Stereocomplexation in novel degradable amphiphilic block copolymer micelles of poly(ethylene oxide) and poly(benzyl α-malate)

Ryan J. Pounder, Helen Willcock, Nga Sze Ieong, Rachel K. O'Reilly and Andrew P. Dove*

Department of Chemistry, University of Warwick, Coventry, CV4 7AL, UK. Tel:+44(0)24 76524107 E-mail:a.p.dove@warwick.ac.uk

Supplementary Information

Experimental Details

Materials. Chloroform was dried over CaH_2, distilled, degassed and stored under a nitrogen atmosphere. All alcohol and amine initiators were dried over suitable dry agents and were distilled, degassed and/or sublimed as required. 2-hydroxy-succinic acid 4-benzyl ester was synthesized as previously reported.[1] All other chemicals and solvents were obtained from Aldrich and used as received.

General Considerations. All synthetic manipulations were performed under moisture- and oxygen-free conditions either in a nitrogen-filled glovebox or by standard Schlenk techniques. Gel-permeation chromatography (GPC) was used to determine the molecular weights and polydispersities of the synthesised polymers. GPC in THF was conducted on a system comprised of a Varian 390-LC-Multi detector suite fitted with differential refractive index (DRI), light scattering (LS) and ultra-violet (UV) detectors equipped with a guard column (Varian Polymer Laboratories PLGel 5 μM, 50 × 7.5 mm) and two mixed D columns (Varian Polymer Laboratories PLGel 5 μM, 300 × 7.5 mm). The mobile phase was tetrahydrofuran with 5% triethylamine eluent at a flow rate of 1.0 mL min^{-1}, and samples were calibrated against Varian Polymer laboratories Easi-Vials linear poly(styrene) standards

(162-2.4 × 10^5 g.mol^{-1}) using Cirrus v3.3. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX-300, DPX-400, AC400, or DRX-500 spectrometer at 293 K unless stated otherwise. Chemical shifts are reported as δ in parts per million (ppm) and referenced to the chemical shift of the residual solvent resonances ($CDCl_3$ ^{1}H: δ = 7.26 ppm; ^{13}C δ = 77.16 ppm). Elemental analyses were performed in duplicate by Warwick Analytical Services. Average solution hydrodynamic diameters (D_h) and size distributions of the PEO-*b*-PBMA micelles in aqueous solution were determined by dynamic light scattering (DLS). The DLS measurements were taken on a Malvern Nano S Zetasizer Nano Series instrument operating at 25 °C with a 635-nm laser module using a cumulants fit analysis method. All determinations were made in triplicate (with 12 runs recorded). Transmission electron microscopy (TEM) samples were prepared by drop deposition and dried *via* blotching onto copper/carbon grids that had been treated with oxygen plasma to increase the surface hydrophilicity. The particles were stained using a dilute 5% solution of uranyl acetate and examined with a transmission electron microscope (JEOL TEM-1200), operating at 100 kV. Micrographs were collected at magnifications varying from 80K to 120K and calibrated digitally. Histograms of number-average particle diameters (D_{av}) and standard deviations were generated from the analysis of a minimum of 100 particles from at least three different micrographs. For atomic force microscopy (AFM) analysis, a Veeco MultiMode AFM was used with a Nanoscope IIIa controller and Quadrex module (Digital Instruments, Veeco Metrology Group; Santa Barbara, CA). The tips wre silicon with nomical force constant and resonant frequency of 3.5 N/m and 75 kHz (NCS18/no Al from MikroMasch). The samples were prepared by placing a drop of the polymer micelle solution (1 mg/mL) onto freshly cleaved mica. Each sample was allowed to air dry prior to AFM analysis. To compare the relative stability of the stereocomplex versus enantiopure micelle solution, the aforementioned AFM samples were subsequently placed in an oven set at 140 °C for 30 minutes prior to further AFM analysis. Critical micelle concentration determinations were performed using fluorescence spectroscopy on a Cary Eclipse single-beam Perkin-Elmer LS55 fluorometer. Specific rotation measurements for were recorded in $CHCl_3$ on a Perkin-Elmer 241 polarimeter using a sodium source (λ = 589 nm) and a 1 cm rotation cell.

Synthesis of 5-(R)-[(benzyloxycarbonyl)methyl]-1,3-dioxolane-2,4-dione, (D-malOCA)

The synthesis of *D*-malOCA was carried out in an identical manner to that of *L*-malOCA, previously reported.[2] To a suspension of 2-hydroxy-succinic acid 4-benzyl ester, (4.78 g,

0.021 mol, 1 equiv) in dry THF (150 mL) was added diphosgene (3.1 mL, 0.026 mol, 1.2 equiv) under a nitrogen atmosphere. The resulting mixture was then treated with activated carbon and left to stir at room temperature for 18 h. The solution was then filtered off the activated carbon and concentrated in *vacuo*. The resulting residue was washed with pentanes (2 x 100 mL) and recrystallised from Et_2O/petroleum ether (b.p. 40-60 °C) and dried over 4Å molecular sieves to yield *D*-malOCA as a white solid. (3.46 g, 13.7 mmol 65%)
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.42-7.33 (5H, m, -C$H_{aromatic}$); 5.14 (2H, s, -CH_2Ar); 5.09 (1H, ABX, $^3J_{A\text{-}X}$ = 4.02 Hz, $^3J_{B\text{-}X}$ = 3.58 Hz, -C*H*CH_2COOCH_2Ar); 3.22 (1H, ABX, $^2J_{A\text{-}B}$ = 18.28 Hz, $^3J_{A\text{-}X}$ = 4.02 Hz, -CH_2COOCH_2Ar), 3.16 (1H, ABX, $^2J_{A\text{-}B}$ = 18.28 Hz, $^3J_{B\text{-}X}$ = 3.58 Hz, -CH_2COOCH_2Ar). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 167.8 (-CH_2*C*$OOCH_2$Ar); 166.7 (-OCOO*C*OCH-); 145.4 (-O*C*OOCOCH-); 134.4 (-$C_{ipso\ aromatic}$); 128.9 (-$C_{meta\ aromatic}$); 128.8 (-$C_{para\ aromatic}$); 128.7 (-$C_{ortho\ aromatic}$); 75.0 (-OCOOCO*C*H-); 68.2 (-*C*H_2Ar); 34.4 (-CH*C*H_2COOCH_2Ar). Elemental Analysis: Calculated (Found) C: 57.6 (57.2); H: 4.0 (4.1). $[\alpha]_D^{33}$ = +20.9° ($CHCl_3$, *c* = 5.98 g L^{-1}).

