Electronic Supplementary Information for

Semi-crystalline block copolymer bicontinuous nanospheres for thermoresponsive controlled release

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SI1 Experimental

Materials

The poly(ethylene oxide)-*block*-poly(octadecyl methacrylate) sample PEO_{45}-*b*-$PODMA_{20}$ was synthesised according to previously described procedures.

M_n = 8,950 (from ^1H NMR), M_w/M_n = 1.26 (from SEC versus polystyrene standards)

^1H NMR (270 MHz, $CDCl_3$, ppm) δ: 0.88 (broad peak, 3H, $–(CH_2)_{17}$-C**H**$_3$), 0.97-1.08 (broad peak, C**H**$_3$-C-CH_2-) 1.15-1.40 (-CH_2-(C**H**$_2$)$_{15}$-), 1.53-2.10 (broad peaks, CH_3-C-C**H**$_2$-), 3.38 (singlet, 3H –OC**H**$_3$ PEO), 3.60-3.70 (sharp peak, 4H –(OC**H**$_2$C**H**$_2$)- PEO), 3.85-4.00 (broad peak, 2**H**, CO_2-C**H**$_2$).
^{13}C NMR ($CDCl_3$, ppm) δ: 14.5 (-**C**H_3); 25.99 (-$CO_2CH_2CH_2$**C**H_2-); 28.12 (-CO_2CH_2**C**H_2-); 29.35 (-**C**$H_2(CH_2)_{10}$**C**H_2-); 29.68 (-(**C**$H_2)_{10}$-); 31.86 (-C-**C**H_3); 64.92 (-O**C**H_2-); 70.51 (-(O**C**H_2**C**$H_2)_n$-).
FTIR (cm^{-1}): 2916.0-2849.0 (strong peaks C-H stretches), 1726.5 (strong peak, C=O), 1146.0 (moderate peak C-O stretches).

Tetrahydrofuran (analytical reagent grade) and water (HPLC gradient grade) were purchased from Fisher Scientific; the HPLC grade water was filtered through a PTFE syringe filter (0.45μm) before use. For bulk dialysis reverse osmosis water was used.

Dialysis

Preparation of block copolymer aggregates: The PEO_{45}-*b*-$PODMA_{20}$ (10mg) was dissolved in THF (8ml) and the solution was filtered using a PTFE syringe filter (0.45μm). HPLC grade water (2ml) was added drop-wise to the stirred solution at 10°C, over 90 minutes. The solution was transferred to a dialysis chamber (Quxisep 5ml dialyzer with a Visking dialysis membrane, cut-off 15,000 Daltons), sealed and immersed in 3 L of stirring 10°C deionised water for 3 days to displace the THF. During this time, the water was replaced three times.

Preparation of block copolymer aggregates with encapsulated pyrene: Pyrene (30mg, 1.5mmol) and PEO_{45}-b-$PODMA_{20}$ was dissolved in THF (8ml) and the solution was filtered using a 0.45μm PTFE syringe filter. HPLC grade water (2ml) was added drop-wise to the stirred solution at 10°C, over 90 minutes. The solution was transferred to a dialysis chamber (Quixsep 5ml dialyzer with a Visking dialysis membrane, cut-off 15,000 Daltons), sealed and immersed in 2 L of stirring 10°C deionised water for 3 days to displace the THF. During this time, the water was replaced three times.

Preparation of Pyrene in Water Suspension: Preparation as above (Preparation of block copolymer aggregates with encapsulated pyrene) but with no PEO-*b*-PODMA.

Construction of Pyrene Calibration Curve

Pyrene (100mg, 0.494 mmol) was dissolved in RO water (2L) overnight. This solution (10ml) was serially diluted by 50% repeatedly to obtain a series of solutions with concnetratins ranging from 0.247 mmol dm^{-3} to 9.70 x 10^{-8} mmol dm^{-3}. Fluorescence measurements of these solutions (Figure SI 1) enabled the construction of a calibration curve (Figure SI 2).

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. 20µl 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 solution of 5% uranyl acetate and 1% acetic acid. 20µl of this solution was deposited on the grid and removed after 5s. Excess solution was dabbed away using filter paper.

For cryoTEM analysis sample vitrification was carried out on an automated vitrification robot (FEI Vitrobot™ 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 µl/ml of PEO_{39}-*b*-$PODMA_{17}$ (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. 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).

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 µm 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.

