Supporting Information

# *In situ* formation of tetraphenylethylene nano-structures on microgels inside living cells *via* reduction-responsive self-assembly

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## 1. Materials

Tetraphenylethylene carboxaldehyde (TPE-CHO) was purchased from AIEgen Biotech Co., Ltd (Shenzhen, China). Cystamine dihydrochloride (Cys) and di-*tert*-butyl dicarbonate (($^{t}$Boc)$_2$O) were purchased from Beijing Innochem (Beijing, China). 1,6-Diaminohexane dihydrochloride (Dah), acryloyl chloride, dithiothreitol (DTT), arginine and glutathione reduced form (GSH) were purchased from TCI (Shanghai, China). Poly(ethylene glycol) methacrylate (PEGMA, average Mn 360 Da), fluorescein isothiocyanate (FITC) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich (St. Luis, MO, USA). 2,2-Azobis(2-methylpropionitrile) (AIBN) was purchased from Adamas-beta (Shanghai, China). Tat peptides (H-Tyr-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg-OH) were synthesized by GL Biochem (Shanghai, China). GA solution (25%), triethylamine ($Et_3N$), trifluoroacetic acid (TFA), calcium nitrate tetrahydrate ($Ca(NO_3)_2 4H_2O$), sodium carbonate ($Na_2CO_3$), ethylenediamine tetraacetic acid (EDTA), monopotassium phosphate ($KH_2PO_4$), sodium sulfate ($Na_2SO_4$), sodium hydroxide (NaOH), sodium chloride (NaCl), potassium chloride (KCl), disodium phosphate ($Na_2HPO_4$), anisole, methanol, diethyl ether, ethyl acetate, dichloromethane ($CH_2Cl_2$), trichloromethane ($CHCl_3$), and other organic solvents were purchased from Sinopharm (Beijing, China). 5-Chloromethylfluorescein diacetate (CMFDA) was purchased from Invitrogen (Carlsbad, USA). AIBN was purified by recrystallization in ethanol. Other chemicals were used as received. The water used in all experiments was prepared via a Millipore Milli-Q purification system.

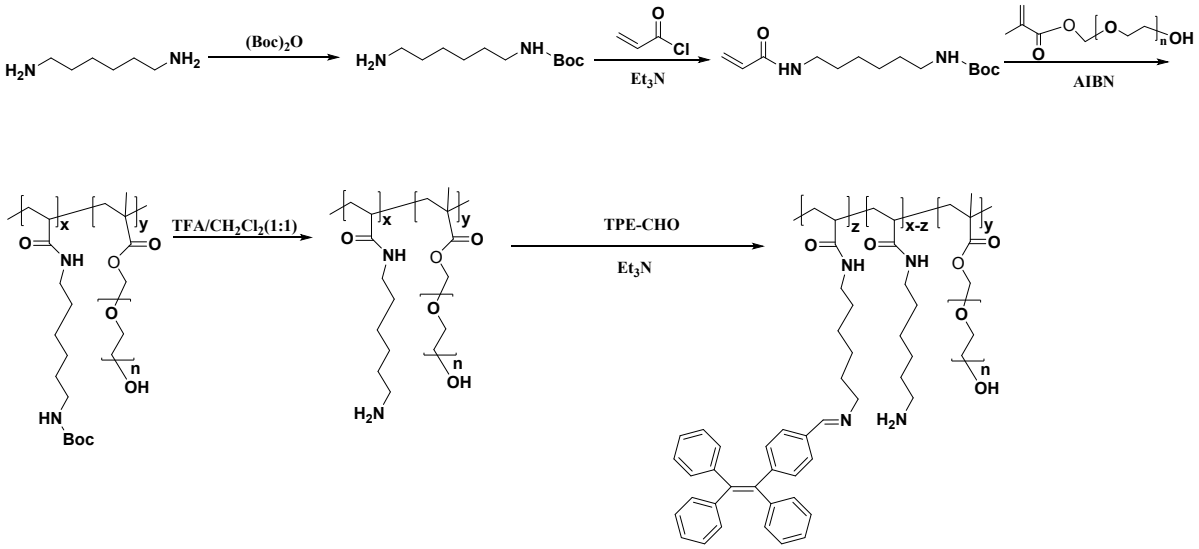
## 2. Characterizations methods of materials

**$^1$H nuclear magnetic resonance ($^1$HNMR) spectroscopy:** The samples were dissolved in corresponding deuterated solvents at a concentration of 10 mg/mL. The $^1$H NMR spectra were obtained on a Bruker DMX500 instrument. **Mass spectra (MS):** The samples were dissolved in chloroform at a concentration of 1 mg/mL. The MS were recorded with a Bruker Esquire 3000plus ion trap mass spectrometer (Brucker-Franzen Analytik GmbH, Bremen, Germany). Nitrogen was used as a nebulizing gas at a pressure of 10 psi and a drying gas at a flow rate of 5 L/min. The drying gas temperature was set at 250 °C and the capillary voltage was set at 4000 V. The solution was infused to the mass spectrometer with a syringe pump at a flow rate of 6 μL/min. **Gel permeation chromatography (GPC):** The samples were dissolved in tetrahydrofuran (THF) at a mass concentration of 5‰, and then the solutions were filtered with 0.22 μm hydrophobic membranes. Molecular weight and its distribution were determined with a Waters1515 instrument at 30 $^o$C by using THF as the eluent (elution time, 25 min). The results were calibrated with polystyrene (PS) as the standard. **Transmission electron microscopy (TEM):** Samples were prepared by placing a drop of the sample suspension onto a carbon-film-coated copper grid and then dried at 37 $^o$C. TEM images were recorded on a Hitachi HT7700 TEM instrument at an acceleration voltage of 120 kV. **Scanning electron microscopy (SEM)**: A drop of the sample suspension was placed onto a clean glass, and dried naturally. The samples were sputtered with gold, and were observed with a Hitachi SU8010 SEM at an operation voltage of 3 kV. **Confocal laser scanning microscopy (CLSM):** The particle suspensions were placed onto clean glass slides, and were observed with a Zeiss LSM 780 system (63× oil immersion using commercial software). The

**ultraviolet-visible (UV–Vis)** absorption spectra were recorded with a Shimadzu UV2550 spectrophotometer. **Fluorescence emission spectra** were recorded with a Shimadzu RF-5301PC instrument (excitation wavelength: 300 nm). For UV-Vis, fluorescence and zeta potential/size measurements, the sample solutions were used directly.