General procedure for preparation of PEO-b-PBMA ([M]/[I] = 25).
A solution of 4-methoxypyridine (4.8 µL, 0.048 mmol, 1 equiv) and MeO-PEO_{5K}-OH macroinitiator (0.24 g, 0.048 mmol, 1 equiv) was added to mal-OCA (300 mg, 1,2 mmol, 25 equiv) in $CHCl_3$ (3.75 mL). The solution was left to stir at room temperature for the allotted time period before being precipitated into ice cold petroleum ether (b.p. 40-60 °C) to yield PEO_{5K}-*b*-$PBMA_{25}$ as a white solid (0.39 g, 0.038 mmol, 82%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.35 - 7.21 (125H, m, -C$H_{aromatic}$), 5.58 – 5.50 (25H, m, -C*H*COO-), 5.13 – 5.05 (50H, m, -CH_2Ar), 3.67 – 3.53 (454H, s, -O(C*H*2)$_2$O-), 3.03 – 2.80 (50H, m, -CH_2COOCH_2Ar). GPC (THF, RI): M_n (PDI) = 16 940 g.mol^{-1} (1.03) for PEO_{5K}-*b*-P(*L*-BMA)$_{25}$.

General procedure for preparation of PEO-b-PBMA polymeric micelles.
Deionised water (60 mL) was added dropwise to a solution of PEO_{5K}-*b*-$PBMA_{25}$ (30 mg, 0.0028 mmol) (M_n = 16 940 g.mol^{-1}, PDI = 1.03) in HPLC grade THF (30 mL) at 25 °C *via* a metering pump at a rate of 10 mL.h^{-1}. After the addition of water was complete, the micelle solution was transferred to a presoaked dialysis membrane tubes (MWCO = 3.5 kDa) and dialysed against nanopure water for 3 days with 5 water changes. The final volume of PEO_{5K}-

b-PBMA$_{25}$ was 100 mL affording a polymer concentration of *ca.* 0.3 mg.mL^{-1}. D_h (DLS) = 18 ± 1 nm; D_{av} (TEM) = 16 ± 5 nm; CMC (Fluorescence microscopy) = 3.61 x 10^{-3} g.L^{-1}.

General procedure for CMC determination of polymeric micelles.[3]

A portion (0.05 mL) of an acetone solution of pyrene at 6 x 10^{-5} mol.L^{-1} was placed into ten 5 mL volumetic flasks and all left to allow the acetone to fully evaporate. Solutions of the polymeric micelles (5 mL) at different concentrations (from 0.3 to 0.0003 mg.mL^{-1}) were added to the volumetic flasks resulting in a pyrene concentration of 6 x 10^{-7} mol.L^{-1}. The solutions were left to stir for two days to ensure equilibrium before being analysed using fluorescence spectroscopy.

SEC Data

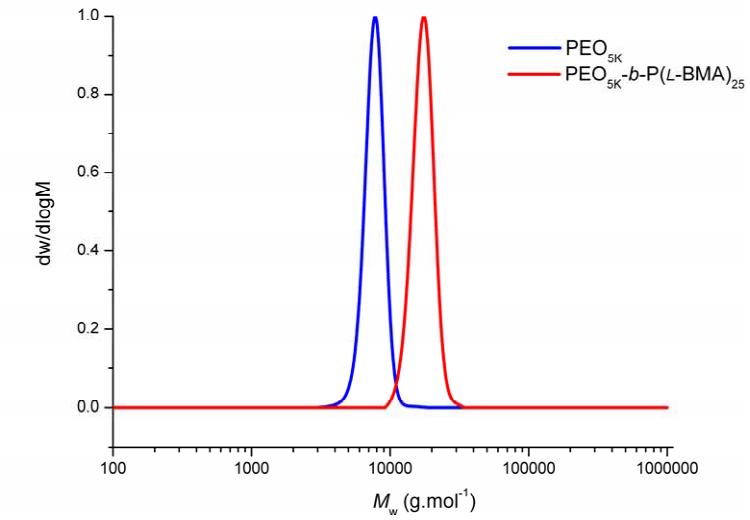

Figure S1. SEC traces of PEO_{5K}-*b*-P(*L*-BMA)$_{25}$ (M_n = 16 940 g.mol^{-1}, PDI = 1.03) (—) prepared by ROP of *L*-malOCA from MeO-PEO_{5K}-OH (M_n = 7 530 g.mol^{-1}, PDI = 1.03) (—).

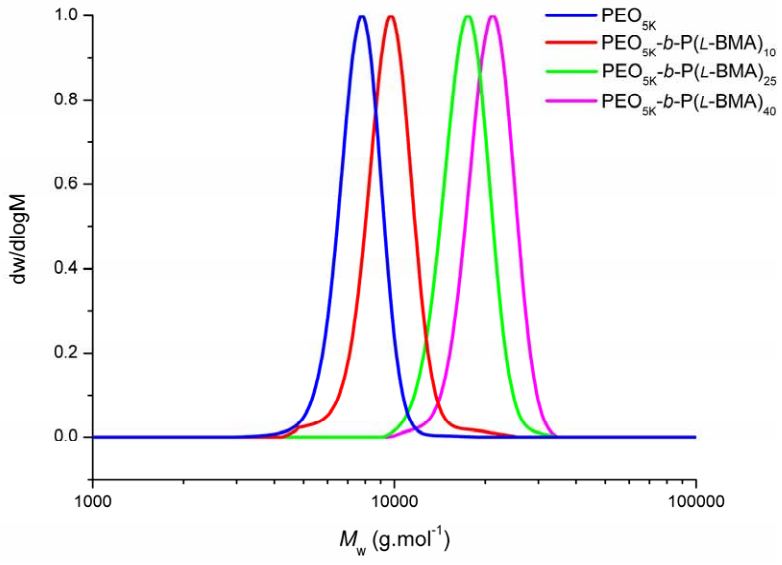

Figure S2. SEC traces of PEO_{5K}-*b*-P(*L*-BMA)$_{10}$ (M_n = 9 400 g.mol^{-1}, PDI = 1.04) (—), PEO_{5K}-*b*-P(*L*-BMA)$_{25}$ (M_n = 16 940 g.mol^{-1}, PDI = 1.03) (—) and PEO_{5K}-*b*-P(*L*-BMA)$_{40}$ (M_n = 20 412 g.mol^{-1}, PDI = 1.03) (—) prepared by ROP of *L*-malOCA from MeO-PEO_{5K}-OH (M_n = 7 530 g.mol^{-1}, PDI = 1.03) (—).

