Supporting Information for

pH-induced Morphology-shifting of DNA-*b*-Poly(propylene oxide) Assemblies

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Electronic Supplementary Material (ESI) for Chemical Communications

Table of Contents

1. Materials and Methods

Unless otherwise stated, the materials were obtained from commercial suppliers and were used without further purification. The poly(propylene glycol) monobutyl ether (PPO), N-diisopropyl-2-cyanoethylchlorophosphoramidite, diisopropylethylamine and solvents and reagents for DNA synthesis were purchased from Aldrich (Tianjing, China). The water used in all of the experiments was Milli-Q deionized (18.2 MΩ.cm).

All of the DNA was synthesized on an ABI-392 via standard solid phase synthesis on controlled pore glass supports. The coupling reactions of DNA and PPO were performed under dry nitrogen atmosphere with anhydrous solvents.

DNA sequence：

DNA: 5'-TTTCCCCTAACCCC-3'

Control sequence: 5'-TTTAGCTC ATAACT-3'

Matrix-assisted laser desorption-ionization (time of flight) mass spectrometry (MALDI-TOF): MALDI-TOF was performed on a Bruker Biflex III MALDI-TOF spectrometer.

Circular dichroism (CD): CD spectra were recorded on a Chirascan Instrument.

TEM measurements: The sample was applied to a carbon grid by adding a 7 μL drop of sample solution to the grid and carefully removing it after 1 min immersion using a filter paper. The grid was allowed to dry for at least 15 min. before applying 5 μL of a 1 wt% uranyl acetate aqueous solution, which was removed after 15 s. The grid was again allowed to dry for at least 15 min. Samples were studied on a JEOL JEM-1011

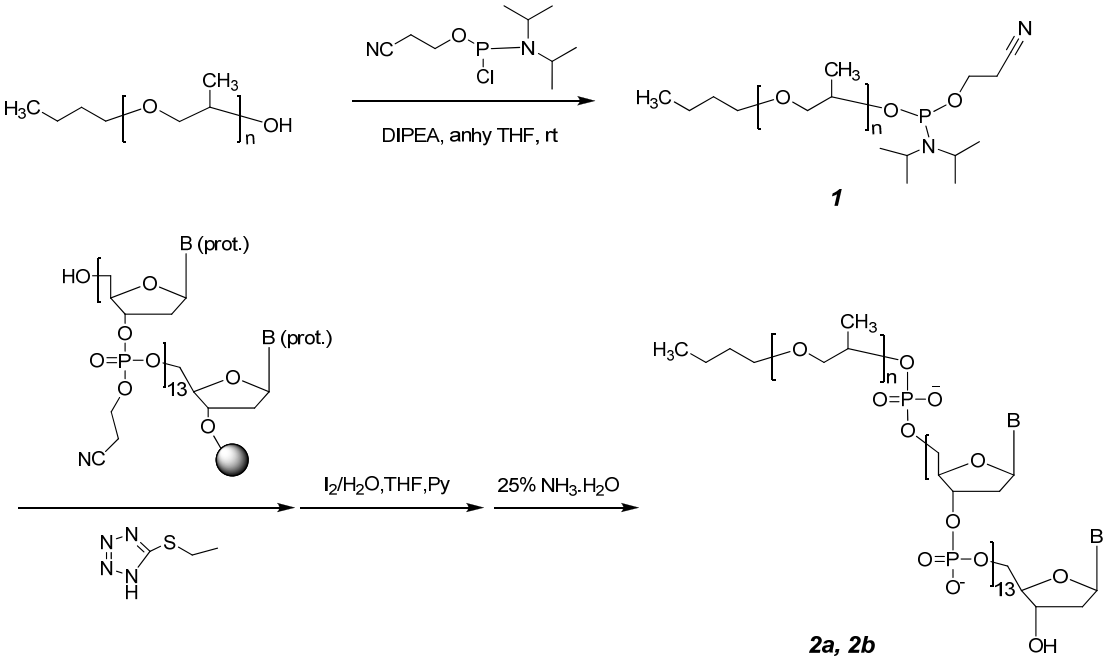
TEM (Jeol, Japan).

