

Review

Polypeptide Brushes Grown *via* Surface-initiated Ring-opening Polymerization of α -Amino Acid *N*-Carboxyanhydrides^{*}

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Abstract Polypeptide brushes are attractive platforms to generate functional and responsive interfaces that are of potential interest due to their possible biodegradability, biocompatibility and tunable secondary structures. Surface-initiated ring-opening polymerization (SI-ROP) of α -amino acid *N*-carboxyanhydrides represents a powerful and versatile strategy to generate polypeptide brushes. This review is an attempt to capture the state-of-the-art in this field and highlights the latest developments in several selected areas. In addition to presenting an overview of the synthetic methods that have been used to generate polypeptide brushes *via* SI-ROP, this article will discuss the preparation of patterned polypeptide brushes, the conformational properties of surface-tethered polypeptides, ways to control chain orientation at surfaces as well as properties and applications of these thin polymer films.

Keywords: Polypeptide brushes; Surface; α -Amino acid *N*-carboxyanhydrides; Ring-opening polymerization; Secondary structure.

INTRODUCTION

Polymer brushes are attractive platforms to modulate the surface properties of various materials and have been explored for a variety of applications, including responsive surfaces, non-biofouling surfaces, antibacterial coating, protein binding and immobilization, *etc*^[1–5]. Polypeptide brushes, *i.e.*, densely grafted, chain-end attached polymer chains composed of amino acids are a very interesting class of polymer brushes. In contrast to traditional polymer brushes, which are usually prepared from vinyl-type monomers, the potential biocompatibility and biodegradability of polypeptide brushes make them promising materials in biological applications. Taking advantage of abundant monomers as building blocks, including twenty natural amino acids and their synthetic derivatives, researchers have prepared various polypeptide brushes with variable chain structures and compositions. Moreover, the ability of polypeptides to form ordered secondary structures, such as α -helices and β -sheets, provides additional opportunities to fabricate polymer brushes with well-defined chain conformation. The conformation of surface grafted polypeptide chains can be modulated by external stimuli, including pH, temperature, ionic strength, which provides additional factors to manipulate the surface properties of polypeptide brushes^[6–9]. In addition, the surface grafted polypeptides with α -helical conformation possess a

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dipole moment and display interesting piezoelectric and electric properties, which are not readily accessible from other polymers^[10, 11].

Similar to vinyl-type polymer brushes, polypeptide brushes can be prepared *via* two strategies, *i.e.*, “grafting-onto” and “grafting-from” strategies. In the “grafting-onto” strategy, functional polypeptide chains can be grafted onto the substrate surface *via* physisorption^[12] or chemical conjugation with surface functional groups^[13–17]. Although the “grafting-onto” strategy is quite straightforward and feasible, this strategy suffers from the low grafting efficiency and density. In addition to the steric repulsion between the polypeptide chains, the unfavorable dipolar interaction between pre-grafted and unbound polypeptide chains would be another serious problem in the preparation of dense array of polypeptide chains. In the “grafting-from” strategy, polypeptide brushes are prepared by surface-initiated ring-opening polymerization (SI-ROP) of α -amino acid *N*-carboxyanhydrides (NCAs) using surface-bound initiators. As compared to the “grafting-onto” approach, the “grafting-from” strategy offers opportunities to fabricate dense polypeptide films with higher chain density and more controlled film thickness. Moreover, “grafting-from” strategy allows the formation of directional aligned helical polypeptide arrays, which is crucial for their piezoelectric and electric properties. This contribution mainly focuses on polypeptide brushes prepared *via* the “grafting-from” approach and is an attempt to cover the developments in this field over the past ~2 decades. The remainder of this article consists of 6 sections. The first section will give an overview of the different synthetic methods that have been used to date to generate polypeptide brushes. This section complements another, recent excellent article that has reviewed the preparation of polypeptide brushes^[18]. After that, we will discuss the different strategies that have been developed to prepare patterned polypeptide brushes. The conformational properties of surface-tethered polypeptides and the possibilities to reversibly change these upon application of external stimuli are the subject of section of conformational properties of polypeptide brushes. The following section is related to conformational properties of polypeptide brushes and highlights methods that can be used to control the orientation of surface-tethered polypeptide chains. Finally, the properties and applications of polypeptide brushes are presented.

SYNTHESIS OF POLYPEPTIDE BRUSHES

Although a variety of polypeptide brushes have been prepared in the past decades, the synthesis of polypeptide brushes with desirable thickness and controlled architecture remains as a challenge. So far, two different types of initiators, *i.e.*, the primary amine and transition metal complex, have been employed for the construction of grafted polypeptide brushes. Compared to transition metal catalysts, the use of primary amine initiators is more popular due to its feasibility and the accessibility of primary amines. A variety of techniques has been used to modify different substrates with primary amines. The subsequent SI-ROP can be conducted in different conditions, including solution, melt and the gas phase. The resulting polypeptide brush thicknesses depend on the polymerization technique and the nature of the NCA monomer. In the following sections, the effects of the initiator, the polymerization technique and the NCA monomers on the brush thickness will be discussed in detail. Table 1 gives an overview of the homopolypeptide brushes that have been reported in literature. For each NCA monomer, Table 1 presents both the polymerization technique that has been used as well as the typical brush thickness. Table 2 gives the block copolypeptides brushes that have been reported, and both the polymerization technique and the block thickness are shown.

Table 1. Homopolypeptide brushes prepared *via* SI-ROP of NCA

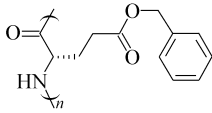
Amino acid	Chemical structure of homopolypeptide	Polymerization techniques	Typical thicknesses
γ -benzyl-L-glutamate		Solution polymerization ^[10, 21, 22, 24, 26, 28, 30–34, 50, 52, 58, 59, 62, 63]	32 nm ^[21]
		Vapor deposition polymerization ^[6, 11, 23, 29, 36–39, 48, 53]	187 nm ^[37]
		Nickel-mediated polymerization ^[43, 44]	3.6 ± 1.8 nm ^[44]

