ADVANCED FUNCTIONAL MATERIALS

Supporting Information

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Bioinspired Reversibly Cross-linked Hydrogels Comprising Polypeptide Micelles Exhibit Enhanced Mechanical Properties

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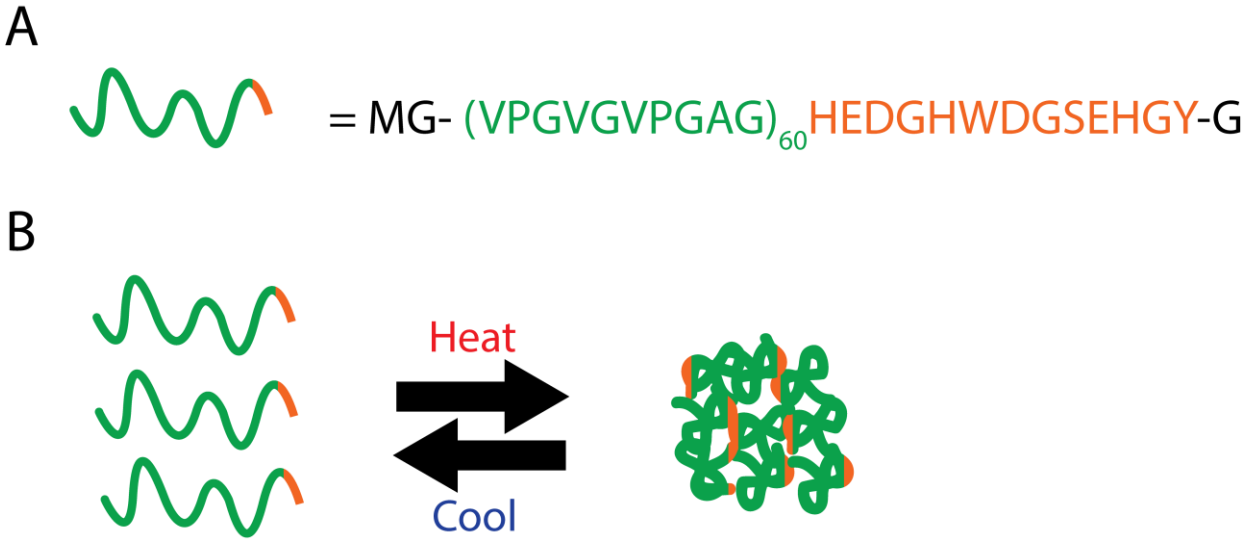

Figure S1. Thermally triggered aggregation of NMF-ELPs into micron-sized aggregates. A) The sequence of the NMF-ELP. B) Schematic diagram of reversible temperature-triggered aggregation and dissociation of polypeptides into large particles.

