# Peptide Amphiphiles and their use in Supramolecular Chemistry

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# Chapter 1

## Introduction: Molecular understanding of the ingredients and inspiration for this thesis

### 1.1 Narrative of a supramolecular chemist

Living systems contain wonderful and inspirational examples of complex and controlled processes which are due to specific interactions between molecules. To accomplish all these different processes in an orthogonal and controlled fashion, nature makes use of an essentially simple toolbox that contains 20 amino acids, a handful of nucleotides, a dozen of lipids and two dozen of sugars. With these basic elements however, a dizzying array of biomolecules are constructed by biological systems. With the aid of these biomolecules life on earth is able to perform an unimaginable amount of intricate tasks simultaneously. The overarching principle upon which this capability is based is self-assembly. Self-assembly concerns the formation of well-defined structures which are stabilized by non-covalent interactions. The most ubiquitous and well-known non-covalent interactions are hydrophobic interactions, hydrogen bonding, π-π interactions, van der Waals interactions and electrostatic interactions. These forces have in common that they are very weak individually, however, cooperatively they are capable of organizing molecules into stable assemblies with the proper spatial structure.

A beautiful example of self-assembly in living systems is the cell membrane, which consist mainly of lipids, proteins and carbohydrates. The self-assembly of lipids into a stable bilayer membrane is governed by hydrophobic interactions between the lipid chains. This is a direct consequence of the fact that lipid-lipid interactions are energetically more favorable than lipid-water interactions. Importantly, cell membranes confine the constituents of cells within a small volume. As a result, reactions performed in bulk will show different reaction rates and equilibrium constants as compared to the cellular values. The differences are caused by the fact that cells are small, have an internal structure and are filled with large numbers of different macromolecules. This macromolecular crowding has been shown to enhance natural processes such as gene expression and it is involved in directing the kinetics of natural processes. Furthermore, cell membranes also supply cells with mechanisms to actively import and export molecules to and from the cell and allows for

communication between cells. Many of these impressively complex and delicate mechanisms, active in the daily operations of living systems, i.e. in you right now, are based upon the work of proteins. Proteins are composed of amino acids and the amino acid sequence, together with environmental factors, determines the folding of the amino acids into an overall structure. The specific location of amino acids within this morphology controls the properties that the protein has and consequently the functions that it can perform, such as transportation of molecules between cells, processing of genetic material and protein-protein binding for the creation of supportive structures, to name a few. The folding of proteins is therefore vital and this process yields regions in proteins with well-defined secondary structures such as β-sheets, β-loops and α-helices. In this thesis processes are investigated in which α-helical protein regions play a vital role. In nature, the assembly of multiple α-helical peptides into structural motifs known as coiled coils is very important. This is not only expressed in the ubiquity of the coiled coil motif, but also in the wide range of functions that they exhibit. Having said this, in all of the processes in which coiled coils play a role, they share a common structural feature: the molecular recognition between two or more α-helices causes the peptide strands to function as “molecular Velcro” that holds together the peptides and the subcellular structures to which they are attached. The specific amino acid sequences can modulate the Velcro binding properties and can also give rise to other, more specific functions of coiled coils.

A particularly exquisite example of a natural process in which coiled coil formation plays a vital role is membrane fusion. Membrane fusion is defined as the merging of two opposing lipid bilayers, during which both the membrane constituents as well as the aqueous cores mix. This process is important for the transport of membrane-impermeable molecules. When such a molecule is encapsulated within a lipid container and membrane fusion between that lipid container and the target container ensues, the membrane impermeable molecule is effectively transported over the membrane of the target container. Membrane fusion in nature is the result of a cascade of events, which allow the final stage, coiled coil formation between helical membrane proteins, to proceed in a controlled manner. When these membrane proteins bind to one another, the membranes are brought into close proximity, upon which membrane fusion takes place. This type of biological processes is often the inspiration for supramolecular chemistry. Initially, the goal is to identify the structure

of the molecules that are involved in natural processes. Next, efforts are directed towards elucidating the mechanisms through which these processes occur. The task of mapping out the processes that these molecules are involved in is an extremely intricate endeavor and therefore model systems are sometimes employed. Synthetic analogues of the native molecules can be synthesized once their structure is known. Subsequently, these molecules and their interactions can be studied with a wide range of techniques that are not compatible with living systems. Furthermore, synthetic organic chemistry allows for the modification of specific sites of the synthetic analogues. This enables chemists to evaluate the influence of each part of the synthetic analogues on the process as a whole, by varying the chemical composition of that part. Another benefit of model systems is that they can be tailored for specific applications. The application of synthetic analogues of natural compounds into living systems so as to influence the chemistry of life, is one of the highlights of supramolecular chemistry.

This thesis reports on my investigations that were inspired by natural processes, most notably membrane fusion. The remainder of this introduction is aimed at describing the inspiration for, and chemical nature of, the molecules and processes that are the topic of this thesis.

## 1.2 From Proteins to Peptides to Peptide Amphiphiles

Proteins are diverse both in structure and morphology, which allows them to execute a wide range of functions. Unfortunately, many proteins are fragile, hard to handle and the extraction of proteins from their natural environment can be laborious. Furthermore, the possibility to chemically tailor proteins for specific applications is limited. Therefore, synthetic peptides, i.e. small regions of proteins, are sometimes used. To illustrate this, let us consider a protein which catalyzes some particular reaction. Typically, the protein contains a binding site to which a guest molecule can bind in a specific manner. The efficient binding of the guest molecule to the protein is based on non-covalent interactions and a specific spatial fit. One could imagine that for this particular function, the complete protein is not an absolute requirement. Instead, if one were able to accurately synthesize the local region which is responsible for the binding of the guest molecule and thereby acquire a more or less identical binding site, this much smaller protein fragment could also perform this particular

function. However, proteins are typically large for a number of reasons. For example, proteins need to fold correctly into a stable morphology and for this, often the whole of the protein is required. Furthermore, correct (re)orientation of the active site once the guest molecule has docked typically requires a more complex structure than just the active site. Nonetheless, simple protein functions can be accurately performed by peptides. Peptides have the advantage that they can be easily synthesized, since the development of solid phase peptide chemistry.[1] Whereas some small peptide motifs self-assemble into nanostructures in which the peptides display an ordered structure, others do not, which depends on the primary amino acid sequence. In order to force the unstructured peptides into well-defined assemblies, can be conjugated to a hydrophobic moiety (e.g. a lipid tail), yielding a peptide amphiphile. A peptide amphiphile typically consists of a peptide segment which is covalently conjugated to a hydrophobic tail. Upon dispersion in aqueous media, the hydrophobic segment induces aggregation of the peptide amphiphiles. When the forces between the hydrophobic tails and between the peptide segments are carefully balanced, this can yield peptide amphiphile nanostructures with a well defined overall morphology in which the peptides form an organized array of functional groups at its surface. A detailed account of peptide amphiphile self-assembly is given in chapter 3.

### 1.2.1 Peptide secondary structure

The functioning of proteins often depends on the accurate local placement of atoms through the aid of well-defined secondary, tertiary and quaternary structures. The secondary structure refers to the highly regulated local substructures of proteins whereas tertiary structures concern the spatial organization of various regions of secondary structures within a single protein molecule. Finally, quaternary structures are used to describe interactions between different proteins or different protein subunits. First, the molecular basis for the most basic level of protein organization, i.e. secondary structure, will be addressed.

The most commonly observed secondary structures in proteins are β-sheets and the α-helices **(Figure 1)**. The formation of both of these secondary structures is driven by hydrogen bonding between amide moieties, which are *intra*molecular for the α-helix and *inter*molecular or *intra*molecular (for long peptides which contain a loop) for the β-sheet.

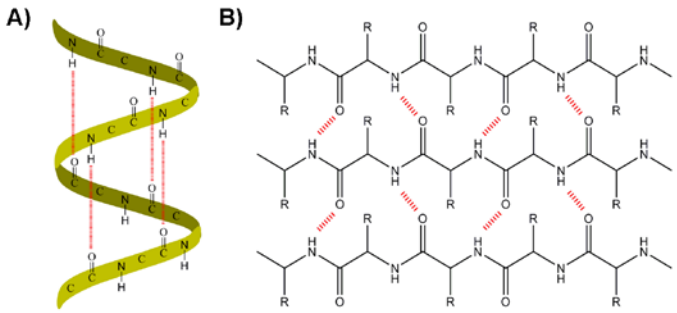


**Figure 1.** *Schematic representation of peptide strands with an α-helical (A) and parallel β-sheet (B) conformation. Hydrogen bonding patterns between the amide bonds are indicated by dotted lines.*

In the α-helical conformation, each amino acid corresponds to a turn of the backbone of 100º. As a result, the helix contains 3.6 amino acid residues per turn. All backbone N-H groups donate a hydrogen bond to C=O groups which are located 4 amino acid residues earlier. In the β-sheet arrangement hydrogen bonds are formed between N-H and C=O groups of adjacent peptide strands. In order to accommodate hydrogen bonding the strands need to be tightly packed, at a distance of ~5 Å. In the following sections these secondary structures will be elaborated on, with the use of examples which illustrate their importance in processes occurring in living systems.

