Chinese Journal of
POLYMER SCIENCE 高分子科学

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Cite this article as:
Zhang Chong, Lu Hua. Helical Nonfouling Polypeptides for Biomedical Applications[J]. *Chinese J. Polym. Sci*, 2022, 40(5): 433-446. doi: 10.1007/s10118-022-2688-2

View online: https://doi.org/10.1007/s10118-022-2688-2

Chinese Journal of
POLYMER SCIENCE

FEATURE ARTICLE

https://doi.org/10.1007/s10118-022-2688-2
Chinese J. Polym. Sci. **2022**, *40*, 433–446

Helical Nonfouling Polypeptides for Biomedical Applications

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Abstract Synthetic polypeptides, also known as poly(*α*-amino acid)s (P*α*AAs), are biomimetic and biodegradable polymers holding great potential for a variety of biomedical applications. Possessing the same peptide bonds as natural proteins, polypeptides can also adopt typical well-defined secondary structures including *α*-helix, which have been shown to significantly impact the physicochemical properties and biological outcomes of materials. In this feature article, we review the state-of-the-art progresses of *α*-helical polypeptides for biomedical applications, with a special emphasis on the manipulation of helix-to-coil dynamic transition, conformation-associated anti-biofouling coatings, cellular uptake regulation, and reducing immunogenicity of polypeptide-protein conjugates. Finally, perspectives on outstanding challenges remained in this field and some important future directions are discussed.

Keywords Poly(*α*-amino acid); Polypeptide; Helix; Nonfouling; PEPylation

Citation: Zhang, C.; Lu, H. Helical nonfouling polypeptides for biomedical applications. *Chinese J. Polym. Sci.* **2022**, *40*, 433–446.

INTRODUCTION

Synthetic polypeptides, also known as poly(*α*-amino acid)s (P*α*AAs), are an important class of biomimetic polymers that possess exactly the same peptide bonds as natural proteins with inherent biocompatibility and biodegradability.[1–9] Owing to their unique structural features and fascinating properties, polypeptides have attracted vast attentions from a variety of areas including but not limited to surface coatings,[10–12] drug delivery,[13–17] protein modification,[18–20] antimicrobial,[21–25] gene therapy[26–28] and tissue engineering.[29–31] Typically made by the ring-opening polymerization (ROP) of amino acid *N*-carboxyanhydride (NCA), polypeptides can be rapidly produced up to kilogram scale with high molecular weight and at a relatively low cost.[32–36] The NCA monomers can be derived from both natural and unnatural amino acids of either $_{L}$- or $_{D}$-enantiomer, which greatly expands the chemical diversity and functionality of polypeptides to meet distinctive application requirements.[37,38]

Thanks to the chirality of amino acid and peptidic backbones, polypeptides are capable of folding into ordered protein-like secondary structures (*e.g.*, *α*-helix and *β*-sheet) with fascinating properties, which are distinctive from conventional polymers that typically bear disordered and irregular conformations.[39] For instance, as the most prevalent structural motifs in natural proteins, *α*-helix is also commonly seen in synthetic polypeptides, especially in derivatives of poly($_{L}$-glutamates) (PGA) and poly($_{L}$-lysine) (PLL). Moreover, the secondary structures can sometimes be switched between ordered and disordered states in a stimuli-responsive and reversible fashion, offering extra parameters to regulate the physicochemical and biological properties of P*α*AAs.[40,41] Earlier studies mainly focused on altering internal structural characters such as charges, chain lengths, polar groups, counterions and hydrophobicity/hydrophilicity of polypeptides to control conformations. Moreover, external triggers such as pH, light, temperature, and redox reagents have also been utilized to modulate the transition of secondary structures.[42–45] In recent years, growing attention has been devoted to the secondary structure-governed functional switches and related applications (Fig. 1).[46] For instance, Cheng and coworkers pioneered ultra-stable ionic helical polypeptides bearing outstanding membrane activities that are highly useful for applications including antimicrobial[47,48] and drug/gene/siRNA delivery.[27,49,50] The team led by Kataoka demonstrated that the helix of poly(ethylene glycol) (PEG)-polyglutamate block copolymer-based nanomedicines can regulate their *in vivo* pharmacological behaviors including half-life and biodistribution.[51,52] Lecommandoux *et al*.,[53] Barz *et al*.,[54] Ding *et al*.,[55–57] Schlaad *et al*.,[58] Lin *et al*.,[59–61] Chen and He *et al*.[62] also reported clear conformation- or chirality-related effects on the self-assembly behaviors of polypeptides. Hammond *et al*.,[63,64] Chen *et al*.,[65] and Zhang *et al*.[66] showed that the secondary structure of polypeptides is an important factor modulating rheological/mechanical properties of polypeptides-based hydrogels or bulk materials. More recently, the helix was found to significantly impact the

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Invited Feature Article for the 40th Anniversary of *Chinese Journal of Polymer Science*
Received December 10, 2021; Accepted December 30, 2021; Published online March 2, 2022

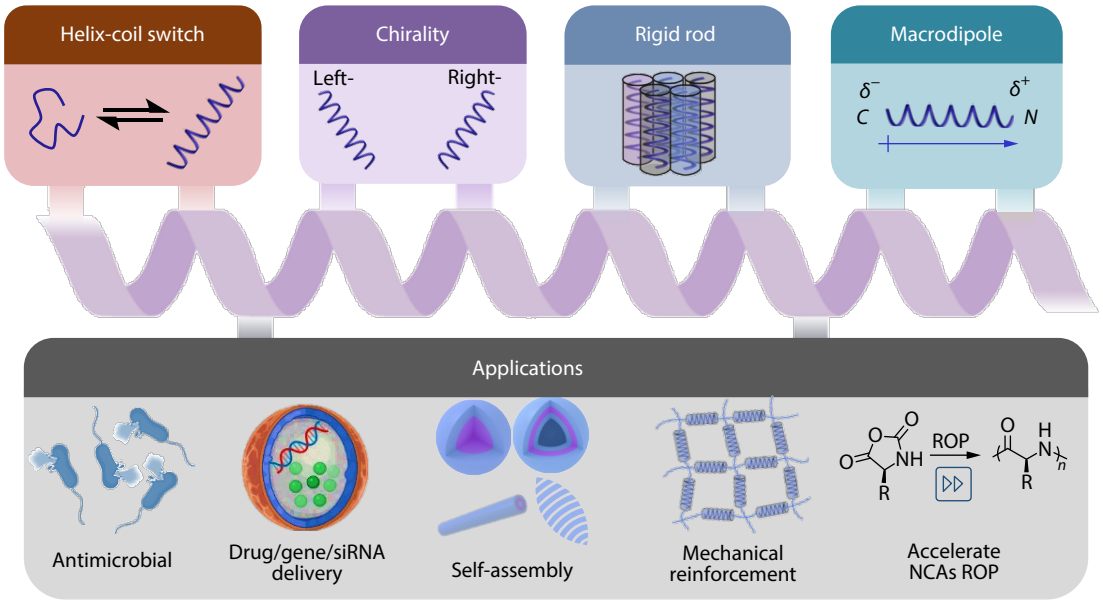

Fig. 1 Schematic representation of the physicochemical properties of *α*-helix and typical applications.

