PhD in Information Technology and Electrical Engineering

Università degli Studi di Napoli Federico II

PhD Student: Giansimone Perrino

XXIX Cycle

Training and Research Activities Report – Third Year

Tutor: Diego di Bernardo – co-Tutor: Mario di Bernardo

1. Information

I received the M.Sc. degree in Control Engineering on October 2013 from the University of Naples Federico II. Currently, I am a Ph.D. student in Information Technology and Electrical Engineering (ITEE) – XXIX cycle – at the University of Naples Federico II.

- Fellowship provided by *Fondazione Telethon.*
- Tutor: Diego di Bernardo.
- Co-Tutor: Mario di Bernardo.

The following table reports the credits summary:

	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	0	9	13	0	3	6	31	10	9	0	0	0	0	0	9	0	0	0	0	0	0	0	0	40	30-70
Seminars	5	1	1	2,2	1,8	0,4	1	7,4	5	1,4	2	1	1,4	1,2	0,8	7,8	5	1,4	1,8	0,4	1	1	1	6,6	22	10-30
Research	35	9	1	1	8	7	3	29	45	1	8	9	8,6	8,8	9,2	45	55	7,6	7,2	7,6	7,5	7,5	7,2	45	118	80-140
	60	10	11	16	9,8	10	10	67	60	11	10	10	10	10	10	61	60	9	9	8	8,5	8,5	8,2	51	180	180

2. Study and Training activities

Seminars

i. **In vivo imaging of adaptive immune responses.**
MD Matteo Iannacone on March 1st 2016.
External Seminar at TIGEM (1 hour)

ii. **One protein complex, three diseases, and all began at Tigem.**
Prof. Giorgio Casari on March 8th 2016
External Seminar at TIGEM (1 hour)

iii. **Investigate the molecular basis of cell fate decisions through functional genomics.**
PhD Davide Cacchiarelli on March 15th 2016
External Seminar at TIGEM (1 hour)

iv. **Programming and reprogramming biological networks**
PhD Lucia Marucci on 18th March 2016
External Seminar at TIGEM (1 hour)

v. **Heat Shock Transcription Factors: From Chemical Biology to Structural Biology to Proteopathy Therapeutics**
PhD Dennis J. Thiele on March 22nd 2016
External Seminar at TIGEM (1 hour)

vi. **p63 signalling in health and disease**
Prof. Caterina Missero on 5th April 2016
External Seminar at TIGEM (1 hour)

vii. **The redox and metabolic signaling controlled by the Rod-derived Cone Viability gene NXNL1 for treating inherited retinal diseases**
PhD Thierry Léveillard on April 26th 2016
External Seminar at TIGEM (1 hour)

viii. **Nutrient sensing by the mTORC1 pathway.**
MD David Sabatini on May 6th 2016
External Seminar at TIGEM (1 hour)

ix. **Deficiency of PI(3,5)P2 biosynthesis leads to neurological disorders and dysmyelination.**
PhD Miriam Meisler on May 9th 2016
External Seminar at TIGEM (1 hour)

x. **Extrinsic control of pluripotent stem cell plasticity: implications in development and disease.**
PhD Gabriella Minchiotti on May 10th 2016.
External Seminar at TIGEM (1 hour)

xi. **Endosomal lipids in trafficking and signaling.**
Prof. Jean Gruenberg on May 17th 2016
External Seminar at TIGEM (1 hour)

xii. **Genetics changes medicine.**
Prof. Han Brunner on May 19th 2016
External Seminar at TIGEM (1 hour)

xiii. **Quantitative proteomics reveals the leukocyte specific protein Sp140 as a new component of repressive chromatin**
PhD Angela Bachi on June 14th 2016
External Seminar at TIGEM (1 hour)

xiv. **Scientific and regulatory challenges for developing gene therapy medicinal products**
PhD Maria Cristina Galli on June 20th 2016
External Seminar at TIGEM (1 hour)

xv. **Four years at Genethon: perspectives and problems for gene therapy of blood and muscle diseases**
PhD Fulvio Mavilio on June 22nd 2016.
External Seminar at TIGEM (1 hour)

xvi. **A biochemical perspective on Primary Hyperoxaluria Type I: exploring new therapeutic strategies from pharmacological chaperones to protein therapeutics**
Barbara Cellini on June 29th 2016
External Seminar at TIGEM (1 hour)