Table 1. Continued

Amino acid	Chemical structure of homopolypeptide	Polymerization techniques	Typical thicknesses
γ -methyl-L-glutamate		Solution polymerization ^[31, 33, 50] Vapor deposition polymerization ^[37, 39, 48] Melt polymerization ^[35]	40 nm ^[31] 69.8 ± 1.0 nm ^[37] 20 nm ^[35]
γ -(2-(2-methoxyethoxy)ethyl)-L-glutamate		Vapor deposition polymerization ^[57]	78 nm ^[57]
γ -(2-(2-methoxyethoxy)ethyl)- <i>rac</i> -glutamate		Vapor deposition polymerization ^[57]	43 nm ^[57]
γ -(2-(2-(2-methoxyethoxy)ethoxy)ethyl)-L-glutamate		Vapor deposition polymerization ^[57]	24 nm ^[57]
β -benzyl-L-aspartate		Vapor deposition polymerization ^[9, 37, 39]	79.0 ± 1.5 nm ^[37]
γ -stearyl-L-glutamate		Solution polymerization ^[27, 30, 50, 52, 54]	5–10 nm ^[30]
γ -4-vinylbenzyl-L-glutamate		Solution polymerization ^[54]	Not reported
β -phenethyl-L-aspartate		Solution polymerization ^[8]	8.2 ± 0.4 nm ^[8]
O-benzyl-L-serine		Vapor deposition polymerization ^[39]	8.0 ± 1.0 nm ^[39]
S-benzyl-L-cysteine		Vapor deposition polymerization ^[39]	13.0 ± 0.5 nm ^[39]
S-tert-butylmercapto-L-cysteine		Solution polymerization ^[59] Nickel-mediated polymerization ^[44]	10–20 nm ^[59] 4.4 ± 1.5 nm ^[44]
O-benzyl-L-tyrosine		Vapor deposition polymerization ^[39]	80.0 ± 11.0 nm ^[39]

Table 1. Continued

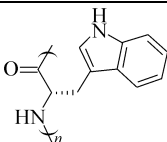
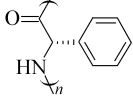
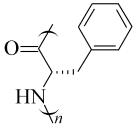
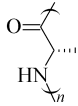
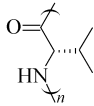
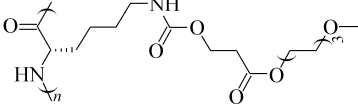
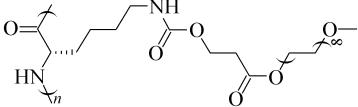
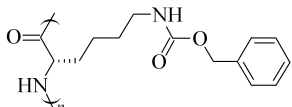
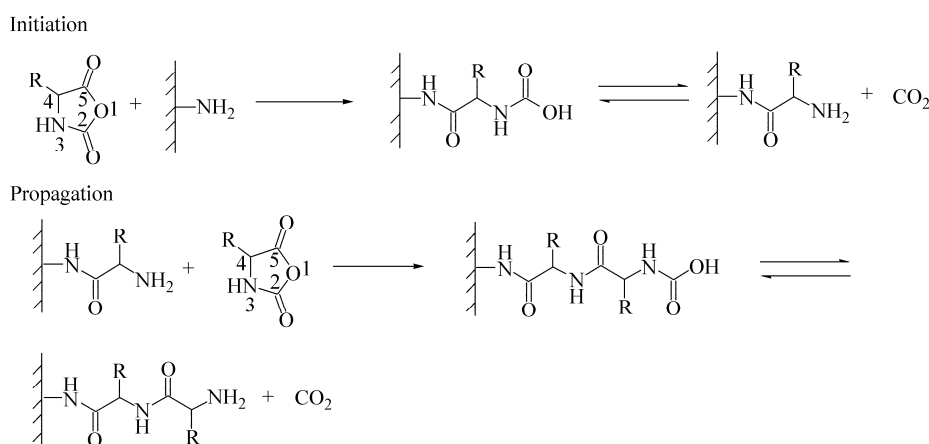
Amino acid	Chemical structure of homopolypeptide	Polymerization techniques	Typical thicknesses
L-tryptophan		Vapor deposition polymerization ^[39]	22.0 ± 1.0 nm ^[39]
L-phenylglycine		Vapor deposition polymerization ^[39]	5.0 ± 0.5 nm ^[39]
L-phenylalanine		Solution polymerization ^[24] Vapor deposition polymerization ^[37, 39]	Not reported 43.6 ± 0.9 nm ^[37]
L-alanine		Solution polymerization ^[24, 60, 64, 65] Vapor deposition polymerization ^[39]	35 nm ^[65] No film formation
L-valine		Vapor deposition polymerization ^[39]	No film formation
N ^ε -(monomethoxy-triethylene glycol-succinate)-L-lysine		Solution polymerization ^[61]	3.8 nm ^[61]
N ^ε -(monomethoxy-oligo(ethylene glycol) ₈ -succinate)-L-lysine		Solution polymerization ^[61]	2.9 nm ^[61]
N ^ε -carbobenzyloxy-L-lysine		Solution polymerization ^[25, 58, 59, 64] Nickel-mediated polymerization ^[44] Vapor deposition polymerization ^[7, 37, 45]	15.5 nm ^[25] 4.2 ± 1.7 nm ^[44] 119 nm ^[7]

Table 2. Block copolypeptide brushes prepared via SI-ROP of NCA

Block copolypeptides brushes	Polymerization techniques	Each block thicknesses
PMLG- <i>b</i> -PBLG	Vapor deposition polymerization ^[48]	PMLG: 49.4 ± 7.7 nm ^[48] PBLG: 44.5 ± 8.3 nm ^[48]
PBLA- <i>b</i> -PBLG	Vapor deposition polymerization ^[9]	PBLA: 60 nm ^[9] PBLG: 60 nm ^[9]
PBLG- <i>b</i> -PTBC	Nickel-mediated polymerization ^[44]	PBLG: 3.6 ± 1.8 nm ^[44] PTBC: 4.4 ± 1.5 nm
PCBL- <i>b</i> -PTBC	Nickel-mediated polymerization ^[44]	PCBL: 4.2 ± 1.7 nm ^[44] PTBC: 4.4 ± 1.5 nm ^[44]
PLPA- <i>b</i> -PBLG	Vapor deposition polymerization ^[37]	PLPA: 38 nm ^[37] PBLG: 21 nm ^[37]
PBLG- <i>b</i> -PCBL	Vapor deposition polymerization ^[37]	PBLG: 60 nm ^[37] PCBL: 55 nm ^[37]
PBLG- <i>b</i> -PMLG	Solution polymerization ^[33]	PBLG: 10 nm ^[33] PMLG: 17 nm ^[33]

