TOWARDS REAL-TIME CONTROL OF GENE EXPRESSION: CONTROLLING THE HOG SIGNALING CASCADE

JANNIS UHLENDORF[1,2], SAMUEL BOTTANI[2], FRANÇOIS FAGES[1], PASCAL HERSEN[2‖], GREGORY BATT[1♯]

[1] *Contraintes research group, Institut National de Recherche en Informatique et en Automatique, INRIA Paris–Rocquencourt, France*
♯*contact: gregory.batt@inria.fr*

[2] *Laboratoire Matière et Systèmes Complexes, Université Paris Diderot and Centre National de la Recherche Scientifique, UMR 7057, Paris, France*
‖*contact: pascal.hersen@univ-paris-diderot.fr*

To decipher the dynamical functioning of cellular processes, the method of choice is to observe the time response of cells subjected to well controlled perturbations in time and amplitude. Efficient methods, based on molecular biology, are available to monitor quantitatively and dynamically many cellular processes. In contrast, it is still a challenge to perturb cellular processes - such as gene expression - in a precise and controlled manner. Here, we propose a first step towards *in vivo* control of gene expression: in real-time, we dynamically control the activity of a yeast signaling cascade thanks to an experimental platform combining a micro-fluidic device, an epi-fluorescence microscope and software implementing control approaches. We experimentally demonstrate the feasibility of this approach, and we investigate computationally some possible improvements of our control strategy using a model of the yeast osmo-adaptation response fitted to our data.

1. Introduction

To understand biology at the system level, one has to study both the *structure* and the *dynamics* of cellular processes [17,18,32]. On the one hand, genetic analyses are required to analyze the structure of signaling pathways and genetic networks. On the other hand, to access to the dynamical functioning of cellular processes, one has to observe the time response of cells to well controlled perturbations. Hence, the information level provided by experiments crucially depends on our capacity to observe *and* perturb biological systems at the cellular level. Efficient experimental tools have been developed to *monitor* both quantitatively and dynamically many cellular processes. Gene expression can be measured through micro-arrays or quantitative RT-PCR and conveniently observed at the single cell level through the combination of fluorescent reporter proteins and FACS techniques or microscopy [18,20,26]. In contrast, it is still a challenge to *perturb* cellular processes in a precise and controlled manner. A commonly used strategy resides on using inducible promoters to modulate the expression of a gene of interest by the addition of a diffusible molecule in the external cellular environment [11, 15,28]. However, even if the activity of the inducible promoter can be modulated quantitatively, there is no guarantee that the target gene will reach a desired constant expression level over a long period of time. Indeed, variations may arise because of modifications of the physiological state of the cell due to internal feedback loops and cellular adaptation. The expression of a transcription factor regulating itself is even more problematic. Moreover, both theoretical [1, 14] and recent experimental [13,23] results demonstrate the need for elaborate, time-varying

perturbations to decipher quantitatively certain dynamics features of cellular responses. This notably includes the numerous biological processes in which the timing of gene expression plays a central role such as the regulation of the cell cycle. To summarize, existing solutions for the artificial control of gene expression are dissatisfying on two counts since, (i) expressing a gene of interest in a well-controlled, sustained way cannot be conveniently realized at the present time, and (ii) the investigation of certain dynamical properties necessitates dynamical, time-varying perturbations of gene expression for which no solution is currently available.

Here, we propose a first step towards *in vivo* control of gene expression. We have implemented an experimental platform for the *in vivo* control of a signaling pathway in *Saccharomyces cerevisiae*. We chose to control the activity of the HOG cascade which is activated in response to hyper-osmotic perturbations and promotes the transcription of osmo-adaptative response genes. Given a desired temporal profile, the activity of the signaling cascade is monitored in real time and deviations from the desired values are dynamically corrected by varying the osmolarity of the cellular environment (Fig. 1). This can be achieved thanks to a dedicated micro-fluidic device. This experimental platform is driven by software, that notably implements control algorithms, responsible for computing how the cellular environment (osmolarity) should be modified to correct the observed deviations from target values.

