Supporting Information

Self-assembling Amphiphilic Poly (propargyl methacrylate) Grafted DNA Copolymer into Multi-strands Helix

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1. Experimental Section

1.1 Material.

Unless otherwise noted, all the chemicals were obtained commercially and used without further purification. The propargyl methacrylate (PMA) was obtained from Alfa Aesar (China) Chemical Co., Ltd. and N,N,N',N",N"-pentamethyl diethylene triamine (PMDETA) was purchased from TCI (Shanghai) Development Co., Ltd. The CuBr was obtained from Aladdin Industrial Inc. Other chemicals and all the organic solvents were obtained from Sinopharm Chemical Reagent Co., Ltd. AIBN was recrystallized with ethanol before use. The water used in the experiment was Milli-Q deionized. All the DNA were purchased from Sangon Biotech (Shanghai) Co., Ltd. DNA sequences were: 5'-N_3-TTGGGAAGTAGCGACAGC-3', complementary DNA: 5'-SH-$(CH_2)_6$-GCTGTCGCTACTTCCCAA-3', non-complementary DNA: 5'-SH-$(CH_2)_6$-TTGGGAAGTAGCGACAGC-3'.

1.2. Methods.

The FT-IR spectra was recorded on Nicolet 5700. The ^{1}H NMR spectra was recorded on Bruker AV 300 Spectrometer. The gel permeation chromatography (GPC) was conducted on Polymer Laboratories PL-GPC220 GPC. THF was used as the mobile phase. Matrix-assisted laser desorption/ ionization time of flight mass spectrometry (MALDI-TOF) was performed on Bruker Autoflex II MALDI-TOF spectrometer with α-cyano-4-hydroxylcinnamic acid (HCCA) as the matrix. The agarose gel electrophoresis was conducted using 2% agarose gel under 80 Volts. The image was taken by Kodak Gel Logic 112 equipped with carestream molecular imaging software. The transmission electron microscopy (TEM) was performed on JEOL JEM-2100 TEM. An excessive amount of the sample solution was loaded on a carbon grid until the solution thoroughly spread on the grid and the filter paper beneath it. The grid was allowed to dry in air for at least 20min. And then, one drop of 2 wt% phosphotungstic acid was loaded on the grid and thoroughly spread. The grid was again allowed to dry in air before conducting tests. The atomic force microscope (AFM) was conducted on Bruker Dimension ICON AFM using ScanAsyst imaging mode. 6μL of the sample solution was drop casted on a freshly cleaved mica surface. The mica was allowed to dry in air for at least 40min before AFM scanning. The circular dichroism (CD) was conducted on Applied Photophysics Chirascan. The fluorescence measurements were acquired on OLYMPUS IX71 Fluorescence Spectrometer.

1.3. Synthesis of Poly(Propargyl Methacrylate) (PPMA)

The solution polymerization approach was applied to the synthesis of poly (propargyl methacrylate) (PPMA). 20 mL THF, 1023 μL (8.1 mmol) propargyl methacrylate (PMA) and 8.9 mg (0.67 %) 2,2-azobisisobutyronitrile (AIBN) was added into a 50 mL three-necked bottle with a reflux tube and a magnetic stirrer. The reactants were allowed to stir for 10 min under nitrogen protection. And then, the reactants were heated to 63 ˚C for 4 h and 20 min. During the polymerization, additional THF was added to make up for the solvent loss caused by evaporation. The resulting polymer was precipitated in ethanol twice to remove unreacted monomers and vacuum dried. The molecular weight was determined by GPC and MALDI-TOF spectrometer. 254 mg PPMA was obtained, yield 25.4 %. M_n = 7 KDa, PDI=1.9 according to GPC measurement.

1.4. Synthesis of PPMA-g-DNA

The "click" reaction was performed as follows. The copper-catalyzed azide-alkyne (CuAAC) reaction[1] was applied for the synthesis of PPMA-g-DNA. 3.1 mg PPMA was dissolved in 775 μL THF. And then, 20 μL of the prepared solution was diluted to 645 μL (1 mM alkynyl). 54 nmol azide-DNA was dissolved in 27 μL water (2 mM

azide). 10 μL Cu(I)-stabilizing ligand N,N,N',N",N"-pentamethyl diethylene triamine (PMDETA), 1.4 mg CuBr and 10 mg sodium ascorbate was added to 490 μL mixed solvent of H2O:DMSO:IPA=2:3:1. Next, 10 μL azide-DNA solution (20 nmol), 20 μL PPMA solution (20 nmol alkynyl, 1 eq.), 20 μL THF and 15 μL mixed solvent containing Cu(I) (300 nmol, 15 eq.), PMDETA (1500 nmol, 75 eq.), sodium ascorbate (1500 nmol, 75 eq.) were added into a 0.5 mL centrifugal tube. The click reaction was proceeded at 55 ˚C for 3 hours. The PPMA-g-DNA was characterized by 2 % agarose gel electrophoresis and further analyzed by carestream molecular imaging software. As shown in Figure S5, the band 3 in lane B marked in red represented the free DNA molecules in solution after reaction, which contributed 89% of the lane. Given the reaction ratio of azide: alkynyl 1:1, approximately 11% of the alkynyl groups were coupled with DNA strands.

1.5. Preparation of DNA labeled 13nm gold nanoparticles.

The AuNPs and the DNA-labelled AuNPs were synthesized following the previous reports. [2] 5 mL of 38.8 mM tisodium citrate was added rapidly into a stirred boiling aqueous solution containing 50 mL of 1mM $HAuCl_4$. The solution turned into black, purple and deep red in sequence within 2 min. After the solution was kept boiling and stirred for 15 min, it was naturally cooled down to room temperature. The concentration of gold nanoparticles (AuNPs) was 13nM calculated by lambert-beer law. The extinction coefficient of $2.7 \times 10^8\ M^{-1}\ cm^{-1}$ at 520 nm was used for the calibration.

