Electronic Supplementary Material (ESI) for Polymer Chemistry

Supporting information

PEGylation and cell imaging applications of AIE based fluorescent organic nanoparticles via ring-opening reaction

Xiaoyong Zhang[a,b,*], Xiqi Zhang[b], Bin Yang[b], Junfeng Hui[b], Meiying Liu[c], Wanyun liu[a], Yiwang Chen[a], Yen Wei[b, *]

[a] Department of Chemistry/Institute of Polymers, Nanchang University, 999 Xuefu Avenue, Nanchang 330031, China. [b] Department of Chemistry and Key Laboratory of Bioorganic Phosphorus Chemistry & Chemical Biology (Ministry of Education), Tsinghua University, Beijing, 100084, P. R. China. [c] 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.

xiaoyongzhang1980@gmail.com; weiyen@tsinghua.edu.cn

1. Experimental

1.1 Materials and measurements

Phenothiazine, 1-bromooctadecane, 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. mPEG-NH_2 (M.W. 2000) was bought from Aladdin company. 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.

UV-Visible absorption spectra were 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-PEG** FONs in phosphate buffer solution (PBS) were determined using a zeta Plus apparatus (ZetaPlus, Brookhaven Instruments, Holtsville, NY). ^{1}H NMR spectra were measured on a JEOL 400 MHz spectrometer [$CDCl_3$ or d_6-DMSO as solvent and tetramethylsilane (TMS) as the internal standard]. HRMS was obtained on Shimadzu LCMS-IT-TOF high resolution mass spectrometry.

1.2 Preparation of RO-OA-PEG FONs

$PhNH_2$ (37 mg, 0.05 mmol), 4,4'-oxydiphthalic anhydride (19 mg, 0.06 mmol) were dissolved in 10 mL DMAc. The above mixture was stirred under air atmosphere at room temperature for 2 h. Then mPEG-NH_2 (40 mg, 0.02 mmol) was added to the above mixture and stirred for 30 min. Then the reaction of polymerization 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 the product.

1.3 Cytotoxicity of $PhNH_2$-OA-PEG FONs

Cell morphology was observed to examine the effects of **PhNH$_2$-OA-PEG** FONs to A549 cells.[1, 2] 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 **PhNH$_2$-OA-PEG** FONs 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 **PhNH$_2$-OA-PEG** FONs on A549 cells was evaluated by cell counting kit-8 (CCK-8) assay based on our previous reports.[3] 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 μg mL^{-1} **PhNH$_2$-OA-PEG** FONs for 10 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 **PhNH$_2$-OA-PEG** FONs), 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).

1.4 Confocal microscopic imaging of cells using PHNH$_2$-OA-PEG FONs

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 1×10^5 cells per dish. On the day of treatment, the cells were incubated with **PhNH$_2$-OA-PEG** FONs 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 **$PhNH_2$-OA-PEG** FONs 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.

Results

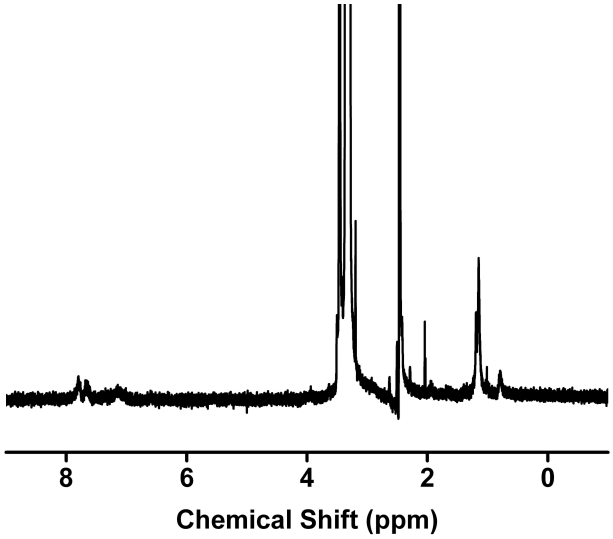

Fig. S1 ^{1}HNMR spectrum of **$PhNH_2$-OA-PEG** dissolved in d_6-DMSO

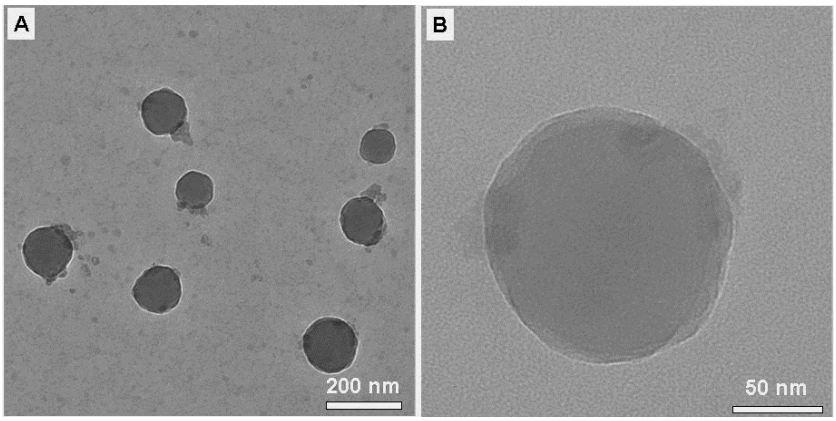

Fig. S2 Representative TEM images of **$PhNH_2$-OA-PEG** FONs with different magnification, (A) scale bar = 200 nm, (B) scale bar = 50 nm.

References

1. H. Qi, M. Liu, L. Xu, L. Feng, l. Tao, Y. Ji, X. Zhang and Y. Wei, *Toxicol. Res.*, 2013, 10.1039/C1033TX50060H.
2. X. Zhang, W. Hu, J. Li, L. Tao and Y. Wei, *Toxicol. Res.*, 2012, **1**, 62-68.
3. X. Zhang, H. Qi, S. Wang, L. Feng, Y. Ji, L. Tao, S. Li and Y. Wei, *Toxicol. Res.*, 2012, **1**, 201-205.