Supporting information

Facile Fabrication and Cell Imaging Applications of Aggregation-induced Emission Dye Based Fluorescent Organic Nanoparticles

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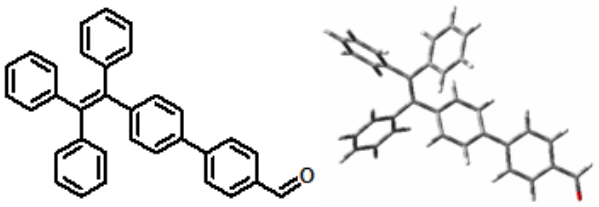
1. Experimental

1.1 Materials and measurements

Benzophenone, n-butyl lithium (n-BuLi) in hexane (2.4 M), 4-formylphenylboronic acid, tetrabutyl ammonium bromide (TBAB), tetrakis(triphenylphosphine) palladium(0) [Pd(PPh3)4], diphenylmethane, 4-bromophenyl phenylmethanone and p-toluenesulfonic acid were purchased from Alfa Aesar and 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. 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 X-ray photoelectron spectra (XPS) were performed on a VGESCALAB 220-IXL spectrometer using an Al Kα 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.

1.2 Preparation of P5-chitosan

The AIE dye (named **P5**) and its intermediates were prepared according to the literature methods.13 The chemical structure of **P5** is given in scheme 2.



Scheme S1 The chemical structure (left) and the optimization geometry (right) of **P5**.

carboxymethyl chitosan (0.20 g) was dissolved in 10 mL of ultra-pure water. **P5** (0.05 g) in 10 mL THF was introduced to the chitosan solution at room temperature. The mixture was adjust to alkaline (pH = 10) and allowed to react for 30 min under stirring. Then sodium borohydride (0.05 g) was added into the mixture, the reaction was carried out for 0.5 h to obtain **P5-chitosan**. And

then the mixture was evaporated to completely remove the organic agent (THF) on a rotary evaporator at 40 °C. To remove the excess sodium borohydride, the **P5-Chitosan** water dispersion was treated by repeated centrifugal washing process for thrice.

1.3 Cytotoxicity of P5-chitosan FONs

Cell morphology was observed to examine the effects of **P5-Chitosan** 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% 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 **P5-chitosan** 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 **P5-chitosan** FONs on A549 cells was evaluated by cell counting kit-8 (CCK-8) assay based on our previous reports. 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} **P5-chitosan** FONs 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 **P5-chitosan** 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 P5-chitosan

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% CO2 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 **P5-chitosan** 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 **P5-chitosan** 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 405 nm.

Results

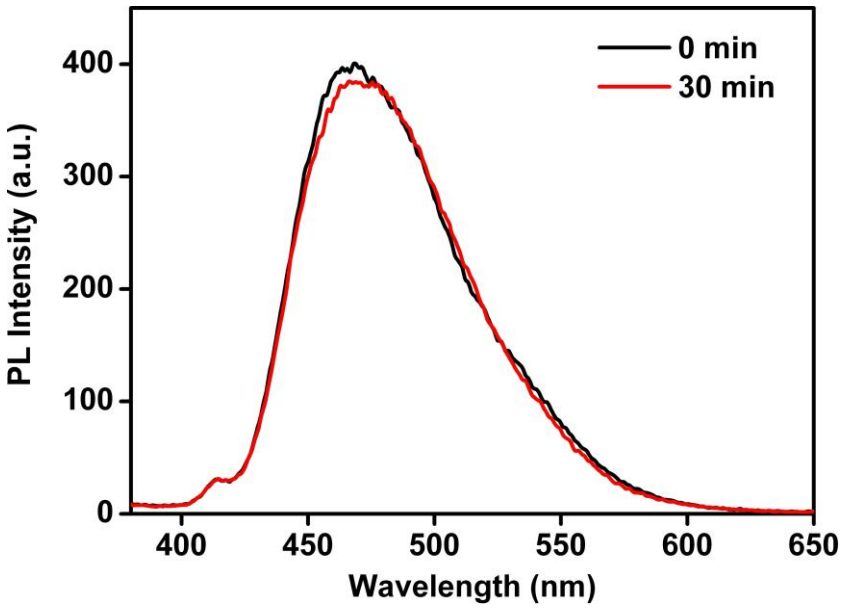


Fig. S1 PL spectra of P5-chitosan FONs before and after they were irradiated UV lamp at 365 nm for 30 min.

Table S1 element contents (%) of **P5-chitosan** FONs based on XPS analysis

	C	O	N
P5	92.66	7.34	0
Chitosan	60.04	35.28	4.68
P5-chitosan	67.59	29.01	3.4