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Supporting information

Luminescence Tunable Fluorescent Organic Nanoparticles from Polyethyleneimine and Maltose: Facile Preparation and Bioimaging Applications

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2. Experimental

2.1 Materials and measurements

Polyethyleneimine (PEI, Mn = 600, alading reagent Inc.), maltose (alading reagent Inc.) was used as received. All other solvents and chemicals were purchased from commercial sources and used directly without further purification.

UV-Visible absorption spectra were recorded on UV/Vis/NIR Perkin-Elmer lambda750 spectrometer (Waltham, MA, USA) using quartz curettes 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 **PEI-Mal** FONs in water was determined using a zeta Plus particle size analyzer (ZetaPlus, Brookhaven Instruments, Holtsville, NY).

Quantum yield was measured according to established procedure in our previous reports. The optical densities were measured on UV-Vis spectra were obtained on a UV/Vis/NIR Perkin-Elmer lambda750 Spectrophotometer. Quinine sulfate in 0.1 M H_2SO_4 (literature quantum yield 0.54 at 360 nm) was chose as a standard. Absolute values are calculated using the standard reference sample that has a fixed and known fluorescence quantum yield value, according to the following equation:

$$\varphi_\chi = \varphi_{std} \frac{I_x}{A_x} \frac{A_{std}}{I_{std}} \frac{\eta_x^2}{\eta_{std}^2}$$

Where φ is the quantum yield, I is the measured integrated emission intensity, and A is the optical density, and η is the refractive index. The subscript "std" refers to the reference fluorophore of known quantum yield. In order to minimize re-absorption effects absorbencies in the 10 mm fluorescence cuvette were kept under 0.1 at the excitation wavelength (360 nm).

The fluorescent stability of **PEI-Mal** FONs was recorded on n a PE LS-55 spectrometer

using time drive model. The excitation wavelength was set at 350 nm with a 15-nm slit width; the emission wavelength was set at 450 nm with a 10-nm slit width.

2.2 Preparation of PEI-Mal FONs

PEI (100 mg) and maltose (200 mg) was dispersed in 20 mL deionized water and heated to 100 °C for 2 h. Then the mixture was purified by dialysis through porous cellulose bag (molecular weight cut off 3500 Da) using ethanol. Finally the product inside the dialysis bag was collected and dried by vacuum oven at 40 °C.

2.3 Cytotoxicity of PEI-Mal FONs

Cell morphology was used to examine the effects of **PEI-Mal** 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 **PEI-Mal** 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 **PEI-Mal** 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} **PEI-Mal** 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 PEI-MAL 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).

2.4 Confocal microscopic imaging of cells using PEI-Mal 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 **PEI-Mal** 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 **PEI-Mal** 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 and 458 nm.

Results

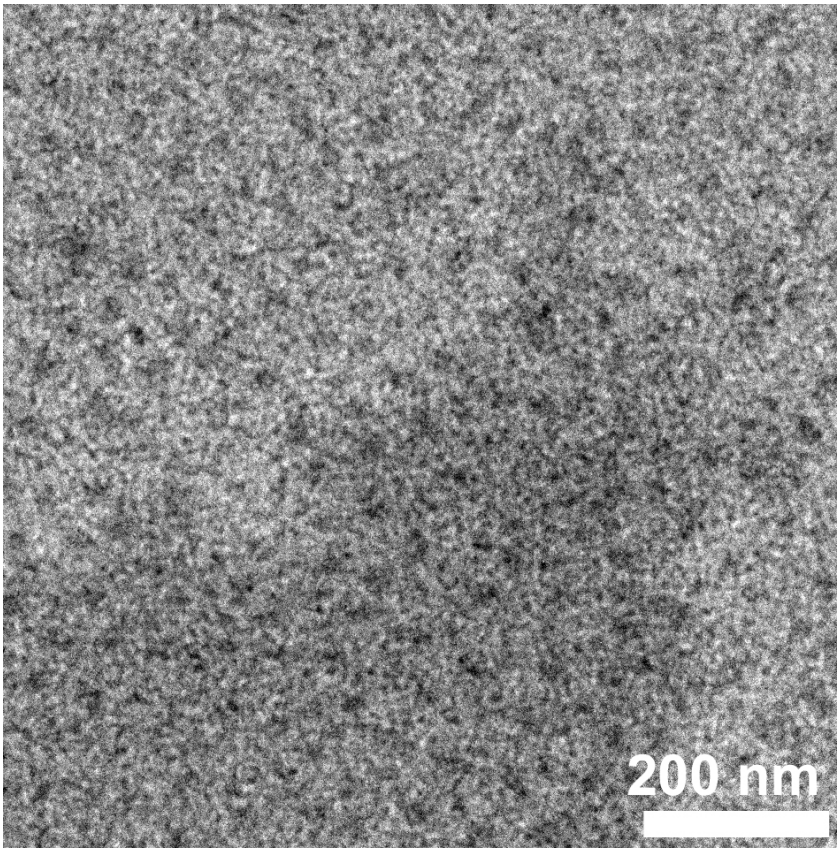

Fig. S1 TEM images of PEI-Mal FONs, scale bar = 200 nm.

