Electronic Supplementary Material (ESI) for ChemComm.

Supplementary Information

for

Influence of Nucleobase Stoichiometry on the Self-Assembly of ABC Triblock Copolymers

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Materials. *n*-Butyl acrylate (*n*BA, 99+%) was purchased from Aldrich and passed through neutral alumina columns before use. α,α'-Azobis(isobutyronitrile) (AIBN, Fluka, 99%) was recrystallized from methanol. 1,4-Butanediol diacrylate (Alfa Aesar, 99%) was used without further purification. Adenine (A, 99%), thymine (T, 99%), triethylamine (TEA, 99%), potassium carbonate (99%), 2-cyano-2-propyl dodecyl trithiocarbonate (CPDT, 97%), and 2,6-di-*tert*-butyl-4-methylphenol (BHT, 99%) were purchased from Aldrich and used without further purification. Hexane (HPLC grade), chloroform ($CHCl_3$, HPLC), tetrahydrofuran (THF, HPLC grade), dimethylsulfoxide (DMSO, HPLC grade) and *N,N*-dimethylformamide (DMF, HPLC grade, anhydrous) were purchased from Fisher Scientific and used as received.

Analytical Methods. ^1H NMR spectra were collected in $CDCl_3$ or DMSO-d_6 on a Varian INOVA spectrometer operating at 400 MHz at 23 °C. Differential scanning calorimetry (DSC) was performed under a nitrogen flush of 50 mL/min at a heating rate of 10 °C/min on a TA instruments Q1000 DSC, which was calibrated using indium (mp = 156.60 °C) and zinc (mp = 419.47 °C) standards. Glass transition temperatures were measured as the midpoint of the transition in the second heating scan. DMA was conducted on a TA Instruments Q800 in tension mode at a frequency of 1 Hz, an oscillatory amplitude of 8 μm, and a static force of 0.01 N. The temperature ramp was 3 °C/min. The glass transition temperature (T_g) was determined at the peak maximum of the tan δ curve.

SAXS experiments were performed using a Rigaku S-Max 3000 3 pinhole SAXS system, equipped with a rotating anode emitting X-ray with a wavelength of 0.154 nm (Cu Kα). The sample-to-detector distance was 1600 mm, and q-range was calibrated using a silver behenate standard. Two-dimensional SAXS patterns were obtained using a fully integrated 2D multiwire, proportional counting, gas-filled detector, with an exposure time of 2 hours. All the SAXS data were analyzed using the SAXSGUI software package to obtain radially integrated SAXS intensity versus scattering vector q, where $q = (4\pi/\lambda)\sin(\theta)$, θ is one half of the scattering angle and λ is the wavelength of X-ray.

Ultrathin sections of PTBA copolymer films were prepared using a CR-X Cryosectioning System (RMC Boeckeler). Ethanol was used as a solvent for cryo-microtome with the diamond knife (Micro Star) temperature set at -85°C and cutting thickness controlled at ~100nm. Sample sections were collected on Cu TEM grids and stained with iodine (99%) overnight. PTBA copolymer films were imaged using a transmission electron microscope (FEI Titan 300) operated at 300 kV. A Veeco MultiMode scanning probe microscope provided AFM phase and height images using the tapping mode. Samples were imaged with Veeco's Nanosensor silicon tips with spring constants of 10-100 N at set-point ratios near 0.6. The contrast and brightness of the AFM phase image in Fig. 1c and the TEM image in Fig. 3 was adjusted for clear images.

