Supplementary Information

Effect of Polymer Chemistry on Globular Protein-Polymer Block Copolymer Self-Assembly

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Synthesis of 2-(2-methoxyethoxy)ethyl acrylate (MEEA) 36 g of 2-(2-methoxyethoxy)ethanol (300 mmol) and 37.9 g of triethylamine (375 mmol) were added to 200 ml of dry dichloromethane at 0°C. 32.5 g of acryloyl chloride (360 mmol) mixed with 60 ml of dry dichloromethane was added to the reaction mixture dropwise. The reaction proceeded overnight at room temperature. The solution was filtered to remove salt and washed twice each with 0.1M HCl(aq), saturated NaHCO₃(aq), and 0.5M NaCl(aq). The solution was dried over anhydrous Na₂SO₄ and the solvent was dried to obtain orange brown liquid. Byproduct was further removed by flash chromatography in silica gel using a 1:1 mixture of hexane and ethyl acetate as eluent. A small amount of hydroquinone was added to the crude product, and vacuum distilled at 300 mTorr at 60°C. The product was dissolved in dichloromethane and washed four times with 0.1M KOH(aq) and once with water to remove hydroquinone. A clear liquid was obtained after drying (37.8g, 72%). ¹H NMR (CDCl₃, δ , ppm): 3.34 (3H, -OCH₃), 3.52 (2H, -OCH₂CH₂OCH₃), 3.61 (2H, -OCH₂CH₂OCH₃), 3.72 (2H, -COOCH₂CH₂O-), 4.27 (2H, -COOCH₂CH₂O-), 5.80 (1H, HHC=CH-), 6.12 (1H, HHC=CH-), 6.41 (1H, H₂C=CH-).



Figure S1. H¹-NMR spectrum of MEEA monomer in CDCl₃ solution.



Figure S2. Plot of differential refractive index of PHPA and POEGA. dn/dc values of PHPA and POEGA are 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 N-dimethyl formamide containing 0.02M LiBr. The dn/dc of PHPA was measured to be 0.0539 ± 0.0014 mL/g (R²=0.9979) and that of POEGA was 0.0418 ± 0.0007 mL/g (R²=0.9991).



Figure S3. GPC traces of (a) PHPA and (b) POEGA homopolymers with three different molar masses used in this study.



Figure S4. H¹-NMR spectrum of deprotected PHPA 27kDa in CDCl₃ solution. The NMR spectrum shows that the polymer consists of 64% of the major isomer and 36% of the minor isomer.



Figure S5. H¹-NMR spectrum of deprotected POEGA 26kDa in CDCl₃ solution. The molar monomer ratio of MEA and MEEA (x:y) incorporated into the polymer is determined based on the ratio of d-type protons and e-type protons shown in the spectrum.



Figure S6. Native protein gel of mCherry-*b*-PHPA and mCherry-*b*-POEGA bioconjugates. Lanes 1-7 represent ladder, mChPH18, mChPH27, mChPH57, mChPOE18, mChPOE26, and mChPOE57, respectively. The molar purities determined by lane and bands analysis tool in Image Lab software were greater than 98%.



Figure S7. SAXS curves of mCherry-*b*-PHPA and mCherry-*b*-POEGA solutions at 30°C and 35°C, respectively. Curves are vertically offset for clarity.



Figure S8. Circular dichroism (CD) spectrum of mCherry and of (a) mCherry-*b*-PHPA and (b) mCherry-*b*-POEGA, after conjugation and after rehydration of solid-state materials. CD spectrum of mCherry was measured in 20 mM Tris-Cl buffer (pH=8.0) while those of the bioconjugates were measured in water. No significant change in secondary structure is observed after conjugation and self-assembly. (c) UV-Vis spectra of mCherry and of mCherry-*b*-PHPA and mCherry-*b*-POEGA normalized to the absorbance at 280 nm. Retention of activity upon conjugation was calculated by comparing the ratio of the chromophore absorption peak of mCherry at 586 nm to the absorption peak at 280 nm (A586/A280) to the A586/A280 ratio of fresh mCherry. (d) Results show that mCherry-*b*-PHPA retains approximately 90% of the initial protein activity after bioconjugation and purification for all polymer coil fractions studied, while mCherry-*b*-POEGA retains approximately 55-90%. (e) After solid-state casting and self-assembly and rehydration, the bioconjugates maintain approximately 70-90% of the activity of the bioconjugates after purification.



Figure S9. SAXS curves of solid state samples in comparison to 80 wt.% solution samples for (a) mChPH18 and (b) mChPOE18. Bulk samples were casted at 4°C and form kinetically trapped structures without annealing, while 80 wt.% bioconjugate solutions show re-entrant ODT behaviours. All samples were measured at 25°C.



Figure S10. Representative SAXS curves for perforated lamellar (PL) phase of 70 wt.% bioconjugate solutions measured at 35°C.

Molecule	Density (g/cm ³)	Molecular Formula	SLD (Å ²)
mCherry	1.35	$C_{1250}H_{1915}N_{341}O_{377}S_{12}$	1.9e-6
PHPA	1.162	$C_6H_{10}O_3$	1.07e-6
POEGA	1.05	$C_{6.9}H_{11.9}O_{3.5}$ (monomer composition of 53/47)	9.08e-7
H_2O	1.00	H ₂ O	-5.6e-7
D_2O	1.107	D_2O	6.37e-6

Density Calculator from the NIST Center for Neutron Research.

Table S1. Scattering length densities (SLDs) of molecules. SLDs were computed using the Scattering Length



Figure S11. Small-angle neutron scattering absolute intensities of (a) mChPH27 and (b) mChPOE26 in different D_2O/H_2O blend compositions at T = 10°C.