variable $\eta$ which should be varied within the feasible region $\mathcal{N}$ if a comparison between $p^{\text{true}}$ and $\bar{p}'$ is to be made.

Replacement by a linear combination

There are several drawbacks with the choice of (4.44) as model structure for the identifiable core expression $v'$. One such drawback is that the model structure (4.44) is not analytical, but is an indirect mapping reusing the old model structure (even though the mapping could, in principle, be substituted directly in the model structure). The benefit of using the old model structure in this way is that the translation, $\Psi$, to the original parameters is easy to establish. It would therefore be interesting to find an analytical reformulation of the original structure that still incorporates the results from the analysis of the covariance matrix, and that still allows for a straightforward back-translation, $\Psi$, to the original parameters.

A way to do this is obtained by a projection of the original parameters $p$ to a space that has removed the unidentifiable directions. Assume first that there is only one singular direction, given by the eigenvector $u_{F;r}$. Consider then a projected parameter vector $\tilde{p}$. The local version of this parameter is orthogonal to $u_{F;r}$, i.e., their scalar product is equal to zero

$$
(\tilde{p} - \bar{p})^T \cdot u_{F;r} = 0
$$

Equation (4.47) therefore describes a linear combination of the $\tilde{p}_i$s that is equal to zero. All parameter vectors $p$ that are mapped to zero by this linear combination thus lie in the space of projected parameters. One may therefore solve for one of the parameters in equation (4.47) and replace that parameter with the resulting linear combination when evaluating the rate expression. Without loss of generality we may solve for the last parameter, and write $p_r = p_r(p_1, \ldots, p_{r-1})$. By choosing the first $r-1$ parameters as the identifiable parameters we have therefore obtained
4.3 Estimation of practically unidentifiable rate expressions

a new reduced model structure \( v' \) given by

\[
v'(p') = v(p_1', p_2', \ldots, p_r' - 1, p_r(p'))
\] (4.48)

This model structure is analytical and result in an identical model structure as the original one except for all occurrences of the parameter \( p_r \) which is replaced by the linear combination \( p_r(p_1, \ldots, p_{r-1}) \). Finally, the model structure is identifiable since the single non-identifiable direction has been removed. If there should be more than one singular directions in the FIM matrix, the same procedure can of course be repeated until identifiability is obtained.

Let us also consider the comparison aspect with the previously obtained in vitro estimates, \( p^{\text{ivt}} \). Again the new model structure has been based on the analysis around the already estimated parameters \( \tilde{p} \) which translates directly to the estimation of the reduced parameters through

\[
\tilde{p}' = (\tilde{p}_1, \ldots, \tilde{p}_{r-1})
\] (4.49)

It should again be pointed out that it is essential to compare the entire confidence regions, if a fair picture of the true relation between the in vivo and in vitro values is to be obtained. To illustrate the close connection between the model structures (4.44) and (4.48) we again choose to do the comparison by computing the back-translation \( \Psi \). The direct back-translation is given by the arguments in (4.48), but again we need to add design variables \( \eta \). If there are \( r' \) identifiable parameters, there will be \( r - r' \) design variables, and they may be chosen as coefficients to the singular directions

\[
\Psi := p' \rightarrow p = (p_1', p_2', \ldots, p_{r'}, p_{r'+1}(p'), \ldots, p_r(p')) + \sum_{i=r'+1}^{r} \eta_i u_{r,i}
\] (4.50)

Note that this is the same type of sum as that in (4.45); the only difference is that the model structure is now analytical.

Finding the real relation

The strength of the two model structures (4.44) and (4.48) is that they only have as many parameters as may be identified from the data, and that they give the same flux as the original model structure in the identifiable directions. The model structures do therefore not have any unnecessary degrees of freedom that may be excited in an in vitro situation but that never are active in the in vivo environment. To achieve model structures with these properties is highly beneficial because it brings us closer to an understanding and a description of what actually happens inside a living cell.

However, there are also several weaknesses of the model structures (4.44) and (4.48). First, the model structures are not simple analytical expressions. Second, the model structures are only based on a local analysis, on the Cov(\( p \)) or the FIM matrices. This means that the optimization over \( \eta \) should only be carried out over a limited region, e.g., only over those \( \eta \) that keep

\[
\| \tilde{y}_v(x, \Psi(p', \eta)) - \tilde{y}_v(x, \Psi(p', 0)) \|
\]
acceptably small. An even more important drawback, however, is that the result is only valid locally around the point \( \hat{p} \) which is not chosen uniquely since \( p \) is not identifiable. This problem is illustrated by a small example at the end of this subsection. All this means that the ability of the model structures in (4.44) and (4.48) to provide good measures for the true distance between the in vivo and in vitro confidence intervals for the parameters is often weak. If the calculation of this difference is the sole purpose of that analysis, it is therefore better to perform a global search in the original space \( \Omega \). Such a global search would, on the other hand, not result in a reduced expression that describes the in vivo setting. We therefore suggest a new way of using the covariance matrix that yields a more globally valid reduced expression. It will thus provide an in vivo expression and be (more) generally applicable in a comparison context.

Assume that there exists a single one-dimensional relation between the parameters that is valid in a larger area around \( \hat{p} \). Let this relation be described by a \( \mathbf{B}(p) \) such that

\[
\mathbf{B}(p) = 0 \quad \text{where} \quad \mathbf{B}(\hat{p}) = 0 \tag{4.51}
\]

and where all \( p \) that fulfils \( \mathbf{B}(p) = 0 \) give a (virtually) identical flux as \( \nu(x, \hat{p}) \) for the given \( x \)-data. Since \( \mathbf{B} \) is one-dimensional equation (4.51) describes a one-dimensional curve in the parameter space. This curve may be parametrized by a single parameter. Let this single parameter be denoted \( s \)

\[
s \rightarrow p(s) \quad \text{where} \quad \mathbf{B}(p(s)) = 0 \quad \text{for all} \quad s \in S \subset \mathbb{R} \tag{4.52}
\]

Let the \( s \) value that gives \( p(s) = \hat{p} \) be denoted \( s_{\hat{p}} \). The tangent to \( \mathbf{B} = 0 \) at \( \hat{p} \) is given by the derivative of \( p(s) \) with respect to \( s \), \( \frac{dp(s)}{ds}|_{s_{\hat{p}}} \). Since \( \mathbf{B} \) describes the only invariant direction at this parameter point, this gradient is a good approximation to the direction of the most singular semi-axes, \( u_{s,r} \) which typically would be very long. We thus have

\[
\frac{dp(s)}{ds}|_{s_{\hat{p}}} \approx \hat{p} + au_{s,r} \quad \text{for some} \quad a \in \mathbb{R} \tag{4.53}
\]

This observation means that the singular semi-axes may not only be used to give the model structures (4.44) and (4.48), they can also be used to extract information about the true nonlinear source of unidentifiability, and thus allow for the derivation of an identifiable model structure \( \nu' \) that has a more global applicability.

The determination of the true relation (4.51) is greatly simplified if it only involves few parameters. This may be detected by the singular eigenvector, that would ideally have non-zero elements for only those parameters that are involved in (4.51). Therefore, by inspection of which elements in the only singular eigenvector that are non-zero, one may detect both how many parameters are involved in the relation, and which these parameters are. Assume now that it has been detected that there are only two parameters involved, \( p_1 \) and \( p_2 \). One may then plot the level curves of the cost function in the \( p_1 \times p_2 \)-plane. The lowest valley, or level curve, in this plot crosses \( (\hat{p}_1, \hat{p}_2) \), and has a gradient that is proportional to the singular eigenvector according to (4.53). Furthermore, the minimum valley will also be a numerically computed approximation of the true relation (4.51). One
may therefore use this plot to obtain a symbolic expression for \( B(p) \) by evaluating different model structures just like one evaluates different model structures to mimic the original experimental data. If \( B(p) \) is a symbolic expression of reasonable complexity this will typically be detected if an exhaustive search is made (since the data with which to fit may be generated with many samples). However, it should be pointed out that there is no guarantee that the true \( B(p) \) is a symbolic relationship. In any case, after a symbolic approximation of \( B(p) \) has been determined this may be used to reparametrize the original rate expression \( v(x,p) \) into a reduced analytical expression \( v'(x,p') \). This reduced expression is then identifiable and with a (generally) more global validity than (4.44) and (4.48). We summarise this last method in the following four step algorithm.

**Algorithm 4.1 Approximating the true \( B(p) \)**

**Input:** A rate expression \( v(x,p) \) that is unidentifiable with respect to a given data set \( \{x_i,v_i\}_{i=1}^N \) due to a one-dimensional invariant relation \( B(p) = B(p_i,p_j) = 0 \).

1. Estimate a \( \hat{p} \) from the data that lies in the region of acceptable parameters
2. Calculate the semi-axes according to (4.43), and see which two elements that are non-zero in the longest semi-axis \( u_{s,r} \)
3. Plot level curves \( V_N(p_i,p_j) = \text{const} \), and identify the lowest valley crossing \( \hat{p} \) and with a gradient given by (4.53)
4. Identify a model structure \( \hat{B} \) that can describe this valley in a satisfactory way

**Output:** An analytical approximation \( \hat{B}(p_i,p_j) \) to be used when identifying an identifiable rate expression \( v'(x,p') \)

**Remark:** The choice of model structure \( \hat{B} \) in Step 4 may be done based on several different criteria. Two apparent such criteria should be that the model structure gives a good agreement with the numerically determined relation on a sufficiently global perspective, and that it implies a simple model structure \( v'(x,p) \). If the true relation \( B(p) \) is not analytical the choice will typically be a trade-off between different such criteria. Finally, we do not provide a general rule for how the reparametrization should be used to parametrize \( v'(x,p') \). However, it should often be the case that the \( B(p) \) may be broken down into \( B'(p) - B'' \), where \( B'' \) is a constant. If this is the case it is our heuristic that the constant is often a good choice for a new identifiable parameter, i.e., to choose \( p'_i = B'' \).

Finally, if the singular eigenvector contains more than two non-zero elements, or if there are more than one singular eigenvectors, the same approach can in principle be applied, even though the complexity grows quickly with such generalisations. Now we will illustrate the proposed methods on two small examples. A larger example including the analysis of real \( \textit{in vivo} \) data is given in Chapter 10.2.2.
Example 4.3  Consider the following reaction rate \( v \) which depends on one state \( x \) and two parameters \( p_1 \) and \( p_2 \)

\[
v = (p_1 + p_2)x
\]  

We assume that data series of \( v \) and \( x \) exists (in Chapter 10.2.2 we review how such time-series may be calculated in practise), and that they are the ones plotted in Figure 4.4. The true values in the model are \( p^0 = (p^0_1, p^0_2) = (2, 3) \), and we assume that the in vitro estimates of the parameters are \( p^{ivt} = (1.6 \pm 0.2, 2.8 \pm 0.2) \). The rate equation is clearly unidentifiable, and let us therefore see how the proposed methods handles the situation, and allows for a comparison of the in vivo estimation with \( p^{ivt} \).

The first step in all the methods is to estimate a parameter \( \hat{p} \). Assume that the estimated values become \( \hat{p} = (1, 4) \). The eigenvectors to FIM at this point are given by the two columns in

\[
\begin{pmatrix}
-0.7071 & 0.7071 \\
0.7071 & 0.7071
\end{pmatrix}
\]  

and the corresponding eigenvalues are 0 (left column) and 0.0620 (right column).

This means that we have detected the unidentifiability, and that it is the left eigenvector that is the singular one. Note that this means that \( FIM^{-1} \) does not exist, and that it is not possible to calculate the uncertainties of \( \hat{p}_1 \) and \( \hat{p}_2 \) directly.
The first method yields the following core model according to (4.44)

\[ v'(x, p') = (p_1(p') + p_2(p'))x \]  
\[ p(p') = \begin{pmatrix} p_1(p') \\ p_2(p') \end{pmatrix} = \begin{pmatrix} 1 \\ 4 \end{pmatrix} + \begin{pmatrix} 0.7071 \\ 0.7071 \end{pmatrix} p' \]  

The parameter in this model may be calculated with an uncertainty, since the associated FIM matrix is one-dimensional and thus clearly invertible. The uncertainty may be estimated to \( p' = 5 \pm 0.2 \). Finally, the back-translation formula \( \Psi \) is given by equation (4.45)

\[ \Psi : (p', \eta) \rightarrow \begin{pmatrix} p_1 \\ p_2 \end{pmatrix} = \begin{pmatrix} 1 \\ 4 \end{pmatrix} + \begin{pmatrix} 0.7071 \\ 0.7071 \end{pmatrix} p' + \begin{pmatrix} -0.7071 \\ 0.7071 \end{pmatrix} \eta \]

and the back-translated uncertainty (with \( \eta \) restricted to vary between \( \pm 2 \)) is plotted in Figure 4.5. As can be seen, \( \Delta_{p'} \) overlaps the back-translated uncertainty region \( \Psi\Delta_{p'}, \eta \), and the distance in (4.46) is therefore zero.

The second method uses the same information as the first, but does only replace one of the original parameters by a linear combination. This is obtained by solving
equation (4.47)

\[
\begin{pmatrix}
p_1 - 1 & p_2 - 4 \\
-0.7071 & 0.7071
\end{pmatrix} = 0
\] (4.58)

for one of the parameters. Solving for \( p_2 \) yields \( p_2 = 3 + p_1 \). We choose \( p' = p_1 \), and \( v' \) is given by equation (4.48) as

\[
v'(p') = (p' + (3 + p'))x = (2p' + 3)x
\] (4.59)

Again we have a one-dimensional FIM matrix, that hence may be inverted, and the associated error in \( p' \), and the back-translated confidence interval given by (4.50)

\[
\Psi := p' \rightarrow p = \begin{pmatrix}
p' - 0.7071 \cdot \eta \\
3 + p' + 0.7071 \cdot \eta
\end{pmatrix}
\] (4.60)

with \( \eta \in (-2, 2) \) is plotted in Figure 4.5. The back-translated confidence interval is almost the same as that for the previous method; it is therefore primarily the forms of the rate expressions \( v' \) that differ.

Both the rate expressions (4.59) and (4.56) describe all the modes that are activated in the in vivo data in Figure 4.4, and if that data describes all the modes that ever will be active in the desired operating point for the cell, they are both valid in vivo expression for the rate. This is interesting information, but as is evident in this simple example, there is an even simpler expression that might be found: \( v' = p'x \). This option could in principle be found by recognising that one could replace \((2p' + 3)\) by a new parameter \( \bar{p} \) in equation (4.59), but we will now show that it may also be found by identifying the true relation \( B(p) = 0 \), as described in Algorithm 4.1. (Yet another option is to use the minimal modelling approach described in Section 4.3.2.)

Steps 1 and 2 in the algorithm have already been carried out and yielded \( \hat{p} = (1, 4)^T \) and \( p_1 \) and \( p_2 \) as the two parameters appearing in the singular eigenvector. The lowest level curve for the cost function in the \( p_1 \times p_2 \) plane is plotted in Figure 4.5. As can be seen it crosses the estimated parameters \( \hat{p} \), and the singular eigenvector acts as a tangent to the lowest level curve at this point. However, for this particular example the valley in the cost function is determined uniquely by this tangent. We therefore conclude that a linear model for \( B(p) \) is sufficient. Since we know the slope and a specific point on the curve we may immediately identify the relation as

\[
B(p) = p_1 + p_2 - 5 = 0
\] (4.61)

Finally, we follow the remark after Algorithm 4.1 and identify \( p_1 + p_2 = p' \).

**Example 4.4** The second example is slightly more complex, and contains a practical identifiability. The rate is still given by one state and two parameters, but this time we have a Michaelis-Menten expression

\[
v(x, p) = \frac{V_{\text{max}}x}{K_M + x}
\] (4.62)
4.3 Estimation of practically unidentifiable rate expressions

and the given data series is plotted in Figure 4.6. As can be seen the time-series for the variable \( x \) is around 0.05. The true parameter values are \( (V_{\text{max}}, K_M) = (5, 0.5) \) and since \( K_M \) is at least an order of magnitude smaller than the state, we have the approximate relation \( V_{\text{max}}/K_M = \hat{p} \). Let us now see how the three presented methods handle this slightly more complicated practical unidentifiability, and how they compare the obtained \textit{in vivo} measurements with the \textit{in vitro} estimates, that we assume were determined as \( p^{\text{ivt}} = (4 \pm 0.3, 1 \pm 0.1)^T \).

As usual the first thing to do is to obtain an estimate \( \hat{p} \) in the original parameters. Assume that the estimation has resulted in \( \hat{p} = (5, 0.5)^T \). The eigenvectors to FIM at \( \hat{p} \) are given by the two columns in

\[
\begin{pmatrix}
-0.9950 & -0.0995 \\
-0.0995 & 0.9950
\end{pmatrix}
\] (4.63)

which corresponds to the eigenvalues 0 (left column) and 0.1166 (right column). We have thus again detected the unidentifiability through the singularity of the FIM matrix, and again the uncertainty of the estimations of the original parameters is infinitely big, according to (4.11). The first method uses the two vectors directly, and obtains the following reduced model structure (according to equation (4.44))

\[
v'(x, p') = \frac{V_{\text{max}}(p')x}{K_M(p') + x}
\]

\[
p(p') = \begin{pmatrix}
V_{\text{max}}(p') \\
K_M(p')
\end{pmatrix} = \begin{pmatrix}
5 \\
0.5
\end{pmatrix} + \begin{pmatrix}
-0.0995 \\
0.9950
\end{pmatrix} p' \tag{4.64b}
\]
and the back-translation is given by equation (4.45) as
\[
\Psi := (p, \eta) \rightarrow p = \left( \begin{array}{c} V_{\text{max}}(p') \\ K_M(p') \end{array} \right) = \left( \begin{array}{c} 5 \\ 0.5 \end{array} \right) + \left( \begin{array}{c} -0.0995 \\ 0.9950 \end{array} \right) p' + \left( \begin{array}{c} -0.0995 \\ -0.9950 \end{array} \right) \eta
\] (4.65)

The uncertainty of the parameter \( p' \) may be estimated, since FIM is one-dimensional and thus clearly invertible, and a reasonable estimate is \( p' = 0 \pm 0.2 \). In Figure 4.7 we see the back-translated uncertainty where \( \eta \in (-2, 2) \).

The second method calculates an analytical formula for one of the parameters by solving (4.47)
\[
\begin{pmatrix} V_{\text{max}} - 5 \\ K_M - 0.5 \end{pmatrix} \begin{pmatrix} -0.9950 \\ -0.9950 \end{pmatrix} = 0
\] (4.66)

Solving for \( V_{\text{max}} \), and replacing \( K_M \) by \( p' \) gives the following reduced expression according to equation (4.48)
\[
V_{\text{max}} = \frac{-K_M + 55}{10} \Rightarrow v'(x, p') = \frac{(55 - p')x}{10p' + 10x}
\] (4.67)
and the following back-translation according to (4.50)

\[
\Psi := p' \rightarrow p = \left(\frac{-p' + 55}{10} - 0.9950 \cdot \eta, p' - 0.0995 \cdot \eta\right)
\] (4.68)

The back-translated uncertainty region is plotted in Figure 4.7 and we have chosen the same \( \eta \in (-2, 2) \).

As can be seen it is not evident from any of the first two methods that the best parametrisation to use is \( p' = \frac{V_{\text{max}}}{K_M} \). This might, on the other hand, easily be obtained by the last method. The true relation lies clearly in the \((p_1, p_2)\)-plane and the lowest level curve for this relation is also plotted in Figure 4.7. As can be seen the tangent is again uniquely determining the entire relation, which is clearly a proportionality curve. This gives the true relation \( B(p) = \frac{V_{\text{max}}}{K_M} - 10 = 0 \), and we again see that the constant in the relation is a good choice for a reduced parameter \( p' \).

These two examples show cases where all methods work, since the singular relation is uniquely given by the gradient and the specific estimate \( \tilde{p} \). It should be pointed out that this is not always the case. Consider for instance the simple system

\[
v(x, p) = p_1 p_2 x
\] (4.69)

This would give a reduced model structure of the form

\[
v(x, p') = p'(\text{const} + p')x
\] (4.70)

and a back-translation of the form

\[
\Psi := p' \rightarrow p = (p' + u_{1,2}\eta, \text{const} + p' + u_{2,2}\eta)
\] (4.71)

where \( u_{i,2} \) is the \( i \)th element in the singular eigenvector. This model structure would clearly have a limited applicability, that would depend strongly on the choice of \( \tilde{p} \) on the invariant manifold \( B(p) = 0 \). Since this choice is arbitrary, it is apparent that widely different models are non-distinguishable considering the \( \text{in vivo} \) data alone. This is one of the reasons why a merging of the information in the \( \text{in vivo} \) data is done with the previous \( \text{in vitro} \) characterisations in the full core-box modelling framework. The limited applicability of the linearised models is also the reason why the finding of the true relation \( B(p) = 0 \) is so beneficial if a valid comparison is to be made. These issues are discussed further in Chapter 7.

### 4.3.2 A minimal modelling approach

We now propose a completely different approach to handle estimation of non-identifiable rate expressions. In the previous subsection we tried different ways to extract as much information about the non-exited modes in the original expression as possible, and then sought different ways to utilise this information to obtain a reduced expression \( v' \) with fewer and identifiable parameters \( p' \). The reduced expression was obtained in a more or less automatic manner through model
reduction. However, even though the reduced expressions $v'$ depended on fewer parameters $p'$, the complexity of the expression could often have increased, e.g., due to the presence of linear combinations. The approach had, however, the benefit that the back-translation $\Psi$ was easy to determine. The method that we propose now is the opposite to this in many ways. The reduced rate $v'$ will not be obtained through model reduction of $v$, but through minimal modelling and hypothesis testing based directly on the experimental data. Further, this has the the strength that the reduced expression is of minimal complexity. Finally, the price for this improvement is that an analytical translation between $v'$ and $v$ will generally be more cumbersome to obtain.

The theory behind the minimal modelling and hypothesis testing is described in detail in Chapter 3.4. The only difference is that we now deal with the identification of single rate expressions instead of complete differential equations. That only leads to some minor modifications and they are described in Section 4.1. Once the reduced model structure is obtained, uniquely estimated parameters $p_0$ are available. If these model structures are validated they are interesting in themselves, because they provide a description of the reaction behaviour in the real cellular environment, without having any unmotivated modes that might be excited only in the in vitro environment. However, the present modelling scenario is primarily concerned with the comparison between the in vitro and in vivo parameter values. To achieve such a comparison it is necessary to determine one of the projections $\Psi$ or $\Phi$.

It might be obvious from a mere inspection of the models which types of reductions that combine the models, and how the translations $\Psi$ and $\Phi$ are to be obtained. However, if this should not be the case, it is still possible to derive the translation $\Phi : p \rightarrow p'$ numerically. This is possible because the reduced parameters $p'$ are identifiable with respect to the given data: $\{x(t_i), v(t_i)\}_{i=1}^N$. The in vitro estimated parameters could be used to generate a similar data set where $v(t)$ is replaced by $v(t, p^{ivt})$. Let this time-series be denoted $Z^N(p^{ivt})$

$$Z^N(p^{ivt}) := \{x(t_i), v(t_i, p^{ivt})\}_{i=1}^N$$

(4.72)

The reduced parameters $p'$ are chosen to be identifiable with respect to such a data set. This means that the identification of $p'$ from this data set provides a unique mapping from $Z^N(p^{ivt})$ to $p'$, and thus also indirectly a unique mapping from $p$ to $p'$. This is a way to numerically determine a mapping $\Phi$, and this is sufficient to obtain a comparison between the $p^{ivt}$ and $p'$. Finally, even though $\Phi$ is only determined as an algorithm by this method, an analytical expression may also be obtained. This is then done in the same way as an analytical expression was obtained for the general nonlinear relation $B(p)$ in equation (4.51), i.e., through modelling based on numerically determined data. The data is this time generated by varying the argument in $Z^N(p)$ in different directions away from $p^{ivt}$. In this way one might also identify those perturbations in the $p$ space that leaves $\Phi(p)$ invariant. These are the directions to use if a back-translation is sought as well (see Chapter 7). We summarise this second approach in an algorithm, and then return to the examples introduced in the previous subsection.

**Algorithm 4.2 Comparison of $p^{ivt}$ and $\tilde{p}'$ through minimal modelling**
Input: In vitro characterisations of a reaction given by \( v \) and \( p^{\text{ivt}} \) and experimental data \( Z^N = \{x(t_i), v(t_i)\}_{i=1}^N \) that is not informative enough to make \( p \) identifiable

1. Determine a reduced expression \( v'(x, p') \) through the minimal modelling approach described in Chapter 3.4

2. Determine the mapping \( \phi : p \to p' \) by the combination of generating the data in equation (4.72) and estimating \( p' \) out of this data

3. Use the mapping to find the minimal distance between the \( p' \) confidence region, \( \Delta_{\phi} \), and the mapped confidence region for \( p^{\text{ivt}}, \phi(\Delta_{p^{\text{ivt}}}) \)

4. (optional) Use numerical perturbation of the numerically determined \( \phi \) to obtain an approximate analytical expression through a new modelling round

Output: A reduced expression \( v' \) describing the in vivo activity of the enzyme as reported by the data, in vivo estimated parameters \( \hat{p}' \), and a comparison between \( p^{\text{ivt}} \) and \( \hat{p}' \). If 4 is done: an analytical expression for \( \phi \)

Remark: Some more details about how to do the final modelling step, also including the determination of the back-translation \( \Psi \) is provided in Chapter 7.

**Example 4.5** Let us now return to Example 4.3. Minimal modelling of the data shown in Figure 4.4 shows easily that the data is best explained by a model structure of the form

\[
v = p'x
\]  

and receives the same estimate for the parameter as in the previous example. However, the major difference between the present situation and Example 4.3 is that we now do not have a determined back-translation \( \Psi \).

We instead perform the forward mapping \( \phi \), by simulating data \( \{x(t_i), v(x(t_i), p^{\text{ivt}})\}_{i=1}^N \), and estimating a new parameter \( p' \) in (4.73). This gives the translated parameter uncertainty \( \phi(\Delta_{p^{\text{ivt}}}) = (4, 4.8) \), which is to be compared to the original uncertainty \( p' \in (4.8, 5.2) \). We therefore again draw the conclusion that the two uncertainties may not be distinguished since the confidence intervals are overlapping. Notice how much easier the comparison is with this approach, since it is done in the space of \( p' \), which has fewer dimensions.

Finally, numerical perturbations using \( \phi \) shows that

\[
\frac{\partial \phi(p)}{\partial p_1} = \frac{\partial \phi(p)}{\partial p_2} = 1 \quad \text{for all } p
\]  

and that

\[
\frac{\partial^2 \phi(p)}{\partial p_1^2} \sim \frac{\partial \phi(p)}{\partial p_2} \sim \frac{\partial \phi(p)}{\partial p_1 \partial p_2} \sim 0 \quad \text{for all } p
\]  

This indicates that the analytical expression for \( \phi \) is linear, and that the coefficients in front of each term is 1, i.e., that \( \phi(p) = p_1 + p_2 \), which is equal to the true analytical expression.
This example shows how an analytical expression may be inferred from a Taylor series expansion. The same may of course in principle be done also for nonlinear expressions, and a table of the Taylor series of the classical functions may be used to facilitate the search for model structures. The remaining parameters in the model structure for $\phi$ may be fitted to a plot with a high resolution, if necessary. It should, however, also be remembered that there is no guarantee that there exists an analytical expression for $\phi$ with a global validity.

### 4.4 Summary of Chapter 4

A priori identifiability analysis is one half of substep 3 in the core-box modelling framework. The other half is to reduce the detected unidentifiabilities, and in this way obtain an identifiable core model. In the case of unidentifiability localised to individual rate expressions it is often quite straightforward to directly utilise the knowledge from the unidentifiability analysis in the model reduction, and we have therefore included such single rate reductions as well in this chapter.

In Section 4.1 we have presented a review of the state-of-the-art methods to determine both structural and practical identifiability in ODEs and individual rate expressions. Loosely speaking a parameter is structurally identifiable if it can, in principle, be uniquely determined from a perfect experiment, and practically identifiable if it can really be estimated from a specific given data set. Structural identifiability is treated using, e.g., differential algebra, and a good implementation to carry out the calculations in practice have been developed by Sedoglavic. If an individual estimation is fast, practical identifiability may be treated using bootstrapping, but otherwise one has to resort to local sensitivity analysis based methods, like the FIM matrix, and other methods to estimate the $\text{Cov}(\hat{p})$ matrix. What is primarily lacking today on the detection part is a non-simulation based method that gives a global measure of practical identifiability.

Another thing that is not presented by the general methods for identifiability analysis is an analysis of the mechanisms leading to the unidentifiability, and a characterisation of those parts of the system that may be estimated from the data. In Section 4.2 we present such results for the special case of unidentifiability in single rate expressions due to conserved moieties. We show that the reason why this occurs, at least in all cases found in the literature, is that the presence of the moiety conservation leads to a rate expression with fewer terms with independent coefficients, $e_{C_0}$, than kinetic parameters $k$. We showed this by a series of transformations, that particularly involved the analysis of the linear transformation matrix $C$. That part of the transformation is revealed to be linear is shown to be beneficial both when identifying the reduced parameters and when back-translating these to the original kinetic parameters. We also present a Mathematica implementation to do all the calculations automatically [5]. Finally, what is primarily missing with the suggested reduced expressions and transformation is a biochemical interpretation, in particular of the reduced expressions and parameters $v'$ and $e'$.

That structurally unidentifiable rate expressions are widely used, and that this seems to be rather unnoticed in the literature is a clear evidence that the concepts of this chapter are not yet widely spread in the systems biology society. This
is probably the reason why there are also few characterisations of the differences between in vivo rate expressions and parameters, and the corresponding in vitro rate expressions and parameters. Such characterisation are important both when using in vitro characterisations in models for the intact cell, and for understanding the general differences of in vitro biochemistry and living cells. However, in order to perform such analysis it is essential to be able to determine what aspects of the kinetic activity that really can be estimated from a given data set.

In Section 4.3 we propose two different methods to approach this question. The first method is based on a characterisation of which parts of the in vitro rate expression, $v$, that are unidentifiable with respect to the given in vivo data. This is done by an analysis of the covariance matrix, which also might lead to a more global characterisation. In any case, these unidentifiable directions are removed, and the confidence regions $\Delta_{\text{in vitro}}$ and $\Delta_{\text{in vivo}}$ are compared in the original space through the back-translation mapping $\Psi$. The alternative approach obtains the reduced expression through minimal modelling, and the comparison is carried out in the reduced space through the (possibly numerically determined) mapping $\phi$. The reduced expression determined through minimal modelling has the advantage that it is simpler than the original expression, and the major drawback is that the relations between $v$ and $v'$ is harder to determine analytically (especially the back-translation $\Psi$). If one is sure that the in vivo data excites all the modes that are active under the relevant conditions, a back-translation is not necessary because the core expression is then equal to the core-box expression. Otherwise, however, it might instead be more beneficial to use the $v'$ characterised by the reduction as the core part of the core-box model, if a core-box expression is sought for the reaction. In any case, the two presented approaches are complementary, and a complete analysis of the given information should therefore include both of them. Finally, it is noteworthy that both the approaches include a general modelling step where good data for the characterisation is available using simulations; it is a challenge for future studies to find good methodologies for these specific modelling problems.
In the previous chapter we dealt with identifiability analysis and the development of reduction methods to remove the unidentifiable parts of individual rate expressions. We will now turn to the more general problem of model reduction in a complete model structure. Model reduction may be done for many purposes, and there are many different types of complexities that one might want to reduce. There is not one single method that is superior for all of these situations, and it has therefore been developed a multitude of different reduction techniques over the years. In Section 5.1 we will review some of the most important such methods, and point out which of them should be the most appropriate in the core-box modelling framework. The remaining two sections present new contributions. Section 5.2 presents a model reduction approach to the understanding of the generation of the complex behaviour. This method is not developed to fit the model reduction step in the core-box modelling framework, but is instead an example of how model reduction may be used as an analysis tool. Section 5.3, on the other hand, presents a method which is developed especially for the reduction step in the core-box modelling framework. It does not guarantee that an identifiable model is obtained, but it does reduce the number of unidentifiable parameters in a model, especially in such models that have big kinetic expressions. Both the presented methods are designed to be applicable to the specific complexities present in a model like the Hynne model for yeast glycolysis. Therefore, in Chapter 10.3 the performance of the methods are demonstrated by applications to this model.
5.1 General theory and review of methods

We now present a short overview of some of the most important methods for model reduction of nonlinear differential equations. It is outside the scope of this dissertation to present a rigorous introduction to the field, and the focus will therefore be on the presentation of the basic ideas underlying each method. References for further reading will be provided in relation to each method. It should also be pointed out that in order to achieve an identifiable core model, it is often necessary to combine different model reduction techniques, and sometimes one even has to resort to the most basic of all methods: that of replacing parameters by guessed values. However, this latter option should generally be avoided if the full benefit of the core-box modelling framework is to be enjoyed. It should also be mentioned that it is not always necessary to achieve a fully identifiable model; if the unidentifiable parts of the model are characterised and controlled this might very well be sufficient.

Lumping

Lumping is the maybe most widely used reduction technique in biochemical modelling. The reason for this is probably that it may be done on an intuitive basis, i.e., without extensive calculations. Further, the resulting model is easy to interpret biochemically; the relation between the states and parameters between the original and reduced models are usually easy to establish, and the number of parameters may be substantially reduced. All these are reasons why lumping is a highly appreciated reduction methodology, and why it is also one of the most appropriate techniques in the core-box modelling framework.

There are several variations of lumping, though, and not all of them are equally useful in the core-box modelling framework. The first variation concerns whether the lumping is proper or improper. The basic principle in all lumping is that each state variable in the reduced model corresponds to one or several state variables in the original model. In proper lumping there will not be any overlaps between the lumped states, i.e., each state variable, $x_i$, in the original model is included in exactly one lumped state $x_0^j$ in the reduced model. This requirement might be violated in improper lumping. However, improper lumping is mainly produced when special automated methods are used. If the lumping is based on biochemical intuition, or by a method that does not yield improper lumps, this problem is avoided. The second variation of lumping concerns whether it is continuous or discrete. Continuous lumping is advantageous if the states in the original system are so many that they may not be modelled in detail. Then the states in the reduced model will be a a continuous variable that may be integrated over some index $i$, that spans all the states in the system. This type of lumping is not particularly appropriate if a full-scale grey-box model exists for the system (at least if it is not larger than the models appearing in this dissertation). In conclusion we can therefore say that our primary interested is in proper and discrete lumping. This kind of lumping is depicted in Figure 5.1a.

Another variation of lumping depends on the complexity of the original model structure. If this only consists of linear interactions between individual molecules
results of a high generality are available. Some such results are derived in Chapter 7.3, where we show how one may do the back-translation step in the case of variable lumping. If the original model structure should contain higher complexity, e.g., reactions involving several substrates or products, or enzyme catalysis, there are fewer general results. This means that the back-translation might not always yield analytical expressions, but might have to involve approximations based either on intuition or on simulation. Such an approach to lumping is taken in Section 5.2, where we combine lumping with parameter estimation to achieve a maximal agreement between the original and the reduced model. Finally, further reading about lumping, apart from these other places in this dissertation, is found in [94] and the references therein.

Time-scale based methods

Two common ways to detect the possibility of lumping are correlation analysis of simulated state trajectories, and analysis of the time-scales in the system. The reactions in the lump are assumed to be very fast, and to equilibrate into a lumped variable that may be treated as a single unit when analysing its interactions with its environment. There are, however, more general methods to deal with differences in time-scales. One common such method is computational singular perturbation (CSP). One then divides the states $x$ into fast states $x_f$ and slow states $x_s$. One also introduces a perturbation parameter $\epsilon$, that enters the differential equations in the following way

$$\frac{dx_s}{dt} = f_s(x_s, x_f, \epsilon) \quad \epsilon \frac{dx_f}{dt} = f_f(x_s, x_f, \epsilon)$$

(5.1)
By letting this perturbation parameter go to zero the second differential equation turns into an algebraic relation. By solving for $x_f$ in this equation we may express $x_f$ as a function of $x_s$.

$$f_f(x_s,x_f,0) = 0 \Rightarrow x = x(x_s) = (x_s, x_f(x_s))$$  \hspace{1cm} (5.2)

The degrees of freedom for $x(x_s)$ is reduced and $x(x_s)$ only describes the slow dynamics in the system. This means that the fast transients in $x_f$ are replaced by direct projections to the corresponding slower manifolds.

There are many examples of successful applications of this approach, e.g., the derivation of the classical Michaelis-Menten expression from its underlying elementary reactions and assumptions about the time-scales. However, in the core-box modelling framework CSP is not ideal. The main reason for this is that CSP does not focus on a reduction of the unidentifiable parameters, but instead on a reduction of the fast dynamics. Further, the parameters describing the fast dynamics are often kept in the model through the algebraic expressions $x_f(x_s)$. Finally, the reduction often involves non-physical coordinate changes (e.g., to optimize the separations between the time-scales for the different states). This makes the states in the reduced model less easy to interpret biochemically, and this is another important drawback with the approach. Nevertheless, time-scale based methods are widely used, and a graphical depiction of the basic idea is given in Figure 5.1c. Finally, further reading about time-scale based methods are available in [94, 142].

**Sensitivity analysis based methods**

Sensitivity analysis based methods (SA methods) consists of two major steps. The first step is the sensitivity analysis. This step checks the quantitative and qualitative impact that each part of the system has on some chosen model output, and those entities that are least important for the generation of the output are ranked the lowest. The second step is based on the result of this analysis: those parts that are ranked as unimportant are sought to be eliminated. However, the ranking is not blindly followed, but is more a guidance in the search for variables to eliminate. Since the effect of combinations of eliminations is not directly given by the combination of the effects of the corresponding individual eliminations, the second step requires clever search schemes and additional precautions. The different elimination combinations are, e.g., followed by a check on the model, and only so many parts are eliminated that the agreement with the original model still is acceptable.

Depending on the complexity in the model, and on the objective with the reduction, different things are reduced. The most common options, though, are elimination of individual terms, individual reactions, and/or individual states. For elimination of terms there exists a *Mathematica* based implementation denoted *AnalogInsydes*. This implementation is based on a theory referred to as numero-symbolic model reduction and it has quite successfully been applied to various technical applications [2]. However, when applying this software to biological systems, care must be taken not to render the models biochemically inconsistent. Such problems occur, for instance, if a term is removed in a given reaction in a given differential equation, but not in the same reaction when it appears in another differential equation. Most of these problems should be helped by implementing a
similar software that works on the reaction and stoichiometry matrices directly. For ranking and elimination of the reactions the problem might be formulated as follows

\[ \dot{x} = N \, D \, v \]  

(5.3)

where \(N\) and \(v\) have their usual interpretation of the stoichiometry matrix and the vector of reactions, respectively (see Chapter 2). In equation (5.3), the symbol \(D\) is also a matrix. The elements of \(D\) are, in the common approach, equal to 1 or 0. Those elements that are 1 correspond to a reaction that is kept, and those elements that are 0 correspond to a reaction that is eliminated. In this way one may work with the eliminations in a straightforward manner, that also allows, for instance, for optimization problems to be formed (see [97] for an example of this). Finally, for elimination of a state, it is necessary that all occurrences of the state are removed as well. The most straightforward way to do this is to eliminate the states after the reactions have been removed, as an after-analysis. Such eliminations are also included in the software AnalogInsydes.

Finally, SA based elimination methods are one of the most tractable methods in the core-box modelling framework. There are some drawbacks, though, and they are associated to the fact that the reduced model will typically not have the same optimum as the original model, when translating results between the models. This is discussed and different solutions are suggested in relation to the back-translation step in Chapter 7.3.

**Balanced Truncation**

In the core-box modelling reduction step the central feature that is optimized, apart from the agreement with the actual time-series, is the degree of identifiability. This means that the above methods should ideally be extended to include such identifiability considerations as well. Balanced truncation is a method that is more especially designed to account for such considerations. The term balancing means that one balances the importance of the inputs and outputs. Usually one balances them equally, i.e., so that equal importance is given to the inputs and the outputs. A partial result in the method is a coordinate change that orders the new variables in descending order according to how much they affect the input-output relation. In equal balancing the transformation ensures that the first variable both is the one that is affected the most by the input and is the variable that affects the output signal the most. The relative importance of the new variables is, e.g., quantified by the Hankel singular values, \(\sigma_{H,i}\), which are given in a diagonal matrix.

\[
\begin{pmatrix}
\sigma_{H,1} & \cdots & 0 \\
\vdots & \ddots & \vdots \\
0 & \cdots & \sigma_{H,n}
\end{pmatrix}
\]  

(5.4)

The balanced states corresponding to eigenvalues several orders of magnitude lower than the first ones can then be eliminated, e.g. using a Galerkin projection, typically without a visible change in the input-output relation. The reduced states and differential equations correspond to the unidentifiable parts of the model.
that way it is easy to remove the unidentifiable parts of the model at a minimum cost in terms of preserving the input-output relation. This method has originally been developed for linear systems, but nonlinear extensions exists as well [51]. The method is highly appreciated in technical applications [77], e.g., in model predictive control [51]. There are also some cases where the method has been used in biochemical modelling situations, e.g., to reduce the environment to the system [74, 75]. However, there is one major drawback with the system in terms of biological modelling, and that is that the variables in the reduced model are not biochemically interpretable. Since this is a requirement we will typically pose on a core model, balanced truncation is, despite of its many virtues, not particularly applicable in the core-box modelling framework. On the other hand, if it would be possible to obtain a straightforward back-translation formula, balanced truncation would be one of the best methods.

5.2 Model reduction for increased understanding of complex behaviour

A recurring feature of biological systems is not only that they are complex, but also that they display complex behaviour. The maybe most common complex behaviour is the oscillatory, and it occurs on many different time-scales. One major cycle that involves virtually all biological system is the circadian rhythm, the rhythm of the day. Examples of larger time-scales are the lunar, the annual, and the several-year cycles. The latter appear in, e.g., predator-prey systems [124]. Examples of shorter time-scales are the oscillations of the blood glucose level, at the time-scale of hours [125], and the cyanide induced oscillations in starved yeast cells, at the time-scale of seconds [32]. Other common complex behaviours in biology are bistability, bursting and chaos [123]. It should be noted that all these complex behaviours appear as the natural operating points of the systems, i.e., it is really the long-term stationary behaviour of these biological systems. A further increase in the complexity occurs when these kind of systems are coupled to each other. The oscillatory system in [32] is actually a system of several million oscillating yeast cells. An example of synchronised subsystems with even more complex behaviour is found in coupled β cells [122], which display coupled bursting systems (see Figure 5.2). Finally, even synchronised chaos, the maybe most complex behaviour imaginable, appears naturally in biological systems [58].

This common occurrence of complex behaviour has, of course, led to much scientific research. Common questions that have been asked are: What is the key interactions giving rise to the complex behaviour? What is its biological function? How is the complex behaviour regulated? How are the subsystems synchronised?

In this work we will focus on a special case of the first of these questions: the question of which mechanisms that give rise to oscillations in a system. This is henceforth referred to as the mechanism problem. There are already several methods developed that tackle this question, and one of the most recent of these was presented in [31]. It builds on an analysis of the eigenvectors of the Jacobian, evaluated at the unstable steady state close to the bifurcation. The method allows
for a grouping of the state variables in two modes. These two modes are then interpreted as the real and imaginary part of $z$ in (2.19). Since the interactions in equation (2.19) are easy to interpret, one can use this mode-interpretation to argue for a specific set of interactions as being the biochemical mechanism for generation of the oscillations.

However, there are two significant shortcomings of this method. First, the mode-interpretation method does not prove its statements, but only makes them plausible. That a given set of variables and interactions are sufficient to generate oscillations is only shown explicitly by isolating these entities, and then showing that the resulting system can oscillate. This is almost impossible experimentally, but quite straightforward in a modelling framework. It is therefore quite natural that this kind of analysis was a common modelling objective during the early developments of bio-modelling [49, 56, 119, 120, 127]. However, in those days the experimental knowledge of parameter and steady state values were very limited, and this made it hard to draw any further conclusions from these early models. The second weakness with the mode-interpretation method in [31] concerns the robustness of the prediction. A quality tag estimating the robustness is obtained by analysis of how the results change upon variations in, e.g., the parameter values, steady state values, or in the model structure.

We will now present a new method for analysis of the mechanism problem. It combines analysis like the mode interpretation presented in [31] with a specific kind
of model reduction. The new method has solved the above mentioned problems with the mode-interpretation method. First we describe the general method, and then we introduce two new reduction methods for the model reduction step. In Chapter 10.3.1 we demonstrate the performance of the new method on the well-studied test case of glycolytic oscillations in yeast.

### 5.2.1 A new method for analysis of the mechanism problem

The new method for identification of mechanisms generating oscillations consists of four steps (see also Figure 5.3): i) analysis of the dynamical structure of the original model; ii) reduction of the model to smaller models with preserved dynamic properties; iii) analysis of the reduced models; iv) comparison of the results for the different models.

The first and third step should use the same methods for analysis of the models' dynamical structures. Our primary choice will be the above mentioned mode-interpretation, but we will also consider another method which ranks the variables and interactions according to their importance for the generation of the oscillations \[110\]. The ranking method will be described in more detail in connection to the second reduction method, which uses the ranking for its reduction procedures. Finally, one could, in principle, also consider using other methods than the ranking method or the mode-interpretation for the analysis steps.

The most complex addition, compared to the mode-interpretation in \[31\], is the reduction step. In the review section we stated that no single reduction method is superior in all situations, but that the usefulness of a method depends on both the type of complexity in the original model, and on the objective with the reduction. Here we will consider biochemical, and typically metabolic models, and we require the reduced models to be both biochemically meaningful, and with preserved basic dynamical properties. There is not any previous method known to us that has been especially developed for this particular purpose. We have therefore developed two new methods that are especially designed for the given objective. They are built on lumping plus optimization, and elimination of dynamic states, respectively, and they are described below.

The comparison step is quite straight-forward, but it is only there that the final result is obtained. What the result is depends both on how far the models have been possible to reduce, and on the relation between the results for the different models. There are several possibilities. If a reduced model is obtained that only consists of the variables and interactions that was predicted to be part of the oscillatory mechanism, then that is a proof that these entities are capable of generating oscillations, i.e., that they make up a possible oscillatory mechanism. This is the same kind of result that often was obtained in the 60's and 70's (see above), except for one important improvement. The present minimal models have been obtained through model reduction of (generally) more realistic models than were available back then, and that means that the fluxes, parameters, and steady state values are more restricted to what is known to be realistic. This strengthens the importance of the results. Furthermore, if the reduced models show the same dynamical structure as the original model, this is evidence of a good robustness of the prediction. The robustness follows from the fact that the same dynamical structure (mechanism)
### Figure 5.3 Overview of the suggest method for identification of the mechanisms generating oscillations.
have been predicted upon variations in both the model structure, parameter values, and steady state values. The robustness and evidence of sufficiency are the two major new improvements compared to the original model-interpretation method.

Note that the two properties are independent of each other. It is quite possible to obtain a strong robustness of the results, without showing that the mechanism is sufficient. Conversely, it could for instance happen that the sufficiency is shown, but that minimal models with other mechanisms are also encountered, or that the models have different dynamical structures. In the latter case, one would have obtained the important information that predicted mechanism is feasible, but that it is only one of several feasible mechanisms, and that one cannot, at this point, tell which mechanism is the correct one. Note finally that all these analyses are only referring to properties of a given model, and that the relation between the model predictions and the real experimental system needs a full treatment using core-box modelling.

5.2.2 Lumping and constrained optimization

The first reduction method is based on lumping and subsequent optimization. The lumping step could in principle be replaced by any other method that results in a reduced biochemically meaningful model structure. We refer to the general review in Section 5.1 for description of different available methods for picking a reduced model structure. The reduced model should, of course, include enough variables and interactions to allow for a sufficiently good agreement after the optimization. The model structure can be picked using the results from the analysis (to prove that the predicted mechanism is feasible), or some other feedbacks that could in principle give rise to oscillations.

While we do not put any specific demands on how the model structure is picked we, on the other hand, suggest a quite specific optimization method. This is because the optimization can be simplified since the reduced model only is required to preserve the basic dynamical properties. These properties are the oscillations themselves, the specific bifurcation that has led to it, and the location of the bifurcation in terms of input signals. Apart from this, we also require that the steady-state values and fluxes should be reasonably preserved. The latter condition follows from the demand that the reduced model should be biochemically meaningful, and the assumption that the original model has biochemically realistic steady-state values and fluxes. The method that we propose is especially designed for high efficiency when these are the prime criteria for the optimization. The method is denoted 'the direct method', and it was used to estimate the Hynne model [62]. The basic idea of the method is to calculate the velocity parameters (mass-action and \( V_{\text{max}} \) kinetic constants) by solving the stationarity condition, and then fitting the remaining intrinsic parameters by continuation methods [62]. This method is especially applicable if there are not so many intrinsic parameters. If one has a reduced model structure with many intrinsic parameters, or a more general agreement between the two models are sought (e.g. based on a least-square comparison), more sophisticated methods are necessary. Two such methods are developed in the next chapter. To sum up we also formulate the method like an algorithm.
Algorithm 5.1 Lumping and optimization

Input: A given model with oscillations

1. Determine a reduced model structure (e.g. through lumping and biochemical intuition)

2. Optimize the parameters using 'the direct method' and simulation data from the original model

Output: A reduced biochemically meaningful model with preserved dynamical properties

5.2.3 Elimination of dynamics

The second method that has been developed for this specific kind of model reduction is based on the elimination of state variables by removing their dynamics. This means that an eliminated variable appears as a parameter in the reduced model. If this can be done without losing the basic dynamical properties, this is a strong indication that the variables is not a part of the feedback mechanism generating the oscillations. In principle one could therefore search through all combinations of eliminations, and in this way identify a minimal model. There are, however, at least three associated problems that needs to be tackled before such a complete search can be applied: i) uniqueness ii) internal consistency iii) combinatorial explosion.

i) The method has problems with uniqueness if the complete search finds several models of the same size. One then has to choose between the different candidates by a new set of selection criteria. If the models are not too many one can easily do this final choice by hand, and then one can also consider all of the models in the analysis step.

ii) The reduced models might have problems with internal consistency if one does not ensure that the preservation of mass is fulfilled. This fulfilment requires that the reaction rates, appearing on the right hand side of an eliminated differential equation, cancels out. This can be ensured in several ways. One option is to use the eliminated differential equation as a quasi-state state assumption, and calculate new rate expressions for a reduced model. However, here we envision biochemical models where part of the complexity lies in the rate expressions, and then this approach is not advantageous (since it leads to even more complex rate expressions). Therefore we suggest to substitute some of the old rate expressions, according to the eliminated differential equation. This solution is further explained for a small system in Example 5.1, and for a realistic model in Chapter 10.3.1.

iii) The number of models to evaluate grows quickly with the number of variables in a model, and this combinatorial explosion makes a complete search unfeasible for large-scale models. This problem is solved by first applying a ranking method to the original model. This ranking was first presented in [110], and the variables are ranked according to their estimated importance for the oscillations. This ranking is in fact a kind of sensitivity analysis, since the effect of each variable on the rest of the system is perturbed by a small perturbation \( \Delta \), and since the variables that have the smallest \( \Delta \) that stops the oscillations are ranked as the most important. The
ranking can be used to obtain a (typically) smaller model, by including variables one by one according to their ranking. Note that this is very similar to an SA-based method. However, this ranking is only semi-quantitative, and one must thus make a complete search also in the obtained smaller model. We sum up this second reduction method in the following algorithm.

