Electronic Supplementary Information (ESI)

Experimental Section

Materials. Poly(ethylene glycol) (PEG, Mn=0.4kDa, 1kDa, 2kDa Sigma-Aldrich), 4, 4′-Methylenebis (cyclohexyl isocyanate) (HMDI, ≥99%, Alfa-Aesar), stannous octanoate (Alfa-Aesar), PCL (Mn=120 kDa, Guanghua Weiye, Guangdong, China), Toluene-p-sulfonic acid, 4-bromobenzophenone, N-formylpiperidine, Titanium tetrachloride, N-butyl lithium, Sodium borohydride, chloroform, Diiodomethane (CH_2I_2), 1,4-butanediol, 1,2-dichloroethane, dichloromethane (DCM), petroleum ether, ethylene glycol, methanol were AR grade, purchased from Guangdong Guanghua Sci-Tech (Guangdong, China) and used as received. Rhodamine Phalloidin was obtained from Invitrogen (Thermo Fisher). Fetal bovine serum (FBS), penicillin, streptomycin, Dulbecco's modified Eagle' medium (DMEM), 0.25 % trypsin-0.22 % ethylene diamine tetraacetic acid (EDTA), 1 × PBS buffer with pH 7.4 (ultrapure grade) were purchased from Hyclone. All other reagents were commercially available and used without further purification unless otherwise noted. Human cervix carcinoma (HeLa), and rat glial cells were provided by Shenzhen university health science center.

Characterization. NMR spectra were collected on Brucker Avance 400 spectrometer. The fluorescence spectra were measured using a Hitachi F-7000 equipped with a xenon lamp excitation source. The crystallinity of the TPE-PU film was determined by X-ray diffraction (XRD, Bruker, D8, Germany), samples were placed in a quartz

sample holder and scanned from 5 ° to 65 ° at a scanning rate of 0.01°/min. The transmission electron microscopy (TEM) images were obtained from a JEOL JEM-1230 transmission electron microscope under an acceleration voltage of 5.0 kV.

Synthesis and characterizations

Synthesis of TPE-2OH. Into a 250 mL two-necked round-bottom flask with a reflux condenser were placed 5.0 g (19.1 mmol) of 4-bromobenzophenone, 2.5g (38.2 mmol) of zinc dust. The flask was evacuated under vacuum and flashed with dry nitrogen three times. 100 mL of THF was then added. The mixture was cooled to -78 ℃ and 3.6 g (19.1 mmol) of $TiCl_4$ was added drop-wise with a syringe. The mixture was slowly warmed to room temperature. After stirred for 0.5 h, the mixture was refluxed for 24 h. The mixture was quenched with 10 % aqueous K_2CO_3 solution and filtered. The filtrate was extracted with dichloromethane three times. The organic layer was washed with purified water and dried over Na_2SO_4. After solvent evaporation, the crude product was purified by silica gel column chromatography using petroleum ether as eluent to obtain the product **2** (white solid). To a stirred solution of **2** (2.0 g) in 50 mL anhydrous THF was added n-BuLi (2.7 mL, 1.6 M in hexane, 4.36 mmol) at -78 ℃ under nitrogen. The mixture was first stirred for 2 h at this temperature and then warmed to room temperature. After stirred for 1 h, the flask was cooled again to -78 ℃ and *N*-formylpiperidine (0.62 g, 5.46 mmol) was injected in one portion. The solution was stirred overnight and warmed to room temperature gradually. The reaction was quenched by adding 100 mL of aqueous hydrochloric acid (2 M solution). The organic layer was separated and the aqueous layer was

extracted with 100 mL ethyl ether three times. The organic layers were collected and dried over Na_2SO_4. After solvent evaporation, the residue was purified by silica gel column chromatography using petroleum ether/ethyl acetate (4/1, v/v) as eluent to give the desired product **3** (yellow solid). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.02 (d, d, *J*=7.70, 1.59 Hz, 4 H) 7.09 - 7.14 (m, 6 H) 7.18 (d, *J*=8.31 Hz, 4 H) 7.58 - 7.68 (m, 4 H) 9.88 - 9.94 (m, 2 H). Into a 100 mL round bottom flask was placed 50 mL THF solution of **3** (1.165 g, 3mmol), followed by addition of 20 mL of methyl alcohol solution of NaBH4 (0.227 g, 6mmol) at room temperature. The mixture was stirred for 0.5 h and then the organic layer was separated and the aqueous layer was extracted with 100 mL dichloromethane. After solvent evaporation, the white solid was the final product **4**. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 4.62 (s, 4 H), 4.69-4.72 (m, 1H), 7.02-7.05 (m, 6H), 7.07-7.17 (m, 12H).

Dihydroxyl terminated prepolymer PCL-diol. Pre-polymer PCL-diol was prepared by transesterification between the purified PCL materials and 1,4-butanediol using *p*-toluenesulfonic acid as catalyst in our previous study.[1-3] Typcially, purified PCL (10 g) was dissolved in 100 mL of chloroform and refluxed for 30 min before *p*-toluenesulfonic acid (4.8 g) and 1,4-butanediol (20 g) were added in subsequently. The reaction was carried out under reflux for 3 h. The resultant solution was washed with distilled water for 3 times, concentrated and dried under reduced pressure. The yield was white waxy solid. ^{1}H NMR ($CDCl_3$, 400MHz, ppm): δ1.15 (d, J) 6 Hz, $CH(C\underline{H}_3)_2$ end group), 1.3 (m, 2H, $C\underline{H}_2$ backbone), 1.6 (m, 4H, $C\underline{H}_2$ backbone), 2.2 (m, 2H, $C\underline{H}_2$ backbone), 3.6 (t, $-C\underline{H}_2OH$ end group), 4.0 (m, 2H, $OC\underline{H}_2$ backbone),

4.9 (sept, J) 6 Hz C<u>H</u>$(CH_3)_2$ end group).[1-3] The obtained PCL-diol structure is consistent with previous reports.[4] GPC: Mn=5887, Mw=7194, PDI=1.22. Hydroxyl Value: 52 mg KOH/g. Acid Value: 0.37 mg KOH/g.

Synthesis of TPE-PUs. Diisocyanate terminated prepolymer PEG-2NCO was prepared according to reported procedure.[5] Briefly, Amount 0.002 mol PEG was dissolved in 10 ml 1,2-dichloroethane in a 50 mL two-neck flask at 105 ℃. Then, any trace of water in the system was removed through azeotropic distillation with amount 4 mL of 1,2-dichloroethane left in the flask, then transferred to a 25 mL isobaric drop funnel, this solution was added drop-wise to a 100 mL 4-neck flask in which a slight excess diisocyanate HMDI (1.155 g, 0.0044 mol) was placed in advance, catalyst stannous octanoate (~0.005 g) were injected sequentially. The reaction was carried out at 50 ℃ for 12 h under a nitrogen atmosphere. PCL/PEG block pre-copolymers were synthesized via a coupling reaction of terminal hydroxyl group of PCL-diol and terminal isocyanate group of PEG-2NCO at equal molar ratio. Amount 0.002 mol PCL-diol was dried by dissolution in 20 mL 1,2-dichloroethane and removed the water by azeotropic distillation, 10 mL solvent was removed. The reminder was transferred to an isobaric drop funnel. The reaction was started when the PCL-diol solution was added drop-wise to the above mentioned ready-prepared PEG-2NCO solution in the 4-neck flask under a nitrogen atmosphere at 70 ℃. After 24 h reaction, the PCL/PEG pre-copolymers were obtained, which is terminated with the isocyanate group. Then product **4** (0.157 g, 0.0004 mol) was dissolved in 1,2-dichloromethane and then added into the pre-copolymer PCL/PEG-diisocyanate; the mixture was

stirred for 48 h at 70 ℃. The production was precipitated in diethyl ether, filtered, and then re-dissolved in dichloromethane, filtered to move the trace amount of insoluble byproduct. In order to eliminate the stannous octanoate residue and the possible low molecular weight oligomers, the filtrate was again precipitated in a mixture of methanol and diethyl ether (1/20, v/v). Product was collected through filtration, washed by distilled water three times followed by drying under vacuum to constant weight at 40 ℃. ^{1}H NMR ($CDCl_3$, 400MHz): δ (ppm) 0.94-0.96 (t, C$\underline{H}_2$(Cy)$_2$), 1.03-1.10 (m, Cy), 1.34-1.46 (m, -OC$\underline{H}_2$CH$_2$CH$_2$CH$_2$CH$_2$C(O)O-), 1.59-1.77 (m, -OCH$_2$C$\underline{H}_2$CH$_2$C$\underline{H}_2$CH$_2$C(O)O-), 2.25-2.37 (t, -OCH$_2$CH$_2$CH$_2$CH$_2$C$\underline{H}_2$C(O)O-), 3.38 (s, CH-NH), 3.57-3.71(s, -C$\underline{H}_2$C$\underline{H}_2$O-), 3.76 (s, N$\underline{H}$), 4.0-4.09 (t, -OC$\underline{H}_2$CH$_2$CH$_2$CH$_2$CH$_2$C(O)O-), 5.0 (s, C$\underline{H}_2$(OC(O)NH)), 4.81 (s, N$\underline{H}$), 7.0(m, aromatic backbone), 7.09 (m, aromatic backbone).

