**Supporting information**

# Polymerizable Aggregation Induced Emission Dye Based fluorescent Nanoparticles For Cell Imaging Applications

Xiaoyong Zhang[a, b,#,*], Xiqi Zhang[b,#], Bin Yang[b], Meiying Liu[c], Wanyun liu[a], Yiwang Chen[a], Yen Wei[b, *]

[a] Department of Chemistry/Institute of Polymers, Nanchang University, 999 Xuefu Avenue, Nanchang 330031, China. [b] Department of Chemistry and Key Laboratory of Bioorganic Phosphorus Chemistry & Chemical Biology (Ministry of Education), Tsinghua University, Beijing, 100084, P. R. China. [c] Beijing National Laboratory for Molecular Sciences (BNLMS), Key Laboratory of Organic Solids, Laboratory of New Materials, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, China.

xiaoyongzhang1980@gmail.com; weiyen@tsinghua.edu.cn

## 1. Experimental

### 1.1 Materials and measurements

All the 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 size distribution of **PhE-PEG** FONs in water and phosphate buffer solution (PBS) were determined using a zeta Plus particle size analyzer (ZetaPlus, Brookhaven Instruments, Holtsville, NY). $^1$H NMR spectra were measured on a JEOL 400 MHzspectrometer [$d_6$-DMSO as solvent andtetramethylsilane (TMS) as the internal standard]. Gel permeation chromatography (GPC) analyses of polymers were performed using DMF as the eluent. The GPC system was a Shimadzu LC-20AD pump system comprising of an auto injector, a MZ-Gel SDplus 10.0 mm guard column (50×8.0 mm, 102 Å) followed by a MZ-Gel SDplus 5.0 μm bead-size columns (50-106 Å, linear) and a Shimadzu RID-10A refractive index detector. The system was calibrated with narrow molecular weight distribution polystyrene standards ranging from 200 to $10^6$ g $mol^{-1}$.

### 1.2 Preparation of PhE-PEG

The AIE dye (**PhE**) wans synthesized according to previous report.[1] **PhE** was incorporated into polymer nanoparticles via reversible addition fragmentation chain transfer polymerization with a water soluble monomer poly(ethlene glycol) monomethyl ether methacylate (PEGMA, Mn = 950). The preparation of **PhE-PEG** nanoparticles was schematically displayed in Scheme 2. In a typical synthesis of **PhE-PEG-20**, PEGMA (0.63 g, 0.67 mmol), **PhE** (0.050 g, 0.074 mmol), CTA (9.7 mg, 0.037 mmol), AIBN (3.0 mg) and dioxane (3 mL) were introduced in schlenk tube and purged by nitrogen flow for 30 min. The above mixture was put into an oil bath maintained at 70 °C for 12 h. Then stop the reaction of polymerization, the purified polymer was obtained via precipitation from dioxane to petroleum ether for three times, and then dried under vacuum for further

characterization. The synthetic procedure of **PhE-PEG-40** was similar to that of **PhE-PEG-20**, whereas the amount of CTA was changed to 4.9 mg.

**1.3 Cytotoxicity of PhE-PEG FONs**

Cell morphology was observed to examine the effects of **PhE-PEG** FONs to A549 cells. Briefly, cells were seeded in 6-well microplates at a density of $1\times10^5$ 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 **PhE-PEG** 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 **PhE-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 $5\times10^4$ cells $mL^{-1}$ in 160 μL of respective media containing 10% FBS. After 24 h of cell attachment, the cells were incubated with 5, 10, 20, 40, 80 μg $mL^{-1}$ **PhE-PEG** 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 **PhE-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).

**1.4 Confocal microscopic imaging of cells using PHE-PEG FONs**

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% $CO_2$ 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 $1\times10^5$ cells per dish. On the day of treatment, the cells were incubated with

**PhE-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 **PhE-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 wavelength of 488 nm.

