**Supporting Information**

# Nanoscale phase separation in sequence-defined peptoid diblock copolymers

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## Experimental Section

### Synthesis of peptoid polymers

Polypeptoids were synthesized on a custom-built automated robotic synthesizer or a commercial Aapptec Apex 396 robotic synthesizer on 100 mg of Rink amide polystyrene resin (0.61 mmol/g, Novabiochem, San Diego, CA). One of the primary amine submonomers, 2-(2-(2-methoxyethoxy)ethoxy)ethyl amine was synthesized with the same protocol as previously described.[1] All the other monomers, solvents, and reagents described here were purchased from commercial sources and used without further purification. The synthesis procedure was a modified version of methods previously described.[2] The Fmoc group on the resin was deprotected with 20% (v/v) 4-methylpiperidine/dimethylformamide before starting the submonomer cycle. An acylation step was then performed on the amino resin by the addition of 1.0 mL of 1.2 M bromoacetic acid in DMF and 0.18 mL of *N*, *N*'-diisopropylcarbodiimide and mixing for 20 min. Displacement of the bromide with various submonomers occurred by adding a 1.0-2.0 M solution of the primary amine in N-methyl-2-pyrrolidone, followed by agitation for 120 min. All the polymers were acetylated on the resin after synthesis using a mixture (2.0 mL

per 100 mg of resin) of 0.4 M acetic anhydride and 0.4 M pyridine in DMF for 30 min. The crude peptoid products were cleaved from the resin by the addition of 50% (v/v) trifluoracetic acid (TFA) in dichloromethane for 20 min, followed by evaporation under a stream of $N_2$. The crude products were then dissolved in 1:1 mixture (v/v) of (50:50 isopropanol: acetonitrile)/water and lyophilized. The polypeptoids were then purified by reverse-phase HPLC on a C4 column (Vydac, 10-15 μm, 22mm x 250 mm) using a linear gradient of 50-100% (50:50 isopropanol:acetonitrile) in water with 0.1% TFA over 60 min at a flow rate of 10 mL/min. The polypeptoids with $\phi_{Nte} < 0.32$ were dissolved in acetonitrile and precipitated from diethyl ether and washed with water for three times.

Each final product was characterized by analytical reverse-phase HPLC using a C4 column (Vydac 214TP, 5 μm, 4.6mm x 150 mm) with 50-100% gradient at 1mL/min over 30min at 60$^{o}$C. Polypeptoid purity was determined using the analytical reverse-phase HPLC conditions detailed above, and the molecular weight was determined by matrix-assisted laser desorption/ionization mass spectrometry (Applied Biosystems MALDI TOF/TOF Analyzer 4800) with a 1:1 (v/v) mixture of polypeptoid [2 mg/mL in 1:1 (50:50 isopropanol:acetonitrile):water] and 1,8,9-dianthracenetriol dissolved in tetrahydrofuran at 10 mg/mL. The final polypeptoids were then lyophilized prior to subsequent measurements.

**Small angle X-ray scattering (SAXS)**

The polypeptoids were loaded into the center of Garolite G-10 spacers with 0.125 μm thickness and a central hole with a diameter of 3.86 mm. All samples were dried in a heated antechamber of an argon glove box, and sealed in the glove box before being well annealed (at various temperatures depending on the block copolymer). Measurements were performed at

beamline 7.3.3 at the Advanced Light Source (ALS) at Lawrence Berkeley National Laboratory and beamline 1-4 at the Stanford Synchrotron Radiation Laboratory (SSRL). Samples were loaded on a custom-built temperature stage and annealed at each temperature for 15~20 min before taking measurements. A silver behenate sample was used as a standard. Full two-dimensional scattering patterns were collected on an ADSC CCD detector. The scattering patterns from ALS were reduced using the Nika program for Igor Pro available from Jan Ilavsky at Argonne National Laboratory[3], and data from SSRL were reduced using a program written by John Pople at SSRL.

**Differential scanning calorimetry**

Differential scanning calorimetry (DSC) experiments were performed to determine the thermal behavior of the synthesized peptoids using a TA Q200 Differential Scanning Calorimeter. In all tests, a scan rate of 10 K/min was used in the temperature range of -60 to 200 $^{o}$C for three heating and cooling cycles.