ROP kinetics of NCAs *via* cooperative interactions of macrodipoles.[67–69] Those progresses have been comprehensively summarized in a number of outstanding review articles published recently.[4,5,26,33,70] To avoid duplication with previous reviews, here, we will mainly discuss the important role of helices in nonfouling polypeptides, *i.e.*, the ability to prevent nonspecific adsorption of biomolecules and microorganisms, and related biomedical applications. Specifically, we will first introduce the design of several polypeptides with dynamic and stimuli-sensitive helix-coil transition behaviors. Then, we will highlight examples in which helical conformation modulates the biological outcomes of nonfouling polypeptides in surface antifouling, *in vitro* cellular uptake, *in vivo* circulation, and immunogenicity mitigation. Finally, we will provide complementary and valuable perspectives on the outstanding challenges and some important future directions of polypeptides for the design of advanced biomimetic materials.

HELIX-COIL DYNAMIC TRANSITION

The stimuli-responsive dynamic and reversible transition of secondary structures in response to changes in environmental conditions is of great interest in mimicking adaptive biological systems.[11,43,70–73] Among which, helix-coil transition is perhaps one of the most well-studied examples.[74–77] Often, in biological systems, the helix-coil transition may suggest denaturing or sometimes activity on-off switch of proteins. Thus, it would be intriguing to mimic, understand, and manipulate such behaviors in synthetic polypeptides. The formation of *α*-helix of polypeptides primarily relies on the well-defined intramolecular hydrogen bonding along the backbone. The physicochemical properties of side groups, which are evenly scattered along and perpendicular to the rigid *α*-helical backbone, can also affect the stability of the helix. A general strategy of controlling the helix-coil transition is thus through altering the status of the side groups to disrupt or restore the main-chain intramolecular hydrogen bonds that are crucial for generating *α*-helix. Many external or internal stimuli including pH, solvent, temperature, light, ionic strength, redox environment, and biomolecules have been used to tune such transitions effectively, which have been summarized in several excellent reviews.[6,78,79]

pH is perhaps the most common factor for regulating the helix-coil transition of polypeptides. Many polypeptides bearing carboxyl,[80,81] primary amines,[82] imidazole,[83,84] triazole[85] and tertiary amines[86] residues have demonstrated their ability of helix-coil transition. When charged, the ordered secondary structures would be destroyed and turn to random coil conformation owing to lateral charge repulsion. PGA and PLL have long been used as building blocks to construct pH-dependent conformation-switchable polypeptides.[44,48,87–93] Cheng, Chen, Yin and co-workers reported that polypeptide bearing negatively charged glutamic acid and positively charged quaternary ammonium showed a random coiled conformation at physiological pH, which enabled coil-to-helix transition under acidic condition.[48] Bonduelle *et al.* reported the reversible helix-to-coil transition at neutral pH by utilizing imidazole-functional PLL to tune the pK_a of PLL[83] and coordination chemistry between poly(L-glutamic acid) and metal ions.[93] Recently, Lu *et al.* developed a series of tertiary amine-functionalized polypeptide electrolytes bearing oligo(ethylene glycol) (EG) linkers (P(EG$_x$DMA-Glu), x=1, 2, 3) *via* the NCA polymerization and thiol-ene chemistry (Fig. 2A).[86] All these ionic polypeptides exhibited a pH-dependent reversible helix-coil switch within the physiological pH range (~5.3–6.5). The authors further investigated the salt effect on secondary structures and observed an interestingly salt-induced coil-to-helix transition, which is speculated to be the unique properties of EG linkers that could endow the polypeptides with fine-tuned amphiphilicity. Interestingly, when the charges of these series polypeptides were switched to zwitterion such as carboxy betaine (CB), the helices of the resulting polypeptides P(CB-EG$_x$Glu) (x=1, 2, 3) were stable with no response to a wide range of

