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"Strukurelle Analyse eines kombinierten Models zur osmotischen Stressantwort in Hefe"

"Structural analysis of a combined model for an osmotic stress response in yeast"

vorgelegt von

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1 Zusammenfassung

Um besser das Zusammenspiel zwischen dem Zellvolumen der Hefe Saccharomyces cerevisiae, wenn diese hyperosmotischen extrazellulären Stress ausgesetzt ist, und der damit verbunden HOG MAPK Signalweg bzw. Plasmamembran-Ionentransport Regulierung zu verstehen, haben wir dafür ein mathematisches Model aus ODEs und algebraischen Gleichungen konstruiert. Dies geschah durch zusammenfügen von drei getesten Einzelmodellen.
Dafür haben wir einen neuartigen Simulationsansatz in Python3 Code mit Hilfe einer lokalen Datenbank implementiert.
Das kombinierte Model verdeutlicht die wechselseitigen Abhängigkeiten zwischen dem Hog Signalweg, der Volumenregulation und der extra-/intrazellulären Ionenkonzentrationen.

2 Abstract

To better understand the interplay between the cell volume of the yeast Saccharomyces cerevisiae when faced with hyperosmotic extracellular stress and the corresponding regulation of the HOG MAPK signaling pathway and the plasma membrane ion transport, we constructed a mathematical model by merging three tested single models. The mathematical model consists out of ODEs and algebraic equations.
For this, a novel simulation approach in python3 code with the help of a local database was implemented.
The combined model clarifies the dependencies between the hog pathway, the volume regulation and the extra-/ intracellular ion concentrations.

Contents

1 Zusammenfassung **I**

2 Abstract **I**

3 Introduction **1**

3.1 System biology 1

3.2 Saccharomyces cerevisiae 1

3.3 State of the art 2

3.4 Theory of the used models 2

3.4.1 Ion model 2

3.4.2 Volume model 3

3.4.3 Hog model 4

4 Material and methods **6**

4.1 Software development 6

5 Results **8**

5.1 Results of single models 8

5.1.1 Ion model 8

5.1.2 Hog model 9

5.1.3 Volume model 10

5.2 Merging of the models 11

5.3 Combined model 13

5.3.1 Single models with the initial values from the combined model . . 16

6 Discussion **18**

6.1 Outlook 19

7 Reference **20**

8 Danksagung **22**

9 Appendix **23**

3 Introduction

3.1 System biology

System biology is used for the extraction of a system wide understanding of living organisms. This includes the interaction of multiple proteins, genes, metabolites et cetera, which are measured in the laboratory. This approach gets more significant in the analysis of executed omics experiments which easily results in data in the gigabyte range. Mathematical modeling has proven to be a promising tool for the study of the complex processes of environmental stress adaptation, to reveal the role of each biological component in the system and to reveal how the system level properties emerged from collected activities of individual components [1].

Currently limitations of the system biology approach are the usages and constructions of mathematical equations which represent the biological system. This is a trade off between reduction of the system of interest without diminishing the information value or reasonableness intended digital twin. Another important problem is that there does not exists a complete biological understanding and knowledge of all system component. The system biological approach is therefore only a heuristic approach.

In-depth insights of an investigated system are e.g. useful for medicine and the biotechnology sector [2] because this results in the improvement of well constructed mathematical models of a cell system which could be useful for the design of target-oriented medications.

Mathematical models are further helpful to test laboratory experiments *in silico* to identify meaningful experiments by construction of DoE (Design of Experiment). This helps to save the resources (e.g. money, time) of the experimentalist and could accelerate the understanding of the underlying biological system.

3.2 Saccharomyces cerevisiae

The yeast Saccharomyces cerevisiae (*S. cerevisiae*) is a unicellular eukaryotic organism and belongs to the class of fungi [3]. It was the first eukaryotic organism where the whole genome had been full sequenced. [4]. *S. cerevisiae* is one of the beast characterized eukaryotic models [3].

3.3 State of the art

In nature, the environment of *S. cerevisiae* varies in factors like temperature, nutrient levels or osmolarity with the time and the cell must adapt with these changes. [5].
To our knowledge, their does not exists a model which combines how *S. cerevisiae* transports ions in response to a extracellular salt stress exposure over the cell membrane with than changing cell volume and hog pathway activity.
Nevertheless, it exists a diversity of models for *S. cerevisiae* which describes many aspects e.g. hog pathway or ion transport over the plasma membrane in good approximations while assuming other important aspects of the system as constant.
In this thesis we constructed a combined model merged from three tested models. The combined model clarifies the dependencies between the hog pathway, the volume regulation and the extra-/ intracellular ion concentrations.

