Electronic Supplementary Material (ESI) for Nanoscale.

Exploiting Poly(α-Hydroxy Acids) for the Acid-Mediated Release of Doxorubicin and Reversible Inside–Out Nanoparticle Self-Assembly

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Nuclear Magnetic Resonance (NMR) Spectroscopy

^{1}H and ^{13}C NMR spectra were recorded on Brucker Avance 500 spectrometers. Chemical shifts (in ppm) were referenced to a trimethylsilane (TMS) standard whose chemical shift is 0 ppm. To avoid possible damage to the NMR probe, Norell® heavy-walled (1.4 mm thick) NMR tubes were used for ^{1}H NMR studies carried out in deuterated triflouroacetic acid (TFA-d). Other NMR studies in common solvents, were carried out using standard 500 MHz Norell® NMR tubes. NMR spectra were analyzed using MestreNova® Research Lab software. The following abbreviations are used in the ^{1}H NMR analyses: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet, *J* = coupling constants (given in Hertz).

Fourier Transform Infrared (FTIR) Spectroscopy

Samples were dried *in vacuo* for 24 hours prior to infrared spectroscopic analysis. Spectra were then recorded on a Bruker ALPHA-P FTIR spectrometer, equipped with Bruker OPUS 7.0 software and a diamond attenuated total reflectance (ATR) accessory, accumulating 32 scans.

Centrifugation, Sample-Drying and Lyophilization

Samples were centrifuged using a Mistral 3000i MSE centrifuge unit, maintained at -5 °C (0 - 6000 rpm). Compounds were dried in a Fistreem vacuum oven that was equipped with a variable temperature control unit (0 °C - 200 °C) and a pressure gauge (0 mbar - 1020 mbar). A Thermoelectron Heto Powerdry LLI500 freeze-dryer, equipped with an Edwards two stage vacuum pump was used for lyophilisation of samples. Samples were lyophilized in distilled water in 50 mL poly(styrene) falcon tubes.

pH Measurements

Solution acidity values and basicity values were measured using a Thermo Scientific UY-58800-04 pH/mV/temperature meter. Sodium hydroxide standard solutions and hydrochloric acid standard solutions were used to adjust the pH of the buffered solutions to the required basic pH value or acidic pH value.

Ultraviolet-Visible (UV-Vis) Spectrophotometry

Absorbance readings (190 - 750 nm) were performed on a dual beam Varian Cary 50 UV0902M112 UV-Vis spectrophotometer (Agilent Technologies), equipped with a xenon pulse lamp and Varian Cary WinUV 3.0 software. Samples were analyzed in UV micro quartz cuvettes (10 mm, 700 µL and 1700 µL, black-walled). 'Simple-reads' at fixed wavelengths were carried out using a Jenway 6305 spectrophotometer (Cole-Parmer Ltd). All of the readings were taken in triplicate.

Preparation of Nanoparticles (Nanoprecipitation)

The creation of particles by the self-aggregation of poly(hydroxy acid)s in an aqueous medium was made possible by nanoprecipitation, using the dropping-in (co-solvent) method (Figure S1). A stock solution of the macromolecule was prepared in DMF. A micropipette was then used to add a predetermined volume of macromolecule solution dropwise into an excess of the aqueous medium (PBS buffer), under vigorous stirring. The obtained suspension was then dialyzed against PBS buffer

for 48 hours. The nanoparticles were then either used in their aqueous suspension after dialysis or lyophilised for further use after reconstitution in the relevant aqueous buffer solutions.

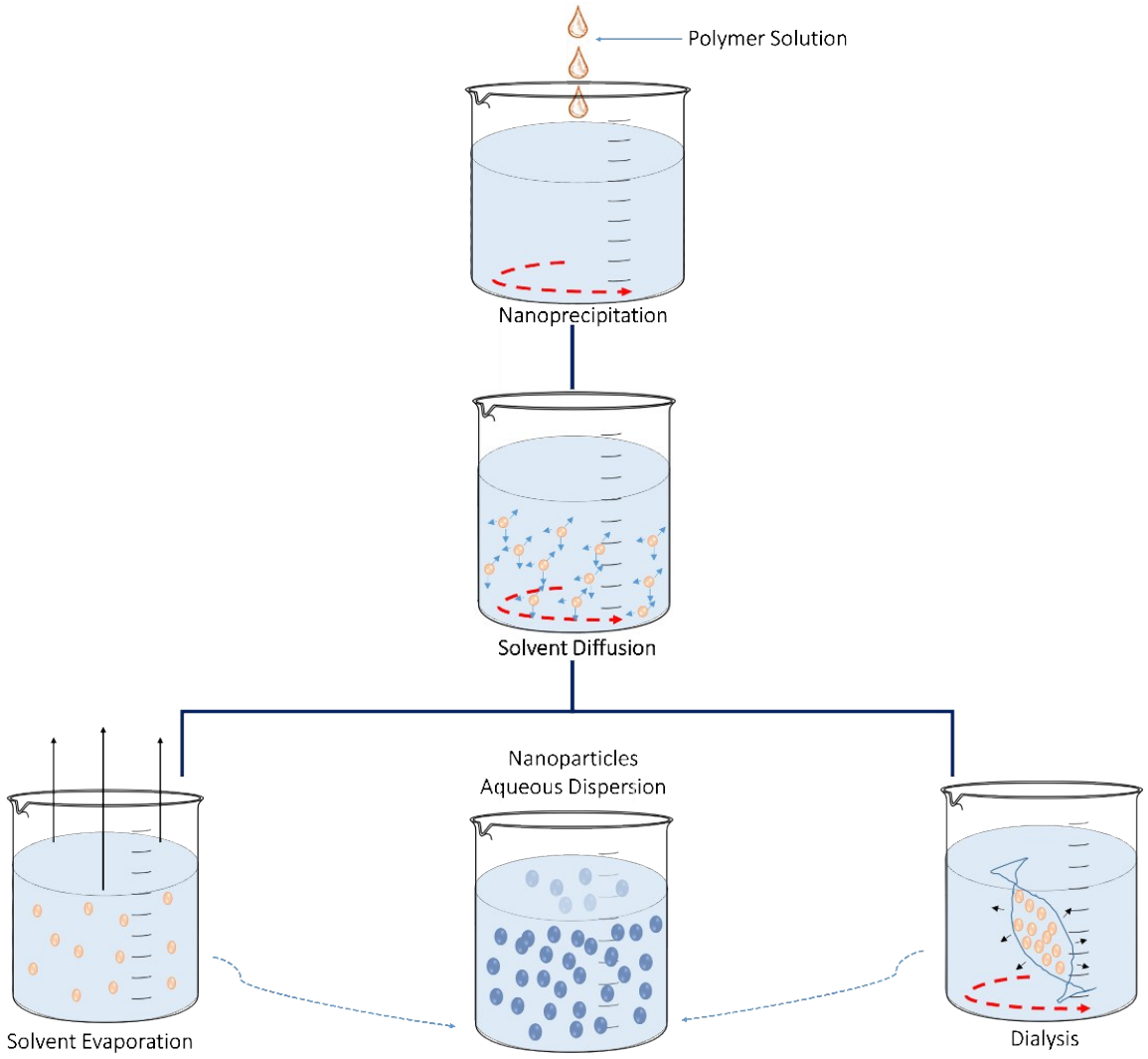


Figure S1. Illustration of the route to the generation of (nano)particles from poly(amino acid)-based macromolecules in solution, using the 'dropping-in' method.