xvii. **Antigen-specific modulation of AAV capsid immunogenicity with tolerogenic nanoparticles**
PhD Federico Mingozzi on July 21st 2016
External Seminar at TIGEM (1 hour)

xviii. **Physiological roles and molecular mechanisms of autophagy**
MD Noboru Mizushima on July 20th 2016
External Seminar at TIGEM (1 hour)

xix. **The nuclear architecture as a paradigm for translational epigenetic studies**
PhD Chiara Lanzuolo on September 20th 2016
External Seminar at TIGEM (1 hour)

xx. **Protein Dosage and Neurological Disorders**
PhD Vincenzo Alessandro Gennarino on October 24th 2016
External Seminar at TIGEM (1 hour)

xxi. **Emerging fluorescence technology to study cell architecture and dynamics**
PhD Jennifer Lippincott–Schwartz on October 21st 2016
External Seminar at TIGEM (1 hour)

xxii. **New technologies to study lipid homeostasis and function**
Prof. Howard Riezman on October 25th 2016
External Seminar at TIGEM (1 hour)

xxiii. **Glycolipid-dependent and lectin-driven construction of endocytic pits: The GL-Lect hypothesis**
PhD Ludger Johannes on October 26th 2016
External Seminar at TIGEM (1 hour)

xxiv. **Genetics and Treatment of Brittle Bone Diseases**
MD Brendan Lee on November 7th 2016
External Seminar at TIGEM (1 hour)

xxv. **From x-rays to x-omes and beyond: genetic disorders of bone as paradigm in human genetics**
Prof. Andrea Superti Furga on November 15th 2016
External Seminar at TIGEM (1 hour)

xxvi. **Understanding the genetics of autism using next-generation sequencing**
Prof. Evan E. Eichler on November 22nd 2016
External Seminar at TIGEM (1 hour)

xxvii. **Endocytic control of collective motility**
PhD Giorgio Scita on November 29th 2016
External Seminar at TIGEM (1 hour)

xxviii. **Conoscere e modificare**
Prof. Edoardo Boncinelli on December 1st 2016
External Seminar at TIGEM (1 hour)

xxix. **Anemie diseritropoietiche dal microscopio alla sequenza**
MD Achille Iolascon on January 25th 2017
External Seminar at TIGEM (1 hour)

xxx. **Drug discovery outside the pharmaceutical industry: an Italian example**
PhD Tiziano Bandiera on February 6th 2017
External Seminar at TIGEM (1 hour)

xxxi. **KCNQ2 encephalopathy: from pathogenetic mechanisms to personalized treatments**
MD Maurizio Taglialatela on February 14th 2017
External Seminar at TIGEM (1 hour)

xxxii. **Quantitative fluorescence imaging of sterol transport through the endocytic pathway**
PhD Daniel Wustner on February 15th 2017
External Seminar at TIGEM (1 hour)

xxxiii. **Disease avatars: cell reprogramming and the functional annotation of human genomes**
MD Giuseppe Testa on February 21st 2017
External Seminar at TIGEM (1 hour)

3. Research activity

Modeling and control of gene expression dynamics in yeast

Synthetic biology is a novel research field, in which biomolecular circuits are assembled in living cells with the final goal of controlling cellular behavior for a number of uses, from energy, to environment, to medicine. However, realisation of synthetic biomolecular circuits is often a lengthy and ad hoc process. Mainly, this is due to nonlinearity, stochasticity, variability and lack of modularity in biomolecular processes. In recent years, control theory has been applied to synthetic biology for tackling several of these problems, leading to promising results.

Control Engineering aims at driving a physical system in order to attain a specific value of a quantity of interest (such as a boiler that needs to warm water to a desired temperature, or a car cruise–control maintaining a constant speed) despite the presence of disturbances. This is achieved by appropriately varying its inputs (switch on or off a heater in the case of the boiler, or accelerating or braking in the cruise–control) as a function of the difference between the measured value of the output and its desired target value (control error). At the core of most control schemes lies a **negative feedback loop**, as depicted in Figure 1. The variable to be

controlled (system output *y*) is measured through a sensor and its value is subtracted from the desired value (control reference *r*). The quantity that is obtained is minimised by the controller, a set of logical and mathematical rules through which an appropriate value of the input *u* is chosen in order to guarantee that the output *y* matches the desired reference *r*. The input *u* is thus applied to the system by an actuator.

