# Extending the self-assembly of coiled-coil hybrids

Robson, M.H.

CHAPTER 8

# POLYMER-PEPTIDE BLOCK COPOLYMERS – AN OVERVIEW AND ASSESSMENT OF SYNTHESIS METHODS

Incorporating peptide blocks into block copolymers opens up new realms of bioactive or smart materials. Because there are such a variety of peptides, polymers, and hybrid architectures that can be imagined, there are many different routes available for the synthesis of these chimera molecules. This Chapter summarizes the contemporary strategies in combining synthesis techniques to create well-defined peptide-polymer hybrids that retain the vital aspects of each disparate block. Living polymerization can be united with the molecular-level control afforded by peptide blocks to yield block copolymers that not only have precisely defined primary structures, but that also interact with other (bio)molecules in a well defined manner.

## INTRODUCTION

Within the last five years the incorporation of peptide segments into block copolymers has been intensively investigated and can be classified into two groups: homopolypeptides, and designed sequences, both of which open up possibilities for novel materials with properties that are unavailable via purely synthetic polymers. One useful aspect of peptide blocks is their inherent ability to adopt stable conformations and self-assemble into precisely defined structures. Homopolypeptides are usually helical, i.e. fold into cylindrical rods, while designed peptides can have cylindrical or sheet-like morphology. These blocks have their shape and size defined to the sub-nanometer scale, which in itself allows unprecedented control over the material morphologies. In addition to spatially well-defined structures, another advantage of peptide blocks is that their structure is determined by noncovalent forces, and they can have reactive side-chains, both factors rendering the blocks ‘smart’, as they can switch conformations or properties upon changing external parameters such as pH, temperature, solvent, or ionic strength. Moreover, designed peptides can be constructed with exquisite control over the patterning of functionality. Based on a large amount of previous experimental research and theoretical modeling, peptide design is advanced enough that in many cases from the position of an amino acid in its primary sequence, its relative position in 3D space can be predicted with a high degree of certainty. This allows one to incorporate targeting, molecular recognition,

nucleation sites etc. into the blocks, creating further vistas of opportunities to polymer science.

As well as contributing to the final material properties, the functional groups of peptides (be they the N- or C-termini, or the side chains of natural or unnatural amino acids) can be used as 'handles', allowing a range of block copolymer synthesis techniques. Indeed one can even utilize the intermolecular interactions between peptides to couple polymer blocks. The advances in synthetic tools allow unprecedented control over structure, composition, and functionality of peptide hybrid materials.[1] Undoubtedly, these developments and future advances will allow the integration of biological design concepts of increasing utility in block copolymers, resulting in materials with properties unprecedented either in nature or synthetically.

This Chapter will review the diverse preparation routes of peptide-polymer block copolymers (Figure 1). The methods for synthesizing well-defined peptide-polymer conjugates are divided into seven strategies: 1 & 2) polypeptides initiated from solution phase polymers or from solid-supported polymers; 3 & 4) controlled radical polymerization initiated from solution phase peptides or from solid-supported peptides; 5) polymerization of macromonomers; 6) convergent synthesis of peptide-polymer hybrids; and lastly 7) noncovalent block copolymers. For each of these synthetic routes a brief overview is given of the types of blocks that it is suited to, along with the foremost advantages and limitations of the particular method. The first documented application of each approach is described, followed by other seminal contributions, and examples focusing on the activity pertaining to each route within the last five years, highlighting the current trends and pitfalls of each of the seven synthetic strategies.

The field of polymer bioconjugates is very broad due to the diversity of biological polymers and synthetic polymers that are available for conjugation. This leads to a wide array of physical, chemical, and biological properties, and hence potential applications. Bioconjugates are reviewed in a broad sense by Lutz and Börner[2] and Velonia,[3] where block copolymers incorporating amino acid, nucleobase, and saccharide based blocks are discussed. For more detailed information on the synthesis of polymer-peptide and polymer-protein block copolymers the reader is directed to reviews of Klok[4] and Nicolas.[5] Polymer-polypeptide block copolymers are concisely reviewed by Deming.[6] For reviews with an emphasis on the supramolecular structure formation of polymer-bioconjugates the reader is directed to Börner,[7,8], Kilbinger,[9] and Van Hest.[10]

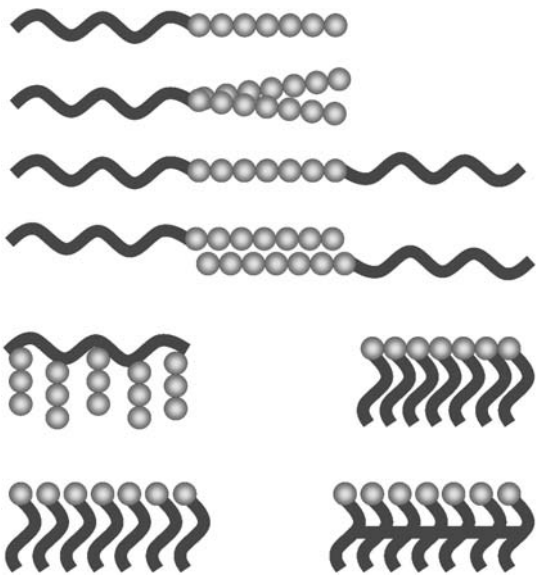

*Figure 1.* A selection of the many peptide-polymer hybrid architectures – linear/branched/pendant, diblock/multiblock, covalent/supramolecular – that are accessible by judicious combinations of synthesis techniques. Examples of methodologies that can be combined are ring-opening polymerization, solid-phase peptide synthesis, dual initiators, reversible addition-fragmentation chain transfer radical polymerization, atom transfer radical polymerization, nitroxide-mediated polymerization, coupling pre-synthesized blocks together, and noncovalent bonds. The balls represent amino acids, the lines represent polymers.

### *1. Polypeptides Initiated from Solution Phase Polymers*

In solution polymers principally act as initiators of homopolypeptides. The most common homopolypeptides are based upon glutamic acid and lysine, as these are known to be the best controlled, but many other monomers can be used.[11] These homopolypeptides, which fold into rods, are predominantly chosen for their well-defined structural organization. Functionality is obtained through the response of the structure to environmental conditions, and by utilizing reactive side-chains.

The most frequently used synthetic methodology for the preparation of homopolypeptide blocks is the ring-opening polymerization (ROP) of protected α-amino acid-N-carboxyanhydrides (α-NCAs) initiated by a primary amino end-functionalized polymer.[11] Indeed this was one of the first routes to be explored in the preparation of polymer-peptide block copolymers, with Gallot and coworkers investigating anionic polymerization followed by ROP of NCAs in the 1970s.[12] This technique allows multi-gram scale synthesis, but has the disadvantage of being beleaguered by chain-breaking transfer and termination reactions, homopolymer contamination, precipitation of the growing polypeptide chain at a certain molecular weight, and the formation of secondary structure, all of which make it difficult to prepare polypeptides with predictable molecular weights and low polydispersity indices (PDI often > 1.2).[6, 13] Increased control over chain length and PDI can be obtained using ammonium initiators,[14] transition metal complexes as

macroinitiators,[15] hexamethlydisilazane,[16] or high vacuum techniques, [17] all of which reduce chain transfer and termination, and have allowed living NCA polymerizations.

Parallel to efforts to improve control over chain growth are investigations into non-linear hybrids. Whether $AB_2$ mikto-arm block copolymers behave differently to AB linear type block copolymers based on polystyrene and poly(glutamic acid) in the solid-phase has recently been investigated.[18] Styrene was polymerized using standard atom transfer radical polymerization (ATRP) conditions and the end-terminus was subsequently modified by an amine- or bifunctional amine group allowing the polymerization of benzyl-L-glutamate (BLG), resulting in linear or branched rod-coil block copolymers with differing organization in the solid-phase.

A greater number of peptides per molecule, and different molecular properties can be obtained by grafting many polypeptides from polymers, either from multiple positions on a linear polymer,[19] or from the branches of dendrimers. For example, polyphenylene dendrimer endgroups were converted from alkynes to amines, which initiated the ROP of up to 16 polylysine chains per polymer dendrimer. Similar polylysine chain lengths were achieved for different degrees of grafting, although the PDI increased from ~ 1.15 to ~ 1.40 as the number of peptide chains increased from 4 to 16.[20]

Both aspects of molecular architecture – control of chain growth, and pattern of chain growth – were addressed by Kim *et al*. They utilized a trifunctional initiator (1,3,5-tris(2-hydroxyethyl)cynuric acid) for the sequential polymerization of polystyrene (PS) and BLG.[21] After the formation of the three PS-arms, a nickel(0) catalyst was prepared at the termini for the living polymerization of the carboxyanhydrides (Figure 2).

