Supporting Information

Enhanced Luminescence and Stability of TFMDSA Nanocrystals via Polymer-Induced crystallization for Bioimaging

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Experimental

Materials

AIE luminogens used in this article were synthesized in our group. MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide) were purchased from Shanghai Beyotime Biotechnology Co., Ltd.. Cell viability (live dead cell staining) assay kit was purchased from Jiangsu KeyGEN Biotechnology Co., Ltd.. All of the other Chemicals and reagents were acquired from commercial sources without further purification, unless otherwise noted. All the solvents were purified according to the standard methods whenever needed. Milli-Q water was collected from a Milli-Q system (Millipore, USA).

Preparation of TFMDSA

A 500 mL two-necked flask was oven-dried. Anthracene (18 g, 0.1 mol) was dissolved in 1,4-dioxane (144 mL) and HCl (24 mL). Paraformaldehyde (15.2 g) was added to this solution, and the mixture was stirred at 110 °C for 2 hours under an HCl atmosphere. After the HCl atmosphere was removed, the reaction mixture was stirred for an additional 4 hours at the same temperature. The mixture was then cooled to room temperature, washed with 1,4-dioxane, and neutralized with water, yielding a yellow powder (compound 1, 12.3 g, 40% yield). Compound 1 (17 g, 50 mmol) was dissolved in P(OC2H5)3 (86.5 mL, 0.5 mol). The reaction mixture was heated to 150 °C in an oil bath and stirred for 18 hours under a nitrogen atmosphere. After cooling to room temperature, the mixture was poured into petroleum ether and filtered, yielding 18 g of a yellow solid (compound 2, 75% yield). Tetraethylanthracene-9,10-diylbis(methylene)diphosphonate (0.600 g, 1.25 mmol) was stirred with a second reagent (0.421 g, 3.76 mmol) in THF (200 mL) under nitrogen. 3,5-Bis(trifluoromethyl)benzaldehyde (2.84 mmol) in THF (50 mL) was added to this solution, which was kept in an ice bath, and the mixture was stirred for 12 hours at room temperature. The resultant precipitate was washed with methanol and filtered, yielding a yellow powder (55% yield).

Preparation of TFMDSA nanoparticles and nanocrystals

TFMDSA nanoparticles (TFMDSA NPs) were prepared using the nanoprecipitation method. First, 1 mg of TFMDSA was mixed with tetrahydrofuran (1 mL) in a 2 mL disposable centrifuge tube and sonicated to achieve a homogenous mixture. Then, 50 μL of the TFMDSA-tetrahydrofuran solution was added to the tetrahydrofuran solution (450 μL), and the mixture was well mixed. Next, a vial containing 5 ml of water was heated to 50°C and placed in the sonication machine. 500 μL of the TFMDSA-tetrahydrofuran solution was rapidly added to the 5 mL of deionized water using a pipette while being sonicated. The final concentration of TFMDSA in the solution is 10 μg/mL. This resulted in the formation of TFMDSA NPs with high efficiency and a narrow size distribution.

Then, we describe the preparation of highly efficient organic fluorescent nanocrystals ($mPEG_{5K}$-PCL_{10K}@TFMDSA NCs) via a simple and scalable "one-pot" ultrasonic emulsification process. 1mg of TFMDSA was weighed using a balance in a 2 ml disposable centrifuge tube, dissolved in 1 ml of tetrahydrofuran, and sonicated until the solution was thoroughly mixed and the concentration was 1 mg/ml. 5 mg of $mPEG_{5K}$-PCL_{10K} was weighed using a balance into a 2 ml disposable centrifuge tube, dissolved in 1 ml of tetrahydrofuran, and sonicated until the solution was thoroughly mixed and the concentration was 5 mg/ml. Prepare another 2 ml centrifuge tube, ladle 750 μL of tetrahydrofuran into the 2 ml tube, then pipette 50 μL of TFMDSA and 200 μL of $mPEG_{5K}$-PCL_{10K} into them, accordingly, and shake slightly until the solutions are thoroughly mixed. Prepare a 10 ml bottle and filling it with 5 ml of ionized water with a pipette. Heat the water temperature of the ultrasonic machine to 50°C, insert the vial with 5 ml of water, and preheat the water temperature in the vial via ultrasonic in advance to 50°C. Use a pipette to swiftly transfer 1 ml of the TFMDSA and $mPEG_{5K}$-PCL_{10K} tetrahydrofuran mixture from the centrifuge tube into 5 ml of the vial under ultrasonic settings. To get $mPEG_{5K}$-PCL_{10K}@TFMDSA, the vial was then put in a fume hood and the surplus tetrahydrofuran solvent slowly evaporated until it was entirely dried out. The prepared $mPEG_{5K}$-PCL_{10K}@TFMDSA aqueous solution was filtered through a 0.22 micron aqueous filter head to obtain a uniform particle size of $mPEG_{5K}$-

PCL_{10K}@TFMDSA NCs. And the concentration of TFMDSA in $mPEG_{5K}$ -$PLLA_{10K}$@TFMDSA NCs was also calibrated 8.2 μg/mL. The mass fraction of 7.58% can be obtained by calculation.

