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Folding Cooperativity of Synthetic Polypeptides with or without "Tertiary" Interactions

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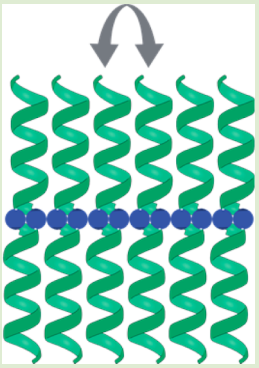
Supporting Information

ABSTRACT: Model-based studies on helix−coil transition and folding cooperativity of synthetic polypeptides have contributed to the understanding of protein folding and stability and to the development of polypeptide-based functional materials. Polypeptide-containing macromolecules with complex architectures, however, remain a challenge in the model-based analysis. Herein, a modified Schellman−Zimm−Bragg model has been utilized to quantitatively analyze the folding cooperativity of polypeptide-containing macromolecules. While the helix−coil transition of homopolypeptides (e.g., poly(ε-benzyloxycarbonyl-L-lysine) (PZLL)) can be described by the classic model, the folding of grafted polypeptide chains in the comb macromolecules (e.g., polynorbornene-*g*-poly(ε-benzyloxycarbonyl-L-lysine) (PN-*g*-PZLL)) cannot be accurately predicted by the existing theories, due to the side-chain interactions between grafted polypeptides in the comb macromolecules. Incorporating nonlocal interaction explicability into the statistical mechanics treatment is found to be instructive to account for the possible "tertiary" interactions of polypeptides in the macromolecules with complex architectures.



For macromolecular folding, cooperativity is a term used to describe the thermodynamics of macromolecular conformation transitions.[1−6] For example, polypeptides such as poly(γ-benzyl-L-glutamate) (PBLG) or poly(ε-benzyloxycarbonyl-L-lysine) (PZLL) can interconvert between α-helix and random coil in response to variations in temperature or solvent composition.[7−12] If a large number of units (e.g., a polypeptide with a high degree of polymerization (DP)) are involved in the folding process, the transitions may be induced by even a small variation of the external parameters—a cooperative behavior. Understanding the mechanism of folding cooperativity of polypeptides has contributed to elucidating the mechanism of protein folding and stability and to developing bioinspired synthetic polymers and functional materials.[13−22]

In their classic work, Schellman,[1] Zimm, and Bragg[2] established the statistical mechanics treatment on the folding cooperativity of individual α-helices, by describing the helix formation with the nucleation parameter (σ) and the equilibrium constant for propagation of a helix (s). The theory has successfully predicted the temperature- or solvent-induced helix−coil transition of a variety of polypeptides. Based on the Schellman models, Ghosh and Dill recently developed a theory[23] that can further treat specific tertiary interactions between individual helices in a macromolecule. The analytical theory correctly described the enhanced folding cooperativity found in three-helix-bundle proteins. The treatment explicitly included the interaction energies, solvent terms, and the chain conformational entropy, which can be evaluated by fitting both the temperature- and solvent-induced transition data with the model. This progress makes it possible to systematically investigate folding cooperativity of experimental model systems containing both local and nonlocal interactions.

We are interested in utilizing a Ghosh−Dill type of approach to analyze the folding cooperativity in synthetic polypeptide-containing macromolecules with a complex architecture[24−26] (e.g., comb-like or brush-like). For example, the densely grafted PZLLs in individual comb-like or brush-like macromolecules can interact with each other (somewhat similar to tertiary interactions in proteins), and this may considerably affect their own folding behaviors. By nuclear magnetic resonance (NMR) based structural analysis, we previously found that the folding cooperativities of polypeptides in the comb macromolecules may significantly deviate from those of linear polypeptides.[27] Herein, we apply the Ghosh−Dill approach to quantitatively analyze the folding behavior of both linear PZLL polymers and PZLL-grafted comb-like macromolecules. We first use the linear PZLLs to determine the helix−coil parameters for the individual helices. Then the parameters are utilized in the model to analyze the interaction energy of grafted PZLL in the comb macromolecules. We found that Ghosh−Dill's approach is instructive in describing the equilibrium properties of PZLLs in comb macromolecules, even though the original model was developed for proteins. Based on this approach, new statistical mechanics treatment may be developed to accurately account

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for the folding behavior of polypeptides in the complex macromolecular architectures, in which both local and nonlocal interactions may exist.

