



PhD | Real time optogenetic control of gene expression

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Cells are complex, autonomous genetic machines with rich information processing capabilities. Understanding the regulatory networks that program cells, and modifying and using these mechanisms for human benefit (health, environment, chemistry, and biotechnology) is the main goal of synthetic biology. In this context, **it is crucial to ensure that engineered cells do precisely what they are supposed to do**, but - at present - robust, safe and high-fidelity synthetic circuits are still incredibly difficult to design and implement. **A major problem is the difficulty of establishing quantitative and predictive models of gene networks and synthetic circuits.** The main limitations are (1) the limited knowledge of the cell state that can be obtained via time lapse fluorescence imaging (at best, only a small number of fluorescent reporters can be used to track a few genes at once) and (2) the existence of stochastic processes (also termed noise) associated with gene transcription, which leads to cell-cell variability. **Another major issue is the fact that a synthetic circuit depends on the physiology and metabolic capability of the host cell. To date, there is no satisfying method to ensure the robustness and fidelity of any synthetic circuits over a long period of time and under a broad range of operating conditions.**

Obtaining inspiration from physical engineering and control theory, an original solution would be to add an external feedback loop control that can - in real-time - monitor the cell state and activate or repress the synthetic program to guarantee proper, robust functioning^{38,39}. **The principle of controlling a dynamic system using a feedback loop has been extensively employed in engineering and is a key feature of most electromechanical tools used in everyday life.** This permits real-time compensation for environmental fluctuations, system variability and unknown dynamics. **Implementing such strategy in biological systems turned out to be a challenge that we recently solved.** In particular, we were the first to successfully force the level of expression of a fluorescent reporter gene in yeast to follow a time varying profile at the population and at the single cell level over multiple cell generations. This puts us in a unique position to develop experimental systems aiming at controlling cellular processes through cell-computer interfaces.

In this context, we are looking for a talented Physicist or Biologist to develop single cell optogenetics for the control of gene expression and signaling pathways using yeast and bacteria as model organisms. The specific objectives of this project are to develop a real time, computer based feedback loop control of gene expression for single yeast cells based on optogenetics and to explore its control capabilities with respect to (1) the stochastic nature of the gene expression, (2) the complexity of the synthetic circuit the gene under control is part of and (3) the fluctuations of the physico-chemical environment.

The successful candidate will join an interdisciplinary team (3 PIs, 4 Postdocs, 6 PhDs), working on biophysics, systems biology and synthetic biology. A Common theme in our research is the study of how information flows in biological systems, from signaling pathways in single cells, to collective processes in embryogenesis and multicellular organisms behaviors. We use microfabrication, microfluidics, genome editing, fluorescence and super resolution microscopy to produce quantitative measurement of living organism dynamical processes. We also use advanced modeling and theoretical tools to extract single cell parameters. Our long term goal is to improve our ability to interact and control cellular processes with a



specific focus on gene expression and cell-computer interfacing. Our team is hosted by the Physics department of the University Paris Diderot / CNRS in Paris and benefits from the support of several facilities and grant agencies.

Relevant Publications of the team:

J. Uhlendorf, A. Miermont, T. Delaveau, G. Charvin, F. Fages, S. Bottani, G. Batt*, **P. Hersen***, Long-term model predictive control of gene expression at the population and single-cell levels; **PNAS**, 109 (35), 14271-6 (2012)

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D. Guet, L.T. Burns, S.Maji, J. Boulanger, **P. Hersen**, S.R. Wentte, J. Salamero and C. Dargemont, Combining high spatial-temporal resolution of Spinach-tagged RNA and gene loci localization to image gene expression in live yeast, **Nature Communications** (2015).

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