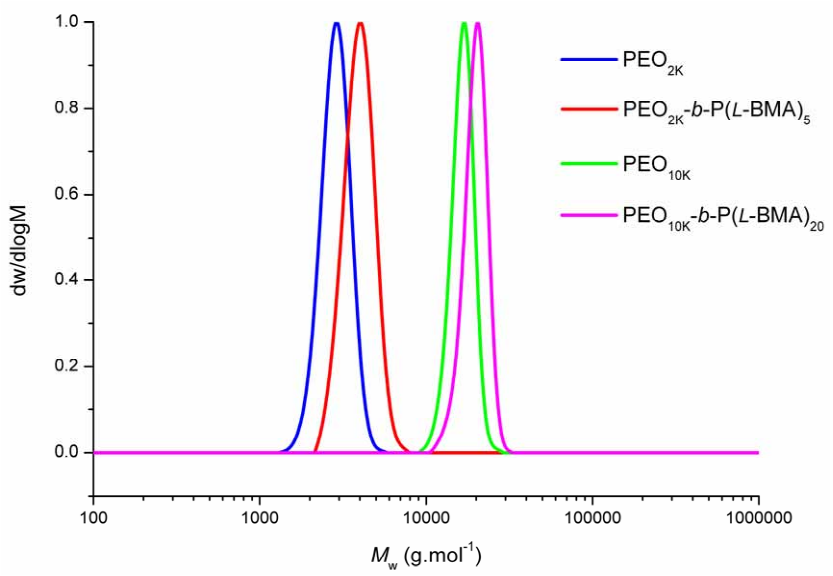

Figure S3. SEC traces of PEO$_{2K}$-*b*-P(*L*-BMA)$_5$ (M_n = 3 850 g.mol^{-1}, PDI = 1.04) (—) and PEO$_{10K}$-*b*-P(*L*-BMA)$_{20}$ (M_n = 19 440 g.mol^{-1}, PDI = 1.03) (—) prepared by ROP of *L*-malOCA from MeO-PEO$_{2K}$-OH (M_n = 2 790 g.mol^{-1}, PDI = 1.04) (—) and MeO-PEO$_{10K}$-OH (M_n = 16 270 g.mol^{-1}, PDI = 1.03) (—) respectively.

Light Scattering Data

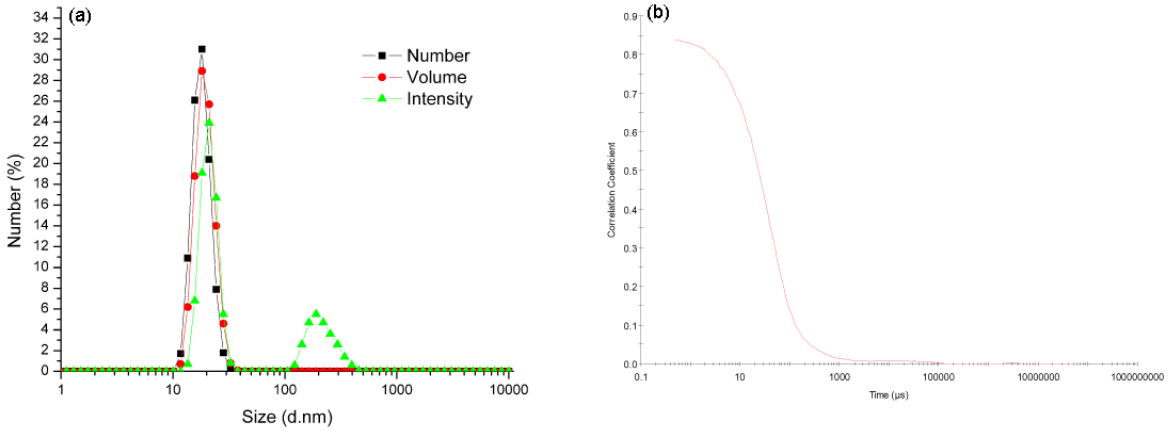

Figure S4. (a) DLS data and (b) Correlation Function for PEO$_{112}$-*b*-P(*L*-BMA)$_{10}$ micelles

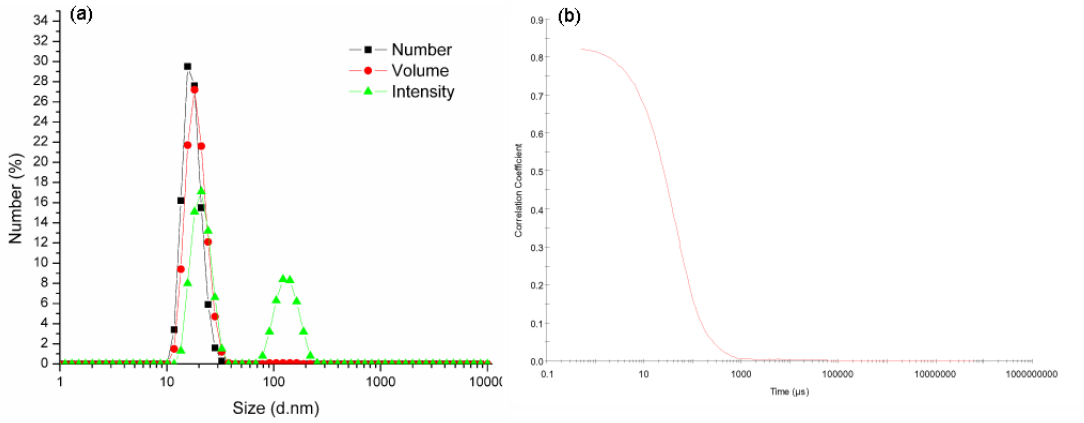

Figure S5. (a) DLS data and (b) Correlation Function for PEO_{112}-*b*-P(*L*-BMA)$_{25}$ micelles

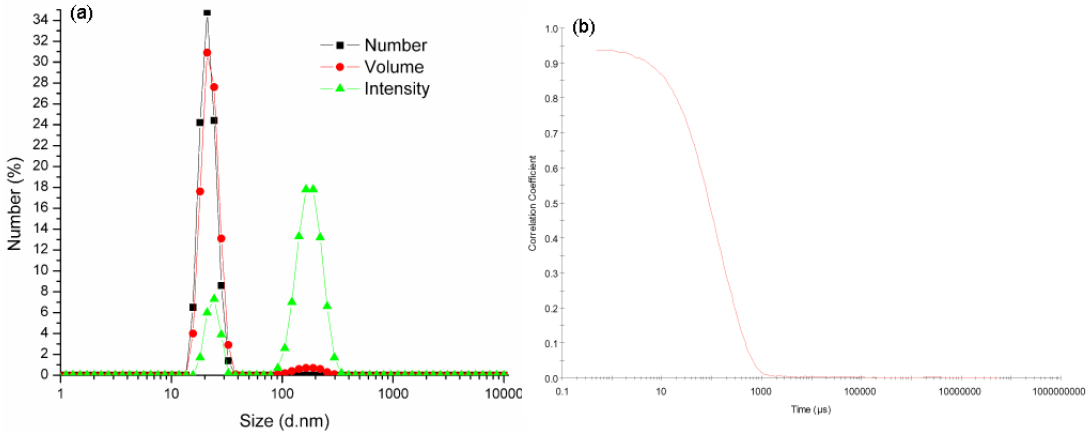

Figure S6. (a) DLS data and (b) Correlation Function for PEO_{112}-*b*-P(L-BMA)$_{40}$ micelles

