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

Rabies virus (RABV) causes fatal encephalitis in human. At the cellular level, infection by RABV induces the formation of cytoplasmic inclusion bodies called Negri's bodies, which have the properties of liquid-like compartments (Nikolic et al. 2017) formed by phase separation and constitute viral factories (Nikolic et al. 2016). The expression of two viral proteins, the nucleoprotein (N) and the phosphoprotein (P), in cultured cells proved to be sufficient to reproduce phase separation and the formation of Negri body-like compartments (Nikolic et al. 2017).

We reconstitute the liquid-liquid phase separation with purified hydrogenated N as well as hydrogenated and deuterated P proteins in the prospect of using small-angle neutron scattering experiments to probe the relative arrangement of biomacromolecules in these densely packed microphases.

To decipher the physico-chemical principles underlying protein-induced liquid-liquid phase separation leading to the self-coacervation of these proteins into membraneless compartments, particularly in the context of viral infection by certain RNA viruses, SANS and SAXS measurements were performed at the Institut Laue-Langevin (ILL) and ESRF. We will present results obtained for proteins in dilute conditions away from phase separation at various ionic strengths as well as over a wide range of concentrations and temperatures, to introduce our proposed mechanism of protein-protein interaction that leads to a fully reversible macroscopic phase transition but only a partially reversible protein quaternary structure.

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