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Supporting Information

Control of targeting ligand display by pH-responsive polymers on gold nanoparticles mediates selective entry into cancer cells

C. Brazzale, F. Mastrotto, P. Moody, P.D. Watson, A. Balasso, A. Malfanti, G. Mantovani, P. Caliceti, C. Alexander, A. T. Jones, S. Salmaso

Materials and methods

Chemicals and equipment. All chemicals and solvents were purchased from commercial sources and used without further purification except 2,2'-azobis(2-methylpropionitrile) (AIBN, Sigma-Aldrich, 98%), which was recrystallized from methanol and tetraethyleneglycol (TEG) that was dried azeotropically from toluene. Folic acid, lipoic acid, tris(2-carboxyethyl)phosphine hydrochloride), sodium hydroxide (NaOH), bicinchoninic acid (BCA), magnesium sulfate, glycidyl methacrylate, *N,N*'-dimethylamino pyridine (DMAP), anhydrous dimethylsulfoxide (DMSO), anhydrous *N,N*'-dimethylformamide (DMF), *N,N'*-dimethylacetamide (DMAC), dichloromethane (DCM), ethyl acetate (EtOAc), methanol (MeOH), diethyl ether (Et_2O), petroleum ether (b.p. 40-60 °C), nitric acid (HNO_3), hydrochloride acid (HCl), fetal bovine serum (FBS), folic free DMEM (FFDMEM), glutamine solution, penicillin-streptomycin-amphotericin B solution, glucose solution, trypsin solution, phosphate buffer (PBS), *N,N'*-dicyclohexylcarbodiimide (DCC), 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (CPADB), triethylamine (TEA), N-hydroxysuccinimide (NHS), 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), ethylenediaminetetraacetic acid (EDTA), sodium citrate dehydrate, tetrachloroauric (III) acid ($HAuCl_4$), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich (St. Louis MO, USA). Sephadex G25 superfine and Sephadex LH 20 resins were obtained from Pharmacia Biotech AB (Uppsala, Sweden). NH_2-PEG_{2kDa}-SH, mPEG_{2kDa}-SH were purchased from Iris Biotech GmbH (Marktredwitz, Germany). Bodipy FL-NHS was purchased from Lumiprobe GmbH (Hannover, Germany). Water for the preparation of all suspensions and solutions was "ultrapure" water (Milli-Q-grade, 0.06 μSiemens cm^{-1}) produced with a Millipore Milli-Q purification system (MA, USA). Salts and buffers were purchased from Fluka Analytical (Buchs SG, Switzerland) and Sigma-Aldrich (St. Louis, MO, USA). Human cervical carcinoma cells (KB) and human breast cancer cells (MCF-7) were acquired from the ATCC cell bank (Manassas, VA, U.S.A.).

Purification by dialysis was performed using Spectra/Por Float-a-lyzer G2 (MW cutoff = 0.5-1 kDa) purchased from SpectrumLabs (Rancho Dominguez, CA).

Reversed phase High-performance liquid chromatographic (RP-HPLC) analyses were carried out using Jasco HPLC system (Tokio, Japan), equipped with two PU-2080 Plus pumps, a UV-2075 Plus detector (set at 363 nm for folic acid and 503 for Bodipy FL detection), a Luna (C18, 5 μ, 300 Å, 250 x 4.6 mm) analytic column from Phenomenex (Torrance, CA, U.S.A.).

Spectrophotometric analyses were carried out with an UV-Vis λ25 Perkin Elmer spectrophotometer (Norworlk, CT, USA) and with Nanodrop 1000 Spectrophotometer (Thermo Scientific Wilmington, DE, USA).

Multiwell plate detections were carried out with Microplate Autoreader purchased from Biotek Instruments inc., mod. EL311SK (Highland, Vermont U.S.A.).

Fluorimetric analyses were performed using a LS 50 B Perkin-Elmer fluorimeter.

FT-IR analysis was carried out using an Agilent Cary 630 FTIR.

ESI-TOF analyses were performed using a Waters LCT-TOF mass spectrometer equipped with a Waters 2795 separations unit and electrospray ionisation. MALDI-TOF analyses were performed using 3,5-dimethoxy-4-hydroxycinnamic acid as matrix with an AB Sciex 4800 Plus MALDI-TOF Analyser (Framingham, MA, USA).
^{1}H and ^{13}C NMR spectrometric analyses were carried out using a Bruker DPX400 Ultrashield spectrometer and NMR spectra were processed with MestreNova 6.2.0 Software (Mestrelab Research SL, Santiago de Compostela, Spain).
Polymer molecular weight and polydispersivity index (Đ) were determined by gel permeation chromatography (GPC) using a Polymer Laboratories GPC 50 system (Polymer Laboratories) equipped with two columns connected in series (Agilent PLgel 5 µm Mixed D, 7,5 x 300 mm) and a RI detector, eluting with DMF + 0.1 % w/w LiBr at flow rate of 1 mL min^{-1}. The molecular weights and polidispersity indices of the polymers were calculated according to a calibration curve obtained with PMMA narrow standards (162-371,000 g mol^{-1}). Data were elaborated with Cirrus GPC/SEC 3.0 software.
Turbidimetric and potentiometric analyses were carried out using a VARIAN Cary 50Bio UV-visible spectrophotometer and 794 Basic Titrino pH meter, Metrohm (Varese, Italy).
Dynamic light scattering (DLS) analyses were performed at 25 °C using a Zetasizer NanoZS (Malvern instruments Ltd, UK) and data were acquired using Zetasizer Software (version 6.12).
Atomic absorption spectroscopy was carried out using a Varian AA240 Zeeman instrument equipped with a GTA120 graphite furnace, Zeeman background corrector and an autosampler (Varian Inc., Palo Alto, CA-USA) to quantify gold nanoparticles in cell samples.
Cells underwent cytofluorimetric analysis using a BD FACSDiva flow cytometer (Becton, Dickinson and Company, Buccinasco, Milan) and data were processed with BD FACSDiva Software package.
Transmission electron microscopy (TEM) was performed using a Tecnai G2 (FEI, Oregon, USA). Samples were placed on copper grid and the water was allowed to dry at room temperature before analysis was performed.
Leica TCS SP5 confocal laser-scanning microscope (Leica Microsystems GmbH, Wetzlar, Germany) equipped with a 488 nm Ar laser, 543/633 nm HeNe laser, 100 × 1.4 NA or 40x 1.4 NA objectives with Leica Type F immersion oil was used for cell microscopic imaging.
TEM and confocal images were processed with ImageJ open source Java image software package (National Institutes of Health, USA).

Synthesis of folate-PEG_{2kDa}-SH (FA-PEG_{2kDa}-SH).
Folic acid (100 mg, 0.226 mmol) was dissolved in anhydrous DMSO (1 mL) and NHS (31.2 mg, 0.271 mmol) and DCC (55.9 mg, 0.271 mmol) were added to the solution. The mixture was stirred overnight in the dark and then filtered to remove the insoluble dicyclohexylurea. The *N*-hydroxysuccinimidyl-ester-activated folic acid (FA-NHS) was isolated by precipitation in cold diethyl ether. The precipitate was filtered, rinsed with cold diethyl ether (40 mL x 5) and then dried under reduced pressure. FA-NHS (50 mg, 0.093 mmol) and NH_2-PEG_{2kDa}-SH (62.0 mg, 0.031 mmol) were dissolved in anhydrous DMSO (2 mL) and added of triethylamine (4.3 µL, 3.1 mg, 0.031 mmol). The reaction mixture was stirred for 12 hrs at room temperature in the dark and then added dropwise to diethyl ether (40 mL). The precipitate was recovered by centrifugation at 1950 x g and dried under vacuum. The crude product was purified from the excess of folic acid by size exclusion chromatography using a Sephadex G-25 resin run with aqueous ammonia solution (pH 9) as mobile phase. The column fractions were tested by UV-Vis spectroscopy (PBS, pH 7.4) at 363 nm and Iodine test to assess folate and PEG, respectively[1]. The fractions positive to both assays were pooled and freeze-dried. The resulting yellow solid residue was then treated with TCEP to regenerate free thiol groups. FA-PEG_{2kDa}-SH (25 mg, 0.010 mmol) and TCEP (25.8 mg, 0.103 mmol) were dissolved in 50 mM acetate buffer at pH 5 and left under stirring for 3 hrs at ambient temperature. The mixture was then dialysed with a Spectra/Por Float-a-lyzer G2 (MW cutoff = 0.5-1 kDa) using a 1 mM HCl, 1 mM

EDTA solution as releasing medium. The dialysis was performed for 2 days and then the FA-PEG_{2kDa}-SH solution was freeze-dried.
The lyophilised FA-PEG_{2kDa}-SH was dissolved in 10 mM PBS at pH 7.4 and analysed by UV-Vis spectroscopy at 363 nm (folate molar extinction coefficient at 363 nm in PBS, pH 7.4 = 6.197 M^{-1} cm^{-1}) and by Iodine test to assess the conjugation efficiency, and by Ellman assay to determine the percentage of free thiol groups[2, 3]. FA-PEG_{2kDa}-SH was characterized by MALDI-TOF mass spectrometry. The purity of the product was evaluated by C_{18} RP-HPLC, eluting with a gradient of 10 mM ammonium acetate buffer, pH 6.5 (eluent A) and acetonitrile (eluent B). The eluent B was increased linearly from 10 to 40% over 40 mins and the UV-Vis detector was set at 363 nm.
^{1}H NMR *(300 MHz, DMSO-d_6, δ, ppm):* δ 8.64 (s, 1H, C*H* of FA), 7.64 (m, 2H, aromatic of FA), 6.65 (m, 2H, aromatic of FA), 4.35 (m, 1H, C*H*(COOH) of FA), 3.50 (s, CH_2O of PEG, ~180H), 2.89 (t, 2H, J = 6.4 Hz, CH_2-S).

FA-PEG_{2kDa}-SH thiol oxidation kinetic under different pH conditions. FA-PEG_{2kDa}-SH (4.9 mg) was dissolved in 1.0 mL of Milli-Q water (2 mM). Two hundred µL of the solution was diluted to 0.5 mM in a test tube with 600 µL of buffers at increasing pH: 20 mM sodium citrate pH 4.0, 20 mM sodium citrate pH 5.0, 20 mM sodium bicarbonate pH 6.0, 20 mM sodium phosphate pH 7.0, 20 mM Tris (hydroxylmethyl) aminomethane pH 8.0, 20 mM sodium tetraborate pH 9.0. In a 96 well plate, at scheduled intervals, 50 µL of each of the samples at different pHs was added to 150 µL of 0.2 M phosphate pH 8.0 containing 1 mM EDTA followed by 30 µL of 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB) in the same buffer. The mixtures were left to react 5 mins and then spectrophotometrically tested at 405 nm with an EL311SK microplate autoreader.[3] The oxidation kinetic of thiols is reported in Figure S2.

Synthesis of Bodipy FL-PEG_{2kDa}-SH (Bdp-PEG_{2kDa}-SH).
Bodipy FL-NHS (Bdp-NHS, 14.6 mg, 37.6 µmol) was dissolved in anhydrous DMSO (400 µL) under stirring and sequentially added of NH_2-PEG_{2kDa}-SH (62.7 mg, 31.3 µmol) and triethylamine (4.4 µL, 31µmol). The reaction was carried out overnight under stirring at room temperature in the dark. The mixture was purified by size-exclusion chromatography using Sephadex LH 20 resin and ethanol as eluent. The column fractions were collected and analysed by UV-Vis spectroscopy (ethanol) at 503 nm and Iodine test (λ=535 nm) for Bodipy FL and PEG assessment, respectively. Fractions positive to both tests were pooled and the solvent removed under reduced pressure. The resulting yellow oil was dissolved in water and freeze-dried. The lyophilised product was analysed by UV-Vis spectroscopy at λ=503 nm (Bodipy FL molar extinction coefficient in ethanol at $\lambda_{503\ nm}$ = 80,000 $M^{-1}cm^{-1}$, as reported by the manufacturer) and by Iodine assay for the quantification of Bodipy FL and PEG concentration, respectively. The conjugation yield, expressed as [Bodipy FL]:[PEG] molar ratio, was estimated to be 94%. Ellman assay was performed to assess the percentage of free thiol groups and showed a 90% of thiol group availability. The purity of the product was evaluated by reverse phase chromatographic analysis using a Jasco HPLC system eluted in a gradient mode with Milli-Q water containing 0.05% of TFA (eluent A) and acetonitrile containing 0.05% of TFA (eluent B). Eluent B was increased linearly from 10 to 90% in 15 mins, and the UV-Vis detector was set at 503 nm.
^{1}H NMR *(400 MHz, DMSO-d_6, δ, ppm)*: δ 7.69 (m, 1H, C(CH_3)CC*H*), 7.08 (d, 1H, J = 4.2 Hz, C(CH_3)CCHCC*H*), 6.35 (d, 1H, J = 4.0 Hz, C(CH_3)CCHCCHC*H*), 6.30 (s, 1H, C(CH_3)C*H*), 3.63 (t, 2H, J = 6.5 Hz, C=ONHCH_2CH_2), 3.51 (s, CH_2O of PEG, ~180H), 3.41 (t, 2H, J = 5.8 Hz, CH_2CH_2S), 3.22 (q, 2H, J = 5.7 Hz, C=ONHCH_2), 3.07 (t, 2H, J = 7.9 Hz, C=OCH_2CH_2), 2.89 (t, 2H, J = 6.4 Hz, CH_2S), 2.61 (m, 2H, C=OCH_2), 2.26 (s, 3H, CHC(CH_3)C), 1.28 (m, 3H, CHC(CH_3)N).

