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Functional polypeptide and hybrid materials: Precision synthesis via α-amino acid *N*-carboxyanhydride polymerization and emerging biomedical applications

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ABSTRACT

Polypeptides derived from naturally occurring α-amino acids have emerged as a unique and versatile family of bio-inspired biomaterials that can be tailor-made for varying biomedical applications such as controlled drug release, gene delivery, tissue engineering and regenerative medicine. In contrast to traditional biodegradable polymers such as aliphatic polyesters and polycarbonates, polypeptides are inherently functional, allow precise control over polarity and charges, show excellent stability against hydrolysis, and are prone to rapid biodegradation in vivo by specific enzymes. Ring-opening polymerization (ROP) of α-amino acid *N*-carboxyanhydrides (NCAs) is the most straightforward and practical approach for large-scale production of high molecular weight polypeptides. In the past decade, a

Abbreviations: AMM, "activated monomer" mechanism; AOB-L-Glu NCA, γ-(4-allyoxylbenzyl)-L-glutamate *N*-carboxyanhydride; APP, 5-(4-aminophenyl)-10,15,20-triphenyl-porphyrin; ATRP, atom transfer radical polymerization; BLA-NCA, β-benzyl-L-aspartate *N*-carboxyanhydride; BLG-NCA, γ-benzyl-L-glutamate *N*-carboxyanhydride; Boc, tert-butoxycarbonyl; BPA, 2-bromo-*N*-(2-aminethyl)-2-methylpropionamide; Bpy, 2,2′-bipyridine; BSA, bovine serum albumin; CCA, cis-1,2-cyclohexanedicarboxylic acid; CLG-NCA, γ-chloropropyl-L-glutamic acid *N*-carboxyanhydride; CLSM, confocal laser scanning microscopy; COD, 1,5-cyclooctadiene; CP, chloropropanyl; CRP, controlled radical polymerization; Cys, L-cysteine; DDT, dodecanethiol; DHBC, double hydrophilic block copolymer; N^{α},N^{ε}-diFmoc Lys, N^{α},N^{ε}-di(9-fluorenylmethoxycarbonyl)-L-lysine; DOPA, L-dihydroxylphenylalamine; DOX, doxorubicin; DTT, dithiothreitol; EG_2-Lys NCA, N^{ε}-2-(2-(2-methoxyethoxy)ethoxyl)acetyl-N^{α}-Z-L-lysine *N*-carboxyanhydride; EO_2MA, 2-(2-methoxyethoxy)ethyl methacrylate; EPR, enhanced permeability and retention; FITC, fluorescein isothiocyanate; Fmoc, 9-fluorenylmethoxycarbonyl; F-PBA, 3-fluoro-4-carboxyphenylboronic acid; GSH, glutathione; HA, hydroxyapatite; HEMA, 2-hydroxylethyl methacrylate; HMDS, hexamethyldisilazane; Hyd, hydrazide; LA, lipoic acid; LCST, lower critical solution temperature; Leu-NCA, L-Leucine *N*-carboxyanhydride; NAM, "normal amine" mechanism; NBC-NCA, *S*-(*o*-nitrobenzyl)-L-cysteine *N*-carboxyanhydride; NGF, nerve growth factor; NMP, nitroxide-mediated polymerization; *N*-TMS, *N*-trimethylsilazane; Nvoc, 6-nitroveratryloxycarbonyl; OEG, oligo(ethylene glycol); PAA, poly(L-aspartic acid); PAla, poly(L-alanine); PAMAM, poly(amido amine); PAOBLG, poly(γ-(4-allyoxylbenzyl)-L-glutamate); PAPB, polyaspartate modified with 4-phenyl-butanol; PAPLG, poly(γ-azidopropylglutamate); PArg, poly(L-arginine); PAsp, poly(L-aspartic acid); PAsp(DET-Aco), poly(*N*-(*N*′-((*N*″-cis-aconityl)-2-amnoethyl)-2-aminoethyl) aspartmide); PAsp(EDA-Cit), poly(*N*-(citraconyl-2-aminoethyl) aspartamide); PBLA, poly(β-benzyl-L-aspartic acid); PBLG, poly(γ-benzyl-L-glutamic acid); PCys, poly(L-cysteine); PDI, polydispersity index; PDMAEMA, poly(2-(dimethylamino)ethyl methacrylate); PDMAM, poly(*N*,*N*′-dimethylacrylamide); PDTA, 2-(2-pyridinyldithio) ethylamine hydrochloride salt; PEG, poly(ethylene glycol); $P(EG_2$-Lys), poly(N^{ε}-2-[2-(2-methoxyethoxy) ethoxyl] acetyl-L-lysine); PEO, poly(ethylene oxide); PEI, polyethylenimine; PGlu, poly(L-glutamic acid); PHArg, poly(L-homoarginine); Phe-NCA, L-phenylalanine *N*-carboxyanhydride; PHis, poly(L-histidine); PLeu, poly(L-leucine); PLGA, poly(lactide-co-glycotide); PLLA, poly(L-lactide); PLys, poly(L-lysine); PMPA, poly((3-morpholinopropyl)aspartamide); PNBC, poly(*S*-(*o*-nitrobenzyl)-L-cysteine); PNIPAM, poly(*N*-isopropylacrylamide); PPhe, poly(L-phenylalanine); PPLG-NCA, γ-propargyl-L-glutamate *N*-carboxyanhydride; PPO, poly(propylene oxide); PS, polystyrene; PTFALys, poly(trifluoroacetyl-L-lysine); PTMC, poly(trimethylene carbonate); PVal, poly(L-valine); PVBLG, poly(γ-(4-vinylbenzyl)-L-glutamate); RAFT, reversible addition-fragmentation chain-transfer; ROP, Ring-opening polymerization; SPPS, solid phase peptide synthesis; tBMLC, *S*-tert-butylmercapto-L-cysteine; TFA, trifluoroacetyl; TFA-Lys NCA, ε-trifluoroacetyl-L-lysine *N*-carboxyanhydride; TMS, trimethylsilyl; 4-VB, 4-vinylbenzyl; VB-Glu NCA, γ-(4-vinylbenzyl)-L-glutamate *N*-carboxyanhydride; VSCys-NCA, vinyl sulfone-substituted L-cysteine *N*-carboxyanhydride; Z, benzyloxycarbonyl; ZLys-NCA, *N*-benzyloxycarbonyl-L-lysine *N*-carboxyanhydride.

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Biomaterials
Drug release
Gene delivery
Tissue engineering

remarkable progress has been made in controlled NCA polymerization, which offers an unprecedented access to precision polypeptide and hybrid materials by combining with living radical polymerization, click chemistry, and/or post-polymerization modification. Notably, several micellar anti-cancer drugs based on poly(ethylene glycol)-polypeptides have been already advanced to the clinical evaluation. In this review paper, we give an overview on de novo design, controlled synthesis and emerging biomedical applications of functional polypeptide and hybrid materials.

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1. Introduction

Proteins and oligopeptides produced by living organisms are the most versatile biological materials that provide structural and mechanical support to cells, tissues and organs (e.g. actin, myosin, collagen), catalyze various biochemical reactions (e.g. enzymes, glutathione), and regulate cell signaling, cell adhesion, and immune responses (e.g. cell surface markers, receptors, peptide hormones) [1–4]. The vastly different functions of proteins and oligopeptides originate from a wide choice of α-amino acid monomers as well as control of peptide sequences [5]. In the past decade, significant effort has been directed to the development of de novo oligo- and polypeptides that mimic natural proteins possessing excellent biocompatibility and biodegradability in vivo for diverse biological, medical and pharmaceutical applications [5–11].

In comparison with aliphatic polyesters and polycarbonates that are the prime synthetic biodegradable polymers currently applied for various biomedical applications, polypeptide materials have several potential advantages, e.g. (i) they are inherently functional providing a variety of reactive groups ranging from hydroxyl, carboxyl, thiol, to amino groups, which render them particularly appealing in design and development of multi-functional bioactive biomaterials; (ii) they offer excellent control over hydrophilic and hydrophobic balance by constituent α-amino acid monomers, compositions, sequences and molecular weights, which allow formation of essentially any supramolecular structures spanning from nanoscale, microscale, to macroscale (Fig. 1); (iii) they can be designed with varying charged groups including imidazole in histidine and guanidino in arginine that play an indispensable role in biological interactions in vivo including

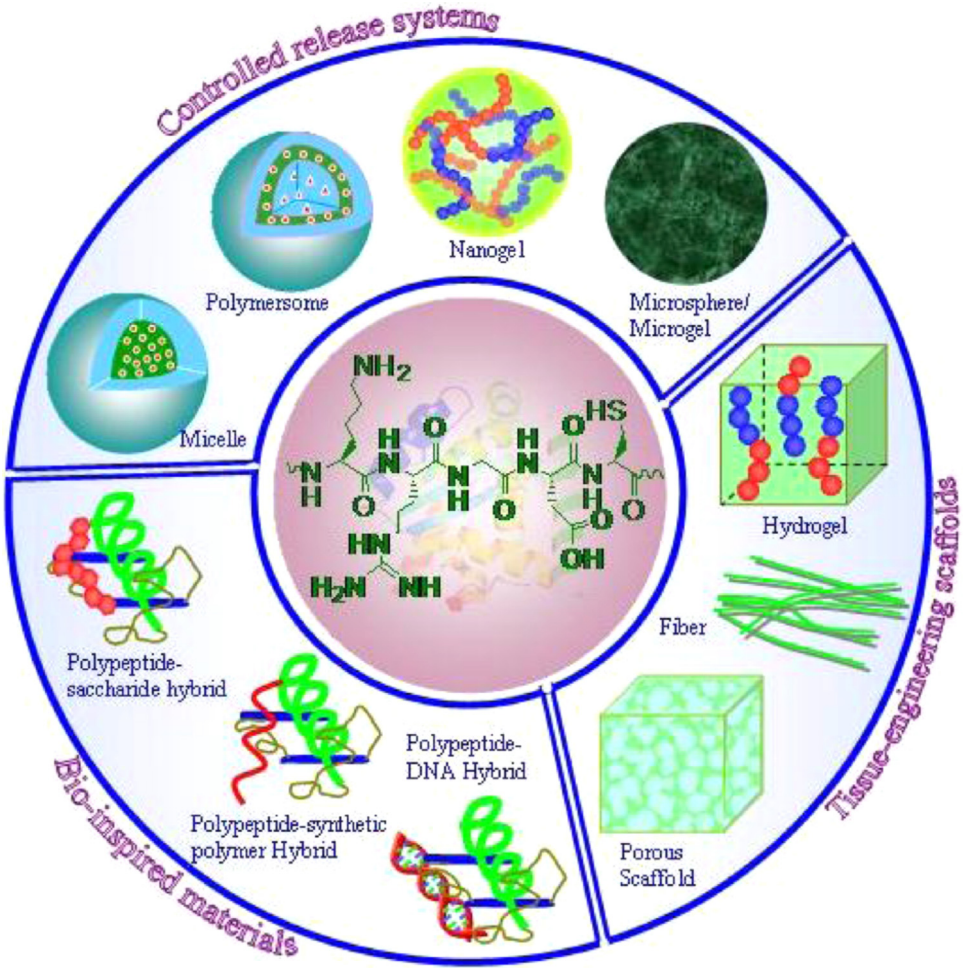

Fig. 1. Emerging biomedical applications of functional polypeptide and hybrid materials.

in cellular and subcellular pathways; and (iv) they have excellent stability against hydrolysis while undergo rapid biodegradation in vivo into natural α-amino acids with the help of specific enzymes. Hence, polypeptides are a versatile family of advanced bioinspired materials that inherit many intriguing properties of proteins such as excellent biocompatibility, versatile structures and functionalities, biodegradability to non-toxic products in vivo, and unique hierarchical assembly property [12–14]. In the past decade, polypeptides have been tailor-made for a wide range of biomedical applications such as controlled drug release, gene delivery, tissue engineering and regenerative medicine (Fig. 1) [15,16]. In particular, polypeptide hybrid materials, which elegantly combine features of polypeptides (versatile functionalities, secondary structure, enzymatic biodegradability, etc.) with synthetic polymers (solubility, processability, nonfouling property, etc.), have significantly broadened the scope of biomaterials [17–20].

Polypeptides are mainly synthesized by protein biosynthesis, solid phase peptide synthesis (SPPS), and α-amino acid *N*-carboxyanhydrides (NCA) polymerization. Protein biosynthesis can be used to produce proteins with precision chain lengths and peptide sequences by introducing an engineered recombinant plasmid into a bacterial host [21], which suffers, however, from low yield, limited post-translational modification, and complex production. SPPS is a routine method that allows peptide backbone modification, incorporation of unnatural amino acids, and access to polypeptides that are difficult to express in bacteria. The chain length and peptide sequence can be well-controlled by successive coupling of amino acids. The maximum chain length is, however, restricted to about 50 amino acid residues due to the statistical accumulation of resin-bound by-products from incomplete reactions and impurities in the solvent, reagents, and protected amino acids used [17]. In addition, the tedious coupling, protection and deprotection reactions result in low productivity, high costs, and possible racemization [22]. NCA polymerization technique is the most widely applied strategy for the synthesis of polypeptide and hybrid materials, as it allows large-scale preparation of high molecular weight polypeptides with no detectable racemization at the chiral centers. Moreover, polypeptide and hybrid materials with various architectures (block copolymers, graft copolymers, random copolymers, star-shaped polymers, and dendrimers), secondary structures and functionalities have been achieved

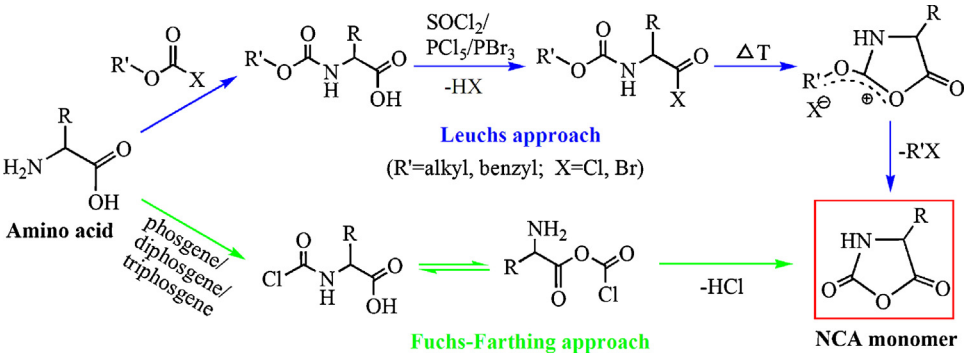

Fig. 2. Synthesis of NCAs by Leuchs approach and Fuchs-Farthing approach.

through the (co)polymerization of diverse NCA monomers [14,23,24]. In this review, we will highlight up-to-date development of precision NCA polymerization techniques, de novo functional polypeptide and hybrid materials, and their emerging biomedical applications.

NCA synthesis began with the Leuchs method in 1906 [25], which involved the reaction of *N*-carbamoyl α-amino acids with thionyl chloride (Fig. 2). Phosphorous pentachloride and phosphorus tribromide were also explored as chlorinating agents due to their higher activity, which enabled to reduce the reaction temperature and avoid decomposition of NCAs. The Fuchs-Farthing method [26,27] involving direct phosgenation of α-amino acids in inert polar solvents (such as ethylacetate, dioxane, tetrahydrofuran, and acetonitrile) with phosgene, diphosgene, or triphosgene (Fig. 2) is most widely used because pure NCA monomers with a good yield and no racemization can be easily obtained. It was reported that washing NCA solutions with water or aqueous sodium bicarbonate at 0 °C and subsequent rapid drying is an efficient method for NCA purification [28]. Hydrochloride scavengers like α-pinene and limonene were proved effective in preventing byproduct formation, especially in the large scale synthesis of L-leucine NCA (Leu-NCA) [29]. Recently, Deming et al. developed a rapid and general method for purification of NCAs using flash column chromatography [30]. This procedure is especially useful for the purification of NCA monomers that cannot or are difficult to recrystallize.

NCA polymerizations are commonly initiated by a nucleophile or base. Depending on the relative nucleophilicity and basicity of initiator, NCA polymerizations have been reported to follow a "normal amine" mechanism (NAM) or an "activated monomer" mechanism (AMM) [14,23]. In case of initiators such as metal alkoxides and tertiary amines, which show stronger basicity than nucleophilicity, NCA polymerization most likely proceeds via an AMM mechanism in which initiation takes place by deprotonation of an NCA monomer and ensuing nucleophilic attack of another NCA monomer. AMM with a high propagation rate could provide polypeptides with relatively high molecular weights though plagued with a comparably high polydispersity index (PDI) due to relatively slow initiation. NAM mechanism is a nucleophilic ring-opening chain growth process where polymer molecular weight grows linearly with monomer conversion if side reactions were absent. Protonic nucleophiles such as primary amines, secondary amines, alcohols and water that display stronger nucleophilicity than basicity promote NCA polymerization most likely via an NAM mechanism. It is noted that sterically unhindered primary amines (e.g. *n*-butylamine, *n*-hexylamine, and benzylamine) are more nucleophilic than the ω-amino groups of the propagating chains, affording faster initiation as compared to propagation. Hence, polypeptides with tailored molecular weights and low PDI could be obtained.

Although NCA polymerization by NAM mechanism using primary amine initiators is promising, it could not provide structurally well-defined polypeptides and copolymers with high molecular weights and low PDIs due to poor control over the reactivity of the growing polymer chain ends. Deming et al. reported living NCA polymerizations using zerovalent nickel and cobalt complexes (e.g. bpyNi(COD) and $(PMe_3)_4Co$) as initiators (Table 1) [31,32], in which polymerization involves activation of NCA monomers into covalent propagating species. The metal residues can be removed from the polymers by precipitation or dialysis of the obtained polypeptides. Schlaad et al. discovered that primary amine hydrochloride salts bring about a controlled NCA polymerization, giving polypeptides with particularly low PDIs (<1.03) (Table 1) [33]. The polymerization is usually incomplete due to low reactivity of amine hydrochloride. Hadijichristidis et al. reported that high vacuum polymerization of γ-benzyl L-glutamate *N*-carboxyanhydride (BLG-NCA) and *N*-benzyloxycarbonyl-L-lysine NCA (ZLys-NCA) using a primary amine as initiator displays a living character (Table 1) [34]. Giani et al. reported that primary amines initiate a living polymerization of ε-trifluoroacetyl-L-lysine NCA (TFA-Lys NCA) in DMF at a low polymerization temperature of 0 °C (Table 1) [35]. Notably, Cheng et al. recently reported that secondary amine hexamethyldisilazane (HMDS) initiates living polymerization of BLG-NCA, giving polypeptides with high yield, prescribed molecular weight, and low PDI (<1.2) (Table 1) [36]. In a subsequent study, various *N*-trimethylsilazane (*N*-TMS) amines were developed for controlled NCA polymerizations [37].

The advancement of controlled/living NCA polymerizations facilitates the design and synthesis of functional polypeptide and hybrid materials with increasing chemical diversity and complexity. In the early time, functional

Table 1
Recent development of controlled/living NCA polymerization methods.

Methods	Initiators	Reaction conditions	Indications	Author/year	Ref.
Transition metal complexes	**(L)$_n$M** (bpyNi(COD), (PMe$_3$)$_4$Co)	RT, 30–60 min	NCAs except proline NCA	Deming/1997, 1999	[31,32]
Primary amine hydrochloride	**R-NH$_2$·HCl** (R = PS, PNIPAM, PEG, PDMAM, 4-VB, butyl)	40–80 °C, 3 days	ZLys/BLG/Phe-NCA	Schlaad et al./2003	[33]
Primary amine and high vacuum	**R-NH$_2$** (R = hexyl, PBLG, PEG)	High vacuum, RT	Most NCAs	Hadjichristidis et al./2004	[34]
Primary amine and low temperature	**R-NH$_2$** (R = hexyl, benzyl, PBLG)	0 °C, 4 or 7 days	Most NCAs	Giani et al./2004	[35]
Silazane derivatives	**R-NHSi(Me)$_3$** (R = allyl, TMS, propargyl, PEG, etc.)	RT, 24 h	BLG/ZLys-NCA, 4-VB/AOB/CP-Glu-NCA,	Cheng et al./2007	[36,37]

Bpy, 2,2′-bipyridine; COD, 1,5-cyclooctadiene; PS, polystyrene; PEG, poly(ethylene glycol); PNIPAM, poly(*N*-isopropylacrylamide); PDMAM, poly(*N*,*N*′-dimethylacrylamide); 4-VB, 4-vinylbenzyl; TMS, trimethylsilyl; AOB, allyloxylbenzyl; CP, chloropropanyl.

polypeptides containing a pendant carboxyl or amine group, i.e. poly(L-glutamic acid) (PGlu), poly(L-aspartic acid) (PAsp) and poly(L-lysine) (PLys), were obtained via polymerization of protected NCA monomers followed by deprotection. The tedious protection/deprotection procedure often gives low overall yields. Furthermore, measures should be taken to minimize polymer degradation during deprotection. In recent years, polypeptides bearing specifically designed functional groups have attracted great interest due to their facile synthesis, high functionality, and tunable physico-chemical characteristics including hydrophilicity, degradation rate and stimuli-sensitivity. In general, functional polypeptides can be synthesized through following three strategies: (i) based on designed functional NCA monomers containing a protected pendant group (such as carboxylic acid, amine, thiol) or containing an exotic functional group (such as oligo(ethylene glycol) (OEG), azido, alkyne, alkene and saccharide); (ii) based on functional initiators combining NCA polymerization with controlled radical polymerization (CRP) or click chemistry; and (iii) via facile post-polymerization modification of functional polypeptides. The current advancement in functional polypeptides has opened doors to many exciting medical and pharmaceutical applications [38–42]. For example, hydrogels prepared from poly(L-lysine)-poly(L-leucine) (PLys-PLeu) diblock copolypeptides are non-toxic, injectable through 30-G needle, and could induce ingrowth of blood vessels as well as nerve fibers in mouse forebrain [39]. Several nano-scale anticancer drug release systems based on PEG-poly(amino acid)s have advanced to different phases of clinical trials [40]. There are excellent reviews on early synthesis and biomedical applications of polypeptides via NCA polymerizations [5,14,23] as well as development of stimuli-sensitive polypeptides for drug and gene delivery [16,43], however, none has focused on functional polypeptide and hybrid materials. This review presents recent advances in functional polypeptide and hybrid materials, with a particular focus on their novel design, synthesis, and emerging biomedical applications. Here, we firstly give a comprehensive overview on synthetic strategies to various side-chain functionalized polypeptides and chain end-functionalized polypeptides. Then, biomedical applications of functional polypeptides and hybrid materials are discussed. At the end, conclusions and future perspectives are presented.

2. Side chain-functionalized polypeptides

2.1. Direct polymerization of side chain-functionalized NCA monomers

2.1.1. NCA monomers containing a protected functional group

Functional polypeptides have been prepared via direct polymerization of NCA monomers that contain a protected pendant group such as carboxylic acid, amine, hydroxyl, thiol, imidazole, and guanidine followed by deprotection. In particular, PGlu, PAsp, PLys have been most synthesized due to facile synthesis and purification of corresponding protected NCA monomers (Fig. 3A–C) and good solubility of thus obtained polypeptides [44,45]. PGlu and PAsp are usually prepared in following four steps: (i) protection of the β/ω-carboxyl groups of aspartic acid and glutamic acid by benzyl groups, (ii) conversion of amino acids into β-benzyl-L-aspartate NCA (BLA-NCA) and BLG-NCA monomers using triphosgene, (iii) preparation of poly(γ-benzyl-L-glutamic acid) (PBLG) and poly(β-benzyl-L-aspartic acid) (PBLA) by polymerization of NCA monomers initiated by primary amine, transition metal complexes, or silazane derivatives, and (iv) removal of benzyl protection groups with strong acid (HBr/HOAc), aqueous base or catalytic hydrogenation to obtain carboxyl functionalized polypeptides (Fig. 4). In a similar way, PLys has been prepared from ω-amine protected L-lysine NCA monomers in which different protective groups including benzyloxycarbonyl (Z), tert-butoxycarbonyl (Boc), trifluoroacetyl (TFA), 9-fluorenylmethoxycarbonyl (Fmoc) and 6-nitroveratryloxycarbonyl (Nvoc) were employed [46].

Although the first synthesis and ring-opening polymerization (ROP) of N^{im}-benzyl-L-histidine NCA could be dated back to 1957 [47], controlled polymerization of N^{im}-DNP-L-histidine NCA (Fig. 3D, DNP: dinitrophenyl) was only reported by Bae et al. in 2003 [48]. N^{im}-benzyl-L-histidine NCA was purified by recrystallization and removal of DNP group from imidazole amine was completed by 2-mercaptoethanol at room temperature. Interestingly, poly(L-histidine) (PHis) with a pK_a of 6.5 has been exploited for tumor extracellular pH-sensitive actuators for ligand exposure [49], and early endosomal pH-induced micelle destabilization followed by endosomal membrane

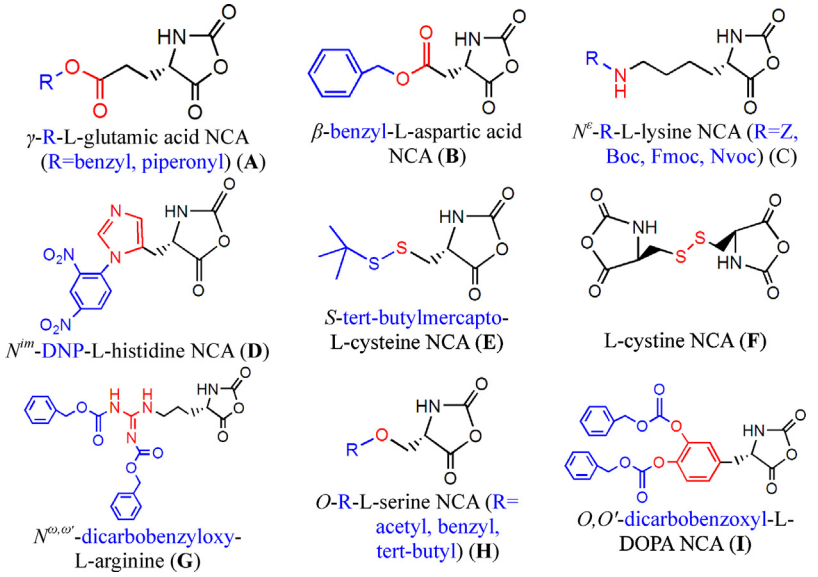

Fig. 3. Functional NCA monomers containing a protected pendant functional group.

disruption due to its proton-sponge effect and fusogenic activity [50].