**Algorithm 5.2 Elimination of dynamics**

**Input:** A given model, $M_1$ showing oscillations

1. Rank the importance of the variables and interactions for the oscillations

2. Add variable after variable from the ranking until an oscillating model, $M_2$, is obtained

3. Make a complete search among the possible sub-models of $M_2$. Identify the minimal model, $M_3$, showing oscillations

**Output:** A reduced biochemically meaningful model, $M_3$, with preserved dynamical properties

**Example 5.1** Consider a small system including the following first three equations

\[
\begin{align*}
\dot{x}_1 &= v_1 + v_2 - v_3 \\
\dot{x}_2 &= -v_1 + v_2 \\
\dot{x}_3 &= v_3
\end{align*}
\]

where the $x_i$s are states and the $v_i$s are reaction rates. Assume that the system is oscillating and that the oscillations are still present even if $x_2$ is held constant. Then one could consider a reduced system with $\dot{x}_2 = 0$. However, in order for such a reduced model to be internally consistent, one must ensure that $v_1 = v_2$. We suggest to do this by replacing $v_1$ by $v_2$, and thus obtaining the following equations:

\[
\begin{align*}
\dot{x}_1 &= 2v_2 - v_3 \\
\dot{x}_2 &= x_{0,2} \\
\dot{x}_3 &= v_3
\end{align*}
\]

This is the method that is used in this dissertation, but we want to point out that one could, in principle, also use the quasi-steady state approximation as well (see Section 5.1 under time-scale based methods).
Summary of Section 5.2

We have reported a novel method for the mechanism problem, i.e., the question of which variables and interactions that generate the complex behaviour in an oscillating system. The method answers the question for a given model. The new approach extends previous analysis methods, like the mode-interpretation method presented in [31]. The mode-interpretation method can come with a prediction of a plausible mechanism, based on analysis of the original model. It does, however, not test whether the predicted mechanism is really capable of generating oscillations in a biochemically meaningful model, and it does not come with a quality tag for the robustness of the prediction. The new method solves both these problems. The crucial step is a model reduction to biochemically meaningful models with preserved basic dynamical properties. The reduced models are then analysed, and the result is compared with the results for the original model. The ability of the predicted mechanism to generate oscillations is tested by a reduction to the corresponding reduced model; the quality tag of the prediction is obtained by a comparison of the dynamical properties of the different models. In Chapter 10.3.1 we demonstrate the method on the 20 dimensional Hynne model for glycolytic oscillations. There we also get a test case evaluation of the properties of the two reduction methods.

5.3 A matlab implementation for reduction of kinetic expressions

We now leave the mechanism problem, and return to the main focus of this chapter: the third step in the core-box modelling framework. One side of this step is to analyse the identifiability of the various models; this was covered in Chapter 4. With these tools established the next step is to reduce those parts of the grey-box model that are unidentifiable, so that an identifiable core model is obtained. We presented some new general methods for how such reductions may be done for a single reaction rate in Chapter 4.3, and reviewed some existing methods for model reduction of a complete model structure in Section 5.1. We will now present a new method for reduction of the number of parameters in a grey-box model. Just like the methods in Chapters 4.2 and 4.3, we focus on reductions of a single rate expression. This time, however, we have automated the processes so much that they may be done for all reactions in a complete model automatically. The new method builds on a reformulation to an estimation problem that is based on the rational expressions from Chapter 4.2, even though we now use them in the more general setting of practical unidentifiability. We first present the new theory in Section 5.3.1. Thereafter, in Section 5.3.2, a software implementation of this theory is presented, and in Chapter 10.3.2 we demonstrate the performance of the theory and implementation on the Hynne model for yeast glycolysis.
5.3.1 Reduction of a linear estimation formulation

Recall the reformulation of a general rate expression \( v(x, p) \) to the rational expression \( v(x, c) \) (see equation (4.23) in Chapter 4.2)

\[
v(x, k) \Rightarrow v(x, c) = \frac{P(x, c)}{Q(x, c)} = \frac{a_1 x^{p_1} + a_2 x^{p_2} + \ldots}{b_1 x^{q_1} + b_2 x^{q_2} + \ldots} \tag{5.5}
\]

Now assume that there exists measurement data \( Z^N \) of \( v \) and \( x \). Let this data be denoted \( v(i) \) and \( x(i) \), where \( i \) is the sample number. The coefficients \( c \) are practically identifiable with respect to this data set if they may be uniquely estimated from the data. The same holds for the original parameters \( p \). We will now derive an ordinary linear least squares formulation for the estimation of \( c \) based on such data. This will allow us to see which parameters that are unidentifiable, and how the unidentifiable directions may easily be reduced.

Multiply both sides of equation (5.5) with the denominator, and sort the remaining numerator on the left hand side so that the former numerator terms come first. Write the relation as a product of two vectors, and finally replace the elements by their corresponding data columnwise. This gives the following equation

\[
\begin{pmatrix}
-x(1)^{p_1} & -x(1)^{p_2} & \ldots & v(1)x(1)^{q_1} & v(1)x(1)^{q_2} & \ldots \\
-x(2)^{p_1} & -x(2)^{p_2} & \ldots & v(2)x(2)^{q_1} & v(2)x(2)^{q_2} & \ldots \\
\vdots & \vdots & \ddots & \vdots & \vdots & \ddots \\
-x(N)^{p_1} & -x(N)^{p_2} & \ldots & v(N)x(N)^{q_1} & v(N)x(N)^{q_2} & \ldots
\end{pmatrix}
\begin{pmatrix}
a_1 \\
a_2 \\
\vdots \\
b_1 \\
b_2 \\
\vdots
\end{pmatrix}
= 
\begin{pmatrix}
0 \\
0 \\
\vdots \\
0 \\
0 \\
\vdots
\end{pmatrix}
\tag{5.6}
\]

Note that the vector with coefficients is equal to \( c \). Let the data matrix in equation (5.6) be denoted \( M \), and let its \( i \)th row be denoted \( u_{M,i} \). Note that there will typically be one coefficient that is equal to a known value, at least if the original rate expression is identifiable. Assume (without loss of generality) that it is the \( j \)th coefficient that is known. Sort the corresponding column \( u_{M,j} \) to the right hand side of equation (5.6). This gives the following equation

\[
\tilde{M} \cdot \bar{c} := \begin{pmatrix}
| & | & | & | & | \\
| & u_{m,1} & \ldots & u_{m,j-1} & u_{m,j+1} & | \\
| & | & | & | & | \\
| & | & | & | & |
\end{pmatrix}
\begin{pmatrix}
c_1 \\
c_{j-1} \\
c_j \\
c_{j+1} \\
\vdots
\end{pmatrix}
= \begin{pmatrix}
-\bar{u}_{m,j}c_j \end{pmatrix}
\tag{5.7}
\]

where the vector on the right hand side consists exclusively of (scaled) data. Note that the symbol \( \bar{c} \) was denoted \( c' \) in Section 4.2. The reason for the change is that we are now considering practical unidentifiability, and while \( c' \) is structurally unidentifiable (if no linear dependencies among the \( x_i \)'s exists) it is not necessarily practically unidentifiable. Equation (5.7) is on the form a linear ordinary least
5.3 A matlab implementation for reduction of kinetic expressions

squares estimation problem. This estimation problem is one of the most well-studied estimation problems, and there exists good theory and software for its solution [50]. One of the characteristics of this equation is that the uncertainty of the estimated parameters may be estimated by the condition number for $\tilde{M}$. The condition number is defined as the fraction between the highest and the lowest singular value obtained by an SVD decomposition of $\tilde{M}$, and the higher the condition number the higher the uncertainty [50]. Therefore, if the lowest singular values are zero, or very close to zero, the parameters are unidentifiable. Hence, if the matrix $\tilde{M}$ is singular, the parameters are unidentifiable.

On the other hand, if the matrix $\tilde{M}$ is singular, there exists linear dependencies among its columns. Let the $i$:th column of $\tilde{M}$ be denoted $u_{\tilde{M},i}$. The linear dependencies among the $u_{\tilde{M},i}$s are given by the last column in the SVD expansion of $\tilde{M}$. Let the (thin) SVD expansion of $\tilde{M}$ be given by the three matrices $U$, $S_{\tilde{M}}$, and $V$ according to

$$\tilde{M} = U \cdot S_{\tilde{M}} \cdot V^T$$  

(5.8)

Let the last column of $V$ be denoted $u_{\tilde{V}}$, with elements given by

$$u_{\tilde{V}} = (u_{\tilde{V},1}, \ldots, u_{\tilde{V},n_t-1})$$  

(5.9)

(Recall that $n_t$ is defined as the number of terms in (5.5)). We thus have the (approximate) relation that $\tilde{M} \cdot u_{\tilde{V}} \approx 0$. Use this linear relation to solve for the last column $u_{\tilde{M},n_t-1}$. Use the obtained result to rewrite $\tilde{M}$ in equation (5.7) into a matrix with $n_t-2$ columns times a matrix which consists of a unity $(n_t-2) \times (n_t-2)$ matrix in the first columns, and the obtained result in the last column. This gives

$$
\begin{pmatrix}
| & | & | \\
\text{u}_{\tilde{M},1} & \ldots & \text{u}_{\tilde{M},n_t-2} \\
| & | & | \\
\end{pmatrix}
\begin{pmatrix}
1 & 0 & \ldots & 0 & -\frac{u_{\tilde{V},1}}{u_{\tilde{V},n_t-1}} \\
0 & 1 & \ldots & 0 & \vdots \\
\vdots & 0 & \ldots & 0 & \vdots \\
0 & 0 & \ldots & 1 & -\frac{u_{\tilde{V},n_t-2}}{u_{\tilde{V},n_t-1}}
\end{pmatrix}
\begin{pmatrix}
\tilde{c}_1 \\
\vdots \\
\tilde{c}_{n_t-1} \\
\end{pmatrix}
\approx
\begin{pmatrix}
\text{y}_{M,j} \\
\end{pmatrix}
$$  

(5.10)

where the approximation is due to the fact that the linear relation might not be exactly fulfilled, i.e., that the last singular value might not be exactly zero. Multiply the last matrices on the right hand side of (5.10) together; this gives

$$
\begin{pmatrix}
| & | & | \\
\text{u}_{\tilde{M},1} & \ldots & \text{u}_{\tilde{M},n_t-2} \\
| & | & | \\
\end{pmatrix}
\begin{pmatrix}
\tilde{c}_1 - \frac{u_{\tilde{V},1}}{u_{\tilde{V},n_t-1}} \tilde{c}_{n_t-1} \\
\vdots \\
\tilde{c}_{n_t-2} - \frac{u_{\tilde{V},n_t-2}}{u_{\tilde{V},n_t-1}} \tilde{c}_{n_t-1} \\
\end{pmatrix}
\approx
\begin{pmatrix}
\text{y}_{M,j} \\
\end{pmatrix}
$$  

(5.11)

Note that this is an equation of the same form as (5.7). There is, however, one important difference. The matrix that corresponds to $\tilde{M}$ has removed the most singular direction. Therefore, equation (5.11) is more likely to provide an identifiable estimation problem. These rewritings is therefore a way to reduce the system,
Figure 5.4 The basic workflow in the implementation of the reduction method. The blocks are described in Section 5.3.2.

and the procedure can of course be repeated until a satisfactory identifiability is obtained, or until the error becomes too large. These are the most central steps in the reduction. Let us now shortly describe how they have been implemented.

5.3.2 The implementation

The basic components in the implementation and their interrelation are depicted in Figure 5.4. We now shortly describe these components one by one. A more detailed description is available in [121], and an example of the application of the program is provided in Chapter 10.3.2.

**Preprocessing:** This step mainly consists of conversions of the input form of the program (which is based on the standard format for a model in the Systems Biology Toolbox for MATLAB) to appropriate internal representations of the matrix $M$ in equation (5.6). Note that the data in the matrix is not experimental data, but data which has been simulated according to the operating points specified by the user. Prior to the complete analysis the program also checks whether any trivial simplifications are possible. Such simplifications include, e.g., putting entire reaction expressions to a constant or to zero.

**Analysis:** The next step analyses the $M$ matrix. The basic analysis part is the sub-division into the smaller matrix $M$ in equation (5.7), and an analysis of its singularity through an SVD expansion. Note, however, that there is an ambiguity in how the sub-divisions may be done. One may, in principle, take away any of the columns in the $M$ matrix (after division with the corresponding coefficient), and the corresponding $M$ matrices may, in principle, all have different singularity properties. From an estimation point of view, it is best to choose the $M$ matrix...
that has the lowest condition number. However, since we are aiming at model reduction, we pick the $M$ matrix with the highest condition number (i.e., with the singular value closest to zero), since this gives the lowest error in the following reduction. Note that this criterion might not always be give a unique $M$ matrix.

**Reduction:** The next step is the reduction of the $\tilde{M}$ matrix using the method described in the previous chapter. In the reduction, as in all the previous reformulations, the relations to the original parameters are kept, and the new parameter values are calculated from the old ones using the analytical expressions. After the reduction has been done the reduced model is compared with the original model. If the error is low enough the reduced model is accepted, and further reductions are attempted through iterations of the previous steps. Otherwise the reduced model is rejected and the original model (from the previous iteration) is sent to the final post-processing step.

**Post-processing:** The post-processing step deals mainly with the presentation of the final model, and analysis of its properties. This includes a displaying of the comparison between a simulation with the original model and the reduced model, and a table of the relations between the new parameters and the old ones.

### 5.4 Summary of Chapter 5

This chapter has dealt with the reduction of models on the form (2.4). The appropriateness of a model reduction technique depends both on the objective with the model reduction, and on the type of complexity in the original model. There are therefore many methods to choose from and in Section 5.1 we reviewed some of the most common choices: lumping, time-scale based methods, sensitivity analysis based methods, and balanced truncation. The emphasis on the review was more on the underlying principles behind the methods, and on when they are most appropriate, then on presenting all the technical details. For the core-box modelling framework, we particularly recommended lumping and sensitivity analysis based methods, even though we pointed out that neither of them are designed to be optimal in the sense of increasing the identifiability.

We then, in Section 5.2, presented a method that used model reduction to reveal the origin of complex behaviour, i.e., to solve the mechanism problem. The method is a four-step approach, and the steps are depicted in Figure 5.3. The central question is whether the analyses seeking to understand the complex behaviour give the same result before and after the reduction. If this is the case, the results have showed a good robustness, and are therefore more trustworthy. Further, if the results may be used to identify the oscillating core, the method provides a proof that the original predictions provide a possible explanation. Note, however, that uniqueness of the result is not proved, and that the method only gives a result that is valid for the model, i.e., without explicitly taking available data into account.

The last method presented a method that is especially designed for the reduc-
tion step in the core-box modelling framework. It reduces the complexity in the individual rate expressions through a reparametrization into a formulation that is linear in the parameters. The most singular directions in this formulation are then easily identified and eliminated. All these reductions are implemented in a MATLAB program, that also returns the relation of the reduced parameters to the original ones. Finally, in Chapter 10.3 we apply both the new methods in this Chapter to the Hynne model for yeast glycolysis.
System Identification

The fourth step in the core-box modelling framework is the system identification step. System identification is a modelling framework that is most widely used in the control engineering society, and its most impressive accomplishments regards linear time-discrete systems [77]. There exist results also for nonlinear ODEs but the main reason for choosing this framework is because of its sound modelling principles, using concepts like identifiability, cross-validation and informative data series. Other reasonable choices would have been any of the similar fields: inverse problems [10, 11], biosimulation [65], dynamic modelling and estimation [37], nonlinear regression [113], and model optimization [109].

According to the classical book on system identification [77] the following 4 components are instrumental in system identification: i) development of experimental design to obtain informative data series; ii) choice of a set of possible models, parametrised through a model structure; iii) choice of selection criterion, and identification of the corresponding model; iv) cross-validation on independent and qualitatively different data. The interrelation of these components is depicted in Figure 6.1 and the multiple arrows back in the ‘system identification loop’ is a restatement of the fact that modelling is not a simple linear algorithm, but a creative process that requires innovative thinking, biological intuition, and a constant re-iteration of the steps and assumptions already taken. It can be seen that system identification involves all steps of quantitative modelling and it could therefore be said that the entire core-box modelling approach is a special approach to system identification. In the previous two chapters we have already developed methods for the art of choosing an appropriate model structure, suitable for the estimation step. This has led us to a core model which has a well-characterised identifiability with respect to the available data. In this fourth step in the core-box modelling
framework we are therefore primarily concerned with the last two steps pointed out in [77]: the identification step and the validation step. In Section 6.1 we will review some of the state-of-the-art options for these steps. Then, in Sections 6.2 and 6.3 we present new results for identification of systems with a Hopf bifurcation. The first result is valid for all systems with an experimentally located Hopf bifurcation, and it reduces the dimension of the kinetic parameters $p_x$. This result is then further improved for systems starting the time-series close to the bifurcation; we show how the initial value parameters $x_0$ may be reparameterized in minimal degrees of freedom, and how they can be solved for in a separate sub-problem without integration, and without the problem of local minima.

Figure 6.1 The system identification loop as depicted in [77].
6.1 General theory and review of methods

Options for formulation of the optimization problem

Throughout this chapter we will use some variation of the standard least squares selection criterion. Remember that the simulated output that corresponds to the time \( t \) and parameter \( p \) is denoted \( \hat{y}(t|p) \), and that the measured output at time \( t \) is denoted \( y(t) \). Remember that the cost function corresponding to the data set \( Z^N \) and the parameter \( p \) is denoted \( V_N(p, Z^N) \), and that the chosen weight at each time-point is denoted \( w(t) \). Then the standard quadratic cost function is written as

\[
V_N(p, Z^N) = \sum_{i=1}^{N} \sum_{j=1}^{n_x} w_i(t_i)(y_j(t_i) - \hat{y}_j(t_i|p))^2
\]  

(6.1)

A common choice for weight function, \( w(t) \), is the inverse of the variance of the experimental noise, but one can also add extra weights on parts of a time-series that are judged to be extra important. If there is extra information that is not easily related to the measured time-series, like for instance previous estimates of metabolite concentrations or of parameter values, this can be added to the cost function as extra terms with their own weighting. Independently of how the cost function has been formulated the selection criterion is chosen as

\[
\hat{p} = \arg \min_{p \in \Omega} V(p, Z^N)
\]  

(6.2)

where \( \Omega \subset \mathbb{R}^r \) is the space of acceptable parameter values.

There are many options for how the model output \( \hat{y}(t|p) \) can be generated. The most common choice in the systems biology community today is to simulate all the outputs for a given parameter \( p \) in a single simulation starting from the given initial values \( x_0 \). This is probably also the most simple approach. However, if \( p \) is not close enough to the correct parameters, \( p^0 \), the simulated output will lie far away from the measured output for the majority of the timeseries. This complicates the search, since this causes the cost function to become large, and the direction in which the true parameters lie to be relatively unrelated to the gradient of the cost function. To improve this problem, one might make use of the past data when creating the predictions. Such methods are common in the prediction-error framework in time-discrete system identification [77], but the same idea is used also in the method of multiple shooting [10, 11, 109]. The method of multiple shooting involves more parameters than a classical single simulation approach does, but the advantage of a more well-behaved cost function is sometimes worth this. Multiple shooting is most beneficial if all, or at least most, of the states in the model can be directly measured. However, if all states can be directly measured with a good data quality many other interpolation based methods suddenly becomes available as well [46, 131], and they often imply an even more tractable estimation problem.

Options for solving the optimization problem

Once the cost function has been chosen the estimated parameters, \( \hat{p} \), are, in principle, uniquely determined by the criterion (6.2) (given identifiability). However,
to find this minimum, in practice, is difficult. This is for instance due to the many
local minima that typically exists in these cost functions. There exists many meth-
ods to choose from for the optimization problem, and they are typically divided in
two groups: local and global methods.

Local methods
Local methods are based on the local shape of the cost function. This shape is
usually formalised by the gradient and by the Hessian, i.e., by the first two terms
in a Taylor expansion around the current estimate \( p^i \)

\[
V_N(p) = V_N(p^i) + \nabla_p V_N(p)|_{p^i} (p - p^i) + (p - p^i)^T H(p^i)(p - p^i)
\] (6.3)

\[
H(p) := \nabla_p^2 V_N(p)|_{p^i}
\] (6.4)

where \( \nabla_p V_N(p) \) is the gradient, and \( H(p) \) is the Hessian. The gradient may be
calculated by numerical perturbation or by solving the sensitivity equations (see
Chapter 4.1). The Hessian may be calculated by extensions of these approaches.
However, such extensions are computationally intensive, and the Hessian is often
approximated as described below. If the exact Hessian is available one might
use the Newton method to solve the optimization problem (6.2). The Newton
method has the advantage that it has a quadratic convergence when being close
to the optimum. Further away from the optimum, however, the convergence is
much slower. In some situations it is usually more advantageous to switch to
other variations of the methods. These variations are commonly referred to as
quasi-Newton methods, and they are usually stepping more in the direction of the
gradient in the beginning of the search, and gradually switches to the direction given
by the Hessian as one approaches the optimum. A common class of quasi-Newton
methods are the Gauss-Newton methods, e.g., the Levenberg-Marquardt method.
The Gauss-Newton methods are especially designed for least squares problem, and
they utilise a special type of approximation when calculating the Hessian:

\[
\frac{\partial^2 V_N}{\partial p_i \partial p_j} \approx \sum_{k=1}^{N} \left( \frac{\partial \hat{y}(t_k|\hat{p})}{\partial p_i} \frac{\partial \hat{y}(t_k|\hat{p})}{\partial p_j} \right)
\] (6.5)

The calculations showing this relation are the same as those in equation (4.16).
After the direction have been chosen, a suitable step length also needs to be chosen.
This is usually solved by a little optimization problem of its own, and common
methods to solve this subproblem are the line-search, the trust region, and the back-
stepping approaches. For an introduction to all these gradient based optimization
methods we refer to the textbook [92].

There are also local optimization methods that are not gradient based. Such
methods are also tractable options, because the cost function \( V_N(p, Z^N) \) is often
not smooth enough for the gradient to give a good approximation of the shape of
the cost function. One way to modify the gradient based methods is to introduce
a minimum step size for the optimization routines, and a minimum perturbation
size for the numerical perturbations. This acts as a kind of filtering of the cost
function. Another local but non-gradient based method is the nonlinear simplex
6.1 General theory and review of methods

method, also referred to as the Nelder-Mead method [91]. This method is based on a polyhedron (or a simplex) that moves through the parameter space. The worst vertex might, e.g., be mirrored to the other side of the polygon to see if it implies a significant improvement. Similarly, if the mirroring should imply an improvement the polygon might extend itself even further in this direction. Other options are shrinking, expanding and restarting of the entire polyhedron. In this way the polygon moves through the parameter space in a way that resembles the gradient based methods, but that is not so sensitive to non-smoothness in the cost function.

Global methods

The big disadvantage of the local methods is that they are reliable on a good initial guess for the parameter. If one does not possess a good enough initial guess, one typically has to resort to global methods. This option, however, should be treated as a last resort, since global methods has a much slower convergence rate, for instance due to the many iterations. The most primitive global method is probably to start in many different places, and then do local searches from all these starting guesses. This approach is referred to as the multi-start or the random search method [89], and it has the benefit of still making use of the high convergence rate of the local methods. However, the method scales badly with the dimension of the search space, Ω, since no particular strategy is taken to fill Ω in an intelligent way.

There are several methods that proposes such more sophisticated global searches. Most of these methods works with an ensemble of parameter guesses, which is updated together. One such method is referred to as simulated annealing. This method has gotten its name from the process of the cooling down of glass. There is a temperature $T_s$ which is high in the beginning of the optimization, and which gradually goes down to zero. This temperature might cause a specific parameter in the ensemble to take on a new parameter value (which it is is more or less randomly assigned), even if that parameter value would correspond to a higher cost function. This is formalised by a probability

$$f = f(V_N(p^i), V_N(p^{i+1}), T_s) = \exp\left(-\frac{V_N(p^{i+1}) - V_N(p^i)}{T_s}\right)$$

where $p^i$ is the old parameter value, and $p^{i+1}$ is the new. However, an improved value of the cost function will always mean an acceptance of the new parameter value (at least in the original formulation of the method). In this way the ensemble can move through the parameter space and find the globally best regions when the temperature $T_s$ is high, and find the best local minimum in those regions when $T_s$ is low.

There are many variations of the global methods that has taken other choices, for instance regarding the choice of whether the new parameter should be accepted or not, or regarding the generation of the new parameter guesses. One also distinguishes between stochastic and deterministic methods, where the latter is distinguished from the former by always returning the same answer from the same initial guesses and optimization settings. There is no general consensus regarding
which methods that work best, but there exists comparison works on examples with simulated data from biologically inspired models. One such comparison was done in [89], and it reported that a method denoted sRES was the most beneficial method, both in terms of finding the correct parameters and in terms of high convergence rate. A later work [106] then improved this method by switching to a local method at the end of the search. Such a switch is the characteristic of a hybrid method, and it might improve the overall optimization time significantly. Finally, it is our own experience that a combination of simulated annealing with the nonlinear simplex method is superior to, e.g., the sRES method, and in this dissertation we have typically used an implementation of this method in the Systems Biology Toolbox for MATLAB.

Options for validating the model

If the core model has been obtained by model reduction of an already optimized grey-box model it might be the case that the core model is already at the optimized parameters. In the formalism of Chapter 7 this is the same as saying that \( \hat{\theta} \) might be equal to \( \Phi(\hat{\theta}) \). If this should be the case, the most important task in step 4 in the core-box modelling framework is to acquire quality tags to the various parts of the core model. Remember that this may, in principle, be done already on the grey-box model, but that in practice, is difficult to give an accurate view of what actually has been estimated from the data.

There are several methods to choose from, and the most important ones has already been described in other places in this dissertation. One such class of methods are those associated with a practical identifiability analysis reviewed in Chapter 4.1. Both the simulation based approaches like bootstrapping, and the sensitivity analysis based methods like the FIM matrix are applicable for this question. Because the same identifiability methods are used one usually distinguishes between them by denoting them \textit{a priori} and \textit{a posteriori} identifiability analysis, respectively. \textit{A priori} identifiability analysis examines whether the model as such seems to be able to give a unique answer, and \textit{a posteriori} identifiability analysis examines the quality of a given estimate \( \hat{\theta} \). Each of the two kinds of identifiability analysis is associated with both different advantages and with different problems.

Another framework that is applicable to the evaluation of the estimated core model is the statistical testing framework reviewed in Chapter 3.4. Such tests were originally developed to answering the question whether the model as a whole should be rejected or accepted. However, as is illustrated in Chapter 11, the methods might also be used as an analysis method to examine the strengths and weaknesses of the different parts of a model as well. Finally, the last approach to the determination of these quality tags is the cross-validation methodology reviewed in Chapter 2.5.1. Remember, however, that even though the successful prediction of a model in a cross-validation experiment might be used as an argument to put more faith in the model, it is not a guarantee that the model as a whole has a high quality. In order to examine which parts of the model that has been most validated by the cross-validation data one should ideally combine biological reasoning with the \textit{a posteriori} identifiability methods mentioned above.
6.2 Improved parameter estimation for systems with an experimentally located Hopf bifurcation

In Chapter 10 we will study glycolytic oscillations in *Saccharomyces cerevisiae*. This system has an experimentally well-determined Hopf bifurcation [32], which was utilised when developing the Hynne model [62]. The method that was used then is denoted 'the direct method', and it is a combination of a calculation using net velocities implying a complex search space, and other algebraic tests that concern the features of the Hopf bifurcation. The 'direct method' has the advantage that it only uses algebraic tests to evaluate the parameters. It also divides the different tests into several categories, where the initial ones are the least computationally expensive, and can thus be used to reject most of the tested parameters in a short time. The major drawbacks of this method is that it only searches over a parameter grid, i.e., that no information is used about the interrelation between the parameters, like for instance in a gradient based method. The direct method is also not applicable to time series, and since time series contains the most informative type of data [77], this is a major drawback of the method.

The results in this section is motivated by, e.g., this example, and especially by the lack of a method that can use all the time-series while still utilising the knowledge about the bifurcation. We report a reformulation of the standard estimation problem (6.2), that includes the knowledge about the experimentally located Hopf bifurcation. We also show two methods that can solve the reformulated problem, and demonstrate the advantage of the presented method on an illustrative example.

6.2.1 Formulation of the optimization problem

Assume that the input to the system (2.4) contains a Hopf bifurcation at a known input signal, denoted $u_b$. We will derive a way to include this information in the standard estimation formulation (6.2).

Start by recalling the basic features of a Hopf bifurcation. The two main features defining a Hopf bifurcation that will later be utilised are

- A Hopf bifurcation point $(x_b, \mu_b)$ for a dynamical system (2.4a) is a non-hyperbolic fix point with exactly two purely imaginary critical eigenvalues (zero real part) to the Jacobian $J = \nabla_x f(x_b, \mu_b)$.

- As the system passes the bifurcation point, through variations in one of the parameters $\mu_{b,i}$, the complex conjugated pair of eigenvalues passes the imaginary axis with non-zero speed. This latter condition is called the crossing-criteria. If one of the critical eigenvalues is denoted $\lambda_1$ this criteria can be written as [135]

$$
\frac{d(\text{Re}(\lambda_1(x_b, \mu_b)))}{d\mu_{b,i}} \neq 0
$$

(6.7)

From the first of these two criteria we can construct the following test for a bifurcation point.

Let $\lambda_i(x, \mu)$ denote the i:th eigenvalue to the Jacobian of (2.4a) evaluated at the point $(x, \mu)$. Further let $F_{\text{Hopf}}$ be a scalar function defined by the following product

$$F_{\text{Hopf}}(x, \mu) := \prod_{i<j}(\lambda_i(x, \mu) + \lambda_j(x, \mu))$$

(6.8)

Then it follows from the first of the two conditions for a Hopf bifurcation, mentioned above, that if $(x_b, \mu_b)$ is a Hopf bifurcation it must fulfill the following relations

$$f(x_b, \mu_b) = 0$$
$$F_{\text{Hopf}}(x_b, \mu_b) = 0$$

(6.9) (6.10)

The first of these two relations is fulfilled if and only if $(x_b, \mu_b)$ is a fix-point, and since a Hopf bifurcation point is also a fix-point (6.9) is fulfilled for all Hopf bifurcation points. The equation (6.10), on the other hand, is not fulfilled by all fix-points. However, since a Hopf bifurcation has one pair of purely imaginary eigenvalues, one factor in (6.8) will be zero. Therefore also the entire product, i.e., $F_{\text{Hopf}}$ will be zero. We thus conclude that both (6.9) and (6.10) will be fulfilled if $(x_b, \mu_b)$ is a Hopf bifurcation point.

Recall that we know that the system is operating at a Hopf bifurcation point for a specific $u_b$. That means that for the estimated parameter $p$ to fulfill the given constraint there must exist a state $x_b$ such that $(x_b, p_x, u_b)$ fulfills (6.9) and (6.10). Let us re-state this in a more compact way. Let $c_{\text{Hopf}}$ denote the $n+1$-dimensional vector containing both the condition in (6.9) and in (6.10). Further let it always be evaluating the $u$ part of the $\mu$ vector at $u_b$, i.e.,

$$c_{\text{Hopf}}^T(x, p) := (f^T(x, p_x, u_b), F^T_{\text{Hopf}}(x, p_x, u_b))^T$$

(6.11)

Now we can reformulate the original estimation problem (6.2) as follows

$$\hat{\mu} := \arg\min_p f(V_N(p, Z^N); \exists x_b \text{ so that } c_{\text{Hopf}}(x_b, p_x) = 0}$$

(6.12)

To utilise the techniques from constrained optimization we need to formulate the constraint into an equality/inequality constraint on the free parameters only. This is done by including the state vectors $x_b$ as additional parameters in the optimization method. That means that we let the the parameter vector $p$ include the following components

$$p = (p_x, p_y, x_0, x_b)$$

(6.13)

With this $p$ vector a final reformulation of the problem can be made.

**Reformulation:** The estimated parameter, $\hat{p}$, defined by (6.13) is identified in the following way:

$$\hat{p} := \arg\min_p f(V_N(p, Z^N))$$

$$c_{\text{Hopf}}^T(x_b, p_x) = 0$$

(6.14a) (6.14b)
6.2 Improved parameter estimation at a Hopf bifurcation - I

The original problem (6.2) has now been transformed to the standard nonlinear optimization problem (6.14a) with the nonlinear constraint (6.14b).

Equation (6.14) is the formulation of the problem that will be used in this chapter. The most apparent drawback of this formulation is that it has introduced \( n \) new parameters, compared to the original estimation problem. As will now be shown, this drawback is outweighed by the presence of the extra condition. The extra condition vector \( c^{\text{Hopf}} \) has \( n + 1 \) dimensions, i.e., one dimension higher than that of the new parameter vector \( x_b \), and therefore the net effect of adding both \( x_b \) and \( c^{\text{Hopf}} \) is a reduction of the degrees of freedom describing the parameter vector.

Both the two presented methods to solve (6.14) will require that the starting point fulfills condition (6.14b). This means that the start estimate should lie on the bifurcation manifold. There is fortunately a rich literature with methods for finding such points [1]. These techniques are called \textit{continuation} techniques, and one of these is now reviewed.

Finding a feasible point

In this section a one-dimensional continuation method is presented. Continuation methods allows for following (continuation) of the attractors (e.g. fix points and limit cycles) as the parameter \( \mu \) varies, i.e., it allows for bifurcation diagrams, like, e.g., Figure 2.4, to be drawn. In general the varied parameters are a subset to the \( \mu \) vector, and can hence be multi-dimensional. For our present purposes, however, we only need to vary one parameter at a time. Let the parameter that is varied be denoted \( \mu_k \). First the three parts of the method are briefly described and then the actual algorithm is given. The method is described as it is implemented in the \textsc{Janet} software (www.fys.dtu.dk/~janet). For a more formal introduction to the subject, and to other similar methods, the reader is referred to [1].

Step I - Extending the system

First the parameter, \( \mu_k \), on which the continuation shall be done has to be chosen. Let then this parameter be considered as a state variable, i.e., let the vector \( x = (x_1, \ldots, x_n) \) be extended to \( x_{\text{ext}} = (x_1, \ldots, x_n, p_k) \), and let the parameter vector be diminished from \( \mu = (\mu_1, \ldots, \mu_s) \) to \( \mu_{\text{dim}} = (\mu_1, \ldots, \mu_{k-1}, \mu_{k+1}, \ldots, \mu_s) \). This is only a redistribution of the same number of parameters and variables and a point \((x, \mu)\) is therefore equal to a point \((x_{\text{ext}}, \mu_{\text{dim}})\) if the corresponding values are the same. Therefore the condition for an extended point \( x_{\text{ext}, f} \) to be a fix point is transformed from (6.9) to

\[
 f(x_{\text{ext}, f}, \mu_{\text{dim}}) = 0 \quad (6.15)
\]

which is the basis for calculating the predictor step.
Step II - The predictor step

The predictor step is calculated by finding a step in \( x_{\text{ext}} \), that does not change the value of \( f \), were it a plane. Therefore it is the solution of the following equation

\[
\begin{pmatrix}
\frac{df}{dx} & \frac{df}{d\mu} \\
0 & 1
\end{pmatrix}
\begin{pmatrix}
\Delta x \\
\Delta \mu_k
\end{pmatrix}
= \begin{pmatrix} 0 \\ 1 \end{pmatrix}
\]

(6.16)

Here the constant 1 is arbitrary and the whole last equation is simply added to obtain a system of equations which has as many unknowns as equations. Since the system also is linear it has an easily obtainable solution. The solution vector is, once obtained, added to the original extended vector to obtain the predicted value, i.e.,

\[
x_{\text{ext}}^{i+1,\text{pred}} = x_{\text{ext}}^i + (\Delta x, \Delta \mu_k) * h
\]

(6.17)

where \( h \) is the somehow chosen step length.

Step III - The corrector step

The predicted value \( x_{\text{ext}}^{i+1,\text{pred}} \) is, however, not necessarily on the bifurcation manifold, i.e., the function \( f(x_{\text{ext}}^{i+1,\text{pred}}, \mu_{\text{dim}}) \) is not necessarily equal to zero. Here Newton-Rhapson iterations finds the \( x_{\text{ext}} \) that solves the equation (6.15) and the outcome of these iterations is the corrected value \( x_{\text{ext}}^{i+1,\text{corr}} \).

To summarise the three steps, making up one continuation step, they are here written as an algorithm. The auxiliary algorithm, Newton(\( x, f, J, \text{acc} \)), means the Newton-Rhapson root-finding algorithm, for the function \( f \), with Jacobian \( J \), starting at \( x \), and with the solution, \( x^* \), satisfying \( |f(x)| < \text{acc} \).

Algorithm 6.1 One Continuation Step

Input: The starting parameter values of the chosen continuation parameter \( \mu_k^i \) as well as of the full parameter vector \( \mu^i \), corresponding fix point \( (x_f^i, \mu^i) \), step length \( h \) (including direction) and correction accuracy \( \text{acc} \).

1. Extending the system: \( x_{\text{ext}} = (x_f, \ldots x_{f,n}, p^i_k) \)
   \( \mu_{\text{dim}} = (\mu_1, \ldots, \mu_{k-1}, \mu_{k+1}, \ldots, \mu_r) \)

2. Calculating the predictor step: Solve

\[
\begin{pmatrix}
\frac{df}{dx_{\text{ext},1}} & \cdots & \frac{df}{dx_{\text{ext},n+1}} \\
0 & \cdots & 0
\end{pmatrix}
\begin{pmatrix}
x_{\text{ext},1} \\
\vdots \\
x_{\text{ext},n+1}
\end{pmatrix}
= \begin{pmatrix} 0 \\ \vdots \\ 1 \end{pmatrix}
\]

(6.18)

3. Taking the predictor step: \( x_{\text{ext,pred}} = x_{\text{ext}} + \Delta x_{\text{ext}} * h 
\)

4. Correcting: \( x_{\text{ext,corr}} = \text{Newton}(x_{\text{ext,pred}}, f(x_{\text{ext}}, p_{\text{dim}}), \nabla x_{\text{ext}}, f(x_{\text{ext}}, \mu_{\text{dim}}), \text{acc}) \)

5. return \( x_{\text{ext,corr}} \)
Figure 6.2  Plot of two continuations done when applying the new methods to the second sample in Section 6.2.4. First a projection down to the bifurcation manifold is done and then an optimization along the manifold is done. The correct optimum, just above the point (1,2), is found from all start guesses (cf. Figure 6.5).

Output: Parameter value $\mu_k^{i+1}$ after one continuation step and the corresponding fix point $(x_f^{i+1}, \mu_k^{i+1})$.

In Figure 6.2 a one-dimensional continuation plot is included as a first step in the algorithm depicted there. It is from the Brusselator example of 6.2.4, and the one-dimensional continuation line is the vertical line going from $(p_A, p_B) = (0.8, 1.8)$ down to $(p_A, p_B) = (0.8, 1.64)$, which is the bifurcating $p_B$ value for this $p_A$.

6.2.2 The first optimization method - by elimination

This section describes the first method to solve (6.14). First, it is explained in three steps, then the steps are combined into an algorithm, and finally, the properties of the method are shown.
Step I: Finding a feasible point

The way the elimination is done here relies on that the initial estimate, \( p^0 = (p_x^0, p_y^0, x_0^0, x_b^0) \), is on, or at least close to, the bifurcation manifold. This means that we want

\[
c^{\text{Hopf}}(p_x^0, x_b^0) = 0
\]  

(6.19)

If this should not be the case for the initial estimate, i.e., if it would be unfeasible, it would have to be projected onto the bifurcation manifold as described in Algorithm 6.1. When \( c^{\text{Hopf}} \) is sufficiently close to zero the exact point on the bifurcation manifold can be found by means of the Newton algorithm. Note that one could not do Newton iterations all the way since the function \( c^{\text{Hopf}} \) only has gradients pointing in towards the bifurcation manifold in closest vicinity of the manifold.

Step II: Reducing the search space

There are two variables to eliminate, \( x_b \) and a chosen parameter from \( p_x \), arbitrary called \( p_x,k \). Then these will be rewritten as functions of the remaining \( p_x \) parameters. Let the parameters be denoted

\[
p = (p_x,1, \ldots, p_x,k(p_x,\text{free}), \ldots, p_y, x_0, x_b(p_x,\text{free}))
\]

where

\[
p_x,\text{free} := (p_x,1, \ldots, p_x,k-1, p_x,k+1, \ldots)
\]  

(6.20)

and where the dependent parameter is chosen to fulfil the constraint (6.11) for each \( p_x,\text{free} \). In practice the dependent parameter \( p_x,k \) will be determined by an iterative process like, e.g., the Newton-Rhapson method. Let the eliminated variables be denoted

\[
p_{\text{elim}}(p_x,\text{free}) := (p_x,k(p_x,\text{free}), x_b(p_x,\text{free}))
\]  

(6.21)

Step III: Ordinary optimization in the reduced space

In this algorithm, the choice of ordinary iteration method in the reduced system is arbitrary. Once the reduction to the reduced space \( (p_x,\text{free}, p_y, x_0) \) has been done the problem is again an ordinary unconstrained optimization problem. In the algorithm, the Levenberg-Marquardt (LM) method has been chosen [92]. By putting all these three steps together the complete algorithm becomes.

Algorithm 6.2 EliminationSolution

Input: Initial estimate of all parameters, \( p^0 = (p_x^0, p_y^0, x_0^0, x_b^0) \), step length (sign gives direction), \( h \), parameter for projection, \( p_x,k \), tolerances \( \text{acc}_1 \), \( \text{acc}_2 \), \( \text{acc}_3 \), perturbation size, \( \text{pert} \).

A Initial projection:
6.2 Improved parameter estimation at a Hopf bifurcation - I

A1 if $c^{\text{Hopf}}(x^0_b, p^0_x) < \text{acc}_1$, then $(x^i_b, p^i_x, k) = (x^0_b, p^0_x)$, goto A4
A2 else $(x^i_b, p^i_x, k) = (x^0_b, p^0_x)$
A3 while $c^{\text{Hopf}}(x^i_b, p^i_x) > \text{acc}_1$ do $(x^{i+1}_b, p^{i+1}_x) = \text{OneContinuationStep}(x^i_b, p^i_x)$
A4 $(x^i_b, p^i_x, k) = \text{Newton}(x^i_b, p^i_x, c^{\text{Hopf}}, \text{NumPert}_{x,b}, p_{x,k} (c^{\text{Hopf}}, \text{pert}), \text{acc}_2)$
A5 $p^i = (p^0_{x,1}, \ldots, p^i_{x,k}, \ldots, p^0_y, x^0_b, x^i_b)$

B Eliminating the $n + 1$ parameters:

- $p = (p_{x,1}, \ldots, p_{x,k}(p_{x,\text{free}}), \ldots, p_y, x_0, x_b(p_{x,\text{free}})$
- $p_{x,\text{free}} := (p_{x,1}, \ldots, p_{x,k-1}, p_{x,k+1}, \ldots)$
- $p_{\text{elim}}(p_{x,\text{free}}) := (p_{x,k}(p_{x,\text{free}}), x_b(p_{x,\text{free}}))$
- where $p_{\text{elim}} = \text{Newton}(x_b, p_{x,k}, c^{\text{Hopf}}, \text{NumPert}_{x,b}(c^{\text{Hopf}}, \text{pert}), \text{acc}_2)$

determines $p_{\text{elim}}$ given $p_{x,\text{free}}$

C Ordinary optimization:

\[
\text{return: } p = \text{LM}([p^i_{x,\text{free}}, p_y, x_0], \text{acc}_3)
\]

Output: estimated parameter $\hat{p}$, where $c^{\text{Hopf}}(\hat{p}, \hat{x}_b) < \text{acc}$, and $V_N(\hat{p}|Z^N)$ has a local minimum, when constrained to the bifurcation manifold.

The two basic features of the method are stated in the following two theorems.

**Theorem 6.1** Soundness of method

If a point $(p_{x,*}, p_{y,*}, x_{0,*})$ is a minimum of the original estimation problem (6.2), and it is on the bifurcation manifold, it is also a minimum of the constrained optimization problem (6.14), given the same time series $Z^N$.

**Proof** Assume $p_* = (p_{x,*}, p_{y,*}, x_{0,*})$ is a minimum of the original estimation problem (6.2), that is also a Hopf bifurcation point. The argumentation in Section 6.2.1 showed that this point will also fulfill $c^{\text{Hopf}}(p_*) = 0$ of (6.14). That the original point is a minimum means that

\[
V_N((p_{x,*}, p_{y,*}, x_{0,*}) \leq V_N((p_{x,*}, p_{y,*}, x_{0,*}) + \delta p, Z^N) \text{ for all } \delta p < \epsilon \quad (6.22)
\]

and hence also the following relation will be fulfilled

\[
V_N((p_{x,*}, p_{y,*}, x_{0,*}) \leq V_N((p_{x,*}, p_{y,*}, x_{0,*}) + \delta p, Z^N) \text{ for all } \delta p < \epsilon
\]

where $\delta p$ are restricted by $c^{\text{Hopf}}(p_{x,*}, p_{y,*}, x_{0,*}) + \delta p) = 0 \quad (6.23)$

That means that the given point will be a minimum also to (6.14). □
**Remark:** Notice that even though the algorithm gives an answer that fulfills (6.14b) this does not mean that the parameter vector describes a Hopf bifurcation. This is because (6.14b) is only a necessary constraint, and the output of the algorithm could also be another non-hyperbolic point than a Hopf bifurcation point. A final check, with, e.g., a center manifold reduction, a transversality check, or a check with time series, for the output parameter $\hat{p}$, must hence be done. This means that one cannot guarantee that all local minima that are outside of the bifurcation manifold, will be eliminated as solutions, even though, for minima far away from the manifold, this will be the case. There might, however, also be added new local minima to the search space, due to the restriction.

**Theorem 6.2** Benefits of method
The Algorithm 6.2 converges to a minimum $(p_{x,s}, x_{b,s})$ on the surface $c^{\text{Hopf}} = 0$ with the same convergence as the method chosen under $C$ in the algorithm. The dimension that the method under $C$ has to work with is one less than the same algorithm would have had to work with when applied to the original non-constrained problem.

**Proof** In the original non-constrained problem $(p_x, p_y, x_0)$ are varied independently of each other. In Algorithm 6.2 only $(p_{x,\text{free}}, p_y, x_0)$ are considered independent of each other, and since $\dim(p_x) = \dim(p_{x,\text{free}}) + 1$, the degrees of freedom for search of minima have been reduced by one. \hfill \Box

### 6.2.3 The second optimization method - a reduced gradient method

The second optimization method presented is a reduced gradient method. Just as the first method this method also reduces the degrees of freedom with $n + 1$ but it does not do it with the same parameters all the time. On the contrary, it does it with all parameters, and differently for each step in the integration. This method will be introduced in the same way as the previous one was, i.e., first with a general description in words, then with the actual algorithm, and finally with statements and proofs of its properties. The initial projection is the same as before, but the rest of the method is taken from [41].

**Step I: Finding a feasible point**

Also this method needs to start at the bifurcation manifold and hence an initial projection has to be done. This is done in exactly the same way as for the previous method using a one dimensional continuation method and a Newton step.

**Step II: The extended Cauchy equation**

The extended Cauchy equation is written (see, e.g., [41]):

$$\frac{dp}{ds} = -[\nabla_p V_N(p, Z^N) + (\nabla_p c(p))^T w(p)] \quad (6.24)$$
Here we demand \( \frac{dc(p)}{ds} = 0 \) for all \( p(s) \) solved by (6.24). Hence

\[
\frac{dc(p)}{ds} = -\nabla_p c(p)[\nabla_p V_N(p, Z^N) + (\nabla_p c(p))^T w(p)] = 0 \quad (6.25)
\]

and if the matrix \( (\nabla_p c(p))(\nabla_p c(p))^T \) is invertible, the matrix \( w \) can be solved to:

\[
w(p) = -[(\nabla_p c(p))(\nabla_p c(p))^T]^{-1}(\nabla_p c(p)\nabla_p V_N(p, Z^N)) \quad (6.26)
\]

Putting this back into equation (6.24) gives

\[
\frac{dp}{ds} = -\nabla_p V_N(p, Z^N) + (\nabla_p c(p))^T[(\nabla_p c(p))(\nabla_p c(p))^T]^{-1}(\nabla_p c(p)\nabla_p V_N(p, Z^N)) = -M(p)\nabla_p V_N(p, Z^N) \quad (6.27)
\]

where the projection operator \( M \) is defined according to:

\[
M(p) = 1 - N(p) \quad (6.28)
\]

\[
N(p) = (\nabla_p c(p))^T[(\nabla_p c(p))(\nabla_p c(p))^T]^{-1}\nabla_p c(p) \quad (6.29)
\]

Putting step I and II together results in the following algorithm.

**Algorithm 6.3 Extended Cauchy**

*Input:* An initial estimate \( p^0 = (p^0_x, p^0_y, x^0_0, x^0_1) \), a continuation parameter \( p_{x,k} \), a step length (and direction) \( h \), tolerances and perturbations \( \text{acc}_1 \) and \( \text{pert}_1 \).

**A Initial projection:**

1. **A1** if \( c^{\text{Hopf}}(x^0_b, p^0_x) < \text{acc}_1 \), then \( (x^i_b, p^i_{x,k}) = (x^0_b, p^0_{x,k}) \), goto **A4**
2. **A2** else \( (x^i_b, p^i_{x,k}) = (x^0_b, p^0_{x,k}) \)
3. **A3** while \( c^{\text{Hopf}}(x^i_b, p^i_x) > \text{acc}_1 \) do (\( (x^i_b, p^i_{x,k}) = \text{OneContinuationStep}(x^i_b, p^i_{x,k}) \))
4. **A4** \( (x^i_b, p^i_{x,k}) = \text{Newton}([x^i_b, p^i_{x,k}], c^{\text{Hopf}}, \text{NumPert}_{x,b}, p_{x,k}, c^{\text{Hopf}}, \text{pert}, \text{acc}_2) \)
5. **A5** \( p^i = (p^i_x, \ldots, p^i_{x,k}, \ldots, p^i_y, x^i_0, x^i_1) \)

**B Solving the Cauchy problem:**

1. **B1** Calculate \( \nabla_p V_N(p, Z^N) = \text{NumPert}_x(V_N(p, Z^N), \text{pert}) \), and \( M(p) \) according to (6.28).
2. **B2** \( p^{i+1}_{\text{pred}} = \text{ODESolver}(p, \text{RHS}(p), \text{acc}_3) \)
3. **B3** \( p^{i+1}_{\text{corr}} = \text{Newton}(p^{i+1}_{\text{pred}}, c^{\text{Hopf}}, \text{NumPert}_{p_{x,ext}}(c^{\text{Hopf}}, \text{pert}), \text{acc}_2) \)
4. **B4** if \( |V_N(p^{i+1}, Z^N) - V_N(p^i, Z^N)| > \text{acc}_4 \) then \( p^i = p^{i+1} \) goto **B1**
Output: An estimated state $\hat{p}$ where $c^{\text{Hopf}}(\hat{p}) < \alpha c_2$

Consider the following set

$$
\Omega_1 := \{ p \in \mathbb{R}^1; V_N(p, Z^N) \leq V_N(p^0, Z^N), \quad c(p) = 0 \} 
$$

(6.30)

Then a convergence theorem can be stated as

**Theorem 6.3 Convergence**

Let the functions defining the problem (6.14) be differentiable on an open set containing $\Omega_1$, where $c(p) = 0$ satisfies the constraint qualifications; let the local minimum of $V_N(p, Z^N)$ be attained on $\Omega_1$ at the unique point $x_*$. Then the solutions of the system (6.27) converge to the point $x_*$ as $s \to \infty$.

**Proof** See [41].

The second theorem tells what the convergence rate is:

**Theorem 6.4 Convergence rate**

The convergence rate of solving a corresponding Cauchy problem, like (6.27), by means of ordinary integration, is linear.