Synthesis of lipoic containing chain transfer agent lipoyl-CTA.
Lipoyl-TEG. Lipoic acid (1.00 g, 4.85 mmol) was dissolved in anhydrous DCM (5 mL) and the solution was cooled in an ice bath. Oxalyl chloride (0.616 g, 4.85 mmol) was added dropwise to the reaction

mixture. The reaction vessel was sealed with a glass stopper and the reaction was left under stirring for 4 hrs at 0 °C. The volatiles were removed under reduced pressure and the residue was redissolved in anhydrous DCM (5 mL); this procedure was repeated twice to remove potential traces of the unreacted oxalyl chloride. In order to prove the lipoyl chloride formation, it was reacted with methanol and the methyl ester derivative underwent ^{1}H NMR analysis which showed the appearance of a peak at 3.67 ppm attributed to the methoxyl ester of lipoic acid.
Tetraethylene glycol (TEG) (9.42 g, 48.5 mmol) was dissolved in anhydrous DCM (5 mL) in the presence of triethylamine (1.06 mL, 7.63 mmol) and a solution of lipoyl chloride (1.09 g, 4.85 mmol) in DCM (4 mL) was added dropwise. The mixture was left under stirring overnight at room temperature, then the solvent was removed under reduced pressure, yielding an oily brown residue, which was purified by flash chromatography on silica gel eluted with a 4:1 (v/v) diethyl ether/ethyl acetate mixture. The lipoyl-TEG intermediate was isolated as a viscous yellow oil (0.39 g, 1.0 mmol, 22 % yield).
ESI-TOF: m/z $[M + H]^+$ calcd for $C_{16}H_{31}O_6S_2$ 383.15; found, 383.09 for $[M + H]^+$. m/z $[M + Na]^+$ calcd for $C_{16}H_{30}NaO_6S_2$ 404.13; found, 404.99.
FT-IR: *v* 3447.98, 2921.37, 1732.44, 1349.02, 1248.27, 1179.35, 1124.19 cm^{-1}.
^{1}H NMR *(400 MHz, $CDCl_3$, δ, ppm)*: δ 4.24 (m, 2H, C(O)OC*H*$_2$), 3.66 (m, 12H, C*H*$_2$O), 3.61 (m, 2H, C*H*$_2$O), 3.57 (m, 1H, C*H*S), 3.15 (m, 2H, C*H*$_2$S), 2.45 (td, 1H, J=12.4, 6.1 Hz, C*H*HCH$_2$S), 2.35 (t, 2H, J = 7.4 Hz, C*H*$_2$C(O)O), 1.91 (td, 1H, J=13.5, 6.9 Hz, CH*H*CH$_2$S), 1.68 (m, 4H, C*H*$_2$), 1.47 (m, 2H, C*H*$_2$).
^{13}C NMR *(100 MHz, $CDCl_3$, δ, ppm)*: δ 173.61 (1C, *C*=O), δ 73.09 (1C, *C*H$_2$O), δ 72.66 (1C, *C*H$_2$O), δ 70.69 (1C, *C*H$_2$O), 70.14 (1*C*, *C*H$_2$O), δ 69.34 (1C, *C*H$_2$O), δ 63.57 (1C, *C*H$_2$O), δ 61.90 (1C, *C*H$_2$O), δ 61.72 (1C, *C*H$_2$O), 56.48 (1C, *C*HS), 40.36 (1C, *C*H$_2$CH$_2$S), 38.62 (1C, *C*H$_2$S), 34.73 (1C, *C*H$_2$), 34.07 (1C, *C*H$_2$), 28.87 (1C, *C*H$_2$), 24.75 (1C, *C*H$_2$).

Lipoyl-CTA. Lipoyl-TEG (0.23 g, 0.60 mmol) and 4-cyano-4-(phenylcarbonothioylthio) pentanoic acid (0.17 g, 0.60 mmol) were dissolved in anhydrous DCM (2 mL) and the mixture was cooled in an ice bath. Separately, DCC (0.15 g, 0.72 mmol) and DMAP (4 mg, 0.03 mmol) were dissolved in anhydrous DCM (1 mL) and added dropwise to the first solution. The resulting mixture was stirred for 48 hrs at room temperature, then the solvent was removed under reduced pressure, and the resulting residue purified by flash chromatography on silica gel, eluting with a 4:1 (v/v) petroleum ether/ethyl acetate mixture. Lipoyl-CTA was isolated as a red oil (0.16 g, yield 42 %).
ESI-TOF: m/z $[M+H]^+$ calcd for $C_{29}H_{42}NO_7S_4$ 644.18; found, 644.18; m/z $[M+Na]^+$ calcd for $C_{29}H_{41}NNaO_7S_4$ 666.17; found, 666.16.
FT-IR: *v* 2927.63, 1733.33, 1383.50, 1290.98, 1235.67, 1182.49, 1110.09, 763.72, 688.20 cm^{-1}.
^{1}H NMR *(400 MHz, $CDCl_3$, δ, ppm)*: δ 7.89 (m, 2H, aromatic), 7.57 (m, 1H, aromatic), 7.40 (m, 2H, aromatic), 4.23 (m, 4H, C(O)OC*H*$_2$CH$_2$O), 3.70 (m, 4H, C(O)OCH$_2$C*H*$_2$O); 3.65 (m, 8H, C*H*$_2$O), 3.56 (m, 1H, C*H*S), 3.15 (m, 2H, C*H*$_2$S), 2.72 (m, 2H, OC(O)C*H*$_2$CH$_2$CCN), 2.62 (m, 1H, C*H*HCCN), 2.45 (m, 2H, C*H*HCH$_2$S+ CH*H*CCN), 2.34 (t, 2H, *J* = 7.4 Hz, CH$_2$CH$_2$C*H*$_2$C(O)O), 1.93 (s, 3H, CH$_3$), 1.88 (m, 1H, CH*H*CH$_2$S), 1.67 (m, 4H, C*H*$_2$), 1.46 (m, 2H, C*H*$_2$).
^{13}C NMR *(100 MHz, $CDCl_3$, δ, ppm)*: δ 222.3 (1C, *C*(S)S), 173.47 (1C, *C*(O)O), 171.64 (1C, *C*(O)O), 144.62 (1C, *C* aromatic), 133.15 (1C, *C*H aromatic), 128.65 (2C, *C*H aromatic), 126.95 (2C, *C*H aromatic), 118.52 (1C, *C*≡N), 70.76 (2C, *C*H$_2$O), 70.72 (1C, *C*H$_2$O), 70.68 (1C, *C*H$_2$O), 69.31 (1C, *C*H$_2$O), 69.11 (1C, *C*H$_2$O), 64.27 (1C, *C*H$_2$O), 63.56 (1C, *C*H$_2$O), 56.47 (1C, *C*HS), 45.87 (1C, *C*CN), 40.35 (1C, *C*H$_2$CH$_2$S), 38.61 (1C, CH$_2$S), 34.72 (1C, *C*H$_2$CHS), 34.07 (1C, *C*H$_2$C(O)O), 33.50 (1C, *C*H$_2$CCN), 29.88 (1C, *C*H$_2$CH$_2$CCN), 28.85 (1C, *C*H$_2$), 24.65 (1C, *C*H$_2$), 24.27 (1C, *C*H$_3$).

Synthesis of lipoyl- [2-(methacryloyloxy)ethyl-3-chloro-4-hydroxybenzoate] chain transfer agent (lipoyl-(MCH)$_{26}$-CTA).
2-(methacryloyloxy)ethyl-3-chloro-4-hydroxybenzoate (MCH) was synthesised as described by Mastrotto *et al*.[4]. MCH (1.5 g, 5.28 mmol), lipoyl-CTA (77 mg, 0.12 mmol) and AIBN (20 mg, 0.050 mmol) were dissolved in anhydrous DMF (9 mL) in a Schlenk tube. After deoxygenation by nitrogen

bubbling for 30 mins, the resulting mixture was heated at 70 °C under stirring. The polymerisation was monitored by ^{1}H NMR in DMSO-d_6 on aliquots withdrawn at regular intervals of time from the reaction flask. The monomer conversion was calculated by comparing the integrals of the polymer aromatic signal at 6.9 ppm, and that of the analogous proton in the residual unreacted monomer at 7.08 ppm.
The polymerisation was quenched at 70 % of conversion (DP=26) by cooling the reaction flask at room temperature, while exposing the reaction mixture to air. The polymer was precipitated in a 1:1 (v/v) diethyl ether/petroleum ether mixture, recovered by centrifugation at 1600 x g, redissolved in DCM and precipitated again in the same solvent mixture. The polymer was isolated by centrifugation, then traces of residual solvent were removed under reduced pressure, yielding lipoyl-$(MCH)_{26}$-CTA polymer intermediate as a pink solid (730 mg, 0.091 mmol, 49 % overall yield).
$M_{n,(^1H\ NMR)}$ = 8 kDa, $M_{n,(SEC)}$ = 28.3 kDa, $Đ_{(SEC)}$ = 1.18.
^{1}H NMR *(400 MHz, DMSO-d_6, δ, ppm)*: δ 11.06 (bs, 1H, O*H*), 7.73 (s, 1H, C*H* aromatic), 7.61 (s, 1H, C*H* aromatic), 6.96 (s, 1H, C*H* aromatic), 4.26 (bs, 2H, C(O)OCH_2), 4.08 (bs, 2H, C(O)OCH_2), 1.74 (m, 2H, C$H_{2,\ backbone}$), 0.83 (m, 3H, C$H_{3,\ backbone}$).

Synthesis of lipoyl-[2-(methacryloyloxy)ethyl-3-chloro-4-hydroxybenzoate]-*b*-[glycerol methacrylate] (lipoyl-[$(MCH)_{26}$-*b*-$(GMA)_{53}$]-CTA).
Glycerol methacrylate (GMA) was synthesised as described by Mastrotto *et al*. [4]. Lipoyl-$(MCH)_{26}$-CTA (0.619 g, 77.1 µmol), GMA (0.815 g, 5.01 mmol) and AIBN (6.3 mg, 38.5 µmol) were dissolved in anhydrous DMF (9 mL) in a Schlenk tube. The solution was degassed by nitrogen bubbling for 30 mins and heated at 70 °C under stirring (t_0). The polymerisation was monitored by ^{1}H NMR in DMSO-d_6 at scheduled intervals, by following the decrease of the integrals of the vinyl protons of GMA (5.74 and 6.13 ppm), using the peak of DMF at 2.97 ppm as an internal standard. The polymerisation was stopped at 81 % of conversion (DP=53) by removing the Schlenk tube from the oil bath and exposing the reaction mixture to the air. Finally, the polymer was isolated by precipitation in a 1:1 (v/v) diethyl ether/petroleum ether mixture, centrifuged and redissolved in methanol and precipitated again in the same solvent mixture. After centrifugation, the precipitate was dried under reduced pressure, yielding lipoyl-[$(MCH)_{26}$-*b*-$(GMA)_{53}$]-CTA (1.01 g, 60.9 µmol, 79 % yield) as a pink solid.
$M_{n,(1H\ NMR)}$ = 16.5 kDa; $M_{n,(SEC)}$ = 33.8 kDa, $Đ_{(SEC)}$ = 1.17.
^{1}H NMR *(400 MHz, DMSO-d_6, δ, ppm)*: δ 11.06 (bs, 1H, O*H*), 7.73 (bs, 1H, C*H* aromatic), 7.64 (s, 1H, C*H* aromatic), 6.96 (s, 1H, C*H* aromatic), 4.92 (s, 2H, O*H*), 4.67 (s, 2H, O*H*), 4.26 (bs, 2H, C(O)OCH_2), 4.09 (bs, 2H, C(O)OCH_2), 3.91 (bd, 4H, OCH_2CHOH) 3.67 (bs, 2H, OCH_2CHOH), 3.49 (bd, 4H, CH_2OH), 1.79 (m, 6H, $CH_{2,\ backbone}$), 0.83 (m, 9H, $CH_{3,\ backbone}$).