Dynamic Light Scattering (DLS) and Zeta Potential Studies

DLS analyses were performed on a Malvern Zetasizer Nano ZSP series instrument that was equipped with a 4 mW He-Ne laser, operating at a wavelength of 633 nm, and an avalanche photodiode (APD) detector. The non-invasive back-scatter-optic arrangement was used to collect the light scattered, at an angle of 173°. Samples were equilibrated for 2 minutes and then analyzed at 37 °C in disposable 12 mm poly(styrene) cuvettes. Data were processed by the cumulative analysis of the experimental correlation function. Then the diameter of the particles was computed from the diffusion coefficients, using the Stokes-Einstein's equation. Measurements were carried out in triplicate. The instrument was furnished with DTS software (Windows 10). Zeta potential studies were carried out on a Malvern Zetasizer Nano ZSP instrument, at pH 7.4, 37 °C. No background electrolyte was added.

A viscosity of 0.891 mPa.s, a dielectric constant equal to 78.6 and Henry function equal to 1.5 were used in the zeta potential computations.

Sample Preparation, Sputter-Coating and Scanning Electron Microscopy (SEM)

With regard to solution-state samples (e.g., nanoparticles), a micropipette was used to extract approximately 20 µL of the sample from the parent suspension. The extracted sample was placed onto an SEM glass cover slip and air-dried in an extractor fume-hood, at ambient temperature. The cover slip was then mounted on an SEM stub using conductive tape.

In order to enhance the surface conductivity, avoiding sample charging up, avoiding thermal damage and improving the electron signal, the samples that were intended for analyses using SEM were sputter-coated with a coherent film of gold for 3 minutes using a rotary-pumped Quorum Q150RS sputter-coater, powered by 20 mA current. The sample size and morphology were then determined using a JEOL JSM-6610LV microscope from Oxford Instruments, equipped with a field emission electron gun as an electron source. The accelerating voltage was varied between 5 - 15 kV and the working distance was varied between 10 mm and 17 mm.

Cell Culturing and Passaging

The appropriate cells were plated in 75 cm^2 Nunclon Δ™ plastic cell culture flasks (T75) in 20 mL of Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10 % (v/v) foetal bovine serum (FBS), L-glutamine (1 mM) and penicillin (100 U/mL)-streptomycin (100 µg/mL) solution. The flasks were then incubated in a humidified incubator at 37 °C and 5 % (v/v) CO_2 in air. The culture medium was changed every 2 days. Upon reaching a 90 % confluence, the cells were passaged using a trypsin/EDTA solution (2 mL) for 5 minutes at 37 °C. An equal volume of culture medium was added to neutralize the trypsin, followed by washing under centrifugation, at 240 g for 10 minutes. Cells that were required for subsequent studies were counted on a Marienfeld-Superior Neubauer-improved haemocytometer using an Olympus CK40 light microscope. Excess cells were cryopreserved in a cryogenic-medium (DMEM supplemented with 20 % (v/v) FBS, L-glutamine (1mM) and penicillin (100 U/mL)-streptomycin (100 µg/mL) solution). The cells were then frozen in an isopropanol bath at a rate of 1 °C/minute in a freezer (-80 °C), and subsequently stored in liquid nitrogen.

The Korsemeyar-Peppas (KP) model

The mechanism for the release of payload that is encapsulated in spherical delivery vehicles can be evaluated by fitting the experimental data to the Korsmeyer-Peppas (KP) model (Equation 1). Using this model, the release exponent (n) can be determined by statistical analysis. For $n \leq 0.45$, the release of the encapsulated cargo follows Fickian diffusion, in which polymer relaxation dominates the rate of diffusion of the encapsulated cargo. Deviation from Fickian diffusion is indicated by values greater than 0.45, whereby the rates of diffusion of the encapsulated cargo and polymer relaxation are comparable. That is, for $0.45 < n < 0.89$ the release can be considered to be non-Fickian (anomalous), possibly because of a variety of factors, including the surface erosion or the bulk erosion of the delivery vehicle.

$$\frac{M_t}{M_\infty} = kt^n \quad (1)$$

Here, M_t and M_∞ represent the cumulative amounts of payload that are released at time t and at infinite time, respectively. Hence, M_t/M_∞ is the fractional payload release at time t, *n* is the release exponent being indicative of the release mechanism and *k* is the rate constant that takes into

account the geometric characteristics of the organogel and the encapsulated cargo. Using Equation 2.3, which is generated by the differentiation of Equation 2, a linear plot can be obtained, whose gradient is the release exponent (*n*), and may be used to determine whether or not the payload release follows a Fickian profile, or a non-Fickian diffusion profile.

$$\text{Log (Release \%)} = \text{Log}\left[\frac{M_t}{M_\infty}\right] = n\,\text{Log}\,t + \text{Log}\,k \qquad (2)$$

Drug Encapsulation Efficiency and Drug Loading Content

The drug encapsulation efficiency (EE) of nanoparticles and the drug loading content (LC) in the nanoparticles were determined using Equation 3 and Equation 4, respectively;

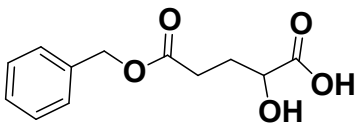
$$Encapsulation\ Efficiency\ (\%) = \frac{Wo - Wn}{Wo} * 100 \qquad (3)$$

$$Drug\ Loading\ Content\ (\%) = \frac{Wo - Wn}{Wnp} * 100 \qquad (4)$$

Here, W_o is the total weight of the drug that was fed during nanoprecipitation, W_n is the net weight of drug that was not encapsulated by the nanoparticles, W_{np} is the weight of the drug-loaded nanoparticles.