Preparation of $mPEG_{5K}$ -$PLLA_{10K}$@TFMDSA nanocrystals $mPEG_{5K}$ -$PLLA_{10K}$@TFMDSA): Similar conditions were used for the fabrication of $mPEG_{5K}$-PCL_{10K}@TFMDSA nanocrystals. The TFMDSA is dissolved in 1 ml of the ketohydrate mold after being added to 2 ml of a single centrifugal tube. The solution is then homogeneously mixed at a concentration of 1 mg/ml using ultrasonic to dissolve the tuberculosis. After dissolving 5 mg of $mPEG_{5K}$-$PLLA_{10K}$ in 1 ml of quadri-hydrogen mucus, the mixture was homogeneously stirred using ultrasonic until the solution had a concentration of 5 mg/ml. A transfer rifle is used to add 50 microliters of TFMDSA and 200 microliters of $mPEG_{5K}$-$PLLA1_{0K}$ of the four hydrogen muffin solution separately to a prepared 2 ml centrifugal tube, which is then added to the 2 ml decentralization tube. Use a transfusion gun to prepare a 10 ml bottle and absorb 5 ml of dision water to heat the water in the ultrasound machine to 50 °C. The 5 ml water bottle will be put inside after the water in the flask has been pre-ultrasonically heated to 50 °C. Under ultrasonic circumstances, the dision cannon will swiftly add 5 ml of deionized water to 1 ml of the TFMDSA and $mPEG_{5K}$-$PLLA_{10K}$ of 4 hydrogen mulling mixed solution and mix the solution for 10 minutes. In order to make the surplus four-hydrogen moisturizer totally volatile, the container is then left in the ventilation cabinet in a static position for approximately 24 hours. At that point, it will be $mPEG_{5K}$-$PLLA_{10K}$@TFMDSA. To create a water solution with a consistent particle diameter of $mPEG_{5K}$-$PLLA_{10K}$@TFMDSA, the prepared water solution of $mPEG_{5K}$-$PLLA_{10K}$@TFMDSA is filtered via a 0.22 micrometer water phase filter head. The concentration of TFMDSA in $mPEG_{5K}$ -PCL_{10K}@TFMDSA NCs was also calibrated 7.4 μg/mL. The mass fraction of 6.89% can be obtained by calculation.

Infrared Spectrum

After determining the infrared spectrum of the solid powder using the potassium bromide press method, a small quantity of polymer powder was added, crushed, and

thoroughly mixed. The resulting mixture was then analyzed on a pressurized 80V FTIR spectrometer. To obtain the infrared spectra, pure potassium bromide was dried for two minutes and then examined on a Bruker Vertex 80V FTIR spectrometer.

Material morphology and particle size characterization

Transmission electron microscopy (TEM) was employed to capture the sample images using a JEOL JEM-2100E instrument. The samples were prepared by depositing the solution onto a carbon-coated 300-mesh copper grid and leaving it to dry at room temperature. Scanning electron microscopy (SEM) images were obtained using a JEOL JSM-6700E instrument. The samples were prepared by depositing the polymer solution onto a silicon wafer and allowing it to dry at room temperature. The dynamic particle size of the nanoparticles was quantified using a Malvern Zetasizer Nano ZS particle size analyzer. The solutions were loaded into a quartz cell for analysis.

Optical property experiments of TFMDSA nanoparticles and nanocrystals

The UV-Vis absorption spectra of solution samples were obtained using an Analytik Jena Specord 210 UV-Vis spectrophotometer. To prepare the solution samples, deionized water and tetrahydrofuran were mixed and then analyzed in a quartz cell. For solid samples, a quartz sheet coated with vacuum grease was employed. The solid sample was pressed onto the sheet with barium sulfate and subjected to diffuse reflection measurement using an integrating sphere attachment.

Fluorescence emission spectra of solution samples were acquired utilizing a Shimadzu RF-5301 PC spectrometer. Solution samples were prepared by using tetrahydrofuran (THF) and deionized water and examined in a quartz cell. For solid sample evaluation, a vacuum grease-coated quartz sheet with a solid attachment was employed.

The fluorescence lifetime of solution samples was measured using the Edinburgh FLS980 steady-state/transient fluorescence spectrometer. Solution samples were prepared by dissolving the samples in tetrahydrofuran and deionized water, and then

they were analyzed in a quartz cell. To analyze solid samples, a quartz sheet coated with vacuum grease was used.