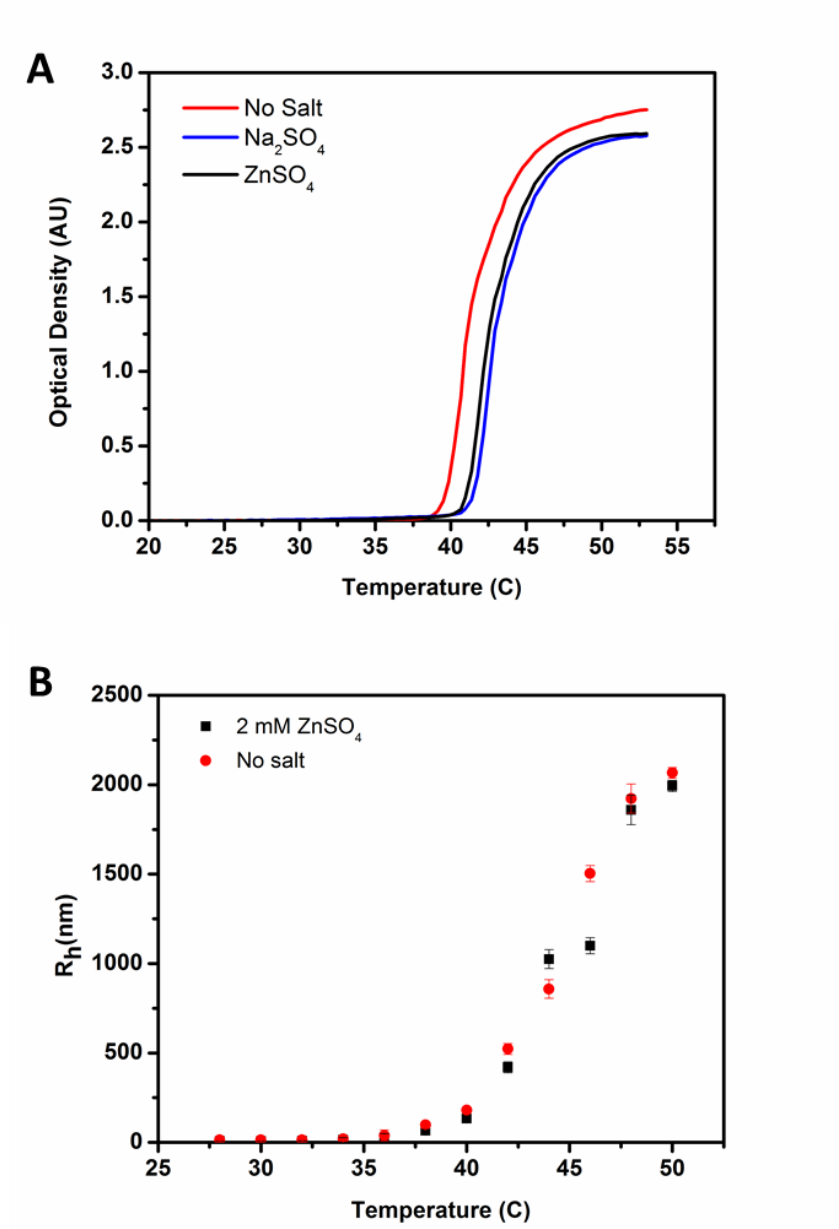

Figure S2. Thermal behavior of NMF-ELP solutions (100 µM, 0.5 % w/v) in the absence and in the presence of salts. A) Optical density as a function of temperature in the absence of salt (red line), in the presence of 2 mM $ZnSO_4$ (blue line), and in 2 mM Na_2SO_4 (black line). B) Hydrodynamic radius (R_h) as a function of temperature with no added salt (red circles), and with 2 mM $ZnSO_4$ (black squares).

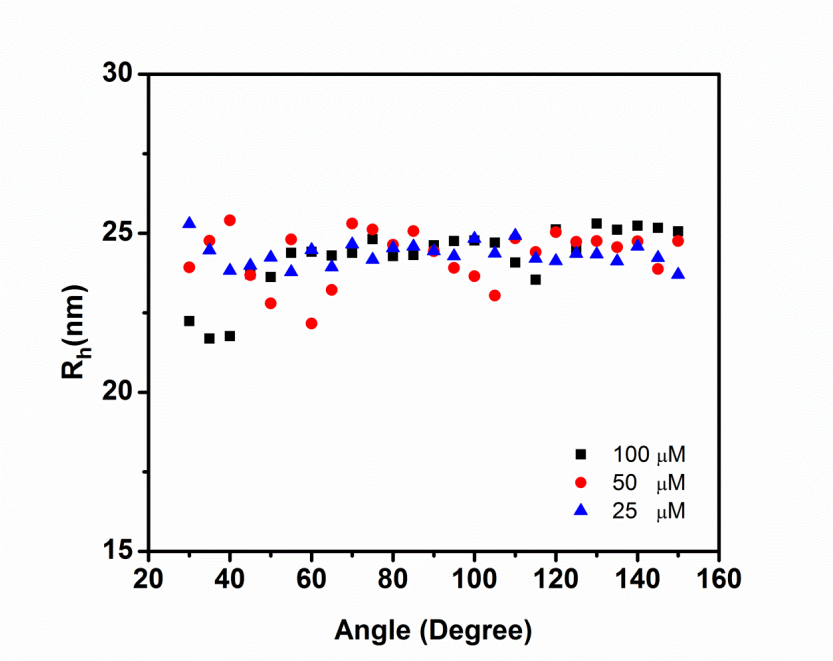

Figure S3. DLS of MF-ELP in water [25μM (blue triangles), 50μM (red circles), and 100 μM (black squares) at scattering angles between 30˚ and 150˚.

