GUNNAR'S CRASH COURSE

IN

SYSTEMS BIOLOGY

In this crash course we will give you the basics of formulating a systems biology model. This is meant as an introduction for the project course TSRT17. This course is centered around typical, but fairly similar projects. All projects' models, are, e.g., implemented as nonlinear ordinary differential equations (ODEs). ODEs are introduced in Section 2. The model structures are 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 3. In Section 4 and 5 we describe how these parts are quantified and put together. The fitting of the model is described in Section 6. These are the steps that will be tested in the first mini-examination (swedish: "dugga"). The remaining steps, to be tested in the second mini-examination are described in remaining parts of the chapter. First, however, let us have a look at an example from the literature.

1 An introductory example

A good way to get a first feeling for what a field is, is to have a look at an example. Here we will use an example from insulin signalling, for exactly this purpose: to see what is needed in a systems biology project, and what systems biology methodologies may provide.

The example is concerned with insulin signalling, and is inspired by the developments in [4, 1]. Insulin signalling occurs via the insulin receptor (IR). The IR signalling processes may be inspected experimentally by following the change in concentration of phosphorylated IR (denoted IR·P), and a typical time-series is presented as vertical lines (which gives one standard deviation, with the mean in the middle) in Figure 2. As is clear from the figure, the degree of phosphorylation increases rapidly upon addition of insulin (100 nM at time zero), reaches a peak value within the first minute, and then goes down again and reaches a steady state value after 5-10 minutes. This behaviour is referred to as an overshoot in the experimental data. These data are one of the three inputs needed for the methods in a systems biology project (Figure 1).

The second input in Figure 1 is prior knowledge. For the IR sub-system this includes, for instance, the facts that IR is phosphorylated much more easily after binding to insulin and that the phosphorylation and dephosphorylation occurs in several catalysed steps. It is also known that IR may leave the membrane and enter the cytosol, a process known as internalisation. The internalisation may also be followed by a return to the membrane, which is known as recycling.

The final type of input in Figure 1 concerns suggested explanations. In systems biology, an explanation should both be able to quantitatively describe the experimental data, and do it in a way that does not violate the prior knowledge, i.e., using a mechanistic model. However, it is important to note that a mechanistic model does not have to explicitly include all the mechanisms that are known to occur. Rather, modelling is often used to achieve a characterisation of which of these mechanisms that are significantly active, and independently important, and which mechanisms that are present but not significantly and/or

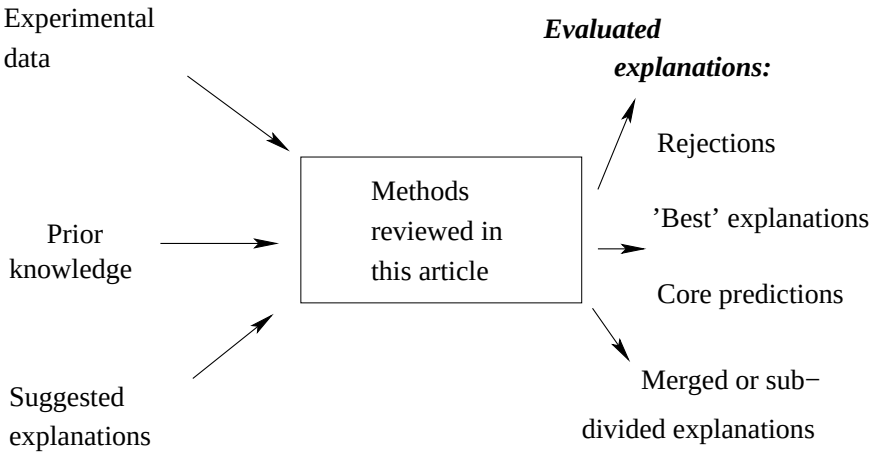

Figure 1: The kind of methods reviewed here: for analysis of given explanations for a given set of experimental data and prior knowledge. Figure taken from [3].

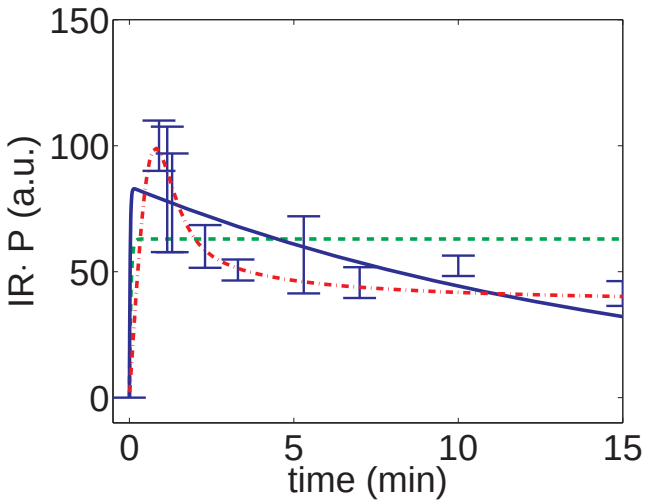

Figure 2: Experimental data and simulations corresponding to the example in Section 1. This review deals with methods for a systematic comparisons between such experimental and simulated data series. The result of these methods is an evaluation and comparison of the corresponding explanations. Importantly, this allows for mechanistic insights to be drawn from such experimental data that would not be obtained without modelling.

uniquely contributing to the experimentally observed behaviour. For instance, it is known that there is an ongoing internalisation and recycling, but it is not known whether these are significantly active already during the first few minutes in a response to insulin, and it is only the first few minutes that is observed in the experimental data. Therefore, it is interesting to consider explanations for these data that contain recycling and then to compare these with corresponding explanations that do not include the recycling. Examples of two such alternative suggested explanations are given in Figure 3.

With all inputs established, the methods in this review can be applied to achieve the outputs displayed in Figure 1. The first phase (Figure 4) checks whether the given hypothesis may serve as an acceptable explanation to the data in Figure 2. This phase consists of several sub-step. The first such sub-step is to translate the graphical drawings in Figure 3 to mathematical models. This translation is the step that opens up for the systematic, quantitative, and automatic analysis of many of the properties that are carried out in the remaining steps. The second sub-step is to optimize the agreement between the model and the data, by forming a cost function, and then optimizing over the parameters. The final sub-step of the first phase is then to evaluate whether the resulting models are able to describe the experimental observations in a satisfactory manner. This is typically done by evaluating the differences between the model predictions and the experimental data for all time-points (referred to as the residuals) and there are several alternatives for how to do this. For the present example, such an analysis shows that the given explanation with both internalisation and recycling can not be rejected (Figure 2, red, dash-dotted line). The analysis also shows that sub-explanations lacking the internalisation can not display the overshoot at all (green, dashed), and that the resulting model with internalisation but without recycling can not display an overshoot with a sufficiently similar shape (blue, solid) [4]. Nevertheless, the hypothesis with internalisation but without recycling is not completely off, and is therefore interesting for an alternative type of analysis as well. This type of analysis analyses whether the slightly better model (here, the one with both internalisation and recycling) is significantly better than a worse one (here, the one without recycling). After it has been decided whether the models should be rejected or not, the rejected models feedback to the first step (potentially warranting reformulations of the model, to see whether that makes them acceptable), and the non-rejected models pass on to the second phase in Figure 4.

