

SUPPORTING INFORMATION:

Morphological transitions in polymer vesicles upon bilayer swelling with small hydrophobic molecules in water

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PEG Macroinitiator (ATRP) synthesis

Materials

α -Hydroxy-Poly(ethylene glycol) (PEG)(ave mol. weight 2×10^3 g mol⁻¹, Aldrich), triethylamine (TEA)(BDH, 98%), Ethyl α -bromoisobutyrate (98%, Aldrich), 2-(dimethylamino) ethyl methacrylate (DMAEMA, 98%, Aldrich), *n*-butyl methacrylate (BMA, 99%, Sigma-Aldrich), tetrahydrofuran (THF) (Romil, "Hidry", 15 ppm water), 2-bromoisobutyryl bromide (Aldrich, 98%), dichloromethane (DCM)(BDH, 99%), CuBr was obtained from Sigma-Aldrich and purified before use, *N*-(*n*-propyl)-2-pyridylmethanimine (PPMI, 95%) was prepared using the procedure reported by Haddleton and co-workers (Haddleton, D. M.; Crossman, M. C.; Dana, B. H.; Duncalf, D. J.; Heming, A. M.; Kukulj, D.; Shooter, A. J. *Macromolecules* **1999**, 32, 2110.), sodium hydrogen carbonate (BDH, 99%), and magnesium carbonate (BDH, 99%) were used as delivered.

Method

The poly(ethyleneglycol) macroinitiator was synthesised by reaction of the hydroxyl group of the PEG with an acid bromide. Dried (dessicator, overnight) PEG (0.01 mol) was dissolved in anhydrous THF with triethylamine (dried over molecular sieves) (0.149 mol) also bromoisobutyryl bromide (0.01mol) added drop-wise from a syringe under nitrogen and the reaction was left to stir for 48 hrs at 25 °C. Next, before being taken up into dichloromethane, (100 ml per 100 ml THF) the solution was washed (x3) with saturated sodium hydrogen carbonate (100 ml per 100 ml organic phase). The organic phase was dried over anhydrous magnesium sulphate and filtered. The solvent was removed under reduced pressure and the resulting liquid precipitated in cold diethyl ether. The crude product was filtered and dried at reduced pressure to give a waxy solid.

NMR spectra were recorded on a Bruker DPX 300 MHz spectrometer at 298K.

¹H NMR (DMSO, δ , ppm): 4.23 (2H, t, -CH₂OCO), 3.64 (2H, t, -CH₂CH₂OCO), 3.51 (176H, m, (CH₂CH₂O)₄₅), 3.36 (3H, s, -OCH₃), 1.89 (6H, s, -CH₃)

¹³C NMR (DMSO, δ , ppm): 170.13, 77.09, 67.37, 64.00, 54.78, 29.68

IR spectra were recorded on a Bruker IR machine at 298K

IR absorption ν (cm^{-1}) 2881, 1731 (C=O), 1466, 1454, 1359, 1340, 1279, 1239, 1146, 1099, 1059, 946, 841, 612 (C-Br), 592, 581, 573, 556, 528, 508

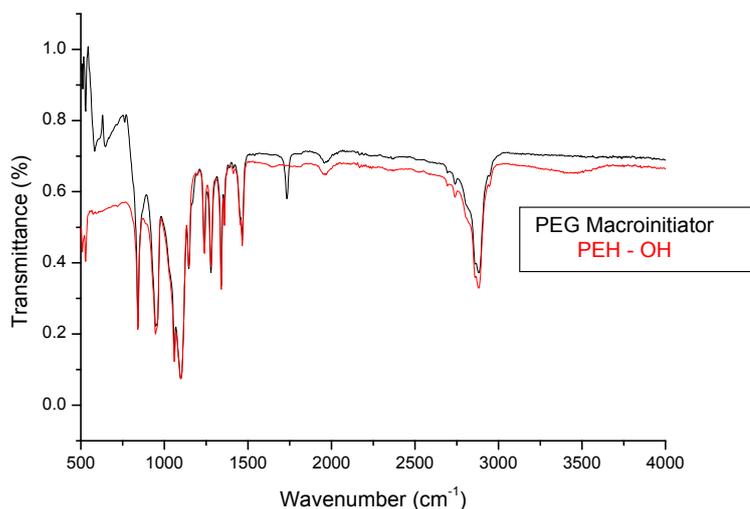
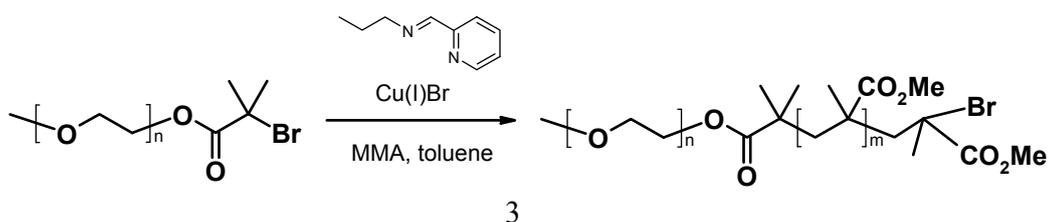


