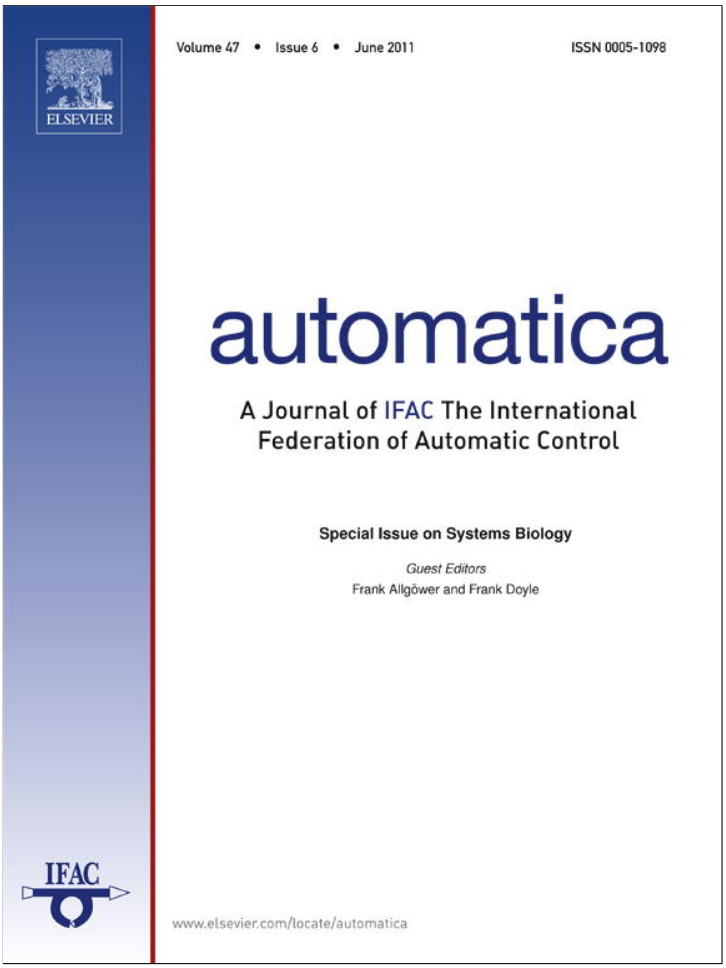

Automatica 47 (2011) 1265–1270

Contents lists available at ScienceDirect

Automatica

journal homepage: www.elsevier.com/locate/automatica

Brief paper

Analysis, design and implementation of a novel scheme for in-vivo control of synthetic gene regulatory networks☆

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ARTICLE INFO

Article history:
Received 8 February 2010
Received in revised form
27 July 2010
Accepted 13 December 2010
Available online 8 April 2011

Keywords:
in vivo control
Gene regulatory networks
Synthetic biology
Microfluidics

ABSTRACT

This paper is concerned with the design and implementation of a simple but effective switching control strategy to regulate the dynamics of synthetic gene networks. The testbed circuit is IRMA, a recently developed network in *S. Cerevisiae* which is proposed as a suitable benchmark problem for control design. The proof-of-concept of an implementation of the control strategy in vivo is presented which is based on the use of microfluidics devices and fluorescence microscopy. Preliminary experimental results are given showing the good matching between the model predictions and the experimental observations.

1. Introduction

Recent advances in synthetic biology have allowed engineering of genetic circuits with a specific desired function. Examples include genetic oscillators and switches (see Mukherji and van Oudenaarden (2009) for a comprehensive review on biosynthetic circuits) and the many novel designs presented every year at the International Genetically Engineered Machine competition (IGEM) at MIT (Goodman, 2008). So far, most of the effort has been devoted to finding effective methods for reverse engineering and modelling of biological circuits (Bansal, Belcastro, Ambesi-Impiombato, & Di Bernardo, 2007). Controlling gene regulatory networks can be a fundamental step for their use in the synthesis of therapeutic strategies as, for example, to restore the correct secretion of insulin from pancreatic cells or the severe phase lag observed in the circadian rhythms of some patients (Bagheri, Stelling, & Doyle, 2008). Some applications have been presented in Dougherty, Pal, Qian, Bittner, and Datta (2010) but a comprehensive approach to the problem of gene network control is still missing. Moreover the implementability of these approaches in a real experimental setting has not been considered so far. Here we propose an integrated control strategy for a novel synthetic network, IRMA (Fig. 1), developed in yeast *S. Cerevisiae*. IRMA (In-vivo assessment of Reverse-engineering and Modelling Approaches) was presented in Cantone et al. (2009), as a testbed synthetic network for the design and validation of reverse engineering and modelling approaches. Specifically we use IRMA for the design and in-silico validation of a novel switched control system to steer the dynamics of its gene products concentrations in a desired manner. We will propose that, taking into account the many technological and biological constraints, ON/OFF control techniques can be successfully used to control gene networks. We will then discuss the major open challenges for the in-vivo implementation of such control laws on living cells and present and validate an in-silico possible solution.