Primary Amine Initiated ROP of NCAs

For SI-ROP of NCAs initiated by primary amines, the mechanism was proposed to proceed mainly through the “normal amine” mechanism^[19]. The initiation and propagation steps are shown in Scheme 1^[19]. In the initiation step, the primary amine acts as a nucleophile to attack the 5-C=O of NCA to give the intermediate carbamic acid. The carbamic acid is not stable and prone to decarboxylation to generate an amine group. The regenerated amino group continues to react with NCA and propagates the polymer chain. Generally, the initiation rate is faster than the propagation rate because the nucleophilicity of the primary amine is stronger than that of the amino group of the propagating species^[19]. As a consequence, the SI-ROP of NCAs initiated by primary amine displays the characteristics of a living polymerization. However, even the presence of trace amount of water and other impurities could prevent the reaction from following the desired pathway. In addition, several side reactions, such as chain transfer and self-cyclization of the propagating chains, can prematurely terminate the propagation.



Scheme 1 Initiation and propagation steps in the SI-ROP of α -amino acid *N*-carboxyanhydrides according to normal amine mechanism

Despite the side reactions in the primary amine initiated ROP, the facile grafting of primary amines on substrate surfaces or interfaces makes them popular initiators for the preparation of polypeptide brushes. Depending on the chemical structure of the anchoring group, primary amines can be grafted on various substrates, including silica, glass, quartz and metal. The most frequently used initiator is 3-aminopropyltriethoxysilane (APS), which is a commercially available aminosilane. APS can be used to modify silicon wafers, glass, quartz and aluminum surfaces, either in solution or in gas phase. Compared to solution grafting of APS, which can lead to multilayer films with defects, the reaction performed in vapor phase results in a strictly monolayer film covered surface^[20]. For the modification of gold substrates, amine terminated thiols or disulfides are generally employed to form a self-assembled initiator monolayer^[11, 21–24].

Primary amines can also be introduced onto multi-walled carbon nanotubes (MWNTs) and polymer membranes. In contrast to the substrates mentioned above, MWNTs and polymer membranes cannot be modified using aminosilane or aminothiols directly. In the case of MWNTs, a multiple-step protocol is required. MWNTs were firstly treated with a mixture of concentrated sulfuric acid and nitric acid to give carboxy modified MWNTs, followed by reaction with a diamine to produce amine modified MWNTs^[25, 26]. For the modification of polymer membranes, amino groups can be introduced onto the surface by treatment with ammonia plasma^[27, 28]. An alternative method employed to introduce amino groups onto poly(vinylidene fluoride) membranes involved dehydrofluorination, bromination and amination^[29].

The subsequent SI-ROP initiated by surface grafted primary amines results in the formation of polypeptide brushes. A variety of polymerization techniques, including solution polymerization, melt polymerization and vapor deposition polymerization have been developed, which will be further discussed below.

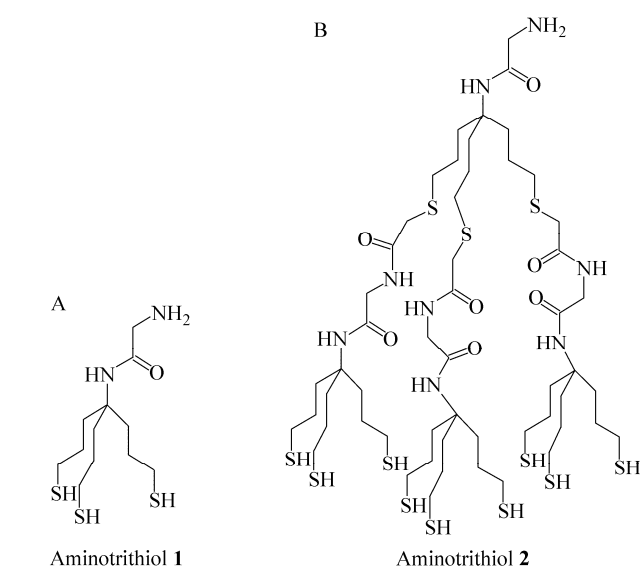
SI-ROP of α -Amino Acid NCAs in Solution

The solution polymerization of NCAs using surface bound initiators is the most straightforward and widely used polymerization technique to prepare polypeptide brushes. Oosterling and Schouten used APS as initiator and successfully grafted random copolyglutamates and copolyaspartates from microparticulate silica and flat surfaces^[30]. In the case of microparticulate silica, all monomers were converted to the grafted polypeptide. However, to graft copolyglutamates and copolyaspartates from flat silicon wafers or glass slides, monomer purification and strictly anhydrous solvent were required to avoid the influence of impurities and moisture on polymerization. Thereafter, Wieringa and Schouten studied the effects of reaction time and monomer concentration on the growth of poly(γ -benzyl L-glutamate) (PBLG) and poly(γ -methyl L-glutamate) (PMLG) brushes from silicon wafers and quartz slides^[31]. Given the “living polymerization” characteristics when primary amine were used as initiator, the polypeptide growth on surface displayed first order kinetics for the first 5 h and then levelled off. The termination of polypeptide propagation was ascribed to the aggregation of free polypeptide chains on the surface rather than the chemical termination. The monomer concentration also played an important role in the polypeptide brush growth. Increasing the monomer concentration promoted the more perpendicular orientation of polypeptide brushes with respect to surface and gave thicker polypeptide film. Under optimized reaction conditions, a PMLG film with thickness up to 40 nm was obtained.

Apart from NCA concentration and reaction time, Niwa and Higashi studied the effect of helix macrodipole moment on the polypeptide brush growth^[22]. A ternary self-assembled monolayer composed of initiator, dummy initiator and preformed helical PBLG was fabricated on a gold surface. By grafting either N-terminal or C-terminal disulfide modified PBLG (PBLG-N-SS or PBLG-C-SS) onto the surface, helix macrodipole moments with opposite direction could be formed before NCA polymerization. When the direction of helix macrodipole moment of the preformed PBLG was anti-parallel to that of polymerizing PBLG chains, the polymerization was accelerated and more perpendicular orientation helical chains were obtained. On the contrary, the polymerization was suppressed due to the energetic disadvantage caused by parallel dipole moment interactions.