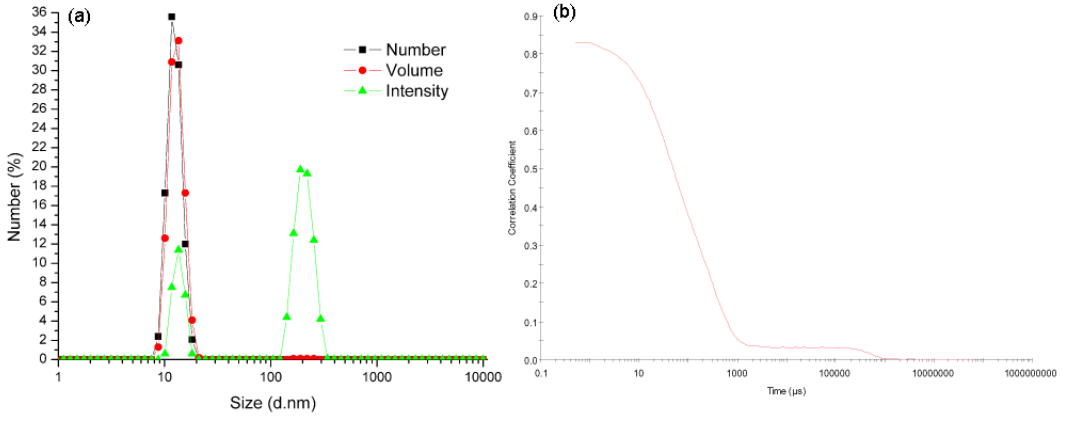

Figure S7. (a) DLS data and (b) Correlation Function for PEO_{45}-*b*-P(*L*-BMA)$_5$ micelles

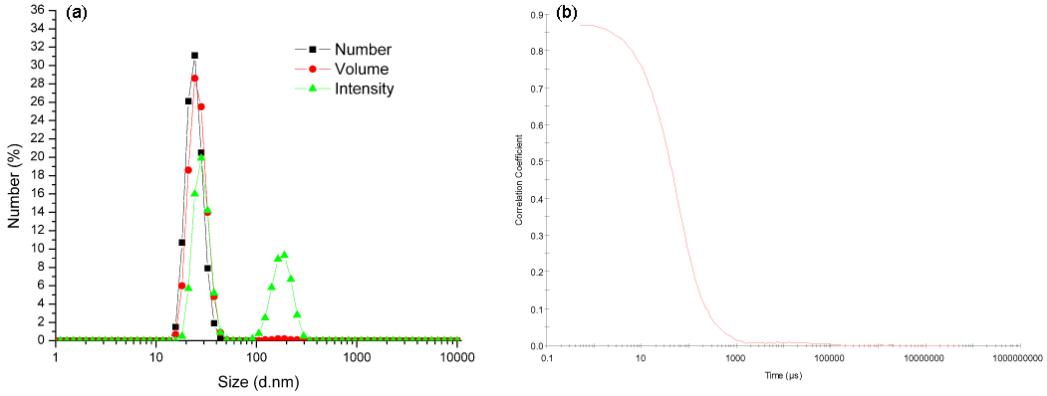

Figure S8. (a) DLS data and (b) Correlation Function for PEO_{227}-*b*-P(*L*-BMA)$_{20}$ micelles

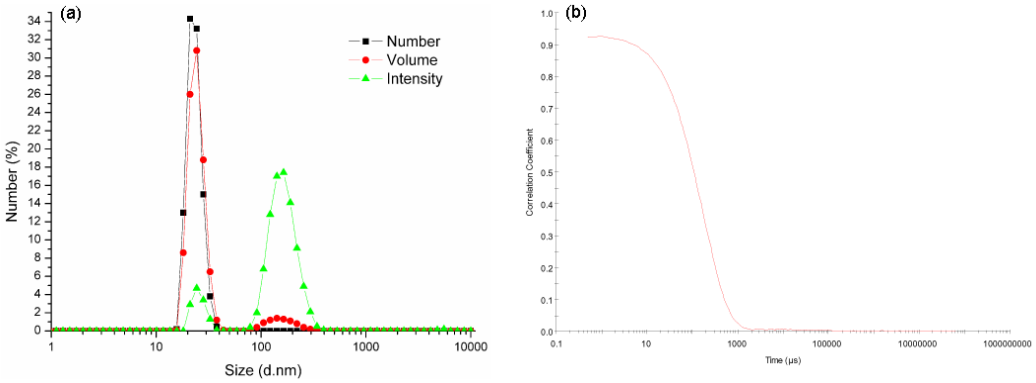

Figure S9. (a) DLS data and (b) Correlation Function for PEO_{112}-*b*-P(*L*-BMA)$_{10}$ + PEO_{112}-*b*-P(*D*-BMA)$_{10}$ stereocomplex micelles

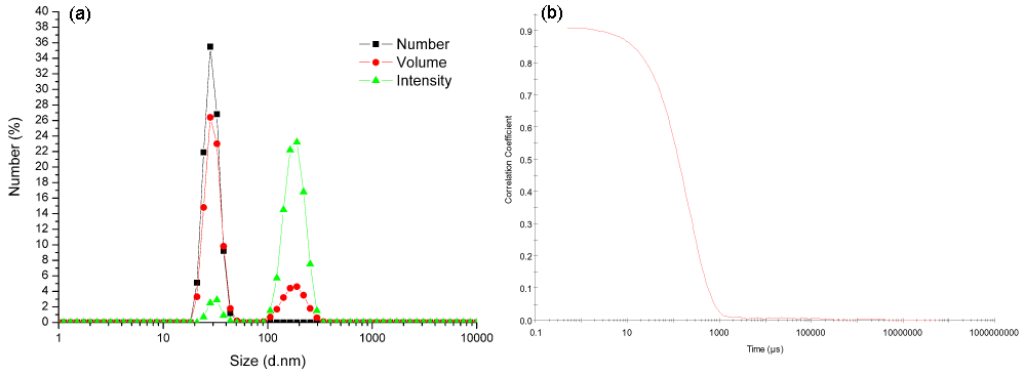

Figure S10. (a) DLS data and (b) Correlation Function for PEO_{112}-b-P(L-BMA)$_{10}$ + PEO_{112}-b-P(D-BMA)$_{10}$ stereocomplex micelles before redispersion

