Cell homeostasis is maintained via strictly regulated processes. One of the important regulation systems is ubiquitin-proteasome proteolytic pathway. Proteins to be degraded are posttranslationally modified with polyubiquitin chains and targeted to the proteasome for degradation.

Ubiquitin-proteasome system consists of several processes: ubiquitination of target substrates via set of enzymes, substrate transfer and degradation in the 26S proteasome. There are two ways of ubiquitinated substrate recognition via proteasome. It is either directly by proteasomal receptors or by protein shuttles. Shuttling factors bind polyubiquitinated target substrate and transfer it to the entrance of proteasomal cavity thanks to their typical domain architecture. The N-terminal ubiquitin-like domain binds to regulatory particle of the proteasome and the C-terminal ubiquitin-associated domain binds polyubiqitinated chains on substrates. This thesis focuses on the human DNA damage-inducible protein homolog 2 (Ddi2), a potential member of protein shuttles of humans, and on the interaction of its ubiquitin-like domain with its putative interaction partner, a proteasomal subunit PSMD2.

PSMD2 has been cloned, expressed and purified in sufficient yields for further experiments. “Cold” as well as isotopically labeled UBL domain of the human Ddi2 was expressed and purified in a sufficient yield for NMR spectroscopy analysis. The structure of UBL from Ddi2 was solved in high precision and compared with solution structures of the mouse ortholog Ddi1-UBL and human ubiquitin.

Key words: ubiquitin-proteasome system, shuttling factors, regulation of cellular processes, nuclear magnetic resonance