

On the Role of Ultrasensitivity in Biomolecular Control Systems

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Abstract—One of the most important design parameters in synthetic biological circuits is the gain of the system. In many naturally occurring biological control systems, however, the precise role of the gain in ensuring accurate control is unclear. In this study, we employ control theory to explore the role of gain in osmoregulation. It has been well-documented that the upstream signalling pathways involved in this system implement high levels of ultrasensitivity, however, the role of such high gain in producing the observed perfect adaptation is not clear. Indeed, it has been argued that a simple integral feedback controller can explain osmoadaptation without the need for high gain. Here, we extend a recently developed proportional controller model for this system with the implementation of ultrasensitivity. We evaluate the performance of the resulting two controllers under different biological assumptions and allowing different levels of gain. We find that a proportional controller implementing ultrasensitivity allows more precise and faster adaptation of cell volume following an osmo-shock. Such an input-output relationship can be tuned as a filter, where the proportional controller couldn't, and thereby allowing responses to signals above a certain threshold. Our results provide insights on the potential role of gain in biological systems, and should be of interest to synthetic biologists attempting to design biomolecular control systems.

I. INTRODUCTION

In recent years, the osmoregulatory response in yeast has emerged as an important model system for studying adaptive, homeostatic responses to environmental disturbances. The underlying molecular control system is well characterized in *Saccharomyces cerevisiae* [1], where it comprises three separate mechanisms that act to adjust the glycerol production in order to keep cells turgor pressure and volume constant in the face of environmental changes: 1) the regulation of the membrane protein Fps1 determining the glycerol export rate; 2) the transcription of several genes, whose proteins are involved in glycerol production, by the activation of the high osmolarity glycerol (HOG) mitogen-activated protein kinase (MAPK) signaling pathway and 3) the HOG kinase dependent regulation of the glycerol via non-transcriptional mechanisms [2].

Despite its biochemical complexity, the osmoregulation system in yeast can be abstracted as a control system comprising of distinct branches as described above. This approach is taken in two recent studies, which aimed to use control models to capture the experimentally observed responses of yeast to osmotic shock and to further predict its structural and dynamic features [2], [3]. These studies

first combined proportional controllers to model the above-described biochemical branches. They then argued for the necessity of at least one branch of such a control model to be an integral controller to achieve adaptive responses in the system, as seen experimentally. The role of integral feedback for perfectly adaptive systems is well-understood [2], [4] and it is highly likely that the osmoregulation system in yeast has indeed a biochemical implementation of integral feedback as seen in other systems [4]–[6]. It is still unknown, however, how evolution of biological control systems such as osmoregulation can proceed to result in integral feedback control. In particular, it is unexplored if alternative control systems other than integral feedback can improve the performance of the system.

Towards answering this question, here we explore the role of ultrasensitivity in osmoregulation. Ultrasensitivity describes a particular form of sensitivity in biological systems, where the system does not respond to incoming signals outside of a certain regime, but responds in a highly sensitive manner within this regime. Such an input-output relationship (i.e. ultrasensitivity) can be described by a specific nonlinear function, is shown to be a ubiquitous feature in several biological systems, and can be biochemically implemented through a variety of mechanisms such as phosphorylation cycles and cooperative binding [7], [8]. Within the yeast osmoregulation system, the HOG MAPK branch is well-documented to be capable of high ultrasensitivity [9], [10] and bistability [11]. Using the recently proportional control model developed by Gennemark et al. [3], we explore here the consequences of such potential ultrasensitivity on the systems performance in achieving homeostasis to osmotic perturbation. We show that incorporating ultrasensitivity in a proportional controller significantly increases system performance and allows additional dynamical features.