Synthesis of Oregon Green labelled lipoyl-[2-(methacryloyloxy)ethyl-3-chloro-4-hydroxybenzoate]-*b*-(glycerol methacrylate) (OG-lipoyl-[$(MCH)_{26}$-*b*-$(GMA)_{50}$]-CTA).
Lipoyl-$(MCH)_{26}$-CTA (0.204 g, 25.4 µmol), GMA (0.265 mg, 1.65 mmol) and AIBN (2.08 mg, 12.7 µmol) were dissolved in anhydrous DMF (6 mL) in a Schlenk tube. The solution was degassed by nitrogen bubbling for 30 min and heated at 70 °C under stirring (t_0). In a separate vessel, Oregon Green methacrylate (OGM) (18.6 mg, 31.7 µmol) synthesised as described by Makwana et al. (Makwana, H., et al. (2017). "Engineered Polymer-Transferrin Conjugates as Self-Assembling Targeted Drug Delivery Systems." Biomacromolecules) was dissolved in 200 µL of anhydrous DMF and the solution was degassed by nitrogen bubbling for 30 min. The polymerisation was monitored by ^{1}H NMR in DMSO-d_6 at scheduled intervals, by comparing the decrease of GMA vinyl proton signals (5.74 and 6.13 ppm) with respect to the DMF peak at 2.97 ppm selected as internal standard. Once reached the 40% of GMA conversion, OGM solution was cannulated into the reaction Schlenk tube. The polymerisation was stopped at 77 % of conversion by removing the Schlenk tube from the oil bath and exposing the reaction mixture to the air. The calculation of the final polymer composition was made with the assumption that GMA and OGM, being both methacrylate monomers, were characterised by a reactivity ratio equal to 1. Thus, we considered that OGM conversion was the same achieved by GMA

in the second step only. The final polymerisation degree was estimated to be 50 composed by 1% of OG and 99% of GMA units. Finally, the polymer was isolated by precipitation in a 1:1 (v/v) diethyl ether/petroleum ether mixture, centrifuged, redissolved in methanol and precipitated again in the same solvent mixture. After centrifugation, the precipitate was dried under reduced pressure, yielding OG-lipoyl-[$(MCH)_{26}$-*b*-$(GMA)_{50}$]-CTA (0.301 g, 18.0 µmol, 71 % yield) as an orange solid.
$M_{n,(1H\ NMR)}$ = 16.5 kDa; $M_{n,(SEC)}$ = 33.5 kDa, $Đ_{(SEC)}$ =1.48.
^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 11.05 (bs, 1H, OH), 7.73 (s, 1H, CH aromatic), 7.61 (s, 1H, CH aromatic), 6.95 (s, 1H, CH aromatic), 4.92 (s, 2H, OH), 4.71 (s, 2H, OH), 4.25 (bs, 2H, $C(O)OCH_2$), 4.10 (s, 4H, OCH_2CHOH), 3.91 (s, 2H, $C(O)OCH_2$), 3.71 (d, J = 29.8 Hz, 4H, CH_2OH), 3.51 (s, 2H, OCH_2CHOH), 1.82 (m, 6H, $CH_{2,\ backbone}$), 0.81 (m, 9H, $CH_{3,\ backbone}$).

Synthesis of lipoyl-(glycerol methacrylate) (lipoyl-$(GMA)_{79}$-CTA).
GMA (1.28 g, 7.99 mmol), lipoyl-CTA (51.9 mg, 80.7 µmol) and AIBN (6.6 mg, 40 µmol) were dissolved in anhydrous DMF into a Schlenk tube and the resulting solution deoxygenated by N_2 bubbling for 30 mins. Then the Schlenk tube was immersed into an oil bath at 70 °C (t_0), and stirred at this temperature for the entire duration of the reaction. The reaction was monitored by ^{1}H NMR in DMSO-d_6 on aliquots withdrawn at regular intervals of time, by following the decrease of the integrals of the monomer vinyl proton signals (6.11 and 5.58 ppm), using the singlet at 7.95 ppm of DMF as a reference. At 80% of conversion, the polymerisation was stopped by lifting the Schlenk tube from the oil bath and exposing the reaction mixture to the air. The polymer was isolated by precipitation in a 1:1 (v/v) diethyl ether/petroleum ether mixture, centrifuged and redissolved in methanol and precipitated again in the same solvent mixture. The precipitate was recovered by centrifugation and dried under reduced pressure and lipoyl-$(GMA)_{79}$-CTA (0.986 g, 74.2 µmol, 92% yield) was isolated as a pink solid.
$M_{n,(^1H\ NMR)}$ = 13.3 kDa; $M_{n,(SEC)}$ = 26.9 kDa, $Đ_{(SEC)}$ = 1.17.
^{1}H NMR *(400 MHz, DMSO-d_6, δ, ppm):* δ 4.92 (s, 1H, O*H*), 4.67 (s, 1H, O*H*), 3.92 (bd, 2H, C(O)O*C*H_2), 3.68 (s, 1H, C*H*OH), 3.40 (m, 2H, CH_2OH), 1.95 (m, 2H, $CH_{2,\ backbone}$), 0.86 (m, 3H, $CH_{3,\ backbone}$).

Removal of the thiocarbonylthio group from polymers to yield lipoyl-[$(MCH)_{26}$-*b*-$(GMA)_{53}$], OG-lipoyl-[$(MCH)_{26}$-*b*-$(GMA)_{50}$] and lipoyl-$(GMA)_{79}$.
The removal of thiocarbonylthio end group from lipoyl-[$(MCH)_{26}$-*b*-$(GMA)_{53}$]-CTA, OG-lipoyl-[$(MCH)_{26}$-*b*-$(GMA)_{50}$]-CTA and lipoyl-$(GMA)_{79}$-CTA to yield the lipoyl-[$(MCH)_{26}$-b-$(GMA)_{53}$], OG-lipoyl-[$(MCH)_{26}$-*b*-$(GMA)_{50}$] and lipoyl-$(GMA)_{79}$ polymers, respectively, was performed according to the method reported by Perrier *et al*.[5]. Typical reaction conditions described below were applied to both polymers.
Lipoyl-$(GMA)_{79}$-CTA (0.986 g, 74.2 µmol) and AIBN (0.366 g, 2.23 mmol) were dissolved in anhydrous DMF (1:30 polymer/AIBN molar ratio) and the resulting solution was heated at 80 °C under stirring. After 4 hrs the reaction mixture was cooled to ambient temperature, and the polymer precipitated by dropwise addition to a 1:1 (v/v) diethyl ether/petroleum ether mixture. Lipoyl-$(GMA)_{79}$ was recovered by centrifugation and reprecipitated in the same solvent mixture for three times. After centrifugation, the precipitate was desiccated under reduced pressure (0.940 g, 71.2 µmol, 96 % of yield).

Fluorimetric analysis of OG-lipoyl-[$(MCH)_{26}$-*b*-$(GMA)_{50}$].
5 mg of OG-lipoyl-[$(MCH)_{26}$-*b*-$(GMA)_{50}$] (0.302 µmol) were dissolved in 1 mL of NaOH 0.02 N and serial dilutions in the concentration range 0-20 µg mL^{-1} were prepared in 10 mM PBS at pH 7.4 and6.5. Each sample was prepared in triplicate. The polymer fluorescence emission spectra were recorded in the wavelength range of 500-600 nm setting the excitation wavelength at 496 nm. The fluorescence intensity at 535 nm was plotted versus the polymer concentration.

Potentiometric titration of lipoyl-[$(MCH)_{26}$-*b*-$(GMA)_{53}$] and OG-lipoyl-[$(MCH)_{26}$-*b*-$(GMA)_{50}$] copolymers.

A 1.0 mg/mL solution of lipoyl-[$(MCH)_{26}$-*b*-$(GMA)_{53}$] and a 1.0 mg/mL solution of OG-lipoyl-[$(MCH)_{26}$-*b*-$(GMA)_{50}$] were prepared in deionised water (10 mL) and the pH was adjusted to 12 with 1 N NaOH. Potentiometric titration was carried out by adding 2 µL aliquots of HCl 1 N under stirring over a pH range of 12-3. The back titration was also performed by adding 2 µL aliquots of 1 N NaOH until pH 12 was reached. pH variations were plotted versus the total volume of titrant obtaining two overlapping sigmoidal profiles. The polymer "apparent pKa" was calculated as the median of the equivalence points of each curve.

Turbidimetric analysis of lipoyl-[$(MCH)_{26}$-*b*-$(GMA)_{53}$] and OG-lipoyl-[$(MCH)_{26}$-*b*-$(GMA)_{50}$].

A 1.0 mg/mL solution of lipoyl-[$(MCH)_{26}$-*b*-$(GMA)_{53}$] and a 1.0 mg/mL solution of OG-lipoyl-[$(MCH)_{26}$-*b*-$(GMA)_{50}$] in deionised water were prepared and the pH of each solution was adjusted to pH 12 by adding 1 N NaOH. The solutions were sequentially added of 10 µL aliquots of 1 N HCl until pH 3.0 was reached. The transmittance at λ=500 nm versus pH was plotted. The relative transmittance at all pH values was expressed as a percentage of the transmittance at pH 12 (100%).

Dynamic light scattering analysis of lipoyl-[$(MCH)_{26}$-*b*-$(GMA)_{53}$]

Lipoyl-[$(MCH)_{26}$-*b*-$(GMA)_{53}$] (20 mg, 1.2 µmol) was dissolved in deionised water (1 mL) and the pH was adjusted to 12 with 1 N NaOH to facilitate dissolution. This solution was diluted to a concentration of 1 mg mL^{-1} with the following buffers: 50 mM borate buffer pH 9, 50 mM phosphate buffer pH 7.4, 50 mM phosphate buffer pH 6.5, 50 mM acetate buffer pH 5. The polymer solutions were analysed by Dynamic Light Scattering Zetasizer Nano equipped with a red (λ=633 nm) laser at a fixed angle of 173°. DTS applications 6.12 software was used to analyse the data which were referred as volume.

Production of gold nanoparticles (AuNPs).

AuNPs were produced according to the Turkevich method by reduction of tetrachloroauric acid with sodium citrate[6]. All the glassware was washed with aqua regia [3:1 (v/v) of (12.2 M Hydrochloric acid)/(14.6 M Nitric acid)] and then rinsed with deionised water before use. An aqueous solution of tetrachloroauric acid (0.25 mM, 100 mL) was prepared and heated to 75 °C under stirring. A volume of 3.0 mL of a trisodium citrate dihydrate solution in water (0.034 M) was added dropwise to the $HAuCl_4$ solution, and the mixture was left under stirring for 1 hour at 75 °C to allow formation of particles. Then, the colloidal suspension was cooled to room temperature and characterised by UV-Vis spectroscopy (Milli-Q water), Dynamic Light Scattering, and Transmission Electron Microscopy.

Dynamic Light Scattering (DLS) analysis.

Naked AuNPs and decorated AuNPs suspended in 10 mM PBS at pH 7.4 or 6.5 underwent size analysis at 25 °C with a Dynamic Light Scattering Zetasizer Nano equipped with a red (λ=633 nm) laser at a fixed angle of 173°. DTS applications 6.12 software was used to analyse the data. All sizes were referred as intensity. For each sample, three DLS measurements were performed with 10 runs per 10 second measurement.

The stability of 2 nM polymer decorated particles in FFDMEM at pH 7.4 and 6.5 was assessed by DLS analysis every 30 min for 4 h.

AuNP concentration assessment.
The molar extinction coefficient in Milli-Q water of gold particle suspensions at λ=506 nm ($\varepsilon_{506\,nm}$) was calculated according to equation (1), as described by Liu et co-workers[7].

$$\ln \varepsilon_{506\,nm} = klnD_H + a \quad (1)$$

where D_H is the hydrodynamic diameter of the nanoparticles (measured by DLS), k and a are two constants whose values are 3.32111 and 10.80505, respectively[8, 9].
The AuNP concentration (M) was then calculated according to the Lambert–Beer Equation using the $\varepsilon_{506\,nm}$.

Fluorimetric analysis of AuNPs decorated with OG-lipoyl-[$(MCH)_{26}$-*b*-$(GMA)_{50}$.
In a vial, 149 µL of a 5.0 mg mL^{-1} OG-lipoyl-[$(MCH)_{26}$-*b*-$(GMA)_{50}$] solution (744 µg, 45.0 nmol) in 0.02 N was added to a freshly prepared 3 nM AuNP suspension (5 mL) to yield a 3000:1 [OG-lipoyl-[$(MCH)_{26}$-b-$(GMA)_{50}$]]:[AuNP] feed molar ratio. The AuNP suspension was incubated at room temperature under rotational stirring overnight in the dark. The particle suspension was centrifuged at 19,720 x g for 30 min, the AuNP pellet was washed twice with Milli-Q water. Particles were redispersed to a final concentration of 3 nM in 10 mM PBS at pH 7.4 or 6.5 and analysed by a LS 50 B Perkin-Elmer fluorimeter. The excitation wavelength was set at 496 nm and fluorescence spectra were recorded in the wavelength range of 500-600 nm.

