Supporting Information for:

Using Dynamic Covalent Chemistry to Drive Morphological Transitions: Controlled Release of Encapsulated Nanoparticles from Block Copolymer Vesicles

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EXPERIMENTAL SECTION

Materials. Glycerol monomethacrylate (GMA; 99.8%) was donated by GEO Specialty Chemicals (Hythe, UK) and was used without further purification. 2-Hydroxypropyl methacrylate (HPMA) and 3-aminophenylboronic acid monohydrate (APBA; 97%) were purchased from Alfa Aesar (UK) and were used as received. 4,4′-Azobis(4-cyanopentanoic acid) (ACVA; V-501; 99%), 2,2'-azobisisobutyronitrile (AIBN), 2-cyano-2-propyl dithiobenzoate (CPDB), ethanol (99%, anhydrous grade), methanol, dichloromethane, ammonium hydroxide solution (28% NH_3 in H_2O) 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). Carbonate buffer solutions (pH 10.0) were purchased from ThermoFisher Scientific (Chelmsford, USA). All solvents were HPLC-grade and purchased from Fisher Scientific (Loughborough, UK).

Synthesis of PGMA$_{45}$ macro-CTA *via* RAFT solution polymerization. GMA (16.8 g, 105 mmol), CPDB (0.415 g, 1.50 mmol), and AIBN (49.0 mg, 0.30 mmol; CPDB/AIBN molar ratio = 5.0) were weighed into a 100 mL round-bottomed flask. Anhydrous ethanol (21.0 g, previously purged with nitrogen for 1 h) was then added to produce a 45% 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. The GMA 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 GMA 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 PGMA macro-CTA was calculated based on ^{1}H NMR spectrum (see Figure S1).

Synthesis of PGMA$_{45}$-PHPMA$_x$ diblock copolymer vesicles *via* RAFT aqueous dispersion polymerization of HPMA. A typical protocol for the synthesis of PGMA$_{45}$-PHPMA$_{170}$ vesicles *via* RAFT aqueous dispersion polymerization of HPMA using a PGMA$_{45}$ macro-CTA is as follows: PGMA$_{45}$ macro-CTA (0.12 g, 16 μmol), HPMA monomer (0.39 g, 2.72 mmol), and ACVA (1.10 mg, 4.0 μmol; macro-CTA/ACVA molar ratio = 4.0) were added into a 25 mL round-bottomed flask, prior to addition of water to produce a 15% 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 70 °C. The reaction mixture was stirred for 3 h to ensure essentially complete conversion of the HPMA monomer,[1] then the polymerization was quenched by exposure to air, followed by cooling to ambient temperature. For the closely related synthesis of PGMA$_{45}$-PHPMA$_{240}$ and PGMA$_{45}$-PHPMA$_{300}$, the mass of added HPMA monomer was 0.56 g (3.84 mmol) and 0.70 g (4.80 mmol), respectively, and the volume of water was also adjusted accordingly in order to maintain a constant 15% w/w solids.

Morphological transitions for PGMA$_{45}$-PHPMA$_x$ diblock copolymer vesicles induced by dynamic covalent chemistry. The initial 15% w/w aqueous vesicle dispersion was diluted to

1.0% w/w using water and then adjusted to pH 10.5 by addition of 0.02 M NaOH solution. APBA was dissolved in either 1 M or 0.1 M NaOH solution to produce a 0.60% w/w APBA aqueous solution at pH 10.5. This solution was stored in the dark prior to use. 0.40 g of the 1.0% w/w aqueous vesicle dispersion was then mixed with the APBA solution at the desired volumetric ratio in a 10 mL vial, and the resulting mixture was further diluted using NaOH solution or water produce a 0.20% w/w aqueous vesicle dispersion at pH 10.5. The sealed vial was stored at room temperature in the dark and aged for the desired time period for turbidimetry, TEM and DLS studies.

Synthesis of silica nanoparticles loaded PGMA$_{58}$-PHPMA$_{250}$ diblock copolymer vesicles. The details for the preparation of PGMA$_{58}$-PHPMA$_{250}$ diblock copolymer vesicles in the presence or absence of an aqueous dispersion of 20 nm diameter glycerol-functional silica nanoparticles were recently reported by Mable *et al.*[2]

Release of silica nanoparticles from PGMA$_{58}$-PHPMA$_{250}$ diblock copolymer vesicles. The silica nanoparticle release experiments were similar to those described for the PGMA$_{45}$-PHPMA$_x$ (where x = 170 or 240) diblock copolymer vesicles, except that NaOH was replaced by aqueous ammonia in all cases. The loaded silica content was 8% w/w as calculated via thermogravimetric analysis (Figure S8).

