PhD inInformation Technology and Electrical Engineering

Università degli Studi di Napoli Federico II

PhD Student: Lorena Postiglione

XXIX Cycle

Training and Research Activities Report – Second Year

Tutor: Diego di Bernardo

Add the following items according to our meeting we had.

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: Dr.Diego di Bernardo

2. Study and Training activities

Seminars

Title: Regulation of two-fold bifurcations in planar piecewise-smoothsystems
Professor: John Hogan
Place and Date: Napoli, June 26th 2015
Credits: 0.2

External courses (Telethon Institute of Genetics and Medicine)

Title: Practical Course – Part B
Professor: MD Alberto Auricchio, PhDVincenzo Nigro, PhD Elvira De Leonibus, and PhD Roman Polishchuk
Place and Date: Napoli, March 2015
Credits: 3

Title: Systems Biology and Functional Genomics – Part B
Professor: Prof. Diego di Bernardo, PhD MargheritaMutarelli, PhD AnnamariaCarissimo, and PhD SandroBanfi
Place and Date: Napoli, March 2015
Credits: 3

Title: Cell Biology of Genetic Diseases – Part B
Professor: MD Antonella De Matteis and MD Brunella Franco
Place and Date: Napoli, March 2015
Credits: 3

External seminars (Telethon Institute of Genetics and Medicine)

Title and professor	Date	Credits
“Spinal and bulbar muscular atrophy: a pure motor neuron or a metabolic disease?”, PhD Maria Pennuto	03/03/15	0.2
“SINEUPs: a new functional class of natural ad synthetic antisense long non-coding RNAs that activate translation”, Prof. Stefano Gustincich	26/03/15	0.2
“Ion channel or phospholipid scramblase? The elusive function of TMEM16F”, Dr. Luis Galietta	10/04/2015	0.2
“Unraveling the Molecular Basis of Polycystic Kidney Disease”, Prof. Alessandra Boletta	14/04/2015	0.2
“AAV vectors as powerful tools for in vivo investigation on gene function”, Prof. Mauro Giacca	21/04/2015	0.2
“Huntington's Disease from evolution to pathology.”, Prof. Elena Cattaneo	28/04/2015	0.2
“Pharmacological induction of autophagy for improved anticancer chemotherapy.”, Prof. Guido Kroemer	05/05/2015	0.2
“Cellular Complexity of Alzheimer’s Disease.”, Prof. Lawrence Rajendran	12/05/2015	0.2
“Investigating novel nutrient sensing functions of the lysosome.”, PhD Roberto Zoncu	13/05/2015	0.2
“Mitochondria, endoplasmic reticulum, lysosomes and the cytoskeleton: what lies at the interface.", PhD Benoît Kornmann	19/05/2015	0.2
“Sphingolipids in human health and disease.", PhD Tony Futerman	26/05/2015	0.2
“Gene therapy for the	15/06/2015	0.2

treatment of inherited arrhythmias.", Prof. Silvia G. Priori		
“Viral infections and unconventional roles of the autophagy machinery.", PhD Fulvio Reggiori	23/06/2015	0.2
“Aberrations in the endolysosomal compartment: Relevance for rare kidney disorders.”, Prof. Olivier Devuyst	30/06/2015	0.2
“The contribution of computational biology to biomedical problems.", PhD Anna Tramontano	02/07/2015	0.2
“Gene therapy for methylmalonicacidemia.", PhD Charles P. Venditti	14/07/2015	0.2
“Integrating the Myc-transctiptional regulatory network with the Wnt signaling pathway in reprogramming the epigenetic state of embryonic and cancer stem cells.", PhD Alessio Zippo	15/07/2015	0.2
“Why are individuals different? Genetic, environmental and stochastic influences on development.", PhD MirkoFrancesconi	20/07/2015	0.2
“G6PD: The implications of a world-wide human genetic polymorphism that is X-Linked.", Prof. Lucio Luzzato	21/07/2015	0.2
“Directed evolution of adeno-associated virus vectors for retinal gene therapy.", Prof. John Gerard Flannery	08/09/2015	0.2
“Transcription factor control in pluripotent stem cells and preimplantation embryonic development.", Prof. Antonio Simeone	15/09/2015	0.2

