Chemistry, structural insight and applications of β-sheet forming lipopeptides

Cavalli, S.

Chapter 1

Bridging Science: Scope and Applications of Peptide-Based Nanostructures from Chemistry to Biology, Materials Science and Engineering

Abstract. Peptides are particularly attractive as molecular "Lego bricks" in the bottom-up fabrication of supramolecular structures based on self-assembly and have potential in many important applications in the field of biotechnology and bioengineering. In the first part of the introduction the main categories of peptide-based amphiphiles as molecular building blocks will be discussed by showing some relevant examples, which demonstrate the importance of amphiphilic peptides as molecular construction moieties for nanostructures. In the second part of the introduction the cross-disciplinary role of peptide-based nanoarchitectures ranging from chemistry to biology, material science and engineering is demonstrated by discussing several examples of applied nanomaterials. Finally the outline of the thesis is given.

1.1 Amphiphilic Peptides as Building Blocks for the Bottom-Up Constructions of Nanometre-Scale Assembled Structures.

The process of self-assembly is based on the spontaneous diffusion and specific interaction among molecules governed by non-covalent interactions, including electrostatic, hydrophobic, van der Waals and metal-ligand interactions, hydrogen bond formation and aromatic π-stacking.[1] Although these interactions are individually weak, if sufficient in number, they can generate highly stable assemblies. Richard Feynman presented in 1959 a lecture entitled "There's plenty of room at the bottom", proposing the idea of a "bottom-up" approach for the fabrication of higher ordered structures via self-assembly using individual atoms and molecules as building blocks.[2] One of the main challenges in supramolecular chemistry involves the issue of forming homogeneous and structurally well-defined architectures with tuneable properties to cover a wide range of possible applications. Therefore, an accurate design and good understanding of the rules governing the molecular assembly of specific monomeric building blocks are key features for the successful engineering of "smart" supramolecular architectures with predictable properties and functions.[1]

During the past decade, many examples of supramolecular assemblies based on peptides as monomeric building blocks have been published and a selection of representative examples will be discussed in detail in the following sections of this Chapter.[3] Peptides are a particularly attractive class of molecules, which can be used as molecular building blocks because their secondary structural folding and stability have already been studied in detail.[4] Amino acids and peptides can be seen as information carriers, which introduce structural "smartness" in nanostructures, particularly due to their ability to respond to external parameters[5] (i.e. changes in solvent, pH and temperature or sensitivity to electronic or photonic energy and to the presence of chelating metals). This is of particular interest when the responsiveness is reversible. Furthermore, the advent of straightforward and fast synthetic methodologies, mainly based on solid phase protocols, offers easy access to a wide variety of (oligo)peptides with virtually any amino acid sequence of about 5-50 residues. Moreover, the possibility to incorporate non-natural amino acids or functional moieties in the peptide sequence is particularly valuable for the introduction of an increased level of functionality in the assemblies.[3] In addition, the intrinsic chiral nature of amino acids can lead to the expression of handedness to a higher hierarchical level.[6] Finally, biologically relevant peptide sequences can be used to generate new materials, at the nanometer-scale with possible applications in the field of biotechnology and bioengineering.[7]

The first part of this introduction gives an overview of four main categories of peptide-based amphiphiles[8] as molecular building blocks and some relevant examples demonstrating the importance of amphiphilic peptides as molecular construction moieties for nanostructures are discussed. First, amphiphilic oligopeptides are examined, followed by an overview on alkylated peptides. A special class includes peptide-phospholipid conjugates. This type of amphiphilic peptides is of particular interest as a new series of lipidated peptides will be discussed in the following chapters of this thesis. A final important category comprises peptide-based block copolymers.[9]

1.1.1 Amphiphilic Oligopeptides

Amphiphilic peptides constituted of only amino acids are organized in amphipathic sequences comprised of both hydrophobic and hydrophilic domains. Zhang and co-workers[10] introduced the concept of "Peptide Lego", based on ionic self-complementary peptides. These peptide building blocks contain two distinct surfaces, one being hydrophilic and the other hydrophobic, similar to the "Lego bricks" that have both pegs and holes positioned in such a well-ordered fashion, allowing precise assembly into a predetermined organization. In aqueous solutions, the hydrophobic side shields itself from water driving the self-assembly of the peptides, comparable to spontaneous protein folding as observed in nature. The unique structural feature of these "Lego bricks" is based on complementary ionic bonds with regular repeats on the hydrophilic surface due to the alternation of positively- and negatively-charged amino acid residues at specific intervals. Lysine (Lys) and arginine (Arg) are typically used as the positively-charged residues, while glutamate (Glu) and aspartate (Asp) are employed to generate the negative charge. The complementary ionic sides have been classified by Zhang into several moduli (i.e. modulus I, II, III, IV, etc.), depending on the alternation of the charges (Figure 1.1). For example, in the case of modulus I, the (+) positively- and (-) negatively-charged amino acid residues are alternated by one (+ - + - + - + - and - + - + - + - in the complementary peptide). Consecutively, for modulus II, III, IV etc., charges are alternated by two, three, four etc. (+ + - - + + - -, + + + - - - and + + + + - - - - etc.). The charge orientation can also be designed with a mixed order to yield entirely different molecules (mixed moduli). The first member of the "peptide Lego" was serendipitously discovered from a segment in a left-handed Z-DNA binding protein in yeast, named Zuotin.[11] The self-complementary sixteen-residue [(Ala-Glu-Ala-Glu-Ala-Lys-Ala-Lys)$_2$] oligopeptides, originally found in a region of alternating hydrophobic and hydrophilic residues in zuotin, interacted strongly with each other to form a stable structure promoted by the hydrated salt

ions.[12] These molecules represent a class of self-assembling β-sheet peptides that spontaneously undergo association, in aqueous solutions, into a macroscopic membrane composed of well-ordered nanofibers. The architecture of the membrane resembled a high-density felt. Scanning electron microscopy (SEM) investigations at low magnification revealed that the structure looked like a flat membrane, which consisted of interwoven individual filaments, as seen at high magnification.

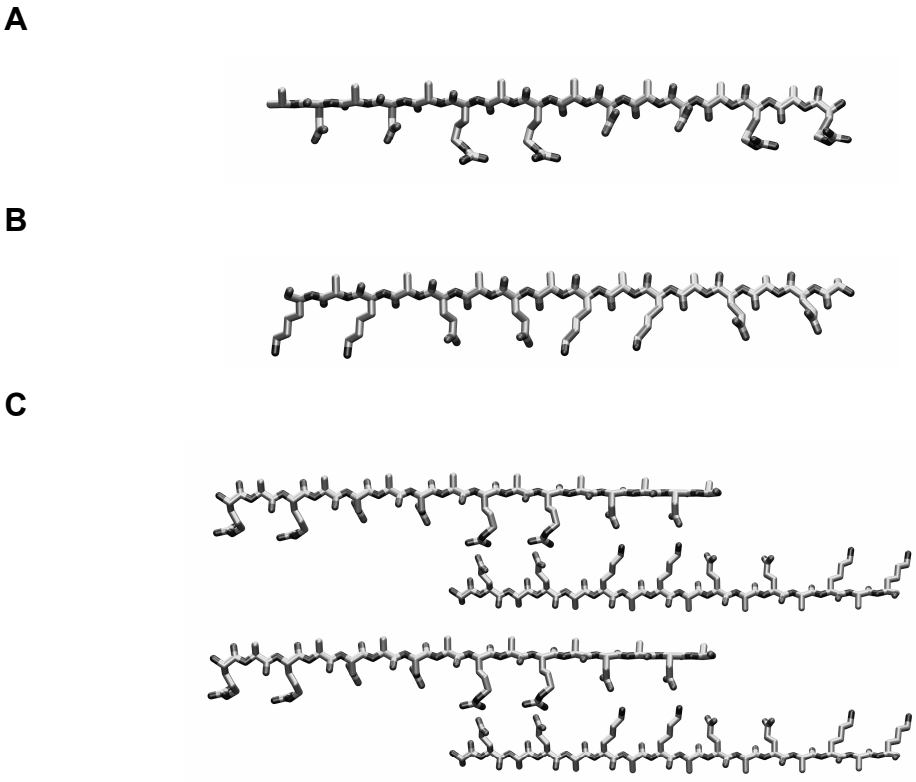


Figure 1.1. Examples of Moduli II. Molecular models of the extended β-strand structures of individual molecules are shown for (A) ARARADADARARADAD (RAD16-II, R, arginine, A, alanine and D, aspartate) and (B) EAEAKAKAEAEAKAKA (EAK16-II, A, alanine, E, glutamate and K, lysine). The distance between the charged side chains along the backbone is approximately 6.8 Å; the methyl groups of alanines are found on one side of the sheet and the charged residues are on the other side. Conventional β-sheet hydrogen bond formation between the oxygens and hydrogens on the nitrogens of the peptide backbones are perpendicular to the page. (C) A proposed staggered assembly of molecular models for EAK16. The complementary ionic bonds and hydrophobic alanines are shown. Although an antiparallel β-sheet is illustrated, a parallel β-sheet model is also possible.[13]

Rajagopal *et al.*[14] recently prepared a class of polypeptides whose ability to self-assemble into hydrogel[40] was dependent on their folded state. Under unfolding conditions soluble peptides were freely flowing in aqueous solutions. When the folded peptides were triggered by external stimuli (i.e. changing the pH), they adopted a β-hairpin conformation

and self-assembled into a highly crosslinked network of fibrils affording mechanically rigid hydrogels[40] (Figure 1.2).

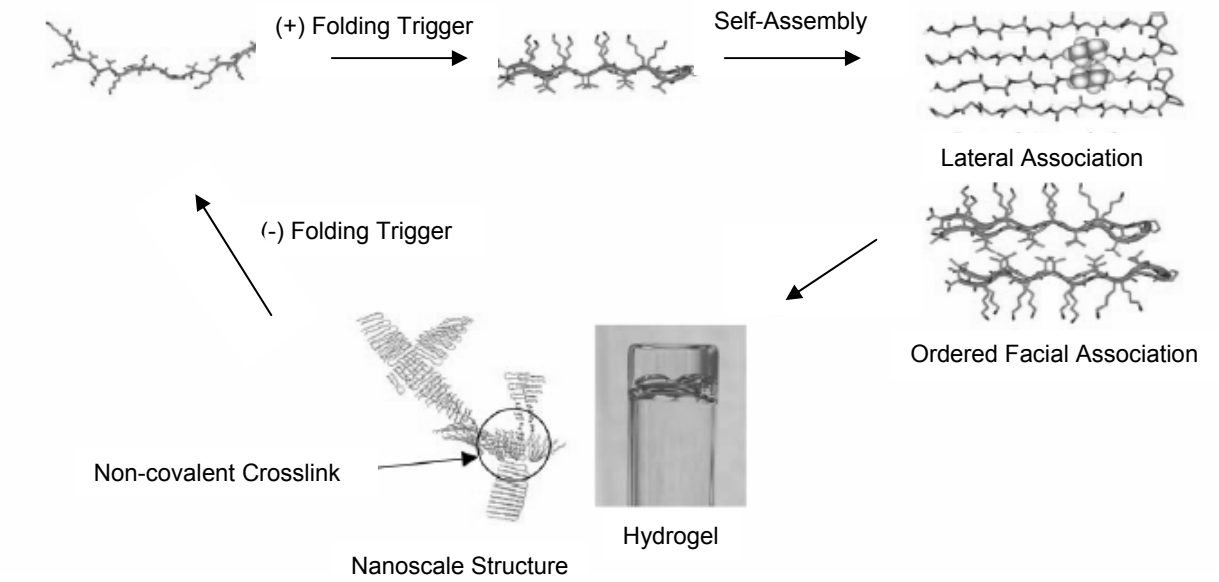


Figure 1.2. Environmentally triggered folding, self-assembly and non-covalent fibril crosslinking leading to hydrogel[40] formation. Crosslinks are formed by the irregular facial self-assembly of hairpins.

