

Coiled-coil biomaterials for biological applications Shen, M.

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Chapter 1

Introduction: Coiled-coil Peptides Based Biomaterials and Membrane Fusion



1.1 Coiled-coil peptides

The coiled-coil motif was first named and described by Crick,¹⁻² and Linus Pauling,³ independently in 1953. After more than 60 years of research, the coiled-coil still arouses the interest of many researchers and has become more and more popular, for both fundamental and applied research.⁴⁻⁵ The coiled-coil is a common structural motif in proteins,⁶ with about 3-5% of amino acids in proteins forming such structures.⁷ This motif is especially common in a large class of fibrous proteins like tropomyosin,⁸⁻⁹ myosin,¹⁰⁻¹¹ intermediate filaments,¹²⁻¹³ and prokaryotic surface proteins,¹⁴⁻¹⁵ where the coiled-coil has been described as the main structural element.

A coiled-coil contains two or more α -helices which wrap around each other and form a left-handed supercoil. Crick described the structure as having "knobs-into-holes" packing since the α -helices twist, at an angle of around 20°, with respect to each other, which interlocks the side chains of the amino acids on neighbouring peptide chains.⁶⁻⁷ The reason for this twist is that, in α -helices, each complete turn of a helix usually contains 3.6 residues, while in a coiled-coil there is a lower number of residues in each turn - 3.5. A repeat of seven residues, which is known as a heptad repeat, is common in many coiled coils. In that case, each heptad in a coiled-coil contains two helical turns. This heptad repeat is usually denoted as (*abcdefg*)_n where each letter represents each position of the coiled-coil and '**n**' is the number of repeats. Heptad repeats can be shown using a helical wheel diagram (Figure 1).



Figure 1. Helical wheel diagram of a parallel heterodimeric coiled-coil.

Figure 1 shows an example of a parallel heterodimeric coiled-coil. In such a structure, positions a and d usually contain hydrophobic amino acids, thus forming the non-polar core of the α -helix. Therefore, hydrophobic interactions and van der Waals interactions could induce coiled-coil formation in an aqueous solution. Positions e and g normally have amino acids containing complementary charged side chains and then the coiled-coil structure can be stabilized by interhelical electrostatic interactions. Since positions b, c and f are exposed to the solvent, hydrophilic amino acids are preferred to increase the solubility of the peptide. Previously, these positions were thought to be less important for defining the properties of the coiled-coil specificity and stability.¹⁶⁻¹⁷

Most coiled coils, both from nature or developed via artificial design, contain two to four helices and these structures have cyclic or dihedral symmetry. ¹⁸ However, coiled coils containing five or more helices have also been reported in numerous studies.¹⁸⁻²¹ Figures 2A-2D show helical wheel diagrams of different structures of coiled coils; those with different oligomeric states or orientations.⁵ All of these structures present 'knobs-in-holes' interactions (figure 2E & 2F).



Figure 2. Differently structured coiled-coils: (A) parallel dimeric coiled-coil; (B) antiparallel dimeric coiled-coil; (C) parallel trimeric coiled-coil; (D) parallel tetrameric coiled-coil. The 'knobs-in-holes' arrangement of a parallel coiled-coil (E) and an antiparallel coiled-coil (F). The parallel coiled-coil contains a and d layers while the antiparallel coiled-coil has mixed ad layers in its 'knobs-in-holes' arrangement. Figures are adapted from references.⁴⁻⁵

Many structural features can influence the stability of coiled coils.^{7, 22} For example, by introducing more hydrophobic residues in the a and d positions, coiled-coil

stability can be increased. Additionally, the salt bridges between residues in g/e' positions can also influence the coiled-coil stability. Most importantly, increasing the length of the coiled-coil forming peptides can not only increase the area of the coiled-coil hydrophobic core but will also increase the number of salt bridges. Many studies have shown that increasing the length of the coiled-coil forming peptides is a facile way to increase the stability of coiled coil.^{7, 22-25} Therefore, coiled-coil stability can be tuned by changing the length of the coiled-coil peptides.

