Electronic Supporting Information

Albumin-Micelles *via* an one-pot Technology Platform for the Delivery of Drugs

Yanyan Jiang, Mingtao Liang, Domenic Svejkar, Hongxu Lu, Gene Hart-Smith, Wei Scarano and Martina H Stenzel

Contents

Materials and Analyses

Chemicals

Unless otherwise specified, all chemicals were reagent grade and used as received. 4',4-azobis(4-cyanopentanoic acid) (Fluka, 98%), 4-(Dimethylamino)pyridine (DMAP, Aldrich, >99%), anhydrous methanol (Sigma-Aldrich, 99.8%), benzyl chloride (Aldrich, 99%), bovine serum albumin (BSA, Sigma, >96%), chloroform-D (CIL, 99.8%), curcumin (Sigma), diethyl ether (Ajax, 98%), dimethylacetamide (DMAc,Sigma-Aldrich, 99.9%), dimethyl sulfoxide (DMSO, Ajax, 98%), elemental sulphur (Ajax, 98%), ethanolamine (Ajax, 97%), ethyl acetate (Ajax, 99%), furan (Aldrich, >99%), hydrochloric acid (Ajax, 31.5% w/w), maleic anhydride (Fluka, >99%), n-hexane (Ajax, >95%), *N*,*N*-dimethylformamide (DMF, Ajax, 99.8%), *N,N'*-dicyclohexylcarbodiimide (DCC, Aldrich, 99%), poly(ethylene glycol) methyl ether methacrylate (PEGMEMA, Mn=300 $g.mol^{-1}$, Aldrich, reagent), potassium ferricyanide (Sigma-Aldrich, 99%), silica gel (Sigma-Aldrich, 60 Å,70-230 mesh), sodium methoxide (Fluka, >97%), sodium phosphate dibasic (Sigma-Aldrich, 98%), sodium phosphate monobasic (Sigma-Aldrich, >99%),sodium hydroxide (Aldrich, 98%), sodium chloride (Univar, reagent), 2,2',2",2"'-(ethane-1,2-diyldinitrilo) tetraacetic acid (EDTA, Sigma-Aldrich, reagent), tetrahydrofuran (THF, Fisher Scientific, HPLC Grade, >99.9%), and toluene (Ajax, 99%) were used as received. 2,2-Azobisisobutyronitrile (Fluka, 98%) was purified by recrystallization from methanol The synthesis of the RAFT agent 4-cyanopentanoic acid dithiobenzoate (CPADB) was described elsewhere. [26] Methyl methacrylate (Aldrich, 99%, <30ppm MEHG inhibitor was purified by passing through a column of activated basic alumina to remove the inhibitor.

Analysis Techniques

Proton Nuclear Magnetic Resonance (^{1}H-NMR) Spectroscopy

All NMR measurements were performed using a Bruker DPX-300 with a ^{1}H/ X inverse broadband z gradient BBI probe at 300 MHz frequencies in deuterated chloroform ($CDCl_3$) using 16 scans as default and HSQC NMR in d-DMSO.

Dynamic Light Scattering (DLS)

DLS was performed by preparation of 1 $mg.mL^{-1}$ aqueous solutions of protein-polymer aggregates filtered through 0.45 µm filters and analysed on a Brookhaven ZetaPlus Particle Sizer at 25 °C with a Dust-Cut-off of 40.

DMAc Gel Permeation Chromatography (DMAc GPC)

DMAc GPC was performed using a Shimadzu modular system containing a DGU-12A degasser, an LC-10AT pump, an SIL-10AD automatic injector, a CTO-10A column oven and a RID-10A refractive index detector. A 50x7.8 mm guard column and four 300x7.8 mm linear columns (500, 10^3, 10^4, 10^5 Å pore size, 5 µm particle size) were used for analyses. *N*,*N*-dimethylacetamide (HPLC grade, 0.05% w/v BHT, 0.03% w/v LiBr) with a flow rate of 1 $mL.min^{-1}$ was used as the mobile phase. The injection volume was 50 µL. The samples were prepared by dissolving 2-3 $mg.mL^{-1}$ of the analyte in *N*,*N*-dimethylacetamide, followed by filtration through a 0.45 µm filter. The unit was calibrated using commercially available linear polystyrene standards (0.5-1000 kDa, Polymer Laboratories). Chromatograms were processed using Cirrus 2.0 software (Polymer Laboratories).

THF Gel Permeation Chromatography (THF GPC)

THF GPC was performed using a Shimadzu modular system containing a DGU-12A degasser, an LC-10AT pump, an SIL-10AD automatic injector, a CTO-10A column oven and a RID-10A refractive index detector. A 50x7.8 mm guard column and four 300x7.8 mm linear columns (500, 10^3, 10^4, 10^5 Å pore size, 5 µm particle size) were used for analyses. Tetrahydrofuran (THF, HPLC Grade) with a flow rate of 1 $mL.min^{-1}$ was used as the mobile phase. The injection volume was 50 µL. The samples were prepared by dissolving 2-3 $mg.mL^{-1}$ of the analyte in tetrahydrofuran, followed by filtration through a 0.45 µm filter. The unit was calibrated using commercially available linear polystyrene standards (0.5-1000 kDa, Polymer Laboratories). Chromatograms were processed using Cirrus 2.0 software (Polymer Laboratories).

Water Gel Permeation Chromatography (Water GPC)

Water GPC (water containing 0.02% w/v NaN_3) was performed using a Shimadzu modular system comprising a DGU-12A solvent degasser, an LC-10AT pump, a CTO-10A column oven, and a RID-10A refractive index detector (flow rate: 0.8 $mL.min^{-1}$). The system was equipped with a Polymer Laboratories 5.0 mm bead-size guard column (50 × 7.8 mm^2)

followed by three 300 × 7.8 mm 2 linear PL columns (30, 40 and 50 respectively in type). Calibration was performed with PEO standards ranging from 500 to 500,000 g/mol.

Transmission Electron Microscopy (TEM)

TEM analyses were performed using a JEOL1400 TEM and a FEI Tecnai-G2 at 80-100 kV beam voltage. Samples were prepared by placing a droplet of solution on formamide and graphite-coated copper grids and draining the excess using filter paper. Stained samples were exposed to uranyl acetate (3% aqueous solution) for 20 seconds once completely dry.

Ultraviolet-Visible Spectroscopy (UV-Vis)

UV-Vis measurements were performed on a double beam VARIAN Cary 300 UV-Vis Spectrophotometer (PerkinElmer Differential Scanning Calorimeter) (<5 Abs, λ = 190-900 nm) over the visible range (λ = 400-800 nm) at 25 °C. Samples containing Curcumin were dissolved in *N*,*N*-dimethylformamide and measured in a quartz cell with a path length of 10 mm.

Laser Scanning Confocal Microscopy

A laser scanning confocal microscope system (Zeiss LSM 780) was used to observe the cell uptaking of the albumin micelle. The system equipped with a Diode 405-30 laser, an argon laser and a DPSS 561-10 laser (excitation and absorbance wavelengths: 405 nm, 488 nm and 561 nm, respectively) connected to a Zeiss Axio Observer.Z1 inverted microscope (oil immersion ×100 /1.4 NA objective). The ZEN2011 imaging software (Zeiss) was used for image acquisition and processing.

Mass spec analysis

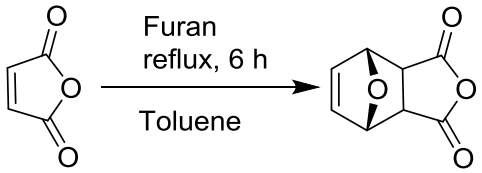
Analyte solutions were prepared for MALDI analysis using 2,5-dihydroxybenzoic acid (DHB) (20 mg/mL in 70:30 acetonitrile:0.1% tetrafluoroacetic acid) as the matrix; samples were dissolved in 70:30 acetonitrile:0.1% tetrafluoroacetic acid (~2 mg/mL). Matrix and sample solutions were mixed 1:1, 0.5 μL of this mixture was spotted on the sample plate, and the spots were dried in air at room temperature.

