Supplemental Material for:

Co-Confinement of Fluorescent Proteins: Spatially Enforced Interactions of GFP and mCherry Encapsulated Within the P22 Capsid

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Percent Occupied Calculation
EGFP(PDB: 2Y0G) h= 4.9nm r= 1.3nm v= 28.1 nm^3

mCherry (PDB: 2H5Q) h= 3.77nm r= 1.38nm v= 22.56 nm^3

Total fusion volume = 50.66 nm^3 x 100 fusions = 5066 nm^3

P22 Procapsid = 40nm interior diameter (PDB: 2XYY), volume = 33510 nm^3
Assuming random packing of spheres, the total volume that can be occupied is 64% of the total volume of the capsid. (Jaeger, H. M. and Nagel, S. R. "Physics of Granular States." *Science* **255**, 1524, 1992.)
Total available capsid volume = 21446 nm^3

5066 nm^3 / 21446 nm^3 = 24% occupied*

*does not take into account the volume contributed by the scaffold protein therefore the fusions take up more than 24% of the capsid volume.

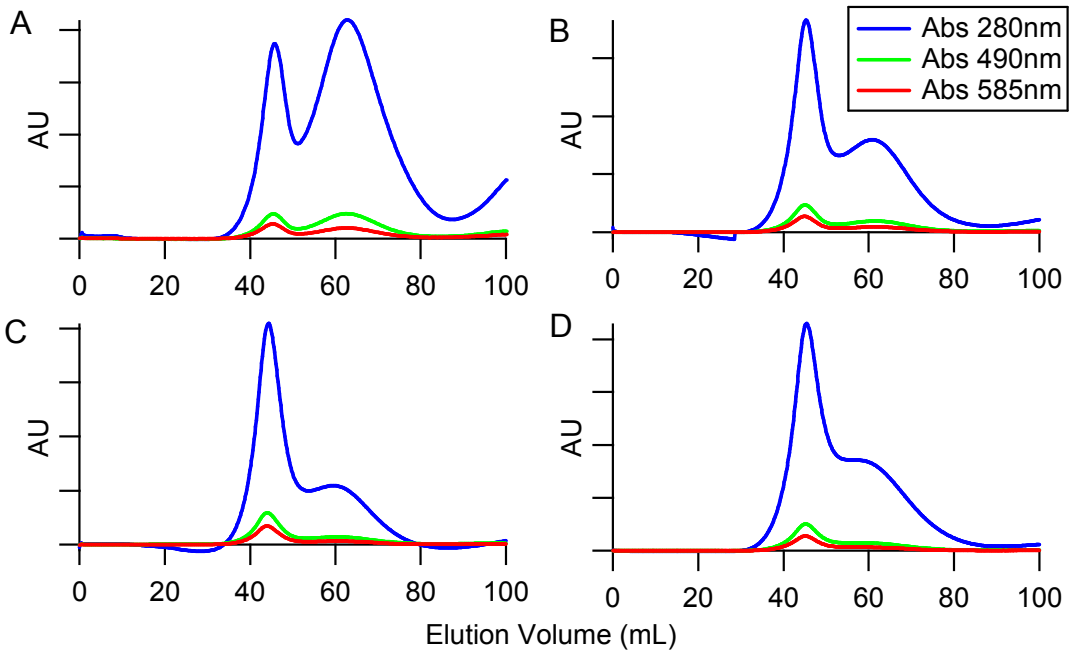

Figure S1: P22 Concatenated samples are purified over a preparative S-500 size exclusion column. This column has the capacity to separate aggregate/mis-formed particles (40 mL elution volume) from the correctly assembled capsids (60 mL elution volume). Fractions from the later half of the 60 mL peak are used for downstream applications. Protein absorbance (280nm) is monitored simultaneously with the GFP absorbance (490nm) and mCherry absorbance (585nm) illustrating that the two fluorescent proteins co-elute with intact capsids. (A) P22 Cat 0gly (B) P22 Cat 6gly (C) P22 Cat 12gly (D) P22 Cat 18gly

