Supporting Information for:

Reversible Loading of Thiol-Modified Curcumin in an Engineered Protein Capsid

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Experimental Methods.

Protein production and purification. Wild-type AaLS was produced from plasmid pMG-AaLS-NoHis and purified as described in the main text.

Electron microscopy. AaLS capsids (0.2 mg/mL each in SEC buffer) were applied to TEM copper grids (100 square mesh formvar coated) and incubated for 1 min to allow adhesion. After 1 min excess protein solution was wicked away with sterile filter paper. Samples were then stained with 2% phosphotungstic acid (PTA) at pH 8.0 for 1 min. Excess staining solution was removed with filter paper. TEM imaging of the negatively stained samples were using a Hitachi 7100 transmission electron microscope at the University of Utah electron microscopy facility.

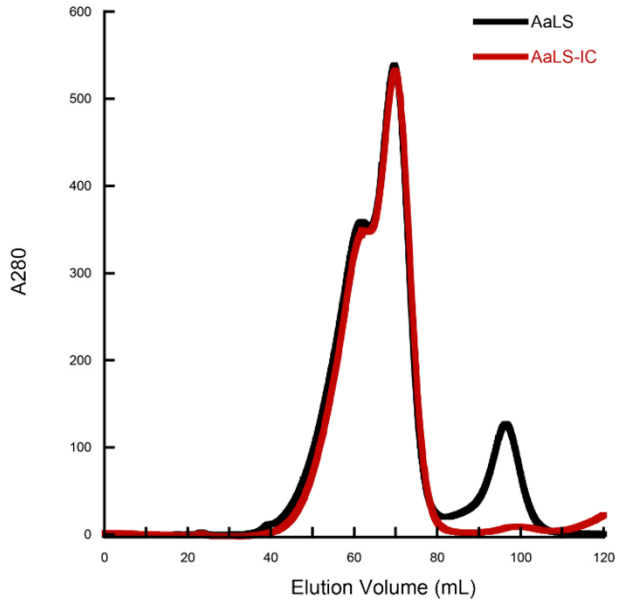

Fig. S1. Size-exclusion chromatograms of AaLS-IC (red) and wild-type AaLS (black).

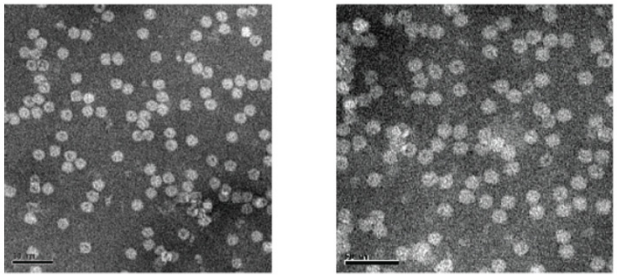

Fig. S2. Negatively stained TEM images of wild-type AaLS capsids (left) and AaLS-IC (right). The scale bars represent 50 nm.

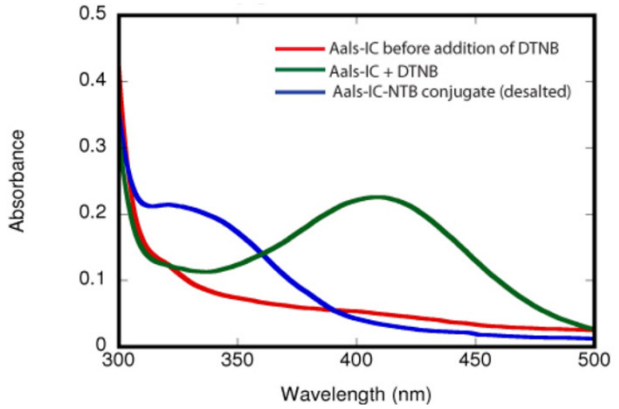

Fig. S3. UV-vis absorbance spectra of AaLS-IC before the addition of DTNB (red), AaLS-IC after the addition of DTNB (green), and AaLS-IC-NTB after the removal of unreacted DTNB and free NTB product (blue).

Table S1. Yield of AaLS-IC-NTB.

Trial #	[AaLS-IC][a] (μM)	[DTNB] (mM)	ΔA_{412}	Yield (%)
1	54	5	0.53	69
2	76	5	0.78	72
3	44	5	0.46	73

[a]Concentrations are given on a protein monomer basis.

Table S2. Yield of AaLS-IC-cur.

Trial #	[AaLS-IC-NTB][a] (μM)	[cur-SH] (mM)	ΔA_{412}	Yield (%)
1	15.8	31.6	0.21	94
2	7.2	14.4	0.10	98

[a]Calculated on a NTB group basis.

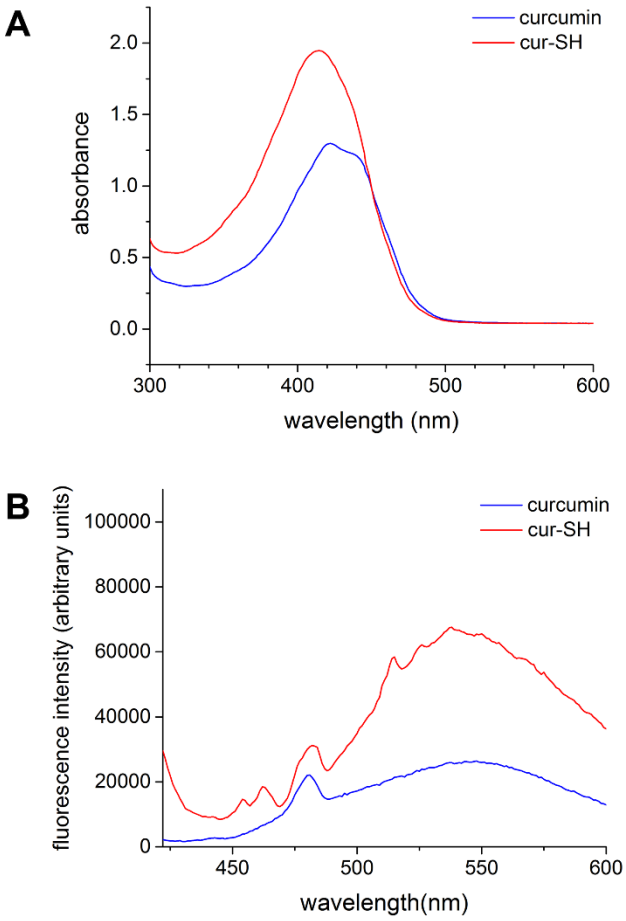

Fig. S4. Comparison of natural curcumin and cur-SH by optical spectroscopy. (A) UV-vis absorbance spectra of curcumin (blue) and cur-SH (red). (B) Fluorescence emission spectra of curcumin (blue) and cur-SH (red) upon excitation at 412 nm. Both molecules were dissolved in methanol by stirring and shaking at room temperature for several minutes. Residual solid particles were apparent in both cases. Undissolved material was removed by filtration prior to analysis.

Table S3. Solubility of AaLS-IC-cur in pure water.

Sample	Theoretical [curcumin] (μM)[a]	Pellet observed?	$A_{412\ nm}$
AaLS-IC-cur	30	No	0.552
AaLS-IC-cur	86	No	1.049
AaLS-IC-cur	430	No	6.462[b]

[a]The theoretical [curcumin] represents the amount of cur-SH conjugated to AaLS-IC that is expected in solution if the curcumin is fully soluble. The solvent is doubly deionized water.
[b]After correction for a 2-fold dilution.