To activate the thiol-DNA, 5 μL TCEP (100 mM) in Tris buffer (20 mM pH 7.3) was added to 20 μL thiol-DNA (100 μM). The resultant solution was incubated for 1 h at room temperature. After incubation, the excess TCEP was removed by 3kD Millipore's Amicon Ultra-0.5 centrifugal filter device. The freshly deprotected and purified DNA was added to 500 μL of gold colloidal solution. The mixed solution was sonicated for 10 s, and then incubated for 20 min with shaking at room temperature. After that, the resultant solution was mixed with 0.1 M phosphate buffer (pH 7.2) and the final concentration of phosphate was maintained at 0.01 M. The solution was sonicated for 10 s, and incubated for 20 min. In the subsequent salt aging process, the concentration of NaCl first increased to 0.05 M using 2 M NaCl. The process was repeated at one more increment of 0.05 M NaCl and for every 0.1 M NaCl increment thereafter until a concentration of 0.5 M NaCl was reached. After each addition of NaCl, the DNA-AuNPs were vortexed and sonicated for 10 s, and then incubated for 20 min. After the salt aging, the mixture was shaken at room temperature overnight. To remove excess DNA, the solution was centrifuged at 13,000 rpm for 20 min, and then redispersed in Tris buffer (20 mM pH 7.3). The step was repeated three times to sufficiently remove excess DNA. The final volume was maintained at 180 μL.

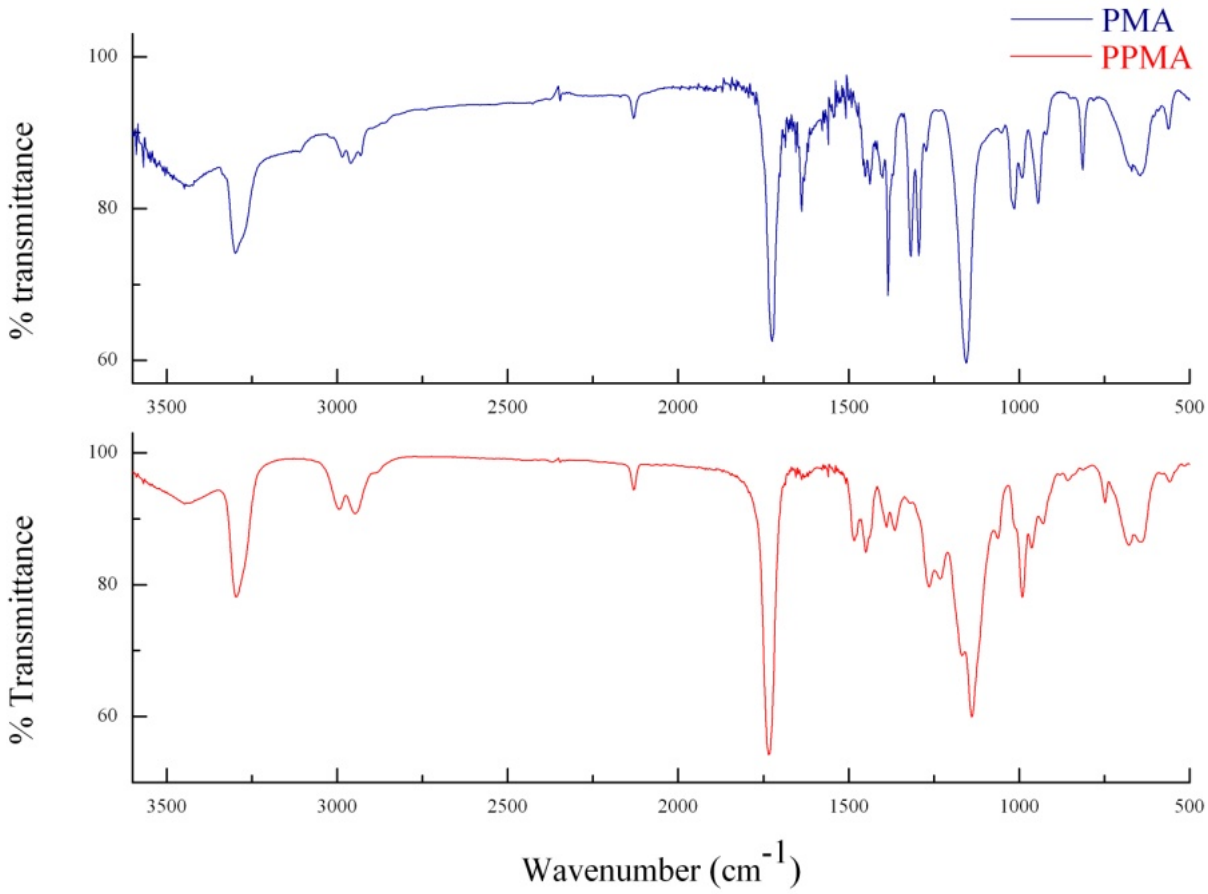
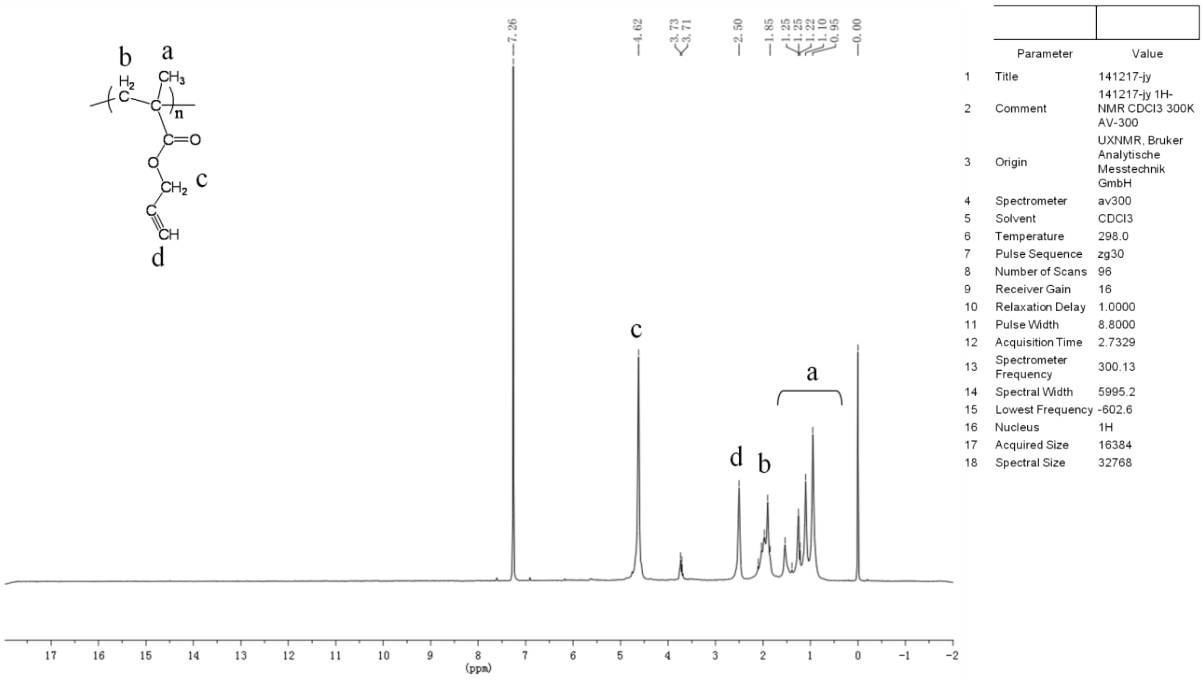
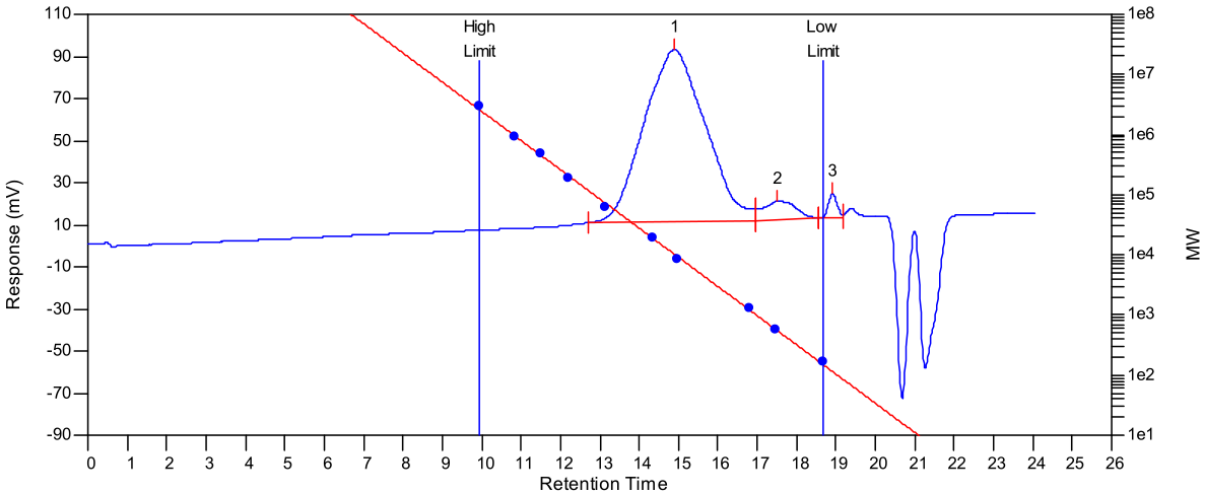