2. A synthetic benchmark for control strategies in biology: IRMA

As mentioned above, IRMA is a synthetic gene network built in yeast *S. Cerevisiae*. It consists of five genes, CBF1, ASH1, SWI5, GAL4 and GAL80 and its topology comprises both transcriptional and protein–protein regulation mechanisms. A detailed discussion of its biological implementation can be found in Cantone et al. (2009). Fig. 1 shows a schematic diagram of the activation/repression links between the five genes. Note that the only external input is the possible presence of galactose (GAL) in the medium whose presence prevented GAL80 from binding GAL4 thereby allowing the latter to activate the GAL10 promoter driving SWI5 transcription. To capture the dynamics of the network the following set of nonlinear

☆ This paper was not presented at any IFAC meeting. This paper was recommended for publication in revised form by Associate Editor Graziano Chesi under the direction of Editor Francis J. Doyle III.

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doi:10.1016/j.automatica.2011.01.073

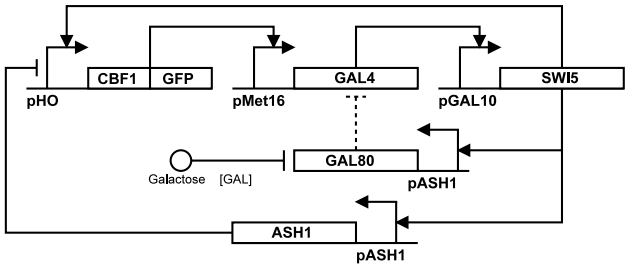


Fig. 1. IRMA schematic circuit. Solid lines model transcriptional interactions while dashed lines are meant to represent protein–protein interactions. (See Cantone et al. (2009) for further details.)

DDEs was presented in Cantone et al. (2009) and is readapted here to the specific technological platform we designed:

$$\frac{dx_1}{dt} = \alpha_1 + v_1 \left(\frac{x_3^{h_1}(t-\tau)}{(k_1^{h_1} + x_3^{h_1}(t-\tau)) \cdot \left(1 + \frac{x_5^{h_2}}{k_2^{h_2}}\right)} \right) - d_1 x_1 \quad (1a)$$

$$\frac{dx_2}{dt} = \alpha_2 + v_2 \left(\frac{x_1^{h_3}}{k_3^{h_3} + x_1^{h_3}} \right) - d_2 x_2 \quad (1b)$$

$$\frac{dx_3}{dt} = \alpha_3 + \hat{v}_3 \left(\frac{x_2^{h_4}}{\hat{k}_4^{h_4} + x_2^{h_4}\left(1 + \frac{x_4^4}{\hat{\gamma}^4}\right)} \right) - d_3 x_3 \quad (1c)$$

$$\frac{dx_4}{dt} = \alpha_4 + v_4 \left(\frac{x_3^{h_5}}{k_5^{h_5} + x_3^{h_5}} \right) - d_4 x_4 \quad (1d)$$

$$\frac{dx_5}{dt} = \alpha_5 + v_5 \left(\frac{x_3^{h_6}}{k_6^{h_6} + x_3^{h_6}} \right) - d_5 x_5 \quad (1e)$$

where $x_1 = [CBF1], x_2 = [GAL4], x_3 = [SWI5], x_4 = [GAL80], x_5 = [ASH1]$ are the system states, all regulations are modelled by Hill functions and the multiple regulation on CBF1 is modelled by a product (AND regulation). A time delay τ is present in the equation for x_1 modelling the transcription of the CBF1 gene which is affected by a 100 min-long time delay due to the sequential recruitment of chromatin-modifying complexes to the HO promoter (which follows binding of SWI5 and other transcription factors) (Cosma, Tanaka, & Nasmyth, 1999). A list of all model parameters can be found in Table S2 in Cantone et al. (2009). Note that some parameters in the model, $\hat{v}_3$, $\hat{k}_4$ and $\hat{\gamma}$, are dependent on whether cells are fed with galactose (inducer compound) or glucose (inert compound).

