# Summary

Type 2 diabetes mellitus (T2D) is a serious threat to global health constituting a major socioeconomic problem. Currently, T2D drugs have many adverse side effects, including severe hypoglycemia. Glycogen metabolism is controlled by a number of enzymes in a complex and dynamic manner. Glycogen phosphorylase (GP) and glycogen phosphorylase kinase (PhK) are two enzymes that play a critical role in glycogen metabolism and in glucose homeostasis. In the framework of this PhD thesis, within the overarching goal to discover new therapeutic agents for T2D, studies on the structure-activity relationship of these two enzymes were performed. GP is a validated target for the discovery of new antihyperglycaemic agents for patients with T2D. In the first part, the inhibitory effect of polyphenolic extracts from 19 samples from byproducts of the industrial juicing process of pomegranate (Punica Granatum) and 23 samples from plants of the Rosaceae family was assessed. The most potent inhibitory extracts showed IC50 values below 10 μg / mL. Employing the affinity crystallography method, the most bioactive molecules of the extracts were determined. For the pomegranate samples, the most bioactive molecules were ellagic and chlorogenic acid (found bound at the inhibition and active site of the enzyme, respectively). For the Rosaceae samples, the most bioactive molecules were glucose, gallic, ellagic and chlorogenic acid found bound at the active, inhibitory, and the new allosteric site, respectively. The binding of chlorogenic acid to GP has not been observed thus far, and structural analysis of the GP-chlorogenic acid complex in the crystal revealed the structural basis of its inhibitory activity. Recent studies have highlighted the usefulness of plant extracts in the production of bio-functional food products and the results of this study constitute an important step towards the design of new biofunctional products for T2D patients. Phk phosphorylates the inactive phosphorylase b converting it to the active phosphorylase a. The enzyme is one of the most complex protein kinases, consisting of four subunits (α, β, γ and δ) with heterodimer stoichiometry (αβγδ)4 and a molecular weight of 1.3 MDa. In the second part of the dissertation, biochemical and biophysical studies of PhK subunits γ and α were performed. Expression studies of human γ truncated subunit of PhK were performed in E. coli strains and then the protein was purified by liquid chromatography methods and crystallization tests were performed. The α subunit of the human muscle PhK was also studied. Initially, gene optimization studies were performed to express it in the E.coli bacterial system. The amplified gene was inserted into the pETM-11 vector and to enhance its solubility, the molecular chaperone Trigger Factor was used. This was followed by chromatographic purification, and biophysical studies to determine the oligomerization state (DLS), as well as experiments to determine the folding and its secondary structure elements (CD), and final the sample was analyzed for its homogeneity and purity through negative stain EM