Well-defined assemblies of β-sheet structures have also been achieved at the air-water interface. Rapaport *et al.*[15] have investigated a family of amphiphilic oligopeptides comprised of alternating hydrophobic and hydrophilic moieties with the generic sequence X-Y-(Z-Y)$_n$-X, where the *N*- and *C*-terminal residues (X) bear charged ammonium and carboxylate groups, respectively, and Y and Z are alternating hydrophilic and hydrophobic amino acids. Variations in amino acid sequence and in the number of dyads (n) participating in hydrogen-bond formation were expected to tune the intermolecular interactions. Peptides Pro-Glu-(Phe-Glu)$_n$-Pro (n = 4, 5 or 7) formed secondary structures composed of two-dimensional self-assembled β-sheet monolayers at the air-water interface confirmed by *in situ* grazing-incidence X-ray diffraction (GIXD) investigations. The alternating hydrophobic phenylalanines and the hydrophilic glutamic acid residues caused the peptide chains to orient as β-pleated sheets parallel to the water surface. The flexibility of the peptide backbone and the repetitive nature of the amino acid sequence may induce dislocation defects (Figure 1.3, a) that limit long-range order to one-dimension (1D), in the direction normal to the peptide backbone (*a* direction). Noteworthy, the two-dimensional (2D) registry of the self-assembled architectures was induced by placement of proline residues at the peptide termini. Without the proline only 1D order was achieved, demonstrating the importance of a careful design in the peptide sequence for the successful self-assembling process. Proline

(Pro), was chosen to prevent the formation of disordered β-sheets along the *a* direction (Figure 1.3, a), on the base of three characteristic features: the tertiary amide, which cannot participate as a donor in the hydrogen bond array; the restricted dihedral angle (Φ) of Pro (ca. -60°) that is significantly different from that of β-sheet peptides (Φ ca. -120 to -150°), therefore making inclusion of Pro in the interior of a β-sheet ribbon sterically unfavourable and the cyclic Pro side chain, which determines geometric constraints, minimizing disorder at the ribbon edge. In addition, attractive electrostatic interactions between the chain termini were expected to juxtapose the β-sheet ribbons along the *b* direction (Figure 1.3, b), facilitating the formation of the two-dimensional order. A schematic representation of the peptide Pro-Glu-(Phe-Glu)$_4$-Pro in the β-pleated conformation and the targeted β-sheet crystalline assembly at the air-water interface is shown in Figure 1.3, c.

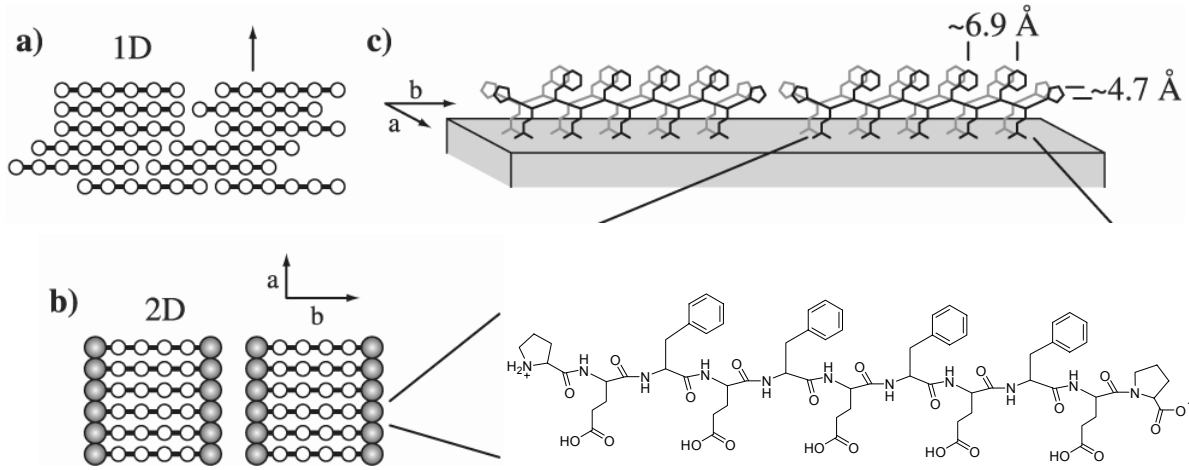


Figure 1.3. Schematic diagrams of β-strand assemblies at the air-water interface (rods and open dots represent peptide backbones and hydrophobic amino acids, respectively). View down the normal to the β-sheet of (a) one-dimensional order and (b) two-dimensional order induced by distinct chain termini. (c) Schematic representation of the peptide Pro-Glu-(Phe-Glu)$_4$-Pro in the β-pleated conformation and the targeted β-sheet crystalline assembly at the air-water interface.

Well-defined β-sheet arrays at the interface were also observed for larger oligopeptides able to form triple-stranded β-sheets.[16] Recently it was shown by Rapaport and co-workers[17] that varying the amino acid sequence, parallel β-sheet assemblies may form as well.

The group of Ghadiri extensively studied the design principles and the preparation strategies to achieve synthetic organic nanotubes, with special emphasis on noncovalent processes such as self-assembly and self-organization and an overview of this work was presented in a recent review.[18] As an example, hollow β-sheet tubular structures could be

formed by the stacking of cyclic peptide rings. The cyclic peptides, containing an even number of alternating D and L-amino acids, in which the amide bonds are perpendicular to the plane of the ring, are shown to aggregate into microcrystalline tubes via a pH-controlled assembly strategy.[19] The sequence of octapeptide cyclo[-(l-Gln-d-Ala-l-Glu-d-Ala)$_2$-] was chosen to impart solubility in basic aqueous solution and thereby to prevent subunit association through coulombic repulsion. Controlled acidification promoted hydrogen bond interactions, thus allowing the self-assembly into hollow tubes composed of ring-shaped subunits stacked through antiparallel β-sheet hydrogen bonding. These structures exhibited a hydrophobic exterior and a hydrophilic interior and could insert into bilayer membranes introducing pores. Varying the number of amino acid residues and, hence, the ring size, modulated the porosity, as the smaller 8-residue rings only transport small ions while the larger 10-membered rings could also transport compounds like glucose and glutamic acid.

1.1.2 Lipidated Oligopeptides

The second category of peptide amphiphiles discussed in this section is constituted by hydrophilic amino acid sequences coupled to hydrophobic alkyl chains at either the *N*- or the *C*-terminus.

The groups of Fields and Tirrell have investigated several amphiphilic peptides, which have been modified at the *N*-terminal with mono-alkyl hydrocarbon chains.[20] In these studies the use of mono-alkyl hydrocarbon chains for inducing protein-like structures such as α-helices and collagen-like triple helices was studied. A potentially α-helical 16-residue peptide sequence, Lys–Ala–Glu–Ile–Glu–Ala–Leu–Lys–Ala–Glu–Ile–Glu–Ala–Leu–Lys–Ala–NH_2, was designed based on a repeating heptad sequence, (Glu–Ile–Glu–Ala–Leu–Lys–Ala)$_n$, known to associate to form a highly α-helical coiled coil structure at a chain length of at least 23 residues.[21] Although the peptide alone did not form a distinct secondary structure in solution, it adopted predominantly an α-helical structure upon acylation at the *N*-terminus with hexanoic acid (C_6) or palmitic acid (C_{16}) mono-alkyl chain, as demonstrated by circular dichroism (CD) spectroscopy. ^{1}H Nuclear Magnetic Resonance (^{1}H-NMR) studies showed that the thermal stability of the peptide structural conformation was already enhanced by *N*-acylation with a C_6 alkyl chain. Increasing the alkyl chain length resulted in even higher thermal stability. This enhancement may partly be correlated to the extent of peptide-amphiphile aggregation.[22] These studies demonstrated that hydrocarbon chains may be useful as general tools for induction and stabilization of protein-like secondary and tertiary

structures in short peptide sequences and might be utilized for studying the mechanisms of peptide folding as well as for the generation of new biomaterials.

Löwik *et al*.[23] demonstrated that certain peptide sequences conjugated by single C_{18} alkyl chains to both the *N*- and *C*-terminus resulted in control over the secondary structure upon incorporation into a liposome membrane. A sequence derived from the CS protein of the malaria parasite *Plasmodium falciparum* was chosen as within the natural protein the Asn-Pro-Asn-Ala repeat is known to adopt a β-turn. Both the unmodified peptide in solution and the analogous peptide with only one alkyl chain showed random coil folding characteristics. However, when the double alkylated peptide was inserted into 1,2-dimyristoyl-sn-glycero-3-phosphoethanol-amine (DSPC) liposomes the peptide folded into a β-hairpin. This simple approach is a convenient way for stabilizing a variety of peptides into their preferred secondary structure on a lipid bilayer and might be employed in the presentation of multiple (hairpin) epitopes.

Lipid-modified proteins play important roles in numerous biological processes like signal transduction and vesicular trafficking, it is particularly interesting therefore to prepare molecule that can allowed the study of these roles in precise molecular detail. The availability of a flexible solid-phase technique for the fast synthesis of lipidated peptide conjugates is of fundamental importance. Ideally such a technique would give access to peptides carrying different combinations of acid and base-labile lipid groups and allow for the introduction of additional reporter and/or linking groups required for application of the target peptides in further biological experiments. In this respect, Waldmann and co-workers[24] developed a method for the efficient solid-phase synthesis of S-farnesylated and S-palmitoylated peptides, which is based on an oxidation-sensitive hydrazide linker that can be cleaved by oxidation with $Cu(OAc)_2$ in the presence of pyridine and acetic acid. Furthermore, biologically fully functional Ras proteins were also prepared by the same group.[25]

Peptide sequences have also been covalently coupled to phospholipids in order to incorporate the lipopeptides into the cell membrane. In general, cell-membrane proteins are anchored to the lipid bilayer by lipidation in co- and post-translational enzymatic processes, including acylation with fatty acids, prenylation,[26] and rather commonly *C*-terminal amidation with glycosylphosphatidylinositols (GPI).[27] It is well established that dual vicinal lipid chains, as present in the di-acyl glycerol moiety of natural GPI anchors are required for an almost irreversible capture of peptides and proteins by lipid bilayers.[28] Musiol *et al.*[29] have shown the preparation of semi-synthetic lipoproteins by *C*-terminal lipidation exploiting the copper(I)-catalyzed Huisgen's 1,3-dipolar cycloaddition (vide infra).[30] A fragment 214-231 of the human prion protein with a *C*-terminal propargylglycine was reacted with an

azido-modified 1,2-dimyristoyl-sn-glycero-3-phosphoethanol-amine (DMPE), mimicking the GPI anchor, to form a stable 1,4-disubstituted 1,2,3-triazole product. The "click" reaction was followed by the ligation of the resulting *N*-cysteinyl-lipopeptides and *N*-terminally fluorescently labeled peptide thioesters.[31] Remarkably, incubation of HeLa cells with the micellar solution of the lipopeptide confirmed its fast uptake of the semisynthetic lipoprotein, as visualized by confocal fluorescence microscopy.

1.1.3 Peptide-Based Block Copolymers

Another class of peptide amphiphiles comprises peptide-based block copolymers. Klok and co-workers[32] synthesized a series of hybrid di- and triblock copolymers, which contained amphiphilic β-strand peptide sequences and poly(ethylene glycol) (PEG) segments. The block copolymers have been prepared via solid-phase synthesis, affording monodisperse peptide segments with a precisely defined α-amino acid sequence. The self-assembly properties of the peptide sequences were retained upon conjugation to PEG and the formation of an ordered superstructure was observed, consisting of alternating layers of PEG domains and peptide domains with an highly organized antiparallel β-sheet structure. This work suggests that the combination of biological structural motifs with synthetic polymers may be a versatile strategy for the development of novel self-assembled materials with complex internal structures and the potential to interface with biology. Smeenk *et al.*[33] described the preparation and assembly of an ABA-type triblock copolymer consisting of a central β-sheet polypeptide block composed of the repetitive $[(AG)_3EG]_n$ sequence conjugated to PEG end blocks. The [(AG)3EG]n β-sheet polypeptides, outfitted with *N*- and *C*-terminal cysteine residues, were constructed by protein engineering. The thiol groups of the cysteines were subsequently selectively alkylated with maleimide-functionalized PEG. Crystallization of the triblock copolymer resulted in well-defined fibrils, which were formed in the β-sheet stacking direction. The authors envisioned that control over the amino acid sequence would offer the possibility of introducing specific amino acid residues at the turns of the β-sheets, thereby creating a regular array of functional moieties at the fibril surface.