The thiol group present in L-cysteine (Cys) can be utilized for a wide variety of reactions such as Michael addition, cross-linking, thiol-ene chemistry and chain transfer in free radical polymerizations. L-cysteine NCA (Cys-NCA) are often prepared using a thiol protecting group like *S*-benzyl-L-cysteine or *S*-Z-L-cysteine [51]. However, the Z groups of poly(S-Z-L-cysteine) obtained could only be removed by Na/NH_3 or HBr/HOAc/TFA and the removal of benzyl was even harder [19,52]. Recently, Heise et al. synthesized *S*-tert-butylmercapto-L-cysteine NCA (tBMLC-NCA) (Fig. 3E), which was copolymerized with BLG-NCA at 0 °C with benzylamine as an initiator to yield a well-defined copolypeptide with a controlled composition and low PDI of 1.2. Notably, selective deprotection of ter-butyl groups in copolypeptides was achieved in the presence

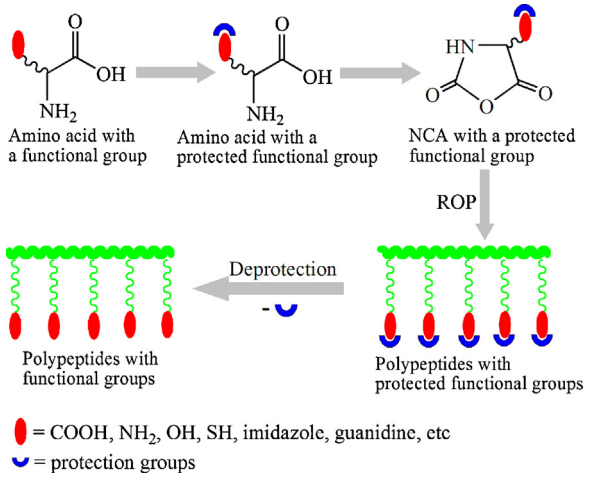

Fig. 4. Synthesis of side chain functionalized polypeptides from NCA monomers containing a protected functional group.

of dithiothreitol (DTT) in DMF at 60 °C [53]. Yan et al. reported synthesis of cystine NCA (Fig. 3F) from *N*,*N*′-dicarbobenzoxy-L-cystine ((Z-cys-OH)$_2$) and PBr_3 in dry dichloromethane. The copolymerization of L-cystine NCA and BLG-NCA using mPEG-NH_2 as an initiator gave a disulfide core cross-linked PEG-polypeptide nanogel with an average size of 250 nm, which was readily de-crosslinked under reductive conditions [54]. Core cross-linked PEG-polypeptide nanogels prepared from copolymerization of L-cystine NCA and L-phenylalanine NCA (Phe-NCA) demonstrated good stability in the circulation condition while accelerating intracellular doxorubicin (DOX) release due to the reduction-sensitivity [55].

Poly(L-arginine) (PArg) has been prepared by polymerization of $N^{\omega,\omega'}$-dicarbobenzyloxy-L-arginine NCA (Z_2-R NCA, Fig. 3G) that was crystalline and synthesized from $N^{\alpha,\omega,\omega'}$-tricarbobenzyloxy-L-arginine (Z_3-R) and thionyl chloride in dioxane, followed by deprotection with hydrogen bromide in glacial acetic acid [56,57]. Alternatively, PArg can be prepared by guanidization of poly(L-ornithine) with 1-guanyl-3,5-dimethylpyrazole or isomethylthiourea [57]. Poly(L-serine) has been obtained via ROP of *O*-acetyl-L-serine NCA, *O*-benzyl-L-serine NCA or *O*-tert-butyl-L-serine NCA (Fig. 3H). However, harsh reagents such as sodium methoxide, HCl or HBr were required for effective deprotection [58–60].

L-Dihydroxylphenylalamine (DOPA) is an important component in adhesive precursor protein that is primarily responsible for its chemisorption to wet surfaces and covalent crosslinking of the adhesives. Pure crystalline *O*,*O*′-dicarbobenzoxyl-L-DOPA NCA (Z_2-DOPA NCA, Fig. 3I) was prepared from *N*,*O*,*O*′-tricarbobenzoxyl-L-DOPA (Z_3-DOPA) and phosphorous pentachloride at 0 to ca. −5 °C in anhydrous ether. Yamamoto et al. synthesized and investigated the cross-linking and adhesive properties of DOPA homopolymers and copolymers with L-lysine, L-glutamic acid or tyrosine [61]. Deming et al. prepared adhesive polymers consisting of DOPA and lysine that are similar

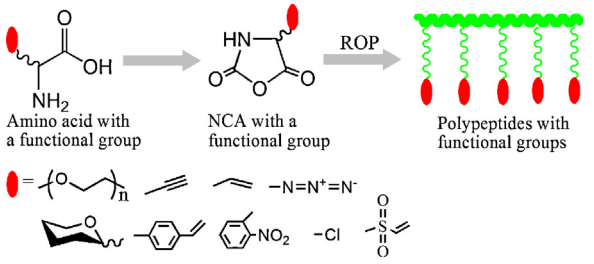

Fig. 5. Synthesis of side chain-functionalized polypeptides from NCA monomers containing a compatible functional group.

to natural marine adhesive proteins displayed moisture-resistant adhesive properties when suitably oxidized [62]. The crosslinking of DOPA polypeptide in water in the presence of an oxidizing agent was also exploited to dramatically improve the membrane stability of vesicles [63].

The direct polymerization of NCA monomers containing a protected functional group provides a straightforward pathway to natural functional polypeptides and copolypeptides. This synthetic strategy, however, suffers drawbacks of complex synthesis involving tedious protection and deprotection of functional groups, low yields, and potential polymer degradation.

2.1.2. Oligo(ethylene glycol)-functionalized NCA monomers

In the past years, various functional polypeptides have been prepared from non-natural functional NCA monomers in which the side chain functional groups do not interfere with NCA polymerization (Fig. 5). This synthetic strategy is very attractive because it avoids laborious protection and deprotection steps and furthermore polypeptides with 100% functionalization can be easily obtained.

Oligo(ethylene glycol) (OEG) has been incorporated into natural amino acids to generate polypeptides and polypeptide hybrids that have displayed thermosensitivity, non-fouling and self-assembly properties. Deming et al. reported synthesis of diethylene glycol-functionalized PLys (poly(EG_2-Lys)) from N^{ε}-2-(2-(2-methoxyethoxy)ethoxyl) acetyl-N^{α}-Z-L-lysine NCA (EG_2-Lys NCA, Fig. 6A) [64]. Poly(EG_2-Lys) is nonionic, water-soluble, completely α-helical in solution under different conditions and similar to pure PEG resistant to proteases. Poly(EG_2-Lys)-b-PLeu block copolymers were shown to self-assemble into spherical vesicles, in which vesicle sizes and structures were primarily determined by the ordered conformations of the polymer segments, in a manner similar to viral capsid assembly [57]. Replacing L-leucine with rac-leucine, Deming et al. synthesized poly(EG_2-Lys)-b-poly(DL-leucine) block copolypeptides possessing an uncommon rodlike hydrophilic segment and a disordered hydrophobic segment that formed highly stable, nanoscale polypeptide micelles [65]. Klok et al. created non-fouling polypeptide brushes with thicknesses of up to 40 Å through surface-initiated polymerization of EG_3-Lys NCA or EG_8-Lys NCA. These OEG-functionalized PLys brushes have an α-helical secondary structure at pH 4–9 and showed effective prevention of nonspecific protein adsorption [66].

Deming et al. also prepared diethylene glycol-functionalized poly(*O*-EG_2-L-serine) and poly(*S*-$EG_{1/2}$-L-cysteine) from *O*-EG_2-L-serine NCA monomer and *S*-$EG_{1/2}$-L-cysteine NCA (Fig. 6B and C), respectively [67]. Interestingly, poly(*O*-EG_2-L-serine) while adopting β-sheet conformation in the solid state was highly water soluble to give random conformation in a wide pH range. Recently, Li et al. prepared OEG-functionalized PGlu (PEG_nGlu, n = 1, 2, 3) by polymerization of OEG_n-L-glutamic acid NCA (Fig. 6D) [68]. These OEG-functionalized PGlus displayed lower critical solution temperature (LCST) behavior in water, and the LCST can be tuned via copolymerization with different NCA monomers. PEG_nGlu copolypeptides were also obtained by ROP of γ-propargyl-L-glutamate NCA (PPLG-NCA, Fig. 6E) and subsequent click reaction between the pendant alkyne groups and EG_2-N_3 or EG_3-N_3 [69]. Dong et al. found that PEG_2Glu mainly adopted an α-helix structure, and could self-assemble into varying nanostructures in aqueous solution from spherical micelles, to worm-like micelles, and to fiber micelles, depending on its molecular weights [70].

2.1.3. Azido, alkyne, or alkene functionalized NCA monomers

Click chemistry is widely used in the modification of biomolecules and synthetic polymers due to its high efficiency, specificity, mild reaction conditions and tolerance to most functional groups. Hammond et al. synthesized poly(γ-propargyl-L-glutamate) (PPLG) from polymerization of PPLG-NCA (Fig. 6E) [71]. The side chain alkyne groups allowed efficient conjugation of PEG-azide or amine-azide covering a range of pK_a and hydrophobicities via click reaction (Fig. 7) [72]. The amine-functionalized polypeptides exhibited the ability to reversibly self-assemble into micelles for nucleic acid encapsulation. Chen et al. reported efficient glycosylation of PPLG with different sugar azides via click reaction to give water-soluble glycopolypeptides (Fig. 7) [73]. Yang et al. demonstrated rapid and highly efficient side-chain functionalization of PPLG via thiol-yne photochemistry to obtain double hydrophilic or amphiphilic block copolymers, which could be used to control the biomineralization of $CaCO_3$ or to form vesicles with a size of ca. 90 nm [74].

Zhang et al. reported synthesis of chloro-functionalized PGlu through polymerization of γ-chloropropyl-L-glutamic acid NCA (CLG-NCA, Fig. 6F) in the presence of HMDS [75,76]. The chloro functional group was converted to azido group that reacted readily with alkyne-containing mannose and PLA-b-PEG via click chemistry to afford functional copolypeptides with quantitative grafting efficiency (Fig. 8A). These functional polypeptides adopted α-helical conformation both in solution and in solid state. The same author prepared azido and allyl dual-functionalized diblock copolypeptides by sequential polymerization of γ-allyl-L-glutamic acid-based NCA (Fig. 6G) and CLG-NCA followed by nucleophilic substitution of chloro groups with NaN_3 [77]. These dual-functional block copolypeptides can be readily functionalized by copper-mediated alkyne-azide cycloaddtion and radical thio-ene addition reactions, respectively, with quantitative efficiency, allowing facile control over polymer bioactivity, solubility and

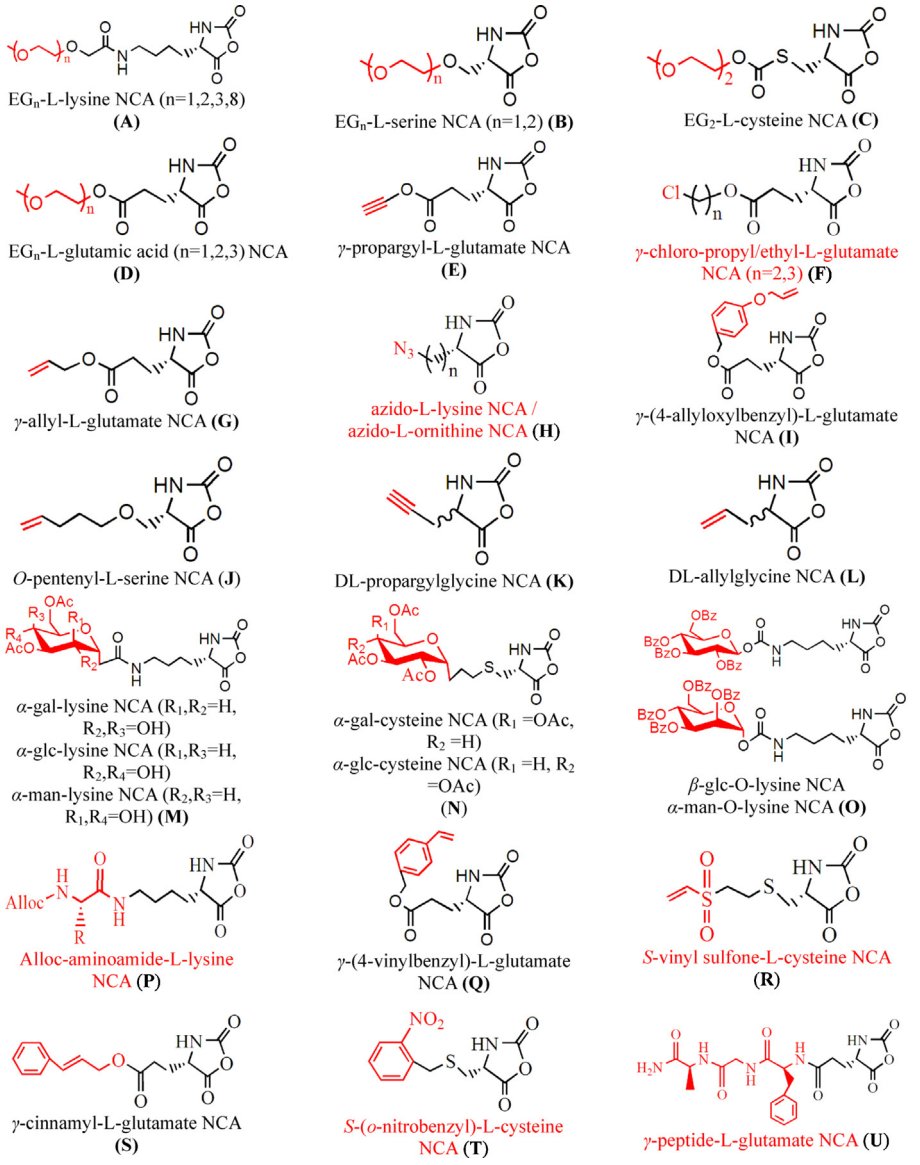

Fig. 6. Side chain-functionalized NCA monomers.

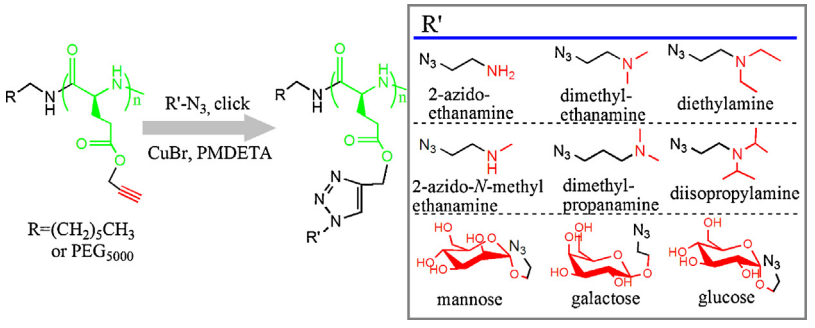

Fig. 7. Click conjugation of poly(γ-propargyl-L-glutamate) (PPLG).
[72,73], Copyright 2011 and 2010, respectively. Reproduced with permission from the Royal Society of Chemistry, and Wiley-VCH Verlag GmbH & Co. KGaA.

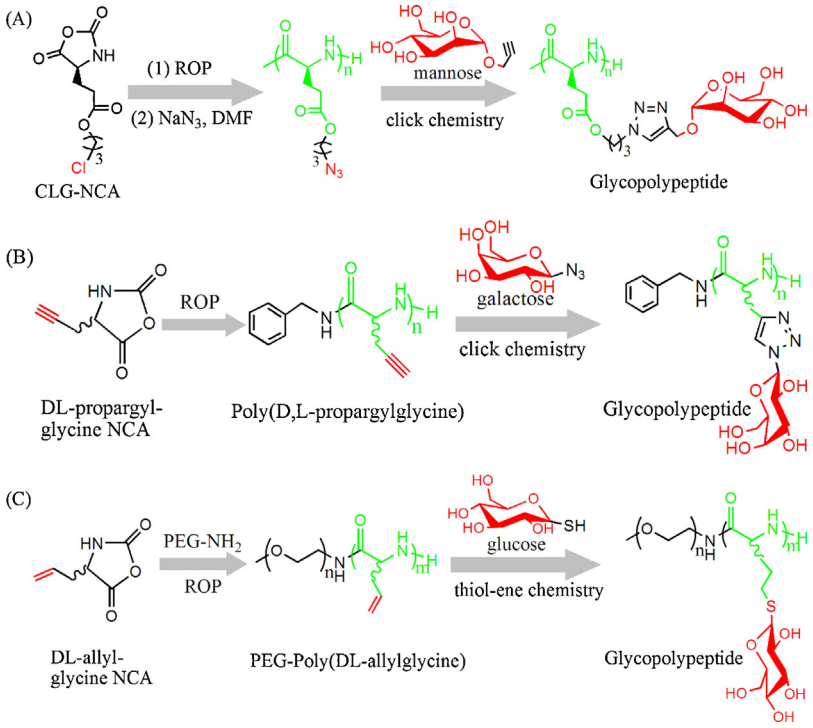

Fig. 8. Synthesis of glycopolypeptides via combining NCA polymerization and click chemistry.

self-assembly properties. Very recently, Deming et al. reported the synthesis of azido-L-lysine and azido-L-ornithine NCA monomers (Fig. 6H) that undergo living polymerization to afford clickable and well-defined azidopolypeptides [78].

Cheng et al. prepared well-defined poly(γ-(4-allyoxylbenzyl)-L-glutamate) (PAOBLG) polypeptides based on γ-(4-allyoxylbenzyl)-L-glutamate NCA monomer (AOB-L-Glu NCA, Fig. 6I), which could be converted into charged polypeptides via thio-ene reactions with 2-aminoethanethiol [79]. Thus obtained charged polypeptides are water soluble and exhibit excellent helical stability due to their elongated hydrophobic side chains and distally situated charges. Similarly, water-soluble poly(L-serine)s with elongated and charged side chains were synthesized via ROP of *O*-pentenyl-L-serine NCA (Fig. 6J) followed by thio-ene reactions [80]. These charged poly(L-serine) derivatives showed stable β-sheet conformations, membrane-penetrating capabilities in different cell lines, and low cytotoxicity.

In addition to γ-propargyl-L-glutamate and *O*-pentenyl-L-serine, alkyne-functionalized non-natural amino acid, DL-propargylglycine (Fig. 6K), was also designed and synthesized to afford alkyne-functionalized polypeptides, which following reacting with azide-functional galactose by click chemistry yielded polypeptide-galactose hybrid materials with a high efficiency (Fig. 8B) [81]. Sun and Schlaad also demonstrated that well-defined poly(DL-allylglycine) homo- and block copolymers prepared from DL-allylglycine NCA (Fig. 6L) could be employed to synthesize glucopolypeptides through radical thio-ene click chemistry without transition metal catalysis (Fig. 8C) [82].

2.1.4. NCA monomers containing a protected saccharide group

Though O-linked glycol-serine NCA monomers have been known since 1960s, their application is hindered by inefficient synthesis, purification and polymerization probably due to steric and H-bonding interactions between the sugar substituents and the NCA rings. Deming et al. prepared new glycosylated-L-lysine NCA monomers (Fig. 6M) using glucose, mannose or galactose in five steps. The glycosylated lysine NCAs could undergo living polymerization to give well-defined high molecular weight glycopolypeptides with 100% glycosylation. The use of C-linked sugars and amide linkage to lysine benefited NCA monomer stability without sacrificing biochemical properties [83]. In a similar manner, glycosylated Cys-NCA monomers (Fig. 6N) were synthesized in high yield by coupling of alkene-terminated C-linked glycosides of D-galactose or D-glucose to Cys using thio-ene "click" chemistry followed by cyclization [84]. Interestingly, oxidation of the side-chain thioether linkages in these polypeptides to sulfone groups in water resulted in helix-to-coil transition without loss of water solubility.

Recently, Gupta et al. have reported a facile three step synthesis of O-glycosylated-lysine NCA (Fig. 6O) using a stable glycosyl donor (propargyl 1,2-orthoester of glucose and mannose) and a commercially available

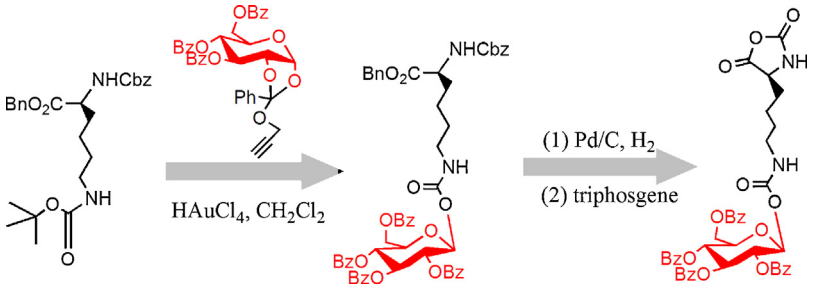

Fig. 9. Synthesis of glucose-*O*-lysine NCA.
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protected amino acid, N^{ε}-Boc-N^{α}-Z-lysine benzyl ester (Fig. 9) [85]. Notably, glycosylation and subsequent deprotection reaction proceeded to completion. These glycosylated NCAs were polymerized to yield well-defined high molecular weight homo- and diblock copolypeptides using amine initiators with the help of 1,8-bis(dimethylamino)naphthalene.

2.1.5. Other functional NCA monomers

Chloroethyl-containing polypeptides were prepared from γ-2-chloroethyl-L-glutamate NCA (CELG-NCA, Fig. 6F) and used for atom transfer radical polymerization (ATRP) of 2-(2-methoxyethoxy)ethyl methacrylate (EO_2MA) [86]. These polypeptide-synthetic polymer hybrids were reported to form stimuli-responsive micelles with α-helical core and thermo-responsive shell in water. Deming et al. prepared high chain density, cylindrical polypeptide brushes based on allyloxycarbonyl-aminoamide L-lysine NCA (alloc-aminoamide-L-lysine NCA, Fig. 6P) through tandem cobalt and nickel catalysis [87]. The alloc-aminoamide groups while inert toward cobalt-initiated NCA polymerization formed active initiating species with nickel for growth of brushes. Cheng et al. reported synthesis of vinyl-containing polypeptides, poly(γ-(4-vinylbenzyl)-L-glutamate) (PVBLG), from corresponding NCA monomers (VB-Glu NCA, Fig. 6Q) [88]. The vinyl groups in PVBLG could further be converted into alcohol, aldehyde, carboxylic acid, allyl functions with high efficiency. Recently, Deng and Zhong et al. designed and prepared vinyl sulfone-functionalized polypeptides based on vinyl sulfone-substituted L-cysteine NCA monomer (VSCys-NCA, Fig. 6R), which provided an unprecedented access to functional polypeptide-based materials including glycopolypeptides, functional polypeptide coatings, and in situ forming polypeptide hydrogels through Michael-type addition chemistry under mild conditions [89].

Jing et al. developed a γ-cinnamyl-L-glutamate NCA monomer (CLG-NCA, Fig. 6S), based on which photo-crosslinked PEG-polypeptide micelles were prepared via photodimerization of the cinnamyl pendant groups [90]. The in vitro paclitaxel release studies indicated that crosslinked micelles exhibited a slow drug release in comparison with the non-crosslinked micelles. Dong et al. prepared photoresponsive poly(*S*-(*o*-nitrobenzyl)-L-cysteine)-PEO (PNBC-PEO) block copolymers from *S*-(*o*-nitrobenzyl)-L-cysteine NCA monomer (NBC-NCA, Fig. 6T) [91]. The release of DOX from PNBC-PEO micelles could be controlled by 365 nm UV irradiation. Recently, Amblard et al. reported that specific peptide-grafted comb polypeptides could be prepared by polymerization of NCA monomer bearing the peptide sequence on its side chain (Fig. 6U) [92].

2.2. Post-polymerization modification

Post-polymerization modification is a powerful strategy to introduce functional groups that are not compatible with direct ROP of the corresponding NCAs [93]. Preparation of side chain functional polypeptides through post-polymerization modification typically involves the polymerization of side chain protected α-amino acid NCAs, followed by deprotection and conversion to desired functional moieties, or by direct aminolysis or transesterification to desired functional groups (Fig. 10).

Jing et al. developed PEG-PLA-PGlu triblock copolymers by ROP of BLG-NCA using PEG-PLA-NH_2 as a macroinitiator, followed by catalytic hydrogenation to remove the protective benzyl groups [94]. The pendant carboxyl groups of PEG-PLA-PGlu were conjugated with cell-adhesive RGD peptide to yield RGD functionalized PEG-PLA-PGlu copolymers. Jing et al. have also reported preparation of bioactive scaffolds or tumor-targeting nanoparticles based on PEG-PLA-PLys and PGlu-PLA-PGlu copolymers by conjugating biomolecules like biotin [95], folic acid [96], and RGD peptides [97]. Chen et al. synthesized PEG-poly(L-glutamic acid) (PEG-PGlu) diblock copolymer by ROP of BLG-NCA with PEG macro-initiators followed by removal of benzyl groups [98]. The pendant carboxyl groups of PEG-PGlu was conjugated with cinnamyl alcohol using DCC/DMAP to afford copolymer mPEG-poly(L-glutamic acid-*co*-γ-cinnamyl-L-glutamate) (mPEG-b-P(Glu/CGlu)) (Fig. 11), which could be employed to fabricate core-crosslinked micelles via photodimerization of the cinnamyloxy pendent groups under UV-irradiation at $\lambda = 254$ nm.

Poly(L-homoarginine)$_{60}$-b-poly(L-leucine)$_{20}$ ($PHArg_{60}$-$PLeu_{20}$) block copolypeptides were prepared by quantitative guanidization of the lysine residues in poly(L-lysine)-b-poly(L-leucine) ($PLys_{60}$-$PLeu_{20}$) with 3,5-dimethyl-1-pyrazolyl-formaminidium nitrate [56]. Notably, vesicles self-assembled from $PHArg_{60}$-$PLeu_{20}$ were shown able to cross the cell membrane and transport encapsulated cargos into different cell lines. In another study, fluorescein isothiocyanate (FITC) was conjugated to $PLys_{60}$-$PLeu_{20}$ for facile imaging of polypeptide vesicles [99]. Thiol

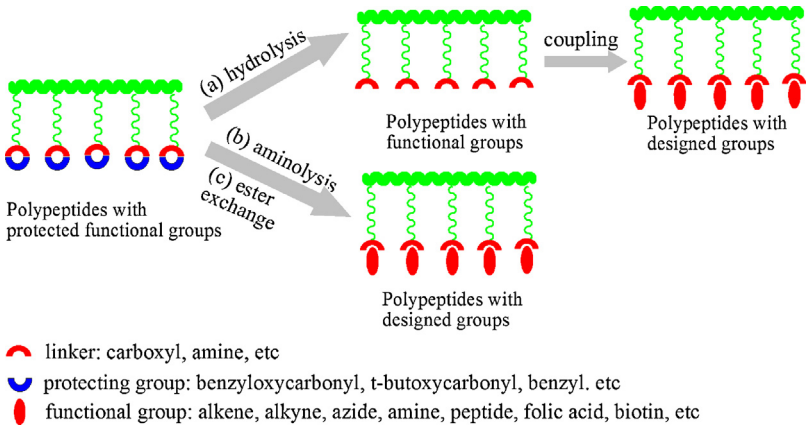

Fig. 10. Side chain-functionalized polypeptides via post-polymerization modification.

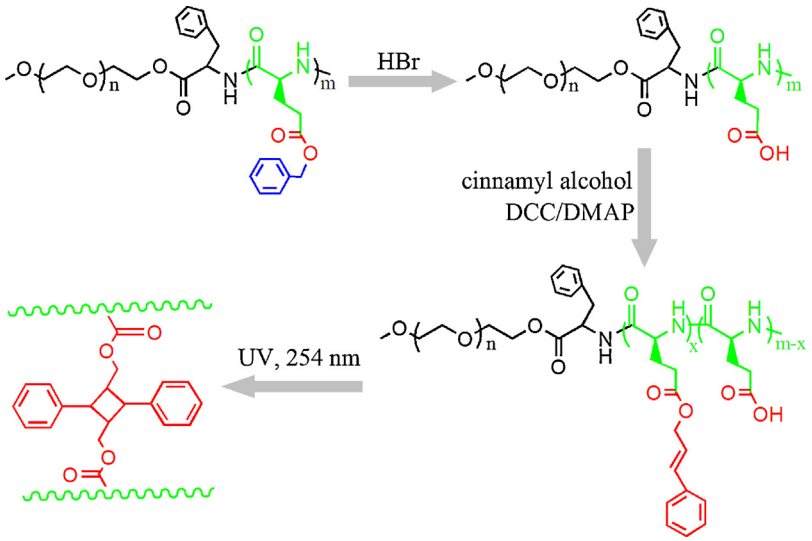

Fig. 11. Synthesis and cycloaddition reaction of polypeptide copolymer mPEG-b-P(Glu/CGlu) containing photopolymerizable cinnamyl groups. [98], Copyright 2011. Reproduced with permission from the Royal Society of Chemistry.