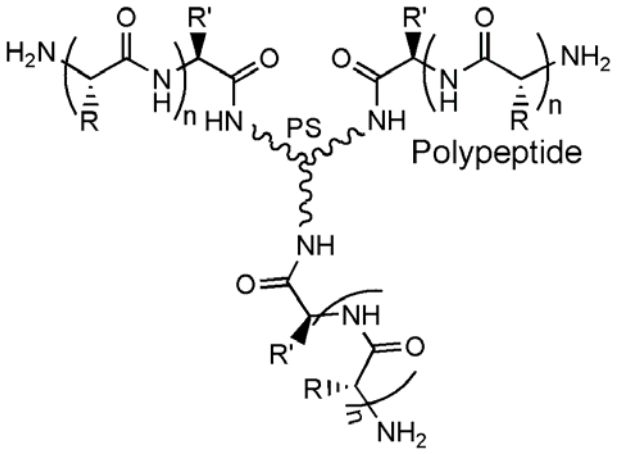


*Figure 2.* The living ROP of BLG-NCA was achieved using amido–amidate nickelacycle initiators. Styrene was polymerized from a trifunctional initiator using ATRP, followed by ROP, resulting in triarm polymer–peptide block copolymers.[21]

Also addressing control over chain growth and chain architecture, Hadjichristidis and coworkers have made applied high vacuum techniques for the living ROP of NCAs initiated from amino-functionalized polystyrene (Figure 3). Using this method large

peptide-polymer hybrids can be produced with low PDIs (e.g. 88000 g/mol, PDI 1.11), and with 100 % yields. Linear and miktoarm peptide-polymer hybrids have been demonstrated using this technique, with the PDI increasing slightly to 1.2 for four arm stars due to steric restrictions on the growing peptide chains.[17]

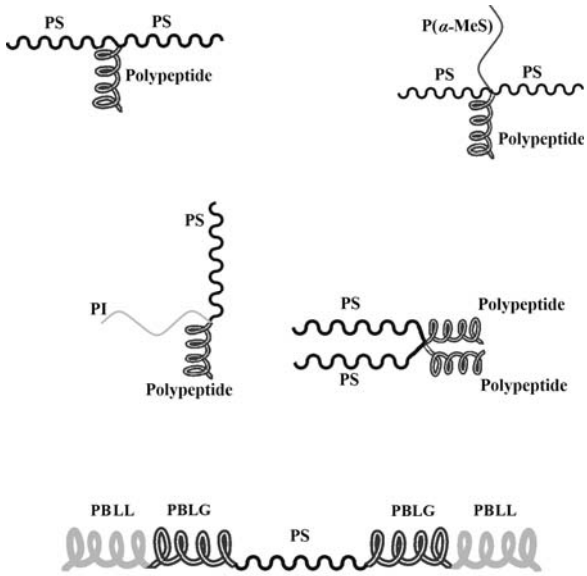


*Figure 3.* Living ROP of BLG-NCA and benzyl-L-lysine-NCA initiated from amino functionalized polystyrene was made possible by using high vacuum techniques. The polystyrene blocks were prepared by living anionic polymerization, also using high vacuum. A range of structures were available by placing the amine functionality either at the PS termini or within the chain.[17]

Regardless of the synthetic advances in preparing homopolypeptides, amino functionalized polymers remain the most commonly used initiators, as they were 30 years ago,[22] as the synthesis is very straight forward. This is illustrated in the work of Zhang *et al* who documented different approaches to the synthesis of double-hydrophilic block copolymers composed of poly(L-glutamate) and poly(N-isopropylacrylamide) (PLG-PNIPAM). A new double-initiator was synthesized bearing a reversible addition-fragmentation chain transfer (RAFT) agent for the poly(N-isopropylacrylamide) synthesis and an amine group for the peptide polymerization.[23] This allows flexibility in the block copolymer synthesis, as either the synthetic or peptide block can be polymerized first, with the NCA polymerization initiated from an amine or ammonium. Of these different methods the traditional route, RAFT polymerization followed by primary amine initiated NCA polymerization gave the best molecular characteristics, with reasonable yields and PDIs (1.2-1.5) for a range of block lengths (n = 27–600, m = 228–360) and block ratios. More recently, a stimuli-responsive zwitterionic hybrid block copolymer was studied.[24] Using the more successful RAFT followed by ROP route a poly(N-isopropylacrylamide)-block-poly(glutamic acid-co-lysine) ($PNIPAM_n(PLG_x$-*co*-$PLL_y)_m$) was synthesized. It was shown that the conformation of the polypeptide block could be controlled as a function of

pH, as at low pH the block copolymer is positively charged due to protonation of the lysine side chain, and at high pH it is negatively charged due to deprotonation of the glutamic acid side chain. In addition the NIPAM block is temperature sensitive resulting in reversible aggregation (Figure 4).

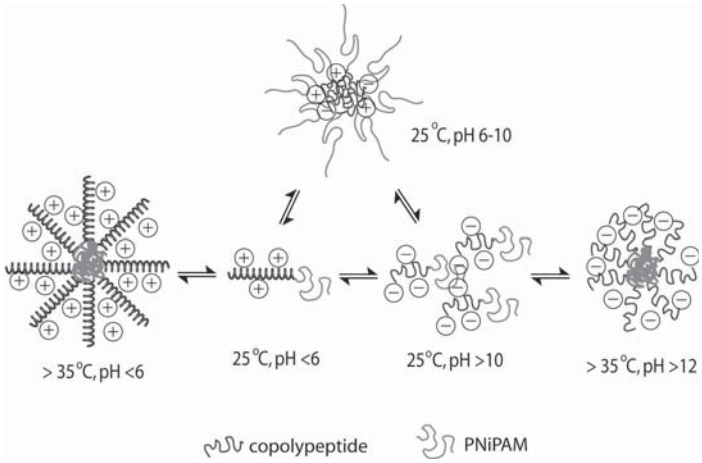


*Figure 4.* Amino terminated PNIPAM was prepared by RAFT polymerization and subsequently used to initiate the ROP of a mixture of benzyl-L-glutamate and benzyl-L-lysine NCAs, resulting in the zwitterionic $PNIPAM_n(PLG_x\text{-}co\text{-}PLL_y)_m$, whose self-assembly was influenced by pH and temperature.[24]

### *2. Solid-Phase Peptide Synthesis from Solid-Supported Polymers*

The majority of advances in peptide-polymer hybrids make use of designed peptides as they have a greater range of efficacies than homopolypeptides. It is not practical to synthesize larger designed peptides in solution; therefore synthesis on a solid support using peptide synthesizers is commonplace. The advantage of this method is the exact control over the amino acid sequence and the improved ease of purification.

In the early 1980s a simple method of using solid-phase peptide synthesis (SPPS) to access peptide-polymer block copolymers was developed by the groups of Mutter and Bayer.[25-27] PS beads that were regularly used for SPPS were loaded with amino-terminated poly(ethylene glycol) (PEG) via an acid labile benzyl-ether linker. SPPS could be conducted from the PEG with high efficiencies, and cleavage at the ether linker liberates the peptide-PEG block copolymer. This was commercialized as PAP resin (Rapp polymere), which has been used by several groups to synthesize peptide-PEG conjugates.[28-33]

A recent example of the use of these pre-loaded resins is the preparation of an amphiphilic block peptide in which a β-sheet forming peptide ($L_4K_8L_4$) was conjugated with the hydrophilic PEG through an short enzyme-cleavable peptide sequence (VPRGS).[34] This is representative of the increasing complexity in the design of the peptide block. These amphiphiles were shown to dissolve into aqueous solutions, with the peptide having α-helical secondary structure. Upon addition of the enzyme thrombin the peptide was

cleaved from the PEG, triggering the propensity of the peptide to fold into β-sheets, and the assembly of long fibers (Figure 5).

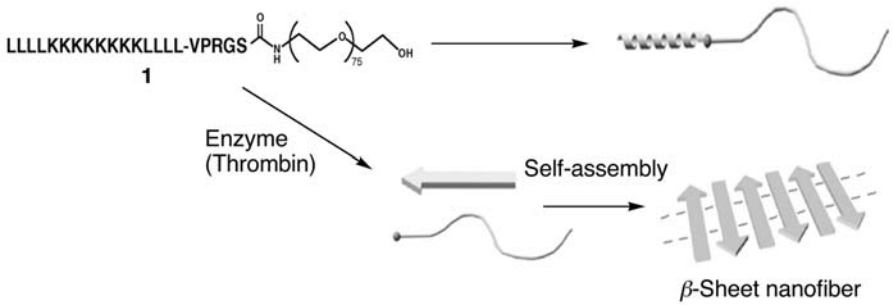


*Figure 5.* SPPS on resin preloaded with PEG afforded peptide-PEG hybrids. Through careful design of the peptide block, the hybrid could be cleaved by an enzyme, leading to the peptide changing secondary structure from an α-helix to a β-sheet, which subsequently aggregated into fibres.[34]

Unfortunately, only resins pre-loaded with PEG are commercially available, and the range of molecular weights available is limited. To access a greater range of hybrids, a polymer can also be attached to the resin followed by SPPS. Polystyrene was attached to a commercially available resin, followed by SPPS of an octapeptide from the base and the N-terminus of the polymer, resulting in di- and triblock copolymers (Figure 6). Typical difficulties in peptide-polymer hybrid synthesis were encountered during the synthesis: even with a long coupling time of 24 hours the first amino acid coupled poorly; additionally, isolation of the hybrid was complicated by the amphiphilic nature of the block copolymer. To reduce this longer PS blocks were used for subsequent investigations.[35]

A drawback to SPPS is that this technique is not suited to the synthesis of many homopolypeptides. This is because using automated peptide synthesis even short homopolypeptides tend to aggregate, resulting in low yields; additionally, peptide synthesizers can only be used to construct peptides up to approximately 40 residues in length before the yield becomes prohibitively poor. It would be possible to produce homopolypeptides by ROP of NCAs from a polymer functionalized solid support, but this has not yet been explored.