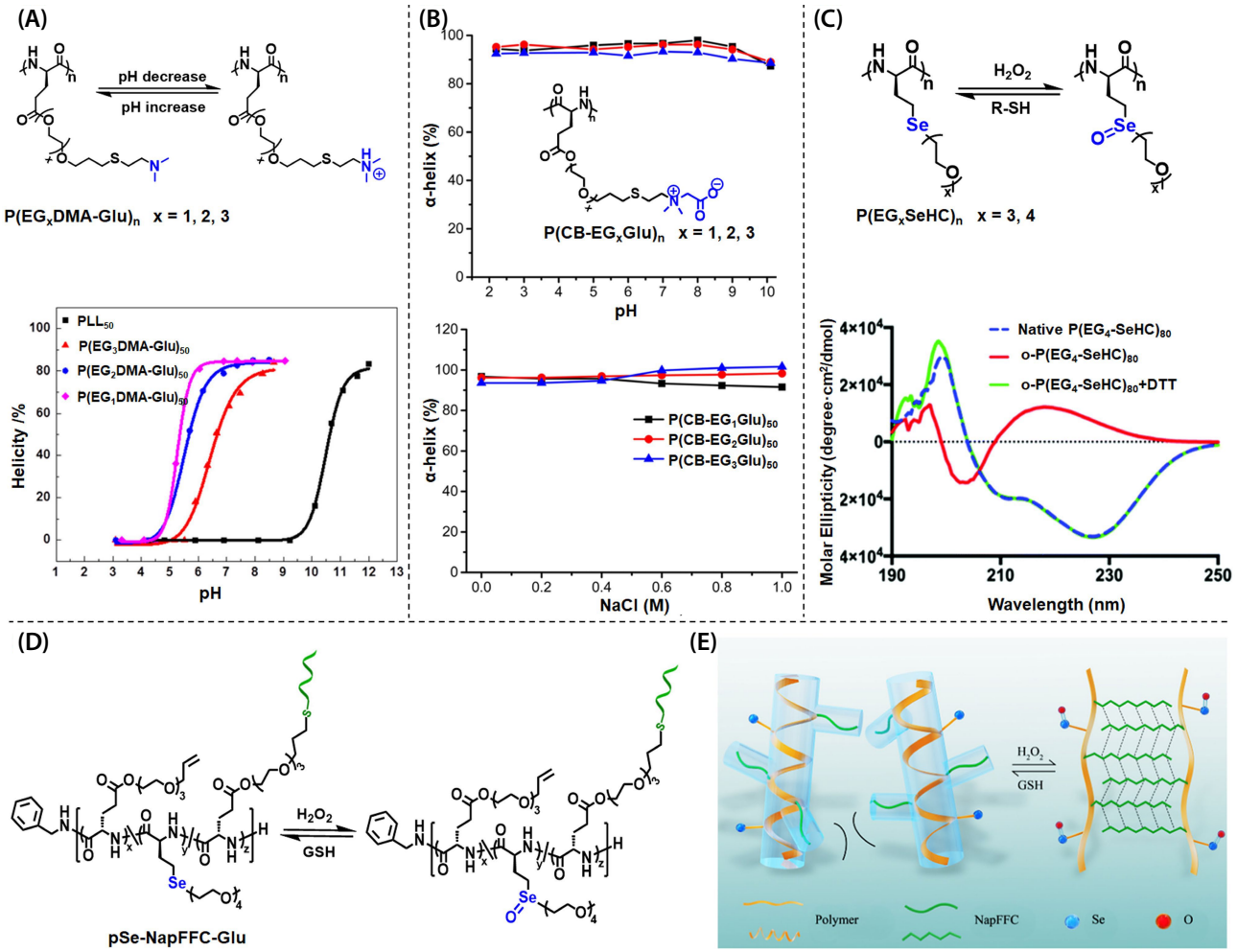

Fig. 2 Dynamic transitions of secondary structures. (A) The chemical structure and pH-dependent helicity of ionic polypeptides P(EG_xDMA-Glu)$_{50}$, x=1–3). (Reproduced with permission from Ref. [86]; Copyright (2018) American Chemical Society). (B) The structure of zwitterionic polypeptides P(CB-EG_xGlu) (x=1, 2, 3) and the helical stability against changes of pH and salt concentrations. (Reproduced with permission from Ref. [94]; Copyright (2018) American Chemical Society). (C) The structure and CD spectra of selenopolypeptides P(EG_4-SeHC)$_{80}$ after H_2O_2 oxidation followed by dithiothreitol (DTT) reduction. (Reproduced with permission from Ref. [100]; Copyright (2019) The Royal Society of Chemistry). (D) The structure changes and (E) schematic illustration of redox-regulated reversible supramolecular assemblies of the brush-like selenopolypeptide pSe-NapFFC-Glu during redox reaction. (Reproduced with permission from Ref. [102]; Copyright (2021) American Chemical Society).

protons (pH 2–10) and salt changes (up to 1.0 mol/L) (Fig. 2B).[94]

Poly($_L$-cysteine) or poly($_L$-methionine) derivatives with thioether linkages in the side chains are commonly employed to prepare redox-sensitive polypeptides with conformation changes.[55,95–99] Presumably, the oxidation of thioether to sulfoxide or sulfone groups can increase the polarity of side-chain and results in disruption of the ordered secondary structures. Deming *et al.*[96] reported the irreversible helix-to-coil transition of sugar based-poly($_L$-cysteine) after complete oxidization to sulfone, while the partially oxidized sulfoxide intermediate was insufficient to destabilize the *α*-helical structure. To accomplish reversible conformation transition, the same group prepared glycol- and EG-functionalized poly($_L$-homocysteine)s,[97] which undergo reversible helix-to-coil transitions upon oxidation to sulfoxide and reduction back. To increase the redox sensitivity, water soluble selenoether-containing polypeptides carrying EG side chains (P(EG_x-SeHC)s, x=3, 4) were prepared by Lu and co-workers *via* the ring-opening polymerization of selenohomocysteine *N*-carboxyanhydrides (SeHC-NCA) (Fig. 2C).[100] The selenium-containing polypeptides possessed reversible redox-induced helix-to-coil transition and were considerably more sensitive compared to previous thioether-based polypeptides due to the higher reactivity of selenium chemistry. P(EG_x-SeHC)s were also thermally responsive with tunable lower critical solution temperature (LCST) near body temperature. These interesting properties made selenopolypeptides P(EG_x-SeHC)s promising as rapid responsive materials under physiological and/or pathological conditions. For example, when conjugated to oxidative-prone proteins such as human alpha-1-antitrypsin (A1T1), P(EG_x-SeHC) was found to protect the protein in *in vivo* from oxidation-induced reactivity loss and in the meantime maintained a prolonged circulation

half-life.[101]

Building on the studies discussed above, Gao, Lu and coworkers exploited redox-responsive dynamic conformation switching provided by selenopolypeptide to develop reversible supramolecular self-assemblies.[102] Brushlike selenopolypeptide (pSe-NapFFC-Glu) carrying selenoether and β-sheet-prone short-peptide naphthyl-Phe-Phe-Cys (NapFFC) were rationally designed (Fig. 2D). The as-prepared pSe-NapFFC-Glu showed a redox-induced reversible transition between backbone α-helix and side chain β-sheet (Fig. 2E). Due to the initial rigid helical backbone of pSe-NapFFC-Glu, supramolecular assembly of NapFFC in the side chains was inhibited, producing irregular aggregate morphology. Interestingly, upon oxidation of selenoether to selenoxide, the backbones switched to flexible and disordered conformation, while, at the same time, activating the β-sheet conformation of NapFFC followed by self-assembling into nanofibrils. Moreover, these reversible conformation and morphology transitions were successfully applied as vehicles for controlled drug release in cancer treatment.