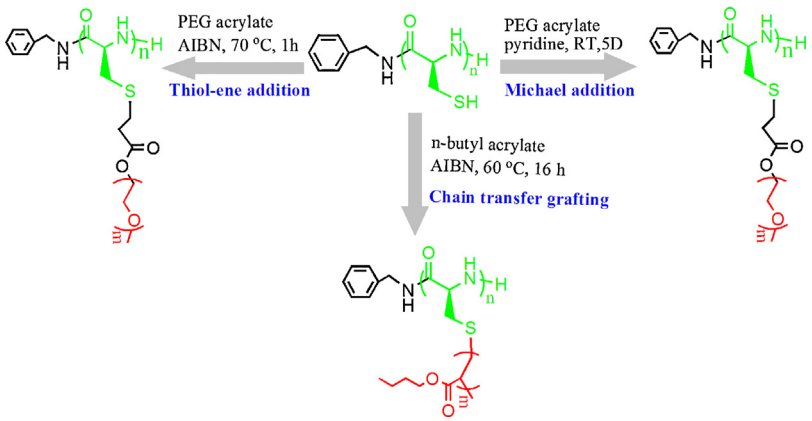

Fig. 12. Synthesis of side chain functional polypeptides by thiol chemistry. [53], Copyright 2009. Reproduced with permission from the Royal Society of Chemistry.

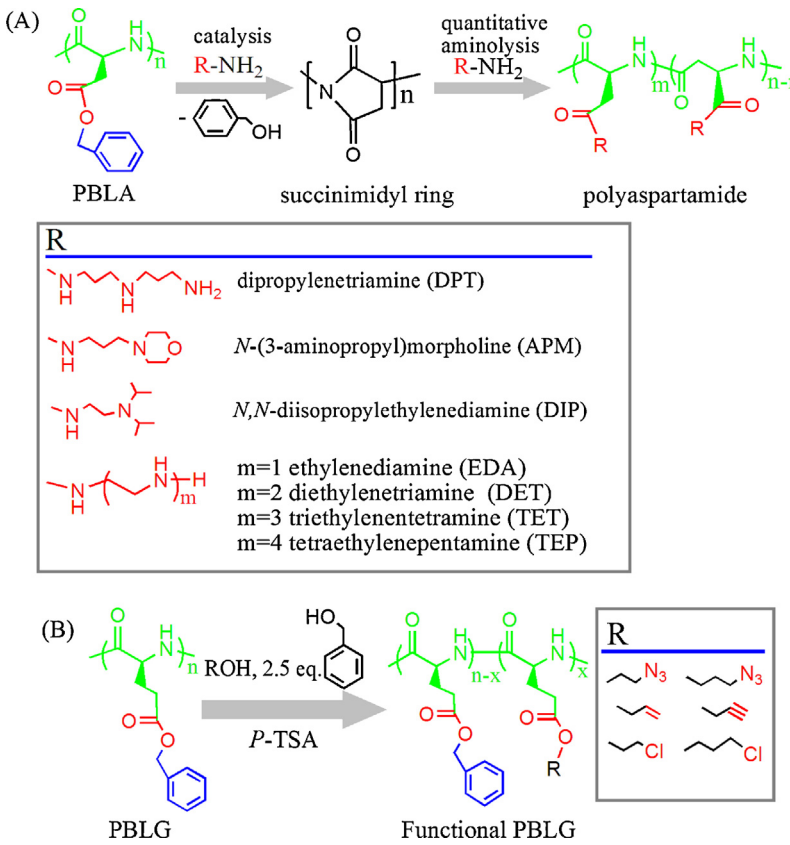

Fig. 13. (A) Synthesis of cationic polyaspartamide copolymers by aminolysis of flanking benzyl ester groups of PBLA [100]; (B) synthesis of functional PBLG through ester exchange reactions.
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chemistry including thio-ene addition, Michael addition, radical reaction and cross-linking reaction was successfully utilized by Heise et al. to prepare well-defined polypeptide copolymers based on poly(L-cysteine) (PCys) (Fig. 12) [53]. While cross-linking reactions and Michael addition to the thiol group are possible, the most promising reactions are thiol-ene addition and radical grafting by chain transfer.

A very attractive post-polymerization modification approach is aminolysis that avoids harsh deprotection process. For example, Kataoka et al. reported facile and quantitative aminolysis of PBLA via first formation of a succinimide intermediate and then conversion to polyaspartamide accompanying the α,β isomerization of the main chain (Fig. 13A) [100]. The stereoregularity of polypeptides was dependant on the polarity of solvents, in which an optical purity of 95% was obtained by carrying out the aminolysis in CH_2Cl_2. Notably, quantitative introduction of various amines like *N,N′*-diisopropylethylenediamine (DIP), 1,2-diaminoethane, 1,5-diaminopentane, *N*-(3-aminopropyl)morpholine (APM) into PBLA could afford pH and temperature dual-sensitive polymers [100,101], cationic polymer for nucleic acid delivery [102], and semipermeable polymesomes [103].

Ester exchange method has also been employed for post-polymerization modification of polypeptides without deprotection process. Huang et al. synthesized functional PBLG copolymers containing chloro, azido, allyl or propargyl groups on the side chains through ester exchange reactions of PBLG with functional alcohols (Fig. 13B) [104]. The degree of substitution varied from 12.1 to 55.8% depending on ratio of alcohol to PBLG, reaction temperature and time. Very recently, Deming et al. reported versatile synthesis of multi-functional and multi-reactive poly(L-methionine) via chemoelective alkylation of thioether groups in methionine residues (Fig. 14) [105]. Poly(L-methionine) was readily prepared with controlled and high molecular weights using $(PMe_3)_4Co$. The alkylation of methionine was reported to exhibit features of a "click" reaction.

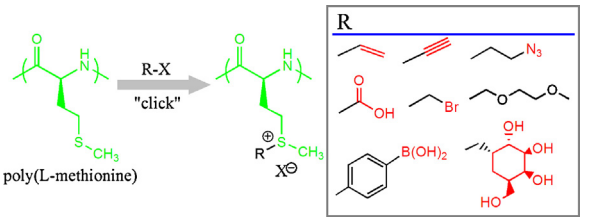

Fig. 14. Preparation of multi-functional and multi-reactive polypeptides via methionine alkylation.
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3. Chain end-functionalized polypeptides

3.1. α-Chain end functionalized polypeptides prepared from designed initiators

α-Chain end functionalized polypeptides can be prepared from designed initiators that combining ROP of NCA with controlled radical polymerization (CRP), click chemistry, or functional molecules (Fig. 15), avoiding tedious synthesis and incomplete transformation of α-chain end in polypeptides. Moreover, the integration of NCA ROP and CRP or click chemistry could straightforwardly generate a variety of block/graft/star polypeptide copolymers.

3.1.1. Dual initiators for NCA polymerization and controlled radical polymerization (CRP)

CRP including atom transfer radical polymerization (ATRP), reversible addition-fragmentation chain-transfer (RAFT), and nitroxide-mediated polymerization (NMP) enables facile synthesis of polymers with well-defined architecture, controlled molecular weight, and low PDI. Dual hetero-functional initiators that are able to bring about ROP of NCA as well as CRP have been employed to synthesize α-chain end functionalized polypeptides and further to prepare various block/star polypeptide hybrids. This hetero-functional initiator strategy has several advantages: (1) avoid tedious synthesis and incomplete transformation of polymer chain ends for CRP, (2) combing various polymerization techniques (ROP/ATRP, ROP/RAFT, ROP/NMP), and (3) possibly conduct dual polymerization in one pot without intermediate purification steps.

Menzel et al. synthesized PBLG-b-PMMA rod-coil block copolymers using a dual initiator containing a Ni amido amidate complex for polymerization of BLG-NCA and a bromide group for ATRP polymerization of methyl methacrylate (Fig. 16A) [106]. Well-defined α-chain end functionalized polypeptides were obtained from BLG-NCA, *O*-benzyl-serine NCA, and ZLys-NCA using 2-bromo-*N*-(2-aminethyl)-2-methylpropionamide (BPA) as an initiator (Fig. 16B) [107]. These bromide-ended polypeptides could be employed for ATRP polymerization of methacrylate to produce plypeptide-polymethacrylate hybrid copolymers. Zhang et al. prepared thermo- and pH-responsive PNIPAM-b-PGlu double hydrophilic block copolymers

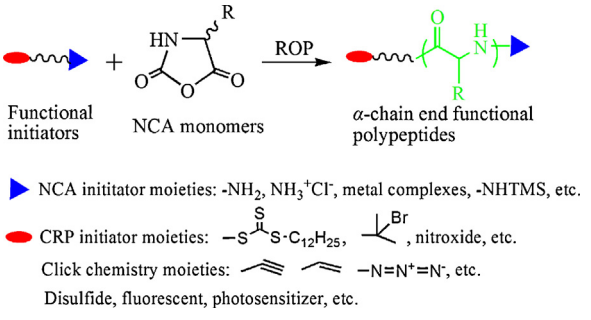

Fig. 15. α-Chain end functionalized polypeptides based on designed initiators.

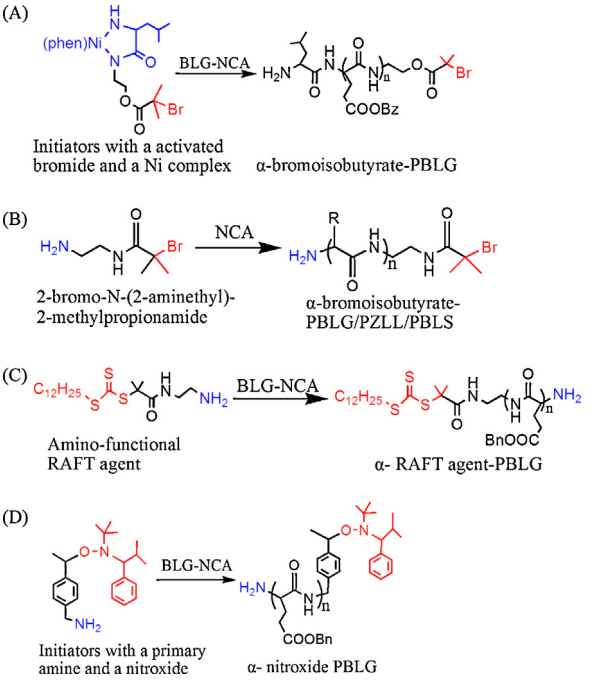

Fig. 16. Dual initiators capable of promoting NCA polymerization and controlled radical polymerization. (A, B) Dual initiators for NCA polymerization and ATRP polymerization; (C) dual initiators for NCA polymerization and RAFT polymerization; and (D) dual initiators for NCA polymerization and NMP polymerization.

(DHBCs) by sequential polymerization of BLG-NCA and NIPAM using amino-functionalized RAFT agent as dual initiator (Fig. 16C) [108]. These PNIPAM-PGlu copolymers formed aggregates with varying morphologies from tree, interconnected round-shape, to fiber depending on solution pH and temperature. PBLG-b-PS block copolymer was obtained with high structural control and low PDI by combining ROP of BLG-NCA and NMP polymerization (a metal free CRP) of styrene using a dual initiator (Fig. 16D) [109]. Interestingly, NMP and NCA polymerization are so compatible that polymerization can be conducted in one pot without intermediate workup. The active nitroxide end-groups in PBLG-b-PS block copolymers could further be crosslinked with divinylbenzene to yield well-defined cross-linked nanoparticles, which following removal of benzyl in PBLG furnished water-dispersible pH-responsive nanoparticles with a PGlu shell and a PS core [110].

3.1.2. Functional initiators containing an azido, alkyne, or alkene group to combine NCA polymerization with click chemistry

α-Azido-PBLG and α-alkyne-PBLG with expected M_w and low PDI could be readily obtained by ROP of BLG-NCA using α-azido-3-aminopropane and α-alkyne propargylamine difunctional initiators, respectively (Fig. 17A and B) [111]. The copper(I)-catalyzed 1,3-dipolar cycloaddition coupling of α-alkyne or azido-PBLG with α-azido or alkyne-poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA) afforded well-defined PBLG-b-PDMAEMA diblock copolymers, which following deprotection gave PGlu-b-PDMAEM

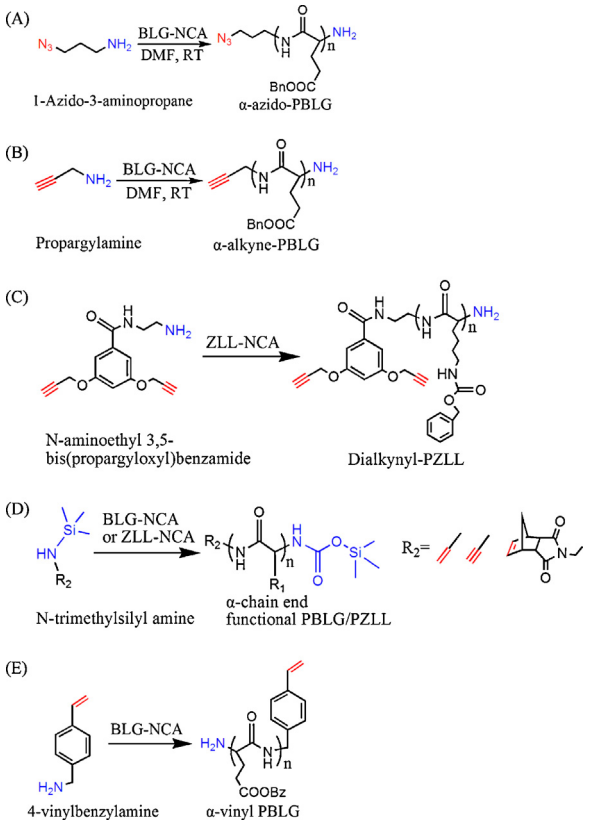

Fig. 17. Synthesis of α-chain end functionalized polypeptides from initiators containing azido groups (A), alkyne groups (B, C, D), or alkene groups (D, E).

DHBCs [112]. The click reaction between α-azido-PBLG and alkyne-terminated dextran or hyaluronan afforded glycoprotein analogs, dextran-b-PBLG and hyaluronan-b-PBLG [113,114]. These glycopolypeptides could be self-assembled into vesicles with a controlled size, excellent colloidal stability, and a high drug loading capacity. The polysaccharide segment serves dual purposes, i.e. as a hydrophilic block critical to vesicle formation and a targeting ligand for cancer therapy [114].

α-Azide or α-alkyne functionalized poly(trifluoroacetyl-L-lysine) (PTFALys) was obtained by ROP of TFALys-NCA using corresponding amino-containing functional initiators, from which PBLG-b-PTFALys copolypeptides were prepared via copper(I)-catalyzed click chemistry with α-alkyne or azido-PBLG [115]. *N*-aminoethyl 3,5-bis(propargyloxyl) benzamide was employed as functional initiator for the ROP of ZLys-NCA to yield dialkynyl-terminated poly(*N*-benzyloxycarbonyl-L-lysine) (dialkynyl-PZLys, Fig. 17C), which following copper(I)-catalyzed cycloaddition click reaction with α-azido-PBLG yield Y-shaped miktoarm star polypeptide copolymer, PZLys-b-$(PBLG)_2$ [116]. Upon removing protecting groups, water-soluble double hydrophilic miktoarm star polypeptide copolymer, PLys-b-$(PGlu)_2$, was obtained. Depending on solution pH, PLys-b-$(PGlu)_2$ copolymers could form PGlu-cored or PLys-cored micelles. Savin et al. developed polypeptide-based A_2B lipid mimetics from α-alkyne-PBLG/PZLys and dodecanethiol (DDT) via thiol-yne chemistry. The copolymer after deprotection containing a peptide block as the head group along with two long alkyl chain DTT 'legs' could be used to produce complex, lipid-mimetic polymersomes with a relatively narrow size distribution [117]. This strategy was extended to conjugate PGlu with natural lipophilic moieties in cell membranes such as cholesterol, which self-assembled into pH-responsive vesicles in aqueous media [118].

Cheng et al. obtained α-allyl polypeptides with controlled molecular weight and narrow PDI through polymerization of BLG-NCA and ZLys-NCA using *N*-trimethylsilyl allylamine as an initiator (Fig. 17D) [37]. Interestingly, TMS-containing functional initiators like propargylamine and *N*-(aminoethylene)-5-norbornene-endo-2,3-dicarboximide were also shown to bring about a controlled BLG-NCA polymerization, providing various α-chain end functionalized polypeptides that allow facile post-polymerization via click chemistry or ring-opening metathesis polymerization. α-Vinyl functionalized PBLG was synthesized by polymerization of BLG-NCA using 4-vinyl benzylamine as an initiator (Fig. 17E) [119]. The vinyl-ended PBLG was crosslinked with divinyl benzene via RAFT polymerization into high molecular weight star polypeptides, which after deprotection could form water soluble pH-responsive nanoparticles.

3.1.3. *Functional initiators containing disulfide, fluorophore or photosensitizer*

Klok et al. reported synthesis of two series of four-arm, star-shaped polypeptides with arm lengths ranging from 10 to 200 α-amino acid residues through the ROP of BLG-NCA and ZLys-NCA using a tetra-amino-substituted perylene fluorophore as an initiator [120]. The removal of protecting groups in polypeptides resulted in water-soluble, perylene-functionalized, star-shaped polypeptides (Fig. 18A) that showed strong fluorescence in aqueous solution. The conformation of the arms in the water-soluble star polypeptides could be reversibly switched from a random coil into an α-helix by adjusting the solution pH. Star-shaped polypeptides have been prepared based on hyperbranched polyethylenimine (PEI) or dendritic poly(amido amine) (PAMAM) as core molecules and investigated for gene delivery, drug release and biomineralization applications [121–126].

5-(4-Aminophenyl)-10,15,20-triphenyl-porphyrin (APP), a photosensitizer for photodynamic therapy, was used as an initiator for the ROP of ZLys-NCA and Leu-NCA, which following deprotection afforded a series of amphiphilc APP-P(Leu-Lys) diblock copolypeptides (Fig. 18B) [127]. These APP-functionalized copolypeptides possessed high fluorescence quantum yield and could self-assemble into micelles with mean particle sizes less than 30 nm in an aqueous medium. These micelles while causing little cytotoxicity in vitro in the dark exhibited high phototoxicity against HepG2 and HeLa cells. Gupta et al. prepared disulfide-functionalized PBLG (Fig. 18C) with low PDIs (<1.25) through polymerization of BLG-NCA in anhydrous chloroform using *N*-lipoyl-1,3-diaminopropane

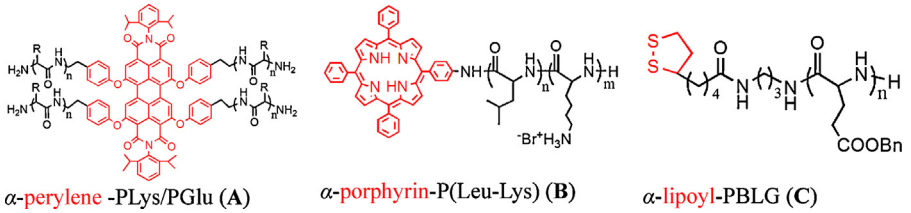

Fig. 18. α-End functional polypeptides based on initiators containing fluorophore, photosensitizer or disulfide.

as an initiator [128,129]. Interestingly, these disulfide-functionalized polypeptides could form self-assembled monolayers on gold substrates at a time scale of minutes.

3.2. ω-Chain end functionalized polypeptides prepared via post-polymerization modification

Polypeptides prepared via NCA polymerization usually possess an amino group at the end, which could be used for polymerization of other NCA monomers to afford block and star copolypeptides. For example, ω-amino polypeptides obtained by ROP of ZLys-NCA or TFA-Lys NCA were conjugated with N^{α},N^{ε}-di(9-fluorenylmethoxycarbonyl)-L-lysine (N^{α},N^{ε}-diFmoc Lys), which following deprotection of the N^{α},N^{ε}-diFmoc groups afforded two primary amine groups. The repetition of the ROP and deprotection resulted in highly branched PLys [130]. Hadjichristidis et al. reported synthesis of well-defined linear and star copolypeptides by treating amine-terminated polypeptides (PBLG or PZLys) or copolypeptides (PBLG-b-PZLys) with diisocyanate or triisocyanates [131].

Functional polypeptides have also been prepared by conjugating functional molecules to ω-amino polypeptides. For instance, Fluorescein isothiocyanate (FITC) was conjugated to the ω-amino end of mPEG-SS-PLeu in the presence of triethylamine to yield FITC-labeled polypeptide hybrid copolymer [132]. These FITC-labeled copolymers self-assembled into micelles with a fluorescent core, facilitating observation of micelle internalization and trafficking in the cells by confocal laser scanning microscopy (CLSM). Deming et al. reported quantitative functionalization of ω-chain ends of polypeptides from NCA polymerizations using electrophiles, such as isocyanates, isothiocyanates, and acid chlorides [133]. The electrophiles can either be small molecules or readily prepared isocyanate-terminated polymers resulting in highly complex PEG-b-PBLG-b-PEG-b-PBLG-b-PEG pentablock copolymers (Fig. 19). Cornelissen et al. observed that ω-chain ends of polypeptides prepared using Deming's catalyst [Ni(bpy)(cod)] were allowed to initiate the isocyanide polymerization to afford PBLG-b-polysiocyanide copolymers, which could be self-assembled into ordered layers of hollow capsules upon drying [134].

4. Biomedical applications of functional polypeptide and hybrid materials

4.1. Polypeptide-saccharide hybrid copolymers as biomimetic analogs of natural glycopolypeptides

Glycopolypeptides are ubiquitous in nature and display a wide range of biological functions, such as mediation of recognition events, protection from proteases, and lubrication in eyes and joints [83]. In the past decade, various glycopolypeptides have been developed to probe carbohydrate-protein interactions and use as biomimetic materials for controlled drug release and tissue engineering. Feng et al. synthesized PLys-polytetrahydrofuran-PLys (PLTL) triblock copolymers by ROP of ZLys-NCA with amine-terminated polytetrahydrofuran as a macroinitiator followed by deprotection and glycopolypeptides by conjugating D-gluconolactone and D-lactobionolactone to PLTL blocks [135]. These glycopolypeptides formed nano-sized aggregates that were able to mediate controlled release of DOX. It should be noted that the saccharides (e.g. galactose) at the surface of nanoaggregates hold the potential for targeting to specific cells.

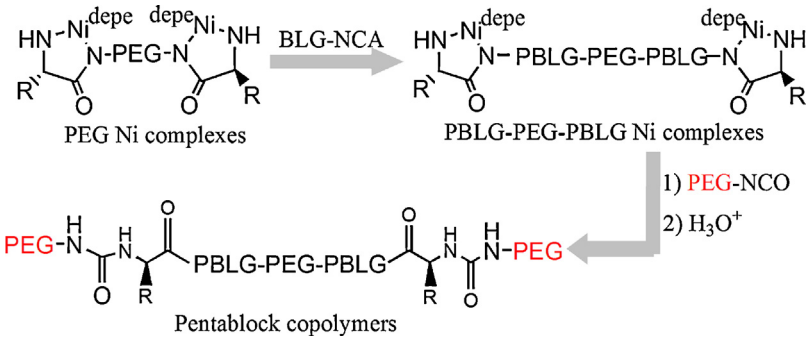

Fig. 19. Synthesis of PEG-b-PBLG-b-PEG-b-PBLG-b-PEG pentablock copolymers using NCA polymerization and isocyanate end-capping. [133], Copyright 2002. Reproduced with permission from the American Chemical Society.

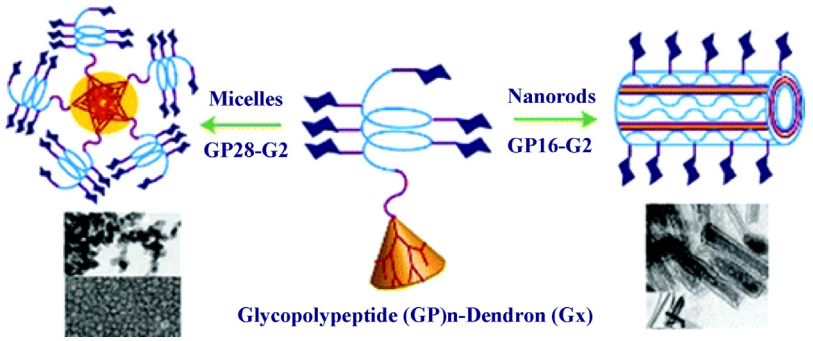

Fig. 20. Schematic illustration of multiple topologies (nanorods and micelles) based on glycopolypeptide-dentron conjugate. [142], Copyright 2012. Reproduced with permission from the American Chemical Society.

Chen et al. reported facile preparation of glycopolypeptides by combining ROP of PPLG-NCA and click "glycosylation" with diverse sugars including D-mannopyranoside, D-glucopyranoside, and D-galactopyranoside [73]. Thus obtained glycopolypeptides displayed almost 100% α-helix structure. The multivalent interactions of glycopolypeptides containing mannose epitope with lectin concavalin A decreased with decreasing mannose eptope densities. Zhang et al. reported that water-soluble mannose-polypeptide conjugates prepared from click reaction between propargyl-containing mannose and poly(γ-azidopropylglutamate) (PAPLG) exhibited α-helical conformations both in aqueous solution and in solid state [75]. Heise et al. reported synthesis of glycopeptides by Huisgen 1,3-dipolar cycloaddition of azide-galactose to alkyne-functionalized poly(DL-propargylglycine) (PPG), poly(γ-benzyl-L-glutamate-co-DL-propargylglycine) (P(BLG-co-PG)), or PBLG-b-poly(DL-propargylglycine) (PBLG-b-PPG) polypeptides [81,136]. Depending on relative block length and nanoprecipitation conditions, PBLG-b-PPG glycopeptide block copolymers self-assembled into spherical and wormlike micelles as well as polymersomes displaying bioactive galactose units at the surface as proven by selective lectin binding experiments. Schlaad and Sun prepared glucopolypeptides by conjugating 1-thiol-β-D-glucopyranose to poly(DL-allylglycine) via thiol-ene chemistry in the absence of transition metal catalyst [82]. Interestingly, glucosylated polypeptides were shown to adopt a random-coil conformation in neutral and basic media while an α-helical conformation under an acidic condition [137]. These glucosylated polypeptides displayed selective binding to the plant lectin concanavalin A.

