Supporting Information for:

Using Host-Guest Chemistry to Tune the Kinetics of Morphological Transitions Undertaken by Block Copolymer Vesicles

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EXPERIMENTAL SECTION

Materials. Glycerol monomethacrylate (GMA; 99.8%) was donated by GEO Specialty Chemicals (Hythe, UK) and used without further purification. 2-Hydroxypropyl methacrylate (HPMA) was purchased from Alfa Aesar (UK) and were used as received. 2,2'-Azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride (VA-044) was purchased from Wako Pure Chemical Industries (Japan) and used as received. Glycidyl methacrylate (GlyMA), 4,4'-Azobis(4-cyanopentanoic acid) (ACVA; V-501; 99%), 2-cyano-2-propyl dithiobenzoate (CPDB), ethanol (99%, anhydrous grade), methanol, dichloromethane, Adamantanamine hydrochloride (Ada-NH_2·HCl) and deuterium oxide (D_2O) were purchased from Sigma-Aldrich (UK) and were used as received. Deuterated methanol (CD_3OD) was purchased from Goss Scientific (Nantwich, UK). All solvents were HPLC-grade and purchased from Fisher Scientific (Loughborough, UK). Azobenzene terminated Methoxypolyethylene glycols (azo-mPEG, molecular weight of mPEG = 1900) and mono-amine modified β-cyclodextrin (NH_2-β-CD) were synthesized according our previous work.[1, 2]

Synthesis of P(GMA$_{53}$-co-GlyMA$_2$) macro-CTA via RAFT solution polymerization. Two steps feeding copolymerization was utilized to produce P(GMA$_{53}$-co-GlyMA$_2$) macro-CTA with GlyMA units locate at the outer region. Step 1): GMA (1.2 g, 7.45 mmol), GlyMA (213 mg, 1.5 mmol), CPDB (0.165 g,

0.745 mmol), and ACVA (41.8 mg, 0.15 mmol) (GMA/GlyMA/CPDB/ACVA molar ratio = 10:2:1:0.2) were weighed into a 100 mL round-bottomed flask. Anhydrous ethanol (1.05 g, previously purged with nitrogen for 30 min) was then added to produce a 60% w/w solution, which was placed in an ice bath and purged under nitrogen for 30 min. The sealed flask was immersed in an oil bath set at 70 °C to initiate the RAFT solution polymerization of GMA and stirred for 2 h at this temperature. Step 2): the second GMA (4.8g, GMA/CPDB molar ratio = 40:1) dissolved in ethanol solution (4.2g, 60% w/w, previously purged with nitrogen for 30 min) was added in the flask with the protective nitrogen atmosphere and reacted for another 2h. The polymerization was then quenched by exposure to air, followed by cooling the reaction mixture to room temperature. Ethanol (25 mL) was added to dilute the reaction solution, followed by precipitation into a ten-fold excess of dichloromethane in order to remove unreacted monomer. The precipitate was isolated via filtration and washed with excess dichloromethane before being dissolved in methanol (50 mL). The crude polymer was precipitated for a second time by addition to excess dichloromethane and isolated by filtration. It was then dissolved in water and freeze-dried for 48 h to afford a pink power. The mean degree of polymerization of this P(GMA_{53}-co-$GlyMA_2$) macro-CTA was calculated based on ^{1}H NMR spectrum (see Figure S1). DMF GPC studies (refractive index detector; calibrated against a series of 10 near-monodisperse poly(methyl methacrylate) standards) indicated an M_n of 13600 g mol^{-1} and an M_w/M_n of 1.14, while an M_n of 6600 g mol^{-1} and an M_w/M_n of 1.13 (see Figure S2).

Synthesis of P(GMA_{53}-co-$GlyMA_2$)-$PHPMA_{250}$ BCPVs via RAFT aqueous dispersion polymerization of HPMA. P(GMA_{53}-co-$GlyMA_2$) macro-CTA (0.18 g, 20 µmol), HPMA monomer (0.72 g, 5 mmol), and VA-044 (1.62 mg, 5.0 µmol; macro-CTA/VA-044 molar ratio = 4.0) were added into a 25 mL round-bottomed flask, prior to addition of water to produce a 20% w/w solution. This reaction solution was purged with nitrogen gas for 30 min at 20 °C prior to immersion into an oil bath set at 50 °C. The reaction mixture was stirred for 3 h to ensure essentially complete conversion of the HPMA monomer (>99% by ^{1}H NMR analysis),[3] then the polymerization was quenched by exposure to air, followed by cooling to ambient temperature.