1.2 Coiled coils for use in biomaterials

Peptide- and protein-based biomaterials have seen rapid development over the last two decades because such biomaterials not only have potential applications in biotechnology but they can also help researchers to learn how natural biomaterials work in biology.²⁶ Coiled coils have become one of the most attractive building blocks for peptide- and protein-based biomaterials due to their excellent self-assembly properties, in addition to their specific recognition and responsiveness. The coiled-coil building block is widely used in drug delivery, protein purification, protein labeling, nanoparticle self-assembly, biosensing, and hydrogel formation, amongst others (Figure 3).⁵



Figure 3. Examples of coiled-coil peptide-based biomaterials: A) drug delivery; B) peptide purification; C) protein labelling; D) nanoparticle self-assembly; E) hydrogel formation, and; F) biosensing. The figure is adapted from reference.⁵

The E/K coiled coil, is a *de novo* designed parallel heterodimeric coiled-coil, developed by Hodges and coworkers.^{22, 27-29} The coiled-coil forming peptides that make up this assembly were named peptide E and peptide K, because they are rich in glutamic acid and lysine respectively. This heterodimer, and others which are similar, have become very popular in a multitude of applications because of its stability and specificity. For example, a two-stage drug-free macromolecular therapeutic system has been developed by Wu et al.³⁰ In this work, the coiled-coil forming peptides E and K, were separately modified with the 1F5 anti-CD20 antibody and an HPMA polymer, respectively. The therapeutic process contains two stages. In the first stage, peptide E modified antibodies selectively bind to CD20 on the surface of Raji B cells. Next, coiled coil formation between peptides K and E crosslinks CD20 receptors due to the action of the HPMA copolymer, resulting in apoptosis. In another study, coiled-coil motifs were used to develop a drug delivery system using the pH-sensitive nature of the K₃/E₃ coiled coil.³¹ Here, peptides E_3 and K_3 were attached to either a synthetic polymer or a drug/dye. By forming a coiled-coil, a polymer drug conjugate was formed which entered cells

via endocytosis. Next, these drugs could be released from the polymer complex due to the lower pH in endosomes/lysosomes compared to the rest of the cell. The pH change results in the dissociation of the K_3/E_3 coiled coil and concomitant drug release.

The coiled-coil motif has also has been used in various hydrogels.³²⁻³⁷ Hill and coworkers designed a thermo-responsive hydrogel using coiled-coil domains as physical crosslinks.³² Due to the reversible self-assembly properties of coiled-coil peptides, the formation and dissociation of the hydrogel can be easily controlled by adjusting the temperature. These hydrogel biomaterials can be used to control small molecule release, which also has potential applications for drug delivery.

1.3 Membrane fusion

Membrane fusion is a fundamental and essential process in life.³⁸⁻⁴² It occurs between cellular plasma membranes, intracellular compartments, cellular membranes and intracellular compartments, and lipid-covered vesicles and cellular membranes.⁴³ Neurotransmitter release.⁴⁴⁻⁴⁵ fertilization.⁴⁶⁻⁴⁷ endocrine hormone secretion,⁴⁸⁻⁴⁹ and enveloped virus infection⁵⁰⁻⁵¹ are examples of biological processes that require membrane fusion. The process of membrane fusion can be described using the fusion-through-hemifusion pathway model, which is shown in Figure 4.52 This process starts from a pre-fusion contact, (i), in which two lipid membranes are forced close to each other. Because of the energy barrier caused by the hydration repulsion between the proximal leaflets of the membranes, a pointlike membrane protrusion state, (ii), is formed which can lower the energy barrier for the transition into the hemifusion stalk state, (iii). The hemifusion stalk state represents the two outer leaflets fused while the inner leaflets are still unfused. The fusion pore can then either directly form from the hemifusion stalk or go through a hemifusion diaphragm state (iv) obtained by hemifusion stalk expansion. After the fusion pore, (v), is formed, complete membrane fusion is achieved. During hemifusion-stalk to fusion pore formation and expansion, high energy barriers exist because of the necessary membrane curvature deformations. Studies have shown that lipid composition influences the curvature of the lipid membrane and assists membrane deformation, thus reducing the energy barrier and facilitating membrane fusion.⁵³⁻⁵⁷ Lipids with short and unsaturated fatty acids can increase the fluidity of the lipid membrane and a higher membrane fluidity has a positive effect on membrane fusion.58-60 Therefore, the lipid composition is a very important parameter in membrane fusion.



