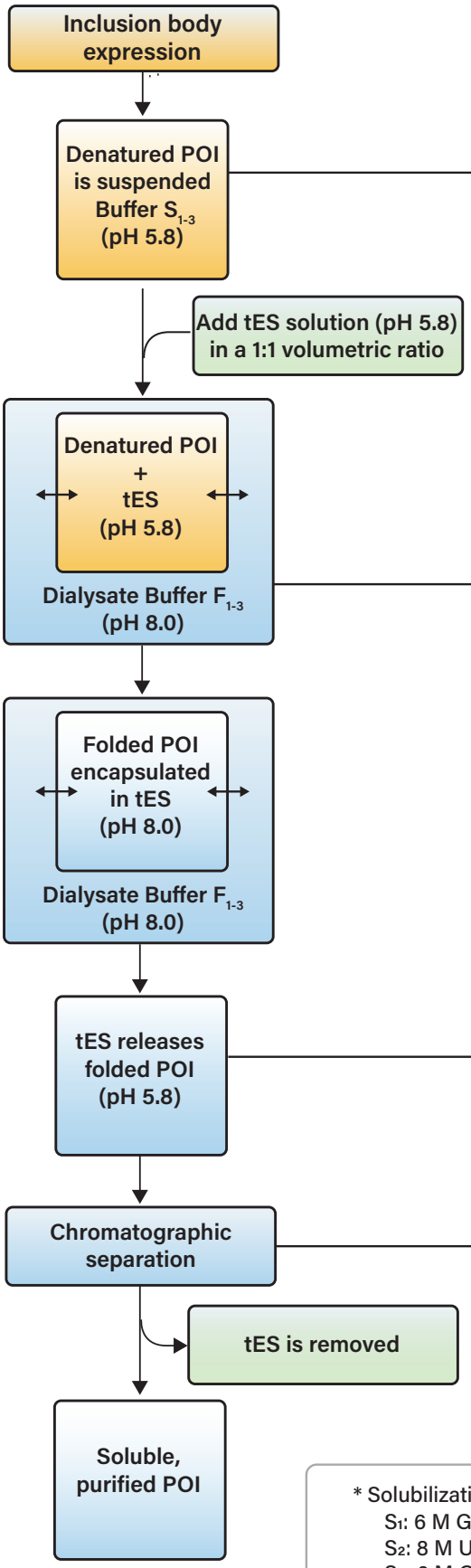
Fig. S1 Generalized protein folding protocol



Procedure
- Inclusion bodies are expressed and isolated from E.coli through standard techniques and suspended in a chaotropic buffer.

Optimization
- One of three solubilization buffers (S1-3)* can be selected based on similarity to published reports of in vitro folding (if any)
- Prepurification of the inclusion body is recomended however use of inclusion body suspension without prepurificaiton can also be tested
- The molar ratio of POI to tES can be estimated based on molecular volume calulations and optimized further for yield.

Procedure
- POI dissolved in solubilization buffer is mixed with tES-F116H (20uM dimer concentration) resulting in a 50% dilution of the solubilization buffer. This

Optimization
- One of three folding buffers is chosen (F1-3)** as the dialysate for the mixture of tES and POI. The length of time for dialysis, temperature and dialysis cutoff can be varied to maximize protein return.
- Protein-specific additives, such as prosthetic groups, can be added to the dialysate.

Procedure
- The pH of the folding mix is adjusted to pH 5.8 to disassociate the shells and release the folded POI.

Optimization
- If the POI is partially released from the tES interior, increased salt concentrations (i.e. 500mM NaCl) can be used in the release buffer

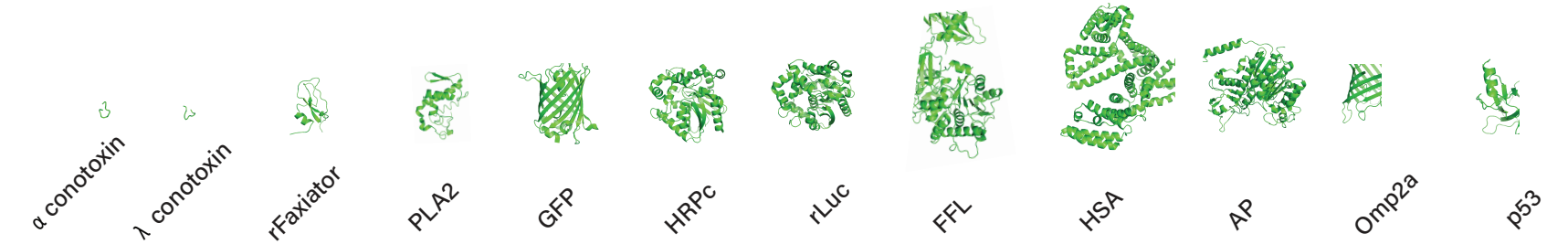
Procedure
- The folded POI is separated from the tES shell components

Optimization
- If the POI is partially released from the tES interior, increased salt concentrations (i.e. 500mM NaCl) can be used in the release buffer

* Solubilization buffers (S_{1-3})
S_1: 6 M GuHCl, 50 mM Tris-HCl, 0.1 M NaCl, pH 8
S_2: 8 M Urea, 50 mM Tris-HCl, 0.1 M NaCl, pH 8
S_3: 6 M GuHCl, 50 mM Tris-HCl, 2mM EDTA, 5 mM DTT, pH 8

** Folding buffers (F_{1-3})
F_1: 50 mM Tris-HCl, 100 mM NaCl, 7% glycerol, pH 8
F_2: 50 mM Tris-HCl, 100 mM NaCl, 2% glycerol, pH 8
F_3: 100 mM Tris-HCl, 2 mM EDTA, 1mM GSSG, 0.8 mM GSH, pH 8.5

