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Synthetic polypeptides: from polymer design to supramolecular assembly and biomedical application

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Synthetic polypeptides from the ring-opening polymerization of *N*-carboxyanhydrides (NCAs) are one of the most important biomaterials. The unique features of these synthetic polypeptides, including their chemical diversity of side chains and their ability to form secondary structures, enable their broad applications in the field of gene delivery, drug delivery, bio-imaging, tissue engineering, and antimicrobials. In this review article, we summarize the recent advances in the design of polypeptide-based supramolecular structures, including complexes with nucleic acids, micelles, vesicles, hybrid nanoparticles, and hydrogels. We also highlight the progress in the chemical design of functional polypeptides, which plays a crucial role to manipulate their assembly behaviours and optimize their biomedical performances. Finally, we conclude the review by discussing the future opportunities in this field, including further studies on the secondary structures and cost-effective synthesis of polypeptide materials.

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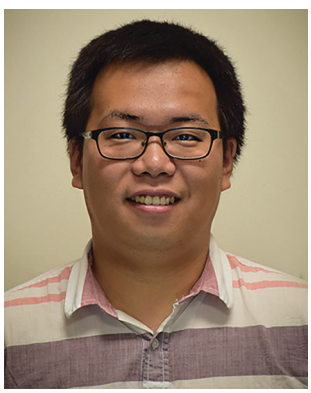
1. Introduction

Proteins, as one of the most important biomacromolecules, not only provide structural support for cells, tissues, and organs, but also participate in a myriad of cellular processes including the catalysis of biochemical reactions, regulation of cellular signals, and transportation of molecules.[1] The versatile functions of proteins originate from their higher ordered structures, which are constructed through hydrogen bonding (H-bonding) within the protein backbones and other non-covalent molecular



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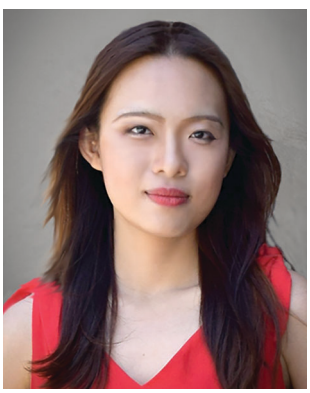

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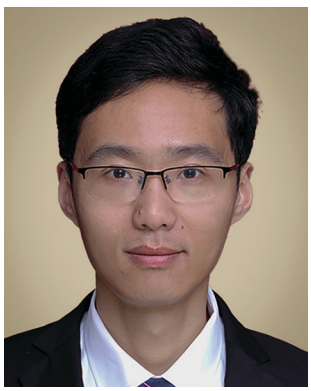
forces from the amino acid side chains (*e.g.*, Coulomb forces and hydrophobic interactions).[1–3] Inspired by nature, numerous efforts have been devoted to the synthesis of protein mimics, which aim to not only construct the higher ordered structures synthetically, but also produce materials for biomedical applications.

Among synthetic protein-mimicking materials, polypeptides are the most studied due to their backbone being similar to those in naturally occurring proteins (*i.e.*, peptide bonds). Furthermore, thanks to their biocompatibility and biodegradability, polypeptide materials have been widely used in various biomedical applications such as drug delivery, gene delivery, and tissue engineering.[4–9] Compared with typical polymers, polypeptides exhibit a great chemical diversity of side chains, having a library of twenty-one natural amino acids and numerous non-natural amino acids one may choose from.[5,6] Functional groups including charged species, sugar moieties, reactive handles, and trigger-responsive units can be easily incorporated into polypeptide materials. In addition, one unique feature of polypeptides is their ability to adopt secondary structures (*e.g.*, α-helices and β-sheets) through intramolecular hydrogen bonds (H-bonds) within peptide backbones. The formation of these secondary structures enables interesting conformation-specific self-assembly behaviours and bioactivity of polypeptides.[5,7,8,10]

Currently, polypeptides are mainly obtained through three methods: microbial synthesis,[11] solid phase peptide synthesis (SPPS),[12] and ring-opening polymerization (ROP) of *N*-carboxyanhydrides (NCAs).[13] While the first two methods are able to produce monodisperse peptide materials with sequence control,



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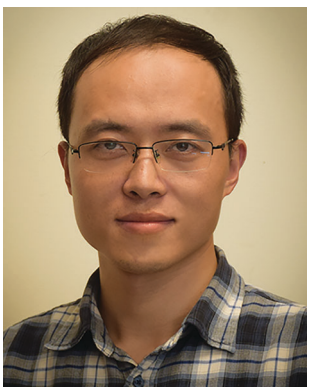

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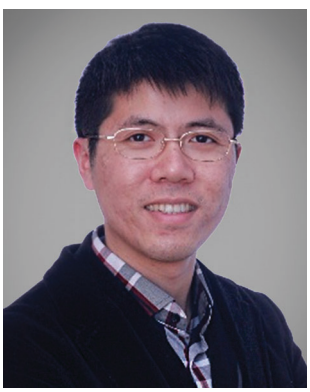

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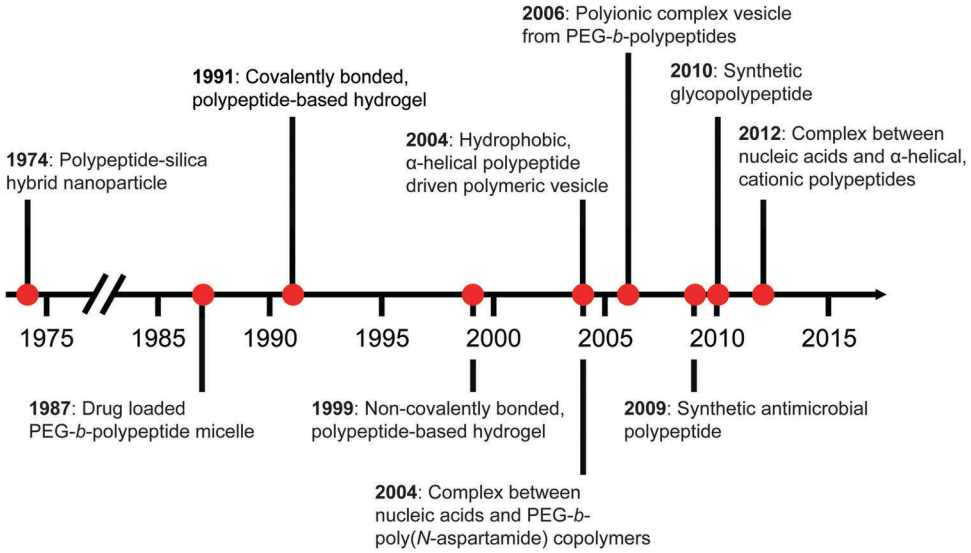
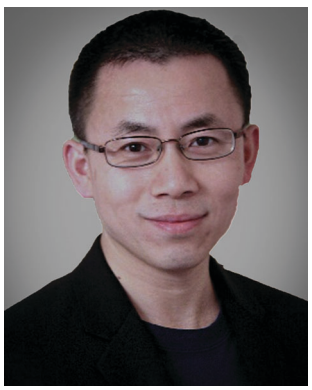


Fig. 1 Timeline summarizing the development of various synthetic polypeptide-based supramolecular structures from NCA chemistry.

the microbial synthesis is only useful for the preparation of peptides with natural amino acid residues, and SPPS is typically limited to short peptides (<50 residues) with low yields.[6] While the ROP of NCAs is known for generating polydisperse polypeptides without precise sequence control, it does have the advantages of offering considerable chemical diversity beyond the twenty-one natural amino acids and enables the large scale synthesis of polypeptides.[5] Advances in NCA chemistry have allowed the design of various supramolecular structures based on polypeptides, such as nucleic acid complexes, micelles, vesicles, hydrogels, and hybrid nanoparticles (NPs) (Fig. 1). Several excellent review articles have been published to highlight the progress in NCA chemistry,[13,14] trigger-responsive polypeptide design,[15,16] self-assembly behaviours,[5,10] and biomedical applications of polypeptides.[4–7,17] In this article, we focus on synthetic polypeptides obtained from the ROP of NCAs, aiming to highlight the most recent advances in the studies of polypeptide-based supramolecular structures and their biomedical applications in the last five years. The chemical design of functional polypeptides is also reviewed and discussed throughout the article, which enables controlled assembly behaviours and essential functionalities for the optimized performances of polypeptide materials.



Jianjun Cheng

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2. Synthetic strategy

Although NCAs were first synthesized back in 1906,[18] polypeptides as versatile materials with desired functionalities were not intensively studied until recent years. Much effort has been devoted to the development of living ROP of NCAs, the synthesis of new functional NCA monomers, and the manipulation of the polypeptide conformation. In this section, we review the synthetic strategies for the preparation of polypeptides with well-defined structures, desired side-chain functionalities, and controlled secondary structures.

2.1 Controlled ROP of NCAs

Controlled polymerization of NCA monomers was first developed by Deming in 1997, when he used organometallic initiators to suppress chain transfer and termination reactions.[19] Well-defined block copolypeptides were successfully prepared with Ni- or Co-based initiators.[19,20] Following this work, several living initiation systems have been developed, including an ammonium salt initiation system (Schlaad, 2003),[21] a high vacuum setup (Hadjichristidis, 2004),[22] a low temperature technique (Giani, 2004),[23] organosilicon initiators (Cheng, 2007),[24–26] and a nitrogen flow strategy (Wooley, 2013) (Fig. 2).[27] These living ROP systems have enabled the preparation of polypeptides with predictable molecular weights (MWs) and a narrow polydispersity index (PDI).

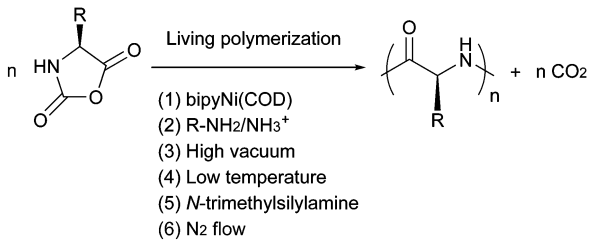

Fig. 2 Summary of living ring opening polymerization (ROP) systems of NCAs.

2.2 Functional NCA monomers

In parallel with the development of controlled ROP of NCA, new NCA monomers were synthesized to prepare polypeptides with functional, non-natural side chains. These new NCA monomers are designed to have either functional groups or reactive handles ready for further post-polymerization modification.[14] In the former case, the functional groups are compatible with the ROP of NCA (or temporarily protected). Polypeptides bearing various functionalities, such as oligo(ethylene glycol)s (OEGs),[28,29] sugar moieties,[30–32] phosphate groups,[33–35] and trigger-responsive units,[29,32,36] are therefore directly obtained after polymerization. In the latter case, reactive units including alkenes,[37–39] alkynes,[40,41] and azides[42] are incorporated into NCA monomers, which may be further reacted with other functional groups after polymerization. The post-modification strategy allows the synthesis of a series of functional polypeptides from one NCA monomer, which is important for the study of structure–function relationship in materials design. In one instance, thirty-one cationic polypeptides were generated from a single NCA monomer, γ-(4-vinylbenzyl)-L-glutamate NCA, to identify the best-performing polypeptide with appropriate hydrophilicity–hydrophobicity balance and charge density for gene delivery applications.[43] Functional NCA monomers and post-modification strategies have already been elegantly summarized in a recent review by Deming.[14] In Fig. 3, we show some representative NCA monomers.

2.3 Secondary structure of polypeptides

The stabilization, regulation, and applications of the α-helical conformation of polypeptides have been intensively studied. Previous works have demonstrated that side-chain interactions have a profound effect on the secondary structures of polypeptides. While hydrophobic interactions usually stabilize the α-helices, side-chain electrostatic interactions often disrupt the helical conformation due to charge repulsion. For instance, poly(L-glutamic acid) (PLG) and poly(L-lysine) (PLL) are well-known ionic polypeptides with random coil conformations when the side chains are charged.[44] Therefore, water-soluble, α-helical polypeptides, which are important for biomedical applications, were designed and synthesized through the elimination of side-chain charge repulsions (Fig. 4). For example, Deming and co-workers prepared α-helical polypeptides with good water solubility by attaching non-ionic, hydrophilic OEG segments on the side chains of polypeptides.[28] A similar strategy was also used with sugar as the non-ionic water-soluble moieties.[30] Interestingly, Krannig and Schlaad demonstrated that the incorporation of only 10 mol% sugar units can significantly enhance the helical stability and water solubility of PLG at acidic pH.[45] In 2011, Cheng, Lin, and co-workers reported the first ionic, α-helical polypeptides by elongating the side-chain lengths.[46] The resulting ionic polypeptides have decreased side-chain charge repulsions and enhanced side-chain hydrophobic interactions compared with traditional polypeptides like PLG and PLL, and therefore adopted stable α-helical structures.

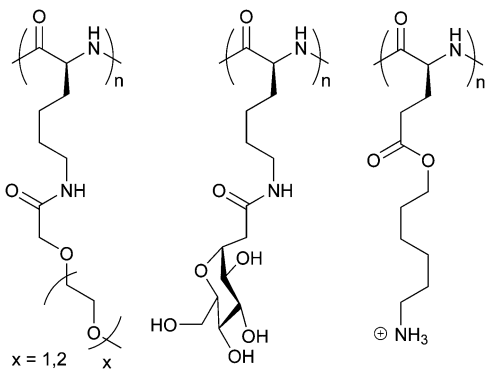

Fig. 4 Chemical structures of representative water soluble, α-helical polypeptides bearing non-ionic side-chain oligo(ethylene glycol)s, non-ionic side-chain sugars, or elongated side chains with terminal charges.

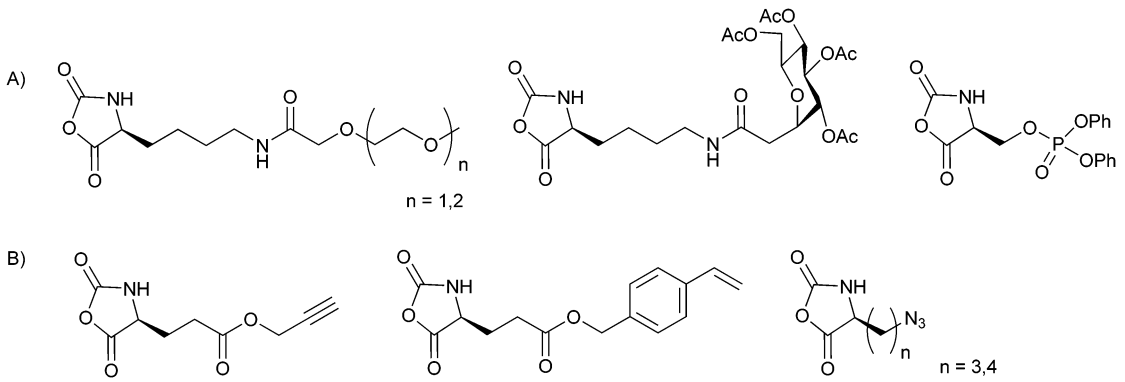

Fig. 3 Chemical structures of representative NCA monomers bearing functional groups (A) or reactive handles (B).

