Macromolecules
Cite This: Macromolecules 2019, 52, 8521−8539
Perspective
pubs.acs.org/Macromolecules

Recent Advances and Future Perspectives of Synthetic Polypeptides from *N*-Carboxyanhydrides

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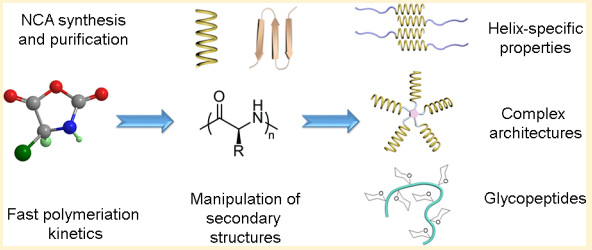
ABSTRACT: Synthetic polypeptides, the analogues of natural proteins, are important biomaterials that have found broad biomedical applications. Ring-opening polymerization of *N*-carboxyanhydrides offers a reliable way to prepare high-molecular-weight polypeptides in large scale with diverse side-chain functionalities. The past two decades have seen significant advances in the polypeptide field, with the development of various controlled polymerization methodologies, the deeper understanding on secondary structures, and the discovery of new assembly behaviors and applications. In this Perspective, we highlight several key advances in the chemical synthesis and materials application of synthetic polypeptides and discuss promising future directions in this area.



INTRODUCTION

Synthetic peptides and polypeptides are promising protein-mimetic materials that have been widely studied for various biomedical applications.[1−5] Inspired by the complex structures and versatile functions of natural proteins, there have been increasing interest in the controlled synthesis and studies of synthetic peptide and polypeptide materials. These materials have the same backbone with proteins (i.e., peptide bond), which enables them to adopt stable secondary structures and offers them remarkable biocompatibility. Synthetic peptide and polypeptide materials are typically prepared through solid-phase peptide synthesis (SPPS),[6] microbial synthesis,[7] or ring-opening polymerization (ROP) of amino acid *N*-carboxyanhydride (NCA) monomers (Figure 1).[8,9] Compared with the other two methods, NCA polymerization offers a reliable way to prepare high-molecular-weight (MW) polypeptides on a large scale. In addition, the ease to incorporate versatile side-chain groups beyond the typical proteinogenic amino acid residues during NCA synthesis offers essentially unlimited opportunities for the preparation of functional polypeptides.[4,10]

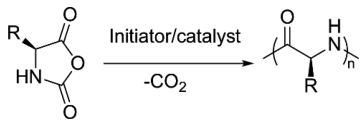


Figure 1. Synthetic route to polypeptide materials through ring-opening polymerization of NCAs.

NCA was first synthesized by Leuch in 1906.[11] The five-member ring molecule was regarded as a reactive precursor of amino acids, which was used for the synthesis of oligopeptides in a stepwise manner,[12] as well as high-MW polypeptides through polymerizations.[13] Numerous studies were later performed to understand the polymerization behavior of NCAs and the conformations of polypeptides.[13−19] A key breakthrough in NCA/polypeptide field is the pioneering work by Deming, where the use of a Ni(0) or Co(0) organometallic catalyst offered the first controlled polymerization of NCAs.[20,21] Following this work, several living polymerization systems have been developed in the past two decades,[22−26] enabling researchers to prepare well-defined polypeptide materials with desired architecture, chain lengths, and dispersity ($Đ$). The advances in polymerization methodologies boost related studies on the self-assembly behaviors,[5,27−30] modulation of conformations,[30,31] and the biomedical applications of synthetic polypeptide materials,[2−5,28,29,32,33] which have been comprehensively summarized in a number of excellent review articles.

The purpose of this Perspective is not to fully discuss all published work in the NCA/polypeptide field. Instead, we aim to highlight some important advances on the chemical synthesis, conformation manipulation, and applications of synthetic polypeptide materials. These studies not only provide insights into the structure−property relationship of proteins and peptides but also pave the way for the further development of synthetic polypeptides as unique and useful biomaterials. Finally, several remaining challenges and unsolved problems in the NCA/polypeptide field are discussed throughout the Perspective, and some promising future directions are suggested.

Received: July 11, 2019
Revised: September 26, 2019
Published: October 7, 2019

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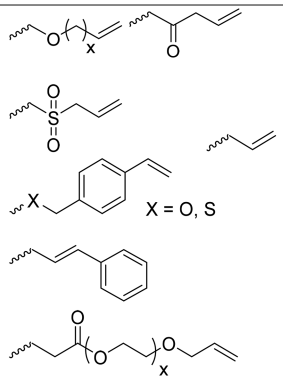
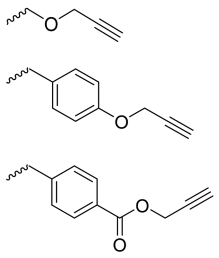
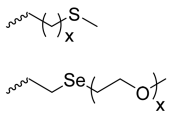
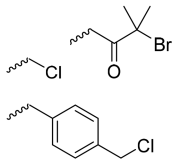
DOI: 10.1021/acs.macromol.9b01450
Macromolecules 2019, 52, 8521−8539

Table 1. Reactive Handles Incorporated on NCA Side Chains and Their Subsequent Postpolymerization Modifications

reactive handles	chemical structures	post-polymerization modifications	refs
Alkene		Photo-induced crosslinking Thiol-ene click reaction Michael addition Ozonolysis	34-42
Alkyne		Huisgen azide-alkyne click reaction Thiol-yne click reaction	43-47
Azide		Huisgen azide-alkyne click reaction	48
Thiol		Thiol-ene click reaction Thiol-yne click reaction	49
Thioether and selenoether		Nucleophilic substitution Oxidation	50-53
Halogen		Nucleophilic substitution Atom transfer radical polymerization (ATRP) initiation	54-59

CHEMICAL SYNTHESIS

NCA Synthesis and Purification. The conformations and functions of synthetic polypeptide materials are dependent on their side-chain structures. Therefore, it is important to develop various NCA monomers bearing different side-chain functionalities to manipulate the secondary structures or to render specific properties of the resulting polypeptides. Currently, there are mainly two strategies to prepare functional polypeptides:[10] the direct polymerization of functional NCA monomers (including those requiring temporary protections during NCA polymerization) and the polymerization of NCA monomers bearing reactive handles followed by postpolymerization modifications. The latter strategy is particularly useful, as it not only enables the attachment of complex functionalities that are incompatible with the polymerization process but also provides a facile way to build a library of polypeptides for the studies of structure−property relationship. NCAs with functional and reactive side chains have been comprehensively reviewed.[10,28] Here we highlight several important reactive functional groups that have been incorporated in NCA side chains (Table 1). These reactive functional groups, coupled with click chemistry or other efficient postpolymerization modifications, offer the chance to access a variety of functional polypeptides. For instance, the incorporated alkene groups allow for the postpolymerization modifications such as thiol−ene click reaction,[34−39] Michael addition,[40] olefin metathesis,[41] and photo-cross-linking.[42] Several important functionalities that may interfere with the polymerization process, including amine,[36,37,40] carboxylic acid,[35−37,40] oligo(ethylene glycol) (OEG),[34] and sugar,[34,40] can be conjugated on polypeptide side chains with high efficiencies. Other than alkene-based NCAs, NCAs with side-chain alkynes,[43−47] azides,[48] thiols,[49] thio- and selenoethers,[50−53] and halogens[54−59] are also widely used for the preparation of functional polypeptides.

On the other hand, the direct polymerization of functional NCA does not have any concern on conjugation efficiency of postpolymerization modifications. Therefore, it is a widely used strategy to prepare functional polypeptide materials.

DOI: 10.1021/acs.macromol.9b01450
Macromolecules 2019, 52, 8521−8539

Because the preparation of functional NCAs with complex side chains requires more difficult synthesis and purification steps, the advances of NCA synthesis and purification methodologies are critical for this strategy. NCA monomers are typically prepared through the phosgenation of amino acid (i.e., Fuchs–Farthing method)[60,61] or the treatment of N^{α}-alkyloxycarbonyl amino acids with halogenating reagents (i.e., Leuch method).[11,62] Recently, Fuse and co-workers took advantage of the flash mixing process in a microflow reactor and developed an improved Fuchs–Farthing protocol.[63] During their synthesis, the initial basic condition enabled a fast conversion of amino acids, and the following basic-to-acidic flash switching (0.1 s) avoided the undesired degradation of resulting NCAs (Figure 2a). Compared with a batch reaction, the microflow strategy

