Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supplementary Information

Self-Assembly of Protein-Zwitterionic Polymer Bioconjugates into Nanostructured Materials

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Materials

3-[*N*-(2-methacroyloyethyl)-*N*,*N*-dimethylammonio]propane sulfonate (DMAPS) monomer (97%, CAS 3637-26-1) was purchased from Sigma-Aldrich. 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (CPP) (min. 97%) was purchased from Strem Chemicals. 2,2'-Azobis[2-(2-imidazolin-2-yl)propane]dihydrochloride (VA-044) was purchased from Wako Chemicals. Trifluoroethanol (ReagentPlus®, ≥99%) was purchased from Sigma-Aldrich. Deuterium oxide (D, 99.8%, sterility tested) was purchased from Cambridge Isotope Laboratories Inc. Other reagents were purchased from Sigma-Aldrich and used without further purification unless otherwise stated.

Calculations of the hydration of protein and polymer

The number of available water molecules per monomer of PDMAPS ($N_{H2O,DMAPS}$) can be obtained from equation 1, where w is the wt.% of bioconjugate in solution, $M_{n,BC}$ is the average molar mass of bioconjugate, DP is the degree of polymerization of PDMAPS, M_{H2O} is the molar mass of water (18.015 g/mol), and f is the fraction of water hydrating polymer domain. In the case of a 50 wt.% solution of mChPD33 ($M_{n,BC}$ 32,800 g/mol and DP 116) and the case of equal hydration (f = 0.5), $N_{H2O,DMAPS}$ is 14.6.

$$N_{H2O,DMAPS} = \frac{\frac{(1-w)}{M_{H2O}} f}{\frac{w}{M_{n,BC}} DP} \quad (1)$$

Similarly, the average number of available water molecules per mCherry molecule ($N_{H2O,mCherry}$) is obtained from the equation 2. 1,691 molecules of water are available for mCherry, which using the bulk density of water of 18.015 g/cm^3 would occupy a volume (V_H) 59.6 nm^3, in the case of mChPD33 and equal hydration between PDMAPS and mCherry domain. The average thickness of the hydration shell of the protein is estimated as a cylindrical shell with this volume. mCherry is assumed to be a cylinder with a length (l) 4.4 nm and radius (R) 1.25 nm based on the crystal structure (PDB 2H5Q).[1] The volume of a hydration layer of mCherry (V_H) with thickness d is given by $V_H = \pi(R+d)^2(l+2d) - \pi R^2 l$, in which d can be determined based on the V_H obtained above. d is estimated to be 0.84 nm.

$$N_{H2O,mCherry} = \frac{\frac{(1-w)}{M_{H2O}}(1-f)}{\frac{w}{M_{n,BC}}} \quad (2)$$

Calculation of Debye lengths.

The Debye lengths (κ^{-1}) of salt solutions with various concentration of NaCl and $(NH_4)_2SO_4$ are calculated based on the equation shown below.

$$\kappa^{-1} = \sqrt{\frac{\varepsilon_0 \varepsilon_r k_B T}{\sum \rho_{\infty,i} e^2 z_i^2}} \quad (3)$$

where ε_0 is vacuum permittivity, ε_r is relative permittivity, k_B is Boltzmann constant, T is temperature in Kelvin, i represents each ionic species, ρ is the number density [m^{-3}], e is the elementary charge, and z is the charge number.