The effect of initiator density on polypeptide brush growth was also studied. Heise *et al.* employed the self-assembled monolayer technique and post modification to produce a mixed amine group monolayer in order to control the initiator density on the surface^[32]. The authors believed a minimum initiator concentration was necessary to obtain a certain thickness and minimum roughness. However, attempts to improve the grafting density of polypeptide by adjusting the initiator density on the surface were unsuccessful. Only brush thickness up to 6.5 nm was obtained. One possible method to generate thick polypeptide brushes is to design an appropriate initiator, which occupies a space consistent with the diameter of the helical polypeptides^[24]. For example, an aminotrithiol **1** (Scheme 2A), which occupies a space on the surface slightly larger than the diameter of the helical polyalanine, was designed for polyalanine brush preparation. By using the aminotrithiol **1** as the initiator, polyalanine brushes with about 600 repeat units (about 100 nm) were successfully prepared. To further extend this method to the preparation of polyphenylalanine, which has a diameter larger than that of polyalanine, it was necessary to design a new initiator which occupied a space to match with the diameter of helical polyphenylalanine. For that purpose, aminothiols **2** with nine thiol terminal groups was synthesized (Scheme 2B). Utilizing this initiator, the preparation of polyphenylalanine brushes with high degree of helicity was successful^[24].

While most polypeptide brushes are homopolymer brushes, it is also possible to graft block copolypeptides from surfaces. During the polymerization, free polypeptide is formed in the solution and intends to form aggregates on top of the surface grafted polypeptide brushes. In this case, the termination of surface propagating chain is mainly due to the blockage of initiation sites by physisorbed free polypeptides instead of permanent termination, such as cyclization. After removing the non-grafted polypeptides, the end groups of the polypeptide brushes can recover activity and re-initiate ROP of NCAs. Accordingly, by removal of the non-grafted polypeptides and followed addition of another NCA monomer, it is possible to graft block copolypeptide brushes from surface. By utilizing this method, block copolypeptide brushes, PBLG-*b*-PMLG, were successfully prepared for the first time^[33].



once the NCA vapor encounters the surface. The distance between the substrate and the NCA monomer, as well as the reaction temperature and time have influences on the brush thickness. Using optimized reaction conditions, PBLG brushes with thickness of 42 nm were obtained within 4 h^[36]. The secondary structure of the PBLG brushes was also studied. All brushes above 4 nm thickness adopted exclusively α -helical conformation according to FTIR characterization.

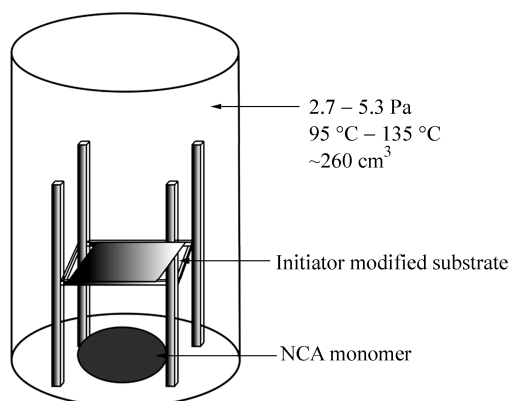


Fig. 1 Schematic diagram of the experimental setup for the surface-initiated vapor deposition polymerization^[36] (Adapted with permission from Ref. [36]; Copyright (1998) American Chemical Society)

Wang and Chang further optimized the SI-VDP method by redesigning the experimental chamber^[37]. Using the new setup, the authors can control both the evaporation temperature of NCA monomers and substrate temperature, which allow to improve the vapor concentration and realize better control of ROP. Through optimization of the reaction parameters, thicker PBLG brush films can be readily obtained within shorter time. The obtained polypeptide brush thickness increased with increase of the substrate temperature from 25 °C to 75 °C. However, further increase the substrate temperature led to the decrease in brush thickness. Specially, a PBLG brush with thickness of 187.4 nm was obtained within half an hour at a substrate temperature of 75 °C. In addition to PBLG, poly-L-phenylalanine (PLPA), PMLG, poly(β -benzyl-L-aspartate) (PBLA) and poly (*N*^ε-carbobenzyloxy-L-lysine) (PCBL) were successfully prepared, which demonstrated the applicability of SI-VDP for a wide range of monomers. SI-VDP also shows its potential in the synthesis of multilayered block copolypeptide brushes. PLPA-*b*-PBLG and PBLG-*b*-PCBL copolypeptide brushes have been successfully prepared using sequential SI-VDP protocol^[37].

More recently, Zheng and Frank developed a SI-VDP system with even further improved pressure and temperature control as compared to the setup reported by Wang and Chang and realized highly efficient grafting of PBLG brushes on silicon wafer at higher pressure^[38]. More importantly, the authors developed a quantitative method to investigate the mechanistic details of the SI-VDP process. By using the new system, the authors could monitor the change of system pressure with reaction time and track the SI-VDP reaction profile. Through controlling the SI-VDP processes, the authors were able to synthesize the PBLG brushes which have either high packing density with mostly α -helical conformation or low packing density with both random coil and α -helical conformation.

