



Elucidation of the S Protein Structure of SARS-CoV-2 Mutants

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SARS-CoV-2 & ACE2

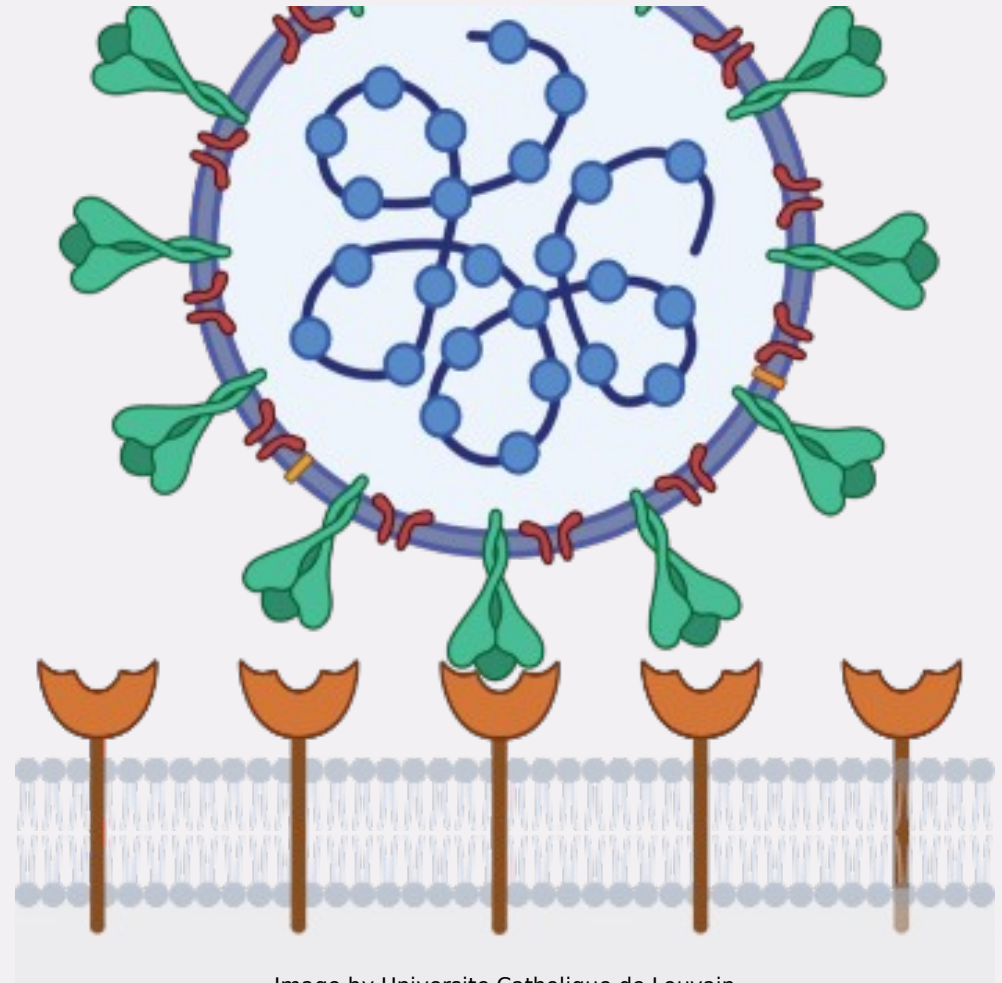


Image by Universite Catholique de Louvain

Relevance

- SARS-CoV-2 is a highly contagious respiratory virus

The virus (now) has several variants, some with increased infectivity

- Previously, elucidation of the S protein structure was crucial

We needed therapeutic solutions to ameliorate death numbers and lessen severity of the disease (vaccines, manufactured antibodies, etc....)