The polymerization of glycosylated NCA monomers is a straightforward synthetic strategy toward glycopolypeptides. Synthesis of *O*-linked glycol-serine NCAs was reported in 1966 though synthesis was inefficient and toxic Hg salts were required for the glycosylation [138,139]. The polymerization of *O*-linked glycol-serine NCAs gave oligomeric glycopolypeptides due to steric and H-bonding interactions between the sugar and the NCA rings. Cameron improved the synthesis of glycoserine/threonine NCA using iodine instead of Hg salts as the promoter, and also developed *S*-linked glycol-cysteine NCA that showed higher stability toward glycosidases [140]. However, no polymerization was reported. Deming et al. reported synthesis of well-defined and high molecular weight glycopolypeptides with 100% glycosylation by living polymerization of *C*-linked glycosylated-L-lysine NCA monomers (**19**) using transition metal [83]. This methodology overcomed the problems in the direct synthesis of glycopolypeptides from NCAs relating to monomer synthesis, purification, and polymerization and generated polypeptides with 100% glycosylation. Recently, Gupta et al. reported improved synthesis of *O*-glycosylated lysine NCA (**20**, **21**) using a stable glycosyl donor and a commercially available protected amino acid [85]. These glycosylated NCAs were polymerized using commercially available simple amine or azide-PEG-amine as an initiator to yield well-defined high molecular weight glycopeptides or PEG-glycopeptide block copolymers in high yields. The poly(α-mannose-*O*-lysine) glycopolypeptide exhibited high binding affinity for lectin concavalin A [141]. Interestingly, amphiphilic glycopolypeptide-dendron conjugate was prepared by "click" reaction between a hydrophobic dendron and glycopolypeptides, which formed gel in dimethylsulfoxide while nanorods or micellar aggregates in aqueous solutions depending on polypeptide chain lengths and dendron generations (Fig. 20) [142].

Lecommandoux et al. synthesized diblock polysaccharide-b-polypeptide glycopolypeptide copolymers like dextran-b-PBLG and hyaluronan-b-PBLG by Huisgen 1,3-dipolar cycloaddition reaction (Fig. 21A) [113,114,143]. Hyaluronan-b-PBLG was able to self-assemble into small vesicles with a high affinity for the surface glycoproteins of living cells (Fig. 21B). Hyaluronan is a specific ligand for CD44 receptor and can be used to target cancer cells overexpressing CD44 glycoproteins. Flow cytometry results demonstrated much higher uptake of DOX-loaded Hyaluronan-b-PBLG polymersomes by CD44-overexpressing MCF-7 cells than U87 cells (negative control). The in vivo studies showed that DOX-loaded polymersomes exhibited more effective growth inhibition of breast tumor in rats as compared to free DOX (Fig. 21C). Jing et al. reported that PLys-grafted chitosan copolymers exhibited much higher gene transfer ability than chitosan and PLys [144,145]. These graft glycopolypeptide copolymers have combined pDNA-binding ability of PLys with good biocompatibility of chitosan. The transfection

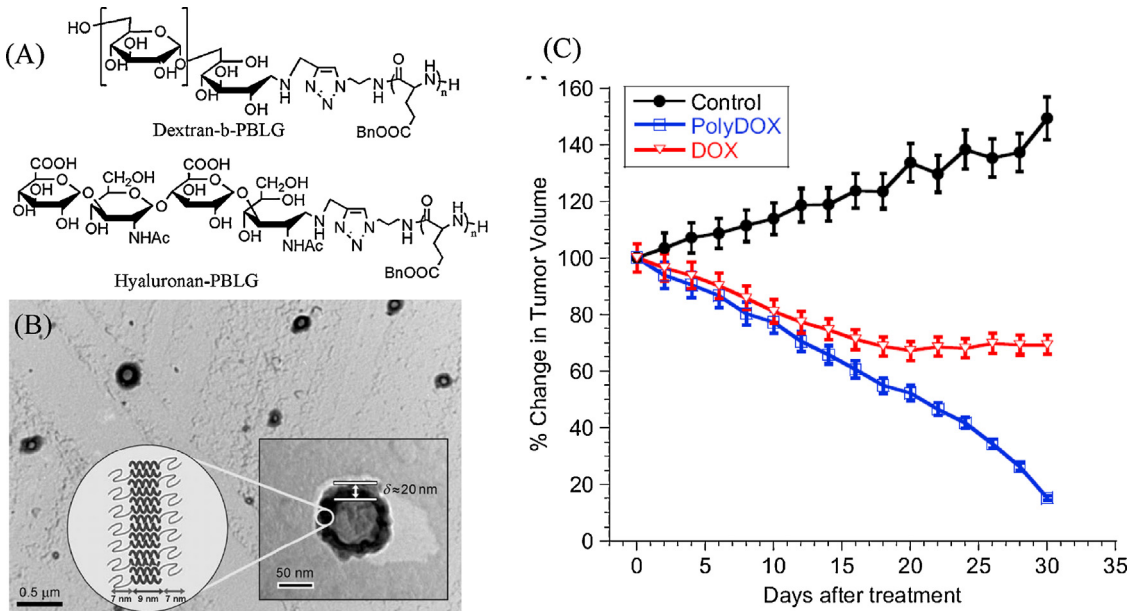

Fig. 21. Polysaccharide-b-polypeptide polymersomes for anticancer drug release. (A) Structures of dextran-b-PBLG and hyaluronan-PBLG block copolymers, (B) TEM image of a dried dispersion of the dextran-b-PBLG copolymer (the inset shows evidence of a vesicle structure with a membrane thickness of approximately 20 nm), (C) tumor progression after single administration of free DOX and DOX-loaded hyaluronan-b-PBLG polymersomes. [113,143], Copyright 2009 and 2010, respectively. Reproduced with permission from Wiley-VCH Verlag GmbH & Co. KGaA and Elsevier Ltd, respectively.

efficiency of these copolymers displayed a positive correlation with PLys chain lengths.

Another synthetic strategy toward glycopolyeptides is NCA polymerization using amine-terminated glycopolymer as a macroinitiator. For example, Dong et al. synthesized (poly(L-glutamate)-poly(2-acryloyloxyethyl-lactoside)-poly(L-glutamate)) (PGlu-PAEL-PGlu) triblock copolymers by ATRP polymerization of a protected lactose-based glycomonomer and ROP of BLG-NCA [146,147]. These triblock glycopolypeptides self-assembled into lactose-installed aggregates that displayed specific interactions with RCA_{120} lectins.

In summary, glycopolypeptides can be designed by facile combination of monosaccharide (glucose, galactose, mannose), oligosaccharide (lactose), or polysaccharide (dextran, hyaluronan, chitosan) with synthetic polypeptides and synthesized by direct polymerization of glycosylated NCA monomers, coupling between polypeptide and glycopolymer, post-polymerization modification of polypeptides with saccharide, or NCA polymerization with glycopolymer as a macroinitiator. These synthetic glycopolypeptides have displayed specific carbohydrate-protein interactions. Moreover, nanoparticles of glycopolypeptides with specific saccharide molecules on the surface can be used for targeted tumor therapy.

4.2. Functional polypeptide-based micelles for drug, protein and gene delivery

4.2.1. PEG-polypeptide hybrid copolymer micelles for drug delivery

PEG-polypeptide copolymer micelles are one of the most promising nanosystems for controlled drug release. Notably, several PEG-polypeptide micellar anticancer drugs developed by Kataoka et al. have advanced to different phases of clinical trials. For example, DOX-loaded PEG-b-PAsp block copolymer micelles (NK911), paclitaxel (PTX)-loaded PEG-b-polyaspartate modified with 4-phenyl-butanol (PEG-b-PAPB) block copolymer micelles (NK105), SN-38 (the active form of irinotecan hydrochloride)-encapsulated PEG-b-PGlu block copolymer micelles (NK012), and cisplatin-incorporated PEG-b-PGlu block copolymer micelles (NC6004) have entered phase II clinical evaluations, while oxaliplatin-incorporated PEG-b-PGlu block copolymer micelles (NC4016) have entered phase I clinical studies [40,148]. These PEG-polypeptide micelles have been reported to decrease drug toxicity, improve drug tolerance, realize subcellular drug targeting, and/or improve drug potency.

Taking advantage of complexation between (1,2-diaminocyclohexane) platinum(II) (DACHPt) and carboxylic groups of PEG-b-PGlu, Kataoka et al. prepared DACHPt-loaded PEG-b-PGlu micelles (Fig. 22A) [149]. CLSM studies revealed that DACHPt/PEG-PGlu micelles were extravasated and deeply penetrated into the tumor tissue (Fig. 22B), internalized inside tumor cells, and dissociated within late endosomes enhancing drug delivery to the nucleus. These DACHPt-loaded micelles exhibited higher antitumor activity than oxaliplatin alone and were shown also effective against oxaliplatin-resistant tumors (Fig. 22C and D). Moreover, DACHPt/PEG-b-PGlu micelles could also efficiently penetrate and accumulate in an orthotopic scirrhous gastric cancer model, leading to effective inhibition of tumor growth and lymph node metastasis [150].

Bae et al. developed PEG-b-PHis diblock copolymers by coupling PEG to poly(N^{im}-DNP-L-histidine) via an amide linkage using the DCC/NHS chemistry, followed by removal of protecting DNP groups [48]. Ultra pH-sensitive

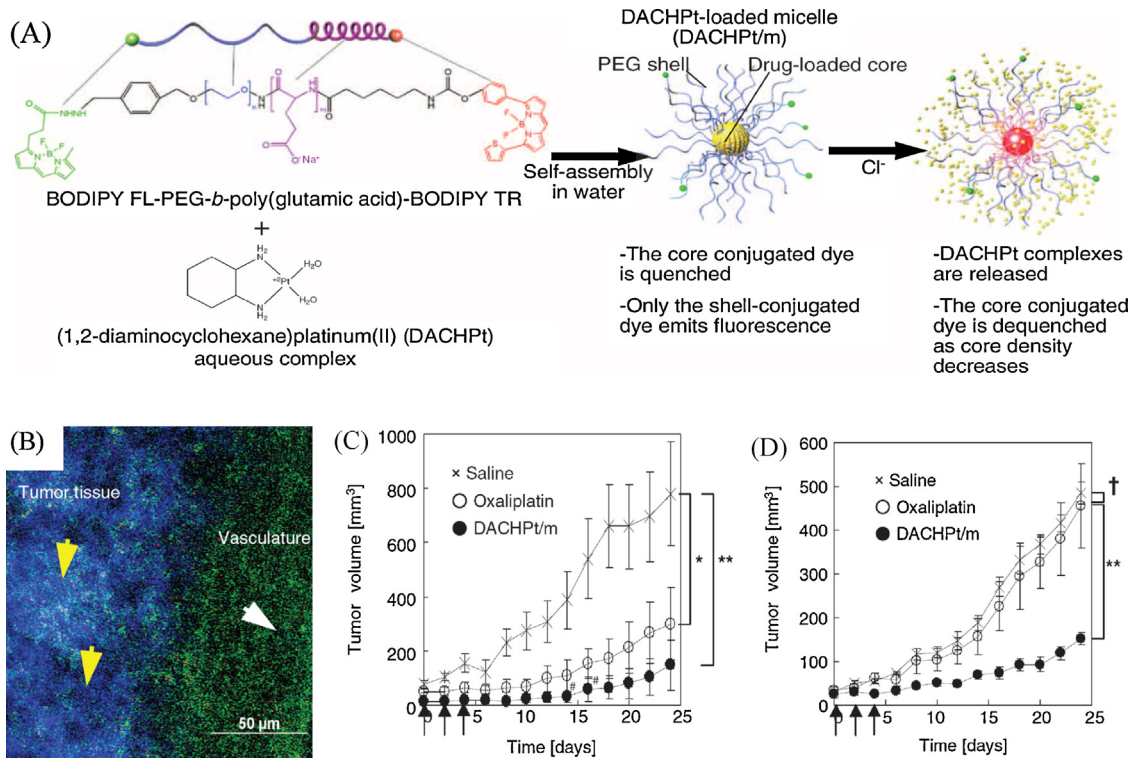

Fig. 22. DACHPt/PEG-PGlu micelles for improving drug potency and efficacy via subcellular targeting. (A) Design of fluorescent-labeled DACHPt/PEG-PGlu micelles for visualization of the localization and drug release in the cell (in the micelle state, only BODIPY (green) emits fluorescence, whereas BODIPY TR (red) remains quenched; as DACHPt is released from DACHPt/PEG-PGlu micelles in chloride ion-containing media, BODIPY TR is dequenched and emits fluorescence); (B) in vivo CLSM observation of DACHPt/PEG-PGlu micelles in blood vessels and tumors at 12 h after intravenous administration (yellow arrows, tumor tissue; white arrow, blood vessel); in vivo effect of DACHPt/PEG-PGlu micelles on subcutaneous HT29 (C) and oxaliplatin-resistant HT29 (HT29/ox) (D) tumor cells (oxaliplatin: 8 mg/kg, DACHPt/PEG-PGlu micelles: 4 mg/kg; $^{*}P < 0.05$, $^{**}P < 0.01$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
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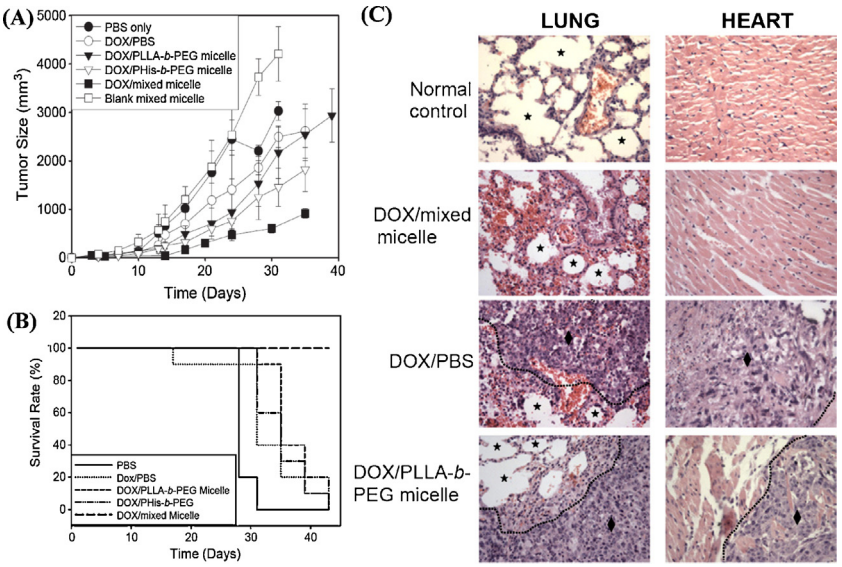

Fig. 23. Folate conjugated pH-sensitive mixed micelles formed from PLLA-b-PEG-folate and PHis-b-PEG copolymers for the prevention of metastasis in a 4T1 murine breast cancer model. (A) Tumor size, (B) survival curves, and (C) histopathologic examination of lung and heart of 4T1 tumor bearing mice following treatment with DOX in PBS, DOX loaded in PLLA-b-PEG micelle, DOX loaded in PHis-b-PEG, and PHis-b-PEG/PLLA-b-PEG-folate micelles (♦: tumor metastasis).
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micelles were prepared by mixing PEG-PHis and PEG-PLA block copolymers. The results showed that 25 wt% PEG-PLA containing mixed micelles were fairly stable at pH 7.0–7.4 but underwent a two-stage destabilization upon decreasing pH, i.e. significant increase in micelle size when pH dropped to 6.8 and disruption of micelles as pH decreased to 6.0 [151,152]. Notably, corresponding folate-decorated DOX-loaded micelles exhibited high cytotoxicity to drug resistant MCF-7 cells in vitro as well as reversal of resistant MCF-7 tumor in mice [152,153]. Recently, folate-decorated DOX-loaded pH-sensitive micelles based on PLLA-b-PEG-folate and PHis-PEG have been investigated for prevention of tumor formation and metastasis [154]. The in vivo studies using a murine mammary carcinoma cell of 4T1 which is one of the most aggressive metastatic cancer cell lines, showed that mixed micelles resulted in retarded tumor growth, no weight loss, no death for 4–5 weeks and no apparent metastasis till 28 days, in comparison with significant metastasis to the lung and heart on day 28 in the mice treated with DOX carried by PBS, PLLA-b-PEG micelles and PHis-b-PEG micelles (Fig. 23). In a similar way, pH-sensitive micelles were also developed from a mixture of poly(L-histidine-co-L-phenylalanine)-b-PEG and PLLA-b-PEG-folate for effective suppression of multidrug resistance (MDR) ovarian tumors in mice for at least 50 days by three i.v. injections at a 3-day interval at a dose of 10 mg of DOX/kg [155,156]. Kataoka et al. conjugated DOX into PEG-PAsp via an acid-labile hydrazone bond that released DOX under endo/lysosomal pH conditions [40,157]. The results showed that 75–85% substitution of hydrazide (Hyd) with DOX gave optimal drug release pattern with clinically relevant stability, while 100% substitution with DOX often inhibited drug release due to a high hydrophobicity. In order to increase the micelle stability during circulation while maintain endosomal pH-triggered release of drugs, pH-sensitive reversibly crosslinked micelles were developed through reaction of carboxyl acid groups of PAsp in PEG-PAsp-poly(L-phenylalanine) (PEG-PAsp-PPhe) micelles with ketal-containing cross-linkers [158]. These ketal-crosslinked micelles showed faster DOX release at endosomal pH than at physiological pH.

Chen et al. prepared pH and thermo-responsive poly(L-glutamic acid-co-(L-glutamate-g-oligo(2-(2-(2-methoxyethoxy)ethoxy)ethyl methacrylate))) (P(Glu-co-(Glu-g-OMEO$_3$MA))) graft copolymers by combining NCA polymerization and ATRP polymerization of MEO$_3$MA [86]. The resulting copolymers could spontaneously self-assemble into micelles in aqueous solutions at pH 7.0 with R_h values of ca. 20.9–176.0 nm, depending on copolymer compositions and temperature.

In addition to pH-sensitive micelles, redox-responsive micelles have recently received great interests for active intracellular drug release due to a high concentration of glutathione (GSH) tripeptides (about 2–10 mM) in the cytosol and cell nucleus, which is 100 to 1000 times higher than that in the extracellular fluids and circulation (about 2–20 μM) [159,160]. For example, reduction-sensitive micelles were prepared by conjugating camptothecin (CPT) to PEG-b-PGlu via a disulfide bond [161]. These micelles showed slow drug release under physiological conditions whereas enhanced drug release in the presence of 3 mM dithiothreitol (DTT). Li et al. reported that reduction-sensitive shell-sheddable micelles based on PEG-SS-PZLys and PEG-SS-PLeu copolypeptides showed accelerated DOX release in the presence of 10 mM DTT and GSH-dependent inhibition of MCF-7 cells [132,162]. Park et al. reported that camptothecin (CPT)-loaded PEG-SS-PBLG micelles released CPT completely within 20 h under 10 mM GSH, resulting in higher cytotoxicity to SCCF7 cancer cells than CPT-loaded reduction-insensitive counterparts [163]. Shuai et al. prepared mPEG-b-PAsp(MEA)-b-PAsp(DIP) triblock copolymer (PAsp(MEA): 2-mercaptoethylamine-grafted PAsp, PAsp(DIP): 2-(diisopropylamino) ethylamine-grafted PAsp, Fig. 24A) by ROP of BLA-NCA followed by click and aminolysis reactions [164]. These polypeptide copolymers self-assembled into highly packed interlayer-crosslinked micelles with reduction and pH dual-sensitivity for intracellular DOX release. Drug leakage could be avoided during micelle storage and significantly reduced during circulation, whereas release of drug was triggered in an acidic and reductant-enriched environment such as in lysosomes. The in vivo studies revealed the DOX-loaded micelles accumulated preferentially in tumors through the enhanced permeability and retention (EPR) effect and exhibited obvious therapeutic effect on decreasing tumor size (Fig. 24C and D). Recently, Zhang et al. reported redox-sensitive shell-crosslinked (PEG-PCys-PPhe) hybrid micelles for controlled DOX release [165]. The in vitro release studies showed that crosslinked micelles could reduce drug loss in the extracellular environment and rapidly release drugs in response to the intracellular GSH level. Deng and Zhong et al. reported that reduction and pH dual-bioresponsive reversibly core-crosslinked polypeptide micelles based on lipoic acid and cis-1,2-cyclohexanedicarboxylic acid decorated PEG-PLL (PEG-P(LL-CCA/LA)) block copolymer could actively load and deliver DOX into the nuclei of cancer cells [166].

Poly(propylene oxide)-b-PLys (PPO-b-PLys) block copolymers were prepared using Huigen's 1,3-dipolar cycloaddition [167]. PPO-b-PLys exhibited a helix-coil transition at around pH 8 and formed micelles or vesicles depending on PLys weight fractions. PPO$_{44}$-PLys$_{62}$ polymersomes exhibited a loading efficiency of 35% and sustained release of DOX·HCl. Lin et al. prepared PGlu-PPO-PGlu triblock copolymers by ROP of BLG-NCA using NH_2-PPO-NH_2 as a macroinitiator followed by deprotection of benzyl groups [168]. These pH and thermo-sensitive copolymers self-assembled into vesicles or spherical micelles depending on PGlu block lengths and solution pH. The size of aggregates decreased with increasing temperature due to dehydration of PPO. Lecommandoux et al. reported synthesis of Jeffamine-PGlu double hydrophilic block copolymers by ROP of BLG-NCA using amino-terminated Jeffamine as a macroinitiator followed by hydrolysis [169]. These copolymers could self-assemble into spherical micelles at a temperature above the LCST of Jeffamine and size of micellar core decreased with increasing temperature with complete core dehydration at 66 °C.

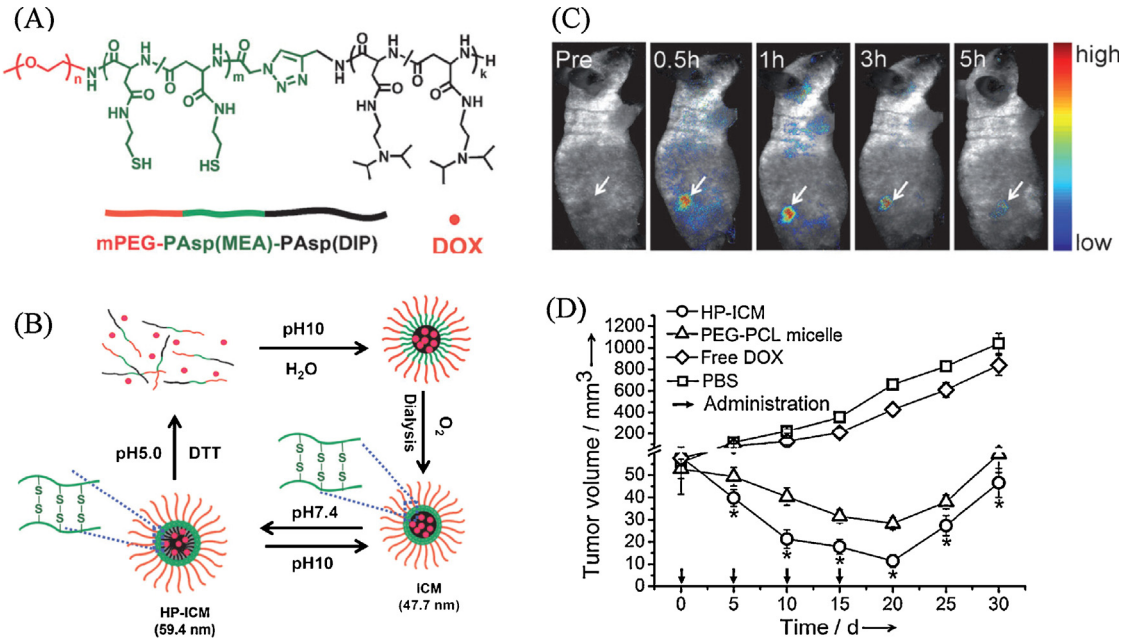

Fig. 24. Interlayer-crosslinked micelles with reduction and pH dual sensitivity for pinpointed intracellular drug release. (A) mPEG-PAsp(MEA)-PAsp(DIP) structure, (B) formation and structural transitions of the dual-sensitive micelles, (C) in vivo DOX fluorescence images showing passive tumor accumulation of DOX-loaded micelles after tail-vein injection into nude mice bearing the Bel-7402 xenograft, (D) tumor growth inhibition in nude mice bearing the Bel-7402 tumor after tail-vein injection of different formulations.
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4.2.2. Polyester-polypeptide hybrid copolymer micelles for drug delivery

Ouchi et al. synthesized poly(L-lactide)-b-PAsp (PLLA-b-PAsp) diblock copolymer through ROP of BLA-NCA using PLLA-NH_2 as a macroinitiator followed by deprotection [170]. PLLA-b-PAsp diblock copolymer formed PLLA-cored micelles with pH-sensitive PAsp shells in aqueous solution and particle sizes depending on relative block lengths of PAsp and PLLA as well as solution pH. The resulting micelles were non-toxic to L929 fibroblast cells [171]. In another study, Poly(lactide-co-glycotide)-b-PGlu (PLGA-b-PGlu) diblock copolymer was prepared by carbodiimide coupling reaction between NH_2-terminated PBLG and COOH-terminated PLGA followed by removal of protecting benzyl groups [172]. The morphologies of PLGA-b-PGlu block copolymer aggregates varied from disordered forms to micelles, and further to vesicles as pH increased from 3 to 9. Jing et al. prepared PEG-PLLA-PGlu triblock copolymers via ROP of BLG-NCA in the presence of PEG-b-PLLA-NH_2 followed by removal of the benzyl protection groups [173]. These copolymers self-assembled into spherical micelles and connected rods in aqueous media depending on PGlu lengths and solution pH [174]. Similarly, PEG-PLLA-PLys triblock copolymer was synthesized and self-assembled into micelles in aqueous solution [175]. The micelles formed following introduction of folic acid into PLys block could deliver hydrophobically modified FITC (mFITC) into folate receptor over-expressing HeLa cells [96]. The ex vivo fluorescence measurements showed that these folate micelles preferably accumulated at the tumor bed indicating good targeting ability.

Bae et al. developed multifunctional micelles based on PLLA-b-PEG-b-PHis-biotin and PHis-b-PEG [49]. The shorter PHis block in PLLA-b-PEG-b-PHis-biotin was located at the interface of hydrophobic core and PEG shell. These micelles were stable at and above pH 7.2. However, at tumor pH (pH 7.0), biotin was exposed on the micelle surface facilitating biotin receptor-mediated endocytosis. When pH was further lowered to below 6.5, micelles were destabilized resulting in disruption of endosomal membrane and enhanced cytosolic drug release. In a similar way, TAT (a non-specific cell penetrating peptide)-decorated micelles were prepared based on PHis-b-PEG and PLLA-b-PEG-b-PHis-TAT [176]. The in vivo studies using drug-resistant A2780/AD human ovarian tumor, drug-sensitive human MCF-7 breast tumor, human A549 lung tumor and human KB epidermoid tumor in nude mice showed significant tumor regression and minimum weight loss by three bolus injection. Jing et al. prepared reversibly shell-crosslinked micelles based on PCys-b-PLLA diblock copolymer (Fig. 25) [177]. These micelles displayed facile loading of rifampicin and drug release was significantly accelerated by DTT [178].

