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Supporting information

Aggregation-Induced Emission Materials Based Fluorescent Organic Nanoparticles: Facile PEGylation and Cell Imaging Applications

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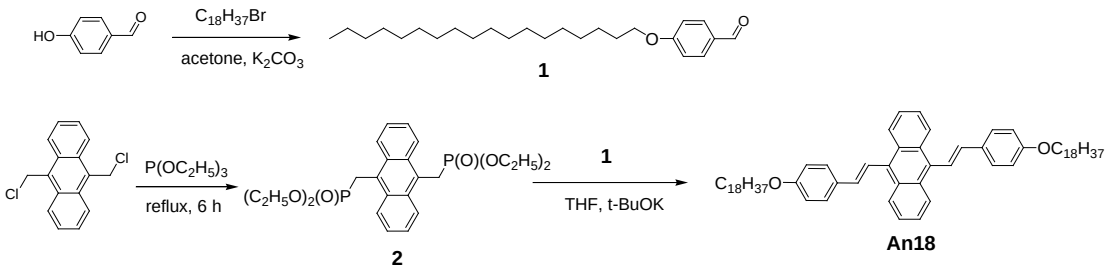
EXPERIMENTAL

1. Materials and measurements:

2,2'-azobisisobutyronitrile (AIBN, J&K Chemical, 98%), the hydrophobic stearyl methacrylate (SMA) monomer was purchased from Aladdin (Shanghai, china, 96%) and poly(ethylene glycol) methyl ether methacrylate (PEGMA, MW: 950 Da, J&K chemica, 98%) were used as received. The chain transfer agent (CTA) was synthesized according to our previous report. 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. 9,10-Bis(chloromethyl)anthracene purchased from TCI and 4-hydroxybenzaldehyde, 1-bromooctadecane purchased from Alfa Aesar were used as received. **An18** were prepared according to our previous report.[1]

^{1}H NMR and ^{13}C NMR spectra were measured on a JEOL 400 MHz spectrometer [$CDCl_3$ or DMSO as solvent and tetramethylsilane (TMS) as the internal standard]. Standard FAB-MS was obtained on ZAB-HS mass spectrometry. Fluorescence spectra were measured on a PE LS-55 spectrometer with a slit width of 3 nm for both excitation and emission. 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 FT-IR spectra were obtained in a transmission mode on a Perkin-Elmer Spectrum 100 spectrometer (Waltham, MA, USA). Typically, 4 scans at a resolution of 1 cm^{-1} were accumulated to obtain one spectrum. Gel permeation chromatography (GPC) analyses of polymers were performed using dimethylformamide as the eluent. The GPC system was a Shimadzu LC-20AD pump system comprising an auto injector, a MZ-Gel SDplus10.0 μm guard column (50 × 8.0 mm, 102A) followed by three MZ-Gel SDplus10.0 μm bead-size columns (105, 103, and 102 A) and a differential refractive index (RI) detector. The system was calibrated with narrow molecular weight distribution polystyrene standards ranging from 200 to 10^6 g mol^{-1}.



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Scheme. S1 Synthetic route of **An18**

2. Synthesis of copolymers

In a typical experiment, PEGMEMA (2.66 g, 2.8 mmol), Stearyl methacrylate (0.41g, 1.2 mmol), the chain transfer agent (CTA) (21.0 mg, 0.08 mmol), AIBN (4.0 mg, 0.016 mmol) and toluene (4.0 mL) were introduced in schlenk tube with a magnetic stir bar and purged by nitrogen flow for 30 min. The final reaction mixture was put into an oil bath maintained at 70 °C for 20 h. Samples were withdrawn periodically for ^{1}H NMR and GPC analyses for conversion and molecular weight determination, respectively. At the end of the polymerization, the purified polymer was obtained via precipitation from methanol to ether for three times, and then dried under vacuum for further characterization. All polymers in current report are obtained with the same approach.

3. Preparation of An18-PEG FONs

The preparation of **An18-PEG** FONs was carried out as follows. Approximately 20 mg of AIE dyes (**An18**) was dissolved in 20 mL of THF and then mixed with 80 mg of copolymers in 20 mL of H_2O in a 100 mL vial. And then the mixture was evaporated to completely remove the organic agent (THF) on a rotary evaporator at 40 °C. To remove the excess copolymers, the **An18-PEG** water dispersion was treated by repeated centrifugal washing process for thrice.