**Proof** See [41].

**Remark:** Hence it might be advisable to end the iterations with a few steps with an ordinary quasi-Newton method.

### 6.2.4 Identification of the Brusselator

We will exemplify the advantage of the method on a system usually referred to as the Brusselator. It consists of two chemicals. Let $x_1$ and $x_2$ denote their concentrations. Let the two parameters governing their dynamics be denoted $p_A$ and $p_B$. Let the sensor noise be denoted $n \sim \mathcal{N}(0, \sigma)$, and assume that the sensor is measuring the variable $x_2$. Then the Brusselator system is formulated in the form (2.4) as [123]:

\begin{align*}
\dot{x}_1 &= p_A + x_1^2 x_2 - p_B x_1 - x_1 
\tag{6.31a} \\
\dot{x}_2 &= p_B x_1 - x_1^2 x_2 
\tag{6.31b} \\
y &= x_2 + n 
\tag{6.31c}
\end{align*}

Their exists analytical expressions for the asymptotic properties of this system. For all parameter combinations there is a fix point at the location $\{x_1, x_2\} = \{p_A, p_B/p_A\}$. When $p_B > 1 + p_A^2$ the fix point is unstable, and when $p_B < 1 + p_A^2$. The fix point becomes unstable in a supercritical Hopf bifurcation, which means that the asymptotic behaviour is either a steady state, or stationary oscillations.
Assume that it is possible to slowly control $p_A$, and $p_B$, but that their absolute values are not yet known. The task is to estimate $p_A$ and $p_B$. Assume that the following two samples have been collected.

**Sample 1:** Collected just before the bifurcation ($(p_A, p_B) = (1, 1.99)$. The time series is shown in the upper plot in Figure 6.3. Initial values $(x_1(0), x_2(0)) = (1, 1.99)$

**Sample 2:** Collected just after the bifurcation ($(p_A, p_B) = (1, 2.01)$. The time series is shown in the lower plot in Figure 6.3. Initial values $(x_1(0), x_2(0)) = (1.00, 1.88)$.

To generate the time series the Runge-Kutta fourth order method has been used with time-step $h = 0.1$. The variance of the noise was $\sigma = 0.1$. Now we will see how well an optimization method performs with and without adding the constraint $c^{\text{Hopf}}$.

\[\begin{array}{c}
\text{Figure 6.3 The first 50 seconds of the time series in Sample 1 (upper) and 2 (lower).}
\end{array}\]
Results before adding $c^{\text{Hopf}}$

We will study the effect of adding the new constraint on the convergence regions, but not on other performance measures like the convergence rates etc. Finding the convergence regions is equivalent to finding the basins of attraction for the correct minimum, when studying the Cauchy formulation of (6.2). With sufficiently short iteration steps this determination will not depend on the optimization method. The equivalent Cauchy formulation is studied, and it is solved with a fourth order Runge-Kutta method. In terms of the original formulation (6.2) this is equivalent to a steepest descent method. The integrator is embedded in a trust-region method to ensure that it does not take too long time-steps.

![Convergence process](image)

**Figure 6.4** The typical behaviour of an estimation process before adding $c^{\text{Hopf}}$.

**Sample 1:** Figure 6.4 shows a typical convergence process. Since there is no dynamics included in this sample, only the fix point value of $x_2$ is measured. Since this value is known to be equal to the fraction $p_B/p_A$, and since both these values are assumed unknown, only the correct fraction can be estimated. For Sample 1 this is equal to 1.99, and as can be seen in Figure 6.4 the algorithm converges
Figure 6.5 The points to which the optimization process has converged before adding $c^{\text{Hopf}}$. As can be seen there are a number of points that have converged to the correct value, $(p_A, p_B) = (1, 2.01)$. It can, however, also clearly be seen that the estimates $p_A$ and $p_B$ are correlated. When adding the constraint $c^{\text{Hopf}}$ all the estimates are correct.

quickly to a fraction value that is very close to the correct one. It then keeps varying $p_A$ and $p_B$ but keeps the fraction constant. Since the true value of $p_A$ is 1, it can also be seen that the algorithm brings $p_A$ in the wrong direction.

Sample 2: Here there is dynamics included in the time-series, although not any transients. Therefore more information than the correct fraction $p_B/p_A$ can be estimated. However, as can be seen in Figure 6.5, it is only the estimations that have started in the vicinity of the correct value $(p_A, p_B) = (1, 2.01)$, that will converge to the global minimum. Initial estimates further away will estimate the fraction $p_A/p_B$ correctly, had the time-series been a steady state. This is understandable when looking at the objective function in Figure 6.6; it has a clear valley corresponding to the correct fraction, and only less distinct global minimum in this valley.
Figure 6.6 The objective function for the second sample. Here the correct value of $x_1(0)$ has been used when calculating the objective function. As can be seen there is a valley along the line $p_B/p_A = 2$, i.e., the same relation as in Sample 1 is still dominant. Now, however, there is also a small global minimum appearing in the valley, and therefore the correct value is obtained for the starting estimates close to the correct one (see Figure 6.5).

Results after adding $c^{Hopf}$

When adding the new parameters $x_b$ together with the new constraint $c^{Hopf}$ and solving the problem with one of the two suggested methods, a typical convergence process looks like the one in Figure 6.2. As can be seen the initial estimate is first projected down to the bifurcation manifold ($p_B = 1+p_A^2$) and then the optimization process carries on, staying on the manifold. In Figure 6.7 the objective function on the bifurcation curve is plotted. As can be seen it is easy to find the only minimum, and therefore all points converged to the same minimum, the correct one. When comparing this result with the uncertain estimation in Figure 6.5, we conclude that the optimization processes have been improved. When comparing Figure 6.7 with Figure 6.6 we understand that the improvement is due to the reduced degree of freedom in the search space.
Objective function $V$ on the bifurcation curve, plotted against the parameter $p_A$. Since there is only one degree of freedom left $p_A$ uniquely determines the objective function. When comparing this objective function with the one obtained without using $c^{\text{Hopf}}$ (see Figure 6.6) one sees that the $c^{\text{Hopf}}$ constraint does indeed simplify the problem.

Summary of Section 6.2

System identification of nonlinear ODEs is a difficult problem. One of the difficulties comes from the local minima in the objective function. This and other problems are simplified if one can reduce the search space. Here have been shown two methods that allow for a reduction in the degrees of freedom describing the $p_x$ parameters, i.e., the parameters appearing in the differential equation (2.4a). The methods are applicable for systems known to operate close to a supercritical Hopf bifurcation. The main idea is to introduce $n$ new parameters $x_b$ describing the fix-point corresponding to the current estimate of the $p_x$ parameters. Then one can introduce $n + 1$ new constraints, $c^{\text{Hopf}}$, and the net result is that one degree of freedom has been reduced. The price for the reduction is that one has to use more advanced optimization methods. The two presented methods are two standard constrained optimization methods: one elimination method and one reduced gradient method. The former has the advantage of translating the problem back to an unconstrained optimization problem, and therefore allowing the user to choose
an appropriate method for the transformed problem. The second method has the advantage of not forcing the user to choose a specific parameter to eliminate. The advantage of the new methods has been shown on the Brusselator.

6.3 Elimination of the initial value parameters when estimating a system close to a Hopf bifurcation

In the previous section we saw how the experimental determination of the location of a Hopf bifurcation can be used to reduce dimension of the \( p_x \) parameters, i.e., those parameters appearing in the differential equations. We will now turn our attention to the \( x_0 \) parameters, i.e., those parameters specifying the initial state \( x(0) \). This \( n \)-dimensional parameter vector will be re-parametrized in two local parameters, using a normal form transformation. These local parameters will then be solved for in an optimization sub-problem, and we will show how this can be done in a straightforward way without the problems with local minima, and without having to numerically integrate the differential equations. Again the advantage of the method will be demonstrated on the Brusselator.

6.3.1 Formulation of the optimization problem

**Parametrizing \( x_0 \) through \( z_1 \) and \( p_\mu \)**

To understand the common idea of the methods presented, first recall the normal form transformation of a Hopf bifurcation. It says that a point, \( x_z \), in the real space, but on the center manifold, can be described as (cf. equation (2.20))

\[
x = x_b + z + \tilde{h}(z_1, p_\mu)
\]

where \( z = z_1 u + z_1 \mathbf{z} \) is a real vector in the center space and \( p_\mu = \mu - \mu_b \) where \( (x_b, \mu_b) \) is the bifurcation point in the state-parameter space. Finally \( \tilde{h} \) is the vector between the center space and the center manifold (see Figure 6.8). That means that if the system is known (or chosen, through the experiment design) to be operating on the center manifold at the time \( t = t_0 \), the initial state parameter vector \( x_0 \) can be parametrized as follows

\[
x_0(p_x, x_b, z_1, p_\mu) = x_b + z + \tilde{h}(z_1, p_\mu)
\]

where \( (x_b, p_x, u(t_{start})) \) would be at the bifurcation point, and \( \mu = (p_x, u(t_{start})) + p_\mu \) would be the real (time varying) value of \( \mu \).

\[\text{The reader that is new to center manifold theory and normal form reductions is referred to, e.g., [135], [88] or [16], and a short review with an example of the calculations involved in a normal form transformation is also given in the appendix.}\]
Dimensionality of the parametrization

In equation (6.33) $x_0$ have been parametrized by four different parameter vectors. Two of these, $x_b$ and $p_x$, are already estimated in each step by the methods described in the previous section, and hence they do not add new complexity, if the new methods are based on the methods of the previous section. The other two, $z_1$ and $p_\mu$, are not a part of the estimation processes described in the previous chapter, and hence they come in as new parameters. Let us now look at the dimension of these two new parameter vectors. For a Hopf bifurcation, $z_1$ is always a complex scalar, and for the one-dimensional bifurcations $z_1$ is a real scalar. The vector $p_\mu$ on the other hand has, in general, the full dimension of $s$, but as we shall see below, experiments can often be designed so that $p_\mu$ can be chosen as one-dimensional. In any case, if $s + 2 \leq n$, the parameter $x_0$ have more dimensions than the new parameters that are needed to parametrize it. Hence it is a reduction in degrees of freedom to exchange $x_0$ for $z_1$ and $p_\mu$, according to (6.33).

Optimization problems caused by straight-forward use of $z_1$ and $p_\mu$

The major problem in utilising this parametrization right away is that there is not a continuous mapping from $x_b$ and $\mu_b$ to $x_0$, i.e., for an infinitesimal change in $x_b$ or $\mu_b$, but with $z_1$ and $p_\mu$ kept still, the change in $x_0(p_x, x_b, z_1, p_\mu)$ will not always be infinitesimal. This problem comes from the fact that the center manifold transformation (6.33) is only a local transformation and that a new transformation is done for each bifurcation point $(x_b, \mu_b)$. This is a problem since the transformation is not unique. For the same bifurcation point, e.g., two different choices of eigenvectors $u$ and $\overline{u}$, in the center manifold transformation, give rise to two different parametrizations of the center manifold, and hence to two different values of $x_0$ given the same $z_1$ and $p_\mu$. Now follows a description of how to come around this problem, and still utilise (6.33) to parametrize $x_0$ in the optimization processes.

The initial value optimization sub-problem

Even though $x_0(p_x, x_b, z_1, p_\mu)$ is not a uniquely determined vector, but is determined only after the choice of parametrization, the center manifold, and the dynamics on it does not depend on the choice of parametrization. The center manifold lies in the original physical space $\mathbb{R}^n$, and the dynamics is determined by the original equation (2.4a). The center manifold reduction (6.32) is just a way of describing this dynamics, and even though the descriptions may vary, the dynamics will be unique. This can be compared to describing a circle in different coordinate systems. The vector $(r, \theta, \phi)$ is not a well-defined vector until one also has determined the coordinate axis, but independently of the choice of coordinate axis, the circle in the real space will always be the same. This means that a minimisation of the objective function over all points on the center manifold

$$\min_{z_1, p_\mu} V(x_0(p_x, x_b, z_1, p_\mu)) \quad (6.34)$$

also leads to a unique value, i.e., to a result independent of the parametrization. The idea is therefore to solve the sub-problem (6.34), over the two parameters
Figure 6.8 The mapping from the center space, where $z = \sum_i z_i u_i$ lies, to the center manifold where the asymptotic dynamics of $x$ occurs.

$z_1 \in \mathbb{C}$ and $p_{\mu} \in \mathbb{R}$, to determine $x_0(p_x, x_b, z_1, p_{\mu})$ in (6.33), for each value of the parameters, $p_x$, $x_b$ and $p_y$. We now summarise this idea in two steps as follows.

1 Replace $x_0$ from being a freely varied parameter to an auxiliary determined by the two parameters $z_1$ and $p_{\mu}$ according to

$$x_0(p_x, x_b, z_1(p_x, p_y, x_b), p_{\mu}(p_x, p_y, x_b)) = x_b + z(p_x, p_y, x_b) + h(z_1(p_x, p_y, x_b), p_{\mu}(p_x, p_y, x_b)), \quad (6.35)$$

2 The two parameters $z_1$ and $p_{\mu}$ are determined from the parameters $p_x, p_y$ and $x_b$ according to:

$$z_1(p_x, p_y, x_b) = \arg \min_{z_1, p_{\mu}} V_N(p_x, p_y, x_0(p_x, x_b, z_1, p_{\mu}), Z^N) \quad (6.36a)$$

$$p_{\mu}(p_x, p_y, x_b) = \arg \min_{p_{\mu}, z_1, p_{\mu}} V_N(p_x, p_y, x_0(p_x, x_b, z_1, p_{\mu}), Z^N) \quad (6.36b)$$

### 6.3.2 The complete algorithm

In this section the idea presented in Section 6.3.1 will be written out as an algorithm, and the basic properties of the method will be shown. In Section 6.2 $x_b$ was determined in two different ways, first through elimination and then through
a reduced gradient method. Here we will also have use of the $x_b$ parameter, and we will determine it by the first of these methods, the elimination method. The algorithm of this section will work with the most general case studied above, and in the next sections we will go to a special case. For the general case the estimated parameters, $\hat{p}$, are found as those solving

$$\hat{p} = \arg \min_{p} V_N(p, Z^N)$$

(6.37)

where

$$p = (p_{x,free}, p_{x,elim}, p_y, x_0(p_{x,free}, p_y, x_b), p_{free}(p_{x,free}))$$

(6.38)

and where (cf Section 6.2.2):

$$p_{x,free} := (p_{x,1}, \ldots, p_{x,k-1}, p_{x,k+1}, \ldots)$$

(6.39)

$$p_{elim}(p_{x,free}) := (p_{x,k}(p_{x,free}), p_b(p_{x,free}))$$

(6.40)

and, by recalling (6.36)

$$x_0(p_{x}, p_y, x_b) := x_b + z(p_{x}, p_y, x_b) +$$

$$+ h(z_1(p_{x}, p_y, x_b), p_{free}(p_{x}, p_y, x_b))$$

(6.41a)

$$z_1(p_{x}, p_y, x_b) := \arg \min_{z_1} V(p_{x}, p_y, x_0(p_{x}, x_b, z_1, p_{mu}), Z^N)$$

(6.41b)

$$p_{mu}(p_{x}, p_y, x_b) := \arg \min_{p_{mu}} V_N(p_{x}, p_y, x_0(p_{x}, x_b, z_1, p_{mu}), Z^N)$$

(6.41c)

The elimination method has the benefit of calling an ordinary optimization method, for the eliminated system, and hence it does not limit the user to using only one type of optimization methods, or to one rate of convergence etc.

Since elimination methods calls an ordinary optimization method for the optimization in the reduced space, the most important thing to specify is how to calculate the full parameter vector, given only the parameters that are considered free to vary. Here the only parameters that are considered independent of each other are $p_{x,free}$ and $p_y$, and given previous estimates of the dependent parameters, $z_{1,0}$, $p_{mu}$ and $p_{elim}$, the dependent parameters will be estimated in the following way:

**Algorithm 6.4** Fullp($p_{x,free}, p_y, p_{elim}^i, z_{1,0}^i, p_{mu}^i, \text{acc}$)

*Input: The freely varied parameters $p_{x,free}$ and $p_y$, initial estimates of the dependent parameters $p_{elim}^i, z_{1,0}^i$, and $p_{mu}^i$, acceptances acc*

1. $p_{elim} = \text{Newton}(p_{elim}^i, e^{\text{Hopf}}, \text{NumPert}_p(p_{elim}^i, e^{\text{Hopf}}, \text{pert}), \text{acc})$

2. $\hat{z}_{1,0}, \hat{p}_{mu} = \text{LM}([z_{1,0}^i, p_{mu}^i]), V_N([p_{x}, p_y, x_0(p_{x}, x_b, z_1, p_{mu})], Z^N), \nabla_{z_{1,0}, p_{mu}} V_N, \text{acc}$

where $x_0(p_{x}, x_b, z_1, p_{mu})$ is decided according to (6.41).

3. Return: $x_0(p_{x}, x_b, \hat{z}_{1,0}, \hat{p}_{mu})$ according to (6.41).
Output: The parameters \( p_{\text{elim}}(p_{\text{x,free}}) \), and \( x_0(p_x, p_y, x_b) \) calculated according to (6.40) to (6.41).

Since the full parameter set \( p \) can hence be determined by the freely varied parameters \( p_{\text{x,free}} \) and \( p_y \) only, \( p \) will for the rest of this subsection be denoted \( p[p_{\text{x,free}}, p_y] \). Hence the objective function can be written \( V_N(p[p_{\text{x,free}}, p_y], Z^N) \) and the following main algorithm will look as follows.

**Algorithm 6.5**

Input: A time series \( Z^N \) collected when the system has, at \( t = 0 \) been operating close to a Hopf bifurcation, a model structure of the form (2.4), initial estimates of the needed parameters \( p_x^{(0)}, p_y, p_{\text{elim}}, z_1 \) and \( p_{\text{pert}}^0 \), acceptances and perturbation sizes, \( \text{acc}_1 \) and \( \text{pert}_1 \).

A Initial projection:

A1 if \( e^{\text{Hopf}}(x_b^0, p_{x}^0) < \text{acc}_1 \), then \( (x_b^i, p_{x,k}^i) = (x_b^0, p_{x,k}^0) \), goto A4
A2 else \( (x_b^i, p_{x,k}^i) = (x_b^0, p_{x,k}^0) \)
A3 while \( e^{\text{Hopf}}(x_b^i, p_{x,k}^i) > \text{acc}_1 \) do \( (x_b^{i+1}, p_{x,k}^{i+1}) = \text{OneContinuationStep}(x_b^i, p_{x,k}^i) \)
A4 \( (x_b^i, p_{x,k}^i) = \text{Newton}(x_b^i, p_{x,k}^i, e^{\text{Hopf}}, p_{x,k}^i, \text{NumPert}_{x_b, p_{x,k}}(e^{\text{Hopf}}, \text{pert}_1), \text{acc}_2) \)
A5 \( p^i = (p_{x,1}^0, \ldots, p_{x,k}^i, \ldots, p_y^0, x_b^0, x_b^i) \)

B Ordinary optimization:

B1 \( (\hat{p}_{x,\text{free}}, \hat{p}_y) = \text{LM}(p_{x,\text{free}}, p_y, V_N(p[p_{x,\text{free}}, p_y]), \text{NumPert}_{x, \text{free}, p_y}(p[p_{x,\text{free}}, p_y], \text{pert}_2), \text{acc}) \)
B2 \( \hat{p} = \text{Fullp}(\hat{p}_{x,\text{free}}, \hat{p}_y, p_{\text{elim}}, z_{1,0}^j, p_{\text{pert}}^j, \text{acc}_3) \), where \( p_{\text{elim}}^j, z_{1,0}^j, p_{\text{pert}}^j \) are obtained from the last step of the LM iterations.
B3 Return \( \hat{p} \)

Output: An estimated parameter \( \hat{p} \), fulfilling \( e^{\text{Hopf}}(\hat{x}_b, \hat{p}_x) < \text{acc}_2 \).

Remark: The \( \mu \) value at each point in time will be \( \mu(t) = (\hat{p}_x, u(t)) + \hat{p}_\mu \).

The soundness of the method is given by the following theorem.

**Theorem 6.5 Soundness**

For time series collected sufficiently close to the bifurcation point, and on the center manifold, the minimum of (6.37) coincides with the minimum of (2.15).

**Proof** That the system operates sufficiently close to the manifold means that the error from approximating the center manifold with an expansion will not be significant. The minima will coincide if

\[
\min_{u,v} f(u,v) = \min_{u} \min_{v} f(u(v), v)
\]

and this is one of the basic results of optimization theory [92].
The benefit of the method is most apparent if the time series have been collected by an experiment of the following type.

**Definition 6.1** By a bifurcation experiment we will mean an experiment where the system at \( t = 0 \) is at a point from where the system can come to the Hopf bifurcation manifold by varying one parameter \( \mu_b \) only. Further the distance from the bifurcation manifold is so small that the center manifold approximation is valid.

The benefit of the method can then be described by the following theorem.

**Theorem 6.6** Benefit
Assume an experiment of the type described in definition 6.1 has given rise to the time series \( Z^N \). Then the algorithm 6.5 will reduce the degrees of freedom describing the \( x_0 \) parameters from \( n \), which is the dimension of the state equations, to 3.

**Proof** Equation (6.41a) gives the relationship. When a bifurcation experiment, as described in Definition 6.1 has been done, \( \mu, \) in equation (6.41a) can be chosen as \( \mu = (0, \ldots, \mu, 0, \ldots) \), i.e., it can be described by one degree of freedom only. Since \( z_1 \) will always be two-dimensional, the total degrees of freedom describing \( x_0 \) in (6.41a) will be 3.

### 6.3.3 Simplifying the initial value sub-problem

For the data used in [62], the initial state was not only on the center manifold, but in the stationary oscillations on the manifold. The bifurcation point could also be reached by small variations of a single known \( u \) (for instance \([\text{Glc}]_x\)). For such cases the optimization sub-problem in step 2 in Algorithm 6.4

\[
\min_{z_1, \mu} V(x_0(p_x, x_0, z_1, \mu))
\]

can be further simplified. This is done in two steps, first by parametrizing \( z_1 \) in \( \mu \) and an additional parameter \( \alpha \), and then by utilising the interpretation of these two parameters when performing the optimization.

### Formulation in minimal degrees of freedom

For quenching data the initial state vector \( x(0) \) lies not only on the center manifold, but in the stationary limit cycle. Let us now restate the dynamic equation, in its normal form, on this manifold and also its explicit solution. Let \( z_1 \in \mathbb{C} \) denote the first center space coordinate, \( \omega_0 \in \mathbb{R} \) the imaginary part of the first critical eigenvalue, and let \( \sigma_1 \in \mathbb{C} \) and \( g_3 \in \mathbb{C} \) be the two complex (scalar) resonant terms. Finally let \( p_\mu \in \mathbb{R} \) be the distance from the bifurcation point in the single control parameter that has been varied in the quenching experiment. Then the dynamics on the center manifold can (with only up to third order terms included) be described by

\[
\dot{z}_1 = (\omega_0 i + p_\mu \sigma_1)z_1 + g_3|z_1|^2z_1
\]  \[
(6.42)
\]
Now let $R \in \mathbb{C}$ and $\alpha \in \mathbb{R}$. An analytical solution to (6.42), describing the stationary behaviour, is given by

$$z_1(t) = \begin{cases} 0 & \text{if } p_\mu \sigma_1 \leq 0 \\ R \exp(i(\omega_1 t + \alpha)) & \text{if } p_\mu \sigma_1 > 0 \end{cases}$$

where $R$, $\omega_1$ and $\alpha$ are determined by

$$0 = p_\mu \sigma_1 + \text{Re}(g_3)|R|^2 \quad (6.43a)$$

$$\omega_1 = \omega_0 + \text{Im}(g_3)|R|^2 \quad (6.43b)$$

$$z_1(0) = R \exp(i\alpha) \quad (6.43c)$$

By deriving the expression for $z_1(t)$ with respect to time, and substituting the result in equation (6.42) one sees that these expression fulfills the dynamics.

Let $Z^{\text{stat}}$ denote the initial stationary part of the oscillations, i.e., if $t^{\text{stat}}$ denotes the time the stationarity ends, then

$$Z^{\text{stat}} = (u(t), y(t))_{t=0}^{t^{\text{stat}}}$$ (6.44)

From equation (6.43c), (2.4b) and equation (6.32) the simulated correspondence to $Z^{\text{stat}}$ can be generated as follows

$$y = y(x, \mu, p_y) \quad (6.45a)$$

$$x = x_b + z + h(z_1, p_\mu) \quad t = 0, \ldots, t^{\text{stat}} \quad (6.45b)$$

$$z_1 = R \exp(i(\omega_1 t + \alpha)) \quad (6.45c)$$

$$0 = p_\mu \sigma_1 + \text{Re}(g_3)|R|^2 \quad (6.45d)$$

$$\omega_1 = \omega_0 + \text{Im}(g_3)|R|^2 \quad (6.45e)$$

and where $p_\mu$ and $\alpha$ are still to be determined. Notice that this means that we can calculate $\tilde{y}(t)$ (in (6.1)) for the whole interval $t = 0, \ldots, t^{\text{stat}}$. Notice also that we only have to compute those rows in equation (6.32) that are needed to form the $x_b$s that can be measured. Loosely we can say that the dynamics is calculated, in the two-dimensional space where it occurs, and then projected to only those directions that are measured. Specifically, we can do this projection for the time $t = t_0 = 0$. This means that we can simplify (6.41) into

$$x_0(p_x, p_y, x_b) := x_b + z(p_x, p_y, x_b) +$$

$$+ h(z_1(p_x, p_y, x_b), p_\mu(p_x, p_y, x_b))$$ (6.46a)

$$z_1(p_x, p_y, x_b) = R(p_x, p_y, x_b) \exp(i\alpha(p_x, p_y, x_b))$$ (6.46b)

$$R(p_x, p_y, x_b) := \arg \min_{R, p_\mu, \alpha} V_N(p_x, p_y, x_0(p_x, x_b, \alpha, p_\mu), Z^{\text{stat}})$$ (6.46c)

$$\alpha(p_x, p_y, x_b) := \arg \min_{\alpha, p_\mu, \alpha} V_N(p_x, p_y, x_0(p_x, x_b, \alpha, p_\mu), Z^{\text{stat}})$$ (6.46d)

$$p_\mu(p_x, p_y, x_b) := \arg \min_{p_\mu, p_\mu, \alpha} V_N(p_x, p_y, x_0(p_x, x_b, \alpha, p_\mu), Z^{\text{stat}})$$ (6.46e)

Notice that the difference between equation (6.41) and (6.46) is that we now only have to minimise over two degrees of freedom, $p_\mu$ and $\alpha$. Previously it was three
degrees of freedom, $p_\mu$ and $z_1$. The two degrees of freedom, $p_\mu$ and $\alpha$, corresponds to two clearly interpretable quantities, the amplitude and the phase, and this can be utilised when solving the optimization problem. Let us now see how this can be done.

The optimization

When solving the optimization sub-problem (6.46c) to (6.46e) we can solve it through an ordinary optimization algorithm, like, e.g., the Levenberg-Marquardt method used earlier. Here, however, we know the analytical solution (equation (6.45c)), and the interpretation of the two degrees of freedom ($R$ is the amplitude and $\alpha$ is the phase) in the normal form equation, and this can be utilised to perform the optimization easier. Other reasons are the desire to have more control of what is going on, and to avoid the problem of getting stuck in local minima.

We will assume that we have determined the amplitude of the oscillations, $A_y$, and the a specific maximum time, $t_{\text{max}}$, from the time-series of measured of one of the measured variables (see Figure 6.9). As can be seen in Figure 6.9, the steady state oscillations in the complex plane are nice, but the oscillations in the physical space are more complex. It is, however, assumed that there is a unique maximum in the measured oscillations, considering one period only. It should also be added that as one approaches the bifurcation, from the unstable side, both the amplitude and the complexity of the oscillations in the measured variable decreases. This comes from the fact that for small $z$, i.e., for small distances from the bifurcation point,
(\(p_x, x_b\)), only the linear terms in the transformation \(h\) will be dominant. This is also the reason why the amplitude will grow monotonically as \(p_\mu\) is increased. These two properties are the reason why \(\alpha\) and \(R\) (in equation (6.43c)) can be determined from the amplitude \(A_y\) and the time-point \(t_{\text{max}}\) by the following simple algorithm.

**Algorithm 6.6** Input: The amplitude \(A_y\), the time at which the measured variable is at its maximum, \(t_{\text{max}}\), the necessary row in the \(h\) function, increments \(\Delta p_\mu\) and \(\Delta \alpha\)

\[
p_\mu = 0, A(p_\mu) = 0
\]

while \(A(p_\mu) < A_y\)

\[
\alpha = 0, y(\alpha) = 0, y_{\text{min}} = \infty, y_{\text{max}} = 0
\]

while \(\alpha \in (0, 2\pi)\)

if \(y(\alpha, p_\mu, t_{\text{max}}) < y_{\text{min}}\)

\(y_{\text{min}} = y(\alpha), \alpha_{\text{min}} = \alpha\)

if \(y(\alpha, p_\mu, t_{\text{max}}) > y_{\text{max}}\)

\(y_{\text{max}} = y(\alpha), \alpha_{\text{max}} = \alpha\)

\(\alpha = \alpha + \Delta \alpha\)

end while

\(A(p_\mu) = y_{\text{max}} - y_{\text{min}}\)

\(p_\mu = p_\mu + \Delta p_\mu\)

end while

\bullet \text{return: } R(p_x, p_y, x_b) = R(p_\mu), \alpha = \alpha_{\text{max}}, p_\mu(p_x, p_y, x_b) = p_\mu

Output: Values of \(R(p_x, p_y, x_b), \alpha(p_x, p_y, x_b)\) and \(p_\mu(p_x, p_y, x_b)\) according to (6.46c) to (6.46e).

**Remark:** Note that there are only two degrees of freedom for the three parameters \(R, p_\mu\) and \(\alpha\). In this algorithm this was solved by only varying \(p_\mu\) and \(\alpha\), and determine \(R(p_\mu)\) according to (6.43a).

The idea behind the algorithm is to utilise that the amplitude grows monotonically as the distance \(p_\mu\) increases. The amplitude for each \(p_\mu\) is simply determined by going through all angles \(\alpha\) and detecting the min and max values, measured in \(y\), and finally by taking the difference between the two. When this is done for increasing distances \(p_\mu\) the amplitude will increase until it eventually hits the measured amplitude. This algorithm is simple and has a clearly defined stopping criterion.

### 6.3.4 Finding the initial values in the Brusselator

Let us now go back to the Brusselator example from Section 6.2.4. In that example two different time-series were collected: one one the stable side of the bifurcation (Sample 1), and one on the unstable (Sample 2). Both of the time series were close to the bifurcation, and this information was shown to be sufficient to determine the correct parameters \((p_A, p_B) \sim (1, 2)\), in both cases. However, in both cases...
the time series were treated as stationary time series, which means that the initial value parameters could be treated by solving \( f(x_0, p_x, u) = 0 \) for each value of the \( p_x \) parameters. That means that there were not estimated any corresponding initial values for Sample 2, and this problem we turn to now. The problem is solved by Algorithm 6.6. By inspection of Sample 2, the measured amplitude, \( A_y \), in Algorithm 6.6 was estimated as \( A_y = 0.3270 \). The value of \( t_{\text{max}} \), in the same algorithm, was estimated as \( t_{\text{max}} = 2.6s \). The estimation of the parameters \( p_x \) and \( x_b \) done earlier gave \( (p_x, x_b) = (1, 2, 1, 2) \), and the normal form transformation around this bifurcation point is explained in the appendix. The relationship between the distance, \( p_u \) from the bifurcation point, and the amplitude of the oscillations, is given in Figure 6.10. As can be seen the amplitude grows monotonically with the distance beyond the interesting region, and the assumption behind Algorithm 6.6 is thus valid. In Figure 6.10 can further be seen that the normal form approximation for distances around the correct distance \( (p_u = 0.01) \) is a good approximation, even though the error eventually increases. The correct distance value was estimated with an accuracy of \( 1E - 4 \) (which was the step length \( \Delta p_u \) ), and the phase \( \alpha \) was estimated to 3.79 rad. The final agreement is shown in Figure 6.11. As can be seen the agreement between the estimated and measured output is good already from the beginning of the time series. The correct initial values have thus been estimated.

\[\text{Figure 6.10} \quad \text{The amplitude of the stationary oscillations as a function of the distance from the bifurcation point. The plot is taken from the problem on the Brusselator described in Section 6.3.4. That the amplitude is growing monotonically with the distance is, however, a generic feature of the Hopf bifurcation. This is the feature utilised in Algorithm 6.6.}\]
Figure 6.11 Plot showing the agreement between the estimated system and the original time series for the second sample introduced in Section 6.2.4. The initial parameters were estimated by Algorithm 6.6.

6.4 Summary of Chapter 6

In this chapter we have dealt with step 4 in the core-box modelling framework, the system identification step. The model structure and data have already been characterised, and the main part of the step is therefore to estimate the parameters, and to estimate the uncertainty with which the different parts of the core model are estimated. We reviewed the state-of-the-art methods for answering these questions in Section 6.1.

The next two sections presented new methods that make use of knowledge concerning a Hopf bifurcation, a common feature in many biological systems. Section 6.2 presented a reduction of the space of the dynamic parameters \( p_x \), and it is valid for all systems where the Hopf bifurcation has been experimentally located. The main idea is to reformulate the additional knowledge in the ordinary constrained estimation problem (6.14). This requires the introduction of \( n \) additional parameters, \( x_b \), but the new constraints are \( n + 1 \), and the net result is therefore a reduction with one degree of freedom in the search space. We presented two classical methods that can work with the reformulated problem, and illustrated the advantage of the suggested approach on an estimation example of the Brusselator.

In Section 6.3 we reported a new method for handling the \( x_0 \) parameters, i.e., those parameters that describes the initial state of the simulation. We have generalised the simple method of starting the system in a steady state to time series starting on an arbitrarily place on the center manifold surrounding a Hopf bifurcation. This is done by reparametrization of \( x_0 \) in minimal degrees of freedom, \( z_1 \) and \( p_\mu \), using equation (6.33). There are problems associated with straightforward usage of the new parameters \( z_1 \) and \( p_\mu \). These have been overcome by solving the
initial value optimization sub-problem (6.36), for each step in the \((p_x, p_y)\) optimization. There exists published data that starts not only on the center manifold, but in stationary oscillations. For systems starting in stationary oscillations the optimization sub-problem may be solved in a straightforward way without integration, and without the problem of local minima. This is possible because of a combination of the center manifold and normal form reduction, which reveals the special structure of the Hopf bifurcation.
The final step in the core-box modelling framework is the back-translation to the core-box model, which is then ready to be analysed. The core-box model is based on the already existing full-scale mechanistic grey-box model and the already existing core model with estimated features and quality tags. The most central aspect of the back-translation step is to back-translate the results from the estimation of the core model to the grey-box model, in particular the most well-estimated features and the quality tags. However, we will see that it is sometimes possible to achieve a complete interchangeability between the core and the grey-box model. If this is possible the two models may actually be treated as two versions of the same model that one changes between by simply 'zooming in' or 'zooming out'. Such a fully integrated core-box model is also advantageous in many other applications than those for which the core-box modelling framework has originally been developed. One such example is hierarchical modelling, i.e., the modelling of systems of systems. Finally, all results in this chapter are new contributions.

In Section 7.1 we look at the general sub-problems involved in the back-translation step, and at some general methods that might be used for the translation between an arbitrary core and grey-box model developed for the same system. Then we look at some special cases where improved solutions are possible. In Section 7.2 we look at two cases involving structural identifiability, the first when it is due to conserved moieties, and the second when symmetry families may be calculated. Then, in Section 7.3, we see how back-translation may be treated for the maybe two most useful reduction techniques in the core-box modelling framework: sensitivity analysis and variable lumping.
7.1 General methods and sub-problems

The major sub-steps involved in the back-translation problem were described in Chapter 3.6, although without introducing any formal notations. Some notation for the central transformations were then introduced in Chapter 4.3, although in a slightly different context: that of comparing the information in the in vivo data with the previous knowledge from the in vitro characterisations. This problem is a special case of the back-translation step, and the other main option is to obtain a merging of the two sources of information. The back-translation step is here described as consisting of four different sub-steps: the first two are involved with the back-translation and merging of the features and quality tags, and the second two are involved with the presentation and analysis of the final core-box model.

7.1.1 Translating the core model behaviour

We now assume that both a core and grey-box model exist for the given system. The model structure for the grey-box model is denoted $M_g$, and it is described by equation (2.4) as it reads. The model structure for the core model is denoted $M_c$, and it is described by the same equations, but where all parameters and states are attached with a prime ($'$). That means that the state space representation of the core model is

$$
\dot{x}'(t) = f'(x'(t), u'(t)) = f'(x'(t), p'_x, u(t)) \quad (7.1a)
$$
$$
y(t) = h'(x'(t), p'_x, u(t), p'_y) \quad (7.1b)
$$
$$
x'(t_0) = x'_0 \quad (7.1c)
$$

and that all parameters are collected in the parameter vector $p'$. Note that the inputs $u$ and the outputs $y$ are not assigned with a prime. The reason why this is not necessary is that the main concern in the development of the core model is its identifiability with respect to the given inputs and outputs, and that these signals are the same for both the models. In some cases (like in Chapter 4.3) the states will be the same in the core and the grey-box model. If this is evident from the situation the prime on the states of the core model will be dropped, i.e., then we write $x$ instead of $x'$.

There are two transformations that are involved in the back-translation step: $\phi$ and $\Psi$. The $\phi$ transformation maps from $M_g$ to $M_c$ and the $\Psi$ transformation from $M_c$ to $M_g$.

$$
\phi : M_g \rightarrow M_c
$$
$$
\Psi : M_c \rightarrow M_g
$$

The mappings symbolises all the content in the model structures, including all states, auxiliary functions etc. In practice, however, we mainly need to be concerned with the mappings of the parameters. The reason for this is that the model structures are autonomous except for a known input function $u(t)$ that is the same in both the models. Therefore, since $p$ includes both $p_x$, $p_y$, and $x_0$, the time-varying signals $x(t)$ and $y(t)$ are uniquely given by $p$ for all $t$. For this reason, we will primarily be concerned with the effect of $\phi$ and $\Psi$ on the parameters.
A first step towards a back-translation is obtained by a mere interpretation of
the forward translation $\phi$. Parts of this interpretation is often immediate because
both $\mathcal{M}_g$ and $\mathcal{M}_c$ are ideally formulated in a biochemically meaningful way, even
though $\mathcal{M}_c$ might not always be the result of a direct reduction of $\mathcal{M}_g$. If $\mathcal{M}_c$
should be the result of such a direct reduction, additional information is typically
available through explicit transformation formulas obtained during the reduction.
This is the case also if identification of the appropriate model reduction scheme is
done after $\mathcal{M}_c$ have been developed.

Methods are also available for the general case, i.e., if $\phi$ may not be deduced
through a prior or posterior model reduction. One such method was proposed in
Chapter 4.3.2. This method is a three-step approach. The first step is to generate
data from $\mathcal{M}_g$. This data is denoted $Z^N(p)$ since it is dependent on $p$. The second
step is to use this data as a basis for a parameter estimation of $p'$. In this way one
may determine an indirect mapping $\phi$ according to

$$\phi : p \rightarrow Z^N(p) \rightarrow p'$$

(7.2)

This may be done for all core models that are practically identifiable with respect
to the original data. However, the actual applicability of this approach extends to
a much wider range of core models than that. The reason for this is that the grey-
box model may generate data without noise and for many more parts of $\mathcal{M}_c$
that the original data set $Z^N$ (all parts that have an interpretation in $\mathcal{M}_g$). As long as
data sets may be generated at the given operating point that make $p'$ identifiable,
the method is applicable.

The last step in the method proposed in Chapter 4.3.2 is to obtain an ana-
lytical expression for the mapping $\phi$. This is done by the creation of data sets
$\{p(i), \phi(p)(i)\}$ that are used as a basis for a novel modelling round. Also this data
set is more informative than an experimental data set. This allows for improved
methods to be applied during, e.g., the estimation phase (see Chapter 6.1). An-
other improvement that is possible because of the high quality of the data is the
possibility of generating a Taylor expansion around the estimated $\bar{p}$. The first
terms in such an expansion reads

$$\phi(p) = \phi(\bar{p}) + \nabla_p \phi(\bar{p}) \cdot (p - \bar{p}) + (p - \bar{p})^T \cdot \nabla^2_p \phi(\bar{p}) \cdot (p - \bar{p}) + \ldots$$

(7.3)

where $\nabla_p$ represent the differential operator, which is obtained through numerical
perturbation. This Taylor expansion may be used to suggest model structures, e.g.,
by identifying those parameters $p_j$ that do not appear in the mapping to a specific
parameter $p'_i$. Another information that is easy to draw from a Taylor expansion
is the possible vanishing of second and higher order terms. Such a vanishing indi-
cates that linear model structures are sufficient in those parts of the relation. The
calculated Taylor series could also be compared to known series in Taylor expan-
sion tables. The Taylor expansion approach is illustrated in Examples 4.5 and 7.1.
Finally, the Taylor expansion could also, by itself, be used as an example to symbolically
represent the mapping $\phi$.

A further step towards a complete back-translation than just interpreting the
results of the core model in terms of the entities in the grey-box model is obtained
by an adaption of the behaviour of the grey-box model so that it agrees with
the behaviour of the core model. Such a back-translation generally involves the characterisation of $\Psi$. However, one might also here get away with a simulation based approach, i.e., without the determination of an analytical expression for $\Psi$. One might, e.g., use the same approach as that described above for the numerical characterisation of $\phi$. One then generates data for the (well-characterised) behaviours in the core model, and uses this data as input for a new optimization of the grey-box model. Again it should be pointed out that such an optimization is unusually straightforward, since the data is of a high quality, and since one can often divide the problem into optimization sub-problems. Even though such an optimization would generally not give a unique result, it would cause the grey-box model to behave like the core model, and thus be an improvement compared to just interpreting the core model behaviour in the grey-box model’s parameters and variables.

Since the numerically determined mapping $\Psi$ is not unique, it is not continuous and certainly not differentiable. There does therefore not exist a Taylor-expansion approach to the analytical characterisation of $\Psi$, like the one described for $\phi$ above. One may, on the other hand, make use of the analytical description for $\phi$. A simple inversion of this expression gives a possible formula, and such inversions may be done in symbolic toolboxes such as Mathematica and MAPLE.

Independently of how an analytical solution to $\Psi$ is obtained, it will involve choices, and the chosen expression is therefore not unique. This is a reflection of the fact that the choice of $p$ is not uniquely determined by $p'$. To formalise these choices we introduce design variables. Let these design variables be collected in the vector $\eta$. The addition of these design variables should make the mapping from $p'$ to $p$ unique, and that means that the dimension of $\eta$ is at least $r-r'$, where $r'$ is the dimension of $p'$. Further, we also require that the design variables should be $\phi$ invariant, i.e., that

$$p' = \phi(\Psi(p', \eta)) \text{ for all } \eta \in \mathcal{N} \subset \mathbb{R}^{r-r'}$$

(7.4)

where $\mathcal{N}$ is the space of allowed design values. In the case of an identifiable core model, the design variables are spanning those directions in the parameter space about which there is no information in the in vivo data (at least from a local perspective, see Section 7.1.2 below). They therefore correspond to a parametrization of the set determined by

$$\{p : B(p) = 0\}$$

(7.5)

where $B(p)$ is the singularity function introduced in Section 4.3.1.

The design variables $\eta$ may also be used as a way to save information about the grey-box model to allow for a back-translation where all parts that have not been estimated by the data are the same as before. To acknowledge such a $p$-dependence in the choice of $\eta$, we write $\eta(p)$. We then assume that $\phi$ is responsible for both these two types of mappings, i.e., that

$$\phi : p \rightarrow (p', \eta) \text{ where } p = \Psi(\phi(p), \eta(p))$$

(7.6)

If we choose $\eta(p)$ in this way it would also be good to require that the mappings $\Psi$ and $\phi$ are continuous and differentiable. If all these requirements are fulfilled
we might do small changes in \( p' \) without it affecting the back-translated value of \( p \) too much compared to the original value. The same holds for changes in \( p \) with respect to \( p' \). That means that one would then have two models that are so interconnected that they could be replaced for each other whenever needed, without loosing the changes one has made. The two models could therefore be considered as two different aspects of the same model, with the only difference being the degree of 'zooming'. Such a fully integrated core-box model would be highly beneficial as a module in a hierarchical model, and would extend the current goals of having libraries of models describing the same system. Let us now, finally, return to the example we introduced in Chapter 3 and perform this first substep of the back-translation again, using the presented techniques and notations.

**Example 7.1** The example in Chapter 3 consists of one state \( x \) and three parameters \( p_1, p_2 \) and \( p_3 \), which together describes the following differential equation

\[
\dot{x} = (p_1 + p_2)x + p_3 \tag{7.7}
\]

There are two types of unidentifiabilities in the example. The first is structural unidentifiability of \( p_1 \) and \( p_2 \), and the second is practical unidentifiability of \( p_3 \). The core model eliminates both of them and is formulated as

\[
\dot{x} = p'x \tag{7.8}
\]

and the associated reduction mapping is easily found to be

\[
\Phi := p \rightarrow p' = \Phi(p) = (p_1 + p_2) \tag{7.9}
\]

However, even if this would not have been found already by inspection, it could have indirectly been deduced from the numerically deduced mapping (7.2). Numeric differentiation of this mapping with respect to the three parameters gives

\[
\frac{\partial \Phi(p)}{\partial p_1} = 1 = \frac{\partial \Phi(p)}{\partial p_2}; \quad \frac{\partial \Phi(p)}{\partial p_3} \approx 0 = \frac{\partial^2 \Phi(p)}{\partial p_1^2} = \frac{\partial^2 \Phi(p)}{\partial p_2^2} = \ldots \tag{7.10}
\]

These Taylor expansion coefficients together with a fitting of the numerically determined curve would gives the same formula for \( \Phi \). A unique back-translation is obtained by the saving of the value of one of the parameters \( p_1 \) or \( p_2 \), and the value of \( p_3 \). Let these values be stored as design variables \( \eta_1 \) and \( \eta_2 \), respectively. The back-translation formula is then given by

\[
\Psi := (p', \eta) \rightarrow (\eta_1(p_1), p' - \eta_1(p_1), \eta_2(p_3)) \tag{7.11}
\]

where the \( \eta \) mappings are equal to the unity mappings, i.e., \( \eta(p_1) = p_1 \). Note that this back-translation formula fulfils both the memory condition (7.6) and the associated continuity and differentiability conditions. This core-box model may therefore be viewed as a fully integrated model where changes are saved when changing from one degree of 'zooming' to another.
Figure 7.1 The uncertainty regions associated with the back-translation step. The left shapes correspond to the original parameters $p$, and the right to the reduced parameters $p'$. The rectangle to the left corresponds to $\Delta_{p_{ivt}}$, and the ellipse to the right corresponds to $\Delta_{p'}$. It is the intersection between the back-translated version of the ellipse, $\Psi(\Delta_{p'}, N)$, and $\Delta_{p_{ivt}}$ that gives the lowest uncertainty. See also Tables 7.1 and 7.2.

7.1.2 Merging the obtained uncertainties

Both $p$ and $p'$ are associated with several types of uncertainties, all of different origins. When presenting and analysing a core-box model it is highly beneficial to account for these different types of uncertainties. These uncertainties are reflected in quality tags associated to the various model predictions. These quality tags should therefore give information not only on the extent of the uncertainty, but also account for the extent to which each of the different sources have contributed to the given degree of certainty. We will now account for how these different sources of certainty may be formalised, characterised, and possibly also merged, and in the next sub-section we will suggest how the results may be presented.

The first uncertainty region that is characterised is that from the *in vitro* estimations, and from all other sources from which the original grey-box model is put together (see Chapter 2). We denote the parameters that are taken from such previous knowledge by $p_{ivt}$, and the associated uncertainty region is denoted by $\Delta_{p_{ivt}}$ (which should be interpreted such that $p_{ivt} \in \Delta_{p_{ivt}}$). This uncertainty region is depicted as the left rectangle in Figure 7.1. Note that this uncertainty region might be very big in some directions, e.g., for those parameters where no previous quantifications have been made.

The region $\Delta_{p_{ivt}}$ is the only region that is completely independent of the *in vivo* data; all the other uncertainty regions have included information from the *in vivo*...
data, in different ways, and to various degrees. The first parameter that involves some such information is denoted \( \hat{p} \), and it is associated with an uncertainty region denoted \( \Delta_{\hat{p}} \). The \( \hat{p} \) parameter is obtained by optimization of the full grey-box model with respect to the *in vivo* data. It would also typically be required that the estimate lies within \( \Delta_{\hat{p},ivt} \), at least if this is possible. An uncertainty region following this optimization is not possible to obtain using the classical sensitivity analysis based methods described in Chapter 4.1 and 6.1. The reason for this is that the associated FIM matrix is singular, something which is equivalent to an infinite uncertainty. However, one might still obtain information, but of a more non-specific nature. This is obtained by variations of some of the parameters to fixed but different values. If this makes it possible/impossible to obtain an acceptable agreement with the data one can draw corresponding conclusions about the possibility that these parameters may have these values. This is illustrated in Example 7.2 below. The region \( \Delta_{\hat{p}} \) is obtained as the remainder of \( \Delta_{p,ivt} \) after all such upper and lower limits have been cut away.

The region \( \Delta_{\hat{p}} \) may be projected to the reduced space \( \Omega' \), where \( p \) lies, using the mapping \( \phi \). If this mapping is done before the system identification step of the core model is performed, the mapped space \( \phi(\Delta_{\hat{p}}) \) may be used to limit the search during this optimization. The mapping \( \phi \) might also be used to obtain a reasonable start estimate for the optimization; simply use the image of \( \hat{p} \). It might even be so that this image gives an optimal solution directly, i.e., that \( \hat{p}' = \phi(\hat{p}) \).

In any case, the main point of reducing the grey-box model is to identify those aspects of the model that may be estimated from the data, and to achieve quality tags describing the degree of certainty by which these estimations may be done. Methods to do this was described in Chapter 4.1. The result is often a confidence ellipsoid, but is in any case a subspace denoted \( \Delta_{\hat{p},0} \subset \Omega' \).

The uncertainty region \( \Delta_{\hat{p},0} \), is exclusively dependent on the information in the *in vivo* data. The interpretation of the uncertainty region, however, is also dependent on the transformations \( \phi \) and \( \Psi \), and these are dependent on assumptions made in the grey-box model, i.e., on the *in vitro* data (and on the global features estimated from the *in vivo* data). Therefore, as long as one wants to make statements about the grey-box model, both types of data have to be incorporated. In any case, the two types of data may be utilised in two qualitatively different ways. One is to compare their information content, and one is to merge them. The comparison approach was taken in Chapter 4.3 and the corresponding measures are

\[
\min_{p,p'} \| \phi(p) - p' \| \quad \text{where } \quad p \in \Delta_{p,ivt}, \ p' \in \Delta_{\hat{p},0} \tag{7.12}
\]

or

\[
\min_{p,p',\eta} \| p - \Psi(p',\eta) \| \quad \text{where } \quad p \in \Delta_{p,ivt}, \ p' \in \Delta_{\hat{p},0}, \ \eta \in \mathcal{N} \tag{7.13}
\]

depending on whether the comparison is done in \( \Omega' \) or in \( \Omega \), respectively. The comparison approach has many advantages, but if a maximally informative model is the goal, the best option is to merge all the available information into a core-box model. The uncertainty of the merged parameters are given by

\[
\Delta_{\hat{p}} \cap \Psi(\Delta_{\hat{p},0},\mathcal{N}) \tag{7.14}
\]
where $\cap$ denotes the intersection of the two parameter regions. Note that such a calculation would generally also involve global searches, just like (7.13). Note also that the space $\mathcal{N}$ is only restricted by the global estimations from the \textit{in vivo} data, and from the assumptions justifying the model reduction. The detailed uncertainty region $\Delta_{p_{\text{ivt}}}$ is therefore not used to characterise $\mathcal{N}$.