3.4 Theory of the used models

3.4.1 Ion model

This model was designed to reproduces the experimentally observed potassium and proton fluxes induced by the external stimuli KCl and glucose in *S. cerevisiae*. It implemented eight well characterized transport proteins relevant to the regulation and maintenance of intracellular alkali-metal cation content [6].

The ion model consists out of a non-equivilibrium thermodynamic (NET) approach to model the transport of ions over the plasma membrane. NET is the analysis of spatial inhomogeneous systems and of time dependent processes. It is holds true if these are not very fast or big inhomogeneous. The ion model assumes that both compartments are well mixed. This way the NET approach is valid.

Per definition, osmolarity means the amount of substances of osmotic active particles per volume V of a solution. Osmotic pressure occurs if two solutions with different osmolarity are separated by a semipermeable membrane, where not all substances can pass the membrane for creating an equilibrium.
S. cerevisiae has a plasma membrane which works as a semipermeable membrane. Water can diffuse freely through it, to adapt to the osmotic changes.

Fluxes over membranes are irreversible processes. This will lead to a production of entropy in the system, which is calculated by the entropy production density σ which is represented with the equation 1

$$\sigma = \vec{J_Q}\left(\frac{1}{T}\right) - \sum_{i=1}^{k} \vec{J_{c_i}} grad\left(\frac{\eta_i}{T}\right) + \sum_{r=1}^{R} J_r \frac{A_r}{T} \geq 0 \tag{1}$$

Equation 1 summaries the production of entropy under the conditions of temperature difference, concentration difference and chemical reactions.
Fundamentally, σ can only be created by fluxes J with it's forces X. In the area around equilibrium we can further assume that a flux J is linearly coupled over a phenomenological coefficient L with it's force X, because at equilibrium all forces and fluxes vanish. Under this condition the following statement 2 holds true:

$$\sigma = \sum_i J_i X_i = \sum_i \sum_j \frac{\delta J_i}{\delta X_j} X_j X_i = \sum_i \sum_j L_{ij} X_j X_i \tag{2}$$

L_{ij} were estimated from the experimental data of starved cells and are independent of the flows and forces [6]. They represents active transporters or channels in the plasma membrane. A high value of L_{ij} indicates that the transport of j is directly coupled to i. After some other assumption made from the ion model (for deeper insight see [6]), the equation for the flux of ion k is:

$$J_k = \sum_{j=1}^{n} L_{kj}(RT \cdot ln\left(\frac{c_k^{in}}{c_k^{out}}\right) + z_k F \Delta\phi) + L_{kAr} A_{Ar} \tag{3}$$

A_{Ar} represents the reaction affinity for the ATP hydrolysis. The hydrolysis of ATP is the only considered chemical reaction in the model.
In the ion model a glycerol stimulus is further simulated. Ion regulation determines many physiological parameters, such as cell volume [1].

3.4.2 Volume model

The volume model only holds true for an individual yeast cell in G1 phase. It can describes the small and steady volume variations during normal growth and the growth caused by hyper- or hypoosmotic shocks [7].
Turgor pressure π_t prevents exaggerated swelling and maintains cell shape [7]. π_t equals the hydrostatic pressure acting on the cell wall. For this the volume model describes the elastically and plastically expansion of the membrane by involving the strain on the

wall
The volume model further describes the water flux J_w between the cell and the environment with

$$J_w = -\frac{d}{dt}V_{os} = G \cdot Lp \cdot (\pi_e + \pi_t - \pi_i) \tag{4}$$

with Lp as the hydraulic conductivity, G the cell surface and the internal, external and turgor pressure (π_i, π_e , π_t). The cell volume V depends essential on the relation of π_i, π_e and π_t due to changes in the osmotic volume V_{os} (water volume) because the model defined the total cell volume $V = V_{os} + V_b$ as the sum of V_{os} and a solid volume V_b which is not affected by water dynamics [7].
The internal and external pressure π depends on the concentration c of the osmotic active substance in the corresponding areas correlated over the equation 5

$$\pi = c \cdot R \cdot T \tag{5}$$

The immediate effect on yeast to an osmotic shock involves water outflow and decreasing volume [8].
Furthermore, the model simulates the changes in the inner osmolyte concentration $\dot{c}_{in}$ with an uptake k_{uptake} and a consumption $k_{consumption}$ term:

$$\dot{c}_{in} = \frac{1}{V}(k_{uptake}G - k_{consumption}V - c_{in}\dot{V})$$

Summarizing, it is highlighted that the cell expansion is mainly influenced by the interaction of

