Supporting Information for

Solid-State Nanostructured Materials from Self-Assembly of a Globular Protein-Polymer Diblock Copolymer

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Sequence of mutant mCherryS131C protein including the 6xHis tag:

MRGSHHHHHHGSMVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAK

LKVTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTV

TQDSSLQDGEFIYKVKLRGTNFPCDGPVMQKKTMGWEASSERMYPEDGALKGEIKQRLKLKD

GGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDELYK

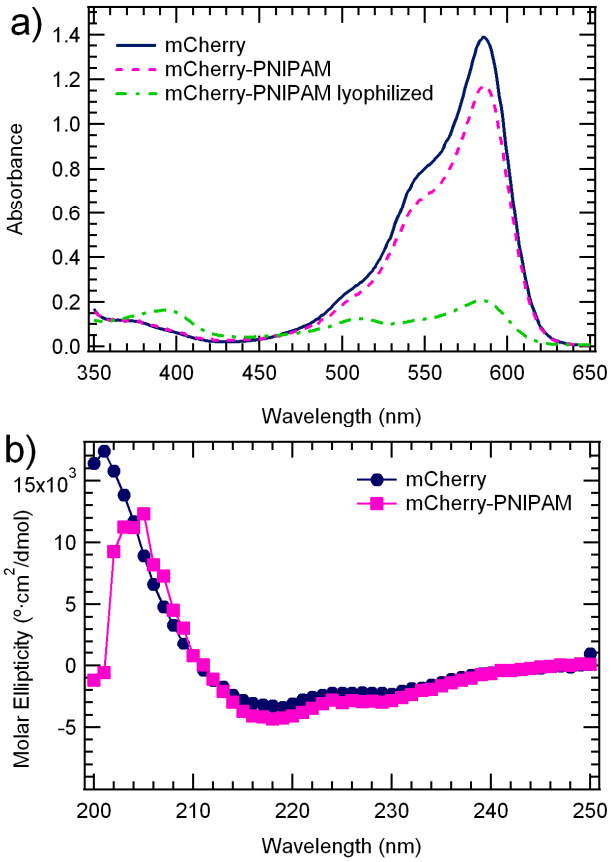

Figure S1. a) UV-vis spectra in solution show minimal change in mCherryS131C chromophore after conjugation to PNIPAM. The spectrum of the mutant mCherryS131C is quantitatively identical to that of the parent protein.[1-2] This is contrasted with the dramatic change observed after lyophilization. b) Circular dichroism spectra of mCherryS131C and its conjugate with PNIPAM in solution indicate the protein fold is not disrupted by conjugation.

Sample	Thermal Transition (°C)
PNIPAM	33.5
mCherryS131C-PNIPAM	38.6

Table S1. Cloud points of mCherryS131C-PNIPAM and the corresponding homopolymer. Solutions were prepared at 1.26 mg/mL PNIPAM and the absorbance at 700 nm was monitored as the temperature was ramped from 20 to 50 ˚C at 0.1 ˚C/min. A PNIPAM molecular weight of 35.5 kg/mol was used for both conjugate and homopolymer measurements. Increasing PNIPAM molecular weight would decrease the transition temperatures.

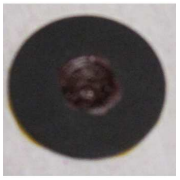

Figure S2. Photograph of mCherry-PNIPAM block copolymer sample cast at room temperature and used for SAXS data acquisition showing the deep red color of mCherry. The sample is 7 mm in diameter and approximately 0.5 mm in height.