Recent studies have demonstrated that the α-helical polypeptides exhibited unique conformation-specific properties that their random coiled analogs did not have.[43,47–49] Detailed helix-associated properties will be discussed in Sections 3.1, 4.2, and 5.1. Motivated by this discovery, polypeptides with helix-to-coil transition behaviours were designed and synthesized, aiming to control the performance of polypeptides through trigger-responsive conformational changes (Fig. 5A). One common strategy to design such polypeptides is to change their charge density, since side-chain charge repulsions are known to destabilize the α-helices. For instance, the side-chain charges of PLG can be shielded by protonation,[45] esterification,[36,50] and metal coordination,[51] leading to the α-helical conformation. Once the protons, ester groups, or the metal ions are removed, the exposure of side-chain charges induces a helix-to-coil transition (Fig. 5B). The second strategy to destabilize the α-helical structure is to increase the polarity of the polypeptide side chains. Kramer and Deming reported the redox-responsive conformational changes of poly(L-cysteine) (PLCys) and poly(L-homocysteine) derivatives, where the oxidation of side-chain thioethers to sulfones resulted in the helix-to-coil transition (Fig. 5C).[32,52] Very recently, Cheng, Yin, Ferguson, and others demonstrated the modulation of polypeptide conformation through donor–acceptor transformation of the side-chain H-bonding ligands. The protonation of side-chain triazoles changed their H-bonding pattern from a donor/acceptor pair to double donors, resulting in the changes in conformation from coil to α-helix. The conformational transition was conclusively demonstrated with both experimental and simulation-based methods (Fig. 5D).[53]

3. Polypeptide/nucleic acid complexes

Gene therapy is the modulation of gene expression by the transfer of genetic materials into specific cells to treat diseases.[54] Typical genetic materials include exogenous nucleic acids such as DNA, messenger RNA (mRNA), small interfering RNA (siRNA), and microRNA (miRNA). Successful gene therapy requires the identification of a therapeutic gene and the transfer of that gene into targeted cells with high efficiency.[55] While viral vectors are highly adopted due to their efficiency, they present serious safety concerns, low loading capacity, and scale up difficulties.[56] Non-viral vectors, including cationic polymers and lipids, are therefore considered as attractive alternatives compared to viral vectors due to their biocompatibility.[7,57] Cationic polypeptides like PLL are one of the first classes of polymers used as non-viral vectors. Previous works demonstrated their ability to condense nucleic acids into complexes.[58,59]

However, PLL-based complexes often showed low transfection efficiency due to their low ability to escape from endosomes.[57] Peptides were therefore often used as supporting materials in gene delivery to add functionalities to other materials.[7,43] Recently, two new approaches were reported to solve the endosomal trapping

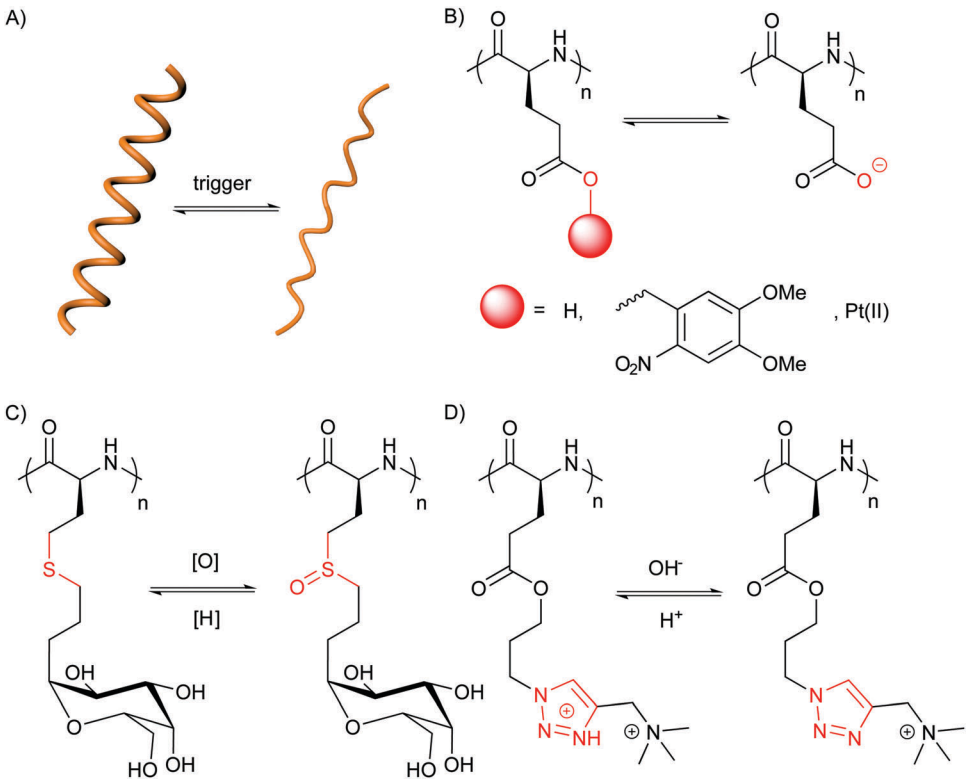

Fig. 5 Synthetic polypeptides with trigger-responsive helix-to-coil transition behaviours. (A) Schematic illustration showing the triggered conformational changes of polypeptides. (B–D) Various strategies to manipulate the conformation of polypeptides based on the changes in side-chain charge density (B), side-chain polarity (C), and side-chain H-bonding pattern (D). The trigger responsive units are highlighted in red.

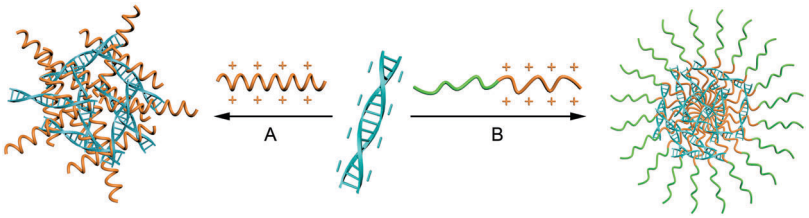

Fig. 6 Schematic illustration showing the complex formation of nucleic acids with α-helical, cationic polypeptides (A) and PEG-*b*-poly(*N*-aspartamide) copolymers (B).

issue of polypeptide materials (Fig. 6). In the first approach, α-helical, cationic polypeptides were used to not only mediate the cell internalization through a non-endocytotic pathway, but also disrupt the endosomal membranes when the complexes were endocytosed.[43] In the second approach, poly(*N*-aspartamide) derivatives bearing low pK_a amino groups were prepared to induce a proton sponge effect for the endosomal escape of complexes.[60] After PEGylation, the poly(ethylene glycol) (PEG)-*b*-poly(*N*-aspartamide) copolymers were used to form polyionic complex (PIC) micelles with nucleic acids for gene delivery.

3.1 Complexes between α-helical, cationic polypeptides and nucleic acids

The use of α-helical, cationic polypeptides as non-viral gene delivery vectors was motivated by the membrane activity of cell penetrating peptides (CPPs). CPPs are able to promote cell internalization and endosomal escape of other vectors, resulting in enhanced transfection efficiency.[61] During the membrane transduction of CPPs, they often form α-helical structures that are closely related to their membrane penetration ability.[62] However, CPPs are often too short in length and lack adequate cationic charges, which limit their application as single delivery vectors. Therefore, the design of α-helical polypeptide vectors can potentially combine the beneficial aspects of both PLL (condensation of nucleic acids as stand-alone vectors) and CPPs (membrane activity). The chemical structures of representative α-helical, cationic polypeptides are summarized in Fig. 7A.

3.1.1 Helix-associated cell-penetrating ability. Based on the development of α-helical, ionic polypeptides,[46] the Cheng group reported a synthetic method to access a library of thirty-one cationic polypeptides with various side-chain amino groups.[43] These polypeptides showed stable α-helical conformations over broad pH ranges (Fig. 8A), but had different side-chain hydrophobic/hydrophilic balances and charge densities. After screening,

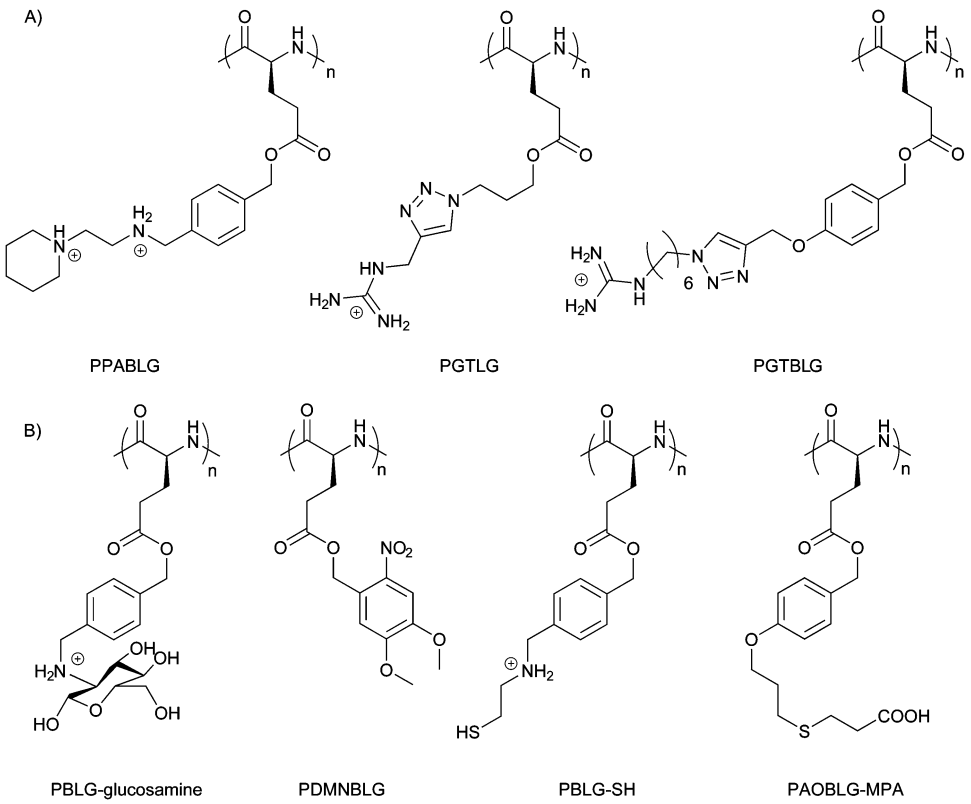

Fig. 7 Chemical structures of α-helical, cationic polypeptides (A) and other polypeptide segments as supporting components (B) for gene delivery.

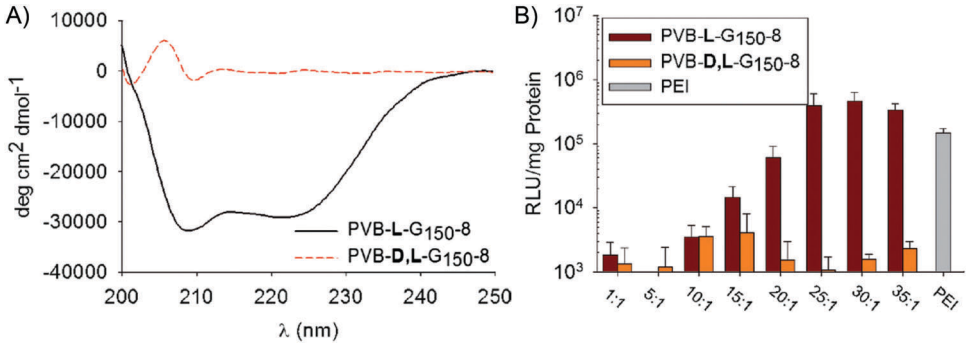

Fig. 8 Helix-associated membrane activity of cationic polypeptides. (A) Circular dichroism spectra of α-helical PPABLG (called PVB-L-G-8 in the figure) and random-coiled PPABDLG (called PVB-D,L-G-8 in the figure) in water. (B) *In vitro* transfection efficiency of DNA complexes with PPABLG and PPABDLG in COS-7 cells at various polypeptide/DNA weight ratios. PEI (25 kDa) at a polymer/DNA weight ratio of 7.5 : 1 was used as a control. Reprinted with permission from ref. 43. Copyright 2012 Wiley-VCH Verlag GmbH & Co. KGaA.

poly(γ-(4-((2-(piperidin-1-yl)ethyl)aminomethyl)benzyl)-L-glutamate) (PPABLG, also called PVBLG-8) (Fig. 7A) was identified as the most potent polypeptide in the library; outperforming the commercially available transfection agent polyethylenimine (PEI, 25 kDa) by 12-fold in terms of transfection efficiency (Fig. 8B). In comparison, its racemic analog PPABDLG (or PVBDLG-8), which adopts a random coil conformation, showed negligible transfection efficiency; verifying the critical role the α-helical structure plays in the enhanced performance (Fig. 8A and B). Further confocal microscopy studies confirmed the endosomal escape of PPABLG through the disruption of endosomal membranes.

The mechanism of helix-associated membrane activity was elucidated with computer simulations recently.[63] The α-helical conformation enables the polypeptides to adopt a core–shell structure, with the peptide backbone core surrounded by side chains that are terminated with cationic groups. The mobility and flexibility of side chains are crucial for the membrane activity of polypeptides, allowing deformable shapes and large side-chain rearrangements during polypeptide–membrane interactions.[63] Random coiled polypeptides, on the other hand, fail to adopt such well-defined adaptive structures, leading to their low membrane activity.

3.1.2 Structure optimization of α-helical polypeptide. The balance of hydrophobic and hydrophilic contents has significant effects on the membrane activity of cell-penetrating polymers.[64,65] Cheng and co-workers developed a class of helical poly(arginine) mimics (HPRMs) based on poly(γ-(3-(4-(guanidinomethyl)-1*H*-1,2,3-triazol-1-yl)propyl)-L-glutamate) (PGTLG) with different hydrophobic side chains (Fig. 7A).[66] Compared with other guanidine-rich CPP mimics with non-peptide backbones that lack ordered secondary structures (such as oligocarbamates[67] and peptoids[68]), HPRMs have both helicity and hydrophobicity incorporated into the design of cationic polymers, thus providing a platform to elucidate structure–property relationship at the molecular level. The results indicated that the elongation of the side chain lengths (P1–P8, Fig. 9A) or the incorporation of hydrophobic alkyl chains at an appropriate fraction (10 mol%) (P9–P14, Fig. 9B) led to significantly improved membrane permeability, further substantiating the need for maintaining the balance between hydrophobic moieties and cationic charges in the design of cell-penetrating polymers (Fig. 9C). The top performing HPRM, P14, outperformed commercial CPPs such as Arg9 and TAT by 1–2 orders of magnitude in terms of cell penetration potency (Fig. 9D). Furthermore, P14 was used as a molecular transporter to deliver both DNA and siRNA (Fig. 9E). Similar side-chain hydrophobicity-related cell-penetrating ability was also reported in another polypeptide system based on poly(γ-(4-(1-(6-guanidinohexyl)-1*H*-1,2,3-triazol-1-yl)methoxybenzyl)-L-glutamate) (PGTBLG) (Fig. 7A).[41]

Additionally, the impact of charge type on the cell-penetrating ability was also studied. Compared with polypeptides with amino side-chains, the guanidine-based polypeptides possessed superior membrane activity,[41] likely due to stronger binding with the phosphate anions of DNA molecules through additional bidentate H-bonds.[69] Phosphonium was also studied as the side-chain cation of polypeptides, which exhibited low toxicity.[70]

3.1.3 Incorporation of other functionalities. Followed by the development of PPABLG as non-viral vectors, various functionalities have been incorporated into this system by the Cheng group, aiming to achieve safe and efficient gene delivery. For instance, a ternary complex system was developed by combining PPABLG with a polypeptide bearing glucosamine side chains, poly(γ-(4-(glucosamine methyl)benzyl)-L-glutamate) (PBLG-glucosamine, Fig. 7B),[71] which significantly improved the biocompatibility without compromising the membrane activity. The same group also designed a series of PEG–PPABLG copolymers with various molecular architectures (diblock, triblock, graft, and star) and investigated their structure related membrane activity.[72] The star-shaped copolymers displayed the highest membrane activity and showed the most potent gene transfection efficiency, likely due to the multivalent polypeptide–membrane interactions. In an attempt to lower the toxicity associated with the poly(cation)s and facilitate the DNA unpacking after transfection, the Cheng group prepared random copolypeptides consisting of PPABLG and poly(γ-(4,5-dimethoxy-2-nitrobenzyl)-L-glutamate) (PDMNBLG, Fig. 7B),[36] a polypeptide with light-responsive side-chain cleavage behaviours. While the random copolypeptides exhibited similar membrane activity compared with PPABLG homopolypeptides, the PDMNBLG residues

