Synthesis and Structural Characterization of Small-Molecule Probes for the Programmed Death 1/Programmed Death Ligand 1 pathway

The research objective of my project is to develop small-molecular weight chemical probes that inhibit the activity of the human programmed death ligand 1 protein (the PD-L1 protein).
Protein-protein interactions (PPIs) are involved in almost every cell functions, therefore are important to explore. The subject of my research is the transmembrane protein PD-L1, which is often overexpressed in many cancer types and immune cells. Binding of PD-L1 to its receptor, the programmed death 1 (PD-1), plays an important role in inhibiting T-cell proliferation, release of cytokines and cytotoxicity. The PD-1/PD-L1 interaction has been used by microorganisms and tumor cells to attenuate the natural immune response. Recent studies have shown that the blockade of the PD-1/PD-L1 interaction can restore T-cell functions, thereby normalization of natural immune response. Blockade PD-1/PD-L1 pathway is possible by targeting both PD-1 and PD-L1, however the literature describes mainly monoclonal antibodies blocking the PD-1/PD-L1 pathway. Therefore, finding small chemical probes which might inhibit PD-L1 would allow us not only to determine the basis of the small molecules binding to PD-L1 but also to understand the mode of action of the PD proteins.

The aim of the proposed research is the synthesis of a line of potential chemical probes for the PD-L1 protein. These probes are designed according to the recently proposed by our group of the active site model of PD-L1 and based on the results from high-throughput NMR-screening. To achieve this objective, I would apply both the stepwise linear synthesis and the multicomponent reactions (MCR). The binding affinity towards PD-L1 will be established by NMR spectroscopy. The method relies on the monitoring of chemical shifts perturbation in 2D SOFAST-HMQC spectra of isotopic labeled protein upon addition of small molecular weight compounds. The ability of synthesized compounds to dissociate the PD1/PD-L1 complex will be evaluated by NMR based AIDA assay (*Antagonist Induced Dissociation Assay-NMR*). The further part of proposed research is associated with co-crystallization of active compounds with the PD-L1 protein in order to determine the structural basis of their interaction with PD-L1.

The development of chemical probes for PD-L1 is at initial stages because of lack of structural information on the modes of interaction between small molecules and their target PD proteins. The development of small-molecule probes binding to PD-L1 should open new perspectives for investigating the signaling in the PD-1/PD-L1 pathway in a broader biological context (cells or organisms). The expected results of proposed research should give an insight into the mechanism of the interaction of the PD proteins, specifically the insights on the interaction of small molecules with the PD-L1 protein.