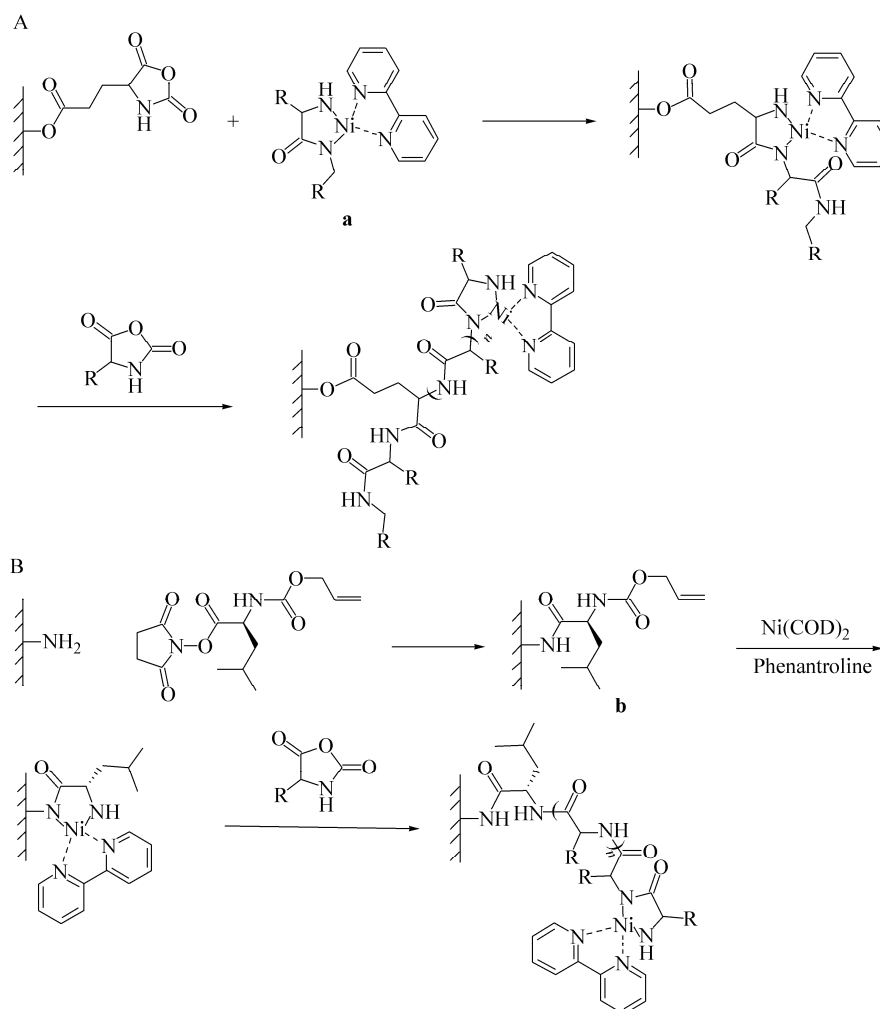
SI-VDP of NCA is a very attractive technique to prepare polypeptide brushes on flat surfaces because it can minimize the oligomerization of NCA caused by moisture or impurities and suppress the side reactions. Compared to other techniques, polypeptide brushes prepared by SI-VDP are much thicker. For example, PBLG brushes with thickness up to 187 nm were obtained under optimized reaction condition. Such a thick PBLG film was not accessible by solution polymerization or melt polymerization. It is worth noting that not all NCA monomers can produce the same thickness. In addition to reaction conditions, the polypeptide brush thickness also depends on NCA monomers and secondary structure of the resulting polypeptides. Generally, the

polypeptides with α -helical or parallel β -sheet conformation give a relative thicker film than those with antiparallel β -sheet or random coil conformation^[39]. Besides, some thermally unstable NCA monomers, such as L-alanine and L-valine, cannot be polymerized efficiently using SI-VDP since SI-VDP usually requires high temperature^[39].

Organonickel Catalyst Mediated NCA Polymerization

An attractive alternative to the primary amine initiated NCA polymerization is the use of organometallic initiators developed by Deming. Compared to primary amines, organonickel and organocobalt initiators can suppress chain transfer and chain termination reactions and allow a “living” NCA polymerization^[40–42]. Although the transition metal catalysts have been extensively used for the solution synthesis of homopolypeptides and copolypeptides, only few reports have investigated their use for the preparation of polypeptide brushes^[43, 44].

In contrast to primary amines, the attachment of the organonickel initiator onto the surface is much more complicated. Two different approaches were developed by Witte and Menzel to graft the organonickel initiator onto polystyrene particles^[43]. In the first strategy, the polymerization is carried out by reacting a surface bound NCA with excess of Ni amido-amidate complex initiator **a** to create growing chain ends (Scheme 3A).



Scheme 3 Strategies for conjugation of organonickel initiator to a surface via (A) the block copolymerization approach and (B) the “alloc-amide” approach for subsequent surface-initiated NCA ring-opening polymerization^[43]

After removing the excess of complex **a**, free NCA monomers were added for the chain propagation. The second strategy is called “Alloc-Amide approach” and uses surface bound Ni amido-amidate complexes as initiator. The initiator was prepared by reacting alloc-amides **b** with $\text{Ni}(\text{COD})_2$ and phenantroline (Scheme 3B). The latter was much more straightforward and showed higher monomer conversion in SI-ROP. Sparks *et al.* utilized the organonickel initiator approach to graft polypeptide brushes from flat surfaces^[44]. Through this method, PBLG, PCBL and poly(s-tert-butylmercapto-L-cysteine) (PTBC) brushes were successfully prepared on silicon wafer. Taking advantages of the living polymerization features, block copolypeptides, such as PBLG-*b*-PTBC and PCBL-*b*-PTBC, were also prepared.

Although narrow polydispersity for surface-grafted polypeptides was obtained using the organonickel mediated SI-ROP of NCA, large amounts of free polypeptide were produced in solution. Less than 20% of the NCA monomers used in the polymerization were grafted on the surface^[43], which indicated the low initiation efficiency of the organonickel initiator in the SI-ROP. In the case of flat surfaces, typically polypeptide brushes with film thicknesses less than 10 nm were obtained. Compared with the primary amine initiated NCA SI-ROP, the polymerization mediated by organometal initiators is still in its infancy. Further researches, including the study on polymerization kinetics, applicability for different NCA monomers, are required in the future to better understand the effects of organometal initiator on polypeptide brush preparation.

PATTERNING POLYPEPTIDE BRUSHES

Patterned polymer brushes, in particular the polypeptide brushes, are of great interest for potential applications in biosensors and biomineralization^[45]. Two different approaches have been proposed to construct patterned polypeptide brushes, *i.e.*, microcontact printing and photolithography.

Microcontact printing (μCP) has been extensively employed for the construction of patterned polymer brushes. In μCP , a patterned initiator molecule layer is printed onto the surface of substrate with the help of an elastomeric PDMS stamp^[46, 47]. Using this method, Kratzmüller *et al.* patterned gold surfaces with 12-mercaptododecylamine, which was subsequently used to initiate the polymerization of NCA monomers and produced a patterned PBLG brushes (Fig. 2)^[21]. The well-defined growth of PBLG brushes on micrometer domains was demonstrated by atomic force microscopy (AFM) and scanning electron microscopy (SEM).