The second phase of the modelling is to evaluate properties of the model, and especially to look for well-determined properties (which are called core predictions) that can be tested experimentally. In this case, we could find such a core prediction for the state x_4, which corresponds to IR that has been internalized and dephosphorylated. The simulated time-series for this state is shown in Figure 5, and as can be seen the state rises from close to zero, to a value that lies around 60-70% of the total amount of receptors. The different curves gives an estimate for the uncertainty of the prediction, and the details of how this is done in practice is explained below. In any case, this is a value that is so extreme that it can be tested by an experiment where the membrane-bound

compartment is separated from the cytosolic compartment. In other words, the prediction feedbacks to the experimental data gathering step, which will generate a new set of inputs to the modelling. The modelling process outlined in Figure 4 is therefore a cycle, which iterates between experimental data gathering plus re-consideration of the existing biological hypothesis, and a data-analysis step which provides two types of insights: i) that some of the hypothesis are insufficient to explain the data, ii) that the non-rejected hypothesis may explain the data only if certain well-characterised properties are fulfilled. For the present example, the experimental test of the internalisation prediction showed that also the internalization hypothesis must be rejected, in favor of even more complex hypotheses. The current state-of-the-art view of this system is that the first few minutes of the response to insulin is governed by a negative feedback from downstream signalling intermediates, and where this feedback is dependent on internalisation, but is not the internalisation in itself. This rather non-trivial view of the system could not have been obtained without this iterative experimental/modelling-approach. In fact, the conclusion required five such iterations, and these are summarized in Figure 6, and described in more detail in [1]. Let us now leave this example, and have a look at how the different steps in the modelling-loop are carried out in practice.

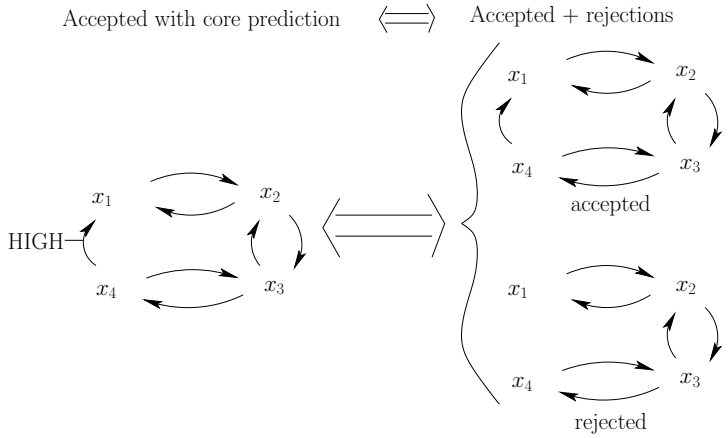

Figure 3: To the right two of the models for the insulin signalling example in Section 1 are depicted. The top one includes both internalisation and recycling after dephosphorylation, but not the lower one. The figure to the left corresponds to an identical way of presenting the same rejection: in terms of core predictions (Section 8). This way depicts a single model with internalisation and recycling, where the core prediction shows that the recycling must have a high (non-zero) rate. x_1 and x_2 corresponds to unphosphorylated and phosphorylated IR, respectively, and x_3 and x_4 corresponds to internalised phosphorylated and dephosphorylated IR, respectively.

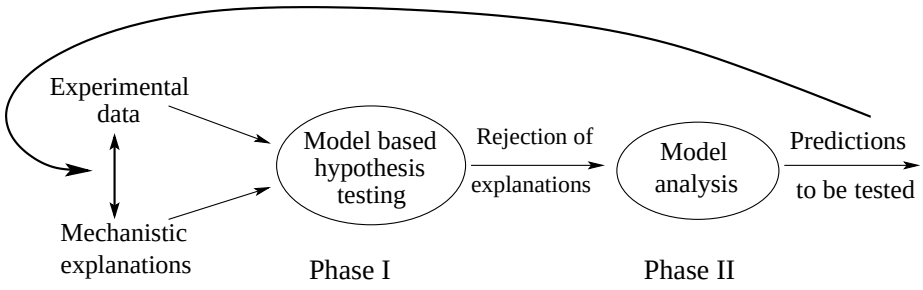

Figure 4: The main conclusions that can be drawn from a model-based data analysis are obtained in two phases. The first phase checks whether the given hypothesis may serve as explanations to the given data. The second phase looks for interesting and uniquely identified predictions that can be tested experimentally.

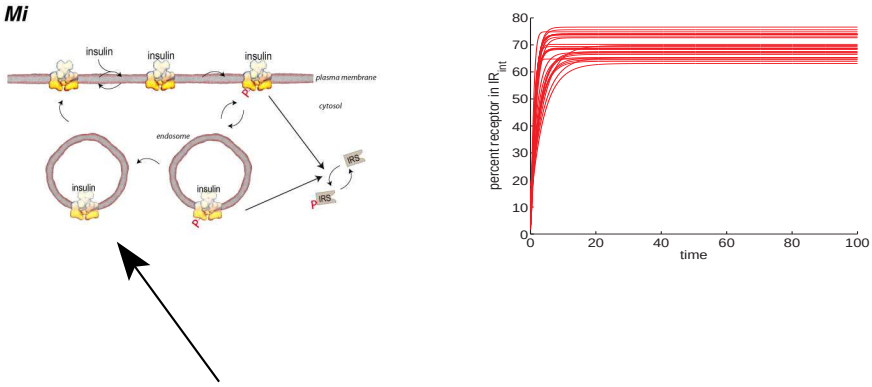

Figure 5: Time-series for state x_4, i.e., the state of IR that has been phosphorylated, internalized, dephosphorylated, but not yet recycled to the membrane. The state is expressed as percent of the total amount of receptors, and as can be seen, the prediction is that around 60-70% of the receptors end up in this state after a few minutes of insulin stimulation. The different lines correspond to simulations with different acceptable parameters, and as can be seen, these different non-rejectable parameter combinations give different predictions, but they are still fairly similar. Most importantly, they are all widely different from the result of the experimental testing, which lies below 5%, and thus leads to a rejection of the model. This is one of the key predictions in [1], and all such conclusions are summarized in Figure 6.

Experimental data	Models				
	Md	*Mm*	*Mf*	*Mi*	*Mfi*
Overshoot	OK	OK	OK	OK	OK
Insulin in medium	Fail	OK	OK	OK	OK
Standard data		OK	OK	OK	OK
Blocking of internalization		Fail	Fail	OK	OK
Extent of internalization				Fail	OK

Figure 6: Summary of five loops in the experimental/modelling-cycle from Figure 4. The columns display different hypotheses, and the rows correspond to different experiments. The table is taken from [1], and as can be seen, the first observation (the overshoot in Figure 2) can be explained by all hypotheses proposed in that paper. Furthermore, the model-based analysis leads to a prediction (concerning the amount of insulin in the medium) that allows for one of the hypotheses to be rejected. After a number of loops, only one explanation remains: one that combines downstream signalling with receptor internalisation in a certain manner. This conclusion could not have been drawn without such an iterative approach involving both experiments and mathematical modelling.

