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Protein- and Peptide-Modified Synthetic Polymeric Biomaterials

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Abstract

This review presents an overview on biohybrid approaches of integrating the structural and functional features of proteins and peptides with synthetic polymers and the resulting unique properties in such hybrids, with a focus on bioresponsive/bioactive systems with biomaterials applications. The review is divided in two broad sections. First, we describe several examples of biohybrids produced by combining versatile synthetic polymers with proteins/enzymes and drugs that have resulted in (1) hybrid materials based on responsive polymers, (2) responsive hydrogels based on enzyme-catalyzed reactions, protein–protein interactions and protein–drug sensing, and (3) dynamic hydrogels based on conformational changes of a protein. Next, we present hybrids produced by combining synthetic polymers with peptides, classified based on the properties of the peptide domain: (1) peptides with different conformations, such as α -helical, coiled-coil, and β -sheet; (2) peptides derived from structural protein domains such as silk, elastin, titin, and collagen; and (3) peptides with other biofunctional properties such as cell-binding domains and enzyme-recognized degradation domains.

Keywords

bio-hybrid; protein; peptide; biomaterials; hydrogel; assembly

INTRODUCTION

The integration of the structural and functional properties of peptides and proteins with the versatility of synthetic polymers has gained significant interest in materials design and application. Although polymers possess a broad and versatile range of properties (including stimuli-responsiveness, biocompatibility, degradability, conductivity, and mechanical strength),¹ many compositions (owing to the availability of many different monomers) and varied architectures (from the wide variety of topologies including linear, block, graft, and hyperbranched polymers),² they are not capable of the folding and recognition of peptides and proteins. The nanoscale structures adopted by synthetic polymers arise mainly from bulk-phase separation and crystallization considerations,³ in contrast to the elegant organization found in the secondary through quaternary structures of peptides and proteins. Moreover, although there have been many advances in the production of well-defined

synthetic polymers, efficient synthetic strategies for preparation of polymers with uniform chain lengths and precisely defined monomer sequences still do not capture the specificity afforded by solid-phase and recombinant methods of peptide and polypeptide/protein synthesis.

The monodispersity and precisely defined primary structures of peptides and proteins impart them with unique secondary and tertiary structures required for hierarchical organization and biological function. In addition, substrate-ligand interactions mediated via protein/peptide domains are often precise and reversible, and the secondary structural folding (and unfolding) events are cooperative. However, peptide and proteins also have limitations; they can be toxic and elicit immune responses, can potentially be easily degraded enzymatically in unpredicted fashion and readily lose their bioactivity. Moreover, peptides and proteins can be costly to produce and difficult to process. Therefore, peptides and proteins with precisely defined primary structure, unique secondary and tertiary structures, and useful biological functions have been conjugated to polymers with versatile range of properties, yielding hybrid molecules with uniquely regulated conformational properties, nanostructural organization and assembly behavior, and various biomaterials applications. The gamut of applications of protein–polymer conjugates has gone beyond biomedical applications to their use for biosensors, phase-separation bio-assays, novel bioseparation protocols, biocatalysis, artificial enzymes, biometrics, light harvesting systems, photonics, and nanoelectronic devices.^{1,4–6}

Hybrids have been prepared by conjugating polymers to many different proteins and peptides. In many cases, the polymer can modify the structural and functional features of the proteins/peptides, and thus appropriate modulation of the properties of the polymers can alter the structure and function of the peptide and protein domains. Such biohybrid conjugates can be broadly classified based on the specific functions of the polymers (e.g., hydrophilicity, inertness, or stimuli-responsiveness), coupled with the structure (secondary, tertiary, and quaternary structure), and/or functions (therapeutic, reversible sensing-actuation, cell binding, cell penetrating, adhesive, etc.) of the proteins or peptides. In the following sections, we will review conjugates in which the combination of polymer properties, with the structure and function of the protein/peptide domain, specifically impacts the properties of the resulting hybrid, with a focus on bioactive systems with biomaterials applications. Readers are directed to other useful reviews for information on general assembly and properties of protein/peptide–polymer conjugates.^{2,3}

DISCUSSION

Protein–Polymer Conjugates

Polymer conjugation to proteins has proved lucrative in enhancing the physiochemical properties and use of proteins for particular applications. Most widely used and described, the attachment of poly(ethylene glycol) (PEG) to various protein-based drugs, enzymes, or antibodies has conferred improved solubility, reduced immunogenicity, increased stability against degradation, increased circulation times, and prolonged biological activity. Therefore, protein PEGylation (attachment of PEG to proteins) has attracted immense attention for commercial biomedical applications in drug delivery and cancer treatment.^{3,7}

With the emergence of biohybrid approaches, polymer–protein conjugates such as PEG-adenosine deaminase (Adagen), PEG-L-asparaginase (Oncaspar), PEG-interferon- α (PEGINTRON), PEG-human growth hormone (Pegvisomant), and others, have progressed through clinical trials and into the market since the 1990s.⁸ Similarly, conjugation of other polymers has found utility in multiple areas. For example, conjugation of poly(styrene-*co*-maleic anhydride) to the antitumor protein neocarzinostatin results in significantly increased accumulation of the drug in cancer tissue as compared to the nonconjugated drug, due to the enhanced permeability and retention effect, and has been approved for liver cancer treatment in Japan.⁹ Lu et al.¹⁰ have prepared methacrylamide-modified macromers of IgG₁ antibody fragments and the drug Mesochlorin e₆, which, when copolymerized with *N*-(2-hydroxypropyl) methacrylamide, provide conjugates with improved targeting and cytotoxicity, respectively, to the human ovarian carcinoma cell line OVCAR-3 when compared with nontargeted polymer-drug conjugates and the free drug.

We describe below several other elegant approaches of combining versatile polymers with proteins/enzymes and drugs that have resulted in (1) hybrid materials based on responsive polymers,^{11–21} (2) responsive hydrogels based on enzyme-catalyzed reactions, protein–protein interactions, and protein–drug sensing,^{22–29} and (3) dynamic hydrogels based on conformational changes of a protein.^{30–34}