A small library of linear PZLL polymers ($PZLL_n$) and PZLL-grafted comb macromolecules with a polynorbornene backbone (PN_x-g-$PZLL_n$) were synthesized (Table 1) via the method we

Table 1. Synthesis of PZLL Homopolymers and PZLL-Grafted Comb-Like Macromolecules with Different DPs

entry	sample	Mn ($\times 10^4$ g/mol)	Mn_{obtain} ($\times 10^4$ g/mol)	PDI
1	$PZLL_{35}$	0.845	0.870	1.08
2	$PZLL_{63}$	1.52	1.57	1.06
3	$PZLL_{89}$	2.15	2.19	1.06
4	$PZLL_{150}$	3.62	3.93	1.12
5	PN_{10}-g-$PZLL_{50}$	13.3	12.1	1.18
6	PN_{10}-g-$PZLL_{70}$	18.7	18.0	1.11
7	PN_{10}-g-$PZLL_{100}$	26.7	25.2	1.06

previously developed,[25,27,28] where n and x are denoted as the DP of the PZLL and the PN backbone, respectively (please see ref 27 and the SI for the detailed procedures for synthesis and characterization of these polymers). We then compare the solvent- and temperature-induced helix−coil transitions of PZLLs in these macromolecules to understand the effect of nonlocal interactions on the folding cooperativity of helical polypeptides.

We have previously described the NMR-based structural analysis[29,30] of helical polypeptides in detail,[27] and a brief experimental procedure to follow the conformational transition of polypeptides in solution can also be found in the SI. Figure 1 shows the helix−coil transitions of the four PZLLs induced by the change of temperatures and by the addition of trifluoroacetic acid (TFA), a strong helix-disrupting solvent. Ghosh−Dill's theory,[23] when treating systems without nonlocal interactions, can be reduced to a simple Schellman-like "zipper" model to describe the transition process between a single helix and its coil state. So we first compare the classic Schellman's model and Ghosh−Dill model for their abilities in describing the helix−coil transition process of simple linear PZLLs and to obtain the model parameters for analysis of PZLL-grafted comb macromolecules.

Schellman's model simply considers a chain that undergoes a transition from a coil to a single helix, with the total partition function

$$q = 1 + \sigma \sum_{i=1}^{N} (N - i + 1)s^i \tag{1}$$

where s is the equilibrium constant for forming a helical unit (H) relative to a coil unit (C) in the end of a helical segment; σ is the nucleation parameter (i.e., σs is the equilibrium constant for initiating the first H after a string of C's); and N is the maximum number of "helical" bonds.[1] Commonly, N is related to the degree of polymerization (DP) of polypeptides by N = DP − 4, as in α-helical polypeptides, a helical hydrogen bond forms between residue i and residue i + 4. The macroscopic properties such as average fractional helicity, θ, can then be predicted from the partition function using the standard expression

$$\theta = \frac{\langle i \rangle}{N} = \frac{1}{N}\frac{d \ln q}{d \ln s} \tag{2}$$