Post-Polymerization functionalization of a 5% w/w Aqueous Dispersion of P(GMA_{53}-co-$GlyMA_2$)-$PHPMA_{250}$ vesicles Using NH_2-*β*-CD. The as-prepared 20% w/w aqueous dispersions of BCPVs described above were diluted with deionized water to 5% w/w solids to allow efficient stirring when conducting postpolymerization derivatization reactions. A typical protocol was as follows: NH_2-*β*-CD (0.036 g, NH_2-*β*-CD/GlyMA molar ratio = 1.0) was added to 9.0 g of a 5% w/w aqueous dispersion of P(GMA_{53}-co-$GlyMA_2$)−$PHPMA_{250}$ diblock copolymer worms. The reaction was conducted at 20 °C for 48h and then dialysis for 2d. DMF GPC studies indicated an M_n of 67700 g mol^{-1} and an M_w/M_n of 1.30 (see Figure S5).

Preparation of mPEG binding to the *β*-CD modified vesicles by host-guest chemistry. Azo-mPEG (9.37 mg, Azo-mPEG/*β*-CD molar ratio = 1.0) was dissolved into the 4.0 g 5% w/w *β*-CD modified vesicles dispersion. This solution was stirred for 2h prior to use. The resulting mixture was further diluted using water produce a 0.10% w/w aqueous vesicle dispersion.

Characterization

NMR Spectroscopy. ^{1}H NMR spectra were recorded in CD_3OD using a 400 MHz Bruker Avance-500 spectrometer (64 scans averaged per spectrum). 2D NOESY NMR spectra were recorded in D_2O on a 500 MHz Bruker Avance III HD spectrometer (typical number of scans per spectrum = 256).

Gel Permeation Chromatography (GPC). Polymer molecular weights and dispersities were determined using a DMF GPC setup operating at 60 °C and comprising two Polymer Laboratories PL gel 5 μm Mixed-C columns connected in series to a Varian 390-LC multidetector suite (only the refractive index detector was utilized) and a Varian 290-LC pump injection module. The GPC eluent was HPLC-grade DMF containing 10 mM LiBr at a flow rate of 1.0 mL min^{-1}. Calibration was conducted using a series of ten near-monodisperse poly(methyl methacrylate) standards (Mn = 625 − 2 480 000 g mol^{-1}). Copolymer solutions (0.70% w/w) were prepared in DMF containing DMSO (1.0% v/v) as a flow rate marker. Chromatograms were analyzed using Varian Cirrus GPC software (version 3.3).

Dynamic Light Scattering (DLS). DLS studies were conducted on 0.10 % w/w copolymer dispersions at 20 °C using a Malvern Instruments Zetasizer Nano series instrument equipped with a 4 mW He–Ne laser (λ = 633 nm) and an avalanche photodiode detector. Scattered light was detected at 173°. The dispersion pH was adjusted using either 0.02 M or 0.1 M NaOH as required. Intensity-average hydrodynamic diameters were calculated via the Stokes–Einstein equation.

Transmission Electron Microscopy (TEM). Copper TEM grids (Agar Scientific, UK) were surface-coated in-house to yield a thin film of amorphous carbon. The grids were then plasma glow-discharged for 30 s to create a hydrophilic surface. Each dispersion (0.10% w/w, 5 μL) was placed on such a grid for 30 s and then blotted with filter paper to remove excess solution. To stain the aggregates, a 5 μL drop of 0.75% w/w uranyl formate solution was placed on the sample-loaded grid for 60 s and then carefully blotted to remove excess stain. The grids were then dried using a vacuum hose. Imaging was performed at 80 kV using a FEI Tecnai Spirit microscope equipped with a Gatan 1kMS600CW CCD camera.

UV−Visible Absorption Spectroscopy. Absorption spectra were recorded between 200 and 800 nm using a Shimadzu UV-1800 spectrophotometer.

