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Article

One-Shot Synthesis of Peptide Amphiphiles with Applications in Directed Graphenic Assembly

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Cite This: *Biomacromolecules* 2020, 21, 3878−3886

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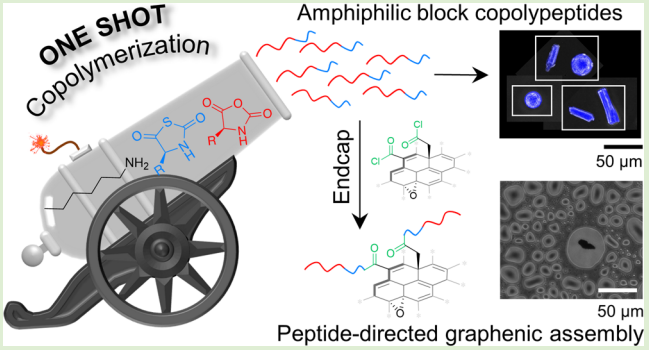
ABSTRACT: High molecular weight, synthetic block copolypeptides that self-assemble are in high demand for biomedical applications. The current standard method for synthesis of block copolypeptides is the controlled ring-opening polymerization (ROP) of α-amino acid N-carboxyanhydride (NCA) monomers, where block architectures can be created by sequential NCA monomer addition. Recently, researchers have focused on developing reaction conditions and initiation systems that make NCA ROP more convenient, particularly for interdisciplinary labs without designated polypeptide facilities. In an effort to further simplify and increase the convenience of polypeptide synthesis, we developed a one-shot copolymerization strategy that allows access to block copolypeptides by capitalizing on the inherently faster reactivity of NCA monomers, compared to NTA (N-*thio*carboxyanhydride) monomers. For the first time, we combine an NCA and NTA monomer in one reaction to kinetically promote block copolypeptide formation, providing a convenient alternative to sequential monomer addition. The controlled nature of this copolymerization technique is supported by a molecular weight that is modulated by the concentration of the initiator and low dispersities. We used this one-shot copolymerization to synthesize p(lysine)-*b*-p(leucine), a known peptide amphiphile (PA). Our one-shot PAs are antimicrobial and can spontaneously form ordered, micron-scale assemblies. Covalent conjugation of one-shot PAs to a graphenic backbone results in a functional graphenic material (FGM) with a self-assembled morphology, paving the way for creation of sophisticated FGM scaffolds with polypeptide-templated, hierarchical order. Overall, we demonstrate that this novel, one-shot copolymerization strategy produces functional copolypeptides with macroscopic sequence control.



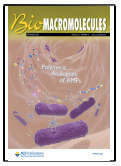
INTRODUCTION

Protein structures dictate function: the complex and diverse architectures that biological proteins adopt allow them to play many roles in the body, such as catalysis, protection, structural support, transport, and storage.[1−3] The inherent structure−function relationship of biological proteins can be recapitulated in the laboratory with biomimetic, synthetic copolypeptides. Numerous reviews summarize how synthetic copolypeptides can be harnessed in biomedical applications such as tissue engineering, gene and drug delivery, and antimicrobial treatments.[4−8] The broad applicability of synthetic copolypeptides has attracted the attention of scientific researchers in the interdisciplinary field of biomaterials.

The standard method to synthesize polypeptides is the ring-opening polymerization (ROP) of cyclic α-amino acid N-carboxyanhydride (NCA) monomers. Using this technique, block copolypeptides can be accessed through sequential NCA monomer addition. Deming successfully developed the first living polymerization of NCAs using organometallic catalysts, marking a significant turning point in the field of polypeptide chemistry.[9−11] This development enabled the synthesis of homo- and block-copolypeptides with unprecedented control over the molecular weight and impressive homogeneity, which are important characteristics for assembly applications. However, this living NCA ROP is initiated by a water- and air-sensitive organometallic catalyst, which is a drawback among interdisciplinary labs without a designated glovebox.

To address this, a plethora of new synthetic methodologies for rapid, controlled NCA ROP have been developed, providing researchers with a wider variety of accessible techniques to synthesize polypeptide biomaterials.[12−14] Primary amine initiation of NCA ROP is a convenient alternative to organometallic initiation because primary amine initiators are readily available and do not require rigorous, air-free reaction conditions. However, amine-initiated NCA ROP is subject to chain-end termination and side

Received: June 22, 2020
Revised: July 10, 2020
Published: July 20, 2020



https://dx.doi.org/10.1021/acs.biomac.0c00962
Biomacromolecules 2020, 21, 3878−3886

reactions that outcompete the rate of propagation, which limits its utility in block copolypeptide synthesis.[15−17] To overcome this limitation, co-catalysts,[18,19] specially designed initiator systems,[20−25] and manipulation of polymerization conditions (N_2 flow,[26] low temperature,[27] low pressure,[28,29] low dielectric constant solvent,[30] and water-in-oil emulsion[31,32]) have been employed to control NCA ROP from a primary amine initiator. Many of these systems have been used to produce high molecular weight polypeptides with macroscopic sequence control in a glovebox-free environment. Despite numerous advancements in the field, opportunities to simplify existing polymerization techniques and increase the convenience of polypeptide synthesis still exist.[33] In this work, we contribute to the continuously growing body of the literature with the first demonstration of kinetically promoted block copolypeptide formation.

The status quo for the synthesis of block copolypeptides via NCA ROP is through sequential addition of NCA monomers. Using this method, block copolypeptides can be designed to combine different secondary structures[9] that participate in hierarchical self-assembly.[34] For example, peptide amphiphiles (PAs), which combine a hydrophilic block with a hydrophobic block,[35] can form vesicle,[36] micelle,[37] and hydrogel[38−40] assemblies that can be harnessed for biomedical applications such as drug delivery.

A more convenient alternative to access block copolymers is by using a pair of monomers with drastically different reactivities to kinetically promote block formation in one shot.[41−45] However, to date, no comonomer pairs have been identified that enable one-shot block formation for copolypeptides.

We developed a one-shot copolymerization strategy to produce block copolypeptides that, for the first time, capitalizes on the faster reactivity of NCA monomers, compared to NTA monomers. α-Amino acid N-thiocarboxyanhydride (NTA) monomers react more slowly than NCA monomers[46] and have been used to synthesize homopeptides and block copolypeptoids[47] with a controlled molecular weight and modest dispersity.[48−50] Although NTAs are generally more expensive and take longer to produce, compared to NCAs, their moisture and thermal stability results in a long shelf life, making them desirable for labs that lack inert-atmosphere storage facilities.[48] As an added benefit, our polymerization strategy requires only a Schlenk line, making it widely accessible to interdisciplinary labs. As such, this strategy allows block copolypeptides to be accessed with unprecedented convenience.

By monitoring reaction kinetics of a copolymerization between Lys(Z)-NCA and Leu-NTA, we demonstrate that the NCA/NTA comonomer pair forms block copolypeptides with a compositional drift. At the beginning of the copolymerization, Lys(Z)-NCA is consumed much faster than Leu-NTA, forming a block that is rich in p(Lys-Z); then, following consumption of Lys(Z)-NCA, the remaining Leu-NTA propagates to form a discrete block of p(Leu). The controlled nature of this copolymerization technique is supported by dispersities between 1.23−1.3 and molecular weight that is modulated by the concentration of the initiator.

To demonstrate the capacity of our synthetic technique to generate functional copolypeptides, we used this one-shot copolymerization strategy to generate p(Lys)-*b*-p(Leu) copolypeptides that exhibit useful properties. These copolypeptides exhibit dose-dependent antimicrobial activity against gram-negative bacteria, *E. coli*. Furthermore, because p(Lys)-*b*-p(Leu) is a peptide amphiphile (PA), our one-shot block copolypeptides can spontaneously form ordered, micron-scale assemblies.

