1 Introduction

The first chapter provides a general introduction to the field covered by this PhD thesis. First, a view of the *nanometer-sized world* is afforded by means of a variety of structures that are obtained using the method of self-assembly. The *basic principles of self-assembly* are then explained and compared to *self-assembly mechanisms as they appear in nature*. Next, we switch to the topic of *drug-delivery* and compare the *classical drug delivery* – as is common for most medications – to *payload delivery as designed by nature*. This gives us the opportunity to combine drug delivery and self-assembly within new *concepts to design novel smart materials* for drug delivery applications, and to show their possible use. Subsequently, we will come to see that these novel materials also need to be *biocompatible* in order to engender as little harm to a patient as possible. This requirement will lead us to the *synthesis of amino acid-based amphiphiles* and I will highlight different synthetic strategies to obtain the molecular building blocks needed to produce self-assembled supramolecular structures. Finally, I will introduce *gramicidin A,* a naturally occurring, short and hydrophobic, membrane-integrating peptide that was used as a basis to produce the amphiphilic peptides that are presented in this thesis. The definition of the *scope of the thesis* closes the chapter.

1.1 Self-assembly

From our daily experiences we know that organization and construction requires considerable effort. Therefore, it would be expedient to use pre-existing building blocks that arrange autonomously to build a desired construct. Such a phenomenon, termed *self-assembly*, actually does exist, exemplified by a variety of different dimensions that range from the organization of galaxies and solar systems down to the organization of small molecules as they assemble into larger aggregates. In this fundamental principle, the organization of pre-existing parts or disordered components forms larger structures or patterns, while the process itself is reversible. Molecular self-assembly is the topic of this thesis and is – as the term implies – an assembly of molecules that leads to the formation of a variety of structures in the nanometer and micrometer scale, while being an important tool for the bottom-up construction in nanotechnology and material science.[1]

1.1.1 The variety of self-assembled structures

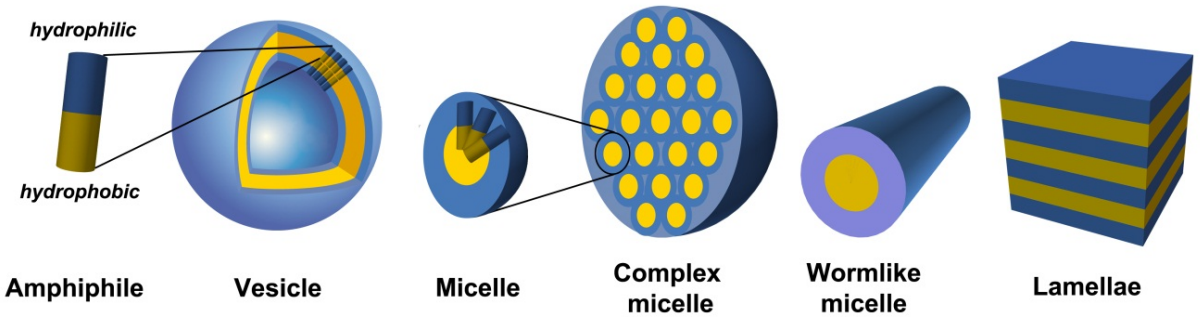

Figure 1-1: A selection of common, self-assembled morphologies and their amphiphilic subunit.

Molecular self-assembly, hereinafter referred to simply as self-assembly, produces a variety of structures of higher order, ranging from the nanometer to the micrometer scale. To obtain such structures, non-covalent intermolecular as well as intramolecular interactions take place. The simplest example is the intermolecular self-assembly of surfactant molecules into micelles in aqueous solution. However, varying the molecular properties (*e.g.* length, mass, shape) can lead to more complex supramolecular assemblies such as, for example, worm-like micelles, complex micelles, lamellae, vesicles, and tubes (see Figure 1-1).[2]

