Published in final edited form as:

J Mater Chem B. 2017 ; 5(42): 8274–8288. doi:10.1039/C7TB02199B.

Responsive Hybrid (Poly)peptide-Polymer Conjugates

Bradford A. Paik^a, Shivshankar R. Mane^b, Xinqiao Jia^{a,c,d}, and Kristi L. Kiick^{a,c,d}

^aDepartment of Materials Science and Engineering, University of Delaware, 201 DuPont Hall, Newark, DE 19716-3106

^bThe Institute For Chemical Technology and Polymer Chemistry, Karlsruhe Institute of Technology, Engesserstr. 18, 76128 Karlsruhe, Germany

^cDepartment of Biomedical Engineering, University of Delaware, 150 Academy Street, 161 Colburn Lab, Newark, DE 19716-3106

^dDelaware Biotechnology Institute, 15 Innovation Way, Newark, DE 19711

Abstract

(Poly)peptide-polymer conjugates continue to garner significant interest in the production of functional materials given their composition of natural and synthetic building blocks that confer select and synergistic properties. Owing to opportunities to design predefined architectures and structures with different morphologies, these hybrid conjugates enable new approaches for producing micro- or nanomaterials. Their modular design enables the incorporation of multiple responsive properties into a single conjugate. This review presents recent advances in (poly)peptide-polymer conjugates for drug-delivery applications, with a specific focus on the utility of the (poly)peptide component in the assembly of particles and nanogels, as well as the role of the peptide in triggered drug release.

Graphical abstract

Introduction

Nanoscale drug delivery vehicles represent a vast and continually growing field employing new strategies and approaches to enhance drug efficacy. Effective delivery systems can

Conflicts of interest

There are no conflicts to declare

prolong drug half-life, improve solubility of hydrophobic drugs, reduce potential immunogenicity, and release drugs in a sustained or stimuli-triggered fashion.^{1,2} Some systems, depending on their formulation and biochemical properties, can also reduce the toxic side effects of drugs and the frequency of drug administration through site-specific delivery, and lowering of the concentration of drug in circulation.^{3–5} In addition, nanocarriers (1–1000 nm) can passively accumulate in specific tissues (such as tumors) through the enhanced permeability and retention (EPR) effect⁵, and for carriers with pH-, thiol-, or enzyme-sensitivity, such accumulation permits targeting via both size and stimuli-responsive delivery.

For decades polymeric drug delivery systems have been extensively studied, and many polymers have been well developed. The variety of polymers, block architectures, self-assembly properties, stimuli-triggered responses, and loading capabilities have all been reviewed in detail elsewhere.^{3,6–10} In addition, the extensive work in polymer conjugation approaches, such as the widely employed conjugation of poly(ethylene glycol) (PEG) to drug or proteins/(poly)peptides has also been reviewed.^{11–13} The natural progression and intersection of polymer science and protein/(poly)peptide science has led to the incorporation of functional (poly)peptides – selected for their unique properties – into these polymers to endow them with useful properties such as biocompatibility and biodegradability, modularity, chemical functionality, control of assembly over multiple length scales, and response to external stimuli. The folding and unfolding behavior also affords flexibility to the backbone, and higher-order assembly of the peptide matrix can be used for guest encapsulation and controlled release.¹⁴ When resistant to degradation,¹⁵ these conjugates can be used to build dynamic enzyme-responsive materials.^{16–19} The use of (poly)peptides also can mediate assembly of predefined morphologies, such as micelles, vesicles, liposomes, and nanoparticles, which are widely used as delivery vehicles.^{20–22} (Poly)peptides have also been extensively used as targeting ligands, the most widely employed example being the integrin-binding sequence RGD,²³ and as a whole, the development of peptide-polymer conjugates has opened new avenues for different biomedical applications (Figure 1).^{24,25} This review aims to highlight the recent advances in the area, and focus on systems that specifically make use of (poly)peptides and their functional role in the assembly, loading, and stimuli responsive nature of the materials for drug delivery purposes. The conclusions section should come in this section at the end of the article, before the acknowledgements.

Synthetic strategies for producing peptide-polymer conjugates

Polymer and peptide synthesis

The development of well-defined peptide-polymer conjugates has been made possible by advances in controlled polymerization methods and chemical coupling reactions. Different polymerization techniques such as atom transfer radical polymerization (ATRP),^{26–30} reversible addition-fragmentation chain transfer polymerization (RAFT),^{29,31} nitroxide mediated polymerization (NMP),^{29,32–35} opening metathesis polymerization (ROMP),^{17,36–39} and ring opening polymerization (ROP),^{40–42} have facilitated the development of tailor-made polymers that possess predictable molecular weight and narrow molecular

weight distributions. The properties of the controlled polymers can then be further enhanced via conjugation of (poly)peptides to tune hierarchical assembly, stimuli response or desired targeting (Figure 2). Conjugation of PEG to (poly)peptides and protein drugs has been most commonly employed,^{11–13} given the significant improvement in circulation times as a result of PEGylation.⁴³ Within the past decade there has been a push to advance beyond PEGylation, as recent literature has documented anti-PEG antibodies that have a detrimental effect on drug efficacy.⁴⁴ A range of PEG-alternatives such as poly(N-vinylpyrrolidone),⁴⁵ poly(glycerol),⁴⁶ poly(zwitterions),⁴⁷ poly(carbonates),⁴⁸ poly(oxazolines),⁴⁹ and poly(saccharides),⁵⁰ have shown promise, and have been reviewed elsewhere.^{51,52} In addition, polyphosphoesters (PPE) represent an important alternative to PEG because of their biocompatibility, biodegradability, similar architecture to biomolecules, and ability to be functionalized.⁵³ Recently, Wooley and co-workers developed a PPE-based nanocarrier which showed great promise in the treatment of lung metastasis of osteosarcoma.⁵⁴ Steinbach et al. have also shown the potential for conjugation of PPE to proteins, enabling protein stabilization, but allowing for degradation of the PPE into biproducts, which are cleared through renal excretion.⁵⁵

Regardless of the type of polymer of interest, the development of controlled polymerization methods has been critical in advancing the production and use of (poly)peptide-polymer conjugates. For example, ATRP methods have been widely used to generate functional polymers that can be easily integrated with peptides and other functionality. For example, ATRP has been employed to prepare a poly(pentafluorophenyl methacrylate) polymer, which was further functionalized with a thiol-terminated peptide (CVPGVG) via a thiol-ene reaction. The main drawback of ATRP is the use of toxic copper catalysts, although this issue can be resolved by the use of ion exchange resins to remove the copper catalyst, ultimately expanding the use of ATRP in biological applications.^{56,57} Given long-standing interest in hybrid biomaterials that are capable of showing unique mechanical behavior, we have employed controlled radical polymerization to produce poly(acrylic acid)-based multiblock polymers with thermoresponsive elastin-like peptide (ELP) domains. Azide-functionalized, telechelic poly(tert-butyl acrylate) (PtBA) was synthesized via ATRP, and was conjugated to a (VPGVG)₂ (VG₂) peptide based on the pentamer repeat unit VPGXG (where X can be any amino acid except proline). The tert-butyl groups were cleaved yielding (VG₂)-poly(acrylic acid) (PAA) multiblocks, and well-defined di- and triblocks.^{58,59}

RAFT-based peptide-polymer hybrid conjugates are also well represented in the literature.^{60,61} RAFT polymerization produces well-defined end-functional polymers by using an appropriate chain transfer agent (CTA) with the advantage that it can be applied toward a wide variety of monomers and conditions without using metal catalysts. De et al. for instance, reported well-defined side-chain peptidic polymers. A short amyloidogenic peptide (LVF), was functionalized with a methacrylate monomer, and subsequently synthesized from a monomethoxy poly(ethylene glycol) (mPEG) macro-chain transfer agent to yield mPEGn-*b*-P(LVF-oxyethyl methacrylate) block copolymers.⁶² In another example, RAFT-based glycopolymers-peptide conjugates were employed to prepare poly(2-(2,3,4,6-tetra-O-acetyl-*b*-D-glucosyloxy)ethyl methacrylate) (PAcGlcEMA). The obtained polymer was further conjugated with the tripeptide glutathione via a thiol-disulfide exchange reaction.⁶³ In one study, a RAFT-based poly(N-vinylpyrrolidone) PVP was functionalized *via* a disulfide

bridge formation between somatostatin (a cyclic peptide hormone) and the end-functionalized PVP.⁶⁴ Although end-group modification of the thiol has utility, a drawback to the use of RAFT is the possibility of cytotoxicity due to trithiocarbonate and dithioester moieties. However, development of facile methods for endgroup removal, as well as post-polymerization end-group modification with azobis(isobutyronitrile) has remedied some of these drawbacks.^{65,66}

Nitroxide-mediated polymerization has also often been employed for its ease of access to functionalized alkoxy-amines for bioconjugation purposes. For instance, amide-functionalized alkoxyamines (a functionality often obtained after conjugation from COOH- and N-succinimidyl-containing alkoxyamines) based on the nitroxide SG1 (N-tert-butyl-N-(1-diethyl phosphono-2,2-dimethylpropyl) nitroxide) have been synthesized.^{32,33} In another approach, peptide-polymer conjugates were produced via a solid-support method involving both step-growth and chain-growth polymerization mechanisms. An amphiphilic triblock copolymer consisting of a protein transduction domain (PTD) peptide, poly(acrylic acid), and poly(methyl acrylate) (PTD-*b*-PAA-*b*-PMA) were prepared using this approach.³⁵ A conjugate with the peptide-based backbone (GK)₄ was synthesized by creating GK repeats, and modifying the lysine side chain with NMP initiators. The side chain initiators were then used to polymerize PNIPAM, yielding a soluble conjugate that undergoes triggerable aggregation. Polystyrene (PS) was also polymerized from the side chain initiator; these conjugates were found to form honeycomb structures.⁶⁷

The speed and fidelity of ROMP, and its yield of monodisperse polymers, has made it valuable in the synthesis of peptide-polymer conjugates as well, mainly by incorporating peptides as side-chains into polymers.^{68,69} Norbornene monomers comprising bioactive peptides, including matrix metalloprotease substrates and RGD-based peptides, have been used to make peptide-polymer conjugates.⁷⁰ Elastin-like peptides,⁷¹ TAT (a well-known cell penetrating peptide derived from human immunodeficiency virus—HIV—transactivator protein), SV40 (a typical NLS peptide), and FxRFxK (a mitochondria-penetrating peptide sequence) have all been conjugated to a norbornene backbone.⁷² Gianneschi et.al reported that a series of norbornyl pentapeptides were prepared with the general structure norbornyl-GX₂PLX₅, where residue 'X' was changed at each of the two positions (2 or 5) to introduce the natural amino acids F, A, V, R, S, K, N, T, M, Q, H, W, C, Y, E, Q, and D. Each peptide monomer, free of protecting groups, was mixed in turn under a standard set of polymerization conditions with the ROMP initiator (IMesH₂)(C₅H₅N)₂(Cl)₂Ru]CHPh, given the remarkable functional group tolerance of this method.^{38,39,73,74}

