Supporting information for:

Can percolation theory explain the gelation behavior of diblock copolymer worms?

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Experimental Section

Materials

Glycerol monomethacrylate (GMA; 99.8%) and 2-hydroxypropyl methacrylate (HPMA) were donated by GEO Specialty Chemicals (Hythe, UK) and were used without further purification. *N*-Phenylmaleimide (NMI; Alfa, 98%) was recrystallized from cyclohexane. Styrene (Aldrich, 99%) was passed through a column of basic alumina to remove inhibitor and then stored at −20 °C prior to use. 2-Cyano-2-propyl dithiobenzoate (CPDB), 4,4′-azobis(4-cyanopentanoic acid) (ACVA or V-501, 99%), 2,2-azobisisiobutyronitrile (AIBN), trimethylsilyldiazomethane (2 M in *n*-hexane), and regenerated cellulose dialysis membrane (Spectra/Por 6, molecular weight cut-off = 1000 Da) were purchased from Sigma-Aldrich (UK) and used as received. NMR solvents d_4-methanol, and d_6-DMSO were purchased from Goss Scientific (Nantwich, UK). Absolute ethanol (maximum water content = 0.1%) and 1,4-dioxane was supplied by VWR International S.A.S (Fontenay-sous-Bois France). All water was deionized grade.

Protocol for the synthesis of a $PGMA_{56}$ macro-CTA *via* RAFT solution polymerization in ethanol

GMA (40.0 g, 250 mmol), CPDB (1.105 g, 5.0 mmol; target DP = 50), and ACVA (0.280 g, 1.00 mmol; CPDB /ACVA molar ratio = 5.0) were accurately weighed into a 250 mL round-bottomed flask. Anhydrous ethanol (previously purged with nitrogen for 1 h) was then added to produce a 40% w/w solution, which was placed in an ice bath and purged under nitrogen for 45 min at 0 °C. The sealed flask was immersed in an oil bath set at 70 °C to initiate the RAFT solution polymerization of GMA and the reaction mixture was stirred for 2 h at this temperature. The polymerization was then quenched at approximately 76% conversion by exposure to air, followed by cooling the reaction mixture to room temperature. Methanol (20 mL) was added to this solution, followed by precipitation into a ten-fold excess of dichloromethane in order to remove unreacted GMA monomer. The precipitate was isolated *via* filtration and washed with excess dichloromethane before being dissolved in methanol (60 mL). The crude polymer was precipitated into excess dichloromethane again and isolated *via* filtration. It was then dissolved in water and freeze-dried overnight to afford a pink solid. ^{1}H NMR studies indicated a mean degree of polymerization of 56 *via* end-group analysis while DMF GPC studies (refractive index detector; calibration relative to a series of near-monodisperse poly(methyl methacrylate) standards) indicated an M_n of 16 100 g mol^{-1} and an M_w/M_n of 1.11.

Protocol for the synthesis of PGMA$_{56}$-PHPMA$_{155}$ diblock copolymer worm gel by RAFT aqueous dispersion polymerization

A PGMA$_{56}$-PHPMA$_{155}$ diblock copolymer worm gel was prepared *via* polymerization-induced self-assembly (PISA) using the following RAFT aqueous dispersion polymerization formulation: PGMA$_{56}$ macro-CTA (0.583 g, 0.063 mmol), HPMA monomer (1.40 g, 9.7 mmol) and ACVA (5.9 mg, 0.021 mmol; PGMA$_{56}$ macro-CTA/ACVA molar ratio = 3.0) were added to a 25 ml round-bottomed flask, followed by addition of water to produce a 15% w/w aqueous solution. This reaction solution was purged with nitrogen gas for 30 min at 20 °C before immersion into an oil bath set at 70 °C. The reaction mixture was stirred for 4 h to ensure essentially complete conversion of the HPMA monomer (> 99% as judged by disappearance of the vinyl signals ay 6.1 ppm using ^{1}H NMR analysis) and was quenched by exposure to air, followed by cooling to ambient temperature. DMF GPC analysis indicated that the diblock copolymer possessed a relatively narrow polydispersity (M_w/M_n = 1.10) and a number-average molecular weight (M_n) of 43 300 g mol^{-1} (relative to a series of near-monodisperse poly(methyl methacrylate) standards).

