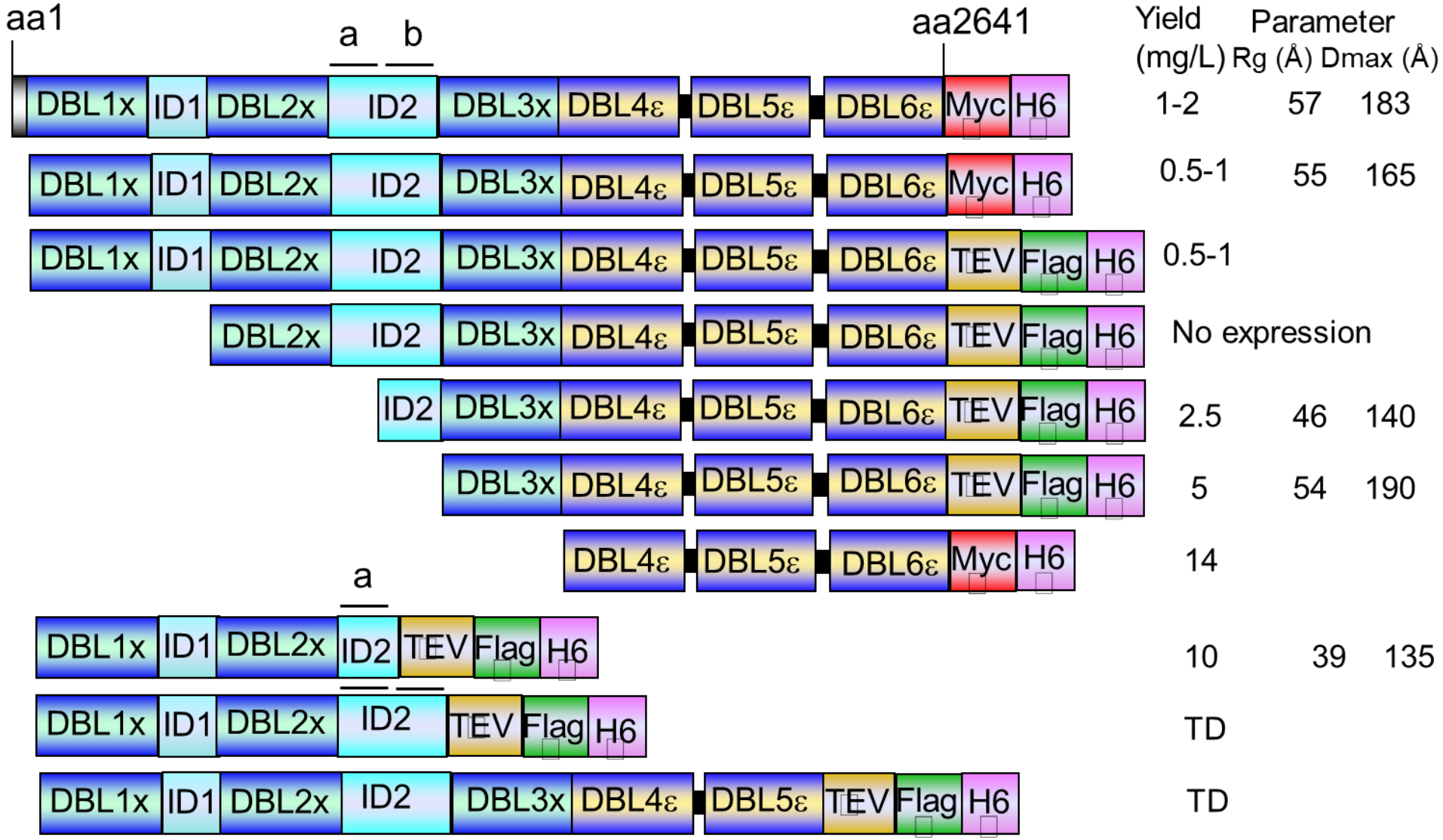
Summary of characterization of Var2CSA

Including data from Spotiton Grids Data Collected at NYSBC

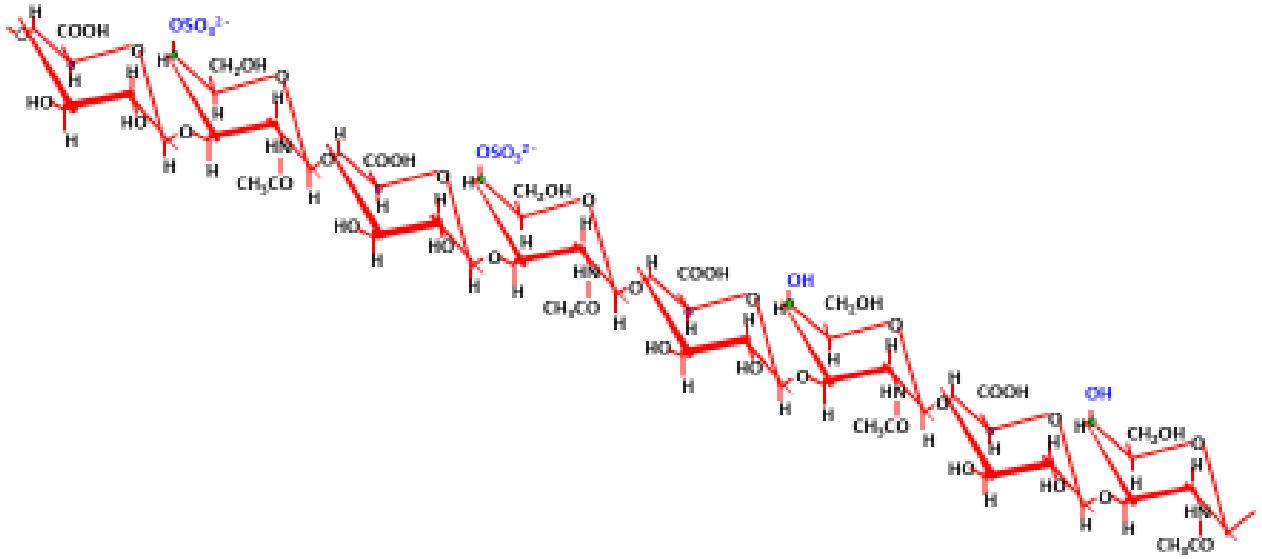
Protein Constructs.



