Supplementary Information: Two-Stepping: Sol-gel-gel transitions in mixed thermoresponsive polymer systems

Mohamad A. Abou-Shamat,[1,2] Alan Reader,[1] Eleanor Hilton,[3] Niamh Haslett,[1,3] Abhishek Rajbanshi,[1] Marcelo Alves da Silva, Najet Mahmoudi,[4] Jacqueline Stair,[1] Jesus Calvo-Castro,[1,5] and Michael T. Cook.[3]

Materials and Methods:

Materials:

2,2′-Bipyridine, 4-Dimethylaminopyridine (DMAP), α-Bromoisobutyryl bromide 98% (BiBB), and Tris[2-(dimethylamino)ethyl]amine 97% (Me6TREN), Poloxamer P407, Copper(I) Bromide, and N-isopropylacrylamide (NIPAM) were purchased from Merck Sigma-Aldridge, UK. Polyethylene Glycol (PEG, 4 kDa) was purchased from Fluka, Germany and determined to have *Mn* 4.6 kDa by ^{1}H NMR. Triethylamine (TEA), was purchased from Fisher Scientific, UK. Dichloromethane anhydrous was purchased from Alfa Aesar, UK. The materials were used without any further purification. Anhydrous dichloromethane was purchased from Fisher Scientific and any traces of water was removed by placing it in freshly-activated 4 A molecular sieves for at least 24 h prior to use.

Synthesis of PEG macroinitiator (PEG-MI):

PEG-MI was synthesised using method published in the literature.[1] All glassware was dried overnight in a 105 °C oven. DMAP (1.17 g, 9.6 mmol) was dissolved in anhydrous dichloromethane (8 mL) and mixed with TEA (0.89 mL, 6.4 mmol) in a round bottom flask and cooled down using an ice bath. BiBB (1.97 mL, 16.0 mmol) in anhydrous dichloromethane (8 mL) then added to the mixture. A solution of PEG (6.4 g; 1.6 mmol) in dichloromethane (160 mL) was then added dropwise over 1 h to the solution while stirring. When the PEG addition finished, the reaction was allowed to rise to room temperature and stirred for 18 h. The solution was filtered, and half of the solvent removed using rotary evaporator. The PEG initiator was precipitated in cold diethyl ether (480 mL) then filtered and washed with the cold diethyl ether. PEG-MI then was dissolved in de-ionised water and dialysed in 3.5 kDa dialysis tube for 24 hours. Finally, the PEG-MI was freeze dried overnight.

Synthesis PEG-b-NIPAM-b-PEG (N10E5) polymer:

ABA tri-block copolymers were synthesised using the di-functionalised PEG-MI. In a general synthesis, copper (I) bromide (35.8 my, 250 µmol) was placed in a sealed flask under nitrogen flow for 30 minutes to remove any oxygen trace in the flask. PEG-MI (0.5 g), Me6TREN (66.8 µL, 250 µmol) and NIPAM (2.50 g, 22 mmol) were added to 10 mL water in a second sealed flask and degassed with nitrogen bubbling for 30 min. After degassing, the monomer, macro-initiator, ligand and solvent mix was transferred to the copper(I) bromide using a degassed syringe. The flask was then stirred for 24 h at room temperature. The mixture then was purified using 3.5 kDa dialysis membrane for 24 h with regular changes of water and freeze dried overnight.

Characterisation

Gel Permeation Chromatography (GPC)

Gel permeation chromatography was performed on an Agilent 1260 GPC equipped with a mixed-D column, running DMF + 0.1 % LiBr at a rate of 1 mL/min. Columns and refractive index detector were held at 30 °C during analysis. The instrument was calibrated with poly(methyl methacrylate) standards and all analysis was conducted in a narrow calibration relative to these standards.