The result of (7.14) is the uncertainty of $\hat{p}$ that fully incorporates both the \textit{in vivo} data and the \textit{in vitro} characterisations. Note that this set is empty if (7.13) yields a non-zero distance. Note also that the corresponding merging in the reduced space

$$\phi(\Delta_{\hat{p}}) \cap \Delta_{\hat{p}'}$$

also gives important information, and where the uncertainties generally are much smaller. Note that the merging does not necessarily lead to an improved quality tag. The condition for such an improvement is given by the following equation

$$\Psi(\Delta_{\hat{p}'}, \mathcal{N}) \setminus \Delta_{\hat{p}} \neq \emptyset$$

(7.16)

where \setminus is the minus sign for sets and the symbol $\emptyset$ represents the empty set. If this condition is not fulfilled, the addition of the \textit{in vitro} information does not give any additional knowledge. Then one may instead just work with the set $\Psi(\Delta_{\hat{p}'}, \mathcal{N})$. The corresponding condition for an improvement caused by the estimations in the core model is given by

$$\Delta_{\hat{p}} \setminus \Psi(\Delta_{\hat{p}'}, \mathcal{N}) \neq \emptyset$$

(7.17)

Let us finally give a warning to a lack of generality in the above expressions. As is evident from the little example in the very end of Chapter 4.3.1, the reduced model $\mathcal{M}_c$ might be dependent on the choice of $\hat{p}$, a choice about which there exists no information in the \textit{in vivo} data. That means that both the mappings $\Psi$ and $\phi$, and hence also the design variables $\eta$ and the corresponding space $\mathcal{N}'$, are dependent on $\hat{p}$, and we should therefore generally write, e.g., $\mathcal{N}(\hat{p})$ instead of $\mathcal{N}$. This means that one should, generally, perform the minimization in (7.13) also over all acceptable estimates $\hat{p}$. If such a minimization of $\hat{p}$ is necessary it is of course more easy to do all calculations in the $\Omega$ space. However, one can often drop the minimization over $\hat{p}$. This should typically be the case, e.g., for reductions based on the assumption that the time-scale in a given reduction is faster than another, or that the flux in one reaction is considerably lower than others effecting the same output, i.e., for the two types of methods described in Section 7.3. One should, however, remember that the results are dependent on the validity of these assumptions. It is therefore important to account for all the assumptions that are involved in the development of the core-box model.

\textbf{Example 7.2} Let us now illustrate the determination of these different uncertainty regions on our little example. The uncertainty of the \textit{in vitro} parameters were given as $p_{\text{ivt}} = (3 \pm 0.2, -0.02 \pm 0.01)$. The next region was not described in Chapter 3 and it is the global requirements that may be put on the parameters. In this case the requirements are that $p_1$ and $p_2$ are both less than 5, and that $p_3$ is less than 0.5. If any of these conditions are violated, the other parameters may
not compensate for that (assuming that all parameters are positive). This information is less restrictive than $p^{\text{ivt}}$ for $p_1$ and $p_3$ even though it does give some first restrictions on the possible $p_2$ values. However, the most important contribution of such a global search is that it gives in vivo based knowledge. The optimization of the parameters do not provide any additional uncertainty information, and the optimization in the reduced space yields $\hat{p}' = 4.48 \pm 0.3$. The back-translation of this directly do not give any additional information above those given by the global search, since the assumptions that make the reduction valid only requires that $p_1x$ or $p_2x$ are much bigger than $p_3$. However, when the merging of the two sources of information is performed according to (7.14) the $p_2$ parameter receives the reduced region $[1.48 \pm 0.5]$. All these regions are presented in Tables 7.1 and 7.2.

7.1.3 The final core-box model

We now suggest how the final core-box model may be presented. As mentioned above, it is essential to account for all the assumptions made in the derivations. Apart from this one must, of course, also present the model structures for the core and grey-box models, just as for an ordinary model. We, however, suggest that one for a core-box model also forms a special table. This table should include detailed information about all the models parameters, and their associated uncertainties. As explained above, specifications of the parameters implies a unique specification of all the features of the model. However, derivation of the translation from the results regarding the parameters to results in specific model features often requires additional analysis. We shortly discuss such analyses in Section 7.1.4.

The core-box model consists of both a core part and a grey-box part. We suggest that the parameters for these two models are included in two separate tables. Two such tables are exemplified in Tables 7.1 and 7.2. Table 7.1 corresponds to the parameters in the grey-box model, and the first column in this table is simply giving the name of the parameter. The second column has the heading $p^{\text{ivt}}$ and it gives the in vitro estimated values and their associated uncertainties. Remember that we also include results from literature searches and simple order of magnitude reasoning in such characterisations. The next column is entitled 'Global search' and it corresponds to information in the in vivo data extracted already before the reduction to the core model is done. Such information is typically only giving upper or lower limits for the parameters, and might often not provide additional information to the $p^{\text{ivt}}$ uncertainties. It is, however, important to specify it separately since it is information from data of a fundamentally different kind. The next column is the uncertainties around the $\hat{p}$ values. This column often gives the same uncertainty regions as the $p^{\text{ivt}}$ column, and the major difference is therefore that the actual parameter values give an optimal agreement with the in vivo data. The next column gives the back-translated uncertainty regions from the results of the estimation on the core model, $\Psi(\Delta_{p'}^{c},\mathcal{N})$. The column after this is the last column with parameter uncertainty regions. This column gives the results of a merging of all the information from all types of data, and is entitled $\Delta_{p^{cb}}$. This should be the most informative and restricted column, and probably the one to use in a standard analysis. However, the previous columns are important too, because they specify the different types of information that have been combined to
form the $\Delta_{p\text{cb}}$ region. It might for instance be interesting to know what one can conclude even if all the used \textit{in vitro} estimates would be inapplicable. The two final columns specify the reduced parameters $p_i^*$ and the design parameters $\eta_j$ that the given $p_j$ is mapped to.

The other type of table is illustrated in Table 7.2, and it is concerned with the parameters in the core model, $p'$. However, these parameters are typically known combinations of the $p_i$s, described by the mapping $\phi$. Therefore, if $\phi$ is known analytically, Table 7.2 also provides information about these parameter combinations in the $p$-space. Note that such information was not provided in Table 7.1. The first column in Table 7.2 gives the name of the parameter. The second column gives the mapped uncertainty $\phi(\Delta_{p\text{ivt}})$ and the third column the mapped uncertainty $\phi(\Delta_{p\text{cb}})$. Note that the we also here may have that $\phi(\Delta_{p\text{cb}}) \neq p^{\text{ivt}}$ even though $\Delta_{p} = \Delta_{p^{\text{ivt}}}$. The fourth column gives the estimated value and uncertainty region obtained from the estimation and analysis of the core model only. The fifth column gives the mapped uncertainty region $\Delta_{p\text{cb}}$. It is this column that give the highest constraints on the parameter combinations in the $p$-space. The last three columns are concerned with the role of the given $p_i^*$ in the mappings $\phi$ and $\Psi$. The first of them gives the formula $\phi_i(p)$ to obtain the parameter, the second column gives the original parameters that are directly effected by the back-translated value of $p'$, and the last column gives all those relations that directly or indirectly involve $p_i^*$, e.g., in uncertainty calculations.

### 7.1.4 Analysis of the core-box model

When the final core-box model is obtained and presented it is ready to be analysed and used in different contexts. There are many methods for analysis, and we have presented some of them in Chapter 2.5.2. The major advantage compared
to the analyses that can be done on the previous models concerns the knowledge about the uncertainties on the various parts of the model. These uncertainty regions should be incorporated in the analysis, to obtain predictions with quality tags. If the analysis involves analytical calculations, the uncertainties can easily be included in these calculations, and if the analysis involves simulation based predictions, corresponding results with an uncertainty tag may, for instance, be obtained by performing the simulations for all the extreme values of the calculated parameter uncertainties. It is outside the scope of this dissertation to go into detail in how specific methods may be generalised to include uncertainty calculations, even though some examples are provided in Part II. An illustration of the benefits when analysing a fully characterised core-box model is also provided in the following example.

**Example 7.3** Assume that one wants to test the possible effects of a drug that lowers the $p_2$ parameter by 50%. It is not possible to test this using only the in vitro characterisations, since there is no suggestion for $p_{2}^{\text{ivt}}$. A result that is based on the in vivo data are the results from the global search. That information alone shows that the maximal reduction of the total decay time for the system (given by $p_1 + p_2$) is <50%. It, however, does not give a lower value. This information is purely based on the in vivo data, and this is important when considering that prediction. By also incorporating the in vitro information one can make the more precise prediction that the reduction is between 10 and 24%. This more precise prediction, however, is based on the belief that the in vitro estimation is accurate (and all predictions are, of course, based on the assumption that the model structure is correct). Finally, the core model alone may not be used, since it does not provide any information on the parameter $p_2$, and the original grey-box model may also not be beneficially used, since it gives a prediction with an infinite uncertainty. Already this simple example therefore clearly shows the much more detailed information that is provided by the core-box model when creating and evaluating model predictions.

### 7.2 Structural unidentifiability

We now turn to the handling of the back-translation step in some specific cases. In this section we consider the special case of structural non-identifiability. These methods would seldom be sufficient by themselves, since few systems have only structural unidentifiabilities. However, there are some fairly well-developed theories for the study of structural unidentifiability (see the review in Chapter 4), and some of these methodologies are closely related to the back-translation problem. One such theory is the derivation of symmetry families, and this is reviewed in Section 7.2.2. First, however, we return to the theory that we developed in Chapter 4.2.
7.2.1 Unidentifiability in single rate expressions due to conserved moieties

In Chapter 4.2 we studied a specific type of unidentifiability in single rate expressions, one due to conserved moieties. The phenomenon had probably been noticed by some scientists, but there did not seem to exist a thorough analysis of why it occurred, or how it could be handled. We solved both these problems in Chapter 4.2.

The analysis was centred around a number of transformations of the original rate expression \( v(x, k) \) in equation (4.20) to \( v'(\bar{x}, \bar{c}') \) in (4.28). The central steps in this transformation are the reformulation to a rational expression \( v(x, c) \), the transformation to another rational expression with only independent variables \( \bar{v}(\bar{x}, \bar{c}) \), and the final elimination of a single coefficient to obtain \( v'(\bar{x}, \bar{c}') \).

Let us now reformulate these transformations into the formalism developed in Section 7.1. The above series of transformations from \( v \) to \( v' \) correspond to the transformation \( \Phi \) from \( p \) to \( p' \) where \( k \) corresponds to \( p \) and \( \bar{c}' \) corresponds to \( p' \) (remember that we write \( k \) instead of \( p \) when we want to emphasise that all parameters \( p \) belong to a given rate expression). We will combine these transformations into a single formula for \( \Phi \). Let \( c_i(p_x) \) denote the \( i \)th coefficient in \( v(x, c) \), where the argument \( p_x \) is included to show that it is only dependent on the kinetic parameters \( p_x \). The coefficients \( c(p_x) \) are then mapped to the new coefficients \( \bar{c} \) through the transformation matrix \( C \). This matrix depends on the concentration \( m \) of the conserved moieties in equation (4.25). Since the concentration of the moiety is constant in time, it is equal to the concentration of the moiety at \( t \), and thus purely a function of \( x_0 \). To symbolise this dependency we write \( C(x_0) \). Let \( C_i \) denote the \( i \)th row in \( C \), and assume that this row corresponds to the identifiable parameter \( p^*_i \). Assume, finally, that the normalisation from \( c \) to \( \bar{c}' \) is done by division with the coefficient \( c_j \). We may then write the entire transformation \( \Phi_1 \) from \( p \) to the identifiable parameter (or coefficient) \( \bar{c}'_i \) in one formula as

\[
\Phi_1 := p \rightarrow p'_i = \bar{c}'_i = \Phi_1(p) = \frac{C_i(x_0) \cdot c(p_x)}{C_j(x_0) \cdot c(p_x)}
\]  \hspace{1cm} (7.18)

where \( \cdot \) denotes the scalar product between two vectors.

The derivation of the forward translation \( \Phi \) involves the scalar product and the analysis of the rank of the matrix \( C \). Further analysis of this matrix gives the null space \( \text{ker}(C) \). The null space gives all the directions in which the solution to \( \bar{c} = Cc \) is undetermined. These directions are therefore also directions in which the back-translation is undetermined, i.e., where design parameters are necessary. We might therefore use an analysis of the \( C \) matrix also when characterising the back-translation \( \Psi \). The first step in the back-translation is from \( \bar{c}' \) to \( \bar{c} \). This is done by multiplying with the factor \( c_j(p_x) \) that was divided away when \( \bar{c}' \) was derived. This step may easily be reversed if the factor \( c_j(p_x) \) is saved in the forward transformation \( \Phi \), i.e., if \( c_j(p_x) \) is a design parameter with memory. We denote this design parameter \( \eta^j \). Note that this first little substep is a back-translation of an unidentifiability problem of its own. This type of unidentifiability (a common factor in the numerator and denominator) may therefore be reversed in a way that allows for back-translation with memory (i.e., that fulfils (7.6)).
The next step in the back-translation requires the solution to the equation \( \vec{c} = C \vec{c} \). A solution to this equation is given by \( \vec{c} = \vec{c}^p + \sum_i \eta_i \vec{u}_{n,i} \) (7.19), where \( \vec{u}_{n,i} \) is the \( i \)th vector spanning the null space \( \ker(C) \), and \( \eta_i \) is the corresponding design variable. There is one additional design parameter needed, and this is due to the fact that \( \vec{c} \) is not identifiable since it contains a coefficient in front of every term in the rate expression. This unidentifiability requires yet another design parameter \( \eta_0 \). We therefore have the following back-translation \( \Psi \) from \( p' \) to \( c \)

\[
\Psi^{p' \rightarrow c} := (p', \eta) \rightarrow c = \eta_0 \left( \begin{array}{c} C^{-1} (p'_1 \eta_1, \ldots, p_{j-1} \eta_j, \ldots, p_r \eta_r) + \sum_i \eta_i \vec{u}_{n,i} \end{array} \right)
\]

Note that the memory requirement (7.6) is not fulfilled for this formulation unless further conditions are added to the formula. This is primarily due to the fact that the particular solution \( \vec{c}^p \) is obtained by the pseudo inverse. Nevertheless, the characterisation of the singular directions is greatly simplified because of the linear property of the transformation, characterised by the matrix \( C \). Note that this linearity is not associated to the linearisation of a nonlinear relation, but a true linearity of an algebraic transformation. The validity of the transformations is therefore global. Finally, if the full back-translation to the original parameters is required, the nonlinear mapping \( p \rightarrow c(p) \) needs to be inverted. Since this is a nonlinear mapping, the characterisation of the singular directions etc is much more complicated, and general results for this step is outside the scope of this dissertation. Finally, let us consider the back-translation of the little example we used to illustrate the other steps of the transformations in Chapter 4.2.

**Example 7.4** The linear transformation equation \( \vec{c} = C \vec{c} \) is for Example 4.2 in Chapter 4.2 given by

\[
\begin{pmatrix} \bar{a}_1 \\ \bar{b}_1 \\ \bar{b}_2 \end{pmatrix} = \vec{c} = C \vec{c} = \begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & m \\ 0 & 0 & 1 & -1 \end{pmatrix} \begin{pmatrix} a_1 \\ b_1 \\ b_2 \\ b_3 \end{pmatrix}
\]

where \( m \) is the concentration of the conserved moiety \( x_1 + x_2 \). The null space to the \( C \) matrix in (7.21) is spanned by the single eigenvector \( \vec{u}_{n,1} = (0, -m, 1, 1)^T \). Therefore, the back-translation from a particular estimate \( \hat{p}' \) to \( c \) is given by equa-
tion (7.20) as

\[
c = \Psi(\hat{p}', \eta) = \eta_0 \begin{pmatrix}
1 & 0 & \frac{\eta}{m} \\
0 & 2 + \frac{\eta}{m} & \frac{\eta}{2 + \frac{\eta}{m}} \\
0 & \frac{\eta}{2 + \frac{\eta}{m}} & -\frac{\eta}{2 + \frac{\eta}{m}}
\end{pmatrix} \cdot \begin{pmatrix}
\hat{p}_1' \eta(\hat{b}_2) \\
\hat{p}_2' \eta(\hat{b}_2) \\
\eta(\hat{b}_2)
\end{pmatrix} + \eta_1 \begin{pmatrix}
0 \\
-m \\
1
\end{pmatrix}
\]

(7.22)

Note that we could find the pseudo-inverse to the \(C\) matrix symbolically. Finally, the original nonlinear transformation \(p \rightarrow c(p)\) is here simply given by

\[
(V_{\text{max}}, K_S, K_P) \rightarrow (a_1, b_1, b_2, b_3) = \begin{pmatrix}
V_{\text{max}}, K_S, 1, \frac{K_S}{K_P}
\end{pmatrix}
\]

which may also be easily inverted analytically. Note that the full back-translation all the way to \(p\) has one design parameter less, since \(\text{dim}(\tilde{c}) = \text{dim}(p) + 1\).

7.2.2 Utilising symmetry families

The back-translation problem for a general structurally unidentifiable nonlinear model is much harder than if some special structure may be utilised. There are, however, developed frameworks that contain theory that might be used also for the general case. A concept that is closely related to the back-translation problem is denoted symmetry families. These families are associated with a mapping \(l_f\) from \(p\) back to itself. These mappings are actually groups. Groups typically appears as the set of all mappings that preserve a given property, and so also this time: the property that is preserved is the output function \(b(x)p\). A mapping is often parametrized by one or several parameters \(\lambda_f\), which means that we may introduce a symmetry mapping \(l_f\) as

\[
l_f := p \rightarrow p(\lambda_f) \quad \text{where} \quad \hat{y}(x|p) = \hat{y}(x|p(\lambda_f)) \quad \text{for all} \ \lambda_f, x
\]

(7.23)

A symmetry family consists of all parameters \(p(\lambda_f)\) that lie on the same manifold spanned by the symmetry parameters \(\lambda_f\). Since the symmetry parameters leave the outputs invariant, they are clearly unidentifiable. In fact, the symmetry parameters completely span the structurally unidentifiable manifolds. Note that this is true for the nonlinear system globally, i.e., without approximations or linearisations.

There is a developed theory for the analysis and characterisation of these symmetry families. There does not seem, yet, to exist a public implementation of the calculation of these families that returns an answer in a reasonable time for a realistically large system. Unofficial implementations, on the other hand, seem to exist [115]. We can therefore expect to be able to determine the symmetry families for structurally unidentifiable systems in some near future.

All this means that symmetry families is a well-developed theory that may soon be used in practice for realistically sized systems. What makes this interesting for the back-translation problem is that the requirement (7.23) means that the symmetry parameters qualify as design parameters \(\eta\). We may therefore, in principle, use the symmetry parameters as design parameters. However, in the
present formulations there is no guarantee that the memory requirement (7.6) may be fulfilled. In fact, the characterisation of the symmetry parameters does not even provide a suggestion for a back-translated parameter \( p = \Psi(p') \). Nevertheless, the theory of symmetry families is one of the theories that is closest related to the back-translation problem, and that very well could be adapted to fit into the core-box modelling framework in the future.

**Example 7.5** Let us now finally illustrate the concept of symmetry parameters on the little example introduced in Section 7.1. A symmetry mapping for this system is given by

\[
l_f := p \rightarrow p(\lambda_f) = (p_1 + \lambda_f, p_2 - \lambda_f, p_3)
\]

which clearly leaves the output invariant for any function. Note that this is a different parametrisation than that in Example 7.1.

### 7.3 Two common reduction methods

We now turn to the more general case of practical unidentifiability. Some methods that work for all such systems were given in Section 7.1. We will now see how improved solutions are possible for two common reduction techniques: sensitivity analysis based methods, and variable lumping. As reviewed already in Chapter 5.1 these are two of the most useful reduction techniques when identifying a core model since they both are good at reducing the number of parameters, and yield a model structure \( M_c \) that is biochemically interpretable. Since we now see that these methods also are good from a back-translation point of view, it is evident that these two reduction methodologies are two of the most tractable options for the whole core-box modelling framework.

#### 7.3.1 Sensitivity analysis based methods

As reviewed in Chapter 5.1 sensitivity analysis based methods (SA methods) are made up of two steps: an analysis step that ranks all variables and interactions according to their importance, and an elimination step that eliminates those variables and interactions that may be eliminated without the model output being affected above a given threshold. We also pointed out that although one in principle may eliminate all entities at any place in the differential equations, care should be taken if the biochemical interpretation and internal consistency is to be preserved.

Since there is no coordinate or variable transformations involved, but only eliminations, the derivations of the \( \Phi \) and \( \Psi \) mappings is straightforward. Assume that all parameters that are eliminated lie at the end of the original parameter vector \( p \). Note that this may be done without loss of generality. Assume further that there are \( r' \) parameters left after the reduction, i.e., that \( \text{dim}(p') = r' \). The forward transformation \( \Phi \) is then simply equal to the unity mapping to \( p' \) for those parameters that remain in the reduced model, and the unity mapping to the design variables \( \eta(p) \) for those parameters that are eliminated

\[
\Phi := p \rightarrow (p_1', \ldots, p_{r'}', \eta_1(p), \ldots, \eta_{r-r'}(p)) = (p_1, \ldots, p_r', p_{r'+1}, \ldots, p_r)
\]  
(7.24)
Note that these design variables will fulfil both the memory condition (7.6) as well as the stricter continuity and differentiability demands.

Since the forward mapping \( \phi \) is equal to two unity mappings, the back-translation mapping \( \Psi \) will of course be equal to a unity mapping as well

\[
\Psi := (\tilde{p}', \eta) \rightarrow p = (\tilde{p}'_1, \ldots, \tilde{p}'_{r'}, \eta_1, \ldots, \eta_{r-r'})
\]

Note that the price of this simplicity is that the optimality of the back-translated parameters will not be kept. There are two obvious solutions to this problem. The first of them is to keep the error tolerance for the difference between \( M_g \) and \( M_c \) low, and leave the reduction of those parts that does not satisfy this requirement for other reduction techniques. The other solution is to replace the eliminated parameters by constant values. In this way the reduced model will still be identifiable, but the optimality in the back-translation will be kept. This latter solution is one of the most common methods to use in traditional handling of unidentifiability, even though the eliminated terms are not always removed on the basis of a sensitivity analysis. This option is illustrated in Example 3.2. Finally, the back-translation of the uncertainties and the associated parameter tables are typically of the same qualitative nature as that for \( p_3 \) in Tables 7.1 and 7.2.

### 7.3.2 Variable lumping

**Back-translation of a lump**

Consider a pair of \( M_g \) and \( M_c \) where one variable in \( M_c \) correspond to a pool of variables in \( M_g \). Such a system is depicted in Figure 7.2. Let the individual variables in the pool be denoted \( S_1 \) to \( S_n \), and let the corresponding concentrations be denoted \( x_1 \) to \( x_n \). Let the pooled variable be denoted \( L \) and let the corresponding concentration be denoted \( x'_L \). Consider an outflow from the lump, and assume that it takes place from the specific variable \( S_i \). Assume further that this outflow is described by mass action kinetics. If this outflow is denoted \( v_o \) we may therefore describe it as

\[
v_o = k_o x_i
\]

where \( k_o \) is the associated kinetic parameter. In the core model the outflow is given by

\[
v'_o = k'_o x'_L
\]

Since this flow is included in both the models, with the same interpretation, the flux through the outflow reactions must be the same (assuming that no general scaling of the fluxes, or other similar changes, have been made). That means that we may put the two expressions equal to each other, and solve for one of the parameters. This gives

\[
k_o x_i = k'_o x'_L \Rightarrow k'_o = \frac{x_i}{x'_L} k_o \quad \text{or} \quad k_o = \frac{k'_o}{x_i/x'_L}
\]

These are the transformations \( \phi \) and \( \Psi \) for \( k_o \) and \( k'_o \). Similar scalings are of course also applicable to an influx \( v_i \). This means that the translation between the parameters that are common between \( M_g \) and \( M_c \) (i.e., the parameters describing
Figure 7.2 The model structure in Example 7.6. There is one inflow and one outflow. The back-translation question is how to translate the parameters to and from the model to the right, which is the corresponding lumped core model. Those translations are given by equation (7.28), and the fractions are given by equation (7.35) or (7.38).

The in and out fluxes to the lump, may be translated analytically if the fractions between the concentration of the corresponding internal variables $x_i$ and the total concentration of the lump $x'_L$ are available. Such fractions are therefore useful auxiliaries in the transformations, and also useful design variables. We denote the associated fractions $\eta_i$

$$\eta_i := \frac{x_i}{x'_L}$$

Note that $\eta_i$ is generally time-varying. However, a lump is usually created because the internal reactions in the lump may be approximated as so fast that they may be assumed to reach an internal steady state immediately, or because the involved variables $x_1, \ldots, x_n$ show a high correlation. In any of these cases, the fraction $\eta_i$ is approximately constant. Finally, since parameters of the type $p'_0$ are the only ones that will be back-translated to any of the variables in the lump, equation (7.28) shows that the relation between the kinetic parameters in the lump and the fraction $\eta_i$ is sufficient for an understanding of the derivation of back-translation formulas for all the parameters. We now derive expressions for this relation under varying assumptions.
Figure 7.3 The three levels of complexity in the lumping derivations. The top figure (a) is a simple cyclic lump, the middle lump (b) has also added some additional reactions (and might be out of chemical equilibrium), and the lower plot (c) has inputs and outputs. For all these model structures analytical expressions to calculate the fraction of the lump in each species exists, but it is only in the a) and b) that those expressions depend solely on the kinetic parameters inside the lump.
Calculating the fraction

First we consider a closed loop system of \( n \) variables with only reversible reactions and equilibrium in all individual reactions. This corresponds to Figure 7.3a, and the equilibrium constraint corresponds to the following set of equations

\[
k_1 x_1 = k_{-1} x_2, \ldots, k_i x_i = k_{-i} x_{i+1}, \ldots, k_n x_n = k_{-n} x_1 \tag{7.30}
\]

which might be written as

\[
K x = 0 \tag{7.31}
\]

where

\[
K = \begin{pmatrix}
k_1 & -k_{-1} & 0 & \ldots & 0 \\
0 & k_2 & -k_{-2} & \ldots & 0 \\
\vdots & \ddots & \ddots & \ddots & \vdots \\
0 & \ldots & 0 & k_{n-1} & -k_{n-1} \\
-k_{-n} & 0 & \ldots & 0 & k_n
\end{pmatrix} \tag{7.32}
\]

In order for equation (7.31) to have a solution the matrix \( K \) must be singular. This is obtained if the determinant of the matrix is zero. The determinant is invariant under linear transformations. Divide all rows in the matrix \( K \) by the off-diagonal term. This yields the following matrix

\[
\begin{pmatrix}
K_1 & -1 & 0 & \ldots & 0 \\
0 & K_2 & -1 & \ldots & 0 \\
\vdots & \ddots & \ddots & \ddots & \vdots \\
0 & \ldots & 0 & K_{n-1} & -1 \\
-1 & 0 & \ldots & 0 & K_n
\end{pmatrix} \tag{7.33}
\]

where \( K_i := k_i/k_{-i} \). The determinant of this matrix is simply given by the product of the diagonal terms minus one. We have therefore found

\[
\prod_{i=1}^{n} K_i - 1 = 0 \Rightarrow \prod_{i=1}^{n} K_i = 1 \tag{7.34}
\]

to be a requirement for the system in Figure 7.3a to have the possibility of all reactions being simultaneously in chemical equilibrium. If this requirement is not fulfilled by the kinetic parameters, simultaneous chemical equilibrium may not be obtained. Assume now that conditions (7.34) is fulfilled by the parameters \( p \). We then have an analytical expression for the design parameters \( \eta_i \) according to

\[
\eta_i = 1 + \sum_{j=1}^{i-1} \prod_{k=1}^{j} K_{i-k}^{-1} + \sum_{j=i+1}^{n-1} \prod_{k=1}^{j-i+1} K_{k+i-1} \tag{7.35}
\]

The expression is derived by forming the sum \( x'_i = \sum x_j \), replacing all concentrations by a constant times \( x_i \), and dividing both sides by \( x_i \). If expression (7.34) is fulfilled we therefore have an analytical expression for \( \eta_i(p_x) \). Note that it actually only consists of the fractions \( k_i = k_i/k_{-i} \).
Even if condition (7.34) is not fulfilled the system might go to a steady state. Such a steady state will have all concentrations constant, but with a non-zero flux going through the system. A corresponding K matrix may still be put up, starting from the more general constraint of \( f(x, p) = 0 \), and this matrix may be transformed into the form (7.33) with the aid of a Gauss-Jordan transformation. The only difference is that the \( K_i \)'s will have been replaced by more or less complex parameter combinations. Once these parameter combinations have been identified, they may be used to calculate the fractions \( \eta_i \) according to equation (7.35). Similar extensions are done if more reactions are added to the system (see Figure 7.3b). For all systems with a lump consisting of a closed loop, with or without diagonal reactions, we may therefore form a corresponding K matrix, transform it to the form (7.33) through linear transformations, and calculate the fractions \( \eta_i \) according to equation (7.35). The only difference is therefore that the calculations in expression (7.35) are more or less tedious. However, with the aid of a computer algebra program, these calculations are still straightforward, and for all systems we have tried never particularly time-consuming (calculations in the MATLAB symbolic toolbox takes less than 5 seconds for Example 7.6 below).

Let us finally consider the case of inputs and outputs from the lump (Figure 7.3c). In the typical lumping situation the inputs to the system occur on a slower time-scale than the internal reactions. Therefore, if one considers the time-scale of the external fluxes, the lump is assumed to find its steady state concentrations momentously, and if one considers the time-scale of the internal states, the external inputs are considered as constants. Let these constant inputs be denoted by the \( n \)-dimensional column vector \( u \). Assume also that there is some outflow, and that the outflow from variable \( x_i \) is described by parameter \( k_{o,i} \). One may then form a corresponding set of differential equations for larger systems by subtracting the \( k_{o,i} \) values from the diagonal of the closed loop K matrix, and by adding the inputs according to

\[
(K - I k_o)x + u = \bar{K}x + u = 0
\]

where \( I \) is the unity matrix and \( k_o \) is the column vector consisting of all the \( k_{o,i} \) values. While \( K \) is always singular, the combined matrix \( \bar{K} \) would typically be invertible. We may therefore find the steady state concentrations, \( x_{ss} \), by solving for \( x \) in equation (7.36)

\[
x_{ss} = \bar{K}^{-1} u
\]

Therefore, we may also find the corresponding fractions \( \eta_i \) according to

\[
\eta_i = \frac{x_{ss,i}}{\sum_{i=1}^{n} x_{ss,i}}
\]

The benefit of this formula for \( \eta_i \) is that it is exact. However, in many situations the approximate solution (7.35) which corresponds to putting the inputs and outputs to zero, yields a good approximation (with an error of less than a percent). This approximate solution has the benefit that it is explicitly only dependent on parameters, and only on parameters inside the lump. We finally illustrate the two ways of calculating the fraction on a small example system.
Two common reduction methods

Example 7.6 The system we consider is illustrated in Figure 7.2, where also all the parameters are given. There is one influx and one outflux. The inflow is described by a constant parameter, and the outflow by mass action kinetics. All the reactions internal to the lump are also described by mass action kinetics, and the initial concentration of \( S_1 \) is 1, and all the others are zero. As can be seen in Figure 7.4, the dynamics of the states has a time-constant which is considerably slower than the time-constant of the fraction dynamics. That means that the system behaves like a lump. Further, the fractions determined by the simulations are correctly predicted by equation (7.38) to be

\[
\eta = (0.1898, 0.3071, 0.2932, 0.1334, 0.0765)^T
\]

(7.39)

The fractions predicted by equation (7.34) (that assumes the inputs and outputs to be zero) are

\[
\eta = (0.1881, 0.3058, 0.2941, 0.1354, 0.0766)^T
\]

(7.40)

These are the same fractions as the true ones within a percent, and this means that one in this example for all practical reasons may disregard the external fluxes effect on the steady state fractions, and only consider them as dependent on the kinetic parameters internal to the lump. It is our experience that this is the typical case, and it is an important simplification of the back-translation equations, given by equation (7.28).
7.4 Summary of Chapter 7

In this chapter we have dealt with the back-translation step in the core-box modelling framework. This step is the last step in the framework, and after this the model is therefore ready to be analysed. The step have been sub-divided in 4 sub-problems and formalised using the two mappings \( \phi \) and \( \Psi \). The latter mapping is generally associated with a number of choices, and these have been characterised by a design parameter \( \eta \). For the characterisation of these two mappings in the general case we have suggested a simulation and optimization based approach, that might also yield analytical expressions for the mappings. Apart from translating the values it is also important to translate the uncertainties, and to characterise where the degree of certainty stems from. The involved sets are depicted in Figure 7.1, and we suggest to present them in tables like Table 7.1 and 7.2.

It is generally better to make use of the special structures that are present in a system, than to use the simulation based approaches suggested for the general case. For instance, in the case of structural unidentifiability in a single rate expression caused by conserved moieties a linear structure in the transformations to an identifiable expression is revealed by the transformation matrix \( C \). This may be used to write up a general analytical expression for \( \phi \) according to (7.18).

The back-translation to \( e \) and \( c \) may also be written up explicitly, even though the memory constraint (7.6) is only fulfilled for the back-translation to \( e \). The full back-translation to \( p \), on the other hand, is a general nonlinear inversion problem, and no special structures has yet been detected. There are, however, methods for general nonlinear identifiability problems that may be re-used in the back-translation problem. One such method is the derivation of symmetry families, since the associated symmetry parameters may be used as design parameters.

For the more general case of practical unidentifiability we present improved methods for two common reduction techniques: SA based methods, and variable lumping. For SA based methods both the \( \phi \) and \( \Psi \) mappings are given by unity transformations, which means that all direct translations are unity mappings. The price of this is that the optimality is not preserved between the systems. To avoid this being a problem one might, e.g., put high constraints on the error from the reduction, or keep the eliminated parameters as constants in the model. We show the structure of the back-translation in a general lumping problem, and identify the fraction of the concentration of the outgoing sub-species to the whole lump as a useful auxiliary in the otherwise analytical expressions for \( \phi \) and \( \Psi \). We give exact formulas for this auxiliary for the case of a cyclic uni-molecular lump with arbitrary in and out ports. We also give an approximation of this formula that is only dependent on the parameters in the lump, and illustrate in an example that the approximation usually gives good results. This means that the design parameters for both lumping and SA based elimination yields design parameters that fulfills the memory constraint (7.6), and that typically also are continuous and differentiable. Therefore, core-box models that are developed using only these two reduction methods will have a total exchangeability between \( M_g \) and \( M_c \), i.e., where parameter modifications are saved when changing between the models. This means that the two models may be considered as two versions of the same model, with the only difference being the degree of 'zooming’. Such models would be
highly beneficial as modules in a hierarchical model. However, the prime objective of the core-box modelling framework is fulfilled already by the combination of the differentiated quality tags with the detailed descriptions of the grey-box model.
Part II

APPLICATIONS TO GLUCOSE HOMEOSTASIS RELATED SYSTEMS
Part I of the dissertation was devoted to the development and presentation of a new modelling framework, core-box modelling. We have now reached Part II, and it will be devoted to the application of these methodologies to the study of four different biological systems. All the chosen systems are related to glucose homeostasis, and in this introductory chapter we give an overview of the whole-body perspective of this homeostasis. The chapter serves as an introduction and a general biological background to the subsequent chapters. Further, we will also present an outline of the big picture, and the long-term research goals, to which all the subsequent chapters contribute. However, no material in this chapter presents new science. Much of the material is general knowledge to people with a biological background, and it is primarily based on the following reviews: [53, 65, 84, 108]. The chapter starts by presenting the organ and hormonal perspective of glucose homeostasis, and then moves on to the cellular perspective. The chapter ends with a review of the diseases associated with an impaired glucose homeostasis, and with an outlook of the long-term research goals that intend to understand and cure these diseases.

8.1 The main regulating organs

Glucose is the most common form of sugar in a human body, and one of the main sources of energy. Glucose is transported to the various parts of the body through the blood. In a healthy individual the concentration of glucose in the blood lies between 4 and 7 mM. This is true independently of the activities that the person undertakes. Since this includes fasting and intense sporting, it is clear that the blood glucose level is tightly regulated. It is this regulation that is referred to as the glucose homeostasis. The regulation is carried out by a complex interplay between
several different organs that can, e.g., sense the blood glucose concentration, effect it by either uptake or release of glucose, or effect the activities of other organs through hormonal signalling. The most central organs to the glucose homeostasis are pancreas, liver, and adipose and muscle tissue. We will now give a short introduction to the specific role of each of these organs. An overview of the organs and their main interactions is also given in Figure 8.1.

**Figure 8.1** The most important regulating organs and some of their main interactions in the whole-body glucose homeostasis. The figure is taken from [61].

**Pancreas**

The pancreas is situated behind the stomach in the abdomen. Its primary role in glucose homeostasis is to sense the blood glucose level, and to secrete hormones that affect the activity of the other organs. One of the most potent regulating hormones is insulin. Insulin is secreted by a specific cell type in the pancreas denoted β cells. The degree of insulin that is secreted by the β cells is proportional to the blood glucose level. There is no insulin secreted when the glucose level is below a certain concentration. The insulin secretion then increases as the glucose level increases beyond this threshold until it eventually reaches a maximal level. The exact mechanisms involved in the glucose sensing and insulin secretion are not fully understood. This is partly due to the fact that they involve a complex interplay.
between the metabolism and the electrophysiology of the \( \beta \) cells. Further, the electrophysiology displays a complex behaviour denoted bursting. Bursting is a combination of oscillations at several time-scales: one at the level of ms, which is denoted spikes, and one at the level of seconds or minutes, which turns on or off periods of spiking (see Figure 5.2). A period of spiking is referred to as a burst, and the bursts are synchronised with the insulin secretion, which means that the secretion is pulsatile. All these complex behaviours have been modelled in some rather extensive grey-box models [82, 83]. However, no further developments of these models are included in this dissertation. Finally, insulin serves as a negative feedback to the glucose level, which means that the effect of insulin on the other organs act to downregulate the blood glucose level.

Another potent hormone secreted by the pancreas is glucagon. It is secreted by another cell type denoted \( \alpha \) cells. The role of glucagon in glucose homeostasis is opposite to that of insulin: glucagon release is increased by a blood glucose decrease, and glucagon increases the other organs tendency to upregulate the blood glucose level. The \( \alpha \) and \( \beta \) cells make up a majority of the cells in the islet of Langerhans, which is the hormonal producing little islets that are situated in the pancreas. The cells are coupled to each other through gap-junctions, and both the metabolism and the electrophysiology is synchronised among the neighbouring cells. This synchronization adds further complexity to the understanding of the cells, and it has also been studied through modelling [122].

Liver

The majority of the insulin secreted by the pancreas is taken up by the liver. The liver is situated directly after the pancreas in the blood flow, and the majority of the released insulin is therefore never part of the main blood flow. Liver cells, also called hepatocytes, have a metabolism that is quite different from other cell types. One important difference is that the liver has the ability to produce glucose from other substrates (see Section 8.2). The liver has therefore not only the ability to upregulate the blood glucose level by decreasing the glucose uptake, but also by increasing the glucose production. Both these functions are regulated by the hormonal controls from insulin and glucagon. The liver is one of the central organs in the glucose homeostasis, but it is not considered further in this dissertation.

Fat cells

Another name for fat cells is adipocytes, and they are spread out in most parts of the body. One of the primary functions of adipocytes is to store fat in the form of triglycerides. The fat is stored in the centre of the cell, and each cell contains a relatively large droplet, which might cause a fat cell to become about a millimeter in diameter (which means that it is visible to the bare eye). However, the actual activities of the cell only occur in the thin outer layer of the cell, a layer that makes up less than a percent of the total volume of the cell. The triglycerides in the droplet may be released into the blood, where it is broken down to free fatty acids (FFA). FFA is another source of energy with an homeostasis of its own. The FFA homeostasis is not as tightly regulated as the glucose homeostasis, which means
that the levels of FFA might vary quite substantially during a day. Nevertheless, there is a tight coupling between the FFA homeostasis and the glucose homeostasis, since they are both part of the energy homeostasis in the body. Glucose might also be converted into FFA, something which is described in Section 8.2 below. The FFA homeostasis is not further studied in this dissertation.

Another important function of the adipocytes is to respond to insulin. This is true in two ways. Adipocytes have their own intracellular response, which might be more or less sensitive to insulin. If this intracellular sensitivity is decreased, this is usually an early sign of an impaired insulin sensitivity in the other tissues as well, something which eventually leads to diabetes. In this way the adipocytes might therefore also be considered as indicators of the current insulin sensitivity in the whole body. One common intracellular response to an increased insulin level is the assembly of glucose transporters to the cell membrane, something which leads to an increased glucose uptake by the cells. This is part of the metabolic response to insulin, and it is mediated by a network of protein-to-protein interactions. Another type of response mediated by this network is referred to as mitogenic. Mitogenic processes in a cell affect, e.g., cell proliferation (i.e., cell division) and cell differentiation (i.e., cell specialisation). The information transfer in these protein networks is referred to as signalling and the mechanisms by which it occurs is described in more detail in Section 8.2 below. The signalling which is initiated by insulin is also the main topic of Chapter 9, where a core-box model is developed.

Yet another important function of the adipocytes is to release hormones. These hormones are denoted adipokines. Four of the most important adipokines are the tumour-necrosis factor-α, leptin, adiponectin, and resistin. All these affect the glucose homeostasis in various ways, e.g., by regulating the food intake, the energy expenditure and the insulin sensitivity. The concentrations of the adipokines are also drastically changed when glucose homeostasis is malfunctioning.

Muscle

Of all the tissues muscle is the one tissue that has the highest direct effect on the blood glucose concentration. The effect is caused by glucose uptake, and up to 75% percent of the insulin-dependent glucose disposal occurs in skeletal muscle alone. Because of this high consumption all muscles are highly optimized to the specific task they are assigned to: long-term steady state contraction, heavy and accelerated contractions, etc. Even though the direct effect on the glucose uptake from muscle is big, the indirect effect through secreted hormones is less pronounced than that of the other three organs (although some hormones are secreted also by muscle tissue). Therefore, muscle tissue is primarily a responsive tissue, and not a regulating tissue. Finally, there is a long-standing contradiction in the conventional interpretation of experimental data in metabolism following contraction under anaerobic conditions. This contradiction is resolved using a modelling approach in Chapter 11.
8.2 The main regulating pathways

Glycolysis

Glycolysis is the maybe most central pathway in the metabolism of a single cell. Due to its importance it is also highly preserved in all eukaryotic cells (i.e., cells with a nucleus), and in Chapter 10 we will study a model for glycolysis in yeast (see also Figure 2.2 for a detailed reaction scheme of the processes described below). The main function of glycolysis is breakdown of glucose (Glc) into pyruvate (Pyr), and the synthesis of adenosine triphosphate (ATP) out of adenosine diphosphate (ADP) and inorganic phosphate (P\text{$_i$}). The synthesis of ATP is an energy consuming process, and the energy is taken from the breakdown of glucose, which is an energy releasing process. The energy that is stored in ATP is then be used as a source of energy in most of the energy consuming reactions in the body. Glycolysis is therefore part of the conversion of the energy stored in the food in the form of sugar, into energy that can be used by the cell in the form of ATP.

ATP is produced within glycolysis if it is considered as a lumped reaction. If one instead considers the various sub-steps in glycolysis two reactions require the breakdown of ATP: the reactions catalysed by the enzymes hexokinase/glucokinase (HK/GK) and phosphofructokinase (PFK). These two enzymes are situated in the upper part of glycolysis. This means that the upper part of glycolysis is ATP consuming, and it is therefore sometimes referred to as the preparatory phase. The lower part of glycolysis is ATP producing, and it is correspondingly referred to as the pay-off phase. There are two reactions that produce ATP in the lower part of glycolysis: phosphoglycerate kinase (PGK) and pyruvate kinase (PK). In between the upper and lower part of glycolysis lies the aldolase reaction (ALD). This reaction splits the six carbon molecule fructose-1,6-bisphosphate (FBP) into the two three carbon molecules glyceraldehyde-3-phosphate (GAP) and dihydroxyacetone phosphate (DHAP). That means that the flux through the reactions in the lower part of glycolysis is approximately twice that of the flux through the upper part of glycolysis. There is therefore four ATP molecules produced per glucose molecule in the lower part of glycolysis and the net result in the lumped glycolysis is therefore a gain of two ATP molecules per glucose molecule.

Glycolysis as described above consists of a linear chain of reaction. In reality, however, there are several branches that lead to different end results than Pyr. Two important such side-branches start at glucose-6-phosphate (G6P). The first of these is the pentose phosphate shunt (PPS). This is important for the production of nicotinamide adenine dinucleotide phosphate (NADPH), and it is important in the activation of neutrophils, which we will encounter in Chapter 12. The second branch starting at G6P is the flux leading to the production of glycogen. Glycogen is a large molecule which consists of many Glc molecules chained after each other. These Glc molecules may then be extracted at a later point, which means that this side-branch is a storage flux which saves the Glc for later. A side-branch that occurs at a later point is the flux to glycerol (Glyc). This flux starts at DHAP, i.e., at the beginning of the lower part of glycolysis. All these side-branches leads to a lower production of Pyr, and to a lower production of ATP.

It was mentioned above that Glc might be produced in the liver. One way in
which this is done is by the extraction of G6P units from the glycogen storages. This may be done in all cell types, but it is only in the liver that the G6P units may be dephosphorylated to pure Glc. The other source of Glc production in the liver is denoted gluconeogenesis. Gluconeogenesis is the reversal of glycolysis, which means that Glc is formed out of two Pyr molecules. However, all individual substeps in gluconeogenesis are not identical to the reversal of a step in glycolysis and it is not always the same enzymes that catalyse the reversed reactions. The flux through gluconeogenesis is also more expensive than the gain in glycolysis. Nevertheless, gluconeogenesis is believed to be an important flux in the glucose homeostasis, and it is also a way to glucogenic proteins into glucose.

**Oxidative phosphorylation**

The pyruvate formed in glycolysis may be further used in several different ways. In yeast there are two primary fates of pyruvate: acetaldehyde (ACA) and ethanol (EtOH), which is the anaerobic branch, and acetyl-CoA, which is the aerobic branch. In humans the two major fates are lactate (Lac), which is the anaerobic branch, and production of acetyl-CoA, which is the aerobic option. The aerobic part eventually leads to oxidative phosphorylation. In Chapter 11 we will study the anaerobic metabolism of muscle, which means that Lac is then the only end product. However, from a general energy production point of view it is the aerobic option that is most important; in glycolysis each Glc gives rise to 2 ATP molecules but from the oxidative phosphorylation 36 ATP molecules can potentially be formed. However, the exact number that is formed, in practice, is significantly lower, since much of the energy available in the process is lost in the mitochondrial uncoupling. The three main sub-processes in this latter process are the TCA cycle, the electron transfer chain, and the ATP synthase.

The TCA cycle is sometimes also called the citric acid cycle or the Krebs cycle. In higher organisms it occurs in the mitochondria, which is a special type of intracellular compartment. The TCA cycle is composed of a number of enzymatically catalysed reactions that are connected in a circle, something which is reflected in the name. The main function of the reactions from our current perspective is the production of NADH and FADH$_2$. These two molecules are used to produce a proton gradient across the inner mitochondrial membrane through the electron transfer chain. The electron transfer chain is a number of reactions that occurs at the inner mitochondrial membrane, and they all have the same purpose: to pump H$^+$ ions out of the mitochondria using the energy released in the oxidation of NADH and FADH$_2$. The obtained proton gradient is then a source of energy which is used to form ATP. This is done by the ATP synthase which is another protein situated in the mitochondrial membrane. This protein lets H$^+$ ions into the mitochondria, and uses a rotor-like technique to transform the released energy into the formation of of ATP from ADP and P$_i$. Finally, one of the main sources for a practical gain which is less than the theoretical 36 ATP molecules is denoted proton slip. It refers to the spontaneous back-transport of the H$^+$ ions by means of the gradient alone.
Figure 8.2 The main catabolic processes of the nutrients glucose, FFA, and proteins in a cell. As can be seen all the processes use the TCA cycle and oxidative phosphorylation in the mitochondria to produce ATP, something which indicates the high interdependency between the processes. Another reason for the interdependency is that some cell types (e.g., liver cells) may reverse many of the catabolic reactions, and thus interconvert, e.g., glucose into fats. Note, however, that the figure is highly schematic and that many details are missing.

Other sources of energy

There are several other important sources of energy in the cell than glucose. One such source is the FFA circulating in the blood, as described above. The energy in these may be released either by beta oxidation, which leads to acetyl-CoA to be used by the TCA cycle. Triglycerids may also be broken down in to glycerol which may also enter glycolysis.

Another catabolic process which ends up in the TCA cycle is the breakdown of proteins. The proteins are broken down by enzymes referred to as protease
enzymes. The breakdown of proteins is, however, not primarily done to produce energy in the cell, but instead to produce new proteins. The contribution from protein catabolism is smaller than that of sugar, but still not neglectable, even during normal operating conditions.

Yet another energy unit that is used for energy storage and transportation in the human body is lactate. If oxygen is present, lactate may be used to form acetyl-CoA which may enter the mitochondria and lead to oxidative phosphorylation and ATP production. The importance of lactate has recently been re-evaluated, and for instance for the energy balance in the brain it is now argued whether the importance of lactate is comparable to that of sugar. The interplay between the different sources of energy is outlined in Figure 8.2.

### Signalling cascades

Almost all metabolic reactions are catalysed by enzymes. This catalysis is usually so important for the reaction rate that the same rate is approximately zero if the correct form of the catalysing enzyme is absent. That means that the regulation of the metabolic activity in a cell may be done through the regulation of the corresponding enzymes. The enzymes in the cell are interacting in a large network of enzymes. This network is highly complex where each enzyme may be involved in interactions with many other enzymes, and where feedbacks and redundant regulations are frequent. The study of the enzymatic regulation of metabolic activity is therefore a highly difficult but yet essential part of the understanding of glucose homeostasis.

The basic building block in a signalling network is the activation and inactivation of a protein, or of an protein complex. Such an activation may be done in several ways. One of the most common ways is phosphorylation. A phosphorylation is catalysed by a kinase, and a phosphorylation equals the addition of a phosphate group to a specific site (location) on the protein. Two common such sites are tyrosine and serine; the former is usually associated with the transmission of information, and the latter is usually associated with regulation of this transmission. Once a signalling intermediate has been phosphorylated its activity to act as an enzyme in other reactions is altered. A phosphorylation often equals an activation of the protein, which means that it might, e.g., act as a kinase in the phosphorylation of another protein, which may then act as a kinase in the phosphorylation of another protein etc. In this way the initial activation proceeds through the network, even though it should again be pointed out that it is not a linear chain of processes. Other common forms of activations are the formation of complexes, where the complex is the active (or inactive) form, or allosteric regulation, where the presence of other key molecules, like Ca$^{2+}$, are affecting the activity of an enzyme.

Finally, it should also be mentioned that the signalling networks also have other end targets than regulation of metabolic activity. Other common end targets are the activation of specific transcription factors (which govern the creation of new proteins), the regulation of when a cell should divide (denoted the cell cycle), or the decision of whether a cell should go into self-destruction (denoted apoptosis). These latter end targets of a signalling network are of little concern in this disserta-
Interplay between signalling and metabolism

To a first approximation one can therefore say that hormonal signalling is a process that is occurring in protein networks which acts as control on the metabolic fluxes in the cell. This simple view is illustrated in the top of Figure 8.3. Further, there is no actual mass-transfer involved in the signalling, as it is in the metabolic networks. This difference is analogous to the difference between a wave and a river. A river is like a metabolic flux, which involves the actual conversion of the initial input (e.g., glucose) to the end product (of pyruvate). The wave, on the other hand, is like a signalling network since it does not convert the initial input (e.g., insulin) to the end product (e.g., a phosphorylated transcription factor), but only transfers the information about the input.

However, the real picture is more complex and involves the regulation and participation of metabolites in the signalling networks. For instance, the enzyme PFK is allosterically regulated by several metabolites, e.g., AMP. AMP is also involved in the activation of an entire signalling network, known as the AMP kinase network. This complicated interplay is illustrated in Figure 8.3. Some other signalling networks that involve the participation of metabolites are the hexosamine signalling pathway and the mTOR signalling pathway. All these networks are relevant for glucose homeostasis, and they are currently receiving an increasing interest in the scientific community. These examples show that the regulation by metabolism on signalling does not only occur indirectly through the secretion of enzymes in other organs, but through the direct activation and participation of metabolites within the same cell. This means that it is necessary to study the combined network – i.e., to consider the metabolism and its regulation as one inseparable whole – also within a single cell.
**Figure 8.3** The relation between metabolism and signalling. To the first approximation metabolism is a flux, much like that in a river, which is regulated by the hormone initiated signalling, which only involves information transfer. In the figure, insulin is the extracellular hormone, and glucose is the extracellular metabolite. However, the real situation is more complex than this. The formed ATP affects the AMP concentration which regulates the glycolytic flux, as well as some of the signalling networks. The control of the signalling networks is mediated, e.g., via the AMP-kinase network, which affects the insulin signalling network. The true picture is therefore that the metabolism and signalling networks should be considered as an inseparable whole, also within a single cell.
8.3 Diseases

Diabetes Mellitus

There are several diseases that are associated with an impaired glucose homeostasis. One of the most known of these diseases is Diabetes Mellitus. It divides in two different types: Type I and Type II diabetes.