HELIX IN ANTI-BIOFOULING SURFACES

Biofouling of materials caused by the undesirable accumulation of biomolecules (*e.g.*, protein, platelet, cell and bacterial) can significantly impair the function and lead to adverse events such as thrombus, implant rejection, bacterial infection, immune response and reduced sensitivity.[103–105] Similarly, it has been increasingly known that protein corona formed on the surface of nanoparticles can determine their eventual fate *in vivo*.[106–109] Surface modification with a layer of anti-biofouling polymers has been proven to be one of the most useful strategies to prevent biofouling.[110–115] PEG is the most widely used and gold standard anti-biofouling polymer owing to its neutral charge, high water-solubility, large excluded volume, and minimum interfacial free energies.[116,117] Jiang *et al.* pioneered various zwitterionic polymers, including $(EK)_n$ polypeptides, with superior antifouling performances than PEG.[118] Polypeptoids, such as polysarcosine, have also been anchored on surface for anti-biofouling purposes.[119] One common character of all these polymers is the disordered and flexible conformation, which was believed to be entropically unfavored in the occurrence of biofouling events.

Recently, it was suggested that conformation-constrained polymeric architectures such as dendrons,[120] loops,[121–123] and cycles,[124,125] can generate denser films with higher steric stabilization and repulsive forces as compared to their linear flexible analogues. These results naturally raised the question of whether a helical polypeptide with rigid conformation could also give rise to good antifouling properties. Klok *et al.*[126] previously prepared oligo(ethylene glycol)-modified poly($_L$-lysine) brushes (PPEG$_x$Lys) *via* surface-initiated ring-opening polymerization (SI-ROP) of OEGylated $_L$-lysine *N*-carboxyanhydrides. Those brushes showed a stable α-helical conformation between pH 4–9 and effective prevention of nonspecific protein adsorption of fibrinogen and bovine serum albumin. Recently, Lu *et al.*[94] synthesized a series of thiol-terminated zwitterionic poly(α-amino acid)s bearing CB and varied lengths of EG linkers, namely P(CB-EG$_x$Glu) (x=1–3, Fig. 2B). It was observed that all these polypeptides exhibited excellent prevention of protein/cell/platelet/bacterial adsorption that outperformed linear PEG, and the antifouling properties were enhanced when the EG length (x) was increased from 1 to 3.

To further investigate the secondary structure effect on antifouling surfaces, Lu and colleagues designed a series of poly(γ-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)esteryl glutamate) (P(EG$_3$Glu)) bearing different conformations by choosing monomers with varying chirality on the α-carbon. Those polymers were anchored to gold surfaces *via* thiol group introduced at either the *C*- or *N*-terminus of the polypeptides (namely, L-C, L-N, DL-C, and DL-N as shown in Figs. 3A and 3B).[127] The adsorption kinetics and antifouling properties were studied using ellipsometer and quartz crystal microbalance with dissipation monitoring (QCM-D) (Fig. 3C and 3D), respectively. It was found that the rigid and constrained α-helical $_L$-P(EG$_3$Glu) (L-C or L-N) self-assembled more rapidly and produced denser adlayers with improved antifouling properties compared to unstructured analogues (DL-C and DL-N) and linear PEGs. Interestingly, further enhancement in the adsorption kinetics and antifouling effect can be achieved by antiparallel orientation through an equal molar mixture of L-C and L-N (L-C/L-N), while unstructured DL-C/DL-N did not show any orientation dependence. The orientation effect observed here is suggested to be a result of the cancellation of repulsive force derived from the macroscopic dipole moment of α-helix. Moreover, the secondary structure effects were also successfully applied to zwitterionic polypeptides derivatives to prepare ultralow fouling surfaces. It should be pointed out that, though, the L-C polypeptide adlayer still adsorbed less proteins than the DL-C one even at the same grafting density of the two polypeptides, suggesting that the helical polypeptide is an intrinsically stealthier material than its random coiled structural analogue.

Helix Regulates Materials-Cell Interaction Behaviors

Controlling the interactions at the biomaterial-cell interfaces is of central importance for the regulation of cellular behavior (*e.g.*, adhesion, proliferation, differentiation and nanoparticles internalization) and prediction of materials performance.[128–131] It is now well understood that these interactions are greatly influenced by physicochemical properties of materials, such as surface chemical compositions, wettability, topographic structures, charge, chirality, rigidity, *etc.*[132–135] As one of the most typical biochemical signatures of life, chirality plays a crucial role in many biological and physiological processes, and thus facilitates the development of chiral biointerface materials with better compatibility and various biofunctions.[136,137] The main-chain chirality of polypeptides can be facilely tuned by simply switching the chirality of monomeric amino acids, which makes it appealing to mimic natural biological systems. Li and co-workers[138] prepared $_{L-}$, $_{D-}$, or $_{DL-}$poly-γ-benzyl-glutamate coated surfaces by SI-ROP of NCAs to study how cells behavior on those conformational isomers. Interestingly, they showed that L929 cells adhered and grew more densely on poly(γ-benzyl-$_L$-glutamate) (PBLG)-grafted substrates than the other two polymer-grafted surfaces.

A deep understanding of the interactions between nanoparticles (NPs) and cells is essential for the development of promising nanomedicines with high therapeutic efficacy and

