University of Basel

Swiss Nanoscience Institute

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# NanoPhase: A multi-scale view of phase separation from cells to nanostructure

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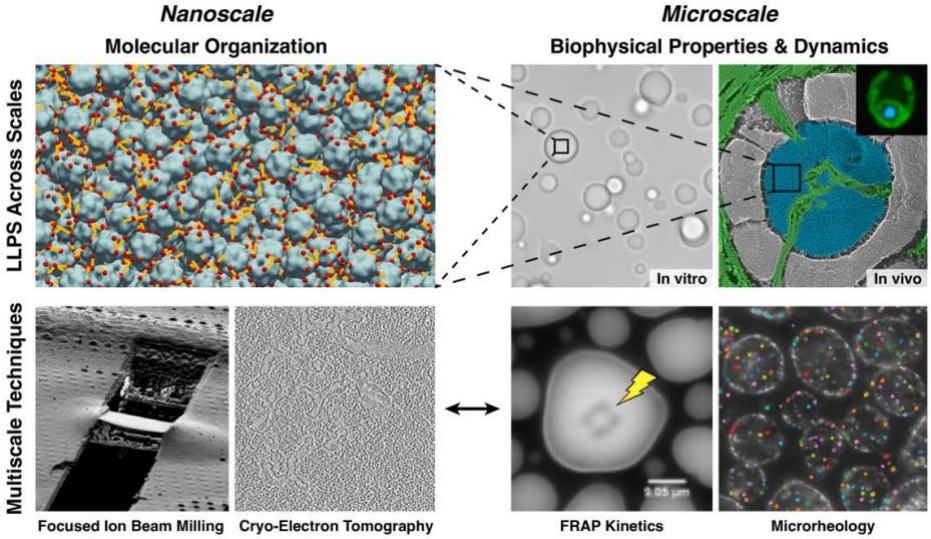
The Engel and Hondele labs, both located at the Biozentrum Basel, are looking for a joint PhD student to work on an innovative and multidisciplinary project at the interface between cryo-electron tomography, biophysics and cell biology. The position should start as soon as possible after January 1st, 2023, so please apply early. We offer an interactive and supportive work environment, and training with state-of-the art techniques focused on highly relevant biological questions.

Come join us at Biozentrum!




**Biomolecular condensation** and **liquid-liquid phase separation (LLPS)** have emerged as major principles of cellular organization and the orchestration of diverse biochemical processes, including RNA processing and carbon fixation. LLPS is based on weak, multivalent interactions that result in highly dynamic, flexible and reversible “membraneless organelles” (MLOs) that vary in size, composition, material properties, regulation and function [1]. Both *in vitro* (when reconstituted from recombinant proteins) and inside cells, biomolecular condensates have mostly been studied at the **microscale** using light microscopy techniques such as fluorescence recovery after photobleaching (FRAP) and passive microrheology.

However, to date, the **nanoscale** molecular architecture of MLOs has been visualized for very few examples – notably including the pyrenoid by the group of Prof. Ben Engel. It remains a fundamental unanswered question whether and how changes in microscale biophysical parameters correlate with architectural changes at the resolution of single protein complexes. Therefore, we propose to develop a multi-scale approach – combining light microscopy, biophysical approaches, and cryo-electron tomography – that bridges the resolution gap between cell biology and structural biology to understand how the molecular organization of LLPS condensates correlates with their biophysical properties both *in vitro* and inside cells.

We will use two condensate model systems that are well established in the Engel and Hondele labs:

**Pyrenoids** are phase-separated chloroplast microcompartments that perform over a quarter of global $CO_2$ fixation. The interaction of Rubisco with its multivalent linker protein EPYC1 drives pyrenoid assembly in the green alga *Chlamydomonas* [2, 3], and is sufficient to form liquid condensates both *in vitro* [4] and upon heterologous expression in plants [5]. The binding interface between Rubisco and EPYC1 has been structurally mapped at single amino acid resolution, and this information was then combined with cryo-ET to reconstruct the first *in vivo* interaction network of a phase-separated condensate [6].

**DDX3X** is an RNA-dependent DEAD-box ATPase that is crucial for translational regulation and stress granule (SG) formation. Interestingly, ATPase-deficient mutants have been described in various medulloblastomas, and cells expressing these mutants have more viscous SG [7].

Both systems – as rare examples in the MLO field – have a functional readout ($CO_2$ fixation for the pyrenoid, translation efficiency for DDX3X-controlled SG), which allows us to measure the biological impact of altering condensate structure and biophysical properties.

**Impact:** This project will innovate by bridging scales between cellular MLOs and nanostructure, directly linking their fundamental biological function and biophysical parameters to their nanometer-scale molecular organization. Thus, it will not only provide novel biological insights – with the promise to e.g. engineer pyrenoids into plants to increase crop yield and resist climate stress [8] – but also develop revolutionary tools that will allow the LLPS field to study any system of interest.


[1] Shin Y, Brangwynne CP. "Liquid phase condensation in cell physiology and disease." Science, 357.6357 (2017).

[2] Mackinder LC, et al. “A repeat protein links Rubisco to form the eukaryotic carbon-concentrating organelle.” PNAS, 113: 5958-5963 (2016).

[3] Freeman Rosenzweig ES, et al. «The Eukaryotic $CO_2$-Concentrating Organelle Is Liquid-like and Exhibits Dynamic Reorganization.” Cell, 171: 162.e119 (2017).

[4] Wunder T, et al. “The phase separation underlying the pyrenoid-based microalgal Rubisco supercharger.” Nat Commun, 9: 5076 (2018).

[5] Atkinson N, et al. "Condensation of Rubisco into a proto-pyrenoid in higher plant chloroplasts.” Nat Commun, 11: 6303 (2020).

[6] He S, et al. “The structural basis of Rubisco phase separation in the pyrenoid.” Nat Plants, 6: 1480-1490 (2020).

[7] Valentin-Vega YA., et al. "Cancer-associated DDX3X mutations drive stress granule assembly and impair global translation." Sci Rep, 6.1: 1-16 (2016).

[8] Hennacy JH, Jonikas MC. “Prospects for Engineering Biophysical $CO_2$ Concentrating Mechanisms into Land Plants to Enhance Yields.” Annu Rev Plant Biol, 29;71:461-485 (2020).