- It now is just as critical, especially as we attempt to understand the reasons for exacerbated infectivity (think the delta variant)

The structures/geometry between variants differ, offering better binding to ACE2

The major difference is the number of H-bonds; more = stronger binding -> useless vaccines

The S Protein

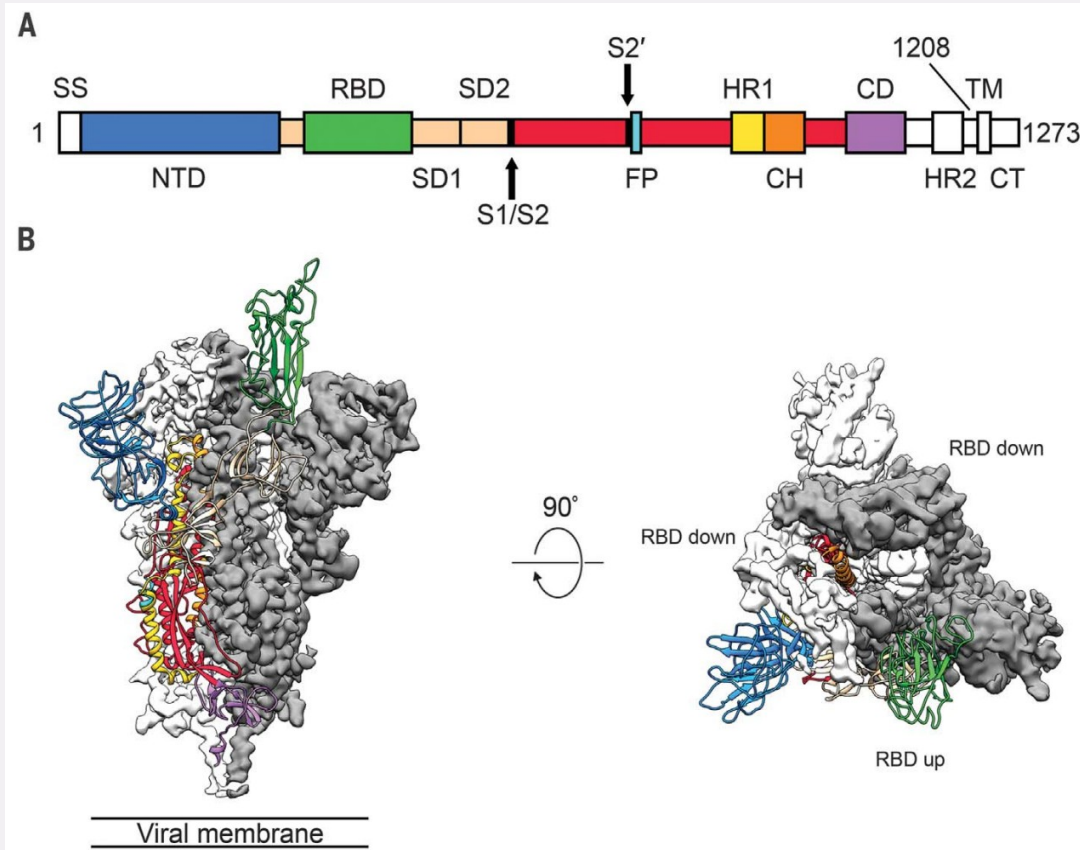


Image by Wrapp, et al

- Trimeric, metastable, glycosylated protein

What does this mean?