4.2.3. Polyacrylate-polypeptide hybrid copolymer micelles for drug delivery

Various stimuli-responsive polypeptide micelles have been constructed based on PNIPAM that presents a LCST of ca. 32 °C. For example, Liu et al. prepared $PNIPAM_{65}$-b-$PGlu_{110}$ diblock copolymer via ROP of BLG-NCA using amino-terminated PNIPAM as a macroinitiator followed by deprotection of benzyl groups [179]. $PNIPAM_{65}$-b-$PGlu_{110}$ copolymer was self-assembled into PGlu-cored micelles

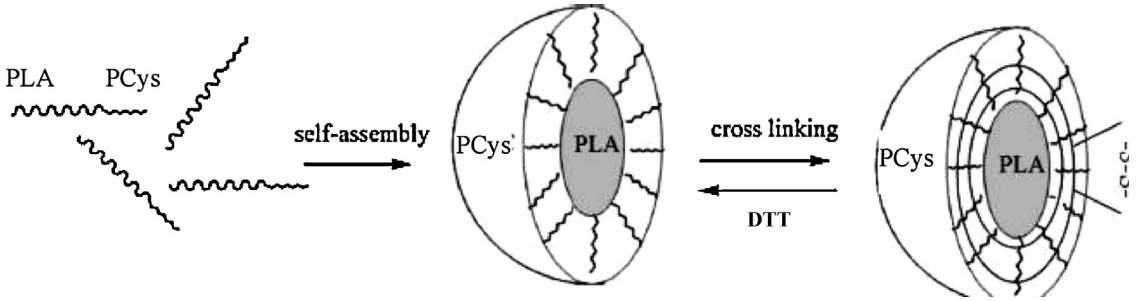

Fig. 25. Schematic representation of the formation of reversible shell cross-linked micelles from PCys-b-PLLA amphiphilic diblock copolymer for drug delivery.
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at acidic pH and room temperature while PNIPAM-cored micelles at basic pH and elevated temperature. Chen et al. reported that PNIPAM-b-P(Glu-co-BLG) diblock copolymers with 30 mol.% BLG content in P(Glu-co-BLG) block exhibited hydrophilic-hydrophobic transition in response to a narrow pH change from physiological pH to endosomal pH [180]. Temperature and pH-responsive PGlu-g-PNIPAM graft copolymers were prepared through grafting amino-terminated PNIPAM to NHS-activated PGlu [181]. These graft copolymers formed core-shell aggregates at pH 4.5–7.4, in which particle sizes decreased with decreasing pH. Chen and Chang demonstrated that PNIPAM-b-PLys diblock copolymer could self-assemble into PLys-cored micelles at basic pH and low temperatures while PNIPAM-cored micelles at acidic pH and high temperature [182,183]. Lecommandoux et al. reported well-defined PDMAEMA-b-PGlu DHBCs could self-assemble into spherical polymeric micelles and polymersomes in aqueous solution by selective triggering a variation of either the pH or the temperature (Fig. 26) [112]. Notably, the pH-sensitivity related to PGlu and the thermosensitivity related to PDMAEMA that possess a LCST of around 40 °C, could be independently varied, which facilitated the variation in size and shape of nanoparticles.

4.2.4. *Copolypeptide micelles for drug and gene delivery*

Deming et al. reported the synthesis of nonionic block copolypeptide, poly(N^{ε}-2-[2-(2-methoxyethoxy) ethoxyl] acetyl-L-lysine)-b-poly(rac-leucine) (P(EG_2-Lys)-b-P(rac-Leu)), which possessed an uncommon rodlike hydrophilic segment and disordered hydrophobic segment, and were able to form highly stable, nanoscale polypeptide micelles [65]. The resulting micelles could efficiently encapsulate cargos such as CPT. Very recently, Cheng et al. developed water soluble cationic α-helical polypeptide via ROP of VB-Glu NCA followed by subsequent ozonolysis and reductive amination [184,185]. The resulting cationic polypeptides, especially 2-(1-piperidinyl)ethanamine decorated PVBLG, mediated effective gene transfection in a variety of cell lines, including immortalized cancer cells and human

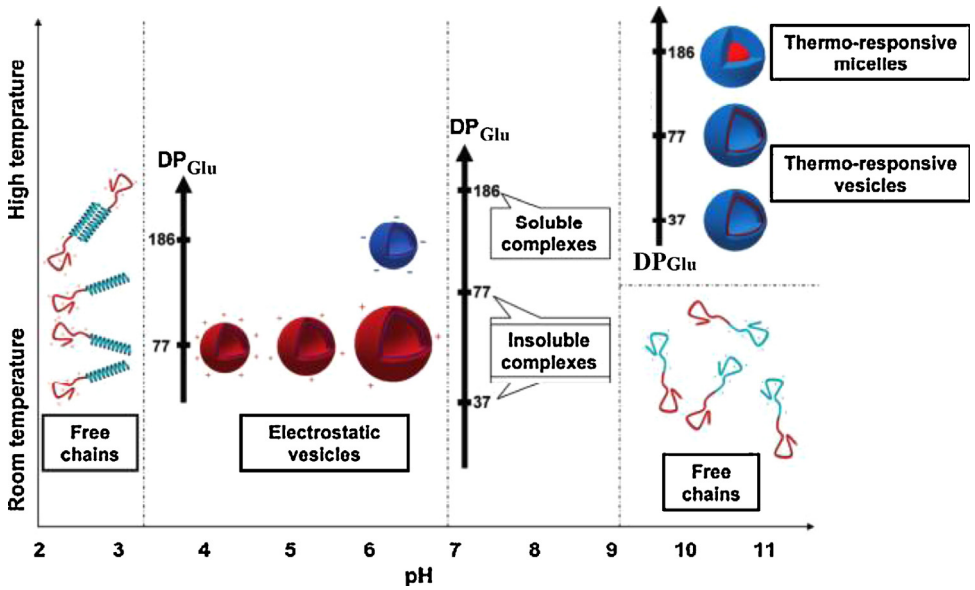

Fig. 26. Schematic representation of the different morphologies obtained from multiresponsive PDMAEMA-b-PGlu block copolymers.
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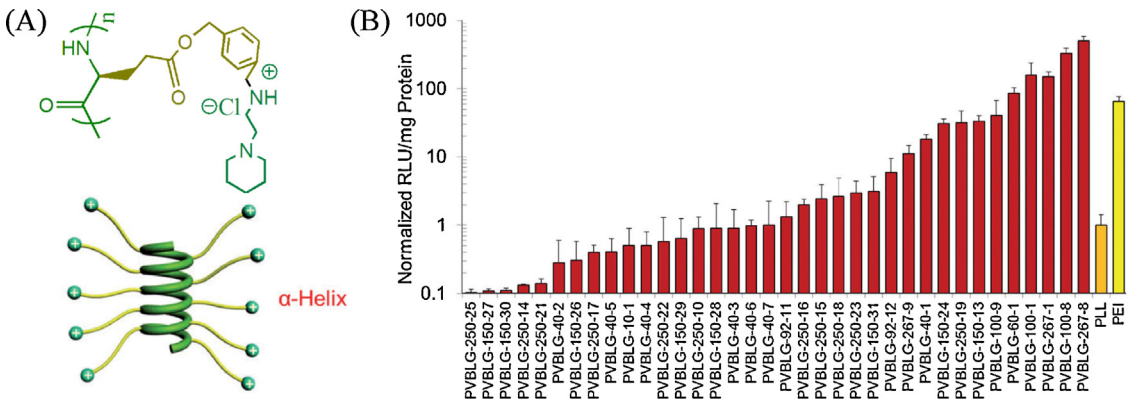

Fig. 27. Reactive and bioactive cationic α-helical PVBLG polypeptide for gene delivery. (A) Structure of 2-(1-piperidinyl)ethanamine decorated PVBLG (PVBLG-100-8 and PVBLG-267-8 in (B)), (B) in vitro transfection of COS-7 cell with cationic PVBLG polypeptides, 22 kDa PLys and 25 kDa PEI were included as controls.
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embryonic stem cells (hESCs), likely due to their α-helicity and membrane destabilization ability (Fig. 27) [185].

4.2.5. PEG-polypeptide hybrid copolymer micelles for protein and gene delivery

PEG-poly(amino acid) polyionic complex (PIC) micelles pioneered by Kataoka's group are highly promising for intracellular protein and nucleic acid release [13,40,186,187]. For example, Kataoka et al. prepared a series of polyaspartamide like PAsp(DET), PAsp(TEP), PAsp(DPT) (Fig. 13A) via the aminolysis of PBLA that facilitated endosomal escape of micelles as well as efficient protein and nucleic acid release inside target cells [40]. Notably, PEG-PAsp(DET) PIC micelles have successfully delivered plasmid DNA in several animal models including a rabbit's clamped carotid artery [188], a mouse skull [189], and a rat lung [190]. PAsp(DPT) containing one more methylene unit between the two amines in the side chain exerted a similar endosome escape but presented severe cytotoxicity, probably due to effective damage of both cytoplasmic and endosomal membranes [102]. Further studies showed that polyaspartamides possessing even-numbered repeating aminoethylene units in the side chains (i.e. PAsp(DET) and PAsp(TEP)) achieved an order of magnitude higher transfection efficiency without marked cytotoxicity than those of polyaspartamides with an odd number of repeating aminoethylene units (i.e. PAsp(EDA) and PAsp(TET), Fig. 13A) [191].

pH-responsive PEG shell-sheddable micelles were developed based on PAsp(Hyd-PEG)-b-PAsp(DET) polypeptide for enhanced gene transfection [192]. The results showed that while majority of the PEG chains could be maintained on the polymer backbone for a long period of time at neutral pH, PEG was rapidly shedded off at acidic pH. These shell-sheddable micelles exhibited 1000 times higher transfection efficiency than those irreversibly PEGylated counterparts. Kataoka et al. prepared a charge-conversion polyanion, PEG-poly(*N*-(citraconyl-2-aminoethyl) aspartamide) (PEG-PAsp(EDA-Cit)), by reacting PEG-PAsp(EDA) with citraconic anhydride [193]. PEG-PAsp(EDA-Cit) formed stable PIC micelles with positively charged lysozyme at pH 7.4 that were dissociated to release proteins at pH 5.5 within 2 h. Ternary polyplexes were prepared from DNA, PAsp(DET) and charge-conversion polypeptide poly(*N*-(*N*′-((*N*″-cis-aconityl)-2-amnoethyl)-2-aminoethyl) aspartmide) (PAsp(DET-Aco)) that expressed negative charges at neutral pH while turned into positive charges at endosomal pH to effectively disrupt endosomes, achieving appreciably high transfection activity with low toxicity [194].

PEG-poly((3-morpholinopropyl) aspartamide)-PLys (PEG-PMPA-PLys) triblock copolymer was developed by the successive ROP of BLA-NCA and ZLys-NCA using mPEG-NH_2 as an initiator, followed by aminolysis of the benzyl ester of PBLA with APM and deprotection of Z groups of PZLys [195]. PEG-PMPA-PLys formed micelles with a three-layered structure, in which PLys with a high pK_a of ca. 9.4 was used to condense DNA, PMPA with a low pK_a of ca. 6.2 was served to disrupt endosomes via the proton sponge effect, and PEG was used to improve the biocompatibility of DNA complexes. The results showed that polyplexes of PEG-PMPA-PLys had low cytotoxicity but comparable transfection efficiency to that of PEI control. Cai et al. reported that cationic PEG-PLys-PLeu polypeptide micelles with a diameter of 40–90 nm achieved two to three times higher transfection efficiency than that of 25 kDa PEI due to their capability of promoting DNA condensation and cell internalization as well as avoiding acidic lysosomes [196]. In another study, PEG-b-PLys-b-PPhe micelles were developed for delivery of both anti-cancer drugs and genes. PEG-b-PLys-b-PPhe copolymers effectively condensed pDNA to afford comparable transfection efficiency in 293T cells to 25 kDa PEI but with lower cytotoxicity [197].

Various reduction-sensitive polypeptide micelles have been designed to achieve active intracellular protein and gene release. For example, PAsp(-SS-siRNA) obtained through exchange reaction between siRNA-SH and 2-pyridinyldithio-functionalized PAsp formed stable PIC micelles with PAsp(DET) that demonstrated competent internalization by cultured cells and efficient siRNA release under intracellular reductive conditions [198]. PEG

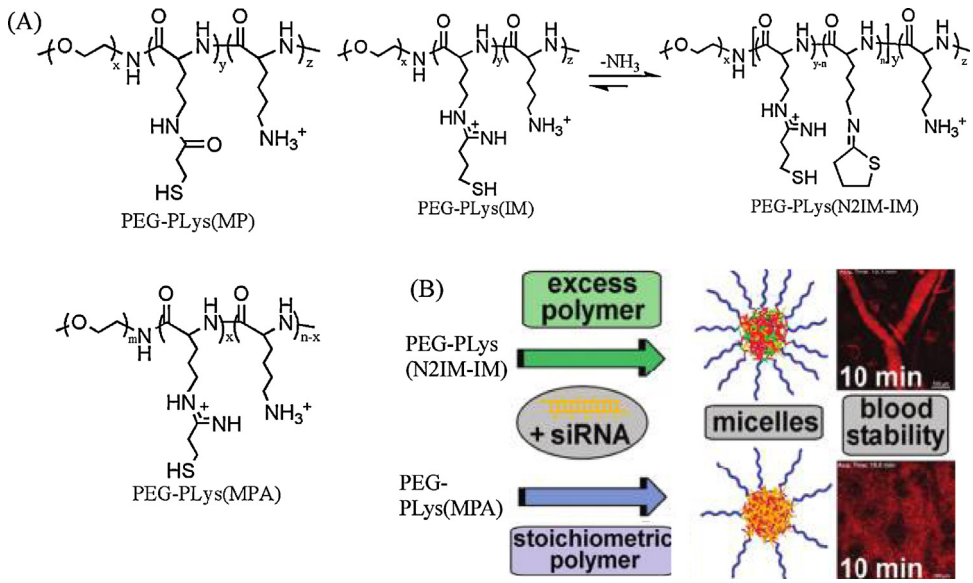

Fig. 28. Block catiomer polyplexes with regulated densities of charge and disulfide cross-linking prepared from thiolated PEG-PLys copolypeptides for gene and siRNA delivery. (A) The structures of thiolated PEG-PLys copolymers including PEG-PLys(MP), PEG-PLys(IM), and PEG-PLys(MPA); (B) the stability of micelles from Cy5-labeled siRNA with PEG-PLys(N2IM-IM) or PEG-PLys(MPA) in the bloodstream observed in mouse ear lobe dermis following *i.v.* injection. [201], Copyright 2011. Reproduced with permission from the American Chemical Society.

detachable polyplex micelles based on PEG-SS-PAsp(DET) block catiomer showed 1–3 orders of magnitude higher gene transfection efficiency than their counterparts without disulfide linkages [199].

Kataoka et al. prepared a series of reversibly core-crosslinked micelles based on thiolated PEG-b-PLys block copolymers to increase micelle stability during circulation without compromising intracellular drug release [200,201]. The reaction of PEG-b-PLys with *N*-succinimidyl 3-(2-pyridyldithio)-propionate (SPDP), 2-iminothiolane (Traut's reagent), and dimethyl 3,3′-dithiobispropionimidate (DTBP) yielded PEG-PLys(MP), PEG-PLys(IM), and PEG-PLys(MPA), respectively (Fig. 28A) [200,201]. The results showed that while polyplexes of PEG-PLys(MP) and PEG-PLys(IM) exhibited similar efficacy in introducing disulfide cross-links to prevent polyplex dissociation in the extracellular conditions, only PEG-PLys(MP) polyplexes achieved effective intracellular pDNA release resulting in approximately 50 times higher transfection efficiency in 293T cells than those of PEG-PLys(IM) likely due to balanced charge density [200]. PEG-PLys(MP) following conjugating with cyclic RGD peptide exhibited further enhanced transfection activity in HeLa cells expressing $\alpha_v\beta_3$ integrins [202]. PEG-PLys(IM) and PEG-PLys(MPA) were investigated for siRNA delivery, in which PEG-PLys(IM)/siRNA showed better stability in buffer and blood stream while PEG-PLys(MPA)/siRNA micelles exhibited higher gene silencing likely due to efficient siRNA release under a reductive condition (Fig. 28B) [201]. In another study, disulfide-crosslinked polyplexes based on cationic PLys containing thiol groups, were coated with a pH-sensitive membrane-active polyanion, PEG-PAsp(DET-aconitic acid) [203]. These polyplexes exhibited low cytotoxicity and enhanced gene transfection efficiency due to improved endosomal escape as observed using CLSM. Murthy et al. developed disulfide-crosslinked PIC micelles based on PEG-poly(L-lysine-dithiopyridine) (PEG-P(Lys-DTP)) copolymer for protein delivery [204]. Notably, proteins were encapsulated under mild conditions without the use of organic solvents. These micelles though stable under extracellular conditions were able to release proteins under intracellular reductive conditions. Recently, Kataoka et al. obtained stable PIC micelles from 3-fluoro-4-carboxyphenylboronic acid (F-PBA) modified PEG-PLys (PEG-P(Lys/F-PBA)) and 1,2 or 1,3-*cis*-diols on a ribose ring of siRNA that are sensitive to ribose concentration for intracellular triphosphate (ATP)-triggered release of siRNA [205].

4.3. Functional polypeptide-based polymersomes for drug, protein and gene delivery

Polymersomes provide a highly promising platform for mimicking biological membranes as well as for controlled drug release. Compared to liposomes prepared from small phospholipids, polymersomes formed from macromolecular amphiphiles have usually thicker membranes, superb colloidal stability, enhanced mechanical strength, and reduced chemical permeability [206,207]. In particular, membranes of polymersomes could be facilely engineered with stimuli (e.g. pH, temperature and/or redox)-sensitivity to modulate their drug release behaviors.

4.3.1. Copolypeptide polymersomes for protein and gene delivery

Jing et al. reported that PLys-b-PPhe block copolymers could directly self-assemble into giant vesicles with a diameter of 1–3 μm in aqueous solution [208]. The carbonylated hemoglobin (CO-Hb) encapsulated in PLys-b-PPhe

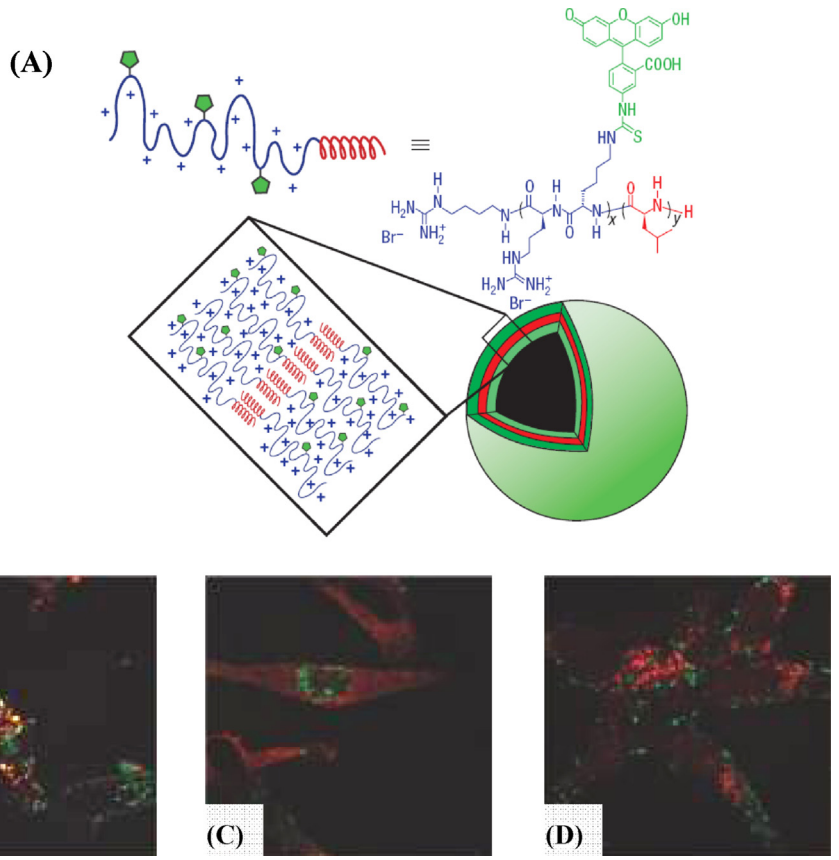

Fig. 29. Structure (A) and intracellular fates (B-D) of charged $PArg_{60}$-$PLeu_{20}$ polypeptide vesicles (green). (B) Endosome antigen-1 (EEA-1, red); (C) lysosomal-associated membrane protein-1 (LAMP-1, red); and (D) EEA-1 (red) with chloroquine treatment. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
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vesicles could be converted into oxygen-binding hemoglobin (O_2-Hb) following 2 h irradiation of visible light [209]. Similarly, Lee et al. prepared PBLG-b-PPhe copolymers via sequential polymerization of BLG-NCA and Phe-NCA using *n*-butylamine·HCl as an initiator [210]. PGlu-b-PPhe block copolypeptides obtained from deprotection of PBLG-b-PPhe could self-assemble into vesicular nanostructures with a diameter of 150–300 nm that displayed pH-sensitivity due to changes in the PGlu ionization state.

Deming et al. reported that uncharged diblock copolypeptide amphiphiles based on P(EG_2-Lys)-PLeu could self-assemble into large vesicles (2–15 μm to 50 μm) and flat membrane sheets depending on lengths of hydrophilic P(EG_2-Lys) block [57]. These vesicles with ethylene glycol coatings were inert toward different ionic media, variations in pH, and presence of large macromolecules such as proteins in serum. The α-helical structure of both hydrophilic and hydrophobic segments yielded very stiff membranes that were impermeable to water, ions or other small molecules. Polypeptide vesicles with membrane fluidity and controlled sizes of tens to hundreds of nanometers were obtained from charged block copolypeptides, $PLys_{60}$-$PLeu_{20}$ and the $PGlu_{60}$-b-$PLeu_{20}$, by extrusion through polycarbonate membranes [211]. These charged polypeptide vesicles were amenable to facile functionalization of vesicle surfaces through chemical conjugation to amine or carboxylic acid. $PArg_{60}$-$PLeu_{20}$ block copolypeptides (Fig. 29A) were shown able to cross the cell membrane and transport encapsulated dextran into epithelial (T84) and endothelial (HULEC-5A) cells [212]. $PArg_{60}$-$PLeu_{20}$ vesicles were taken up by cells mainly via macropinocytosis. Immunostaining experiments demonstrated that most vesicles were trapped within the early endosomes and addition of chloroquine helped release of vesicles into the cytosols (Fig. 29B–D) [99]. Low stability of peptide vesicles has limited their use for encapsulation and controlled release of therapeutic agents. Deming et al. reported that crosslinked vesicles based on L-3,4-dihydroxyphenylalanine (DOPA)-containing amphiphilic copolypeptide $PLys_{60}$-poly($DOPA_x$/Leu_y)$_{20}$ had dramatically improved membrane stability against freeze-drying, organic solvent, osmotic stress and complex media [63].

Hadjichristidis et al. reported that amphiphilic PLys-b-PBLG-b-PLys triblock copolypeptides containing 19–75% PBLG block formed vesicles with a diameter of ca. 130 nm at neutral pH [213]. Notably, these vesicles were pH and temperature-responsive as the secondary structure of PLys

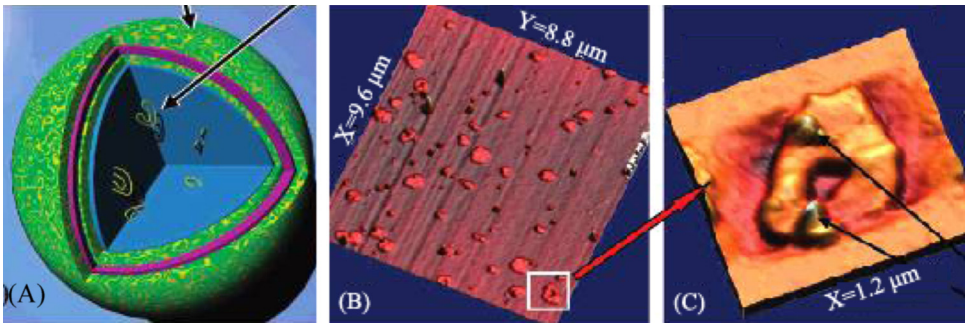

Fig. 30. PLys-b-PBLG-b-PLys vesicles for gene encapsulation. (A) Schematic representation of pDNA encapsulation, (B) typical 3D height picture of AFM of disrupted copolypeptide vesicles loaded with pDNA, (C) magnification of the 3D height picture of a rupture vesicle, (black arrow: encapsulated DNA). [213], Copyright 2007. Reproduced with permission from the American Chemical Society.

changed from a random coil at pH 7.4 to an α-helix at pH 11.5 and 25 °C, and to a β-sheet at pH 11.5 and 37 °C [214]. The encapsulation of DNA showed that DNA was partially encapsulated on the PLys shell and partially inside the vesicles (Fig. 30) [213]. Recently, He et al. reported virosome-like nanoparticles mimicking viral capsids through cooperative hierarchical self-assembly of globular PLys polypeptide dendrimers and carboxyl-functionalized linear PLeu polypeptides via electrostatic interactions and/or hydrogen bonds [215]. These peptidesomes exhibited comparable gene-transfection activity to 25 kDa PEI in the absent of serum. Lecommandoux et al. prepared a short zwitterionic diblock copolypeptide, $PLys_{15}$-b-$PGlu_{15}$ (Fig. 31A) by sequential ROP of trifluoroacetyl-L-Lysine NCA and BLG-NCA using *n*-hexylamine as initiator followed by removal of the protective groups [216]. This copolypeptide while molecularly dispersed in aqueous solution at pH 5–9 was self-assembled into small schizophrenic vesicles at pH < 4 or pH > 10 (Fig. 31B).

4.3.2. Polypeptide hybrid copolymer polymersomes for drug and protein delivery

Kataoka et al. prepared polymer vesicles with a diameter up to 10 μm named PICsomes by simple mixing of a pair of oppositely charged block copolymers, i.e. PEG_{45}-$P(Asp)_{100}$ and PEG_{45}-poly([5-aminopentyl]-α,β-aspartamide)$_{100}$ (PEG_{45}-$P(Asp\text{-}AP)_{100}$) or PEG_{45}-poly([2-aminoethyl]-α,β-aspartamide)$_{100}$ (PEG_{45}-$P(Asp\text{-}AE)_{100}$) in an aqueous medium [103]. These PICsomes had a unique three-layered structure with semipermeability and were stable in proteinous medium. Myoglobin (Mb), which forms stable oxygen adducts in muscle, was readily encapsulated as a compartmentalized protein in these PICsomes [217]. Loaded Mb was smoothly reduced to deoxy Mb by $S_2O_4^{2-}$ that had permeated through the PIC membrane, and reversible oxygenation of the Mb in the PICsome occurred even in the presence of trypsin in the outer medium (Fig. 32). A pair of oppositely charged PEG block aniomer PEG-P(Asp) and homocatiomer Homo-P(Asp-AP) spontaneously formed nano-PICsomes in the range of 100–400 nm in an aqueous medium [218]. Moreover, cross-linking of amino and carboxylic acid groups in the vesicle membrane with EDC allows enhancement of stability and facile tuning of permeability under physiological conditions.

Recently, Kataoka et al. reported polypeptide polymersomes with uniform size (~100 nm) self-assembled through the complexation reaction of DACHPt with a Y-shaped block copolymer of ω-cholesteroyl-PGlu and two-armed PEG (PEGasus-PGlu-Chole) (Fig. 33) [219]. The in vivo studies of antitumor activity in C-26-bearing mice showed that considerable reduction in the tumor growth rate without showing significantly body weight loss was achieved for mice treated with 6 mg/kg of DACHPt-loaded polymersomes, while no inhibition of the tumor growth was observed for free oxaliplatin under otherwise the same conditions. Moreover, DACHPt-loaded polymersomes were also able to encapsulate

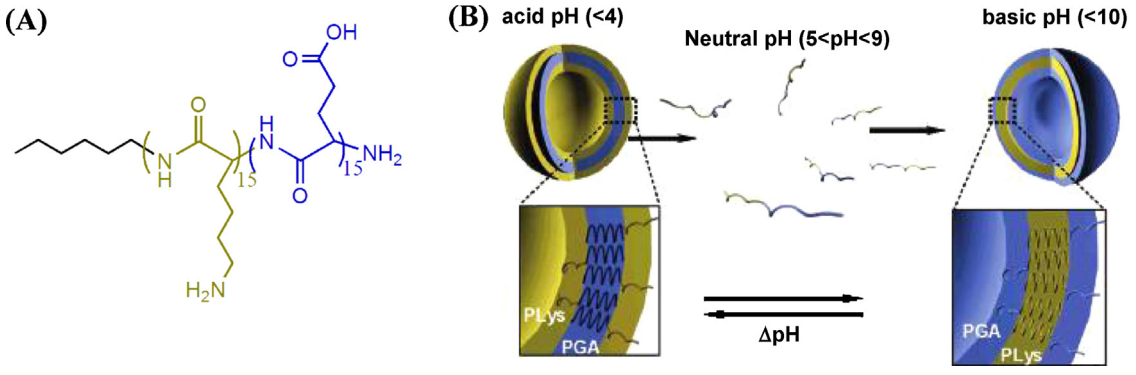

Fig. 31. The structure (A) and self-assembly (B) of $PLys_{15}$-b-$PGlu_{15}$ copolypeptide. [216], Copyright 2005. Reproduced with permission from the American Chemical Society.

