PhD in Information Technology and Electrical Engineering

Università degli Studi di Napoli Federico II

PhD Student: Lorena Postiglione

XXIX Cycle

Training and Research Activities Report – Third Year

Tutor: Diego di Bernardo

Add the following items according to our meeting we have.

Concerning the structure of the document, use the Section number as is. Use the sub-contents indicated with a letter only as a suggestion for your content (a free form text is preferable)

1. Information

Name: Lorena
Surname: Postiglione
Education: MS In Biomedical Engineering – UniversitàdegliStudi di Napoli Federico II
PhD course: XXIX Cycle- ITEE – Università di Napoli Federico II
Fellowship type: MIUR
Tutor: Prof. Diego di Bernardo

2. Study and Training activities

External courses (Telethon Institute of Genetics and Medicine)

Title: Science Communication
Professor: PhD. G. Diez Roux and PhD. P. Cormio
Place and Date: Napoli, Aprile 12-15 2016
Credits: 1

External seminars (Telethon Institute of Genetics and Medicine)

Title and professor	Date	Credits
"In vivo imaging of adaptive immune responses", PhD. Matteo Iannacone	01/03/16	0.2
"One protein complex, three diseases, and all began at Tigem",Prof. Giorgio Casari	08/03/16	0.2
"Investigate the molecular basis of cell fate decisions through functional genomics", Dr. Davide Chiacchiarelli	15/03/2016	0.2
"Programming and reprogramming biological networks", PhD. Lucia Marucci	18/03/2016	0.2
"Heat Shock Transcription Factors: From Chemical Biology to Structural Biology to Proteopathy Therapeutics", PhD. Dennis J. Thiele	22/03/2016	0.2
"p63 signalling in health and disease.", Prof. Caterina	5/04/2016	0.2

Missero		
“Nutrient sensing by the mTORC1 pathway.”, PhD. David Sabatini	06/05/2016	0.2
“Deficiency of PI(3,5)P2 biosynthesis leads to neurological disorders and dysmyelinationon”, PhD. Miriam Meisler	09/05/2016	0.2
“Extrinsic control of pluripotent stem cell plasticity: implications in development and disease.”, PhD Gabriella Minchiotti	10/05/2016	0.2
“Endosomal lipids in trafficking and signaling.", Prof. Prof. Jean Gruenberg	17/05/2016	0.2
“Endothelial cell plasticity. The blood brain barrier and its pathological modifications.", PhD Elisabetta Dejana	31/05/2016	0.2
“Quantitative proteomics reveals the leukocyte specific protein Sp140 as a new component of repressive chromatin.", PhD. Angela Bachi	14/06/2016	0.2
“A biochemical perspective on Primary Hyperoxaluria Type I: exploring new therapeutic strategies from pharmacological chaperones to protein therapeutics.”, PhD. Barbara Cellini	29/06/2016	0.2
“Physiological roles and molecular mechanisms of autophagy”, PhD. Noboru Mizushima	20/07/2016	0.2
“Antigen-specific modulation of AAV capsid immunogenicity with tolerogenic nanoparticles.", PhD Federico Mingozzi	21/07/2016	0.2
Angelo Maramai (Direttore Generale, FAI, Fondo Ambiente Italiano, Milano) and Niccolò Contucci (Direttore Generale, AIRC, Associazione	22/07/2016	0.2

Italiana per la Ricerca sul Cancro, Milano)		
“Emerging fluorescence technology to study cell architecture and dynamics.", PhD. Jennifer Lippincott-Schwartz	21/10/2016	0.2
"Protein Dosage and Neurological Disorders.", PhD. Vincenzo Alessandro Gennarino	24/10/2016	0.2
“New technologies to study lipid homeostasis and function” . Prof. Howard Riezman	25/10/2016	0.2
“Glycolipid-dependent and lectin-driven construction of endocytic pits: The GL-Lect hypothesis”, PhD. Ludger Johannes	26/10/2016	0.2
“Genetics and Treatment of Brittle Bone Diseases.", PhD. Brendan Lee on November	07/11/2016	0.2
“From x-rays to x-omes and beyond: genetic disorders of bone as paradigm in human genetics.", Prof. Andrea Superti Furga	15/11/2016	0.2
“Endocytic control of collective motility." ,PhD. Giorgio Scita	29/11/2016	0.2
“Conoscere modificare.”, Prof. Edoardo Boncinelli on December 1st 2016	1/12/2016	0.2
“Anemie diseritropoietiche dal microscopio alla sequenza”, PhD. Achille Iolascon	25/01/2017	0.2
Drug discovery outside the pharmaceutical industry: an Italian example.”, PhD Tiziano Bandiera	06/02/2017	0.2
“KCNQ2 encephalopathy: from pathogenetic mechanisms to personalized treatments.”, PhD. Maurizio Taglialatela	14/02/2017	0.2

