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Protruding of nano-spikes on cholesterol-containing microgels by reduction-responsive self-assembly in cell milieu and its influences on cell functions

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Materials

Cystamine dihydrochloride (Cys) and di-*tert*-butyl dicarbonate ((^{t}Boc)$_2$O) were purchased from Innochem. 1,6-Diaminohexane dihydrochloride (DAH), acryloyl chloride, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DM-TMM), dithiothreitol (DTT), succinic anhydride, 4-dimethylaminopyridine (DMAP) and glutathione reduced form (GSH) were purchased from TCI Development Co., Ltd (Shanghai, China). Poly (ethylene glycol) methacrylate (PEGMA, 360 Da), cholesterol, fluorescein isothiocyanate (FITC), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and 4,6-diamidino-2 -phenylindole dihydrochloride (DAPI) were purchased from Sigma-Aldrich. Rhodamine-conjugated phalloidin was bought from Thermo Fisher Scientific. 2,2-Azobis(2-methylpropionitrile) (AIBN) was purchased from Adamas-beta. Tat peptides (H-Tyr-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg-OH) were synthesized by GL Biochem Ltd. (Shanghai, China). Triethylamine (Et_3N), trifluoroacetic acid (TFA), monopotassium phosphate (KH_2PO_4), sodium sulfate (Na_2SO_4), sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), dichloromethane (CH_2Cl_2), trichloromethane ($CHCl_3$), methanol (MeOH), methylbenzene and other organic solvents were purchased from Sinopharm Chemical Reagent Company (Beijing, China). CH_2Cl_2 was dried by heating reflux in the presence of calcium hydride (CaH_2). Other chemicals were used as received. ELISA kit for TNF-α, IL-6 and IL-1β were bought from Boster, China. The ER-Tracker Blue-White DPX was purchased from Yesen, China. The Annexin V-FITC/PI Apoptosis Detection Kit was bought from 7 Sea Biotech, China. The water used in all experiments was prepared via a Millipore Milli-Q purification system.

Characterizations

^{1}H nuclear magnetic resonance (^{1}HNMR) spectroscopy: The dried samples were dissolved in corresponding deuterated solvent 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 data were determined with a Waters1515 instrument at 30 °C by using THF as the eluent (elution time, 25 min). The results were calibrated with polystyrene (PS) as the standard. **Scanning electron microscopy (SEM):** Samples were prepared by placing a drop of the sample suspension onto a clean glass, and then dried at 37 °C. The samples were sputtered with gold, and were observed with a Hitachi S-4800 SEM at an acceleration voltage of 3 kV. **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 °C. TEM images were recorded on a Hitachi HT7700 TEM instrument at an acceleration voltage of 120 kV. **Confocal laser scanning microscopy (CLSM):** The particle suspensions were placed onto clean glass slides, and were observed with a Leica TCS SP5 system (63× oil immersion using commercial software). **X-ray photoelectron spectroscopy (XPS):** Samples were prepared by dropping the sample suspension onto a clean silicon wafer and covering the clean silicon wafer completely. Then the samples were dried completely at 37 °C and were analyzed with an Escalab 250Xi instrument (Thermo Fisher Scientific, UK). **Polarizing microscopy:** Samples were prepared by placing a drop of the sample suspension onto a clean glass slide and were observed with a ECLIPSE E600W POL (NIKON) polarizing microscope.

Synthesis of copolymers CSEG-g-Chol and ADEG-g-Chol

1. Synthesis of cholesterol-3-hemisuccinate ester (Chol-COOH)

Cholesterol (1.5g, 3.6 mmol), succinic anhydride (0.36g, 3.6 mmol) and DMAP (10mg, 0.073 mmol) were dissolved in 20 mL of methylbenzene in a 50 mL round-bottomed flask. Then the reaction mixture was refluxed at 115 °C for 12 h. After being cooled down to room temperature, the solvent was removed by rotary evaporation, and the residual solid was redissolved in CH_2Cl_2 (25 mL). The solution was extracted with HCl (0.1 M) and water (100 mL × 3) respectively to remove succinic anhydride and DMAP. The collected organic phase was dried over Na_2SO_4 and then filtered. The crude product was purified by column chromatography (silica gel) with CH_2Cl_2 and MeOH mixed solvent as eluent in a gradient way to obtain pure product, $R_{f(product)}$= 0.1 (CH_2Cl_2:MeOH= 100:1). After drying, Chol-COOH was yielded as a white solid (0.5g, 27%). ^{1}H NMR (500 MHz, $CDCl_3$, Figure S1a): δ= 2.60 (t, 2H, -C**H**$_2$COOH), 2.67 (t, 2H, -C**H**$_2$CH$_2$COOH), 4.59 (s, 1H, C**H**-OCO), 5.37 (s, 1H, double bond of cholesterol). ^{13}C NMR (500 MHz, $CDCl_3$, Figure S1b): δ= 39.53 (s, 4C, -**C**H$_2$-

CH_2-COOH), 56.15, 56.69 (s, 2C), 74.54 (s, 1C, **C**H-OCO), 122.61, 139.43 (s, 2C, double bond of cholesterol), 171.53 (s, 1H, -**C**OO-), 178.14 (s, 1C, -**C**OOH).

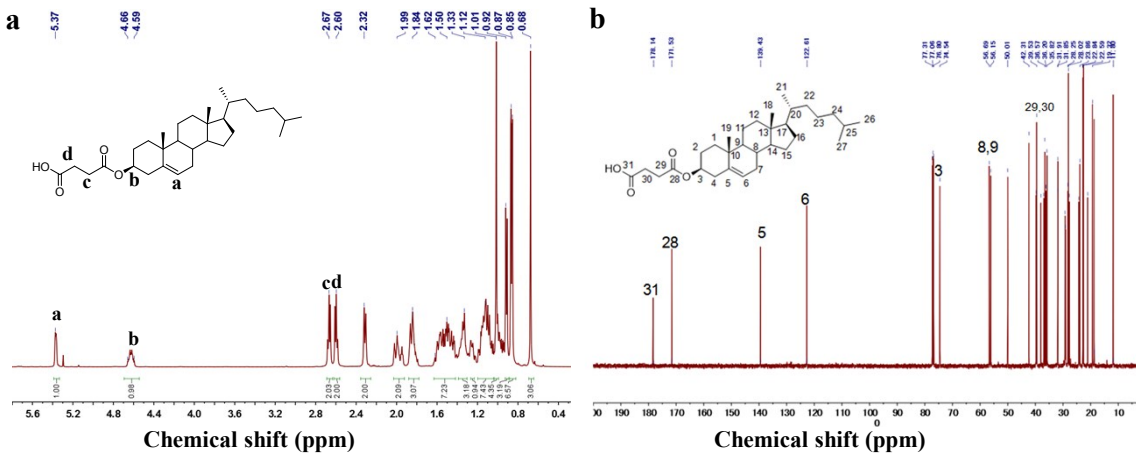


Fig. S1 (a) ^{1}H NMR and (b) ^{13}C NMR spectra of Chol-COOH ($CDCl_3$).

Synthetic routes employed for the preparation of reduction-responsive copolymers CSEG-g-Chol is shown in Scheme 1.

2. Synthesis of Ac-Cys-^{t}Boc

Ac-Cys-^{t}Boc was synthesized in two steps according to a reported approach with some optimizations [1, 2].

Synthesis of N-*tert*-butoxycarbonyl cystamine (Cys-^{t}Boc). Cystamine dihydrochloride (5.00 g, 22.20 mmol) was added into anhydrous methanol (MeOH) (50 ml) in a 250 mL round-bottomed flask and the mixture was cooled down with an ice bath. Then triethylamine (6.74 g, 66.61 mmol) was added and the mixture was magnetically stirred until it turned transparent. Di-*tert*-butyl dicarbonate (4.86 g, 22.20 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 cystamine dihydrochloride solution over 4 h in an ice bath.

After maintained in the ice bath overnight, the reaction solution was evaporated under vacuum to remove MeOH completely, and then 1 M KH_2PO_4 aqueous solution (150 mL, pH 4.2) was added. After removal of the insoluble solids, the aqueous phase was extracted with diethyl ether (3 × 100 mL) to remove N ,N'-di-tert-butyloxycarbonyl cystamine. The pH value was then adjusted to 9 with 1 M NaOH, following with extraction with ethyl acetate (7 × 100 mL). The collected organic phase was dried over Na_2SO_4 and then filtered. After the solvent was removed, Cys-^{t}Boc was yielded as a slight yellow oil (1.94 g, 35%). ^{1}H NMR (500 MHz, $CDCl_3$, Figure S2a): δ = 1.38 (s, 9H, Boc C**H$_3$**), 2.70 (t,

2H, CH_2-S), 2.73 (t, 2H, C**H₂**-S), 2.95 (t, 2H, NH_2-C**H₂**), 3.37 (t, 2H, CONH-C**H₂**). MS (ESI/APCI) ($C_9H_{20}N_2S_2O_2$: exact mass = 252.4): calcd m/z for [M+H$^+$], 253.4; found 253.2 (Figure S2b).