## 3. Supplementary experimental methods

### 3.1 Synthesis of ADEG-g-TPE




**Scheme S1** Synthesis route and molecular structure of the tetraphenylethylene-containing amphiphilic copolymer ADEG-g-TPE.

The control amphiphilic polymers (ADEG-g-TPE) without reduction-responsiveness were synthesized with similarly as shown in Scheme S1 by using the following procedures.

**Synthesis of N-*tert*-butoxycarbonyl-N'-acryloyl-diaminohexane (Ac-Dah-$^t$Boc)**

1,6-Diaminohexane dihydrochloride (8.40 g, 44..40mmol) was added into anhydrous methanol (MeOH) (100 mL) in a 500 mL round-bottomed flask. After the mixture was cooled down with an ice bath, trimethylamine (13.48 g, 13.22 mmol) was added. The mixture was magnetically stirred until the solution turned transparent. Di-*tert*-butyl dicarbonate (4.86 g,

44.45 mmol) was dissolved in MeOH (100 mL), and was cooled down under -20 $^{o}$C for 15 min. Then the di-*tert*-butyl dicarbonate solution was added dropwise into the above 1,6-diaminohexane dihydrochloride solution over 4 h in an ice bath. After being maintained in the ice bath overnight, the solvent was completely removed under reduced pressure. 150 mL $CHCl_3$ was added to dissolve the organize products. After the insoluble solids were filtered, the acquired reaction product was reacted with 5% (w/v) $Na_2CO_3$ aqueous solution (2 × 100 mL). The remained organic phase was concentrated under vacuum before 1 M $KH_2PO_4$ aqueous solution (300 mL, pH 4.2) was added. After removal of the insoluble solids, the aqueous phase was extracted with diethyl ether (3 × 200 mL) to remove N ,N'-di-*tert*-butyloxycarbonyl diaminohexane. The pH of water phase was adjusted to 9 with 1 M NaOH, and then was extracted with ethyl acetate (7 × 200 mL). The collected organic phase was dried over $Na_2SO_4$ and filtered. After the solvent was removed, Dah-$^{t}$Boc was yielded as slight yellow oil (2.00 g, 24.0%).

Dah-$^{t}$Boc (2.00 g, 9.24 mmol) and triethylamine (1.96 g, 19.40 mmol) were dissolved in 80 mL chloroform. After cooled down under -20 $^{o}$C, it was dropwise added to a magnetically stirred solution of acryloyl chloride (1.66 g, 62.80 mmol) in chloroform (80 mL) placed in an ice bath over 2.5 h. After being maintained for 24 h, the reaction solution was evaporated under vacuum to remove the solvent. The residue was washed with water (240 mL), and then extracted with chloroform (3 × 100 mL). The combined organic phase was dried over $Na_2SO_4$, and was then filtered. After removal of the solvent, Ac-DAH-$^{t}$Boc was yielded as a light yellow solid (3.00 g, 81.6%). $^{1}$H NMR (500 MHz, $CDCl_3$): δ= 1.27 (s, 4H, CONH-$(CH_2)_2$-C**H**$_2$-C**H**$_2$-), 1.38 (s, 11H, Boc C**H**$_3$, $CH_2CHCONHCH_2$-C**H**$_2$-), 1.47 (t, 2H, $OCONHCH_2$-

C**H$_2$-**), 3.02 (s, 2H, CONH-C**H$_2$-**), 3.24 (t, 2H, -C**H$_2$**-NHCOO), 4.52 (1H, amide proton), 5.55, 6.07 (m, 2H, vinyl proton), 6.20 (m, 1H, vinyl proton).

**Synthesis of poly[(N-*tert*-butoxycarbonyl-N'-acryloyl-diaminohexane)-co-[poly(ethylene glycol)]] (ADBEG)**

ADBEG was synthesized by free radical polymerization of Ac-Dah-$^t$Boc and poly(ethylene glycol) methacrylate (PEGMA, average Mn 360 Da). Ac-Dah-$^t$Boc (152.00 mg, 0.562 mmol), PEGMA (67.50 mg, 0.188 mmol) and AIBN (43.72 mg, 0.272 mmol) were dissolved in 12 mL anisole in a 25 mL Schlenk tube. The mixture was degassed by a cyclic freeze-pump-thaw method for three times. Then the Schlenk tube was backfilled with nitrogen and sealed. The reaction system was heated at 65 $^o$C for 12 h under magnetic stirring. The product was purified by precipitation in cold ether for three times and dried in vacuum for 24 h to yield ADBEG as a transparent viscous solid (76.42 mg, 35%). $^1$H NMR spectrum (500 MHz, $CDCl_3$, Figure S6) of ADBEG shows the characteristic peaks of [Ac-Dah-$^t$Boc] (Boc C**H$_3$**, δ= 1.44; CONH-$(CH_2)_2$-C**H$_2$**, δ= 1.32; C**H$_2$**-N, δ= 3.08) and PEGMA (-COO-$CH_2$, δ= 4.12; -O-$CH_2$, δ= 3.66 ), and the proton peaks of $CH_2$=CH in Ac-Dah-$^t$Boc monomers disappeared, indicating successful copolymerization of Ac-Dah-$^t$Boc and PEGMA. The copolymerization ratio of [Ac-Dah-$^t$Boc] to [PEGMA] in ADBEG was determined to be 2:1 (mol/mol) based on integrals of Boc C**H$_3$** peak and -COO-C**H$_2$** peak. The average molecular weight and molecular weight distribution of ADBEG were determined with GPC by using THF as the eluent, revealing an $M_n$ of 13.3 kDa and $M_w/M_n$ of 1.24 (Figure S7).