1. the control of the internal osmolarity c_{in}, which together with π_t drives J_w and therefore V_{os}

2. the elasto-plastic deformation of the cell wall due to π_t. $J_w < 0$ expands the cell wall and increase therefore π_t

3.4.3 Hog model

The hog pathway in *S. cerevisiae* has a significant role in the adaption process after an osmotic stress exposure. It normalize the volume of the cell and with that the water balance with an accumulation of the osmolyte glycerol inside, by closing the glycerol membrane transporter Fps1 [9] [8] and the production of glycerol.
The hog model is composed out of the Hog1 Mitogen Activated Protein Kinase (MAPK) cascade. This cascade is well conserved even in higher eukaryotes including humans [8].

The Hog1 MAPK is activated in the response to an increase in extracellular osmolarity [9]. All other non-osmotic stresses (e.g. temperature stress) which are known to also activate the HOG pathway [9] are not represented in this model. The MAPK pathways are important for transmitting and processing signals from the cell membrane into the cell [8].
Hog1 MAPK is activated by the upstream Pbs2 MAP kinase kinase (MAPKK) by phosphorylation. The phosphorylated Hog1c (Hog1PPc) translocates into the nucleus where it activates different transcription factors by phosphorylation [5] [10].

The model is composed out of these key components for the regulation of the osmo-adaption process.
It consists of ODEs of the following form:

$$\frac{d[P]}{dt} = f(\vec{P}) - V_{ratio} \cdot [P] \tag{6}$$

Equation 6 describes in general with the first term at the right hand side the changes of a concentration due to a chemical process and with the second term the changes in the concentration due to changes in volume [1].
Osmotic stress impulses with precise concentration level were used. This approach holds true because the experimental data sets used for the parameter fitting were yielded from microfluidic devices. Microfluidic devices allow a rapid medium change, because the cells are exposed to a constant, switchable flow of external media which comes from different reservoirs [10].

Finally, two important assumptions for this model were made:

1. no cell growth
2. before osmotic stress, the system is in a steady state

4 Material and methods

4.1 Software development

A simulation of the combined model can take several minutes. The thereafter calculation and handling of these results are not granted to work in the process of a program development. We designed a simple workflow that prevents to repeat a simulation with already simulated settings:

1. simulation ⇒ save data
2. fetch data ⇒ analysis

For the construction of this workflow a PostgreSQL database was implemented and connected with the python script. Pure python3 code was the choice for this thesis.
A concept from CDISC for clinical trials was used for the data storing architecture. This allows to construct useful column names, data types and table in the database.
Because a simulation of ODEs and algebraic equations can have pending sets of initial values, parameter values or equations terms, the concept of CDISC was expanded to the area of system biology by designing new data storage procedures. This helps to keep track for all model settings and changes and use any of them for a new simulation. The intention for the implementation of CDISC to system biology was the fact, that the FDA is moving towards CDISC standards for regulatory submissions [11].
In figure 1 we can see the proposed workflow.

With this approach, we can analysis and try new codes snippets with the results of a long simulation in few seconds.
Furthermore, the storage of the model in a JSON file was designed in a way, that a natively typed equation in a .txt file format like

$$\frac{d}{dt}Na_{in} = \frac{J_{Na} \cdot G}{V_{os}} \cdot 10^6 - Na_{in} \cdot V_{ratio}$$

gets automatically separated in the terms $\frac{J_{Na} \cdot G}{V_{os}} \cdot 10^6$ and $-Na_{in} \cdot V_{ratio}$. This allows us to simulate each term component and analyze its contribution to the result of the equation in relation to the time.

Figure 1: workflow for the analysis of a model in the designed software

5 Results

5.1 Results of single models

Before merging the three models together it must be controlled whether each model is implemented correctly for itself. The figures of the models simulation results are the guide for this. Sometimes there are discrepancies between the published model and the figure which should represents the model. Hereafter, the implementation process for each of the models is described.
To better retrace the implementation steps it is recommended to read the paper for the hog [10], ion [6] and volume model [7].