Yield (mg/L)	SAXS Parameter Rg (Å)	Dmax (Å)
1-2	57	183
0.5-1	55	165
0.5-1		
No expression		
2.5	46	140
5	54	190
14		
10	39	135
TD		
TD		

Var2CSA targets placental specific carbohydrates.

Structural features that are involved in IRBC binding

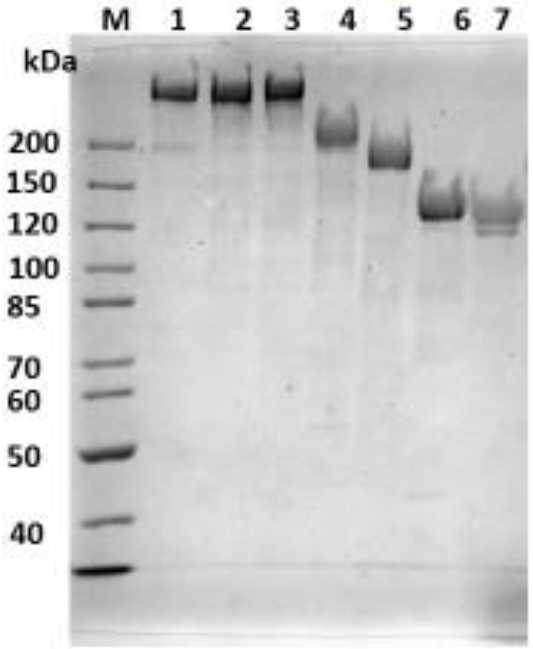


- **The 12 saccharide motif is the minimum chain length**
- **Fully sulfated C4S does not support strong binding**
- **Two sulfate groups are sufficient for maximum binding strength**

Only intact full-length Var2CSA binds with high affinity and specificity this substrate.

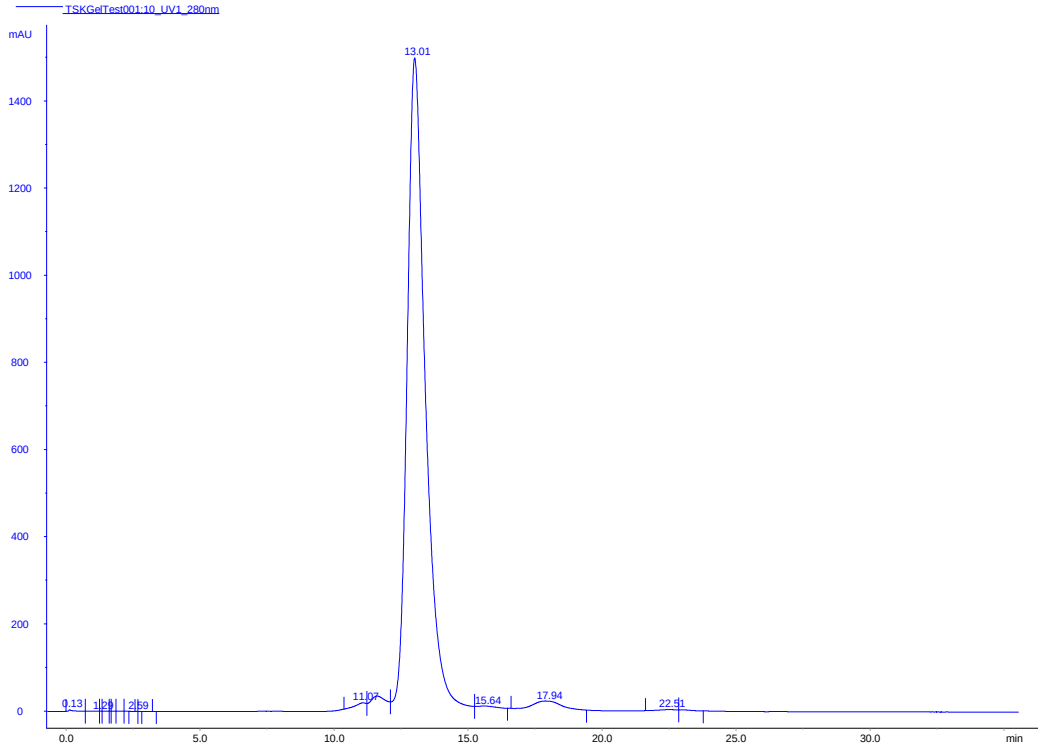
Purified Var2CSA constructs.