Synthesis of Glu(Bz) OCA

Synthesis of Y-Benzyl-2-Hydroxyglutaric Acid



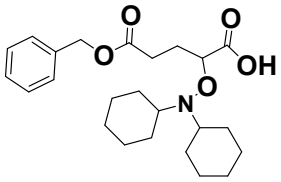
Glu(Bz) (5 g, 21.1 mmol) was dissolved in a solution of 1 M sulfuric acid and acetone (100 mL, 1:1 v/v). The solution was cooled to 0 °C in an ice bath. Sodium nitrite (4.40 g, 63.2 mmol) was dissolved in deionized water (10 mL). The solution was added dropwise to the cooled reaction medium, over a period of 30 minutes. The reaction medium was maintained at 0 °C for 2 hours, and then at room temperature for 18 hours. Deionized water (500 mL) was added and the mixture extracted three times with ethyl acetate (300 mL). The organic layers were then combined and washed three times with deionized water (500 mL), followed by saturated brine (500 mL) and dried over magnesium sulfate for one hour. The drying agent was discarded by filtering under vacuum and the product subsequently isolated from ethyl acetate by rotary evaporation. The crude hydroxyacid was purified by flash chromatography (Eluent: DCM (95)/MeOH (4.5)/AcOH (0.5) to give a light yellow oil. The hydroxy acid slowly crystallized upon standing.

Yield: 2.10 g, 8.83 mmol, 41.9%. ESI-MS (261.1 M + Na^+).

^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 7.25 - 7.31 (m, 5H, Ph), 5.06 (s, 2H, C$\underline{H_2}$Ph), 4.26 - 4.24 (dd, 1H, CH, *J* = 4.25 Hz), 2.59 - 2.46 (m, C$\underline{H_2}CO_2$, *J* = 2.51 Hz), 2.20 - 1.96 (m, 2H, C$\underline{H_2}CHCO_2$).

Synthesis of γ-Benzyl-2-Hydroxyglutaric Acid Dicyclohexylamine Salt

γ-Benzyl-2-hydroxyglutaric acid (3.34 g, 14.0 mmol) was dissolved in anhydrous diethyl ether (50 mL). The solution was injected into a round bottom flask and it was cooled in an ice bath. Dicyclohexylamine (2.54 g, 14.0 mmol) was added dropwise to the cooled solution. The reaction medium was removed from the ice bath and stirred at room temperature for 45 minutes. The precipitated salt was filtered and then it washed several times using cold diethyl ether. The salt was dried, *in vacuo.*

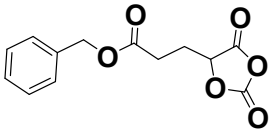


Yield: 3.34 g, 8.00 mmol, 57.1%.

^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 7.28 7.24 (m, 5H, Ph), 5.04 (s, 2H, CH_2Ph), 3.86 - 3.84 (dd, 1H, CH, *J* = 3.85 Hz), 2.98 - 2.93 (m, 2H, NCH), 2.56 - 2.36 (m, 2H, $CH_2CH_2CO_2$), 2.11 - 2.09 (m, 2H, CH_2CHCO_2), 2.00 - 1.48 (m, 22H, CH_2, *cyclohexyl*). ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 174.59 (COOH), 172.94 ($CH_2CO(O)$), 137.09 (Ar), 128.50 - 127.92 (Ar), 79.72 (αCH), 66.15 ($BzCH_2O$), 61.35 (N(CH)), 30.92 (s), 30.10 ($(CH_2)_2$, *cyclohexyl*), 26.32 ($\alpha CHCH_2$), 25.99 (CH_2, *cyclohexyl*), 24.77 ($(CH_2)_2$, *cyclohexyl*). ESI-MS (417.25), Elemental Analysis; *Theoretical:* Carbon 69.04%, Hydrogen 8.45%, Nitrogen 3.35%. *Found:* Carbon 69.07%, Hydrogen 8.42%, Nitrogen 3.38%.

Salt Cyclisation to form an OCA Monomer

γ-benzyl-2-hydroxyglutaric acid dicyclohexylamine salt (3.33, 8 mmol) was added to a suspension of poly(styrene)-supported diisopropylethylamine resin, in anhydrous diethyl ether (60mL). Disphosgene (4.80 g, 24 mmol) was added. The reaction medium was degassed several times and then it was stirred under a nitrogen flow, at room temperature, for 18 hours. The volume of the filtered crude product was then reduced to one-third of its initial volume using rotary evaporation. The crude solution was added dropwise to cold anhydrous THF/pentane (1/9, v/v) to crystallize the OCA. The OCA was collected, washed several times with cold pentane and then dried under vacuum. Yield: 1.06 g, 3.45 mmol, 50.2% (Light yellow oil that crystallizes on standing).

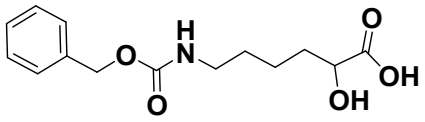


^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 7.39 - 7.34 (m, 5H, Ph), (dd, 1H, CH, *J* = 5.22 Hz), 5.15 (s, 2H, CH_2Ph), 2.65 - 2.56 (m, 2H, CH_2CO_2, *J* = 2.61 Hz), (m, CH_2CHCO_2). ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 172 ($\alpha CHCO_2$), 167 (CO_2CH_2Ph), 148 ($\alpha CHCO_3$), 135 ($(CH)_5C$ *aromatic*), 128 ($(CH)_5$ *aromatic*), 78 (αCH) 67 (CH_2Ph), 28 (CO_2CH_2), 26 (CH_2CH). FTIR:ν_{max}/cm^{-1} (oil): 3033 (C-H, Ar stretch), 2896 (C-H, alkyl), 1853, 1776 (C=O, amide and ester overlap), 1457 (C-O), 732 (aromatic 'oop' bends). Elemental Analysis; *Theoretical:* Carbon 59.09%, Hydrogen 4.58%; *Found:* Carbon 59.12%, Hydrogen 4.59%. Melting point range: 142 °C – 145 °C.

Synthesis of Lys(Cbz) OCA

Synthesis of 6-(Benzyloxycarbonylamino)-2-Hydroxyhexanoic Acid

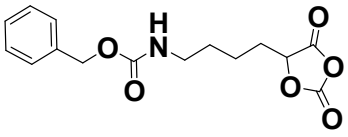
A similar procedure to that described for the synthesis of γ-Benzyl-2-Hydroxyglutaric Acid was followed. However, the reaction was worked up in diethyl ether.



Yield: 4.74 g, 16.8 mmol, 93.7% (Yellow powder).

^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 7.40 - 7.19 (m, 5H, Ph), 5.43 (s, 2H, CH_2Ph), 4.16 - 4.14 (dd, 1H, CH, *J* = 4.15 Hz), 1.79 - 1.57 (m, 2H, CH_2CHCO_2), 1.47 - 1.26 (m, 4H, CH_2CH_2).

Cyclisation into Lys(Cbz) OCA



6-(benzyloxycarbonylamino)-2-hydroxyhexanoic acid (4.70 g, 16.7 mmol) was dissolved in anhydrous THF. The solution was added to a round bottom flask, which contained activated charcoal (0.2 g, 16.7 mmol). Diphosgene (13.2 g, 66.8 mmol) and triethylamine (300 µL, 16.7 mmol) were injected dropwise into the reaction medium, under constant stirring. Further steps were carried out, as described for the synthesis of Glu(Bz) OCA, with the exception that pure Lys(Cbz) OCA was obtained by recrystallization of crude product in diethyl ether:diisopropyl ether solution (1:5 v/v), at -18 °C, for 48 hours.