Fig. S2



	1.35 kDa	1.24 kDa	12 kDa	17 kDa	27 kDa	34 kDa	36 kDa	60 kDa	66 kDa	52 kDa	39 kDa	43.7 KDa
PI of protein	7.8	8	8.3	4.8	5.80	6.30	5.86	6.69	5.67	4.63	4.3	6.33
Molucular Volume	1116 A^3	1116 A^3	15615 A^3	32509 A^3	: 41198 A^3	41198 A^3	43585 A^3	70007 A^3	80426 A^3	64545 A^3	46001 A^3	52819 A^3
Charge at pH 7.0	0.8	1	3.5	-5.5	-6.2	-1.2	-13.4	-1	-15.7	-38.6	-22.6	-3.3
Disulfide (#)	2	2	3	7	0	4	0	0	17	2	0	4
Optimal tES	tES(-)	tES(-/+)	tES(-)	tES(+)	tES(+)	tES(+)	tES(+)	tES(-/+)	tES(+)	tES(+)	tES(+)	tES(+,-/+)
tES:POI encapsulation (max ratio)	N/A	N/A	1:3	N/A	N/A	1:1	1:2	N/A	N/A	N/A	N/A	N/A
tES:POI (max stabilization)	60:150	60:150	60:15	60:15	60:10	60:10	60:10	60:5	60:5	60:5	60:5	60:10
tES:POI (max fxnl yeild)	1:60	1:60	1:6	1:6	1:4	1:4	1:4	1:8	1:8	1:8	1:8	1:4
POI multimer	No	No	No	No	No	No	No	No	No	Yes	Yes	Yes
Source	Molluscs	Molluscs	Reptiles	Reptiles	Cnidarians	Plants	Cnidarians	Arthropods	Human	Arthropods	Bacteria	Human
Percentage Secondary Structure	N.A	N.A	β strand : 14.9	β strand : 5.9	β strand : 45	β strand : 1.9	β strand : 16.5	β strand : 19.3	β strand : 0	β strand : 16	β strand : 55.6	β strand : 20.6
Cysteine Density (%)	33	40	9	10	0.8	2.5	1	0.9	6	0.8	0.6	2.5
pI	8.89	7.18	8.58	5.42	5.8	5.66	5.76	6.72	5.67	4.63	4.57	6.42

Fig. S2| Comparative characteristics of monomeric and multimeric POIs

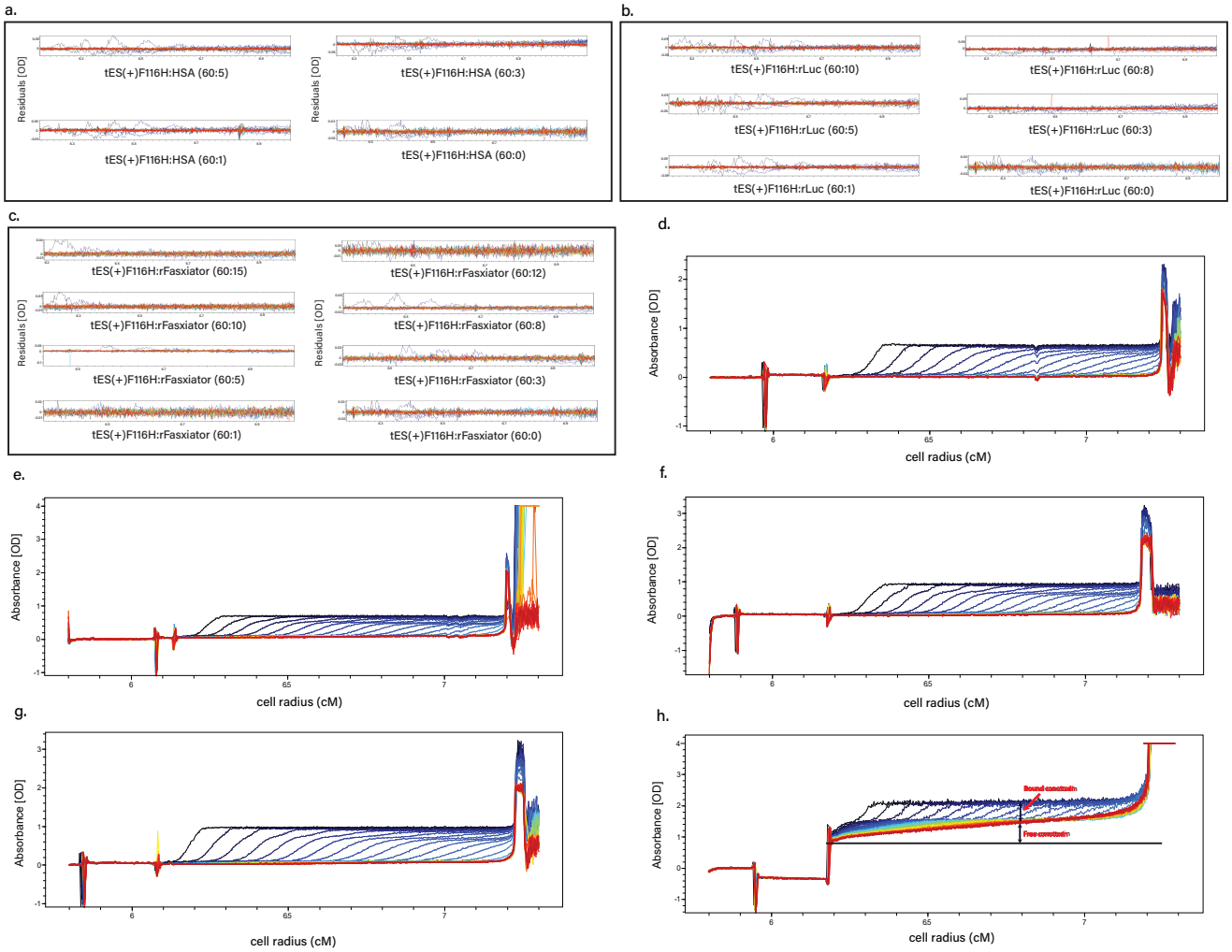


Fig. S3| Analysis of encapsulation process with increasing POI concentration using Analytical Ultracentrifuge (AUC): Residuals plots (a,b,c) and absorbance profiles of (d) tES(+)F116H, (e) tES(+)F116H:HSA, (f) tES(+)F116H:rLuc, (g) tES(-)F116H:rFasxiator, (h) tES(+)F116H:λ conotoxin at optimal molar ratios registered at 280 nm , in time intervals of 7 min for total experimental time of approximately 7 h. AUC profile for λ conotoxin shows decreasing sedimentation of tES encapsulated conotoxin and increasing presence of free conotoxin as the run progresses.

