

Project title: Analysis of the gene expression dynamics by periodic forcing of transcriptional regulators.

Supervisor: Professor Alfonso Jaramillo

Introduction

A fundamental goal in biology is to describe cellular processes in living cells at the molecular level. Analogous to the molecular vibrations due to intermolecular interactions, gene networks are expected to undergo oscillations in gene expression levels. Thus, probing them with external oscillatory forcing enables the mechanistic details of the underlying network to be determined[1]. This approach has rarely been attempted partially because of the requirement for single-cell and chemostatic controls, which implies the use of microfluidic techniques. The Jaramillo group has developed a new technology that quantitatively characterizes the dynamics transcriptional regulation[2]. This uses microfluidics-driven time-lapse microscopy to monitor the gene expression of single genes under oscillatory gene forcing. However, the molecular description of cellular processes in living cells is impeded by the lack of mathematical techniques that are able to quantify precisely the transcript dynamics in spite of existing noise.

Objectives

We aim at analyzing the frequency response to obtain the transcription, translation and degradation rates of genes and their products from a novel approach that requires fewer replicates than using step stimuli. The student will develop a model for our RNA system[3] able to predict its dynamics under an oscillatory forcing. This will extrapolate the analysis of resonances customarily used to dissect the structure of matter from physics and engineering to living cells, which will provide a new technique to probe gene regulation in a living cell. Periodic forcing has also been used in non-linear chemical systems to understand the origins of non-equilibrium pattern formation. This analysis is also extensively used in electrical engineering, where an electric circuit can be debugged through the use of oscillatory currents (for the periodic forcing) and an oscilloscope (for the sensing). Would it be possible to characterize and debug a living cell's RNA regulation (transcriptional and post-transcriptional) with an "RNA generator" and an "RNA oscilloscope"?

Importance

Characterization using resonances would allow inferring less noisy molecular model of transcription regulation than currently available. We will be able to check and identify regulations in living cells, to monitor their response in space and time, one gene at a time. By studying pairwise and multiple-gene interactions, we will provide a new way to characterize transcriptional rates, RNA degradation in cells, and transcriptional regulators at the molecular level. This is a long-standing challenge in systems biology, but also a limiting step in synthetic biology. We expect that the use of oscillatory signals to probe gene networks will become a standard characterization practice in the future.

Background to be assimilated and techniques required

The student will use our existing data, obtained by microscopy experiments and microfluidics chips to infer an ODE model able to predict the response of the biological circuit under novel forcing dynamics. The student will train the model with a minimal set of meaningful parameters to later make predictions under many untrained fluctuating environments. The student will fit an ODE model for sRNA-sRNA interactions to the time-lapse data, using a linear noise approximation to fit the variance in the response. The model will then be generalised to include stochastic dynamics.

Deliverables

Software using a stochastic model able to predict the dynamics of our synthetic RNA circuits under an oscillatory forcing.

End users and real world applications

Biologists willing to understand cell dynamics at the molecular level.

Follow-up PhD project

The proposed research will be extended to an experimental validation in living cells. Where we will aim (1) to develop a new methodology that forces the expression of individual regulatory genes to oscillate and that systematically measures the response of other genes to this oscillation at the single-cell and single-molecule levels, and (2) to understand the mechanisms of gene regulation through the use of this novel approach. We will manipulate the expression of each regulatory gene one by one and we will analyze the dynamics of regulated genes using microfluidics. This will require learning basic experimental molecular biology, microfluidics, and quantitative live cell imaging. This work will be done in collaboration with the MIT (USA).

References

- [1] O. Lipan, W.H. Wong, The use of oscillatory signals in the study of genetic networks, *Proc Natl Acad Sci U S A.* 102 (2005) 7063–7068. doi:10.1073/pnas.0403790102.
- [2] G. Rodrigo, B. Kirov, S. Shen, A. Jaramillo, Theoretical and experimental analysis of the forced LacI-AraC oscillator with a minimal gene regulatory model, *Chaos.* 23 (2013) 025109. doi:10.1063/1.4809786.
- [3] G. Rodrigo, T.E. Landrain, A. Jaramillo, De novo automated design of small RNA circuits for engineering synthetic riboregulation in living cells, *Proc Natl Acad Sci U S A.* 109 (2012) 15271–15276. doi:10.1073/pnas.1203831109.

Resources required

The lab is well funded and the students have all the support of postdocs, PhD students and technicians. I will personally meet daily with the student.