NOVEL METHODS FOR STRUCTURAL BIOLOGY OF LARGE SYMMETRIC PROTEIN ASSEMBLIES BY SOLID-STATE NUCLEAR MAGNETIC RESONANCE WITH ULTRAFAST MAGIC-ANGLE SPINNING AND PROTON DETECTION

Proteins are essential building blocks in living organisms. They regulate cell functions, synthetize and destroy other proteins, transport ions and water through cell membranes etc. For decades scientists have been struggling to gain insight at the atomic level to understand structure and functions of the incredibly varied world of proteins.

One of the interesting observations is that nature tries to save resources and reuse the genetic information. In so doing, large molecular machines are frequently built of smaller proteins, arranged according to high symmetry. An extreme case are viral capsids, which contain hundreds copies of a protein monomer. In bacteria and higher organisms, complex molecular machines such as DNA replisomes and proteasomes often contain ring-shaped symmetric assemblies (trimers, hexamers, heptamers, dodecamers).

Proteins are very dynamic entities, and their function is often realized through transient interactions with other proteins, RNA or DNA. Therefore, static images of single proteins, known from leading structural methods (X-ray crystallography, cryoelectron microscopy and nuclear magnetic resonance (NMR)), are often insufficient to understand their mode of operation. The last method, NMR, exploits subtle magnetism of nuclei of ^{1}H atoms, abundant in biomolecules, to monitor their chemical environment. In this respect, NMR is a unique method to investigate protein-protein or protein-nucleic acids interactions, since it is very sensitive to changes of local structure and dynamics.

In this project we propose to apply NMR to large symmetric protein self-assemblies. In the conventional NMR performed on proteins in solution, the difficulty is caused by the slow tumbling of large proteins, leading to a rapid decay of NMR signal. We thus propose to rely on the novel methods for studying biomolecules immobilized in space which behave as in the solid-state (although macroscopically looking like a gel). One of the most straightforward and non-invasive methods to confine molecules is the sedimentation under very strong centrifugal forces. The effective gravity, as high as 150,000 g in an ultracetrifuge or a few million g in a spinning rotor, keeps molecules in spatial proximity preventing their rotation, without the need for specific intermolecular interactions. At the same time, proteins remain in a near-native highly hydrated environment, in which they are free to interact with other molecules as it occurred in solution.

The “solid” phase built of crowded proteins needs to be rotated rapidly to improve spectroscopic NMR properties. Nowadays, the material is packed into tiny rotors, as small as 1 millimeter of outer diameter, and spun by a focused air jet as fast as 100,000 times per second, making them the fastest rotating mechanical objects ever used by a man. The rotation is performed in a stator titled by the so-called magic-angle (≈54°) with respect to the strong external magnetic field that is generated by a cryogenically cooled superconducting magnet. Equipped with these sophisticated devices, we use radiofrequency waves to acquire information on the local environment of the nuclear magnetic moments.

The specific goal of the project is to develop a strategy for interpretation of complex NMR spectra of large proteins, which simultaneously show hundreds to thousands resonance lines over a limited range of frequencies. We will design new experiments to resolve signal overlap, and support the analysis of experimental data with computational approaches, taking advantage of the availability of the 3D structural models. Additionally, smart biosynthetic methods will be employed to restrict the NMR visible resonances to only one part of the large protein. In this way, we will preserve a site-specific resolution of NMR spectra, where every signal can be attributed to a specific atom in a molecule.

This will enable us to monitor binding and identify interaction interface in one of the component of bacterial DNA replication machinery, a helicase DnaB and its interacting partner DnaGC which, fuelled by ATP, unwind the double-stranded DNA helix and initiate the replication. We will also show the power of NMR on a proteasome 20S from archea, an assembly that unfolds and degrades redundant or misfolded proteins. An additional important outcome of the project will be strengthening of NMR as a highly efficient method for structural and functional investigations of large proteins and their assemblies, which should attract attention of structural biologists and bioengineers in answering their everyday scientific questions.