Summary

Cell penetrating peptides (CPPs) are short peptides containing 5 – 30 amino acids residues. CPPs are able to cross plasma membranes without disturbing its function, and even assist other biological molecules in translocation into cell.

The aim of this doctoral dissertation was synthesis and biological evaluation of novel class of cell penetrating peptidomimetics. A set of compounds containing various numbers of L-2,3-diaminopropionic acid (Dap) residues connected through the peptide bond was synthesized. Each β-amino group of Dap residue was coupled with oxa-acids functionalized with a guanidine, $–NH_2$ or –OH moiety. Synthesized peptidomimetics were fluorescently labeled or contained free N-terminus.

Cytotoxicity tests confirmed that compounds at the concentration used in the cell experiments (10 μM) are not toxic. First, the ability to penetrate the cell membrane of each synthesized compound was investigated. Obtained peptidomimetics were incubated with breast cell lines: healthy – HB2, cancer – MDA-MB-231 for 24 hours and their uptake by the cell efficiency was evaluated under fluorescent microscope. Peptidomimetics consisting of six or eight guanidine moieties were able to effectively cross the cell membrane. In this process the crucial factor is the number of guanidine groups. Replacement of any guanidine group in peptidomimetics structure resulted in the shift of their biological activity. The exchange to amino group weakened their penetrating properties and the replacement to hydroxyl group resulted in complete activity loss. Most potent peptidomimetics were capable of locating themselves inside various cell lines of breast, skin, bladder, murine macrophage and rat podocytes. Mixed mechanism is used during penetration of biological membranes which is the combination of endocytosis and direct transport. Additionally, this group of compounds did not influence a cell cycle of above-mentioned cell lines.

In the next step, interaction between most potent peptidomimetics and nucleic acid were investigated by means of MST, SPR and electrophoresis. Tested compounds interact with ssDNA and dsDNA in micromolar range. The strength of this interaction correlates with the number of guanidine groups present in peptidomimetics sequence. Further the interaction of selected compounds with plasmid DNA was tested by AFM technique. The obtained results indicated that peptidomimetics with multiple guanidine moieties caused formation of large structures, which complexity depends on number of guanidine groups. In consequence such compounds were able to condensate DNA and diminish its charge and could mediate its transport to the cell.

What is more tested peptidomimetics can also mediate protein intracellular transport. It is effective for labelled inert protein and enzymatic active molecule.