The quantum efficiencies for both solution and solid states were determined using an Edinburgh FLS980 steady-state/transient fluorescence spectrometer equipped with an integrating sphere attachment.

Stability experiments of TFMDSA nanoparticles and nanocrystals

The photostability of TFMDSA nanocrystals and nanoparticles was assessed by monitoring their UV absorption spectra and fluorescence emission spectra over 7 days at room temperature, in a closed environment. A UV spectrophotometer and a fluorescence spectrometer were used for this purpose.

Cell culture

HeLa cells were purchased from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, Shanghai, China. The cells were propagated to confluence in Dulbecco's modified Eagle's medium (DMEM, GIBCO) supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin (Sigma) and heat-inactivated fetal bovine serum (FBS, GIBCO), and maintained at 37oC in a humidified atmosphere of 5% CO2 for further cell experiments.

Biocompatibility by MTT Assay

Cells harvested in a logarithmic growth phase were seeded in 96-well plates at a density of 8×103 cells per well and incubated in DMEM for 24 h. The medium was then replaced by 200 μL of DMEM containing predetermined concentrations of TFMDSA NPs, and then incubated for 24 h, followed by MTT assays to measure the live cells. Cell viabilities were determined by reading the absorbance of the plates at 490 nm with a microplate reader. The cells incubated with DMEM were used as the control. The cell viability (%) =A sample /A control ×100%. The procedures were the same for that of $mPEG_{5K}$-PCL_{10K}@TFMDSA NCs and $mPEG_{5K}$-$PLLA_{10K}$@TFMDSA NCs.

Cell imaging experiments

The Hela cell line was selected for our cell imaging experiments. The cells were cultured in DMEM medium supplemented with 10% fetal bovine serum for 24 hours at 37°C with 5% CO2 in a cell incubator until they reached 90% confluency. The cells were then passaged and seeded in 6-well plates. After 24 hours of culture in a cell incubator, the cells were incubated with three dyes ($mPEG_{5K}$-PCL_{10K}@TFMDSA NCs, $mPEG_{5K}$ -$PLLA_{10K}$@TFMDSA, and TFMDSA NPs) for 1 hour, 3 hours and 6 hours at 37°C with 5% CO2. The dyes were diluted in PBS buffer solution, and each dye was added in 200 μL at a concentration of 5 μg/mL. Following the incubation, the cells were washed three times with PBS and fixed in 4% paraformaldehyde for 15 minutes, followed by another three PBS washes. Fluorescent confocal images of cells were obtained using Olympus FV1000 laser scanning confocal microscope (CLSM) test. Confocal fluorescence microscopy was performed using an excitation wavelength of 488 nm to visualize the cells. Confocal fluorescence microscopy uses excitation light at 488 nm and receives light from 550 nm onwards to observe cells.

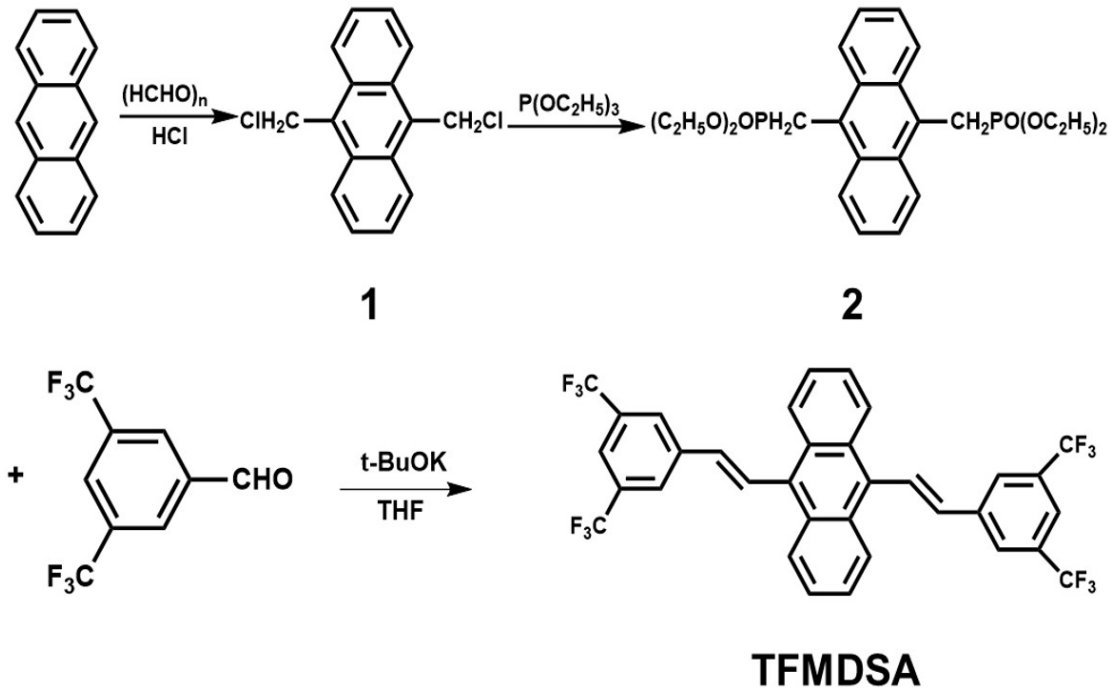

Figure S1. The synthetic route of TFMDSA molecule.

