Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information for

Supramolecular-Micelle-Directed Preparation of Uniform Magnetic Nanofibers with Length Tunability, Colloidal Stability and Capacity for Surface Functionalization

Mingwei Tian, Chen Ma, *Xiaoyu Huang*,* *Guolin Lu*, *Chun Feng**

Key Laboratory of Synthetic and Self-Assembly Chemistry for Organic Functional Molecules, Center for Excellence in Molecular Synthesis, Shanghai Institute of Organic Chemistry, University of Chinese Academy of Sciences, Chinese Academy of Sciences, 345 Lingling Road, Shanghai 200032, People's Republic of China

* To whom correspondence should be addressed, E-mail: cfeng@mail.sioc.ac.cn (Tel: +86-21-54925606, Fax: +86-21-64166128), xyhuang@mail.sioc.ac.cn (Tel: +86-21-54925310, Fax: +86-21- 64166128).

SUPPORTING EXPERIMENTAL DETAILS

Materials

2,2'-Azobis(isobutyronitrile) (AIBN, 98%, Aldrich) was recrystallized from anhydrous ethanol. 2-Vinylpridine (2-VP, 97%, Aldrich) was passed through a basic alumina column and distilled under reduced pressure from CaH_2 prior to use. Copper(I) chloride (CuCl, Aladdin, 99%) was purified by stirring overnight over CH_3CO_2H at room temperature, followed by washing the solid with ethanol, diethyl ether and acetone prior to drying *in vacuo* at 40°C overnight. Tetraethylorthosilicate (TEOS, 98%, Aldrich), 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA, Aldrich, 97%), trimethyl[3-(triethoxysilyl)propyl]ammonium chloride (TMTESA, 98%, TCI), DNA (from calf thymus, 98%, Macklin), ethanol (≥99.8%, Aladdin) and tetrahydrofuran (THF, 99.9%, Aladdin) were used as received without further purification. 2-Mercaptosuccinic acid capped Fe_3O_4 nanoparticles (d = 10 nm) was purchased from Beijing Banda Juban Sci. & Tech. Co. Ltd. Other reagents not specially mentioned were purchased from Aladdin and used as received without further purification. Alkyne-terminated OPV_5 and azide-functionalized chain transfer agent (CTA) were prepared according to a previous report.[1]

Instrumentation

^1H (400 MHz) NMR analyses were performed on a JEOL JNM-ECZ400 spectrometer in $CDCl_3$ and CD_2Cl_2, tetramethylsilane (TMS) was used as internal standard. Relative molecular weights and molecular weight distributions were

measured by conventional gel permeation chromatography (GPC) using a system equipped with a Waters 1515 Isocratic HPLC pump, a Waters 2414 refractive index detector, a Waters 2487 dual λ absorbance detector and a set of Waters Styragel columns (HR3 (500- 30,000), HR4 (5,000-600,000) and HR5 (50,000-4,000,000), 7.8×300 mm, particle size: 5 μm). GPC measurements were carried out at 35°C using THF as eluent with a flow rate of 1.0 mL/min. The system was calibrated with linear polystyrene standards.

Transmission electron microscopy (TEM)

TEM images were obtained by a JEOL JEM-2100 instrument operated at 80 kV. A drop of micellar solution (10 μL) was placed on a Formvar and carbon-coated copper grid for 30 s and then the edge of the drop was touched by a filter paper to absorb most of the liquid on the grid. The grid was allowed to dry at room temperature. For each sample, length distributions of micelles were determined by tracing more than 100 individual micelles, and width distributions were determined by making measurements at least 100 different positions on several micelles and analysis using the ImageJ software from National Institutes of Health. Values of number-average (L_n) and weight-average (L_w) length of micelles were calculated as follows:

$$L_n = \frac{\sum_{i=1}^{N} N_i L_i}{\sum_{i=1}^{N} N_i} \quad (1)$$

$$L_w = \frac{\sum_{i=1}^{N} N_i L_i^2}{\sum_{i=1}^{N} N_i L_i} \quad (2)$$

where N_i is the number of micelles of length L_i, and N is the number of calculated

micelles in each sample. The distribution of micellar length is characterized by both L_w/L_n and standard deviation of the length distribution (σ).

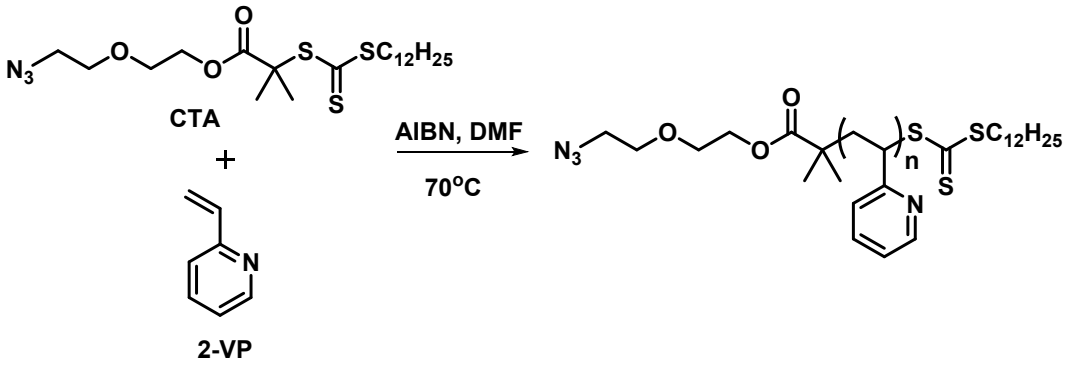
Dynamic light scattering (DLS)