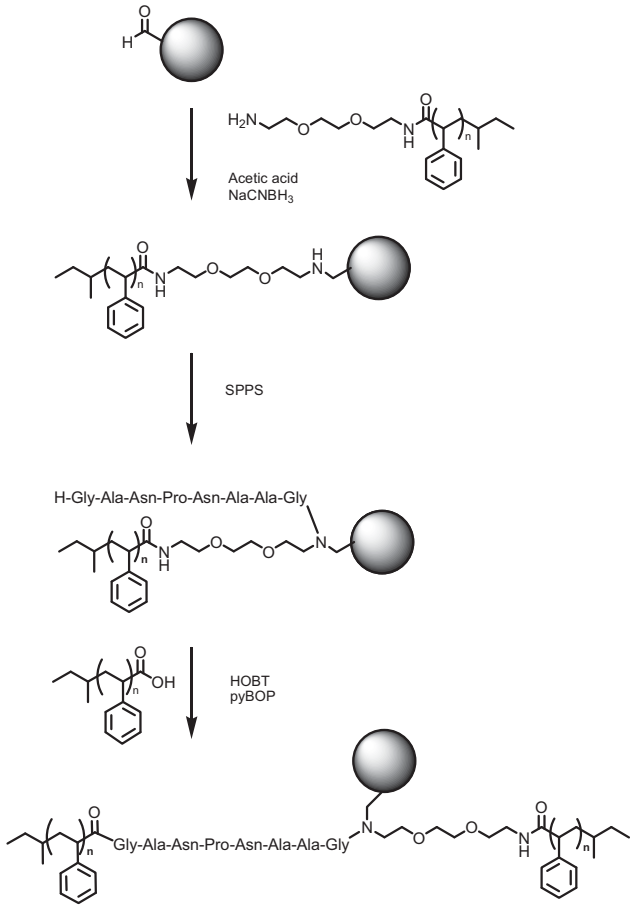


*Figure 6.* PS was attached to a resin via a secondary amine, which was used for SPPS. After the peptide was synthesized the hybrid could be cleaved or carboxylated PS coupled to the N-terminus of the peptide, resulting in a triblock hybrid. Adapted from [35].

### *3. Controlled Radical Polymerization Initiated from Solution Phase Peptides*

A versatile route to well-defined peptide-polymer bioconjugates is to first construct the peptide block, which is then used to initiate the polymerization of the synthetic block in solution. In this way homopolypeptides or designed peptide blocks can be used, and the polymer can be attached at the N- or C-terminus, from an internal amino acid, or from multiple amino acids. A popular route to the synthesis of peptide-polymers is based on the following steps: 1) synthesis of the (oligo)peptide and introduction of the initiating group using solid-phase peptide synthesis; 2) Cleavage from the resin resulting in (de)protected peptide; 3) controlled radical polymerization (ATRP, RAFT, nitroxide-mediated polymerization (NMP)) in solution resulting in the hybrid peptide-polymer.

This frequently used pathway to polymer-peptide block copolymers is quite recent, being first demonstrated by Börner and co-workers in 2004, whereby a pentapeptide was synthesized by SPPS, which was then used in solution as a macroinitiator for the ATRP of *n*-butyl acrylate. The resulting block copolymer exhibited a low PDI of 1.19, and a controllable Mn.[36] More recently, Börner *et al* presented a convenient approach for the synthesis of bioactive peptide-polymer conjugates using RAFT polymerization rather than

ATRP.[37] The N-terminus of a small oligopeptide was modified with a chain-transfer agent based on trithiocarbonates. Polymerization of *n*-butylacrylate yielded polymers with narrow polydispersity (~1.1) and good control over the molecular weight.

The properties of peptide blocks that open up a new range of materials can also be a hindrance during the synthesis of the peptide-polymer hybrids. Recently poly(*n*-butyl acrylate) blocks were synthesized from a $(TV)_5$ peptide segment which has the propensity to form amyloid type aggregates.[38] This is symptomatic of many amphiphilic block copolymers, which are difficult to work with due to an inherent tendency to unwanted large scale organization. Nevertheless, the multiple configurations available to peptide blocks offer opportunities to circumvent this. To prevent the formation of undesirable β-sheet aggregates during the RAFT polymerization of *n*-butyl acrylate, which could hinder the polymerization, reversible pseudoproline defects were introduced. After polymerization the native undisturbed peptide segment was reestablished by changing the pH, which resulted in the aggregation of the polymer-peptide hybrid into large beta-sheet domains and a fibril superstructure (Figure 7).

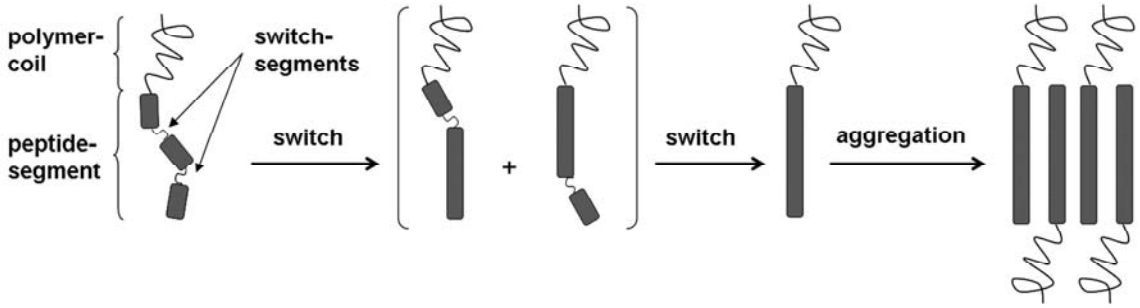


*Figure 7.* Aggregation during peptide initiated RAFT polymerization was prevented by incorporating pH-controlled defects into the peptide secondary structure.[38]

The polymers in all of the examples in this section have been initiated from the modified N-termini of peptides cleaved after SPPS. Maynard and co-workers recently chose to use a different approach by placing the initiating group at one of the amino acids in the peptide sequence.[39] Two types of ATRP-initiators were introduced in the side chain of tyrosine and serine respectively. These modified amino acids could be treated as per ordinary amino acids in SPPS protocols using Fmoc-chemistry. The Fmoc protected serine derivative bearing a 2-bromoisobutyrate moiety was investigated for the controlled polymerization of poly(hydroxyethyl methacrylate) HEMA and an N-acetylglucosamine-modified HEMA (Figure 8). In both cases well defined peptide-polymer hybrids were obtained (PDI < 1.3).

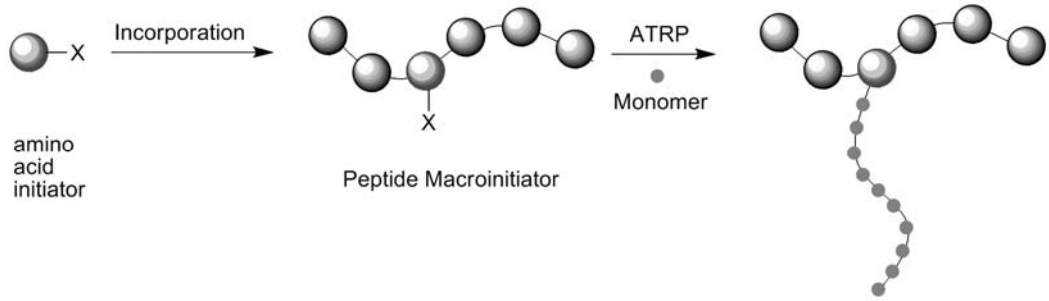


*Figure 8.* The range of polymer-peptide architectures is extended by initiating the polymerization from the middle of the peptide block.[39]

Another approach for non-N-terminal polymer attachment involved a peptide sequence able to form β-hairpin motifs that was modified with two ATRP initiating groups.[40] The peptide sequence contained two serine residues, one at the C- and one at the N-terminus. While still resin bound, these hydroxyl functions were modified with 2-bromoisobutyric acid. After cleavage from the resin, methyl methacrylate was polymerized resulting in an ABA triblock copolymer hybrid. Unfortunately, the peptide was no longer able to adopt its desired β-hairpin motif, showing that the polymer blocks have to be chosen with care in order to not prevent the peptides from assembling in the designed controlled manner.