MALDI mass spectra were recorded on a Bruker ultrafleXtreme MALDI–TOF/TOF spectrometer (Bruker, Bremen, Germany) using the following instrument parameters: accelerating potential = 25 kV, pulsed ion extraction = 450 ns, and laser frequency = 2000 Hz. The data from approximately 5000 shots were signal averaged to obtain a final spectrum. All data were processed using the Bruker flexAnalysis (version 3.4) software package.

Synthesis

Synthesis of end-functionalised RAFT agent

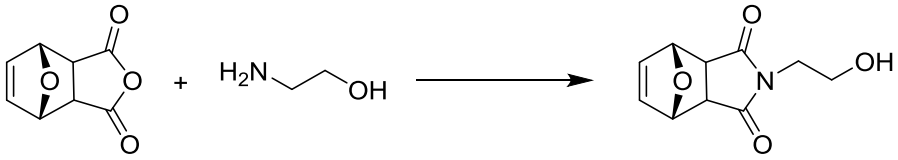
Step 1: Synthesis of 4,10-Dioxatricyclo[5.2.1.02,6]dec-8-ene-3,5-dione



Maleic anhydride (30.0 g, 306 mmol) was suspended in 150 mL of toluene and the mixture warmed to 80 °C. Furan (33.4 mL, 459 mmol) was added via syringe and the turbid solution stirred for 24 hours in an oil bath at this temperature. The mixture was then cooled to ambient temperature and the stirring stopped. After 1 hour the resulting white crystals were collected by filtration and washed with 2x30 mL of petroleum ether to obtain 44.4 g (267 mmol, 87% yield) of the product as small white needles.

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 3.17 (s, 2H, CH), 5.45 (t, 2H, J=1.0 Hz, CHO), 6.57 (t, 2H, J=1.0 Hz, CH_{vinyl})

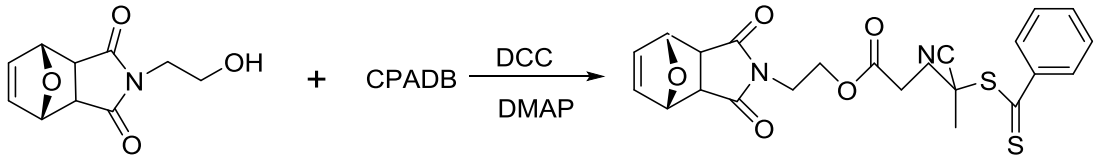
Step 2: Synthesis of 4-(2-Hydroxy-ethyl)-10-oxa-4-aza-tricyclo[5.2.1.02,6]-dec-8-ene-3,5-dione



To a dry 100 mL round-bottomed flask, 4, 10-dioxatricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (10.0 g, 60.2 mmol), anhydrous methanol (20 mL) and ethanolamine (3.68 g, 60.2 mmol) were added. The mixture was brought to reflux under stirring and the solution turned dark orange. After 24 hours, the reaction mixture was cooled to room temperature and product began to crystallize after 2 hours. The mixture was stored in the freezer overnight, and the precipitate was collected by vacuum filtration. The filtrate volume was reduced by rotary evaporation and allowed to crystallize, and a second crop of crystals was collected (yield 59%).

^{1}H NMR (300 MHz, $CDCl_3$) δ = 6.535 (s, 2H), 5.108 (s, 2H), 3.402 (s, 4H), 2.912 (s, 2H)

Step 3: Synthesis of protected-maleimide CPADB RAFT agent MCPADB



Protected maleimide alcohol (0.25 g, 1.2 mmol), CPADB (0.29 g, 1.1 mmol), and DMAP (14mg, 0.11 mmol) were dissolved in dry THF (20 mL). Then DCC (0.27 g, 1.3 mmol) was added and the reaction mixture was stirred at room temperature for 12 hours. The reaction was monitored by TLC (hexane:EtOAc = 2:1) and the third spot was the product. The reaction mixture was filtered to remove the white precipitate. The filtrate was concentrated enough to afford crude red oil and the crude was further purified by silica column chromatography (using 3:1 hexane:EtOAc to wash out the first two side product and 1:1 hexane:EtOAc to wash out the target compound). If the product was still contaminated with dicyclohexyl urea (DCU), cold acetonitrile was used to precipitate DCU to afford the final product (0.34g).

$CDCl_3$ ^1H NMR (300 MHz, $CDCl_3$) δ = 7.75 (t, 2H), 7.42 (t, 1H), 7.25 (t, 2H) 6.38 (t, 2H), 5.12 (t, 2H), 4.13 (t, 2H), 3.60 (t, 2H), 2.70 (m, 2H), 2.2-2.5 (m, 4H), 1.78 (s, 3H)

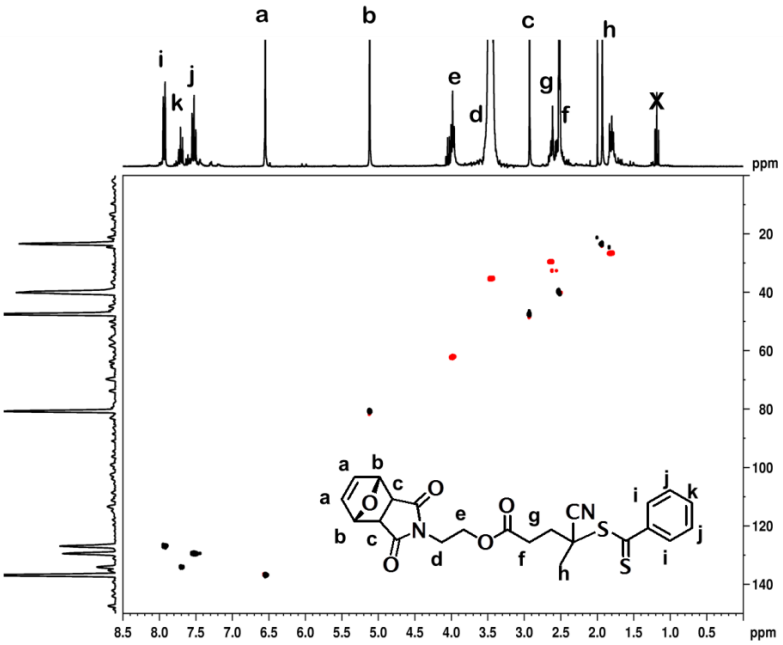
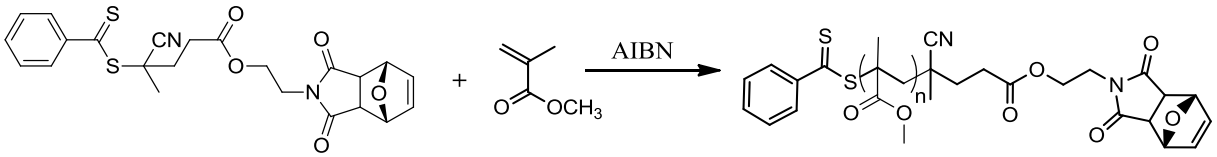


Figure S1. *2D HSQC NMR of a furan protected maleimide-CPADB RAFT agent, MCPADB (Black : –CH– and –CH_3, Red : –CH_2-)*

Synthesis and analysis of PMMA-BSA conjugates

Polymerisation of Methyl Methacrylate with protected-maleimide RAFT agent *MCPADB*



