Supporting Information for JACS manuscript:

Testing the Vesicular Morphology to Destruction: Birth and Death of Diblock Copolymer Vesicles Prepared via Polymerization-Induced Self-Assembly

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Experimental

Materials

Glycerol monomethacrylate (GMA; 99.8%) was kindly donated by GEO Specialty Chemicals (Hythe, UK) and used without further purification. 2-Hydroxypropyl methacrylate (HPMA, 97%) was purchased from Alfa Aesar (Heysham, UK) and used without further purification. 2-Cyano-2-propyl dithiobenzoate (CPDB), 4,4′-azobis(4-cyanopentanoic acid) (ACVA; V-501; 99%) D_2O and anhydrous ethanol (99%), were purchased from Sigma-Aldrich UK and used as received. In the case of the CPDB, the manufacturer's stated purity was 97%, but ^{1}H NMR analysis indicated a somewhat lower (and variable) purity of 75−90%. The actual purity of each CPDB batch was taken in account when calculating the target degree of polymerisation for the PGMA block. Deuterated methanol (CD_3OD) was purchased from Goss Scientific (Nantwich, UK). All solvents were of HPLC quality and were purchased from Fisher Scientific (Loughborough, UK).

$PGMA_{55}$ macro-CTA synthesis

CPDB RAFT agent (3.90 mmol, 0.864 g, purchased from Sigma-Aldrich with 80% purity as judged by ^{1}H NMR spectroscopy) and GMA monomer (156.1 mmol, 25.0 g) were weighed into a 100 mL round-bottomed flask and purged under N_2 for 30 min. ACVA initiator (0.78 mmol, 218.6 mg, CTA/ACVA molar ratio = 5.0) and anhydrous ethanol (49.6 mL), which had been purged with N_2 for 30 min, were then added, and the resulting red solution was purged for a further 10 min. The flask was subsequently sealed and immersed into an oil bath set at 70 °C. After 100 min, the polymerization was quenched by immersion in liquid nitrogen and dilution with methanol (100 mL). A final GMA conversion of 82 % was determined by ^{1}H NMR. The methanolic solution was precipitated into a ten-fold excess of

dichloromethane. After filtering and washing with dichloromethane, the crude homopolymer was dissolved in water and the residual dichloromethane was evaporated under vacuum. The resulting aqueous solution was freeze-dried overnight to yield a pink powder. ^{1}H NMR analysis of the pure solid indicated a mean degree of polymerization of 55 for this PGMA macro-CTA. Its M_n and M_w/M_n were 15 600 g mol^{-1} and 1.10, respectively, as judged by DMF GPC (using a refractive index detector and a series of near-monodisperse poly(methyl methacrylate) calibration standards).

RAFT Aqueous Dispersion Polymerization of HPMA using the $PGMA_{55}$ macro-CTA

A typical protocol for the synthesis of $PGMA_{55}$−$PHPMA_{400}$ is as follows: $PGMA_{55}$ macro-CTA (0.076 g, 0.0087 mmol) and HPMA monomer (0.500 g, 3.47 mmol; target DP = 400) were weighed into a 25 mL round-bottomed flask and purged with N_2 for 20 min. ACVA was added (0.8 mg, 0.0029 mmol, CTA/ACVA molar ratio = 3.0) and purged with N_2 for a further 5 min. Deionized water (5.20 mL, producing a 10.0% w/w aqueous solution), which had been previously purged with N_2 for 30 min, was then added and the solution was deoxygenated for a further 5 min prior to immersion in an oil bath set at 70 °C. The reaction solution was stirred for 3 h before the RAFT polymerization was quenched by exposure to air. For the sake of brevity, we denote the PGMA and PHPMA blocks as simply 'G' and 'H' in this article. For example, the above $PGMA_{55}$−$PHPMA_{400}$ diblock is referred to as G_{55}−H_{400}.

Polymer Characterization

^{1}H NMR Spectroscopy. All NMR spectra were recorded using a 400 MHz Bruker Avance-400 spectrometer (64 scans averaged per spectrum).

Gel Permeation Chromatography (GPC). Copolymer molecular weights and polydispersities 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 (refractive index detector only) 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}. DMSO was used as a flow-rate marker. Calibration was conducted using a series of ten near-monodisperse poly(methyl methacrylate) standards (M_n = 625− 618,000 g mol^{-1}). Chromatograms were analysed using Varian Cirrus GPC software (version 3.3).