Synthesis of a PMAA$_{81}$ macro-CTA *via* RAFT solution polymerization in ethanol

In a 100 mL round-bottomed flask, CPDB RAFT agent (0.551 g, 2.49 mmol), AIBN (0.041 g, 0.249 mmol; CPDB/AIBN molar ratio = 10.0) and methacrylic acid (MAA; 15.0 g, 174 mmol) were dissolved in ethanol (15.0 g) to obtain a 50% w/w solution. The reaction mixture was degassed using a dry nitrogen purge for 40 min at 0 °C before being placed into a preheated oil bath at 60 °C. After 5 h (69% MAA conversion), the polymerization was quenched by cooling the reaction mixture to 20 °C and subsequently exposing it to air. Ethanol (30 mL) was then added to dilute the solution, and unreacted MAA monomer was removed by precipitation into a tenfold excess of diethyl ether. This purification protocol was repeated a further four times. A pink solid was obtained after drying under vacuum (11.2 g, 55% yield). ^{1}H NMR spectroscopy indicated a mean degree of polymerization of 81 for the PMAA macro-CTA (as calculated by comparing the integrated signals assigned to the aromatic protons at 7.2−8.0 ppm with those owing to the methacrylic polymer backbone at 0.4−2.5 ppm). After exhaustive methylation with excess trimethylsilyldiazomethane, THF GPC analysis (calibration with ten near-monodisperse poly(methyl methacrylate) standards) indicated an M_n of 10 800 g mol^{-1} and an M_w/M_n of 1.18.

Synthesis of PMAA$_{81}$-P(St-alt-NMI)$_{430}$ diblock copolymer worm gel by RAFT dispersion alternating copolymerization in a 50/50 ethanol/1,4-dioxane mixture

PMAA$_{81}$ macro-CTA (93.6 mg, 0.013 mmol), AIBN (0.438 mg, 0.00267 mmol; PMAA$_{81}$ macro-CTA/AIBN molar ratio = 5.0), styrene (297 mg, 2.86 mmol) and *N*-phenylmaleimide (395 mg, 2.86 mmol) were dissolved in a 50/50 w/w ethanol/1,4-dioxane mixture to make up a 20% w/w reaction mixture. This solution was sealed in a small Schlenk flask and purged with nitrogen for 15 min at 20 °C, before being immersed in a preheated oil bath at 70 °C. The RAFT dispersion alternating copolymerization was allowed to proceed for 10 h to ensure at least 90% comonomer conversion and then quenched by exposure to air. THF GPC analysis (calibration with ten near-monodisperse poly(methyl methacrylate) standards) indicated an M_n of 41 400 g mol^{-1} and an M_w/M_n of 1.43.

^{1}H NMR spectroscopy

All ^{1}H NMR spectra were recorded in either d$_4$-methanol or d$_6$-DMSO using a 400 MHz Bruker Avance-400 spectrometer. Typically 64 scans were averaged per spectrum.

Transmission Electron Microscopy (TEM)

Copper/palladium TEM grids (Agar Scientific, UK) were surface-coated to yield a thin film of amorphous carbon. The grids were then plasma glow-discharged for 30 seconds to create a hydrophilic surface. A small volume (10 µL) of a dilute copolymer solution (0.1% w/w) was placed on the freshly prepared grids for one minute and then blotted with filter paper to remove excess solution. To stain the aggregates, a 0.75% w/v uranyl formate solution (10 µL) was placed *via* micropipette on the sample-loaded grid for 20 seconds and then carefully blotted to remove excess stain. Each grid was then carefully dried using a vacuum hose. Imaging was performed on either a Phillips CM100 instrument at 100 kV equipped with a Gatan 1k CCD camera or a FEI Tecnai Spirit microscope equipped with a Gatan 1kMS600CW CCD camera. Mean worm widths were estimated for at least 100 particles using ImageJ software.

