Towards Real-Time Control of Gene Expression: *in silico* Analysis ⋆

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Abstract: Motivated by experimental work in our lab, we consider here the problem of the real-time control of the expression of a single gene at the cellular level in yeast. We provide a description of the biological problem and a mathematical formulation. We develop a model predictive control strategy tailored to the specificities of the biological problem and assess *in silico* its effectiveness and its robustness to biological variability.

1. INTRODUCTION

One major goal of systems biology is to understand the dynamical functioning of biological systems at the cellular level. One common approach to investigate the dynamics of a system is to observe its response to perturbations. For the development of a quantitative understanding (and of quantitative models), precision in the observation *and* in the perturbation are both important. With the development of fluorescent markers, our capacity to observe cellular processes at the single cell level has recently greatly improved (Muzzey and van Oudenaarden, 2009). In contrast, our capacity to perturb cellular processes in a well-controlled, quantitative manner remains very limited. This is particularly striking if one considers time-varying perturbations, that are however highly informative on system's dynamics. This can notably be explained by the large variability of individual cell responses to a same external stimulation (Colman-Lerner et al., 2005; Newman et al., 2006).

To improve our capacity to perturb cellular processes via the expression of a given protein with a chosen temporal expression profile, we develop a platform for the real time control of gene expression (Uhlendorf et al., 2010). In short, this platform allows for applying short osmotic stresses to yeast cells, that trigger gene expression via the activation of the so-called Hyper Osmotic Glycerol (HOG) signal transduction pathway, and for observing in real time the cellular response. In Uhlendorf et al. (2010), we describe preliminary experimental results on the control of the activity of the signal transduction pathway, obtained using a simple proportional-integral (PI) controller. However, the control of the full system, being more complex and much slower (with inertia), will likely require more elaborate control methods.

In this paper, we propose a control strategy tailored to the specificities of our biological problem. In particular, we decompose the system into two subsystems, corresponding to signal transduction and gene expression, and having significantly different response times. We exploit the 'cascaded' structure of the system to propose a two-layer model predictive control strategy. Additionally, to skirt the rapid cell adaptation to stress, we adopt a pulse-modulated strategy to control gene expression. The practical feasibility and the robustness of the proposed approach with respect to noise in the gene expression is tested *in silico* using a simple, switched linear model of the osmostress response in yeast.

The paper is organized as follows. In section 2, we briefly present the control platform and the controlled system. This brief description of the experimental setup motivates important modeling and control assumptions made in the following section. In section 3, we detail the proposed control strategy. Then, we test its feasibility and robustness with respect to biological variability on two simple control problems. The last section summarizes our work and discusses results in the context of related work.

2. THE CONTROLLED SYSTEM AND THE CONTROL PLATFORM

The overall objective is to place the expression of a protein under the control of an external signal and to control this expression in a precise temporal manner. This necessitates signal transduction and gene expression. We have chosen to exploit a natural signal transduction and gene expression pathway: the High Osmolarity Glycerol (HOG) pathway in the yeast *Saccharomyces cerevisiae*.

2.1 Exploiting the natural osmostress response

The HOG pathway senses osmolar pressure changes in the environment. The activation of this pathway orchestrates cellular osmotic stress responses that maintain water homeostasis (Hohmann and Mager, 2003). More precisely, two osmo-sensor proteins (Sln1 and Sho1) transduce the signal to the Hog1 protein via a phosphoryla-