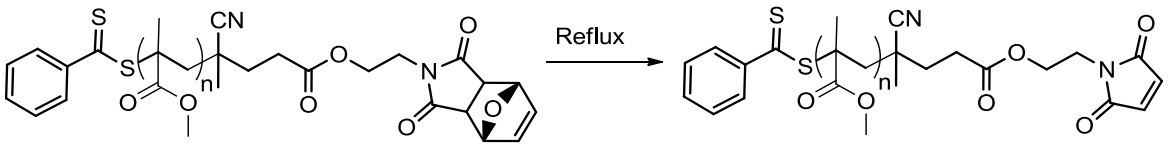
Protected-maleimide RAFT agent, AIBN initiator and methyl methacrylate monomer were dissolved in toluene in a cospak equipped with a magnetic stirrer bar. The solution was degassed by purging with nitrogen for 30 min in an ice bath, and the polymerisation was carried out at 70 °C for 20 hours under an inert atmosphere. The polymerization was terminated by placing the flask in an ice bath for 5 min and introducing air. The mixture was dried in an aluminium pan in a vacuum oven overnight at 40 °C. The number average molecular weight (M_n) and polydispersity index (M_w/M_n) were measured by gel permeation chromatography (GPC) using DMAc as solvent.

^{1}H NMR (300 MHz, $CDCl_3$) δ = 6.30 (s, 1H), 5.72 (s, 1H), 3.78 (t, 3H), 1.30 (s, 1H)

Table S1: *Polymerization of MMA in the presence of MCPADB (reaction time 15h)*

Sample	RAFT (mg) (MW = 470g/mol)	AIBN (mg) (MW = 164g/mol)	MMA (g) (Mw = 100g/mol)	Toluene (mL)	M_n (Da)	PDI
1	98	4.3	7.05	2	42500	1.15
2	93	4.2	3.71	10	20000	1.10
3	92	4.2	1.94	10	12000	1.13
4	225	8.7	2.40	10	8500	1.12
5	110	4.2	0.98	5	5400	1.13

Deprotection of End-Functionalised Poly(Methyl Methacrylate)



1.3 g polymer was dissolved in 50 mL toluene and the solution was brought to reflux at 110 °C for 7 hours. Therefore, the Diers-Alder protection reaction of the maleimide moiety attached to the RAFT agent on the ends of the polymer chains would be reversed. The solvent was evaporated and further dried in a vacuum oven at 40 °C overnight to afford the target maleimide-terminated PMMA. The formation of maleimide group was confirmed by ^{1}H-NMR with the presence of new proton peak at near 6.76. Care need to be taken at this stage since long reaction times or high temperature can lead to the formation of dimers that are visible as a high molecular weight shoulder.

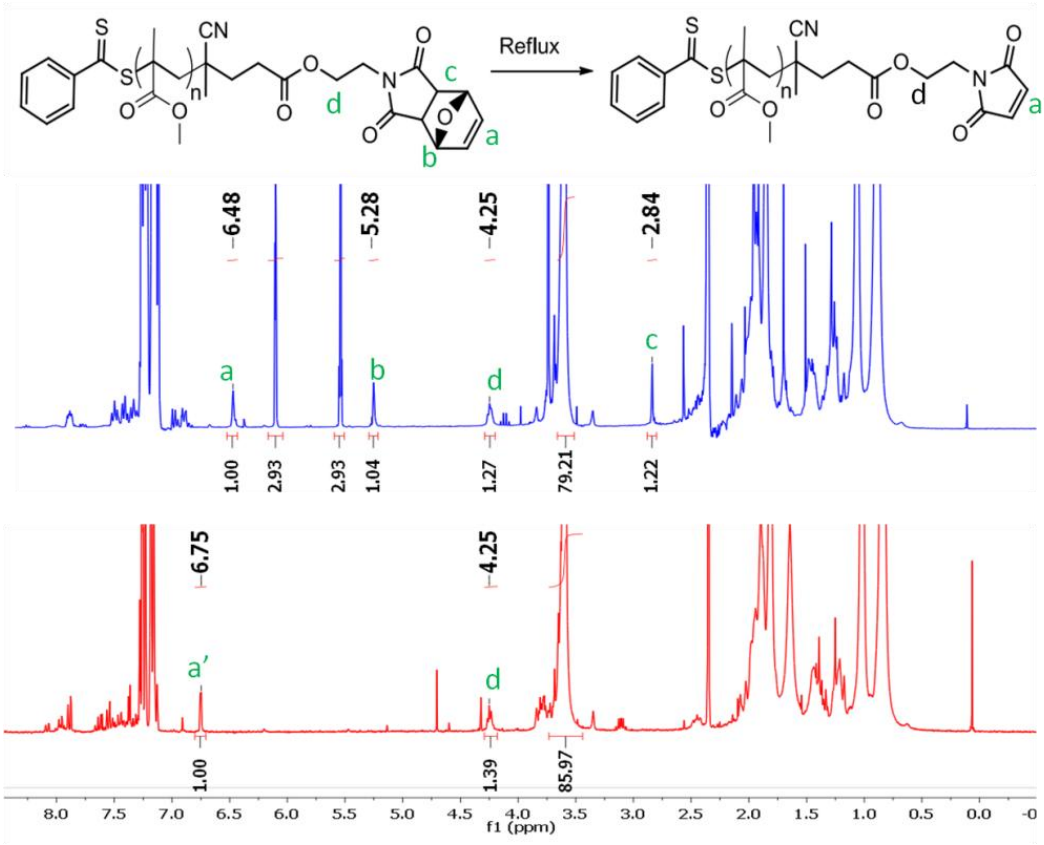


Figure S2. *^{1}H-NMR Spectra of Crude PMMA (blue) and deprotected PMMA (red). The removal of the peaks at 5.28 and 6.48 ppm and the appearance of the peak at 6.75 indicate successful deprotection of the maleimide end-group.*

Conjugation of Bovine Serum Albumin (BSA) to Poly(Methyl Methacrylate) Polymer Chains

Polymer (4.3 mg, 0.8 μmol) was dissolved in 2 mL of DMSO and a solution of bovine serum albumin (54 mg, 0.8 μmol) in 8 mL of PB buffer (0.1 M, pH 7.0) was added dropwise in the

rate of 0.2 mL/h via syringe pump into the polymer solution under stirring. The mixture was stirred for 48 hours and subsequently dialyzed against MilliQ water using a MWCO = 6000-8000 dialysis membrane. After dialysis, the mixture was subjected to a brief sonication for 5 min and centrifugation (2000g × 5min) to remove un-reacted PMMA polymer, and passed through a 0.45 μm (Millipore) filter to remove large particles. The supernatant containing albumin-PMMA conjugate (both filtered and non-filtered) was finally collected and analysed by DLS and TEM.

