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Review

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PEG–Peptide Conjugates

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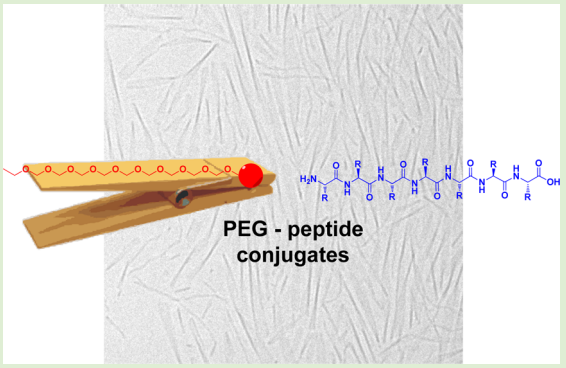
ABSTRACT: The remarkable diversity of the self-assembly behavior of PEG–peptides is reviewed, including self-assemblies formed by PEG–peptides with β-sheet and α-helical (coiled-coil) peptide sequences. The modes of self-assembly in solution and in the solid state are discussed. Additionally, applications in bionanotechnology and synthetic materials science are summarized.



1. INTRODUCTION

Attachment of PEG to peptides or proteins, so-called PEGylation, offers improved water solubility and stability as well as reduced clearance through the kidneys, leading to a longer circulation time.[1] Several reviews on polymer–peptide conjugates discuss examples of self-assembling PEG–peptide conjugates,[2−4] and reviews discussing applications of PEGylation of peptides and proteins for applications in biotechnology are available.[5−8]

This review is focused on PEG–peptide conjugates and does not cover methods of protein PEGylation or applications of PEGylated proteins. These topics have been extensively reviewed elsewhere.[9−12] The application of PEGylation in drug delivery has been reviewed, including lists of commercially developed PEGylated proteins for therapeutics.[5,7,8,13] General reviews on polymer–peptide conjugates also feature discussion of PEG–peptide conjugates.[1,14−16] A book on bioconjugation techniques[17] discusses many chemistries that can be used to prepare PEG–peptide conjugates. This review focuses on the self-assembly of PEG–peptide conjugates, with a brief discussion of the relevant preparation chemistries, as these are reviewed in more detail in the above-mentioned publications.

The following notation is used here for PEG with different molar mass: in PEG*x*k, *x* denotes the molar mass in kilograms per mole, and in PEG$_n$, *n* denotes the average degree of polymerization. Amino acids are abbreviated with single- or three-letter codes.

This review is organized as follows. Methods to synthesize PEG–peptide conjugates are outlined in Section 2. Sections 3 and 4 discuss the self-assembly of PEG–peptide diblock conjugates containing β-sheet or α-helical peptides, respectively. The self-assembly of PEG–peptide conjugates with more complex triblock and multiblock architectures is reviewed in Section 5. In Section 6, the class of PEG–peptides containing synthetic polypeptides such as poly(γ-benzyl L-glutamate) (PBLG) is considered. PEG crystallization effects on self-assembly are examined in Section 7. The final sections introduce selected applications of PEG–peptides. Section 8 concerns enzyme-responsive PEG–peptide conjugates, whereas Section 9 summarizes studies on PEG–peptides for drug delivery. Lastly, a brief summary and outlook are included in Section 10.

2. SYNTHETIC METHODS

2.1. Coupling. *2.1.1. Coupling Chemistries.* Examples of some of the common coupling chemistries are shown in Figure 1. As will become apparent in the following discussion, this is not an exhaustive list of methods used to link PEG polymers and peptides.

Amine groups at the N terminus or on lysine side chains are common sites for PEG attachment using hydroxyl- or aldehyde-functionalized PEG. First-generation methods relied on activated hydroxyl groups on end-functionalized PEGs.[18,19] A widely used method in current use involves *N*-hydroxysuccinimide (NHS) esters, which are highly reactive toward amines at physiological pH (Figure 1a).[20] Potential hydrolysis of the ester bond of succinylated PEG can be avoided by use of a linking carbonate group.[1,21,22] NHS-terminated PEG was used to prepare alternating multiblock copolymers of PEG and coiled-coil peptides using NHS–PEG3.4k–NHS.[23]

Other methods to activate the reaction of PEG and amines have been reviewed.[1] Rather than preparing polymers with reactive end groups, in situ activation using carbodiimides is possible,[24] and this has been used to couple the carboxylic group of succinylated PEG to various peptides.[1] Aldehyde-terminated polymers may be reacted with N-terminal amines (Figure 1b).[1,25−27] In one example, poly(oligo-(ethylene

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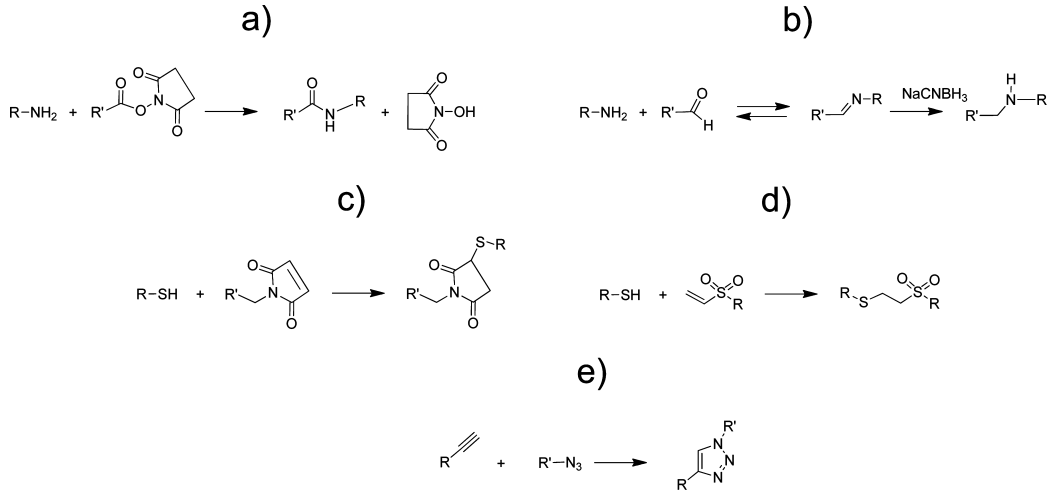

Figure 1. Representative coupling chemistries (adapted from ref. 17). In general, R denotes a peptide chain, and R′, a PEG chain, although in some cases these are interchangeable (e.g., reaction e). (a) Reaction of an amine with an NHS ester forms an amide bond, (b) reaction of an amine with an aldehyde produces a Schiff base that can be reduced by borohydrides to produce a secondary amine linkage, (c) reaction of a thiol (in a cysteine residue) with a maleimide derivative produces a thioether bond, (d) reaction of a thiol-containing peptide with a vinylsulfone-modified PEG produces a thioether bond, and (e) Cu-catalyzed alkyne−azide click reaction produces a triazole. Adapted with permission from ref 17. Copyright 2008 Elsevier.

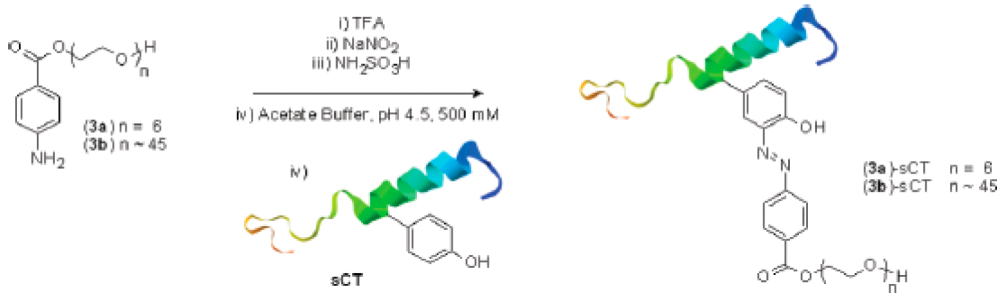

Figure 2. Coupling of PEG via diazonium linkage to Tyr22 in salmon calcitonin (sCT).[42] Reproduced from ref 42. Copyright 2012 American Chemical Society.

glycol) methacrylate) (POEGMA) or PEG was coupled to the 3432 Da peptide hormone salmon calcitonin.[28] POEGMA contains oligomeric ethylene oxide side chains and has some distinct properties to linear PEG while retaining advantages such as biocompatibility. For instance, copolymerization of different oligo-ethylene gycol acrylates leads to the formation of thermosensitive polymers with lower critical solution temperature (LCST) behavior.[29] In the conjugates of PEG or POEGMA with salmon calcitonin, the bioactivity was not influenced by the M_n of POEGMA (containing PEG1.1k) in the range 6.5−109 kDa.[30] Later, this group used dibromomaleimides for site-specific linkage to the disulfide bridge in salmon calcitonin.[31] The same model peptide was also conjugated to poly((monomethoxy ethylene glycol) (meth)acrylates) via the disulfide using water-soluble organic phosphines as catalysts.[32] An amine-terminated polymer may be attached to an aldehyde-functionalized resin, from which peptide synthesis is performed.[33]