Dynamic Light Scattering (DLS). Intensity-average hydrodynamic diameters of the dispersions were obtained by DLS using a Malvern Zetasizer NanoZS instrument, which

detects backscattered light at 173°. Aqueous copolymer dispersions of 0.1 - 0.20 % w/v were analyzed using plastic disposable cuvettes, and all data were averaged over three consecutive runs.

Transmission Electron Microscopy (TEM). Aggregate solutions were diluted fifty-fold at 20 °C to generate 0.20% w/w dispersions. Copper/palladium TEM grids (Agar Scientific, UK) were surface-coated in-house to yield a thin film of amorphous carbon. The grids were then treated with a plasma glow discharge for 30 s to create a hydrophilic surface. Each aqueous diblock copolymer dispersion (0.20% w/w, 12 μL) was placed onto a freshly treated grid for 1 min and then blotted with filter paper to remove excess solution. To stain the deposited nanoparticles, a 0.75% w/w aqueous solution of uranyl formate (9 μL) was placed via micropipet on the sample-loaded grid for 20 s and then carefully blotted to remove excess stain. Each grid was then carefully dried using a vacuum hose. Imaging was performed using a FEI Tecnai Spirit TEM instrument equipped with a Gatan 1kMS600CW CCD camera operating at 120 kV.

Small Angle X-Ray Scattering SAXS patterns were recorded at two synchrotron sources (Diamond Light Source, station I22, Didcot, UK and ESRF, station BM26, Grenoble, France) using monochromatic X-ray radiation (wavelength, λ, 0.1001 nm and 0.1033,respectively) and a 2D Pilatus CCD detector (2M and 1M, respectively). The camera length setup in both cases was covering q range from 0.02 nm^{-1} to 1.9 nm^{-1}, where $q = \frac{4\pi \sin\theta}{\lambda}$ is the length of the scattering vector and θ is a half of the scattering angle. A liquid cell composed of two mica windows (each of 25 μm thickness) separated by a polytetrafluoroethylene spacer of 1 mm thickness was used as a sample holder. 2D scattering data were integrated using Nika SAS macros for Igor Pro (integration) to obtain 1D SAXS profiles. These profiles were further reduced (normalization and background subtraction) and fitted to appropriate models using Irena SAS macros for Igor Pro[1]. The SAXS measurements were conducted on an aqueous dispersion of $PGMA_{55}$-$PHPMA_x$ (x = 200 – 2000, see Table 1 in the main text) copolymer diluted from as-synthesized 20 % w/w to 1 % w/w prior to data collection.

Charge detection mass spectrometry.

Experiments were performed on a custom-built charge detection-mass spectrometer with an electrospray source. This instrument has been previously described in detail.[2] Briefly, aqueous solutions of vesicles at 10% w/w were diluted 100-fold in deionized water and

gently vortexed (1 min, 10 Hz) before injection to the electrospray source. Solutions were typically injected at flow rates of 200 μL/h, and entered the electrospray chamber through a 0.1 mm internal diameter stainless steel capillary tube located inside the needle tip. Nitrogen drying gas was injected between the end cap and the transfer glass capillary and flew through a heater typically set to 200 °C. The vacuum interface was composed of a glass transfer capillary that passes the ions into the first stage of the vacuum system, an end cap, a skimmer between the first and second vacuum stages, a hexapole ion guide and an exit lens. The charge detection device was used in a single pass mode, and was built according to the specifications reported by Keaton and Stradling.[3] The signal induced on the tube was picked up by a JFET transistor and was amplified by a low-noise, charge-sensitive preamplifier and then shaped and differentiated by a home-built amplifier. The signal was recorded with a waveform digitizer card that recorded the entire waveform for each ion passing through the detector tube at a sampling rate of 10 MHz. The data were transferred to a desk-top computer where they were analyzed to compute the charge and mass of each ion. A user program calculated the time between the maxima of positive and negative pulses, the amplitudes of the two pulses and the ratio between their absolute values. Events, for which the absolute value of the amplitude ratio between the first and the second pulses was superior to 1.5 or inferior to 0.75, were automatically excluded. These events may result from either ion that entered but did not exit the detector (due to fragmentation or disappearance in the charge detector) or from two or more ions entering the charge detector during a time-of-flight measurement.