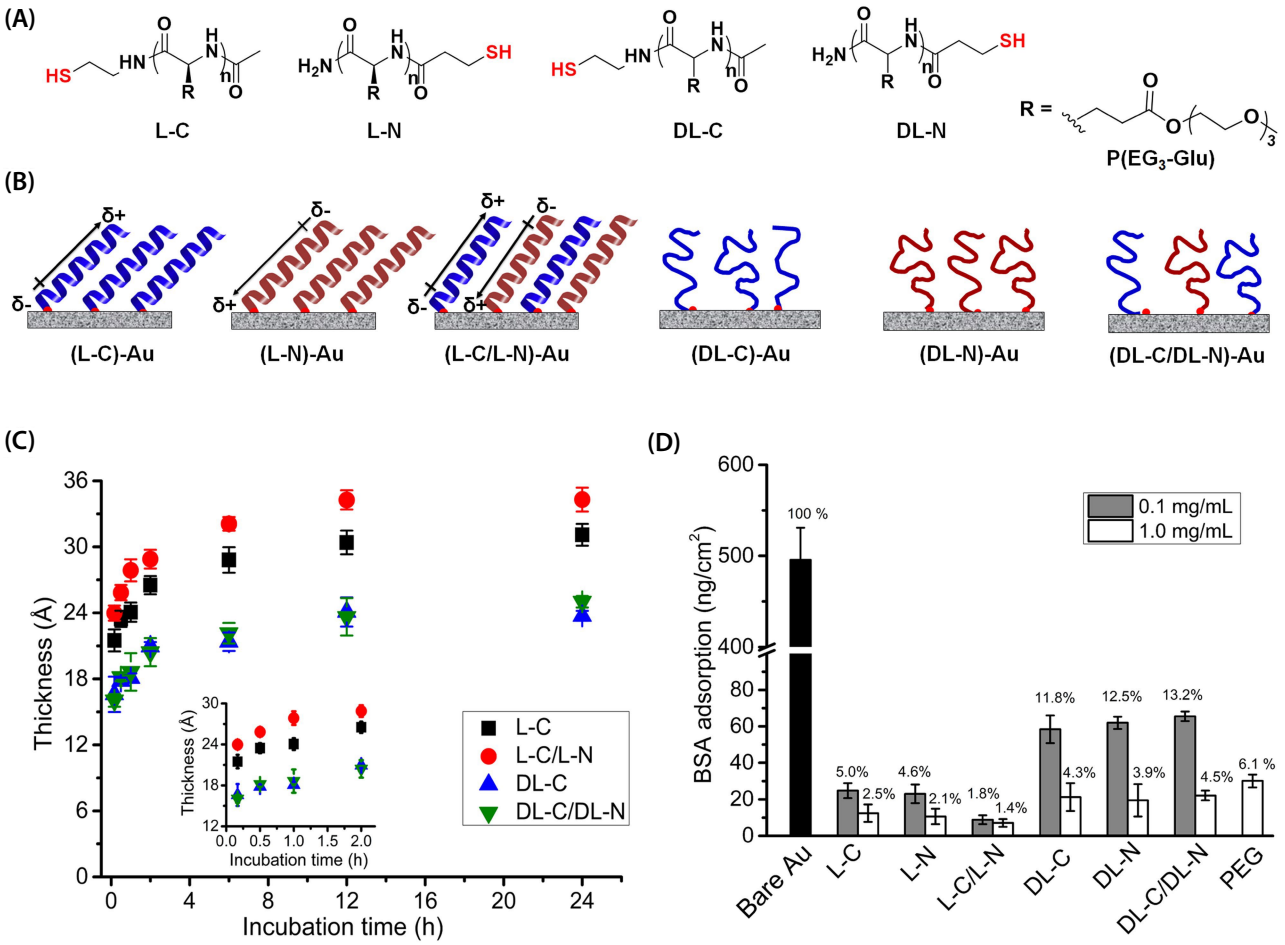

Fig. 3 (A) Chemical structures of P(EG_3Glu)s bearing different conformations with the anchoring thiol group at either *C* or *N*-terminus (L-C, L-N, DL-C, and DL-N). (B) Cartoon illustration of different P(EG_3Glu) adlayers on gold surfaces. (C) The ellipsometric thickness of P(EG_3Glu) adlayers as a function of incubation time (polymer concentration: 1.0 mg/mL). (D) The amount of BSA adsorbed on P(EG_3Glu)-coated gold surfaces prepared at 0.1 and 1.0 mg/mL polypeptide concentration. (Reproduced with permission from Ref. [127]; Copyright (2018) Elsevier).

low toxicity. As a result, cell-penetrating peptides (CPPs) have been extensively used as delivery vectors because of their excellent membrane activities that can transport different cargoes into cells.[139,140] Inspired by the intrinsic positive charges (arginine and lysine residues) and helical structure of natural CPPs,[141,142] Cheng and co-workers developed a series of amphiphilic *α*-helical polypeptides CPPs-mimics with long hydrophobic side-chains (~11–18 *σ*-bonds) that terminated with different cationic groups such as amine, guanidium, quaternary ammoniums, and phosphoniums, *etc*.[45,47,50,85,143,144] Briefly, the cationic polypeptides with rigid *α*-helical conformation demonstrated superior cell-penetrating capability compared to their random-coiled counterparts with identical chemical constitution. Computer simulation revealed that the side chains of cationic *α*-helical polypeptides could undergo significant rearrangement in response to different environments and induce membrane-destabilizing negative Gaussian curvature (NGC) for membrane disruption, while the disordered polypeptides did not have such ability.[145] Since then, the cationic helical polypeptides have been used extensively for the delivery of nucleic acids and demonstrated extraordinary helix-associated transfection efficiency for both plasmid genes and siRNAs. Lu *et al*.[146] screened libraries of cationic helical polypeptides bearing various pyridine derivatives and alkyl chains and successfully expanded the applications of these cationic polypeptides for protein delivery.

The above studies emphasize the essential contribution of side-chain cationic groups in mediating helix-dependent cell and materials interactions. Beyond that, another interesting structural feature of *α*-helix is the macrodipole with a partial negative and positive charge at *C*- and *N*-terminus, respectively, which is the result of the vectorial sum of the unidirectionally aligned hydrogen bonding dipoles along the helical axis. The macrodipole has been previously shown to play an important role in controlling peptide/protein structures and functions,[147–149] regulating surface self-assembly behaviors,[150] as well as inducing unprecedented redox,[151] electrical conductivity,[152] piezoelectric,[153] and electro-optical properties, [154] *etc*. To get further insight into the effect of conformation and macrodipole orientation on cellular internalization behaviors, neutral nonfouling P(EG_3Glu)s bearing different secondary structures were grafted onto gold nanoparticles (AuNPs) by Lu and co-workers (Fig. 4A).[155] AuNPs coated with parallel aligned *α*-helical $_L$-P(EG_3Glu) displayed a