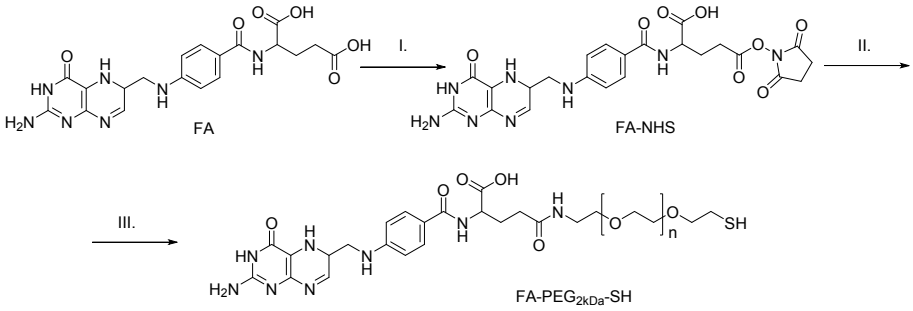
Transmission electron microscopy (TEM) analysis of AuNPs.
The naked particle samples (2 nM) were suspended in 10 mM PBS, pH 7.4, placed on a carbon coated copper grid, the buffer was allowed to dry at room temperature and samples underwent TEM imaging with a Tecnai G2 microscope. The average diameter of particles was calculated by measuring 100 individual particles with ImageJ software.
Polymer decorated AuNPs were suspended in 10 mM PBS at pH 7.4 or 6.5, negatively stained with 2% uranyl acetate dissolved in distilled water. TEM grids with particle samples were incubated with the staining medium for 5 minutes at room temperature and analysed by TEM imaging as described above.

Cell culture and viability test.
KB cells (human cervical carcinoma) were grown at 37 °C, in 5% CO_2 humidified atmosphere, using FFDMEM medium supplemented with 15% FBS, 2 mM L-glutamine, 100 IU mL^{-1} penicillin, 100 µg mL^{-1} streptomycin and 0.25 µg mL^{-1} of amphotericin B (Sigma-Aldrich). MCF-7 cells (human breast adenocarcinoma) were grown at 37 °C, in 5% CO_2 humidified atmosphere, using RPMI-1640 medium supplemented with 10% FBS, 100 IU mL^{-1} penicillin, 100 µg mL^{-1} streptomycin and 0.25 µg mL^{-1} of amphotericin B.
Cell uptake studies and viability assay were performed using $NaHCO_3$-free DMEM based medium supplemented with 10 mM Na_2HPO_4 at pH 7.4 and 6.5.
Cell viability assay. MTT assay was carried out to assess cell viability after incubation with gold nanoparticles. MCF-7 cells in RPMI-1640 containing 10% fetal bovine serum (FBS) and KB cells in FFDMEM added of 15% of FBS were seeded in a 96-well plate at a density of 5 x 10^3 cells per well (200 µL/well). After 24 hrs, the medium was replaced with particle suspensions in FFDMEM at concentrations in the 0.2-2 nM range at pH 7.4 and pH 6.5. Cells were incubated with particles for 24 hrs. Afterwards, the medium was discharged and wells were washed with PBS at pH 7.4 (100 µL x 3). MTT solution (0.5 mg mL^{-1}, 200 µL) in FFDMEM at pH 7.4 was added into each well and the plates were incubated for 3 hrs at 37 °C. The medium was then removed and DMSO (200 µL) was added to each well. The plate was gently shaken to dissolve the formazane crystals. The absorbance at 570 nm was measured by an EL311SK microplate autoreader (Bio-Tek Instruments, Winooski, VT-USA). The cell viability was expressed as viability percentage referred to untreated cells.

Results

Synthesis and characterization of folate-PEG$_{2kDa}$-SH

The folate-PEG$_{2kDa}$-SH (FA-PEG$_{2kDa}$-SH) was synthesised as shown in Scheme S1.



Scheme S1. Synthesis of FA-PEG$_{2kDa}$-SH. I. DCC/NHS, DMSO. II. NH_2-PEG-SH, Et_3N; III. TCEP, acetate buffer pH 5.0.

After purification and treatment with TCEP, Ellman assay was performed and a 96% chain-end fidelity in thiol groups was found. MALDI-TOF mass spectrometric analysis of the conjugate showed a bell shape profile centered at 2416.17 m/z, which was in agreement with the expected molecular weight of FA-PEG$_{2kDa}$-SH and confirmed the purity of the conjugate and absence of both the NH_2-PEG$_{2kDa}$-SH starting material and the FA-PEG-SS-PEG-FA dimer (Figure S1). Moreover, RP-HPLC analysis of the purified product showed that the content of free folate was below 0.1 mol% and the spectrophotometric analysis revealed a folate/PEG molar ratio that was consistent with a conjugation efficiency of 98%. Notably, folic acid possesses two carboxylic groups of which the one in the α position is required for the folate receptor binding. It is known from the literature that, after NHS ester activation, the less hindered γ–carboxylic group of folate will preferentially react with amino terminating polymers. On average, about 70% of the γ–carboxylic groups and 30% of α-carboxylic groups are involved in conjugation reactions [10, 11].

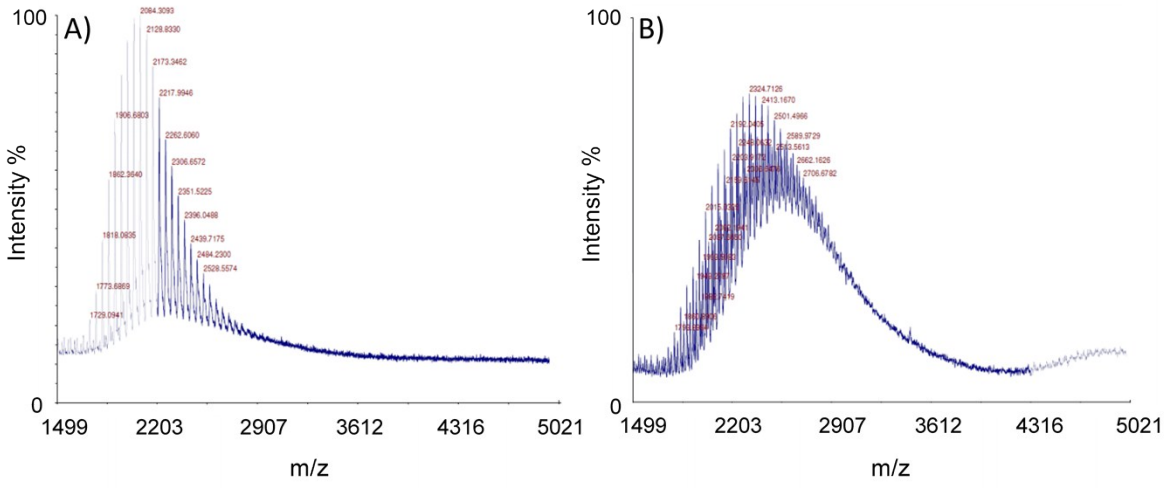


Figure S1. MALDI-TOF spectrum profile of (A) NH_2-PEG$_{2kDa}$-SH and (B) FA-PEG$_{2kDa}$-SH.

The condition for FA-PEG_{2kDa}-SH purification was set up after preliminary investigation of its stability at different pH values (Figure S2).

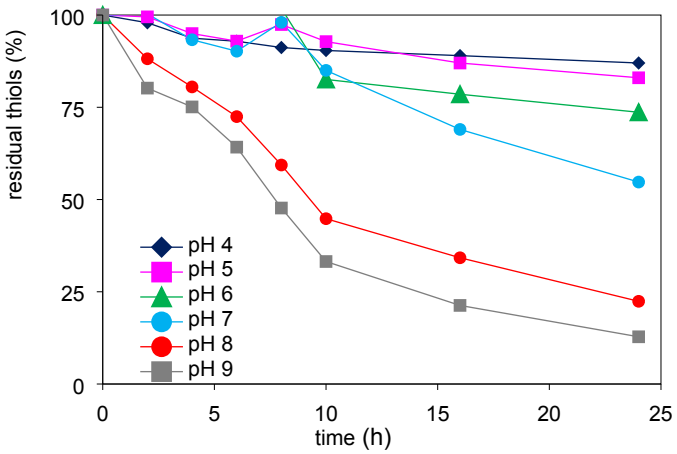
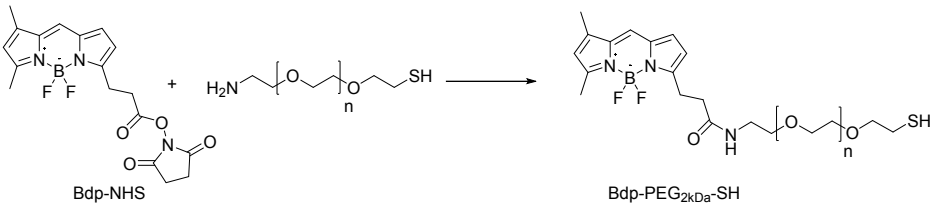


Figure S2. Kinetic profile of thiol group oxidation of FA-PEG_{2kDa}-SH (B) at pH 4.0 (♦), 5.0 (■), 6.0 (▲), 7.0 (●), 8.0 (●), 9.0 (■).

Synthesis and characterization of Bodipy FL-PEG_{2kDa}-SH

Bdp-PEG_{2kDa}-SH was obtained by conjugating *N*-hydroxysuccinimidyl ester activated Bodipy FL (Bdp-NHS) to NH_2-PEG_{2kDa}-SH (Scheme S2).



Scheme S2. Synthesis of Bdp-PEG_{2kDa}-SH.

Similarly to the preparation of FA-PEG_{2kDa}-SH, Bdp-NHS conjugation to NH_2-PEG_{2kDa}-SH was performed using a molar excess of the activated fluorophore to promote the derivatisation of the NH_2-PEG_{2kDa}-SH amino groups. Spectrophotometric analysis of the purified product showed a conjugation yield of 94% and RP-HPLC showed that the free Bodipy FL was below of 0.1 mol%. MALDI-TOF analysis showed a bell shaped profile centered at 2227.6 m/z (Figure S3), in agreement with the expected molecular weight of Bdp-PEG_{2kDa}-SH (theoretical MW: 2276.12 Da) while no signal of the NH_2-PEG_{2kDa}-SH starting material and the dimer Bodipy FL-PEG-SS-PEG-Bodipy FL was detected. Ellman analysis showed 90% thiol group availability.

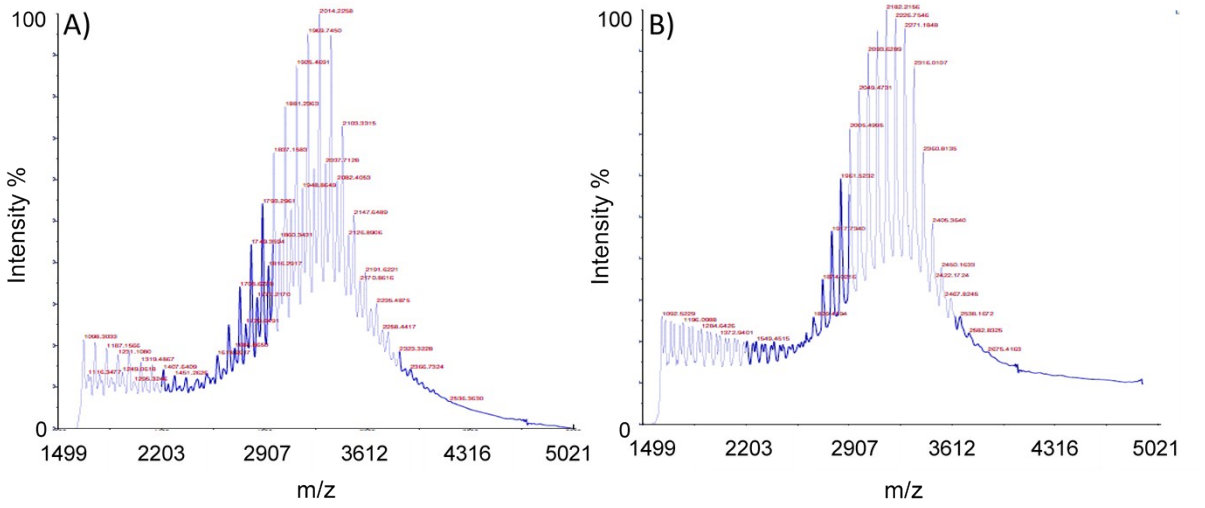


Figure S3. MALDI-TOF spectrum of (A) NH_2-PEG_{2kDa}-SH and (B) Bdp-PEG_{2kDa}-SH.