Briefly, ROP has been extensively used to prepare polypeptides that can easily be obtained through controlled polymerization of α -amino acid N-carboxyanhydrides (NCAs).⁷⁵ ROP has potential for the development of peptide-based stimuli-responsive materials with unique properties such as tunable conformations, chemical functionalities, and 3D nanostructures. In particular, L-glutamic acid NCA has been a widely used monomer in ROP aimed at producing conjugates. Diblocks consisting of PEG and poly(L-glutamic acid) (PLGA) have been used for micelle formation for delivery of platinum drugs,^{76–80} and conjugation of cisplatin to the carboxylic acid side chain results in subsequent vesicle formation.⁸¹ Incorporation of a poly(L-leucine) into a PEG-PLeu-PLGA triblock allowed for controlled

release of a loaded drug due to the pH-responsive conformational change of the PLGA block. In another example, Wooley and co-workers have also used NCA ROPs to develop di- and triblock conjugates consisting of oligopeptides whose secondary structure can drive the formation of gels.^{82,83}

Finally, the synthesis of peptides can be achieved by several approaches, dependent on length and complexity of the peptide. As previously discussed, ROP can be used to synthesize peptides of various lengths but low compositional and/or sequence complexity, where one to a few amino acids would be used. For more complex sequences that do not extend beyond 50 amino acids in length, well-developed solid-phase peptide synthesis (SPPS) methods are employed. For example, ELPs,^{59,84–90} collagen-like peptides (CLPs),^{90–93} and helical, alanine-rich peptides,^{94,95} have been easily incorporated by our group and others into polymer conjugates. Others have explored the synthesis of amyloidogenic domains,^{96,97} coiled-coil sequences,^{98,99} and peptides for use as targeting ligands.^{100–102} Longer (poly)peptide sequences are produced via recombinant protein expression, providing great control over amino acid sequence for larger polypeptides. PEG has been coupled to alanine-rich, recombinantly produced polypeptides to enable the formation of fibrils upon changes in pH and temperature,¹⁰³ and it has also been conjugated with recombinant elastin-like polypeptides (ELPp) to yield particles.¹⁰⁴ Poly(oligo(ethylene glycol) methyl ether methacrylate) (poly(OEGMA)) was synthesized from a central ELPp block to form a gel.¹⁰⁵ Poly(oxazoline) (POx) has also been conjugated to an ELP to form particles for drug delivery.¹⁰⁶ Another recent study explored the conjugation of ELPp to poly(lysine), poly(arginine), and poly(ethyleneimine), as well as to poly(2-vinyl-4,4-dimethyl azlactone) to form nanoparticles with potential in drug delivery.¹⁰⁷ While SPPS, ROP and recombinant methods are successful, the development and application of hydrophobic peptides and their inclusion into peptide-polymer conjugates remains a synthetic challenge.⁴²

Chemical Conjugation Approaches

The development of biorthogonal coupling chemistries has had a profound effect on the expansion of bioconjugation methods, enabling the synthesis of an enormous variety of hybrid materials. These chemistries are highly efficient and form products in high yields, and their site-specific reactivity allows for precision in the synthesis of distinct biomolecular conjugates (Figure 3). The copper I-catalyzed alkyne-azide cycloaddition (CuAAC), Cu-free strain-promoted azide-alkyne cycloaddition (SPAAC), Diels-Alder reaction, thiol-ene chemistry, and tetrazine ligation have all been employed to create a wide variety of hybrid biomaterials.

CuAAC reactions, developed by Sharpless and co-workers,¹⁰⁸ are frequently used because of their high yield, fast reaction time, regiospecificity, and high efficiency.^{109–113} Development of ligands has also enabled CuAAC reactions to proceed with high efficiency in aqueous solutions as well as organic.¹¹⁴ Our group has employed these methods in the synthesis of peptide-polymer conjugated hybrid materials;¹¹⁵ the PAA-VG2 di-, tri- and multiblocks mentioned above were conjugated via CuAAC chemistry with great fidelity. The synthesized hybrid copolymer has potential utility for drug loading through hydrogen bonding and hydrophobic interactions.^{89,59} Similarly, an alanine-rich peptide (AKAAKA)₂

and PEG-containing multiblock copolymer were synthesized via CuAAC reaction.¹¹⁶ In another study, the functional oligopeptide GGRGDG was conjugated to poly(oligo(ethylene glycol)acrylate) (POEGA) yielding well-defined, bioactive conjugates.¹¹⁷ The integration of RGD-peptide motifs and their incorporation via CuAAC has been reviewed by Colombo and Bianchi.¹¹⁸ The conjugation of poly(butyl acrylate), poly(styrene) or PAA to a cyclic peptide via CuAAC, their self-assembly into nanotubes, and the effect of grafting density has also been explored.^{119,120} PEG conjugation via CuAAC and its effect on the fibrillization of amyloid-based β -sheet forming peptides was investigated to inform the synthesis of peptides used for supramolecular structures.¹²¹

Though CuAAC is widely used, it can be difficult to remove the Cu catalyst from the products, which can result in biocompatibility and toxicity issues. To overcome these limitations, Bertozzi and coworkers introduced a Cu-free, SPAAC that exploits the ring strain of cyclooctynes.^{122–126} SPAAC has commonly been used for the labeling of proteins, and in some cases, PEGylation of proteins.¹²⁷ In one study, ELP block copolymers were recombinantly expressed with lysines introduced at the N-terminus, which were subsequently functionalized with azides. The LCST-like behavior of the ELPs was exploited to produce nanoparticles which were then crosslinked using SPAAC, and could sequester the hydrophobic dye 1,8-ANS.¹²⁸ In another example, a difunctional cRGD peptide derivative was functionalized with an N_3 -PEG₄-azadibenzocyclooctatriazole (ADIBOT) via SPAAC for positron emission tomography (PET); the addition of the small PEG linker increased the binding affinity to the $\alpha_v\beta_3$ receptor.¹²⁹ In terms of reactivity, ring strain as well as electron withdrawing functionality (such as fluorine substituents) can induce a rapid reaction of cyclooctynes with azide. The most important limitation of this reaction is the rate of the reaction, which is 10-to-100 times slower than a typical CuAAC reaction. Other challenges include the hydrophobicity of the reactants as well as the instability of cyclooctynes in the physiological environment.¹³⁰

Thiol-ene reactions are also employed for conjugation, as well as polymerization, and forming networks, and generally categorized can occur via a Michael-type addition in the presence of a nucleophile or via free-radical addition of a thiol initiated by light or heat.^{131–137} These thiol-ene reactions have been used in conjugation reactions with functional groups such as maleimides, acrylates, norbornenes, and alkenes.^{138–142} Michael-type addition chemistry was used to covalently link cysteines in the sequence GRGDSPC, as well as glutathione *S*-transferase (GST) tagged III_{9–10} (GST-III_{9–10}) to the acrylate groups of PEG diacrylate (PEGDA) hydrogels. Hydrogels functionalized with the III_{9–10} stimulated more robust cell adhesions than the RGD-functionalized hydrogels.¹⁴³ Tsurkan et al functionalized a starPEG-Heparin hydrogel with an RGD-peptide sequence using Michael-type addition. The method allowed for precise control over the peptide concentration within the hydrogel, and three types of RGD sequences were used to study the effect of their flanking residues as well as three-dimensional structure on cell adhesion and viability. Their results indicated that at a 1 mol peptide/mol heparin, cyclo-RGDYC was the most effective system, yielding confluent cell layer, compared to lower concentrations of RGD, or the non-cyclical peptides.¹⁴⁴ UV-initiated thiol-ene chemistry has been used for conjugation of an RGD peptide to a linear poly(allyl glycidyl ether) (PAGE) for platelet adhesion. An N-terminal cysteine was added to the RGD sequence, which was subsequently reacted with

PAGE, although the peptide conjugation to PAGE was reportedly low.¹⁴⁵ In one study, a peptide sequences based on the CDR H3 region of IgG1 b12, a neutralizing anti-HIV-1 antibody, was conjugated to the side chains of a poly(pentafluorophenyl methacrylate) (PPFMA) via heat initiated thiol-ene chemistry. Though the conjugate system was not as effective as the IgG1 b12 antibody by itself, the platform shows potential as an interesting therapeutic alternative.¹⁴⁶ Diblocks were designed by Bacinello et al., consisting of PVGLIG peptides conjugated via UV-initiated thiol-ene chemistry to poly(trimethylene carbonate) (PTMC). Well defined vesicular nanoparticles were formed with morphologies that could be tuned by varying the length of the hydrophobic PTMC block. Enzymatic cleavage of the peptide was used to promote particle degradation.¹⁴⁷ In a further study, a triblock comprising PLGA-*b*-PVGLIG-*b*-PTMC was synthesized using UV-initiated thiol-ene coupling methods. Vesicles were assembled using nanoprecipitation methods, and were used for the encapsulation of imipramine, which is used to treat depression; release profiles showed that the vesicles were responsive to pH, temperature, and enzymatic stimuli.¹⁴⁸ Reportedly, the conjugation of the larger molecular weight species (PLGA-*b*-PVGLIG) to the PTMC resulted in low yields, and required a constant supply of initiator, which may be a drawback to using UV-initiated thiol-ene chemistry for larger molecules.

Recently, tetrazine ligation (an inverse electron Diels-Alder reaction between s-tetrazine and trans cyclooctene (TCO)) has emerged as a versatile conjugation method owing to its exceptionally rapid reaction rates;¹⁴⁹ second order rates of reaction of $3.3 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$ in aqueous conditions at room temperature have been achieved.^{150–153} Furthermore, literature suggests that TCO offers better reaction rates than other dienophiles such as alkene, norbornene, cyclooctynes etc.^{154–157} Pioneered by the Fox group, the Fox and Jia groups subsequently developed the tetrazine ligation for the production of polymers and polymer conjugates.¹⁵⁸ Jia and coworkers further reported the formation of block copolymers by interfacial bioorthogonal polymerization. The multiblock copolymer fibers were obtained by rapid reaction between sTCO and diphenyl-s-tetrazine (GGWGRGDSPG peptide tagged with PEG) PEG derivatives with immiscible liquid-liquid interfaces.¹⁵⁹ These studies indicate clear use for the tetrazine ligation reactions owing to their fast reaction time, high yield, lack of toxic side products, and the lack of catalyst required in the aqueous environment.

