

Supporting Information

Temperature responsive nanospheres with bicontinuous internal structures from a semi-crystalline amphiphilic block copolymer

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Contents

Page

S2 SI1 Experimental

S6 **Scheme S1:** Synthesis of PEO macroinitiator and PEO₃₉-*b*-PODMA₁₇ block copolymer.

Figure S1: ¹H NMR spectrum of the PEO macroinitiator in CDCl₃.

Figure S2: ¹³C NMR spectrum of the PEO macroinitiator in CDCl₃.

S7 **Figure S3 :** ¹H NMR spectrum of PEO₃₉-*b*-PODMA₁₇ in CDCl₃.

Figure S4: ¹³C NMR spectrum of PEO₃₉-*b*-PODMA₁₇ in CDCl₃.

S8 **Figure S5:** SEC traces of the PEO macroinitiator and PEO₃₉-*b*-ODMA₁₇.

Table S1: Molecular weight parameters of PEO and PEO₃₉-*b*-PODMA₁₇.

S9 SI2 DSC

Figure S6: DSC scans of PEO₃₉-*b*-PODMA₁₇ (in bulk) measured during 1st heating and 2nd heating runs. Inset, crystallisation during cooling.

Discussion of DSC Results

S10 SI3 DLS

Figure S7: Particle diameters of PEO₃₉-*b*-PODMA₁₇ aggregates taken with increasing temperature.

S11 SI4 CryoTEM

Figure S8: cryoTEM of 1wt % solution of PEO₃₉-*b*-PODMA₁₇ aggregates at 4°C

Figure S9: cryoTEM of 1wt % solution of PEO₃₉-*b*-PODMA₁₇ aggregate at 45°C

S12 **Figure S10:** cryoTEM images of 5wt % solution of PEO₃₉-*b*-PODMA₁₇ aggregates vitrified at different temperatures.

S13 **Figure S11:** gallery of cryoTEM images of 5wt % solution of PEO₃₉-*b*-PODMA₁₇ aggregates vitrified at 4°C.

S14 **Figure S12:** gallery of cryoTEM images of 5wt % solution of PEO₃₉-*b*-PODMA₁₇ aggregates vitrified at 22°C.

Supporting Information

- S15** **Figure S13:** gallery of cryoTEM images of 5wt % solution of PEO₃₉-*b*-PODMA₁₇ aggregates vitrified at 45°C.
- S16** **Figure S14:** cryoET of a 5wt % solution of PEO₃₉-*b*-PODMA₁₇ aggregates at 45°C
- S17** **Figure S15:** cryoET of 5wt % solution of PEO₃₉-*b*-PODMA₁₇ aggregates at 4°C
- S18** **SI5 Incorporation of pyrene**
Figure S16: Negative staining TEM pictures of PEO₃₉-*b*-PODMA₁₇ aggregates: a) and b) without pyrene; c) and d) with pyrene.
- S19** **Figure S17:** Fluorescence spectra at various temperatures for pyrene encapsulated in PEO₃₉-*b*-PODMA₁₇ micelles with significant fluorescence peaks labelled.
Figure S18: Variation with temperature of ratios of the I_{III}:I_I (I_{Em383}/I_{Em372}) bands and the I_{exc}:I_{mon} (I_{Ex483}/I_{Mon372}) bands of pyrene encapsulated in PEO₃₉-*b*-PODMA₁₇ micelles.
- S20** **References**

Supporting Information

SI1 Experimental

Materials and Apparatus

Poly (ethylene glycol) methyl ether (molecular weights ca. 2000 g mol⁻¹), 2-bromoisobutyryl bromide (98%), dimethylamino pyridine (99%), triethylamine (99%), octadecyl methacrylate (ODMA) and copper (I) bromide (98%) were all purchased without further purification from Sigma-Aldrich. Aluminium oxide (activated, neutral, for column chromatography 50-200 μm) and magnesium sulphate (97%, anhydrous) were purchased from Acros Organics. Sodium bicarbonate (analytical reagent grade) was purchased from Fisher Scientific. Hydrochloric acid (35.4%; sp. gr. 1.18) was purchased from BDH Chemicals. N-(n-octyl)-2-pyridyl(methanime) was synthesised according to literature¹.

Methanol (analytical reagent grade), tetrahydrofuran (analytical reagent grade), isopropanol, ethanol (analytical reagent) and water (HPLC gradient grade) were all purchased from Fisher Scientific. Dichloromethane (analytical reagent grade) was purchased from Fisher Scientific, and before use was dried and distilled over calcium hydride. σ xylene was purchased from BDH Lab Supplies. The deuterated solvent D₁-chloroform (99.8%) was used as received from Cambridge Isotope Laboratories Incorporated.

Characterisation

¹H NMR spectra were recorded using a JEOL GX-270 FT spectrometer (270 MHz) at 25°C in solutions of deuterated chloroform (CDCl₃). ¹H NMR was used to ascertain structure and follow monomer conversion of the polymers. Molecular weight averages were calculated via size exclusion chromatography (SEC) using two 5 μm mixed C PLgel columns at 40°C, calibrated using poly(methyl methacrylate) standards. The samples in THF were detected by a Shodex RI-101 refractive index detector. Infra-red spectra were collected using a Thermo Nicolet Avatar 360 FT-IR spectrometer.

Thermal Analysis

A Perkin Elmer differential scanning calorimeter (PE DSC 7) calibrated with Indium (mp = 156.1, ΔH = 28.3 J/g) was used to determine the thermal energies of the block copolymers, at a scanning rate of 10°C/min. The temperatures for melting and crystallisation were determined from the peak maxima of the heating and cooling curves.

Dialysis

10mg of block copolymer was dissolved in 4ml THF and left stirring in an oil bath at 35°C. 6ml water was added drop-wise to the stirring solution over 90mins. The solution was transferred to dialysis tubing, sealed and immersed in 3 L of stirring 35°C deionised water for 24 hours to displace the THF. During this time, the water was replaced twice. For fluorescence studies the block copolymer was dissolved in a 5x10⁻⁵ M solution of pyrene in THF, and the method followed as stated above.

Fluorescence

Excitation and emission spectra were collected using a Varian CARY Eclipse fluorescence spectrophotometer and a Perkin Elmer LS 50 B luminescence spectrometer.

Optical and Size Measurements

Transmission electron microscopy (TEM) was carried out using a JEOL JEM (200-FX) machine, operating at 120kV. 20 μl of the dialysed sample was deposited onto a carbon-covered copper grid, left for 30s and removed via suction. The grid was then stained with a

Supporting Information

solution of 5% uranyl acetate and 1% acetic acid. 20 μ l of this solution was deposited on the grid and removed after 5s. Excess solution was dabbed away using filter paper. Dynamic light scattering (DLS) measurements were carried out on a Malvern High Performance Particle Sizer (HPPS HPP5001) with a laser at a wavelength of 633nm. 1ml of the dialysed solution was taken, filtered using a 1.2 μ m filter and placed in a clean cuvette. The desired temperature was set and the sample left at this temperature for 15mins before the runs were conducted. At each temperature ten size readings were obtained and an average of these taken.

CryoTEM

Sample vitrification was carried out on an automated vitrification robot (FEI VitrobotTM Mark III) for plunging in liquid ethane. CryoTEM Cu R2/2 Quantifoil Jena Grids (Quantifoil Micro tools GmbH) were surface plasma treated using a Cressington 208 carbon coater prior to use. For vitrification, 3 μ l/ml of PEO₃₉-*b*-PODMA₁₇ (1 mg/ml in water), equilibrated to 4 °C or to 45 °C, was applied to the cryoTEM grids inside the vitrobot chamber which was conditioned to 100 % humidity and 4 °C or 45 °C.

For 2D imaging and tomography, samples were studied on the TU/e CryoTitan (FEI, www.cryotem.nl), equipped with a with a field emission gun (FEG) operating at 300 kV. Images were recorded using a 2k x 2k Gatan CCD camera equipped with a post column Gatan Energy Filter (GIF).

The 3-dimensional reconstructions were preformed with the software Inspect 3D v.3.0 (FEI Company). For the segmentation and visualization of the 3D volume, Amira 4.1.0 (Mercury Computer Systems) was used.

Tomography conditions:

1wt % solution PEO₃₉-*b*-PODMA₁₇

PEO₃₉-*b*-PODMA₁₇ at 4 °C:

From -70° to +70°, with 2° increments from 0° to +45 and to -45° and 1° increment from +45°/-45° to +70°/- 70°.

Magnification 19000x.

Defocus -5 μ m

Total dose = 100 e⁻.Å⁻²

PEO₃₉-*b*-PODMA₁₇ at 45 °C:

From -70° to +67°, with 2° increments from 0° to +45 and to -45° and 1° increment from +45°/-45° to +67°/- 70°.

Magnification 15000x.

Defocus -5 μ m

Total dose = 100 e⁻.Å⁻²

5wt % solution PEO₃₉-*b*-PODMA₁₇

PEO₃₉-*b*-PODMA₁₇ at 4 °C:

From -65° to +65°, with 1.5° increments.

