

Modelling Yeast Osmoregulation at Different Levels of Resolution

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Abstract

Real-world systems can be modelled at different levels of resolution. The choice is typically based on the amount and quality of available data, prior information of the system, and the research question in focus. In molecular biology, it is often the case that a single system is modelled at different levels of resolution, using various modelling techniques. To exemplify, we give an overview of proposed mathematical models of the response to osmotic stress in yeast. These models mainly differ in the choice of mathematical representation (e.g. Bayesian networks, ordinary differential equations or agent-based models), to what extent modelling is data-driven, and predictability. Based on this overview, we discuss how models with different levels of resolution can complement each other to gain insight into the system, the chronological order of the models in comparison to their resolution, and how hybrid models can be designed to achieve a reasonable balance between model resolution and model complexity for the specific modelling problem.

1 Introduction

An essential issue in all modelling is to define the scope of the model. This involves specifying which subsystems and which variables should constitute the model. A natural goal is to find a system that is reasonably well isolated under the considered experimental conditions. Obviously, for biochemical systems this is a very difficult task, since all processes in the living cell are more or less dependent on each other. This is particularly challenging for experimental scenarios ranging in the order of hours. One way to identify variables that are adjusting to an experimental perturbation is to run genome-wide experiments, like microarrays.

Another important issue in modelling is to consider what amount and quality of experimental data and prior information is available. This influences the choice of modelling approach as well as the level of detail of the model. A va-

riety of modelling approaches with different precision are used for modelling and analysis of biological systems. In general, a more precise approach requires more precise and extensive data to be identified. Naturally, a more precise approach also offers more realistic and useful predictions.

A common approach in modelling of biochemical systems is to manually select the model structure and parameter constraints and then estimating the parameters automatically. Alternatively, both the structure and the parameters of the model can be inferred simultaneously by an automatic identification algorithm [1].

It is difficult to present a general methodology for how to construct a model of a particular system. Instead, it can be useful to exemplify by briefly describing methodologies chosen to model a particular system.

In this paper, we describe some approaches that have been applied to model osmoregulation in yeast. Osmoregulation involves the biophysical and biochemical responses of a yeast cell when it is exposed to an osmotic shock, see Figure 1 for an overview.

The osmoregulation system in yeast is an interesting target for mathematical modelling for several reasons:

- The basic structure of the system is relatively well-characterized.
- The dynamics of the system are highly complex. Several feedback loops operating on different time-scales are included.
- Basic strategies of cellular adaptation are conserved from bacteria to humans. Therefore, the system involves several components that are of general biological and medical interest (osmosensors, signalling pathways etc.).

The outline of this paper is as follows. Section 2 introduces the biophysics and biochemistry of osmoregulation. Sections 3-6 cover different modelling approaches to the osmoregulation system. In particular, Section 3 deals with Bayesian networks, Section 4 considers simple models of