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tion cascade. Once phosphorylated, Hog1 triggers different osmo-adaptative responses that essentially favor the accumulation of glycerol in the cell, thus restoring the osmotic balance of the cell with its environment. Firstly, Hog1 regulates negatively Fps1 glycerol channels, thereby preventing glycerol export (Beese et al., 2009). Secondly, Hog1 directly activates enzymes involved in glycerol production (Westfall et al., 2008). Thirdly, Hog1 translocates into the nucleus and regulates, directly or indirectly, the expression of a large number of genes, including genes coding for glycerol producing enzymes (e.g. Gpd1; O'Rourke and Herskowitz, 2004). One can distinguish a short-term adaptation response, that rapidly promotes glycerol accumulation in the cell and a long-term adaptation response that results in preparing the cell to face prolonged periods of osmotic stress by triggering a large transcription program. Once the osmotic balance is restored, Hog1 is dephosphorylated and, in case of an excess in internal glycerol concentration, Fps1 channels reopen allowing glycerol to leak out (Beese et al., 2009). This pathway is schematically represented in Figure 1(left). For our application, we slightly modify the natural pathway. In addition to fusing Hog1 to a fluorescent marker that allows to detect its nuclear localization and hence quantify its activity, we fuse under the control of an osmo-responsive promoter the protein of interest, called X, to a second fluorescent marker that allows the quantification of the concentration of protein X.

Our motivation for using this pathway is threefold. Firstly, it has been extensively experimentally studied and quantitative models are available (Capaldi et al., 2008; Hersen et al., 2008; Klipp et al., 2005; Macia et al., 2009; Mettetal et al., 2008; Muzzey et al., 2009; Zi et al., 2010). Secondly, 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: we have experimentally access to an important variable (Ferrigno et al., 1998). 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 (Hersen et al., 2008). 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. This is important since the microfluidic device we use (see below) does not allow for mixing solutions, whereas our control approach uses a continuous input variable for the external osmolarity.

2.2 The integrated platform

Because in comparison to open loop control, closed loop control approaches are generally less sensitive to model uncertainties and can compensate for external disturbances, feedback control seems unavoidable for our application. However, feedback control implies a real-time requirement: *in vivo* measurements, image analysis, computation of control strategies, and actuation on the cell environment must all be integrated and performed faster than the typical response time of the system.

The control platform is represented in Figure 1(right). Time lapse fluorescent microscopy allows for monitoring the HOG pathway activity and gene expression at the single cell level. Using the micro-fluidic device developed by Hersen and colleagues, the cellular environment can be precisely controlled (2008). Not only this device allows a fast and well-controlled change of the cellular environment, but also, it guarantees that with the exception of its osmolarity the cell environment is otherwise held constant. In its current state, the platform integrates a microscope controller, a microfluidic pressure controller, and software for image analysis and PI control (Uhlendorf et al., 2010).

3. CONTROL APPROACH

We propose to use a model based control strategy. Therefore, we first present a model of the system. Then, we present and motivate the three main features of our control strategy: using a two-layer control, pulse modulation and a model predictive control approach. Lastly, we propose a control algorithm implementing the proposed approach.

3.1 Model developments

Many models of the yeast stress response have been developed (Hersen et al., 2008; Klipp et al., 2005; Mettetal et al., 2008; Muzzey et al., 2009; Zi et al., 2010). Because only a very limited number of variables can be measured simultaneously *in vivo* in single cells, and to simplify as much as possible parameter search, and even more importantly, state estimation problems, we have chosen to use a very simple four-variable switched linear model.

- if $osm_e \geq osm_i$: (hyperosmotic conditions)

$$\dot{osm}_i = \kappa_o hog - \gamma_o osm_i \quad (1)$$
$$\dot{hog} = \kappa_g(osm_e - osm_i) - \gamma_g hog \quad (2)$$
$$\dot{rna} = \kappa_m hog - \gamma_m rna \quad (3)$$
$$\dot{p} = \kappa_p rna - \gamma_p p \quad (4)$$

- if $osm_e < osm_i$: (hypoosmotic conditions)

$$\dot{osm}_i = \kappa_o hog - (\gamma_o + \gamma'_o) osm_i \quad (1')$$
$$\dot{hog} = -\gamma_g hog \quad (2')$$
$$\dot{rna} = \kappa_m hog - \gamma_m rna \quad (3)$$
$$\dot{p} = \kappa_p rna - \gamma_p p \quad (4)$$