5.1.1 Ion model

The challenges with the implementation of the ion model were, that the presented equations, initial values and parameters did not result in the intended system behavior.
After an in-depth analysis of the equation there were two anomalies:

1. the calculation of the change of the inner proton ion concentration has *Bf* as an undefined parameter
2. the fluxes have the wrong units

For solving the problem with the undefined parameter the ODE was constructed by deriving the formula for the calculation of the pH value for diluted solution equation 7

$$pH = -log_{10}([H^+])$$

$$\frac{d}{dt}pH = \frac{d}{dt}(-log_{10}([H^+])) = -\frac{1}{ln(10)}\frac{d}{dt}ln([H^+]) = -\frac{1}{ln(10)}\frac{1}{H^+}\frac{dH^+}{dt} \tag{7}$$

and combine it with the equation 8 for the change of the pH Value as a result of proton flux ([12])

$$\frac{d}{dt}pH_{in} = \frac{J_H \cdot Surface}{V_{in} \cdot pbc} \tag{8}$$

The equation 9

$$-\frac{1}{ln(10)}\frac{1}{H^+}\frac{dH^+}{dt} = \frac{J_H \cdot Surface}{V_{in} \cdot pbc} \Rightarrow \frac{dH^+}{dt} = -\frac{J_H \cdot Surface \cdot ln(10) \cdot H^+}{V_{in} \cdot pbc} \tag{9}$$

was used for modeling the change of the inner proton concentration. *pbc* is the proton buffer capacity.

To solve the problem with the wrong units it was identified, that the equation for the reported fluxes only missed the unit Kelvin (K) in its numerator. Multiplying the equation with the temperature T solved this problem and resulted in the equation 3. This model represents the model with the initial values and global quantities and volumes from Table 4 in the ion paper [6]. The phenomenological and stoichiometric coefficients were adopted from the original Copasi ion model file. In figure 2 the implemented ion model is visualized.

Figure 2: left: membrane voltage in the ion paper for different NaCl stimulus (0.01 mM - 10 mM) at time $t = 300s$ and glucose at $t = 600s$; right: the simulated implemented ion model for the same stimulus range

5.1.2 Hog model

For the implementation of the hog model, the *Table S1-S3* and the Matlab code for the used NaCl impulse in *Code S1* in the supporting informations of the paper were implemented in the python3 environment. No difficulties were encountered. The only problems occur with the handling of the intended stimulus time length. See figure 3 for the validation of the implemented model.

Figure 3: Hog1PPn expression after a 0.2 M NaCl single pulse stimulus for $t = 0 - 300s$; left: hog model from the paper; right: the simulated implemented hog model

5.1.3 Volume model

The volume model could not be implemented with only the corresponding paper because the model wants to describes the change of the non-osmolytic volume V_b but missed to note down an equation for this.

After consultation with my supervisor and one of the author of the model about this information gap, it was recommended that I use the already implemented volume model in their local network "YCM" as a template. After translating this template in my own software structure, no problems were encountered. In the appendix I documented the used ODE, algebraic equation, parameter and initial values for this model, visualized in figure 4.

Figure 4: no stimulus; left: volume model from the paper; right: the simulated implemented volume model

5.2 Merging of the models

The process of merging models is a tricky task. There exists some good guideline [13] for this. Hereafter, the used essential steps are sketched:

1. equations: One of the first steps in the process of model merging is to control whether there is a conflict between assumptions of the description of the biological systems. Conflicts must be resolved by combining equation terms or discard some informations

2. names: Find all the overlaps in the variable and parameter naming and convert them to a common set of names

3. units: Standardize all used units for the parameter and initial values to a common set. In this process new factors where give to individual values if the unit changes requires that.

Changes in the equation design (ODE and algebraic equation) were done in the process of merging the models and are presented below in table 1:

Table 1: changes in the equation design

	changes	reason
internal / external osmolyte concentration	$c = \sum([Ionen] + [\text{other osmolyte}])$	simplification
internal / external pressure	$\pi = c \cdot R \cdot T$	simplification
ion x changes	$\frac{J_x \cdot CellSurface}{CellVolume} \cdot 10^6$	unit harmonization without changing flux J_x calculation
sorbitol stimulus	$\frac{d}{dt}[Sorbitol] = 0$	implementation of sorbitol stimulus with the assumption that sorbitol can not diffuse over the plasma membrane; in ODE format because this generates less memory data
uptake / dilution of osmotic active compounds	removed	replaced with the equations for ion and glycerol

The NaCl stimulus approach from the hog model was not adopted. The response to a stimulus takes place with stopping the ODE solver and adding the stimulus concentration to the last values of the affected substances.
In the whole process of merging we must consider their biochemical interpretation to maintain the plausibility of the model [13]. No parameter value was changed in its biological interpretation.