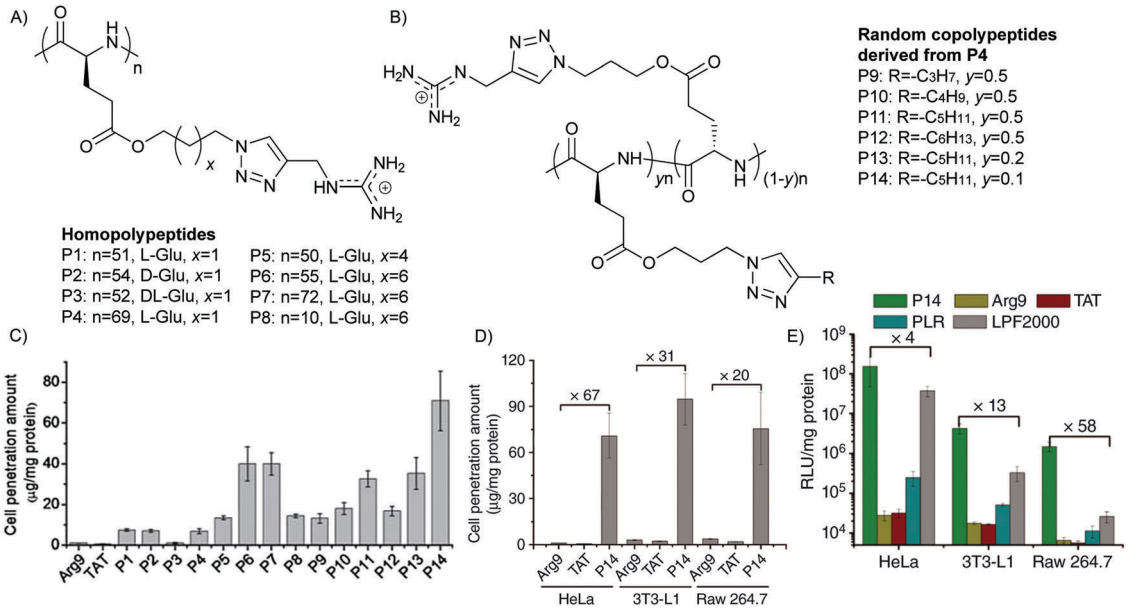

Fig. 9 Impact of helicity and hydrophobicity/hydrophilicity balance on the membrane activity of cationic polypeptides. (A and B) Chemical structures of helical poly(arginine) mimics (HPRMs). (C) Uptake of Rhodamine B (RhB) labelled HPRMs in HeLa cells following incubation at 37 °C for 2 h. Commercial CPPs including Arg9 and TAT were used as control groups. (D) Uptake of RhB labelled P14 in HeLa, 3T3-L1, and Raw 264.7 cells following incubation at 37 °C for 2 h. Arg9 and TAT were used as controls. (E) Transfection efficiencies of P14/DNA complexes in HeLa, 3T3-L1, and Raw 264.7 cells. Arg9, TAT, poly(L-arginine) (PLR), and lipofectamine 2000 (LPF2000) were used as controls. Reprinted with permission from ref. 66. Copyright 2013 Royal Society of Chemistry.

converted into anionic PLG units upon UV irradiation, leading to the disruption of the α-helical conformation and the decrease of charge density. Therefore, the UV treatment in the post-transfection state facilitated the intracellular release of DNA and eliminated long-term material toxicity.

Compared with DNA, the delivery of siRNA using PPABLG is more difficult due to the insufficient charges of siRNA and the rigidity of polypeptides.[73,74] In order to achieve efficient siRNA delivery, Cheng, Yin, and co-workers used both chemical and electrostatic cross-linking strategies to prepare stable polypeptide/siRNA complexes without compromising the α-helical secondary structure. In the first approach, random copolypeptides with PPABLG and poly(γ-(4-((2-mercaptoethyl)aminomethyl)benzyl)-L-glutamate) (PBLG-SH, Fig. 7B) were prepared, which were used to stabilize the complexes with siRNA through the oxidation of side-chain thiol groups from PBLG-SH into disulfides. The resulting complexes showed good protection of siRNA against nuclease digestion, and maintained excellent membrane activity to deliver siRNA into the targeted cells.[73] In the second approach, an α-helical, anionic polypeptide, poly(γ-(4-((2-carboxyethyl)-thiopropoxy)benzyl)-L-glutamate) (PAOBLG-MPA), was used as the electrostatic cross-linker, which also stabilized the PPABLG/siRNA complexes.[74] The successful delivery of siRNA against tumour necrosis factor-α (TNF-α) was verified by the effective systemic downregulation of TNF-α.

PPABLG was also used as a membrane-active component in a supramolecular self-assembled nanoparticle (SSNP) system for the oral delivery of TNF-α siRNA.[75] The SSNPs were constructed through the electrostatic and hydrophobic interactions of several building blocks, including PPABLG for membrane activity, oleyl trimethyl chitosan (OTMC) for DNA condensation, oleyl-PEG-mannose (OPM) for mannose targeting, oleyl-PEG-cysteine (OPC) for cell binding, sodium tripolyphosphate (TPP) for electrostatic cross-linking, and TNF-α siRNA as the cargo (Fig. 10A). Specifically, PPABLG mediated efficient membrane permeation of SSNPs in both normal enterocytes and M cells, resulting in an augmented intestinal absorption level of the siRNA cargos (Fig. 10B). In addition, PPABLG also facilitated the uptake level of SSNPs and the subsequent RNA interference (RNAi) in macrophages. Due to the infiltration of transfected gut-associated macrophages into systemic reticulo-endothelial tissues, the authors demonstrated successful systemic TNF-α knockdown against lipopolysaccharide (LPS)-induced hepatic injury (Fig. 10C).[75] In a similar approach, PPABLG was incorporated into the self-assembled nanocomplexes for DNA delivery.[76]

3.2 Complexes between PEG-*b*-cationic polypeptides and nucleic acids

Inspired by the correlation between the buffering capacity of amino groups and the endosomal escape (*e.g.*, the comparison between PLL and PEI), Kataoka and co-workers designed *N*-substituted polyaspartamides bearing various amino side chains with tunable pK_a values.[77–79] Coupled with the studies on the PIC micelles from PEG-*b*-catiomer,[80] the authors developed a series of PEG-*b*-poly(*N*-aspartamide) copolymers as non-viral gene delivery carriers. In the presence of PEG segments, the resulting complexes with DNA exhibited improved solubility and enhanced nuclease resistance.[57,80] The chemical structures of PEG-*b*-poly(*N*-aspartamide) copolymers are summarized in Fig. 11.

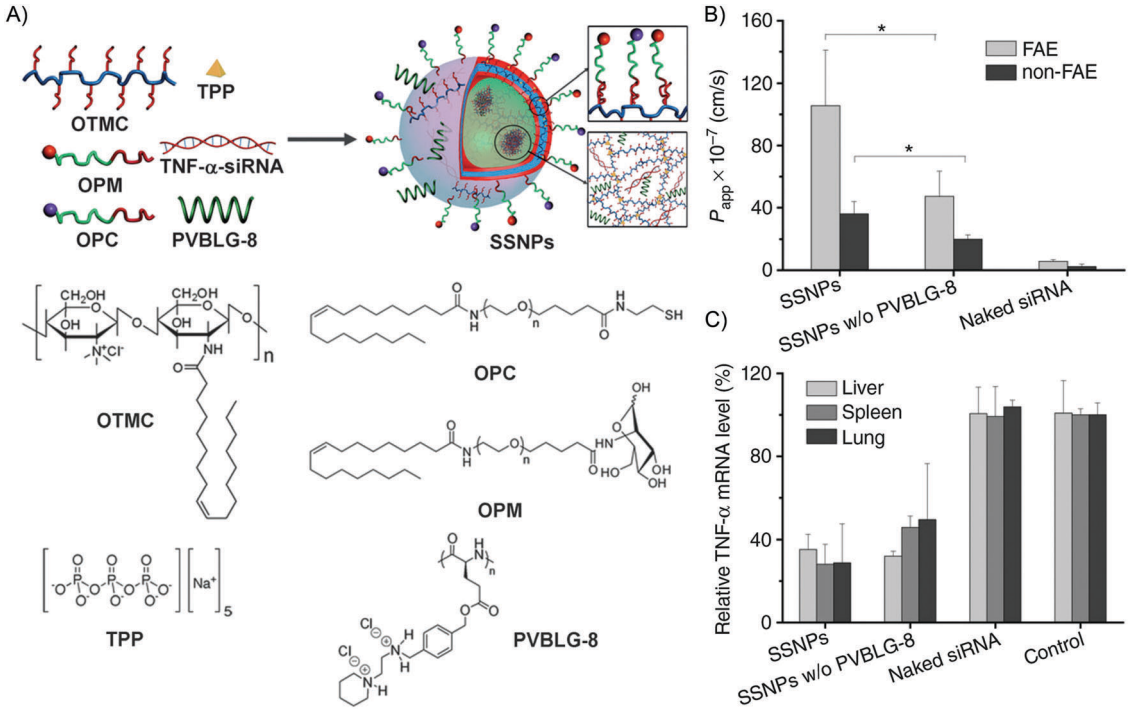

Fig. 10 The use of α-helical, cationic polypeptides as the membrane-active components in a supramolecular self-assembled nanoparticle (SSNP) system. (A) Schematic illustration showing the formation of SSNPs for siRNA delivery. (B) Intestinal absorption of SSNPs containing Cy3 labelled siRNA. Results are shown by the apparent permeability coefficient (P_{app}) of Cy3-siRNA across human follicle-associated epithelia (FAE) and non-FAE models. (C) Relative TNF-α mRNA levels in mouse liver, spleen, and lung 24 h after oral gavage of SSNPs at 200 μg kg^{-1}. Reprinted with permission from ref. 75. Copyright 2013 Wiley-VCH Verlag GmbH & Co. KGaA.

Among these poly(*N*-aspartamide)s, poly(*N*′-(*N*-(2-aminoethyl)-2-aminoethyl)aspartamide) (PAsp(DET)) with two aminoethylene units on the side chains is mostly studied due to its high buffering capacity at pH 5.5, which is directly related to the endosomal escape ability (Fig. 11).[79]

Over the last five years, the Kataoka group has put lots of efforts to optimize the existing PEG-*b*-poly(*N*-aspartamide)-based PIC micelles, including the optimization of the copolymer structures, the stabilization of the complexes, and the introduction of other functionalities. The incorporated components and their supporting roles in the complexes are summarized in Table 1.

3.2.1 Structure optimization of PEG-*b*-cationic polypeptide. Poly(*N*-aspartamide)s bearing different numbers of side-chain aminoethylene units showed distinct pH-dependent protonation behaviours. It is the buffering capacity at endosomal pH (~5.5) that determines the transfection efficiency of poly(*N*-aspartamide)s.[79] In a recent study, Kataoka and co-workers demonstrated that the charge status of side chains at pH 7.4 was crucial for the condensation of siRNA.[81] PAsp(DET), with only one cation on the side-chain at pH 7.4, was not able to form stable complexes with siRNA in serum containing media; while PAsp(TEP), which contains two positive charges at pH 7.4 (Fig. 11), was able to form stable complexes under similar conditions due to its multivalent interaction with siRNA. In addition, the packaging of DNA molecules in complexes plays an essential role in the gene expression, with a folded packaging structure showing higher transfection efficiency compared with a collapsed packaging structure.[82,83] For example, the toroidal packaging of DNA within the complexes was obtained by manipulating the concentration of NaCl, outperforming the rod-like structures in terms of gene transfer efficacy.[83]

While the PEG shielding prevents non-specific binding and lowers toxicity, the presence of PEG segments hampers the membrane interaction as well as the intracellular process of the polyplexes.[84] To tackle this problem, Kataoka, Nakano, and co-workers synthesized new PEG-*b*-PAsp(DET) copolymers with a disulfide spacer between two blocks. The cleavage of disulfide bonds under reductive conditions induced the detachment of PEG moieties, facilitating the endosomal escape of DNA.[85–87] On the other hand, the homopolypeptide PAsp(DET) was incorporated into the PIC micelles, which balanced the PEG shielding and the functioning of poly(*N*-aspartamide) segments at appropriate ratios, resulting in higher gene expression.[84,88,89]

3.2.2 Stabilization of complexes. The dissociation of polyplexes in biological environments is a key issue in the development of gene carriers. This issue is more serious for the fabrication of complexes containing siRNA, given its short length and insufficient charges. To solve this issue, Kataoka, Miyata, and co-workers modified the polypeptide blocks with thiol groups to stabilize the complexes through the formation of disulfide bonds.[90,91] The reduction of disulfides in the cytoplasm facilitated the release of siRNA. In a similar approach, phenylboronic acid (PBA) groups were incorporated onto the side chains of PLL segments (PEG-*b*-PLL/FPBA, Fig. 11), which were able to form ester bonds with

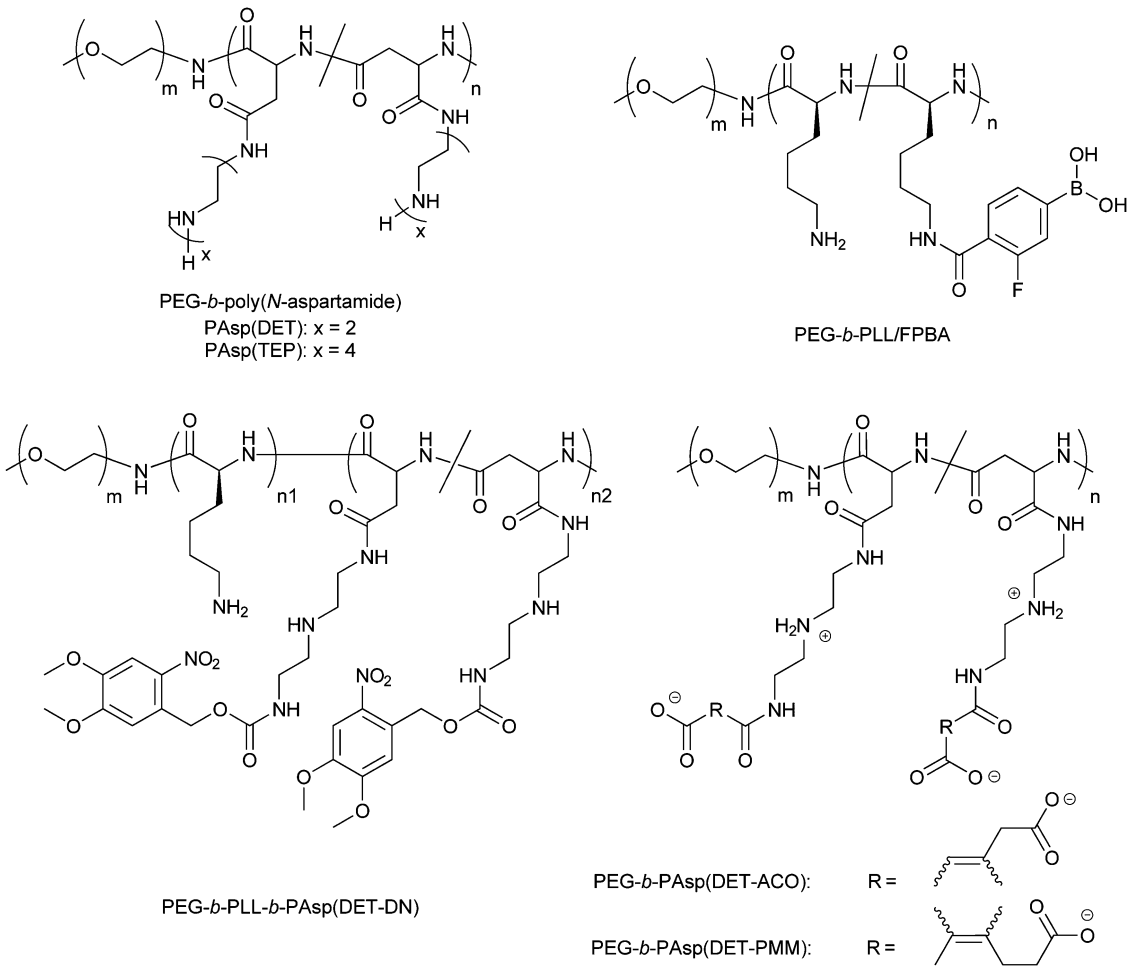

Fig. 11 Chemical structures of PEG-*b*-poly(*N*-aspartamide) or PEG-*b*-poly(L-lysine) copolymers for gene delivery.