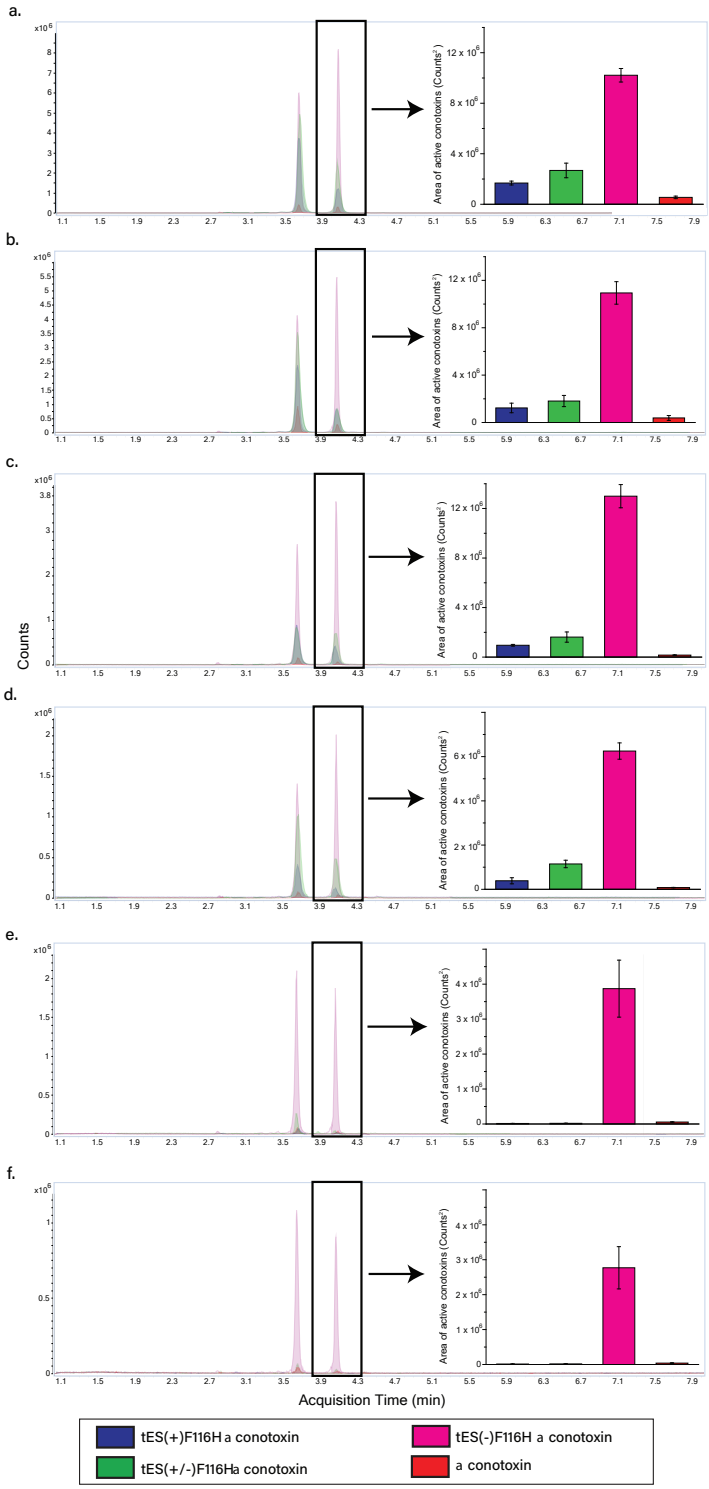


Fig. S4 | Chromatographic profiles of α conotoxin folded in presence and absence of tES: Chromatographic profiles for α conotoxin folded in-vitro without tES or within tES(+)F116H, tES(+/-)F116H or tES(-)F116H respectively with different molar ratios of tES subunits:α conotoxins from (a) 60:37.5, (b) 60:75, (c) 60:112.5, (d) 60:150, (e) 60:225 and (f) 60:300 respectively. Relative yield of globular (active) conformation when conotoxin is folded in presence and absence of tES is shown as a histogram.