Synthesis and processing conditions. A three-step RAFT polymerization afforded a series of ABC block copolymers with complementary nucleobase-functionalized external blocks and poly(*n*BA) central block, following procedures from Long *et al.*[1] Polymerization of adenine acrylate (AdA) and thymine acrylate (ThA) monomers allowed the synthesis of adenine- and thymine-functionalized external blocks, respectively. ^{1}H NMR spectroscopy determined the number-average molecular weight (M_n) of all poly(ThA-*b*-*n*BA-*b*-AdA) block copolymers, abbreviated as PTBA (Table S1). PTBA triblock copolymers were solution cast from DMSO into non-patterned PTFE molds, dried, and annealed to form free-standing films ranging from 0.2 mm to 0.5 mm in thickness, in accordance with previously reported procedures.[1]

Thermal properties. Thermogravimetric analysis revealed $T_{d,5wt\%}$ weight loss temperatures of PTBA copolymer ranged from 280 °C to 290 °C, and differential scanning calorimetry showed two T_g's for each PTBA copolymer near -45 °C and 70 °C respectively (Fig. S1,S2, Table S2). The lower T_g corresponded to the poly(*n*BA) soft phase, and the higher T_g ranged from 60 °C to 71 °C with varying adenine to thymine ratio, as poly(AdA) showed slightly higher T_g than poly(ThA).[2] Each PTBA copolymer exhibited only one T_g above room temperature, as the hydrogen bonding between adenine- and thymine-functionalized blocks contributed to a single hard phase, consistent with earlier work. [1]

Table S1. PTBA triblock copolymer compositions, thymine-adenine (T-A) ratios, nucleobase block weight percent, and morphologies, determined using AFM and SAXS.

Poly(ThA) (kDa)	DP (ThA)	Poly(*n*BA) (kDa)	DP (*n*BA)	Poly(AdA) (kDa)	DP (AdA)	T-A ratio	M_n total	NB wt%	Morphology
9.4	28.9	4.3	333.6	10.1	30.3	1.0	23.8	82	No order
9.4	28.9	20.0	156.3	7.8	23.3	1.2	37.2	46	Disordered Lamellae
9.4	28.9	19.3	150.8	6.8	20	1.4	35.5	46	Disordered Lamellae
9.4	28.9	20.0	156.3	3.4	10.2	2.8	32.8	39	Ordered Lamellae
9.4	28.9	26.0	203.1	10.0	29.9	1.0	45.4	43	Disordered Lamellae
9.4	28.9	27.2	212.5	5.3	16	1.8	41.9	35	Ordered Lamellae
9.4	28.9	33.8	264.1	10.7	32.1	0.9	53.9	37	Disordered biphasic
9.4	28.9	33.8	264.1	6.6	19.8	1.5	49.8	32	Disordered Lamellae
9.4	28.9	31.5	246.1	4.6	14	2.1	45.5	31	Ordered Lamellae

9.4	28.9	39.3	307.0	11.6	34.7	0.8	60.3	35	Disordered Lamellae
9.4	28.9	39.3	307.0	6.8	20.4	1.4	55.5	29	Disordered Lamellae
5.8	17.9	23.6	184.4	1.8	5.55	3.2	31.2	24	Ordered Lamellae
5.8	17.9	11.5	89.8	10.5	31.6	0.6	27.8	59	Cylinders

