Supporting information

Biocompatible fluorescent polymeric nanoparticles based on AIE dye and phospholipid monomer

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1. Experimental

1.1 Materials and measurements

11-Bromoundecanol, 4-bromobenzaldehyde and triethyl phosphite purchased from Alfa Aesar company and 9,10-bis(chloromethyl)anthracene purchased from TCI company were used as received. All other agents and solvents were purchased from commercial sources and used directly without further purification. Ultra-pure water was used in the experiments.

Gel permeation chromatography (GPC) analyses of polymers were performed using DMF as the eluent. The GPC system was a Shimadzu LC-20AD pump system comprising of an auto injector, a MZ-Gel SDplus 10.0 mm guard column (50×8.0 mm, 10 Å) followed by a MZ-Gel SDplus 5.0 μm bead-size columns (50-10 Å, linear) and a Shimadzu RID-10A refractive index detector. The system was calibrated with narrow molecular weight distribution polystyrene standards ranging from 200 to 10 g mol⁻¹. ¹H NMR spectrum was measured on a Mercury-Plus 300 MHz spectrometer [d₆-DMSO as solvent and tetramethylsilane (TMS) as the internal standard]. The FT-IR spectra were obtained in a transmission mode on a Shimadzu Spectrum 8400 spectrometer (Japan). Typically, 8 scans at a resolution of 1 cm⁻¹ were accumulated to obtain one spectrum. The X-ray photoelectron spectra (XPS) were performed on a VGESCALAB 220-IXL spectrometer using an Al Ka X-ray source (1486.6 eV). The energy scale was internally calibrated by referencing to the binding energy (Eb) of the C1s peak of a carbon contaminant at 284.6 eV. UV-Visible absorption spectra were recorded on UV/Vis/NIR 2600 spectrometer (Shimadzu, Japan) using quartz cuvettes of 1 cm path length. Fluorescence spectra were measured on an F-4600 spectrometer with a slit width of 3 nm for both excitation and emission. Transmission electron microscopy (TEM) images were recorded on a HT7700 microscope (Hitachi, Japan) operated at 100 kV, the TEM specimens were made by placing a drop of the nanoparticles suspension on a carbon-coated copper grid. The size distribution of An-MTP FPNs in phosphate buffer solution (PBS) were determined using a zeta Plus apparatus (ZetaPlus, Brookhaven Instruments, Holtsville, NY).

1.2 Synthesis of An

Tetraethyl anthracene-9,10-diylbis(methylene)diphosphonate and 4-(undec-10-enyloxy)benzaldehyde were synthesized according to the previous literature.
Tetraethyl anthracene-9,10-diylbis(methylene)diphosphonate (0.10 g, 0.21 mmol) and 4-(undec-10-enyloxy)benzaldehyde (0.137 g, 0.50 mmol) were dissolved in THF (20mL), and then t-BuOK (0.2 g) was added under nitrogen. The solution was stirred at room temperature overnight. After removing the solvent under reduced pressure, the residue was recrystallized with THF/EtOH to afford An (0.14 g, 93% yield). 1H NMR (300 MHz, CDCl3) δ (ppm): 1.28-1.41 (m, 24H), 1.78-1.85 (m, 4H), 2.01-2.08 (m, 4H), 4.02 (t, 4H), 4.90-5.05 (m, 4H), 5.74-5.90 (m, 2H), 6.95 (d, 2H), 6.98 (d, 4H), 7.45 (q, 4H), 7.60 (d, 4H), 7.78 (d, 2H), 8.39 (q, 4H).