Magnification 19000x.

Defocus -10 μ m

Total dose = 40 e⁻.Å⁻²

PEO₃₉-*b*-PODMA₁₇ at 45 °C:

From -66° to +66°, with 1.5° increments.

Magnification 11500x.

Defocus -15 μ m

Total dose = 40 e⁻.Å⁻²

Supporting Information

Syntheses

Preparation of Ethylene Glycol Methyl 2-Bromo 2-Methyl Propanoate (PEO macroinitiator)

A modification of a literature method was followed.² A solution of PEGME M_n ca. 2000 g mol^{-1} (10g, 5 mmol) in dichloromethane was added dropwise to a stirred mixture of 2-bromoisobutyl bromide (1.24ml, 10 mmol), triethylamine (1.4ml, 10 mmol), and dimethylamino pyridine (1.22g, 10 mmol) in dichloromethane at 0°C for 1h under nitrogen. The solution was stirred for a further 18 h at room temperature. A third of the reaction solvent was evaporated off and the resultant yellow precipitate was filtered off. The remaining solution was made up to 100ml with dichloromethane and transferred to a separating funnel. The solution was washed several times with a saturated sodium bicarbonate solution and then with a 10% hydrochloric acid solution. The organic layer was collected and dried over anhydrous magnesium sulphate for an hour, filtered and the dichloromethane evaporated off. Finally, the resultant yellow solid was left in the vacuum oven at 50°C for two hours.

The structure was confirmed using ^1H , ^{13}C NMR and Fourier Transform Infrared (FTIR) spectroscopy, and molecular weight parameters calculated using size exclusion chromatography (SEC).

^1H NMR (270 MHz, CDCl_3 , ppm) δ : 1.80 (singlet, 6H, $(\text{CH}_3)_2\text{C}-$), 3.36 (singlet, 3H, $-\text{OCH}_3$), 3.59 (broad peak, 4H $-\text{OCH}_2\text{CH}_2-$), 3.84 (triplet, 2H, $-\text{CH}_2\text{O}-$), 4.28 (triplet, 2H, $\text{O}=\text{COCH}_2-$). **^{13}C NMR** (270 MHz, CDCl_3 , ppm) δ : 30.75 [$(\text{CH}_3)_2$], 55.69 (C-Br), 59.03 ($-\text{OCH}_3$), 65.13 ($\text{O}=\text{COCH}_2-$), 68.73 ($-\text{CH}_2\text{O}-$), 70.57 ($-\text{CH}_2\text{CH}_2\text{O}-$), 71.92 ($-\text{CH}_2-\text{OCH}_3$), 171.59 ($-\text{C}=\text{O}$). **FTIR** (cm^{-1}): 2882.9 (strong peak C-H stretches), 1734.5 (moderate peak $\text{C}=\text{O}$), 1146.6 (weak peak C-O ester stretch), 1099.9 (strong peak C-O ether stretches), 528.3 (weak peak C-Br).

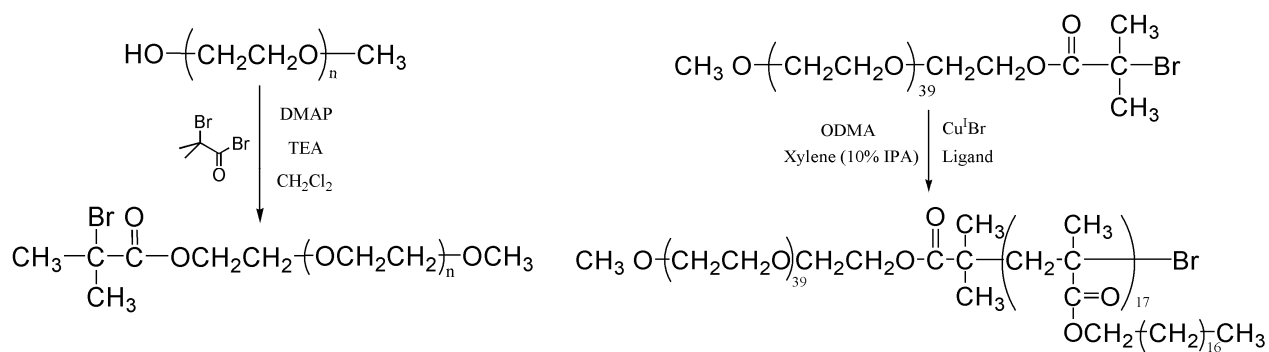
Preparation of PEO_{39-b}-ODMA₁₇

The block copolymer was synthesised via ATRP³ as follows: The PEO macroinitiator (0.84g, 0.44 mmol) was placed in a schlenk tube along with a magnetic stirrer, and dissolved in 2ml of a solution of xylene with 10% isopropanol. To this solution were added octadecyl methacrylate (also dissolved in 2 ml of the above solution) (2.5g, 7.4 mmol), N-(n-octyl)-2-pyridyl(methanimine) (0.19g, 0.88 mmol) and Cu(I)Br (22.5mg, 0.16 mmol). The schlenk tube was then sealed and the solution degassed with nitrogen for 30mins. The reaction mixture was placed in an oil bath at 95°C for 48 hours. The reaction mixture was then exposed to air, diluted in THF and run through an alumina column. The polymer was isolated from precipitation into methanol.

The structure was confirmed using ^1H , ^{13}C NMR and FTIR spectroscopy, and molecular weight parameters calculated using SEC.

^1H NMR (270 MHz, CDCl_3 , ppm) δ : 0.88 (broad peak, 3H, $-(\text{CH}_2)_{17}-\text{CH}_3$), 1.03-1.4 (broad peaks, $\text{CH}_3-\text{C}-\text{CH}_2-$ and $-\text{CH}_2-(\text{CH}_2)_{15}-$), 1.26 (broad peak, 30H $-(\text{CH}_2)_{15}-$), 1.53-2.10 (broad peaks, $\text{CH}_3-\text{C}-\text{CH}_2-$), 3.38 (singlet, 3H $-\text{OCH}_3$ PEO), 3.64 (broad peak, 4H $-(\text{OCH}_2\text{CH}_2)-$ PEO), 3.91 (broad peak, 2H, $\text{O}=\text{CO}-\text{CH}_2$). **^{13}C NMR** (CDCl_3 , ppm) δ : 14.05 ($-\text{CH}_3$); 18.26 ($\text{CH}_3-\text{C}-\text{CH}_2-$); 25.99 ($-\text{OCH}_2\text{CH}_2\text{CH}_2$); 28.12 ($-\text{OCH}_2\text{CH}_2-$); 29.35 ($-\text{CH}_2(\text{CH}_2)_{10}\text{CH}_2-$); 29.68 ($-(\text{CH}_2)_{10}-$); 31.86 ($-\text{CH}_2\text{CH}_2\text{CH}_3$); 64.92 ($-\text{OCH}_2-$); 70.51 ($-(\text{OCH}_2\text{CH}_2)-$). **FTIR** (cm^{-1}): 2916.0-2849.0 (strong peaks C-H stretches), 1726.5 (strong peak, $\text{C}=\text{O}$), 1146.0 (moderate peak C-O stretches).

Supporting Information



Scheme S1: Synthesis of PEO macroinitiator and PEO-PODMA block copolymer.

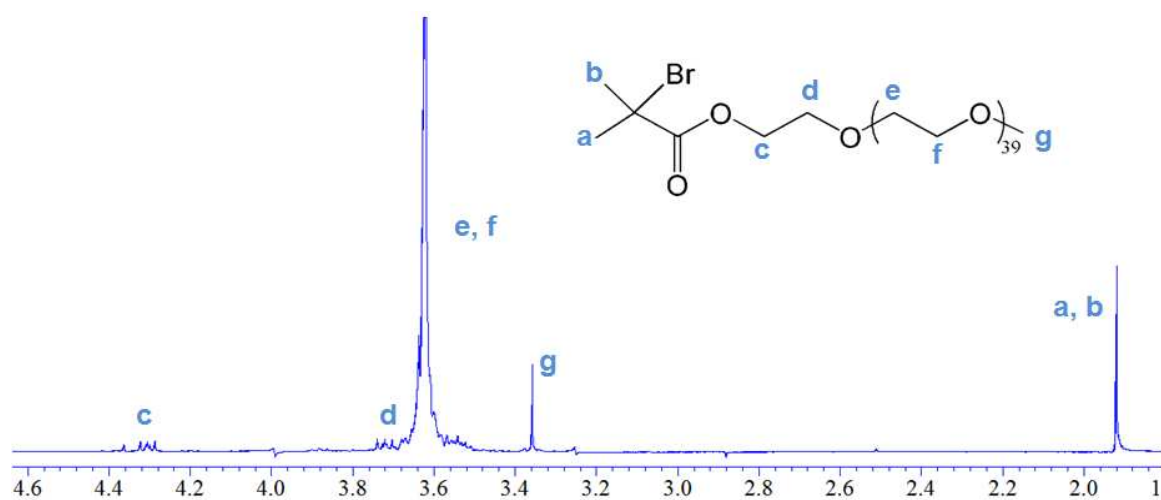


Figure S1: ^1H NMR spectrum of the PEO macroinitiator in CDCl_3 .

