Abstract

Within recent years, so-called cell-penetrating peptides (CPPs) emerged as potential delivery system for various cargoes. Their ability to overcome biological membranes and transport their cargoes in a non-invasive manner is a major prerequisite of these delivery vehicles. In fact, the interaction and interplay of CPPs with biological membranes is crucial for their activity and, thus, their drug delivery performance. In this regard, a distinct and stable secondary structure is proposed as major hallmark for an efficient cellular uptake, facilitating an optimal lipid-peptide interaction. Indeed, the secondary structure and the resulting properties of the CPPs are influenced by numerous factors. Thereby, the incorporation of hydrophobic moieties might support the formation of secondary structures and thus the lipid-peptide interaction. So-called carboranes (CB) are associated within this class of hydrophobic molecules and have the potential to increase the lipophilicity of peptides remarkably. In this regard, novel CB-CPP conjugates, having one or more carborane moieties incorporated, were synthesized and evaluated towards their physico-chemical properties as well as their biological activity. Interestingly, the introduction of carboranes resulted in a conformational change of the peptides dependent on the amount of carboranes attached. The results highlight the possibility to modulate and fine-tune the secondary structure of CPPs and therefore their membrane-activity and interaction potential. As a consequence, the cellular uptake efficiency was significantly enhanced, which underlines the great potential of carboranes to increase the peptide internalization and to enhance the potential delivery of cargo molecules.

However, besides the introduction of hydrophobic moieties, also exchanges within the amino acid sequence of a CPP represents an interesting approach to modulate their properties. Therefore, the CPP sC18 and its truncated version sC18* were investigated in more detail and potential positions were identified, which might benefit from an amino acid exchange. In this way, two additional sC18-derived peptides, namely $sC18^{\Delta E}$ and $sC18^{*R,L}$ were synthesized and investigated towards their biological activity. In general, the amino acid exchanges within sC18 resulted in novel variants with a more pronounced amphipathic character and were determined to adopt an alpha-helical character, which improved the cellular uptake. Indeed, the novel sC18 variants were internalized to a higher extent compared to their parent peptides. Furthermore, uptake studies within three dimensional cell models underlined the impact of membrane-peptide interaction for a deeper penetration into multi-layered spheroids. As a result, $sC18^{\Delta E}$ was highlighted as novel promising bioactive peptide, which might potentially be used for the development of a future drug delivery system. Consequently, the exchange of amino acids within a peptide sequence displays a convenient strategy to enhance the amphipathicity and thus the performance of CPPs, which plays a key role in cellular uptake and the delivery of cargo molecules.

In summary, within this thesis, the modulation of the structure-activity relationship of cell-penetrating peptides was highlighted and the resulting peptides or peptide-conjugates were investigated towards their biophysical and biological properties. This was achieved by either extrinsic incorporation of a hydrophobic moiety or by intrinsic amino acid substitutions. Both approaches display promising strategies to fine-tune and enhance the properties of cell-penetrating peptides.