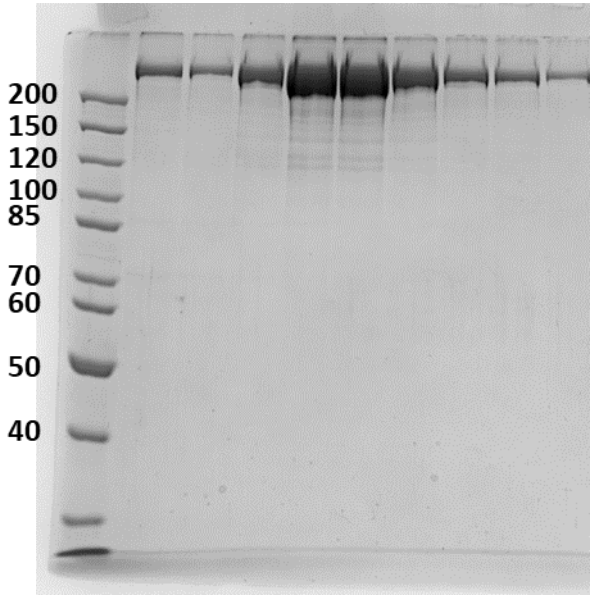
SDS-PAGE of purified Var2CSA constructs



	Size kDa	Carbohydrate binding
1: DBL1x-DBL6ε	315	+++ Specific to CSA
2: Δ59DBL1x-DBL6ε	308	+++ Specific to CSA
3: Δ59DBL1x-DBL6ε with FLAG tag	308	+++ Specific to CSA
4: ID2b-DBL6ε	191	+ Specific to CSA
5: DBL3x-DBL6ε	170	-
6: DBL4ε-DBL6ε	133	-
7: DBL1x- ID2a	121	++ Non specific bind other carbohydrates

Final Step in Purification of Full length Var2CSA





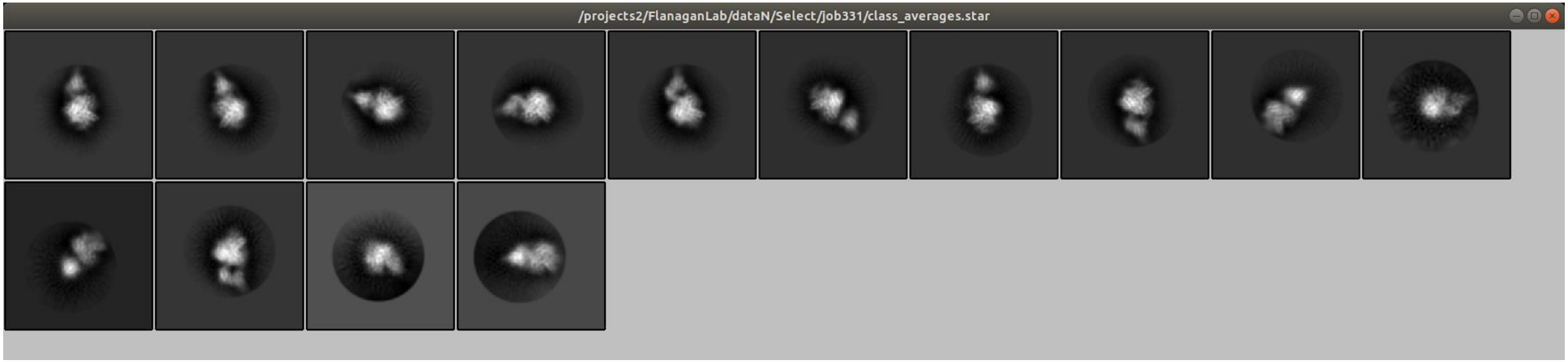
Under native conditions (blue native gel) only a single band is observed.

Two peak fraction, from each SEC run, 330µL, is used for cryoEM sample preparation