**X-ray Diffraction**

X-ray diffraction (XRD) was performed at beamline 8.3.1 at the Advanced Light Source located at Lawrence Berkeley National Laboratory. Samples were made under the same condition as those for SAXS. Approximately 1 mg of sample was loaded onto a nylon loop and measured.

**Density determination**

The density of poly-*N*-(2-ethylhexyl)glycine (pNeh) and poly-*N*-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)glycine (pNte) was measured to be 0.97±0.01 g/cm$^3$ and 1.23±0.01 g/cm$^3$ respectively using a combination of a density gradient column and mass/volume method at room temperature. An aqueous sucrose gradient and toluene/tetrachloride carbon gradient were used in the density gradient column method.[4] In the method of mass/volume, the polymer was hot pressed into a washer and weighed before/after the loading of the sample. The cylinder volume equation was used for the space volume calculation. The measured densities were used to calculate the volume fraction of the polypeptoids.

**Circular dichroism (CD)**

CD measurements were performed on a J-185 CD spectrometer (Jasco Inc., Easton, MD). Stock solutions of the polypeptoids were made in vials using 5 mg/mL of peptoid powder in acetonitrile. The stock solutions were then diluted to a concentration of 0.1 mg/mL before acquiring CD spectra. CD spectra were acquired using a quartz cell (Hellma USA, Plainview, NY) with a path length of 1 mm. A scan rate of 50 nm/min was used, and 3 measurements were averaged for each compound.

**Polarized optical microscope**

Approximately 1-2 mg of samples were quickly loaded onto a glass slide from sealed cells in the glove box. The sample apparatus was placed in a Leica DM 4500P microscope, and data were collected using a 5× lens. All the samples except the one with $\phi_{Nte} = 0.65$ were bright when observed under crossed polarizers. This is consistent with previous work on the optical properties of lamellar diblock copolymers.[5]

**Calculations of polydispersity indices**

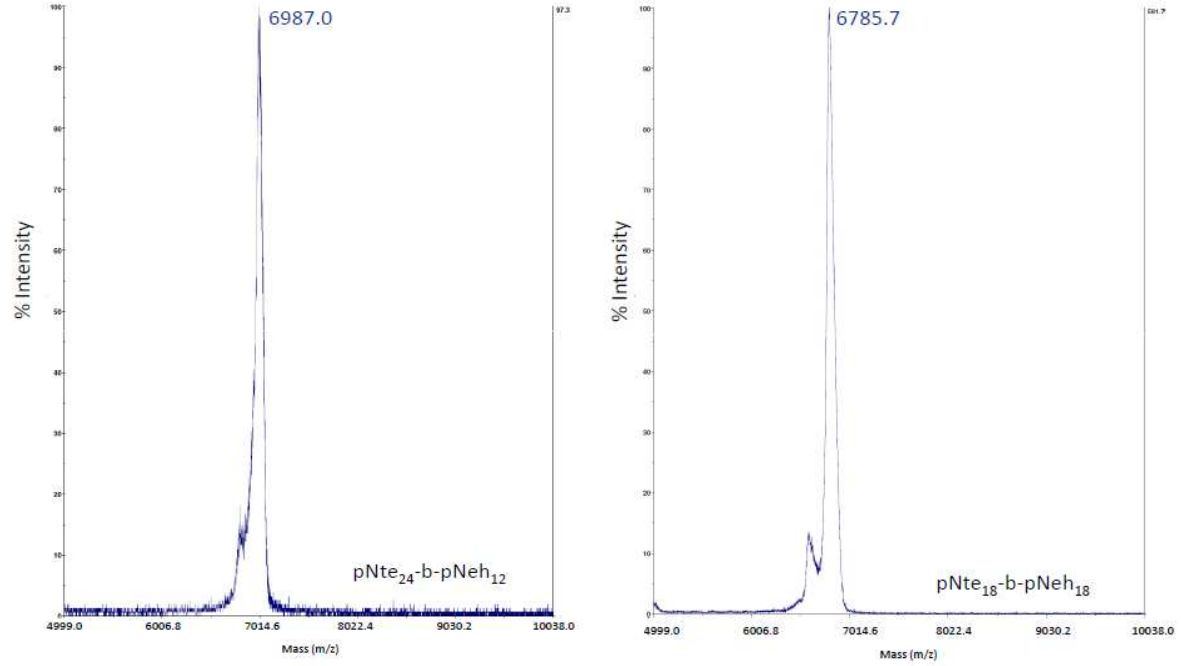
Polydispersity indices (PDIs) are calculated based on the results of MALDI and analytical HPLC.