Cryo-TEM measurements: samples were prepared in a controlled environment vitrification system (CEVS) at 28 °C. A micropipetee was used to load 5 μL of the samples onto a lacey support TEM grid that was held with tweezers. The excess solution was blotted with a piece of filter paper, which formed a thin film that was suspended on the grid. After waiting for about 10 s to release any stress induced during blotting, the samples were quickly plunged into a reservoir of liquid ethane (cooled by liquid nitrogen) at its melting temperature. The vitrified samples were then stored in liquid nitrogen until they were transferred to a cryogenic sample holder (Gatan 626) and examined with a JEM 2200FS TEM (200 keV) at about -174 °C. The images were recorded on a Gatan multiscan CCD and processed into a digital micrograph.

Fluorescence measurements: the experiments were conducted using a PerkinElmer SL55 Fluorescence Spectrometer.

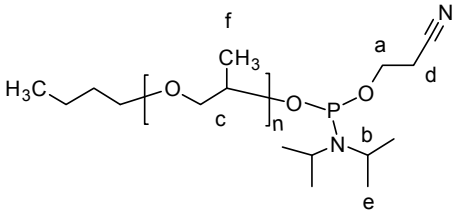
2. Synthesis and Characterization of DNA-*b*-PPO

2.1 Synthetic route of the conjugate hybrids of DNA-*b*-PPO[1]



Scheme S1 Synthesis of DNA-*b*-PPO. B (prot.) = protected nucleobase. PPO (M_n ~ 2000 g mol^{-1}). DNA: 5'-TTTCCCCTAACCCC-3'; DNA control sequence: 5'-TTTAGCTCATAACT-3'. ***2a*** stands for DNA-*b*-PPO, ***2b*** stands for Control DNA hybrids.

2.2 Compound ***1***



Compound ***1*** was synthesized by using Poly(propylene glycol) monobutyl ether with a molecular weight of 2000 g mol^{-1}, following the previous literatures[1], and it was characterized by ^{1}H NMR, ^{13}C NMR, ^{31}P NMR.

^{31}P NMR (162 MHz, $CDCl_3$): 146 ppm

^{1}H-NMR (300 MHz, $CDCl_3$): 3.88 (t, 2H, J = 2.9 Hz, a), 3.55-3.67 (broad, 2H, b) 3.51-3.36 (broad, 102H, c), 2.61 (t, 2H, J=2.4 Hz, d), 1.16 (d, 12H, e), 1.09 (broad,

102H, f)

^{13}C-NMR (75MHz, CDCl3): 13.86, 17.02, 20.09, 20.65, 24.27, 24.39, 42.62, 73.06, 75.02, 117.48

2.3 Compound **DNA-*b*-PPO** and its Control

General Procedure for the preparation of DNA-*b*-PPO hybrids: The CPG-loaded DNA was synthesized using ABI 392 DNA synthesizer at a 1 μmol scale using a standard phosphoramidite DNA synthesis protocol. The DNA-loaded CPG (1 μmol) was transferred into a vial, then 5-ethylthiotetrazole (100 μmol) and compound ***1*** (50 μmol) were added consequently. After dried *in vacuo*, 0.5 mL anhydrous CH_3CN was added under dry nitrogen protection. The reaction mixture was allowed to stay overnight under room temperature. Then the CPG was washed twice with anhydrous CH_3CN, followed by oxidation with iodine and water in CH_3CN. After cleavage by concentrated ammonia solution at 55 °C for 3 hrs, the crude product was purified by 10% denaturing polyacrylamide gel electrophoresis (PAGE) with 0.5 × TBE buffer as the running buffer. The identification of the conjugates was achieved by UV shadowing. The respective bands were excised from the gel and incubated in deionized water for 12 hrs. After centrifugation, the supernatant was desalted with C18 column and dried by lyophilization. The purity of DNA-*b*-PPO and its control sample was assessed by 20% PAGE, followed by staining with Stains All. The samples were stored at -20 °C before use. The products were analyzed by MALDI-TOF.