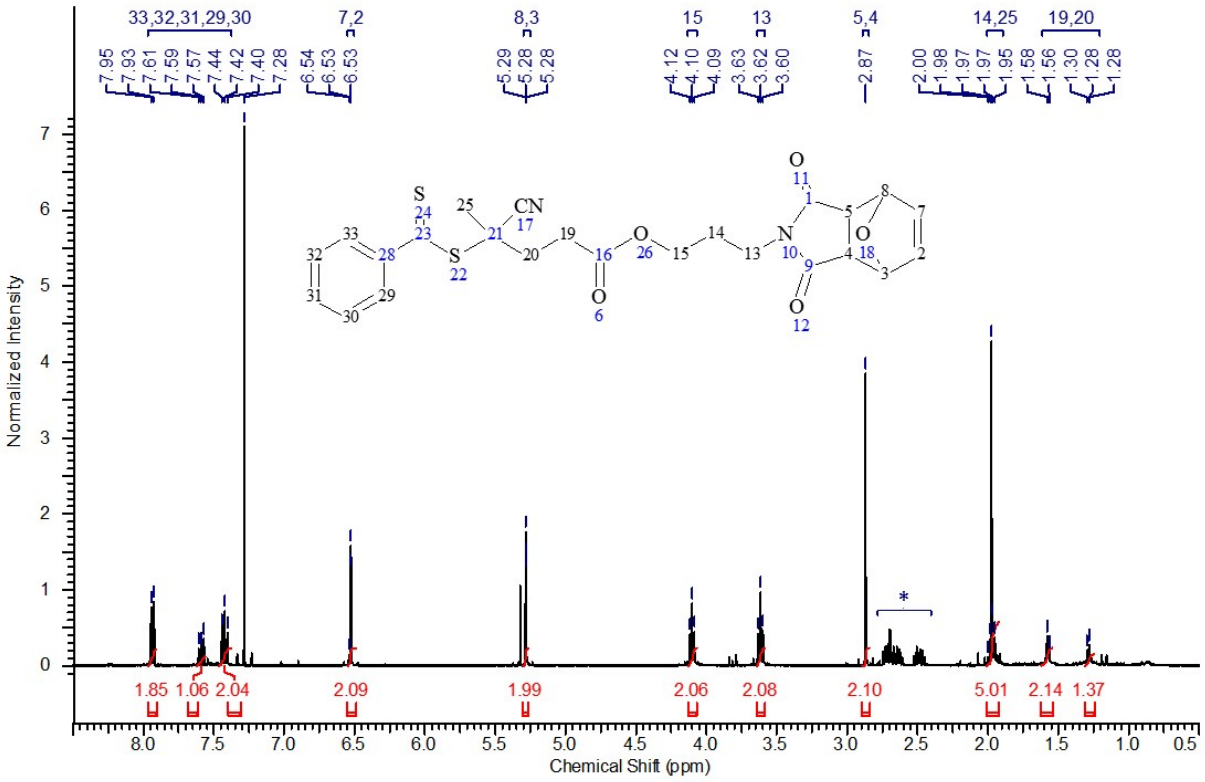

Figure S1. H^1-NMR spectrum of CPP-imide in $CDCl_3$ solution. The peaks at δ2.4-2.8 (labeled with *) originate from unreacted CPP which would result in a small fraction of polymer without the protected maleimide end group. This polymer without a maleimide end group would not react with proteins and thus would be removed in successive purification as well as unreacted hompolymers with a maleimide end group.