The live cellular imaging. Human cervix carcinoma HeLa cells (1 × 10^4 cell/mL) were seeded in 6-well plates and grown for 24 h in DMEM containing 10 % fetal bovine serum (FBS), 1 % penicillin and 1 % streptomycin in an incubator with humidified CO_2/air (5:95) atmosphere at 37 ℃. Then HeLa cells were stained with a serum-free DMEM containing TPE-PU-400, TPE-PU-1000, and TPE-PU-2000 (1 × 10^{-3} g/mL) for 45 min, respectively. After washed three times with pre-warmed 1 × PBS buffer to eliminate the excess TPE-PU, the cells were imaged under an Olympus BX 41 inverted fluorescence microscope (Olympus Japan) using combination of excitation and emission filter: excitation filter = 330-380 nm, dichroic mirror = 400 nm and emission filter = 480 nm long pass.

Subcellular microfilaments imaging in living rat glial cells. Rat glial cells (1 × 10^4 cell/mL) were seed into 6-well plates and grown for 24 h in DMEM containing 10 % fetal bovine serum (FBS), 1 % penicillin and 1 % streptomycin in an incubator with humidified CO_2/air (5:95) atmosphere at 37 ℃. Then the cells were incubated with a serum-fresh DMEM medium containing TPE-PU-1000 at the concentration of 1 × 10^{-3} g/mL for 45 min. Subsequently the original medium was removed, and the cells were washed three times with pre-warmed 1 × PBS buffer. The cells were imaged by confocal laser scanning microscope (CLSM, Leica, TCS SP5, Germany) with imaging software (Lasaf-Wfip). Rat glial cells cultured with Rhodamine Phalloidin in serum-fresh DMEM medium (0.1 μM) for 45 min were also imaged.

Cellular uptake and endocytosis pathway. Rat glial cells (1 × 10^4 cell/mL) were seeded into 6-well plates and grown in DMEM containing 10 % fetal bovine serum (FBS), 1 % penicillin and 1 % streptomycin in an incubator with humidified CO_2/air (5:95) atmosphere at 37 ℃ for 24 h. The cells were then incubated with the DMEM medium containing TPE-PU-1000 at the concentration of 1 × 10^{-3} g/mL for 4 h at 37 or 4 ℃. Subsequently, the cells were carefully washed three times by pre-warmed 1 × PBS and detached by 0.25 % trypsin-0.22 % EDTA, followed by centrifugation (800 rpm for 3 min) to harvest the cells, then the cells were re-suspended by serum-fresh DMEM to obtain single cell suspension. Finally, the single cell suspension was seeded into a new 96-well plate at 1.0 × 10^4 cells/cm^2. The fluorescence intensity of TPE-PU nanoparticles at 505 nm in each well was then

measured by microplate reader (SpectraMax L, Molecular Devices, China) with excitation wavelength of 365 nm. The relative cellular uptake efficiency under different conditions was expressed as the ratio of the fluorescence in the corresponding sample wells to that of the control wells.

To elucidate their potential endocytosis pathway, the rat glial cells were pretreated with different chemical inhibitors of clathrin-mediated endocytosis (4 % w/v of sucrose), and caveolae-mediated endocytosis (200 μM of genistein) at concentrations which were not toxic to the cells. [6,7] Following pre-incubation for 30 min, the inhibitor solutions were removed, and freshly prepared TPE-PU-1000 (1×10^{-3} g/mL) in serum-fresh DMEM containing the same inhibitor concentrations mentioned above were added and further incubated for 4 h. After incubation, the cells were washed three times with pre-warmed 1 × PBS, detached by 0.25 % trypsin-0.22 % EDTA, followed by centrifugation (800 rpm for 3 min) to harvest the cells, then the cells were re-suspended by serum-fresh DMEM to obtain single cell suspension. Finally, the single cell suspension was seeded into a new 96-well plate at 1.0×10^4 cells/cm^2. The fluorescence intensity of TPE-PU nanoparticles at 505 nm in each well was then measured by microplate reader (SpectraMax L, Molecular Devices, China) with excitation wavelength of 365 nm. The relative cellular uptake efficiency under different conditions was expressed as the ratio of the fluorescence in the corresponding sample wells to that of the control wells.

Cytotoxicity of TPE-PU-1000. Cell count kit-8 (CCK-8) assay was employed to quantitatively assess the cell viability. After rat glial cells were cultured in the serum-

free medium containing TPE-PUs at the concentration of 1×10^{-3} g/mL for 12, 24, 36, and 48 h at 37 ℃, the original medium was replaced by 500 μL fresh DMEM contains 50 μL CCK-8. It was incubated for 3 h at 37 ℃ to form water dissoluble formazan. Then the above formazan solutions were taken from each sample and added to one 96-well plate, six parallel replicates were prepared. The absorbance at 450 nm was determined using microplate reader (SpectraMax L, Molecular Devices, China), DMEM containing CCK-8 was used as a control.

Subcellular microfilaments imaging in fixed and permeabilized rat glial cells. Rat glial cells (1×10^4 cell/mL) were seed into 6-well plates and grown for 24 h in DMEM containing 10 % fetal bovine serum (FBS), 1 % penicillin and 1 % streptomycin in an incubator with humidified CO_2/air (5:95) atmosphere at 37 ℃. Then the cells were fixed with 4 % paraformaldehyde for 10min and permeabilized with 0.1 % triton X-100 for 5 min at 25 ℃. The fixed and permeabilized cells were further blocked by 1 % BSA in 1 × PBS for 30 min and washed three times with 1 × PBS. The cells were then incubated with Rhodamine Phalloidin in serum-fresh DMEM medium (0.1 μM) for 30 min at 25 ℃. After the cells were washed with 1 × PBS, imaging was carried out on CLSM. The fluorescence of Rhodamine Phalloidin was collected with 565-620 nm band-pass filter upon 543 nm excitation.

Colocalization of TPE-PU and Rhodamine Phalloidin in rat glial cells. Rat glial cells (1×10^4 cell/mL) were seed into 6-well plates and grown for 24 h in DMEM containing 10 % fetal bovine serum (FBS), 1 % penicillin and 1 % streptomycin in an incubator with humidified CO_2/air (5:95) atmosphere at 37 ℃.

Then the cells were incubated with a serum-fresh DMEM medium containing TPE-PU-1000 at the concentration of 1×10^{-3} g/mL for 45 min, the cells were washed three times with pre-warmed 1 × PBS to eliminate the excess TPE-PU. The cells were then fixed with 4 % paraformaldehyde for 10min and permeabilized with 0.1 % triton X-100 for 5 min at 25 ℃. After washing with 1 × PBS three times, then the cells were incubated with Rhodamine Phalloidin in 1 × PBS (0.1 μM) for 45 min at 25 ℃. After the cells were washed with 1 × PBS, imaging was carried out on CLSM. The fluorescence of TPE-PU-1000 was collected with a 470-510 nm band-pass filter upon 364 nm excitation (1 m W laser power) and that of Rhodamine Phalloidin was collected with 565-620 nm band-pass filter upon 543 nm excitation (1 m W laser power). Under these experimental conditions, the fluorescence of TPE-PU-1000 is not detectable with the 565-620 nm band-pass filter and that of Rhodamine Phalloidin is not detectable with the 470-510 nm range.

Photo-stability Studies. The confocal samples of rat glial cells incubated with TPE-PU-1000 or stained with Rhodamine Phalloidin were prepared according to previously described procedures. The CLSM images of each sample were recorded at 2 min interval under continuous laser scanning at excitation wavelength of 364 nm for TPE-PU-1000 and 541 nm form Rhodamine Phalloidin. The fluorescence intensity of each image was analyzed by Image J software. The photo-stability of TPE-PU-1000 and Rhodamine Phalloidin was expressed by the ratio of fluorescence intensity of each sample after excitation for a designated time interval to its initial value as a function of exposure time.

Endosome staining. In detail, rat glial cells (10^5 cell mL^{-1}) were seeded on glass-bottomed dishes and cultivated for 16 h in a humidified CO_2/air (5:95) atmosphere at 37 ℃. One hour prior to the Rab5-GFP (an early endosome marker) staining, the cells were rinsed with phosphate buffered saline (PBS) and the medium was changed to a serum-fresh DMEM medium containing TPE-PU-1000 at the concentration of 1×10^{-3} g/mL for 45 min, the cells were washed three times with pre-warmed 1 × PBS to eliminate the excess TPE-PU. Subsequently, the medium was replaced by serum-fresh DMEM medium supplemented with DAPI (from Thermo Fisher Scientific) at 30 nM for nucleus staining. Subsequently, cells were methanol fixed (5 min) and incubated with the Rab5-GFP antibody (an early endosome marker, $1 \mu g mL^{-1}$) for 1 h at room temperature. The second antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG used at a 1/1000 dilution for 1 h. Imaging was performed with CLSM (CLSM, Leica, TCS SP5, Germany) applying a 40 × 10 NA plan apochromat objective. For cell imaging, cells were grown on high precision cover glasses (NEST, 20 × 20 mm, 170 ± 5 μm certified thickness) at a density of 5×10^4 cells mL^{-1}. The fluorescence of TPE-PU-1000 was collected with a 470-505 nm band-pass filter upon 364 nm excitation (1 m W laser power). The fluorescence of Rab5-GFP antibody was collected with a 510-530 nm band-pass filter upon 488 nm excitation (1 m W laser power). The fluorescence of DAPI was collected with a 450-460 nm band-pass filter upon 341 nm excitation (1 m W laser power).