DLS measurements were performed at room temperature (23°C) on a Malvern Nano-ZS90 Zetasizer at a scattering angle of 173°. Intensity distributions (CONTIN plots) were calculated with the instrument software.

Polymer Synthesis

Synthesis of azide-terminated poly(2-vinylpyridine)

Azide-terminated poly(2-vinylpyridine) was prepared by RAFT homopolymerization using an azide-functionalized CTA (Scheme S1).[1]



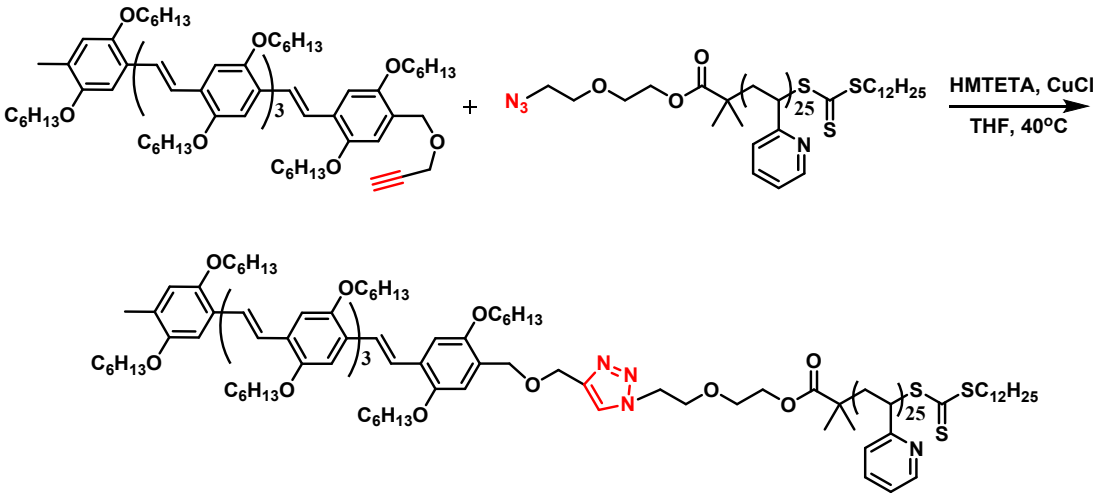
Scheme S1. Synthesis of azide-terminated poly(2-vinylpyridine).

2-VP (2.475 g, 23.54 mmol), CTA (0.255 g, 0.53 mmol) and AIBN (25.5 mg, 0.16 mmol) were introduced into a 25 mL Schlenk flask. After the mixture was degassed and kept under N_2, dry DMF (5 mL) was added via a gastight syringe. Three freeze-pump-thaw cycles were conducted before the flask was immersed into an oil bath set at 70°C. The polymerization lasted 8 h and it was terminated by freezing the whole

system with liquid N_2. The reaction mixture was precipitated in cold *n*-hexane. The crude product was purified by dissolution in THF and precipitation in cold *n*-hexane three times followed by drying *in vacuo* overnight to give P2VP-N_3 (1.237 g, 75.1%) as a light yellow solid, which was subjected to GPC (Figure S1) and ^{1}H NMR measurements. The "absolute" molecular weight of P2VP-N_3 was confirmed by ^{1}H NMR after the "click" reaction with OPV_5 (Figure S2). ^{1}H NMR (CD_2Cl_2): δ (ppm): 0.87 (t, 3H, CH_2CH_3), 1.23 (s, 6H, $C(CH_3)_2$), 1.36-2.05 (m, 18H, $CH_2(CH_2)_9CH_3$), 2.30 (m, 2H, $CS_3CH_2CH_2CH_2$), 3.20 (t, 2H, $N_3CH_2CH_2$), 3.29 (t, 2H, CS_3CH_2), 3.44 (t, 1H, $N_3CH_2CH_2$), 3.55 (t, 2H, $N_3CH_2CH_2OCH_2CH_2O_2C$), 6.09-8.54 (m, 4H, pyridine). GPC: M_n^{GPC} = 3440 g/mol, M_w/M_n =1.12.

Synthesis of OPV_5-b-$P2VP_{25}$

Cu-catalyzed alkyne azide cycloaddition (CuAAC) reaction was employed to couple alkyne-terminated OPV_5 with azide-terminated $P2VP_{25}$ to give a diblock copolymer of OPV_5-*b*-$P2VP_{25}$ (Scheme S2).[1]



Scheme S2. Synthesis of OPV_5-*b*-$P2VP_{25}$ diblock copolymer.