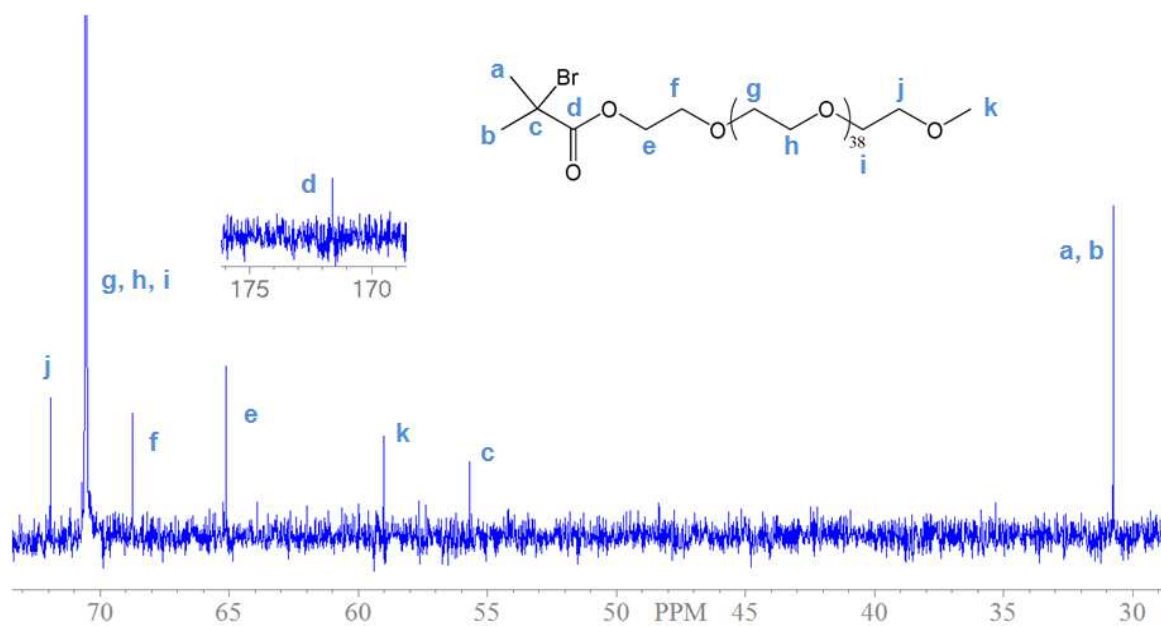


Figure S2: ^{13}C NMR spectrum of the PEO macroinitiator in CDCl_3 .

Supporting Information

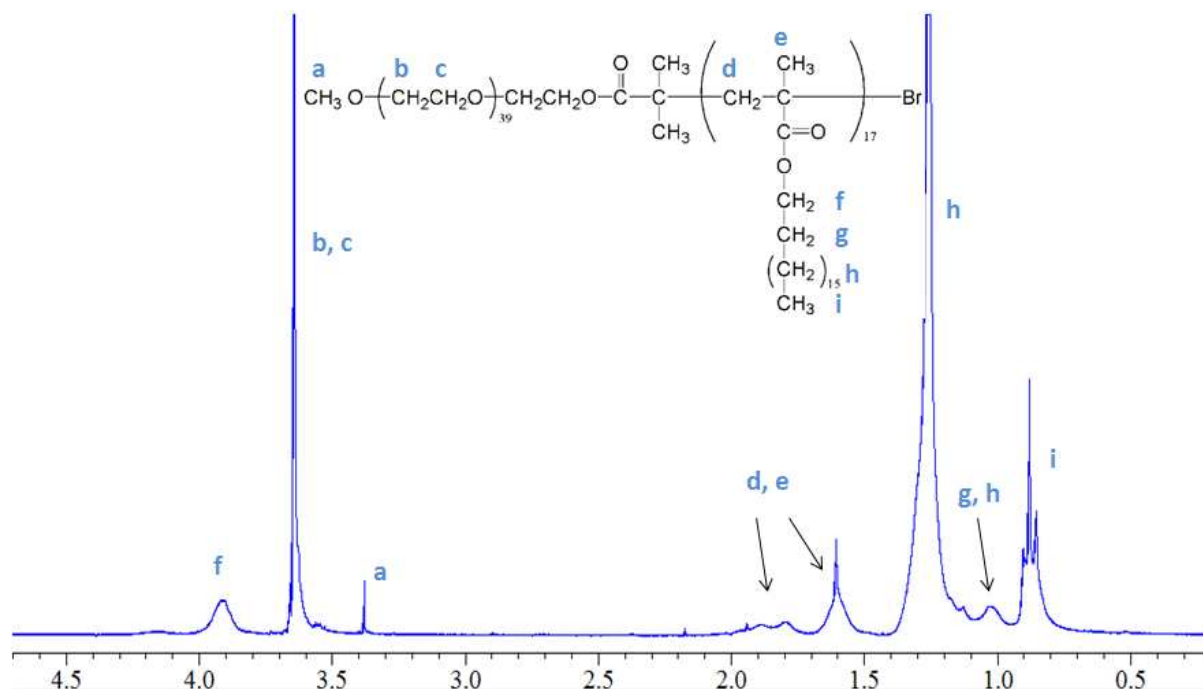


Figure S3 : ¹H NMR spectrum of PEO₃₉-b-PODMA₁₇ in CDCl₃.

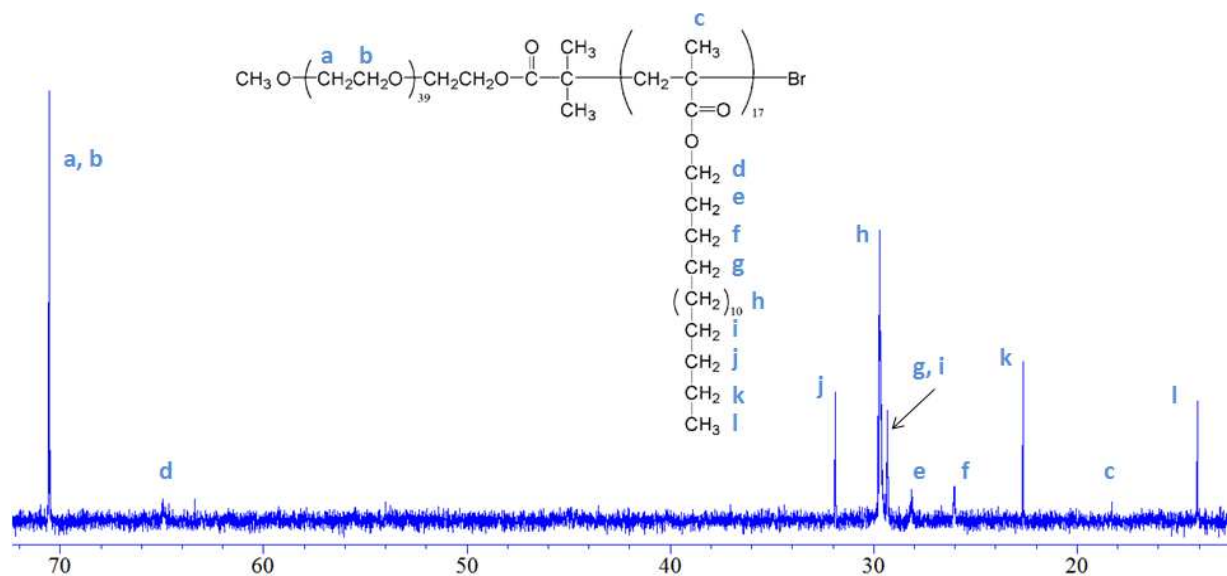


Figure S4: ¹³C NMR spectrum of PEO₃₉-b-PODMA₁₇ in CDCl₃.

