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Coiled-coil mediated liposomal fusion: Asymmetric behaving peptide fusogens

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CHAPTER I

GENERAL INTRODUCTION TO MEMBRANE FUSION

INTRODUCTION

Membrane fusion, a vital process in all living organisms is triggered by special zipper-like proteins and is key for understanding cellular logistics. These processes include exocytosis, fusion of small enveloped viruses with cells and cell-cell fusion.¹ Whilst the exact mechanism of membrane fusion is still a matter of debate, it is widely accepted that it proceeds in four stages:² (Figure 1) opposing membranes are brought into close proximity (I); the membrane is disrupted at the site of contact (II); outer membranes are merged resulting in hemifusion (III/IV); and full fusion is achieved, facilitating content mixing between the two fused compartments via pore formation (V). Since lipid bilayers possess an intrinsic stability against any structural deformations by a powerful hydrophobic effect, membrane remodeling via highly curved intermediates requires energy. This energy barrier prohibits spontaneous and non-targeted vesicle fusion in biological systems³⁻⁴ and specialized proteins are employed to overcome the energy-gap and to guide these processes.⁵

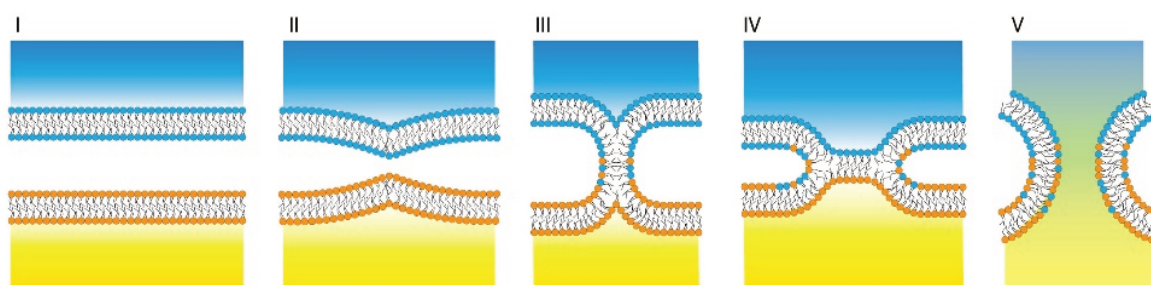


Figure 1. *Stages of the fusion cascade. Membranes are brought in close apposition (I) and approach each other in a point-like protrusion (II). A stalk intermediate is formed by merging of the outer membrane layers (III). This intermediate widens (IV) resulting in pore formation and full fusion (V).*

SNARE (soluble N-ethylmaleimide-sensitive factor (NSF) attachment protein receptor) proteins are a well conserved class of fusion proteins, which have been implicated as central in most, if not all, intracellular vesicle trafficking events studied so far.⁶⁻⁸ SNARE proteins are located on both target membranes and transport vesicles, and zipper into a parallel four-helix bundle,⁹⁻¹¹ bringing the opposing membranes into close contact and guiding the fusion process, as shown in Figure 2.¹²⁻¹³ Furthermore, several other membrane tethered proteins are known to initiate merging of the membranes via quick and specific Ca^{2+} triggered membrane destabilization.¹⁴⁻¹⁸ Upon fusion, the complex undergoes ATP catalyzed disassembly to recycle the SNAREs for a new fusion