Calibration in charge was performed using a test capacitor that allowed a known amount of charge to be pulsed onto the pick-up tube. The test pulses were generated with a shaping-pulse generator so that the time-dependent signal response could be determined as well. The charge on a particle was then directly deduced from this calibration and from the average value of the voltage intensity of the two pulses generated by the particle on the detector. The mass-to-charge m/z ratio of an ion is determined from the time-of-flight Δt (i.e. the time delay between the positive and negative pulses that correspond to the entrance and the exit from the detector tube). The ion velocity v_m is given by:

$$v_m = \frac{L}{\Delta t}$$

and

$$\frac{m}{z} = \frac{2eV}{v_m^2 - v_g^2}$$

where L is the length of the detector tube (i.e. 3.75 cm), m is the mass, z is the number of charges and V is the electrostatic acceleration voltage. A correction is required to account for the initial kinetic energy imparted to the ion by the free jet expansion of the gas prior to acceleration by the electric field: v_g is the ion velocity due to the free gas expansion. It is determined by grounding all electrostatic lenses and timing the passage of the ion through the detector. These procedures allow internal calibration of the charge detection mass spectrometer. A final external calibration was performed using NIST traceable size standards (70, 100, 150, 200, and 300 nm polystyrene nanospheres supplied by Polysciences Europe GmbH).

In charge detection mass spectrometry, the mass m of nanoobjects is calculated using:

$$m = \frac{2zeV}{v_m^2 - v_g^2}$$

Mass histograms are constructed from a statistically relevant number N of single mass measurements for each sample (typically N > 5000). Such histograms are fitted with a log normal function, from which the mean mass is extracted.

References

[1] Ilavsky J.; and Jemian P. R. *Journal of Applied Crystallography*, 2009, **42**, 347-353

[2] Doussineau, T.; Bao, C. Y.; Clavier, C.; Dagany, X.; Kerleroux, M.; Antoine, R.; Dugourd, P. *Rev Sci Instrum* **2011**, *82*, 084104 ; T. Doussineau et al., *ACS MacroLetters* **2012**, *1*, 414-17

[3] Keaton, P. W.; Idzorek, G. C.; Rowton Sr, L. J.; Seagrave, J. D.; Stradling, G. L. ;Bergeson, S. D. ; Collopy, M. T.; Curling Jr, H. L.; McColl, D. B.; Smith, J. D.; *Int J Impact Eng* **1990**, *10*, 295-308

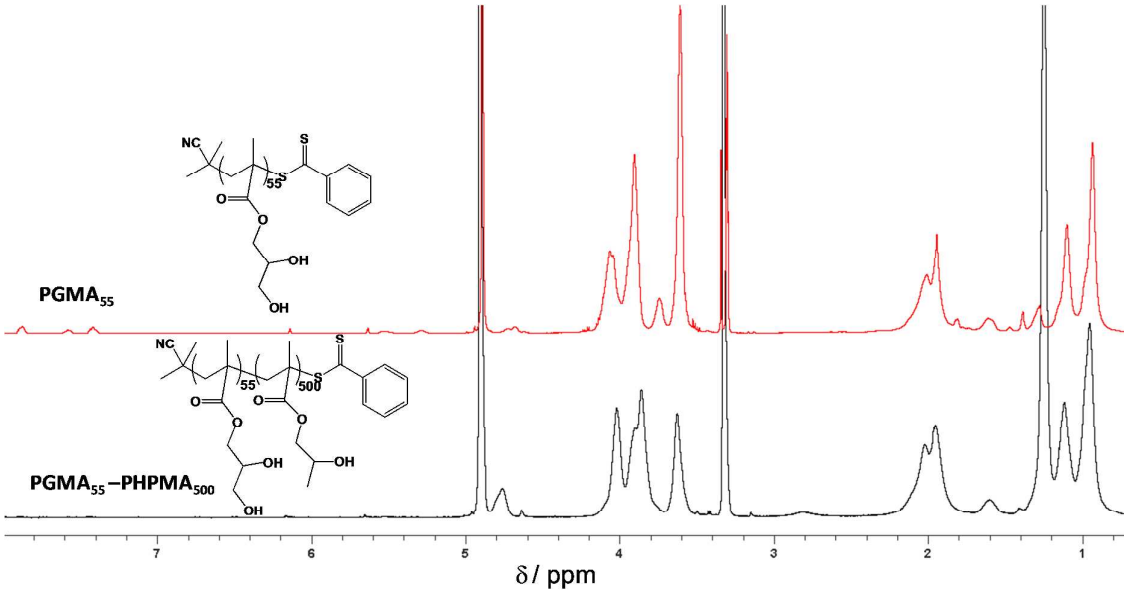

Figure S1. ^{1}H NMR spectra recorded in CD_3OD for the $PGMA_{55}$ macro-CTA and the $PGMA_{55}$-$PHPMA_{500}$ diblock copolymer prepared by RAFT-mediated PISA using an aqueous dispersion polymerization formulation.