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). ^{11}B NMR spectra were recorded in deionized water containing 10% D_2O at the desired pH using quartz NMR tubes on a 500 MHz Bruker Avance III HD spectrometer operating at 160.46 MHz (typical number of scans per spectrum = 256).

Gel Permeation Chromatography (GPC). Polymer molecular weights and dispersities were determined using a DMF GPC set-up 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 (M_n = 625 − 2 480 000 g mol^{-1}). Aqueous copolymer dispersions were freeze-dried overnight to obtain powders. 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 0.20% 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 (0.20% 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.

Turbidimetry Studies. Absorbance spectra were recorded at 20 °C every 10 min over 15 h using a Shimadzu UV-1800 spectrometer operating at a fixed wavelength of 450 nm.

Rheology. Storage (G′) and loss (G″) moduli were determined at 20 °C for a 10% w/w aqueous dispersion of $PGMA_{45}$–$PHPMA_{170}$ vesicles at pH 10.5 using a TA Instruments AR-G2 rheometer. A cone-and-plate geometry (40 mm 2° aluminum cone) was used for these measurements, which were conducted at a fixed strain of 1.0% and an angular frequency of 1.0 rad s^{-1}.

Small-angle X-ray scattering (SAXS)

SAXS patterns were recorded at a synchrotron facility (ESRF, station ID02, Grenoble, France) using monochromatic X-ray radiation (wavelength λ = 0.0995 nm; sample-to-detector distance = 5.0042 m; q ranged from 0.015 to 1.5 nm^{-1}, where $q = 4\pi \sin \theta/\lambda$ is the length of the scattering vector and θ is one-half of the scattering angle) and a Ravonix MX-170HS CCD detector. A glass capillary of 2.0 mm diameter was used as a sample holder. Scattering data were reduced using standard routines provided by beamline personnel and were further analyzed using Irena SAS macros for Igor Pro.[3] Water was used for the absolute intensity calibration. Time-resolved measurements were conducted on a 10% w/w aqueous dispersion of $PGMA_{45}$-$PHPMA_{170}$ vesicles at pH 10.5 immediately after adding ABPA.

Thermogravimetric analysis (TGA). Analyses were conducted on freeze-dried samples that were heated in air to 800 °C at a heating rate of 15 °C min^{-1} using a TA Instruments Q500 instrument.

Supporting Figures

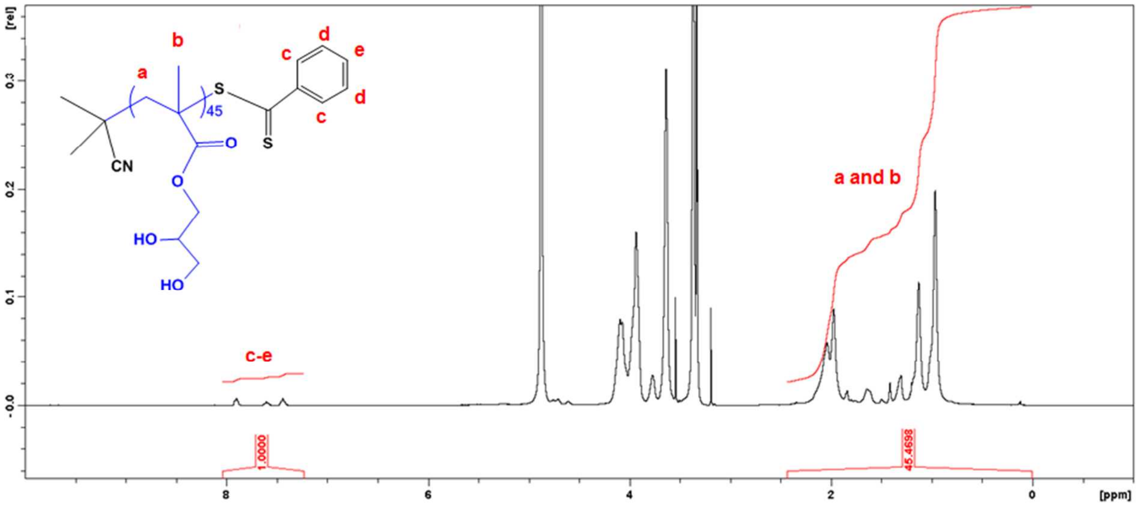

Figure S1. ^{1}H NMR spectrum of the purified PGMA$_{45}$ macro-CTA recorded in CD$_3$OD.