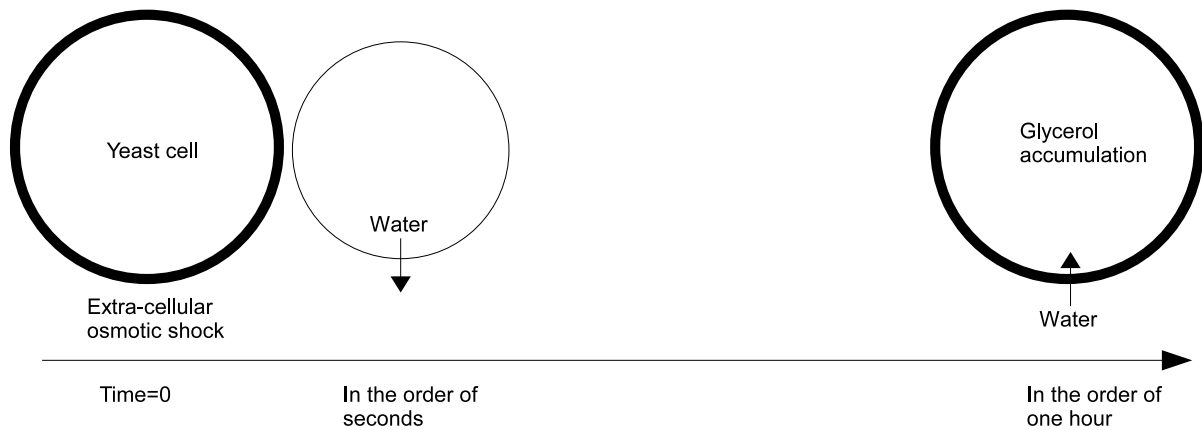


Figure 1. A basic overview of osmoregulation in yeast [2, 3]. An extra-cellular osmotic shock, e.g. the addition of 0.5M NaCl to the medium, rapidly initiates a water flow out of the cell leading to loss of turgor pressure and volume decrease. The cell adapts by accumulating glycerol in order to regain water and thereby volume and turgor pressure. In the figure, turgor pressure is indicated by thickness of the cell membrane.

ordinary differential equations (ODEs), Section 5 presents more detailed ODE models, and Section 6 covers agent-based models. The paper ends with a discussion.

2 Osmoregulation in yeast

To understand osmoregulation it is useful to consider a simplified cell, containing a water solution of large molecules (e.g. proteins and sugars) and small inorganic ions. We further assume that the cell membrane is semi-permeable, such that large molecules are unable to pass the membrane, while water and small ions can freely pass. In principle, the ions would then have equal concentration inside and outside the cell at equilibrium. However, large molecules in the cell are often highly charged and attract many small inorganic ions. Therefore, the concentration of ions is greater inside than outside the cell at equilibrium.

Based on this simple cell model we can give a conceptual explanation of two fundamental variables in osmoregulation: *osmotic pressure* and *turgor pressure*. On a basic level, osmotic pressure is proportional to the *concentration* of molecules other than water in a solution. Hence, a large protein contributes as much as a small ion to the osmotic pressure. Since the concentration of ions is greater inside than outside the cell at equilibrium, the cell has a higher intra-cellular than extra-cellular osmotic pressure. This causes an outward pressure on the plasma membrane. Due to this difference water will flow into the cell. In isolation, this would cause the cell to swell and potentially lead to cell rupture. This is a fundamental problem that any cell must master. Basic solutions are to actively pump out ions,

to actively extrude water or to prevent the cell to swell by a cell wall.

The yeast cell uses the latter solution and has a cell wall with less elasticity than the plasma membrane. Basically, the cell wall resists the expansion of the cell, and creates an inward pressure on the cell contents. This pressure is called turgor pressure, defined as the difference in the hydrostatic pressure between the inside and the outside of the cell. At equilibrium, the osmotic pressure difference is balanced by the turgor pressure and the cell volume is constant with no net flow of water.

An *hyper-osmotic shock* is a sudden increase in the extra-cellular osmotic pressure, for instance due to the addition of salt to the cell medium as illustrated in Figure 1. The immediate effect on yeast to an osmotic shock involves water outflow and decreasing volume. In this way, a new equilibrium is reached, in which the higher extra-cellular osmotic pressure is balanced by an increased intra-cellular osmotic pressure (due to the reduced volume), and reduction of turgor pressure (due to reduced size of the cell wall). We will refer to these processes as the *biophysical system* of the cell.

Generally, the cell strives to keep volume and turgor pressure constant and independent of environmental changes. It therefore has a control system responding to these changes by accumulating glycerol and thereby increasing the intra-cellular osmotic pressure in order to regain its previous size [2, 3]. This process is called *osmoregulation*.