Alkyne-terminated OPV_5 (41 mg, 26.1 μmol), $P2VP_{25}$-N_3 (357 mg, 114.9 μmol), CuCl (8.2 mg, 0.083 mmol), HMTETA (21 μL, 0.078 mmol) and dry THF (5 mL) were added into a 25 mL Schlenk tube (flame-dried three times under vacuum before using). After degassed by three freeze-pump-thaw cycles, the tube was immersed into an oil bath set at 40°C for 2 days. The solvent was evaporated and the crude product was purified by silica column chromatography (gradient eluent with $V_{CH2Cl2}/V_{methanol}$ from 100/1 to 10/1) to remove the unreacted alkyne-terminated OPV_5 and CuCl. The crude product was further purified by dissolution in THF and precipitation in cold acetonitrile three times to remove excess P2VP-N_3, followed by drying *in vacuo* overnight to obtain OPV_5-*b*-$P2VP_{25}$ (26 mg, 21.3 %) as an orange solid. The purified diblock copolymer was subjected to GPC analysis (Figure S1), and the number-average degree of polymerization of $P2VP_{25}$ was determined by comparing the integration ratio of peak 'a' to peak 'e', 'f' in ^{1}H NMR spectrum (Figure S2). ^{1}H NMR (CD_2Cl_2): M_n^{NMR} = 4676 g/mol. GPC: M_n^{GPC} = 4700 g/mol, M_w/M_n =1.08.

Self-Assembly Experiments

Self-assembly of OPV_5-b-$P2VP_{25}$ diblock copolymer in ethanol

OPV_5-*b*-$P2VP_{25}$ (1 mg) was directly suspended in 10 mL of ethanol to obtain an OPV_5-*b*-$P2VP_{25}$ solution (0.1 mg/mL). The mixture was then heated at 80°C for 30 min, followed by aging at room temperature (23°C) for 24 h. Then, A drop of solution was placed on a Formvar and carbon-coated copper grid and examined by TEM (Figure 1B).

Preparation of seed micelles of OPV_5-b-$P2VP_{25}$

The original long fiber-like micelles of OPV_5-*b*-$P2VP_{25}$ (0.1 mg/mL in ethanol) were fragmented into short seed micelles via sonication (BRANSON model 1510 70 W ultrasonic cleaning bath) at 0°C for 4 h. A drop of solution was placed on a Formvar and carbon-coated copper grid and examined by TEM (Figure 1C).

Self-seeding of seed fiber-like micelles of OPV_5-b-$P2VP_{25}$

The seed fiber-like micelles formed by OPV_5-*b*-$P2VP_{25}$ (L_n = 54 nm, L_w/L_n = 1.04, 0.1 mg/mL in ethanol) was thermal annealed at different temperatures ranging from 40°C to 50°C. Aliquots of seed micelles (0.1 mg/mL in ethanol) were added into several 2 mL vials and immersed into the preset water baths with different temperatures for 0.5 h. The vials were removed from water bath, followed by aging at room temperature (23°C) for 24 h. A drop of each solution was placed on a Formvar and carbon-coated copper grid and examined by TEM (Figures 1D-G and S3).

Preparation of OPV_5-b-$P2VP_{25}$/Fe_3O_4 nanofibers

2-Mercaptosuccinic acid-coated Fe_3O_4 NPs (40 μL, 0.4 mg/mL in ethanol) was added dropwise into 0.25 mL of as-prepared fiber-like micelles of OPV_5-*b*-$P2VP_{25}$ (0.1 mg/mL in ethanol) by a micro-syringe. The mixture was then placed in a shaking table and shaken for 3.5 h at a rotation speed of 200 r/min. The resulting sample was subjected to TEM analysis (Figures 2A and S4).

Preparation of OPV_5-b-$P2VP_{25}$/Fe_3O_4@SiO_2 nanofibers

Aqueous HCl (50 μL, 0.01 M) was added slowly into the solution of OPV_5-*b*-$P2VP_{25}$/Fe_3O_4 nanofibers (~0.1 mg/mL in ethanol, 0.25 mL), followed by adding 17 μL of TEOS (0.45 M in ethanol) dropwise into the mixture by a microsyringe. The mixture was then placed in a shaking table and shaken for 24 h at a rotation speed of 200 r/min. The resulting sample was subjected to TEM analysis (Figures 2B and S4). To remove excess TEOS, a magnet was put beside the resulting sample for 20 min, followed by removing the almost clear supernatant with a syringe and redispersing the yellow-like colloids in 100 μL of ethanol.

