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Microcalorimetric and spectroscopic studies on the mechanism of interaction between novel peptoids and lipid bilayers - effect of length, charge and N-terminal end group

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Biomacromolecules as proteins and nucleic acids are promising drug candidates. However, one problem with biomacromolecules is that they usually have to pass the cell membrane to exert their effect. Utilization of cell penetrating peptides (CPPs) might be a way to transport biomacromolecules across the cell membrane. It is becoming increasingly evident that CPP uptake pathways may vary depending on the physico-chemical properties of the CPP and the cargo they deliver, the specific cell types and the specific experimental conditions. Nevertheless, the interaction between CPPs and membrane is the very first step of the internalization. Analysis of the CPPs interaction with liposomes is expected to provide information about the CPPs interaction with the cell membrane. We have performed a thermodynamic characterization and spectroscopic of the binding between a series of novel CPPs and anionic liposomes. Recently, we described a new class of CPPs, which seem to show superior biological effect compared to the well described CPPs. The molecular design of these alpha-peptide-beta-peptoid chimeras is based on alternating repeats of (-amino acids and (-peptoid residues. The rationale was to benefit from the structure-promoting effects and lipophilicity from the unnatural chiral (-peptoid residues, and the (-amino acid residues providing cationic properties and hydrogen bonding possibilities. The chimeras are very stable toward proteolysis, non-hemolytic, possess antibacterial activity and promising cell-penetrating potential. Interpretation of the data obtained in ITC-experiments showed that an increased number of basic residues in the novel CPPs sequence resulted in a more favorable interaction with the anionic liposomes. Additional experiments revealed that a hydrophobic interaction was a part of the binding. From CD spectra it was concluded, that no major structural changes occurred in the novel CPPs when they were in the presence of anionic liposomes. The initial electrostatic attraction in CPPs internalization mechanism was confirmed by comparing Gibbs free energy ((*G*) with the number of basic residues. Furthermore, it is proposed that the hydrophobic interaction registered could be between hydrophobic groups on the novel CPP and the hydrophobic region of the liposome. Another possibility could be simultaneously increased lipid-lipid interaction in the hydrophobic region of the liposome. In conclusion, when comparing the novel CPPs with results obtained for the well described CPP penetratin it seems, that the binding to anionic liposomes is more favorable for all novel CPPs investigated.

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Studies towards improved cell-penetrating peptide-promoted macromolecular drug delivery

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The general concept of drug delivery facilitated by cell-penetrating peptides (CPPs) is well-known; however its practical utility for delivery of biopharmaceuticals necessitates further development concerning *in vivo* stability and efficiency of these peptidic carriers. In the present project, the aim is to increase the stability towards enzymatic degradation as well as to improve membrane translocation properties by incorporating novel unnatural amino acids into the naturally occurring CPP penetratin. The CPP efficiency of these penetratin analogues will be tested upon conjugation to a therapeutic biomacromolecule. Nine novel and unique amino acid building blocks have been synthesized from enantiopure aziridines to form amino acids with additional cationic charges as compared to natural amino acids. An increased number of cationic charges in CPPs have been shown to improve the interaction between CPPs and the cell membrane. The novel amino acids will be incorporated into penetratin to increase its cationic charge and to generate more efficient and stable CPPs. The enzymatic stability of penetratin is estimated by testing its resistance towards degradation by intestinal juice from rats. The metabolites are analyzed by an Orbitrap MS to identify the initial sites of cleavage and the largest non-degradable fragment as well. Thereby the optimal sites for incorporation of the novel amino acids may be revealed. The modified penetratin molecules will be tested for stability and CPP efficiency.

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New configuration of an *in vitro* blood–brain barrier model

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It is an undeniable fact that neuroscience has an urgent need for a reliable and translatable *in vitro* model to investigate the human blood–brain barrier (B3). The use of human primary cerebral capillary endothelial cells is considered to provide such a model. The aim of the present study was to compare a B3-model based on two novel immortalized human primary brain endothelial cell (hBEC) lines. The human cerebral cortex microvascular endothelial cell (hCMEC-D3) and the human brain capillary endothelial cell line (NKIM-6) were used. These cell lines were used to investigate the potential transport of large molecules across the cell monolayer. The B3 is unique in that it consists of highly selective endothelial cell interface that create tight junctions around the capillaries separating the bloodstream from the brain parenchyma. Brain endothelial cells in association with astrocytes display complex tight junctions, polarized expression of enzymes, transporters and receptors. In order to take advantage of the influence associated with astrocytes we established an *in vitro* co-culture model of hBECs with primary human astrocytes. The co-culture was performed either by growing the cells on either side of a permeable membrane or growth in direct contact. Using a cell-based kinetic profiling approach