Lipoyl-TEG characterisation

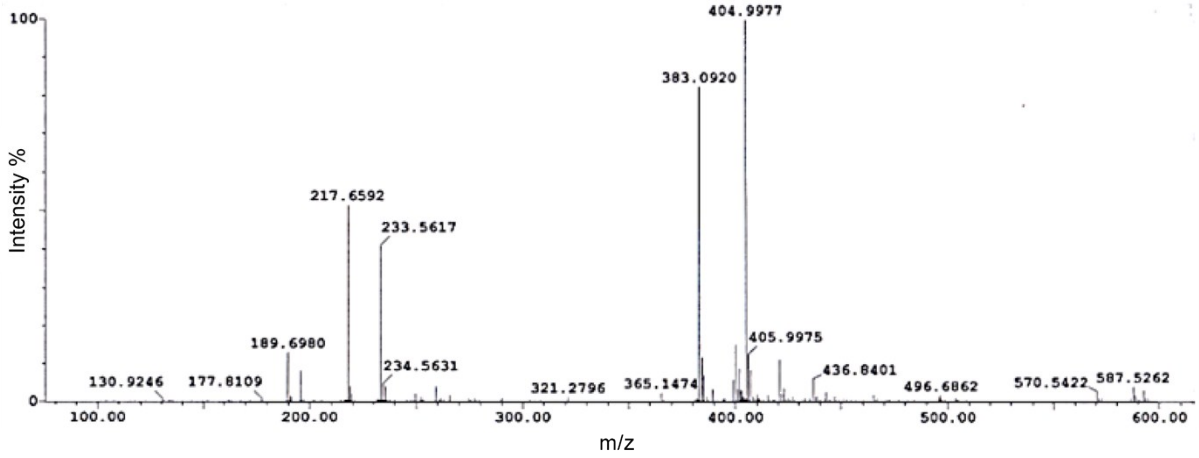


Figure S4. ESI-TOF mass spectrum of lipoyl-TEG.

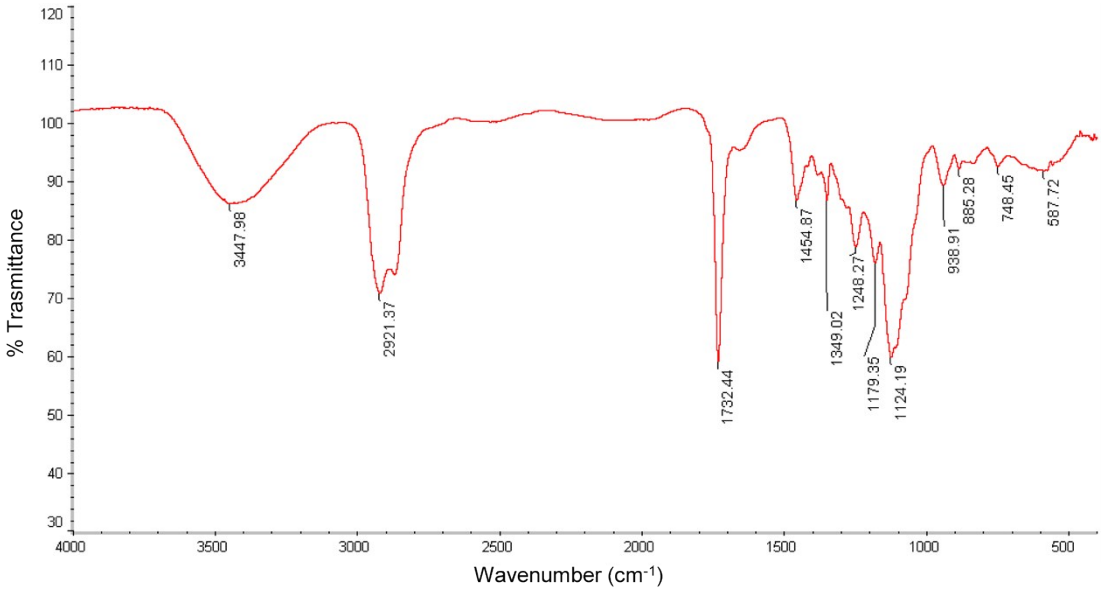


Figure S5. FTIR spectrum of lipoyl-TEG.

The ^{1}H NMR spectrum confirmed a 1:1 molar ratio between the TEG (signal of 12 methylene protons at 3.66 ppm) and the lipoyl group (signal of 2 methylene protons at 1.47 ppm, Figure S6).

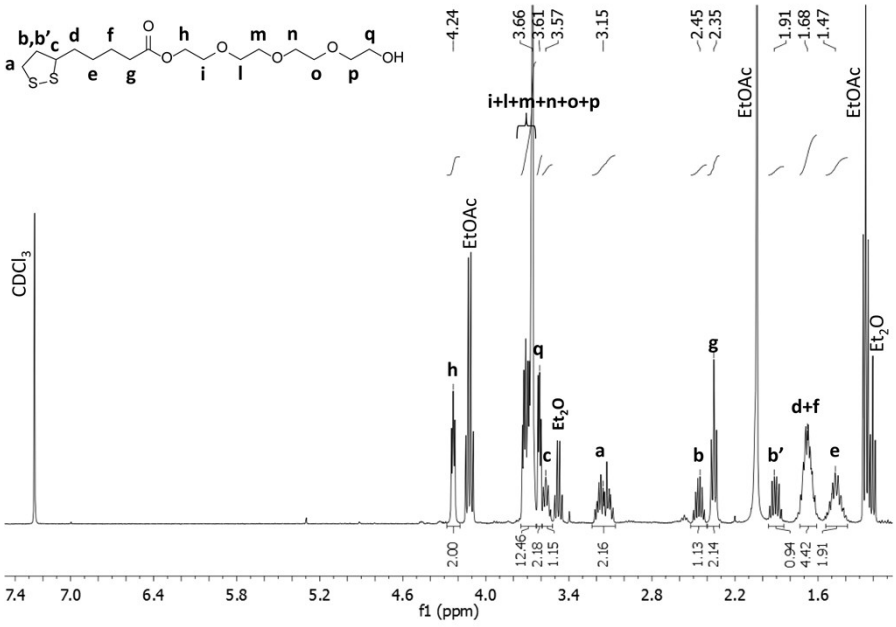


Figure S6. ^{1}H NMR spectrum of lipoyl-TEG in $CDCl_3$ with peak assignment.

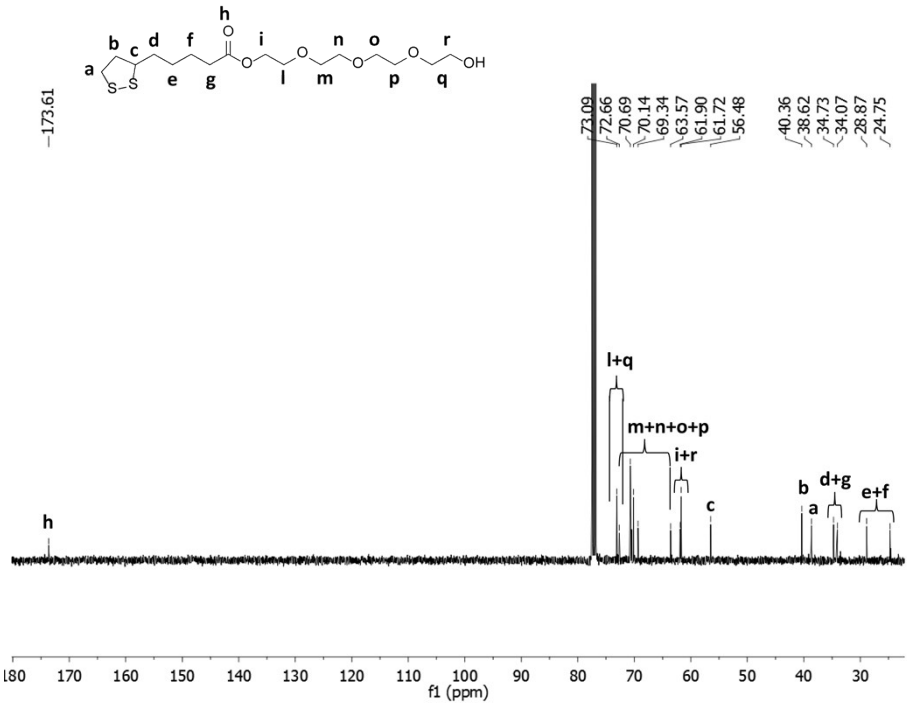


Figure S7. ^{13}C NMR spectrum of lipoyl-TEG in $CDCl_3$ with peak assignment.

Lipoyl-CTA characterisation

The ESI-TOF analysis of the lipoyl-CTA reported in Figure S8 showed an ion at 644.18 m/z (m/z: $[M + H]^+$ calcd 644.18). The ^1H NMR spectrum in Figure S10 confirmed the purity of the conjugate as the molar ratio between the 4-cyano-4-(phenylcarbonothioylthio) pentanoic acid (signals of 5 aromatic protons at 7.92-7.38 ppm) and the TEG (signal of 8 methylene protons at 3.65 ppm) was 1:1.

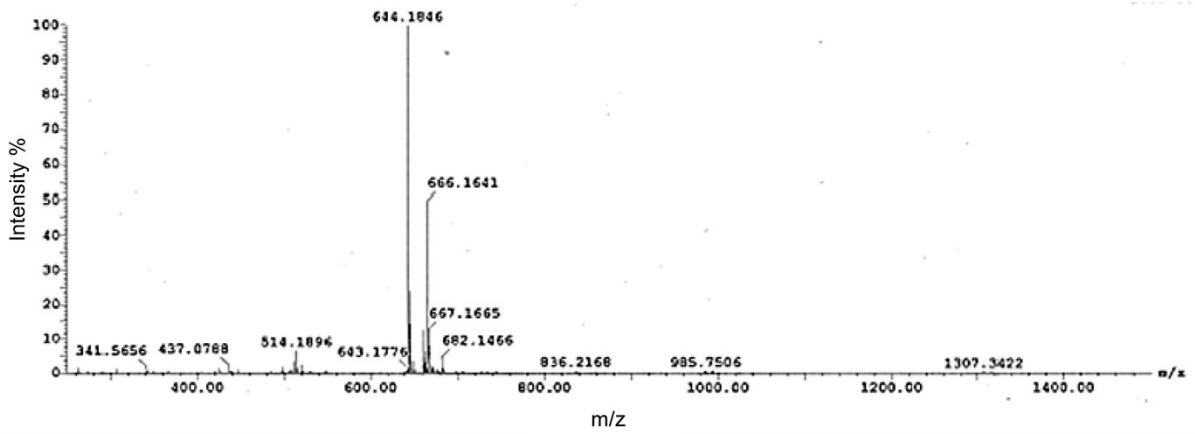


Figure S8. ESI-TOF mass spectrum of lipoyl-CTA.

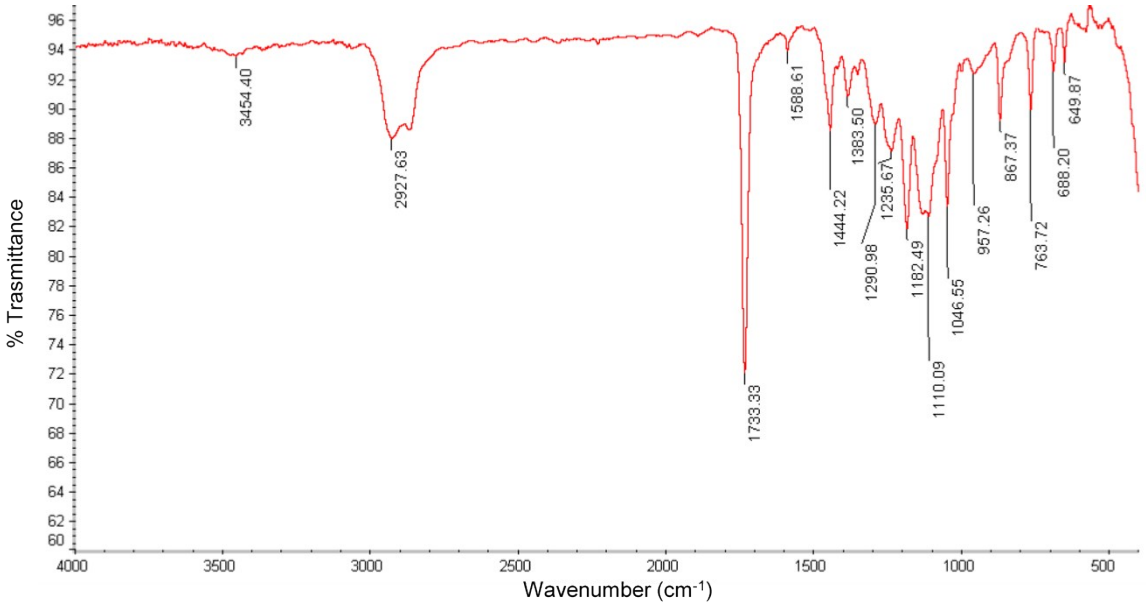


Figure S9. FTIR spectrum of lipoyl-CTA.