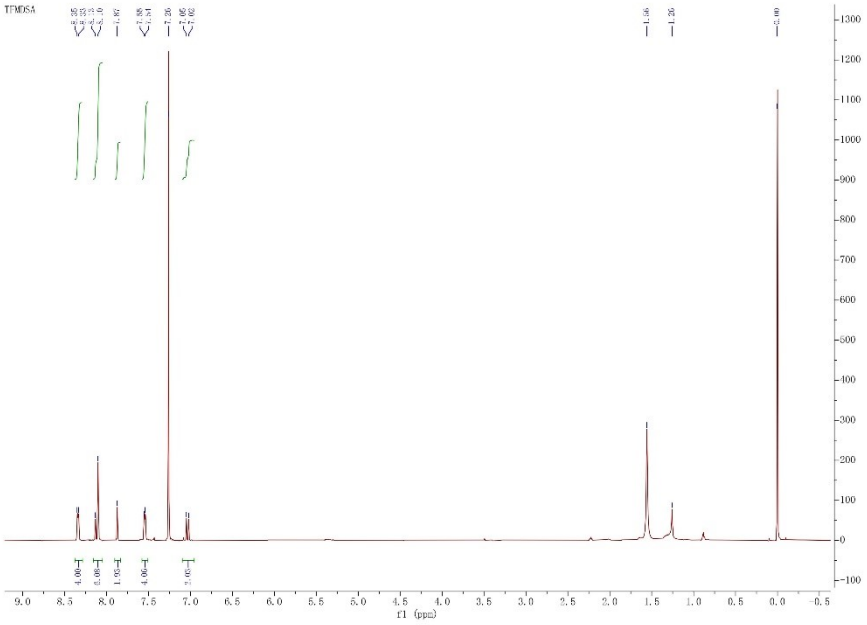

Figure S2. ^{1}H NMR spectra of TFMDSA.

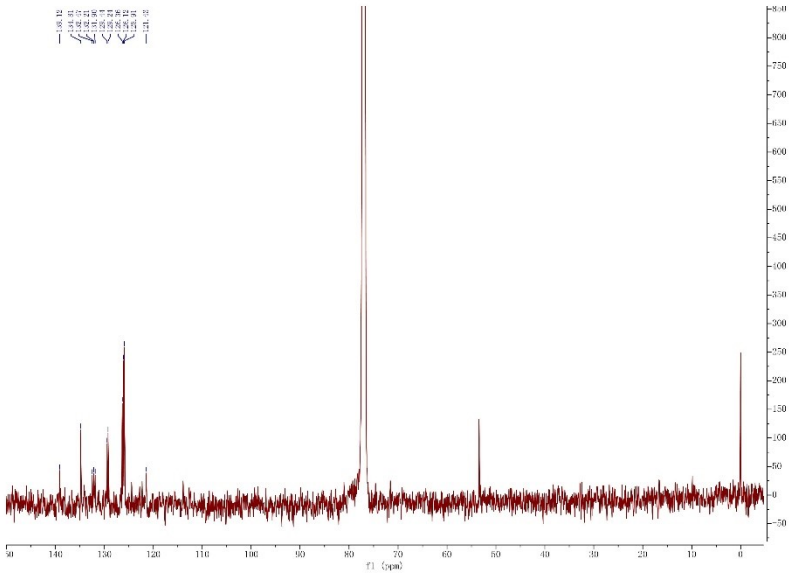

Figure S3. ^{13}C NMR spectra of TFMDSA.