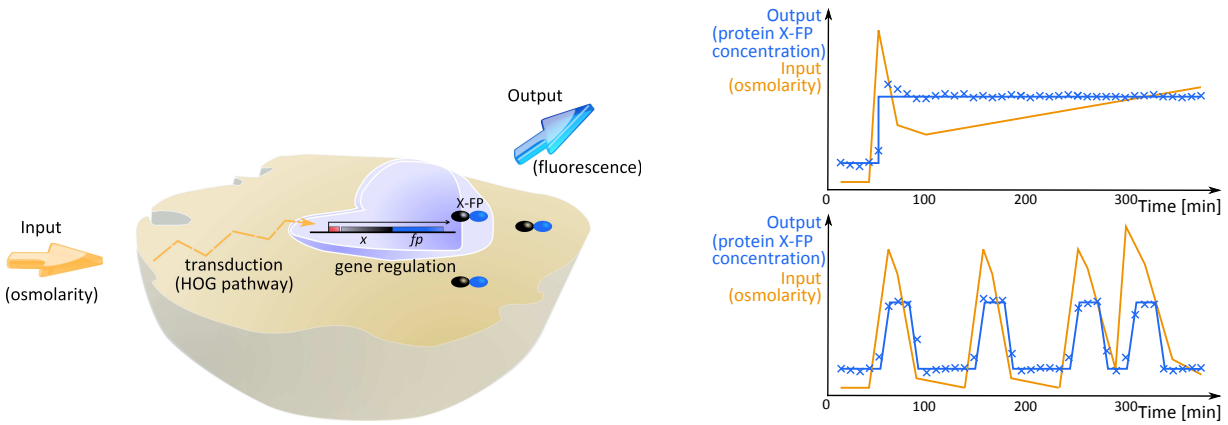

Fig. 1: The control problem. (a) Schematic Input/Output description of the cell. (b) Schematic representation of a desired output (blue), an applied input (orange) and the obtained output (blue crosses), for two different situations. In the first case (top), the goal is to dynamically maintain the concentration of a target protein X, fused to a fluorescent protein (FP), at a constant level. In the second case (bottom), the goal is to create a complex perturbation signal by varying the concentration of the protein X with time.

The work presented here differs significantly from previous applications of control theory in systems and synthetic biology contexts. So far, control theory contributions consisted essentially to shed a new light on biological phenomena, notably by suggesting underlying organization principles in biology [7,8]. An illustrative example is the use of the notion of integral feedback control to explain the robust perfect adaptation observed in bacterial chemotaxis [34]. Other insightful examples are given in a recent textbook [14]. Control theory has also been used in optimal experimental design applications [1,22]. But quite surprizingly, only a few

(theoretical) studies focused on the actual control on a biomolecular process, e.g. [2,6,16,24]. Moreover, to the best of our knowledge, control theory has not yet been applied *in vivo* for the *actual feedback control of biological cellular processes at the single cell level*.

The paper is organized as follows. In section 2, we present in details the proposed platform for real-time control of the HOG signaling cascade activity and gene expression. In Section 3, we present preliminary experimental results obtained when controlling the nuclear localization of the Hog1 protein. This represents an essential first step towards controlling gene expression. In Section 4, we discuss possible improvements of our control approach using a simple model of the osmotic stress response. Conclusions are provided in the last section.

2. A platform for real-time control of gene expression

2.1. *An integrated real-time control platform*

Central to control theory is the notion of *feedback control* [30]. The idea is to compute the inputs to apply at the next time instant in function of outputs previously obtained. This way, knowledge of past errors is used to improve the control. In comparison to open loop control where the control strategy is computed beforehand, closed loop control approaches are generally less sensitive to model uncertainties and can compensate for external disturbances. These two features are highly desirable for any biological application. However, performing a control in real-time necessitates a tight integration between measurement device, control software and actuator.

As described in Figure 2, the HOG pathway activity can be monitored at the single cell level using time lapse fluorescent microscopy. The cellular environment can be controlled using the micro-fluidic device developed by Hersen and colleagues [13]. Not only this device allows a fast and well-controlled change of the cellular environment, but also, it guarantees that with the exception of the input signal the cell environment is otherwise held constant. We implemented algorithms for image analysis, state estimation and input computation in a Matlab program that communicates with and drives the microscope via MicroManager [31] and the micro-fluidic pressure controller.

2.2. *Using HOG signaling cascade*

To link external environmental changes to gene expression, we use a natural signaling pathway: the Hyper Osmolar Glycerol (HOG) pathway in the yeast *Saccharomyces cerevisiae*. This MAP kinase pathway is used to sense osmolar pressure changes in the environment and to trigger osmotic stress responses that maintain water homeostasis [29]. More precisely, two osmo-sensor proteins (Sln1 and Sho1) transduce the signal to the Hog1 protein via a phosphorylation cascade. Once phosphorylated, Hog1 promotes the osmo-adaptative response in at least three different ways. Firstly, Hog1 translocates into the nucleus and alters, directly or indirectly, the expression of a large number of genes [27]. Secondly, Hog1 has also a cytoplasmic activity since it regulates negatively glycerol export by inhibiting the activity of the Fps1 glycerol channel [3]. Thirdly, Hog1 activates glycerol producing enzymes, notably Gpd1 [33]. Hence, the osmo-adaptative response involves at least three natural feedback loops.