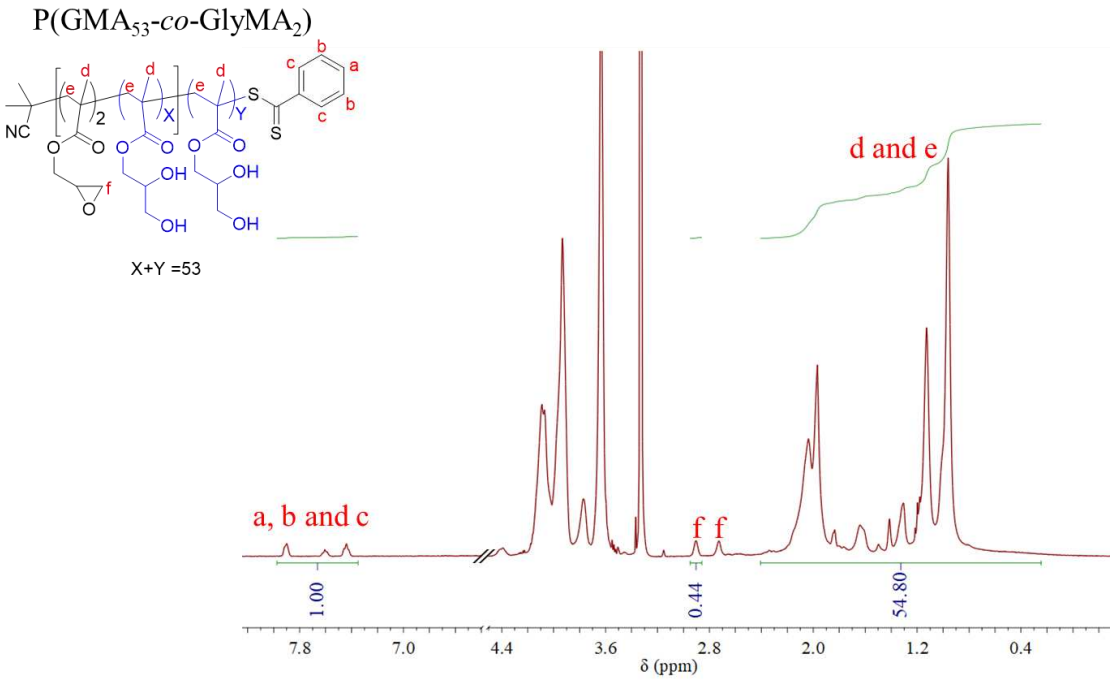

Figure S1. ^{1}H NMR spectrum of the purified P(GMA$_{53}$-co-GlyMA$_2$) macro-CTA recorded in CD_3OD.

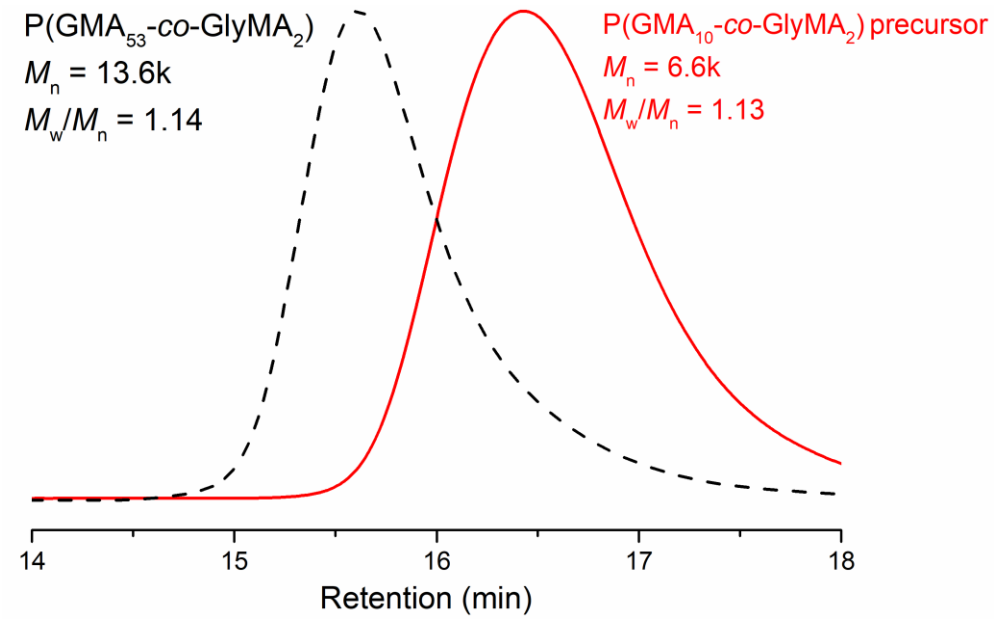

Figure S2. DMF GPC curves obtained for the P(GMA$_{53}$-co-GlyMA$_2$) macro-CTA from its P(GMA$_{10}$-co-GlyMA$_2$) precursor.