**Synthesis of Poly [(N-acryloyl-diaminohexane)-co-[poly (ethylene glycol)]] (ADEG)**

To the solution of ADBEG (76.42 mg) in $CH_2Cl_2$ (4 mL) placed in an ice bath, trifluoroacetic acid (2 mL) was added in dropwise. The reaction was maintained under magnetic stirring for 4 h. Then the solvent was evaporated under vacuum. The ADEG free of $^t$Boc groups was yielded as a transparent viscous solid.

**Synthesis of the amphiphilic ADEG-g-TPE copolymers**

TPE-CHO was conjugated to ADEG to obtain ADEG-g-TPE via a one-step procedure. First, ADEG (containing 0.260 mmol $–NH_2$), TPE-CHO (14.70 mg, 0.041 mmol) and triethylamine (100 mg, 0.990 mmol) were dissolved in anhydrous methanol (MeOH) (10 mL) in a 50 mL round-bottomed flask placed in a 37 $^o$C water bath. The reaction was maintained under magnetic stirring for 24 h. The product was purified by precipitation in cold ether for three times to yield the ADEG-g-TPE as a yellow solid. $^1$H NMR spectrum (500 MHz, $CDCl_3$, Figure S8) of ADEG-g-TPE shows characteristic peaks of TPE and ADEG, indicating successful conjugation of TPE-CHO onto ADEG. The structural unit ratio ([Ac-Dah-TPE] : [Ac-Dah] : [PEGMA]) in the ADEG-g-TPE was calculated to be 1 : 17.8 : 9.4. Thus the loading content of TPE was calculated to be 4.8 wt%, similar to that in the CSEG-g-TPE MGs (4.9 wt%).