Advances in chemical polypeptide syntheses include the polymerization of α-amino acid-*N*-carboxyanhydride (NCA) monomers.[34] The use of transition metal initiators in NCA polymerizations has allowed the preparation of very well-defined homopolypeptides and led to facile routes into peptide block copolymer materials. Recently, Kros and Cornelissen[35] described the synthesis and self-assembly of hybrid block copolymers composed of a poly(γ-benzyl L-glutamate) block (PBLG) and two different polyisocyanide blocks, namely,

poly((S)-(−)-α-methylbenzyl isocyanide) (PMBI) and poly(L-isocyanoalanyl-L-alanine methyl ester) (L,L-PIAA). The diblock copolymers were synthesized by the nickel-catalyzed living polymerization[36] of γ-benzyl L-glutamate *N*-carboxyanhydride (Bn-Glu, NCA, *vide infra*) according to the procedure of Deming and co-workers[37] followed by the addition of (S)-(−)-α-methylbenzyl isocyanide (MBI) or L-isocyanoalanyl-L-alanine methyl ester (L,L-IAA) to the reaction mixture. This whole new class of rod–rod block copolymers exhibited multiple structural motifs (for example, an α-helical peptide segment combined with a β-helical polyisocyanopeptide segment) and consequently unique properties. Remarkably, The PBLG-block-L,L-PIAA has three secondary structural motifs within one macromolecule, that is, an α-helical polypeptide segment and a polyisocyanide helix with side arms organized in a parallel β-sheet. Preliminary self-assembly studies were performed in organic solutions and the formation of polymersomes[38] (with a uniform diameter of 7.5 mm) was observed upon fast drying of a solution of PBLG-block-L,L-PIAA revealed by confocal laser scanning microscopy. However, slow evaporation resulted in the formation of closed films, which suggested that the polymersomes[38] were a kinetically trapped architecture. Polarized optical microscopy studies showed that the polymers were aligned and confirmed that the polymersomes[38] were hollow.

Deming and co-workers[39] synthesized diblock copolypeptide amphiphiles using the transition metal-mediated-amino-acid *N*-carboxyanhydride polymerizations described above, which allowed control over polypeptide chain length and composition. Many of these diblock amphiphiles were found to form rigid hydrogels[40] in water. The gelation process of the copolypeptides was found to be dependent not only on the amphiphilic nature of the polypeptides, but also on the type of secondary structures present in the chain, as α-helices or β-strands favoured the hydrogel[40] formation, while random coil domains inhibited gelation. The poly(L-lysine·HBr) and poly(L-glutamate sodium salt) domains, being highly charged polyelectrolytes at neutral pH, dissolved readily in water. The hydrophobic domains, when sufficiently large, could adopt regular conformations that aggregate and were insoluble in water, namely, rod-like α-helices for poly(L-leucine) and crystalline β-sheets for poly(L-valine). Copolypeptides of identical compositions, but of random sequences, were found never to form hydrogels[40] at all.

Coiled coil-based hydrogels[40] represent an interesting area that continuously attract the attention of many research groups especially for potential biomedical applications. Selected examples of hydrogels[40] prepared exploiting the coiled coil motif are given below.

The coiled coil[41] is α left-handed superhelix of two or more right-handed α-helices and it has been identified in proteins ranging from muscle proteins to DNA transcription factors. It

has a characteristic amino-acid heptad repeating units designated as a to g in one helix and a' to g' in the other. The hydrophobic residues occupy positions at the interface of the two helices (a, d and a', d'), whereas e, g and e', g', which are solvent exposed, are generally polar residues that give specificity between the two helices through electrostatic interactions (Figure 1.4).

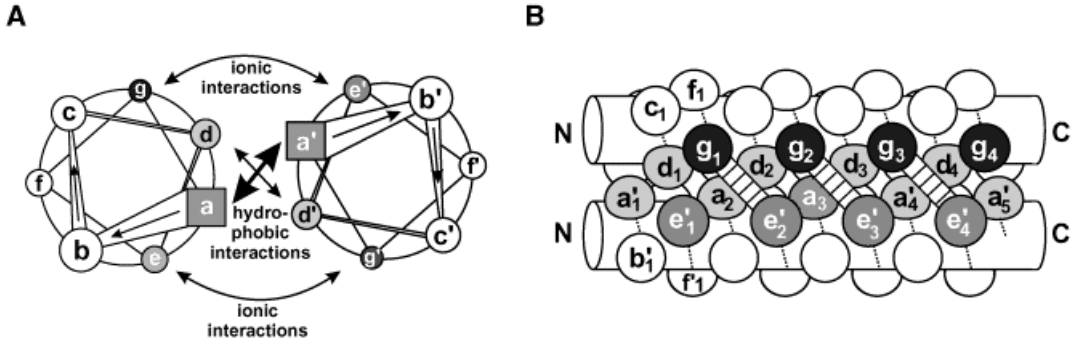


Figure 1.4. A parallel dimeric coiled coil in a schematic representation. The helical wheel diagram in (A) top view down the axis of the α-helices from *N*-terminus to *C*-terminus. Panel (B) provides a side view. The residues are labeled a-g in one helix and a'-g' in the other.

For example the group of Kopeček[42] exploited the coiled coil motif for the preparation of hybrid hydrogels.[40] Based on the observation that upon minor alteration of the primary structure many native and *de novo* designed coiled coils can undergo conformational transition induced by changes in temperature, pH, ionic strength and solvent, Kopeček's group studied engineered coiled coil sequences as crosslinkers in synthetic polymer chains. For the primary chains of their hybrid hydrogels,[40] a linear hydrophilic copolymer of *N*-(2-hydroxypropyl)-methacrylamide (HPMA) and a metal-chelating monomer *N*-(*N'*,*N'*-dicarboxymethylaminopropyl)methacrylamide (DAMA) were prepared by radical copolymerization. A metal complex was formed by the pendant metal-chelating ligand-iminodiacetate (IDA)-Ni^{2+} and the terminal histidine residues (His tag) of the coiled coils. Using this approach, genetically engineered His-tagged coiled coils were connected to the polymethacrylamide polymer in a convenient manner resulting in the formation of a hydrogel[40] (Figure 1.5). This hybrid system preserved the benefit of using synthetic polymer backbones that are well characterized, easy to manufacture and biocompatible. The temperature-responsiveness of the hybrid hydrogels[40] was investigated and the gel structural transition was found to be related to the temperature-induced conformational change in the coiled coil motif.

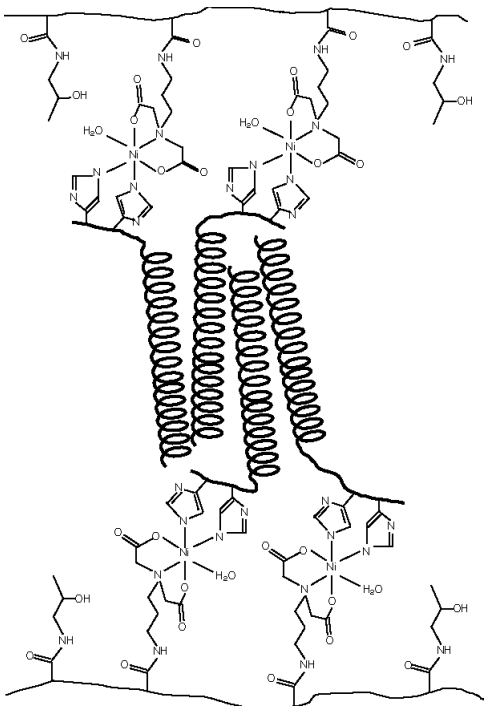


Figure 1.5. Structural representation of the hybrid hydrogel[40] primary chains and the attachment of His-tagged coiledcoil proteins.

Among others also Harden's and Tirrell's groups extensively explore the area of coiled coil-based hydrogels,[40] as reported in very recent works. As an example, in the former group the self-assembly of hydrogels[40] composed of acidic and basic leucine zipper associating domains and a soluble disordered coil block containing three copies of the Arg-Gly-Asp (RGD) integrin[43] binding sequence (vide infra) were studied.[44] The RGD sequences embedded in the disordered coil region supported the adhesion, spreading and polarization of human fibroblast cells on protein coated surfaces. Such hydrogel-forming bioactive proteins have potential for cell and tissue culture applications. In the latter group recombinant DNA methods were used to create artificial proteins that undergo reversible gelation in response to changes in pH or temperature.[45] The proteins consisted of terminal leucine zipper domains next to a flexible water soluble polyelectrolyte segment. Very recently, proteins bearing dissimilar helical coiled coil end domains were found to degrade much more slower than hydrogels[40] formed from those bearing the same end domains.[46] The mild conditions under which gel formation can be achieved (near-neutral pH and near-ambient temperature) and the control over the erosion rate suggest that these materials have potential in bioengineering applications for encapsulation or controlled release of drugs.

Although the current research efforts have already led to an enhanced understanding of the criteria that govern the assembly processes in amphiphilic peptides, due to the complexity of these processes, more investigation is required to gain a better insight into the way aggregation and peptide secondary structure influence each other. The construction of tailor made self-assembling peptides, with high levels of structural and functional control has a high potential, especially in the biomedical and materials science fields. Therefore the activities within this area have been intensified considerably with the aim to prepare functional types of amphiphilic peptide architectures.

Amphiphilic peptides as self-assembling smart nanometer-scale building blocks have an extensive potential because sequence manipulations enable the specific fabrication of a vast number of different structures that can be developed for many important applications, ranging from chemistry to material science and engineering (i.e. nanomaterials have been successfully employed in catalysis, tissue repair, patterning and for the preparation of optical and electronic devices). Finding specific methodology to build "smartness" into peptide-based nanomaterials in order to incorporate special responsiveness is a particularly fascinating emerging area. In the next part of the introduction several examples of recent innovations which incorporate "smart" peptides into tuneable hybrid materials will be discussed, demonstrating the multifunctional role of amphiphilic peptide-based nanoarchitectures.

1.2 General Synthetic Strategies

From a chemical perspective, recent advances in synthetic strategies have allowed the preparation of (poly)peptides and (poly)peptide hybrids, which are able to assemble in a controlled fashion into supramolecular architectures and materials that mimic the structure and function of proteins in a controlled manner. These synthetic methods can be divided in three main classes: solid-phase synthesis, protein engineering and ring-opening polymerization.[47]

Solid-phase peptide synthesis (SPPS) is a powerful method for the preparation of small to medium-sized peptides. In contrast to the ring-opening polymerization, SPPS allows the preparation of monodisperse peptides with precise control of the primary structure. Although the yields in each of the reaction steps are very high (>98%), they are not quantitative, resulting in the formation of shorter sequences. The concentration of undesired side products increases exponentially with chain length. As a consequence, small and medium-sized peptides are easily obtained in high yields and purities, however, yields rapidly

drop and purification becomes more difficult with increasing chain length. One way to overcome the limitations of SPPS with respect to the size of the peptides that can be prepared is the thioester-mediated native chemical ligation (NCL)[31] of unprotected peptide segments. This method relies on the chemoselectivity of the reaction between a peptide-α-thioester and another segment containing an *N*-terminal cysteine residue. NCL has been successfully used for the total chemical synthesis of a variety of proteins. Recently, protocols have been developed that allow NCL reactions to be carried out on solid supports. This procedure does not only simplify the purification of the resin-bound product, but also provides a facile route for the synthesis of proteins composed of a larger number of peptide segments. SPPS is also useful for the synthesis of hybrid molecules containing, for example, hydrophobic alkyl or polymeric chains conjugated to peptide sequences. In some cases, the solid-phase supported synthesis, purification and analysis of these amphiphilic (poly)peptides is highly challenging. Strategies were developed to facilitate the synthesis of "difficult peptide sequences" (i.e. amyloid peptides), by integrating defined structure defects into peptides.[48] In a recent example reported by Hentschel *et al.,*[49] a reversible ester segment was temporarily introduced in the peptide sequence in order to disrupt the amidic backbone. The "switch" ester was obtained by modification of the standard Fmoc protocols using a Boc-protected threonine derivative with an unprotected hydroxyl side chain functionality (Boc-Thr-OH), followed by the coupling of Fmoc-Val-OH to the β-hydroxyl group, disrupting the amidic backbone and therefore suppressing the aggregation tendency. The peptide backbone was subsequently re-established via a selective pH dependent rearrangement (O → N acyl switch).