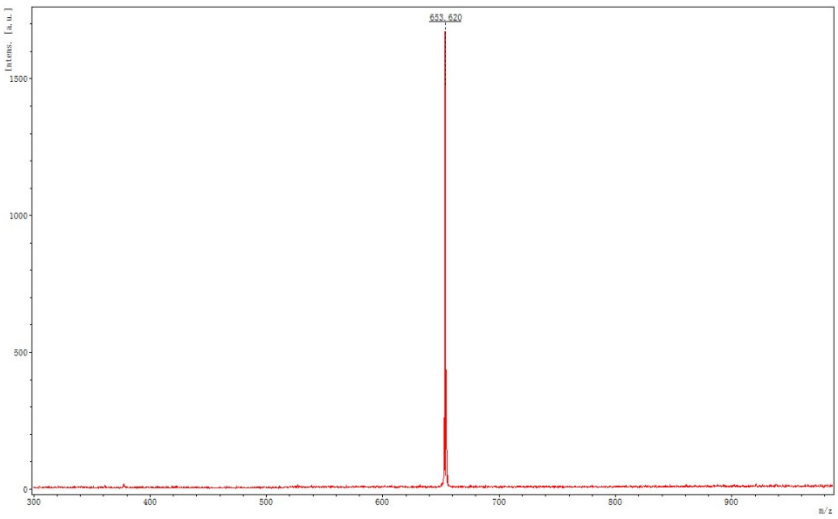

Figure S4. The mass spectrometry of TFMDSA.

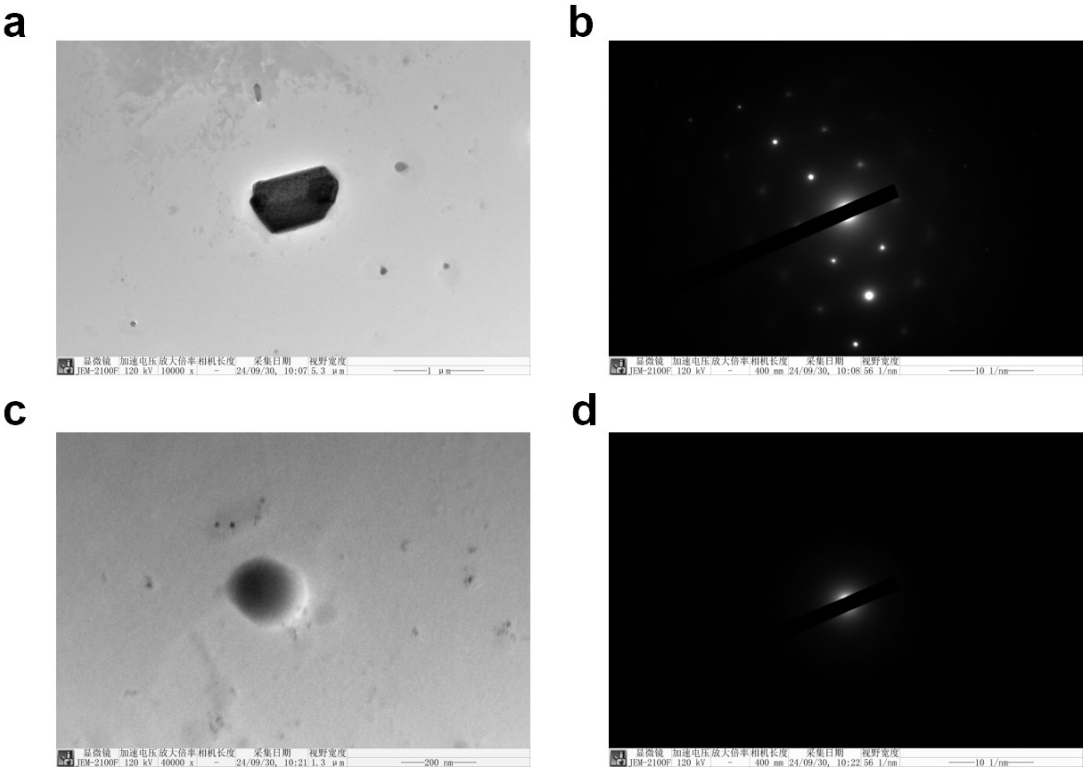

Figure S5. TEM images and SAED of the mPEG5K-PCL10K nanoparticles (a, b), and the mPEG5K-PLLA10K nanoparticles (c, d).

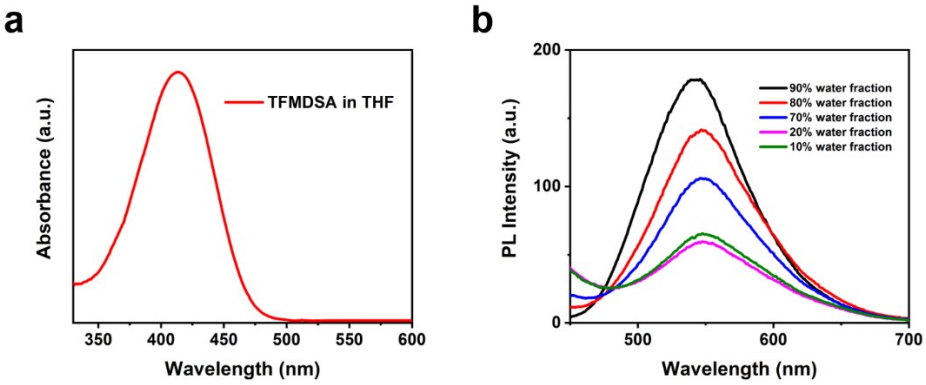

Figure S6. (a) The absorbance spectra of the TFMDSA; (b) PL spectra of TFMDSA in THF and THF/water mixtures with different water fractions.