MALDI-TOF MS: DNA-*b*-PPO 5498 m/z

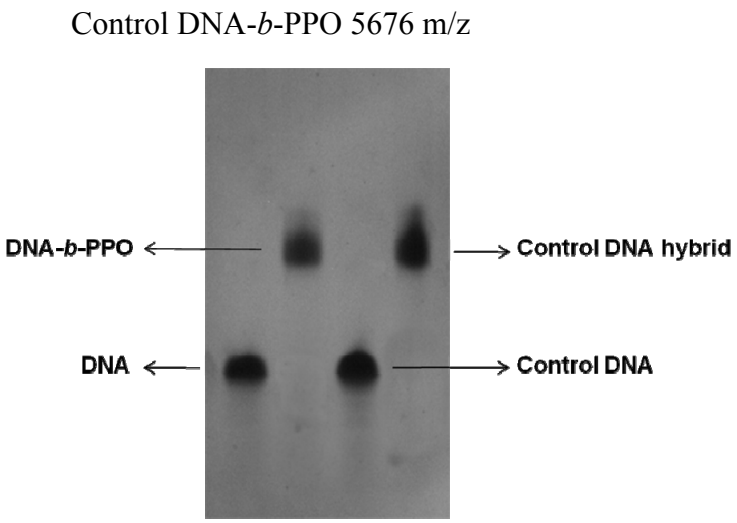


Figure S1 20% Denaturing polyacrylamide gel electrophoresis (Acr=acrylamide, Bis=N, N'-methylenebisacrylamide; Acr/Bis 19:1).

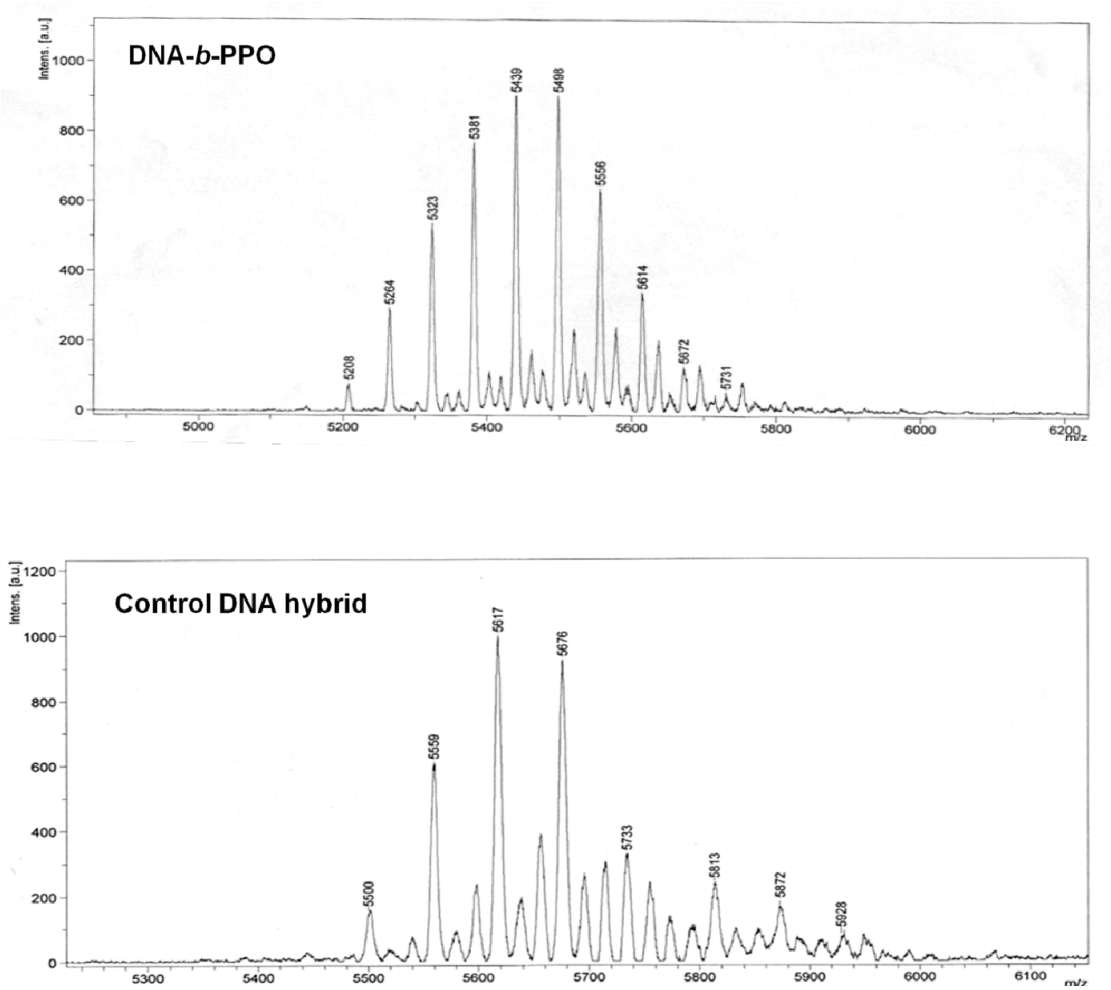


Figure S2 MALDI-TOF spectra of compound DNA-*b*-PPO and control sample. (Matrix: α-Cyano-4-hydroxycinnamic acid)