### 1.2.2 β-sheet forming peptides

It is well known that designed peptides which are comprised of alternating hydrophobic and hydrophilic amino acid residues have a tendency to form β-sheet domains[2, 3] and it is likely that side-chain interactions make a substantial contribution to β-sheet stability. First of all, statistical surveys reveal a nonrandom pair wise distribution of amino acids in interstrand positions in β-sheets.[4-6] Typically, a hydrophobic amino acid residue in one of the peptide strands will pair with another hydrophobic amino acid in the adjacent peptide strand, which suggests that the side chains can interact favorably. Also, theoretical analyses suggest that specific interactions between side chains play an important role in determining β-sheet stability.[7] For instance, oppositely charged amino acid residues are often paired on adjacent peptide strands as the charges can than interact favorably to stabilize the β-sheet. Finally, amino acid residues with hydrophobic side chains are often paired, as

their interactions can decrease the surface area of the hydrophobic side chain that is exposed to the aqueous exterior.[8]

Typically, proteins which harbor β-sheet regions are soluble, whereas the isolated β-sheet regions and designed β-sheet forming peptides tend to aggregate.[9] As the edges of a β-sheet are already optimally aligned to interact with incoming β-strands, unprotected β-sheets tend to lead to precipitation. Therefore, nature has constructed various mechanisms through which β-sheet formation can be controlled. β-barrels for instance, avoid edges altogether as the hydrogen bonding ensues around the barrel cylinder **(Figure 2A)**.[10] Another mechanism is employed by β-helix proteins, which protect their β-sheet domains by decorating them with loops which are of another secondary structure **(Figure 2B)**.[11] Also, proline residues[12] and strategically located amino acids with charged side chains are able to act as so-called β-sheet breakers.[13]

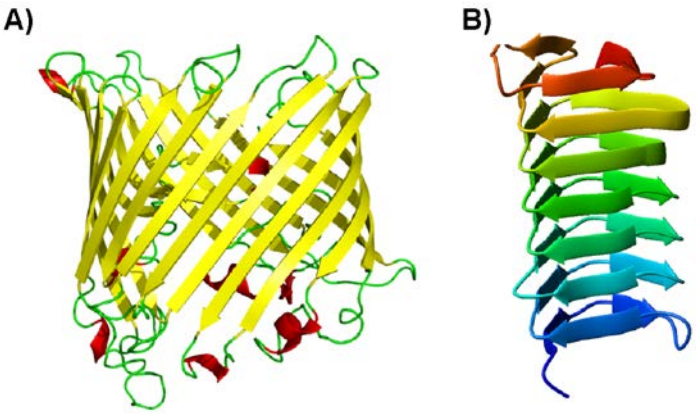


**Figure 2**. *Representations based on crystal structures of A) a β-barrel and B) a β-helix protein.*

### 1.2.3 Dysfunctional β-sheet formation in nature

A thorough understanding of the factors that determine β-sheet formation is very relevant, as the uninhibited growth of β-sheets causes the formation of amyloid fibers which are related to Alzheimer's disease.[14] The amyloid A4 or β peptide is a major component of the extracellular amyloid deposits that are a characteristic feature of Alzheimer's disease.[15] The definition of amyloid includes filaments of any polypeptide with a diameter of ~10 nm, which have a cross-β-sheet structure. The aggregation of the β-peptide into fibrils does not occur in a linear fashion, instead, distinct intermediates are formed **(Figure 3)**.[16, 17] Whereas native Aβ adopts a

random-coil structure, the formation of fibrils is initiated when Aβ undergoes a conformational change to a misfolded intermediate. This intermediate subsequently adopts a conformation in which it is rich in β-sheet structures. Due to the β-sheet character, this intermediate is unstable, and aggregates in a rate-limiting step into higher-order oligomers composed of multiple monomer units. Recruitment of additional monomers results in the formation of protofibrils, which assemble further into insoluble fibrils.

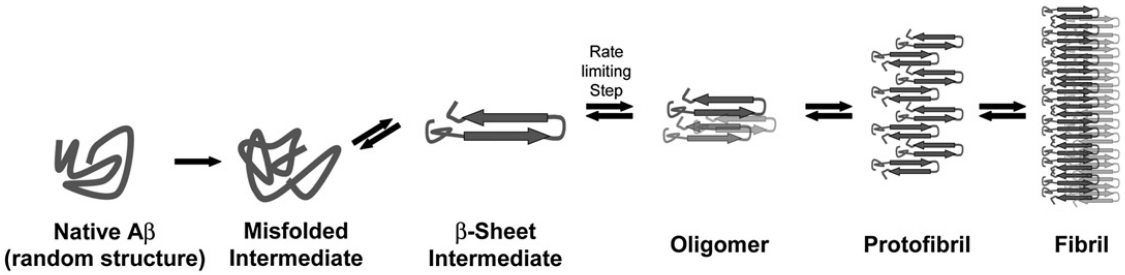


**Figure 3**. *Schematic representation of amyloid-β aggregation. Fibrillogenesis is initiated when Aβ undergoes misfolding which lead to more beta-sheet rich structures. These structures are then prone to aggregate into oligomers and eventually fibrils. Figure adapted from ref 17.*

### 1.2.4 Functional β-sheet formation in nature

β-sheet domains in proteins are involved in a wide variety of recognition events, which can be divided into three major groups: protein-DNA, protein-RNA and protein-protein recognition. The recognition of specific DNA sequences by proteins is of vital importance, as this is required for gene regulation. The source of the specificity is based on recognition by the protein of the pattern of functional groups on the edges of the base pairs in the DNA sequence through hydrogen bonding, together with a contribution from the sequence dependent conformational preferences of the DNA backbone.[17, 18] β-sheet structures are able to bind to both the minor and major groove. TATA binding proteins (TBP) use a large β-sheet surface to recognize DNA sequences by binding in the minor groove.[19, 20] The protein-DNA interactions are constituted by hydrogen bonds, van der Waals contacts and phenylalanine-base stacking.[20] Insertion of the concave, ten-stranded β-sheet of TBP into the minor groove requires profound DNA distortion, such as unwinding and bending. Binding of β-sheet proteins to the smaller major groove entails fewer β-strands and results in much smaller DNA distortion. For example, proteins MetJ[21] and arc[22] form

homodimers in which each monomer contributes a single β-strand for DNA binding. In this protein-DNA assembly the β-strands are oriented parallel with respect to the sugar-phosphate backbone and its side chains interact with the base pairs.

The RNA recognition motif (RRM) is the most abundant RNA-binding domain in higher vertebrates.[23] Furthermore, in terms of both structure and biochemistry, it is the most studied RNA binding domain.[24] Typically, the RRM is ~90 amino acids long with a typical $\beta_1\alpha_1\beta_2\beta_3\alpha_2\beta_4$ topology that forms a four-stranded β-sheet packed against two α-helices. RRMs bind a variable number of nucleotides, ranging from a minimum of two[25, 26] to a maximum of eight.[27] Most commonly, three aromatic side chains located in strands $\beta_3$ and $\beta_1$ accommodate binding. The bases of the 5' and 3' nucleotides are then able to stack on these aromatic rings. The third aromatic ring is often inserted between the two sugar rings of the dinucleotide **(Figure 4)**.[28,29] However, there are many variations to this basic theme. This high variety of interactions makes the mechanistic role and the function of the RRM difficult to decipher.

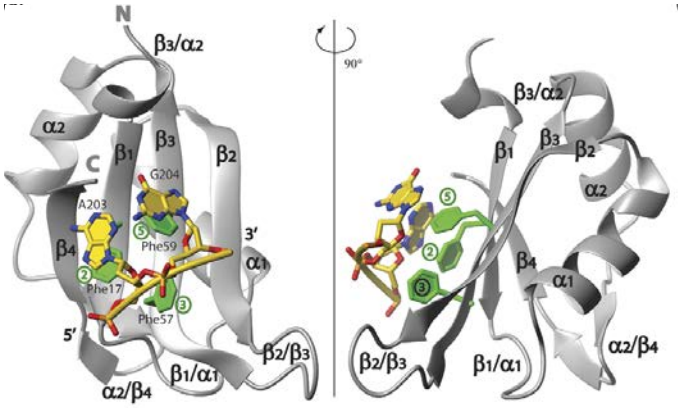


**Figure 4**. *Structure of a RNA binding protein with single stranded telomeric DNA as a model for nucleic acid binding. Figure adapted from ref 29.*

Protein-protein interactions have essential roles at almost every level of organization and communication in living cells. It is believed that proteins have, on average, 6-8 interacting partners.[30, 31] It has been argued that the interactions between β-sheet proteins can be classified into three different categories:[32] i) β-sheet augmentation, here one of the proteins contributes a β-strand, which binds to the edge of a β-sheet in the other protein; ii) β-strand insertion and fold complementation, where a peptide loop or appendage can add onto a pre-folded binding domain; iii) β-strand zippering,

two unfolded loop regions of apposing proteins come into contact to form a two-stranded β-sheet or β-zipper. A particularly appealing example of the latter was observed by the recruitment of Smad2 to the Smad anchor for receptor activation (SARA). The β-zipper is thought to add binding affinity for the Smad2-SARA complex, although it is believed that the specificity for this complex is derived from α-helical regions in the SARA protein **(Figure 5)**.[31]

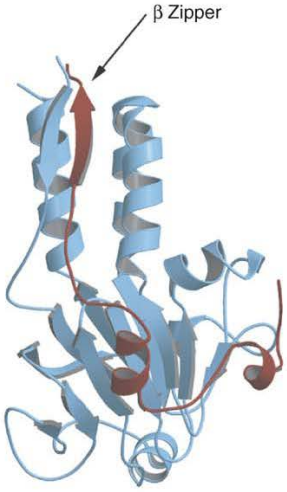


**Figure 5**. *Secondary-structure representation of the complex between Smad2 MH2 domain and the Smad anchor for receptor activation (SARA). Figure adapted from ref 31.*

The previously mentioned examples in which β-sheets play a central role reveal that this motif is important in natural binding processes. Furthermore, the physical character of the binding is diverse, depending on side interactions for DNA and RNA binding and hydrogen bonding for protein binding.