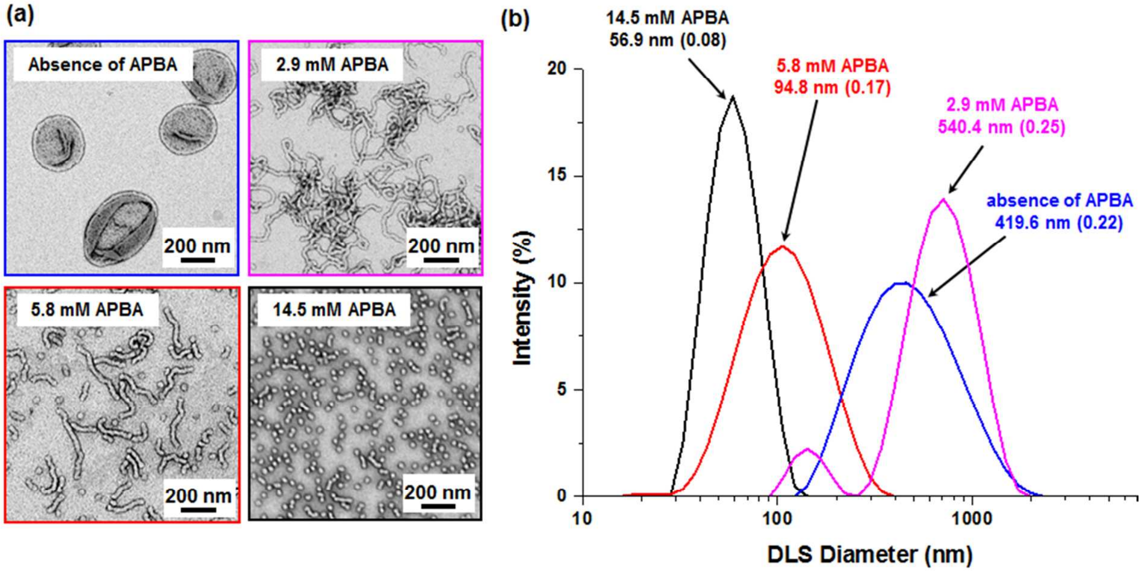

Figure S2. (a) TEM images and (b) DLS data obtained for 0.20% w/w PGMA$_{45}$-PHPMA$_{170}$ vesicles aged for 15 h at pH 10.5 and 20 °C in the absence or presence of various concentrations of APBA.