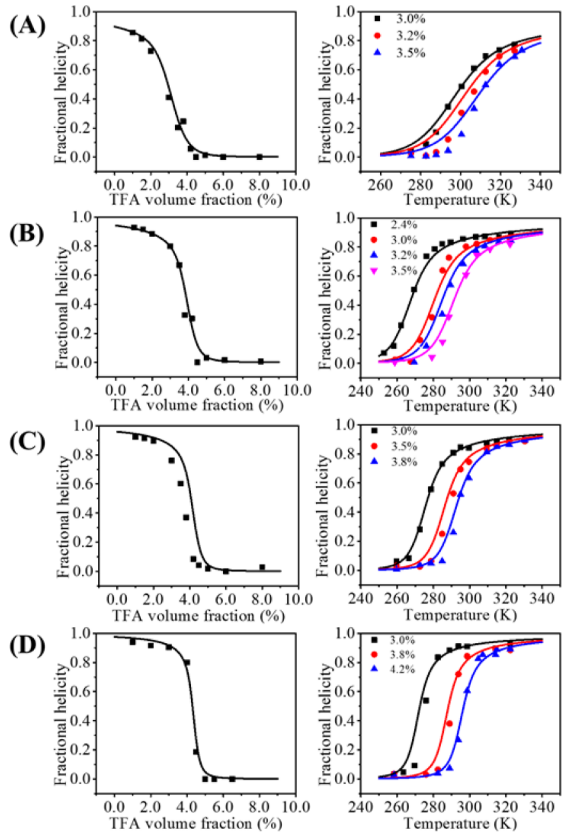

Figure 1. TFA- and temperature-induced helix−coil transitions of (A) $PZLL_{35}$, (B) $PZLL_{63}$, (C) $PZLL_{89}$, and (D) $PZLL_{150}$ in deuterochloroform. Filled symbols represent the experimental data, and solid lines represent the global fit of all the data using eqs 1, 2, and 3.

To connect the model predictions with the experimental results, we can make explicitly the temperature and solvent composition dependence by expressing s in three terms, considering the effects from enthalpy (ΔH^0), entropy (ΔS), and the addition of helix-disrupting solvent (m)

$$s = e^{-(\Delta H^\circ - T\Delta S + mc)/kT} \tag{3}$$

where T and c are temperature and concentration, respectively. As classic theories usually treat σ as being a temperature-independent coefficient, we make the same assumption here in Schellman's model to keep the consistency. We then combined eqs 1, 2, and 3 and obtained a global fit of the equations to the helix−coil transitions of the four PZLL samples (Figure 1), with a single set of parameters as $\sigma = 2.24 \times 10^{-4}$, $\Delta H^0 = 708$ cal/mol, $\Delta S = 3.39$ cal/(K·mol), and $m = 69.7$ cal/(mol·vol %). Apparently, the Schellman's model is able to describe the folding cooperativity of PZLL with a good confidence level, except some deviations found in $PZLL_{35}$ at the higher TFA concentrations.

When considering the polypeptides that undergo a transition from a coil to a single helix, the Ghosh−Dill model[23] adds the total count of all the polymer chain conformations into the Schellman's partition function to obtain

$$q = (z - 1)^{N+2}\left(1 + \sigma \sum_{i=1}^{N} (N - i + 1)s^i\right) \tag{4}$$

where z is the number of rotameric configurations accessible to each backbone "virtual" bond. The model also makes s explicitly

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dependent on temperature and solvent and, in addition, treats σ as a free energy term to reflect that the nucleation of helix is thermally activated

$$s = e^{-(\varepsilon_{hb}^0 + mc)/kT}/(z-1) \quad (5)$$

$$\sigma = e^{-\varepsilon_{nuc}/kT}/(z-1)^2 \quad (6)$$

The entropic component cost is considered in the $z-1$ and $(z-1)^2$ factor in the equations, respectively. In practice, however, the chain conformation term $((z-1)^{N+2})$ in the partition function is canceled out when calculating the average fractional helicity (θ), and the temperature dependence of σ is often too weak to be accurately determined. If we treated σ still as a constant, then the Ghosh–Dill model is identical to Schellman's model for the simple linear PZLLs, with ε_{hb}^0 = 708 cal/mol, ε_{nuc} = 3038 cal/mol, z = 1.18, m = and 69.7 cal/(mol·vol %). It allows for an identical fit of the data in Figure 1 by combining eqs 4 and 5 (Figure S48).