FIGURE S1. Infrared spectrum of PEG Macroinitiator and PEG-OH starting material.

The above figure, S1, shows the before and after traces of PEG-OH and PEG-macroinitiator. Note the appearance of the peak at 1733 cm^{-1} due to (C=O) and the disappearance of the broad OH peak 3100 to 3600 cm^{-1} as the acid bromide replaces the terminal hydroxyl group. The PEG C-Br peak appears at 612 cm^{-1} . This confirms the existence of the product as distinct from the starting material.

PEG-PMMA Synthesis

The product was used as an initiator in ATRP using conditions reported by Perrier and Haddleton (Perrier, S., Haddleton, D.M. *European Polymer Journal* **2004**, 40, 2277). Experiments were performed at $80 \text{ }^\circ\text{C}$ with *N*(*n*-propyl)-2-pyridylmethanamine as ligand.



3.4.3.1 Materials

Methyl methacrylate (Aldrich, 99%), toluene (Fisher, 99%) and diethylether (Fisher, 98%.) were used as received. Copper bromide (Aldrich, 98%) was purified according to the method of Keller and Wycoff (Veatch, S. L., Keller, S.L. Biophysical Journal 2003, 85, 3074) . N-(n-propyl)-2-pyridyl methanimine (Propyl ligand) and PEG-macroinitiator were used as prepared.

Method

The PEG macroinitiator (3 g) and copper bromide (0.201 g) were added to a 200 ml schlenk with a magnetic stirrer bar. The flask was sealed with a rubber septum, placed under vacuum, and then placed under nitrogen. Monomer and toluene (50% v/v) were degassed prior to use (by bubbling nitrogen through them) and then added via syringe. The solution was freeze-pump-thawed (x3) to further remove oxygen and the schlenk was placed into an oil bath at 80 °C, whereupon propyl ligand (0.44 ml) was added to initiate reaction. The reaction was monitored by samples taken at regular intervals which were analysed by NMR and also GPC. Once the reaction had reached the desired conversion (50 – 75%), the schlenk was removed from the bath, 50 ml and toluene was added to the polymer solution and filtered over a basic alumina column to remove the copper and ligand. The filtered solution was collected and excess solvent removed before precipitation into cold diethyl ether. Polymer was filtered and dried at reduced pressure.

PBMA-PDMAEMA Synthesis by ATRP

In a typical ATRP procedure, a Schlenk tube was charged with solvent, purified monomer, the initiator, and the Cu (I) catalyst such as CuBr. The mixture was de-aerated by three freeze- pump-thaw cycles, placed under a nitrogen gas atmosphere, and subsequently immersed into a preheated oil bath of 90°C. Next the ligand, PPMI was injected into the system by syringe to start the reaction. After the polymerization, the tube was rapidly cooled and exposed to air. The polymer was purified by precipitation in methanol at -30°C and further dried under vacuum. To synthesize pBMA, 10g of toluene,

BMA (10g, 70mmol), Ethyl α -bromoisobutyrate (0.185g, 0.95mmol), CuBr (0.138g, 0.96mmol) were charged in the Schlenk tube. After the system reached 90°C, PPMI (0.3ml, 1.94mmol) was injected and the solution turned to dark brown. The reaction was performed for 4 hours before quenching with cold water. M_n calc = 10000 Da. M_n (GPC) = 11605. PDI (GPC) = 1.14. To synthesize the block copolymer, the same protocol was applied except using the pBMA product as macroinitiator and DMAEMA as monomer. M_n calc = 15000 Da. M_n (GPC) = 14748. PDI (GPC) = 1.10.