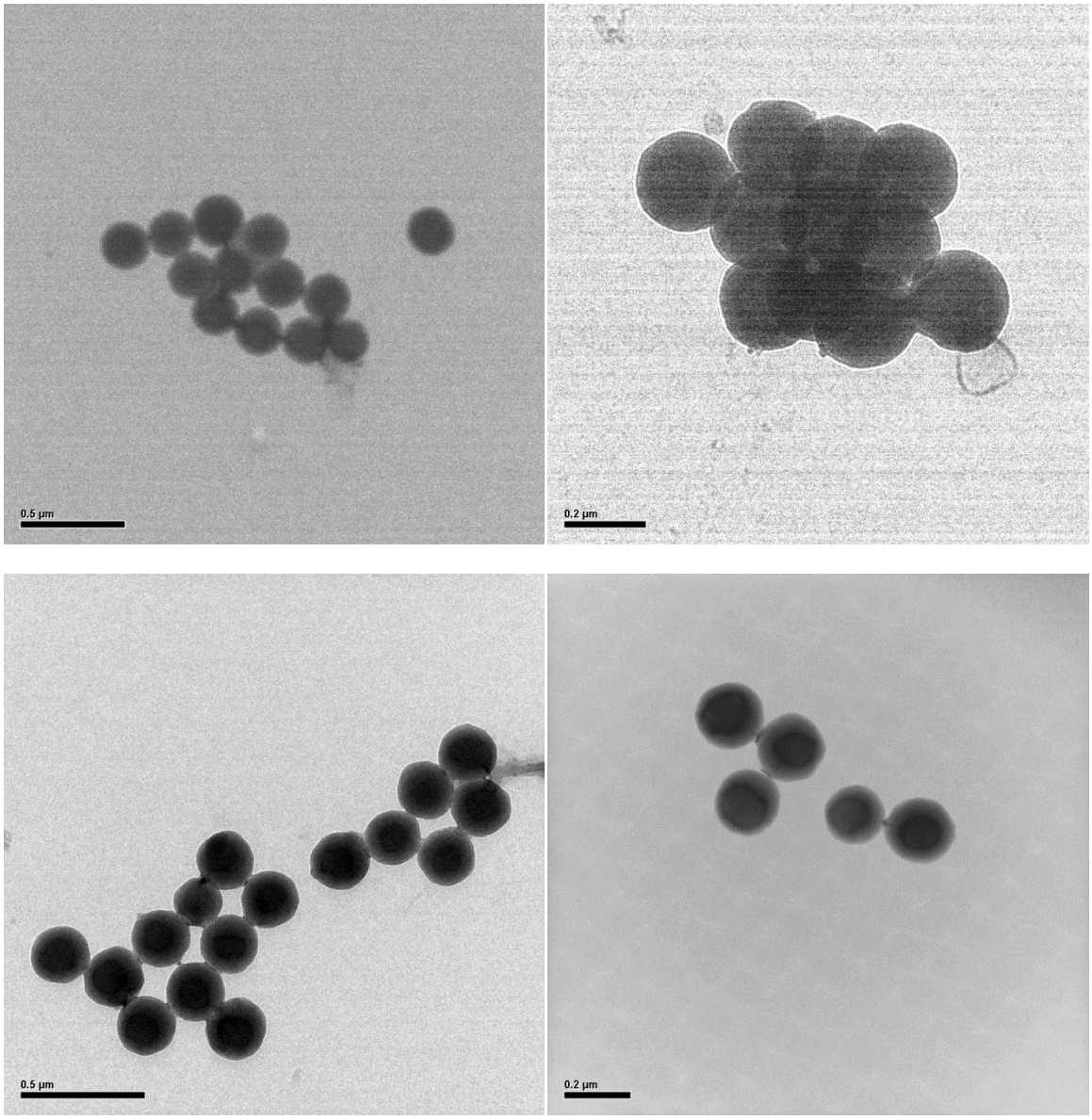

Figure SI 1: TEM micrographs of PEO-*b*-PODMA bicontinuous nanospheres prepared at 10°C at 0.1 wt% in water.

