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Supplemental Information

Bottom-Up Evolution of Vesicles from Disks to High-Genus Polymersomes

Claudia Contini, Russell Pearson, Linge Wang, Lea Messager, Jens Gaitzsch, Loris Rizzello, Lorena Ruiz-Perez, and Giuseppe Battaglia

1 SUPPLEMENTAL FIGURES

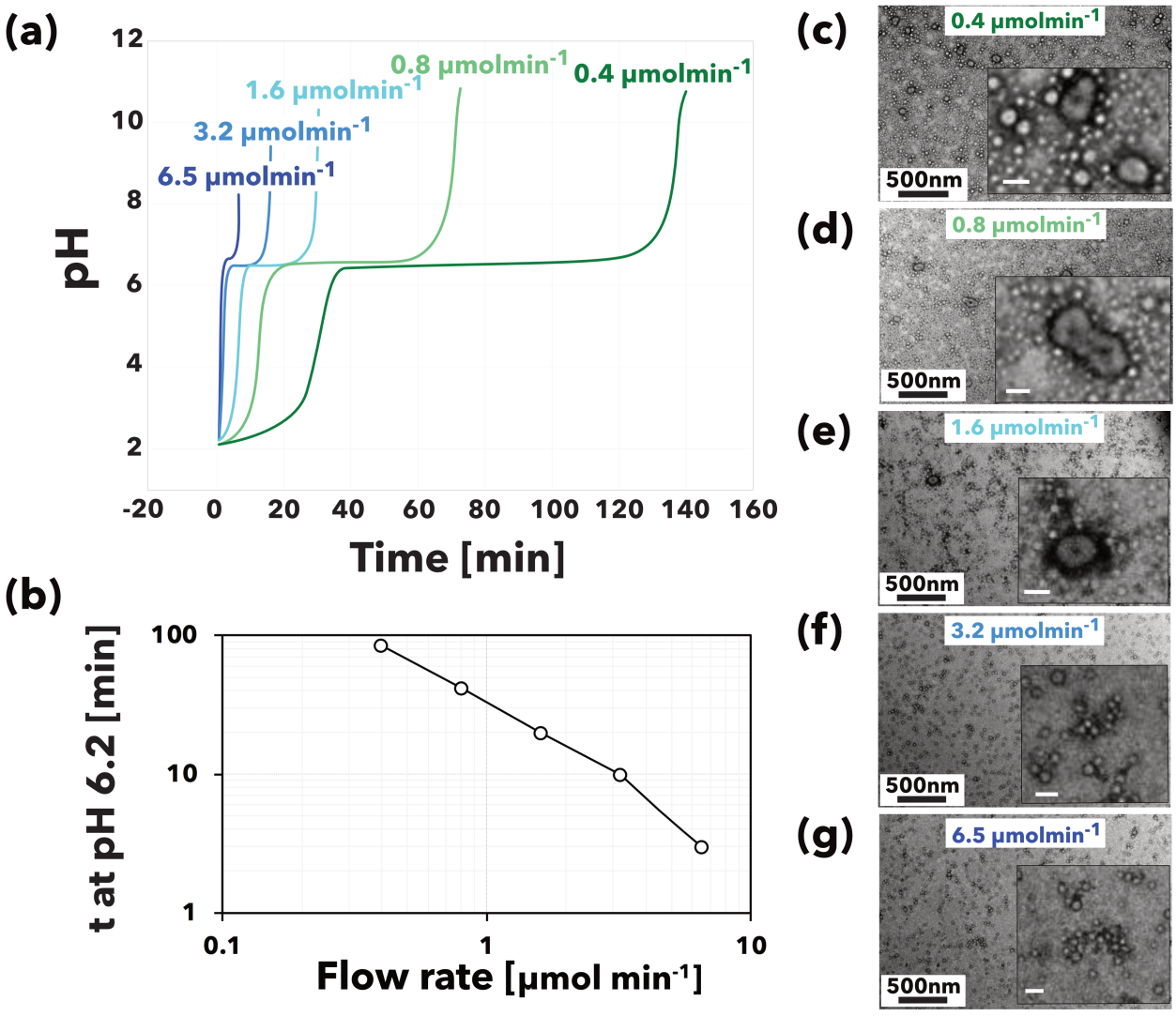

Figure 1 Related to Figure 2 pH titration. Titration curves plotted as pH versus time using different flow rates of NaOH solution addition to $PMPC_{25}$-$PDPA_{70}$ A solutions **(a)**. The time plateau obtained from the titration curves trend lines versus the NaOH's flow rate of injection calculated graphically from the titration curves **(b)**. TEM micrographs of the sample obtained at different flow-rates **c-g**. The small inset scale bar is 50nm

