Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting information

Ultra-stable biocompatible cross-linked fluorescent polymeric nanoparticles using AIE chain transfer agent

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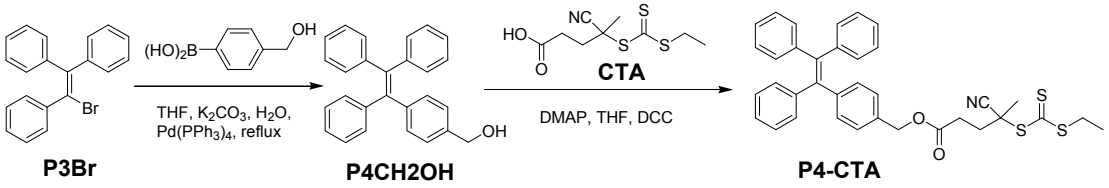
1. Experimental

1.1 Materials and measurements

1-bromo-1,2,2-triphenylethene, 4-(hydroxymethyl)phenylboronic acid, 4-dimethylamino-pyridine (DMAP), tetrakis(triphenylphosphine) palladium(0), N,N'-dicyclohexylcarbodiimide (DCC), azobisisobutyronitrile, poly(ethylene glycol) monomethyl ether methacylate (PEGMA, M_n = 950), and diethylene glycol dimethacrylate (DEGDM) purchased from Alfa Aesar were used as received. All other agents and solvents were purchased from commercial sources and used directly without further purification. Ultra-pure water was used in the experiments.

^{1}H NMR spectra were measured on a Mercury-Plus 300 MHz spectrometer [$CDCl_3$ or d_6-DMSO as solvent and tetramethylsilane (TMS) as the internal standard]. UV-Visible absorption spectra were recorded on UV/Vis/NIR 2600 spectrometer (Shimadzu, Japan) using quartz cuvettes of 1 cm path length. Fluorescence spectra were measured on an F-4600 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 Shimadzu Spectrum 8400 spectrometer (Japan). 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 HT7700 microscope (Hitachi, Japan) 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 and zeta potential measurement of **P4-PEG** FPNs in phosphate buffer solution (PBS) were determined using a zeta Plus apparatus (ZetaPlus, Brookhaven Instruments, Holtsville, NY). 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, 10^2 Å) followed by a MZ-Gel SDplus 5.0 μm bead-size columns (50-10^6 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 Synthesis of P4-CTA



Scheme S1 Synthetic route of **P4-CTA**.

The synthetic route of **P4-CTA** was shown in Scheme S1. 1-bromo-1,2,2-triphenylethene (**P3Br**, 1.0 g, 3.0 mmol) and 4-(hydroxymethyl)phenylboronic acid (0.55 g, 3.6 mmol) were dissolved in a mixture of tetrahydrofuran (40 mL) and 2 M potassium carbonate aqueous solution (20 mL). The mixture was stirred at room temperature for 0.5 h under Ar gas followed by adding tetrakis(triphenylphosphine) palladium(0) (0.010 g) and then heating to the reflux temperature overnight. After removing the solvent under reduced pressure, the residue was chromatographed on a silica gel column with pure dichloromethane as eluent to give **P4CH2OH** (0.90 g, 83% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.56 (s, 1H), 4.54 (s, 2H), 6.91-7.11 (m, 19H).

P4CH2OH (0.36 g, 1.0 mmol), DMAP (0.050 g, 0.45 mmol), CTA (0.32 g, 1.2 mmol) and DCC (0.27 g, 1.3 mmol) were dissolved in tetrahydrofuran (20 mL). The mixture was stirred at room temperature for 6 h under Ar gas, followed by filtrating the resulting precipitate and then removing the solvent under reduced pressure, the residue was chromatographed on a silica gel column with dichloromethane/petroleum ether (1:3) as eluent to afford **P4-CTA** (0.50 g, 82% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.36 (t, 3H), 1.86 (s, 3H), 2.37-2.57 (m, 2H), 2.63-2.70 (m, 2H), 3.34 (q, 2H), 5.05 (s, 2H), 7.00-7.14 (m, 19 H).