The number of non-terminal polymer blocks synthesized from each peptide is further increased in the following example in which the side chains of three lysine residues in a cyclic octapeptide were modified to initiate ATRP. Cyclic oligopeptides composed of an alternating sequence of D- and L-amino acids have been shown to assemble in solution to form nanotubes with a well-defined diameter, which are held together by a large number of hydrogen bonds between the individual peptides.[41] This noncovalent stacking is analogous to the polymerization of monomers to form a linear synthetic polymer. Biesalski *et al* described the synthesis of peptide-polymer hybrid nanotubes by modifying a cyclic octapeptide with three initiation sites for the controlled radical polymerization by the ATRP technique, resulting in cyclic peptides with up to three pendant polymer chains (Figure 9).[42] The initial grafts were PNIPAM, polymerized in water,[42] but due to fast reactions, control over the molecular characteristics (e.g., graft density and molar mass of peptide-attached PNIPAM) was not trivial in these studies. Better control was achieved when conducting the ATRP in 2-propanol and adding a sacrificial initiator to the polymerizations, which favorably interfered with the ATRP equilibrium and that produced ‘‘free’’ polymer that could be analyzed with respect to the evolution of molar mass and molar mass distribution (up to ~ 4500 g/mol, PDI 1.2-1.4).[43] Larger degrees of polymerization were obtained for poly(*n*-butyl acrylate), synthesized in dimethyl sulfoxide (up to ~ 30000 g/mol, PDI 1.2-1.4). In this solvent the cyclicpeptides were not preassembled[44] It is not clear if this factor lead to the longer polymers however, as with PS it was shown that the degree of polymerization was similar when initiated from the nanotubes (in acetone) or from free initiator in solution. The PS was close to the theoretically expected molar mass (Mn up to ~ 6000 g/mol), with low PDIs of 1.05–1.10.[45]

With all three polymer-peptides an increase in the length of the grafted polymer lead to a consecutive reduction in the length of the supramolecular nanotubes.[45]

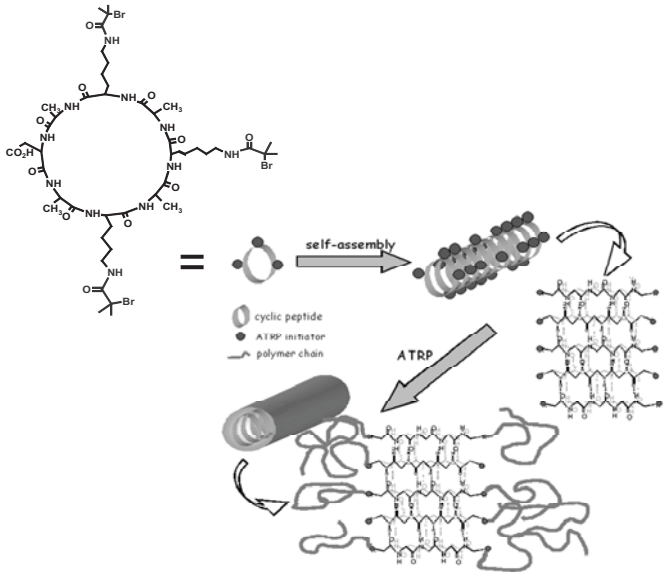


*Figure 9.* Cyclic peptides with pendant polymers stack into noncovalent 'polypeptides'. The length of the stacks decreases upon increasing the molar mass of the polymer chains. The polymer can be synthesized from aggregated or monomeric cyclic peptides.[45]

All of the examples in this section have employed designed, monodisperse peptides. In order to further control the hybrid properties it is therefore desirable to improve the synthesis of the polymer. In an interesting study the influence of the initiator was investigated as it has been shown in the past that the choice of initiator is critical in ATRP.[46] In many cases, the N-terminus of a peptide is modified with 2-bromoisobutyryl bromide to yield an amide-based initiator. However, polymerization of methacrylate monomers resulted in polymers with molecular weights higher than expected and higher polydispersity compared to ester-based initiators (Figure 10). It was suggested that not all peptides successfully initiate polymerization or that significant termination took place. Therefore initiators coupled to a peptide via an ester linkage should be studied in more detail.

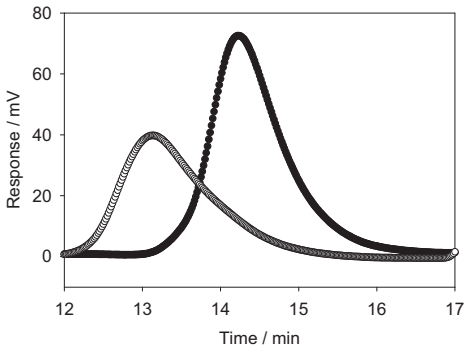


*Figure 10.* The ATRP of methacrylic monomers using ester-based initiators was more controlled than when using standard amide-based peptide initiators.[46]

Recently, several groups have started to explore the use of dual initiators to construct polypeptide-polymer hybrids in solution. Menzel, Heise and coworkers designed a bifunctional initiator for the sequential nickel mediated polymerization of amino acid NCAs and ATRP of methyl methacrylates (Figure 11).[47] Polymerization of benzyl-L-glutamate using the nickel initiator resulted in a controlled polymerization, although the experimental molecular weights were higher compared to the theoretical expected molecular weight. This was explained by the presence of a small amount of inactive impurities, which has also been reported by Deming for similar catalysts.[48] The obtained poly(γ-benzyl L-glutamate) (PBLG) macroinitiator was subsequently applied in the ATRP of methyl methacrylate. Initial studies were done in dimethyl sulfoxide, however better results were obtained in dimethyl formamide with a linear increase in molecular weight as a function of time.[47] Removal of metals after polymerization can be important in some biomedical applications.[49] More recently the group switched back to using amines as the initiator for NCAs combined with a nitroxide group in a bifunctional initiator for the controlled radical polymerization of styrenes.[50] The polymerization of BLG was studied in several solvents and at different temperatures. Previously it was shown that NCAs could be polymerized in a living fashion as long as the temperature was kept at 0 °C.[51] Also in this case the best results were obtained at low temperature resulting in defined polymers with low PDIs (1.1).

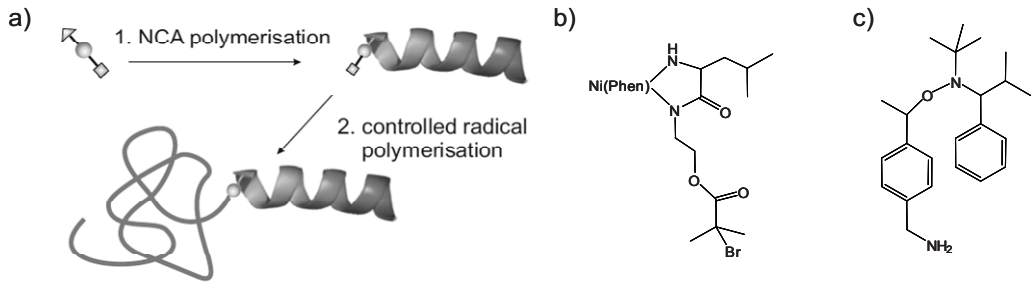


*Figure 11* a) Synthesis of rod–coil polypeptide block copolymers starting from the bifunctional initiator b)[47] or c).[50]

### *4. Controlled Radical Polymerization Initiated from Solid-Supported Peptides*

The possibility of combining solid-phase peptide protocols with controlled radical polymerizations while the peptide is still anchored to a resin has also only recently been investigated. In these studies an amino acid sequence is synthesized bearing a polymerization initiator at the N-terminus, which is used to grow the second block on the resin. A clear advantage of these methods is that side products such as homopolymers and unreacted monomers can simply be removed by washing steps.

This path to peptide-polymer block copolymer was first demonstrated by Wooley *et al* in 2003.[52] The protein transduction domain of the HIV-1 TAT protein, which is a 15-mer oligopeptide, was synthesized from which NMP was conducted starting from the N-

terminus, to yield the synthetic polyacrylate segments in the polymer-peptide hybrid. Using an extension of the NMP procedure it was shown that large amphiphilic triblock copolymers could also be obtained in good yields (Figure 12).[52] Washburn and co-workers used the same methodology to prepare GRGDS-poly(2-hydroxyethyl methacrylate) by ATRP protocols. After cleavage of the polymer-peptide from the resin using a trifluoroacetic acid wash, a clean product was obtained.[53] Previous studies have shown that the ester moieties in (meth)acrylates are not affected under these conditions.[54] In a subsequent investigation of Wooley and coworkers amphiphilic polymer-peptides were prepared via NMP and ATRP composed of an antimicrobial peptide headgroup (tritrpticin) and a poly(acrylic acid) block (tritrpticin-b-PAA), and a polystyrene block (tritrpticin-b-PAA-b-PS).[55] Dispersion into aqueous buffer yielded micellar assemblies with peptides exposed at its surface, which were shown to possess antimicrobial activity.

