Abstract

As a standard, both laboratory experiments and simulations of proteins, especially enzymes, are conducted in diluted solutions like buffers or water with ions. However the cytoplasm of a living cell, where the proteins function in nature, provides quite a different environment, with around 30% of the volume taken by macromolecules like RNA, proteins and lipids. Such level of crowding can affect diffusion and conformational dynamics of the proteins, and thus also indirectly affect their activity.

In this thesis I describe investigation of how does crowded environment affect the NS3/4A protease from Hepatitis C virus, which is an example of a protein containing an intrinsically disordered region (IDPr). Such proteins make up almost a half of all proteins, and even larger proportion of viral enzymes. Viruses exploit IDPr to widen a range of functionalities within a very limited number of proteins, by binding to and taking over cellular structures and proteins of the host. Unstructured protein fragments gain more ordered conformations upon approaching binding partners or under changing environment, and so they might be especially prone to be affected by crowding.

To better understand the molecular mechanisms of the effects of crowding on NS3/4A, I combined analysis of laboratory experiments and simulations conducted in analogical conditions. In both cases the protease was observed in water and in the presence of polyethylene-glycol (PEG) and polysucrose. These polymers are among the most often used crowder molecules in the laboratory assays, but hardly ever included in molecular dynamics (MD) simulations. In order to divide observed effects into those caused by volume exclusion and by protein-crowder interactions, I additionally simulated the protease surrounded with coarse-grained crowders of my design.

Thanks to combining observations in two scales - of billions of proteins in the activity assay and one protein in the simulations, I was able to propose a molecular mechanism behind the opposite effects of PEG and Ficoll on the NS3/4A activity, and discover that crowded environment may influence biological functions of the protease, by stabilising a precursor of transmembrane helix at the terminus of NS4A protein - this way facilitating the process of anchoring the protease to the host membrane. I also found that polysucrose crowders, widely thought to be an inert, non-interacting crowder, actually engage in multiple contacts and hydrogen bonds with the studied protein.

New technical methods described in the thesis include parameterisation of all-atom PEG and polysucrose models, that maintain realistic diffusion in the simulations of multiple molecules, and also a design of a spherical bead-shell coarse-grained crowder, that can be used in multi-scale MD simulations with explicit water.