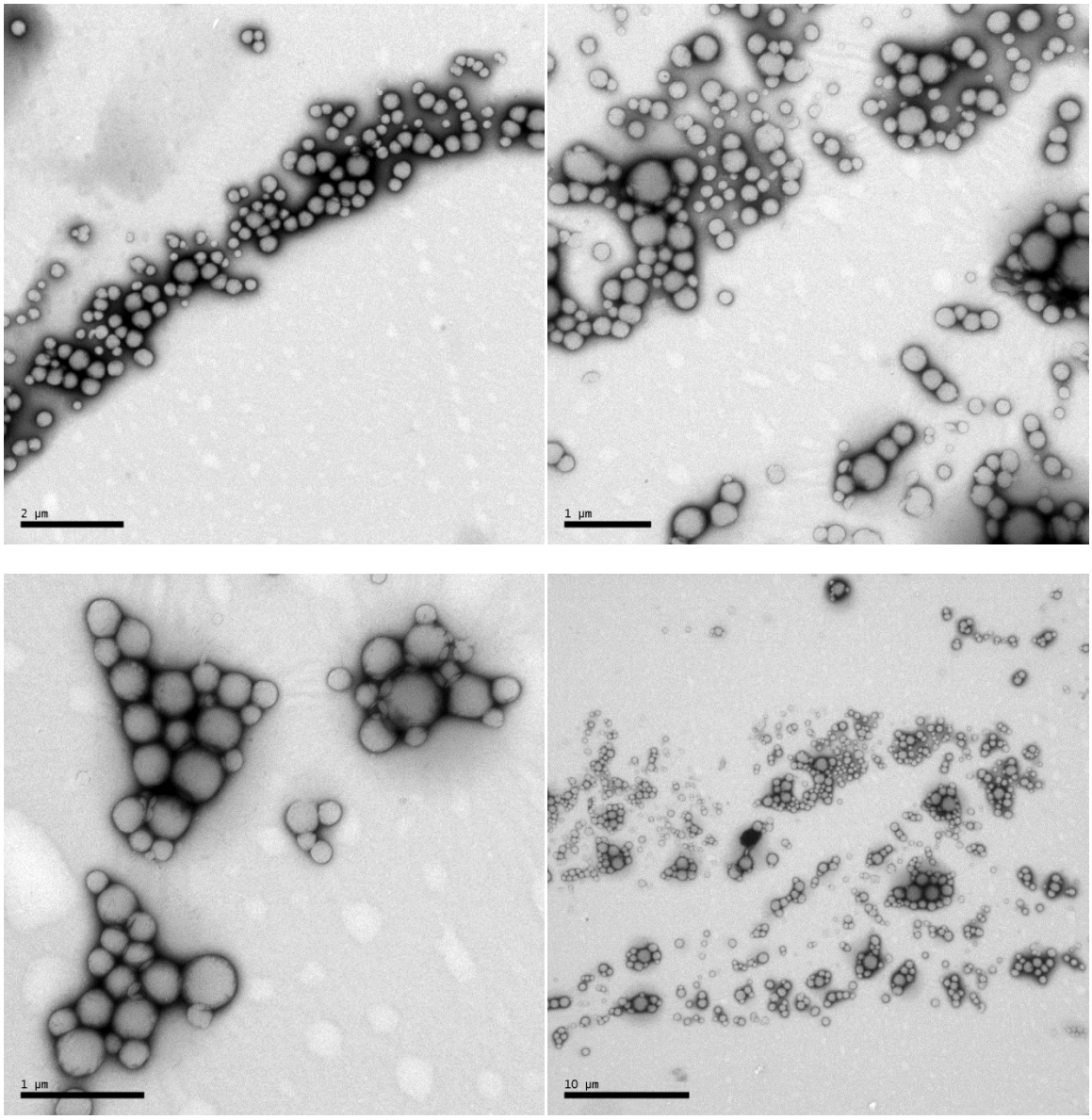

Figure SI 2: TEM micrographs of pyrene containing PEO-*b*-PODMA bicontinuous nanospheres prepared at 10°C at 0.1 wt% in water.

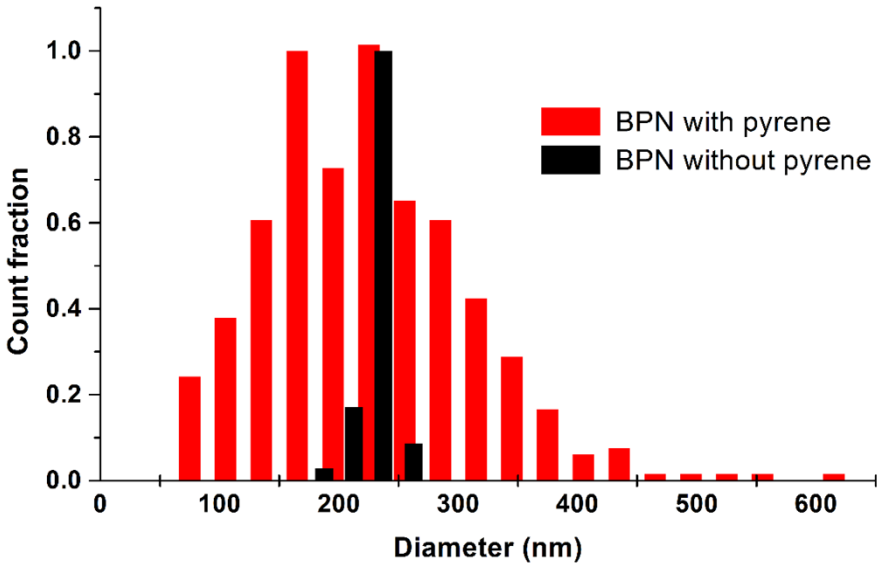

Figure SI 3: Histograms of particle diameters from TEM analysis of PEO-*b*-PODMA bicontinuous nanospheres with and without pyrene.