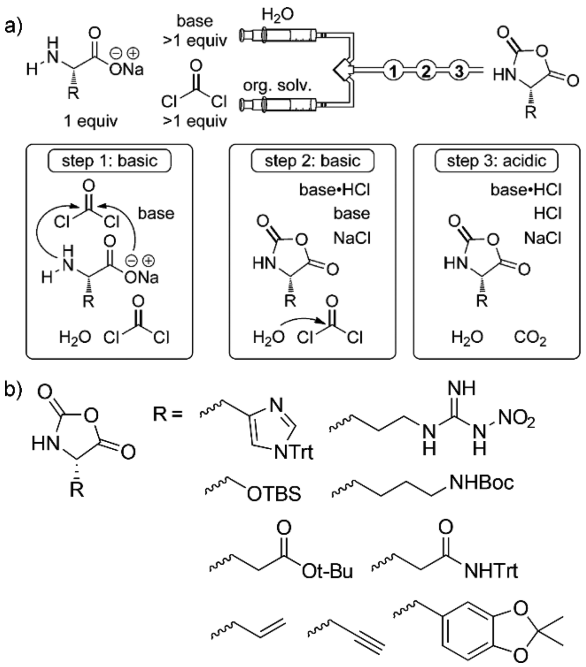


Figure 2. Synthesis of NCAs with microflow strategy. (a) Scheme illustrating the basic-to-acidic flash switching in a microflow reactor. (b) Representative NCAs with acid-labile side chains that were readily synthesized with the microflow strategy. Reproduced with permission from ref 63. Copyright 2018 WILEY-VCH Verlag GmbH & Co. KGaA.

enabled the rapid preparation of various NCAs at room temperature. Different than the preparation of NCAs with acid labile-side chains in a batch reactor, which requires acid scavengers (e.g., α-pinene and triethylamine (TEA)) to remove acidic byproducts generated during NCA synthesis,[64−66] the NCA synthesis in a microflow reactor employed a flash dilution step to protect the NCAs from being overexposed in an acidic environment, making it a facile and efficient method for the preparation of NCAs with acid-labile side chains (Figure 2b).

During NCA synthesis, acidic and/or electrophilic impurities are generated, which may inhibit the subsequent polymerization steps by quenching the basic and/or nucleophilic initiators, even in a trace amount.[23] Conventional purification method involves repetitive recrystallization with anhydrous solvent, which is tedious and time-consuming. In addition, NCAs with poor crystallinity cannot be effectively purified with the recrystallization method. Alternatively, washing the solution of NCAs with aqueous base removes the impurities,[67] but the introduction of water gradually degrades NCAs.[68] In an attempt to develop a more general strategy for NCA purification, Deming and Kramer used flash column chromatography to remove the impurities in an effective manner.[69] A wide range of NCA monomers, either crystalline or noncrystalline, were successfully purified with improved yields. This strategy is particularly useful for NCAs with complex and biologically relevant side-chain functionalities, including OEG-based NCAs and glycosylated NCAs.[62,70] In fact, the poor crystallinity of these NCAs makes flash chromatography the only practical method for their purifications.

In recent years, α-amino acid *N*-thiocarboxyanhydrides (NTAs), the thio analogues of NCAs, have drawn considerable attention due to their higher stability against moisture and heat compared to NCAs. While the synthesis of NTA was first achieved in 1950,[71] the use of NTA monomers to prepare well-defined polypeptide materials is limited due to their low reactivity.[72] Zhang and co-workers reported the interfacial ring-opening polymerization (iROP) of NTA monomers in a controlled manner in heated nonpolar solvents (e.g., hexane or heptane), where the soluble monomers reacted with insoluble propagating polypeptide chain ends (Figure 3a).[73] The iROP facilitated the elimination of COS to retain an active amine terminus, rather than the release of H_2S that would terminate the propagating chains, leading to well-defined polypeptides with high monomer conversion and controlled MW (Figure 3b). Recently, the same group also reported another controlled ROP of NTA in the presence of organic acid, which also facilitated the COS release.[74] Because of the moisture stability of NTA monomer, the ROP can be conducted in air that generate similar results compared with that in air-free conditions. In addition, polypeptide materials with pendant

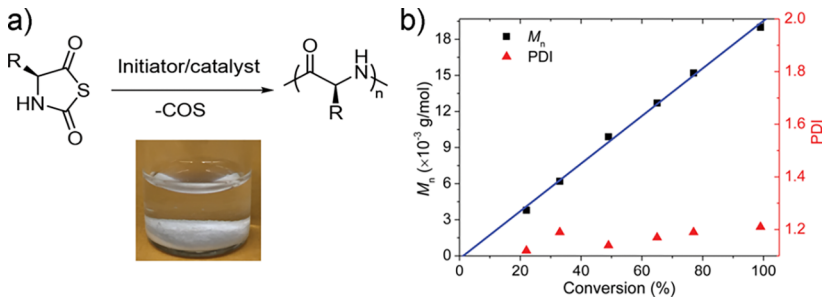


Figure 3. Preparation of polypeptides through iROP of NTA. (a) Synthetic route to polypeptides from NTA. (b) MW and dispersity control of iROP of NTA in hexane at 50 °C. Reproduced with permission from ref 73.

DOI: 10.1021/acs.macromol.9b01450
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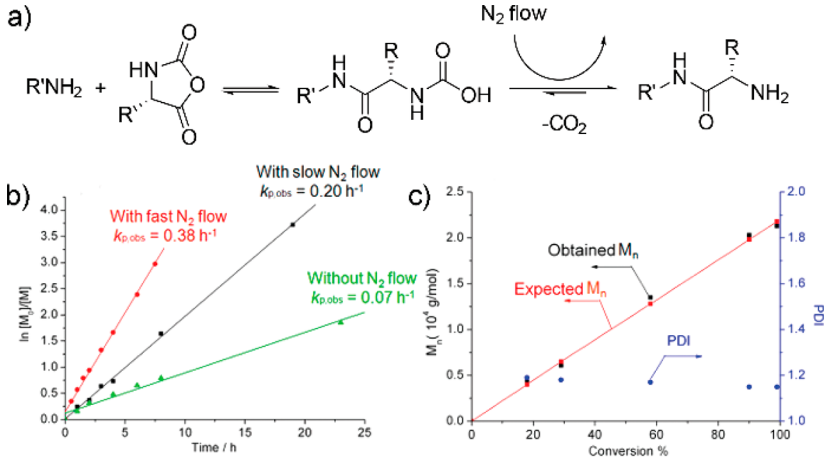

Figure 4. Use of N_2 flow for the acceleration of NCA polymerization. (a) Removal of CO_2 with N_2 flow during NCA polymerization. (b) Kinetics studies of ROP of BLG-NCA initiated by *n*-hexylamine under different rates of N_2 flow. The polymerization was accelerated with N_2 flow, with faster polymerization observed at higher flow rate. (c) Characterization of resulting polypeptides at different monomer conversion that indicates a living polymerization feature. Reproduced with permission from ref 81.

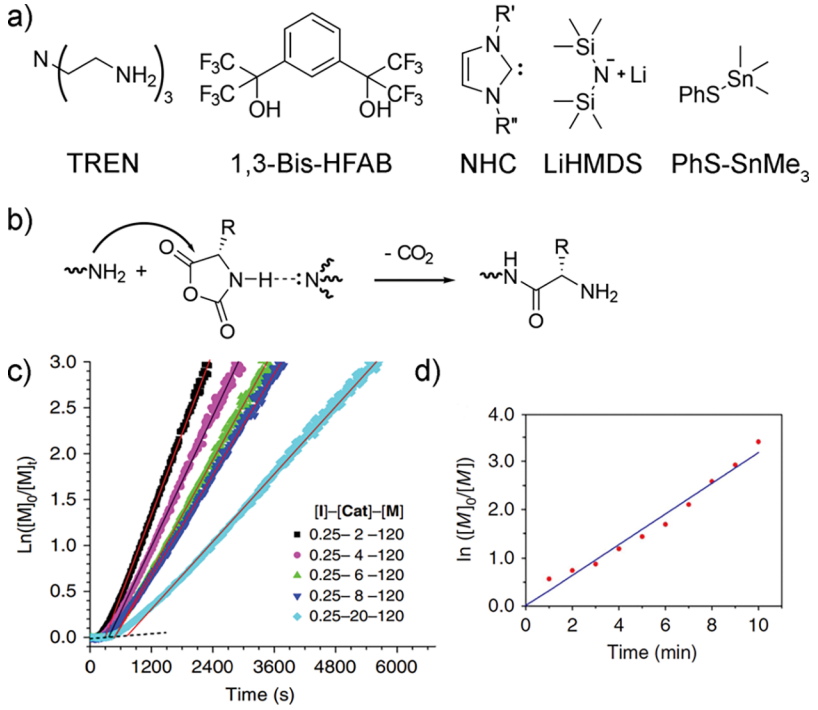

Figure 5. Initiator systems that enable fast polymerization of NCAs. (a) Chemical structures of the initiators. (b) Proposed AAMMA mechanism to elucidate the acceleration with TREN. (c) Polymerization kinetics of BLG-NCA in DCM in the presence of 1,3-Bis-HFAB. Reproduced with permission from ref 84. Copyright 2019 Springer Nature. (d) Conversion of BLG-NCA in NHC mediated polymerization. More than 95% NCA was polymerized within 10 min. Reproduced with permission from ref 87. Copyright 2018 Springer Nature.

hydroxyl groups can be directly prepared with ROP of corresponding NTA monomer without protection, as the NTA ring exhibited great tolerance toward hydroxyl groups.[75]