Initial values were changed because, as we can see in figure 5
multiple components are used in several models. These components have different quantities in the initial assumptions. One of the main difference was the assumption of the cell volume V_{cell} in the hog model ($V_{cell} = 58fL$) and the volume model ($V_{cell} \approx 0.27fL$). To solve this problem we simulated the volume model until V_{cell} also has the value of $58fL$. We assumed the ODE values at this time point as the initial values for the corresponding substance in the merged model.

We removed the volume related variables and π_t from the hog model because the calcu-

Figure 5: intersections between the three single models parameter and variables; colored: significant different assumptions for same intended behavior

lation of π_t in the volume model was based on updated insights.

5.3 Combined model

In figure 6 the interplay between the key components in the combined model in response to a hyperosmotic extracellular shock are visualized. For the analysis and validation of the merged model we choose the nuclear phosphorylated Hog1 (Hog1PPn) as the control substance. Hog1PPn is also used in the hog model as the output because it regulates the expression of hundred of genes [10]. We borrowed the idea of a dose response curve with a calculation of the area under the curve (AUC) of Hog1PPn from the theme field pharmacokinetic as a visualized output, showed below in figure 7.

Figure 6: interplay between the key components in the combined model

It is indicated that a NaCl stimulus results in a higher overall expression of Hog1PPn in contrast to KCl. Furthermore, figure 7 visualizes that a sorbitol shock results in even a slight higher overall expression of Hog1PPn per the amount of osmotic active particles. The simulation time was set to 200s, with an initial glucose stimulus at $t = 1s$ and the unique hyperosmotic shock at $t = 30s$.

As we can see in figure 8, multiple stimulus at predefined times are possible with the model and with the software environment. A second stimulus does not results in the same volume V and turgor pressure π_t drop as the first one. Cause of clarity, only a set of ODE are visualized. They represent the other ODEs, because they are the output of multiple interacting components.

Figure 7: Response of Hog1PPn; initial Glucose at $t = 1s$; hyperosmotic shock at $t = 30s$

Figure 8: visualized results of a selected set of ODEs from the combined model

5.3.1 Single models with the initial values from the combined model

We further asked us how each of the original paper model would behave, if they have the initial values of the combined model inherited.

Every simulation was executed under the condition of a 200 mM NaCl impulse at $t = 30s$:

Figure 9 illustrates that the initial values from the combined model for the volume

Figure 9: Responses of the volume V

model ODE components does not fit at all. The model ODEs quickly reach a steady state far below from the combined model. It does not exists a drop in volume when faced with a stimulus. The volume model assumes the external osmolyte concentration c_e and corresponding external pressure π_e as a constant. It simply does not has an interface for noticing a rise in c_e in a running simulation under the used software development conditions.

On the other hand, figure 10 and 11 show similarly results between the corresponding models. It is shown in figure 11 that in the combined model the ion fluxes representing membrane potential Deltaphi shows a more dynamically behavior in the interplay with volume and hog pathway regulation. Deltaphi from the ion model drops only slightly. After the adaption to the second stimulus, Deltaphi behaves approximately the same.

Figure 10: Response of Hog1PPn; hog model with 200 mM NaCl shock for $t = 30 - 40s$

Figure 11: Response of the membrane potential Deltaphi; a second stimulus of 200 mM NaCl at $t = 100s$

6 Discussion

Distinctions between the peaks of the membrane potential in figure 2 seems to have its root in the different time grid setting of the ODE-Solver in Copasi, which was used for the ion model simulation in the paper [6].
Martina Fröhlich (one of the author of the ion model) noted in her dissertation, that the ion model should only be used for analyses with starved cells [12]. This should be keep in mind if the data are compared with data from non starved *S. cerevisiae* cells. The cells in the volume and hog model were not starved before the experimental analysis [10][7].