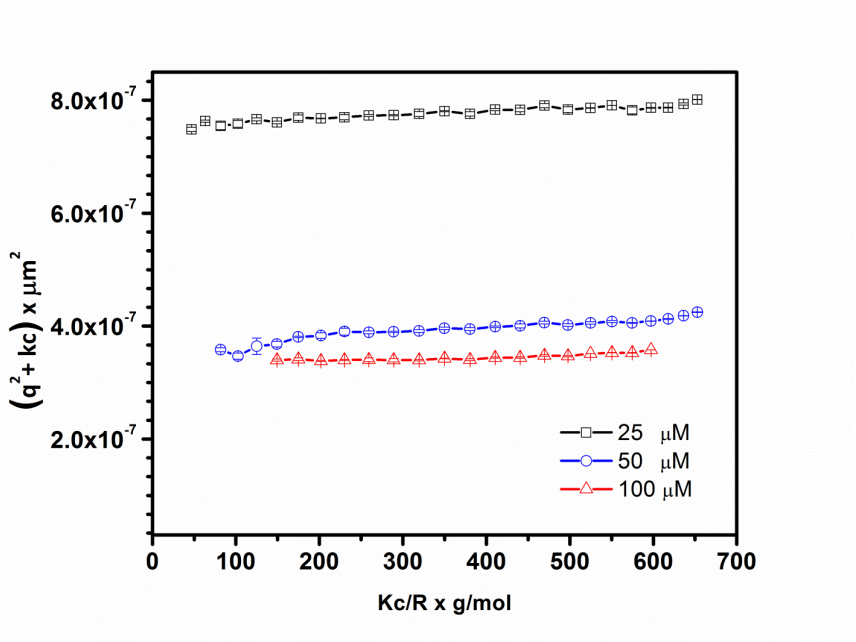

Figure S4. Static light scattering data for MF-ELP at 25 µM (squares), 50 µM (triangles), and 100 µM (diamonds). In this plot q is the scattering vector, k is an arbitrary constant, K is optical contrast, R is the Rayleigh constant, and c is concentration.

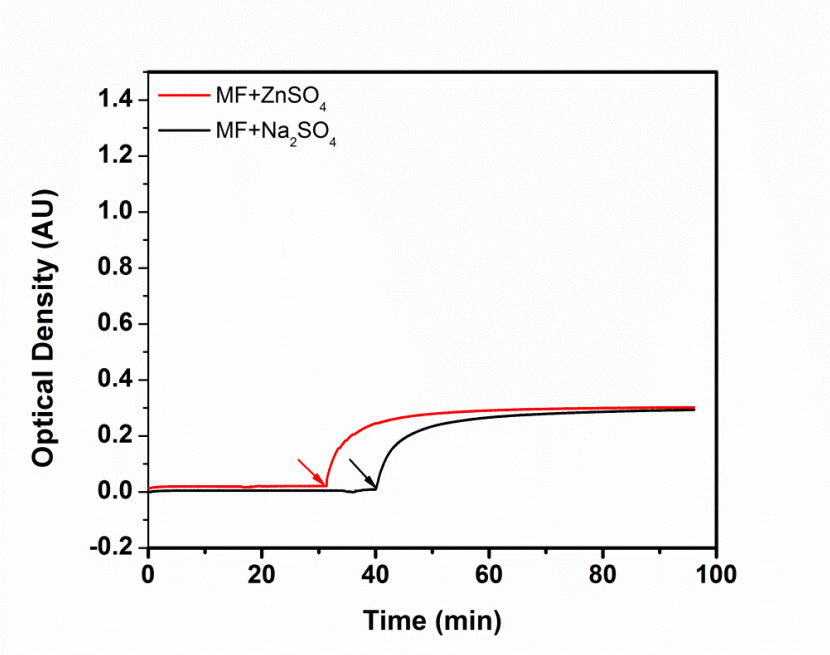

Figure S5. Micelle formation in a 10 % w/v solution of MF-ELP at room temperature (25ºC) upon addition of 20 mM $ZnSO_4$ or Na_2SO_4 and the arrows show the time at which the salts were added. The slight increase in optical density (to ≈0.3) is indicative of the formation of nanoscopic micelles (see Figure 1D).