Polymerization with Accelerated Rate. The development of controlled polymerization of NCAs has greatly enriched the toolbox for researchers to prepare desired polypeptide materials. Despite the excellent control over MWs and dispersity, the polymerization rates of these conventional polymerization methods are usually slow, taking up to several days to finish. With the prolonged polymerization time, side reactions such as chain terminations and monomer degradation become significant. This is particularly true for the polymerization of NCAs with γ-glutamate side chains, one of the most widely used types of NCAs, where the amidation reaction between the terminal amino groups and the side-chain esters deactivate the propagating polypeptide chains.[9,26,76] In addition, stringent water-free conditions are required, as water-induced polymerizations or degradations of NCAs have similar rates, if not faster, compared with the slow polymerization process.[77] While initiators with strong basicity (e.g., TEA) lead to rapid consumption of NCAs through activated monomer mechanism (AMM), the resulting polypeptides usually have uncontrolled MWs and broad dispersity.[78] Therefore, the development of a controlled and rapid polymerization would

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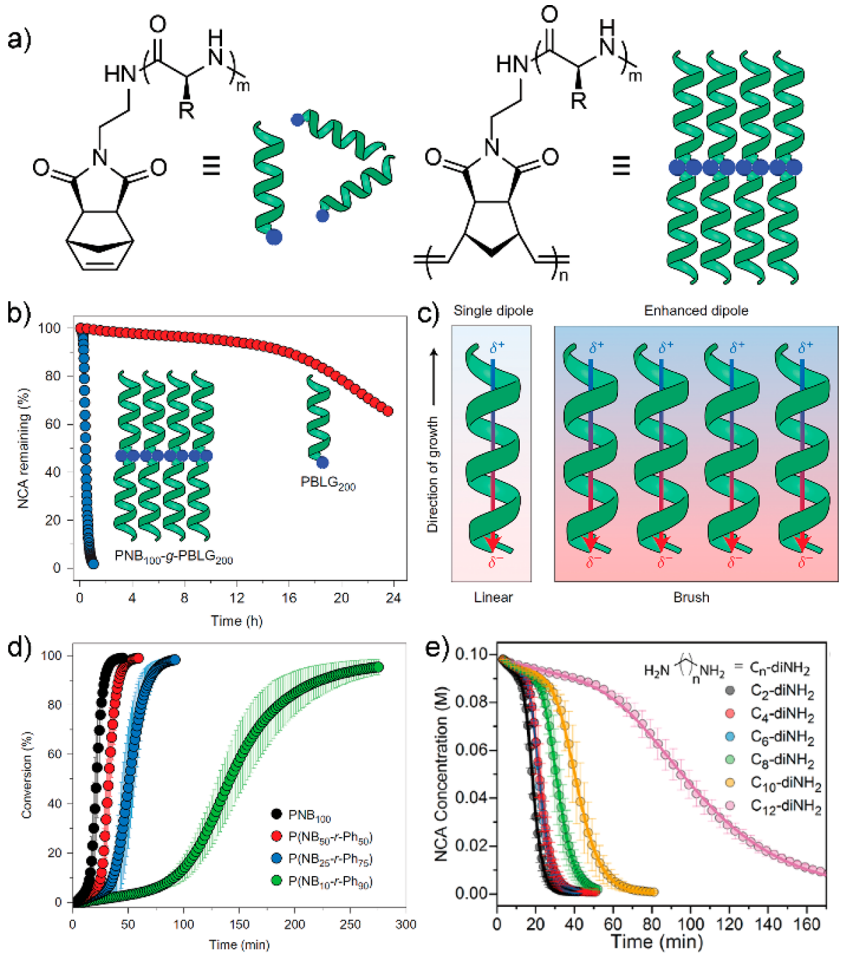

Figure 6. Rapid, cooperative polymerization of NCAs in DCM. (a) Chemical structures and schematic illustrations of single-chain and brush polypeptides. (b) Conversion of BLG-NCA in DCM using single-site initiator or brushlike macroinitiator. Polymerization rate initiated by the macroinitiator was much faster than that by the single-chain analogue. (c) Illustration of macrodipole of a propagating α-helical polypeptide. The macrodipole of α-helices in the brush polypeptides exhibited enhanced interactions. (d) Conversion of BLG-NCA using macroinitiators with different densities of initiation sites on the backbone. Lower initiator density led to a decreased polymerization rate. Reproduced with permission from ref 92. Copyright 2017 Springer Nature. (e) Conversion of BLG-NCA using different diamine initiators with various alkyl spacers. Shorter alkyl length between two amino groups resulted in a faster polymerization rate. Reproduced with permission from ref 93.

further improve the preparation of polypeptides, boosting the downstream conformational and biomedical studies of these materials.

The release of CO_2 has a significant impact on NCA polymerization. Several groups have reported facilitated polymerization of NCA by efficient removal of CO_2 through high vacuum or N_2 purging.[23,26,79,80] In 2013, Wooley and co-workers used precisely controlled N_2 flow and reported that the removal of CO_2 with N_2 flow accelerated the polymerization of γ-benzyl-L-glutamate NCA (BLG-NCA) initiated by *n*-hexylamine, producing polypeptides with desired MWs and narrow dispersity. The use of N_2 flow facilitated the decarboxylation of carbamic acid intermediates and the release of nucleophilic amino groups (Figure 4a), promoting the chain-propagation process. Interestingly, the polymerization rate could be tuned by changing the N_2 flow speed, with faster NCA consumption observed at higher speed (Figure 4b).[81] Living polymerization under N_2 flow was further demonstrated (Figure 4c), in sharp contrast with that without N_2 flow, where ill-defined polypeptides with broader dispersity were obtained due to significant side reactions.

Accelerated polymerization of NCAs can also be achieved by new initiation systems. Hadjichristidis's group developed a series of initiators with both primary and tertiary/secondary amines (Figure 5a).[82,83] When the structure of the initiator was properly designed (e.g., triethylaminetriamine, TREN), the polymerization exhibited the living feature of primary amine initiators through a normal amine mechanism (NAM). Moreover, the fast rate of the polymerization, which is characteristic in tertiary amine initiation with AAM, was observed. The steric hindrance close to the tertiary amine was essential to achieve controlled polymerization, as initiators with less sterics (e.g., *N*,*N*-dimethylethylenediamine) showed poor control over MWs and dispersity. The authors proposed a new polymerization mechanism, accelerated amine mechanism by monomer activation (AAMMA), to elucidate the polymerization process (Figure 5b).[82] While the central tertiary amine group in TREN cannot effectively abstract protons from NCA for an initiation with AMM, it plays an important role in activating NCA monomers through hydrogen bonds (H-bonds). Similarly, initiators containing primary and secondary amines exhibited rate acceleration, producing linear polypeptide materials.[83] Very recently, a catalytic system for NCA

DOI: 10.1021/acs.macromol.9b01450
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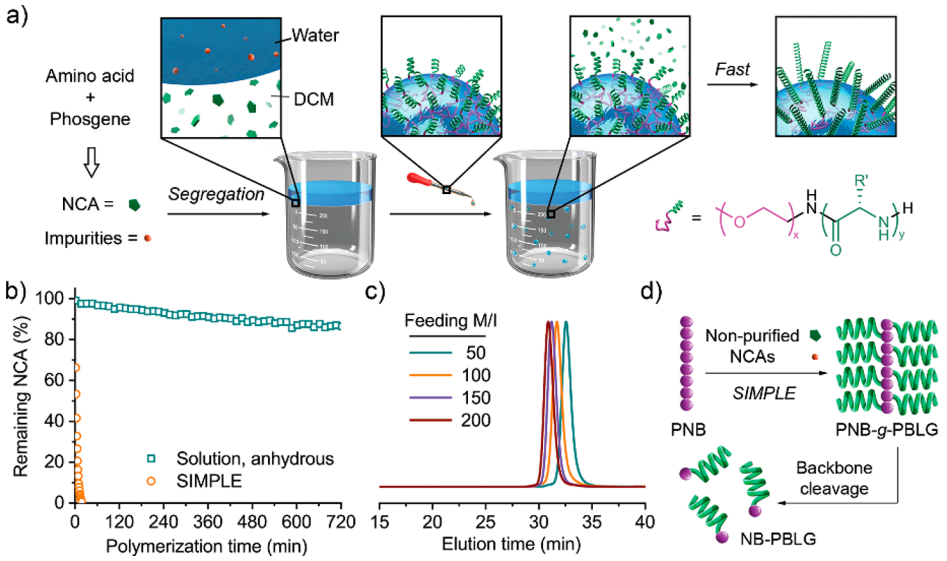

Figure 7. SIMPLE polymerization of nonpurified NCAs. (a) Schematic representation of SIMPLE polymerization of nonpurified NCAs in a w/o emulsion. The segregation for *in situ* removal of impurities and the fast polymerization to outpace side reactions are the key designs for this strategy. (b) Conversion of nonpurified BLG-NCA in SIMPLE polymerization and in anhydrous DCM solution. The aqueous phase plays an important role to remove impurities. (c) GPC characterization showing remarkable control of SIMPLE polymerization with nonpurified NCAs at various $[M]_0/[I]_0$ ratio. (d) Scheme illustrating the synthesis of linear homo-polypeptides with SIMPLE strategy using a brushlike macroinitiator. Reproduced with permission from ref 77. Copyright 2019 National Academy of Sciences.