II. MODEL

The mathematical description is based on the work presented in [3] and is brought forward in the following paragraphs. Apart from allowing the controller to be non-linear under certain conditions, which is explained in more detail below, the rationale behind the mathematical formalism and the underlying biological assumptions are, if not explicitly stated, based on the study of Gennemark et al. [3]. A diagram of the model is given in Fig. 1.

a) *The biophysical module:* At any given time t , the internal osmotic pressure $P_i(t)$, the external osmotic pressure $P_e(t)$ and the turgor pressure $P_t(t)$ are determining the flow of water across the cell membrane, which is proportional to $(P_i(t) - P_e(t) - P_t(t))$. Assuming that the cell volume is only

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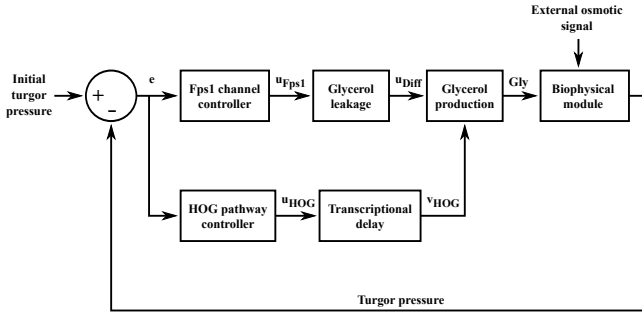


Fig. 1. Mathematical model of the osmoregulation process. This figure has been adapted from [3].

affected by the inflow and outflow of water, we then can express the change in volume as

$$\frac{dV}{dt} = k_{p1}(P_i(t) - P_e(t) - P_t(t)),$$

with k_{p1} denoting a hydraulic water permeability constant. At equilibrium (equil.), this reduces to

$$P_i = P_e + P_t \quad (\text{equil.}).$$

The only osmolyte considered explicitly in the model is glycerol (*Gly*), and the intra-cellular osmotic pressure, according to van't Hoff's law is expressed as

$$P_i(t) = \frac{s + Gly(t)}{V(t) - V_b}, \quad (1)$$

with s being the concentration of the sum of osmolytes other than glycerol present in the cell, and V_b being the non-osmotic volume of the cell, subsuming non-polar cellular components, such as membranes. The turgor pressure is linearly dependent on the volume according to [12], in the following manner:

$$P_t(t) = \varepsilon \left(\frac{V(t)}{V(0)} - 1 \right) + P_t(0), \quad (2)$$

where $V(0)$ is the initial volume, $P_t(0)$ is the initial turgor pressure, and ε is the volumetric elastic modulus. By expressing the volume at which $P_t = 0$ with the notation $V^{P_t=0}$, (2) can be rewritten as

$$P_t(t) = \begin{cases} P_t(0) \frac{V(t) - V^{P_t=0}}{V(0) - V^{P_t=0}}, & V(t) > V^{P_t=0} \\ 0, & \text{otherwise.} \end{cases}$$

b) The controller modules: There are two branches of control in the model: one is considering the closure of Fps1 glycerol transporter channels as a reaction to osmotic shock, and the second is the activation of the HOG pathway, leading to glycerol production after a time delay. The input signal e arriving at the controllers is expressed as

$$e(t) = P_t(0) - P_t(t), \quad (3)$$

which is the difference in turgor pressure. The output of

the Fps1 branch, which corresponds to the response of the transporter channels, is given by

$$u_{Fps1}(t) = \begin{cases} k_{p2} \frac{P_t(0) - e(t)}{P_t(0)}, & e(t) > 0 \\ k_{p2}, & \text{otherwise.} \end{cases}$$

The output of the HOG branch, which corresponds to the HOG pathway dependent glycerol production, is expressed as

$$u_{HOG}(t) = \begin{cases} k_{HOG} \cdot f(e), & e(t) > 0 \\ 0, & \text{otherwise,} \end{cases} \quad (4)$$

where the control function is given by

$$f(e) = \frac{e(t)^n}{\beta e(t)^n + K^n}, \quad (5)$$

with $\beta = 1$ and K and n being the nonlinear Hill function variables. We modify the control law for the HOG pathway, compared to the model of Gennemark et al. [3]. This part of the model formalism is the main difference to the Gennemark et al. model, allowing for a non-linear response. This is inspired by the indication that MAPK systems, of which the HOG pathway is an example, show Hill type responses [9]. The performance of a nonlinear controller is contrasted to the proportional controller given in Gennemark et al., which is one where $\beta = 0$ and $K = n = 1$.