**Results**

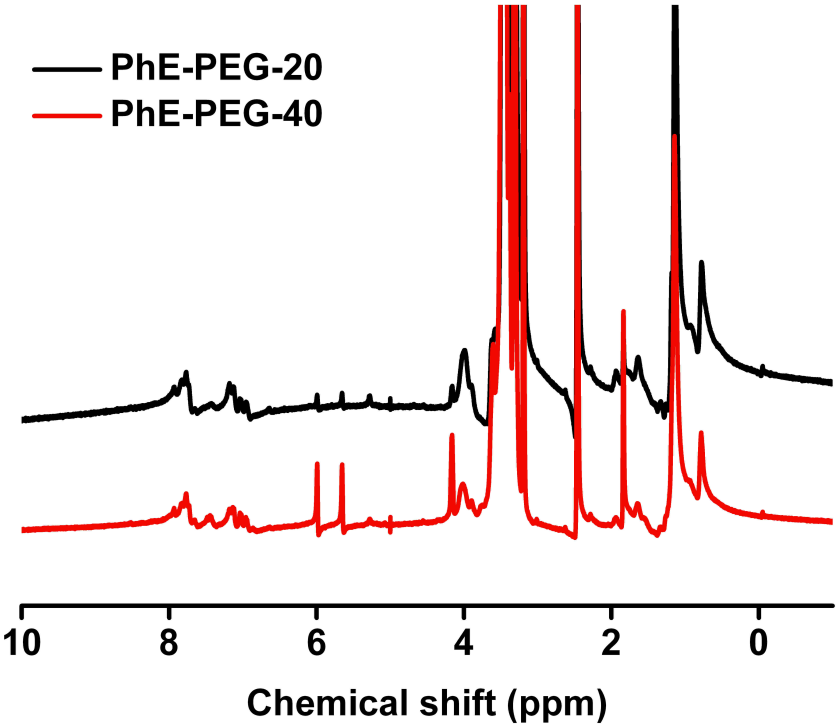


**Fig. S1** $^{1}$HNMR spectra of **PhE-PEG-20** and **PhE-PEG-40 in DMSO**.

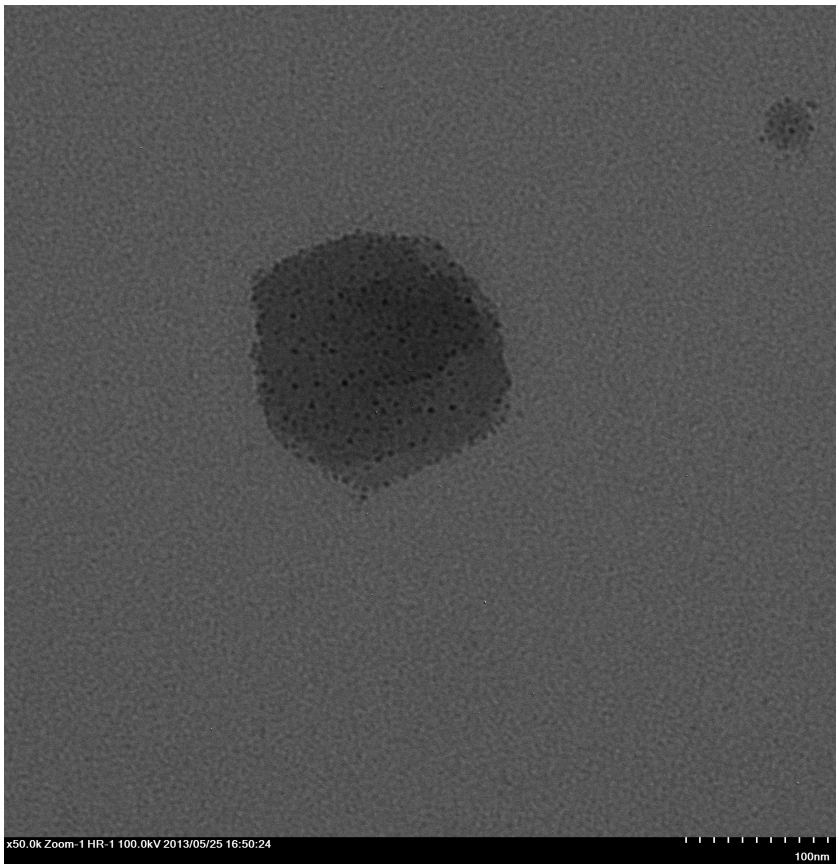


**Fig. S2** Representative TEM image of **PhE-PEG-20**, scale bar = 100 nm.


**Fig. S3** Photographs of **PhE-PEG-20** (left bottle) and **PhE-PEG-40** (right bottle) water dispersion.

