ACS Macro Letters

Letter

Cite This: ACS Macro Lett. 2019, 8, 1517−1521

pubs.acs.org/macroletters

Facile Synthesis of Helical Multiblock Copolypeptides: Minimal Side Reactions with Accelerated Polymerization of *N*-Carboxyanhydrides

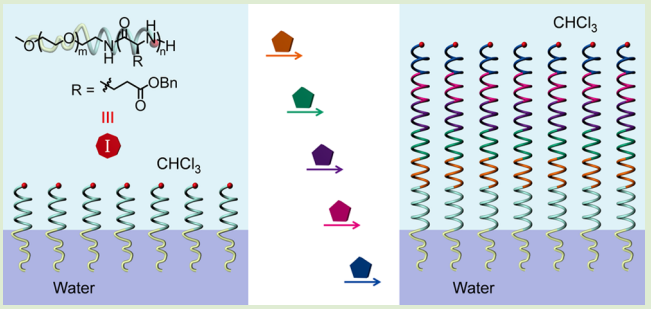
Xuefang Wang,[†,‡,§,#] Ziyuan Song,[*,†,#] Zhengzhong Tan,[†] Lingyang Zhu,[∥] Tianrui Xue,[⊥] Shixian Lv,[†] Zihuan Fu,[⊥] Xuetao Zheng,[⊥] Jie Ren,[*,‡,§] and Jianjun Cheng[*,†,⊥]

[†]Department of Materials Science and Engineering, [∥]NMR Laboratory, School of Chemical Sciences, and [⊥]Department of Chemistry, University of Illinois at Urbana−Champaign, Urbana, Illinois 61801, United States

[‡]Key Laboratory of Advanced Civil Engineering Materials, Ministry of Education, and [§]Institute of Nano and Biopolymeric Materials, School of Materials Science and Engineering, Tongji University, Shanghai 201804, China

Supporting Information

ABSTRACT: Multiblock copolypeptides have attracted broad interests because their potential to form ordered structures and possess protein-mimetic functions. Controlled synthesis of multiblock copolypeptides through the sequential addition of *N*-carboxyanhydrides (NCAs), especially with the block number higher than five, however, is challenging and rarely reported due to competing side reactions during the polymerization process. Herein, we report the unprecedented synthesis of block copolypeptides with up to 20 blocks, enabled by ultrafast polypeptide chain propagation in a water/chloroform emulsion system that outpaces side reactions and ensures high end-group fidelity. Well-defined multiblock copolypeptides with desired block numbers, block lengths, and block sequences, as well as very low dispersity were readily attainable in a few hours. This method paves the way for the fast production of a large number of sequence-regulated multiblock copolypeptide materials, which may exhibit interesting assembly behaviors and biomedical applications.



Multiblock copolymers have drawn increasing attentions in recent years due to their unique self-assembly behaviors and materials properties.[1−3] By sequential addition of monomers and control of end-group fidelity,[4−9] sequenced-block regulation is achieved along the polymer chain,[10] which provides a facile strategy to access materials with promising, block-sequence-dependent folding behaviors and biomimetic functions.[11−13] Given the ability to form stable secondary structures with a handful of amino acids as their building blocks, multiblock copolypeptides can potentially offer more versatile self-assembled structures than other type of block copolymers and adopt protein-like biological functions.[14−19]

The preparation of multiblock copolypeptides from the ring-opening polymerization (ROP) of *N*-carboxyanhydrides (NCAs), however, is limited to low block numbers, mainly due to the side reactions of amino propagating chain end that includes undesired polymerization with different mechanisms, amidation with side-chain esters, the reactions with NCA anions and *N*,*N*-dimethylformamide (DMF) solvent, and the chain transfer to NCAs.[20−26] These side reactions lead to dead chains, cyclic polypeptides, and homopolypeptide species, which are difficult to separate and contaminate the final multiblock copolypeptide materials.[27−29] Additionally, the repetitive additions of NCA monomers, especially for high block numbers, lead to accumulation of acidic/electrophilic impurities from NCA synthesis, which may inhibit the chain propagation and lead to more significant side reactions.[30] Previous preparation of multiblock copolypeptides used the metal catalysts and the low temperature technique to minimize the side reactions.[31−33] While these reports successfully produced well-defined multiblock copolypeptides, the obtained copolymers were still limited to low block numbers (usually less than 5) or required several days to complete the synthesis.

The acceleration of polymerization rate is a useful strategy to reduce the contribution of side reactions, which have been used to prepare well-defined polymers that are difficult to synthesize using polymerization with normal rate.[34,35] In particular, several accelerated ROP of NCAs were reported,[30,36−43] which were successfully applied to outpace water-induced side reactions.[30,39] Inspired by these results, we reasoned that an accelerated ROP of NCAs may render the chain propagations more favorable over various side reactions during the polypeptide growth, thereby minimizing the generation of undesired contaminates and producing well-defined multiblock copolypeptides.