Responsive peptide-mediated assembly

Peptide-polymer conjugates have been widely studied, as mentioned above, owing to the fact that assembly can be directed in response to stimuli such as light,^{160–162} pH,^{62,163–169} temperature,^{170–172} reduction,¹⁷³ and enzymatic cleavage,^{174–176} all which greatly enhance a conjugate's versatility across applications in biological and pharmacological areas and allow on-demand or targeted release of therapeutic molecules^{140,142,177–183} In addition to external stimuli, amphiphilic interactions are often employed to drive the self-assembly of peptide-based materials. Peptide amphiphiles are a promising class of material in which the addition of an alkyl chain to a peptide leads to assembly.^{184–186} The mechanism for assembly of peptide amphiphiles is most commonly derived from the addition of an alkyl chain, although other approaches employing the inherent hydrophobicity of the peptide sequence have been developed. In addition to the amphiphilic and stimuli-responsive

properties that the (poly)peptide domain can introduce to a conjugate, the (poly)peptide domains can also facilitate sequestration of desired cargo via encapsulation,¹⁸⁷ association,¹⁸⁸ or covalent attachment.¹⁸⁹ Tuning the responsiveness of these conjugates by varying the identity of the peptide domain can thus enable control over circulation time, targeting and drug release. Here, examples of systems driven by the (poly)peptide incorporated into the conjugate are highlighted; a summary of some of these examples is provided in Table 1.

Amphiphilic driven assembly

Xu and coworkers have developed a series of amphiphilic peptide-polymer conjugates.^{99, 190–193} Shu et al. explored coiled-coil helix bundle forming peptides in which hydrophobic PS was conjugated onto the N-terminus.⁹⁹ These conjugates were able to form micelles ca. 10 nm in diameter in the presence of surfactant. This study found, in comparison to helical bundles that were conjugated with PEG, that PS denatured the helices and thus cofactor binding was severely limited.⁹⁹ Dube et al. further explored the effect of various conjugated hydrophobic polymers, PS, PMMA, and PNIPAAm, finding that the peptide structures was greatly affected by the hydrophobicity of the polymer. An intriguing aspect was the use of PNIPAAm, and the ability to disrupt the helicity of the bundle with increasing temperature due to the LCST properties of the polymer.¹⁹⁰ Whereas the previous work used a four-helix bundle, Dong et al. employed a 3-helix bundle conjugated with a PEG molecule, and functionalized with two alkyl chains at the N-terminus (Figure 4). This conjugate amphiphile was able to form micelles with diameters ca. 10 nm, and was able to encapsulate DOX (drug loading 7–8%) and other drug and dye molecules. Even more promising was the amphiphile's ability to co-assemble with ⁶⁴Cu ions; these loaded micelles were injected into mice bearing neu deletion (NDL) model tumors (a model for human breast cancer). Positron emission tomography (PET) was used 48 hours after injection to demonstrate the high concentration in the blood, with minimal liver and spleen accumulation. The β -phase blood circulation half-life of the micelles was estimated to be ~29.5 h; this long circulation half-life was attributed to the size, stability and surface chemistry of the micelle.¹⁹¹ One further study showed that the increase of the alkyl chain length led to higher stability of the micelles, and slower and extended cargo release.¹⁹² This 3-helix amphiphile was labeled with ⁶⁴Cu and demonstrated a superior ability to accumulate in glioblastoma within a rat model, as compared to a PEGylated liposome.¹⁹³ The size, stability, blood circulation half-life and ability to be used for imaging or carrying a drug payload, shows the versatility of this 3-helix amphiphile and its potential in drug delivery systems.

pH-responsive assembly

Poly(L-glutamic acid) (PLGA), poly(L-lysine) (PLL), poly(L-histidine) (PHis) have been used in peptide-polymer conjugates, conferring pH-sensitivity to drug delivery systems, providing amphiphilicity for forming particles, and presenting reactive sites for further functionalization. The polypeptides alone have shown drug carrier capabilities through complexation with fibrillar DOX,^{194,195} and in direct conjugation of DOX to PLGA.¹⁶⁴ However, to achieve higher, direct dosage of drug to cancer cells, high-efficiency encapsulation of drugs and uptake into cells is desired. Having shown their promise alone,

the conjugation of these peptides to drugs has allowed for particle formation, with tunable pH responsiveness.

Li et al. conjugated PLGA with PEG chains to form nanomicelles for DOX and superparamagnetic iron oxide nanoparticles (SPIONs). The carboxylic acid side chains of the PLGA were functionalized with N,N-diisopropyl (DIP) for approximately 50% functionalization. The ionic interaction between the free carboxylic acid side chains and the tertiary amine DIP allowed for pH-responsive drug release. At pH 7, the carboxylic acid and tertiary amine form ionic crosslinks, with less than 20% of the loaded DOX being released after 30 days. At pH 5.0, DOX release was greatly accelerated, with 41.5% released being released after 2 days. Functionalization of folate to the PEG chain for targeting did increase cellular uptake, but the folate molecule significantly inhibited the encapsulation of DOX in the conjugate core, resulting in a reduction in loading from 39% in the allyl-functionalized PEG to 3.2% for the folate-functionalized conjugate.¹⁹⁶ More recently, variations in the grafting density of DIP onto the PGA was explored, with higher grafting densities achieving enhanced release rates of DOX.¹⁹⁷

Poly(histidine) (PHis) has also been used in pH-responsive conjugates as a “proton-sponge”, enabling swelling at acidic pH and release of the conjugate’s payload.^{198–203} As the blood stream has a pH of 7.4, and tumor environments a pH of 5, PHis-based conjugates are an ideal component for an injectable drug delivery systems that, upon introduction to the tumor microenvironment, release a chosen drug. Recently, Johnson et. al. synthesized triblocks comprising poly(ethylene glycol) methyl ether acrylate-*b*-PLL-*b*-PHis [p(PEGA)-*b*-PLL-*b*-PHis], which were able to form vesicles ~200 nm in diameter (Figure 5). PLL was chosen for its promotion of vesicle clearance from the blood compartment, and PHis used for its ability to act as a proton-sponge. Various PHis block lengths were used within the triblocks with DOX loading content increasing with PHis length. These vesicles showed biocompatibility, and were successfully internalized into NIH 3T3 and CT26 cells; the vesicles preferentially released DOX in the acidic environment of the cells.²⁰⁴ Sun et. al. used a poly(itaconic acid)-poly-PEG-PHis (PIA-PEG-PHis) triblock to form micelles (with diameters ca. 180 nm) which could encapsulate DOX. PIA was used as a backbone polymer, with PEG and PHis acting as hydrophilic and hydrophobic side chains, respectively. DOX was encapsulated in the hydrophobic core, and under acidic conditions, 100% drug release was observed within 50 hours.¹⁸⁷ Bagherifam et. al. synthesized polysebacic anhydride (PSA) and modified it with PHis, to take advantage of the biocompatibility and controlled erosion of PSA, as well as the pH triggerability of PHis. The nanocapsules were also modified with PEG to improve transport performance. An interesting finding was that PEGylation actually led to less cell encapsulation as compared to non-PEGylated nanocapsules. The dual responsive nature of the PSA and PHis makes this a promising drug delivery vehicle.²⁰⁵

Polyion complex (PIC) micelles, in which assembly is directed by the electrostatic interaction between a pair of oppositely charged polymers or molecules, have also been employed. Chen et al. explored the use of side-chain modification of PLL in a PIC system for pH-responsive drug delivery. A PIC micelle consisting of methoxy-PEG-*b*-PLL (mPEG-*b*-PLL) was prepared via ROP of N(e)-Benzyloxycarbonyl-L-lysine (ZLL) by using mPEG-

NH₂ as a macroinitiator. The side chains of the PLL were subsequently modified via a ring-opening reaction (ROR) with four different anhydrides: succinic anhydride (SA), cis-cyclohexene-1,2-dicarboxylic anhydride (CDA), *cis*-aconitic anhydride (CA), and dimethyl-maleic anhydride (DMMA). The hydrolysis of these amide bonds was then characterized under various pH conditions. Each PIC variant formed micelles of sub-100 nm diameter at pH 7.4, and at pH 5.3, underwent an increase in diameter of up to several hundred nanometers, and then complete degradation after 4 hours. Increased acidity led to hydrolysis of amide bonds and increased DOX release rates across all variants. The variants demonstrated a gradient in pH-sensitivity, with the DMMA PIC variant having the highest acid sensitivity, and thus the highest cumulative DOX release rate. CA and DMMA variants also showed improved intracellular targeting DOX release and cytotoxicities over free DOX. The CDA variant micelles were chosen for testing in a H22 hepatoma-bearing BALB/c mouse model and exhibited higher antitumor efficacy, and lower systematic toxicity, when compared to free DOX. The facile synthesis of these PIC micelles, and their pH-responsiveness, suggests their promise as a drug delivery vehicle.²⁰⁶

Enzyme-responsive assembly

Enzymatically responsive polymer-peptide conjugates are widely used in PEG-based bulk hydrogels. Here, we review examples of microparticles formed from hydrogels, as well as nanoparticles formed by self-assembly of polymersomes. Some common peptide sequences well established in the literature include AAPVR, AAP(Nva), and AAAAAAAAAAK, which are degraded by the enzyme elastase,^{207–209} and GGLGPAGGK,²⁰⁹ GPQGIQQQ, GPQGIAGQ and GPQGILGQ, which are degraded by collagenase and the matrix metalloproteinases MMP 1–9.^{174,210,211,212} Recently, Secret et al. reported matrix metalloproteinase-responsive microparticle hydrogels (diameters of 2.8 to 4 μm) in which the peptide Gly-Leu-Lys was incorporated in the polymer backbone of a PEG-diacrylate macromer to form a bis-poly(ethylene glycol) acrylate Gly-Leu-Lys conjugate that specifically responds to MMP2, which is overexpressed in pulmonary diseases such as lung cancer and tuberculosis. Furthermore, in order to increase the sensitivity to matrix metalloproteinase, the GPQGIFGQK peptide was employed; this longer peptide permitted degradation of the microparticles at 10-fold lower concentrations than microparticles comprising the GLK peptide.²¹³

In an additional example, novel four-armed poly(*ε*-adamantane-L-lysine)₂-*b*-poly(ethylene glycol)-*b*-poly(*ε*-adamantane-L-lysine)₂ (PLys(Ad)₂-*b*-PEG-*b*-PLys(Ad)₂) drug carriers were prepared via the ring-opening polymerization of amino acid N-carboxyanhydrides. These copolymers formed core-shell micelles in aqueous solutions, and the *in vitro* release of entrapped DOX from the micelles was accelerated by the addition of β-CD.²¹⁴ As previously mentioned, Bacinello et al developed polymersomes comprising the enzymatically degradable peptide PVGLIG, which showed enzymatic responsiveness when mixed with MMP1, demonstrating promising particle degradation and drug release (Figure 6).^{147,148}

Temperature-responsive assembly

Thermoresponsive peptides and recombinant polypeptides have been extensively studied, and are a promising material used in conjugates for temperature dependent drug delivery. Elastin-like polypeptides (ELPs) have been a commonly employed biomaterial used in drug delivery vehicles owing to its lower critical solution temperature (LCST)-like behavior. Various ELPs, based on the pentamer repeat unit VPGXG (where X can be any amino acid besides proline), have been used to encapsulate therapeutic peptides and other drugs.^{215–217} Collagen-like polypeptides (CLPs) have also been explored due to their thermoresponsive triple helical secondary structure, and have been used for gels in tissue regeneration.²¹⁸ Here we discuss the use of shorter peptides mimicking the thermoresponsiveness of their polypeptide counterparts.