## 4. Supplementary results

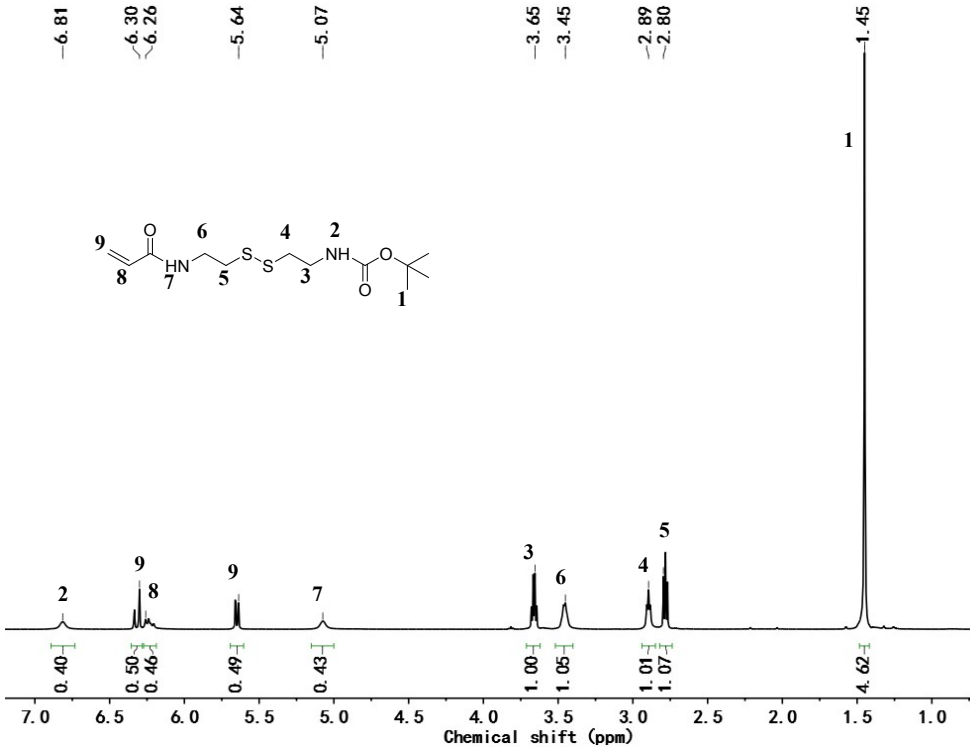



**Figure S1** $^{1}$H NMR spectrum of Ac-Cys-$^{t}$Boc ($CDCl_3$).

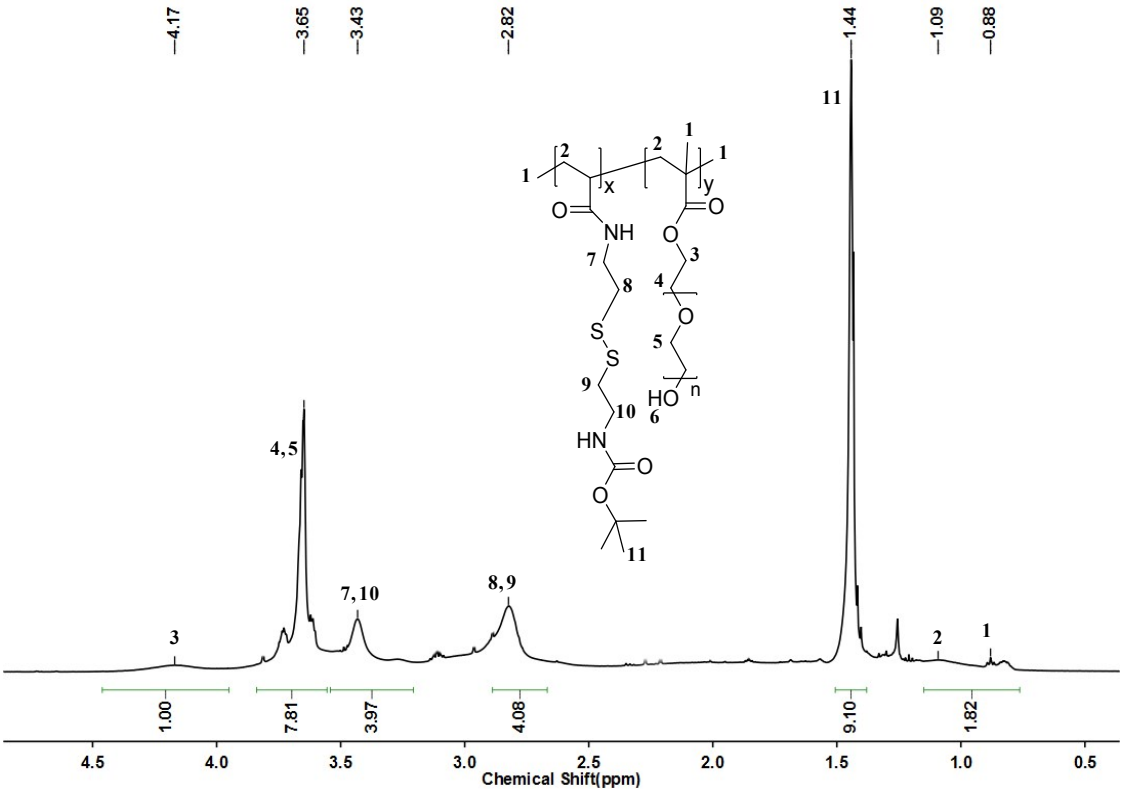
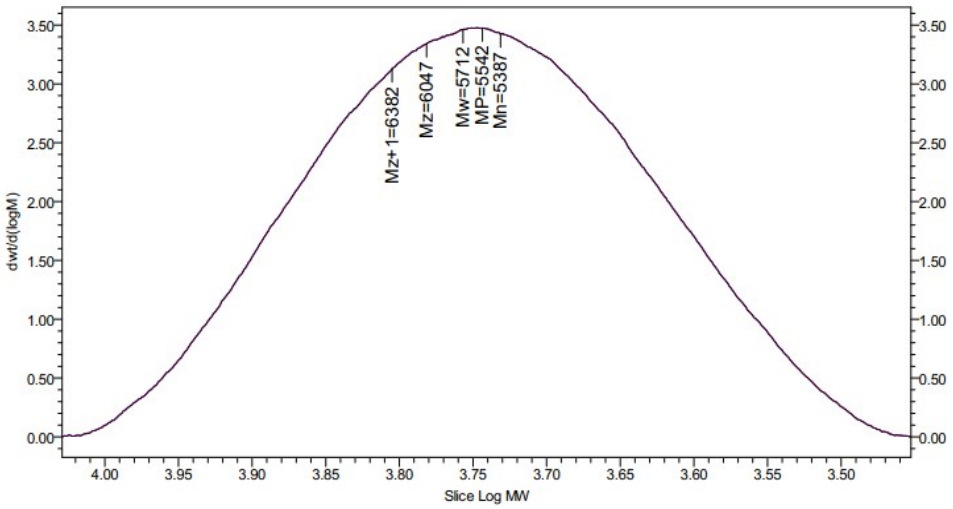



**Figure S2** $^{1}$H NMR spectrum of CSBEG ($CDCl_3$) copolymers.




| Mn | Mw | Mp | Mz | Mz+1 | PDI | Mz/Mw | Mz+1/Mw |
|---|---|---|---|---|---|---|---|
| 5387 | 5712 | 5542 | 6047 | 6382 | 1.060319 | 1.058724 | 1.117343 |