Cysteines are an attractive target for conjugation of polymers to proteins because only a few are typically available to react with;[1] however, further discussion of protein conjugation is outside the scope of this review. A number of thiol-reactive groups can be used to couple end-functionalized polymers to cysteine-terminated peptides (Figure 1c,d). One widely used chemistry employs maleimides, which react selectively with the thiols of cysteine residues by Michael addition in the pH range 6.5−7.5 (Figure 1c).[15] To prepare atom-transfer radical polymerization (ATRP) initiators containing maleimides, protection is required.[1] Amine-terminated PEG has been converted to a maleimide by reaction with maleic anhydride.[34] Maleimide−PEGs have been coupled to cysteine flanked silk-like peptide $(EG)_3EG$.[35] In another case, maleimide polymers including POEGMA were coupled to the model thiol-containing peptide reduced glutathione (γ-ECG) as well as the protein bovine serum albumin.[36] The maleimide was incorporated into an initiator for ATRP to produce α-functionalized polymers. Maleimide-modified RAFT chain-transfer agents (CTAs) have also been used to synthesize poly(ethylene glycol) methyl ether acrylate (PEGA) for conjugation to proteins such as lysozyme.[37,38]

Another chemistry that can be used to link polymers to cysteine residues employs vinyl sulfone-terminated polymers (Figure 1d); for example, this approach has been used to couple PEGA to proteins.[39] A PEGA−PS−PEGA triblock copolymer with pyridyl sulfide end groups, prepared by RAFT using a novel CTA, has been linked to glutathione as a model tripeptide. The triblock forms micelles, and the conjugate forms peptide-decorated micelles.[40]

Thiol-ene chemistry may also be employed to link alkene-terminated polymers and cysteine-containing peptides. For

instance, copolymers of di(ethylene glycol) methyl ether methacrylate (DEGMEMA) and allyl methacrylate have been linked to α-keratin using this chemistry.[41]

Diazonium derivatives may be coupled to tyrosine, and this has been exploited to link a diazo-functional PEG (PEG_6) to the pentapeptide (D-Ala2)-leucine enkephalin (an endogenous opioid peptide) as well as diazo-functional PEG_6 and PEG_{45} to salmon calcitonin (Figure 2).[42] This method was also employed using POEGMA instead of PEG.

Carboxylated PEG may be linked to oligopeptides via NHS activation with *N*,*N*′-dicyclohexylcarbodiimide (DCC).[43] This method was used to prepare conjugates of short PEG chains (PEG350−750) with hydrophobic tetra- and hexapeptides. The conjugates formed micelles with thermosensitive properties because of the LCST behavior.

Oxime formation by reaction of aminooxy end-functionalized polymers and levulinyl-modified proteins or peptides may also be used to prepare conjugates, such as those containing POEGMA.[44] The method has also been used to conjugate branched PEG-based polymers to peptides (small proteins) such as an anti-HIV protein[45,46] (Figure 3) or synthetic erythropoietic proteins.[47]

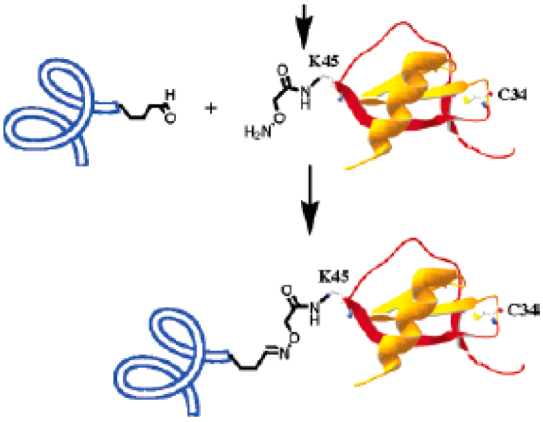

Figure 3. Coupling of aldehyde-functionalized PEG to an isopropylidene-functionalized 34-residue anti-HIV peptide at lysine 45 via oxime exchange using methoxylamine.[46] Reproduced from ref 46. Copyright 2005 American Chemical Society.

On-resin coupling of PEG−CH_2−COOH to N-terminal peptide resins has been carried out via acylation, occurring rapidly and with high conversion in the case of lower molar mass PEG (750 g mol^{-1}) but not for PEG10k (PEG5k could be coupled to unhindered N-terminal Gly but not hindered Ile).[48] Di-PEGylation was also possible using C-terminal PEGylated amino acids (ornithine or lysine), and this method was used to prepare di-PEGylated interleukin-2 peptide fragments. Lysine side chain/C-terminal PEG conjugates were also prepared. The authors also showed that PEG end-functionalized with methylnorleucine (Nle) is a good reagent for N-terminal PEGylation using BOP activation.[49,50] The method was used to prepare a number of PEG−$OCHH_2CO$−Nle$(NANP)_3$ conjugates with PEG5000[49] as well as a conjugate of PEG5000 and a 13-residue peptide fragment of interleukin-2.[50]

A mixed grafted PLL poly(L-lysine) conjugate was prepared by coupling PEG2k with an NHS end group via the lysine amine as a side chain.[51] In addition, a fraction of PEG chains were functionalized with RGD-based peptides via vinylsulfone groups coupled to cysteine residues. The incorporation of the RGD cell adhesion motif supported the growth of human dermal fibroblasts while blocking adsorption of serum proteins. In a related context, PEG5000 has been grafted via a lysine residue in fluorophore-labeled RGD peptides for applications in bioimaging.[52] PEGylation was found to enhance fluorescence quantum yield while reducing interactions between fluorophores and biomolecules in cells.

2.1.2. Click Coupling. The well-known [3 + 2] cycloaddition click reaction (Figure 1e) between alkynes and azides has been widely used in the synthesis of bioconjugates.[53]

By using click reactions, grafting efficiencies approaching 100% are possible, as demonstrated with poly(γ-propargyl-L-glutamate), to which azide-terminated PEG with molar masses in the range 750−5000 g mol^{-1} has been attached (Figure 4).[54] The conjugates adopt a α-helical secondary structure. Alkyne−azide click chemistry was used to link PEGA to a GGRGDG peptide.[55]

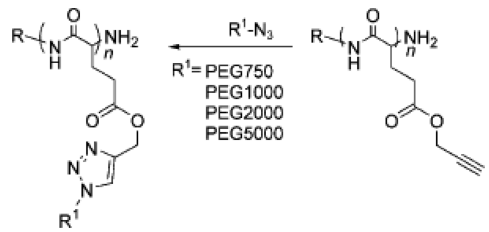

Figure 4. Clicking of PEG to poly(γ-propargyl-L-glutamate).[54] Reproduced with permission from ref 54. Copyright 2009 WILEY-VCH Verlag GmbH & Co. KGaA. http://onlinelibrary.wiley.com/doi/10.1002/anie.200904070/abstract.

Peptidomimetics have also been linked via click ligation to PEG, for example, using alkyne-functionalized peptidomimetics and α,ω-diazido-PEG.[56] Other examples of click reactions are provided in the following sections.

2.1.3. Noncovalent Coupling. Reversible coupling can be achieved using noncovalent chemistries. For example proteins (or peptides) may be tagged with the commonly used (in affinity chromatography) hexahistidine motif, which is recognized by the complementary nickel−nitriloacetic acid (Ni-NTA) complex on end-modified PEG.[57] This has been proposed as a facile, reversible PEGylation method for the screening of therapeutic proteins in vivo.

The interaction between heparin and a heparin-binding growth factor (VEGF) has been used to prepare noncovalent hydrogels of heparin-terminated four-arm PEG and VEGF.[58] The presence of VEGF receptors on endothelial cells lead to erosion of the hydrogels, producing a biomaterial responsive to a specific receptor cue.

The biotin−streptavidin complex has been used to couple polymers and proteins noncovalently;[1] however, we are not aware of the use of this high-binding-affinity system to prepare PEG−peptide conjugates.