1.1.2 Self-assembly of amphiphilic molecules

Most self-assembly is enabled by using *amphiphilic* molecules. The amphiphilic (*amphi:* of both kinds; *philic:* having an affinity) properties are produced by combining at least two subunits, one possessing hydrophilic properties, the other having a hydrophobic character. The self-assembly occurring in aqueous solution is mainly driven by the low solubility of the hydrophobic part. Aggregation of the hydrophobic parts, and thus their shielding from the aqueous environment, leads to a hydrophobic surface minimization of the aggregate and results in minimized free energy. The structures of the thereby obtained supramolecular aggregates strongly depend on the position and availability of non-covalent interactions (van der Waals forces, electrostatic-, and π-π interactions) as well as the shape and flexibility of the molecules.[3]

Described in more detail, the aggregation of an amphiphilic molecule can be separated into three terms influencing the free energy of an amphiphilic self-assembly in dilute, aqueous solutions: (1) the hydrophobic contribution from the hydrophobic parts that aggregate inside the structure; (2) the surface contribution of the hydrophilic parts, reflecting the tendency to arrange so as to minimize the effect of the surrounding water on the hydrophobic parts enabled by hydration, electrostatic repulsion, and steric hindrance and (3) a packing term, describing the geometrical shape of the molecule and the possibility of their spatial arrangement. Surface and packing contribution are expressed in the surfactant parameter N_s, defined as $\nu_0/l_0 a_e$ with ν_0 for the volume of the hydrophobic part of the molecule, l_0 being the length and a_e the equilibrium area per molecule at the aggregate surface, which is in fact the effective area of the hydrophilic group. The surfactant parameter is often used to explain and predict the curvature of the assemblies and

the resulting structure, e.g. for spherical micelles (N_s = 0.33), infinite cylinders (N_s = 0.5), planar bilayers and vesicles (N_s = 1), bicontinuous structures ($N_s \geq 1$) and inverted micelles (N_S >1).[3-4]

Another attempt to predict resulting structures is the hydrophilic to hydrophobic ratio ($f_{hydrophilic}$), which expresses the tendency to form vesicles ($f_{hydrophilic}$ = 35% ± 10%), inverted microstructures $f_{hydrophilic}$ < 25%) and micelles ($f_{hydrophilic}$ > 45%).[5]

As mentioned above, the low solubility of the hydrophobic part is a major driving force for the self-assembly. Being referred to as attractive forces among apolar solutes in water, the important factor is actually the increase in entropy due to the liberation of water from the hydration shell during aggregation. However, the thermodynamic factors giving rise to this phenomenon, commonly referred to as the hydrophobic effect [6], are complex and still not fully understood.

As derived from the above driving forces, the structures can also be influenced by environmental stimuli, *e.g.* pH[7], ionic strength[7d-f, 8], temperature[9], counter ions[9c, 10], light[9b, 11], and oxidative stress[12], which all influence either the stabilizing interactions, the shape of the molecule, or the molecular composition itself. This fact can either be used to control or to trigger the destruction of the self-assembled structures[13]. The latter, in particular, is of interest in the triggered release of encapsulated payloads (*e.g.* in drugs delivery). Due to the so produced capability of the resulting materials to react to environmental stimuli, the materials are often referred to as “smart”. The versatile variables thereby obtained enable manifold possibilities to control the self-assembly, but, on the other hand also make it hard to rationally predict self-assembled morphologies from scratch.

1.1.3 Hierarchically organized structures in nature

Hierarchical organization, as is often the case in self-assembly, also occurs in nature, where, for example, spider silks are produced by salt- and shear force-triggered self-assembly of proteins[10b]. But also the production of functional enzymes and of molecular machines in each cell are most often constructs of hierarchical organization and the key parameter for life[14]. Surprisingly, the variety of functions is mostly enabled by proteins and peptides that consist of only 20 natural amino acids (AA). These serve multiple functions as acids, bases, thiols, aromatic rings, etc., while spanning the wide range from hydrophilic to hydrophobic, resulting in almost endless possibilities of combinations in linear sequences. However, these linear sequences (primary structures) do not, in such state, allow for the broad functions of molecular machines. Only the folding of the sequence into a three-dimensional secondary structure – which is, in fact, self-assembly – imparts a functional structure to the biopolymers. The necessary rotations of the molecules happen on the N-C_α-bonds and the C_α-C-bonds, the related angles of rotation are expressed as Φ and Ψ, respectively. Other rotations are impossible, due to the specific electronic structure of the peptide bond. Steric hindrance also limits remaining possibilities to about 25%, reflected in the Ramachandran plot (Figure 1-2). With natural occurring L-amino acids, three secondary structures are possible: α-helix, β-sheet, and random coil. The alternation of L- and D-amino acids (rare) also enables wider β-helical confirmations, in which all residues point to the outside of the helix.[15]