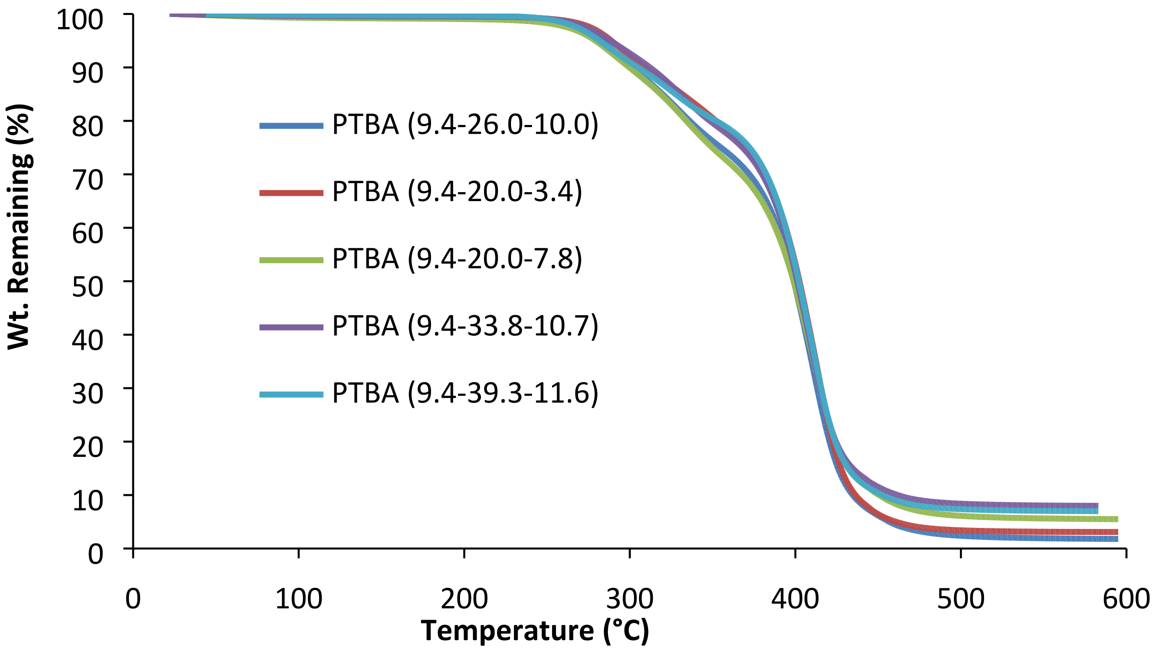


Figure S1. Representative TGA thermograms of PTBA copolymers.

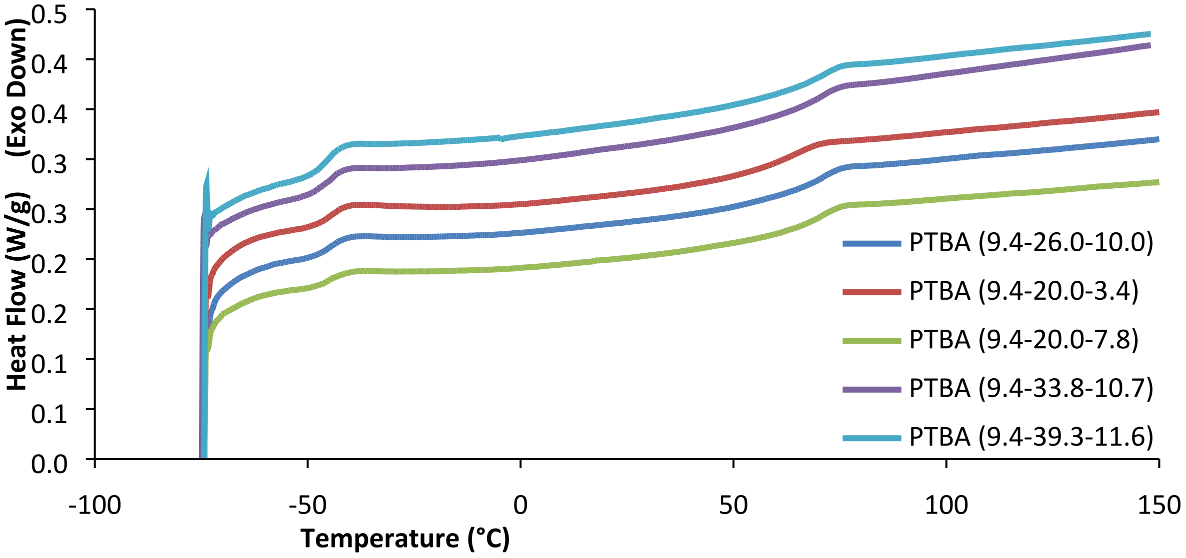


Figure S2. Representative DSC traces of PTBA copolymers.

Table S2. Compositions and thermal properties of representative PTBA copolymers.

