Interaction of influenza virus fusion peptides with lipid bilayers – project overview.

Influenza is one of the five most lethal infectious diseases faced by humans, being responsible for up to 600000 deaths per year world wide. Prevalence of influenza virus in animal reservoir and its high mutation rate facilitating human adaptation pose a constant threat of pandemic outbreak, with potentially catastrophic consequences. One of viable therapeutic strategies against influenza, as well as other enveloped viruses, is targeting their entry into host cells, in particular its stage involving the fusion of viral and cellular membranes.

In the case of influenza virus, membrane fusion is mediated by a hemagglutinin (HA) protein anchored at its surface. This trimeric protein inserts its N-terminal fragments, known as fusion peptides (HAfp), into the target membrane. Than, by undergoing a large conformational change, in a jackknife motion it drags viral and host membranes to close proximity, enabling their subsequent fusion. In this process HAfps act not merely as molecular grappling hooks, but also actively contribute to lipid mixing and early stalk formation. Intriguingly, in the form of synthetic $20 - 23$ amino acid long peptides HAfps are already able to fuse liposomes without the aid of the remaining HA structure. The relevance of HAfp-mediated fusion model to the process induced by complete HA system is indicated by similar fusion abrogating or preserving effects of mutations in the HAfp sequence in both cases.

Given significant threat to public health posed by influenza, HA-mediated fusion has been extensively studied. Virus-induced membrane remodelling was characterised by cryo-electron tomography, atomistic structures of juxtamembrane region of HA trimer in pre and postfusion states were solved by X-ray crystallography, and conformations of isolated, membrane-bound HAfps were determined by nuclear magnetic resonance. In addition a number of studies documented functional effects of amino acid sequence alterations in HA. Still, however, the details of fusion process, including the HAfp-membrane interaction and its role in early stages of fusion process remain elusive, since no experimental method can trace membrane-bound macromolecular systems with sufficient spatial and temporal resolution. The availability of extensive, yet still fragmented experimental data on the one hand, and fast growing power and maturity of computational approaches on the other hand, make for a great opportunity to bridge the gaps and resolve the details of HAfp role in the fusion process.

In this project we will conduct extensive computer simulations of HAfp-membrane systems in fully atomistic resolution coupled to experimental studies. We will characterise configurations (conformations, insertion depths, and orientations) adopted by HAfp within lipid bilayers of various compositions. By confronting them with experimental data that describe fusogenic activity in corresponding systems we will gain insights into structural details of HAfp-lipid interaction that are required for membrane fusion. Our particular attention will be focussed on the effect of varying cholesterol concentration on HAfp activity. Its investigation is important, since modulation of cholesterol metabolism is one of cellular defence mechanisms agains viral penetration, whose details are not yet understood.

Part of the project will be devoted to the characterisation of geometries and membrane placement of HAfp trimers tethered to a rigid HA coiled coil core. This will shed light on the mechanism of cooperative peptide effect on membrane perturbation. It will also allow studies of structural relations between the intramembrane HA segment and its juxtamembrane rigid core during structural rearrangements that the complete protein must execute during fusion process *in vivo*. Finally, by conducting molecular dynamics simulations of HAfp inserted into closely apposed lipid bilayers we will attempt to capture early stages of the fusion process leading to the formation of intermembrane stalk structure. It will help to determine which of the many putative fusion mechanisms considered in the literature, but not yet verified, is the most feasible.

Aside from contributing to better characterisation of HAfp-mediated fusion mechanism, structural insights gained by conducting the project will aid in the development of drugs targeting influenza fusion machinery. The understanding of HA-mediated fusion will also open the possibilities for designing fusion proteins that would allow the construction of cell specific liposome-based cargo delivery systems. Furthermore, owing to general similarities between the mode of action of viral and cellular fusion proteins, any advances in the field of viral fusion will support our knowledge of related processes such as intracellular vesicle trafficking, neurotransmitter release, or eggs fertilisation by sperm cells.