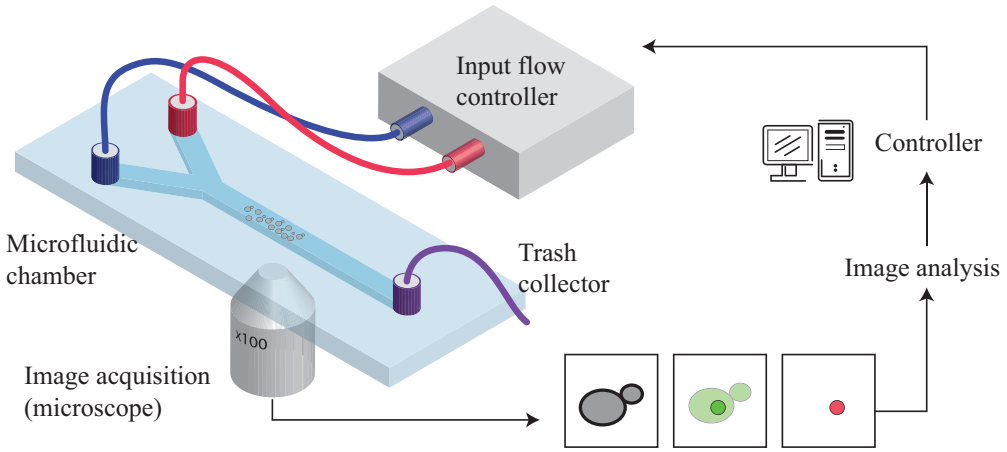

Fig. 2: The integrated control platform. The main elements of the feedback loop are (i) a microfluidic device allowing a rapid control of the cellular environment, (ii) a microscope for phase contrast and fluorescence measurements, (iii) yeast cells with Hog1, a nuclear marker (Htb2) and the protein of interest (X) fused to compatible fluorescent markers, and (iv) Matlab software for image analysis and controller implementation.

Our motivation for using this pathway is triple. Firstly, it has been extensively experimentally studied and quantitative models are available [5,13,19,21,23,25,35]. Second, the output of the signal transduction pathway can be experimentally quantified. Indeed, if Hog1 is fused to a fluorescent protein, its nuclear localization can be quantified and provides a measure of the Hog1 activity [9]. Thirdly, it has been experimentally shown that for fast osmolarity changes, the pathway integrates the signal: the transduction pathway acts as a low-pass filter with a bandwidth approximatively equal to 5×10^{-3} Hz [13]. This property allows us to *emulate* an analog control by rapidly switching (frequencies greater than 0.1 Hz) between two media: the normal growth medium and a sorbitol enriched ($\sim 1\,M$) medium. For example, a two minute osmotic stress corresponding to a $0.4\,M$ sorbitol intensity is obtained by flowing cells 12 times with normal medium during $6\,s$. and with sorbitol-rich medium during $4\,s$.

In this paper, we use a yeast strain with Hog1 fused to GFP and the nuclear protein Htb2 fused to mCherry [13]. The latter is used to conveniently localize the nuclear region. We define the relative Hog1 nuclear localization $h(t)$ as the ratio of the mean fluorescence pixel intensities of Hog1-GFP in the nucleus and in the cytoplasm.

$$h(t) = \frac{\langle Pixel\ intensity \rangle_{nuc}}{\langle Pixel\ intensity \rangle_{cyto}}$$

The normalized Hog1 nuclear localization $h_n(t)$ is then simply $h_n(t) = h(t)/h(t_0)$. These definitions are motivated by the fact that this gives measures that are relatively robust with respect to fluorescent protein photo-bleaching and cell-to-cell variations.

3. Controlling transcription factor nuclear localization using a simple control approach

In this section, we present preliminary results obtained on controlling the Hog1 nuclear localization. The control of Hog1 nuclear activity is a prerequisite for utilizing the Hog pathway

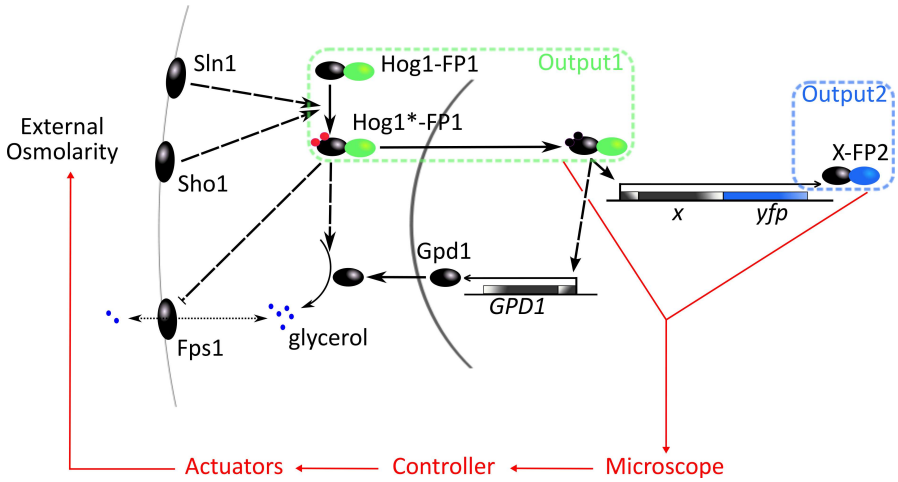

Fig. 3: Schematic representation of the HOG pathway with natural and engineered feedbacks. Solid and dashed arrows indicate direct and indirect effects, respectively. For a detailed description, see the main text. FP1 and FP2 in the figure denote two different fluorescent proteins.

to control gene expression. As a matter of fact, controlling the duration of activated Hog1 residence in the nucleus will lead to burst of expression for the genes which are placed under Hog1 dependent promoter. There are different options how to encode a certain gene expression profile. We could either work with a constantly high signal and adjust the amount of Hog1 in the nucleus (amplitude modulation), or we could successively activate the Hog pathway for a short duration and control the frequency of these activations (frequency modulation). To test these two alternative strategies, we consider two problems: maintaining a given constant level of Hog1 nuclear localization over a long time period, or obtaining pulses of Hog1 nuclear localization in a repeated manner. These results have been obtained using the simplest control approach: a PID controller.