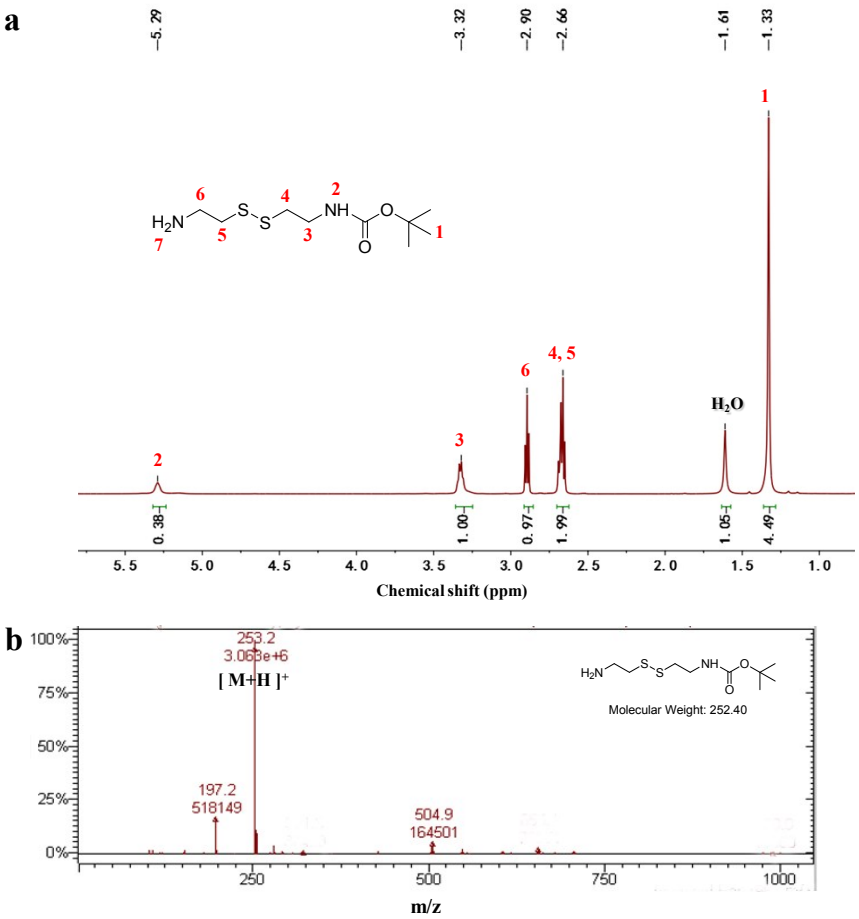


Fig. S2 (a) ^{1}H NMR spectrum of Cys-^{t}Boc ($CDCl_3$) and (b) mass spectrum of Cys-^{t}Boc (ESI).

Synthesis of N-*tert*-butoxycarbonyl-N'-acryloyl-cystamine (Ac-Cys-^{t}Boc). Cys-^{t}Boc (1.00 g, 3.97 mmol) and triethylamine (0.84 g, 8.30 mmol) were dissolved in 20 mL chloroform. After cooled down under -20 ^{o}C, it was dropwise added to a magnetically stirred solution of acryloyl chloride (0.71 g, 7.85 mmol) in chloroform (20 mL) placed in an ice bath over 2.5 h. After maintained for 24 h, the reaction solution was evaporated under vacuum to remove the solvent. The residue was washed with water (50 mL), and extracted with chloroform (3 × 20 mL). The combined organic phase was dried over Na_2SO_4 and filtered. After removal of the solvent, Ac-Cys-^{t}Boc was yielded as a light yellow solid (0.98 g, 81.7%). ^{1}H NMR (500 MHz, $CDCl_3$, Figure S3a): δ= 1.44 (s, 9H, Boc C**H₃**), 2.77 (t, 2H, C**H₂**-S), 2.88 (t, 2H,C**H₂**-S), 3.45 (t, 2H, -NH-C**H₂**), 3.65 (t, 2H, -C**H₂**-NH-), 5.01 (1H, amide proton), 5.64 (m, 1H, vinyl proton), 6.18-6.359 (m,2H, vinyl proton), 6.72 (1H, amide proton). MS (ESI/APCI) ($C_{12}H_{22}N_2S_2O_3$: exact mass = 306.4): calcd m/z for [M+Na$^+$], 329.4; found 328.9 (Figure S3b).

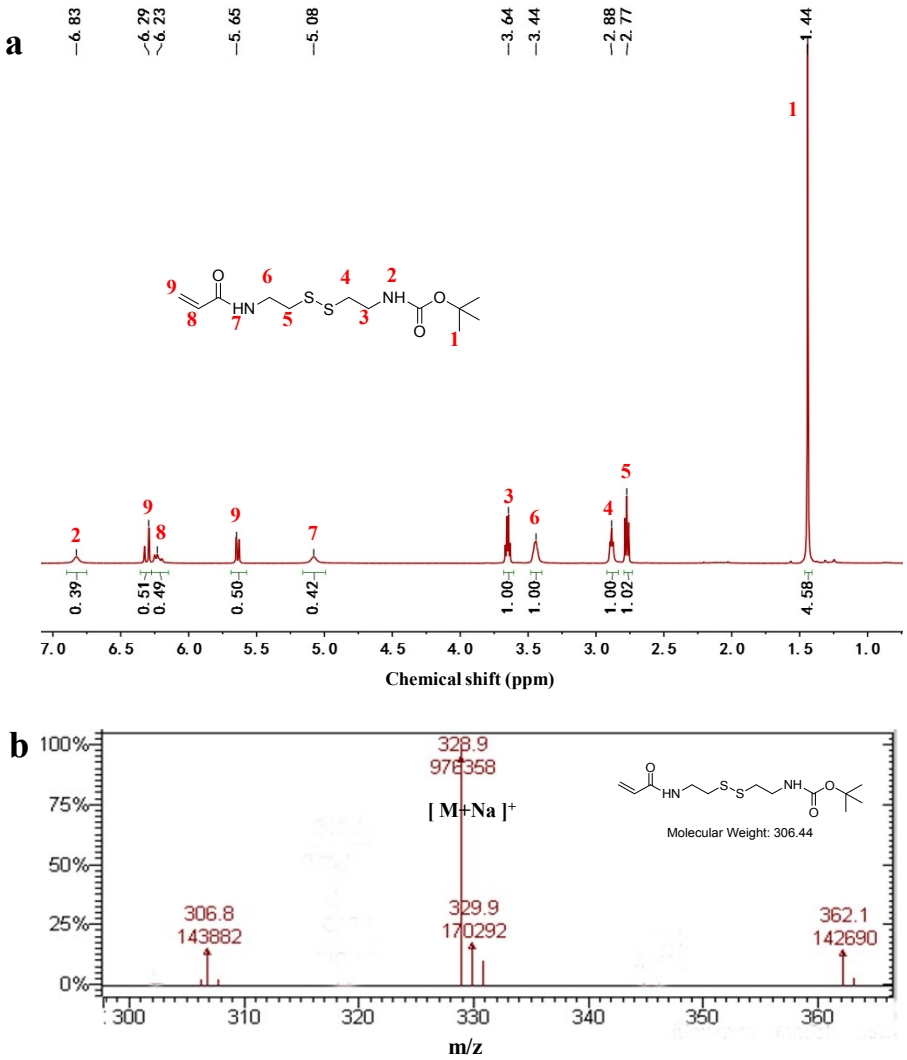


Fig. S3 (a) ^{1}H NMR spectrum of Ac-Cys-^{t}Boc ($CDCl_3$) and (b) mass spectrum of Ac-Cys-^{t}Boc (ESI).

3. Synthesis of CSEG copolymers

Synthesis of poly[(N-*tert*-butoxycarbonyl-N'-acryloyl-cystamine)-co-[poly(ethylene glycol)]] (CSBEG). CSBEG was synthesized by free radical polymerization of Ac-Cys-^{t}Boc and poly (ethylene glycol) methacrylate (PEGMA). Ac-Cys-^{t}Boc (150 mg, 0.49 mmol), PEGMA (35.3 mg, 0.098mmol) and AIBN (1.45 mg, 0.0088 mmol) were dissolved in 11 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 give CSEBG as a transparent viscous solid (68 mg, 36.7%). ^{1}H NMR (500 MHz, $CDCl_3$, Figure S4) spectrum of CSBEG shows the characteristic peaks of [Ac-Cys-^{t}Boc] (Boc C**H$_3$**, δ= 1.43; C**H$_2$**-S, δ= 2.79; C**H$_2$**-N, δ= 3.40) and PEGMA (-COO-CH_2, δ= 4.08; -O-CH_2, δ= 3.63), and the

proton peaks of CH_2=CH in Ac-Cys-^{t}Boc monomers disappeared, indicating successful copolymerization of Ac-Cys-^{t}Boc and PEGMA. The copolymerization ratio of [Ac-Cys-^{t}Boc] to [PEGMA] in CSBEG was determined to be 2.7:1 (mol/mol) based on integrals of Boc C**H$_3$** peak and -COO-C**H$_2$** peak. The molecular weight and molecular weight distribution of CSBEG were determined by GPC by using THF as the eluent, revealing an Mn of 3.57 kDa and Mw/Mn of 1.36 (Figure S5).