Type I diabetes is a disease that is usually developed already in the early years, and almost exclusively before the age of 30. Its primary symptom is that the β cells that produce insulin are being destroyed. This is done by the body’s own immune system, because the β cells are for some reason considered hostile. Since the beta cells are destroyed, no more insulin is being produced, and this of course leads to severe problems with the glucose homeostasis. Since the downregulation of the glucose level is impossible using insulin, a common symptom of the disease is increased levels of blood glucose. This is toxic to the body, and will in the long run cause severe damages to the inner organs, including blindness and sometimes even death. The good part of Type I diabetes is that it is relatively straightforward to treat; regular injections of insulin, and regular meals keeps the glucose homeostasis functioning in an artificial way and can often allow for the patients to live a relatively normal life.

Type II diabetes was originally referred to as a disease that almost exclusively occurred to elderly people. In the later years, however, the disease has become common in younger and younger years, and now it even occurs among children and teenagers. Diabetes is becoming a global health problem, with rapidly increasing numbers of patients, and it is primarily the Type II diabetes that is increasing in numbers. One reason for that is that the Type II diabetes is primarily associated with the lifestyle, while Type I diabetes is primarily associated with genetics.

The exact mechanisms that lead to Type II diabetes are not known. It is known, however, that the digestion of unhealthy food, lack of regular exercise and obesity are all strong factors leading to the development of the disease. To end these behaviours is also an effective form of treatment. It is also known that an important precursor to the fully developed diabetes is insulin insensitivity. In the early stages of development this decreased sensitivity can be balanced by an increased production of insulin. As the disease progresses, however, this becomes more and more difficult, and the result is increasingly unhealthy levels of both insulin (denoted insulinemia) and glucose (denoted hyperglycemia). Further, the increased production of insulin puts higher strains on the β cells, and if this goes on for extended periods of time (several years) the β cells will eventually start to malfunction. This is when the diabetes is considered to be in a fully developed form, and it is in some ways even more problematic than the Type I diabetes. There is no insulin in the body, and it is almost pointless to inject it into the body, due to the high insulin resistance.

Obesity and the metabolic syndrome

Diabetes Mellitus is just one of many diseases that are associated with an impaired energy homeostasis. Obesity, which means excessive overweight, is such a disease.
Obesity is also tightly connected with diabetes since obese people have a much greater tendency to get diabetes. This is rather natural since obesity puts strong demands on the regulating organs. Nevertheless, the exact mechanisms that couples the two diseases are not known. It should also be mentioned that even though obesity often leads to diabetes, the opposite is not true. In fact, one of the symptoms of diabetes is weight loss.

There is no general consensus on an exact definition of what Type II diabetes is. There are also several other names for the disease, for instance Non-Insulin Dependent Diabetes Mellitus (NIDDM), maturity onset diabetes and adult onset diabetes. Further, diabetes is associated with several symptoms, like hyperglycemia and insulinemia which actually are diseases in themselves. All these diseases are also associated with other symptoms like high blood pressure, proinflammatory state, blood fat disorders etc. People with a majority of these symptoms are said to suffer from the metabolic syndrome. The metabolic syndrome is therefore a state which includes diabetes, but also all other problems due to an impaired glucose homeostasis.

The metabolic syndrome might not only lead to diabetes but to a wide variety of other problems, not directly involved in the regulation of the blood glucose. An elevated blood glucose level might, e.g., lead to an increased activity of the immune defence system. This follows since a neutrophil (which is a kind of white blood cell, see Chapter 12) which is supposed to be activated by the presence of invading pathogens, might be spontaneously activated by a high blood glucose level. In Chapter 12 we study the sensitivity of a model for activated neutrophils upon various kinds of perturbations. Finally, the metabolic syndrome might lead to even more distant symptoms like coronary heart disease and cancer.

8.4 Summary and outlook

Glucose homeostasis is a systems property

Glucose homeostasis – i.e., the control of the blood glucose level – is evident by the fact that the blood glucose level stays between 4 and 7 mM in a healthy body, independently of which activities that are undertaken. Part of the control is hormonal and two of the most important hormones are insulin and glucagon. These hormones affect both the glucose production in the liver, and the glucose uptake in the peripheral tissues: primarily muscle, liver and adipose tissue. Insulin and glucagon are secreted by the pancreas, and other important hormones are secreted by the adipose tissue. Adipocytes are also important because they are instrumental in the FFA homeostasis, something which is tightly co-regulated with the glucose homeostasis. Other important sources of energy to the body are proteins and lactate, and a complete understanding of glucose homeostasis must include the regulation of all these nutrients combined.

On the intracellular level the different nutrients are typically broken down to acetyl-CoA which enters the TCA cycle which leads to ATP production through oxidative phosphorylation. Since the catabolism of both glucose, fat, and proteins, share these latter steps, and since several of the catabolic processes may be re-
versed to convert one nutrient into another, a full understanding of intracellular glucose metabolism should include the metabolism of the other nutrients as well. Likewise, the signalling which is initiated by hormones to up- and down-regulate the enzymes catalysing the various properties must also ultimately be studied together with the metabolism itself. This follows since some signalling involves the active participation of metabolites inside the same cell. We draw the conclusions that glucose homeostasis is a systems property, both on the intracellular and on the whole-body level. Finally, just like glucose homeostasis is a systems property that cannot be satisfactorily understood by a mere consideration of the various sub-processes, so are the associated diseases: most notably Type II Diabetes and the metabolic syndrome.

**Towards an hierarchical model for glucose homeostasis**

That glucose homeostasis is a systems property means that it cannot be satisfactorily understood by conventional reductionist methods alone. This is one of the main reasons why the current medications of the associated diseases are ineffective and relatively non-specific. Better results should be possible to achieve using a systems biology approach. However, since the system of study is so big the study has to be undertaken in steps.

One important such step is the development of a well-functioning model for the role of each of the involved cell types, e.g., fat cells, α and β cells, liver cells, and muscle cells. A precursor to this is the development of models for the most important intracellular functional modules, like the insulin signalling in adipocytes, and the regulation of the glycolytic flux in muscle etc. To combine models for small subsystems into larger, and gradually more and more comprehensive, models is referred to as a bottom-up approach.

The complementing approach is referred to as a top-down approach, and this approach has its own important sub-steps. The first of these is probably to establish a steady-state model for the major metabolic and hormonal fluxes in the body under some specific constant and well-defined operating conditions. After such steady-state flux models have been developed for each of the major operating states (before/after meal, rest/exercise, etc), one could develop models for the transient behaviours, first for small perturbations around each operating state, and then for larger perturbations, e.g., moving from one operating state to another. When this has been established a dynamic whole-body model have been obtained, and this in itself will give great insights into the understanding of glucose homeostasis and its associated diseases.

However, even though the whole-body model and the models for the various subsystems give insights on their own, a fully applicable model is not obtained until the models are merged. A whole-body model on the top level only lacks the necessary resolution to understand the detailed mechanisms that are malfunctioning in the diseases, and the detailed mechanisms a potential drug could target. Likewise, an isolated model for one of the sub-systems lacks the connection to the other subsystems; and it is these connections that allows for a translation of the importance of a mechanism for a subsystem to the importance of a mechanism to the actual whole-body homeostasis. The ultimate goal is therefore to merge the models for
the whole body with the models for the various sub-systems. Such a combined model is referred to as a hierarchical model, and the ultimate goal is therefore to obtain and work with hierarchical models for glucose homeostasis in a human body. This dissertation is a step towards the achievement of this goal.

**Outlook at the remaining chapters of Part II**

The following Chapters 9 to 12 all deal with various sub-systems related to glucose homeostasis. Chapter 9 studies insulin signalling in human fat cells, for which a full core-box model is developed. The next chapter looks at glycolysis in the important model system *Saccharomyces cerevisiae*. Several of the new theoretical methods were inspired by the Hynne model for this system, and their performance is now demonstrated. In Chapter 11 we again look at glycolysis, but this time in muscle. As mentioned above, muscle glycolysis is the most important consumer of glucose, and the chapter shows the resolution to an important contradiction that has long been debated about the control of the glycolytic flux following anaerobic contraction. Finally, in Chapter 12 a metabolic system that is affected by diabetes is studied: neutrophils. Neutrophils are over-activated in the case of hyperglycemia, and in this chapter we study the sensitivity of the activated state to various kinds of perturbations. This study is also preparatory for the development of a core-box model for the system. Finally, even though the long-term goal is to construct a hierarchical model for the whole-body glucose homeostasis, the models in all these chapters are primarily developed to study specific research questions associated with the different sub-systems.
We will now turn to the development of the first of the three new models developed in this dissertation. It is a model for insulin receptor activation and internalisation in human fat cells, and the developments will include many of the different steps in the core-box modelling framework. As described in the previous chapter, insulin signalling is an important subsystem in the understanding of glucose homeostasis and Type II Diabetes. Despite this, there are not many good models for the system, and it is therefore judged that it is best to develop a minimal model from scratch. This is done in Section 9.2, and since a hypothesis testing approach is chosen, several non-trivial conclusions follow from the model developments. The developed minimal model is then used as a basis for model reduction, and for extensions to a full-scale grey-box model. Translations between all models are also given, which means that many of the criteria of a core-box model will be obtained. The translations and the final analysis are included in 9.3.

9.1 Background

9.1.1 Biological background

Insulin is detected by the cells using a specific insulin receptor (IR). This receptor then mediates this information to other parts of the cell, for instance to the cell nucleus and to the glucose transporters, using a complex network of protein-to-protein interactions. A common way to transfer a signal in such networks is phosphorylation, i.e., the addition of a phosphate group. An enzyme that is responsible for the catalysis of phosphorylation is referred to as a kinase. The insulin receptor is a bit special, because it consists of two ’arms’ that acts as kinases for
each other. We therefore say that IR undergoes autophosphorylation. There are several sites upon which a phosphate group may bind, and they all have more or less different biological effects. In this work we will only consider phosphorylation on tyrosine residues, but there are evidences that serine phosphorylation also is relevant for glucose homeostasis [28]. Autophosphorylation of IR is, in principle, always possible, but in practice it only occurs when an insulin molecule is bound to the extracellular part of the receptor. The phosphorylation of IR acts as an on-switch for the subsequent signalling machinery as described in the previous chapter. A phosphorylated IR is denoted by IR-\(P\), and IR-\(P\) is a kinase to several other proteins, e.g., the insulin receptor substrates (IRS), see Figure 9.1. The receptor might also be turned off, and there are two main mechanisms for how this may be done. The first mechanism is dephosphorylation, and the proteins responsible for the dephosphorylation are referred to as protein tyrosine phosphatases (PTP). The other mechanism by which the receptor might be turned off is endocytosis.

Apart from acting as a kinase the receptor may also do other things, once phosphorylated. It might for instance bind to a second insulin molecule, and it might undergo endocytosis. Endocytosis is the process by which a membrane bound receptor leaves the membrane in a little vesicle, and the process is also referred to as internalisation. The exact role of this internalisation is not known, but there are several hypotheses. One of these is that the internalisation acts to down-regulate further signalling by routing the internalised receptor to lysosomal degradation (destruction of the receptors). Another hypothesis is that the internalisation turns off the signal by providing access to dephosphorylating/deactivating protein phosphatases, and because they become unavailable for new insulin molecules. A third hypothesis is that the internalisation provides for efficient signalling, or compartmentalised signalling, by providing good access to downstream signalling intermediates. Of course, any combination of these is also possible. It is hard to distinguish between these different hypothesis experimentally, and a systems biology approach is therefore of interest. However, in this chapter we do not include a description of the actual signalling. We instead focus on the dynamics of the IR activation and internalisation. However, the IR activation may be considered as a fairly isolated subsystem, and the models developed in this chapter may therefore easily be extended to larger models, where the present models acts as a module.

### 9.1.2 Previous modelling of the system

Insulin signalling is not one of the most modelled systems in the past, even though some previous models do exist. There exists a series of models developed by Sedeghat and Quon. The latest of these models [114], includes most of the steps in Figure 9.1, and it could thus be a potential grey-box model for the system. However, when compared to the data, the disagreements are big both quantitatively and qualitatively. Further, there are several time-constants that are strange in the model (e.g., the degradation is in the order of years, and the internalisation in the order of days), and most of the concentrations correspond to less than one molecule per cell. Therefore, those models are judged to be associated with some fundamental problems that need to be handled, and they do not provide a better alternative than developing minimal models from scratch.
9.1 Background

It should also be mentioned that there are some other models for similar systems, e.g., for the EGF receptor [112] which has a similar signalling pattern as IR. They, e.g., both activate the MAPK-kinase response, through the activation of RAF and SOS-Grb2, and they are both tyrosine phosphorylated and have the possibility for internalisation. However, the internalisation processes are fundamentally different since IR is located in caveolae and EGF in clathrin-coated pits, which undergo endocytoses through completely different mechanisms. Further, the EGF receptor is dimerised after binding the ligand, while IR is dimerised at an earlier stage. Finally, the given data set has a quite significant overshoot at a very short time-scale, and this kind of data has not been present for the developments of the EGF models. All these factors have led us to the conclusion that it is better to construct a minimal model from scratch, than to simplify an existing model using model reduction.

Finally, it should be mentioned that there is a recent article by Hori [60], that also takes a minimal modelling approach to the modelling of the IR subsystem. There are, however, several arguments why these models too are inappropriate in the current study. First, that article looks at data from hepatocytes. Second, the data is also occurring on a quite different time-scale; their first measured time-point after the step is at 10 minutes, and the most interesting dynamics within our data has occurred already after 3 minutes. Finally, there is no overshoot in their data, and this overshoot is the main source of information in our data set. All these differences together leads to quite different mechanisms, model structures, and parameter values in the corresponding models. We therefore judge it to be best to disregard also these previous modelling efforts, and start from scratch.
9.2 Development of a minimal model using hypothesis testing

Experimental data

All the experimental data that have been used for the model developments have been collected on human adipocytes using Western blotting. Western blotting only allows for measurements of relative changes, i.e., the signals come with an unknown scaling factor. We have measured phosphorylated and total IR. The phosphorylation signal has been divided by the signal for the total concentration, to achieve a more robust measurement signal. There is thus only one signal to use for the modelling, one describing the relative phosphorylation degree of IR.

The experimental data for the model developments are plotted in Figure 9.2. The data is collected during 15 minutes at 10 time points following a step in the external insulin concentration. There is no extracellular insulin concentration before the step, and the step, which is taken at \( t = 0 \), is taken to an insulin level of 0.1\( \mu \)M. As can be seen, the cellular response is higher in the beginning than in the end. This particular behaviour is henceforth referred to as the overshoot in the data, and it will be used to draw several non-trivial conclusions regarding the system.
Figure 9.3 Six of the examined model structures. The three upper ones are $M_1$, $M_2$, and $M_3$ (left to right), and the three lower ones are $M_{1i}$, $M_{2i}$ and $M_{3i}$. The grey shape corresponds to the membrane.

Model development

The model development occurs through an iterative process leading to a minimal model for the given system and data. Some of the most important model structures for the IR subsystem are outlined in Figure 9.3. The most simple model structure is given in the upper left corner, and the final model for the IR dynamics is given in the lower right corner. The upper row of models are without internalisation, and the lower ones with internalisation. A model is rejected if its ability to describe the system and data is unsatisfactory, or if a more complex model can achieve a statistically significant improvement. The process ends when a model is achieved that gives a satisfactory description of the system and data, and where a more complex model does not significantly improve the fit.

A model with simple reversible kinetics

The simplest model we consider is denoted $M_1$. It is given by a reversible phosphorylation and dephosphorylation of IR, where both the reactions occur with mass action kinetics and without intermediate complex formations. The interaction graph for $M_1$ is depicted as the upper left plot in Figure 9.3.

A parameter optimization is used to optimize the agreement between the data and the current model structure. What is judged to be optimal is specified by a cost function. Different choices are available. Here we choose a least squares based framework, where each time point and signal is associated with an individual
Figure 9.4 The agreement between $\mathcal{M}_1$ and the mean values in the data. As can be seen the agreement is not very convincing, and the clear overshoot in the data is completely absent in the model.

weight. The optimization problem is solved using a combination of the global simulated annealing method, and the local but not gradient based nonlinear simplex method (see Chapter 6.1).

The result of the optimization of the present two-state model structure is plotted in Figure 9.4. As can be seen, the agreement is not particularly convincing, and the clearly pronounced overshoot in the transient data is totally absent in the model. When an agreement between a model and its estimation data is unacceptably poor, there are several possible explanations to consider:

1. The search has failed due to, e.g., problems with local minima
2. The cost function does not accurately describe what is judged to be most important in the data
3. There is something wrong with the data
4. The model structure is incapable of explaining the data

It is only in the case of option 4, that the model structure can be rejected. In general all the other options should therefore be rejected first. However, for this specific model structure it is possible to show that it is option number four in a more direct way. This is done by an analysis of the requirements for an overshoot.

The overshoot is significantly present in all three experiments. Therefore it is judged to be a trustworthy part of the data set, and something that a final model
must be able to capture. (This means that we reject possibility number three above.) Using methods from linear theory, it is straightforward to show that it is not possible to generate an overshoot with the given model structure. If the formulation of the input signal is changed, the system becomes a linear set of differential equations. This system is then written on an input-output form, and the corresponding zeros in the transfer function are analysed (see [24] for details concerning these transformations). The zeros and complex poles in the transfer function describes the basic qualities of an overshoot in a model, as a function of the parameter values. However, for this particular model structure, it turns out that there are no zeros or complex poles present for any parameter values. This means that this model structure is incapable of producing an overshoot. It is thus apparent that the mismatch between the model and the data in Figure 9.4 must be due to option number 4 above. The model structure $M_1$ is thus rejected.

**Addition of complex intermediates**

The reason that model structure $M_1$ cannot produce an overshoot is most likely due to its lack of delays in the feedback; the directly phosphorylated state is directly influencing the reproduction of the unphosphorylated state. In the next model structure, $M_2$, a delay has been added through an intermediate state in the phosphorylation. This means that there is one more state before the input

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*Figure 9.5* The agreement between $M_3$ and the mean values in the data. As can be seen there is a slight overshoot at the right place, but it is way too small. This is nevertheless the best agreement for a model without internalisation.
signal is returned to itself, and this should give possibilities for an overshoot in the system. This is also observed for some parameter values, in some states. However, the state which has been measured, IR-P, does not show any overshoot for any parameter values made in a parameter search. This prediction is also verified analytically using the transfer function analysis above. The model structure $M_2$ is thus also rejected because of its inability to generate an overshoot in the transient IR-P simulations.

The fact that an overshoot was observed in some states shows that the introduction of intermediate states are successful in generating the necessary delay. Another place for complex formation occurs in the dephosphorylation; this complex is present in model structure $M_3$. $M_3$ is exactly the same model structure as $M_2$, just with another interpretation of the state variables. In the new model structure $M_3$ there are two states that corresponds to the measured signal: IR-P and IR-P-ins-PTP. The measured signal is proportional to the sum of the concentration of these two species. In this model structure IR-P gives an overshoot for some parameter combinations, but not IR-P-ins-PTP (which is exactly the same result as for $M_2$). This means that the sum of the two signals can produce an overshoot, even though it will be less pronounced since half the signal is without an overshoot. The important question is therefore: Can this overshoot have the same shape as the measured one?

This question is first treated through a parameter estimation approach. A result of such an estimation is shown in Figure 9.5. As can be seen the agreement is qualitatively correct, i.e., an overshoot occurs at around $t = 1 \text{ min}$. The quantitative agreement, on the other hand, is less than optimal. We therefore need to consider possibility 1 and 2 above above. The apparent mismatch between the model and the data could, e.g., be due to the fact that a local minima has been found, or because the cost function does not give a good representation of what the eye thinks is important. Both these problems are common when doing system identification, and have thus been treated extensively. Several different weights have been tried, in order to put more or less emphasis on various aspects of the experimental data. Further, the simulated annealing has been started in several different places, and with a high temperature. Neither of these efforts have made a significant improvement compared to the match in Figure 9.5. Since the model only contains four parameters this should be enough to cover all its possible behaviours; we therefore conclude that the agreement in Figure 9.5 is the best agreement that is possible with the model structure $M_3$.

The question about the agreement is then treated using statistical testing. Statistical tests are a sound way to examine whether a given model shows predictions that lie within the statistical uncertainty in the data, and an introduction was given in Chapter 3.4. Such tests are typically of one of the following two types: i) tests that are done on a single model, and ii) tests that are done to compare two models. We will now perform a test of the first kind, and then, in the next subsection, a test of the second kind.

The test we will do now is a $\chi^2$ test and the test function is given by the sum of the square of the normalised residuals as described in equation (3.5). The normalisation is done by a division with the standard deviation of the measurement noise. Since the model structure $M_3$ is a mean value model, which does not take
9.2 Development of a minimal model using hypothesis testing

Figure 9.6 The cumulative density function for $\chi^2$ of order 8.

experiment to experiment variations into account, the most appropriate estimate for the measurement noise is obtained by considering the statistical variation in each time point. With these assumptions, the model is clearly rejected also by the $\chi^2$ test. All of the model structures $M_1$, $M_2$, or $M_3$ are therefore rejected.

**Addition of receptor internalisation**

Several model structures with small variations to $M_3$ were tested. Such examples include the addition of reversible steps in some of the velocities. Another thing that was tried is the possibility for multiple phosphorylation of IR, something which is known to be a non-neglectable part of the total IR phosphorylation (a model like $M_3$ would thus assume that all such states are lumped together), see Section 9.3 below. However, neither of these variations led to a fit that was significantly better than the one in Figure 9.5.

The addition of internalisation, on the other hand, gives significant improvements. Two initial model structures were tried, $M_{1i}$ and $M_{2i}$, using various extensions of the $M_1$ model structure. The first of these, $M_{1i}$, included a direct dephosphorylation of IR-P through an internalisation reaction. This model structure did not have the ability to achieve an overshoot in the relevant model outputs. The second model, $M_{2i}$, structure included an internalised intermediate, IR$_i$-P, but it could still not produce an overshoot in the relevant model output. Both these model structures were therefore easily rejected in the same way as, e.g., $M_1$.

The next model structure, however, shows the ability to give an overshoot. This model structure, $M_{3i}$, includes two internalised states: IR$_i$ and IR$_i$-P. A parameter optimization was done, and the resulting agreement is shown in Figure 9.7. As
can be seen the agreement looks much more convincing than those without internalisation. This impression is supported by the comparison of the corresponding cost functions at the optimum: \( V_N(M_3(b_p)) = 2850 \) and \( V_N(M_{3i}(b_p)) = 150 \), which means that the cost function is reduced more than a factor 10. Further the model structure passes a \( \chi^2 \) test. It is thus the first non-rejected model structure.

The question is then if there are model structures that give rise to an even better agreement. In this respect it is also important to ask whether such an improvement really is statistically significant. This is important since, in the general case, there is a relation between the model complexity and the best possible agreement with a given set of data. This is especially important when considering even more flexible model structures, like neural networks, since these can be made to fit almost any data arbitrarily well with a sufficiently high model order. This leads to the risk of over-fitting, and if there is not much data available for validation, this should definitely be taken into account. In our case, the problem of over-fitting should not be that severe, since we are working with physically based model structures. They typically have a limit to their flexibility (as could be seen, e.g., from the fact that all models without internalisation could be rejected).

There are several methods for comparing two models, where these issues are taken into account. One of them is the likelihood ratio test, and it is shortly described in Chapter 3.4. Another test is the Akaike Information Criterion (AIC), and this is the option we will choose now. AIC was especially developed to situations where the amount of data is scarce, i.e., where no validation data is present. The test gives a trade-off between model complexity and agreement. The reasons
Development of a minimal model using hypothesis testing

for the trade-off is to achieve as small model as possible, and to avoid over-fitting. The AIC is computed according to

$$\log((1 + 2 \frac{r}{N} V_N))$$  \hspace{1cm} (9.1)

where \(r\) denotes the number of (identifiable) parameters, \(N\) the number of measurement points, and \(V_N\) the cost function. The cost function should be unweighted but scaled with the number of samples, i.e.,

$$V = \frac{1}{N} \sum_{k=1}^{N} (y(t_k) - \hat{y}(t_k, \hat{p}))^2$$  \hspace{1cm} (9.2)

where \(y\) and \(\hat{y}\) have their usual interpretation of the measured and simulated output, respectively. A related measure, also introduced by Akaike, is the Final Prediction Error (FPE) and it is given by

$$\frac{1 + \frac{r}{N}}{1 - \frac{r}{N}} V_N$$  \hspace{1cm} (9.3)

When comparing to model structures it is the model with the lowest value of these criteria that is chosen as the superior model. Neither of these tests give a significant improvement for any of the more complex model structures tried.

**Rejection of complex models with internalisation**

Above we showed that all models without internalisation could be rejected (except for one particular model, which needs additional reasoning, see Section 9.3 below), but that a rather simple model with internalisation could give a quite convincing fit with the data. It is therefore interesting whether there are complex models including internalisation that are not extensions of the \(M_{3i}\) model structure that also may be rejected based on the agreement alone. We investigated this using the same methods as above.

The analyses showed that quite many models with internalisation could be rejected. In fact, all tested models without the recycling from the free intracellular IR gave a visibly worse agreement with the data. Since these models included more interactions and parameters than \(M_{3i}\), and had a higher cost function, they were also rejected by the statistical tests. One such model structure is depicted in Figure 9.8, and the corresponding agreement is given in Figure 9.9. It is noteworthy that this model has both a reversible phosphorylation and internalisation, and contains the intracellular dephosphorylation. It is therefore clear that the possible route of internalisation and recycling without dephosphorylation is not sufficient to generate the given behaviour. It is remarkable that so much information can be extracted from so few data points, data points that gives information about approximately two kinetic parameters (see below). This clearly shows the strength of the hypothesis testing approach.
**Figure 9.8** A rejected model structure with many interactions but no recycling of the free IR.

**Figure 9.9** The agreement between the model structure in Figure 9.8 and the data. As can be seen the agreement is fairly good, but still worse than that of the chosen minimal models ($M_3$ and $M_c$). This illustrates that we seem to be able to reject all model structures without the recycling of the internalised receptors.
9.3 Towards a core-box model

At this point we have found a model $\mathcal{M}_{3i}$, which both gives an acceptable agreement with the data, and that is not worse than any other more complex model structure in a statistically significant way. We will now analyse this model and try to extend it to a full core-box model.

**In silico experiments with $\mathcal{M}_{3i}$**

Figure 9.10 shows simulations of some of the key components in the $\mathcal{M}_{3i}$ model. The upper plots show the time traces for the two phosphorylated versions of IR-P. As can be seen they are following each other closely. Further, they are of a similar magnitude, and do therefore both contribute to a significant part of the measured IR-P signal. This means that it is hard to distinguish between the kinase activity from the internalised and membrane bound IR-P under the present working conditions. This therefore indicates that one should probably look for other input signals, if a study should be made to investigate the role of the internalisation in the kinase activity.

There are several other observations that can be done from the simulated curves in Figure 9.10. It can for instance be observed that the total amount of phosphorylated receptors is significantly lower than the total amount of receptors. This means that it is predicted that it is only a small portion of the receptors that are phosphorylated at a time. It is also interesting to see how much of the receptors are internalised in the model, and at what time scale this happens. Since IR-P and IR$_{int}$-P follow each other so closely, the internalisation of the phosphorylated
receptors is fast, and a significant part of the phosphorylated receptors leave the membrane for internalisation. The flux to the internalisation of IR-P is much higher than the flux leading to direct dephosphorylation. The receptors are dephosphorylated (since the rapid rise in the total IR-P is quickly lowered), and this is therefore mainly due to the intra-cellular dephosphorylation. A large part of the dephosphorylated receptors returns to the membrane, but the long-term trend is anyway that the pole of free IR leaves the membrane and enters the cytosol. However, it should be noted that the main mechanisms for this is through the intermediate phosphorylated state. This long-term changes are of the same time-scale as the slow decline in the data (taking some 3-5 minutes). All this means that there is a slow trend for the internalisation of the free receptors, even though the internalisation of the phosphorylated receptors is rapid.

Towards an identifiable core model

These differences in time-scale and magnitude of the different fluxes allows for model reductions of the $M_{3i}$ model. In Chapter 7.3 we described two types of model reductions that are especially fit for the core-box modelling framework: sensitivity analysis based elimination, and variable lumping. Both these methods are applicable to the $M_{3i}$ model. The flux in the dephosphorylation at the membrane is so low that it may be eliminated without visibly effecting the output of the model. Likewise, the two phosphorylated states IR$_P$ and IR$_I$ are so highly correlated that they may be pooled into a single state with a good approximation. This leads to the top model structure depicted in Figure 9.11. An optimization with this model structure leads to the agreement displayed in Figure 9.12, and as can be seen the agreement is just as good as for the $M_{3i}$ model structure. We have therefore reduced one state and two parameters without sacrificing the agreement with the data, and using only the reduction methods with known back-translations. The resulting model structure contains only three states and four parameters and is henceforth referred to as the core model structure $M_c$.

However, the parameters in the $M_c$ model does not seem to be practically identifiable. The 50% uncertainty regions for the parameters were calculated using equation (4.11), and the result is given in Table 9.1. As can be seen, the uncertainty region for all four parameters are larger than the nominal values, and this indicates that the uncertainty is larger than the method can handle (since negative values of, e.g., the $k_P$ parameter clearly is unacceptable). By fixing one or two of the parameters in the model to their estimated values, the uncertainty regions are quickly reduced to about 30% of the nominal values (for $k_{ID}$ and $k_R$). However, such a model reduction does not give any clear indication to what it is that has really been estimated. The best thing in this situation would be to establish the nonlinear uncertainty regions in the model, and use an analysis of this region to find out what the identified features are for the system. Another approach would be to normalise the model so that the scaling parameter vanishes. Unfortunately, such analyses are outside the scope of this dissertation.

However, a robustness analysis has nevertheless been performed by comparing simulations for different acceptable parameter regions in the different acceptable model structures. This analysis indicates that several of the above mentioned
Figure 9.11: Three possible model structures in the core-box model. The top one is the core model, the minimal one is the one chosen from the minimal modelling, and the lower one is an example of a full-scale grey-box model for the system. Translations between all model structures is possible using only the methods from Chapter 7.3, which means that the models will be fully interconvertible, and that the resulting core-box model will have the possibility of 'zooming'. Finally, the hand-drawn line encircles the variables that are pooled together into the IR·P variable in $\mathcal{M}_c$. 
Figure 9.12 Agreement between the data and the $M_c$ model structure.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Nom. Value</th>
<th>$\delta_4$</th>
<th>$\delta_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_P$</td>
<td>7.956</td>
<td>141.9</td>
<td>39.2</td>
</tr>
<tr>
<td>$k_{1D}$</td>
<td>0.892</td>
<td>1.30</td>
<td>0.25</td>
</tr>
<tr>
<td>$k_R$</td>
<td>0.350</td>
<td>0.87</td>
<td>0.16</td>
</tr>
<tr>
<td>$k_Y$</td>
<td>17.9</td>
<td>39</td>
<td>fixed</td>
</tr>
</tbody>
</table>

Table 9.1 Values and uncertainty regions for the parameters in the $M_c$ model. As can be seen, if one of the parameters is fixed (here the unknown scaling factor), two of the other parameters may be fairly well estimated. An uncertainty greater than the nominal value is of course not realistic, and this simply indicates that the uncertainty is greater than the current method can deal with.

observations seem to have a high robustness. One of the robust features seems to be that the internalised unphosphorylated receptors grew with the time constant of 3-5 minutes. Another robust feature seems to be that both the internalised and membrane bound receptors display similar shapes at similar concentrations, which is then of course also similar to the measured IR-P signal. This high correlation between the states is a support for the lumping that has been done to obtain $M_c$. There are, however, also some dissimilarities between the different models. These unrobust predictions by the model concerns the maximum levels in absolute terms of the concentrations. In some cases, the total phosphorylated states accounted for some 70% of the total amount of receptors (at the peak value), while in some
other cases the total amount of phosphorylated receptors never accounted for more than 1-2% of the total amount of receptors. Likewise, the values of the common parameters between the models (\(k_P, k_D\) and \(k_R\)) show variations with at least an order of magnitude.

Finally, these robust properties may be used to reject the possibility of an interpretation of, e.g., the \(M_c\) model structure with the internalised free receptors representing the membrane bound receptors that are dephosphorylated but still bound to the protein phosphatase. This is possible because such a model would have a large and growing pool of this highly transitory state. Since this is a robust prediction of the models, and since such a phenomenon is quite unlikely biochemically, we may therefore reject this interpretation of the acceptable model structures. These latter rejections illustrates that modelling is a recursive process, where decisions and assumptions has to be re-examined several times, as new information is revealed by the analysis.

**Grey-box models and translations**

Finally, the translation between \(M_c\) and \(M_{3i}\) is straightforward, since only two parameters and one state are missing, and since only the methods in Chapter 7.3 have been used. The formulas for \(\Phi\) and \(\Psi\) are therefore given by equations (7.24), (7.25) and (7.28). It is possible to extend the translations to even larger grey-box models. One such model is depicted at the bottom in Figure 9.11, and it is referred to as \(M_g\). The formulas for \(\Phi\) and \(\Psi\) for this larger model is given by the same equations as for the translations to and from \(M_{3i}\): the reversible reactions and the degradation and synthesis of the internalised receptors have such a low flow that they may be eliminated, and the different phosphorylated states may be treated as a single lump. This clearly shows that the reductions and extensions that are possible with only the well-characterised reduction techniques (lumping and SA-based elimination) may yield quite substantial translations. However, a full analysis of the translations, especially of the translations of the uncertainties, is outside the scope of this dissertation.

**Summary of Chapter 9**

This chapter has presented a modelling approach to the study of insulin receptor activation and internalisation in human fat cells. The main results are due to a minimal modelling approach using hypothesis testing, although some steps towards a complete core-box model also have been taken. The hypothesis testings show that all models structures without internalisation give an unacceptable agreement with the data. Furthermore, even complex models including both internalisation and intra-cellular dephosphorylation results in an unacceptable agreement with the data. However, if the recycling of the free receptors is included, a simple model structure with only three states and three kinetic parameters is sufficient to explain the data. The three-state model may serve as a core model, even though its identifiability is not possible to establish using the traditional local sensitivity based methods. Nevertheless, a back-translation from the core model to relatively
comprehensive models is possible to establish using only reduction methods with known back-translation formulas. This means that the resulting core-box model does not yet have a well-characterised identifiable core, but that it may anyway serve as a module with 'zooming' in future hierarchical models describing insulin signalling and glucose homeostasis.
Improving the Identifiability of a Model for Yeast Glycolysis

We will now return to the system and model that we started our developments with: the Hynne model describing yeast glycolysis. As was described in Chapter 2 yeast glycolysis has been studied in much detail, both in terms of individual kinetic studies of the different enzymes, and in terms of \textit{in vivo} measurements and models describing the whole system. In a work by Teusink [130], an almost complete set of enzyme characterisations \textit{in vitro} was put together into a white-box model which was then compared with \textit{in vivo} measurements. The agreement was quite poor, and this is an indication that white-box modelling (i.e., no subsequent optimization) is not sufficient for these complex systems. In a subsequent work by Hynne the same data were re-used, some new data describing \textit{in vivo} oscillations around a Hopf bifurcation were collected, and, most importantly, a model optimization was done. The resulting grey-box model (the Hynne model) showed a much better agreement with the data. The agreement was good also for separate and qualitatively different validation data.

However, as we pointed out in Chapter 3, the agreement between the Hynne model and the validation data is only a good beginning, and not the end of a modelling development. This is due to the fact that the Hynne model is highly over-parametrized with respect to the available data, and that it is therefore not yet possible to draw any specific conclusions regarding the reliability of various model predictions. To be able to get such quality tags to predictions of a model, without reducing the study to minimal modelling, we proposed the core-box modelling framework. Its main steps after the development of the grey-box model are: identifiability analysis, model reduction, system identification and back-translation. Thereafter, the remaining chapters in part I, Chapters 4 to 7, were devoted to reviews and new developments within these separate research fields. With this
accomplished we are now ready to return to the Hynne model, for demonstrations and testing of some of the new methods.

A sound modelling work is typically associated with one or several specific questions, that the model should help answering. In this chapter we answer several questions, since several new methods are applied. One of the biological questions is the mechanism problem for the oscillations. This problem has already been analysed for the Hynne model, and our contribution will be a more robust analysis result. A review of the previous research around this question, and of previous yeast glycolysis research in general, is given in Section 10.1. Another question we pose is what the difference is between the existing in vitro characterisations of the enzymes, and the information that can be extracted from data about the corresponding in vivo actions. The relation between in vivo and in vitro is at the heart of the understanding of life, and it is therefore a very interesting problem. However, the given rate expressions are not identifiable with respect to the available in vivo data. This problem is handled using the methods developed in Chapter 4, and the result is presented in Section 10.2. The mechanism problem is solved with the methods in Chapter 5.2, and the results are presented in Section 10.3.1. Finally, we apply the MATLAB implementation developed in Chapter 5.3 to the Hynne model in Section 10.3.2.

10.1 Background

10.1.1 Biological background

Saccharomyces cerevisiae, or “baker’s yeast”, is one of the most well studied model organisms. There are many reasons for this. Some reasons are related to the fact that it is easy to do experiments on yeast. Yeast cells are cheap, easy to grow in a well-defined way, and there are no ethical issues. From a modelling perspective it is also highly advantageous that there is a high degree of consensus between different labs regarding the way the cells are prepared. Many data sets are therefore comparable and have a high degree of reproducibility not only between different days, but between different labs. Another important reason why yeast is such an attractive model system is because it has a high similarity to mammalian cells. Yeast is a simple single cell organism, but it is nevertheless eukaryotic (i.e., it has a cell nucleus). There are furthermore many biological modules that are highly preserved. Much work can therefore be done on yeast cells, even when the ultimate goal is to understand, e.g., a human disease. Two enzymes that are functionally and structurally related, but situated in different species, are denoted homologs. Important homolog couples between yeast and mammalian systems are, e.g., the Snf (yeast) vs AMP-kinase (humans) couple, and different versions of the MAPK-cascade (see Figure 10.1 and Chapters 8 and 9). There are actually processes that are even more preserved than enzymatic networks, namely the metabolic reactions. A highly preserved metabolic network is the most central one of them all: glycolysis.

Yeast glycolysis is probably one of the most well-studied biological systems. Virtually every enzyme in glycolysis has been well-characterised in numerous in
vitro experiments, and there are several unusually good data sets with intracellular in vivo measurements of intact yeast cells (see [62] and the references therein). One phenomenon appearing in glycolysis is particularly well-studied: the temporal oscillations in starved yeast cells treated with glucose and cyanide [8, 32, 71, 99, 103, 136] (for recent reviews, see [102, 137]). They are depicted in Figure 10.2, and as can be seen the period of the oscillations is about 20 sec. These oscillations occur both in batch (a closed reactor) and in a continuously stirred tank reactor (CSTR). In a CSTR all substances, including the cells, are continuously replaced. That is especially beneficial from a modelling perspective, since the circumstances can be kept constant for as long as one desires, and since the user has total control of the cellular environment. This includes the oscillations, which can be made to run for at least several days [32]. That also means that the experiment reproducibility in a CSTR is high. Another advantage with this particular model system is due to the preparation of the cells. The preparation has been developed to turn off as many functions of the cells as possible, except for glycolysis; and although there are still other processes going on, glycolysis can be considered as a relatively isolated subsystem.

It should be mentioned that there are other oscillatory phenomena in yeast cells, appearing under other preparations and on other time-scales [69, 78–80, 129]. Some of these oscillations are linked to various other biological functions, like the cell-
cycle (the cycle by which a cell divides, see e.g. [79]). There is to date no evidence that the above mentioned cyanide-induced glycolytic oscillations [32] are linked to any other biological cycle. There are nevertheless several interesting questions regarding them that have received high scientific interest: Why are the oscillations appearing, is it because they are optimal in some sense, or is it just non-significant outcome of the nonlinear interactions in the system? Which interactions constitute the oscillating core generating the oscillations, and which parts of the system are just following the oscillations? How are the several billion cells synchronised? What regulates the features of the oscillations? etc. etc. All these questions have been extensively studied, and often using modelling.

10.1.2 Previous modelling

Much of the early modelling work were devoted to the study of individual enzymes, or to minimal models for glycolysis as a whole. Since glycolysis is so central, virtually all enzymes have been well-characterised in numerous in vitro experiments. It has been going on for a long time, but especially the 60’s and 70’s were very active in this field. Such in vitro could, e.g., be performed by comparing the best fits from different kinetic expressions with in vitro data. There are, however, also many other types of studies. It should be noted that even though many of the enzymatic steps have been fully characterised, like for instance the phosphoglucoisomerase reaction (PGI), there are some steps which kinetic expressions still are debated within the society. One such step is the glucose transporter, and this is partly due to the fact that this step can not be examined in in vitro studies [99,105]. There are also studies of the ability of the in vitro characterised kinetic expressions to explain the in vivo behaviour [7,141]. Such studies are a kind of miniature of the
study that Teusink and Hynne did for the whole glycolysis [62, 130]. We initiate
an updated version of such a study for PGI in Section 10.2.2.

The minimal models describing glycolysis as a whole were primarily developed
during the same period in time as the above described kinetic characterisations,
during the 60’s and 70’s [49, 56, 119, 120, 127]. These models were often consider-
ing the qualitative behaviour, and a single phenomenon. One phenomenon that
was extensively modelled is the above described cyanide-induced oscillations, ap-
pearing with a frequency of approximately 20-30 seconds. These minimal models
were primarily intended to serve two purposes: evidence that a certain set of in-
teractions can generate oscillations, and studies of generic features of oscillations.
One of the main limitations with these early minimal models are that they are
not based on realistic parameter values, steady state fluxes etc (since these were
then not yet experimentally determined). This gave the prediction that a given
set of interactions can generate oscillations much less significance, since almost any
system with a feedback can generate oscillations, if the parameters may take any
values [123]. Glycolysis has several feedbacks, and there were thus many different
proposed minimal models, with many different predictions of possible mechanisms.
Some examples include allosteric regulation of PFK, the ATP autocatalytic stoi-
chiometry and the feedbacks in the lower parts of glycolysis (see [81] for a recent
review). In Section 10.3.1 we present an updated version of the minimal modelling
approach, where the minimal models are obtained by application of various model
reduction methods to the relatively realistic Hynne model.

As science evolved during the 80’s and 90’s, the glycolytic models grew in com-
prehension, partly due to the increased availability of reliable in vivo data. This
eventually led to the comprehensive models by Teusink and Hynne, which have
been described above and in Chapter 2. These developments allowed for an in-
creased understanding of the mechanism problem. A recent example is [81]. In [81]
it was proposed that the feedback from the lower ATP producing parts of glycolysis,
back to the ATP consuming parts, mediated primarily through the stoichiometry
of ATP and through the allosteric regulation of PFK, were the central interac-
tions generating the oscillations. We now prove the feasibility, and strengthen the
credibility, of this prediction through model reduction. We will also reduce the
unidentifiable parts of the Hynne model in various ways, and thereby bringing the
Hynne model closer to becoming a core-box model. All this will be done using the
methods developed in Chapters 4 and 5.

10.2 Handling of unidentifiable rate expressions

10.2.1 Structural non-identifiability in the dehydrogenase
reactions

In Chapter 4.2 we showed that the presence of all variables from a conserved moiety
in the same rate expression might lead to structural unidentifiability in an other-
wise identifiable rate expression. We will now shortly show that this problem is
present also for two reactions in the Hynne model, and also suggest new kinetic
identifiable expressions that may replace the existing ones, without introducing any
approximations or loss of information.

There are two conserved moieties in the Hynne model, one for the nicotinamide nucleotides and one for the adenosine nucleotides, i.e.

\[
\text{[NADH]} + \text{[NAD]} = m_1 \quad (10.1a)
\]
\[
\text{[ATP]} + \text{[ADP]} + \text{[AMP]} = m_2 \quad (10.1b)
\]

where \(m_1\) and \(m_2\) are the two total concentrations. These conserved moieties are not explicitly formulated in the model, but are implicitly present in the stoichiometric matrix. There is no kinetic expression in the model where \([\text{ATP}], [\text{ADP}]\) and \([\text{AMP}]\) appear together, and this specific problem with identifiability does therefore not appear for the adenosine nucleotides. There are three reactions including the inter-conversion of the nicotinamides: glyceraldehyde-3-phosphate-dehydrogenase (GAPDH), a lumped reaction forming glycerol from dihydroxyacetonephosphate (lpGlyc), and the alcohol dehydrogenase (ADH). The reaction ADH is formulated as an irreversible reaction, and only \([\text{NADH}]\) appears in the kinetic expression. Since not all variables from the conserved moiety are included, the ADH reaction is not covered by the theory developed in 4.2. However, both GAPDH and lpGlyc have both \([\text{NADH}]\) and \([\text{NAD}[+]\) in their kinetic expressions

\[
v_{\text{GAPDH}} = \frac{V_{\text{max}} ([\text{GAP}][\text{NAD}[+])}{K_{\text{GAP}}K_{\text{NAD}} (1 + \frac{[\text{GAP}]}{K_{\text{GAP}}} + \frac{[\text{BPG}]}{K_{\text{BPG}}} (1 + \frac{[\text{NAD}[+]}{K_{\text{NAD}}} + \frac{[\text{NADH}]}{K_{\text{NADH}}}))} \quad (10.2a)
\]
\[
v_{\text{lpGlyc}} = \frac{V_{\text{max}} [\text{DHAP}]}{K_{\text{DHAP}} (1 + \frac{K_{\text{NADH}}}{[\text{NADH}]} (1 + \frac{[\text{NAD}]}{K_{\text{NAD}}})) + [\text{DHAP}] (1 + \frac{K_{\text{NADH}}}{[\text{NADH}]} (1 + \frac{[\text{NAD}]}{K_{\text{NAD}}}))} \quad (10.2b)
\]

(Note that the indexes specifying which reaction the parameters belong to have been dropped.) Both these expressions could therefore potentially be unidentifiable due to conserved moieties as described in Chapter 4.2. Sedoglavíc’ algorithm shows that the complete Hynne model is unidentifiable even for the case when everything can be measured. Further, if all state variables can be measured (i.e., \(y = x\)) the transcendence degree is 2 and it is only the parameters that appears in GAPDH and lpGlyc that are unidentifiable. Sedoglavíc’ algorithm gives this result with a high probability, and does not provide an explanation for why it occurs, or how identifiable rate expressions may be chosen. Using the method developed in Chapter 4.2 we may answer all these questions. The analysis shows that the reason why these two rate expressions become unidentifiable due to the conserved moieties is that the constraints from the moiety makes the actual number of terms (with independent coefficient) appearing in the rate expressions one less than the number of original parameters. This is formally verified by showing that \(\text{rank}(C) = \text{dim}(k)\) in the formalism of Chapter 4.2. Using the \(C\) matrix we may also suggest the following two rate expressions as structurally identifiable alternatives to the original
Handling of unidentifiable rate expressions

\[ v_{\text{GAPDH}} = \frac{[\text{GAP}][\text{NAD}] - [\text{BPG}][\text{NADH}]}{K_{\text{eq}}(1 + \frac{[\text{GAP}]}{K_{\text{GAP}}} + \frac{[\text{BPG}]}{K_{\text{BPG}}})(k_1 + k_2[\text{NADH}])} \]  

(10.3a)

\[ v_{\text{lpGlyc}} = \frac{[\text{DHAP}][\text{NADH}]}{k_3 + k_4[\text{NADH}] + k_5[\text{DHAP}] + k_6[\text{DHAP}][\text{NADH}]} \]  

(10.3b)

where

\[ k_1 = \frac{K_{\text{NAD}}}{V_{\text{max}}} \left(1 + \frac{m_1}{K_{\text{NAD}}} \right) \]  

(10.4a)

\[ k_2 = \frac{K_{\text{NAD}}}{V_{\text{max}}} \left(\frac{1}{K_{\text{NAD}}} - \frac{1}{K_{\text{NAD}}} \right) \]  

(10.4b)

\[ k_3 = \frac{K_{\text{DHAP}}K_{\text{NADH}}}{V_{\text{max}}} \left(1 + \frac{m_1}{K_{\text{NAD}}} \right) \]  

(10.4c)

\[ k_4 = \frac{K_{\text{DHAP}}K_{\text{NADH}}}{V_{\text{max}}} \left(1 - \frac{K_{\text{NADH}}}{K_{\text{NAD}}} \right) \]  

(10.4d)

\[ k_5 = \frac{K_{\text{NADH}}}{V_{\text{max}}} \left(1 + \frac{m_1}{K_{\text{NAD}}} \right) \]  

(10.4e)

\[ k_6 = \frac{1}{V_{\text{max}}} \left(1 - \frac{K_{\text{NADH}}}{K_{\text{NAD}}} \right) \]  

(10.4f)

The detailed calculations are included in [5]. Note that these formulas allow for the values of the identifiable parameters to be directly translated from the original kinetic parameters. In the formalism of Chapter 7 this is the same as saying that (10.4) describes the forward mapping \( \phi \).

These results mean that the presence of the conserved moiety (10.1a) is the only source of structural unidentifiability if all metabolites can be measured, i.e., if \( y = x \). In the real experimental setup only NADH can be measured with a high time-resolution, and only with an unknown scaling constant \( k_{\text{scaling}} \). It is therefore interesting to note that even with \( y = k_{\text{scaling}}[\text{NADH}] \) there are only new unobservable parameters and states in the reactions associated with the membrane transport of glycerol and ethanol. These new unidentifiabilities are most likely due to the fact that no extracellular concentrations are measured. The problem with conserved moieties is therefore the only complex source of structural unidentifiability in the Hynne model, and the only source that leads to structural unidentifiability inside glycolysis.

10.2.2 Estimating in vivo parameters in the phosphoglucoisomerase reaction

We have now seen how the Hynne model may be made structurally identifiable when \( y = x \). This did not require any major reductions, but only some minor modifications in the dehydrogenase reactions. We will now turn to the more general...
problem of practical unidentifiability. For such a complex model as the Hynne model, there is a big difference between structural and practical identifiability (even though there did not turn out to be a big difference in structural identifiability by replacing $y = x$ for a more realistic assumption). We will now consider the problem of estimating the parameters in a single rate expression, and in Section 10.3 we will consider the problem of finding core models for the whole system.

Background

The reaction we will consider is the phosphoglucoisomerase reaction (PGI). The reaction converts glucose-6-phosphate (G6P) into fructose-6-phosphate (F6P). Traditionally this reaction has been considered as a fast reaction (i.e., close to chemical equilibrium), and it is a relatively non-complex reaction. For instance, the enzyme does not require any co-factors (like ATP or NADH), and it is traditionally described by simple reversible Michaelis-Menten expression

$$v_{\text{PGI}} = \frac{v_{\text{max}}}{K_{\text{G6P}}} \left( \frac{[\text{G6P}]}{K_{\text{G6P}}} - \frac{[\text{F6P}]}{K_{\text{F6P}}} \right)$$

(10.5)

where $K_{\text{G6P}}$ and $K_{\text{F6P}}$ are the Michaelis-Menten constants for the substrate and product, respectively. This is a relatively uncomplicated, but yet enzyme catalysed, rate expression with only four unknown parameters. This is one reason why this is a good test case for the \textit{in vivo} estimation methods developed in Chapter 4.3.