**Figure S3** GPC characterization of CSBEG. THF as the eluent, and PS as the standard.

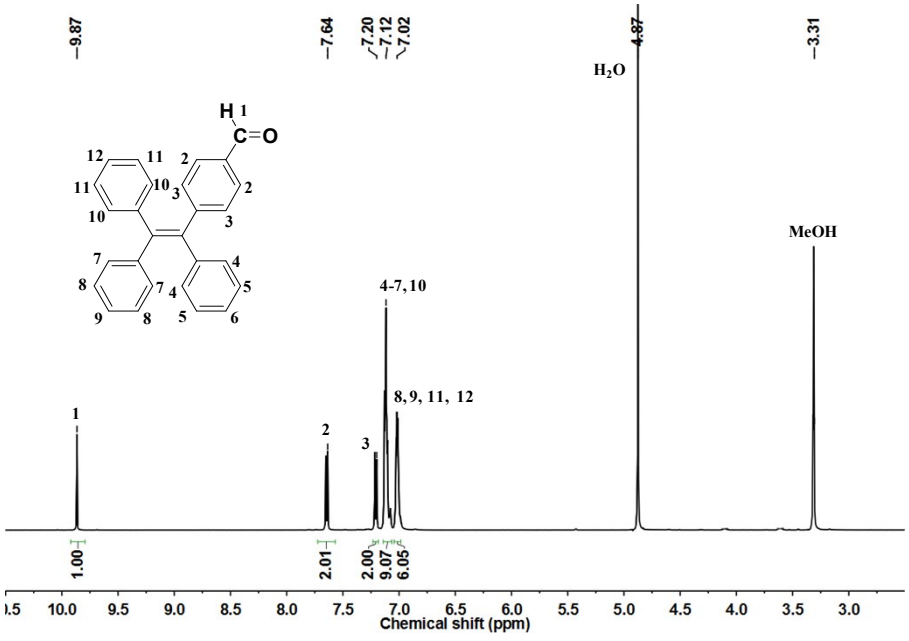



**Figure S4** $^{1}H$ NMR spectrum of TPE-CHO ($CD_3OD$).

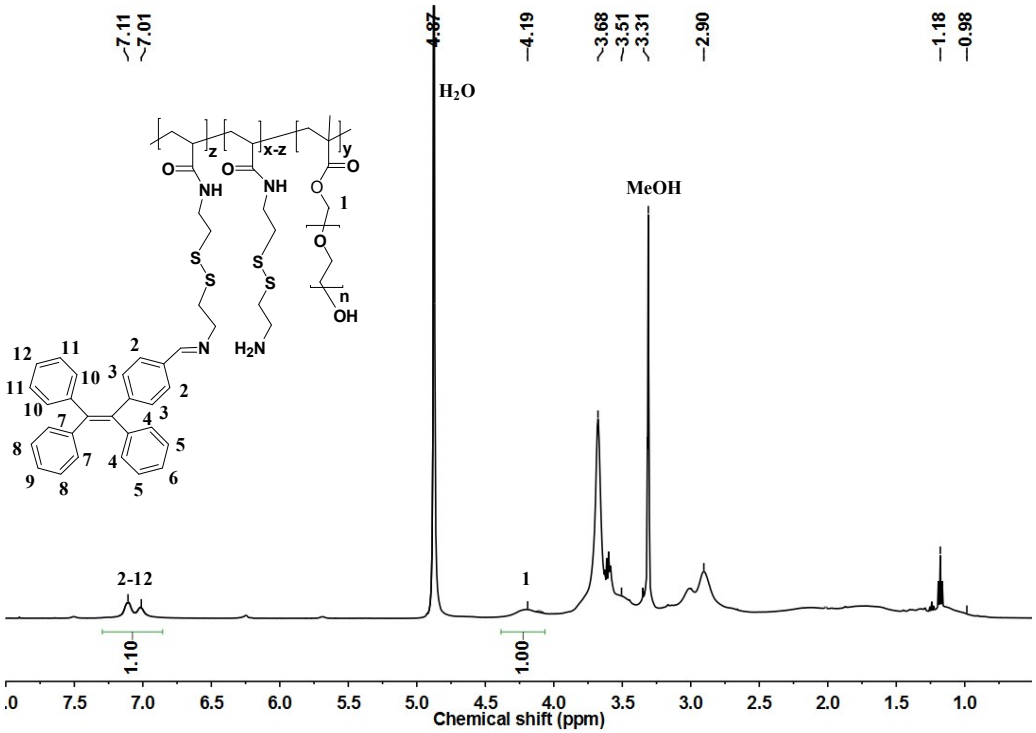



**Figure S5** $^{1}$H NMR spectrum of CSEG-g-TPE ($CD_3OD$).

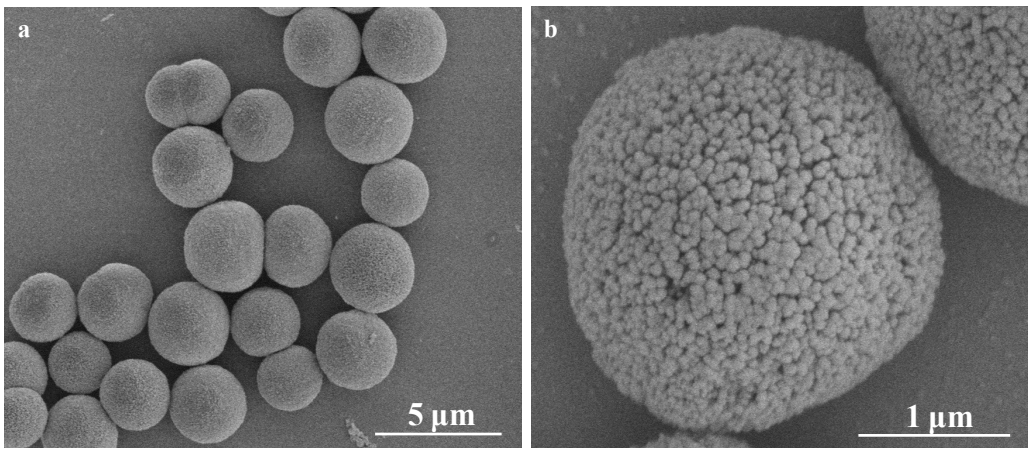



**Figure S6** (a, b) SEM images of $CaCO_3$ template microparticles.

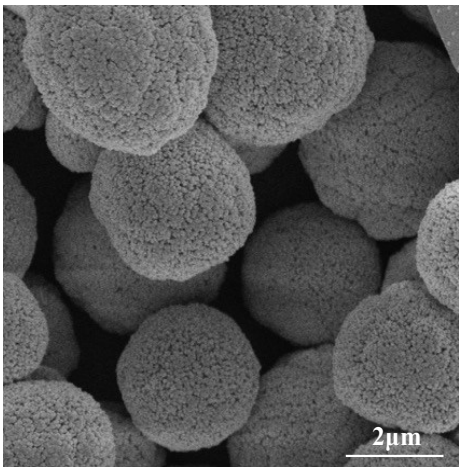



**Figure S7** SEM image of (CSEG-g-TPE)-loaded $CaCO_3$ microparticles.

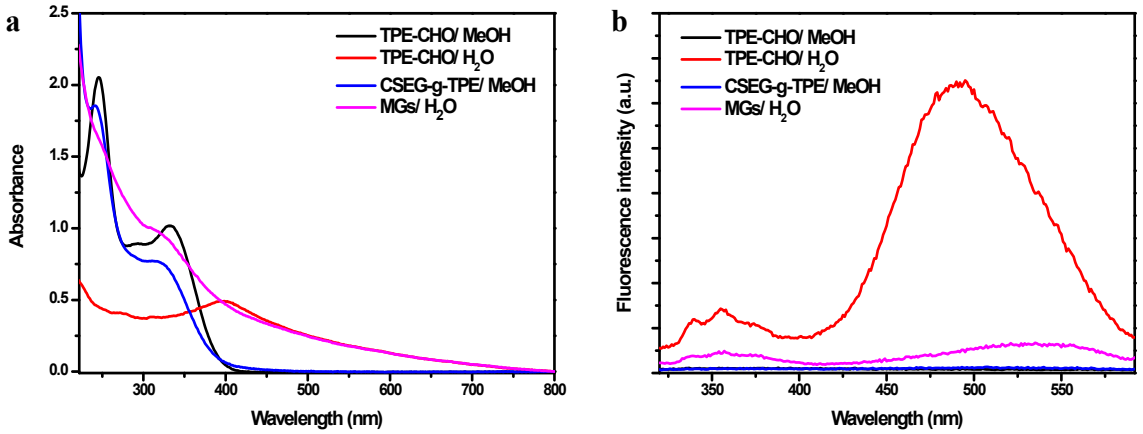



**Figure S8** (a) UV-Vis absorbance spectra and (b) fluorescence emission spectra (excitation wavelength, 300 nm) of CSEG-g-TPE MGs (0.5 mg $mL^{-1}$) and TPE-CHO aggregates (0.0215 mg $mL^{-1}$) in water; and CSEG-g-TPE (0.5 mg $mL^{-1}$) and TPE-CHO (0.0215 mg $mL^{-1}$) in methanol.