The time delay accounting for transcription and translation in the HOG pathway is approximated by

$$\frac{d\tilde{u}_{HOG}}{dt} = \frac{1}{T_d}(u_{HOG}(t) - \tilde{u}_{HOG}(t)),$$

with $\tilde{u}_{HOG}(t)$ being the time delayed variable and T_d being the amount of time delay considered. The controllers are constrained by restricting the HOG controller to positive values and by the assumption that the Fps1 controller is dependent on glycerol concentration differences between the extracellular and intracellular volumes.

c) The glycerol module: Diffusion of glycerol over the Fps1 channel is modelled as

$$u_{Diff}(t) = u_{Fps1}(t) \left(\frac{Gly(t)}{V(t) - V_b} - \frac{Gly_e(t)}{V_e} \right),$$

with V_e being the extra-cellular volume and Gly_e being the glycerol concentration in the extra-cellular compartment. Intra-cellular glycerol Gly production is expressed, combining the output of the two controllers described above, as

$$\frac{dGly}{dt} = \tilde{u}_{HOG}(t) - u_{Diff}(t)$$

and extra-cellular glycerol, depending only on the diffusion over the Fps1 channel, is described by

$$\frac{dGly_e}{dt} = u_{Diff}(t).$$

III. PARAMETER ESTIMATION

The model contains 16 parameters as reported in Table II. However, four of these are dependent parameters which we

TABLE I

MODEL PARAMETERS: ALL VOLUMES ARE SCALED SUCH THAT THE INITIAL VOLUME OF THE CELL IS 1. BOTH Gly AND Gly_e REPRESENT NUMBER OF MOLECULES (MOL SCALED BY $V(0)$).

Parameters		Bounds
k_{p1}	Water perm. coeff.	[0.0052 160] Osm ⁻¹
k_{p2}	Fps1 control const.	[0 10]
T_d	Time delay	[5 30] min
k_{HOG}	HOG control const.	[0 2] Osm ⁻¹
K	Hill const.	[0 .01 2]
n	Hill exponent	[0 4]
Fixed parameters		Value
$Gly(0)$	Initial Gly	2×10^{-4}
$P_i(0)$	Initial P_i	.636 Osm
$P_e(0)$	Initial P_e	.24 Osm
V_b	Non osmotic volume	.368
$V^{P_i=0}$	V when $P_i = 0$.8
V_e	External volume	4.79×10^3
Dependent parameters		Value
$V(0)$	Initial V - relative volume	1
$Gly_e(0)$	Initial Gly_e	$\frac{V_e Gly(0)}{(V(0) - V_b)}$
$P_i(0)$	Initial P_i	$P_i(0) - P_e(0)$
s	No. of osmolytes other than Gly	$P_i(0)(V(0) - V_b) - Gly(0)$

do not need to constraint. The other parameters are estimated by simulating the model with different osmotic shocks and minimising the error, defined by the equation (3), and time adaptation corresponding to the time required by the cell to reach again approximately the volume before the stress (see the definition in the next section). For the optimization, we use a Genetic Algorithm (GA) [13], that combines the most well-known type of evolutionary algorithms with local algorithms [14], [15]. We used the function *ga* from the MATLAB Global Optimization Toolbox [16] and *fmincon* [17] as hybrid function. By the optimisation procedure some parameters do not significantly change their values, therefore, they are fixed equal to the values estimated in [3], except for $V^{P_i=0}$, which is set to 0.8, value of the volume at zero P_i according to a recent study presented in [18].

The cost function used for the parameter estimation is given by

$$\min_x J, \quad (6)$$

where

$$J = J_p + J_v + J_t \quad (7)$$

is a sum of three scalar functions: J_p is the turgor pressure error, J_v is the difference between the desired and the effective volume and J_t is the response time of the system after the perturbation.

