Master in Photonics – "PHOTONICS BCN"
Master ERASMUS Mundus "EuroPhotonics"

MASTER THESIS PROPOSAL

Starting full time from April 2025
Presentation at the end of July or beginning of September 2025

Laboratory: Single Molecule Biophotonics group
Institution: ICFO- Institute of Photonic Sciences
City, Country: Castelldefels, Barcelona, Spain

Title of the master thesis: Single molecule dynamic approaches to assess entropy production of living cells

Name of the master thesis supervisor and co-supervisor: Maria Garcia-Parajo
(for external proposals a co-supervisor from the Master program and a collaboration agreement is needed)
Email address: maria.garcia-parajo@icfo.eu
Phone number: +34 93 5534158
Mail address: ICFO, Mediterranean Technology Park, 08860, Castelldefels, Barcelona, Spain

Keywords: single molecule fluorescence imaging, single particle tracking, entropy production, thermodynamics, cortical actin, membrane receptors.

Summary of the subject (maximum 1 page):

Living cells maintain themselves out-of-equilibrium by constantly absorbing and dissipating energy, residing in states known as non-equilibrium steady states (NESS). The constant dissipation of energy in the form of heat is known as the entropy production rate σ. Its measurement provides access to NESS and to understanding how life works. Yet, despite its importance, measuring σ at the molecular, sub-cellular and full cell levels has never been attempted.

Recently, one of our collaborators introduced an approach that combines experimental measurements of forces or nanometric displacements combined with simple theoretical models of active Brownian motion[1]. The main outcome is the so-called *variance sum rule* (VSR), a mathematical equality encoding the balance between the energy injected into a system, the part used for housekeeping tasks, and the part that is dissipated as heat (σ)[1]. Thus, by essentially measuring the displacements of a molecular probe as its diffuses in a living cell, one should be able to estimate the local entropy production σ in a living cell system. In this project, we aim at exploiting single molecule imaging and tracking approaches to measure the diffusion of receptors located in the plasma membrane and to infer from the particle displacements the entropy production occurring at the cell cortex.

The eukaryotic cell cortex is a key driver of cell migration morphogenesis and cell division, that comprises the plasma membrane and the underlying cortical cytoskeleton. In particular, the actin cytoskeleton is formed by actin filaments (F-actin), the motor protein myosin-II and other actin-binding proteins that perform mechanical work via the consumption of ATP with the concomitant generation of heat. Importantly, transmembrane proteins located in the plasma membrane interact with surrounding lipids and cholesterol as well as with

components of the external and internal cell machinery, i.e., the actin cytoskeleton. Those interactions result in the spatiotemporal compartmentalization of receptors and play a key role regulating receptor function.

In this project, we will monitor the diffusion of the prototypical transmembrane receptor CD44 that directly interacts with the cortical actin cytoskeleton using single molecule imaging and tracking approaches under varying ATP conditions. Lateral displacements of CD44 positions will be analysed using different algorithms to derive their diffusion behaviour under varying ATP conditions. Moreover, we will apply a novel single molecule methodology developed in our group termed HiDenMaps in combination with a Rivers algorithm to describe spatiotemporal maps of cortical actin and myosin-II activity under different ATP conditions[2,3]. In parallel, molecular displacements will be used as input to the VSR formalism in collaboration with the group of Prof. F. Ritort at the UB. Our final aim is to obtain for the first time an entropy production map of the cell cortex.

References:

1. I. di Terlizzi, et al., Variance sum rule for entropy production. **Science** 383, 971-976 (2024).
2. P. Sil, N. Mateos et al., Dynamic actin-mediated nanoscale clustering of CD44 regulates its mesoscale organization at the plasma membrane. **Mol. Biol. Cell**, 31, 561 (2020).
3. N. Mateos et al., High-density single-molecule maps reveal transient membrane receptor interactions within a dynamically varying environment. arXiv preprint **arXiv:2307.07334**.

Objectives:

1.- Becoming familiar with single particle tracking approaches based on fluorescence imaging by means of literature reading and experiments.
2.- Recording the diffusion of individual CD44 molecules on the plasma membrane of living cells at different fluorescent labelling densities.
3.- Analyse the diffusion of individual CD44 using standard algorithms developed in our Lab.
4.- Reconstruct HiDenMaps of CD44 with high labelling conditions & analyse the data using the Rivers algorithm.
5.- Perform similar experiments and analysis under varying conditions of ATP.
6.- Exploit CD44 molecular displacements under different ATP conditions for VSR methodology (in collaboration with Ritort Lab at the UB).

Additional information (if needed):

* Required skills:
1) Preferably a Master student with a strong Physics background and affinity for biophysics.
2) Experimental skills are highly desirable.
3) No previous experience in Biology is needed for the project, although it is expected that the student rapidly shows interest and develops experimental skills in the biological topic related to the project.