Target PHPMA DP (x)	Conversion[a] (%)	M_n [b] x 10^3 g mol^{-1}	M_w /M_n [b]
0	82	15.6	1.10
200	100	58.6	1.20
300	100	66.00	1.09
400	100	73.0	1.14
500	100	81.6	1.18
600	100	116.7	1.31
700	100	128.6	1.29
800	98	158.8	1.39
1000	99	204.0	1.43
1500	88	282.5	1.58
2000	90	565.0	2.96

a Determined by ^{1}H NMR spectroscopy.

b Determined by DMF GPC vs. a series of near-monodisperse PMMA calibration standards.

Table S1. Monomer conversions and DMF GPC data obtained for (i) the G_{55} macro-CTA prepared by RAFT solution polymerization of GMA in ethanol (see first entry) and (ii) a series of G_{55}-H_x block copolymers prepared by RAFT aqueous dispersion polymerization.

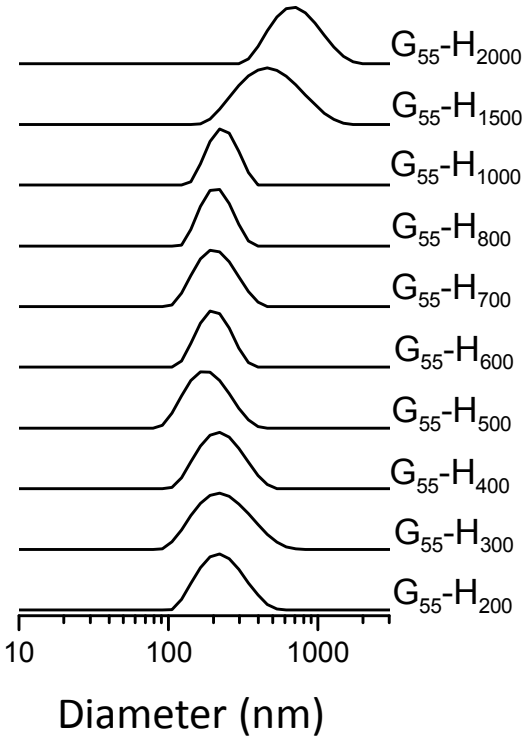

Figure S2. DLS intensity-average particle size distributions obtained for 0.10 % w/w dispersions of G_{55}-H_x vesicles in water.

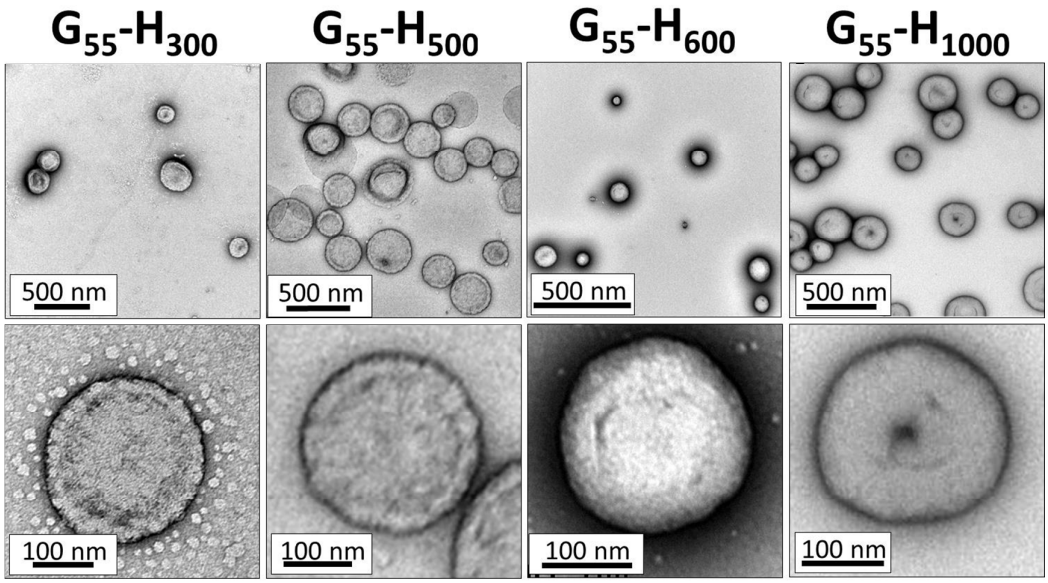

Figure S3. TEM images obtained for G_{55}-H_{300}, G_{55}-H_{500}, G_{55}-H_{600} and G_{55}-H_{1000} showing the increasing membrane wall thickness.

