Cytosolic delivery of therapeutic peptides based on receptor-mediated endocytosis

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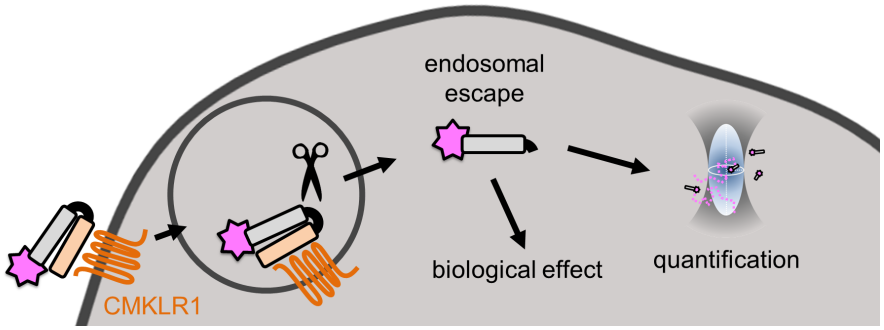
Therapeutic peptides are promising tools to address intracellular protein-protein interactions (PPIs), which are challenging targets for conventional drug moieties. However, effective cytosolic delivery still proves to be a major problem in the development of therapeutic peptides targeting intracellular PPIs. Additionally, common strategies to improve membrane permeability of peptides can result in off-target effects or toxicity. Therefore, selective and efficient delivery systems are highly desirable.

Here we present a shuttle system for peptide cargos utilizing the chemokine like receptor 1 (CMKLR1). CMKLR1 is highly expressed in a variety of tissues and an efficiently internalizing nonapeptide ligand with nanomolar affinity, chemerin-9, has been identified.[1] A cyclic variant of chemerin-9 has been shown to be metabolically stable and might be useful as a shuttle system for therapeutics.[2] Our data demonstrates that large peptide cargos can be N-terminally attached to chemerin-9 while still maintaining excellent internalization behavior. We then investigated the intracellular fate of the internalized peptides with a strong focus on proteolytic degradation in the endosomal pathway and the delivery of intact peptide to the cytosol. Our data shows that unnatural amino acids can be utilized to confer resistance against proteases to survive the conditions in the endosomal pathway. After internalization, fluorescence correlation spectroscopy (FCS) can be used to directly quantify cytosolic concentrations of fluorescently labeled cargos, giving us direct insights into the accumulation of peptide into cells bearing CMKLR1. Overall, our results advance the understanding of targeted application of therapeutic peptides for intracellular targets.



[1] V. Wittamer et al., J. Biol. Chem. **2004**, 279, 9956-9962
[2] T. F. Fischer et al., Cancers **2021**, 13, 3788