

# ABSTRACT

Calorimetric methods are used to studying the mechanism of regulation and control of biological processes at the molecular level. Isothermal titration calorimetry (ITC) is used for monitoring the heat exchange of molecular interactions in an environment with constant temperature. This method is also preferred because of its ability of direct measurement of thermodynamic parameters associated with complex formation. ITC measures mostly the energetics of biochemical reactions or molecular interactions protein–protein, protein–ligand, or enzyme–substrate. Main goals of this bachelor thesis were: (I) to acquire working skills with nano isothermal titration calorimeter; (II) to prepare the DNA-binding domain of human transcription factor FOXO4 (FOXO4-DBD); and (III) to study thermodynamic aspects of the interaction between FOXO4–DBD and the double-stranded DNA containing specific binding motif. FOXO4 protein is one of the four members of a “O” subgroup of forkhead transcription factors FOX, which play an important role in many cellular processes including stress resistance, age control and oncogenesis. Forkhead DNA-binding domain recognizes sequences containing the core motif 5'-(A/C)AA(C/T)A-3' with the binding stoichiometry 1:1. Results of this bachelor thesis show that FOXO4–DBD binds the double-stranded DNA with a dissociation constant  $K_D = 3,58 \cdot 10^{-7} \text{ mol} \cdot \text{dm}^{-3}$ . In addition, values of the standard enthalpy and entropy change of this interaction were obtained as well.