3.1. *PID control*

A proportional-integral-derivative (PID) controller is a generic closed-loop control algorithm, generic meaning that is does not require any structural knowledge about the controlled system [30]. Due to its simplicity this type of control is very often applied in engineering applications. A PID controller measures the deviations ("errors") of measured states from target states, and uses this information to compute the control. The applied control u at time t is the weighted sum of the error, $e(t)$, its derivative and the integral of past errors $e(\tau)$, $\tau \in [0, t]$:

$$u(t) = k_p \cdot e(t) + k_i \cdot \int_0^t e(\tau)d\tau + k_d \cdot \frac{d}{dt}e(t)$$

where k_p, k_i and k_d are the proportional, integral and derivative gains.

In our case the error $e(t)$ is the difference between the measured normalized Hog1 nuclear localization $h_n(t)$ and its reference value at the corresponding time point. Because we consider tracking problems, only the recent past errors are relevant. Therefore, we integrate the error only on the interval $[t-\Delta, t]$, where Δ is approximatively 2 minutes. We tuned the controller gains manually using a trial and error approach. The derivative term, and to a lesser extend,

the proportional term are responsible for implementing a fast system response to target value changes. However large values for these parameters favor oscillations and loss of stability. In practice, we found that setting the derivative gain to zero and using values for k_p and k_i close to 2 and 1.5 leads to a good compromise between response time and stability in our experimental setting.

3.2. *Experimental results*

We designed two control experiments to test the possible strategies discussed above: using amplitude or frequency encoding. The first type of experiment is to try to maintain the system output at a constant target level (Fig 4 left). Quantitatively, the relative Hog1 nuclear localization should remain 20% higher than its nominal value in unstressed cells. The second type of experiment is to try to obtain repeatedly trapezoidal motifs. The amplitude of output variations also corresponds to a 20% increase above nominal value (Fig 4 right).

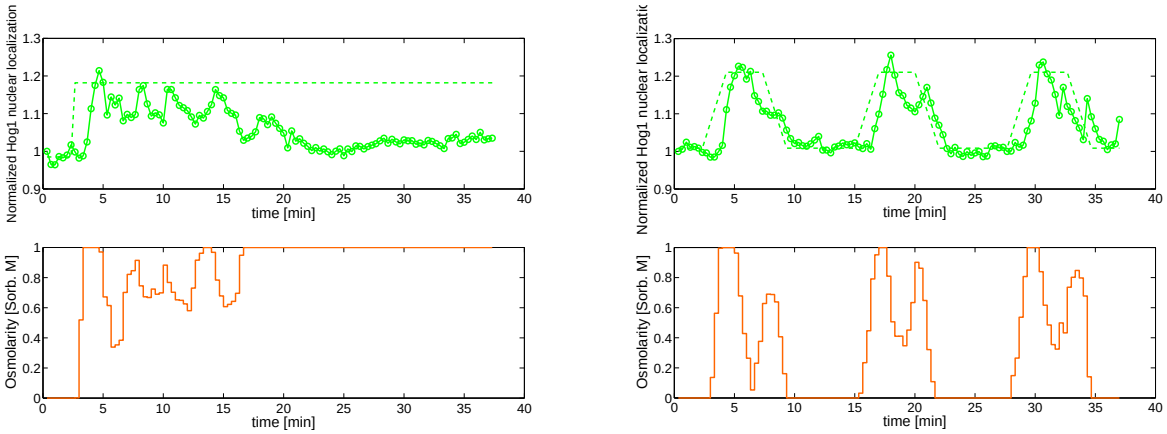

Fig. 4: Experimental results for the control of Hog1 nuclear localization. Left: Controlling the amplitude of Hog1 localization does only work for short durations due to internal feedback and cellular adaptation to sustained hyper osmotic conditions. Right: With a frequency encoded signal, the cell is able to reset between successive shocks and follows the reference values for the whole experiment.

Our experimental results clearly show that the control is effective in yeast cells. Consider for example the step experiment at time 2, when the target value changes. Following this change, the controller applies an osmotic stress, resulting after a 1-2 minute delay in an increase in the Hog1 nuclear localization. Then the system overshoots and the controller decreases the osmolarity of the environment. Oscillations ensue around a level below the target value during approximatively 15 minutes, during which increasing inputs are applied. Finally, even the maximal input is not sufficient to prevent the system from drifting away towards its nominal level.

The interpretation of these control results is simple. Because of internal natural feedbacks, the cells adapt (notably produce glycerol) and become insensitive to high osmolarity environments. Therefore, unless all internal feedback loops are inactivated, the amplitude-based control strategy seem not feasible. The inability of the controller to maintain the output at the

target value in osmo-sensitive cells can be explained by the initiation of an osmo-adaptative response causing cells to drift away from the target value, together with the use of a rather narrow integration window in our PID controller.

Concerning the repeated motif experiment, it is fair to say that despite time lags and a relatively noisy behavior, the controller succeeds in producing the desired time varying output (Fig 4 right). As it appears on the plots, the 6 minute time separation between the 8 minute long motifs seem sufficient to fully reset the system to its normal, osmo-sensitive state. Based on these experimental results, the frequency encoding strategy for gene expression seems promising. However, before dealing with the actual control of gene expression, improvements in our control approach are needed. The capacity of the controller to predict rather than just to react -this would help dealing with the lag problem-, and the capacity to filter noise out -this would make the control more robust- are two features of significant interest.