We then utilize the same set of thermodynamic parameters obtained from Schellman's model to predict the folding cooperativity of grafted PZLLs in comb macromolecules and found relatively large discrepancy with the experimental data (Figure 2). We note here that the discrepancy is not due to the

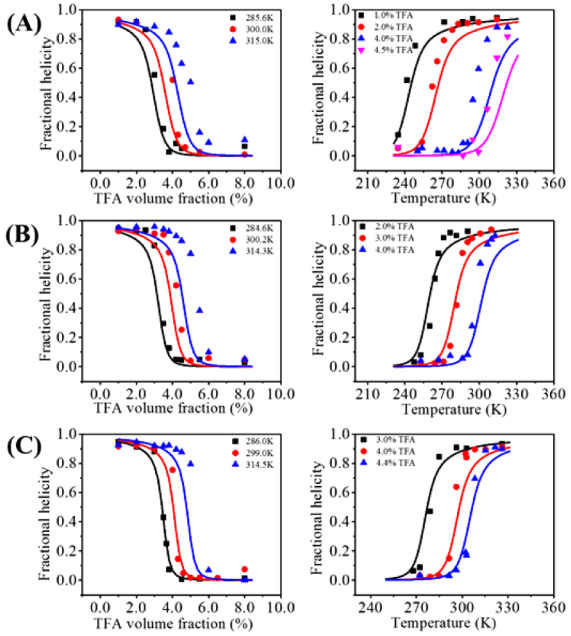

Figure 2. TFA- and temperature-induced helix–coil transitions of (A) PN_{10}-g-$PZLL_{50}$, (B) PN_{10}-g-$PZLL_{70}$, and (C) PN_{10}-g-$PZLL_{100}$ in d-chloroform. Filled symbols represent the experimental data; solid lines represent the prediction based on eqs 1–3, with the thermodynamic parameters obtained from linear PZLL polymers in Figure 1.

change of nucleation parameter, which may change as the consequence of tethering of the polypeptide chains to the PN backbone. Varying the value of σ does not improve the fitting (Figure S56). In addition, varying the value of N used in the model for each sample does not improve much the overall fitting to the experimental data either (Figure S57). Rather, the apparently higher folding cooperativity observed in all three comb macromolecules seems to result from interactions from the densely grafted PZLLs within the same comb macromolecules. Nuclear Overhauser enhancement spectroscopy (NOESY) experiments were performed to further reveal the interactions of PZLLs in the comb macromolecules.

In NOESY, the exchanges of magnetization can be observed as cross (off-diagonal) peaks. Cross peaks connecting several of the resonances were observed for the aromatic and benzylic methylene protons in the grafted PZLL (Figure 3a and Figures

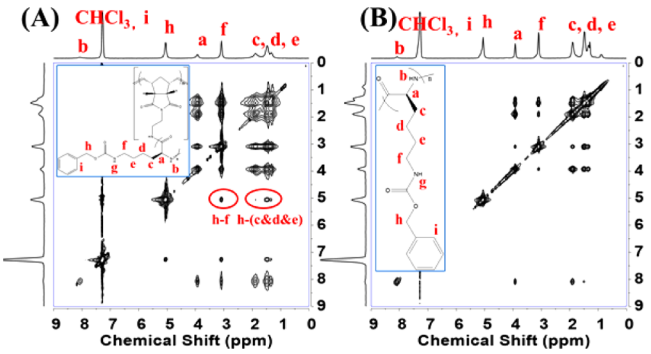

Figure 3. NOESY spectra of (A) PN_{10}-g-$PZLL_{50}$ and (B) $PZLL_{63}$ obtained by the $(\pi/2)$-t_1-$(\pi/2)$-τ_m-$(\pi/2)$-t_2 pulse sequence.