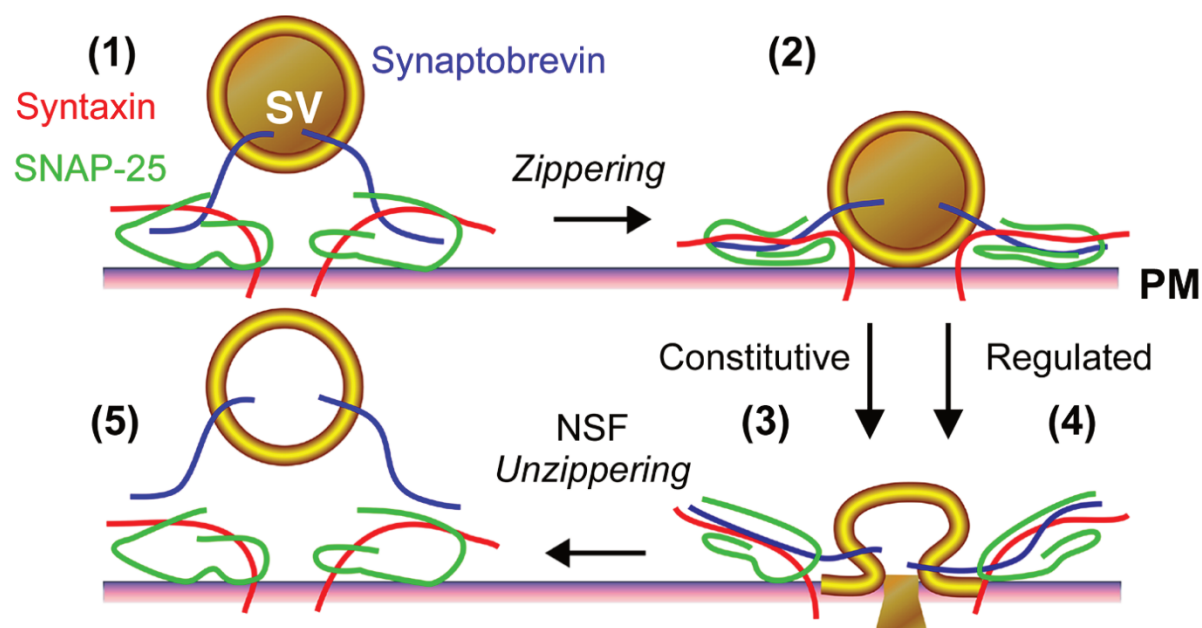


Figure 2. The proposed model of the SNARE mediated synaptic fusion cycle. Synaptobrevin forms a partial complex with Syntaxin and SNAP 25 (1). Full coiled coil formation brings the synaptic vesicle close to the plasma membrane (2) and full fusion occurs either spontaneously (3) or evoked by Ca^{2+} (4). Finally, NSF ATPase catalyzes the disassembly of the SNARE complex and the recycling of the vesicles. Figure adapted from Lagow et al.¹⁴⁻¹⁵

event.¹⁶⁻¹⁹ It is commonly accepted that the lower limit to induce membrane fusion involves only 3 SNARE complexes, and even a minimum of only 1 SNARE complex has been reported,²⁰ indicating the large amount of energy released with the formation of the tetrameric coiled-coil complex. Although the basic principles of SNARE mediated vesicle fusion are identified, many details are still unknown.

ARTIFICIAL STATE-OF-THE-ART MEMBRANE FUSION

Understanding and mimicking directed membrane fusion, i.e. targeted and leakage-free content mixing between two different enclosed compartments, opens ways to applications in drug delivery and biotechnology, and is thus an interesting target for supramolecular and biomaterials chemists. In recent years, simplified model systems have been reported in which membrane fusion is mediated by designed supramolecular model systems such as carbo-hydrates,²¹⁻²² peptides,²³⁻²⁷ DNA,²⁸⁻²⁹ small molecule recognition,³⁰⁻³⁵ and light responsive systems.³⁶⁻³⁷ Despite the immense efforts in this field, efficient content mixing of fusogen-functionalized liposomes remains challenging.