3. Circular Dichroism (CD)

Supplementary Figure 3: Thermal denaturation spectrum (normalized) for the pristine intermolecular *i*-motif DNA and DNA-*b*-PPO in 50 mM β-morpholinoethanesulfonic acid (MES) buffer (pH 5.0, with 50 mM NaCl) at a temperature ramp rate of 0.5 °C/min at the wavelength of 285 nm. The melting temperature of pristine *i*-motif DNA was found to be about 45.8 °C and the melting temperature of i-motif of the triblock copolymer at pH 5.0 was 76 °C (due to the disassembly of *i*-motif structure). According to our previous study,[2] the increased stability might be attributed to the hydrophobic interactions of the PPO domains at high temperature, which suggests the two PPO domains in the assembled structure are at the same end of the bimolecular i-motif structure. Therefore, this assembled structure could be considered as a supra-amphiphilic molecule with a different hydrophilic/hydrophobic ratio comparing to the diblock copolymer.

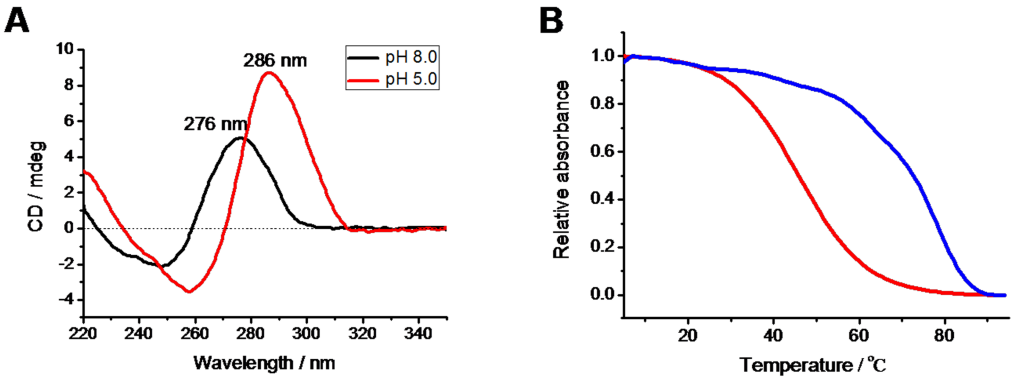


Figure S3 (A) CD spectra of pristine *i*-motif DNA; (B) thermal denaturation spectrum (normalized) at pH 5.0, including pristine *i*-motif DNA (red line) and the triblock copolymers (blue line).

4. Measurement of Critical Micelle Concentration (CMC)

Acetone solution of Nile Red (0.036 mM) 5 μL was added to every tube and sonicated for 10 min. Then the solvent was evaporated under vacuum overnight. The solutions of DNA-*b*-PPO with various concentrations ranging from 0.05 to 200 μM were prepared separately in pH 5.0 and pH 8.0 buffer. The solutions were retained at 4 ^{o}C overnight, heated to 36 °C and kept for 30 min, then slowly cooled to room temperature for 18 h. Fluorescence spectra were recorded at room temperature using an excitation wavelength of 550 nm. The CMCs of the DNA diblock copolymer at pH 8.0 and the DNA triblock copolymer at pH 5.0 were determined to be 16 μM and 30 μM, respectively, see **Figure S4**.

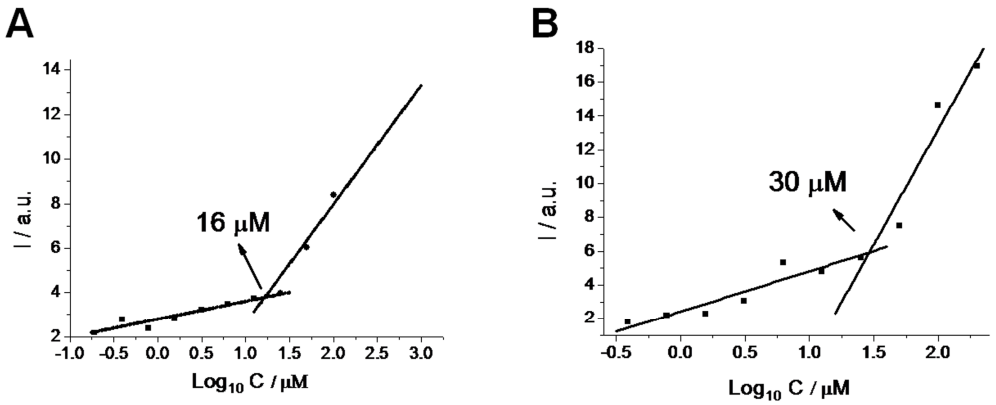


Figure S4 CMC determination data of DNA-*b*-PPO (A) diblock copolymer at pH 8.0, and (B) DNA triblock copolymer at pH 5.0.