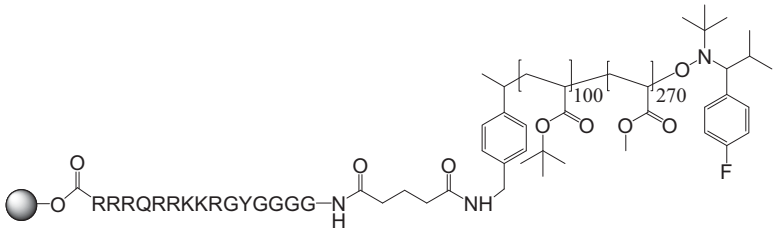


*Figure 12.* Polymers initiated from the N-terminus of the peptides that are anchored to the resin can still achieve high molecular weights, and impurities can be readily washed away.[52]

### *5. Polymerization of Macromonomers*

In many cases peptides are desired in block copolymers for their bioactive properties. The incorporation of peptide sequences as side chains of polymers has the advantage over main chain peptide-polymer block copolymers that it is possible to introduce a much higher concentration of peptide sequences, and therefore bioactive moieties, in the polymer. Side chain peptide polymers can be synthesized via polymerization of macromonomers which include peptides or by attaching the desired sequence to a pre-formed polymer backbone. An advantage of polymerization of macromonomers is that each unit is identical, resulting in well defined hybrid molecules. The disadvantage is that it is necessary to synthesize a complex monomer. To date it has not been possible with either method to synthesize long polymers with larger peptides, most likely due to steric hindrance. In the past, it has been shown that side-chain peptide-polymer hybrids could be prepared via conventional free-radical polymerization.[56-58] However, the very nature of this polymerization method does not allow for the synthesis of well-defined polymeric architectures. Therefore a different approach was explored by van Hest and coworkers who studied the possibility of the controlled polymerization of methacrylates with oligopeptides in the side chain. Inspired by elastin, a VPGVG derivative of methacrylate was polymerized using a difunctional PEG macroinitiator and ATRP protocols.[59,60] This led to polymers with lower critical solution temperature behavior due to the elastin-based

peptide sequence. However, the degree of polymerization was rather low (DPn < 10). To obtain higher molecular weight polymers, elastin-based side-chain polymers were prepared using RAFT polymerization starting from a methacrylate derivative of VPGVG.[59,61] Well-defined homopolymers were obtained with Mn varying between 25000 and 62000 g/mol and low PDIs (1.03-1.23). However, the experimental values for Mn deviated significantly from theoretical values, which was attributed to the complex architecture of the monomer. RAFT polymerization allows for the easy introduction of functional groups at the termini, and as an example, the thioester group was modified using a previously described method[62] which involves the treatment of the polymer with an excess of initiator (4-cyanopentanoic acid dithiobenzoate) resulting in two carboxylate endgroups. To investigate the scope of peptide complexity that can be used, a cyclic decameric peptide (gramicidin-S) modified with a methacrylate group was polymerized (Figure 13).[63] This peptide is known for its antibiotic properties and typically adopts a β-sheet conformation, with both intra- and intermolecular hydrogen bonding. Using ATRP conditions a well-defined polymer was obtained after 15.5 hours with low a PDI (1.09), however the degree of polymerization was rather low (average DPn 13). This shows that this method of preparing polymers with peptides in the side chain is only accessible for small oligopeptides as larger peptides (>10 amino acids) hinder an efficient polymerization.

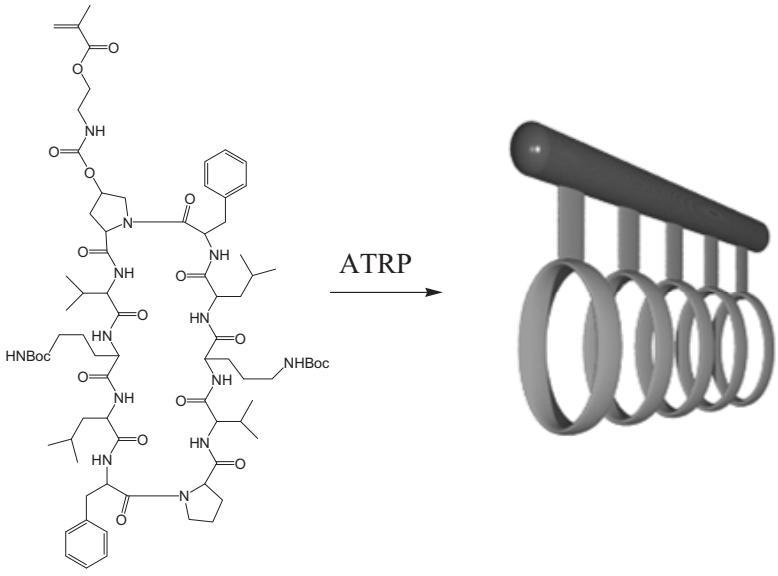


*Figure 13.* The cyclic decapeptide gramicidin S was modified with a methacrylate handle and polymerized by ATRP resulting in polymethacrylate with pendant gramicidin S. The secondary structure of the peptide moiety was retained within the resulting polymer.[63]

Frauenrath and coworkers produced a complex monomer with four elements: a tetrapeptide that forms β-sheets to noncovalently organize the monomers, a diacetylene moiety that can be polymerized once the monomers are organized, a short aliphatic coil segment to prevent global order, and different endgroups which modify the peptide-driven assembly. The peptide induced aggregation lead to supramolecular polymers that were

microns long, and this structure was retained when the diacetylene groups were cross-linked by UV irradiation, leading to a backbone polymer with pendant peptides (Figure 14).[64-69]

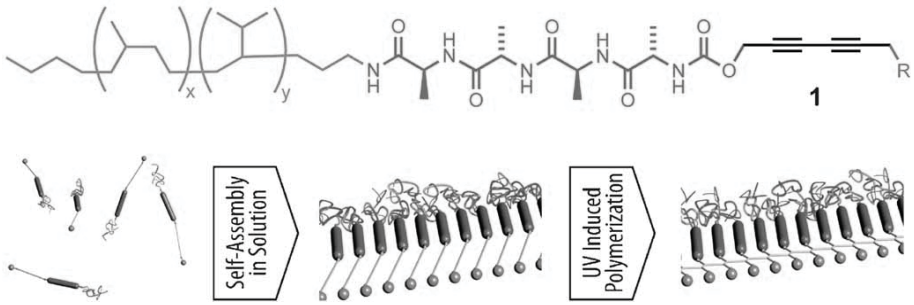


*Figure 14.* Monomers with four functional units assembled into noncovalent nanorods via peptide interactions, and were then covalently linked by UV polymerization of the diacetylene groups.[64]

### *6. Convergent Synthesis of Peptide-Polymer Hybrids*

The direct coupling of a pre-formed peptide/polymer block to another has been used since 1955,[70] initially covalently linking polymers to proteins,[70-72] and later to prepare peptide–polymer conjugates.[73] In general the difficulty of this approach lies in the reduced accessibility of functional groups on macromolecules relative to small molecules, which can limit reaction conversion, and also in the isolation of the desired conjugate from a reaction mixture containing macromolecular starting materials and/or by-products.[4] Coupling strategies are consequently most suitable for the synthesis of polymer-peptide hybrids with low to moderate molecular weights.[37] In contrast the polymerization from a peptide approach allows for the preparation of high molecular weight conjugates (Mn > 30000 g/mol).[38,55]

A 'tidy' method of conjugating peptide and polymer blocks is via an amide bond in the same manner that the peptide was built up. This has usually been achieved by coupling a carboxylated polymer to the N-terminus of the peptide. This is a very straight forward method as it does not require any modification of the peptide, and any amino acid can be used. Kros *et al* synthesized peptide-PS hybrids by coupling acid terminated-PS (Mn = 2000 g/mol) to the N-terminus of the resin-bound peptide using standard amino acid coupling conditions.[31] The weight of the coupled PS chains was ~ 1000 g/mol showing that the shorter chains coupled more efficiently, exemplifying the inherent limitations of coupling blocks together when one block is attached to a resin. In contrast Cornelissen *et al* were able to couple $PS_{35}$-COOH to the N-terminus of a resin-bound peptide and obtained a Mn higher than theoretically expected. In this case it was observed that the reaction was slow and incomplete.[35] A reason for these conflicting reports could be the fact that different resins were used which have different porosities and degrees of functionalization. The same conjugation procedure was demonstrated recently with a single length polymer, resulting in monodisperse PEG-peptide hybrids. Fmoc-PEG-COOH was synthesized with 17 or 29 ethylene glycol monomers and coupled to a resin-bound

peptide. This approach results in well defined bioconjugates that may have different biological properties from their polydisperse counterparts. However, as with the previous two examples, the coupling was not quantitative as only 60% of the peptide blocks were conjugated with PEG after a coupling time of three days.[74]