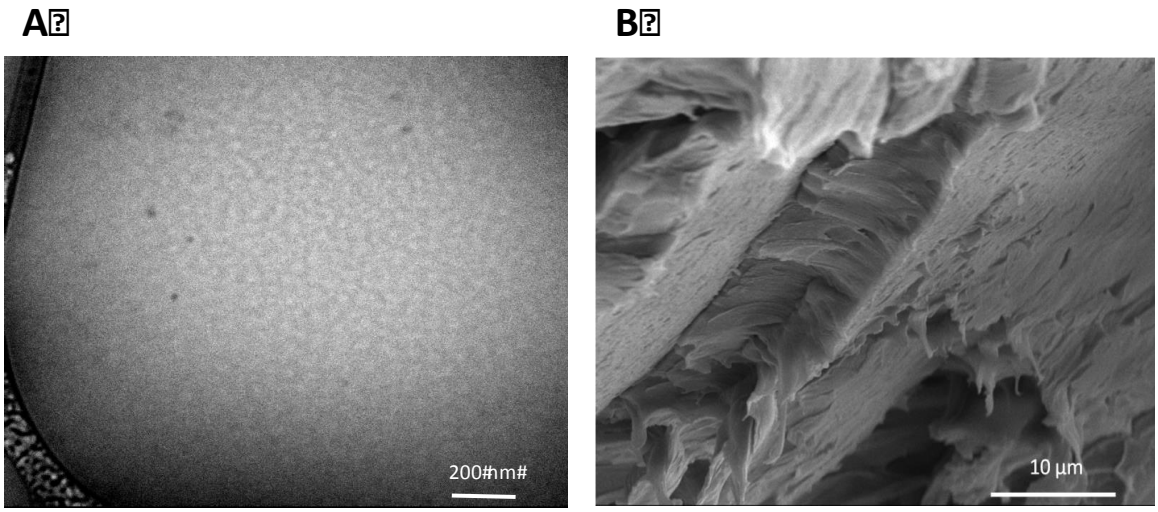

Figure S6. Characterization of the microstructure of NMF-ELP coacervates by electron microscopy. A) Cryo-TEM of coacervate (1 % w/v NMF-ELP solution vitrified 10 s after addition of zinc sulfate (2 mM) at 45 °C). B) SEM of a 10 % w/v NMF-ELP coacervate freeze-dried 12 hr after the addition of $ZnSO_4$ (20 mM) at 45 °C.

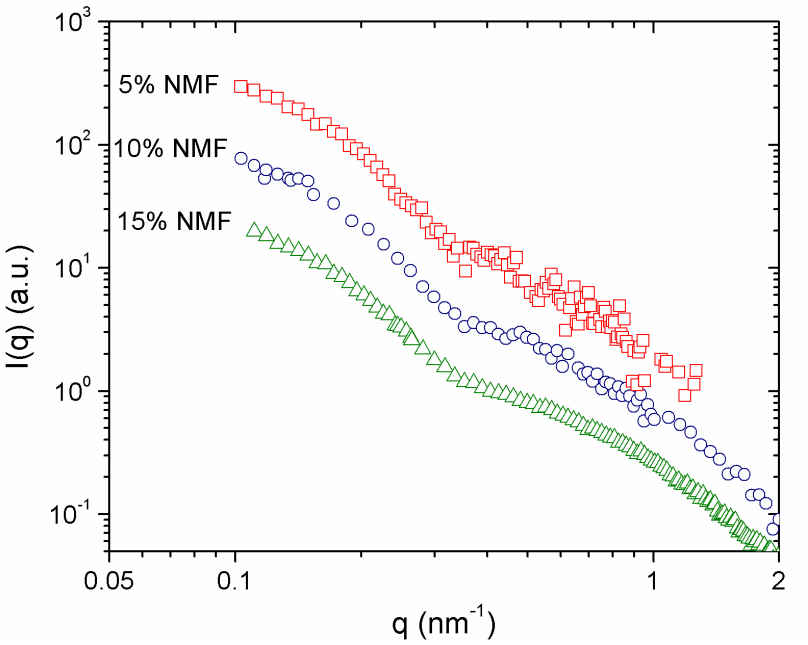

Figure S7. SAXS intensity profiles for NMF-ELP coacervate formed at 45°C from three different concentrations of NMF-ELP. Data were taken after the addition of zinc sulfate (10 mM). Squares: 5 % w/v, circles: 10 % w/v triangles: 15 % w/v; the data has been offset for clarity of presentation.