1.3 Preparation of P4-PEG FPNs

P4-CTA was incorporated into crosslinked polymer nanoparticles as a chain transfer agent *via* reversible addition fragmentation chain transfer polymerization with a water soluble monomer poly(ethlene glycol) monomethyl ether methacylate (PEGMA, M_n = 950) and diethylene glycol dimethacrylate (DEGDM) as a crosslinker. The preparation of **P4-PEG** FPNs was schematically displayed in scheme 2. In a typical synthesis of **P4-PEG-1**, PEGMA (950 mg, 1.0 mmol), DEGDM (73 mg, 0.30 mmol), **P4-CTA** (61 mg, 0.10 mmol), AIBN (3.3 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 **P4-PEG-2** was similar to that of **P4-PEG-1**, whereas the amount of PEGMA was adjusted to 1900 mg.

1.4 Cytotoxicity of P4-PEG FPNs

Cell morphology was observed to examine the effects of **P4-PEG** FPNs 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 **P4-PEG** FPNs 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 **P4-PEG** FPNs on A549 cells was evaluated by cell counting kit-8 (CCK-8) assay. 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} **P4-PEG** FPNs 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 **P4-PEG** FPNs), 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).

1.5 Confocal microscopic imaging of cells using P4-PEG FPNs

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 1×10^5 cells per dish. On the day of treatment, the cells were incubated with

P4-PEG FPNs 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 **P4-PEG** FPNs 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.

2. Results

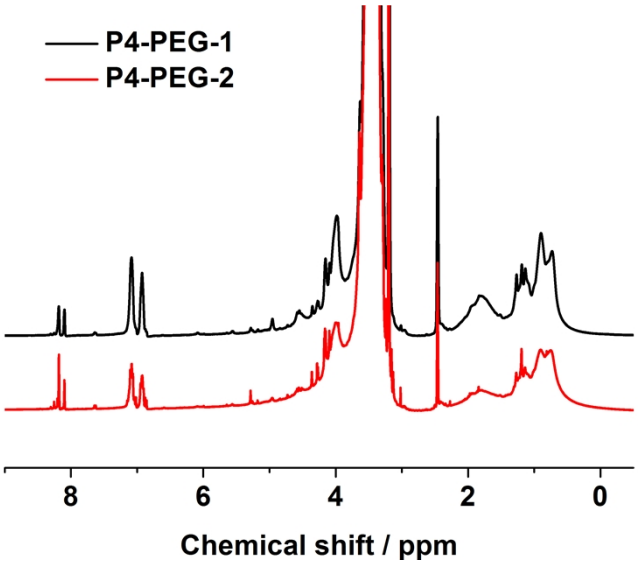


Fig. S1 ^1H NMR spectra of **P4-PEG** dissolved in d_6-DMSO.

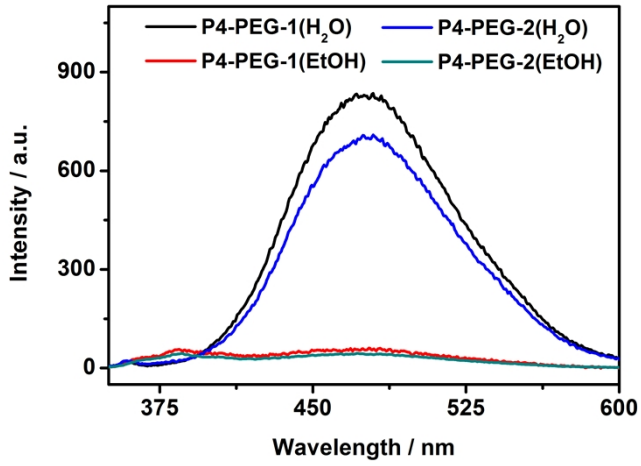


Fig. S2 Fluorescence emission spectra of **P4-PEG** FPNs in H_2O and EtOH, the concentrations of **P4-PEG** FPNs were 0.1 mg mL^{-1}.

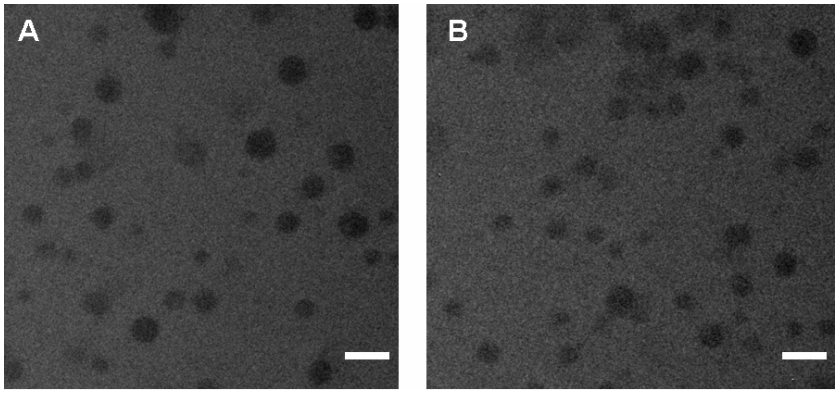


Fig. S3 TEM image of **P4-PEG-1** (A) and **P4-PEG-2** (B) FPNs dispersed in water, scale bar = 100 nm.