PTBA Composition (kDa)	9.4-26.0-10.0	9.4-20.0-3.4	9.4-20.0-7.8	9.4-33.8-10.7	9.4-39.3-11.6
Total (kDa)	45.4	32.8	37.2	53.9	60.3
NB mol%	23%	20%	25%	19%	17%
NB wt %	43%	39%	46%	37%	35%
DP	29-203-30	29-156-10	29-156-23	29-264-32	29-307-35
$T_{d,\ 5wt\%}$ (°C)	283	289	280	281	282
T_g (°C)	-45, 71	-45, 64	-46, 71	-44, 71	-45, 71

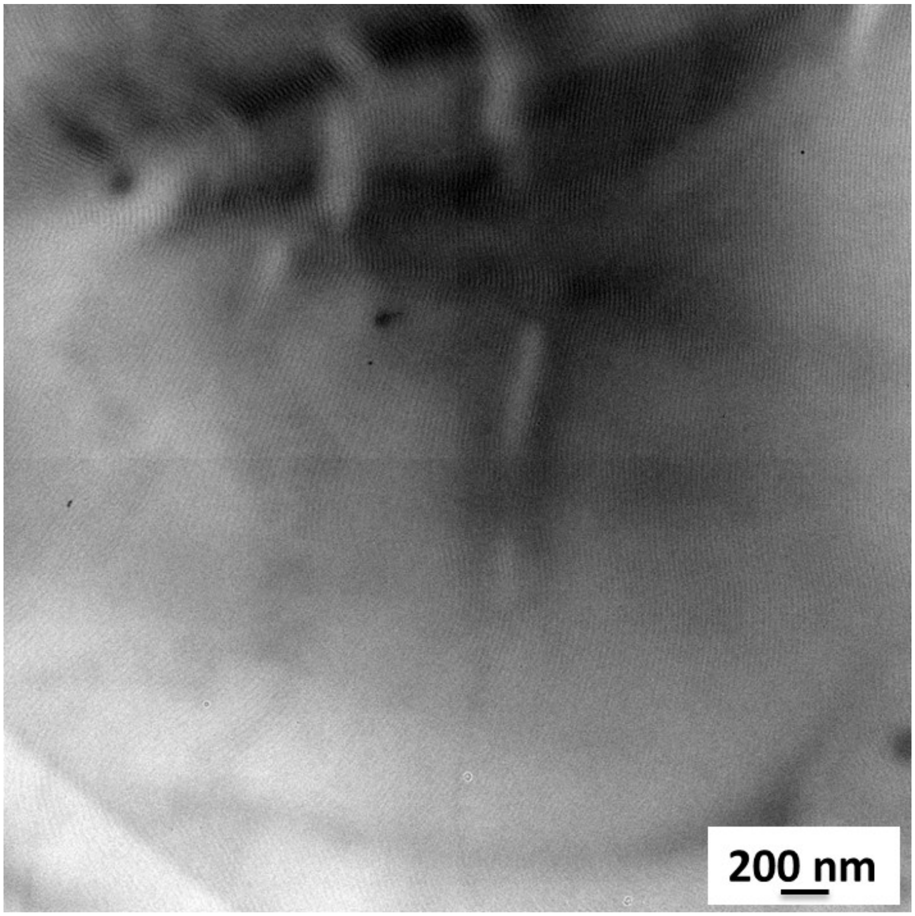
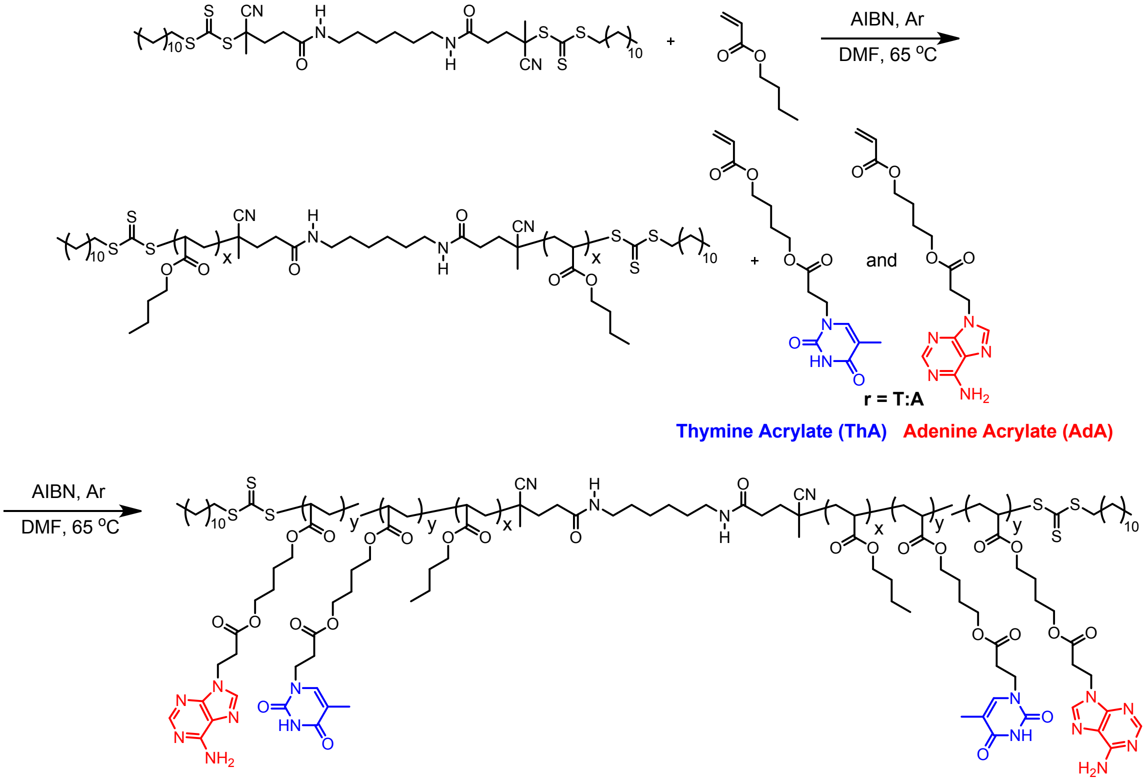