Responsive Hybrids Modulated by the Properties of Responsive Polymers—

Some polymers display temperature-dependent and reversible solubility behavior. For example, poly(*N*-isopropylacrylamide) undergoes a collapse in size via hydrophobic association of the constituting monomer units resulting in coacervation upon increasing temperature (also called lower critical solution temperature or LCST behavior). Using the LCST behavior of oligo(*N*-isopropyl-acrylamide) (NIPAAM) to design responsive biohybrids has been explored in several studies by Hoffman, Stayton, and coworkers. In an early work, Michael-type addition reactions were used to site-specifically conjugate maleimide-terminated oligo-NIPAAM to genetically engineered cytochrome b5, engineered to contain a unique cysteine residue.¹¹ The resulting hybrid displayed LCST behavior similar to the free oligomer and could be reversibly precipitated from solution above the LCST. In other work,¹³ a mutant streptavidin was engineered to contain a single cysteine near the outer edge of the biotin binding pocket for site-specific conjugation of a vinyl-sulfone end-modified poly(*N*-isopropylacrylamide) chain. Normal binding of biotin to the PNIPAAM-conjugated streptavidin occurred below the LCST (32°C) of the PNIPAAM, while at 37°C binding decreased to 16.2% of the original value, due to the collapse of the PNIPAAM chains, which sterically blocked the biotin-binding site. Several applications of such system can be envisioned, including molecular gates and switches and triggered release of bound ligands. In a similar vein, Pennadam et al.¹⁴ conjugated PNIPAAM to a large multisubunit DNA motor (EcoRI24I R-M enzyme) to control methyltransferase activity of the multienzyme complex, and hence subsequent DNA modification, to mimic the natural DNA modification process in vivo. Several other studies have indicated that biomolecules covalently attached to stimuli-responsive polymers can be selectively extracted from a mixture upon increasing temperature. Therefore, bioconjugates based on NIPAAM have been studied for affinity precipitation of polysaccharides, proteins, and peptides.^{15–17}

Various other temperature-^{18,20,21} and pH-sensitive¹⁹ polymers have been used for covalent conjugation to enzymes to develop smart hybrid systems to control enzyme activity and substrate access.

In addition to thermally responsive polymers, photo-responsive polymer-enzyme switches¹² have also been reported by Hoffman, Stayton, and coworkers in which the previously described steric blocking of substrate binding site was achieved via the photoinduced collapse of a copolymer of *N,N*-dimethyl acrylamide and azophenyl-containing monomers. The model enzyme used in this study was endoglucanase 12A (EG 12A), which catalyzes the hydrolysis of internal β (1–4) linkages of cellulose. The polymer in this polymer-enzyme conjugate acted as a switch to modulate the activity EG 12A, in response to distinct wavelengths of light. When the polymer was in the compact form (under illumination by visible light), substrate access and enzyme activity were completely restricted in solution, whereas when it was in its extended form (under illumination by far UV light), the conjugates displayed ~60% of the activity of the unconjugated enzyme. The photoswitching was used reversibly to cycle between the enzyme's active and inactive state with a fast response time of ~1 min. Such conjugates could be useful in several solution-phase enzyme assays and bioprocessing applications.

Responsive Hybrid Hydrogels Modulated by Activities of the Protein Domain

—Perhaps, the most widely studied responsive enzymatic systems are those responsive to glucose. Many versions of glucose-responsive hydrogels use a pH-sensitive hydrogel synthesized from different polymers, with immobilized/entrapped glucose oxidase (GOD).^{25–29} These materials are generally responsive to glucose owing to the reduction in pH that occurs upon the conversion of glucose to gluconic acid, which causes a volume change in the hydrogel leading to release of biomolecules (especially insulin). As a representative example, glucose-responsive hydrogels developed by Hassan et al.²² (Figure 1a) used GOD in a poly(methacrylic acid-*g*-ethylene glycol) network. These materials are pH-sensitive owing to the intermolecular complexes formed by the hydrogen bonding between carboxylic acid and ether groups. Upon introduction of glucose, the grafted GOD catalyzes the formation of gluconic acid, resulting in a decrease in pH and thus collapse of the gel. This collapse physically ejects the entrapped drug, insulin. As the concentration of glucose decreases upon the action of the released insulin, the resulting decrease in gluconic acid concentration (and hence increase in pH) causes the gel to swell. This mechanism can be used for pulsatile and controlled release of insulin or other drugs in response to glucose concentration.

Antigen-responsive hydrogels developed by Miyata et al.²³ (Figure 1b) consist of polyacrylamide-based semi-interpenetrating hydrogels grafted with a specific antigen and the corresponding antibody. The antigen-antibody interactions form reversible noncovalent cross-links that reinforce the hydrogel network. On addition of free antigen, the crosslinks formed via interchain antigen-antibody interactions are disrupted due to competitive binding, leading to swelling of the hydrogel. Although the hydrogels reported to date have demonstrated rather small volume changes, with further development, this concept may find application in drug delivery via antigen sensing.

Drug-sensing hydrogels reported by Ehrbar et al.²⁴ (Figure 1c) use drug-dependent association and dissociation of protein motifs in hybrid hydrogels as a functional switch to trigger the release of pharmaceutical drugs. Specifically, polyacrylamide polymers were functionalized with nitrilotriacetic acid (NTA) groups chelated with Ni^{2+} ions, so that the polymers would bind genetically engineered, hexahistidine-tagged, *E. coli* gyrase subunit B (GyrB). The antibiotic coumermycin dimerizes the pendant GyrB, forming physical cross-links in the hydrogel. The storage modulus of the coumermycin-dimerized GyrB-based physical gels was improved upon subsequent chemical crosslinking via an amine-specific bifunctional crosslinking agent dimethylsuberimidate. Upon addition of a competitive inhibitor antibiotic, novobiocin, the coumermycin was liberated from the gel, resulting in dissociation of the gel. Biosynthetically produced VEGF₁₂₁ equipped with a hexahistidine motif was also immobilized onto the pendant NTA motifs along with the GyrB to permit sustained release of VEGF₁₂₁ upon addition of novobiocin (GyrB is released as well), which led to a significant increase in the proliferation of human umbilical vein endothelial cells. Hydrogels that deliver drugs based on a pharmacological agent may prove more patient-compliant.

Dynamic Hydrogels Based on Conformational Changes of Protein Domains—

The conformational changes in proteins have also been used to trigger volume changes in hydrogels. For example, Ehrick et al.³⁰ developed stimuli-responsive hybrid hydrogels (Figure 2a) by integrating a genetically engineered calmodulin (CaM) and the corresponding ligand phenothiazine within poly(acrylamide)-based hydrogels. CaM undergoes a conformational change from an open dumbbell conformation in the presence of Ca^{2+} to a more compact conformation when protein- Ca^{2+} complexes are further bound to phenothiazine. Upon removal of Ca^{2+} with a chelating agent, or upon addition of the competitive ligand chlorpromazine, the hydrogel swelled due to the associated change in conformation of CaM and the disruption of the phenothiazine physical crosslinks; this volume change was reversible. The designed hydrogels were used for controlled transport of vitamin B₁₂ and were also incorporated in microfluidic systems to control flow from the reservoir.

In a similar vein, Murphy and coworkers^{31,32} have prepared photocross-linked acrylate-terminated PEG-CaM-PEG-based hydrogels (Figure 2b) expanding on the previous work.³² The terminal tyrosine residues of wild-type CaM were mutated to cysteines and the modified CaM used as a cross-linker in a PEG diacrylate polymer hydrogel. The hinged motion of the CaM units within the hydrogel (from ~ 50 Å in extended conformation to ~ 15 Å upon collapse), with the introduction of the ligand trifluoperazine (TFP) in the presence of Ca^{2+} , resulted in reversible macroscopic volume changes (up to 65% of the initial volume) of the hydrogels. By changing the molecular weight of PEG and its fractional mass content in the hydrogel, the crosslinking density of the hydrogel could be modulated to achieve a decrease in volume up to 90%.³⁵ These protein-based hydrogels also exhibited unique reversible changes in optical transparency, which was used as high-throughput label-free biosensing platform for the detection of the drug TFP or other CaM ligands.