- Undergoes significant changes for viral fusion to host receptor

*Facilitated by RBD's hinge-like movements
(down = inaccessible)*

- This image depicts various 2D structures and 3D motifs


Alpha helices and beta-pleated sheets

Different representations – colours vs grey

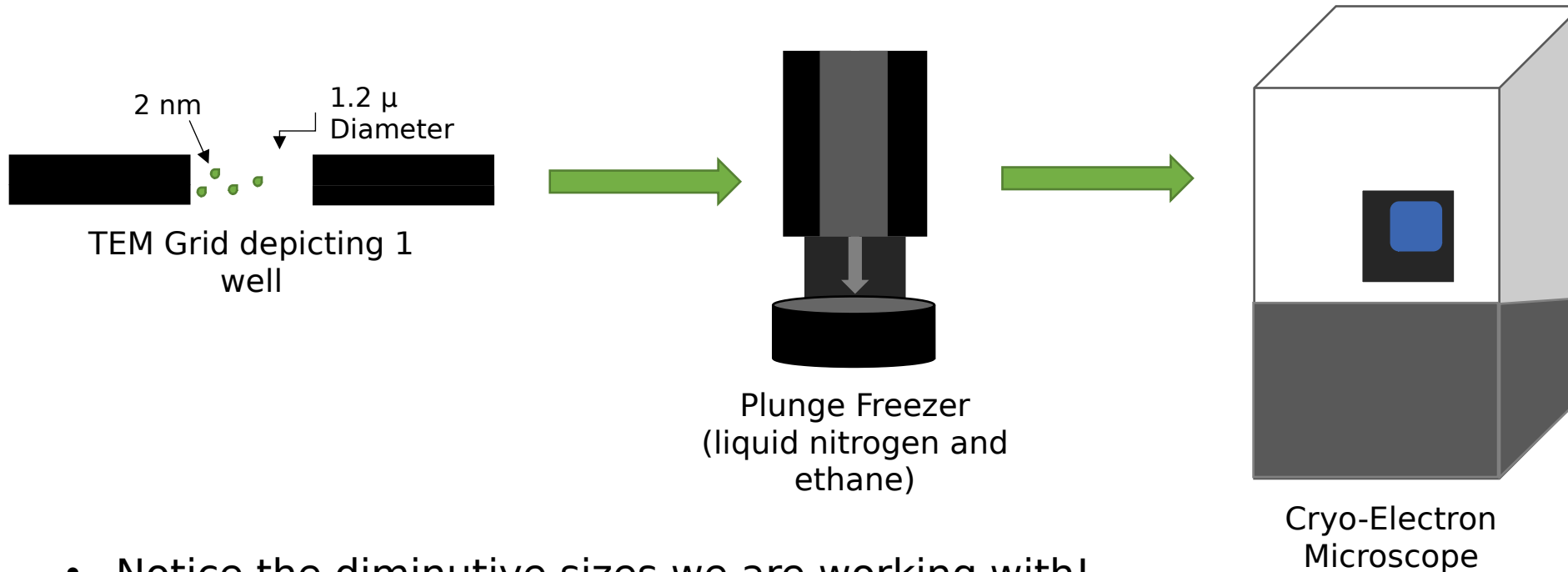
Goals

My goal is to create a high-resolution 3D reconstruction of the S protein using cryoSPARC

cryoSPARC is a program that analyses micrograph images to produce 3D models of microscopic particles