Figure S3. TEM image of PTBA (9.4-31.5-4.6) film.

Two symmetric ABA triblock copolymers carrying both thymine and adenine on the external blocks served as controls to investigate the necessity of asymmetric triblock structure in forming ordered lamellar morphology. A two-step RAFT polymerization with a difunctional chain transfer agent[3] yielded two ABA triblock copolymers poly((ThA-*co*-AdA)-*b*-*n*BA-*b*-(ThA-*co*-AdA)), abbreviated as P(TA-B-TA) using a modified procedure from Long *et al.* (Scheme S1).[2] The external blocks were random copolymers of ThA and AdA, considering the identical structure of these two monomers near their acrylic backbones and the absence of templating effect in polar solvents.[2,4] The initial monomer charge ratios of 1.0 and 2.0 matched with T-A ratios of 1.0 and 1.9 in the purified block copolymers, respectively. M_n of P(TA-B-TA) with T-A 1.0 was

12.5-44.8-12.5 kDa and M_n of P(TA-B-TA) with T-A 1.9 was 9.4-44.8-9.4 kDa. The nucleobase blocks comprised 35 wt% and 30 wt% for P(TA-B-TA) with T-A 1.0 and T-A 1.9, respectively.



Scheme S1. RAFT polymerization of poly((ThA-*co*-AdA)-*b*-*n*BA-*b*-(ThA-*co*-AdA)) ABA triblock copolymers.