Supporting Information

SI2 DSC

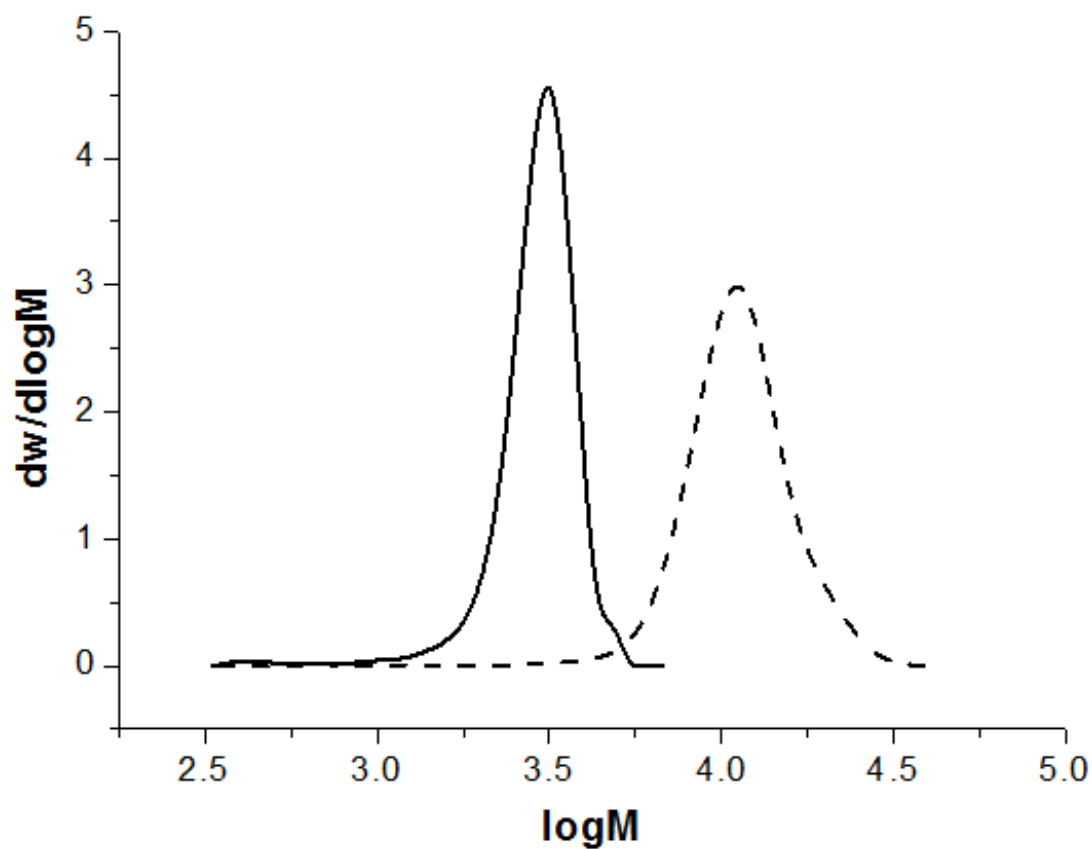


Figure S5: SEC traces of the PEO macroinitiator and PEO₃₉-*b*-ODMA₁₇.

Table S1: Molecular weight parameters of PEO and PEO₃₉-*b*-PODMA₁₇.

	M_n^a	M_n^b	M_w^b	M_w/M_n^b
PEO Macroinitiator	1,934	2,846	3,020	1.06
PEO₃₉-<i>b</i>-PODMA₁₇	7,680	10,754	11,937	1.11

a: calculated via ¹H NMR spectroscopy

b: calculated via SEC

Supporting Information

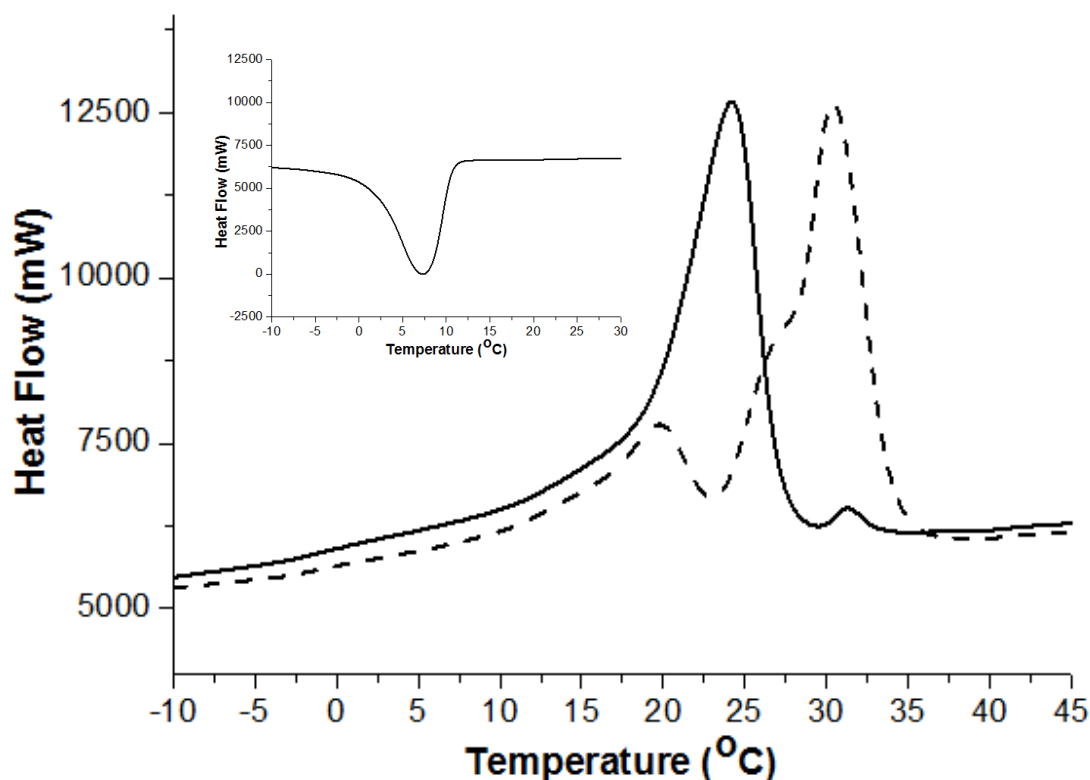


Figure S6: DSC scans of PEO₃₉-*b*-PODMA₁₇ (in bulk) measured during 1st heating and — 2nd heating runs. Inset, crystallisation during cooling (T_c)

Discussion of DSC Results

Thermal analysis of the polymer was carried out using DSC (Figure SI6). Analysis of the separate semi-crystalline components displayed melting transitions with $T_{\text{onset}} = 17.4^\circ\text{C}$ ($\Delta H = 39.7 \text{ Jg}^{-1}$) and 40.5°C ($\Delta H = 141.6 \text{ Jg}^{-1}$) for PODMA (DP=45) and the PEO macroinitiator (PEO = 39 units), respectively. Whereas the PEO transition is in agreement with literature values, the PODMA melts at a considerably lower value than reported for higher MW samples (e.g. $T_m = 39^\circ\text{C}$ for PODMA $M_n = 210,000$),^{4,5} most likely a consequence of its low molecular weight inhibiting extensive crystallisation.

The initial heating run revealed two melting transitions at 19.8°C and 30.4°C possibly corresponding to the PODMA and PEO blocks respectively. This is due to a temporary separation of the PEO and PODMA components of the block copolymer, probably induced by the precipitation of the polymer into methanol during its purification (PEO being more soluble in methanol than PODMA). This first heating run was therefore used to eradicate the thermal behaviour induced by environmental/ experimental exposure. The sample was then cooled at a rate of $-10^\circ\text{C}/\text{min}$ and crystallisation occurred at 7.3°C , which closely corresponds to the T_c of the PODMA₄₅ homopolymer at 8°C .

The second heating run for PEO₃₉-*b*-PODMA₁₇ showed two melting transitions: a large transition with $T_{\text{onset}} = 18.2$, ($\Delta H = 38.3 \text{ Jg}^{-1}$) and a very small transition with $T_{\text{onset}} = 29.9$, ($\Delta H = 0.45 \text{ Jg}^{-1}$) which were attributed to the PODMA and PEO blocks, respectively. The data for the PODMA block are very similar to those recorded of the pure component, indicating a microphase separated structure before the transition. In contrast, the low melting enthalpy recorded for the PEO block suggests that only a very small fraction of the PEO exists in a microphase separated state after PODMA melting. Hence the two polymer blocks become miscible above $\sim 18^\circ\text{C}$.