2.2. Peptide Synthesis from PEG Chains ("Grafting to"). *N*-Carboxyanhydride (NCA) polymerization from PEG macroinitiators enables the synthesis of a range of polymer−peptide conjugates.[59,60] NCA of homopolypeptides such as poly(L-proline) as well as copolymers from PEG macroinitiators has been demonstrated, and self-assembly in the solid state was noted.[61] The polyproline II secondary structure was adopted in water. NCA polymerization has also been used to polymerize L-alanine from amino-terminated PEG.[62] It has also been used to prepare PEG-poly(Z-L-lysine) diblocks with

dodecylamine or α-naphthylamine (as a fluorophore) between the two blocks.[63]

A variety of chemistries have been employed using PEG as a cleavable support for peptide synthesis (prior to cleavage, PEG−peptide conjugates are produced).[64] PEGylated resins are available commercially (see below). The PEG chain is connected to the peptide via a linker, of which examples include those that are photocleavable, acid- or base-labile, or thiol-labile. A method to prepare 9-fluorenylmethyl chloroformate (Fmoc)−PEG amino acids to incorporate PEG spacers within peptides, compatible with solid-phase synthesis methods, has been employed by several groups using PEG−amino acids with 3−8 spacers.[65−67] This builds on earlier work in which shorter ethylene glycol spacers were incorporated into disulfide-bridged peptides.[68]

A method to convert synthesized PEG (up to EG_{29}) diols into Fmoc−amino acids compatible with solid-phase peptide synthesis has been reported.[69] These were used to synthesize (on resin) a peptide−PEG_{17}−folate conjugate incorporating a folic acid cysteine-targeting ligand as well as an N-terminal amphipathic peptide with high transfection efficiency. The peptide−PEG_{17} conjugate was coupled to the cysteine-folate ligand using maleimide coupling chemistry. Monodisperse Fmoc−PEG−COOH with a range of PEG (PEG_2−PEG_{36}) are now available commercially (from Quanta Biodesign)[17] and have been used to prepare PEG−peptide conjugates such as PEG−FFKLVFF−COOH.[70]

PEG-functionalized resins are commercially available under the trade name Tentagel PAP (from Rapp Polymere).[71−73] These comprise cross-linked polystyrene beads to which PEG chains are preattached with a labile benzyl ether linkages. A range of PEG molar mass beads is available. The method was originally developed to attach PEG to improve the solubility of hydrophobic peptides or lipopeptides,[71] although recent attention has focused on the interesting self-assembly properties arising from the amphiphilicity of PEG−peptide conjugates.

Mutter et al. developed liquid-phase synthesis of peptides up to 20 residues from linear PEG (5−20k) supports,[74] although the method has yet to find widespread application.[75] These authors prepared PEG with several acid- and photocleavable linkers for the synthesis of a series of test peptides.

PEG chains have been used as supports for liquid-phase peptide synthesis, as reviewed in detail elsewhere.[64] In general, the synthesis has been carried out in organic solvents, although some examples in water have been reported (using 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide (EDC) instead of DCC). Figure 5 shows a direct esterification method to produce, in this case, a bifunctional conjugate. This method has been used with PEG up to PEG20k and for up to 14-residue peptides.[76] Fragments of the peptide secretin have been synthesized on a PEG10k support, which provides a solubilizing protecting group.[77−79] The stepwise synthesis of a fragment of insulin B was performed using a PEG3k support.[80] Liquid-phase methods were used to prepare PEG−peptides incorporating PEG10k and oligo-glycine peptides with 1−9 glycine repeats (β-sheet structures were observed for conjugates with longer glycine sequences at low dilution in chlorinated solvents).[81] Similarly, t-Boc-X_n-G-PEG conjugates were prepared with X = Ala, n = 1−8 or X = Val, n = 1−6.[82]

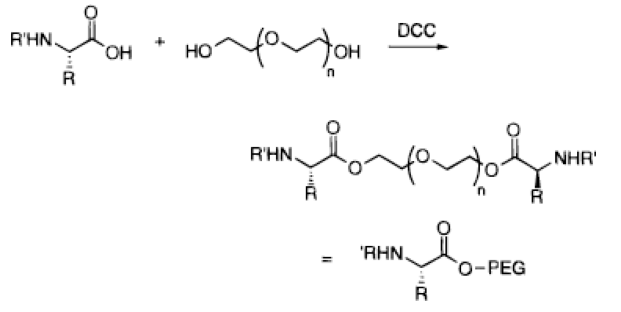

Figure 5. Direct esterification of PEG with amino acids using dicyclohexylcarbodiimide (DCC).[64] Reproduced from ref 64. Copyright 1997 American Chemical Society.

2.3. Polymer Synthesis from Peptide ("Grafting from"). In the grafting from method, initiators for polymerization methods such as ATRP may be incorporated at the peptide termini. This method has been widely used to prepare protein−polymer conjugates,[83] although this is outside the scope of the present review. The technique has also been used for polymer−peptide conjugates.[84] In one example, two different ATRP initiators were attached at the termini of a model matrix metalloprotease (MMP) substrate 11-residue peptide, and POEGMA was polymerized from the C terminus and poly(*N*-isopropylacrylamide) (PNIPAM) was polymerized from the N terminus.[85] This conjugate self-assembled into micelles under suitable conditions in aqueous solution, and enzymatic degradation of the peptide linker was demonstrated.

A reversible addition−fragmentation transfer (RAFT) agent has been appended to a solid-phase-synthesized peptide and used as a macroinitiator for RAFT and also via functionality shift for ATRP, which may be used to polymerize PEG (although this study involved polymerization of *n*-butyl acrylate).[86]

2.4. Synthesis of PEG Side-Chain Polymer−Peptide Conjugates. As an alternative to the attachment of linear PEG to peptides, it is often synthetically convenient to use PEG-rich polymers bearing PEG or oligo-ethylene glycol side chains such as acrylates or methacrylates, which are conveniently prepared by living radical polymerization methods such as RAFT (and ATRP, although this is also used directly to synthesize PEG).[16] Polymers with PEG "grafts" enable the incorporation of PEG of a defined length at a controlled density along the polymer backbone. Furthermore, certain PEG acrylates show very well-defined LCST behavior (i.e., they undergo a sharp coil−globule transition on heating). Incorporation of PEG in the monomer ("grafting through") is generally advantageous compared to grafting to, where quantitative functionalization can be hard to achieve.

Short PEG chains have been grafted to PBLG,[87] and PEG chains with M_w = 1k, 2k, and 3k have been grafted to poly(L-lysine),[88] with both showing moderate grafting densities. Poly(L-glutamates) with oligo-ethylene glycol (1, 2, or 3 repeats) have been prepared by NCA polymerization of ethylene glycol-modified amino acid monomers.[89] The PEGylated polymers exhibit LCST behavior in water. The LCST transition can be tuned via the composition (PEGylated monomer content) of the copolymer or the enantiomeric composition.[89] Cysteine-based C and CF peptides have been attached to polybutadiene chains in a PEO−polybutadiene diblock via free-radical addition.[90] Spherical or worm-like micelles or vesicles were observed depending on the hydrophobicity of the conjugate copolymers.

Langer's group studied polyhistidine polymers with PEG grafts (PEG5k) for gene delivery (plasmid DNA) and

compared these conjugates to linear PEG−polyhistidine diblocks.[91] Steric hindrance because of PEG led to a direct relationship between PEG content and the size of the complexes formed. The transfection efficiency was good, and cytotoxicity to macrophages was low.

2.5. Synthesis of Tethered PEG peptides. PEGylated peptides may be tethered to solid supports, for instance, to create functionalized surfaces for cells. Ulijn's group developed a system comprising amine-terminated PEG tethered to silica surfaces in which initially blocked cell adhesion peptide motifs were subsequently exposed via enzymatic cleavage of the terminal blocking group.[92,93] In a first example, PEGA-coated surfaces were created with reactive amine groups for the sequential coupling with Fmoc−amino acids to produce PEGA−DGRF−Fmoc. Because proteases such as α-chymotrypsin cleave on the carboxylic side of phenylalanine residues, they can be used to remove the blocking Fmoc unit, as was demonstrated, leading to the spreading of osteoblast cells.[92] In a second example, Fmoc−amino acids were then coupled to the PEG−amine to produce PEG−DGRAAFmoc.[93] The Fmoc unit serves as a blocking group that can be removed by enzymatic cleavage at the alanine residues using elastase, thus exposing the cell adhesion RGD motifs. PEG/RGD-based peptide conjugates grafted to solid substrates have been studied as supports for cell growth and differentiation.[51,93]

3. SELF-ASSEMBLY OF β-SHEET PEPTIDE−PEG CONJUGATES

Lynn and co-workers investigated the fibrillization of conjugates of PEG with amyloid β (Aβ) peptide fragments (Figure 6).[94] Specifically, they studied the self-assembly of Aβ(10−35)−PEG3k. In contrast to the Aβ(10−35) peptide itself, conjugation to PEG was found to enhance the solubility and led to concentration-dependent reversible fibrillization. The fibril dimensions (of the peptide core and PEG corona) were determined via contrast matching small-angle neutron scattering (SANS) experiments,[95] and the pH dependence of these parameters was also examined.[96]

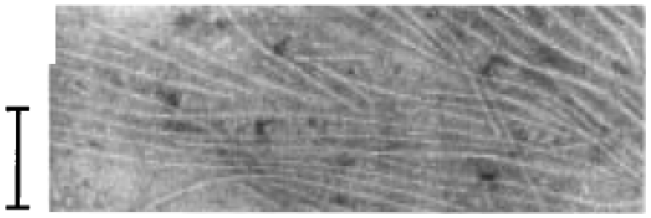

Figure 6. TEM image of fibrils formed by Aβ(10−35)−PEG3000.[94] The scale bar is 200 nm. Reproduced from ref 94. Copyright 1998 American Chemical Society.