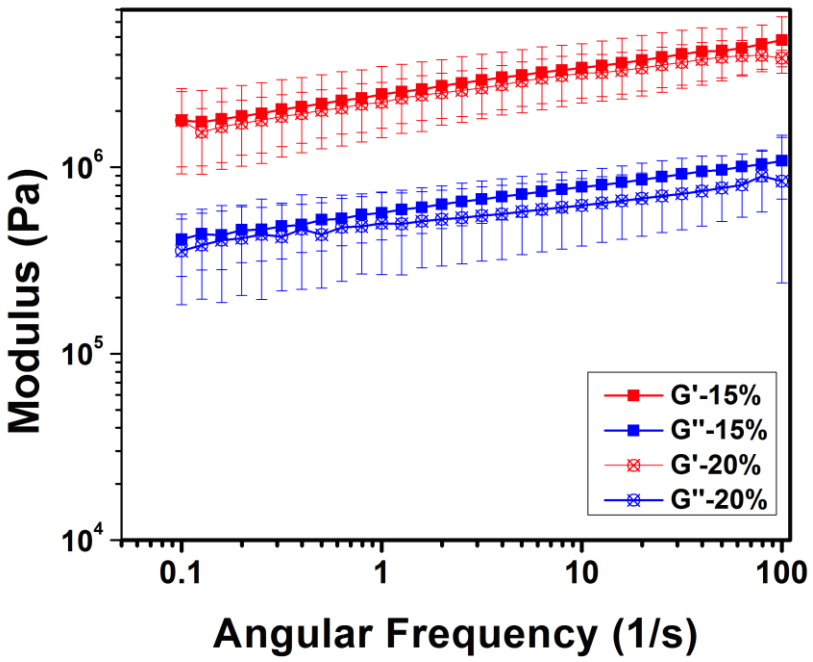

Figure S8. Storage (red) and loss (blue) moduli of 15 % w/v and 20 % w/v MiGels made by incubation of MF-ELP with 30 mM and 40 mM zinc sulfate respectively for 12 hr at 45°C Each point on the plot is an average value of three independent measurements and error bars are calculated standard deviations of the three measurements.

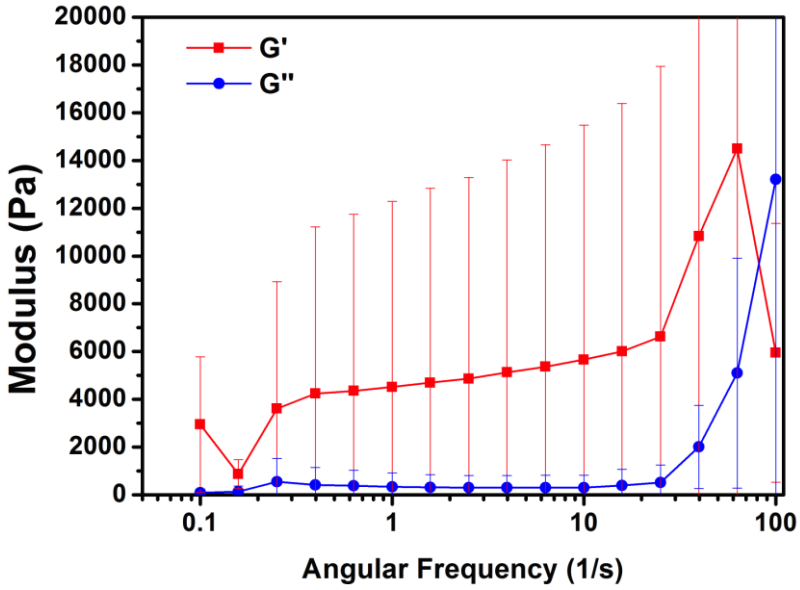

Figure S9. Storage and loss moduli of 10 % w/v NMF-ELP coacervates formed by addition of 20 mM Zn^{2+} to polypeptide solutions at 45°C. The solutions were kept at 45°C for 24 hrs before experiments.

