

Life on Earth depends on photosynthesis. As a result of this process, plants produce organic molecules, fueling nearly all Earth's ecosystems, and oxygen we breathe. Photosynthesis is a complicated network of processes and reactions converting sunlight energy into chemical energy, with chlorophyll molecules playing a crucial role. Single-cell organisms started to synthesize chlorophyll molecules more than 2.7 billion years ago and since that time the chlorophyll biosynthetic pathway keeps evolving, even today.

In our research we focus on one, unique reaction of the chlorophyll biosynthetic pathway. The enzyme responsible for it, LPOR, is active only when illuminated. When in darkness, however, it interacts with lipid membrane forming large networks composed of both proteins and lipids, namely prolamellar bodies. With the use of spectroscopy, electron microscopy and molecular biology techniques we have shown, that LPOR variants originating from bacteria and plants differ. Bacterial LPOR forms only short, elongated complexes in darkness, and their activity is not dependent on the presence of the lipids. On the other hand, plant LPOR variants form elongated tube-shaped complexes, that spontaneously rearrange themselves into prolamellar bodies. Interestingly, most of angiosperms have two different copies of LPOR: one is active independently on the lipids, the other is active almost exclusively when bound to the lipid membrane.

In this project we want to explain how the properties of LPOR changed in the course of evolution in different groups of organisms. We will determine what physiological role the interaction with the lipids plays. As a result of the experiments, we will determine which residues are responsible for the interaction with the lipids, for the substrates binding, for the light-dependent properties, and for the formation of multisubunit complexes.

To meet our goals, we will make *E. coli* cells producing all of the components required by LPOR to form complexes. At the same time, we will make libraries of thousands of LPOR genes with randomly introduced mutations. By combining the modified bacterial cells, the LPOR libraries and a high-throughput screening technique we will find and determine the effect of hundreds of mutations on the properties of LPOR. Such an approach is called directed evolution and it was awarded a Nobel Prize in 2018.

All of the data collected in the project will be analyzed by a neural network with the use of machine learning technology. As a result, we will develop an algorithm that will be able to predict the properties of newly discovered LPOR variants and to generate a sequence of non-natural LPOR variant having desired properties. The algorithm will be publicly available to everyone in the Internet.