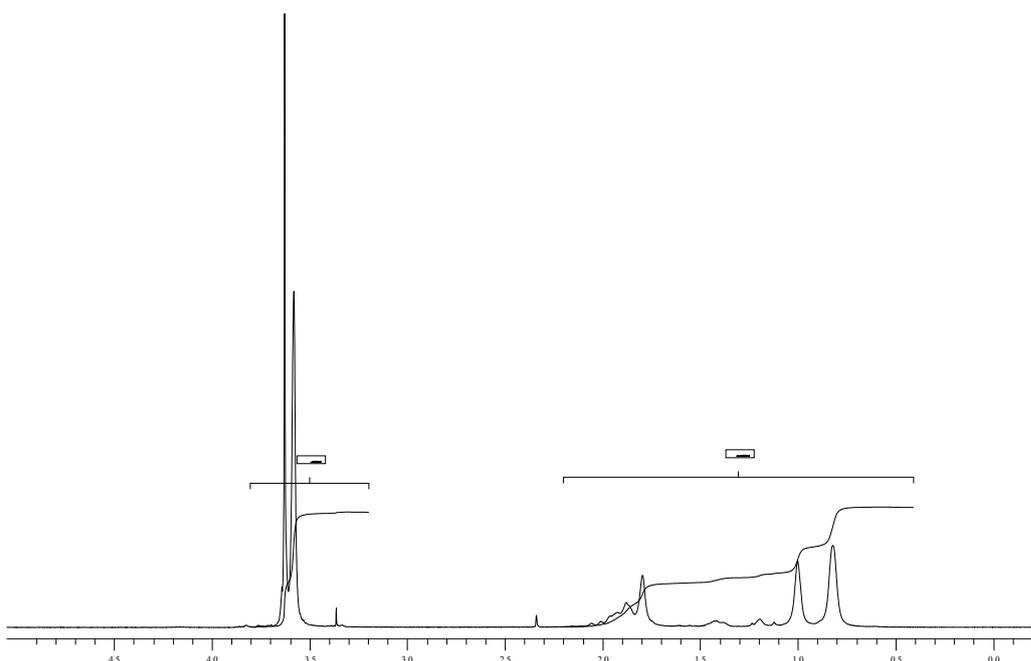


Figure S2. ^1H NMR of PEG₄₅-*b*-PMMA₁₆₄

Polymersome formation

To prepare polymer colloidal dispersions, 0.2 g of block copolymer was dissolved in THF (20 ml for 10 g L⁻¹, 200 ml for 1 g L⁻¹) and water was added slowly with stirring (0.2 ml/min for 1 wt %, 2 ml/min for 0.1 wt %) using an MCP-CPF Process IP 65 pump from IsmaTec. 100 μ l samples were taken at every 10% interval and analysed by NS-TEM and/or (Cryo)-TEM. PEG-PMMA polymersomes were produced as previously

described using the reverse solvent addition method to a final solvent composition of 80% water: 20% THF at a concentration of 0.2 g L^{-1} . PBMA-PDMAEMA polymersomes were prepared in the same way but with an initial concentration of 2 g L^{-1} and final solvent ratio of 90% water: 10% THF.

Polymersome dialysis

The polymersome solutions (50 ml) at initial concentration 0.2 g L^{-1} PEG₄₅-*b*-PMMA₁₆₄ were dialysed in tubing which had been prepared by boiling for 20 minutes and thoroughly washed with RO grade water. The dialysis tubing was tied and clipped at the top and bottom and placed in a 2 L beaker of water (RO grade). The solution was left to dialyse and water was replaced daily for 7 days to encourage the maximum removal of THF.

Polymersome swelling

Dialysis tubing containing dialysed polymersome solution (25 ml) was suspended inside a glass tube partly submerged in fresh RO grade water to prevent direct contact between the monomer layer and dialysis tubing.

A total of 100 ml monomer (methyl methacrylate) was added carefully by pipette down the side of the (2 litre) beaker to minimise splashing or monomer penetrating too far below the surface of the water