Micellar stability in THF

A magnet was put beside the ethanol solution of OPV_5-*b*-$P2VP_{25}$/Fe_3O_4@SiO_2 nanofibers (Figure S4B, ~0.1 mg/mL, 0.25 mL), followed by removing the supernatant with a syringe and redispersing the yellow-like colloids in 100 μL of THF. For the ethanol solution of OPV_5-*b*-$P2VP_{25}$/Fe_3O_4 (Figure S4A, ~0.1 mg/mL, 0.25 mL), similar strategy was used for transferring the micelles from ethanol to THF. The resulting samples were subjected to TEM analysis (Figure S5).

Preparation of -NMe_3^+ decorated OPV_5-b-$P2VP_{25}$/Fe_3O_4@SiO_2 nanofibers

An ethanol solution of TMTESA (60 μL, 115 mg/mL) was added slowly into the ethanol solution of OPV_5-*b*-$P2VP_{25}$/Fe_3O_4@SiO_2 nanofibers (Figure S4A, ~0.1 mg/mL, 1.5 mL). The mixture was then placed in a shaking table and shaken for 24 h

at a rotation speed of 200 r/min. Then, a magnet was put beside the resulting sample for 20 min, followed by removing the supernatant with a syringe and redispersing the yellow-like colloids in 1.2 mL of H_2O. The resulting sample was subjected to DLS and zeta potential analysis (Figures 3A-B).

Immobilization of DNA onto -NMe_3^+ decorated OPV_5-b-$P2VP_{25}$/Fe_3O_4@SiO_2 nanofibers

DNA solution (60 μL, 0.01 mg/mL in H_2O, DNA from calf thymus) was added into the aqueous solution of as-prepared -NMe_3^+ decorated OPV_5-*b*-$P2VP_{25}$/Fe_3O_4@SiO_2 nanofibers (Figure 3C, 1.2 mL). The mixture was then placed in a shaking table and shaken for 0.5 h at a rotation speed of 200 r/min. The resulting sample was subjected to TEM, DLS and zeta potential analysis (Figures 3A-D).

Relaxivity Measurements

Fe contents in -NMe_3^+ decorated OPV_5-*b*-$P2VP_{25}$/Fe_3O_4@SiO_2 nanofibers and free Fe_3O_4 nanoparticles were firstly measured by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES, Prodigy). Relaxivity measurements were carried out on a 7.0 T Bruker Biospec animal MRI instrument. Aqueous solutions containing -NMe_3^+ decorated OPV_5-*b*-$P2VP_{25}$/Fe_3O_4@SiO_2 nanofibers or free Fe_3O_4 nanoparticles with different Fe contents were prepared, and 200 μL of each solution was transferred into an Eppendorf tube. The detailed parameters for T_2 measurements were set as follows: TE = 40 ms, TR =2000 ms for T_2-weighted imaging.

SUPPORTING FIGURES

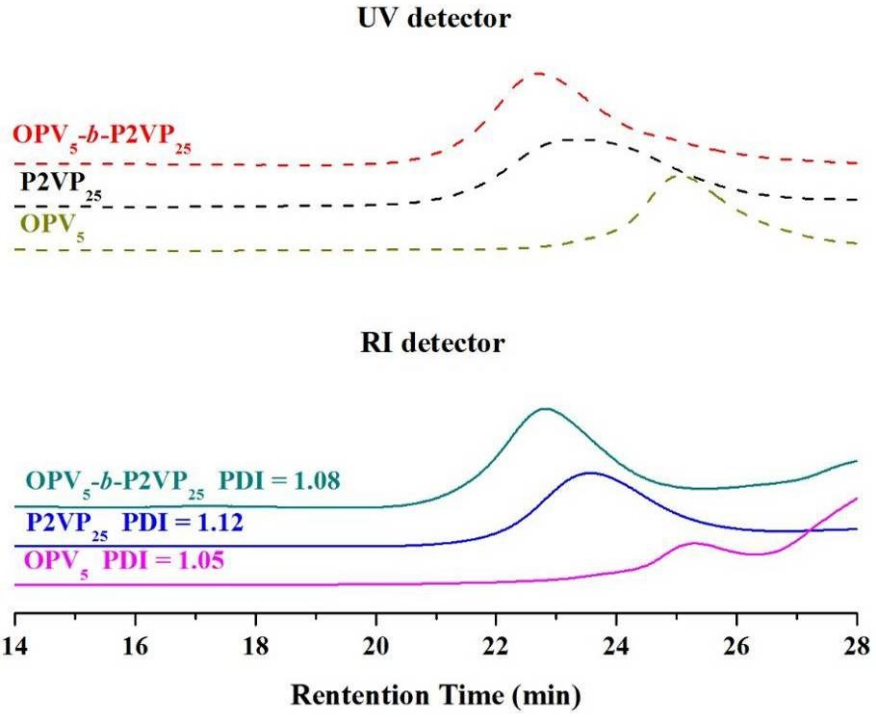


Figure S1. GPC curves of OPV_5, $P2VP_{25}$ and OPV_5-*b*-$P2VP_{25}$ in THF.

