ABSTRACT:

Natural killer (NK) cells are an intensively studied part of immune system, possessing unique ability to recognize and induce death of tumor and virus-infected cells without prior antigen sensitization. Their function is regulated by a fine balance of signals induced by multiple activating and inhibitory cell surface receptors and their interaction with the ligands present on the target cell. Recent research in their C-type lectin-like receptors repertoire has shown that ligands of some of these previously orphan receptors lie within their own family, describing a lectin-lectin interaction. This is the case of human inhibitory receptor NKRP1 (gene KLRB1) and its ligand LLT1 (gene CLEC2D). Previous studies have shown that overproduction of LLT1 in cancer cells or lower production of NKRP1 in NK cells is connected to cancerous manifestations.

This master's thesis shows a successful production of the extracellular part of LLT1 utilizing a mammalian expression system based on transient transfection of modified human embryonic kidney (HEK) cell lines. It was found that the five cystein residues contained within the lectin domain of LLT1 tend to cause misfolding and formation of aggregates. Stabilization of the domain was achieved by restoration of the sixth cystein residue at the evolutionary conserved position utilizing a site directed mutagenesis approach. The reconstruction of disulfide bond was verified by mass spectrometry. The mutation of His 176 to Cys 176 led to significant improvement in yield and homogeneity of product that enabled successful crystallization in two different crystalline forms and solution of the structure at 2.0 Å.

(The thesis is written in Czech.)