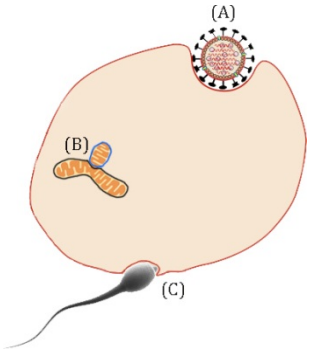
Chapter 1

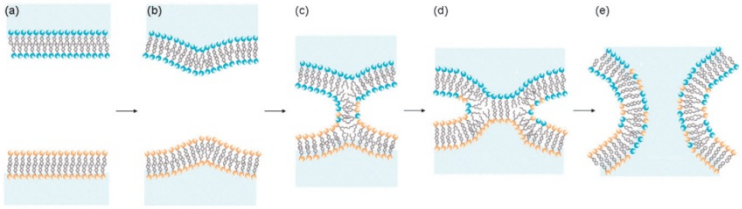
Introduction

Membrane fusion is one of the most fundamental processes in living organisms.[1] Main performances of natural membrane fusion are for example virus-host cell fusion, intracellular fusion (e.g. mitochondria fusion) and extracellular membrane fusion (e.g. sperm-egg fusion) (Scheme 1).[2-4]



Scheme 1. Diagrammatic sketch of different kinds of natural membrane fusion. (A) indicates pathogen with host cell fusion. (B) indicates mitochondria fusion as an example of intracellular organelles fusion. (C) indicates sperm oocytes fusion as an example of extracellular fusion of eukaryotic cells.

In order to achieve fusion, the two opposing membranes are first brought into close proximity, following by surface docking, and the formation of a stalk intermediate connecting the membranes. Before cargo transfer can occur, the stalk intermediate further develops in a hemifusion state, which is followed by a pore formation and expansion. (Scheme 2).[5, 6] However, all these processes are energy driven. Studies show that membrane fusion proteins are in charge of the energy supply in whole process of membrane fusion.[7]



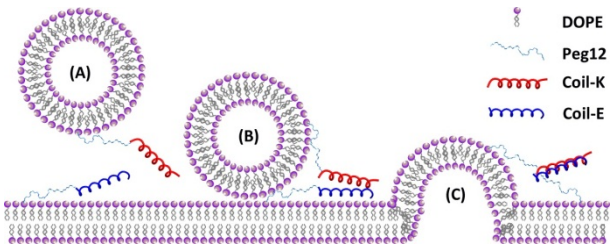
Scheme 2. (A) Two opposing membranes in the pre-fusion state. (B) A point-like membrane protrusion minimizes the energy of the hydration repulsion between the proximal leaflets of the membranes coming into immediate contact. (C) A hemifusion stalk with proximal leaflets fused and distal leaflets fused and distal leaflets unfused. (D) Stalk expansion yields the hemifusion diaphragm. (E) A fusion pore forms either in the hemifusion diaphragm bilayer or directly from the stalk.[5]

The SNARE (soluble N-ethyl maleimide sensitive factor attachment protein receptors) protein complex family is known to play a vital role in facilitating most intracellular and

exocytic membrane fusion.[8-10] However, understanding of membrane fusion at the molecular level is at a rather primitive stage due to the complexity of native fusogenic systems. According to Nobel prize laureate Sűdhof, how SNARE proteins promote fusion remains a major question in cell biology.[11] Do SNAREs only bring the opposing membranes together, or is there another function to promote membrane fusion?