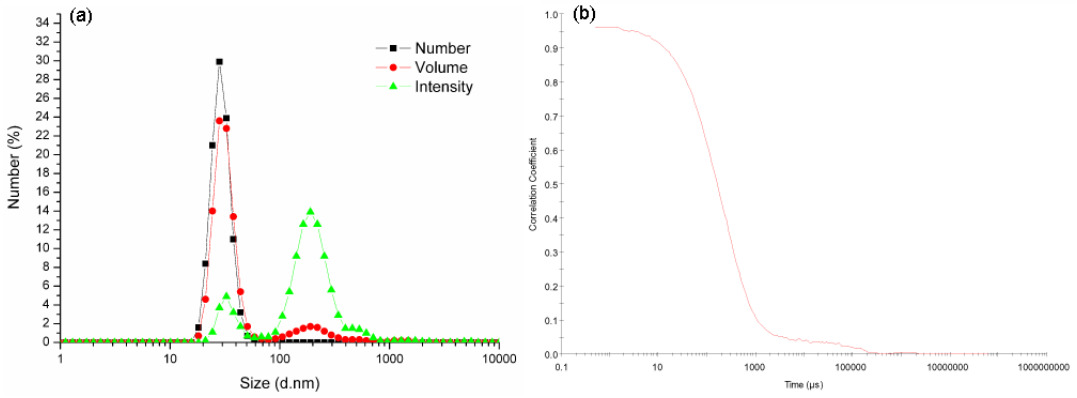

Figure S11. (a) DLS data and (b) Correlation Function for PEO_{112}-*b*-P(*L*-BMA)$_{10}$ + PEO_{112}-*b*-P(*D*-BMA)$_{10}$ stereocomplex micelles after redispersion

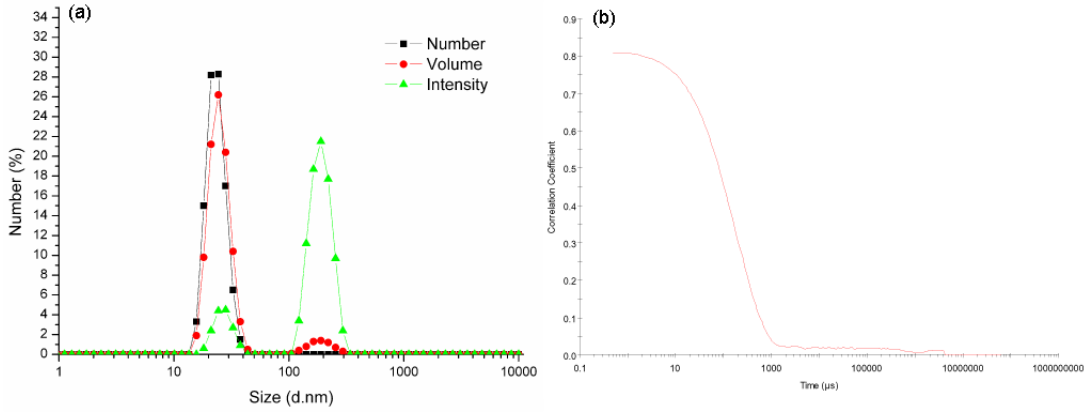

Figure S12. (a) DLS data and (b) Correlation Function for PEO_{112}-*b*-P(L-BMA)$_{10}$ micelles before redispersion

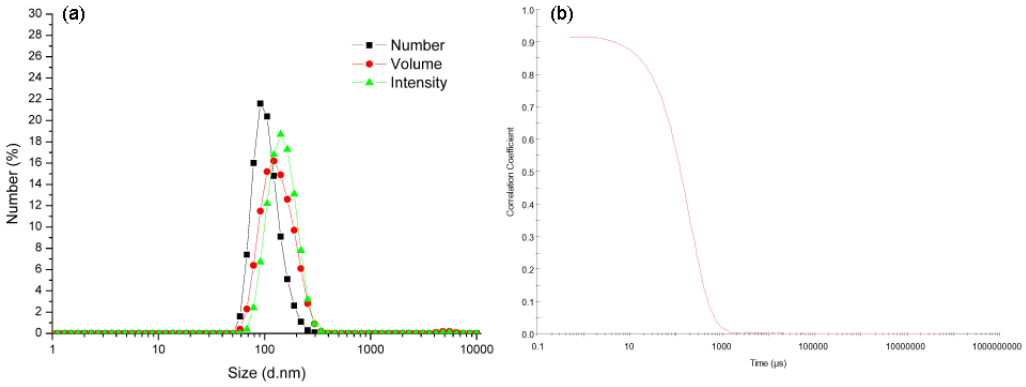

Figure S13. (a) DLS data and (b) Correlation Function for PEO_{112}-b-P(*L*-BMA)$_{10}$ micelles after redispersion

TEM Data

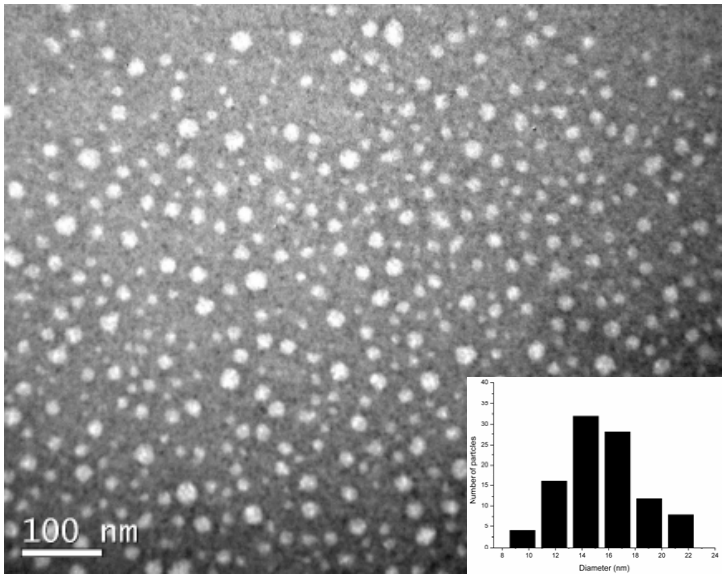

Figure S14. TEM image of the micelles prepared from PEO_{112}-*b*-P(*L*-BMA)$_{10}$ (D_{av} = 16 ± 5 nm) *via* the solvent switch method. Scale bar shown is 100 nm. Samples were stained with uranyl acetate (2% solution), drop deposited onto a carbon-coated copper grid and allowed to dry under ambient conditions. Inset: TEM size distribution histogram.