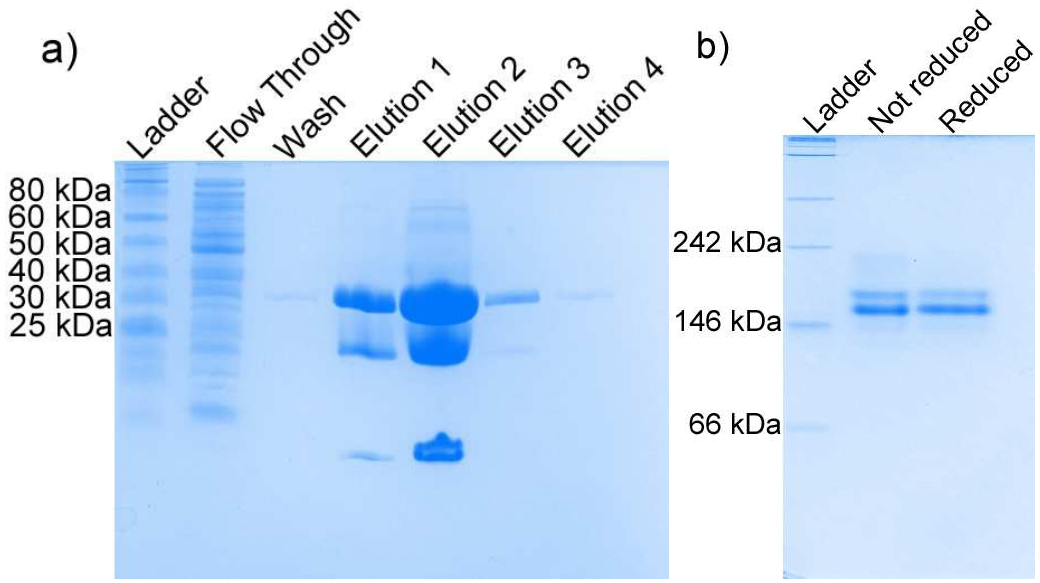

Figure S3. a) SDS PAGE denaturing gel showing mCherryS131C purity. The two smaller molecular weight segments are the result of the chromophore acylimine bond breaking during protein boiling. b) Native gel showing that mCherry remains intact throughout the purification process. A higher molecular weight band shows the presence of dimers formed through disulfide linkages; the dimers are more prevalent under non-reducing conditions. Reduced mCherry was obtained by adding an equimolar amount of β-mercaptoethanol and allowing the reaction to occur at room temperature for one hour.

Table S2. Quantitative analysis of circular dichroism spectra using CD Pro software.

Sample	Method	Fraction helix	Fraction sheet	Fraction turn	Fraction unordered	Average fraction helix	Average fraction sheet	Average fraction turn	Average fraction unordered
mCherryS131C	CONTINLL	0.057	0.503	0.183	0.257	0.041	0.497	0.192	0.270
	SELCON3	0.071	0.501	0.142	0.286				
	CSDDTR	-0.004	0.486	0.251	0.267				
mCherryS131C-PNIPAM	CONTINLL	0.027	0.393	0.202	0.378	-0.003	0.433	0.219	0.351
	SELCON3								
	CSDDTR	-0.033	0.473	0.235	0.325				
Room temperature cast	CONTINLL	0.021	0.394	0.198	0.386	-0.067	0.462	0.224	0.381
	SELCON3	0.026	0.436	0.204	0.334				
	CSDDTR	-0.248	0.557	0.271	0.421				
40 C cast	CONTINLL	0.029	0.390	0.203	0.377	0.001	0.428	0.215	0.356
	SELCON3	-0.006	0.427	0.213	0.366				
	CSDDTR	-0.019	0.467	0.228	0.324				
4 C anneal	CONTINLL	0.020	0.381	0.196	0.404	-0.016	0.413	0.206	0.396
	SELCON3	-0.036	0.403	0.179	0.454				
	CSDDTR	-0.032	0.456	0.244	0.332				
Room temperature anneal	CONTINLL	0.033	0.508	0.192	0.267	0.029	0.480	0.215	0.276
	SELCON3	0.037	0.480	0.217	0.266				
	CSDDTR	0.018	0.452	0.235	0.295				

Volume Fraction Calculation. The volume fraction of PNIPAM in the mCherry-PNIPAM block copolymer was calculated using mCherry crystallographic data[2] along with a PNIPAM density[3] of 1.05 g/cm^3. First, the mCherry density is calculated.

$$\rho = \frac{MW \cdot z}{V \cdot N_A}$$

The molar mass, MW, is 28,134.48 g/mol including the 6xHis tag. Z, the number of formula units per unit cell, is 3. N_A is Avogadro's number, and the volume, V, is calculated using the dimensions of the unit cell.

$$V = a \cdot b \cdot c \cdot \sin(\beta)$$

where a = 4.876 nm, b = 4.285 nm, c = 6.106 nm, and β = 112.31°.

The volume fraction of PNIPAM is then calculated using the following formula:

$$\varphi_{PNIPAM} = \frac{\frac{MW_{PNIPAM}}{\rho_{PNIPAM}}}{\frac{MW_{PNIPAM}}{\rho_{PNIPAM}} + \frac{MW_{mCherry}}{\rho_{mCherry}}}$$

1. Shaner, N. C.; Campbell, R. E.; Steinbach, P. A.; Giepmans, B. N. G.; Palmer, A. E.; Tsien, R. Y. Improved Monomeric Red, Orange and Yellow Fluorescent Proteins Derived from *Discosoma* sp. Red Fluorescent Protein. *Nat. Biotechnol.* **2004,** *22*, 1567-1572.

2. Shu, X. K.; Shaner, N. C.; Yarbrough, C. A.; Tsien, R. Y.; Remington, S. J. Novel Chromophores and Buried Charges Control Color in mFruits. *Biochemistry* **2006,** *45*, 9639-9647.

3. Zhang, L.; Daniels, E. S.; Dimonie, V. L.; Klein, A. Synthesis and Characterization of PNIPAM/PS Core/Shell Particles. *J. Appl. Polym. Sci.* **2010,** *118*, 2502-2511.