The control system consists of several components. First, glycerol accumulates due to activation of the High Osmolarity Glycerol (HOG) pathway. The pathway consists

of a sensing system, a cascade of protein kinases and output systems such as transcriptional regulators [3]. To give an overview, a static pathway diagram is given in Figure 2. At least three membrane proteins, Cdc42, Sho1 and Sln1, are activated by osmotic stress, presumably by changes in turgor pressure [5]. Two different branches, the Cdc42-Sho1 branch and the Sln1 branch, merge at the protein Pbs2, which in turn activates Hog1, the last kinase in the pathway. Hog1 activates cytosolic effectors and enters the nucleus and induces transcription. This, in turn, leads to intracellular glycerol accumulation that counters effects of the osmotic shock by increasing intra-cellular osmotic pressure.

A second component in the control system is the aquaglyceroporin Fps1, that closes upon hyper-osmotic shock and thereby prevents outflow of glycerol.

To investigate these different aspects of osmoregulation, genetics and molecular biology are used in numerous ways. Cells are exposed to high osmolarity medium and the response to the hyper-osmotic stress is analysed. For example, the phosphorylation (activation) state and the localization of Hog1 is measured to elucidate the kinetics and the duration of the response. Moreover, mRNA expression patterns of genes dependent on activated Hog1 (such as *GPD1* and *STL1*) are studied. In order to understand the physiological response to the stress, intra-cellular and total amount of glycerol, as well as cell volume, are measured.

A general observation of the experiments that have been performed hitherto is that the collection of possible system modifications using genetically modified strains is very rich and advanced. Such modifications give valuable insights into the system and can actually be necessary in order to completely understand certain systems, e.g. systems with mixed fast and slow kinetics and systems including feedback loops.

We will now discuss various approaches to model the response to osmotic stress in yeast.

3 Bayesian networks

In a Bayesian networks the model structure is represented by a directed acyclic graph, whose vertices correspond to random variables, and the edges correspond to conditional dependencies [4, 7]. The probability of variable X_i can be calculated from an associated probability function that is evaluated for the variables corresponding to the direct regulators of X_i in the graph.

Since a Bayesian network approach deals with stochastic aspects of the system and noisy measurements, it may be a natural approach for many system biology problems. Moreover, efficient algorithms exist that perform inference and learning in Bayesian network. However, the formalism does not explicitly take into account the dynamics of regulatory systems.

Gat-Viks and Shamir [8] present a Bayesian network model of the response to osmotic shock in yeast. The variables represent mRNAs, proteins, metabolites and phenotypes, and each variable can be in one of several (usually three) discrete states. The model structure was constructed manually from the literature, and the parameters were inferred from more than 100 publicly available gene expression profiles obtained by microarrays. Since all data are continuous, inference depends strongly on discretization thresholds. Gat-Viks and Shamir partly solves this problem by optimizing the discretization function together with the model parameters.

In order to generate new biological hypotheses, the original (manually constructed) model structure was automatically refined and expanded to improve the fit to data. The main limitations of the proposed approach are that the model describes the steady-state of the system, and that discrete variables are used.

4 Simple ODE models

ODEs have been widely used to model biological systems. In general, a system is described as

$$X'_i(t) = f_i(\mathbf{X}, \mathbf{I}), \quad i = 1 \dots n, \quad (1)$$

where $\mathbf{X} = (X_1 \dots X_n)$ is the state vector of real-valued concentrations and $\mathbf{I} = (I_1 \dots I_m)$ is a vector of input variables, and f_i are typically non-linear functions. These functions usually include several parameters (rate constants) that can either be experimentally determined or estimated from various data. We generally note that it is difficult to measure kinetic rate constants experimentally and that parameter estimation is a complex optimization problem for ODE models of realistic size.

The main feature of a system of ODEs is that it can be simulated in order to obtain deterministic time series for the variables. Standard numerical methods exist for this purpose. The input to such a simulation is the ODEs, values for the parameters and initial values for all variables.