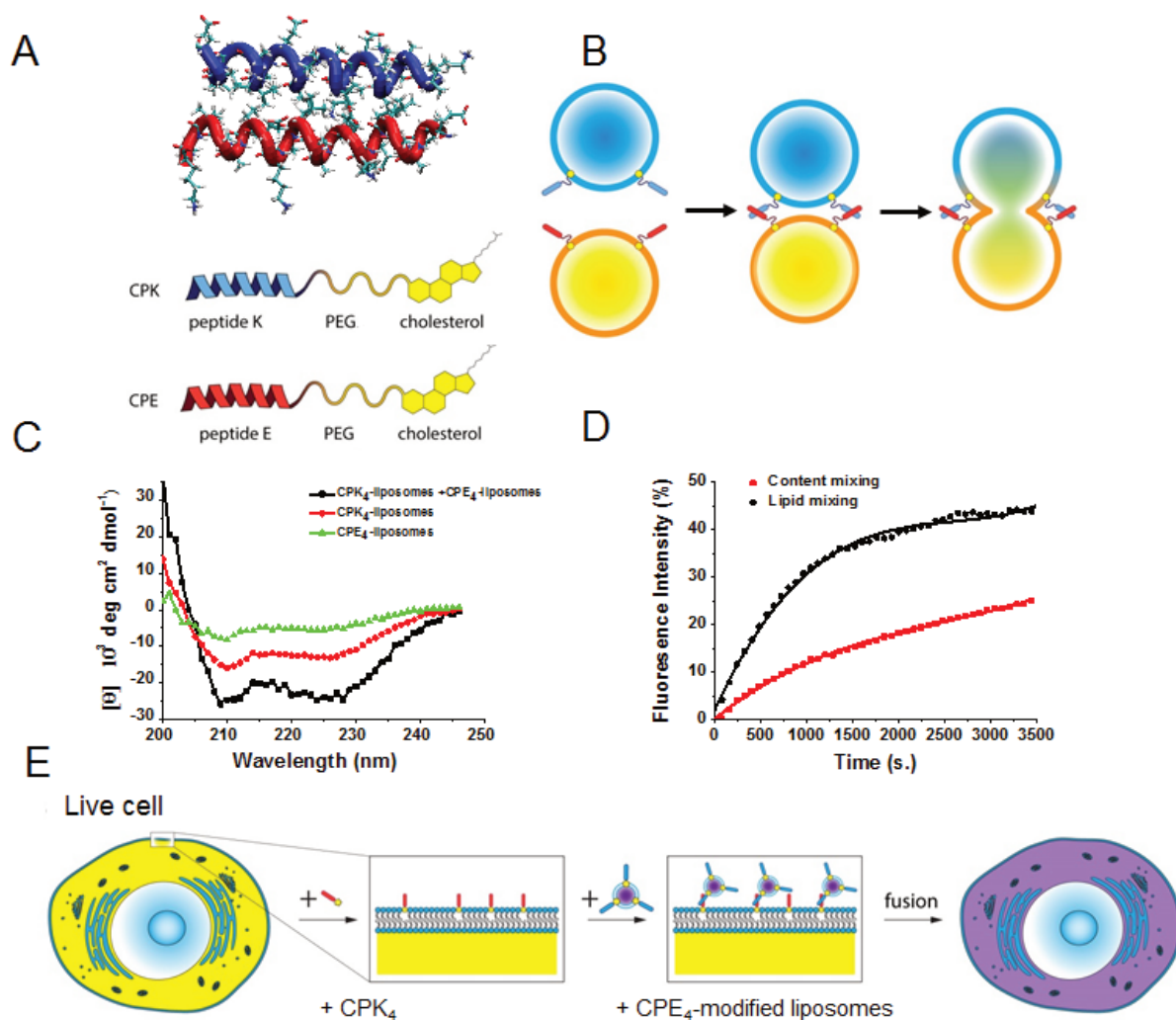


Figure 3. Schematic representation of (A) coiled-coil structure between peptides E and K, (B) targeted liposome fusion mediated by coiled-coil formation between CPE₄ modified liposomes and CPK₄ modified liposomes, (C) CD spectra of CPE₄ modified liposomes, and CPK₄ modified liposomes and an equimolar mixture thereof. [Total lipid] = 0.5 mM with 1 mol % lipopeptide in PBS. (D) Lipid mixing and content mixing between CPE₄-liposomes and CPK₄-liposomes. Fluorescence traces showing lipid mixing between CPE₄ and CPK₄ decorated liposomes, as measured through an increase in NBD fluorescence. [Total lipid] = 0.1 mM with 1 mol % of lipopeptide, in PBS; and showing content mixing between sulphorhodamine loaded (20 mM), CPE₄ decorated liposomes and nonfluorescent, CPK₄ decorated liposomes. [Total lipid] = 0.25 mM with 1 mol % lipopeptide in PBS. (E) Scheme of fusion between cell and liposomes. Figure reprinted from Yang et al.³⁸