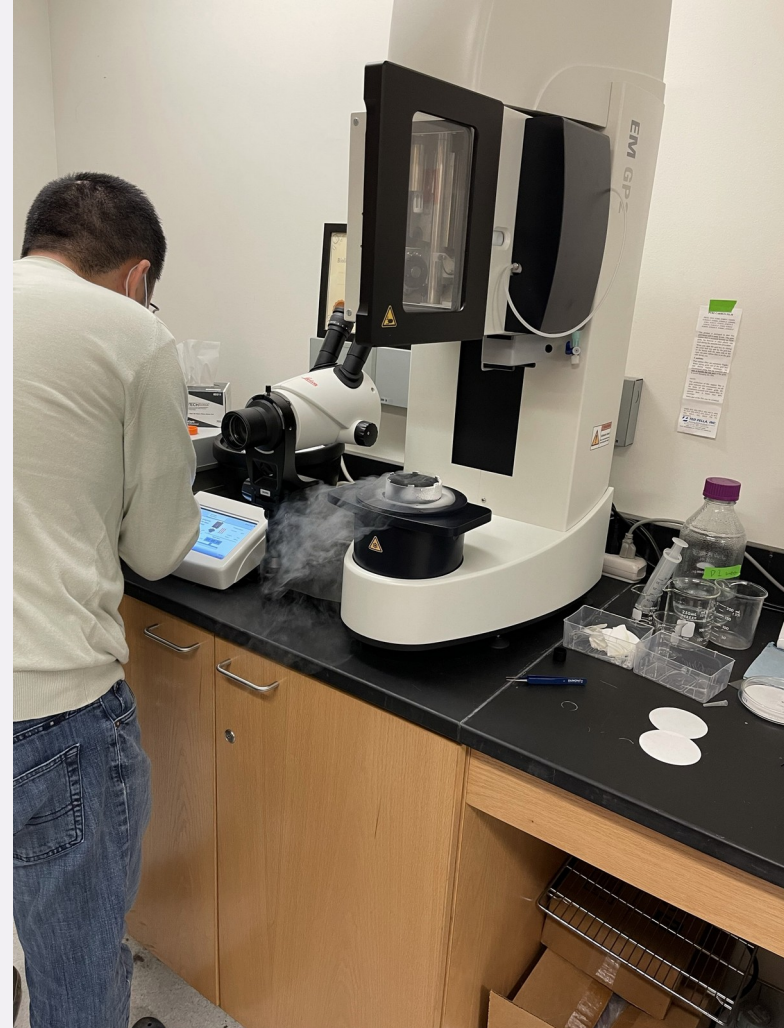


Cryo-EM Workflow

