Supporting information

Glycosylated Aggregation Induced Emission Dye Based Fluorescent Organic Nanoparticles: Preparation and Bioimaging Applications

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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. 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 of **$PhNH_2$-OA-Glu** FONs in water and phosphate buffer solution (PBS) were determined using a zeta Plus apparatus (ZetaPlus, Brookhaven Instruments, Holtsville, NY).

1.2 Preparation of $PhNH_2$-OA-Glu 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. Glu (5 mg, 0.002 mmol) was dispersed in 5 mL of DMF and stirred at 60 °C for 12 h. Then the Glu solution (in DMF) was added to the above mixture and stirred for 30 min. Then the reaction 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-Glu FONs

Cell morphology was used to examine the effects of **$PhNH_2$-OA-Glu** 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 **$PhNH_2$-OA-Glu** 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-Glu** FONs on A549 cells was evaluated by cell counting kit-8 (CCK-8) assay based on our previous reports.[1] 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-Glu** 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 Dulbecco's modified eagle medium (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-Glu** 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-Glu FONs

A549 cells were cultured in 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-Glu** 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-Glu** 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 Optical images of **PhNH$_2$-OA-Glu** FONs water water for more than one week. Insets are optical images of **PhNH$_2$-OA-Glu** FONs in water under UV lamp (λ = 365 nm).

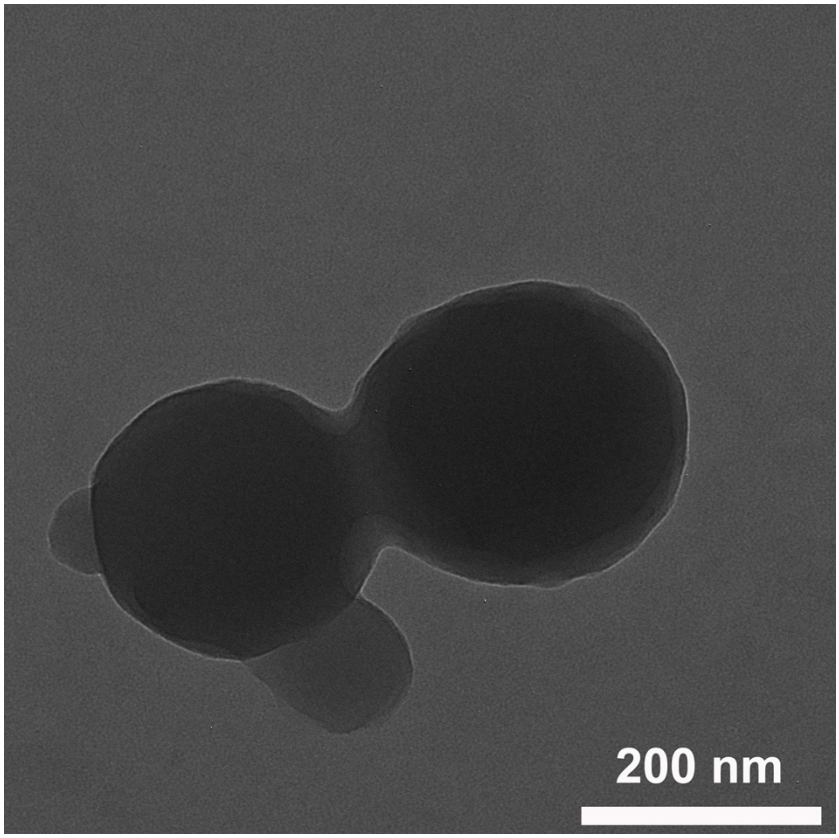

Fig. S2 Representative TEM image of **PhNH$_2$-OA-Glu** FONs.

References

1. X. Zhang, H. Qi, S. Wang, L. Feng, Y. Ji, L. Tao, S. Li and Y. Wei, *Toxicol. Res.*, 2012, **1**, 201-205.