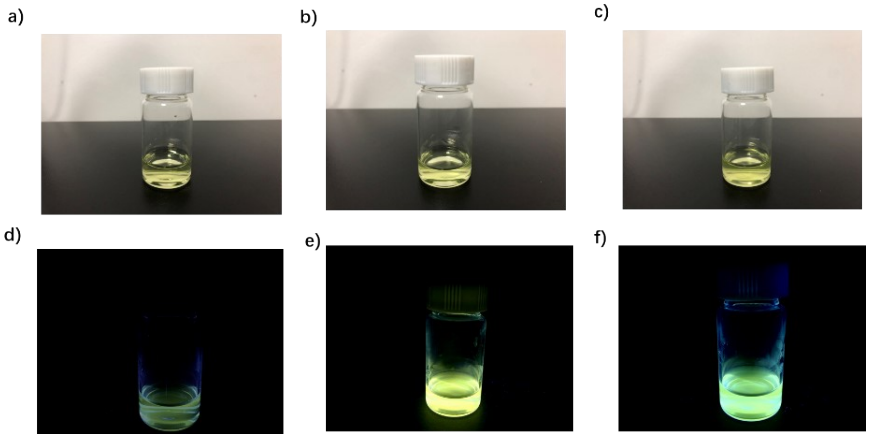

Figure S7. The photographs of (a) TFMDSA NPs, (b) $mPEG_{5K}$-PCL_{10K}@TFMDSA NCs, and (c) $mPEG_{5K}$-$PLLA_{10K}$@TFMDSA NCs under room light； The photographs of (d) TFMDSA NPs, (e) $mPEG_{5K}$-PCL_{10K}@TFMDSA NCs, and (f) $mPEG_{5K}$-$PLLA_{10K}$@TFMDSA under UV light.

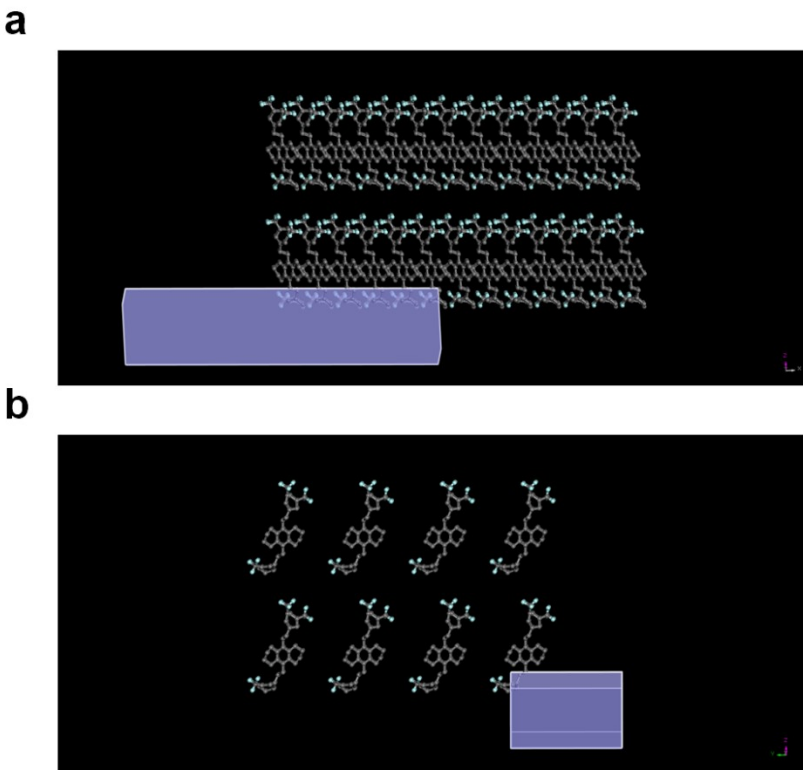

Figure S8. Theoretically predicted equilibrium morphology of TFMDSA.

