Project title: Liquid-liquid phase separation of RNA splicing-related proteins and their regulation

PI: Nasrollah Rezaei-Ghaleh

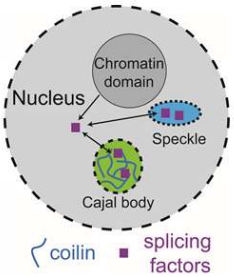
Laboratory: Structural Dynamics of Proteins in Health and Disease, Unit of Immunology and General Pathology, DMM

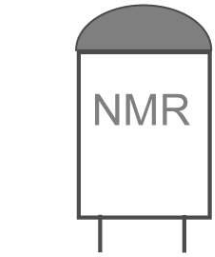
Main Abstract: Cellular activities frequently occur within membrane-less compartments, which are formed through a physicochemical process termed liquid-liquid phase separation (LLPS). The physiological function of these biomolecular condensates is regulated by various cellular signals through mechanisms such as posttranslational modifications (PTMs). Increasing evidence is accumulated supporting a link between phase separation and diseases such as cancers, infections and neurodegenerative diseases. The proposed research project will investigate proteins involved in the formation of nuclear bodies, such as Cajal bodies (CB) and nuclear speckles, which are prototypical examples of highly dynamic cellular bodies and act as the major sites for the storage, modification and assembly of RNA splicing factors. It is suggested that these nuclear bodies are formed through LLPS and the exchange of the splicing factors between them plays a key role in the compositional dynamics of spliceosome, a highly dynamic ribonucleoprotein assembly involved in RNA splicing. Of particular focus in this project will be the protein coilin (UniProt: P38432), which has no known function other than being the scaffold of CBs. Coilin is a 576 residue long protein, containing a disordered domain coupled to an N-terminal folded domain with a propensity for coilin-coilin self-interaction. Coilin has a large content of glycine (8%), and we have previously shown that the large nanoseconds dynamics of glycine residues in an RG-rich fragment of coilin is largely preserved within the phase-separated coilin droplets, potentially supporting the rapid component exchange dynamics in them. Coilin is also enriched in lysine (10%) and serine (13%), which can be modified through several PTMs at different stages of the life cycle of CBs, regulating their assembly/disassembly during mitosis and component exchange dynamics. In addition, coilin contains 35 prolines, which makes it a potential substrate for nuclear cyclophilins with peptidyl-prolyl isomerase (PPIase) activity.

Aims: The general objective of the project is to understand the molecular mechanisms governing the LLPS of RNA splicing-related biomolecular condensates. Some specific objectives are:

1- To determine the phase behavior of coilin in *in vitro* conditions in un-modified and modified forms;
2- To characterize the high-resolution structure and dynamics of the intrinsically disordered regions of coilin in different phases and investigate the effect of relevant PTMs on them;
3- To determine molecular factors underlying biophysical properties of condensates formed by coilin;
4- To determine the rate of component exchange between coilin condensates and their environment and characterize factors influencing them;
5- To monitor and characterize the maturation process of coilin condensates;
6- To determine the internal structure of fresh and aged coilin condensates;
7- To characterize the effect of PPIase activity on biophysical properties of coilin condensates;
8- To monitor and characterize the *in-vivo* LLPS of wild-type and PTM-mimicking mutants of coilin;
9- To develop new NMR methodologies, especially in relation with 19F and singlet-state NMR, to expand our experimental access to the structural dynamics of proteins undergoing LLPS.

Techniques: NMR spectroscopy, fluorescence and circular dichroism spectroscopy, confocal microscopy, protein expression and purification, biochemical analysis, computational methods.





NMR method development