Lysosome staining. Live cell imaging was performed for uptake studies. In detail, rat glial cells (10^5 cell mL^{-1}) were seeded on glass-bottomed dishes and

cultivated for 16 h in a humidified CO_2/air (5:95) atmosphere at 37 ℃. One hour prior to the TPE-PU-1000 nanoparticles addition, the cells were rinsed with phosphate buffered saline (PBS) and the medium was replaced by serum-fresh DMEM medium supplemented with DAPI (from Thermo Fisher Scientific) at 30 nM for nucleus staining, Lyso Tracker® Green DND-26 (from Thermo Fisher Scientific) at 50 nM for lysosome staining. Subsequently, medium was replaced by fresh serum-free DMEM containing TPE-PU-1000 nanoparticles (1×10^3 g/mL) for 45 min, the cells were washed three times with pre-warmed 1 × PBS to eliminate the excess TPE-PU. Imaging was performed with CLSM (CLSM, Leica, TCS SP5, Germany) applying a 40 × 10 NA plan apochromat objective. For cell imaging, cells were grown on high precision cover glasses (NEST, 20 × 20 mm, 170 ± 5μm certified thickness) at a density of 5×10^4 cells mL^{-1}. The fluorescence of TPE-PU-1000 was collected with a 470-505 nm band-pass filter upon 364 nm excitation (1 m W laser power). The fluorescence of Lyso Tracker® Green was collected with a 510-520 nm band-pass filter upon 488 nm excitation (1 m W laser power). The fluorescence of DAPI was collected with a 450-460 nm band-pass filter upon 341 nm excitation (1 m W laser power).

Viscoelastic characterization. The rheological properties were characterized at 25 ℃ on a strain-controlled rheometer (MCR 302, Anton Paar) with a cone and plate geometry (60 mm diameter and 2° cone angel) for concentrated samples and the double cylinder geometry for less viscous samples. Steady-state shear viscosity was measured in the shear rate range of 0.1 to 100 s^{-1}. The elastic (G') and viscous (G'')

moduli were determined as a function of increasing stress amplitude at a frequency of 1 Hz and the crossover of G' and G'' was used to estimate the yield stress.

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Figure Captions:

Fig. S1. The Molecular structure and ^{1}H NMR spectrum of TPE-2CHO and TPE-2OH.

Fig. S2. Synthesis of linear block fluorescent polyurethane copolymers based on PCL-diol, PEG, and TPE-2OH.

Fig. S3. ^{1}H NMR spectrum of TPE-PU in $CDCl_3$.

Fig. S4. (A) PL spectra of TPE-PU-1000 in THF/water mixtures with different fractions of water (f_w), λ=360 nm. (Inset) Plot of the relative PL intensity (I/I_0) of TPE-PU at 505 nm vs the composition of the THF/water mixture (f_w). I_0 = PL intensity of TPE-PU-1000 in THF solution. (B) Fluorescent photographs of the TPE-PU-1000 THF/water mixtures with different fractions of water (f_w) taken under UV illumination.

Fig. S5. TEM images of TPE-PU-1000 nanoparticles. Bar scale, 100 nm.

Fig. S6. TEM images of various TPE-PU nanoparticles were immersed in PBS pH 7.4 and 5.0 for 1 h, respectively. Bar scale, 10 nm.

Fig. S7. XRD patterns of PCL, TPE-PU-400, TPE-PU-1000 and TPE-PU-2000 films.

Fig. S8. Confocal laser scanning microscopy observation the subcellular microfilaments imaging in rat glial cells. (A1) confocal images of the rat glial cells after incubation with 1×10^{-3} g/mL TPE-PU-1000 in culture medium for 45 min at 37 ℃ (λ=364 nm, 1 m W laser power). (B1) confocal images of the rat glial cells after incubation with Rhodamine Phalloidin in culture medium (0.1 μM) for 45 min at 37 ℃ (λ=543 nm, 1 m W laser power). (C1) confocal images of the fixed and permeabilized rat glial cells stained by Rhodamine Phalloidin (λ=543 nm, 1 m W laser power). (A2) - (C2) DAPI, (A3) - (C3) Merged images. Bar scale, 20 μm (applicable to all images).

Fig. S9. Cell viability of rat glial cells incubation with 1×10^{-3} g/mL TPE-PU-1000 for 12, 24, 36, and 48 h at 37℃.

Fig. S10. Nanoparticles accumulation in early endosomes and late endo-lysosomes. (A), fluorescence images of rat glial cells expressing Rab5-GFP (Alexa Fluor® 488) incubated with 1×10^{-3} g/mL TPE-PU-1000 nanoparticles for 1 h. The excitation/emission of TPE-PU-1000, Alexa Fluor® 488, and DAPI lines was 364/470-505 nm, 488/510-530 nm, and 341/450-460 nm, respectively. Red triangle shows spatial overlap between TPE-PU-1000 nanoparticles and early endosomes. (B), fluorescence images of TPE-PU-1000 nanoparticles and Lyso tracker® (green) in rat glial cells after cell incubation with 1×10^{-3} g/mL TPE-PU-1000 nanoparticles for 45 min. The excitation/emission of TPE-PU-1000,

Lyso tracker®, and DAPI lines was 364/470-505 nm, 488/510-520 nm, and 341/450-460 nm, respectively. Bar scale 20 μm (applicable to all images). (C), superposition of TPE-PU-1000 nanoparticles and Alexa Fluor® 488 intensity profiles along the line scan is shown in Fig.S10A, shows TPE-PU-1000 nanoparticles accumulation in the early endosomes. (D), superposition of TPE-PU-1000 nanoparticles and Lyso tracker® intensity profiles along the line scan is shown in Fig.S10B, shows a small number of TPE-PU-1000 nanoparticles accumulation in the late endo-/lysosomes.

Fig. S11. Fluorescent characterizations of TPE-PUs. (A) and (B), photographs of various TPE-PU films taken under room lighting and UV illumination, respectively. (C), fluorescent images of the HeLa cells cultured in the presence of various TPE-PU nanoparticles with different DLs for 45 mins at 37 ℃. [TPE-PU] =1 × 10^{-3} mg/mL, pH=7.4. Scale bar, 10 μm (applicable to all images).

Fig. S12. The sub-cellular microfilaments colocalization of TPE-PU and Rhodamine Phalloidin in rat glial cells. The Excitation/Emission of TPE-PU (sample: TPE-PU-1000), Rhodamine Phalloidin, and DAPI lines was 364/470-505 nm, 543/565-620 nm, and 341/450-460 nm, respectively. Bar scale 20 μm (applicable to all images). (A), the original Fig.3. (B), the newly supplementary images of sub-cellular microfilaments colocalization of TPE-PU and Rhodamine Phalloidin in

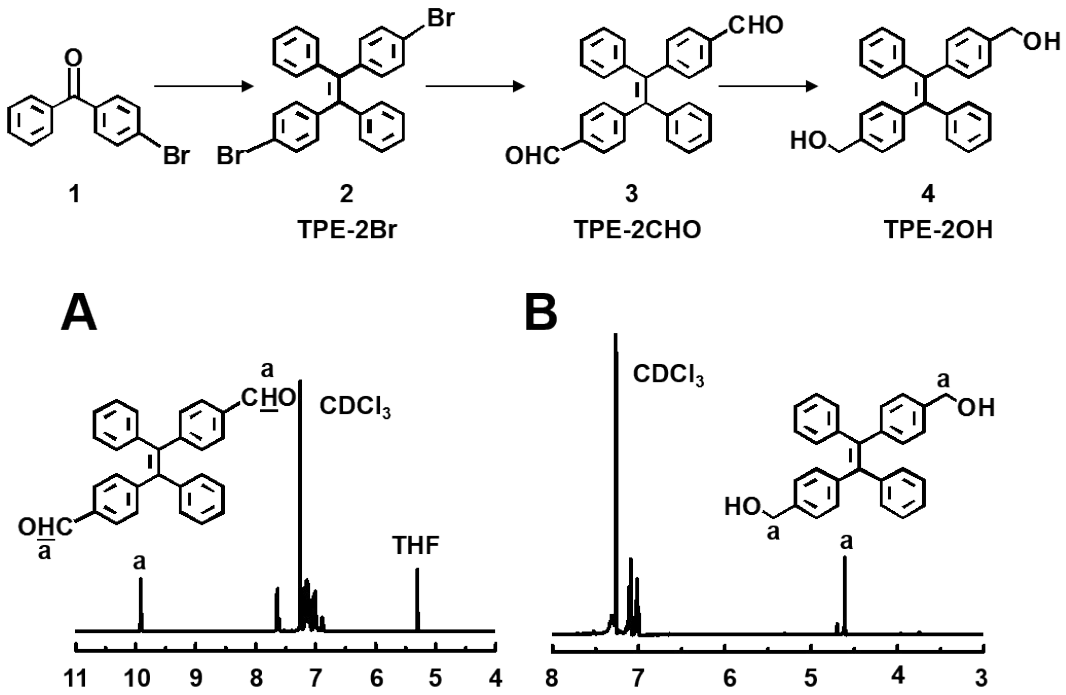
rat glial cells. (C), the superposition of TPE-PU-1000 nanoparticles and Rhodamine Phalloidin intensity profiles along the line scan shown in Fig.S12A. (D), the averaged fluorescence intensity of TPE-PU-1000 nanoparticles and Rhodamine Phalloidin in microfilaments for the single measurement shown in Fig.S12A. (E), superposition of TPE-PU-1000 nanoparticles and Rhodamine Phalloidin intensity profiles along the line scan shown in Fig.S12B. (F), the averaged fluorescence intensity of TPE-PU-1000 nanoparticles and Rhodamine Phalloidin in microfilaments for the single measurement shown in Fig.S12B. Error bars are standard deviation. n=3.