"Quantitative fluorescence imaging of sterol transport through the endocytic pathway", PhD Daniel Wustner	15/02/2017	0.2

		Credits year 1								Credits year 2								Credits year 3									
			1	2	3	4	5	6			1	2	3	4	5	6			1	2	3	4	5	6			
		Estimated	bimonth	bimonth	bimonth	bimonth	bimonth	bimonth	Summary	Estimated	bimonth	bimonth	bimonth	bimonth	bimonth	bimonth	Summary	Estimated	bimonth	bimonth	bimonth	bimonth	bimonth	bimonth	Summary	Total	Check
	Modules	20		9	7		3	6	25	10	9						9	0	1						1	35	30-70
	Seminars	5	1	1	0.2	1.8	0.4	1	5.4	5	0.2	2	1	1.4	4.2	0.8	9.6	5	1.2	1.6	0.8	0.8	0.8	0.8	6	21	10-30
	Research	35	9	1	1	8	7	4	30	45	1	8	9	8.6	5.8	9.2	42	55	7.8	8.4	9.2	9.2	9	9.2	53	124	80-140
		60	10	11	8.2	9.8	10	11	60	60	10	10	10	10	10	10	60	60	10	10	10	10	9.8	10	60	180	180

3. Research activity

Feedback Control of Gene Expression in Mammalian Cells

Mammalian cells are dynamical systems. They detect, adapt and respond to time-varying inputs such as environmental cues, secreted molecules, and mechanical stimuli. These processes are controlled by networks of genes, proteins, small molecules, and their mutual interactions, the so-called gene regulatory networks, showing complex topologies. Understanding how these networks work is essential to identify triggering events both in common disease as well as in rare genetic disorders. Control Theory makes available several tools that can be applied to explore the mechanisms driving gene networks. Recently, several successful attempts to apply the Control Theory to steer gene expression from inducible promoters have been reported in the literature, but only in lesser eukaryotes [1, 2, 3, 4, 5, 6].
Very few attempts have been made at applying Control Theory to mammalian cells due to their increased complexity.
In [7] we presented a proof-of-principle study to demonstrate that microfluidics based control of gene expression from a tetracycline-inducible promoter in mammalian cells is feasible. We showed that it is indeed possible to force a population of mammalian cells harbouring the inducible promoter to express a predetermined level of a protein of interest by automatically administering pulses of tetracycline whose duration is computed in real time.
We designed and compared the performance of two controllers: a simple relay, and a Proportional-Integral controller with a Pulse Width Modulator scheme.
We demonstrated that, despite the oscillations around the set-point value, these controllers are suitable to control gene expression in mammalian cells.
However the low performance of the relay and PI control strategies is due to slow transcriptional dynamics in mammalian cells. Therefore, I chose to deal with the inherent inertia of the biological system by applying model-based control strategies such as the Model Predictive Control (MPC) [2, 3, 5]. Indeed, the MPC allows to reduce or eliminate the oscillations around the reference value thanks to its predictive nature. As a test bed for MPC Controller, I considered *tetO7*-Ub$^{v\,76}$GFP network shown in Figure 1.