The synthesis of polypeptides by bacterial expression of artificial genes, also referred to as protein engineering, is a very attractive strategy, since it allows the preparation of high molecular weight and perfectly monodisperse polypeptides with a precisely defined primary structure.[50] Protein engineering has been successfully used for the synthesis of natural structural proteins such as silk, collagen and elastin as well as for the preparation of *de novo* designed proteins. Protein engineering is not restricted to proteogenic α-amino acids and various methods are available that allow the incorporation of unnatural analogues.[51] In this way, it has become possible to prepare artificial proteins that carry a variety of non proteogenic functional groups. The growing improvements in expression of recombinant protein polymers allowed to expand the use of protein-based biomaterials both in the investigation of basic cellular processes and in therapeutic applications.[52]

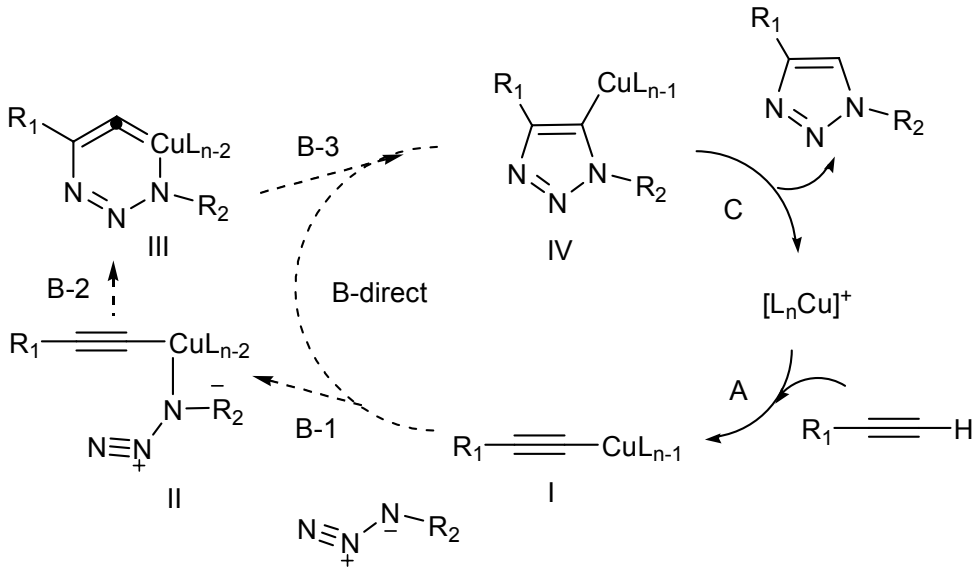
Polypeptides can also be prepared by ring-opening polymerization of α- and β-amino acid *N*-carboxyanhydrides (NCA)[53] and β-lactams[54] affording poly(amino acid)s. Generally, the ring-opening polymerizations can be performed following well-established protocols, thus

allowing a straightforward and high yielding synthesis of polypeptides with a broad molecular weight range at large scale. On the other end, using ring-opening polymerization strategies it is not possible to control the exact primary peptide sequence, in contrast to the solid phase protocol. Polydispersities have been achieved as low as 1.03.[55] However, high purity monomers are required. In addition, side reactions may occur, which complicate the preparation of optically pure polypeptides with predictable molecular weights and narrow molecular weight distributions, also hampering the formation of well-defined block copolymers. A number of these drawbacks have been overcome by using transition metal initiators including for example nickel[37,39] and cobalt[56] complexes.

A versatile methodology to prepare peptide-based hybrid polymeric biomaterials exploits the atom transfer radical polymerization (ATRP), which allows the synthesis of polymers with well-controlled molecular weight and molecular weight distributions without the strict requirements on a water- and oxygen-free environment necessary for other types of living polymerizations.[57] ATRP has been shown to be able to polymerize a wide range of bioinspired monomers such as peptide-based monomers that have been used to synthesize block copolymers either in solution[58] or on solid-support.[59] In a recent example, Ayres *et al.*[60] used ATRP to polymerise a monomer based on the cyclic decapeptide gramicidin S, bearing a methacrylate moiety at the side chain of an hydroxy-proline residue (which was introduced at the place of one of the proline residues in the β-turn). Gramicidin S is a large cyclic peptide that is well known for its antibiotic properties[61] (vide infra) and its ability to form inter- and intramolecular β-sheets.[62] Interestingly, the ability to achieve controlled polymerization of such a bulky, biologically relevant, peptide-based monomer represents a new approach towards the preparation of well-defined, antimicrobial polymeric biomaterials.

Several other chemical strategies have been used in order to synthesize conjugates of non-peptidic segments, such as lipid or polymer tails, to peptides. Connectivity has also been achieved for example using amide[63] or thiol-maleimide coupling[64,65] as well as by imine[66] or hydrazone linkage. A recent approach exploits the use of the [3+2] cycloaddition[30,67] between azides and alkynes as a highly useful chemical handle for conjugation either in a non copper-mediated[68,69] or copper(I)-catalyzed[70,71,72] manner. The so called “click” chemistry has recently emerged as a powerful tool for the synthesis of bioconjugates mainly due to the fact that the copper(I)-catalyzed [2+3] cycloaddition compared to the non-catalyzed reaction, occurs with dramatic rate increase and exclusive regioselectivity in aqueous media and at room temperature.[73]

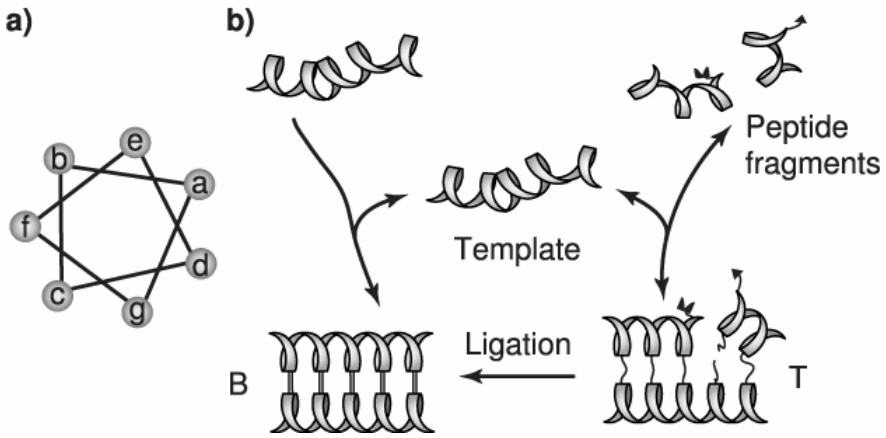
The proposed mechanism is described in Scheme 1.1.[73,74]



Scheme 1.1. Proposed mechanism of the catalytic cycle of the copper(I)-catalyzed Huisgen's 1,3-dipolar cycloaddition.

The sequence begins with the coordination of the alkyne to the Cu(I) species, displacing one of the ligands (step A). With water as a ligand the displacement process is exothermic, in good agreement with the experimental observation that the reaction proceeds much faster in aqueous solutions and does not require an amine base. Conversion of the alkyne to the acetylide **I** is well known to be involved in many C-C bond forming reactions in which Cu acetylide species are intermediates. Extensive density functional theory calculations[74a] offer compelling evidence that strongly disfavours the concerted [2+3] cycloaddition (B-direct) and points to a stepwise, annealing sequence (B-1 → B-2 → B-3). The azide replaces one of the ligands and binds to the copper atom via the nitrogen proximal to carbon, forming intermediate **II**. After that, the distal nitrogen of the azide in **II** attacks the C-2 carbon of the acetylide, forming the unusual six-membered copper(III) metallacycle **III**. From **III**, the barrier for ring contraction, which forms the triazolyl-copper derivative **IV**, is very low. Proteolysis of **IV** releases the 1,4-disubstituted 1,2,3-triazole product, thereby completing the catalytic cycle.

Another interesting synthetic strategy is related to self-replication. Self-assembly of peptides has been used as a driving force for chemical catalysis in amide bonds formation, resulting in self-replication.[75] The discovery that peptides have replicative properties has led to the design of a wide range of autocatalytic systems based on the coiled coil motif and exploiting the Kent's native chemical ligation[31] (Scheme 1.2).



Scheme 1.2. Self-replication cycle based on the coiled coil motif. (a) Helical wheel representation of a coiled coil peptide showing the heptad repeat. (b) The reaction cycle for a self-replicating peptide with its fragments.

In self-replication, the product (e.g. peptide) acts as a template to preorganize precursors (e.g. peptide fragments) based on its own sequence in order to catalyze the formation of the product itself. As a result, a new product is formed that is identical to the template. The template's ability to form a ternary complex with the two peptide fragments is mediated by interhelical hydrophobic interactions which, in turn, may promote the chemical ligation process. After dissociation, the newly synthesized peptide could then act as a template for a new set of fragments, resulting in exponential product growth. Lately, improvement in the design of self-replicating peptides have been directed towards the enhancement of the autocatalytic efficiency.[76] Ideas have also been discussed to promote the use of self-replicating peptides in novel biomaterial and biomedical applications.[77]

1.3 Applications of Peptide-Based Nanostructures

The advances in synthetic tools allow unprecedented control over composition, structure, and organization of artificial proteins and peptide hybrid materials. Undoubtedly, these developments and future advances will allow the integration of biological design concepts in materials science and lead to protein-inspired new materials.[78] Applications of self-assembling peptide systems as simple and versatile molecular building blocks can provide new opportunities in biotechnology and engineering. An overview of the possible applications is given in the following sections of this introduction.

1.3.1 Nanoreactors and Catalysts

Amphiphilic peptides belong to the class of low-molecular weight amphiphiles (typical examples are phospholipids with average molecular volume ~ 0.5 nm^3 and molecular weight ~ 1 kDa). A more recently introduced class of super-amphiphiles consists of hydrophilic-hydrophobic block-co-copolymers (e.g. diblock polymer of polystyrene and a polyisocyanopeptide with molecular volume ~ 6.5 nm^3 and molecular weight ~ 6 kDa). Combining synthetic polymers with enzymes as head group could lead to the development of a new class of giant amphiphiles with catalytic properties. In terms of both molecular volume and molecular weight the next generation of amphiphiles, the giant amphiphiles, are considerably larger than their low-molecular weight and polymeric counterparts (e.g. the n = 40 polystyrene-lipase biohybrid with molecular volume ~ 25 nm^3 and molecular weight ~ 40 kDa). Velonia *et al.*[79] coupled the enzyme Lipase B from *Candida Antarctica* to an end maleimido-modified polystyrene. Remarkably, these giant amphiphiles self-assembled into fibres in a comparable manner to their low-molecular weight counterparts upon dispersion in aqueous solutions. It was proposed that the fibres were composed of micellar rods with a hydrophobic polystyrene core with the protein, acting as the hydrophilic head group, exposed to the aqueous environment. However, it was found that the catalytic activity of the enzyme was reduced 15-fold, which was ascribed to a destabilizing effect of the hydrophobic polystyrene tail on the active conformation and a possible lower accessibility of the active site. A better understanding of the rules governing the assembly of these giant amphiphiles could lead to improvement in their activities.

Stimulus-responsive polymersomes[38] based on polybutadiene-*b*-poly(γ-L-glutamic acid) were investigated as possible nanoreactors.[80] The size of the polymersome[38] molecules could be reversibly altered by changing both the pH and the ion strength. Vriezema *et al.*[81] described the encapsulation of *Candida Antarctica* lipase B (CAL B) enzymes inside polymersomes[38] of polystyrene-*b*-poly(isocyano-L-alanine(2-thiophen-3-yl-ethyl)amide) (PS-PIAT). It was demonstrated that the enclosed CAL B enzymes were still active and that the polymersome[38] membrane was permeable to low molecular weight substrates, for example, 6,8-difluoro-4-methylumbelliferyl octanoate (DiFMU octanoate). Upon hydrolysis of the ester bond of this substrate, a fluorescent coumarin-type of product was formed, allowing the monitoring of the enzyme activity. Noteworthy, the membrane of the PS-PIAT vesicles was chiral and therefore potentially selective toward chiral substrates or chiral products.