In solution the quantitative coupling of a polymer block to a peptide was achieved with amino acid coupling conditions, albeit the polymer chain was also relatively short (Mn = 2100 g/mol, PDI = 1.10).[75] Interestingly the polymer, carboxylated poly(*n*-butyl acrylate), was coupled not to the N-terminus of a linear peptide, but to the primary amines of lysine side chains in a cyclic octapeptide. Also in solution, the concept of convergent synthesis using amino acid coupling conditions was extended to polymerization. Amide bonds were formed between dicarboxy PEG and amino terminated oligoalanine-$PEG_5$-oligoalanine, resulting in mulitblock copolymers that were inspired by spider silk.[76]

Again making use of amide bond formation, preformed oligopeptide blocks have been coupled to solid-supported polymers. In an interesting approach polymethacrylate brushes were formed on glass slides by ATRP polymerization, and then the end groups were converted to an activated ester using *p*-nitrophenyl chloroformate.[77] Similarly to SPPS, this application has the advantage that side products and reagents can be washed away. Amino acid coupling conditions were then used to couple bioactive hepta- and octapeptides to the polymer brushes via their N-terminal amine groups.[78]

Another method of coupling preformed blocks is the Michael addition of the thiols from cysteine side chains of peptides onto activated alkenes on polymers (for example PEG diacrylate[79,80] PEG acrylate bound to macroparticles,[80] or maleimide moieties on the surface of polyphenylene dendrimers[20]). The advantage of this coupling technique is that the reaction is very selective, can occur in quickly in physiological conditions, and does not require the use of metallic catalysts. PEG diacrylate and peptides containing thiols were copolymerized by photo illumination to form hydrogels linked by polyacrylate and having peptide pendants, bound via Michael addition to the PEG diacrylate. The coupling rate depends on the amino acid sequence, but was always complete within 1 minute.[81] This method is promising but has not been widely used to create peptide-polymer hybrids.

The [3+2] cycloaddition[82-84] between azides and strained[85] or terminal[86] alkynes has recently emerged as a chemical handle for conjugation in a non copper-mediated[87-89] and copper(I)-catalyzed[90,91] manner, also known as the “click” reaction. This is potentially a very useful coupling handle for peptide-polymers: both azides and alkynes are unreactive towards functional groups present in biomolecules, the cycloaddition product is highly thermally and hydrolytically stable, the copper(I) catalyzed “click” reaction can occur efficiently at room temperature, and metal-free azide-alkyne cycloaddition is possible, as reviewed by Lutz,[92] which is important because in some applications the presence of copper salts negatively influences the properties of peptides/proteins. This approach is

suitable for a wide range of biomolecular applications. For example proteins,[93] enzymes, [94] virus particles[95] and cells,[96] have been selectively modified using this method.
In 2005 Cornelissen *et al* were the first to apply click chemistry to the convergent synthesis of peptide-polymer block copolymers. Azide-terminated polystyrene was conjugated to small oligopeptides, and to proteins resulting in giant amphiphiles.[97] In order to induce spontaneous [3+2] cycloaddition it was envisaged that strained and electron-deficient alkyne derivatives (Figure 15a) should be activated enough for the click reaction to proceed at room temperature.[98] In a step towards this, poly(ethylene glycol) was modified with such an activated alkyne and reacted with azide-bearing oligopeptides resulting in a 80% yield after 36 hours at 37 °C (Figure 15b).

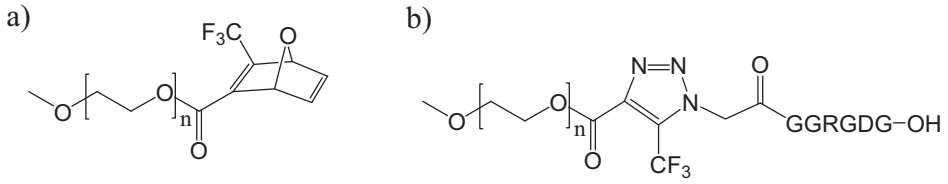


*Figure 15.* a) By using strained and electron deficient oxa-bridged bicyclic systems the use of copper catalysts is circumvented in the coupling of a peptide-polymer hybrid via the "click" reaction b).[98]

Pegylation, the bioconjugation of poly(ethylene glycol) with biomolecules, is one of the most studied and applied biomedical fields to date.[73] Recent studies have shown that brush-like PEG macromolecules are as biocompatible as their linear counterparts. Therefore Lutz *et al* studied the functionalization of the bromine chain-ends of poly(oligo(ethylene glycol) acrylate) prepared by ATRP.[99] The polymerization was stopped at low monomer conversion in order to obtain a polymer with a high degree of end-functionalization (~90%). At higher monomer conversions increasing amounts polymers are formed without a functional endgroup, making separation between these polymers necessary.[100] The bromine group was substituted for an azide group which was subsequently used to "click" an alkyne-modified peptide resulting in a polymer-peptide hybrid. Yields were typically in the order of 75%.[99]
Since its introduction in polymer science, [3+2] cycloaddition has already been exploited for the synthesis of a wide variety of synthetic polymer architectures including end-functionalized polymers, block copolymers, cyclic polymers, graft copolymers, star-shaped copolymers, dendrimers and crosslinked materials, as recently reviewed by Lutz,[101] Binder,[102] and Schubert,[103] and it is envisaged that this techniques will be increasingly applied in the creation of peptide-polymer conjugates.

### *7. Noncovalent Peptide-Polymer Hybrids*

The degree of control over the organization of peptide-polymer block copolymers can be increased not only by using the single molecule properties of peptides (well-defined shape and functionality), but also by utilizing their supramolecular interactions. This was demonstrated in 2008 whereby two block copolymers containing coiled-coil forming peptides, PS-G(EIAALEK)$_3$ (PS-E) and (KIAALKE)$_3$G-PEG (K-PEG) were connected via the specific coiled-coil complex to form a noncovalent tri-block copolymer (Figure 16). The assembly of these block copolymers into rod-like micelles in solution involved both coiled-coil formation and polystyrene aggregation.[31] All peptide-polymer hybrids self-assemble to some degree, whether it be the polymer wrapping around the peptide, or aggregation driven by the polymer, the peptide, or both blocks. An emerging area is to control when peptide-polymer amphiphiles aggregate by utilizing designed peptide blocks. This possibility will likely be further explored in the coming years.

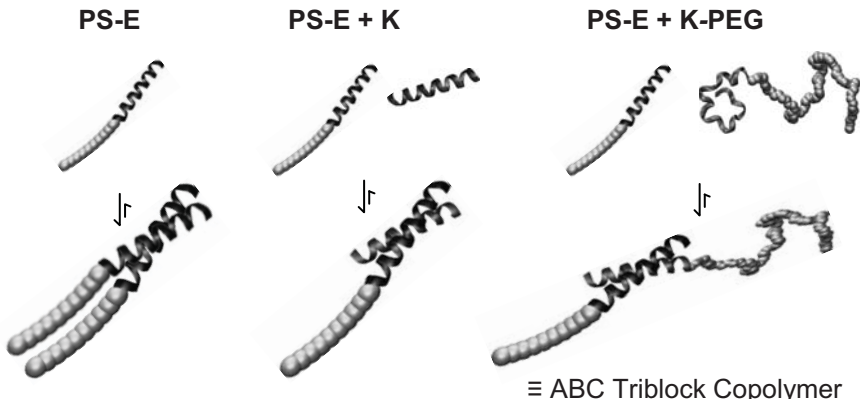


*Figure 16.* The blocks, and hence self-assembling properties, of peptide-polymer block copolymers can readily and reversibly be changed via the noncovalent coiled-coil interaction of the peptide block.[31] Schematic representation of the hierarchical self-assembly of the hybrids PS-E and K-PEG containing complementary peptide blocks.