We selected the water-in-oil (w/o) emulsion system containing amine-terminated poly(ethylene glycol)-*block*-poly(γ-benzyl-L-glutamate) (PEG−PBLG) macroinitiator (Figure

Received: October 10, 2019
Accepted: October 30, 2019

DOI: 10.1021/acsmacrolett.9b00784
ACS Macro Lett. 2019, 8, 1517−1521

1) to enable the fast polymerization kinetics.[30] The emulsion system was selected due to its ultrafast polymerization kinetics

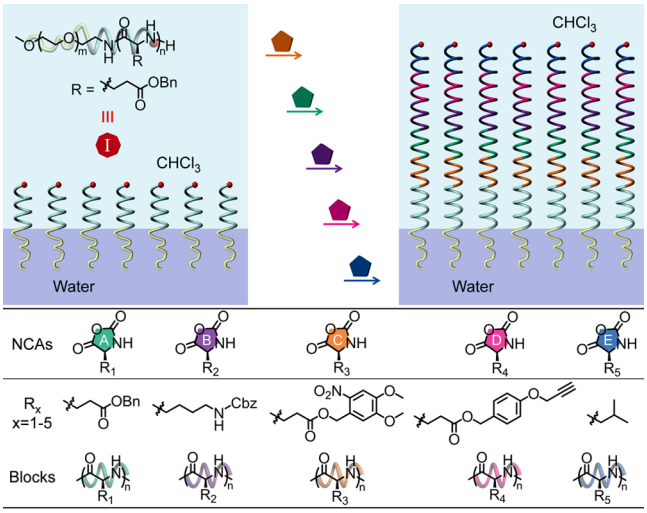


Figure 1. Scheme illustrating the synthesis of multiblock copolypeptides via rapid polymerization of NCAs.

and superior control over molecular weights (MWs). The rapid polymerization outpaces water-induced NCA degradations, allowing the preparation of well-defined polypeptide materials even in the presence of water. The added benefit for the emulsion system is the presence of aqueous phase that helps remove acidic and electrophilic impurities that may compete with the NCA to react with amine propagating groups and undesirably terminate the multistep polymerization. Herein, we report the successful preparation of multiblock, narrowly dispersed ($Đ$ < 1.1) copolypeptides with previously unattained high number of blocks (up to 20) and fast polymerization rates (on average <15 min/block), by taking advantage of the accelerated polymerization in w/o emulsion, which helps outpace different side reactions of propagating amino groups and ensures high end-group fidelity.

To demonstrate the importance of polymerization rate on end-group fidelity, we tested the polymerization of γ-benzyl-L-glutamate NCA (BLG-NCA) to synthesize a triblock copolypeptides in our w/o emulsion system versus in DMF, a typical solvent for NCA polymerization. The w/o emulsion containing PEG−PBLG macroinitiators (MW of PEG and PBLG block is 5 kDa and 9.5 kDa, respectively, Figure S1) was prepared by emulsifying a water/chloroform mixture, into which a chloroform solution of BLG-NCA ($[M]_0/[I]_0$ = 50 for each block) was sequentially added at the completion of synthesis of the previous block. NCA monomers were rapidly consumed, reaching >99% conversion within 15 min for all three blocks (Figure 2a, Figure S2a, and Table S1). In contrast, polymerization conducted in anhydrous DMF solution with PEG−PBLG macroinitiators was much slower, taking 8, 18, and 48 h to finish for the first, second, and third block, respectively (Figure 2a, Figure S2b, and Table S2). The increase in polymerization time was presumably due to the accumulation of acidic/electrophilic impurities after multiple additions of NCA monomers. During such elongated polymerization period in DMF, it is very likely that side reactions happen at the chain terminus that prevent the further growth of polypeptide chains. In order to quantify the end-group fidelity, terminal amines of the resulting polymers were labeled