In our lab, we successfully modified the specific heterodimeric coiled-coil pair E (EIAALEK)₃ and K (KIAALKE)₃ to mediate the fusion of liposomes, as shown in Figure 3.³⁹⁻⁴¹ Peptides are tethered to the liposomal membrane by a flexible PEG-linker conjugated to a lipid anchor, usually a membrane constituent.⁴² Recently, we reported the targeted and leakage-free delivery of various molecules, including the cytotoxic drug doxorubicin, both *in vitro* and *in vivo* via fusion of small unilamellar liposomes with live cells or zebra fish embryo's.^{38, 43} This method allows for quick and efficient delivery of

drugs and (bio)macromolecules without cell damage and is expected to have many applications *in vitro* and *in vivo*.

COILED-COIL MEDIATED LIPOSOMAL FUSION

However, years of research revealed that the mechanism is less straightforward than initially proposed,⁴¹ and that similarly designed peptides play different roles in the fusion mechanism.⁴⁴ Furthermore, study of the relationship between structure and behavior of membrane-tethered peptides and the unambiguous assignment of these factors in the complex and short living fusion machinery is inherently difficult.

The role of the lipopeptides in the fusion process is revealed by studying relationships between rational lipopeptide modifications and their resulting influence on fusion efficiency. The consequences of the anchor type were previously investigated,⁴² and it was shown that only DOPE and cholesterol anchors yielded significant fusion, with the latter being the most efficient. Increasing the length of the peptide (which increases coiled-coil binding strength and peptide-membrane interactions) also had a positive effect on fusion efficiency.⁴¹ Conversely, parallel or anti-parallel coiled-coil orientation,⁴⁵ and coiled-coil oligomer state,⁴⁶ did not influence the rate of fusion. The asymmetric roles of the peptides in the fusion process was recently discovered.⁴⁴ Peptide K interacts with the liposomal membrane, resulting in membrane destabilization and the initiation of the fusion process, whereas peptide E facilitates coiled-coil formation, thereby forcing the opposing liposomes in close proximity and concomitant fusion. These different roles lead to competing interactions, resulting in an equilibrium between K – E coiled-coil formation and K – membrane interactions. These results gave us new insights in the fusion mechanism and it is proposed that the membrane immersion of K is of major importance for efficient membrane fusion.

SCOPE OF THIS THESIS

In all previous studies the PEG₁₂ linker between the peptide and the anchor was kept constant. However, the length of this spacer could highly influence the roles of both peptides and a systematic study could reveal the dynamics of the peptides in the fusion mechanism. Therefore, the influence of the PEG-spacer length and the anchor type on the fusion efficiency and on the peptide-membrane interaction was investigated. In this thesis cholesterol anchored peptides were used and compared to previous research conducted with DOPE-anchored peptides. The results of these studies are described in **Chapter II** (liposome fusion) and **Chapter III** (peptide-membrane interaction).

To better understand the role of homo-coiled-coil interaction of peptide K, structure – behavior relationships of K-dimers were investigated via different covalently bound K-K dimers, as described in **Chapter IV**. These peptide-dimers also mediate liposomal fusion between CPE decorated vesicles, demonstrating the versatility of fusion systems with anchored and non-anchored fusogens.

Next, coiled-coil peptide mediated liposome fusion was optimized in terms of fusion efficiency by using a range of coiled-coil peptide pairs. Therefore, a set of orthogonal coiled-coil peptides is introduced in **Chapter V**, and the characteristics of these heterodimeric coiled-coil peptides were studied. Subsequently, cholesterol anchored lipopeptide derivatives were synthesized, and the properties of the peptides when tethered to liposomal membranes was investigated. Furthermore, the ability of these peptides to mediate liposomal fusion is measured and the results are related to the described peptide characteristics. Finally, **Chapter VI** summarizes and interconnects the findings in the present work.