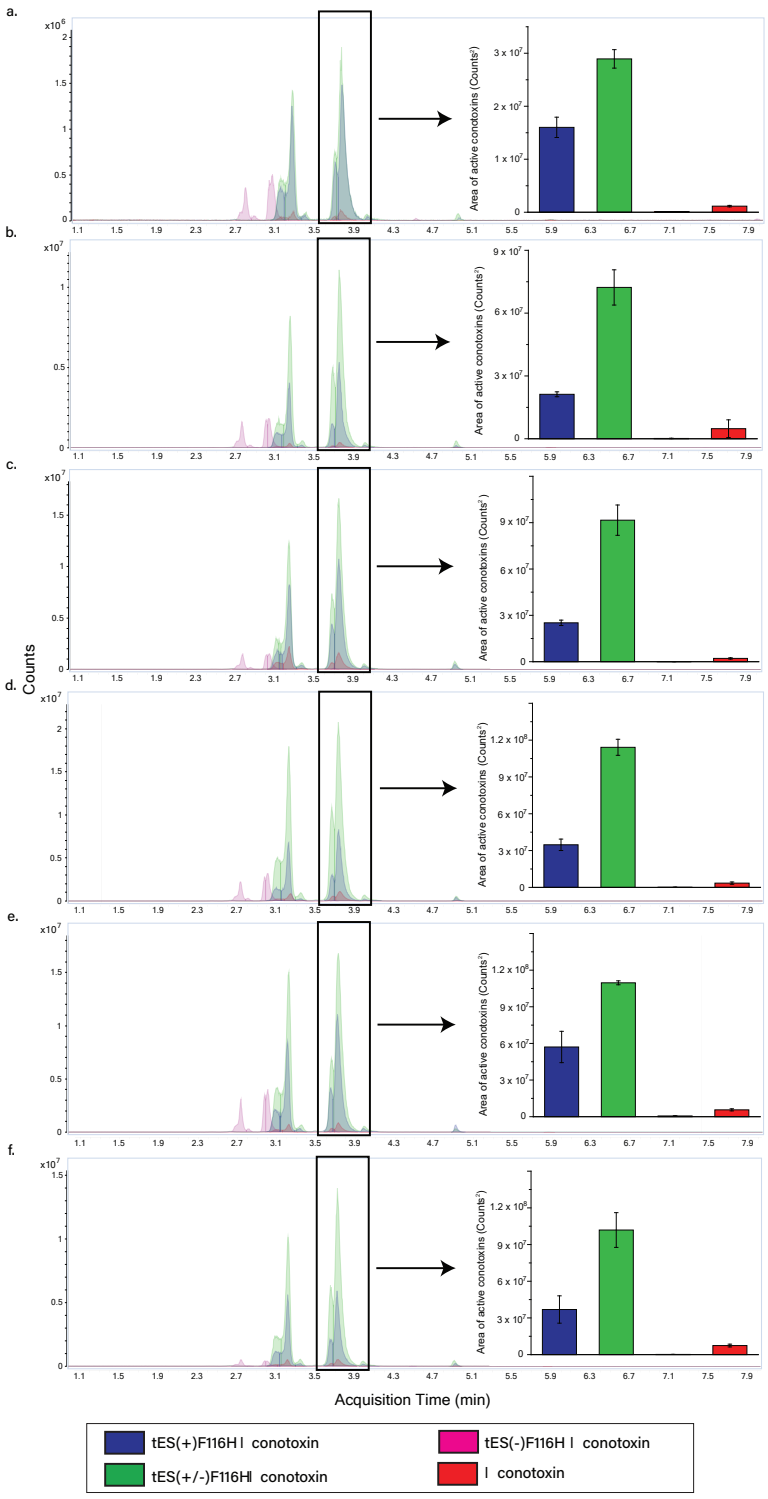


Fig. S5| Chromatographic profiles of λ conotoxin folded in presence and absence of tES: Chromatographic profiles for λ conotoxin folded in-vitro without tES or within tES(+)F116H, tES(+/-)F116H or tES(-)F116H respectively with different molar ratios of tES subunits:λ conotoxins from (a) 60:37.5, (b) 60:75, (c) 60:112.5, (d) 60:150, (e) 60:225 and (f) 60:300 respectively. Relative yield of ribbon (active) conformation when conotoxin is folded in presence and absence of tES is shown as a histogram.