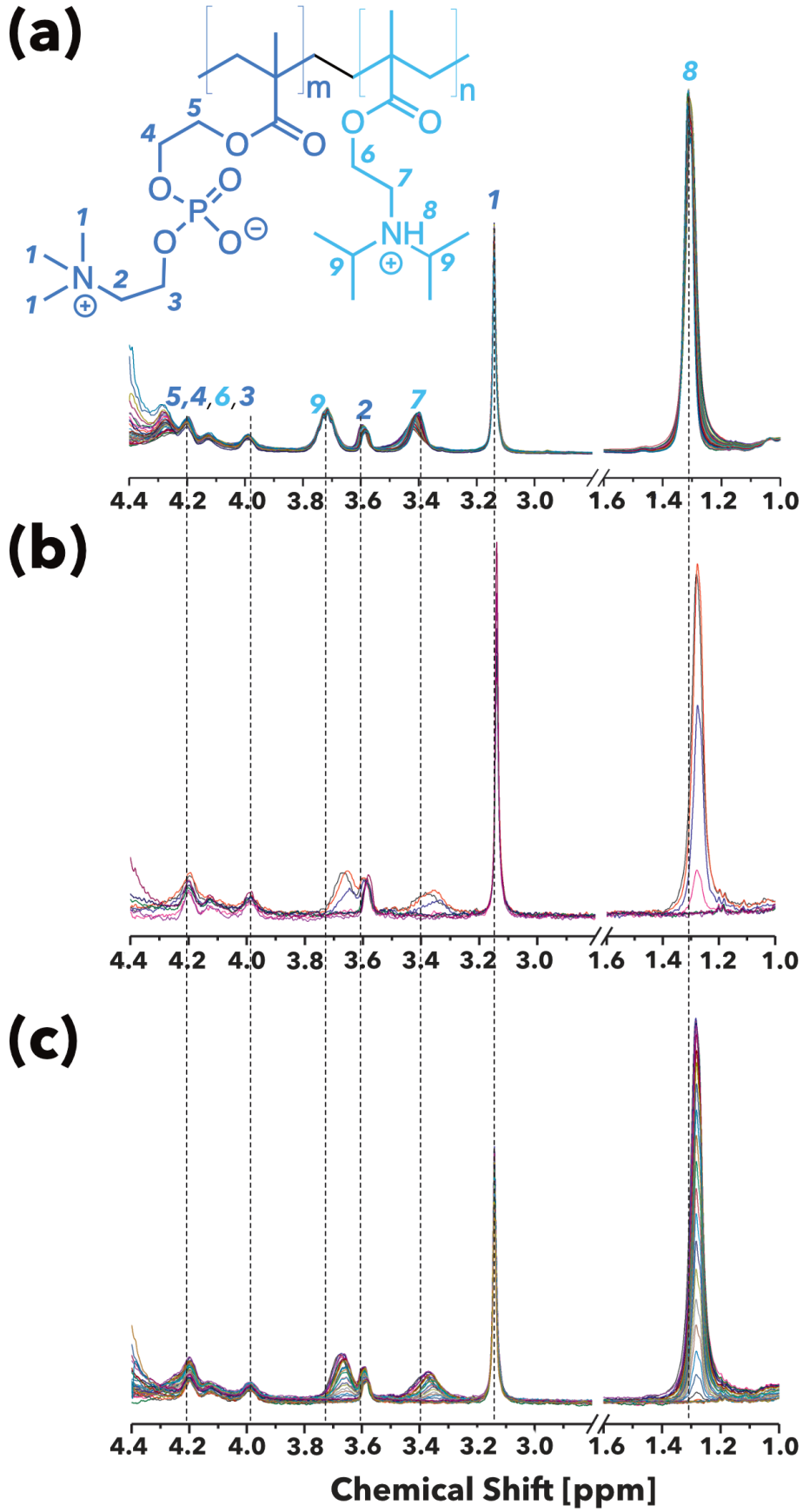

Figure 2 Related to Figure 3. NMR titration spectra as a function of temperature. $PMPC_{25}$-$PDPA_{70}$ dispersion during slow heating at pH=2 **(a)**, fast **(b)** and slow **(c)** heating at pH=7.

1.1 TRANSPARENT METHODS

Materials. MPC monomer (99.9 % purity) was donated by Biocompatibles U.K. Ltd.. Anhydrous ethanol (99 %), anhydrous methanol (99.8 %), DPA, copper(I) bromide (99.9 %), 2,2'-bipyridine (99 %), tris (2-carboxyethyl) phosphine hydrochloride (TCEP, 98 %), dry triethylamine and phosphotungstic acid (PTA) were purchased from Sigma Aldrich UK. The silica gel 60(63-200 nm) used to remove the ATRP catalyst CuBr was purchased from E. Merck (Darmstadt, Germany). HPLC grade dichloromethane and methanol was purchased from Fisher Scientific (Loughborough, UK). All the above were used as received. Phosphate-buffered saline (PBS) was prepared from tablets obtained from Oxoid (Basingstoke, UK). Semi-permeable cellulose dialysis tubing (Spectra/Por 6 MWCO 1,000) was purchased from Fisher Scientific (Loughborough, UK).