4. Design of an improved control approach

The major advantage of the PID controller is that it does not rely on a model of the system. This makes it particularly easy to deploy. However, performances achieved using model-based control approaches are generally superior. In this section, we use the simple model proposed by Muzzey and colleagues [25], fitted to our data, to compare performances obtained with the PID controller and a model based control approach.

4.1. *Development of a simple linear model*

Numerous models have been developed for the osmotic stress response [5,13,19,21,23,25,35]. Because of its capacity to capture essential aspects of the HOG pathway, including notably the cell adaptation, and of its mathematical simplicity, we reuse the three variables linear model developed by Muzzey and colleagues [25]. In short, the state of the system is described by three variables, s_1, s_2, and s_3, corresponding to the nuclear Hog1 enrichment, its time integral, and glycerol relative concentration, respectively, and one input, u, corresponding to the external osmolarity. Since the osmo-stresses studied in [25] are caused by a different osmolyte (salt versus sorbitol), we introduce a factor σ to rescale the input u, if needed.

$$\dot{s}_1 = k_h\,(\sigma\,u - s_3) - \gamma_h\,s_1 \tag{1}$$

$$\dot{s}_2 = \alpha_d\,s_1 \tag{2}$$

$$\dot{s}_3 = s_2 + \alpha_i\,(\sigma\,u - s_3) - \gamma_g\,s_3 \tag{3}$$

In the above model, $\sigma\,u - s_3$ corresponds to the net osmolarity effectively sensed by the cell. In hyper-osmotic conditions, the production of intracellular glycerol (s_3) and the Hog1 nuclear localization (s_1) are increased. The increased Hog1 nuclear localization increases s_2 and hence s_3. Therefore one distinguishes a *direct* and an *indirect* effect of hyper-osmotic stress on glycerol accumulation [25].

To fit the model parameters to our system we perform two types of experiments in which cells are exposed to hyper-osmotic stresses differing either in magnitude or duration. The first set of experiments is used primarily to estimate the relation between osmotic stress and Hog1 localization, whereas the second set of experiments is used primarily to investigate the cell

dynamical adaptation to osmotic stress. One should note that we experimentally measure the normalized Hog1 nuclear *localization* $h_n(t)$, whereas the variable $s_1(t)$ in the Muzzey model corresponds to the Hog1 nuclear *enrichment*. However, it holds that $h_n(t) = s_1(t) + 1$ [25]. In the sequel, to allow for comparison with the experimental results of Section 3, we present all our results -experimental and computational- using $h_n(t)$.

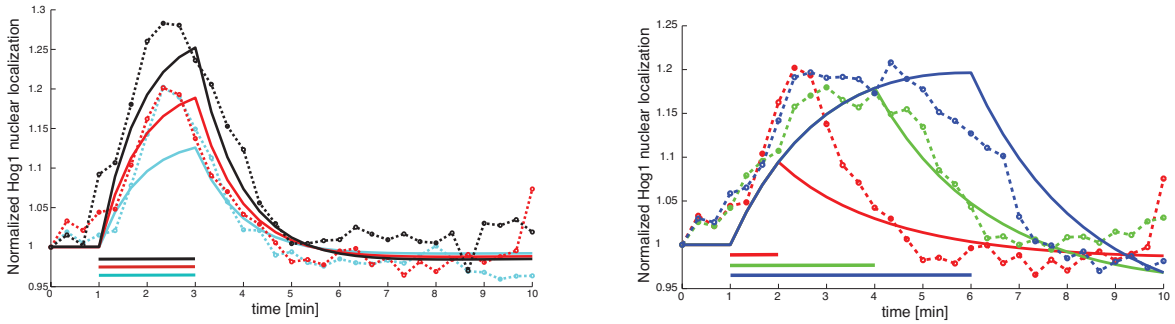

Fig. 5: Cell response to different hyper-osmotic stresses. Left: Stresses of different intensities. Cyan, red, and black plots correspond to a 0.4, 0.6, or 0.8 M stress applied during 2 minutes. Right: Stresses of different durations. Red, green and blue plots correspond to 0.6 M stress applied during 2, 4, or 6 minutes. Dashed and solid lines represent experimental data and model predictions, respectively.

To find parameter values for our model, we use the state-of-the-art non-linear optimization tool CMAES implementing a covariance matrix adaptation evolution strategy [12]. The objective function to minimize is the sum of a mean square error term, where the error is the difference between measured and predicted values for s_1, and a penalty term enforcing the positiveness of parameters and initial conditions. Parameter estimates are then manually fine-tuned (see Table 1).

k_h	γ_h	γ_g	α_h	α_g	σ
1.984	0.9225	0.5950	0.1612	0.0106	0.2

Table 1: Parameter values fitted to the experimental data shown in Fig. 5. All parameter units are min^{-1}, excepted for the dimensionless parameter σ.

As can be seen from the plots shown in Fig. 5, the model is able to capture qualitatively, and up to some degree, quantitatively, the behavior of yeast cells subjected to hyper-osmotic stresses. This is commendable given the extreme simplicity of the model.