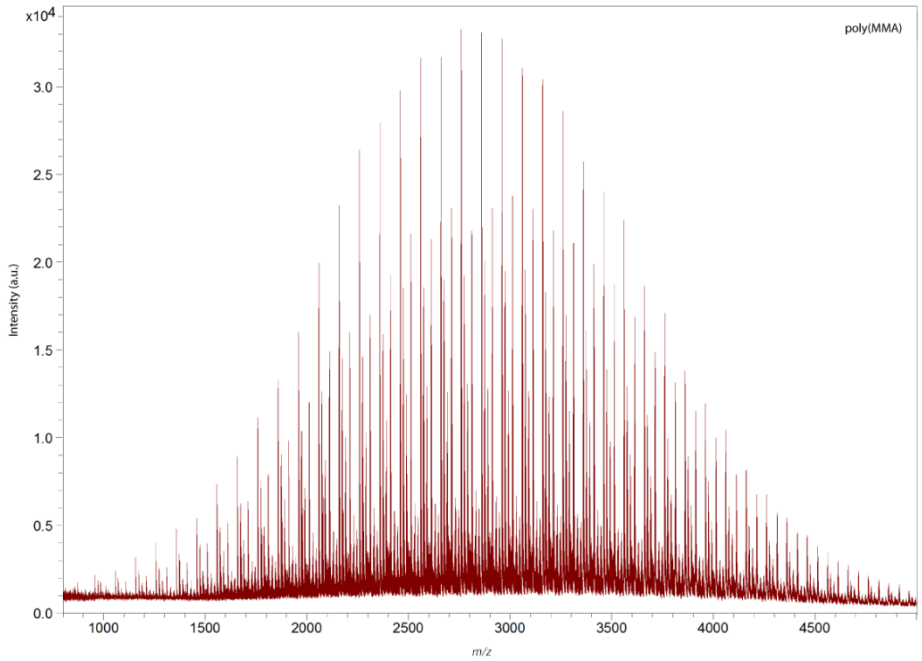


Figure S3 *MALDI-TOF spectrum of PMMA. 2,5-Dihydroxybenzoic acid (DHB) was used as the matrix.*

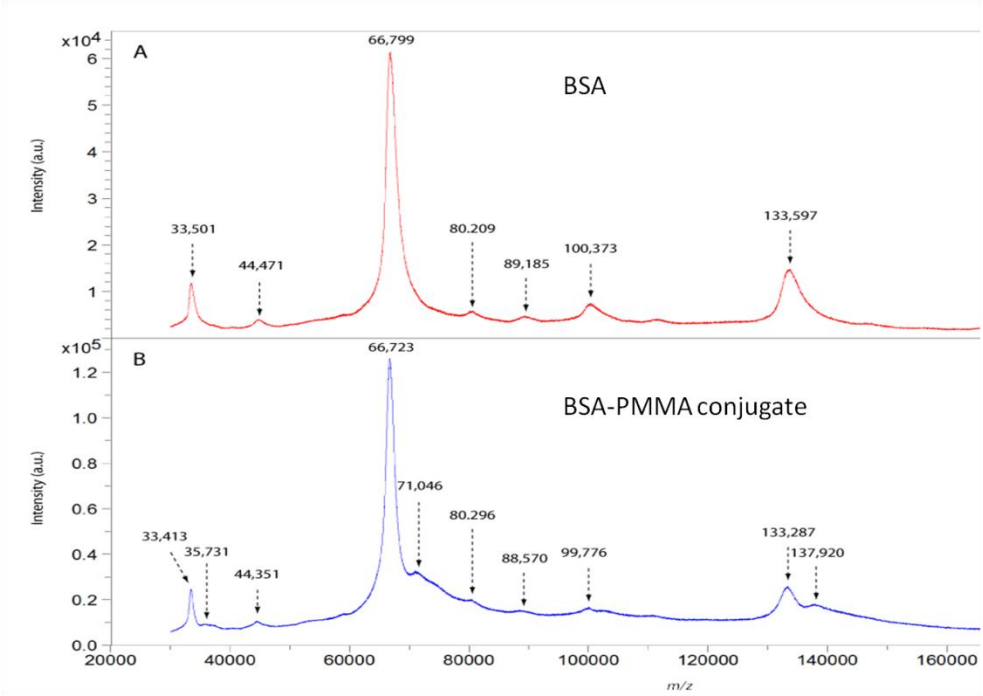


Figure S4 *MALDI-TOF spectrum of BSA (A) and BSA-PMMA conjugate (B). 2,5-Dihydroxybenzoic acid (DHB) was used as the matrix.*

The BSA and PMMA conjugates without curcumin was carefully dialysed using a tubular membrane (MWCO = 3500) in MilliQ water and then freeze dried. The product was divided into two parts. One part was dissolved in THF to analyse the amount of residual PMMA still in the mixture; and the other part was dissolved in water to determine the amount of free BSA. At the same time, the initial amount of PMMA and BSA has also been measured prior to reaction with THF and Water GPC respectively. Suspension of the final product in THF or water led to the phase separation of the BSA polymer conjugate, free BSA (soluble in water) and the unreacted PMMA (soluble in THF). From the THF GPC result, equimolar amount of albumin and PMMA led to a conjugation efficiency of about 86.6 %, whereas the conjugation rate from the water GPC is 58%. The difference of these two results is likely due to the difficulty to fully dissolve free PMMA from the particle. The results show in Fig. S9 and S10. A control experiment has also been carried out by mixing BSA and PMMA without maleimide endgroup and the same measurement has been made with THF GPC. From the Figure S11, it is clear that no similar nano aggregation happened if no maleimide endgroup exists.

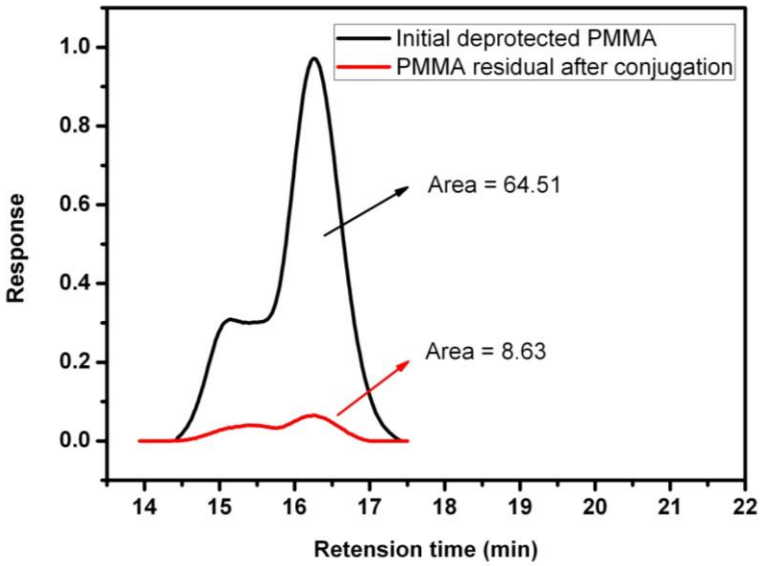


Figure S5. *THF GPC traces of the conjugation of BSA and PMMA (molar ratio= 1;1): Initial PMMA (black line) and PMMA residual after conjugation (red line) indicating a conjugation efficiency of 87%, which may be the result of incomplete dissolution of PMMA in THF. (Please note: the sample used here was of poor quality and was only employed for demonstration purposes in this experiment. A typical PMMA distribution is depicted in Fig. S7)*

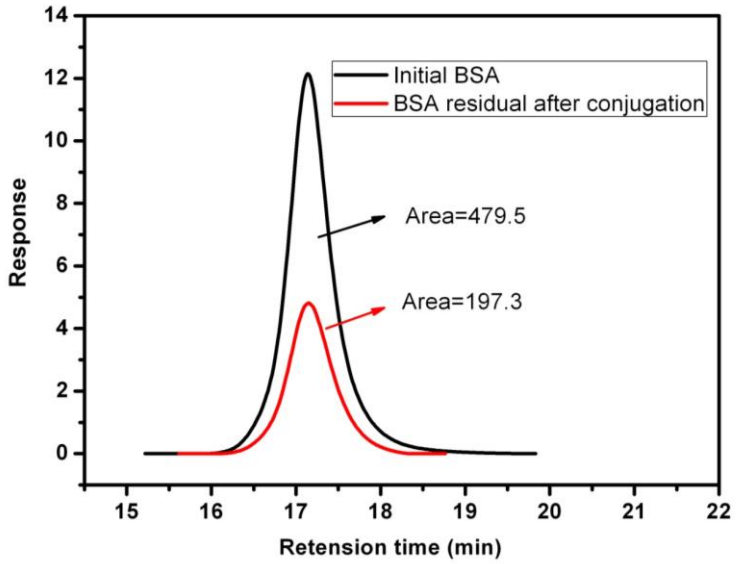


Figure S6. *Water GPC traces of the conjugation of BSA and PMMA (molar ratio= 1;1): Initial BSA (black line) and BSA residual after conjugation (red line). The conjugation efficiency is around 58%, which is also in agreement that not all BSA molecules are active for conjugation.*