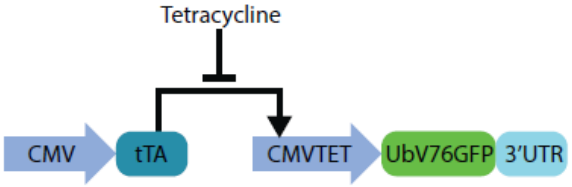

Figure 1: tetO7 -Ub^{v76}GFP network

In *tetO7*-Ub $^{V\ 76}$GFP cells, the destabilized fluorescent reporter protein Ub$^{V\ 76}$GFP is expressed under the control of the CMVTET promoter [17, 53], which harbours seven tet-responsive elements (*tetO7*) upstream of a minimal CMV promoter, embedded in CHO cells, constitutively expressing the tetracycline Transactivator (tTA) protein. In cells grown in standard growth medium, tTA protein is able to bind the *CMVTET* promoter causing Ub$^{V\ 76}$GFP to be maximally expressed. Upon addition of tetracycline, or its homologous doxycycline, to the culture medium, tTA detaches from the *CMVTET* promoter thus preventing the expression of Ub$^{V\ 76}$GFP. The 3'UTR of *Hes1* gene sequence is cloned downstream of the Ub$^{V\ 76}$GFP to increase its degradation rate.
Starting from the input-output data shown in Figure 2, I derived the following set of three linear differential equations describing the production and degradation of the mRNA of reporter protein (x_1), the unfolded reporter protein (x_2) and the folded reporter protein (x_3). Specifically, I assumed distinct dynamics for the unfolded (inactive) and folded (active) forms of the Ub$^{V\ 76}$GFP reporter protein in order to take into account Ub$^{V\ 76}$GFP protein maturation time needed for correct protein folding.

$$\frac{dx_1}{dt} = -d_1 x_1 + \beta_1 u$$

$$\frac{dx_2}{dt} = \alpha_2 x_1 - d_2 x_2$$

$$\frac{dx_3}{dt} = \alpha_3 x_2 - d_3 x_3$$

In the previous dynamical model, u is the only external input to the model and it is assumed to be equal to 1 when cells are fed with standard medium, and 0 when tetracycline is provided to the cells (Figure 2 B). d_1 is a linear degradation coefficient for the mRNA and the input coefficient b is its production rate. The coefficients d_2 and d_3 are the degradation rates of the unfolded Ub$^{V\ 76}$GFP and folded Ub^{V76}GFP protein, and represent the translation rate and the folding rate respectively.

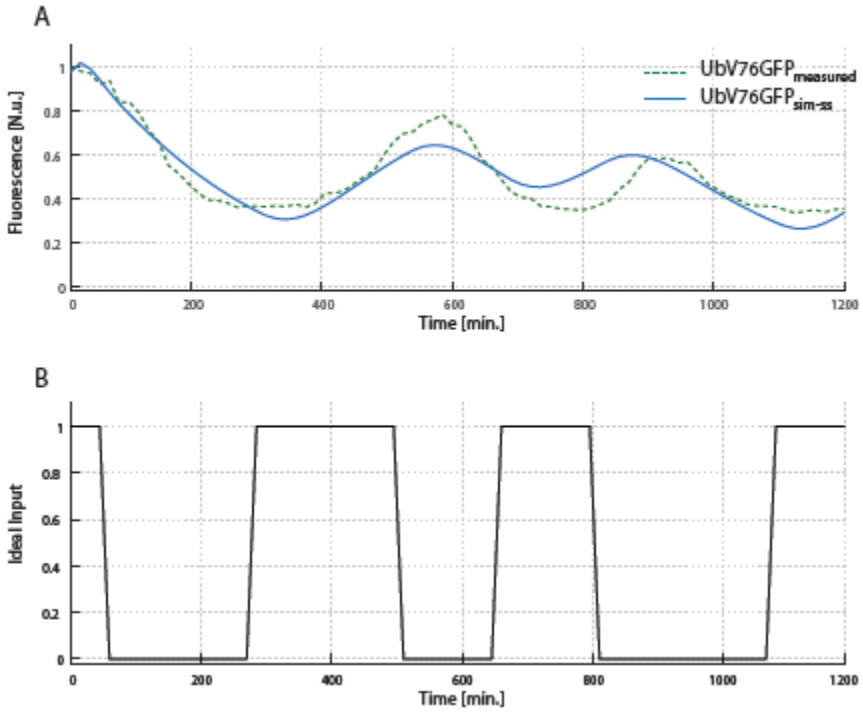

Figure 2: Grey box identification of the Ub^{V76}GFP model

The model parameters (Table 1) were obtained by using the grey-box identification technique described in [34, 59], and implemented in the MATLAB System Idenfication toolbox (Mathworks Matlab R2016b) with the

function greyest, on the input-output data in Figure 2, where the output is the average cells fluorescence of the reporter protein (x3) and the input represents the tetracycline rich or standard medium (u).