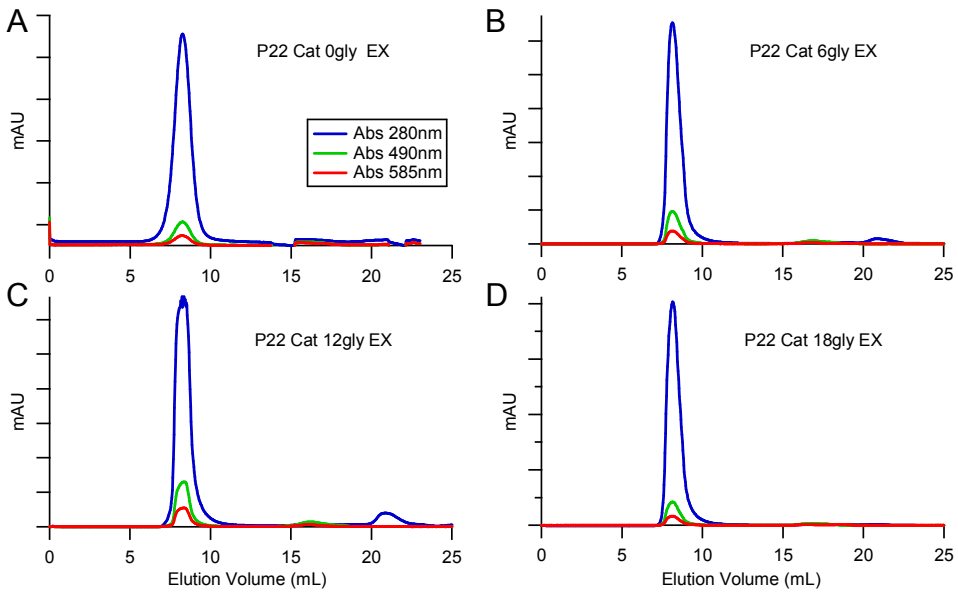

Figure S2: Size exclusion (S500) purified capsids were further purified after heating to 65°C by Sup6 size exclusion chromatography. The capsid elutes in the void volume of ~8 mL. The GFP-mCherry fusion is still encapsulated within the capsid as illustrated by the co-elution of the GFP absorbance (490nm, green line) and the mCherry absorbance (585nm, red line) with the capsid peak (~8mL elution volume).

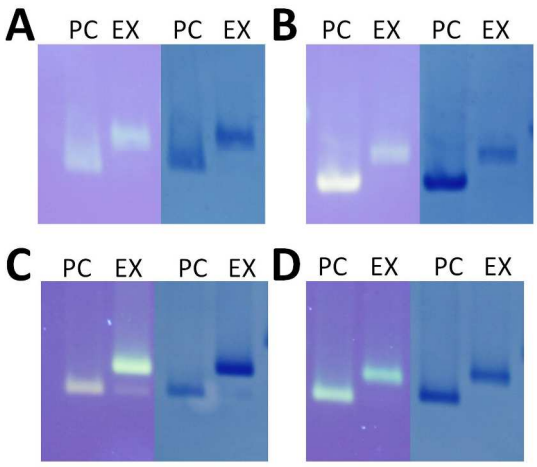

Figure S3: Non-denaturing agarose shift gels showing the procapsid (PC) and the shift to expanded capsid (EX) in all samples. The GFP-mCherry fusion is retained in both the PC and EX capsid, in all samples, as seen by the co-migration of the fluorescence signal, seen by UV illumination (left panel), and the coomassie protein stain (right panel). (A) P22 Cat 0gly (B) P22 Cat 6gly (C) P22 Cat 12gly (D) P22 Cat 18gly