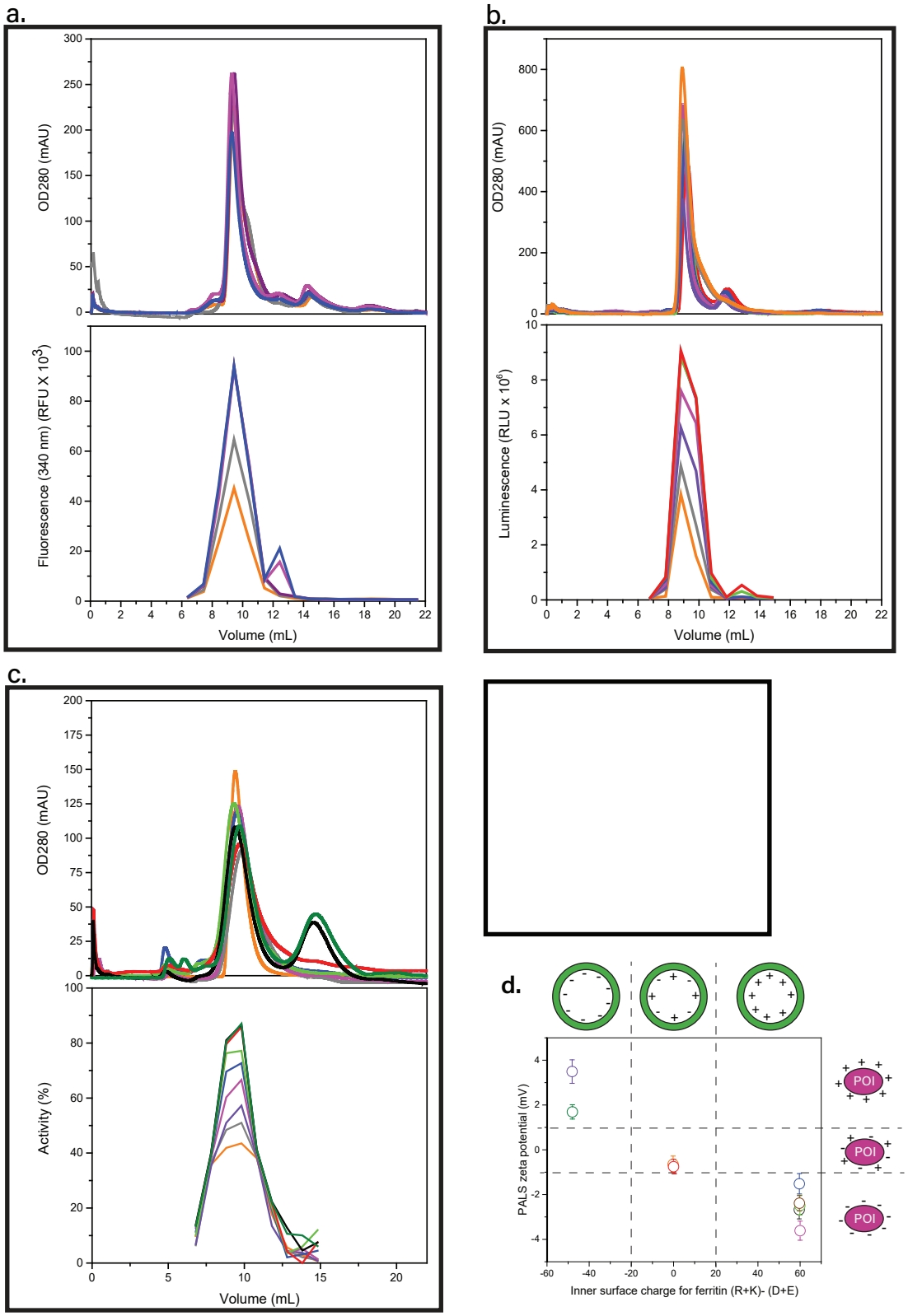


Fig. S6 | Optimization of molar ratios of tES subunits to POI: Size-exclusion profiles (upper panel) of (a) tES(+)F116H:HSA (b) tES(+)F116H:rLuc (c) tES(-)F116H:rFasxiator with different molar ratios of tES subunits and POI. Each fraction of size-exclusion chromatography was analysed for POI activity for all molar ratios tested (lower panel). POI activity coincides with tES peak, suggesting its encapsulation inside tES assembly. All experiments were performed in triplicates, error bar represents ± standard deviation. (d) Charge matching of tES-F116H and the measured zeta potential of the POI predict optimal folding. Combinations with rank order highest functional yield are shown for nine monomeric POI's. tES-F116H(+), tES-F116H(+/-), tES-F116H(-) and POI-alone are indicated as (+), (+/-), (-) and h6, respectively.

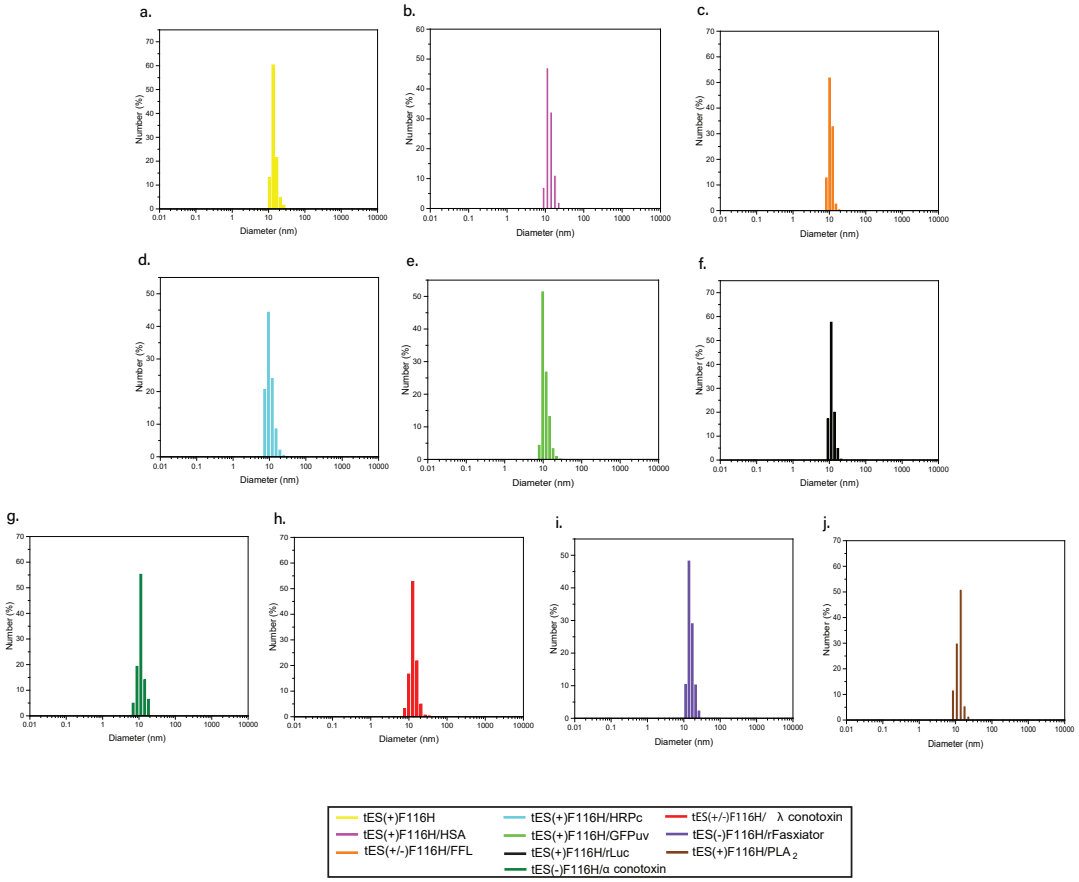


Fig. S7 | Characterization of tES nanoparticles using Dynamic Light Scattering (DLS): DLS experiments of tES nanoparticles showed no change in the hydrodynamic diameter (12 nm approx.) of POI encapsulated tES suggesting internalization of POI within tES.