This assembly behavior was harnessed to template the assembly of a graphenic conjugate structure. Using a polypeptide end-capping technique developed by our group,[51] we demonstrate that covalent conjugation of the one-shot PAs to a graphenic backbone results in a functional graphenic material (FGM) with a self-assembled morphology. Due to their unique cell adhesion properties, electrical conductivity, and bioactive functionalization capacity, FGMs are good candidates for regenerative healing therapies.[52] Our work shows that polypeptides can template graphenic assemblies. This paves the way for creation of sophisticated FGM scaffolds with polypeptide-promoted, hierarchical order.

METHODS

Copolypeptide Synthesis and Deprotection. In a typical copolymerization, Lys(Z)-NCA and Leu-NTA were added to a flame-dried round bottom flask and vacuum-backfilled thrice with N_2. While maintaining an inert atmosphere, the monomers were dissolved in dry dimethylformamide (DMF), and the copolymerization was initiated with hexylamine from a stock solution in dry DMF (concentration quantitatively determined by ^{1}H-NMR using recrystallized benzoic acid as an internal standard). The initial monomer concentration (NCA + NTA) was maintained at 0.45 M for all copolymerizations. The copolymerization was stirred under N_2; or, to accelerate the polymerization, the reaction vessel may be put under light vacuum (approximately 300 mbar). After stirring the copolymerization until all monomer was consumed (as observed by ^{1}H-NMR), the copolypeptide was precipitated into ethyl ether at 0 °C, vortexed, and centrifuged at 2420 × g (Z 366, HERMLE Labortechnik GmbH, Wehingen, Germany) for 10 min to pellet a white solid (99% yield). The copolypeptide was then dried under vacuum.

In a typical deprotection, the copolypeptide (95.9 mg) was dissolved in TFA (∼1 mL). HBr (∼1.4 mL) was added to the solution. After stirring overnight, the solution was precipitated dropwise into ice cold THF, vortexed, and centrifuged at 2420 × g for 10 min to give a white solid with a bright yellow supernatant. The deprotected copolypeptide was dried under vacuum.

^{1}H-NMR Kinetics Experiments. *Experimental Setup.* In a typical kinetics experiment, Lys(Z)-NCA and Leu-NTA were added as solids to an oven-dried round bottom flask and vacuum back-filled thrice with N_2. The comonomers were then dissolved in dry DMF. A T_0 aliquot (0.1 mL) was removed from the solution prior to initiation. Then, while stirring under N_2, hexylamine was added from a stock solution in dry DMF. The total volume of neat DMF (added to dissolve the comonomers) and hexylamine initiator solution was such that the total monomer (NCA + NTA) concentration was 0.45 M at the start of the copolymerization. Using an air-free technique, aliquots (0.1 mL) were removed from the reaction at various time points throughout the copolymerization.

Data Acquisition. After extracting an aliquot (0.1 mL) from the propagating copolymerization, the aliquot was immediately cut with 0.6 mL of dimethylsulfoxide-d_6 (DMSO-d_6) and analyzed by ^{1}H-NMR (500 MHz, 1 scan). The concentration of each monomer in the reaction aliquot is proportional to the absolute integral of the proton in the NCA or NTA ring (Figure S1). To allow comparison across different kinetic time points, the absolute integration of each monomer peak was first divided by the absolute integration of the solvent peak (DMF, 7.860−8.000 ppm) to account for slight variations in the volume of the reaction aliquot. Then, the concentration of the NCA and NTA at each time point was calculated using a proportion to the normalized monomer integrations from the T_0 time point.

Determination of Kinetic Parameters. Apparent rate constants for the Lys(Z)-NCA and Leu-NTA comonomers were calculated using the linear regression of first-order, semilogarithmic plots of monomer

https://dx.doi.org/10.1021/acs.biomac.0c00962
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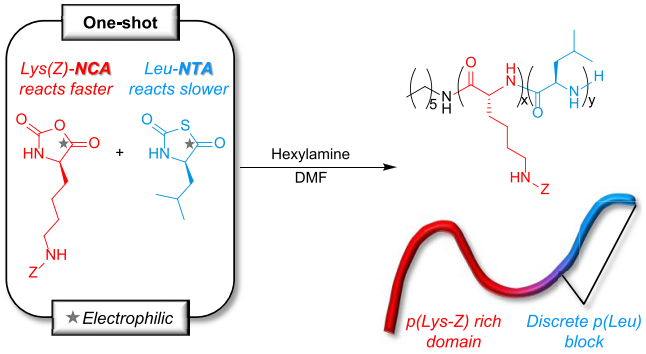
consumption over time (Figure S2). Reactivity ratios of the comonomers were calculated using a Mayo–Lewis nonlinear fitting procedure (Figure S3).[53] Details and calculations can be found in the Supporting Information.

RESULTS AND DISCUSSION

Design of the One-Shot Copolymerization Strategy. Here, we outline the design considerations of our one-shot copolymerization strategy based on three main polymerization parameters: (1) identity of the electrophile in the α-amino acid monomer, (2) the type of initiator, and (3) the identity of the amino acid side chain in each comonomer.

The reactivity difference between NCA and NTA monomers is due to their differing electrophilicity. The electrophilicity of the anhydride in these cyclic monomers is dictated by the electronegativity of the central heteroatom: an oxygen in the NCA and a sulfur in the NTA. Oxygen is more electronegative than sulfur;[54] thus, the NCA anhydride is more electrophilic and more reactive than the NTA anhydride (Scheme 1). We leveraged this inherent reactivity difference to develop the first amino acid-based comonomer pair that would enable a convenient, one-shot synthesis of block copolypeptides.

Scheme 1. Differing electrophilicities of α-amino acid N-carboxyanhydride (NCA) and N-thiocarboxyanhydride (NTA) monomers in their respective anhydride allow the NCA/NTA comonomer pair to be polymerized in one shot to give a copolypeptide with a domain that is rich in the NCA amino acid followed by a discrete block of the NTA amino acid



We developed our one-shot block copolypeptide synthesis with a convenient primary amine initiator for its numerous advantages. First, primary amine initiators are readily available and often inexpensive.[55] Furthermore, primary amine initiators are tolerant to ambient conditions, unlike metal[9,11,34] and silyl[56,57] initiators, which require rigorous air- and water-free conditions for synthesis. In fact, many recent developments in the field of NCA ROP have focused on initiation from a primary amine using conditions that enable a controlled polymerization without a glovebox.[25,26,31]

The identity of the amino acid side chain in each comonomer (NCA and NTA, respectively) will dictate the macroscopic amino acid sequence of the resulting copolypeptide and thus the functionality of the material. We chose to study Lys(Z)-NCA and Leu-NTA, as this comonomer system results in a known PA, p(Lys)-*b*-p(Leu). This block copolypeptide has been synthesized using conventional sequential addition methods[35,58] and is known to exhibit antimicrobial capacity[59,60] and form interesting intermolecular assemblies.[38,61−63] Related research has found that blocklike, tapered copolymers synthesized by a one-shot method possess the same macroscopic order as copolymers synthesized by sequential monomer addition,[44] suggesting that our new copolymerization strategy could allow facile access to functional PAs in one shot.