Rheology Studies

Rheology measurements were conducted using an AR-G2 rheometer (TA instruments) equipped with a variable temperature Peltier plate. A core-and-plate geometry (40 mm 2^o aluminium cone) was used for all experiments. The storage (G’) and loss (G”) moduli for the $PMAA_{81}$-P(St-*alt*-NMI)$_{430}$ and $PGMA_{56}$-$PHPMA_{155}$ worm gels were determined at various copolymer concentrations by performing % strain sweeps in the range of 0.1 to 100% using a constant angular frequency of 1.0 rad s^{-1}. The critical gelation concentration (CGC) was determined by the cross-over point for the G’ and G” curves.

Shear-Induced Polarized Light Imaging (SIPLI)

Given their well-known thermoresponsive character,[1] shear alignment experiments were conducted on an aqueous dispersion of 5.0% w/w $PGMA_{56}$-$PHPMA_{155}$ worms using a mechano-optical rheometer (Anton Paar Physica MCR301 with SIPLI attachment). Measurements were performed between 15 and 18 °C using a plate-plate geometry composed of a 25 mm polished steel plate and a fused quartz plate connected to a variable temperature Peltier system. The gap between plates was set at 1 mm for all experiments. An additional Peltier hood was used to ensure good control over the sample temperature. Sample illumination was achieved using an Edmund Optics 150 W MI-150 high-intensity fiber optic white light source. The polarizer and analyzer axes were crossed at 90° in order to obtain polarized light images (PLIs), which were recorded using a color CCD camera (Lumenera Lu165c). The point at which the Maltese cross appeared most bright indicated the formation of highly linear worms.

Small Angle X-ray Scattering (SAXS)

SAXS patterns were recorded for dilute dispersions of PGMA$_{56}$-PHPMA$_{155}$ and PMAA$_{81}$-P(St-*alt*-NMI)$_{430}$ worms at a synchrotron source (Diamond Light Source, station I22, Didcot, UK) using monochromatic X-ray radiation (wavelength, $\lambda = 0.124$ nm, with q ranging from 0.015 to 1.3 nm^{-1} , where $q = 4\pi \sin \theta/\lambda$ is the length of the scattering vector and θ is one-half of the scattering angle) and a 2D Pilatus 2M pixel detector (Dectris, Switzerland). Glass capillaries of 2.0 mm diameter were used as a sample holder. Measurements were conducted on 1.0% v/v dispersions at optimum temperatures where the worms were most linear, as indicated by SIPLI experiments. SAXS data were reduced (integration, normalization and absolute intensity calibration using SAXS patterns of deionized water assuming that the differential scattering cross-section of water is 0.0162 cm^{-1}) using DAWN software developed and supplied by Diamond Light Source.[2] Data were then analysed (background subtraction, data modelling and fitting) using Irena SAS macros for Igor Pro.[3]

Gel Permeation Chromatography (GPC)

PGMA$_{56}$ macro-CTA and PGMA$_{56}$-PHPMA$_{155}$ analysis by DMF GPC

Molecular weight distributions were assessed using an Agilent 1260 Infinity set-up comprising two Polymer Laboratories PL gel 5 µm Mixed-C columns, a refractive index detector operating at 60 °C. The GPC eluent was HPLC-grade DMF containing 10 mM LiBr at a flow rate of 1.0 mL min^{-1}. Calibration was conducted using a series of near-monodisperse poly(methyl methacrylate) standards.

