2023-12-19

International PhD Program IC-PhD

# PhD thesis project

2024 Call for application

# Diffusion of active transporters in bio membranes

## General information

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| **Call** | 2024 |
| **Reference** | 2024-11-REGMI_BASSEREAU |
| **Keyword(s)** | Membrane protein clustering; conformations; non-equilibrium activity of proteins, single-molecule biophysics |

## Director(s) and team

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| **Thesis director(s)** | Raju Regmi & Patricia Bassereau |
| **Research team** | Membranes and Cellular Functions |
| **Research department** | UMR 168 – Physical Chemistry Curie |

## Description of the PhD thesis project

**Abstract**

Fundamental cell physiology originates from conformational changes and diffusion dynamics of membrane proteins embedded in the lipid bilayer. However, studying the interplay between nanoscale protein conformations and membrane mechanics is inherently challenging in cell-based experiments. In addition, the effect of membrane properties on the conformational cycles, and thus in the protein's activity remains an open question. In this thesis, we aim to investigate the diffusion dynamics and conformational heterogeneity of multidrug resistance ABC transporter BmrA as a function of its conformation, post-hydrolytic (closed) and apo (open). Diffusion analysis might reveal the formation of protein clusters in the different conditions. To this end, the PhD student will perform single-particle tracking measurements and single-molecule FRET experiments on BmrA reconstituted in giant unilamellar vesicles (GUVs) with controlled and varying membrane tensions. Combining advanced optical methods with biochemical approaches, this project will bring fundamental insights into the intrinsic diffusion dynamics of an active membrane transporter and its clustering in relation to membrane mechanics and conformational cycles; all possibly influencing the cell function.

**Background**

Our team has pioneered studies on membrane protein dynamics using micropipette-aspirated GUV systems combined with single-molecule tracking. More recently, during her PhD, A. Damm obtained evidence of membrane curvature-induced enhanced opening of protein conformations and thus perturbing its activity. The new PhD student will benefit from these established biochemical assays and custom built single-molecule microscopes, to tackle open questions in membrane biophysics to systematically decouple the role of intrinsic protein dynamics from protein-protein interactions and membrane-mediated effects.

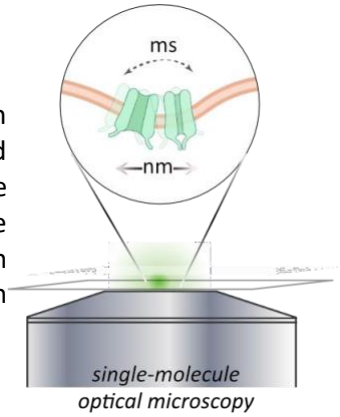
**Objectives**

The proposed PhD project has two main research objectives with focus on investigating the,

1. **Effect of membrane tension on BmrA catalytic cycle** (and thus its activity): We will employ GUV assay at very low protein density to probe the effect of membrane stretching on the dynamics of conformational changes of single transporter. Using single-molecule FRET coupled to single molecule tracking, we will measure the ATP dependence of the opening and closing rates for varying tensions relevant for physiological conditions. These results will be compared to a theoretical model developed by Pierre Sens's group.
2. **Effect of protein conformational dynamics on its diffusion in membranes:** Our team have discovered that proteins with similar sizes, but different shapes can have different motilities when the membrane tension is lowered. However, the mobility of a protein that constantly switches conformation is not known. To this end, we aim to measure the effect of protein activity on the mobility of single BmrA in membranes using single-particle tracking. Moreover, we expect that membrane deformations induced by the conformational switches might generate active protein clusters in the membrane, revealed by the protein mobility.

**Experimental approaches**

The PhD candidate will benefit from training in experimental biophysics. In particular, concepts and tools regarding the use of biomimetic systems combined with state-of-the-art single-molecule fluorescence spectroscopies will be employed to study spatial-temporal dynamics of membrane proteins (also see Figure below). The candidate will also work in close collaborations with theoreticians to provide physical models/frameworks of protein fluctuating/clustering while undergoing conformational switches in membranes.




## International, interdisciplinary & intersectoral aspects of the project

The PhD candidate will benefit from the existing network, both national and international teams including computational microscopy and machine learning approaches for single-molecule data analysis. This project will be developed in close collaboration with Daniel Lévy (membrane protein expert) within the department and with the theoreticians of the lab.

We employ biochemistry (for in-vitro protein reconstitution), synthetic biology (such as click-chemistry for site-specific fluorescence labeling), and advanced optical methods (single-molecule FRET, single particle tracking, fluorescence correlation spectroscopy) to explore the influence of conformations and protein diffusion in bio membranes. Thus, the PhD candidate will benefit from a highly interdisciplinary and multinational research environment located in the historic neighborhood of Paris.

## Recent publications

1. Damm A. *Interplay between the conformational dynamics of a transmembrane protein and the mechanical properties of its surrounding membrane* (Doctoral dissertation, Sorbonne Université, 2019), updated results arriving soon in bioRxiv.
https://theses.hal.science/tel-03330142

2. Srinivasan S, Regmi R, Lin X, Dreyer CA, Chen X, Quinn SD, He W, Coleman MA, Carraway III KL, Zhang B, Schlau-Cohen GS. Ligand-induced transmembrane conformational coupling in monomeric EGFR. Nature Communications. 2022;13(1):3709.
https://www.nature.com/articles/s41467-022-31299-z

3. Regmi R, Srinivasan S, Latham AP, Kukshal V, Cui W, Zhang B, Bose R, Schlau-Cohen GS. Phosphorylation-dependent conformations of the disordered carboxyl-terminus domain in the epidermal growth factor receptor. The journal of physical chemistry letters. 2020;11(23):10037-44. https://pubs.acs.org/doi/abs/10.1021/acs.jpclett.0c02327

4. Quemeneur F, Sigurdsson JK, Renner M, Atzberger PJ, Bassereau P, Lacoste D. Shape matters in protein mobility within membranes. Proceedings of the National Academy of Sciences. 2014;111(14):5083-7. https://www.pnas.org/doi/abs/10.1073/pnas.1321054111

5. Tsai FC, Simunovic M, Sorre B, Bertin A, Manzi J, Callan-Jones A, Bassereau P. Comparing physical mechanisms for membrane curvature-driven sorting of BAR-domain proteins. Soft Matter. 2021;17(16):4254-65. https://pubs.rsc.org/en/content/articlelanding/2021/sm/d0sm01573c/unauth

## Expected profile of the candidate

Interdisciplinary profile with exposure to either of the following research domains:

- Biochemistry of membrane proteins, and model membrane systems
- Fluorescence microscopy and/or advanced optical techniques such as single particle tracking and single molecule FRET experiments

Expected courses/training include topics on soft matter, biophysics, optics, etc.