IV. RESULTS

To explore the role of ultrasensitivity in osmoregulation, we further developed a previously described proportional

control model of this system (see Model section). This model is previously shown to capture the characteristic features of the osmoregulation system observed in the model organism *S. cerevisiae* [3]. In our re-implementation of the model, we particularly considered the observed ultrasensitivity in the HOG branch of the system. This branch was originally modeled as a proportional control in the model, which we have replaced here by a Hill-type function to model ultrasensitivity (see Model section). We then compared the performance of this new model against the original model. In particular, we evaluated the two different controllers - proportional (Pr) and ultrasensitive (Us) - by simulating their dynamics with different stress inputs (see Fig. 2) and optimizing their parameters for optimum response (i.e. minimal deviation of cell volume and turgor pressure in presence of an osmo-schock, see Parameter Estimation section for details). We repeated this procedure for different levels of overall sensitivity (i.e. gain) of the HOG branch and different types of osmo-shock sequences and quantified the final (i.e. optimal) performance of the two controllers using two different performance indices: adaptation precision and adaptation time. The adaptation precision is defined as

$$X_a = \prod_i X_{s,i}, \quad (8)$$

where $X_{s,i}$ is the steady state value of the variable X (volume V or turgor pressure P_i) after the i -th perturbation. Since the initial volume is set to unity, this measure gives 1 for perfect adaptation. Deviations from 1 indicate inability of the system to perfectly adapt volume to pre-perturbation levels. The time adaptation, T_a , defined as

$$T_a = \sum_i t_{a,i}, \quad (9)$$

where $t_{a,i}$ is the time required by the system to reach the 85% of the volume V after the i -th osmotic stress.

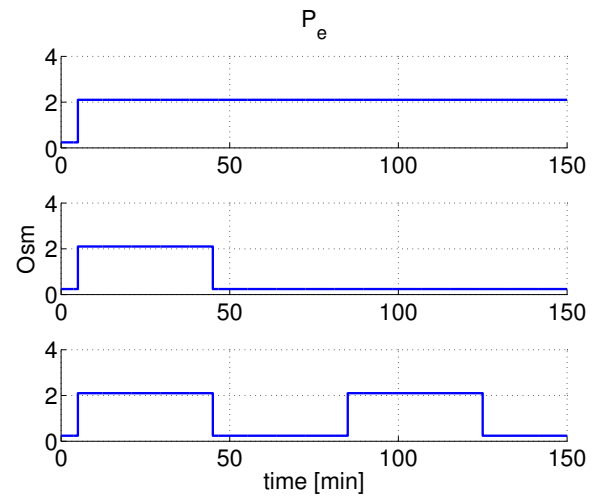


Fig. 2. Different osmotic stress. Upper plot: a constant step of 1M NaCl at $t = 5$ min corresponding to an increase of P_e equal to 1.96 Osm. Middle plot: single pulse signal at $t=5$ min with duration of 40 min of 1M NaCl. Lower plot: double pulse signal at $t_1 = 5$ min and $t_2 = 85$ min, both with duration of 40 min and amplitude of 1M NaCl.

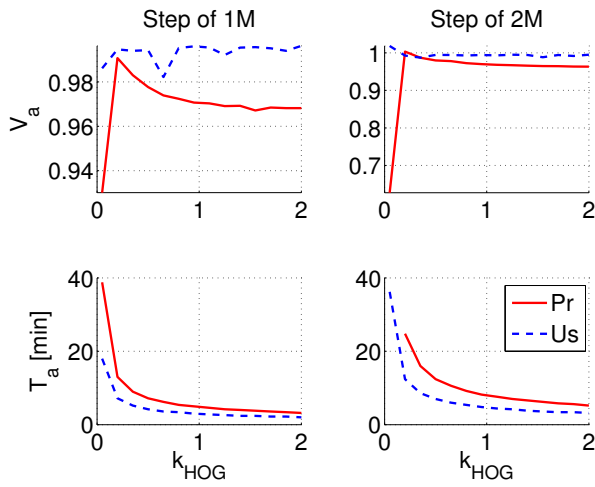


Fig. 3. Results with two constant step signals of 1M and 2M of NaCl.