A current limitation of the combined model is that the concentration of Cl_{in} and $Glyc_{in}$ grows limitless (figure 8) because the cell can not restore its full turgor pressure and the expression for Hog1PPn does not turn off.
The solution should be a better parametrization or a better initial value set for the ODEs. The used setting for the combined model is the best result of multiple manual attempts to get the simulated system near to a steady state in the absence of any stimulus..

It is assumed that there are not any temperature gradient ($grad(T) = 0$) which would results in a heat flux. Only under well controlled experimental conditions this assumption holds true.

One of the problems in merging kinetic models from different sources and experimental conditions is the parameterization process of the merged model [14]. With no data available it was a try and error process to find a good parameter value for a substance, which is in a interplay with many variables. Available data sets could help in this process but even with this the parameterizations task remains a challenge. [1].

In figure 3 the response of Hog1PPn for a single NaCl impulse for the hog model is not the same as in the paper. Nevertheless, the simulated behavior reproduces experimental data for this stimulus. It is suspected, that not the final version of the model were visualized for the paper.

6.1 Outlook

Even tough I am aware of that a full understanding of complex nonlinear systems might not be ever possible [15], I would like to expand the combined model to the point where I am able to nearly control plasma membrane transport mechanism in eukaryotic single cells. I propose, that a precise analysis of the extracellular environment could allow me then to get an idea what happens inside the cell to get this measured secretion. For this, secretory pathways (SEC) must be implemented to expand the stress-response network for the desired approach.
Another further work aspect is the improvement of the model vision control with the help of a database.
A general problem with a personal designed programming environment is that it quickly needs multiple programs to be installed on the server and several configuration to be made. This way it is not easy to run a simulation on another computer system. To address this problem, the software Docker will be implemented in the near future. With Docker you allow other users to run your program and its dependencies.

I propose that this would allow us to perform an efficient way for a structural analysis of complex mathematical systems, because it would save resources if you can simulate easily from every computer system in the same environment even at the same time. This is called computer cluster and could enhances us to find an optimal configuration for a model with the help of machine learning. This should be my main task for the future with the focus on DoE (Design of Experiment).

7 Reference

[1] Ruian Ke, Piers J. Ingram, and Ken Haynes. An integrative model of ion regulation in yeast. *PLoS Computational Biology*, 9(1):e1002879, jan 2013.

[2] Samik Ghosh, Yukiko Matsuoka, Yoshiyuki Asai, Kun-Yi Hsin, and Hiroaki Kitano. Toward an integrated software platform for systems pharmacology. *Biopharmaceutics & Drug Disposition*, 34(9):508–526, dec 2013.

[3] Serge Feyder, Johan-Owen De Craene, Séverine Bär, Dimitri Bertazzi, and Sylvie Friant. Membrane trafficking in the yeast Saccharomyces cerevisiae model. *International Journal of Molecular Sciences*, 16(1):1509–1525, jan 2015.

[4] André Goffeau, Bart G Barrell, Howard Bussey, RW Davis, Bernard Dujon, Heinz Feldmann, Francis Galibert, JD Hoheisel, Cr Jacq, Michael Johnston, et al. Life with 6000 genes. *Science*, 274(5287):546–567, 1996.

[5] Jannis Uhlendorf. *Real-time feedback control of gene expression.* PhD thesis, Université Paris-Diberot, 2013.

[6] Susanne Gerber, Martina Fröhlich, Hella Lichtenberg-Fraté, Sergey Shabala, Lana Shabala, and Edda Klipp. A thermodynamic model of monovalent cation homeostasis in the yeast Saccharomyces cerevisiae. *PLOS Computational Biology*, 12(1):e1004703, jan 2016.

[7] Tom Altenburg, Björn Goldenbogen, Jannis Uhlendorf, and Edda Klipp. Osmolyte homeostasis controls single cell growth rate and maximum cell size of Saccharomyces cerevisiae. unpublished, April 2018.

[8] Peter Gennemark, Bodil Nordlander, Stefan Hohmann, and Dag Wedelin. A simple mathematical model of adaptation to high osmolarity in yeast. *In Silio Biology*, 2006.

[9] H. Saito and F. Posas. Response to hyperosmotic stress. *Genetics*, 192(2):289–318, oct 2012.

[10] Zhike Zi, Wolfram Liebermeister, and Edda Klipp. A quantitative study of the hog1 MAPK response to fluctuating osmotic stress in Saccharomyces cerevisiae. *PLoS ONE*, 5(3):e9522, mar 2010.