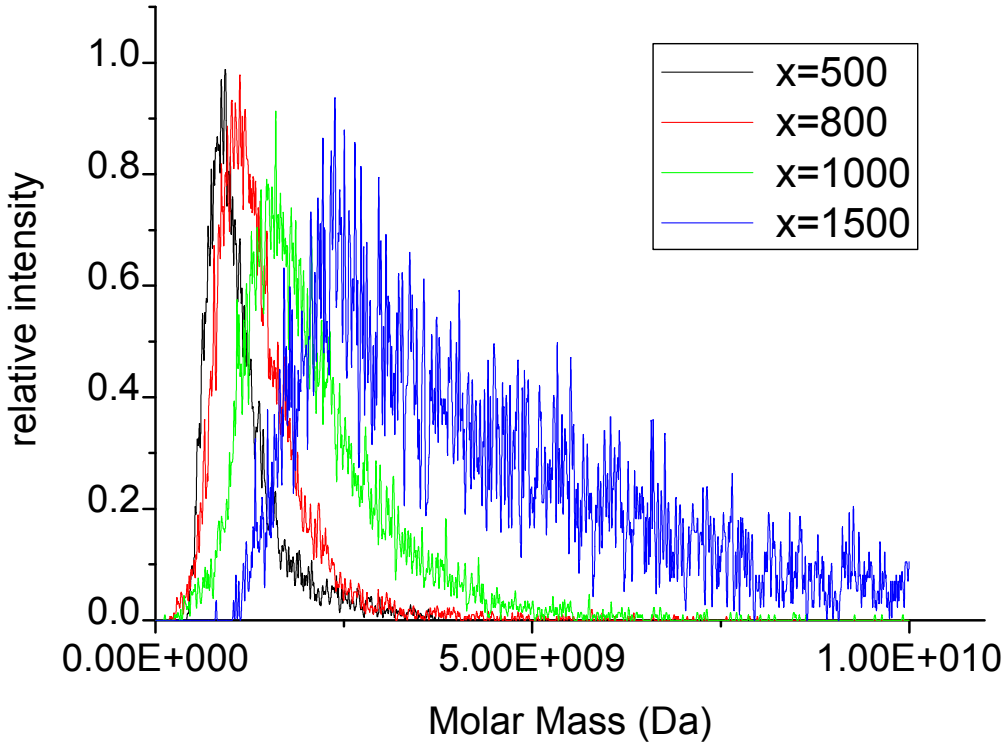

Figure S4. Number-average mass distributions obtained for a series of G_{55}-H_x vesicle dispersions using CD-MS.