ROP was developed based on a fluorinated alchohol, 1,3-bis(2-hydroxyhexafluoroisopropyl)benzene (1,3-bis-HFAB) (Figure 5a), which could form complex H-bonds with the initiator, the monomer, and the growing chain.[84] The hydrogen bonding (H-bonding) interactions activated the NCA monomers while dynamically deactivate the overactive initiator and propagating polymer ends, resulting in the fast polymerization kinetics and well-defined polypeptide (Figure 5c).

Initiating systems enabling fast polymerization of NCAs were also developed based on the knowledge of ROP of other heterocyclic monomers. For instance, *N*-heterocyclic carbene (NHC) (Figure 5a), a zwitterionic catalyst for ROP of lactides and *N*-substituted *N*-carboxyanhydrides,[85,86] has been used to polymerize NCAs.[87] The NHC-mediated polymerization proceed rapidly in the presence and absence of primary amines (Figure 4d), resulting in well-defined linear and cyclic polypeptides, respectively. In another example, Liu and co-workers developed a superfast polymerization of NCAs using lithium hexamethyldisilazide (LiHMDS) as the initiator (Figure 5a),[88] which was previously used for polymerization of β-lactams.[89] Much faster polymerization was observed compared with the conventional initiators, especially with high $[M]_0/[I]_0$ ratios (i.e., for the synthesis of high-MW polypeptides). In addition, the authors demonstrated the feasibility of open vessel polymerization of NCAs with the LiHMDS system,[88] presumably due to the fast polymerization rates that outpace water-induced side reactions. This work highlights the importance of accelerated polymerization of NCAs, which downplays the requirement for stringent water-free setups (e.g., glovebox and Schlenk line).

On the basis of the previous work to use Lewis acid agents as the initiators,[24,90] Lu and co-workers developed a new initiator, (trimethylstannyl)phenyl sulfide (PhS-$SnMe_3$), for rapid preparation of polypeptides (Figure 5a).[91] The fast polymerization was attributed to the S−Sn Lewis pair that resulted in a more reactive trimethylstannyl group during the propagation. PhS-$SnMe_3$ facilitated the synthesis of high-MW polypeptide materials in a fast manner in mixed THF/DMF. In contrast, the polymerization initiated by its analogue, (trimethylsilyl)-phenyl sulfide, took much longer time to finish.

Recently, Cheng, Lin, and co-workers developed the covalent, cooperative polymerization (CCP) of NCAs in solvents with low dielectric constants. Unlike the polymerization in traditional solvents like *N*,*N*-dimethylformamide (DMF) or tetrahydrofuran (THF), the cooperative interactions of α-helical macrodipoles in close proximity facilitate the chain propagation process, resulting in an accelerated ROP of NCAs in solvents with low dielectric constants, including dichloromethane (DCM), chloroform, or 1,2-dichloroethane. For example, the propagation rate from a brushlike macroinitiator PNB is more than 1000 times faster compared with a single-chain analogue (Figure 6a−c).[92] The proximity-induced rate acceleration was further evidenced by polymerizing NCAs with macroinitiators with different initiator densities. A significant decrease of polymerization rate was observed with lower density of initiating sites, even when the concentrations of initiators were maintained the same (Figure 6d).[92] In a following study, the same team reported such a cooperative effect even with simple diamino initiators.[93] Faster polymerization was observed with shorter alkyl length between the primary amine initiating sites (Figure 6e), with significantly diminished rate when the distance between two amino groups were larger than 11 σ-bonds, further validating the relationship between polymerization rates and distances of macrodipoles.

The rate acceleration of CCP was not limited to neighboring initiators with covalent linkages. A self-assembled array of poly(ethylene glycol)-*block*-poly(γ-benzyl-L-glutamate) amine (PEG−PBLG) enabled fast polymerization of NCAs in a water-in-oil (w/o) emulsion (Figure 7a).[77] The conversion of BLG-NCA reached >99% after 20 min, outpacing water-induced side reactions (i.e., undesired polymerization and hydrolysis). Quantitative kinetic modeling studies indicated that <0.1% of BLG-NCA was consumed by water, highlighting the remarkable role rate acceleration played in the emulsion

DOI: 10.1021/acs.macromol.9b01450
Macromolecules 2019, 52, 8521−8539

system. Furthermore, the aqueous phase was further used for the *in situ* removal of impurities through segregation (Figure 7a), leading to unprecedentedly fast and controlled polymerization with nonpurified NCAs (Figure 7b,c). Conversely, no polymerization was observed after 12 h for nonpurified NCAs in an anhydrous solution (Figure 7b), confirming the detrimental inhibitory effect of trace amount of impurities for NCA polymerization.[77] In addition, the strategy reported in this work, named segregation-induced monomer purification and initiator localization promoted rate enhancement (SIMPLE), can be further extended to other rate acceleration systems, producing polypeptide materials with various architectures from nonpurified NCAs (Figure 7d).[77] As a major breakthrough in the NCA/polypeptide field, this strategy not only circumvents the anhydrous setups during NCA synthesis, storage, and polymerization but also skips the tedious NCA purification process. While the scope of NCA monomers is to be tested for this SIMPLE strategy, the concept to take advantage of fast kinetics can be used for the design of new polymerization system to suppress other side reactions.

Manipulation of Secondary Structures. One of the unique features of polypeptide materials, compared to other synthetic polymers, is their ability to form stable secondary structures.[30] The secondary structures of homopolypeptides containing natural amino acid residues have been well studied. Generally speaking, polypeptides bearing hydrophobic side chains adopt ordered α-helical or β-sheet conformation, where the exact secondary structure depends on the α-helix/β-sheet propensity of the amino acids (e.g., poly(L-leucine) (PLLeu) is α-helical, and poly(L-valine) (PLVal) adopts a β-sheet conformation).[94,95] On the other hand, poly(L-glutamic acid) (PLG) and poly(L-lysine) (PLL) adopt random coil conformation when they are dissolved in aqueous solution and charged, as the charge repulsions between side chains prevent the formation of ordered secondary structures.[96]

The pH-responsive helix−coil transitions of PLG and PLL were first reported 50 years ago[97,98] and have been used to tune the self-assembly behaviors of polypeptide-based copolymers.[99,100] Recently, significant efforts have been devoted to the development of non-natural polypeptides with ordered secondary structures, aiming to not only elucidate the impact of side-chain interactions on polypeptide conformation but also facilitate the design of conformationally switchable polypeptides.[30] With the advances in developing functional NCAs and NCAs with reactive handles, a variety of functionalities can be incorporated onto the polypeptide side chains, allowing detailed studies on the structure−property relationship. It has to be noted that most secondary structure reports of synthetic polypeptides have been focused on α-helices, as β-sheet polypeptides usually have solubility issues.

Until now, three types of side-chain functionalities have been studied as "α-helical disruptors", including charges,[36,96,101] polar groups,[102] and H-bonding ligands with a specific binary H-bonding (BHB) pattern (Figure 8a).[103] The incorporation of these groups onto polypeptide side chains, when at proper distance to the backbone peptides, results in the disruption of helical conformation and the formation of random coils. On the contrary, the "silencing" of these disruptors resumes the helical structure of the polypeptides.[53,101,103] These disruptors offer a great opportunity to manipulate the secondary structure of synthetic polypeptides through stimuli-responsive changes of side-chain

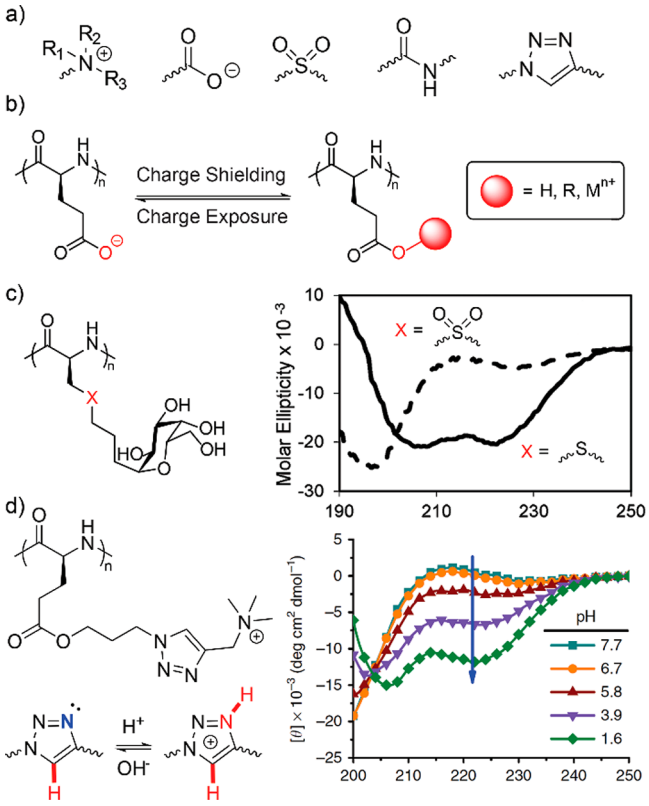

Figure 8. Modulation of helix−coil transition of synthetic polypeptides. (a) Representative α-helical disruptors. (b) Illustration of charge shielding or exposure on polypeptide side chains to manipulate secondary structure. (c) Chemical structure and circular dichroism spectra showing redox-responsive helix−coil transition of glycopolypeptides. Helical structure was disrupted when the side-chain thioether linkage was oxidized to sulfone. Reproduced with permission from ref 102. (d) Chemical structure, H-bonding pattern analysis, and circular dichroism spectra showing pH-responsive helix−coil transition of triazole-based polypeptides. H-bonding donors are highlighted in red, and H-bonding acceptors are highlighted in blue. As the pH decreased, the transfer of H-bonding pattern of side-chain triazole led to the recovery of α-helical conformation. Reproduced with permission from ref 103. Copyright 2017 Springer Nature.