Yield: 4.52 g, 14.6 mmol, 87.6% (White waxy crystals).

^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 7.43 - 7.19 (m, 5H, Ph), 5.10 – 4.81 (m, 3H, Ph-C$\underline{H}_2$, αC$\underline{H}$, *J* = 5.06 Hz), 3.75 - 3.57 (t, 1H, CON$\underline{H}$, *J* = 4.19 Hz), 3.12 (d, 2H, NHC$\underline{H}_2$, *J* = 3.15 Hz), 2.03 - 1.73 (m, 2H, CHC$\underline{H}_2$), 1.64 - 1.29 (m, 4H, C$\underline{H}_2$C$\underline{H}_2$). ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 178 (αCH$\underline{C}O_2$), 167.1 ($\underline{C}O_2CH_2Ph$), 157 (αCHO$\underline{C}O_2$), 148.2, ($\underline{C}CH_2OCONH$), 128.6 (Ar$\underline{C}$H), 79.6 (α$\underline{C}$H), 67.9 (Ph$\underline{C}H_2OCO$), 40.3 (OCONH$\underline{C}H_2$), 30.3 (NHCH$_2\underline{C}H_2$), 29.1 (αCH$\underline{C}H_2$), 21.29 (NHCH$_2$CH$_2\underline{C}H_2$). FTIR:ν_{max}/cm^{-1} (oil): 3601 (NH, amide stretch), 3029 (C-H, Ar stretch), 2787 (C-H, alkyl stretch), 1862, 1783 (C=O, amide and ester overlap), 758 (aromatic 'oop' bends). Elemental Analysis: *Theoretical:* Carbon 58.63%, Hydrogen 5.58%; Nitrogen 4.56%; *Found:* Carbon 58.65%, Hydrogen 5.59%, Nitrogen 4.52%. Melting point range: 214 °C – 217 °C.

Synthesis of Poly[(Lys(Cbz)LA)$_m$-*b*-(Glu(Bz)LA)$_n$]

Lys(Cbz) OCA (562 mg, 1.82 mmol) was dissolved in anhydrous DMF (10 mL), in a flame-dried and nitrogen-purged Schlenk tube that was equipped with a magnetic stirrer bar. 4-dimethylaminopyridine (DMAP) (22.3 mg, 0.18 mmol) was dissolved in anhydrous DMF (2 mL). The solution was injected into the Schlenk tube. Then, a solution of 2-methyl-1-propanol (4.50 mg, 0.06 mmol) in anhydrous DMF (5 mL) was added to initiate the reaction. The reaction medium was stirred at room temperature, for 120 hours under a nitrogen flow. Aliquots (0.1 mL) were extracted from the reaction medium at predetermined time intervals, added to 0.5 mL deuterated chloroform and analyzed by ^{1}H NMR, to ascertain the degree of polymerization. When the OCA monomer was fully polymerized, the poly(L-Lys(Cbz)LA)$_m$ macromolecule was isolated by precipitation in cold diethyl ether and then centrifugation. The macromolecule was subsequently re-dissolved in anhydrous DMF (10 mL). The solution was injected into a flame-dried and nitrogen-purged Schlenk tube. DMAP (22.3 mg, 0.18 mmol) was dissolved in anhydrous DMF (2 mL) and added to the reaction medium. Then Glu(Bz) OCA (481.1 mg, 1.82 mmol) was dissolved in anhydrous DMF (5 mL) and injected gently into the reaction medium. The reaction medium was degassed and then stirred at room temperature, for a further 120 hours, after which the Glu(Bz) OCA had fully polymerized. The reaction medium was diluted with distilled water (5 mL) and emptied into a dialysis tubing membrane (2000 Da, MWCO) and dialyzed against distilled water for 96 hours, replenishing the dialysate every 12 hours. The diblock polyester was eventually isolated by lyophilization.

Yield: 65 wt. %. ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 7.65 - 7.35 ($C_6\underline{H}_5$), 5.24 - 5.09 (Ph-C$\underline{H}_2$O), 4.98 (αC$\underline{H}$(CO)O), 4.28 (N$\underline{H}$COO), 3.65 ((CH$_3$)$_2$CHC$\underline{H}_2$), 3.49 - 3.05 (NHC$\underline{H}_2$), 2.75 - 2.39 (C$\underline{H}_2$)$_2$COO), 2.29 ((CH$_3$)$_2$C$\underline{H}$), 2.11 - 1.01 (αCH(C$\underline{H}_2$)$_3$, 0.88 (C$\underline{H}_3$)$_2$). FTIR:ν_{max}/cm^{-1} (solid): 690 - 800 cm^{-1} (aromatic C-H bending), 1744 (C=O ester stretch), 2942 cm^{-1} (C-H alkyl stretch).

Deprotection of Poly[(Lys(Cbz)LA)$_m$-*b*-(Glu(Bz)LA)$_n$]

Poly[(Lys(Cbz)LA)$_m$-*b*-(Glu(Bz)LA)$_n$] (320 mg) was dissolved in THF:methanol (1:1 v/v, 30 mL). The solution was injected into a sealed round bottomed flask, that was furnished with a 10% Pd/C (64 mg, 20 wt. % of poly(ester)). The reaction medium was purged several times using hydrogen, and then stirred under hydrogen flow for 72 hours, at room temperature. The Pd/C catalyst was isolated from the reaction medium by gravitational filtration. The filtrate was concentrated by rotary evaporation, yielding the crude deprotected block copolyester. The polymer was then dialyzed against deionized water for 48 hours and then lyophilized. Yield: 185 mg, 57.8 wt %). ^{1}H NMR (500 MHz, D_2O, δ, ppm): 4.89 - 4.86 (αC<u>H</u>(CO)O), 4.35 - 4.28 (N<u>H</u>COO), 3.74 - 3.65 ($(CH_3)_2CHC\underline{H}_2$), 3.16-3.07 (NHC<u>H</u>$_2$), 2.69 - 2.51 (C<u>H</u>$_2$)$_2$COO), 2.19 ($(CH_3)_2C\underline{H}$), 2.01 - 1.21 (αCH(C<u>H</u>$_2$)$_3$, 0.81 (C<u>H</u>$_3$)$_2$). FTIR:ν_{max}/cm^{-1} (solid): 1752 (C=O ester stretch), 2499 - 3300 cm^{-1} (carboxylic acid OH stretch + C-H alkyl stretch).