Figure 4. Lipid membrane fusion via the fusion-through-hemifusion pathway. The figure is adapted from reference.⁵²

1.4 SNARE assisted membrane fusion

SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptors) proteins play a key role in mediating membrane fusion in eukaryotic cells.⁶¹⁻⁶² SNARE proteins were first characterized in the late 1980s, and these SNAREs were found to mediate a variety of intracellular trafficking processes.⁶³⁻⁶⁴ For instance, efficient membrane fusion between synaptic vesicles and presynaptic plasma membranes, a process induced by SNARE proteins, allows fast neurotransmitter release and thus efficient synaptic transmission.44-45, 65 The SNARE complex contains two parts: the transport vesicle SNARE (v-SNARE) and target membrane SNARE (t-SNARE). Usually, the SNARE complex contains one v-SNARE with two or three t-SNAREs. The first identified structure of a SNARE complex in the presynaptic neuron was composed of vesicle-associated membrane protein (VAMP), syntaxin 1 and SNAP-25.⁶⁶ VAMP is located on the synaptic vesicle and therefore acts as the v-SNARE while syntaxin and SNAP-25 are located on the presynaptic plasma membrane and are known as t-SNAREs. VAMP and syntaxin contain transmembrane domains (TMD), which anchor them into the lipid membrane, while SNAP-25 is connected to membranes via covalent bonds between palmitoyl side chains of the membrane and multiple cysteine side chains of the protein. Some studies have shown that the TMD of the SNARE proteins within the complex could reduce the energy barrier and facilitate fusion pore formation.⁶⁷ By forming a four-helix coiled-coil structure (Figure 5), the v-SNARE and t-SNAREs could interact with each other and bridge two membranes thus facilitating fusion. Other SNARE proteins have similar structures and mediate membrane fusion in quite similar manners.



Figure 5. A) Membrane fusion induced by a SNARE complex which contains a tetrameric coiled-coil motif formed by syntaxin, and SNAP-25 on target membrane and synaptobrevin (also known as VAMP) on the synaptic vesicle. The fusion pore is formed by the coiled-coil complex 'zipping up' from the N- to the C-terminus and dragging the two membranes into close proximity. B) The tetramer coiled-coil complex of the SNARE protein. The figure is adapted from reference.⁶⁸

1.5 Membrane fusion triggered by lipid-anchored coiled coils

In our group, we have designed a coiled-coil peptide mediated membrane fusion system inspired by SNARE proteins.⁶⁹⁻⁷⁰ A lipid anchor was conjugated to the coiled-coil forming peptides K and E by a short poly(ethylene glycol) (PEG) spacer (Figure 6). In one study examining the effect of the lipid anchor, cholesterol was found to be the best anchor as it triggers liposome membrane fusion with the highest efficiency.⁷¹ The spacer length has also been investigated and the results show that short coiled-coil peptides prefer a longer spacer in order to reach each other and form a coiled-coil interaction, while longer coiled-coil peptides do not need such a long spacer.⁷² The length of coiled-coil peptides was also found to play an important role in fusogenicity: four heptad coiled coils can trigger both liposome-liposome and cell-liposome membrane fusion.^{71, 73-74} The K/E coiled-coil triggered membrane fusion system was also found to have potential

applications in drug delivery.74-76

Mechanistic studies show that the stability of the coiled-coil and the membrane affinity of peptide K are two crucial factors that affect fusogenicity.⁷⁷⁻⁷⁹ As previously mentioned, several energy barriers exist during the membrane fusion process. A more stable coiled-coil can bind the two separate membranes more tightly and overcome these energy barriers more easily. Furthermore, peptide K interacts with membranes and induces a positive curvature, thereby destabilizing the membrane, lowering the energy barrier for fusion pore formation.⁷⁷



Figure 6. Cartoon pictures illustrating A) the structure of CPK and CPE, B) liposome membrane fusion triggered by CPK and CPE, C) cell-liposome membrane fusion triggered by CPK₄ and CPE₄.