[11] Carey Smoak, Smitha Krishnamurthy, Mansi Singh, and Sy Truong. Forging new sdtm standards for in-vitro diagnostic (ivd) devices: A use-case, 2014.

[12] Martina Fröhlich. *Computational and experimental investigation of the mechanisms involved in cellular water transport and (cat)ion homeostasis*. PhD thesis, Freie Universität Berlin, 2012.

[13] Wolfram Liebermeister. Validity and combination of biochemical models. 2008.

[14] Zhuo Wang, Samuel A. Danziger, Benjamin D. Heavner, Shuyi Ma, Jennifer J. Smith, Song Li, Thurston Herricks, Evangelos Simeonidis, Nitin S. Baliga, John D. Aitchison, and Nathan D. Price. Combining inferred regulatory and reconstructed metabolic networks enhances phenotype prediction in yeast. *PLOS Computational Biology*, 13(5):e1005489, may 2017.

[15] Guillaume Noell, Rosa Faner, and Alvar Agustí. From systems biology to p4 medicine: applications in respiratory medicine. *European Respiratory Review*, 27(147):170110, feb 2018.

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9 Appendix

Table 2: initial conditions of the volume model

state variable	initial condition	annotation
r_{os}	$1.188\ \mu m$	osmotic radius
r_b	$0.496\ \mu m$	solid components radius
r	$1.684\ \mu m$	cell radius
π_t	$2 \cdot 10^5\ Pa$	turgor pressure
R_{ref}	$1.361\ \mu m$	relaxed cell radius
c_i	$6.389 \cdot 10^{-12}\ mmol$	internal amount of osmolytes

Table 3: complete list of volume model parameter values

parameter	value	annotation
d	$0.115\ \mu m$	cell wall thickness
phi	$10^{-4}\ Pa^{-1} \cdot s^{-1}$	cell wall extensibility
π_c	$2 \cdot 10^5\ Pa$	critical turgor pressure
π_e	$6 \cdot 10^5\ Pa$	external pressure
E	$3.44 \cdot 10^6\ Pa$	Youngs modulus (elastic modulus)
Lp	$1.19 \cdot 10^{-6}\ \mu m \cdot Pa^{-1} \cdot s^{-1}$	hydraulic conductivity
nu	$0.5\ dimensionless$	Poissons ratio
k_{uptake}	$2 \cdot 10^{-16}\ mmol \cdot s^{-1} \cdot \mu m^{-2}$	osmolyte uptake rate
$k_{consumption}$	$2.5 \cdot 10^{-16}\ mmol \cdot s^{-1} \cdot \mu m^{-3}$	osmolyte dilution rate
T	$303.15\ K$	temperature
R	$8.314\ J \cdot K^{-1} \cdot mol^{-1}$	gas constant

The values for the initial conditions and the parameters are rounded.

Table 4: complete list of ordinary differential equations and algebraic equations in the volume model

$V = \frac{4}{3} \cdot \pi \cdot 10^{-15} \cdot r^3$
$\pi_i = \frac{c_i}{V} \cdot R \cdot T$
$V_{ref} = \frac{4}{3} \cdot \pi \cdot 10^{-15} \cdot R_{ref}^3$
$G = 4 \cdot \pi \cdot r^2$
$R_{ref} = \frac{r}{(1+(1-nu)\cdot(\pi_t \cdot r)/(E \cdot 2 \cdot d))}$
$R_{refdt} = \frac{phi \cdot R_{ref} \cdot r}{2 \cdot d} \cdot max(\pi_t - \pi_c, 0)$
$r_{osdt} = -Lp \cdot (\pi_t + \pi_e - \pi_i)$
$r_{bdt} = 0.2 \cdot R_{refdt}$
$r_{dt} = r_{osdt} + r_{bdt}$
$\frac{d}{dt} R_{ref} = R_{refdt}$
$\frac{d}{dt} c_i = k_{uptake} \cdot G - k_{consumption} \cdot V \cdot 10^{15}$
$\frac{d}{dt} \pi_t = \frac{E \cdot 2 \cdot d}{1-nu} \cdot (\frac{r_{dt}}{r^2} - \frac{R_{refdt}}{R_{ref} \cdot r}) - \frac{r_{dt}}{r} \cdot \pi_t$
$\frac{d}{dt} r = r_{dt}$
$\frac{d}{dt} r_{os} = r_{osdt}$
$\frac{d}{dt} r_b = r_{bdt}$