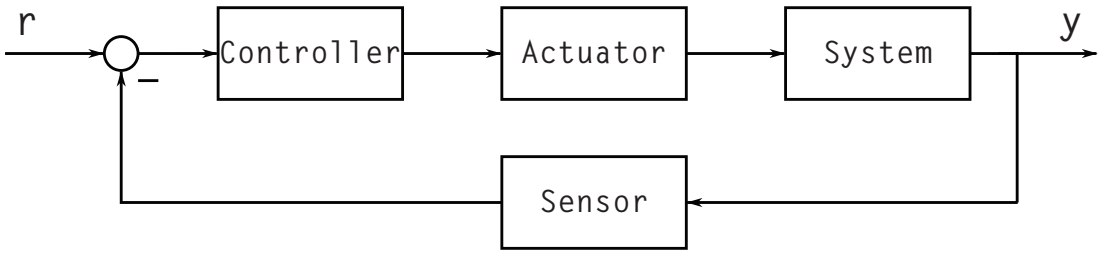

Figure 1

Implementations of negative feedback in synthetic biology fall into two different categories:

- **embedded feedback control**, which has both the controller and the process realised inside the cell by means of biomolecular processes;
- **external feedback control**, which has the whole cell as the process to be controlled, while the controller is implemented in a computer workstation.

External feedback control is an application of control theory to synthetic biology. It has been conceived to overcome the limitations arising from implementing an entire feedback control loop inside a cell (i.e. embedded feedback control). Indeed, the feedback control loop is realised outside the cell. So far, external feedback control has been extensively applied to control growing conditions of cells in chemostats in terms of temperature and/or CO_2 and it is a current feature of bench–top and industrial chemostats. Only recently, however, the application of control theory principles has been exploited to regulate molecular events in living cells, thanks to innovative microfluidics and optogenetics platforms. A multitude of successful attempts to control **gene expression** (the process by which information from a gene, a discrete sequence of DNA nucleotides, is used in synthesis of proteins), or even signaling pathways, have been described in the literature. They mainly differ in the control input (osmotic pressure, light, small–molecules) and the control strategy adopted.

The **aim** of my research activity is to explore and extend the methodology to model and control gene expression in population of living cells by applying concepts borrowed from control theory.

During the first two years of research activity, I carried out a comparative analysis of different control strategies that can be used to control gene expression from the galactose-responsive promoter in yeast *Saccharomyces cerevisiae*. So far, a similar comparison has never performed in literature, thus representing an important contribution to the field of control theory in synthetic biology. I carried out the analysis by comparing three control strategies: proportional-integral (PI) control, model–predictive–control (MPC), and zero–averaged–dynamics (ZAD) control. I demonstrated that both MPC and ZAD control strategies can be successfully employed to control gene expression from the galactose–inducible promoter to generate any desired time–varying concentration of the reporter protein (GFP). Instead, the PI controller performed similarly

to the MPC and ZAD strategies only in the setpoint control task, whereas it was the worst performer in the case of signal–tracking experiments.

During the third year of research activity, I exploited the potential of external feedback control of gene expression to study in yeast a pathological hallmark of Parkinson's disease, i.e. the aggregation of the human α–synuclein protein. PD, as other neurodegenerative disorsers, is characterised by the progressive disruption of specific neuronal population partly due to the formation of abnormal protein aggregates that interfere with normal cell functions. A neuropathological hallmark of PD is the aggregation of the α–synuclein protein in intraneuronal proteinaceous inclusions, termed Lewy bodies (LBs) or Lewy neurites, that are toxic for neurons. Since the α–synuclein accumulation contributes to PD pathogenesis, it is important to investigate and understand the dynamics of α–synuclein aggregation.

So far, a quantitative understanding of the dynamics of α–synuclein protein aggregation in living cells is lacking. Thus, I investigated the aggregation dynamics of the human α–synuclein protein in yeast by exploiting the potentiality of the external feedback control. The aim was to attain a quantitative understanding of the dynamics of α–synuclein protein's aggregation by carefully regulating its expression and following its dynamics in real–time in living cells (cf. Figure 2). By regulating the expression level of the α–synuclein protein at different setpoints, I can assess quantitatively the threshold protein expression level and the dynamics that lead to the formation of α–synuclein aggregates.