Therefore, a bottom-up approach is proposed by several groups using synthetic analogues inspired by the natural fusion machinery in order to gain insight in coiled coil mediated membrane fusion. In this approach the chemical structure and composition of synthetic analogues can be systematically varied in order to study the influence of each segment on the fusion process. Thus, studying membrane fusion of this biomimetic model system will yield valuable information on the mechanism on a molecular level resulting in a better general understanding of coiled coil mediated membrane fusion. Inspired by this this fascinating process, our group mimicked the intricate natural SNARE proteins mediated membrane fusion into a simple coiled coil peptide complex mediated liposome fusion model.[12]

The beauty of the reduced membrane fusion system is that all the fusion parts are artificial. In our model system, the natural membrane bilayer is replaced by a liposomal bilayer, and the SNARE protein coiled coil tetramer is replaced by heterodimeric coiled coil. Previous work has shown that this model system can achieve a high efficiency in targeted content mixing, which is the hallmark of natural fusion.[12-19] Thus our model is a highly controllable supramolecular membrane fusion system. The study not only simplifies the membrane fusion study, but also opens new possibilities for membrane fusion applications, for example it may use as a drug delivery system or nanoreactors.



Scheme 1. Schematic diagram of peptide coiled coil induced membrane fusion. (A) liposome membrane is drawn in close proximity to lipid bilayer by peptide Coil-K (red) and Coil-E (blue) electrostatic attraction. (B) Liposome is docking on surface of lipid bilayer by forming of CC-K/E coiled coil. (C) Accomplishment of membrane fusion between liposome and lipid bilayer. DOPE=1,2-dioleoyl-sn-glycero-3-phosphoethanolamine; Peg12=12-polyethylene glycol. Coil-K= Ac-$(KIAALKE)_3$-$CONH_2$; Coil-E= Ac-$(EIAALEK)_3$-$CONH_2$.

The aim of this thesis was to optimize the reduced membrane fusion model system resulting in more efficient content mixing in liposomal membrane fusion studies. One of the most essential parts to trigger our fusion model is peptide coiled coil complex. Therefore, this thesis starts with coiled coil zipping peptides studies, and following by the coiled coil mediated membrane fusion studies, in title of 'zipping into fusion'.

Chapter 2 investigates the coiled coil peptide quaternary structure by paramagnetic NMR spectroscopy. In this chapter, a new approach of investigating coiled coil self-assembly has been described. The innovation of the method is based on a combination of site-directed spin labeling and fluorescent aromatic amino acid labeling on peptides. Using this approach, coiled coil interactions can be studied by paramagnetic 1H-NMR and compared with steady state fluorescence measurements.

Chapter 3 describes the design and characterization of an antiparallel tetrameric coiled coil complex. The coiled coil quaternary structure was determined using the approach described in Chapter 2 and confirmed with from experimental and theoretical modeling. Finally, the fusogenicity of the antiparallel tetrameric coiled coil was studied and compared with the original liposome fusion model.

In Chapter 4, we tried to manipulate membrane fusion rate and efficiency by tuning either fusogen or lipid concentration. DLS and optical microscopy revealed that there are two fusion regimes – the fusion of thousands of liposomes through multiple fusion rounds into giant liposomes up to 10 μm in diameter, and the fusion of two liposomes. This mapping of the rate and route of liposome fusion under different conditions gives a detailed understanding of the capacity of the reduced SNARE model to fuse liposome membranes. This understanding paves the way for future applications of the minimal model such as controlled nanoreactor mixing and the directed delivery of drugs to cells.

Chapter 5 describes attempts to control the rate of coiled coil driven membrane fusion. In this chapter, the thermal stability of the coiled coil motif was varied by changing the length of the peptides by using either two, three or four heptad repeat units. This study shows that the rate of liposome fusion can be manipulated by tuning coiled coil binding energy.

In Chapter 6 the liposome fusion efficiency was increased by decreasing the tendency of the peptides to aggregate in the prefusion state. In this chapter, the charges of the peptides was varied by single amino acid mutations at specific positions. The binding energies of these new coiled coil peptides were determined and the fusogenicity was determined

revealing that amino acid mutations at positions not critical for coiled coil formation can influence the tendency to aggregate in the pre-fusion stage. Further, the membrane fusion efficiency was investigated.

Chapter 7 summarizes the findings of this work and gives and a general discussion is given.

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