1.3.2 RGD Functionalized Materials

The tripeptide motif Arg-Gly-Asp (RGD) sequence, so-called "universal recognition site", has been identified as minimal essential cell adhesion sequence.[82] Since its discovery, numerous materials have been RGD functionalized for academic or medical applications.[83] RGD-related peptides are known to contribute to various cellular functions such as adhesion, invasion and to inhibit tumor metastasis.[84] However, peptide-based drugs are generally rapidly hydrolyzed and eliminated from the bloodstream. In a recent example, RGD-modified liposomes were shown to enable the half-lives and affinity of the unmodified peptide, resulting in enhancement of antimetastatic activity.[85] Liposomal RGD was prepared using lipophilic derivatives of the peptide, which could easily be synthesized and incorporated into the liposomal bilayer. In an other example, Yagi *et al.*[86] have prepared liposomes whose surface is modified with peptides containing a five-time repeat of the GRGDS sequence, as found in the cell adhesion sequence of fibronectin. The peptide was lipidated by incorporation at the *N*-terminus of an aspartic acid residue modified with two C_{16} alkyl chains. The availability of the peptides on the surface was confirmed with immuno-electron microscopy studies, employing a specific antibody to the peptide that could be visualized with gold colloids. The liposomes were shown to bind mouse fibroblast cells and the association took place through interaction of the exposed peptide and the corresponding cell surface receptor. Most approaches to display RGD-containing peptides immobilize the peptide by covalently linking it through the *N*-terminus, leaving the carboxy-terminus free. It is a well-known fact, however, that the RGD sequence exists in a conformational constrained loop in proteins such as fibronectin. It has been demonstrated that cyclic peptides which contain the RGD sequence can display higher affinities than their unconstrained linear counterparts. Pakalns *et al.*[87] have shown that the same conformational constraint can be obtained by attaching doubly alkylated glutamic acid derivatives to both the *N*- and *C*-terminus. These peptide amphiphiles were utilized to prepare self-assembled monolayers which could be deposited as Langmuir–Blodgett films on a surface. On these surfaces functionalized with looped RGD amphiphiles, melanoma cells were able to spread in a concentration dependent manner. A similar approach has been followed to enhance the activity of the RGD sequence towards integrin[43] receptors. Marchi-Artzner *et al.*[88] have attached a cyclized RGD containing pentapeptide to lipid alkyl chains connected through a short ethylene glycol spacer. A supported membrane containing these amphiphiles selectively adhered to endothelial cells of the human umbilical cord. Moreover, giant vesicles functionalized with cyclic RGD peptides adhered to the same endothelial cells, a process that could be inhibited by adding the

corresponding soluble peptide. This suggests a specific interaction between the bilayer anchored peptide and the integrin[43] receptors of the cells.

1.3.3 Tissue Regeneration Materials

Culturing cells in three-dimensions has received a growing interest in the past few years.[89] To grow in 3D culture, cells need to be embedded in a structure that mimics the extracellular matrix (ECM) of structural proteins and other biological molecules found in real, living tissues. Many researchers use a commercially available material called Matrigel,[90] which consists of structural proteins such as laminin and collagen, plus growth factors and enzymes, all taken from mouse tumours. Matrigel displays lower critical solution temperature (LCST) and it is a liquid below 4 °C. Cells can easily be mixed in it and find a place to grow in the porous solidified gel obtained upon gently warming.

Zhang and co-workers[91] have developed a series of amphiphilic peptides that form stable hydrogels[40] at low peptide concentrations (0.1-1%). They are characterized by an alternating sequence of hydrophobic and hydrophilic residues, in which the hydrophilic residues, in turn, alternate between being positively and negatively charged, such as in $(KLDL)_n$, $(EAKA)_n$ and $(RADA)_n$. The alternation between polar and non-polar residues promoted the formation of a β-strand building block with hydrophobic and hydrophilic faces. The self-assembly process to produce a hydrogel[40] can be triggered rapidly when the ionic strength exceeded a certain threshold or the pH was adjusted to provide a zero net charge on the peptide. These types of peptides have been shown to be non-cytotoxic and of potential use in the repair of cartilage tissue. Chondrocytes were encapsulated within the hydrogel[40] scaffold produced by the peptide Ac-$(KLDL)_3$-$CONH_2$.[92] The scaffold was shown to maintain differentiated chondrocytes and to stimulate the synthesis and accumulation of extracellular matrix.

In another example, self-assembling peptides are being developed as scaffolds for tissue regeneration purposes, including cartilage repair and promotion of nerve cell growth.[93] A major benefit of synthetic materials is that they minimize the risk of biological contamination. Self-assembling peptides also frequently show favorable properties concerning biocompatibility, immunogenicity and biodegradability, producing non-toxic waste products. Laminin is an extracellular matrix protein that influences neurite outgrowth. A peptide amphiphile shown to promote the re-growth of nerve cells in rats was made by including a neurite-promoting laminin epitope tag, IKVAV. Another construct, containing a heparin-binding site, showed preliminary results in being able to promote angiogenesis, the

growth of blood vessels. These types of peptide amphiphiles have been further modified with biotin and a Gd^{3+} metal-chelating moiety suitable for detection by magnetic resonance imaging (MRI).

1.3.4 Drug-Delivery Vehicles

Molecular and supramolecular drug-delivery systems have attracted great attention as pharmaceutical formulations for the administration of otherwise only poorly soluble, rapidly degradable, or even toxic molecules. Synthetic carriers, including peptide-based delivery systems, have been developed in several laboratories as promising alternatives to using inactivated viruses as gene vectors. Peptide-based vectors are particularly amenable to rational

design and development in this field due to their exceptional adaptability. Zhang *et al.*[94] developed a series of surfactant peptides comprising a hydrophobic tail attached to a polar head group consisting of one to two positively charged residues at the *C*- or *N*-terminus, one example being LLLLLLKK. These peptides self-assemble in water to produce nanovesicles and nanotubes that have been used as DNA delivery vehicles. When placed in a solution of DNA, the positively charged peptides self-assembled into a tube, encapsulating the negatively charged DNA. In some cases, the DNA was delivered to growing cells. Keller *et al.*[95] introduced a dipalmitoylated cell penetrating peptide as a potent lead for the intracellular delivery of hydrophobic drugs using bilayer-mimetic nanocarriers. These supramolecular vehicles share unique properties, as they combine the advantages of both liposomes and micelles offering a hydrophobic environment, given by the palmitoyl tails, in spite of their small size. *In vitro* experiments demonstrated the rapid cellular internalization of a carboxyfluorescein-labeled derivative of the amphiphilic peptide into immortalized mouse brain capillary endothelial cells as visualized by confocal laser scanning microscopy.

Bellomo *et al.*[96] recently published on pH-sensitive polymersomes[38] based on a diblock copolypeptide. Stimuli responsive release studies were demonstrated by fluorescence. A Fura-2 dye was entrapped in the vesicles in the presence of external calcium and a frequency shift for maximum emission intensity was observed when the pH dropped from 10.6 to 3.0, demonstrating the calcium binding by the dye upon disruption of the bilayer.

1.3.5 Antimicrobial Biomaterials

Fatty acid conjugation may improve the usefulness of peptides as antimicrobial agents by enhancing their ability to form secondary structures upon interacting with the bacterial membranes. In a work reported by Tirrell and co-workers,[97] three peptides, YGAA[KKAAKAA]$_2$ (AKK), KLFKRHLKWKII (SC4), and YG[AKAKAAKA]$_2$ (KAK), were conjugated with lauric acid and tested for the effect on their structure, antibacterial activity, and eukaryotic cell toxicity. The currently accepted model for the mechanism of action of helical antimicrobial peptides involves peptides that are typically unfolded in solution but fold in the presence of membranes to form an amphipathic structure.[98] The formation of an amphipathic structure seems to be required for membrane binding and lytic activity.[99] When the concentration of peptide bound to the membrane reaches a critical level, the peptides either insert into the membrane to form pores, or destabilize the membrane by disrupting the its curvature.[100] The conjugated AKK and SC4 peptides showed increased antimicrobial activity relative to unconjugated peptides, but the conjugated KAK peptide did not. The circular dichroism spectrum of AKK showed a significantly larger increase in its α-helical content in the conjugated form compared to KAK in a vesicle solution composed of phosphatidylethanolamine/phosphatidylglycerol, mimicking bacterial membranes. The KAK and AKK peptides and their corresponding fatty acid conjugates showed little change in their structure in the presence of phosphatidylcholine vesicles, which mimic the cell membrane of eukaryotic cells. The hemolytic activity of the KAK and AKK peptides and conjugates was low. However, the SC4 fatty acid conjugate showed a large increase in hemolytic activity and a corresponding increase in helical content in the presence of phosphatidylcholine vesicles. These results support the model of antimicrobial peptide haemolytic and antimicrobial activity being linked to changes in secondary structure as the peptides interact with lipid membranes.

1.3.6 Templates for Mineralization

Amphiphilic peptide-based systems can be used to create a new class of materials at the molecular scale using self-assembly with a high impact in several fields of research. One example is the controlled mineralization of inorganic crystals.

Hartgerink *et al.*[101] exploited the self-assembly of single alkyl chain amphiphilic peptide for the preparation of tailor-made nanostructured composite materials suited for controlled biomineralization towards bone-like materials.

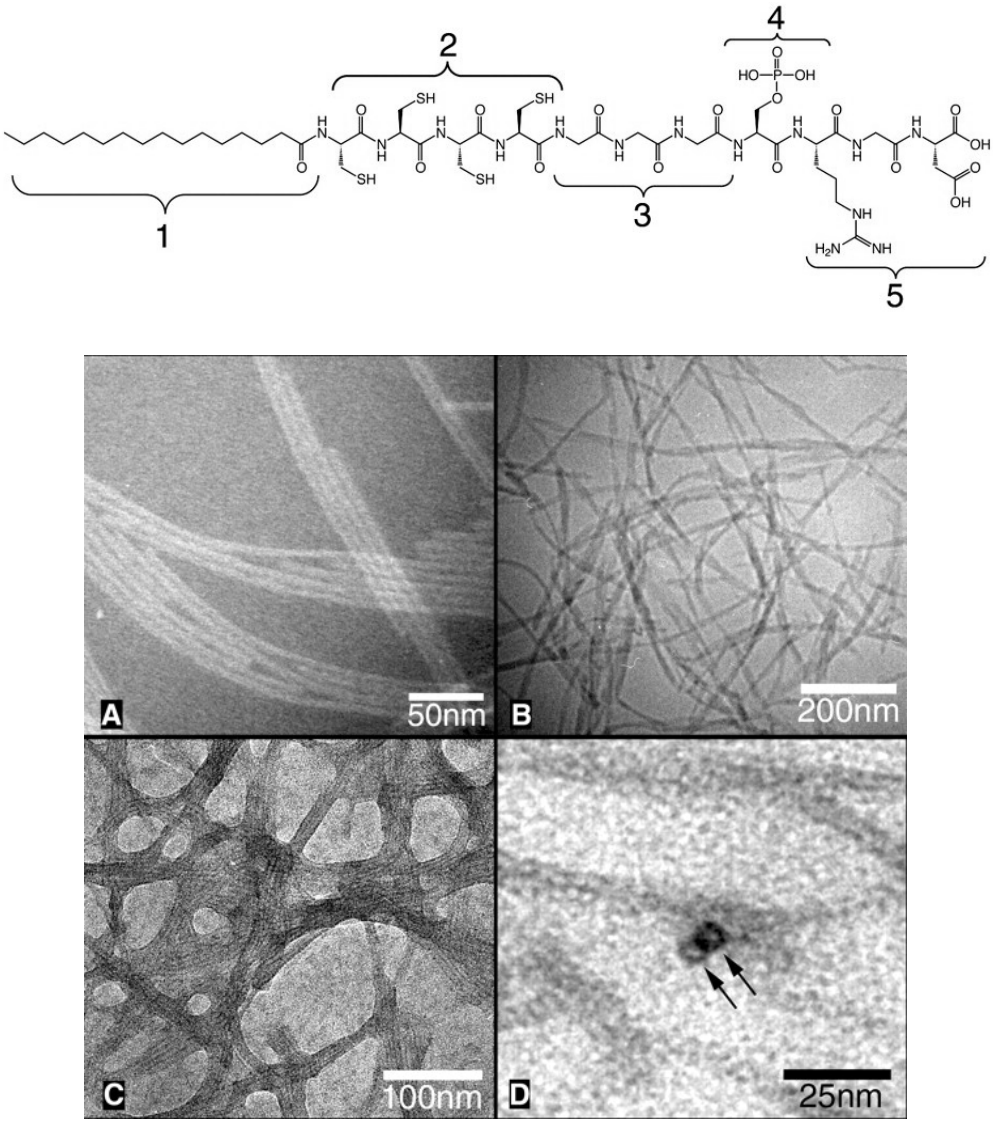


Figure 1.6. (top) Chemical structure of the peptide amphiphile, highlighting the key structural features. Region 1 is a long alkyl tail that conveys hydrophobic character to the molecule and, when combined with the peptide region, makes the molecule amphiphilic. Region 2 is composed of four consecutive cysteine residues that when oxidized may form disufide bonds to polymerize the self-assembled structure. Region 3 is a flexible linker region of three glycine residues to provide the hydrophilic head group flexibility from the more rigid crosslinked region. Region 4 is a single phosphorylated serine residue that is designed to interact strongly with calcium ions and help direct mineralization of hydroxyapatite. Region 5 displays the cell adhesion ligand RGD. (Bottom) (**A**) Negative stain (phosphotungstic acid) TEM of the self-assembled nanofibers before covalent capture. Fibers are arranged in ribbon-like parallel arrays. (**B**) Vitreous ice cryo-TEM of the fibers reveals the diameter of the fibers in their native hydrated state to be 7.661 nm. (**C**) Positive stain (uranyl acetate) TEM of the self-assembled nanofibers after oxidative crosslinking shows electron dense regions due to the stain that localized on the periphery of the fibers. (**D**) Thin section TEM of positively stained (uranyl acetate) nanofibers after oxidative crosslinking and embedding in epoxy resin. Two fibers are observed in cross section (arrows), showing the lack of staining in the interior of the fiber.