Another reason why PGI is a good test case for these methods is that the reaction is tractable from a measurement point of view. There are existing measurements techniques to measure $[\text{G6P}]$ and $[\text{F6P}]$ intracellularly with absolute values. Further, the flux through the reaction may also be estimated intracellularly. The reason for this is that the flux through the reaction is approximately equal to the flux through glycolysis. The flux through glycolysis is relatively straightforward to measure, since it is equal to the glucose uptake in the cell, and that can simply be estimated by measuring the extracellular glucose concentration.

However, there are some approximations associated with these measurements. First, the absolute values of the concentrations are only obtained by estimating the fraction between the intracellular and extracellular volumes in the cell suspension, and this is an estimation associated with considerable errors. Second, the flux through PGI is only the same as the flux through glycolysis under stationary conditions. If the rate of glucose uptake is estimated by numerical derivation of the extracellular glucose concentration there are errors associated both to the numerical derivation and to the non-stationarity. However, it should be mentioned that the glucose uptake has a slower time-scale than the dynamics inside of glycolysis. This is the reason why it can be assumed that the flux through PGI is quasi-stationary and equal to the glucose uptake, even though the glucose uptake is varying over time. These second types of errors would be improved if one would instead work in a CSTR, with a constant glucose inflow and uptake for each measurement. Third, the flux through PGI is not the same as the glucose uptake even under stationarity conditions. There is always a flux to storage and to the pentose pathway, and
10.2 Handling of unidentifiable rate expressions

measurements of these fluxes are also associated with errors. Nevertheless, despite all these sources of errors, measurements are available for both the flux $v_{PGI}$ and for the concentrations involved in the kinetic expression: $[G6P]$ and $[F6P]$. Therefore, the conditions of Chapter 4.3 are fulfilled and the methods developed in that chapter may therefore be used to obtain an in vivo characterisation for PGI.

**Results on simulated data**

We start by solving the problem for simulated data, because there we may, e.g., turn off the noise level and test the algorithms more easily. Simulated data containing $v_{PGI}$, $[G6P]$ and $[F6P]$ under steady state conditions is included in Figure 10.3. The data has been obtained using continuation of the Teusink model (which has the same rate expression for PGI as the Hynne model), and the external glucose concentration has been varied between such values that reasonable fluxes have been obtained. This data is of the same qualitative character as the available experimental data. We may make the data even more realistic by introducing noise, and also then we will have the important advantage of knowing and controlling all the effects the noise has on the data.

It is outside the scope of this dissertation to do a full analysis of the expres-
Chapter 10  Improving the Identifiability of a Model for Yeast Glycolysis

Figure 10.4 Comparison between the different types of model structures and the original data.

sion and data, and we therefore restrict ourselves to using the second method of Chapter 4.3, that was presented in Chapter 4.3.2. This method takes a minimal modelling approach to the characterisation of the core expression, and makes all the characterisations and comparisons in the 'language' of the parameters in the reduced model \( p' \in \Omega' \).

We start the minimal modelling with the three options for simple mass action kinetics

\[
\begin{align*}
\mathcal{M}_1: \quad v_{\text{PGI}} &= \ k_1^f [\text{G6P}] \\
\mathcal{M}_2: \quad v_{\text{PGI}} &= \ k_2^b [\text{F6P}] \\
\mathcal{M}_3: \quad v_{\text{PGI}} &= \ k_3^f [\text{G6P}] - k_3^b [\text{F6P}]
\end{align*}
\]

where the subscripts \( f \) and \( b \) refer to forward and backward, respectively, and where the superscript numbers refer to the number of the model structure. Remember also that \( \mathcal{M} \) is the symbol for model structure. The agreement between \( \mathcal{M}_2 \) and the estimation data is shown in Figure 10.4. As can be seen the agreement is quite
10.2 Handling of unidentifiable rate expressions

poor. This is verified by a $\chi^2$ test which shows all the mass action kinetics based model structures $\mathcal{M}_1$ to $\mathcal{M}_3$ may be rejected.

With these simple models rejected we turn to the most common simple non-linear options, the irreversible Michaelis-Menten expressions. With conventional notations these expressions are given by (see Chapter 2)

$$ \mathcal{M}_4 : \quad v_{\text{PGI}} = \frac{V^4_{\text{max}} [\text{G6P}]}{K^4_{\text{M}} + [\text{G6P}]} $$

$$ \mathcal{M}_5 : \quad v_{\text{PGI}} = \frac{V^5_{\text{max}} [\text{F6P}]}{K^5_{\text{M}} + [\text{F6P}]} $$

As can be seen in Figure 10.4 the agreement between these model structures and the simulated data from the full model is much better than that for the linear model structures $\mathcal{M}_1$, $\mathcal{M}_2$ and $\mathcal{M}_3$. This observation is confirmed by a $\chi^2$ test that shows that both $\mathcal{M}_4$ and $\mathcal{M}_5$ passes the test.

Even with these models established as acceptable models, we need to investigate whether there are other model structures that give a statistically improved agreement. For this we try the following three extended model structures

$$ \mathcal{M}_6 : \quad v_{\text{PGI}} = \frac{V^6_{\text{max}} ([\text{G6P}] - \frac{[\text{F6P}]}{K^8_{\text{eq}}})}{K^6_{\text{M}} + [\text{G6P}]} $$

$$ \mathcal{M}_7 : \quad v_{\text{PGI}} = \frac{V^7_{\text{max}} [\text{G6P}]}{1 + \frac{[\text{G6P}]}{K^8_{\text{G6P}}} + \frac{[\text{F6P}]}{K^8_{\text{F6P}}}} $$

$$ \mathcal{M}_8 : \quad v_{\text{PGI}} = \frac{V^8_{\text{max}} ([\text{G6P}] - \frac{[\text{F6P}]}{K^8_{\text{eq}}})}{1 + \frac{[\text{G6P}]}{K^8_{\text{G6P}}} + \frac{[\text{F6P}]}{K^8_{\text{F6P}}}} $$

All of these agreements are similar to that of $\mathcal{M}_4$ and $\mathcal{M}_5$. This is verified by an AIC test shows that the additional of the complexity in the models does not improve the agreement with the data enough to justify these models over $\mathcal{M}_4$ and $\mathcal{M}_5$. Since these latter model structures also include the true model structure it is likely, therefore, that either of the model structures $\mathcal{M}_4$ and $\mathcal{M}_5$ are the ones to choose. Note, however, that there still might exist a model structure that does give an improved agreement, even though it most likely one with only one or two parameters. Finally, the choice between $\mathcal{M}_4$ and $\mathcal{M}_5$ could be made by a statistical test (which here would mean to compare the cost functions). However, we instead decide to make the choice by biochemical reasoning. $\mathcal{M}_4$ has a rate expression that is dependent solely on the substrate, and $\mathcal{M}_5$ has a rate expression that depends solely on the product. We find a substrate dependency more convincing, since the net velocity of $v_{\text{PGI}}$ is greater than zero for the whole data series. We thus choose $\mathcal{M}_4$ as the minimal model for this data.
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Results on experimental data

Let us now turn to the real data, and to the problem of comparing the previous in vitro characterisations for PGI with the current in vivo estimations. It is outside the scope of this thesis to do a full analysis. An initial analysis, however, reveals that the a linear model structure seems to almost as capable of describing the experimental data as a nonlinear model structure. This is probably due to the fact that the experimental system is even less excited than the simulated steady-state data. Since the numerically determined forward translation $\phi$ in equation (7.2) is based on the assumption that the core model is identifiable, we therefore use $M_1$ above when comparing the the in vivo and in vitro characterisations. The in vivo estimated parameters for this model structure is

$$k'_f = 0.021$$ (10.6)

Some of the in vitro parameters for this system are [130]

$$(V_{\text{max}}, K_{G6P}, K_{F6P}, K_{eq}) = (p_{1}^{\text{ivt}}, p_{2}^{\text{ivt}}, p_{3}^{\text{ivt}}, p_{4}^{\text{ivt}}) = (340.20, 1.4, 0.3, 0.29)$$ (10.7)

and the translated value of these values according to equation (7.2) are

$$k'_f(p^{\text{ivt}}) = \phi(p^{\text{ivt}}) = (0.4)$$ (10.8)

This is therefore a first indication of the difference between the in vivo and in vitro activity of reactions. However, there are many questions that needs to be resolved before one knows how to relate to the obtained difference, for instance regarding the uncertainty regions of the two estimates. Unfortunately such analyses lie outside the scope of this dissertation.

10.3 Core-models

We now turn to the problem of finding a core model corresponding to the complete Hynne model. We do this in two different ways: i) identification of the oscillating core, and ii) by applying the MATLAB program developed in Chapter 5.3. In the first of these steps we get a preliminary answer to the mechanism question mentioned above: What is the mechanism that generates the experimentally observed oscillations? This result will only be valid for the Hynne model. However, the analysis also produces several suggestions for oscillating core-models. Such models, together with tools like the MATLAB program of Chapter 5.3, are useful for the development of an identifiable core-model, and for the long-term goal of developing a core-box model. Such a model would allow us to answer the mechanism problem in a reliable way also for the real system.

10.3.1 The 'real' oscillating core model

We will now apply the methods developed in Chapter 5.2 to the Hynne model. This will result in biochemically meaningful reduced models, that still captures the basic dynamical behaviour of the Hynne model: oscillations close to a Hopf bifurcation.
The smallest of these models is only 3-dimensional, and it is thus comparable in size to the minimal models developed in the 60's and 70's (as reviewed in Section 10.1.2 above). Those models suffered from the weakness of non-realistic parameter and steady state values, and could therefore only give predictions of possible core-models. The now obtained models have inherited much of the fluxes and steady state values of the Hynne model, and since this model is quite realistic in these respects, the new models will be significantly improved compared to the previous ones. We can therefore say that we will be able to judge which of the many previous suggestions, that seems to have been the 'real' oscillating core model. We present the analysis in the four steps of Figure 5.3: initial analysis, reduction with conserved dynamical properties, new analysis, comparison. We end with an analysis of the biochemical properties of the models and with a discussion of the results.

**Step I: Initial analysis**

As analysis methods we use the mode-interpretation method, and a ranking method. The mode-interpretation method was first presented in [31], and it is based on an amplitude-phase plot of all the metabolites, obtained through either experimental (or simulated) time-series data, or through the eigenvectors spanning the center space. The ranking method was first presented in [110], and it is based on a sensitivity analysis of the linearised system: a state variables is ranked according to how small perturbation is needed to the affect of the variable to be able to eliminate the oscillations. Both these methods have already been applied to models for glycolytic oscillations, in [81] and [110], respectively. Both the results pointed to the same mechanism: the feedback from the lower ATP producing steps of glycolysis back to the upper ATP consuming steps. The result for the Hynne model, is the same and it is shown in Figures 10.9 and 10.10, respectively. A more detailed analysis of the figures is given in step III below, when the results for all the models are compared.

**Step II: Reduction with conserved dynamical properties**

Figure 10.5 provides an overview of the three new models obtained through the two model reduction techniques. The first is obtained through the lumping and optimization method (L), the second through the elimination of dynamics method (E), and the final model is obtained through the application of the E method to the first reduced model. The models are referred to as 20L8D, 20E6D, and 20LE3D, respectively. The models are named according to the formula 'XYZ', where X is the dimension of the parent model, Y is the method or methods applied to it, and Z is the dimension of the reduced model.

**Construction of the 20L8D model**

The L method obtains the model structure by lumping (or by any other formal or 'intuitive' method), and the reduced model is then optimized using the direct method of optimization (see Chapter 5.2).

For the development of the model structure, we reuse much of the work done by Wolf and Heinrich [139]. They have presented a 7D model, and we adopt it directly
accept for the following modifications $^1$: extracellular glucose was introduced as an additional species, glucose transporter kinetics ($v = \text{GlcTrans}$) and glucose flows in and out of the reactor ($v = \text{inGlc}$) were added, a glycogen-producing side branch was added ($v = \text{storage}$), and the removal of extracellular acetaldehyde (ACA) ($v = \text{outACA}$) was changed so that it is now formally composed of the ACA leaving the reactor with the outflow, and the ACA being removed by reactions in the extracellular medium. This resulted in the 20L8D model structure shown in Figure 10.6. The reaction rates are given in Table A.1 in the appendix.

Neither the original 7D nor the new 8D model structure have dynamical properties which are similar to the 20D model (or the experiments) when the parameter values of Wolf and Heinrich are inserted. This is not unexpected, and it is the reason why an optimization step is a part of this method. Just as in ref. [62] we choose $[\text{Glc}]_0$ as the bifurcation parameter. It turned out that the intrinsic parameters, which are the only remaining free parameters in the optimization, did not change many of the oscillatory properties, like the Stuart-Landau parameters. Changes occurred, on the other hand, if the operating point was changed. The reason why the operating point can change in the reduced model for a given operating point in the original model, is because the interpretation of the lumped states is not unique. For the model developed here, a reasonable interpretation is $[\text{Glc}]_{20L8D} = [\text{Glc}] + [\text{G6P}] + [\text{F6P}]$, $[\text{trioseP}]_{20L8D} = [\text{FBP}] + [\text{DHAP}] + [\text{GAP}]$ and $[\text{ACA}]_{20L8D} = [\text{Pyr}] + [\text{ACA}]$. This was the chosen interpretation, since it gave the best agreement in terms of frequency. Some more details of how the search in the few intrinsic parameters was performed is given in [30], and the final set of parameters (11 velocity parameters and six intrinsic parameters) is found in the appendix.

$^1$Brusch et al.[14] have previously developed a modified version of the model by Wolf and Heinrich with similar purpose as ours. We, however, find this model unsuitable due to problems concerning the formulation of the model.
Construction of the 20E6D model

The elimination of dynamics methods removes the dynamics of all states where this can be done, without loosing the basic dynamical properties. To help narrow down the search, a ranking is done first (see Chapter 5.2).

The result of the ranking is shown in Figure 10.10a. By adding states in descending order and stopping at the appearance of oscillations, a 9-dimensional model is obtained. A complete search among the 9-dimensional model’s sub-models shows that there is a unique model with 6 dimensions that oscillates. Since this model is identical to the 20D model with fixed concentrations of 14 species, the reduced model will not be self-consistent unless the corresponding 14 conservation-of-mass relations are fulfilled. Some of these relations are external to the reduced model (cf. Table A.2), and do not call for any action. Other relations have both internal
and external rates. For example,
\[
\frac{d}{dt}[\text{NADH}] = 0 = v_{\text{glycerol}} + v_{\text{ADH}} - v_{\text{GAPDH}} \quad (10.9)
\]
has the external reaction rate \( v_{\text{ADH}} \) while \( v_{\text{glycerol}} \) and \( v_{\text{GAPDH}} \) are internal. We deal with these cases simply by assuming that the external rates balance the equations. In the remaining two relations
\[
\frac{d}{dt}[\text{PEP}] = 0 = v_{lp\text{PEP}} - v_{PK} \quad (10.10)
\]
\[
\frac{d}{dt}[\text{G6P}] = \frac{d}{dt}[\text{F6P}] = 0 = v_{\text{HK}} - v_{\text{storage}} - v_{\text{PFK}} \quad (10.11)
\]
all rates are internal in the model, and these relations therefore need more careful attention. Eq. (10.10) demands that \( v_{lp\text{PEP}} \) is substituted by \( v_{PK} \) or vice versa, and eq. (10.11) demands substitution of either \( v_{\text{HK}} \), \( v_{\text{storage}} \) or \( v_{\text{PFK}} \). We tested the six possible combinations of substitutions. The choice of solutions is unique, since only one of the combinations retains the ability to oscillate. This combination is substitution of \( v_{PK} \) with \( v_{lp\text{PEP}} \) and of \( v_{\text{HK}} \) with \( v_{\text{storage}} + v_{\text{PFK}} \). The resulting model

**Figure 10.7** Reaction network of the 20E6D model.
is the 20E6D model defined by Table A.2 and the rate expressions in Table A.3. These tables along with the final set of parameters is found in the appendix.

![Reaction network of the 20LE3D model.](image)

**Figure 10.8 Reaction network of the 20LE3D model.**

### Construction of the 20LE3D model

The 20LE3D model is obtained by application of the E method to the 20L8D model. The chosen operating point is defined by \([\text{Glc}_x]_0 = 24\ \text{mM}\). A complete search among the sub-models of 20L8D reveals that there is only one oscillating model of 3 dimensions at the chosen operating point. The self-consistency of this model, denoted 20LE3D, is automatically ensured since all five conservation-of-mass relations have external parts. The model structure is shown in Table A.4 and Figure 10.8. The same model could be identified using the ranking directly.

### Step III: New analysis of the models

We first perform various analyses of the different models’ dynamical properties. They fall into three classes: bifurcation aspects, mode-interpretation and variable ranking.

**Stuart-Landau parameters and location of Hopf bifurcations**

All models have been chosen to oscillate, and to be in the vicinity of the bifurcation, using inflow of substrate as bifurcation parameter. In the cases where the \([\text{Glc}_x]_0\) parameter has been eliminated, the parameter which biochemically corresponds
most closely to it is used instead. All the Hopf bifurcations are supercritical and Table 10.1 shows the Stuart-Landau parameters.

The 20L8D model matches the original model quite well, which is not strange since the parameter optimization was partly performed using these criteria. The 20E6D model also matches the original model quite well at its lower ($C_{\text{G6P}} = 11.4\mu\text{M}$) Hopf bifurcation; this is more surprising since the elimination of dynamics method does not include any parameter optimisation. In the 20E6D there is an upper bifurcation, at ($C_{\text{G6P}} = 5.6\text{ mM}$), and this is in qualitative disagreement with the original model. Also for the 3D model, which also was constructed by the E method, we note that the Stuart-Landau parameters are close to those of the parent model, i.e., the 20L8D model.

<table>
<thead>
<tr>
<th>model</th>
<th>location</th>
<th>$\omega_0$</th>
<th>$\text{Re}(\sigma)$</th>
<th>$\text{Im}(\sigma)$</th>
<th>$\text{Re}(g)/\text{Im}(g)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>20D</td>
<td>$[\text{Glc}_x]_0 = 18.5\text{ mM}$</td>
<td>10 min$^{-1}$</td>
<td>0.058 min$^{-1}$</td>
<td>-0.11 min$^{-1}$</td>
<td>0.71</td>
</tr>
<tr>
<td>20L8D</td>
<td>$[\text{Glc}_x]_0 = 18.5\text{ mM}$</td>
<td>17 min$^{-1}$</td>
<td>0.056 min$^{-1}$</td>
<td>0.096 min$^{-1}$</td>
<td>0.68</td>
</tr>
<tr>
<td>20E6D</td>
<td>$C_{\text{G6P}} = 11.4\mu\text{M}$</td>
<td>15 min$^{-1}$</td>
<td>$(16 \text{ min}^{-1})$</td>
<td>$(2.4 \text{ min}^{-1})$</td>
<td>0.42</td>
</tr>
<tr>
<td>20E6D</td>
<td>$C_{\text{G6P}} = 5.61\text{ mM}$</td>
<td>8.9 min$^{-1}$</td>
<td>$(-4.8 \text{ min}^{-1})$</td>
<td>$(-1.1 \text{ min}^{-1})$</td>
<td>0.065</td>
</tr>
<tr>
<td>20LE3D</td>
<td>$C_{\text{Glc}} = 6.12\text{ mM}$</td>
<td>18 min$^{-1}$</td>
<td>$(0.67 \text{ min}^{-1})$</td>
<td>$(1.3 \text{ min}^{-1})$</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Table 10.1 Stuart-Landau parameters of the models.

Mode-interpretation analysis

Another measure of the models’ dynamical properties is given by their polar phase plane plots (Figure 10.9). As described in detail in [31], such plots indicate the biochemical composition of the Stuart-Landau modes, i.e. the sets of metabolites which correspond to either the real or the imaginary parts of $z$ in eq. (2.19). This, in turn, indicates the nature of the interactions underlying the dynamical structure of the system. The “real” mode is an activator, promoting the formation of the “imaginary” mode, and the “imaginary” mode is an inhibitor repressing the “real” mode. Figure 10.9 shows that all four models have similar polar phase plane plots, indicating that the underlying dynamical structures of the models are similar. With the interpretation given in the plots, the activating mode corresponds to low energy charge, and the inhibitory mode corresponds to substrate for the lower part of glycolysis. Low energy charge promotes substrate for the lower part of glycolysis via allosteric activation of phosphofructokinase (PFK), and substrate for the lower part of glycolysis inhibits low energy charge by increasing ATP production in the phosphoglycerate kinase (PGK) and pyruvate kinase (PK) reactions [81]. In conclusion, Figure 10.9 shows that all the models display similar dynamical structures according to the mode-interpretation method.

Ranking analysis

We now rank the states according to their importance for the generation of the oscillations in the linearised system, using a variation of the method from [110]. The
main difference is that we consider all ways in which the oscillations can disappear, including the possibility for merging of the two unstable complex conjugated poles at the real axis. We carried out the analysis at the operating point corresponding to $[\text{Glc}]_0 = 24$ mM. The computations were performed using the Systems Biology Toolbox for MATLAB.

The importance rankings of the species are shown in Figure 10.10. The most important species of the 20D model (Figure 10.10a) are ADP and ATP, reflecting that these species are hubs of the reaction network. The high importance ranking of BPG probably reflects its importance for ATP production in the lower part of glycolysis. The following six species are ranked almost equally important. This fits their localisation around the central part of glycolysis. This matches the results from the polar phase plane plot analysis above, and the conclusions of, e.g., ref. [81]. In broad terms, the ranking is conserved in the model reductions.

**Biochemical analysis of the models**

Now follows analyses of some of the biochemical properties of the models. This is used for evaluation of the general applicability of the reduction methods, and for evaluation of the general properties of the obtained models.

**Flux control**

The flux control pattern is an important property of a metabolic system. We determine this pattern using metabolic control analysis (MCA, see Chapter 2.5). The analyses are carried out at the (lower) Hopf bifurcation points of the models. The
Figure 10.10 Importance ranking of species. $\epsilon_x$ is the smallest scalar perturbation of the linear feedback of species $s$ which causes the unstable complex conjugate eigenvalues of the Jacobian to disappear [110]. Hence, $1/|\epsilon|$ is a measure of importance. A large value of the importance measure indicates that the stability of the system is very sensitive to the feedback of the species.

MCA calculations were performed with the Systems Biology Toolbox for MATLAB. We define glycolytic flux as flux through the PFK reaction.

The 20D model shows supply control of the glycolytic flux (Figure 10.11a): most of the flux control resides with the hexokinase (HK) reaction and the mechanical flow rate of the reactor, $k_0$. The negative flux control coefficient of alcohol dehydrogenase (ADH) arises because increased ADH flux results in a higher ATP yield per glucose molecule, and this allows more glucose-6-P to be converted into glycogen. Since the overall flux is supply controlled, more glycogen production results in less PFK flux. The flux control pattern of the 20L8D model (Figure 10.11b) is similar to that of the 20D model. The flux control pattern of the 20E6D model (Figure 10.11c) is, however, very different from that of its parent model: the glycolytic flux is demand controlled, most importantly by the ATPase reaction. The flux control exhibited by the glycerol branch is also a manifestation of demand control, since increased flux in the glycerol branch decreases the ATP yield. The change from supply to demand control can readily be understood as a consequence of the model reduction, since the mechanical flow of the reactor, $k_0$, has been eliminated from the model, and the kinetics of the HK reaction have been substituted...
by the kinetics of the PFK and the glycogen producing branch. The flux control pattern of the 20LE3D model (Figure 10.11d) is also very different from its parent model. The lumped HK-PFK reaction has a significant share of the flux control. As a consequence of the model reduction, the storage reaction is in fact an ATPase reaction. The ADP produced here activates the flux-controlling HK-PFK reaction, so that the overall flux control pattern is a mixture of supply and demand control.

Figure 10.11  Flux control patterns of the 20D model (a), the 20L8D model (b), the 20E6D model (c) and the 20LE3D model (d). The heights of the bars indicate the magnitude of the flux control coefficients, and the colour coding indicates the signs: black is positive and white is negative. All flux control patterns are calculated at the (lower) Hopf bifurcations of the models (see Table 10.1).

Control of frequency, stability and amplitude

The importance of the different reactions for the oscillatory dynamics can be mapped out by doing sensitivity analysis at the (lower) Hopf bifurcation of the models. By performing the analysis in the framework of the Stuart-Landau equation (2.19), it can be shown [31] that control of amplitude is equivalent to control
of stability, and that reactions with a high share of stability control will, generally, also have significant frequency control. In contrast, reactions controlling frequency will not necessarily have large stability control. The sensitivity analyses were performed with the continuation software cont [70], customised perl scripts and Mathematica (Wolfram Research, Champaign, IL) as described in ref. [31].

The stability sensitivity analysis of the 20D model (Figure 10.12a) shows that PFK is the major destabilising reaction, whereas the ATP-consuming reactions HK, storage and ATPase are the major stabilising reactions. As expected [31], the frequency control pattern (Figure 10.13a) involves more reactions than the stability control pattern, most notably the redox reactions (glyceraldehydephosphate dehydrogenase (GAPDH), ADH and the glycerol branch) and the specific flow rate of the reactor, $k_0$. (The results for the 20D model have previously been published [81].) In comparison, the stability control pattern of the 20L8D model (Figure 10.12b) reveals a less important role for PFK in the stabilisation of the
Figure 10.13 Frequency control patterns of the 20D model (a), the 20L8D model (b), the 20E6D model (c) and the 20LE3D model (d). The heights of the bars indicate the magnitude of the frequency control coefficients, and the colour coding indicates the signs: black is positive and white is negative. All frequency control patterns are calculated at the (lower) Hopf bifurcations of the models (see Table 10.1).

stationary state; the glucose transporter is now equally important. Among the stabilising reactions, GAPDH is now more important than in the 20D model. As in the 20D model, the redox feedback loop has a fair share of the frequency control (Figure 10.13b). In the 20E6D model, stability control is dominated by GAPDH as the most important stabilising reaction, and the ATPase as the major destabilising reaction (Figure 10.12c). Hence, the effect on stability of the ATP-consuming reactions is now opposite of that seen in the 20D and 20L8D models. The frequency control pattern is a mirror image of the stability control pattern (Figure 10.13c). The 20LE3D model shows a stability control pattern where the major destabilising reaction is PFK, and where the most important stabilising reactions are GAPDH and storage (Figure 10.12d). The frequency control pattern (Figure 10.13d) resembles that of the 20E6D model, in particular when taking into account that the storage reaction functions as an ATPase.
Step IV: Comparison and discussion

We have now obtained reduced models, whose dynamical and biochemical structure have been analysed. Let us now evaluate the success of the new mechanism method, the properties of the two new reduction methods, and the general applicability of the new models.

The main conclusion from the analysis of the dynamical structure of all the reduced models is that their dynamical structures are similar. All models are close to a supercritical Hopf bifurcation, and all the mode-interpretations and importance rankings are similar (Figs. 10.9 and 10.10). Furthermore, both the mode-interpretation and the importance ranking corresponds to the same biochemical mechanism: the negative feedback mediated by the ATP/ADP allosteric regulation of PFK from the lower ATP producing back to the upper parts of glycolysis. This analysis result was predicted already in [81], which applied the mode-interpretation method to the Hynne model. This result has now been supported by the new method, and the main improvement is that the new result has a verified robustness. The main reason for this is that the same conclusions has been obtained in models with different parameter values, steady state values, and, maybe most importantly, different model structures. Furthermore, this can be concluded since the reduction methods only guarantees the preservation of the basic dynamical behaviour (oscillations), not the preservation of the dynamical structure (mechanism). Another result is due to that the most reduced model, 20LE3D, contains almost exclusively the interactions in the suggested mechanism. This means that the second possible result has also been obtained: the verification of the feasibility of the mechanism. As mentioned in Chapter 5.2 these kind of results have been obtained many times before (see, e.g, [49, 56, 119, 120, 127]), and the main advantage with the present results is that the new minimal models are obtained through model reduction of a realistic model, and do therefore have more realistic parameter and steady values. It should, finally, be remembered that this mechanism has been proposed, and argued for, in many different ways. The results are therefore rather expected. The main purpose of the application has also been to exemplify the usage of the methods, and the example has also been chosen since it is advantageous to know the result in advance.

While it must be concluded that the new reduction methods have served their intended purpose well - to preserve the basic dynamical properties in biochemically meaningful models - the ability of the methods to preserve the biochemical properties of the methods is much less impressive. This is especially true for the E method. It, e.g., produced demand controlled flux control pattern of two models with supply controlled flux patterns (see Figure 10.11 and the associated discussion). This is, however, not surprising given the fact that the E method does not take any biochemistry into account, and since the method substitutes rate expressions. That the lumping and optimization method seems to be better at preserving the biochemical properties is also not surprising, since a more detailed adjustment is done via the optimization, and since biochemical knowledge is added during the construction of the model structure.

When comparing the three reduced models as general glycolysis models, we can thus say that they are all good for analysis of the dynamical structure, but that
### Table 10.2

The different models obtained using the MATLAB implementation for model reduction. The original Hynne model has 61 kinetic parameters and is referred to as H61P. If all reactions (except HK and PFK) are reparametrized into rational expressions there are 76 parameters. This does not imply any approximation. If GAPDH, ALD and lpGlyc are reduced with the settings described in columns 3-5, a 52 parameter model (H52P) is obtained. Finally, the reduction of GlcTrans leads to a 48 parameter model (H48P), and the reduction of PGI and TIM leads to a 44 parameter model (H44P).

<table>
<thead>
<tr>
<th>name</th>
<th>reaction</th>
<th>rentol</th>
<th>negtol</th>
<th>contol</th>
<th>parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>H61P</td>
<td>22 reactions</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>61</td>
</tr>
<tr>
<td>H76P</td>
<td>GAPDH</td>
<td>0</td>
<td>10^-5</td>
<td>0</td>
<td>-3</td>
</tr>
<tr>
<td></td>
<td>ALD</td>
<td>0</td>
<td>10^-5</td>
<td>0</td>
<td>-4</td>
</tr>
<tr>
<td></td>
<td>lpGlyc</td>
<td>0</td>
<td>0</td>
<td>0.05</td>
<td>-2</td>
</tr>
<tr>
<td>H52P</td>
<td>GcTrans</td>
<td>0</td>
<td>0</td>
<td>0.05</td>
<td>-4</td>
</tr>
<tr>
<td>H-48P</td>
<td>PGI</td>
<td>0</td>
<td>0.05</td>
<td>0</td>
<td>-2</td>
</tr>
<tr>
<td></td>
<td>TIM</td>
<td>0</td>
<td>0.5</td>
<td>0</td>
<td>-2</td>
</tr>
<tr>
<td>H44P</td>
<td></td>
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</tr>
</tbody>
</table>

it is the 20L8D model that is the best candidate for general analysis. This is also the only reduced model that contains the NAD\(^+\)/NADH redox control loop, which means that 20L8D is the best model candidate for an identifiable core model (see below), and for modelling of the cell synchronisation phenomenon [29, 55, 81, 137, 139].

In conclusion we can thus say that both reduction methods have been successful in the fulfilment of the given objective: to produce reduced but biochemically meaningful models that reproduce the basic dynamic properties. The strength of the elimination of variables method is that it is algorithmic, and that it does not require any input in the form of biochemical knowledge. A major advantage of the lumping method, on the other hand, seems to be that it results in models with more well-preserved biochemical properties. We have also used the general method of Chapter 5.2 to both strengthen the robustness of the mechanism prediction, and to show that it actually qualifies as a mechanism under realistic circumstances. This result is only valid for the Hynne model. For a result that is valid for the biological systems, a complete core-box modelling approach is necessary. The first step in this approach is the reduction to an identifiable core model.
10.3.2 Towards an identifiable core model

Results of the MATLAB implementation

We will now apply the model reduction method we developed in Chapter 5.3 to the Hynne model. We will see that this will reduce about a third of the parameters in the model. This, however, is not sufficient to make the model identifiable, and we will therefore end with a discussion concerning possible sub-problems that need to be handled before a core model can be obtained.

When running the MATLAB program that we developed in Chapter 5.3 one enters the model together with an error criterion and a specification of which reactions one seeks to simplify. We started by entering the Hynne model, and by specifying an error limit that was so low that no reductions would be accepted. This was done to detect fundamental problems with the reactions. Two reactions did show such problems: HK and PFK. The reason why HK showed such problems was that one of the parameters were equal to zero in the original model. Since the original parameters appear in combinations which include fractions, the situation of division with zero appears in some of the calculations. The reason why PFK is problematic is that it contains an exponential which needs special considerations not included in the present implementation. Apart from these two reactions, these initial tests were successful for the model, and a new model structure with coefficients instead of the original parameters was obtained. The new model agreed with the original model also at very high accuracy demands, and the new model is structurally identifiable, apart from the parameters in the dehydrogenase reactions, as explained in Chapter 10.2.1 above. However, the number of parameters has actually increased. In the original Hynne model there were 61 parameters, and in the new model 76. This shows the quite common phenomenon that physically derived in vitro expressions are structurally under-parametrized, i.e., there could be more parameters in the expressions without losing structural identifiability.

As the next step we choose to reduce the parameters in those reactions that had the most number of coefficients. Those reactions were GAPDH, ALD and lpGlyc. This yielded a net reduction of 9 parameters, i.e., the reduced model had only 52 parameters. Note that this means that there were actually 24 coefficients reduced, since there were more coefficients than original parameters. The error associated with these reactions were marginal. Note that these reductions also took care of the structural unidentifiabilities detected in Chapter 10.2.1 above. The next reaction that was reduced was the glucose transporter, GlcTrans. The reduction of this reaction did imply some visual changes. Strangely these changes were most visible in the end product, pyruvate, and as can be seen in Figure 10.15, there is no difference in the phase, but a constant offset has been introduced. The difference shown in the figures are the maximum errors, in the steady state working conditions. Two more reactions where eliminated: PGI and TIM. Both these reaction expressions were reduced with 2 parameters each, and for the PGI expression this fits perfectly with the results we obtained in Section 10.2.2. The reductions of PGI and TIM did not increase the differences in absolute values between the different values, but it did introduce a phase shift in some of the variables, see Figure 10.16. After all these reductions the reduced model had 44 parameters, and this is equal to a reduction
of about a third of the parameters in the model. The different models, and the settings used in the program to obtain them are summarised in Table 10.2.

Discussion

The above results obtained for the MATLAB implementation shows that it is possible to get a considerable reduction in the number of parameters in the model by only focusing on the individual reactions. It is outside the scope of this dissertation to do a full analysis of how far one can get with this approach alone. It should also be added that the present model reduction approach does not include an optimization of the model as a whole, but only an optimization of each individual rate expression. A subsequent optimization of the model as a whole would probably improve the possibilities for reductions, even though it would make the back-translation formulas less valid.

Another option to obtain a model with smaller rate expressions would be to start off the developments from another model. One could for instance work with the 20L8D model developed in Section 10.3.1, which is both small and contains much of the essential biochemistry of the system. It might, however, be so that there are details that are lacking in that model structure, and that would have to be modelled in more detail. All this depends on what the purpose with the core model should be, and what data one considers to be available. It is not until that is obtained that one knows the exact criteria the the core model should fulfill. To
Chapter 10 Improving the Identifiability of a Model for Yeast Glycolysis

Figure 10.15 Time-series of Pyr comparing the original Hynne model, H61P, with the model that has also reduced the glucose transporter, H48P. As can be seen a slight offset has appeared.

Summary of Chapter 10

The Hynne model for yeast glycolysis has served as a source of inspiration for many of the developments in Part I of the dissertation. This model has been chosen for several reasons: the system is well-studied and there exists good data; the model is a grey-box model that gives a good description of the system and accurate predictions also of qualitatively different validation data; the model is highly complex and needs to be reduced both for estimation and for analysis purposes.

Part of the complexity in the Hynne model is due to its complex rate expressions. In Chapter 4 we developed new methods for identifiability analysis and reductions of such expressions. We showed that conserved moieties might lead to unidentifiable rate expressions, especially in dehydrogenase reactions. This has now been shown to be the case also for the Hynne model, and we have introduced alternative rate expressions for those reactions that are structurally identifiable. In Chapter 4 we also presented new methods for the development of practically identifiable rate expressions, that can be used to compare in vivo with in vitro characterisations.

have that clear in an early stage of the modelling is essential in any modelling work, whether it is based on formal modelling methodologies, or on mere biochemical intuition.
We have now used these methods to suggest a simpler expression for PGI, and to compare in vitro and in vivo values for the kinetic parameters.

Another part of the complexity in the Hynne model lies in its complex behaviour. In Chapter 5 we developed new methods for analysis of the mechanisms producing this behaviour. These methods have now been applied to the Hynne model, and we have shown explicitly that the previous predictions really do point to a possible oscillatory core, and that the predictions are robust towards variations in model complexity.

However, the final goal is to achieve a core-box model for the system. This requires the development of an identifiable core model, the estimation and uncertainty characterisation of this model, and the eventual back-translations $\phi$ and $\Psi$. In Chapter 5.3 we presented a method to help in such developments, and this method has now been applied to the Hynne model. This has shown that the developed MATLAB program may be used to significantly reduce the number of unidentifiable parameters in the model, and provide an interpretation $\phi$. We have also shortly discussed some of the remaining steps needed to develop a core model that is fully identifiable.
Solving the Riddle of Glycolytic Control in Muscle

We now present the third and last new modelling work presented in this dissertation. This model describes the central metabolism of human muscle cells, and it is valid before, during, and after anaerobic contraction. The new model is not a full core-box model, but a mechanistically based minimal model, which is developed primarily for the purpose of hypothesis testing. It is based on measurements collected using nuclear magnetic resonance (NMR).

NMR data have several advantages: it allows for many samples during one time course, it can measure many time courses simultaneously, the time courses contain absolute values without an unknown scaling factor, the technique is non-invasive, and finally, the technique can be performed without harming the subject and is therefore without ethical complications. Our present data have been collected during a pulse response. Although a pulse is not the most informative kind of input signal, non-trivial information is available. The given data point to a problem with the current understanding of the system, a problem that has been known for 25 years.

However, the problem has so far primarily been argued on a qualitative and reasoning basis; we now want to test these arguments quantitatively, and systematically. This will be done by construction of a model structure from the known hypotheses, a subsequent model optimization, and an evaluation and analysis of the final model. Such a modelling work will either show more quantitatively that the qualitative prediction was right, or it will falsify the prediction. If the prediction should turn out to be wrong, that will be another evidence that modelling is necessary to evaluate data describing complex biological systems. If the prediction should turn out to be right – if a model containing only the conventional mechanisms would be unable to explain the data – the developed model might be
used as a basis for a following modelling work, with the intention of coming with suggestions for what the missing control might be. Therefore, all possible outcomes of the work are of interest, and our situation as modellers is ideal.

In Section 11.1 we review the relevant biological background, and explain which features of the NMR data are believed to be impossible to explain with current biochemical assumptions. In Section 11.2 the new model structure is developed, and in Section 11.3 the model is optimized to the data. The remainder of the chapter is devoted to evaluation of the obtained model, and to a discussion of the results.

11.1 Background

11.1.1 Biological background

The term 'muscle' comes from the Latin word *musculus*. This is the diminutive of the word *mus*, which means mouse, and the term was chosen because the flexing of a muscle resembled the movement of a mouse. Muscle tissue exists in three different categories, skeletal, cardiac and smooth tissue, and together they accounts for both the majority of the body weight, and the majority of the daily energy consumption, in a human body. An understanding of muscle metabolism is therefore an essential ingredient in the understanding of glucose homeostasis (see Chapter 8). Muscle metabolism, however, is widely different in different tissue types, and in different situations. In this chapter we study skeletal muscle in the situation of anaerobic contraction.

A skeletal muscle is under voluntary control, while muscle from the other two tissue types are involuntary, i.e., not under the conscious control of the individual. This means that the subject (the person subject to the experiment) can contract and relax the muscle at a given signal. This is of course advantageous when a contraction study is made. The contraction will be performed after the blood supply to the muscle has been stopped. Such situations are generally referred to as ischemic. To us, however, the major effect of this will be that the cells are working without oxygen, and therefore we typically just refer to the experiments as anaerobic. To us, however, the major effect of this will be that the cells are working without oxygen, and therefore we typically just refer to the experiments as anaerobic. That the cells are working without oxygen means, e.g., that the mitochondrial oxidative phosphorylation is switched off. Further, it also means that lactate (Lac) is the only thing formed out of pyruvate (Pyr), and that lactate can not be used as a fuel for energy. Finally, that there is no blood supply means that there is no access to external glucose. Therefore, there are only two mechanisms for ATP production in the case of no oxygen: glycogenolysis followed by glycolysis, and the reversed creatine kinase reaction.

Glycogenolysis consists of two steps: the catabolism and phosphorylation of glycogen giving a separate glucose unite (G1P), and the reconfiguration of G1P into glucose-6-phosphate (G6P). In the sequel we will refer to these steps together by the enzyme glycogen phosphorylase (GP), although we want to point out that there are actually two enzymes involved. The end result, G6P, is then available as a substrate for glycolysis, see Figure 11.1. Creatine kinase (CK) is the second enzyme involving ATP production in the case of no oxygen. The additional phosphate in
this reaction is given by the substrate phosphate creatine (PCr), see Figure 11.1. It is generally believed that the ATP production through CK covers the rapid drops of [ATP]/[ADP] ratio, but that an increased glycolytic flux takes care of the long-term changes. Due to this particular property of handling short-term exercises, creatine (Cr) is used as a part of the diet by certain kind of athletes. After the [ATP]/[ADP] level have been restored in the cell, PCr is reformed.

The situation we consider in this chapter involve muscle contraction. Since we are considering skeletal muscle, the contraction is typically initiated by a conscious decision. This decision is then mediated to the cell through various electrical impulses. These lead to the opening of calcium channels which quickly elevates the intracellular concentration of free calcium ions, [Ca$^{2+}$]. This initiates a signalling cascade involving cyclic-AMP, which eventually leads to the various responses in the cell. The relevant responses for this study are the activation of GP, and the activation of ATP-consuming processes. This is depicted in Figure 11.1, where the various ATP-consuming processes are lumped together into a single ATPase reaction. Even though there are many steps involved in the translation of the conscious decision to activation of the two reactions, they occur within a second. The onset of the contraction is thus to be considered as momentary compared to the time-scale in our experimental data.
11.1.2 The data

NMR measurements

Our available data have been collected using nuclear magnetic resonance (NMR). NMR was discovered as early as 1945 by Harvard physicists Purcell, Torrey and Pound, and it has since then developed into a multi-purpose method, which is used in many different applications. It is, e.g., used for imaging of brain-activity, for food quality imaging, and for solid state studies of properties of a material. The principle that the method is based on, and which the above physicists discovered how to make use of, is based on the fact that a nucleus can be excited by electromagnetic particles (photons). Such excited molecules are then eventually de-excited, upon which they emit another photon. Both the uptake and the release of a photon obeys the same simple principle: the wavelength of the photon corresponds to the difference in energy levels of the molecule (see Figure 11.2). Since each molecule has an, in principle, unique excitation spectra, the emitted photons can be used to characterise all the constituent molecules in a given sample. However, if the given sample contains too many diverse substances, it is not practically possible to distinguish between all the constituent molecules.

There are many strengths with the NMR method. The excitations are processes that occur more or less independently of each other. Similarly, photons of different wavelengths can be measured independently of each other. Therefore, light of all frequencies can be applied simultaneously, after which all concentrations can be measured simultaneously. One can thus obtain simultaneous time courses. This is of course a major strength of the method, but there are many others. The measurements are performed without making any kind of harm to the cells or to the subject. NMR is therefore both fully non-invasive and without ethical complications. This also means that all the measurements from a given time-series are taken from the same cells (which was not the case, e.g., for the Western blots used in Chapters 9). Further, even though the absolute values of the measured signals varies from experiment to experiment, the relations between the peaks are believed to be experiment independent (from a measurement point-of-view, and apart from the noise). Therefore, if the absolute start value of one of the measured signals is known, all the absolute values of all the other measured concentrations can be considered as known as well. We have seen in the previous two chapters that absolute values are not always available, and that it is greatly advantageous when they are. Finally, the process that we investigate in this study takes four minutes, and this allows for some 50 samples in each time course, and during one single day several time courses can be collected. With biological standards, this is good data availability. All these are properties considers as strengths of the NMR method.

There are some problems associated with NMR as well. First, it cannot be used on all organisms. Second, the given data is typically quite noisy. The high noise level and the limited sampling speed together means that each individual time course is not particularly informative. This can partly be helped by averaging over many time courses. Averaging can be done in several ways, but each of them is associated with problems of its own; later in the chapter we consider different options. Further, the noisy data means that the data do not allow for usage of
Figure 11.2 The frequency, ν, of an emitted or absorbed photon is given by the relation \( \Delta E = h\nu \), where \( h \) is Planck's constant, and \( \Delta E \) is the difference between the energy levels of the state before and after the interaction.

To sum up: NMR has mainly advantageous for our particular model system; it non-invasively delivers simultaneously collected time courses given in absolute concentrations; the main problems are associated with the high measurement noise.
The experimental input signal

Each experiment is performed in the same way and consists of the following three phases: anaerobic rest, anaerobic exercise, and anaerobic recovery. First the blood to the relevant muscle is stopped using a special ring. The muscle is therefore deprived of its oxygen, and all processes quickly turn anaerobic. After an initial settling down of three minutes, the measurements are started. For the first five minutes (where only the last two of them are measured) the subject relaxes the muscles completely. Then the subject is asked to exercise the muscle by pulling an ergonomical handle. This goes on for one minute. Finally the subject is asked to go back to the relaxed state, and the measurements goes on for one final minute. Thus, a single time course is four minutes long.

This means that the three phases of the input signal to the system are: relax, work, and relax again. Ideally, this is therefore a pulse input. There is, however, a complex chain of events leading from the decision to relax or work to the actual effect on the metabolism (see Section 11.1.1 above). The end result of this chain of events is a change in intracellular calcium concentration, $[\text{Ca}^{2+}]$. Calcium is then activating the two enzymes CK and GP (see Figure 11.1). The absolute calcium levels before, during, and after the contraction are not known, and it might also vary from experiment to experiment. This is, of course, a source of uncertainty when constructing the model. We can, on the other hand, assume that the switches between the different levels occur momentously, at least compared to the time-scale of the rest of the experiment.

To sum up: the real input signal, $\text{Ca}^{2+}$, is not measured and only its qualitative behaviour is known; a reasonable first approximation is that it is 0 during the first two minutes, 1 during the third minute, and 0 again during the final, fourth minute (see Figure 11.4).
The five measurement signals

For each experiment we obtain five different time courses, i.e., we measure five independent signals. In principle, it should be possible to collect arbitrarily many time courses with NMR, since each measurement point in an NMR is represented by a continuous spectra. However, there are only a finite number of distinguishable peaks in the spectra, and each peak has a certain 'broadness' (see Figure 11.3). There is therefore a practical limit to how many independent signals that can be measured. Further, there are overlaps between the contributions from different constituent molecules, and each peak generally corresponds to a mixture of many molecules with similar chemical properties. For instance, it might be difficult to distinguish between different molecules composed of the same atoms and with the same charge, since this could mean that they contribute to the same microscopical peak. Finally, the peaks might be partially overlapping and it could thus be hard to account for the contribution from each peak.

Given all these problems, there still remains five independent, and relatively trustworthy signals, that are of interest to us. The simplest interpretation of them is the following: ATP, PCr, P, PME, and pH. Let us now go through the different signals one-by-one, in some further detail.

ATP: The ATP-signal consists mostly of noise centred around an average value. This is the case since ATP is kept constant by the cells, being the main source of directly available energy. The ATP-signal is therefore not particularly informative, except for one important exception. The absolute value of ATP during the initial resting phase has been independently measured on many occasions, and it has been found to have a relatively constant value of about 5.5 mM. Since all signals are scaled with the same unknown constant, this ATP value can be used to obtain measurements directly in concentrations for all the signals. This is the main usage of the ATP signal. It should finally be said that ATP is present in three different peaks, and could thus potentially be given by three different signals: \( \alpha \), \( \beta \), and \( \gamma \). These three signals should ideally give the same value. We use the average of the \( \alpha \) and \( \beta \) signals to calculate the scaling constant, and it is the \( \beta \) signal that is plotted in Figure 11.4.

P: The phosphate signal is one of the most expressive signals, varying almost a factor 3 within a single time course. There is little phosphate present before the contraction, and the reliability of this part of the signal is thus poor compared to the other parts.

PME: The phosphomonoester (PME) signal has a qualitative shape which is quite similar to that of phosphate. One problem with the PME signal is that it is made up of a complex mixture of many similar molecules (basically all singly phosphorylated sugars). The signal is believed to primarily correspond to G6P. A smaller contribution comes from F6P, and in this context, the biochemical role of F6P and G6P are the same. The mixture between these two contributions is therefore non-problematical. Another argument for this is that G6P and F6P are highly correlated (cf. also with the encountered
identifiability problems for PGI in Chapter 10.2.2). There are, however, also contributions from other sources, e.g., AMP. How these signals change over time, compared to G6P and F6P is not fully known, and since these remaining contributions do make up a non-negligible part of the PME signal, this has to be accounted for, somehow, in the modelling.

**PCr:** The PCr signal also has a qualitative shape similar to that of P and PME, though mirrored. It is not believed to be composed of constituents with biochemically different functions. It is the shape of the PCr signal, especially during the final relaxation phase, that is judged to be the most difficult signal to explain using only conventional biochemical hypotheses (see below).

**pH:** This signal is measured as the concentration of $H^+$, even though it will typically be converted to pH using the formula $pH = -\log_{10}[H^+]$. The concentration of $H^+$ is also not measured directly, but through the position (frequency) of the P peak. The variations in this signal are, from an absolute point-of-view, quite small. Nevertheless, the particular shape of the signal is judged to be trustworthy, especially during the contraction when the P peak is easy to identify.

### 11.1.3 Contradictions between data and conventional biochemistry

**What happens; and what should happen?**

Let us now inspect the given data a little bit more closely, in order to get a qualitative picture of what is going on, and to understand which features that seems contradictory to the current biochemical understandings. All the time courses are relatively constant during the first relaxation phase. There is a small flux through glycolysis, which produces some of the ATP consumed in the general cellular processes. This small flux through glycolysis corresponds to a small consumption of substrate (G6P and F6P), which is believed to be balanced by an equal flux through GP. Some part of the ATP consumption is also balanced by a basal CK flux. This is seen in the small but constant decrease of PCr. All in all, the first relaxation phase is believed to be well-understood, and it is straight-forward to find a biochemical interpretation of this part of the data set.

The second part of the data set is the exercise phase, occurring between $t = 2$ and $t = 3$. This is the dynamically most expressive phase in the data. Over the one minute contraction there occur a 3-fold increase in [P], a 4-fold increase of [PME], and a 75% reduction of [PCr]. The reduction of [PCr] is most rapid in the beginning of the contraction. This is in agreement with the biochemical understanding of what should happen; CK is supposed to cover the rapid drops in the [ATP]/[ADP] ratio, and an increased flux through glycolysis is supposed to cover the long-term changes. The muscle cells’ adjustments to gradually turn on a higher glycolytic flux are also seen in the PME signal; PME primarily represents substrate for glycolysis, and an increasing substrate availability (through an increased GP flux) implies an increasing flux through glycolysis. The rapid increase in P directly after the
onset of the contraction, on the other hand, is believed to be due to the rapidly increased ATP consumption. Nevertheless, P is also a substrate for glycolysis and thus also another up-regulator of the glycolytic flux. Just like PME it is also monotonically growing during the work phase. Finally, the shape of the pH signal during the contraction is believed to be due to a combination of CK, glycolysis, and LDH, where the effect from the two latter is dominant during the later part of the contraction. All in all, the contraction part of the time course is the dynamically most dramatic, but there are no fundamental conflicts between the observations and the current biochemical understandings of the system.