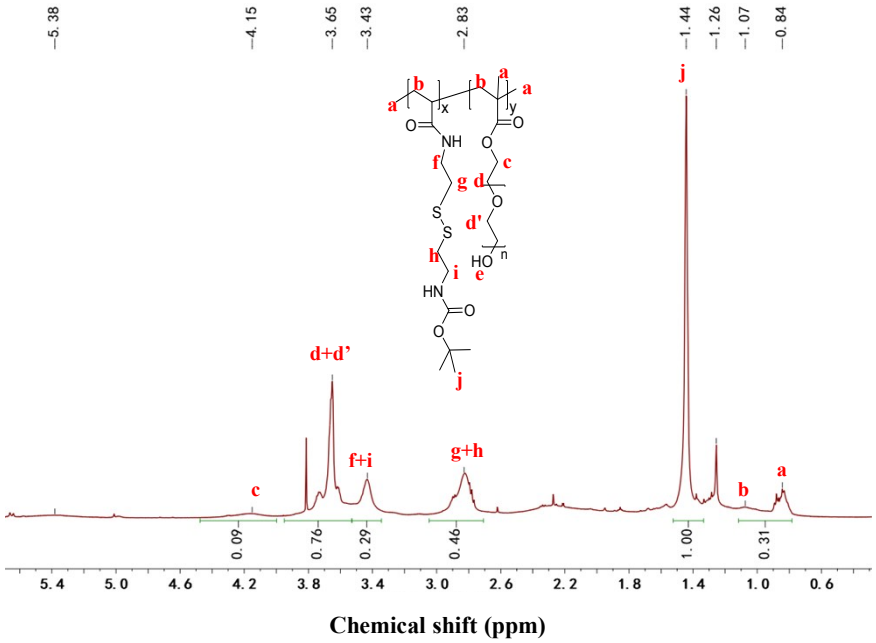


Fig. S4 ^{1}H NMR spectrum of the copolymers CSBEG ($CDCl_3$).

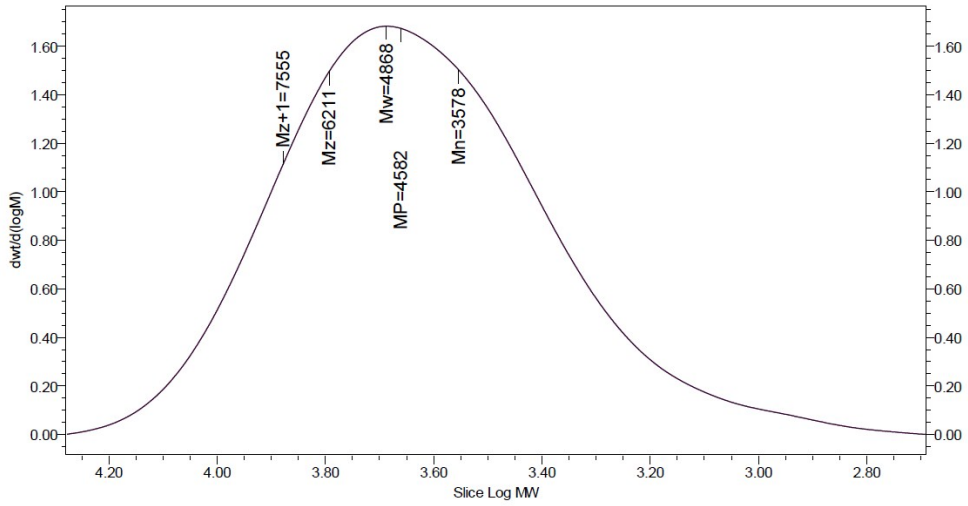


Fig. S5 GPC characterization (THF as the eluent, and PS as the standard) of CSBEG.

Synthesis of poly[(N-acryloyl-cystamine)-co-[poly(ethylene glycol)]] (CSEG). To the solution of CSBEG (68 mg) in CH_2Cl_2 (4 mL) placed in an ice bath, trifluoroacetic acid (2 mL) was added dropwise. The reaction was maintained under magnetic stirring for 4 h. Then the solution was

evaporated under vacuum to remove the solvents. The CSEG free of ^{t}Boc groups was yielded as a transparent viscous solid. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of CSEG shows that the characteristic peak of the protection Boc groups at δ= 1.43 disappeared, indicating successful removal of Boc groups. Therefore, the $–NH_2$ groups were regained, which could be utilized to conjugate cholesterol groups in the following procedure.

4. Synthesis of the amphiphilic copolymers CSEG-g-Chol

Chol-COOH molecules were conjugated to CSEG to obtain CSEG-g-Chol via a one-step procedure. First, CSEG (containing 0.177 mmol $–NH_2$), chol-COOH (8.6 mg, 0.017 mmol), DMTMM (53.9 mg, 0.195 mmol) and triethylamine (22.4 mg, 0.22 mmol) were dissolved in anhydrous methanol (MeOH) (12 mL) in a 50 ml round-bottomed flask placed in a 37 °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 CSEG-g-Chol as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$, Figure S6) spectrum of CSEG-g-Chol shows characteristic peaks of Chol and CSEG, indicating successful conjugation of Chol-COOH onto CSEG.

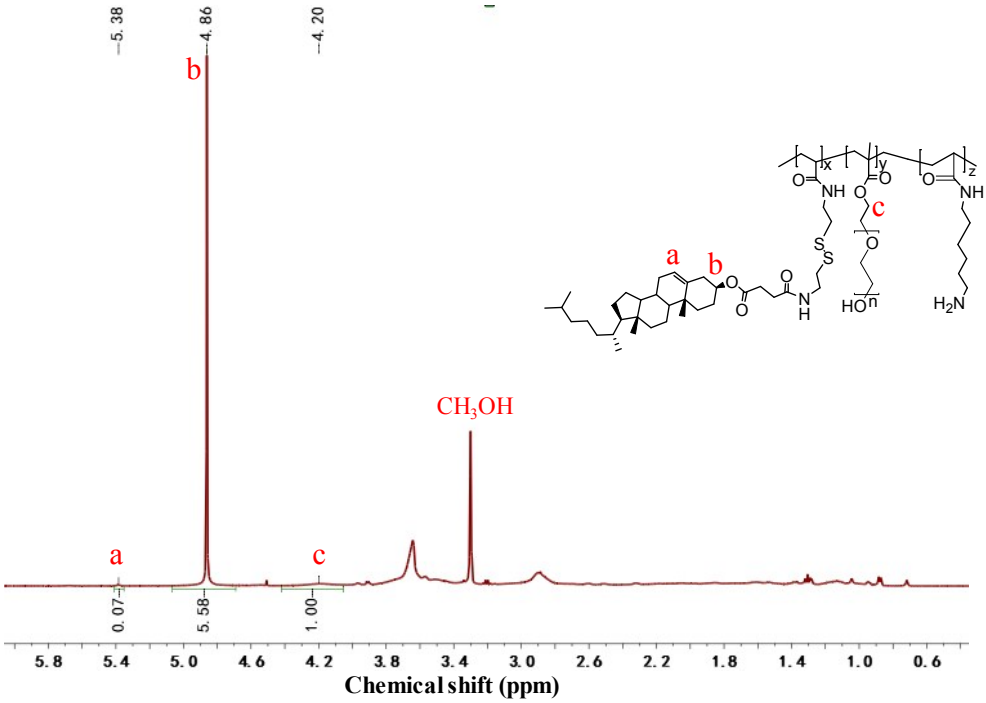
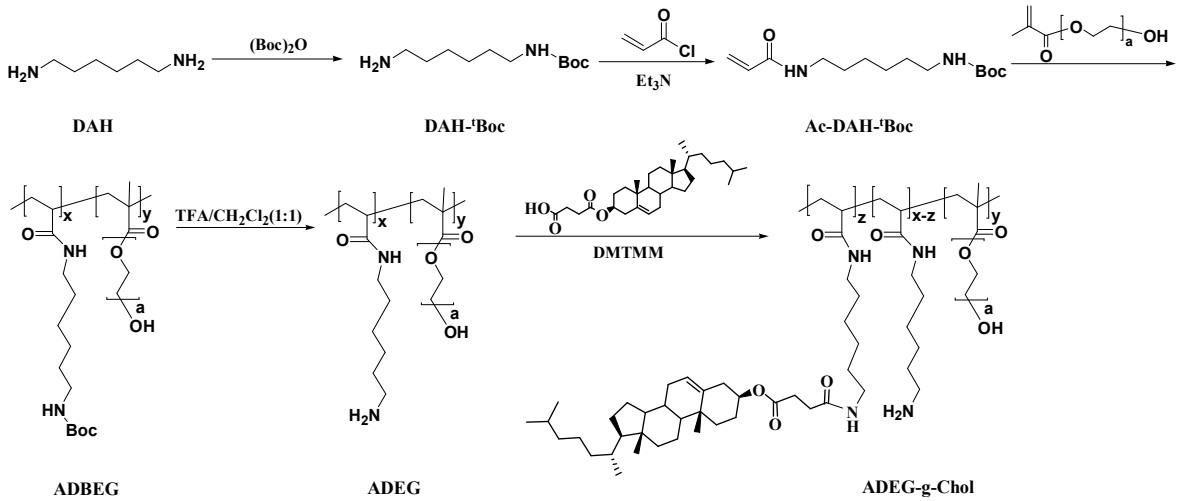


Fig. S6 ^{1}H NMR spectrum of CSEG-g-Chol ($CDCl_3$).