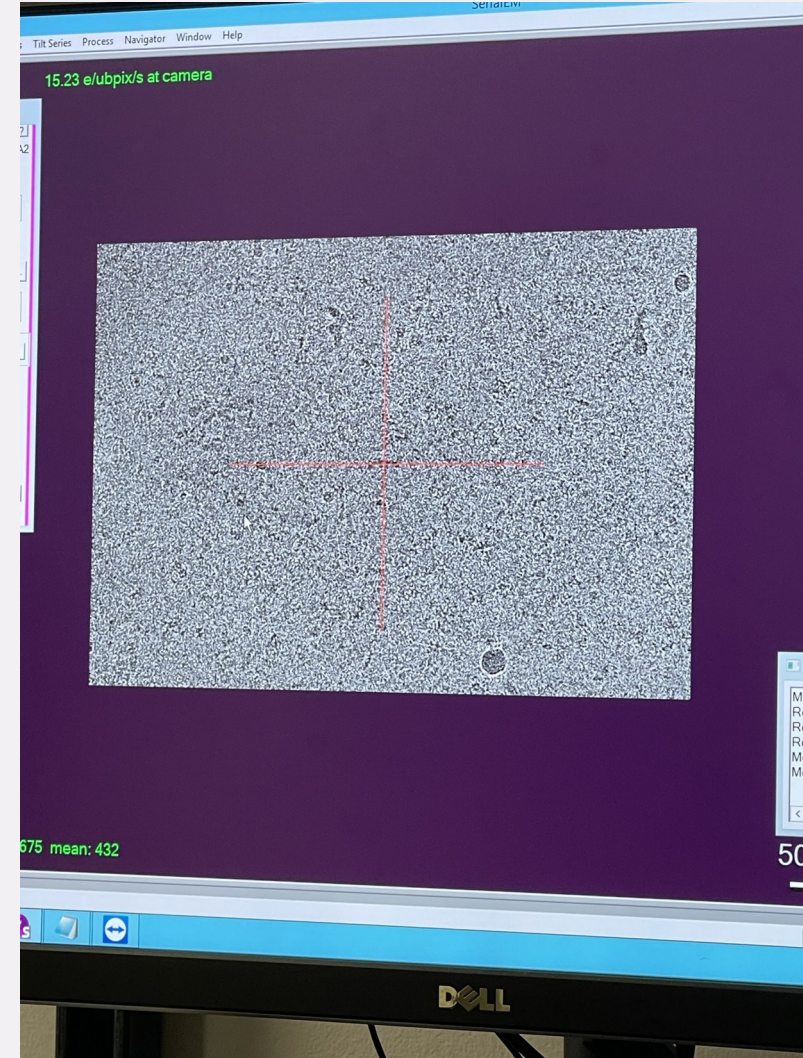
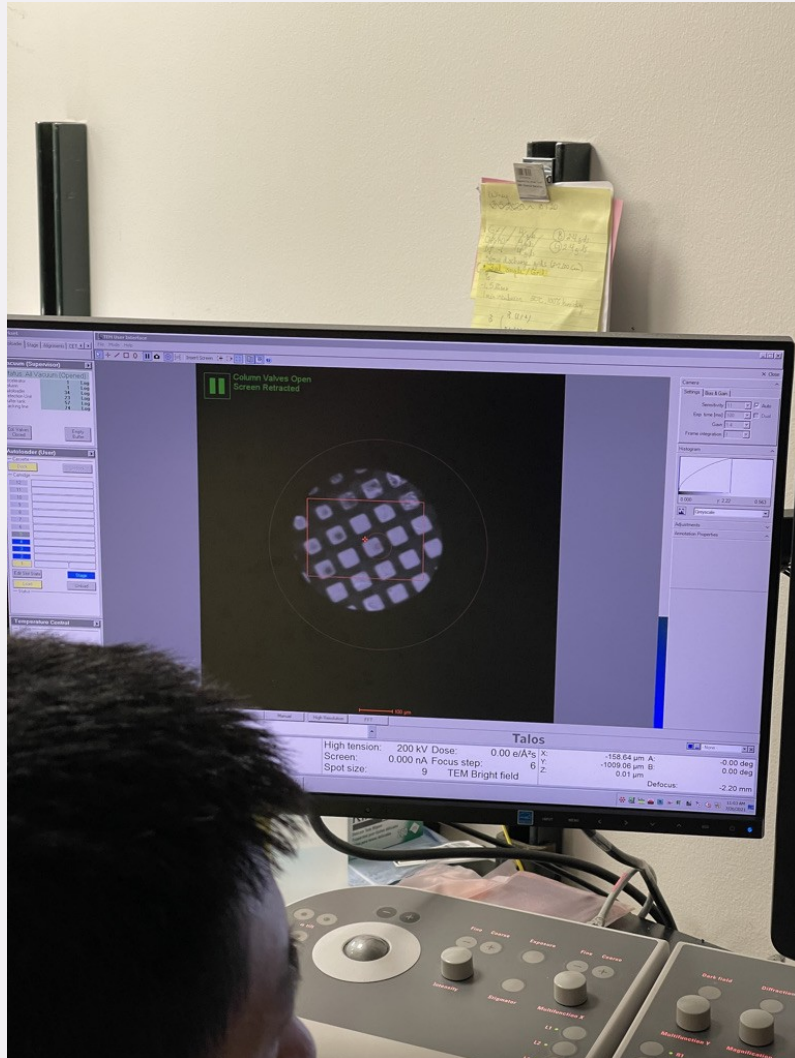