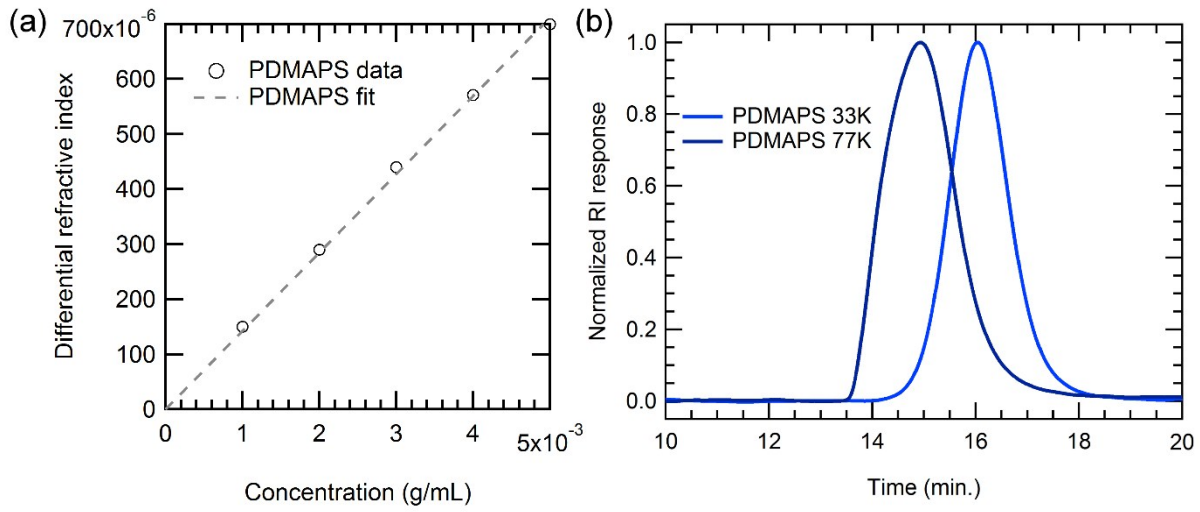

Figure S2. Characterization of PDMAPS. (a) Plot of differential refractive index of PDMAPS. dn/dc value is measured using a built-in batch mode software provided by Wyatt Technology. 1,2,3,4, and 5 mg/mL of polymer solutions were prepared in 0.5 M NaCl solution. The dn/dc was measured to be of 0.1423 ± 0.0024 mL/g (R^2=0.9979). (b) GPC trace of PDMAPS used in this study.

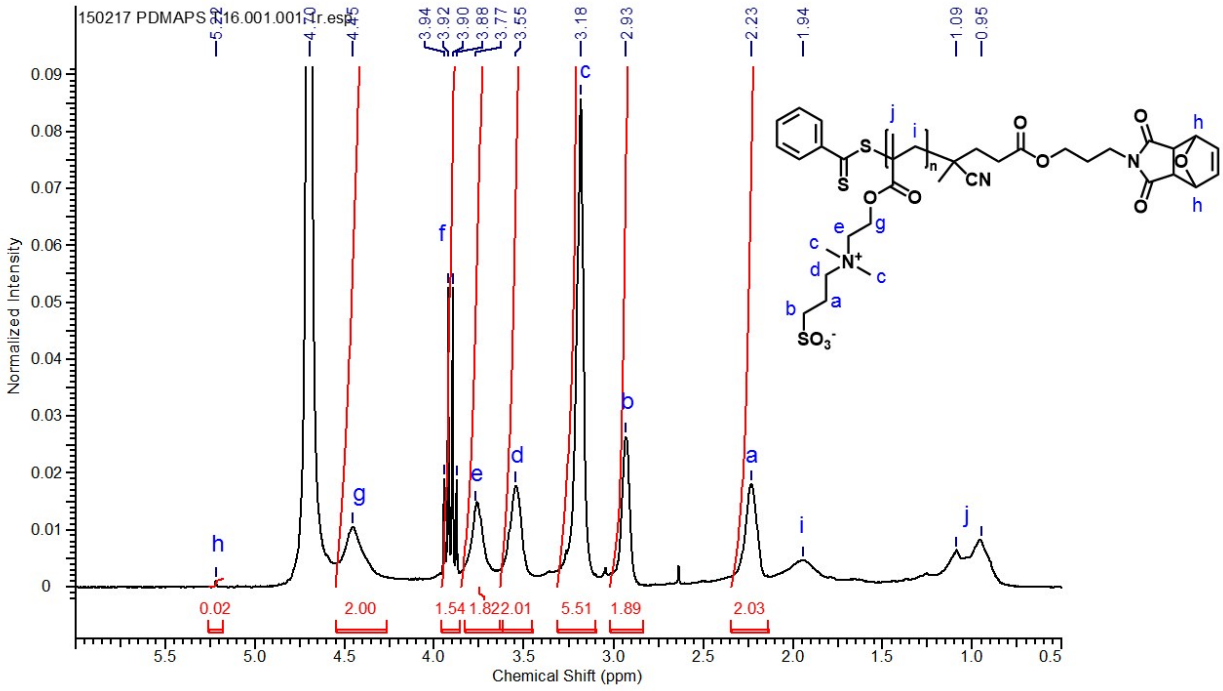

Figure S3. ^{1}H-NMR spectrum of PDMAPS with M_n 32,880 g/mol dissolved in D_2O.