“V-ATPase and SNAREs: New functions for old lysosomal proteins.", PhD Thomas Vaccari	22/09/2015	0.2
“Where we were, where we are, and where we are going in treating lysosomal storage diseases.", Prof. Mark Haskins	23/09/2015	0.2
"Selective Autophagy in the Fight Against Aging.", MD Ana Maria Cuervo	6/10/2015	0.2
"From Cells to Mice: Unrevealing the Molecular Pathophysiology of MPS IIIC.", PhD Alexey V. Pshezhetsky on	08/10/2015	0.2
“Cell and Gene Therapy for Hemophilia A.", Prof. Antonia Follenzi	27/10/2015	0.2
“De novo mutations in genetic disease.", Prof. JorisVeltman	3/11/2015	0.2
“Transcription factors and miRNAs in eye development.", PhD Ruth Ashery-Padan	24/11/2015	0.2
“Single molecule approaches for studying gene expression in intact mammalian tissues.",PhD ShalevItzkovitz	01/12/2015	0.2
“Computer simulations of molecular binding phenomena.”, PhD Vittorio Limongelli	03/12/2015	0.2
“Role of Golgi Structure in Haemostasis.”, PhD Francesco Ferraro	15/12/2015	0.2
“Drosophila in the study of ALS pathogenesis.”, PhD GiuseppaPennetta	18/12/2015	0.2
“Non-mutational adaptive changes in melanoma driving the establishment of drug resistance.”, Prof. GennaroCiliberto	12/01/2016	0.2
“Who with whom? Versatile interactions by bZIP transcription factors.”, PhD Matthias Wilmanns	18/01/2016	0.2

"CLUH gives a clue about regulation of mitochondrial metabolism.", Prof. ElenarRugarli	26/01/2016	0.2
"ER stress and ER homeostasis.", PhD Eelco van Anken on February 9th 2016. External seminar (TIGEM)	09/02/2016	0.2

	Credits year 1								Credits year 2							
	Estimated	1 bimonth	2 bimonth	3 bimonth	4 bimonth	5 bimonth	6 bimonth	Summary	Estimated	1 bimonth	2 bimonth	3 bimonth	4 bimonth	5 bimonth	6 bimonth	Summary
Modules	20		9	7		3	6	25	10	9						9
Seminars	5	1	1	0.2	1.8	0.4	1	5.4	5	4.2	2	1	1.4	4.2	0.8	14
Research	35	9	1	1	8	7	4	30	45	1	8	9	8.6	5.8	9.2	42
	60	10	11	8.2	9.8	10	11	60	60	14	10	10	10	10	10	64

3. Research activity

Microfluidic-based automatic control of gene expression in mammalian cells.

Automatic control of gene expression in living cells is paramount importance to characterize both endogenous gene regulatory networks and synthetic circuits. In addition, such a technology can be used to maintain the expression of synthetic circuit components in an optimal range in order to ensure reliable performance.
In 2011, a semi-automated control strategy was implemented to regulate gene expression in yeast from the GAL1 promoter, using a synthetic light-inducible transcription factor [Milias-Argeitis2011]. A more advanced fully automated strategy was described in 2012, to control the expression of a reporter protein from the Hog1-responsive promoter using a microfluidics platform [Uhlendorf2012] with osmotic pressure as control input. The first control strategy to regulate gene expression from GAL1 promoter using galactose and glucose as control was developed in 2014 [Menolascina2014] by means of an ad-hoc microfluidic platform.
The application of control engineering to mammalian systems is still at its infancy. This is a very challenging task, since these cells exhibit complex and adaptive behaviour and are very sensitive to external perturbations. Moreover, transcription in mammalian cells is up to one order of magnitude slower than in bacteria or simple eukaryotes, therefore the time-scale involved in the control of gene expression are much longer (days instead of hours) .
During last two years I developed a microfluidics-based feedback control strategy to quantitatively regulate gene expression from a Tetracycline-inducible promoter in mammalian cells. I demonstrated that it is possible to force a population of cells, harbouring the inducible promoter, to express a predetermined level of a protein of interest by automatically administering pulses of Tetracycline to the cells, whose duration is computed in real-time by a control algorithm.
The control platform, described in Figure 1, was set up to monitor in real-time fluorescent protein levels and to provide time-varying concentrations of an inducer molecule.