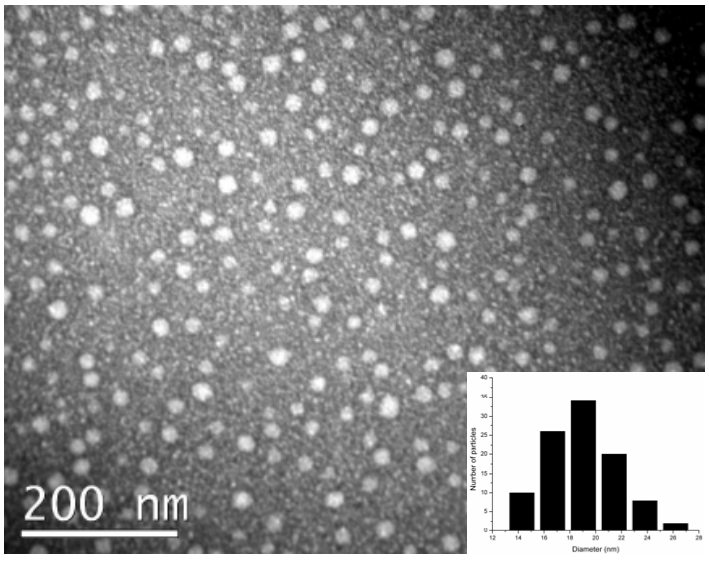

Figure S15. TEM image of the micelles prepared from PEO_{112}-*b*-P(*L*-BMA)$_{40}$ (D_{av} = 19 ± 5 nm) the solvent switch method. Scale bar shown is 200 nm. Samples were stained with uranyl acetate (2% solution), drop deposited onto a carbon-coated copper grid and allowed to dry under ambient conditions. Inset: TEM size distribution histogram.

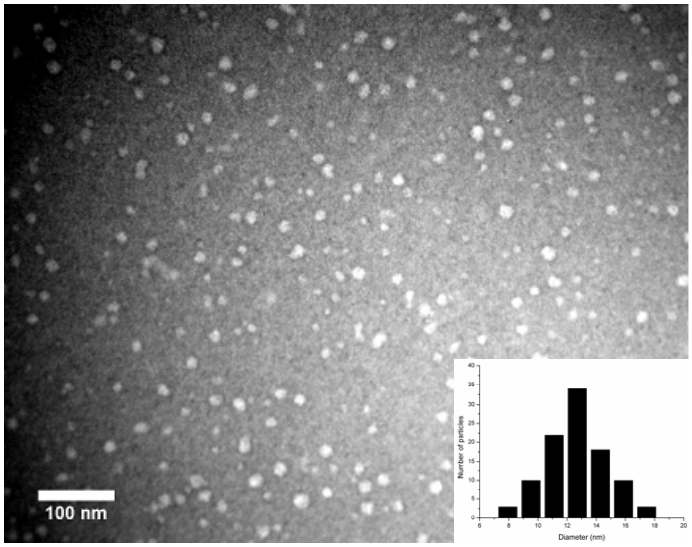

Figure S16. TEM image of the micelles prepared from PEO_{45}-*b*-P(*L*-BMA)$_5$ (D_{av} = 13 ± 5 nm) *via* the solvent switch method. Scale bar shown is 100 nm. Samples were stained with uranyl acetate (2% solution), drop deposited onto a carbon-coated copper grid and allowed to dry under ambient conditions. Inset: TEM size distribution histogram.

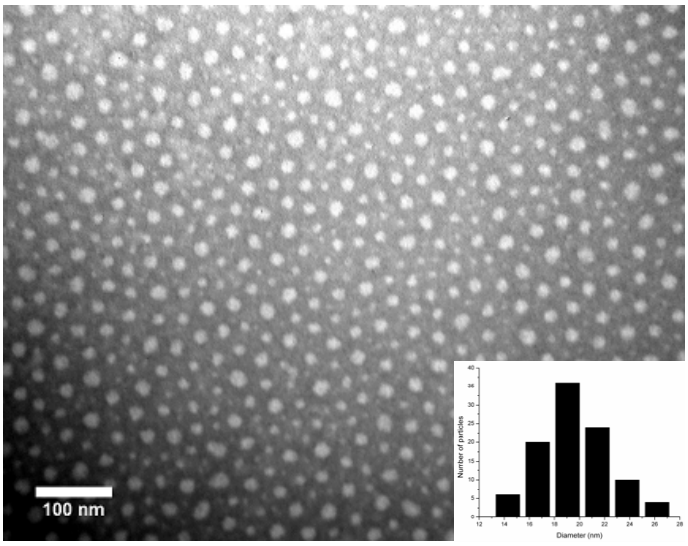

Figure S17. TEM image of the micelles prepared from PEO_{227}-*b*-P(*L*-BMA)$_{40}$ (D_{av} = 20 ± 6 nm) *via* the solvent switch method. Scale bar shown is 100 nm. Samples were stained with uranyl acetate (2% solution), drop deposited onto a carbon-coated copper grid and allowed to dry under ambient conditions. Inset: TEM size distribution histogram.

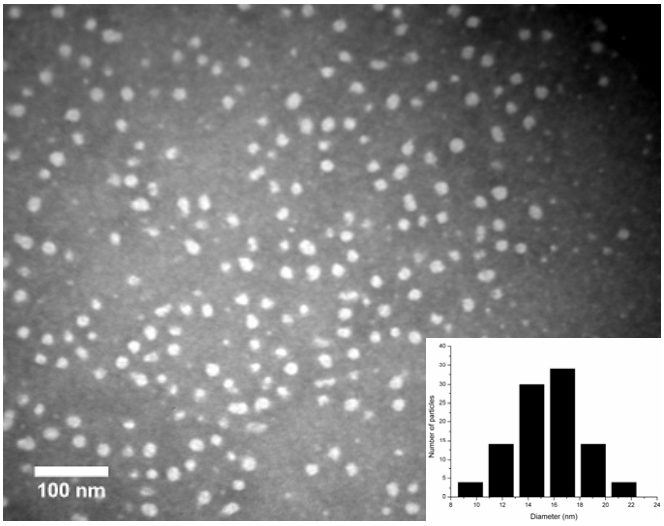

Figure S18. TEM image of the micelles prepared from PEO_{112}-*b*-P(*D*-BMA)$_{10}$ (D_{av} = 16 ± 4 nm) *via* the solvent switch method. Scale bar shown is 100 nm. Samples were stained with uranyl acetate (2% solution), drop deposited onto a carbon-coated copper grid and allowed to dry under ambient conditions. Inset: TEM size distribution histogram.