Fig. S13. The quantitative fluorescence analysis of the TPE-PUs. (A) and (B), the averaged fluorescence intensity and hue fluorescence data of this series of TPE-PUs films from original Fig.2B. (C) and (D), the averaged fluorescence intensity and hue fluorescence data of this series of TPE-PUs nanoparticles from original Fig.2C. Error bars are standard deviation. n=3.

Fig. S14. Linear storage and loss modulus as a function of frequency for TPE-PU-1000 (1×10^{-3} g/mL) nanoparticles at T=25 ℃.

Table.S1. Composition and molecular distribution information of block polyurethane copolymers based on PCL, PEG and TPE.

353 **Figures**



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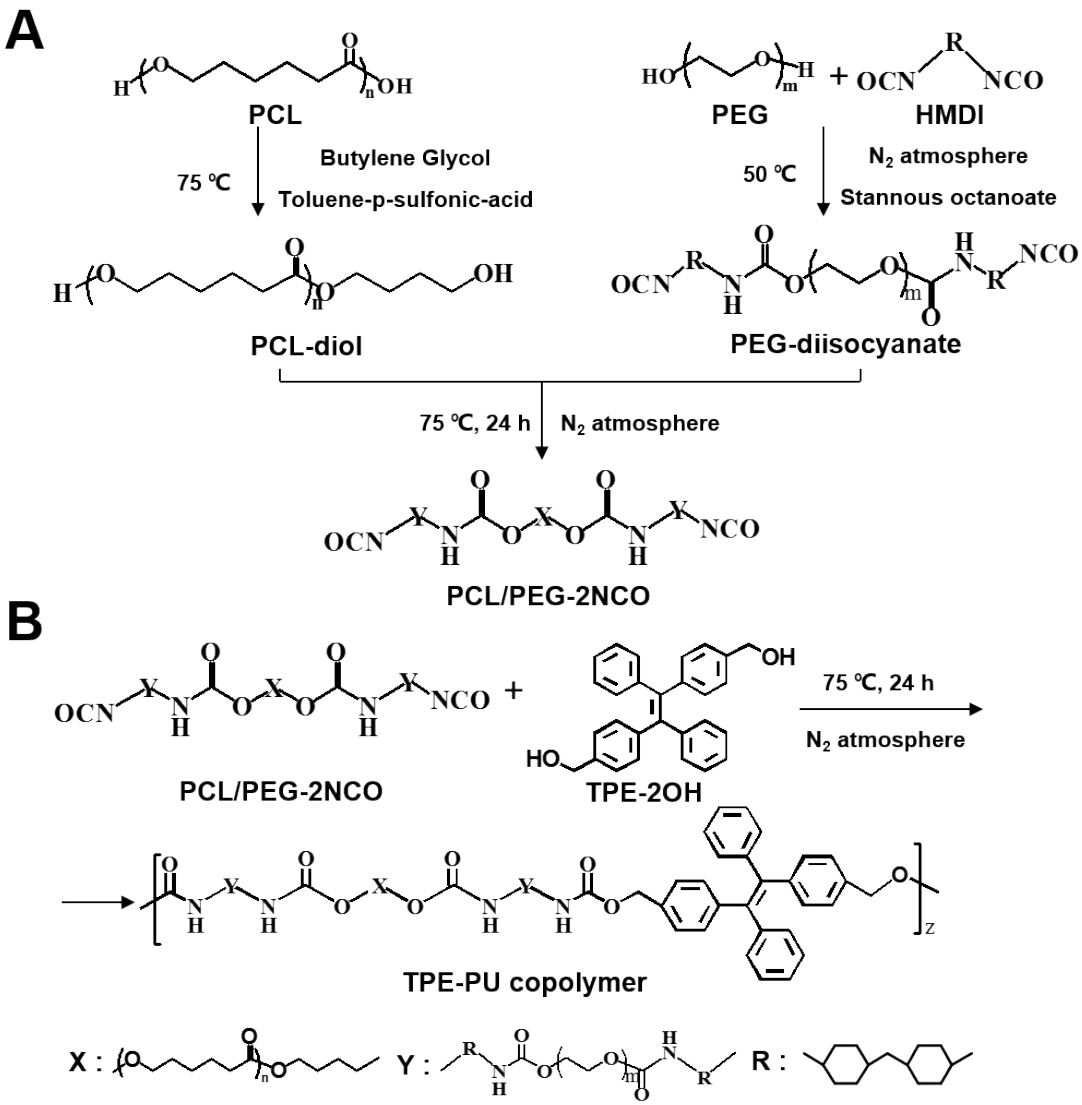
355 **Fig. S1.** The Molecular structure and ^{1}H NMR spectrum of TPE-2CHO

356 and TPE-2OH.



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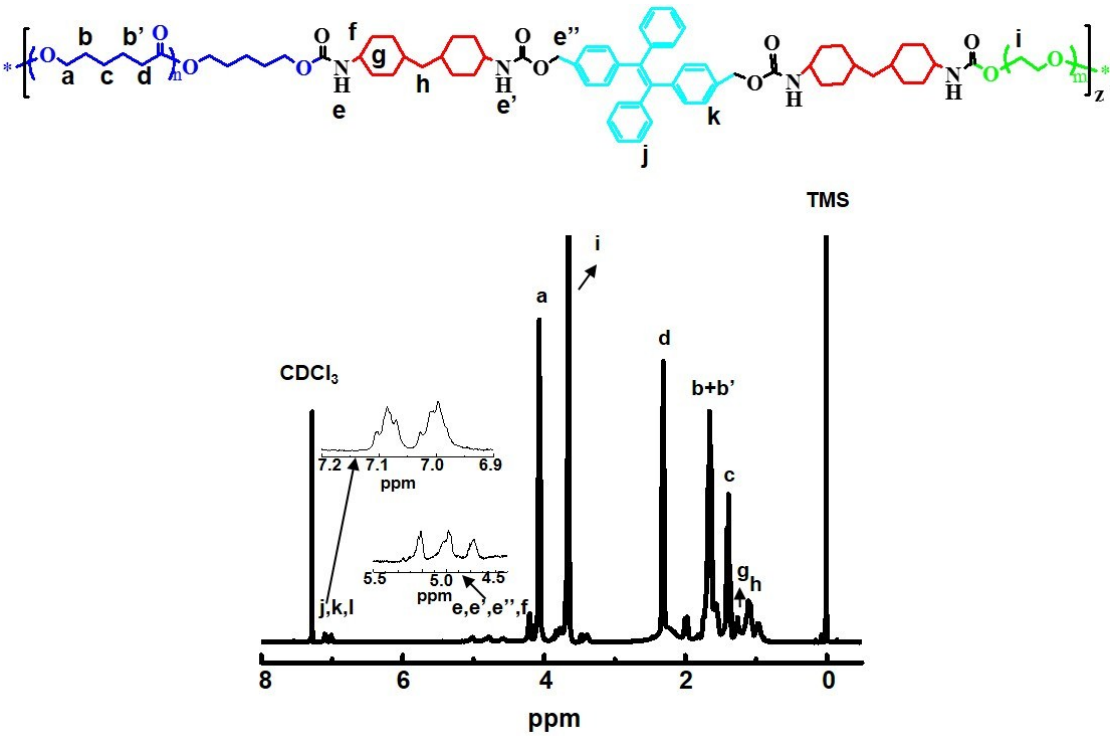
358 **Fig. S2.** Synthesis of linear block fluorescent polyurethane copolymers

359 based on PCL-diol, PEG, and TPE-2OH.



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361 **Fig. S3.** ^{1}H NMR spectrum of TPE-PU in $CDCl_3$.