### 1.2.5 Synthetic peptides that form β-sheets

Model peptides that fold into β-sheet structures can be a valuable tool to mimic and study β-sheet formation.[33, 34] Furthermore, designed β-sheet peptides can be used for novel functions **(Chapter 2)**, of which a few examples will be addressed here. Stupp and coworkers developed peptide amphiphiles that assemble into fibers, in which the peptides adopt a β-sheet conformation. These fibers have been used for a variety of functions, such as the formation of hydroxyapatite crystals[35] and the selective differentiation of neural progenitor cells to neurons.[36] Further research on β-sheet peptides has focused on understanding and ultimately inhibiting amyloid formation, in

search of therapeutics for diseases such as Alzheimer's, Parkinson's and type II diabetes. Nowick and coworkers designed amyloid β-sheet mimics which were able to bind β-sheet proteins and subsequently prevent further aggregation.[37] This example underlines the importance of synthetic model systems, as they can aid the molecular understanding of β-sheet formation, which is the first step towards controlling it. A final example of functions that designed β-sheet peptides can fulfill concerns biomineralization. As peptides that are comprised of alternating hydrophobic and hydrophilic amino acid residues tend to adopt β-sheet structures, this results in ordered arrays of functional (charged) groups. These charged groups can be used as a nucleation point for biomineralization of, for example calcium carbonate crystals. Previous work in our group has shown that it was possible to influence the morphology of calcium carbonate crystals, by growing these crystals under a monolayer of lipidated β-sheet forming peptides **(Chapter 3)**.[4]

### 1.2.6 Helical peptides

Naturally occurring L-amino acids can fold into a right-handed α-helical conformation. The tendency of amino acids to adopt a helical conformation is referred to as their helical propensity. The conformational preferences of amino acids were accurately predicted remarkably early on, starting with the Ramachandran plot in 1963.[38] These predictions were based on relatively simple geometrical considerations by using van der Waals radii of chemical groups within amino acids to determine the dihedral angels φ and ψ.[38, 39] More recently, these predictions regarding structure and conformational preferences were later largely validated in protein crystal structures.[40-42] Certain amino acids, such as alanine and arginine have a much higher helical propensity, i.e. tendency to fold into a helix, than for example glycine and proline. In general, a few factors are known which are detrimental to helix formation. A rigid backbone (as in proline) will induce strain in the helix,[43] an aromatic side chain will lead to greater entropy loss than a non-aromatic side chain[44, 45] and hydrogen bonding between side chain and backbone atoms will also destabilize the helix.[46] Favorable interactions include hydrogen bonding or hydrophobic interactions between side chains and hydrophobic interactions between side chains and the backbone of the helix.[47]

### 1.2.7 Coiled coils

When two or more α-helices bind together to form a complex, this is called a helical bundle. When the helical bundle displays a so-called "knobs in holes" character and consists of helices that are comprised of heptad repeat units, this is referred to as a coiled coil.[48, 49] Although there are variations in the topology and oligomer state of coiled coils, here, the focus will be solely on heterodimeric coiled coils. Coiled coils are typically characterized by a heptad repeat, denoted **abcdefg**.[50] Due to the helical character of the individual peptides, it is possible to place amino acids at specific sites of the helix. As is shown in **Figure 6**, positions **a** and **d** are typically occupied by hydrophobic amino acid residues. This results in the formation of a hydrophobic face in each of the helices, which are subsequently able to bind to one another. Furthermore, it has been found that alternating leucine and isoleucine amino acid residues in the core of the coiled coil, create a continuous hydrophobic core through a knobs into holes design, which greatly stabilizes the coiled coil.[51, 52] On the **e** and **g** positions charged amino acids are placed, which increase the solubility, destabilize homocoils and further stabilize heterocoils. On positions **b** and **c** amino acids which have a high α-helical propensity, such as alanine, are located to ensure that the separate peptides form helices. The amino acids on the **f** position are chosen as such so it reduces the overall charge of the helices.

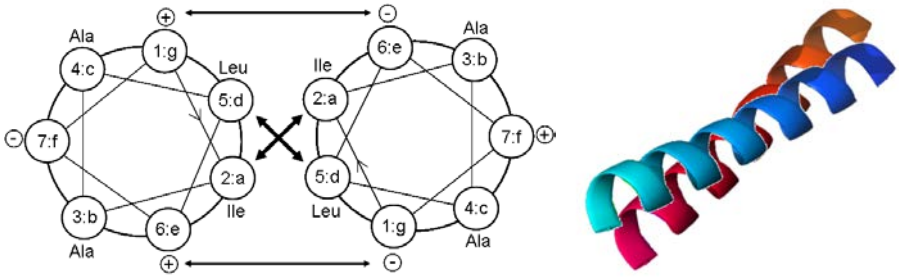


**Figure 6.** *Left: Helical wheel diagram of a heterodimeric coiled coil. Right: 3-Dimensional representation of a heterodimeric coiled coil.*

The functions that coiled coils perform in living systems vary and are a direct consequence of their physicochemical properties. For instance, coiled coils have a rod-like morphology and it is therefore hardly surprising that these entities can exhibit a role as a structural component of the cell.[53] For example, a particularly stable coiled coil construct is found at the cell surface of the bacterial species *Staphylothermus*

*marinus*, which thrives at an environmental temperature of 92 ºC.[54] The 70 nm long proteins form a meshwork at the cell surface through end-to-end interactions and the coiled coils are anchored into the surface at the C-terminus.[55] The meshwork is thought to act as a cytoskeleton[56] which stabilizes lipids and proteins of the cytoplasmic membrane **(Figure 7)**.[57]

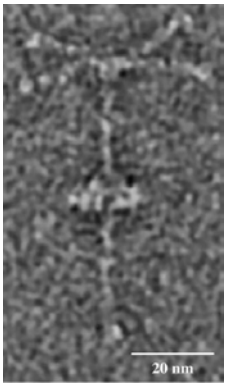


**Figure 7**. *Negative-stained TEM image of the tetrabrachion protein. It consists of a 70 nm long coiled coil, 4 β-sheet arms at the top and two noncovalently bound proteases. Figure adapted from ref 56.*

Other processes in which coiled coils play a vital role include muscle contraction (Myosin II) and the transport of cargo over actin filaments by kinesin.[53]

### 1.2.8 Peptides at interfaces

Many biologically relevant interactions occur at membrane interfaces. Therefore, the properties of peptides and peptide amphiphiles at interfaces have been relatively well studied. It has been observed that the confinement of peptide amphiphiles to an interface (e.g. lipid membrane or air-water interface) typically increases its secondary structure. This phenomenon is most likely due to a combination of factors: 1) increased local peptide concentration,[58] 2) decreased conformational freedom[59] and 3) interactions between the peptide and the membrane.[60] For a more detailed description of peptide amphiphile self-assembly and their behavior at interfaces, see **chapter 3**.

In the next section the process of membrane fusion is described and the role of coiled coils in this process is discussed.