4.2. *Comparison of different control approaches*

Equipped with a model of our system, we can computationally simulate the system response and compare various control approaches. Given the time-consuming aspect of experiments, working on simulated but realistic data allows us to rapidly test alternative control approaches. When computationally testing a model-based control approach, one uses the same model in the simulator and in the model based controller. That is, the model based controller knows

perfectly the system dynamics. To make fair comparisons, we assume that only the output (and not the full state) is visible by the controller and we add (Gaussian) noise to the system output.

We present here a model predictive control (MPC) approach. The objective of MPC is to minimize the difference between the simulated and the target outputs by using a receding horizon strategy: given an estimate of the current state of the system, a control strategy to be applied during a short time horizon is searched for, and applied for a short period of time. Then, the approach is applied again, with the estimation of the new state, and the computation of a control strategy for a new short time horizon. This receding horizon strategy yields an effective feedback control [10]. Because MPC applies to linear and nonlinear systems, this approach can easily be extended to deal with future improved models. An other motivation for using MPC rather than the conventional control approach for linear system output tracking, based on a linear quadratic gaussian controller [30], is that simple non-linear constraints (e.g. bounded input) can easily be integrated in this framework.

For our application, we implement an MPC approach using Kalman filtering and a simple search strategy. The use of a Kalman filter is a standard approach to estimate the full state of a linear system based on (noisy) observations [30]. Then, at time t, we search for three input values, u_1, u_2, and u_3, that when applied on the time intervals $[t, t+1]$, $]t+1, t+2]$, and $]t+2, t+3]$, respectively, minimize the squared error, again defined as the distance between the target and the simulated outputs, on the time interval $[t, t+3]$. u_1 is applied on $[t, t+1]$ and the procedure is restarted at time $t+1$. At each iteration, we use CMAES, a global optimization, tool to search for the three input osmolarities u_1, u_2, and u_3. Naturally, in our setting, the input (osmolarity) is necessarily positive and bounded. Therefore, we limit the search to the interval of feasible osmolarities. The computational effort remains limited, since less than one second is needed for each iteration. For comparison, the timestep duration of the control loop in our experiments is $20\,s$. So using MPC does not challenges the real-time requirement.

We also consider here the PID controller presented in Section 3, but applied on simulated data as explained in this section. All these computational procedures have been implemented in Matlab.

The results obtained with the two control strategies and the for two different control problems are shown in Figure 6. Regarding the difficulty to maintain pathway activity over a prolonged period and the feasibility of creating repeated short time activity patterns, the results obtained with both control approaches are fully consistent with our experimental findings. The comparison of the results obtained with the PID controller on the experimental (Fig 4) and simulated data (Fig 6) shows that the PID performs better in the second case. This might be explained by a higher complexity of cellular variability (ie the "noise" is not just plain Gaussian). As expected, the lag and incomplete drift compensation are also observed on simulated data, albeit attenuated. In contrast, the model predictive results show neither. This corroborates the fact that they originate -at least partly- from the reactive rather than predictive nature of the PID controller. Moreover, the control is also much more regular in the MPC experiments. Very likely, this comes from the use of Kalman filtering. One should note that this is not due to an improper parametrization of the PID. Indeed the relatively

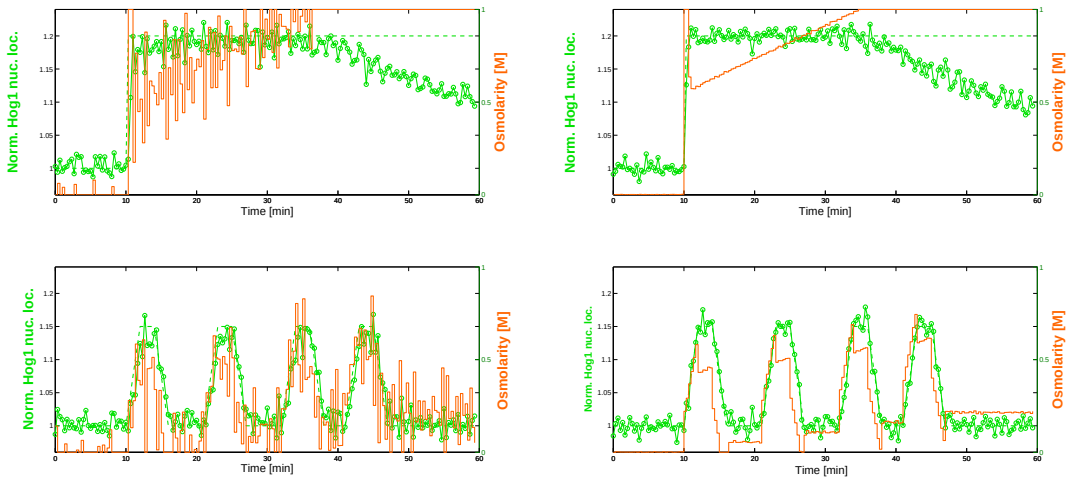

Fig. 6: Comparison of PID (left) and MPC (right) control strategies for two different control problems and on simulated data. *Norm. Hog1 nuc. loc.* stands for normalized Hog1 nuclear localization.

high proportional gain that cause large input changes is needed to ensure a fast response.