Conversely, the final recovery phase is dynamically undramatic but it, nevertheless, contains some features believed to be in contraction with our current biochemical understandings. That there is not that much happening during this phase means, e.g., that the high concentrations of P and PME stays high, at least compared to the original levels. Since both these are substrates and major regulators of glycolytic flux, one would expect that glycolysis would keep running with a high flux also during the recovery phase. However, since there is virtually no flux

**Figure 11.4** The NMR data. It consists of five measured signals, and one non-measured input signal, and is collected in nine experiments. The thick line corresponds to the mean value at each time point.
through GP, the concentrations of P and PME would be drastically reduced by such a high flux; this is not observed. Further, a high glycolytic flux would restore the lowered [ATP]/[ADP] ratio, which in turn would increase the PCr level. This is also not observed to any extent that even remotely corresponds to the rapid changes during the work phase. Therefore, it seems like glycolysis flux is low during the recovery phase. This contradicts the above mentioned measurements of the flux regulators, which indicate an equally high flux as during the work phase. This contradiction was hinted above, and it is the major problem with the interpretation of this data.

Possible explanations

There are different possible explanations to the problem; some of them are quite plausible, and some are more far-fetched. We will now mention two of them: i) there exists an unknown overruling control of glycolysis, e.g., from calcium, and ii) there is something wrong with the above qualitative reasonings.

i) The major problem is that glycolysis seems to be virtually shut off even though all its major regulators indicate a high flux. This could be explained by the fact that there exists an, as yet, unknown control of the glycolytic flux. This would be a possible explanation if this new control would be able to overrule the other regulators, and if the new control would down-regulate the glycolytic flux, in the case of anaerobic recovery. There are several possible suggestions for where such a regulator could lie: one of them is \([Ca^{2+}]\). Calcium is known to regulate many different functions in the cell, e.g., some of the enzymes in the TCA cycle, the secretion of insulin in beta cells, and the onset of the contraction of the muscle cells (see Chapter 8). Since calcium is already known to be such a versatile regulator, it is thus always a candidate when searching for a new source of control. Calcium also fits the two restrictions in this specific case: \(Ca^{2+}\) is not one of the previously known regulators of the glycolytic flux, and its concentration is rapidly down-regulated when the muscle contraction ends. In any case, the specific mechanisms behind the regulation would have to be determined, since there are probably intermediates involved as well (see Figure 11.5). Finally, it should be noted that if it could be shown that there exists an unknown and overruling control of such a central pathway as glycolysis, that would be a major biochemical discovery.

ii) Another plausible explanation is that the arguments for the presented contradiction are, somehow, faulty. The biochemistry of a cell is a large and complex network of many nonlinear interactions. In the arguments leading to the conclusions that there is a contradiction, most of these interactions have been considered as irrelevant for the phenomenon. Likewise, many complex relationships have been drastically simplified using various assumptions. This is true for some of the complementing arguments to those mentioned above. There is, e.g., one more known important control of glycolytic flux that was not mentioned above: ADP. There is no directly available ADP signal, but an approximate time course for ADP can be calculated from the original five signals. This is done using the assumption that CK is in equilibrium, that \([Cr] = 0.2[PCr]\) during the resting phase, and that the sum of \([PCr]\) and \([Cr]\) is constant during the whole experiment. Given these assumptions, the approximate ADP time course has high levels during the recovery
phase. Since high ADP is up-regulating the glycolytic flux, this supports the arguments for the above contradiction. The same kind of supporting evidence exists for other non-measured regulators, and for other ways of measuring the glycolytic flux. These kind of assumptions make it possible to consider the interactions in such a complex network without using the aid of a model. However, it might very well be the case that some of these assumptions are not valid. Then some of the arguments for the apparent contradiction would fall, and it would no longer be possible to draw the conclusion that the current biochemical understandings are unable to explain the given data.

All in all, the question that we seek to answer in this chapter is the following: Can we based on the given NMR data really draw the conclusion that there is a need for previously unknown regulators? Qualitative reasoning seems to say so, but we will now test this prediction with a modelling approach. It is thus primarily the possible explanation ii) above, that we consider in this particular study.

11.2 Choosing the model structure

The modelling approach consists of three steps: i) choice of model structure ii) optimization of the parameters and, iii) evaluation of the obtained model. We now treat the first of these steps, and leave the remaining two for the following two sections.
Choosing an interaction graph

A common option when choosing a model structure is to base it on an already existing model (see, e.g., Chapter 10.3.1). For anaerobic muscle metabolism only one previously published model exists. This model was developed by Lambeth and Kushmerick [73], and it includes glycogenolysis, glycolysis, CK, adenylate kinase (AK), and ATPase. The model is quite detailed, and it is thus not optimal for the kind of hypothesis testing we primarily seek now, where a more or less complete search in parameter space is warranted. One option could be to reduce the Lambeth model, just as in the core-box modelling approach. There is, however, one important difference between the present situation and the standard core-box reduction step: the present grey-box model does not sufficiently well describe the experimentally observed behaviour. Therefore we choose to construct a new, and simpler model structure from scratch.

Our intent is to construct a biochemically meaningful model that includes all the measured variables, with as few extra states as possible. Further, the reduced model should use as few parameters as possible, while still including the major regulatory controls of the central pathways.

The chosen model structure is given in Figure 11.1. The measurements of the substrate for glycolysis are available in the PME signal. PME is thus included as a state in the model. The signal, however, consists of some unknown parts that is not substrate for glycolysis. The exact fraction between these parts is not known. When the PME state is used in a reaction rate this has to be accounted for. This is done by subtraction with a constant parameter, denoted $H_{\text{diff}}$, and the resulting auxiliary is referred to as G6P.

$$\text{[G6P]} := \text{[PME]} - H_{\text{diff}}$$

The formation of G6P through GP is lumped into one step, which we assume is not dependent on glycogen. The breakdown of G6P ends by lactate, under anaerobic conditions, and we only assume one intermediate step: Pyr. This is assumed since the lactate dehydrogenase reaction (LDH) needs to be singled out from a redox balance perspective, and because of its important, and qualitatively different, influence on pH. That all the rest of glycolysis is lumped into one step is a big simplification, but since a minimal model is sought, it is a reasonable first approximation. Glycolysis as a whole has the following cofactors: NADH/NAD$^+$, ATP/ADP, P, and H$^+$. LDH has NADH/NAD$^+$ and H$^+$ as cofactors. The cofactors are included as dynamic states in the model. All processes that consume ATP are lumped together into a single reaction denoted ATPase. The final measured variable, PCr, is included, and it is assumed that it is only affected by CK. Note that there are more nodes in Figure 11.1 than there are independent states in the corresponding model; ATP/ADP, NADH/NAD/Pyr, and PCr/Cr make up conserved moieties, and each of these groups only correspond to one independent state variable. Finally, Ca$^{2+}$ is included as an input signal to the model, acting as modifier to GP and CK.

To sum up, our chosen model structure is given in Figure 11.1: it contains 7 independent states, and only two of these cannot be measured: NADH (or NAD$^+$ or Pyr) and Lac.
11.2 Choosing the model structure

Choosing rate expressions

The rate expressions are chosen to include all central regulations, using as few parameters as possible. A regulation that might be more or less active is most simply described by a Michaelis-Menten expression. Let such a regulation by $A$ be denoted $\text{MM}(A)$. Let a product of such regulations by $A_1, \ldots, A_n$ be denoted $\text{MM}(A_1, \ldots, A_n)$

$$\text{MM}(A_1, \ldots, A_n) := \left(\frac{[A_1]}{K_{A_1} + [A_1]}\right) \times \cdots \times \left(\frac{[A_n]}{K_{A_n} + [A_n]}\right)$$

A single Michaelis-Menten expression also includes a maximum velocity constant $V_{\text{max}}$, but since we here often multiply several regulatory expressions after each other, these constants are lumped together into a single velocity parameter, given explicitly. Let us now turn to the problem of formulating the different rate expressions.

The rate of glycolysis is only described by the phenomenological regulations given by the expression above. The major regulators are assumed to be the substrates ADP, G6P, P, and NAD$^+$; the rate is thus given by

$$v_{\text{Glyc}} = V_{\text{max}} \text{MM}(\text{ADP}, \text{G6P}, \text{NAD}^+, \text{P}) \quad (11.1)$$

The reason why such a phenomenological expression is chosen is because so many reactions after each other have been lumped together that it simply isn’t feasible to preserve the mechanistic information, as is done when deriving the original Michaelis-Menten expression (see Chapter 2.2). The LDH reaction, on the other hand, consists of lumped elementary reactions, and its reduced form have been described many times. Here we chose a kinetic expression from the literature given by

$$v_{\text{LDH}} = \frac{V_{\text{max}} \left[\text{Pyr} \cdot \text{NADH} - \frac{[\text{NAD}^+ \cdot \text{Lac}]}{K_{eq}}\right]}{K_{\text{Lac}} K_{\text{NAD}} \left(1 + \frac{[\text{Pyr}]}{K_{\text{Pyr}}} + \frac{[\text{NADH}]}{K_{\text{NADH}}} + \frac{[\text{Pyr} \cdot \text{NADH}]}{K_{\text{Pyr}} K_{\text{NADH}}} \right) + K_{\text{NAD}} [\text{Lac}] + K_{\text{Lac}} [\text{NAD}] + [\text{Lac}] \cdot [\text{NAD}] }$$

The reaction rate for CK is given by mass-action kinetics

$$v_{\text{CK}} = V_{\text{max}} \left(\text{PCr} \cdot [\text{ADP}] \cdot \left[H^+\right] - \frac{[\text{ATP}] \cdot [\text{Cr}]}{K_{eq}}\right) \quad (11.2)$$

The reaction rate for the lumped ATPase reactions is assumed to be at a constant low level in the case of no contraction. The higher flux, activated by the contraction, is supposed to be dependent on ATP. If the low level is given by the parameter $v_{\text{rest}}$, the expression becomes

$$v_{\text{ATPase}} = v_{\text{rest}} + V_{\text{max}} \text{MM}(\text{ATP}) \cdot u_{\text{Ca}^{2+}}$$

We will generally assume that the Michaelis-Menten constant is so low that this, in practice, will be an on-off switch. In any case, $u_{\text{Ca}^{2+}}$ is always assumed to be
given by such a switch

\[ u_{\text{Ca}^{2+}} = \begin{cases} 
0 & \text{if } t < 2 \text{ or } t > 3 \\
1 & \text{if } 2 < t < 3 
\end{cases} \]

The final flux, \( v_{\text{GP}} \), is also turned drastically on by calcium, even though also this flux is assumed to be non-zero in the absence of contraction. The calcium dependent flux is assumed to be regulated by AMP, but we assume that this regulation is more steep than a classical Michaelis-Menten relation, and therefore use a Hill type expression. We assume that the rate is saturated for high concentrations of \( P \). The calcium dependent flux of this reversible reaction is then given by

\[ v_{\text{GP},1} = V_{\text{max}} \left( \frac{[P] - \frac{[G6P]}{K_{eq}}}{K_{P} + [P]} \right) \cdot \left( \frac{[\text{AMP}]^{g}}{K_{AMP} + [\text{AMP}]^{g}} \right) u_{\text{Ca}^{2+}} \]

The calcium independent flux is due to a different form of the GP enzyme, whose activity is independent of calcium. We assume that this flux is described by

\[ v_{\text{GP},2} = \frac{V_{\text{basal}} [P] - \frac{[G6P]}{K_{eq}}}{K_{P} + [P]} \]

and the total flux through GP is given by the sum of the two fluxes

\[ v_{\text{GP}} = v_{\text{GP},1} + v_{\text{GP},2} \]

Finally, the concentration of AMP is given by ATP and ADP and the assumption that AK is at equilibrium; this gives

\[ [\text{AMP}] = \frac{[\text{ADP}]^2}{[\text{ATP}] \cdot K_{eq}} \]

Handling the variable pH

We pointed out already in Chapter 2 that models with varying pH need special considerations. In all previous models the changes in pH have been judged to be so small that these effects can be neglected. However, in the present biological system we can see in the experimental data that the cells’ pH is lowered from approximately 7.1 to 6.5; this is too much to be neglected.

Generally, the generalisations needed to account for pH variations in a system are of two kinds: i) each reaction in a system does not only change the different concentrations in a system, they also change the pH, and ii) the rate constants in all the reactions depend on pH. In the present study we only consider the first of these effects, and assume that the second effect is small enough to be neglected. The neglect is motivated by the fact that there are no available formulas for how reaction rates change with pH in vivo, and by the fact that we are primarily constructing a minimal model, by which we should be able to make some rather course predictions. Finally, the qualitative reasonings that we seek to formalise
with the given model have not taken the kinetic parameters’ dependence on pH into account. All this means that the calculations we now describe are primarily a way to calculate pH from the given reactions, and only indirectly (through the appearance of $[\text{H}^+]$ in some of the rate expressions) a way to incorporate the effects of pH on the rest of the system.

The reason why a reaction does not only change the concentrations in a medium, but also the pH, is that each substance is associated with a different acid dissociation constant, $K_a$. This constant denotes the equilibrium value when an acid $\text{HA}$ reacts with water to form $\text{H}_3\text{O}^+$:

$$\text{HA} + \text{H}_2\text{O} \rightleftharpoons \text{A}^- + \text{H}_3\text{O}^+$$

In the sequel we will refer to $\text{H}_3\text{O}^+$ as $\text{H}^+$. If the subscript $\text{ss}$ denotes steady state we can thus define the $K_a$ value as

$$K_a := \frac{[\text{H}^+]_{\text{ss}} \cdot [\text{A}^-]_{\text{ss}}}{[\text{HA}]_{\text{ss}}}$$

Note that the concentration of water is dropped out from the definition. Minus of the 10-logarithm of this value is referred to as $pK_a$

$$pK_a := -\log_{10} K_a$$

Note that this is analogous to the definition of pH. Thus, the $pK_a$ value refers to the pH that the specific acid $\text{A}$ strives to. Therefore, different molecular compositions with different $K_a$ values imply an altered pH. This is the reason why reactions (generally) change the pH too, when they convert substrates into products. Note that this happens independently of whether $\text{H}^+$ is present as a substrate or product in the given reaction.

All this means that the differential equation for $[\text{H}^+]$ will include all the reactions in the model. The ordinary reaction rates for the model were defined above. These rates will have to be adjusted to fit into the differential equation for $[\text{H}^+]$, since the effect on $[\text{H}^+]$ will also depend on the $pK_a$ values of the substrates and products. Let these modifications be contained in a multiplicative factor, and let this factor be denoted $S_R$, where $R$ is the name of the reaction. This multiplicative factor is then given by

$$S_R = \frac{\alpha_{A_1}}{\alpha_{A_1} + 1} + \cdots + \frac{\alpha_{A_i}}{\alpha_{A_i} + 1} - \left( \frac{\alpha_{B_1}}{\alpha_{B_1} + 1} + \cdots + \frac{\alpha_{B_j}}{\alpha_{B_j} + 1} \right)$$

where

$$\alpha_x = 10^{-p\text{H}^- - pK_a}$$

and where $R$ is a reaction with substances $A_1$ to $A_i$ and products $B_1$ to $B_j$

$$R : A_1 + \cdots + A_i \xrightarrow{v_R} B_1 + \cdots + B_j$$

To form the final differential equation for $[\text{H}^+]$ these terms are added together, and the sum is multiplied by $[\text{H}^+]$ and the cell’s buffer capacity, $S_{\text{buf}}$. We refer to the appendix in [19] for a more complete description of these derivations.

---

1. There are completely analogous definitions for bases.
The complete model structure

When the variable pH has been handled, the other reactions are formed according to equation (2.10) as usual. The resulting system of equations is given by

\[
\begin{align*}
\frac{d}{dt}[G6P] &= v_{GP} - v_{Glyc} \\
\frac{d}{dt}[P] &= -v_{GP} - 2v_{Glyc} + v_{ATPase} \\
\frac{d}{dt}[PCr] &= -v_{CK} \\
\frac{d}{dt}[Cr] &= v_{CK} \\
\frac{d}{dt}[ATP] &= 3v_{Glyc} - v_{ATPase} + v_{CK} \\
\frac{d}{dt}[ADP] &= -3v_{Glyc} + v_{ATPase} - v_{CK} \\
\frac{d}{dt}[Pyr] &= 2v_{Glyc} - v_{LDH} \\
\frac{d}{dt}[NADH] &= 2v_{Glyc} - v_{LDH} \\
\frac{d}{dt}[NAD^+] &= -2v_{Glyc} + v_{LDH} \\
\frac{d}{dt}[Lac] &= v_{LDH} \\
\frac{d}{dt}[H^+] &= [H^+]_{S_{buf}}(-s_{GP}v_{GP} - s_{LDH}v_{LDH} + s_{Glyc}v_{Glyc} - s_{ATPase}v_{ATPase} - s_{CK}v_{CK}) 
\end{align*}
\]

Note that the equations for PCr and Cr, the equations for Pyr, NADH and NAD\(^{+}\), and the equations for ATP and ADP all have linear dependencies. Each of these three groups of variables can thus be replaced by a single state variable, and corresponding auxiliaries. The full set of equations, reaction rates, and corresponding parameter values are given in the appendix of [19].

11.3 Optimizing the model agreement with the data

We now use various methods to optimize the agreement between the model and the experimental data, and in the next section we evaluate whether this agreement is sufficiently poor to ensure the predicted rejection. The optimization is done by varying the parameters in the model. It is, however, not a classical parameter estimation, since the actual parameter values are not of interest. It is therefore also not necessary to bother about analysing the identifiability of the model.

Ensuring initial stationarity

The first thing that was noted from initial estimations was that it is not automatically ensured that the initial phase of the simulation behaves nicely, even though it is believed to be easy to understand biochemically. The main problem is that the model tends to be more dynamic than the virtually constant experimental data. It is not possible to ensure that the model is at a steady state during the complete first two minutes. It is, on the other hand, possible to ensure that the time-derivatives of the [PME], [P], [PCr] and [ATP] signals are zero at \(t = 0\), by replacing some parameters by auxiliaries. This gives a good approximation of a steady state for the first two minutes, and we therefore adopt this solution; it is derived as follows.
From eq. (11.4) it is clear that the differential equation for [PCr] is zero if the forward and backward reactions in $v_{\text{CK}}$ (given in eq. (11.2)) cancel each other. Solving this relation for [ADP] gives the following expression for [ADP] at $t = 0$

$$[\text{ADP}](0) = \frac{[\text{ATP}](0) \cdot [\text{Cr}](0)}{K_{\text{eq}} \cdot [\text{PCr}](0) \cdot [H^+](0)}$$

If this initial value of [ADP] is chosen, the time derivative of [PCr](0) is zero at $t = 0$. The differential equation for [G6P] is zero if

$$v_{\text{GP}}(0) = v_{\text{Glyc}}(0) \quad (11.5)$$

At $t = 0$, the flux through GP is given by equation (11.3). Insert this relation in eq. (11.5) and solve for $V_{\text{basal}}$. This gives

$$V_{\text{basal}} = \frac{v_{\text{Glyc}}(0) \cdot (K_P + [P](0))}{[P](0) - [\text{G6P}](0)/K_{eq}}$$

and if this value for $V_{\text{basal}}$ is chosen, the time derivative of [G6P] is ensured to be zero at $t = 0$. The above calculations ensured that $v_{\text{GP}} = v_{\text{Glyc}}$. Therefore, the time derivative of [P] is zero at $t = 0$ if $v_{\text{ATPase}} = 3v_{\text{Glyc}}$. At $t = 0$, the flux through the lumped ATPase reaction is given by a constant parameter $v_{\text{basal}}$. The phosphate equation is thus solved by always choosing $v_{\text{basal}}$ as equal to three times the glycolytic flux at time zero.

$$v_{\text{basal}} = 3v_{\text{Glyc}}(0)$$

Note finally that the two relations $v_{\text{CK}}(0) = 0$ and $v_{\text{ATPase}}(0) = 3v_{\text{Glyc}}(0)$ automatically ensures that the ATP differential equation is zero at $t = 0$.

### The optimization

The above calculations ensures initial values with time derivative zero at $t = 0$ for all measured concentrations except for $[H^+]$, which was not problematic during the initial phase. Therefore, the initial states of all measured variables were simply chosen as the corresponding mean values for the initial phase of the time course, or a value close to it. The remaining, unmeasured, initial values were chosen to realistic values, where it was also ensured that the values did not give rise to fast transients in the beginning of the simulations. This was ensured by manual adjustments, prior to the formal optimization; no initial values were included as ordinary parameters by the automatic optimization routines.

The above initial values refer to mean values over the nine experiments. The developed model structure has no experiment specific parameters, and it is thus a mean value model. Since it seemed like there were some experiment to experiment variations in the data that could not be explained by the difference in initial value alone the model structure can not explain the experiment variations given in the data. Therefore, we formed the mean of all the nine experiments at each time point, and based the optimization on such an averaged data set. An alternative to
this would have been to form the cost function on the individual data sets, and in
this way do the averaging with the aid of the model. This method for averaging
could be considered as an advanced filtering method. If the model would have been
able to handle more experiment to experiment variations than the differences in
initial value, that method for averaging the data would be clearly superior to the
current choice.

The formal optimization was performed over all parameters, except those spec-
cific parameters restricted by the approximation of the initial steady state. The
primary choice of optimization method have been the global optimization routine
simulated annealing, combined with the local, but not gradient-based, nonlinear
simplex method (see Chapter 6.1). Usage of purely local optimization methods,
primarily the simplex method, was tested at first, but the conclusion was that
they typically got stuck in local minima. On the other hand, the simplex method
did not give a unique result, which indicated that it also did not find a unique
global optimum. The parameters used by the optimization method are given in
the appendix.

It turned out that the ordinary unweighted least square cost function did not
give an agreement that was acceptable. This was believed to be due to the long
initial steady state, which gave a too high relative importance to these parts of
the time courses, compared to the parts that are considered as biochemically in-
teresting. These are primarily the two other parts, the contraction phase and the
anaerobic recovery phase. Further, it is judged that the different time courses are
unequally important for the agreement. To compensate for all these subjective
assignments to the different degrees of importance to the various parts of the time
courses, scalings and a weighted least square were introduced.

The scalings were done as follows. The three signals [P], [PME] and [PCr] were
divided by the greatest value in the time course. This was not appropriate for the
pH signal, since it had so little relative variation. Therefore the following formula
was used

$$\text{pH}^\text{scaled}(t) = \frac{\text{pH}(t) - \text{pH}_{\text{min}}}{\text{pH}_{\text{max}} - \text{pH}_{\text{min}}}$$

where pH_{max} and pH_{min} refer to the greatest and lowest pH value found in the time
series, respectively. This scaling results in a pH signal that varies between zero and
one. This variation is similar to the variation in the three other time courses. The
fifth curve, for ATP, was not judged to contain any dynamic information, and it
was therefore replaced by the mean value during the optimization.

After these scalings, each phase (initial rest, contraction, and anaerobic recov-
ery) and each time course were given an individual weight in the cost function.
Different weights were tried, and they all resulted in different model agreement
with the data. The subjective judgement of the quality of these fits were judged by
simulations with the corresponding optimized model. Each model that was judged
to improve the agreement were chosen as a starting guess for the next choice of
weights; in this way a final model and set of weights were iteratively obtained. The
final set of weights are given in Table 11.1, and the agreement between the final
model and the data is shown in Figure 11.6.
11.4 Testing the model

We now turn to the problem of evaluating the final model, to see whether it is possible to reject it. We will do this formally using statistical tests, and qualitatively using simulations of certain key properties. First, however, let us make some initial observations.

The agreement between the experimental data and the final model is plotted in Figure 11.6. As is seen, the major qualitative features of the data are captured

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|c|c|}
\hline
 & PME & P & PCr & ATP & pH \\
\hline
Rest & 1 & 1 & 1 & 1 & 1 \\
Contraction & 2 & 3 & 3 & 1 & 3 \\
Recovery & 2 & 2 & 9 & 1 & 2 \\
\hline
\end{tabular}
\caption{The weightings used in the cost function for the optimization when the final model was obtained.}
\end{table}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure11.6.png}
\caption{Comparisons between the experimental data and simulations with the model.}
\end{figure}
completely by the model. Both the data and the simulations are relatively constant during the initial resting phase; both the data and the simulations show a big response to the onset of the contraction; and both the data and the simulations slow down to a relatively constant behaviour when the contraction is over. This in itself is remarkable (especially the agreement during the recovery phase) and above the expectations of what was judged to be possible to achieve with the given model structure, according to the qualitative biochemical reasonings in Section 11.1.3.

Moreover, there are also some more subtle agreements between the simulations and the data. For instance, the response of \([P]\) to the onset of the contraction is more rapid in the beginning than at the end of the work phase. The same capturing of the concave- vs. convexness in the data is captured by the model also in, e.g., the work phase for PCr, even though the exact quantitative agreement there is worse. Finally, an agreement that is as good as it can be based on the given data is the ATP curve; the ATP level is constant to the exact same degree in the simulations as can be observed in the time courses.

However, there are also some discrepancies in the agreement, where the model simulations temporarily leave the experimental time courses altogether, or where the quantitative agreement must be judged to be less than optimal. Two examples where the simulations temporarily leave the experimental time courses are found in the pH curve for \(t \in [2,2.3]\) and in the PCr curve for \(t \in [2.2,2.5]\). One example where the quantitative agreement between the data must be said to give an in-optimal impression is for instance given by the recovery phase in the PCr curve. Let us now use some formal statistical tests to evaluate formally and quantitatively whether these discrepancies are enough to reject the model with a reasonable confidence level, or whether it is fairly plausible that the disagreements are just the outcome of this particular noise realisation.

**Statistical tests**

To evaluate the model formally we will now apply various variations of the \(\chi^2\) test. The basic theory behind this test was reviewed in some detail in Chapter 3.4. There it is explained that the corresponding null hypothesis \(H_0\) assumes that the estimated model structure, \(\mathcal{M}(\hat{\theta})\), is the ‘true’ model, \(\mathcal{M}_0\). That means that the given data is assumed to have been generated by the model output \(\hat{y}(t|\hat{\theta})\) for some specific realisation of additive measurement noise.

\[
y(t) = \hat{y}(t|\hat{\theta}) + \epsilon(t)
\]

One can therefore make statistical tests of the residuals \(\epsilon(t|\hat{\theta})\), to see whether they fulfil the assumed properties of the measurement noise. In the case of the \(\chi^2\) test it is assumed that the measurement noise is Gaussian with mean value zero. Gaussian variables with mean value zero that are divided by their standard deviation follow a \(\mathcal{N}(0,1)\) distribution. The sum of squares of such random variables follow a \(\chi^2\) distribution, where the degrees of freedom in the distribution is given by the number of terms in the sum. Therefore one can simply construct the following sum

\[
T = \sum_{t=1}^{N} \left( \frac{\epsilon(t|\hat{\theta})}{\sigma(t)} \right)^2 = \sum_{t=1}^{N} \left( \frac{\hat{y}(t|\hat{\theta}) - y(t)}{\sigma(t)} \right)^2
\]  

(11.6)
11.4 Testing the model

To be able to form the test quantity (11.6) the standard deviation of the experimental noise at each time point must be estimated. The model that has been developed above is a mean value model, which has been optimized to agree with the mean value at each time point; that means that the most natural choice for standard deviation is the one obtained in the same way: by considering the statistical variation obtained at each time point. It is important to note that such an estimate of the standard deviation would be inappropriate if the model would describe the individual time courses. This problem is, however, mostly to be considered as a limitation of the model; given a mean value model, the noise should be obtained in this or in a similar fashion. A variation of this approach would be to average the noise level obtained in each time point over each phase in the time course. Each of the five time courses would then be associated with three noise levels: one for the initial resting phase, one for the contraction, and one for the final recovery phase.

In all the tests a 95% confidence level is chosen. That means that even if the null hypothesis is true, the test will reject the model one time out of twenty. This is the lowest limit typically used (other common limits are 99% and 99.9%). The reason for this is that we want to consider as big possibility for rejection as is statistically meaningful, since the agreement with the data was judged to be good by visual comparison.

The test was applied in several ways, and all the results are collected in Table 11.2. The first row describes the result when the test was applied to all the given data. This is the most appropriate way to test the model, and as can be seen the model passes the test with a wide marginal. This is the most essential test, which the model thus has passed with ease. The remaining rows describes the test applied to parts of the data, i.e., to individual time courses or to the individual phases in the experiment. This is not a statistically correct way to reject the model as such,

<table>
<thead>
<tr>
<th>Part of data</th>
<th>$T$</th>
<th>95% limit</th>
<th>Passed?</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>136.498</td>
<td>282.511</td>
<td>yes</td>
</tr>
<tr>
<td>PME</td>
<td>15.0258</td>
<td>66.3386</td>
<td>yes</td>
</tr>
<tr>
<td>P</td>
<td>24.8975</td>
<td>66.3386</td>
<td>yes</td>
</tr>
<tr>
<td>PCr</td>
<td>36.4055</td>
<td>66.3386</td>
<td>yes</td>
</tr>
<tr>
<td>ATP</td>
<td>5.23854</td>
<td>66.3386</td>
<td>yes</td>
</tr>
<tr>
<td>pH</td>
<td>54.9311</td>
<td>66.3386</td>
<td>yes</td>
</tr>
<tr>
<td>Relaxation</td>
<td>30.8263</td>
<td>152.094</td>
<td>yes</td>
</tr>
<tr>
<td>Contraction</td>
<td>86.2537</td>
<td>79.0819</td>
<td>no</td>
</tr>
<tr>
<td>Recovery</td>
<td>19.4185</td>
<td>79.0819</td>
<td>yes</td>
</tr>
</tbody>
</table>

Table 11.2 Results from the different $\chi^2$ tests.

and see whether it gives a value that is plausible according to the $\chi^2_N$ distribution. If the null hypothesis is wrong, the residuals will generally be larger than if the null hypothesis holds. As the model error increases the above sum will eventually exceed the chosen limit for what is plausible for a corresponding $\chi^2$ test; this leads to the rejection of the null hypothesis. Those are the basic principles behind the $\chi^2$ test (see Chapter 3.4 for a more comprehensive introduction).
but it might nevertheless give valuable insights into the strengths and weaknesses of the model, seen from the perspective of a statistical $\chi^2$ test. The next five rows in Table 11.2 give the result of the $\chi^2$ test applied to the individual five signals. As can be seen, all the individual signals pass the test, although some with more marginal than others. The signal with most problems is the pH signal, and the reasons for this is discussed below. The final three rows give the result when all the signals are applied, but only to one of the three phases in the experiment. Both the initial relaxation phase and the recovery phase are significantly lower than the limit for rejection. However, the contraction is slightly above the 95% limit, and if this was all the model had predicted it would have been rejected by the test. The reasons for the model’s problems during the contraction phase lie mostly in the pH and PCr curves, and this too is analysed in the discussion below.

To sum up: the model as such passed a statistical $\chi^2$ test; smaller tests were applied for analysis, and the main problems according to test were found to lie in the pH and PCr curves during the contraction.

11.5 Discussion

Understanding the main difference between the new and old predictions

The tests in the previous section show that the present mean value model can explain the data so well that it is not possible to reject it with a $\chi^2$ test using the standard levels for statistical significance. This result stands in contrast to the belief obtained from biochemical reasonings done with the data. This probably means that some of the simplifying assumptions needed to make this reasonings without a model were not fulfilled. It is therefore interesting to go back and consider some of these assumptions, to come with a suggestion for how the reasonings might have gone wrong. It is also interesting to see what a possible explanation to the apparent contradiction might be.

First, however, it should be pointed out that the present study will not be able to make any accurate predictions to what any mechanisms ought to be, in order to achieve the current agreement with the data. This is due to the fact that the model is not (shown to be) identifiable with respect to the available data. There might therefore be an infinite number of parameter combinations in the model that gives rise to an identical agreement with the data. Further, it might also be so that some of these parameter combinations correspond to fundamentally different biochemical mechanisms, giving rise to the same agreement with data. Therefore, all we can do at the present stage is to analyse what the mechanisms giving rise to the observed behaviour are in the present model, without making any statements about the generality of these understandings.

The apparent contradiction in the previous analysis of the data is centred around the flux of glycolysis when the contraction stops: it seems like the flux is almost completely switched off, while all its known regulators seems to indicate that it should have a high flux. Since the model seems to have come around this problem, one of the two contradicting deductions must have been wrong. A simulation of the glycolytic flux is shown in Figure 11.7, and it clearly shows that the
Figure 11.7 The glycolytic flux in the obtained model. It is interesting to see that it is almost completely switched off directly at the onset of the recovery phase. This agrees with the predictions from the qualitative reasonings.

glycolytic flux is indeed almost completely turned off immediately at the onset of the recovery phase.

That means that there must be a mechanism in the model by which the glycolytic flux has been turned off. The glycolytic flux in the model is regulated by four substances: PME, P, NAD\(^+\), and ADP. These regulatory effects all lie between zero and one, and they are plotted in Figure 11.8. When interpreting the plot it should be remembered that these values are multiplied together; it is thus the relative changes that counts, and the absolute values are not particularly important. The only signal that has a significant relative change at \(t = 3\) is the ADP-regulation factor. This factor, on the other hand, almost completely vanishes, and it is thus apparently responsible for the switching off of the glycolytic flux at the termination of the contraction.

The ADP curve was also taken into account when doing the qualitative reasonings leading to the contradiction. Then, however, the ADP curve was calculated
Figure 11.8 Simulations for the four major regulators of glycolytic flux in the model. These values are multiplied together, which means that relative change is the important factor. It can thus be seen that it is the rapid change in [ADP] that switches off glycolysis at $t = 3$.

through various assumptions (described in Section 11.1.3 above), and the resulting ADP curve was predicted to have a qualitative shape similar to that of, e.g., phosphate. The ADP curve calculated by the previous assumptions, and the ADP curve predicted by the model are thus fundamentally different. This difference between the ADP curves in the two frameworks (qualitative reason vs. quantitative modelling) seems to be the main reason why the corresponding predictions are so fundamentally different. This is an example of the dramatic differences in interpretation that can occur, if one of the many used assumptions are disregarded.

Further analysis of the model

While still remembering that the different model predictions are not uniquely determined by the data, and that we have no quality tag for which predictions to believe more or less, it is still interesting to analyse the model a bit further, to
understand the main mechanisms leading to the observed behaviour.

It could, e.g., be interesting to understand what happens at the onset of the contraction. The previous biochemical reasonings led to the conclusion that CK responds first, and that glycolysis takes over more and more the longer the contraction lasts. The glycolytic flux and its regulators are given in Figures 11.7 and 11.8. As can be seen, the flux is rapidly activated by ADP at the start of the contraction, but even though ADP is then lowered glycolysis keeps growing. The latter is due to the fact that the PME and P factors are increasing more than the ADP factor is decreasing. The CK flux is plotted in Figure 11.9. As can be seen it is also rapidly switched on at the onset of the contraction. It is switched on to a higher rate than the glycolytic flux, but when taking the stoichiometry into account it is approximately equal to glycolysis in its contribution to the ATP production. As the contraction then goes on, the glycolytic flux is gradually turned on by the increase in PME and P, while the CK flux is gradually turned down, since it is virtually only effected by [ADP], which is slowly decreasing. Thus, the previous reasonings regarding the interplay between $v_{\text{glyc}}$ and $v_{\text{CK}}$ during the contraction seem to be supported by the model.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure119.png}
\caption{The flux through the creatine kinase reaction.}
\end{figure}
There is a conceptually important implication of this novel interpretation of the experimental data. The conventional interpretation of ADP is that it gives an indication of the availability of energy in the cell. Under these specific conditions, however, it also serves an indicator of the major metabolic fluxes in the cell. At the initial relaxation phase the fluxes are low, as is the ADP level. When the contraction starts the fluxes through the ATPase reactions increase drastically, something which triggers an increase in the fluxes of all the other reactions as well. A key mediator for this, although not the only one, is ADP, and in any case the ADP concentration is high during the entire contraction phase, i.e., when the fluxes are high. Finally, when the flux through the ATPase reactions is drastically reduced, the other fluxes are also drastically reduced. Again ADP and AMP are the two key mediators for the change, and in any case the ADP level is again low at the same time as the fluxes are low. This means that there is a total correlation between the fluxes in the cell and the ADP and AMP levels. This correlation implies that any of these metabolites might be used as a flux sensor in the cell. Measuring fluxes in a cell is otherwise notoriously difficult, and this observation is therefore an important implication of the novel interpretation. It might be used by the cell as a means of information transfer, and might also be useful for future experimental developments.

To conclude the analysis of the model behaviour we can thus say that the rapid changes in calcium to the two ATP producing reactions are mainly mediated by [ADP], both at the onset and at the termination of the contraction. After the onset of the contraction, the ADP concentration slowly declines. This causes the CK flux to drop as well. This in combination with the increasing up-regulatory effects on glycolysis from P and PME, causes the glycolytic flux to be more and more responsible for the total ATP production in the cell. The main differences between the previous qualitative reasonings and the present model predictions stem from the fact that in the model it is ADP that determines the non-equilibrium CK flux, and not the equilibrated CK that determines ADP.

Limitations of the model

The primary goal of the given model was to see whether the standard biochemical assumptions can explain the main features in the given NMR data. The model has shown that this is indeed possible, and a possible mechanism for how this can be achieved has just been given. With this established, let us now finally consider the more general applicability of the model as such, i.e., the model considered as a general model for anaerobic muscle contraction, under these particular experimental conditions.

There are some quantitative disagreements between the model and the data. Some of these have already been detected, either by inspection or through the various $\chi^2$ tests. One such disagreement is present just after the onset of the contraction in the pH curve. The pH curve was found to be the individual curve with the worst $\chi^2$ agreement, and it can be seen in the residual plot in Figure 11.10, that the residuals between $t = 2$ and $t = 2.3$ are contributing to the majority of this high value. A possible biochemical interpretation of this is that CK in the model is too little activated by the onset of the contraction. It should, on the other hand be,
noted that some of the individual curves are moving downwards during this little period in time, which means that the qualitative disagreement is not present for all the time courses. The belief that the model has too little activation in the CK is further supported by the PCr curve: the simulations in the model show a falling PCr trend which is significantly less steep than the corresponding experimental trend. The insufficient activation of CK therefore seems like a plausible explanation to much of the disagreements during the work phase.

Some other quantitative disagreements that are apparent in the data concern the inability to keep the steady states, especially in the $[P]$ curve during the initial relaxation phase, and in the $[PCr]$ curve during the final recovery phase. A better agreement was sought for some of the most critical disagreements between the model and the data. This was done by putting a higher weight on such parts. With the disagreements and weight combinations tried, such modifications did not give a significantly improved agreement on the desired parts, and typically it also resulted in a much worse agreement in other aspects of the data. This indicates that the remaining quantitative disagreements might be outside the capacity of the present model structure. Therefore, if such agreements are sought, new reactions,
states and/or rate expressions are most likely necessary. It should, however, be pointed out that the disagreements between the model and the data is mostly a matter of further exaggerating features that are already qualitatively present in this model. It is, e.g., desired to keep the glycolytic flux even lower during the final recovery phase. Therefore it is probably possible to use the same mechanisms that has been generating the qualitatively correct behaviour in the present model, but further extending the switch-like behaviours. This might, e.g., be possible to achieve by replacing some of the Michaelis-Menten regulations by Hill type expressions. Another apparent suggestion would be to let AMP regulate the glycolytic flux as well. This is known to happen (e.g., by allosteric regulation of PFK) and a linear relative change in ADP equals a quadratic relative change in AMP. The AMP regulation would thus lead to a Hill type regulation. It should, however, be pointed out that if one seeks to make a quantitative model that fully captures these behaviours, it would be beneficial to design experiments giving data which displays these features more prominently.

The above arguments were all concerned with the kind of disagreements between the data and the model, considered as a mean value model. A fundamentally different kind of shortcoming in the present model is its inability to explain the experiment-to-experiment variations. It can clearly be seen in some parts of some time-series that it is an inaccurate approximation to consider each experiment sample as given by a mean value and independent noise. Consider, e.g., the beginning of the PCr curve. There it is clear that in one experiment virtually all measurements were above the others, and in another experiment virtually all measurements below the others. One could consider these particular experiments as outliers, and remove them from the data. It would, however, be more satisfying to be able to describe the variations by introducing some experiment specific parameters, ideally ones with a clear biochemical interpretation. Note that this would also give another interpretation of the noise, e.g., when forming test functions like the ones above. One would then instead have to subtract a filtered version of the data, or in some other way consider the noise in each time course, without comparing with the average value for all time courses. Even though these experiment specific studies are highly interesting, and have a relevance when forming statistical tests, such considerations are outside the scope of the present study.

Summary and conclusions

This chapter started out with a review of a problem that has been observed for 25 years in anaerobic muscle metabolism. The main problem was stated to be that it seems like glycolysis is shut off during anaerobic recovery, even though all the known major regulators of glycolytic flux seems to be high. This conclusion, however, had never been tested with a systematic quantitative approach, but had only been argued using biochemical reasoning and various simplifying assumptions. The main objective with the study in this chapter have been to systematically and quantitatively test whether it is really possible to draw the conclusion that there is such a contradiction between observation and known biochemistry by using a modelling approach.
A minimal but biochemically meaningful model was constructed; it was developed to include states corresponding to a given set of NMR measurements, but with as few extra states as possible, and it was developed to include all the essential biochemical interactions, using as few parameters as possible. The model agreement with the data was optimised using an iterative improvement of the cost function, which was minimised with respect to the parameters using a combination of simulated annealing and a nonlinear simplex method. The final model was evaluated qualitatively by comparing simulations of certain key properties with the data and known features of the system, and quantitatively using various variations of a $\chi^2$ test. The obtained model had succeeded with the capturing of the major features in the data, during all its three phases: a dynamically calm initial rest phase, a dynamically expressive work phase, and a mysteriously calm recovery phase. This observation was also confirmed quantitatively using the $\chi^2$ tests.

The main conclusions of the chapter is therefore that the biochemical reasonings concerning the data were not accurate; it is not possible to draw the conclusion that the experimental observations contradicts the known biochemistry. The reasons for this turned out to lie in some of the simplified assumptions that are necessary to reason about such a complex biochemically system. One such assumption involved the calculations of the non-measured state ADP, which led to the conclusion that ADP must be high also during the recovery phase. The main reason why the model was able to turn off glycolysis so efficiently was that ADP was equally much lowered, and the assumptions behind the ADP calculations might therefore have been erroneous. It therefore seems like we have solved a 25 year old problem regarding the control of glycolysis in muscle. It is also noteworthy that an implication of the new interpretation is that AMP or ADP might be used as flux indicators.

This study is therefore an example of the fact that qualitative reasonings might lead to wrong conclusions, simply because it is too difficult to account for all the nonlinear interactions in a complex biochemical system without using too severe assumptions. We there conclude that modelling has once again shown to be a necessary ingredient in successful biochemical analysis; not as a complement to experiments and data – but as an aid to their interpretation.
This is the final chapter presenting modelling and analysis of specific systems related to glucose homeostasis. Just like the previous two chapters, this analysis concerns the central metabolism of the chosen cellular system, which in this chapter is activated neutrophils. In the chosen model, however, it is not glycolysis, but the hexose monophosphate shunt and a peroxidase system, that is believed to be most relevant to the observed behaviour. Another difference is that neutrophils not are involved in the regulation of glucose homeostasis, but instead are effected by it. It is well established that the immune defence system starts to malfunction in diabetic patients. This malfunction has most likely something to do with the disturbance tolerance of its metabolism.

In a modelling setting such phenomena are studied through model robustness analysis, which seeks to answer how sensitive a model behaviour is to different kinds of perturbations. The interpretation of the result from the model analysis is, as always, non-trivial, and depends on the both the outcome of the analysis and on the prior knowledge about the system. If both the model and the real system are highly robust against a specific type of disturbances this is a strength of the model. If the model should happen to be highly unrobust this can either be an artifact of the model, or an indication of a fragility of the real system, such as the mentioned fragility of neutrophil activity towards disturbances in the glucose homeostasis. If the latter situation is the case, model analysis might help gain an understanding of the more specific source of the fragility, and thus help find suggestions for drugs and treatments. The typical situation, however, is that models are much more unrobust than the systems they are describing. This is problematic both when developing and analysing a model, and because it means that all the stabilizing regulations in the system are not yet fully understood. The property of functional robustness is
very central to living systems, and it has therefore even been proposed as a possible
definition of a living system [57]. We therefore end this introduction by claiming
that the understanding of robustness is at the heart of the understanding life itself
(compare also with the discussions in Chapter 1).

In this chapter we compare two different measures for model robustness. The
first is obtained by measuring the sensitivity to variations in individual parameters
and is the most commonly used measure. In contrast to this we use a different
measure, which is based on perturbations in the model structure, also including
delays. We show that the latter method does show important fragilities in the
chosen model, that are not at all detected by the parametric analysis. It is shown
that the fragilities missed by the parametric analysis are really there in the model,
and the main conclusion is therefore that one should exercise extreme care when
interpreting an analysis of model robustness, that is based entirely on parametric
sensitivity (like for instance an MCA analysis). The chosen model has also shown
itself to be hard to reduce, and the present analysis might therefore also indicate
which parts of the model that has to be remodelled before this can be done.

12.1 Background

The oscillatory metabolism of activated neutrophils

The model we have chosen to study describes an activated neutrophil. Neutrophils
are polymorphonuclear white blood cells that constitute a major part of our pri-
mary defence against invading pathogens, such as bacteria, yeast, and fungi. A
neutrophil senses the presence of these unwanted entities through changes in the
chemical environment, typically by the binding of complement factors, bacteria de-
"ved peptides etc., to specific membrane receptors [138]. This leads to activation of
the neutrophil which involves a series of events. Among the most important of these
is a re-organisation of its cytoskeleton to an oval shaped form, and a movement
of the neutrophils in the gradient towards higher concentration of the stimulatory
substance. The activation also dramatically increases the production of NADPH
through the hexose monophosphate shunt (HMS), and starts the production of
the enzyme NADPH oxidase. This enzyme is situated in the membrane between
the cytosol and the extracellular space or, more commonly, between the cytosol
and an intracellular compartment containing toxic substances. Upon activation
this compartment is formed by the merging of smaller compartments, for instance
azurophilic granula, which gathers at the leading edge of the neutrophil. If the
pathogen is small enough it is engulfed in this large compartment, which is then
called a phagosome. One of the sources for the formation of the toxic substances, is
the formation of superoxide (O$_2^-$), which is formed in a two-compartment reaction,
which is catalysed by the NADPH oxidase and which uses NADPH as substrate
on the cytosolic side

$$
\text{NADPH} + 2 \text{O}_2 \xrightarrow{\text{NADPH oxidase}} \text{NADP}^+ + 2 \text{O}_2^-
$$

Superoxide is then used to form other reactive oxygen species (ROS), through a
series of reactions involving the protein myeloperoxidase. The different granules
that discharges their content of bactericidal proteins and enzymes through fusion with the phagosome, is another important source of toxic substances, used in the killing of the bacteria. All these events are outlined in Figure 12.1.

![Figure 12.1](image)

*Figure 12.1 Some of the most important steps in neutrophil activation.*

**The Olsen model**

The model that we study in this chapter was first presented in [95], which will henceforth be referred to as the Olsen model. The Olsen model describes the central metabolism of an activated neutrophil. The model has 16 states and 25 parameters. All states correspond to concentrations of different substances, and they are divided between two compartments: the cytosol and the phagosome. There are two conserved moieties in the model, and there are therefore only 14 independent states. The main feature that is captured in the model is the temporal oscillations that is observed, e.g., when measuring NAD(P)H or ROS through fluorescence techniques. In these experiments one can also observe spatial waves that move along the direction of the cell [67], but since the Olsen model only contains two compartments, such behaviour can not be captured. Apart from displaying oscillations with the right frequency, and with all concentrations in reasonable orders of magnitude, the model has also been used to make some non-trivial predictions. One such prediction concerns the relation between melatonin (MLTH) and the amplitude of the oscillations [95].
Previous indications of high sensitivity

In [95] it is claimed that all components included in the model are necessary to explain the observed oscillations. Indeed, the necessity of several of the included mechanisms was even shown experimentally. The authors conclude that if one of the mechanisms included in the model is taken away, the qualitative behaviour changes completely. This is a first indication that the model might be highly sensitive to perturbations.

Another indication that the model has a low robustness is given in [142]. In this article a new method for model reduction using a low-dimensional manifold technique with time-varying decompositions is presented. The method is applied to a peroxidase-oxidase (PO) model. This model is a predecessor to the Olsen model, and all reactions regarding the peroxidases (i.e. those in the phagosome) as well as, e.g., the production term for NADPH are virtually identical in the two models. The reduction method divides the variables in two modes, one active and one inactive, according to the rate of their dynamics. The inactive mode is eliminated in the reduced model. Since the method is continually updating the division in modes, variables might belong to the active mode during one phase of the simulation, and to the inactive mode during another. This time-varying division into modes is good for computational reasons, since one can then typically consider a smaller active mode during each phase in the simulation. Further, if some variables belong to an inactive mode during the whole simulation this indicates that it is possible to eliminate that variable from the original equations, while still keeping most of the dynamics. However, the analysis showed that all variables are present almost equally long time in the active mode during a simulation (see e.g. Figure 6 in [142]). This is thus another indication that there are no components in the model that can be taken away, without significantly changing the behaviour of the model, i.e., that the model shows poor robustness.

A final indication of the sensitivity of the model to changes is given in the preceding Licentiate Thesis [16]. There it is shown that a division of the Olsen model in compartments, does not lead to the wave phenomena observed experimentally, but instead to a constant gradient in, e.g., the NADPH concentration. This gradient is much bigger than the amplitude of the oscillations, also for so high diffusion that there is virtually no phase-shift between the oscillations in the different compartments. This again shows that a relatively small perturbation might lead to a completely different model behaviour. Finally, in [16] it is also argued that this specific problem might be helped by a more detailed HMS model.

12.2 Parametric robustness

The parametric robustness of the model is examined first. The parametric sensitivity of the amplitude and frequency is studied by MCA (see Chapter 2.5.2). Since it is interesting to see the parametric sensitivity with respect to all parameters, and since the observed quantity is time-varying, only the response coefficients $R_X^p$ is appropriate.

$$ R_X^p = \frac{\partial X/X}{\partial \ln p} = \frac{\partial \ln X}{\partial \ln p} $$  \hspace{1cm} (12.1)
The sensitivity of the parameters with respect to stability is also examined, but this is done using a more global search, specifying the maximal perturbation that can be done before the oscillations stop completely. The distances are obtained with a Java application using the continuation methods available in the JANET software ([www.fys.dtu.dk/~Janet](http://www.fys.dtu.dk/~Janet)), and only local bifurcations are considered. The response coefficients are obtained by a matlab script, using the Systems Biology Toolbox for Matlab [111]. Both codes are available on request.

**Results**

The parameter values were chosen as those described in [95], with $k_{12} = 3 \times 10^{-5}$ and $k_{18} = 0.009$. The initial values $x_0$ were chosen as the unstable steady-state underlying the stable limit cycle. The result from the nonlinear analysis is given in Table 12.1. As can be seen from the table, all parameters can be varied significantly without the metabolic oscillations disappearing. The largest sensitivity, with this measure, is found for the parameters $k_{12}$ and $k_{13}$ which both can be varied about $\pm 20\%$ without removing the metabolic oscillations.

![Amplitude sensitivity](image)

**Figure 12.2** Absolute values of the amplitude response coefficients in the original Olsen model. The parameter sensitivities are sorted in decreasing order from left to right.