4.1 A two-variable model

Mettetal et al. [9] infers a simple model from measurements of Hog1 localization in cells exposed to square-wave oscillations in input NaCl concentration. The model space is defined by a canonical form, a second-order linear time-invariant (LTI) model, complemented with a non-linear element. The corresponding ODE representation of the inferred model can be obtained as

$$y'(t) = (A_0 u(t) - x(t)) - \gamma y(t), \quad (2)$$

$$x'(t) = \alpha (A_0 u(t) - x(t)) + \beta y(t), \quad (3)$$

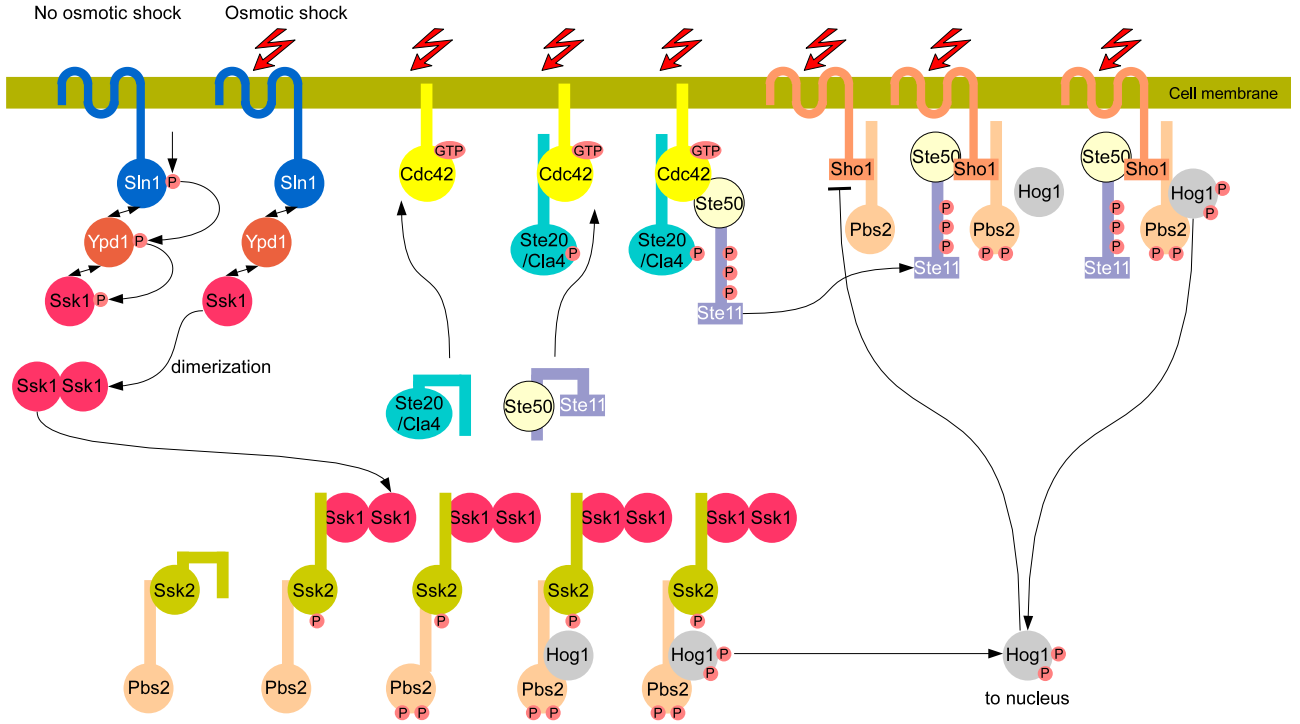


Figure 2. Overview of the HOG pathway redrawn from [6]. Some causality has been incorporated by re-drawing certain complexes in series and indicating molecule bindings by arrows.

where $u(t)$ is the input signal corresponding to NaCl concentration, x is an internal state variable that can be interpreted as the intra-cellular osmolyte concentration, and y is the enrichment of nuclear phosphorylated Hog1 above some base level. We note that both the model structure (the form of the equations) and the four parameters (A_0 , γ , α , and β) were inferred from data.