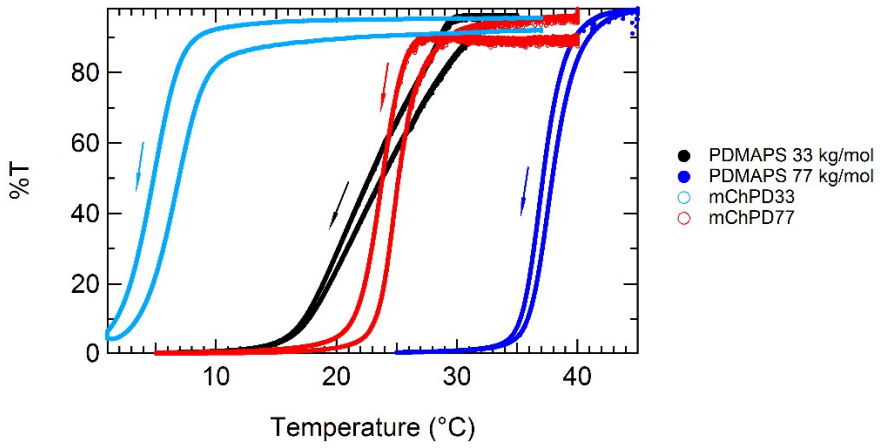

Figure S4. Thermal transition of PDMAPS homopolymers and corresponding bioconjugates. 10 mg/mL polymer solution in Milli-Q water was cooled down first and heated. Cooling traces are marked with arrows pointing downwards. The differences between cloud point measured from 10% reduction in transmittance and that from 50% reduction are 12.3, 1.8, 2.8, and 1.8 °C for PDMAPS 33 kg/mol, PDMAPS 77 kgm/mol, mChPD33, and mChPD77, respectively, which represents the breadth of their thermal transitions. The broad transition of PDMAPS 33 kg/mol homopolymer is thought to be due to kinetic effects, as the slope changes with a ramp rate.

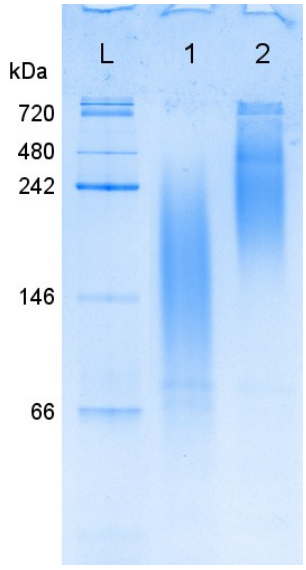

Figure S5. Native gel of purified products. Lane L, lane 1, and lane 2 represent protein ladder, mChPD33 conjugate, and mChPD77 conjugate, respectively. Lanes between the ladder and bioconjugates are omitted for clarity.