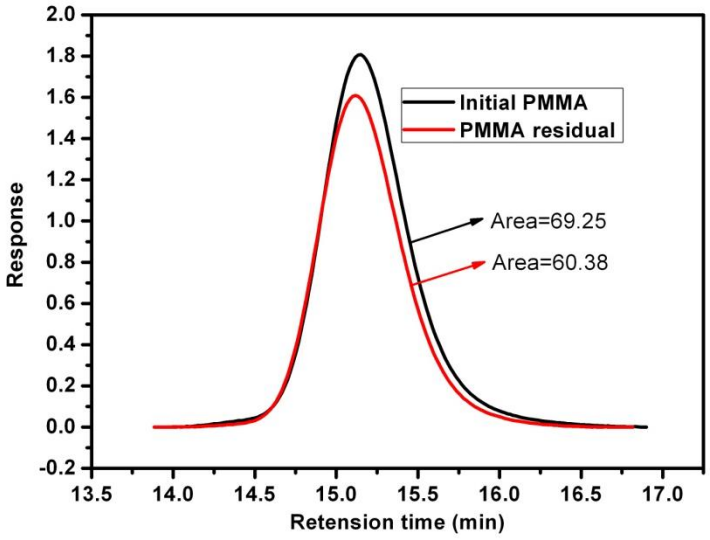


Figure S7. *THF GPC traces of the conjugation of BSA and PMMA without maleimide end group (molar ratio= 1;1): Initial PMMA (black line) and PMMA residual after conjugation (red line), which indicates that no conjugation happens in this mixture.*

Synthesis of Curcumin-Encapsulated Polymer-Protein Micelles

A stock solution was prepared by dissolving curcumin (8 mg) dissolved in DMSO (10 mL) to give concentration of 0.8 mg/mL and small aliquots were removed via micro-pipette. Polymer (4.3mg, 0.8 μmol) and curcumin (0.16 μmol) were dissolved in 2 mL of DMSO and a solution of bovine serum albumin (54 mg, 0.8 μmol) in 8 mL of PB buffer (0.1 M, pH 7.0) was added dropwise in the rate of 0.2 mL/h via syringe pump into this solution under stirring. The mixture was stirred for 48 hours and subsequently dialyzed against deionised water using a MWCO = 6000-8000 dialysis membrane. After dialysis, the mixture was subjected to a brief sonication for 5 min and centrifugation (2000g × 5min) to remove un-reacted PMMA polymer, and passed through a 0.45 μm (Millipore) filter to remove large particles. The supernatant containing albumin-PMMA conjugate (both filtered and non-filtered) was finally collected and analysed by DLS and TEM. A calibration curve was created testing the absorbance of a range of concentrations of curcumin dissolved in DMF (0.001 – 0.03 $mg.mL^{-1}$) through UV-Vis Spectroscopy (λ = 430 nm), and the polymer-protein conjugate sample was analyzed with the results compared to this graph.

Table S2. *Absolute mass of polymer, albumin and curcumin in the preparation of drug loaded albumin micelles*

Sample Name/ Description	Curcumin: Polymer Molar Ratio	Mass of Polymer /mg	Mass of Albumin /mg	Volume of DMSO /mL	Volume of PBS Buffer /mL	Mass of Curcumin /mg	DLS Average Number Distribution /nm
15 P/C	1:15	10	115.7	2	8	0.0667	112.4
15 P/C	1:15	10.3	117.2	4	6	0.067	119.6
15 P/C	1:15	9.9	117.5	3	7	0.067	50.8
10 P/C	1:10	10.0	117.0	3	7	0.1	59.0
5 P/C	1:5	10	120.8	3	7	0.200	91.3

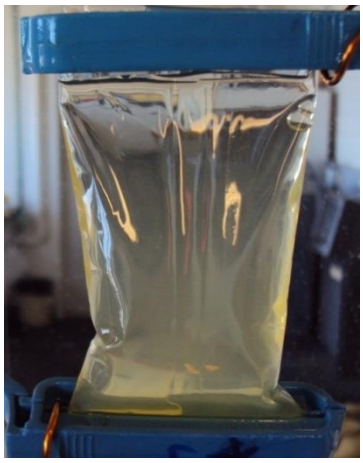


Figure S8. *Image of a dialysis membrane containing polymer-protein aggregates with a 1:5 curcumin to polymer ratio*

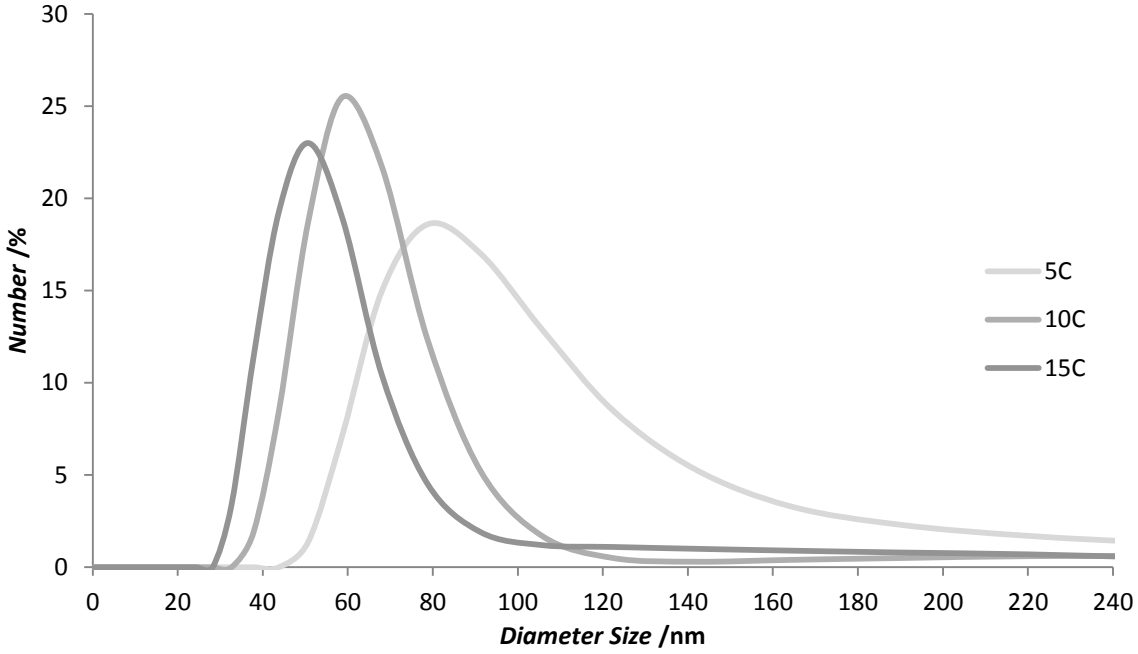


Figure S9. *DLS Average Number Distribution of PMMA-3000 aggregates with varying curcumin: polymer ratios, 1:5 (5C), 1:10 (10C) and 1:15 (15C)*