Further exploiting the mechanism of protein conformational change upon substrate binding, Yuan et al.³³ developed smart hydrogels using the enzyme adenylate kinase (Figure 2c).

Specifically, an HPMA scaffold with pendant maleimide groups was crosslinked into a hydrogel by reaction with thiol groups (from two spatially separated cysteines) present on a triple mutant derivative of the *E. coli* enzyme adenylate kinase (AKtm). Upon addition of the substrate (ATP, adenosine triphosphate), the AKtm in the hydrogel recognized the substrate and catalyzed the chemical reaction-transfer of a phosphate group. The expected nanoscale conformational change of AKtm upon ATP binding, from open to closed form, concomitantly resulted in a macroscopic volume transition of the hydrogel. Potential applications of deswelling of the hydrogel upon enzyme-substrate recognition can be used for drug delivery, microfluidic valves, and mechanical actuators. Taken together, the studies presented earlier indicate the promise of reversible macroscopic shape changes in hybrid hydrogels via nanoscale conformational changes of a protein domain; many applications can be envisioned for such materials given the versatility of polymer design and the potential for engineering the specificity of the protein domains. For further information, readers are directed to a detailed review of the bioinspired design of dynamic materials.³⁴

Challenges in Preparing Protein–Polymer Conjugates—Although polymer modification of proteins, especially PEGylation, has proved substantially useful, the enzyme activity and in vivo fate of the modified protein have been found to depend on number, length, and architecture (linear, branched, or dendrimeric) of the polymer chains that are attached to the protein as well as on the site of polymer attachment.^{36–40} Particularly, if the polymer is nonspecifically attached to the active center, it can result in significant loss of biological activity. The use of commercially available polydisperse polymers with various degrees of functionality has often resulted in introduction of heterogeneity in the resulting hybrid with multiple PEG conjugation or protein crosslinking; hence a consequent variability and loss in the biological activity results.^{41,42} For medical or pharmaceutical applications, FDA approval requires precisely defined conjugates.⁴³ Therefore, a significant challenge in the design of polymer–protein conjugates is that optimization is required for each biomolecule (especially considering the size, function, tertiary structure, and the availability of the specific functionality on the biomolecule). To overcome this, control of chemical conditions such as use of precise protein-to-polymer ratios, as well as the development of site-specific conjugation methods, remains areas of active research.⁴⁴

For example, site-selective conjugation strategies have been expanded via introduction of specific chemoselective handles for selective coupling with a high-functional group tolerance. The Maynard group,^{45–49} Francis group,^{50–54} and others^{55–58} have demonstrated several elegant approaches for synthesizing well-defined protein–polymer conjugates and controlling the protein behavior in the resulting hybrids; for example, using grafting-to and grafting-from approaches using controlled radical polymerization methods such as atom transfer radical polymerization (ATRP) and reversible addition-fragmentation chain transfer (RAFT). Readers are directed to very useful reviews on various synthetic approaches to prepare protein–polymer conjugates.^{1,47,59,60}

Perhaps, the most widely used site-specific conjugation strategy uses Michael-type addition reactions between thiols of cysteine residues and maleimide- or vinyl sulfone-functionalized semitelechelic polymers. The success of this conjugation relies on the relatively low-natural abundance of cysteine on proteins (1.7% of all amino acids in globular proteins),³ mild

conditions of reaction in water, and high-reactivity rates of Michael-type addition reactions, thus minimizing any side reactions. Other site-specific conjugation strategies include reductive alkylation of a protein's amine terminus with aldehyde-functionalized PEG,^{61,62} and using enzyme-catalyzed reactions with glutaminase.⁶³ A very promising, yet underutilized strategy, using genetic engineering approaches with non-natural amino acid incorporation offers very chemoselective reactions based on the Staudinger ligation^{64–66} or click chemistry reactions^{67–69} as an opportunity to site specifically modify proteins. In particular, the Huisgen [3 + 2] azide-alkyne cycloaddition reactions are highly specific, can be carried out in aqueous medium, and are fully bio-orthogonal (nonreactive to other amino acids present) without any side reactions. Another approach can be to incorporate controlled radical polymerization initiators directly as a part of the incorporated non-natural amino acid, which offers possibility of carrying out polymerization directly from the protein surface. Such approaches introduce high specificity in protein modification, low polydispersity, and a well-defined nature of the conjugate.

Peptide–Polymer Conjugates

The studies above indicate the versatility of engineering hybrid materials with the properties of proteins, whereas challenges in controlling sites of protein modification and in maintaining protein structure have fueled continued interest in the application of peptides in hybrid materials. It has also been convenient and useful to mimic the specific secondary and tertiary structure and the function of natural proteins by using short-peptide domains derived from them. The studies discussed in the following sections indicate that the peptides incorporated into hybrid materials are able to retain their secondary structure and, in some cases, are further stabilized upon polymer conjugation. The peptide domains have been shown to guide the hierarchical organization of the hybrid leading to formation of a variety of higher-order structures. As discussed below, the biofunctional properties of the peptide domains have also been used for tissue-engineering applications,^{70–77} as cell-penetrating scaffolds,^{78–80} for imparting antimicrobial activities^{81,82} and bioadhesive properties.⁸³

In the following section, we will discuss hybrids classified on the peptide domain with different conformations, such as α -helical coiled-coil and β -sheets; peptides derived from structural protein domains such as silk, elastin, titin, and collagen; and other biofunctional domains such as cell-binding domains and enzyme-recognized degradation domains. Other hybrids based on cyclic peptides and peptide amphiphiles have also been introduced; these have been reviewed elsewhere.^{84,85}

Polymer Conjugates with Peptides of Different Conformations. Helical and Coiled-Coil Peptide–Polymer Conjugates—Homopolypeptide–polymer conjugates based on α -helical (e-benzyloxycarbonyl-L-lysine), c-benzyl-glutamate, and L-leucine homopolypeptides have been prepared via ring opening polymerization of appropriate α -amino acid *N*-carboxyanhydrides initiated by primary amines, alkoxides, or transition metal catalysts.^{86–88} This topic has been described in detail elsewhere.^{2,89} Here, we will discuss polymer conjugates containing short peptides with helical and coiled-coil conformations, for use mainly in hydrogel-based applications; a summary of some of these designs is presented in Figure 3.

The laboratories of Jia and Kiick have explored high-molecular weight peptide–polymer multiblock polymers due to their potential for higher-order assembly, hydrogel formation, and unimolecular micellization for use in tissue engineering and drug-delivery applications. In polymers aimed at mimicking elastin, telechelic, alkyne-terminated α -helical peptides with the sequence $(AKA_3KA)_n$ (inspired by the cross-linking domain of native elastin) were conjugated to telechelic, azide-terminated PEG to synthesize multiblock hybrid polymers.⁹⁰ Crosslinking of the hybrid multiblock copolymers via reaction with hexamethylene diisocyanate through the lysine residues resulted in elastomeric hydrogels with a compressive modulus of 0.12 MPa under hydrated conditions, which is comparable to that of a commercial polyurethane elastomer (Tecoflex SG80A). These elastomeric hydrogels were cytocompatible as monitored via the growth and proliferation of primary porcine vocal-fold fibroblasts.