2 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 [23].

Some alternative, and more general, choices would have been partial differential equations (PDEs) [9], or differential delay equations (DDEs) [7]. 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) [17], 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 [18]. 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 [2]. 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

$$\dot{x} = f(x, p_x, u) \tag{1}$$

Sometimes it will be beneficial to consider the u and p_x vector together. Let this pair be denoted μ

$$\mu = (p_x, u)$$

Note that μ 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 μ, x, and perhaps some additional parameters p_y. Let this functional relationship be described by h

$$y = h(x, \mu, p_y) \tag{2}$$

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

$$x(t_0) = x_0 \tag{3}$$

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 (1), (2), and (3) fully specifies 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

$$\dot{x}(t) = f(x(t), \mu(t)) = f(x(t), p_x, u(t)) \tag{4a}$$

$$y(t) = h(x(t), p_x, u(t), p_y) \tag{4b}$$

$$x(t_0) = x_0 \tag{4c}$$

Notice that equation (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

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

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

$$\mathcal{M} : p \to \widehat{y}^{\mathcal{M}}(t|p)$$

where the superscript is optional, and simply identifies the model structure generating the simulated output. An equation system of the form (4) describes the most general model structure when modelled by ODEs. A common objective in systems biology is to use experimental data to choose, or estimate, the parameters to have specific values, denoted $\widehat{p}$. This leads to an *estimated model* $\mathcal{M}(\widehat{p})$. 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.

3 Describing the parts

We will now turn our attention to how the building blocks of the models are formulated. In this course 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, ..., S_i, and let the products be denoted P_1, ..., P_j. The stoichiometric coefficients specifies the relative numbers of reactants and products that are involved in a reaction. Let these coefficients be denoted ν_1, ..., ν_{i+j}. The reaction can then be written

$$\nu_1 S_1 + \cdots + \nu_i S_i \rightleftharpoons \nu_{i+1} P_1 + \cdots + \nu_{i+j} P_j \tag{5}$$

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, ..., M_l

$$v = v([S_1], \ldots, [S_i], [P_1], \ldots, [P_j], [M_1], \ldots, [M_l], k) \tag{6}$$

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

Example 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 [15], and is a good description of elementary reactions, i.e., reactions which are not the result of lumping other reactions together. In mass action kinetics, 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

$$A + B \underset{k_b}{\overset{k_f}{\rightleftharpoons}} C + D \tag{7}$$

the rate expression is simply

$$v = k_f[A][B] - k_b[C][D] \tag{8}$$

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 (7).

Example 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 $V_{\max}$. 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.

$$v = \frac{V_{\max}[\mathrm{S}]}{K_{\mathrm{M}} + [\mathrm{S}]} \tag{9}$$

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. The principles behind (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 $[\mathrm{A}]_j$ 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. (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. 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.

4 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 (4). This process often involves 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 (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 (6). To indicate modifications, like those from the M_is in (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 7, 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 the Example 3 below. Since this represen-

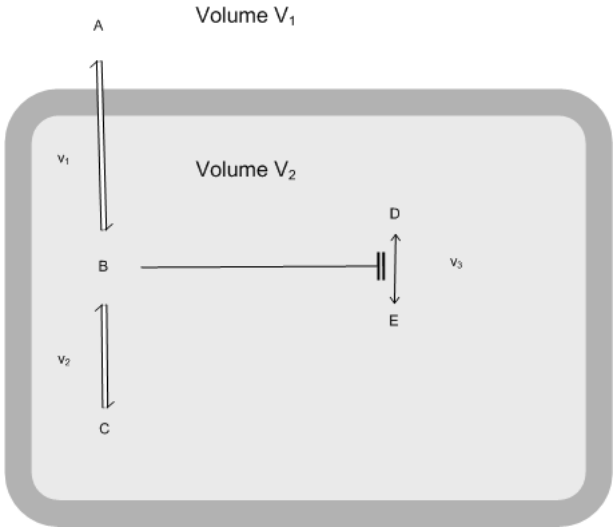

Figure 7: Example of an interaction graph.

tation 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 the model are: i) decisions regarding the boundaries 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 the projects appearing in this course, you will see various examples of both detailed and simplified models.

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. Note, however, that biologists drawing such graphs, may resort to simplified notions, leaving out crucial information regarding the *reactions* (as opposed to the overall flows of information) present in the system.

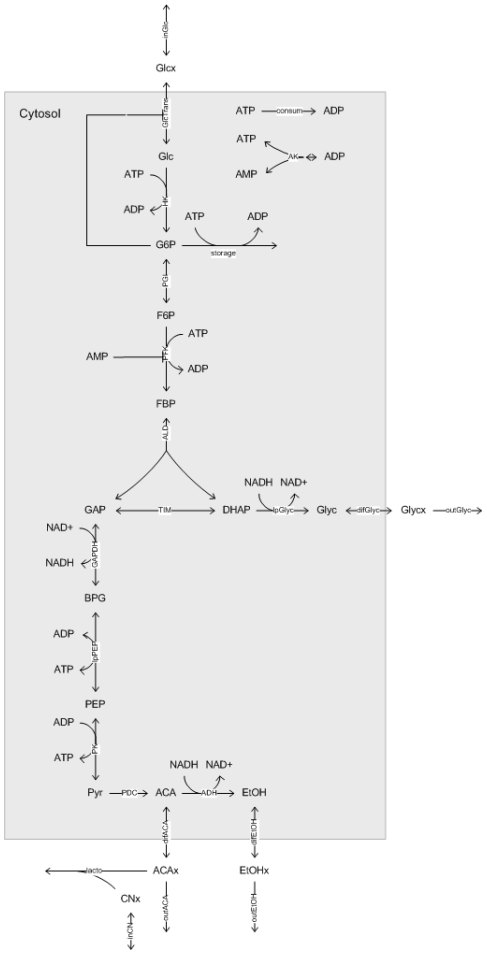

Figure 8: Example of an interaction graph showing only the included variables (here metabolites), and their interactions (here reactions and in- and out-flows). This interaction graph corresponds to a model of yeast glycolysis [13], and it was created in the software PathwayLab. When an interaction graph is modelled in such a software each arrow is 'clickable', and if all reactions are filled in of the form (6), the generation of a an ODE model (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` [5] and MathModelica (mathcore.com). Another common alternative for obtaining the model structure (4) from an interaction graph, is to use the stoichiometry matrix.

The stoichiometry matrix

We will now see how the stoichiometry indexes ν_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 ν_{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

$$N = \begin{pmatrix} \nu_{11} & \nu_{12} & \dots \\ \nu_{21} & \nu_{22} & \vdots \\ \vdots & \vdots & \ddots \end{pmatrix}$$

Collect the reaction rates v_i in a vector v

$$v = (v_1, v_2, \dots)^T$$

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

$$\dot{x} = Nv \tag{10}$$

There are a number of situations in which it is not as straightforward as in eq. (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 course we will encounter situations with different volumes, but where the different volumes at least are constant in time. This allows for a simpler approach than the general one, with time-varying volumes (see, e.g., [10] for a general treatment).