## CONCLUSIONS

Recent developments in peptide-polymer block copolymer synthesis are two-pronged: one focus is on increasing the molecular level control of the hybrids – control over the block length and polydispersity of synthetic polymers and homopolypeptides on the one hand, and control over the intra- and intermolecular interactions of designed peptide blocks on the other; and the other focus is on broadening the available architectures of peptide-polymer block copolymers. Just as there are many polymers and peptides with widely differing properties available to make up these chimera molecules, there are likewise many different synthesis methods available to create the hybrids. Each synthesis method has its own particular advantages and disadvantages with regards to the conjugation of these different blocks. To summarize the field to date: polymerization in solution, either of synthetic polymers or homopolypeptides can yield large polymers; solid-phase synthesis has been investigated for shorter designed peptides; coupling of preformed blocks is best suited to short blocks; the more elaborate architectures have all been demonstrated with either short peptides or short polymers - grafting of long polymers or peptides is difficult; and only short polymers have been attached noncovalently. In fact many of the hybrids discussed in this review have less than 10 monomers in a block, pushing the definition of ‘polymer’. That is not to say that increasing the size as well as the architectural complexity is not possible, it remains a challenge. This challenge exemplifies the development of this field of research – to increase the fecundity of the hybrids by further exploring peptide-polymer space. With such an increasing wealth of procedures at hand, multifaceted blocks will be able to be combined at will such that the hybrids resonate with new characteristics with contemporary and unforeseen relevance.

## REFERENCES


1. Langer, R.; Tirrell, D. A. *Nature* **2004,** 428, (6982), 487-492.
2. Lutz, J. F.; Borner, H. G. *Prog. Polym. Sci.* **2008,** 33, (1), 1-39.
3. Le Drournaguet, B.; Velonia, K. *Macromol. Rapid Commun.* **2008,** 29, (12-13), 1073-1089.
4. Gauthier, M. A.; Klok, H. A. *Chem. Commun.* **2008**, (23), 2591-2611.
5. Nicolas, J.; Mantovani, G.; Haddleton, D. M. *Macromol. Rapid Commun.* **2007,** 28, (10), 1083-1111.
6. Deming, T. J., Polypeptide and polypeptide hybrid copolymer synthesis via NCA polymerization. In *Peptide Hybrid Polymers*, Springer-Verlag Berlin: Berlin, 2006; Vol. 202, pp 1-18.
7. Borner, H. G. *Macromolecular Chemistry And Physics* **2007,** 208, (2), 124-130.
8. Borner, H. G.; Schlaad, H. *Soft Matter* **2007,** 3, (4), 394-408.
9. Konig, H. M.; Kilbinger, A. F. M. *Angew. Chem.-Int. Edit.* **2007,** 46, (44), 8334-8340.
10. Van Hest, J. C. M. *Polymer Reviews* **2007,** 47, (1), 63-92.
11. Smeenk, J. M.; Lowik, D.; van Hest, J. C. M. *Curr. Org. Chem.* **2005,** 9, (12), 1115-1125.
12. Billot, J. P.; Douy, A.; Gallot, B. *Makromol. Chem.* **1977,** 178, (6), 1641-1650.
13. Kricheldorf, H. R. *Angew. Chem.-Int. Edit.* **2006,** 45, (35), 5752-5784.
14. Dimitrov, I.; Schlaad, H. *Chem. Commun.* **2003**, (23), 2944-2945.
15. Deming, T. J. *Nature* **1997,** 390, (6658), 386-389.
16. Lu, H.; Cheng, J. J. *J. Am. Chem. Soc.* **2008,** 130, (38), 12562-12563.
17. Karatzas, A.; Iatrou, H.; Hadjichristidis, N.; Inoue, K.; Sugiyama, K.; Hirao, A. *Biomacromolecules* **2008,** 9, (7), 2072-2080.
18. Babin, J.; Taton, D.; Brinkmann, M.; Lecommandoux, S. *Macromolecules* **2008,** 41, (4), 1384-1392.
19. Yu, H. J.; Chen, X. S.; Lu, T. C.; Sun, J.; Tian, H. Y.; Hu, J.; Wang, Y.; Zhang, P. B.; Jing, X. B. *Biomacromolecules* **2007,** 8, (5), 1425-1435.
20. Mihov, G.; Grebel-Koehler, D.; Lubbert, A.; Vandermeulen, G. W. M.; Herrmann, A.; Klok, H. A.; Mullen, K. *Bioconjugate Chem.* **2005,** 16, (2), 283-293.
21. Abraham, S.; Ha, C. S.; Kim, I. *J. Polym. Sci. Pol. Chem.* **2006,** 44, (9), 2774-2783.
22. Gallot, B. *Prog. Polym. Sci.* **1996,** 21, (6), 1035-1088.
23. Zhang, X. Q.; Li, J. G.; Li, W.; Zhang, A. *Biomacromolecules* **2007,** 8, (11), 3557-3567.
24. Li, J. G.; Wang, T.; Wu, D. L.; Zhang, X. Q.; Yan, J. T.; Du, S.; Guo, Y. F.; Wang, J. T.; Zhang, A. *Biomacromolecules* **2008,** 9, (10), 2670-2676.
25. Becker, H.; Lucas, H. W.; Maul, J.; Pillai, V. N. R.; Anzinger, H.; Mutter, M. *Makromo. Chem. Rapid* **1982,** 3, (4), 217-223.
26. Bayer, E.; Dengler, M.; Hemmasi, B. *Int. J. Pept. Protein Res.* **1985,** 25, (2), 178-186.
27. Bayer, E. H., B.; Albert, K.; Rapp, W.; Dengler, M. In *Peptides, Structure and Function.*, Proceedings of the 8th American PeptideSymposium, Pierce Chemical Company: Rockford, IL, 1983; Hruby, V. J., Rich, D. H., Ed. Pierce Chemical Company: Rockford, IL, 1983; p 87–90.
28. Burkoth, T. S.; Benzinger, T. L. S.; Jones, D. N. M.; Hallenga, K.; Meredith, S. C.; Lynn, D. G. *J. Am. Chem. Soc.* **1998,** 120, (30), 7655-7656.
29. Rosler, A.; Klok, H.-A.; Hamley, I. W.; Castelletto, V.; Mykhaylyk, O. O. *Biomacromolecules* **2003,** 4, (4), 859-863.
30. Hamley, I. W.; Krysmann, M. J. *Langmuir* **2008,** 24, (15), 8210-8214.
31. Marsden, H. R.; Korobko, A. V.; van Leeuwen, E. N. M.; Pouget, E. M.; Veen, S. J.; Sommerdijk, N. A. J. M.; Kros, A. *J. Am. Chem. Soc.* **2008,** 130, (29), 9386-9393.
32. Eckhardt, D.; Groenewolt, M.; Krause, E.; Borner, H. G. *Chem. Commun.* **2005**, (22), 2814-2816.
33. Rosler, A.; Klok, H. A.; Hamley, I. W.; Castelletto, V.; Mykhaylyk, O. O. *Biomacromolecules* **2003,** 4, (4), 859-863.
34. Koga, T.; Kitamura, K.; Higashi, N. *Chem. Commun.* **2006**, (47), 4897-4899.
35. Reynhout, I. C.; Lowik, D.; van Hest, J. C. M.; Cornelissen, J.; Nolte, R. J. M. *Chem. Commun.* **2005**, (5), 602-604.
36. Rettig, H.; Krause, E.; Borner, H. G. *Macromol. Rapid Commun.* **2004,** 25, (13), 1251-1256.
37. Hentschel, J.; Bleek, K.; Ernst, O.; Lutz, J. F.; Borner, H. G. *Macromolecules* **2008,** 41, (4), 1073-1075.
38. Hentschel, J.; ten Cate, M. G. J.; Borner, H. G. *Macromolecules* **2007,** 40, (26), 9224-9232.
39. Broyer, R. M.; Quaker, G. M.; Maynard, H. D. *J. Am. Chem. Soc.* **2008,** 130, (3), 1041-1047.