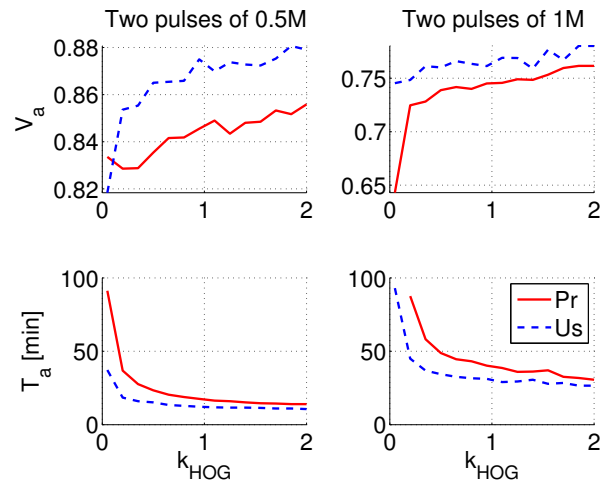


Fig. 5. Results with two double pulse signals of 0.5M and 1M of NaCl.

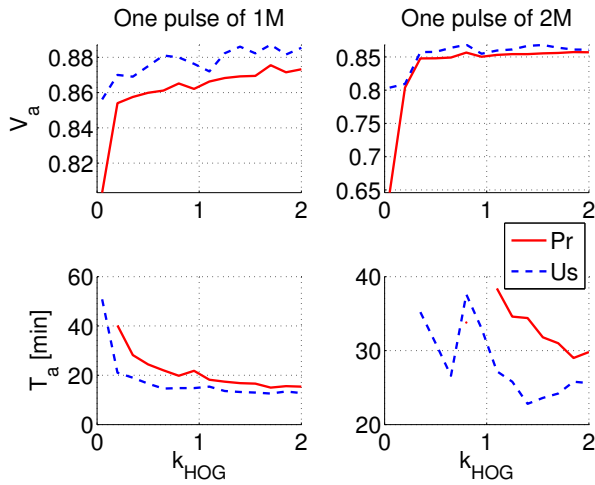


Fig. 4. Results with two single pulse signals of 1M and 2M of NaCl.

Figs. 3–5 show the results of this analysis. We find that for all the different osmo-shock sequences considered - constant step, single pulse and double pulse -, the ultrasensitive controller achieves better and faster adaptation irrespective of the level of overall gain. The better performance was particularly significant when overall gain was limited to lower values, where the ultrasensitive controller achieved almost 2-fold faster responses. We can understand this result simply by considering the input and output of the HOG controller within the full control model (see Fig. 1). By incorporating a Hill function within this branch, we effectively achieve a steeper response from this branch compared to a linear function for any given error (see equations (4) and (5)). Thus, the controller acts faster and more strongly, allowing quick and full recovery of the system.

This insight is in line with the optimized parameters for both controllers (see Table II). In most cases, we find that the optimal parameters for the ultrasensitive controller result in a very steep Hill function that produces maximal outputs

TABLE II
OPTIMIZED PARAMETERS FOR A GIVEN k_{HOG} WITH A DOUBLE PULSE SIGNAL OF 1M.

k_{HOG}	Optimized Parameters - Pr/Us						Us	
	k_{p1}		T_d		k_{p2}		K	n
	Pr	Us	Pr	Us	Pr	Us		
.65	93	155	5	5	.43	.96	.17	3.53
1.1	159	124	5	5	.69	1.17	.23	3.78
1.55	.36	134	5	5	.9	1.61	.23	3.8
2	155	159	5	5	1	1.54	.25	3

for even small error values. Of the other free parameters of the model, we note that certain parameters get optimized differently for the two controllers. For example, the permeability coefficient, which controls water flow in the model (see Model section) is usually optimized to higher values in the ultrasensitive controller compared to the proportional controller. This parameter affects the sensitivity of the system, as faster water movement can allow both a high volume reduction for a given osmo-shock but also fast recovery. Given its fast dynamics, the ultrasensitive controller can “afford” this parameter to become higher compared to the proportional controller.

