CHAPTER 8

Summary and perspective

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Chapter 2 describes the importance of the poly(ethylene glycol) linker, PEG, in our membrane fusion model system. The length of the flexible PEG-linker is critical and significantly influences the kinetics and the final yield of liposomal fusion as shown by the differences in the dynamic light scattering (DLS) measurements, lipid mixing and content mixing assays. From this study it is clear that the tetra(ethylene glycol) linker is the optimal length in combination with the DOPE anchor to obtain efficient fusion between liposomes. A longer linker reduces the fusion efficiency, while when the linker is too short or absent the ability of coiled coil formation is lowered resulting in low rates of membrane fusion. PEG_4 linker aids an optimal membrane fusion between liposomes due to an efficient coiled coil formation and providing a most favorable distance between the two opposing membranes, which gave rise to less aggregation and a higher rate of membrane fusion. When the PEG spacer was replaced by a natural peptide spacer derived from Synaptobrevin and Syntaxin proteins, stable liposomes could not be obtained due to aggregation (data not shown). This suggests that there is an interaction between natural peptide spacer and lipid bilayers. This should be investigated more thoroughly yielding a better understanding of the function of the linker region in membrane fusion.

Chapter 3 describes initial efforts towards fusion between liposomes and live cells using the membrane fusion model system. Peptide amphiphiles composed of a cholesterol anchor conjugated via a PEG spacer to the coiled coil forming peptide were designed to transiently modify the surface of live cells and zebrafish embryos. Cell membrane modification was fast and occurred within 5-15 minutes. This method can be used to introduce artificial receptors on biological membranes. Furthermore, by employing an unnatural coiled coil peptide pair, a wide range of materials can be docked on the membranes of both cells and zebrafish embryos. Thus, this generic method holds much potential as an elegant tool in surface engineering and drug delivery. Currently, the delivery of small molecules to the cell cytoplasm as a result of direct membrane fusion instead of traditional endosome mediated pathway is being studied. Special attention should be given towards choosing the optimal lipid mixture to overcome the energy barrier associated with membrane fusion. Steric hindrance by membrane glycoproteins could be another reason why in these studies no liposome-cell fusion was observed. Pretreatment of cells with proteases like trypsin might solve this problem. In summary, this tool holds promise to deliver therapeutics like nucleic acids directly to the cell cytoplasm circumventing the endocytotic pathway.

In **chapter 4 and 5**, a nanoparticle platform was developed to present antigens to the immune system. It was shown that polymersomes can act as an adjuvant.

The efficiency of these nanoparticles as a vaccine delivery tool was increased by synthesizing two new peptide amphiphiles, $PBLG_{30}$-TAT and $PBLG_{30}$-K. Co-delivery of immunostimulatory TLR9 ligand CpG and subunit hemmaglutinin (HA) using these peptide amphiphiles resulted in an enhanced Th1 immune response in mice against the HA antigen, as reflected by elevated levels of IgG2a/c. This Th1 response is important to target intracellular pathogens, thus this study opens an avenue for vaccine development against viral pathogens using peptide-based nanoparticles. This formulation also demonstrated a higher immunogenicity when compared to the commonly used adjuvant alum ($Al(OH)_3$). More studies are required to obtain a better understanding of the self-assembly of the polypeptide amphiphiles and the effect of the resulting nanoparticles on the immunogenicity of the associated antigens.

In **chapter 6**, we have successfully demonstrated the automated solid phase synthesis of a complete library of random copolypeptides from Glu, Lys and Ala monomers with good reproducibility and excellent control over the degree of polymerization, polydispersity and composition. Due to the step-by-step addition procedure a very low polydispersity index (PDI) was obtained, which are inaccessible using traditional polymerization methods. Moreover, the randomness within the peptides was maintained throughout the growing copolymer chains. The control over composition gave access to a library of polymers with a precisely defined total charge which can range from approximately –15 to +15 per chain. The random character of the copolypeptides results in a disordered conformation almost completely devoid of any secondary structure. Furthermore, the copolypeptides are largely present as unimers in solution and only the copolypeptides with a high degree of hydrophobic amino acids showed some signs of aggregation. Thus, a versatile class of copolypeptides with tunable composition was synthesized that can be used in for example biomineralization experiments. The presented method allows for the systematic variation of charge and hydrophobicity, while interference due to a defined secondary structure or aggregation is largely absent. Furthermore, the scope of the synthetic method is not restricted to the three monomers used in this study and several hundreds of milligrams of the copolypeptides can be conveniently obtained from a single batch. The synthetic flexibility and convenience of the automated procedure, but also the precise control over polydispersity and molecular weight makes this the method of choice over ring-opening polymerization for the synthesis of amino acid-based polymer libraries.

In chapter 7, a new toxicity assay in zebrafish based on the oral delivery of a model drug using a dextran-based hydrogel is presented. Four-day old zebrafish embryos were shown to take up drug loaded hydrogels via the oral route. Sodium

valproate was used as a model drug in this study. Previously, it was demonstrated that this highly soluble drug was not taken up effectively by zebrafish embryos resulting in a lower than expected toxicity. However, when this drug is encapsulated into a dextran-based hydrogel, the uptake into the gut is enhanced resulting in an increased zebrafish lethality and thereby revealing the inherent toxicity of sodium valproate. This simple approach solves the issue of poor drug uptake by zebrafish embryos of water-soluble drugs. In addition, cyclodextrin moieties conjugated to the dextran-backbone also enables the solubilization of hydrophobic drugs via the formation of a host-guest complex. This raises the effective concentration and increases the uptake efficiency into the zebrafish embryos. The same results were obtained when a albumin-dextran hydrogel was used to deliver sodium valproate. In conclusion this method enhances the effectiveness of toxicity screening in zebrafish embryos.