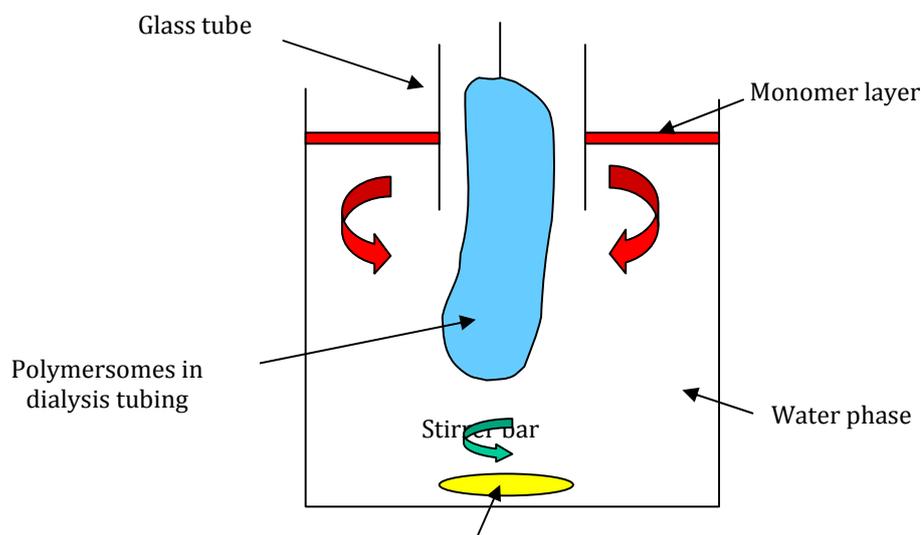


Figure S3. Schematic of polymersome swelling apparatus.

The water was stirred slowly to encourage migration of the monomer into the dialysis tubing containing the polymersomes via the water phase. The top of the beaker and glass tube were covered with a sheet of aluminium foil to minimise monomer loss due to evaporation and maintain osmotic pressure.

The monomer was allowed to diffuse in this way for five days to allow saturation of the hydrophobic leaflet of the polymersome.

Cryo-TEM preparation

Lacey carbon grids (Agar scientific, lacey carbon on copper) were placed into a humid chamber and 5 μ l of sample pipetted onto the grid. After blotting the sample grid was plunged into a pot of liquid nitrogen cooled liquid ethane (-168 °C) using a Cryoplunge (Birkbeck college). After freezing, the grid was transferred to a storage box under liquid nitrogen and stored in a liquid nitrogen dewar. Transfer to microscope goniometer was performed under liquid nitrogen and microscopy performed at -180 °C under vacuum. Cryo-TEM measurements were performed on a JEM-2011 FasTEM transmission electron microscope at an accelerating voltage of 200 kV. Images were recorded on a Gatan Ultrascan 1000 CCD camera (2048 x 2048 pixel, 15 Å per pixel resolution)

Cryo-SEM preparation

Cryogenic scanning electron microscopy was performed on ZEISS SUPRA 55-VP equipped with cold stage and sample preparation chamber. Accelerating voltage was set to 1-3kV to avoid burning sample and platinum target was used for sputter coating. For the Cryo-SEM sample preparation, typically, the specimen stub was tapped on the surface of the solution to take the sample by capillary action till fulfilled. The stub was then rapidly frozen in the solid nitrogen (<-210 °C) which obtained by slash the liquid nitrogen (-196 °C) under vacuum. The sample was stabilized on the pre-frozen stub adaptor and transferred under vacuum to the cold stage of the preparation chamber, which is mounted

on the SEM chamber. Both the anti-contaminate plates in the chamber and microscope were cooled down to -186°C while both the cold stages were set to -125°C . After fracturing the ice on the surface, the sample was then sublimated at -95°C for 1 minute. Sputter coating was applied using platinum target after cooling the sample back down to -125°C . The stub adaptor was subsequently transferred under vacuum into the SEM chamber where it is easily located on the cold stage specifically tailored to the SEM