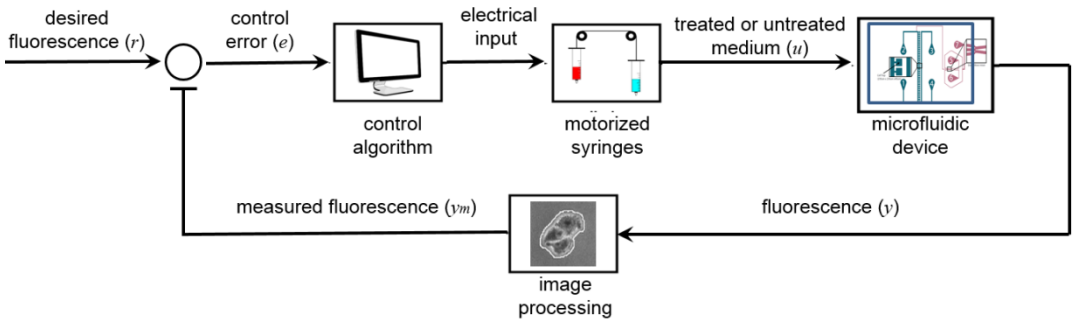

Figure 1: Control Platform

Cells are grown in a microfluidic device within a temperature- and CO_2-controlled environment under an inverted fluorescence microscope. Images are acquired every 15 minutes (y), and quantification of fluorescence is performed via an image-segmentation algorithm. The actual level of fluorescence (y_m) is compared to the desired value (r) and the control error (e) is computed. As Figure 1 shows, the control algorithm computes the input (u, the necessary amount of inducer molecule) to be provided to the cells in order to minimize the control error (e) and then moves the two syringes containing either untreated medium or Tetracycline-treated medium accordingly, as to close the loop in a feedback mechanism. The syringes are connected to chamber with cell via capillary tubes.

In my early *in vitro* experiments I decided to use a binary control input that is either no inducer (i.e., just culture medium) or a single predetermined concentration of the inducer molecule is provided.

As a test bed for assessing the feasibility of automatic control of gene expression in mammalian cells, I chose CHO Tet-OFF cell line (already available in the laboratory [Siciliano et al., 2011]). In this biological system (Figure 2), the CMV promoter drives the expression of the tTA protein, which drives the transcription of d2EYFP from a CMV-TET promoter.

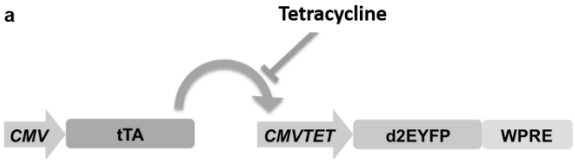

Figure 2: Biological model

This system is switched off by adding Tetracycline, therefore it can be described as a single input-single output (SISO) dynamical system. The input u(t) is represented by the presence or absence of Tetracycline in the growth medium and the output y(t) is the measured average level of d2EYFP in the cell population.

First I performed in *vitro* set-point control experiments a set-point regulation, in which the cell population is forced to express over the time a fixed amount of fluorescence (control reference r(t)). To this end, I first developed two control strategies based on Relay and Proportional-Integral controller (PI).

The Relay controller requires only the computation of the control error at each sampling time (kT), where T=15 min, whose sign dictates which input will be provided to the cells. Specifically, cells will be treated with standard growing medium for the next 15 min, if $e=(r-y_m)>0$, or Tetracycline-containing medium otherwise. Moreover to avoid the chattering phenomenon, I added a 5% hysteresis interval to the controller, corresponding to a percentage tolerance interval around the set-point in which the algorithm ignores the control error value.

The PI controller produces a control input which can assume any positive or negative numerical value. However, cells can only be treated either with Tetracycline or standard medium. To solve this problem, I applied a Pulse Width Modulation (PWM) coding scheme. In PWM, at each sampling time, the control input

is converted to a pulse of Tetracycline (ON) whose duration is proportional to the control input value. The Tetracycline pulse can vary from 0 min to 15 min (i.e. the sampling time), hence 0 min corresponds to the minimum control input value and 15 min to its maximum value.
Figure 3a,b shows the results of the *in vitro* control experiments for the relay and PI controllers respectively. The set-point control task was to reach and maintain a set point (cyan line) equivalent to 50% of the fluorescence value (cyan line) measured during the calibration phase. This value is obtained by imaging cells in the absence of Tetracycline for 180 minutes at 15 min intervals, and then calculating the time-averaged value of fluorescence across the images. The red line under each graph represents the respective Tetracycline input provided to cells in the microfluidic device. The green line represents the measured mean fluorescence of the imaged fields.