Nuclear Magnetic Resonance (NMR) spectroscopy

^{1}H NMR were performed using 600 MHz magnet from JEOL (system ECA 600) NMR spectrometer with Delta 4.3.6 software. All samples were measured in D_2O. All spectra were analysed using MestReNova by Mestrelab Research. The average Mn of the PEG macro-initiators and tri-block copolymers were calculated from ^{1}H NMR. In the case of PEG-MI, the Mn was calculated by using the ratio of the integral for the two terminal CH_3 groups at 1.79 ppm to the integral of the remaining CH_2 groups at 3.53 ppm present in the polymer back bone. For the ABA tri-block copolymers Mn were estimated by calculating the ratio of the calculated CH_2 groups at 3.53 ppm peak integral to that of the peaks for the NIPAM CH_3 at 0.98 ppm group.

Fourier Transform Infra-red (FT-IR) spectroscopy analysis of PEG macroinitiator and ABA block copolymers:

IR spectroscopy was performed on the polymer powder using Perkin Elmer Fourier Transform Infra-red (FTIR) Spectrometer Frontier with a Perkin Elmer Universal attenuated total reflectance (ATR) Sample Accessory. A wavelength range of 650-4000 cm^{-1} was used with a resolution of 4 cm^{-1}. The instrument was cleaned with isopropyl alcohol before and after use.

Rheological study of P407 and ABA block copolymer additives formulations:

ABA formulations were prepared using the following steps: ABA polymer (0.4 g) was added to deionised water (2 mL) and stirred for half an hour in an ice bath before being left in the fridge overnight.

Mixed formulations were prepared as follows, ABA polymer was added as additive at 0.10, 0.25, 0.50, 0.75 and 1.00 g to deionised water (10 mL) along with P407 (3 g). The mixture was placed in an ice bath and stirred approximately for 30 minutes until the polymers are completely dissolved. Then the formulations were left in the fridge at 4 °C overnight.

Temperature ramp rheometry experiments were performed using a TA instruments AR1500ex rheometer fitted with a solvent trap using a stainless steel parallel plate geometry (40 mm in diameter), a 600 µm gap, and air pressure at 30 bar. Temperature ramp measurements were carried out by leaving the gel to equilibrate for 5 min at 15 °C on the rheometer temperature-controlled stage, then temperature ramp measurements were carried out at a frequency of 1 Hz and oscillatory stress of 1 Pa with temperatures increased from 15 to 40 °C at a rate of 1 °C/min.

Frequency sweep was performed for formulations at 1 Pa stress, between 0.1 and 1000 Hz, and temperatures at 15, 20, 25, 30 and 37 °C.

Stress sweeps were performed between 0.01 and 1000 Pa oscillatory stress at a frequency of 1 Hz and temperatures at 37 °C.

Small-angle neutron scattering (SANS) analysis for the ABA copolymer and mixed formulations:

Small-angle neutron scattering (SANS) measurements were conducted using the time-of-flight diffractometer instrument SANS2d at the STFC ISIS Neutron and Muon Source (Didcot, UK).[1] Incident wavelengths from 1.75 to 12.5 A° were used with a sample-to-detector distance of 12 m, corresponding to a total scattering vector range Q from $1.5X10^{-3}$ to 0.25 $A^{°-1}$. The sample temperature was controlled using an external circulating thermal bath (Julabo, DE). Samples were loaded in 1 mm path length, 1 cm wide optical quartz cells. Three formulations were prepared for SANS analyses in D_2O at ABA 7.5 % w/v, P407 30 % w/v, a mixture of both at the same percentage, and dilute mixed systems of 3/0.75 wt% and 3/1.5 wt% P407/N10E5. The 2D raw data were processed using wavelength-dependent corrections of the incident spectrum, detector efficiency and measured sample transmission, in the instrument reduction software Mantid.[2] The scattering intensity was then converted to the differential scattering cross-section in absolute units (cm^{-1}) using ISIS standard procedures. [G.D. Wignall, F.S. Bates, Absolute calibration of small-angle neutron scattering data, J. Appl. Cryst. 20 (1987) 28–40, https://doi.org/10.1107/ s0021889887087181.]. SANS data were fitted using SASView. In a general approach to fitting, the volume fraction of the object was approximated to the weight fraction, considering the similar density of PEG (1.13 g/cm^3) and D_2O (1.11 g/cm^3). The background intensity was estimated as the limit of the intensity in the high q region. Initial fitting was then conducted with the Levenberg-Marquart algorithm. Once an approximate fit was achieved, polydispersity was added to smear the model and the data refit. For low q analysis, the region $2 \times 10^{-3} < q < 1 \times 10^{-2}$ was isolated and fit to a power law model. For analysis of Bragg peaks in the high q region, the low q region was neglected and data fit solely in the region $1 \times 10^{-1} < q$ using the face-centred cubic (FCC) paracrystal model.