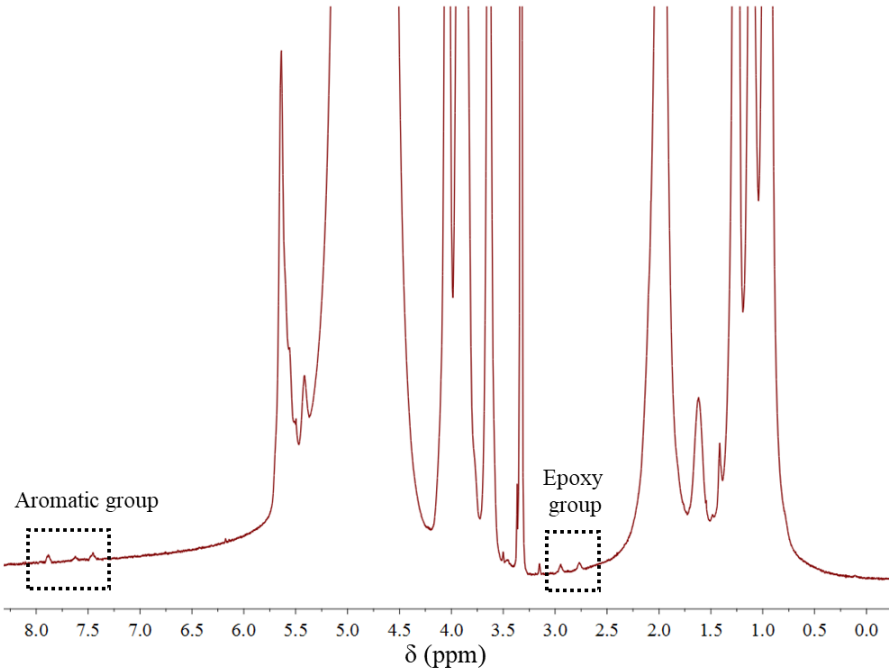

Figure S3. ^{1}H NMR spectrum of the pure P(GMA$_{53}$-co-GlyMA$_{2}$)-PHPMA$_{250}$ diblock copolymer stirred at 20 ℃ for 48h recorded in CD_3OD.

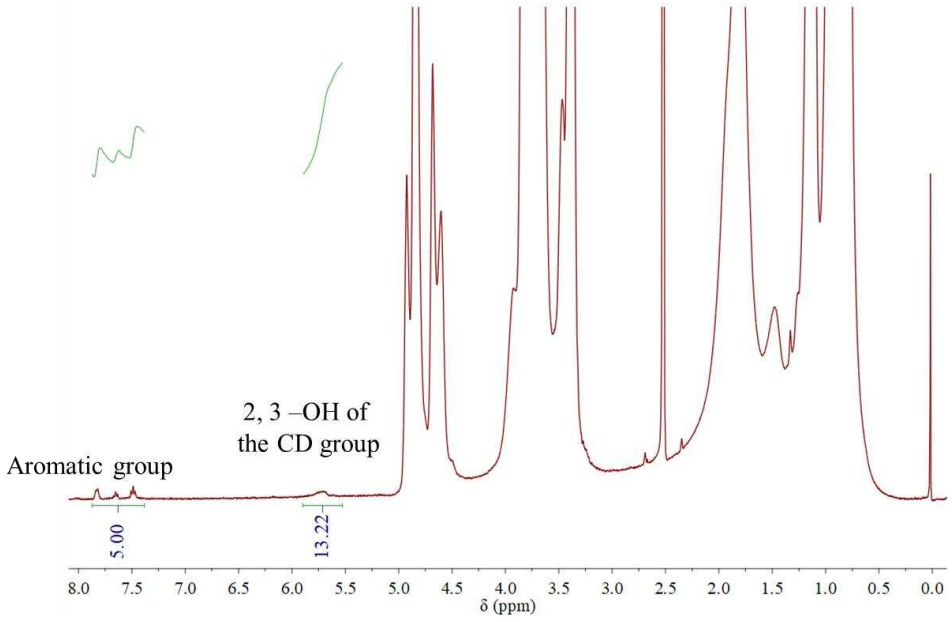

Figure S4. ^{1}H NMR spectrum of the purified P(GMA$_{53}$-co-β-CD)-PHPMA$_{250}$ recorded in d_6-DMSO.