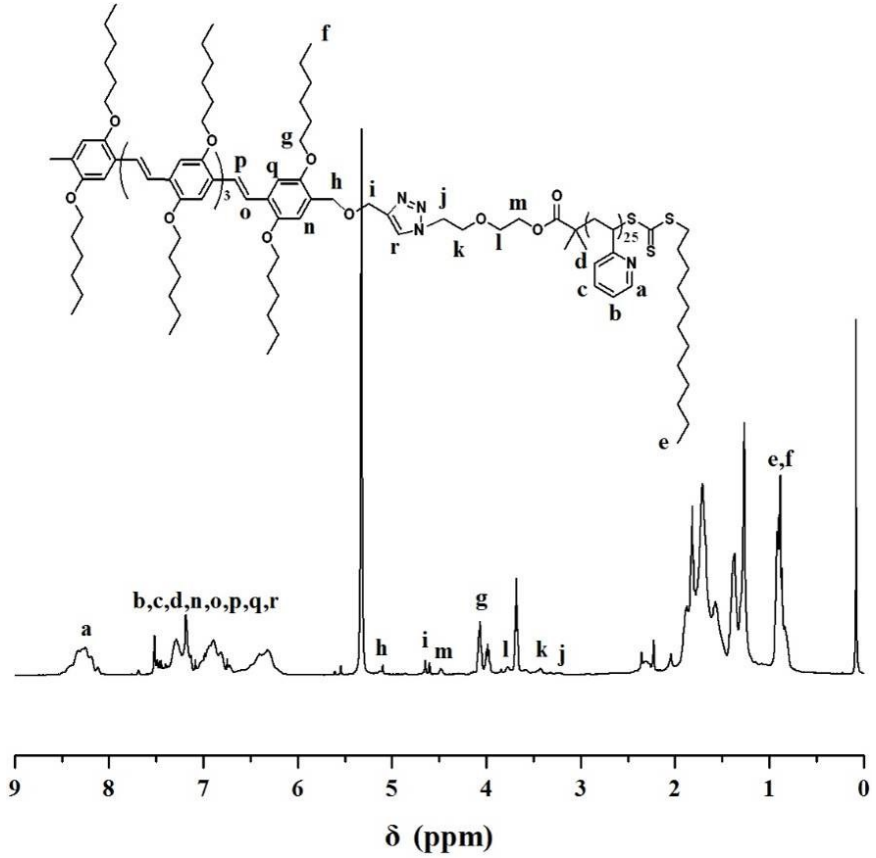


Figure S2. ^{1}H NMR spectrum of OPV_5-*b*-$P2VP_{25}$ in CD_2Cl_2 (DP_{2VP}=33*S_a/S_{e+f}).