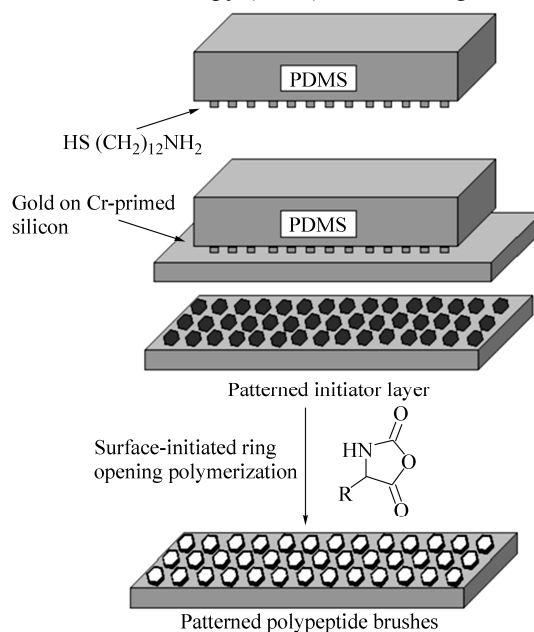


Fig. 2 Schematic representation of the preparation of thin patterned polypeptide layers by microprinting and surface-initiated polymerization^[21] (Adapted with permission from Ref. [21]; Copyright 1999 WILEY-VCH Verlag GmbH, Weinheim, Fed. Rep. of Germany.)

Photolithography is one of the most frequently used methods to fabricate patterned thin films. A light sensitive chemical photoresist is spin cast onto a substrate, and a pattern from a photomask is transferred onto the photoresist with the help of light. Combining the SI-VDP and photolithography, Wang and Chang successfully fabricated multilevel, laterally and vertically patterned PMLG-*b*-PBLG brush films^[48]. This strategy is outlined in Fig. 3. Key to the success of this strategy is the compatibility between photolithography and solvent free SI-VDP protocol. This can be extended to other polypeptide brushes.

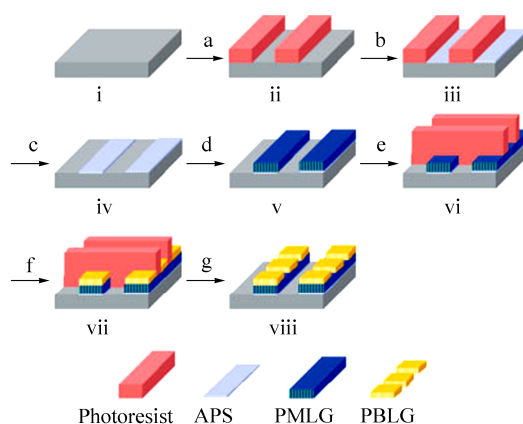


Fig. 3 Schematic illustration of the formation of patterned surface-grafted PMLG and PMLG-*b*-PBLG films on a silicon (100) oxide surface: (a) Deposition of photoresist stripes on a clean silicon wafer; (b) Vapor silanization of APS that reacts only with the unmasked SiO₂; (c) Removal of photoresist and non-specific absorbed materials; (d) SI-VDP of MLG-NCA from APS layer, and the subsequent removal of physisorbed materials; (e) Deposition of photoresist stripes at 90° to the previously deposited stripes; (f) SI-ROP of BLG-NCA initiated from the exposed PMLG regions; (g) Removal of photoresist and physisorbed materials^[48] (Reprinted with permission from ref [48]; Copyright 2003 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim)

CONFORMATIONAL PROPERTIES OF POLYPEPTIDE BRUSHES

In contrast to vinyl-type polymer brushes, polypeptide brushes can adopt ordered secondary structures such as α -helices and β -sheets. The ability to manipulate these secondary structures by external stimuli, such as pH, surfactants, ions, solvent, makes polypeptide brushes an ideal candidate for preparation of stimuli responsive surface coatings. As compared with free polypeptides, chain end of polypeptides grafted to a surface is immobilized. While surface-grafted polypeptide chains are able to adopt regular secondary structures that can be changed by using external stimuli, the immobilization of the polymer chain ends in polypeptide brush films does lead to differences in conformational properties. The conformational properties of polypeptide brushes are not only of fundamental interest, but also from an application point of view. In the following section, the conformational properties of various polypeptide brushes will be discussed in detail.

Poly(L-glutamic acid) Brushes

Poly(L-glutamic acid) (PLGA) is generally prepared by debenzoylation of PBLG with anhydrous hydrogen bromide in glacial acetic acid. Considering the acetic acid may cleave the —Si—O— bonds, a mixture of hydrogen bromide and benzene was used in the study of Wang and Chang^[6]. Three different external stimuli, including pH, surfactants (decylammonium chloride (DAC)) and divalent metal ions (Ca²⁺) were used to investigate the conformational properties of PLGA brushes. CD spectra showed that the PLGA brushes adopted an α -helical conformation at pH < 5.75 and a random coil when pH > 7. This conformational change was accompanied by an increase in brush thickness as confirmed by ellipsometry. Upon addition of DAC or Ca²⁺ into a pH = 8 buffer, the PLGA brushes transformed from random coil to α -helix. A large difference was observed in the conformation transition rates upon adding DAC or Ca²⁺. The transition induced by DAC was much slower

than the transition induced by Ca^{2+} . The former took about 5 min while the latter transition completed within 20 s due to the faster diffusion of Ca^{2+} within PLGA brushes.

Poly(L-lysine) Brushes

Poly(L-lysine) (PLL) is another widely investigated pH responsive polypeptide. In addition to α -helical and random coil conformation, PLL brushes can also adopt β -sheet conformation. PLL brushes can be prepared by debenzoylation of PCBL with anhydrous hydrogen bromide in benzene. Different external stimuli, including pH, surfactants (sodium dodecyl sulfate (SDS)) and anions (ClO_4^-), were used to induce conformational transitions of PLL brushes^[7]. At $\text{pH} < 7$, PLL brushes possess a random coil conformation while they adopt an α -helical conformation when $\text{pH} > 9$. Further increasing the pH up to 11 caused formation of β -sheets although the coexistence of α -helical conformation cannot be excluded. Upon addition of SDS to a $\text{pH} = 7$ buffer, the PLL brushes transformed from random coil to β -sheet. The complexation of PLL with ClO_4^- led to transition of random coil to α -helix.