functionalities.[30] For instance, the most studied disruptor, side-chain charges, can be easily exposed through protonation/deprotonation,[104] deprotection,[105,106] and loss of coordinated metal ions,[107] triggering helix-to-coil transitions in an aqueous environment (Figure 8b). Conversely, reactions including hydrolysis of charged groups,[108] *in situ* amidation,[109] metal coordination,[110] and salt screening,[111] lead to the removal of charge repulsions that induces the formation of helices (Figure 8b). Similarly, chemical modification of side-chain polar groups and H-bonding ligands were also used to design functional polypeptides with helix−coil transition behaviors (Figure 8c,d).[102,103]

MATERIALS APPLICATIONS

Polypeptides with Complex Architectures. Polymers with complex architectures other than linear structure have drawn increasing attention due to their unique materials properties, assembly behaviors, and biomedical applications.[112−115] For synthetic polypeptides, several studies have indicated that the architectures of these materials have significant impacts on their biomedical performance.[116−119]

DOI: 10.1021/acs.macromol.9b01450
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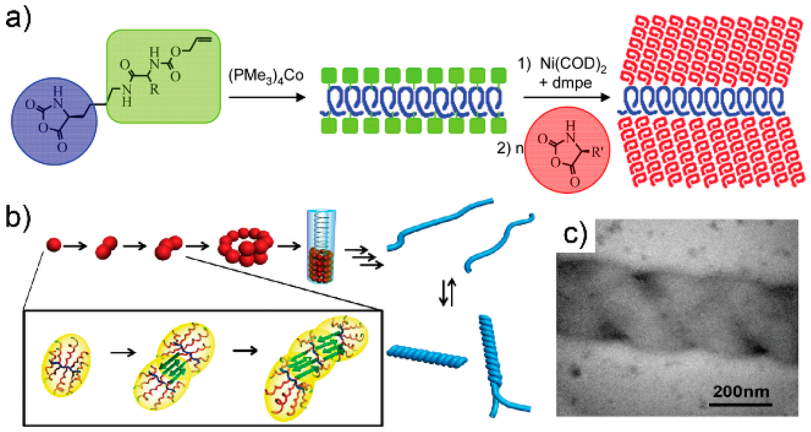

Figure 9. Preparation and assembly of polypeptide-based brushes. (a) Scheme illustrating the synthesis of entirely polypeptide-based brushes through a tandem catalysis. Reproduced with permission from ref 139. (b) Illustration showing the supramolecular polymerization of polypeptide brushes. (c) Transmission electron microscopy (TEM) image of the obtained tubular superstructure. Reproduced with permission from ref 141.

Star polymers have been intensively studied due to their compact structures, spatially defined domains, and relatively easy synthesis.[114,120] Specifically, star-shaped polypeptides were prepared from ROP of NCAs with various multifunctional initiators (i.e., core-first strategy)[121,122] or the convergent cross-linking of linear polypeptides (i.e., arm-first strategy).[123,124] Notably, amine-terminated poly(amido amine) (PAMAM) and poly(propylene imine) (PPI) dendrimers are widely used as multifunctional initiators due to their defined structures. These star polypeptides have found a variety of applications as unimolecular nanocarriers, including drug delivery,[125,126] gene delivery,[117,119,127] and tissue engineering.[128] We refer to a comprehensive review article by Heise and co-workers,[120] where the synthetic strategies and biomedical applications of star polypeptides were well discussed. Additionally, miktoarm star polymers bearing polypeptide chains were prepared by combining ROP of NCAs with other polymerization techniques like controlled radical polymerization and anionic polymerization.[129,130] Entirely polypeptide-based miktoarm star polymers were also prepared through postpolymerization conjugation.[131] Because of the rodlike feature of helical polypeptide arms, these miktoarm star polymers exhibited interesting hierarchical self-assembly behaviors.[132−134]

Inspired by the preparation of hyperbranched polymers through the polymerization of AB* inimer, Dong and co-workers recently reported the controlled synthesis of hyperbranched polypeptide through UV-triggered ROP of photocaged lysine-NCA derivative.[135,136] The UV irradiation generated active AB* NCA inimer *in situ*, forming hyperbranched poly(L-lysine) with tunable MW and degree of branching (DB).

Polypeptide-based molecular brushes were prepared through "grafting onto",[43] "grafting from",[57,92,137−139] and "graft through" methods.[137,140] Coupled with radical polymerization, ring-opening metathesis polymerization (ROMP), and click chemistry, several polypeptide-based hybrid brush materials were successfully prepared. Besides these hybrid copolymer brushes, Deming's group prepared entirely polypeptide-based brushes in one pot through a tandem catalysis procedure.[139] Specifically, NCAs bearing allyloxycarbonyl-α-aminoamido side chains were first polymerized with $(PMe_3)_4Co$ initiators. These allyloxycarbonyl-α-aminoamido groups were then *in situ* activated by Ni, serving as the new initiating sites for the growth of brushes (Figure 9a). Well-defined, cylindrical polypeptide brushes were obtained with this strategy, which may have interesting materials properties considering the high grafting density and the ability to adopt ordered secondary structure for both backbones and side chains. While the assembly behaviors and functions of these polypeptide brushes require further exploration, Lin, Cheng, and co-workers reported the formation of tubular supramolecular structures through the multichain interactions of polypeptide brushes (Figure 9b,c).[141] The branched feature of the brushes is critical to provide specific and directional interactions between the polymers.

In addition, the above-mentioned NHC initiators were used to synthesize cyclic polypeptides.[87] The cyclic topology was convincingly demonstrated by mass spectrometry and viscometry. Considering the remarkable differences between cyclic and linear structures found in other polymers,[142] further studies on the cyclic polypeptides may open new opportunities for the design of functional polypeptide materials.

A recent example using oil-in-water (o/w) miniemulsion system provides a facile strategy to prepare entirely polypeptide-based nanoparticles (NPs).[143] One of the key condition was to use TEA for fast polymerization kinetics through AMM mechanism. Entirely polypeptide-based macroporous hydrogels were also prepared by Qiao and co-workers through cross-linking of PLG-*r*-PLL or PLL-*b*-poly(DL-valine).[144,145]

Helix-Specific Materials Properties. Polypeptides have shown interesting helix-specific materials properties, which have not been observed from their random-coiled analogues, even with the same degree of polymerization (DP) and side-chain structures. Therefore, the functions of polypeptide can be modulated not only through the changes of chain lengths and side-chain functionalities, which are usually used for synthetic polymers lacking ordered conformations, but also through the control over secondary structures.[30] These conformation-associated properties are usually validated by comparing polypeptides from chiral NCA monomers (i.e., NCAs synthesized from L-amino acid or D-amino acid only) with those from racemic NCA monomers (i.e., NCAs synthesized from a mixture of L-amino acid and D-amino acid, in 1:1 molar ratio). Helix-specific materials properties of synthetic polypeptides have been well summarized in the recently published review articles.[30,31] Here we only highlight