Results and Discussion

Polymer characterisation using NMR is a well-established method to monitor the polymerisation reaction, to determine end-group composition and to confirm successful synthesis, in addition, it is a reliable technique to calculate the polymer's number average molecular weight and the degree of polymerisation (DP).[3] Polyethylene glycol macroinitiator (PEGMI) synthesis was confirmed by ^{1}H NMR, with PEG and PEGMI NMR spectra given below in Figure S1. PEG ^{1}H NMR exhibits two peaks, one associated with the $-CH_2-$ in the polymer backbone, presented as singlet peak at between 3.5 and 3.6 ppm due to the equivalent protons of the two methylene groups,[4] and one at around 4.6 assigned to the water residue in the D_2O NMR solvent.[5] For the PEGMI, same peaks as PEG were found in addition to a singlet peak at 1.79 ppm assigned to the methyl group at the end of the polymer chain which confirms the addition of the macroinitiator to the ends of the polymer chain.

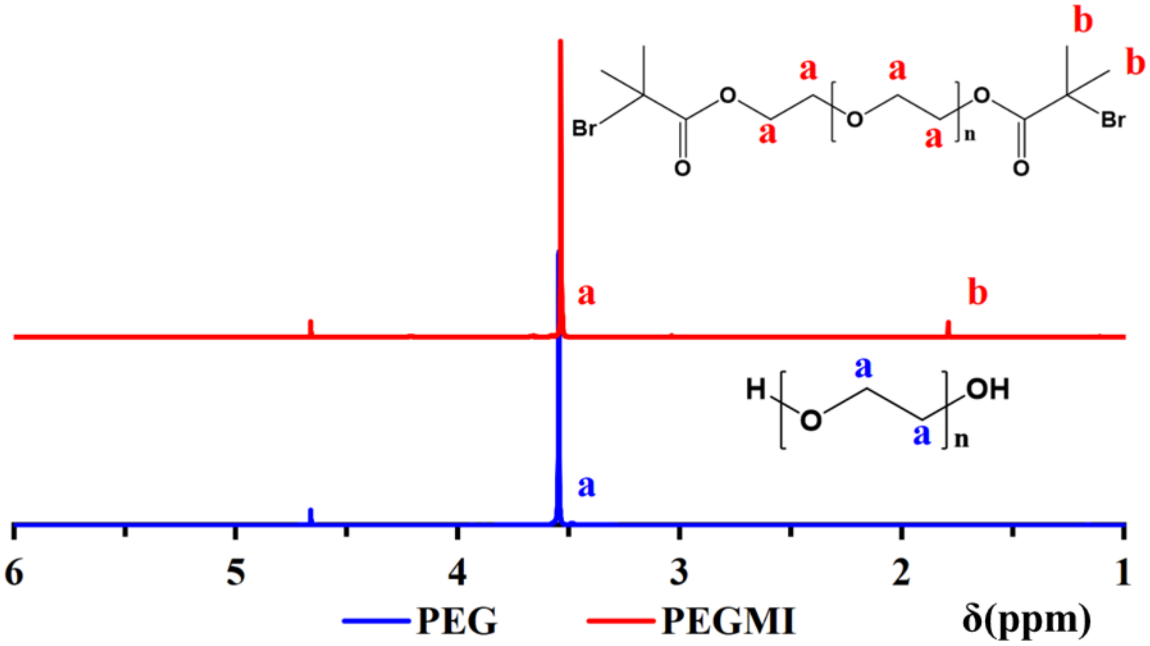

Figure S1: NMR spectra for PEG (blue) and the synthesised PEG-MI (red) in D_2O at 600 Hz

