Fluorescence tools to characterize Liquid-Liquid Phase Separation

Liquid-liquid phase separation (LLPS) is a thermodynamically driven phenomenon during which a homogeneous solution de-mixes to form two separate liquid phases. One appears as dense droplet like structures dispersed in a second more diluted phase. During the last decade, LLPS has emerged in the field of cellular biology as a fundamental phenomenon driving the assembly of intracellular biomolecular condensates also called membrane-less organelles (MLOs). Among these the nucleolus, Cajal bodies, P bodies and stress granules are the most known.

MLOs represent multicomponent system that displays droplet-like properties such as ability to flow and coalesce. Due to their transient and dynamic nature, these organelles play major roles in gene regulation and signaling pathways or serve as storage compartments making the biological material immediately available. Hence, MLOs internal environment is optimally tuned to ensure efficient biochemical reactions together with an optimal exchange rate with its external environment. Consequently, the deregulation of LLPS process is associated with pathologies including cancer, neurodegenerative and infectious diseases. At present, the biophysical understanding of LLPS, as a process orchestrating the MLOs assembly and internal organization, is only emerging and the experimental tools used to characterize the LLPS *"in vitro"* and *"in cellulo"* are limited.

The aim of this PhD project is to develop quantitative fluorescence imaging to shed new light on the internal dynamics and the physicochemical properties of the condensates. We propose to expand conventional fluorescence imaging and single molecule localization microscopy using environment-sensitive dyes (solvatochromic probes and molecular rotors) to measure the polarity and/or the viscosity of condensates. Such ambitious experiments will allow us to monitor the biophysical properties of the condensates with improved spatial and temporal resolutions. In parallel, LLPS will be studied at molecular level by physicochemical characterization of *in vitro* reconstituted condensates to interpret the data obtained *in cellulo* and ultimately to uncover the relationship between the structure, dynamics and the biophysical properties of the condensates.

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Supervisors:

Halina ANTON halina.anton@unistra.fr
Pascal DIDIER pascal.didier@unistra.fr

Laboratory of Bioimaging and Pathologies https://lbp.unistra.fr/en/
Team "Photoactive materials and bioimaging"