In this model, osm_e, osm_i, hog, p, and rna represent respectively the external osmolarity, that is the input, the internal osmolarity whose variations essentially result from glycerol synthesis and degradation/export, the activity of the Hog1 protein, reflected by its nuclear localization, and the concentrations of the target protein and of its messenger RNA. Like in the Mettetal and Muzzey models (2008; 2009), we assume that Hog1 activation is proportional to the intensity of the hyperosmotic stress, $osm_e - osm_i$. Using results from Mettetal et al. (2008), we neglect the Hog1-independant glycerol synthesis and simply assume that glycerol synthesis is proportional to Hog1 activity. We assume that the Fps1 glycerol channels are either open or closed, in hypoosmotic or hyperosmotic conditions, respectively. The term $\gamma'_o\, osm_i$ corresponds to glycerol diffusion out of the cell through Fps1 channels (assuming a negligible external glycerol concentration), whereas the term $\gamma_o\, osm_i$ corresponds to glycerol degradation in the cell. It was expected that $\gamma'_o \gg \gamma_o$. When fitting model

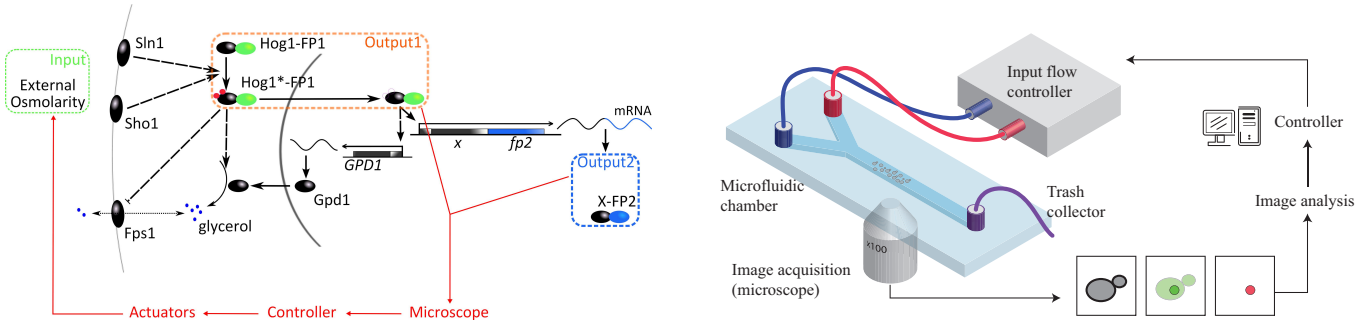

Fig. 1. **Left**: Schematic representation of the HOG pathway with natural and engineered feedbacks. Dashed arrows indicate indirect effects. For a detailed description, see the main text. FP1 and FP2 in the figure denote two different fluorescent proteins. **Right**: The integrated control platform. The main elements of the feedback loop are (i) a micro-fluidic 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.

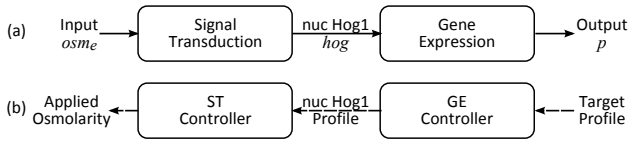

Fig. 2. **(a)** Decomposition of the osmotic stress response system into two subsystems: the relatively fast signal transduction system and the relatively slow gene expression system. Known and experimentally measurable quantities are represented. **(b)** Decomposition of the original control problem into two simpler control problems.

parameters to experimental data generated in our lab, we indeed found that $\gamma_o' \gg \gamma_o \approx 0$. For gene expression, we use a simple reaction rate model in which mRNA synthesis is proportional to the nuclear concentration of the regulator of gene expression Hog1, and protein synthesis is proportional to mRNA concentration. mRNA and protein degradations are proportional to their concentrations (see for example Wilkinson (2009) for a similar model). In both conditions, the first two equations represent signal transduction and (short term) cell adaptation, whereas the last two represent gene expression. Therefore, we will refer to the first, switched linear model (1)-(2) and (1')-(2') as the (fast) signal transduction model and to the second, linear model (3)-(4) as the (slow) gene expression model. This gives rise to the schematic representation given in Figure 2(a), where we decompose the full system in two subsystems: one responsible for signal transduction, and one responsible for gene expression.