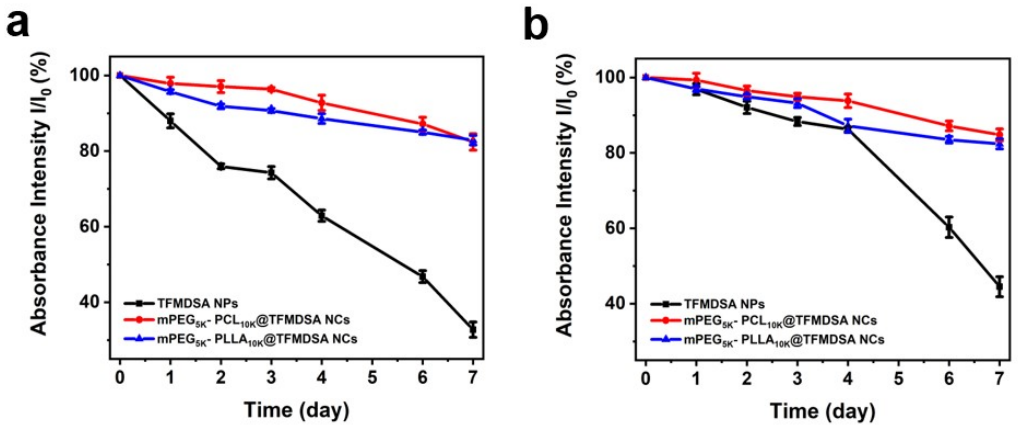

Figure S9. The stability of the (g) absorbance intensity and (h) fluorescence intensity of $mPEG_{5K}$-$PLLA_{10K}$@TFMDSA NCs, $mPEG_{5K}$-PCL_{10K}@TFMDSA NCs, and TFMDSA NPs over seven days.

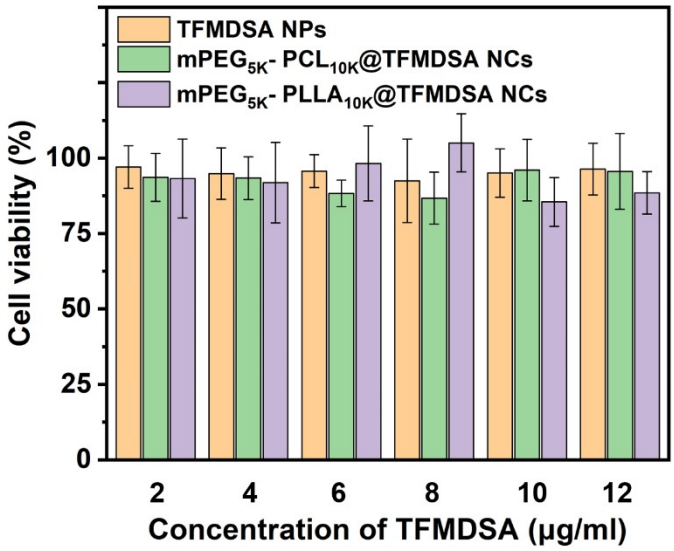

Figure S10. Cell viability diagram of Hela cells co-cultured with different concentrations of TFMDSA NPs, $mPEG_{5K}$-PCL_{10K}@TFMDSA NCs, and $mPEG_{5K}$-$PLLA_{10K}$@TFMDSA NCs for 24h.

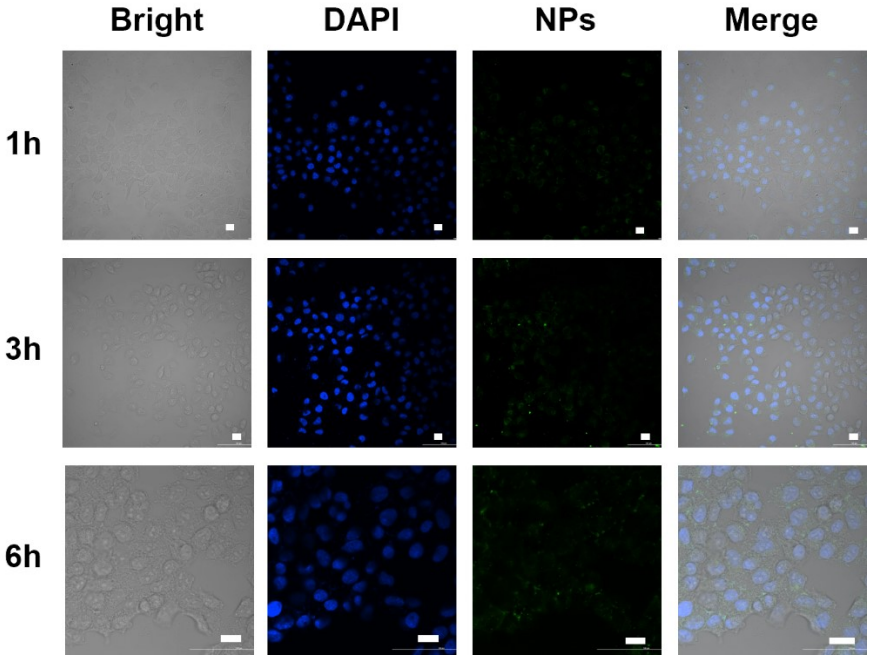

Figure S11. CLSM images of HeLa cells incubated with the TFMDSA NPs for 1 h, 3 h and 6 h at 37°C. Cells are viewed in the blue channel for DAPI, and the green channel for TFMDSA. Scale bars represent 20 μm in all images.