There are many ways to generalise eq. (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 $V_{vol,r}$. Similarly, denote the volume in which x_i

resides by $V_{vol,i}$. Construct the following $n \times n$ matrix V_{vol}

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

The generalisation of (10) is then given by [13]

$$V_{vol}\dot{x} = Nv \tag{11}$$

An alternative approach is to let the states denote absolute quantities, instead of concentrations. This means that the fluxes out from one compartment and into the next will be the same (note that this is not the case when modelling concentrations belonging to compartments in different volumes). In that case, the rate constants will, however, be dependent on the involved volumes, and the values can therefore not as easily be translated from one system to another, even though the same moulecules are involved.

Formulating a reaction network in the form (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 (i.e., constant entities) by comparing the rank of N, $\text{rank}(N)$, with the dimension of x, $\dim(x)$. If $\text{rank}(N)$ is less than $\dim(x)$ that is evidence that there are conservation laws in the system, and that the system could actually be described by fewer differential equations. These conservation laws can also be deduced by analysis of this matrix, which is done, e.g., by the command `SBreducemodel` in the Systems Biology Toolbox for `MATLAB` [21]. Let us now consider a small example that contains all the grey-box modelling steps considered so far.

Example 3

Consider the simple system depicted in Figure 7. 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 7 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

$$\begin{aligned} v_1: &\quad \text{A} \rightleftharpoons \text{B} \\ v_2: &\quad \text{B} \rightleftharpoons 2\text{C} \end{aligned}$$

which is equivalent to the following stoichiometric matrix

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

For this matrix we have $\dim(x) > \text{rank}(N)$, 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 (11) then gives the following differential equations

$$\begin{aligned} \frac{d}{dt}[\text{A}] &= -\frac{V_2}{V_1} v_1 \\ \frac{d}{dt}[\text{B}] &= v_1 - v_2 \\ \frac{d}{dt}[\text{C}] &= 2v_2 \end{aligned}$$

It is now easy to see that

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

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.

$$[\text{A}]V_1/V_2 + [\text{B}] + 1/2[\text{C}] = m \tag{12}$$

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. (12). Assume that the reaction rates for v_1 and v_2 are described by simple reaction kinetics, with k_i and k_{-i} denoting the forward and backward rate constant for reaction i, respectively. This gives the following ODEs

$$\frac{d}{dt}[\text{A}] = -\frac{V_2}{V_1}(k_1[\text{A}] - k_{-1}[\text{B}]) \tag{13a}$$

$$\frac{d}{dt}[\text{B}] = (k_1[\text{A}] - k_{-1}[\text{B}]) - (k_2[\text{B}] - k_{-2}[\text{C}]) \tag{13b}$$

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

By identifying x with ([A],[B]), and p_x with $(V_1, V_2, k_1, k_{-1}, k_2, k_{-2}, m)$ eq. (13) is found to be of the form (4a). We have thus obtained the non-trivial part of a model structure for this system. The two remaining equations in (4) are easily obtained from knowledge about how the measurements relate to the state variables, and of how the initial values are chosen.

5 Quantifying the parts

Before the model can be used, e.g., in a simulation, one needs parameter values for the kinetic expressions (6), and initial values for the variables (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 *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 (9), but much more complicated. As example we give the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) reaction in the Hynne model [13] which is described by a reversible Michaelis-Menten expression with two non-competing substrate-product couples

$$v_{GAPDH} = \frac{V_{\max}([\mathrm{GAP}][\mathrm{NAD^+}] - \frac{[\mathrm{BPG}][\mathrm{NADH}]}{K_{8eq}})}{K_{8GAP}K_{8NAD}(1 + \frac{[\mathrm{GAP}]}{K_{8GAP}} + \frac{[\mathrm{BPG}]}{k_{8BPG}})(1 + \frac{[\mathrm{NAD^+}]}{K_{8NAD}} + \frac{[\mathrm{NADH}]}{K_{8NADH}})} \quad (14)$$

Although an expression of this kind contains fewer parameters than the underlying network of elementary reactions, there are still many unknown parameters. Note 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 examined in *in vitro* experiments. One such example is the glucose transporter [20], 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.

6 Optimizing and analysing the obtained model

According to the so-called 'biochemical promise' [24] 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 (14)) and how they are related (for instance by an interaction graph of the type in Figure 8), 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 biochemistry' [24]. 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 [18]. 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, are central to modelling, and is also touched upon in relation to the model evaluation and core predictions sections below.

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, and two common non-invasive methods are auto-fluorescence after exposure to ordinary light, and nuclear magnetic resonance (NMR).

6.1 Fitting the model to data

The major conclusion from the Teusink study [24] 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 (4a) and/or (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). 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 [24] by Hynne, Danø and Sørensen [13]. 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 [6]. The most important difference between [24] and [13], 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 [13] 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 $y(t) \in Z^N$ and simulated outputs $\widehat{y}(t|p)$. For an experiment with n_y measurement signals this becomes

$$V_N(p, Z^N) = \sum_{i=1}^{N} \sum_{j=1}^{n_y} (y_j(t_i) - \widehat{y}_j(t_i|p))^2$$

There are many variations of this approach, for instance by adding prior knowledge regarding parameter values or important behaviours as additional terms to the cost function. In any case the parameter is chosen that lies within the region of allowed parameter values, denoted Ω, and minimizes the cost function. 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 $\widehat{p}$, is determined by

$$\widehat{p} = \arg\min_{p} V_N(p, Z^N) \tag{15}$$

and the corresponding model is given by $\mathcal{M}(\widehat{p})$. In relation to the core predictions below, we review different global and local search strategies for solving this minimization problem.

7 Statistical assessment of the quality of the model

We now turn to the problem of evaluating a single hypothesis $\mathcal{M}$ with respect to the given data Z^N, in particular whether the agreement between the model simulations and experimental data is good enough. In other words, we want to know whether the estimated model should be rejected or not. From the introduction of $\mathcal{M}$ above, an obviously important entity to consider for the evaluation of $\mathcal{M}$ is the difference between the measured and predicted data points. We denote such a difference e

$$\varepsilon^{\mathcal{M}}(t, p) := y(t) - \widehat{y}^{\mathcal{M}}(t, p)$$

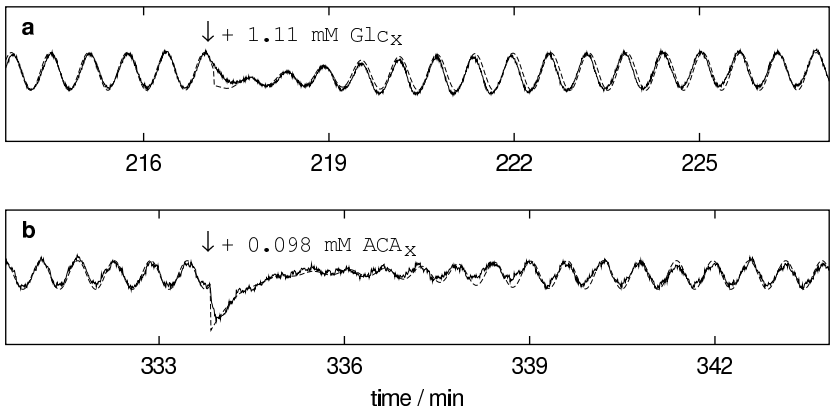

Figure 9: Agreement between the Hynne model and validation data from a quenching experiment.

and it is referred to as a residual. Residuals are depicted in Figure 10. If the residuals are large, and especially if they are large compared to the uncertainty in the data, the model does not provide a good explanation for the data. The size of the residuals is tested in a χ^2 test, which is presented in Section 7.2 below. Likewise, if a large majority of the residuals are similar to their neighbours, e.g., if the simulations lie on the same side of the experimental data for large parts of the data set, the model does not explain the data in an optimal way. This latter property is tested by methods in Section 7.3. The difference between the two types of tests is illustrated in Figure 10. Tests like the χ^2 test, which analyses the size of the residuals, would typically accept the right part of the data series, but reject the left one, and correlation-based methods like the whiteness or run test (Section 7.3), would typically reject the left part, but accept that to the right.