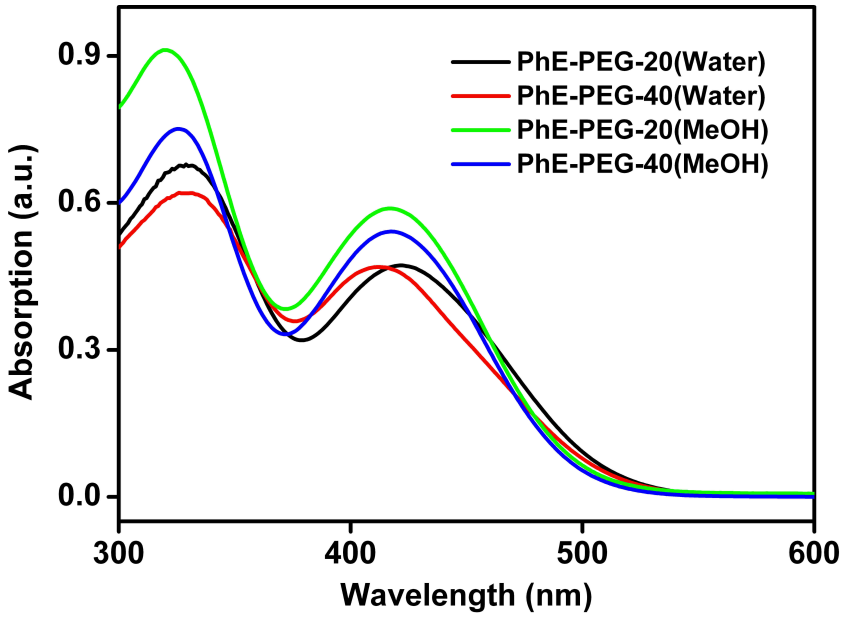


**Fig. S4** UV-Vis spectra of **PhE-PEG-20** and **PhE-PEG-40**.

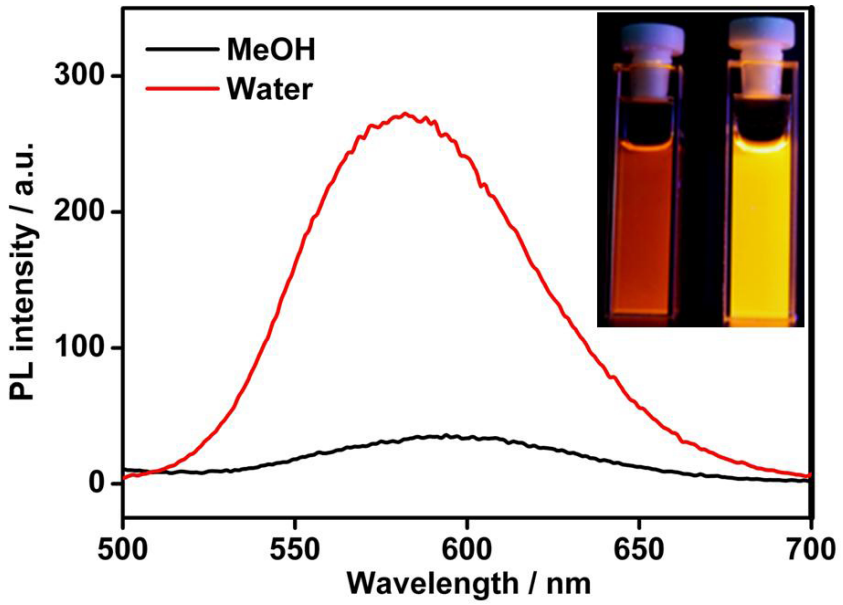


**Fig. S5** PL spectra of **PhE-PEG-40** (in MeOH and water), insets are their photographs of **PhE-PEG-40** in water (right bottle) and in MeOH (left bottle) under UV lamp (λ = 365 nm)