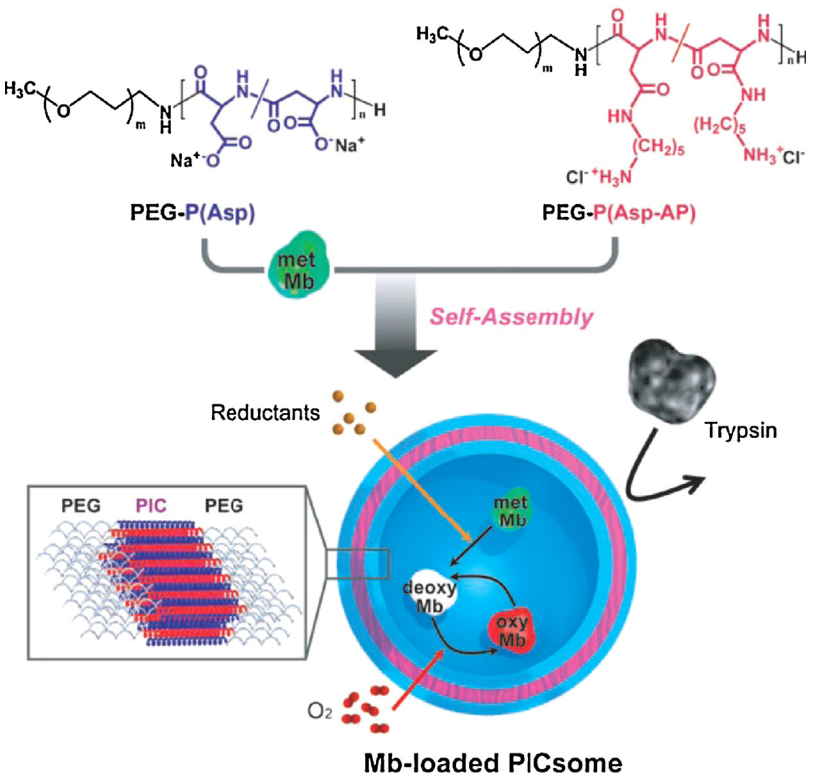

Fig. 32. Reversible Mb oxygenation inside the PICsomes self-assembled from a pair of oppositely charged block ionomers.
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water-soluble agents like Alexa 680-labeled dextran, which allowed in vivo noninvasive tumor imaging by near-infrared fluorescence imaging. Lecommandoux et al. synthesized amphiphilic poly(trimethylene carbonate)-b-PGlu (PTMC-b-PGlu) copolymers by ROP of BLG-NCA using amino-functionalized PTMC (PTMC-NH_2) as an initiator and subsequent hydrogenation [220,221]. PTMC-b-PGlu copolymers self-assembled in water into pH-responsive, size-tunable and stable vesicles. Interestingly, $PTMC_{22}$-b-$PGlu_{14}$ vesicles exhibited reversible thermo-responsive behavior in aqueous solution due to change of PTMC conformation with temperature [222]. Vesicle budding and fission were observed when temperature was increased to above the PTMC melting temperature (34 °C) and fusion events occurred when temperature was decreased. PTMC-PGlu vesicles co-loaded with DOX and superparamagnetic iron oxide nanoparticles (USPIO) revealed improved cytostatic effects toward HeLa cells when a high frequency *AC* magnetic field was applied [223].

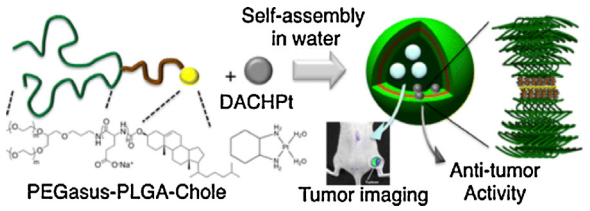

Fig. 33. Polypeptide polymersomes formed through the complexation reaction of DACHPt with a Y-shaped PEGasus-PGlu-Chole block copolymer.
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4.4. Functional polypeptide-basde nanogels for drug delivery

Various PEG-polypeptide copolymers were covalently crosslinked to prepare nanogels for controlled drug and protein release. For example, Bae et al. prepared pH-responsive PEG-PAsp polypeptide nanogels by crosslinking of PAsp side chain with 1,6-hexanediamine [224]. These nanogels remained stable in the pharmaceutically relevant pH ranges between 4 and 9 and were able to load DOX·HCl. The drug release was accelerated in acidic conditions (pH 5.0) due to increased solubility of DOX. Chen et al. demonstrated that pH-responsive PEG-b-P(Glu/CGlu) nanogels crosslinked via the photodimerization of the cinnamyloxy groups could be utilized for rifampin delivery [98]. The in vitro release studies demonstrated that drug release was much faster at pH 7.4 than at pH 4.0. pH- and thermo-responsive polypeptide microgels with a diameter of 570 nm were obtained by copolymerization of NIPAM with 2-hydroxylethyl methacrylate (HEMA) functionalized PGlu (P(Glu-g-HEMA)), and demonstrated shrinkable behaviors upon increasing temperature or decreasing pH [225]. In another study, pH- and thermo-responsive nanogels were developed based on P(Glu-g-HEMA/PNIPAM) graft copolypeptides, in which

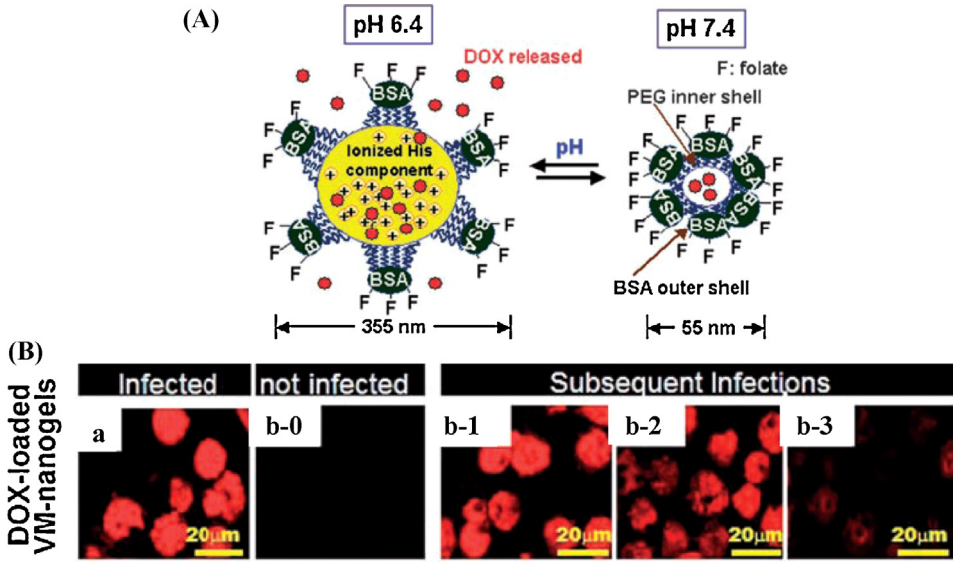

Fig. 34. (A) The structure of virus-mimetic nanogels consisting of a hydrophobic core P(His_{32}-co-Phe_6) and two layers of hydrophilic shells (PEG and BSA), (B) migration of DOX-loaded nanogel from infected A2780/AD cells to untreated cells (a: cells pretreated with DOX-loaded nanogel for 4 h, b-0: no infected cells, b-1: co-culture with a for 20 h, b-2: co-culture with b-1 for 20 h, b-3: co-culture with b-2 for 20 h).
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nanogels shrinked when increasing temperature to above their LCST (33–36 °C) or decreasing pH from 10.0 to 6.0 [226].

Reduction-sensitive core-crosslinked PEG-polypeptide nanogels were obtained by copolymerization of Cys-NCA and BLG-NCA/Phe-NCA using PEG-NH_2 as a macroinitiator [54,55]. The results showed that DOX-loaded nanogels gave enhanced intracellular DOX release and higher cellular proliferation inhibition to GSH pretreated HeLa cells as compared to non-pretreated cells [55]. Chen et al. reported that pH-responsive disulfide-crosslinked PEG-P(Cys-co-Glu) nanogels displayed improved DOX loading via electrostatic interaction [227].

A virus-mimetic nanogel vehicle consisting of a P(His_{32}-co-Phe_6) hydrophobic polymer core, a PEG hydrophilic inner shell and a bovine serum albumin (BSA) capsid-like outer shell was developed by Bae and coworkers (Fig. 34A) [50]. The nanogel sizes could be reversibly switched from 55 nm to 355 nm by cycling pH between pH > 7.4 (extracellular and cytosolic pH) and pH < 6.4 (endosomal pH) due to reversible deionization/ionization of PHis, which allowed control of drug release in an on-off manner. Interestingly, DOX-loaded nanogels following effectively kill tumor cells could migrate to neighboring cells to carry on anti-tumor effect as viruses do (Fig. 34B).

4.5. Funtional polypeptide hydrogels for tissue engineering and drug delivery

Deming et al. reported that amphiphilic diblock copolypeptides consisting of a charged, water soluble domain (PLys or PGlu) and a α-helical hydrophobic domain (PLeu) or β-strand hydrophobic domain (poly(L-valine), PVal) could be self-assembled into hydrogels by direct dissolution in water (Fig. 35A-B) [228–230]. The resulting hydrogels though containing over 95% water possessed a network structure with nanoscale to microscale porosity and significant material rigidity. Their physical properties including hydrogel strength, porosity, stability, as well as functionality could be tuned independent of each other. Moreover, they were injectable through a 30-G needle and were non-toxic to cells [231]. Interestingly, $PLys_m$-$PLeu_n$-$PLys_o$ triblock copolypeptides and $PLys_m$-$PLeu_n$-$PLys_o$-$PLeu_n$-$PLys_m$ pentablock copolypeptides were shown to from hydrogels with greater strength, better salt tolerance and more controllable morphology than diblock copolypeptides at similar concentrations [232,233]. The in vivo studies on PLys-PLeu copolypeptide hydrogels showed that hydrogel depot formed by directly injecting into mouse forebrain elicited no more gliosis, inflammation, or toxicity to neurons, myelin or axons than did physiological saline [39]. Notably, depot of PLys-PLeu exhibited time-dependent in-growth of blood vessels, glial cells, and nerve fibers (Fig. 35C–F). PLys-PLeu and PGlu-PLeu hydrogels were also investigated for sustained local delivery of bioactive nerve growth factor (NGF) in the central nervous system, which was shown to maintain hypertrophy of local forebrain cholinergic neurons for at least 4 weeks and induce hypertrophy a further distant away (up to 5 mm) from injection sites [234].

Jeong et al. developed thermal-reversible hydrogels based on PEG-poly(L-alanine) (PEG-PAla) [235] and PAla-poloxamer-PAla copolymers [236]. These polypeptide copolymers exhibited sol-gel transition temperature in the range of 20–40 °C. There existed various amounts of nanofibers depending on secondary structure and initial polymer concentration. These polypeptide hydrogels were proven to be excellent 3D matrices to preserve the phenotypes as well as enhance the proliferation and differentiation of chondrocytes [237,238]. PEG-P(Ala-co-Phe) copolypeptides with significantly lower sol-gel transition temperature and concentration as compared to PEG-PAla

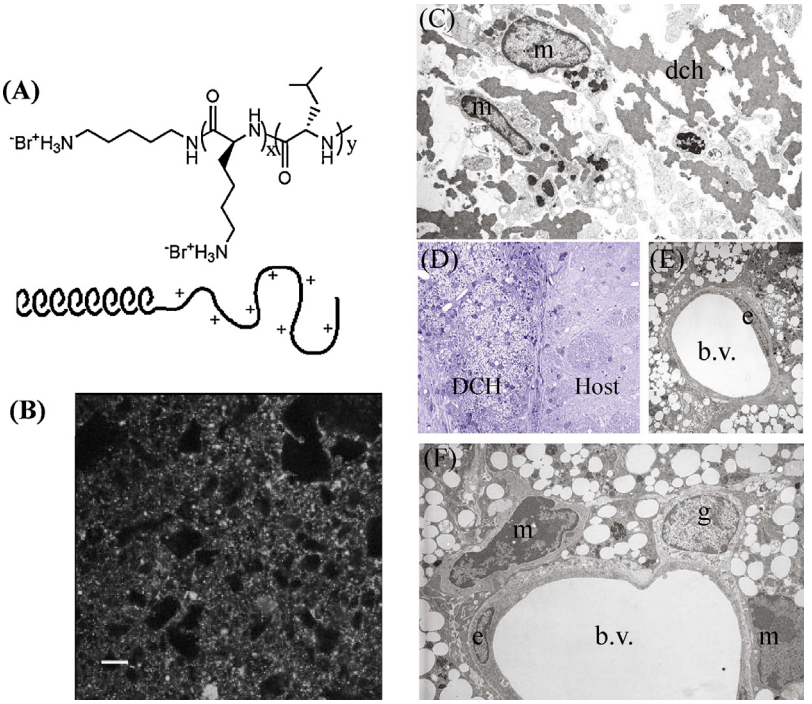

Fig. 35. Hydrogel scaffolds from self-assembling block copolypeptide amphiphiles. (A) Structure of diblock copolypeptide PLys-PLeu; (B) confocal micrograph of PLys-PLeu at 1.0 wt% in H_2O with heterogeneous microstructure visualized using $DiOC_{18}$ hydrophobic dye, scale bar, 20 μm; (C) electron microscopic image of 3% PLys-PLeu at 2 weeks after injection into the center of the caudate putamen nucleus (CPN) of the mouse forebrain; (D) light-microscopic survey image of a PLys-PLeu deposit after 8 weeks and examined in a semi-thin (0.3 um) plastic section stained with toluidine blue, note that many cells and blood vessels are scattered throughout the deposit; (E, F) magnified electron microscopic images of ultrathin sections of the tissue shown in B. (m indicates microglia, b.v. indicates blood vessel, e indicates endothelial cells, * indicates intracellular vacuoles).
[39,228], Copyright 2009 and 2002, respectively. Reproduced with permission from Elsevier Ltd and Nature Publishing Group, respectively.

were used for sustained insulin release [239]. Interestingly, the results showed a hypoglycemic effect over 18 days after a single injection into diabetic rats.

Dong et al. reported a versatile strategy for fabricating supramolecular polypeptide-based normal micellar hydrogels as well as reverse micellar hydrogels based on PGlu-b-poly(ethylene oxide) (PGlu-PEO) copolypeptides (Fig. 36) [240]. PGlu-PEO was synthesized via combination of ROP of BLG-NCA and click chemistry followed by the removal of protecting benzyl groups. Notably, these hydrogels exhibited temperature and pH sensitivity, and the reverse micellar hydrogels achieved sustained DOX release for 45 days. The polypeptide-shelled reverse micelles were obtained from dendron-like PGlu-PEO (Dm-PGlu-PEO) block copolymers and α-cyclodextrin (α-CD) in alkaline solution by supramolecular self-assembly [241]. The micelles of Dm-PGlu-PEO copolymer exhibited a higher DOX loading capacity and longer time (~70 days) of sustained drug release than linear PGlu-PEO counterparts.

Chen et al. fabricated thermo- and pH-responsive polypeptide hybrid hydrogels based on PGlu-g-P(NIPAM-co-HEMA) graft copolymers that were prepared from PGlu and amine-terminated P(NIPAM-co-HEMA) via carbodiimide chemistry [242]. The in vitro protein release studies demonstrated that hydrogels with a lower crosslinking density and P(NIPAM-co-HEMA) content had a faster release rate at pH 7.4 and drug release rate was slower at pH 4.0 than that at pH 7.4. In a following study, stimuli-responsive PGlu-hydroxypropycellulose (PGlu-HPC) hybrid hydrogels were prepared by free-radical copolymerization of pH-sensitive P(Glu-g-HEMA) and temperature-sensitive acrylic acid grafted hydroxypropycellulose (HPC) (HPC-g-AA) [243]. Interesting, release of BSA from PGlu-HPC hydrogels was very slow in artificial gastric juice (pH 1.2) and significantly accelerated in artificial intestinal liquid (pH 6.8) due to protonation of the P(Glu-g-HEMA).

4.6. Functional polypeptide and hybrid materials for tissue engineering scaffolds

Whittaker et al. developed PEG-PLA-PLys triblock copolymer for enhancement of osteoblast attachment and growth [244]. Human osteoblast tests revealed that PLLA films modified with PEG-PLA-PLys were much more effective in promoting cell adhesion and proliferation than the parent PLLA films and PLLA films modified with PEG-PLLA diblock copolymers. Moreover, the free amino groups of PEG-PLA-PLys can potentially be used to immobilize cell recognition motifs or growth factors to induce specific cellular responses. Jing et al. reported that matrix based on PLLA and PEG-PLLA-P(Glu-g-RGD), which was obtained by grafting RGD tripeptide onto PEG-PLA-PGlu using carbodiimide chemistry, exhibited significantly

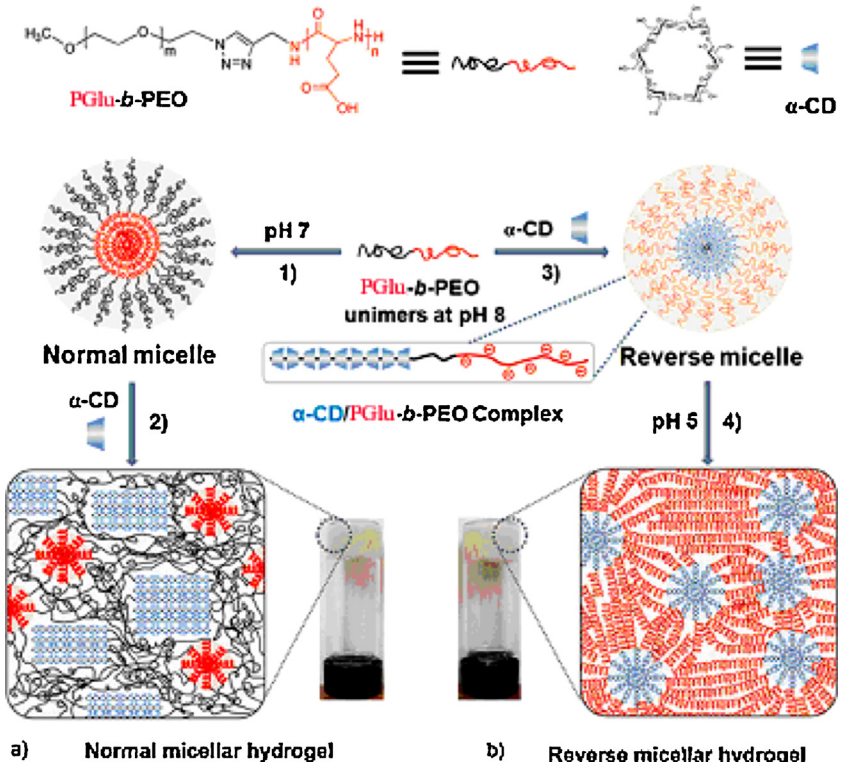

Fig. 36. Schematic illustration of the proposed structures and gelation mechanism of supramolecular hydrogels: (a) normal micellar hydrogels, and (b) reverse micellar hydrogels. Stages: (1) the micellization of copolymer, (2) the normal micellar hydrogel induced by supramolecular inclusion complexation, (3) the reverse micellization of copolymer, and (4) the reverse micellar hydrogelation.
[240], Copyright 2010. Reproduced with permission from Wiley-VCH Verlag GmbH & Co. KGaA.

improved adhesion, spreading and proliferation of human chondrocytes and 3T3 cells compared to the parent PLLA counterparts [94]. In a follow-up study, porous scaffolds were prepared from PEG-PLLA-P(Glu-RGD), PLGA and hydroxyapatite (HA) using solvent casting/particulate leaching method (Fig. 37) [245]. The in vivo studies on rabbit radius bone defects demonstrated that PEG-PLLA-P(Glu-RGD) induced increased bone formation, better fusion interface and enhanced bone ingrowth. Recently, Yin et al. empolyed polyelectrolyte complex scaffolds based on PGlu and chitosan for delivery of adipose-derived stem cells to repair full thickness articular cartilage defects [246]. Histological observations showed that articular defects were recovered with newly formed cartilage 6 weeks post-implantation, and the regenerated cartilage had integrated well with the surrounding native cartilage and subchondral bone in 12 weeks.

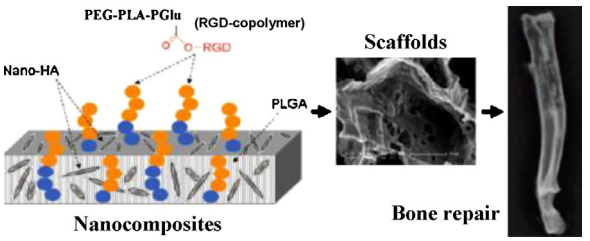

Fig. 37. Schematic illustration of PEG-PLA-P(Glu-RGD)/PLGA/HA composite scaffolds for the repair of rabbit bone defects.
[245], Copyright 2011. Reproduced with permission from the American Chemical Society.

5. Conclusions and future perspectives

The past decade has witnessed significant progress in the de novo design and synthesis of functional polypeptide and hybrid materials with precise control over molecular weights, architectures and functions. These protein-mimicking polymers offer several unique features such as excellent biocompatibility, in vivo biodegradability, versatile functionality, as well as bioactivity. Notably, various advanced nano-carriers including micelles, polymersomes, and nanogels have been developed based on functional polypeptides and hybrid materials for controlled and/or targeted delivery of anti-cancer drugs, proteins and nucleic acids, among which several micellar drugs (e.g. NK911, NK105, NK012, and NC6004) have entered different phases of clinical trials. The polypeptide hydrogels with elegant control over gel porosity and strength, degradation rate, adhesive property, growth factor release rate, etc. have great potentials as depots for sustained local drug or protein release as well as functional scaffolds for tissue engineering. The synthetic glycopolypeptides, similar to their natural analogs, display fast and selective biorecognition of proteins and furthermore drug nanocarriers based on glycopolypeptides possess high affinity for the surface

glycoproteins of living cells. It should be further noted that functional polypeptides are also promising in various biomedical coatings, e.g. mussel adhesive-mimicking polypeptides have been developed for stent coatings [247], protein-releasing multilayer polypeptide coatings for the prevention of infections [248], and coating of adenoviruses with polypeptides to augment their gene transduction and in vivo stability [249].

The development of functional polypeptide and hybrid materials for biomedical and pharmaceutical applications, however, remains at its infancy. Despite the fact that a variety of functional polypeptides have been constructed, most of them are very primitive and have no obvious biological activity. In principle, with proper design, virus-mimicking multifunctional vehicles and cell-responsive tissue scaffolds could be developed based on functional polypeptide and hybrid materials for targeted and controlled delivery of pharmaceutics and tissue regeneration, respectively. This might be realized by combining functional polypeptides with complementary functional synthetic polymers or designing de novo multifunctional polypeptides. For example, by combining membrane-disrupting cationic α-helical polypeptide with oleyl-conjugated chitosan and oleyl-PEG-mannose, multifunctional nonviral gene delivery systems that outperform commercial transfection reagents by 1–2 orders of magnitude in vitro and by 1.5–4 fold in vivo have recently been reported [250]. It has been found that polypeptide nanocarriers responsive to one single stimulus though show in general a better release profile than their non-responsive counterparts are not optimal in terms of therapeutic efficacy. In the future, dual and multi-responsive polypeptide nanocarriers will attract increasing attention as they promise to offer greater temporal and spatial control over drug release [251]. To be applied as tissue scaffolds, multifunctional polypeptides and hybrid materials that possess good mechanical property, appropriate biodegradation rate as well as cell-instructive behavior should be developed.

It should further be noted that there is lack of adequate biocompatibility and biodegradation studies on functional polypeptide and hybrid materials in vivo. Functional polypeptide and hybrid materials are structurally different from natural polypeptides, which possibly results in altered biocompatibility and enzymatic degradation behaviors. In addition, the synthesis of functional polypeptides might also introduce potentially cytotoxic elements such as copper catalysts in the click chemistry. In the future, special cares should be taken to maintain biocompatibility and functions of natural polypeptides in the design and development of novel functional polypeptide and hybrid materials. The in vivo fate of functional polypeptide and hybrid materials should be investigated in detail. We are convinced that functional polypeptide and hybrid materials as advanced biomaterials will play a more and more important role in biomedical technology.

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References

[1] Gomes S, Leonor IB, Mano JF, Reis RL, Kaplan DL. Natural and genetically engineered proteins for tissue engineering. Prog Polym Sci 2012;37:1–17.
[2] Maji SK, Perrin MH, Sawaya MR, Jessberger S, Vadodaria K, Rissman RA, Singru PS, Nilsson KPR, Simon R, Schubert D, Eisenberg D, Rivier J, Sawchenko P, Vale W, Riek R. Functional amyloids as natural storage of peptide hormones in pituitary secretory granules. Science 2009;325:328–32.
[3] Dobson CM. Protein folding and misfolding. Nature 2003;426:884–90.
[4] Lutz JF, Boerner HG. Modern trends in polymer bioconjugates design. Prog Polym Sci 2008;33:1–39.
[5] Deming TJ. Synthetic polypeptides for biomedical applications. Prog Polym Sci 2007;32:858–75.
[6] Liu L, Xu K, Wang H, Tan JPK, Fan W, Venkatraman SS, Li L, Yang YY. Self-assembled cationic peptide nanoparticles as an efficient antimicrobial agent. Nat Nanotechnol 2009;4:457–63.
[7] Lowik DWPM, Leunissen EHP, van den Heuvel M, Hansen MB, van Hest JCM. Stimulus responsive peptide based materials. Chem Soc Rev 2010;39:3394–412.
[8] Dueber JE, Wu GC, Malmirchegini GR, Moon TS, Petzold CJ, Ullal AV, Prather KLJ, Keasling JD. Synthetic protein scaffolds provide modular control over metabolic flux. Nat Biotechnol 2009;27:753–9.
[9] Banwell EF, Abelardo ES, Adams DJ, Birchall MA, Corrigan A, Donald AM, Kirkland M, Serpell LC, Butler MF, Woolfson DN. Rational design and application of responsive alpha-helical peptide hydrogels. Nat Mater 2009;8:596–600.
[10] Zaccai NR, Chi B, Thomson AR, Boyle AL, Bartlett GJ, Bruning M, Linden N, Sessions RB, Booth PJ, Brady RL, Woolfson DN. A de novo peptide hexamer with a mutable channel. Nat Chem Biol 2011;7:935–41.
[11] Nederberg F, Zhang Y, Tan JPK, Xu K, Wang H, Yang C, Gao S, Guo XD, Fukushima K, Li L, Hedrick JL, Yang YY. Biodegradable nanostructures with selective lysis of microbial membranes. Nat Chem 2011;3:409–14.
[12] Cai C, Wang L, Lin J. Self-assembly of polypeptide-based copolymers into diverse aggregates. Chem Commun 2011;47:11189–203.
[13] Bae Y, Kataoka K. Intelligent polymeric micelles from functional poly(ethylene glycol)-poly(amino acid) block copolymers. Adv Drug Delivery Rev 2009;61:768–84.
[14] Hadjichristidis N, Iatrou H, Pitsikalis M, Sakellariou G. Synthesis of well-defined polypeptide-based materials via the ring-opening polymerization of alpha-amino acid *N*-carboxyanhydrides. Chem Rev 2009;109:5528–78.
[15] Duncan R. The dawning era of polymer therapeutics. Nat Rev Drug Discov 2003;2:347–60.
[16] He C, Zhuang X, Tang Z, Tian H, Chen X. Stimuli-sensitive synthetic polypeptide-based materials for drug and gene delivery. Adv Healthcare Mater 2012;1:48–78.
[17] Klok HA. Peptide/protein-synthetic polymer conjugates: quo vadis. Macromolecules 2009;42:7990–8000.
[18] Carlsen A, Lecommandoux S. Self-assembly of polypeptide-based block copolymer amphiphiles. Curr Opin Colloid Interface Sci 2009;14:329–39.
[19] Cha JN, Stucky GD, Morse DE, Deming TJ. Biomimetic synthesis of ordered silica structures mediated by block copolypeptides. Nature 2000;403:289–92.
[20] Canalle LA, Lowik DWPM, van Hest JCM. Polypeptide–polymer bioconjugates. Chem Soc Rev 2010;39:329–53.
[21] van Hest JCM, Tirrell DA. Protein-based materials, toward a new level of structural control. Chem Commun 2001:1897–904.
[22] Bacsa B, Horvati K, Bosze S, Andreae F, Kappe CO. Solid-phase synthesis of difficult peptide sequences at elevated temperatures: a critical comparison of microwave and conventional heating technologies. J Org Chem 2008;73:7532–42.
[23] Deming TJ. Polypeptide and polypeptide hybrid copolymer synthesis via NCA polymerization. Adv Polym Sci 2006;202:1–18.