The peptide was carefully designed bearing five different regions in order to introduce important features such as the hydrophobic character for promoting self-assembly imparted by the alkyl chain (Figure 1.6). Cysteines were introduced to stabilize the aggregates, while the glycines inferred some flexibility between the crosslinked area and the hydrophilic head group. The phosphorylated serine could interact strongly with calcium ions to direct mineralization and finally an RGD sequence was attached as a cell adhesion ligand. This design allowed the fibers to be susceptible to pH-dependent crosslinking. At a pH of 8 and with all cysteines reduced, the peptide amphiphiles were highly soluble. Upon acidification the compound became insoluble forming a fiber network stabilized by formation of disulfide bonds through oxidation and this process was found to be reversible. Mineralization of hydroxyapatite was directed by the obtained structure, forming a composite material with an alignment that is also observed between collagen fibrils and hydroxyapatite in bone. This highly dynamic amphiphilic peptide system appears to be an ideal scaffold for biomineralization and tissue engineering applications.

The group of Kelly[102] investigated the use of peptidomimetics that assembled into 2D β-hairpin monolayers at the air-water interface for the nucleation of CdS nanocrystals via the {01.0} face. From this study it appeared that the degree of lattice matching played a major role in controlling the face nucleated and the crystallite size. Volkmer *et al.*[103] employed amphiphilic peptides, comprising alternating hydrophilic (Asp) and hydrophobic (Phe) amino acid residues, as acidic peptides designed to imitate the epitopes of acidic proteins from calcified tissues. $CaCO_3$ crystallization experiments have shown that the amphiphilic peptides H–(Phe–Asp)$_2$–OH and H–(Phe–Asp)$_4$–OH specifically interact with distinct crystal faces of calcite. The morphological features of calcite crystals grown in their presence were very similar to calcite crystals grown from solutions containing natural acidic proteins.

In this journey from chemistry to biology and materials science, molecular self-assembling peptides pave the way to the next frontier: new technologically relevant engineered nanostructures. Recent advancements in patterning technologies have significantly enhanced the ability to spatially control surfaces at the micrometer level. Common patterning methods include photolithography[104] and soft lithographic approaches such as microcontact printing,[105] microfluidic patterning[106] and micromolding.[107] These techniques have been widely used for the high fidelity patterning of rigid substrates, such as modified silicon or glass[108] and recently of deformable, solvated, biocompatible platforms such as hydrogels.[40] In this way well-defined surfaces have been prepared, which can be useful for the construction

of photosensitive switches, nanowires or light-harvesting devices. Finally, an interesting emerging field exploits the use of peptide-based well-defined structures for the fabrication of molecular machines or robots.

1.3.7 Photosensitive Switches and Nanowires

Chemical strategies to build photosensitive switches into peptides as modulators of structure have been pioneered by Bredenbeck *et al.*[109] They have shown that the inclusion of various azobenzene derivatives as crosslinkers of amino acid residues at well-defined positions can modulate helix formation upon irradiation with light of specific frequencies. This concept has been applied to the regulation of tertiary structures, including coiled coils and also provides a means for studying the kinetics of protein folding. Inclusion of such a switch in biomaterials designs would be useful in creating photo-responsive materials.

The possibility of fabricating conducting nanowires by molecular means using peptide scaffolds is of particular interest to the electronics industry. Nanotubes made from self-assembling peptides are used as templates for mineralization of metals interesting for conductive purposes (e.g. copper). Once the organic scaffold has been removed, a pure conducting wire, immobilized on a surface, is left behind. Matsui and co-workers[110] have been able to prepare nanowires of copper and nickel from peptide nanotubes. Reches and Gazit[111] have demonstrated that the short dipeptide $(Phe)_2$ was able to form nanotubes, which were removed either enzymatically, chemically or through heat burning after diffusion of silver ions into the tubes, followed by the silver reduction with citric acid leaving silver wires.

Schreibel *et al.*[112] described the use of self-assembling amyloid protein fibers to construct nanowire elements. Self-assembly of a prion determinant from Saccharomyces cerevisiae, the *N*-terminal and middle region (NM) of Sup35p, produced 10-nm-wide protein fibers that were stable under a wide variety of conditions. The length could be varied in the range of 60 nm to several hundred micrometers by controlling the conditions during assembly. A genetically modified NM variant that presented reactive, surface accessible cysteine residues was used to covalently link NM fibers to colloidal gold particles. These fibers were placed across gold electrodes and additional metal was deposited by highly specific chemical enhancement of the colloidal gold by reductive deposition of metallic silver and gold from the corresponding salts. These biotemplated metal wires, with a diameter of ~ 100 nm, demonstrated the conductive properties of a solid metal wire, such as low

resistance and ohmic behavior. With such materials it should be possible to harness the extraordinary diversity and specificity of protein functions to nanoscale electrical circuitry.

1.3.8 Molecular Machines

Finally, an exciting emerging field is the development of molecular machines or robots that can be turned on and off in response to a signal. Most of this work has, thus far, been carried out using large proteins (i.e. motors proteins such as myosin, kinesins and dyneins) or small interlocked catenane- and rotaxane-based organic molecules and was recently described in a review by Kinbara *et al.*[113] To investigate the influence of supramolecular architecture on biomotor cooperativity, the group of Tirrell[114] engineered a model multimotor system that allows the precise regulation of intermotor coupling. The model was composed of a rigid block, comprised of strongly associated acidic and basic leucine zipper domains that anchored motor proteins at specific distances along the polymer backbone. The artificial protein scaffolds incorporated a basic zipper into the polymer backbone, whereas the complementary acidic zipper was fused to the *C*-terminus of a truncated kinesin-1 motor. The flexible polymer block was derived from the elastomeric poly(VPGV$_\alpha$G) structural motif of the protein elastin and conferred well-characterized mechanical compliance on the assembly. Every fifth α-valine residue was replaced by a phenylalanine residue in the elastin sequence. This substitution provided a means to control the thermoresponsive behavior of the polymers, as discussed in more detail below. Variation in the number of diblock repeats in the polymer provided discrete control over the number of coupled motors, which in the present series of experiments ranged from one to three. The *C*-terminus of each scaffold was labelled with biotin to allow the motor assemblies to be tethered to streptavidin coated surfaces. These results indicated that the artificial proteins provided a structural framework that allowed motors to push and pull on one another to enhance activity through control of the supramolecular architecture of multimotor assemblies, providing a means to reconfigure mechanisms of collective biomotor transport.

Supramolecular chemistry in general aims to construct defined structures based on non-covalent interactions. The manner in which molecules self-assemble and their interaction energies, shapes and ultimate functions can be programmed at the molecular level. Examples illustrated herein have shown that amphiphilic peptide-based hybrids can be used to construct nanometer-scale structures. Challenging is to *a priori* design molecules that self-assemble in a predictive manner into targeted three-dimensional supramolecular structures. With this ability

in hand, the next step is to incorporate function. The increasing demand for miniaturization in both academia and industry necessitates progress and addresses questions to all scientific disciplines, from chemistry to biology, physics and materials science, pointing out the cross-disciplinary role of peptide-based amphiphiles.

1.4 Aim and Outline of the Thesis

The first part of the research described in this thesis aimed to synthesize and investigate the physical properties of a new series of amphiphilic lipopeptides (ALPs), comprising of an alternating hydrophilic and hydrophobic amino acid residue sequence coupled to a phospholipid tail (DOPE) to form supramolecular assemblies composed of β-sheet arrays decorated by lipid tails. In **Chapter 2** the straightforward synthetic approach based on solid phase synthesis, followed by an efficient purification protocol to prepare the lipid-peptide conjugates (tetra-ALP, hexa-ALP and octa-ALP) is described. Structural insight into the organization of the amphiphilic lipopeptides into β-sheet monolayers at the air-water interface is provided by surface pressure *versus* area (π-A) isotherms, circular dichroism (CD), Fourier transform infrared spectroscopy (FTIR) and Brewster angle microscopy (BAM). In-situ grazing-incidence X-ray diffraction (GIXD) studies reveal that lipopeptides, consisting of six or eight amino acids residues, form a new type of two-dimensional self-organized monolayers that exhibit β-sheet ribbons segregated by lipid tails. Furthermore, the conclusions drawn from the experimental findings are supported by a representative model based on molecular dynamics simulations of an ALP at the vacuum-water interface. **Chapter 3** describes the application of ALP monolayers as well-defined two-dimensional templates for the nucleation of calcium carbonate. The influence of the lenght of the peptide head group on the biomineralization is investigated. For comparison, non-lipidated peptides are studied as well. The introduction of the phospholipid moiety to the octapeptide $(Leu\text{-}Glu)_4$ motif is shown to enhance the amphiphilic behavior of the molecule and also to increase the flexibility of the monolayer, without compromising the β-sheet structure. This leads to a distinct change in the templating behavior and the differences in the ability of templates to interact with the developing mineral phase are discussed. In **Chapter 4** the assembly of the ALPs upon dispersion in aqueous media is studied. The ability to obtain well-defined three-dimensional structures which exhibit on their surface the well-defined two-dimensional β-sheet architecture of the monolayer, elucidated in Chapter 2, is investigated. The formation of β-sheet fibers in solution and upon deposition of the monolayer of octa-ALP on solid support (mica) is visualized by atomic force microscopy (AFM) and electron microscopy

(EM). Spherical structures are observed when octa-ALP is mixed with 1,2-Dioleoyl-sn-Glycero-3-Phosphoethanolcholine (DOPC). The coexistence of spherical and elongated ribbons complicated the CD analysis. However, the β-pleated sheet folding is shown to be dominant. A simpler method to prepare lipid vesicles decorated by β-sheet peptides is the post-modification of liposomes. An example of this approach is given in **Chapter 5**. In this chapter, a new generic method for the efficient *in situ* modification of liposome surface is described. The [2+3] cycloaddition, Cu(I) catalyzed, between azide and acetylene moieties forming a 1,4-disubstituted 1,2,3-triazole ring ("click" reaction) is studied for this purpose. Furthermore, a simple colorimetric assay is developed for monitoring the reaction. CD spectroscopy is used to follow the reaction in time, exploiting the random coil to β-sheet conformational change in the $(Leu\text{-}Glu)_4$ motif due to the conjugation to the surface of liposomes. The versatility and scope of this chemical approach for surface modification of vesicles is described and also investigated for non-peptidic biologically relevant compounds. As an example, immunogenic liposomes were prepared by "clicking" toll-like receptor ligands to the vesicle outer membrane. Ultimately, the work described in **Chapter 6** combines the synthesis of a lipidated Gramicidin S (GS) analogue and the structural evaluation of the monolayers of lipidated and non-lipidated GS analogues at the air-water interface. Furthermore, monolayers are transferred on microscope glass slides towards the development of antimicrobial surfaces.

1.5 References and Notes

[1] a) Whitesides, G. M.; Grzybowski, B. *Science* **2002**, *295*, 2418-2421. b) Whitesides, G. M.; Mathias, J. P.; Seto, C. T. *Science* **1991**, *254*, 1312-1319. d) Lehn, J. M. *Science* **2002**, 295, 2400-2403.

[2] Feynman. R. *Engineering and Science* **1960**, 22-36.

[3] a) Fairman, R.; Åkerfeldt, K. S. *Curr. Opin. Struc. Biol.* **2005**, *15*, 453-463. b) Rajagopal, K.; Schneider, J. P. *Curr. Opin. Struc. Biol.* **2004**, *14*, 480-486.

[4] a) Chen, P. *Colloids and Surfaces A: Physicochem. Eng. Aspects* **2005**, *261*, 3-24. b) Zhao, X.; Zhang, S. *TRENDS Biotechnol.* **2004**, *22*, 470-476. d) Venkatraman, J.; Shankaramma, S. C.; Balaram, P. *Chem. Rew.* **2001**, *101*, 3131-3152.