REFERENCES

1. Jahn, R.; Lang, T.; Sudhof, T. C., Membrane fusion. *Cell* **2003**, *112* (4), 519-533.
2. Chernomordik, L. V.; Kozlov, M. M., Mechanics of membrane fusion. *Nat. Struct. Mol. Biol.* **2008**, *15* (7), 675-683.
3. Jahn, R.; Grubmuller, H., Membrane fusion. *Curr. Opin. Cell Biol.* **2002**, *14* (4), 488-495.
4. Francois-Martin, C.; Rothman, J. E.; Pincet, F., Low energy cost for optimal speed and control of membrane fusion. *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114* (6), 1238-1241.
5. Bonifacino, J. S.; Glick, B. S., The mechanisms of vesicle budding and fusion. *Cell* **2004**, *116* (2), 153-66.
6. Weber, T.; Zemelman, B. V.; McNew, J. A.; Westermann, B.; Gmachl, M.; Parlati, F.; Sollner, T. H.; Rothman, J. E., SNAREpins: Minimal machinery for membrane fusion. *Cell* **1998**, *92* (6), 759-772.
7. Jahn, R.; Scheller, R. H., SNAREs--engines for membrane fusion. *Nat. Rev. Mol. Cell Biol.* **2006**, *7* (9), 631-43.
8. Han, J.; Pluhackova, K.; Bockmann, R. A., The Multifaceted Role of SNARE Proteins in Membrane Fusion. *Front. Membr. Physiol. Membr. Biophys.* **2017**, *8*, 5.
9. Lou, X.; Shin, Y. K., SNARE zippering. *Biosci. Rep.* **2016**, *36* (3).
10. Zhang, Y., Energetics, kinetics, and pathway of SNARE folding and assembly revealed by optical tweezers. *Protein Sci.* **2017**.
11. Zorman, S.; Rebane, A. A.; Ma, L.; Yang, G.; Molski, M. A.; Coleman, J.; Pincet, F.; Rothman, J. E.; Zhang, Y., Common intermediates and kinetics, but different energetics, in the assembly of SNARE proteins. *Elife* **2014**, *3*, e03348.
12. Risselada, H. J.; Grubmuller, H., How SNARE molecules mediate membrane fusion: recent insights from molecular simulations. *Curr. Opin. Struct. Biol.* **2012**, *22* (2), 187-96.
13. Hong, W.; Lev, S., Tethering the assembly of SNARE complexes. *Trends Cell Biol.* **2014**, *24* (1), 35-43.
14. Lagow, R. D.; Bao, H.; Cohen, E. N.; Daniels, R. W.; Zuzek, A.; Williams, W. H.; Macleod, G. T.; Sutton, R. B.; Zhang, B., Modification of a hydrophobic layer by a point mutation in syntaxin 1A regulates the rate of synaptic vesicle fusion. *Plos Biology* **2007**, *5* (4), 800-817.
15. Lin, R. C.; Scheller, R. H., Structural organization of the synaptic exocytosis core complex. *Neuron* **1997**, *19* (5), 1087-94.
16. Winter, U.; Chen, X.; Fasshauer, D., A conserved membrane attachment site in alpha-SNAP facilitates N-ethylmaleimide-sensitive factor (NSF)-driven SNARE complex disassembly. *J. Biol. Chem.* **2009**, *284* (46), 31817-26.
17. Baker, R. W.; Hughson, F. M., Chaperoning SNARE assembly and disassembly. *Nat. Rev. Mol. Cell Biol.* **2016**, *17* (8), 465-79.
18. Ryu, J. K.; Jahn, R.; Yoon, T. Y., Review: Progresses in understanding N-ethylmaleimide sensitive factor (NSF) mediated disassembly of SNARE complexes. *Biopolymers* **2016**, *105* (8), 518-31.
19. Zhao, M.; Wu, S.; Zhou, Q.; Vivona, S.; Cipriano, D. J.; Cheng, Y.; Brunger, A. T., Mechanistic insights into the recycling machine of the SNARE complex. *Nature* **2015**, *518* (7537), 61-7.
20. van den Bogaart, G.; Holt, M. G.; Bunt, G.; Riedel, D.; Wouters, F. S.; Jahn, R., One SNARE complex is sufficient for membrane fusion. *Nat. Struct. Mol. Biol.* **2010**, *17* (3), 358-64.
21. Kauscher, U.; Ravoo, B. J., Mannose-decorated cyclodextrin vesicles: The interplay of multivalency and surface density in lectin-carbohydrate recognition. *Beilstein J. Org. Chem.* **2012**, *8*, 1543-1551.