Figure S1. FT-IR spectra of PMA (blue curve) and PPMA (red curve). The stretching vibration of C≡C ($\upsilon_{C\equiv C}$), ≡C-H ($\upsilon_{\equiv C-H}$) and C=C ($\upsilon_{C=C}$) occurred at 2130 cm^{-1}, 3298 cm^{-1} and 1637 cm^{-1} respectively. Comparing the spectra of PMA and PPMA, the $\upsilon_{C=C}$ absorption disappeared in PPMA, which indicated the successful polymerization between carbon-carbon double bonds. The absorptions of $\upsilon_{C\equiv C}$ and $\upsilon_{\equiv C-H}$ that remained the same after polymerization showed the integrity of alkynyls.



	Parameter	Value
1	Title	141217-jy
2	Comment	141217-jy 1H-NMR CDCl3 300K AV-300
3	Origin	UXNMR, Bruker Analytische Messtechnik GmbH
4	Spectrometer	av300
5	Solvent	CDCl3
6	Temperature	298.0
7	Pulse Sequence	zg30
8	Number of Scans	96
9	Receiver Gain	16
10	Relaxation Delay	1.0000
11	Pulse Width	8.8000
12	Acquisition Time	2.7329
13	Spectrometer Frequency	300.13
14	Spectral Width	5995.2
15	Lowest Frequency	-602.6
16	Nucleus	1H
17	Acquired Size	16384
18	Spectral Size	32768

Figure S2. ^{1}H NMR spectrum of PPMA. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 4.62, 3.73, 3.71, 2.50, 2.10, 2.03, 1.97, 1.90, 1.85, 1.54, 1.39, 1.25, 1.25, 1.22, 1.10, 0.95.



MW Averages

Peak No	Mp	Mn	Mw	Mz	Mz+1	Mv	PD
1	10335	7043	13212	22383	33423	12104	1.87591
2	554	472	526	605	670	512	1.11441
3	113	114	113	115	117	112	0.991228

Processed Peaks

Peak No	Name	Start RT (mins)	Max RT (mins)	End RT (mins)	Pk Height (mV)	% Height	Area (mV.secs)	% Area
1		12.72	14.88	16.97	81.7484	80.0922	8816.58	92.7981
2		16.97	17.50	18.57	8.91844	8.73776	503.666	5.30129
3		18.57	18.92	19.18	11.401	11.17	180.569	1.90056

Figure S3. GPC chromatogram of PPMA (M_n=7K, M_w=13K, PDI=1.9)