Table 1 Summary of the incorporated functionalities and their supporting roles in the polypeptide/nucleic acid complexes

Functionalities[a,b]	Location	Supporting roles	Ref.
Disulfide	Between two blocks	PEG detachment	85–87
Thiol	Side chains of polypeptide block	Redox-sensitive cross-linker	90 and 91
PBA	Side chains of polypeptide block	ATP-sensitive cross-linker	92
Cholesteryl; PAsp(DET-DN)	Polypeptide chain terminus	Stabilization of complexes	93–95
Silica layer; PNIPAM layer	Between PEG shell and PIC core	Stabilization of complexes	87 and 96
cRGD	PEG chain terminus	Cell targeting; change intracellular kinetics	93, 94 and 97
PAsp(DET-ACO); PAsp(DET-PMM)	Side chains of polypeptide block	Charge conversion units	90, 91 and 98–100

[a] The chemical structures of PAsp(DET-DN), PAsp(DET-ACO), and PAsp(DET-PMM) can be found in Fig. 11. [b] PBA = phenylboronic acid; cRGD = cyclo-Arg-Gly-Asp peptide.

the ribose rings of siRNA, leading to stable polypeptide/siRNA complexes. Upon the addition of adenosine triphosphate (ATP), the complexes were destabilized due to competitive binding of ribose moieties on ATP with PBA units. The concentration difference of ATP across the plasma membrane validated such ATP-responsive design, which facilitates the intracellular release of siRNA.[92]

The enhanced stability of complexes was also achieved through the introduction of hydrophobic segments. For example, cholesteryl moieties[93,94] and dimethoxy nitrobenzyl protected PAsp(DET) blocks (PAsp(DET-DN), Fig. 11)[95] located at the polypeptide chain terminus improved the stability of polyplexes through hydrophobic interactions. In addition to the hydrophobic interactions, inorganic materials such as silica were also used to form an interlayer, providing enhanced stability of the polyplexes.[87] With a similar strategy, a hydrophobic polymeric interlayer was formed between PEG shells and PIC cores based on the temperature-responsive collapsing of poly(*N*-isopropylacrylamide) (PNIPAM).[96]

3.2.3 Functionalization of complexes. In an attempt to compensate for the reduced interactions between PIC micelles and cell membranes due to PEG shielding, cyclo-Arg-Gly-Asp

peptide (cRGD) was conjugated at the PEG chain terminus to mediate targeted delivery of the polyplexes.[93,94,97] The cRGD units not only served as the targeting ligands to enhance the cellular uptake of PIC micelles, but also changed the intracellular kinetics of the polyplexes for more efficient gene delivery.

Additionally, trigger-responsive groups were conjugated on the side chains of polypeptide segments in order to achieve controlled release of nucleic acid cargos. The pH-sensitive, charge-conversional groups, including *cis*-aconitic (ACO)[90,98,99] and 2-propionic-3-methyl maleic (PMM) moieties,[91,99,100] were attached onto PAsp(DET) side chains (Fig. 11). The charge conversion from poly(anion)s to poly(cation)s not only destabilized the polyplexes in the cytoplasm, but also mediated the endosomal escape with the recovered PAsp(DET) segments.

3.3 Complexes between polypeptide-based copolymers and nucleic acids

Polypeptides were used not only as poly(cation)s to condense nucleic acids, but also as supporting components for other non-viral vectors. For instance, the Chen group previously prepared PEI-poly(γ-benzyl-L-glutamate) (PBLG) to improve the performance of PEI.[101,102] While hyper-branched PEI exhibits high transfection efficiency due to the "proton sponge effect" to mediate endosomal escape, the cytotoxicity and non-degradability issue hamper its application.[103] The PEI–polypeptide conjugates are therefore advantageous considering the biodegradability and biocompatibility of polypeptide materials. Recently, the same group evaluated more polypeptides with various side chain structures.

Extended from the PEI–PBLG work, Tian, Zhu, and co-workers reported the preparation of PEI conjugates with hydrophobic poly(L-phenylalanine) (PLPhe)[104] and poly(L-alanine) (PLAla),[105] yielding amphiphilic polymer structures. These amphiphilic structures showed enhanced gene expression not only because of the improved affinity with cell membranes, but also due to the altered shape and size of the complexes with added hydrophobic interactions. Additionally, hydrophilic polypeptides, including PLL,[106] PLL-*r*-PLG,[107] and poly(*N*-(2-hydroxyethyl)-L-glutamine) (PHEG),[108] were also used to modify PEI to achieve reduced toxicity and better gene delivery efficiency. The PEI–polypeptide conjugates have been further decorated with other functionalities (*e.g.*, targeting ligands, PEG).[109] With a similar strategy, PEI–polypeptide charge complexes[110,111] and polypeptide–PEI graft polymers[112] were also prepared and evaluated as gene carriers.

4. Drug-loaded polypeptide micelles

Amphiphilic block copolymers can form core–shell type micelle structures in aqueous medium, which are able to load cargos in the hydrophobic cores.[17,113] In the past several decades, polymeric micelles have attracted enormous attention as drug carriers for cancer therapy.[114,115] Compared with free drugs, drug-loaded polymeric micelles exhibit better aqueous solubility, improved *ex/in vivo* stability, and prolonged blood circulation time, which enable better performances of drug molecules in biological environments.[17] Moreover, the size of most polymeric micelles (<200 nm) allows the accumulation of drugs at tumour sites through an enhanced permeability and retention (EPR) effect.[116]

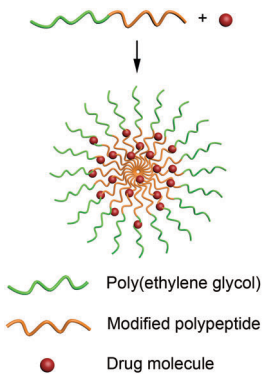

Fig. 12 Schematic illustration showing the formation of drug-loaded micelles with PEG-*b*-polypeptide copolymers.

Among all micelle-forming copolymers developed, PEG-*b*-polypeptide copolymers are one of the most promising candidates (Fig. 12).[117,118] The PEG shell shields the micelles from the capture of reticuloendothelial systems (RES) and thus prolongs their circulation time in the blood stream. On the other hand, the chemical diversity of polypeptides enables the incorporation of various functionalities for interactions with drug molecules. Therefore, the formation of micelles and the loading of drug molecules are well manipulated through the chemical design of polypeptide blocks. Therapeutic drugs can be loaded through covalent conjugation[119–121] as well as non-covalent interactions. Depending on the specific structures of drug molecules and polypeptide segments, the non-covalent molecular interactions to drive the formation of micelles include hydrophobic interactions, metal coordination, and electrostatic interactions. Recently reported drug-loaded PEG-*b*-polypeptide micelles are summarized in Table 2.

4.1 Micelles formed through hydrophobic interactions

Hydrophobic drugs can be easily incorporated into PEG-*b*-polypeptide micelles through the hydrophobic interactions with the polypeptide segments, including PBLG,[122,123] PLPhe,[124] poly(ε-carbobenzyloxy-L-lysine) (PZLL),[125] poly(L-leucine) (PLLeu),[126,127] and poly(DL-leucine) (PDLLeu).[126,127] With a similar mechanism, the drugs can also be covalently conjugated onto the polypeptide side chains, driving the self-assembly to form micelles through hydrophobic interactions.[128–130] The selection of hydrophobic groups on polypeptide side chains plays an important role in determining the drug loading, micelle stability, and particle size.[131] Therefore, polypeptides containing carboxyl, hydroxyl, and amino groups are commonly used as the precursors due to the facile modification with drugs or various hydrophobic moieties. For instance, 4-phenyl-1-butanol modified PEG-*b*-poly(α,β-aspartic acid) (PAsp) (Fig. 13) was used to encapsulate paclitaxel (PTX), which showed lower allergenicity, reduced side effects, and enhanced antitumour activity compared with free PTX.[132]

Table 2 Summary of drug-loaded PEG-*b*-polypeptide micelles

Polymers[a]	Drug molecules[b]	Driving forces[c]	Ref.
PEG-*b*-PBLG	DOX, RAP	H	122 and 123
PEG-*b*-PZLL	DOX	H	125
PEG-*b*-PLLeu/PDLLeu	DOX	H	126 and 127
Modified PEG-*b*-PAsp	DOX, EPI, PTX	H	128–130 and 134
Modified PEG-*b*-PHEA	DMXAA, DOX, PTX, PPT	H	133
Modified PEG-*b*-PLL	PTX, CPT	H	135–137
Cross-linked PEG/PLL-*b*-(PLPhe-*r*-PLCys)	DOX, HCPT	H	138 and 139
Cross-linked PEG-*b*-PPLG	DOX	H	140 and 141
PEG-*b*-PLG	DACHPt, CDDP	M	48 and 142–148
PLG-*g*-mPEG	CDDP	M	149–151
PEG-*b*-PLG	DOX	E	153
Modified PEG-*b*-PLL	DOX	E	155
PEG-*b*-(PLG-*r*-PLPhe)	DOX	E	156
PEG-*b*-P(Lys-CCA/LA)	DOX	E	157
PEG-*b*-(PLL/PLG-*r*-PLCys)	DOX, CAD, DAD	E	158–160
PEG-*b*-PLG-*b*-PLL/DOCA	DOX and PTX	H, E	163
PEG-*b*-PLG-*b*-PLPhe	PTX and CDDP	H, M	164

[a] The chemical structures of representative polymers can be found in Fig. 13, 15, 17 and 18. [b] DOX = doxorubicin, RAP = rapamycin, EPI = epirubicin, PTX = paclitaxel, DMXAA = 5,6-dimethylxanthenone-4-acetic acid, PPT = podophyllotoxin, CPT = camptothecin, HCPT = 10-hydroxy-CPT, DACHPt = dichloro(diaminocyclohexane)platinum(II), CDDP = *cis*-dichlorodiammine platinum(II), CAD = *cis*-aconityl-DOX, DAD = 2,3-dimethyl-maleyl-DOX. [c] H stands for hydrophobic interactions, M stands for metal coordination, and E stands for electrostatic interactions.

Combining both covalent and non-covalent drug loading strategies, Chen, Zhong, Cheng, and others developed a co-delivery system based on PEG-*b*-poly(*N*-(2-hydroxyethyl)aspartamide) (PHEA) (Fig. 13) to bypass the tumour penetration obstacles for nanomedicines.[133] A vascular-disrupting agent, 5,6-dimethylxanthenone-4-acetic acid (DMXAA), was covalently conjugated onto PHEA side chains followed by the loading of doxorubicin (DOX) through hydrophobic interactions. At the tumour site, the sustained release of DMXAA inhibited the interior tumour cells by destroying tumour vessels, and the release of DOX killed the exterior tumour cells at the tumour rim. Therefore, the co-delivery system suppressed tumour growth with no need to penetrate into the inner tumour regions, leading to desired anticancer activity.

Incorporation of trigger-responsive linkers into micelles facilitates the intracellular release of cargos. For instance, Kataoka, Cabral, and others developed a pH-responsive micelle system to co-deliver both a cytotoxic agent, epirubicin (Epi), and an inhibitor toward recalcitrant cancer stem cells (CSCs), staurosporine (STS). Epi was covalently bound to the PEG-*b*-PAsp through an acid-labile hydrazone bond, and STS was loaded into the Epi-stabilized micelle core through hydrophobic interactions (Fig. 14A).[134] Due to the cooperative interactions between Epi and STS, the acidic pH-sensitive hydrolysis of the hydrazone

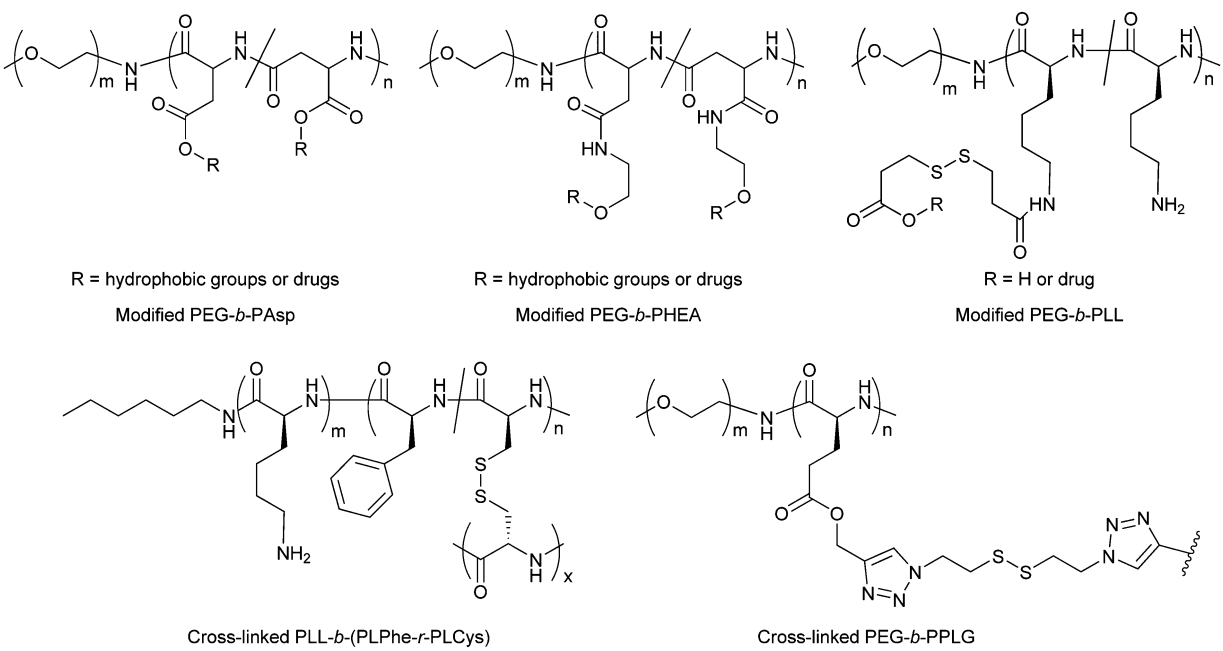

Fig. 13 Chemical structures of amphiphilic PEG-*b*-polypeptide copolymers for the encapsulation of hydrophobic drugs.

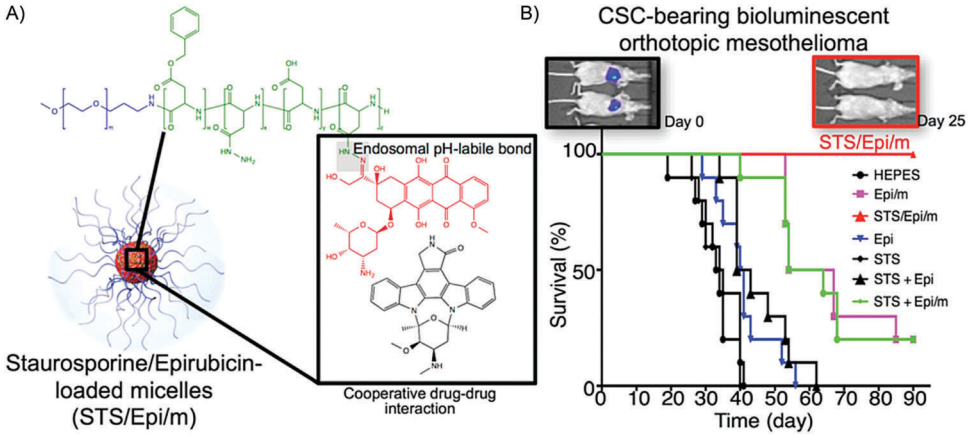

Fig. 14 Co-delivery of staurosporine (STS) and epirubicin (Epi) with pH sensitive PEG-*b*-polypeptide micelles eradicating orthotopic mesothelioma tumours. (A) Schematic illustration showing the loading of Epi and STS into the micelles (STS/Epi/m). (B) Kaplan–Meier survival curve of mice bearing orthotopic mesothelioma tumours. HEPES buffer, free drugs (Epi, STS, STS + Epi), Epi-loaded micelles (Epi/m), and STS plus Epi/m were used as controls. Reprinted with permission from ref. 134. Copyright 2016 American Chemical Society.

bond facilitated the coordinated release of both drugs. The resulting micelles, called STS/Epi/m, were able to eliminate orthotopic mesothelioma xenografts bearing a recalcitrant CSC subpopulation due to the synergistic efficacy. The survival rate of mice treated with STS/Epi/m was significantly improved compared with control groups (Fig. 14B). With a similar strategy, disulfide bonds[135–137] were also incorporated into PEG-*b*-PLL to enable triggered release of conjugated drug molecules under reductive conditions.