Statistics Data collected at NYSBC

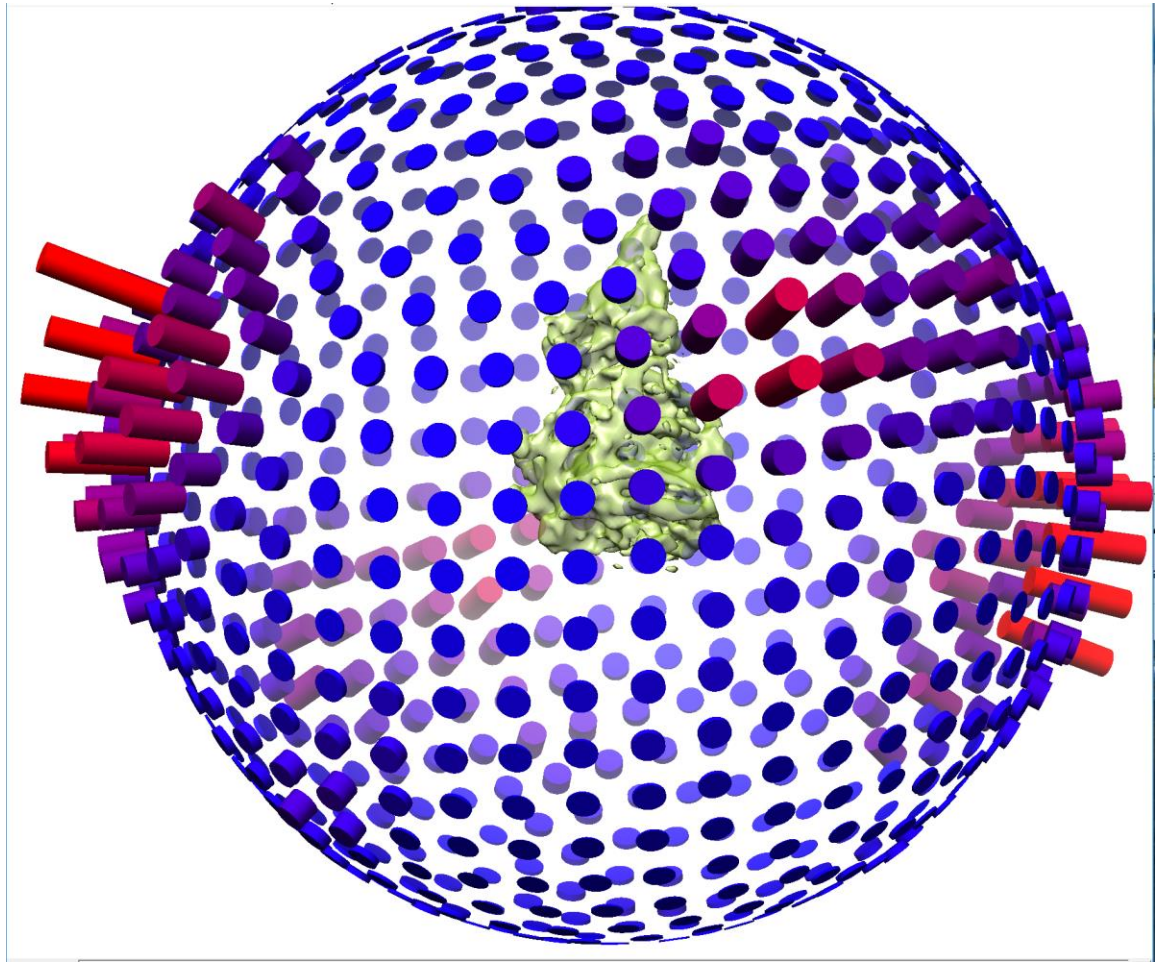
- 1880 images
  - 1300 used due to problems with data collection.
- Manual Picking of ~3k particles
  - 2D Classification to find representative views for template.
- Autopicking with Gautomatch with templates
  - ~250k particles
    - 75k after several rounds of 2D/3D classification.
    - Tried topaz on subset of images, may actually work better than Gautomatch.
- 3D Classification/Refinement/CTF refinement (2x) (9A resolution).
- 3D Classification with mask and no alignment followed by 3D refinement lead to current 6.8A model.

