Revisiting the Helical Cooperativity of Synthetic Polypeptides in Solution

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Calculation of critical chain length ($n^*$) above which two helical sections are statistically preferred than single helices in solution.

**Figure S1.** The $n$-$s$ plane for $\sigma=10^{-4}$. The boundary of the transition is chosen to be $\theta=0.1$ and $\theta=0.9$. The diagonal line indicates the conditions where half of the chains in the solution contain a single unbroken helix, the other half contain two helical sections connected with a coil segment. Solid line represents calculation results without taking the approximation in calculating the partition function of Zimm-Bragg. Dash line represents the calculation based on the Eq. (2) and (9) described in the manuscript.
Molecular weight determination of PBLG samples.

Figure S2 Overlay of the normalized light scattering GPC traces of PBLGₙ.
TFA and temperature induced helix-coil transitions studies for PBLGs. (Figure S3-S33). Solvent and temperature induced helix-coil transition studies were carried out on a Bruker DRX 500 MHzs spectrometer. To prevent the potential aggregation of PBLG chains, at least 1% TFA-d has been added into the solution. Methods of Goodman and Marborough were applied to study TFA/Temperature induced helix-coil transition of PBLGs. For TFA induced helix-coil transition studies, PBLG containing macromolecules at different solvent compositions were studied at 300K. While for temperature induced helix-coil transition, PBLG containing macromolecules at predetermined solvent compositions were measured at different temperatures. All the temperature calibrations were performed by measuring the OH resonances and CH₈ resonances in either Methanol or Ethylene glycol. The chemical shifts at 4.0 ppm and 4.6 ppm are used to identify the α-helix and random coil structures, respectively. The fractional helicity was calculated by dividing the total α-CH content by the α-CH that existing in helical form. On increasing the volume fraction of TFA in CDCl₃/TFA-d or decreasing the temperature of the solution, the α-helix conformation in homo-PBLGs diminishes and eventually disappears, as can be seen from the reduced intensity of α-CH peak at 4.0 ppm. The TFA/Temperature induced helix-coil transitions are more apparent when plotting the helical contents of PBLGs as a function of TFA volume fraction and temperature.
Figure S3 $^1$H NMR spectra of TFA induced helix-to-coil transition of PBLG$_{42}$ in TFA-d/CDCl$_3$ mixture.
Figure S4 $^1$H NMR spectra of Temperature induced helix-to-coil transition of PBLG$_{42}$ in TFA-d/CDCl$_3$ mixture. The TFA volume concentration is 5.0%.

Figure S5 $^1$H NMR spectra of Temperature induced helix-to-coil transition of PBLG$_{42}$ in TFA-d/CDCl$_3$ mixture. The TFA volume concentration is 6.0%.
Figure S6 $^1$H NMR spectra of Temperature induced helix-to-coil transition of PBLG$_{42}$ in TFA-d/CDCl$_3$ mixture. The TFA volume concentration is 7.0%.

Figure S7 Temperature induced helix-to-coil transition of PBLG$_{42}$ in TFA-d/CDCl$_3$ mixture.
Figure S8 $^1$H NMR spectra of TFA induced helix-to-coil transition of PBLG$_{90}$ in TFA-d/CDCl$_3$ mixture.
**Figure S9** $^1$H NMR spectra of Temperature induced helix-to-coil transition of PBLG$_{90}$ in TFA-d/CDCl$_3$ mixture. The TFA volume concentration is 6.0%.

**Figure S10** $^1$H NMR spectra of Temperature induced helix-to-coil transition of PBLG$_{90}$ in TFA-d/CDCl$_3$ mixture. The TFA volume concentration is 7.0%.
**Figure S11** $^1$H NMR spectra of Temperature induced helix-to-coil transition of PBLG$_{90}$ in TFA-d/CDCl$_3$ mixture. The TFA volume concentration is 8.0%.

**Figure S12** $^1$H NMR spectra of Temperature induced helix-to-coil transition of PBLG$_{90}$ in TFA-d/CDCl$_3$ mixture.
**Figure S13** $^1$H NMR spectra of TFA induced helix-to-coil transition of PBLG$_{147}$ in TFA-d/CDCl$_3$ mixture.
Figure S14 $^1$H NMR spectra of Temperature induced helix-to-coil transition of PBLG$_{147}$ in TFA-d/CDCl$_3$ mixture. The TFA volume concentration is 6.0%

Figure S15 $^1$H NMR spectra of Temperature induced helix-to-coil transition of PBLG$_{147}$ in TFA-d/CDCl$_3$ mixture. The TFA volume concentration is 7.0%
Figure S16 $^1$H NMR spectra of Temperature induced helix-to-coil transition of PBLG$_{147}$ in TFA-d/CDCl$_3$ mixture. The TFA volume concentration is 8.0%.

