

A. GliPΔCt: gels, domains, SAXS and chemistry

Final stock of GliPΔCt (180 kDa) on 10% SDS-PAGE, SAXS envelope fitted to a homology model of GliPΔCt yields a diameter of 147 Å. Domain arrangement and reaction catalyzed by GliPΔCt.

B. 2D classification

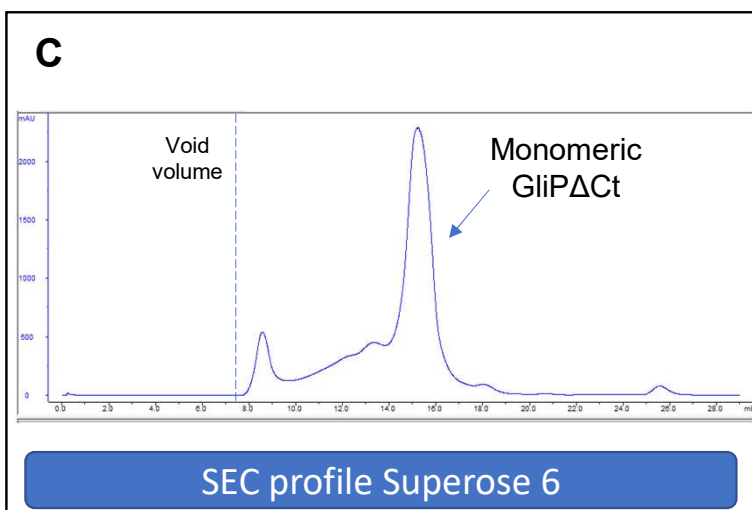
2D-class averages of the full length GliP-wt in optimized conditions that set the baseline for GliPΔCt cryoEM data acquisition with Titan-Krios.

C. High MW range SEC

Superose 6 column elution profile shows a primary peak distinct from the oligomeric / aggregated fractions (next to void volume); performed each time before grids preparation for cryo-EM.

D. Grids

Grid setup and expected outcomes for exploring conformational states.



D

Grid #	Conc (mg/ml)	Substrates	Potential Outcome
1	0.5	None	Open State - Baseline conformational space
2	0.5	L-Phe, L-Ser, AMP.PNP	Adenylation State both A domains
3	0.5	L-Phe, AMP.PNP	Adenylation State A1 domain
4	0.5	L-Ser, AMP.PNP	Adenylation State A2 domain
5	0.5	ATP	ATP bound state - Substrates entry mechanism
6	0.5	L-Phe, L-Ser	Aminoacid bound state - substrate entry mechanism
7	0.2	None	Open State - Baseline conformational space
8	0.2	L-Phe, L-Ser, AMP.PNP	Adenylation State both A domains

Grids and expected outcomes