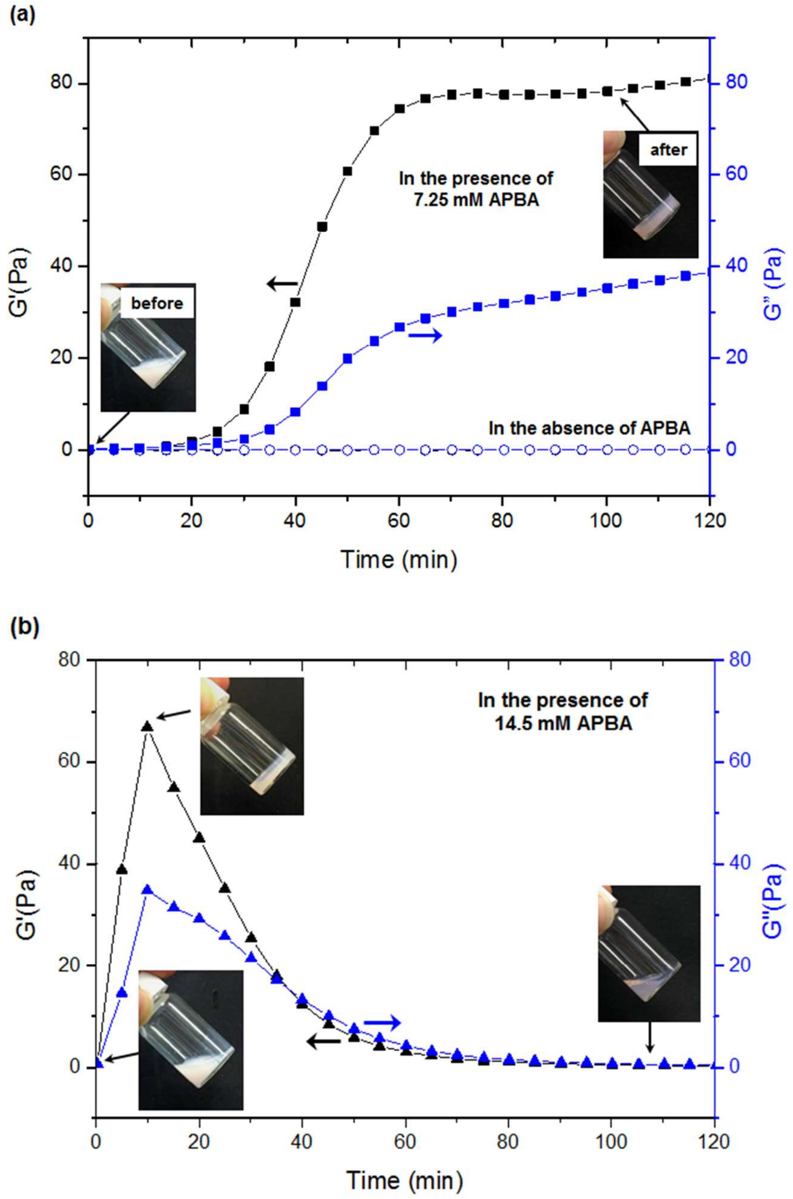

Figure S3. Time-dependent rheology studies (G' = black curve and G'' = blue curve) recorded at 20 °C for 10% w/w $PGMA_{45}$-$PHPMA_{170}$ diblock copolymer nano-objects at pH 10.5, (a) in the absence (open circles) or presence of 7.25 mM APBA (filled squares), and (b) in the presence of 14.5 mM APBA.

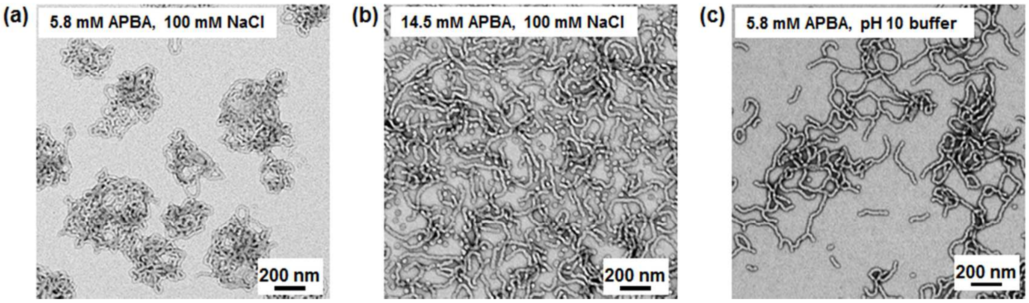

Figure S4. Representative TEM images obtained for 0.20% w/w PGMA$_{45}$-PHPMA$_{170}$ diblock copolymer nano-objects in the presence of: (a) 5.8 mM APBA and 100 mM NaCl at pH 10.5, (b) 14.5 mM APBA and 100 mM NaCl at pH 10.5, and (c) 5.8 mM APBA in pH 10.0 buffer solution, which were recorded at 20 °C after ageing for 24 h.