The model contains two feedback loops, one that is independent on Hog1 (the first term in x' is independent on y), and one that is dependent on Hog1 induced protein synthesis and subsequent glycerol accumulation (the second term of x' depends on y).

The model is able to predict the Hog1 response to various input signals, both in wild-type and for genetically modified strains. However, the low dimension of the model makes biological interpretation hard. Muzzey et al. [10] later proposed a revised model taking glycerol production into account.

4.2 Controlling the biophysical system

We will now increase the level of detail by explicitly model the biophysical system, together with a simplistic control model representing osmoregulation. The purpose is to get an intuitive understanding of the osmoregulation

system.

A simple manually constructed biophysical model for osmoregulation is given in [11, 12] and includes a system of equations for intra-cellular osmotic pressure, extra-cellular osmotic pressure, the volume of the cell and the turgor pressure. The biophysical description allows us to explicitly link glycerol production to the turgor pressure and consequently to the input signal of the pathway.

In a simplistic control model we only consider the HOG pathway and its effect on intra-cellular glycerol production. We note that accumulation of glycerol works as a feedback response to osmotic shock, since the intra-cellular osmotic pressure is dependent on intra-cellular glycerol. An overview of the model is given in Figure 3.

To model the turgor pressure sensors, the HOG pathway, transcription and translation in a very simple way, we apply a single time delayed control function corresponding to all these steps. We let the difference between turgor pressure and a reference turgor level be the input (e) to this controller as

$$e(t) = \text{Ref. turgor pressure} - \text{Turgor pressure}(t). \quad (4)$$

We consider the simplest possible controller (u) that adjusts e by a constant K as

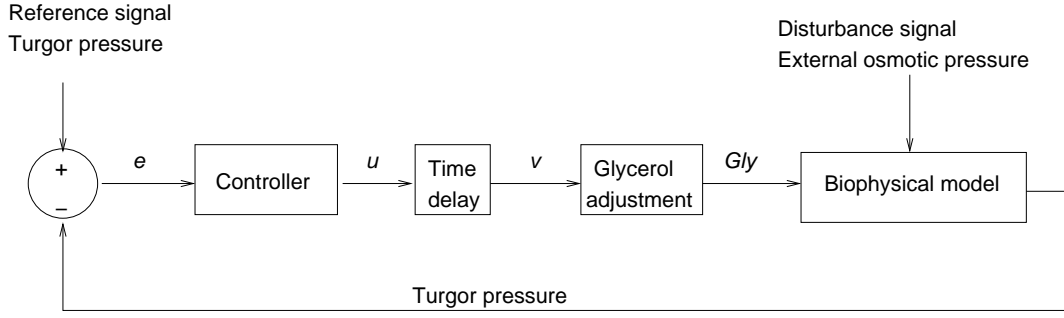


Figure 3. A simple model controlling the biophysical system. The glycerol level is adjusted by the integral of a proportional and time delayed controller.

$$u(t) = K e(t). \quad (5)$$

To make the model more realistic, we also include a time delay (t_d) corresponding to the time it takes to initiate glycerol accumulation, i.e. transcription and translation of enzymes. The time delayed control signal (v) is obtained as

$$v(t) = u(t - t_d). \quad (6)$$

Finally, we let the rate of change of glycerol, Gly , be dependent on the control signal as

$$Gly'(t) = v. \quad (7)$$

We use this model to simulate an experiment where the input signal is an osmotic shock of 0.5M NaCl, see Figure 4. We note the input signal of increased external osmotic pressure at $t = 0$, followed by the rapid changes towards a new equilibrium in the biophysical variables. First, the pressure imbalance causes a drop in volume, which leads to a decrease in turgor pressure and an increase in intra-cellular osmotic pressure. Turgor pressure is abolished and the system reaches a new equilibrium (where the internal and external osmotic pressures are equal) only a few seconds after the applied stress. The control model initiates glycerol production immediately after the time delay has expired (10 minutes after the stress in this simulation), which in turn results in increasing intra-cellular osmotic pressure. Consequently, water flows back into the cell and both volume and turgor pressure are slowly increasing to their original values. In particular, about 33 minutes after the stress, turgor pressure starts to increase, while the increase in volume slows down. At this point we also see a slight increase in the rate of glycerol accumulation. This is because glycerol is plotted as concentration and therefore is dependent on volume.

