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## Synthetic modification of fusogenic coiled coil peptides

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## Summary and Perspectives

Coiled coil peptides have found applications in synthetic biology because of their biocompatibility, high binding strength and selectivity for their binding partner. In this thesis, a synthetic membrane fusion system based on the heterodimeric coiled coil peptides 'E' and 'K' is investigated. Structural variants of these peptides are prepared and tested in fusion assays, to uncover the mechanism behind coiled-coil based membrane fusion. To improve upon the functionality of the system, the introduction of a photoactive azobenzene moiety is investigated. Coupling photoisomerization to coiled coil activity should allow for precise spatiotemporal control of biomolecules and nanomaterials containing coiled-coil peptides.

As a mimic of naturally occurring side-chain palmitoylation, in **Chapter 2** peptide derivatives were prepared with the lipid anchor positioned in the center of the peptide sequence (**fCPK** and **fCPE**), in contrast to attachment at the N-terminus. Liposomes containing these novel peptides showed reduced efficiency in fusion assays and liposomes with **fCPE** were unstable. These findings were attributed to homomeric peptide interactions between different lipopeptide containing liposomes. Homomeric interactions of peptide E were previously assumed to have either no effect, or a negative effect, on fusion efficiency. The observed instability demonstrates the role of homomeric peptide interactions in stabilizing the lipopeptide on the liposome surface before fusion can take place. Furthermore the conclusion can be drawn that terminally anchored coiled-coil peptides are more effective at inducing membrane fusion than peptides anchored in the center of the sequence.

Previously, the membrane interactions of peptide K were considered one of the key features that allows for high fusion efficiency of the coiled-coil system. In **Chapter 3** this theory is further investigated via the preparation of intramolecular crosslinked (stapled) derivatives of peptide K. These stapled peptides are structural isomers that differ in the location and ring size of the macrocycle, achieved by the use of different dibromoxylene crosslinkers. This strategy was effective in increasing peptide  $\alpha$ -helicity, coiled-coil binding strength and stability to denaturation, with calorimetric experiments revealing that this occurs through a preorganization mechanism. Liposomal fusion studies with stapled lipopeptides

demonstrated a large increase in content mixing, which was directly related to the increase in coiled-coil binding strength. This provides the conclusion that the previously proposed theory was incorrect for this peptide system and coiled-coil binding is the major driving force behind membrane fusion. The lipid membrane interactions might still be beneficial to the process, but for further optimization of this system they should not be the main focus.

Because membrane fusion efficiency was strongly dependent on coiled-coil binding strength, active control over coiled-coil peptides was hypothesized as a novel strategy for controlling fusion efficiency. To test this hypothesis, in **Chapter 4** stapled peptides were prepared with the dibromoxylene crosslinker substituted by a photoactive azobenzene moiety. Photocontrol over peptide structure was shown for peptide K, and three-heptad coiled coils could also be switched between different folded states using light illumination. These results were promising, but the crosslinking strategy was very low yielding, therefore a different intramolecular crosslinking strategy is suggested for further investigation of this hypothesis.

An alternative coiled coil photomodulation strategy was investigated in **Chapter 5**, not relying on crosslinking, but on the incorporation of azobenzene-based amino acids. Three azobenzene amino acids were prepared, two literature examples based on phenylalanine and one novel amino acid based on phenylglycine (**APgly**). Derivatives of peptide K containing these amino acids were easy to prepare, and showed effective photoisomerization. Out of the three photoactive amino acids, **APgly** showed both the highest overall coiled-coil binding and the largest difference in structure and binding between the two different isomers. This difference was hypothesized to originate from the methylene group present in phenylalanine, which positions the diazo group outside of the hydrophobic core. Molecular dynamics simulations support this theory and also displayed less distance changes between the two isomers of **APgly**. Together, these data show the novel **APgly** as an effective amino acid for coiled-coil photocontrol, acting through the disruption of the hydrophobic core by changing polarity after photoisomerization.

This mechanism of photocontrol was hypothesized to extend to other peptides that self-assemble by forming a hydrophobic domain. In **Chapter 6** we tested the ability of a phenylalanine-based azobenzene amino acid (**APhe**) to control folding and self-assembly of a  $\beta$ -structured peptide known to form self-assembled peptide fibers. Both peptide structure and self-assembly characteristics could be controlled through **APhe** isomerization, with its position in the peptide also observed to affect

peptide fiber oligomerization. To test if this change in self-assembly could be used to change peptide activity, catalytic histidine residues were introduced in the peptide sequence. In ester hydrolysis experiments, photoisomerization was observed to affect the organocatalytic reaction rates of the peptides, but the exact mechanism through which this occurs is not yet clear. This chapter confirms the initial hypothesis, that the photoisomerization of azobenzene-based amino acids can be used to control self-assembly of  $\beta$ -structured peptides, in addition to  $\alpha$ -helical peptides.

The work presented in this thesis directly impacts the development of synthetic membrane fusion systems. Peptide stapling was shown to be an effective technique to increase coiled-coil binding, which, in turn was shown to be the major contributor to the effectiveness of the E/K coiled-coil membrane fusion system. It also provides a new hypothesis; that regulation of synthetic membrane fusion can be achieved through active control over coiled-coil formation. Furthermore, the presented work on peptide photocontrol of both  $\alpha$ -helical coiled-coils and  $\beta$ -structured peptides provides a mechanistic background, and a novel amino acid, that can be used to introduce photo-responsive activity in many other peptide or protein systems. These techniques function as tools for the generation of active materials in synthetic biology, and might help shine a light on the complex protein interactions observed in the natural world.