The results for the response coefficient of the amplitude, $R^A_p$, is shown in Figure 12.2, and the results are similar for the period. In the figure the parameters have been sorted according to the median value of all amplitude sensitivities (since each variable gives rise to an individual sensitivity). The median is the middle line in
Table 12.1 Parameter regions with sustained oscillations when perturbing one parameter at a time. The unperturbed state is the Olsen model with NADPH influx ($k_{12}$) equal to 3.0E-5M/s, and the superoxide diffusion constant ($k_{18}$) equal to 0.009.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Nominal Value</th>
<th>Lower Limit</th>
<th>Upper Limit</th>
<th>Distance in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_1$</td>
<td>5.0E+07</td>
<td>1.2E+07</td>
<td>1.5E+07</td>
<td>76.00</td>
</tr>
<tr>
<td>$k_{-1}$</td>
<td>58</td>
<td>0</td>
<td>7.2E+03</td>
<td>100</td>
</tr>
<tr>
<td>$k_2$</td>
<td>1.0E+07</td>
<td>6.3E+04</td>
<td>4.9E+09</td>
<td>99.37</td>
</tr>
<tr>
<td>$k_3$</td>
<td>4.0E+03</td>
<td>2.3E+03</td>
<td>1.9E+04</td>
<td>42.50</td>
</tr>
<tr>
<td>$k_4$</td>
<td>2.0E+07</td>
<td>2.5E+06</td>
<td>8.5E+07</td>
<td>87.50</td>
</tr>
<tr>
<td>$k_5$</td>
<td>1.0E+07</td>
<td>0</td>
<td>1.3E+10</td>
<td>100</td>
</tr>
<tr>
<td>$k_6$</td>
<td>1.0E+05</td>
<td>1.6E+04</td>
<td>6.7E+05</td>
<td>84.00</td>
</tr>
<tr>
<td>$k_7$</td>
<td>1.0</td>
<td>0</td>
<td>9.6E+02</td>
<td>100</td>
</tr>
<tr>
<td>$k_8$</td>
<td>5.0E+07</td>
<td>5.4E+06</td>
<td>1.6E+08</td>
<td>89.20</td>
</tr>
<tr>
<td>$k_9$</td>
<td>5.0E+08</td>
<td>1.2E+04</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>$k_{10}$</td>
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<td>5.4E+03</td>
<td>1.0E+19</td>
<td>99.95</td>
</tr>
<tr>
<td>$k_{11}$</td>
<td>6.0E+07</td>
<td>5.2E+06</td>
<td>4.4E+09</td>
<td>91.33</td>
</tr>
<tr>
<td>$k_{12}$</td>
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<td>2.1E-05</td>
<td>3.5E-05</td>
<td>16.67</td>
</tr>
<tr>
<td>$k_{13}$</td>
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<td>1.0E-05</td>
<td>1.8E-05</td>
<td>20.00</td>
</tr>
<tr>
<td>$k_{-13}$</td>
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<td>0</td>
<td>0.19</td>
<td>100</td>
</tr>
<tr>
<td>$k_{14}$</td>
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<td>1.4</td>
<td>3.1E+15</td>
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</tr>
<tr>
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</tr>
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</tr>
<tr>
<td>$k_{17}$</td>
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<td>2.2E+15</td>
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</tr>
<tr>
<td>$k_{18}$</td>
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<td>0</td>
<td>3.2E+15</td>
<td>100</td>
</tr>
<tr>
<td>$V$</td>
<td>288E-06</td>
<td>6.9E-06</td>
<td>0.08</td>
<td>97.60</td>
</tr>
<tr>
<td>$L$</td>
<td>550</td>
<td>1.1</td>
<td>3.4E+08</td>
<td>99.80</td>
</tr>
<tr>
<td>$K_O$</td>
<td>1.5E-06</td>
<td>0</td>
<td>2E-04</td>
<td>100</td>
</tr>
<tr>
<td>$K_{NADPH}$</td>
<td>60E-06</td>
<td>1.0E-06</td>
<td>0.054</td>
<td>98.33</td>
</tr>
</tbody>
</table>

each box and the other two lines display the maximum and minimum sensitivity (except for the effect on the amplitude of melatonin in the cytosol by parameters $k_{16}$ and $k_{17}$ which has been discarded for clarity reasons). The corresponding analysis of the period gave similar results, and we conclude that also the linear parametric sensitivity analysis does not reveal any extremely sensitive parts in the model, but that $k_{12}$ and $k_{13}$ are the most sensitive parameters.

A more detailed HMS model

The two parameters which leads to the highest model sensitivity, $k_{12}$ and $k_{13}$, are also the two parameters appearing in the simplest kinetic expressions. It is therefore interesting to see whether a more detailed description of the corresponding processes changes the corresponding model sensitivity. We choose to check this
Figure 12.3 Absolute values of the amplitude response coefficients in the Olsen model with a more detailed HMS reaction. Observe that no parameters corresponding to the old \( k_{12} \) parameter, in Figure 12.2, are present among the important ones.

on the \( k_{12} \) parameter, since it was also reported to create the problems in the spatial extension [16]. The \( k_{12} \) parameter describes the fairly complex hexose monophosphate shunt (HMS) and in the original Olsen model this whole process, which is the major source of NADPH production, is described exclusively by \( k_{12} \), i.e., as a constant inflow. The main biochemical steps in HMS catalysis glucose-6-phosphate (G6P) to ribulose-5-phosphate (R5P), and converts two NADP\(^+\) into two NADPH.

\[
\text{G6P} + 2 \text{NADP}^+ \xrightarrow{\text{HMS}} \ldots \xrightarrow{\text{HMS}} \text{R5P} + 2 \text{NADPH}
\]

In the extended model this shunt is described in three enzymatic steps, and the production of G6P is modelled by a constant flux through glycolysis. The model with the new HMS description is based on a similar model developed by Ursula Kummer\(^1\), and it is available on request.

The amplitude sensitivity for the extended model is displayed in Figure 12.3. In the new model \( k_{12} \) still corresponds to a part of the NADPH production, and the new parts from the extended HMS description are described by parameters with indexes starting with 0, like \( V_{02}, k_{02} \) etc. As can be seen none of these parameters are ranked among the most important parameters, and we thus conclude that an unrealistically high sensitivity in a single parameter, can be reduced by a more

\(^1\) personal communication, August 2005
detailed modeling of the processes that the parameter represents. This analysis can by no means by used to draw any general conclusions, but the phenomenon is interesting because it means that sensitivity is not exclusively dependent on which processes are included in a model, but also on how they are described.

12.3 Structural robustness

Now we will consider the structural robustness of the same model. When referring to the structure of a model, we mean the same kind of graph-theoretical structure that was introduced in Chapter 2.3, i.e., with nodes corresponding to the concentrations, and with edges corresponding to reactions and transports. An illustration of the network structure of the Olsen model is shown in Figure 12.4. Only the 14 independent variables are included. As can be seen from the figure, there is a relatively high degree of connectivity in the model, i.e., each node is directly connected to a relatively large number of nodes. One can also see that there are a large number of feedbacks in the model.

![Figure 12.4 Graphical representation of the Olsen model.](image)

We apply the method presented in [110], and shortly reviewed in Chapter 7. That means that we analyse the unstable fix point, that becomes unstable in the Hopf bifurcation. There are no autocatalytic feedbacks in the model, and the oscillating instability is therefore caused by the feedback interactions in the model. This is formally verified by disconnecting all feedback in the linearized network and considering the eigenvalue loci of the corresponding loop-gain. According to the generalised Nyquist criterion, the feedback will induce an oscillating instability if some eigenvalue locus encircles the point +1, and the crossing of the positive real axis to the right of +1 is for a non-zero frequency. The loop-gain of the network is
12.3 Structural robustness

obtained from the linearized state space model

\[ \Delta \dot{x} = \tilde{J} \Delta x + (J - \tilde{J}) \Delta u(t) \]  

(12.2)

where \( J \) is the Jacobian obtained at the unstable fix point, and \( \tilde{J} \) is a diagonal matrix containing the diagonal elements of \( J \). The signal \( \Delta u(t) \) is the feedback signal, and the closed loop system (the original model) is obtained by letting

\[ \Delta u(t) = \Delta x(t) \]

In transfer matrix form, the corresponding loop gain of the network is then

\[ L(s) = (sI - \tilde{J})^{-1}(J - \tilde{J}) \]

**Results**

For the Olsen model we find only one eigenlocus that encircles the +1 point, and this eigenlocus is shown in Figure 12.5. The crossing of the real axis corresponds to the frequency \( \omega_0 = 0.323 \) rad/s, i.e., to a period of 19.5 s, which is close to original frequency (see Figure 12.8). The crossing of the real axis is at 1.003, and hence very close to the critical point +1, indicating that there exist some very small perturbation of the network that will stabilize the steady-state and thereby remove the limit cycle corresponding to the metabolic oscillations. Thus, we should expect the limit cycle in the model to be highly fragile with respect to some specific perturbation of the model structure.

![Critical characteristic locus of the Olsen model.](image)

Figure 12.5 Critical characteristic locus of the Olsen model.

To quantify the instability margin of the underlying steady-state, we employ the structural singular value \( \mu_s \) [39]. Thus, we add a perturbation to the feedback signal \( \Delta u(t) \) of the following form

\[ \Delta u_p(t) = (I + \Delta_t) \Delta u(t) : \quad \Delta_t = \text{diag}(\delta_t) \]
where $\Delta I$ is a diagonal and complex valued perturbation matrix. The perturbation corresponds to adding a relative perturbation to the direct effect of each metabolite on the other metabolites. Using the structured singular value we can compute the smallest size $|\delta_1| = 1/\mu_s$ of the perturbation that moves one eigenlocus of the loop-gain $L$ to the critical point +1 at a given frequency. Since the perturbation in principle may move some locus other than the critical one to +1, it is necessary to verify that the perturbation in fact moves the critical locus to +1. At the nominal steady-state we find that the structured singular value $\mu_s = 380$ (maximum over frequency) and we define the corresponding instability margin to be

$$\delta_1 = 1/\mu_s = 0.0026$$

Thus, a relative perturbation of less than 0.3% in the feedback signals will stabilize the network steady-state, and hence remove the oscillations from the model (assuming no other limit cycle exists, at the given operating point). To consider whether improved robustness can be obtained for other parameter values than the nominal ones, we compute the stability margin along the one-parameter continuation curve connecting the two Hopf points of the nonlinear model for each parameter. The result for the parameter $k_{12}$ is shown in Figure 12.6, and as can be seen the nominal value $k_{12} = 30$ provides a close to maximum robustness in terms of the $\mu_s$ measure. Similar results are obtained for all other parameters in the model, which means that the nominal parameters used in [95] give close to maximal robustness for the given model structure. Thus, the model structure appears to be relatively unrobust regardless of the parameter values used. We realize that a definite conclusion would require simultaneous variations of all the parameters to optimize the robustness, a rather exhaustive task not included in this work.

Because the $\mu_s$-computations in principle are based on applying the same mag-
12.3 Structural robustness

Figure 12.7 Real relative perturbations of each metabolite required to stabilise the Olsen model.

Figure 12.8 Comparison between the original Olsen model, and the reduced 12 state model.

magnitude perturbation to all feedback channels, it does not provide any information on which part of the network which is most sensitive to the perturbation. To obtain such information we employ two measures proposed in [110]. The first measure is based on adding a real valued perturbation to one feedback signal at a time

\[ \Delta u_{pi} = (1 + \epsilon_i) \Delta u_i \quad \Delta u_{pj} = \Delta u_{i, j} \neq i \]

and determine the size of the perturbation \( \epsilon_i \) needed to stabilize the network. The resulting \( \epsilon_i \) values for the nominal parameter values are shown in Figure 12.7. As
can be seen from the figure, the instability of the network is most sensitive with respect to perturbations in the feedback of components 5 (peroxide in phagosome) and 14 (free oxygen in cytoplasm). However, the instability displays a relative large sensitivity with respect to all components apart from components 8 (MLTH in phagosome) and 10 (NADPH in cytoplasm). This result is verified by removing the dynamics of these components, just as we did for the glycolysis models in Chapters 5 and 10.3.1. The resulting model is compared with the original one in Figure 12.8, and as can be seen the two models give quite identical results.

The second structural robustness measure proposed in [110] is based on adding a complex valued perturbation to one element of the loop-gain $L_{ij}$ at the time

$$L_{p,ij} = L_{ij}(1 + \delta_{ij}); \quad L_{p,kl} = L_{kl}, \; kl \neq ij$$

and determining the size of $|\delta_{ij}|$ needed to stabilize the network. If no perturbation of a particular $L_{ij}$ can stabilize the network, then the corresponding $\delta_{ij}$ is put to $\delta_{ij} = \infty$. This perturbation corresponds to perturbing the direct effect of one compound $j$ on another compound $i$. As shown in [110], the $\delta_{ij}$-measure is straightforward to compute from the state-space description of the network. The $\delta_{ij}$ measure for the 15 most sensitive elements of the loop-gain $L$ are shown in Figure 12.9. As can be seen from the figure, the most sensitive interactions involve components 1, 2 and 5.

![Figure 12.9 Magnitudes of relative perturbations of the interaction strengths, needed to stabilize the system.](image)

12.4 Discussion

The result from the structural analysis indicated that the model was highly sensitive to structured perturbations, and pointed to the model’s two most fragile
interactions as being the effect of the H$_2$O$_2$ (component 5) on col (component 2), and the effect of per$^{3+}$ (component 1) on coI, which are all occurring in the phagosome. This is in complete contrast to the results from the parametric robustness analysis which did not indicate a particularly high sensitivity for most of the metabolites, and which indicated that the most fragile reactions were the creation of the NADPH and O$_2$, which are both occurring in the cytoplasm.

The parametric robustness was improved by the extended Olsen model, with a more detailed HMS description. However, this extended model did not show any improved structural robustness, even though it revealed that all the new states could be replaced by constant parameters at the steady state values, without it affecting the structural robustness. This is another indication that the most unrobust parts pointed out by the structural analysis is not at all detected by the parametric analysis. If this is the case, it is a strong indication that one should not rely solely on parametric analysis, when evaluating the robustness of a model. It must, however, first be made clear that the result obtained by the structural analysis really is pointing to a property of the model, and not just to an artifact of the analysis method.

To test this we introduced a delay in the system. A delay corresponds to an imaginary perturbation in the structural analysis. The structural analysis indicates that a delay of $\theta = 0.04$ s should stabilize the system if included in the effect to of per$^{3+}$ on coI. This predicted delay is quite small compared, e.g., to the total period of the oscillations which is about 20 s. The original model is transformed to a nonlinear delay equation, by rewriting the first reaction rate into

$$R_1(t) = k_1[H_2O_2]_p(t)[per^{3+}]_p(t - \theta) - k_{-1}[CoI]_p(t)$$

In Figure 12.10 we see the Nyquist plot for three different delays: $\theta = \{0, 0.045, 0.075\}$ s. As can be seen $\theta = 0.045$ is just sufficient to stabilize the system. The linear structural robustness analysis has thus not only successfully predicted that a small perturbation in the nonlinear model is sufficient to stabilize the system, it has also accurately predicted where the perturbation should be made, and how big it should be. As a final verification that the above perturbation does not only stabilize this specific steady state, but also removes the limit cycle, behaviour bifurcation diagrams for the three delays are computed. As can be seen in Figure 12.11, for $\theta = 0.0045$ s, the limit cycle disappears for all values of $k_{12} > 30$ (the nominal value used during the analysis), and if the delay is increased to $\theta = 0.0075$ the steady state is stabilized for all values of $k_{12}$. This shows that the parametric analysis has failed in detecting a highly fragile aspect of the model, which completely alters the model behaviour even on a global perspective.

Conclusions

Model robustness analysis is an important form of model analysis and, since living systems can be defined as 'robust towards external changes', an understanding of robustness is central in the understanding of life itself. One model that has shown indications of poor robustness is the Olsen model describing the central metabolism of activated neutrophils. A parametric sensitivity analysis, however, indicates that
Chapter 12  Parametric versus Structural Robustness of a Model for
Activated Neutrophils

Figure 12.10 Nyquist plots corresponding to different delays.

Figure 12.11 Bifurcation diagram corresponding to different delays.
the model is quite robust with respect to variations in most of its parameters. This analysis also gives the two most sensitive parameter as those describing the inflow of oxygen, and the production of NADPH. Both these correspond to the most simple descriptions of the model, and for the latter a more detailed process description does give rise to a more robust model. This indicates that parametric robustness is not only dependent on the processes included, but also on how they have been described. We have compared these results with an analysis of the structural robustness of the model. This shows that the model is highly sensitive to perturbations in most of its metabolites. It further predicts that the most fragile interactions in the model are situated in the phagosome. We verified the latter predictions by including a delay in one of interactions reported as highly fragile. The location and magnitude that was needed to completely alter the model behaviour (remove the oscillations) with a small perturbation was predicted with a high accuracy. This means that the structural robustness analysis have detected a real, and highly fragile, weakness of the model, that was completely undetected by the parametric analysis. This high fragility of the model might be the reason why previous attempts at reducing the model complexity have failed. The main conclusion, however, is that analysis of model robustness based entirely on parametric sensitivity, such as MCA, should be interpreted with great care, and that it should ideally be complemented with other types of robustness analyses.
Chapter 12  Parametric versus Structural Robustness of a Model for Activated Neutrophils
This dissertation is entitled 'Core-box modelling – theoretical contributions and applications to glucose homeostasis related systems', and it is divided in two parts: one for theoretical developments, and one for modelling examples. The first part presents a new modelling framework, core-box modelling, and presents existing and new methods for its major sub-steps. The second part presents applications of these methodologies to the model based analyses of four different sub-systems related to glucose homeostasis.

**Theoretical contributions**

The main intent with the core-box modelling framework is to combine the strengths of minimal modelling with the strengths of mechanistic grey-box modelling. Some of the strengths of minimal modelling are related to the fact that a minimal number of parameters are used; their identifiability and uncertainty with respect to an estimation based on *in vivo* data can thus be determined, and a full search in a hypothesis testing approach is thus feasible. Some of the main strengths of mechanistic grey-box modelling is that all the known mechanisms can be included in the model; much of this information is discarded in a minimal model, and this information is important, e.g., when testing different drug scenarios. In a core-box modelling framework, both a minimal and a grey-box model are developed and a translation between them is obtained. This leads to a combined model with all the detailed mechanisms of a grey-box model, as well as the knowledge about the quality of its various predictions, obtained for the minimal model. Finally, if a fully integrated core-box model is obtained, the grey-box and core model may actually be considered as two different versions of the same model, with the only difference being the degree of 'zooming'.
Even though it is important to remember that modelling is a creative process, which cannot be formalised completely, the most important sub-problems in the core-box modelling framework are depicted in Figure 3.1. If there exists a good grey-box model for the system, it is often advantageous to obtain the identifiable core model through identifiability analysis and reduction of this model. We reviewed the state-of-the-art methods for both structural and practical identifiability analysis in Chapter 4.1. For analysis of the mechanisms leading to the unidentifiability, and for reparametrizations into identifiable core models few results are available. In Chapter 4.2 we show such results for unidentifiability in a single rate expressions due to conserved moieties. The results reveal a linear property of the transformations to the identifiable parameters, $\hat{c}$, and this property is utilized both when characterising the translations $\phi$ and $\Psi$. In Chapter 4.3 two new approaches for identification of single rate expressions in the case of practical unidentifiability are presented. The first approach is based on an analysis of the FIM matrix, and yields an identifiable rate expressions with a straightforward back-translation $\Psi$. The second approach generates simpler expressions through minimal modelling, and allows for a comparison with previous in vitro characterisations through the forward translation $\phi$.

Chapter 5 deals with the general problem of reducing a biochemical model. First, in Chapter 5.1, a review of existing methods is given, and the lumping and SA based methods are pointed out as the ones most suited for the core-box modelling framework, even though an identifiability analysis needs to be incorporated in the methods. In Chapter 5.2 we present a model reduction based method for the mechanism problem, i.e., the characterisation of the oscillating core in a model. The key idea is to compare analyses done before and after the reduction. However, the reduction itself also gives information about the feasibility of the original predictions. Thereafter, in Chapter 5.3, we presented a MATLAB implementation for reducing the rate expressions in a complete model. The theory behind the implementation is based on a reformulation into an ordinary linear least squares estimation problem, and the program also returns an interpretation in terms of the original parameters. Both the new reduction methods are developed for models with complexities like the Hynne model for yeast glycolysis, and in Chapter 10.3 it is shown that the obtained reductions are quite significant.

Once the model structure of a core model has been obtained it is ready to be estimated from the available data; this problem is treated in Chapter 6. First a review of the state-of-the-art methods is given. It is concluded that one of the big problems is associated with the size of the search space. A method that allows for a reduction of the $p_x$ parameters is presented in Chapter 6.2. The method is applicable for all systems with an experimentally located Hopf bifurcation. The key step is to translate the information about the bifurcation into a standard constrained optimization problem. Further improvements are given if the initial part of the experiment is collected in the vicinity of the bifurcation. The initial value parameters $x_0$ may then be parametrized in minimal degrees of freedom, and solved for in a separate sub-problem. For the case of initial stationary oscillations, we show how the optimization sub-problem may be solved in a straightforward manner without problems with local minima. This is possible because of a combination of a central manifold and normal form transformation, which reveals the special structure of
the Hopf bifurcation. Finally, the advantage of the methods are demonstrated on
the Brusselator.

The final step in the core-box modelling framework is the back-translation step. This
seems to be a novel research field, and part of the contribution of Chapter 7
is to introduce a basis for future research within this field. The central transfor-
mations are $\phi : p \mapsto p'$ and $\Psi : (p'; \eta) \mapsto p$, which maps to and from the core
model, respectively. For the general case we present simulation based approaches
for the characterisation of these mappings. These numerically determined map-
pings might also be converted into analytical expressions, e.g., based on an analysis
of the Taylor expansions, which might also serve as model structures in themselves.
However, there are several situations where improved solutions are possible. For
structural unidentifiability on may reuse the theory from symmetry families, and
if the structural unidentifiability is due to conserved moieties, an analysis of the
$C$ matrix may be used to obtained explicit formulas for the both the forward and
back-translations. For practical unidentifiabilities eliminated by SA based meth-
ods $\phi$ and $\Psi$ are given by unity transformations. Another reduction method that
yields analytical translation formulas is variable lumping; we give both the exact
transformation formulas and simplified expressions that are only dependent on the
kinetic parameters inside the lump. The high accuracy of the simplified expres-
sions are illustrated on a 6-state lump example. For both these reduction methods
$M_g$ and $M_c$ are so integrated that they may be considered as different degrees
of ‘zooming’ of the same model. Finally, we illustrate the advantage of having the
detailed quality tags of the core-box model compared to both the grey-box and
minimal models on a biosimulation test case.

Modelling work

The second part of the dissertation applies the modelling methodologies developed
in part I to various systems related to glucose homeostasis. The whole body per-
spective is presented in Chapter 8, and each of the following four chapters contains
modelling of some of its most important sub-systems.

Chapter 9 is devoted to the development of a core-box model for insulin receptor
activation and internalisation in human fat cells. A core model is developed using
a hypothesis testing approach. Much of the information in the data set turns out
to lie in a rapid overshoot in the data. A reformulation of the model structures in
transfer function form allows for easy discrimination of models that can not display
an overshoot in the relevant output for any parameter value. This rejects most of
the simpler models, and further analysis shows that all model structures without
internalisation are incapable of giving a satisfactory quantitative agreement with
the data. It is shown that a satisfactory agreement requires both internalisation,
intracellular dephosphorylation and recycling of the internalised free receptors to
the membrane. If this is included, it is sufficient with a model with three states
and three kinetic parameters to describe the data. An analytical back-translation
from this model to comprehensive grey-box models is established using the meth-
ods in Chapter 7.3, which means that a ‘zoomable’ model is obtained. All three
kinetic parameters in the core model are not identifiable together, but some robust
predictions are nevertheless obtained through global searches. One such robust
prediction is that the pool of free intracellular receptors is growing to substantial levels with a time-scale of 3-5 minutes, and that the internalisation of the phosphorylated receptors instead follows the time-scale of the rapid overshoot in the data.

Yeast glycolysis is one of the most well-studied systems, and it therefore serves as a good test case for the state-of-the-art in systems biology. Several new methods for this system, often with the type of complexity in the Hynne model in mind, are presented in Part I of the dissertation. Most of these methods are applied to the Hynne model in Chapter 10. First, a structural unidentifiability in the model is characterised and eliminated using the methods from Chapter 4.2. Thereafter practical identifiability with respect to steady state data is obtained in the PGI reaction using the methods of Chapter 4.3. A new in vivo expression is proposed, and the estimated parameters are compared with the previous in vitro parameters through the forward translation $\Phi$. Thereafter, the new method for analysis of the mechanisms problem developed in Chapter 5.2 is applied to the Hynne model. This shows that the primary mechanism leading to the oscillations is the feedback from the lower ATP producing steps to the upper ADP consuming steps, especially through the auto-catalytic stoichiometry and through the allosteric regulation of PFK. Further, this mechanism is shown to be sufficient to yield an oscillating core by the reduction to a three-state model. The last application to the Hynne model is with the MATLAB program developed in Chapter 5.3. This shows that a third of the parameters in the model may easily be reduced by the program.

Muscle metabolism is one of the most important players in the glucose homeostasis, since it accounts for a majority of the consumption. Despite this, metabolism regarding anaerobic muscle has an unanswered question regarding the control of anaerobic glycolysis following contraction. We solve this 25 year old problem through a modelling approach. A minimal model is put up based only on the standard biochemical assumptions. The model is then optimized with respect to a given set of NMR data, and the final agreement is evaluated. It is found that an agreement is obtained that falls within the uncertainties in the data, measured, e.g., with a $\chi^2$ test. The reason why this is possible is that a previous error had been made in the ADP calculations. This error was to assume that the CK reaction was at equilibrium also during the contraction. If this unnecessary assumption is removed, the long-standing contradiction between the observations and the biochemical understanding is resolved through control of glycolysis through ADP and AMP. This new interpretation of the data also implies that ADP and AMP (but not $P_i$) may be used as flux indicators at the given operating point.

In Chapter 12 a neutrophil model is analysed with respect to stability. The model has previously been shown to be unrobust, since it, e.g., is hard to reduce any of its 14 independent states. The parametric robustness does not display an unusual fragility. A structural analysis of the model, on the other hand, shows that the model is highly fragile to some specific complex perturbations ($\mu < 0.001$). Further, some modifications in the model that give a significantly improved parametric robustness do not at all change the structural robustness. It is thus clear that there is a fundamental difference between the structural and parametric robustness. That the structural robustness analysis gives the correct robustness is further supported by a successful prediction of a critical perturbation in the model (eliminating the
oscillations) which is accurate to the third digit. This work therefore shows that
a parametric robustness analysis should be interpreted with care, and ideally be
complemented with other types of analyses.

All these modelling examples show how a model based approach can be used,
and is often necessary, to draw correct and nontrivial conclusions from in vivo
data. It is shown that a minimal model often is sufficient to draw the relevant
conclusions but that translations to a full-scale grey-box model might be possible
to establish already with the present back-translation results. Several of the new
methods developed in Part I have also been illustrated on real models, and their
performance have been demonstrated. All the chosen models are related to glucose
homeostasis, and the present results should therefore bring science some further
steps towards the modelling, and eventually also the understanding, of the whole-
body glucose homeostasis.
Bibliography


[70] M. Kohout, I. Schreiber, and M. Kubícek. A computational tool for nonlinear

[71] K. H. Kreuzberg and A. Betz. Amplitude and period length of yeast NADH
oscillations fermenting on different sugars in dependence of growth phase,
1979.

[72] Peter Kunkel and Volker Mehrmann. *Differential-Algebraic Equations: Ana-

[73] M. Lambeth and M. Kushmerick. A computational model for glycogenolysis

[74] W. Liebermeister. Dimension reduction by balanced truncation applied to
a model of glycolysis. In *Proceedings of the 4th workshop on computation
Logos-Verlag.

[75] W. Liebermeister, U. Baur, and E. Klipp. Biochemical network models sim-

[76] C.-T. Lin. Structural controllability. *IEEE Transactions on Automatic Con-


tory oscillations in yeast: clock-driven mitochondrial cycles of energization.

tory oscillations in yeast: mitochondrial reactive oxygen species, apoptosis

of mitochondrial energization driven by the ultradian clock in a continuous

[81] M.F. Madsen, S. Dana, and P.G. Sørensen. On the mechanisms of glycolytic


[83] G. Magnus and J. Keizer. Model of beta-cell mitochondrial Ca^{2+} handling
and bursting electrical activity. I cytosolic and plasma membrane variables.


Notation

Symbols

\(N, Z, Q\) The sets of natural, integer and rational numbers.
\(R, C\) The sets of real and complex numbers.
\(x\) The \(n\)-dimensional vector with variables in an ODE
\(y\) The \(n_y\)-dimensional vector with measured outputs from a system
\(u\) The known and controllable inputs to a system
\(f\) The vector of functions to an ODE
\(h\) The vector of functions for the outputs from an ODE
\(p_x\) The parameters appearing in the ODEs
\(p_y\) The parameters appearing in the output equations only
\(\mu\) The \(s\)-dimensional vector containing both \(u\) and \(p_x\)
\(x_0\) The parameters describing the initial state to an ODE
\(v\) A reaction rate
\(N\) The stoichiometry matrix
\(\gamma_{ij}\) An element in the stoichiometry matrix
\(k\) A vector with kinetic parameters
\(V_{\text{max}}\) The maximum velocity of a Michaelis-Menten rate expression
\(K_M\) The Michaelis-Menten constant specifying the substrate concentration giving \(v = 0.5V_{\text{max}}\)
<table>
<thead>
<tr>
<th>Notation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$m$</td>
<td>The concentration of a conserved moiety</td>
</tr>
<tr>
<td>$\hat{y}(t</td>
<td>p)$</td>
</tr>
<tr>
<td>$w(t)$</td>
<td>The weight in the error function for the data at time $t$</td>
</tr>
<tr>
<td>$Z^N$</td>
<td>A data series collected at $N$ time points</td>
</tr>
<tr>
<td>$V_N(p, Z^N)$</td>
<td>The cost function to minimise when estimating the parameters</td>
</tr>
<tr>
<td>$\varOmega$</td>
<td>The set of all acceptable parameter values</td>
</tr>
<tr>
<td>$r$</td>
<td>Number of parameters in the system (individual rate expression or system of ODEs)</td>
</tr>
<tr>
<td>$d$</td>
<td>The measurement noise</td>
</tr>
<tr>
<td>$J$</td>
<td>The jacobian $df/dx$</td>
</tr>
<tr>
<td>$I$</td>
<td>The Unity matrix</td>
</tr>
<tr>
<td>$\mu_s$</td>
<td>Structured singular value</td>
</tr>
<tr>
<td>$L$</td>
<td>A transfer function from $u$ to $y$ or of the loop gain</td>
</tr>
<tr>
<td>$E(x)$</td>
<td>Expectation value of $x$</td>
</tr>
<tr>
<td>$\mathcal{L}(p)$</td>
<td>The likelihood function for the given parameter $p$</td>
</tr>
<tr>
<td>$M$</td>
<td>A model structure, i.e., a mapping from $p$ to specific models</td>
</tr>
<tr>
<td>$M_0$</td>
<td>The 'true' model structure</td>
</tr>
<tr>
<td>$x^0$</td>
<td>The 'true' state space vector</td>
</tr>
<tr>
<td>$p^0$</td>
<td>The 'true' parameter vector</td>
</tr>
<tr>
<td>$T$</td>
<td>A statistical test</td>
</tr>
<tr>
<td>$\mathcal{L}$</td>
<td>A likelihood function</td>
</tr>
<tr>
<td>$l$</td>
<td>The logarithm of a likelihood function</td>
</tr>
<tr>
<td>$C$</td>
<td>The covariance matrix</td>
</tr>
<tr>
<td>$H$</td>
<td>The Hessian (typically of the cost function $V$ at the optimum)</td>
</tr>
<tr>
<td>$c$</td>
<td>The coefficients to the terms in $v(x,c)$ in eq. (4.23)</td>
</tr>
<tr>
<td>$n_t$</td>
<td>The number of terms in the rational expression $v(x,c)$ in eq. (4.23)</td>
</tr>
<tr>
<td>$P$</td>
<td>The polynomial in the numerator of (4.23)</td>
</tr>
<tr>
<td>$Q$</td>
<td>The polynomial in the denominator of (4.23)</td>
</tr>
<tr>
<td>$p^{ivt}$</td>
<td>In vitro estimates of the parameters $p$</td>
</tr>
<tr>
<td>$\Delta \hat{p}$</td>
<td>Uncertainty region associated with the estimated $\hat{p}$</td>
</tr>
<tr>
<td>$\eta$</td>
<td>Design parameter in the back-translation</td>
</tr>
<tr>
<td>$\mathcal{N}$</td>
<td>The space of acceptable design-parameters</td>
</tr>
<tr>
<td>$f$</td>
<td>A probability density function</td>
</tr>
<tr>
<td>$F$</td>
<td>A cumulative density function</td>
</tr>
<tr>
<td>$z$</td>
<td>A vector lying in the center space</td>
</tr>
<tr>
<td>$z_1$</td>
<td>The coefficient that uniquely determines $z$</td>
</tr>
<tr>
<td>$x_b$</td>
<td>An extra parameter specifying the state at the bifurcation point</td>
</tr>
<tr>
<td>$\mu_b$</td>
<td></td>
</tr>
<tr>
<td>$\hat{h}$</td>
<td>The vector between the true state $x$ and the image of $x$ on the center manifold</td>
</tr>
</tbody>
</table>
\( p_\mu \) The distance from the bifurcation point (usually a scalar)

\( p' \) The parameter vector in the core model

\( M_c \) The model structure of the core model

\( M_g \) The model structure of the grey-box model

\( \sigma^2 \) The variance (typically of the measurement noise)

\( df \) Degrees of freedom

\( \varepsilon_i(t) \) The \( i \)th residual at the time \( t \)

\( B(p) \) A singular relation whose level curves are approximate invariant manifolds of the output

\( \phi \) The forward translation from \( p \) to \( p' \) (and maybe also \( \eta(p) \))

\( \Psi \) The back-translation from \( p' \) and \( \eta \) to \( p \)

\( p^{ivt} \) Vector with \textit{in vitro} estimated parameter values

**Operators and Functions**

\( A \subset B \) \( A \) is a subset of \( B \).

\( A \subsetneq B \) \( A \) is a proper subset of \( B \).

\( A \cap B \) Intersection of sets \( A \) and \( B \).

\( A \cup B \) Union of sets \( A \) and \( B \).

\( A \setminus B \) The set \( \{ x \mid x \in A \text{ and } x \notin B \} \).

\( A := B \) \( A \) is defined by \( B \).

\( \arg_{p_i} \min_{p \in A} f(p) \) The (sub)-argument \( p_i \) obtained by minimisation of \( f(p) \) with \( p \in A \).

**Acronyms**

- **ODE** Ordinary Differential Equation
- **PDE** Partial Differential Equation
- **AIC** Akaike Information Criterion
- **FPE** Final prediction error
- **NMR** Nuclear Magnetic Resonance
- **IR** Insulin Receptor
- **IRS** Insulin Receptor Substrate
- **NADH** Nicotinamide Adenine Dinucleotide
- **HMS** Hexose Monophosphate Shunt
- **NADPH** Nicotinamide Adenine Dinucleotide Phosphate
- **MLTH** Melatonin
- **PDF** Probability Density Function
- **CDF** Cumulative Density Function
- **FIM** Fisher Information Matrix
- **G6P** Glucose-6-phosphate
- **F6P** Fructose-6-phosphate
- **PGI** Phosphoglucoisomerase
<table>
<thead>
<tr>
<th>Notation</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyr</td>
<td>Pyruvate</td>
</tr>
<tr>
<td>Lac</td>
<td>Lactate</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
</tr>
<tr>
<td>AMP</td>
<td>Adenosine monophosphate</td>
</tr>
<tr>
<td>CK</td>
<td>Creatine kinase</td>
</tr>
<tr>
<td>LDH</td>
<td>Lactate dehydrogenase</td>
</tr>
<tr>
<td>HK</td>
<td>Hexokinase</td>
</tr>
<tr>
<td>GK</td>
<td>Glucokinase</td>
</tr>
<tr>
<td>PFK</td>
<td>Phosphofructokinase</td>
</tr>
<tr>
<td>PTP</td>
<td>Protein tyrosine phosphatase</td>
</tr>
<tr>
<td>Glc</td>
<td>Glucose</td>
</tr>
<tr>
<td>Glyc</td>
<td>Glycerol</td>
</tr>
<tr>
<td>GAP</td>
<td>Glyceraldehyde 3-phosphate</td>
</tr>
<tr>
<td>DHAP</td>
<td>Dihydroxyacetone phosphate</td>
</tr>
<tr>
<td>BPG</td>
<td>1,3-bisphosphoglycerate</td>
</tr>
<tr>
<td>FBP</td>
<td>Fructose 1,6-bisphosphate</td>
</tr>
<tr>
<td>PEP</td>
<td>Phosphoenol pyruvate</td>
</tr>
<tr>
<td>ADH</td>
<td>Alcohol dehydrogenase</td>
</tr>
<tr>
<td>AK</td>
<td>Adenylate kinase</td>
</tr>
<tr>
<td>GAPDH</td>
<td>Glyceraldehyde 3-phosphate dehydrogenase</td>
</tr>
<tr>
<td>ENO</td>
<td>Enolase</td>
</tr>
<tr>
<td>TIM</td>
<td>Triosephosphate isomerase</td>
</tr>
<tr>
<td>ACA</td>
<td>Acetaldehyde</td>
</tr>
<tr>
<td>PME</td>
<td>Phosphomonoesters</td>
</tr>
<tr>
<td>HP</td>
<td>Hexosephosphates</td>
</tr>
<tr>
<td>TCA</td>
<td>Tricarboxylic acid (appears in TCA cycle)</td>
</tr>
<tr>
<td>FFA</td>
<td>Free fatty acids</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>Calcium</td>
</tr>
</tbody>
</table>
Yeast Glycolysis Models

This appendix includes tables over the models in Chapter 10.3. The tables are referenced in the main text.

<table>
<thead>
<tr>
<th>reaction r</th>
<th>rate expression ( v_r )</th>
</tr>
</thead>
<tbody>
<tr>
<td>inGlc: ( \xrightarrow{\Rightarrow} ) Glc(_x)</td>
<td>( k_0 ( [Glc_x]_0 - [Glc_x] ) )</td>
</tr>
<tr>
<td>GlcTrans: Glc(_x) ( \rightarrow) Glc</td>
<td>( V_1 \frac{[Glc_x]}{[Glc_x][ATP]} )</td>
</tr>
<tr>
<td>HK-PFK: Glc + 2 ATP ( \rightarrow) 2 trioseP + 2 ADP</td>
<td>( V_2 \frac{[Glc_x]}{K_{trans} + [Glc_x]} + \frac{[ATP]}{K_i} )</td>
</tr>
<tr>
<td>GAPDH: trioseP + NAD(^+) ( \rightarrow) BPG + NADH</td>
<td>( k_3[\text{trioseP}][\text{NAD}^+] )</td>
</tr>
<tr>
<td>lowpart: BPG + 2 ADP ( \rightarrow) ACA + 2 ATP</td>
<td>( k_4[BPG][ADP] )</td>
</tr>
<tr>
<td>ADH: ACA + NADH ( \rightarrow) NAD(^+)</td>
<td>( k_5[\text{ACA}][\text{NADH}] )</td>
</tr>
<tr>
<td>ATPase: ATP ( \rightarrow) ADP</td>
<td>( k_6[\text{ATP}] )</td>
</tr>
<tr>
<td>storage: Glc + 2 ATP ( \rightarrow) 2 ADP</td>
<td>( k_7[Glc][ATP] )</td>
</tr>
<tr>
<td>glycerol: trioseP + NADH ( \rightarrow) NAD(^+)</td>
<td>( k_8[\text{trioseP}][\text{NADH}] )</td>
</tr>
<tr>
<td>difACA: ACA ( \xrightarrow{\Rightarrow} ) ACA(_x)</td>
<td>( k_9 ( [\text{ACA}] - [\text{ACA}_x] ) )</td>
</tr>
<tr>
<td>outACA: ACA(_x) ( \rightarrow)</td>
<td>( (k_9 + k_{10}) [\text{ACA}_x] )</td>
</tr>
</tbody>
</table>

*Table A.1* The reactions and reaction rates in the 20L8D model.
<table>
<thead>
<tr>
<th>r</th>
<th>reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>HK(^\d)</td>
<td>ATP $\rightarrow$ ADP</td>
</tr>
<tr>
<td>PFK</td>
<td>ATP $\rightarrow$ ADP + FBP</td>
</tr>
<tr>
<td>ALD</td>
<td>FBP $\rightleftharpoons$ GAP + DHAP</td>
</tr>
<tr>
<td>TIM</td>
<td>DHAP $\rightleftharpoons$ GAP</td>
</tr>
<tr>
<td>GAPDH</td>
<td>GAP $\rightleftharpoons$ BPG</td>
</tr>
<tr>
<td>pPEP</td>
<td>ADP + BPG $\rightleftharpoons$ ATP</td>
</tr>
<tr>
<td>PK(^\dd)</td>
<td>ADP $\rightleftharpoons$ ATP</td>
</tr>
<tr>
<td>glycerol</td>
<td>DHAP $\rightarrow$</td>
</tr>
<tr>
<td>storage</td>
<td>ATP $\rightarrow$ ADP</td>
</tr>
<tr>
<td>ATPase</td>
<td>ATP $\rightarrow$ ADP</td>
</tr>
<tr>
<td>AK</td>
<td>ATP + AMP $\rightleftharpoons$ 2 ADP</td>
</tr>
</tbody>
</table>

Table A.2 The model structure of the 20E6D model. \(^\d\): The rate expression of the hexokinase reaction has been substituted according to \(v_{HK} = v_{storage} + v_{PFK}\). \(^\dd\): The rate equation of the pyruvate kinase reaction has been substituted according to \(v_{PK} = v_{pPEP}\).
The rate expressions of the 20E6D model. The reaction names refer to Table A.2. The model reduction has allowed us to lump some of the parameters (indicated by ~), but the underlying parameters are as in the parent 20D model.

\begin{table}
\centering
\begin{tabular}{lll}
\hline
reaction & rate expression $v_r$ \\
\hline
HK-PFK: & $\frac{V_{\text{em}}}{K_5 + [\text{ATP}]} + k_{22}[\text{ATP}]$ \\
PKF: & $\frac{V_{\text{em}}}{K_5 + [\text{ATP}]}$ \\
ALD: & $k_{6|FBP} + [FBP] + k_{6|GAP} + K_6 + [GAP] + [DHAP] + [GAP] + [DHAP] - k_{6|FBP} + [FBP] + k_{6|GAP}$ \\
TIM: & $V_{\text{em}} \left( [\text{DHAP}] - [\text{GAP}] \right)$ \\
GAPDH: & $V_{\text{em}} \left( [\text{GAP}] - [\text{BPG}] / K_3 \right)$ \\
lpPEP: & $k_{9f} [\text{BPG}][\text{ADP}] - k_{9r} [\text{ATP}]$ \\
PK: & $k_{9f} [\text{BPG}][\text{ADP}] - k_{9r} [\text{ATP}]$ \\
glycerol: & $V_{\text{em}} [\text{DHAP}] / K_{15} + [\text{DHAP}]$ \\
storage: & $k_{22} [\text{ATP}]$ \\
ATPase: & $k_{23} [\text{ATP}]$ \\
AK: & $k_{24f} [\text{AMP}][\text{ATP}] - k_{24r} [\text{ADP}]^2$ \\
\hline
\end{tabular}
\caption{The rate expressions of the 20E6D model. The reaction names refer to Table A.2. The model reduction has allowed us to lump some of the parameters (indicated by ~), but the underlying parameters are as in the parent 20D model.}
\end{table}

The model structure of the 20LE3D model. The model reduction has allowed us to lump some of the parameters (indicated by ~), but the underlying parameters are as in the parent 20L8D model.

\begin{table}
\centering
\begin{tabular}{lll}
\hline
reaction & rate expression $v_r$ \\
\hline
HK-PFK: & 2 ATP $\rightarrow$ 2 trioseP + 2 ADP $\frac{V_3 [\text{ATP}]}{1 + \left( \frac{[\text{ATP}]}{V_3} \right)^h}$ \\
GAPDH: & trioseP $\rightarrow$ BPG $k_3 [\text{trioseP}]$ \\
lowpart: & BPG + 2 ADP $\rightarrow$ 2 ATP $k_4 [\text{BPG}][\text{ADP}]$ \\
ATPase: & ATP $\rightarrow$ ADP $k_6 [\text{ATP}]$ \\
storage: & 2 ATP $\rightarrow$ 2 ADP $k_7 [\text{ATP}]$ \\
glycerol: & trioseP $\rightarrow$ $k_8 [\text{trioseP}]$ \\
\hline
\end{tabular}
\caption{The model structure of the 20LE3D model. The model reduction has allowed us to lump some of the parameters (indicated by ~), but the underlying parameters are as in the parent 20L8D model.}
\end{table}
B

Normal Form Transformations

B.1 The transformation table

In Chapter 6.3 we used a normal form transformation to reparametrize the initial value parameters $x_0$ in minimal degrees of freedom. To help the reader check his implementations of these transformations we here include an example with the Brusselator with much of the detailed calculations included. Recall that the goal of the transformation is to find the $h$ function needed to translate the normal form coordinate, $z_1$, to the original coordinates according to equation (6.32), and to find the resonant terms, $g_3$ and $\sigma_1$, needed to determine the time-evolution of the normal form coordinates according to the amplitude equation (6.42). (note that we have dropped to tilde over $h$ compared to the notation in equation (2.20)) Detailed derivations of the algorithms determining these unknowns are given in [88] and [16], and the resulting algorithm is summarised in Table B.1. Section A in the table reviews equation (6.32) with the $h$ function described by its Taylor expansion coefficients $h_{ijk}$. Note that we assume that $p_\mu = \mu$ in these expressions. Section B in the table contains the linear equations needed to calculate these Taylor coefficients. Section C in the table describes how to calculate the resonant coefficients, needed to solve the dynamics of the complex coordinate, $z_1$, through the amplitude equation. The amplitude equation is given in section D in the table. $F_{xx}(u,v)$ and
all similar terms appearing in Table B.1 are defined through equation (B.1)

\[
F_{xx}(u, v) = \sum_{i, j=1}^{n} \frac{\partial^2 f}{\partial x_i \partial x_j} |_{x_b, \mu_b} u_i v_j
\]

\[
F_{xxx}(u, v, w) = \sum_{i, j, k=1}^{n} \frac{\partial^3 f}{\partial x_i \partial x_j \partial x_k} |_{x_b, \mu_b} u_i v_j w_k
\]

\[
F_{\mu} \cdot \mu = \sum_{i=1}^{s} \frac{\partial f}{\partial \mu_i} |_{x_b, \mu_b} u_i
\]

\[
F_{x\mu}(u, \mu) = \sum_{i=1}^{n} \sum_{j=1}^{s} \frac{\partial^2 f}{\partial x_i \partial \mu_j} |_{x_b, \mu_b} u_i \mu_j
\]

where the index on e.g. \(u_i\) means that it is the \(i\):th element of the \(u\) vector. Note also that \(\mu\) often is chosen as one-dimensional, i.e., that \(s = 1\).

### B.2 Normal form transformation of the Brusselator

The equations for the Brusselator are given in equations (6.31a) and the chosen point, around which the transformation is performed, is chosen as \((x_1, x_2, p_1, p_2) = (1, 2, 1, 2)\).

At the chosen point the Jacobian, \(J\), of the right hand side function, \(f\), of the Brusselator is

\[
J(1, 2, 1, 2) = \nabla_x f(1, 2, 1, 2) = \begin{pmatrix} 1 & 1 \\ -2 & -1 \end{pmatrix}
\]

which has two eigenvalues \(\lambda = \pm i\). The right and left eigenvectors of the eigenvalue \(+i\) are

\[
v = (-1 - i, 2)^T
\]

\[
v^* = \frac{1}{2}(2i, 1 + i)
\]

In Table B.1 it can be seen that the second order term equations require the terms
B.2 Normal form transformation of the Brusselator

Table B.1 In this table, taken from [88], all the information that is needed to make a transformation between an arbitrary system with a Hopf bifurcation, and the normal form coordinates, is collected. Section A in the table gives the formula for calculating the original $x$ vector, when all the other equations have been solved. Section B in the table contains the linear equations needed to calculate the $h_{ijk}$s appearing in A. Section C in the table describes how to calculate the resonant coefficients, needed to solve the amplitude equation, i.e., the dynamic equation for $z_1$. The actual amplitude equation is given in section D in the table. The expression $F_{xx}(u,v)$ and all similar terms are Taylor expansions of the $f$ function, as defined in equation (B.1).

$\Phi_{200} = \frac{1}{2} F_{xx}(v,v)$ and $\Phi_{110} = F_{xx}(v,\overline{v})^1$, they become

$$\frac{1}{2} F_{xx}(v,v) = \sum_{i,j=1}^{2} \frac{\partial^2 f}{\partial x_i \partial x_j} |_{(1,2,1,2)} v_i v_j =$$

$$= \frac{1}{2} \begin{pmatrix} 2 & 2 \\ -2 & -2 \end{pmatrix} (-1 - i)(-1 - i) + \frac{1}{2} \begin{pmatrix} 2 & 1 \\ -2 & -1 \end{pmatrix} (-1 - i)2 +$$

$$+ \frac{1}{2} \begin{pmatrix} 2 & 1 \\ -2 & -1 \end{pmatrix} (-1 - i)2 + \frac{1}{2} \begin{pmatrix} 0 & 0 \\ 0 & 0 \end{pmatrix} 2 \cdot 2 = (-4, 4)^T \quad (B.5)$$

$$F_{xx}(v,\overline{v}) = (0, 0)^T \quad (B.6)$$

$^1$The introduction of the auxiliaries $\Phi_{ijk}$ is taken from the full derivation in [88], but their interpretation is not essential for understanding this example.
With these terms, the top three linear equations in Table B.1 for $h_{200}$, $h_{020}$ and $h_{110}$ become

\[
\begin{pmatrix}
1 & 1 \\
-2 & -1 \\
\end{pmatrix}
- 2i
\begin{pmatrix}
1 & 0 \\
0 & 1 \\
\end{pmatrix}
\begin{pmatrix}
\begin{pmatrix} h_{200,1} \\ h_{200,2} \end{pmatrix} \\
\begin{pmatrix} h_{020,1} \\ h_{020,2} \end{pmatrix} \\
\end{pmatrix}
= 
\begin{pmatrix}
-4 \\
4 \\
\end{pmatrix}
\]  
\begin{pmatrix}
\begin{pmatrix} h_{110,1} \\ h_{110,2} \end{pmatrix} \\
\end{pmatrix}
= 
\begin{pmatrix}
0 \\
0 \\
\end{pmatrix}
\]  
(B.7)

and once solved, they give

\(h_{200} = \mathbf{F}_{020} = \frac{1}{3}(8i, -4 - 8i)^T\)  
(B.9)

\(h_{110} = (0, 0)^T\)  
(B.10)

The third order terms (starting with $z_1^2$ in Table B.1) require in the same way

\[\phi_{300} = F_{xx}(v, h_{200}) - \frac{1}{6}F_{xxx}(v, v, v) = (8 + 12i, -8 - 12i)^T\]

\[Q \cdot \phi_{210} = Q \cdot (F_{xx}(v, h_{110}) + F_{xx}(v, h_{200}) + \frac{1}{2}F_{xxx}(v, v, v)) = \frac{1}{3}(8 + 10i, 2 - 18i)^T\]

to put up the $z_1^2$ equations in Table B.1. The solutions to these equations are

\[h_{300} = \mathbf{F}_{300} = \frac{1}{2}(9 - 6i, -7 + 9i)^T\]

\[h_{210} = \mathbf{F}_{210} = \frac{1}{3}(5 - 4i, -10 - i)^T\]

The unfolding terms ($\mu$ and $\mu z_1$ equations in Table B.1) require

\[Q \cdot \phi_{101} = Q \cdot (F_{xx}(v) + F_{xx}(v, h_{001})) = \frac{1}{2}(-1, 2)^T\]  
(B.12)

These unfolding equations, once put up, gives

\[h_{101} = \mathbf{F}_{101} = \frac{1}{4}(-1 + i, 2)^T\]  
(B.13)

\[h_{001} = (0, 1)^T\]  
(B.14)

Finally the two resonant terms of $g$ become

\[g_3 = v^* \cdot \phi_{210} = (-3 - \frac{i}{3})\]  
(B.15)

\[\sigma_1 = v^* \cdot \phi_{101} = \frac{1}{2}\]  
(B.16)

With these terms calculated, the transformation under $A$ in Table B.1 maps the normal form coordinates $(z_1, \mu) = (0.1 + 0i, 0)$ on the original coordinates $(x_1, x_2) = (0.812, 2.36033)$.  