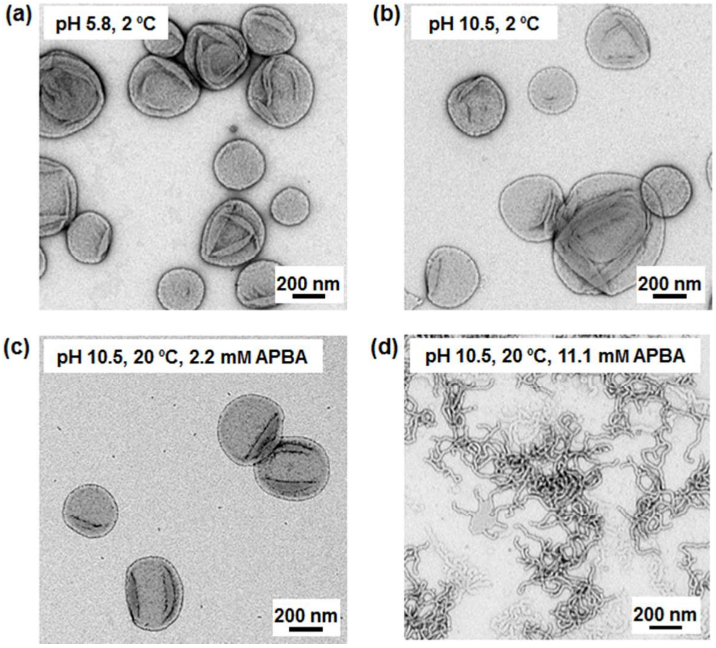

Figure S5. Representative TEM images obtained for 0.20% w/w PGMA$_{45}$-PHPMA$_{240}$ diblock copolymer vesicles after 20 h ageing under various conditions: (a) at 2 °C and pH 5.8 in the absence of any APBA, (b) at 2 °C and pH 10.5 in the absence of any APBA; (c) at 20 °C and pH 10.5 in the presence of 2.2 mM APBA; (d) at 20 °C and pH 10.5 in the presence of 11.1 mM APBA.

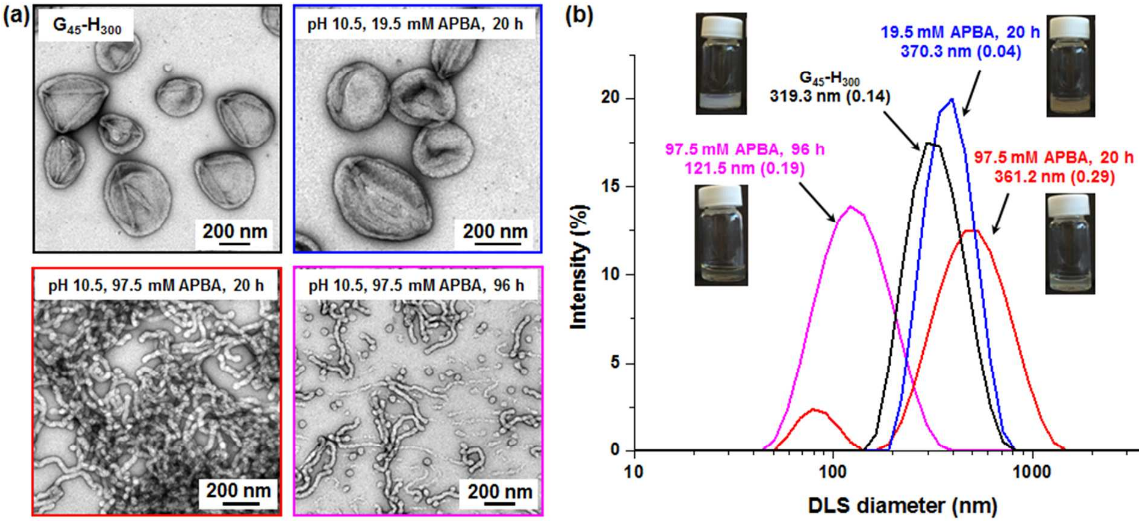

Figure S6. (a) Representative TEM images and (b) corresponding DLS data recorded for 0.20% w/w aqueous dispersions of PGMA$_{45}$-PHPMA$_{300}$ vesicles in presence of varied APBA and aged for different time at pH 10.5.