In addition to small molecules and ions, polyelectrolytes can also alter PLL conformation. Yang and Chang investigated the conformational transition of PLL brushes induced by three anionic polymer, *i.e.*, poly(acrylic acid) (PAA), poly(L-glutamic acid) (PLGA) and poly(L-aspartic acid) (PLAA) at neutral pH^[49]. Interestingly, the minor differences among PAA, PLGA and PLAA resulted in different secondary structure of the complexes. For example, PLL brushes transform from a random coil to an α -helix and then to a β -sheet after complex formation with PAA and PLGA, respectively, while the addition of PLAA did not change PLL conformation. These conformational changes were accompanied by changes in brush thickness as indicated by ellipsometry.

Poly(β -phenethyl-L-aspartate) (PELA) Brushes

In addition to conformational transitions from one to another type of secondary structure, a variety of polypeptide brushes have been prepared which show changes in the helix screw sense under the influence of appropriate stimuli. Upon annealing at 150 °C for 30 min, for example, PPELA brush conformation transforms from a right-handed α -helix to a left-handed π -helix. The left-handed π -helix conformation can be reversibly reverted to the original right-handed α -helix by immersing the PPELA film into helicogenic solvents, such as chloroform, 1,2-dichloroethane, and 1,1,2,2-tetrachloroethane^[8].

Poly(β -benzyl-L-aspartate) (PBLA) Brushes

PBLA brushes are also able to reversibly switch helix screw sense between the right handed and left handed α -helical conformation. PBLA brushes adopt a left handed α -helical conformation upon exposure to dichloroacetic acid vapor and are converted into a right handed α -helical conformation upon exposure to *N,N*-dimethylformamide vapor. To further investigate the helix screw conversion of PBLA, surface grafted PBLA-*b*-PBLG copolypeptide films were prepared. In contrast to PBLA brushes, both chain termini of PBLA block were fixed in the case of PBLA-*b*-PBLG copolypeptide brush. Compared to the left handed α -helix, the right handed α -helix was preferred in PBLA-*b*-PBLG copolypeptide brush, and the right-to-left screw sense conversion of PBLA block was consequently restricted. Apart from solvent, the conformational transition of PBLA brushes can also be triggered by temperature variation. When the PBLA film is heated, the conformation of PBLA brushes first changes from a right handed or a left handed α -helix to a left handed ω -helix at 120 °C and is then irreversibly converted to a β -sheet at 180 °C^[9].

Although surface-tethered polypeptide brushes show similar transition behavior as free polypeptides, surface-tethered polypeptide chains do show some differences. For example, both free PLGA and surface grafted PLGA transform from an α -helix to a random coil with increasing solution pH. However, compared with free PLGA, the inflection point of transition curve for surface grafted PLGA shifts from $\text{pH} = 5.74$ (for free PLGA) to $\text{pH} = 6.25$. On the contrary, the transition inflection point for PLL brushes shifts to more acidic pH values relative to free PLL. Both results indicate that α -helical conformation is more stable than random coil conformation for the surface grafted polypeptide chains. In addition, the transition kinetics of the grafted polypeptide brushes are different from that of free polypeptides. As mentioned above, the transition of PLGA

brushes from a random coil to an α -helix induced by DAC is very slow due to the slow diffusion of DAC into PLGA brushes while the same transition for free PLGA in solution happens in just a few seconds. On the contrary, the surface grafted PLL brushes transform from α -helical conformation to β -sheet conformation immediately once increasing pH up to 11. In contrast, the conformation transition is much slower and takes at least 2 h for free PLL chains given the mean concentration of about 25–50 mg/mL. The reason may be ascribed to the reduced freedom and much higher local PLL concentration in PLL brushes as compared to those in solution.

CONTROLLING THE ORIENTATION OF α -HELICAL POLYPEPTIDE BRUSHES

An important characteristic of surface-tethered polypeptides, and especially helical polypeptides, is the orientation of the polypeptide chains with respect to the surface to which they are anchored. The helix orientation is characterized by the tilt angle θ between the helix axis and the substrate normal (Fig. 4) and is generally characterized using FTIR spectroscopy. Considering that the transition dipole moment of amide I is almost parallel to the backbone of the helix while that of amide II is almost perpendicular to the backbone of the helix, the tilt angle θ can be calculated from the ratio (D) of the amide I absorbance to amide II absorbance^[12, 13, 50, 51]. Depending on the strategies used for preparation of polypeptide brushes, the tilt angle θ of α -helical polypeptide typically ranges from 64° to 48° ^[36, 50, 52]. This means that the surface-tethered α -helical polypeptide chains very often are not perfectly perpendicular to the surface. However, for applications involving piezoelectric or electric properties of polypeptide brushes, a perpendicular orientation of the α -helical polypeptide with respect to the surface is critically important^[10, 11].

Wang and Chang proposed a simple “solvent quenching” approach to promote the perpendicular orientation of PBLG brushes^[53]. As shown in Fig. 4, the approach consisted of two steps: firstly stretching out the grafted PBLG chains by treatment with a good solvent, such as chloroform, subsequent immersing the PBLG brushes into a non-solvent, like acetone. In the non-solvent, the PBLG chains formed periodic aggregates to minimize the interaction between the polypeptide chains and solvent. At the same time, the perpendicular orientation of helical PBLG was frozen by the non-solvent quench even after drying. Through this approach, the tilt angle θ of PBLG brushes decreased from 49° to 3° .

Although the “solvent quenching” approach is straightforward and feasible, this approach seems ineffective for relatively thin films (~ 15 nm), and the perpendicular orientation is not retained in good solvents. To solve these problems, Luijten *et al.* proposed a chemical crosslinking procedure to permanently fix the perpendicular orientation of surface-tethered polypeptide chains^[54]. For this purpose, ternary statistic polypeptide brushes containing polymerizable side groups were prepared from γ -methyl-L-glutamate NCA, γ -stearyl-L-glutamate NCA and γ -4-vinylbenzyl-L-glutamate NCA. By crosslinking the ternary polypeptide using radical polymerization in a good solvent, permanently perpendicular oriented helical polypeptide brushes were obtained as indicated by a tilt angle θ of 11° . However, the effects of the crosslinking on the polypeptide brush properties, such as piezoelectric and electric properties, were not investigated.

