# Abstract

Protein-protein interactions are involved in various biological processes and detailed characterization of their structural basis by the means of structural biology is often instrumental for rigorous understanding of underlying molecular mechanisms. This information is important not only for fundamental biology but also plays an important role in search for sites amenable for therapeutic intervention. Nuclear magnetic resonance spectroscopy is alongside X-ray crystallography and single-particle cryo-electron microscopy one of the key high-resolution techniques in structural biology. Although its applicability to larger systems has a well-known physical limit, it offers unique capabilities in addressing highly dynamic or inherently heterogeneous systems.

In this doctoral thesis, the solution-based NMR approach was used for detailed structural characterization of selected biologically important proteins and their complexes that provided important insights into their biological roles. In three distinct projects, I (i) studied the relationship between the structural effects of particular modifications in the insulin-like growth factor II (IGF-II) and their selectivity to the insulin axis receptors; (ii) the specific binding mechanism of the SH3 domain from the Crk-associated substrate (CAS); (iii) and the structural basis for the membrane recruitment of Phosphatidylinositol 4-kinase beta (PI4KIIIβ). For the structural studies of IGF-II analogues, I developed an efficient purification protocol, prepared a series of modified proteins with altered preference for the cognate receptors and structurally characterized the analogues that showed markedly reduced binding towards insulin receptor. The obtained structural information suggested a key role for the semi-flexible C-domain from IGF-II in receptor binding specificity. In the project focused on how the CAS SH3 domain recognizes its ligands, I solved the structures of the free CAS SH3 domain and in complex with two polyproline peptides motifs derived from either tyrosine phosphatase PTP-PEST or cytoskeletal protein Vinculin. The structural data shed the light on the detailed CAS SH3 binding mechanism, suggested that the CAS SH3 ligand binding is negatively regulated by a Src-mediated phosphorylation and led to identification of the two novel CAS cellular binding partners. In the search for the determinants of the PI4KIIIβ membrane targeting, I determined the structure of the complex formed between the N-terminal region of PI4KIIIβ kinase and PolyQ domain of ACBD3 that revealed the detailed mechanism underlying the PI4KIIIβ membrane recruitment.