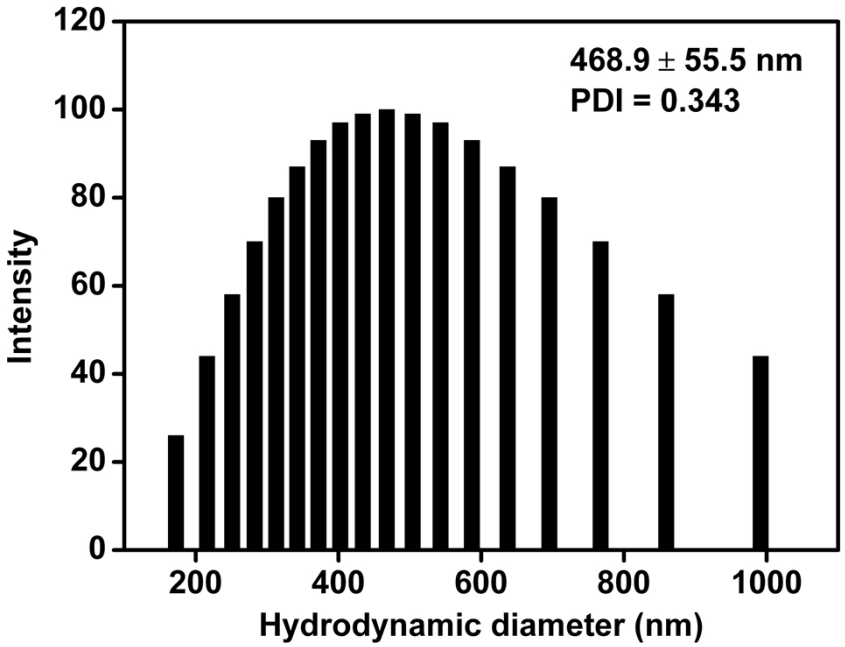

Fig. S2 TEM images of PEI-Mal FONs, scale bar = 200 nm.

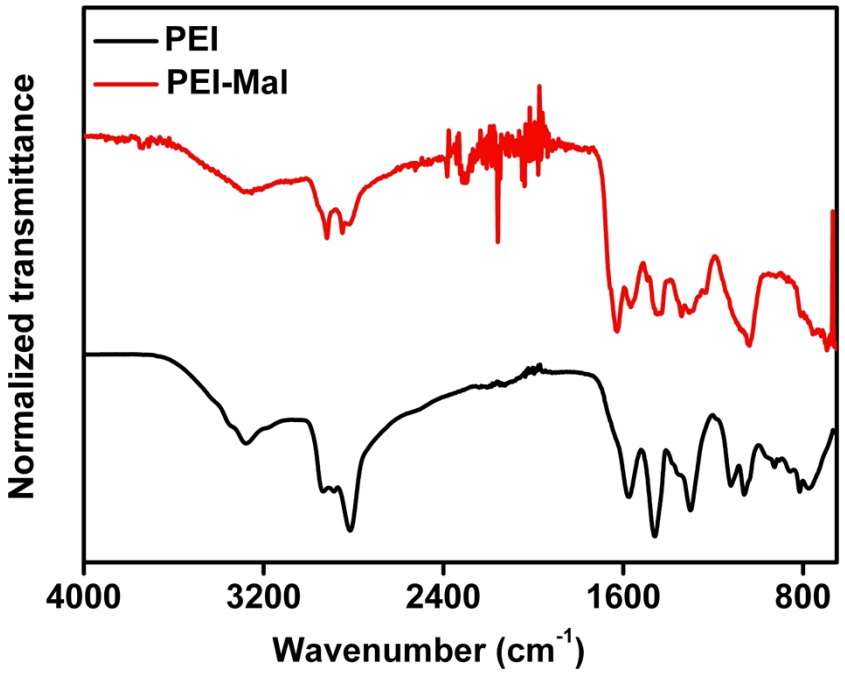

Fig. S3 FT-IR spectra of PEI and **PEI-Mal** FONs.

Fig. S4 Optical images of **PEI-Mal** FONs water dispersion for more than one week (left bottle). The right bottle is the optical images of **PEI-Mal** FONs in water under UV lamp (λ = 365 nm).