40. Ayres, L.; Hans, P.; Adams, J.; Lowik, D.; van Hest, J. C. M. *J. Polym. Sci. Pol. Chem.* **2005,** 43, (24), 6355-6366.
41. Ghadiri, M. R.; Granja, J. R.; Milligan, R. A.; McRee, D. E.; Khazanovich, N. *Nature* **1993,** 366, (6453), 324-327.
42. Couet, J.; Jeyaprakash, J. D.; Samuel, S.; Kopyshev, A.; Santer, S.; Biesalski, M. *Angew. Chem.-Int. Edit.* **2005,** 44, (21), 3297-3301.
43. Couet, J.; Biesalski, M. *Macromolecules* **2006,** 39, (21), 7258-7268.
44. Loschonsky, S.; Couet, J.; Biesalski, M. *Macromol. Rapid Commun.* **2008,** 29, (4), 309-315.
45. Couet, J.; Biesalski, M. *Small* **2008,** 4, (7), 1008-1016.
46. Adams, D. J.; Young, I. *J. Polym. Sci. Pol. Chem.* **2008,** 46, (18), 6082-6090.
47. Steig, S.; Cornelius, F.; Witte, P.; Staal, B. B. P.; Koning, C. E.; Heise, A.; Menzel, H. *Chem. Commun.* **2005**, (43), 5420-5422.
48. Deming, T. J.; Curtin, S. A. *J. Am. Chem. Soc.* **2000,** 122, (24), 5710-5717.
49. Gil, E. S.; Hudson, S. A. *Prog. Polym. Sci.* **2004,** 29, (12), 1173-1222.
50. Knoop, R. J. I.; Habraken, G. J. M.; Gogibus, N.; Steig, S.; Menzel, H.; Koning, C. E.; Heise, A. *J. Polym. Sci. Pol. Chem.* **2008,** 46, (9), 3068-3077.
51. Vayaboury, W.; Giani, O.; Cottet, H.; Deratani, A.; Schue, F. *Macromol. Rapid Commun.* **2004,** 25, (13), 1221-1224.
52. Becker, M. L.; Liu, J. Q.; Wooley, K. L. *Chem. Commun.* **2003**, (2), 180-181.
53. Mei, Y.; Beers, K. L.; Byrd, H. C. M.; Vanderhart, D. L.; Washburn, N. R. *J. Am. Chem. Soc.* **2004,** 126, (11), 3472-3476.
54. Angot, S.; Ayres, N.; Bon, S. A. F.; Haddleton, D. M. *Macromolecules* **2001,** 34, (4), 768-774.
55. Becker, M. L.; Liu, J. Q.; Wooley, K. L. *Biomacromolecules* **2005,** 6, (1), 220-228.
56. Murata, H.; Sanda, F.; Endo, T. *Macromolecules* **1996,** 29, (17), 5535-5538.
57. Murata, H.; Sanda, F.; Endo, T. *Macromolecules* **1997,** 30, (10), 2902-2906.
58. Lubbert, A.; Nguyen, T. Q.; Sun, F.; Sheiko, S. S.; Klok, H. A. *Macromolecules* **2005,** 38, (6), 2064-2071.
59. Ayres, L.; Vos, M. R. J.; Adams, P.; Shklyarevskiy, I. O.; van Hest, J. C. M. *Macromolecules* **2003,** 36, (16), 5967-5973.
60. Ayres, L.; Koch, K.; Adams, P.; van Hest, J. C. M. *Macromolecules* **2005,** 38, (5), 1699-1704.
61. Fernandez-Trillo, F.; Dureault, A.; Bayley, J. P. M.; van Hest, J. C. M.; Thies, J. C.; Michon, T.; Weberskirch, R.; Cameron, N. R. *Macromolecules* **2007,** 40, (17), 6094-6099.
62. Perrier, S.; Takolpuckdee, P.; Mars, C. A. *Macromolecules* **2005,** 38, (6), 2033-2036.
63. 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, (16), 1336-1340.
64. Jahnke, E.; Millerioux, A. S.; Severin, N.; Rabe, J. P.; Frauenrath, H. *Macromol Biosci* **2007,** 7, (2), 136-143.
65. Jahnke, E.; Lieberwirth, I.; Severin, N.; Rabe, J. P.; Frauenrath, H. *Angew. Chem.-Int. Edit.* **2006,** 45, (32), 5383-5386.
66. Weiss, J.; Jahnke, E.; Frauenrath, H. *Macromol. Rapid Commun.* **2008,** 29, (4), 330-339.
67. Jahnke, E.; Severin, N.; Kreutzkamp, P.; Rabe, J. P.; Frauenrath, H. *Adv. Mater.* **2008,** 20, (3), 409-414.
68. Jahnke, E.; Weiss, J.; Neuhaus, S.; Hoheisel, T. N.; Frauenrath, H. *Chem.-Eur. J.* **2009,** 15, (2), 388-404.
69. Weiss, J.; Jahnke, E.; Severin, N.; Rabe, J. P.; Frauenrath, H. *Nano Lett.* **2008,** 8, (6), 1660-1666.
70. Jatzkewitz, H. *Z. Naturforsch. Pt. B* **1955,** 10, (1), 27-31.
71. Mitz, M. A.; Summaria, L. J. *Nature* **1961,** 189, (476), 576-&.
72. Abuchowski, A.; Vanes, T.; Palczuk, N. C.; Davis, F. F. *Journal Of Biological Chemistry* **1977,** 252, (11), 3578-3581.
73. Duncan, R. *Nat. Rev. Drug Discov.* **2003,** 2, (5), 347-360.
74. Niculescu-Duvaz, D.; Getaz, J.; Springer, C. J. *Bioconjugate Chem.* **2008,** 19, (4), 973-981.
75. ten Cate, M. G. J.; Severin, N.; Borner, H. G. *Macromolecules* **2006,** 39, (23), 7831-7838.
76. Rathore, O.; Sogah, D. Y. *J. Am. Chem. Soc.* **2001,** 123, (22), 5231-5239.
77. Tugulu, S.; Arnold, A.; Sielaff, I.; Johnsson, K.; Klok, H. A. *Biomacromolecules* **2005,** 6, (3), 1602-1607.
78. Tugulu, S.; Silacci, P.; Stergiopulos, N.; Klok, H. A. *Biomaterials* **2007,** 28, (16), 2536-2546.
79. Lutolf, M. P.; Tirelli, N.; Cerritelli, S.; Cavalli, L.; Hubbell, J. A. *Bioconjugate Chem.* **2001,** 12, (6), 1051-1056.

80. Heggli, M.; Tirelli, N.; Zisch, A.; Hubbell, J. A. *Bioconjugate Chem.* **2003,** 14, (5), 967-973.
81. Salinas, C. N.; Anseth, K. S. *Macromolecules* **2008,** 41, (16), 6019-6026.
82. Huisgen, R. *Pure Appl. Chem.* **1989,** 61, (4), 613-628.
83. Huisgen, R., *1,3-Dipolr Cycloaddition Chemistry*. Wiley: New York, 1984; Vol. 1, p 1-176.
84. Meldal, M.; Tornoe, C. W. *Chem. Rev.* **2008,** 108, (8), 2952-3015.
85. Agard, N. J.; Prescher, J. A.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2004,** 126, (46), 15046-15047.
86. Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Edit.* **2002,** 41, (14), 2596-2599.
87. Mock, W. L.; Irra, T. A.; Wepsiec, J. P.; Manimaran, T. L. *J. Org. Chem.* **1983,** 48, (20), 3619-3620.
88. Mock, W. L.; Irra, T. A.; Wepsiec, J. P.; Adhya, M. *J. Org. Chem.* **1989,** 54, (22), 5302-5308.
89. Lewis, W. G.; Green, L. G.; Grynszpan, F.; Radic, Z.; Carlier, P. R.; Taylor, P.; Finn, M. G.; Sharpless, K. B. *Angew. Chem.-Int. Edit.* **2002,** 41, (6), 1053-1057.
90. Feng, T.; Tsao, M. L.; Schultz, P. G. *J. Am. Chem. Soc.* **2004,** 126, (49), 15962-15963.
91. Cavalli, S.; Tipton, A. R.; Overhand, M.; Kros, A. *Chem. Commun.* **2006**, (30), 3193-3195.
92. Lutz, J. F. *Angew. Chem.-Int. Edit.* **2008,** 47, (12), 2182-2184.
93. Deiters, A.; Cropp, T. A.; Summerer, D.; Mukherji, M.; Schultz, P. G. *Bioorg. Med. Chem. Let.* **2004,** 14, (23), 5743-5745.
94. Speers, A. E.; Cravatt, B. F. *Chem. Biol.* **2004,** 11, (4), 535-546.
95. Wang, Q.; Chan, T. R.; Hilgraf, R.; Fokin, V. V.; Sharpless, K. B.; Finn, M. G. *J. Am. Chem. Soc.* **2003,** 125, (11), 3192-3193.
96. Link, A. J.; Vink, M. K. S.; Tirrell, D. A. *J. Am. Chem. Soc.* **2004,** 126, (34), 10598-10602.
97. Dirks, A. J. T.; van Berkel, S. S.; Hatzakis, N. S.; Opsteen, J. A.; van Delft, F. L.; Cornelissen, J.; Rowan, A. E.; van Hest, J. C. M.; Rutjes, F.; Nolte, R. J. M. *Chem. Commun.* **2005**, (33), 4172-4174.
98. van Berkel, S. S.; Dirks, A. T. J.; Debets, M. F.; van Delft, F. L.; Cornelissen, J.; Nolte, R. J. M.; Rutjes, F. *ChemBioChem* **2007,** 8, (13), 1504-1508.
99. Lutz, J. F.; Borner, H. G.; Weichenhan, K. *Macromolecules* **2006,** 39, (19), 6376-6383.
100. Hong, S. C.; Lutz, J. F.; Inoue, Y.; Strissel, C.; Nuyken, O.; Matyjaszewski, K. *Macromolecules* **2003,** 36, (4), 1075-1082.
101. Lutz, J. F. *Angew. Chem.-Int. Edit.* **2007,** 46, (7), 1018-1025.
102. Binder, W. H.; Sachsenhofer, R. *Macromol. Rapid Commun.* **2007,** 28, (1), 15-54.
103. Fournier, D.; Hoogenboom, R.; Schubert, U. S. *Chem. Soc. Rev.* **2007,** 36, (8), 1369-1380.