To summarize, the model predictive control approach is superior on all counts to the PID controller, at the cost of a very limited computational overhead. However, one should stress that the quality of a model based controller ultimately depends on the quality of the model of the system. So to effectively apply MPC on yeast cells, significant modeling work might be needed. But then one will have the effective proof that the main features of the osmo-adaptative response are captured in sufficient details.

5. Discussion

We presented an integrated experimental platform and demonstrated the feasibility of controlling the nuclear localization of the protein Hog1. Stated differently, we have shown how to create a dynamically controlled inducible promoter. As a matter of fact, it should be possible to place any gene under the control of a Hog1-dependent promoter and then to force its expression by controlling Hog1 nuclear localization. Consequently, this contribution describes a first, crucial step towards real-time control of gene expression.

Using the HOG pathway has several advantages, the most important ones being its quick activation and de-activation which are crucial to ensure efficient dynamics of the control loop, and the established correlation between nuclear localization and activity. It is to be noted though, that contrarily to known inducible promoters such as the Tet system, activating the HOG pathway also affects the cell physiological state, since many genes are transcribed to ensure proper cellular response to an hyper osmotic environment. For real applications, one should achieve a clear separation between controlling gene expression dynamically and altering the physiological state. This might require engineering the HOG cascade, or using other alternative signaling pathways with similar dynamics and nuclear translocation.

Interestingly, our results suggest that for our application, it is preferable to use *frequency*

encoding to control gene expression. Indeed, because of fast, non-genetic adaptation feedbacks, the output of the signaling cascade can not be held constant over a prolonged period. A Frequency encoding strategy is widely used by neural networks which computing activity relies on action potential pulses. Although it is generally assumed that gene regulation is naturally controlled by amplitude modulation, a recent study by Elowitz's team showed that the expression of some genes in yeast are regulated by the frequency of expression bursts led by the transcription factor Crz1 [4]. The authors proposed that the functional role of frequency modulation is to ease the coordination of the expression of multiple target genes. Based on our results, one can propose an alternative role of regulation by frequency modulation: it allows for both a rapid non-genetic response and a slower transcriptional response leading to a complete adaptation to a given stress.

In a future work, we will use a model-based control approach to improve our results on Hog1 nuclear localization. Moreover, we will progress towards our main goal, that is, gene expression control, by studying a candidate gene fused to a fluorescent tag under the direct control of Hog1. The control platform will be adapted to read as outputs both the localization of Hog1 and the actual expression level of the gene of interest.

We anticipate that this platform to tune in real-time the level of expression of a gene of interest will be a useful tool for the biologist to better understand living processes in single cells. Quoting Feynman saying 'what I cannot built, I cannot understand', synthetic biologists propose that building systems helps to better understand them. Here, we propose that controlling them is an effective way to assess our understanding: what I cannot control, I have not understood.

References

1. J.F. Apgar, J.E. Toettcher, D. Endy, F.M. White, and B. Tidor. Stimulus design for model selection and validation in cell signaling. *PLoS Computational Biology*, 4(2):e30, 2008.
2. S. Azuma, E. Yanagisawa, and J. Imura. Controllability analysis of biosystems based on piecewise affine systems approach. *IEEE Transactions on Circuits and Systems and IEEE Transactions on Automatic Control*, 53, 2008. Joint special issue on Systems Biology.
3. S. E. Beese, T. Negishi, and D.E. Levin. Identification of positive regulators of the yeast Fps1 glycerol channel. *PLoS Genetics*, 5(11):e1000738, 2009.
4. L. Cai, C.K. Dalal, and M.B. Elowitz. Frequency-modulated nuclear localization bursts coordinate gene regulation. *Nature*, 455(7212):485–490, 2008.
5. A.P. Capaldi, T. Kaplan, Y. Liu, N. Habib, A. Regev, N. Friedman, and E. K O'Shea. Structure and function of a transcriptional network activated by the MAPK Hog1. *Nature Genetics*, 40(11):1300–1306, 2008.
6. M. Chaves and J.-L. Gouzé. Exact control of genetic networks in a qualitative framework: the bistable switch example. Technical Report RR-7359, INRIA Sophia-Antipolis, 2010.
7. M.E. Csete and J.C. Doyle. Reverse engineering of biological complexity. *Science*, 295(5560):1664–1669, 2002.
8. H. El-Samad, H. Kurata, J.C. Doyle, C.A. Gross, and M. Khammash. Surviving heat shock: Control strategies for robustness and performance. *Proceedings of the National Academy of Sciences of the USA*, 102(8):2736–2741, 2005.
9. P. Ferrigno, F. Posas, D. Koepp, H. Saito, and P.A. Silver. Regulated nucleo/cytoplasmic exchange of HOG1 MAPK requires the importin beta homologs NMD5 and XPO1. *EMBO Journal*, 17(19):5606–5614, 1998.