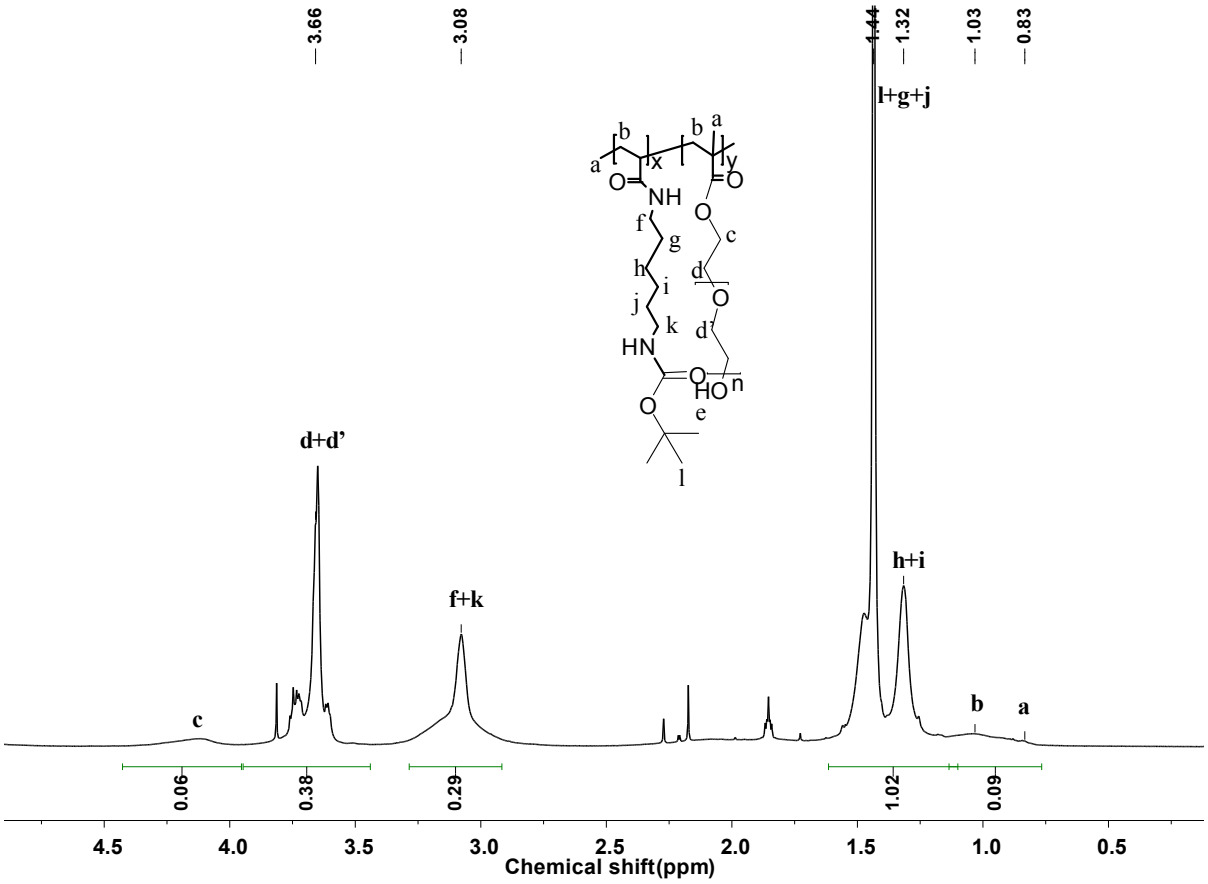
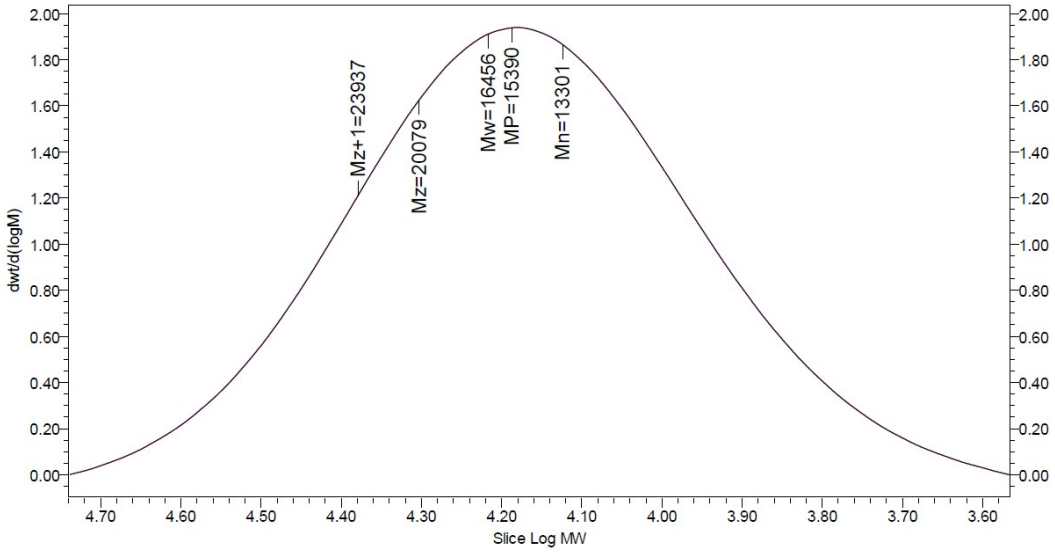



**Figure S9** $^{1}$H NMR spectrum of ADBEG ($CDCl_3$) copolymers.




| Mn | Mw | Mp | Mz | Mz+1 | PDI | Mz/Mw | Mz+1/Mw |
|---|---|---|---|---|---|---|---|
| 13301 | 16456 | 15390 | 20079 | 23937 | 1.237149 | 1.220179 | 1.454659 |

