Internship project Master 2
Year 2018-2019

Laboratory/Institute: ILL/IBS

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Title of the project:

Understanding how coacervation of two viral proteins drives the formation of membrane-less viral factories

Background

Micron-scale membrane-less compartments are found in cells infected by several types of negative-sense RNA viruses (rabies virus, Ebola virus,...) forming **viral factories** in which viral replication and assembly occur (Negri 1903; Lahaye 2009; Hoenen 2012). Recent studies have shown that some **membrane-less compartments** have typical properties of liquids and are formed by **liquid-liquid phase separation** (Hyman 2014). The separation is typically induced by **multivalent interactions** between proteins composed of multiple modular interaction domains and/or proteins containing intrinsically disordered regions (IDRs), or by RNA or DNA molecules that provide multiple binding sites for other nucleic acid molecules or proteins (Brangwynne 2015; Banani 2017). It results in the co-existence of a condensed phase in which the interacting macromolecules are concentrated and a second phase in which they are diluted. However, little is known about the organisation of the condensed liquid phase, about its physicochemical properties or about the dependence of the process on the nature of the attractive interactions, their valence, interaction strength, and interaction range.

Rabies virus (RV) infection induces the formation of cytoplasmic inclusions, which are called Negri's bodies (NBs) (Negri 1903). Previously, it has been shown that co-expression of only N and P in cells is sufficient to induce the formation of cytoplasmic inclusions similar to NBs (Chenik 1994; Nikolic 2017). Our hypothesis is that for rabies virus the separation is induced by multivalent interactions between the viral nucleoprotein (N) and phosphoprotein (P) in the presence of RNA.

This multidisciplinary project is at the interface between soft-matter physics and biophysics (biochemistry) and it relies on a collaboration between a team of molecular virologist at the IBS and a team of the Large Scale Structure group at the ILL.

Objectives

Our primary goal for the M2 internship is to reconstitute this biological coacervation process *in vitro* with highly purified proteins and to investigate the role of different solution parameters on this process. The overall goal of the project (PhD thesis) is to use **neutron scattering and neutron diffraction experiments** on different instruments at the ILL to probe these molecular processes at different time and length scales and to use complementary methods to better understand the assembly of intracellular membrane-less compartments and to decipher the aspects of protein chemistry and of polymer physics that lead to liquid-like states.

Methods

- protein expression, purification, quality control and concentration
- phase diagrams from visual observations, by variation of the composition and

temperature
- differential scanning calorimetry and isothermal titration calorimetry
- measure and analysis of Dynamic Light Scattering, Static Light Scattering and Small Angle Neutron Scattering

Relevant publications

Liquid-liquid phase separation in dilute solutions of poly(styrene sulfonate) with multivalent cations: Phase diagrams, chain morphology, and impact of temperature. Hansch M, Hämisch B, Schweins R, Prévost S, Huber K. J Chem Phys. (2018)148:014901. doi: 10.1063/1.5006618.

Inward growth by nucleation: Multiscale self-assembly of ordered membranes. Landman J, Ouhajji S, Prévost S, Narayanan T, Groenewold J, Philipse AP, Kegel WK, Petukhov AV. Sci Adv. (2018) 4):eaat1817. doi: 10.1126/sciadv.aat1817.

Ensemble Structure of the Highly Flexible Complex Formed between Vesicular Stomatitis Virus Unassembled Nucleoprotein and its Phosphoprotein Chaperone. Yabukarski F, Leyrat C, Martinez N, Communie G, Ivanov I, Ribeiro EA Jr, Buisson M, Gerard FC, Bourhis JM, Jensen MR, Bernadó P, Blackledge M, Jamin M. J Mol Biol. (2016) 428:2671-2694. doi: 10.1016/j.jmb.2016.04.010.

Ensemble structure of the modular and flexible full-length vesicular stomatitis virus phosphoprotein. Leyrat C, Schneider R, Ribeiro EA Jr, Yabukarski F, Yao M, Gérard FC, Jensen MR, Ruigrok RW, Blackledge M, Jamin M. J Mol Biol. (2012) 423:182-197. doi: 10.1016/j.jmb.2012.07.003.