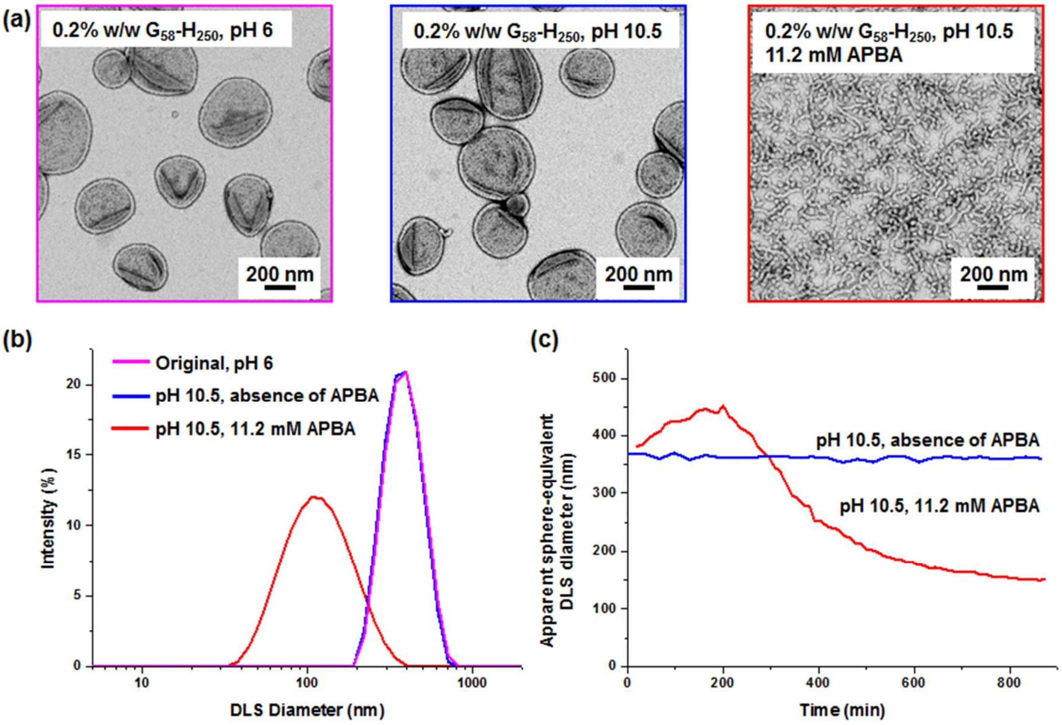

Figure S7. (a) Representative TEM images and (b) corresponding DLS data recorded for a 0.20% w/w aqueous dispersion of $PGMA_{58}$-$PHPMA_{250}$ vesicles before and 24 h after inducing a vesicle-to-worm transition *via* addition of 11.2 mM APBA at pH 10.5. (c) Evolution of the apparent sphere-equivalent DLS average diameter recorded for $PGMA_{58}$-$PHPMA_{250}$ diblock copolymer nano-objects with ageing time in the absence and presence of 11.2 mM APBA at pH 10.5.

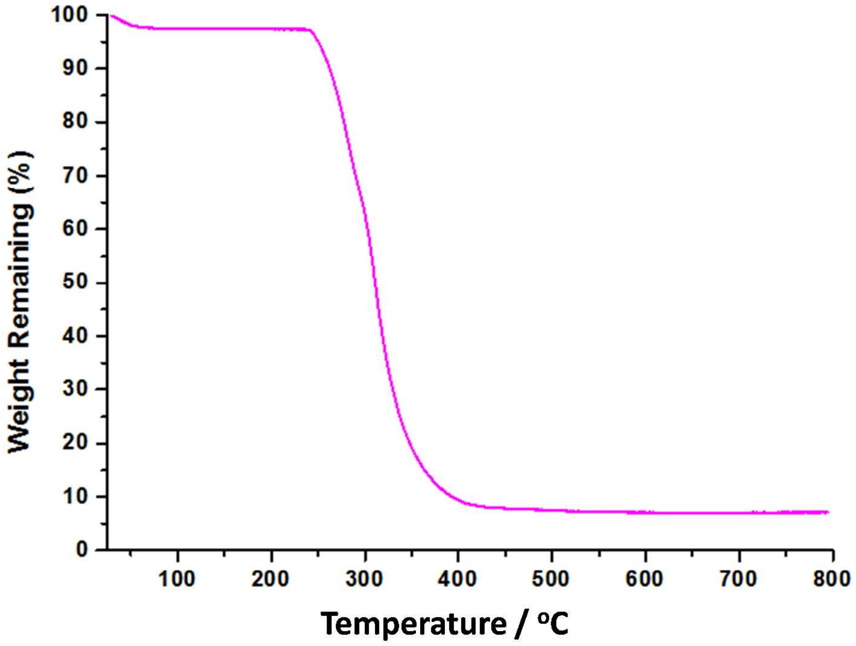

Figure S8. Thermogravimetric analysis curve recorded for silica-loaded $PGMA_{58}$-$PHPMA_{250}$ diblock copolymer vesicles after centrifugation to remove the excess silica nanoparticles. The residual mass observed at 600 °C is attributed to the incombustible encapsulated silica nanoparticles within the vesicles.

References

(1) Warren, N. J.; Mykhaylyk, O. O.; Ryan, A. J.; Williams, M.; Doussineau, T.; Dugourd, P.; Antoine, R.; Portale, G.; Armes, S. P. *J. Am. Chem. Soc.* **2015**, *137*, 1929.

(2) Mable, C. J.; Gibson, R. R.; Prevost, S.; McKenzie, B. E.; Mykhaylyk, O. O.; Armes, S. P. *J. Am. Chem. Soc.* **2015**, *137*, 16098.

(3) Ilavsky, J.; Jemian, P. R. *J. Appl. Crystallogr.* **2009**, *42*, 347.