Assessment of pH-Dependent Zwitterionic Behaviour

A polyester solution (3 mg/mL) was prepared in anhydrous DMF and filtered through a 0.2 µm syringe filter. Then, aqueous buffer solutions, i.e., an acetate buffer (pH 4.5), a PBS buffer (pH 7.4) and a 4-(cyclohexylamino)-1-butanesulfonic acid (CABS) buffer (pH 11.2) were also filtered through 0.2 µm syringe filters. The filtered polyester solution (1 mL) was added dropwise to the vigorously stirred pH 4.5, pH 7.4 and pH 11.2 aqueous buffer solutions (10 mL), respectively. The prepared samples were collected immediately into disposable poly(styrene)-based cuvettes, and the samples were analyzed using DLS, at 37 °C, to ascertain polymer aggregation, aggregate sizes and zeta potential values. Samples were also collected immediately after nanoprecipitation, and deposited onto SEM stubs. These were air-dried, sputter-coated with gold and subsequently analysed using SEM.

Dox-Loading and Release from Poly[(Lys(Cbz)LA)$_m$-*b*-(Glu(Bz)LA)$_n$] NPs

Dox-loaded poly[(Lys(Cbz)LA)$_{26.6}$-*b*-(Glu(Bz)LA)$_{28.3}$] NPs were prepared by the co-nanoprecipitation of the Dox free-base and poly[(Lys(Cbz)LA)$_{26.6}$-*b*-(Glu(Bz)LA)$_{28.3}$] in a pH 7.4 PBS buffer. The NPs were then dialyzed against the PBS buffer for 72 hours and subsequently lyophilized. Dox-loaded NPs were subsequently reconstituted in an acetate buffer (pH 4.5) only and in a PBS buffer (pH 7.4) only. The release profile of Dox from the dispersions was assessed using the dialysis method. In addition, free Dox was reconstituted in a acetate buffer (pH 4.5) only, and also in a PBS buffer (pH 7.4) only. The release profile of Dox from the respective NPs was then assessed.

Cytotoxicity Assessment of Blank NPs and Dox-Loaded NPs

T47D and MCF-7 cells were supplied by the European Collection of Authenticated Cell Cultures (ECACC) and were cultured in DMEM (Invitrogen), supplemented with 10% (v/v) FCS (Sigma) at 37 °C in 5% CO_2. The cells were certified mycoplasma-free and were short tandem, repeat-profiled for verification. 1000 T47D cells and 2000 MCF-7 cells were plated in quadruplicate per well, in 96-well microplates. After 24 hours, the Dox-loaded NPs were added to the cells at varying Dox concentrations. In addition, equivalent loadings of blank NPs (polymer) only, and free Dox only were added to cells in independent wells. The experimental sets-up were incubated for 72 hours, at 37 °C, in a humidified 5% CO_2-containing atmosphere. Then, the culture medium was replaced with a 0.5 mg/mL MTT-containing medium and the sets-up were incubated further for 3 hours, at 37 °C. The medium was replaced with DMSO. Then absorbance readings were recorded at 620 nm, using a Mithras LB 940 plate-reader. To obtain IC_{50} values, data were fitted to the four-parameter log (inhibitor) versus response curve in GraphPad Prism (software version 7.02).

MDA-MB-453 and MDA-MB-231 cells were obtained from ECACC and cultured in DMEM (Invitrogen) supplemented with 10% (v/v) FCS (Sigma) at 37 °C in 5% CO_2. The cells were certified mycoplasma-free and were STR profiled for verification. 10,000 cells were plated per well in 96-well plates. 24 hours later, Dox-loaded polymers were added to the cells in quadruplicate at each concentration. Equivalent concentrations of polymer alone were also added to cells alongside free Dox. Cells were incubated with the polymers and drug for 72 hours before the medium was replaced with 0.5 mg/mL MTT-containing medium. After incubation for 3 hours at 37 °C, the medium was removed and DMSO was added. The absorbance at 620 nm of each well was read on a plate-reader (BertholdTech Mithras). Each entire experiment was carried out in triplicate. To obtain an IC_{50} value, the results were fitted with a four-parameter log(inhibitor) vs. response curve using GraphPad Prism software version 7.02.

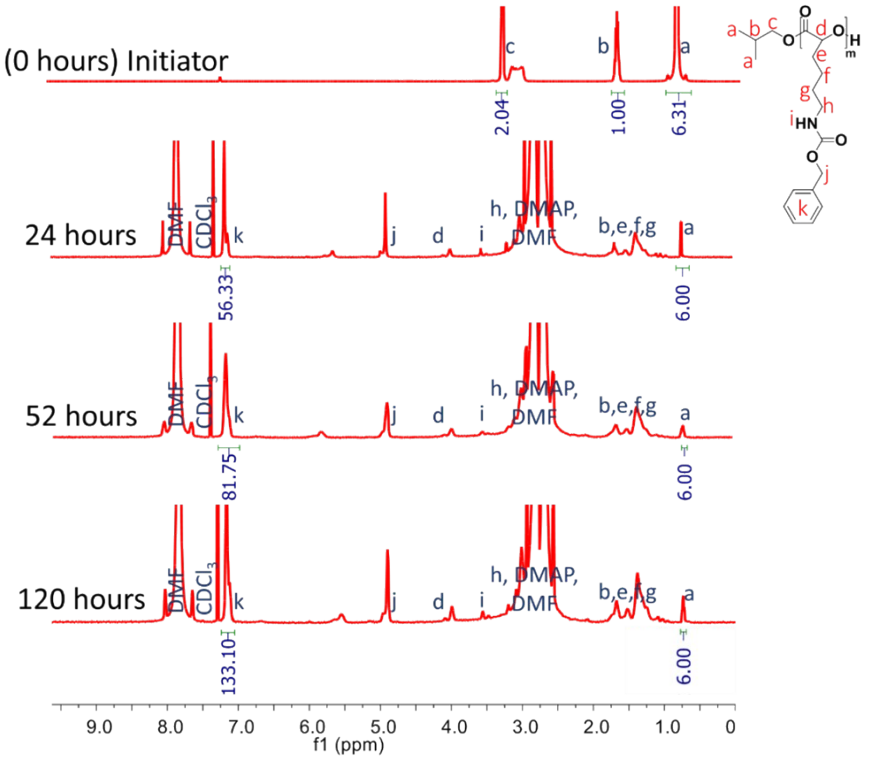


Figure S2. ^{1}H NMR spectra obtained from the analyses of reaction fractions that were extracted from the ROP of Lys(Cbz) OCA from an isobutanol molecule.