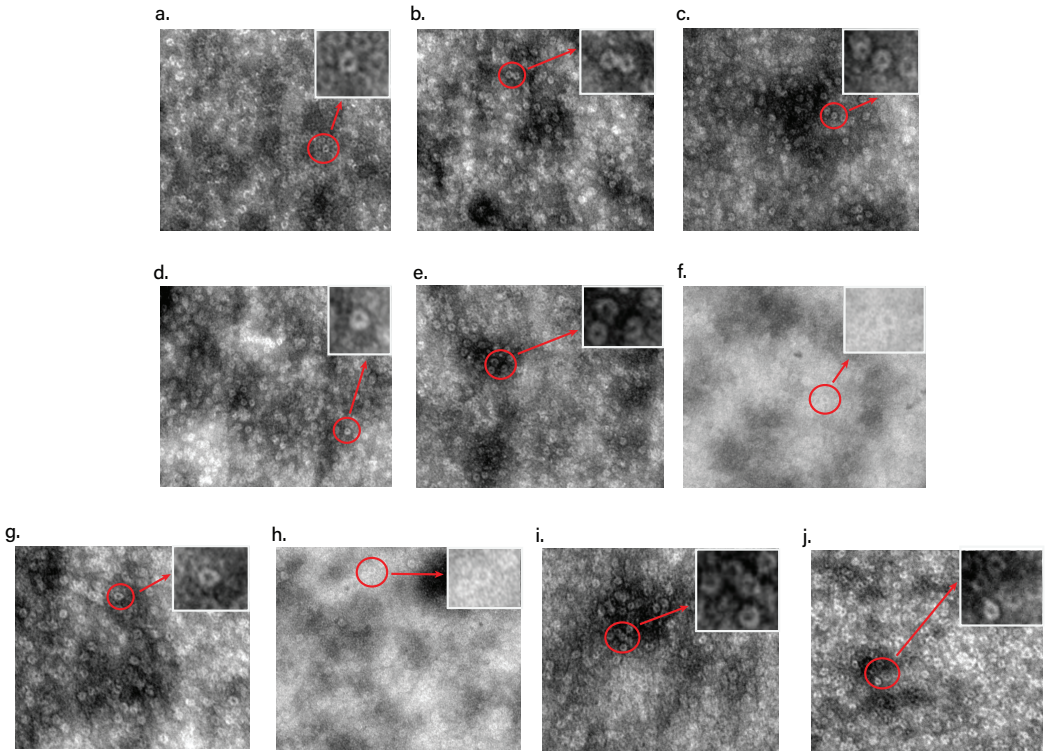


Fig. S8 | Characterization of tES nanoparticles using Transmission Electron Microscopy (TEM): TEM experiments of tES nanoparticles showed no change in the morphology and hydrodynamic diameter (12 nm approx.) of POI encapsulated tES suggesting internalization of POI within tES.

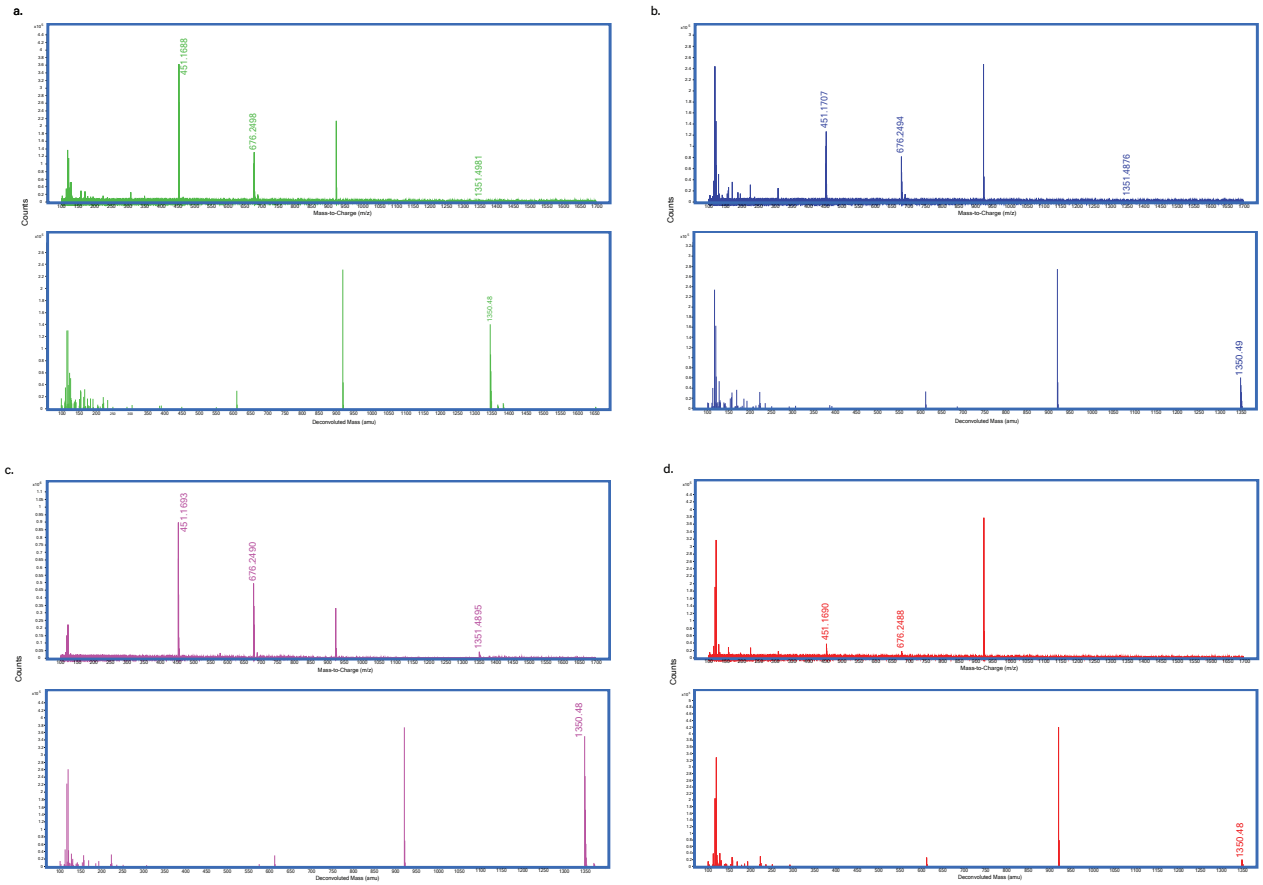


Fig. S9 | Mass spectrometry profiles of globular conformation of α conotoxin folded in presence and absence of tES: Raw mass/charge spectrum (m/z) (upper panel) and deconvoluted mass spectrum showing inferred molecular masses (lower panel) of α conotoxin folded in-vitro in presence of (a) tES(+)F116H, (b) tES(+/-)F116H and (c) tES(-)F116H and in (d) absence of tES were determined by QTOF. The protein is predominantly +3 charged, appearing with m/z of 451 amu. Protein has inferred molecular mass of 1350.48 amu which closely matches with the predicted molecular mass of 1350.92 amu. In all cases, molecular weight of the oxidized α conotoxin shows a reduction of four mass units, reflective of the formation of the two disulfide bridges. m/z at 121 and 922 were from mass calibrant. All experiments were performed in triplicates.