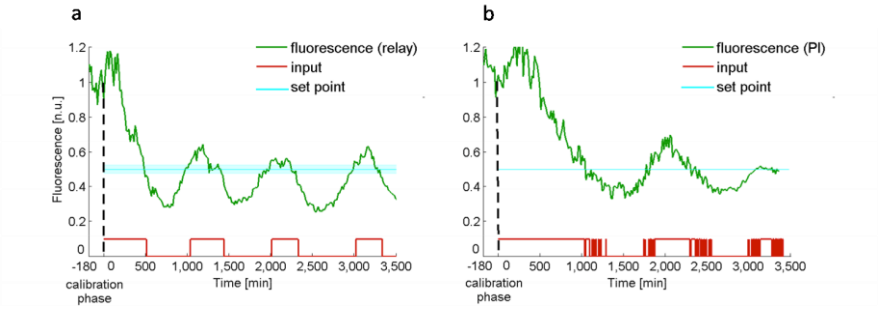

Figure 3*: In vitro* set point control experiments of gene expression. a) Relay controller b) Pi-PWM controller

The Figure 3 shows that the system is unable to settle on the reference value, but it persistently oscillates around it for the whole length of the experiment (3500 min). In the case of the PI controller in Figure 3b, oscillations have a decreasing amplitude with time but the system presents the longer initial delay following Tetracycline administration before fluorescence starts to decrease. Nevertheless, the controller is able to react accordingly, and the fluorescence value of the cells is slowly brought towards the desired set point. This slight difference in the initial dynamics could be due to intrinsic variability caused by experimental conditions and cell batches.
The fact that transcription occurs at very large timescales in mammalian cells is responsible for the low performance of the presented control strategies**.**
Control performances can be improved using a model-based control algorithms, such as Model Predictive Control as suggested by *in silico* results shows in Figure 4. Specifically, using these advanced controllers, it might be possible to reduce or eliminate oscillations around the desired set-point and to force cells to track a desired time-varying fluorescence value.

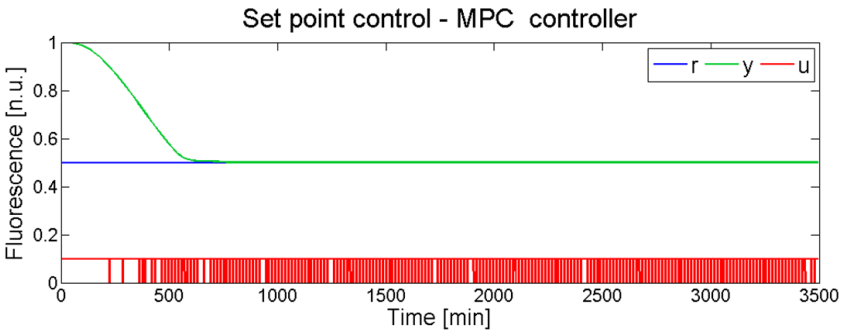

Figure 4: In silico set point control experiment of gene expression. MPC.

For this reason my next work will be directed to perform some in vitro MPC control experiment and to perform a tracking control to some time-variant reference value. Moreover in order to further improve the control performance, I will consider another biological model that exhibits faster transcriptional dynamic then that I presented before. Therefore my ongoing work first aims to identify a mathematical model of the new biological system and then I will develop mathematical model based control strategies.

From switched to continuous control inputs for improved real-time control of mammalian cells.

