



Universiteit
Leiden
The Netherlands

Coiled-coil mediated liposomal fusion: Asymmetric behaving peptide fusogens

Daudey, G.A.

Citation

Daudey, G. A. (2018, December 5). *Coiled-coil mediated liposomal fusion: Asymmetric behaving peptide fusogens*. Retrieved from <https://hdl.handle.net/1887/67109>

Version: Not Applicable (or Unknown)

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/67109>

Note: To cite this publication please use the final published version (if applicable).

Cover Page



Universiteit Leiden



The handle <http://hdl.handle.net/1887/67109> holds various files of this Leiden University dissertation.

Author: Daudey, G.A.

Title: Coiled-coil mediated liposomal fusion: Asymmetric behaving peptide fusogens

Issue Date: 2018-12-05

CHAPTER VI

SUMMARY AND PERSPECTIVES

SUMMARY

In daily life, the accurate logistics of nutrients is very important for keeping our communities in order. The American writer Alfred H. Lewis illustrated this relationship 100 years ago by stating “There are only nine meals between mankind and anarchy”. Within our bodies and even in every cell, this principle also applies. When necessary nutrients are not available, or are delivered at wrong places, cells or organs will die a sudden death. Therefore, biological life has massive and extremely fast logistic systems with highly specific molecular zip codes: folded proteins named SNARE proteins. The study of these coiled-coil forming proteins, and the art of constructing synthetic coiled-coil mimics of those SNARE proteins, is of high value for many different scientific fields of research. In biological studies, researchers seek to understand the molecular mechanisms of targeted delivery. Understanding of these processes and the roles of the involved proteins leads to the construction of functional mimics of SNARE proteins. This lays the foundation for chemists to develop drug delivery systems or repair occurring faults. In materials sciences, these SNARE mimics can be used as a bottom-up approach to construct new materials with control over the ordering of the building blocks.

In this thesis five different complementary coiled-coil peptide pairs are used as functional mimics of SNARE proteins to study molecular recognition processes between small liposomes. Peptides are tethered to these particles via a poly(ethylene glycol) (i.e. PEG) spacer with variable length and a conjugated cholesterol anchor. Molecular recognition of the peptides leads with liposomes to immediate fusion. Studying the efficiency of fusion and aggregation processes provides insight as to the underlying molecular mechanism of the involved peptides.

In **Chapter I**, SNARE mediated membrane fusion is explained, and the use of our coiled-coil peptides E and K as an *in vivo* drug delivery system is described. The influence of the PEG-spacer length and an in-depth study of the fusion mechanism of E and K mediated liposomal fusion were the focus of **Chapter II**. We observed a significant influence of the PEG-spacer length and the anchor type on the fusion efficiency. For peptide E, more elongated spacers yielded higher fusion efficiencies, while for peptide K

an optimal PEG₈/PEG₁₂ spacer length was found. The combination of these findings with the knowledge that peptide K can immerse in the membrane, unraveled the dynamics of the peptide roleplay in the initial stages of the process of merging membranes. The following mechanism for the initial stages of E-K mediated liposomal fusion is proposed: Lipopeptide K is fully immersed in the liposomal membrane, while lipopeptide E adopts a largely unfolded state and does not interact with the membrane. Interliposomal coiled-coil formation occurs when liposomes interact and lipopeptide E binds with lipopeptide K to form a transient heterodimeric coiled-coil. Since the peptide K-membrane interaction is favored over coiled-coil formation, peptides are in an equilibrium, switching between the different states. Upon initial coiled-coil formation, K is in close proximity to both membranes and hence can interact with either. Immersing itself in the opposite membrane will force both membranes into even closer proximity, destabilizing both membranes by forming protrusions with concomitant dehydration of the opposing membranes providing the necessary initial conditions for the membrane fusion.

In **Chapter III**, the conformation of membrane tethered peptides is studied with infrared spectroscopy at the air-water interface, and we found an unexpected and significant influence of the PEG-spacer length on the conformation of the peptides. Peptide E doesn't interact with its supporting membrane, but differences in squeeze-out pressure and peptide orientation with altering spacer length revealed that elongated spacers somehow stabilize weak peptide-peptide interactions, only occurring when peptides are immobilized at a surface. For peptide K, a strong peptide-membrane interaction was found for both short and elongated spacers. A minor squeeze-out upon compression of monolayers containing both E and K demonstrated the simultaneous occurrence of both membrane immersed K and coiled-coil bound K, which wasn't observed before.

Since peptide K tends to homo-associate in a concentration dependent manner, covalent peptide dimers were synthesized to study and exploit this behavior, as described in **Chapter IV**. It was found that the position and the spacer-type influences the equilibrium of the peptide-peptide interaction between inter- and intramolecular interactions, and binding strengths could be obtained by CD spectroscopy. Dimers with a short linker were found to trigger and mediate fusion of lipopeptide CP₁₂E decorated

liposomes efficiently, which shows the suitability of a recognition motif with both membrane tethered and non-anchored fusogens for liposomal fusion.

The possibility of liposomal fusion based on orthogonal recognition of a set of coiled-coil peptide pairs was examined in **Chapter V**. This study showed the significant influence of peptide conformations, peptide – membrane interactions, and coiled-coil binding strengths on fusion efficiency. When one of the peptides in a pair show some degree of membrane interaction, fusion of liposomes functionalized with these peptides is highly efficient. Also, more helical peptides were more efficient in mediating fusion than weakly helical peptides, but coiled-coil binding strength did not have an pronounced effect on peptide helicity. Furthermore, a weak interaction between the positively charged peptide 1_K and the negatively charged peptide E was measured. This non-designed coiled-coil peptide pair was even more efficient in mediating fusion compared to the designed E - K peptide pair. These results revealed that peptide mediated fusion highly depends on the helicity and on the peptide membrane interactions of the involved peptides and shed new light on the mechanisms of membrane fusion.

PERSPECTIVES

This research has deepened our insight in the mechanism of membrane fusion induced by complementary pairs of lipopeptides able to form coiled-coil assemblies, and provides directions for future investigations. The finding that the anchor and spacer-length of the lipopeptides dramatically influence their fusogenic activity revealed the peptide – roleplay in the fusion mechanism, but also changed the view on lipopeptide design. Large molecules with distinct conjugated moieties display molecular dynamics that differ from the sum of the individual parts, and intramolecular effects have to be addressed. These intramolecular effects on molecular dynamics are also applicable for other conjugated molecules, and not limited to lipopeptides.

The increased fusion efficiency of peptides showing a peptide-membrane interaction provides evidence for the importance of membrane destabilization in the fusion process. The important question here is how these different peptides (1_K, 3_K and K) incorporate in the membrane, and what the effect is of the involved peptide association constants on peptide conformations and fusion efficiency. Membrane fusion is a highly dynamic process, and understanding of the dynamic parameters of the used lipopeptides is vital for designing artificial fusion systems with high fusion efficiencies, which is essential for developing successful drug delivery systems.

The demonstrated orthogonal fusion assay enables the development of multicomponent targeted fusion *in vitro* and *in vivo*. Many issues have to be addressed in this journey, such as peptide compatibility with live cells, peptide accessibility on cellular membranes and selective peptide functionalization of target membranes. The accomplishment of targeted orthogonal fusion of different drug loaded liposomes with live tissues will redefine currently used treatments and circumvent most of the known side effects of today's drugs.