Copolymerization Kinetics Demonstrate Block Formation. Consumption of Lys(Z)-NCA and Leu-NTA was monitored over the course of the copolymerization using ^{1}H-NMR. When both Lys(Z)-NCA and Leu-NTA are present at the beginning of the reaction (Phase I), Lys(Z)-NCA is consumed at a faster rate than Leu-NTA, resulting in a domain of the copolypeptide that is rich in p(Lys-Z) (Figure 1A). Following the complete consumption of the Lys(Z)-NCA (Phase II), the remaining Leu-NTA is consumed, forming a discrete block of p(Leu) (Figure 1A). Mayo–Lewis nonlinear fitting provided reactivity ratios of 4.38 for Lys(Z)-NCA and 0.71 for Leu-NTA (Figure 1B, Figure S3), which indicates block formation in two ways. First, comonomer pairs where $r_1 \times r_2 > 1$ typically give rise to block copolymers[41] or copolymers with a compositional drift. For the Lys(Z)-NCA and Leu-NTA comonomer pair, $r_{NCA} \times r_{NTA} = 3.11$, demonstrating block copolypeptide formation. Second, Lys(Z)-NCA and Leu-NTA favor homo- and cross-propagation, respectively, which suggests preferential block formation. The reactivity ratio of

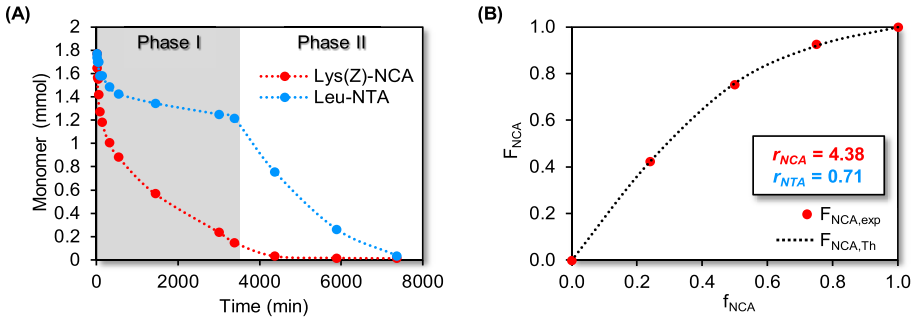


Figure 1. (A) Consumption of Lys(Z)-NCA and Leu-NTA comonomers was monitored via ^{1}H-NMR (500 MHz, DMSO-d_6), where $[M]_o/[I]$ = 70. In Phase I of the copolymerization, when both the NCA and NTA comonomers are present, Lys(Z)-NCA is preferentially added to the growing copolypeptide chain. In Phase II of the polymerization, when only the NTA is present, Leu-NTA is added to the copolypeptide chain to form a discrete block of p(Leu). (B) Mayo–Lewis plot of copolymer composition at 10% total monomer conversion (F_{NCA}) versus initial monomer feed ratio (f_{NCA}) allows determination of reactivity ratios for the NCA and NTA comonomers, which suggest block formation in the resulting copolypeptide.

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Biomacromolecules 2020, 21, 3878−3886

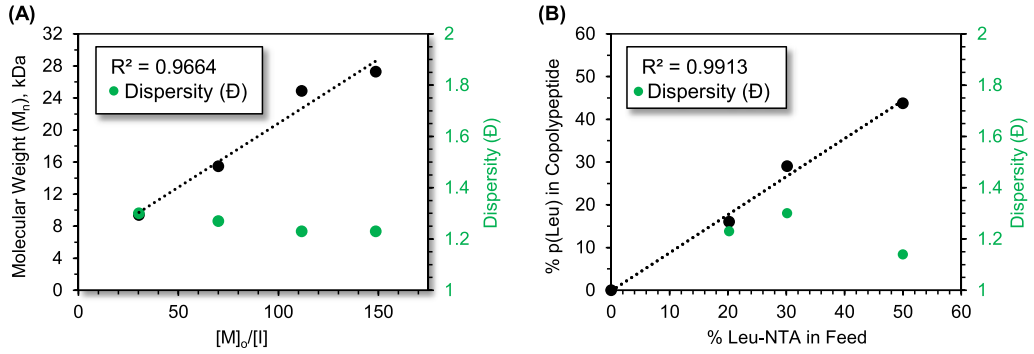

Figure 2. (A) Control of molecular weight (M_n) is demonstrated through variation of the monomer-to-initiator ratio ($[M]_o/[I]$). Comonomer feed ratios were held constant (20% Leu-NTA) to control for the difference in the molecular weight of Lys(Z) and Leu residues. M_n and dispersity (Đ) were determined by GPC (Figure S10). (B) Copolypeptide composition (ratio of lysine-to-leucine residues) is controlled by varying the comonomer feed ratio. Here, copolypeptides were synthesized with varying NCA/NTA feed ratios and constant $[M]_o/[I] = 150$. The percent p(Leu) in the copolypeptide was calculated by ^{1}H-NMR (Figure S4).

Lys(Z)-NCA is greater than one, meaning that homopropagation is favored. In other words, when a unit of Lys(Z)-NCA is added to the copolypeptide chain, successive addition of a Lys(Z)-NCA unit is favored over addition of a Leu-NTA unit. Conversely, the reactivity ratio of Leu-NTA is less than one, meaning that cross-propagation is preferred. This means that if a Leu-NTA unit is added to the copolypeptide chain in Phase I of the copolymerization, subsequent monomer addition will likely be Lys(Z)-NCA rather than another Leu-NTA. This homo- and heteropropagation relationship explains why Lys(Z)-NCA is depleted faster than Leu-NTA when both comonomers are present, no matter what the feed ratio is; yet, when only Leu-NTA remains in the reaction (Phase II), propagation of this monomer is favorable.

Copolymerization Is Controlled. We have demonstrated overall control of the copolymerization through molecular weight and copolypeptide composition studies. Molecular weight is controlled by the monomer-to-initiator ratio ($[M]_o/[I]$) with a strong linear trend ($R^2 = 0.9664$) up to a degree of polymerization of 150 (Figure 2A). Copolypeptide composition is reliably controlled by the comonomer feed ratio with a strong linear trend between targeted and actual percent p(Lys-Z) in the block copolypeptide ($R^2 = 0.9913$) (Figure 2B). The $[M]_o/[I]$ and the comonomer feed ratio dictate the length of the discrete p(Leu) block at the N-terminus of each copolypeptide. The length of the p(Leu) block can be calculated using a comonomer conversion versus time plot (Figure S5, Table S1). Generally, as $[M]_o/[I]$ and the feed ratio of Leu-NTA decrease, the length of the discrete p(Leu) block decreases.

This copolymerization strategy exhibits a lack of homopeptide impurities, demonstrated by the unimodal molecular weight distribution given by gel permeation chromatography (GPC) (Figure S10). Qualitatively, a lack of p(Leu) homopeptides is suggested by the solubility of the copolymerization solution throughout the course of the reaction. Homopeptides of p(Leu) are insoluble in the reaction solvent, DMF; therefore, continued solubility and lack of turbidity as our copolymerizations progressed indicate the absence of p(Leu) homopeptide impurities.

One-Shot PAs Form Ordered Assemblies. We chose to investigate the morphology of our blocklike copolypeptides to determine if assembly was possible given the gradient sequence within the nondiscrete p(Lys) domain. Generally, p(Lys)-*b*-p(Leu) PAs assemble in water due to intermolecular interactions between the hydrophobic p(Leu) blocks, which favor aggregation rather than contact with the hydrophilic solvent.[38,62] Because the p(Leu) block drives assembly and our one-shot block copolypeptides possess a discrete p(Leu) block, we hypothesized that our copolypeptides would be able to form ordered assemblies. This ability to self-assemble reinforces the utility of our synthetic technique.

The morphology of the PA assembly is influenced by the length of the discrete p(Leu) block, which exhibits more α-helix content with a higher degree of polymerization (DP).[64] Longer p(Leu) blocks have a greater α-helix content, which allows them to pack together into a highly anisotropic membrane that either forms a stiff sheet or curves into a spherical vesicle; meanwhile, shorter p(Leu) blocks, which exhibit more random coil content, lack an ordered packing preference and form micelles or irregular aggregates.[65] These self-assembly trends allow us to explain and predict the type of assembly formed by the one-shot PAs based on the length of their discrete p(Leu) block.

