Membrane proteins mediate fundamental biological processes, such as sensing of chemical signals, signal transduction, coordination of interactions between cells, and transport across biological membranes. The latter is maintained, among others, by proteins from the ABC family (ATP-binding cassette proteins). ABC transporters are common in all living organisms, but in plants they are exceptionally numerous. It is believed that this is the result of plant adaptation the terrestrial lifestyle. One of the important adaptation elements was the formation of a specialized and diverse secondary metabolism, which required the development of a dedicated distribution system, including, *inter alia*, ABC transporters.

An example of such a transporter is ABCG46 from the model legume plant *Medicago truncatula*. This protein is involved in the proper functioning of the Medicago defense system during pathogen attack by modulating the phenylpropanoid pathway, which produces medicarpin (the main defense compound). In our previous studies, we were able to identify molecules transported by MtABCG46. These are low molecular weight compounds that are the early precursors of medicarpine namely, p-coumaric acid and liquiritigenin. Interestingly, the MtABCG46 protein exhibits high selectivity for transported molecules. We have checked that other compounds, despite their high structural similarity to the above-mentioned ones, p-coumaric acid and liquiritigenin, are not transported by this protein. We know that one of the elements determining the selectivity of the MtABCG46 protein is the structure of tunnels leading to the interior of the transporter. However, knowledge regarding the direct interaction of transported molecules with the investigated protein during transport through biological membranes still remains a mystery.

Our preliminary results show that our proposed solutions based on dedicated genetic constructs introduced into BY2 tobacco suspension cells provide hope for the efficient production and purification of the MtABCG46 protein and thus allow to perform detailed analyzes of protein-ligand interactions and structural relationships. Beside the scaling-up protein production and purification, we are planning to reconstitute purified MtABCG46 in lipid vesicles, which will enable us to perform biochemical activity tests and direct transport experiments and therefore determine the impact of the presence of various molecules on the activity of the tested protein. Additionally, based on biophysical methods, we will try to examine direct protein-ligand interactions, and using cryo-electron microscopy, we want to image the structure of the MtABCG46 protein and its complexes with ligands during the transport process.

The results obtained on the basis of the proposed research (combination of structural analysis and transport biology) may become a source of detailed knowledge about the mechanism of action of plant ABC proteins, including the selective binding of transported molecules or the course of conformational changes during transport. They may also contribute to the development of new solutions, e.g. in the biotechnological production of secondary metabolites or obtaining new varieties of legumes with desirable features, such as: better nutritional values or greater natural resistance to diseases based on increased secretion of phenolic compounds, without the need to introduce GMOs.