For the signal transduction model, we selected parameter values that minimize the mean square deviation between model prediction and experimental data produced in our lab (Uhlendorf et al., 2010), using a global optimization tool, implementing a covariance matrix adaptation evolution strategy (CMA-ES; Hansen and Ostermeier 2001). For the gene expression model, we used realistic parameter values. More precisely, we used degradation parameters values that correspond to mRNA and protein half-lives of 5 and 20 minutes (typically corresponding to a protein fused to a destabilized CFP (Hackett et al., 2006)). For production parameters, we used values that yield, in presence of a prolonged gene activation (i.e. $hog = 25$ in our context), a mean protein number of 250 and a standard deviation of 10% if one uses the stochastic interpretation of our gene expression reaction rate model (Ghaemmaghami et al. (2003); Friedman et al. (2006); see for example Wilkinson (2009) for the correspondence between the ODE and the discrete stochastic interpretations of reaction rate models). Note that the mean value for the number of protein X is somewhat arbitrary, since our target profiles are defined relatively to this value.

3.2 Control strategy

The specific, 'cascaded' structure of our system and the possibility to observe the *hog* variable motivated us to decompose our original control problem into two simpler control problems (Figure 2b). Firstly, given the target protein profile, one looks for a desired Hog1 profile, considered as the input of the gene expression system. And secondly, given the desired Hog1 profile, considered this time as the output of the signal transduction system, one looks for external osmolarities to apply. In each case, only the model of the subsystem needs to be taken into account. This approach shares analogies with backstepping control strategies (Sepulchre et al., 1997).

Moreover, these two systems have different time scales. Indeed, response times for Hog1 signal transduction are typically in the order of one or 2 minutes, whereas response times for gene expression in yeast are much slower, typically on the order of 20 to 40 minutes. This allows us to develop a model predictive control strategy (Findeisen et al., 2007) in which the gene expression controller uses long-term predictions with large sampling times ($\approx$ 10 minutes), and the signal transduction controller uses short term predictions with high sampling times ($\approx$ 20 seconds).

A last important feature of the system is that cells adapt to osmotic stresses: natural negative feedback loops restore the cellular osmotic balance via glycerol production. For example, it has been experimentally demonstrated that to obtain a constant nuclear Hog1 concentration, and hence a constant protein synthesis rate, one has to apply a constantly increasing ('ramp') input (Muzzey et al., 2009). This fact, combined with the boundedness of the input implies that such strategies cannot be applied over long periods. Instead, we have experimentally shown - using a simple PI controler- that trapezoidal-like motifs of Hog1 nuclear localization can be repeatedly obtained,

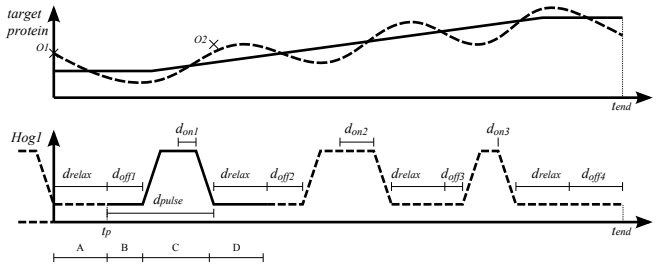

Fig. 3. Model predictive control for the GE controller. **(Bottom)** Hog1 profile with $n = 3$ motifs, defined by $d_{off} = (2, 2, 1, 4)$ and $d_{on} = (1, 2, 0)$ times. **(Top)** Corresponding protein profile (dotted line) with its reference profile (solid lines). This profile has been computed during period A, based on the observation $O1$. During periods B and D, no stress will applied ($osm_e = 0$), whereas in period C, the signal transduction controller will be used to apply osmotic stresses yielding the desired Hog1 profile. Based on measurement $O2$, the control strategy will be updated during period D. t_p denotes the start time of the current pulse.