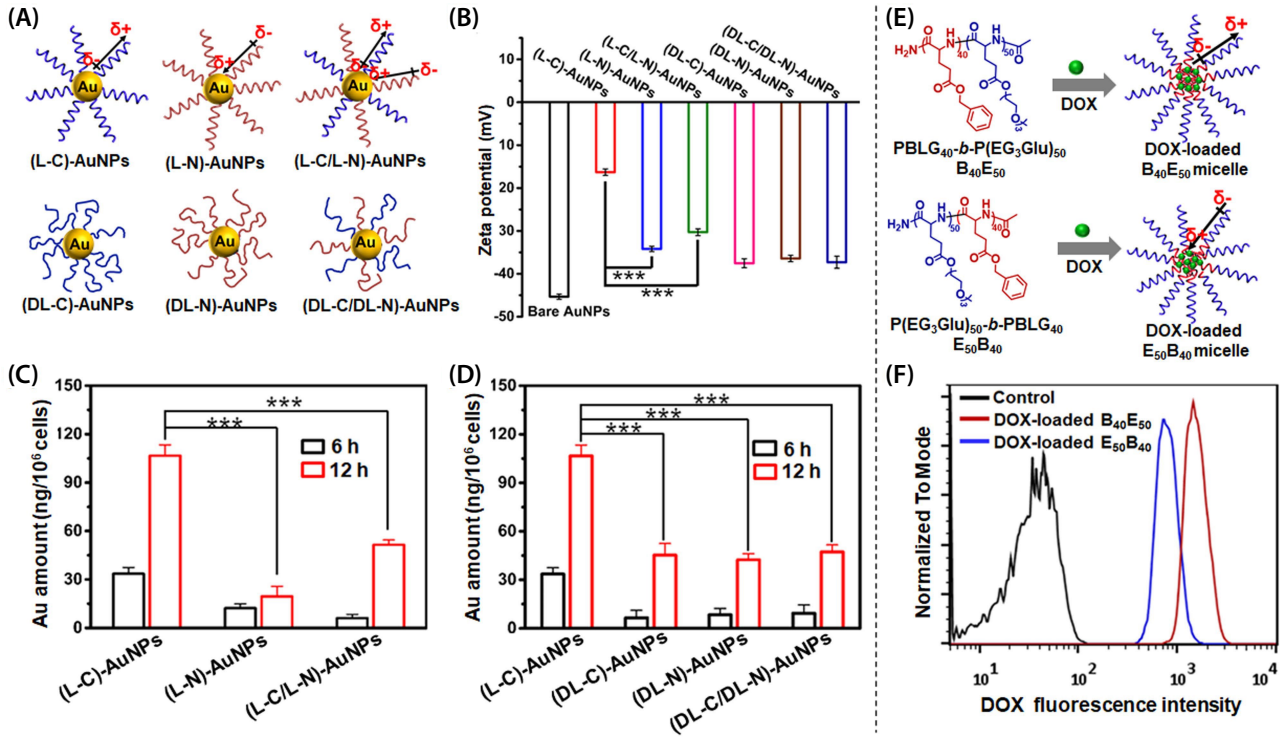

Fig. 4 (A) Cartoon illustration of AuNPs modified with P(EG_3Glu)s with different conformations and anchoring orientations. Arrows indicate the moment of helix macrodipole. (B) Zeta potential of P(EG_3Glu)s-coated AuNPs in H_2O. (C) and (D) the internalization level of various P(EG_3Glu)s-coated AuNPs after incubation with Hela cells for 6 or 12 h. (E) Schematic illustration and (F) flow cytometric histogram profiles of the HeLa cells incubated with the DOX-loaded $E_{50}B_{40}$ and $B_{40}E_{50}$ polypeptide micelles. (Reproduced with permission from Ref. [155]; Copyright (2019) Springer).

relatively larger hydrodynamic size and higher grafting density than those coated with unstructured ones. Moreover, the surface grafting density can be further increased by anchoring antiparallel orientated helices on nanoparticles ((L-C/L-N)-AuNPs). These results are in line with what was previously found in Fig. 3. Notably, the authors also found that the orientation of surface-grafted polypeptides changed the zeta potential and cellular internalization behaviors of AuNPs. For example, (L-C)-AuNPs possessed a greater zeta potential because the partial positive macrodipole (*N*-terminus) was placed at the outer layer surface (Fig. 4B), which resulted in greater cellular uptake (2.0–5.5 fold higher) than other P(EG_3Glu)-coated AuNPs (Figs. 4C and 4D). Dissipative particle dynamics (DPD) simulations indicated that the helix (L-C)-AuNPs with outermost positive macrodipole can be wrapped and internalized rapidly through long range electronic attractive force with the lipid membrane. Besides, P(EG_3Glu)s were also grafted onto AuNRs and a very similar cell internalization pattern was observed. Interestingly, this orientation effect was also successfully applied to block copolypeptide-based nanomedicines. For this, two doxorubicin (DOX)-loaded micelles were designed based on PBLG_{40}-*b*-P($_L$-EG_3Glu)$_{50}$ ($B_{40}E_{50}$) and P($_L$-EG_3Glu)$_{50}$-*b*-PBLG_{40} ($E_{50}B_{40}$), respectively. The two micelles were similar in all other structural parameters (molecular weights, dispersity, sizes, and DOX content) but differ only in the orientation of the polypeptides (Fig. 4E). DOX-loaded $B_{40}E_{50}$ with the *N*-terminus at the outer layer showed higher zeta potential and enhanced cellular uptake than DOX-loaded $B_{40}E_{50}$ (Fig. 4F), further highlighting the importance of surface engineering of polypeptide-grafted nanomedicines.

Helical Nonfouling Polypeptides for Protein Modification (Protein PEPylation)

Protein-polymer conjugation has been identified as a clinic-validated approach to overcome some major limitations of therapeutic proteins such as rapid renal clearance, susceptibility to proteolysis, and strong immunogenicity.[156–160] Thus far, the modification of protein drugs with poly(ethylene glycol) (PEGylation) showed great clinical success and more than a dozen PEGylated proteins have been approved for the treatments of diseases such as hepatitis, neutropenia, acromegaly, hemophilia, and rheumatoid.[161,162] However, an increasing body of evidence has shown that the non-biodegradable PEG can elicit anti-PEG antibodies and immune response, which finally results in the so-called accelerated blood clearance (ABC) effect and decreased efficacy of PEGylated therapeutics upon repeated injections.[163,164] Among all the developed PEG alternatives, synthetic nonfouling polypeptides represent a class of promising biomaterials for protein conjugation owing to the biomimetic protease-degradable backbone and unique secondary structures.[20,165]