1.3 Preparation of An-MTP FPNs

To prepare An-MTP copolymers, a hydrophilic phospholipid monomer of MTP and a new AIE dye based dimer (An) were copolymerized at the existence of CTA via RAFT polymerization. The preparation of An-MTP FPNs was schematically shown in scheme 2. CTA (20 mg, 0.076 mmol), An (55 mg, 0.076 mmol), MTP (224 mg, 0.76 mmol), AIBN (5.0 mg) and DMF (5 mL) were introduced in schlenk tube and purged by nitrogen flow for 30 min. The above mixture was put into an oil bath maintained at 70 °C for 12 h. Then stopped the reaction of polymerization, and dialyzed against tap water for 24 h and ethanol for 6 h using 7000 Da Mw cutoff dialysis membranes. Finally, the solution in dialysis bag was carried out by freeze-drying to obtain An-MTP FPNs.

1.4 Cytotoxicity of An-MTP FPNs

Cell morphology was observed to examine the effects of An-MTP FPNs to A549 cells. Briefly, cells were seeded in 6-well microplates at a density of 1×10^5 cells mL⁻¹ in 2 mL of respective media containing 10% fetal bovine serum (FBS). After cell attachment, plates were washed with PBS and cells were treated with complete cell culture medium, or different concentrations of An-MTP FPNs prepared in 10% FBS containing media for 24 h. Then all samples were washed with PBS three times to remove the uninternalized nanoparticles. The morphology of cells was observed by using an optical microscopy (Leica, Germany), the overall magnification was ×100.
The cell viability of An-MTP FPNs on A549 cells was evaluated by cell counting kit-8 (CCK-8) assay. Briefly, cells were seeded in 96-well microplates at a density of $5 \times 10^4$ cells mL$^{-1}$ in 160 μL of respective media containing 10% FBS. After 24 h of cell attachment, the cells were incubated with 10, 20, 40, 80, 120 μg mL$^{-1}$ An-MTP FPNs for 8 and 24 h. Then nanoparticles were removed and cells were washed with PBS three times. 10 μL of CCK-8 dye and 100 μL of DMEM cell culture medium were added to each well and incubated for 2 h at 37 °C. Plates were then analyzed with a microplate reader (VictorIII, Perkin-Elmer). Measurements of formazan dye absorbance were carried out at 450 nm, with the reference wavelength at 620 nm. The values were proportional to the number of live cells. The percent reduction of CCK-8 dye was compared to controls (cells not exposure to An-MTP FPNs), which represented 100% CCK-8 reduction. Three replicate wells were used per microplate, and the experiment was repeated three times. Cell survival was expressed as absorbance relative to that of untreated controls. Results are presented as mean ± standard deviation (SD).

1.5 Confocal microscopic imaging of cells using An-MTP FPNs

A549 cells were cultured in Dulbecco’s modified eagle medium (DMEM) supplemented with 10% heat-inactivated FBS, 2 mM glutamine, 100 U mL$^{-1}$ penicillin, and 100 μg mL$^{-1}$ of streptomycin. Cell culture was maintained at 37 °C in a humidified condition of 95% air and 5% CO$_2$ in culture medium. Culture medium was changed every three days for maintaining the exponential growth of the cells. On the day prior to treatment, cells were seeded in a glass bottom dish with a density of $1 \times 10^5$ cells per dish. On the day of treatment, the cells were incubated with An-MTP FPNs at a final concentration of 10 μg mL$^{-1}$ for 3 h at 37 °C. Afterward, the cells were washed three times with PBS to remove the An-MTP FPNs and then fixed with 4% paraformaldehyde for 10 min at room temperature. Cell images were taken with a confocal laser scanning microscope (CLSM) Zesis 710 3-channel (Zesis, Germany) with the excitation wavelength of 405 nm.

2. Results
Fig. S1 The AIE characteristic of An (λ_{Ex} = 405 nm): (A) PL spectra of An in THF–water mixtures with different water fractions; (B) the changes in PL peak intensity of An in different water fraction mixtures.

Fig. S2 ^1^H NMR spectrum of An-MTP dissolved in d_6-DMSO.
Fig. S3 XPS spectra of MTP and An-MTP FPNs: (A) C 1s spectra; (B) N 1s spectra; (C) O 1s spectra; (D) P 2p spectra.