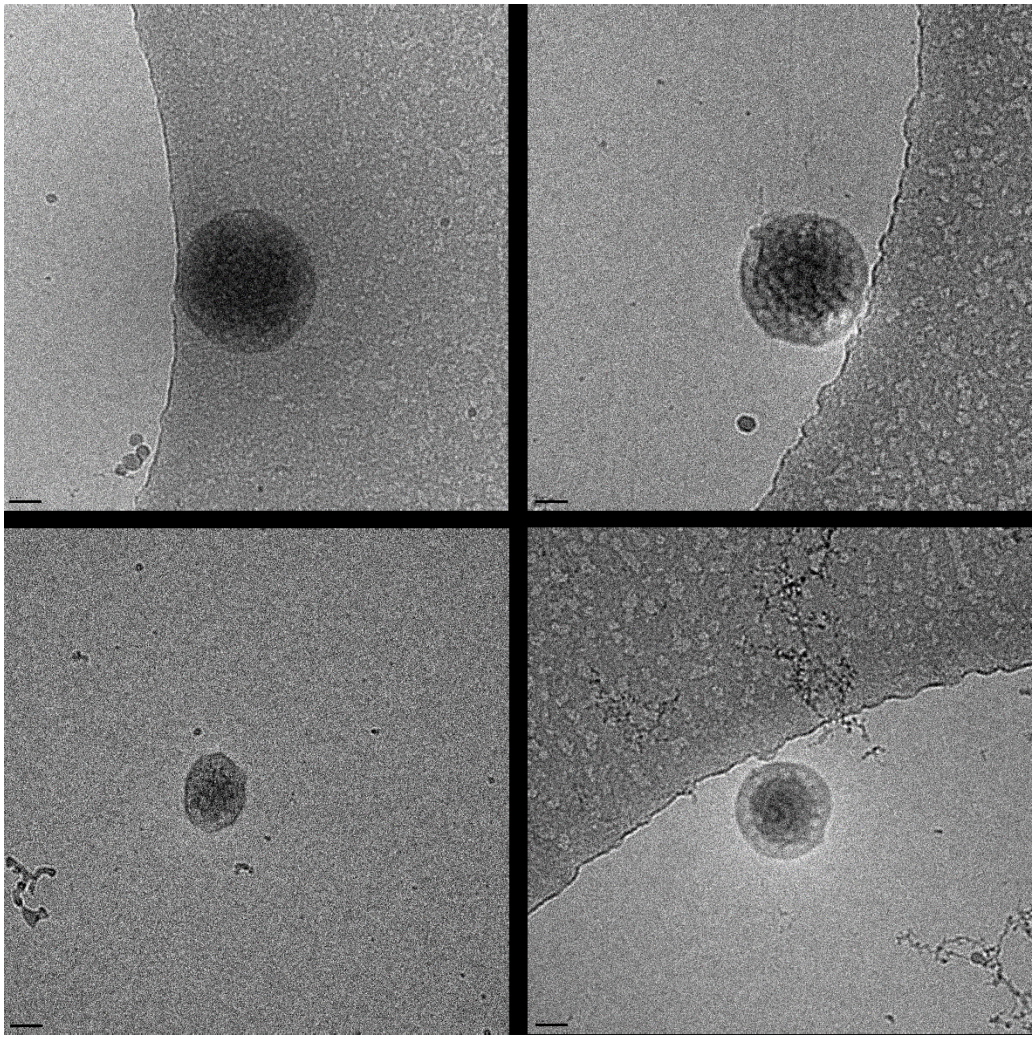

Figure SI 4: Cryo-TEM micrographs of BPN containing pyrene. Scale bar = 50 nm.

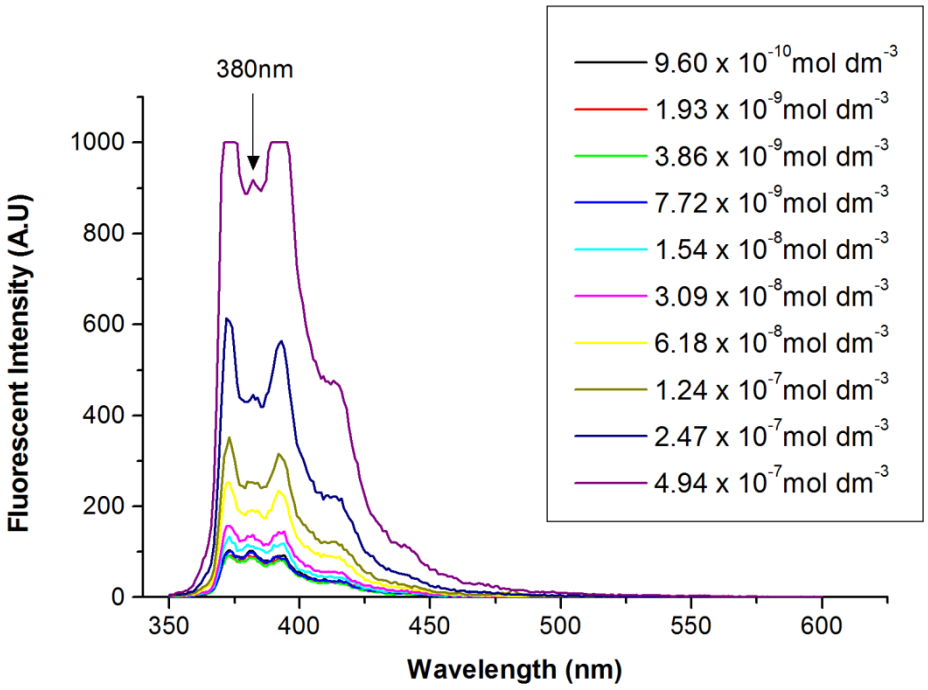

Figure SI 5: Fluorescent traces of pyrene in water at various concentrations.