Conjugation to dendrimers of short ELPs and CLPs, for generation of drug delivery vehicles, has also been explored. Short CLP segments, (Pro-Pro-Gly)₅, have been conjugated to an ethylene diamine-core polyamidoamine (PAMAM) dendrimer, and the hallmark triple helix formation of the CLP was observed; the ability to form the triple helix was attributed to the local clustering of the short peptides. Rose Bengal (RB) was used as a model cargo for encapsulation and the release of the RB was effectively suppressed at 4 °C, as compared to release at higher temperatures. This suppression was attributed to the CLP enhancement of the binding of the RB to the dendrimer, as well as the decreased permeability of RB at the dendrimer surface.⁸⁷ The conjugation of a the short ELP-derived peptide VPGVG onto the dendrimer was also explored. A β -turn conformation of the VPGVG was observed with increasing temperature, indicating that conjugation of the ELP to the dendrimer did not inhibit the temperature-dependent conformation change of the ELP. As in the case of the CLP conjugate, they also attributed this elastin-like characteristic to the local clustering of peptides at the dendrimer surface, inducing the LCST-like behavior, although the ELP alone would not be expected to undergo the thermally induced transition. The encapsulation and release of RB was also characterized, but there was no significant difference between the ELP-functionalized dendrimer and the dendrimer alone.²¹⁹ In another study, the effects of increasing the number of peptide repeats and increasing dendrimer generation were explored. Doubling the length of the peptide repeat unit (to ELP₂) led to lower phase transition temperatures, in agreement with ELP literature.^{215,220,221} Increasing dendrimer generation in combination with ELP₂ led to even lower phase transition temperatures, corroborating the local clustering hypothesis.⁸⁸ Fukushima et al. employed this ELP₂ dendrimer system to encapsulate gold nanoparticles (AuNPs) for photo-induced cytotoxicity, achieving higher photocytotoxicity with the ELP₂ dendrimer system as compared to a PEG-functionalized dendrimer with AuNPs. This higher toxicity in the ELP system was attributed to the increased hydrophobic interaction of the dendrimer with the HeLa cells.^{222,223} The grafting of VPGVG to PLL side chains, and a PLL based dendrimer (PLD) has also been explored by Niidome and coworkers. Curiously, the linear ELP-PLL conjugates exhibited a lower transition temperature than that observed for the ELP-PLD dendrimer, which may have resulted from the α -helical conformation observed for the PLL, which would be more hydrophobic than the random-coil PLL and thus induce the phase transition at lower temperature.²²⁴ In general, the extensive work on short ELP or CLP segments shows the

promise of exploiting high, local concentration to achieve thermoresponsive conjugates without the need to recombinantly express large polypeptides.

Our group has recently developed a thermoresponsive ELP-CLP diblock that self-assembles into well-defined vesicles. The solid-phase synthesis of the peptides, and use of CuAAC click chemistry protocols, enabled facile synthesis and conjugation of peptide blocks. A significant lowering of the phase transition temperature of the ELP domains occurs as a result of the anchoring of ELPs to triple helical CLPs, which results in a higher local concentration of ELPs, similar to the work reported above. However, the ELP-CLP vesicles undergo a resolubilization at higher temperatures (Figure 7), due to the unfolding of the triple helix. The facile synthesis and conjugation, and noncovalent control over self-assembly demonstrate opportunities for dual-temperature responsiveness and the potential of this diblock as a drug delivery vehicle.⁹⁰

Thermoresponsive behavior in peptide conjugates has also been employed to alter nanoparticle diameter via modulation of the strength of hydrophobic interactions in other peptide-based systems. Ko et al. explored ionic complexes prepared from a PEG-PLL-poly(L-alanine) (PEG-PLL-PA) triblock and hyaluronic acid (HA) and their formation into nanogels. Nanogels formed because of the ionic complexation of the positively charged PLL block and the negatively charged HA, resulting in a hydrophobic core along with the PA block, with a hydrophilic PEG outer shell. The extent of internalization of fluorescein isothiocyanate-bovine serum albumin (FITC-BSA), used as a model macromolecular drug, was dependent upon the ratio of polymer to HA. Higher ratios of HA led to negative zeta-potential (ζ^-) values which yielded low internalization efficiencies. Ratios that yielded zeta potentials near zero (ζ^0) achieved the best internalization efficiency, as well as showing improved relative cell viability. Compositions with ζ^0 also demonstrated a decrease in nanogel diameter with increased temperature from 20–37 °C; the charge neutrality allowed for increased hydrophobic interactions, yielding nanogels on the order of 120–180 nm (220 nm prior to heating). Endocytosis inhibitors were used to determine that the ζ^0 system enters the cytosol through clathrin-mediated endocytosis.²²⁵

Reduction-responsive assembly

Reduction-responsive materials that are sensitive to reducing environments *in vivo* can be further utilized as one of the stimuli to release encapsulated drug molecules. Stimuli-sensitive polymers composed of amphiphilic block copolymers based on poly(ethylene glycol)-*b*-poly(lysine)-*b*-poly(caprolactone), bearing a disulfide bond (PEG-*b*-PLys-SS-PCL), offer a simple chemical linkage for imparting necessary sensitivity. This copolymer emerged as a promising nanocarrier to encapsulate and trigger release of the anticancer drug camptothecin (CPT). The copolymers bearing the bio-reducible linker show significant potential as carriers for intracellular dual-drug delivery.²²⁶ In another example, dithiothreitol (DTT) has been used to reduce the disulfide bond. The core of the cross-linked star (CCS) polymers was degraded by cleavage of the disulfide bridge with DTT, yielding the star's linear poly((*e*-Z-L-lysine)-*b*-(L-cysteine)) (P(ZLL-*b*-LC)) block copolymer components which were characterized by GPC.²²⁷

In human plasma, the presence of glutathione, GSH-free reduced homocysteine (0.1–0.35 μM), and free cysteine (ca. 5 μM), can be utilized as alternative triggers for reductive cleavage of disulfide bonds. The disulfide-linked block copolymers of methoxy polyethylene glycol (mPEG) and poly(3-benzyloxycarbonyl-L-lysine) (PZLL) were prepared through ring-opening polymerization. The copolymers self-assembled into micelles in phosphate buffered saline (PBS) at pH 7.4. DOX was loaded into the micelles and its *in vitro* release with GSH was demonstrated.²²⁸

Similarly, Huang and coworkers reported a novel drug carrier based on keratin-poly(ethylene glycol) (keratin-*g*-PEG) copolymers synthesized via thiol-ene click chemistry and demonstrated dual triggerable release properties. The DOX content in the keratin-*g*-PEG copolymer nanoparticles was measured to be up to ca. 18% (w/w). Dual-stimulus DOX release was observed at intracellular GSH concentrations (10 mM), and additional release from the nanoparticles was demonstrated upon treatment with trypsin.²²⁹ Moreover, similar GSH-responsive systems have been used to deliver the gemcitabine prodrug (TPE-GEM-RGD) with aggregation-induced emission (AIE) properties for targeted and image-guided cancer therapy.²³⁰

In addition, aryl-thiol/maleimide chemistry has proven valuable for similar degradation, owing to the reversible nature of Michael-type adducts comprising aryl thioether succinimides in the presence of glutathione (GSH), as we have studied.^{140–142,231–233} Similarly, Wieduwild et al. reported the use of a series of tagged peptide-polymer conjugates (KA7-starPEG/heparin hydrogel) for antithrombin III (ATIII) release.²³⁴ In another approach, a cysteine-linked antibody drug conjugated with an N-phenyl maleimide was reported. In this case, N-phenyl maleimide functionality was incorporated into the cytotoxic agent valinecitrulline-*p*-aminobenzyloxycarbonyl-monomethyl-aurostatin-E (Val-Cit-PAB-MMAE) to permit delivery in response to GSH.²³⁵ In general, while reduction-responsive peptide-polymer conjugates have great potential in intracellular drug delivery, most of the studies demonstrated the disulfide bond cleavage after *in vitro* treatment with DTT or GSH. The presence of free cysteine and homocysteine in human plasma offers an alternative trigger, but, unfortunately, this approach may result in the cleavage of the disulfide bond before it reaches the specific target, which could result in premature drug release.

Magnetic-responsive assembly

Peptide-polymers conjugates can also be used to sequester magnetically responsive materials and yield sophisticated drug delivery vehicles. For instance, Held and coworkers demonstrated the synthesis of the lysine and aspartic acid-based block copolypeptide poly(EG2-Lys)100-b-poly(Asp)309 copolymer. The block-copolymer was combined with maghemite ($\gamma\text{-Fe}_2\text{O}_3$) nanoparticles, which resulted in the formation of water-soluble block copolypeptide-magnetic nanoparticle clusters. The architecture of the micelle results from the electrostatic association of the poly(Asp) with the maghemite nanoparticle forming the core of the micelle (with approximately 20 nanoparticles), with the hydrophilic poly(EG2-Lys) comprising the surface. This behavior offers physiological stability with a 20 times higher magnetic moment as compared to a single nanoparticle allowing for great potential in drug delivery.¹⁸⁸

Further, Lecommandoux and coworkers reported a synthesis of biodegradable polyglutamic acid-based poly(trimethylene carbonate)-*b*-poly(L-glutamic acid) (PTMC-*b*-PGA) block copolymers. The self-assembly of the conjugates resulted in vesicles with a controlled size between 100–400 nm in diameter. The hydrophobic maghemite (γ -Fe₂O₃) nanoparticles, as well as doxorubicin, were encapsulated using a one-step nanoprecipitation process into the amphiphilic conjugate, with the hydrophilic PGA providing biocompatibility. These prodrug encapsulated vesicles were quite stable and showed suitable properties for doxorubicin release after applying a magnetic field.²³⁶ Furthermore, the same group previously reported polymersome membranes of PB-*b*-PGA where the hydrophobic ultra-small superparamagnetic iron oxide (USPIOs) was loaded, and their release was observed at the reversible variation of the membrane thickness, that was induced by the applied magnetic field.²³⁷ Magnetically responsive delivery offers important possibilities in increasing drug delivery efficiency and targeting, as the applied magnetic field holds the micelles intact and effectively drives them into tumors to release the drug.