Parameter	Description	Value
$d_1\ [min^{-1}]$	degradation rate of $Ub^{V76}GFP$ mRNA	0.0256
$d_2\ [min^{-1}]$	degradation rate of $Ub^{V76}GFP$ unfolded protein	0.0257
$d_3\ [min^{-1}]$	degradation rate of $Ub^{V76}GFP$ folded protein	0.0045
$\beta_1\ [min^{-1}]$	production rate of $Ub^{V76}GFP$ mRNA	0.0029
$\alpha_2\ [min^{-1}]$	production rate of $Ub^{V76}GFP$ unfolded protein	0.0501
$\alpha_3\ [min^{-1}]$	production rate of $Ub^{V76}GFP$ folded protein	0.0209

Table 1: Parameters of *TetO7*-$Ub^{V76}GFP$ with a discrete input

In order to implement a control strategy to steer $Ub^{V76}GFP$ expression, I employed the experimental platform described in Figure 3 and used also in [7] it consists of a closed loop control platform based on miicrofluidic device, featuring a computer implementation of the control algorithm and an inverted fluorescence microscope.
The *tetO7*-$Ub^{V76}GFP$ cells are grown in the microfluidic device and are imaged by a timelapse epifluorescence microscope. The computer uses the images to quantify cells fluorescence and compare it with the desired fluorescence amount at each sampling time. On the basis of the control error, the control algorithm computes the control action and varies the height of two motorized syringes filled with either tetracycline rich medium or standard medium. Hydrostatic pressure generated by the relative difference in the heights of the two syringes drives the flow in the microfluidic device and determines the type of growth medium cells will sense in the chamber. The system output y(t) is the measured average level of fluorescence of the $Ub^{V76}GFP$ protein over the cell population, which can be used as a proxy of the protein concentration. In the control experiment, the cell fluorescence is imaged at 15 minutes intervals.

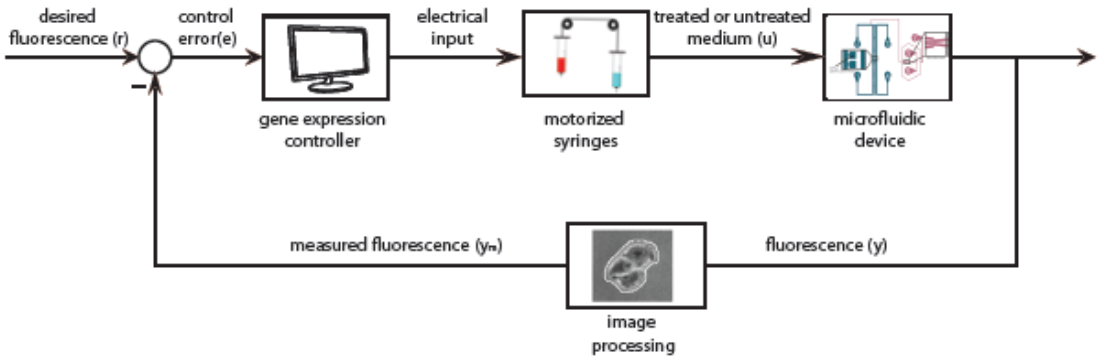

Figure 3: Control Platform

In order to regulate the $Ub^{V76}GFP$ protein expression in CHO cells, I decided to implement a Model Predictive Control (MPC) feedback law with a discrete control input. At each sampling time the solution to the optimal control problem solved by MPC controller is the optimal duty cycle ($d_k = t_{on}/T$) to be provided to the cells. Specifically The MPC decides for how long the cells have to be fed with standard medium within the sampling period T.

Figure 4 shows the results of numerical simulation and in vitro MPC control. The control task in a set-point regulation forcing the cell population to express an average fluorescence equal to 50% of their maximum fluorescence level calculated during the calibration phase (180min. at the beginning of the experiments in which the cells are fed with standard medium and thus they express their maximum fluorescence). The results show that the developed MPC control strategy is able to achieve and maintain the set-point task without any oscillation around the set-point value.