Supporting Information

SI3 DLS

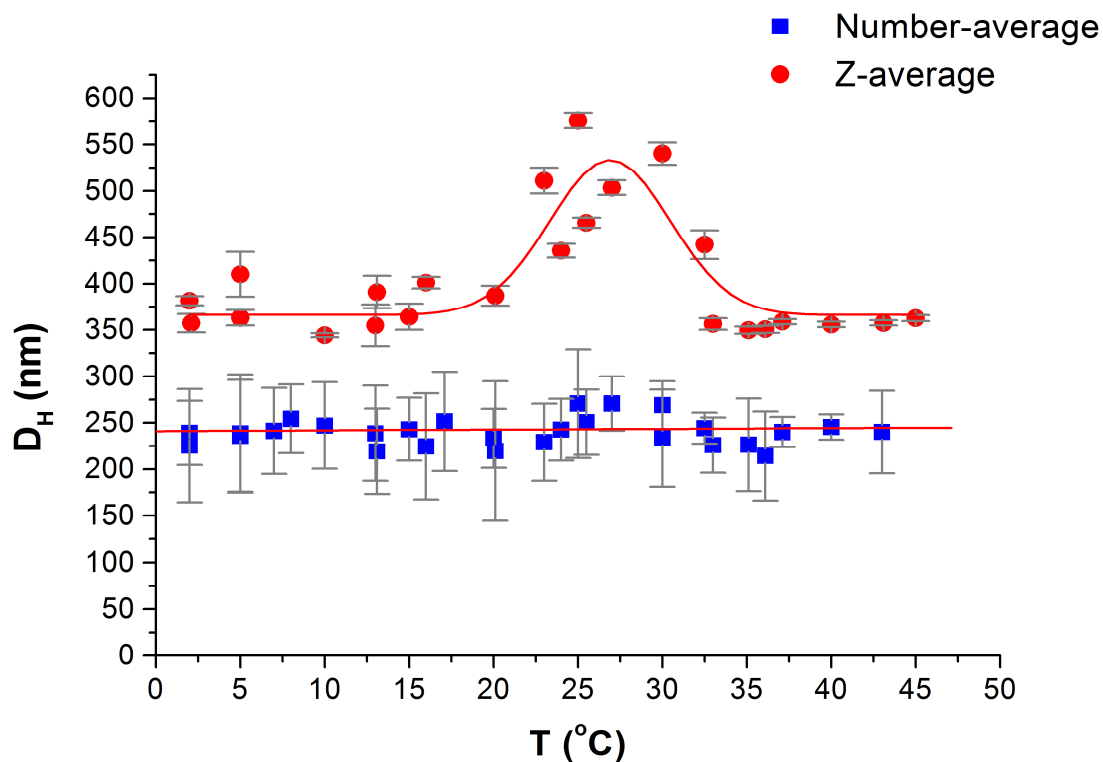


Figure S7: Number and z-average particle diameters of PEO₃₉-*b*-PODMA₁₇ aggregates taken with increasing temperature. Each size measurement was obtained from an average of ten readings at each temperature. Error bars represent the standard deviation of the hydrodynamic diameter (D_H) recorded at each temperature.

Supporting Information

SI4 CryoTEM

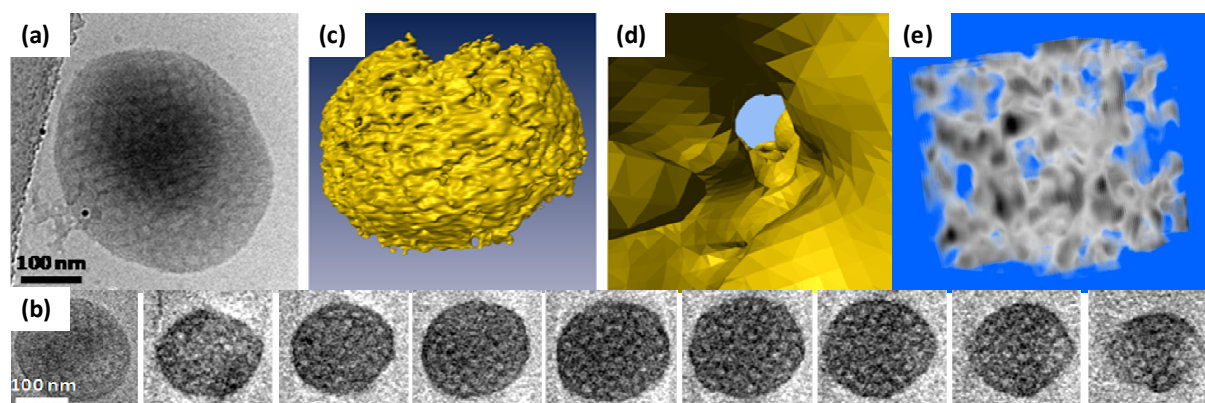


Figure S8: cryoTEM of 1wt % solution of $\text{PEO}_{39}\text{-}b\text{-PODMA}_{17}$ aggregates at 4°C : (a) 2D projection image of a larger aggregate; (b) gallery showing an 2D projection image (1st image) of a smaller aggregate and z slices of a 3D reconstruction of a tilt series of the same particle; (c-e) computer aided visualization of the reconstruction in (b) showing (c) the shape of the aggregate, (d) the connection of the internal structure with the surrounding solution and (e) the bicontinuous nature of the internal aggregate structure.

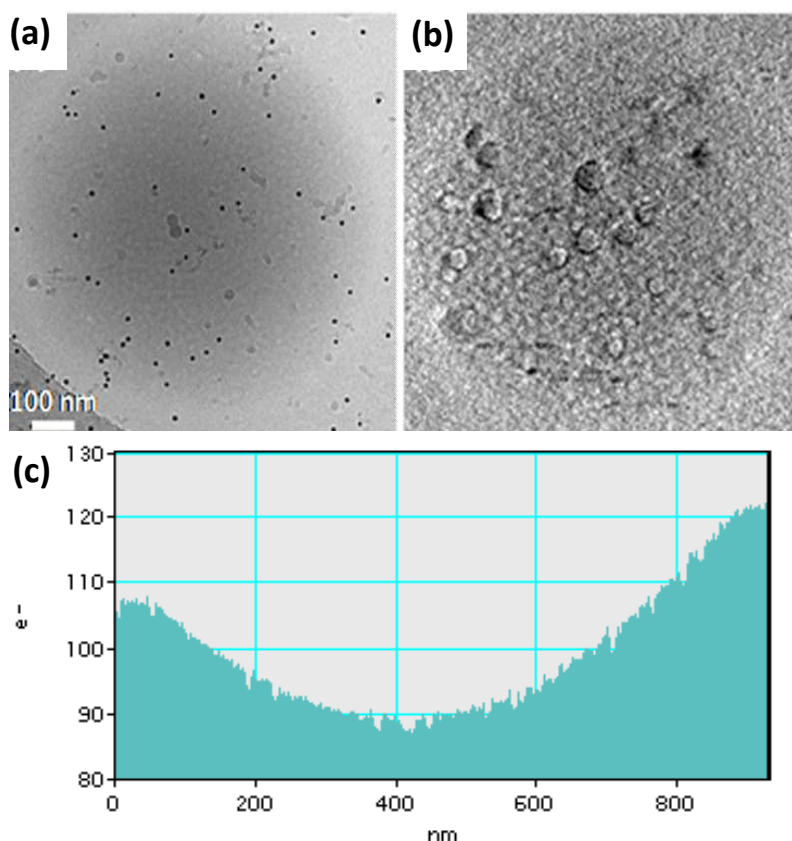


Figure S9: cryoTEM of 1wt % solution of $\text{PEO}_{39}\text{-}b\text{-PODMA}_{17}$ aggregate at 45°C: (a) 2D projection image of one of the aggregates, dark spots are gold nanoparticles used as fiducial markers for tomography; (b) z-slice of a 3D reconstruction of a tilt series of the same particle, showing the internal aggregate structure; (c) electron density profile through the aggregate in (a)

Supporting Information

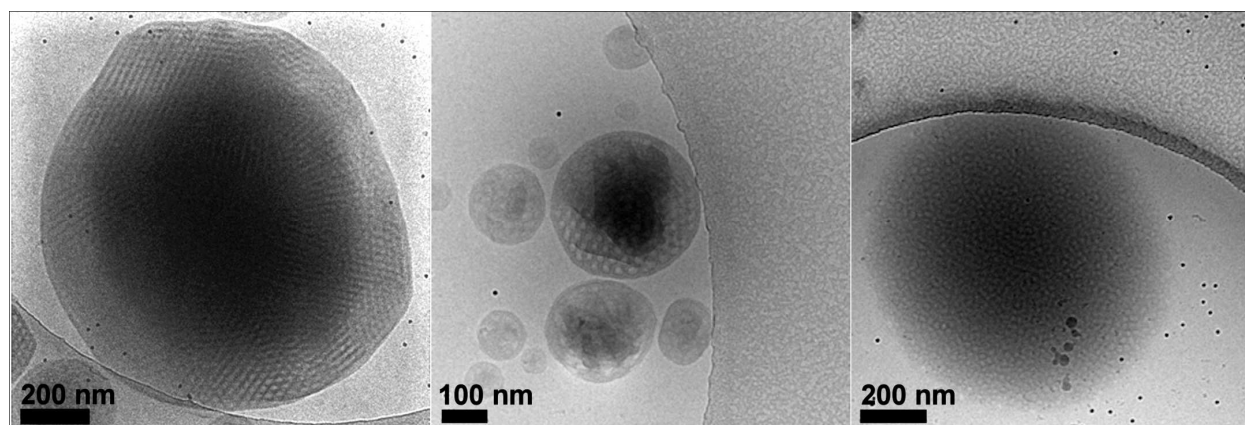


Figure S10: cryoTEM 2D projection images of 5wt % solution of PEO₃₉-*b*-PODMA₁₇ aggregates vitrified at (a) 4 °C, (b) 22 °C and (c) 45 °C.