The synthesis of the ABA polymer was also confirmed with ^1H NMR. PEG-MI, NIPAM and PNIPAM-*b*-PEG-*b*-PNIPAM NMR spectra are presented in Figure S2. NIPAM's NMR exhibited five peaks. Three peaks assigned to the vinyl protons, which completely disappear after the ATRP polymerisation, at c.a. 6.0, 5.9 and 5.5. The other alkyl -CH and $-CH_2$ protons as multiplets at 3.8 and 1.0 ppm. The ratio of the CH_3 integral to the CH_2 of ethylene oxide was 1:34.45, indicating 93% conversion with an estimated DP of 97.

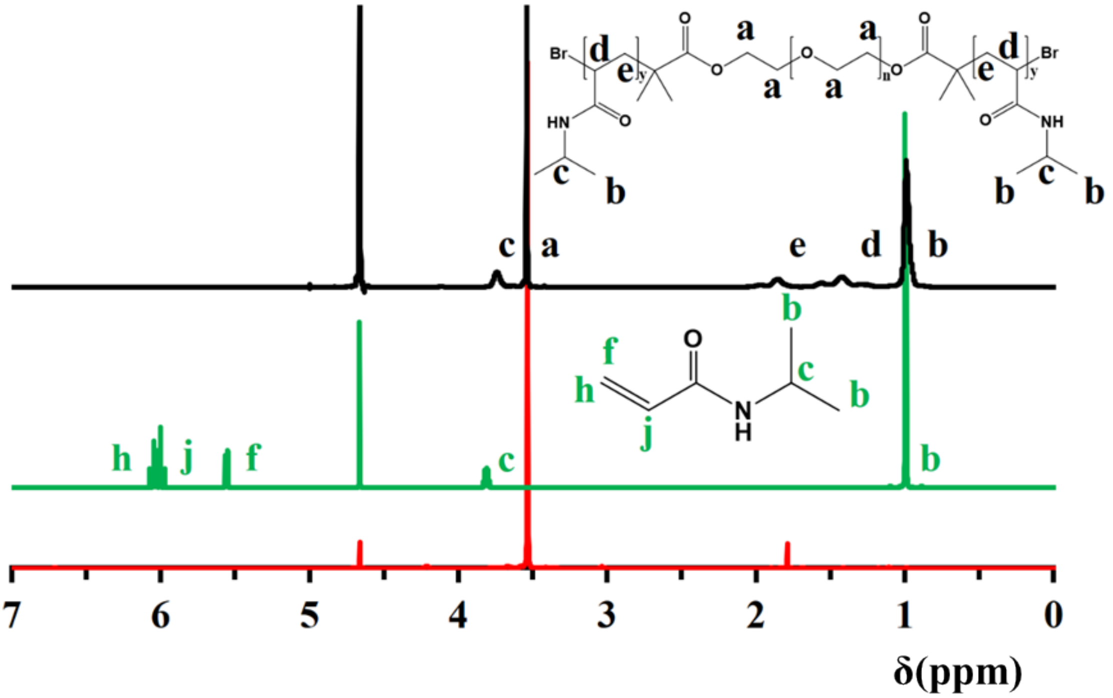

Figure S2: ^{1}H NMR spectra for PEGMI (red), NIPAM raw material (green), and the synthesised ABA polymer (black)

The degree of polymerisation for the ABA polymers were calculated using a reported method in the literature.[3] Briefly, since the areas under the resonance peaks are proportional to the relative molar concentration of the species in the sample it is possible to determine the molecular weight and the degree of polymerisation of the polymer using NMR peak integration.[3] The integration values and peaks shifts are presented in Table S1. Gel-permeation chromatography was also conducted (figure S3) to determine M_n and PDI, which were 33.9 kDa (relative to PMMA standards) and 1.8, respectively.