[5] Pagel, K.; Vagt, T.; Koksch, B. *Org. Biomol. Chem.* **2005**, *3*, 3843-3850.

[6] Cornelissen, J. J. L. M.; Rowan, A. E.; Nolte, R. J. M.; Sommerdijk, N. A. J. M. *Chem. Rew.* **2001**, *101*, 4039-4070.

[7] a) Gao, X.; Matsui, H. *Adv. Mater.* **2005**, *17*, 2037-2050. b) Zhang, S.; Marini, D. M.; Hwang, W.; Santoso, S. *Curr. Opin. Chem. Biol.* **2002**, *6*, 865-871.

[8] Löwik, D. W. P. M.; van Hest, J. C. M. *Chem. Soc. Rev*. **2004**, *33*, 234-245.

[9] Vandermeulen, G. W. M.; Klok, H.-A. *Macromol. Biosci.* **2004**, *4*, 383-398.

[10] Zhang, S. *Nat. Biotechnol.* **2003**, *21*, 1171-1178.

[11] Zuo means left in Chinese while tin means protein in biology. Zuotin is a yeast protein that was initially identified for its ability to bind preferentially to left-handed Z-DNA.

[12] Zhang, S.; Holmes, T.; Lockshin, C.; Rich, A. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 3334-3338.

[13] Zhang, S.; Altman, M. *React. Funct. Polym.* **1999**, *41*, 91-102.

[14] Rajagopal, K.; Ozbas, B.; Pochan, D. J.; Schneider, J. P. *Eur. Biophys. J.* **2006**, *35*, 162-169.

[15] Rapaport, H.; Kjaer, K.; Jensen, T. R.; Leiserowitz, L. Tirrell, D. A. *J. Am. Chem. Soc.* **2000**, *122,* 12523-12529.

[16] Rapaport, H.; Möller, G.; Knobler, C. M.; Jensen, T. R.; Kjaer, K.; Leiserowitz, L. Tirrell, D. A. *J. Am. Chem. Soc.* **2002**, *124,* 9342-9343.

[17] Sneer, R.; Weygand, M. J.; Kjaer, K.; Tirrell, D. A.; Rapaport, H. *ChemPhysChem* **2004**, *5*, 747-750.

[18] Bong, D. T.; Clark, T. D.; Granja, J. R.; Ghadiri, M. R. *Angew. Chem. Int. Ed.* **2001**, *40*, 988-1011.

[19] Ghadiri, M. R.; Granja, J. R.; Milligan, R. A.; McRee, D. E.; Khazanovich, N. *Nature* **1993**, *366*, 324-327.

[20] a) Forns, P.; Lauer-Fields, J. L.; Gao, S.; Fields, G. B. *Biopolymers* **2000**, *54*, 531-546. b) Yu, Y.-C.; Tirrell, M.; Fields, G. B. *J. Am. Chem. Soc.* **1998**, *120*, 9979-9987.

[21] Su, J. Y.; Hodges, R. S.; Hay, C. M. *Biochemistry* **1994**, *33*, 15501-15510.

[22] No detailed information is available on what kind of aggregation were obtained, as these aggregates were not investigated by electron microscopy (EM) or atomic force microscopy (AFM).

[23] Löwik, D. W. P. M.; Linhardt, J. G.; Adams, P. J. H. M.; van Hest, J. C. M. *Org. Biomol. Chem.* **2003**, *1*, 1827-1829.

[24] Ludolph, B.; Eisele, F.; Waldmann, H *J. Am. Chem. Soc.* **2002**, *124*, 5954-5955.

[25] a) Kuhn, K.; Owen, D. J.; Waldmann, H.; Wittinghofer, A.; Kuhlmann, J. *Nature* **2000**, *403*, 223-226. b) Kuhn, K.; Owen, D. J.; Bader, B.; Wittinghofer, A.; Kuhlmann, J.; Waldmann, H. *J. Am. Chem. Soc.* **2001**, *123*, 1023-1035.

[26] Prenylation or isoprenylation or lipidation is the addition of hydrophobic molecules to a protein. It is usually assumed that prenyl groups facilitate attachment to cell membranes, similar to lipid anchor like the GPI anchor, though direct evidence is missing. Prenyl groups have been shown to be important for protein-protein binding through specialized prenyl-binding domains. Protein prenylation involves the transfer of either a farnesyl or a geranylgeranyl moity to *C*-terminal cystein(s) of the target protein. There are three enzymes that carry out prenylation in the cell. Farnesyltransferase and geranylgeranyltransferase I and Rab geranylgeranyltransferase, or Geranylgeranyl transferase II.

[27] a) Casey, P. J. *Science* **1995**, *268*, 221-225. b) Bhatnagar, R. S.; Gordon, J. I. *Trends Cell. Biol.* **1997**, *7*, 14-20. c) Methods in Enzymology Vol. 250: Lipid Modification of Proteins (Eds.: P. J. Casey, J. E. Buss), Academic Press, New York, **1995**. d) Ikezawa, H. *Biol. Pharm. Bull.* **2002**, *25*, 409-417. e) Kellam, B.; De Bank, P. A.; Shakesheff, K. M. *Chem. Soc. Rev.* **2003**, *32*, 327- 337.

[28] a) C. Tanford, The Hydrophobic Effect: Formation of Micelles and Biological Membranes, 2nd ed., Wiley, New York, 1980. b) Shahinian S.; Silvius, J. R. *Biochemistry* **1995**, *34*, 3813-3822. c) Lutz, J.; Romano-Götsch, R.; Escrieut, C.; Fourmy, D.; Mathä, B.; Müller, G.; Kessler, H.; Moroder, L. *Biopolymers* **1997**, *41*, 799-817.

[29] Musiol, H.-J.; Dong, S.; Kaiser, M.; Bausinger, R.; Zumbusch, A.; Bertsch, U.; Moroder, L. *ChemBioChem* **2005**, *6*, 625-628.

[30] Huisgen, R. In *1,3-Dipolar Cycloaddition Chemistry*; Padwa, A., Ed.; Wiley: New York, 1984; Vol. 1, pp 1-176.

[31] a) Dawson, P.E.; Muir, T. W. ; Clarklewis, I.; Kent, S. B. H. *Science* **1994**, *266*, 776-779. b) Otaka, A.; Ueda, S.; Tomita, K.; Yano, Y.; Tamamura, H.; Matsuzaki, K.; Fujii, N. *Chem. Commun.* **2004**, *15*,1722-1723.

[32] Rösler, A.; Klok, H.-A.; Hamley, I. W.; Castelletto, V.; Mykhaylyk, O. O. *Biomacromolecules* **2003**, *4*, 859-863.

[33] Smeenk, J. M.; Otten, M. B. J.; Thies, J.; Tirrell, D. A.; Stunnenberg, H. G.; van Hest, J. C. M. *Angew. Chem. Int. Ed.* **2005**, *44*, 1968-1971.

[34] Deming, T. J. *Adv. Mater.* **1997**, *9*, 299-311.

[35] Kros, A.; Jesse, W.; Metselaar, G. A.; Cornelissen, J. J. L. M. *Angew. Chem. Int. Ed.* **2005**, *44*, 4349-4352.

[36] In polymer chemistry, living polymerization is a form of addition polymerization where the ability of a growing polymer chain to terminate has been removed. Living polymerization is a popular method for synthesizing block copolymers since the polymer can be synthesized in stages, each stage containing a different monomer. Additional advantages are predetermined molar mass and control over end-groups.

[37] a) Deming, T. J.; Curtin, S. A. *J. Am. Chem. Soc.* **2000**, *122*, 5710-5717. b) Deming, T. J. *Nature* **1997**, *390*, 386-389.

[38] Polymersomes are bilayered membranes of amphiphilic synthetic polymers that are similar to liposomes, which use naturally occurring lipids. While having most of the properties of natural liposomes, polymersomes exhibit increased stability and reduced permeability. Furthermore, the use of synthetic polymers enables designers to manipulate the characteristics of these capsules, including controlled release.

[39] Nowak, A. P.; Breedveld, V.; Pakstis, L.; Ozbas, B.; Pine, D. J.; Pochan, D.; Deming, T. J. *Nature*, **2002**, *417*, 424-428.

[40] Hydrogel is a network of polymer chains that are water-soluble, sometimes found as a colloidal gel in which water is the dispersion medium. Hydrogels are superabsorbent (they can contain over 99% water) natural or

synthetic polymers. Hydrogels possess also a degree of flexibility very similar to natural tissue, due to their significant water content.

[41] a) Mason, J. M.; Arndt, K. M. *ChemBioChem* **2004**, *5*, 170-176. b) Wayne, D. K.; Cyril, M.K.; Hodges, R. S. *J. Mol. Biol.* **1998**, *283*, 993-1012.

[42] Wang, C.; Stewart, R. J., Kopeček, J. *Nature* **1999**, *397*, 417-420.

[43] An integrin, or integrin receptor, is an integral membrane protein in the plasma membrane of cells. It plays a role in the attachment of a cell to the extracellular matrix (ECM) and in signal transduction from the ECM to the cell.

[44] Mi, L.; Fischer, S.; Chung, B.; Sundelacruz, S.; Harden, J. L. *Biomacromol.* **2006**, *7*, 38-47.

[45] Petka, W. A.; Harden, J. L.; McGrath, K. P.; Wirtz, D.; Tirrell, D. A. *Science* **1998**, *281*, 389-391.

[46] Shen, W.; Zhang, K.; Kornfield, J. A.; Tirrell, D. A. *Nature materials* **2006**, *5*, 153-158.

[47] Klok, H.-A. *Angew. Chem. Int. Ed.* **2002**, *41*, 1509-1513.

[48] Mutter, M.; Chandravarkar, A.; Boyat, C.; Lopez, J.; Dos Santos, S.; Mandal, B.; Mimna, R.; Murat, K.; Patiny, L.; Saucede, L.; Tuchscherer, G. *Angew. Chem. Int. Ed.* **2004**, *43*, 4172-4178.

[49] Hentschel, J.; Krause, E.; Borner, H. G. *J. Am. Chem. Soc.* **2006**, *128*, 7722-7723.

[50] van Hest, J. C. M.; Tirrell, D. A. *Chem. Commun.* **2001**, *19*, 1897-1904.

[51] a) Dougherty, D. A. *Curr. Opin. Chem. Biol.* **2000**, *4*, 645-652. b) Hohsaka, T.; Kajihara, D.; Ashizuka, Y.; Murakami, H.; Sisido, M. *J. Am. Chem. Soc.* **1999**, *121*, 34-40.

[52] Maskarinec, S. A.; Tirrell, D. A. *Curr. Opin. Biotech.* **2005**, *16*, 422-426.

[53] a) H. R. Kricheldorf, α-Amino acid *N*-carboxyanhydridesand related heterocycles, Springer, Berlin, 1987; b) T. J. Deming *J. Polym. Sci. Part A* **2000**, *38*, 3011-3018.

[54] For a recent review, see Hashimoto, K. *Prog. Polym. Sci.* **2000**, *25*, 1411-1462.

[55] Dimitrov, I.; Schlaad, H. *Chem. Commun.* **2003**, *23*, 2944-2945.

[56] Deming, T. J. *Macromol.* **1999**, *32*, 4500-4502.

[57] Matyjaszewski, K.; Xia, J. *Chem. Rev.* **2001**, *101*, 2921-2990.

[58] a) Ayres, L.; Adams, H. H. M.; Löwik, D. W. P. M.; van Hest, J. C. M. *Biomacromolecules* **2005**, *6*, 825-831. b) Ayres, L.; Koch, K.; Adams, H. H. M.; van Hest, J. C. M. M*acromolecules* **2005**, *38*, 1699-1704. c) Ayres, L.; Vos, M. R. J.; Adams, H. H. M.; Shklyarevskiy, I. O.; van Hest, J. C. M. M*acromolecules* **2003**, *36*, 5967-5973.

[59] Mei, Y.; Beers, K. L.; Byrd, H. C. M.; VanderHart, D. L.; Washburn, N. R. *J. Am. Chem. Soc.* **2004**, *126*, 3472-3476.

[60] Ayres, L.; Grotenbreg, G. M.; van der Marel, G. A.; Overkleeft, H. S.; Overhand, M.; van Hest, J. C. M. *Macromol. Rapid Commun.* **2005**, *26*, 1336-1340.