3. A synthetic testbed for gene network control

We can view IRMA as an input–output system where the input u models the presence of galactose and the output is the concentration of one of its genes, for example CBF1 (x_1). Moreover, galactose can either be provided to the cells or not, therefore the control input (interpreted as percentage values of the inducer compound in the total volume of fluid reaching cells) is restricted to be either ON ($u \neq 0$) or OFF ($u = 0$). The system output $y = x_1$ cannot be measured directly as a concentration. Instead, the cells were synthetically engineered so that CBF1 is fused with GFP, the green fluorescent protein. In this way, higher concentrations of CBF1 are associated to higher levels of fluorescence (Gordon et al., 2007). It should be noted that in our control strategy we are assuming similar dynamics for both the CBF1 and GFP proteins. (Other options are also available in the literature where, for example, GFP evolution is modelled by one or two additional ODEs (Tigges, Marquez-Lago, Stelling, & Fussenegger, 2009)). From a control perspective, the IRMA network is, therefore, a highly nonlinear, hybrid, time-delayed dynamical system.

4. Switched control design

We now move to control design. The aim is to regulate the amount of one of the gene products in the network, namely CBF1, to some desired value $x_{1d}(t)$ by modulating, in a feedback manner, the control input $u(t)$ (the amount of galactose being fed to the network). It would be possible at this stage to consider the most sophisticated control laws but the challenge when dealing with biological systems is the presence of many unavoidable constraints, high levels of noise, uncertainties and unmodelled dynamics. Cells are living organisms and they resist external actions very effectively. The main design constraints are: (1) currently it is only possible to either feed or not galactose to cells but it is difficult to modulate in real time its exact concentration levels as cells will tend to consume glucose since it is their primary carbon source and they obtain energy from it at a lower energetical cost (Willey, Sherwood, & Woolverton, 2007). Moreover GLU genes repress GAL genes as outlined in Bennett et al. (2008); (2) we can only observe the output via the GFP fluorescence; (3) the control action should be robust to parameter variations (cells differ from each other), noise and external disturbances. Looking at the above set of constraints, it is impossible to avoid the analogy with the problems faced in the early design of feedback control strategies for power electronic circuits (Kassakian, Schlecht, & Verghese, 1991). The simplest and most successful control technique used in this context is PWM (Pulse Width Modulation) control. In what follows, we wish to propose that PWM can be an effective and viable strategy to control biological circuits and systems. (For details on PWM see Kassakian et al. (1991).)

4.1. Design and parameter tuning

Let $\eta(t) = \alpha + \beta(t \mod T)$ be a sawtooth signal and say $e(t) = x_1(t) - x_{1d}(t)$ the control error; then $u(t) = 0$ if $\eta(t) - \varphi(e(t), t) > 0$ and $u(t) = U$ otherwise, where $\varphi(e(t), t)$ is some appropriately chosen function of the control error (that will be specified later) and U is the available non-zero level of the galactose concentration. We now have to choose the control parameters and the modulating signal $\varphi(e(t), t)$. To control IRMA, we chose $\alpha = 0$, $\beta = 10E-5$ and $T = 10$ min, so that the amplitude of the sawtooth wave is 10% of the CBF1 level at the steady state in glucose and the period is twice the settling time of CBF1. Moreover, as is commonly done in control of power systems, to avoid multiple pulsing an SR flip–flop controlled by a set signal (fired at the beginning of each period) and reset by the following switch off is virtually added to the control logic (Kassakian et al., 1991). The behaviour of the theoretical model of IRMA controlled by the PWM action is shown in Fig. 2. We notice that, as expected, the control performance is poor with wild fluctuations about the reference signal. Indeed, the model of IRMA is highly nonlinear and, more notably, presents a time-delay on the CBF1 dynamics. In order to directly compensate the presence of delays, a classical approach is to use a Smith Predictor (Smith, 1957). This simple but effective technique has been recently proposed to design controllers in highly uncertain environments as, for instance, in congestion control of communication networks (see Mascolo, Cavendish, and Gerla (1997) for further details). The Smith predictor schematic shown in Fig. 3 and described in Smith (1957) consists of an approximate linearised version of the plant model $G_P(s)$ and a linear filter block F (in our scheme this block has a transfer function with a 20 min time constant chosen in agreement with Smith (1957) to capture low-frequency disturbances). The control performance, omitted here for the