In addition, trigger-responsive moieties were also incorporated as cross-linking units, allowing the destabilization of PEG-*b*-polypeptide micelles at desired tumour sites. Poly(L-cystine)s (PLCys), for example, are commonly used as cross-linkable polypeptide components with their pendent thiol groups.[138,139] Chen, Hou, Ding, and co-workers designed positively charged nanogels with PLL-*b*-(PLPhe-*r*-PLCys) for the delivery of 10-hydroxycamptothecin (HCPT) to bladder tumours (Fig. 13).[139] The resulting nanogels showed rapid release of encapsulated HCPT in bladder cancer cells due to the reductive intracellular microenvironment. In a similar approach, bis(2-azidoethyl)disulfide was also used to cross-link PEG-*b*-poly(γ-propargyl-L-glutamate) (PPLG) for redox-responsive destabilization of micelles (Fig. 13).[140,141]

4.2 Micelles formed through metal coordination

In order to deliver platinum (Pt) drugs with PEG-*b*-polypeptide micelles, polypeptides with pendent carboxyl side chains (*e.g.*, PLG) were used to load metal complexes. PLG was able to efficiently coordinate with Pt(II) drugs, leading to formation of stable cross-linked micelles with a narrow size distribution.[118] The reversible ligand exchange reaction of Pt(II) enables the release of active Pt complexes from micelles under physiological conditions, leading to desired antitumour activity.[118] Compared with free Pt complexes, Pt(II) incorporated micelles showed prolonged blood circulation time, significantly enhanced tumour accumulation, and superior *in vivo* antitumour efficacy with reduced side-effects.

PEG-*b*-PLG micelles (Fig. 15) were used to load *cis*-dichlorodiammineplatinum(II) (CDDP) or dichloro(diaminocyclohexane)platinum(II) (DACHPt), which demonstrated improved efficacies against various subcutaneous tumour models as well as tumour metastasis.[142–145] The functionalization with targeting ligands on the surface of PEG-*b*-PLG micelles further improved their antitumour performance.[146–148] In an attempt to investigate the assembly process, Kataoka and Nishiyama groups demonstrated that the enhanced antitumour efficacy of CDDP-loaded PEG-*b*-PLG and PEG-*b*-poly(D-glutamic acid) (PDG) micelles (called L-CDDP/m and D-CDDP/m) originated from the formation of ordered α-helical bundles in the core (Fig. 16A and B).[48] The bundled assembly of the α-helical nanostructures resulted in a gradual, erosion-like disintegration process of the micelles and sustained release of cisplatins under physiological conditions (Fig. 16C). Therefore, the undesired disintegration during blood circulation and premature drug release were greatly minimized. Compared with the micelles from racemic

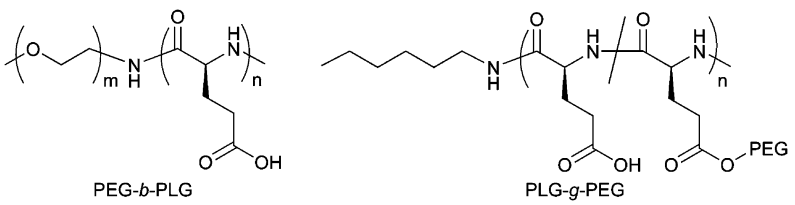

Fig. 15 Chemical structures of PEGylated PLG copolymers for the loading of Pt(II) drugs through metal coordination interactions.

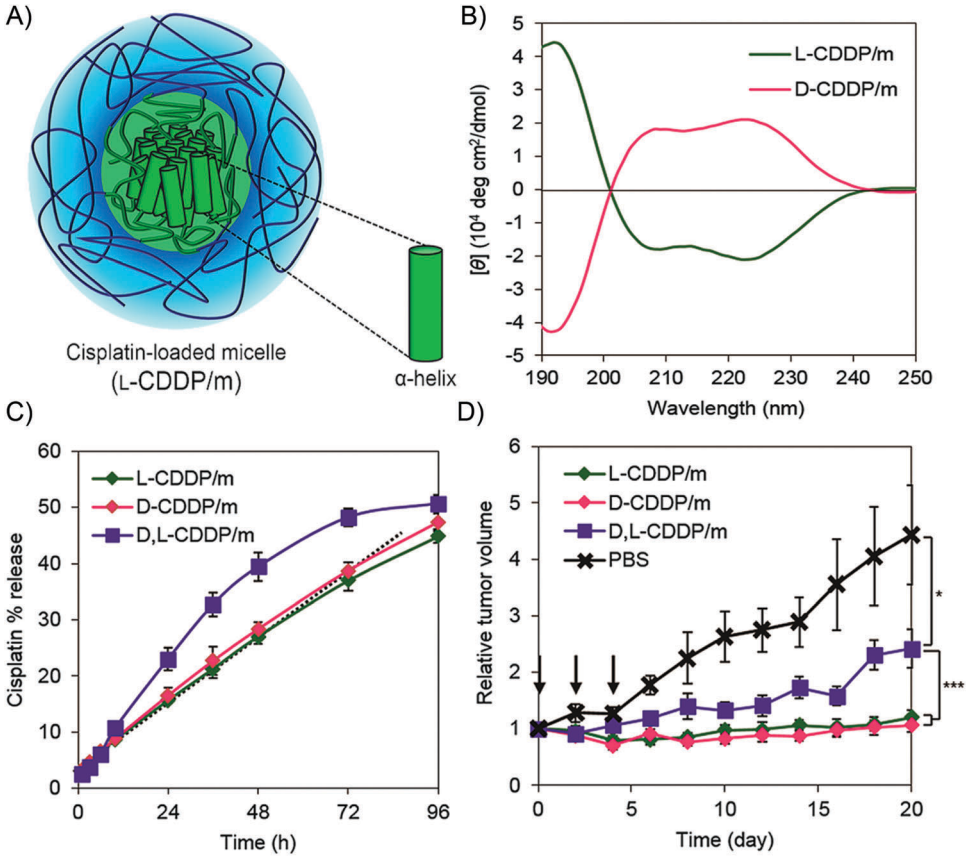

Fig. 16 The impact of bundled α-helical nanostructures on the performance of *cis*-dichlorodiammine platinum(II) (CDDP) loaded PEG-*b*-polypeptide micelles (CDDP/m). (A) Schematic illustration showing the formation of α-helical bundles in the core of L-CDDP/m. (B) Circular dichroism spectra of L-CDDP/m and D-CDDP/m. (C) Release profile of CDDP from CDDP/m under physiological conditions. (D) Antitumour efficacy of CDDP/m. PBS buffer was used as a control. Reprinted with permission from ref. 48. Copyright 2014 American Chemical Society.

PEG-*b*-poly(DL-glutamic acid) (PDLG) (called D,L-CDDP/m), L-CDDP/m and D-CDDP/m exhibited enhanced tumour accumulation and reduced nonspecific distribution to the liver and spleen, which contributed to their improved antitumour efficacies (Fig. 16D).

Recently, Pt(II)-loaded micelles from the PEG-grafted PLG copolymers (PLG-*g*-PEG, Fig. 15) were reported.[149,150] Compared with the block copolymer PEG-*b*-PLG, PLG-*g*-PEG offered more flexible control of the micelle structures (*e.g.*, PEG density). It has been shown that several structural parameters, including MWs of PLG, weight ratios of PEG to PLG, and the chain lengths of PEG, play important roles in regulating the blood circulation time of CDDP-loaded micelles.[151] Significantly enhanced area under the concentration–time curve (AUC) for CDDP-loaded micelles was observed in both plasma (up to 31-fold) and tumour (up to sixfold) compared with free CDDP. *In vivo* studies demonstrated the improved tolerance and antitumour efficacy of CDDP-loaded PLG-*g*-PEG micelles. Based on this system, the same group designed a cooperative platform for the delivery of CDDP by altering the tumour microenvironment, which showed superior antitumour efficacy and low systemic toxicity as compared with non-cooperative controls.[152]

4.3 Micelles formed through electrostatic interactions

Charged, hydrophilic drugs were loaded into PEG-*b*-polypeptide micelles through electrostatic interactions with charged polypeptide segments. This strategy to drive the formation of micelles is advantageous since the formulation is done in an aqueous environment without the need for organic solvents. For instance, the Chen group developed PIC micelles consisting of PEG-*b*-PLG and DOX·HCl through electrostatic interactions (Fig. 17).[153,154] These micelles exhibited a pH-sensitive release profile of DOX due to the lowered ionization of PLG at acidic pH. With a similar strategy, modified PLLs with anionic side chains were also used to complex with DOX·HCl (Fig. 17), which underwent an acid-triggered charge conversion and subsequent release of encapsulated drugs.[155]

In order to solve the stability issue against high-dilution, hydrophobic segments were incorporated into the micelles. For instance, Chen, Liu, and others designed a PEG-*b*-(PLG-*r*-PLPhe) copolymer for the delivery of DOX through charge complexation between the PLG block and DOX·HCl (Fig. 17).[156] The incorporation of phenylalanine not only stabilized the micelles through hydrophobic interactions, but also contributed to an enhanced cellular uptake level. On the other hand, cross-linking is a common strategy to stabilize micelles. Zhong and Deng lab

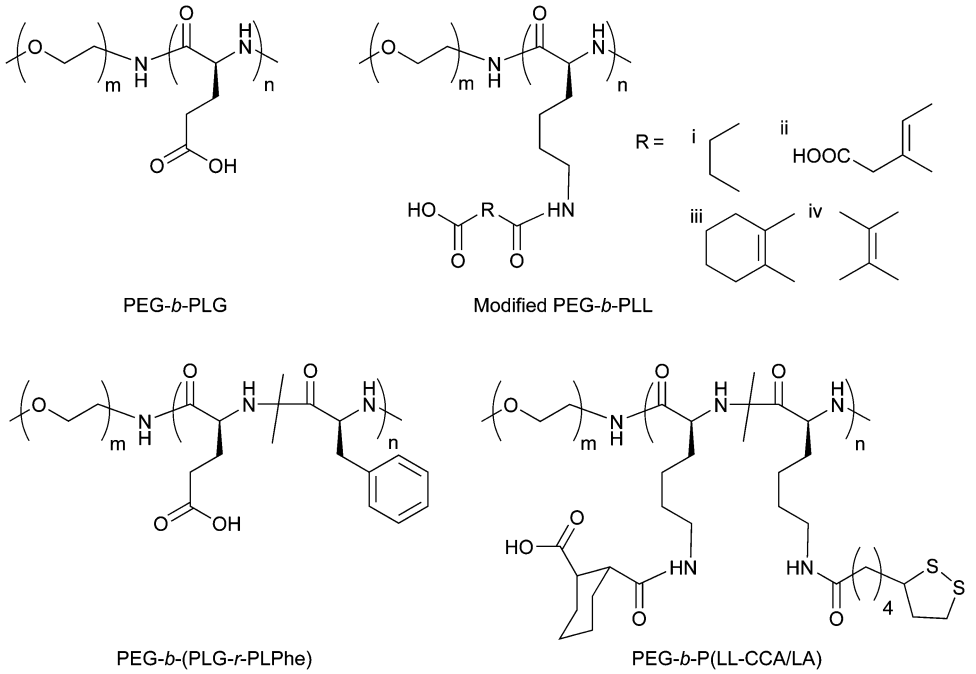

Fig. 17 Chemical structures of PEG-*b*-polypeptide copolymers with charged polypeptide segments for drug encapsulation through electrostatic interactions.

reported reversibly core-cross-linked micelles from lipoic acid (LA) and *cis*-1,2-cyclohexanedicarboxylic acid (CCA) decorated PEG-*b*-PLL (Fig. 17).[157] These micelles exhibited good stability against dilution, while quickly disassembled in the presence of dithiothreitol (DTT). The release of encapsulated DOX was accelerated under acidic and reductive conditions, resulting in significant cytotoxicity to tumour cells. Other similar polypeptide micelles based on the cross-linking of PLCys units were also reported.[158–160]

4.4 Micelles formed with multiple molecular interactions

More than one drug can be incorporated into PEG-*b*-polypeptide micelles through distinct molecular interactions,[161,162] which enabled the simultaneous co-delivery to the targeted sites and controlled release profile of various drugs. Chen, Zhang, and co-workers reported a deoxycholate (DOCA) modified PEG-*b*-PLG-*b*-PLL triblock copolymer for the co-delivery of DOX and PTX (Fig. 18).[163] The amphiphilic copolymer spontaneously self-assembled in aqueous solutions to form stable micelles with a PEG outer corona, a PLG middle layer, and a hydrophobic PLL/DOCA inner core. DOX·HCl was complexed with the anionic PLG units and PTX was loaded into the core through hydrophobic interactions with modified PLL segments. A similar design was applied to a PEG-*b*-PLG-*b*-PLPhe triblock copolymer for the delivery of CDDP (metal coordination with PLG segments) and PTX (hydrophobic interaction with PLPhe segments) (Fig. 18).[164]

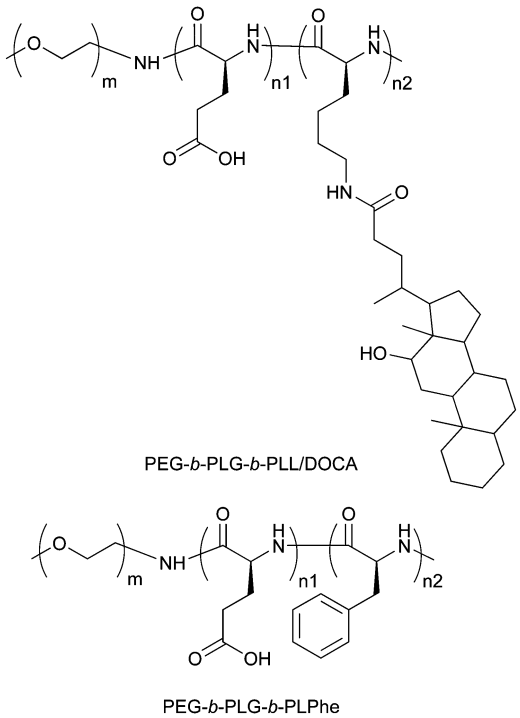

Fig. 18 Chemical structures of PEG-*b*-polypeptide copolymers designed for the co-delivery of drug molecules through various molecular interactions.

5. Polypeptide vesicles

Polymeric vesicles (also named polymersomes), formed through the self-assembly of polymeric amphiphiles, are hollow spheres with a bilayer polymeric wall and an enclosed internal cavity.[165] Compared with micelles, vesicles are advantageous in their ability to load both hydrophilic (in the aqueous cavity) and hydrophobic (in the polymer membrane) cargos.[166,167] Therefore, polymer vesicles have been intensively studied for drug delivery applications.