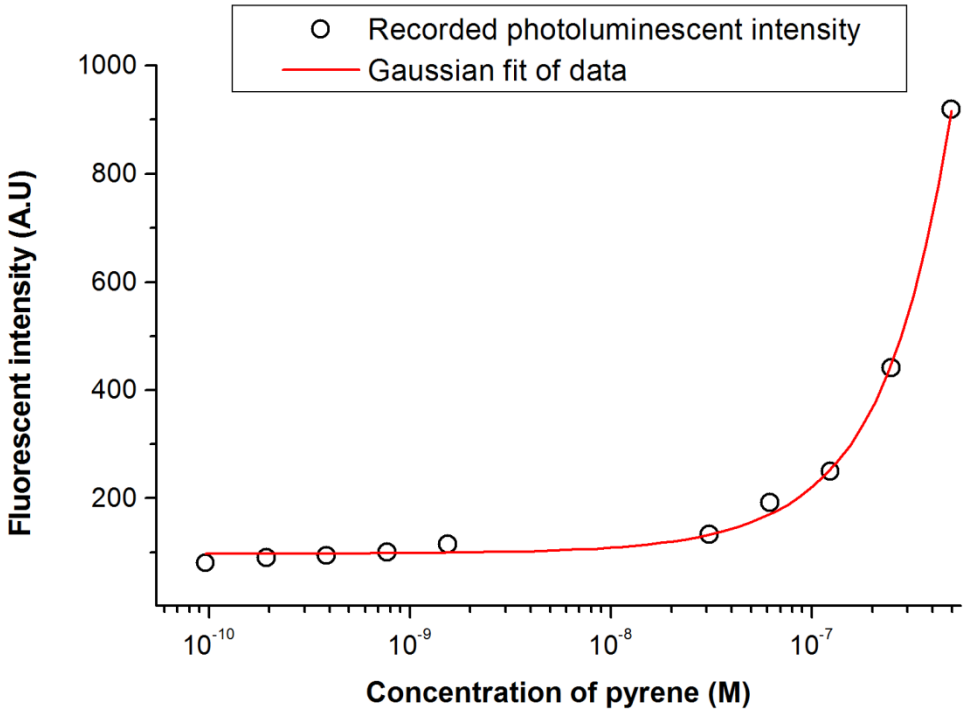

Figure SI 6: Calibration curve for pyrene in RO water.

Table SI 1. Data used to estimate release efficiencies of pyrene from BPNs at various temperatures.

T °C	T (h)	Curve integral 350-600 nm[a]	% Py in BPN_b	Conc. in solution x 10^7 mol dm^{-3} [c]	Mols in solution at end x 10^7	Mols in BPN at end x 10^6 [d]	Mols in BPN at start x 10^6 [e]	Grams in BPN at start x 10^4
--	0	73261	100	--	--	--		--
10	275	50088	68.4	1.93	5.80	1.25	1.83	1.83
20	250	52620	71.8	2.35	7.05	1.80	2.50	2.50
25	375	43800	59.8	3.02	9.07	1.35	2.26	2.26
30	360	34995	47.8	3.81	11.4	1.04	2.19	2.19
40	360	21363	29.2	4.52	13.5	0.56	1.91	1.91
							Grams pyrene in 10mg BPN =	4.32×10^{-4}

a. Total areas under photoluminescent spectra between 350 and 600 nm (see Figure 3b). b. Calculated from area at end of dialysis at time and temperature stated, divided by area prior to dialysis. c. Calculated from calibration curve, Figure SI X. d. Calculated from (Mols in solution) x [(100-(%Py in BPN))/(%Py in BPM)]. E. Calculated from Mols in BPN at end + Mols in solution at end.

Construction of fractional release profiles

Upper limit solubility values of pyrene at various temperatures estimated from upper asymptote of sigmoidal (Boltzmann) curve fits illustrated in Figure 3a. Equation and results given below

$$y = \frac{A_1 - A_2}{1 + e^{(x - x_0)idx}} + A_2$$

Sigmoidal(Boltzman) fit to Data1_Conc10		
Chisqr	1.45585E-17	
---	---	---
Init(A1)	-3.5882E-8	8.66E-9
Final(A2)	2.7796E-7	2.78E-8
XatY50(x0)	253.22	16.3
Width(dx)	109.72	13.6
---	---	---
XatY20	101.12708	
XatY80	405.32226	

Sigmoidal(Boltzman) fit to Data1_Conc20		
Chisqr	4.29513E-18	
---	---	---
Init(A1)	-2.7064E-8	2.74E-9
Final(A2)	2.7183E-7	4.82E-9
XatY50(x0)	200.27	2.30
Width(dx)	78.670	2.74
---	---	---
XatY20	91.20985	
XatY80	309.32847	

Sigmoidal(Boltzman) fit to Data1_Conc25		
Chisqr	1.19458E-17	
---	---	---
Init(A1)	-8.8165E-8	1.36E-8
Final(A2)	3.4785E-7	8.85E-9
XatY50(x0)	148.44	4.51
Width(dx)	100.57	6.56
---	---	---
XatY20	9.02578	
XatY80	287.85383	

Sigmoidal(Boltzman) fit to Data1_Conc30		
Chisqr	2.34185E-17	
---	---	---
Init(A1)	-8.1391E-8	1.12E-8
Final(A2)	4.1522E-7	6.39E-9
XatY50(x0)	102.80	2.33
Width(dx)	56.977	2.76
---	---	---
XatY20	23.80919	
XatY80	181.78433	