For the one-shot PAs reported herein, the length of the discrete p(Leu) block can be tuned from 5 to 34 units by varying the $[M]_o/[I]$ and comonomer feed ratio. This tunability allows us to access different types of assembly. When a low molecular weight ($[M]_o/[I] = 70$) and low feed ratio of Leu-NTA (21%) is targeted, the discrete p(Leu) block is DP 5 (Table 1). This p(Leu) block is too short to adopt an α-helix conformation, resulting in the formation of disordered aggregates (Figure 3). Increasing the molecular weight of the PA to $[M]_o/[I] = 149$ while retaining the low Leu-NTA feed (20%) results in a discrete p(Leu) block of DP 12 (Table 1). At DP 12, the p(Leu) block is still too short to adopt an α-helix conformation, but it is long enough to drive ordered, macroscopic assembly.[64,65] As such, the flexible, random coil p(Leu) block drives this PA to assemble into micelles (Figure 3).

Increasing the comonomer feed of Leu-NTA allows us to further increase the length of the discrete p(Leu) block within the PA, which changes the type of self-assembly. When the length of the p(Leu) block exedes DP 15, the α-helical content begins to dominate, resulting in a rigid hydrophobic domain.[64,65] This rigidity, coupled with the anisotropic packing of the α-helices, drives assembly into either vesicle or sheet structures (Figure 3).[65] This is observed in PAs with 50% Leu-NTA feed, where the length of the discrete p(Leu) block is

https://dx.doi.org/10.1021/acs.biomac.0c00962
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Table 1. Summary of Structural and Assembly Characterization of One-Shot PAs[a]

$[M]_o/[I]$	M_n (kDa)	Đ	% p(Leu)	DP of discrete p(Leu) block	assembly
70	15.47	1.27	21	5	Irregular aggregates
149	27.29	1.23	20	12	Circular (micelle)
68	15.30	1.34	50	15	Sheet
153	18.27	1.14	50	34	Circular (vesicle), sheet

[a]Molecular Weight (Mn) and dispersity (Đ) of protected p(Lys-Z)-*b*-p(Leu) copolypeptides were determined using gel permeation chromatography (GPC) with PMMA standards. The percent (%) p(Leu) was determined using ^{1}H-NMR of the final copolypeptide. The degree of polymerization (DP) of the discrete p(Leu) block was calculated using representative ^{1}H-NMR kinetics experiments (Figure S5), as described in the Supporting Information. The assembly of the corresponding deprotected PAs was evaluated by microscopy as 1 wt % solutions in 99% DI H_2O, 1% TFA. Representative microscopy images are shown in Figure 3.

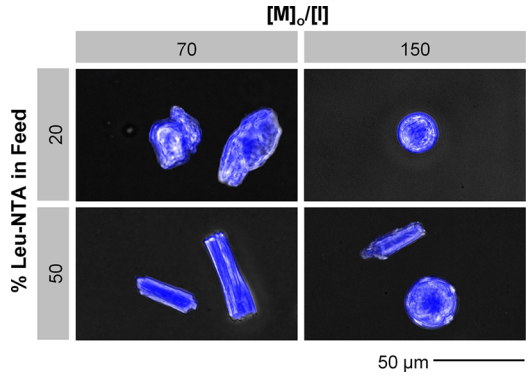

Figure 3. One-shot peptide-amphiphiles (PAs) assemble at 1 wt % in a solution of 99% DI H_2O and 1% TFA. PA assemblies, represented in blue, were visualized by staining with FM-4-64 fluorescent dye (excitation 558 nm, emission 734 nm).

either DP 15 or 34, for PAs with $[M]_o/[I]$ = 68 or $[M]_o/[I]$ = 153, respectively (Table 1).

One-Shot PAs are Antimicrobial. Our one-shot PAs perform effectively as antimicrobial agents, again supporting the utility of this synthetic technique to produce functional copolypeptides. Block and statistical copolypeptides of p(Lys)-*co*-p(Leu) have been previously identified as effective antimicrobial agents against many species of bacteria as well as yeast.[59,60] These copolypeptides are antibacterial by employing a pore-forming mechanism: the cationic residues are adsorbed to the anionic bacterial membrane, while the hydrophobic residues penetrate into the bacterial membrane causing cell death.[66] Here, we demonstrate that our one-shot PAs exhibit dose-dependent antimicrobial activity against gram negative bacteria, *Escherichia coli* (*E. coli*).

A mixture of streptomycin and penicillin (at concentrations of 100 μg/mL for streptomycin and 100 U/mL or ∼60 μg/mL for penicillin) was chosen as the positive control. This combination is widely used in cell culture because it conclusively eliminates bacterial contamination.[67,68]

Universally, at 2 mg/mL, all one-shot PAs that were tested show equally efficient antimicrobial activity as the positive control, reaching >99% reduction in live cells with no statistical difference. Previously studied p(Lys)-*b*-p(Leu) copolypeptides reach >99% bacterial reduction at 0.01 mg/mL.[60] While our blocky PAs require a higher concentration to achieve antimicrobial efficacy, our results at lower concentrations provide insight into the characteristics of PAs that enable improved antibacterial activity.

At lower concentrations, PA composition had a more apparent effect on antimicrobial activity. At 0.2 mg/mL, a higher p(Leu) content leads to enhanced antimicrobial activity: with 50% pLeu, $p(Lys)_{75}$-*b*-$p(Leu)_{75}$ had significantly more antimicrobial activity than the other tested PAs, which had either 20 or 30% pLeu (Figure 4). At 0.02 mg/mL, lower

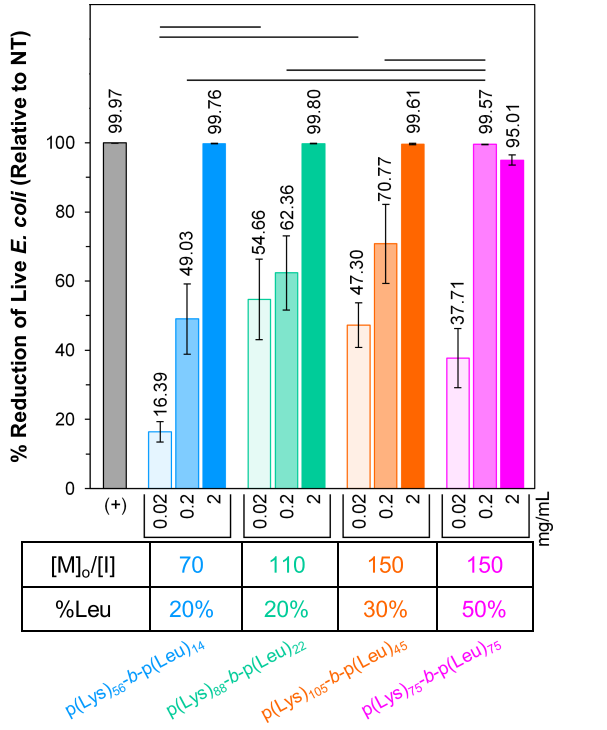

Figure 4. One-shot peptide amphiphiles with varying copolypeptide compositions and molecular weights reduce the number of live *E. coli*. Percent reduction of live *E. coli* is a function of the bacteriostatic and bactericidal abilities of the copolypeptides, relative to the no treatment condition, which is designated as 0% reduction of live cells. The positive control (+) was 100 U/mL (∼60 μg/mL) penicillin with 100 μg/mL streptomycin. Horizontal bars above the graph indicate statistical significance ($p<0.05$).