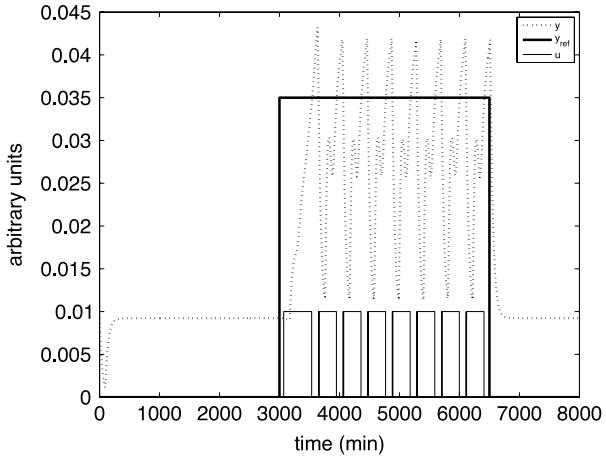


Fig. 2. Setpoint regulation in the presence of PWM controller. The setpoint signal (y_{ref}) is represented with a thicker solid line while the actual system output (y) is reported in a dotted line. The solid thinner signal is a scaled version (1:10) of the control input (u).

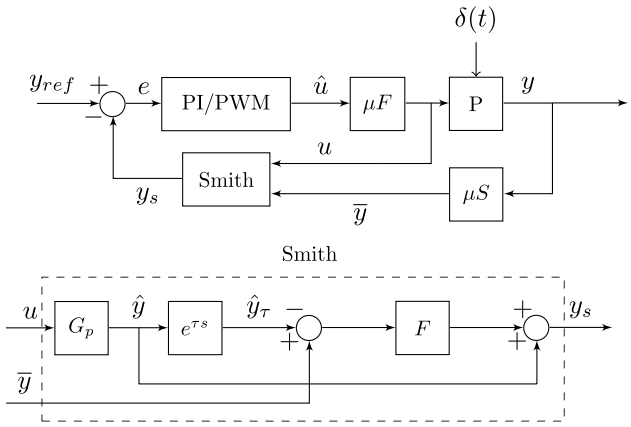


Fig. 3. Proposed control schematic. The upper block diagram represents the whole control scheme documented in this work while the lower block magnifies the Smith predictor control block referred to in the previous schematic.

sake of brevity (see Supplementary material for more details), shows a definite suppression of the output oscillatory performance when the Smith predictor is present but exhibits some non-zero asymptotic tracking error due to the presence of nonlinear terms and unmodelled dynamics. To better compensate the nonlinear terms, inspired again from power electronics, we then choose φ as the output of a PI control law on the error $e(t)$, with the gains empirically set to $K_P = 1$ and $K_I = 3$. These gains where found iteratively by means of a classical trial-and-error approach driven by a gradient-like estimation of the variation of the integral squared error (ISE). The final theoretical control scheme is shown in Fig. 3 where the IRMA network (the "plant") is labelled as P. Note that the figure also contains a microfluidics actuator block (μF) and one for the fluorescence microscopy sensor (μS) which will be taken as ideal to start with and then appropriately modelled in Section 5.2. The performance of this strategy is depicted in Fig. 4. (Note that the control input is set to zero whenever the set-point value is smaller than the basal transcriptional level of CBF1 to avoid unwanted phenomena for low values of the concentration of CBF1). Finally, to test the robustness of this approach, we perturbed off-line randomly, five parameters (namely k_4, α_2, v_1, d_3, h_4) of the model by adding to each a random offset with zero mean and 10% of the nominal value as standard deviation. The resulting control performance is found to be still acceptable despite the uncertainties and is omitted here for the sake of brevity.

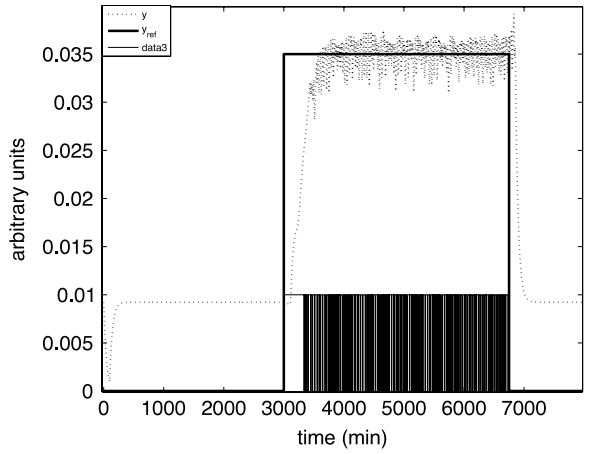


Fig. 4. Setpoint regulation performance when the PWM is complemented with a Smith Predictor and a PI controller.