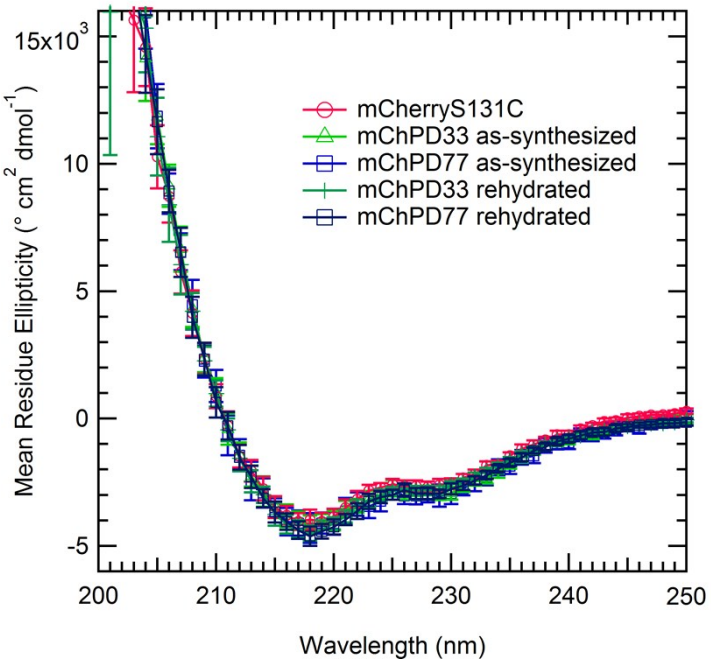

Figure S6. CD spectra of mCherryS131C and bioconjugates measured at 25 °C. mCherryS131C is measured in 20 mM Tris Cl (pH 8.0), and the bioconjugates are measured in 50 mM NaCl solution. Signal from 20 mM Tris Cl (pH 8.0) is subtracted from mCherryS131C data, and signal from 50 mM NaCl solution is subtracted from bioconjugate data.

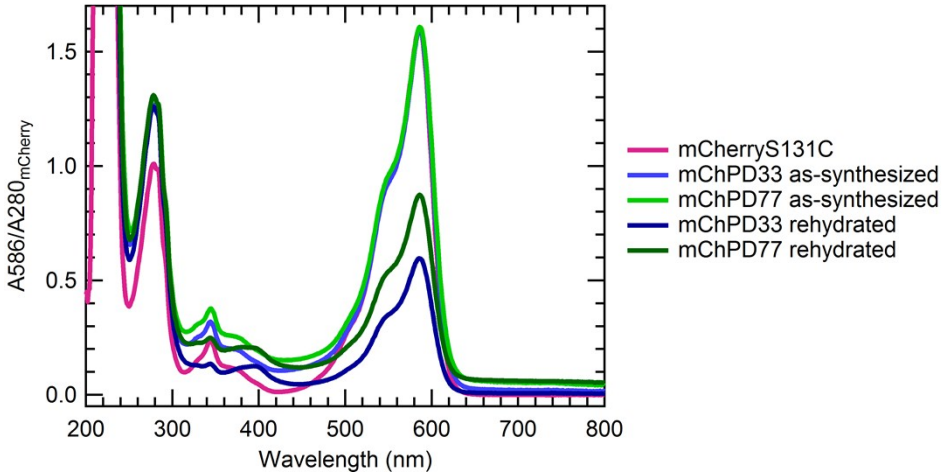

Figure S7. UV-vis spectra of mCherryS131C, mChPD33 conjugate, and mChPD77 conjugate. Protein activity of bioconjugates is analyzed by accounting for PDMAPS absorption at 280 nm with molar attenuation coefficients 8,190 M^{-1} cm^{-1} and 9,715 M^{-1} cm^{-1} for PDMAPS 33 kg/mol and 77 kg/mol polymers, respectively. The signal is normalized using an estimated absorbance of mCherry at 280 nm ($A280_{mCherry}$). Plot of $A586/A280_{mCherry}$ show that 98% of mCherry activity is preserved in mChPD33. The activity of mCherry in mChPD77 conjugate is estimated to be 109% of the original mCherry activity. mChPD33 and mChPD77 retain 38% and 54% of activity of as-synthesized conjugates after drying and rehydration after accounting for polymer absorption.

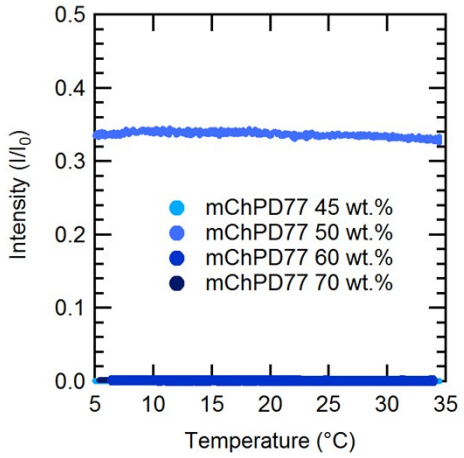

Figure S8. Birefringence measurement of mChPD77 solutions in water as a function of temperature. The 50 wt.% curve is reported in the main section but plotted here again for comparison.