5. Dynamic Light Scattering (DLS)

The effective hydrodynamic diameter of the micelle was measured by dynamic light scattering (DLS) at 25 °C using Nano ZS90 ZEN3690, Malvern Instruments Ltd., United Kingdom. 40 μM DNA-*b*-PPO of pH 8.0 solutions were retained at 4 ^{o}C overnight, heated to 36 °C and kept for 30 min, then slowly cooled to room temperature overnight., then were filtrated with Millex syringe filter units (pore size 0.22 μm, filter diam. 33 mm) before the measurement. The measurements were carried out in triplicate.

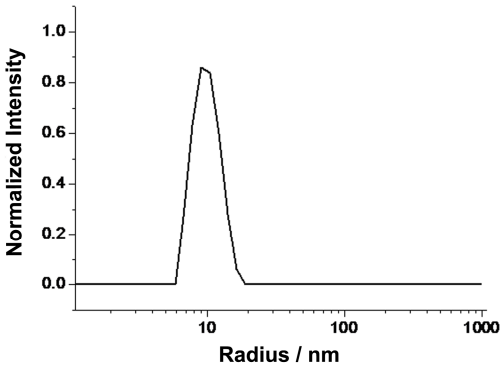


Figure S5 Dynamic light scattering data of micelles of DNA-*b*-PPO at pH 8.

6. TEM Tests

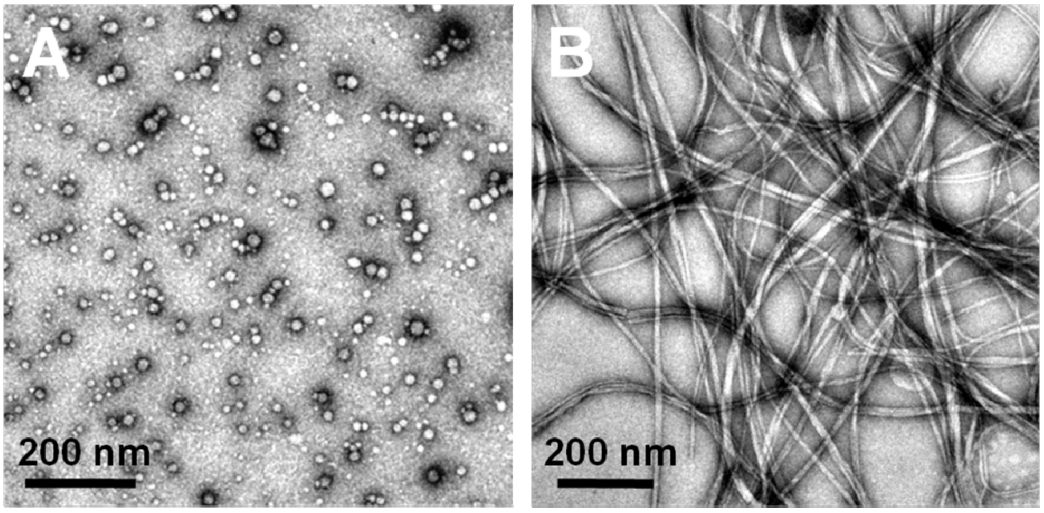


Figure S6 TEM images of DNA-*b*-PPO assembles at room temperature for 45 days (A) spherical micelles at pH 8.0;(B) nanofibers at pH 5.0.