Jiang and co-workers[18] employed a polypeptide with high density zwitterionic carboxybetaine groups (PepCB) to randomly modify uricase in 2018 and the resultant uricase-PepCB conjugate showed improved pharmacokinetics and immunological properties compared with uricase-PEG conjugates. The polypeptide PepCB also showed high biosafety and less toxicity in direct comparison with PEG of similar hydro-

dynamic sizes. Nevertheless, the authors did not mention the conformation of PepCB in their study. Starting from 2016, Lu *et al.* reported a series of works conjugating synthetic polypeptides to proteins in site-specific, macrocyclic, or random labeling fashions, which they termed as protein PEPylation.[20,166–171] To increase the conjugation efficiency, the Lu group established a concise protein PEPylation strategy by developing initiators trimethylsilyl phenylsulfide (PhS-TMS)[172] and trimethylstannyl phenylsulfide (PhS-$SnMe_3$).[173] The new initiators not only enabled controlled ROP, more importantly, they *in situ* generated a reactive phenyl thioester that can be used for site-specific conjugation with proteins bearing *N*-terminal cysteine *via* the chemoselective native chemical ligation (NCL). Taking advantage of this approach, nonfouling polypeptide $_{L}$-P(EG_3Glu) and $_{DL}$-P(EG_3Glu) with similar chemical structures but differ in conformation were separately conjugated to the *N*-terminus of interferon (IFN), respectively (Fig. 5A).[20,167] Interestingly, the resulting helical $_{L}$-P(EG_3Glu) conjugates showed improved binding affinity, *in vitro* antiproliferative activity, and *in vivo* efficacy with significantly less antidrug and antipolymer antibodies compared to unstructured $_{DL}$-P(EG_3Glu) and PEG conjugates (Fig. 5B), suggesting the critical role of helical structure in minimizing the immune responses. This study provided a solid ground for the utility of helical synthetic polypeptides as PEG alternatives for protein modification. By altering the protein from IFN to human growth hormone (GH), they observed very similar immunogenicity results by using the conjugation chemistry and the same polymers (Fig. 5B). The *in vivo* low immunogenicity results of the helical polypeptide $_{L}$-P(EG_3Glu) compared with $_{DL}$-P(EG_3Glu) and PEG were in good agreement with their surface nonfouling performances as described in Fig. 3. Next, the same group employed the zwitterionic helical polypeptides P(CB-EG_3Glu) (see Fig. 2B) to randomly modify asparaginase (ASNase), a notoriously immunogenic protein in clinic (Fig. 5C).[168] Apart from retaining fully bioactivity and extending circulating half-life, the zwitterionic conjugate P(CB-EG_3Glu)-ASNase elicited negligible antibodies (Fig. 5D) and gave no sign of ABC effect upon repeated administration, which remarkably outperformed PEG-ASNase. The authors attributed the outstanding immune escaping properties of P(CB-EG_3Glu)-ASNase to the urchin-like nanostructure originated from the rigidity of nonfouling zwitterionic helical polypeptide, which may help the formation of a stronger repuls-