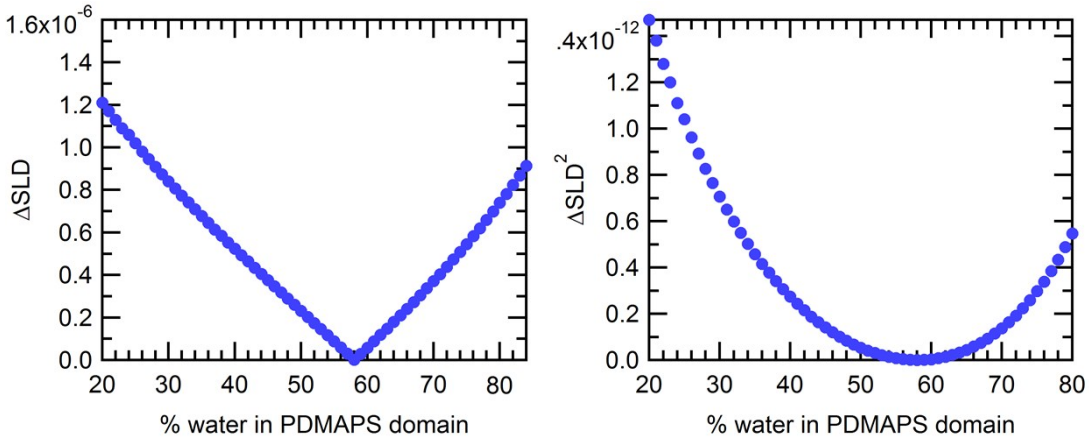

Figure S9. A plot of calculated X-ray scattering contrast (ΔSLD) and ΔSLD^2 as water distribution changes.

Table S1. Scattering length density of each component calculated at 8 keV and 12 keV from https://www.ncnr.nist.gov/resources/activation/

	Compound Formula	**Mass density (g/cm^3)**	**X-ray SLD (real part) (10^{-6} $Å^2$)**
mCherryS131C	$C_{1250}H_{1915}N_{341}O_{377}S_{12}$	1.35	12.245
PDMAPS	$C_{11}H_{21}NO_5S$	1.37	12.521
Water	H2O	1.00	9.444

Table S2. Solubility of mCherryS131C and PDMAPS homopolymer in different salt solutions.

Solubility*	**Deionized water**	**25 mM NaCl**	**1 M NaCl**	**1 M $(NH_4)_2SO_4$**
mCherryS131C	Good (> 200 mg/mL)	Good (> 200 mg/mL)	Not tested	Poor (<10 mg/mL)
PDMAPS (M_n 32.9 kg/mol)	Poor (T_t 27.5 °C at 10 mg/mL)	Moderate (T_t 13.6 °C at 10 mg/mL)	Good (>100 mg/mL)	Moderate-Good (50-100 mg/mL)

T_t: Thermal transition temperature due to UCCST behaviour

*Solubility is measured at room temperature.

