Description for general public (in English)

Membrane integral proteins play vital roles in the functioning of our body, as they are enzymes, nutrient transporters, ion channels, or receptors. In the case of illnesses and metabolic disorders they become, therefore, major therapeutic targets. Understanding the action of integral membrane proteins and factors affecting their activities is of crucial importance. Today in the time of the pandemic no one needs to be convinced that basic research conducted *in vitro,* i.e. outside the living organism, is an essential stage preceding any therapeutic treatment. Unfortunately, the studies of membrane proteins are still limited due to the fact that outside the membrane the protein undergoes structural changes and loses fast its activity. Studies in natural membranes are very difficult because of the complexity of the membrane, diversity of proteins and other components present in it, and the interactions between them. Removing the protein from the native environment, e.g. using detergents, and placing it in a synthetic membrane or polymer disk quickly leads to a loss of activity and large structural changes as well. Studies of a membrane protein in fully functional state remains, therefore, a challenge.

The aim of this project is to design an environment in which membrane proteins can maintain their natural structures and functionalities. Liquid crystalline lipid nanoparticles – cubosomes create such an environment. The bicontinuous cubic phase consists of a network of water channels surrounded by lipid bilayers and fits to the structure of a membrane protein (Fig. 1). The hydrophobic internal parts of the protein are embedded in the lipid walls of the cubosome channels, and the extra- and intracellular ends of the protein molecule are located in the water channels, which ensures contact with the aquatic environment as in the case of the natural membrane of the biological cell. Appropriate doping with lipids that have very large polar headgroups, e.g. sugar groups, allows to increase the width of the channels adjusting the structure of the cubosome to the structural needs of a larger proteins. At the same time, the very large internal lipid surface ($400 m^2/ g$) promotes the incorporation of large amounts of protein, significantly larger than in liposomes or other models of biological membranes (Fig. 1). The cubosome containing the membrane protein - proteocubosome, is therefore, a suitable system for the physical and chemical studies of the protein e.g. location and orientation in the membrane and activity. It allows discovering compounds active in the protein inhibition or activation, and finding optimal regulators of protein functioning. The protein stability in the proteocubosome not only facilitates storage and research over time, the cubosome is a convenient small carrier transporting the protein to a given place and it also facilitates fusion with biological and biomimetic membranes. In this way, biosensitive platforms can be constructed, e.g. on conductive substrates, which may become useful for drug determination or screening the candidates for therapeutic agents.

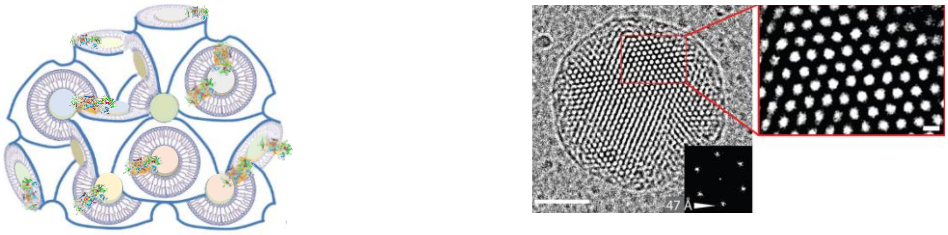

Fig.1 Liquid – crystalline lipid phase with built-in protein molecules and cryo-TEM image of the cubosome

In this project, we design liquid crystalline lipid nanoparticles for embedding two important proteins. The first one is HMG-CoA reductase, an enzyme responsible for the synthesis of cholesterol. A common reason of cardiovascular diseases are the elevated levels of cholesterol especially LDL (low density lipoprotein) in blood. When cholesterol is overproduced, statins (inhibitors) are used to modulate its production. Proteocubosomes containing the enzyme will allow to monitor its activity and determine the impact of various statins on its functionning. The research will allow to select statins that most effectively inhibit, but at the same time minimally disturb the structure of the entire lipid membrane (which is one of the possible reasons for the adverse side effects of statins). Another protein encapsulated by the cubosome will be the mitochondrial potassium channel with cytoprotective properties. Its pore forming subunit, ROMK, will be examined for its activity in potassium transport, also under the action of several activators and channel inhibitors. The research facilities of the Faculty of Chemistry University of Warsaw enable detailed structural studies of proteocubosomes, e.g. by cryo-microscopy (Fig. 1) or X-ray scattering, and - after being built into lipid layers - to determine the location of the protein in the membrane by infrared spectroscopy and atomic force microscopy, and then to assess changes in protein activity under the influence of selected activators / inhibitors, i.e. potential drugs. The method of storing and delivering membrane proteins in the form of a thermodynamically stable liquid crystalline nanoparticle in an aqueous solution will also protect even sensitive membrane proteins from the chemical and biological degradation typical under medical application conditions.