The synthetic routes employed for the preparation of nonreduction-responsive copolymer ADEG-g-Chol is shown in Scheme S1.



Scheme S1 Schematic illustration of synthesis of the amphiphilic copolymer ADEG-g-Chol.

5. Synthesis of Ac-DAH-^{t}Boc

Ac-DAH-^{t}Boc was synthesized in two steps according to a reported approach with some optimizations [1, 2].

Synthesis of N-*tert*-butoxycarbonyl diaminohexane (DAH-^{t}Boc). 1,6-Diaminohexane dihydrochloride (4.1989 g, 22.20mmol) was added into anhydrous methanol (MeOH) (50 mL) in a 250 mL round-bottomed flask, and the mixture was cooled down with an ice bath. Then trimethylamine (6.74 g, 66.61 mmol) was added, and the mixture was magnetically stirred until it turned transparent. Di-*tert*-butyl dicarbonate (4.86 g, 22.20 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 maintained in the ice bath overnight, the reaction solution was evaporated under vacuum to remove MeOH completely, and then 150 mL $CHCl_3$ was added. After removal of the insoluble solids, the acquired reaction product was extracted with 5% (w/v) Na_2CO_3 aqueous solution (2 × 100 mL). The remained organic phase was concentrated under vacuum, following addition of 1 M KH_2PO_4 aqueous solution (150 mL, pH 4.2). After removal of the insoluble solids, the aqueous phase was extracted with diethyl ether (3 × 100 mL) to remove N ,N'-di-*tert*-butyloxycarbonyl diaminohexane. The solution pH was adjusted to 10 with 1 M NaOH, and then was extracted with ethyl acetate (7 × 100 mL). The collected organic phase was dried over Na_2SO_4 and filtered. After the solvent was removed, DAH-^{t}Boc was yielded as a slighted yellow oil (0.5 g, 12%). ^{1}H NMR (500 MHz, $CDCl_3$,

Figure S8a): δ = 1.31(s, 4H, NH_2-$(CH_2)_2$-C**H**$_2$-C**H**$_2$), 1.42 (s, 13H, Boc C**H**$_3$，NH_2-CH_2-C**H**$_2$), 2.68 (t, 2H, NH_2-C**H**$_2$), 3.09 (s, 2H, CONH-C**H**$_2$). MS (ESI/APCI) ($C_{11}H_{24}N_2O_2$: exact mass = 216.32): calcd m/z for [M+H$^+$], 217.32; found 217.13 (Figure S8b).

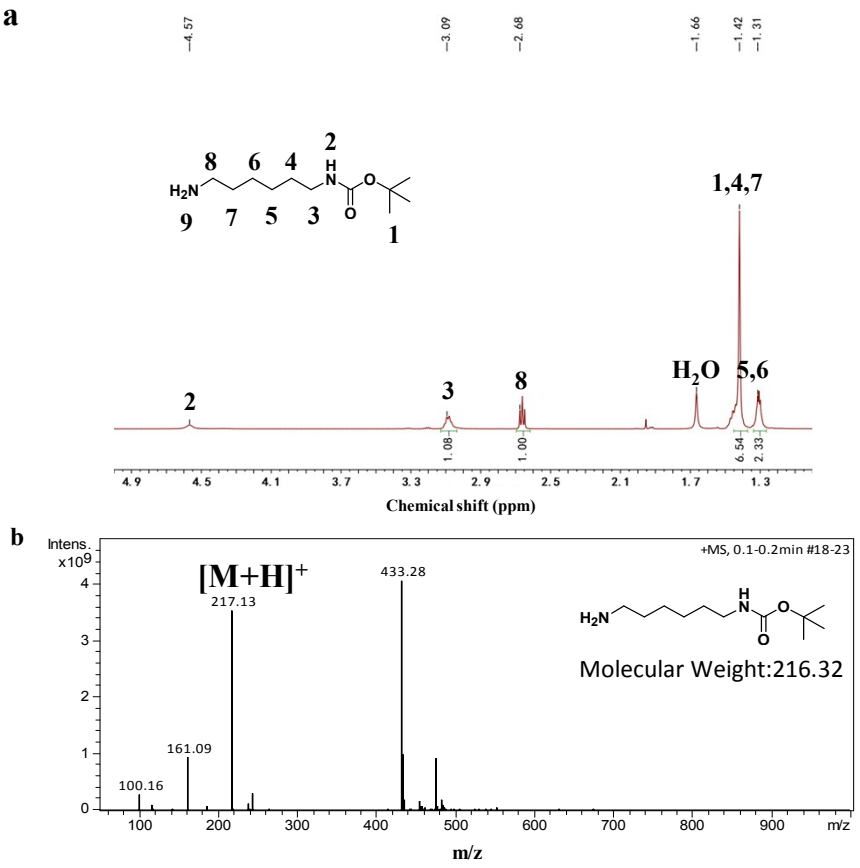


Fig. S8 (a) ^{1}H NMR spectrum of DAH-^{t}Boc ($CDCl_3$) and (b) mass spectrum of DAH-^{t}Boc (ESI).

Synthesis of N-*tert*-butoxycarbonyl-N'-acryloyl-diaminohexane (Ac-DAH-^{t}Boc). DAH-^{t}Boc (1.00 g, 4.622 mmol) and triethylamine (0.98 g, 9.7 mmol) were dissolved in 40 mL chloroform and cooled down under -20 ^{o}C. Then the mixture was dropwise added to a magnetically stirred solution of acryloyl chloride (0.83 g, 9.24 mmol) in chloroform (40 mL) placed in an ice bath over 2.5 h. After maintained for 24 h, the reaction solution was evaporated under vacuum to remove the solvent. The residue was washed with water (120 mL), and then extracted with chloroform (2 × 50 mL). The combined organic phase was dried over Na_2SO_4 and filtered. After removal of the solvent, Ac-DAH-^{t}Boc was yielded as a light yellow solid (1.5 g, 81.6%). ^{1}H NMR (500 MHz, $CDCl_3$, Figure S9a): δ= 1.28 (s, 4H, CONH-$(CH_2)_2$-C**H**$_2$-C**H**$_2$-), 1.37 (s, 11H, Boc C**H**$_3$, $CH_2CHCONHCH_2$-C**H**$_2$-), 1.47 (t, 2H, $OCONHCH_2$-C**H**$_2$-), 3.04 (s, 2H, CONH-C**H**$_2$-), 3.24 (t, 2H, -C**H**$_2$-NHCOO), 4.53 (1H, amide proton), 5.56, 6.06 (m, 2H, vinyl proton), 6.19 (m, 1H, vinyl proton). MS (ESI/APCI) ($C_{14}H_{26}N_2O_3$: exact mass

= 270.37): calcd m/z for [M+Na$^+$], 293.35, found 293.11 (Figure S9b).