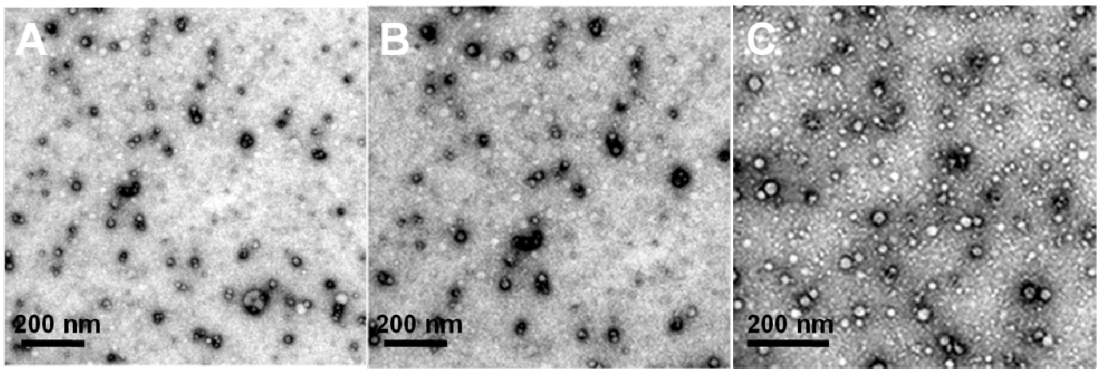


Figure S7 TEM images of 40 μM control sample at different pH values and the shape shifting upon pH change. (A) at pH 8.0; (B) at pH 5.0; (C) at pH 8.0 by directly adding NaOH to the pH 5.0 solutions.

7. Nile Red Encapsulation Experiments

In the fluorescence microscopy experiment, the coverslips were cleaned by sonication in detergent, ultrapure water, acetone, ethanol, NaOH/ethanol and ultrapure water for 30 min each. Then they were boiled in 1/3 H_2O_2/H_2SO_4 for 2 hrs. The clean coverslips were stored in ultrapure water until use. Immediately prior to use, two coverslips were dried by nitrogen flow. 7 μL of a solution were deposited onto one slide and covered by a second one. The DNA solution was drawn under the capillary forces created by the two slides and sealed with vacuum grease. Samples were imaged on an Olympus Reflected Fluorescence System (the microscope was Olympus B × 51). Blue light (420-480 nm) was filtered from a mercury arc lamp (Olympus U-RFL-T) for excitation. The emitted fluorescence passed through a dichroic mirror (DM 500). Images were recorded to a computer via a cooled CCD camera (Q-IMAGING RETIGA 2000R) using the corresponding software (Image-Pro Express Version 6.0).

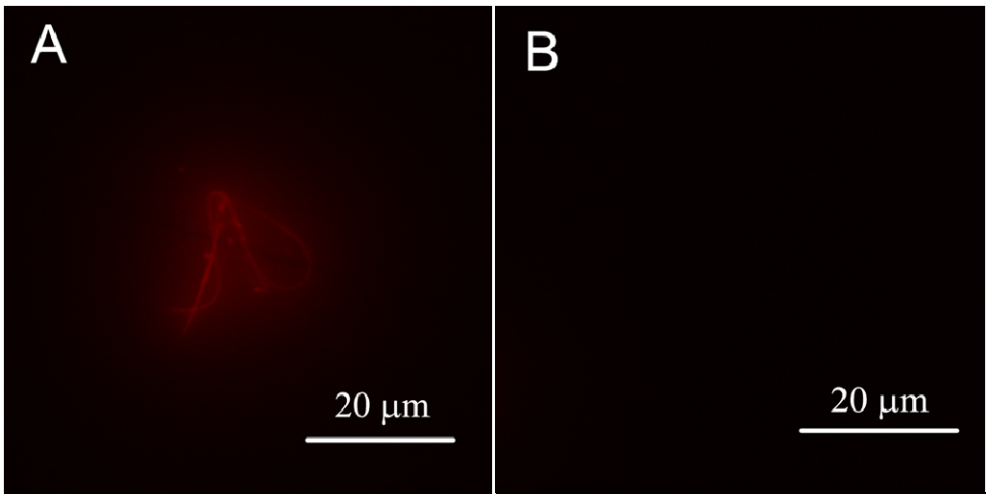


Figure S8 Fluorescent images of DNA-*b*-PPO assemblies after Nile Red encapsulation at pH (A) 5.0 and (B) 8.0.

References:

[1] a) Z. Li, Y. Zhang, P. Fullhart, C. A. Mirkin, *Nano Lett.*, 2004, **4**, 1055; b) F. E. Alemdaroglu, K. Ding, R. Berger, A. Herrmann, *Angew. Chem., Int. Ed*., 2006, **45**, 4206; c) L. Y. Wang, Y. Feng, Y. W. Sun, Z. B. Li, Z. Q. Yang, Y. -M. He, Q. -H. Fan, D. S. Liu, *Soft Matter*, 2011, **7**, 7187.

[2] Y. W. Sun, H. J. Liu, L. J. Xu, L. Y. Wang, Q. -H. Fan, D. S. Liu, *Langmuir*, 2010, **26**, 12496.