PMAA$_{81}$ macro-CTA and PMAA$_{81}$-P(St-alt-NMI)$_{430}$ analysis by THF GPC

Prior to analysis by GPC, both polymers were modified by exhaustive methylation of the carboxylic acid groups in the PMAA block. Molecular weight distributions were assessed using an Agilent 1260 Infinity set-up comprising two Polymer Laboratories PL gel 5 µm Mixed-C columns, a refractive index detector operating at 30 °C. The GPC eluent was HPLC-grade THF containing 2.0% v/v triethylamine and 0.05% w/v butylhydroxytoluene (BHT) at a flow rate of 1.0 mL min^{-1}. Calibration was conducted using a series of near-monodisperse poly(methyl methacrylate) standards.

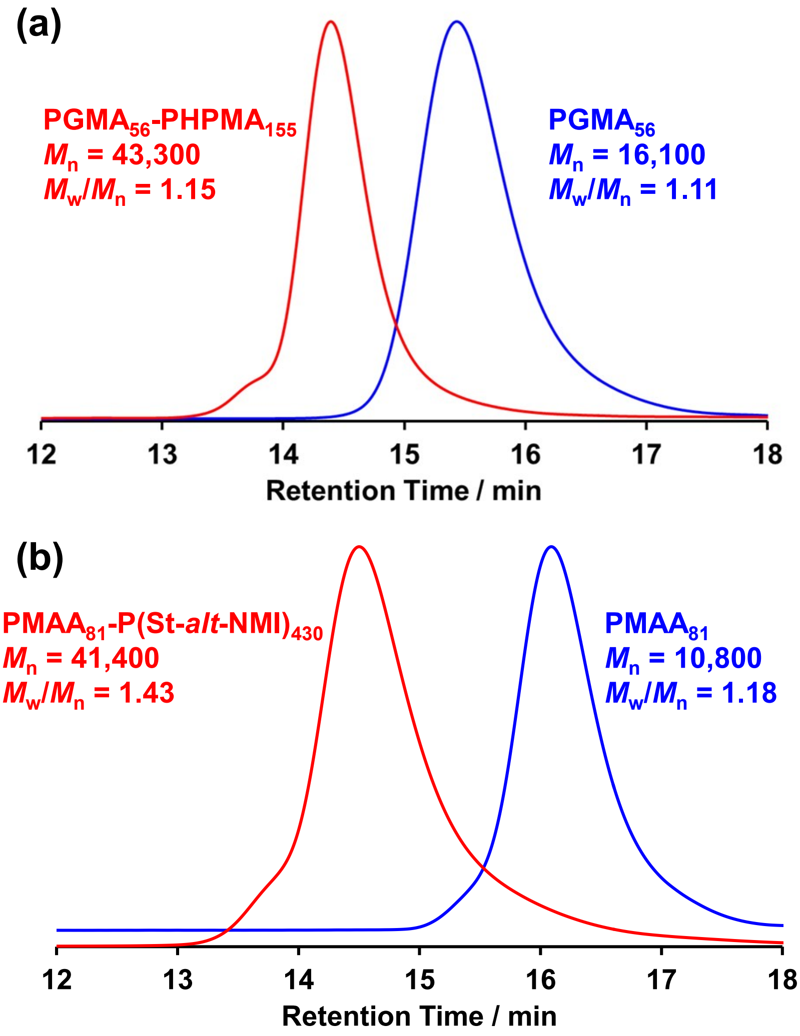


Figure S1. Gel permeation chromatograms obtained for (a) $PGMA_{56}$ macro-CTA (red) and $PGMA_{56}$-$PHPMA_{155}$ diblock copolymer (blue) in DMF and (c) methylated $PMAA_{81}$ macro CTA (red) and methylated $PMAA_{81}$-P(St-alt-NMI)$_{430}$ diblock copolymer (blue) in THF. Note all molecular weight data are expressed relative to a series of near-monodisperse poly(methyl methacrylate) standards.