Table S1: ABA block segment's degree of polymerisation and the average molecular weight in addition to the total average polymer molecular weight calculated using NMR.

	PNIPAM block DP	PNIPAM block Mn /kDa	PEG block DP	PEG block Mn / kDa	ABA Polymer Mn / kDa
N10P5	108	12	97	5	17

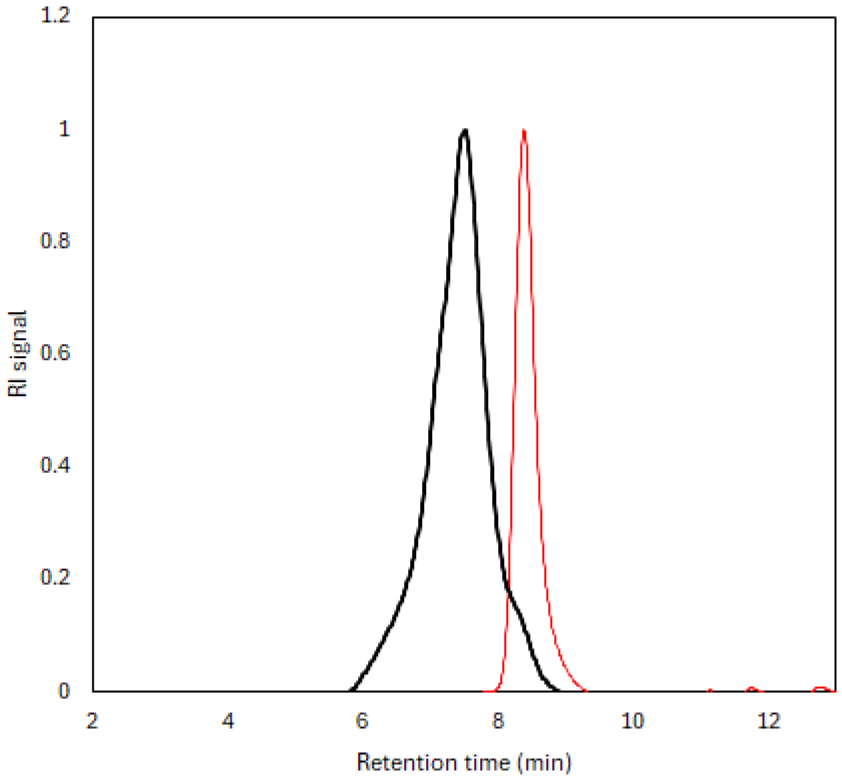

Figure S3. Gel-permeation chromatography of N10E5 (black) and PEG macroinitiator (red).