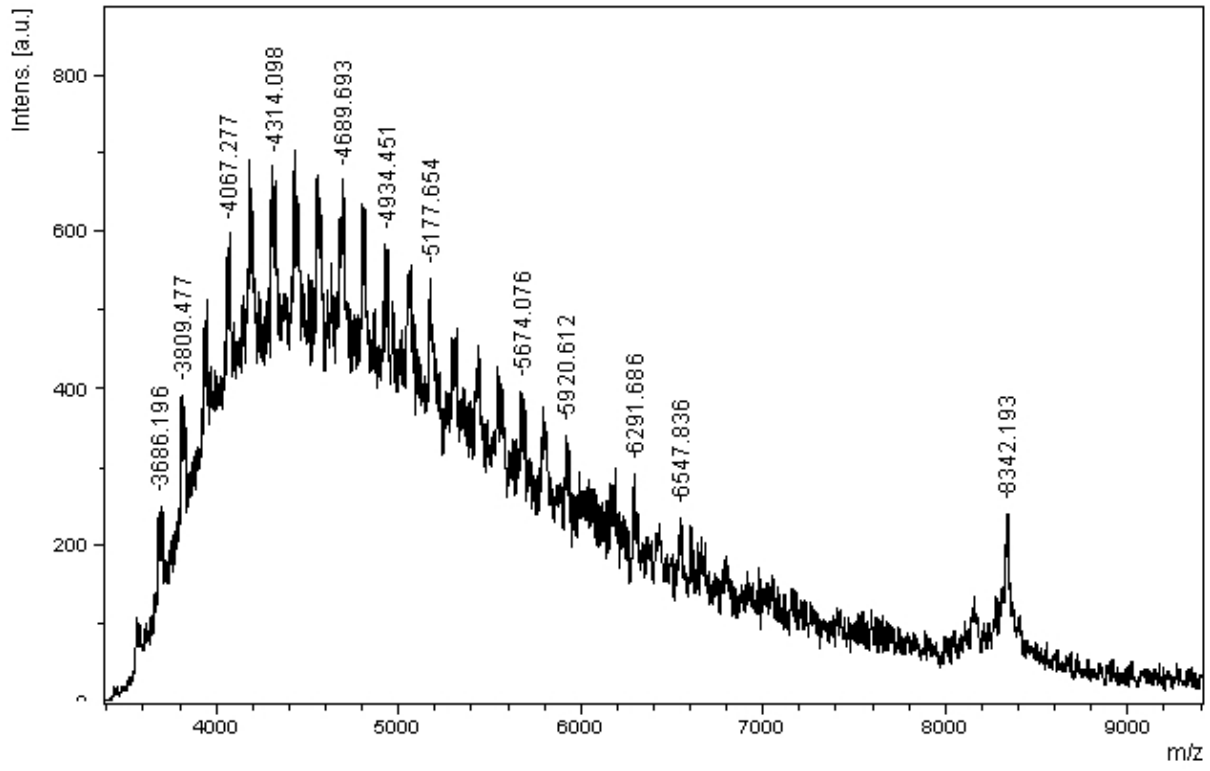
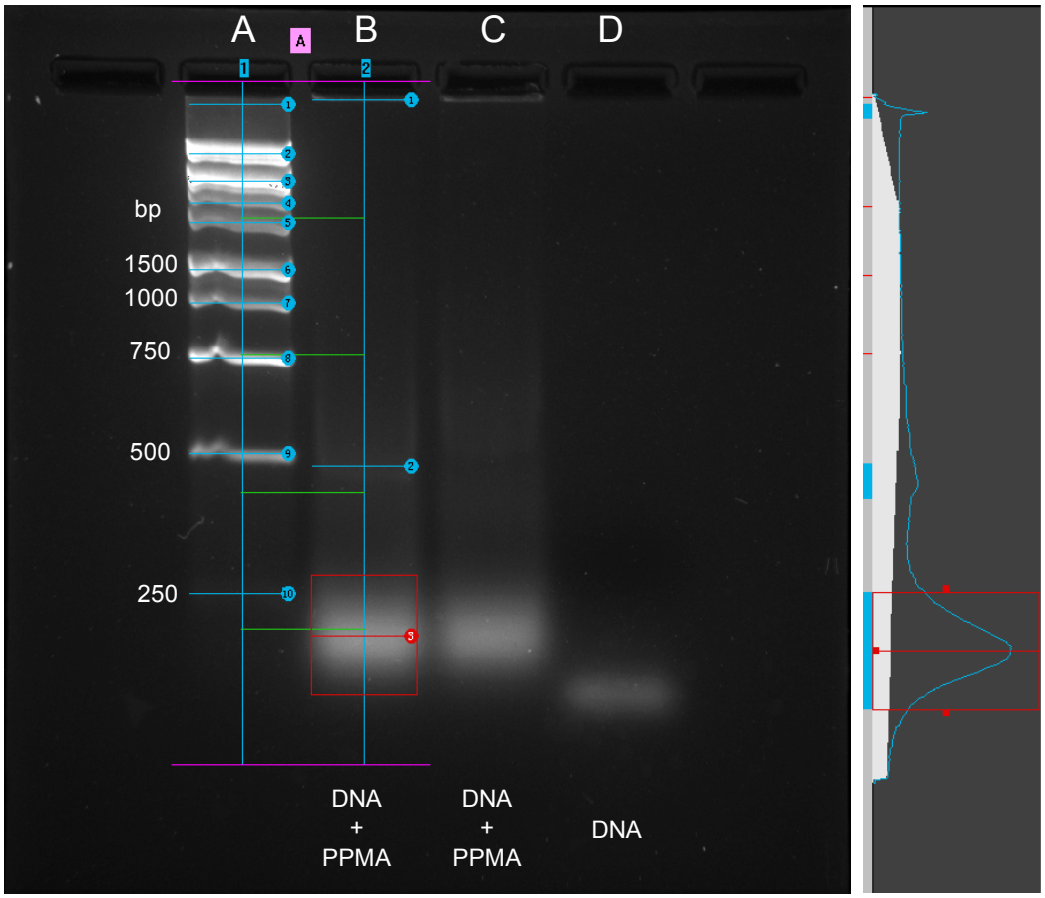


Figure S4. MALDI-TOF spectrum of the synthesized polymer PPMA.



2: Untitled

	Band Int.	Net Int.	Sum Int.	Rel. Int.	Mean Int.	Mean Back.	Max Int.
1	45.70	31015.80	67707.0	0.0423	50.38	27.30	110.00
2	18.88	51746.22	191413.0	0.0706	60.42	44.09	74.00
3	110.05	650433.27	1053886.0	0.8871	101.65	38.91	161.00

Figure S5. Agarose gel electrophoresis of PPMA-*g*-DNA. Lane A is the DNA ladder. Lane B and C are the click reaction solution. Lane D is the azide-DNA. On the right is the profile window corresponding to lane B. In the bottom is the lane analysis data window for lane B. Lane B and C are the reaction solution, compared to azide-DNA in lane D, the PPMA-*g*-DNA block copolymer formed new bands in the middle and in the well. The main items listed in lane analysis data window represent the following band information. Band Int.: the intensity value at the peak of the profile. Net Int.: the sum of the background – subtracted pixel values in the band rectangle. Sum Int.: the sum of all the pixel intensities in the band rectangle. Rel. Int.: the percent intensity contribution of a band within a same lane. Mean Int.: the average intensity of the pixel in the band rectangle.

