Peptide Amphiphiles and their use in Supramolecular Chemistry

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Chapter 8

A. Summary and Perspectives

This thesis is a narrative that describes contributions that fall within the bounds of supramolecular chemistry. In this field, which often derives its inspiration from natural processes, self-assembly takes a pivotal role. The natural products which are mimicked in supramolecular chemistry include proteins, lipids, sugars and DNA. This can lead to so-called smart materials and applications in the areas of materials science and drug delivery. Here, the self-assembly properties of peptide amphiphiles were studied at the surface of liposomes, cyclodextrin vesicles, cells and zebrafish embryos

In **chapter 2**, a β-sheet forming peptide, $(leu\text{-}glu)_4$, was functionalized with an adamantane moiety. These peptide amphiphiles could be used to decorate the surface of cyclodextrin vesicles as inclusion complex formation between adamantane and cyclodextrin ensued. These oligopeptide decorated vesicles proved to have very interesting properties. At neutral pH, the glutamic acid residues were largely deprotonated and electrostatic repulsion between the peptide strands prevents β-sheet formation. Upon lowering the pH to 5.0 however, a fraction of the negatively charged carboxylic acids were protonated and a transition in the secondary structure of the peptides took place from random coil to a β-sheet. This transition was found to be reversible and furthermore, β-sheet formation led to a morphological transition of the whole assembly from spheres to fibers. This transition was also found to be reversible, i.e. upon raising the pH back to 7.4 the spherical assemblies reappeared. Finally, the transition from spheres to fibers could be used to trigger release from the assembly into the aqueous exterior. In conclusion, a switchable nanodevice was designed, based on peptide-peptide interactions, which could release its cargo.

Chapter 3 reviews the self-assembly of peptide amphiphiles (PAs) into well-defined, functional nanostructures. In this review, only PAs consisting of two distinct blocks are considered: a peptide block and a hydrophobic block. The hydrophobic block is often a lipid or alkyl tail, or, less commonly, a polymer or polypeptide. This hydrophobic block induces aggregation of the PAs upon dispersion in aqueous media. Examples are discussed to illustrate the different effects that the hydrophobic block

can have on the self-assembly of the PAs: i) induction of a well-defined secondary structure of the peptide segment; ii) moderation of the peptide-peptide interactions, balancing peptide interactions with hydrophobic interactions can yield dynamic nanostructures instead of rigid peptide aggregates; iii) prevention of peptide interactions, which occurs when the hydrophobic anchors are sufficiently large and the hydrophobic interactions overwhelm the peptide-peptide interactions. Subsequently, the influence of peptide-peptide interactions on the morphology of whole assemblies was discussed. The final part of this review highlights applications of PAs, which originate from the careful balancing of all the repulsive and attractive forces that govern peptide amphiphile self-assembly and yield structures that can be used for example in materials science and drug delivery.

With **chapter 4** the realm of membrane fusion was entered. A synthetic model system, derived from native SNARE proteins and based on coiled coil forming peptides, was used to induce fusion between liposomes. The fusogenic peptides have the basic structure anchor-spacer-coiled coil forming peptide. The hydrophobic anchor confines the peptides to the surface of the liposomes. The set of complementary coiled coil forming peptides E and K act as the recognition domain and coiled coil formation forces the liposomes into close proximity. The spacer was constituted by PEG_{12}, which was incorporated to give peptide E and K sufficient freedom of motion to enable efficient coiled coil formation. In this contribution the influence of the hydrophobic anchor on the rate of fusion was examined. This was performed by synthesizing a small library of lipidated peptides with the anchors being: DOPE, cholesterol, palmitic acid, palmitoleic acid, decanoic acid and adamantane. In a typical experiment, two batches of liposomes were prepared, one in which the K peptide and one in which the E peptide was confined to the liposomal surface. Upon combining the separate batches of peptide decorated liposomes, peptides E and K form coiled coil complexes, after which membrane fusion takes place. It was found that both DOPE and cholesterol anchored peptides cause efficient lipid mixing, whereas the other anchors only showed moderate lipid mixing rates. These findings were confirmed when full fusion was studied using content mixing experiments. It was shown that the cholesterol anchor induces full fusion even more efficiently than DOPE. Another important advantage of the cholesterol anchor is that the cholesterol modified peptides could be used to modify liposomes *in situ*.

Typically, E and K decorated liposomes were prepared by 1) mixing lipids and lipopeptides in organic solvent, 2) evaporating the solvent, 3) hydrating this lipid layer with PBS and finally 4) sonication. For the *in situ* modification, the cholesterol peptides were added to plain liposomes and spontaneous insertion of the cholesterol modified peptides into the liposomal membranes ensued, which resulted in highly fusogenic liposomes. This in situ modification of membranes is highly interesting, as this is a strong indication that the addition of the cholesterol peptides to cells might lead to membrane insertion of these peptides which can enable coiled coil formation at the surface of live cells. These applications are further explored in **chapter 7**.