[24] Tian H, Tang Z, Zhuang X, Chen X, Jing X. Biodegradable synthetic polymers: preparation, functionalization and biomedical application. Prog Polym Sci 2012;37:237–80.
[25] Kricheldorf HR. Polypeptides and 100 years of chemistry of alpha-amino acid *N*-carboxyanhydrides. Angew Chem Int Ed 2006;45:5752–84.
[26] Farthing AC, Reynolds RJW. Anhydro-*N*-carboxy-DL-beta-phenylalanine. Nature 1950;165:647–57.
[27] Coleman D, Farthing AC. Synthetic polypeptides. 2. Properties of oxazolid-2-5-diones and an initial study of the preparation of polypeptides therefrom. J Chem Soc 1950:3218–22.
[28] Poche DS, Moore MJ, Bowles JL. An unconventional method for purifying the *N*-carboxyanhydride derivatives of gamma-alkyl-L-glutamates. Synth Commun 1999;29:843–54.
[29] Smeets NMB, van der Weide PLJ, Meuldijk J, Vekemans J, Hulshof LA. A scalable synthesis of L-leucine-*N*-carboxyanhydride. Org Process Res Dev 2005;9:757–63.
[30] Kramer JR, Deming TJ. General method for purification of alpha-amino acid-*N*-carboxyanhydrides using flash chromatography. Biomacromolecules 2010;11:3668–72.
[31] Deming TJ. Facile synthesis of block copolypeptides of defined architecture. Nature 1997;390:386–9.
[32] Deming TJ. Cobalt and iron initiators for the controlled polymerization of alpha-amino acid-*N*-carboxyanhydrides. Macromolecules 1999;32:4500–2.
[33] Dimitrov I, Schlaad H. Synthesis of nearly monodisperse polystyrene-polypeptide block copolymers via polymerisation of *N*-carboxyanhydrides. Chem Commun 2003:2944–5.
[34] Aliferis T, Iatrou H, Hadjichristidis N. Living polypeptides. Biomacromolecules 2004;5:1653–6.
[35] Vayaboury W, Giani O, Cottet H, Deratani A, Schue F. Living polymerization of alpha-amino acid *N*-carboxyanhydrides (NCA) upon decreasing the reaction temperature. Macromol Rapid Commun 2004;25:1221–4.
[36] Lu H, Cheng J. Hexamethyldisilazane-mediated controlled polymerization of alpha-amino acid *N*-carboxyanhydrides. J Am Chem Soc 2007;129:14114–5.
[37] Lu H, Cheng J. *N*-Trimethylsilyl amines for controlled ring-opening polymerization of amino acid *N*-carboxyanhydrides and facile end group functionalization of polypeptides. J Am Chem Soc 2008;130:12562–3.
[38] Hanson JA, Chang CB, Graves SM, Li Z, Mason TG, Deming TJ. Nanoscale double emulsions stabilized by single-component block copolypeptides. Nature 2008;455:85–8.
[39] Yang CY, Song B, Ao Y, Nowak AP, Abelowitz RB, Korsak RA, Havton LA, Deming TJ, Sofroniew MV. Biocompatibility of amphiphilic diblock copolypeptide hydrogels in the central nervous system. Biomaterials 2009;30:2881–98.
[40] Miyata K, Christie RJ, Kataoka K. Polymeric micelles for nano-scale drug delivery. React Funct Polym 2011;71:227–34.
[41] Hanson JA, Deming TJ. Functionalized nanoscale through microscale polypeptide stabilized emulsions for display of biomolecules. Polym Chem 2011;2:1473–5.
[42] Engler AC, Shukla A, Puranam S, Buss HG, Jreige N, Hammond PT. Effects of side group functionality and molecular weight on the activity of synthetic antimicrobial polypeptides. Biomacromolecules 2011;12:1666–74.
[43] Huang J, Heise A. Stimuli responsive synthetic polypeptides derived from *N*-carboxyanhydride (NCA) polymerisation. Chem Soc Rev 2013;42:7373–90.
[44] Deming TJ. Polypeptide and polypeptide hybrid copolymer synthesis via NCA polymerization. In: Klok HA, Helmut S, editors. Peptide hybrid polymers. Berlin/Heidelberg: Springer-Verlag; 2006. p. 1–18.
[45] Klok HA, Lecommandoux S. Solid-state structure, organization and properties of peptide – synthetic hybrid block copolymers. In: Klok HA, Schlaad H, editors. Peptide hybrid polymers. Berlin/Heidelberg: Springer-Verlag; 2006. p. 75–111.
[46] Hernandez JR, Klok HA. Synthesis and ring-opening (Co) polymerization of L-lysine *N*-carboxyanhydrides containing labile side-chain protective groups. J Polym Sci Part A Polym Chem 2003;41:1167–87.
[47] Patchornik A, Berger A, Katchalski E. Poly-L-histidine. J Am Chem Soc 1957;79:5227–30.
[48] Lee ES, Shin HJ, Na K, Bae YH. Poly(L-histidine)-PEG block copolymer micelles and pH-induced destabilization. J Control Release 2003;90:363–74.
[49] Lee ES, Na K, Bae YH. Super pH-sensitive multifunctional polymeric micelle. Nano Lett 2005;5:325–9.
[50] Lee ES, Kim D, Youn YS, Oh KT, Bae YH. A virus-mimetic nanogel vehicle. Angew Chem Int Ed 2008;47:2418–21.
[51] Berger A, Noguchi J, Katchalski E. Poly-L-cysteine. J Am Chem Soc 1956;78:4483–8.
[52] Abraham S, Kim I, Batt CA. A facile preparative method for aggregation-free gold nanoparticles using poly(styrene-block-cysteine). Angew Chem Int Ed 2007;46:5720–3.
[53] Habraken GJM, Koning CE, Heuts JPA, Heise A. Thiol chemistry on well-defined synthetic polypeptides. Chem Commun 2009:3612–4.
[54] Xing T, Lai B, Ye X, Yan L. Disulfide core cross-linked PEGylated polypeptide nanogel prepared by a one-step ring opening copolymerization of *N*-carboxyanhydrides for drug delivery. Macromol Biosci 2011;11:962–9.
[55] Ding J, Shi F, Xiao C, Lin L, Chen L, He C, Zhuang X, Chen X. One-step preparation of reduction-responsive poly(ethylene glycol)-poly(amino acid)s nanogels as efficient intracellular drug delivery platforms. Polym Chem 2011;2:2857–64.
[56] Hayakawa T, Kondo Y, Yamamoto H, Murakami Y. The synthesis of poly-L-arginine hydrobromide and copolymers of L-arginine and other amino acids. Bull Chem Soc Jpn 1969;42:479–82.
[57] Bellomo EG, Wyrsta MD, Pakstis L, Pochan DJ, Deming TJ. Stimuli-responsive polypeptide vesicles by conformation-specific assembly. Nat Mater 2004;3:244–8.
[58] Fasman GD, Blout ER. The synthesis and the conformation of poly-L-serine and poly-*O*-acetyl-L-serine. J Am Chem Soc 1960;82:2262–7.
[59] Tooney NM, Fasman GD. Synthesis of poly-*O*-tert-butyl-serine and poly-L-serine. Biopolymers 1968;6:81–96.
[60] Deng M, Wu J, Reinhart-King CA, Chu CC. Biodegradable functional poly(ester amide)s with pendant hydroxyl functional groups: synthesis, characterization, fabrication and in vitro cellular response. Acta Biomater 2011;7:1504–15.
[61] Yamamoto H, Hayakawa T. Letter: sense of helix poly-*O*,*O*′-dicarbobenzoxy-L-DOPA in solution. Macromolecules 1976;9:532–4.
[62] Yu ME, Deming TJ. Synthetic polypeptide mimics of marine adhesives. Macromolecules 1998;31:4739–45.
[63] Holowka EP, Deming TJ. Synthesis and cross linking of L-DOPA containing polypeptide vesicles. Macromol Biosci 2010;10:496–502.
[64] Yu M, Nowak AP, Deming TJ, Pochan DJ. Methylated mono- and diethyleneglycol functionalized polylysines: nonionic, alpha-helical, water-soluble polypeptides. J Am Chem Soc 1999;121:12210–1.
[65] Hanson JA, Li Z, Deming TJ. Nonionic block copolypeptide micelles containing a hydrophobic rac-leucine core. Macromolecules 2010;43:6268–9.
[66] Wang J, Gibson MI, Barbey R, Xiao SJ, Klok HA. Non-fouling polypeptide brushes via surface-initiated polymerization of N(epsilon)-oligo(ethylene glycol)succinate-L-lysine N-carboxyanhydride. Macromol Rapid Commun 2009;30:845–50.
[67] Hwang JY, Deming TJ. Methylated mono- and di(ethylene glycol)-functionalized beta-sheet forming polypeptides. Biomacromolecules 2001;2:17–21.
[68] Chen C, Wang Z, Li Z. Thermoresponsive polypeptides from pegylated poly-L-glutamates. Biomacromolecules 2011;12:2859–63.
[69] Cheng Y, He C, Xiao C, Ding J, Zhuang X, Chen X. Versatile synthesis of temperature-sensitive polypeptides by click grafting of oligo(ethylene glycol). Polym Chem 2011;2:2627–34.
[70] Liao Y, Dong CM. Synthesis, conformation transition, liquid crystal phase, and self-assembled morphology of thermosensitive homopolypeptide. J Polym Sci Part A Polym Chem 2012;50:1834–43.
[71] Engler AC, Lee Hi, Hammond PT. Highly efficient "grafting onto" a polypeptide backbone using click chemistry. Angew Chem Int Ed 2009;48:9334–8.
[72] Engler AC, Bonner DK, Buss HG, Cheung EY, Hammond PT. The synthetic tuning of clickable pH responsive cationic polypeptides and block copolypeptides. Soft Matter 2011;7:5627–37.
[73] Xiao CS, Zhao CW, He P, Tang ZH, Chen XS, Jing XB. Facile synthesis of glycopolypeptides by combination of ring-opening polymerization of an alkyne-substituted *N*-carboxyanhydride and click "glycosylation". Macromol Rapid Commun 2010;31:991–7.
[74] Huang Y, Zeng Y, Yang J, Zeng Z, Zhu F, Chen X. Facile functionalization of polypeptides by thiol-yne photochemistry for biomimetic materials synthesis. Chem Commun 2011;47:7509–11.
[75] Tang H, Zhang D. General route toward side-chain-functionalized alpha-helical polypeptides. Biomacromolecules 2010;11:1585–92.
[76] Tang H, Li Y, Lahasky SH, Sheiko SS, Zhang D. Core-shell molecular bottlebrushes with helical polypeptide backbone: synthesis,

characterization, and solution conformations. Macromolecules 2011;44:1491–9.
[77] Tang H, Zhang D. Multi-functionalization of helical block copoly(alpha-peptide)s by orthogonal chemistry. Polym Chem 2011;2:1542–51.
[78] Rhodes AJ, Deming TJ. Soluble, clickable polypeptides from azide-containing *N*-carboxyanhydride monomers. ACS Macro Lett 2013;2:351–4.
[79] Zhang Y, Lu H, Lin Y, Cheng J. Water-soluble polypeptides with elongated, charged side chains adopt ultrastable helical conformations. Macromolecules 2011;44:6641–4.
[80] Tang H, Yin L, Lu H, Cheng J. Water-soluble poly(L-serine)s with elongated and charged side-chains: synthesis, conformations, and cell-penetrating properties. Biomacromolecules 2012;13:2609–15.
[81] Huang J, Habraken G, Audouin F, Heise A. Hydrolytically stable bioactive synthetic glycopeptide homo- and copolymers by combination of NCA polymerization and click reaction. Macromolecules 2010;43:6050–7.
[82] Sun J, Schlaad H. Thiol-ene clickable polypeptides. Macromolecules 2010;43:4445–8.
[83] Kramer JR, Deming TJ. Glycopolypeptides via living polymerization of glycosylated-L-lysine *N*-carboxyanhydrides. J Am Chem Soc 2010;132:15068–71.
[84] Kramer JR, Deming TJ. Glycopolypeptides with a redox-triggered helix-to-coil transition. J Am Chem Soc 2012;134:4112–5.
[85] Pati D, Shaikh AY, Hotha S, Sen Gupta S. Synthesis of glycopolypeptides by the ring opening polymerization of *O*-glycosylated-alpha-amino acid *N*-carboxyanhydride (NCA). Polym Chem 2011;2:805–11.
[86] Ding J, Xiao C, Zhao L, Cheng Y, Ma L, Tang Z, Zhuang X, Chen X. Poly(L-glutamic acid) grafted with oligo(2-(2-(2-methoxyethoxy)ethoxy) ethyl methacrylate): thermal phase transition, secondary structure, and self-assembly. J Polym Sci Part A Polym Chem 2011;49:2665–76.
[87] Rhodes AJ, Deming TJ. Tandem catalysis for the preparation of cylindrical polypeptide brushes. J Am Chem Soc 2012;134:19463–7.
[88] Lu H, Bai Y, Wang J, Gabrielson NP, Wang F, Lin Y, Cheng J. Ring-opening polymerization of gamma-(4-vinylbenzyl)-L-glutamate *N*-carboxyanhydride for the synthesis of functional polypeptides. Macromolecules 2011;44:6237–40.
[89] Zhou J, Chen P, Deng C, Meng F, Cheng R, Zhong Z. A simple and versatile synthetic strategy to functional polypeptides via vinyl sulfone-substituted L-cysteine *N*-carboxyanhydride. Macromolecules 2013;46:6723–30.
[90] Yan L, Yang L, He H, Hu X, Xie Z, Huang Y, Jing X. Photo-cross-linked mPEG-poly(gamma-cinnamyl-L-glutamate) micelles as stable drug carriers. Polym Chem 2012;3:1300–7.
[91] Liu G, Dong CM. Photoresponsive poly(*S*-(*o*-nitrobenzyl)-L-cysteine)-b-PEO from a L-cysteine *N*-carboxyanhydride monomer: synthesis, self-assembly, and phototriggered drug release. Biomacromolecules 2012;13:1573–83.
[92] Enomoto H, Nottelet B, Al Halifa S, Enjalbal C, Dupre M, Tailhades J, Coudane J, Subra G, Martinez J, Amblard M. Synthesis of peptide-grafted comb polypeptides via polymerisation of NCA-peptides. Chem Commun 2013;49:409–11.
[93] Gauthier MA, Gibson MI, Klok HA. Synthesis of functional polymers by post-polymerization modification. Angew Chem Int Ed 2009;48:48–58.
[94] Deng C, Tian HY, Zhang PB, Sun J, Chen XS, Jing XB. Synthesis and characterization of RGD peptide grafted poly(ethylene glycol)-b-poly(L-lactide)-b-poly(L-glutamic acid) triblock copolymer. Biomacromolecules 2006;7:590–6.
[95] Lu T, Chen X, Shi Q, Wang Y, Zhang P, Jing X. The immobilization of proteins on biodegradable fibers via biotin-streptavidin bridges. Acta Biomater 2008;4:1770–7.
[96] Lu T, Sun J, Chen X, Zhang P, Jing X. Folate-conjugated micelles and their folate-receptor-mediated endocytosis. Macromol Biosci 2009;9:1059–68.
[97] Deng C, Chen X, Sun J, Lu T, Wang W, Jing X. RGD peptide grafted biodegradable amphiphilic triblock copolymer poly(glutamic acid)-b-poly(L-lactide)-b-poly(glutamic acid): synthesis and self-assembly. J Polym Sci Part A Polym Chem 2007;45:3218–30.
[98] Ding JX, Zhuang XL, Xiao CS, Cheng YL, Zhao L, He CL, Tang ZH, Chen XS. Preparation of photo-cross-linked pH-responsive polypeptide nanogels as potential carriers for controlled drug delivery. J Mater Chem 2011;21:11383–91.
[99] Sun VZ, Li Z, Deming TJ, Kamei DT. Intracellular fates of cell-penetrating block copolypeptide vesicles. Biomacromolecules 2011;12:10–3.
[100] Nakanishi M, Park JS, Jang WD, Oba M, Kataoka K. Study of the quantitative aminolysis reaction of poly(beta-benzyl L-aspartate) (PBLA) as a platform polymer for functionality materials. React Funct Polym 2007;67:1361–72.
[101] Arnida, Nishiyama N, Kanayama N, Jang WD, Yamasaki Y, Kataoka K. PEGylated gene nanocarriers based on block catiomers bearing ethylenediamine repeating units directed to remarkable enhancement of photochemical transfection. J Control Release 2006;115:208–15.
[102] Miyata K, Oba M, Nakanishi M, Fukushima S, Yamasaki Y, Koyama H, Nishiyama N, Kataoka K. Polyplexes from poly(aspartamide) bearing 1,2-diaminoethane side chains induce pH-selective, endosomal membrane destabilization with amplified transfection and negligible cytotoxicity. J Am Chem Soc 2008;130:16287–94.
[103] Koide A, Kishimura A, Osada K, Jang WD, Yamasaki Y, Kataoka K. Semipermeable polymer vesicle (PICsome) self-assembled in aqueous medium from a pair of oppositely charged block copolymers: physiologically stable micro-/nanocontainers of water-soluble macromolecules. J Am Chem Soc 2006;128:5988–9.
[104] Guo J, Huang Y, Jing X, Chen X. Synthesis and characterization of functional poly(gamma-benzyl-L-glutamate) (PBLG) as a hydrophobic precursor. Polymer 2009;50:2847–55.
[105] Kramer JR, Deming TJ. Preparation of multifunctional and multireactive polypeptides via methionine alkylation. Biomacromolecules 2012;13:1719–23.
[106] Steig S, Cornelius F, Witte P, Staal BBP, Koning CE, Heise A, Menzel H. Synthesis of polypeptide based rod-coil block copolymers. Chem Commun 2005:5420–2.
[107] Habraken GJM, Koning CE, Heise A. Peptide block copolymers by *N*-carboxyanhydride ring-opening polymerization and atom transfer radical polymerization: the effect of amide macroinitiators. J Polym Sci Part A Polym Chem 2009;47:6883–93.
[108] Zhang X, Li J, Li W, Zhang A. Synthesis and characterization of thermo- and pH-responsive double-hydrophilic diblock copolypeptides. Biomacromolecules 2007;8:3557–67.
[109] Knoop RJI, Habraken GJM, Gogibus N, Steig S, Menzel H, Koning CE, Heise A. Synthesis of poly(benzyl glutamate-b-styrene) rod-coil block copolymers by dual initiation in one pot. J Polym Sci Part A Polym Chem 2008;46:3068–77.
[110] Li J, Wang T, Wu D, Zhang X, Yan J, Du S, Guo Y, Wang J, Zhang A. Stimuli-responsive zwitterionic block copolypeptides: poly(*N*-isopropylacrylamide)-block-poly(lysine-co-glutamic acid). Biomacromolecules 2008;9:2670–6.
[111] Agut W, Taton D, Lecommandoux S. A versatile synthetic approach to polypeptide based rod-coil block copolymers by click chemistry. Macromolecules 2007;40:5653–61.
[112] Agut W, Brulet A, Schatz C, Taton D, Lecommandoux S. pH and temperature responsive polymeric micelles and polymersomes by self-assembly of poly[2-(dimethylamino)ethyl methacrylate]-b-poly(glutamic acid) double hydrophilic block copolymers. Langmuir 2010;26:10546–54.
[113] Schatz C, Louguet S, Le Meins JF, Lecommandoux S. Polysaccharide-block-polypeptide copolymer vesicles: towards synthetic viral capsids. Angew Chem Int Ed 2009;48:2572–5.
[114] Upadhyay KK, Le Meins JF, Misra A, Voisin P, Bouchaud V, Ibarboure E, Schatz C, Lecommandoux S. Biomimetic doxorubicin loaded polymersomes from hyaluronan-block-poly(gamma-benzyl glutamate) copolymers. Biomacromolecules 2009;10:2802–8.
[115] Agut W, Agnaou R, Lecommandoux S, Taton D. Synthesis of block copolypeptides by click chemistry. Macromol Rapid Commun 2008;29:1147–55.
[116] Rao J, Zhang Y, Zhang J, Liu S. Facile preparation of well-defined AB(2) Y-shaped miktoarm star polypeptide copolymer via the combination of ring-opening polymerization and click chemistry. Biomacromolecules 2008;9:2586–93.
[117] Naik SS, Chan JW, Comer C, Hoyle CE, Savin DA. Thiol-yne 'click' chemistry as a route to functional lipid mimetics. Polym Chem 2011;2:303–5.
[118] Ray JG, Ly JT, Savin DA. Peptide-based lipid mimetics with tunable core properties via thiol-alkyne chemistry. Polym Chem 2011;2:1536–41.
[119] Audouin F, Knoop RJI, Huang J, Heise A. Star polymers by cross-linking of linear poly(benzyl-L-glutamate) macromonomers via free-radical and RAFT polymerization. A simple route toward peptide-stabilized nanoparticles. J Polym Sci Part A Polym Chem 2010;48:4602–10.