Conclusion and Future Perspectives

In summary, we have highlighted peptide-polymer conjugates in which functionality is tailored primarily by the incorporated peptide. Peptide incorporation has shown the ability to control the size and architecture of the conjugates. The adoption of peptides into conjugates has also enabled drug-delivery to be triggered via pH, temperature, enzyme, and in particular, be responsive to biologically relevant stimuli. Moreover, peptide-polymer conjugates show exceptional stability, allow for convenient functionalization with cell-targeting ligands, and are easily synthesized via a variety of strategies. Many have shown utility in multiple cancer cell lines, including, breast,¹⁹² colon,²⁰⁴ and cervical,^{222,223} as well as in animal models.^{192,193,206}

Many opportunities and challenges remain in the development of peptide-polymer conjugates for desired application. The use of computational models has expanded significantly to permit identification of a large number of peptide sequences amenable for sequence-dependent assembly, as well as elucidation of peptide-polymer interactions, allowing insight into potential assembly pathways. The complementary expansion of experimental techniques, including scattering, spectroscopic, and interfacial, will also enable more detailed understanding of the specific benefits and outcomes of using peptides to direct conjugate assembly and activity. While di- and triblock conjugates have traditionally been employed for conjugates, owing to their synthetic accessibility, the development of larger multiblock systems that capture the large number of blocks found in structural proteins, should prove useful for further control over self-assembly. While PEG has been the gold-standard in peptide and protein conjugation, other polymers such as poly[oligo(ethylene glycol) methyl methacrylate] (POEGMA), as well as the aforementioned PPE, may prove useful in overcoming common biological barriers (degradation, biocompatibility, circulation time, cytotoxicity) in *in vivo* studies. With continued focus on the utility of specific peptide sequences in conjugates, the development of conjugation techniques, experimental characterization of self-assembled structures, and fine-tuning of peptide sequences *via* computational models will all play major roles in expanding the field.

Acknowledgments

We gratefully acknowledge financial support from National Science Foundation (CHE 1213728 and DMR 1206310), the National Institutes of Health (RO1 EB006006, 1 P30 GM103519 (instrument resources) and 1 P30 GM110758 (instrument resources)) and the DuPont Company (DuPont Young Professor to XJ). Its contents are the sole responsibility of the authors and do not necessarily reflect the views of the funding agencies.

References

1. Langer R. *Nature*. 1998; 392:5–10. [PubMed: 9579855]
2. Langer R, Tirrell DA. *Nature*. 2004; 428:487–492. [PubMed: 15057821]
3. Nishiyama N, Kataoka K. *Pharmacol Ther*. 2006; 112:630–648. [PubMed: 16815554]
4. Timko BP, Dvir T, Kohane DS. *Adv Mater*. 2010; 22:4925–4943. [PubMed: 20818618]
5. Petros RA, DeSimone JM. *Nat Rev Drug Discov*. 2010; 9:615–627. [PubMed: 20616808]
6. Rösler A, Vandermeulen GW, Klok HA. *Adv Drug Deliv Rev*. 2001; 53:95–108. [PubMed: 11733119]
7. Kataoka K, Harada A, Nagasaki Y. *Adv Drug Deliv Rev*. 2001; 47:113–131. [PubMed: 11251249]
8. Torchilin VP. *Pharm Res*. 2007; 24:1–16. [PubMed: 17109211]
9. Kumari A, Yadav SK, Yadav SC. *Colloids Surfaces B Biointerfaces*. 2010; 75:1–18. [PubMed: 19782542]
10. Zhu Y, Yang B, Chen S, Du J. *Prog Polym Sci*. 2017; 64:1–22.
11. Harris JM, Chess RB. *Nat Rev Drug Discov*. 2003; 2:214–221. [PubMed: 12612647]
12. Veronese FM, Pasut G. *Drug Discov Today*. 2005; 10:1451–1458. [PubMed: 16243265]
13. Jevšvar S, Kunstelj M, Porekar VG. *Biotechnol J*. 2010; 5:113–128. [PubMed: 20069580]
14. Panda JJ, Mishra A, Basu A, Chauhan VS. *Biomacromolecules*. 2008; 9:2244–2250. [PubMed: 18624454]
15. Ruoslahti E, Bhatia SN, Sailor MJ. *J Cell Biol*. 2010; 188:759–768. [PubMed: 20231381]
16. Chien MP, Rush AM, Thompson MP, Gianneschi NC. *Angew Chemie - Int Ed*. 2010; 49:5076–5080.
17. Ku TH, Chien MP, Thompson MP, Sinkovits RS, Olson NH, Baker TS, Gianneschi NC. *J Am Chem Soc*. 2011; 133:8392–8395. [PubMed: 21462979]
18. Amir RJ, Zhong S, Pochan DJ, Hawker CJ. *J Am Chem Soc*. 2009; 131:13949–13951. [PubMed: 19739628]
19. Wang C, Chen Q, Wang Z, Zhang X. *Angew Chemie - Int Ed*. 2010; 49:8612–8615.
20. Larson N, Gormley A, Frazier N, Ghandehari H. *J Control Release*. 2013; 170:41–50. [PubMed: 23602864]
21. Greish K, Ray A, Bauer H, Larson N, Malugin A, Pike D, Haider M, Ghandehari H. *J Control Release*. 2011; 151:263–270. [PubMed: 21223983]
22. Zhang B, Sun X, Mei H, Wang Y, Liao Z, Chen J, Zhang Q, Hu Y, Pang Z, Jiang X. *Biomaterials*. 2013; 34:9171–9182. [PubMed: 24008043]
23. Temming K, Schiffelers RM, Molema G, Kok RJ. *Drug Resist Updat*. 2005; 8:381–402. [PubMed: 16309948]
24. Shi J, Votruba AR, Farokhzad OC, Langer R. *Nano Lett*. 2010; 10:3223–3230. [PubMed: 20726522]
25. Bae Y, Nishiyama N, Fukushima S, Koyama H, Yasuhiro M, Kataoka K. *Bioconjug Chem*. 2005; 16:122–130. [PubMed: 15656583]
26. De Graaf AJ, Mastrobattista E, Vermonden T, Van Nostrum CF, Rijkers DTS, Liskamp RMJ, Hennink WE. *Macromolecules*. 2012; 45:842–851.
27. Deng X, Smeets NMB, Sicard C, Wang J, Brennan JD, Filipe CDM, Hoare T. *J Am Chem Soc*. 2014; 136:12852–12855. [PubMed: 25170805]
28. Broyer RM, Quaker GM, Maynard HD. *J Am Chem Soc*. 2008; 130:1041–1047. [PubMed: 18161975]

29. Pan X, Tasdelen MA, Laun J, Junkers T, Yagci Y, Matyjaszewski K. *Prog Polym Sci.* 2016; 62:73–125.
30. Boyer C, Corrigan NA, Jung K, Nguyen D, Nguyen TK, Adnan NNM, Oliver S, Shanmugam S, Yeow J. *Chem Rev.* 2016; 116:1803–1949. [PubMed: 26535452]
31. Chernikova EV, Sivtsov EV. *Polym Sci Ser B.* 2017; 59:117–146.
32. Guégain E, Delplace V, Trimaille T, Gigmes D, Siri D, Marque SRA, Guillaneuf Y, Nicolas J. *Polym Chem.* 2015; 6:5693–5704.
33. Chenal M, Boursier C, Guillaneuf Y, Taverna M, Couvreur P, Nicolas J. *Polym Chem.* 2011; 2:1523–1530.
34. Becker ML, Liu J, Wooley KL. *Biomacromolecules.* 2005; 6:220–228. [PubMed: 15638524]
35. Becker ML, Liu J, Wooley KL. *Chem Commun.* 2003; 1:180–181.
36. Wang Z, Li Y, Huang Y, Thompson MP, LeGuyader CLM, Sahu S, Gianneschi NC. *Chem Commun.* 2015; 51:17108–17111.
37. Barnhill SA, Bell NC, Patterson JP, Olds DP, Gianneschi NC. *Macromolecules.* 2015; 48:1152–1161.
38. Kammeyer JK, Blum AP, Adamiak L, Hahn ME, Gianneschi NC. *Polym Chem.* 2013; 41:3929–3933. [PubMed: 24015154]
39. Hahn ME, Randolph LM, Adamiak L, Thompson MP, Gianneschi NC. *Chem Commun.* 2013; 49:2873–2875.
40. Tardy A, Nicolas J, Gigmes D, Lefay C, Guillaneuf Y. *Chem Rev.* 2017; 117:1319–1406. [PubMed: 28085265]
41. Boerner HG. *Prog Polym Sci.* 2009; 34:811–851.
42. Shu JY, Panganiban B, Xu T. *Annu Rev Phys Chem.* 2013; 64:631–657. [PubMed: 23331303]
43. Abuchowski A, Mccoy JR, Palczuk NC, Es TVAN, Davis FF. 1977; 252:3582–3586.
44. Zhang P, Sun F, Liu S, Jiang S. *J Control Release.* 2016; 244:184–193. [PubMed: 27369864]
45. Zelikin AN, Such GK, Postma A, Caruso F. *Society.* 2007; 8:2950–2953.
46. Imran Ul-Haq M, Lai BFL, Chapanian R, Kizhakkedathu JN. *Biomaterials.* 2012; 33:9135–9147. [PubMed: 23020861]
47. Lewis A, Tang Y, Brocchini S, Choi JW, Godwin A. *Bioconjug Chem.* 2008; 19:2144–2155. [PubMed: 18973286]
48. Engler AC, Ke X, Gao S, Chan JMW, Coady DJ, Ono RJ, Lubbers R, Nelson A, Yang YY, Hedrick JL. *Macromolecules.* 2015; 48:1673–1678.
49. Mero A, Fang Z, Pasut G, Veronese FM, Viegas TX. *J Control Release.* 2012; 159:353–361. [PubMed: 22405905]
50. Lee S, Saito K, Lee HR, Lee MJ, Shibasaki Y, Oishi Y, Kim BS. *Biomacromolecules.* 2012; 13:1190–1196. [PubMed: 22414172]
51. Qi Y, Chilkoti A. *Curr Opin Chem Biol.* 2015; 28:181–193. [PubMed: 26356631]
52. Pelegri-O'Day EM, Maynard HD. *Acc Chem Res.* 2016; 49:1777–1785. [PubMed: 27588677]
53. Zhao Z, Wang J, Mao H, Leong KW. *Adv Drug Deliv Rev.* 2003; 55:483–499. [PubMed: 12706047]
54. Zhang S, Zou J, Elsabahy M, Karwa A, Li A, Moore DA, Dorshow RB, Wooley KL. *Chem Sci.* 2013; 4:2122. [PubMed: 25152808]
55. T. Steinbach and F. R. Wurm, 2016.
56. Isabelle Ydens PD, Moins Sébastien, Botteman François, Dubois P. *E-Polymers.* 2004; 39:1–7.
57. Zhang H, Abeln CH, Fijten MWM, Schubert US. *E-Polymers.* 2006:1–9.
58. Grieshaber SE, Paik BA, Bai S, Kiick KL, Jia X. *Soft Matter.* 2013; 9:1589–1599. [PubMed: 23976897]
59. Paik BA, Blanco MA, Jia X, Roberts CJ, Kiick KL. *Soft Matter.* 2015; 11:1839–1850. [PubMed: 25611563]
60. Cobo I, Li M, Sumerlin BS, Perrier S. *Nat Mater.* 2014; 14:143–159. [PubMed: 25401924]
61. Li M, De P, Li H, Sumerlin BS. *Polym Chem.* 2010; 1:854.

