M2 internship 2022

Spatial mapping of molecular crowding in living cells via phase separation induced by optogenetics

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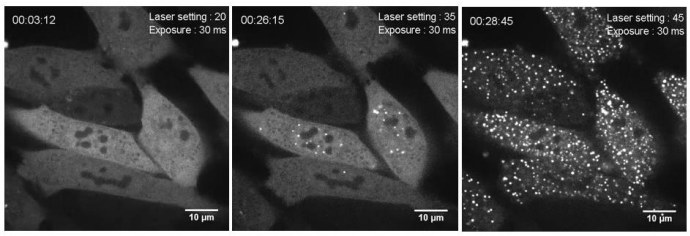
Abstract:

The interior of cells is a medium heavily encumbered by the high density of macromolecules. The space accessible to biological molecules is a crucial parameter because it controls their diffusion as well as their local concentrations, and therefore it controls the reactivity of biological processes. This molecular hindrance is probably inhomogeneous at the subcellular scale, and it is possible that the biochemical activity of the cell is spatially regulated by local hindrance. It is currently very difficult to measure molecular crowding with spatial resolution. The subject of this internship will be to develop a new method, based on the optogenetic control of liquid-liquid phase separation, to map spatially the molecular crowding in a living cell.

Liquid-liquid phase separation is a non-linear process: above a critical concentration, a molecule separates from its solvent and forms droplets. Recently, work has shown that genetically encoded proteins, initially soluble in the cytosol, can be activated by light to induce phase separation. We will use such proteins, and by finely adjusting the amount of light we will reach the critical threshold of concentration. We will then be able to see where in the cells the droplets form first - and therefore identify the most crowded places of the cell interior. By gradually increasing the amount of light, we will then classify cell regions according to their local crowding.

Experimental techniques

The intern will learn the basics of cell culture, transfection, live cell fluorescence microscopy as well as optogenetics.



Example of phase separation induced by optogenetics (after Shin et al, Cell 2017): initially the protein is soluble in the cytosol, then at the threshold of phase separation droplets are formed - in this example preferably in the nucleus - and finally beyond the threshold the demixing is complete.