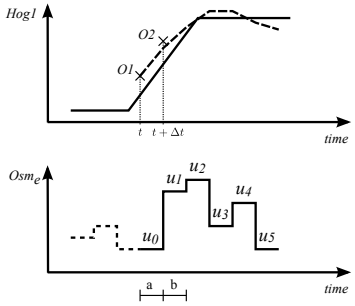

Fig. 4. Model predictive control for the ST controller. External osmolarity profile corresponding to $osm_e = (u_i)_{i\in[0,5]}$, computed during period a, based on observation $O1$. u_0 will be effectively applied during period b, and a new osmolarity profile will be computed based on observation $O2$.

provided that these motifs are separated by sufficient time ($\approx$ 5 minutes in our conditions, Uhlendorf et al. (2010)). Therefore, we adopted a control strategy based on pulse-modulated signals for gene expression (Gelig and Churilov, 1998). This pulse-modulated approach exploits the fact that in absence of stress, glycerol channels open and let glycerol leak out of the cell, which effectively 'resets' the cell to its initial state. More precisely, Hog1 profiles will be defined by the number n of trapezoidal motifs, and each motif will be defined by the durations of its *off* state, d_{off}, and of its *on* state, d_{on}, as represented in Figure 3. To these times, we add fixed times for increase and decrease, and minimal durations of the *off* plateau, d_{relax}, and of the *on* plateau. For our computational studies, these fixed durations equal 2, 2, 5 and 1 minute, respectively. We also require that all d_{on} durations last less than 6 minutes to limit cell adaptation problems.

To summarize, we will develop a gene expression controller that, given a target protein profile, computes a desired pulse-modulated nuclear Hog1 profile (Figure 3), and a signal transduction controler that computes the osmolarity to apply to get the desired nuclear Hog1 profile (Figure 4).

3.3 Control algorithm

In this section, we propose a control algorithm implementing the control strategy presented above. The algorithm exploits two functions, searchHogProfile and searchOsmProfile, that we introduce first.

For the gene expression controller, a control strategy is defined by a nuclear Hog1 profile, that is, by the number of peaks n and the *on* and *off* durations of the peaks, $d_{on} \in \mathbb{R}^n_{\geq 0}$ and $d_{off} \in \mathbb{R}^{n+1}_{\geq 0}$, using the notations introduced in Figure 3. Then, given the target protein profile on a given time horizon (typically 100 minutes), the best control strategy at time t is the one that minimizes the mean square distance between the target protein profile and the protein profile that one obtains when applying the Hog1 profile on the input of subsystem 2, during the given time horizon. Good -although not necessarily optimal- solutions can be obtained by using global optimization tools that search for the best parameters n, d_{on} and d_{off}. This is implemented by the function $[n, d_{on}, d_{off}] = \text{searchHogProfile}(t, s_{ge}, target)$, with t the current time, s_{ge} the state of the gene expression subsystem at time t, and *target* the target protein profile. In practice, we consider a limited set of possible values for n, and for each value, we search for d_{on} and d_{off} parameters. We assume that these computations are executed in parallel, e.g. using a multicore processor.

For the signal transduction controller, a control strategy on the time horizon $[t, t + m\,\Delta t[$ is defined by m external osmolarity values $u = (u_1, \ldots, u_m) \in [0, 1]^m$. Given a desired nuclear Hog1 profile, the best control strategy is the one that minimizes the mean square error between the nuclear Hog1 profile obtained by applying the u_i osmolarities, each during Δt, and the desired nuclear Hog1 values, on the time interval $[t, t + m\,\Delta t[$. Again in practice, good solutions can be obtained by using global optimization tools that search for the best parameters $u_1, \ldots, u_m$. This is implemented by the function $u = \text{searchOsmProfile}(t, s_{st}, t_p, n, d_{on}, d_{off})$, with t the current time, s_{st} the state of the signal transduction subsystem at time t, and t_p, n, d_{on}, d_{off} defining the desired Hog1 profile, t_p being the start time of the first pulse.