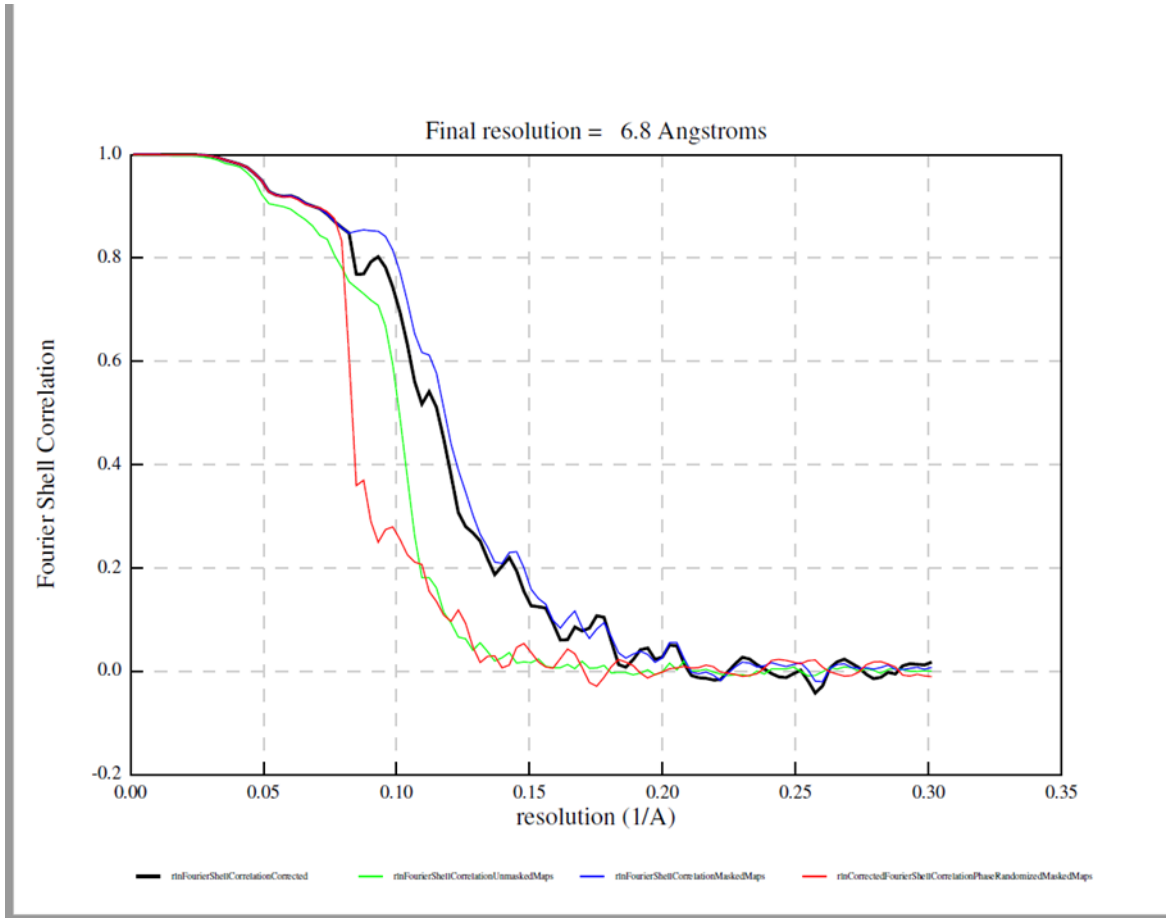
2D Classes used for 3D classification



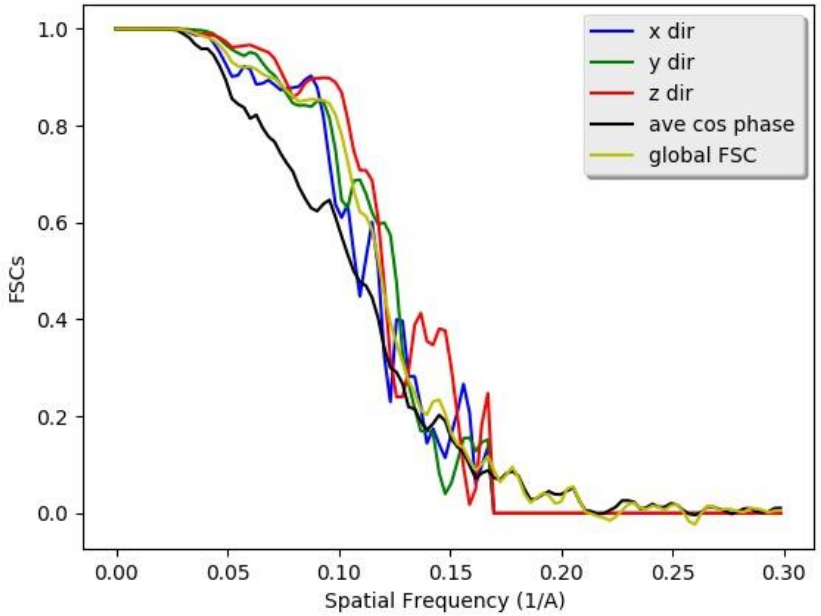
Particles in these classes (120k) from the second round of 2D classification were then used for 3D classification. Three of the five 3D classes gave similar structures with similar resolution 13A. The particles from these were Re-extracted and used for refinement (10.5A final) CTF refinement. The final map was produced using masked 3D classification with a single class and without particle alignment (reported resolution 9.7A) Followed by 3D refinement (8.7A) and post processing yielding a map with 6.7A reported resolution (see below).

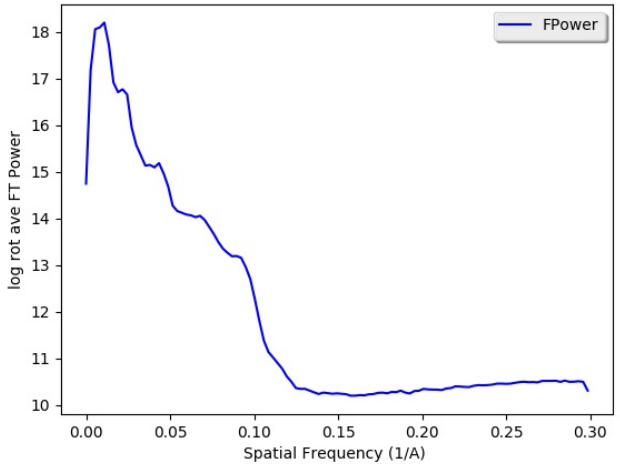
Final Relion Map Post Processed.