S53–55), showing that these protons are in close contact with β-CH and γ-CH, resulting in an exchange of magnetization (within ~0.5 nm). For homo PZLL samples, no cross peaks were observed for the aromatic or benzylic methylene protons in the experimental conditions (Figure 3b and Figures S49–52), showing that nonlocal chain interactions only exist for grafted PZLLs in the comb macromolecules. As Schellman's model does not consider the type of nonlocal interactions based on the macromolecular architecture, it is not a surprise that the model cannot describe the helix-to-coil transition for the grafted PZLLs in the comb macromolecules.

We then examine whether the helix–coil transitions of grafted PZLLs in comb macromolecules can be better described by a Ghosh–Dill type of approach that treats the nonlocal interactions from the contacts between the two neighboring helices as a binding equilibrium. By assuming each helix–helix contact has a contact energy ε_{hh} corresponding to an equilibrium constant $r = \exp\left(\frac{-\varepsilon_{hh} + T\Delta S^*}{kT}\right)$, the partition function for the grafted chains with possibility to form a two-helix bundle with neighboring chains becomes

$$q = 1 + \sigma \sum_{i=1}^{N} (N - i + 1)s^i + \sigma \sum_{i=1}^{N/4} \sum_{j=1}^{N/4} (r^{\min(i,j)} - 1)s^{4i} \quad (7)$$

The second summation is operated over the number of helical turns (about one-fourth of the helical bonds), due to the nature of contacts between two α-helices. To simplify, we assume that adding helix-disrupting solvent would change the contact energy between helices approximately linearly

$$\varepsilon_{hh} = \varepsilon_{hh}^0 + mc \quad (8)$$

where ε_{hh}^0 is the contact energy in the absence of helix-disrupting solvent. We then compare the predictions from this model with the experiments of PZLL-grafted comb macromolecules, using the helix–coil parameters, σ, ΔH^0, ΔS, and m, measured from linear PZLLs to obtain an optimized value of ε_{hh}^0 and ΔS^*. Figure 4 shows a global fit of eqs 2, 7, and 8 to the helix–coil transitions of the three PN-g-PZLL samples, with a single set of parameters as $\varepsilon_{hh}^0 = 2.02 \times 10^3$ cal/mol and $\Delta S^* =$

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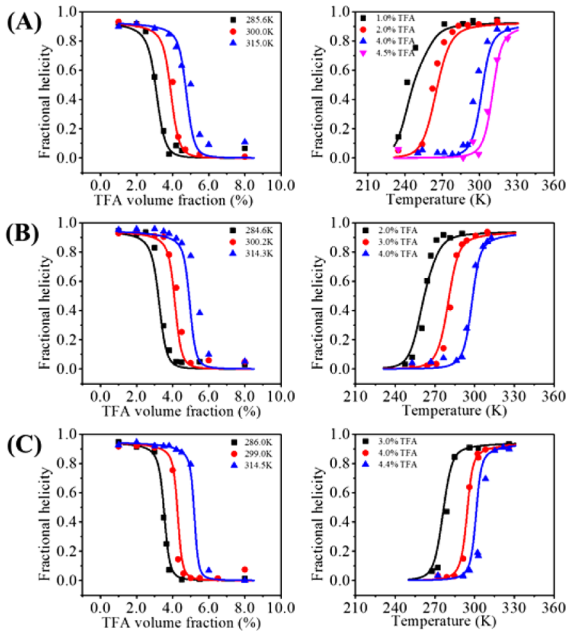

Figure 4. TFA- and temperature-induced helix−coil transitions of (A) PN_{10}-*g*-$PZLL_{50}$, (B) PN_{10}-*g*-$PZLL_{70}$, and (C) PN_{10}-*g*-$PZLL_{100}$ in chloroform. Solid lines represent the global fit of all the data with the interaction parameters described in eqs 7 and 8, in addition to the thermodynamic parameters obtained from linear PZLL polymers in Figure 1.