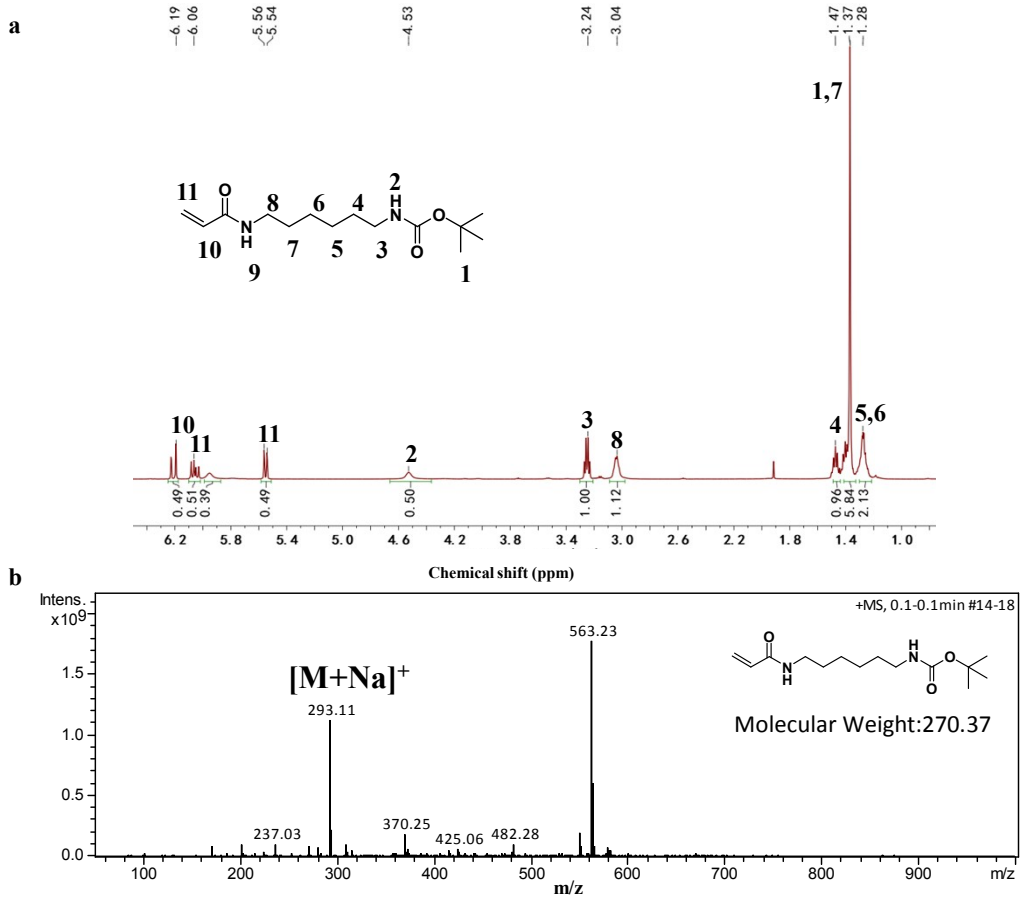


Fig. S9 (a) ^{1}H NMR spectrum of Ac-DAH-^{t}Boc ($CDCl_3$) and (b) mass spectrum of Ac-DAH-^{t}Boc (ESI).

6. Synthesis of ADEG copolymers

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). Ac-DAH-^{t}Boc (132.5 mg, 0.49 mmol), PEGMA (58.8 mg, 0.16 mmol) and AIBN (38.62 mg, 0.24 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 give ADBEG as a transparent viscous solid (59 mg, 31%). ^{1}H NMR (500 MHz, $CDCl_3$, Figure S10) spectrum of ADBEG shows the characteristic peaks of [Ac-DAH-^{t}Boc] (Boc C$\mathbf{H_3}$, δ= 1.43; CONH-$(CH_2)_2$-C$\mathbf{H_2}$, δ= 1.31; C$\mathbf{H_2}$-N, δ= 3.08) and PEGMA (-COO-CH_2, δ= 4.11; -O-CH_2, δ= 3.65), 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 1.96:1 (mol/mol) based on integrals of Boc C**H$_3$** peak and -COO-C**H$_2$** peak. The molecular weight and molecular weight distribution of ADBEG were determined by GPC by using THF as the eluent, revealing an Mn of 11.7 kDa and Mw/Mn of 1.19 (Figure S11).

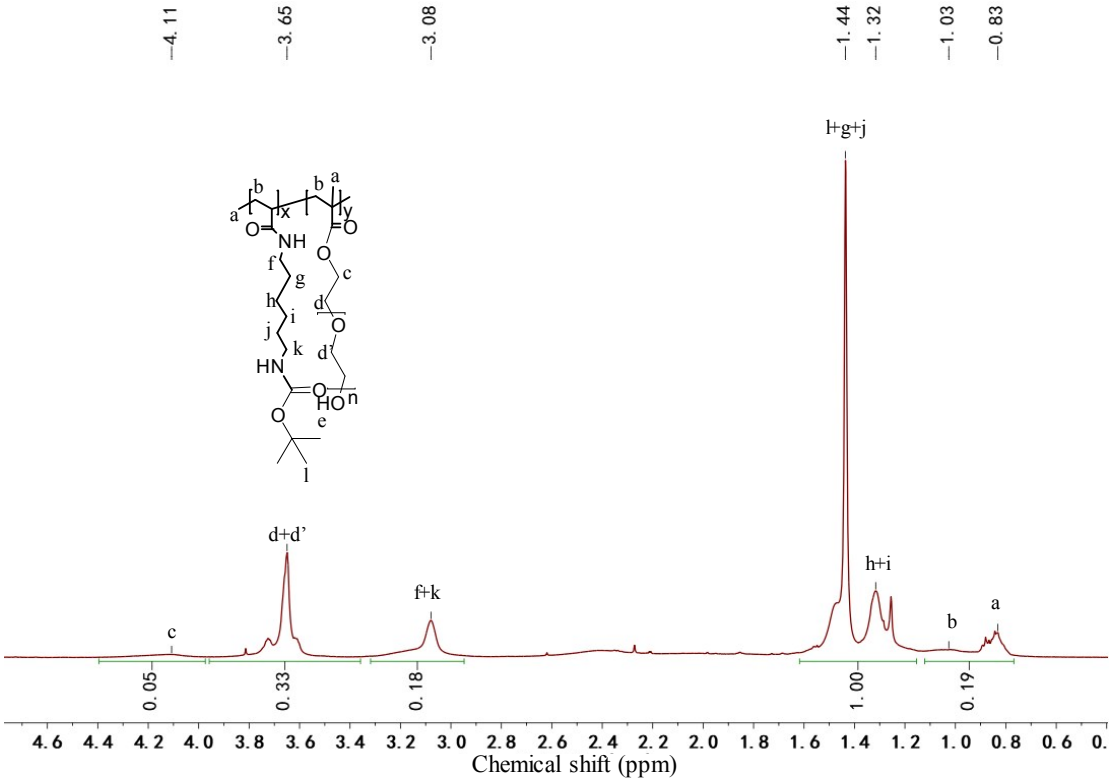


Fig. S10 ^{1}H NMR spectrum of copolymers ADBEG ($CDCl_3$).

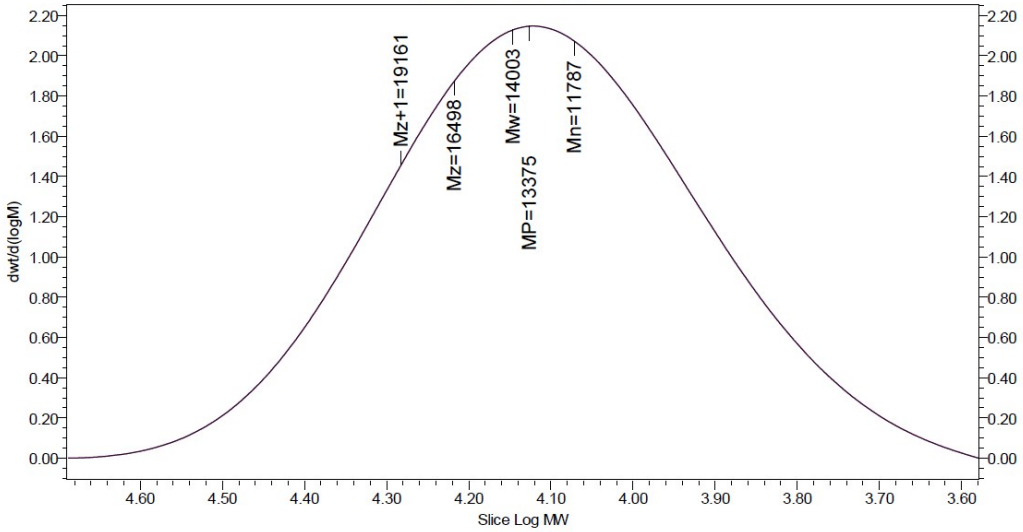


Fig. S11 GPC characterization (THF as the eluent, and PS as the standard) of ADBEG.

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

solution of ADBEG (59 mg) in CH_2Cl_2 (4 mL) placed in an ice bath, trifluoroacetic acid (2 mL) was added dropwise. The reaction was maintained under magnetic stirring for 4 h. Then the solution was evaporated under vacuum to remove the solvents. Then ADEG free of ^{t}Boc groups was yielded as a transparent viscous solid.

7. Synthesis of the amphiphilic copolymer ADEG-g-Chol

Chol-COOH was conjugated to ADEG to obtain ADEG-g-Chol via a one-step procedure. First, ADEG (containing 0.167 mmol $–NH_2$), Chol-COOH (8.11 mg, 0.016 mmol), DM-TMM (50.8 mg, 0.184 mmol) and triethylamine (21.1 mg, 0.21 mmol) were dissolved in anhydrous methanol (MeOH) (12 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-Chol as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$, Figure S12) spectrum of ADEG-g-Chol shows characteristic peaks of Chol and ADEG, indicating successful conjugation of Chol-COOH onto ADEG.

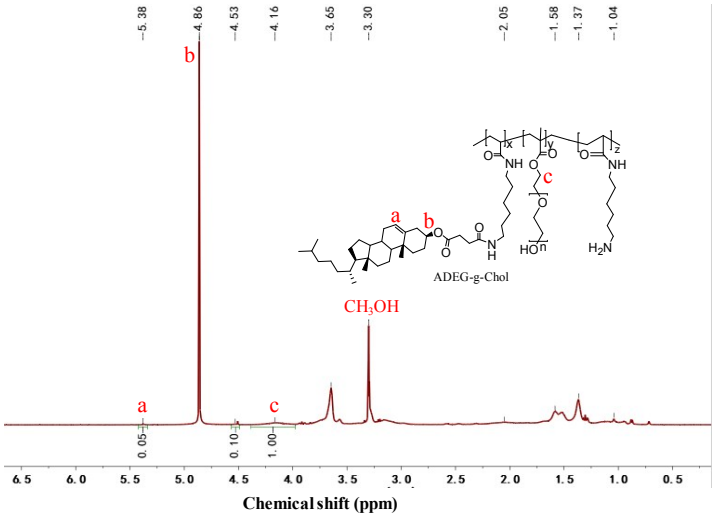


Fig. S12 ^{1}H NMR spectrum of ADEG-g-Chol ($CDCl_3$).