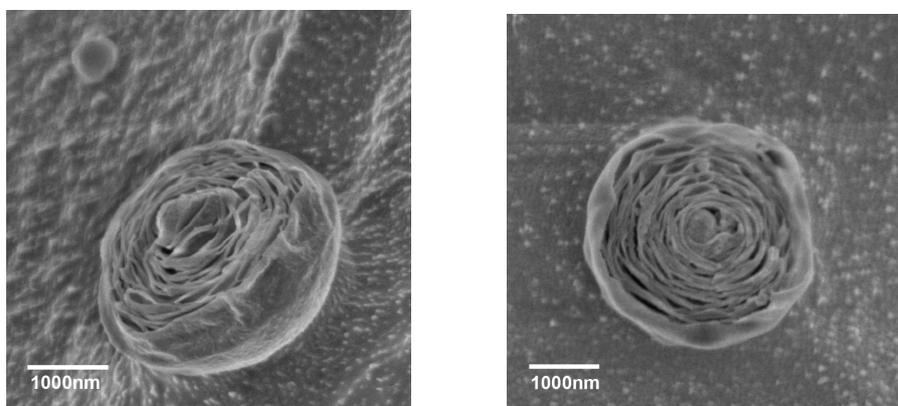


Figure S4. Giant multilamellar polymersomes formed from PEG-PMMA

Simulations

In order to investigate the microscopic details of the membrane/polymersome swelling coarse-grained molecular simulations of a model polymer bilayer were performed. The individual polymer chains consisted of 10 DPD beads, 4 hydrophilic (denoted as type A) and 6 hydrophobic (type B); as the hydrophobic ratio $f=0.6$ such chains would be expected to form stable bilayers in solution. A pre-assembled bilayer, consisting of 100 polymer chains (3000 polymer beads in total), was initially placed in 21000 solvent beads (type S). To mimic the effect of exposure of the bilayer to hydrophobic monomers at regular intervals a number of solvent beads are changed to hydrophobic beads. The density of the system was set to $\rho = 3r_c^{-3}$, where r_c is the non-bonded interaction range (see below), giving a total volume $V = 8000r_c$. The cross-sectional area was set to $A=383.7r_c$, the area for which the initial bilayer had zero surface tension.

In discussion of the simulation results, quantities are given in reduced units, i.e. lengths in units of r_c , mass in units of bead mass m , energies in $k_B T$, and time in units $\tau=(mr_c^2/k_B T)^{1/2}$, with other units derived from these.

The system was simulated using dissipative particle dynamics (DPD) [P. J. Hoogerbrugge and J. M. V. A. Koelman, *Europhys. Lett.*, **19**, 155-160 (1992)]. The interaction between beads was given by $\mathbf{F}_{ij} = \mathbf{F}_{ij}^C + \mathbf{F}_{ij}^D + \mathbf{F}_{ij}^R$ where the terms on the right hand side are the conservative, dissipative, and random forces respectively. The conservative force has two components; the first is the non-bonded interaction, which has the usual soft, repulsive form $\mathbf{F}_{ij} = a_{ij} (1 - r_{ij}/r_c)\mathbf{r}_{ij}$ where $r_{ij} = |r_i - r_j| = |\mathbf{r}_{ij}|$ is the separation between i and j , $\mathbf{r}_{ij} = r_{ij}/r_{ij}$, and r_c is the interaction range (which defines the length unit for the simulations). The interaction parameters (listed in Table X) were taken from [V Ortiz, SO Nielsen, DE Discher, ML Klein, R Lipowsky, and J Shillcock, *J Phys Chem B*, **109**, 17708-17714 (2005)].

In addition to the non-bonded interaction, neighboring beads in the polymers are bound together via a harmonic potential $\mathbf{F}_{ij} = -k_{bond} \mathbf{r}_{ij}$ where $k_{bond}=4k_B T$ [R D Groot and P Warren, *J Chem Phys*, 107, 4423-4435 (1997)].

The dissipative and random forces are given by $\mathbf{F}_{ij} = -\lambda w_{ij}^2 (\mathbf{r}_{ij})^2 (\mathbf{r}_{ij} \cdot \mathbf{v}_{ij}) \mathbf{r}_{ij}$ and $\mathbf{F}_{ij} = \sigma w(\mathbf{r}_{ij}) \xi \delta t^{-1/2} \mathbf{r}_{ij}$ where $w(r) = (1 - r/r_c)$, $\lambda = 3k_B T \tau r_c^{-2}$ is the damping parameter, σ is the random noise strength (related to λ by $\sigma^2 = 2k_B T \lambda$), ξ is a Gaussian random number of zero mean and unit variance and δt is the integration timestep [P Espanol and P Warren, *Europhys. Lett.* **30**, 191-196 (1995)].

The simulations were performed using LAMMPS simulation package [S. J. Plimpton, *J Comp. Phys.*, **117**, 1 (1995)] All simulations were performed at $k_B T = 1$ with a timestep of $\delta t = 0.02\tau$. The initial simulation configuration consisted of a polymer bilayer in a pure W solvent, which was simulated for 1.5×10^6 timesteps (10^6 timesteps for equilibration and 5×10^5 timesteps for data gathering). Successive cycles of hydrophobic particle addition were then performed. For each cycle 500 W beads (chosen at random) were exchanged for M beads. The system was then simulated 1.5×10^6 timesteps at each loading.

	A	B	S	M
A	78	86.7	79.3	86.7
B	86.7	78	100	78
S	79.3	100	78	100
M	86.7	78	100	78

Table: Non-bonded interaction parameters (in $k_B T$).