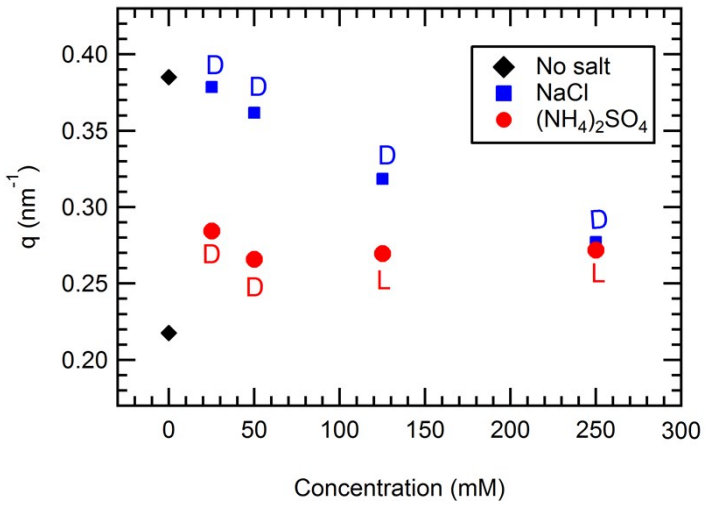

Figure S10. Peak positions at the maximum intensity as a function of salt concentrations of mChPD33 bioconjugate at 50 wt.%. Phase formed at each point is indicated as a letter, D denoting disordered phase, and L denoting a lamellar phase. The first two peaks from mChPD33 in pure water are indicated in black diamond markers.

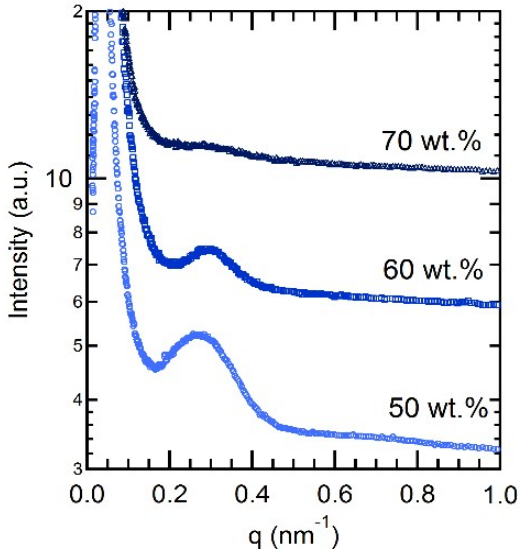

Figure S11. SAXS curves of mChPD33 solutions in the presence of 250 mM NaCl.

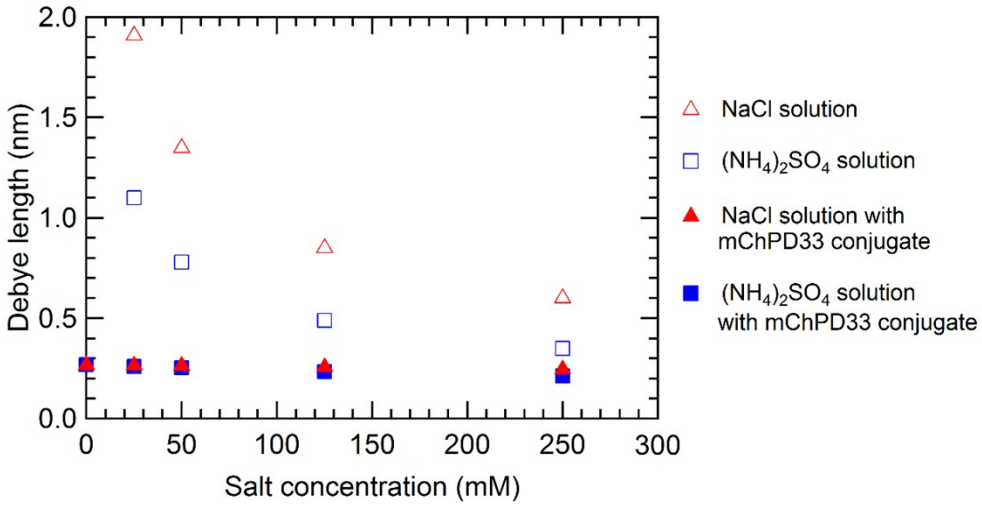

Figure S12. The Debye lengths in bioconjugate solutions in the presence of either NaCl or $(NH_4)_2SO_4$.

References

1 X. Shu, N. C. Shaner, C. A. Yarbrough, R. Y. Tsien and S. J. Remington, *Biochemistry*, 2006, **45**, 9639–47.

2 D. N. Schulz, D. G. Peiffer, P. K. Agarwal, J. Larabee, J. J. Kaladas, L. Soni, B. Handwerker and R. T. Garner, *Polymer*, 1986, **27**, 1734–1742.