62. Kumar S, Acharya R, Chatterji U, De P. *Polym Chem.* 2014; 5:6039–6050.
63. Shi H, Liu L, Wang X, Li J. *Polym Chem.* 2012; 3:1182–1188.
64. McDowall L, Stenzel MH. *Polym Chem.* 2014; 5:1772–1781.
65. Perrier S, Takolpuckdee P, Mars CA. *Macromolecules.* 2005; 38:2033–2036.
66. Benajmin LM, Fairbanks D, Gunatillake Pathiraja A. *Adv Drug Deliv Rev.* 2015; 91:141–152. [PubMed: 26050529]
67. Möller M, Hentschel C, Chi L, Studer A. *Org Biomol Chem.* 2011; 9:2403. [PubMed: 21321771]
68. Gauthier MA, Klok HA. *Chem Commun.* 2008:2591.
69. Sutthasupa S, Shiotsuki M, Sanda F. *Polym J.* 2010; 42:905–915.
70. Patel PR, Kiser RC, Lu YY, Fong E, Ho WC, Tirrell DA, Grubbs RH. *Biomacromolecules.* 2012; 13:2546–2553. [PubMed: 22783892]
71. Conrad RM, Grubbs RH. *Angew Chemie - Int Ed.* 2009; 48:8328–8330.
72. Feng K, Xie N, Chen B, Tung CH, Wu LZ. *Biomacromolecules.* 2016 acs.biomac.5b01450.
73. Chien MP, Thompson MP, Lin EC, Gianneschi NC. *Chem Sci.* 2012; 3:2690–2694. [PubMed: 23585924]
74. Blum AP, Kammeyer JK, Gianneschi NC. *Chem Sci.* 2016; 7:989–994. [PubMed: 26925209]
75. Wibowo SH, Sulistio A, Wong EHH, Blencowe A, Qiao GG. *Chem Commun (Camb).* 2014; 50:4971–4988. [PubMed: 24577357]
76. Song W, Li M, Tang Z, Li Q, Yang Y, Liu H, Duan T, Hong H, Chen X. *Macromol Biosci.* 2012; 12:1514–1523. [PubMed: 23070837]
77. Miura Y, Takenaka T, Toh K, Wu S, Nishihara H, Kano MR, Ino Y, Nomoto T, Matsumoto Y, Koyama H, Cabral H, Nishiyama N, Kataoka K. *ACS Nano.* 2013; 7:8583–8592. [PubMed: 24028526]
78. Plummer R, Wilson RH, Calvert H, Boddy AV, Griffin M, Sludden J, Tilby MJ, Eatock M, Pearson DG, Ottley CJ, Matsumura Y, Kataoka K, Nishiyama T. *Br J Cancer.* 2011; 104:593–598. [PubMed: 21285987]
79. Yu H, Tang Z, Zhang D, Song W, Zhang Y, Yang Y, Ahmad Z, Chen X. *J Control Release.* 2015; 205:89–97. [PubMed: 25529533]
80. Li G, Wu J, Wang B, Yan S, Zhang K, Ding J, Yin J. *Biomacromolecules.* 2015; 16:3508–3518. [PubMed: 26414083]
81. Shirbin SJ, Ladewig K, Fu Q, Klimak M, Zhang X, Duan W, Qiao GG. *Biomacromolecules.* 2015; 16:2463–2474. [PubMed: 26166192]
82. He X, Fan J, Zhang F, Li R, Pollack KA, Raymond JE, Zou J, Wooley KL. *J Mater Chem B.* 2014; 2:8123–8130. [PubMed: 25485113]
83. Fan J, Zou J, He X, Zhang F, Zhang S, Raymond JE, Wooley KL. *Chem Sci.* 2014; 5:141–150.
84. Ayres L, Vos MRJ, Adams PJHM, Shklyarevskiy IO, Van Hest JCM. *Macromolecules.* 2003; 36:5967–5973.
85. Ayres L, Koch K, Adams PHHM, Van Hest JCM. *Macromolecules.* 2005; 38:1699–1704.
86. Fernández-Trillo F, Duréault A, Bayley JPM, Van Hest JCM, Thies JC, Michon T, Weberskirch R, Cameron NR. *Macromolecules.* 2007; 40:6094–6099.
87. Kojima C, Tsumura S, Harada A, Kono K. 2009; 131:6052–6053.
88. Kojima C, Irie K. *Biopolymers.* 2013; 100:714–721. [PubMed: 23893507]
89. Grieshaber SE, Paik BA, Bai S, Kiick KL, Jia X. *Soft Matter.* 2013; 9:1589–1599. [PubMed: 23976897]
90. Luo T, Kiick KL. *J Am Chem Soc.* 2015; 137:15362–15365. [PubMed: 26633746]
91. Yu YC, Berndt P, Tirrell M, Fields GB. *J Am Chem Soc.* 1996; 118:12515–12520.
92. Jiang J, Tong X, Zhao Y. *J Am Chem Soc.* 2005; 127:8290–8291. [PubMed: 15941255]
93. Luo T, He L, Theato P, Kiick KL. *Macromol Biosci.* 2015; 15:111–123. [PubMed: 25393381]
94. Shimada T, Lee S, Bates FS, Hotta A, Tirrell M. *J Phys Chem B.* 2009; 113:13711–13714. [PubMed: 19572667]
95. Bhagwat N, Kiick KL. *J Mater Chem C.* 2013; 1:4836.

96. Nilsson MR, Nguyen LL, Raleigh DP. *Anal Biochem.* 2001; 288:76–82. [PubMed: 11141308]
97. Elgersma RC, Kroon-Batenburg LMJ, Posthuma G, Meeldijk JD, Rijkers DTS, Liskamp RMJ. *Eur J Med Chem.* 2014; 88:55–65. [PubMed: 25087966]
98. Dong H, Hartgerink JD. *Biomacromolecules.* 2006; 7:691–695. [PubMed: 16529401]
99. Shu JY, Huang YJ, Tan C, Presley AD, Chang J, Xu T. *Biomacromolecules.* 2010; 11:1443–1452. [PubMed: 20465287]
100. Heckmann D, Kessler H. *Methods Enzymol.* 2007; 426:463–503. [PubMed: 17697896]
101. Zhang XX, Eden HS, Chen X. *J Control Release.* 2012; 159:2–13. [PubMed: 22056916]
102. Wang J, Masehi-Lano JJ, Chung EJ. *Biomater Sci.* 2017
103. Top A, Roberts CJ, Kiick KL. *Biomacromolecules.* 2011; 12:2184–2192. [PubMed: 21553871]
104. Van Eldijk MB, Smits FCM, Vermue N, Debets MF, Scho S, Van Hest JCM. 2014
105. Gao X, He C, Xiao C, Zhuang X, Chen X. *Polym (United Kingdom).* 2013; 54:1786–1793.
106. Nawroth JF, McDaniel JR, Chilkoti A, Jordan R, Luxenhofer R. *Macromol Biosci.* 2016; 16:322–333. [PubMed: 26756582]
107. Weeks CA, Aden B, Kilbey SM, Janorkar AV. *ACS Biomater Sci Eng.* 2016; 2:2196–2206.
108. Rostovtsev VV, Green LG, Fokin VV, Sharpless KB. *Angew Chemie - Int Ed.* 2002; 41:2596–2599.
109. Hong V, Presolski S, Ma C, Finn MG. *Angew Chemie Int Ed.* 2009; 48:9879–9883.
110. Kolb HC, Finn MG, Sharpless KB. *Angew Chem Int Ed Engl.* 2001; 40:2004–2021. [PubMed: 11433435]
111. Johnson JA, Lu YY, Burts AO, Lim YH, Finn MG, Koberstein JT, Turro NJ, Tirrell DA, Grubbs RH. *J Am Chem Soc.* 2011; 133:559–566. [PubMed: 21142161]
112. Mane SR, Sarkar S, Rao VN, Sathyan A, Shunmugam R. *RSC Adv.* 2015; 5:74159–74161.
113. Obermeyer AC, Olsen BD. *ACS Macro Lett.* 2015; 4:101–110.
114. Presolski SL, Hong VP, Finn MG. *Curr Protoc Chem Biol.* 2011; 3:153–162. [PubMed: 22844652]
115. Jia X, Kiick KL. *Macromol Biosci.* 2009; 9:140–156. [PubMed: 19107720]
116. Grieshaber SE, Nie T, Yan C, Zhong S, Teller SS, Clifton RJ, Pochan DJ, Kiick KL, Jia X. *Macromol Chem Phys.* 2011; 212:229–239. [PubMed: 21359141]
117. Lutz JF, Börner HG, Weichenhan K. *Macromolecules.* 2006; 39:6376–6383.
118. Colombo M, Bianchi A. *Molecules.* 2010; 15:178–197. [PubMed: 20110882]
119. Chapman R, Jolliffe KA, Perrier S. *Polym Chem.* 2011; 2:1956.
120. Poon CK, Chapman R, Jolliffe KA, Perrier S. *Polym Chem.* 2012; 3:1820.
121. Dehn S, Castelletto V, Hamley IW, Perrier S. *Biomacromolecules.* 2012; 13:2739–2747. [PubMed: 22770360]
122. De Almeida G, Sletten EM, Nakamura H, Palaniappan KK, Bertozzi CR. *Angew Chemie - Int Ed.* 2012; 51:2443–2447.
123. Sletten EM, Bertozzi CR. *Acc Chem Res.* 2011; 44:666–676. [PubMed: 21838330]
124. Agard NJ, Prescher JA, Bertozzi CR. *J Am Chem Soc.* 2004; 126:15046–7. [PubMed: 15547999]
125. Shieh P, Dien VT, Beahm BJ, Castellano JM, Wyss-Coray T, Bertozzi CR. *J Am Chem Soc.* 2015; 137:7145–7151. [PubMed: 25902190]
126. Robinson PV, de Almeida-Escobedo G, de Groot AE, McKechnie JL, Bertozzi CR. *J Am Chem Soc.* 2015; 137:10452–10455. [PubMed: 26280358]
127. Debets MF, van Berkel SS, Schoffelen S, Rutjes FPJT, van Hest JCM, van Delft FL. *Chem Commun.* 2010; 46:97–99.
128. Smits FCM, Castelijns WWA, Van Hest JCM. *Eur Polym J.* 2015; 62:386–393.
129. Kim HL, Sachin K, Jeong HJ, Choi W, Lee HS, Kim DW. *ACS Med Chem Lett.* 2015; 6:402–407. [PubMed: 25893040]
130. Jewett JC, Bertozzi CR. *Chem Soc Rev.* 2010; 39:1272–1279. [PubMed: 20349533]
131. De Graaf AJ, Kooijman M, Hennink WE, Mastrobattista E. *Bioconjug Chem.* 2009; 20:1281–1295. [PubMed: 19191567]

