ABSTRACT

The ability of proteins to bind other molecules in response to various stimuli in their microenvironment serves as a platform for extensive regulatory networks coordinating downstream cell actions. The correct function of these signaling pathways depends mostly on noncovalent interactions often affecting the structure of proteins and protein complexes. Understanding the molecular mechanism of a protein function in cell signaling therefore often depends on our knowledge of a three-dimensional structure.

In this doctoral thesis, I present the work that led to the understanding of several protein-protein and protein-ligand interactions implicated in cell signaling at the molecular level. I applied nuclear magnetic resonance spectroscopy, small angle X-ray scattering and other biophysical methods to determine the molecular basis of inhibition of four signaling proteins: Calcium/Calmodulin (Ca^{2+}/CaM)-dependent protein kinase kinase 2 (CaMKK2); protease Caspase-2; Forkhead transcription factor FOXO3, and Apoptosis signal-regulating protein kinase 1 (ASK1). In particular, I investigated the distinct roles of 14-3-3 and Ca^{2+}/CaM in the regulation of CaMKK2 activity. I also studied in detail the mechanism how 14-3-3 interferes with the caspase-2 oligomerization and its nuclear localization as well as provided a basis for transcriptional activity modulation of FOXO transcription factors by investigating a small-molecule compound binding to the DNA-binding domain of FOXO3. The structural studies of the complex between TRX binding domain of ASK1 and TRX revealed that TRX interacts with ASK1 through its redox active site and the oxidation induces structural changes in the ASK1 TRX binding interface, suggesting that the ASK1 oxidation is an important regulatory signal for the complex dissociation.

This doctoral thesis provides a comprehensive insight into the intriguing relationship between understanding the molecular mechanisms of activity modulation of signaling proteins and the knowledge of structural details of their complex interactions. The integrative interdisciplinary approach used in this work points at the new opportunities in the world of biological structures, where the traditional methods have already reached their limits.