Sigmoidal(Boltzman) fit to Data1_Conc40		
Chisqr	3.53314E-17	
---	---	---
Init(A1)	-1.212E-6	4.44E-7
Final(A2)	4.6071E-7	3.35E-9
XatY50(x0)	-51.717	22.8
Width(dx)	55.102	3.46
---	---	---
XatY20	-128.10532	
XatY80	24.67097	

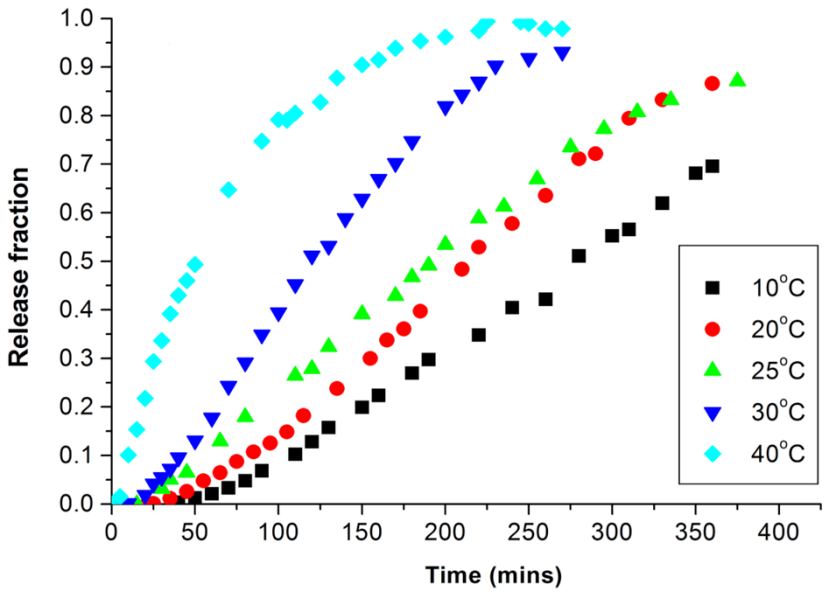

Figure SI 7: Fractional release profiles of pyrene from PEO-PODMA bicontinuous nanospheres.

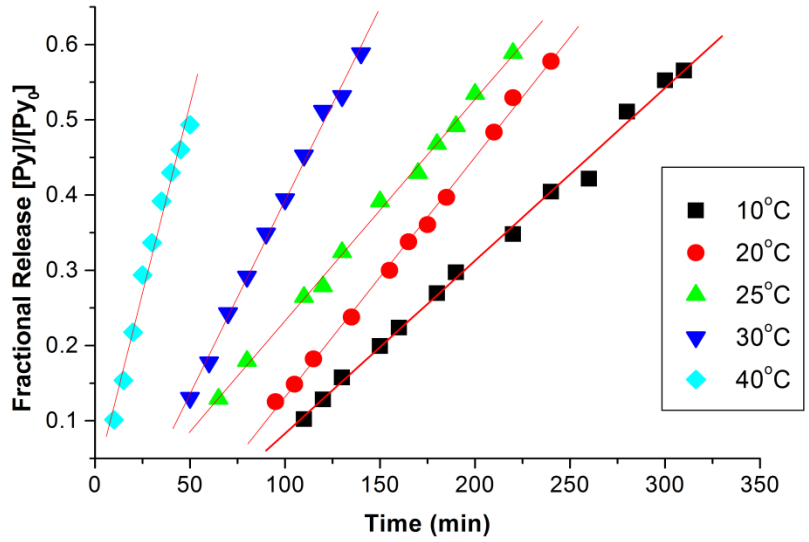

Figure 8I 7: Linear fits to fractional release profiles of pyrene from PEO-PODMA bicontinuous nanospheres (0.1 to 0.6 only).