The SAXS profile of P(TA-B-TA) T-A 1.0 showed broad scattering maxima with periodic intensity oscillations that roughly matched q, $\sqrt{3}$q, $\sqrt{4}$q, $\sqrt{7}$q, and $\sqrt{9}$q peak positions for hexagonally packed cylindrical morphology (Figure S6). The AFM phase image also depicted a cylindrical surface morphology with a distribution of inter-domain spacing, agreeing with the SAXS results. On the AFM phase image, nucleobase-functionalized external blocks self-assembled into the cylindrical hard domains (light areas), distributed in the poly(*n*BA) soft matrix (dark areas), and all cylinders pointed towards the film surface. The morphology of P(TA-B-TA) T-A 1.0 matched closely with the supramolecular blend of poly(AdA-*b*-*n*BA-*b*-AdA) and poly(ThA-*b*-*n*BA-*b*-ThA) with similar block length and T-A ratio,[2] while the PTBA analog with

T-A of 1.2 and 35 NB wt% exhibited a mixed cylindrical-lamellar morphology. SAXS and AFM results of P(TA-B-TA) T-A 1.9 showed a mixed cylindrical-lamellar morphology, distinctively different from the ordered lamellar morphology of PTBA (9.4-31.5-4.6) with similar T-A ratio and NB wt%. As a result, the presence of both thymine and adenine on a single polymer chain with optimal T-A ratio proved insufficient for ordered lamellae formation. The less ordered morphologies of symmetric ABA controls compared to PTBA likely resulted from significant amount of intramolecular hydrogen bonding. The randomly distributed thymine and adenine pendant groups predominately associated with their adjacent partners from the same block, which resulted in microphase-separated morphology of typical non-associating ABA block copolymers. The asymmetric PTBA block copolymer structure facilitated the intermolecular recognition between adenine and thymine units from different polymer chains, which proved crucial for ordered lamellae formation. However, determining the ratio of intra- versus intermolecular hydrogen bonding of P(TA-B-TA) and PTBA remained challenging.

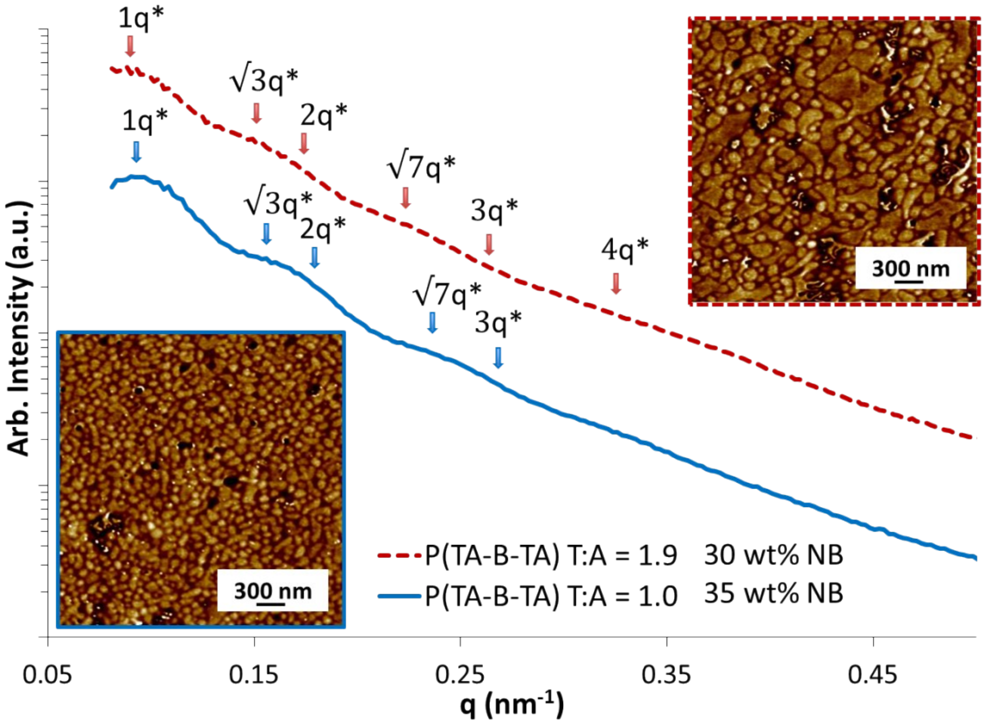


Figure S4. SAXS scattering profiles and AFM phase images of P(TA-B-TA) films with varying thymine-adenine ratio.