8.03 cal/(K·mol). With the introduction of the contact energy term into the partition function, the model now is able to describe the folding cooperativity of grafted PZLL in comb macromolecules with a good agreement. While it is to be proved yet whether this approach will be generally applicable to other polypeptides or different macromolecular architectures, considering the contact energy from the structural components with self-similarity appears to be a valid approach to modeling the cooperative behaviors in the macromolecules with complex architecture and extensive local crowding.

Inspired by Dill's recent work, we explicitly include enthalpic, entropic, and solvent terms in Schellman's equation to analyze temperature- and solvent-induced helix−coil transitions simultaneously. The approach is applicable to homopolypeptides with a range of different DPs and allows for the determination of the folding parameters of PZLLs accurately. In comb macromolecules containing polypeptide grafts, however, the nonlocal interactions between the grafted polypeptides preclude the accurate prediction of their folding cooperativity using the existing theories. Incorporating Ghosh−Dill type of approach to treat the nonlocal interactions as a binding equilibrium considerably improves the agreement between the predictions from the model with experiments. We note that the comb polymers we examined have only about 10 grafted chains per macromolecule; the dependence of the folding cooperativity on the extent of crowding in the brush-like macromolecules with a large number of grafted chains (e.g., >100) is the subject of future studies. Nevertheless, this exercise, while preliminary, suggests that a new statistical mechanics model may be established based on the Ghosh−Dill theory to successfully treat this type of "tertiary" interaction in polypeptide-containing macromolecules of complex architectures.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmacrolett.7b00324.

Synthesis and characterization of polymers; TFA-/temperature-induced helix−coil transitions for PZLL containing polymers; model-based analysis of TFA- and temperature-induced helix−coil transitions of linear PZLLs; NOESY study of PZLL containing polymers; effect of variation of σ on the fitting of PZLL-grafted comb macromolecules; and effect of variation of chain length on the fitting of PZLL-grafted comb macromolecules (PDF)

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‖Y.R. and H.F contributed equally to this work.

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Schellman, J. A. The factors affecting the stability of hydrogen-bonded polypeptide structures in solution. *J. Phys. Chem.* **1959**, *62*, 1485−1494.

(2) Zimm, B. H.; Bragg, J. K. Theory of the phase transition between helix and random coil in polypeptide chains. *J. Chem. Phys.* **1959**, *31*, 526−35.

(3) Lifson, S.; Roig, A. On the theory of helix-coil transition in polypeptides. *J. Chem. Phys.* **1961**, *34*, 1963−74.

(4) Nagai, K. Configurational Changes of Polypeptide Molecules in the Helix-Coil Transition Region, I. *J. Phys. Soc. Jpn.* **1960**, *15*, 407−16.

(5) Teramoto, A.; Fujita, H. In *Macroconformation of Polymers*; Springer Berlin Heidelberg: Berlin, Heidelberg, 1975; pp 65−149.

(6) Cantor, C. R.; Schimmel, P. R. *Biophysical Chemistry, Part III*; W.H. Freeman: San Francisco, 1980.

(7) Doty, P.; Wada, A.; Yang, J.; Blout, E. R. Polypeptides. VIII. Molecular configurations of poly-L-glutamic acid in water-dioxane solution. *J. Polym. Sci.* **1957**, *23*, 851−61.

(8) Doty, P.; Yang, J. T. Polypeptides. VII. Poly-γ-benzyl-L-glutamate: The helix-coil transition in solution. *J. Am. Chem. Soc.* **1956**, *78*, 498−500.

(9) Zimm, B. H.; Doty, P.; Iso, K. DETERMINATION OF THE PARAMETERS FOR HELIX FORMATION IN POLY-γ-BENZYL-L-GLUTAMATE. *Proc. Natl. Acad. Sci. U. S. A.* **1959**, *45*, 1601−7.

(10) Blout, E. R.; Lenormant, H. Reversible Configurational Changes in Poly-L-Lysine Hydrochloride Induced by Water. *Nature* **1957**, *179*, 960−3.

(11) Davidson, B.; Fasman, G. D. The conformational transitions of uncharged poly-L-lysine. α helix-random coil-β structure. *Biochemistry* **1967**, *6*, 1616−29.