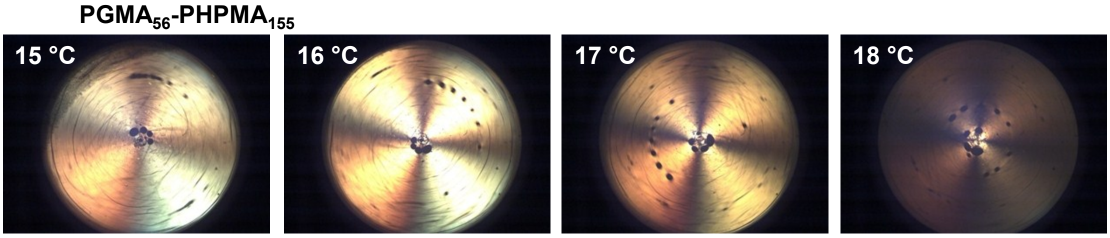


Figure S2. Shear-induced polarized light images (SIPLIs) collected at varying temperatures at a maximum shear rate of 20 s^{-1} for a 5.0% w/w aqueous dispersion of $PGMA_{56}$-$PHPMA_{155}$ worms. The appearance of a characteristic Maltese cross indicates shear-induced worm alignment**.**

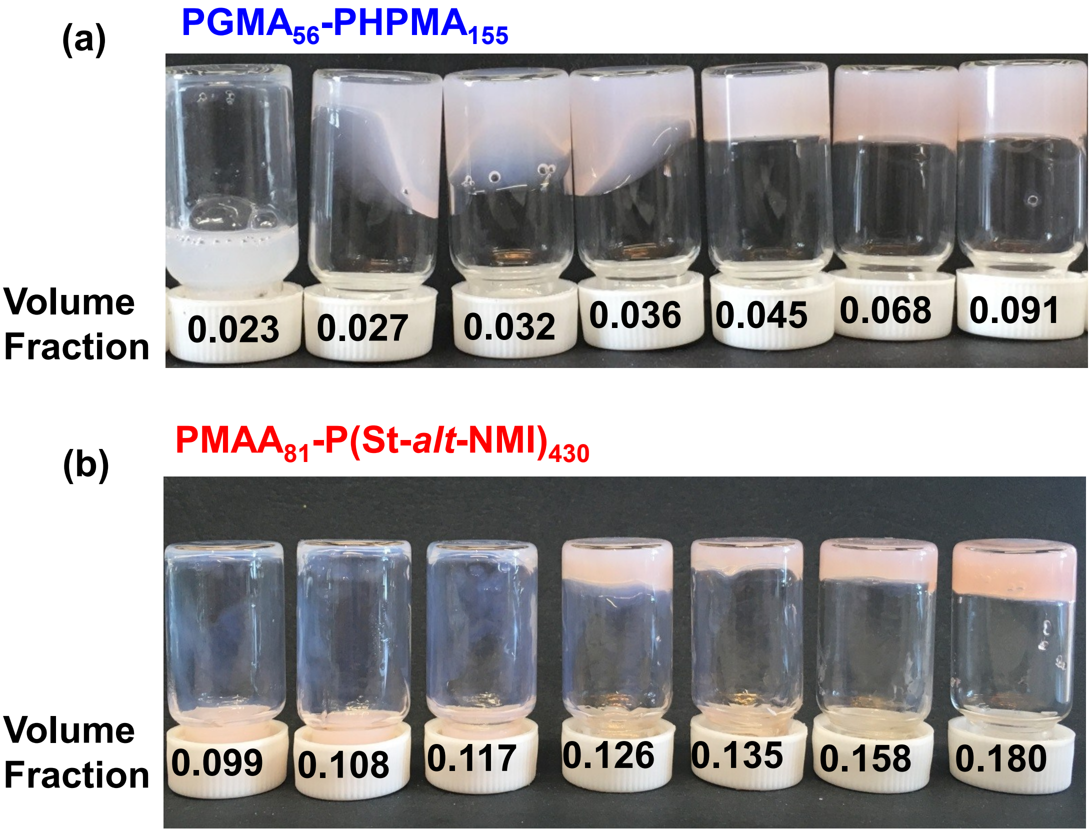


Figure S3. Tube inversion tests conducted at approximately 17 °C for a series of (a) $PGMA_{56}$-$PHPMA_{155}$ worms and (b) $PMAA_{81}$-P(St-alt-NMI)$_{430}$ worms at the stated copolymer volume fraction.