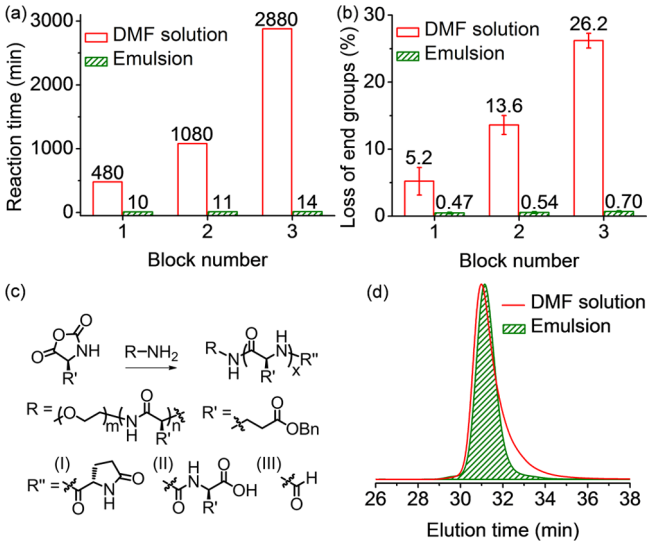


Figure 2. (a, b) Reaction time (a) and loss of end-group fidelity (b) during triblock copolypeptide synthesis in a w/o emulsion and an anhydrous DMF solution. (c) The formation of pyroglutamate (I), hydantoic acids (II), and *N*-formyl groups (III) leads to the loss of end-group fidelity. (d) Normalized GPC-LS traces of triblock copolypeptides synthesized in w/o emulsion and DMF solution. $[M]_0/[I]_0$ = 50 for each block, $[I]_0$ = 0.97 mM and 5.1 mM for the w/o emulsion and the DMF solution, respectively.

with 4-(dimethylamino)azobenzene-4′-isothiocyanate, an UV-active dye, and analyzed via UV−vis spectrometry.[44] The loss of the end groups for DMF polymerization was approximately (5.2 ± 2.1)%, (13.6 ± 1.4)%, and (26.2 ± 1.1)% for the first, second, and third block, respectively (Figure 2b), which was attributed to various side reactions that lead to the formation of pyroglutamates (I),[26] hydantoic acids (II),[20] and *N*-formyl groups (III)[24] (Figure 2c), which can no longer polymerize NCA monomers. The significantly elongated polymerization time and great loss of end groups for triblock copolypeptide synthesis in DMF make it difficult, if not impossible, to further extend the chains. On the contrary, more than 99% terminal amines were retained after triblock copolypeptide synthesis in the w/o emulsion system (Figure 2b), substantiating the importance of rapid polymerization on minimizing side reactions and maintaining high end-group fidelity. In addition, obvious low-MW tailing was observed on the gel permeation chromatography−light scattering (GPC-LS) trace of each intermediate block copolypeptides synthesized in DMF (Figures 2d and S3), further suggesting the existence of polymeric contaminates resulted from the side reactions. Due to the chain termination, the obtained MW in DMF was larger than the designed value, with obvious shifts observed for higher block numbers (Table S2).

The highly reserved end-group fidelity during the polymerization in the emulsion prompted us to further extend the system to the synthesis of multiblock copolypeptides with higher block numbers. A block length of 10 was selected for the initial polymerization studies, as shorter block lengths (<10) resulted in missing blocks during synthesis.[45] After repetitive, sequential addition of BLG-NCA at $[M]_0/[I]_0$ = 10 at an interval of 10−20 min, icosablock (i.e., 20-block) copolypeptides were synthesized with complete NCA conversion in each step and an isolated yield of 85% for the final polymer. The GPC analysis of the resulting purified icosablock

DOI: 10.1021/acsmacrolett.9b00784
ACS Macro Lett. 2019, 8, 1517−1521

copolypeptides revealed a narrow, monomodal peak with no obvious oligomeric species ($Đ$ = 1.07), indicating minimal loss of end groups and well-controlled polymerization process. The obtained number-averaged MW, M_n = 58.6 kDa, agrees perfectly with the designed MW (59.0 kDa).

In order to further understand the polymerization process, NMR was used to monitor the polymerization kinetics during the synthesis of icosablock copolypeptides (Figure 3a). The

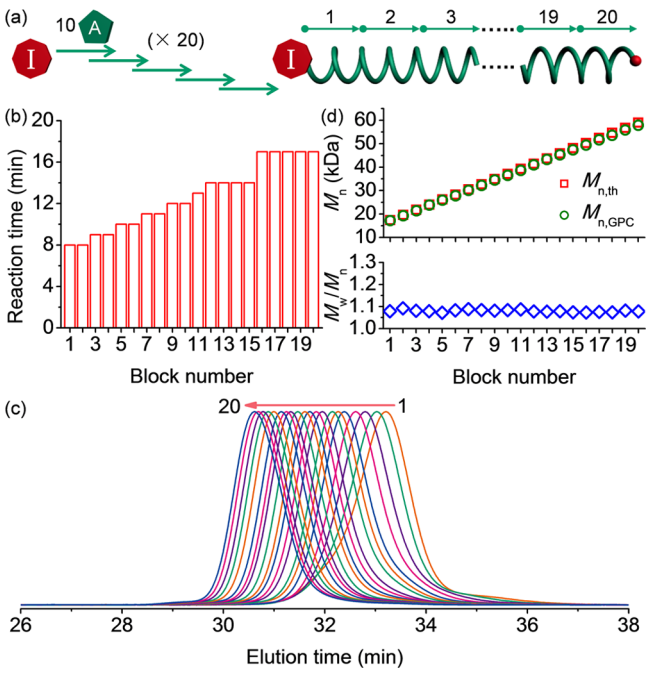


Figure 3. (a) Schematic illustration of the synthesis of icosablock copolypeptides through sequential addition of BLG NCA. (b) Reaction time for the synthesis of each block reaching >99% NCA conversion. (c) Normalized GPC-LS traces of intermediate copolypeptides after the synthesis of each block. (d) Comparison of the theoretical ($M_{n,th}$) and the experimental ($M_{n,GPC}$) MWs and the obtained dispersity ($Đ$ = M_w/M_n) of intermediate copolypeptides after the synthesis of each block. $[M]_0/[I]_0$ = 10 for each block, $[I]_0$ = 0.97 mM.