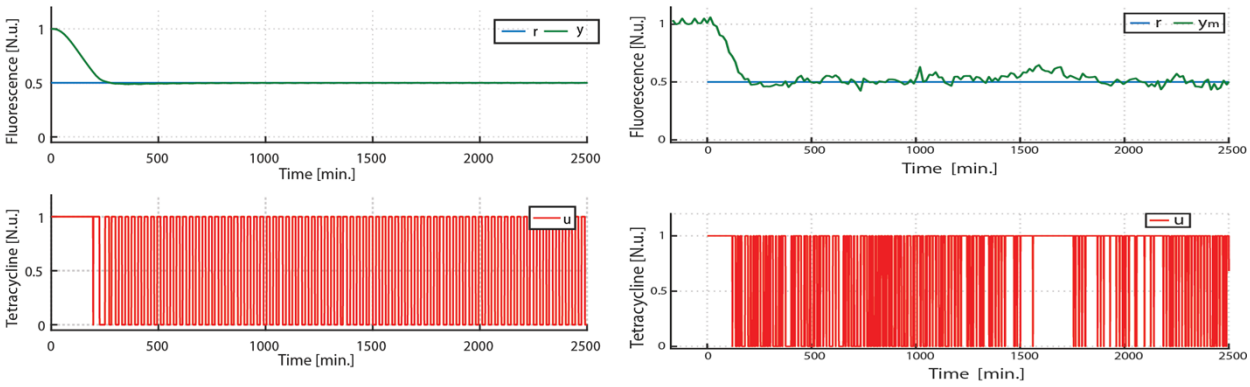

Figure 4: A) Numerical simulations of control tetO7-UbV 76GFP model by means of MPC with discrete control input. B) In vitro control of tetO7-UbV 76GFP expression by means of MPC with discrete control input

The experimental results described in [7] and obtained controlling *tetO7*-Ub^{V76}GFP convincingly demonstrate that the expression of a protein can be controlled in vitro in real-time, using an inducer molecule, by applying a discrete actuation signal, i.e. either standard medium or medium with tetracycline at concentration of 100 ng/ml can be provided to the cells. However, it is important to note that for the biological models in exam the concentration of tetracycline to be supplied to the cells could be graded, obtaining thus a continuous control input which may lead to improved control performances. This would require a highly refined calibration of the actuator that can be avoided by introducing in the experimental platform an active control of tetracycline concentration in the cell chambers as shown in Figure 5.

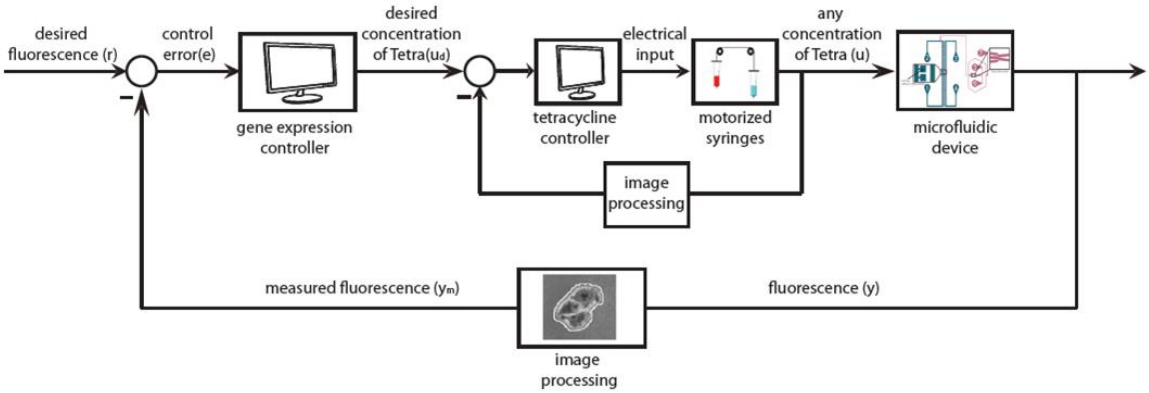

Figure 5: Technological control platform for continuous control input

In order to design the tetracycline controller, I first derived the dynamical model describing the accumulation of tetracycline in the microfluidic device by starting from the data shown in Figure 6. The strategy I followed is to dynamically modulate the differential heights between the two syringes, as input to the system (Figure 6B), and to follow the dynamics of the tetracycline accumulation in the cells chambers (Figure 6A) in response to such an input.