PA length leads to lower antimicrobial activity: $p(Lys)_{56}$-*b*-$p(Leu)_{14}$ (DP 70) was significantly less antimicrobial than $p(Lys)_{88}$-*b*-$p(Leu)_{22}$ (DP 110) and $p(Lys)_{105}$-*b*-$p(Leu)_{45}$ (DP 150) (Figure 4). These trends indicate that the material design (copolypeptide length and composition) can be optimized to achieve a greater antimicrobial activity.

Covalent Peptide Amphiphile−Graphenic Conjugate (PA−G) Directs Graphenic Assembly. Functional graphenic materials (FGMs) can be useful in biomedicine due to their biocompatibility, electrical conductivity, mechanical strength, and bioactive functionalization capacity.[52] However, many of these desirable properties can only be realized in ordered structures, where graphenic sheets are aligned.[69−73] Few spontaneous, bottom-up assembly methods exist to arrange graphenic sheets into ordered 3D structures, and the types of architectures that can be accessed are limited.[74−76]

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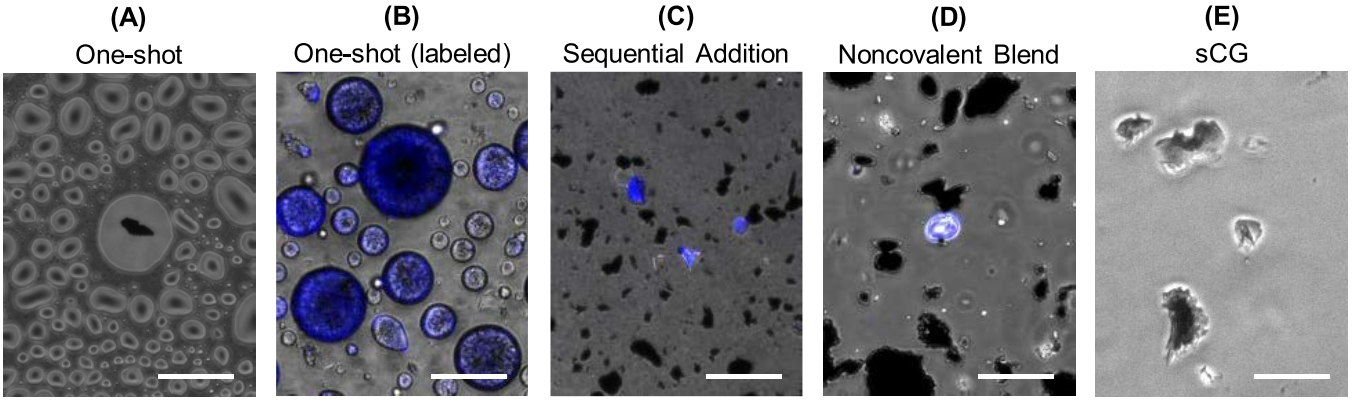

Figure 5. (A, B) One-shot peptide amphiphiles (PA) are end-capped with a graphenic material to give PA−G, where the PA templates the assembly of the graphenic material. (C) When PA is synthesized by sequential NCA monomer addition, end-capping with the graphenic material does not result in directed graphenic assembly. (D) Similarly, noncovalent blends of one-shot PA with the graphenic material result in no directed assembly. (E) Optical image of the pure graphenic material (sCG) prior to endcapping with PA is given for comparison of morphology. Scale bars represent 50 μm. Note that the graphenic material is black; and in (B−D), the PA is labeled with FM-4-64 fluorescent dye (represented in blue). Solution preparation is described in the Supporting Information.

To address this void, we designed a strategy to template the bottom-up assembly of an FGM by covalently binding our self-assembling PA to a graphenic surface, giving a PA−graphenic conjugate material (PA−G). To synthesize this PA−G conjugate, we modified a polypeptide end-capping procedure developed by our group.[51] Polypeptide end-capping with a graphenic material is more effective than grafting from due to adventitious functional groups and associated water on the graphenic material that interfere with NCA or NTA ROP.[77] Furthermore, end-capping an active polypeptide chain end overcomes steric limitations associated with traditional grafting methods.[78] Briefly, a protected PA of p(Lys-Z)-*b*-p(Leu) was synthesized using the one-shot copolymerization strategy described herein (Scheme S2). Then, the protected PA, which has a nucleophilic propagating end-group, was end-capped with an electrophilic graphenic material prepared via a Claisen modification of graphene oxide.[51,77] This reaction resulted in a mixture of covalently bound PA−G with unbound PA. This PA−G/PA mixture was then treated with acid to deprotect the lysine residues, exposing positively charged amines.

The PA−G/PA composite was characterized by Fourier transform infrared spectroscopy (FTIR) and thermogravimetric analysis (TGA). FTIR clearly shows that the PA−G/PA material contains both copolypeptide and graphenic components: amide peaks at 1650 and 1524 cm^{-1} indicate the presence of copolypeptide, and the peak at 1246 cm^{-1} represents C−O stretching on the graphenic material,[79] which are not present in the neat PA (Figure S13). Using TGA, the weight percent of the graphenic backbone within the PA−G/PA mixture was determined to be 6.39% (Figure S6).

We observed directed assembly of PA−G conjugate structures when copolypeptides were prepared using our one-shot synthesis followed by our polypeptide end-capping strategy (Figure 5A). To verify that the graphenic assembly was driven by the PA, we used a lipophilic fluorescent dye, which associates with the leucine block of the PA. Here, we observe the association of the blue dye with the spherical assemblies, demonstrating that the graphenic material is encapsulated by a PA shell (Figure 5B).

Both the one-shot PA synthetic method and covalent conjugation to the graphenic material are necessary to produce a PA−G conjugate with directed graphenic assembly. We performed two control experiments to demonstrate this. In one control, we synthesized a PA using conventional sequential addition of NCA monomers and attempted to end-cap the resulting copolypeptide with the graphenic material. Here, the graphenic material aggregated outside of the PA assemblies, exhibiting no directed graphenic assembly (Figure 5C). This result is likely due to an unsuccessful PA end-capping, resulting in a noncovalent blend of PA + G. This is a reasonable hypothesis because, when sequential addition is used to make the PA using primary amine initiation at room temperature, many chains become terminated in the delay period between addition of the second NCA monomer type (when the concentration of the first NCA monomer is very low relative to the concentration of propagating chain ends). Terminated polypeptide chains cannot participate in the graphenic end-capping reaction. On the other hand, when our one-shot technique is used to synthesize the PA, the chain ends are still active following the copolymerization and can participate in the graphenic end-capping reaction. In the second control, noncovalently-bound, one-shot PA was mixed with a graphenic moiety (PA + G). Here, a similar result to the sequential addition PA−G was observed; there was no directed graphenic assembly (Figure 5D).

These preliminary results represent an important proof-of-concept that graphenic assemblies can be directed using self-assembling copolypeptides. This graphenic assembly control can be advanced by using copolypeptides that participate in more complex and larger length-scale assemblies. This would create three-dimensional constructs that incorporate the natural order of the copolypeptide assembly with enhanced therapeutic capacity,[52] mechanical strength,[80] and electrical conductivity[81] from the graphenic component.

■ CONCLUSIONS

We developed a glovebox-free, one-shot synthesis of block copolypeptides using a ring-opening polymerization that capitalizes on the difference in the reactivity of cyclic α-amino acid NCA and NTA comonomers. Our copolymerization strategy provides an alternative to sequential NCA monomer addition and allows convenient preparation of

https://dx.doi.org/10.1021/acs.biomac.0c00962
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block copolypeptides with a controlled molecular weight and copolypeptide homogeneity.

We used this one-shot ring-opening copolymerization to synthesize antimicrobial peptide amphiphiles (PAs) that can form ordered assemblies. Furthermore, when covalently conjugated to a graphenic material to produce PA−G, graphenic assembly can be directed by the co-assembly of neat PA with PA−G.