Such differences between optimal parameters of the two controllers suggest that implementation of ultrasensitivity might allow more freedom in the other parameters of the model or allow them to be in a more favorable regime. To test the former possibility, we have run a simple sensitivity analysis for the two controllers. Given a certain gain, and Hill function parameters, we evaluated the adaptation precision and time of the two controllers for a set of 100 randomly generated parameters. We found that compared to the proportional controller, the ultrasensitive controller achieved much more robust behavioral performance according to these two criteria (Figs. 6–7).

As discussed above, the performance increase of the ultrasensitive controller over the proportional one stems from

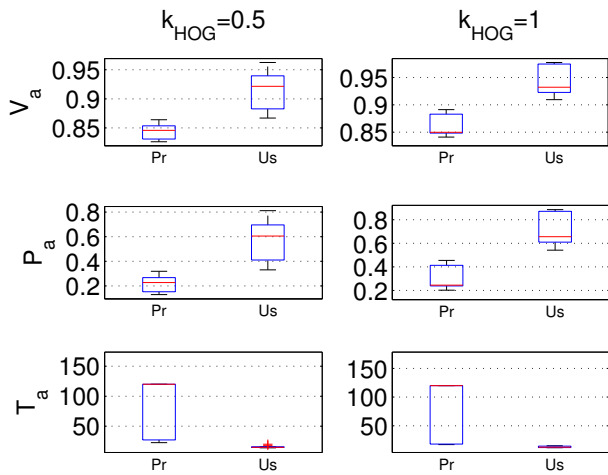


Fig. 6. Sensitivity analysis by fixing k_{HOG} and Hill function parameters ($K = 0.05$, $n = 3.5$) and applying one pulse signal of 1M. The first column of each boxplot reports the results for the proportional (Pr) controller, the second column the results for the ultrasensitive (Us) controller.

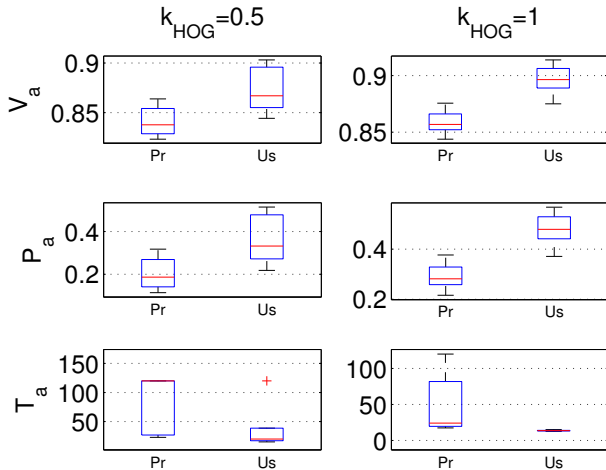


Fig. 7. Sensitivity analysis by fixing k_{HOG} and the Hill function parameters ($K = 0.2$, $n = 2$) and applying one pulse signal of 1M. The first column of each boxplot reports the results for the proportional (Pr) controller, the second column the results for the ultrasensitive (Us) controller.

its high sensitivity to the error due to the Hill function. The incorporation of the Hill function, however, should also allow development of thresholds in the system. In particular, the ultrasensitive controller should be tunable to respond only to signals of certain magnitude or duration. To test this hypothesis, we devised an alternative cost function for the optimization procedure and optimized the system towards functioning as a filter. The new cost function is given by $J_n = J - J_{glyc}$, where J is defined by the equation (7) and J_{glyc} represents the glycerol production upon the signal of limited duration. Fig. 8 shows the performance for a signal with a first short and then long duration pulse. The ultrasensitive controller ignores the first pulse and responds to the second through the Hill parameters, whereas the proportional