AFM Data

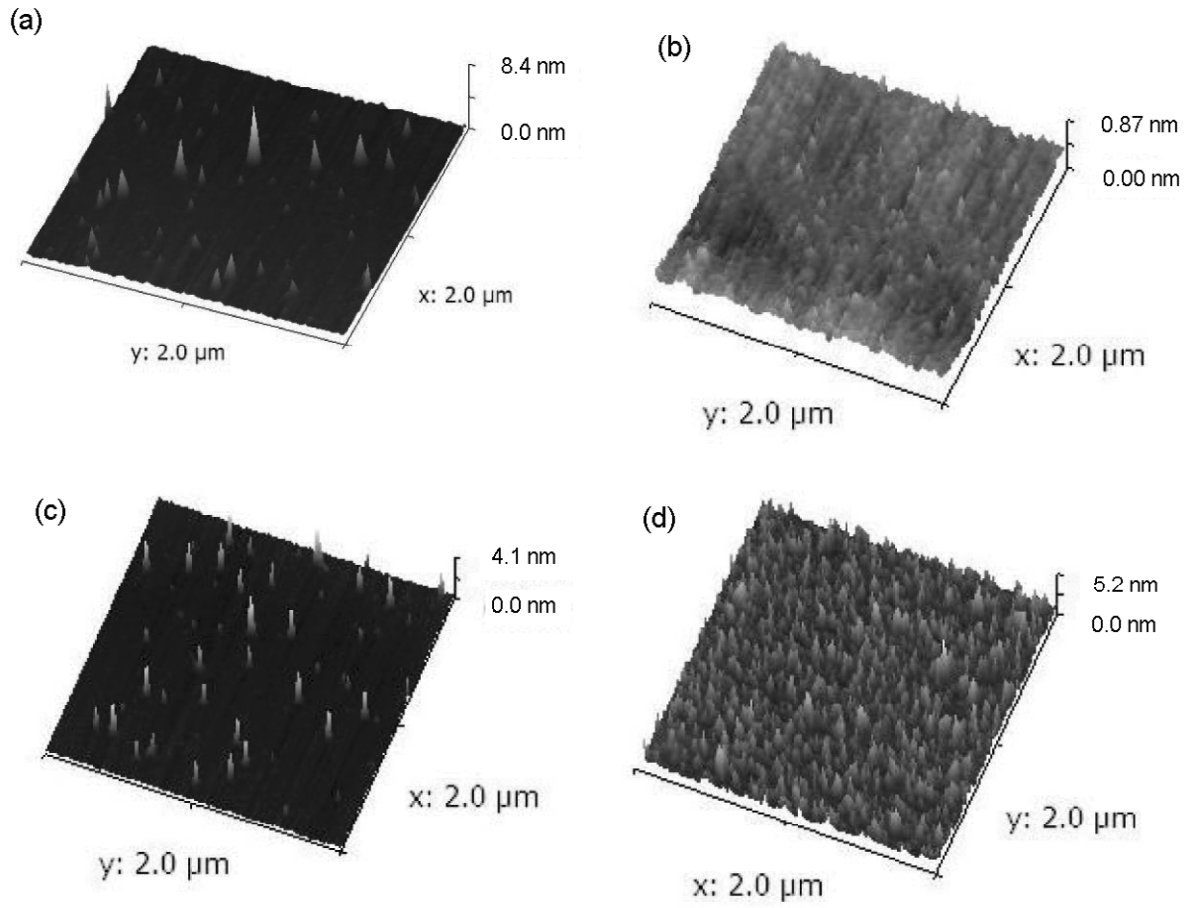

Figure S19. AFM analysis of (a) PEO_{112}-*b*-P(*L*-BMA)$_{10}$ micelles before heating; (b) PEO_{112}-*b*-P(*L*-BMA)$_{10}$ micelles after heating at 140 °C for 30 mins; (c) Micelles formed from an equimolar mixture of PEO_{112}-*b*-P(*L*-BMA)$_{10}$ and PEO_{112}-*b*-P(*D*-BMA)$_{10}$ before heating; (d) Micelles formed from an equimolar mixture of PEO_{112}-*b*-P(*L*-BMA)$_{10}$ and PEO_{112}-*b*-P(*D*-BMA)$_{10}$ after heating at 140 °C for 30 mins.

CMC Data

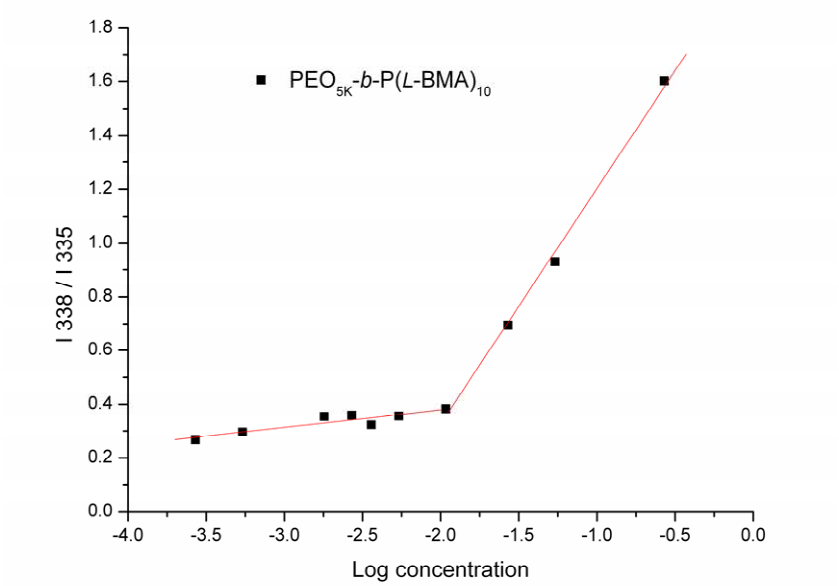

Figure S20. Concentration dependence of pyrene I_{338}/I_{335} intensity ratio for PEO_{112}-*b*-P(*L*-BMA)$_{10}$ in water at room temperature. (Diblock: M_n = 9 400 g.mol^{-1}, PDI = 1.04; and [pyrene]$_0$ = 6 x 10^{-7} M). Inflection point at 1.23 x 10^{-2} g.L^{-1}.

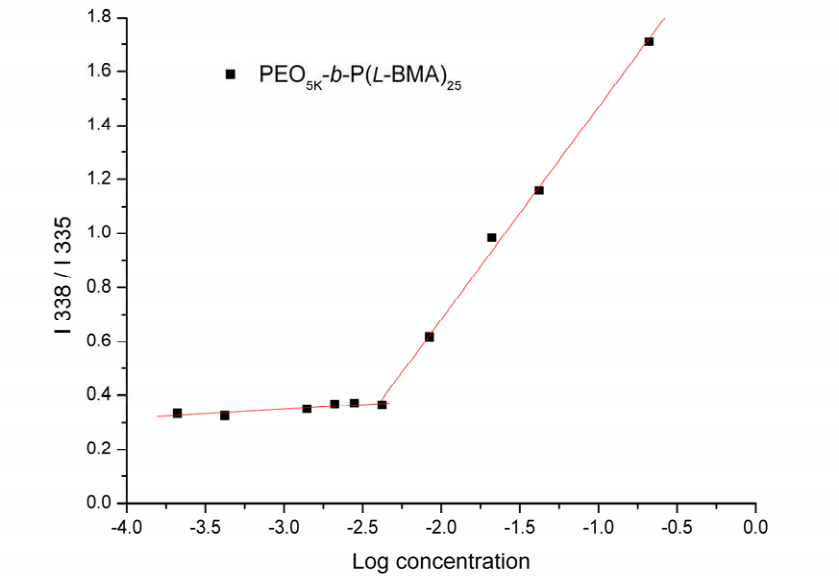

Figure S21. Concentration dependence of pyrene I_{338}/I_{335} intensity ratio for PEO_{112}-*b*-P(*L*-BMA)$_{25}$ in water at room temperature. (Diblock: M_n = 16 940 g.mol^{-1}, PDI = 1.03; and [pyrene]$_0$ = 6 x 10^{-7} M). Inflection point at 3.61 x 10^{-3} g.L^{-1}.