4. Cytotoxicity of An18-PEG FONs

Cell morphology was observed to examine the effects of **An18-PEG** FONs 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% FBS. After cell attachment, plates were washed with PBS and the cells were treated with complete cell culture medium, or different concentrations of fluoridated **An18-PEG** FONs prepared in 10% FBS containing media for 24 h. Then all samples were washed with PBS three times to remove the uninternalized FONs. The morphology of cells was observed by using an optical microscopy (Leica, Germany), the overall magnification was ×100.

The cell viability of **An18-PEG** FONs on A549 cells was evaluated by cell counting kit-8 (CCK-8) assay based on our previous reports.[2] 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, 160 μg mL^{-1} **An18-PEG** FONs 24 h. Then nanoparticles were removed and cells were washed with PBS three times. 10 μL of CCK-8

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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 **An18-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).

4. Confocal microscopic imaging of cells using An18-PEG FONs

A549 and NIH-3T3 cells were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (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 **An18-PEG** FONs at a final concentration of 40 μg mL^{-1} for 3 h at 37 °C. Afterward, the cells were washed three times with PBS to remove the **An18-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 wavelengths of 405 nm.

Results and Discussions

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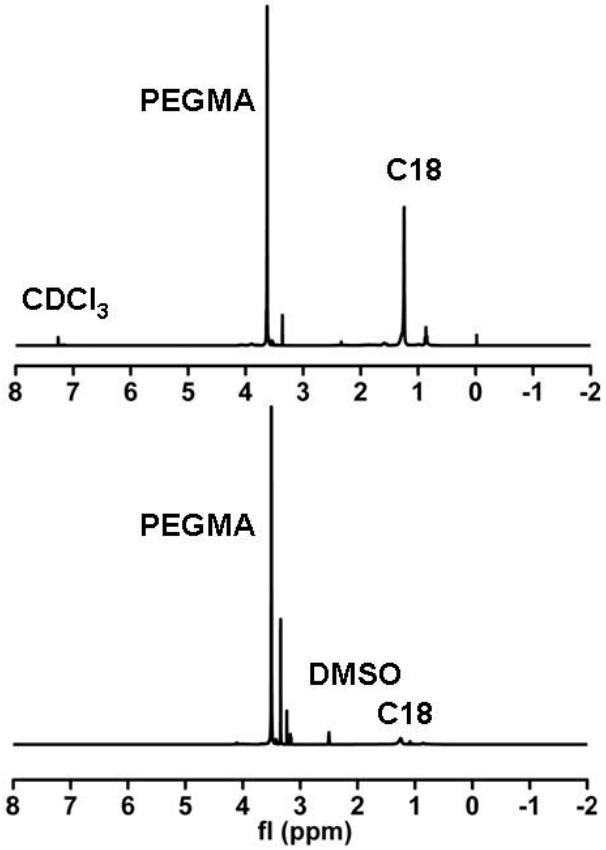

Fig. S1 ^{1}H NMR spectra of PEG-3 in $CDCl_3$ and PEG-7 in DMSO.

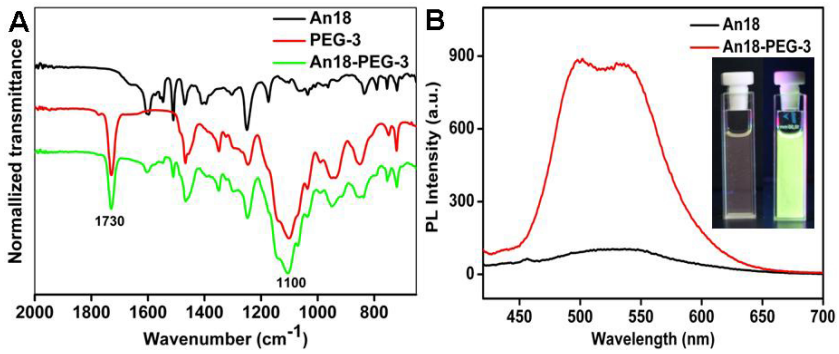

Fig. S2 (A) normallized IR spectra of **An18**, PEG-3 and **An18-PEG-3**, strong stretching vibration bands of C=O which located at 1730 cm^{-1} and C-O stretching vibration bands which located at 1100 cm^{-1} were observed in the sample of **An18-PEG-3**, suggesting **An18-PEG-3** nanoparticles were formed. (B) PL spectra of **An18** (in THF) and **An18-PEG-3** (in H_2O), the excitation wavelength is 405 nm. Insets are fluorescent images of **An18** (in THF) and **An18-PEG-3** dispersion under UV lamp (λ=365 nm).

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Fig. S3 Water dispersion of **An18-PEG-3** and **An18-PEG-7**, the concentration of **An18-PEG** FONs is 1 mg mL^{-1}.