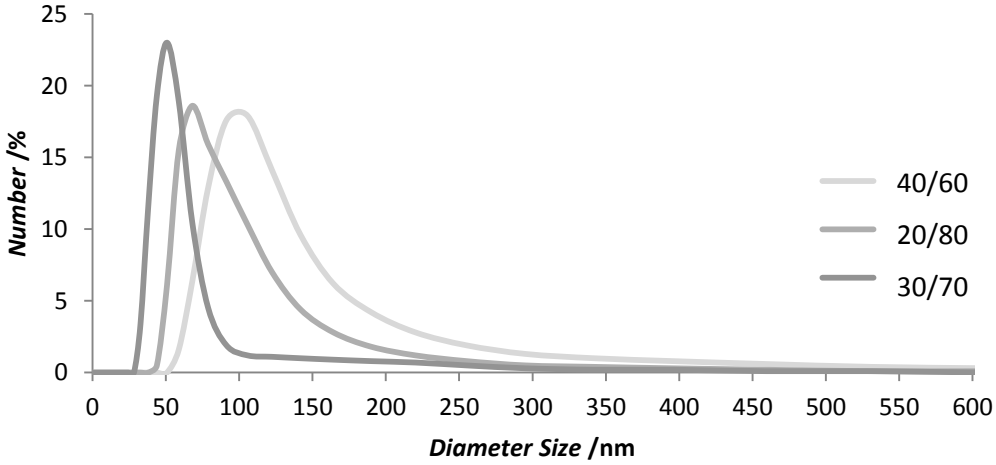


Figure S10: *Hydrodynamic diameter of albumin-micelle particles loaded with curcumin in a 1:15 ratio with polymer, prepared using various ratios of DMSO/water*

Measurement of Drug Loading Efficiency of Protein-Polymer conjugated micelles

The polymer-protein micelle with curcumin loaded in was used for the measurement of encapsulation efficiency of curcumin. After 48 hours of dialysis in deionised water, 100 μL

of sample was removed from the dialysis membrane and passed through a 0.45 μm (Millipore) filter to remove precipitated drug that would provide false encapsulation efficiency. After freeze drying, approximately 3 mL of DMF was added to re-dissolve the sample and UV-Vis Spectroscopy was undertaken, with the absorbance compared to the calibration curve created in Figure S6, and the concentration back-calculated to determine the amount of curcumin inside the conjugated micelles. The encapsulation efficiency and entrapment efficiency was then calculated according to **Equation 1**, with encapsulated curcumin determined dividing the total amount of curcumin used.

Encapsulation Efficiency of Curcumin within Polymer-Protein Conjugated Micelles

$$Drug\ Encapsulation\ \% = \frac{Encapsulated\ curcumin}{Total\ amount\ of\ curcumin\ used}\ x\ 100 \qquad \text{Equation 1}$$

$$Entrapment\ efficiency = \frac{Total\ amount\ of\ curcumin}{Total\ amount\ of\ polymer}\ x\ 100 \qquad \text{Equation 2}$$

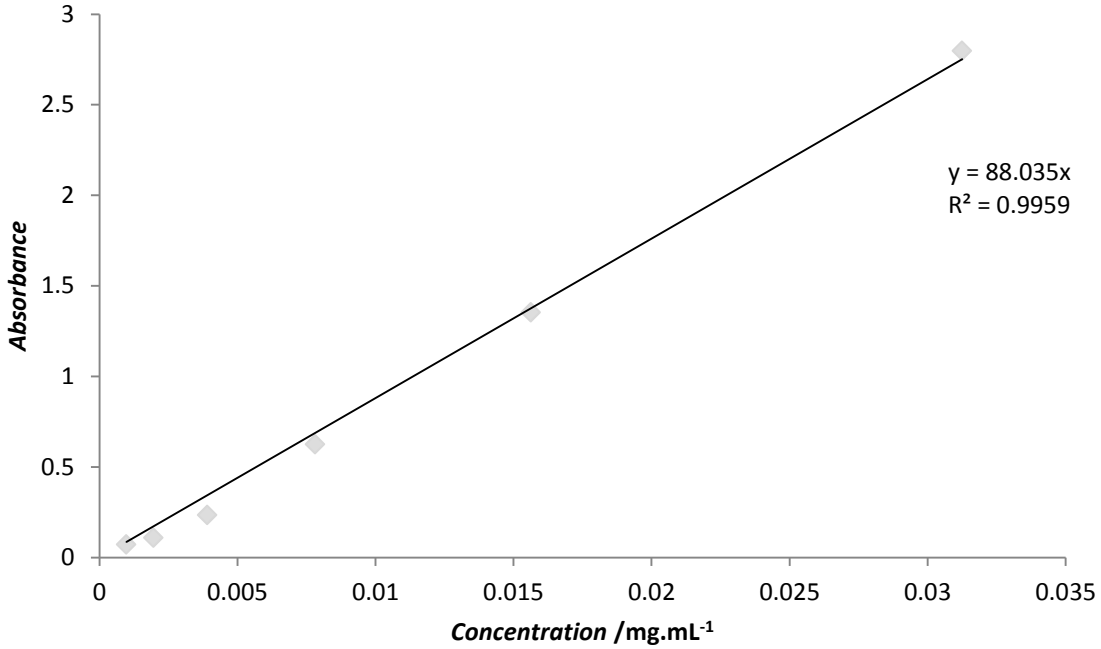


Figure S11. *Calibration curve generated from Curcumin dissolved in DMF absorbed at 430 nm. A linear trendline that runs through the origin was applied, with an R^2 value of 0.9959 indicating a very strong correlation*

Measurement of Drug Release Rate from Protein-Polymer conjugated micelles

2 mL of the polymer-protein curcumin carrying micelle solution was dialysed in 200 mL of phosphate buffer (pH=7.4) at 37^{o}C. Aliquots of the samples which were taken out from the membrane tube at different time points over the period of 96 hours were then freeze dried and redissolved in DMF. The samples were then analysed via UV-Vis spectrometry and their absorbance was compared to the standard curve.

Synthesis of P(OEGMEMA-co-MAA)-b-PMMA control polymer

CPADB RAFT agent (18.6 mg, 66.6 μmol), AIBN initiator (2.19 mg, 13 μmol) and OEGMEMA monomer (10 g, 0.03mol) and 5 mol-% MAA (0.129 g, 1.5 mmol) were dissolved in toluene (14.8 mL, 2.25 M) in a cospak equipped with a magnetic stirrer bar. The solution was degassed by purging with nitrogen for 30 min in ice bath, and the polymerisation was carried out in an oil bath at 70 °C for 4 hours under an inert atmosphere. After quenching the reaction and introducing air in the vial, the reaction mixture was added dropwise into a large excess of mixture of diethyl ether. The precipitated Poly(OEGMEMA-co-MAA) was then dried overnight in a vacuum oven at 40 °C. The number average molecular weight (M_n) and polydispersity index (M_w/M_n) were measured by gel permeation chromatography (GPC) using DMAc as solvent.

^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.33 (s, 1H), 5.67 (s, 1H), 4.19 (m, 2H)

Poly(OEGMEMA-co-MAA) was used as macroRAFT initiator (0.5 g, 6.87 μmol), and was dissolved in toluene (0.21 mL, 1M) with AIBN initiator (0.23 mg, 1.37 μmol) and MMA monomer (0.021 g, 0.21 mmol) in a flask equipped with stirrer bar. The solution was degassed by purging with nitrogen for 30 min in ice bath, and the polymerisation was carried out at 70 °C for 20 hours under an inert atmosphere. After quenching the reaction with liquid nitrogen, the reaction mixture was dissolved in toluene and purified by precipitation in diethyl ether. The solvent was decanted off and the product dried in a vacuum oven overnight at 40 °C. The number average molecular weight (M_n) and polydispersity index (M_w/M_n) were measured by gel permeation chromatography (GPC) using DMAc as solvent.