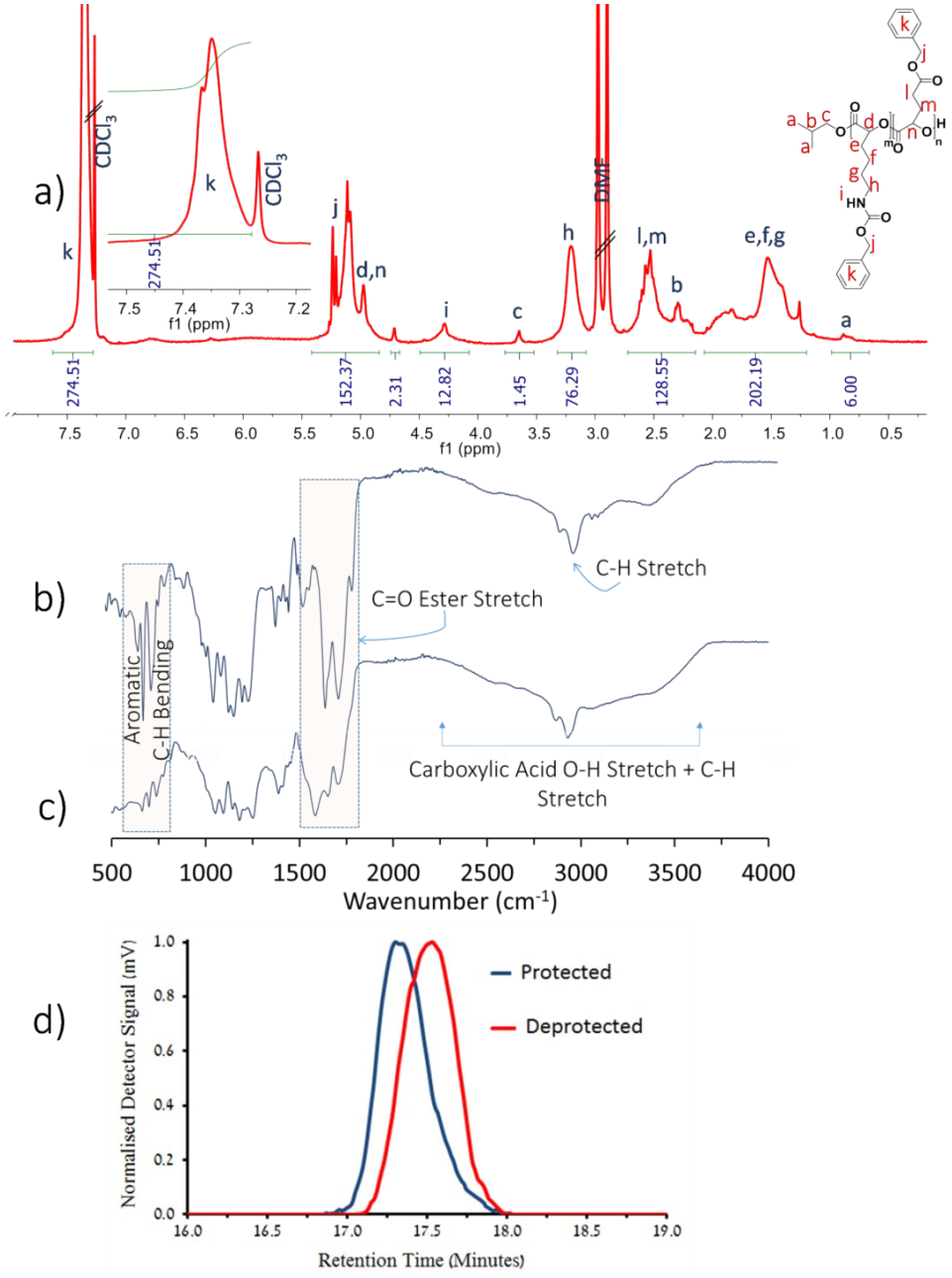


Figure S3. (a) ^{1}H NMR spectrum of poly[(Lys(Cbz)LA)$_{26.6}$-*b*-(Glu(Bz)LA)$_{28.3}$], FTIR spectra of poly[(Lys(Cbz)LA)$_{26.6}$-*b*-(Glu(Bz)LA)$_{28.3}$] before (b) and after poly(ester) deprotection (c), and (d) GPC chromatograms obtained from the analyses of poly[(Lys(Cbz)LA)$_{m}$-*b*-(Glu(Bz)LA)$_{n}$] macromolecules before after poly(ester) deprotection and after poly(ester) deprotection.

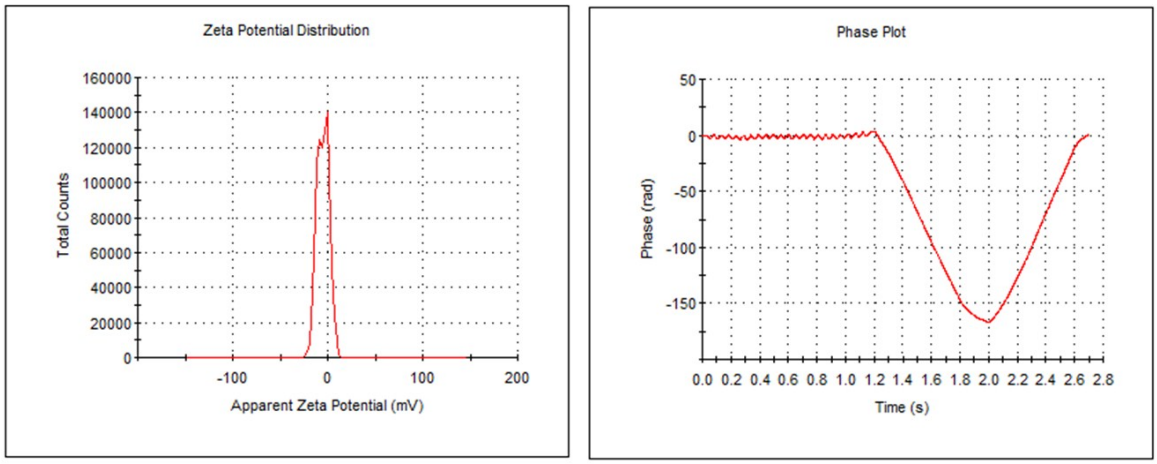


Figure S4. Representative graphs showing zeta potential distribution (*left*) and a 'good quality' phase plot (*right*) of Poly[(Lys(Cbz)LA)$_{26.6}$-*b*-(Glu(Bz)LA)$_{28.3}$] dispersion at **pH 4.2** (37 °C), suggesting that the particles are monomodal and stable (Data: n = 3). A viscosity of 0.891 cP, a dielectric constant of 78.6, and Henry function of 1.5 were used for the zeta potential calculations.

