ABSTRACT

Protein-protein interactions (PPIs) play a crucial role in almost all biological processes. Many proteins require a number of dynamic interactions with other proteins and/or biomolecules to function. Proteomic studies have suggested that human protein-protein interactome consists of several hundred thousands of protein complexes. A detailed insight into these PPIs is essential for a complete understanding of the processes mediated by these protein complexes. Because many PPIs are involved in disease-related signaling pathways, such PPIs are important targets for pharmaceutical interventions, especially in situations where a more conventional target (e.g. the active site of an enzyme, the binding site of a receptor) cannot be used.

This doctoral thesis focuses on 14-3-3 proteins, a family of eukaryotic adaptor and scaffolding proteins involved in the regulation of many signaling pathways. The 14-3-3 proteins function as interaction hubs and critical regulators of many enzymes, receptors and structural proteins. The main aim was to structurally characterize selected 14-3-3 protein complexes and investigate their stabilization by small molecule compounds. Using combination of protein crystallography, differential scanning fluorimetry, fluorescence polarization and analytical ultracentrifugation, the PPIs between 14-3-3 and two physiologically important binding partners the Ca^{2+}/calmodulin-dependent protein kinase kinase 2 (CaMKK2) and the nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (IκBα) have been characterized. The stabilization of PPIs between 14-3-3 and CaMKK2 by fusicoccins have been investigated and we showed that the targeting of the fusicoccin binding site by small-molecule compounds could be an alternative way how to suppress CaMKK2 activity by stabilizing its phosphorylation-dependent inhibited state. In addition, the screening of a fragment library designed to target the 14-3-3 protein surface enabled us to identify three molecules that bind to two different surfaces of the 14-3-3 protein outside the usual binding groove, thus highlighting new possibilities for selective modulation of 14-3-3 complexes.