- Notice the diminutive sizes we are working with!
We attempt to generate ≤ 5 Angstrom (0.5 nm) resolution images, which are obtainable with electron microscopy
- After these steps, we can process the images in cryoSPARC (or other EM processing software)

Cryo-EM Preparation



Imaging



cryoSPARC

Image Preprocessing

Import, Motion
Correction, CTF
Estimation

Selecting Exposures and Particles

Select
Micrographs,
Blob Picker,
Inspect Picks,
Extract Picks

Particle Refinement

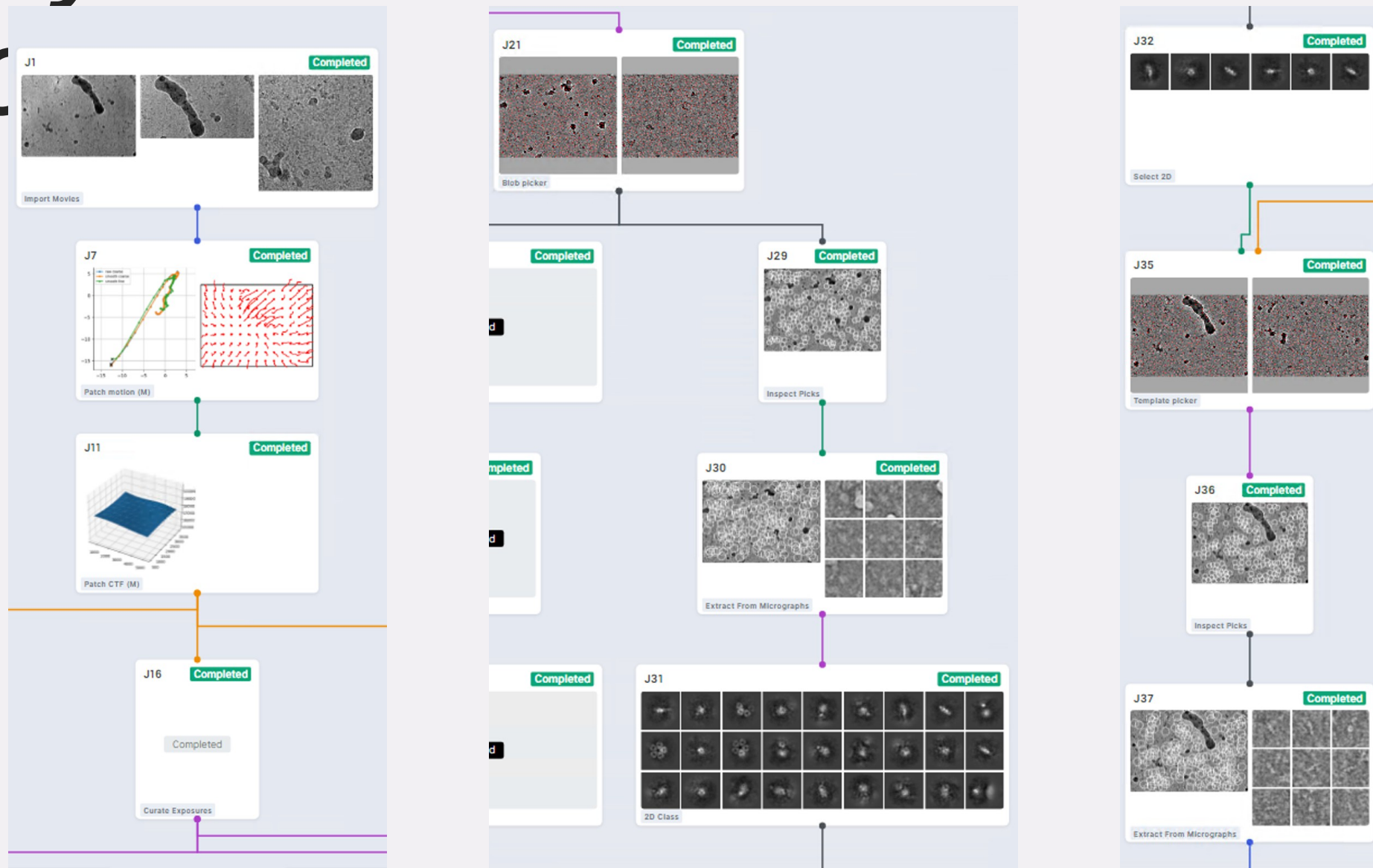
2D Classification,
Select 2D
Classes (several
rounds)