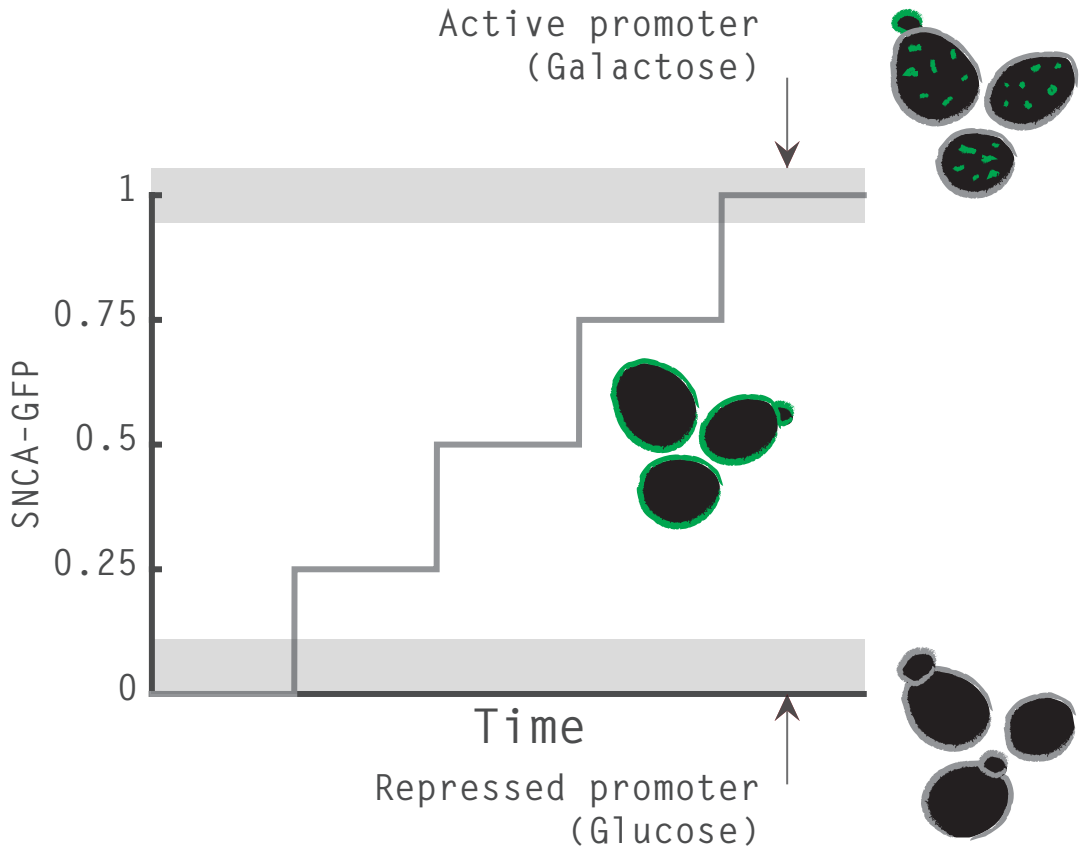

Figure 2

I thus performed a quantitative analysis of the aggregation dynamics of the mutant form A53T of the human α–synuclein protein. I have discovered that the accumulation of the mutant form is characterised by a threshold level. Below this level, the protein does not aggregate, whereas above it, the protein accumulates in cells, and the aggregated corps are visible as cytoplasmic inclusions.

I also continued the characterisation of gene expression from the endogenous galactose–responsive promoter both at single cell and at population level. In particular, I first performed an analysis on single cell data of gene expression, characterising both the intrinsic and extrinsic noise. I then verified that a mixed–effects dynamical model can correctly describe the variability in fluorescence level both in individual cells and at the population level (cf. Figure 3).

