

Supporting information

Novel biocompatible cross-linked fluorescent polymeric nanoparticles based on an AIE monomer

Xiqi Zhang^{*a}, Xiaoyong Zhang^a, Bin Yang^a, Junfeng Hui^a, Meiyong Liu^b, Zhenguo Chi^c, Siwei Liu^c, Jiarui Xu^c, Yen Wei^{*a}

^a Department of Chemistry and the Tsinghua Center for Frontier Polymer Research, Tsinghua University, Beijing, 100084, P. R. China. ^b Beijing National Laboratory for Molecular Sciences (BNLMS), Key Laboratory of Organic Solids, Laboratory of New Materials, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, China. ^c PCFM Lab, DSAPM Lab and KLGHEI of Environment and Energy Chemistry, FCM Institute, State Key Laboratory of Optoelectronic Materials and Technologies, School of Chemistry and Chemical Engineering, Sun Yat-sen University, Guangzhou 510275, China.

sychyzhang@126.com; weiyen@tsinghua.edu.cn

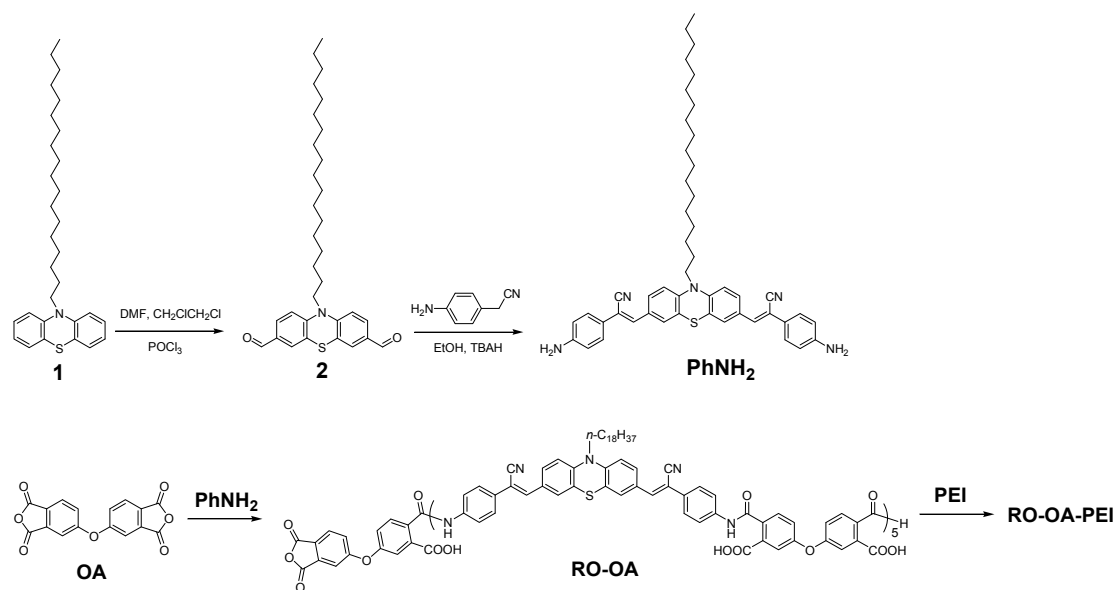
1. Experimental

1.1 Materials and measurements

Phenothiazine, 3-bromoprop-1-ene, N,N-dimethylformamide (DMF), 1,2-dichloroethane, phosphoryl chloride, 4-aminobenzyl cyanide, tetrabutylammonium hydroxide (0.8M in methanol), N,N-dimethylacetamide (DMAc), 4,4'-Oxydiphthalic anhydride purchased from Alfa Aesar were used as received. All other agents and solvents were purchased from commercial sources and used directly without further purification. Tetrahydrofuran (THF) was distilled from sodium/benzophenone. Ultra-pure water was used in the experiments.