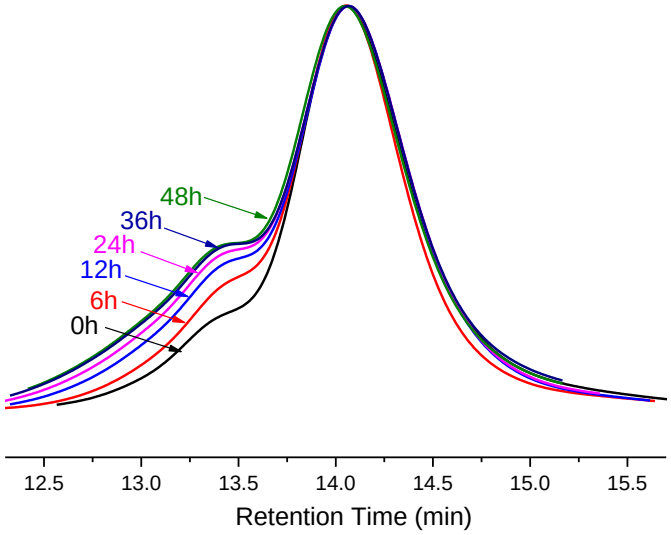

Figure S5. (b) DMF GPC curves obtained at various time points for the reaction between the P(GMA$_{53}$-co-GlyMA$_2$)-PHPMA$_{250}$ diblock copolymer and NH$_2$-*β*-CD.

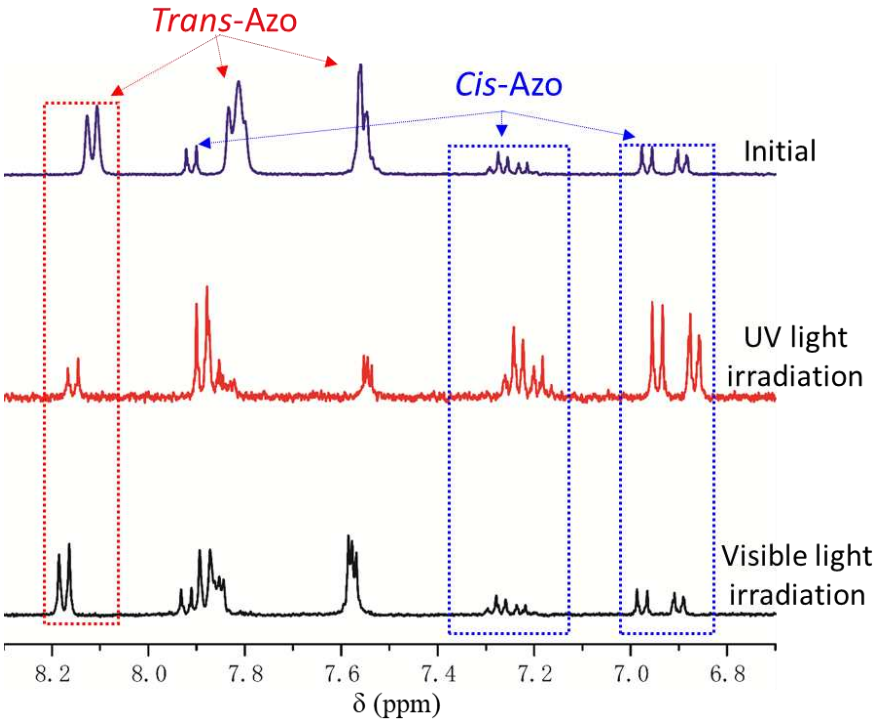

Figure S6. ^{1}H NMR spectra for the reversible photoisomerization of the azo group in D$_2$O at 20 °C after irradiation with UV and visible light, respectively.

