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 – Second 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

i. "*Elettromagnetismo e Relatività*" (5 CFU)
Lecturer: Prof. Amedeo Capozzoli

ii. "*Data Science and Optimization*" (1.2 CFU)
Lecturer: Prof. Manlio Gaudioso, Prof. Laura Palagi, Prof. Enza Messina

iii. "*Workshop on Modeling, Analysis, and Control of Complex Networks and Cyber-Physical Systems (MACCC2019)*" (2.6 CFU)

b. Seminars

- "Research Work in Active Perception and Robot Interactive Learning Lab in IIT" (0.4 CFU)
  Lecturer: Dr. Fei Chen
  Date: 17/04/2019
- "Robotics in medical applications: An overview of the current medical robotics market from the industry's point of view" (0.6 CFU)
  Lecturer: Vincenzo Schettino
  Date: 30/04/2019
- "Designer matter: meta-material interactions with light, radiowaves and sound optical phenomena" (0.2 CFU)
  Lecturer: Andrea Alù
  Date: 20/06/2019

3. Research activity

a. Title:

Analysis and Control of Bacterial Populations in Synthetic Biology

b. Research description:

Synthetic Biology is an interdisciplinary field that embraces several disciplines to build artificial biological systems for research, engineering and medical applications. These artificial systems are based on the concept of Gene Regulatory Networks, which are a collection of molecular regulators that interact with each other and with other substances in the host cell to govern the gene expression levels of some proteins of interest.
The Genetic Toggle Switch [1] is one of the fundamental Gene Regulatory Networks that is composed of two mutually repressive genes. 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.

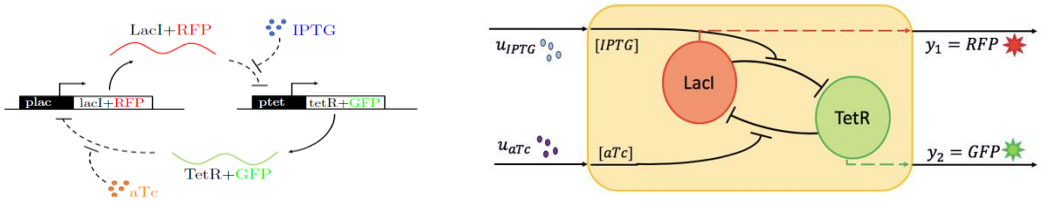

Figure 1 – Left: A network composed by two mutually repressive genes. Black boxes represent the promoters of the genes (white boxes); the proteins (red and green curve lines) exert a repressive action (dashed line) on the competitive promoter. External inducers (aTc and IPTG) reduce this repressive behavior.
Right: Schematic MIMO representation of the Genetic Toggle Switch. Inputs are concentrations of molecules of aTc and IPTG in the growth medium (outside the host cells), outputs are fluorescence measures proportional to the concentration of proteins.

The above mentioned example can be modelled in a deterministic way through an ODE system, that shows a bi-stable system having 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.

Controlling the Genetic Toggle Switch about 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].
Beside some *in-silico* preliminary results [4-5], open-loop *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. This last strategy, however, has shown poor robustness properties. In particular, the off-line choice of the characteristics of the signals (amplitude, period and duty-cycle) might lead the population falling towards one of the two stable equilibria.
We developed several algorithms to carefully select the characteristics of the above-mentioned pulsatile inputs. An average model, proposed in [6], can be exploited to select the amplitudes of the inputs, with the duty-cycle modulated in a closed-loop way. We compared different control strategies [7] in an extensive set of *in-silico* experiments that includes the most advanced agent-based simulation tool for cell level dynamics [8]. The results of our comparison showed that Model Predictive Control offers better performances when compared to a PI-PWM strategy we developed. However, this second strategy is a suitable alternative from the point of view of the *in-vivo* implementation, considering the computational burden of the Genetic Algorithm upon which the MPC stands.
We are now focusing on the development of a host machine to conduct *in-vivo* experiments. We proposed a low-cost and open-source prototype [9] of a Turbidostat which showed good preliminary results in the regulation of the optical density of the population of cells, which is the first mandatory step to regulate the inputs.