132. Syrett JA, Jones MW, Haddleton DM. Chem Commun (Camb). 2010; 46:7181–7183. [PubMed: 20820534]
133. McCall JD, Anseth KS. Biomacromolecules. 2012; 13:2410–2417. [PubMed: 22741550]
134. Caldwell S, Johnson DW, Didsbury MP, Murray BA, Wu JJ, Przyborski SA, Cameron NR. Soft Matter. 2012; 8:10344.
135. Meyer A, Vasseur JJ, Morvan F. European J Org Chem. 2013:465–473.
136. Li LY, He WD, Li J, Zhang BY, Pan TT, Sun XL, Ding ZL. Biomacromolecules. 2010; 11:1882–1890. [PubMed: 20536122]
137. Lin CC, Ki CS, Shih H. J Appl Polym Sci. 2015; 132:1–11. [PubMed: 25866416]
138. Phelps EA, Enemchukwu NO, Fiore VF, Sy JC, Murthy N, Sulchek TA, Barker TH, García AJ. Adv Mater. 2012; 24:64–70. [PubMed: 22174081]
139. Lei Y, Gojgini S, Lam J, Segura T. Biomaterials. 2011; 32:39–47. [PubMed: 20933268]
140. Baldwin AD, Kiick KL. Bioconjug Chem. 2011; 22:1946–1953. [PubMed: 21863904]
141. Baldwin AD, Robinson KG, Militar JL, Derby CD, Kiick KL, Akins RE. J Biomed Mater Res Part A. 2012; 100:2106–2118.
142. Baldwin AD, Kiick KL. Polym Chem. 2013; 4:133–143. [PubMed: 23766781]
143. Zhang C, Hekmatfer S, Karuri NW. J Biomed Mater Res - Part A. 2014; 102:170–179.
144. Tsurkan MV, Chwalek K, Schoder M, Freudenberg U, Werner C. Bioconjug Chem. 2014; 25:1942–1950. [PubMed: 25297697]
145. O'Connor A, Marsat JN, Mitrugno A, Flahive T, Moran N, Brayden D, Devocelle M. Molecules. 2014; 19:17559–17577. [PubMed: 25361422]
146. Danial M, Root MJ, Klok HA. Biomacromolecules. 2012
147. Bacinello D, Garanger E, Taton D, Tam KC, Lecommandoux S. Biomacromolecules. 2014; 15:1882–1888. [PubMed: 24670109]
148. Bacinello D, Garanger E, Taton D, Tam KC, Lecommandoux S. Eur Polym J. 2015; 62:363–373.
149. Blackman ML, Royzen M, Fox JM. J Am Chem Soc. 2008; 130:13518–9. [PubMed: 18798613]
150. Lang K, Davis L, Wallace S, Mahesh M, Cox DJ, Blackman ML, Fox JM, Chin JW. J Am Chem Soc. 2012; 134:10317–10320. [PubMed: 22694658]
151. Še kut J, Yang J, Devaraj NK. Nucleic Acids Res. 2013; 41:e148–e148. [PubMed: 23775794]
152. Selvaraj R, Fox JM. Curr Opin Chem Biol. 2013; 17:753–760. [PubMed: 23978373]
153. Taylor MT, Blackman ML, Dmitrenko O, Fox JM. J Am Chem Soc. 2011; 133:9646–9649. [PubMed: 21599005]
154. Devaraj NK, Weissleder R, Hilderbrand SA. Bioconjug Chem. 2008; 19:2297–2299. [PubMed: 19053305]
155. Chen W, Wang D, Dai C, Hamelberg D, Wang B. Chem Commun. 2012; 48:1736.
156. Niederwieser A, Späte AK, Nguyen LD, Jüngst C, Reutter W, Wittmann V. Angew Chem, Int Ed. 2013; 52:4265–4268.
157. Karver MR, Weissleder R, Hilderbrand SA. Angew Chem, Int Ed. 2012; 51:920–922.
158. Zhang H, Dicker KT, Xu X, Jia X, Fox JM. ACS Macro Lett. 2014:727–731. [PubMed: 25177528]
159. Liu S, Zhang H, Remy RA, Deng F, Mackay ME, Fox JM, Jia X. Adv Mater. 2015; 27:2783–2790. [PubMed: 25824805]
160. Brieke C, Rohrbach F, Gottschalk A, Mayer G, Heckel A. Angew Chemie Int Ed. 2012; 51:8446–8476.
161. Petersen S, Alonso JM, Specht A, Duodu P, Goeldner M, del Campo A. Angew Chemie Int Ed. 2008; 47:3192–3195.
162. Tan X, Li BB, Lu X, Jia F, Santori C, Menon P, Li H, Zhang B, Zhao JJ, Zhang K. J Am Chem Soc. 2015; 137:6112–6115. [PubMed: 25924099]
163. She W, Luo K, Zhang C, Wang G, Geng Y, Li L, He B, Gu Z. Biomaterials. 2013; 34:1613–1623. [PubMed: 23195490]
164. Yang S, Zhu F, Wang Q, Liang F, Qu X, Gan Z, Yang Z. J Mater Chem B. 2015; 3:4043–4051.

165. Wang SSS, How SC, Chen YD, Tsai YH, Jan JS. *J Mater Chem B*. 2015; 3:5220–5231.
166. Goel R, Gopal S, Gupta A. *J Mater Chem B*. 2015; 3:5849–5857.
167. Zhong J, Li L, Zhu X, Guan S, Yang Q, Zhou Z, Zhang Z, Huang Y. *Biomaterials*. 2015; 65:43–55. [PubMed: 26142775]
168. Lindsey S, Piatt JH, Worthington P, Sönmez C, Satheye S, Schneider JP, Pochan DJ, Langhans SA. *Biomacromolecules*. 2015; 16:2672–2683. [PubMed: 26225909]
169. Lee JS, Groothuis T, Cusan C, Mink D, Feijen J. *Biomaterials*. 2011; 32:9144–9153. [PubMed: 21872328]
170. Lam CN, Olsen BD. *Soft Matter*. 2013; 9:2393–2402.
171. Chang D, Lam CN, Tang S, Olsen BD. *Polym Chem*. 2014; 5:4884–4895.
172. Glassman MJ, Chan J, Olsen BD. *Adv Funct Mater*. 2013; 23:1182–1193. [PubMed: 25568642]
173. Holm R, Klinker K, Weber B, Barz M. *Macromol Rapid Commun*. 2015; 36:2083–2091. [PubMed: 26344836]
174. Kalafatovic D, Nobis M, Javid N, Frederix PWJM, Anderson KI, Saunders BR, Ulijn RV. *Biomater Sci*. 2015; 3:246–249. [PubMed: 26218115]
175. Pan H, Yang J, Kopec P. 2011; 12:247–252.
176. Li N, Li N, Yi Q, Luo K, Guo C, Pan D, Gu Z. *Biomaterials*. 2014; 35:9529–9545. [PubMed: 25145854]
177. Singh S, Topuz F, Hahn K, Albrecht K, Groll J. *Angew Chemie - Int Ed*. 2013; 52:3000–3003.
178. Chen W, Zheng M, Meng F, Cheng R, Deng C, Feijen J, Zhong Z. *Biomacromolecules*. 2013; 14:1214–1222. [PubMed: 23477570]
179. Kharkar PM, Kiiick KL, Kloxin AM. *Chem Soc Rev*. 2013; 42:7335–7372. [PubMed: 23609001]
180. Lin LY, Karwa A, Kostelc JG, Lee NS, Dorshow RB, Wooley KL. *Mol Pharm*. 2012; 9:2248–2255. [PubMed: 22742525]
181. Yesilyurt V, Ramireddy R, Thayumanavan S. *Angew Chemie - Int Ed*. 2011; 50:3038–3042.
182. Zhuang J, Gordon MR, Ventura J, Li L, Thayumanavan S. *Chem Soc Rev*. 2013; 42:7421–35. [PubMed: 23765263]
183. Chacko RT, Ventura J, Zhuang J, Thayumanavan S. *Adv Drug Deliv Rev*. 2012; 64:836–851. [PubMed: 22342438]
184. Honggang Cui SIS, Webber Matthew J. *Pept Sci*. 2010; 94:1–18.
185. Leite D, Barbu E, Pilkington G, Lalatsa A. *Curr Top Med Chem*. 2015; 15:2277–2289. [PubMed: 26043734]
186. Hamley IW, Castelletto V. *Bioconjug Chem*. 2017; 28:731–739. [PubMed: 27348697]
187. Sun Y, Li Y, Huang H, Wang Y, Sa Z, Wang J, Chen X. *J Macromol Sci Part A*. 2015; 52:925–933.
188. Euliss LE, Grancharov SG, O'Brien S, Deming TJ, Stucky GD, Murray CB, Held GA. *Nano Lett*. 2003; 3:1489–1493.
189. Ai S, Jia T, Ai W, Duan J, Liu Y, Chen J, Liu X, Yang F, Tian Y, Huang Z. *Br J Pharmacol*. 2013; 168:1719–1735. [PubMed: 23146125]
190. Dube N, Presley AD, Shu JY, Xu T. *Macromol Rapid Commun*. 2011; 32:344–353. [PubMed: 21433182]
191. Dong H, Dube N, Shu JY, Seo JW, Mahakian LM, Ferrara KW, Xu T. *ACS Nano*. 2012; 6:5320–5329. [PubMed: 22545944]
192. Dube N, Seo JW, Dong H, Shu JY, Lund R, Mahakian LM, Ferrara KW, Xu T. *Biomacromolecules*. 2014; 15:2963–2970. [PubMed: 24988250]
193. Seo JW, Ang J, Mahakian LM, Tam S, Fite B, Ingham ES, Beyer J, Forsayeth J, Bankiewicz KS, Xu T, Ferrara KW. *J Control Release*. 2015; 220:51–60. [PubMed: 26437259]
194. Zhu L, Yang S, Qu X, Zhu F, Liang Y, Liang F, Wang Q, Li J, Li Z, Yang Z. *Polym Chem*. 2014; 5:5700.
195. Yang S, Zhu F, Wang Q, Liang F, Qu X, Gan Z, Yang Z. *J Mater Chem B*. 2016; 4:7283–7292.
196. Xian Li BL, Li Hao, Yi Wei, Chen Jianyu. *Int J Nanomedicine*. 2013:3019–3031. [PubMed: 23976852]