Supporting Information

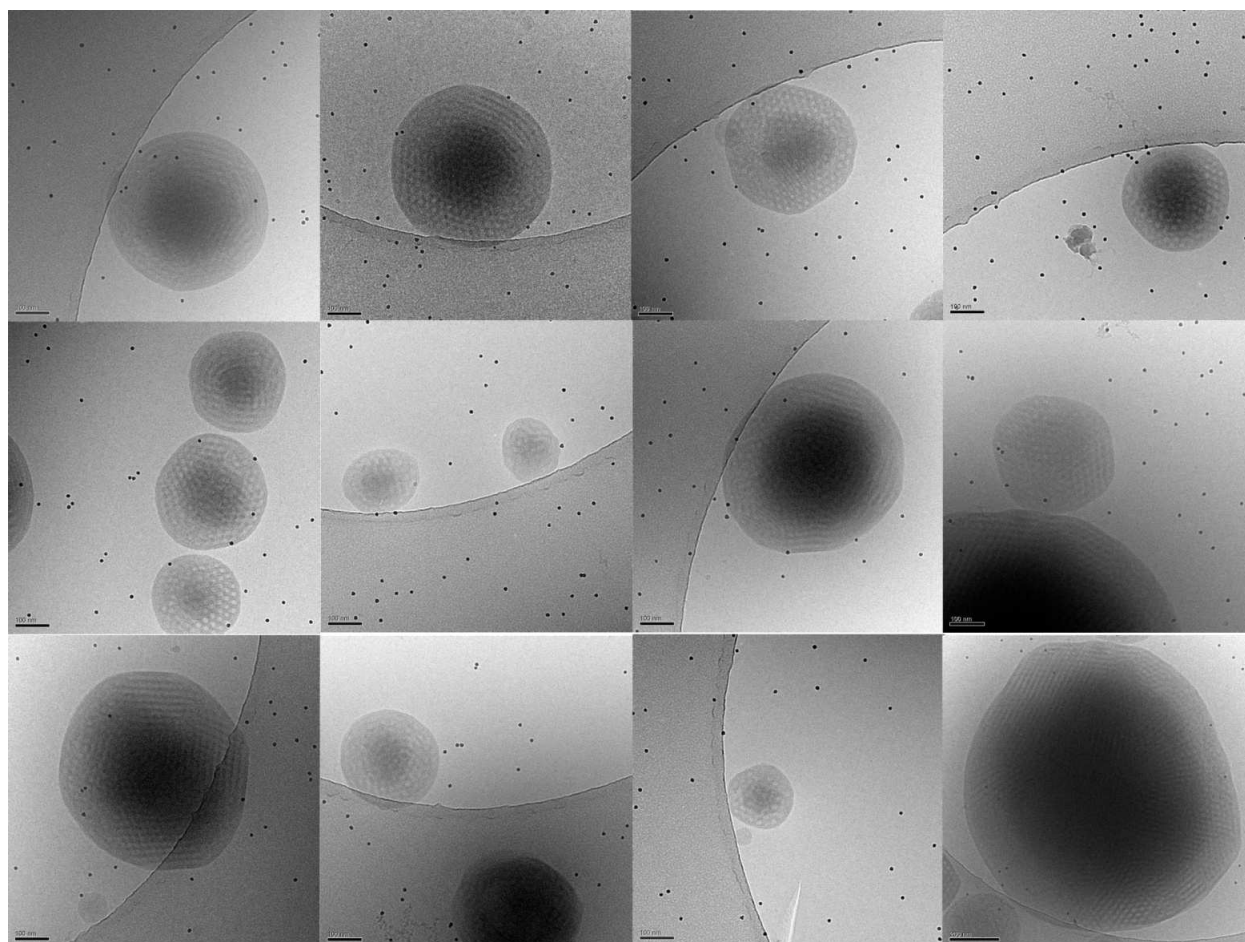


Figure S11: cryoTEM 2D projection images of 5wt % solution of PEO₃₉-*b*-PODMA₁₇ aggregates vitrified at 4 °C,

Supporting Information

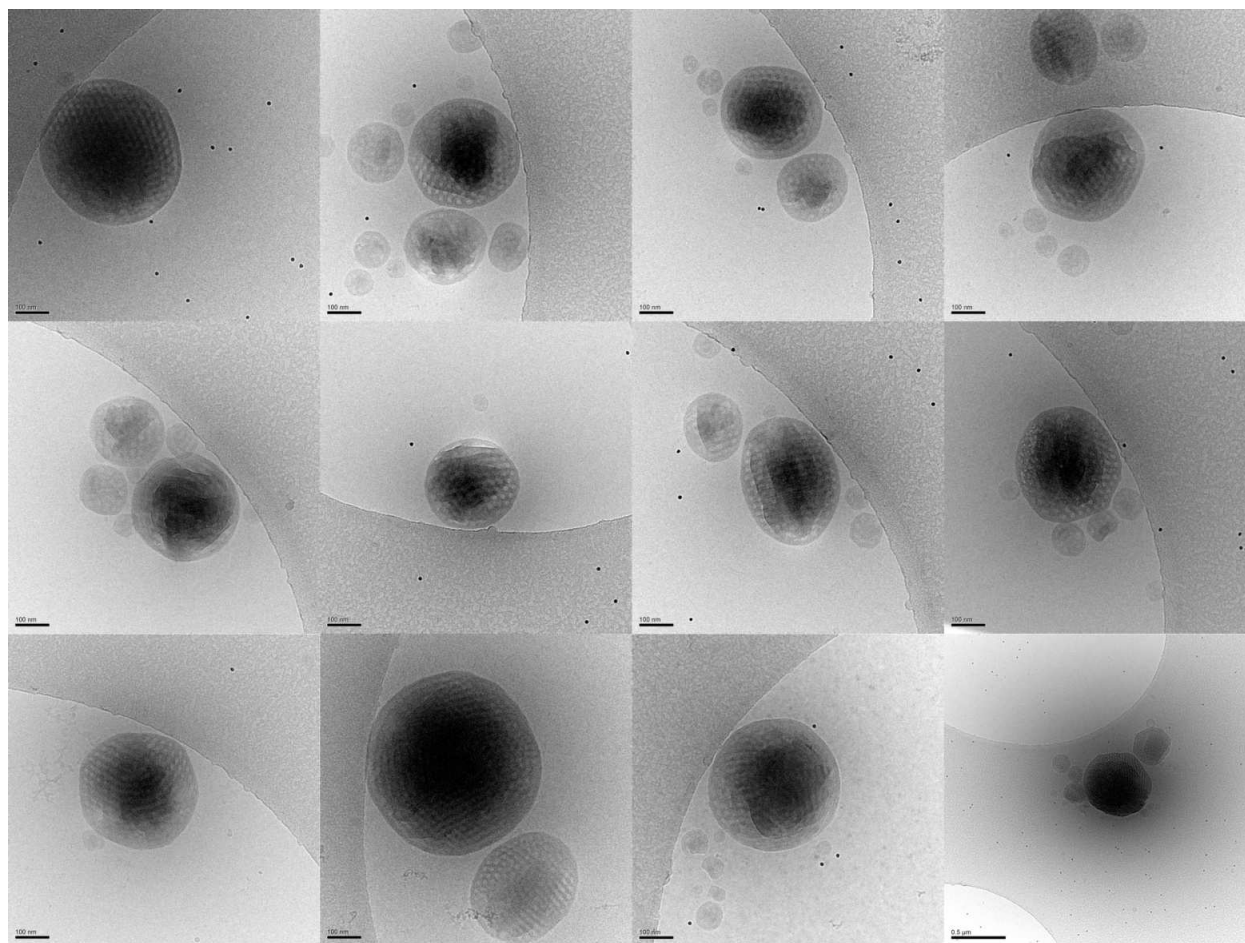


Figure S12: cryoTEM 2D projection images of 5wt % solution of PEO₃₉-*b*-PODMA₁₇ aggregates vitrified at 22 °C,