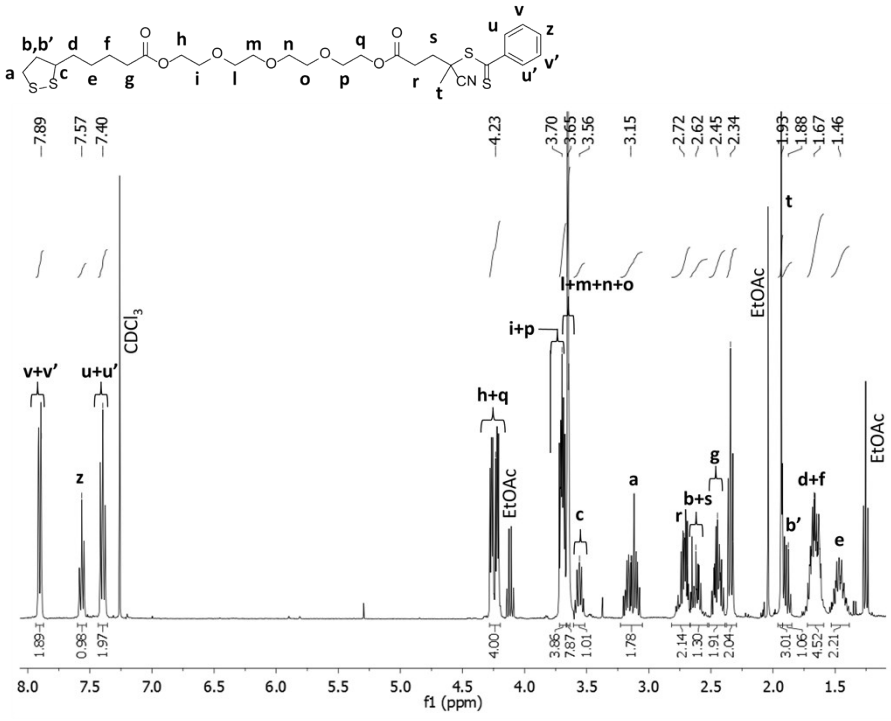


Figure S10. ^{1}H NMR spectrum of lipoyl-CTA in $CDCl_3$ with peak assignment.

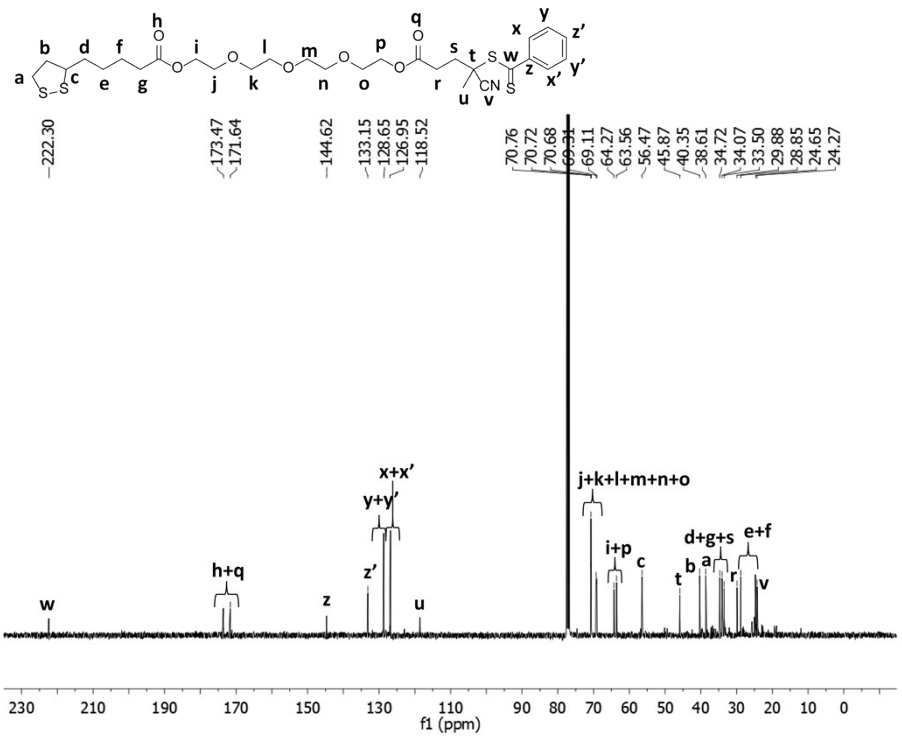


Figure S11. ^{13}C NMR in $CDCl_3$ of lipoyl-CTA with peak assignment.

Characterization of the pH-responsive Lipoyl-[(MCH)$_{26}$-*b*-(GMA)$_{53}$] copolymer

The M_n obtained from the ^{1}H NMR analysis of lipoyl-(MCH)$_{26}$-CTA and lipoyl-[(MCH)$_{26}$-*b*-(GMA)$_{53}$] were found to be 8 kDa and 16.5 kDa, respectively (Figure S12 and S14). The polydispersity (Đ) of lipoyl-(MCH)$_{26}$-CTA and lipoyl-[(MCH)$_{26}$-*b*-(GMA)$_{53}$] determined by GPC were 1.18 and 1.17, respectively (Figure S13 and S15).

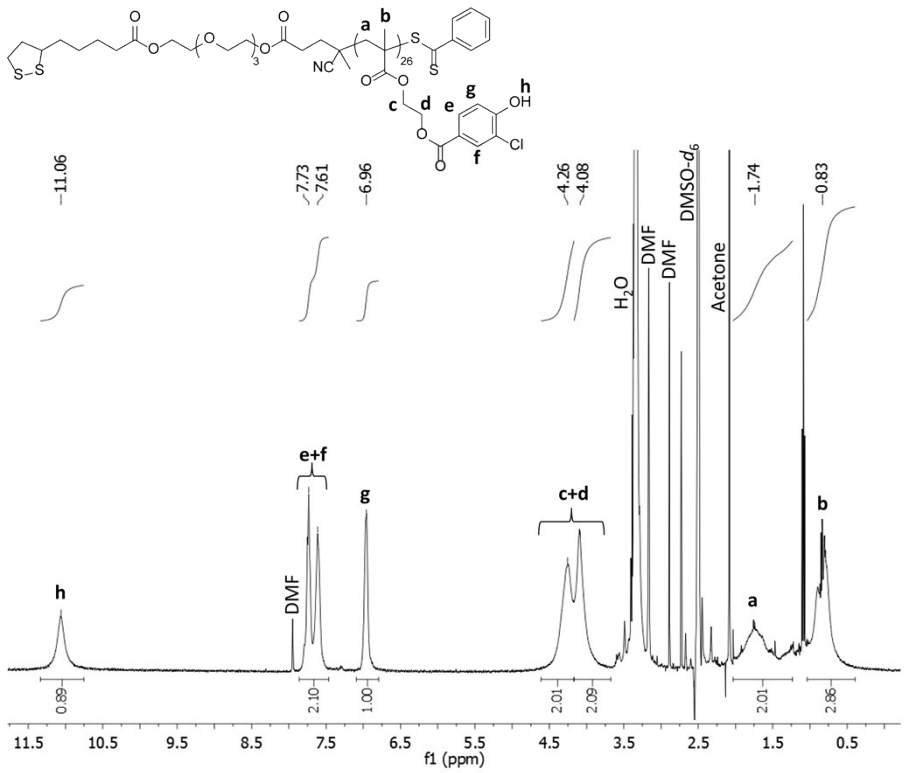


Figure S12. ^{1}H-NMR spectrum of lipoyl-(MCH)$_{26}$-CTA performed in DMSO-d_6 with peak assignment.

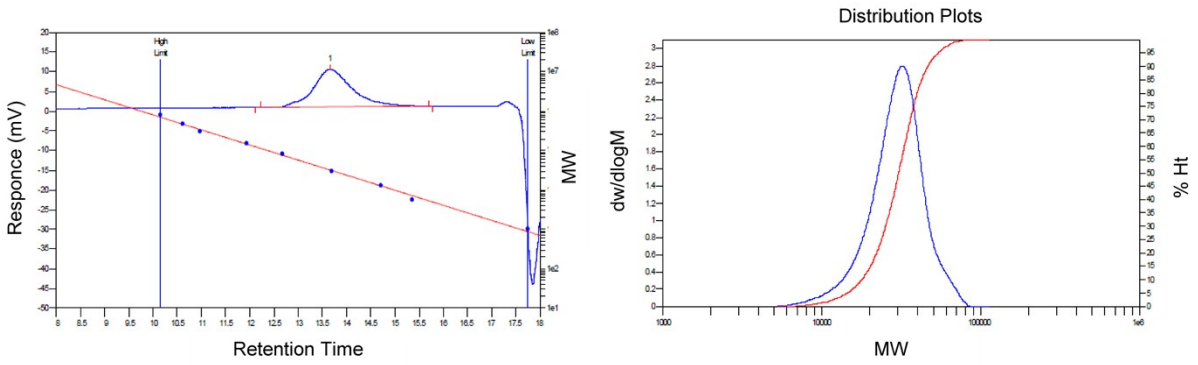


Figure S13. GPC profile of lipoyl-poly(MCH)$_{26}$-CTA eluted with DMF + 0.1 % w/w LiBr.

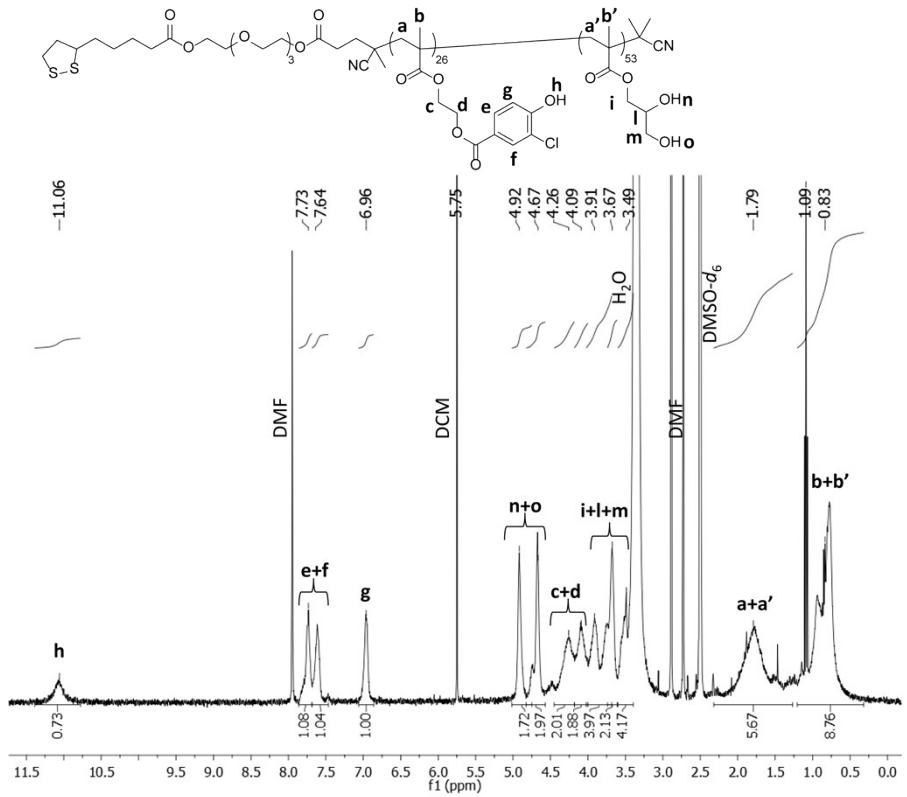


Figure S14. ^{1}H-NMR spectrum of lipoyl-[(MCH)$_{26}$-*b*-(GMA)$_{53}$] performed in DMSO-d_6 with peak assignment.

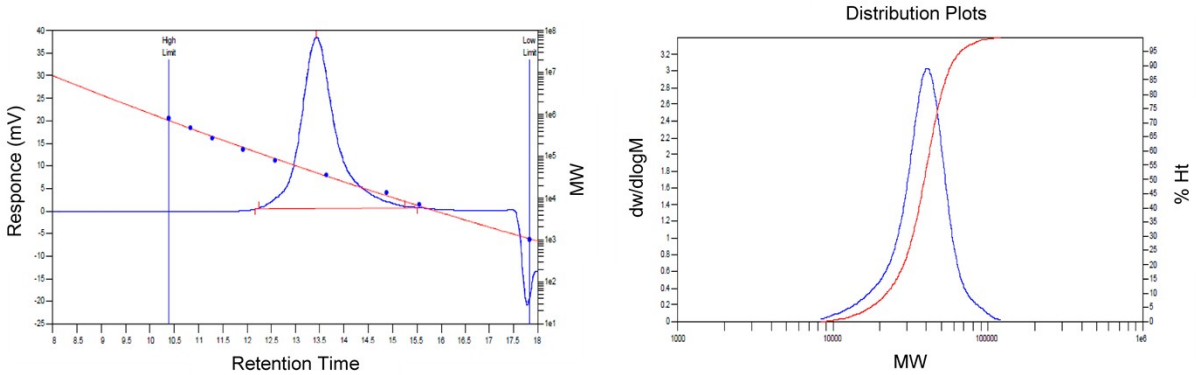
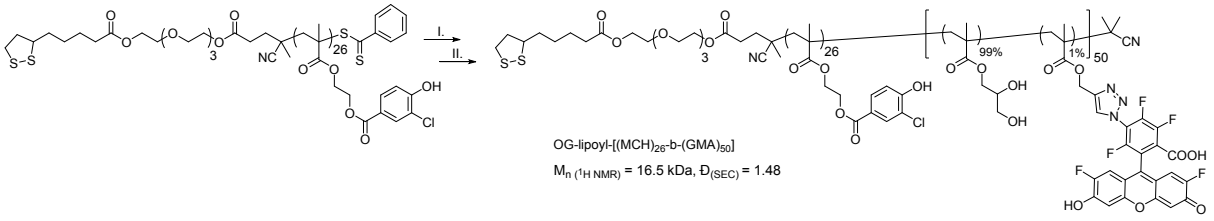


Figure S15. GPC profile of lipoyl-[(MCH)$_{26}$-*b*-(GMA)$_{53}$] eluted with DMF + 0.1 % w/w LiBr.