Having defined the two functions searchHogProfile and searchOsmProfile, we can now describe our main algorithm (Algorithm 1). Note that the real-time requirement implies that measurements, image analysis, state estimation, and control strategy updates are terminated before the alloted time (d_{relax} for GE control and Δt for ST control).

4. TESTING THE CONTROL APPROACH ON SIMULATED DATA

Because testing control approaches on real cells is very time consuming, we first test the proposed approach on a simulated system. We present here these computational results.

We define the following two control problems that might be relevant for various applications.

- Problem 1: maintaining the concentration of protein X at a given level
- Problem 2: obtaining a sine wave temporal profile for the concentration of protein X

Control Algorithm 1 Each iteration in the external *while* loop corresponds to applying one pulse and computing the next one, and each iteration in the internal *while* loop corresponds to applying an osmotic stress during a small Δt and computing the next one. Tasks performed in a synchronous manner are represented by a || sign. t denotes the current time.

// initialization prior to control experiment, using known parameters *target* and s^0_{ge}.
$[n, d_{on}, d_{off}] = \text{SEARCHHOGPROFILE}(0, s^0_{ge}, target)$
// control experiment starts
while $n \neq 0$ **do**
  $t_p = t$
  // B: no stress during $[t_p, t_p + d_{off1}[$
  $u_1 = 0$; **apply** u_1; **wait for** d_{off1};
  // C: active control during time interval $[t_p + d_{off1}, t_p + d_{pulse}]$
  while $t + \Delta t < t_p + d_{pulse}$ **do**
    apply u_1; **wait for** Δt || **measure** s_{st}; $[u_1, \ldots, u_m] = \text{SEARCHOSMPROFILE}(t, s_{st}, t_p, n, d_{on}, d_{off})$
  end while
  // D: no stress and global strategy update during time interval $[t_p + d_{pulse}, t_p + d_{pulse} + d_{relax}]$
  apply 0; **wait for** d_{relax} || **measure** s_{ge}; $[n, d_{on}, d_{off}] = \text{SEARCHHOGPROFILE}(t, s_{ge}, target)$
end while
apply $u = 0$; **wait for** d_{off1}
// control experiment ends with $t = t_{end}$

Solving the first problem *in vivo* would allow us to study the effect of well-controlled, steady perturbations, whereas solving the second control problem would allow us to study signal processing capabilities of gene networks, as it was done recently for signal transduction cascades (Hersen et al., 2008; Mettetal et al., 2008).

To test the robustness of our control approach with respect to the large variability of biological processes, we use a stochastic interpretation of the gene expression reaction rate model, and the Gillespie algorithm for the simulation of system behavior (Wilkinson, 2009). For the sake of simplicity, we ignore observation and state estimation problems. For real-life applications, these two issues also need to be appropriately solved.

In Figure 5, we present simulated results corresponding to control experiments for Problem 1 and 2, and using either a deterministic or a stochastic model for gene expression. For the stochastic cases, the examples provided are representative of typical results in terms of mean square deviation with respect to target profiles. More precisely, the root mean square (rms) distance for the solutions shown for Problem 1 and 2 are respectively 10.6 and 8.8, while the mean rms over 10 runs is respectively 11.6 and 8.5.

These results demonstrate the feasibility of the pulse modulated control strategy proposed, and shows its robustness with respect to large biological variabilities, since for both problems, the concentration of the protein under control remains within admissible bounds around its target value. Indeed, even if these results seem unimpressive for traditional control problems, obtaining simular results *in vivo* would be a genuine tour de force.

Moreover, the proposed computation procedure, relying on global optimization to implement the MPC scheme, conforms with our real-time requirement, in the sense that, for each iteration, the computational times of the SEARCHOSMPROFILE and SEARCHHOGPROFILE procedures is less than 300 and 20 seconds, respectively.