In **chapter 5**, another important factor for membrane fusion, the orientation of the coiled coil complex, was studied. In nature, SNARE proteins form a 4-helix coiled coil complex in which all the helices have a parallel orientation with respect to one another. It has been found that in biological systems, membrane fusion only occurs when the orientation of the coiled coil complex resembles that of a zipper. Our model system for membrane fusion, as used in the previous chapter, resembles that zipper-like coiled coil orientation, as parallel coiled coil formation ensues between peptides that are anchored into the liposomal membrane at identical peptide termini. Here, the influence of the orientation of the coiled coil complex in membrane fusion in our model system was investigated. This was examined by comparing zipper like to non-zipper like coiled coil orientation. By conjugating the cholesterol-PEG_{12} segment to opposite peptide termini, this phenomenon could be studied. It was found through both lipid and content mixing experiments that fusion events occurred efficiently for the zipper like and non-zipper-like coiled coil orientations. This strongly indicates that this orientation does not influence membrane fusion in our model system. This was confirmed when cholesterol-PEG_{12} was conjugated to both termini of the K peptide. When this lipidated peptide was used in membrane fusion experiments, lipid and content mixing rates were similar to the single anchored K peptides. It is possible that the spatial dimensions of our coiled coil motif is not sufficiently large for its orientation to play a significant role.

Chapter 6 gives a detailed narrative of a complex, dynamic self-assembly process in which two types of vesicles are forced, by using the E/K peptide pair, to form hybrid vesicles. It was attempted to induce membrane fusion between β-cyclodextrin

vesicles (CDVs), as introduced in **chapter 2**, and liposomes. In a typical experiment, CDVs were mixed with CPE decorated liposomes. Next, adamantane modified peptide K (APK) was added to the mixture. APK formed an inclusion complex at the surface of the CDVs and subsequent coiled coil formation between peptide E and K forced the CDVs and liposomes into close proximity. Through lipid mixing, inner leaflet lipid mixing and content mixing experiments it was observed that the interactions between these two distinct types of vesicles can most aptly be characterized as hemifusion. In hemifusion, the amphiphiles in the outer leaflets are mixed, whereas the inner leaflets stay in tact and therefore no mixing of the aqueous interiors takes place. Detailed investigations concerning the overall morphology of the resulting hybrid structures and the structure of their membranes were performed with the aid of cryo-TEM tomography. These studies revealed that multilayer vesicular species were formed. Furthermore, contact points between the membranes were found. Surprisingly however, the CDVs by themselves showed structures which were similar to the mixed vesicles. Future cryo-TEM studies will be aimed at corroborating lipid mixing between CDVs and liposomes by studying the membrane granularity of the starting compounds as well as the mixed vesicles.

The final part, **chapter 7**, builds on **chapter 4** in which it was observed that membranes were activated by adding an aqueous solution of lipidated peptides. Here it is shown that the cholesterol modified peptides CPE and CPK could be incorporated into biological membranes in both *in vitro* and *in vivo* environments through spontaneous insertion, induced by hydrophobic interactions. Subsequent addition of the complementary peptide led to efficient coiled coil formation at the surface of CHO cells (*in* vitro) and zebrafish embryos (*in vivo).* As the coiled coil motif can be used to connect distinct molecular constructs together, this peptide-peptide binding was used to dock liposomes to both CHO cells and zebrafish embryos.

The question that remains is: how to continue this line of research? In this thesis a set of coiled coil forming peptides was used to 1) induce membrane fusion between liposomes 2) form hybrid vesicles and 3) dock liposomes at the surface of cells and zebrafish embryos. Concerning each of these findings there are important steps to be taken. First, elucidating the mechanism through which membrane fusion occurs would be very valuable. For this, there needs to be a fundamental understanding about what

happens between coiled coil formation and content mixing. Here, interactions between the peptides and lipids will play a vital role and these need to be studied in detail. Furthermore, the use of fusion as an elegant way to employ liposomes as microreactors has not been explored as of yet. Two reactants can be encapsulated in separate batches of liposomes can come together through membrane fusion, which enables doing reactions in an enclosed environment.

Coiled coil formation at the surface of biological membranes and liposome docking as presented in chapter 7 opens up many possibilities to study membrane processes and influence the chemistry operating at these membranes. A logical next step is to fuse liposomes with cells. This would enable the delivery of therapeutic agents directly into the cytoplasm, thereby preventing the natural metabolic pathways through which these therapeutic agents are often degraded before they can reach their target. The approach to achieving this is not straightforward, as there are many variables in our system that can be altered such as the composition of the peptide amphiphiles, liposomes and buffer. Although it might take substantial time and effort to find the righ combination, in my opinion the rewards would be magnificent. Furthermore, specificity towards certain cell types should be incorporated in the system. This might be achieved by making mixed micelles, consisting of the lipidated peptides and lipidated aptamers. Aptamers can be designed as such that they can be targeted towards specific cell types. A future tale concerning this system might sound something like this. A zebrafish embryo is injected with the aid of microinjection with mixed micelles containing the lipidated peptides and lipidated aptamers. The micelles target a specific cell type, and these cells contain the lipidated peptides in their membranes. Subsequently, liposomes containing the complementary peptide are injected. These liposomes target the pre-treated cells based on the specific interactions between the complementary peptides and the liposomes fuse with the cells, thereby delivering their cargo into the cell cytoplasm. It is my hope that this vision will be realized within the forseeable future.