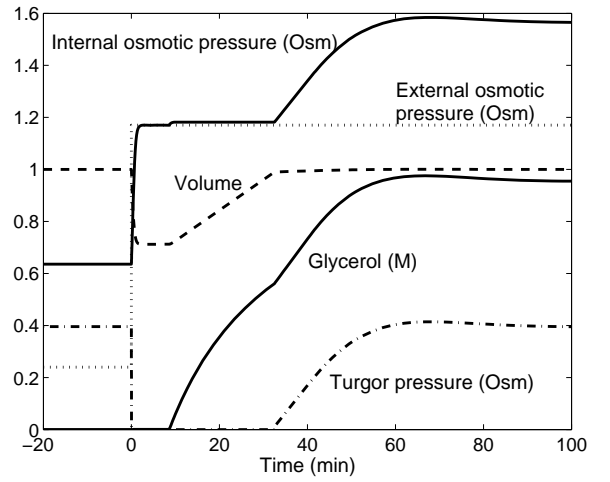


Figure 4. Simulated data from the biophysical model in combination with (7). The input signal is an osmotic shock of 0.5M NaCl and reasonable model parameters are taken from [11].

The model presented in [11] can be seen as a refined version of the didactic control model described above. The following new processes are included:

- Both intra- and extra-cellular glycerol are considered in the model.
- Changes in turgor pressure is independently sensed by Sln1 and Fps1. The second affects the diffusion constant for glycerol over the cell membrane.

The model contains 4 ODEs and 10 parameters, allowing enough flexibility to capture the most fundamental processes that are discovered in yeast osmoregulation. Us-

ing the simplest possible sensor mechanisms (linear dependence on the difference between turgor pressure and reference turgor pressure), the model can reasonably well predict intra- and extra-cellular glycerol concentrations for various input signals and mutant strains.

A similar, but slightly more complex model is proposed by Zi et al. [14]. In this model, the proteins Pbs2 and Hog1 are explicitly modelled with two states (unphosphorylated and phosphorylated), and besides, Hog1 exists in both the cytosol and the nucleus. In total, there are 10 ODEs and 22 parameters that are estimated from data.

5 Detailed ODE models

Hitherto, the control system has been modelled in a very simplistic way - far from the level of detail indicated in Figure 2. We will now describe a model proposed in [12] taking more molecular details of the control system into account. Briefly, the model includes the biophysical system as previously described, the HOG signalling pathway, transcription/translation, carbohydrate metabolism and glycerol production. The model structure was constructed manually, and the parameters were numerically optimized to fit data.

The model of the HOG pathway considers the Sln1-branch of the pathway in isolation, since it is known that this branch gives a similar response as the complete pathway including the Cdc42-Sho1-branch. We note that this is very useful since the sensor protein of that branch is not identified. The transmembrane protein Sln1 senses turgor pressure by adjusting its rate of auto-phosphorylation.

In the HOG pathway, Pbs2 is believed to act as a scaffold protein. A scaffold protein binds several other protein species, and can rapidly transmit signals and facilitate signal transduction. However, due to lack of data this aspect was not included in the model.

The cell is assumed to contain two compartments, the nucleus and the cytosol. Double phosphorylated Hog1 may enter the nucleus and is considered to be a transcription factor in the nucleus compartment.