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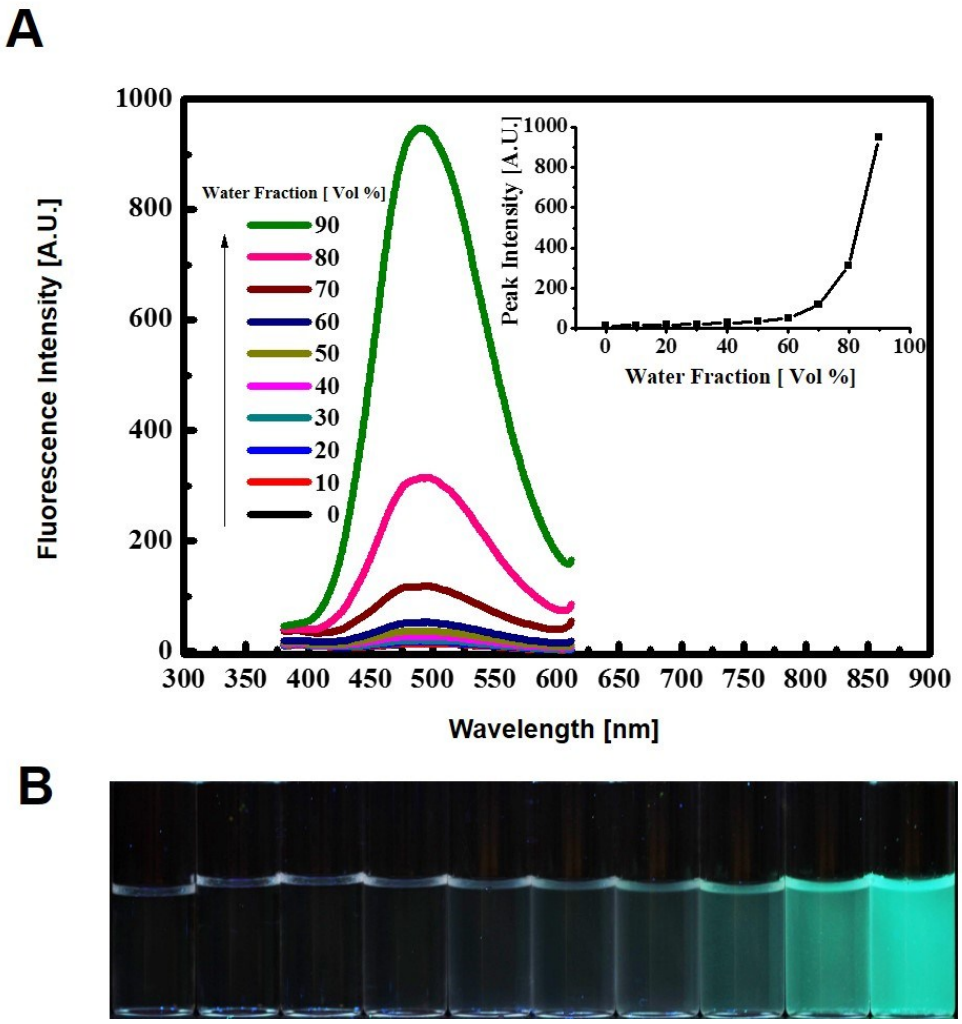


Fig. S4. (A) PL spectra of TPE-PU-1000 in THF/water mixtures with different fractions of water (f_w), λ=360 nm. (Inset) Plot of the relative PL intensity (I/I_0) of TPE-PU at 505 nm vs the composition of the THF/water mixture (f_w). I_0 = PL intensity of TPE-PU-1000 in THF solution. (B) Fluorescent photographs of the TPE-PU-1000 THF/water mixtures with different fractions of water (f_w) taken under UV illumination.

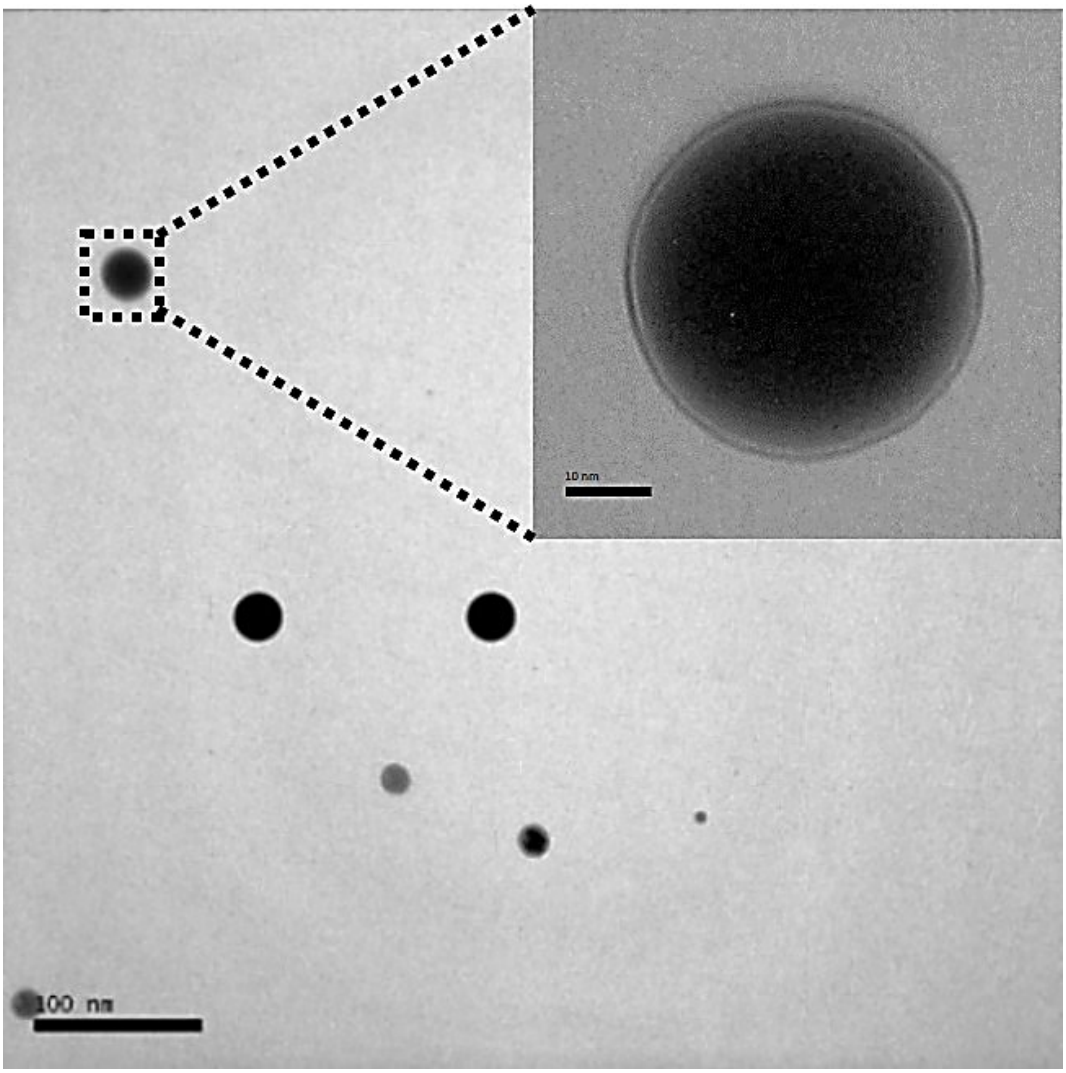


Fig. S5. TEM images of TPE-PU-1000 nanoparticles. Bar scale, 100 nm.

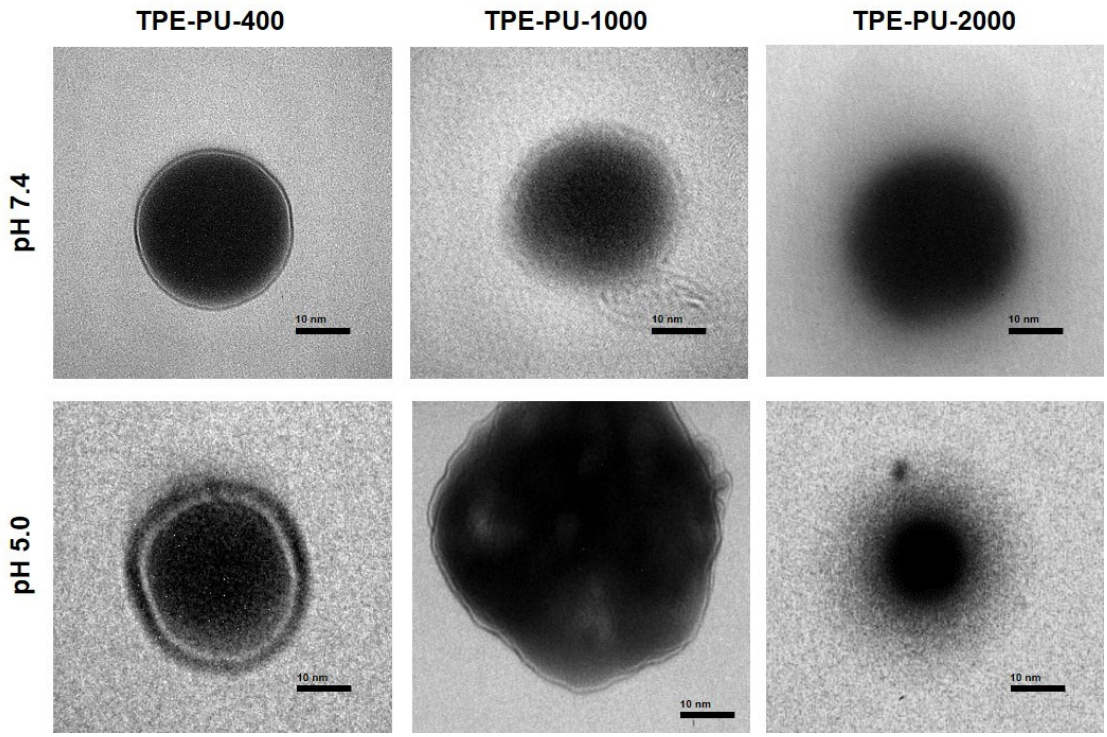


Fig. S6. TEM images of various TPE-PU nanoparticles were immersed in PBS pH 7.4 and 5.0 for 1 h, respectively. Bar scale, 10 nm.