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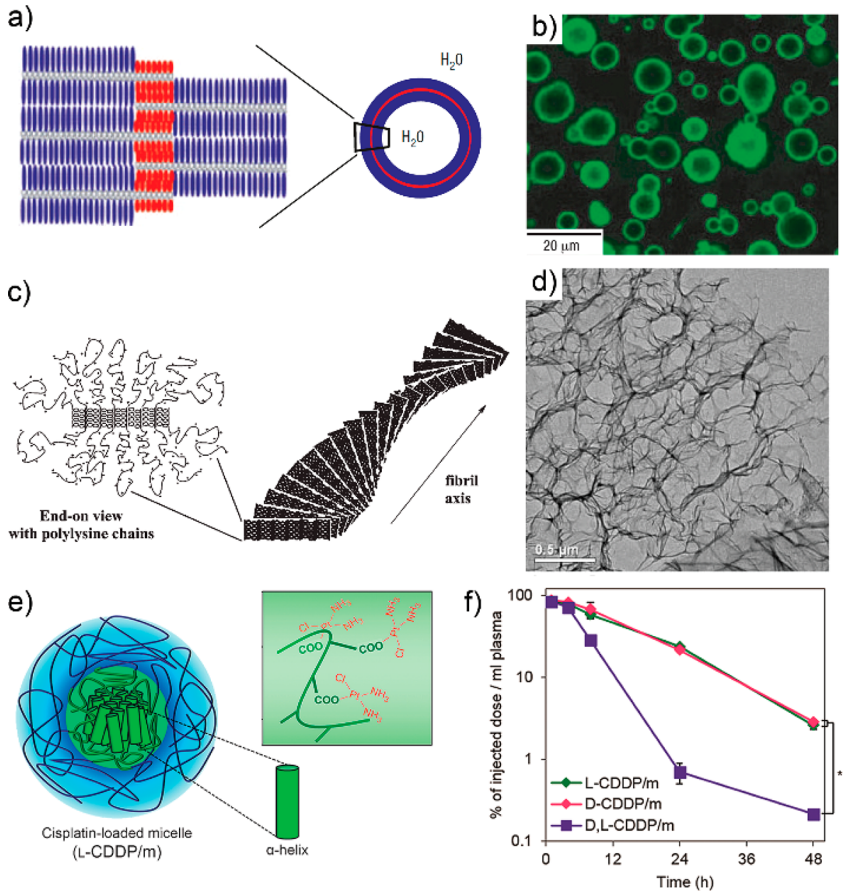

Figure 10. Assembly of helices in aqueous environment. (a) Illustration showing anisotropic alignment of amphiphilic α-helical polypeptides into vesicles. (b) Laser confocal scanning microscopy (LCSM) image of the vesicles from poly(N^{ε}-2-(2-(2-methoxyethoxy)ethoxy)acetyl-L-lysine)-*block*-PLLeu. Reproduced with permission from ref 146. Copyright 2004 Springer Nature. (c) Scheme illustrating the proposed packing of block co-polypeptide amphiphiles into twisted fibrillar tapes with the helices packed perpendicular to the fibril axes. (d) Cryogenic TEM image of the three-dimensional hydrogel networks from PLL-*b*-PLLeu. Reproduced with permission from ref 153. Copyright 2005 Royal Society of Chemistry. (e) Illustration of proposed bundling of cisplatin-loaded polypeptide micelles from PEG-*b*-PLG. (f) Concentration of cisplatin in plasma over time after intravenous administration into mice. The α-helical core showed improved drug release profile compared to the random coil counterpart. Reproduced with permission from ref 154.

some representative examples and discuss the possible molecular mechanisms. We hope the discussions will provide insights into the discovery of new functions of polypeptides with ordered secondary structures.

Hydrophobic polypeptide helices have shown completely different aqueous self-assembly behaviors compared with random-coiled polymers. Amphiphilic block copolymers, with an α-helical polypeptide as the hydrophobic segments and a variety of hydrophilic blocks with different polymer types, charge densities, and conformations, formed vesicle morphologies in aqueous environment even at high hydrophilic-to-hydrophobic ratios (Figure 10a,b).[106,107,146−149] Block copolymers with the same composition but a random-coiled hydrophobic block, on the contrary, failed to form the lamellar structures.[106,146] Using polypeptides with pH-sensitive helix−coil transition behaviors, Lecommandoux's group designed polypeptide vesicles with reversible inside-out micellization behavior.[148] The protonated PLG and deprotonated PLL served as the helical, hydrophobic block under acidic and basic conditions, respectively, driving the formation of vesicular morphology. In addition, on the basis of oxidation-triggered conformational change of cholesterol-modified poly(L-cysteine) (PLCys) from β-sheet to α-helix, Ding and co-workers reported the secondary structure directed micelle-to-vesicle morphological transition, which was then used for drug delivery application.[150]

The high tendency of helical polypeptides to form lamellar assemblies originates from their low conformational entropy,[151] enabling the anisotropic, side-by-side packing of helical rods and stabilizing the bilayers (Figure 10a).[146] With a similar reason, the coiling of hydrophobic helices was used as noncovalent cross-linkers that enable the formation of hydrogels (Figure 10c,d).[152,153] Additionally, the bundled, lateral alignment of α-helices was also observed for platinum coordinated PLG in the core of the micelles, which significantly changed the release profile of the cisplatin drugs due to the compact, bundled core (Figure 10e,f).[154] The anisotropic packing of helices was also used for catalysis in solvents with low dielectric constants (*vide ante*), where the interactions between helical macrodipoles in proximity facilitated the growth of helices through accelerated NCA polymerization.[92]

Besides the packing behaviors, soluble, monomeric α-helices also exhibited interesting conformation-specific properties. For example, α-helical polypeptides possess well-defined spatial domains (i.e., "core" region containing peptide backbones and

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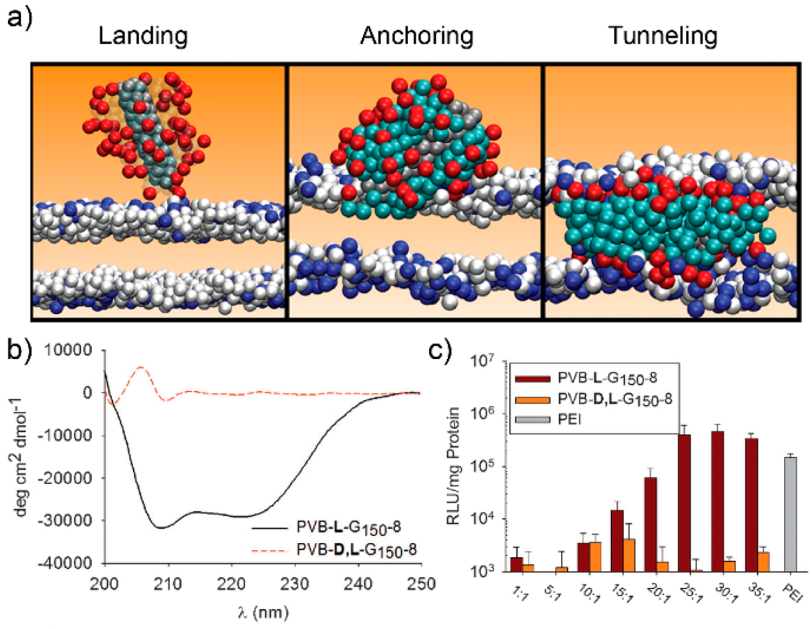

Figure 11. Helix-specific cell-penetrating ability of cationic polypeptides. (a) Simulation images of the interaction of a cationic, α-helical polypeptide bearing flexible side chains with cell membrane. Reproduced with permission from ref 155. (b) Circular dichroism spectra of a cationic, α-helical polypeptide, poly(γ-(4-((2-(piperidin-1-yl)ethyl)aminomethyl)benzyl)-L-glutamate) (PPABLG, also called PVBLG-8), and its random-coiled analogue. (c) The *in vitro* transfection of the helical and coiled polypeptides in COS-7 cells. Polyethylenimine (PEI) was used as a positive control. Reproduced with permission from ref 156. Copyright 2012 WILEY-VCH Verlag GmbH & Co. KGaA.

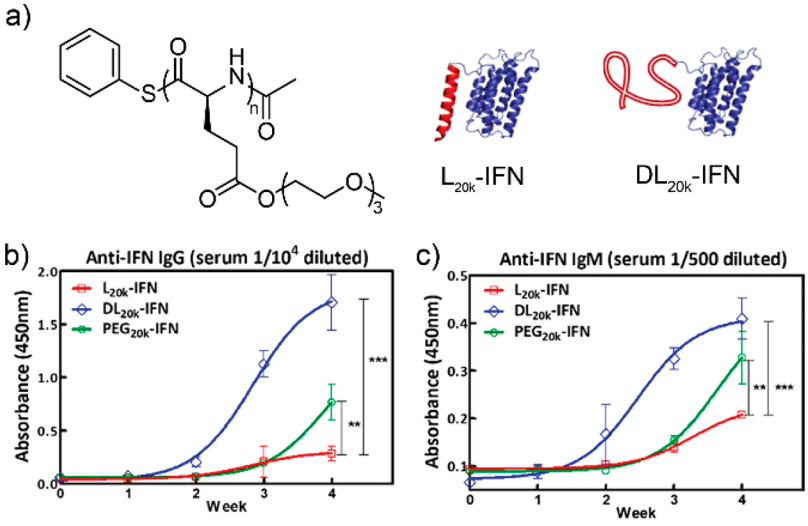

Figure 12. Proteins conjugated with OEG-functionalized α-helical polypeptides showed reduced antidrug antibody production. (a) Chemical structure of OEG-functionalized α-helical polypeptides, L_{20k}, and the illustration of polypeptide–protein conjugates. (b, c) Anti-IFN IgG (c) and IgM (c) contents in the sera immunized with various polymer–IFN conjugates measured by enzyme-linked immunosorbent assay (ELISA). The L_{20k}–IFN induced the lowest immune response compared with its nonhelical analogue and PEG–IFN conjugates. Reproduced with permission from ref 168.