Conjugates comprising an N-terminal alkyl chain attached to the amyloid-forming hexapeptide KTVIIE and C-terminal PEG3000 were observed to form fibrils. These could be disassembled by removal of the alkyl chains via a UV-active photolabile nitrobenzyl group.[97]

A diblock conjugate incorporating the peptide QQKFQFQFEQQ and a PEG3.7k block self-assembles into β-sheet fibrils.[98] The peptide is designed as a transglutaminase substrate for enzymatic biofunctionalization of the supramolecular structure. A diblock conjugate containing PEG440 and the truncated peptide KFQFQFQ also formed fibrils (the corresponding peptide−PEG−peptide conjugate was not soluble in water), although the analogous peptide-terminated triblock did not (because of insolubility in aqueous solution). The morphology of fibrils formed by the PEG conjugates differed from those of the parent peptides.[98]

PEGylation can hinder β-sheet formation. This is exemplified by a study on a designed alanine-rich peptide.[99] The peptide self-assembles into helices under ambient conditions at acidic pH but converts into β-sheets at high temperature. PEGylated peptides (conjugated to PEG5k or PEG10k) show similar behavior; however, β-sheet formation at elevated temperature is slowed, and there is reduced cooperativity in the thermally induced unfolding.[99]

Nematic and hexagonal columnar-phase formation in aqueous solution by the peptide−PEG conjugate FFKLVFF−PEG3k was also noted.[73,100,101] The peptide is based on a fragment of the amyloid β peptide, KLVFF, Aβ(16−20) extended at the N terminus by two phenylalanine residues. This peptide amphiphile forms core−shell cylindrical fibrils. The PEG coronas around the peptide fibrils are expected to mediate the purely repulsive interactions because attractive interactions can result from interpenetration of coronal chains. It is interesting to consider how the balance of electrostatic and steric (soft elasticity from polymer corona) interactions influence the packing constraints that lead to the nematic and hexagonal columnar phases observed in these PEG−peptide conjugates. Nematic ordering has also been reported for a

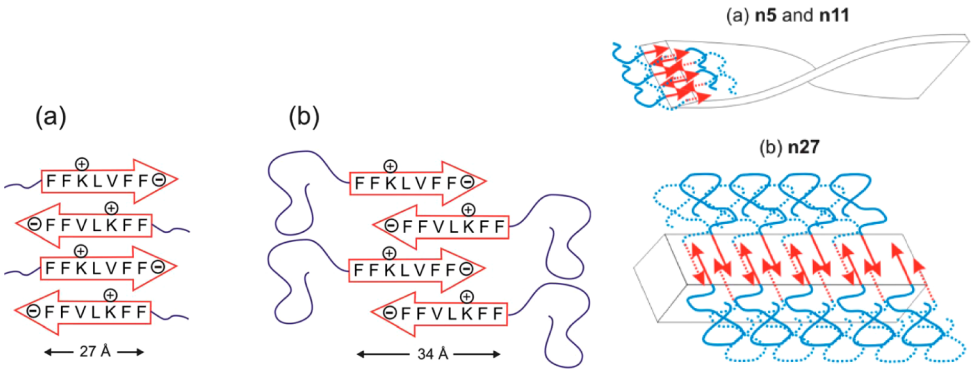

Figure 7. (a, b) Schemes for the packing of FFKLVFF β-strands based on X-ray diffraction for $(EG)_n$−FFKLVFF−COOH with (a) n = 5 and 11 and (b) n = 27. (c) Scheme for self-assembled structure based on stacking of β-sheets shown in panels a and b.[70] Reproduced with permission from ref 70. Copyright 2012 The Royal Society of Chemistry. http://pubs.rsc.org/en/content/articlehtml/2012/sm/c2sm25546d.

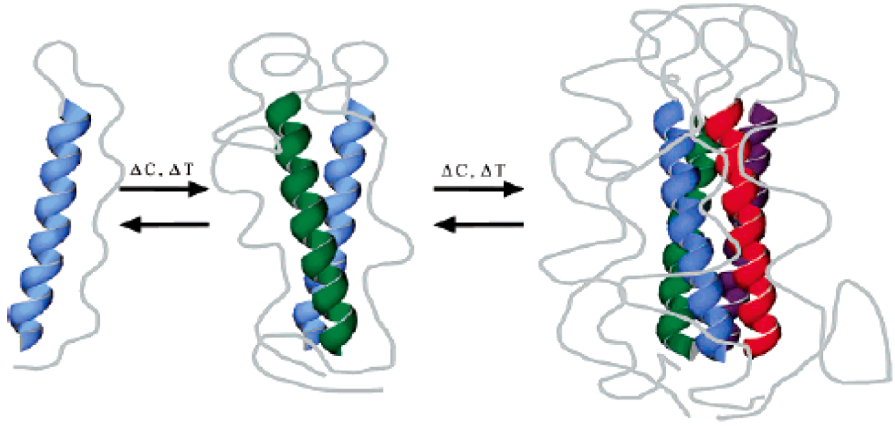

Figure 8. Proposed model for the self-assembly of PEG−peptide conjugates containing the coiled-coil peptide GEAK(LAEIEAK)$_2$−LAEIY−Am.[110] Reproduced from ref 110. Copyright 2003 American Chemical Society.

PEG−peptide amphiphile containing a two-tailed peptide motif attached to a rigid aromatic branch point.[102] This forms tape-like aggregates that show nematic ordering at higher concentration.

The effect of PEG molar mass on the self-assembly of FFKLVFF−PEG with PEG1k and PEG2k (both C-terminal PEG) as well as PEG10k−FFKLVFF (N-terminal PEG) was also investigated.[103] The three FFKLVFF−PEG hybrids form fibrils comprising a FFKLVFF core and a PEG corona. The β-sheet secondary structure of the peptide is retained in the FFKLVFF fibril core. At sufficiently high concentration, FFKLVFF−PEG1k and FFKLVFF−PEG2k form a nematic phase, whereas PEG10k−FFKLVFF exhibits a hexagonal columnar phase. Simultaneous small-angle neutron scattering/shear flow experiments were performed to study the shear flow alignment of the nematic and hexagonal liquid crystal phases. On drying, PEG crystallization occurs without disruption of the FFKLVFF β-sheet structure, leading to characteristic peaks in the X-ray diffraction pattern and FTIR spectra (also see Section 7). The stability of β-sheet structures was also studied in blends of the FFKLVFF−PEG conjugates with poly(acrylic acid) (PAA), which can hydrogen bond to PEG, potentially modifying self-assembly. Although PEG crystallization was observed only up to 25% PAA content in the blends, the FFKLVFF β-sheet structure is retained up to 75% PAA.

The influence of the PEG chain length on the self-assembly of N-terminal PEGylated FFKLVFF−PEG conjugates has been examined.[70] Three (EG)$_n$−FFKLVFF−COOH conjugates were studied, where EG denotes ethylene glycol and n = 5, 11, or 27 is the PEG degree of polymerization. Importantly, these samples are based on commercially available (Quanta biodesign) monodisperse ethylene glycol oligomers. For these model conjugates, where PEG polydispersity effects are eliminated, X-ray diffraction revealed different packing motifs dependent on PEG chain length (Figure 7). This is correlated to remarkable differences in self-assembled nanostructures depending on PEG chain length. The control of strand registry points to a subtle interplay between aromatic stacking and electrostatic and amphiphilic interactions.

The influence of PEG molar mass on the self-assembly of FFFF−PEG has been examined. Nanotubes were observed for a conjugate with a low PEG molar mass (350 g mol^{-1}),[104] whereas at higher PEG molar mass (studied in the range 1.2−5k), fibrils are observed.[104,105] The nanotubes comprise antiparallel β-sheets that are stabilized by $\pi-\pi$ stacking of the aromatic residues.[106] Soft hydrogels arising from nanotube entanglements are reported at higher concentration.[106]

The self-assembly into β-sheet fibrils of the conjugate DGRFFF−PEG containing the RGD cell adhesion motif (attached N terminally), three F residues to ensure amphiphilicity, and PEG3k in aqueous solution has been observed.[107] The adhesion, viability, and proliferation of human corneal fibroblasts were examined for films of the conjugate on tissue culture plates (TCP) as well as low-attachment plates. On TCP, DGRFFF−PEG3k films prepared at sufficiently low concentration are viable, and cell proliferation was observed. However, on low-attachment surfaces, neither cell adhesion nor proliferation was observed, indicating that the RGD motif was not available to enhance cell adhesion. This was ascribed to the core−shell architecture of the self-assembled fibrils with a peptide core surrounded by a PEG shell, which hinders access to the RGD unit.

Tape structures were observed for a PEG−peptide containing PEO68 and a peptide incorporating a (TV)$_4$−ester−VG "switch" peptide sequence that forms β-sheet tape structures upon increasing the pH to 6.2 in aqueous solution because of an O → N acyl switch.[108] The authors observed fibrils for a related conjugate comprising PEG linked via a dibenzofuran-based linker to two tails containing the VTVT peptide.[109] It was pointed out that short peptide end groups can be used to direct the ordering of PEO into fibrillar structures.