The HOG signalling pathway triggers transcription and translation of several genes. The biochemical details of this activation are not understood to the same degree as the HOG signalling pathway. These processes are therefore simplified and only two types of mRNA species and two types of proteins are considered. The first type corresponds to metabolic enzymes, such as *GLK1*, *GPD1* and *GPD2*, and the second corresponds to phosphatases, such as *PTP2* and *PTP3*.

Modelling of carbohydrate metabolism and glycerol production were based on previously published models. The dependence of carbohydrate metabolism and glycerol production on the HOG signalling pathway was included by letting the rates of several reactions be linearly dependent

on the Hog1-induced protein. Besides, the dependence of glycerol transport on Fps1 was included.

The concentrations of all species in the cytosol were dependent on the cell volume (which is a dependent variable in the biophysical model). The complete model as given in [12] includes 35 ODEs and 70 parameters.

We finally note that another model using a similar level of detail have subsequently been reported in [13].

6 Agent-based models

The previously described ODE models of the HOG pathway have used simplifying assumptions to avoid the combinatorial problem of complexes. Such assumptions are hard to make and may obscure or remove essential properties of the system. The HOG pathway includes at least three scaffold proteins that form complexes, and involves reactions that are strictly dependent on the set of species bound to a certain complex. The scaffold proteins lead to a combinatorial increase in the number of possible states. For example, Pbs2 is a scaffold protein in the HOG pathway that contains at least three binding sites: for Sho1, Ssk2/22 and Hog1, respectively (Figure 2). These species can in turn be bound by other proteins (Ste11-Ste50 in case of Sho1 and Ssk1 in case of Ssk2/22). Moreover, each of the proteins involved can be in different states of activation or phosphorylation. A full enumeration of all states would require 1900 state variables [6].

There are several different modelling languages that support modelling of complexes without enumerating all states [15, 16, 17, 18]. Here, we consider Kappa [17].

Kappa is a process calculus in the tradition started by the Calculus of Communicating Systems [19], allowing description and analysis of concurrent systems. In Kappa, a rule-based representation is used, and state variables are not explicitly enumerated. Instead, the model is fully represented by a list of agents (e.g. proteins) and their individual docking sites and activation sites (e.g. phosphorylation), and a list of rules corresponding to a generalised form of a chemical reaction.

One main advantage of a rule-based representation is that the complete state of an agent binding to a scaffold protein does not need to be specified. Instead of 1900 state variables, only a few Kappa rules are required to implicitly describe the full state space of the above mentioned scaffold protein Pbs2 [6].

To describe the Kappa syntax, a simple example taken from [6] is given here. To denote that Sho1 has a state called x that is activated (a) and, furthermore, that Sho1 has a docking site for Ste11, we write

$Sho1(x^{\sim}a, Ste11)$

Similarly, to denote that Ste11 is phosphorylated (p) and has docking sites for both Sho1 and Cdc42, we write

```
Ste11(x~p, Sho1, Cdc42)
```

To model the association of active Sho1 to Ste11, it is reasonable to require that Ste11 is not bound to Cdc42, and that the phosphorylation state of Ste11 is arbitrary. We write the rule as

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Sho1(x~a, Ste11), Ste11(Sho1, Cdc42) ->
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Sho1(x~a, Ste11!), Ste11(Sho1!, Cdc42) @ 2.0
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Docking is indicated by an index after the reserved symbol !, and the kinetic rate of the rule is defined at the end, after the reserved symbol @. Note that the phosphorylation state of Ste11 is simply omitted, and that the binding site for Cdc42 on Ste11 is unoccupied on both sides of the arrow.

In agent-based modelling, each variable represents the number of molecules. The state changes discretely, but how and when is determined stochastically [20]. One simulation corresponds to one realization of the stochastic process. By repeating the simulation many times, an approximation to the probability distribution of the system over time is obtained.