197. Li H, Li X, Zhang C, Sun Q, Yi W, Wang X, Cheng D, Chen S, Liang B, Shuai X. *J Biomed Nanotechnol.* 2016; 12:1258–1269. [PubMed: 27319219]
198. Liu R, Li D, He B, Xu X, Sheng M, Lai Y, Wang G, Gu Z. *J Control Release.* 2011; 152:49–56. [PubMed: 21397642]
199. Liu R, He B, Li D, Lai Y, Tang JZ, Gu Z. *Polymer (Guildf).* 2012; 53:1473–1482.
200. Li F, Fan L, Li W, Duan X, Qiao Y, Wu H. *Polym Eng Sci.* 2015:277–286.
201. John JV, Uthaman S, Augustine R, Manickavasagam Lekshmi K, Park I-K, Kim I. *J Polym Sci Part A Polym Chem.* 2017; 55:2061–2070.
202. Herranz-Blanco B, Shahbazi MA, Correia AR, Balasubramanian V, Kohout T, Hirvonen J, Santos HA. *Adv Healthc Mater.* 2016; 5:1904–1916. [PubMed: 27245691]
203. Zhou Z, Badkas A, Stevenson M, Lee JY, Leung YK. *Int J Pharm.* 2015; 487:81–90. [PubMed: 25865568]
204. Johnson RP, Uthaman S, John JV, Lee HR, Lee SJ, Park H, Park IK, Suh H, Kim I. *ACS Appl Mater Interfaces.* 2015; 7:21770–21779. [PubMed: 26375278]
205. Bagherifam S, Skjeldal FM, Griffiths G, Mælandsmo GM, Engebråten O, Nyström B, Hasirci V, Hasirci N. *Pharm Res.* 2015; 32:1249–1263. [PubMed: 25288014]
206. Chen J, Ding J, Zhang Y, Xiao C, Zhuang X, Chen X. *Polym Chem.* 2015; 6:397–405.
207. Aimetti AA, Machen AJ, Anseth KS. *Biomaterials.* 2009; 30:6048–6054. [PubMed: 19674784]
208. Aimetti AA, Tibbitt MW, Anseth KS. *Biomacromolecules.* 2009; 10:1484–1489. [PubMed: 19408953]
209. Mann BK, Gobin AS, Tsai AT, Schmedlen RH, West JL. *Biomaterials.* 2001; 22:3045–3051. [PubMed: 11575479]
210. Knipe JM, Chen F, Peppas NA. *Biomacromolecules.* 2015; 16:962–972. [PubMed: 25674922]
211. Nagase H, Fields GB. *Pept Sci.* 1996; 40:399–416.
212. Lutolf MP, Lauer-Fields JL, Schmoekel HG, Metters AT, Weber FE, Fields GB, Hubbell JA. *Proc Natl Acad Sci U S A.* 2003; 100:5413–5418. [PubMed: 12686696]
213. Secret E, Kelly SJ, Crannell KE, Andrew JS. *ACS Appl Mater Interfaces.* 2014; 6:10313–10321. [PubMed: 24926532]
214. Wang K, Liu Y, Li C, Cheng S, Zhuo R, Zhang X. *ACS Macro Lett.* 2013; 2:201–205.
215. MacEwan SR, Chilkoti A. *Biopolymers.* 2010; 94:60–77. [PubMed: 20091871]
216. Bidwell GL, Raucher D. *Adv Drug Deliv Rev.* 2010; 62:1486–1496. [PubMed: 20478348]
217. Rodriguez-Cabello JC, Arias FJ, Rodrigo MA, Girotti A. *Adv Drug Deliv Rev.* 2016; 97:85–100. [PubMed: 26705126]
218. Pastuszka MK, Mackay JA. *Wiley Interdiscip Rev Nanomedicine Nanobiotechnology.* 2016; 8:123–138. [PubMed: 26112277]
219. Chie Kojima KI. *Pept Sci.* 2013; 100:714–721.
220. Nuhn H, Klok HAA. *Eur Polym J.* 2011; 47:782–791.
221. Chilkoti A, Christensen T, MacKay JA. *Curr Opin Chem Biol.* 2006; 10:652–657. [PubMed: 17055770]
222. Fukushima D, Sk UH, Sakamoto Y, Nakase I, Kojima C. *Colloids Surfaces B Biointerfaces.* 2015; 132:155–160. [PubMed: 26037705]
223. Fukushima D, Kojima C. *J Photopolym Sci Technol.* 2016; 29:519–523.
224. Kojima C, Sk UH, Fukushima D, Irie K, Akazawa N, Umeda M, Niidome T. *RSC Adv.* 2015; 5:104900–104906.
225. Ko DY, Moon HJ, Jeong B. *J Mater Chem B.* 2015; 3:3525–3530.
226. Thambi T, Deepagan VG, Ko H, Lee DS, Park JH. *J Mater Chem.* 2012; 22:22028.
227. Sulistio A, Widjaya A, Blencowe A, Zhang X, Qiao G. *Chem Commun.* 2011; 47:1151–1153.
228. Ding J, Chen J, Li D, Xiao C, Zhang J, He C, Zhuang X, Chen X. *J Mater Chem B.* 2013; 1:69–81.
229. Li Q, Zhu L, Liu R, Huang D, Jin X, Che N, Li Z, Qu X, Kang H, Huang Y. *J Mater Chem.* 2012; 22:19964–19973.

230. Han H, Jin Q, Wang Y, Chen Y, Ji J. Chem Commun. 2015; 51:17435–17438.
231. Liang Y, Kiick KL. Polym Chem. 2014; 5:1728–1736.
232. Liang Y, Kiick KL. Acta Biomater. 2014; 10:1588–1600. [PubMed: 23911941]
233. Kharkar PM, Kiick KL, Kloxin AM. Polym Chem. 2015; 6:5565–5574. [PubMed: 26284125]
234. Wieduwild R, Lin W, Boden A, Kretschmer K, Zhang Y. Biomacromolecules. 2014; 15:2058–2066. [PubMed: 24825401]
235. Christie RJ, Fleming R, Bezabeh B, Woods R, Mao S, Harper J, Joseph A, Wang Q, Xu ZQ, Wu H, Gao C, Dimasi N. J Control Release. 2015; 220:660–670. [PubMed: 26387744]
236. Sanson C, Diou O, Thévenot J, Ibarboure E, Soum A, Brûlet A, Miraux S, Thiaudière E, Tan S, Brisson A, Dupuis V, Sandre O, Lecommandoux S. ACS Nano. 2011; 5:1122–1140. [PubMed: 21218795]
237. Lecommandoux S, Sandre O, Chécot F, Rodriguez-Hernandez J, Perzynski R. Adv Mater. 2005; 17:712–718.

Fig. 1. Schematic of peptide polymer conjugation, and their subsequent loading with cargo. The assembly of such conjugate systems can be driven through amphiphilicity, pH, temperature or enzymatic response. A wide variety of vehicles have been developed for drug delivery, shown here are examples of microgels, micelles and dendrimers. Upon degradation triggered by endogenous or exogenous stimuli, cargo can be delivered to a desired site.

Fig. 2. Controlled polymerization techniques commonly employed in the production of peptide-polymer conjugates. These techniques have allowed for specific tuning of polymer and peptide parent materials. They have also enabled many new PEG-alternative polymers to be developed.

Fig. 3.

A wide range of functional groups and conjugation techniques are available for peptide-polymer conjugation. This has enabled the development of materials with site-specific conjugation, allowing for protein labeling, drug conjugation, and design of complex materials for drug delivery vehicles.

Fig. 4.

(a) Illustration of a 3-helix bundle forming peptide with alkyl chains located at the N-terminus, and PEG covalently linked to the side chain of the helix. (b) Association of the amphiphile, with 3-helix bundle, and PEG flanking the outside of the bundle. (c) Schematic of the micelle, where the core is made of the alkyl chains and the shell is comprised of the 3-helix bundle. Reproduced with permission from ref¹⁸⁶. Copyright © 2014, American Chemical Society.

Fig. 5. Schematic depicting the assembly of the triblock $p(\text{PEGA})\text{-}b\text{-}p(\text{Lys})\text{-}b\text{-}p(\text{His})$ and encapsulation of DOX. After uptake of the vesicle by tumor cells, the acidic pH mediates the disassembly of the vesicle and release of drug. Reproduced with permission from ref¹⁹⁹. Copyright © 2015, American Chemical Society.

Fig. 6. TEM micrographs and a schematic of the conjugate structure. (a) Vesicles assembled from shorter PTMC₁₃-PVGLIG, (b) micelle nanoparticles formed by PTMC₂₁-PVGLIG, and (c) core-shell nanoparticles assembled from the longest variant, PTMC₃₃-PVGLIG. The PTMC and PVGLIG peptide are represented by the blue and green components, respectively. Reproduced with permission from ref¹⁴². Copyright © 2014, American Chemical Society.

Fig. 7.

Schematic demonstrating the formation of ELP-CLP vesicles upon increasing temperature, by induction of an LCST transition in the ELP domain which promotes vesicular assembly. With increasing temperature, the CLP triple helix unfolds and the vesicles disassemble. Reproduced with permission from ref⁸⁵. Copyright © 2015, American Chemical Society.