22. Voskuhl, J.; Stuart, M. C. A.; Ravoo, B. J., Sugar-Decorated Sugar Vesicles: Lectin-Carbohydrate Recognition at the Surface of Cyclodextrin Vesicles. *Chem. - Eur. J.* **2010**, *16* (9), 2790-2796.
23. Rauschenberg, M.; Bomke, S.; Karst, U.; Ravoo, B. J., Dynamic Peptides as Biomimetic Carbohydrate Receptors. *Angew. Chem., Int. Ed.* **2010**, *49* (40), 7340-7345.
24. Nomura, F.; Inaba, T.; Ishikawa, S.; Nagata, M.; Takahashi, S.; Hotani, H.; Takiguchi, K., Microscopic observations reveal that fusogenic peptides induce liposome shrinkage prior to membrane fusion. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101* (10), 3420-5.
25. Kashiwada, A.; Matsuda, K.; Mizuno, T.; Tanaka, T., Construction of a pH-responsive artificial membrane fusion system by using designed coiled-coil polypeptides. *Chem. - Eur. J.* **2008**, *14* (24), 7343-7350.
26. Gong, Y.; Luo, Y.; Bong, D., Membrane activation: selective vesicle fusion via small molecule recognition. *J. Am. Chem. Soc.* **2006**, *128* (45), 14430-14431.
27. Lygina, A. S.; Meyenberg, K.; Jahn, R.; Diederichsen, U., Transmembrane Domain Peptide/Peptide Nucleic Acid Hybrid as a Model of a SNARE Protein in Vesicle Fusion. *Angew. Chem., Int. Ed.* **2011**, *50* (37), 8597-8601.
28. Chan, Y. H.; van Lengerich, B.; Boxer, S. G., Lipid-anchored DNA mediates vesicle fusion as observed by lipid and content mixing. *Biointerphases* **2008**, *3* (2), FA17.
29. Stengel, G.; Simonsson, L.; Campbell, R. A.; Hook, F., Determinants for membrane fusion induced by cholesterol-modified DNA zippers. *J. Phys. Chem. B* **2008**, *112* (28), 8264-8274.
30. Ma, M. M.; Bong, D., Controlled Fusion of Synthetic Lipid Membrane Vesicles. *Acc. Chem. Res.* **2013**, *46* (12), 2988-2997.
31. Kashiwada, A.; Tsuboi, M.; Mizuno, T.; Nagasaki, T.; Matsuda, K., Target-selective vesicle fusion system with pH-selectivity and responsiveness. *Soft Matter* **2009**, *5* (23), 4719-4725.
32. Kashiwada, A.; Tsuboi, M.; Takamura, N.; Brandenburg, E.; Matsuda, K.; Kokschi, B., Design and Characterization of Endosomal-pH-Responsive Coiled Coils for Constructing an Artificial Membrane Fusion System. *Chem. - Eur. J.* **2011**, *17* (22), 6179-6186.
33. Marchi-Artzner, V.; Gulik-Krzywicki, T.; Guedeau-Boudeville, M. A.; Gosse, C.; Sanderson, J. M.; Dedieu, J. C.; Lehn, J. M., Selective adhesion, lipid exchange and membrane-fusion processes between vesicles of various sizes bearing complementary molecular recognition groups. *ChemPhysChem* **2001**, *2* (6), 367-376.
34. Richard, A.; Marchi-Artzner, V.; Lalloz, M. N.; Brienne, M. J.; Artzner, F.; Gulik-Krzywicki, T.; Guedeau-Boudeville, M. A.; Lehn, J. M., Fusogenic supramolecular vesicle systems induced by metal ion binding to amphiphilic ligands. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101* (43), 15279-15284.
35. Voskuhl, J.; Fenske, T.; Stuart, M. C.; Wibbeling, B.; Schmuck, C.; Ravoo, B. J., Molecular recognition of vesicles: host-guest interactions combined with specific dimerization of zwitterions. *Chemistry* **2010**, *16* (28), 8300-8306.
36. Nalluri, S. K. M.; Ravoo, B. J., Light-Responsive Molecular Recognition and Adhesion of Vesicles. *Angew. Chem., Int. Edit.* **2010**, *49* (31), 5371-5374.
37. Nalluri, S. K. M.; Bultema, J. B.; Boekema, E. J.; Ravoo, B. J., Photoresponsive Molecular Recognition and Adhesion of Vesicles in a Competitive Ternary Supramolecular System. *Chem. - Eur. J.* **2011**, *17* (37), 10297-10303.
38. Yang, J.; Bahreman, A.; Daudey, G.; Bussmann, J.; Olsthoorn, R. C.; Kros, A., Drug Delivery via Cell Membrane Fusion Using Lipopeptide Modified Liposomes. *ACS Cent. Sci.* **2016**, *2* (9), 621-630.
39. Marsden, H. R.; Elbers, N. A.; Bomans, P. H. H.; Sommerdijk, N.; Kros, A., A Reduced SNARE Model for Membrane Fusion. *Angew. Chem., Int. Ed.* **2009**, *48* (13), 2330-2333.