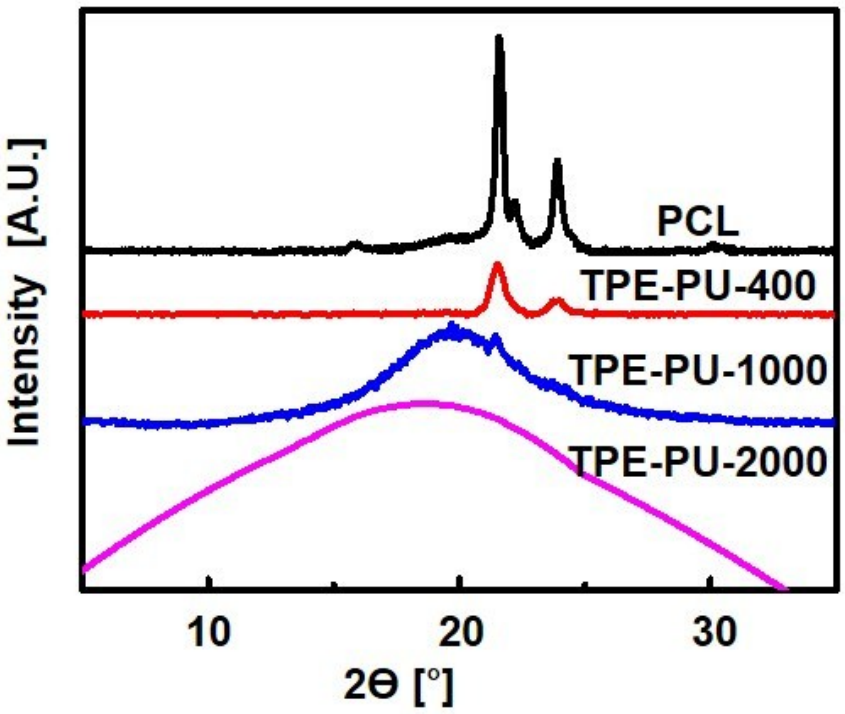


Fig. S7. XRD patterns of PCL, TPE-PU-400, TPE-PU-1000 and TPE-

PU-2000 films.

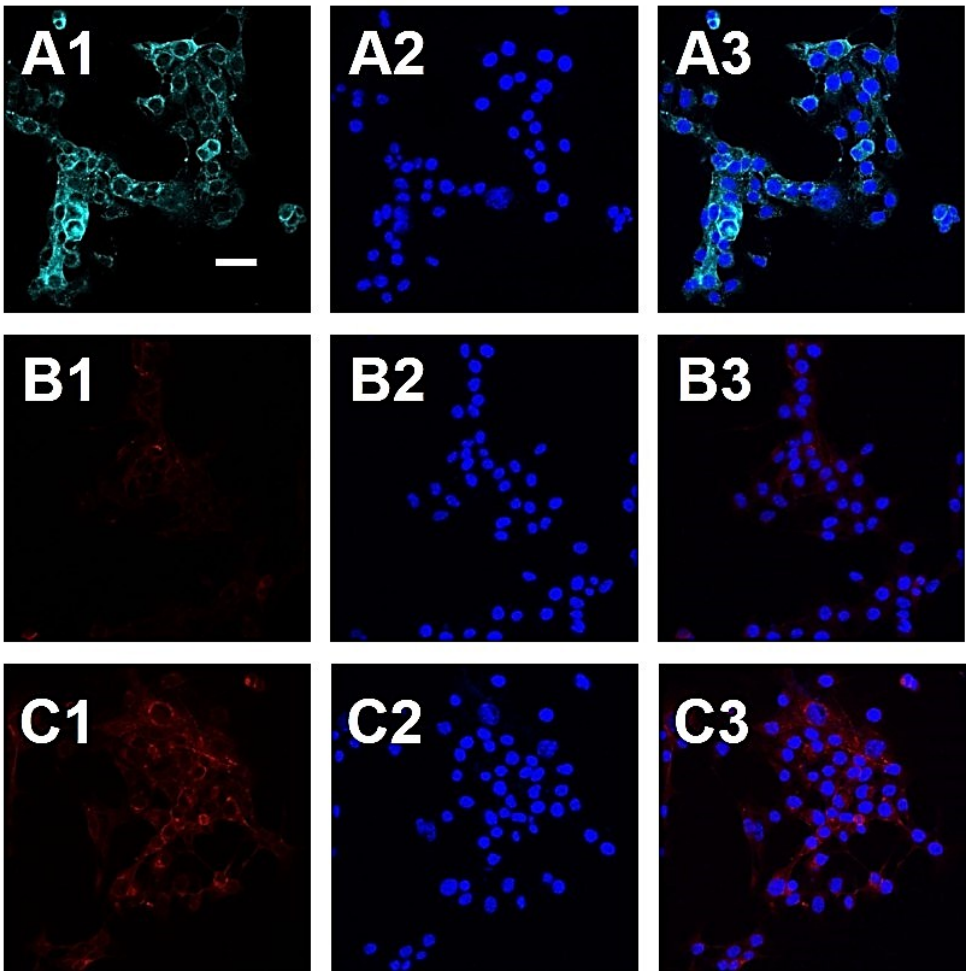


Fig. S8. Confocal laser scanning microscopy observation the subcellular microfilaments imaging in rat glial cells. (A1) confocal images of the rat glial cells after incubation with 1×10^{-3} g/mL TPE-PU-1000 in culture medium for 45 min at 37 ℃ (λ=364 nm, 1 m W laser power). (B1) confocal images of the rat glial cells after incubation with Rhodamine Phalloidin in culture medium (0.1 μM) for 45 min at 37 ℃ (λ=543 nm, 1 m W laser power). (C1) confocal images of the fixed and permeabilized rat glial cells stained by Rhodamine Phalloidin (λ=543 nm, 1 m W laser power). (A2) - (C2) DAPI, (A3) - (C3) Merged images. Bar scale, 20 μm

(applicable to all images).

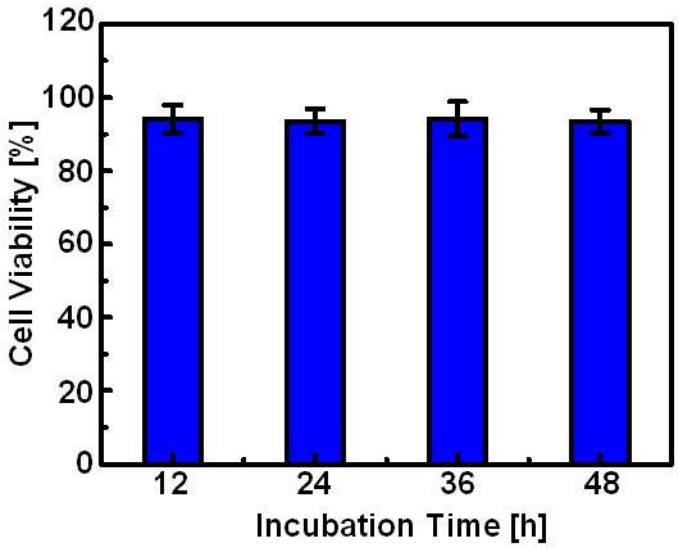
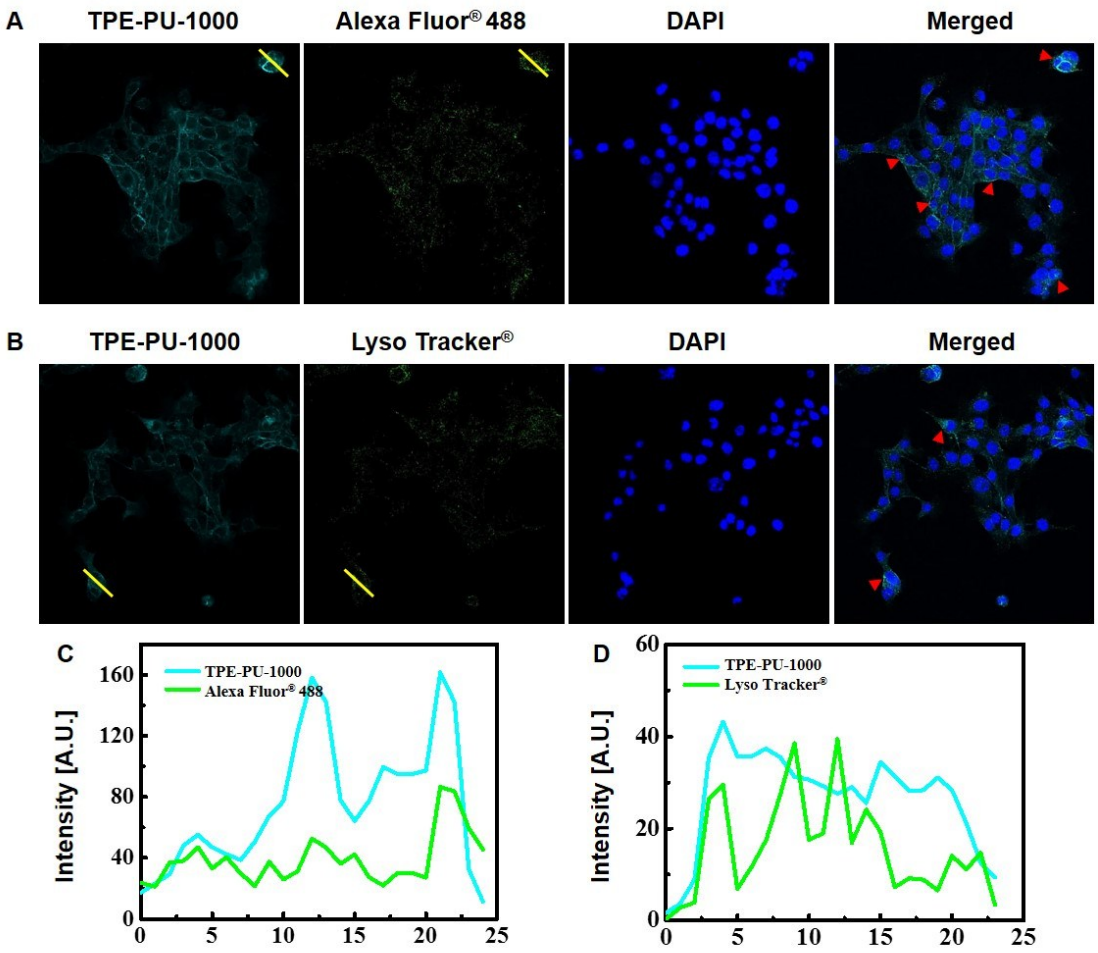