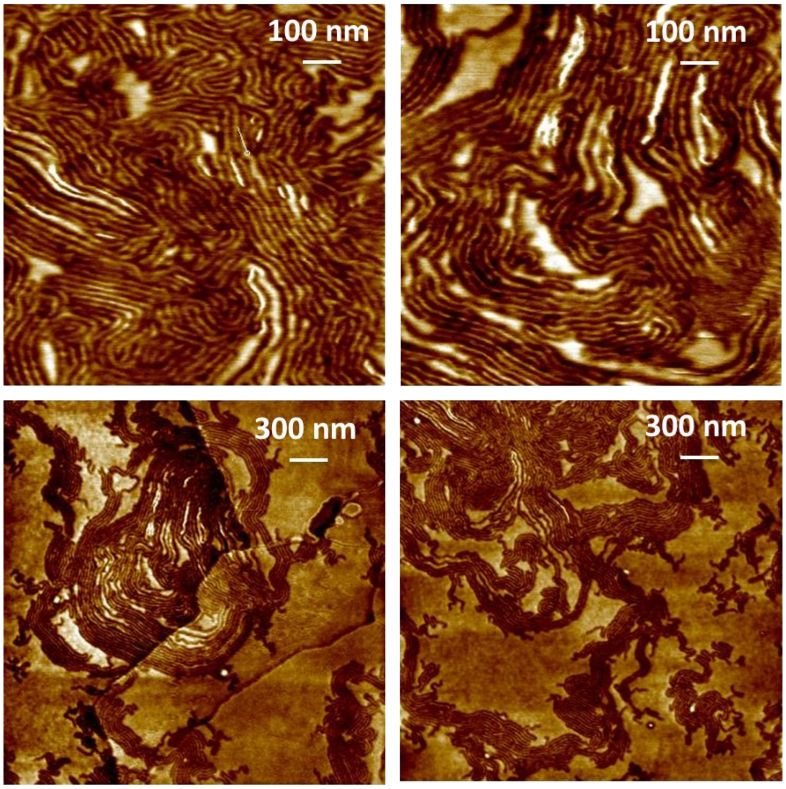


Figure S5. AFM phase images of PTBA (5.8-23.6-1.9) film surface. AFM phase images failed to show lamellar on the PBTA film surface except for PTBA (5.8-23.6-1.9) with 25 wt% NB. It is presumed that the nucleobase-containing hard domain favored the surface to air, and increasing the poly(*n*BA) content forced the soft phase to be present at the film surface. As a result, only PTBA with low NB wt% exhibited lamellae morphology on the film surface.