4. SELF-ASSEMBLY OF COILED-COIL AND α-HELICAL PEPTIDE−PEG CONJUGATES

Klok and co-workers have observed that the coiled-coil structure of designed peptides can be retained upon conjugation to PEG.[110] The peptides are based on a de novo sequence designed by Hodges et al.[111] and incorporate two heptad LAEIEAK sequences conjugated to PEG with n = 15 or 40. An equilibrium between unimers and dimeric and tetrameric coiled-coil aggregates was proposed, with increases in concentration or temperature favoring the more aggregated state (Figure 8).[110] The close folding of PEG around the coiled-coil structures was indicated by electron paramagnetic resonance (EPR), which also pointed to a parallel alignment of the helices, as least for the dimeric species.[112]

A later study focused on the secondary structure in a related series of coiled-coil peptides with substitutions among the charged residues, which compared PEG conjugates (PEG$_n$, n =

15 and 40) and the parent peptides.[113] The stability of the secondary structure against changes in concentration and pH was analyzed. Substitutions of E and K residues did not influence the ability of the peptides and conjugates to form coiled-coil structures. The biocompatibility of the conjugates was also examined via hemolysis assays. The stability of PEGylated conjugates (compared to the parent peptide) based on this type of coiled-coil peptide against changes in pH and concentration was studied via CD spectroscopy.[114] In addition, several switch peptides,[115] which are designed to switch between α-helical and β-sheet structure depending on pH, were investigated in comparison with their PEG conjugates.[114] The conjugates of the coiled-coil peptides exhibited reduced concentration-dependent changes in α-helix content, although the coiled-coil structure was retained. The switch peptide-PEG conjugates exhibited enhanced stability of the coiled-coil structure against pH increase. Solid-state structures were also examined by TEM and AFM.

The formation of heterodimers between pairs of E- and R-rich coiled-coil peptides was not impaired by their incorporation into alternating multiblock PEG copolymers.[23]

The influence of PEG2k on the secondary structure of a series of peptides $(VSSLESK)_n$ with n = 3−6 was examined using CD spectroscopy.[116] The conjugates with longer peptides n = 5 and 6 adopted a two-stranded α-helical structure in aqueous solution. PEG was found not to interfere with the formation of α-helices for the shorter conjugates as well. The thermal stability of the conjugate with n = 6 was higher than that of the corresponding peptide.

The conformation of the PEG chain attached laterally to coiled-coil 3- or 4-helix bundle peptides (30-mers) via cysteine residues using maleimide-functionalized PEG was examined by SANS.[117] The form factor was described by a cylinder (for the coiled-coil structure) and a Gaussian coil for the PEG conformation.

Complexation of PEG−K and PS−E containing α-helical peptides termed E ($G(EIAALEK)_3$) and K ($((KIAALKE)_3G$) with opposite charges led to the formation of heterocoiled coils and thus a noncovalently linked PS−E/K−PEG triblock (Figure 9), which self-assembles into rod-like micelles.[118] In related work, the coiled-coil conjugate K−PEG_{77} was prepared as part of a study on complexation with diblocks containing oppositely charged coiled-coil sequences $(EIAALEK)_3$ and PBLG.[119] Vesicle formation was observed in the case of complexes formed with lower molar mass ($PBLG_{36}$) diblocks.

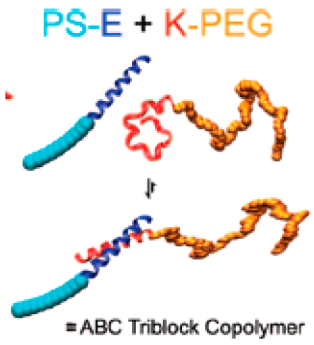

Figure 9. Heterocoiled-coil formation between glutamic acid and lysine-containing helical peptides attached to polystyrene and PEG chains leads to the formation of a noncovalent ABC triblock copolymer.[118] Reproduced from ref 118. Copyright 2008 American Chemical Society.

Oligo-ethylene glycol (EG_2) has been attached to a protein with a grafted minimal p53 tumor suppressor peptide sequence to improve the solubility of the α-helix-stabilized self-assembled peptide nanostructure.[120]

5. SELF-ASSEMBLY OF PEPTIDE−PEG−PEPTIDE, PEG−PEPTIDE−PEG, AND RELATED CONJUGATES

Telechelic peptide−PEG−peptide conjugates containing hydrophobic peptides offer the possibility to create hydrogels by physical association of the terminal peptide domains. This has been further enhanced with disulfide cross-linking (Figure 10) of conjugates based on terminal peptides incorporating designed six-heptad leucine zipper coil motifs.[121] The unstructured midblock comprises $((AG_3)PEG)_{10}$. Intermolecular interactions between these triblock constructs was later probed by AFM force measurements under extension/retraction, and the adhesive interactions between molecules were found to increase upon lowering the pH.[122] The influence of the end blocks on the erosion rate of this type of leucine zipper peptide hydrogel was examined.[123] Erosion was much slower for hydrogels constructed from triblocks with dissimilar end blocks compared to those with the same end groups, an effect ascribed to reduced chain looping in the heterotelechelic constructs.[123] Similar constructs were later developed to create cell adhesive materials by incorporation of the RGDS cell adhesion motif or heparin-binding motifs into the terminal coiled-coil peptide domains.[124] Furthermore, cross-linked coatings could be produced by 1-ethyl-3-(3-(dimethylamino)-propyl) carbodiimide (EDC)-mediated cross-linking of proximal Glu and Lys residues.

A triblock comprising PEG end blocks and a central peptide block of silk-like tandem $(AG)_3EG$ repeats forms fibrils in aqueous solution irrespective of PEG with a molar mass in the range 750−5000 g mol^{-1} is used.[35]

In another example, collagen-mimetic peptides were attached to four-arm PEG. Hydrogels were formed via physical cross-links mediated by thermally reversible triple helical assembly of collagen-mimetic peptides.[125] Hydrogel formation by a peptide−PEG−peptide conjugate containing peptides derived from the coiled-coil region of fibrin has been investigated.[126] The hydrogels may have application in tissue engineering and/or wound healing.

A thermoplastic hydrogel has been produced from a hexablock copolymer containing PEO and PBLG, with a complex architecture comprising four PBLG-bearing arms, two of which also contain PEO.[127] The thermoplastic properties may be due to microphase separation within the concentrated hydrogels.

The self-assembly in aqueous solution of peptide−PEG−peptide conjugates comprising aromatic dipeptides linked telechelically to PEG1.5k has been examined.[128] The role of capping Fmoc (*N*-fluorenyl-9-methoxycarbonyl) units at one or both termini was also examined. A self-assembled β-sheet fibril-based hydrogel was identified for a conjugate containing dityrosine end groups (and a C-terminal Fmoc unit), which exhibits a gel−sol transition near body temperature (Figure 11).[128] This thermoresponsive PEG-based biofunctional hydrogel is expected to have diverse potential uses in delivery or diagnostics for biomedical applications. Another group later prepared similar telechelic conjugates of desaminotyrosine or desaminotyrosyl-tyrosine with a linear PEG3k midblock and

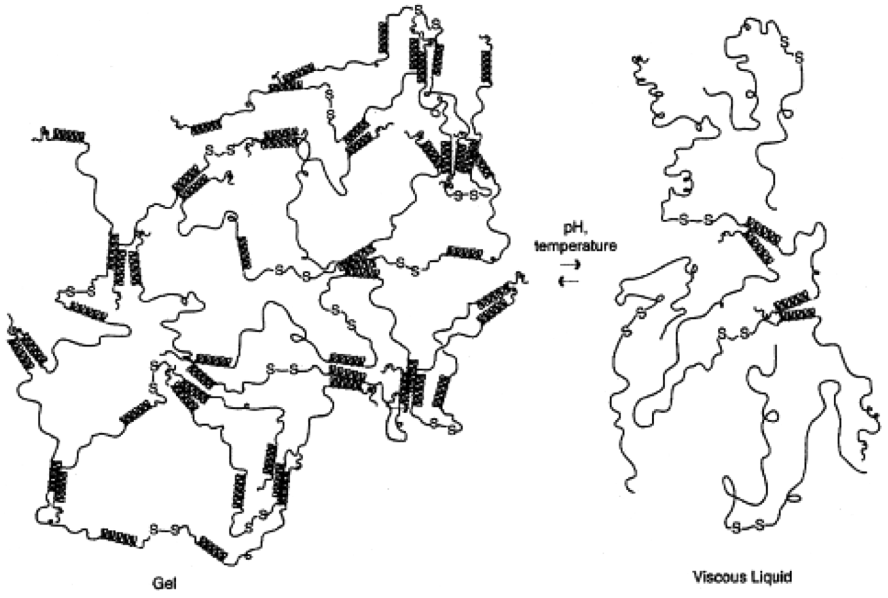

Figure 10. Proposed structure of gels formed by a peptide−PEG−peptide conjugate containing α-helix end blocks and C-terminal cysteine residues.[121] The hydrogel breaks down upon increasing pH or temperature. Reprinted with permission from ref 121. Copyright 1998 AAAS. http://www.sciencemag.org/content/281/5375/389.full.html.