^{1}H NMR (300 MHz, $CDCl_3$) δ = 6.30 (s, 1H), 5.72 (s, 1H), 3.78 (t, 3H), 1.30 (s, 1H)

Preparation of P(OEGMEMA-co-MAA)-*block*-PMMA micelles

$PEGMEMA_{104}$-MAA_5-MMA_{74} was prepared to form micelles in the controlled experiment to evaluate the effect of the albumin shell and cellular uptake. The block copolymer micelle was

obtained by dissolving 30 mg of polymer in 2 ml of DMSO. A stock solution of curcumin was prepared (0.8 mg/mL) and 74 µL was added into the polymer solution. Distilled water (4mL) was added into the mixed solution dropwise at the rate of 0.2 mL/h. Then, the sample was dialysed against distilled water suing membrane (MWCO: 12000-14000) for 24 hrs to remove the DMSO. The distilled water was replaced every 3 h. The hydrodynamic diameter D_h of micelles and the PdI was measured with Dynamic Light Scattering (DLS). Samples were filtrated before analysis

Cell uptake study using laser scanning confocal microscopy

Human ovarian carcinoma Ovcar-3 cells were seeded in 35mm Fluorodish (World Precision Instruments) at a density of 60,000 per dish and cultured for 3 days with RPMI 1640 medium supplemented with 10% fetal bovine serum. Micelles solution was loaded to Ovcar-3 cells at a working concentration of 50 µg/mL and incubated at 37 ^{o}C for 2 hrs. After incubation, the cells were washed twice with phosphate buffered saline (PBS, pH 7.4). Then the cells were stained with 100 nM LysoTracker Red DND-99 (Invitrogen) for 1 min. The dye solution was quickly removed and the cells were gently washed with PBS. Finally, the cells were mounted in PBS and observed under a laser scanning confocal microscope system (Zeiss LSM 780).. The ZEN2011 imaging software (Zeiss) was used for image acquisition and processing.

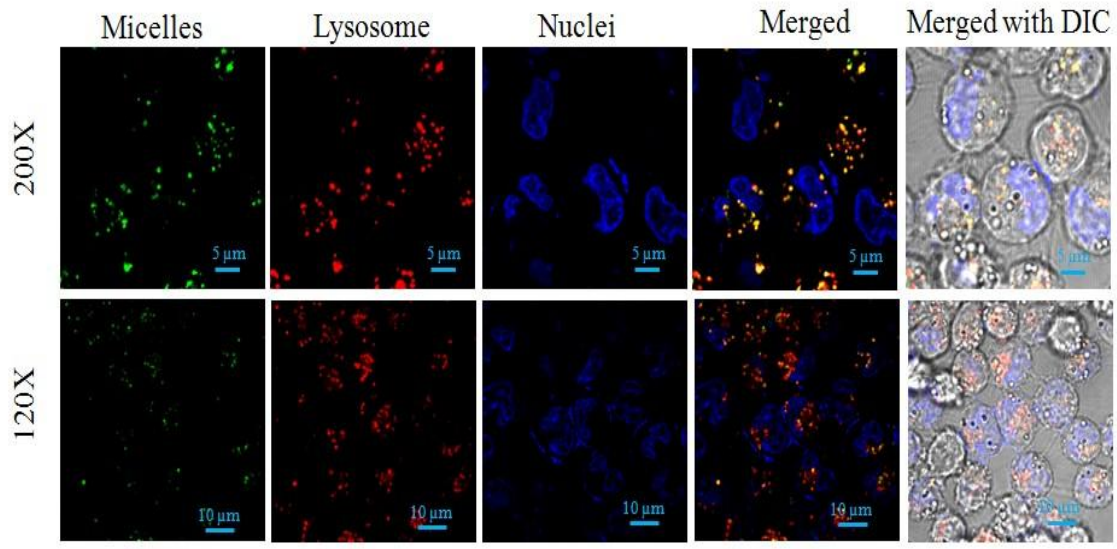


Figure S12. *Confocal microphotographs of Ovcar-3 cells after incubation with micelles at 37 ^{o}C for 2 hours at magnifications of 200X (upper part) and 120X (lower part). Micelles (Green) were labelled with curcumin loaded. Cell nuclei (blue) were stained with Hoechst 33342. Lysosomes (Red) were stained with LysoTracker Red DND-99. Scale bar is 5 µm for the 200X images and 10 µm for the 120X images.*

In vitro Cytotoxicity Test

The cytotoxicity of the albumin conjugated PMMA micelles with the encapsulation of curcumin and the $PEGMEMA_{104}$-MAA_5-MMA_{74} block copolymer micelles with the encapsulation of curcumin was measured by a standard sulforhodamine B colorimetric proliferation assay (SRB assay). The SRB assay was established by the U.S. National Cancer Institute for rapid, sensitive, and inexpensive screening of antitumor drugs in microtiter plates. The AsPC human pancreas adenocarcinoma cells, the A2780 human ovarian cancer cells and the Chinese hamster ovary (CHO) non cancerous cells were seeded at the corresponding density of 4000 and 8000 cells per well in 96-well microtiter plates followed by the addition 200 μL of culture medium per well and incubated at 37 ° C in a 5% CO_2 for 24 h. The medium was then replaced with fresh medium (200 μL) containing gradient concentrations of these two types of curcumin-loaded micelles and incubated in the same conditions for 48 h. Non-treated cells were used as controls.

After 48 h incubation, cells were fixed with trichloroacetic acid 10% w/v (TCA) by incubated at 4 ° C for 40 min, and then washed five times with tap water to get rid of the TCA and culture medium reminder. Next, TCA- fixed cells were stained for 30 min with 0.4% (w/v) SRB dissolved in 1% acetic acid. At the end of the staining period, SRB outside the cells was removed by washing the plates with 1% acetic acid. The plates were left to air-dry overnight followed by the addition of 100 μL 10 Mm Tris buffer per well to dissolve the dye in the cells. The absorbance at 570 nm of each well was measured using a microtiter plate reader scanning spectrophotometer. IC50 inhibitory concentrations were estimated by regression analysis.