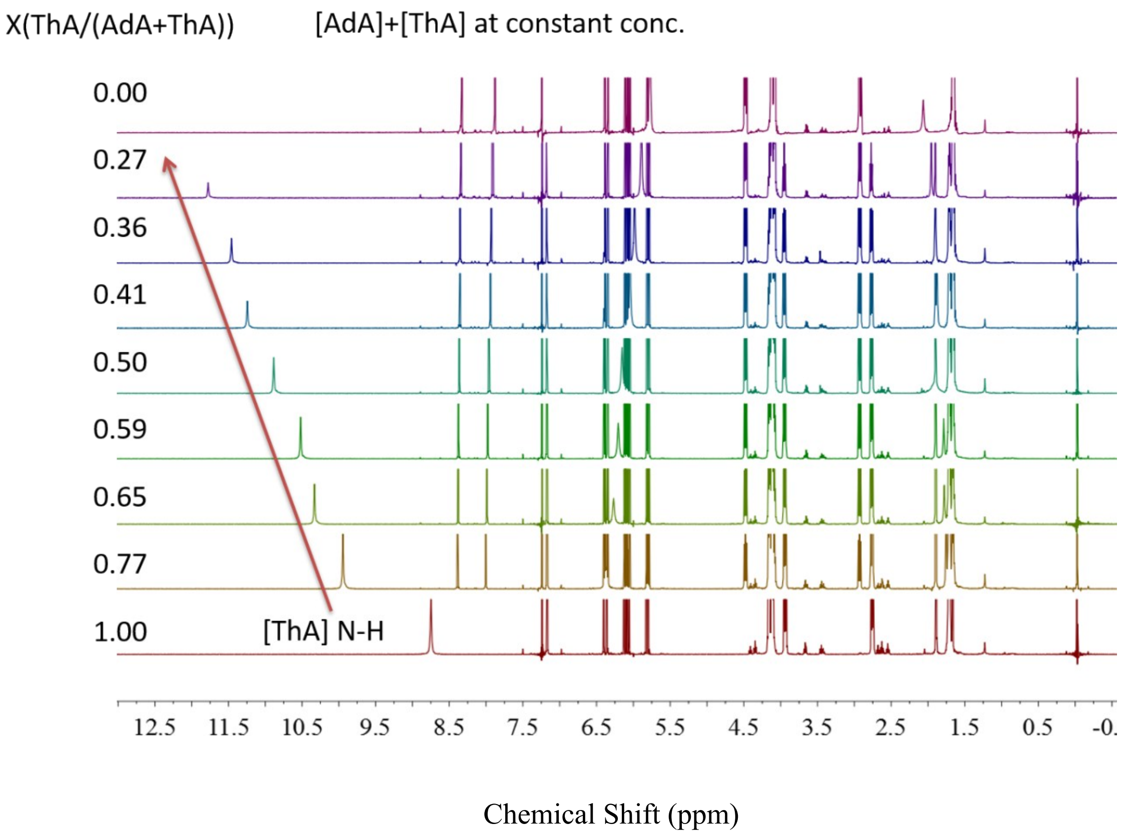


Figure S6. ^{1}H NMR spectra of the AdA and ThA mixture in $CDCl_3$ with varying thymine to adenine ratio at 23 °C.

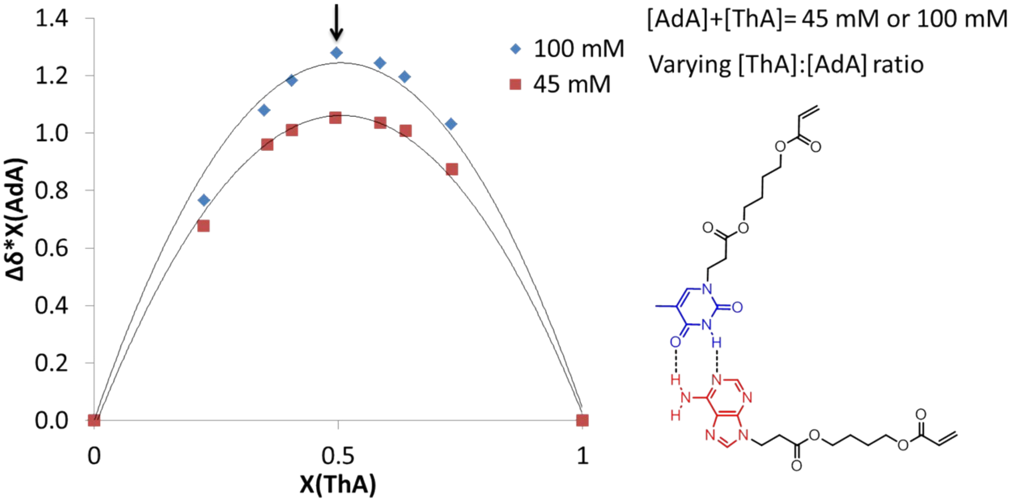


Figure S7. Job's plot of AdA and ThA in $CDCl_3$ with overall concentration of 45 mM and 100 mM. The solubility of ThA and AdA in nonpolar solvents limited their titration at a higher concentration.

Reference

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