[61] a) Prenner, E. J.; Lewis, R.; McElhaney, R. N. *Biochim. Biophys. Acta* **1999**, *1462*, 201-221. b) Izuyima, N.; Kato, T.; Aoyagi, H.; Waki, M.; Kondo, M. ‘‘Synthetic Aspects of Biologically Active Cyclic Peptides-Gramicidin S and Tyrocidines’’, Halstead (Wiley), New York **1979**.

[62] a) Hull, S. E.; Karlsson, R.; Main, P.; Woolfson, M. M.; Dodson, E. J. *Nature* **1978**, *275*, 206-207. b) Stern, A.; Gibbons, W. A.; Craig, L. C. *Proc. Natl. Acad. Sci*. USA **1968**, *61*, 734-741.

[63] Kung, V. T.; Redemann, C. T. *Biochim. Biophys. Acta* **1986**, *862*, 435-439.

[64] Martin, F. J.; Papahadjopoulos, D. *J. Biol. Chem.* **1982**, *257*, 286-288.

[65] Fleiner, M.; Benzinger, P.; Fichert, T.; Massing, U. *Bioconjugate Chem.* **2001***, 12*, 470-475.

[66] Nakano, Y., Mori, M., Nishinohara, S., Takita, Y., Naito, S., Kato, H., Taneichi, M., Komuro, K., Uchoda, T. *Bioconjugate Chem.* **2001**, *12*, 391-395.

[67] Huisgen, R. *Pure Appl. Chem.* **1989**, *61*, 613-628.

[68] Agard, N. J.; Prescher, J. A.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2004**, *126,* 15046-15047.

[69] a) Mock, W. L.; Irra, T. A.; Wepsiec, J. P.; Manimaran, T. L. *J. Org. Chem.* **1983**, *48*, 3619-3620. b) Mock, W. L.; Irra, T. A.; Wepsiec, J. P.; Adhya, M. *J. Org. Chem.* **1989**, *54*, 5302-5308. c) Lewis, W. G.; Green, L. G.; Grynszpan, F.; Radic, Z.; Carlier, P. R.; Taylor, P.; Finn, M. G.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **2002**, *41*, 1053-1057. d) Wang, Z.-X.; Qin, H.-L. *Chem. Commun.* **2003**, *19*, 2450-2451.

[70] a) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **2002**, *41*, 2596-2599. b) Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057-3062.

[71] Tian, F., Tsao, M.-L., Schultz, P. G. *J. Am. Chem. Soc.* **2004**, *126*, 15962-15963.

[72] Cavalli, S.; Tipton, A. R.; Overhand, M.; Kros, A. *Chem. Commun.* **2006**, *30*, 3193-3195.

[73] a) Bock, V. D.; Hiemstra, H.; van Maarseveen, J. H. *Eur. J. Org. Chem.* **2006**, *1*, 51-68. b) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **2002**, *41*, 2596-2599.

[74] a) Himo, F.; Lovell, T.; Hilgraf, R.; Rostovtsev, V. V.; Noodleman, L.; Sharpless, K. B.; Fokin, V. V. *J. Am. Chem. Soc.* **2005**, *127*, 210-216. b) Rodionov, V. O.; Fokin, V. V.; Finn, M. G. *Angew. Chem. Int. Ed.* **2005**, *44*, 2210-2215.

[75] Issac, R.; Ham, Y.-W.; Chmielewski, J. *Curr. Opin. Struc. Biol.* **2001**, *11*, 458-463.

[76] a) Ghosh1, I.; Chmielewski, J. *Curr. Opin. Chem. Biol.* **2004**, *8*, 640-644. b) Li, X.; Chmielewski, J. *Org. Biomol. Chem.* **2003**, *1*, 901-904. c) Li, X.; Chmielewski, J. *J. Am. Chem. Soc.* **2003**, *125*, 11820-11821.

[77] Ryadnov, M. G., Ceyhan, B.; Niemeyer, C. M.; Woolfson, D. N. *J. Am. Chem. Soc.* **2003**, *125*, 9388-9394.

[78] Langer, R.; Tirrell, D. A. *Nature* **2004**, *428*, 487-492.

[79] Velonia, K.; Rowan, A. E.; Nolte, R. J. M. *J. Am. Chem. Soc.* **2002**, *124*, 4224-4225.

[80] a) Chécot, F.; Lecommandoux, S.; Klok, H.-A.; Gnanou, Y. *Eur. Phys. J. E* **2003**, *10*, 25-35. b) Chécot, F.; Lecommandoux, S.; Gnanou, Y. ; Klok, H.-A. *Angew. Chem. Int. Ed.* **2002**, *41*, 1339-1343.

[81] Vriezema, D. M.; Hoogboom, J.; Velonia, K.; Takazawa, K.; Christianen, P. C. M.; Maan, J. C.; Rowan, A. E.; Nolte, R. J. M. *Angew. Chem. Int. Ed.* **2003**, *42*, 772-776.

[82] Pierschbacher, M. D.; Ruoslahti, E. *Nature* **1984**, *309*, 33-33.

[83] In-depth review on the RGD motif and RGD-modified materials most recently published: a) Gentilucci, L.; Tolomelli, A.; Squassabia, F. *Curr. Med. Chem.* **2006**, *13*, 2449-2466. b) Hersel, U.; Dahmen, C.; Kessler, H. *Biomaterials* **2003**, *24*, 4385-4415.

[84] Oku, N.; Koike, C.; Tokudome, Y.; Okada, S.; Nishikawa, N.; Tsukada, H.; Kiso, M.; Hasegawa, A.; Fujii, H.; Murata, J.; Saiki I. *Adv. drug deliver. rev.* **1997**, *24*, 215-223.

[85] Asai, T.; Oku, N. *Methods Enzymol.* **2005**, *391*, 163-176.

[86] Yagi, N.; Ogawa, Y.; Kodaka, M.; Okada, T.; Tomohiro, T.; Konakahara, T.; Okuno, H. *Chem. Commun.* **1999**, *17*, 1687-1688.

[87] Pakalns, T.; Haverstick, K. L.; Fields, G. B.; McCarthy, J. B.; Mooradian, D. L.; Tirrell, M. *Biomaterials* **1999**, *20*, 2265-2279.

[88] Marchi-Artzner, V.; Hellerer, B. L. U.; Kantlehner, M.; Kessler, H.; Sackmann, E. *Chem. Eur. J.* **2001**, *7*, 1095-1101.

[89] Abbott, A *Nature* **2003**, *424*, 870-872.

[90] a) Kleinman, H. K.; McGarvey, M.L.; Hassell, J.R.; Star, V.L.; Cannon, F.B.; Laurie, G.W.; Martin, G. R. *Biochemistry* **1986**, *25*, 312-318. b) Kleinman, H. K.; McGarvey, M. L.; Liotta, L. A.; Robey, P. G.; Tryggvason, K.; Martin, G. R. *Biochemistry* **1982**, *21*, 6188-6193.

[91] a) Holmes, T. C.; de Lacalle, S.; Su, X.; Liu, G.; Rich, A.; Zhang, S. G. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 6728-6833. b) Zhang, S. G.; Holmes, T. C.; DiPersio, C. M.; Hynes, R. O.; Su, X.; Rich, A. *Biomaterials* **1995**, *16*, 1385-1393. c) Zhang, S. G.; Holmes, T. C.; Lockshin, C.; Rich, A. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 3334-3338.

[92] Kisiday, J.; Jin, M.; Kurz, B.; Hung, H., Semino, C.; Zhang, S. G.; Grodzinsky, A. J. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 9996-10001.

[93] Silva, G. A.; Czeisler, C.; Niece, K. L.; Beniash, E.; Harrington, D. A.; Kessler, J. A.; Stupp, S. I. *Science* **2004**, *303*, 1352-1355.

[94] a) von Maltzahn, G.; Vauthey, S.; Santoso, S.; Zhang, S. *Langmuir* **2003**, *19*, 4332-4337. b) Gorman, J. *Sci News* **2003**, *163*, 43-44. c) Zhao, X.; Zhang, S. *Trends Biotechnol.* **2004**, *22*, 470-476.

[95] Keller, S.; Sauer, I.; Strauss, H.; Gast, K.; Dathe, M.; Bienert, M. *Angew. Chem. Int. Ed.* **2005**, *44*, 5252-5255.

[96] Bellomo, E. G.; Wyrsta, M. D.; Pakstis, L.; Pochan, D. J.; Deming, T. J. *Nat. Mater.* **2004**, *3*, 244-248.

[97] Chu-Kung, A. F.; Bozzelli, K. N.; Lockwood, N. A.; Haseman, J. R.; Mayo, K. H.; Tirrell, M. V. *Bioconjugate Chem.* **2004**, *15,* 530-535.

[98] a) Hancock, R. E. W.; Rozek, A. *FEMS Microbiol. Lett.* **2002**, *206*, 143-149. b) Zhang, L. J.; Rozek, A.; Hancock, R. E. W. *J. Biol. Chem.* **2001**, *276*, 35714-35722.

[99] a) Giangaspero, A.; Sandri, L.; Tossi, A. *Eur. J. Biochem.* **2001**, *268*, 5589-5600. b) Blondelle, S. E.; Houghten, R. A. *Biochemistry* **1992**, *31*, 12688-12694.

[100] Shai, Y. *Biopolymers* **2002**, *66*, 236-248.

[101] Hartgerink, J. D.; Beniash, E.; Stupp, S. I. *Science* **2001**, *294*, 1684-1688.

[102] Bekele, H.; Fendler, J. H.; Kelly, J. W. *J. Am. Chem. Soc.* **1999**, *121,* 7266-7267.

[103] Volkmer, D.; Fricke, M.; Huber, T.; Sewald, N. *Chem. Commun.* **2004**, *16*, 1872-1873.

[104] a) Ryan, D.; Parviz, B. A.; Linder, V.; Semetey, V.; Sia, S. K.; Su, J.; Mrksich, M.; Whitesides, G. M. *Langmuir* **2004**, *20*, 9080-9088. b) Herbert, C. B.; McLernon, T. L.; Hypolite, C. L.; Adams, D. N.; Pikus, L; Huang, C.-C.; Fields, G. B.; Letourneau, P. C.; Distefano, M. D.; Hu, W. S. *Chem. Biol.* **1997**, *4*, 731-737.

[105] Chen, C. S.; Mrksich, M.; Huang, S.; Whitesides, G. M.; Ingber, D. E. *Biotechnol. Progr.* **1998**, *14*, 356-363.

[106] a) Takayama, S.; McDonald, J. C.; Ostuni, E.; Liang, M. N.; Kenis, P. J. A.; Ismagilov, R. F.; Whitesides, G. M. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 5545-5548. b) Delamarche, E.; Bernard, A., Schmid; H.; Michel, B.; Biebuyck, H. *Science* **1998**, *276*, 779-781.

[107] a) Suh, K. Y.; Seong, J.; Khademhosseini, A.; Laibinis, P. E.; Langer, R. *Biomaterials* **2004**, *25*, 557-563. b) Khademhosseini, A.; Jon, S.; Suh, K. Y.; Tran, T.-N. T.; Eng, G.; Yeh, J.; Seong, J.; Langer, R. *Adv. Mater.* **2003**, *15*, 1995-2000.

[108] a) Xia, Y.; Whitesides, G. M. *Angew. Chem. Int. Ed.* **1998**, *37*, 550-575. b) Kane, R. S.; Takayama, S.; Ostuni, E.; Ingber, D. E.; Whitesides, G. M. *Biomaterials* **1999**, *20*, 2363-2376.

[109] Bredenbeck, J.; Helbing, J.; Kumita, J. R.; Woolley, G. A.; Hamm, P. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 2379-2384.

[110] a) Djalali, R.; Chen, Y. F.; Matsui, H. *J. Am. Chem. Soc.* **2002**, *124*, 13660-13661. b) Matsui, H.; Porrata, P.; Douberly, G. E. *Nano Lett.* **2001**, *1*, 461-464.

[111] Reches, M.; Gazit, E. *Science* **2003**, *300*, 625-627.

[112] Scheibel, T.; Parthasarathy, R.; Sawicki, G.; Lin, X.-M.; Jaeger, H. ; Lindquist, S. L. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 4527-4532.

[113] Kinbara, K.; Aida, T. *Chem Rev* **2005**, *105*, 1377-1400.

[114] Diehl, M. R.; Zhang, K.; Lee, H. J.; Tirrell, D. A. *Science* **2006**, *311*, 1468-1471.