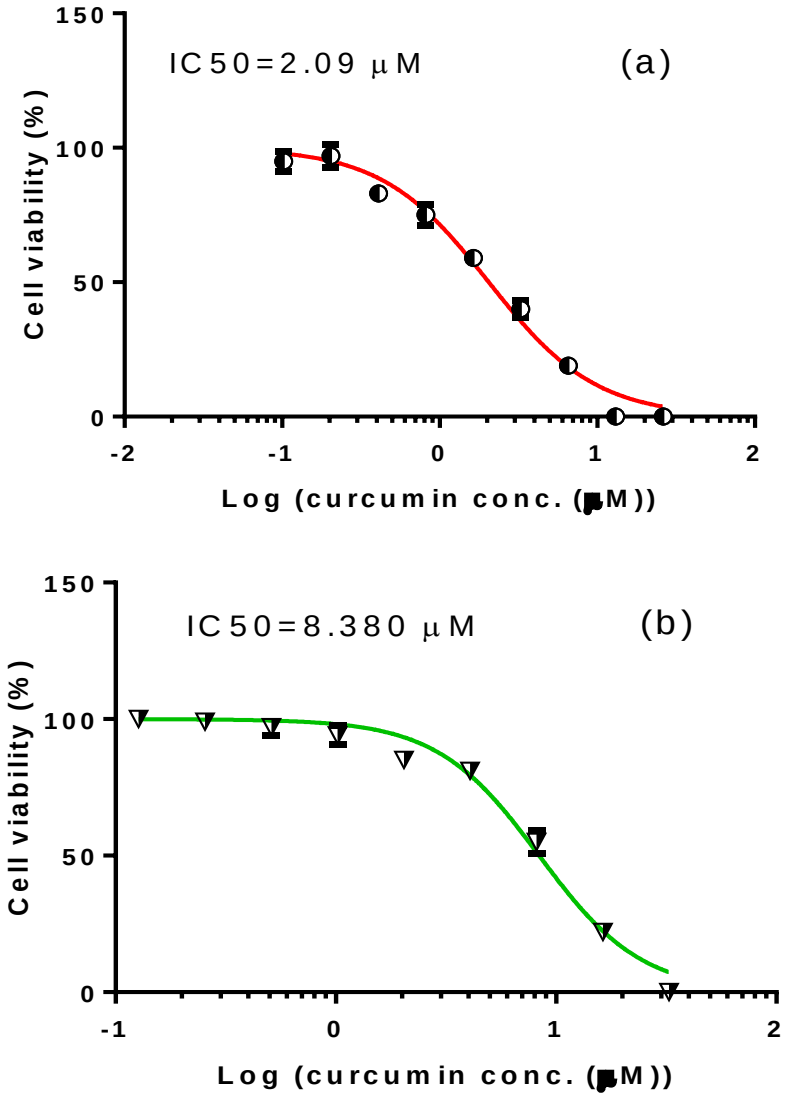


Figure S13. *Cytotoxicity profile of the curcumin-carrying micelle against A2780 cells for 48 hours. (a) Albumin micelle (b) P(OEGMEMA-co-MAA)-b-PMMA.*

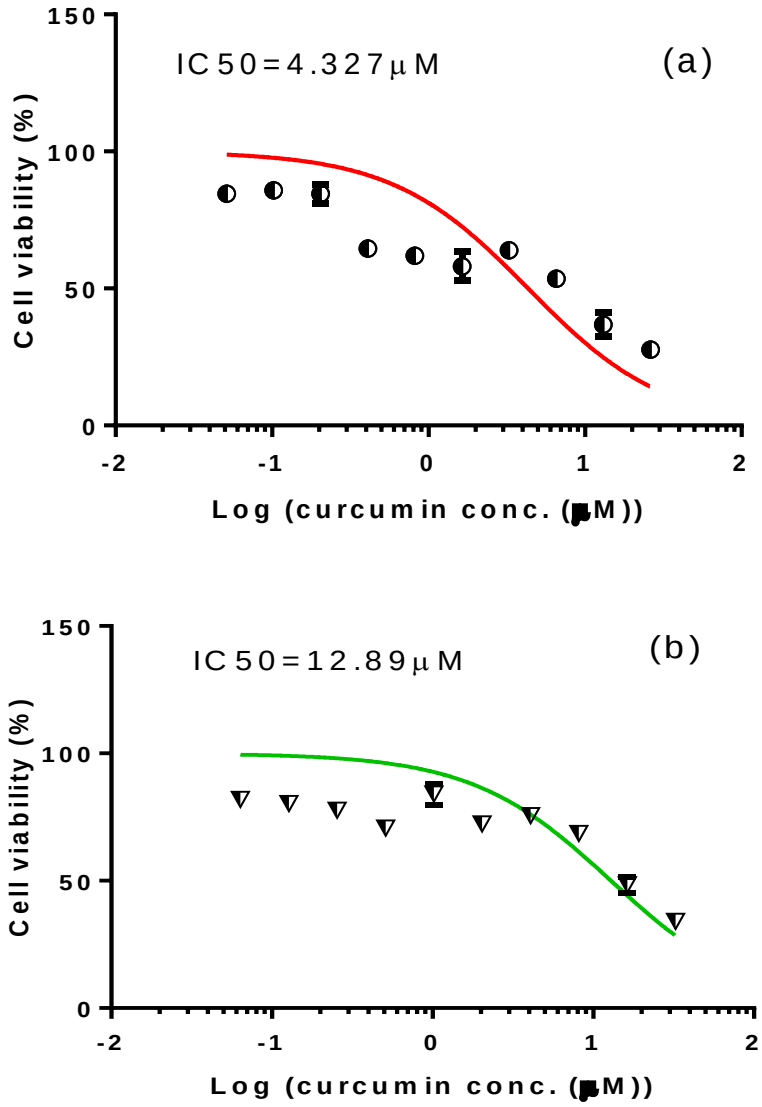


Figure S14. *Cytotoxicity profile of the curcumin-carrying micelle against AsPC cells for 48 hours. (a) Albumin micelle (b) P(OEGMEMA-co-MAA)-b-PMMA.*

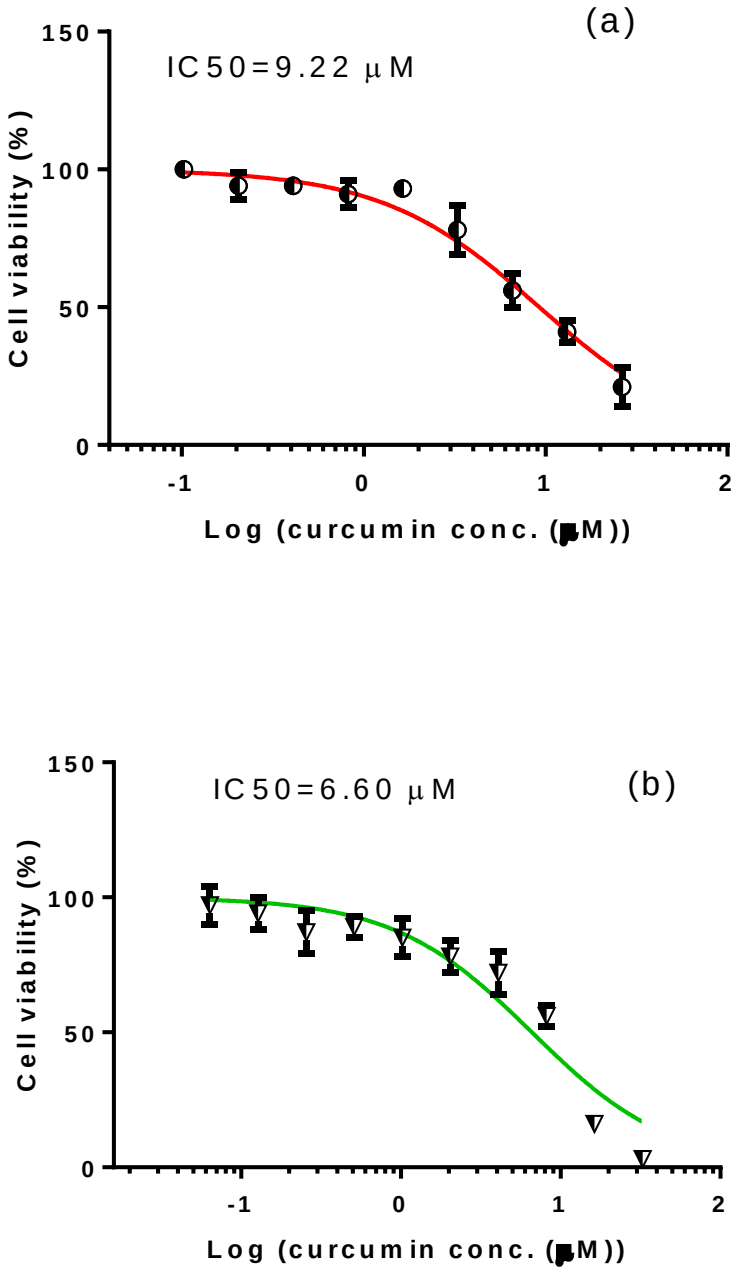


Figure S15. *Cytotoxicity profile of the curcumin-carrying micelle against CHO cells for 48 hours. (a) Albumin micelle (b) P(OEGMEMA-co-MAA)-b-PMMA*