## 1.3 Membrane Fusion

### 1.3.1 SNARE proteins

Membrane fusion in nature occurs through a cascade of intricate reactions, first however, it will be examined how it is that two lipid bilayers come into close proximity. Liposome fusion can be caused by a variety of factors, among which are the addition of calcium or polyethylene glycol and high membrane curvature. However, these fusion processes are often accompanied by significant lysis.[61-63] In living systems there is a variety of different fusion processes like cell-to-cell fusion,[64-66] the entry stage of enveloped viruses[67-69] and fusion events in exocytosis, protein trafficking and mitochondrial remodeling.[70-73] These different fusion processes are induced by different proteins and also the bilayer composition may vary. For simplicity and for the purpose of this introduction, the focus will solely be on neurotransmitter release, the machinery for which is comprised of soluble N-ethylamide sensitive factor attachment protein receptors, abbreviated as SNAREs.[74]

### 1.3.2 Synaptic Vesicle Fusion

Synaptic vesicle fusion lies at the heart of information processing in the brain. It is a fast process, which is extremely well controlled in both temporal and spatial dimensions. In the first event of this cycle, synaptic vesicles are filled with neurotransmitters through active transport. In a subsequent step, these vesicles dock at the active zone, after which they undergo an initial priming step at the cost of ATP. Then, upon the release of $Ca^{2+}$ (via active transport using calcium channels), fusion pore opening ensues and the synaptic vesicles undergo endocytosis and recycle via various mechanisms **(Figure 8)**.[75]

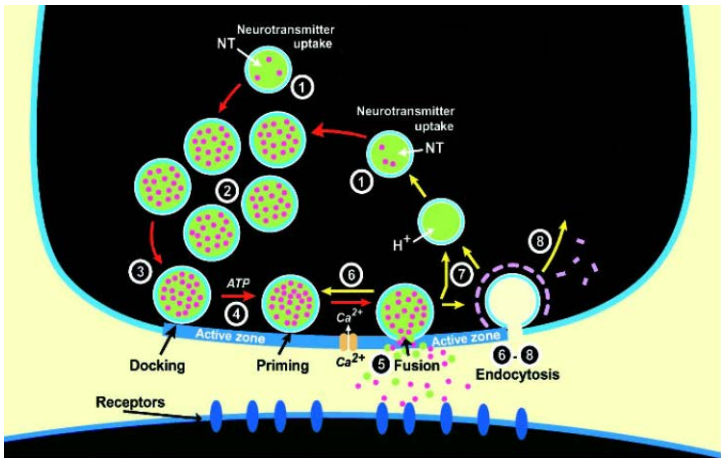


**Figure 8.** *Schematic representation of the synaptic vesicle cycle. In the first step, synaptic vesicles are filled with neurotransmitters and form clusters. Then, the vesicles dock at the active zone through the assembly of SNARE complexes, after which release of* $Ca^{2+}$ *is sufficient to initiate membrane fusion. After membrane fusion, the vesicles are recycled. Figure adapted from ref 75.*

SNARE proteins are involved in the docking, priming and fusion of the synaptic vesicles. The SNARE hypothesis postulates that this protein complex is composed of 2 categories, v-SNAREs (synaptobrevin, present in the synaptic vesicles,) and t-SNAREs (syntaxin-1 and SNAP-25, present in the presynaptic plasma membrane,).[76, 77] Individual SNARE proteins are unfolded, but together they assemble into a remarkably stable four-helix bundle **(Figure 9)**. Even though the class of SNARE proteins is very diverse, the common denominator is a ~70 amino acid residue "SNARE motif" which is build up from heptad repeats.[78] It is this common part of SNARE proteins that is involved in the formation of the four-helix bundle.[79] Of these four helices, three are present in the presynaptic plasma membrane, syntaxin-1 delivers a single helix and SNAP-25 contributes two. The remaining helix, synaptobrevin, is anchored in the membrane of the synaptic vesicles.

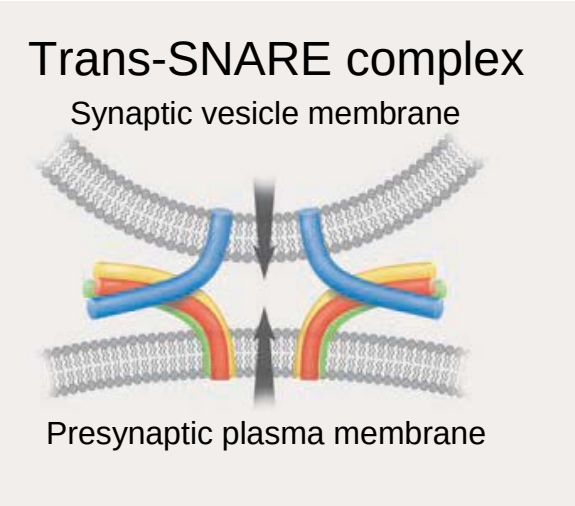


**Figure 9**. *The zippering model for SNARE induced membrane fusion. Three helices anchored in one membrane (the t-SNARE) assemble with the fourth helix anchored in the other membrane (v-SNARE) to form trans-SNARE complexes, or SNARE pins. Assembly proceeds progressively from the N-termini toward the C termini. Figure adapted from ref 80.*

Binding starts at the N-termini of the proteins and subsequently "zippers up" in the direction of the C-termini. This brings the opposing membranes in close proximity.[80, 81] Each SNARE complex releases approximately 35 $k_BT$ of energy upon the binding of the helices.[82] It has been estimated that the energy required for membrane fusion is around 50-100 $k_BT$,[83] so the formation of 2-3 SNARE complexes in a suitable arrangement should in theory be sufficient to initiate membrane fusion.[84] Having said this, it remains unclear how the energy gained from the assembly of the SNARE complex is transferred to the membrane. Once fusion has occurred, the unfolding of the SNARE proteins is carried out by a specialized adenosine triphosphatase (ATPase), NSF, and an adaptor protein, SNAP, the latter of which binds directly to the SNARE complex.[85, 86] The regeneration of the SNARE proteins has a cost of 3-6 ATPs, or 35-70 $k_BT$.

The proteins that are involved in the membrane fusion process and how these SNARE proteins are regenerated has been discussed in previous sections. For membrane fusion to occur in a timely and controlled manner however, regulatory mechanisms are needed. One of these mechanisms involves Sec1/Munc18-like, or SM-like, proteins.[87] SM proteins associate with SNARE proteins in multiple ways, including clasp binding of the 4-helix bundle. It seems likely that SM proteins organize trans-

SNARE complexes both spatially and temporally and it has been shown that membrane fusion does not occur when the SM protein is lacking. Furthermore, they bind to individual syntaxin-1, which yields a complex in which the SNARE-motif of syntaxin is partly occupied which disables the formation of the 4-helix SNARE complex. The two described interactions between SM proteins and SNARE proteins show that SM both up- and down regulates membrane fusion.

Two other proteins which are involved in fusion regulation are complexin and synaptotagmin. These proteins have been described as being 'grappling' proteins, which means that they can controllably activate a system, while keeping it in a locked state. From this reactive but stable state, a small trigger can cause rapid membrane fusion. SNAREs are first activated and then clamped by complexin and are finally triggered by the release of $Ca^{2+}$ to bind to synaptotagmin, which reverses the action of complexin and allows fusion to be completed.[88]

### 1.3.3 Membrane geometry

It has been argued that during fusion the involved membranes proceed through various different geometrical phases **(Figure 10)**.[89] In a first step, two opposing bilayers come into close contact. Then, a so-called point like protrusion is formed, which minimizes hydration repulsion between the outer leaflets of the bilayers. In the hemifusion stalk, the outer leaflet lipids of the bilayer structures started to mix, whereas the inner leaflets remain intact. The stalk subsequently expands to a diaphragm, after which a fusion pore is formed which completes the fusion process resulting in content mixing.

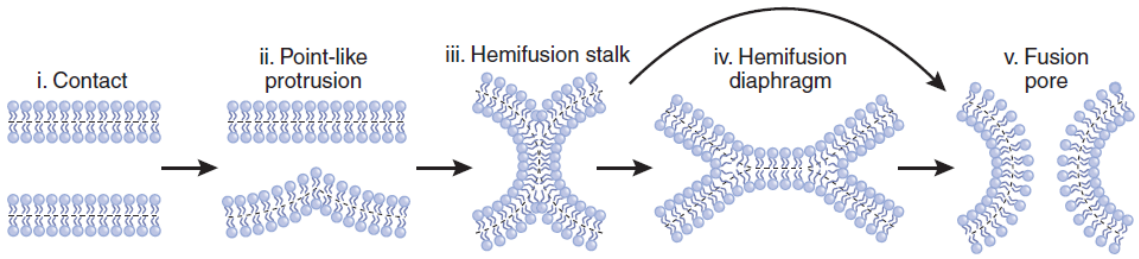


**Figure 10.** *Schematic representation of a fusion event which takes place through a hemifusion intermediate. Figure adapted from ref 89.*

The tendency of lipid bilayers to fuse has been found to depend on the lipid composition. This phenomenon is related to the effective spontaneous curvature of the lipids, which is defined as the curvature of a monolayer formed spontaneously by this lipid in the absence of any constraints. This property is determined by the molecular structure and lipid interactions within the monolayer. Lipids that are shaped like an inverted cone, such as lysophosphatidylcholine (LPC), tend to self-assemble into positively curved monolayers, whose surfaces bulge in the direction of the polar heads.[90] In contrast, lipids such as unsaturated phosphatidylethanolamine (PE) and diacylglycerol (DAG) have a propensity to form monolayers whose surfaces bulge in the direction of the hydrocarbon chains.[91] These lipids have the shape of a cone and show negative curvature. The third class of lipids is comprised of amphiphiles that tend to form flat monolayers and this have a curvature which is close to zero. These lipids, for example dioleoylphosphatidylcholine (DOPC) have the shape of a cylinder **(Figure 11)**.

