PhD in Information Technology and Electrical Engineering

Università degli Studi di Napoli Federico II

PhD Student: Agostino Guarino

XXXIII Cycle

Training and Research Activities Report – First Year

Tutor: Mario di Bernardo

1. Information

I received on 02/10/2017 the M. Sc. degree cum laude in Ingegneria dell'Automazione from University of Naples "Federico II". Currently I'm a PhD student of the XXXIII cycle in ITEE.

Fellowship type: Borsa su Progetto Europeo COSYBIO

Tutor: Prof. Mario di Bernardo

2. Study and Training activities

a. Courses

- "*How to publish a scientific paper*" (0.4 CFU)
  Lecturer: Aliaksandr Birukou & Elisa Magistrelli
- "*Mathematical and numerical models for multi-physics applications*" (1.5 CFU)
  Lecturer: Alfio Quarteroni
- *"Delay differential equations (DDEs) and their applications"* (3 CFU)
  Lecturer: John Hogan
- *"Analisi e Controllo di Reti e Sistemi Complessi"* (6 CFU)
  Lecturer: Pietro De Lellis
- *"Systems and synthetic biology" (6 CFU)*
  Lecturer: Diego di Bernardo
- *"Geometric Theory of Soft Robots" (4 CFU)*
  Lecturer: Stanislao Grazioso
- *"Elettromagnetismo e Relatività" (5 CFU)*
  Lecturer: Amedeo Capozzoli

b. Seminars

- *"Approssimazione di problemi alle derivate parziali e applicazioni"* (1 CFU)
  Lecturer: Alfio Quarteroni
  Date: 11/05/2018 - 5h
- *"Tailoring waves at the extreme with metamaterials"* (0.5 CFU)
  Lecturer: Nader Engheta
  Date: 31/05/2018 - 2.5h
- *"Non-zero-index photonics"* (0.5 CFU)
  Lecturer: Nader Engheta
  Date: 01/06/2018 - 2.5h
- *"From engineering to mathematics (and the way round): two nonlinear case-studies"* (0.6 CFU)
  Lecturer: Josep Olm
  Date: 05/06/2018 - 3h
- *"Discovering the network topology of complex systems"* (0.2 CFU)
  Lecturer: Daniel A. Burbano-L.
  Date: 05/06/2018 - 1h
- *"The Napoli Federico II IEEE Student Branch"* (0.2 CFU)
  Lecturer: Stefano Marrone
  Date: 17/07/2018 - 1h
- *"Domains of attraction and manifolds in a gear model"* (0.2 CFU)
  Lecturer: Petri Piiroinen
  Date: 05/11/18 - 1h

- *"Parallel and Distributed Computing with MATLAB"* (0.4 CFU)
  Lecturer: Stefano Marrone
  Date: 21/11/2018 - 2h
- *SINCRO Group Meetings*
  Weekly 2 hours meeting.

3. Research activity

a. Title:

Analysis and Control of Bacterial Populations in Synthetic Biology

b. Research description:

The Genetic Toggle Switch is a genetic network composed of two mutually repressive genes which is the subject to increasing attention since its proposal in [1]. Each promoter activates the production of a protein that represses the other, consequently at steady-state only one gene is fully expressed. External inducers can be used to make the system switch between its stable states: they sequestrate the repressor of one the two proteins enhancing its expression.

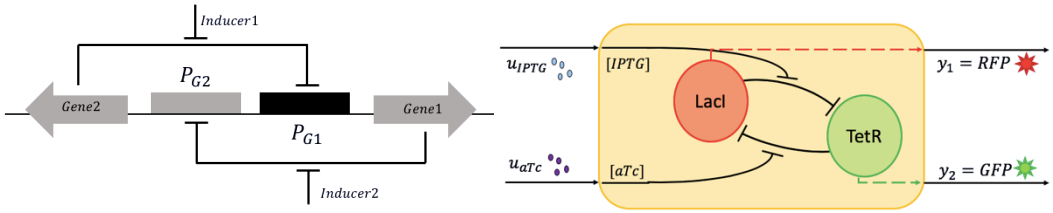

Figure 1 – Left: A network composed by two mutually repressive genes. Boxes represent the promoters of the genes, arrows the proteins and the solid black line the actions of repression.
Right: Schematic MIMO representation of the Genetic Toggle Switch. Inputs are concentrations of molecules of aTc and IPTG in the growth medium, outputs are fluorescence measures proportional to the concentration of proteins.

A system with these properties can be seen, from a systems engineering view point, as a bi-stable system with two stable equilibria and an unstable one. The stable equilibria represent points in which one protein is fully expressed while the other is latent; in a neighbourhood of the unstable equilibrium, conversely, the two proteins are expressed at an intermediate level.

The problem of controlling the Genetic Toggle Switch in a neighbourhood of its unstable equilibrium point is considered as the genetic equivalent of the stabilization of the inverted pendulum [2]. Moreover, it is believed that a mechanism like the one occurring in the toggle switch plays a fundamental role in fate decision and differentiation of stem cells. Therefore, the control strategies developed to keep the toggle switch in an undecided state may be also used to postpone fate decision in stem cells [3].
Several *in-silico* techniques have been proposed including Stochastic Motion Planning [4] and Piecewise Linear Switched Control [5]. *In-vivo* experiments carried out in [2] showed that the use of mutually exclusive pulse waves could be helpful to balance a population of toggle switches in a region between the two stable equilibrium points. All the strategies mentioned so far fall in the class of external controllers: a control architecture made up by sensors, computer and actuators closes the control loop in an artificial manner.