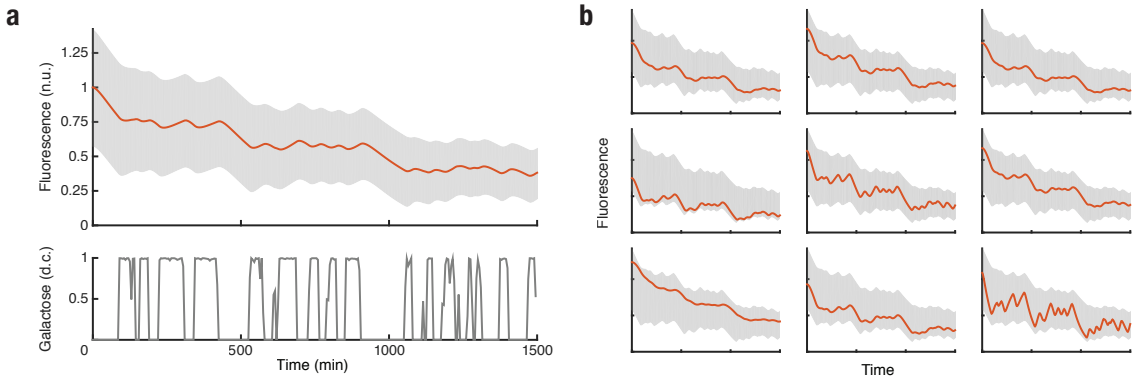

Figure 3

Finally, I proposed a novel model–predictive–control approach, based on single cell models, for predicting the overall behaviour of a cell population (cf. Figure 4). I decided to name it, maybe improperly, Mixed–effects Model–Predictive–Control (MEMPC).

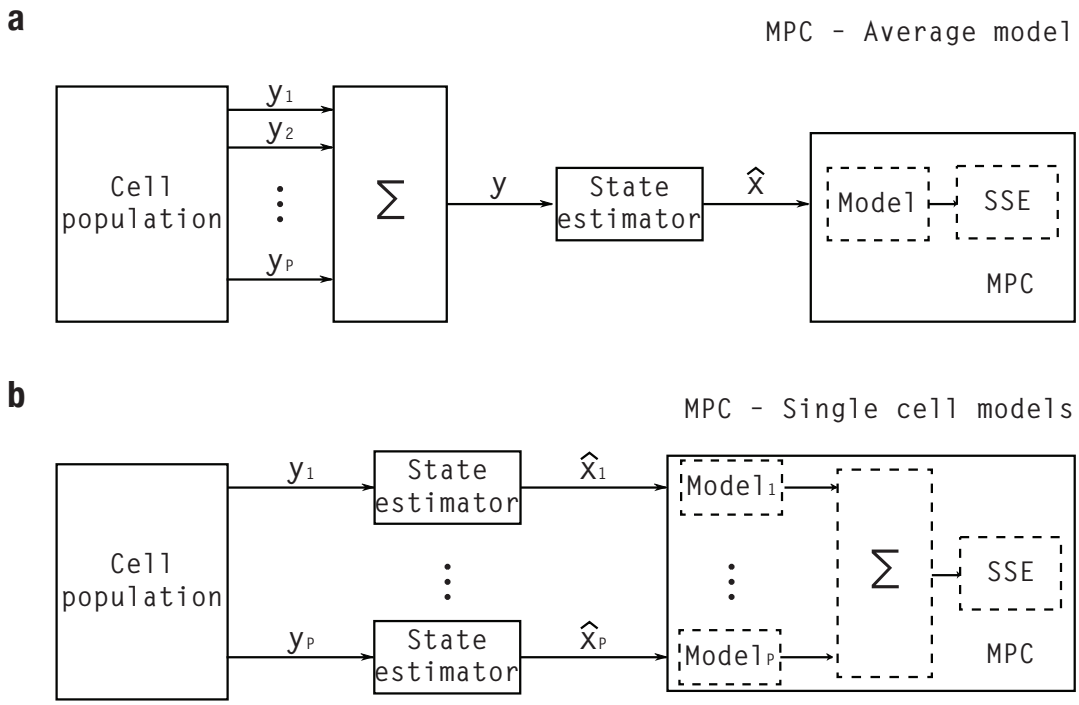

Figure 4

Numerical simulations confirmed that the proposed strategy could successfully achieve the regulation of gene expression from the galactose–inducible promoter in yeast cells (cf. Figure 5). Despite the control performances of the MEMPC and MPC controllers being similar, at least in terms of reference tracking, MEMPC is much more flexible and could have a great impact in practical applications. For example, the proposed MEMPC strategy can be used to control only a subpopulation of cells, by excluding in real–time unhealthy cells that have extreme behaviors (no expression or full expression of the reporter protein independently of the induction medium concentration). MEMPC could also be useful when additional safety constrains must be satisfied, such as preventing any cell from expressing the protein being controlled above a certain toxic threshold. In this case, the MEMPC could be used to steer average population dynamics, but deviations from the control objective would be allowed if one cell (or a given percentage) is predicted to exceed the safety threshold. In addition to the above applications, the MEMPC strategy enables the prediction of the variance of the population over time, and if the experimental system were to allow more than one control input, it could be used to control the variance of the population as well as its mean.