1.6 Aim and outline of this thesis

In this thesis, we used the K/E coiled-coil motifs to facilitate various novel applications.

In **chapter 2**, a magnetic-activated cell sorting (MACS) system based on the K_3/E_3 coiled-coil is described. For this project, iron oxide particles (IOPs) were functionalized with coiled-coil forming peptides and coiled-coil formation on the IOPs surface was demonstrated. Using these coiled-coil forming peptide-functionalized IOPs and an external magnetic field, cell sorting assays were performed with three different cell lines, showing that coiled-coil decorated cells can be separated from non coiled-coil decorated cells. Finally, we will show that cells that express peptide K_3 on the cell membrane can be enriched using this

approach.

In **chapter 3**, we compare the fusogenicity of different coiled-coil pairs formed by different lengths of K and E peptides. Using circular dichroism (CD) spectroscopy and dynamic light scattering (DLS), the secondary structure and stability of the individual K and E peptides, together with their coiled-coil complexes has been studied in order to probe the relationship between coiled-coil length and stability. We subsequently investigated the fusogenicity of these coiled coils by performing lipid-mixing and content-mixing assays using liposomes. After demonstrating their fusogenicity in liposome systems, we prove that all K-lipopeptides can be used to decorate cell membranes, and we subsequently performed liposome-cell docking and evaluated the docking efficiency by flow cytometry. Finally, we selected the coiled-coil pairs which exhibited good liposome-cell docking and used them for cell-liposome membrane fusion studies. These studies showed that liposome fusion efficiency can be obtained by comparing the PI fluorescence intensity inside the cells.

The membrane affinity of peptide K is crucial for coiled-coil triggered membrane fusion because it can induce positive curvature of the lipid membrane, which lowers the energetic barrier and thus facilitates fusion pore formation. In **chapter 4**, we describe a membrane fusion system comprising different dimeric K₄ peptides, which interact with E_4 peptides resulting in membrane fusion. The secondary structure of three different K₄ dimers, and their self-assembly with E_4 , was investigated. These K₄ dimers form either temperature-dependent aggregates or concentration-dependent homodimers in buffered solutions. Furthermore, all K₄ dimers are capable of forming coiled coils with E_4 . The membrane affinity of these three K₄ dimers was studied by performing a tryptophan fluorescence titration assay, before the fusogenicity of different K₄ dimer-E₄ coiled coils was examined by liposome fusion assays. Finally, we studied cell-liposome membrane fusion triggered by these coiled coils.

Since a K₄ dimer-E₄ coiled-coil shows enhanced fusogenicity for both liposomeliposome and cell-liposome membrane fusion (**chapter 4**), in **chapter 5** we used this coiled-coil complex to trigger 1-form cell-cell membrane fusion. L-forms are cell wall-deficient bacteria that can survive without their original cell wall.⁸⁰⁻⁸¹ Therefore, the study of 1-form fusion may be a novel tool to study the origin of life questions.⁸² Two phenotypes of 1-forms containing different fluorescence markers and different antibiotic resistance markers were produced. We studied the possibility of decorating l-form membranes with E_4 via a membrane labelling assay. Then, l-form cell-cell fusion was performed using the K₄ dimer-E₄ coiled-coil pair and the fusion efficiency was determined. In order to obtain stable l-forms expressing double markers, a fused l-form enrichment protocol was developed. After enrichment, more than 97% of double marker expressing l-forms were obtained in the total l-form population. Finally, we investigated the fused l-form cell division in medium and chromosome segregation in the solid-state.

In **chapter 6**, the main results and conclusions of the studies described in this thesis are summarized and future perspectives of coiled-coil based biomaterials are discussed.

REFERENCES

(1) Crick, F. The packing of α-helices: simple coiled coils. Acta crystallographica 1953, 6, 689.

(2) Crick, F. H. The Fourier transform of a coiled-coil. Acta crystallographica 1953, 6, 685.

(3) Pauling, L.; Corey, R. B. Compound helical configurations of polypeptide chains: structure of proteins of the α -keratin type. *Nature* **1953**, *171*, 59.