Calculation of composite density and volume fraction

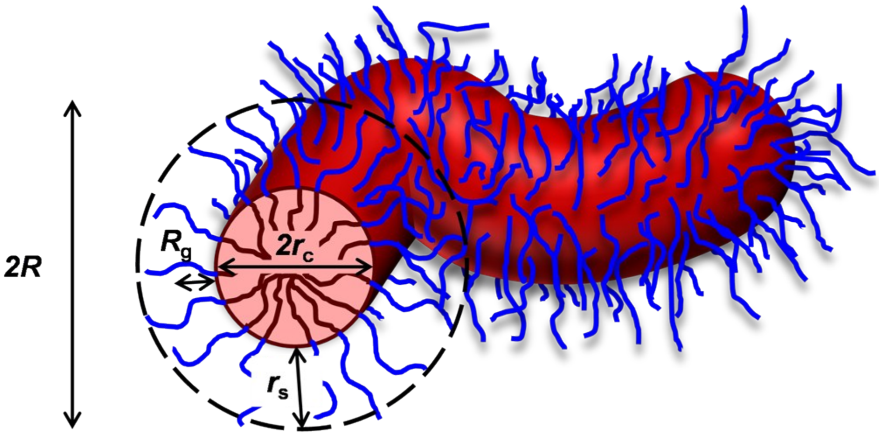
The effective density (ρ_e) of a composite material such as a core-shell cylinder can be determined using the following equation:

$$\rho_e = \frac{\rho_1 \rho_2}{M_1 \rho_2 + M_2 \rho_1} \tag{S1}$$

where M_1, ρ_1 and M_2, ρ_2 are the mass fractions and densities of the cylindrical core and the outer shell layer, respectively. In the case of $PGMA_{56}$-$PHPMA_{155}$ worms, ρ_1 is determined to be 1.21 g cm^{-3} by helium pycnometry and ρ_2 is estimated to be equal to the density of water (1.00 g cm^{-3}). Prior to determining M_{f1} and M_{f2}, the masses of the worm core (m_1) and the worm shell (m_2) must be calculated. Here, $m_1 = \pi r_c^2 L \rho_1$, where r_c is the mean worm core radius and L is the mean worm contour length, which are determined to be 8.5 nm and 1100 nm respectively by SAXS (see data fit to the SAXS pattern shown in Figure 2). Similarly, $m_2 = [\pi(r_c + r_s)^2 L - \pi r_c^{\,2} L]\rho_2$, where r_s is the mean stabilizer layer thickness, which is estimated to be approximately twice the radius of gyration, R_g (or 3.6 nm), determined for the $PGMA_{56}$ stabilizer block. Thus the ρ_e for the $PGMA_{56}$-$PHPMA_{155}$ worms is calculated to be 1.10 g cm^{-3} using equation (S1**).** Consequently, the experimental CGC is reduced from 2.75% w/w to 2.50% v/v, or a volume fraction of 0.025.

Similarly, ρ_e for the $PMAA_{81}$-P(St-*alt*-NMI)$_{430}$ worms is calculated to be 1.11 g cm^{-3}. In this case, ρ_1 is determined to be 1.32 g cm^{-3} by helium pycnometry and ρ_2 is approximated as the density of the 50/50 w/w ethanol/1,4-dioxane mixture (or 0.913 g cm^{-3}). From SAXS analyses, we find that r_c = 20.0 nm, r_s = 6.6 nm and L = 296 nm. Consequently, the experimental CGC is reduced from 12.5% w/w to a volume fraction of 0.113.