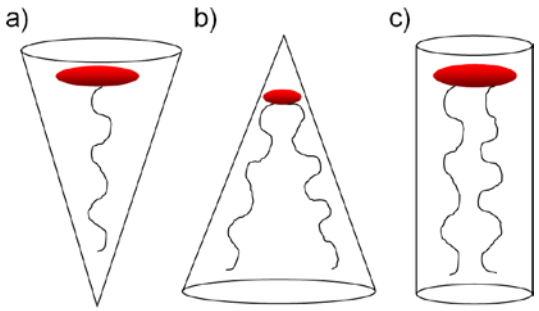


**Figure 11**. *Lipids with a) positive, b) negative and c) neutral curvature propensities.*

The finding that inverted cone-shaped LPC and cone-shaped PE inhibit and promote hemifusion, respectively, indicates that hemifusion involves formation of intermediates of net negative curvatures. The pore formation however, shows the reverse correlation, it is inhibited by lipids like PE and facilitated by lipids like LPC, indicating that the pore edge has a net positive curvature. Another factor which plays a role in determining the fusogenity of bilayer membranes is membrane tension. Smaller liposomes exhibit higher membrane tension and it was shown that relief of membrane tension pushes fusion beyond the hemifusion state towards fusion pore formation and expansion.[92, 93]

### 1.3.4 Model systems for membrane fusion

As demonstrated in the previous paragraphs, several of the components that are involved in native membrane fusion have been identified and their molecular structures are well documented. However, the exact mechanism through which membrane fusion proceeds is still unknown. For example, the interactions between proteins and lipids during the several stages of fusion are poorly understood. One strategy to elucidate this mechanism is by using model systems. By employing synthetic analogues of the native fusion machinery, insights into the complex chain of events that lead to membrane fusion can be obtained. Furthermore, such synthetic analogues can be easily varied in chemical structure. This might yield detailed information on the mechanisms of fusion. Typically, membrane fusion that is induced by synthetic fusogens is studied with the aid of liposomes.[94] Liposomes are used here as simple analogues of cell membranes that can be easily prepared and controlled for size and composition.

## 1.4 Liposomes

Liposomes are dynamic supramolecular structures which consist of a bilayer membrane and an aqueous interior. Typically, the term liposomes is reserved for vesicles which are composed of natural phospholipids, while the term vesicles is a more general expression for closed bilayer entities. Essentially, liposomes can be viewed as a simple model system for cell membranes.[95] Cell membranes consist of numerous different molecules such as lipids, proteins and carbohydrates, whereas liposomes typically consist of one or a few different lipids. However, research has shown that liposomal membranes share similarity with their natural counterparts and as it is almost trivially easy to prepare or to introduce chemical modifications. Therefore, liposomes can be used to study (bio)-physical processes which occur in the cell membrane.

### 1.4.1 Liposome Structure

The lipids of which liposomes consist contain two distinct parts, a hydrophilic head group and a hydrophobic tail. Upon dispersion in aqueous media, the head group will interact favorably with the water molecules surrounding it. The (un)saturated alkyl tail however, prefers interactions with other tails to interactions with water molecules. Therefore, an assembly arises which both maximizes head group-water and tail-tail

interactions and minimizes tail-water interactions. For lipids to arrange into bilayer liposomes, the overall geometry of these lipids needs to resemble that of a cylinder **(Figure 12)**. Typically, when the tail consists of a single alkyl chain, or when the head group is rather large, the lipids assemble into a micellar structure. When the hydrophobic part of the lipid consists of a double alkyl chain, the chains no longer fit into the micellar geometry and they will typically assemble into liposomes.[96]

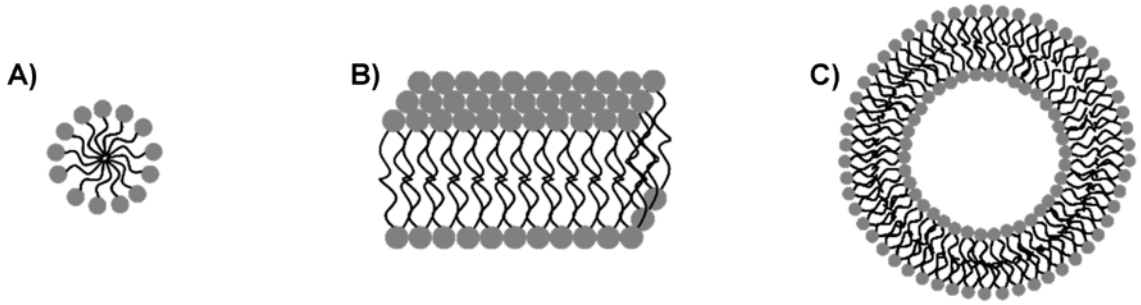


**Figure 12**. *Assemblies of lipids: A) micelles, B) bilayers and C) liposomes.*

The physical characteristics of liposomes are directly determined by the properties of the lipids. The length of the tails influence the rigidity of the membranes, the longer the chains the stiffer the membranes become. Also, the chains can be saturated or unsaturated, linear, cyclic or branched, aromatic or aliphatic or fluorinated and all of these characteristics influence the properties of the liposomes.

### 1.4.2 Applications of liposomes

Liposome research has received significant attention in recent decades, which is mainly due to their potential application as pharmaceutical carriers.[97] Generally speaking, peptide, protein and gene based drug delivery fails when traditional drug administrations, such as oral and topical transmucosal and inhalations are being used, as these therapeutics are susceptible to enzymatic degradation or they lack the ability to be taken up in the system circulation. Ideally, a drug delivery system should be able to: 1) deliver the drug to the desired target (e.g. an organ or specific cell type) with 2) control over the released drug concentration and 3) protect the drug from degradation. Liposomes are an interesting candidate as a drug delivery system, as water soluble drugs can be encapsulated into their aqueous interiors, thereby protecting the drugs from degradation. Furthermore, hydrophobic drugs can be inserted into the membrane. The hydrophobic membrane can be used to decorate the surface of the liposomes with functional molecules by conjugation of the functional molecule to a

hydrophobic anchor. This anchor inserts into the membrane of the liposome and the functional part of the hybrid then extends from the membrane into the aqueous exterior. The evolution of liposomes has come a long way, as is illustrated in **Figure 13**. Originally, liposomes simply contained drugs in the aqueous interior and membrane. A severe drawback of these formulations was the rapid elimination from the blood stream and capture of the liposomes by the reticulo-endothelial system resulting in high drug levels mainly in the liver. Many efforts have been aimed at reducing these drawbacks. Increased accumulation of drugs into the targeted tissues and organs was achieved by decoration of the liposomes with ligands capable of binding to specific cell of types. However, the circulation time of the liposomes was insufficient, since most of the liposomes still accumulated in the liver. In a next step of development, liposomes were decorated with biocompatible polymers, most successfully with polyethylene glycol (PEG). These flexible polymer chains form a protective layer around the liposomes and it was observed that the clearance rate of liposomes was significantly lowered.[98, 99] Some formulations of these long-circulation liposomes were evaluated in clinical trials.[100, 101] However when both ligand and PEG modification were combined, steric hindrance of the ligands was observed and specific binding of liposomes to targeted tissues was lost.[102] This was resolved by attaching the ligands at the periphery of the PEG brush, thereby extending out of the polymer layer and resulting in binding to the targeted cells.[102, 103]

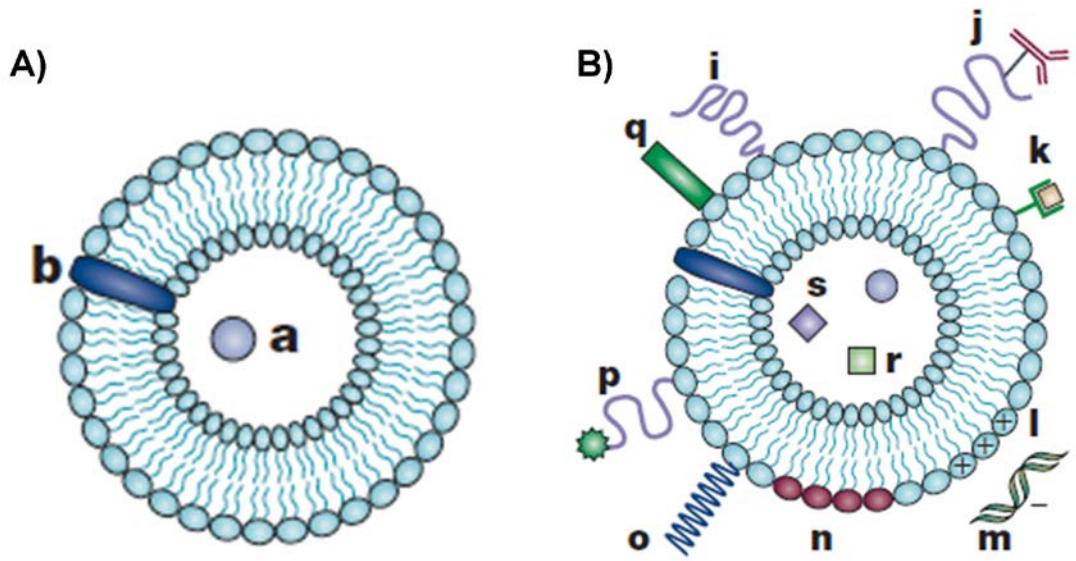


**Figure 13**. *Cartoon representation of the evolution of liposomes. (A) Traditional liposomes with encapsulated hydrophilic drugs (a) and membrane inserted hydrophobic drugs (b). Modern liposomes with protective polymers (i) or protective*