"shell" region with side chains), which has been used to modulate the interactions of polypeptides with plasma membranes. Typically, charged, α-helical polypeptides have long (>11 σ-bonds), hydrophobic, alkyl side chains with the charges located at the side-chain terminus, which strengthens the side-chain hydrophobic interactions and weakens the charge repulsions for the stabilization of helices.[36] When the side chains are positively charged, the helical polypeptides approach the anionic cell membrane in a stepwise "landing–anchoring–tunneling" manner (Figure 11a), where the flexibility of the side-chain alkyl spacers plays an important role.[155] As a result, the cationic polypeptides mediate efficient cell penetrations through pore-formation mechanism, as evidenced by the formation of negative Gaussian curvature (NGC) characterized by small-angle X-ray scattering (SAXS).[155] In contrast, coiled polypeptide analogues do not have defined structures and are therefore unable to generate NGC, leading to their poor cell penetrating ability.[156−158] This unique feature of cationic, α-helical polypeptides has been used for various biomedical applications, including cell-penetrating peptides (CPPs),[157] nonviral gene delivery vectors (Figure 11b,c),[46,156,159−164] and antibacterial polymers,[108,158,165] de-

DOI: 10.1021/acs.macromol.9b01450
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pending on the specific side-chain functionality, charge density, and hydrophobicity.

Soluble, α-helical polypeptides bearing OEG side chains were used by Lu and co-workers to improve the nonfouling property of a surface[166] and enhance cellular uptake efficiency of nanoparticles[167] in a helix-dependent manner, highlighting the importance the secondary structure plays in surface engineering. Interestingly, the authors observed significant impact of the orientations of helical polypeptides in both studies, which is important to guide the future design of surface-coated polypeptides. In addition, the OEG-grafted polypeptides were also conjugated on therapeutic proteins to study the resulting immune reaction.[168] By use of interferon (IFN) as the model protein, the protein−polypeptide conjugates not only exhibited higher binding affinity and antiproliferative activity but also showed reduced immune response compared with proteins attached to an unstructured, random-coiled polypeptide analogue or PEG (Figure 12). Similar helix-specific, lower production of antidrug antibodies was also observed for another polypeptide conjugate with human growth hormone (GH). While the molecular mechanism is not fully understood, this discovery highlights the role of ordered polymer conformation in the design of therapeutics, shedding light on some new functions of helical polypeptide materials.[169]

Inspired by the conformation-specific properties of polypeptide materials, conformationally switchable polypeptides have been designed, whose performance can be regulated in response to stimuli-triggered changes in secondary structures. For instance, controlled assembly of polypeptides was achieved through coil-to-helix transition induced by *in situ* amidation of side chains (Figure 13).[109] Conversely, the dissociation of

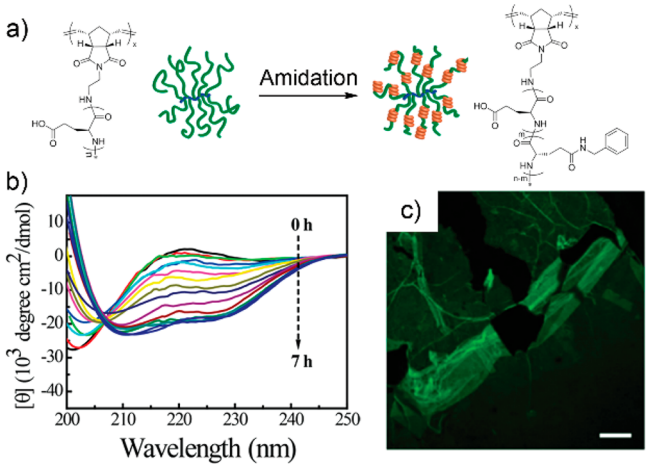

Figure 13. Triggered assemblies of conformationally switchable polypeptides. (a) Scheme illustrating coil-to-helix transition induced by *in situ* amidation of PLG. (b) Circular dichroism demonstrating the change of secondary structures. (c) LCSM image of assembled membrane supramolecular structures stained with Thioflavin T. Scale bar = 20 μm. Reproduced with permission from ref 109.

polypeptide vesicles was triggered through helix-to-coil transition of the hydrophobic segments, which was further used for the release of encapsulated cargos.[146,170] Based on the helix-specific cell penetrating ability, cationic polypeptides with photoresponsive helix-to-coil transition behaviors were first used to complex with DNA and mediate penetration of plasma membrane, the subsequent UV irradiation after cell uptake reduced the cytotoxicity of polypeptides, as the degraded coiled form exhibited lower pore-formation ability (Figure 14). In addition, the accompanied decrease of positive charge density also contributed to the decrease of toxicity and facilitated the unpacking of DNA complexes at the same time.[105] With a similar approach, stimuli-responsive, conformationally switchable polypeptides were used as smart cell penetrating polymers mediating acid-triggered endosomal escape[103] as well as enzyme-activated antibacterial polymers.[108]

While helix-dependent properties of synthetic polypeptides have been widely studied, β-sheet-relevant properties gained much less focus, mainly due to the difficulty in synthesis, despite the importance of this secondary structure.[30,171] One example worth mentioning is from Qiao and co-workers, where β-sheet forming polypeptides were formed through surface-initiated ROP of L-valine NCA on silica nanoparticles.[172] After removal of the silica template, the interchain H-bonding stabilized polypeptide shells, which were further used to trap or conjugate various materials.

Glycopolypeptides. Glycopolypeptides represent a unique class of polypeptide materials with pendant side-chain saccharides, which mimic the structures and functions of glycoproteins.[173,174] Compared with other glycopolymers with non-peptide backbones, the biocompatibility, biodegradability, and the ability to form ordered secondary structures of glycopolypeptides make them promising for biomimicry and biomedical applications.[175,176]

The past decade has seen the significant progress in the synthesis of glycopolypeptides, which has been summarized in two comprehensive review articles.[175,176] With the advances of NCA chemistry, the sugar moieties were successfully attached through postpolymerization modification methods via click chemistry[45,48,55,104,177,178] or other efficient conjugation methods (Figure 15a).[50,179−181] On the other hand, glycopolypeptides were also obtained through the polymerization of NCAs bearing saccharide side chains (Figure 15b).[70,102,182−187] Thanks to the chromatography purification technique, glycosylated NCA monomers can be synthesized in high quality and polymerized in a living manner with the transition metal initiation system.[183] Notably, the synthetic glycopolypeptides with naturally occurring side chains were also successfully prepared from NCA polymerization (Figure 15c).[70,182,187] For instance, synthetic polypeptides with serine residues bearing α-GalNAc by a natural *O*-linkage were prepared through chromatography-purified glyco-NCAs and controlled polymerizations.[70] Detailed characterization indicated the dense *O*-glycosylation resulted in a highly organized and rigid backbone structure.

Glycopolypeptides with multiple saccharide side chains exhibited enhanced affinity for protein receptors due to the multivalency.[186,188,189] Similarly, the enhanced molecular recognition of self-assembled glycopolypeptides was also observed,[64,149,190−192] demonstrating their potential as inhibitors or therapeutics. Recently, Bertozzi and co-workers reported the use of glycopolypeptides as innate immune cell activators of dectin-1 and dectin-2, the glycan-binding proteins presented on antigen-presenting cells (APCs) (Figure 16a,b).[187] When glycopolypeptides bearing β-laminaribiose and β-laminaritriose side chains were attached on spherical beads, the resulting conjugates induced receptor-specific inflammatory responses that are comparable to the bacterially derived dectin-1 agonist Curdlan (Figure 16c,d).

DOI: 10.1021/acs.macromol.9b01450
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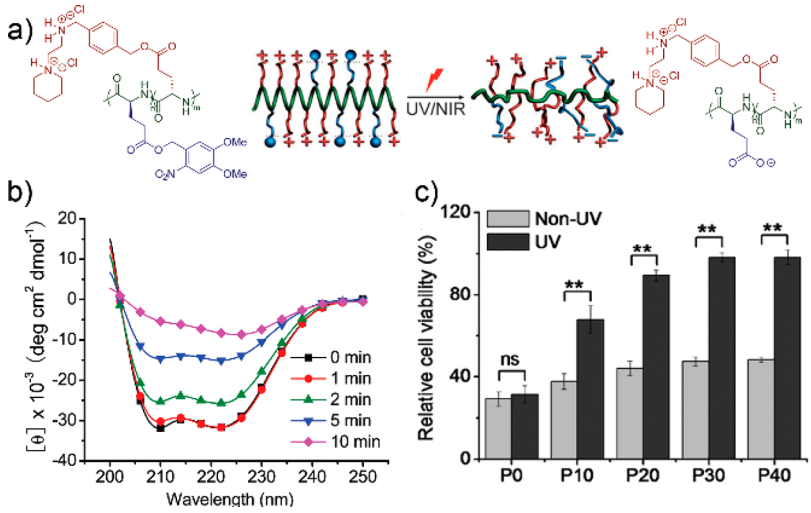

Figure 14. Triggered toxicity reduction and DNA unpacking using conformationally switchable polypeptides. (a) Illustration showing UV/NIR-triggered helix-to-coil transition of cationic polypeptides. (b) Circular dichroism demonstrating the change of secondary structures. (c) Cytotoxicity of polypeptide/DNA complexes with or without UV irradiation. The cytotoxicity of the complexes significantly decreased after UV-induced secondary structure transition. Reproduced with permission from ref 105. Copyright 2013 WILEY-VCH Verlag GmbH & Co. KGaA.