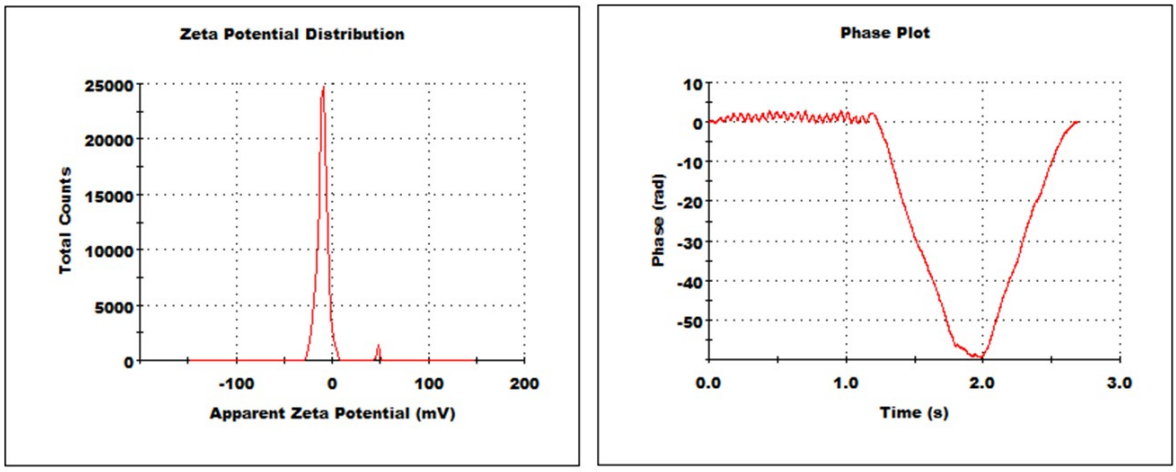


Figure S5. Representative graphs showing zeta potential distribution (*left*) and a 'good quality' phase plot (*right*) of Poly[(Lys(Cbz)LA)$_{26.6}$-*b*-(Glu(Bz)LA)$_{28.3}$] dispersion at **pH 7.4** (37 °C), suggesting that the particles are monomodal and stable (Data: n = 3). A viscosity of 0.891 cP, a dielectric constant of 78.6, and Henry function of 1.5 were used for the zeta potential calculations.

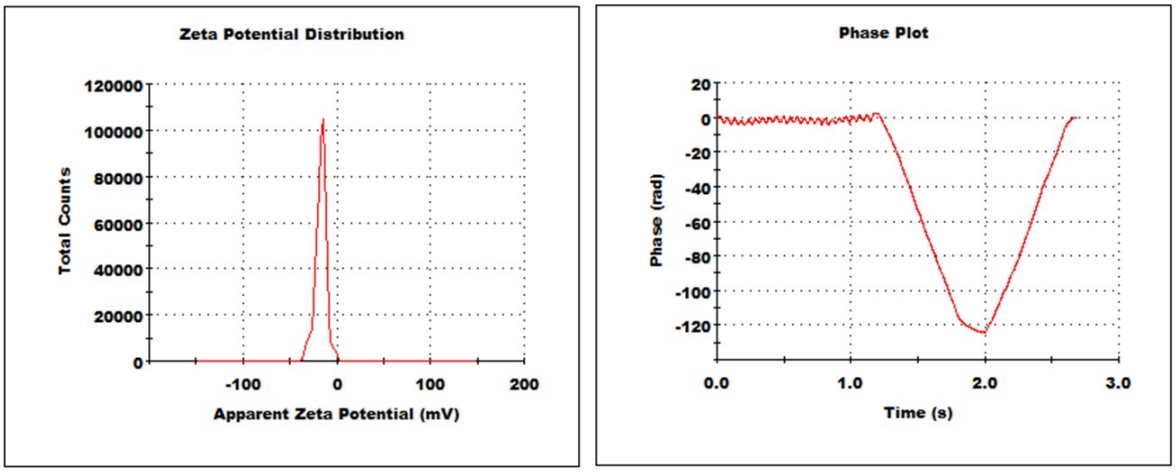


Figure S6. Representative graphs showing zeta potential distribution (*left*) and a 'good quality' phase plot (*right*) of Poly[(Lys(Cbz)LA)$_{26.6}$-*b*-(Glu(Bz)LA)$_{28.3}$] dispersion at **pH 11.2** (37 °C), suggesting that the particles are monomodal and stable (Data: n = 3). A viscosity of 0.891 cP, a dielectric constant of 78.6, and Henry function of 1.5 were used for the zeta potential calculations.

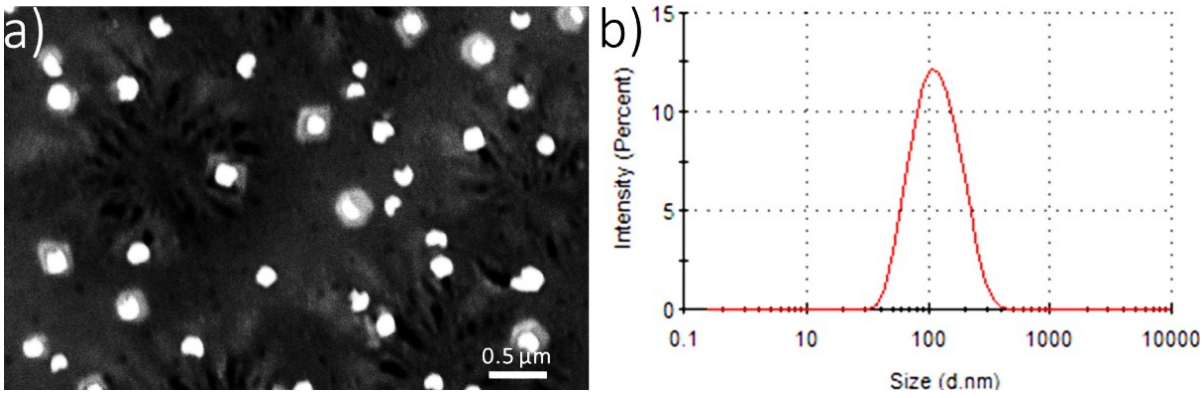


Figure S7. (a) SEM microphotograph and (b) DLS chart, revealing the size-distribution of Dox-loaded poly[(Lys(Cbz)LA)$_{26.6}$-*b*-(Glu(Bz)LA)$_{28.3}$] NPs. Scale bar represents 0.5 µm.