40. Marsden, H. R.; Tomatsu, I.; Kros, A., Model systems for membrane fusion. *Chem. Soc. Rev.* **2011**, *40* (3), 1572-1585.
41. Zheng, T.; Voskuhl, J.; Versluis, F.; Zope, H. R.; Tomatsu, I.; Marsden, H. R.; Kros, A., Controlling the rate of coiled coil driven membrane fusion. *Chem. Commun. (Cambridge, U. K.)* **2013**, *49* (35), 3649-3651.
42. Versluis, F.; Voskuhl, J.; van Kolck, B.; Zope, H.; Bremmer, M.; Albregtse, T.; Kros, A., In situ modification of plain liposomes with lipidated coiled coil forming peptides induces membrane fusion. *J. Am. Chem. Soc.* **2013**, *135* (21), 8057-8062.
43. Yang, J.; Shimada, Y.; Olsthoorn, R. C. L.; Snaar-Jagalska, B. E.; Spaink, H. P.; Kros, A., Application of Coiled Coil Peptides in Liposomal Anticancer Drug Delivery Using a Zebrafish Xenograft Model. *ACS Nano* **2016**, *10* (8), 7428-7435.
44. Rabe, M.; Aisenbrey, C.; Pluhackova, K.; de Wert, V.; Boyle, A. L.; Bruggeman, D. F.; Kirsch, S. A.; Bockmann, R. A.; Kros, A.; Raap, J.; Bechinger, B., A Coiled-Coil Peptide Shaping Lipid Bilayers upon Fusion. *Biophys. J.* **2016**, *111* (10), 2162-2175.
45. Versluis, F.; Dominguez, J.; Voskuhl, J.; Kros, A., Coiled-coil driven membrane fusion: zipper-like vs. non-zipper-like peptide orientation. *Faraday Discuss.* **2013**, *166*, 349-359.
46. Zheng, T. T.; Bulacu, M.; Daudey, G.; Versluis, F.; Voskuhl, J.; Martelli, G.; Raap, J.; Sevink, G. J. A.; Kros, A.; Boyle, A. L., A non-zipper-like tetrameric coiled coil promotes membrane fusion. *RSC Adv.* **2016**, *6* (10), 7990-7998.