Preparation of microgels (MGs)

1. Preparation of $CaCO_3$ microparticles template

Spherical $CaCO_3$ microparticles were prepared by mineralization of $Ca(NO_3)_2$ and Na_2CO_3 solutions. Briefly, 5 mL 0.22 mol/L $Ca(NO_3)_2$ solution was rapidly poured into 5 mL 0.33 mol/L Na_2CO_3 solution under magnetic stirring at 1200 rpm. 30 s later, the solution was kept undisturbed for 5 min. $CaCO_3$ microparticles with a diameter around 2 μm were obtained, which were washed three

times with water and ethanol. They were stored in ethanol (10 mL) before use.

2. Preparation of CSEG-g-Chol MGs and ADEG-g-Chol MGs

The $CaCO_3$ microparticles suspension (1 mL) was centrifuged at 1500 rpm for 3 min to remove the supernatant ethanol, and then the $CaCO_3$ microparticles were placed in a vacuum oven to remove ethanol completely. The amphiphilic copolymers CSEG-g-Chol or ADEG-g-Chol MeOH solution (10 mg / mL, 200 μL × 5) was added into the $CaCO_3$ microparticles under vacuum. The excess polymers were dissolved in 100 μL ethanol and were removed by centrifugation at 1500 rpm for 3 min. The particles were then dispersed in 25% glutaraldehyde (GA) aqueous solution (100 μL) for 2 h in a 37 ^oC water bath, followed by three washings in MeOH and water, respectively. Arginine aqueous solution (20 mg / mL, 1 mL) was added to the GA-crosslinked particles to react with the excess aldehyde groups of GA. After maintained in a 37 ^oC water bath for 1 h, the particles were washed with water three times, and were then incubated in 2 mL 0.2 M EDTA (pH= 7.29) solution for1 h in a 37 ^oC water bath to decompose the $CaCO_3$ cores. The obtained microgels were washed with water three times and dispersed in 1 mL water, then modified with Tat peptides. Finally, the microgels were centrifuged at 5000 rpm for10 min to remove the excess Tat peptides, and then dispersed in 2 mL water and stored at 4 ^oC before use.

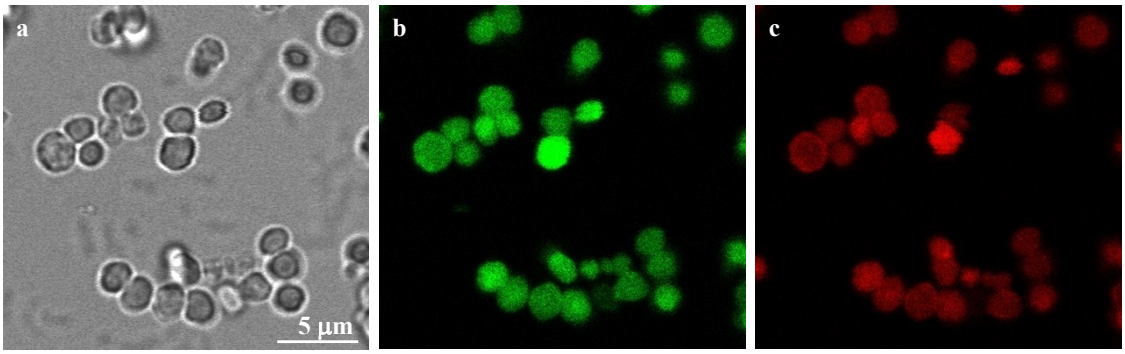


Fig. S7 (a-c) CLSM images of CSEG-g-Chol MGs. The MGs were excited with 488 and 543 nm laser to obtain green (b) and red (c) autofluorescence, respectively.

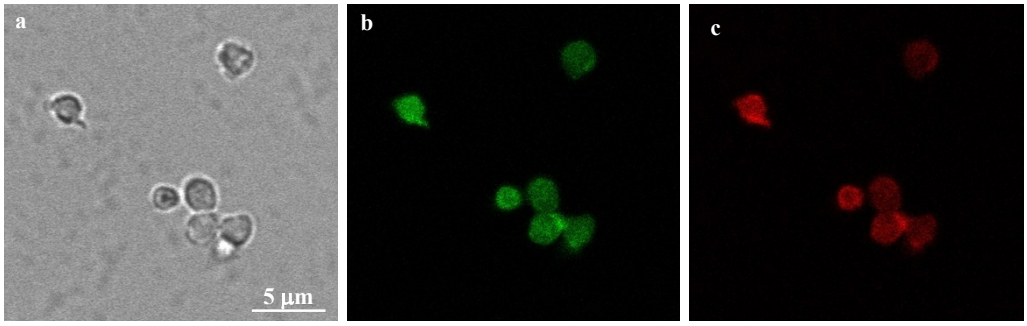


Fig. S13 (a-c) CLSM images of ADEG-g-Chol MGs. The MGs were excited with 488 and 543 nm laser to obtain green (b) and red (c) autofluorescence, respectively.

3. The decomposition-assembly performance of MGs induced by reductants

GSH (10 mM, 100 μL) or DTT (10 mM, 100 μL) aqueous solution was added into MGs suspension (100 μL) placed in a 37 °C water bath, which was further incubated for 0 h, 0.5 h, 1 h, 3 h, 6 h, 12 h, and 24 h, respectively. The decomposition-assembled performance was monitored by TEM.

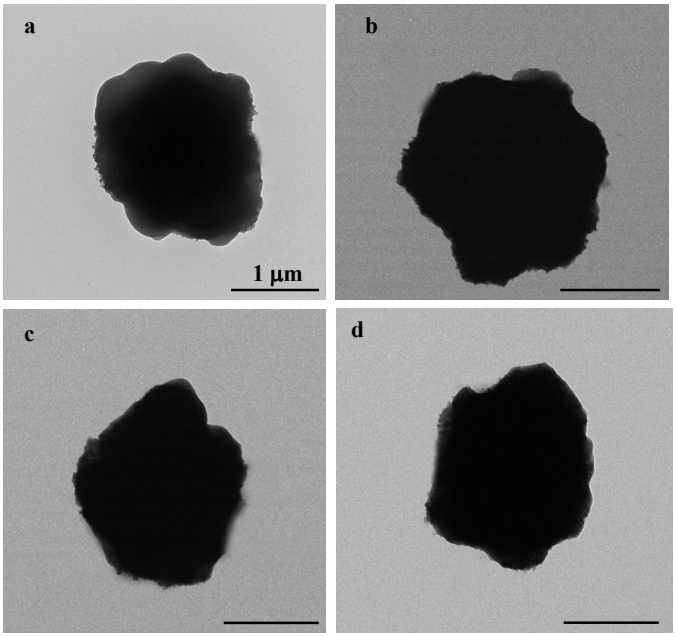


Fig. S14 (a-d) TEM images of ADEG-g-Chol MGs after being incubated in glutathione solution (5 mM) for 0 (a), 0.5 (b), 3 (c) and 24 h (d), respectively.

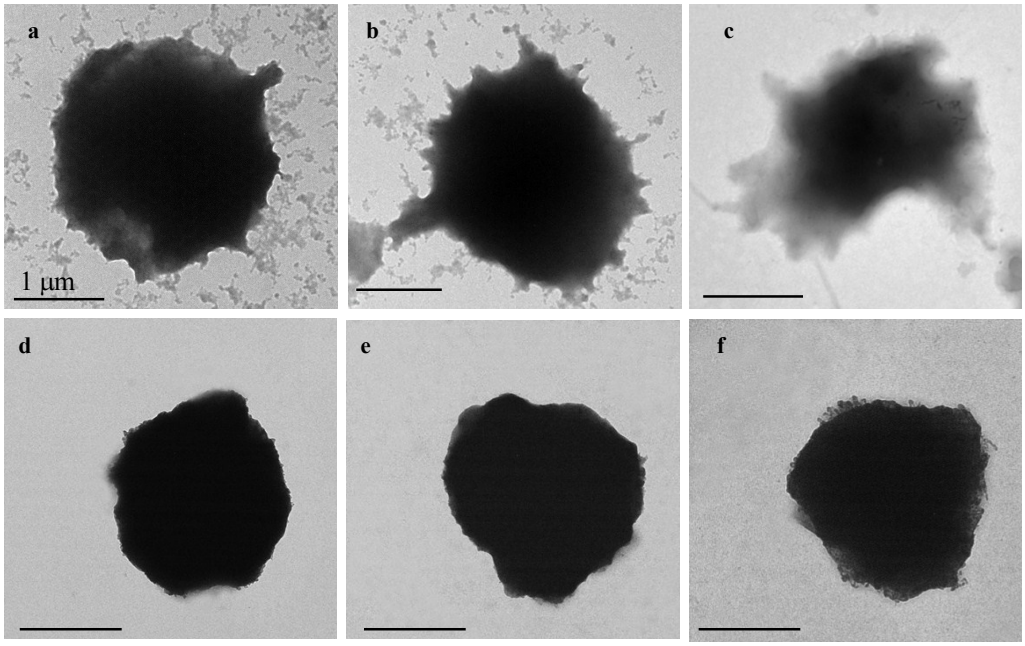


Fig. S15 (a-c) TEM images showing the protruding process of nano-spikes on the surface of CSEG-g-Chol MGs after being incubated in DTT solution (5 mM) for 0.5 (a), 3 (b) and 24 h (c), respectively. (d-f) TEM images of ADEG-g-Chol MGs after being incubated in DTT solution (5 mM) for 0.5 (d), 3 (e) and 24 h (f), respectively.