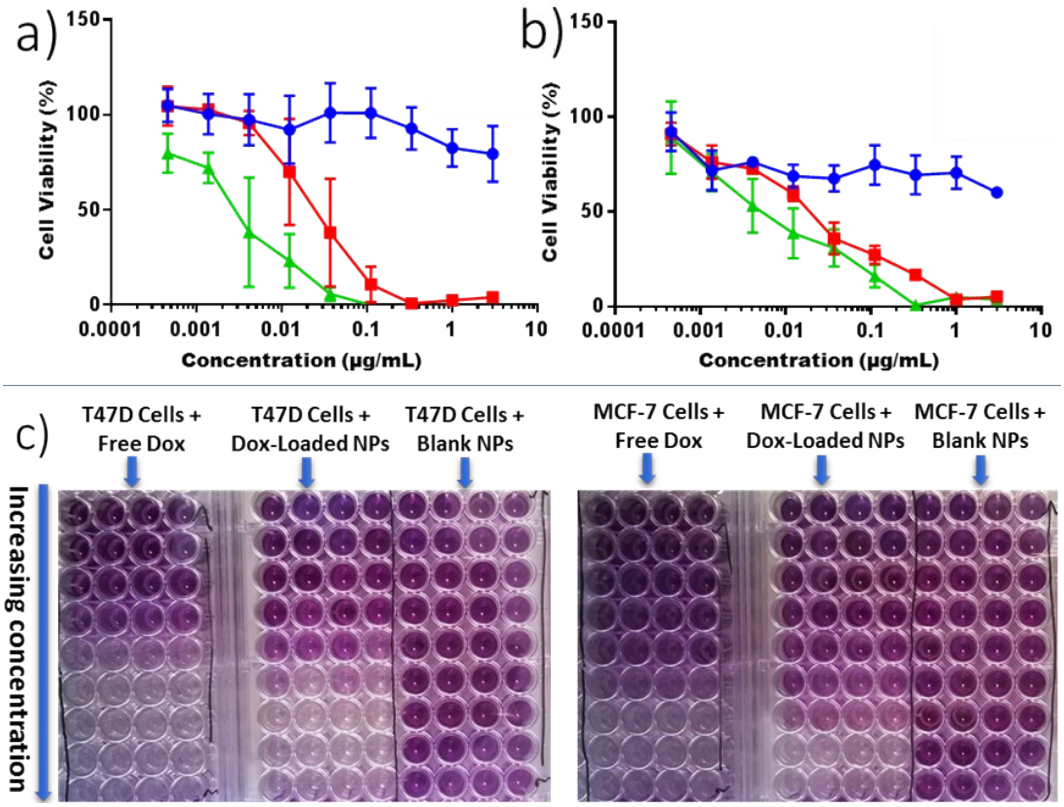


Figure S8. The effect of blank polymer NPs (●), Dox-loaded polymer NPs (■) and free Dox (▼) on the viability of (a) T47D human breast cancer cells and (b) MCF-7 human breast cancer cells. Data is presented as mean ± SD (n = 4). (c) Representative macro images obtained from the colorimetric MTT assay in which the purple colouring indicates the presence of viable cells.

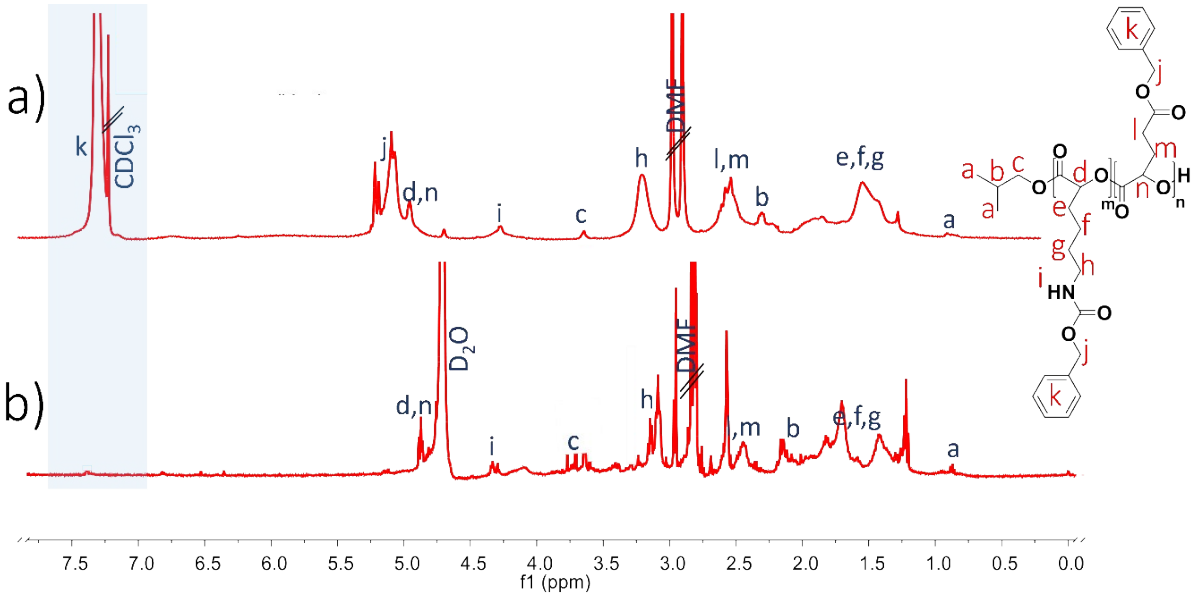


Figure S9. ^{1}H NMR spectra of (a) poly[(Lys(Cbz)LA)$_m$-*b*-(Glu(Bz)LA)$_n$] prior to carrying out deprotection and (b) the deprotected poly[(LysLA)$_m$-*b*-(GluLA)$_n$], revealing the absence of Cbz and Bz ester-protecting groups, which were removed by Pd/C catalytic hydrogenolysis.

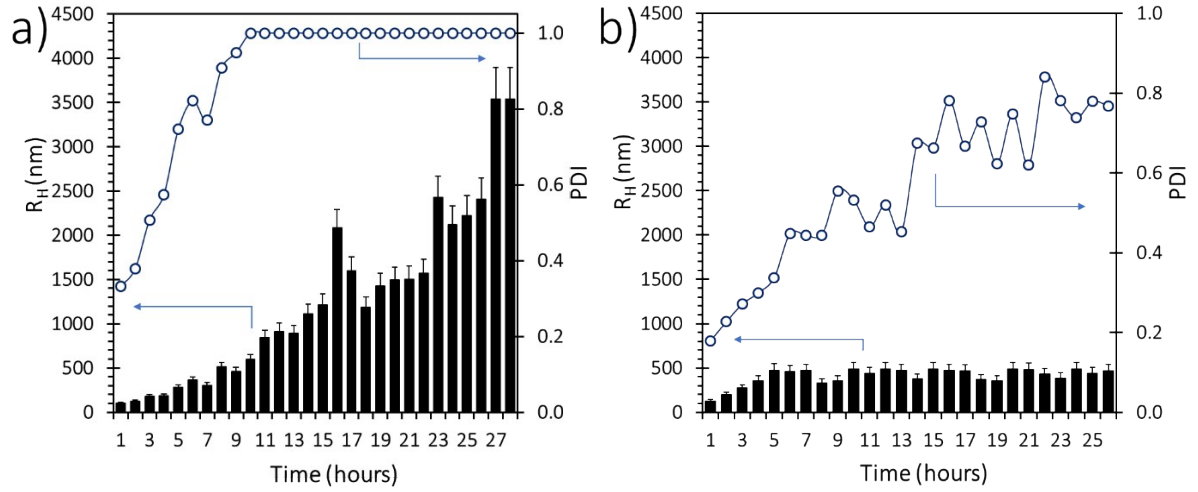


Figure S10. The evolution of the size of aggregates and the PDI values of poly[(LysLA)$_{26.6}$-*b*-(GluLA)$_{28.3}$] NPs that were incubated at (a) pH 4.5, at 37 °C and at (b) pH 11.2, at 37 °C.