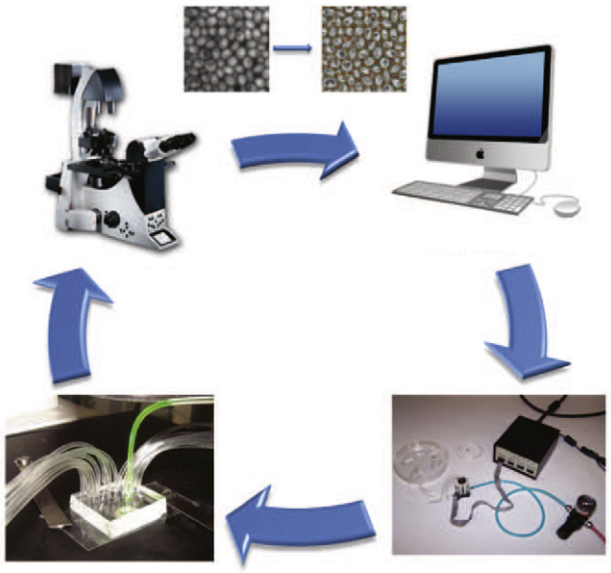


Fig. 5. Overview of the platform designed for the implementation of control experiments.

5. Virtual in-vivo implementation via microfluidics

In this section, we propose a novel approach to implement in-vivo the PWM control action introduced earlier. A general schematic representation of the main approach illustrating the integration of microfluidics, microscopy and the living system under control is shown in Fig. 5. This is an alternative approach to the common flask based experiments allowing (a) much higher flexibility in terms of the modulation of the inducer/inert compound concentration sensed by the cells; (b) considerable saving in terms of the running costs due to the very low volumes (in the order of magnitude of μL of media per experiment) and (c) both single cell and/or population experiments (Gulati et al., 2009). The key idea is to use microfluidic devices to overcome the unavoidable limitations of macro-scale reactors that would make the switching between two media (galactose and glucose) too complicated (involving washing and filtration steps that can require up to several minutes). Real-time microscopy is used to sense the fluorescence of the cells.

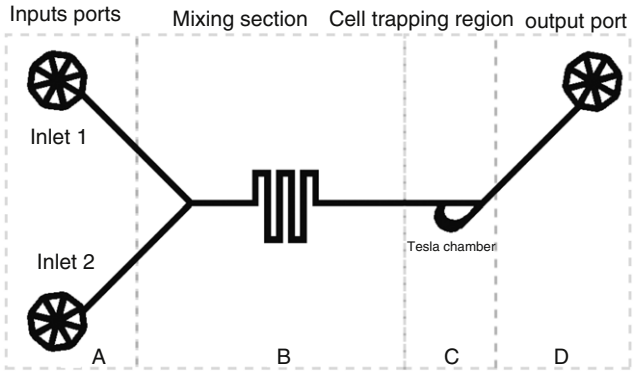


Fig. 6. Overview of the device designed to perform the control experiments outlined above. The four sections of the device are highlighted: the input ports (A) are used to supply the two media supplemented either with glucose or galactose. The mixing section (B) allows complete mixing of the two streams coming from the two inlets while the cell trapping region features the Tesla chamber (C) (Bennett et al., 2008) that allows cell trapping to happen by deriving a secondary flow from the main stream. Finally, media are extracted from the device through the output port (D).

5.1. Microfluidic device design

In order to design a suitable device we need: (i) a mixing strategy to obtain a uniform solution of both the compounds at the inlet; (ii) a trapping region where cells are supposed to be affected by the feedback control strategy; (iii) an appropriate network of channels and chambers to easily accomplish both cell loading and cell feeding. The device we propose here, named "Yeast Controller" is presented and described in Fig. 6. The specific configuration of the Tesla chamber where cells are trapped allows a dramatic flow speed decrease at the channel split downstream from the mixing section as it can be seen in Fig. 7(e). A finite element model was numerically implemented in COMSOL Multiphysics 3.5a (Comsol AB, Stockholm) to simulate both the feeding of the inducer compound to the cells and their loading in the device. To feed the cells a pressure equal to 105 KPa is applied to "Inlet 1" in the case of Fig. 7(a) and (b), whereas the "Inlet 2", as well as the outlet, are kept at 100 KPa. In this way a 100% galactose-to-glucose mixing ratio ($u = U$) is obtained. These two pressures are generated by a programmable micropump (KDScientific, US). A 0% mixing ratio ($U = 0$) is instead obtained by reversing these pressures from "Inlet 1" to "Inlet 2" and vice versa (see Fig. 7(c) and (d)). In Fig. 7(e) a simulation of the cell trapping is presented. Cells are simulated as massless particles (represented by white dots in Fig. 7(e)). As it can be seen in Fig. 7(f), a strong agreement between in-silico simulations and experimental results is observed. This confirms that finite element models can be usefully employed to predict the dynamic behaviour of the fluids in the device we designed.