rapid decrease of α-H signals (4.34 to 4.41 ppm) from BLG-NCA and the increase of α-H signals (3.80 to 4.00 ppm) from resulting PBLG indicate a fast polymerization process (Figure S4), with over 99% NCA consumed in 8 min for the first block (Figure 3b). With the addition of NCA monomers into the polymerization mixture, the polymerization time increased slightly with higher block numbers (Table S3), which was attributed to the decrease in concentration of the propagating chains. Nevertheless, the synthesis of the later blocks was still very fast (<20 min; Figure 3b), enabling the completion of the icosablock copolypeptides synthesis within 5 h (Table S3 and Figure S5a). Analysis of the terminal amines at the completion of the icosablock copolypeptide synthesis revealed that more than 97% end group was retained, suggesting that copolypeptides with even higher block number are very likely attainable. The circular dichroism (CD) analysis revealed the retainment of α-helical conformation after the entire synthesis (Figure S5b), which is important to keep the fast polymerization kinetics throughout the polymerization. Furthermore, GPC-LS analysis after each chain extension revealed obvious peak shift toward higher MWs with negligible low-MW tailings (Figures 3c and S6). The obtained MWs agree well with the expected MWs (Figure 3d), which is consistent with the quantitative conversion of NCAs. The narrow dispersity observed for all intermediate copolypeptides ($Đ$ < 1.1) suggested the well-controlled polymerization throughout the entire polymerization progress (Figure 3d and Table S3).

We next explored the synthesis of the multiblock copolypeptides with NCA monomers bearing different side-chain functionalities. NCA monomers with strong α-helical propensity, including glutamate, lysine, leucine, and their functional derivatives, can be used for the synthesis of functional block copolypeptides. Decablock copolypeptides with ABABABABAB sequence were prepared by alternating, sequential addition of BLG-NCA and ε-carboxybenzyl-L-lysine NCA (ZLL-NCA; Figure 4a). The incorporation of ZLL-NCA

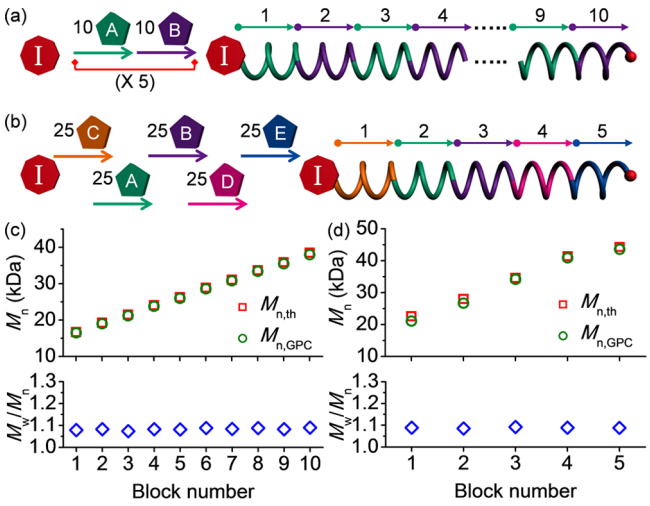


Figure 4. (a, b) Schematic illustration of the synthesis of AB type decablock copolypeptides (a) and ABCDE type pentablock copolypeptides (b). (c, d) Comparison of the theoretical ($M_{n,th}$) and the experimental ($M_{n,GPC}$) MWs and the obtained dispersity ($Đ$ = M_w/M_n) of intermediate copolypeptides after the synthesis of each block for decablock (c) and pentablock (d) copolypeptides. $[I]_0$ = 0.97 mM.