2. Formation of Nanofiber and Morphology Control

2.1 Addtional TEM and AFM images

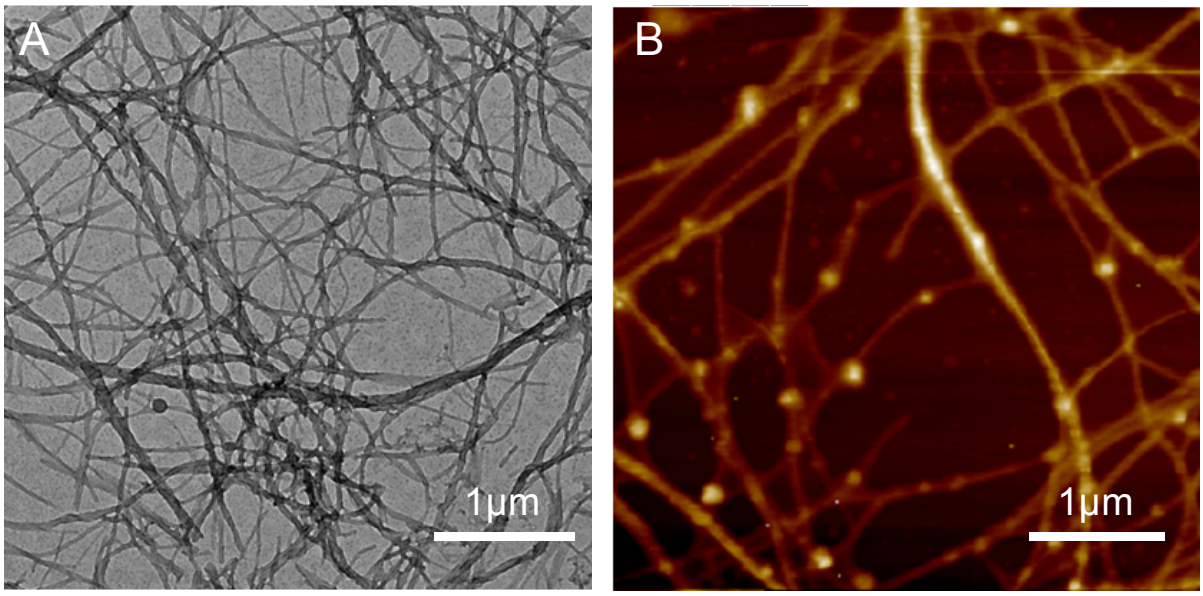


Figure S6. TEM and AFM images of PPMA-*g*-DNA nanofibers in pure water. The TEM (A) and AFM (B) images show that the nanofibers were in averaged orientations and tended to wrap around each other.

The obtained PPMA-*g*-DNA in organic solvent was diluted with 200 μL water. Next, the small molecules were removed and the solvent was changed into water through a 3kD Millipore's Amicon Ultra-0.5 centrifugal filter device at five steps. In the first three steps, each time 80 μL PPMA-*g*-DNA solution and 300 μL water was added in the amicon and allowed to spin at 10,000 rpm for 30 min. In the last two steps, 300 μL water was added and allowed to spin. Finally, about 60 μL PPMA-*g*-DNA solution in water was obtained. The PPMA-*g*-DNA was allowed to assemble at 15˚C for three days to form the nanofibers completely (Figure S6).

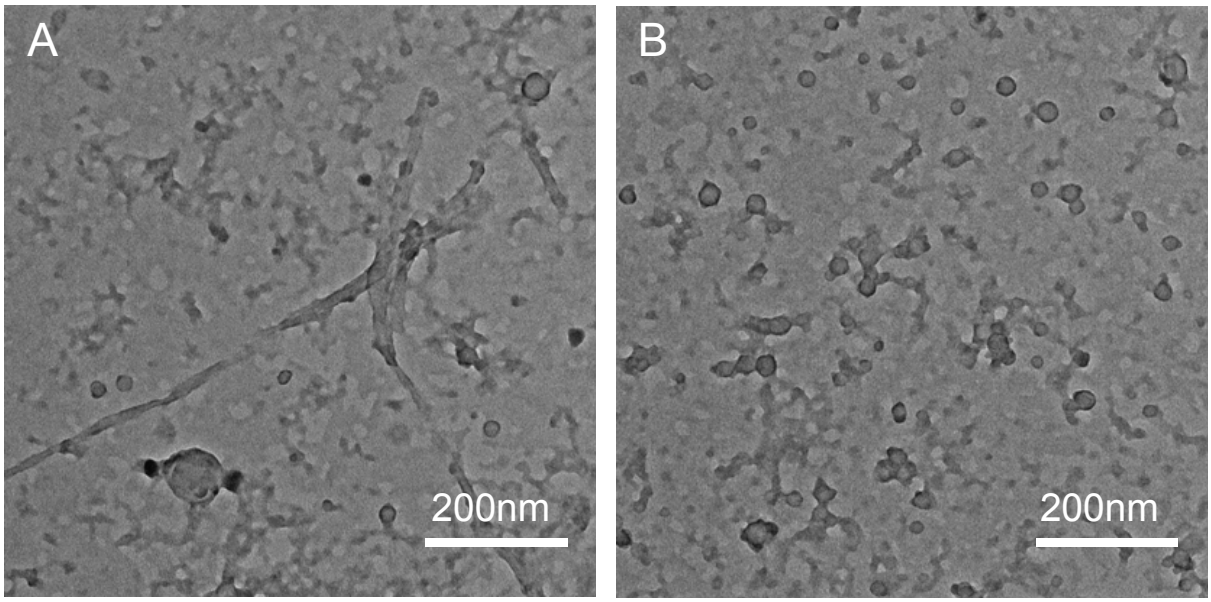


Figure S7. TEM images of the intermediate state of the nanofiber formation. The photos were taken after PPMA-*g*-DNA changed solvent into water immediately. Amorphous aggregates, nanoparticles and the hint of nanofibers coexisted, which revealed the possible mechanism of PPMA-*g*-DNA self-assembling.

We also observed the intermediate state of the nanofiber formation, the TEM images in Figure S7 were taken immediately after PPMA-*g*-DNA changed solvent into water. Amorphous aggregates, nanoparticles and the hint of nanofibers were observed. It shows that the amphiphilic copolymer tends to form nanoparticles first and then the nanoparticles gradually collide and fuse into nanofibers.