^1H NMR spectrum was measured on a JEOL 400 MHz spectrometer [d_6 -DMSO as solvent and tetramethylsilane (TMS) as the internal standard]. UV-Visible absorption spectrum was recorded on UV/Vis/NIR Perkin-Elmer lambda750 spectrometer (Waltham, MA, USA) using quartz cuvettes of 1 cm path length. Fluorescence spectra were measured on a PE LS-55 spectrometer with a slit width of 3 nm for both excitation and emission. The FT-IR spectra were obtained in a transmission mode on a Perkin-Elmer Spectrum 100 spectrometer (Waltham, MA, USA). Typically, 8 scans at a resolution of 1 cm^{-1} were accumulated to obtain one spectrum. Transmission electron microscopy (TEM) images were recorded on a JEM-1200EX microscope 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 and zeta potential measurement of **RO-OA-PEI** FPNs in phosphate buffer solution (PBS) were determined using a zeta Plus apparatus (ZetaPlus, Brookhaven Instruments, Holtsville, NY). 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 ($50\times 8.0\text{ mm}$, 10^2 Å) followed by a MZ-Gel SDplus 5.0 μm bead-size columns ($50\text{-}10^6\text{ Å}$, linear) and a Shimadzu RID-10A refractive index detector. The system was calibrated with narrow molecular weight distribution polystyrene standards ranging from 200 to 10^6 g mol^{-1} .

1.2 Preparation of FPNs



PhNH₂ was synthesized according to our previous literature.¹ In the synthesis of **RO-OA-PEI**, PhNH₂ (37 mg, 0.05 mmol) and 4,4'-oxydipthalic anhydride (19 mg, 0.06 mmol) were dissolved in 10 mL DMAc. The above mixture was stirred under air atmosphere at room temperature for 2h. Followed by adding branched PEI (M_w = 600, 3.0 mg, 0.005 mmol) which was dissolved in 3 mL DMAc. The above mixture was stirred at room temperature for another 0.5 h. Then the reaction of cross-linking was stopped and dialyzed against tap water for 24 h and ethanol for 6 h using 7000 Da Mw cutoff dialysis membranes. Finally, thus solution in dialysis bag was carried out by freeze-drying to obtain **RO-OA-PEI** FPNs. The syntheses of **RO-OA-PEI-0.33** and **RO-OA-PEI-3** FPNs were similar to that of **RO-OA-PEI** FPNs, the only difference was that the PEI feed ratio was 1.0 mg and 9.0 mg, which was 0.33 and 3 folds compared to **RO-OA-PEI**, respectively.

1.3 Cytotoxicity of RO-OA-PEI FPNs

Cell morphology was observed to examine the effects of **RO-OA-PEI** FPNs to A549 cells. Briefly, cells were seeded in 6-well microplates at a density of 1×10⁵ 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 **RO-OA-PEI** 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 **RO-OA-PEI** 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 5×10^4 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 $\mu\text{g mL}^{-1}$ **RO-OA-PEI** 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 **RO-OA-PEI** 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 $\pm$ standard deviation (SD).

1.4 Confocal microscopic imaging of cells using **RO-OA-PEI** 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 $\mu\text{g mL}^{-1}$ 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 1×10^5 cells per dish. On the day of treatment, the cells were incubated with **RO-OA-PEI** FPNs at a final concentration of 10 $\mu\text{g mL}^{-1}$ for 3 h at 37 °C. Afterward, the cells were washed three times with PBS to remove the **RO-OA-PEI** 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 543 nm.