(4) Truebestein, L.; Leonard, T. A. Coiled-coils: The long and short of it. Bioessays 2016, 38, 903.

(5) Apostolovic, B.; Danial, M.; Klok, H. A. Coiled-coils: attractive protein folding motifs for the fabrication of self-assembled, responsive and bioactive materials. *Chem Soc Rev* **2010**, *39*, 3541.

(6) Lupas, A. N.; Bassler, J. Coiled-coils - A Model System for the 21st Century. *Trends Biochem Sci* **2017**, *42*, 130.

(7) Mason, J. M.; Arndt, K. M. Coiled-coil domains: stability, specificity, and biological implications. *Chembiochem* **2004**, *5*, 170.

(8) McLachlan, A.; Stewart, M. Tropomyosin coiled-coil interactions: evidence for an unstaggered structure. *Journal of molecular biology* **1975**, *98*, 293.

(9) Parry, D. Analysis of the primary sequence of α -tropomyosin from rabbit skeletal muscle. *Journal of molecular biology* **1975**, *98*, 519.

(10) Root, D. D.; Yadavalli, V. K.; Forbes, J. G.; Wang, K. Coiled-coil nanomechanics and uncoiling and unfolding of the superhelix and α -helices of myosin. *Biophysical journal* **2006**, *90*, 2852.

(11) Zhou, G.-P. The structural determinations of the leucine zipper coiled-coil domains of the cGMP-dependent protein kinase I α and its interaction with the myosin binding subunit of the myosin light chains phosphase. *Protein and peptide letters* **2011**, *18*, 966.

(12) Parry, D. A.; Steven, A. C.; Steinert, P. M. The coiled-coil molecules of intermediate filaments consist of two parallel chains in exact axial register. *Biochemical and biophysical research communications* **1985**, *127*, 1012.

(13) Steinert, P. The two-chain coiled-coil molecule of native epidermal keratin intermediate

filaments is a type I-type II heterodimer. Journal of Biological Chemistry 1990, 265, 8766.

(14) Söderholm, N.; Javadi, A.; Flores, I. S.; Flärdh, K.; Sandblad, L. Affinity to cellulose is a shared property among coiled-coil domains of intermediate filaments and prokaryotic intermediate filament-like proteins. *Scientific reports* **2018**, *8*, 1.

(15) Soppa, J. Prokaryotic structural maintenance of chromosomes (SMC) proteins: distribution, phylogeny, and comparison with MukBs and additional prokaryotic and eukaryotic coiled-coil proteins. *Gene* **2001**, *278*, 253.

(16) Kaplan, J. B.; Reinke, A. W.; Keating, A. E. Increasing the affinity of selective bZIP-binding peptides through surface residue redesign. *Protein Sci* **2014**, *23*, 940.

(17) Drobnak, I.; Gradisar, H.; Ljubetic, A.; Merljak, E.; Jerala, R. Modulation of Coiled-Coil Dimer Stability through Surface Residues while Preserving Pairing Specificity. *J Am Chem Soc* **2017**, *139*, 8229.

(18) Rhys, G. G.; Wood, C. W.; Lang, E. J. M.; Mulholland, A. J.; Brady, R. L.; Thomson, A. R.; Woolfson, D. N. Maintaining and breaking symmetry in homomeric coiled-coil assemblies. *Nat Commun* **2018**, *9*, 4132.

(19) Zaccai, N. R.; Chi, B.; Thomson, A. R.; Boyle, A. L.; Bartlett, G. J.; Bruning, M.; Linden, N.; Sessions, R. B.; Booth, P. J.; Brady, R. L.; Woolfson, D. N. A de novo peptide hexamer with a mutable channel. *Nat Chem Biol* **2011**, *7*, 935.

(20) Thomas, F.; Dawson, W. M.; Lang, E. J. M.; Burton, A. J.; Bartlett, G. J.; Rhys, G. G.; Mulholland, A. J.; Woolfson, D. N. De Novo-Designed alpha-Helical Barrels as Receptors for Small Molecules. *ACS Synth Biol* **2018**, *7*, 1808.