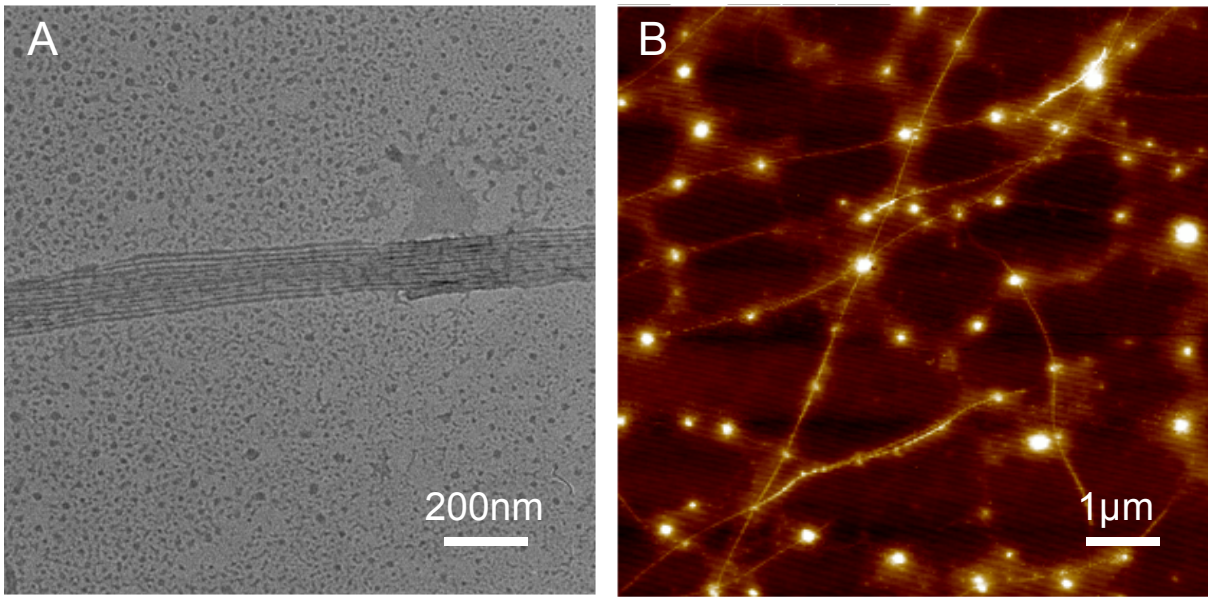


Figure S8. TEM and AFM images of PPMA-*g*-DNA nanofibers assembled in 20% THF. The TEM (A) and AFM (B) images show that the nanofibers appeared in extended length and oriented parallel to each other.

In the 20 % THF group, the PPMA-*g*-DNA solution was condensed to about 50 μL in the final ultrafiltration step and THF was added to reach a final concentration of 20%. The PPMA-*g*-DNA solution was then allowed to assemble at 15 ˚C for three days. Single nanofibers in extended length were formed (Figure S8).

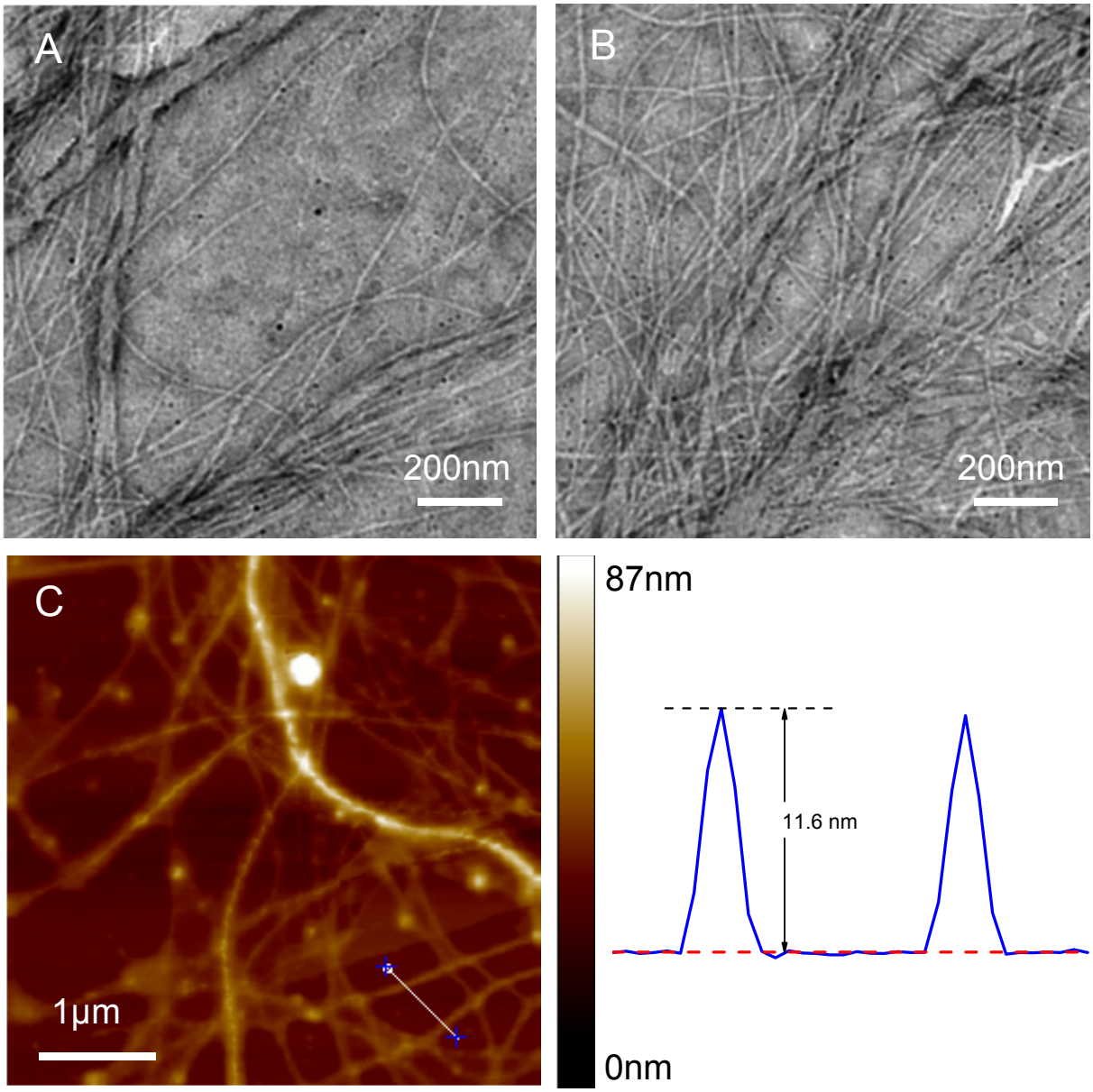


Figure S9. TEM and AFM images of PPMA-*g*-DNA nanofibers assembled in 40 % THF. The TEM (A and B) and AFM (C) images show that the nanofibers began to wrap around in the same direction to form multi-strand helices.