[120] Klok HA, Hernandez JR, Becker S, Mullen K. Star-shaped fluorescent polypeptides. J Polym Sci Part A Polym Chem 2001;39:1572–83.
[121] Tian HY, Deng C, Lin H, Sun JR, Deng MX, Chen XS, Jing XB. Biodegradable cationic PEG-PEI-PBLG hyperbranched block copolymer: synthesis and micelle characterization. Biomaterials 2005;26:4209–17.
[122] Byrne M, Thornton PD, Cryan SA, Heise A. Star polypeptides by NCA polymerisation from dendritic initiators: synthesis and enzyme controlled payload release. Polym Chem 2012;3:2825–31.
[123] Wang Q, Yu J, Yan Y, Xu S, Wang F, Li Q, Wang J, Zhang X, Liu D. Controlled biomimetic silica formation using star-shaped poly(L-lysine). Polym Chem 2012;3:1284–90.
[124] Fu C, Lin L, Shi H, Zheng D, Wang W, Gao S, Zhao Y, Tian H, Zhu X, Chen X. Hydrophobic poly (amino acid) modified PEI mediated delivery of rev-casp-3 for cancer therapy. Biomaterials 2012;33:4589–96.
[125] Xia J, Chen L, Chen J, Tian H, Li F, Zhu X, Li G, Chen X. Hydrophobic polyphenylalanine-grafted hyperbranched polyethylenimine and its in vitro gene transfection. Macromol Biosci 2011;11:211–8.
[126] Chen L, Tian H, Chen J, Chen X, Huang Y, Jing X. Multi-armed poly(L-glutamic acid)-graft-oligoethylenimine copolymers as efficient nonviral gene delivery vectors. J Gene Med 2010;12:64–76.
[127] Li ZY, Wang HY, Li C, Zhang XL, Wu XJ, Qin SY, Zhang XZ, Zhuo RX. Porphyrin-functionalized amphiphilic diblock copolypeptides for photodynamic therapy. J Polym Sci Part A Polym Chem 2011;49:286–92.
[128] Williams AJ, Gupta VK. Self-assembly of a rodlike polypeptide on solid surfaces: role of solvent, molecular weight, and time of assembly. J Phys Chem B 2001;105:5223–30.
[129] Williams AJ, Gupta VK. Structure and formation of self-assembled monolayers of helical poly (gamma-benzyl L-glutamate) by surface plasmon resonance and infrared spectroscopy. Thin Solid Films 2003;423:228–34.
[130] Rodriguez-Hernandez J, Gatti M, Klok HA. Highly branched poly(L-lysine). Biomacromolecules 2003;4:249–58.
[131] Aliferis T, Iatrou H, Hadjichristidis N. Well-defined linear multiblock and branched polypeptides by linking chemistry. J Polym Sci Part A Polym Chem 2005;43:4670–3.
[132] Ren TB, Xia WJ, Dong HQ, Li YY. Sheddable micelles based on disulfide-linked hybrid PEG-polypeptide copolymer for intracellular drug delivery. Polymer 2011;52:3580–6.
[133] Brzezinska KR, Curtin SA, Deming TJ. Polypeptide end-capping using functionalized isocyanates: preparation of pentablock copolymers. Macromolecules 2002;35:2970–6.
[134] Kros A, Jesse W, Metselaar GA, Cornelissen J. Synthesis and self-assembly of rod-rod hybrid poly(gamma-benzyl L-glutamate)-block-polyisocyanide copolymers. Angew Chem Int Ed 2005;44:4349–52.
[135] Tian Z, Wang M, Zhang AY, Feng ZG. Preparation and evaluation of novel amphiphilic glycopeptide block copolymers as carriers for controlled drug release. Polymer 2008;49:446–54.
[136] Huang J, Bonduelle C, Thevenot J, Lecommandoux S, Heise A. Biologically active polymersomes from amphiphilic glycopeptides. J Am Chem Soc 2012;134:119–22.
[137] Krannig KS, Schlaad H. pH-responsive bioactive glycopolypeptides with enhanced helicity and solubility in aqueous solution. J Am Chem Soc 2012;134:18542–5.
[138] Rude E, Westphal O, Hurwitz E, Fuchs S, Sela M. Synthesis and antigenic properties of sugar-polypeptide conjugates. Immunochemistry 1966;3:137–51.
[139] Aoi K, Tsutsumiuchi K, Okada M. Glycopeptide synthesis by an alpha-amino-acid *N*-carboxyanhydride (NCA) method – ring-opening polymerization of a sugar-substituted NCA. Macromolecules 1994;27:875–7.
[140] Gibson MI, Hunt GJ, Cameron NR. Improved synthesis of *O*-linked, and first synthesis of *S*-linked, carbohydrate functionalised *N*-carboxyanhydrides (glycoNCAs). Org Biomol Chem 2007;5:2756–7.
[141] Pati D, Shaikh AY, Das S, Nareddy PK, Swamy MJ, Hotha S, Gupta SS. Controlled synthesis of o-glycopolypeptide polymers and their molecular recognition by lectins. Biomacromolecules 2012;13:1287–95.
[142] Pati D, Kalva N, Das S, Kumaraswamy G, Sen Gupta S, Ambade AV. Multiple topologies from glycopolypeptide-dendron conjugate self-assembly: nanorods, micelles, and organogels. J Am Chem Soc 2012;134:7796–802.
[143] Upadhyay KK, Bhatt AN, Mishra AK, Dwarakanath BS, Jain S, Schatz C, Le Meins JF, Farooque A, Chandraiah G, Jain AK, Misra A, Lecommandoux S. The intracellular drug delivery and anti tumor activity of doxorubicin loaded poly(gamma-benzyl L-glutamate)-b-hyaluronan polymersomes. Biomaterials 2010;31:2882–92.
[144] Yu H, Deng C, Tian H, Lu T, Chen X, Jing X. Chemo-physical and biological evaluation of poly(L-lysine)-grafted chitosan copolymers used for highly efficient gene delivery. Macromol Biosci 2011;11:352–61.
[145] Yu H, Chen X, Lu T, Sun J, Tian H, Hu J, Wang Y, Zhang P, Jing X. Poly(L-lysine)-graft-chitosan copolymers: synthesis, characterization, and gene transfection effect. Biomacromolecules 2007;8:1425–35.
[146] Dong CM, Faucher KM, Chaikof EL. Synthesis and properties of biomimetic poly(L-glutamate)-b-poly(2-acryloyloxyethyllactoside)-b-poly(L-glutamate) triblock copolymers. J Polym Sci Part A Polym Chem 2004;42:5754–65.
[147] Dong CM, Sun XL, Faucher KM, Apkarian RP, Chaikof EL. Synthesis and characterization of glycopolymer-polypeptide triblock copolymers. Biomacromolecules 2004;5:224–31.
[148] Matsumura Y, Kataoka K. Preclinical and clinical studies of anticancer agent-incorporating polymer micelles. Cancer Sci 2009;100:572–9.
[149] Murakami M, Cabral H, Matsumoto Y, Wu S, Kano MR, Yamori T, Nishiyama N, Kataoka K. Improving drug potency and efficacy by nanocarrier-mediated subcellular targeting. Sci Transl Med 2011;3:64ra2/1-12.
[150] Rafi M, Cabral H, Kano MR, Mi P, Iwata C, Yashiro M, Hirakawa K, Miyazono K, Nishiyama N, Kataoka K. Polymeric micelles incorporating (1,2-diaminocyclohexane) platinum (II) suppress the growth of orthotopic scirrhous gastric tumors and their lymph node metastasis. J Control Release 2012;159:189–96.
[151] Yin H, Lee ES, Kim D, Lee KH, Oh KT, Bae YH. Physicochemical characteristics of pH-sensitive poly(L-histidine)-b-poly (ethylene glycol)/poly(L-lactide)-b-poly(ethylene glycol) mixed micelles. J Control Release 2008;126:130–8.
[152] Lee ES, Na K, Bae YH. Polymeric micelle for tumor pH and folate-mediated targeting. J Control Release 2003;91:103–13.
[153] Lee ES, Na K, Bae YH. Doxorubicin loaded pH-sensitive polymeric micelles for reversal of resistant MCF-7 tumor. J Control Release 2005;103:405–18.
[154] Gao ZG, Tian L, Hu J, Park lS, Bae YH. Prevention of metastasis in a 4T1 murine breast cancer model by doxorubicin carried by folate conjugated pH sensitive polymeric micelles. J Control Release 2011;152:84–9.
[155] Kim D, Gao ZG, Lee ES, Bae YH. In vivo evaluation of doxorubicin-loaded polymeric micelles targeting folate receptors and early endosomal pH in drug-resistant ovarian cancer. Mol Pharm 2009;6:1353–62.
[156] Kim D, Lee ES, Oh KT, Gao ZG, Bae YH. Doxorubicin-loaded polymeric micelle overcomes multidrug resistance of cancer by double-targeting folate receptor and early endosomal pH. Small 2008;4:2043–50.
[157] Bae Y, Fukushima S, Harada A, Kataoka K. Design of environment-sensitive supramolecular assemblies for intracellular drug delivery: polymeric micelles that are responsive to intracellular pH change. Angew Chem Int Ed 2003;42:4640–3.
[158] Lee SJ, Min KH, Lee HJ, Koo AN, Rim HP, Jeon BJ, Jeong SY, Heo JS, Lee SC. Ketal cross-linked poly(ethylene glycol)-poly(amino acid)s copolymer micelles for efficient intracellular delivery of doxorubicin. Biomacromolecules 2011;12:1224–33.
[159] Cheng R, Feng F, Meng F, Deng C, Feijen J, Zhong Z. Glutathione-responsive nano-vehicles as a promising platform for targeted intracellular drug and gene delivery. J Control Release 2011;152:2–12.
[160] Meng F, Hennink WE, Zhong Z. Reduction-sensitive polymers and bioconjugates for biomedical applications. Biomaterials 2009;30:2180–98.
[161] Cabral H, Nakanishi M, Kumagai M, Jang WD, Nishiyama N, Kataoka K. A photo-activated targeting chemotherapy using glutathione sensitive camptothecin-loaded polymeric micelles. Pharm Res 2009;26:82–92.
[162] Wen HY, Dong HQ, Xie WJ, Li YY, Wang K, Pauletti GM, Shi DL. Rapidly disassembling nanomicelles with disulfide-linked PEG shells for glutathione-mediated intracellular drug delivery. Chem Commun 2011;47:3550–2.
[163] Thambi T, Yoon HY, Kim K, Kwon IC, Yoo CK, Park JH. Bioreducible block copolymers based on poly(ethylene glycol) and poly(gamma-benzyl L-glutamate) for intracellular delivery of camptothecin. Bioconjug Chem 2011;22:1924–31.
[164] Dai J, Lin S, Cheng D, Zou S, Shuai X. Interlayer-crosslinked micelle with partially hydrated core showing reduction and pH dual

sensitivity for pinpointed intracellular drug release. Angew Chem Int Ed 2011;50:9404–8.
[165] Wang K, Luo GF, Liu Y, Li C, Cheng SX, Zhuo RX, Zhang XZ. Redox-sensitive shell cross-linked PEG-polypeptide hybrid micelles for controlled drug release. Polym Chem 2012;3:1084–90.
[166] Wu L, Zou Y, Deng C, Cheng R, Meng F, Zhong Z. Intracellular release of doxorubicin from core-crosslinked polypeptide micelles triggered by both pH and reduction conditions. Biomaterials 2013;34:5262–72.
[167] Naik SS, Ray JG, Savin DA. Temperature- and pH-responsive self-assembly of poly(propylene oxide)-b-poly(lysine) block copolymers in aqueous solution. Langmuir 2011;27:7231–40.
[168] Cai C, Zhang L, Lin J, Wang L. Self-assembly behavior of pH- and thermosensitive amphiphilic triblock copolymers in solution: experimental studies and self-consistent field theory simulations. J Phys Chem B 2008;112:12666–73.
[169] Agut W, Brulet A, Taton D, Lecommandoux S. Thermoresponsive micelles from jeffamine-b-poly(L-glutamic acid) double hydrophilic block copolymers. Langmuir 2007;23:11526–33.
[170] Arimura H, Ohya Y, Ouchi T. The formation of biodegradable polymeric micelles from newly synthesized poly(aspartic acid)-block-polylactide AB-type diblock copolymers. Macromol Rapid Commun 2004;25:743–7.
[171] Arimura H, Ohya Y, Ouchi T. Formation of core-shell type biodegradable polymeric micelles from amphiphilic poly(aspartic acid)-block-polylactide diblock copolymer. Biomacromolecules 2005;6:720–5.
[172] Yang Y, Cai J, Zhuang X, Guo Z, Jing X, Chen X. pH-dependent self-assembly of amphiphilic poly(L-glutamic acid)-block-poly(lactic-co-glycolic acid) copolymers. Polymer 2010;51:2676–82.
[173] Deng C, Rong GZ, Tian HY, Tang ZH, Chen XS, Jing XB. Synthesis and characterization of poly(ethylene glycol)-b-poly (L-lactide)-b-poly(L-glutamic acid) triblock copolymer. Polymer 2005;46:653–9.
[174] Sun J, Deng C, Chen X, Yu H, Tian H, Sun J, Jing X. Self-assembly of polypeptide-containing ABC-type triblock copolymers in aqueous solution and its pH dependence. Biomacromolecules 2007;8:1013–7.
[175] Deng C, Chen X, Yu H, Sun J, Lu T, Jing X. A biodegradable triblock copolymer poly(ethylene glycol)-b-poly(L-lactide)-b-poly(L-lysine): synthesis, self-assembly and RGD peptide modification. Polymer 2007;48:139–49.
[176] Lee ES, Gao Z, Kim D, Park K, Kwon IC, Bae YH. Super pH-sensitive multifunctional polymeric micelle for tumor pH(e) specific TAT exposure and multidrug resistance. J Controlled Release 2008;129:228–36.
[177] Sun J, Chen X, Lu T, Liu S, Tian H, Guo Z, Jing X. Formation of reversible shell cross-linked micelles from the biodegradable amphiphilic diblock copolymer poly(L-cysteine)-block-poly(L-lactide). Langmuir 2008;24:10099–106.
[178] Sun J, Chen X, Wei J, Yan L, Jing X. Application of the biodegradable diblock copolymer poly(L-lactide)-block-poly(L-cysteine): drug delivery and protein conjugation. J Appl Polym Sci 2010;118:1738–42.
[179] Rao J, Luo Z, Ge Z, Liu H, Liu S. "Schizophrenic" micellization associated with coil-to-helix transitions based on polypeptide hybrid double hydrophilic rod-coil diblock copolymer. Biomacromolecules 2007;8:3871–8.
[180] He C, Zhao C, Chen X, Guo Z, Zhuang X, Jing X. Novel pH- and temperature-responsive block copolymers with tunable pH-responsive range. Macromol Rapid Commun 2008;29:490–7.
[181] He C, Zhao C, Guo X, Guo Z, Chen X, Zhuang X, Liu S, Jing X. Novel temperature- and pH-responsive graft copolymers composed of poly(L-glutamic acid) and poly(*N*-isopropylacrylamide). J Polym Sci Part A Polym Chem 2008;46:4140–50.
[182] Zhao C, Zhuang X, He C, Chen X, Jing X. Synthesis of novel thermo- and pH-responsive poly(L-lysine)-based copolymer and its micellization in water. Macromol Rapid Commun 2008;29:1810–6.
[183] Huang CJ, Chang FC. Polypeptide diblock copolymers: syntheses and properties of poly(*N*-isopropylacrylamide)-b-polylysine. Macromolecules 2008;41:7041–52.
[184] Lu H, Wang J, Bai Y, Lang JW, Liu S, Lin Y, Cheng J. Ionic polypeptides with unusual helical stability. Nat Commun 2011;2:206/1-9.
[185] Gabrielson NP, Lu H, Yin L, Li D, Wang F, Cheng J. Reactive and bioactive cationic a-helical polypeptide template for nonviral gene delivery. Angew Chem Int Ed 2012;51:1143–7.
[186] Osada K, Christie RJ, Kataoka K. Polymeric micelles from poly(ethylene glycol)-poly(amino acid) block copolymer for drug and gene delivery. J R Soc Interface 2009;6:S325–39.
[187] Cabral H, Nishiyama N, Kataoka K. Supramolecular nanodevices: from design validation to theranostic nanomedicine. Acc Chem Res 2011;44:999–1008.
[188] Akagi D, Oba M, Koyama H, Nishiyama N, Fukushima S, Miyata T, Nagawa H, Kataoka K. Biocompatible micellar nanovectors achieve efficient gene transfer to vascular lesions without cytotoxicity and thrombus formation. Gene Ther 2007;14:1029–38.
[189] Itaka K, Ohba S, Miyata K, Kawaguchi H, Nakamura K, Takato T, Chung UI, Kataoka K. Bone regeneration by regulated in vivo gene transfer using biocompatible polyplex nanomicelles. Mol Ther 2007;15:1655–62.
[190] Harada-Shiba M, Takamisawa I, Miyata K, Ishii T, Nishiyama N, Itaka K, Kangawa K, Yoshihara F, Asada Y, Hatakeyama K, Nagaya N, Kataoka K. Intratracheal gene transfer of adrenomedullin using polyplex nanomicelles attenuates monocrotaline-induced pulmonary hypertension in rats. Mol Ther 2009;17:1180–6.
[191] Uchida H, Miyata K, Oba M, Ishii T, Suma T, Itaka K, Nishiyama N, Kataoka K. Odd-even effect of repeating aminoethylene units in the side chain of *N*-substituted polyaspartamides on gene transfection profiles. J Am Chem Soc 2011;133:15524–32.
[192] Lai TC, Bae Y, Yoshida T, Kataoka K, Kwon GS. pH-sensitive multi-PEGylated block copolymer as a bioresponsive pDNA delivery vector. Pharm Res 2010;27:2260–73.
[193] Lee Y, Fukushima S, Bae Y, Hiki S, Ishii T, Kataoka K. A protein nanocarrier from charge-conversion polymer in response to endosomal pH. J Am Chem Soc 2007;129:5362–3.
[194] Lee Y, Miyata K, Oba M, Ishii T, Fukushima S, Han M, Koyama H, Nishiyama N, Kataoka K. Charge-conversion ternary polyplex with endosome disruption moiety: a technique for efficient and safe gene delivery. Angew Chem Int Ed 2008;47:5163–6.
[195] Fukushima S, Miyata K, Nishiyama N, Kanayama N, Yamasaki Y, Kataoka K. PEGylated polyplex micelles from triblock catiomers with spatially ordered layering of condensed pDNA and buffering units for enhanced intracellular gene delivery. J Am Chem Soc 2005;127:2810–1.
[196] Deng J, Gao N, Wang Y, Yi H, Fang S, Ma Y, Cai L. Self-assembled cationic micelles based on PEG-PLL-PLLeu hybrid polypeptides as highly effective gene vectors. Biomacromolecules 2012;13:3795–804.
[197] Li YY, Hua SH, Xiao W, Wang HY, Luo XH, Li C, Cheng SX, Zhang XZ, Zhuo RX. Dual-vectors of anti-cancer drugs and genes based on pH-sensitive micelles self-assembled from hybrid polypeptide copolymers. J Mater Chem 2011;21:3100–6.
[198] Takemoto H, Ishii A, Miyata K, Nakanishi M, Oba M, Ishii T, Yamasaki Y, Nishiyama N, Kataoka K. Polyion complex stability and gene silencing efficiency with a siRNA-grafted polymer delivery system. Biomaterials 2010;31:8097–105.
[199] Takae S, Miyata K, Oba M, Ishii T, Nishiyama N, Itaka K, Yamasaki Y, Koyama H, Kataoka K. PEG-detachable polyplex micelles based on disulfide-linked block catiomers as bioresponsive nonviral gene vectors. J Am Chem Soc 2008;130:6001–9.
[200] Miyata K, Kakizawa Y, Nishiyama N, Harada A, Yamasaki Y, Koyama H, Kataoka K. Block catiomer polyplexes with regulated densities of charge and disulfide cross-linking directed to enhance gene expression. J Am Chem Soc 2004;126:2355–61.
[201] Christie RJ, Miyata K, Matsumoto Y, Nomoto T, Menasco D, Lai TC, Pennisi M, Osada K, Fukushima S, Nishiyama N, Yamasaki Y, Kataoka K. Effect of polymer structure on micelles formed between siRNA and cationic block copolymer comprising thiols and amidines. Biomacromolecules 2011;12:3174–85.
[202] Oba M, Aoyagi K, Miyata K, Matsumoto Y, Itaka K, Nishiyama N, Yarnasaki Y, Koyama H, Kataoka K. Polyplex micelles with cyclic RGD peptide ligands and disulfide cross-links directing to the enhanced transfection via controlled intracellular trafficking. Mol Pharm 2008;5:1080–92.
[203] Sanjoh M, Miyata K, Christie RJ, Ishii T, Maeda Y, Pittella F, Hiki S, Nishiyama N, Kataoka K. Dual environment-responsive polyplex carriers for enhanced intracellular delivery of plasmid DNA. Biomacromolecules 2012;13:3641–9.
[204] Heffernan MJ, Murthy N. Disulfide-crosslinked polyion micelles for delivery of protein therapeutics. Ann Biomed Eng 2009;37:1993–2002.
[205] Naito M, Ishii T, Matsumoto A, Miyata K, Miyahara Y, Kataoka K. A phenylboronate-functionalized polyion complex micelle for ATP-triggered release of siRNA. Angew Chem Int Ed 2012;51:10751–5.
[206] Meng F, Zhong Z. Polymersomes spanning from nano- to microscales: advanced vehicles for controlled drug delivery and robust vesicles for virus and cell mimicking. J Phys Chem Lett 2011;2:1533–9.

[207] Meng F, Zhong Z, Feijen J. Stimuli-responsive polymersomes for programmed drug delivery. Biomacromolecules 2009;10:197–209.
[208] Sun J, Chen X, Deng C, Yu H, Xie Z, Jing X. Direct formation of giant vesicles from synthetic polypeptides. Langmuir 2007;23:8308–15.
[209] Sun J, Huang Y, Shi Q, Chen X, Jing X. Oxygen carrier based on hemoglobin/poly(L-lysine)-block-poly(L-phenylalanine) vesicles. Langmuir 2009;25:13726–9.
[210] Kim MS, Dayananda K, Choi EY, Park HJ, Kim JS, Lee DS. Synthesis and characterization of poly(L-glutamic acid)-block-poly(L-phenylalanine). Polymer 2009;50:2252–7.
[211] Holowka EP, Pochan DJ, Deming TJ. Charged polypeptide vesicles with controllable diameter. J Am Chem Soc 2005;127:12423–8.
[212] Holowka EP, Sun VZ, Kamei DT, Deming TJ. Polyarginine segments in block copolypeptides drive both vesicular assembly and intracellular delivery. Nat Mater 2007;6:52–7.
[213] Iatrou H, Frielinghaus H, Hanski S, Ferderigos N, Ruokolainen J, Ikkala O, Richter D, Mays J, Hadjichristidis N. Architecturally induced multiresponsive vesicles from formation of gene vehicles, well-defined polypeptides. Biomacromolecules 2007;8:2173–81.
[214] Dzwolak W, Muraki T, Kato M, Taniguchi Y. Chain-length dependence of alpha-helix to beta-sheet transition in polylysine: model of protein aggregation studied by temperature-tuned FTIR spectroscopy. Biopolymers 2004;73:463–9.
[215] Xu X, Yuan H, Chang J, He B, Gu Z. Cooperative hierarchical self-assembly of peptide dendrimers and linear polypeptides into nanoarchitectures mimicking viral capsids. Angew Chem Int Ed 2012;51:3130–3.
[216] Rodriguez-Hernandez J, Lecommandoux S. Reversible inside-out micellization of pH-responsive and water-soluble vesicles based on polypeptide diblock copolymers. J Am Chem Soc 2005;127:2026–7.
[217] Kishimura A, Koide A, Osada K, Yamasaki Y, Kataoka K. Encapsulation of myoglobin in PEGylated polyion complex vesicles made from a pair of oppositely charged block lonomers: a physiologically available oxygen carrier. Angew Chem Int Ed 2007;46:6085–8.
[218] Anraku Y, Kishimura A, Oba M, Yamasaki Y, Kataoka K. Spontaneous formation of nanosized unilamellar polyion complex vesicles with tunable size and properties. J Am Chem Soc 2010;132:1631–6.
[219] Osada K, Cabral H, Mochida Y, Lee S, Nagata K, Matsuura T, Yamamoto M, Anraku Y, Kishimura A, Nishiyama N, Kataoka K. Bioactive polymeric metallosomes self-assembled through block copolymer–metal complexation. J Am Chem Soc 2012;134:13172–5.
[220] Le Hellaye M, Fortin N, Guilloteau J, Soum A, Lecommandoux S, Guillaume SM. Biodegradable polycarbonate-b-polypeptide and polyester-b-polypeptide block copolymers: synthesis and nanoparticle formation towards biomaterials. Biomacromolecules 2008;9:1924–33.
[221] Sanson C, Schatz C, Le Meins JF, Brulet A, Soum A, Lecommandoux S. Biocompatible and biodegradable poly(trimethylene carbonate)-b-poly (L-glutamic acid) polymersomes: size control and stability. Langmuir 2010;26:2751–60.
[222] Sanson C, Le Meins JF, Schatz C, Soum A, Lecommandoux S. Temperature responsive poly(trimethylene carbonate)-block-poly(L-glutamic acid) copolymer: polymersomes fusion and fission. Soft Matter 2010;6:1722–30.
[223] Oliveira H, Perez-Andres E, Thevenot J, Sandre O, Berra E, Lecommandoux S. Magnetic field triggered drug release from polymersomes for cancer therapeutics. J Control Release 2013;169:165–70.
[224] Lee HJ, Bae Y. Cross-linked nanoassemblies from poly(ethylene glycol)-poly(aspartate) block copolymers as stable supramolecular templates for particulate drug delivery. Biomacromolecules 2011;12:2686–96.
[225] Zhao C, Gao X, He P, Xiao C, Zhuang X, Chen X. Facile synthesis of thermo- and pH-responsive biodegradable microgels. Colloid Polym Sci 2011;289:447–51.
[226] Zhao C, He P, Xiao C, Gao X, Zhuang X, Chen X. Synthesis of temperature and pH-responsive crosslinked micelles from polypeptide-based graft copolymer. J Colloid Interface Sci 2011;359:436–42.
[227] Shi F, Ding J, Xiao C, Zhuang X, He C, Chen L, Chen X. Intracellular microenvironment responsive PEGylated polypeptide nanogels with ionizable cores for efficient doxorubicin loading and triggered release. J Mater Chem 2012;22:14168–79.
[228] Nowak AP, Breedveld V, Pakstis L, Ozbas B, Pine DJ, Pochan D, Deming TJ. Rapidly recovering hydrogel scaffolds from self-assembling diblock copolypeptide amphiphiles. Nature 2002;417:424–8.
[229] Nowak AP, Breedveld V, Pine DJ, Deming TJ. Unusual salt stability in highly charged diblock co-polypeptide hydrogels. J Am Chem Soc 2003;125:15666–70.
[230] Deming TJ. Polypeptide hydrogels via a unique assembly mechanism. Soft Matter 2005;1:28–35.
[231] Pakstis LM, Ozbas B, Hales KD, Nowak AP, Deming TJ, Pochan D. Effect of chemistry and morphology on the biofunctionality of self-assembling diblock copolypeptide hydrogels. Biomacromolecules 2004;5:312–8.
[232] Nowak AP, Sato J, Breedveld V, Deming TJ. Hydrogel formation in amphiphilic triblock copolypeptides. Supramol Chem 2006;18:423–7.
[233] Li Z, Deming TJ. Tunable hydrogel morphology via self-assembly of amphiphilic pentablock copolypeptides. Soft Matter 2010;6:2546–51.
[234] Song B, Song J, Zhang S, Anderson MA, Ao Y, Yang CY, Deming TJ, Sofroniew MV. Sustained local delivery of bioactive nerve growth factor in the central nervous system via tunable diblock copolypeptide hydrogel depots. Biomaterials 2012;33:9105–16.
[235] Choi YY, Joo MK, Sohn YS, Jeong B. Significance of secondary structure in nanostructure formation and thermosensitivity of polypeptide block copolymers. Soft Matter 2008;4:2383–7.
[236] Oh HJ, Joo MK, Sohn YS, Jeong B. Secondary structure effect of polypeptide on reverse thermal gelation and degradation of L/DL-poly(alanine)-poloxamer-L/DL-poly(alanine) copolymers. Macromolecules 2008;41:8204–9.
[237] Choi BG, Park MH, Cho SH, Joo MK, Oh HJ, Kim EH, Park K, Han DK, Jeong B. Thermal gelling polyalanine-poloxamine-polyalanine aqueous solution for chondrocytes 3D culture: initial concentration effect. Soft Matter 2011;7:456–62.
[238] Choi BG, Park MH, Cho SH, Joo MK, Oh HJ, Kim EH, Park K, Han DK, Jeong B. In situ thermal gelling polypeptide for chondrocytes 3D culture. Biomaterials 2010;31:9266–72.
[239] Jeong Y, Joo MK, Bahk KH, Choi YY, Kim HT, Kim WK, Lee HJ, Sohn YS, Jeong B. Enzymatically degradable temperature-sensitive polypeptide as a new in-situ gelling biomaterial. J Control Release 2009;137:25–30.
[240] Chen Y, Pang XH, Dong CM. Dual stimuli-responsive supramolecular polypeptide-based hydrogel and reverse micellar hydrogel mediated by host–guest chemistry. Adv Funct Mater 2010;20:579–86.
[241] Chen Y, Dong CM. pH-sensitive supramolecular polypeptide-based micelles and reverse micelles mediated by hydrogen-bonding interactions or host-guest chemistry: characterization and in vitro controlled drug release. J Phys Chem B 2010;114:7461–8.
[242] Zhao C, Zhuang X, He P, Xiao C, He C, Sun J, Chen X, Jing X. Synthesis of biodegradable thermo- and pH-responsive hydrogels for controlled drug release. Polymer 2009;50:4308–16.
[243] Zhang Z, Chen L, Deng M, Bai Y, Chen X, Jing X. Biodegradable thermo- and pH-responsive hydrogels for oral drug delivery. J Polym Sci Part A Polym Chem 2011;49:2941–51.
[244] Peng H, Xiao Y, Mao X, Chen L, Crawford R, Whittaker AK. Amphiphilic triblock copolymers of methoxy-poly(ethylene glycol)-b-poly(L-lactide)-b-poly(L-lysine) for enhancement of osteoblast attachment and growth. Biomacromolecules 2009;10:95–104.
[245] Zhang P, Wu H, Wu H, Lu Z, Deng C, Hong Z, Jing X, Chen X. RGD-conjugated copolymer incorporated into composite of poly(lactide-co-glycotide) and poly(L-lactide)-grafted nanohydroxyapatite for bone tissue engineering. Biomacromolecules 2011;12:2667–80.
[246] Zhang K, Zhang Y, Yan S, Gong L, Wang J, Chen X, Cui L, Yin J. Repair of an articular cartilage defect using adipose-derived stem cells loaded on a polyelectrolyte complex scaffold based on poly(L-glutamic acid) and chitosan. Acta Biomater 2013;9:7276–88.
[247] Yin M, Yuan Y, Liu C, Wang J. Development of mussel adhesive polypeptide mimics coating for in-situ inducing re-endothelialization of intravascular stent devices. Biomaterials 2009;30:2764–73.
[248] Li B, Jiang B, Boyce BM, Lindsey BA. Multilayer polypeptide nanoscale coatings incorporating IL-12 for the prevention of biomedical device-associated infections. Biomaterials 2009;30:2552–8.
[249] Jiang ZK, Koh SBS, Sato M, Atanasov IC, Johnson M, Zhou ZH, Deming TJ, Wu L. Engineering polypeptide coatings to augment gene transduction and in vivo stability of adenoviruses. J Control Release 2013;166:75–85.
[250] Yin L, Song Z, Kim KH, Zheng N, Gabrielson NP, Cheng J. Non-viral gene delivery via membrane-penetrating, mannose-targeting supramolecular self-assembled nanocomplexes. Adv Mater 2013;25:3063–70.
[251] Cheng R, Meng F, Deng C, Klok HA, Zhong Z. Dual and multi-stimuli responsive polymeric nanoparticles for programmed site-specific drug delivery. Biomaterials 2013;34:3647–57.