In last months I also focused my attention on the actuator system of the control platform showed in Figure 1.
The actuator system is represented by a couple of motorized syringes containing tetracycline-treated and untreated medium. The control algorithm changes the relative heights of two syringes to give to the cell the necessary amount of inducer molecule.
In order to minimize the possibility of technical errors, in my previous experiments I decided to use a binary control input, that is, either no inducer is provided (i.e. just culture medium) or a single predetermined concentration of the inducer molecule.
However, in principle, the difference in hydrostatic pressure between the two syringes can be used to provide any desired concentration of the inducer molecule by mixing the two fluids by properly adjusting the heights of the two syringes. Small errors in positioning the syringes, which may be caused by the mechanical inaccuracies of motors, pulleys and belts supporting them, may accumulate over time leading to large discrepancies between the desired concentration of the molecule in the microfluidic cell chamber and its actual value.
To overcome this problem and pass from switched to continuous control input in order to improve the control performance of gene expression, I developed a controller that makes the actual concentration of input molecule surely equal to the desired concentration calculated by the gene expression controller.
Thus gene expression control platform presented in Figure 1 is endowed with a new controller as Figure 5 shows. To measure the concentration of the inducer molecule, a fluorescent dye is added to the syringes containing tetracycline. The fluorescence of the dye is imaged by the microscope at regular sampling time of 1 minute and converted to a quantitative value *via* an image segmentation algorithm.

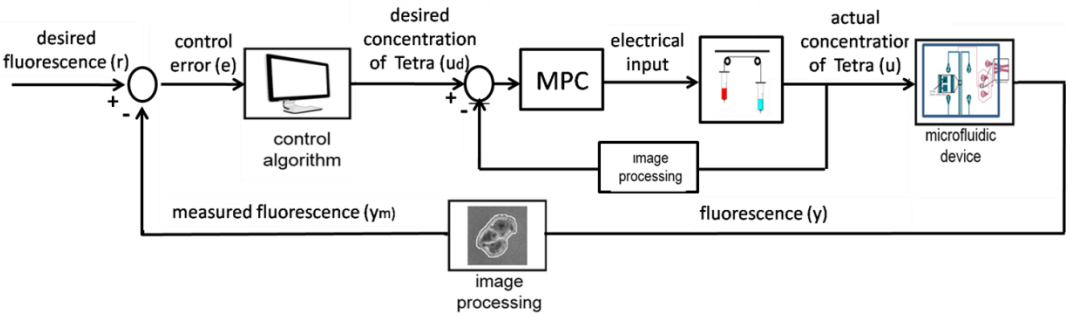

Figure 5: Control platform

In order to develop a model based control strategy, first I identified a mathematical model describing the concentration of tetracycline in chamber depending on the movement of two syringes.

$$\frac{dx}{dt} = -0.2234x(t) + 0.0506u(t)$$
$$y(t) = 4.946x(t)$$

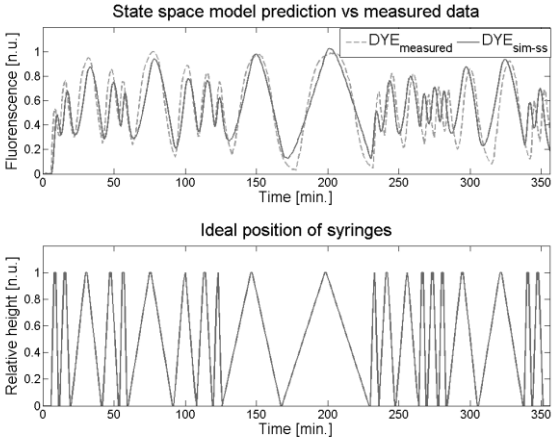

Figure 6: Experimental data and ideal input

The Figure 6 describes the result of a black box identification. The bottom panel shows the ideal input represented by the ideal relative position of two syringes. Specifically the value 1 means that the syringe with Tetracycline is at its highest position and the syringe with untreated medium is at lowest position. When the syringes are located in the alternative positions the signal is equal to 0. In the upper panel the solid line represents the output of the state space model identified with PEM algorithm in response to the input used for the identification signal represented in the bottom plot, the dashed line is Tetracycline+DYE fluorescence measured during the experiment.

After a validation of the identified mathematical model, I developed a MPC based strategy to control the actual concentration of tetracycline.

However the autofluorescence of dye (no modelled by the mathematical model) interferes with detection of specific fluorescent signal. But it can be regarded as a constant disturbance and I added an integral action on the control strategy (Figure 7) in order to ensure a zero control error. In the figure 7, u is the actual concentration of Tetracycline and $\hat{u}_d$ is the desired concentration calculated by control algorithm of gene expression.