In addition to this work on helical peptide–polymer multiblock polymers, we have also investigated the formation of multiblock polymers comprising glutamic-acid rich and arginine-rich heterodimeric coiled-coil peptide partners equipped with lysine at the *f*-positions of the terminal heptads. Many studies of coiled-coil peptide–polymer conjugates, reported by the groups of Kopecek,⁹¹ Klok,^{92,93} and Degrado⁹⁴ have shown that the coiled-coil conformation of the peptide is retained upon conjugation with PEG either at the peptide termini or at the *f*-positions of the coiled coil. In our studies, peptides were conjugated to homobifunctional PEG *N*-hydroxysuccinimide esters to synthesize multiblock copolymers of the form (peptide-PEG)_{*m*}, where the peptide was conjugated to the polymer through a lysine at the *f*-position of the coiled coil.⁹⁵ Multiblock polymers of high-molecular weights were possible to isolate after purification; polymers with *m* ~ 10, corresponding to molecular weights greater than 100 kDa (suggested via size exclusion chromatography), were formed. The multiblock copolymers exhibited electrostatically driven coiled-coil heterodimer formation (Figure 3a), confirming that PEG conjugation at the *f*-positions of coiled-coil heptads does not significantly impair coiled-coil formation even in the context of a multiblock polymer. Interestingly, coiled-coil homodimer formation, not observed in the isolated peptides, was also observed. The formation of the homodimeric coiled coils resulted in the production of isolated aggregates of low polydispersity, suggesting that the potential use of these types of multiblock conjugates in the design of responsive drug-delivery vehicles.

In another design strategy, Collier and coworkers⁹⁶ have prepared rod-coil-rod triblock polymers (Figure 3b) to form elastic hydrogels. The peptide domain of these polymers, although adopted from the coiled-coil region of human fibrin, was chosen owing to its coiled-coil association behavior; the polymers were not intended to capture any structural or biological properties of fibrin. Specifically, the N-terminal cysteine of a coiled-coil peptide was reacted with monofunctional or homobifunctional maleimide-terminated PEG to generate diblock and triblock hybrid copolymers. Their design differs from earlier designs in the fact that in the triblocks the coiled-coil domain flanked the central PEG linker. CD spectra for the peptide, diblock, and triblock displayed characteristics of the α -helical coiled coil with similar mean residue ellipticities, indicating that PEG conjugation had negligible effect on the secondary structure of the peptide domain. Triblocks formed elastic hydrogels

at 12 wt %, whereas the diblocks formed nonviscous solutions, suggesting that the hydrogel formation occurred through the crosslinking of the PEG chains via the dimeric and tetrameric association of the terminal coiled-coil domains.

Some of the earliest studies of hydrogel formation from coiled-coil peptide–polymer conjugates involved coiled coils as pendant groups from polymer chains, as reported by Wang et al.⁹⁷ In these studies, hybrid hydrogels were prepared by metal-complexation-mediated, noncovalent cross-linking of a synthetic polymer backbone via association of biosynthetically produced, histidine-tagged coiled-coil domains (Figure 3c). The synthetic polymer backbone was based on copolymer system of a hydrophilic *N*-(2-hydroxy-propyl)methacrylamide and *N*-(3-(*N'*, *N'*-dicarboxymethyl)-aminopropyl)methacrylamide [poly(HPMA-*co*-DAMA)] and carried pendant metal-chelating groups of iminodiacetic acid (IDA) to permit gelation upon association of the histidinetagged coiled coils with the copolymer chain. The hydrogels displayed volume transitions at the melting temperature of the coiled-coil domain and eventually dissolved at high temperatures and were responsive to the stronger metalchelating ligand imidazole. In additional studies of these types of systems,⁹⁸ hybrid graft copolymers (Figure 3d) were produced by covalently grafting coiled-coil peptides on poly(HPMA-*co*-DAMA). These studies suggested a minimal length of the coiled-coil domains of four heptad repeats were required to support hydrogel formation. Heterodimeric coiled-coil domains have also been similarly used to mediate the assembly of the hybrid graft copolymers into hydrogels.^{98,99}

Hybrids of β -Sheet Peptide–Polymer Conjugates—Self-assembly of β -strands into β -sheet fibrils and higher-order assemblies to create nanostructured hybrid materials and hydrogels has been pursued for use in tissue engineering and other biomedical applications.^{100–102} Conjugation of PEG to β -sheet peptides results in hybrids that retain β -sheet conformation and higher-order assembly that yields fibrils upon stacking of the β -sheet domains, but that are of higher hydrophilicity. For example, Klok et al.¹⁰³ have studied conjugates of PEG with the amphiphilic peptide (GALEAALKLAELAAG and GAALKAALELAAG). These peptides adopt predominantly a β -sheet conformation at pH 7, whereas at pH 2 and 11, partial α -helical secondary structure was indicated. In contrast to the behavior of the peptides, the diblock hybrid showed partial α -helical character independent of pH. Transmission electron microscopy (TEM) and atomic force microscopy (AFM) revealed fibrillar structures for hybrids, which were suggested to be bundles of rodlike aggregates.

Taking similar advantage of conformational changes in polypeptides to alter assembly, we have designed genetically engineered alanine-rich polypeptides, which adopt a monomeric α -helical conformation at pH 7.4, but that aggregate and subsequently convert to β -sheet fibrils at elevated temperatures under acidic conditions (pH 2.3).¹⁰⁴ The N-terminus of cyanogen-bromide-cleaved alanine-rich polypeptides is readily PEGylated via treatment with propionaldehyde-functionalized PEG (5 kDa). Evaluation of the PEG conjugates of the polypeptide suggests that PEGylation does not significantly alter the conformation of the polypeptide nor the size of aggregates, although the solubility of the aggregates is improved. The functional utility of the hybrid system is currently under investigation.

Altering the solubility and assembly of β -sheet aggregates via polymer conjugation has also been used to modulate fibrillogenesis in multiple investigations. Investigating the steps of irreversible fibrillogenesis and deposition of amyloid plaques linked to Alzheimer's disease, Burkoth et al.^{105–107} pioneered the demonstration that PEG conjugation to a β -amyloid peptide fragment prohibited nonspecific aggregate formation. Work by Collier and Messersmith¹⁰⁸ also indicated that PEG attachment to fibril-forming, de novo-designed β -sheet peptides (Figures 4a and 4b) prevented lateral aggregation of the peptide domain in the hybrid to produce fibrils of uniform width. In addition, Radu et al.¹⁰⁹ recently reported the effect of conjugating poly[*N*-(2-hydroxypropyl)methacrylamide], which has a random-coil conformation (in contrast to the extended conformation of PEG), to a β -sheet peptide QQRFWQFEQQ (previously designed by Aggeli et al.¹¹⁰), via thiol–maleimide chemistry (Figure 4a). The peptide adopted antiparallel β -sheet conformation at pH = 2, when incubated for 3 days at room temperature, whereas the peptides in the hybrid were still random coil under these conditions. The β -sheet conformation of the peptide domain could be imposed in the hybrid only after favorable electrostatic interactions between the oppositely charged glutamic acid and arginine residues at higher pH (pH = 11). Micrometer long fibrils with width of 20–25 nm were observed for the peptide, whereas thinner fibrils of width 10–15 nm were observed for the hybrid.