However, the control approach presented in [2] is a heuristic open-loop strategy, which is not based on any mathematical model of the switch and thus has poor robustness properties. Our research is exploiting the observation made in [2] that pulse width modulated inputs can enhance coherence across the population to design an effective control strategy that forces a network of toggle switches to oscillate in a neighbourhood of the unstable equilibria. We developed a time average system that allows us to regulate precisely the mean value of the oscillations the system exhibits over a fixed value, when diffusion dynamics is neglected. To

deal with the effects of diffusion – which can be portrayed as a delay in the control loop -, we designed two external control strategies that showed good performances *in-silico*. The first strategy we proposed, a PI-PWM control, corrects online the duty-cycle of the periodic pulse waves thanks to a PI controller. The second one is a Model Predictive Controller that solves an optimization problem, via Genetic Algorithms, in order to evaluate the duty-cycle of the mutually exclusive pulse waves, whose amplitude are still evaluated using the time average system.

Our long-term goal, however, is to move towards an approach which is known as Multicellular Control [6]. In this scenario, a population of switches (control target) can be driven by another population of cells, which acts as a controller. The key role is played by the diffusion process: quorum-sensing molecules diffuses across the membrane passing from the cell to the solution and vice versa. They can be used the vehicle used by the controller population that senses the state of the target and, thanks to its inner genetic circuit, produces molecules that play the role of the control action.

Currently, in collaboration with Dr. Barbara Shannon and partners at TIGEM and the University of Bristol, we developed a prototype of a Turbidostat [7]. This machine is essentially a bioreactor that controls the concentration of cells in a solution closing the loop over an optical density measure. Indeed, as the bacteria reproduce in the reactor, the turbidity of the growth medium grows. This measure can therefore be used to regulate the cell population number by removing medium from the reactor and adding fresh one. This modular and flexible machine can be upgraded to run multicellular control experiments.

[1] T. S. Gardner, C. R. Cantor, and J. J. Collins, “Construction of a genetic toggle switch in Escherichia coli,” Nature, vol. 403, no. 6767, p. 339, 2000.

[2] J.-B. Lugagne, S. Sosa Carrillo, M. Kirch, A. Ko¨hler, G. Batt, and P. Hersen, “Balancing a genetic toggle switch by real-time feedback control and periodic forcing,” Nature Communications, vol. 8, no. 1, p. 1671, 2017.

[3] M. Andrecut, J.D. Halley, D.A. Winkler and S. Huang” A general model for binary cell fate decision gene circuits with degeneracy: indeterminacy and switch behavior in the absence of cooperativity”. PloS one, 6(5), p.e19358, 2011.

[4] P. M. Esfahani, “Analysis of Controlled Biological Switches via Stochastic Motion Planning,” Proc. of the European Control Conference, no. 1, pp. 93–98, 2013.

[5] M. Chaves and J.-L. Gouze´, “Exact control of genetic networks in
a qualitative framework: the bistable switch example,” Automatica, vol. 47, no. 6, pp. 1105–1112, 2011.

[6] D. Del Vecchio, A.J. Dy, and Y. Qian, *“Control theory meets synthetic biology”* Journal of the Royal Society Interface, 2016.

[7] C. N. Takahashi, A. W. Miller, F. Ekness, M. J. Dunham, and E. Klavins, *“A low cost, customizable turbidostat for use in synthetic circuit characterization”* ACS Synthetic Biology, 4(1), 32–38, 2015.

Collaborations:

- Prof. Diego di Bernardo
  TIGEM – Telethon Institute of Genetics and Medicine
- Dr. Barbara Shannon
  Bristol Centre for Synthetic Biology
  University of Bristol

4. Products

a. **Publications**

i. **Already published:**

- D. Fiore, A. Guarino, M. di Bernardo *"Analysis and control of genetic toggle switches subject to periodic multi-input stimulation"*, IEEE Control System Letters 3 (2), 278-283, 2018.

ii. **Submitted:**

- A. Guarino, D. Fiore, M. di Bernardo, *"In-silico Feedback Control of a MIMO Synthetic Toggle Switch via Pulse-Width Modulation",* 17th European Control Conference (ECC'19), Naples, Italy.

5. Conference and Seminars:

- COSYBIO European Project, 1st Annual Meeting
  Pozzuoli (NA) – 19-21/09/2018

6. Activity abroad

a. Period of research and development at the Department of Engineering Mathematics of University of Bristol (United Kingdom) in collaboration with Dr Lucia Marucci
From 31.01.2018 to 19.02.2018

7. Tutorship

Weekly 2 hours tutorship for the B.Sc. course "Controlli Automatici" (Cod. 02826), held by Prof. Mario di Bernardo.

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Tutor: Mario di Bernardo mario.dibernardo@unina.it

Cycle XXXIII

								Credits year 2									**Credits year 3**									
	1	2	3	4	5	6			1	2	3	4	5	6			1	2	3	4	5	6			
	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	0	0	6	3	0	1.9	**10.9**	**15**	5						**0**	**5**							**0**	**10.9**	**30-70**
Seminars	0	0	2.8	0.2	0	0.6	**3.6**	**5**							**0**	**4**							**0**	**3.6**	**10-30**
Research	10	10	1.2	6.8	10	7.5	**45.5**	**43**							**0**	**53**							**0**	**39,5**	**80-140**
	10	10	10	10	10	10	**60**	**60**	0	0	0	0	0	0	**0**	**0**	0	0	0	0	0	0	**0**	**60**	**180**