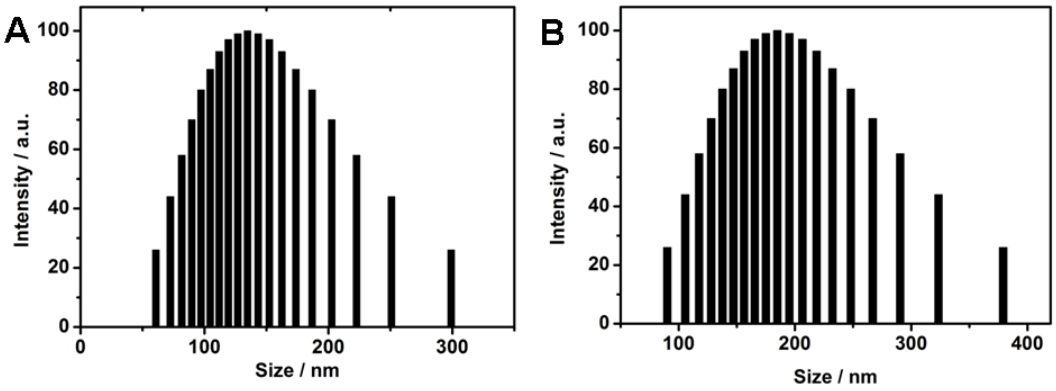


Fig. S4 Dynamic light scattering results for (A) **P4-PEG-1** and (B) **P4-PEG-2** FPNs in PBS, the concentrations of **P4-PEG** FPNs were 0.1 μg mL^{-1}.

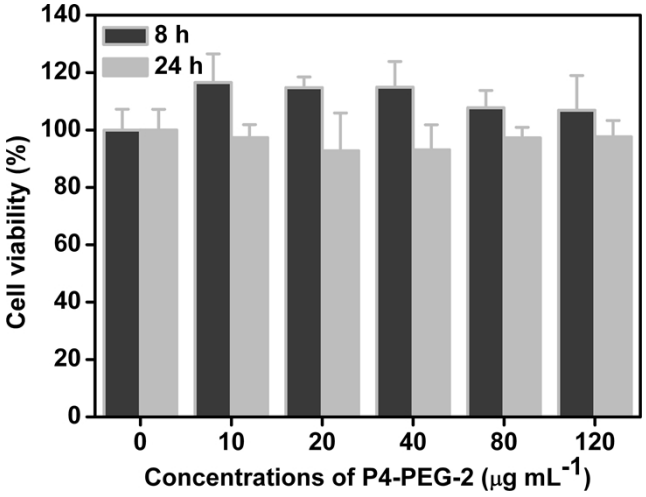


Fig. S5 Cell viability of **P4-PEG-2** FPNs for 8 h and 24 h.

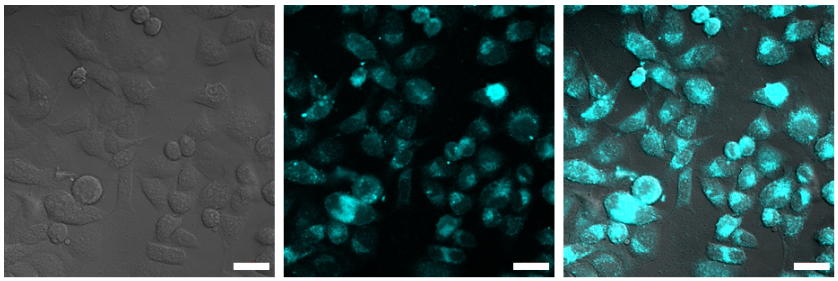


Fig. S6 CLSM images of A549 cells incubated with 10 μg mL^{-1} of **P4-PEG-2** FPNs for 3 h. (A) bright field, (B) excited with 405 nm laser, (C) merged image of A and B. Scale bar = 20 μm.