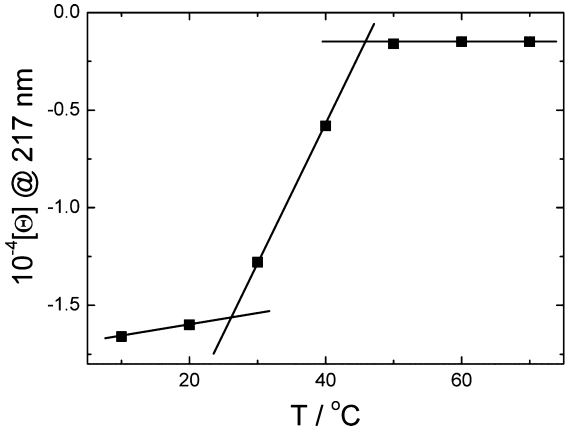

Figure 11. Temperature dependence of molar ellipticity at 217 nm (measuring β-sheet content) of an aqueous solution (1.3 wt %) of conjugate NH_2−YY−PEG_{35}−YY−Fmoc.[128] There is a large decrease in β-sheet content centered around 37 °C. Reproduced with permission from ref 128. Copyright 2011 WILEY-VCH Verlag GmbH & Co. KGaA. http://onlinelibrary.wiley.com/doi/10.1002/mabi.201100022/abstract.

also four-armed−PEG conjugate. A critical aggregation concentration was noted for the linear midblock conjugate, pointing toward self-assembly at high concentration.[129]

As mentioned in Section 2.2, Fmoc−PEG amino acids have been developed that incorporate oligo-ethylene glycol spacers. Boumrah et al. developed the synthesis of these residues, consistent with solid-phase peptide synthesis methods, and incorporated triethylene glycol repeats into their disulfide-bridged atrial natriuretic factor peptide analogues.[68] A tyrosine kinase-based tetrapeptide has been incorporated within pYTGL−$(ethylene\ glycol)_n$−pYETL conjugates (pY, phosphorylated tyrosine) with n = 4 or 6.[67] The spacer leads to an affinity between the two linked phosphopeptides that is comparable to that between the two sequences in the full native peptide, showing that the PEG spacer can substitute for the intervening amino acids. PEG spacers have been incorporated into multivalent (four-arm dendron) peptide constructs with peripheral cyclo(RGDfE) integrin-targeting units attached via hexaethylene glycol spacers linked to terminal lysine units.[65] Peptides incorporating PEG spacers have been developed for applications in gene delivery. A DNA-binding lysine sequence (K_{16}) was separated from a disulfide-bridged integrin-binding sequence (CRRETAWAC) by oligo-ethylene glycol spacers with 3−8 repeats.[66] The PEG spacer delivered enhanced stability in buffer compared to control (peptide lacking PEG spacer).

A $L_4K_8L_4$VPRGS−PEO conjugate containing a VPRGS substrate for thrombin self-assembles into β-sheet fibrils in response to addition of the enzyme (in contrast to a peptide containing a scrambled peptide sequence).[130] Heterotelechelic conjugate PS−L_4−PEO (PS, polystyrene), a so-called peptide-inserted triblock copolymer with a tetraleucine β-sheet-forming midblock, forms vesicles in aqueous solution.[131] This peptide was prepared starting from a Tentagel PAP PEGylated resin (Section 2.2). ATRP of PS was then performed from a brominated L_4−PEG macroinitiator.

The solid-state morphology of a silk-based multiblock ABCD copolymer where A, GAGA; B, EO_5; C, AGAG; and D, EO_{13} has been investigated (**P1**, Figure 12).[132] In these copolymers, PEG replaces the amorphous domains within the native silk structure (Figure 12a). Microphase separation was noted with 20−50 nm peptide domains within a continuous PEO matrix. In later work, the solid-state structure of ABCD multiblocks containing the *Bombyx mori* (silk worm) sequence GAGA was compared to that of multiblocks containing the *Nephila clavipes* spider silk-type sequence AAAAAA (**P2** and **P3**, Figure 12). The formation of antiparallel β-sheets was observed in both cases. The copolymers containing the oligo-alanine sequence exhibited a higher modulus than those of the GAGA copolymers, and this was ascribed to the presence of physical cross-links.[133]

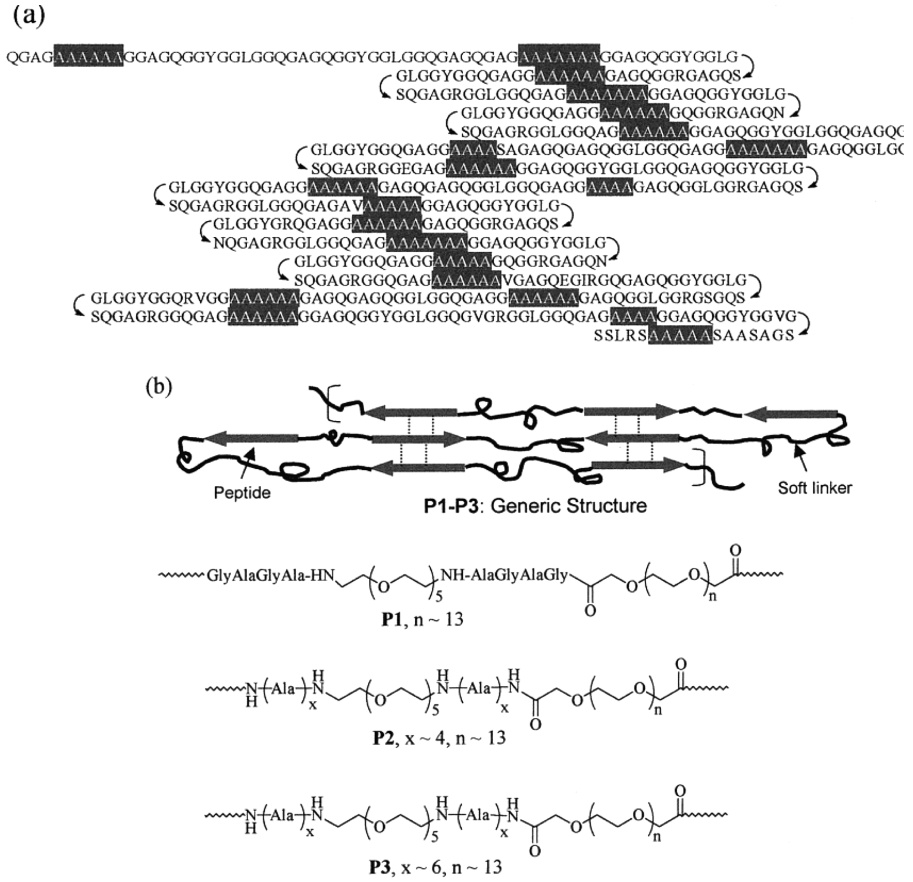

Figure 12. (a) Native sequence of *N. clavipes* major ampullate showing the alanine-rich β-sheets (highlighted) and glycine-rich amorphous regions. (b) Generic structure of designed silk-inspired segmented multiblock copolymers **P1**−**P3** in which the amorphous segments are replaced by flexible PEG blocks.[133] Reproduced from ref 133. Copyright 2001 American Chemical Society.

6. SELF-ASSEMBLY OF PEG−PEPTIDE CONJUGATES CONTAINING SYNTHETIC HOMOPOLYPEPTIDES

6.1. Self-Assembly in the Solid State. In the solid state, PBLG−PEG conjugates undergo microphase separation of the components of the block copolymers. This leads to hierarchical ordering because of the additional shorter length scale periodicities resulting from the packing of the PBLG, its secondary structure, and the crystal structure of PEG (if the molar mass is sufficiently large). Floudas et al. reported that the phase behavior of PBLG−PEG−PBLG triblocks depends on the composition, specified as the peptide volume fraction, f.[134] For copolymers with low peptide volume fractions ($f < 0.4$), microphase separation was noted along with α-helical or β-sheet ordering of PBLG (depending on chain length) and chain-folding of crystalline PEG.[134] In contrast, for $f > 0.4$, no regular microphase-separated structures were noted, and only an α-helical secondary structure of PBLG was observed. Our group subsequently examined the microphase-separated morphology within the same series of triblock copolymers via SAXS, AFM, and TEM experiments.[135] A phase diagram was presented, including ordered structures that were observed for some samples with $f > 0.4$. The PBLG adopts a mixture of α-helical and β-sheet structure for low chain lengths ($n < 18$), but it is purely α-helical when the degree of polymerization is larger.

Microphase separation into a lamellar structure was observed in the solid state (solvent cast samples) for a series of PBLG−PEO-PBLG triblocks containing 25−75% PBLG.[136] The WAXS pattern was dominated by the contribution from the α-helical PBLG. This group also observed microphase separation in the films cast from a PCLL−PEO−PCLL (PCLL, poly(ε-benzyloxycarbonyl-L-lysine)) triblock.[137] Lamellar ordering has been reported in the solid state of di- and triblock copolymers of PEO and poly(DL-valine-*co*-DL-leucine).[138]

Ordering in the solid state has been observed for complexes of a PEG−PLGA (PLGA, poly(L-glutamic acid)) diblock with *n*-alkylamines (octadecylamine, dodecylamine, and octylamine).[139] Hierarchical ordering was observed, ranging from the length scale associated with PEG crystallization to the PLGA−alkylamine complexes (lamellar structure for the octadecylamine complex) as well as microphase separation of the block copolymer (also lamellar).