Klok and coworkers^{111,112} prepared hybrids via PEG attachment, at either the C-terminus or both the N- and C-termini, of a de novo-designed amphiphilic β -strand peptide (Figure 4c). Their results suggested that the self-assembling property of the β -strand peptide is retained upon conjugation in both the solid state¹¹¹ as well as in solution.¹¹² A highly organized lamellar superstructure consisting of alternating PEG and peptide domains in the solid state is difficult to achieve via assembly of conventional block copolymers. It was observed that PEG conjugation stabilized the β -strand secondary structure in solution compared to the peptide and rendered insensitivity of the peptide secondary structure toward pH changes.

β -Sheet peptides have been interesting structural motif, because even short sequences are able to display useful conformational properties. Using template-preorganized short peptides,¹¹³ the Bönnner group^{114–116} synthesized peptide–polymer hybrids (Figure 4d) on a three-functional carbazole-based template, which was functionalized with a PEO chain on one side, and two strands of the (Val-Thr)₂ tetrapeptide on the other side. This tetrapeptide organizer in water formed interdigitated, antiparallel β -sheets as a core, with PEO extended as a shell, leading to formation of nanostructured tapes of up to 2 μ m in length, 1.4 nm in height, and 13–14 nm in width. The hydroxyl group of the threonine residue was further used to promote biosilicification, resulting in well-defined composite fibers with six distinguishable levels of hierarchical design. Nanoindentation experiments revealed an indentation hardness of 0.99 $\pm$ 0.2 GPa, which is approximately one-third the hardness of natural sponge spicules and $\sim$ 13% that of optical glass fibers.¹¹⁶ In another variation, this group^{117,118} used the *o*-acyl migration strategy in a peptide–polymer conjugate [using poly(*n*-butyl acrylate)] and PEG polymers with (VT)₄ and (VT)₅ peptides to achieve pH-triggered conformational change and assembly behavior (Figure 4e).

Conjugation with Peptides Derived from Structural Protein Domains. Hybrids Containing Silk-Based Peptide Domains— β -Sheet peptide domains based on *Nephila*

clavipes silk-inspired alanine repeats or *Bombyx mori* silk-inspired GAGA tetrapeptide repeats have also been used to create polymer–peptide conjugates.^{119–126} The mechanical properties of polymer conjugates have been modified via the incorporation of silk-based peptide units in a multiblock polymer context. The Sogah group¹¹⁹ conjugated amorphous poly (ethylene glycol) (PEG) to the silk-inspired consensus peptide sequence GAGA or A_{4–8}, to prepare segmented block copolymers (Figure 5a) to mimic natural silk. The β -sheet formation of the peptide domain was retained in the presence of the amorphous PEG blocks. Stress–strain curves for the films and fibers of the hybrid copolymers indicated that the *N. clavipes* silk-inspired polyalanine-containing block copolymers exhibited superior mechanical properties when compared with the *Bombyx mori* silk-inspired GAGA tetrapeptide-containing block copolymers and also as compared to regenerated *N. clavipes* silk. An aromatic template was also used to nucleate and preform β -sheet structure with PEG chains between peptides (Figure 5b).¹²⁰ For both designs, phase-separated structures were observed via TEM and AFM. Superstructures formed via agglomeration of peptide domains with the polyether dispersed between them revealed morphological features with sizes on the order of 100–150 nm. The mechanical properties of the cast films and fibers could be modulated by varying the nature and length of the building blocks.

The fibril formation of silklike polypeptides has also been modified via conjugation with PEGs of various molecular weights. van Hest and coworkers^{122,123} synthesized an ABA triblock hybrid, PEG-[(AG)₃EG]_{*n*}-PEG, where *n* = 10 and 20 (Figure 5c). The central polypeptide block equipped with terminal cysteine residues was conjugated to maleimide-functionalized PEG to generate the triblock, with a rationale of restricting macroscopic crystallization of the polypeptide block, allowing the formation of fibrils with dimensions well correlated with the dimensions of the polypeptide chain. They observed that the antiparallel β -sheet structure is retained in the hybrid; with PEG chain lengths similar to polypeptide blocks, a well-defined fibrillar structure was observed, whereas, for larger PEG chains, no fibrillar structures were observed probably due to steric hindrance. The use of β -sheet domains as structure-directing units has also been explored for polymers in which the peptides are appended as sidechains. Ayres et al.¹²⁴ synthesized ABA tri-block copolymers containing *Bombyx mori* silk-inspired GAGA tetrapeptide as a β -sheet forming side chains on the B block (Figure 5d). Their results demonstrated an antiparallel β -sheet conformation for the peptides in the triblock copolymers, suggesting that the conformational behavior is retained even when the peptide is appended as a side chain.

Hybrids Containing Elastin, Titin, or Mussel Adhesive Peptide Domains—To mimic the elastomeric and conformational properties of some of the natural proteins such as elastin and titin, and/or the adhesive properties of mussel adhesive proteins (MAPs), the consensus sequences of these proteins have been conjugated with synthetic polymers to create new hybrid materials and widen the general applicability of such functional domains. Elastin is a major constituent of elastic tissues and extracellular matrix, with mammalian elastins comprising mainly VPGVG sequences that exhibit an inverse temperature transition (LCST) with a conformational change from random coil to type II β -turn with increasing temperature. Riersen et al.¹²⁷ had shown that even a single repeat of the VPGVG sequence undergoes a structural conformational change from random coil to type II β -turn. To

investigate if the transition behavior observed in elastin would be retained by polymers equipped with VPGVG-based peptide pendant groups, Ayres et al.^{128,129} synthesized, via ATRP methods, ABA hybrid triblock copolymers with the central PEG domain flanked by poly(methacrylate) domains containing side chains of elastin-based VPGVG pentapeptides. Temperature-dependent CD spectra of the homopolymer and the triblock showed conformational changes characteristic of the random coil to type II β -turn transition upon increasing temperature, similar to that reported for elastins. Turbidity measurements indicated no increase in turbidity for the homopolymer, whereas a significant increase was observed for the triblocks, suggesting the phase-separation of the VPGVG-containing domains.