*polymers with a targeting ligand (j), a diagnostic label (k), positively charged lipids (l) which allow for complexation with DNA (m), stimuli sensitive lipids (n), stimuli sensitive polymers (o), cell penetrating peptides (p) and viral components (q). Figure adapted from ref 98.*

There are several liposomal drugs which have been approved for clinical application or are currently undergoing clinical evaluation. Improved survival rates were observed in patients with breast-carcinoma metastases upon treated with PEG decorated liposomes which contained the anticancer agent doxorubicin.[104, 105] Also, clinical research has shown the improved efficacy of encapsulating doxorubicin in PEG liposomes against inoperable hepatocellular carcinoma, cutaneous T-cell lymphoma and sarcoma.[106, 107] These and other examples show that liposomes can be used for targeted drug delivery. However, a fundamental problem that has not been resolved is the mechanism of uptake of liposomes by cells. In all described cases cell uptake follows endocytotic pathways. The cellular factors that regulate nanoparticle uptake are complex and not always understood,[108-110] what is known however is that extracellular components are engulfed by the cell membrane, followed by entrapment and degradation in endosomes and subsequently lysosomes, from which only a small fraction is released into the cytoplasm.[111-113] However, it has been suggested that nanoparticles that have a buffering capacity can cause the endosome to swell, resulting in endosomal escape.[114] Furthermore, the use of cationic lipids in the nanoparticle might be beneficial as these positively charged lipids can interact with the negatively charged lipids of the endosome, thereby destabilizing the endosomal membrane.[115] Nonetheless, as of today, drug delivery mediated by endocytosis is in many cases very efficient. A possible approach to directly deliver therapeutic agents into the cytoplasm is to mimic the process of viral entry into cells or the process used by cells to transport molecules, membrane fusion. Liposomes can be loaded with therapeutics and subsequent membrane fusion of these liposomes with cells results in direct drug delivery.

In the next paragraphs, a type of vesicle composed of non-lipid amphiphiles is discussed. These vesicles, which are denoted cyclodextrin vesicles are highly interesting as cyclodextrin allows for the possibility to perform host-guest chemistry at the vesicle surface. Thereby, biological processes taking place at membranes can be

mimicked and studied. First, properties of unmodified cyclodextrins are discussed, after which vesicles composed of amphiphilic cyclodextrins are addressed.

### 1.5 Cyclodextrins

Cyclodextrins are cyclic oligosaccharides consisting of six (α-cyclodextrin), seven (β-cyclodextrin) or eight (γ-cyclodextrin) glucopyrannose rings. These rings are linked by α-(1,4) bonds **(Figure 14)**.

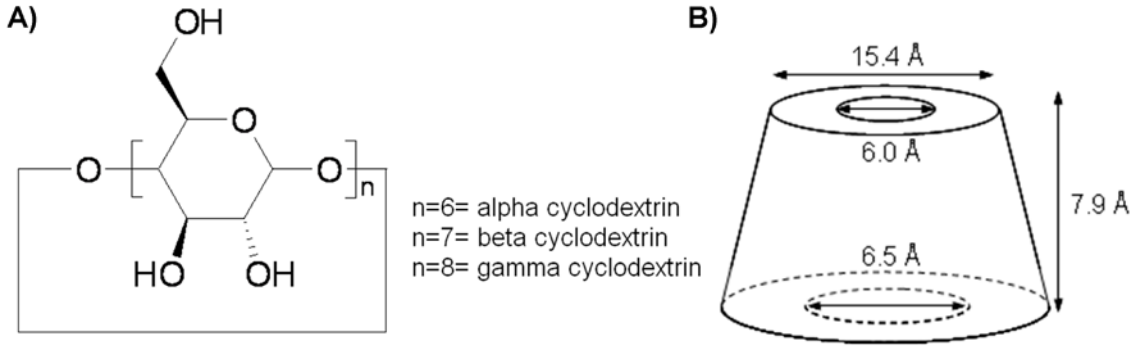


**Figure 14.** *a) Chemical structure of alpha, beta and gamma cyclodextrin. b) 3-Dimensional structure of cyclodextrins, with the measurements for β-cyclodextrin.*

This class of compounds was discovered late in the 19$^{th}$ century by Villiers.[116] According to Villiers, a small amount of crystalline material was obtained from starch digest of *Bacilus amylobacter*, however, other authors have suggested that the culture used by Villiers was impure and that the cyclodextrins were in fact produced by a contamination called *Bacillus macerans*. Some 20 years later, this strain was shown to produce also large amounts of crystalline dextrins. At this point both α- and β-cyclodextrins were isolated. Their 3D structure was determined in 1948 with the aid of X-ray crystallography.[117] Through these experiments it could be determined that: 1) the overall structure has the shape of a truncated cone, 2) the secondary hydroxyl groups ($C_2$ and $C_3$) are located on the wider edge of the ring and the primary hydroxyl groups ($C_6$) on the other edge and 3) the apolar hydrogens ($C_3$ and $C_5$) are located in the cavity. Through this hydrophobic cavity, cyclodextrins are able to form inclusion complexes with a wide range of guest molecules, with two of the requirements for binding being that the guest molecule has: 1) the appropriate size to fit in the cyclodextrin cavity and 2) the guest should be hydrophobic.[118-122]

### 1.5.1 Inclusion complex formation

The most notable feature of cyclodextrins is their ability to engage in host-guest complexation, i.e. to form inclusion complexes, with a wide range of compounds. The hydrophobic cavity of the cyclodextrins is an environment in which hydrophobic compounds of the right dimensions can bind. The main driving forces for inclusion complex formation are van der Waals and hydrophobic interactions,[123, 124] although hydrogen bonding and steric effects also play minor roles.[125]

Cyclodextrins are widely used in industrial products, technologies and analytical methods. The negligible cytotoxic effects of cyclodextrins make them also suitable for applications concerning drug administration, food and flavors, cosmetics, packing, textiles, separation processes, environmental protection, fermentation and catalysis.

Due to complex formation with cyclodextrin, the physicochemical properties of the guest molecules can be altered. This can have several beneficial effects: 1) Stabilization of light- or oxygen-sensitive substances;[126] 2) Modification of the chemical reactivity of guest molecules; [127] 3) Fixation of very volatile substances;[128] 4) Improvement of solubility of compounds;[129] 5) Protection of guest molecules from degradation by micro-organisms;[130] 6) Masking of bad smell or taste;[131] and 7) Masking of colors of compounds.[132]

Pioneering work on self-assembled monolayers (SAMs) of cyclodextrins on solid substrates has been performed in recent years, for example by the group of Reinhoudt, Huskens, and coworkers. As β-cyclodextrin can be used as a receptor, immobilization of this receptor was investigated with sensor development in mind.[133] Initial measurements on simple, monovalent host-guest interactions revealed that surface immobilization did not alter the recognition properties of cyclodextrin as compared to in solution.[134] Next it was found that addition of adamantyl-functionalized poly(propyl imine) (PPI) dendrimers led to permanent attachment to the β-cyclodextrin SAMs due to multivalent adamantane-cyclodextrin interactions.[135] This enabled researchers to build intricate, stable, supramolecular assemblies from these receptor-functionalized assemblies. One approach to achieve complex architecture using multivalent host guest interactions is denoted layer-by-layer **(Figure 15).** Starting from a β-cyclodextrin SAM, adamantyl-terminated PPI was added, which

bound strongly to the monolayer. As many of the adamantane residues were not bound to the surface, subsequent addition of cyclodextrin functionalized gold particles resulted in the deposition of another layer. The previous two steps can be repeated to give rise to a multilayered supramolecular construct.[136] These systems can mimic biological host-guest processes as these are often multivalent. Furthermore, the degree of freedom is enormous. Not only various substrates can be used to assemble the cyclodextrin SAMs on, the choice of guest molecules is extremely large, just as long as a residue is incorporated that will bind to the cyclodextrin SAM. Therefore a wide variety of processes on these receptor-functionalized surfaces can be studied.

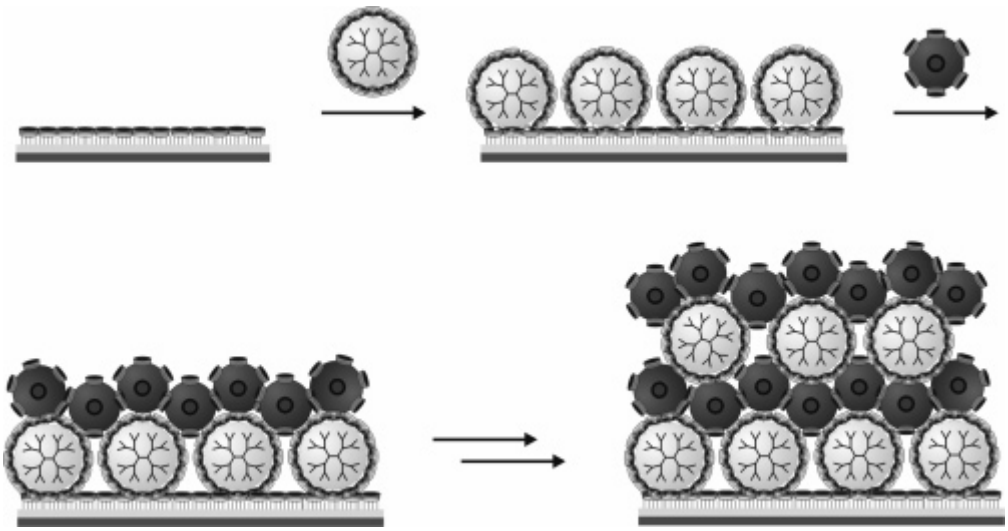

**Figure 15.** *Adamantyl-terminated PPI and cyclodextrin-functionalized gold nanoparticles are assembled alternatingly onto cyclodextrin SAMs, giving rise to the layer-by-layer scheme. Figure adapted from ref 136.*

### 1.5.2 Cyclodextrin vesicles

Darcy and Ravoo were the first to describe the formation of bilayer vesicles, consisting solely of amphiphilic β-cyclodextrin **(Figure 16)**.[137] The bilayer consists of interdigitated amphiphilic cyclodextrin molecules in which the cyclodextrin cavities face both outwards and inwards, towards the aqueous media.