In the future, this copolymerization technique can likely be adapted to synthesize block copolypeptides with other NCA/NTA comonomer pairs to access a variety of ordered structures. Our technique is suitable for interdisciplinary labs without a designated glovebox for polypeptide synthesis and could be implemented to broaden the scope of block copolypeptide applications.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.biomac.0c00962.

Materials; instrumentation; synthetic methods; ^{1}H-NMR kinetic data and calculations; ^{1}H-NMR spectra of monomers and copolypeptides; FTIR and TGA of the PA−G conjugate material compared to parent materials (neat PA and G); GPCs of one-shot and sequential addition copolypeptides; supplemental bacterial data; and bacterial culture and analysis methods (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

For providing use of their facilities, we thank R. Gil (NMR, funded in part by NSF grant # CHE-9808188, CHE-1039870, and CHE-1726525), M. Bruchez (microplate reader), and K. Matyjaszewski (GPC, light scattering), all at Carnegie Mellon University.

■ ABBREVIATIONS

NCA,N-carboxyanhydride; NTA,N-thiocarboxyanhydride; PA,peptide amphiphile; FGM,functional graphenic material; ROP,ring-opening polymerization; DMF,dimethylformamide; NMR,nuclear magnetic resonance; PA−G,peptide amphiphile−graphenic conjugate

■ REFERENCES

(1) Petsko, G. A.; Ringe, D. *Protein Structure and Function*; New Science Press, 2004, pp. 3.

(2) Gomes, S.; Leonor, I. B.; Mano, J. F.; Reis, R. L.; Kaplan, D. L. Natural and Genetically Engineered Proteins for Tissue Engineering. *Prog. Polym. Sci.* **2012**, *37*, 1−17.

(3) Maji, S. K.; Perrin, M. H.; Sawaya, M. R.; Jessberger, S.; Vadodaria, K.; Rissman, R. A.; Singru, P. S.; Nilsson, K. P. R.; Simon, R.; Schubert, D.; Eisenberg, D.; Rivier, J.; Sawchenko, P.; Vale, W.; Riek, R. Functional Amyloids As Natural Storage of Peptide Hormones in Pituitary Secretory Granules. *Science* **2009**, *325*, 328−332.

(4) Song, Z.; Han, Z.; Lv, S.; Chen, C.; Chen, L.; Yin, L.; Cheng, J. Synthetic Polypeptides: From Polymer Design to Supramolecular Assembly and Biomedical Application. *Chem. Soc. Rev.* **2017**, *46*, 6570−6599.

(5) Lu, H.; Wang, J.; Song, Z.; Yin, L.; Zhang, Y.; Tang, H.; Tu, C.; Lin, Y.; Cheng, J. Recent Advances in Amino Acid N-Carboxyanhydrides and Synthetic Polypeptides: Chemistry, Self-Assembly and Biological Applications. *Chem. Commun.* **2013**, *50*, 139−155.

(6) Deng, C.; Wu, J.; Cheng, R.; Meng, F.; Klok, H.-A.; Zhong, Z. Functional Polypeptide and Hybrid Materials: Precision Synthesis via α-Amino Acid N-Carboxyanhydride Polymerization and Emerging Biomedical Applications. *Prog. Polym. Sci.* **2014**, *39*, 330−364.

(7) Deming, T. J. Synthetic Polypeptides for Biomedical Applications. *Prog. Polym. Sci.* **2007**, *32*, 858−875.

(8) Ge, C.; Ye, H.; Wu, F.; Zhu, J.; Song, Z.; Liu, Y.; Yin, L., Biological Applications of Water-Soluble Polypeptides with Ordered Secondary Structures *J. Mater. Chem. B* **2020**, In press

(9) Deming, T. J. Facile Synthesis of Block Copolypeptides of Defined Architecture. *Nature* **1997**, *390*, 386.

(10) Deming, T. J. Amino Acid Derived Nickelacycles: Intermediates in Nickel-Mediated Polypeptide Synthesis. *J. Am. Chem. Soc.* **1998**, *120*, 4240−4241.

(11) Deming, T. J. Cobalt and Iron Initiators for the Controlled Polymerization of α-Amino Acid-N-Carboxyanhydrides. *Macromolecules* **1999**, *32*, 4500−4502.

(12) Song, Z.; Tan, Z.; Cheng, J. Recent Advances and Future Perspectives of Synthetic Polypeptides from N-Carboxyanhydrides. *Macromolecules* **2019**, *52*, 8521−8539.

(13) Mazo, A. R.; Allison-Logan, S.; Karimi, F.; Chan, N. J.-A.; Qiu, W.; Duan, W.; O'Brien-Simpson, N. M.; Qiao, G. G. Ring Opening Polymerization of α-Amino Acids: Advances in Synthesis, Architecture and Applications of Polypeptides and Their Hybrids *Chem. Soc. Rev.* **2020**, In press

(14) Liu, Y.; Li, D.; Ding, J.; Chen, X. Controlled Synthesis of Polypeptides *Chin. Chem. Lett.* **2020**, In press

(15) Cheng, J.; Deming, T. J. Synthesis of Polypeptides by Ring-Opening Polymerization of α-Amino Acid N-Carboxyanhydrides. In *Peptide-Based Materials*; Deming, T., Ed.; Springer Berlin Heidelberg: Berlin, Heidelberg, 2011; Vol. *310*, pp 1−26.

(16) Cardinaux, F.; Howard, J. C.; Taylor, G. T.; Scheraga, H. A. Block Copolymers of Amino Acids. I. Synthesis and Structure of Copolymers of L-Alanine or L-Phenylalanine with D,L-Lysine-D7 or D,L-Lysine. *Biopolymers* **1977**, *16*, 2005−2028.

(17) Sekiguchi, H. Mechanism of N-Carboxy-α-Amino Acid Anhydride (NCA) Polymerization. *Pure Appl. Chem. Chim. Pure. Appl. Berl.* **1981**, *53*, 1689−1714.

(18) Zhang, H.; Nie, Y.; Zhi, X.; Du, H.; Yang, J. Controlled Ring-Opening Polymerization of α-Amino Acid N -Carboxy-Anhydride by Frustrated Amine/Borane Lewis Pairs. *Chem. Commun.* **2017**, *53*, 5155−5158.

(19) 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*, 3590.