does not compromise the fast polymerization kinetics, which ensures the synthesis of well-defined decablock copolypeptides within 2 h with negligible side reactions (Figure S7 and Table S4). In addition, pentablock copolypeptides bearing five different side-chain functionalities, with a sequence of ABCDE, were synthesized using BLG-NCA, ZLL-NCA, γ-(4,5-dimethoxy-2-nitrobenzyl)-L-glutamate NCA (DMNB-NCA), γ-(4-propargyloxybenzyl)-L-glutamate NCA (POB-NCA), and L-leucine NCA (Leu-NCA; Figure 4b). The incorporation of photoresponsive and alkyne-functionalized side chains are useful for postpolymerization modifications of multiblock copolypeptides.[46−49] A longer block length was easily tuned by changing the feeding $[M]_0/[I]_0$ ratios to 25 for each block. Even with the increased block lengths, we were still able to finish the synthesis of pentablock copolypeptides in 1 h (Figure S8 and Table S5). Similar with the icosablock copolypeptide synthesis, the chain propagation after each addition of monomers was validated by GPC-LS with clear shift toward high MWs (Figure S9). The obtained MWs agree well with the designed MWs for both syntheses, with low dispersity ($Đ$ < 1.1) observed for all copolypeptide intermediates and final products (Figure 4c,d). The robustness

DOI: 10.1021/acsmacrolett.9b00784
ACS Macro Lett. 2019, 8, 1517−1521

Table 1. Synthesis of Multiblock Copolypeptides Using Five Different NCA Monomers[a]

entry	block sequence	A*/B*/C*/D*/E*[b] (%)	A/B/C/D/E[c] (%)	M_n/M_n*[d,e] (kDa)	$Đ$[e]	t_{total}[f] (min)
1	$B_{50}D_{50}C_{50}$	-/33/33/33/-	-/33/35/32/-	57.6/57.8	1.09	62
2	$C_{25}A_{25}B_{25}D_{25}E_{25}$	20/20/20/20/20	20/19/20/18/23	43.6/44.3	1.08	60
3	$A_{100}C_{100}B_{100}D_{100}$	25/25/25/25/-	26/24/24/26/-	132.5/122.6	1.10	192
4	$C_{10}A_{10}B_{10}D_{10}E_{10}$	20/20/20/20/20	21/19/19/19/22	23.5/26.4	1.08	42
5	$C_{10}B_{20}E_{30}D_{40}A_{50}$	33/13/7/27/20	34/12/7/24/23	47.7/48.6	1.09	81
6	$C_{10}A_{10}B_{10}D_{10}E_{10}A_{10}E_{10}A_{10}D_{10}B_{10}$	30/20/10/20/20	30/20/10/19/21	34.5/37.2	1.07	94
7	$C_{10}B_{20}E_{30}D_{40}A_{50}D_{40}E_{30}B_{20}C_{10}$	20/16/8/32/24	19/15/8/30/28	69.8/71.4	1.09	183

[a]Polymerizations were conducted at room temperature in a w/o emulsion initiated by interfacially anchored PEG−PBLG (water/chloroform = 1:50, w/w; pH = 7.0). A: BLG-NCA; B: ZLL-NCA; C: DMNB-NCA; D: POB-NCA; E: Leu-NCA. [b]The expected composition. [c]The obtained final composition by ^{1}H NMR analysis. [d]Obtained MW/designed MW*. [e]Determined by GPC. [f]Determined by FTIR.

and versatility of this strategy was further evidenced by the syntheses of several additional multiblock copolypeptides with variable block numbers (3−10 blocks), block lengths (10−100 units), and block sequences with five different NCAs (Table 1). All syntheses completed within 3.5 h with minimal loss of end-group fidelity. The final copolypeptides were characterized by GPC (Figure S10) and NMR (Figure S11), which revealed the obtained MW and composition as expected, respectively. The rapid and well-controlled polymerization of the NCAs with different side-chain structures during the synthesis of multiblock copolypeptides suggests that our strategy holds great potentials to be extended and applied to a richer variety of NCA monomers, which is important to prepare complex, highly functionalized multiblock copolypeptides.

In summary, we were able to prepare well-defined multiblock copolypeptides with unattained high number of blocks (up to 20) in a fast and reliable manner by sequential addition of NCA monomers into a w/o emulsion initiation system. The fast kinetics (<15 min/block synthesis) outpaces the side reactions and the chain terminations during NCA polymerization, resulting in minimal loss of the end-group fidelity. Multiblock copolypeptides with desired block sequences, predictable MWs, and low dispersity ($Đ$ < 1.1) were easily attainable from a variety of NCAs, demonstrating the robustness of the strategy. This work enables the synthesis of multiblock copolypeptides with versatile sequences, in particular with very high block numbers (>10 or 20) that are otherwise difficult or impossible to obtain, providing essentially an unlimited library of protein-mimetic polypeptide biomaterials.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmacrolett.9b00784.

Materials, experimental procedures, polymerization kinetics, and detailed multiblock copolypeptide analysis (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: jianjunc@illinois.edu.
*E-mail: zsong5@illinois.edu.
*E-mail: renjie6598@163.com.