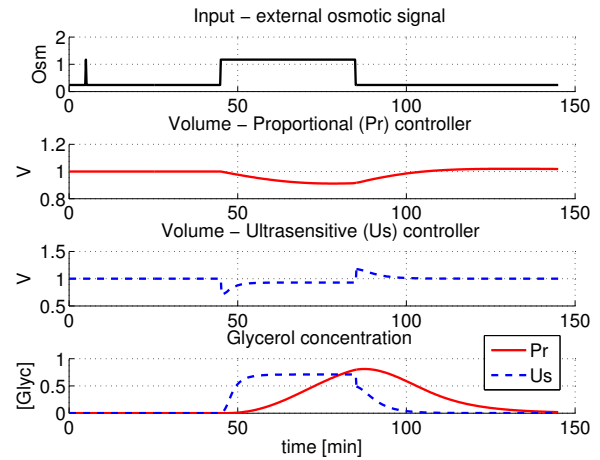


Fig. 8. Response to a signal with a short and long pulse duration, assuming $k_{HOG} = 0.2$. Upper plot: external osmotic signal. Second plot: the volume response for the proportional controller model. Third plot: the volume response for the ultrasensitive controller model. Lower plot: the glycerol concentration for both the models

controller model is not able to respond to the second signal (the permeability coefficient kp_1 , that affects the sensitivity of the system, is equal to the lower bound).

V. CONCLUSIONS

Control theory provides a highly useful approach to abstract complex biological systems that seem to operate with similar goals as engineered control systems. The osmoregulation system in yeast is a prime example of this, where a complex biochemical signalling and regulatory network allows cell volume to maintain homeostasis in face of osmotic shock. While this system has been abstracted by conventional proportional and integral feedback controllers, these models do not shed light on how the biochemical complexity in the system could have arisen in evolution and whether its distinct features have particular functional roles. In particular, the yeast osmoregulation system employs at least two main and distinct branches; an ultrasensitive one that regulates glycerol production (HOG branch) and another that regulates glycerol exchange across the membrane.

Using a previously developed proportional control model incorporating these two branches, we showed here that ultrasensitivity in the HOG branch allows better overall performance. We find that the primary effect of ultrasensitivity in the HOG branch is an increase in the response speed of the system and consequently in its adaptation precision. In addition to this, however, we find that ultrasensitivity provides also a non-trivial flexibility to the system parameters. By increasing the speed of overall system responses, ultrasensitivity in the HOG branch allows sensitivity to be increased in the other branch of the system. In the absence of ultrasensitivity, fast (i.e. highly sensitive) regulation of the glycerol exchange branch limits the cells adaptability through the HOG branch (i.e. glycerol production). With

ultrasensitivity in glycerol production, the other system parameters can be increased or varied more freely, without compromising performance. Moreover, by increasing the gain of the HOG branch, the system with a proportional HOG controller is able to improve the performance in terms of adaptation but there is a presence of overshoot in the system response, whereas ultrasensitivity in the HOG branch allows to avoid this phenomena (we do not consider the overshoot to compute the performance). Note that for large values of the error ($e > 1$), a proportional branch may have a higher gain than ultrasensitive one and, if $K > 1$, the gain of the proportional controller will always be higher, but it is not the case because the error never goes above 1 given the system parameters (the absolute maximum value of the error is $P_t(0)$).

The ultrasensitive response in the HOG branch also allows tuning of the overall system response towards certain signal regimes. In other words, the control system can be tuned to filter out signals below a threshold and respond only when volume decreases cross this threshold. Considering that glycerol production is potentially highly costly for the cell, this ability of the system could given an evolutionary advantage by allowing cells to ignore short lived or low doses of osmo-shock.

In summary, our analysis provides the first steps towards using control theory based approaches to decipher the potential roles of specific biochemical features in complex regulatory systems seen in biology. Such understanding can provide on one hand, insights into evolutionary steps that led to current, complex biological systems and on the other hand, allow better (re)engineering of de novo biological systems in synthetic biology.

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