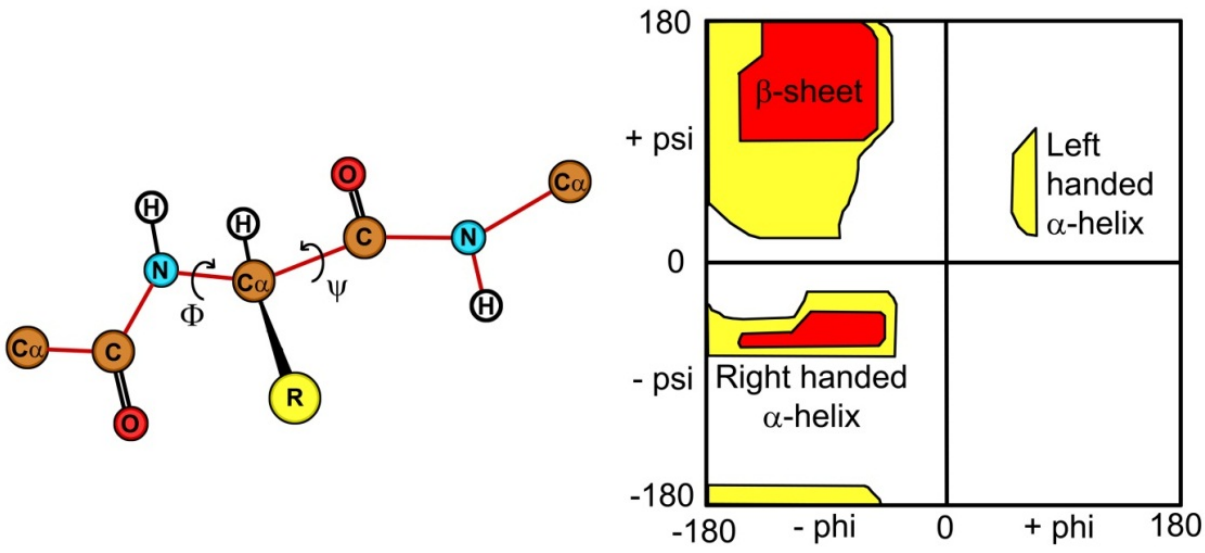

Figure 1-2: Illustration of the peptides phi- and psi-angles with the red line forming the repeating backbone of the peptide (left). The Ramachandran plot representing the possible angel combinations and the resulting secondary structures (right).

The complete folding of a protein – comprising several regions of different secondary structures – is called the tertiary structure and forms three-dimensional building blocks, which can further assemble into more complex tertiary structures.

The oxygen transporter, hemoglobin, is a good example of an assembly consisting of four subunits. Another more complex example is the hierarchically organized thermosome, a chaperonin from the thermophilic organism *Thermoplasma acidophilum*. It is a spherical assembly, consisting of two stacked, eight-membered rings, where each member is built of alternating α and β subunits.[16] Ironically, this self-assembled construct of two half-spheres assists proteins in folding.

Further examples are viruses, which are also the product of hierarchical organization, their sole task being the protection of their own DNA/RNA in the interior and delivering it into other cells. Recently, a self-assembled protective capsule of a virus (capsid) was produced by a synthetic 24-mer peptide[17]. This is a good example of how nature's self-assembly strategies can be understood and used in designing self-assembled structures.