Fragment-based screening using NMR spectroscopy for identification of chemical probes that interact with the non-canonical ubiquitin-like protein Hub1

Protein-protein interactions (PPIs) are involved in almost every cell functions, thus are important to explore. The subject of my research is small protein Hub1 (known also as the UBL5 protein), which has recently been shown to control alternative splicing by non-covalent binding to the HIND domain of the Snu66 spliceosomal protein. Since malfunctions in alternative splicing can cause multiple diseases like, for example: hypercholesterolemia, premature aging, neurodegenerative diseases as well as cancer, finding chemical probes which can inhibit the Hub1/Snu66 interaction can allow us not only to understand the mode-of-action of Hub1 in living organisms but also to begin novel approches to anticancer therapy in the future.

Finding small molecular weight organic compound that can selectively interact with target protein is difficult. Every single interactions like, for example: hydrogen bonding, ionic or hydrophobic effects must be considered. It is important to create compounds which will posses functional groups that can fit to the active pocket of target protein. The procedure called the "fragment-based screening" is used to develop chemical fragments which can be used further to create larger molecules. The library of small molecular weight compounds is tested in high throughput screening for this purpose. My research methodology relies on the development of chemical probes using NMR spectroscopy. It is planned to test 600 fragments. Perturbation in the NMR chemical shifts in the 2D SOFAS HMQC spectra of N15-labeled Hub1 monitors the interactions between tested compound and the protein.

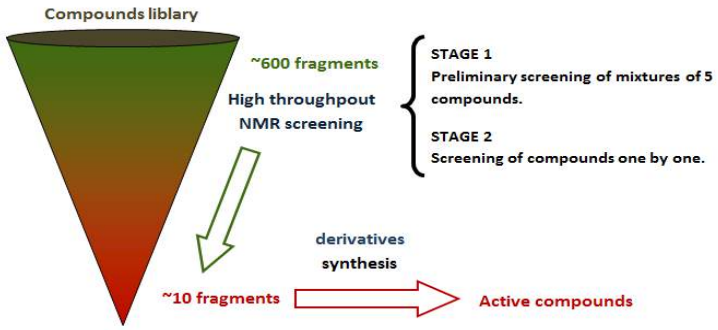

Figure 1. Schematic presentation of fragment-based screening with application of NMR techniques.

Fragments selected in the high throughput-NMR screening usually show weak binding. To improve their affinity in binding to Hub1, optimization of such compounds is required. I am going to use two approaches for this purpose: one will be based on the '*in silico*' screening and the second one on X-ray structure analysis of the compound-protein complexes. Attempts will be made to synthesize the designed compounds using multi component reaction (MCR) chemistry.

I expect that the proposed research should provide new insights into the mechanism of interactions between Hub1 and other proteins. Since there are no reports about small molecules that can bind to Hub1, new chemical probes for Hub1 could open opportunities for investigating the function of human Hub1' function *in vitro* and *in vivo.*