The morphology and mechanical properties in the solid state of a PBLA−PEO−PBLA (PBLA, poly(benzyl L-aspartate)) triblock have been examined.[140] The PBLA chains adopted a α-helical conformation, whereas the PEG also crystallized, although an increase in β-sheet content of the PBLA chains was noted upon cooling following heating above the PEO melting temperature.

Microphase separation in the solid state was suggested as a result of the observation of two distinct thermal transitions by DSC for a poly(L-alanine)−PEG conjugates.[62]

6.2. Self-Assembly in Solution. In solution, PEG−polyelectrolyte diblocks based on charged synthetic peptides can self-assemble into vesicles. In one study, the kinetics of the

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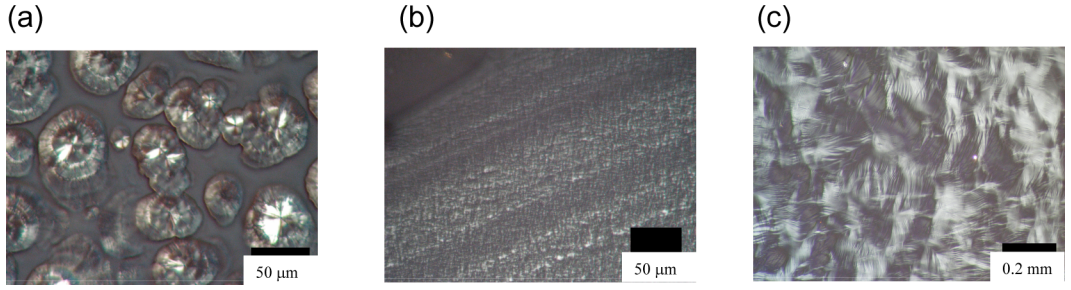

Figure 13. Polarized optical microscopy images showing spherulite formation for (a) KLVFF−PEG but not (b) AAKLVFF−PEG or (c) FFKLVFF−PEG.[150] Reproduced from ref 150. Copyright 2008 American Chemical Society.

growth of PEG−poly(aspartic acid) unilamellar vesicles via 2D supramolecular polymerization has been monitored by dynamic light scattering.[141] A four-armed H-shaped $(PzLL)_2$−PEG−$(PzLL)_2$ conjugate (PzLL, poly(ε-benzyloxylcarbonyl-L-lysine)) self-assembles into vesicles in aqueous solution.[142] Furthermore, the vesicles can be loaded with the hydrophobic drug doxorobucin and delivered to human breast cancer cells.

PEG−peptide diblock copolymers can form polyion complex micelles (PICs) by pairwise association of diblocks containing oppositely charged blocks. In one example, PICs were observed in aqueous solutions of PEG−poly(aspartic acid) (P(Asp)) and PEG−poly(lysine) (P(Lys)).[143] Vesicle formation by complexation of a pair of diblocks containing oppositely charged blocks has been observed.[144] The vesicles, termed PICsomes, were formed by complexation of PEG−P(Asp) (anionic) with PEG−P(Asp-AE) or PEG−P(Asp-AP), where these cationic blocks are prepared by aminolysis of poly(β-benyl-L-aspartate). These vesicles were shown, by fluorescence imaging, to be semipermeable. Triblock copolymers can self-assemble into three-layer micelles. In one example, micelle-forming PEG−PMPA−PLL copolymers were investigated for DNA condensation.[145] The PLL forms the micelle core, with a poly(3-morpholinopropyl) aspartamide (PMPA) buffering inner layer and a biocompatible PEG outer layer. Enhanced transfection of DNA into HeLa cells was observed for the three-layer micelles compared to those from PEG−PLL.

A thermoresponsive conjugate of PEG and poly(γ-(2-methoxyethoxy)esteryl-L-glutamate) has been prepared by ring-opening polymerization.[146] Extended annealing times drive a transition in peptide secondary structure from helical to β-sheet, leading to a concomitant nanostructure transition from worm-like micelles to nanoribbons.

The conformational properties (α-helical structure) of PBLA in a PEO−PBLA diblock in organic solvents has been examined by NMR and optical rotation measurements.[147]

Charged polypeptide blocks within self-assembling block copolymers can form complexes with oppositely charged species, leading to novel nanostructures. Micelles were observed for PEO−PLL diblocks forming complexes with retinoic acid. The α-helix formed by PLL is stabilized over a wider pH range within the complexes.[148] This was also observed in the solid state and is in contrast to the mixed α-helix/β-sheet structure for the PLL block in the uncomplexed copolymer. The micelle core contains a smectic-like PLL−retinoic acid complex. Nanoparticles were observed upon complexation of a PEO−PGlu (PGlu, poly(L-glutamate)) conjugate with diminazene.[149] Under pH 7.4 conditions where the PGlu adopts a random-coil structure, complexation led to a transition to α-helical structure.

7. PEG CRYSTALLIZATION EFFECTS ON SELF-ASSEMBLY

For PEG of sufficiently high molar mass, crystallization is observed in the dry state. This can influence the nanostructure observed for PEG−peptide conjugates, for example, by TEM, where the specimen is dried. We have found that PEG crystallization can overwhelm peptide fibrillization if the latter is not strong. This was investigated for a series of PEG−peptide conjugates: FFKLVFF−PEG3k, AAKLVFF−PEG3k, and KLVFF−PEG3k. Fibrillization, as characterized in particular by the presence of a cross-β amyloid structure as well as by the macroscopic morphology, was disrupted for the conjugate containing the weak fibrillizer KLVFF, whereas fibrils were retained (on drying) for the conjugates containing the stronger fibrillizers AAKLVFF and FFKLVFF containing additional hydrophobic AA or FF units.[150,151] The formation of spherulites resulting from PEG crystallization is observed for the KLVFF conjugate but not for the other two (Figure 13).[151] The fibrillization strength depends on the number of hydrophobic, particularly aromatic, residues; indeed, several tables summarizing different assessment methods of β-sheet propensity show that phenylalanine particularly favors this secondary structure.[152−155] For AAKLVFF−PEG and FFKLVFF−PEG, the alignment of peptide fibrils also drives the orientation of the attached PEG chains.[150] These results highlight the importance of the antagonistic effects of PEG crystallization and peptide fibril formation in the self-assembly of PEG−peptide conjugates.

As mentioned in Section 3, the self-assembly and bioactivity of the peptide−polymer conjugate DGRFFF−PEG3k has been investigated.[107] At sufficiently high concentration, self-assembled β-sheet fibrillar nanostructures were observed. The fibrils are observed despite PEG crystallization which occurs on drying. This suggests that DGRFFF has an aggregation tendency that is sufficiently strong not to be hindered by PEG crystallization.

8. ENZYME-RESPONSIVE PEG−PEPTIDE CONJUGATES

An enzyme-responsive hydrogel has been prepared by Cu-catalyzed click chemistry from four- or eight-armed PEG capped with alkyne end groups and a bis-azido-funtionalized protease-sensitive peptide.[156] The peptide sequence D-Ala-Phe-Lys is sensitive to plasmin and trypsin, leading to biodegradable hydrogels.

A PEG−peptide micelle system that can be enzymatically cleaved, leading to the release of unaggregated amyloid-based peptides, has been reported.[157] The βAβAKLVFF−PEG3k conjugate incorporates the βAβAKLVFF peptide based on the

amyloid β peptide sequence Aβ(16−20) with two N-terminal β-alanine residues. The enzyme α-chymotrypsin cleaves the conjugate to produce βAβAKLVF and F−PEG3k (Figure 14).[157] The hexapeptide does not aggregate into β-sheet structures, in contrast to the heptapeptide βAβAKLVFF that forms well-defined β-sheet ribbons.[158,159]

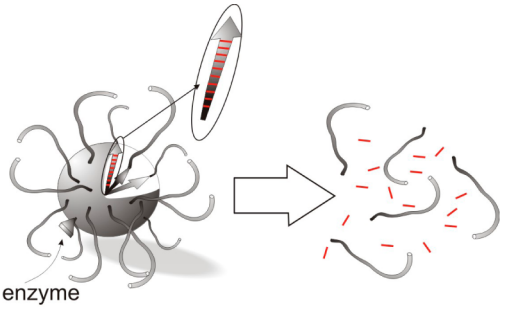

Figure 14. α-Chymotrypsin cleaves βAβAKLVFF−PEG3000 (βA denotes β-alanine), which self-assembles into spherical micelles with a PEG corona to produce F−PEG3000 and βAβAKLVFF, of which neither self-assemble.[157] Reproduced from ref 157. Copyright 2010 American Chemical Society.