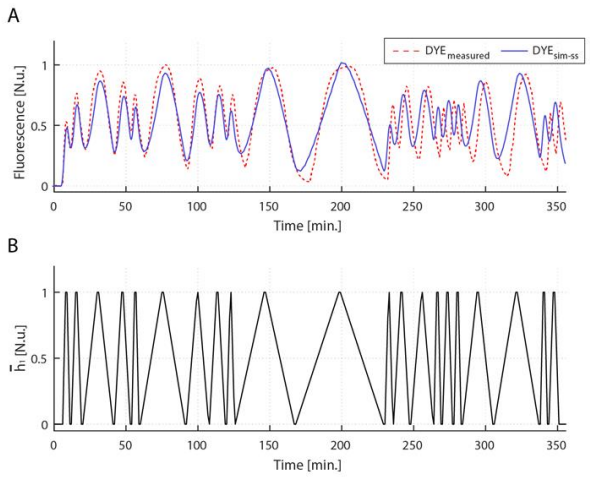

Figure 6: Black-box identification of tetracycline accumulation dynamics in cells chambers.

I first identified the following simple one dimensional state-space linear model

$$\dot{x} = ax + bu, \quad x(0) = x_0, \quad u \in [0, 1]$$
$$y = cx$$

$$a = -0.2234,$$

$$b = 0.0506,$$

$$c = 4.9643,$$

$$x_0 = 1.12$$

Then I developed a MPC strategy to control the tetracycline concentration in microfluidic device. Simulated and experimental results of MPC control of tetracycline concentration are shown in Figure 7.

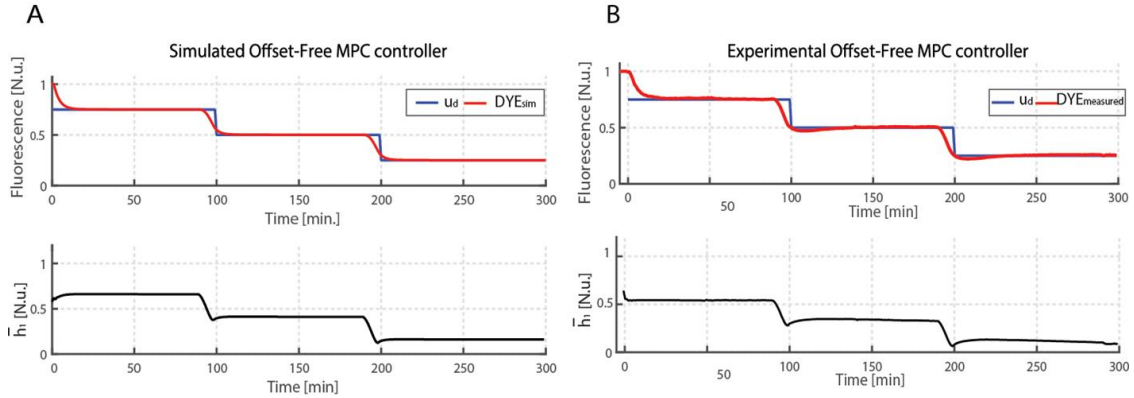

Figure 7: Offset-free MPC staircase tracking control of tetracycline accumulation

Once obtained a working tetracycline concentration controller, I moved to develop a MOC control strategy with a continuous control input to steer the gene expression of *tetO7*-Ub^{V76}GFP cells. In order to obtain this task , I identified a dynamical model of *tetO7*-Ub^{V76}GFP network with a conitnuous control input. Indeed the *tetO7*-Ub^{V76}GFP system can be also described by the following model in which the effect of inducer molecule (tetracycline) on the *tetO7* -Ub$^{V\,76}$GFP system is now modelled by the Hill function [8].

$$\frac{dx_1}{dt} = -d_1 x_1 + \beta_1 \left(\frac{k^n}{k^n + u^n} + \gamma \right) \quad u \in [0,1]$$
$$\frac{dx_2}{dt} = \alpha_2 x_1 - d_2 x_2$$
$$\frac{dx_3}{dt} = \alpha_3 x_2 - d_3 x_3$$

where x_1 is the concentration of Ub$^{V\,76}$GFP mRNA, x_2 and x_3 are the concentrations of Ub$^{V\,76}$GFP unfolded protein and Ub$^{V\,76}$GFP folded protein, respectively. In order to obtain the model parameters, I performed two steps identification: (i) first I identified the coefficients of the Hill function and (ii) then the other model parameters were identified by using a grey-box identification approach from experimental data. The model parameters are reported in Table 2.