ORCID

Ziyuan Song: 0000-0002-3165-3712
Jie Ren: 0000-0002-3744-1470
Jianjun Cheng: 0000-0003-2561-9291

Author Contributions

#These authors contributed equally.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

J.C. acknowledges the support of National Science Foundation (CHE-1709820) and partial support of National Institutes of Health (1R01CA207584). X.W., a visiting student from Tongji University, China, acknowledges the support from China Scholarship Council for his studies in Professor Jianjun Cheng's laboratory at UIUC.

■ REFERENCES

(1) Bates, F. S.; Hillmyer, M. A.; Lodge, T. P.; Bates, C. M.; Delaney, K. T.; Fredrickson, G. H. Multiblock polymers: panacea or pandora's box? *Science* **2012**, *336* (6080), 434−440.

(2) Zhang, Q.; Collins, J.; Anastasaki, A.; Wallis, R.; Mitchell, D. A.; Becer, C. R.; Haddleton, D. M. Sequence-controlled multi-block glycopolymers to inhibit DC-SIGN-gp120 binding. *Angew. Chem., Int. Ed.* **2013**, *52* (16), 4435−4439.

(3) Elacqua, E.; Manning, K. B.; Lye, D. S.; Pomarico, S. K.; Morgia, F.; Weck, M. Supramolecular multiblock copolymers featuring complex secondary structures. *J. Am. Chem. Soc.* **2017**, *139* (35), 12240−12250.

(4) Soeriyadi, A. H.; Boyer, C.; Nyström, F.; Zetterlund, P. B.; Whittaker, M. R. High-order multiblock copolymers via iterative Cu(0)-mediated radical polymerizations (SET-LRP): toward biological precision. *J. Am. Chem. Soc.* **2011**, *133* (29), 11128−11131.

(5) Gody, G.; Maschmeyer, T.; Zetterlund, P. B.; Perrier, S. Rapid and quantitative one-pot synthesis of sequence-controlled polymers by radical polymerization. *Nat. Commun.* **2013**, *4*, 2505.

(6) Chuang, Y.-M.; Ethirajan, A.; Junkers, T. Photoinduced sequence-controlled copper-mediated polymerization: synthesis of decablock copolymers. *ACS Macro Lett.* **2014**, *3* (8), 732−737.

(7) Engelis, N. G.; Anastasaki, A.; Nurumbetov, G.; Truong, N. P.; Nikolaou, V.; Shegiwal, A.; Whittaker, M. R.; Davis, T. P.; Haddleton, D. M. Sequence-controlled methacrylic multiblock copolymers via sulfur-free RAFT emulsion polymerization. *Nat. Chem.* **2017**, *9*, 171−178.

(8) Anastasaki, A.; Oschmann, B.; Willenbacher, J.; Melker, A.; Van Son, M. H. C.; Truong, N. P.; Schulze, M. W.; Discekici, E. H.; McGrath, A. J.; Davis, T. P.; Bates, C. M.; Hawker, C. J. One-pot synthesis of ABCDE multiblock copolymers with hydrophobic, hydrophilic, and semi-fluorinated segments. *Angew. Chem., Int. Ed.* **2017**, *56* (46), 14483−14487.

(9) Clothier, G. K. K.; Guimarães, T. R.; Khan, M.; Moad, G.; Perrier, S.; Zetterlund, P. B. Exploitation of the nanoreactor concept for efficient synthesis of multiblock copolymers via macroRAFT-mediated emulsion polymerization. *ACS Macro Lett.* **2019**, *8* (8), 989−995.