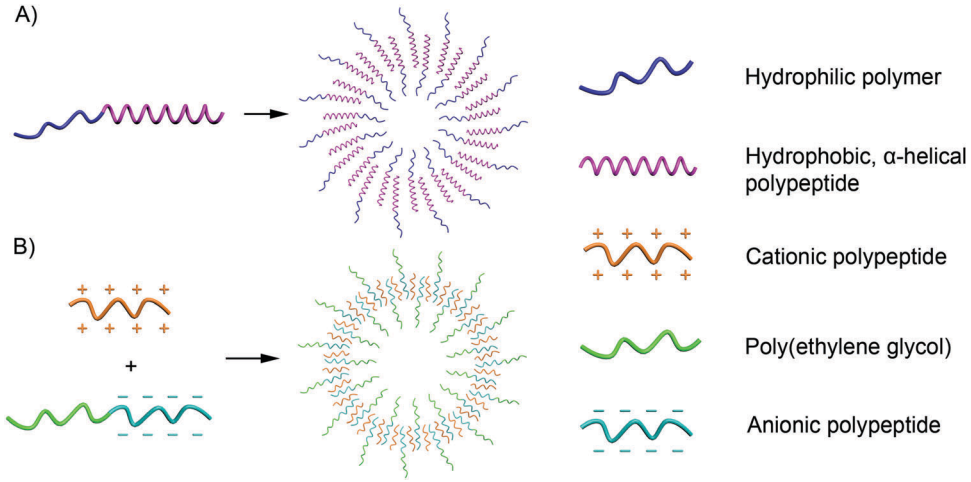

Fig. 19 Schematic illustration showing the formation of polymeric vesicles from block copolymers bearing hydrophobic, α-helical polypeptide segments (A) and PICsomes from two polymers containing charged polypeptide segments with opposite charges (B).

Polypeptide-based copolymers are widely used for vesicle preparation as drug carriers. Polypeptide vesicle related papers have been previously reviewed by Carlsen and Lecommandoux.[10] Generally, polypeptide vesicles were formed mainly through two mechanisms (Fig. 19). First, if one polypeptide block was hydrophobic and α-helical, the anisotropic packing of the α-helices enabled the formation of vesicle morphology even at very high hydrophilic-to-hydrophobic ratios.[47] Second, if one polypeptide block was charged, the charge complexation with other charged polypeptides or small molecules facilitated the vesicular assembly (polyion complex vesicle, PICsome).[168] Moreover, polypeptides are also used as the hydrophilic blocks of vesicles. Table 3 summarizes the compositions and key features of polypeptide-based vesicles developed in the last five years.

An ideal drug carrier should not only be stable and robust to prevent any premature leakage, but also quickly disassemble upon trigger to release the encapsulated drugs at a target site.[169] Several trigger-responsive polypeptide vesicles were therefore developed. Until now, there have been mainly four mechanisms to disrupt or disassemble polypeptide vesicles: (1) structural changes of hydrophobic blocks upon trigger,[50,170,171] (2) structural changes of hydrophilic blocks upon trigger,[172] (3) cleavage of the linkage between hydrophilic and hydrophobic blocks,[173] and (4) disruption of vesicle membranes.[174,175] Related papers will be reviewed in the following sections.

5.1 Polypeptide vesicles formed through the anisotropic packing of α-helical, hydrophobic polypeptides

Hydrophobic polypeptides, such as PBLG, PLLeu, and PLLeu-*r*-PLPhe (Fig. 20A), are regarded as rigid rods due to their α-helical conformation. The self-assembly behaviour of rod-like polymers is quite different from that of traditional coiled polymers, since they prefer side-by-side alignment along the helical axis that favours lamellar assemblies.[176] Therefore, copolymers with stiff, rod-like, hydrophobic polypeptide blocks are able to self-assemble into vesicular morphologies even at very high hydrophilic-to-hydrophobic ratios, at which composition the random coiled copolymers can only form micelles.[47] The conformation of polymers, or in other words the stiffness of polymers, became another important parameter to control the solution self-assembly behaviours of polymeric amphiphiles. The following studies confirmed the conformation-specific self-assembly of polypeptide-based block copolymers, and further revealed that only the stiffness of the hydrophobic block was essential for vesicular morphology.[177–179] Charged polypeptide vesicles were developed with coiled PLG, PLL, or poly(L-arginine)

Table 3 Summary of the compositions and key features of polypeptide-based vesicles

Hydrophobic block[a]	Hydrophilic block[a]	Key features	Ref.
PBLG, PLLeu or PLLeu-*r*-PLPhe	Ionic polypeptides	Ionic vesicles; cell penetrating	180–187
	PLMetO	Trigger-responsive vesicles (enzyme)	172
	Glycopolypeptides	Glycopolypeptide vesicles; lectin binding	188 and 189
PBLG	Hyaluronan	Drug-loaded vesicles; cancer cell targeting	190
PDMNBLG	PEG	Trigger responsive vesicles (UV)	50
PLLeu	PEG and PLG	Trigger responsive chimaeric vesicles; drug delivery	191
PLG (protonated)	PEG-*b*-PLL	Trigger responsive vesicles (pH)	171
Polyion complex	PEG	PICsomes by charge complexation; drug delivery	175, 194 and 196–200
PLG + DACHPt	PEG	Metallosomes; drug delivery	170
PTMC	PLG	Drug delivery	173 and 174

[a] The chemical structures of representative polymers can be found in Fig. 20 and 22.

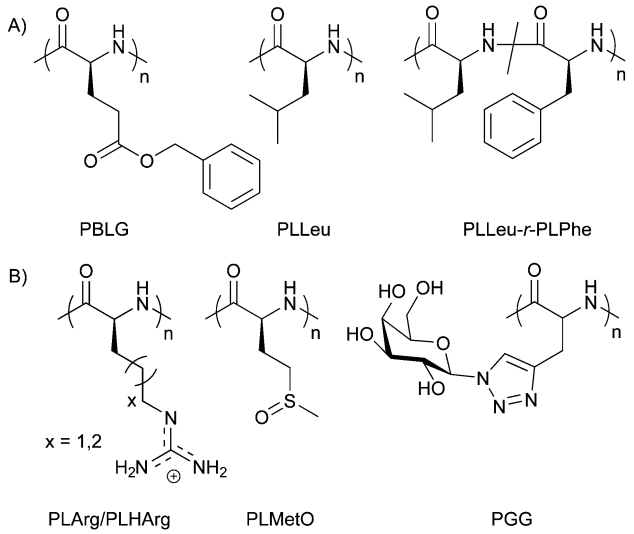

Fig. 20 Chemical structures of the hydrophobic polypeptide blocks (A) and hydrophilic polypeptide blocks (B) of vesicle-forming block copolypeptides.

(PLArg) as the hydrophilic blocks.[177–179] In the recent five years, lots of efforts have been devoted to incorporate functional groups on vesicle-forming polymers, aiming to utilize the polypeptide vesicles for biomedical applications.

5.1.1 Ionic copolypeptide vesicles. Extended from their ionic copolypeptide vesicle work,[177] Kamei, Deming and co-workers investigated the potential of these vesicles as drug carriers.[180–187] For instance, PLArg-*b*-PLLeu copolypeptide vesicles possessed a cationic, guanidine-rich surface that not only formed complexes with DNA, but also mediated cell internalization.[181] To reduce cytotoxicity issues, these guanidine-rich vesicles were further improved by introducing an anionic or neutral polypeptide segment as the second hydrophilic block.[180,185] The resulting triblock copolypeptide-based vesicles exhibited lower cytotoxicity compared with the original PLArg-*b*-PLLeu or poly(L-homoarginine) (PLHArg)-*b*-PLLeu vesicles, while maintaining similar cell-penetrating ability due to their guanidine-rich surfaces.[180,185]

In addition to cationic polypeptide vesicles, anionic PLG-*b*-PLLeu vesicles were also used for drug delivery applications. The PLG-*b*-PLLeu vesicles with anionic surface exhibited minimal cytotoxicity, with ~100% relative survival of cells even in the presence of 200 μg mL^{-1} vesicles.[182] In order to utilize these vesicles for drug delivery, transferrin was conjugated to facilitate the cellular uptake[182] and PEG was attached for steric stability of the vesicles.[184] The resulting vesicles were successfully applied for the delivery of DOX.[184]

Non-ionic polypeptide vesicles bearing poly(L-methionine sulfoxide) (PLMetO) segments exhibited trigger-responsive changes in water solubility (Fig. 20B), which was used to design enzyme-triggered cargo release from vesicles.[172] In the presence of reductase, the random coiled, water-soluble methionine sulfoxide residues were reduced into α-helical, water-insoluble methionine units. The change in both conformation and hydrophilicity resulted in the disassembly of polypeptide vesicles.

5.1.2 Glycopolypeptide-based copolypeptide vesicles. Other than ionic polypeptides, glycopolypeptides can also be used as the hydrophilic blocks to prepare copolypeptide vesicles. Lecommandoux, Heise, and co-workers reported the vesicular morphology from the self-assembly of poly(β-(1-galactosyl-1*H*-1,2,3-triazol-4-yl)-DL-alanine) (PGG)-*b*-PBLG, where PGG segments contained galactose units (Fig. 20B and 21A).[188] The authors showed that the morphologies of the assemblies were controlled by the order of solvent addition. The vesicular morphology was only observed when the dimethyl sulfoxide (DMSO, good solvent) solution of copolypeptides was added into water (poor solvent) (Fig. 21B). Furthermore, the bioactivity of glycopolypeptide vesicles was demonstrated by lectin binding experiments.[188] The galactose functionalized vesicles selectively bind with *Ricinus communis* agglutinin (RCA_{120}), resulting in instantaneous precipitation upon mixing. Concanavalin A (Con A) that selectively binds with mannosyl and glycosyl residues, on the other hand, showed negligible change in turbidity upon mixing (Fig. 21C).

The Deming group studied the impact of the conformation of hydrophilic glycopolypeptide blocks on the self-assembly morphology.[189] Vesicular morphology was observed from the assembly of copolypeptides with random coiled, hydrophilic glycopolypeptide blocks. Copolypeptides with rigid, α-helical, hydrophilic glycopolypeptide blocks, however, failed to self-assemble into vesicles due to the lack of chain flexibility. The ability to selectively bind with RCA_{120} over Con A was also demonstrated.[189]

5.1.3 Hybrid polypeptide vesicles. Other than polypeptides, water-soluble polymers including hyaluronan (HYA)[190] and PEG[50,171,191] were also used as hydrophilic blocks for vesicle preparation. For example, HYA-*b*-PBLG vesicles developed by the Lecommandoux group have been used to load DOX.[190] The HYA-rich surface of the vesicles helped in targeting cancer cells with over-expressed CD44 glycoproteins, enabling higher uptake level in cancer cells compared with free drugs.

Cheng, Leal, and co-workers reported PEG-*b*-PDMNBLG self-assembled into vesicular structure with densely packed multilayer membranes.[50] The short PEG segments were buried between the hydrophobic polypeptide layers, forming multilayer structures with no water molecules between the bilayers. The vesicles thus had a thick membrane of ~40 nm. The hydrophobic polypeptide blocks were light-responsive, which changed to hydrophilic PLG upon UV irradiation, resulting in the disassembly of vesicles. PEG was also used in the preparation of chimaeric polymersomes from PEG-*b*-PLLeu-*b*-PLG triblock copolymers.[191] After self-assembly, PEG moieties served as the outer leaflet for the shielding effect, while the PLG segments formed the inner leaflet to establish electrostatic interactions with loaded DOX. The resulting DOX-loaded vesicles exhibited pH-responsive drug release and high antitumour activity.

Due to the pH-dependent conformation and water-solubility, PLG and PLL were used for the design of pH-responsive vesicles. For instance, PEG-*b*-PLL-*b*-PLG formed vesicle structures at acidic pH where the terminal PLG blocks were α-helical,[171] with PEG and charged PLL blocks serving as the hydrophilic shells. At high pH values with α-helical PLL segments in the middle,

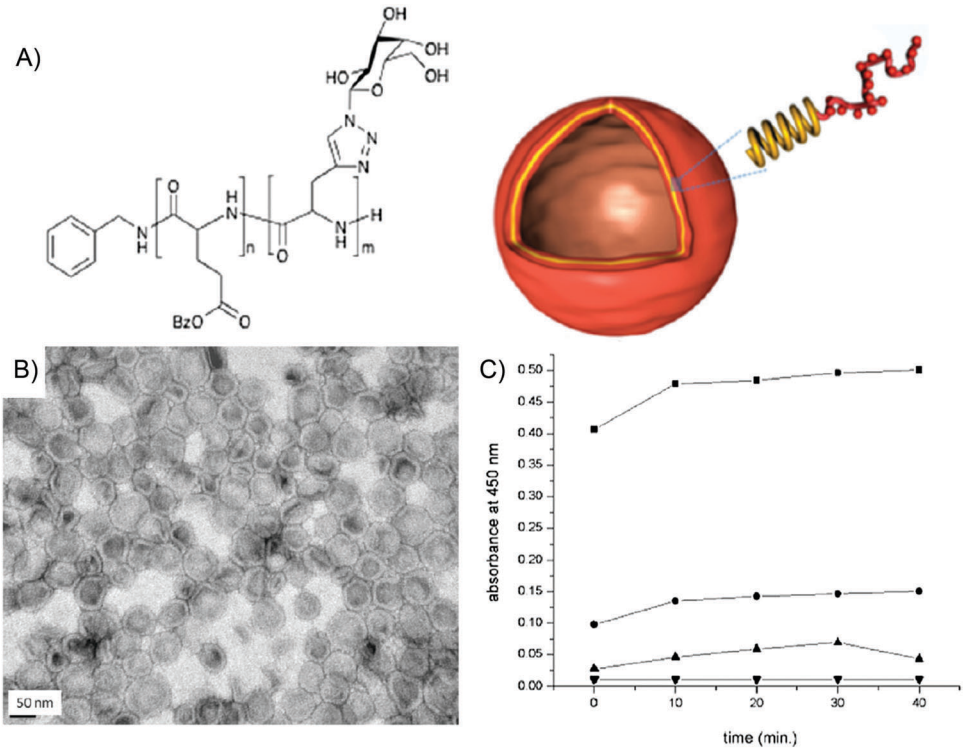

Fig. 21 Self-assembly behaviour and bioactivity of glycopolypeptide-based vesicles. (A) Chemical structures of PGG-*b*-PBLG and schematic illustration showing the self-assembly behaviour. (B) TEM image of PGG-*b*-PBLG vesicles. (C) Lectin binding studies of PGG-*b*-PBLG vesicles. Absorbance at 450 nm in the presence of RCA_{120} (square and circle symbols) and Con A (up triangle symbols) in DI water. Vesicles with no lectin (down triangle symbols) were used as a control. Reprinted with permission from ref. 188. Copyright 2012 American Chemical Society.

however, the copolymers preferred staying in the solution as single chains. The middle hydrophobic PLL was shielded by two outer hydrophilic segments so that the single chain was stable in the aqueous phase.

5.2 Polypeptide vesicles formed through charge complexation

PICsomes were first reported by the Kataoka group, which were prepared by the mixing of oppositely charged polymers in an aqueous environment.[168,192] Compared with traditional polymersomes formed through the self-assembly of amphiphilic block copolymers, PICsomes require no organic solvents for the fabrication, enabling easy encapsulation of water-soluble cargos for biomedical applications.[168] PEG-*b*-polypeptides are the most common building blocks to prepare PICsomes, where polypeptide blocks are charged for complexation. The polypeptide blocks with opposite charges form the PIC membranes, while the PEG segments serve as the inner and outer shells. Typically, PICsomes were formulated between two PEG-based block ionomers, or one PEG-based block ionomer and one homoionomer (Fig. 22).[168,192]

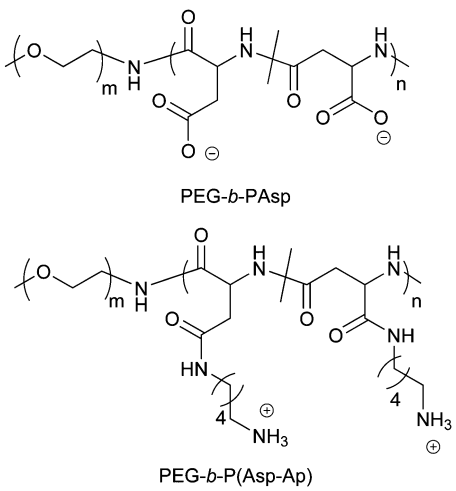

Fig. 22 Chemical structures of representative PEGylated charged polypeptides for the formation of PICsomes.