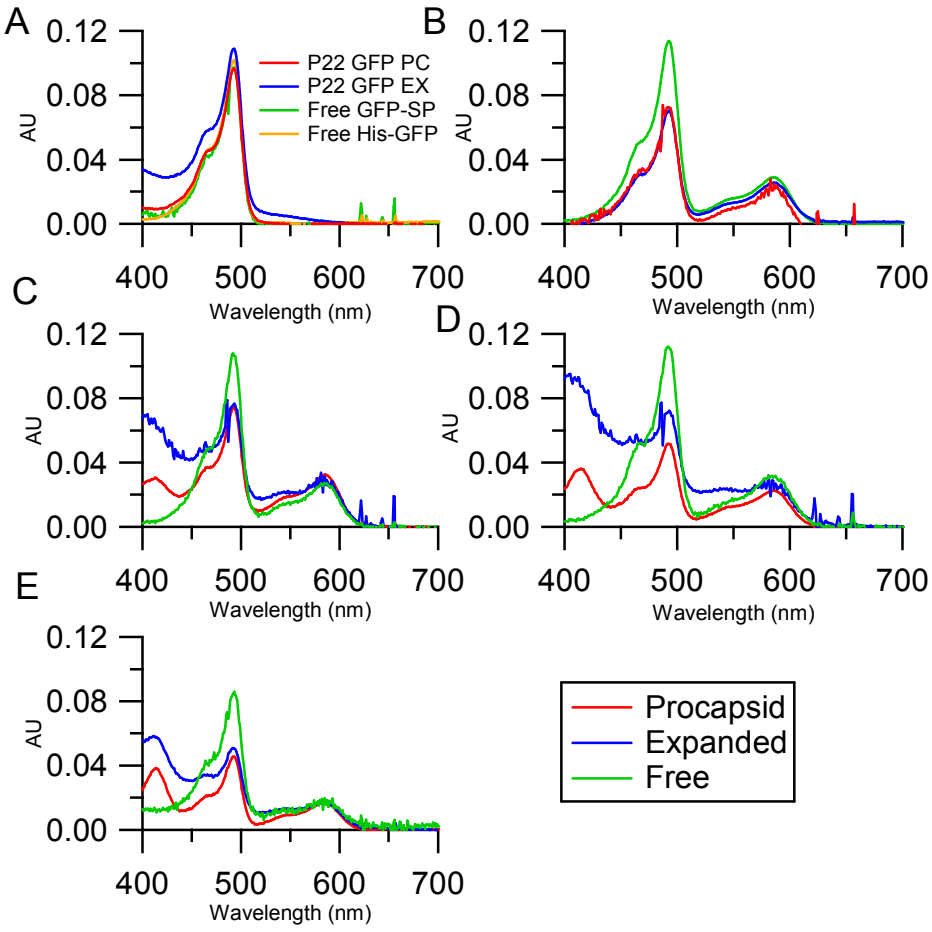

Figure S4: Scattering corrected UV-Vis spectra of all encapsulated and free in solution fusions. Procapsid traces are in red, expanded capsid traces in blue, and free fusions are in green. . (A) P22 GFP only (B) P22 Cat 0gly (C) P22 Cat 6gly, (D) P22 Cat 12gly, (E) P22 Cat 18gly.

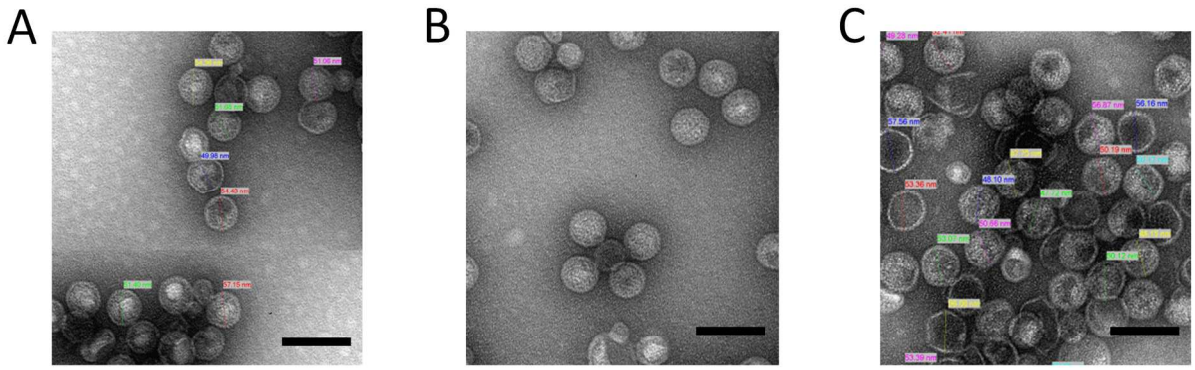

Figure S5: Transmission electron micrograph of all the. (A) P22 Cat6gly (B) P22 Cat12gly (C) P22 Cat18gly. Black bar represents 100nm. Colored text in panels A and C are arbitrary measured values of the particles.