Titin is a large modular protein containing immunoglobulin (Ig)-like domains that are elastic in nature and important for contraction and elasticity in muscle tissue. The Ig domain maintains a β -sandwich structure of the immunoglobulin fold. With a goal of creating hybrid hydrogels with environmental responsiveness, Chen et al.¹³⁰ cross-linked an acrylamide copolymer with the immunoglobulin(Ig)-like module I28 of the human cardiac muscle protein titin. The copolymer carried pendant metal-chelating groups of IDA,⁹⁷ and like the coiled-coil-containing materials described earlier, hydrogels were formed when the histidine-tagged I28 was mixed with Ni^{2+} -chelated acrylamide-based copolymer. These hydrogels exhibited positive temperature responsiveness and swelled isotropically more than three times their initial volume at temperatures above the melting temperature of the β -sheet. The temperature response of such hydrogels could be adjusted by using different Ig modules or a combination of modules that have different melting temperatures. The mild hydrogel assembly conditions and the environment-responsive swelling profile offer potential for controlled delivery of therapeutic proteins.

MAPs have been a source of inspiration to materials design, as the MAPs secreted by mussels enable the organism to adhere to a wide variety of wet surfaces. Specifically, the adhesive property of MAPs has been linked to the catechol group of L-3,4-dihydroxyphenylalanine (DOPA) present in the decapeptide sequence Ala-Lys-Pro-Ser-Tyr-DHP-Hyp-Thr-DOPA-Lys (where DHP = dihydroxyproline). In an interesting application of adhesive domains to form non-adhesive surfaces, the Messersmith group has conjugated DOPA-based peptide sequences with PEG to impart adhesive properties to the otherwise nonfouling PEG.⁸³ Immobilization of the peptide-PEG conjugate via the adhesive properties of peptide on various substrates yielded surfaces resistant to both protein and cell adhesion.

Hybrids Containing Collagen-Based Peptide Domains—Collagen is one of the most abundant fibrous proteins found in body tissues, organs, and extracellular matrix and is responsible for various biological functions.^{131,132} Therefore, collagen has widely been used in tissue-engineering scaffolds and as biomedical reconstruction materials.¹³³ To immobilize collagen in PEG-based synthetic hydrogels, Lee et al.¹³⁴ conjugated acryloyl-PEG-*N*-hydroxysuccinimide to the amine terminus of a collagen-mimetic peptide (CMP) with the sequence NH_2 -(Pro-Hyp-Gly)₇-Tyr-OH to prepare an acryloyl-PEG-CMP macromonomer, which was photopolymerized with PEG-diacrylate to form hydrogels. This hydrogel bound externally added type I collagen molecules via strand invasion with the CMP chains in the

hydrogel. Results from these studies indicated that the inclusion of CMP in the hydrogel enhanced tissue production of cells in the PEG hydrogel.

In continued efforts to produce synthetic and recombinant materials functionalized with the biological and structural properties of collagen-containing proteins, we have designed a hydrophilic, hydroxyproline-free, and thermally stable collagen-mimetic peptide via the incorporation of triple-helix-stabilizing charged triplets.¹³⁵ Interestingly, this collagen-mimetic peptide also assembles into nanorods and microfibrillar structures as observed via TEM. Given the high-thermal stability of the triple helical structure of a cysteine-containing variant of this peptide and its propensity for higher-order assembly, the peptide was functionalized at both ends with a thermally responsive polymer, poly(diethylene glycol methyl ether methacrylate), to form a hybrid tri-block polymer.¹³⁶ CD characterization of the triblock confirms that the triple helical structure of the peptide, and the dual responsiveness of both blocks, is retained. It is anticipated that the polymer-collagen peptide-polymer triblocks will exhibit hierarchical assembly guided by the triple helical collagen peptide block to form useful nanostructures for various biology and device applications.

Conjugates Containing Cell Adhesion and Degradation Domains—The integrin-binding, fibronectin-derived, cell-adhesive peptide RGD has been incorporated widely in polymeric scaffolds for tissue-engineering applications.¹³⁷ For example, the Hubbell¹³⁸ group initially formed these types of hydrogels by copolymerizing acrylate-functionalized RGD sequences with PEG diacrylate upon photoinitiation. Their results suggested that RGD peptides immobilized via appropriately long spacer arms could mediate cell adhesion and spreading even on the nonadhesive PEG. Many other groups have applied RGD immobilization onto polymeric hydrogels to promote cell adhesion and proliferation.^{139–143} Because RGD can bind to a large number of cell types, specific cell-binding selective motifs have also been used. For example, the neurite-adhesion promoting, laminin epitope IKVAV incorporated into peptide-based amphiphiles has been used to trigger neural progenitor cells into neurons while discouraging the development of astrocytes.¹⁴⁴ Because tissue development requires simultaneous extracellular matrix production and hydrogel degradation, biodegradable polymers functionalized with cell-adhesive sequences have been developed. For example, Barrera et al.¹⁴⁵ synthesized the biodegradable copolymer poly(L-lactic acid-co-L-lysine) and functionalized the lysines with the GRGDY peptide sequence. Similar RGD peptide modification of hydrolytically degradable polymers has also been reported by other groups.^{146,147} However, for polymeric hydrogels, which are not hydrolytically degradable, incorporating enzymatically cleavable peptide sequences have proven to be useful. Enzymatically triggered degradation is also attractive, because enzymes are highly specific toward the substrate, and hence the release of multiple therapeutics can be tuned. Such systems can potentially mimic the three-dimensional degradation characteristic of native ECM during cell migration and hence can be used for tissue engineering and wound healing applications.

West and Hubbell¹⁴⁸ prepared a photocrosslinked hydrogel from acryloyl-terminated BAB triblock copolymer systems; the A block comprised PEG and the B block comprised an enzymatically degradable oligopeptide sequence. Acryloyl-terminated (Ala-Pro-Gly-Leu)-

PEG-(Ala-Pro-Gly-Leu) macromers formed hydrogels that specifically degraded in the presence of the matrix-metalloproteinase (MMP) I, whereas acryloyl-terminated (Val-Arg-Asn)-PEG-(Val-Arg-Asn) formed hydrogels that specifically degraded in the presence of plasmin. In further work,⁷⁰ it was observed that incorporating the cell-adhesive peptide RGD in such enzymatically degradable hydrogel systems offered smooth muscle cell adhesion, growth, and migration characteristics of potential use in tissue-engineering applications. Expanding the concept of incorporating both the cell binding and enzymatically cleavable domain for creating polymeric cell responsive hydrogels,^{73–76} the Hubbell group partially grafted monocysteine-functionalized adhesion peptides on vinyl sulfone-functionalized four-arm PEG, and then the remaining vinyl sulfone groups were used in crosslinking via biscysteine MMP substrate peptides. The presence of MMPs rendered the matrix degradable via cell-secreted MMPs. Primary human fibroblast cells could proteolytically invade this matrix dependent on MMP substrate activity, concentration of integrin binding domains, and the hydrogel crosslinking density. The recombinant human bone morphogenetic protein-2 could be delivered through these gels, which promoted bone regeneration. These gels also allowed differentiation of pluripotent cardiac progenitors into mature cardiac cells by systemic modulation of matrix elasticity, MMP-sensitivity, and the concentration of cell-adhesive ligand.⁷⁷

Rizzi et al.^{71,72} prepared hybrid hydrogels via Michael-type conjugate addition of vinyl sulfone groups of end-functionalized PEG with the thiol groups of cysteine residue incorporated at desired crosslinking sites within the recombinant protein polymer. The biosynthetically produced recombinant protein polymer with a modular design comprising a cell-adhesive fibronectin fragment domain, as well as substrates for plasmin- and MMP-mediated degradation, was used to mimic the cell binding and migration properties of the natural extracellular matrix. Biochemical assays and in vitro cell-culture experiments on these elastic hydrogels confirmed cell-adhesion properties and degradation via target enzymes. When optimized, the hydrogels supported bone healing in a rat cavariar model, with release of rhBMP-2 upon proteolytic degradation. Although not a synthetic conjugate, from a design perspective, this study highlights the potential of modular design in tailoring the resultant matrix toward its intended biological function.