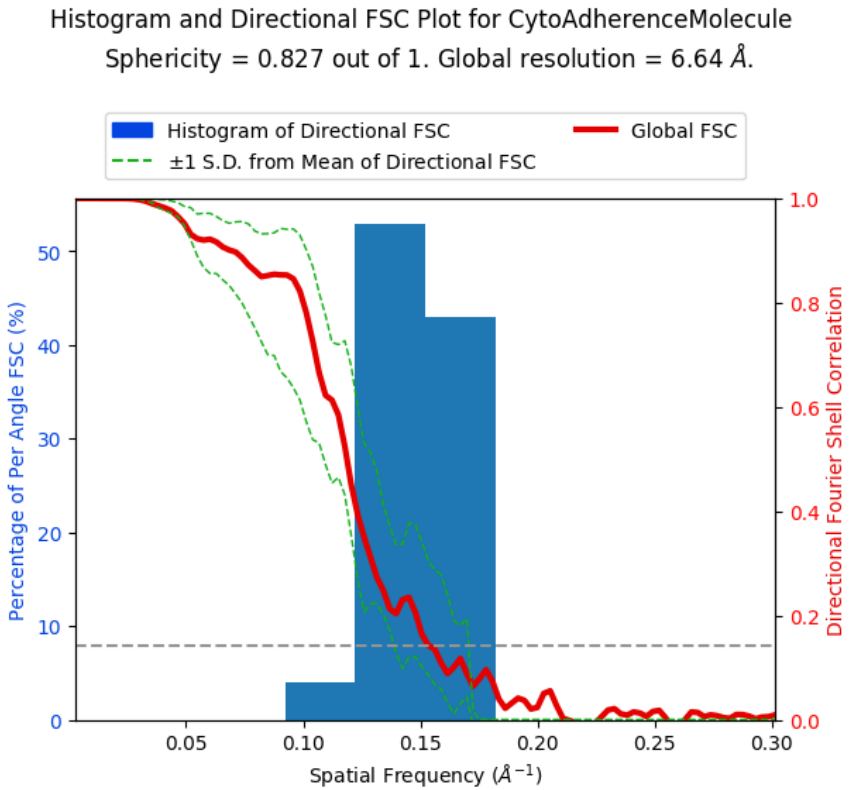




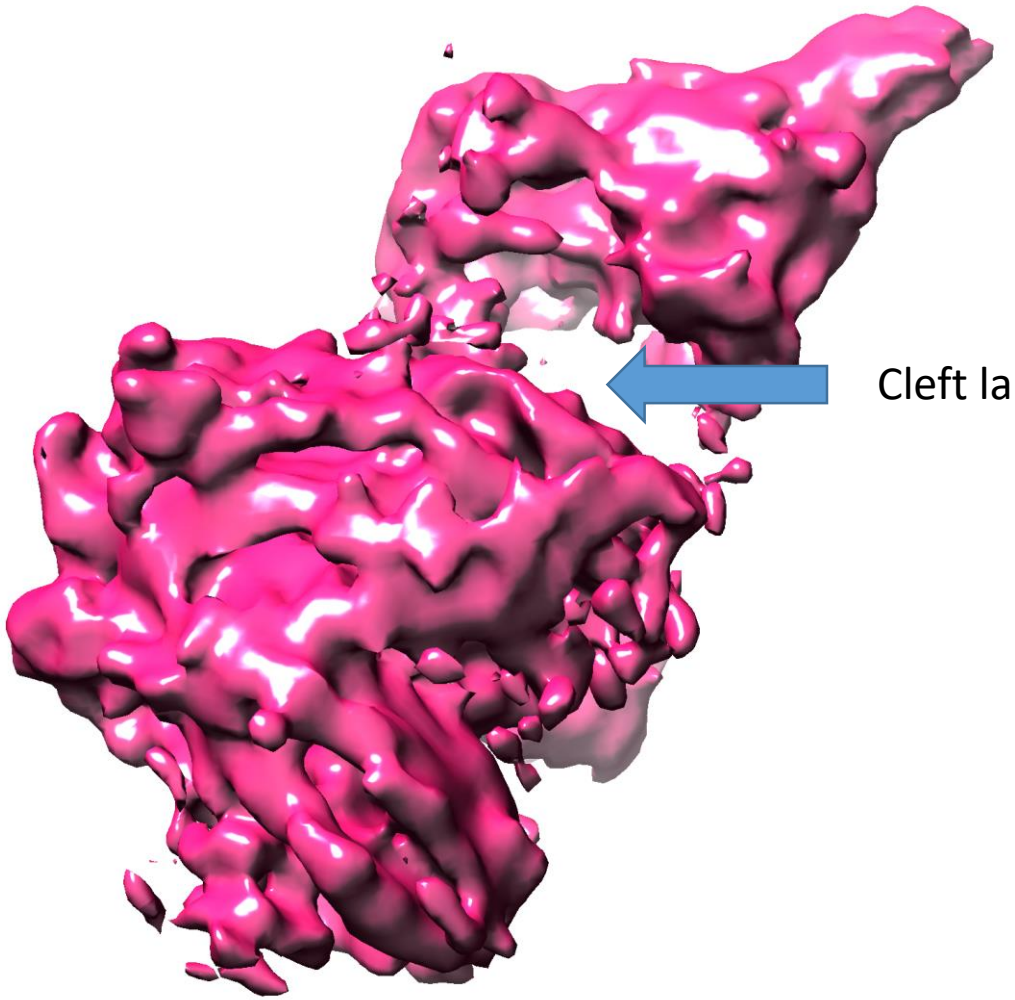
Directional FSC Current Dataset.