7.1 The null hypothesis: that the tested model is the 'true' model

We now turn to a more formal treatment of the subject. A common assumption in theoretical derivations (e.g., in [18]) is that the data has been generated by a system that behaves like the chosen model structure for some parameter, p^0, and for some realisation of the noise $e(t)$

$$y(t_i) = \widehat{y}^{\mathcal{M}}(t_i, p^0) + e(t_i) \quad \forall\ i \in [1, N] \tag{16}$$

If the $e(t)$s are independent, they are sometimes also referred to as the innovations, since they constitute the part of the system that never can be predicted from past data. It should also be noted that the noise here is assumed to be

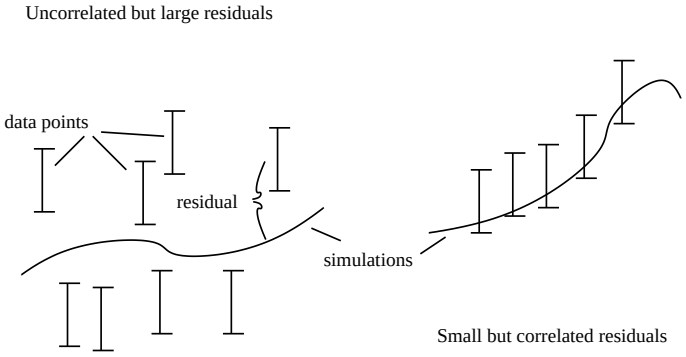

Figure 10: Two sections of experimental data series and simulations. The data points y are shown with one standard deviation. As can be seen in the first part (to the left) the simulations lie outside the uncertainty in the data for all data points. Nevertheless, they lie on both sides of the simulation curve, and with no obvious correlation. Conversely, the second part of the data series shows a close agreement between the data and simulations, but all data points lie on the same side of the simulations. Typically, situations like that to the left are rejected by a χ^2 test but pass a whiteness test, and situations like that to the right pass a χ^2 test but would be rejected by a whiteness test.

additive, and only affecting the measurements. In reality, noise will also appear in the underlying dynamics, but adding noise to the differential equations is still unusual in systems biology.

The assumption (16) can also be tested. According to the standard traditions of testing one can, however, not prove that this, or any, hypothesis is correct, but only examine whether the hypothesis can be rejected [19, 12]. In a statistical testing setting, a null hypothesis is formulated. This null hypothesis corresponds to the tested property being true. The null hypothesis is also associated with a test entity, $\mathcal{T}$. The value of $\mathcal{T}$ depends on the data Z^N. If this value is above a certain threshold, $\delta_{\mathcal{T}}$, the null hypothesis is rejected, with a given significance α_δ [12]. Such a rejection is a strong statement, since it means that the tested property with large probability does not hold, which in this particular case means that the tested hypothesis $\mathcal{M}$ is unable to provide a satisfactory explanation for the data. On the other hand, if $\mathcal{T} < \delta_{\mathcal{T}}$, one simply says that the test was unable to reject the potential explanation from the given data, which is a much weaker statement. In particular, one does not claim that failure to reject the null hypothesis means that it is true, i.e., that $\mathcal{M}$ is the best, or correct, explanation. Nevertheless, passing such a test is a positive indication of the quality of the model.

7.2 Testing the size of the residuals - the χ^2 test

With all the notations in place, (16) together with the hypothesis that $p^0 = \widehat{p}$ can be re-stated as

$$\varepsilon^M(t_j, \widehat{p}) \quad \text{follows the same distribution as} \quad e(t_j) \quad \forall t \in [1, N] \tag{17}$$

which is a common null hypothesis. The most obvious thing one can do to evaluate the residuals is to plot them and to calculate some general statistical properties, like max and mean values etc. This will give an important intuitive feeling for the quality of the model, and for whether it is reasonable to expect that (17) will hold, and that $\mathcal{M}$ is a non-rejectable explanation for the data. However, for given assumptions of the statistical properties of the experimental noise $e(t)$, it is also possible to construct more formal statistical tests. The easiest case is the assumption of independent, identically distributed noise terms following a zero mean normal distribution, $e(t) \in N(0, \sigma^2(t))$. Then, the null hypothesis implies that each term $(y(t) - \widehat{y}(t, p))/\sigma(t)$ follows a standard normal distribution, $N(0, 1)$, and this in turn means that the sum of squares of such terms should follow a χ^2 distribution [11]; this sum is therefore a suitable test function

$$\mathcal{T}_{\chi^2} = \sum_{i,j} \frac{(y_i(t_j) - \widehat{y}_i^M(t_j))^2}{\sigma_i^2(t_j)} \in \chi^2(d) \tag{18}$$

and it is commonly referred to as the χ^2 test. The symbol d denotes the degrees of freedom for the χ^2 distribution, and this number deserves some special attention. In case the test is performed on independent validation data, the residuals should be truly independent, and d is equal to N_{val}, the number of data points in the validation data set, Z^N_{val} [14, 22]. Then the number d is known without approximation.

A common situation, however, is that one does not have enough data points to save a separate data set for validation, i.e., that both the parameter estimation and the test are performed on the same set of data, Z^N. Then one might have the problem of over-fitting. For instance, consider a flexible model structure that potentially could have $e = 0$ for all data points in the estimation data. For such a model structure, $\mathcal{T}_{\chi^2}$ could consequently go to zero, even though the chosen model might behave very poorly on another data set. This is the problem of over-fitting, and it is illustrated in Figure 11. In this case, the residuals cannot be assumed to be independent. In summary, this means that if $Z^N_{\text{test}} = Z^N_{\text{est}}$, one should replace the null hypothesis (17) by (16), and find another distribution than $\chi^2(N_{\text{val}})$ for the χ^2 test (18).