(20) Lv, S.; Kim, H.; Song, Z.; Feng, L.; Yang, Y.; Baumgartner, R.; Tseng, K.-Y.; Dillon, S. J.; Leal, C.; Yin, L.; Cheng, J. Unimolecular Polypeptide Micelles via Ultrafast Polymerization of N-Carboxyanhydrides. *J. Am. Chem. Soc.* **2020**, *142*, 8570−8574.
(21) Zhao, W.; Gnanou, Y.; Hadjichristidis, N. From Competition to Cooperation: A Highly Efficient Strategy towards Well-Defined (Co)Polypeptides. *Chem. Commun.* **2015**, *51*, 3663−3666.
(22) Zhao, W.; Gnanou, Y.; Hadjichristidis, N. Fast and Living Ring-Opening Polymerization of α-Amino Acid N-Carboxyanhydrides Triggered by an "Alliance" of Primary and Secondary Amines at Room Temperature. *Biomacromolecules* **2015**, *16*, 1352−1357.
(23) Vacogne, C. D.; Schlaad, H. Primary Ammonium/Tertiary Amine-Mediated Controlled Ring Opening Polymerisation of Amino Acid N-Carboxyanhydrides. *Chem. Commun.* **2015**, *51*, 15645−15648.
(24) 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.
(25) Grazon, C.; Salas-Ambrosio, P.; Ibarboure, E.; Buol, A.; Garanger, E.; Grinstaff, M. W.; Lecommandoux, S.; Bonduelle, C. Aqueous Ring-Opening Polymerization-Induced Self-Assembly (ROPISA) of N-Carboxyanhydrides. *Angew. Chem., Int. Ed. Engl.* **2020**, *59*, 622−626.
(26) 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*, 4223−4226.
(27) 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*, 514−524.
(28) 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*, 1322−1330.
(29) Pickel, D. L.; Politakos, N.; Avgeropoulos, A.; Messman, J. M. A Mechanistic Study of α-(Amino Acid)-N-Carboxyanhydride Polymerization: Comparing Initiation and Termination Events in High-Vacuum and Traditional Polymerization Techniques. *Macromolecules* **2009**, *42*, 7781−7788.
(30) Song, Z.; Fu, H.; Baumgartner, R.; Zhu, L.; Shih, K.-C.; Xia, Y.; Zheng, X.; Yin, L.; Chipot, C.; Lin, Y.; Cheng, J. Enzyme-Mimetic Self-Catalyzed Polymerization of Polypeptide Helices. *Nat. Commun.* **2019**, *10*, 5470.
(31) 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*, 10658−10663.
(32) Wang, X.; Song, Z.; Tan, Z.; Zhu, L.; Xue, T.; Lv, S.; Fu, Z.; Zheng, X.; Ren, J.; Cheng, J. Facile Synthesis of Helical Multiblock Copolypeptides: Minimal Side Reactions with Accelerated Polymerization of N-Carboxyanhydrides. *ACS Macro Lett.* **2019**, *8*, 1517−1521.
(33) Song, Z.; Fu, H.; Wang, R.; Pacheco, L. A.; Wang, X.; Lin, Y.; Cheng, J. Secondary Structures in Synthetic Polypeptides from N-eCarboxyanhydrides: Design, Modulation, Association, and Material Applications. *Chem. Soc. Rev.* **2018**, *47*, 7401−7425.
(34) Deming, T. J. Living Polymerization of α-Amino Acid-N-Carboxyanhydrides. *J. Polym. Sci. A Polym. Chem.* **2000**, *38*, 3011−3018.
(35) Deming, T. J. Preparation and Development of Block Copolypeptide Vesicles and Hydrogels for Biological and Medical Applications. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* **2014**, *6*, 283−297.
(36) Bellomo, E. G.; Wyrsta, M. D.; Pakstis, L.; Pochan, D. J.; Deming, T. J. Stimuli-Responsive Polypeptide Vesicles by Conformation-Specific Assembly. *Nat. Mater.* **2004**, *3*, 244−248.
(37) Hanson, J. A.; Li, Z.; Deming, T. J. Nonionic Block Copolypeptide Micelles Containing a Hydrophobic Rac-Leucine Core. *Macromolecules* **2010**, *43*, 6268−6269.
(38) Nowak, A. P.; Breedveld, V.; Pakstis, L.; Ozbas, B.; Pine, D. J.; Pochan, D.; Deming, T. J. Rapidly Recovering Hydrogel Scaffolds from Self-Assembling Diblock Copolypeptide Amphiphiles. *Nature* **2002**, *417*, 424−428.
(39) Zhang, S.; Alvarez, D. J.; Sofroniew, M. V.; Deming, T. J. Design and Synthesis of Nonionic Copolypeptide Hydrogels with Reversible Thermoresponsive and Tunable Physical Properties. *Biomacromolecules* **2015**, *16*, 1331−1340.
(40) Yang, C.-Y.; Song, B.; Ao, Y.; Nowak, A. P.; Abelowitz, R. B.; Korsak, R. A.; Havton, L. A.; Deming, T. J.; Sofroniew, M. V. Biocompatibility of Amphiphilic Diblock Copolypeptide Hydrogels in the Central Nervous System. *Biomaterials* **2009**, *30*, 2881−2898.
(41) Paul, C. H.; Timothy, P. L.. *Polymer Chemistry*, 2nd ed; Taylor & Francis Group: Boca Raton, FL, 2007.
(42) Grune, E.; Johann, T.; Appold, M.; Wahlen, C.; Blankenburg, J.; Leibig, D.; Müller, A. H. E.; Gallei, M.; Frey, H. One-Step Block Copolymer Synthesis versus Sequential Monomer Addition: A Fundamental Study Reveals That One Methyl Group Makes a Difference. *Macromolecules* **2018**, *51*, 3527−3537.
(43) Ariga, T.; Takata, T.; Endo, T. Block Copolymerization of Cyclic Carbonate and Oxetanes in One-Shot Feeding. *Macromolecules* **1993**, *26*, 7106−7107.
(44) Shin, S.; Yoon, K.-Y.; Choi, T.-L. Simple Preparation of Various Nanostructures via in Situ Nanoparticlization of Polyacetylene Blocklike Copolymers by One-Shot Polymerization. *Macromolecules* **2015**, *48*, 1390−1397.
(45) Wu, R.; AL-Azemi, T. F.; Bisht, K. S. One-Shot Block Copolymerization of a Functional Seven-Membered Cyclic Carbonate Derived from l-Tartaric Acid with ε-Caprolactone. *Macromolecules* **2009**, *42*, 2401−2410.
(46) Dewey, R. S.; Schoenewaldt, E. F.; Joshua, H.; Paleveda, W. J.; Schwam, H.; Barkemeyer, H.; Arison, B. H.; Veber, D. F.; Denkewalter, R. G.; Hirschmann, R. Synthesis of Peptides in Aqueous Medium. V. Preparation and Use of 2,5-Thiazolidinediones (NTA's). Use of the 13C-H Nuclear Magnetic Resonance Signal as Internal Standard for Quantitative Studies. *J. Am. Chem. Soc.* **1968**, *90*, 3254−3255.
(47) Deng, Y.; Chen, H.; Tao, X.; Cao, F.; Trépout, S.; Ling, J.; Li, M.-H. Oxidation-Sensitive Polymersomes Based on Amphiphilic Diblock Copolypeptoids. *Biomacromolecules* **2019**, *20*, 3435−3444.
(48) Cao, J.; Siefker, D.; Chan, B. A.; Yu, T.; Lu, L.; Saputra, M. A.; Fronczek, F. R.; Xie, W.; Zhang, D. Interfacial Ring-Opening Polymerization of Amino-Acid-Derived N-Thiocarboxyanhydrides Toward Well-Defined Polypeptides. *ACS Macro Lett.* **2017**, *6*, 836−840.
(49) Siefker, D.; Williams, A. Z.; Stanley, G. G.; Zhang, D. Organic Acid Promoted Controlled Ring-Opening Polymerization of α-Amino Acid-Derived N-Thiocarboxyanhydrides (NTAs) toward Well-Defined Polypeptides. *ACS Macro Lett.* **2018**, *7*, 1272−1277.
(50) Tao, X.; Li, M.-H.; Ling, J. α-Amino Acid N-Thiocarboxyanhydrides: A Novel Synthetic Approach toward Poly(α-Amino Acid)s. *Eur. Polym. J.* **2018**, *109*, 26−42.
(51) Eckhart, K. E.; Holt, B. D.; Laurencin, M. G.; Sydlik, S. A. Covalent Conjugation of Bioactive Peptides to Graphene Oxide for Biomedical Applications. *Biomater. Sci.* **2019**, *7*, 3876−3885.
(52) Wright, Z. M.; Arnold, A. M.; Holt, B. D.; Eckhart, K. E.; Sydlik, S. A. Functional Graphenic Materials, Graphene Oxide, and Graphene as Scaffolds for Bone Regeneration. *Regen. Eng. Transl. Med.* **2019**, *5*, 190−209.
(53) Zhang, J.; Gellman, S. H.; Stahl, S. S. Kinetics of Anionic Ring-Opening Polymerization of Variously Substituted β-Lactams: Homopolymerization and Copolymerization. *Macromolecules* **2010**, *43*, 5618−5626.