Supporting Information

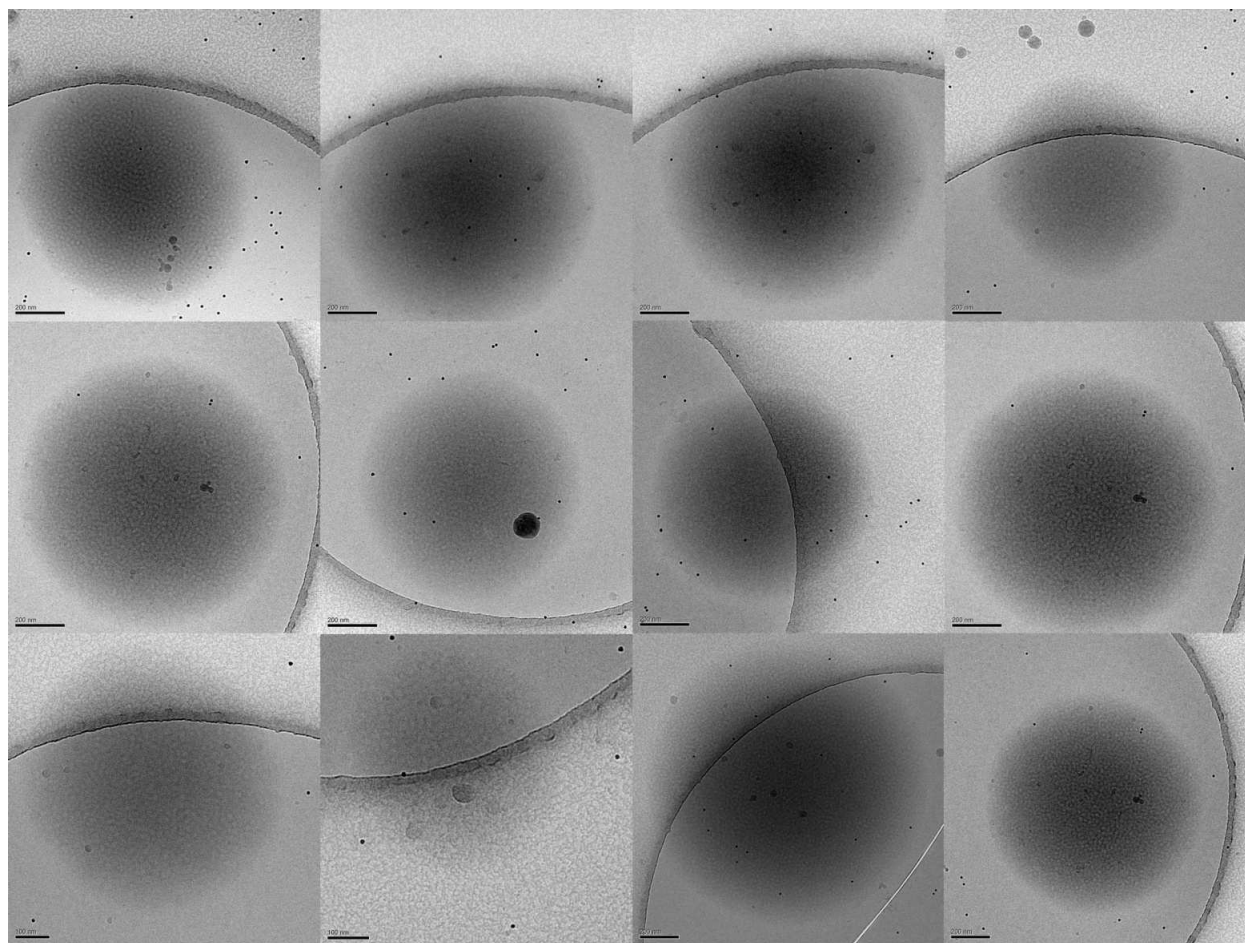


Figure S13: cryoTEM 2D projection images of 5wt % solution of PEO₃₉-*b*-PODMA₁₇ aggregates vitrified at 45 °C,

Supporting Information

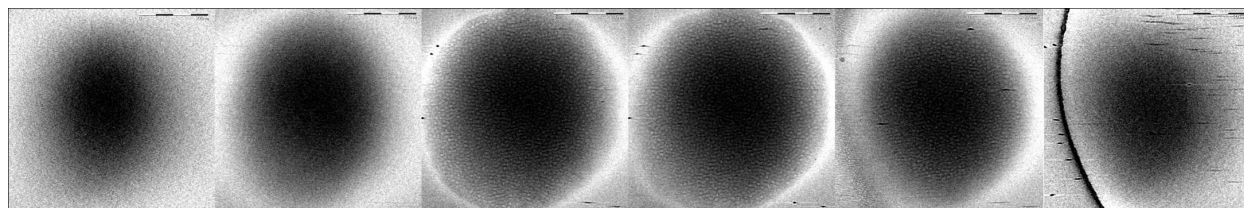


Figure S14: cryoET of a 5wt % solution of PEO₃₉-*b*-PODMA₁₇ aggregates at 45°C : gallery of z slices (top to bottom, left to right) of a 3D reconstruction of a tilt series of a particle showing its internal structure.

Supporting Information

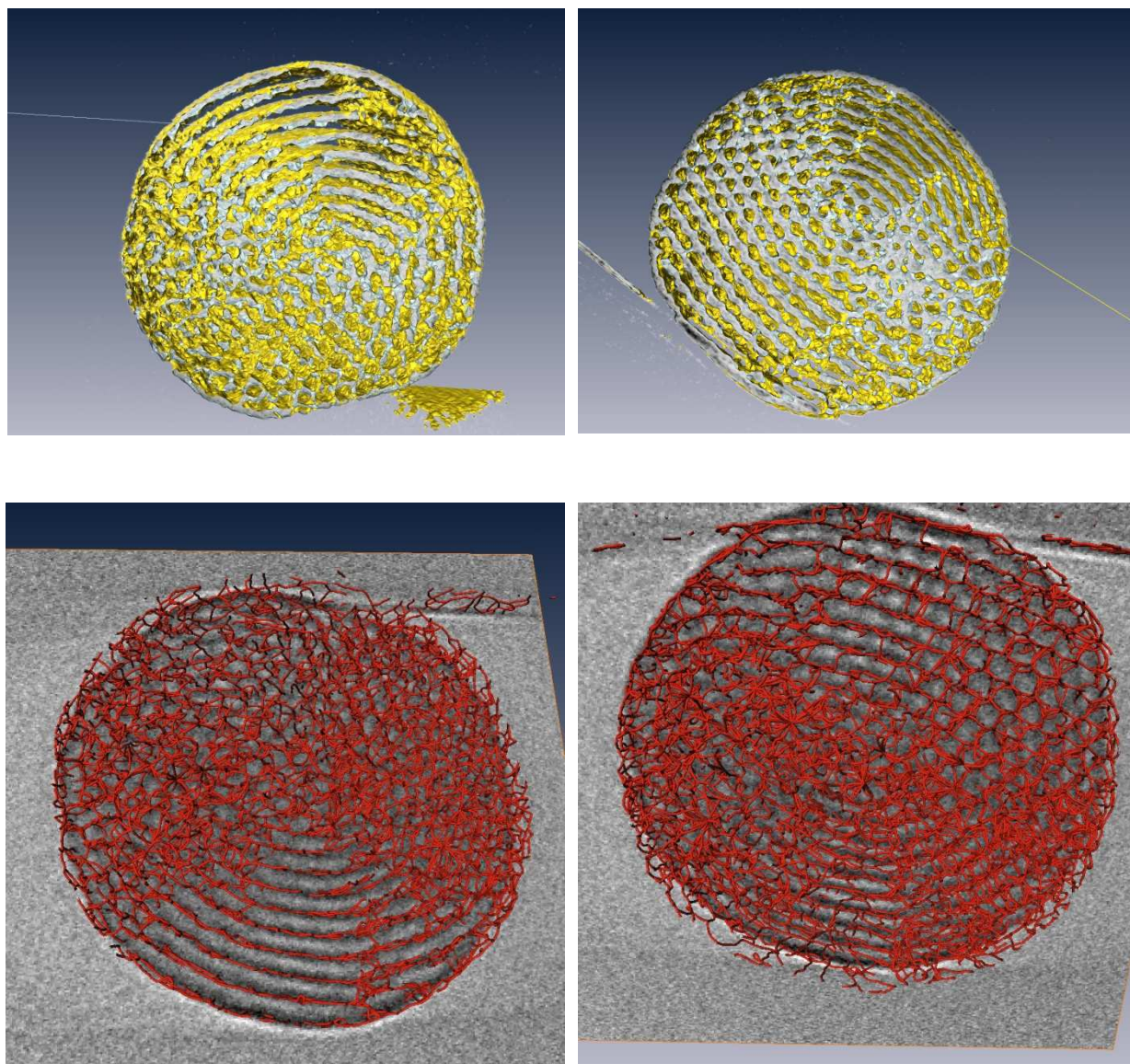


Figure S15: cryoET of 5wt % solution of PEO₃₉-*b*-PODMA₁₇ aggregates at 4°C: (top) computer aided visualization of a cryoelectron tomograms under different angles showing the coexistence of lamellar and bicontinuous regions; (bottom) overlays of a cross-section of the 3D reconstruction and the skeletonised view on the organic material (corresponding to the top images) showing the bicontinuous structure.