**Figure S10** GPC characterization of ADBEG. THF as the eluent, and PS as the standard.

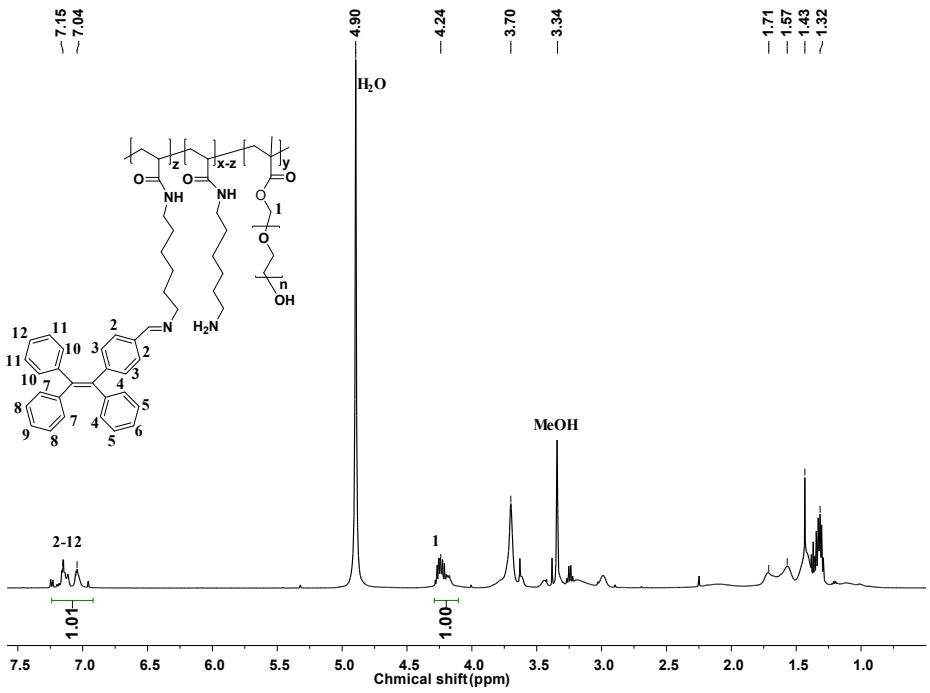



**Figure S11** $^{1}$H NMR spectrum of ADEG-g-TPE ($CD_3OD$).

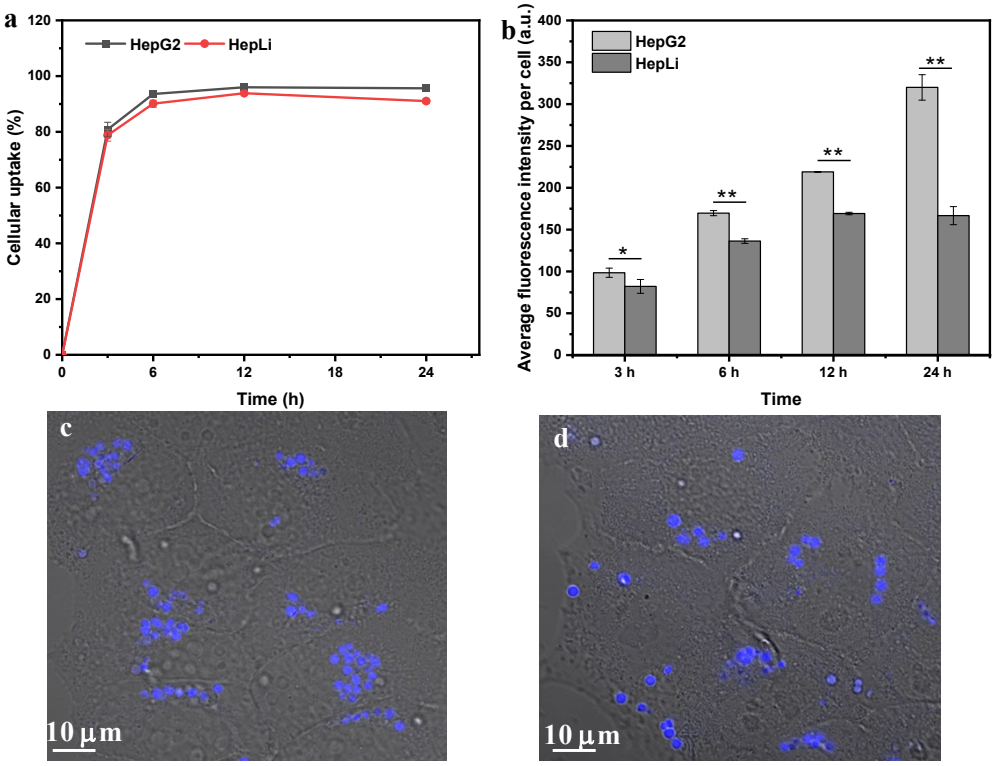



**Figure S12** Characterization of cellular uptake of ADEG-g-TPE MGs. (a, b) Flow cytometry results showing the ratio of cells that internalized MGs as a function of incubation time (a), and the cellular uptake amount of ADEG-g-TPE MGs (b) after co-incubation for designated time at a particle-to-cell ratio of 15 : 1. The MGs in flow cytometry measurements were labeled with FITC, while in all the other experiments were not labeled. (c, d) CLSM images showing the uptake performance of ADEG-g-TPE MGs after being incubated with HepG2 (c) and HepLi (d) cells at a particle-to-cell ratio of 15 : 1 for 24 h, respectively.