Following a similar concept, it has been shown that PEG−peptide micelles with a peptide substrate (GPLGVRG) for MMP2 can be cleaved enzymatically, thus removing the PEG coating and leaving polyaspartamide-based nanoparticles that can form polyplexes with DNA.[160] These PEG-free polyplex micelles can be taken up by cells (with high endosomal escape) for gene delivery applications.

9. PEG−PEPTIDES FOR DRUG DELIVERY

It is now well-established that attachment of PEG improves the circulation time of PEGylated molecules as well as reducing renal clearance. Extended circulation lifetimes result from reduced recognition by the host response system (reduced immunogenicity) and reduced enzymatic degradation. This concept has been widely employed in the development of therapeutic materials, a subject that will not be reviewed here; instead, the focus will be solely on PEG−peptides used for drug delivery applications.

A review by Torchilin provides a number of examples where PEG−peptide micelles have been loaded with pharmaceutical compounds for drug delivery applications.[161] Kataoka and co-workers have also reviewed PEGylated block copolymers, including those incorporating polypeptides, in drug delivery and other biological applications.[162−165]

PEG−peptide conjugates containing poly(aspartic acid) (P(Asp)) have been employed by the Kataoka group and others in several studies on delivery of model drugs. These conjugates form PIC micelles that can be used to entrap drugs, enzymes, and other molecules through electrostatic interactions with the polyanionic peptide block. The enzyme lysozyme (positively charged in aqueous solution) was trapped within such micelles.[166] Later, hydrophobic aromatic groups were attached to the N-terminus of PEG−P(Asp) polymers to enhance association between the PIC micelles and lysozyme; this led to enhanced stability against increased NaCl concentration.[167]

Encapsulation within PIC micelles can improve the solubility of poorly soluble or hydrophobic molecules. For example, the anticancer drug adriamycin forms complexes through interactions between its amine group and the carboxyl unit of poly(aspartic acid), leading to its incorporation within micelles.[168−170] Reduced toxicity as well as enhanced stability in buffer was observed for the PIC micelles. This group later developed cleavable PIC micelles based on PEG−SS−P(Asp(DET)), where the disulfide linker can be reduced by glutathione in the cytoplasm and P(Asp(DET) denotes poly(aspartic acid) modified with *N*-(2-aminoethyl)-2-aminoethyl) or reductants such as dithiothreitol (Figure 15).[171] In earlier work, the Kataoka group used disulfide formation to produce PIC micelles from complexation of PEG−P(thiol-Lys) containing thiolated lysine and PEG−P(Asp).[172] The core could be cross-linked in the presence of oxygen or dissociated in the presence of a reductant such as DTT. Similar to poly(aspartic acid) diblocks, conjugates of PEG with poly(glutamic acid) (PLGA) have been used in a study on the delivery of the anticancer drug doxorubicin.[173] Following a similar concept, a hydrazone unit has been used as an acid-sensitive linker to release adriamycin from PEG−p(Asp-Hyd-ADR) conjugates (Hyd, hydrazone; ADR, adriamycin) in endosomes or lysosomes.[174] Complexation of a two-arm PEG−PLGA−cholesterol conjugate with a platinum-containing anticancer agent leads to the formation of metal-containing polymersomes.[175]

Several groups have investigated PLL−PEG conjugates for drug delivery, exploiting, in particular, the high cationic charge of the PLL for condensation of (anionic) DNA. This system offers the biocompatibility of the poly(L-lysine) block as well as the amphiphilicity of the diblock, which enables micellization. The binding of plasmid DNA was investigated in a series of papers on PEGylated polyplexes for gene therapy.[176−178] A series of diblocks with a fixed PEG12k block was studied, and the conformation of the PEG was found to depend on the PLL segment length. In another example, Hua et al. used PLL−PEG−PLL for slow-release drug delivery of encapsulated paclitaxel.[179] PLL−PEG diblock copolymers have been used as micellar drug delivery systems for DNA, showing an increased resistance to nucleases of plasmid DNA encapsulated in the core because of electrostatic interaction with PLL.[180]

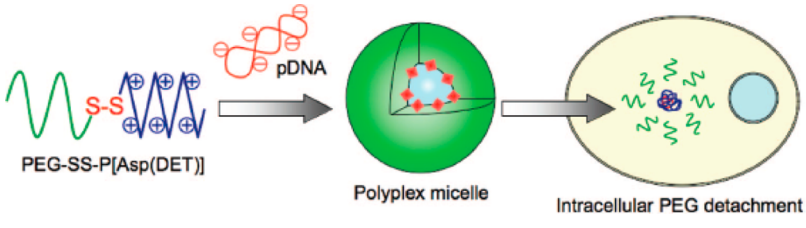

Figure 15. Cleavable disulfide linked PEG−poly(Asp(DET)) conjugate forms polyion complex micelles with plasmid DNA.[171] Glutathione in the cytoplasm causes PEG release. Reproduced from ref 171. Copyright 2008 American Chemical Society.

PLL-based PLL−PEG diblocks (containing poly(ε,*N*-(triiodobenzoy1)-L-lysine)) formed micelles that can be used as long-lived carriers for the contrast medium used for X-ray computed tomography.[181] PEG−PLL diblocks form PIC micelles with anionic dendrimer porphyrins, and this led to enhanced intracellular photodynamic efficiency.[182] Photodynamic therapy has been proposed as a means to treat disease, such as cancer, via light-triggered localized toxicity of sequestered nanoparticles or compounds.

Micelle forming PEG−peptides incorporating synthetic peptides such as PBLG or PBLA have similarly been used to encapsulate a variety of poorly water-soluble drugs. PEG−PBLG diblocks have been observed to self-assemble in aqueous solution, and the core of the micelles can be loaded with the benzodiazepine drug clonazepam.[183] The release rate decreases with increasing PBLG chain length. A PEG−PBLA diblock has been used to solubilize indomethacin, a nonsteroidal anti-inflammatory drug. The release rate was found to increase with pH in the range 1.2−7.4.[184]

In another application, PEGylated prodrugs have been prepared that incorporate insect-derived proline-rich antimicrobial peptides toward the development of novel antibacterial compounds.[185] The peptide prodrugs are released from the conjugate by serum proteases.

As shown for synthetic PEG diblock copolymers, the shape of nanostructures has a profound influence on circulation time in vivo: extended cylindrical micelles (sometimes termed filomicelles) have greatly extended circulation times compared to spherical particles, although the latter are more readily taken up by cells.[186] The enhanced persistence of fibrillar nanostructures may be relevant to PEG−peptide conjugates, which are often fibrillar, although a systematic study of this effect has yet to be carried out.

10. SUMMARY AND OUTLOOK

PEGylation of peptides offers advantages for biomedical applications such as drug delivery or the development of enzyme-responsive self-assembling biomaterials. PEG-based hydrogels containing peptide motifs have been shown to have excellent utility in the development of substrates for cell culture, with their ultimate application in areas such as tissue engineering although this is outside the scope of the present review.

A range of coupling chemistries have been developed that enable a rich diversity of PEG−peptides to be prepared, which also facilitates the synthesis of PEG−peptide conjugates with different architectures (starblock polymers and multiblock polymers). Grafting to PEG and grafting from reactions offer distinct capabilities. Peptides can be synthesized from PEG macroinitiators (grafting to), for example, via NCA polymerization incorporating noncanonical amino acids (PBLG, etc.), enabling longer chain lengths to be accessed than is possible with standard stepwise coupling peptide synthesis methods. However, PEG-based resins are available commercially and are compatible with Fmoc−peptide synthesis. Liquid-phase peptide synthesis has been demonstrated but is not currently widely used. Grafting from reactions involve transforming a protein peptide into a macroinitiator. Currently, this approach is used extensively to prepare protein-based macroinitiators for living radical polymerization methods such as ATRP or RAFT, as detailed earlier. Grafting through reactions enable the lateral attachment of PEG chains to provide PEG-stabilized chains. Furthermore, POEGMA-type copolymers with PEG side chains on a methacrylate or acrylate backbone show LCST behavior, which may be useful in the development of thermoresponsive peptide-functionalized materials.

β-Sheet PEG−peptide conjugates generally self-assemble into fibrillar structures in solution. PEGylation of coiled coils confers greater stability against changes in conditions (pH, temperature, concentration) without perturbing the secondary structure.

In the solid state, microphase separation is observed for β-sheet-based peptides such as those containing silk-like sequences. Lamellar and other morphologies are observed. However, PEG crystallization can overwhelm any microphase-separated structure if the peptide has a low β-sheet-forming propensity.

Many aspects of PEG−peptide conjugates suggest exceptional potential for further research. Examples include remodellable biomaterials, for example, photoresponsive hydrogels for cell growth and differentiation or polyion complex micelles/vesicles for drug delivery. The control of self-assembled nanostructure and exploitation of different modes of self-assembly in biomedical applications (e.g., influence of aggregate shape on encapsulated drug activity) are topics that greatly merit further research. Overall, this is likely to be a burgeoning field of activity in the coming years.

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Notes

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