2. Results

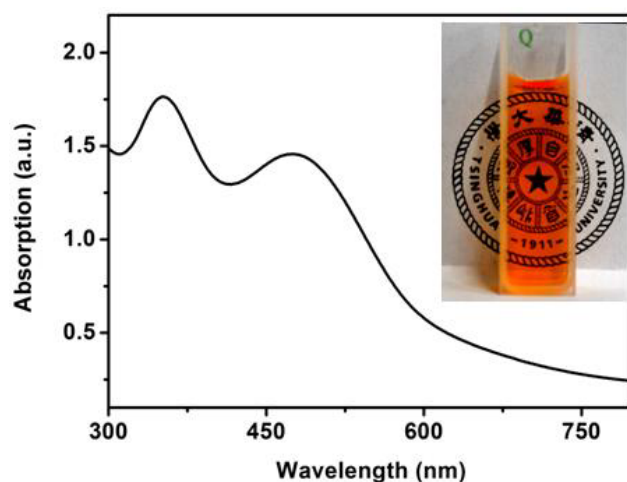


Fig. S1 UV-Vis spectrum of **RO-OA-PEI** FPNs, inset is the visible image of thus FPNs in water using the logo of “Tsinghua University” as background.

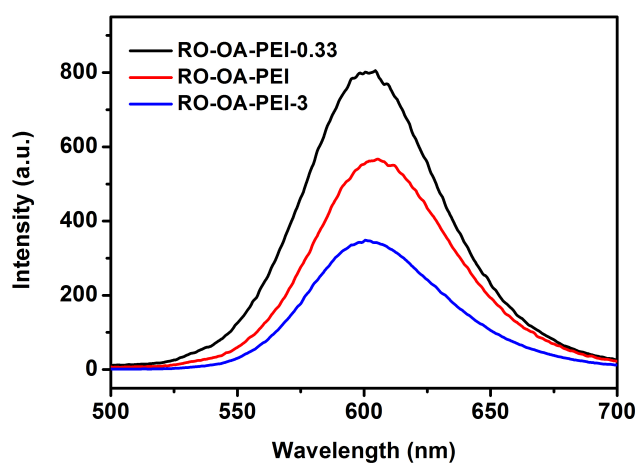


Fig. S2 The emission spectra of **RO-OA-PEI-0.33**, **RO-OA-PEI** and **RO-OA-PEI-3** FPNs. The concentration of FPNs is $10 \mu\text{g mL}^{-1}$.

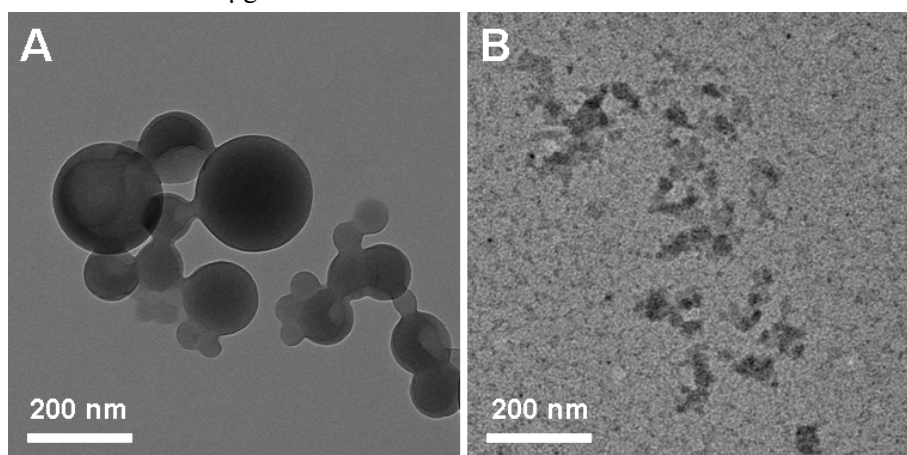


Fig. S3 TEM images of (A) **RO-OA-PEI-0.33** and (B) **RO-OA-PEI-3** FPNs dispersed in water, scale bar = 200 nm.

Reference

1. X. Zhang, X. Zhang, B. Yang, J. Hui, M. Liu, Z. Chi, S. Liu, J. Xu and Y. Wei, *Polym. Chem.*, 2014, DOI:10.1039/C1033PY01143G.