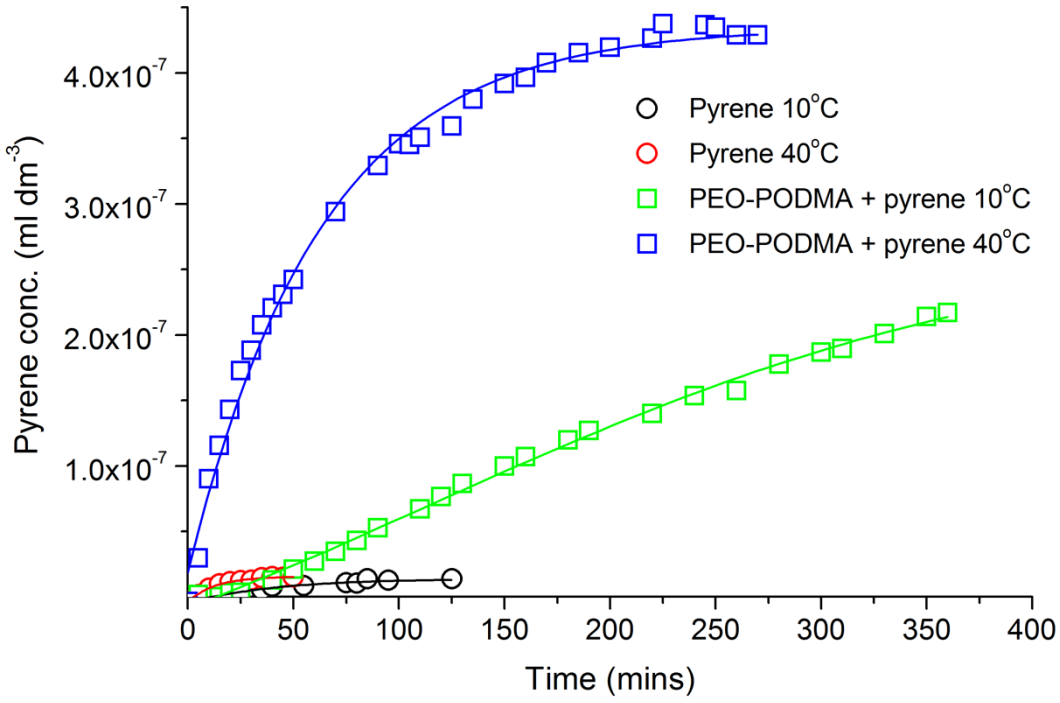

Figure SI 9: Comparison of release profiles of pyrene in PEO-PODMA bicontinuous nanospheres against that for non-encapsulated pyrene in RO water; linear concentration scale.

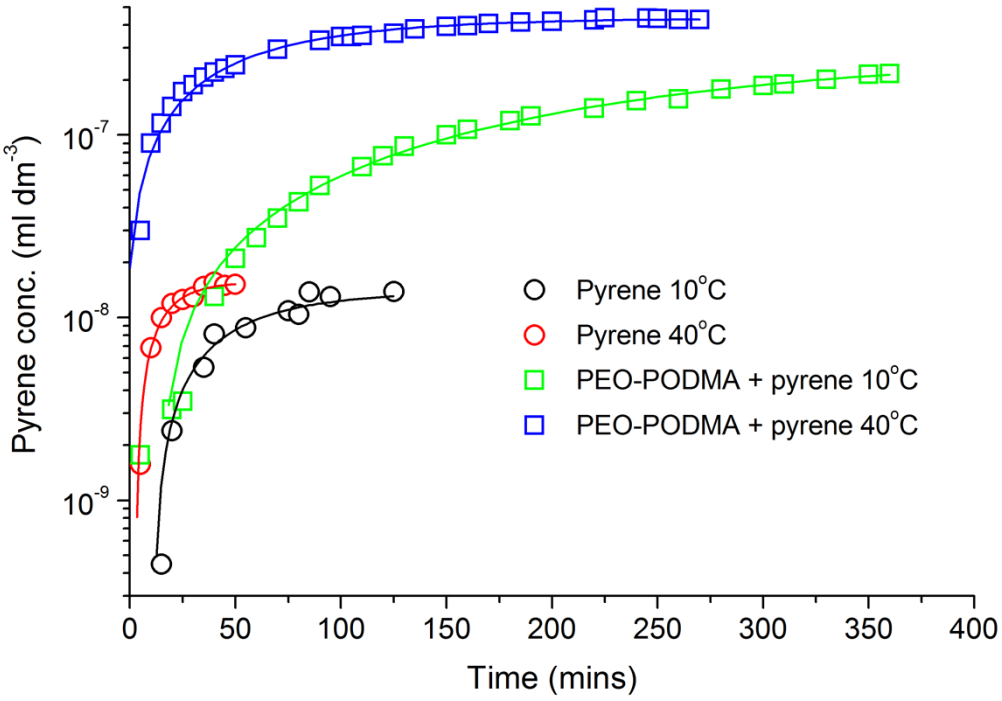

Figure SI 10: Comparison of release profiles of pyrene in PEO-PODMA bicontinuous nanospheres against that for non-encapsulated pyrene in RO water; log concentration scale.

