Structural and functional analysis of regulation of the lipid-transport protein ORP8

Among the major components of biological cells are semipermeable membranes. The plasma membrane (PM) separates the interior of the cell from its environment. Other membranes enclose intracellular organelles such as the endoplasmic reticulum (ER), the Golgi apparatus, and mitochondria. All of these membranes are composed of diverse macromolecules – mostly lipids and proteins of different types – and the unique lipid composition of a given membrane is essential for its proper functioning within the cell.

Most lipids are synthesized in the ER and must be transported against their concentration gradients to proper target membranes. The intracellular transport of lipids between various organelles is carried out by specialized proteins. Oxysterol-binding protein (OSBP)-related proteins (ORPs) mediate lipid transfer at membrane contact sites, such as the ER-PM contact sites, which ensures the transport of specific lipids from the place of their synthesis (ER) to target membranes (PM, Golgi apparatus, mitochondria), and also maintains the proper lipid composition of cellular membranes. As such, the process of lipid transfer by ORPs must be tightly controlled.

There are several types of ORPs in human cells. Almost all of them contain three characteristic structural elements, i.e., a lipid binding domain, a lipid transport domain, and a linker between these two domains. The flexibility of this linker plays an important role in the process of lipid transfer by ORPs. However, it also impedes solving the atomic structures of full-length ORPs using conventional methods of structural biology. While the atomic structures of the individual domains of ORP proteins have been solved for most of ORP family members, there is no understanding on how these domains communicate and cooperate. For this reason, the molecular mechanisms of ORP-mediated lipid transfer remain obscure.

As a representative protein of the ORP family we have selected the ORP8 protein because it has a typical domain composition and can be expressed in *E. coli* in amounts needed for biophysical experiments. The overall goal of our project is to elucidate – at the molecular level – the mechanisms of lipid transfer from donor to target membranes by the ORP8 proteins. To attain this goal we will combine several complementary biophysical methods, including X-ray crystallography, small angle X-ray scattering, single-molecule Förster resonance energy transfer, and molecular dynamics simulations. We will use artificial lipid membranes to precisely monitor the dynamics of ORP8 during the process of lipid transfer. This research project will be carried out in close collaboration with the Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences in Prague.