Fig. S9. Cell viability of rat glial cells incubation with 1×10^{-3} g/mL TPE-PU-1000 for 12, 24, 36, and 48 h at 37℃.



410

411 **Fig. S10. Nanoparticles accumulation in early endosomes and late**
412 **endo-lysosomes.** (A), fluorescence images of rat glial cells expressing
413 Rab5-GFP (Alexa Fluor® 488) incubated with 1×10^{-3} g/mL TPE-PU-
414 1000 nanoparticles for 1 h. The excitation/emission of TPE-PU-1000,
415 Alexa Fluor® 488, and DAPI lines was 364/470-505 nm, 488/510-530
416 nm, and 341/450-460 nm, respectively. Red triangle shows spatial
417 overlap between TPE-PU-1000 nanoparticles and early endosomes. (B),
418 fluorescence images of TPE-PU-1000 nanoparticles and Lyso tracker®
419 (green) in rat glial cells after cell incubation with 1×10^{-3} g/mL TPE-PU-
420 1000 nanoparticles for 45 min. The excitation/emission of TPE-PU-1000,

Lyso tracker®, and DAPI lines was 364/470-505 nm, 488/510-520 nm, and 341/450-460 nm, respectively. Bar scale 20 μm (applicable to all images). (C), superposition of TPE-PU-1000 nanoparticles and Alexa Fluor® 488 intensity profiles along the line scan is shown in Fig.S10A, shows TPE-PU-1000 nanoparticles accumulation in the early endosomes. (D), superposition of TPE-PU-1000 nanoparticles and Lyso tracker® intensity profiles along the line scan is shown in Fig.S10B, shows a small number of TPE-PU-1000 nanoparticles accumulation in the late endo-/lysosomes.

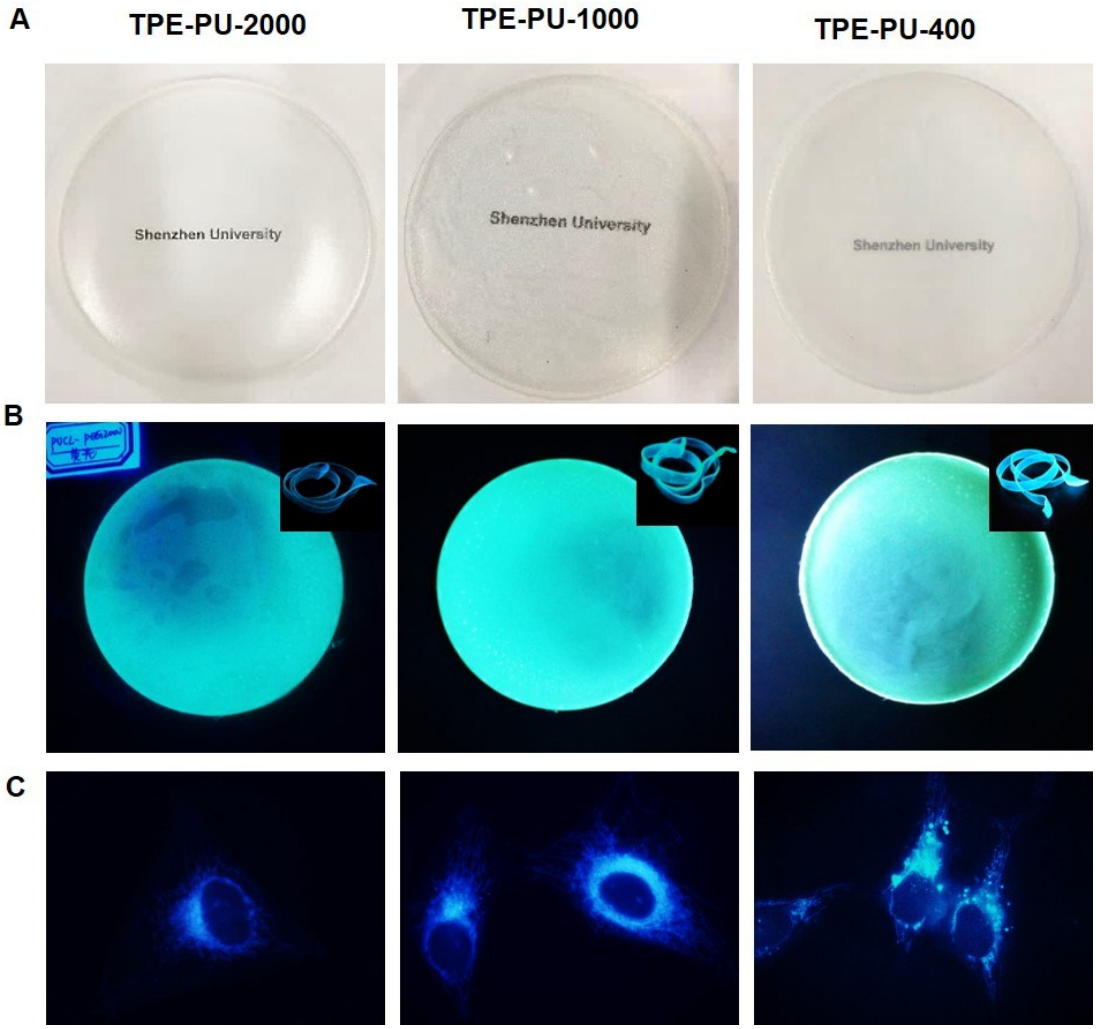
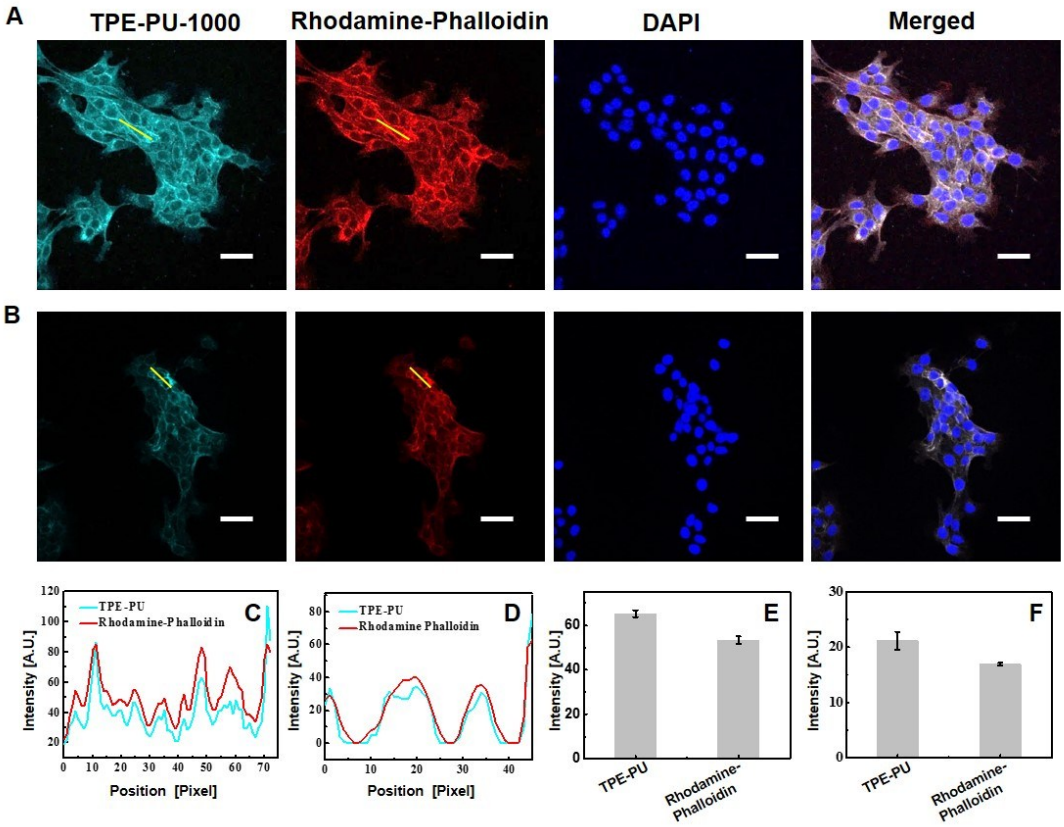