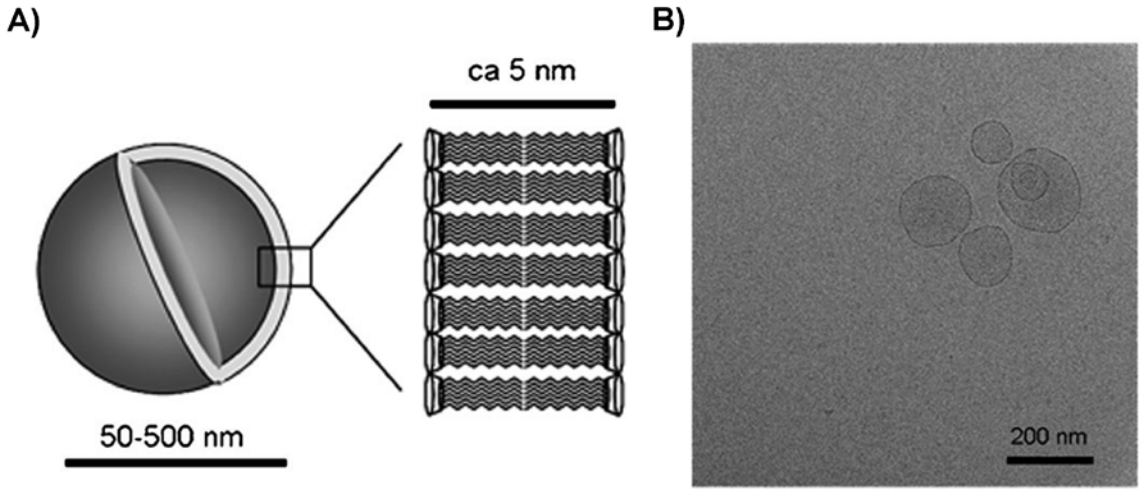


**Figure 16**. (a) *Schematic representation of a bilayer vesicle, composed of amphiphilic β-cyclodextrin. (b) Cryo-TEM images of cyclodextrin vesicles. Figure adapted from ref 137.*

The most interesting property that these vesicles display is that they have a high density of embedded receptor molecules that bind hydrophobic guest molecules such as cholesteryl, butylbenzyl and adamantyl derivatives. These binding moieties can be coupled to a wide range of functional units, which then cover the surface of the cyclodextrin vesicles.[138-141] Recently, Ravoo reported the fully reversible capture and release of proteins at the surface of cyclodextrin vesicles.[142] To achieve this, maltose and lactose were covalently conjugated to an azobenzene moiety, yielding G1 and G2, respectively. This moiety can be reversibly isomerized from *trans* to *cis* by irradiation at 350 nm and from *cis* to *trans* by irradiation at 455 nm. The *trans* isomer forms an inclusion complex with cyclodextrin, whereas the *cis* isomer does not. Consequently, G1 and G2 could be bound and unbound by irradiating the assembly with 350 nm and 455 nm light, respectively. It was found that lectins could bind to the maltose and lactose moieties and that the whole complex could be reversibly captured and released from the cyclodextrin vesicle surface **(Figure 17)**.

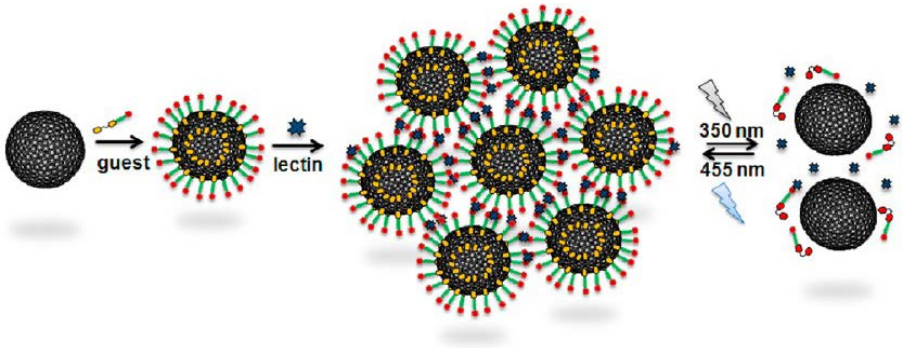


**Figure 17**. *The light responsive formation and dissociation of a ternary complex of cyclodextrin vesicles, azobenzene-lactose (or maltose) and lectins. Figure adapted from ref 142.*

Another example that shows the capabilities of cyclodextrin vesicles to bind and release biologically relevant molecules was shown by the reversible binding of a azobenzene modified, positively charged spermine moiety to the cyclodextrin vesicles.[143] As in the previous example, the light triggered cis-trans transition of azobenzene was used to bind and release the azobenzene-spermine molecule. These positively charged spermine residues at the surface of the cyclodextrin vesicles could then be used to bind DNA. As multivalent binding between DNA and spermine occurred, aggregation of the cyclodextrin vesicles was observed. Upon irradiating the assembly with light of 455 nm, the spermine-DNA conjugates were released from the vesicle surface and the aggregation was reversed **(Figure 18)**.

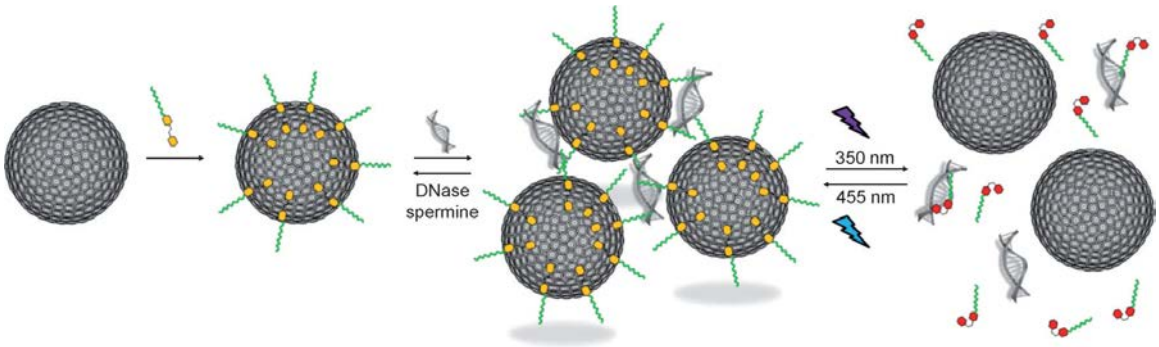


**Figure 18**. *The light responsive formation and dissociation of a ternary complex of cyclodextrin vesicles, azobenzene-spermine and DNA. Figure adapted from ref 143.*

### 1.6 Stimulus responsive systems

Living systems are able to adjust to a change in their direct environment. For example, membrane fusion between synaptic vesicles and the presynaptic plasma membrane was finally triggered by the external release of calcium. The assembly was able to respond to an environmental chemical change. Other stimuli that have been used to trigger responses in synthetic systems are for instance light, pH and temperature. Although there many stimuli responsive systems,[144] the focus will be on vesicular systems. Zhu and coworkers prepared polymersomes consisting of the diblock copolymer $PEG_{45}$-*b*-$P(DMA\text{-}Azo)_{47}$.[145] The PEG chains were directed towards the aqueous environment, whereas the hydrophobic region of the membrane was formed by P(DMA-Azo). UV absorption measurements showed a blue shift upon aggregation, indicating that the DMA-Azo chromophores are π-conjugated. Furthermore, fluorescence increased by a factor 130 as compared to fully dissolved P(DMA-Azo) in THF, which indicates that aggregation caused the fluorescence increase.[146, 147] The resulting vesicles were highly fluorescent and ~120 nm in diameter. Upon acidification to pH 4 however, the particle size increased to ~230 nm, and a loss of fluorescence was observed. The addition of HCl partially protonated the P(DMA-Azo) segments which subsequently increased in hydrophilicity. As a result, the repulsive interchain electrostatic forces caused the vesicles to expand due to a lower interaction free energy, explaining the vesicle swelling. Upon increasing the pH, the original fluorescence intensity and vesicle sizes were obtained **(Figure 19)**.