Figure S17 Temperature induced helix-to-coil transition of PBLG$_{147}$ in TFA-d/CDCl$_3$ mixture.
Figure S18 $^1$H NMR spectra of TFA induced helix-to-coil transition of PBLG$_{232}$ in TFA-d/CDCl$_3$ mixture.
Figure S19 $^1$H NMR spectra of Temperature induced helix-to-coil transition of PBLG$_{232}$ in TFA-d/CDCl$_3$ mixture. The TFA volume concentration is 6.0%.

Figure S20 $^1$H NMR spectra of Temperature induced helix-to-coil transition of PBLG$_{232}$ in TFA-d/CDCl$_3$ mixture. The TFA volume concentration is 7.0%.
Figure S21 Temperature induced helix-to-coil transition of PBLG<sub>232</sub> in TFA-d/CDCl<sub>3</sub> mixture.
Figure S22 $^1$H NMR spectra of TFA induced helix-to-coil transition of PBLG$_{308}$ in TFA-d/CDCl$_3$ mixture.
Figure S23 $^1$H NMR spectra of Temperature induced helix-to-coil transition of PBLG$_{308}$ in TFA-d/CDCl$_3$ mixture. The TFA volume concentration is 6.0%.

Figure S24 $^1$H NMR spectra of Temperature induced helix-to-coil transition of PBLG$_{308}$ in TFA-d/CDCl$_3$ mixture. The TFA volume concentration is 7.0%.
Figure S25 Temperature induced helix-to-coil transition of PBLG$_{232}$ in TFA-d/CDCl$_3$ mixture.
Figure S26 $^1$H NMR spectra of TFA induced helix-to-coil transition of PBLG$_{484}$ in TFA-d/CDCl$_3$ mixture.
Figure S27 ¹H NMR spectra of Temperature induced helix-to-coil transition of PBLG₄₈₄ in TFA-d/CDCl₃ mixture. The TFA volume concentration is 6.0%.

Figure S28 ¹H NMR spectra of Temperature induced helix-to-coil transition of PBLG₄₈₄ in TFA-d/CDCl₃ mixture. The TFA volume concentration is 7.0%.
Figure S29 Temperature induced helix-to-coil transition of PBLG$_{484}$ in TFA-d/CDCl$_3$ mixture.
Figure S30 $^1$H NMR spectra of TFA induced helix-to-coil transition of PBLG$_{1228}$ in TFA-d/CDCl$_3$ mixture.
Figure S31 $^1$H NMR spectra of Temperature induced helix-to-coil transition of PBLG$_{1228}$ in TFA-d/CDCl$_3$ mixture. The TFA volume concentration is 6.0%.

Figure S32 $^1$H NMR spectra of Temperature induced helix-to-coil transition of PBLG$_{1228}$ in TFA-d/CDCl$_3$ mixture. The TFA volume concentration is 7.0%.
Figure S33 Temperature induced helix-to-coil transition of PBLG$_{1228}$ in TFA-d/CDCl$_3$ mixture.
NOESY study of linear PBLGs. (Figure S34-S36). Polymers for NOESY experiments were dissolved in 98:2 d-CDCl3:d-TFA and sealed in NMR tubes to prevent solvent evaporation. 2D NOESY experiments were performed on a Bruke DRX-500 MHz spectrometer with the (π/2)-t1-(π/2)-τm-(π/2)-t2 pulse sequence. 2048K spectra were acquired with a sweep width of 6510 Hz in each dimension. The π/2 pulse width was 8.4 μs, τm was 100 ms, and the delay between acquisitions was 2 s.

Figure S34 2D NOESY spectra of PBLG232 obtained by the (π/2) -t1-(π/2)-τm-(π/2)-t2 pulse sequence
Figure S35 2D NOESY spectra of PBLG$_{308}$ obtained by the (π/2) -t1-(π/2)-τm-(π/2)-t2 pulse sequence
Figure S36 Model-based analysis of temperature-induced helix-coil transition of two PBLG samples (DP of 232 and 308) at specific solvent conditions. (A) CDCl₃:TFA-d = 94:6, the solid lines were based on Zimm-Bragg model with σ = 5.26 ×10⁻⁵ and ΔHₒ = 5.84 kJ/mol, but the value of n was set to be 222 for PBLG232 and 219 for PBLG308 to get a good curve fit; (B) CDCl₃:TFA-d = 93:7, the solid lines were based on Zimm-Bragg model with σ = 2.74 ×10⁻⁵ and ΔHₒ = 5.37 kJ/mol, but the value of n was set to be 222 and 219 for PBLG232 and PBLG308 to get a good curve fit.