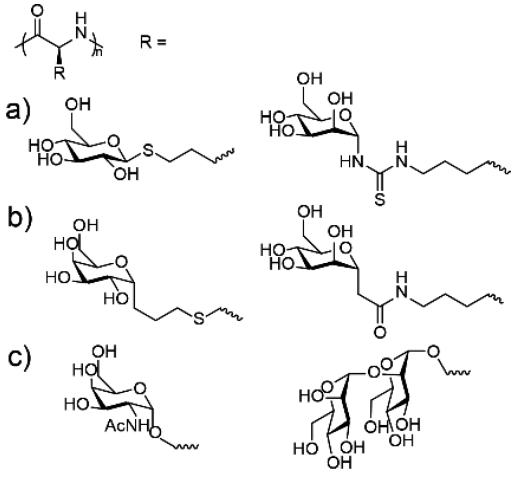

Figure 15. Chemical structures of representative glycopolypeptides prepared by NCA polymerization method. (a) Representative glycopolypeptides prepared by polymerizing NCAs with reactive handles followed by postpolymerization modification. (b) Representative glycopolypeptides synthesized by direction polymerization of glycosylated NCAs. (c) Representative glycopolypeptides synthesized by direction polymerization of glycosylated NCAs bearing natural linkages.

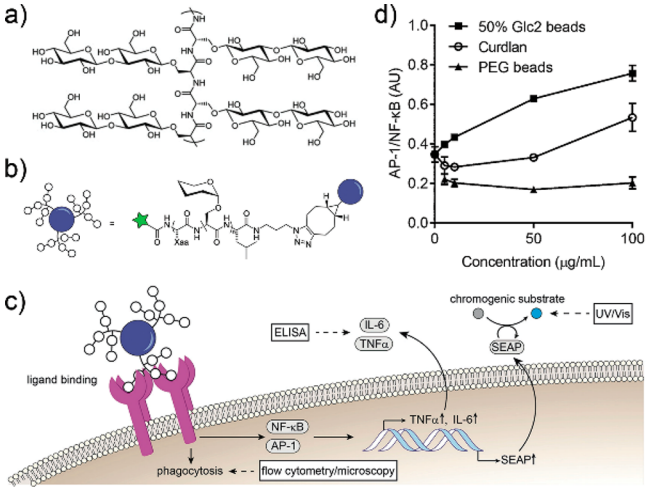

Figure 16. Activation of antigen-presenting cells with glycopolypeptides. (a) Chemical structure of glycopolypeptides based on natural β-1,3-glucan agonists of dectin-1. (b) Preparation of glycopolypeptide–bead conjugates to mimic the microbial cells bearing natural ligands. (c) Scheme illustrating the cellular responses induced by the binding of glycopolypeptides with lectin receptors. (d) Dose response curve showing AP-1/NF-κB activation in RAW-Blue cells. PEG-coated beads and the canonical agonist Curdlan were used as negative and positive controls, respectively. Reproduced with permission from ref 187. Copyright 2018 WILEY-VCH Verlag GmbH & Co. KGaA.

FUTURE OUTLOOK

Synthetic polypeptides from NCA polymerization are promising polymers that not only offer new biomaterials with unique properties but also mimic natural proteins and help the understanding of protein functions. Despite the exciting advances highlighted in this Perspective, the properties and functions of synthetic polypeptides are still far from matching that of proteins. Further developments on chemical synthesis, conformation modulation, higher-ordered assemblies, and biomedical applications of polypeptides are needed to fully realize the potential of these materials.

With the progress on NCA/polypeptide chemistry in the past two decades, synthetic polypeptide materials can be prepared with predictable MWs, defined terminal and side-chain functionalities, and narrow dispersity. Specifically, the chromatography purification methods,[69] the open vessel polymerization technique,[77,88] and the direct polymerization of nonpurified NCAs[77] greatly simplify the polypeptide synthesis procedures, making the preparation of functional NCAs accessible to nonexperts and opening infinite opportunities for downstream studies on materials properties and functions. It is therefore a great opportunity to prepare more complicated polypeptide materials with spatially defined three-dimensional structures and various functionality, which may exhibit protein-like functions. However, the poor control over monomer sequence is still a challenge for ROP of NCA. Learning from nature and other synthetic polymers,[193]

DOI: 10.1021/acs.macromol.9b01450
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achieving sequence regulation in synthetic polypeptides may provide chances,[65] making it possible for polypeptide materials to serve as low-cost alternatives of protein materials and peptide materials from SPPS.

While the secondary structures of polypeptides with proteinogenic amino acid residues are well studied, the conformations of non-natural polypeptides remain poorly understood. Specifically, it is difficult, if not impossible, to predict the conformation of new, non-natural polypeptide materials based on their side-chain structures. Therefore, fundamental understandings on the side-chain interactions, including charge interactions, H-bonding, hydrophobic interactions, and dipole−dipole interactions, are required to elucidate their impact on the secondary structure. Besides α-helix, the β-sheet structure plays an important role in regulating protein interactions and aggregations[171] but is seldom studied for synthetic polypeptide materials. Further understanding on the stabilization and disruption of β-sheets,[194,195] as well as β-sheet-specific materials functions, are promising future directions. We believe the research works on the modulations and applications of secondary structures will inspire the design of new polypeptide materials with unique materials functions. Additionally, the functions of globular proteins rely on the construction of their higher-ordered, three-dimensional structures, which are formed by the supramolecular interactions of secondary structures. However, the controlled interactions and associations of secondary structures into three-dimensional structures and their properties and functions remain largely unexplored.[92,141] By taking advantages of the current knowledge on architecture designs and conformation modulations, it is possible to push one step further and synthetically reconstruct the higher-ordered structures of proteins in polypeptides.

Finally, besides the use of polypeptide materials as nanocarriers, synthetic polypeptide can potentially be used as standalone therapeutics. One notable example is glatiramer acetate (GA), a statistical copolypeptide with four natural amino acid residues.[196] GA is an approved drug for the immunomodulatory treatment of multiple sclerosis, the most common inflammatory demyelinating disease of the central nervous system (CNS).[197] The recent studies in glycopolypeptides represent an exciting example to show the potential of synthetic polypeptides,[187] which may open new avenues for applying polypeptide materials for the regulation of cell signaling and functions.

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DOI: 10.1021/acs.macromol.9b01450
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■ ACKNOWLEDGMENTS

The authors thank and acknowledge the financial support from National Science Foundation (CHE-1709820).

■ ABBREVIATIONS

SPPS, solid-state peptide synthesis; ROP, ring-opening polymerization; NCA, *N*-carboxyanhydride; MW, molecular weight; OEG, oligo(ethylene glycol); ATRP, atom transfer radical polymerization; TEA, triethylamine; iROP, interfacial ring-opening polymerization; NTA, *N*-thiocarboxyanhydrides; AMM, activated monomer mechanism; BLG-NCA, γ-benzyl-L-glutamate NCA; TREN, triethylaminetriamine; NAM, normal amine mechanism; AAMMA, accelerated amine mechanism by monomer activation; H-bond, hydrogen bond; 1,3-Bis-HFAB, 1,3-bis(2-hydroxyhexafluoroisopropyl)benzene; H-bonding, hydrogen bonding; NHC, *N*-heterocyclic carbene; LiHMDS, lithium hexamethyldisilazide; PhS-$SnMe_3$, (trimethylstannyl)-phenyl sulfide; CCP, covalent cooperative polymerization; DMF, *N*,*N*-dimethylformamide; THF, tetrahydrofuran; DCM, dichloromethane; PEG−PBLG, poly(ethylene glycol)-*block*-poly(γ-benzyl-L-glutamate) amine; w/o, water-in-oil; SIMPLE, segregation-induced monomer purification and initiator localization promoted rate enhancement; PLLeu, poly(L-leucine); PLVal, poly(L-valine); PLG, poly(L-glutamic acid); PLL, poly(L-lysine); DB, degree of branching; BHB, binary hydrogen bonding; PAMAM, poly(amido amine); PPI, polypropylenimine; ROMP, ring-opening metathesis polymerization; TEM, transmission electron microscopy; o/w, oil-in-water; NP, nanoparticle; DP, degree of polymerization; PLCys, poly(L-cysteine); LCSM, laser confocal scanning microscopy; NGC, negative Gaussian curvature; SAXS, small-angle X-ray scattering; CPPs, cell-penetrating peptides; PPABLG, poly(γ-(4-((2-(piperidin-1-yl)ethyl)aminomethyl)benzyl)-L-glutamate); PEI, poly(ethylene imine); IFN, interferon; GH, human growth hormone; ELISA, enzyme-linked immunosorbent assay; APCs, antigen-presenting cells; GA, glatiramer acetate; CNS, central nervous system.

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DOI: 10.1021/acs.macromol.9b01450
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DOI: 10.1021/acs.macromol.9b01450
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