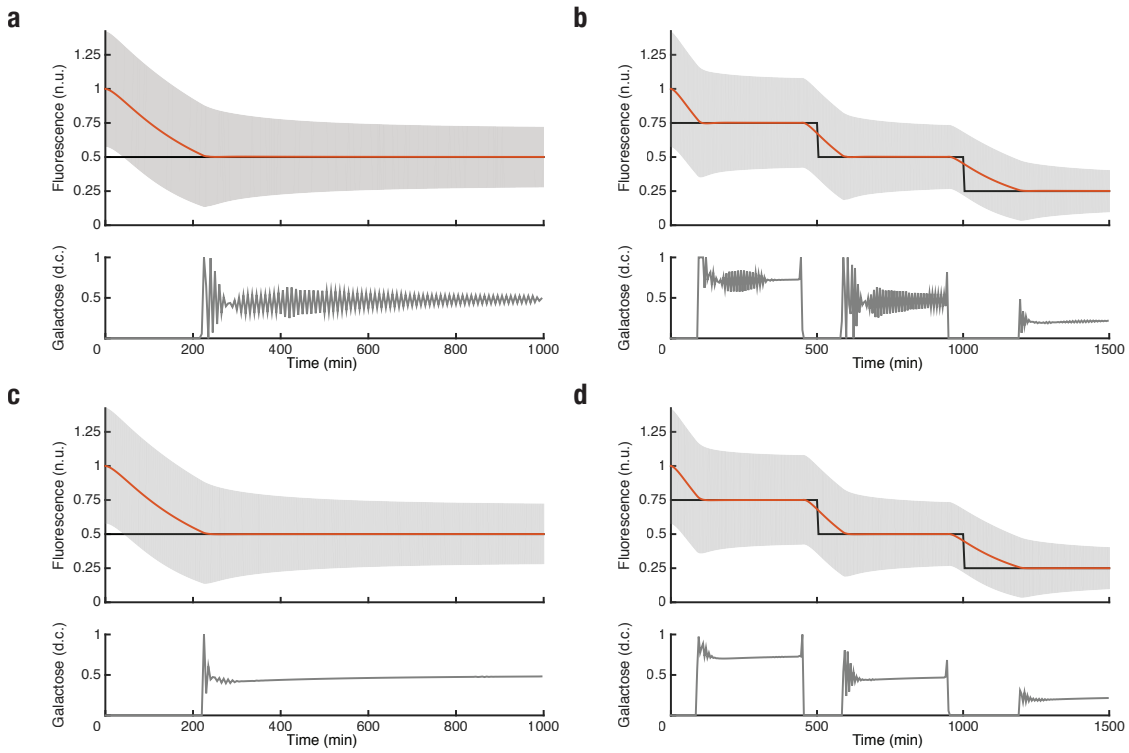

Figure 5

4. Products

Peer Reviewed Conference Papers

i. Giansimone Perrino, and Diego di Bernardo. **Modelling, simulation and control of single cell expression dynamics of the galactose-inducible promoter in yeast.** In Proc. of 2016 IEEE 55th Conference on Decision and Control (CDC 2016).

ii. Giansimone Perrino, Cathal Wilson, Marco Santorelli, and Diego di Bernardo. **Control of gene expression for the study of neurodegenerative disorders: a proof-of-principle experimental study.** In Proc. of 6th IFAC Conference on Foundations of Systems Biology in Engineering (FOSBE 2016).

5. Conferences and Seminars

i. ***2016 IEEE 55th Conference on Decision and Control (CDC2016)*** on December 2016 in Las Vegas, Nevada, USA. Oral presentation entitled "*Modelling, simulation and control of single cell expression dynamics of the galactose-inducible promoter in yeast.*"

ii. ***6th IFAC Conference on Foundations of Systems Biology in Engineering (FOSBE 2016)*** on October 2016 in Magdeburg, Germany. Oral presentation entitled *"Control of gene expression for the study of neurodegenerative disorders: a proof-of-principle experimental study."*

iii. *"Control of gene expression for the study of neurodegenerative disorders".* Poster presentation held at the **V Congresso del Gruppo Nazionale di Bioingegneria (GNB)** on June 2016 in Naples, Italy.

6. Activity abroad

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7. Tutorship

Assistant of the course ***System Analysis for Bioengineering*** (Cod. U1575) held by Prof. Diego di Bernardo for Laurea Magistrale in *Industrial Bioengineering* (8 hours).