Particle Reconstruction

Ab-initio
Reconstruction,
Refinement

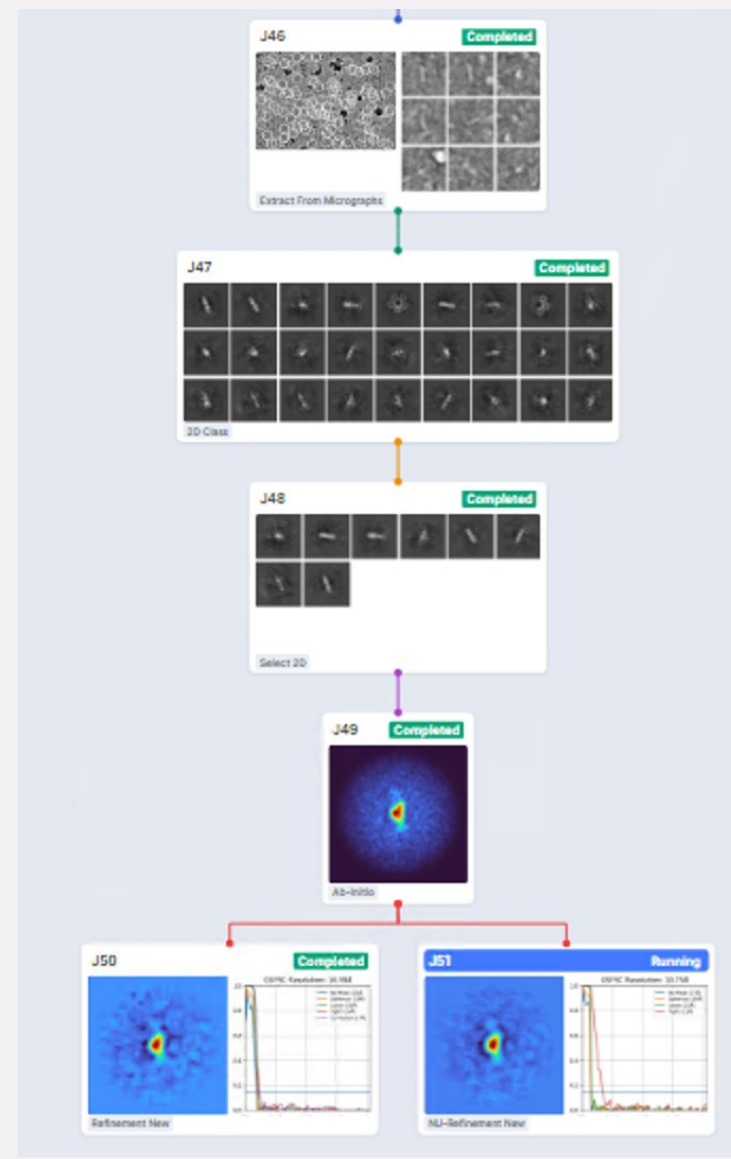
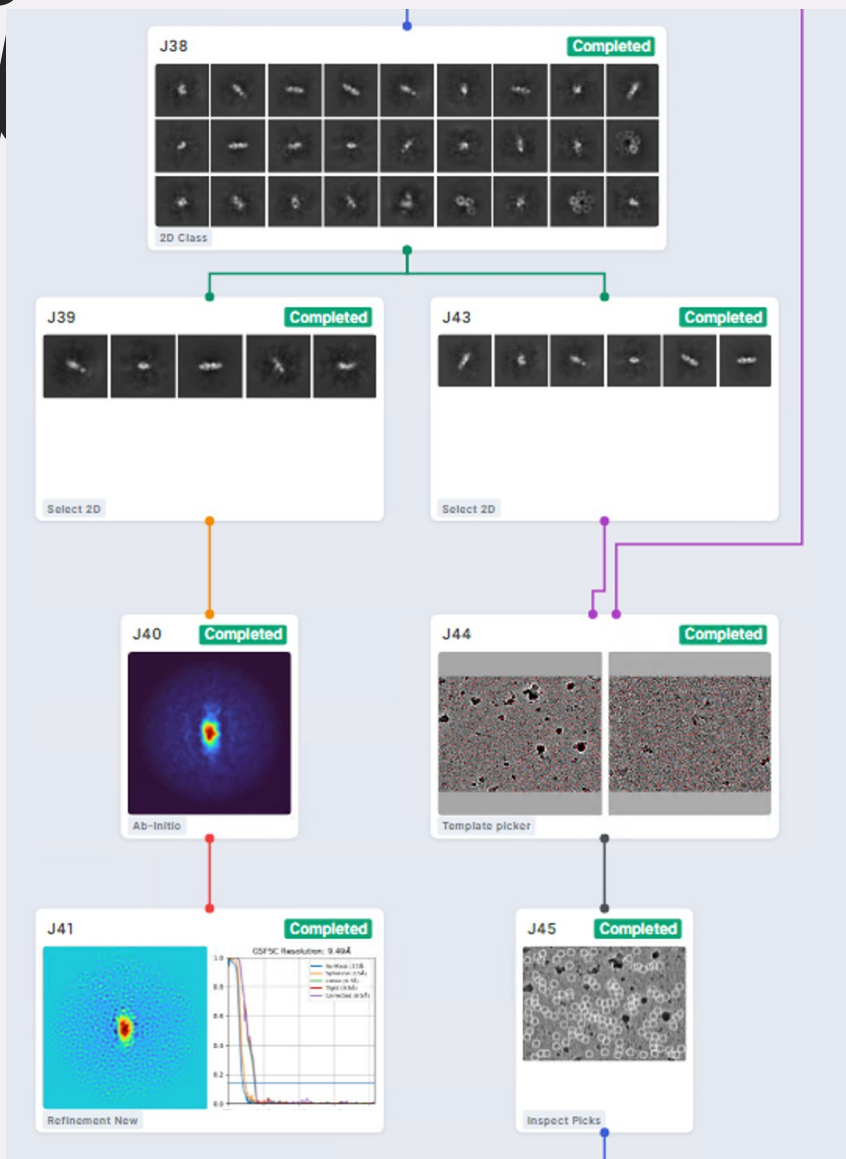
cryoSPARC