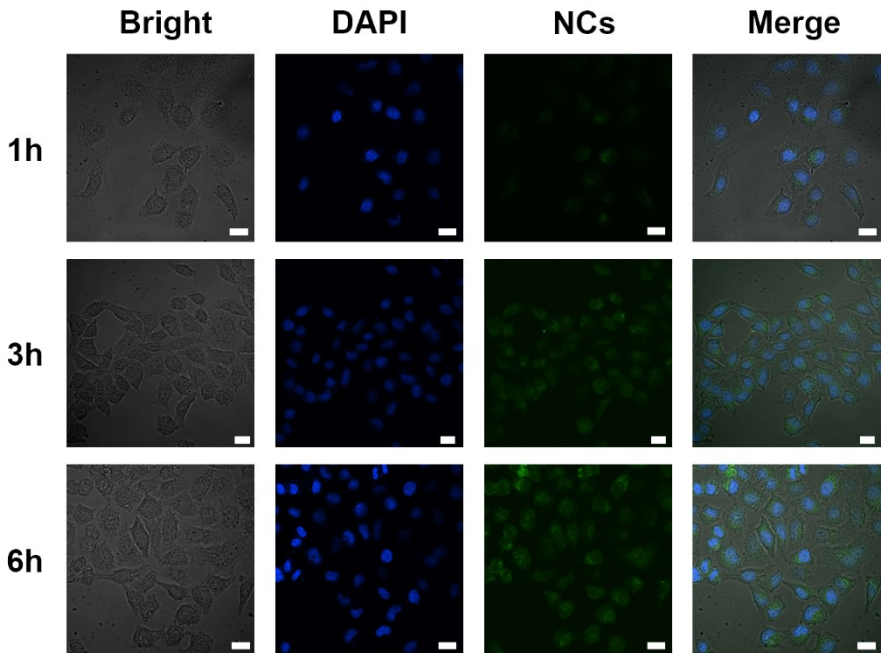

Figure S12. CLSM images of HeLa cells incubated with the $mPEG_{5K}$ -PCL_{10K} @ TFMDSA NCs for 1 h, 3 h and 6 h at 37oC. Cells are viewed in the blue channel for DAPI, and the green channel for TFMDSA. Scale bars represent 20 μm in all images.

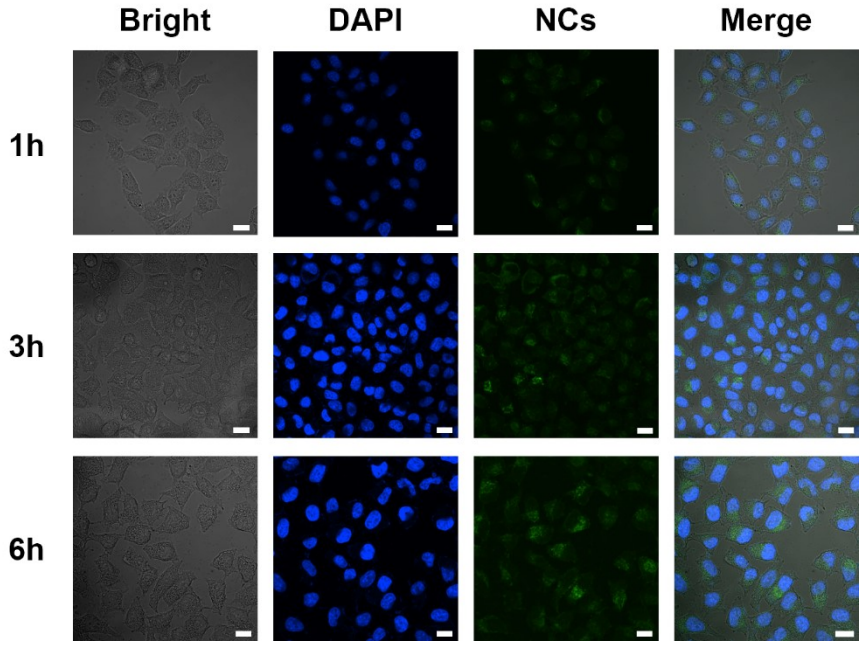

Figure S13. CLSM images of HeLa cells incubated with the $mPEG_{5K}$ -$PLLA_{10K}$ @ TFMDSA NCs for 1 h, 3 h and 6 h at 37oC. Cells are viewed in the blue channel for DAPI, and the green channel for TFMDSA. Scale bars represent 20 μm in all images.