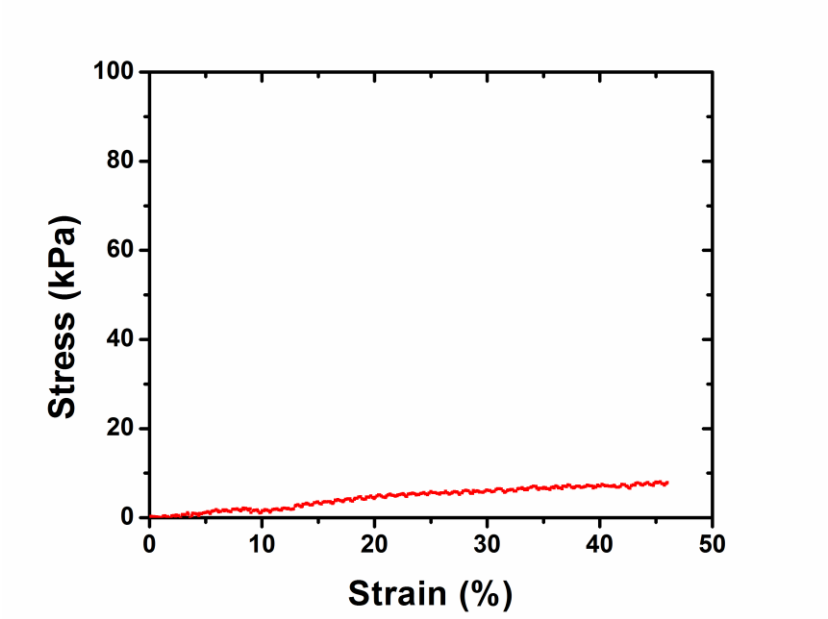

Figure S10. Compressive stress-strain curve for 10 % w/v NMF-ELP coacervate after incubation with 20 mM zinc sulfate for 6 hrs at 45°C.

Scattering theory and data modeling:

At a given scattering vector (q), the scattered intensity (I) of a suspension of particles is given by

$$I(q) = NV_P^2 \Delta\rho_s^2 P(q) S(q) \quad (1)$$

where N is the number density of the scattering objects, V_p is the volume of one scattering particle, $\Delta\rho_s$ is the scattering contrast of a particle against the matrix, P(q) is the form factor determined by the shape and size of the particles and S(q) is the structure factor originating from the inter-particle interactions.[53] In this work, we focus solely on analyzing the form factor and interpret the inter-particle correlations empirically without model fitting.

We approximated the scattering originating from micelles in MiGels on the basis of the form factor of a spherical core-shell model. The form factor of such a core-shell structure is be given by

$$P_{sph-shell}(q) = |F(q, R + dR, \Delta\rho_{shell-water}) - F(q, R, \Delta\rho_{shell-core})|^2 \quad (2)$$

$$F(q, R, \Delta\rho) = \frac{4}{3}\pi R^3 \Delta\rho J_1(qR) \quad (3)$$

where R is the core radius, *d*R is the shell thickness, $J_1(qR)$ is the first order spherical Bessel function, $\Delta\rho_{shell\text{-}water}$ and $\Delta\rho_{shell\text{-}core}$ are the scattering length densities contrast between shell-water and shell-core, respectively. In MiGels, the spherical shell is composed of polymer chains and the solvent penetration is well known for such assemblies, [54] hence a linear decay in the scattering length density away from the core has been assumed and is given by[55]

$$\rho_{shell}(r) = \left(\frac{x_{R+dR}\rho_{chains} - x_R\rho_R}{dR}\right) r - \left(\frac{x_{R+dR}\rho_{R+dR} - x_R\rho_R}{dR}\right) R + \rho_R \quad (4)$$

where x_R and x_{R+dR} are the fractions of solvent at distances R and R+dR from the center of the core-shell particle, respectively.

The polypeptide chains within the hydrogel can exist in two distinct configurations: as self-assembled micelles and as non-assembled free polymer chains. The scattering originating from both forms can be modeled based on Gaussian chains and the form factor of such chains is given by[56]

$$P_{Gauss}(q) = 2 \times I(q = 0) \times \frac{e^{-q^2Rg^2} + q^2Rg^2 - 1}{(q^2Rg^2)^2} \quad (5)$$

where I(q = 0) is the forward scattering due to polypeptide chains and R_g is the radius of gyration of the chains. The overall scattering intensity of MiGels ($I_{shell\text{-}gauss}$) is given by the sum of the individual contributions of free chains and chains in the core-shell morphology and can be estimated by equations (1), (2) and (5).

ρ_{core} (cm^{-2})	x_R	x_{R+dR}	dR (nm)	R (nm)	R_g (nm)	I_0
1.32×10^{-4}	0.6	0.95	5.3	15.7	2.7	2.5

Table S1. Summary of the parameters used to fit the X-ray scattering data for the 5 % w/v MiGels.