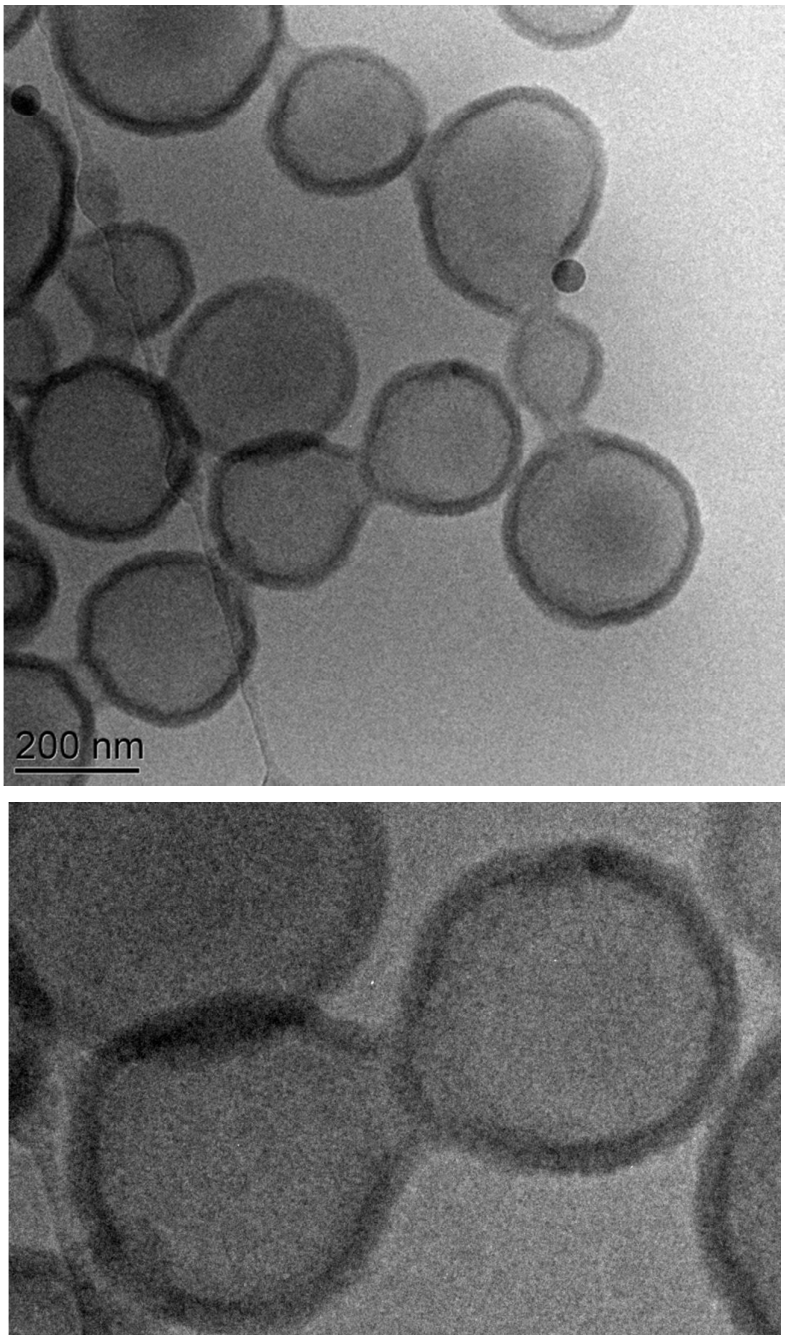

Figure S5. Cryo-TEM images showing a pair of partially fused G_{55}-H_{800} vesicles.

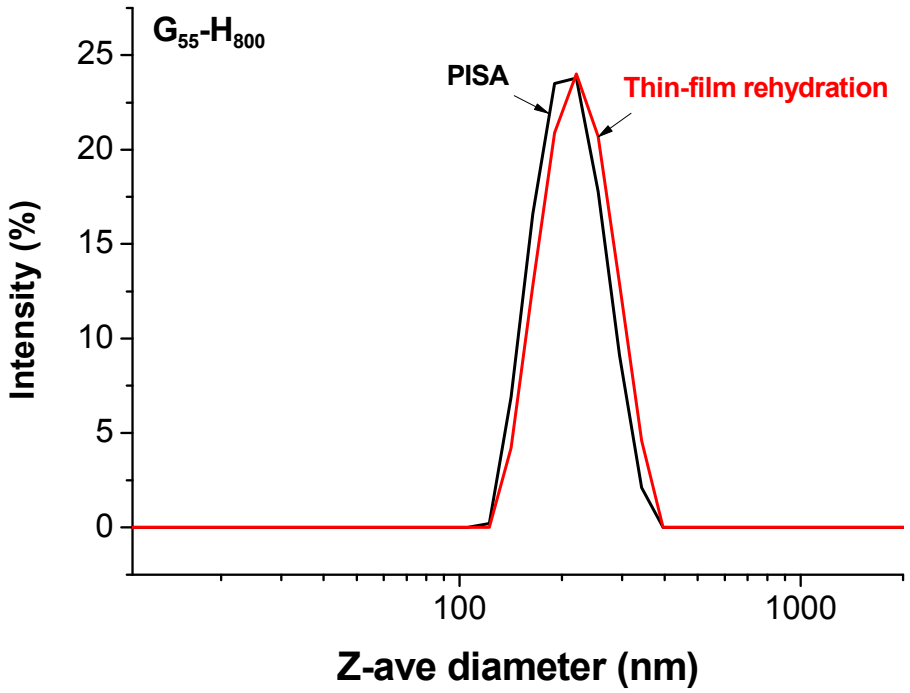

Figure S6. Representative DLS traces obtained for a 10 % w/w dispersion of G_{55}-H_{800} (i) after PISA and (ii) after thin film rehydration in water. In the latter case, the film was cast from a methanolic copolymer solution and the dispersion was stirred for 2 weeks.