Parameter	Description	Value
$d_1\,[min^{-1}]$	degradation rate of Ub^{V76}GFP mRNA	0.0098
$d_2\,[min^{-1}]$	degradation rate of Ub^{V76}GFP unfolded protein	0.0199
$d_3\,[min^{-1}]$	degradation rate of Ub^{V76}GFP folded protein	0.0204
$\alpha_2\,[min^{-1}]$	production rate of Ub^{V76}GFP unfolded protein	0.0139
$\alpha_3\,[min^{-1}]$	folding rate of Ub^{V76}GFP protein	0.0172
$\beta_1\,[min^{-1}]$	CMVTET maximum transcriptional rate	0.0117
$\gamma\,[a.u.]$	CMVTET leakiness	0.011
$k\,[a.u.]$	Tetracycline concentration to achieve $\frac{\beta_1}{2}$	0.62
n	Hill coefficient	3.45

Table 2: Parameters of *TetO7*-Ub^{V76}GFP system with a continuous input

In order to implement a control strategy to steer expression of the Ub$^{V\,76}$GFP with a continuous control input, I took advantage of the integrated experimental platform described in Figure 5. As Figure 5 shows, the original platform (Figure 3) is endowed with an inner loop (tetracycline concentration control loop) in which the measured level of tetracycline (u) is compared to the desired tetracycline concentration (u_d), i.e. the continuous control input computed by the gene expression controller. The tetracycline controller modulates the relative height of the syringes on the linear rails in order to provide cells with the desired amount of tetracycline. As gene expression controller I develop a MPC strategy.

I numerically tested the control strategy with the continuous actuation by simulating the whole experimental setup consisting of both the tetracycline concentration controller and the model predictive controller of gene expression with continuous control input. The control strategy was simulated to perform both set-point regulation and signal-tracking of the model output and the results of the simulations are shown in Figure 8.

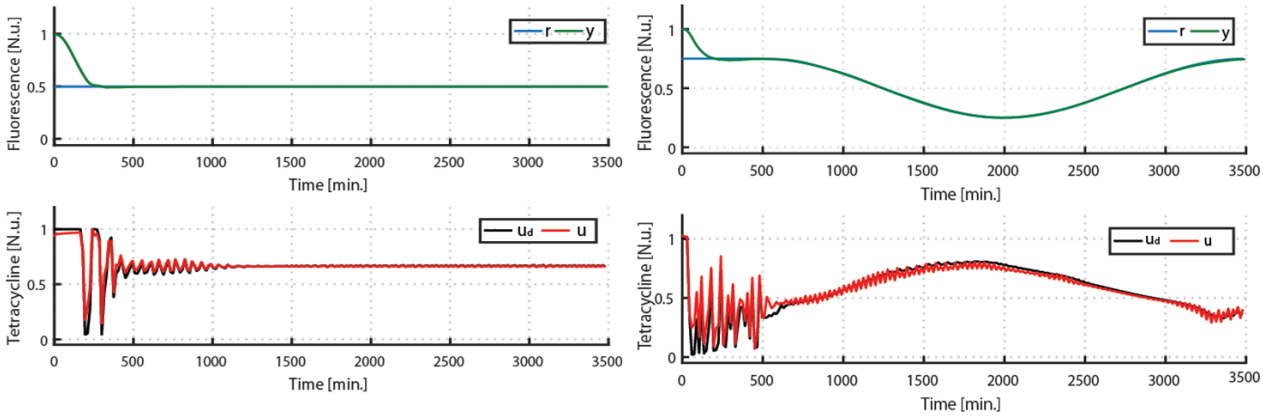

Figure 8: Numerical simulations of tetO7-UbV 76GFP network with a continuous control input.