Supporting Information

SI5 Incorporation of pyrene

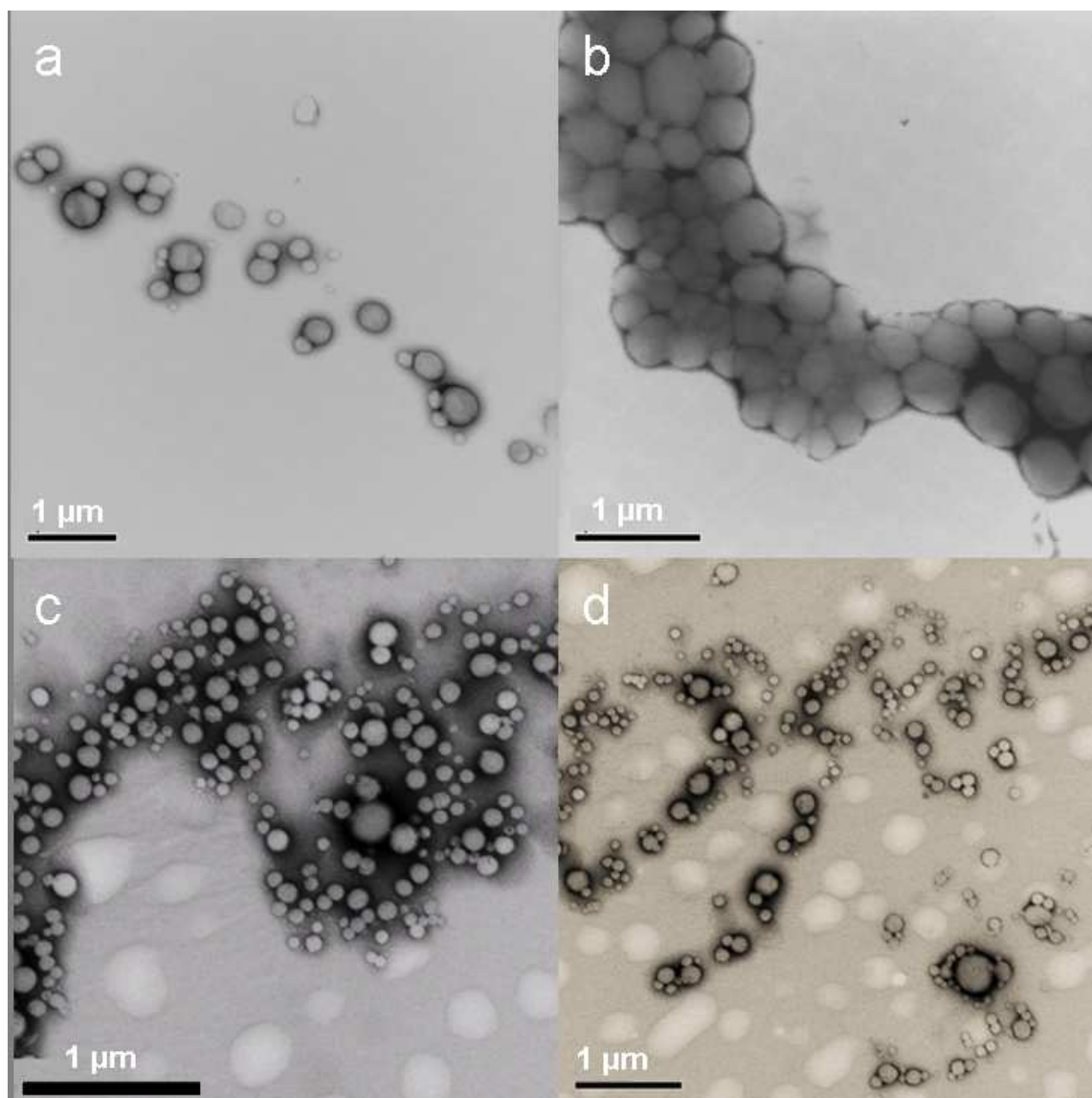


Figure S16: Negative staining TEM pictures of 1 wt% PEO₃₉-*b*-PODMA₁₇ aggregate solutions: (a) and (b) without pyrene; (c) and (d) with pyrene.

Supporting Information

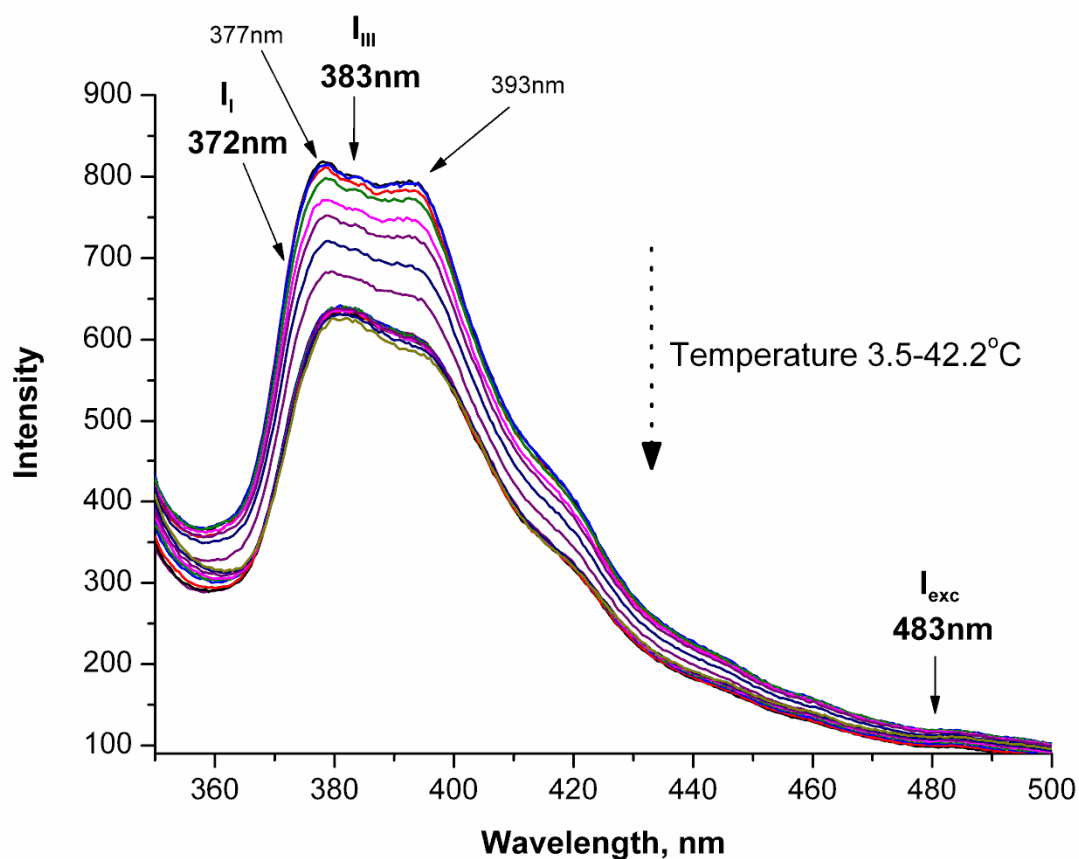


Figure S17: Fluorescence spectra at various temperatures for pyrene encapsulated in PEO₃₉-*b*-PODMA₁₇ micelles with significant fluorescence peaks labelled.

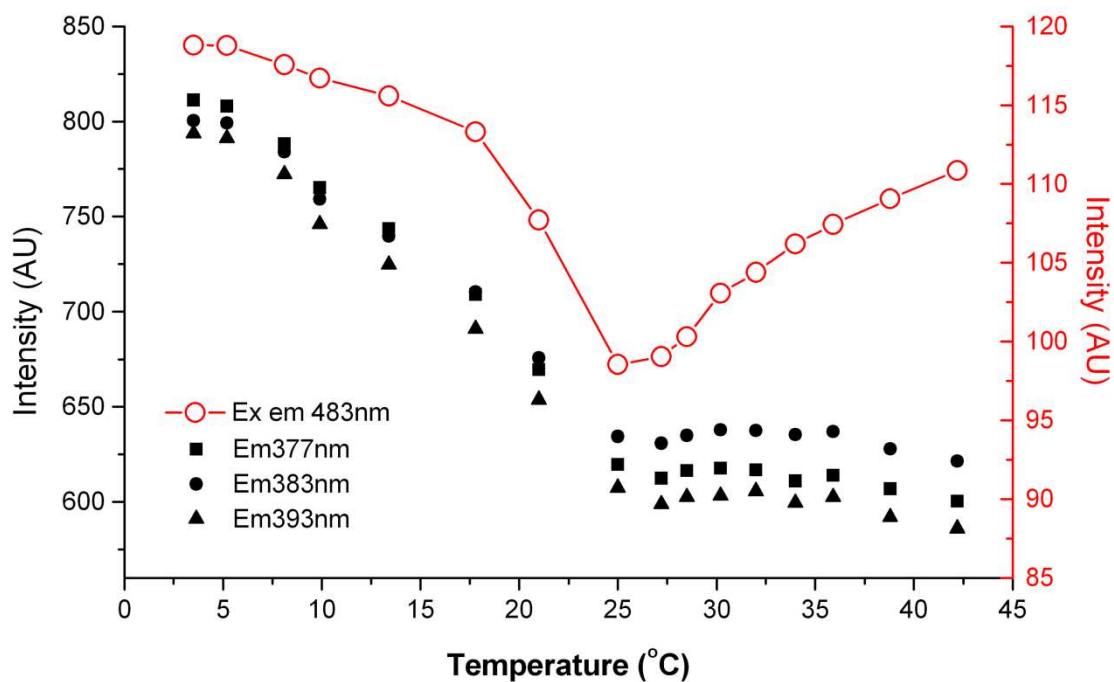


Figure S18: Variation with temperature of ratios of the $I_{III}:I_I$ (I_{Em383}/I_{Em372}) bands and the $I_{exc}:I_{mon}$ (I_{Ex483}/I_{Mon372}) bands of pyrene encapsulated in PEO₃₉-*b*-PODMA₁₇ micelles.

Supporting Information

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