Table S1. Size analyses by DLS of 1 mg mL^{-1} solutions of lipoyl-[(MCH)$_{26}$-*b*-(GMA)$_{53}$] at different pHs. Size distribution was referred as volume. The population percentage of each species is reported in brackets.

pH 9	pH 7.4	pH 6.5	pH 5
4.88±0.57 (100%)	11.74±0.49 (100%)	61.45±4.37 (100%)	193.87±61.16 (91.1%)
			1838.64±210.88 (8.9%)



Scheme S3. Synthesis of OG-lipoyl-[$(MCH)_{26}$-*b*-$(GMA)_{50}$]. I. GMA, OGM, AIBN, DMF 70 °C. II. AIBN, DMF 70°C.

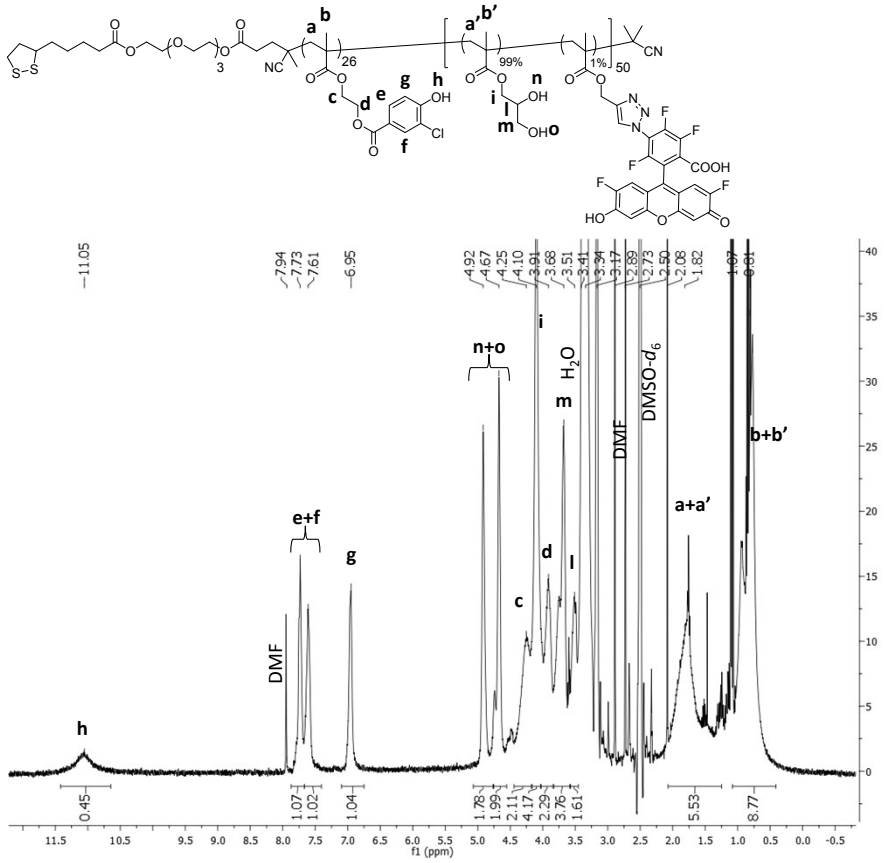


Figure S16. ^{1}H-NMR spectrum of OG-lipoyl-[$(MCH)_{26}$-*b*-$(GMA)_{50}$] in DMSO-d_6,with peaks assignment.

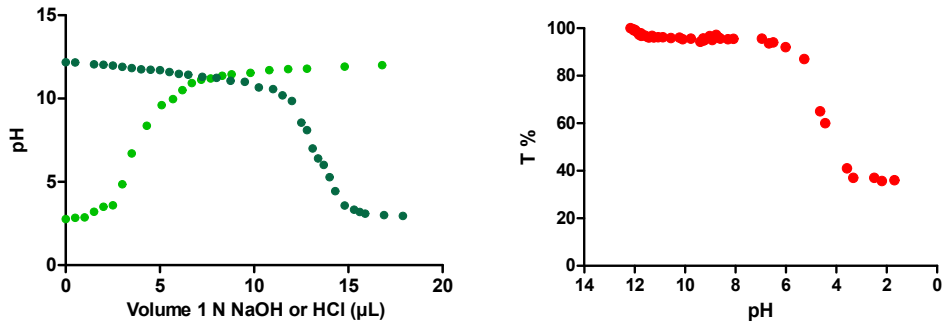


Figure S17. (A) Potentiometric Titration (●) and back titration (●) profiles of OG-lipoyl-[$(MCH)_{26}$-*b*-$(GMA)_{50}$] (B) Turbidimetric profile (●) of OG-lipoyl-[$(MCH)_{26}$-*b*-$(GMA)_{50}$].

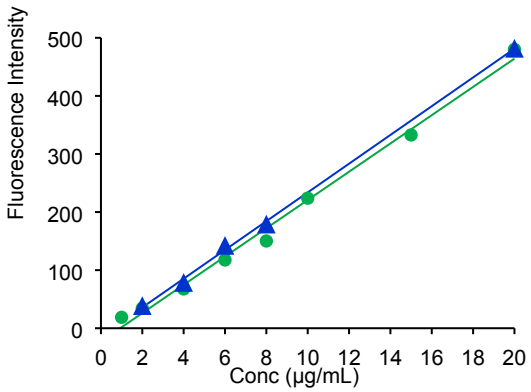


Figure S18. Fluorescence intensity of OG-lipoyl-[$(MCH)_{26}$-*b*-$(GMA)_{50}$] at 535 nm in 10 mM phosphate buffer at pH 7.4 (▲) and pH 6.5 (●) at increasing polymer concentration.

Characterization of the non-pH-responsive lipoyl-$(GMA)_{79}$ polymer

The M_n obtained from the ^1H NMR analysis of lipoyl-$(GMA)_{79}$ was found to be 13.3 kDa (Figure S19) and the polydispersity (Đ) determined by GPC was 1.17 (Figure S20).

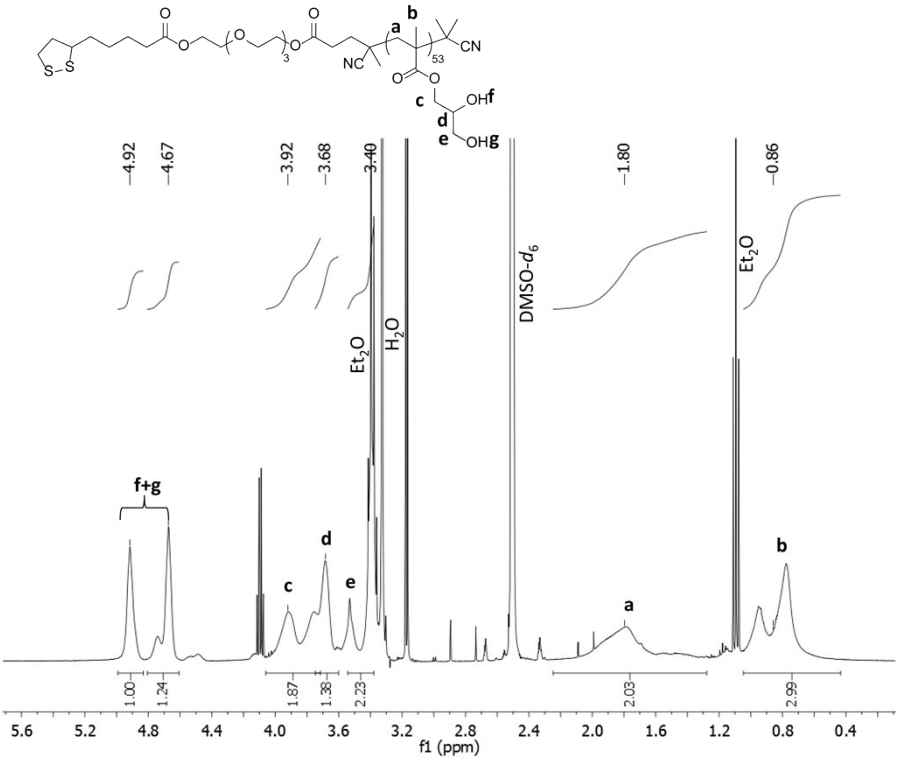


Figure S19. ^1H-NMR spectrum of lipoyl-$(GMA)_{79}$ performed in DMSO-d_6 with peak assignment.

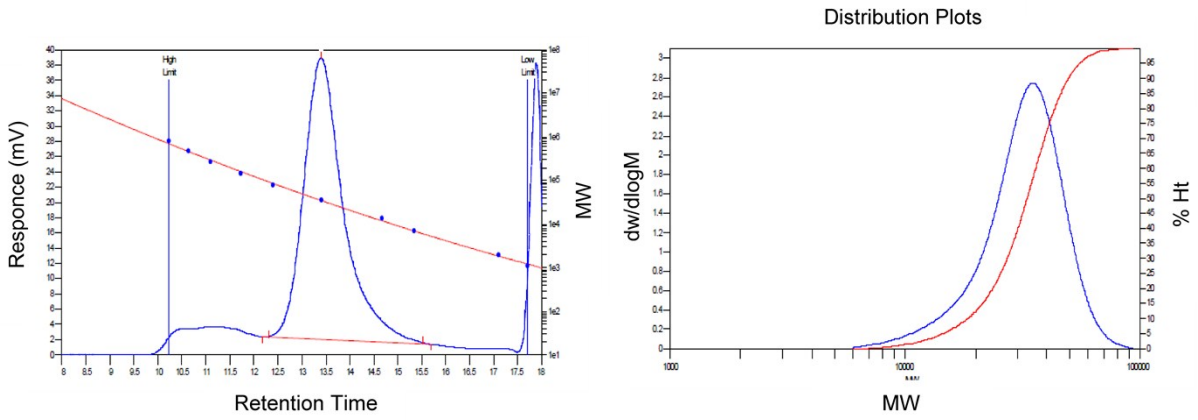


Figure S20. GPC profile of lipoyl-$(GMA)_{79}$ eluted with DMF + 0.1 % w/w LiBr.

AuNP characterization

Table S2. Sizes by DLS of naked AuNPs in 10 mM PBS at pH 7.4 and folate-targeted pH-responsive AuNPs, non-targeted pH-responsive AuNPs and folate-targeted non-pH-responsive AuNPs in 10 mM PBS at pH 7.4 or 6.5. Size distribution was referred as number, volume and intensity.

Sample	Number (d. nm)	Volume (d. nm)	Intensity (d. nm)	PDI
Naked AuNPs pH 7.4	14.5±1.6	15.4±1.8	19.5±3.8	0.08±0.01
FA pH-resp. AuNPs pH 7.4	34.2±3.1	38.2±13.8	54.4±20.1	0.34±0.08
FA pH-resp. AuNPs pH 6.5	47.9±5.4	58.5±6.7	77.2±18.7	0.33±0.10
Non-FA pH-resp. AuNPs pH 7.4	31.4±4.2	40.3±12.9	52.5±16.5	0.31±0.01
Non-FA pH-resp. AuNPs pH 6.5	44.5±7.4	56.6±11.5	80.3±30.0	0.34±0.05
FA non-pH-resp. AuNPs pH 7.4	35.3±2.6	40.8±14.4	56.6±19.9	0.29±0.04
FA non-pH-resp. AuNPs pH 6.5	31.3±2.1	40.1±14.7	57.1±20.9	0.25±0.02