In the 40 % THF group, the PPMA-*g*-DNA solution was condensed to about 40 μL in the final ultrafiltration step and THF was added to reach a final concentration of 40%. The PPMA-*g*-DNA solution was then allowed to assemble at 15 ˚C for three days. Single nanofibers tend to wrap around to form multi-strand helices (Figure S9).

2.2 Circular Dichroism (CD)

The circular dichroism for the PPMA-*g*-DNA block copolymer was measured by using water, 20% THF and 40% THF as the solvent respectively. 50 μL of the well-assembled block copolymer was diluted to 150 μL with the corresponding solvent before conducting CD measurement.

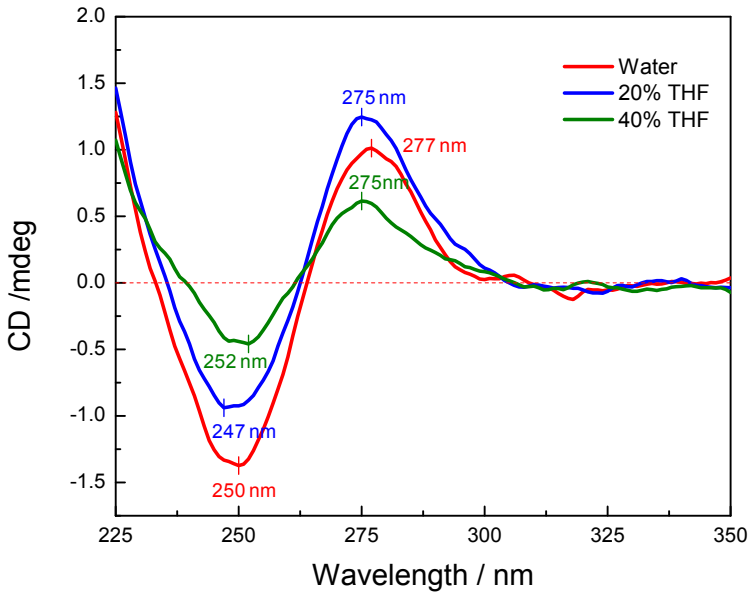


Figure S10. CD spectra of PPMA-*g*-DNA nanofibers formed in different solvents.

3. PPMA-*g*-DNA Assembling with DNA Labeled Gold Nanoparticles

10μL of the well assembled nanofibers in water was added into 90 μL of 13 nm DNA- AuNPs and then allowed to anneal for 1min at each 1˚C decrement from 72 ˚C to 25 ˚C (Figure S11A). A control experiment was conducted under the same condition using non-complementary DNA labelled AuNPs (Figure S11B).

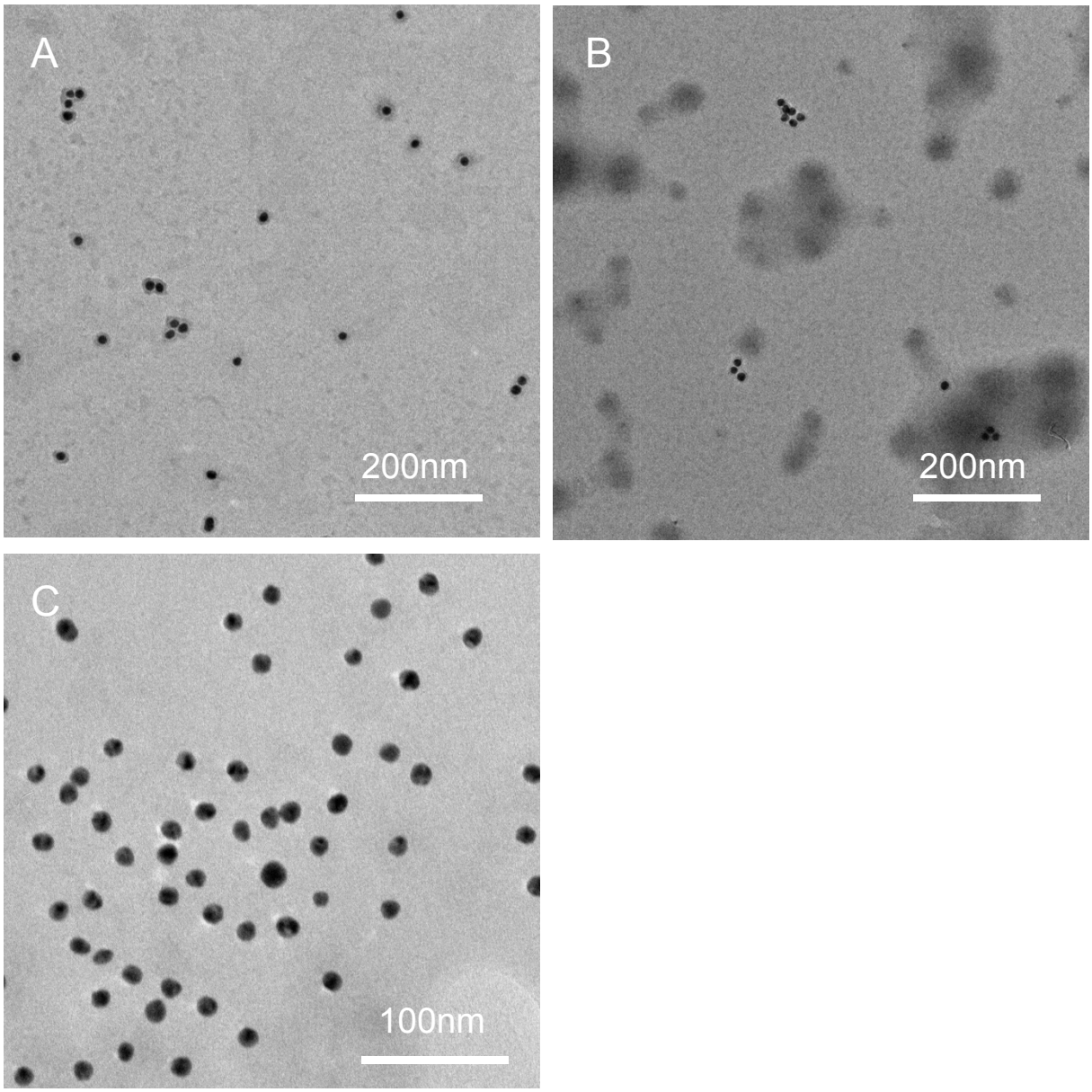


Figure S11. TEM images of the PPMA-*g*-DNA assembly with DNA labeled 13 nm AuNPs and control. (A) shows the assembly of PPMA-g-DNA cluster around single AuNP obtained from complementary DNA labelled AuNPs. (B) shows the control experiment using non-complementary DNA labelled AuNPs. No organic layers can be seen around the AuNPs and the nanofibers are destroyed during the annealing process. (C) shows DNA labeled 13 nm AuNPs alone.