5.2. In-silico validation

In order to validate in-silico the proposed scheme, the first step is to obtain an appropriate model of all of its technological

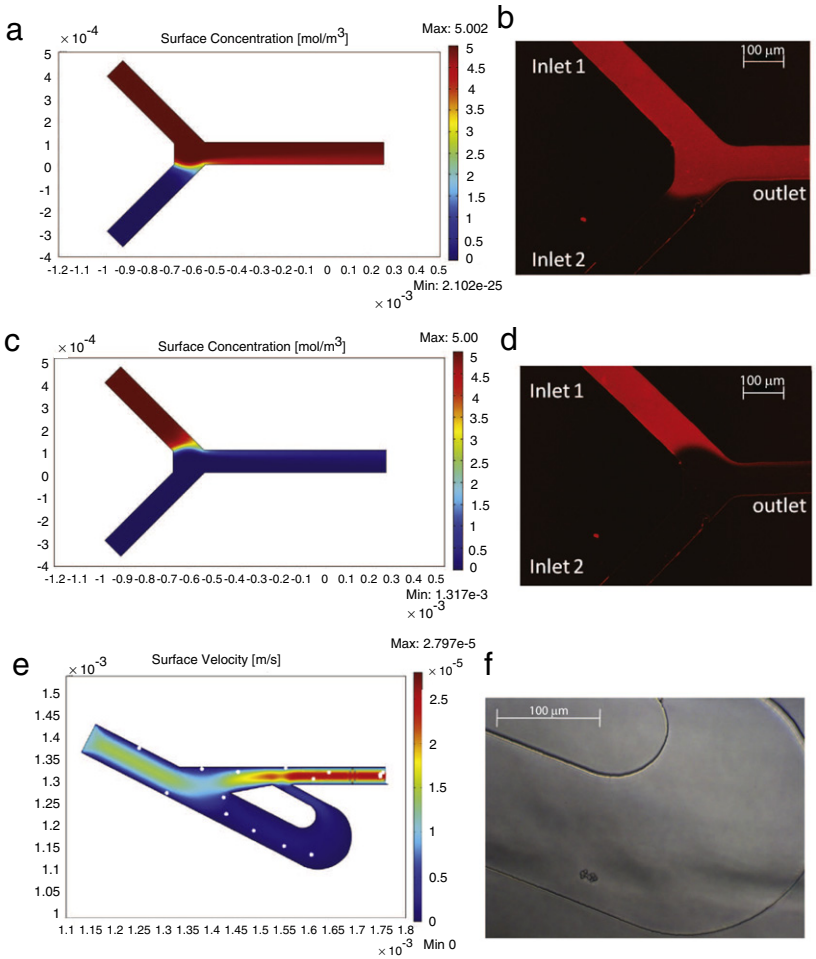
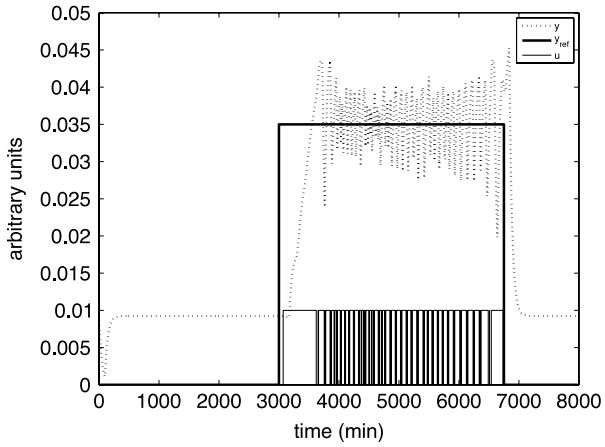
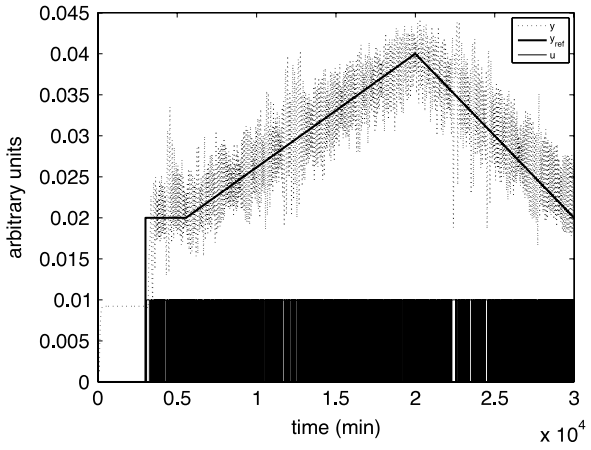