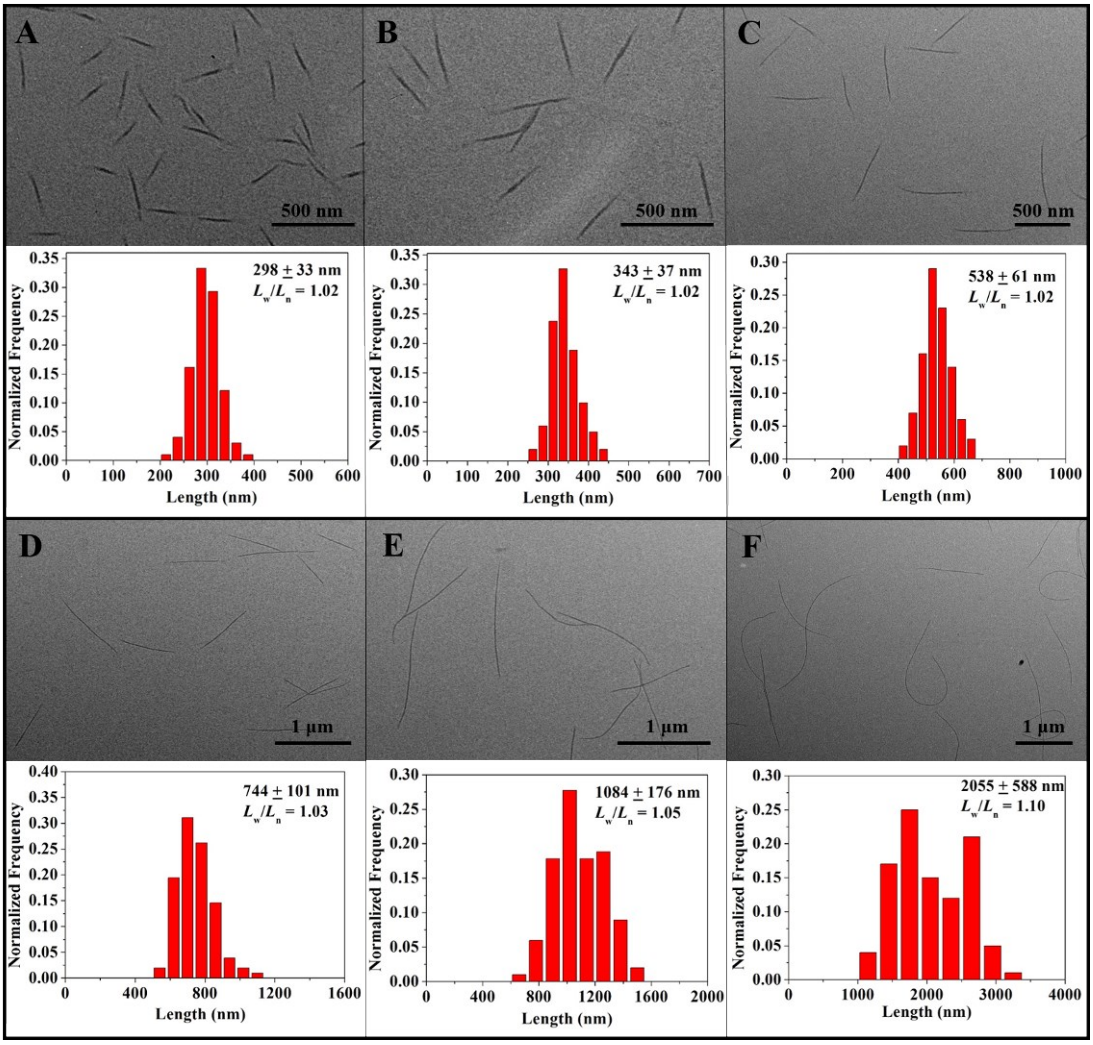


Figure S3. TEM images of uniform micelles of OPV_5-*b*-$P2VP_{25}$ formed by heating seed micelles at (A) 40°C, (B) 42°C, (C) 44°C, (D) 46°C, (E) 48°C and (F) 50°C, followed by cooling/aging at 23°C for 24 h.