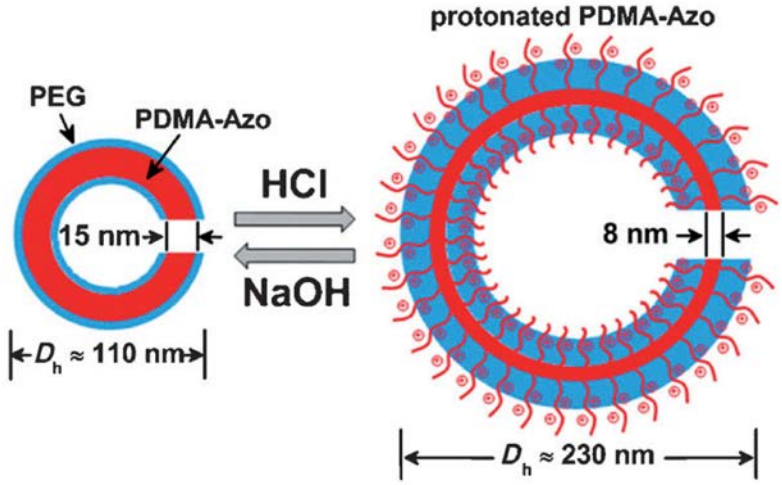


**Figure 19**. *Schematic representation of the pH-induced reversible "breathing" process of the vesicles upon the protonation and deprotonation of PDMA-Azo blocks in response to the pH value of the solution. Figure adapted from ref 145.*

Hubbel reported another example of polymeric vesicles,[148] composed of an A-B-A type block copolymer. The A blocks were constituted of poly(ethylene glycol) and the B block was poly(propylene sulfide). Upon dispersion in aqueous media, $PEG_{16}$-$PPS_{50}$-$PEG_{16}$ was shown to form spherical and oblong type aggregates of a few hundred nanometers in size. Upon the addition of $H_2O_2$, oxidation of the propylene sulfide moieties to the more hydrophilic propylene sulfoxide and subsequently to the even more propylene sulfone occurred. This caused disintegration of the vesicles and a transition to wormlike and spherical micelles ensued **(Figure 20)**. As drugs can be easily encapsulated into the polymeric vesicles, this system might be used as a potential drug delivery system.

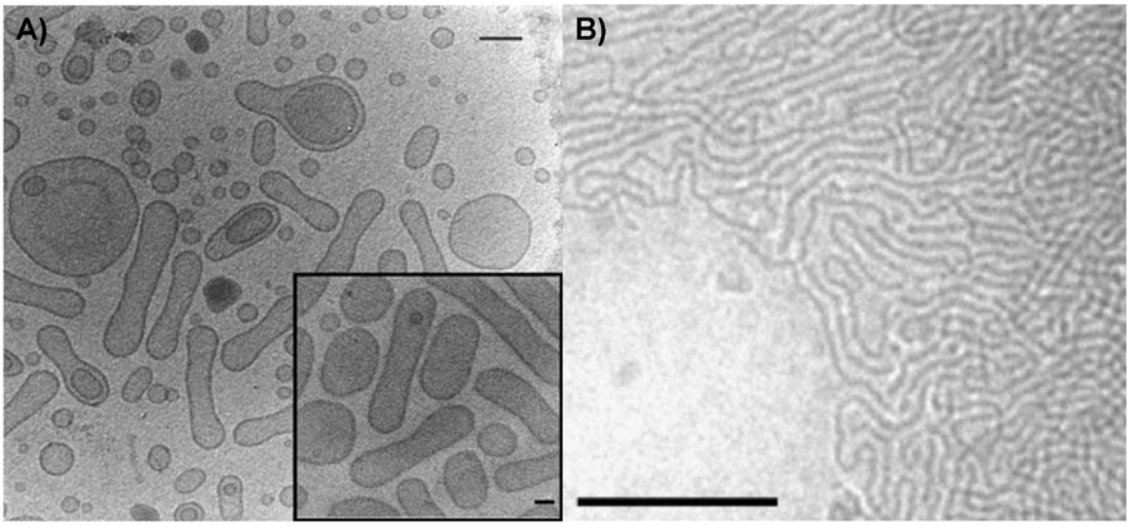


**Figure 20**. *Cryo-TEM images showing A) unilamellar polymersomes with either spherical or oblong morphology. The scale bar represents 100 nm. B) Upon the addition of 10 vol% $H_2O_2$ worm-like micelles several micrometres long appeared after a few minutes. The scale bar represents 250 nm. Figure adapted from ref 148.*

The previously mentioned examples illustrate the importance of systems which can be manipulated with the aid of an external stimulus. These types of systems are able to respond to a change in the environment and therefore there is an additional level of control over the self-assembly. These systems will allow for the formation of more intricate materials and mimicking of nature, in which an appropriate molecular response to external stimuli is highly important.

## 1.7 Scope of this thesis

This thesis describes the characterization of assemblies composed of vesicles and peptides. Both entities have been widely studied separately and have been found of high interest in for example materials science and drug delivery. My objective was to design peptides which, when confined to the surface of a vesicle, would yield highly structured smart materials, i.e. materials that can execute a specific function by design. The curiosity driven, fundamental research reported on in this thesis describes routes to novel, functional systems which can potentially be applied in the aforementioned realms of science called materials science and drug delivery.

In **chapter 2**, a set of short oligopeptides were designed which bound non-covalently to β-cyclodextrin vesicles (CDVs). It was found that these peptides were only able to fold into well-defined β-sheets at the surface of the CDVs. Subsequently, a morphological transition from spherical to fiber-like assemblies was observed. The transition both in secondary structure as well as morphology was found to be reversible as a function of pH. These pH responsive nanostructures might be suitable candidates for drug delivery systems, as a large part of the content of the assemblies was released upon fiber formation.

**Chapter 3** is dedicated to a review of the current literature on the self-assembly of peptide amphiphiles. Most important in the discussion is the influence of a hydrophobic anchor on the peptide secondary structure and morphology of the assemblies. First it is noted that for example an alkyl chain can induce the formation of the secondary structure of peptide amphiphiles. However, when the hydrophobic interactions between the tails are too large, this might hinder or prevent the formation of well-defined nanostructures. Also, when the interactions between the peptides are too large, rigid, non-responsive structures are obtained. Therefore, a careful balancing of all the non-covalent forces is required to yield well-defined and responsive nanostructures. Finally, a range of applications of peptide amphiphiles and their nanostructures are discussed.

**Chapter 4** focuses on membrane fusion, driven by a complementary set of coiled coil forming peptides denoted “E” and “K.” Previous work showed that when these peptides were 1) covalently conjugated to a phospholipid tail and 2) incorporated in separate batches of liposomes that upon mixing of these peptide decorated liposomes membrane fusion ensued. In this chapter, the chemistry of the hydrophobic membrane anchor was varied to understand the role of this transmembrane domain on membrane

fusion, as well as to optimize and expand the scope of the model system for membrane fusion. It was found that the hydrophobicity of the anchor is an important factor, as peptides with a less hydrophobic anchor did not induce membrane fusion efficiently. Furthermore, it was found that a cholesterol anchor performed significantly better than the initially used DOPE anchor, as is shown by both lipid and content mixing experiments. Importantly, by simply adding a micellar solution of cholesterol modified peptides to plain liposomes, it was possible to modify vesicles in situ through spontaneous insertion of the cholesterol anchor into the liposomal membranes. These liposomes were subsequently able to fuse. The ability of the cholesterol modified peptides to insert into preformed membranes was studied further in natural membranes in chapter 7.

In **chapter 5**, the importance of the binding orientation of peptides E and K in inducing fusion between liposomes was examined. In living systems, membrane fusion occurs only through the zipper-like binding of coiled coil forming proteins. This zipper-like orientation arises due to 1) parallel coiled coil formation and 2) anchoring of the proteins in the membrane at identical peptide termini. The importance of the coiled coil orientation in our model system was studied by coupling the cholesterol anchors on opposite peptide termini. Also a K peptide was synthesized with cholesterol anchors on both termini.

In **chapter 6**, coiled-coil forming peptides E and K are used to study fusion between traditional phospholipid liposomes and non-natural cyclodextrin based vesicles. Peptide K was conjugated to an adamantane moiety in order to decorate the CDVs with K peptides. The E peptide was confined to the surface of liposomes through a phospholipid anchor. Upon mixing of the two populations of vesicles, peptides E and K were able to form coiled coils. It was observed that subsequently to coiled coil formation hemifusion occurred and multilayered hybrid structures were formed.

Finally, **chapter 7** describes efforts to form coiled coils at the surface of live cells and zebrafish embryo membranes with cholesterol modified E and K peptides. It is shown that by simply adding a solution of the cholesterol modified peptides to cells or zebrafish embryos, a ‘handle’ is obtained to which molecules or aggregates of interest can be bound, whenever they carry the complementary K peptide. Efficient insertion of the cholesterol peptides into cell membranes was observed. Furthermore, coiled coil formation ensued upon addition of the complementary peptide. Finally, when the

complementary peptide was inserted into liposomes, docking of these liposomes occurred at the surface of CHO cells and zebrafish embryos.

**Chapter 8** offers a summary of the most important findings and lessons contained in this thesis. Furthermore, suggestions for future studies that are based upon this work are given.

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