PMPC-PDPA synthesis. The PMPC-PDPA copolymers were synthesised using the already published protocol (Lomas et al., 2007). In a typical ATRP synthesis procedure for $PMPC_{25}$-$PDPA_{70}$, a solution containing an equivalent of morpholinoethyl-bromoisobutyric acid ester (ME-Br) was mixed in a round-bottom flask with MPC (25 eq.). The mixture was then dissolved in a few mililiters of ethanol and purged with nitrogen. Subsequently, a solid mixture of 2,2-bipyridine (2 eq.) and Cu(I)Br (1 eq.) was added under a constant nitrogen flow. The reaction mixture was stirred for 60 minutes to yield a highly viscous brown solution. Meanwhile, a solution of DPA (70 eq.) was prepared and purged with nitrogen in a separate flask before addition. Then, the reaction mixture was left overnight at room temperature. The mixture gradually turned green after dilution with ethanol, indicating the catalyst oxidation and passed through silica. The solution was then dialysed (MWCO 1,000 Da) against dichloromethane, methanol and water. The polymer was then freeze-dried under vacuum. PMPC25-PDPA72 was solubilised in a mixture of CDCl3/MeOD (3:1) and analyse in 1H NMR analysis to confirm the success of the reaction.

Preparation of PMPC-PDPA dispersions. PMPCx-PDPAy copolymers were solubilised at a concentration of 40μM, at room temperature in acidified phosphate buffer saline (PBS, pH 2) solution in order to dissolve all the block copolymer chains (unimers). For the pH switch, this was raised adding drop-wise a 1 M NaOH solution. The temperature of the solution was then dropped to 5°C and the pH was raised to 7 by adding the required 1 M NaOH solution.

UV-Vis Spectroscopy analysis. UV-vis spectroscopy experiments were carried out on a JASCO XX spectrophotometer equipped with a temperature controller. Solutions of PMPCx-PDPAy were prepared at low temperature with a concentration of 40μM, and placed in the UV chamber. Absorbance was recorded from 5 to 60°C at a constant rate of 0.3 to 3°C/min.

Transmission Electron Microscopy (TEM) imaging. TEM imaging was performed using a JEOL 2100 TEM microscope at 200 kV, equipped with a Gatan CCD camera. The polymersomes were stained using a phosphotungstic acid (PTA) solution at 0.75 % (w/v). This PTA solution was prepared by dissolving 37.5 mg of PTA in boiling distilled water (5 mL). The pH was adjusted to 7.0 by adding a few drops of 5 M NaOH under continuous stirring. The PTA solution was then filtered through a 0.2 μm membrane. Copper grids were glow-discharged for 40 seconds in order to render their surface hydrophilic. Then 5 μL of copolymer dispersion (concentration 0.5 mg mL^{-1}) was deposited onto the grids for one minute. After that, the grids were blotted with filter paper and immersed into the PTA staining solution for 5 s for positive staining. Then the grids were blotted again and dried under vacuum for 1 min. Cryogenic TEM specimens were prepared by fast immersing a pre-sample-engrossed grid in liquid ethane using the Gatan Cryoplunge® 3 system. The grid is quickly placed in a cryogenic stage and kept at -170 °C and imaged using a GATAN cryogenic holder.

Differential Scanning Calorimetry (DSC) analysis. The analysis was carried out using a VP-DSC MicroCalorimeter with a sample cell of 0.5 mL. The sample were prepared as previously described at pH 7 and 5°C, and analyse at a rate of $1°Cm^{-1}$ from 5 to 60 °C and vice versa.

^{1}H NMR spectroscopy. NMR spectroscopy of PMPC-PDPA after synthesis was carried out on a Bruker AV600 spectrometer. ^{1}H-NMR was also used to monitor the intensity evolution of DPA and MPC protons peaks as function of temperature by using a Bruker 400 MHz instrument. In a typical experiment, block copolymer solutions in deuterated PBS using a the same protocol described above for all the PMPC-PDPA dispersions. NMR spectra were then recorded while the temperature was increased at 40 °C with a rate of 0.2 and 1° $min^{-}1$. The proton intensity of the peak 7, 8 and 9 were normalised setting the maximum value to 1. All the following intensities were set in relation to this value to give a distinct intensity correlation as a function of the temperature change. The average and standard deviation values were then calculated and used to illustrate the change in intensities.

References

Lomas, H., I. Canton, S. MacNeil, J. Du, S. P. Armes, A. J. Ryan, A. L. Lewis, and G. Battaglia (2007). Biomimetic ph sensitive polymersomes for efficient dna encapsulation and delivery. *Adv. Mater. 19*(23), 4238–4243.