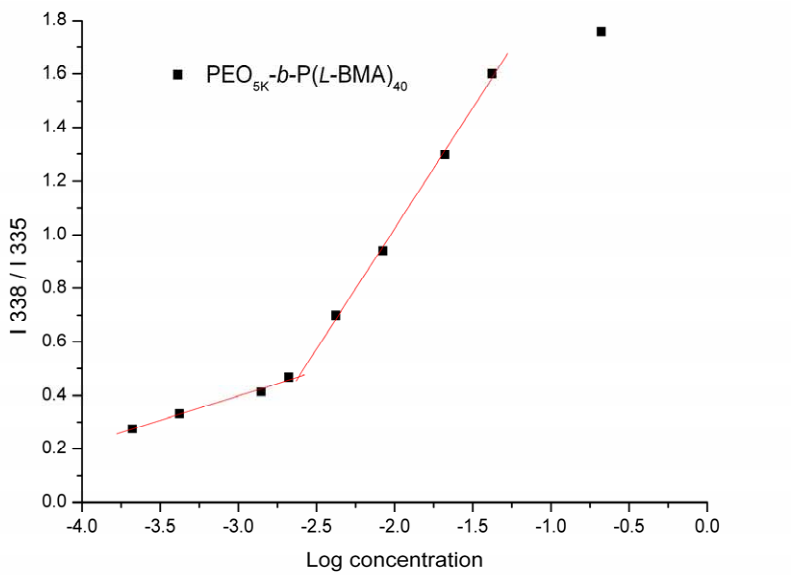

Figure S22. Concentration dependence of pyrene I_{338}/I_{335} intensity ratio for PEO_{112}-*b*-P(*L*-BMA)$_{40}$ in water at room temperature. (Diblock: M_n = 20 412 g.mol^{-1}, PDI = 1.03; and [pyrene]$_0$ = 6 x 10^{-7} M). Inflection point at 2.33 x 10^{-3} g.L^{-1}.

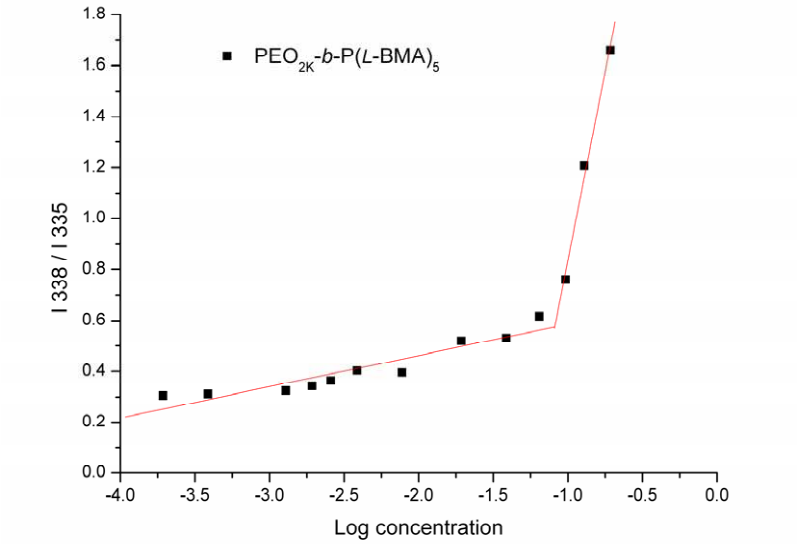

Figure S23. Concentration dependence of pyrene I_{338}/I_{335} intensity ratio for PEO_{45}-*b*-P(*L*-BMA)$_5$ in water at room temperature. (Diblock: M_n = 3 850 g.mol^{-1}, PDI = 1.04; and [pyrene]$_0$ = 6 x 10^{-7} M). Inflection point at 6.16 x 10^{-2} g.L^{-1}.

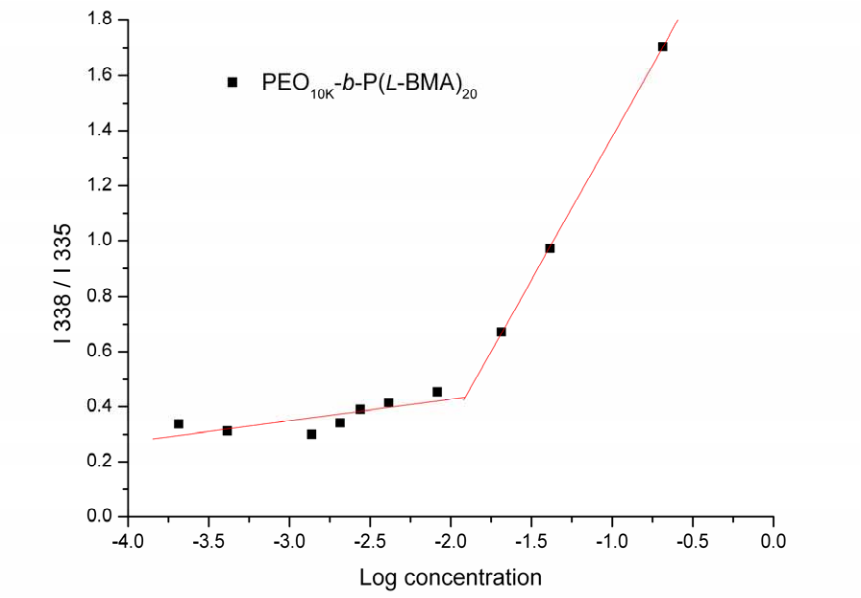

Figure S24. Concentration dependence of pyrene I_{338}/I_{335} intensity ratio for PEO_{227}-*b*-P(*L*-BMA)$_{20}$ in water at room temperature. (Diblock: M_n = 19 440 g.mol^{-1}, PDI = 1.03; and [pyrene]$_0$ = 6 x 10^{-7} M). Inflection point at 1.00 x 10^{-2} g.L^{-1}.

References

1) Pounder, R. J.; Dove, A. P. *Biomacromolecules* **2010**, *11*, 1930.

2) Pounder, R. J.; Fox, D. J.; Barker, I. A.; Bennison, M. J.; Dove, A. P. *Polym Chem.* In Press

3) Wilhelm, M.; Zhao, C. L.; Wang, Y.; Xu, R.; Winnik, M. A.; Mura, J. L.; Riess, G.; Croucher, M. D. *Macromolecules* **1991**, *24*, 1033.