5. DISCUSSION

In this work, we have presented a specific control problem: controlling *in vivo* the expression of a protein such that its cellular concentration follows a target temporal profile. This control problem is directly motivated by current research in our lab. We have also proposed a control strategy tailored to the specificities of our biological application. It's main ingredients are the use of a two-layer model predictive control approach and of a pulse-modulated strategy for the control of gene expression. Using a simple switched linear model of the system, we have shown that the proposed approach satisfies our real-time requirements and is robust with respect to the significant noisiness of gene expression processes.

To the best of our knowledge, the specific problem of the precise control of the expression of a single gene has not been previously studied. However, several approaches have been developed for the control of gene networks. These approaches differ notably by the class of models that is considered. One can notably mention the works of Farcot and Gouzé (2008) and of Edwards et al. (2010), developed for qualitative piecewise affine models, of Datta et al. (2007), for probabilistic Boolean models, of Yu et al. (2010) for Sum models, and of Tumova et al. (2010), for piecewise affine models. Although we use here a simple switched linear model (actually being in the class studied by Tumova et al. (2010)), our MPC approach does not exploit any specific mathematical property of the model. Because the predictive power of our model will be essential to get good performance for the control of the real biological system, being not bound to any specific mathematical formalism is likely to be a significant asset. Further works naturally involve applying the proposed framework to the control of the actual biological system. Iterations between experiments, model developments and control strategy improvements will likely be needed.

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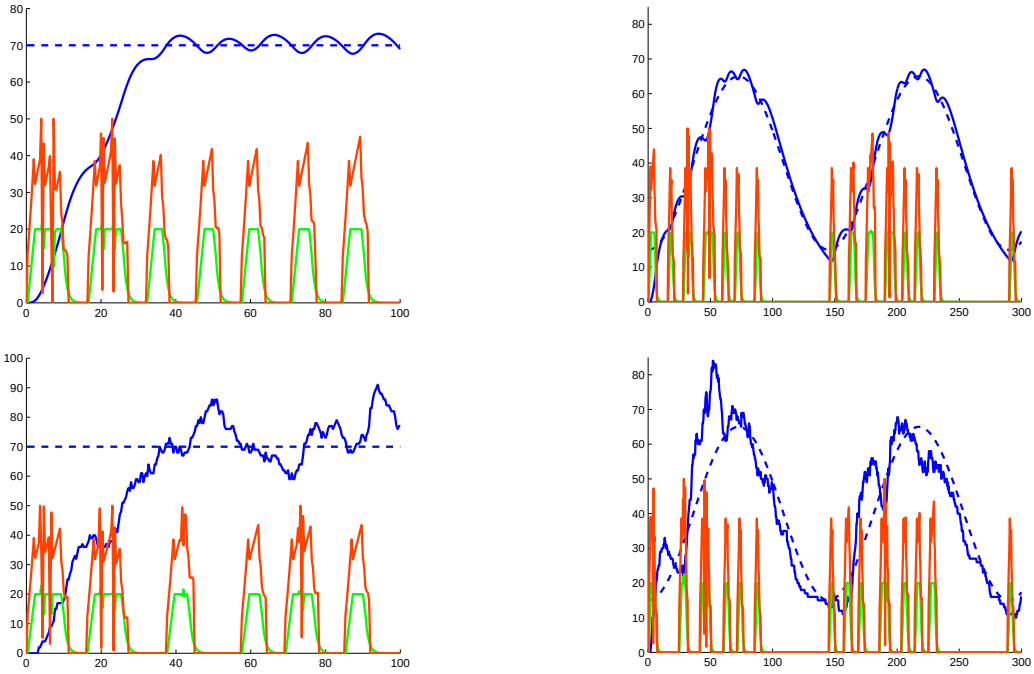

Fig. 5. Simulated control experiments. In all plots, dotted blue, orange, green, and solid blue represent the target protein profile, the applied osmolarity (x50), the nuclear concentration of Hog1, and the concentration of the X protein. System behavior is either computed using a deterministic (ODE, top) or a stochastic (Gillespie, bottom) model. Time is in minutes.

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