Fig. 7. Computational fluid dynamic simulations. Finite elements model predictions and experimental validation are presented side-by-side for the cases of 0% and 100% galactose (supplemented in this experiment with a fluorescent dye, Sulforhodamine 101) to glucose ratio.



(a) Setpoint control experiment.



(b) Signal tracking control experiment.

Fig. 8. Closed loop simulations of the control algorithm. The signal y_{ref} is the setpoint we want our system to force its fluorescently tagged protein time evolution to adapt to. The signal u is the scaled version (1:10) of the modulated control input while y is the evolution of the output signal (CBF1-GFP protein concentration).

components (see Fig. 5), including the microfluidics circuit, the microscopy-based sensing device and the biological actuators. The resulting in-silico control scheme, has been implemented in MATLAB/Simulink. We assume the control signal $\hat{u}$ which is mapped to a pressure level for both the reservoirs connected to the device and then transduced by the microfluidics in the actual input signal u. We considered $u = 1$ when the galactose concentration $[Gal]$ computed in the Tesla chamber met the condition $[Gal] \geq 2\%$ and $u = 0$ otherwise. To obtain a dynamical model of the microfluidic device we extracted a linearised model from the finite element simulator. This model was exported by using a specific function of COMSOL Multiphysics 3.5a. The linearisation was carried out on the steady state flow of the fluid being characterised by a 100 μm s^{-1} fluid speed in the main channels and 1 μm s^{-1} in the Tesla chamber. These conditions were chosen in order to prevent any effect of shear and mechanical stress on the cells. A transfer function of order 100 was found heuristically to give a good approximation of the microfluidics device. To model the transducer device based on the use of microscopy and image segmentation we followed the approach discussed in Gordon et al. (2007) and modelled the device as a static filter (taking the form $\bar{y} = ky + c$ where y is the actual amount of proteins to be estimated, $\bar{y}$ is the amount of fluorescence in arbitrary units k and c parameters usually depend on the electro–optical properties of the microscope used (for our microscope we estimated them to be $k = 0.1325$ and $c = -0.078$)). In-silico regulation and a tracking experiment are shown in Fig. 8(a) and (b). We notice that despite the presence of lags and noise due to the presence of microfluidics channels and chambers, the static filter associated to fluorescence transducers and the uncertainty on the cell parameters, the control performance matches closely the one predicted in the theoretical control scheme. Note that the frequency of the switching between the two media is within the admissible physical range of the actuator. Finally, a preliminary experimental result is shown in Fig. 9, where the response of the (open-loop) IRMA network in-vivo is compared with that predicted by the in-silico model when a square-wave galactose input is fed to the cells. In this case, cells were fed with galactose for 180 min, then glucose for an extra 180 min and finally galactose was administered again till the end of the experiment. Three images were taken at intervals of 5 min. The bright field image was used to segment cells while fluorescent fields for both the green and red spectra were acquired to quantify the amount of CBF1-GFP produced by the cells and the galactose concentration. (Sulforhodamine 101 (Sigma-Aldrich, Germany) has been added to galactose at the concentration of 1 μg L^{-1}.) Fluorescence was

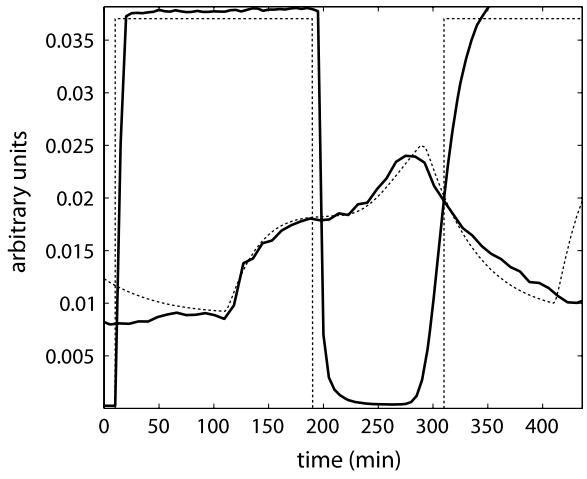


Fig. 9. Preliminary experimental result. The simulated input (thinner dashed-line square wave) and quantified galactose concentration (thicker solid-line square wave) are plotted against the experimental GFP (thicker solid line) and simulated CBF1-GFP (thinner dashed line). Clogging due to cell growth after time $t = 350$ min partially disrupted the galactose concentration control implemented in the platform.

quantified by a segmentation algorithm written in MATLAB and aimed at finding clusters of yeast cells to compute the mean fluorescence over the whole yeast floccule. The experimental results further confirm the viability of the proposed model to capture the experimental observations.

