Supporting information

Surfactant Modification of Aggregation-Induced Emission Material as Biocompatible Nanoparticles: Facile preparation and cell imaging

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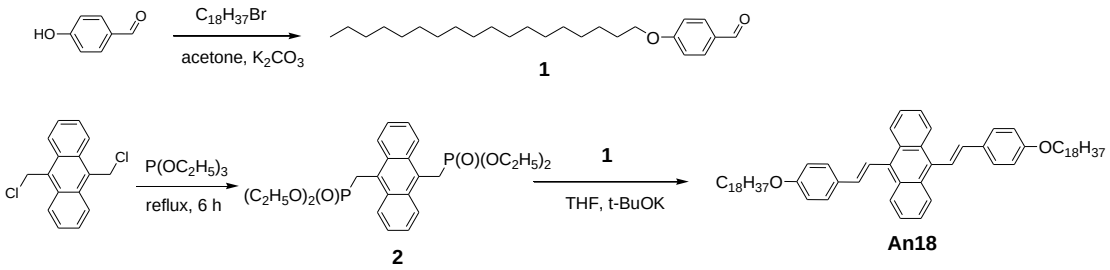
EXPERIMENTAL

1. Materials and measurements:

9,10-Bis(chloromethyl)anthracene purchased from TCI and 4-hydroxybenzaldehyde, 1-bromooctadecane 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. Intermediates **1**[1] and **2**[2] were prepared according to the literature methods.

^{1}H NMR and ^{13}C NMR spectra were measured on a JEOL 400 MHz spectrometer [$CDCl_3$ 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.



Scheme. S1 Synthetic route of **An18**

1 (0.45 g, 1.20 mmol) and **2** (0.24 g, 0.50 mmol) were dissolved in THF (20 mL), then *t*-BuOK (0.2 g) was added under Ar gas. The solution was stirred at room temperature overnight. After removing the solvent under reduced pressure, the residue was recrystallized with THF/EtOH to give **An18**. Yield 87%。 ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.91 (t, 6H, J = 8.8 *Hz*, -CH_3), 1.17-1.56 (m, 60H, -CH_2-), 1.85 (quintet, 4H, J = 9.2 *Hz*, -CH_2-), 4.02 (t, 4H, J = 8.8 *Hz*, -CH_2-O-), 6.84 (s, 1H, Ar-H), 6.90 (s, 1H, Ar-H), 6.99 (d, 4H, J = 11.6 *Hz*, -CH=CH-), 7.47 (quartet, 4H, J = 4.4 *Hz*, Ar-H), 7.61 (d, 4H, J = 11.6 *Hz*, Ar-H), 7.76 (s, 1H, Ar-H), 7.81 (s, 1H, Ar-H), 8.40 (quartet, 4H, J = 4.4 *Hz*, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 159.3, 137.1, 133.0, 130.2, 129.8, 128.0, 126.8, 125.3,

123.0, 115.1, 68.5, 32.3, 30.1, 29.8, 29.7, 29.6, 26.5, 23.1, 14.5; IR (cm^{-1}): 2954, 2917, 2849, 1599, 1549, 1511, 1470, 1394, 1303, 1250, 1174, 1110, 1033, 1020, 962, 835, 791, 754, 719; MS (FAB) calcd. for $C_{66}H_{94}O_2$ 918, found 918.

2. Preparation of An18-F127 NPs:

The preparation of **An18-F127** NPs was carried out as follows. Approximately 20 mg of AIE dyes (**An**) was dissolved in 20 mL of THF and then mixed with 50 mg of Pluronic F127 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 Pluronic F127, the **An18-F127** water dispersion was treated by repeated centrifugal washing process for thrice.

3. Cytotoxicity of An18-F127 NPs:

Cell morphology was observed to examine the effects of **An18-F127** NPs 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-F127** NPs prepared in 10% FBS containing media for 24 h. Then all samples were washed with PBS three times to remove the uninternalized NPs. The morphology of cells was observed by using an optical microscopy (Leica, Germany), the overall magnification was ×100.

The cell viability of **An18-F127** NPs 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, 60, 100 μg mL^{-1} **An18-F127** NPs 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 **An18-F127** NPs), 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-F127 NPs:

A549 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-F127** NPs 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-F127** NPs and then fixed with 4% paraformaldehyde for 10 min at room temperature. Cell images were taken with a Laser Scanning Confocal Microscope (LCSM) Zesis 710 3-channel (Zesis, Germany) with the excitation wavelengths of 405 nm.

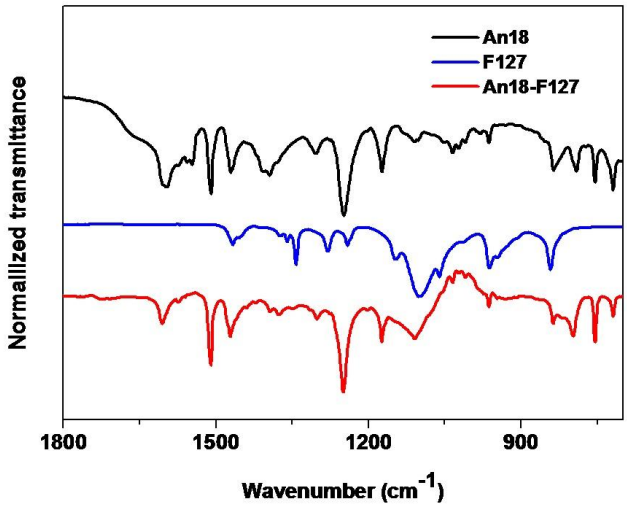


Fig. S1 FT-IR spectra of **An18**, **F127** and **An18-F127**.

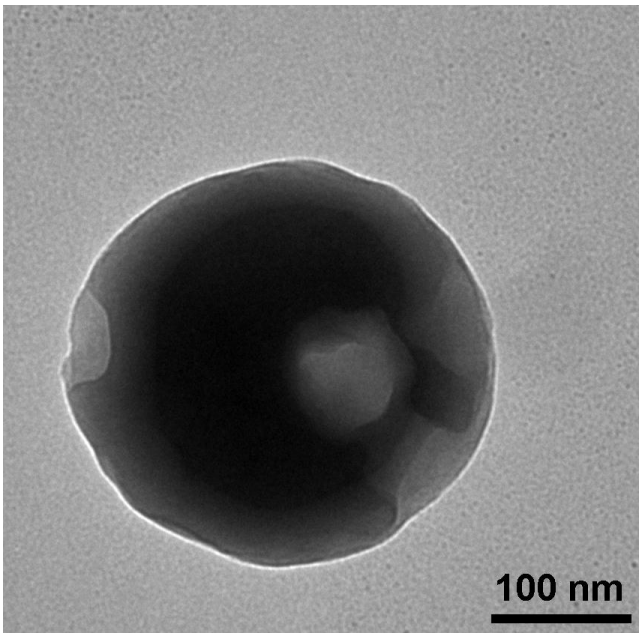


Fig. S2 TEM image of **An18-F127** NPs dispersed in water.

References

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2. H. C. Zhang, E. Q. Guo, Y. L. Zhang, P. H. Ren and W. J. Yang, *Chem. Mater.*, 2009, **21**, 5125-5135.
3. X. Zhang, H. Qi, S. Wang, L. Feng, Y. Ji, L. Tao, S. Li and Y. Wei, *Toxicol. Res.*, 2012, 10.1039/C1032TX20035J.