

**Project title:** Computational design of RNA-based oscillatory circuits in living cells using Monte Carlo methods.

**Supervisor:** Professor Alfonso Jaramillo

### **Introduction**

The design of synthetic regulatory circuits with defined behaviours enables the reprogramming cells for particular applications. However, the path between sequence and function remains challenging to map due to the unpredictability of protein function, preventing the general application of design methods from quantitative models to engineer gene circuits in living cells. The engineering of devices made of small RNAs (sRNAs), non-coding RNAs with regulatory functions, would allow a higher functional predictability from physicochemical models. Recent studies have exploited these properties, using computational predictions of intra-/inter-molecular conformations to engineer sRNAs showing multifunctional behaviour.

### **Objectives**

The objective of this project consists of designing a synthetic regulatory circuit in *E. coli* with oscillatory behaviour. This has only been possible before with transcription factors, but never before with RNA. The reason is that oscillators require non-linear behaviour, usually absent in riboregulators (sRNA able to regulate gene expression). Our group has engineered the first cooperative riboregulators that synergistically bind to create a dimer able to activate gene expression non-linearly.

### **Importance**

Such RNA circuits may change the current paradigm of using transcription factors to create synthetic regulatory circuits, which is very limited by the transcription factor known in Nature. RNA are much smaller than proteins and, contrary to them, they can be designed computationally de novo<sup>1,2</sup>. Our RNA oscillator prototype will be the first circuit of a new category of synthetic gene circuits relying on standardised small RNAs parts<sup>3</sup>.

### **Background to be assimilated and techniques required**

The student will be assumed to be familiar with statistical mechanics and C++. The student will extend existing C++ code to implement new biophysical models of RNA function. She/he will learn new applications of statistical mechanics to re-engineer living bacteria. She/he will interact with researchers involved in experimental molecular biology, directed evolution, 3D printing and Arduino.

### **Deliverables**

A set of nucleotide sequences of sRNA able to spontaneously behave as an oscillatory system in bacteria according to biophysical models.

### **End users**

Synthetic Biologists using synthetic regulatory circuits to solve applied problems in medicine, energy and environment.

### **Follow-up PhD project**

The proposed research will be extended to an experimental validation in living cells. This will require learning experimental molecular biology, microfluidics, and quantitative live cell imaging. This work will be done in collaboration with the MIT (USA).

### **References**

1. Rodrigo, G., Landrain, T. E. & Jaramillo, A. De novo automated design of small RNA circuits for engineering synthetic riboregulation in living cells. *Proc Natl Acad Sci U S A* **109**, 15271–15276 (2012).
2. Rodrigo, G., Landrain, T. E., Majer, E., Daròs, J.-A. & Jaramillo, A. Full design automation of multi-state RNA devices to program gene expression using energy-based optimization. *PLoS Comp Biol* **9**, e1003172 (2013).
3. Rodrigo, G., Landrain, T. E., Shen, S. & Jaramillo, A. A new frontier in synthetic biology: automated design of small RNA devices in bacteria. *Trends Genet* **29**, 529–536 (2013).

### **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.