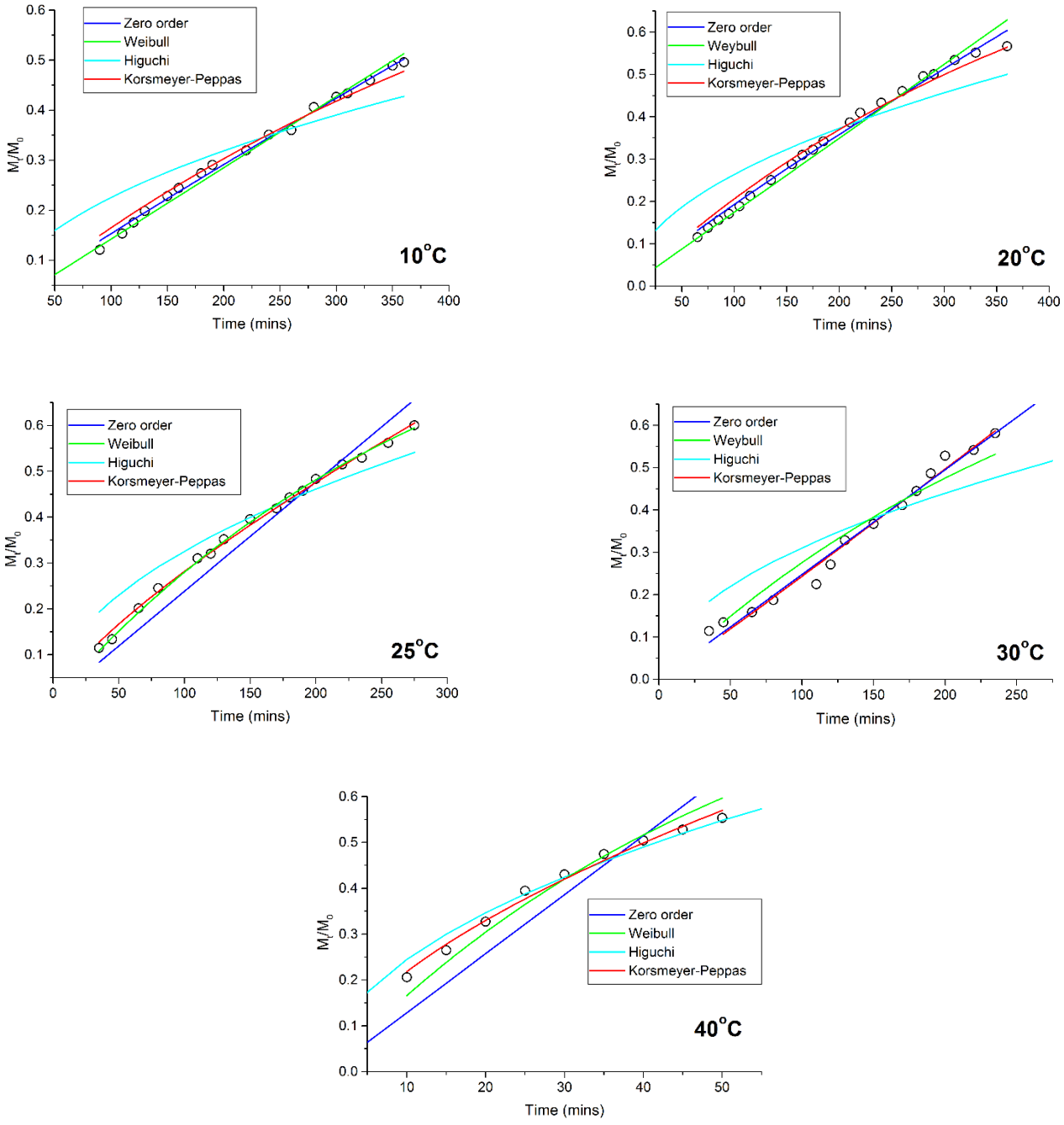

Figure SI 11: Results of curve fitting for matching release models to release of pyrene from PEO-PODMA bicontinuous nanospheres at 10, 20, 25, 30 and 40°C.

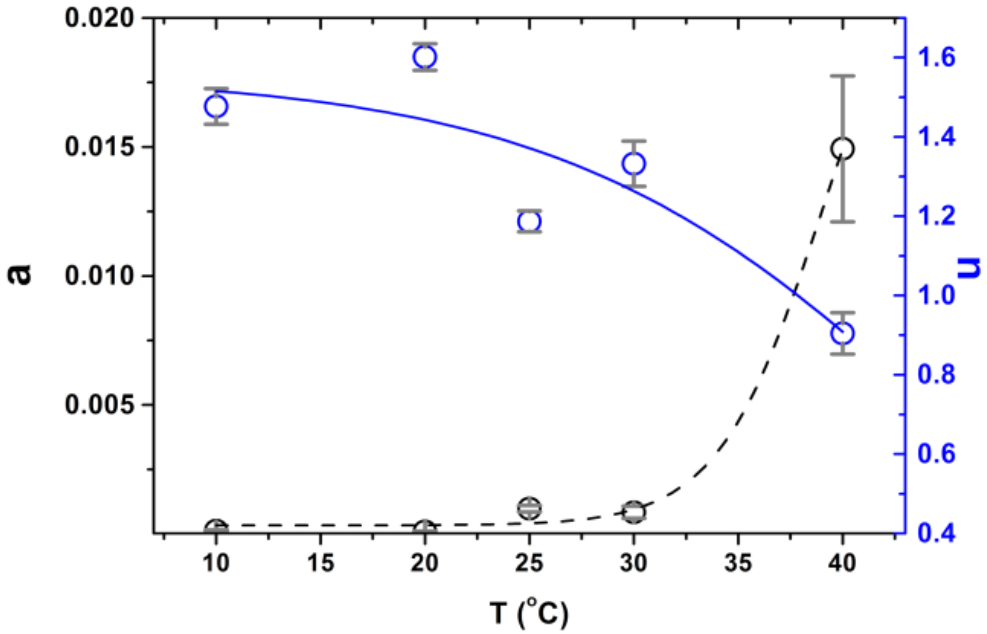

Figure SI 12. Variation in *a* (structural and geometrical component) and *n* with temperature following the Korsmeyer-Peppas model of controlled release.