(21) Dawson, W. M.; Martin, F. J. O.; Rhys, G. G.; Shelley, K. L.; Brady, R. L.; Woolfson, D. N. Coiled-coils 9-to-5: rational de novo design of α -helical barrels with tunable oligomeric states. *Chemical Science* **2021**, *12*, 6923.

(22) Litowski, J. R.; Hodges, R. S. Designing heterodimeric two-stranded alpha-helical coiled-coils. Effects of hydrophobicity and alpha-helical propensity on protein folding, stability, and specificity. *J Biol Chem* **2002**, *277*, 37272.

(23) Kwok, S. C.; Hodges, R. S. Effect of chain length on coiled-coil stability: decreasing stability with increasing chain length. *Biopolymers* **2004**, *76*, 378.

(24) Sadeghi, S.; Emberly, E. Length-dependent force characteristics of coiled-coils. *Phys Rev E Stat Nonlin Soft Matter Phys* **2009**, *80*, 061909.

(25) Fairman, R.; Chao, H. G.; Mueller, L.; Lavoie, T. B.; Shen, L.; Novotny, J.; Matsueda, G. R. Characterization of a new four-chain coiled-coil: Influence of chain length on stability. *Protein Science* **1995**, *4*, 1457.

(26) Woolfson, D. N. Building fibrous biomaterials from alpha-helical and collagen-like coiled-coil peptides. *Biopolymers* **2010**, *94*, 118.

(27) Chao, H.; Bautista, D. L.; Litowski, J.; Irvin, R. T.; Hodges, R. S. Use of a heterodimeric coiledcoil system for biosensor application and affinity purification. *Journal of chromatography. B, Biomedical sciences and applications* **1998**, *715*, 307.

(28) Tripet, B.; Yu, L.; Bautista, D. L.; Wong, W. Y.; Irvin, R. T.; Hodges, R. S. Engineering a de

novo designed coiled-coil heterodimerization domain for the rapid detection, purification and characterization of recombinantly expressed peptides and proteins. *Protein Eng* **1997**, *10*, 299.

(29) Chao, H.; Houston, M. E.; Grothe, S.; Kay, C. M.; O'Connor-McCourt, M.; Irvin, R. T.; Hodges, R. S. Kinetic study on the formation of a de novo designed heterodimeric coiled-coil: use of surface plasmon resonance to monitor the association and dissociation of polypeptide chains. *Biochemistry* **1996**, *35*, 12175.

(30) Wu, K.; Liu, J.; Johnson, R. N.; Yang, J.; Kopecek, J. Drug-free macromolecular therapeutics: induction of apoptosis by coiled-coil-mediated cross-linking of antigens on the cell surface. *Angew Chem Int Ed Engl* **2010**, *49*, 1451.

(31) Apostolovic, B.; Deacon, S. P.; Duncan, R.; Klok, H.-A. Hybrid polymer therapeutics incorporating bioresponsive, coiled-coil peptide linkers. *Biomacromolecules* **2010**, *11*, 1187.

(32) Hill, L. K.; Meleties, M.; Katyal, P.; Xie, X.; Delgado-Fukushima, E.; Jihad, T.; Liu, C.-F.; O'Neill, S.; Tu, R. S.; Renfrew, P. D. Thermoresponsive protein-engineered coiled-coil hydrogel for sustained small molecule release. *Biomacromolecules* **2019**, *20*, 3340.

(33) Jing, P.; Rudra, J. S.; Herr, A. B.; Collier, J. H. Self-assembling peptide-polymer hydrogels designed from the coiled-coil region of fibrin. *Biomacromolecules* **2008**, *9*, 2438.

(34) Wang, C.; Stewart, R. J.; KopeČek, J. Hybrid hydrogels assembled from synthetic polymers and coiled-coil protein domains. *Nature* **1999**, *397*, 417.

(35) Wang, C.; Kopeček, J.; Stewart, R. J. Hybrid hydrogels cross-linked by genetically engineered coiled-coil block proteins. *Biomacromolecules* **2001**, *2*, 912.

(36) Fletcher, N. L.; Lockett, C. V.; Dexter, A. F. A pH-responsive coiled-coil peptide hydrogel. *Soft Matter* **2011**, *7*, 10210.