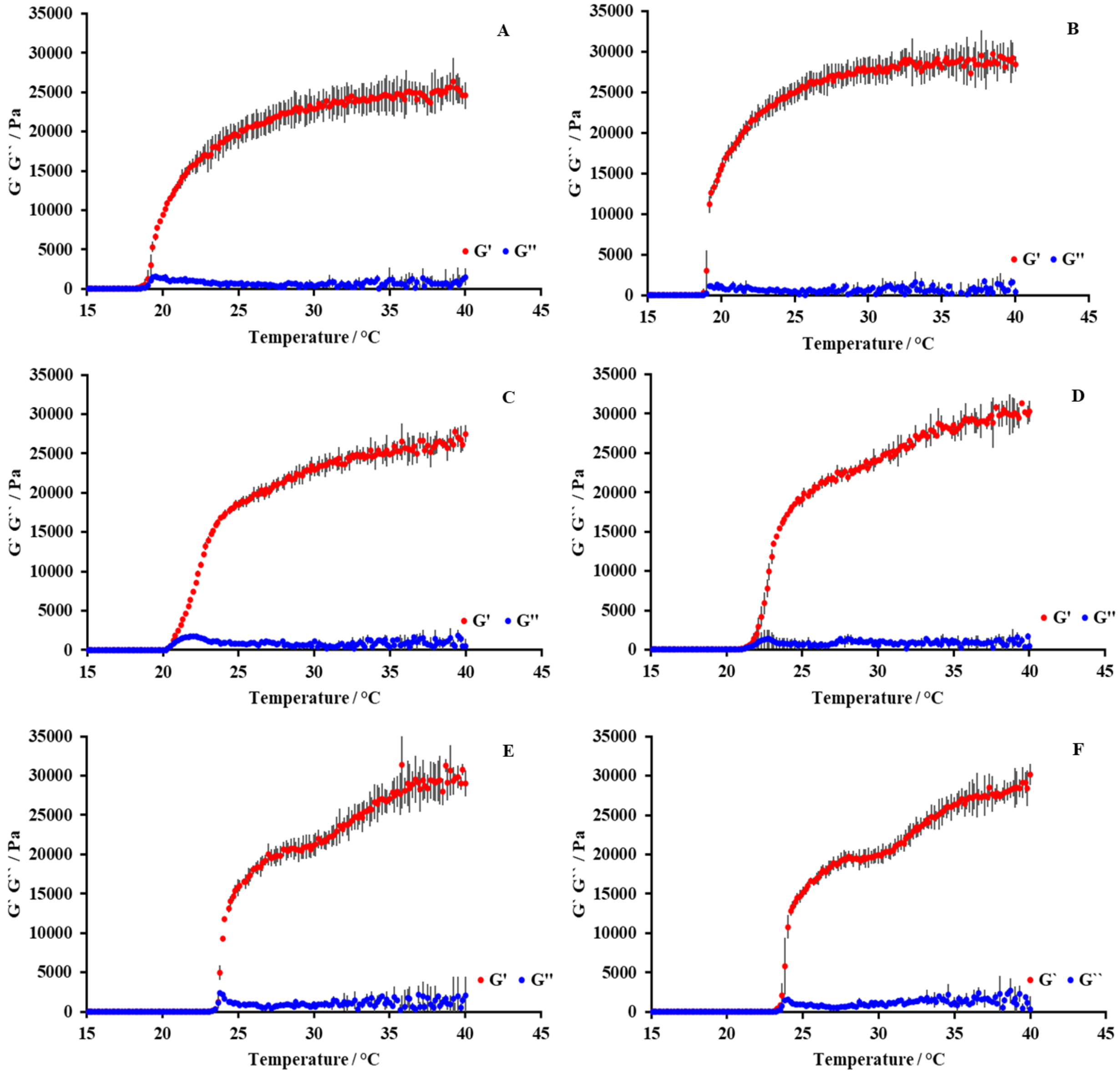

Figure S4. Temperature ramp rheograms of 30 wt% P407 (A) with 1 (B), 2.5 (C), 5 (D), 7.5 (E) and 10 (F) wt% N10E5. Data shown with G′ (red) and G″ (blue) including standard deviation (n=3).