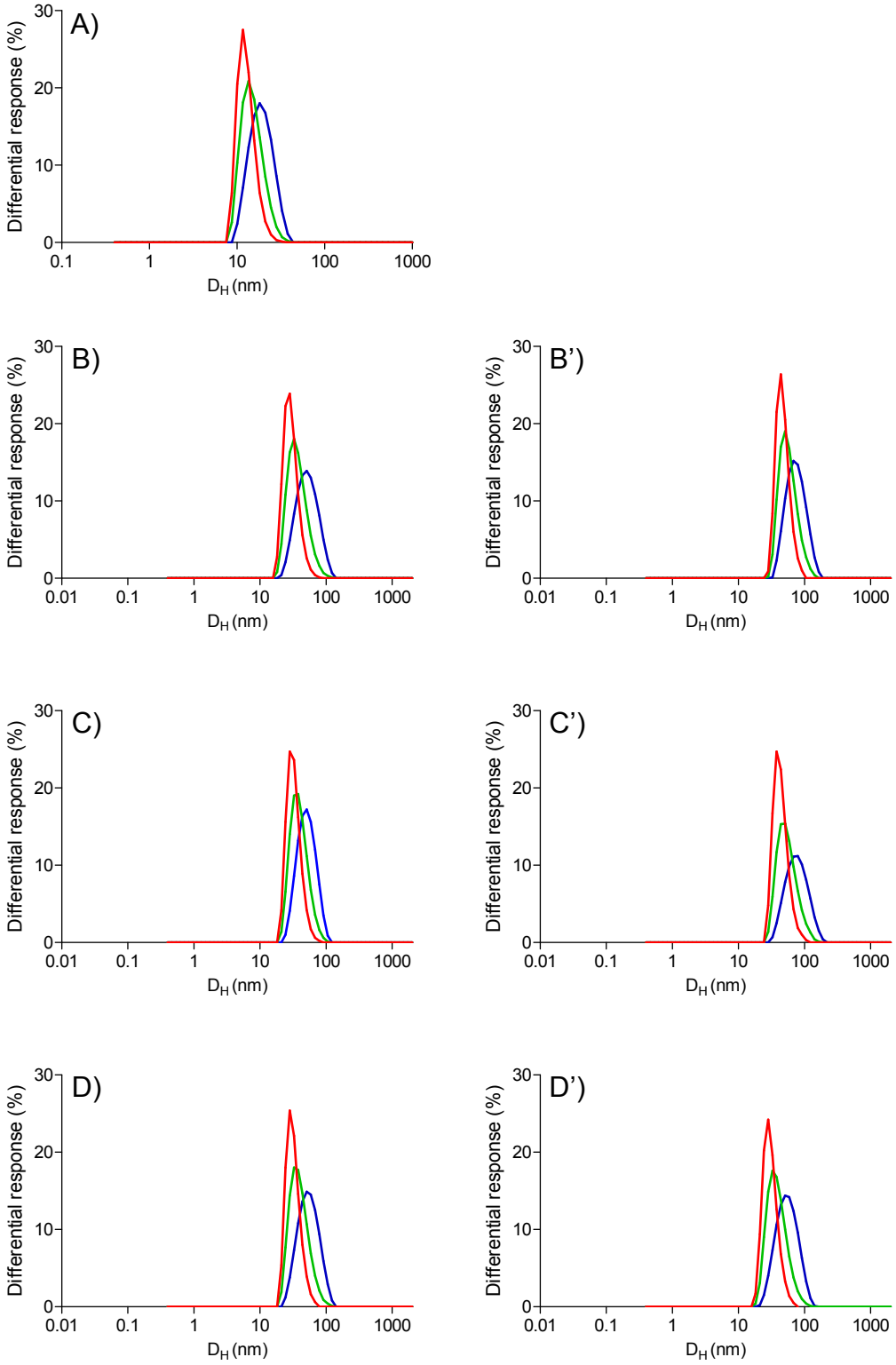


Figure S21. Size distribution profiles of naked AuNPs (A) and folate-targeted pH-responsive AuNPs (B,B'), non-targeted pH-responsive AuNPs (C,C') and folate-targeted non-pH-responsive AuNPs (D,D') in 10 mM PBS at pH 7.4 (A, B, C, D) and 6.5 (B', C', D') expressed in Number % (■), Volume % (■) and Intensity % (■).

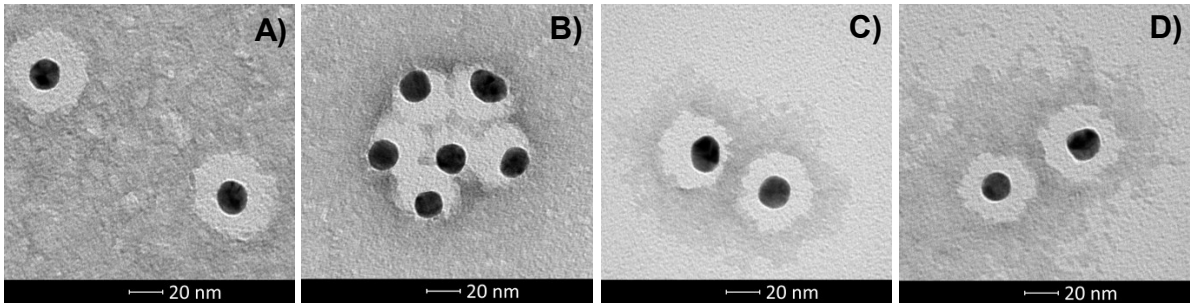


Figure S22. TEM images of folate-targeted pH-responsive AuNPs in 10 mM PBS at pH 7.4 (A) and 6.5 (B). TEM images of folate-targeted non-pH-responsive AuNPs in10 mM PBS at pH 7.4 (C) and 6.5 (D).

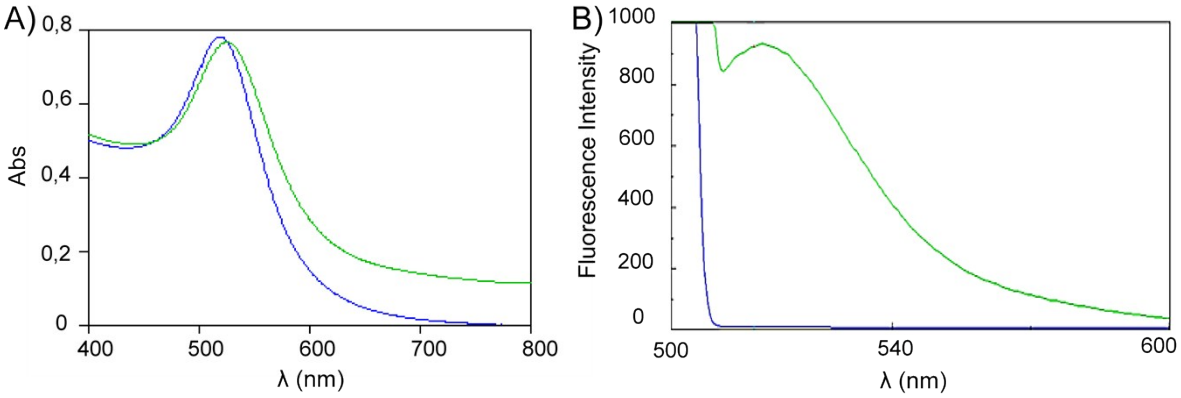


Figure S23. Absorption (A) and fluorescence (B) spectra of naked AuNPs (—) and folate-targeted pH-responsive AuNPs (—) in Milli-Q water.

***In vitro* toxicity characterization**

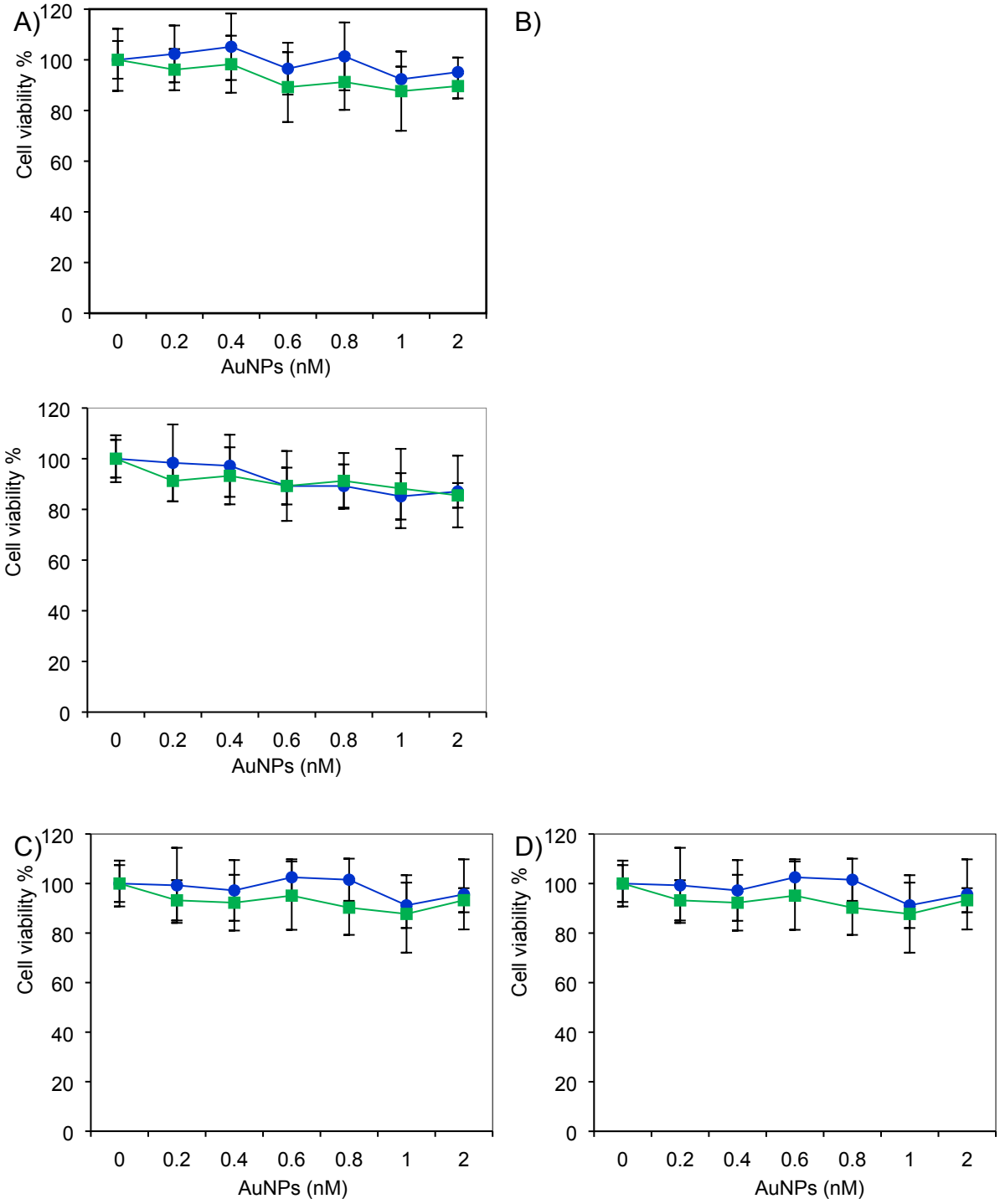


Figure S24. MTT cell viability profile of KB cells (A and B) and MCF-7 cells (C and D) incubated with increasing concentration of non-targeted pH-responsive AuNPs (A and C) and folate-targeted non-pH-responsive AuNPs (B and D) at pH 7.4 (●) and 6.5 (■) for 24 h.

Cell uptake study

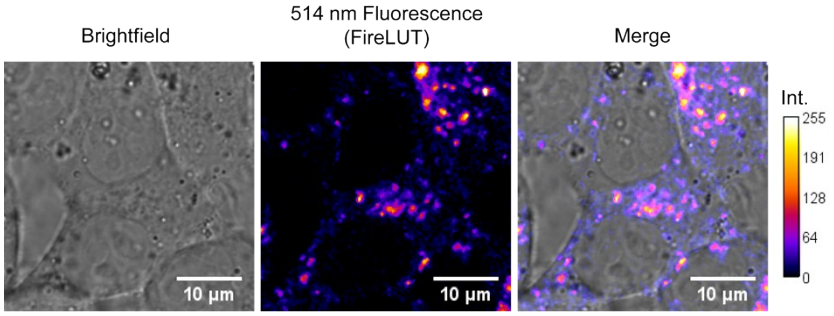


Figure S25. Representative single slice confocal microscopic image of folate-targeted pH-responsive AuNPs incubated with KB cells at pH 6.5. *Fire LUT* using ImageJ software was applied to the confocal images to emphasise differences in fluorescence intensity. Scale bars: 10 µm.

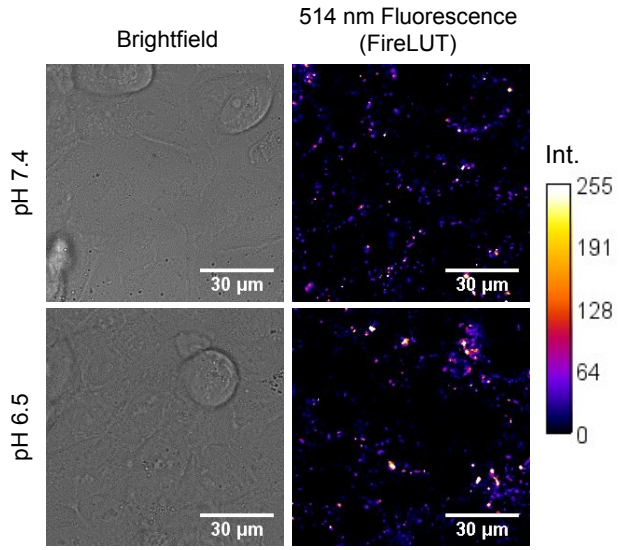


Figure S26. Confocal microscopic images of and MCF-7 cells incubated with folate-targeted pH-responsive AuNPs at pH 7.4 and 6.5. *Fire LUT* using ImageJ software was applied to the confocal images to display particle density within cells. Scale bars: 30 µm.

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