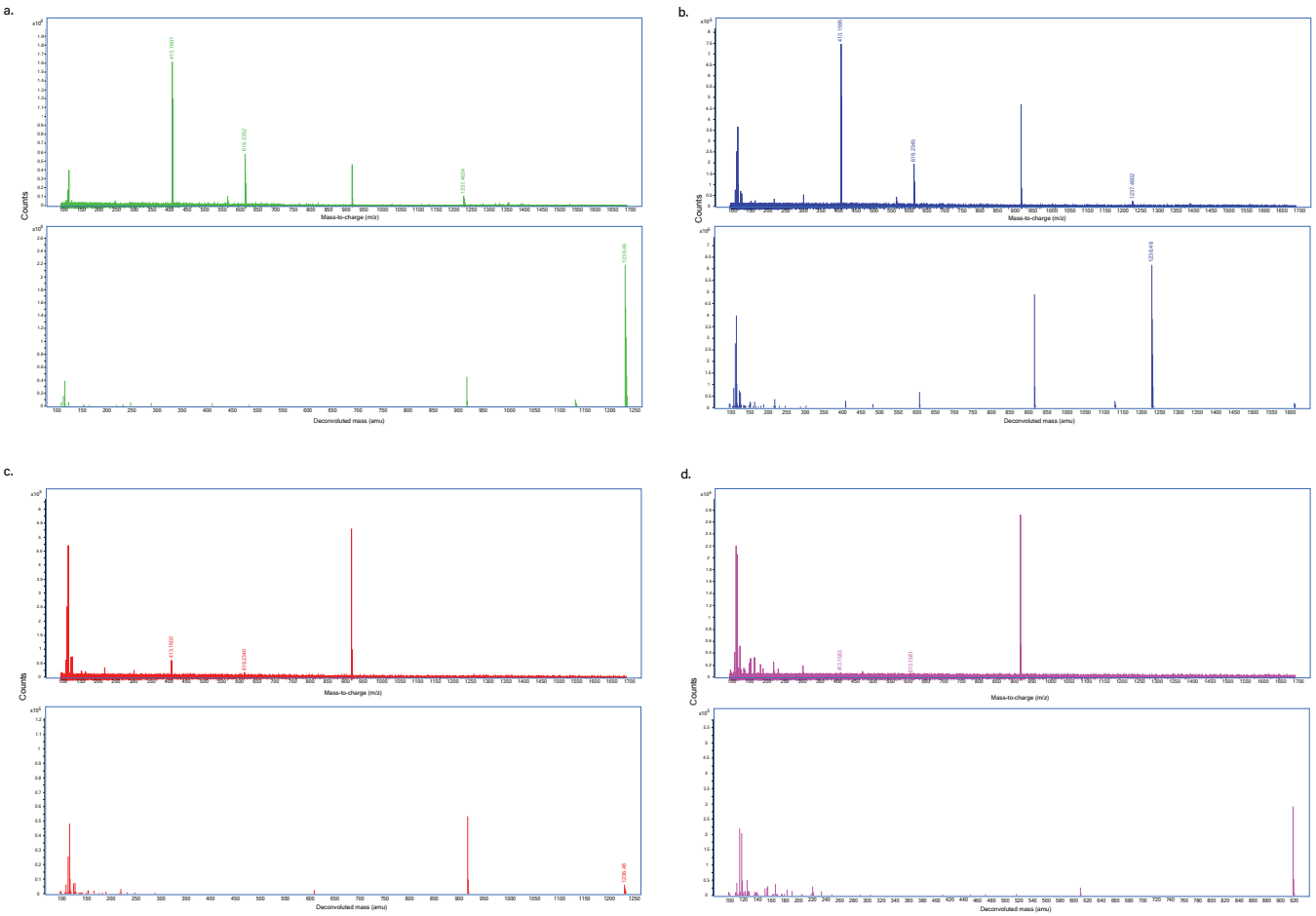


Fig. S10 | Mass spectrometry profiles of ribbon conformation of λ conotoxin folded in presence and absence of tES: Raw mass/charge spectrum (m/z) (upper panel) and deconvoluted mass spectrum showing inferred molecular masses (lower panel) of λ conotoxin folded in-vitro in presence of (a) tES(+)F116H, (b) tES(+/-)F116H and (c) tES(-)F116H and in (d) absence of tES were determined by QTOF. The protein is predominantly +3 charged, appearing with m/z of 413 amu. Protein has inferred molecular mass of 1236.46 amu which closely matches with the predicted molecular mass of 1236.90 amu. In all cases, molecular weight of the oxidized λ conotoxin shows a reduction of four mass units, reflective of the formation of the two disulfide bridges. m/z at 121 and 922 were from mass calibrant. All experiments were performed in triplicates.