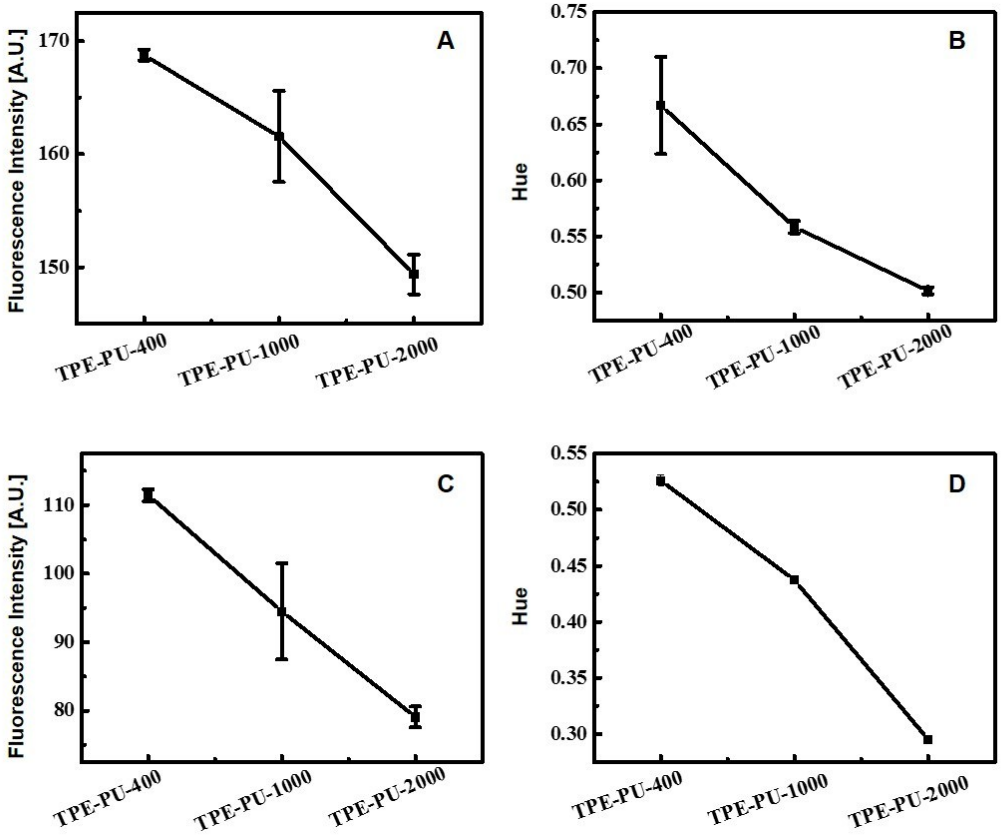
Fig. S11. Fluorescent characterizations of TPE-PUs. (A) and (B), the newly supplementary photographs of various TPE-PU films taken under room lighting and UV illumination, respectively. (C), fluorescent images of the HeLa cells cultured in the presence of various TPE-PU nanoparticles with different DLs for 45 mins at 37 ℃. [TPE-PU] =1 × 10^{-3} mg/mL, pH=7.4. Scale bar, 10 μm (applicable to all images).



453

454 **Fig. S12. The sub-cellular microfilaments colocalization of TPE-PU**
455 **and Rhodamine Phalloidin in rat glial cells.** The Excitation/Emission
456 of TPE-PU (sample: TPE-PU-1000), Rhodamine Phalloidin, and DAPI
457 lines was 364/470-505 nm, 543/565-620 nm, and 341/450-460 nm,
458 respectively. Bar scale 20 μm (applicable to all images). (A), the original
459 Fig.3. (B), the newly supplementary images of sub-cellular
460 microfilaments colocalization of TPE-PU and Rhodamine Phalloidin in
461 rat glial cells. (C), the superposition of TPE-PU-1000 nanoparticles and
462 Rhodamine Phalloidin intensity profiles along the line scan shown in
463 Fig.S12A. (D), the averaged fluorescence intensity of TPE-PU-1000
464 nanoparticles and Rhodamine Phalloidin in microfilaments for the single
465 measurement shown in Fig.S12A. (E), superposition of TPE-PU-1000

nanoparticles and Rhodamine Phalloidin intensity profiles along the line scan shown in Fig.S12B. (F), the averaged fluorescence intensity of TPE-PU-1000 nanoparticles and Rhodamine Phalloidin in microfilaments for the single measurement shown in Fig.S12B. Error bars are standard deviation. n=3.



487

488 **Fig. S13. The quantitative fluorescence analysis of the TPE-PUs.** (A)
489 and (B), the averaged fluorescence intensity and hue fluorescence data of
490 this series of TPE-PUs films from original Fig.2B. (C) and (D), the
491 averaged fluorescence intensity and hue fluorescence data of this series of
492 TPE-PUs nanoparticles from original Fig.2C. Error bars are standard
493 deviation. n=3.

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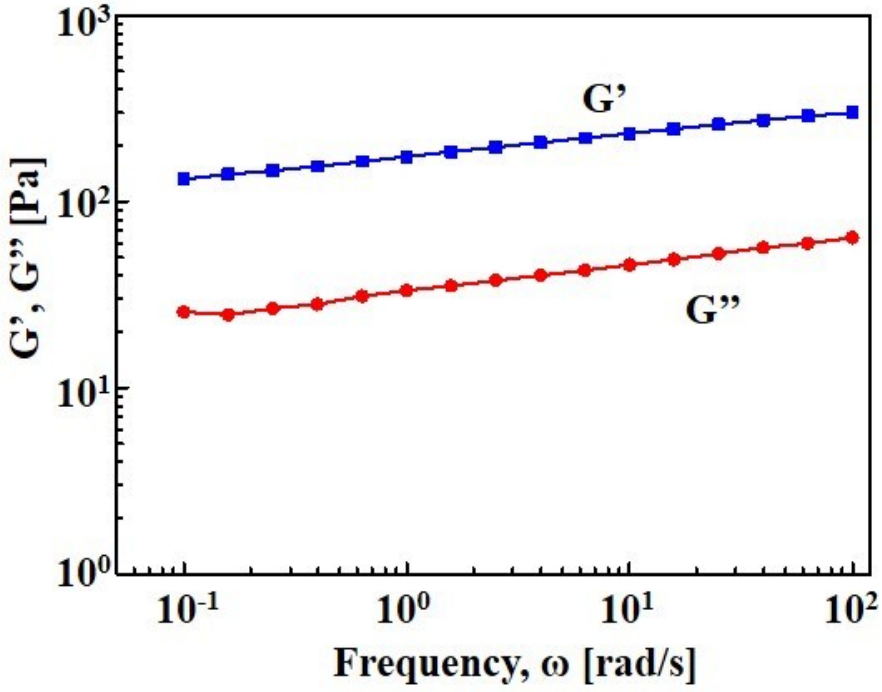

Fig. S14. Linear storage and loss modulus as a function of frequency for TPE-PU-1000 (1×10^{-3} g/mL) nanoparticles at T=25 ℃.

TableS1. Composition and molecular distribution information of block polyurethane copolymers based on PCL, PEG and TPE.

Sample	R[a]	W[b]/%	W[c]/%	M[d]	PDI[e]
TPE-PUE-400	11:5:5:1	10.76	2.108	56300	1.39
TPE-PUE-1000	11:5:5:1	23.45	1.838	72400	1.61
TPE-PUE-2000	11:5:5:1	37.99	1.489	59600	1.46

[a] **R:** HMDI/PCL-diol/PEG/TPE molar ratio in feed.

[b] **W:** The mass percentage of PEG content in feed.

[c] **W:** The mass percentage of TPE content in feed.

[d] **M:** Weight average molecular weight, determined by GPC using THF as mobile phase.

[e] **PDI:** M_w/M_n, determined by GPC using THF as mobile phase.