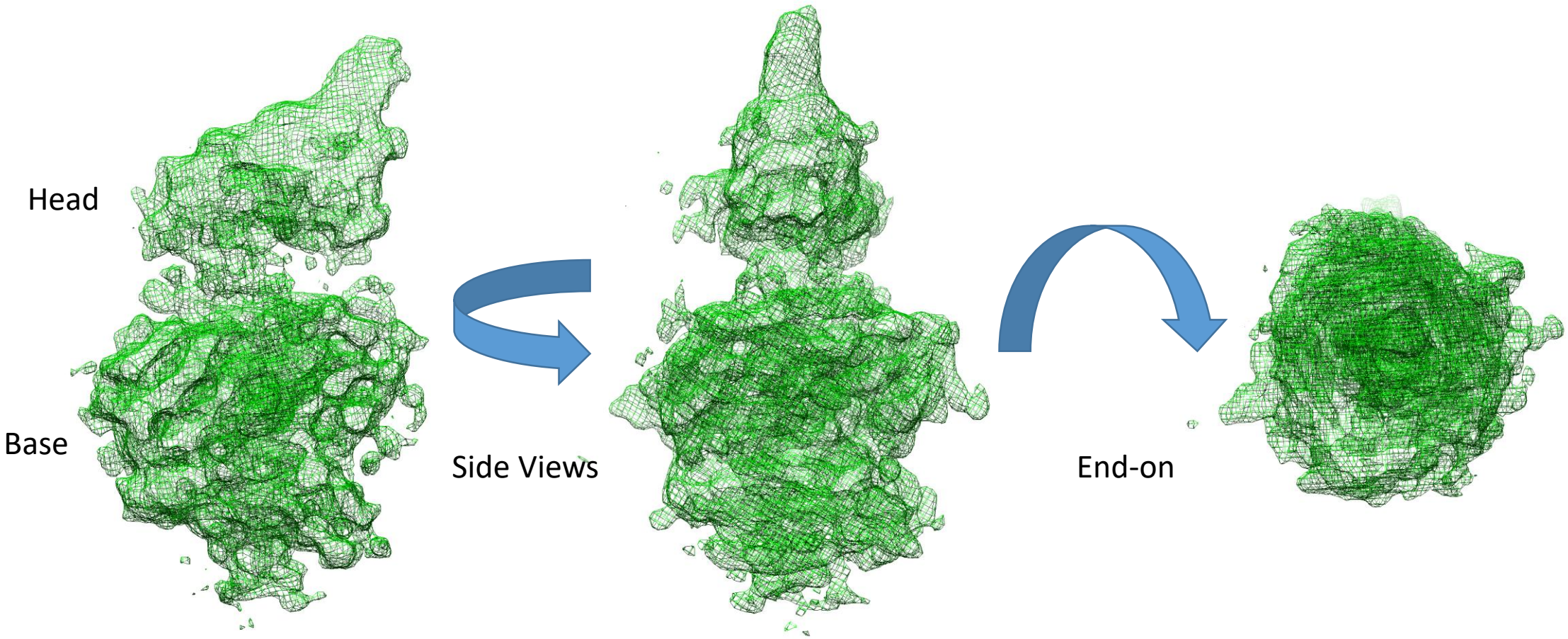


Cleft between head and base seen in all reconstructions independent of resolution

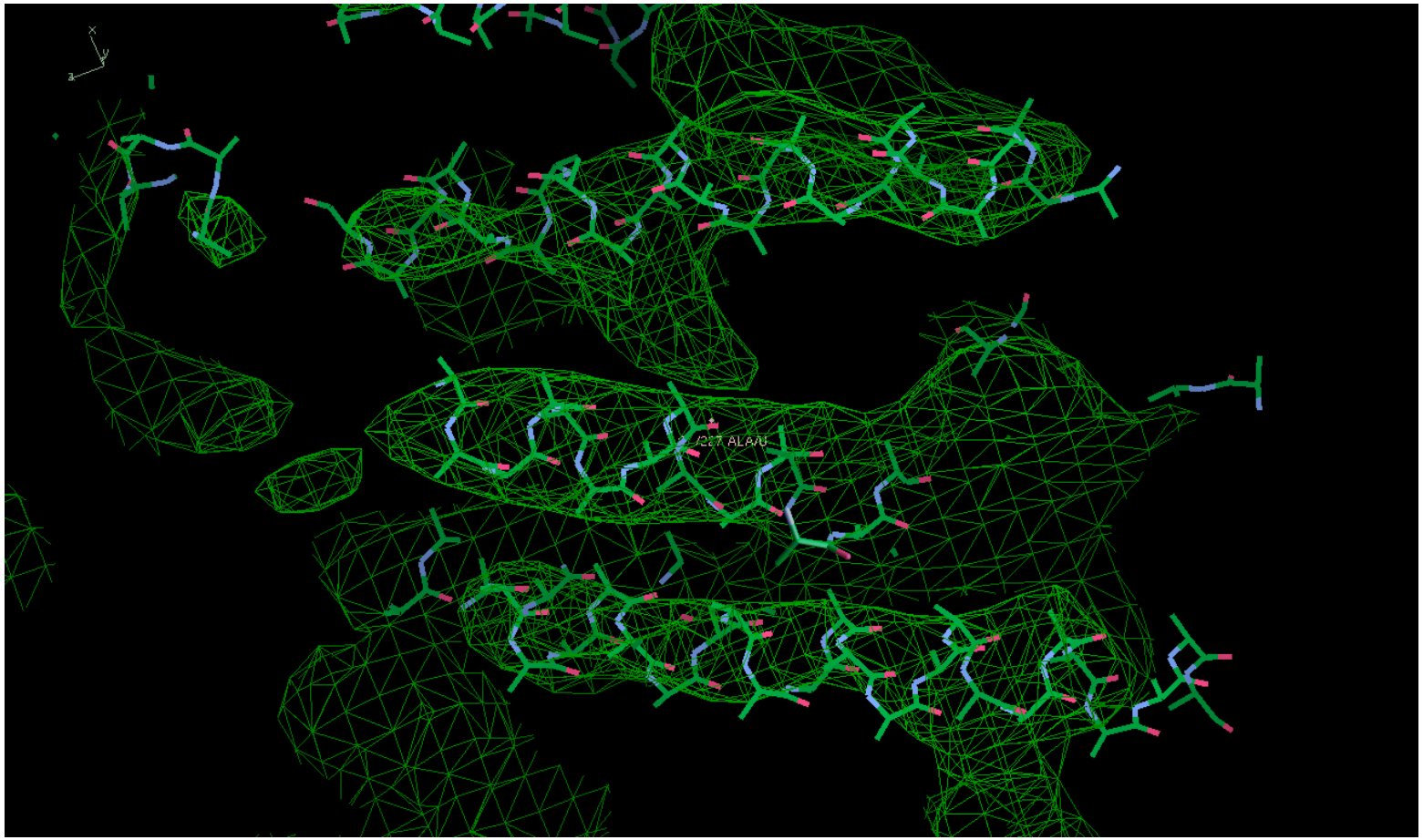


Cleft large enough for carbohydrate

Overall Density at ~6.8Å Resolution



Section of Electron Density Autobuilt by Phenix/Coot.