If the model structure is linear in the parameters, and all parameters are identifiable, each parameter that has been fitted to the data can be used to eliminate one term in (18), i.e. one term (e.g., $(y_1(t_4) - \widehat{y}_1(t_4))^2/\sigma_1^2(t_4)$) can be expressed using the other terms and the parameters. When all parameters have been used up, the remaining terms are again normally distributed and independent. This means that the degrees of freedom can then be chosen as

$$d = N - r \quad \text{where} \quad r = dim(p) \tag{19}$$

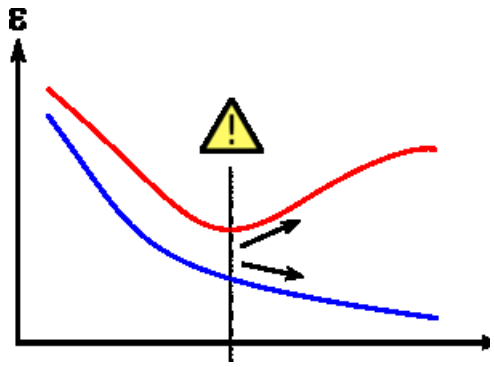

Figure 11: The blue curve is the increasing agreement to estimation data, with increasing model complexity (i.e., flexibility). The red curve shows that there comes a point where the agreement with new validation data gets worse. This is the problem of over-fitting.

This result is exact and holds, at least locally, also for systems which are non-linear in the parameters, like (4) [14, 22]. Note that this compensation with r is done for the same reason as why the calculation of variance from a data series has a minus one in the denominator, if the mean value has been calculated from the data series as well.

However, equation (19) does not hold for unidentifiable systems, i.e., where the data is not sufficient to uniquely estimate all parameters. Since virtually all models in systems biology are unidentifiable, the true degrees of freedom therefore lies somewhere between N and $N - r$. A more detailed discussion regarding this issue can be found in [3].

7.3 Testing the correlation between the residuals

Although the χ^2 test (18) is justified by an assumption of independence of the residuals, it primarily tests the size of the residuals. We will now look at two other tests that more directly examine the correlation between the residuals.

The first test is referred to as the run test. The number of runs R_u is defined as the number of sign changes in the sequence of residuals, and it is compared to the expected number of runs, $N/2$ (since it is assumed that the mean of the uncorrelated Gaussian noise is equal to zero) [8]. An assessment of the significance of the deviation from this number is given by a comparison of

$$\frac{R_u - N/2}{\sqrt{N/2}}$$

and the cumulative N(0,1) distribution for large N and a cumulative binomial distribution for small N [8].

The second test is referred to as a whiteness test. Its null hypothesis is that the residuals are uncorrelated. The test is therefore based on the correlation coefficients $\mathcal{R}(\tau)$, which are defined as follows

$$\mathcal{R}_i(\tau) := \frac{1}{N_i} \sum_{j=1}^{N_i} e_i(t_j) e_i(t_{j-\tau})$$

where N_i is the number of data points with index i. Using these coefficients one may now test the null hypothesis by testing whether the test function $\mathcal{T}_{white}$

$$\mathcal{T}_{white} := \frac{N}{\mathcal{R}(0)^2} \sum_{\tau=1}^{M} \mathcal{R}(\tau)^2 \in \chi^2(M)$$

follows a χ^2 distribution [8].

7.4 Tests that compares two models

There exists another important class of methods, which does not test whether a single model should be rejected based on a poor agreement with the data, but because another model is signicantly better. One of the most common such methods is the likelihood ratio test. The test function, $\mathcal{T}_{lr}$, and the corresponding distribution under standard conditions is given by

$$\mathcal{T}_{lr} = 2(l_1 - l_2) \in \chi^2(d_1 - d_2) \tag{20}$$

where l_i is the logarithm of the likelihood function for model $\mathcal{M}_i(\widehat{p}_i)$, and where d_i is given by $\dim(p_i)$ for $i = 1, 2$. Recall that the likelihood function may be estimated by the cost function, where the residuals are normalised by the standard deviation of the noise, just as for the chi-square test.

However, also when using these model comparison tests, one must recall the fact that these tests are derived based on a number of assumptions which typically are not fulfilled. For instance, the likelihood ratio test assumes that the models are nested (that one of the models is a special case of the other), that one has infinitely many data points, and that the experimental noise is gaussian. Since such assumptions are virtually never fulfilled, one may sometimes want to consider more general – but computationally more demanding – approaches, such as the bootstrap method outlined in [3].

8 Phase II: identification of experimentally testable core predictions

We have now learned all the sub-steps of Phase I in the modelling loop, outlined in Figure 4. This means that we have learned to formulate a model from the given biological hypothesis, to fit the model to data, and to judge whether the resulting agreement is good enough to be acceptable. Both outcomes of this

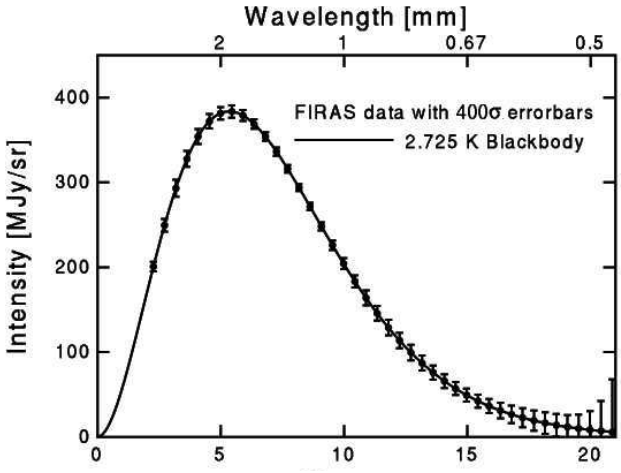

Figure 12: Agreement between the physical model in Eq. 21 and experimental data. The solid line is the model simulations, and the data points show mean plus/minus experimental spread. Note that the plotted spread is 400 standard deviations.

process are interesting. If the model is rejected, this is a strong conclusion, and a valuable insight: it means that some crucial component is missing in the model. The rejections therefore feedback to the beginning of the loop, by forcing the modeller to consider new and modified hypotheses. If it is concluded from Phase I that the model is not rejected, this is a weaker conclusion (since it will probably be revised at some point in the future), but it does mean that the model passes on to Phase II: identification of experimentally testable core predictions.

8.1 The problem of looking at ordinary simulations for a single parameter

First it is important to understand why this step is not as easy as it first might seem. After all, we have already learned to simulate and generate predicted outputs from the model. Would it not simply be sufficient to take the simulate the model for the estimated parameters, and look for interesting predictions? To realize why this is not the case, it might be instructive to compare the situation in systems biology with the historically more common situation of modelling a physical system.

In Figure 12 we show the model agreement for one of the more well-known examples in physics: the black-body heat radiation expression. As can be seen the agreement between the data and the model is good, and the proposed model

$$I(\lambda, T) = \frac{2hc^2}{\lambda^5} \frac{1}{e^{\frac{hc}{\lambda kT}} - 1} \tag{21}$$

is indeed accepted. This model can also be used to look for predictions in a straightforward manner. However, to realise why this is possible here, but not in a biological system, one should notice two things:

- The data in this physical example is of a much higher quality. The experimental spread in the figure is actually 400 standard deviations, which means that the data is determined to many digits. No biological data is that good. Furthermore, biological data usually do not allow you to measure all the interesting states in the model, but usually only a few signals which corresponds to sums of states multiplied with an unknown scaling parameter.
- The parameters in the physical model contains only universal constants like the speed of light (c), and Planck's and Boltsmann's constants (h and k). These few parameters can be determined once and for all, both from the present data and from other sources. Parameters in a biological model, however, are many more, and cannot be determined once and for all, but depend on many conditions, such as species, temperature, pH, and may even be different from cell to cell, or from day to day.