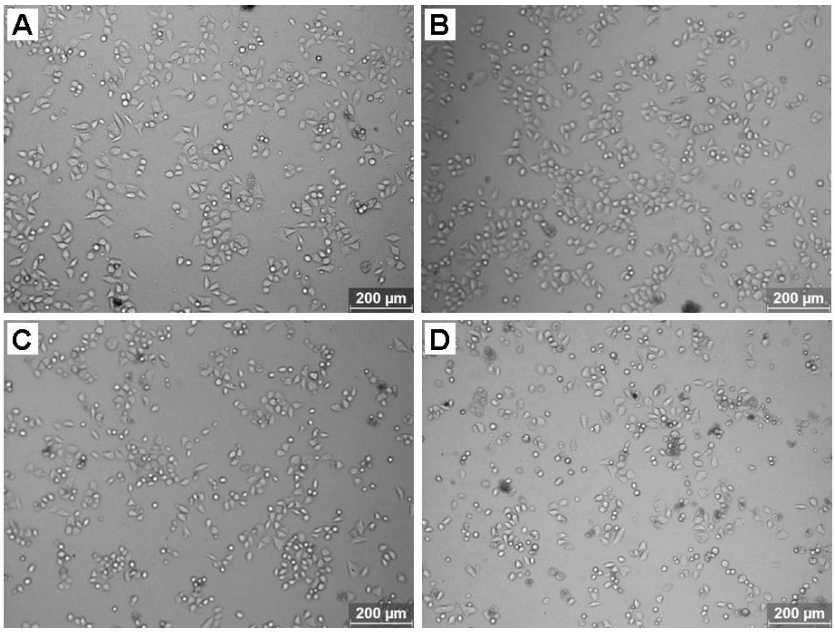

Fig. S4 optical microscopy images of A549 cells incubated with different concentrations of of **An18-PEG-3** for 24 h. (A) control cells, (B) 20 μg mL^{-1},(C) 40 μg mL^{-1}, (D) 160μg mL^{-1}.

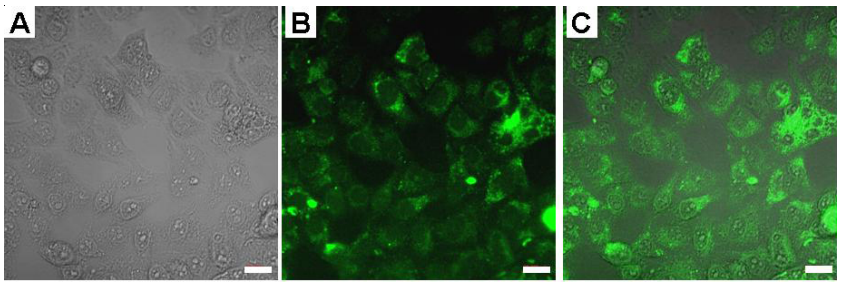

Fig. S5 CLSM images of A549 cells incubated with 40 μg mL^{-1} of **An18-PEG-3** for 3 h. (A) bright field, (B) excited with 405 nm laser, (C) merge image of a and b. Scale bar = 20 μm.

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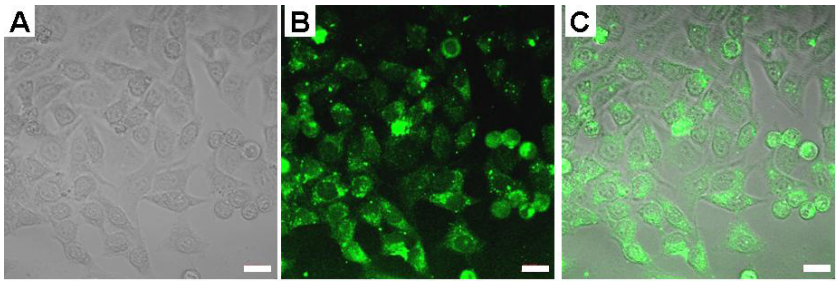

Fig. S6 CLSM images of NIH-3T3 cells incubated with 40 μg mL^{-1} of **An18-PEG-7** for 3 h. (A) bright field, (B) excited with 405 nm laser, (C) merge image of a and b. Scale bar = 20 μm.

References

1. X. Zhang, X. Zhang, S. Wang, M. Liu, L. Tao and Y. Wei, *Nanoscale*, 2013, **5**, 147-150.
2. X. Zhang, H. Qi, S. Wang, L. Feng, Y. Ji, L. Tao, S. Li and Y. Wei, *Toxicol. Res.*, 2012, **1**, 201-205.