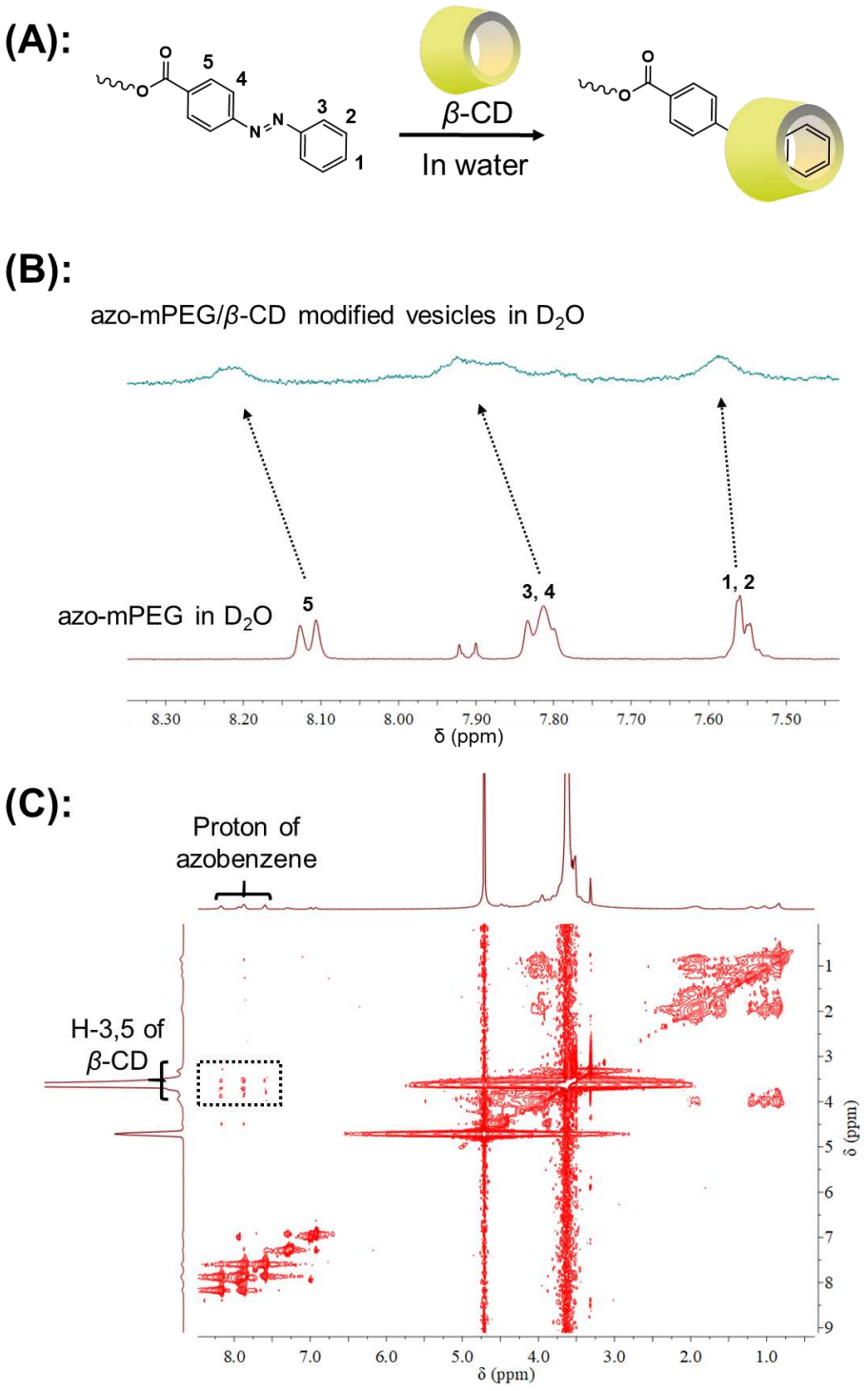

Figure S7. (A) Schematic cartoon depicting the formation of a *β*-CD/azobenzene host-guest complex in aqueous solution. (B) ^{1}H NMR spectra recorded in D_2O for azo-mPEG alone and also for an aqueous dispersion of *β*-CD-functionalized vesicles in the presence of azo-mPEG ([azo-mPEG] = [*β*-CD] = 2.5 mM). (C) Corresponding 2D NOESY spectra recorded for an aqueous dispersion of *β*-CD-functionalized vesicles in the presence of azo-mPEG ([azo-mPEG] = [*β*-CD] = 2.5 mM).

References

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2. Liu, Y. Y.; Fan, X. D.; Gao, L. Synthesis and Characterization of β-Cyclodextrin Based Functional Monomers and its Copolymers with N-isopropylacrylamide. Macromolecular Bioscience **2003,** 3, 715-719.
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