(12) Bamford, C. H.; Elliott, A.; Hanby, W. E. *Synthetic Polypeptides*; Academic Press: New York, 1956.

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ACS Macro Lett. 2017, 6, 733−737

(13) Bryson, J. W.; Betz, S. F.; Lu, H. S.; Suich, D. J.; Zhou, H. X.; O'Neil, K. T.; DeGrado, W. F. Protein design: A hierarchic approach. *Science* **1995**, *270*, 935−41.
(14) Chakrabartty, A.; Baldwin, R. L. Stability of α-Helices. *Adv. Protein Chem.* **1995**, *46*, 141−76.
(15) DeGrado, W. F.; Wasserman, Z. R.; Lear, J. D. Protein design, a minimalist approach. *Science* **1989**, *243*, 622−8.
(16) Dill, K. A. Polymer principles and protein folding. *Protein Sci.* **1999**, *8*, 1166−80.
(17) Floudas, G.; Spiess, H. W. Self-assembly and dynamics of polypeptides. *Macromol. Rapid Commun.* **2009**, *30*, 278−98.
(18) Gans, P. J.; Lyu, P. C.; Manning, M. C.; Woody, R. W.; Kallenbach, N. R. The helix−coil transition in heterogeneous peptides with specific side-chain interactions: Theory and comparison with CD spectral data. *Biopolymers* **1991**, *31*, 1605−14.
(19) Poland, D.; A, S. H. *Theory of Helix-Coil Transitions in Biopolymers*; Academic Press: New York, 1970.
(20) Qian, H.; Schellman, J. A. Helix-coil theories: A comparative study for finite length polypeptides. *J. Phys. Chem.* **1992**, *96*, 3987−94.
(21) Shu, J. Y.; Panganiban, B.; Xu, T. *Annu. Rev. Phys. Chem.* **2013**, *64*, 631−57.
(22) van Hest, J. C. M.; Tirrell, D. A. Protein-based materials, toward a new level of structural control. *Chem. Commun.* **2001**, *19*, 1897−904.
(23) Ghosh, K.; Dill, K. A. Theory for protein folding cooperativity: Helix bundles. *J. Am. Chem. Soc.* **2009**, *131*, 2306−12.
(24) 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*, 5528−78.
(25) Lu, H.; Wang, J.; Lin, Y.; Cheng, J. One-pot synthesis of brush-like polymers via integrated ring-opening metathesis polymerization and polymerization of amino acid N-carboxyanhydrides. *J. Am. Chem. Soc.* **2009**, *131*, 13582−3.
(26) Baumgartner, R.; Fu, H.; Song, Z.; Lin, Y.; Cheng, J. Cooperative polymerization of α-helices induced by macromolecular architecture. *Nat. Chem.* **2017**, DOI: 10.1038/nchem.2712.
(27) Wang, J.; Lu, H.; Ren, Y.; Zhang, Y.; Morton, M.; Cheng, J.; Lin, Y. Interrupted helical structure of grafted polypeptides in brush-like macromolecules. *Macromolecules* **2011**, *44*, 8699−708.
(28) Lu, H.; Cheng, J. Hexamethyldisilazane-Mediated Controlled Polymerization of α-Amino Acid N-Carboxyanhydrides. *J. Am. Chem. Soc.* **2007**, *129*, 14114−5.
(29) Bradbury, E. M.; Carpenter, B. G.; Crane-Robinson, C.; Rattle, H. W. E. Application of high resolution nuclear magnetic resonance to conformational analyses of polypeptides in solution. *Nature* **1968**, *220*, 69−72.
(30) Bradbury, E. M.; Crane-Robinson, C.; Goldman, H.; Rattle, H. W. E. Proton magnetic resonance and the helix-coil transition. *Nature* **1968**, *217*, 812−6.

DOI: 10.1021/acsmacrolett.7b00324
ACS Macro Lett. 2017, 6, 733−737