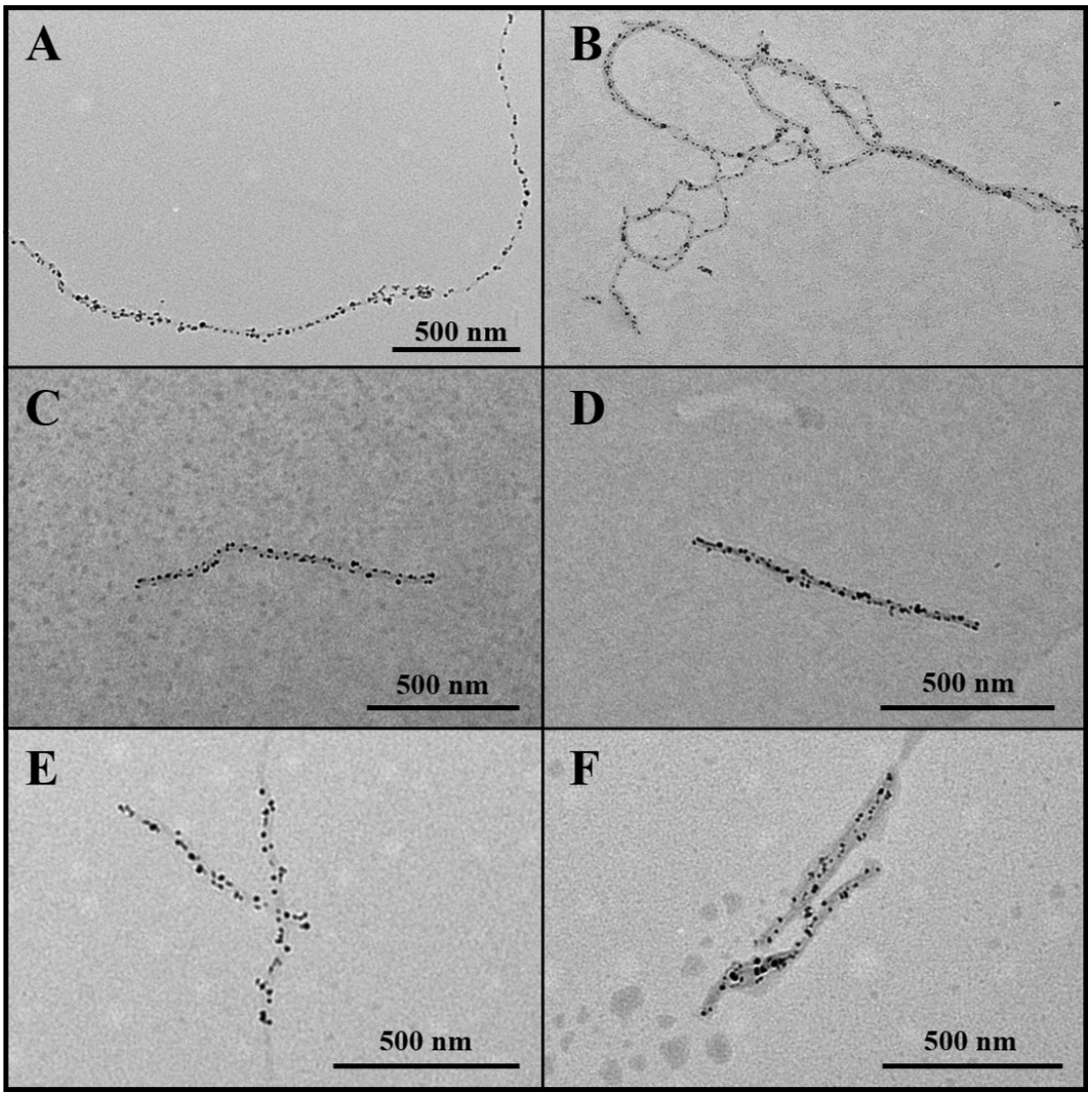


Figure S4. TEM images of polydisperse OPV_5-*b*-$P2VP_{25}/Fe_3O_4$ nanofibers (A) before and (B) after silica coating. TEM images of uniform OPV_5-*b*-$P2VP_{25}/Fe_3O_4$ nanofibers with L_n of 1084 nm (C) before and (D) after silica coating, L_n of 538 nm (E) before and (F) after silica coating.

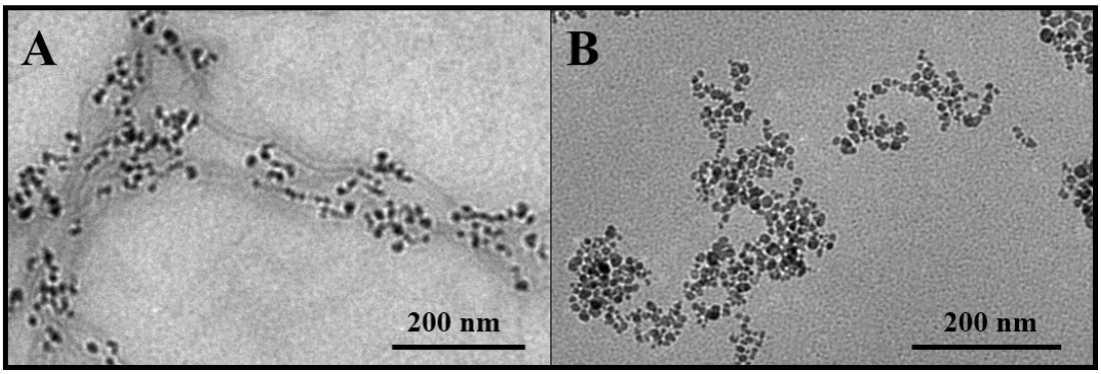


Figure S5. TEM images of (A) OPV_5-*b*-$P2VP_{25}$/Fe_3O_4@SiO_2 and (B) OPV_5-*b*-$P2VP_{27}$/Fe_3O_4 nanofibers after transferring into THF.

