

Biophysical investigation of the interplay between biomolecular condensates and lipid membranes: the case of Clathrin-Mediated Endocytosis

Supervisor: Pierre-Emmanuel Milhiet. pierre-emmanuel.milhiet@cnrs.fr

Co-supervisor: Andreas Santamaria (post-doc). andreas.santamaria@cnrs.fr
Centre de Biologie Structurale, University of Montpellier, INSERM, CNRS, Montpellier, France

Introduction

Biomolecular condensates are liquid assemblies of biological material (proteins, DNA, RNA) that are formed via liquid-liquid phase separation. Briefly, transient and multiple interactions between biomolecules drive the formation of assemblies organized in a dynamic network of molecules. Therefore, condensates can be viewed as droplets with a specific molecular composition that play an essential role in subcellular compartmentalization and the regulation of cellular processes, including clathrin-mediated endocytosis (CME). CME is essential for nutrient uptake and cellular homeostasis. It is the primary internalization route for receptor tyrosine kinases (RTKs), whose dysregulated signalling is a hallmark of cancer. Among CME initiation proteins, FCHo1/2 and Eps15 are key regulators whose absence severely impairs endocytosis. FCHo1/2 and Eps15 form biomolecular condensates at the plasma membrane, which trigger CME. The physical properties of these condensates regulate CME kinetics and efficiency, supporting a **paradigm in which the interplay between membranes and biomolecular condensates controls membrane remodelling**. However, the **molecular mechanisms underlying condensate-membrane interactions** and the consequent membrane remodelling, **remain poorly understood**. This project aims to investigate CME regulation through FCHo1/2-Eps15 condensation, focusing on (1) determining **condensate properties** (surface tension and viscosity), which govern condensate biological functions, and (2) investigating **condensate-driven membrane remodelling**, essential for a successful endocytic event. The student will work side-by-side with experts in the field of condensates and membranes, using advanced microscopy techniques such as atomic force microscopy (AFM; imaging, force spectroscopy) and Fluorescence microscopy (wide-field, and in correlation with AFM).

Project research axes

The production, purification and fluorescent labelling of Eps15 are routinely performed.

Axis 1: biophysical characterization of CME condensates

Surface tension and viscosity govern condensate behaviour and membrane interactions and are therefore expected to influence CME progression. This axis will focus on characterizing the material properties of Eps15-FCHo1/2 condensates and determine how they vary with membrane and protein composition. Condensate properties will be investigated using fluorescence recovery after photobleaching (FRAP), passive microrheology, fusion experiments (**Figure 1A**), and Fluorescence Recovery After Probe-Induced Dewetting (FRAP-ID), a novel methodology based on AFM and fluorescence microscopy that has been developed in the lab.

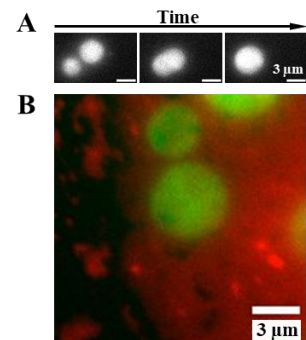


Figure 1 Wide-field fluorescence microscopy showing (A) fusion event of Eps15 condensates. (B) Eps15 condensates marked with Atto488 (green) lying on a model membrane (bilayer) marked with Rhodamine B (red). Scale bars are 3 μm.

Axis 2: Membrane remodelling activity of CME condensates

This axis will determine how condensate composition and material properties regulate membrane remodelling activity. I expect to establish quantitative relationships between condensate surface tension, membrane morphology and structure, lipid packing, and membrane curvature. AFM and fluorescence microscopy will be employed to investigate the morphology of membranes and condensates (**Figure 1B**). Finally, neutron reflectometry (NR) will be used to characterize how condensates influence membrane structure at the Ångström scale. It is a reciprocal-space technique within the expertise of the lab members that can provide information on membrane thickness and water content.

Feasibility & Uniqueness

Preliminary data acquired so far (AFM, fluorescence microscopy, and NR) support the project's feasibility. Although techniques such as AFM and NR are rarely used to investigate condensates and

condensate-membrane interactions, they can provide key information for a thorough investigation of the condensate-membrane interplay.

Student responsibilities and activities

The candidate will prepare condensates with different protein compositions (Eps15 and Eps15+FCHo1/2) and use AFM and fluorescence microscopy to determine condensate surface tension and viscosity. The student will prepare model membranes with different compositions and evaluate how membrane binding influences condensate properties.

Finally, we have submitted three proposals to use the neutron reflectometer at the Institut Laue-Langevin (ILL) in Grenoble. If accepted, the student will join us in performing experiments (beamtime). We strongly believe that the beamtime experience would enrich the student's scientific background.

References

- Milhiet, P-E, et al. *The Scientific World Journal*. **2003**;3(1):59-74.
Owen DJ, et al. *Annu Rev Cell Dev Biol*. **2004**;20(1):153-191.
Milhiet, P-E, et al. *Biophys J*. **2006**;91(9):3268-3275.
Milhiet, P-E, et al. *PLoS One*. **2010**;5(10): e13240.
Day KJ, et al. *Nat Cell Biol*. **2021**;23(4):366-376.
Vial, A. et al. *Nanoscale*. **2021**;13:12484-12493.
Hutin, S. et al. *PNAS*. **2023**;120.
Linsenmeier, M. et al. *Nat Chem*. **2023**;15:1340-1349.
Santamaria, A, et al. *Biophys J*. **2024**;123(19):3366-3374.
Malady, B.T. et al. *Nature Comm*. **2025**;16(1):9139.