10. R. Findeisen, F. Allögwer, and L. Biegler. *Assessment and Future Directions of Nonlinear Model Predictive Control*, volume 358 of *LNCIS*. Springer, 2007.
11. M. Gossen, S. Freundlieb, G. Bender, G. Muller, W. Hillen, and H. Bujard. Transcriptional activation by tetracyclines in mammalian cells. *Science*, 268(5218):1766–1769, 1995.
12. N. Hansen and A. Ostermeier. Completely derandomized self-adaptation in evolution strategies. *Evolutionary Computation*, 9(2):159–195, 2001.
13. P. Hersen, M. N. McClean, L. Mahadevan, and S. Ramanathan. Signal processing by the HOG MAP kinase pathway. *Proceedings of the National Academy of Sciences of the USA*, 105(20):7165–7170, 2008.
14. P.A. Iglesias and B.P. Ingalls. *Control Theory and Systems Biology*. MIT Press, 2009.
15. F. Jacob and J. Monod. Genetic regulatory mechanisms in the synthesis of proteins. *Journal of Molecular Biology*, 3:318–356, 1961.
16. A.A. Julius, A. Halasz, M.S. Sakar, H. Rubin, V. Kumar, and G.J. Pappas. Stochastic modeling and control of biological systems: the lactose regulation system of *E. coli*. *IEEE Transactions on Circuits and Systems and IEEE Transactions on Automatic Control*, 53, 2008. Joint special issue on Systems Biology.
17. H. Kitano. Systems biology: A brief overview. *Science*, 295(5560):1662–1664, 2002.
18. E. Klipp, R. Herwig, A. Kowald, C. Wierling, and H. Lehrach. *Systems biology in practice: Concepts, implementation and application*. Wiley Press, 2005.
19. E. Klipp, B. Nordlander, R. Kruger, P. Gennemark, and S. Hohmann. Integrative model of the response of yeast to osmotic shock. *Nature Biotechnology*, 23(8):975–982, 2005.
20. K.M. Klucher, M.J. Gerlach, and G.Q. Daley. A novel method to isolate cells with conditional gene expression using fluorescence activated cell sorting. *Nucleic Acids Research*, 25(23):4858–4860, 1997.
21. J. Macia, S. Regot, T. Peeters, N. Conde, R. Solé, and F. Posas. Dynamic signaling in the Hog1 MAPK pathway relies on high basal signal transduction. *Science Signaling*, 2(63):ra13, 2009.
22. F. Menolascina, D. Bellomo, T. Maiwald, V. Bevilacqua, C. Ciminelli, A. Paradiso, and S. Tommasi. Developing optimal input design strategies in cancer systems biology with applications to microfluidic device engineering. *BMC Bioinformatics*, 10(Suppl 12):S4, 2009.
23. J.T. Mettetal, D. Muzzey, C. Gomez-Uribe, and A. van Oudenaarden. The frequency dependence of osmo-adaptation in Saccharomyces cerevisiae. *Science*, 319(5862):482–484, 2008.
24. D. Mitra, E. J. Parnell, J. W. Landon, Y. Yu, and D. J. Stillman. SWI/SNF binding to the HO promoter requires histone acetylation and stimulates TATA-binding protein recruitment. *Molecular and Cellular Biology*, 26(11):4095–4110, 2006.
25. D. Muzzey, C.A. Gomez-Uribe, J.T. Mettetal, and A. van Oudenaarden. A systems-level analysis of perfect adaptation in yeast osmoregulation. *Cell*, 138(1):160–171, 2009.
26. D. Muzzey and A. van Oudenaarden. Quantitative time-lapse fluorescence microscopy in single cells. *Annual Review of Cell and Developmental Biology*, 25(1):301–327, 2009.
27. S.M. O'Rourke and I. Herskowitz. Unique and redundant roles for HOG MAPK pathway components as revealed by whole-genome expression analysis. *Molecular Biology of the Cell*, 15(2):532–542, 2004.
28. T.M. Roberts, R. Kacich, and M. Ptashne. A general method for maximizing the expression of a cloned gene. *Proceedings of the National Academy of Sciences of the USA*, 76(2):760–764, 1979.
29. W.H. Mager S. Hohmann. *Yeast stress responses*. Topics in Current Genetics. Springer, 2003.
30. E.D. Sontag. *Mathematical Control Theory. Deterministic Finite-Dimensional Systems*. Springer, 1998.
31. N. Stuurman and A. Edelstein. μmanager: the open source microscopy software v.1.3, 2010. http://www.micro-manager.org.
32. Z. Szallasi, J. Stelling, and V. Periwal, editors. *System Modeling in Cellular Biology: From Concepts to Nuts and Bolts*. MIT Press, 2006.
33. P.J. Westfall, J.C. Patterson, R.E. Chen, and J. Thorner. Stress resistance and signal fidelity independent of nuclear MAPK function. *Proceedings of the National Academy of Sciences of the USA*, 105(34):12212–12217, 2008.
34. T.-M. Yi, Y. Huang, M.I. Simon, and J.C. Doyle. Robust perfect adaptation in bacterial chemotaxis through integral feedback control. *Proceedings of the National Academy of Sciences of the USA*, 97(9):4649–4653, 2000.
35. Z. Zi, W. Liebermeister, and E. Klipp. A quantitative study of the Hog1 MAPK response to fluctuating osmotic stress in Saccharomyces cerevisiae. *PLoS ONE*, 5(3):e9522, 2010.