(37) Hellmund, K. S.; Von Lospichl, B.; Böttcher, C.; Ludwig, K.; Keiderling, U.; Noirez, L.; Weiß, A.; Mikolajczak, D. J.; Gradzielski, M.; Koksch, B. Functionalized peptide hydrogels as tunable extracellular matrix mimics for biological applications. *Peptide Science* **2021**, *113*, e24201.

(38) Wickner, W.; Schekman, R. Membrane fusion. *Nature structural & molecular biology* **2008**, *15*, 658.

(39) Blumenthal, R.; Clague, M. J.; Durell, S. R.; Epand, R. M. Membrane fusion. *Chemical Reviews* **2003**, *103*, 53.

(40) Jahn, R.; Lang, T.; Südhof, T. C. Membrane fusion. Cell 2003, 112, 519.

(41) White, J. M. Membrane fusion. Science 1992, 258, 917.

(42) Wickner, W.; Schekman, R. Membrane fusion. Nat Struct Mol Biol 2008, 15, 658.

(43) Martens, S.; McMahon, H. T. Mechanisms of membrane fusion: disparate players and common principles. *Nat Rev Mol Cell Biol* **2008**, *9*, 543.

(44) Scheller, R. H. In search of the molecular mechanism of intracellular membrane fusion and neurotransmitter release. *Nature medicine* **2013**, *19*, 1232.

(45) Yang, Z.; Gou, L.; Chen, S.; Li, N.; Zhang, S.; Zhang, L. Membrane Fusion Involved in Neurotransmission: Glimpse from Electron Microscope and Molecular Simulation. *Front Mol Neurosci* **2017**, *10*, 168.

(46) Primakoff, P.; Myles, D. G. Cell-cell membrane fusion during mammalian fertilization. *FEBS Lett* **2007**, *581*, 2174.

(47) Wassarman, P. M.; Litscher, E. S. Mammalian fertilization is dependent on multiple membrane fusion events. *Cell Fusion* **2008**, *475*, 99.

(48) Lang, J. Molecular mechanisms and regulation of insulin exocytosis as a paradigm of endocrine secretion. *European journal of biochemistry* **1999**, *259*, 3.

(49) Jena, B. P. Cell secretion and membrane fusion. Domestic animal endocrinology 2005, 29, 145.

(50) Harrison, S. C. Viral membrane fusion. Virology 2015, 479, 498.

(51) Harrison, S. C. Viral membrane fusion. Nature structural & molecular biology 2008, 15, 690.

(52) Chernomordik, L. V.; Kozlov, M. M. Mechanics of membrane fusion. *Nat Struct Mol Biol* **2008**, *15*, 675.

(53) Irie, A.; Yamamoto, K.; Miki, Y.; Murakami, M. Phosphatidylethanolamine dynamics are required for osteoclast fusion. *Sci Rep* **2017**, *7*, 46715.

(54) Harayama, T.; Riezman, H. Understanding the diversity of membrane lipid composition. *Nat Rev Mol Cell Biol* **2018**, *19*, 281.

(55) Meher, G.; Chakraborty, H. Membrane Composition Modulates Fusion by Altering Membrane Properties and Fusion Peptide Structure. *J Membr Biol* **2019**, *252*, 261.

(56) Casares, D.; Escribá, P. V.; Rosselló, C. A. Membrane lipid composition: Effect on membrane and organelle structure, function and compartmentalization and therapeutic avenues. *International journal of molecular sciences* **2019**, *20*, 2167.

(57) Hamai, C.; Yang, T.; Kataoka, S.; Cremer, P. S.; Musser, S. M. Effect of average phospholipid curvature on supported bilayer formation on glass by vesicle fusion. *Biophys J* **2006**, *90*, 1241.

(58) Carlini, E.; Palmerini, C. A.; Cosmi, E. V.; Arienti, G. Fusion of sperm with prostasomes: effects on membrane fluidity. *Archives of biochemistry and biophysics* **1997**, *343*, 6.

(59) Prives, J.; Shinitzky, M. Increased membrane fluidity precedes fusion of muscle cells. *Nature* **1977**, 268, 761.