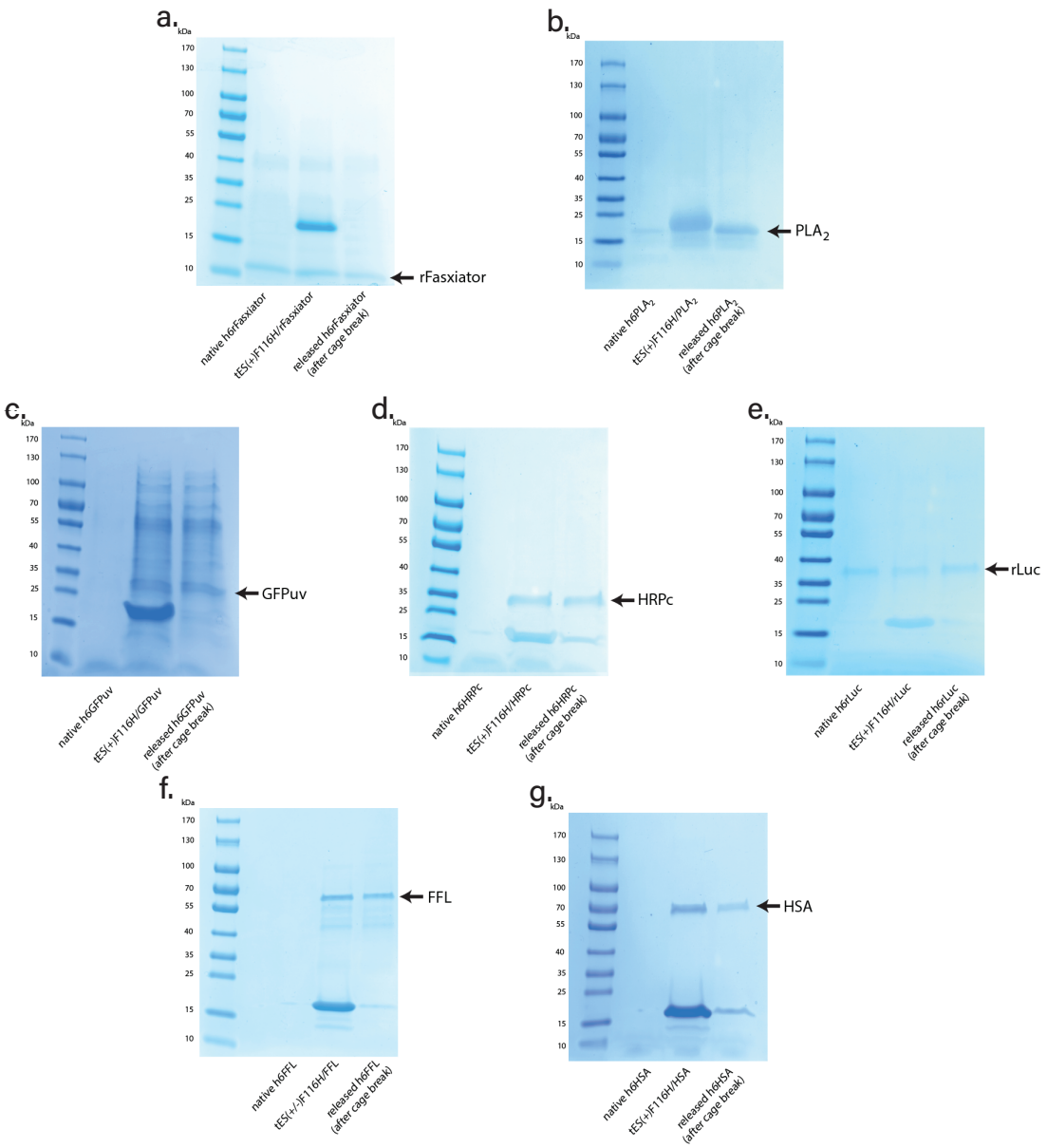


Fig. S11 | Determining yield of in-vitro folded POI in presence and absence of shells: SDS-PAGE gel of in-vitro folded POI in absence of tES, in-vitro folded POI in presence of tES and POI released and purified from tES after in-vitro folding for (a) rFasxiator, (b) PLA2, (c) GFPuv, (d) HRPc, (e) rLuc, (f) FFL and (g) HSA.

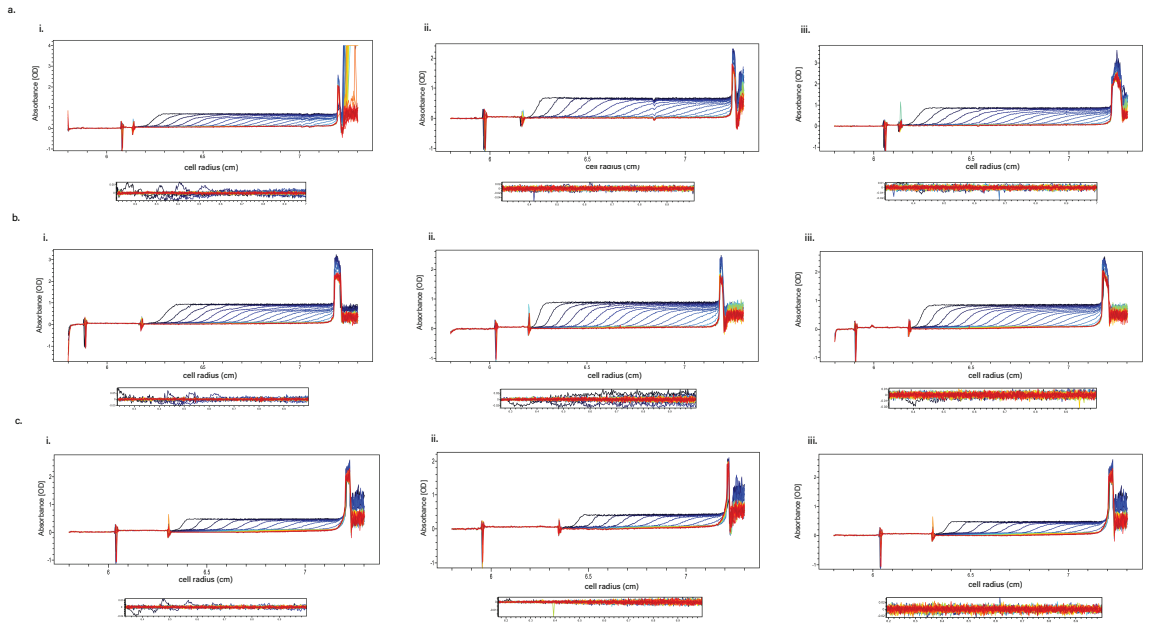


Fig. S12 | Analysis of encapsulation process with different internal charge of tES-F116H using Analytical Ultracentrifuge (AUC): Absorbance profiles (upper panel) and residuals plots (lower panel) of (a) HSA, (b) rLuc and (c) rFasxiator encapsulated within (i) tES(+)F116H, (ii) tES(+/-)F116H and (iii) tES(-)F116H respectively at optimal molar ratios registered at 280 nm , in time intervals of 7 min for total experimental time of 7 h approximately.