https://dx.doi.org/10.1021/acs.biomac.0c00962
Biomacromolecules 2020, 21, 3878−3886

(54) Brown, B.; Foote, C. *Organic Chemistry*, 3rd ed.; Brooks/Cole, 2002, pp. 6−7.
(55) Tao, X.; Zheng, B.; Bai, T.; Zhu, B.; Ling, J. Hydroxyl Group Tolerated Polymerization of N-Substituted Glycine N-Thiocarboxyanhydride Mediated by Aminoalcohols: A Simple Way to α-Hydroxyl-ω-Aminotelechelic Polypeptoids. *Macromolecules* **2017**, *50*, 3066−3077.
(56) Yuan, J.; Sun, Y.; Wang, J.; Lu, H. Phenyl Trimethylsilyl Sulfide-Mediated Controlled Ring-Opening Polymerization of α-Amino Acid N-Carboxyanhydrides. *Biomacromolecules* **2016**, *17*, 891−896.
(57) Lu, H.; Cheng, J. N-Trimethylsilyl Amines for Controlled Ring-Opening Polymerization of Amino Acid N-Carboxyanhydrides and Facile End Group Functionalization of Polypeptides. *J. Am. Chem. Soc.* **2008**, *130*, 12562−12563.
(58) Goury, V.; Jhurry, D.; Bhaw-Luximon, A. Synthesis of Sugar-Based Polypeptides. *Des. Monomers Polym.* **2008**, *11*, 357−369.
(59) Bevilacqua, M. P.; Benitez, D.; Deming, T. J.; Hanson, J. A.; Koziol, L. Compositions and Uses of Materials with High Antimicrobial Activity and Low Toxicity. US 9,017,730 B2, April 28, 2015.
(60) Bevilacqua, M. P.; Huang, D. J.; Wall, B. D.; Lane, S. J.; Edwards, C. K.; Hanson, J. A.; Benitez, D.; Solomkin, J. S.; Deming, T. J. Amino Acid Block Copolymers with Broad Antimicrobial Activity and Barrier Properties. *Macromol. Biosci.* **2017**, *17*, 1600492.
(61) Mason, T. G.; Deming, T. J.; Hanson, J. A.; Chang, C. B.; Graves, S. M. Copolymer-Stabilized Emulsions. EP2178517B1, January 25, 2017.
(62) Breedveld, V.; Nowak, A. P.; Sato, J.; Deming, T. J.; Pine, D. J. Rheology of Block Copolypeptide Solutions: Hydrogels with Tunable Properties. *Macromolecules* **2004**, *37*, 3943−3953.
(63) Hanson, J. A.; Chang, C. B.; Graves, S. M.; Li, Z.; Mason, T. G.; Deming, T. J. Nanoscale Double Emulsions Stabilized by Single-Component Block Copolypeptides. *Nature* **2008**, *455*, 85−88.
(64) Kricheldorf, H. R.; Mueller, D. Secondary Structure of Peptides. 3. Carbon-13 NMR Cross Polarization/Magic Angle Spinning Spectroscopic Characterization of Solid Polypeptides. *Macromolecules* **1983**, *16*, 615−623.
(65) Holowka, E. P.; Pochan, D. J.; Deming, T. J. Charged Polypeptide Vesicles with Controllable Diameter. *J. Am. Chem. Soc.* **2005**, *127*, 12423−12428.
(66) Su, X.; Zhou, X.; Tan, Z.; Zhou, C. Highly Efficient Antibacterial Diblock Copolypeptides Based on Lysine and Phenylalanine. *Biopolymers* **2017**, *107*, No. e23041.
(67) Jannadi, H.; Correa, W.; Zhang, Z.; Brandenburg, K.; Oueslati, R.; Rouabhia, M. Antimicrobial Peptides Pep19−2.5 and Pep19-4LF Inhibit Streptococcus Mutans Growth and Biofilm Formation. *Microb. Pathog.* **2019**, *133*, 103546.
(68) Ryu, A. H.; Eckalbar, W. L.; Kreimer, A.; Yosef, N.; Ahituv, N. Use Antibiotics in Cell Culture with Caution: Genome-Wide Identification of Antibiotic-Induced Changes in Gene Expression and Regulation. *Sci. Rep.* **2017**, *7*, 1−9.
(69) Qiu, B.; Xing, M.; Zhang, J. Recent Advances in Three-Dimensional Graphene Based Materials for Catalysis Applications. *Chem. Soc. Rev.* **2018**, *47*, 2165−2216.
(70) Geim, A. K.; Novoselov, K. S. The Rise of Graphene. *Nat. Mater.* **2007**, *6*, 183−191.
(71) Li, D.; Liu, Y.; Ma, H.; Wang, Y.; Wang, L.; Xie, Z. Preparation and Properties of Aligned Graphene Composites. *RSC Adv.* **2015**, *5*, 31670−31676.
(72) Yin, S.; Niu, Z.; Chen, X. Assembly of Graphene Sheets into 3D Macroscopic Structures. *Small* **2012**, *8*, 2458−2463.
(73) Ma, Y.; Chen, Y. Three-Dimensional Graphene Networks: Synthesis, Properties and Applications. *Natl. Sci. Rev.* **2015**, *2*, 40−53.
(74) Han, T. H.; Lee, W. J.; Lee, D. H.; Kim, J. E.; Choi, E.-Y.; Kim, S. O. Peptide/Graphene Hybrid Assembly into Core/Shell Nanowires. *Adv. Mater.* **2010**, *22*, 2060−2064.
(75) Ohtomo, M.; Jippo, H.; Hayashi, H.; Yamaguchi, J.; Ohfuchi, M.; Yamada, H.; Sato, S. Interpolymer Self-Assembly of Bottom-up Graphene Nanoribbons Fabricated from Fluorinated Precursors. *ACS Appl. Mater. Interfaces* **2018**, *10*, 31623−31630.
(76) Tour, J. M. Top-Down versus Bottom-Up Fabrication of Graphene-Based Electronics. *Chem. Mater.* **2014**, *26*, 163−171.
(77) Holt, B. D.; Arnold, A. M.; Sydlik, S. A. Peptide-Functionalized Reduced Graphene Oxide as a Bioactive Mechanically Robust Tissue Regeneration Scaffold. *Polym. Int.* **2017**, *66*, 1190−1198.
(78) Rubio, N.; Au, H.; Leese, H. S.; Hu, S.; Clancy, A. J.; Shaffer, M. S. P. Grafting from versus Grafting to Approaches for the Functionalization of Graphene Nanoplatelets with Poly(Methyl Methacrylate). *Macromolecules* **2017**, *50*, 7070−7079.
(79) Monteserín, C.; Blanco, M.; Aranzabe, E.; Aranzabe, A.; Laza, J. M.; Larrañaga-Varga, A.; Vilas, J. L. Effects of Graphene Oxide and Chemically-Reduced Graphene Oxide on the Dynamic Mechanical Properties of Epoxy Amine Composites. *Polymer* **2017**, *9*, 449.
(80) Lee, C.; Wei, X.; Kysar, J. W.; Hone, J. Measurement of the Elastic Properties and Intrinsic Strength of Monolayer Graphene. *Science* **2008**, *321*, 385−388.
(81) Menaa, F.; Abdelghani, A.; Menaa, B. Graphene Nanomaterials as Biocompatible and Conductive Scaffolds for Stem Cells: Impact for Tissue Engineering and Regenerative Medicine. *J. Tissue Eng. Regen. Med.* **2015**, *9*, 1321−1338.

https://dx.doi.org/10.1021/acs.biomac.0c00962
Biomacromolecules 2020, 21, 3878−3886