Almany and Seliktar¹⁴⁹ prepared hybrid hydrogels by integrating PEG with natural fibrinogen. Specifically, a precursor solution consisting of PEGylated fibrinogen (prepared by reacting PEG diacrylate with the multiple thiol residues on the cyanogen bromide cleaved fibrinogen fragment) and PEG diacrylate was photopolymerized in the presence of a photoinitiator. Although the fibrinogen portion of the hybrid provided a natural biological domain for endothelial cell and smooth cell adhesion and proteolytic degradation, the molecular weight and the content of PEG could be modulated for manipulating the elastic modulus of the hydrogel. The cellular morphology and the rate of cellular invasion were demonstrated to be related to the molecular architecture and network structure of the 3D PEG-fibrinogen hydrogel matrixes.^{150,151} The hydrogels implanted into segmental rat tibial defects promoted new bone formation after 5 weeks, which corresponded to the biodegradation pattern of the hydrogel as suggested by histological and X-ray results.¹⁵² The optimal composition of PEG and fibrinogen content in the PEGylated fibrinogen

hydrogels also supported extensive neurite outgrowth of invading glial cells from encapsulated dorsal root ganglion cells in the presence of nerve growth factor.¹⁵³

Thornton et al.,¹⁵⁴ have described a new PEG-based hydrogel, modified with enzymatically degradable, pendant zwitterionic tetrapeptides (DAAR, attached at the R-containing terminus), which upon specific enzymatic hydrolysis lead to charge-induced swelling of the hydrogel and the controlled release of physically entrapped guest molecules. The enzyme thermolysin, which catalyzes the hydrolysis of the peptide bond between the dialanine sequence, is of the appropriate size to enter the hydrogel pores and access the substrate, causing enzymolysis of the substrate (AA) in the hydrogels and leaving positively charged pendant arginine residues, which caused the gel to swell and release the highly cationic glycoprotein avidin. However, anionic albumin was retained, probably due to its electrostatic attraction with the hydrogel interior. Hence, a different peptide RRAADD was conjugated to the gels, which upon enzymatic cleavage left the hydrogel with a highly negatively charged interior that would release the anionic protein.¹⁵⁵ The described nondissolving hydrogel system demonstrated the potential of releasing charge-matched selective guest molecules upon specific enzymatic cleavage of the sensing and actuating peptide domain within the hydrogel.

Challenges in Preparing Peptide–Polymer Conjugates—Despite the recent surge in preparing peptide–polymer conjugates, peptide synthesis of long peptide sequences is often limited by current peptide synthesis technology. Solid-phase peptide synthesis is a multistep process involving coupling of the activated acid group of the incoming amino acid to amine group of the preceding amino acid in the sequence, and deprotection of the Fmoc (9-fluorenyl methoxycarbonyl) or Boc (tert-butoxycarbonyl) protected *N*-α-amine. If the average yield for each amino acid linkage is 95%, the overall yield of recovery for the repetitive sequential synthesis of, for example, 25 amino acid long-peptide sequence would be $(0.95)^{25} = 27\%$, along with several deletion sequences.

Another important consideration while preparing peptide–polymer conjugates is the need for orthogonal protection of the side chains of different amino acids in the peptide sequence, which might also interfere with normal conjugation strategies. For example, in preparing peptide–polymer conjugates using the terminal amine, presence of lysine in the peptide sequence also presents a potential reactive site. To overcome this, either fully protected peptide sequences (prepared by selective cleavage of the fully protected peptide sequence from the resin without deprotecting the protecting groups on the side chains of the constituent amino acids; using special commercially available resins like 2-chlorotrityl chloride resin) or orthogonally protected side chains of the amino acids is needed. Another strategy is to link the polymer on the resin-bound protected peptide; however, it is difficult to purify the conjugate with the desired full-length sequence from the conjugates with several deletion sequences of the peptide. It becomes especially difficult to purify conjugates of peptide sequences with one or few amino acid deletions via the commonly used high-performance liquid chromatography techniques. Recombinant methods represent an alternative method of producing linear, monodisperse, peptide, and polypeptide sequences; however, these methods do not provide facile approaches for site-selective reaction of sequences containing multiple chemically reactive groups. As mentioned earlier, the

incorporation of non-natural amino acids with chemo-selective handles offers attractive alternatives, although the efficiency of incorporation and ability to co-incorporate a natural amino acid and its chemically reactive counterpart require optimization and must be evaluated on a case-by-case basis.

CONCLUSION

The formation of protein- and peptide-containing polymers has afforded many unique materials. The conjugation of proteins with polymers has resulted in smart materials such as responsive hydrogels based on enzymecatalyzed reactions, protein–protein interactions, and protein–drug sensing. The conformational behavior of proteins has also been used for the creation of dynamic hybrid materials that undergo volume changes in response to protein-specific stimuli. Attachment of PEG to various proteins as drugs/enzyme has conferred improved therapeutically relevant functions, such as improved solubility, reduced immunogenicity, stability against degradation (increased circulation time), and prolonged biological activity. Attachment of stimuli-responsive polymers to peptides and proteins has resulted in the creation of stimuli-responsive smart materials.

The several examples of peptide–polymer conjugate discussed in this review have provided almost universal evidence that the secondary structure of the peptide is retained in the hybrids. Various peptides used in such hybrids, with α -helical, coiled-coil, and β -sheet structures, as well as peptides modeled after domains of structural proteins such as elastin, silk, and collagen, are able to display the native peptide's secondary structure and assembly. Thus, such peptide domains have endowed the hybrids with unique secondary structure, conformational behavior, and mechanical properties, and, in several cases, have resulted in well-defined hierarchical organization with useful potential applications as nanostructured materials. Various peptides such as cell binding, targeting, and penetrating peptides, enzyme-cleavable peptide substrates, and target-recognizing epitopes have also been used for synthesizing bioresponsive and bioactive hybrids for bio-medical applications. The conjugation of polymers, mostly PEG, to these several types of peptides has affected the aggregation behavior of coiled-coil domains, reduced the nonspecific aggregation of β -amyloid peptides, provided superior mechanical properties, governed a variety of elegant nanostructure assemblies, led to creation of protein and cell-adhesion-resistant surfaces, and, in several examples, led to the creation of smart stimuli-responsive hydrogels.