DOI: 10.1021/acsmacrolett.9b00784
ACS Macro Lett. 2019, 8, 1517−1521

(10) Lutz, J.-F.; Ouchi, M.; Liu, D. R.; Sawamoto, M. Sequence-controlled polymers. *Science* **2013**, *341* (6146), 1238149.
(11) Marcotte, E. M.; Pellegrini, M.; Ng, H.-L.; Rice, D. W.; Yeates, T. O.; Eisenberg, D. Detecting protein function and protein-protein interactions from genome sequences. *Science* **1999**, *285* (5428), 751−753.
(12) Dobson, C. M. Protein folding and misfolding. *Nature* **2003**, *426* (6968), 884−890.
(13) Judzewitsch, P. R.; Nguyen, T.-K.; Shanmugam, S.; Wong, E. H. H.; Boyer, C. Towards sequence-controlled antimicrobial polymers: effect of polymer block order on antimicrobial activity. *Angew. Chem., Int. Ed.* **2018**, *57* (17), 4559−4564.
(14) Carlsen, A.; Lecommandoux, S. Self-assembly of polypeptide-based block copolymer amphiphiles. *Curr. Opin. Colloid Interface Sci.* **2009**, *14* (5), 329−339.
(15) Fan, J.; Zou, J.; He, X.; Zhang, F.; Zhang, S.; Raymond, J. E.; Wooley, K. L. Tunable mechano-responsive organogels by ring-opening copolymerizations of *N*-carboxyanhydrides. *Chem. Sci.* **2014**, *5* (1), 141−150.
(16) Song, Z.; Fu, H.; Wang, R.; Pacheco, L. A.; Wang, X.; Lin, Y.; Cheng, J. Secondary structures in synthetic polypeptides from *N*-carboxyanhydrides: design, modulation, association, and material applications. *Chem. Soc. Rev.* **2018**, *47* (19), 7401−7425.
(17) Shen, W.; He, P.; Xiao, C.; Chen, X. From antimicrobial peptides to antimicrobial poly(α-amino acid)s. *Adv. Healthcare Mater.* **2018**, *7* (20), 1800354.
(18) Hou, Y.; Zhou, Y.; Wang, H.; Sun, J.; Wang, R.; Sheng, K.; Yuan, J.; Hu, Y.; Chao, Y.; Liu, Z.; Lu, H. Therapeutic protein PEPylation: the helix of nonfouling synthetic polypeptides minimizes antidrug antibody generation. *ACS Cent. Sci.* **2019**, *5* (2), 229−236.
(19) Sun, Y.; Deming, T. J. Self-healing multiblock copolypeptide hydrogels via polyion complexation. *ACS Macro Lett.* **2019**, *8* (5), 553−557.
(20) Sela, M.; Berger, A. The mechanism of polymerization of *N*-carboxy-α-amino acid anhydrides. *J. Am. Chem. Soc.* **1953**, *75* (24), 6350−6351.
(21) Lundberg, R. D.; Doty, P. Polypeptides. XVII. A study of the kinetics of the primary amine-initiated polymerization of *N*-carboxy-anhydrides with special reference to configurational and stereo-chemical effects. *J. Am. Chem. Soc.* **1957**, *79* (15), 3961−3972.
(22) Kricheldorf, H. R. *α-Aminoacid-N-carboxy-anhydrides and Related Heterocycles: Syntheses, Properties, Peptide Synthesis, Polymerization*; Springer-Verlag, 1987.
(23) Aliferis, T.; Iatrou, H.; Hadjichristidis, N. Living polypeptides. *Biomacromolecules* **2004**, *5* (5), 1653−1656.
(24) Vayaboury, W.; Giani, O.; Cottet, H.; Deratani, A.; Schué, F. Living polymerization of α-amino acid *N*-carboxyanhydrides (NCA) upon decreasing the reaction temperature. *Macromol. Rapid Commun.* **2004**, *25* (13), 1221−1224.
(25) Hadjichristidis, N.; Iatrou, H.; Pitsikalis, M.; Sakellariou, G. Synthesis of well-defined polypeptide-based materials via the ring-opening polymerization of α-amino acid *N*-carboxyanhydrides. *Chem. Rev.* **2009**, *109* (11), 5528−5578.
(26) Habraken, G. J. M.; Peeters, M.; Dietz, C. H. J. T.; Koning, C. E.; Heise, A. How controlled and versatile is *N*-carboxy anhydride (NCA) polymerization at 0 °C? Effect of temperature on homo-, block- and graft (co)polymerization. *Polym. Chem.* **2010**, *1* (4), 514−524.
(27) Kricheldorf, H. R.; v. Lossow, C.; Schwarz, G. Primary amine and solvent-induced polymerizations of L- or D,L-phenylalanine *N*-carboxyanhydride. *Macromol. Chem. Phys.* **2005**, *206* (2), 282−290.
(28) Kricheldorf, H. R.; von Lossow, C.; Schwarz, G. Cyclic polypeptides by solvent-induced polymerizations of α-amino acid *N*-carboxyanhydrides. *Macromolecules* **2005**, *38* (13), 5513−5518.
(29) Deming, T. J. Polypeptide and polypeptide hybrid copolymer synthesis via NCA polymerization. In *Peptide Hybrid Polymers*; Klok, H.-A., Schlaad, H., Eds.; Springer: Berlin, Heidelberg, 2006; pp 1−18.