The results obtained by the numerical analysis show that the MPC based control strategy with continuous control input is feasible and advantageous over the MPC with discrete control input. Indeed interestingly the continuous control input MPC strategy is able to achieve an optimal performance with a very small variations of tetracycline concentration. Furthermore from a biological point of view, slower is the variation in the tetracycline concentration (variation of control input), lower is the stress occurring to the cells.
In order to verify whether the outcome of the numerical simulations can be confirmed experimentally controlling living cells using microfluidics, I performed preliminary in vitro set-point control experiment shown in Figure 9.

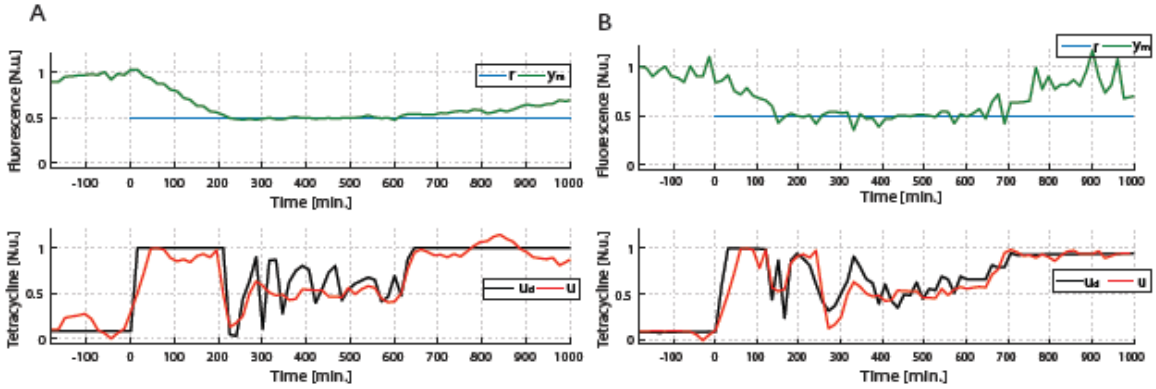

Figure 9: in vitro set-point contol of tetO7-UbV 76GFP network with a continuous control input

Bibliography

[1] Tal Danino, Octavio Mondragón-Palomino, Lev Tsimring, and Jeff Hasty. A synchronized quorum of genetic clocks. Nature, 463(7279):326–330, 2010.

[2] Andreas Milias-Argeitis, Sean Summers, Jacob Stewart-Ornstein, Ignacio Zuleta, David Pincus, Hana El-Samad, Mustafa Khammash, and John Lygeros. In silico feedback for in vivo regulation of a gene expression circuit. Nature biotechnology, 29(12):1114–1116, 2011.

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[4] Filippo Menolascina, Gianfranco Fiore, Emanuele Orabona, Luca De Stefano, Mike Ferry, Jeff Hasty, Mario di Bernardo, and Diego di Bernardo. In-vivo real-time control of protein expression from endogenous and synthetic gene networks. PLoS Comput Biol, 10(5):e1003625, 2014.

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[7] Chiara Fracassi, Lorena Postiglione, Gianfranco Fiore, and Diego di Bernardo. Automatic control of gene expression in mammalian cells. ACS synthetic biology, 5(4):296–302, 2015

[8] Uri Alon. An introduction to systems biology: design principles of biological circuits. CRC press, 2006

4. Products
    a. Publications
        i. Lorena Postiglione, Marco Santorelli, Barbara Tumaini, and Diego di Bernardo. From a discrete to continuous actuation for improved real-time control of gene expression in mammalian cells. IFAC-PapersOnLine, 49(26):14 – 19, October 2016
5. Conferences and Seminars

    - 6th International Conference on Foundations of Systems Biology in Engineering. Magdeburgh, Germany, October 9-12,2016. **Oral Presentation** “From a Discrete to Continuous Actuation for Improved Real-Time Control of Gene Expression in Mammalian Cells.”
    - V Congresso. Gruppo Nazionale di Bioingegneria (GNB 20-22 Giugno 2016, Napoli). **Poster Presentation “**Microfluidic-based automatic control of gene expression in mammalian cells”

6. Tutorship

    - Assistant for exercises of the Laurea Magistrale course “System Analysis for Bioengineering” (Cod. U1576), held by Prof. Diego di Bernardo, 15 hours.
    - Assistant for exercises of the B.Sc. course “Modelli per la previsione e l’ottimizzazione” (Cod. 33800), held by Prof. Diego di Bernardo, 8 hours.