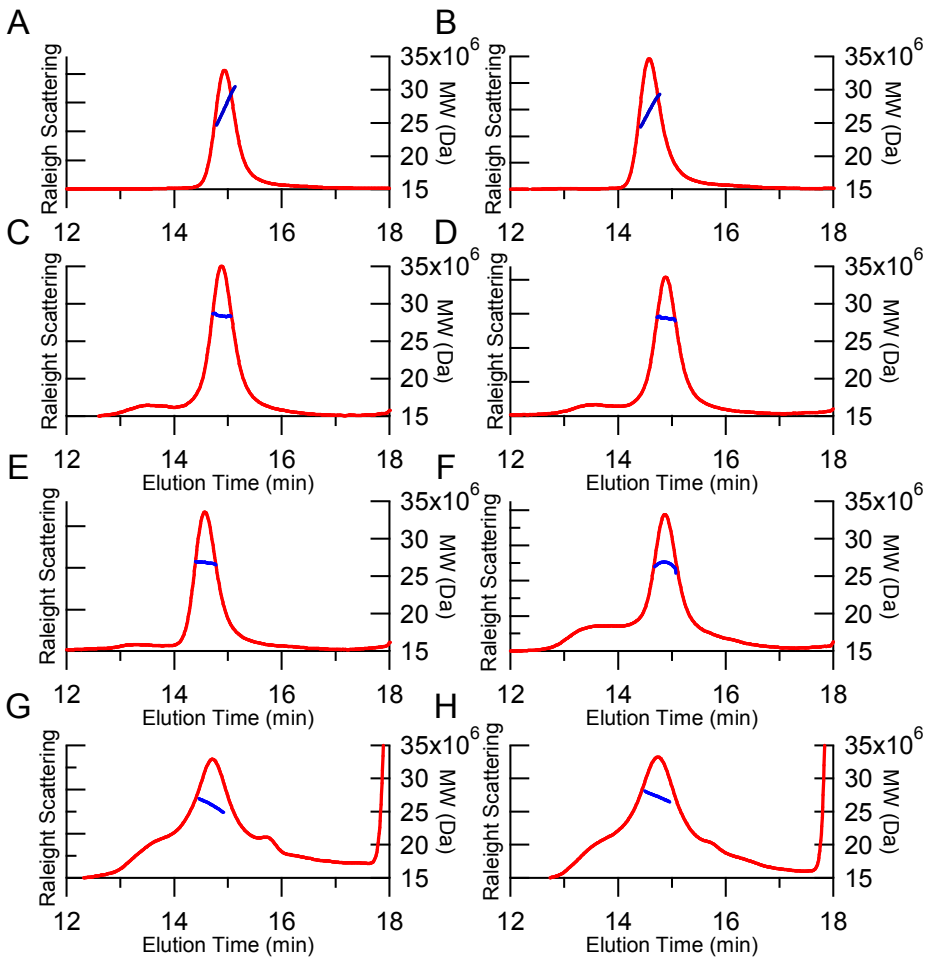

Figure S6: High pressure liquid chromatography coupled to multi-angle light scattering data for the P22 Cat glycine linker series in the procapsid and expanded capsid morphology. Raleigh scattering versus elution time is plotted in red with the total molecular weight across the capsid peak in blue. A) P22 Cat 0gly, procapsid B) P22 Cat 0gly, expanded C) P22 Cat 6gly, procapsid D) P22 Cat 6gly, expanded E) P22 Cat 12gly, procapsid F) P22 Cat 12gly, expanded G) P22 Cat 18gly, procapsid H) P22 Cat 18gly, expanded