Thus, the development of hybrid materials, by combining the useful properties of peptides, proteins, and polymers, has offered new avenues in materials research and tremendous potential for various applications beyond biology and medicine. Continued active areas of research that will yield uniquely responsive and useful materials include the creation of dynamic, reversible, and responsive materials based on the use of actuating protein domains that can be engineered to respond to specific cues outside of those designated by evolution. Lessons from the unique conformational properties of peptides/proteins will be used for the development of peptide/protein-mimetic polymers, which can adopt similar secondary or tertiary structures or show similar biofunctional properties. Enzyme-substrate recognition has inspired and resulted in configurational biomimetic imprinting within hydrogels.¹⁵⁶ These approaches will be certain to result in materials that are increasingly responsive to

specific cellular cues and that may direct the formation of more biologically faithful tissue mimics. In the future, the ultimate goals for polymer conjugates with modular design could be to mimic the elegantly complex multilayered microstructural organization of biological system such as cell wall or tissue structures. A multicomponent hybrid system could be generated to exhibit responsive structural reorganization mediated via the polymer domain, which could then directly (or indirectly) mediate the structural organization and/or biological functions of the peptide/protein domain. Such elegance may offer new synthetic systems capable of recognition, decision-making, and regeneration.

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FIGURE 1.

Schematic illustration of responsive hybrid hydrogels modulated by the activities of the protein domain. (a) Mechanism of shrinkage of a glucose-sensitive, pH-responsive polymer network consisting of poly(methacrylic acid-*g*-ethylene glycol). Glucose oxidase (GOD) immobilized in the polymer network catalyzes the oxidation of glucose (G) to gluconic acid (GluA) in the presence of oxygen, which causes protonation of carboxylic acid groups, leading to the collapse of hydrogel. Reproduced with permission from Ref. 22. © 1997 American Chemical Society. (b) Swelling mechanism of hydrogel formed from polyacrylamide-based semi-interpenetrating network grafted with specific antigen and corresponding antibody. Upon competitive binding of the free antigen, preformed physical crosslinks in the hydrogels are disrupted, leading to reversible swelling. Reproduced with permission from Ref. 23. © 1999 Nature Publishing Group. (c) Gelation of polyacrylamide-based hydrogels containing a genetically engineered bacterial gyrase subunit B (GyrB) is triggered upon introduction of the GyrB dimerizing antibiotic coumermycin; gels are subsequently dissolved upon competitive binding of antibiotic novobiocin. Reproduced with permission from Ref. 24. © 2008 Nature Publishing Group.

FIGURE 2.

Schematic illustration of responsive hybrid hydrogels based on conformational changes of protein domains. (a) Swelling mechanism of a polyacrylamide-based hydrogel coupled with genetically engineered calmodulin (CaM) and the corresponding ligand phenothiazine. Original state: hydrogel saturated with Ca^{2+} had phenothiazine-binding site accessible and immobilized phenothiazine bound to it forming noncovalent cross-links. Swollen state: Ca^{2+} removed from the CaM-binding site upon introduction of Ca^{2+} -chelating agent ethylene glycol-bis(β -aminoethyl ether)-*N, N, N', N'*-tetraacetic acid leads to the disruption of cross-links and swelling of the hydrogel. Reproduced with permission from Ref. 30. © 2005 Nature Publishing Group. (b) Mechanism of deswelling of photopolymerized PEG-CaM-PEG hydrogel. Hydrogel underwent significant volume reduction upon the conformational change of CaM from an extended conformation (in Ca^{2+} buffer) to a closed conformation (in Ca^{2+} buffer with the ligand trifluoperazine). Reproduced with permission from Ref. 31. © 2007 Wiley. (c) HPMa-based hydrogels cross-linked with adenylate kinase (AKtm)/DTT. The expected nanoscale conformational change of AKtm upon substrate (ATP, adenosine triphosphate) binding, from open to closed form, concomitantly resulted in a macroscopic volume transition of the hydrogel. Reproduced with permission from Ref. 33. © 2008 American Chemical Society.

FIGURE 3.

Schematic illustration of various hybrids generated via conjugation of α -helical coiled-coil peptides to synthetic polymers. (a) Alternating multiblock hybrids synthesized via conjugation of PEG with coiled-coil peptides through the *f*-position on the heptads. Top, blue-colored schematic of the coiled coil represents arginine-rich coiled-coil partner, whereas the bottom, green one represents glutamic acid-rich coiled-coil partner. The conjugation of PEG triggered homo-oligomer formation in arginine-rich coiled-coil-PEG conjugate, which is monomeric in the absence of PEG. (b) Fibrin-derived coiled coil conjugated to a central PEG block. The oligomeric association of the coiled-coil end blocks mediates cross-linking of the hybrids to form hydrogel. (c) A synthetic HPMA-based copolymer carrying pendant metal-chelating group of iminodiacetic acid is cross-linked upon the tetrameric association of the histidine-tagged coiled-coil peptides, leading to gelation. (d) Hybrid graft copolymers are produced by grafting coiled-coil peptides on HPMA-based copolymer. Homodimeric association of the coiled-coil peptides leads to cross-linking of the polymer chains and gelation.

FIGURE 4.

Schematic illustration of various hybrids generated via conjugation of β -sheet peptides to synthetic polymers. PEG or HPMA-based copolymer conjugated to the β -sheet forming peptides (represented by blue arrow) at the (a) C terminus, (b) N terminus, and (c) both the termini. These peptide–polymer conjugates could form fibrils, mediated by the peptide domain. Schematic (d) represents a three-functional carbazole-based template, which was functionalized with a PEO chain on one side, and two strands of the short (Val-Thr)₂ tetrapeptide on the other side. This tetrapeptide organizer in water-formed interdigitated, antiparallel β -sheets as a core, with PEO chains extended as a shell. Schematic (e) represents poly(*n*-butyl acrylate) conjugated to β -sheet forming peptides. The peptide block had defect in the primary sequence, which restricted β -sheet formation. However, upon increasing the pH, *O*-*N*-acyl migration corrects the primary structure to generate intact β -sheet forming peptide and exhibits higher-order assembly to form fibrils and fibrillar network.

FIGURE 5.

Schematic illustration of various hybrids generated via conjugation of silklike β -sheet peptides or polypeptides to synthetic polymers. PEG chains conjugated to (a) silk-inspired β -sheet peptide sequence (indicated by blue arrow) (b) silk-inspired β -sheet peptide sequence preorganized on an aromatic hairpin template to prepare segmented multiblock copolymer. These multiblock hybrid forms microphase-separated architecture displaying nanodomains of the aggregated peptide block interspread within the PEG phase. Schematic (c) represents a hybrid triblock system in which PEG chains were conjugated to central β -sheet forming silklike polypeptide. The peptide block permitted formation of fibrils with dimensions well correlated with the dimensions of the polypeptide chain. Schematic (d) represents a triblock hybrid system with the central block containing β -sheet peptide as pendant side chains. The conformational behavior of silk-inspired β -sheet peptide sequence was still retained in such a design.