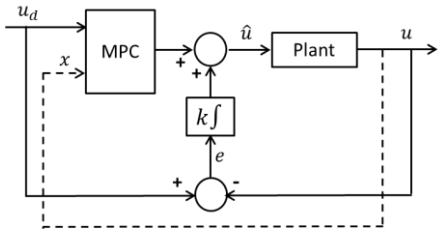

Figure 7: Control strategy for input concentration.

Figure 8 shows the result of experimental control of Tetracycline concentration. In the upper panel the red line is the measure of fluorescence and the blue signal is the desired value. The bottom plot shows the input calculated by the controller.

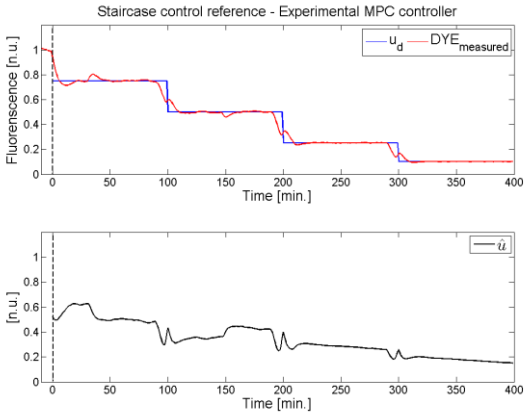

Figure 8: Experimental tracking control of Tetracycline concentration

Then I decided to test beforehand the performance of the complete control strategy (Figure 5) *in silico*. Figure 9 shows the results of the simulation. The simulations reveal how the continuous control input improves the performance of gene expression control respect to the switched control input.

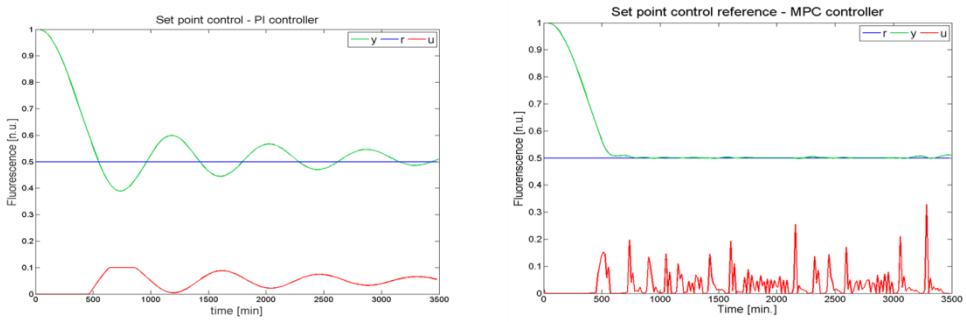

Figure 9: *In silico* control experiment of gene expression with continuous input control. PI and MPC controllers

My next work will be directed to perform *in vitro* tracking control of gene expression in mammalian cells by using the control strategy showed in Figure 5. Furthermore, the presented control strategy with a continuous control input can be used to study the effects of gene dosage in disease.

Bibliography

[1] 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, 2011.

[2] Jannis Uhlendorf, Agnes Miermont, Thierry Delaveau, Gilles Charvin, Franc_ois Fages, Samuel Bottani, Gregory Batt, and Pascal Hersen. Long-term model predictive control of gene expression at the population and single-cell levels. Proceedings of the National Academy of Sciences, 2012.

[3] 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 computational biology, 2014.

[4] Velia Siciliano, Filippo Menolascina, Lucia Marucci, Chiara Fracassi, Immacolata Garzilli, Maria Nicoletta Moretti, and Diego di Bernardo. Construction and modelling of an inducible positive feedback loop stably integrated in a mammalian cell-line. PLoS computational biology, 2011.

4. Products
    a. Publications
        i. Automatic Control of Gene Expression in Mammalian Cells.
        (Fracassi C, **Postiglione L**, Fiore G, di Bernardo D)
        ACS synthetic biology, 2015

5. Conferences and Seminars

- Design, Optimization and control in system and synthetic Biology DOC'15 (Paris, November 12-13, 2015). **Oral Presentation** "Automatic control of gene expression in mammalian cells"

- The Second International Mammalian Synthetic Biology Workshop (Boston, April 25-26, 2015). **Poster Presentation** "Microfluidics-based Automatic Control of gene expression in mammalian cells"

6. Tutorship

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.