Calculation of the effective cross-sectional radius for sterically-stabilized worms



The effective worm cross-section radius, R, can be determined using the following equation:

$$R = r_c + r_s \tag{S2}$$

Where r_c is the mean radius of the worm core and r_s is the mean radius of the worm shell (assuming a Gaussian coil conformation for the stabilizer chains, r_s is approximately equal to twice the radius of gyration, R_g). Both r_c and R_g were determined using SAXS analysis. In the case of the PGMA$_{56}$ stabilizer, R_g was determined to be 1.80 nm, which is in good agreement with SAXS data reported by Akpinar and co-workers for a series of four PGMA$_x$ (x = 28 to 98) homopolymers in aqueous solution.[4]

Systematic underestimation of the theoretical percolation volume fraction, ϕ_c

Chatterjee[5] derived equation (S3) from equation (S4) by assuming that the standard deviation of the rod cross-sectional radius, σ_R, was equal to zero: this means that the rods are assumed to have a perfectly monodisperse cross-sectional radius, R.

$$\emptyset_C \approx \left(\frac{R}{L_W}\right)\left(\frac{1+\frac{\sigma_R^2}{R^2}}{1+\frac{\sigma_R^2}{4R^2}}\right) \tag{S3}$$

$$\emptyset_C \approx \frac{R}{L_w} \tag{S4}$$

However, if δ = $(\sigma_R/R)^2$, then equation (S4) can be written in the following form:

$$\emptyset_C \approx \left(\frac{R}{L_W}\right)\left(\frac{1+\ \delta}{1+\frac{\delta}{4}}\right) = \left(\frac{R}{L_W}\right)\left(1+\frac{3\delta}{4+\delta}\right) \tag{S5}$$

The worm cross-sectional radius, *R*, is indeed fairly well-defined as judged by small-angle X-ray scattering (see main text). Nevertheless, *R* does possess a finite standard deviation and therefore δ ≥ 0. Thus it is clear from equation (S5) that the above assumption must necessarily result in a systematic *underestimation* in the theoretical numerical value for ϕ_c.

Worm-like micelle SAXS model

In general, the X-ray intensity scattered by a dispersion of nano-objects [usually represented by the scattering cross section per unit sample volume, $\frac{d\Sigma}{d\Omega}(q)$] can be expressed as:

$$\frac{d\Sigma}{d\Omega}(q) = NS(q)\int_0^\infty \dots \int_0^\infty F(q,r_1,\dots,r_k)^2 \Psi(r_1,\dots,r_k) dr_1 \dots dr_k \tag{S6}$$

where $F(q,r_1,\dots,r_k)$ is their form factor, $r_1,\dots,r_k$ is a set of *k* parameters describing the structural morphology, $\Psi(r_1,\dots,r_k)$ is the distribution function, S(*q*) is the structure factor and *N* is the nano-object number density per unit volume expressed as:

$$N = \frac{\varphi}{\int_0^\infty \dots \int_0^\infty V(r_{1,}\dots,r_k)\Psi(r_{1,}\dots,r_k)dr_1\dots dr_k} \tag{S7}$$

where $V(r_{1,}\dots,r_k)$ is the volume of the nano-object and φ is their volume fraction in the dispersion.

The worm-like micelle form factor for Equation (S is given by:[6]

$$F_{w\_mic}(q) = {N_w}^2{\beta_s}^2F_{sw}(q) + N_w{\beta_c}^2F_c(q,R_g) + N \tag{S8}$$

