**Structural insight into the peroxisomal protein import – the PEX translocon**

Summary for the general public

The cell constitutes the basic organizational unit of living organisms. Cell interior is divided into a number of compartments (organelles) that separate various biochemical processes. These processes are possible thanks to proteins that facilitate (catalyse) a prevailing part of chemical reactions in living organisms. Most organelles do not have the ability to produce proteins, so they must obtain the proteins from the cellular compartments where protein production takes place. The production of proteins to be delivered to most organelles takes place in the endoplasmic reticulum (ER), and transport occurs by vesicle exchange. Peroxisomes are an exception in this respect - the production of peroxisomal proteins takes place in the cytoplasm and transport into the interior of the peroxisome is facilitated by a protein system called the PEX translocon.

Peroxisomes play an important physiological role. To maintain the function of the peroxisomes, undisturbed transport of proteins from the cytoplasm is essential. Diverse disorders of this transport manifest as a group of human diseases known as peroxisome biogenesis disorders (PDBs). Understanding the molecular basis of this group of diseases through investigation of the PEX translocon will facilitate future development of mitigation strategies against PDBs. At the same time, therapeutic benefits may also result from pharmacological interference in the peroxisomal transport - when the transport is selectively interrupted in the cells of human parasites. Therefore, understanding the peroxisomal transport of proteins is an important topic of modern science.

Proteins intended for peroxisomal transport (hereafter "cargo") are labelled with either of two types of signals: PTS1 or PTS2. PTS1 is recognized in the cytoplasm by the PEX5 receptor, and PTS2 by the PEX5 complex with PEX7 co-receptor. The cargo/PEX5 or cargo/PEX5/PEX7 complexes then dock on the receptors anchored in the peroxisome membrane (PEX14 and PEX13). The next step involves cargo translocation across the membrane surrounding the peroxisome. The mechanism of translocation is currently virtually unexplored. **The aim of this project is to characterize on a structural level the cargo recognition process, docking at the peroxisomal membrane, and the process of protein translocation across the peroxisome membrane.** Figuratively speaking, the aim of the project is to 'observe' how the cargo is identified among other proteins, how it docks on the peroxisome surface, and what the machinery catalysing the membrane translocation process looks like.

To achieve the above-mentioned objectives of the project the most advanced achievements of structural biology will be utilized: X-ray crystallography, cryo-electron microscopy (cryo-EM) and cryo-electron tomography (cryo-ET). The first two methods provide insight into the investigated systems at the atomic level. Cryo-electron tomography has a lower resolution, but allows to image the structures that are much larger spatially. Combined, these methods, complemented by biochemical and cellular studies, will allow to develop a consistent model of peroxisomal protein import.

The primary expected scientific achievement of the project encompasses comprehensive insight into the mechanisms of recognition of PTS1 and PTS2 tagged proteins, the structural basis of the docking of the recognition complex at the peroxisome surface and, most significantly, the description of the translocation mechanism and of the protein machinery involved in translocation. In a broader perspective, the results of this project will elucidate some of the structural foundations of peroxisome biogenesis disorders, enabling the scientific community to pursue new efforts to develop mitigation strategies. Further, the forward-reaching translational impact of the project encompasses provision of structural basis for future rational design of inhibitors against protozoal infections.