For these reasons, the parameters in a physical model may be assumed to be known, or determined with a high accuracy. The parameters in a typical systems biology model, however, are rarely neither known, or uniquely determined. This non-uniqueness problem is referred to as unidentifiability, and it is the main reason why it is rarely enough to consider a single simulation for a single parameter set, to find truly interesting predictions in a systems biology model.

8.2 A core prediction is a uniquely identified part of an unidentifiable model

We will now introduce two concepts that will allow us to distinguish between two levels of confidence that we may have in a model prediction. The first concept is referred to as a beach statement, and the second concept is referred to as a core prediction

Def: A model prediction that only states its assessment to the level of "It might be like this, but it might also be in some other way" is referred to as a beach statement.

Def: A model prediction that states its assessment on the level of "This model property must be fulfilled if the given model should describe the given data" is referred to as a core prediction.

Let us understand the difference between these two types of predictions by considering the following little example:

$$\dot{x} = -(p_1 + p_2)x \tag{22}$$

$$y = x \tag{23}$$

As is clear from the section on model construction above, this example describes a system with one state governed by two processes. These two processes both

describe a decay, which are governed by the parameters p_1 and p_2, respectively. Finally, the state x is directly measured, without any additional scaling parameters or other assumed distortions to the measurements. This is a very small model, with close to ideal measurement assumptions. However, already for this case it is clear that even in the case of a perfect agreement between the model and experimental data, and even if the data is obtained without noise and the model is tested against validation data, the accuracy of all aspects of the model can not be guaranteed. The problem lies in distinguishing between the two processes, governed by the two parameters p_1 and p_2. Even though the model has passed all validation tests, predictions concerning the detailed contributions of these two processes may be arbitrarily wrong, i.e., you have no confidence at all in such predictions. Such predictions are hence beach statements. However, predictions concerning the sum of the two parameters, $p_1 + p_2$, may not be arbitrarily wrong, but will be as well-determined as the data is informative (which depends on how excited the system has been, and what the signal/noise ratio is). Hence, if the data is informative enough, statements concerning the sum $p_1 + p_2$ therefore qualify as core predictions. Note that we could find such a well-determined core prediction even though not all aspects of the model could be uniquely determined.

Finally, in the above example, the problems can be easily detected, but in more realistic examples, the problems may typically be more hidden. Another simple, but slightly less obvious example is the case of a model with a saturation, but where the data is not exposing the saturation in the system. In such cases, a similar situation appears, and the values of the individual parameters (for instance $V_{\max}$ and K_{M}) would correspond to beach statements, but certain relations between them (for instance $\frac{V_{\max}}{K_{\mathrm{M}}}$) would correspond to core predictions. Note that the problem of non-uniqueness in models is amplified by a high noise level, by lack of excitation of the system, and by a failure to measure all states and process directly in the model.

8.3 How to find interesting core predictions in practice

Let us now see how these core predictions can be determined in practice. The approach we will explain here is outlined in Figure 13, which is an extension of Figure 4. As can be seen, the lower half of the figure corresponds to Phase II, and takes the experimental data and an acceptable model from Phase I as inputs. It then determines not only one acceptable parameter, but all of them, and then looks for shared properties, which are the same for all such acceptable parameters. Those shared properties equal the core predictions.

Let us just shortly re-consider the small example above, before we turn to the issue of carrying this out in practice. We have already understood that the parameters p_1 and p_2 may take very different values individually, i.e., that they are unidentifiable. However, independently of which values the individual parameters take, the sum of the two parameters would always be (roughly) the same. The sum would therefore be such a shared property among all acceptable parameters, and would thus qualify as a core prediction.

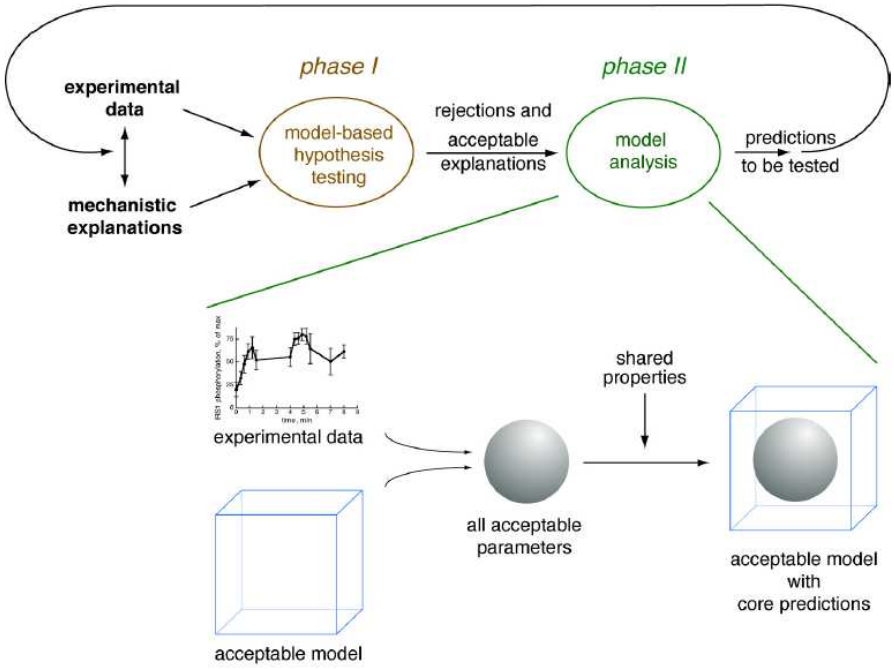

Figure 13: A revised version of the modelling loop in Figure 4. The main point is that core predictions are obtained by looking for shared properties among the set of all acceptable parameters.

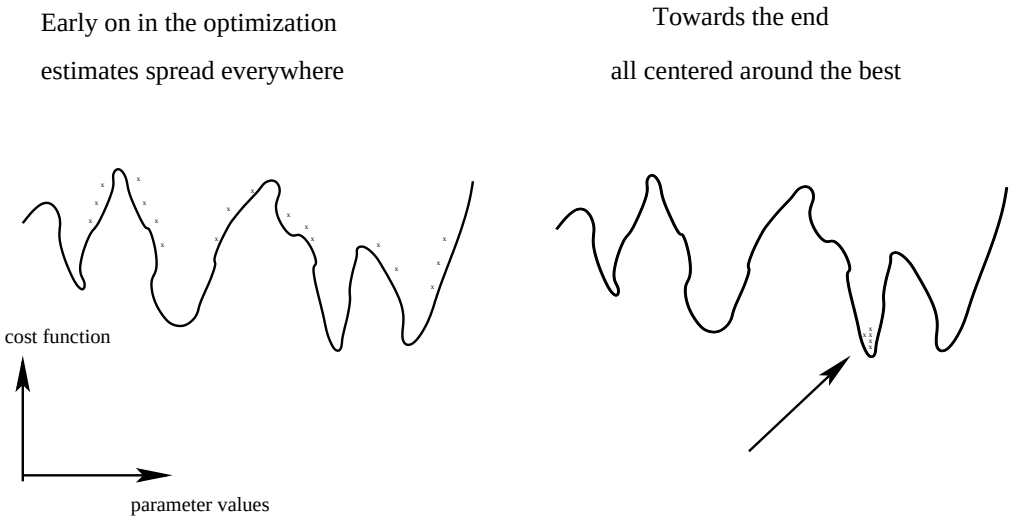

Figure 14: The cost function landscape, and the behaviour of a classical global optimization algorithm such as simulated annealing, which normally tries to find the global optimum.