where the core block and the corona block X-ray scattering length contrast is given by $\beta_s = V_s(\xi_s - \xi_{sol})$ and $\beta_c = V_c(\xi_c - \xi_{sol})$, respectively. Here ξ_s, ξ_c and ξ_{sol} are the X-ray scattering length densities of the core block (ξ_s: ξ_{PHPMA} = 11.11 x 10^{10} cm^{-2} or $\xi_{P(St\text{-}alt\text{-}NMI)}$ = 10.81 x 10^{-10} cm^{-2}), the corona block (ξ_c: ξ_{PGMA} = 11.94 x 10^{10} cm^{-2} or ξ_{PMAA} = 11.88 x 10^{-10} cm^{-2}) and the solvent (ξ_{sol}: ξ_{H20} = 9.42 x 10^{10} cm^{-2} or $\xi_{EtOH/dioxane}$ = 8.41 x 10^{-10} cm^{-2}), while V_s and V_c are volumes of the core block (V_{PHPMA} or $V_{P(St\text{-}alt\text{-}NMI)}$) and the corona block ($V_{PGMA}$ or V_{PMAA}). Each of these volumes was obtained from $V = \frac{M_{n,pol}}{N_A\rho}$ using the solid-state densities of PGMA (ρ_{PGMA} = 1.31 g cm^{-3}), PHPMA (ρ_{PHPMA} = 1.21 g cm^{-3}), PMAA (ρ_{PMAA} = 1.31 g cm^{-3}) and P(St-*alt*-NMI) ($\rho_{P(St\text{-}alt\text{-}NMI)}$ = 1.21 g cm^{-3}) as determined by helium pycnometry, where $M_{n,pol}$ corresponds to the number-average molecular weight of the block determined by ^{1}H NMR spectroscopy. The self-correlation term for the worm-like micelle core or radius R_{sw} is:

$$F_{sw}(q) = F_{worm}(q,L_w,b_w){A_{cs_{worm}}}^2(q,R_{sw}) \tag{S9}$$

which is a product of a core cross-section term:

$$F_{cs_{worm}}(q,R_g) = {A_{cs_{worm}}}^2(q,R_{sw}) = \left[2\frac{J_1(qR_{sw})}{qR_{sw}}\right]^2 \tag{S10}$$

where J_1 is the first-order Bessel function of the first kind, and a form factor $F_{worm}(q,L_w,b_w)$ for self-avoiding semi-flexible chains represents the worm-like micelle, where b_w is the worm Kuhn length and L_w is the mean worm contour length. A complete expression for the chain form factor can be found elsewhere.[7] The self-correlation term for the corona block is given by the Debye function:

$$F_c(q,R_g) = \frac{2\left[\exp\left(-q^2{R_g}^2\right) - 1 + q^2{R_g}^2\right]}{q^4{R_g}^4} \tag{S11}$$

where R_g is the radius of gyration of the coronal block. The interference cross-term between the worm micelle core and the corona chain is given by:

$$S_{sc}(q) = \Psi^2(qR_g){J_0}^2\left[q\left(R_{sw} + R_g\right)\right]F_{worm}(q,L_w,b_w) \tag{S12}$$

where $\Psi(qR_g) = \frac{1 - exp\left(-q^2{R_g}^2\right)}{(qR_g)^2}$ is the form factor amplitude of the corona chain, R_g is the radius of gyration of the corona block and J_0 is the zero-order Bessel function of the first kind. The interference term between the worm corona chains is:

$$S_{cc}(q) = \Psi\left(qR_g\right)A_{cs\_worm}J_0\left[q\left(R_{sw} + R_g\right)\right]F_{worm}(q,L_w,b_w) \tag{S13}$$

It is also assumed that $S(q) = 1$ at sufficiently low copolymer concentrations (e.g. 1.0% v/v).

In an attempt to estimate the associated uncertainty in the calculated contour length for each type of diblock copolymer worm, each L_w value was systematically varied in order to examine how this affected the quality of the fit to the corresponding SAXS pattern. For example, for the relatively long flexible PGMA-PHPMA worms with an L_w of 1100 nm, this parameter was varied by ± 100. Unfortunately, this approach proved ineffective because of the limited q range over which the scattering data were acquired. This led to extremely poor sensitivity, so no error estimate could be obtained.

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