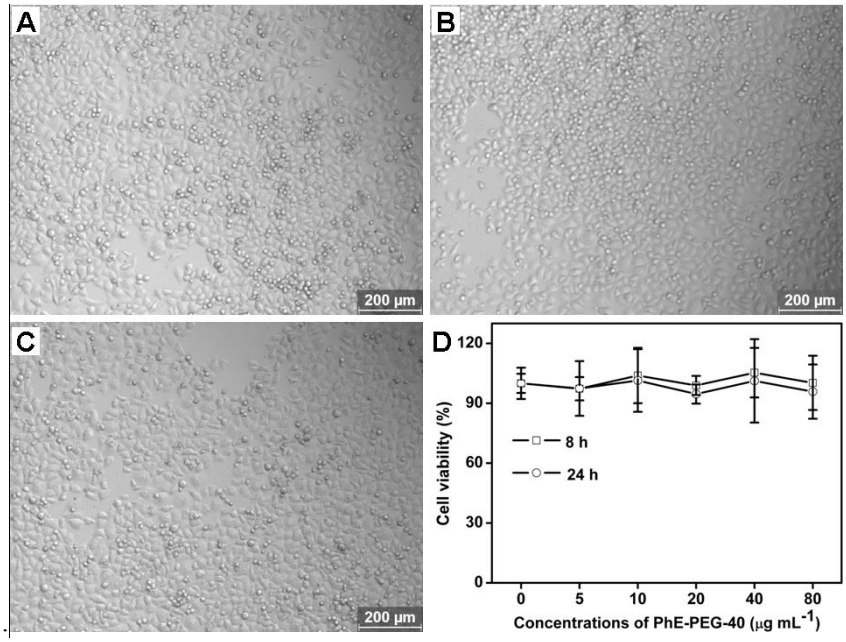


**Fig. S6** Biocompatibility evaluations of **PhE-PEG-40**. (A-C) optical microscopy images of A549 cells incubated with different concentrations of **PhE-PEG-40** for 24 h, (A) control cells, (B) 20 μg $mL^{-1}$, (C) 80 μg $mL^{-1}$, (d) cell viability of **PhE-PEG-40** with A549 cells.

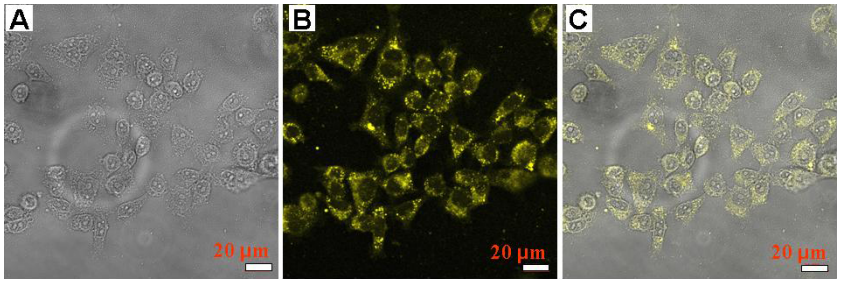


**Fig. S7** CLSM images of A549 cells incubated with 40 μg $mL^{-1}$ of **PhE-PEG-40** for 3 h. (A) bright field, (B) excited with 488 nm laser, (C) merge image of A and B. Scale bar = 20 μm.

## Reference

1. X. Zhang, X. Zhang, B. Yang, M. Liu, W. Liu, Y. Chen and Y. Wei, *Polym. Chem.*, 2013, 10.1039/C1033PY00984J.
2. X. Zhang, H. Qi, S. Wang, L. Feng, Y. Ji, L. Tao, S. Li and Y. Wei, *Toxicol. Res.*, 2012, **1**, 201-205.