Our long-term goal, however, is to move towards Multicellular Control [10], having a population of switches (control target) driven by another population of cells (controllers). Multicellular Control is considered a milestone in the field of Synthetic Biology. However, a reliable experimental platform for *in-vivo* experiments lacks, limiting results to *in-silico* experiments. We are currently studying how to extend the flexible and modular Turbidostat we assembled to provide such a promising machine.

[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. 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.

[7] A. Guarino, D. Fiore, D. Salzano, M. di Bernardo, *"Balancing cell populations endowed with a synthetic toggle switch via adaptive pulsatile feedback control",* bioRxiv, 2019.

[8] T. E. Gorochowski, A. Matyjaszkiewicz, T. Todd, N. Oak, K. Kowalska, S. Reid, K. Tsaneva-Atanasova, N. Savery, C. Grierson, and M. di Bernardo, "*BSim: an agent-based tool for modeling bacterial populations in systems and synthetic Biology*", PLOS One 7, e42790, 2012.

[9] A. Guarino, B. Shannon, L. Marucci, C. Grierson, N. Savery, M. di Bernardo, *"A low cost, open source Turbidostat design for in-vivo control experiments in Synthetic Biology"*, 8th IFAC Conference on Foundations of Systems Biology in Engineering (FOSBE2019), Valencia, Spain.

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

Collaborations:

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

4. Products

a. Publications

i. Already published:

1. 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.

2. 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.
3. A. Guarino, B. Shannon, L. Marucci, C. Grierson, N. Savery, M. di Bernardo, *"A low cost, open source Turbidostat design for in-vivo control experiments in Synthetic Biology"*, 8th IFAC Conference on Foundations of Systems Biology in Engineering (FOSBE2019), Valencia, Spain.

ii. Submitted:

1. A. Guarino, D. Fiore, D. Salzano, M. di Bernardo, *"Balancing cell populations endowed with a synthetic toggle switch via adaptive pulsatile feedback control",* ACS Synthetic Biology.

5. Conference and Seminars:

- 17th European Control Conference (ECC'19)
  Naples, Italy – 25-28/06/2019
- Workshop on Modeling, Analysis, and Control of Complex Networks and Cyber-Physical Systems (MACCC2019)
  Ischia (NA), Italy – 29-30/06/2019
- COSYBIO European Project, 2nd Annual Meeting
  Valencia (Spain) – 14-15/10/2019
- 8th FOSBE IFAC Conference
  Valencia (Spain) – 15-18/10/2019

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 08.02.2019 to 30.04.2019

7. Tutorship

Weekly 2 hours tutorship for the M.Sc. course "Sistemi di Controllo per la Bioingegneria" (Cod. U2243), held by Prof. Mario di Bernardo.

Student: Agostino Guarino **Tutor: Mario di Bernardo** **Cycle XXXIII**

agostino.guarino@unina.it mario.dibernardo@unina.it

	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**	bimonth	bimonth	bimonth	bimonth	bimonth	bimonth	**Summary**	bimonth	bimonth	bimonth	bimonth	bimonth	bimonth	**Summary**	**Total**
Modules	0	0	6	3	0	1,9	**10,9**	6,2	0	2,6	0	0	0	**8,8**							**0**	**19,7**
Seminars	0	0	2,8	0,2	0	0,6	**3,6**	0	1	0,2	0	0	0	**1,2**							**0**	**5**
Research	10	10	1,2	6,8	10	7,5	**45,5**	3,8	9	7,2	10	10	10	**50,0**							**0**	**95,3**
	10	10	10	10	10	10	**60**	10	10	10	10	10	10	**60**	0	0	0	0	0	0	**0**	**120**


Ph.D
itee
INFORMATION tECHNOLOGY
eLECTRICAL eNGINEERING


UNIVERSITÀ DEGLI STUDI DI NAPOLI
FEDERICO II