Table S1 Atomic concentrations detected by XPS and calculated C/O ratios of MGs before and after GSH reduction.

	CSEG-g-Chol MGs-GSH	**CSEG-g-Chol MGs**	**ADEG-g-Chol MGs-GSH**	**ADEG-g-Chol MGs**
C%	47.31	42.67	72.44	71.47
O%	42.60	44.71	20.60	19.85
S%	7.73	3.51	0.79	0.6
C/O	1.11	0.95	3.52	3.60

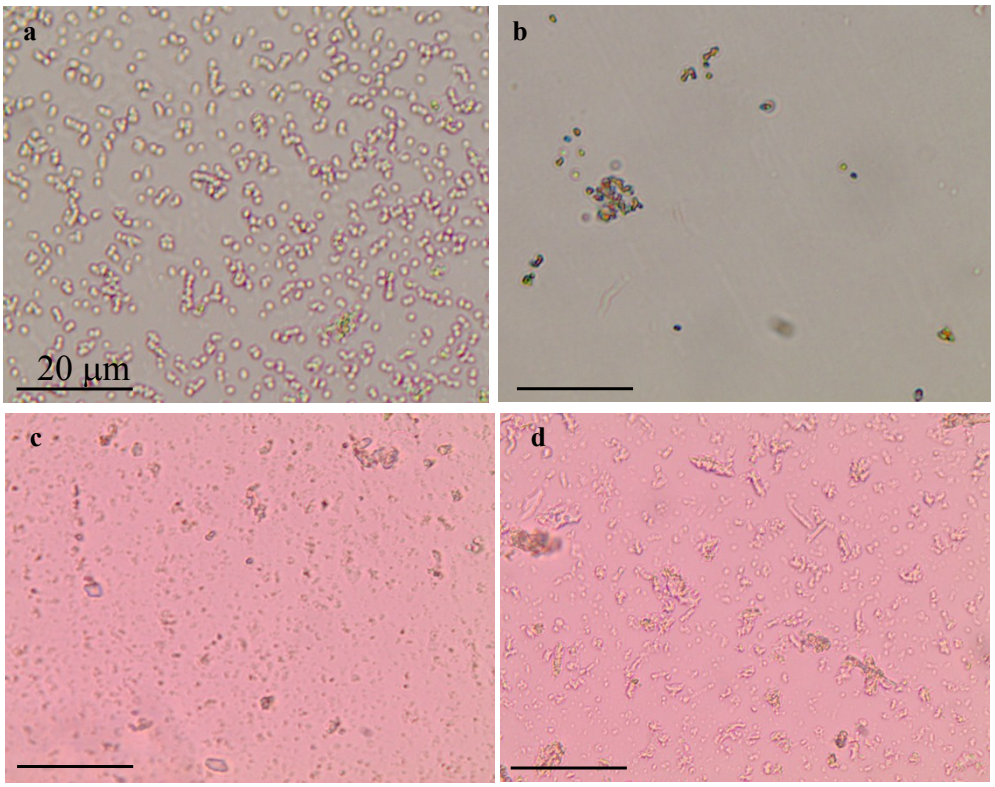


Fig. S16 Polarized microscopy images of CSEG-g-Chol MGs (a, b) and ADEG-g-Chol MGs (c, d) before (a, c) and after (b, d) being incubated in GSH solution (5 mM) for 24 h.

Cell experiments

1. Cell culture

The human liver hepatocellular carcinoma cells (HepG2 cells) and mouse monocyte-macrophage cells (RAW264.7 cells) were purchased from the Cell Bank of Typical Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Primary human normal hepatocytes (HepLi cells) were obtained from the First Affiliated Hospital, College of Medicine, Zhejiang University. The HepG2 cells were cultured in high-glucose Dulbecco's modified eagle medium (DMEM) (Gibco, USA). The HepLi cells were cultured in low-glucose DMEM (Gibco, USA). The medium was supplemented with 10% fetal bovine serum (FBS, Gibco), 100 U/mL penicillin and 100 μg/mL streptomycin. The RAW264.7 cells were cultured in high-glucose DMEM (Gibco, USA) containing 10% fetal bovine serum (FBS). The FBS was inactivated by heating at 56 °C for 30 min before use. The cells were incubated in an incubator at 37 °C supplied with 5% CO_2 and 100% humidity.

2. Cellular uptake of CSEG-g-Chol MGs and ADEG-g-Chol MGs

The difference of cellular uptake performance of CSEG-g-Chol MGs and ADEG-g-Chol MGs was analyzed with flow cytometry and CLSM. For the flow cytometry measurements, HepG2, HepLi or RAW264.7 cells were seeded at a density of 7.5×10^4 cells per well on 24-well plates, and were allowed to attach for 24 h. Then CSEG-g-Chol MGs and ADEG-g-Chol MGs were added to each well (particles: cells = 15:1). The cells and particles were co-incubated for different time (6 h, 12 h and 24 h). After being washed with PBS three times to remove the free particles, the cells were detached with trypsinization and dispersed in PBS at a concentration of about 1.6×10^5/mL. The average fluorescence intensity per cell and the ratio of cells with fluorescence was measured with flow cytometry (FACS Calibur, BD).

For the CLSM measurements, the HepG2, HepLi or RAW264.7 cells were seeded at a density of 5×10^4 on a 20-mm cell culture dish with a glass bottom, and were cultured for 24 h. Then the cells were incubated with CSEG-g-Chol MGs and ADEG-g-Chol MGs at a particle-to-cell ratio of 15:1 for 24 h. After being washed with PBS three times to remove the free particles, 1 mL of cell culture medium without phenol red indicator and fetal bovine serum was added into each well. Images were acquired by a Leica TCS SP5 CLSM (63×/1.4 NA oil immersion objective using commercial software). MGs were excited at 488 and 543 nm, respectively.

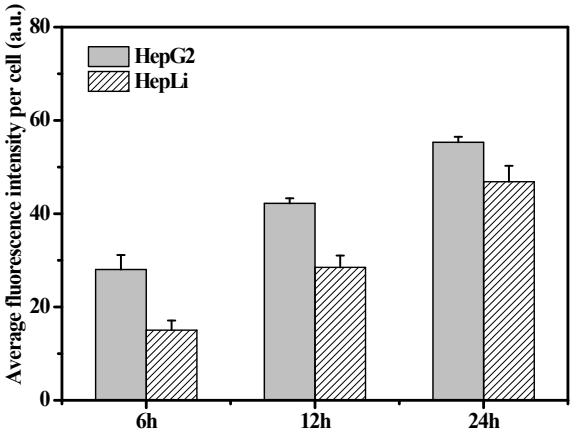


Fig. S17 The cellular uptake amount of ADEG-g-Chol MGs after co-incubation for designated time at a particle-to-cell ratio of 15 : 1. Data are expressed as the mean±SD, *n =3*, **p < 0.05*, ***p < 0.01*.

3. The decomposition-assembly performance of MGs in cells

The decomposition-assembly performance of MGs in cells was observed by TEM. The HepG2, HepLi, A549, or SMC cells were seeded at a density of 3×10^5 per well on 6-well plates, and were

cultured for 24 h. Then the cells were incubated with CSEG-g-Chol MGs and ADEG-g-Chol MGs at a particle-to-cell ratio of 15:1 for 24 h. After being washed with PBS three times to remove the free particles, the cells were detached with 0.3 mL trypsinization and were prefixed with 2.5% GA at 4 °C for 24 h. After being washed twice with phosphate-buffered saline (PBS, 0.1 M, pH= 7.4), the samples were post-fixed with 1% osmium tetraoxide (Fluka,Seelze, Germany) at 4 °C for 1 h and washed three times with PBS, each for 15min, respectively. They were then dehydrated by a graded series of ethanol (50, 70, 80, 90, 95, and 100%), each for 15min, and transferred to absolute acetone for 20 min. After incubated in 1:1 mixture of absolute acetone and the final Spurr resin mixture for 1 h at room temperature, they were transferred to 1:3 mixture of absolute acetone and the final resin mixture for 3 h, and finally to the final Spurr resin mixture overnight. The solidified resin with samples was placed in Eppendorf tubes containing embedding medium and heated at 70 °C for about 9 h, followed by ultrathin sectioning with a Reichert ultra microtome. After stained by uranyl acetate and alkaline lead citrate for 15 min, they were observed under a JEM-1230EX TEM (JEOL, Tokyo, Japan) at an accelerating voltage of 80 kV.