Kühn et al. [6] presents a detailed generic model of the HOG system without simplifying assumptions, showing the molecular interactions known from the literature. The model is defined in Kappa and takes complexes into account, and summarises existing knowledge in an unambiguous and detailed representation. It can thus be used to anchor discussions about the HOG system. For a model at this level of detail, the parameters can not be identified given current data.

7 Discussion

The choice of modelling approach is usually based on the amount and quality of available data, prior information of the system, and the research question in focus. We have given an overview of proposed mathematical models of the response to osmotic stress in yeast. These models mainly differ in the choice of mathematical representation, to what extent modelling is data-driven, and predictability.

Since many models of the same system are available it is interesting to compare these with each other. The models are similar in the sense that they share some main characteristics and often give the same qualitative predictions. The difference lies in the level of detail at which the processes are modelled, and the precision of the predictions. In the most simple models, hardly no molecular details are included, while the most detailed models aims at including all known biology.

Models of Bayesian networks and ODEs with few variables are relatively easy to handle: standard routines are available for parameter estimation, and for sufficient amount of data also for structure identification. On the other hand, simplifying assumptions like discrete states and composite variables representing several biochemical species, make the connection between data and model less intuitive. For instance, representing a gene deletion in one of the simple ODE models is not straight-forward.

Detailed ODE models are harder to construct and maintain. Parameter estimation is often a bottle-neck, since it requires repeated numerical integration of a large model to search the high dimensional space of parameters. The available amount of time-series data is limited, but this can partly be compensated for by employing data from modified systems (gene deletions, overexpression etc.). Furthermore, prior knowledge from the biological literature can help restricting the space of possible models and parameters.

For the agent-based model of the HOG pathway, the majority of the kinetic rate constants are currently not observable. However, we generally note that models are important not only for simulations but also for communicating the system in a compact and precise way.

In principle, it would be interesting to perform quantitative comparative analyses of different models, using a complexity measure like Akaike Information Criterion. Such a measure would reveal to what price of increased complexity it is reasonable to increase the goodness-of-fit to data. Unfortunately, there is no single accepted measure for this kind of problems. Besides, it is very difficult to compare models when different data sets have been employed in the construction of the models. For instance, the most detailed ODE model [12] was based on the identified structure of the system, and hence, implicitly uses the data from which the structure is determined. Such data is typically not obtained from time series experiments of protein concentrations but rather from protein-protein interaction experiments and experiments measuring cell growth in various mutant strains. Although this kind of data can be directly employed in model identification, it may be difficult and tedious to extract the data from the literature. We would also like to point out that the structural information obtained from these experiments only tells whether variables interact, not the mechanism of interaction.

It is interesting to note that simple and detailed models of osmoregulation have been constructed in parallel. There is no dependence between the chronological order of the proposed models and their level of detail, as could be expected since the amount of data continuously increases. One explanation for this is that it can be useful to consider a simple model when developing a more detailed model, partly because the main characteristics of the system can more easily be observed but also because the simple model can be pa-

parameterized with higher confidence than the detailed model. For instance, a simple model of osmoregulation can indicate realistic levels of glycerol given a certain input signal, and in this way reducing the model space of a more detailed model.

Clearly, modelling the protein interactions of the HOG pathway alone does not suffice to properly model the dynamics of osmoregulation, since signalling is naturally integrated with the environment, changes in cell size, gene regulation and metabolic changes. Therefore, to properly model the dynamics of osmoregulation, several model constructs must be integrated. Here one can think of mixing different modelling approaches. Such hybrid models can be designed to achieve a reasonable balance between model resolution and model complexity for a specific modelling problem. For example, part of the agent-based model could be complemented with an ODE model of the biophysical system. Furthermore, hybrid simulation methods (deterministic and stochastic) are of interest when combining metabolic systems with signalling pathways in one model.

To facilitate hybrid modelling, two fields of research are of major importance. First, standardization of model representation, like the Systems Biology Markup Language, and second, standard methods and software for conveniently modelling and representing prior knowledge and data.

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