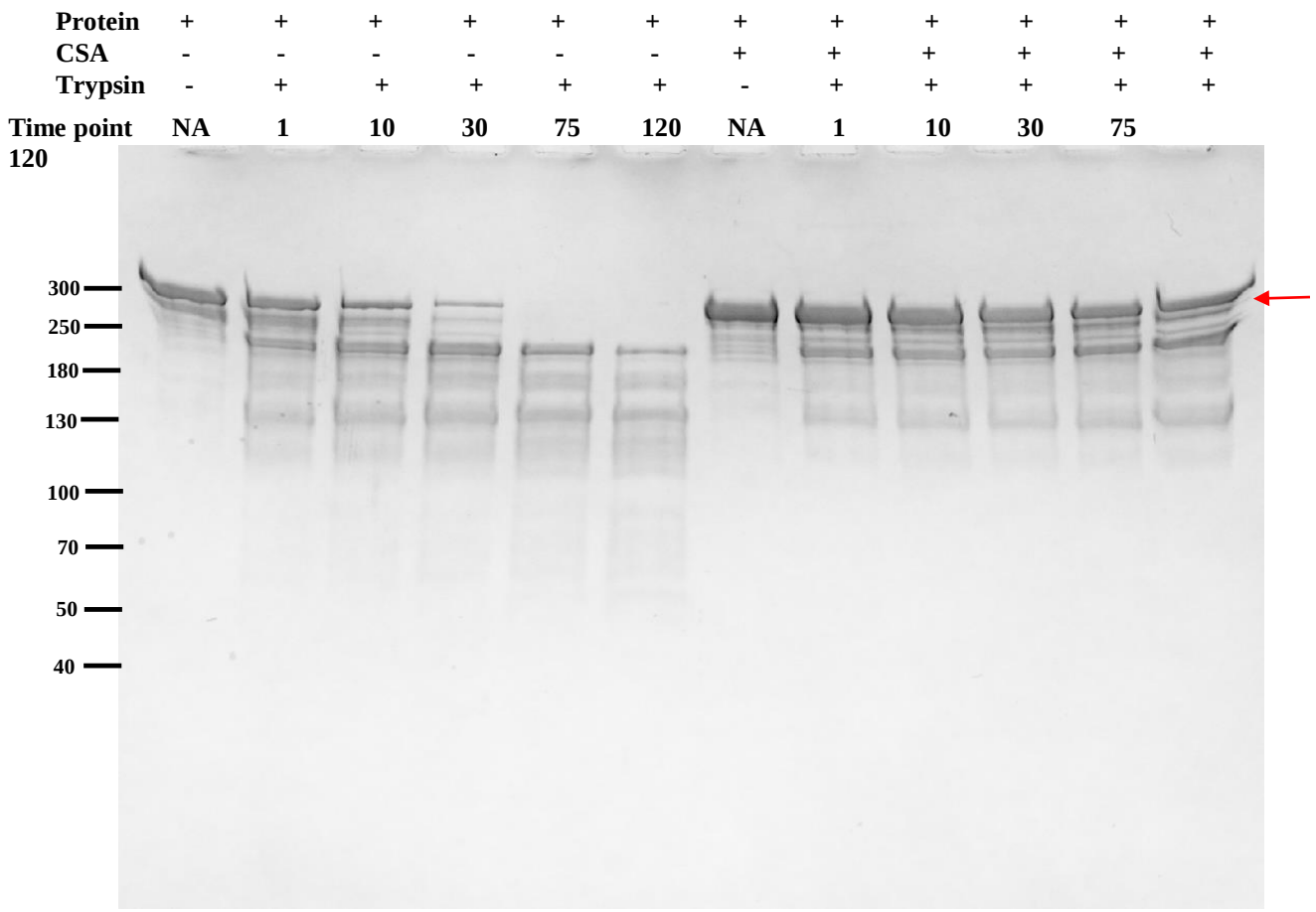


~ 12 helices of the quality shown here were identified in Phenix, and another 6 secondary structural elements of lesser quality were also identified. All of the these were found in the Base of the molecule.

Recent advances in grids preparation and new biochemical developments.

- Prepared grids with chameleon partially characterized them.
- Prepared gold grids with Spotiton. These have yet to be tested.
- Purified CSA (Var2CSA substrate) 12-14 sugars in length (See Slide 3).
  - We have previously shown that this length is optimal for binding.
    - Despite heterogeneity (at the single chain level), in the location of the sulfates groups, this preparation binds with ~5nM affinity to full length Var2CSA and provides significant stabilization in protease protection assays.
    - We have synthesized short CSA oligomers with specifically placed sulfates, currently we have a CSA 8 mer with 2 adjacent SO_4 groups that binds with 0.5µM affinity and seek to extend this to produce a set of dodecamers to fully define the optimal sulfate positions in this ligand.
- We have engineered monoclonal antibody binding sites into Var2CSA.
  - Of the 8 constructs tested, two one in Dbl2 and the other in Dbl6, were identified that did not affect protein stability, global folding (negative stain), carbohydrate binding and had high affinity for the cognate monoclonal antibody. The full-length antibodies can be seen in negative stain bridging two modified Var2CSA molecules but the samples have not been characterized with Fab fragments or for production cryo-cooled samples.

Chondroitin sulfate binding to Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1/VAR2CSA) protein increase protease resistant



The Purified VAR2CSA protein was incubated with and without CSA (faction number 30) in 1: 20 molar ratio for one hour at 37^{o} C. Trypsin (1:500) was added to each sample (without/with CSA) and incubation and aliquots collected at different time intervals such as 1, 10, 30, 75 and 120 minutes were removed reduced using loading dye containing β-mercaptoethanol followed by SDS-PAGE gel electrophoresis respectively. The results of VAR2CSA protein showed protease resistance to tryptic digestion in presence of chondroitin sulfate in post 10 minute aliquots (Marked in red color).