$PDI = M_W/M_N = (M_n * W_n\% + M_{n-1} * W_{n-1}\% + \ldots)*( W_n\% / M_n + W_{n-1}\% / M_{n-1} + \ldots)$

$M_n$ is the mass of n-mer (the peptoid polymer that we designed); $M_{n-1}$ is the mass of (n-1)-mer; …

$W_n\%$ is the weight fraction of n-mer (purity from analytical HPLC); $W_{n-1}\%$ is the weight fraction of (n-1)-mer; …

$W_n\% + W_{n-1}\% + \ldots = 100\%$



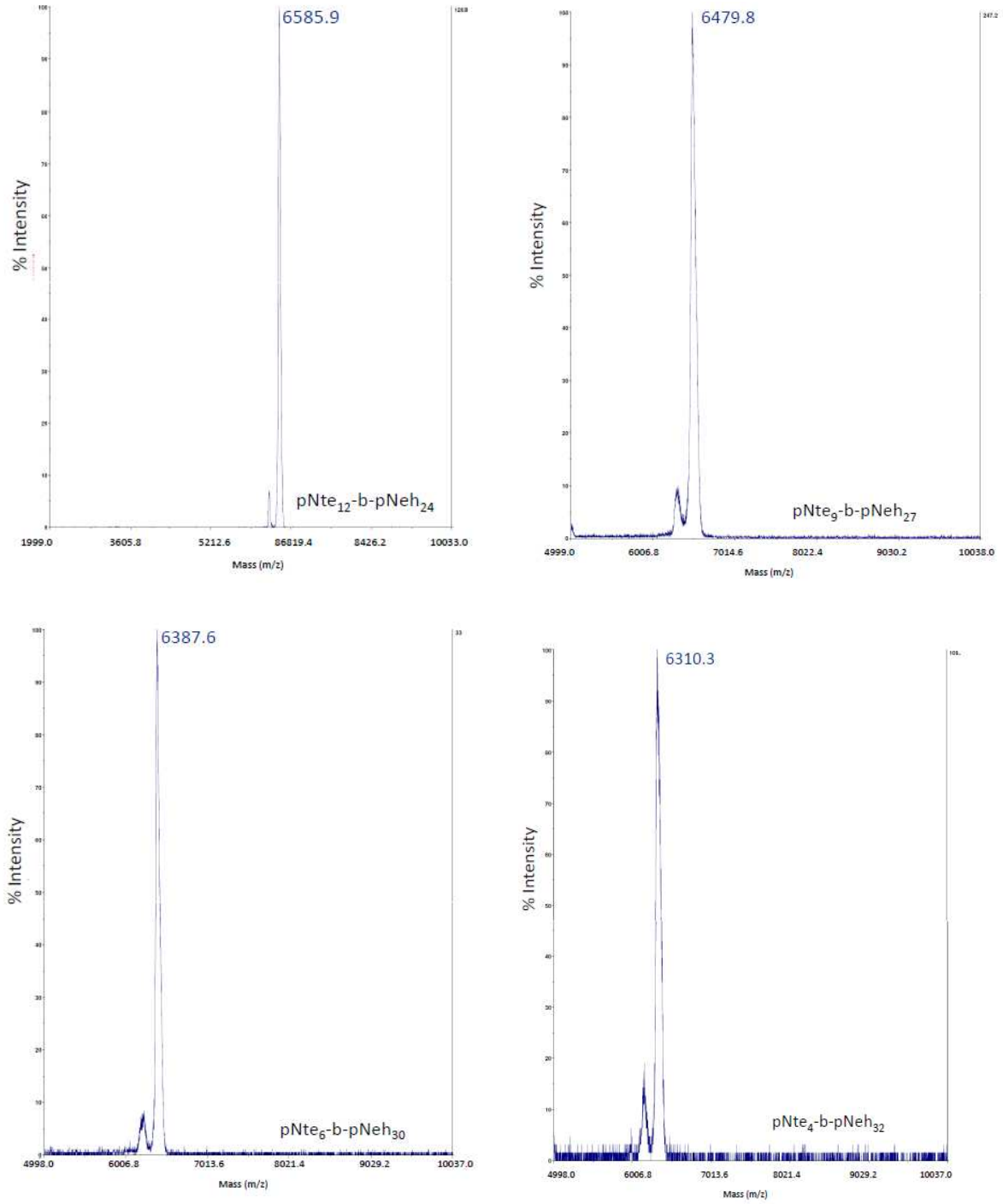



**Figure S1.** MALDI mass spectrum of diblock copolymer pNte-b-pNeh. The observed molecular weight is increased by 23 due to sodium from the matrix used.