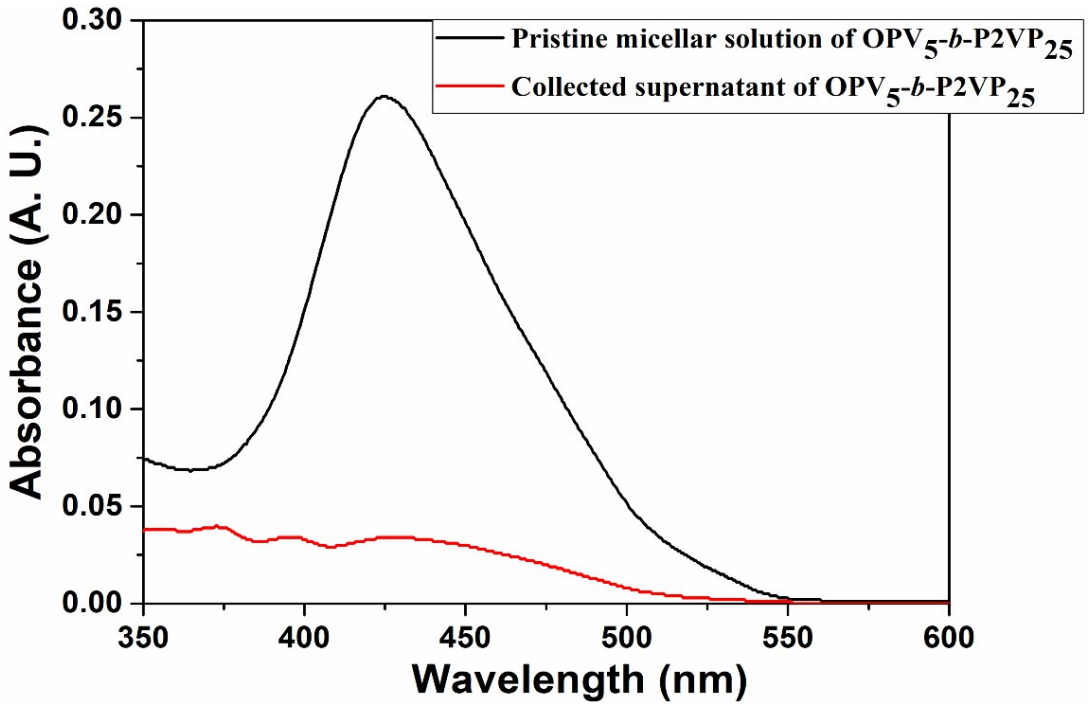


Figure S6. UV/vis absorption spectra of pristine micellar solution of OPV_5-*b*-$P2VP_{25}$ and collected supernatant of solution of nanofibers of OPV_5-*b*-$P2VP_{25}$/Fe_3O_4@SiO_2 after dispersing in THF. After the nanofibers were dispersed in THF, the supernatant was collected and then subject to UV/vis absorption measurement. A typical absorbance with a peak at 428 nm attributed to OPV_5 segments of OPV_5-*b*-$P2VP_{25}$ appeared for the solution of collected supernatant, which indicated the release of

OPV_5-*b*-$P2VP_{25}$ from the nanofibers upon dispersing in THF. The absorbance intensity of collected supernatant at 428 nm was about 0.035. On the contrary, the absorbance intensity of solution of pristine micelles of OPV_5-*b*-$P2VP_{25}$ at 428 nm without surface decoration was about 0.259. These results indicated that there were about 14% (= 0.035/0.259*100%) of OPV_5-*b*-$P2VP_{25}$ released from the nanofibers after dispersing in THF.

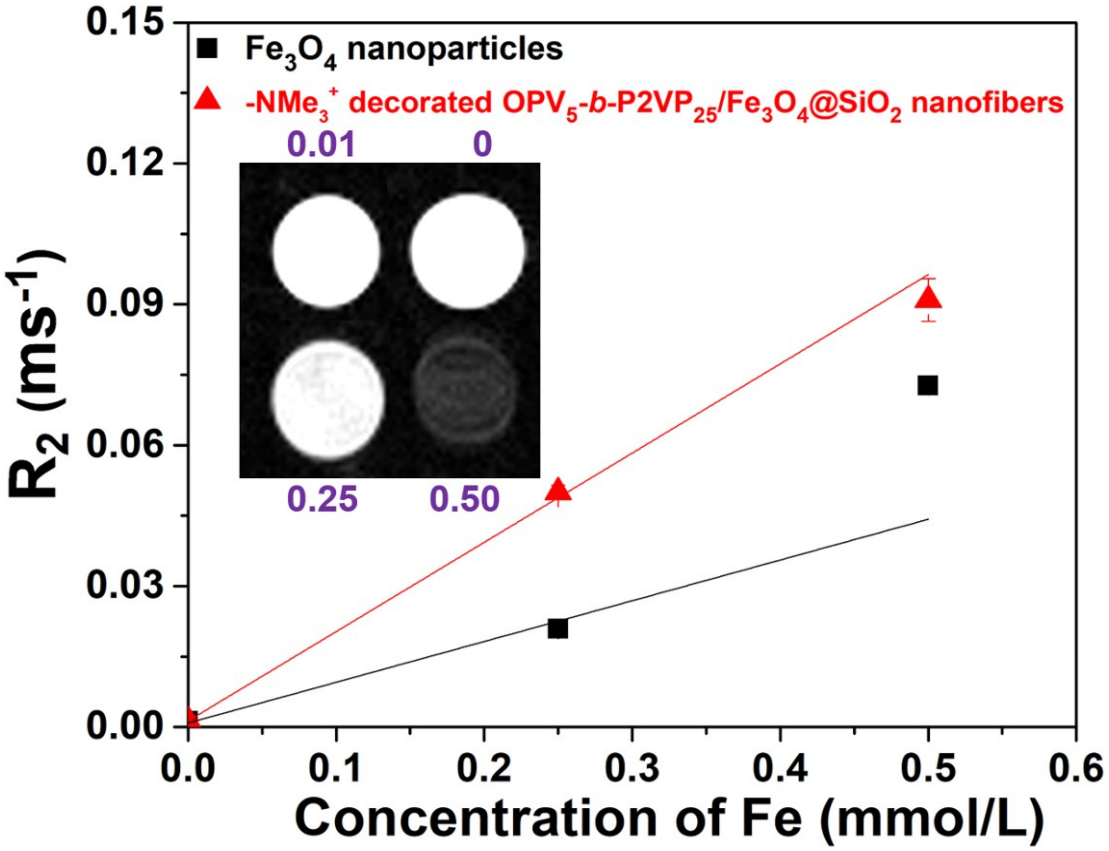


Figure S7. Plots of R_2 MRI signal over Fe_3O_4 nanoparticles and -NMe_3^+ decorated OPV_5-*b*-$P2VP_{25}$/Fe_3O_4@SiO_2 with series concentrations of Fe. The inset shows T_2-weighted MRI phantom images of Fe_3O_4 nanoparticles with series concentrations of Fe.

References:

1. D. L. Tao, Z. Q. Wang, X. Y. Huang, M. W. Tian, G. L. Lu, I. Manners, M. A. Winnik, C. Feng, *Angew. Chem. Int. Ed.* 2020, **59**, 8232-8239.