4. Nile Red Encapsulation Experiments

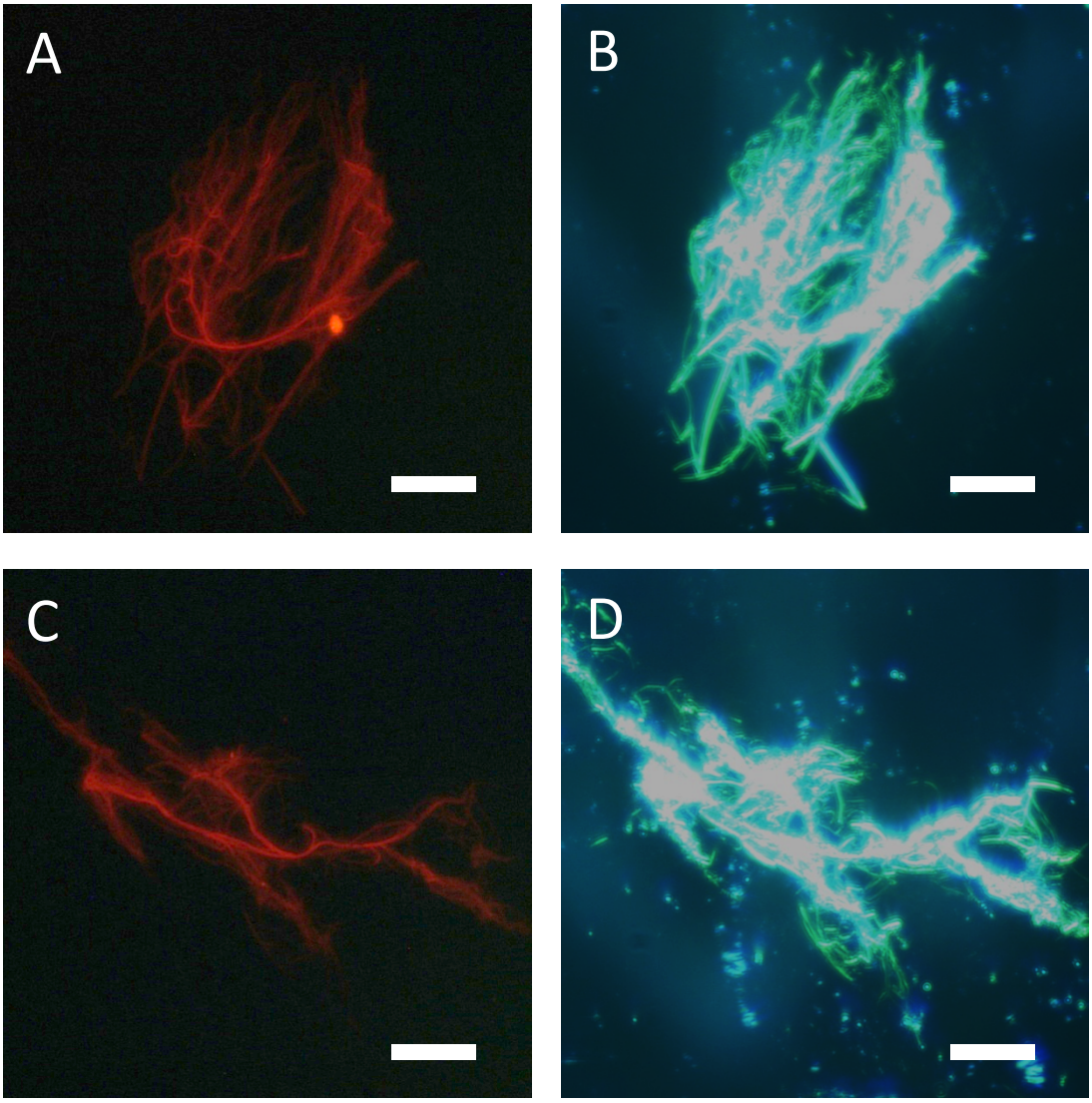


Figure S12. Fluorescent images of PPMA-*g*-DNA nanofibers with Nile Red encapsulation under green light (A, C) and visible light (B, D). The scale bars are 10 μm.

To the 0.5 mL vial containing 40 μL of the well assembled nanofiber solution, 10μL of the Nile Red in acetone (0.038 mM) was added. The acetone was allowed to evaporate overnight by opening the microcentrifuge tube cap and the final volume of the solution became about 40 μL[3]. 5 μL of the resultant solution was diluted to 50μL directly for the fluorescence spectroscopy observation.

The glass slides used in the experiment were cleaned by sonication in detergent, water, acetone, ethanol, water for 30 min each in sequence. The clean glass slides were stored in water until use. Prior to use, the glass slides were dried by nitrogen flow. 7 μL of the prepared sample was loaded on a glass slide and covered with a cover slip, and then used for the fluorescence spectroscopy observation immediately. The fluorescence spectra was acquired on OLYMPUS IX71 Fluorescence Spectrometer and the excitation wavelength is 543 nm.

5. References

[1] J. Gierlich, G. A. Burley, P. M. Gramlich, D. M. Hammond and T. Carell, *Org. Lett.*, **2006**, *8*, 3639.
[2] Z.-M. Zhang, Y.-F. Meng, M.-L. Zhu, Z.-F. Wang and Y. Jiang, *RSC Adv.*, **2014**, *4*, 47268.
[3] L. Wang, Y. Feng, Y. Sun, Z. Li, Z. Yang, Y.-M. He, Q.-H. Fan and D. Liu, *Soft Matter*, **2011**, *7*, 7187.