(60) Howell, J.; Ahkong, Q.; Cramp, F. Membrane fluidity and membrane fusion. *J. Pathol* **1972**, *104*, 115.

(61) Jahn, R.; Scheller, R. H. SNAREs--engines for membrane fusion. *Nat Rev Mol Cell Biol* **2006**, *7*, 631.

(62) Bock, J. B.; Scheller, R. H. SNARE proteins mediate lipid bilayer fusion. *Proceedings of the National Academy of Sciences* **1999**, *96*, 12227.

(63) Wang, T.; Li, L.; Hong, W. SNARE proteins in membrane trafficking. Traffic 2017, 18, 767.

(64) Jung, J. J.; Inamdar, S. M.; Tiwari, A.; Choudhury, A. Regulation of intracellular membrane trafficking and cell dynamics by syntaxin-6. *Biosci Rep* **2012**, *32*, 383.

(65) Ramakrishnan, N. A.; Drescher, M. J.; Drescher, D. G. The SNARE complex in neuronal and sensory cells. *Mol Cell Neurosci* **2012**, *50*, 58.

(66) Sutton, R. B.; Fasshauer, D.; Jahn, R.; Brunger, A. T. Crystal structure of a SNARE complex

involved in synaptic exocytosis at 2.4 Å resolution. Nature 1998, 395, 347.

(67) Smirnova, Y. G.; Risselada, H. J.; Müller, M. Thermodynamically reversible paths of the first fusion intermediate reveal an important role for membrane anchors of fusion proteins. *Proceedings of the National Academy of Sciences* **2019**, *116*, 2571.

(68) Zucker, R. S.; Kullmann, D. M.; Kaeser, P. S. Release of neurotransmitters. In *From Molecules to Networks*; Elsevier: 2014; pp 443.

(69) Robson Marsden, H.; Kros, A. Self-assembly of coiled-coils in synthetic biology: inspiration and progress. *Angew Chem Int Ed Engl* **2010**, *49*, 2988.

(70) Robson Marsden, H.; Korobko, A. V.; Zheng, T.; Voskuhl, J.; Kros, A. Controlled liposome fusion mediated by SNARE protein mimics. *Biomater Sci* **2013**, *1*, 1046.

(71) 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*, 8057.

(72) Daudey, G. A.; Zope, H. R.; Voskuhl, J.; Kros, A.; Boyle, A. L. Membrane-fusogen distance is critical for efficient coiled-coil-peptide-mediated liposome fusion. *Langmuir* **2017**, *33*, 12443.

(73) Zope, H. R.; Versluis, F.; Ordas, A.; Voskuhl, J.; Spaink, H. P.; Kros, A. In vitro and in vivo supramolecular modification of biomembranes using a lipidated coiled-coil motif. *Angewandte Chemie International Edition* **2013**, *52*, 14247.

(74) 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*, 621.

(75) Yang, J.; Shimada, Y.; Olsthoorn, R. C.; 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*, 7428.

(76) Kong, L.; Askes, S. H.; Bonnet, S.; Kros, A.; Campbell, F. Temporal control of membrane fusion through photolabile PEGylation of liposome membranes. *Angewandte Chemie* **2016**, *128*, 1418.

(77) 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*, 2162.

(78) Rabe, M.; Schwieger, C.; Zope, H. R.; Versluis, F.; Kros, A. Membrane interactions of fusogenic coiled-coil peptides: implications for lipopeptide mediated vesicle fusion. *Langmuir* **2014**, *30*, 7724.

(79) 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 (Camb)* **2013**, *49*, 3649.

(80) Errington, J. L-form bacteria, cell walls and the origins of life. Open Biol 2013, 3, 120143.

(81) Errington, J.; Mickiewicz, K.; Kawai, Y.; Wu, L. J. L-form bacteria, chronic diseases and the origins of life. *Philosophical Transactions of the Royal Society B: Biological Sciences* **2016**, *371*, 20150494.

(82) Shitut, S.; Bergman, G. Ö.; Kros, A.; Rozen, D. E.; Claessen, D. Use of permanent wall-deficient cells as a system for the discovery of new-to-nature metabolites. *Microorganisms* **2020**, *8*, 1897.