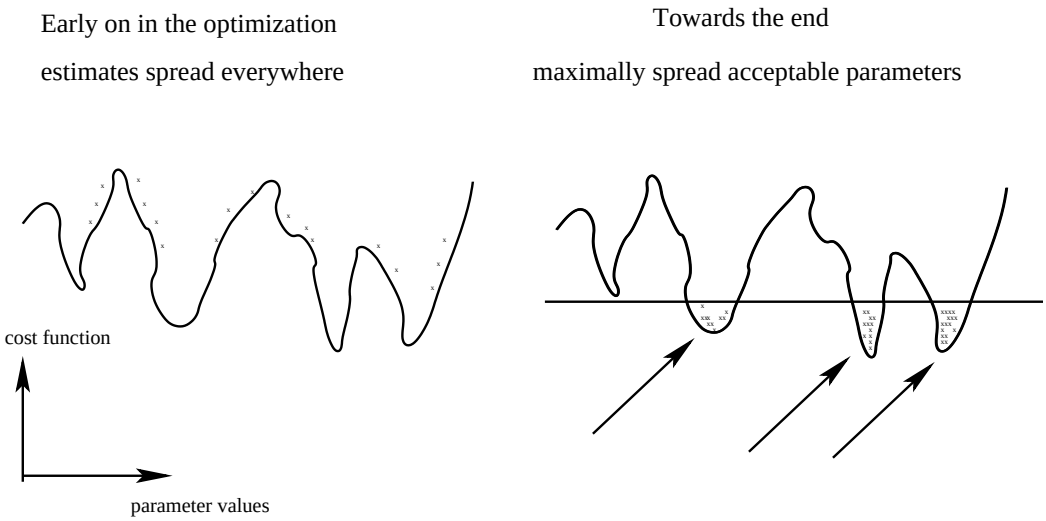

Figure 15: The cost function landscape, and the behaviour of a modified global optimization algorithm, which seeks to identify a good approximation of the entire space of acceptable parameters.

The main challenge is therefore how to determine the set of acceptable parameters, and how to look for interesting and shared predictions within this set. To solve the first of these two sub-tasks, let us first have a slightly closer look at the optimization step introduced already in the model fitting in Phase I. Then we introduced a cost function V(p), which implies a landscape with p on the x-axes and each parameter combination being mapped to a single cost V(p). Such a landscape is depicted in Figure 14, which also depicts the idea of the global optimization algorithm Simulated Annealing. Simulated Annealing is global because it is able to search up-hill in the landscape. In the beginning of the optimization it is searching in parallell places (corresponding to the little 'x's in the figure) and each little 'x' is able to climb any hill. However, as the optimization goes by, Simulated Annealing searches more and more locally, and eventually all 'x's end up close to the best parameter that has been found. When we search for core predictions, we have therefore simply modified Simulated Annealing to stop searching downhill when it finds parameters whos cost is below an acceptable level, and then instead start searching with the aim of finding as different parameters as possible. This process is outlined in Figure 15, and it thus results in an approximation of the set of all acceptable parameters.

Once such a point-approximation of the space of acceptable parameters has been identified, one may essentially proceed as previously: except for the fact that one now has to simulate the model for the entire point-cloud of acceptable parameters. In practice, the point-approximation obtained by Simulated Annealing (or some other optimization method) will be sampled to obtain a representative sub-set, which implies a number of simulations that lies within the feasible computer power. One such method is to take the extreme parameters in all directions, i.e., the parameter sets which has at least one of the parameter values as being unusually big or small. This sub-sampling of the point-approximated space of acceptable parameters is, however, in reality a sub-problem of its own.

When it comes to examining the simulations for interesting predictions, it becomes increasingly important to again consider the biology. Recall that all the modelling steps discussed in this Crash Course are actually only a tool to analyse the given data, and that all of these different sub-steps has as their sole purpose to extract as much and as accurate information as is possible from the given data and prior knowledge. One should therefore look for predictions that has a clear biological interpretation. Furthermore, the predictions becomes especially interesting if they are experimentally testable, and if the crucial behaviour predicted by the model is expected to be discernible from the collected data, when comparing the results with competing predictions and/or default behaviours. Such considerations must be done in close discussion with the biologists and those who have a wide experience of doing such experiments, and a deep knowledge concerning what is known and what would be interesting to know, concerning the studied systems. Even though there is a whole research field devoted to the topic of model-based experiment design (see e.g. [16]), we will here end by mentioning that there are three types of reasons one might have for wanting to do an experiment based on a core-prediction oriented model

analysis

1. Because a core prediction allows you to check/validate the model
2. If two different models predict two different core predictions, the corresponding experiment would distinguish between them
3. Because a specific part of the model is too uncertain and needs to be clarified

All of these reasons are valid, but it is important that you know which one you advocate, and that this is clearly communicated in the final report of the project.

9 Summary

In this chapter we have introduced some of the most important components in model-based data-analysis, which is at the heart of systems biology. The overall goal of this analysis is to extract as much and as accurate information as is possible from the given data, assumptions, and prior knowledge. There are several types of insights that might be obtained from such an analysis, but we have here focused on the two strongest types of conclusions that can be drawn. These are represented by Phase I and Phase II in Figure 4. Phase I finds out whether the given hypothesis can serve as a mechanistic explanation to the given data in a manner which is also consistent with the prior knowledge. Phase I is carried out by translating the given hypotheses to mathematical models, by fitting these models to the data by optimizing over the parameter space using a cost function, and by evaluating the obtained model agreement with the (ideally: validation) data, which also may be done using statistical tests. Rejected models are important because they point towards the necessity for non-included mechanisms and they feedback to the model hypothesis formulation step. The non-rejected models pass on to Phase II. Phase II is non-trivial in systems biology, because the parameters can seldom be uniquely determined from the given data. This non-uniqueness is problematic because it means that without further analysis, model predictions will only be of the status of a beach-statement: "It might be as the model says, but it might also be in some other way". It is therefore highly valuable to do a core-prediction analysis, where one seeks such model properties that must be fulfilled if the given model should be able to explain the data. Formally, such core-predictions may be understood as shared properties among all acceptable parameters, and the space of acceptable parameters may be obtained by the usage of modified versions of global optimization algorithms such as Simulated Annealing. When looking for interesting core-predictions, one should also recall to have a close contact with the biologists, to ensure that the chosen core predictions are both biologically interesting and experimentally testable. This final step thus feedbacks to the data collection step again, and the experimental/modelling cycle of Figure 4 is complete. Note, finally, that each phase in this cycle improves upon the current

knowledge regarding the system, and that an example where numerous loops in this cycle has been used to draw non-trivial conclusions regarding insulin signalling can be found in [1].

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