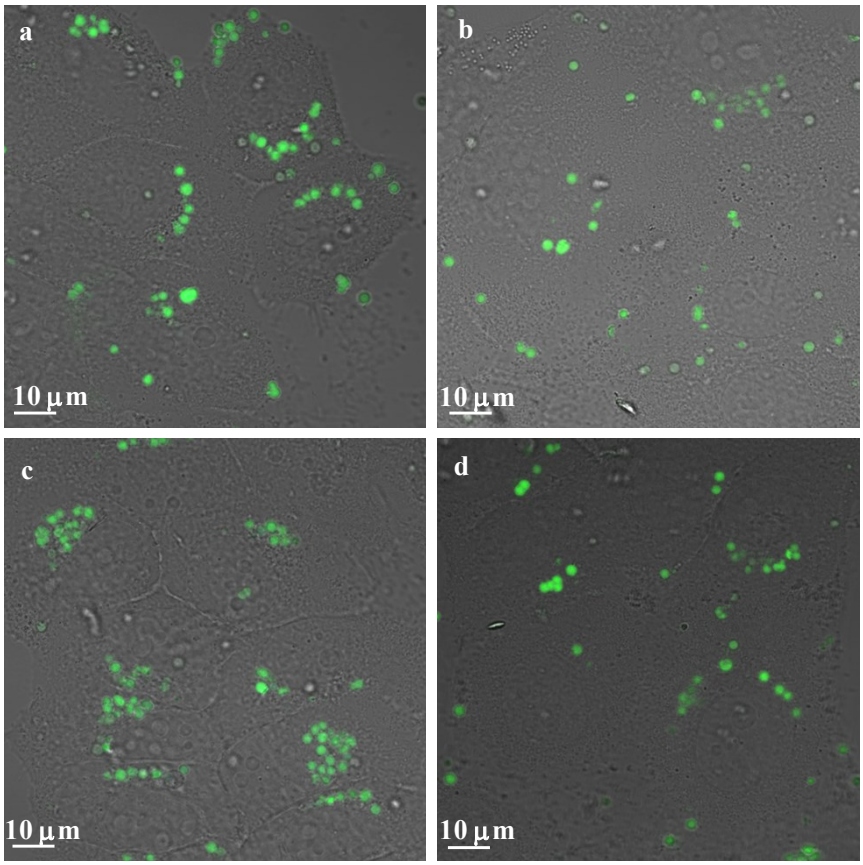



**Figure S13** (a-d) CLSM images showing the internalization of CSEG-g-TPE MGs (a, b) or ADEG-g-TPE MGs (c, d) after being incubated with HepG2 (a, c) and HepLi (b, d) cells at a particle-to-cell ratio of 15:1 for 24 h, respectively. The green fluorescence came from the autofluorescence of MGs.

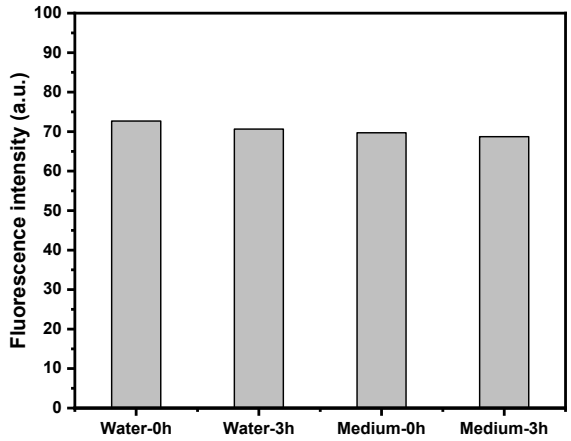



**Figure S14** Fluorescence intensity of TPE in CSEG-g-TPE MGs after being incubated in DMEM medium (containing 10% fetal bovine serum, without phenol red) and water for the indicated time at 37 °C. The excitation and emission wavelengths were 300 nm and 500 nm, respectively.

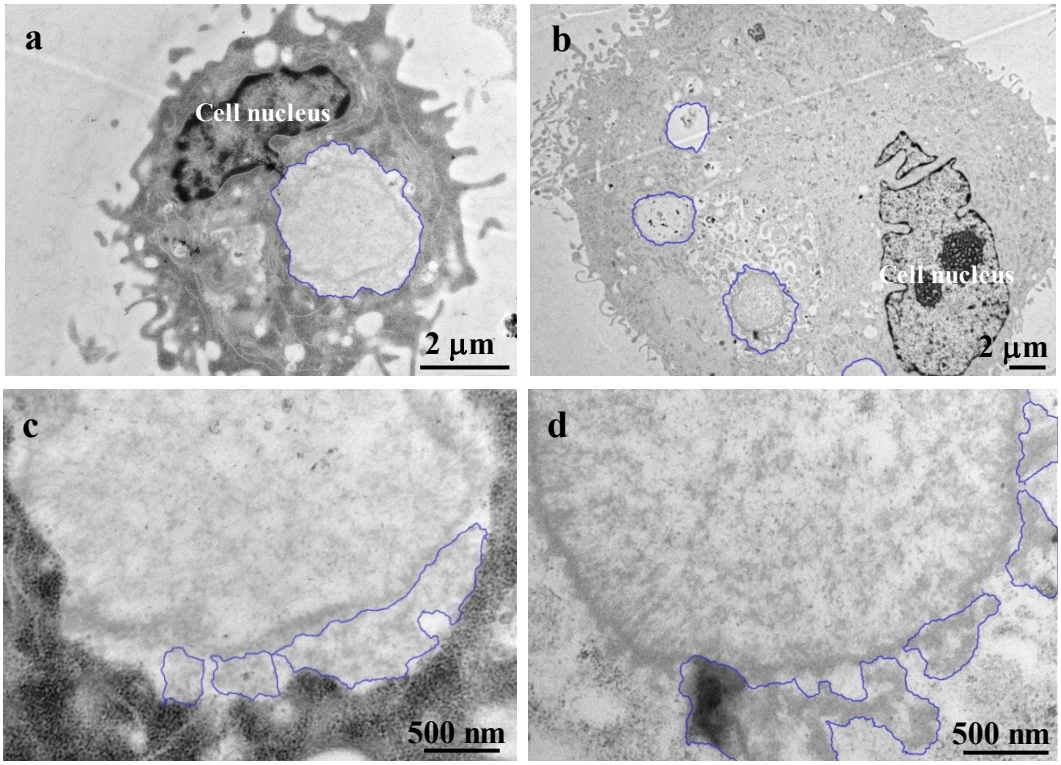



**Figure S15** (a-d) Cross-sectional (ultramicrotomy) TEM images showing the protruding of nano-spikes on the surface of CSEG-g-TPE MGs after being co-incubated with A549 (a, c) and SMC (b, d) cells for 24 h at a particle-to-cell ratio of 15: 1, respectively. The MGs (a, b) and the producing nanostructures (c, d) are outlined with the blue solid lines.