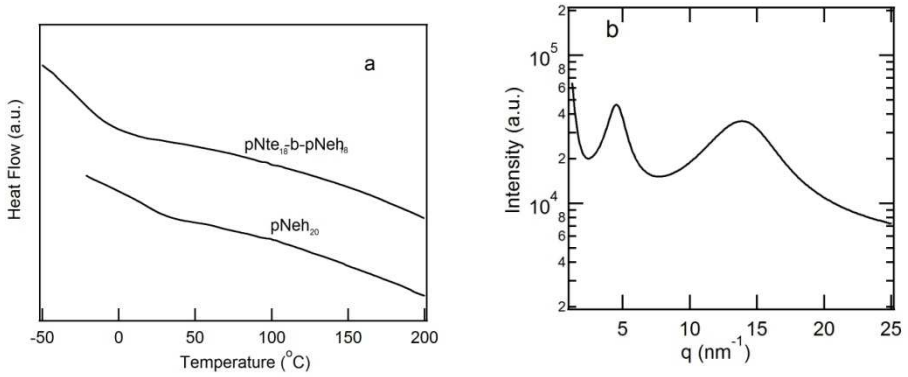



**Figure S2.** (a) DSC endotherm of $pNte_{18}$-b-$pNeh_{18}$. The lack of a melting peak indicates an amorphous structure. (b) XRD spectra of $pNte_{18}$-b-$pNeh_{18}$ at room temperature. Broad peaks indicates a disordered structure in high q range.

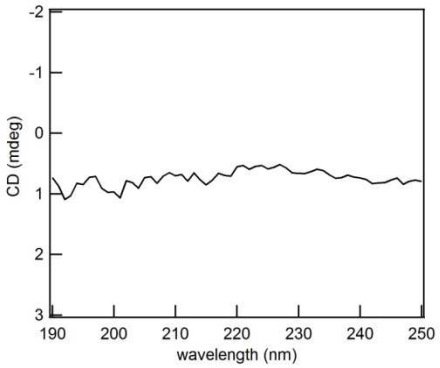



**Figure S3.** CD Spectra of $pNte_{18}$-b-$pNeh_{18}$.

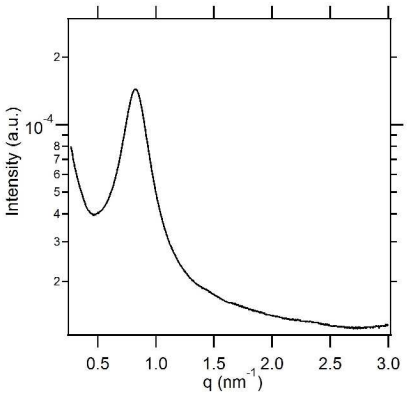



**Figure S4**. SAXS profiles at room temperature for a blend of pNte-*b*-pNeh polymer samples. Three purified peptoids, with $\phi_{Nte}$ of 0.49, 0.32, 0.24, were combined at molar ratio of 1:3:1. This creates a defined heterogenous mixture with an average $\phi_{Nte}$ of 0.34. The broad peak observed in the SAXS profile indicates a disordered structure for the blend. This indicates that the lamellar ordering observed is closely coupled to the high purity of the polypeptoids.

**References**:

(1) Sun, J.; Stone, G. M.; Balsara, N. P.; Zuckermann, R. N. *Macromolecules* **2012**, *45*, 5151-5156.
(2) Zuckermann, R. N. *Curr. Opin. Struc. Bio.* **1993**, *3*, 580-584.
(3) Ilavsky, J. *J. Appl. Crystallogr.* **2012**, *45*, 324-328.
(4) Rosales, A. M.; McCulloch, B. L.; Zuckermann, R. N.; Segalman, R. A. *Macromolecules* **2012**, *45*, 6027-6035.
(5) Balsara, N. P.; Garetz, B. A.; Dai, H. J. *Macromolecules* **1992**, *25*, 6072-6074.