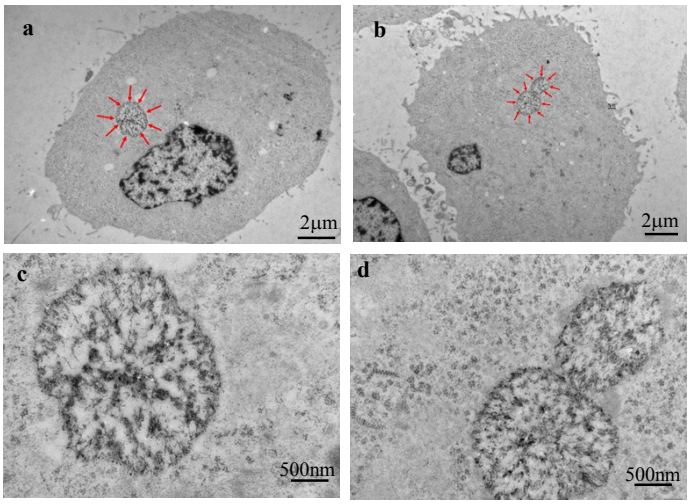


Fig. S18 (a-d) Cross-sectional (ultramicrotomy) TEM images of ADEG-g-Chol MGs after being co-incubated with HepG2 (a, c) and HepLi (b, d) cells for 24 h at a particle-to-cell ratio of 15: 1, respectively. The MGs are outlined by the inward arrows.

4. Cell viability assay

Cell viability was determined with MTT assay. HepG2, HepLi or RAW264.7 cells were seeded at a density of 5×10^3 cells per well on 96-well plates and were incubated for 24 h. Then the cells were

incubated with CSEG-g-Chol MGs or ADEG-g-Chol MGs at a particle to cell ratio of 15:1, 10:1 and 5:1 for 24 h, followed by washing three times with PBS to remove the free particles, respectively. Thereafter, 100 μL of cell culture medium without phenol red indicator and 20 μL MTT/PBS solution (5 mg/mL) were added into each well. After 3.5 h incubation at 37 °C, the MTT medium solution was removed carefully, and 100 μL dimethyl sulfoxide (DMSO) was added into each well to dissolve the purple formazan crystals generated by the mitochondria dehydrogenase. After incubation at 37 °C for 15 min, 50 μL formazan/DMSO solution was pipetted into a well of another new 96-well plate. The absorbance at 565 nm was measured by a microplate reader (M200 Pro, Tecan). Cell viability was expressed as the ratio of absorbance of the experimental groups to that of the control group in which the cells were incubated with cell culture medium only. Data were expressed as average ± SD (n=5).

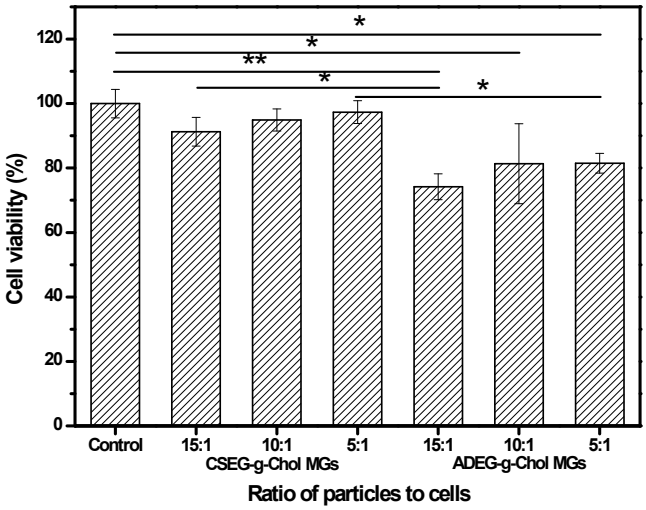


Fig. S19 Cytotoxicity of CSEG-g-Chol MGs and ADEG-g-Chol MGs after being co-incubated with RAW264.7 cells at a particle-to-cell ratio of 15:1, 10:1 and 5:1 for 24 h, respectively. Data are expressed as the mean±SD, $n = 5$, $^{*}p < 0.05$, $^{**}p < 0.01$.

5. Cytoskeleton staining

The HepG2 and HepLi cells were seeded at a density of 5×10^4 on a 20-mm cell culture dish with a glass bottom, and were cultured for 24 h. Then the cells were incubated with CSEG-g-Chol MGs and ADEG-g-Chol MGs at a particle-to-cell ratio of 15:1 for 24 h. After being carefully washed with PBS three times to remove the free particles, the cells were fixed with 4% paraformaldehyde in PBS at 37 °C for 30 min. The cells were permeabilized with 0.5% Triton X-100/PBS solution at 37 °C for 5 min, and further incubated in 1% BSA/PBS solution at room temperature for 60 min to block nonspecific

adsorption. The cells were subsequently treated with 1 μL rhodamine-conjugated phalloidin and 1 μL DAPI in 500 μL 1% BSA/PBS solution for 60 min at 37 °C in dark. Finally, the cells were observed under CLSM (Zeiss LSM 780, 63×/1.4 NA oil immersion objective).

6. Endoplasmic reticulum (ER) staining

The HepG2 and HepLi cells were seeded at a density of 5×10^4 on a 20-mm cell culture dish with a glass bottom, and were cultured for 24 h. Then the cells were incubated with CSEG-g-Chol MGs and ADEG-g-Chol MGs at a particle-to-cell ratio of 15:1 for 24 h. After being carefully washed with PBS three times to remove the free particles, the cells were incubated with 100 nM ER-Tracker Blue-White DPX at 37 °C for 30 min, and finally observed under CLSM. The ER-Tracker Blue-White DPX was diluted with Hank's Balanced Salt Solution (HBSS) (containing Ca^{2+} and Mg^{2+}) to yield a final concentration of 100 nM.

7. ELISA test

The RAW264.7 cells were seeded onto 48-well plates at a density of 2×10^4 cells/well and maintained for 24 h. Then the cells were incubated with CSEG-g-Chol MGs and ADEG-g-Chol MGs at a particle-to-cell ratio of 15:1 for 24 h. The cell culture supernatants were collected and stored at -80 °C for enzyme immunoassay.

The secretion of cytokines including TNF-α, IL-6 and IL-1β by RAW264.7 cells was measured with a standard quantitative sandwich enzyme-linked immune-sorbent assay (ELISA) technology. The specific antibodies that can capture the target analyte (various cytokines) were pre-coated on the 96-well plates. The standards or samples were pipetted into the wells, and any cytokines present were bound by the immobilized antibodies. Then a detector antibody (biotinylated detection antibody) that can bind to a different epitope on the cytokines was added to complete the sandwich. The streptavidin molecules conjugated to horseradish peroxidase (HRP) were added, and 3,3′,5,5′-tetramethylbenzidine (TMB) color developing agent was used to develop a particular color, which is proportional to the amount of cytokines captured in the initial step. The color development was stopped by adding acid to the wells, and the intensity of the color was measured at 450 nm by a microplate reader (M200 Pro, Tecan). The cytokine concentration was determined by comparing the O.D. of samples to the standard curves. Three parallel samples were measured for each specimen by using the same protocol.

8. Cell apoptosis assay

The cell apoptosis was tested by using Annexin V-FITC/PI Apoptosis Detection Kit. The

RAW264.7 cells were seeded onto 24-well plates at a density of 7.5 × 10^4 cells/well and were incubated for 24 h. Then CSEG-g-Chol MGs and ADEG-g-Chol MGs were added to each well (particles: cells = 15:1) and were incubated with cells for 24 h. Then the supernatant was removed. The remaining cells were carefully washed with PBS for three times to remove the free particles. The cells were then detached with trypsinization, and were combined with the supernatant that may contain apoptosis cells. After centrifugation to collect the cells, they were washed with PBS to remove the trypsin. The cells were then dispersed in 400 μL binding buffer and were incubated with 5 μL Annexin V-FITC at room temperature for 15 min in dark, followed with 10 μL Propidium Iodide (PI) staining in ice-bath for 5 min in dark. The ratio of apoptosis cells was finally measured with flow cytometry (FACS Calibur, BD). Cells that are in early apoptosis are FITC Annexin V positive and PI negative; and cells that are in late apoptosis or already dead are both FITC Annexin V and PI positive. Data were analyzed by adding the ratio of apoptosis cells in early and late apoptosis.

References

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2. R. S. Shirazi, K. K. Ewert, C. Leal, R. N. Majzoub, N. F. Bouxsein and C. R. Safinya, *Bba-Biomembranes*, 2011, **1808**, 2156-2166.