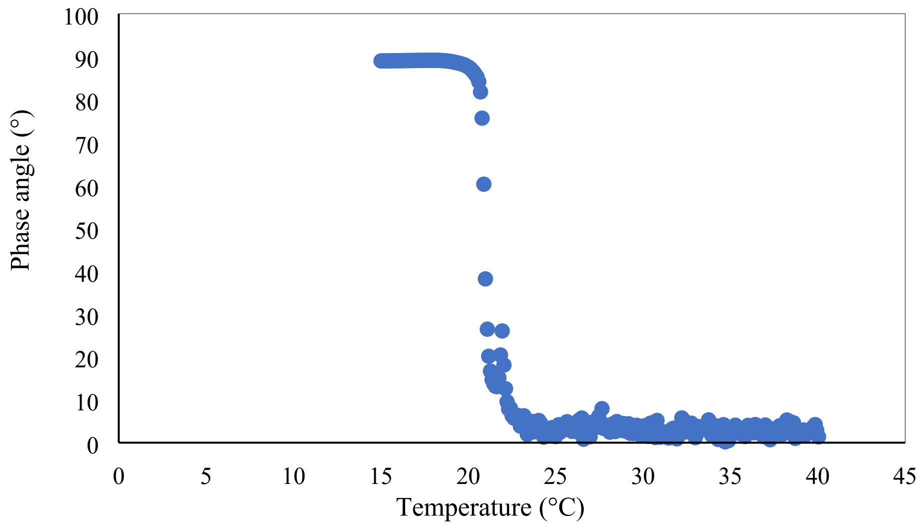

Figure S5. Phase angle on rheological temperature ramp for 7.5/30 wt% N10E5/P407 mixture measured at 1Hz.

Table S2. Fitting parameters for N10E5 at 25°C.

sasview_parameter_values	Value
model_name: poly_gaussian_coil	
scale	1
background	0.07
A_scale	6.569E-08
A_power	3.144
B_scale	0.075
B_i_zero	20
B_rg	55.962
B_polydispersity	3.4934

Table S3. Fitting parameters for N10E5 at 37°C.

sasview_parameter_values	Value
model_name: CylPlusPGC	
scale	1
background	0.07
A_scale	0.0062093
A_sld	0.9
A_sld_solvent	6.37
A_radius	168.95
A_length	20867
B_scale	0.048347
B_i_zero	20

B_rg	54
B_polydispersity	1.8
A_radius.width	0.2
A_length.width	0

Table S4. Fitting parameters for N10E5 at 40°C.

sasview_parameter_values	**Value**
model_name: CylPlusPGC	
scale	1
background	0.07
A_scale	0.011858
A_sld	0.9
A_sld_solvent	6.37
A_radius	148.85
A_length	1096.4
B_scale	0.0075
B_i_zero	20
B_rg	54
B_polydispersity	1.8
A_radius.width	0.2
A_length.width	0

Table S5. Fitting parameters for N10E5 at 50°C.

sasview_parameter_values	**Value**
model_name: FlexCylPlusPGC	
scale	1
background	0.07
A_scale	0.0062093
A_length	17420
A_kuhn_length	94.778
A_radius	124.9
A_sld	0.9
A_sld_solvent	6.37
B_scale	0
B_i_zero	20
B_rg	54
B_polydispersity	1.8
A_length.width	0
A_kuhn_length.width	0
A_radius.width	0.2

1 R. K. Heenan, S. E. Rogers, D. Turner, A. E. Terry, J. Treadgold and S. M. King, *Neutron News*, 2011, 22, 19–21.

2 http://www.mantidpro ject.org/ISIS_SANS, ISIS SANS Data Reduction in Mantid.

3 J. U. Izunobi and C. L. Higginbotham, *J Chem Educ*, 2011, **88**, 1098–1104.

4 B. R. Dauner and D. L. Pringle, *J Chem Educ*, 2014, **91**, 743–746.

5 G. R. Fulmer, A. J. M. Miller, N. H. Sherden, H. E. Gottlieb, A. Nudelman, B. M. Stoltz, J. E. Bercaw and K. I. Goldberg, *Supporting Information NMR Chemical Shifts of Trace Impurities: Common Laboratory Solvents, Organics, and Gases in Deuterated Solvents Relevant to the Organometallic Chemist*, .