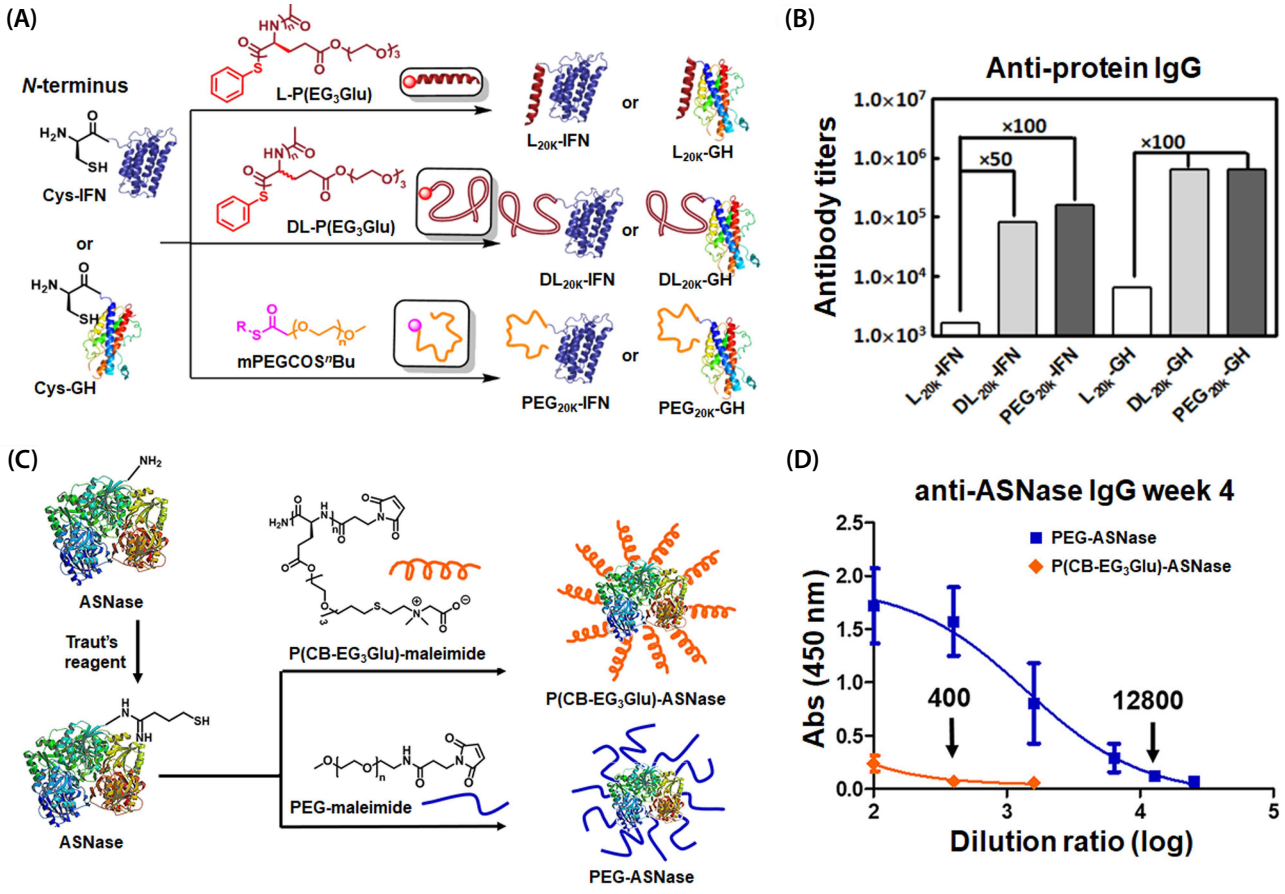

Fig. 5 (A) Scheme of the site-specific conjugation of synthetic polypeptides P(EG_3Glu) (helical and unstructured) or PEG to the *N*-terminus of IFN and GH *via* native chemical ligation. The molecular weights of all polymers were ~20 kDa. (B) The anti-protein IgG antibody titers after the 4th immunization of various protein-polymer conjugates. (Reproduced with permission from Ref. [20]; Copyright (2019) American Chemical Society.); (C) Schematic illustration of the synthesis of P(CB-EG_3Glu)-ASNase and PEG-ASNase. (D) The anti-ASNase IgG levels in serially diluted sera drawn from mice being weekly immunized with P(CB-EG_3Glu)-ASNase or PEG-ASNase for 4 weeks, respectively. (Reproduced with permission from Ref. [168]; Copyright (2021) Elsevier).

https://doi.org/10.1007/s10118-022-2688-2

ive layer towards immune system attacking. Taken together, these results appeared to indicate that the helix-empowered low immunogenicity of nonfouling polypeptides is universal to some extent and independent of the side-chain groups of polypeptides, conjugation chemistry or protein category.

CONCLUSIONS AND PROSPECTS

Scientists have long been fascinated by the secondary structures of polypeptides to mimic the functions of natural proteins. In fact, the full validation of *α*-helix of proteins, initially proposed by Pauling in 1951,[174] was aided by resolving the X-ray diffraction data of synthetic polypeptides such as poly(*γ*-methyl-$_L$-glutamate) and PBLG.[175,176] Over the past few decades, the development of new ROP methodologies has enabled rapid and controlled preparation of synthetic polypeptides with well-defined secondary structures and related biological applications. We presented here a brief and focused overview of recent progresses on the *α*-helical nonfouling polypeptides for biomedical applications, with a special emphasis on the manipulation of helix-to-coil transition, and conformation-associated antifouling coatings, cellular uptake regulation, and immunogenicity mitigation. Looking to the future, however, there are still considerable challenges and opportunities that lie ahead as follows.

From a material synthesis point of view, the production of polypeptides with low-cost and large-scale remains a great challenge. The tedious and stringent anhydrous conditions for NCAs preparation and ROP have strongly discouraged and frustrated both non-experts and experts from working on these materials. This can be reflected by the fact that, compared to other degradable polymers such as polyesters, the polypeptide field has largely lagged behind from the perspectives of both the number of academic research papers and the development degree of mass industrial manufacturing. To facilitate easier polypeptide synthesis, Ling and Zhang *et al*. made significant progresses in replacing the moisture-sensitive NCAs using amino acid *N*-thiocarboxyanhydrides (NTAs).[177,178] Endo[179,180] and Liu[181] showed that polypeptides can also be made by using *N*-phenoxycarbonyl-activated amino acids as precursors of NCAs without using the toxic phosgene or triphosgene reagents. It is also worth highlighting that by employing epoxy compounds as ultra-fast hydrogen chloride scavengers, moisture-tolerant NCA monomer synthesis has been realized up to decagram scales.[35] This robust protocol showed particular advantages in preparing challenging unprotected NCAs over conventional hydrogen chloride scavengers such as *α*-pinene and (+)-limonene.[182] Recent pioneer works have also shown that the ROP of NCAs can be carried out in open vessels with aqueous solution.[34,53,183–185] More recently, Lu *et al*. discovered the ROP of Proline NCA (ProNCA) can be dramatically accelerated in mixed acetonitrile and water, in which water played multiple critical roles from improving the solubility of poly-$_L$-proline to assisting proton shift in rate-determining step of ROP.[186] The surprising observation underscored the complexity of water in NCA chemistry and pointed out the necessity of thinking out-of-box to turn detrimental factors into beneficial components. All these works, although rather preliminary, are important steps toward greener and more economic production of synthetic polypeptides both in the lab and at industry scale.

Polypeptides that can undergo conformational switches, *e.g*., helix-to-coil, are highly interesting in offering opportunities in fabricating intelligent materials and devices. Challenges, however, also exist concerning the design and characterization of synthetic polypeptides with reliable dynamic secondary structure transitions under specific physiological or pathological environments. Such transitions, better triggered by biological relevant signals such as glutathione or enzymes, should be realized in a tissue or cell selective manner to facilitate targeted delivery or on-demand function switches. The dynamics of the transition should also be taken into consideration for many biological applications. Since the short retention time of exogenous materials *in vivo*, the designed materials need to complete such transitions very rapidly or even instantaneously before clearance. For this, researchers should be more careful when translating the *in vitro* experimental results to live animals. Moreover, while considerable progress has been made towards helix-to-coil transition and its related biological effects, the regulation of secondary structures between helix and *β*-sheet may open up enormous new opportunities.

Last but not least, although helical polypeptides, such as $_L$-P(EG_3Glu) and $_L$-P(CB-EG_3Glu), have shown promising properties outperforming PEG in some nonclinical studies, many outstanding questions still remained from both fundamental and clinical aspects. For example, what is (are) the molecular origin(s) of the superior antifouling properties and low immunogenicity of the rigid helical polypeptides as compared to those flexible polymers? How safe are those helical polypeptides upon repeated injections and at high dosages? How do the helical polypeptide-modified proteins interact with various immune cells *in vivo*? How do the polypeptide-protein conjugates distribute and metabolite in live animals? Can the secondary structure-facilitated low immunogenicity phenomenon be extended to other types of helices such as polyproline-II (PPII) helix? To answer those questions, significantly more studies from molecular design, simulation, biophysics, to *in vivo* pharmacological and biosafety evaluations are all necessary.

In summary, this feature article highlighted recent advances of *α*-helical nonfouling polypeptides for biomedical applications. It can be envisaged that the secondary structures will continue to serve as a bridge between chemistry and biology to help researchers better mimic the sophisticated structures and functions of proteins through rational design of polypeptides at the molecular level.

BIOGRAPHY

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https://doi.org/10.1007/s10118-022-2688-2

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NOTES

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was financially supported by the National Key Research and Development Program of China (No. 2019YFA0904203) and the National Natural Science Fund for Distinguished Young Scholars (No. 22125101).

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