5.2.1 Morphology control of PIC materials. Recently, Kataoka, Kishimura, and co-workers examined the impact of temperature, the composition of the block ionomers, and the side-chain structure of homoionomers on the self-assembly behaviours of PIC materials.[193,194] In a binary system with block aniomers and homocatiomers, longer aniomer segments favoured the formation of PICsomes with a critical weight fraction of PEG (f_{PEG}) of ~10%, and homocatiomers with longer side chains preferred the assembly into PICsomes.[194] Block aniomers with short polypeptide blocks ($f_{PEG} > 10\%$) or homocatiomers with short aliphatic spacers, on the other hand, only form PIC micelles.

5.2.2 Polymersome formation between ionomers and other charged molecules. The building blocks for the preparation of PICsomes are not limited to charged polypeptides. Other charged

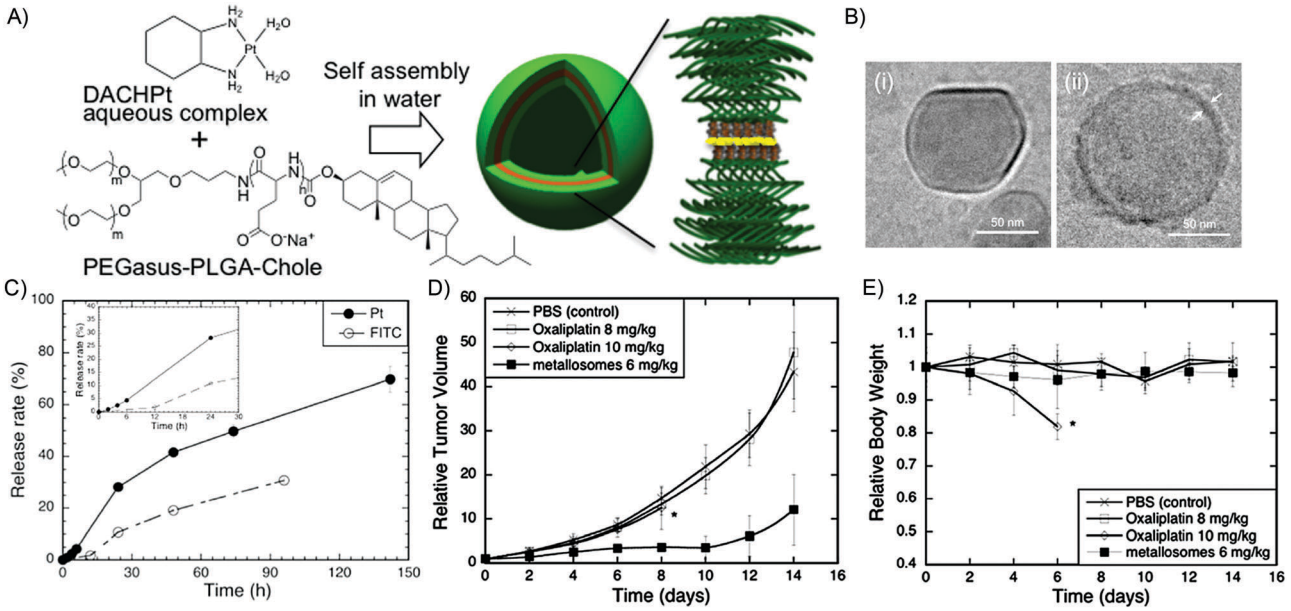

Fig. 23 Formation of metallosomes and their application for drug delivery. (A) Schematic illustration showing the formation of metallosomes through metal coordination. (B) Cryogenic phase-contrast TEM images of metallosomes after 0 h (i) and 48 h (ii) incubation. (C) Release profiles of DACHPt and encapsulated fluorescein labelled dextran from metallosomes. (D and E) Antitumour activity of metallosomes by relative tumour volume (D) and relative body weight (E). PBS buffer and free platinum drug analog (oxaliplatin) were used as controls. Reprinted with permission from ref. 170. Copyright 2012 American Chemical Society.

molecules have also been used to form PIC with ionomers, resulting in the assembly of polymersomes. For example, Kataoka's lab reported the fabrication of metallosomes, which were formed by metal coordination bonds between PLG segments and a Pt-based anticancer agent, DACHPt (Fig. 23A).[170] The coordination induced the conformational transition of PLG into an α-helix, facilitating the self-assembly into vesicular structures (Fig. 23B). Further studies indicated that Y-shaped PEG segments and a cholesterol unit at the PLG chain terminus were essential for the vesicle formation, since they provided steric repulsions and additional hydrophobic interactions, respectively. Interestingly, DACHPt was gradually released under physiological conditions, inducing a change in the permeability of metallosome membranes. A delayed release of the encapsulated cargos (dextran, 10 kDa) in the aqueous cavity was therefore observed, with an induction period of ~12 h (Fig. 23C). The antitumour activity of metallosomes has been demonstrated *in vivo*, which outperformed the free platinum drug analog due to the enhanced accumulation in the tumour tissues (Fig. 23D and E). With a similar strategy, cationic drug DOX·HCl was used to form charge complexes with anionic polypeptides for vesicular assemblies.[195]

5.2.3 Biomedical application of PICsomes. Due to their facile preparation without the need for organic solvents, PICsomes have been used to encapsulate various cargos, including inorganic NPs,[196,197] amphiphilic photosensitizers (PSs),[175] and enzymes.[198–200] Specifically, cross-linked PICsomes formed by mixing PEG-*b*-PAsp and homo poly(5-aminopentyl-α,β-aspartamide) (P(Asp-AP)) are the most used carriers for the encapsulation and delivery of various cargos (Fig. 22). Take PS-loaded PICsomes as an example; after the PICsomes were internalized by the cells, the release of encapsulated PSs was observed when the cells were irradiated with near infrared (NIR) light.[175] The release of PSs not only resulted in higher photocytotoxicity compared with free PSs, but also induced photochemical disruption of PICsome membranes as well as the endolysosomal membranes. PS-loaded PICsomes were therefore useful carriers with trigger-responsive membrane disruption that may facilitate the cytoplasmic delivery of other encapsulated cargos.

Enzyme-loaded PICsomes were used as *in vivo* nanoreactors.[199,200] The mild conditions used to prepare PICsomes were less likely to deactivate the fragile enzyme molecules, and the semipermeable PIC membranes enabled the uptake of substrates and the release of products while retaining enzymes in the enclosed aqueous cavity.[199] The enzymatic activity of β-galactosidase-loaded PICsomes was maintained even four days after administration, demonstrating their long-term stability under physiological conditions.[199]

5.3 Other polypeptide-based polymersomes

The good water solubility of PLG at neutral pH makes it good hydrophilic blocks for vesicle assembly. Lecommandoux and co-workers reported the preparation of vesicles from poly(trimethylene carbonate) (PTMC)-*b*-PLG as drug carriers.[201] These vesicles were recently used to encapsulate both DOX and superparamagnetic iron oxide NPs, which showed enhanced DOX release kinetics under a magnetic field.[174] Further modification of the polymers by incorporating a PVGLIG peptide spacer enabled the disassembly of vesicles in the presence of tumour-associated enzyme matrix metalloproteinase 2 (MMP-2).[173]

6. Other polypeptide-based materials

6.1 Glycopolypeptides

Glycosylation of proteins, which is the modification of proteins with carbohydrates, endows the proteins with a wide range of biological functions such as cell signaling, adhesion, and lubrication.[202,203] Synthetic glycopolypeptides, as simplified synthetic counterparts, show great potential for understanding and mimicking the structures and functions of glycoproteins.[188,204–206] Compared with single carbohydrate molecules, glycopolypeptides bearing side-chain carbohydrates showed dramatically enhanced affinity to carbohydrate binding protein receptors due to their multivalency.[207–211] For instance, Becer, Heise, and others prepared a series of glycopolypeptides bearing mannose and galactose side-chains, which were used to interact with dendritic cell lectins, inhibiting their bindings with human immunodeficiency virus (HIV).[209] As demonstrated in a star-shaped glycopolypeptide system, the efficiency and kinetics of lectin binding can be manipulated by the polymer architectures and the degrees of glycosylation.[211]

In addition, block copolypeptides bearing glycopolypeptide segments were also used for self-assembly into micelles[212–218] or vesicles,[188,189] where the sugar moieties were on the surface of the assemblies to bind with protein receptors. Glycopolypeptide vesicles were already covered in Section 5.1.2. Through the post-modification of polypeptides, a series of PBLG-*b*-glycopolypeptides with various architectures were synthesized depending on the selection of saccharide precursors (Fig. 24). Amphiphilic copolymers with linear,[217] tree-like,[212] dendritic,[216] and graft structures[217] were obtained, whose self-assembly behaviour and lectin-binding activity were studied. When immunosugar inhibitors were conjugated as the peripheral ligand of polymeric micelles, the resulting micelles exhibited increased inhibition potency towards Jack bean α-mannosidase up to three orders of magnitude compared with the corresponding monovalent analogue.[215]

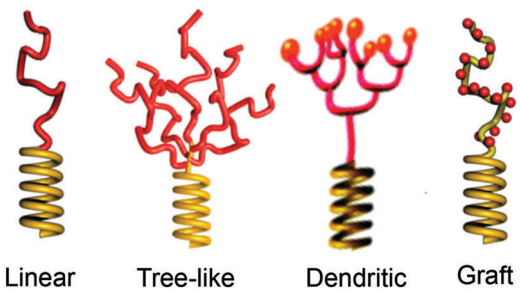

Fig. 24 Amphiphilic PBLG-*b*-glycopolypeptides with various architectures including linear, tree-like, dendritic, and graft structures. Saccharide moieties are highlighted in red, and α-helical, hydrophobic PBLGs are highlighted in yellow. Reprinted with permission from ref. 212, 216 and 217. Copyright (2012, 2015, 2016) Royal Society of Chemistry.

6.2 Polypeptide-based hybrid materials

Hybrid materials containing both inorganic and organic components yield properties shared by each individual component (for inorganic: optical, magnetic, and other properties, size control, *etc.*; for organic: biocompatibility, trigger-responsiveness, *etc.*), making them a promising platform for imaging and therapeutic applications.[219] Synthetic polypeptides, as one of the most important biocompatible polymers, have recently been used to coat inorganic NPs, induce the assembly of discrete metal complexes, and direct the growth of minerals.

6.2.1 Polypeptide-coated inorganic NPs. Polypeptides may be coated onto the surfaces of inorganic NPs by either covalent conjugation or physical adsorption. The covalent conjugation strategy has been reviewed recently by Borase and Heise,[220] which contains both grafting from and grafting onto methods. The grafting from method required the presence of amino initiators on the surface, which was achieved by pre-treating the inorganic NPs with aminopropyltriethoxysilane (APTS) (Fig. 25A). The APTS modification has been applied to silica[221] and iron oxide NPs,[222,223] where the amine-functionalized NPs were used for the surface initiation of various NCA monomers. For instance, Heise, Brougham, and co-workers reported the preparation of glycopolypeptide coated magnetic NPs through the surface initiation of alkyne-based NCA monomers and subsequent glycosylation with click chemistry.[222] The resulting NPs showed good colloidal stability due to the glycopolypeptide layer, which was important for magnetic resonance imaging (MRI) applications. In addition, the surface amino groups were also introduced by anchoring dopamine on the surface of the inorganic NPs (Fig. 25B).[224,225] Other than the grafting from method, the grafting onto method was also used to prepare polypeptide-based hybrid NPs by reacting LA terminated PEG-*b*-PLL with Au NPs.[226]

Compared with the covalent conjugation strategy, physical adsorption methods provide an easy way to prepare hybrid NPs without multistep chemical synthesis. For example, Schatz,

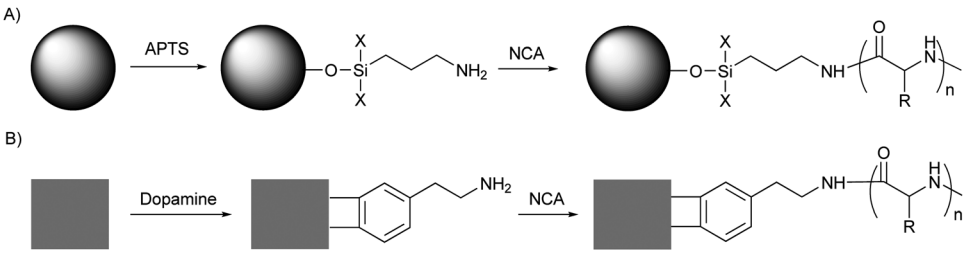

Fig. 25 Synthetic strategies to prepare polypeptide coated inorganic NPs with surface initiated polymerization of NCA monomers. The surface amino groups are introduced through the aminopropyltriethoxysilane (APTS) modification strategy (A) or dopamine anchoring strategy (B).

Lecommandoux, and others reported the preparation of hybrid NPs with silica coated magnetic NPs and (poly(ethylene oxide)-*r*-poly(propylene oxide) (PEO-*r*-PPO))-*b*-PLL copolymers.[227] The electrostatic interactions between negatively charged silica and positively charged PLL segments enabled an anchor-buoy conformation of the copolymers on the silica surface with good colloidal stability. Furthermore, the thermo-responsiveness and the drug loading capacity can be tuned by varying the fraction of PPO units in the copolymer. The loaded DOX was successfully released by applying a magnetic field, which induced the structural change of polymer coatings.

6.2.2 Other polypeptide-based hybrid materials. Deming's lab reported the formation of polypeptide–metal composites that were composed of discrete metal complexes and copolypeptide amphiphiles. Depending on their charge status, the metal complexes either formed charge interactions with the charged polypeptide segments (*e.g.*, $[Au(CN)_2]^-$ interacting with PLL)[228,229] or resided in the hydrophobic region from the self-assembly of hydrophobic polypeptide blocks (*e.g.*, $[Fe(ppi)_2(NCS)_2]$ residing in the PLLeu hydrophobic domain).[230] The long-range interactions between metal ions within the composites resulted in unique features. For example, the composites consisting of $[Fe(ppi)_2(NCS)_2]$ and PLLeu-*b*-PLG amphiphiles displayed an unusual spin crossover phenomenon with a lower critical solution temperature (LCST)-type transition.[230]

Polypeptide-based copolymers have been used to direct the growth of minerals for a long time.[231,232] Typically, the copolymer contained an anionic block (calcium binding units) and a non-ionic block (steric stabilizer), and was used to direct the growth of calcium carbonate[233–235] and calcium phosphate.[236–240] Due to their unique biodegradability and biocompatibility,[241] calcium phosphate-based nanocarriers have been used to load photosensitizers,[240] gadolinium chelates,[236,237,239] and manganese(II) cations[238] for photodynamic therapy, neutron capture therapy, and MRI applications, respectively.

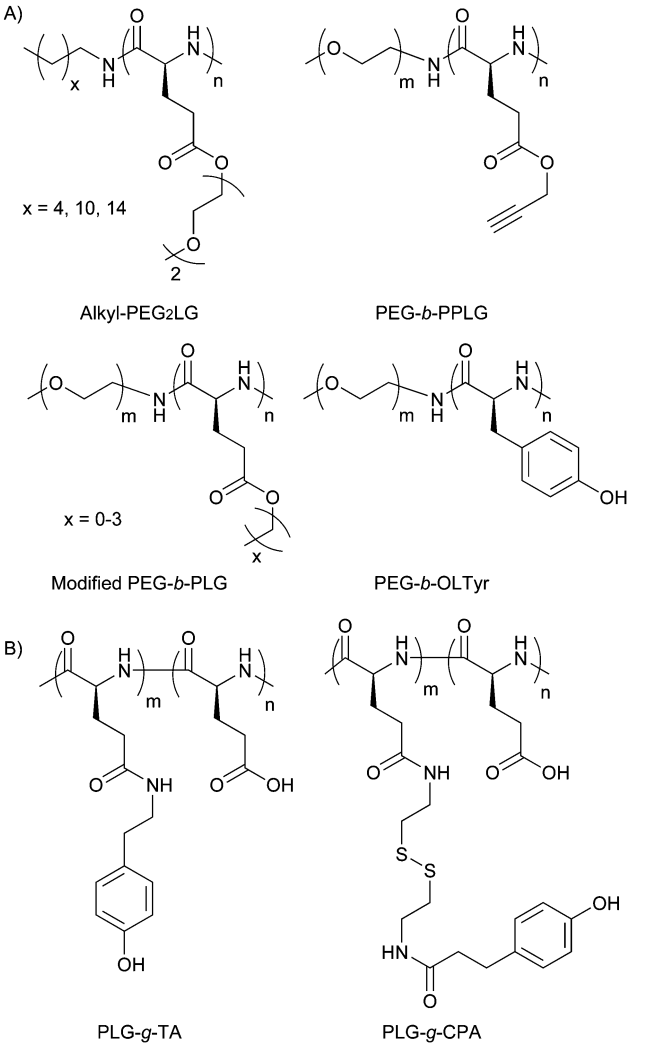

Fig. 26 Chemical structures of polypeptides for the formation of hydrogels through non-covalent bonding (A) or covalent bonding (B).