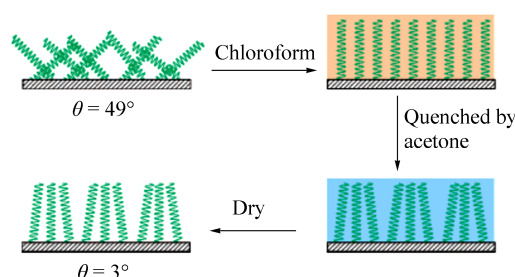


Fig. 4 Schematic model of the alignment of surface-grafted PBLG rods by the “solvent quenching” treatment^[53] (Adapted with permission from Ref. [53]; Copyright (2003) American Chemical Society)

PROPERTIES AND APPLICATIONS

Piezoelectric and Electric Properties

Their ordered secondary structure, especially the α -helical conformation, can endow the polypeptide brushes with interesting physical properties. In polypeptide brushes composed of α -helical polypeptides, the dipole moments of individual repeat units add up to constitute a large dipole moment, making them a potential piezoelectric material. Jaworek *et al.* for the first time demonstrated that surface-grafted PBLG brushes had the piezoelectric properties^[10]. Using a Nomarski optical interferometer, the authors were able to measure changes in film thickness as a function of applied electric field. A quite large persistent polarization was calculated from the experimental data. Using a Langmuir-Blodgett assembled PBLG film as a control, the authors demonstrated that the inverse-piezoelectric effect of the grafted PBLG film was a direct result of the polar alignment of the polypeptide chains. Although the inverse-piezoelectric coefficient of the grafted PBLG film was not high enough, the voltage sensitivity was comparable with commercial piezoelectric materials.

The large dipole moment existing in the helical polypeptide brushes also generates an internal electric field in the direction from N terminus to C terminus. The electron transfer is strongly dependent on the direction of external electric field, making polypeptide brushes suitable as potential organic diodes. The orientation of helical polypeptide brushes affects both the strength of the internal electric field and the electrical conductivity of the polypeptide brushes. Wu *et al.* investigated the effect of PBLG brush orientation on the electric properties by comparing the electrical conductivity of three PBLG films with different chain orientations^[11]: (i) a spin-coated PBLG film (SC_{PBLG}); (ii) a surface grafted PBLG film (UQ_{t-PBLG}) and (iii) a surface grafted PBLG film that had been subjected to a subsequent solvent quenching (Q_{t-PBLG}). The tilt angles θ of SC_{PBLG} , UQ_{t-PBLG} and Q_{t-PBLG} were 75° , 57° and 13° , respectively. The three PBLG films displayed distinguished electrical behavior. The SC_{PBLG} film behaved as an insulator, while UQ_{t-PBLG} and Q_{t-PBLG} showed asymmetric I - V curve. Compared to UQ_{t-PBLG} , the more perpendicular chain orientation of Q_{t-PBLG} led to a much higher rectification ratio, which was comparable with organic diodes.

Stimuli-responsive Polypeptide Brushes

As mentioned above, the conformation of surface-attached polypeptides can be manipulated using appropriate external stimuli, making them of interest to fabricate stimuli-responsive surface coatings. For example, PLGA brushes transform from an α -helical to a random coil conformation and simultaneously increase film thickness when the pH is increased from 4 to 8. Capitalizing upon this, Ito *et al.* fabricated a polymer membrane that showed pH-dependent water permeability by grafting PLGA brushes onto a porous poly(tetrafluoroethylene) membrane^[28].

Recently, our group developed shape-persistent, thermo-responsive polypeptide brushes, which were obtained by SI-ROP of γ -di- or tri(ethylene glycol) modified L-glutamic acid (L-EG₂Glu or L-EG₃Glu) NCAs^[55, 56]. The resulting poly(L-EG_xGlu) ($x = 2, 3$) brushes retained their α -helical conformation while the pendent oligo(ethylene glycol) side chain collapsed upon temperature increase^[57]. This is in contrast to the vinyl-type thermo-responsive polymer brushes, such as poly(*N*-isopropylacrylamide) (PNIPAM) where the polymer main chain collapses upon increasing temperature above the LCST.

Biological Applications

Due to their potential biocompatibility and biodegradability, polypeptide brushes are of interest for bioconjugation, biomineralization and chiral separation. For example, fluorescein isothiocyanate (FITC) and enhanced green fluorescent protein (eGFP) have been successfully conjugated with PLL and PLGA brushes^[58, 59]. The biosynthesis of silica materials has drawn a lot of attention due to their mild reaction condition and precise controlled nanostructure in the past decades. Wu and coworkers developed a novel method to synthesize (patterned) silica films on substrates using grafted PLL brushes as templates^[45]. The growth of the silica film could be precisely controlled by the PLL brush thickness and grafting density. Polypeptide brushes have also been investigated for the separation of chiral molecules^[29]. PLGA and triethylene glycol monomethyl ether modified poly-L-glutamates (PLTEG) were both grafted on poly(vinylidene fluoride) (PVDF) ultrafiltration membranes. The polypeptide brushes grafted PVDF membrane showed enantioselective

permeability for different chiral molecules, including tryptophan, phenylalanine, tyrosine, propranolol, atenolol and ibuprofen.

In addition to the applications introduced above, polypeptide brushes have also been explored for drug delivery, protein enrichment^[60] and non-biofouling surface coating^[61].

CONCLUSIONS AND PERSPECTIVES

Polypeptide brushes possess intriguing properties, including secondary structures that can be manipulated by external stimuli, piezoelectric and electric properties, *etc.* Potential applications of polypeptide brushes are manifold. However, compared to the large number of reports on the vinyl-type polymer brushes, research on polypeptide brushes is still in its infancy. Several polymerization techniques have been developed to prepare polypeptide brushes. Among them, SI-VDP shows many advantages over other techniques. For example, better control and much thicker films can be obtained *via* SI-VDP. However, SI-VDP is not applicable to thermally unstable monomers, such as alanine and valine NCAs. As a consequence, there is a need for more efficient and feasible strategies to further improve the control over film thickness, surface roughness, monomer scope and compatibility and chain orientation. Until now, most researches have concentrated on the canonical NCA monomers, such as glutamate NCAs. Another area of interest is to expand the number of non-natural NCA monomers that can be used to prepare polypeptide brushes to include functionalities, which could further increase the range of possible applications of these polypeptide films. While some efforts have been made to investigate potential applications of polypeptide brushes, there is still plenty of room for further development. Due to the possible advantages in biocompatibility and biodegradability, polypeptide brushes seem to be interesting candidates for use in cell culture and tissue engineering. Other potential applications, including antibacterial coating, chromatography supports, also seem to be worthwhile pursuing.

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