6. Conclusions

We proposed an integrated experimental and theoretical strategy to control the behaviour of gene regulatory network in a living cell. Our strategy is flexible and can thus be easily extended to include other control algorithms. Nevertheless, many issues remain open both in terms of model uncertainty and parametric variability: in order to address these problems, adaptive and robust control strategies are required. Despite these limitations, this work constitutes the first experimentally feasible strategy to control gene networks. In-silico results provide evidence for the success of the proposed approach in a real-case scenario. An open-loop preliminary experimental result is shown to further validate the viability of the in-silico modelling procedure. Ongoing work is aimed at achieving a full experimental implementation

and validation of the proposed technique which will be presented elsewhere.

References

Bagheri, N., Stelling, J., & Doyle, F. J. (2008). Circadian phase resetting via single and multiple control targets. *PLoS Computational Biology*, *4*(7).

Bansal, M., Belcastro, V., Ambesi-Impiombato, A., & Di Bernardo, D. (2007). How to infer gene networks from expression profiles. *Mol. Syst. Biol.*, *3*(February).

Bennett, M. R., Pang, W. L., Ostroff, N. A., Baumgartner, B. L., Nayak, S., Tsimring, L. S., & Hasty, J. (2008). Metabolic gene regulation in a dynamically changing environment. *Nature*, *454*(7208), 1119–1122.

Cantone, I., Marucci, L., Iorio, F., Ricci, M. A., Belcastro, V., Bansal, M., Santini, S., di Bernardo, M., di Bernardo, D., & Cosma, M. P. (2009). A yeast synthetic network for in vivo assessment of reverse-engineering and modeling approaches. *Cell*, *137*(1), 172–181.

Cosma, M. P., Tanaka, T., & Nasmyth, K. (1999). Ordered recruitment of transcription and chromatin remodeling factors to a cell cycle and developmentally regulated promoter. *Cell*, *97*(3), 299–311.

Dougherty, E. R., Pal, R., Qian, X., Bittner, M. L., & Datta, A. (2010). Stationary and structural control in gene regulatory networks: basic concepts. *International Journal of Systems Science*, *41*(1), 5.

Goodman, C. (2008). Engineering ingenuity at iGEM. *Nat. Chem. Biol.*, *4*(1), 13.

Gordon, A., Colman-Lerner, A., Chin, T. E., Benjamin, K. R., Yu, R. C., & Brent, R. (2007). Single-cell quantification of molecules and rates using open-source microscope-based cytometry. *Nature Methods*, *4*(2), 175–181.

Gulati, S., Rouilly, V., Niu, X., Chappell, J., Kitney, R. I., Edel, J. B., Freemont, P. S., & deMello, A. J. (2009). Opportunities for microfluidic technologies in synthetic biology. *Journal of the Royal Society, Interface/the Royal Society*, *6*(Suppl. 4), S493–S506.

Kassakian, J. G., Schlecht, M. F., & Verghese, G. C. (1991). *Principles of power electronics* (facsimile ed.). Prentice Hall.

Mascolo, S., Cavendish, D., & Gerla, M. (1997). ATM rate-based congestion control using a smith predictor. *Performance Evaluation*, *31*(1–2), 51–65.

Mukherji, S., & van Oudenaarden, A. (2009). Synthetic biology: understanding biological design from synthetic circuits. *Nature Reviews Genetics*, *10*(12), 859–871.

Smith, O. J. M. (1957). Closed control of loops with dead time. *Chemical Engineering Progress*, *57*, 217–219.

Supplementary material. http://dibernardo.tigem.it/menolascina/automatica10_sup_mat.doc.

Tigges, M., Marquez-Lago, T. T., Stelling, J., & Fussenegger, M. (2009). A tunable synthetic mammalian oscillator. *Nature*, *457*(7227), 309–312.

Willey, J., Sherwood, L., & Woolverton, C. (2007). *Microbiology* (7 ed.). McGraw-Hill.

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