6.3 Polypeptide hydrogels

Hydrogels are made of cross-linked polymeric networks that contain a large amount of water.[242] Natural macromolecules, such as proteins,[243] polysaccharides[244] and DNA,[245,246] as well as synthetic macromolecules[247,248] are known to form hydrogels. Protein hydrogels possess complicated mechanisms of self-assembly, which inspires the design of hydrogels based on peptide materials.[249–251] Therefore, scientists have developed various synthetic polypeptide-based hydrogels in order to not only unravel the gelation mechanism of protein gels, but also to provide therapeutically beneficial outcomes considering the biocompatibility and biodegradability of polypeptide materials.[252,253]

Polypeptide hydrogels have been widely used in drug delivery,[254] cell therapy,[255–258] and tissue engineering.[259] Based on the gelation mechanism, polypeptide hydrogels can be categorized into two groups. First, the ability of polypeptides to adopt secondary structures allows direct gelation through the association of β-sheets or the coiling of α-helices (Fig. 26A). Second, the chemical diversity of the side chains of polypeptides enables the attachment of various functional groups for chemical cross-linking (Fig. 26B).

6.3.1 Polypeptide hydrogels from non-covalent gelation. Non-covalent bonded polypeptide hydrogels are preferred for injectable hydrogel applications because of their shear-thinning characteristics.[260] Polypeptides with β-sheet conformations are more commonly utilized as the building blocks for hydrogels.[261–266] The Li group discovered that poly(γ-(2-methoxyethoxy)ethyl-L-glutamate)s (PEG_2LG) with a short degree of polymerization (DP, <15) tended to form β-sheet in aqueous solution.[264,265] A series of PEG_2LG amphiphiles with various alkyl chains on the backbone terminus were therefore prepared for hydrogel formation (Fig. 26A and 27A). Cryogenic TEM and atomic force microscopy (AFM) results showed that all amphiphiles formed fibril networks with uniform widths and heights. The polypeptide segments played a key role in the nanoribbon structure: the β-sheet conformation acted as the building blocks and OEG side chains contributed to the water solubility. The shear-thinning and rapid recovery properties of the hydrogels made them promising materials for biomedical applications.[266]

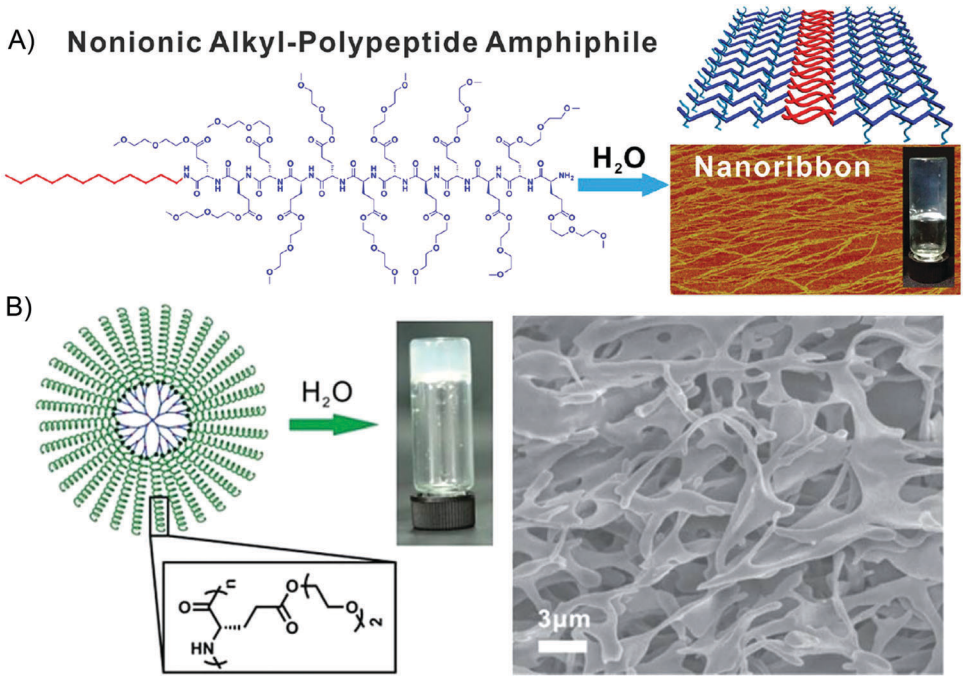

Fig. 27 Hydrogel formation from polypeptide-based materials through non-covalent bonding. (A) Gelation mechanism of β-sheet alkyl-PEG_2LG amphiphiles. The AFM image of nanoribbons is shown on the right side. Reprinted with permission from ref. 264. Copyright 2013 American Chemical Society. (B) Gelation mechanism of α-helical, star-shaped PEG_2LG. The SEM image of fibril assemblies is shown on the right side. Reprinted with permission from ref. 267. Copyright 2015 Royal Society of Chemistry.

Besides OEG modified PLG, alkyl chain modified PLG,[261] PPLG,[262] oligo(L-tyrosine) (OLTyr),[263] and oligo(DL-allylglycine)[266] were also able to form β-sheets (Fig. 26A), and the block copolymers of these polypeptides with PEG segments underwent sol–gel transitions in response to changes in temperature.

Additionally, the hydrophobic α-helix is another important building block to form non-covalent hydrogels.[267,268] Recently, Li and co-workers synthesized star-shaped PEG_2LG that spontaneously formed a hydrogel at low concentration (Fig. 27B). The low critical gelation concentration was attributed to the formation of nanofibers by the oriented parallel or anti-parallel packing of rigid α-helices.[267]

Non-covalently bonded hydrogels are able to restore the gel phase once injected, making them important candidates for *in vivo* applications.[260] For instance, amphiphilic diblock copolypeptide hydrogels (DCH) prepared by Sofroniew, Deming, and coworkers were used as depots for sustained release of encapsulated cargos. DCH were biocompatible and fully degradable over several weeks, with no detectable toxicity and good integration with central nervous system (CNS) tissues.[255,256] The release of cargos can be easily manipulated through the polymer design including copolypeptide compositions, polypeptide lengths, and the type of hydrophobic side chains.[256]

6.3.2 Polypeptide hydrogels from covalent gelation. Chen, He, Li, and co-workers have prepared various covalently bonded polypeptide hydrogels based on enzymatic cross-linking chemistry.[269–271] For example, the phenol groups on the side chains of tyramine modified PLG (PLG-*g*-TA, Fig. 26B) reacted with each other to form intermolecular cross-linking in the presence of H_2O_2 with the catalysis of horseradish peroxidase (HRP).[269,271] The resulting hydrogels were evaluated as an artificial extracellular matrix (ECM) for three-dimensional cell cultures. In a similar approach, disulfide bond-modified phloretic acid was conjugated on the side chains of PLG (PLG-*g*-CPA, Fig. 26B) for gelation.[270] The incorporated disulfide linkers induced the trigger-responsive degradation of the hydrogels, enabling easy collection of cells after culturing. In addition, injectable polypeptide hydrogels were also achieved by the mixing of two polymers for *in situ* gel formation.[259,272] For instance, the mixing of hydrazide- and aldehyde-modified PLG resulted in rapid hydrogel formation, and the resulting hydrogels exhibited good mechanical stability and cell ingrowth for cartilage regeneration.[259]

6.4 Antimicrobial polypeptides

Antimicrobial peptides (AMPs) produced by multicellular organisms serve as a defense against pathogenic microbes.[273] AMPs typically possess an amphipathic structure with cationic charges, allowing them to attach and insert into membrane bilayers. Current AMPs are usually obtained from microbial screening or SPPS that are highly expensive and labor-intensive.[274] Therefore, the ROP of NCAs provides promising alternatives to produce AMPs with tunable compositions, secondary structures, and architectures.[275,276]

The balanced amphipathicity of AMPs enables appropriate interaction with cell membranes.[273] Copolypeptides with both hydrophilic (*e.g.*, PLL) and hydrophobic segments (*e.g.*, PLAla, PLLeu, or PLPhe) were therefore prepared for antimicrobial evaluation.[275]

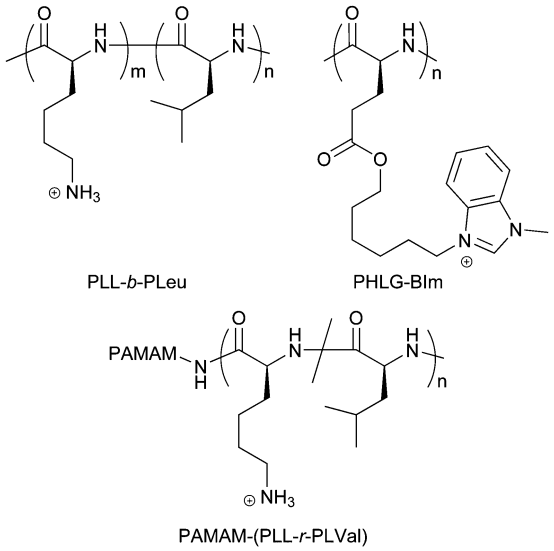

Fig. 28 Chemical structures of antimicrobial polypeptides synthesized from NCA polymerization.

For instance, Deming and co-workers synthesized PLL-*b*-PLLeu that can form hydrogels in aqueous environments (Fig. 28). The resulting hydrogels displayed broad-spectrum antimicrobial activity and showed promising properties to prevent the microbial contamination of wounds.[277] Apart from amphipathicity, the conformation of AMPs is another important parameter to regulate the antimicrobial activity.[278] Inspired by the conformation-associated activity, Cheng, Wong, Ferguson, and others designed an α-helical antimicrobial polypeptide with radial amphiphilicity (PHLG-BIm, Fig. 28).[279] The helical polymers displayed excellent antibacterial activity compared with their random coiled analogs, indicating the crucial role of the α-helical structure in membrane interaction. The radial amphiphilicity design not only minimized the hydrophobic interaction with blood cells, but also protected the polypeptides from rapid enzymatic degradation, which is a typical drawback for AMPs.

In addition, the macromolecular architectures of polypeptides significantly contributed to their activities. Qiao, Reynolds, and co-workers prepared structurally nanoengineered antimicrobial peptide polymers (SNAPPs, Fig. 28) consisting of a poly(amido amine) (PAMAM) dendritic core and multiple PLL-*r*-poly(L-valine) (PLVal) arms.[280] The star-shaped SNAPPs stayed as unimolecular structures in aqueous environments, and were highly effective in combatting multidrug-resistant Gram-negative bacteria. Different from typical AMPs, the efficacy of SNAPPs resulted from a multi-modal antimicrobial mechanism, including the disruption of outer membranes and cytoplasmic membranes, unregulated ion movement across the cytoplasmic membrane, and the induction of an apoptotic-like death pathway.

6.5 Other polypeptide-based materials

Synthetic polypeptides have also been used to coat adenoviral particles,[281] stabilize drug-loaded protein cages,[282] formulate pH-responsive complexes with proteins,[283] form fibril-shaped aggregates with DOX,[284] or fabricate unimolecular CPT–brush polymer conjugates.[285] The polypeptide modification stabilized the actives under physiological conditions and enhanced cell internalization. Recently, Lu and co-workers reported site-specific conjugation of proteins with heterotelechelic polypeptides.[286] The phenyl thioester terminus and the polyglycine moieties of polypeptides allowed the native chemical ligation and sortase A-mediated ligation, respectively. Various protein–polypeptide conjugates with well-defined topological structures, including cyclic conjugates, were obtained, which showed enhanced stability compared with native proteins.

7. Conclusions and perspectives

As a class of materials with excellent biocompatibility, biodegradability, chemical diversity, and capability to adopt higher ordered structures, synthetic polypeptides are widely recognized as one of the most important bio-mimicking polymers for biomedical applications. In this review article, we have shown how scientists were able to optimize the biomedical performances of polypeptide materials through rational design and controlled self-assembly techniques in the last two decades.

Advances in polypeptide chemistry have enabled the incorporation of various functionalities into the side chains of synthetic polypeptides. These added functionalities help improve the binding with cargos, direct the formation of ordered self-assemblies, stabilize the formed supramolecular structures, enhance the interaction with cells, or control the cargo release on-demand. In the design of polypeptides to incorporate proper functional groups onto polypeptide side chains for optimized outcomes, we have gained additional understanding in terms of the desired molecular forces for cargo loading and material assembly, how the functional groups are installed on polypeptide side chains, and the optimal composition in the case of copolymers. The functional groups should be selected based on the type of cargos (*e.g.*, nucleic acids or hydrophobic drugs) as well as the expected assembly mechanism (*e.g.*, charge complexation or hydrophobic interaction). If the functional groups are not compatible with NCA polymerization (*e.g.*, amino, hydroxyl, and guanidino groups), they need to be protected during NCA synthesis or incorporated through post-polymerization modifications. The screening of copolymer compositions is often necessary to achieve controlled assembly behavior, best cargo loading, and good water solubility of resulting assemblies.

Depending on the chemical structures of polypeptide side chains and cargos, supramolecular structures were constructed through various molecular interactions including hydrophobic interactions, charge complexation, metal coordination, and hydrogen bonding interactions. Several factors were considered during the self-assembly process: the stability of supramolecular structures under physiological conditions, the protection of cargos against possible degradation (*e.g.*, hydrolysis and enzymatic degradation), and the release profile of the encapsulated cargos. After the preliminary evaluation of the supramolecular assembly, the chemical design of polypeptides can be revisited to

add other functionalities such as cross-linkable moieties, targeting ligands, and trigger-responsive segments.

Despite the great advances in polypeptide chemistry and supramolecular assembly, there is still a long way to go to make synthetic polypeptides as a broadly used platform of biomedical materials and therapeutics, which is not only to provide a deeper understanding of the structure–property relationships of naturally occurring proteins, but also to synthetically produce protein-mimicking materials on a large scale for biomedical applications. To date, polypeptide-based materials have been found to be still far from matching the structures and functions of proteins, partly because of the lack of sequence control and monodispersity of synthetic polypeptides compared with proteins, which are critical for the precise construction of higher ordered structures. It is thus inspiring to see the recent advances in the design, regulation, and applications of the secondary structures of synthetic polypeptides, which is a big step toward the mimicking of structures and functions of proteins. Therefore, the manipulation of higher ordered structures of synthetic polypeptides, as well as further understanding of the relationship between higher ordered structures and polypeptide functions, will be important future directions for polypeptide scientists. To facilitate broader application of polypeptides, the high costs and tedious procedures for the synthesis, purification and handling of moisture-sensitive NCA monomers, as well as the stringent requirements for controlled polypeptide synthesis such as NCA with high purity and anhydrous polymerization conditions, are critical barriers for synthetic polypeptides. The need for more economical pathways to produce polypeptide materials, such as improving the moisture resistance of polypeptides and simplifying the processes of NCA and polypeptide chemistry, provides important and challenging tasks to polypeptide chemists.

Conflicts of interest

There are no conflicts to declare.

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