Plants use solar energy to grow and conduct all necessary physiological processes. The process of energy conversion, known as photosynthesis, is a chain of reactions of electron and proton transfers taking place in specific proteins that work as molecular machines. One of such proteins, cytochrome b_6f, is a complex that not only participates in reactions of photosynthesis, but also is postulated to be involved in regulation of this process. This is because cytochrome b_6f may control a switch between the two possible paths that are available for the electrons as they are transferred trough the chains of specific metal centers during photosynthesis. While it is agreed that such the switch is necessary to secure energetic efficiency of photosynthesis in response to various light conditions, the molecular mechanism of regulation remains unknown.

In our recent work we described a high-resolution structure of plant cytochrome b_6f which unexpectedly revealed a novel protein partner of this complex: the thylakoid soluble phosphoprotein 9 (TSP9). Intriguingly, TSP9 occupies the region proximal to a site where the putative electron-donating protein partners of cytochrome b_6f are proposed to bind. The binding/unbinding of these partners is believed to play a role in the switch between the two electron paths. Furthermore, TSP9 itself can undergo phosphorylation – a specific chemical reaction known to be generally associated with regulatory processes in the living cells. In this context, the discovery of TSP9 as a novel partner of cytochrome b_6f opens an entirely new perspective for investigating regulatory function of cytochrome b_6f in photosynthesis. We hypothesize that TSP9 provides a missing structural link for the functional connection between cytochrome b_6f and other elements involved in control of photosynthetic electron transfer. With this general notion, we envisage several intriguing but mechanistically plausible and experimentally testable concepts. These concepts consider either reversible or permanent association of TSP9 with cytochrome b_6f and also consider specific states under which the binding of electron-donating partners is either favored or discouraged.

In this project we propose to test these new concepts using advanced spectroscopic approaches (optical, electron paramagnetic resonance (EPR), cryogenic electron microscopy (cryo-EM)) in combination with biochemical analyses and protein engineering. Towards this end we will describe at molecular level how TSP9 binds to cytochrome b_6f, how the interaction of TSP9 with cytochrome b_6f influences properties of the enzyme, its catalytic activity and abilities to bind other protein partners involved in photosynthetic electron transfer. We will also determine what are the factors influencing binding of TSP9 to cytochrome b_6f and examine dynamics of the interaction. In addition, we will explore possible structural changes taking place within TSP9 and their regulatory consequences, as well as the dynamics of binding of TSP9 to the lipid bilayer. We will also test whether TSP9 itself can directly interact with other cytochrome b_6f protein partners.

Altogether, we aim at providing an extended and detailed description of the primary molecular processes associated with the interaction of TSP9 and cytochrome b_6f and their mechanistic consequences. We believe that such basic knowledge, in view of the fact that TSP9 has just been discovered as the partner of cytochrome b_6f, comes as natural prerequisite for further studies addressing mechanistic basis of the regulation of photosynthesis and the contribution of cytochrome b_6f in this process. Ultimately, the knowledge gained by implementation of this project will contribute to better understanding how plants efficiently use light energy and adopt to environmental changes at molecular level. In a long run, the discovered mechanistic principles might turn out useful in providing hints for targeted development of enhanced photosynthetic organisms to increase efficiency in plant production.