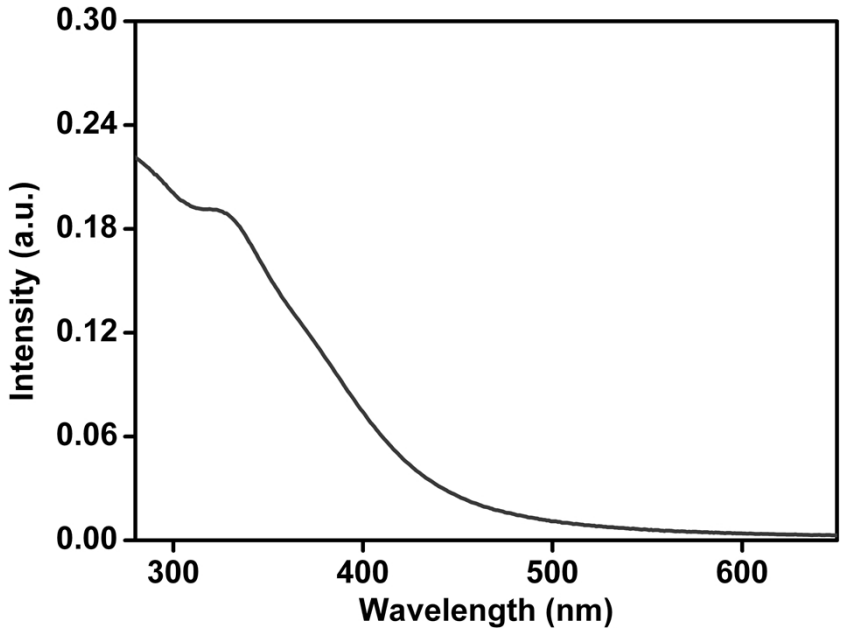

Fig. S5 UV-vis spectrum of **PEI-Mal** FONs.

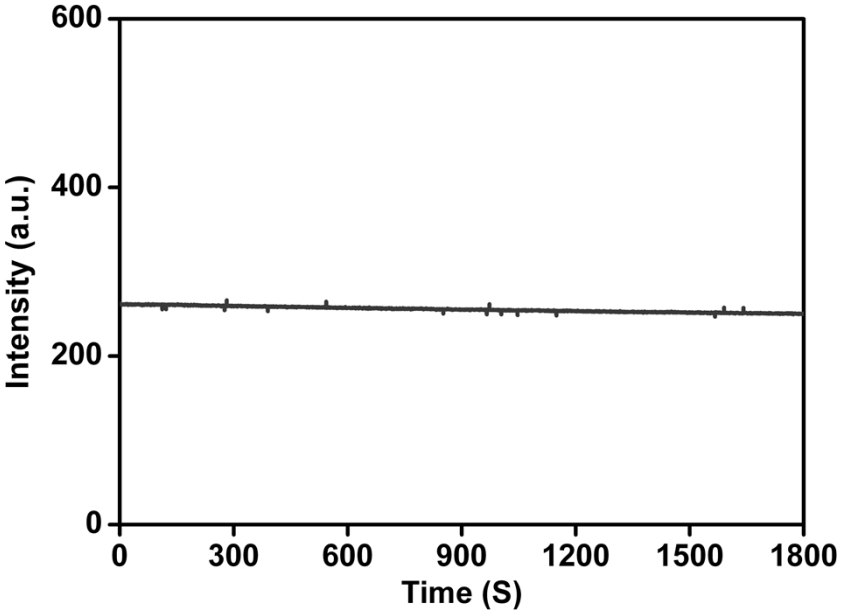

Fig. S6 Photostability of **PEI-Mal** FONs measured by time drived model using FL spectroscopy.

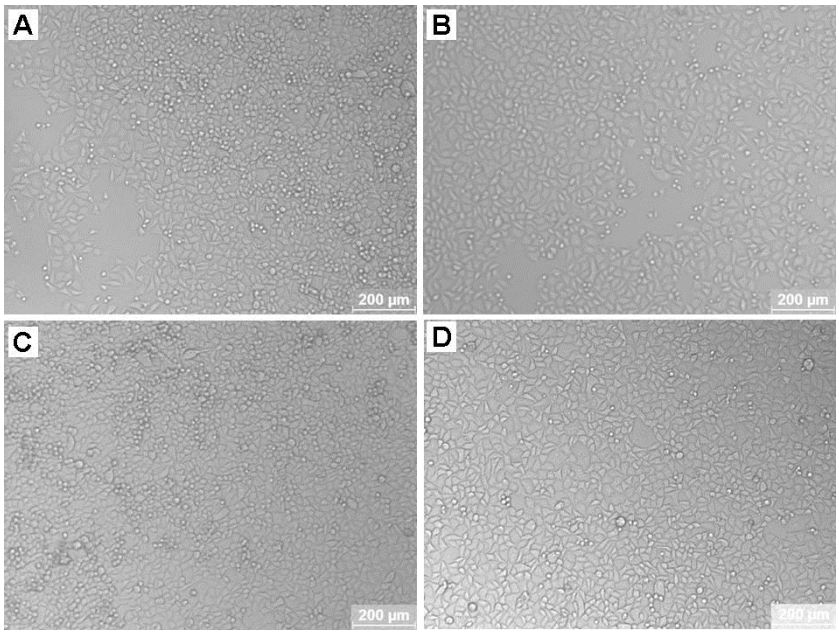

Fig. S7 Optical microscopy images of A549 cells incubated with different concentrations of **PEI-Mal** FONs for 24 h, (A) control cells, (B) 40 μg mL^{-1}, (C) 80 μg mL^{-1}, (D) 120 μg mL^{-1}.

Reference

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