(30) Song, Z.; Fu, H.; Wang, J.; Hui, J.; Xue, T.; Pacheco, L. A.; Yan, H.; Baumgartner, R.; Wang, Z.; Xia, Y.; Wang, X.; Yin, L.; Chen, C.; Rodríguez-López, J.; Ferguson, A. L.; Lin, Y.; Cheng, J. Synthesis of polypeptides via bioinspired polymerization of in situ purified *N*-carboxyanhydrides. *Proc. Natl. Acad. Sci. U. S. A.* **2019**, *116* (22), 10658−10663.
(31) Li, Z.; Deming, T. J. Tunable hydrogel morphology via self-assembly of amphiphilic pentablock copolypeptides. *Soft Matter* **2010**, *6* (11), 2546−2551.
(32) Habraken, G. J. M.; Wilsens, K. H. R. M.; Koning, C. E.; Heise, A. Optimization of *N*-carboxyanhydride (NCA) polymerization by variation of reaction temperature and pressure. *Polym. Chem.* **2011**, *2* (6), 1322−1330.
(33) Lavilla, C.; Byrne, M.; Heise, A. Block-sequence-specific polypeptides from α-amino acid *N*-carboxyanhydrides: synthesis and influence on polypeptide properties. *Macromolecules* **2016**, *49* (8), 2942−2947.
(34) Yonezumi, M.; Takaku, R.; Kanaoka, S.; Aoshima, S. Living cationic polymerization of α-methyl vinyl ethers using $SnCl_4$. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46* (6), 2202−2211.
(35) Fantin, M.; Isse, A. A.; Venzo, A.; Gennaro, A.; Matyjaszewski, K. Atom transfer radical polymerization of methacrylic acid: a won challenge. *J. Am. Chem. Soc.* **2016**, *138* (23), 7216−7219.
(36) Zou, J.; Fan, J.; He, X.; Zhang, S.; Wang, H.; Wooley, K. L. A facile glovebox-free strategy to significantly accelerate the syntheses of well-defined polypeptides by *N*-carboxyanhydride (NCA) ring-opening polymerizations. *Macromolecules* **2013**, *46* (10), 4223−4226.
(37) Zhao, W.; Gnanou, Y.; Hadjichristidis, N. From competition to cooperation: a highly efficient strategy towards well-defined (co)-polypeptides. *Chem. Commun.* **2015**, *51* (17), 3663−3666.
(38) Baumgartner, R.; Fu, H.; Song, Z.; Lin, Y.; Cheng, J. Cooperative polymerization of α-helices induced by macromolecular architecture. *Nat. Chem.* **2017**, *9*, 614−622.
(39) Wu, Y.; Zhang, D.; Ma, P.; Zhou, R.; Hua, L.; Liu, R. Lithium hexamethyldisilazide initiated superfast ring opening polymerization of alpha-amino acid *N*-carboxyanhydrides. *Nat. Commun.* **2018**, *9* (1), 5297.
(40) Zhang, Y.; Liu, R.; Jin, H.; Song, W.; Augustine, R.; Kim, I. Straightforward access to linear and cyclic polypeptides. *Commun. Chem.* **2018**, *1* (1), 40.
(41) Yuan, J.; Zhang, Y.; Li, Z.; Wang, Y.; Lu, H. A S-Sn Lewis pair-mediated ring-opening polymerization of α-amino acid *N*-carboxyanhydrides: fast kinetics, high molecular weight, and facile bioconjugation. *ACS Macro Lett.* **2018**, *7* (8), 892−897.
(42) Chen, C.; Fu, H.; Baumgartner, R.; Song, Z.; Lin, Y.; Cheng, J. Proximity-induced cooperative polymerization in "hinged" helical polypeptides. *J. Am. Chem. Soc.* **2019**, *141*, 8680−8683.
(43) Zhao, W.; Lv, Y.; Li, J.; Feng, Z.; Ni, Y.; Hadjichristidis, N. Fast and selective organocatalytic ring-opening polymerization by fluorinated alcohol without a cocatalyst. *Nat. Commun.* **2019**, *10* (1), 3590.
(44) Chang, J. Y. Amino-terminal analysis of polypeptide using dimethylaminoazobenzene isothiocyanate. *Anal. Biochem.* **1980**, *102* (2), 384−392.
(45) Gody, G.; Zetterlund, P. B.; Perrier, S.; Harrisson, S. The limits of precision monomer placement in chain growth polymerization. *Nat. Commun.* **2016**, *7*, 10514.
(46) Deming, T. J. Synthesis of side-chain modified polypeptides. *Chem. Rev.* **2016**, *116* (3), 786−808.
(47) Yin, L.; Tang, H.; Kim, K. H.; Zheng, N.; Song, Z.; Gabrielson, N. P.; Lu, H.; Cheng, J. Light-responsive helical polypeptides capable of reducing toxicity and unpacking DNA: toward nonviral gene delivery. *Angew. Chem., Int. Ed.* **2013**, *52* (35), 9182−9186.
(48) Zhang, R.; Zheng, N.; Song, Z.; Yin, L.; Cheng, J. The effect of side-chain functionality and hydrophobicity on the gene delivery capabilities of cationic helical polypeptides. *Biomaterials* **2014**, *35* (10), 3443−3454.
(49) Song, Z.; Kim, H.; Ba, X.; Baumgartner, R.; Lee, J. S.; Tang, H.; Leal, C.; Cheng, J. Polypeptide vesicles with densely packed multilayer membranes. *Soft Matter* **2015**, *11* (20), 4091−4098.

DOI: 10.1021/acsmacrolett.9b00784
ACS Macro Lett. 2019, 8, 1517−1521