Jobs

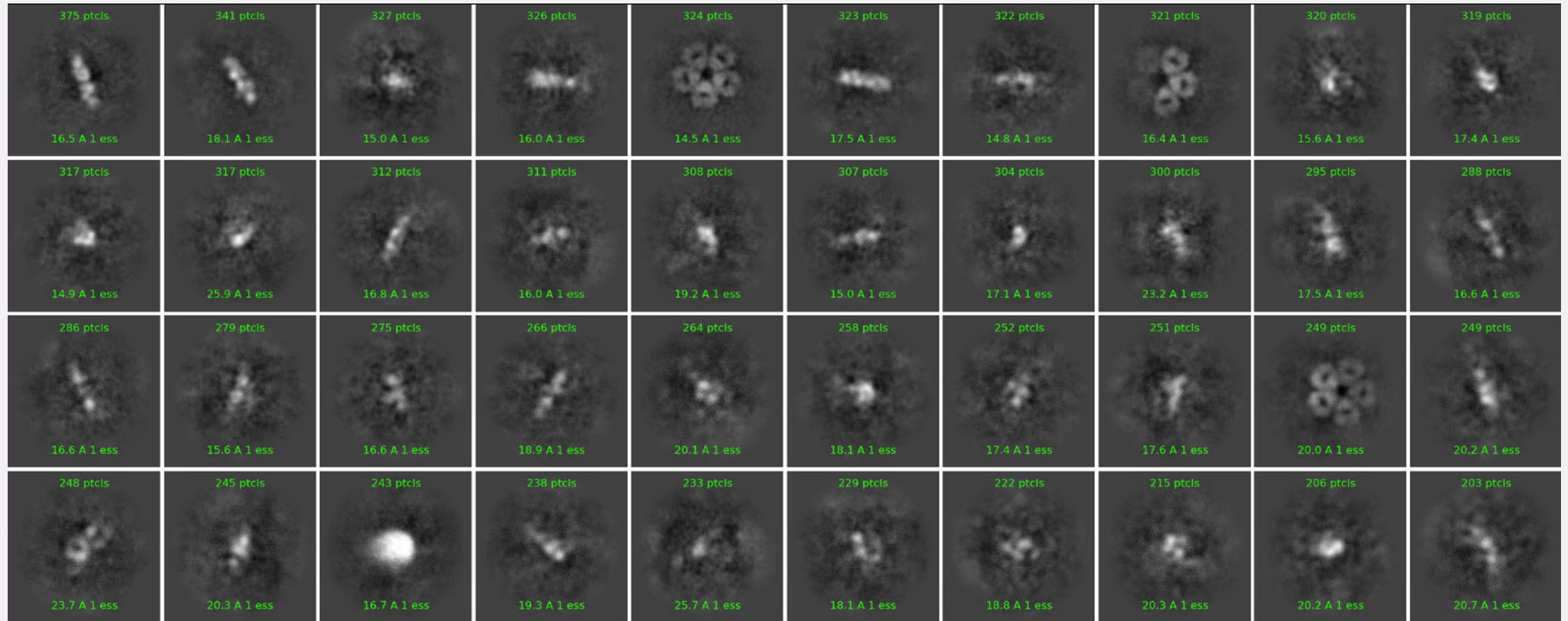


cryoSPARC

Join



2D Classification



Mathematical Methods

- Ab-initio reconstruction relies on a Bayesian framework¹

argmax is the set of points where p is maximised

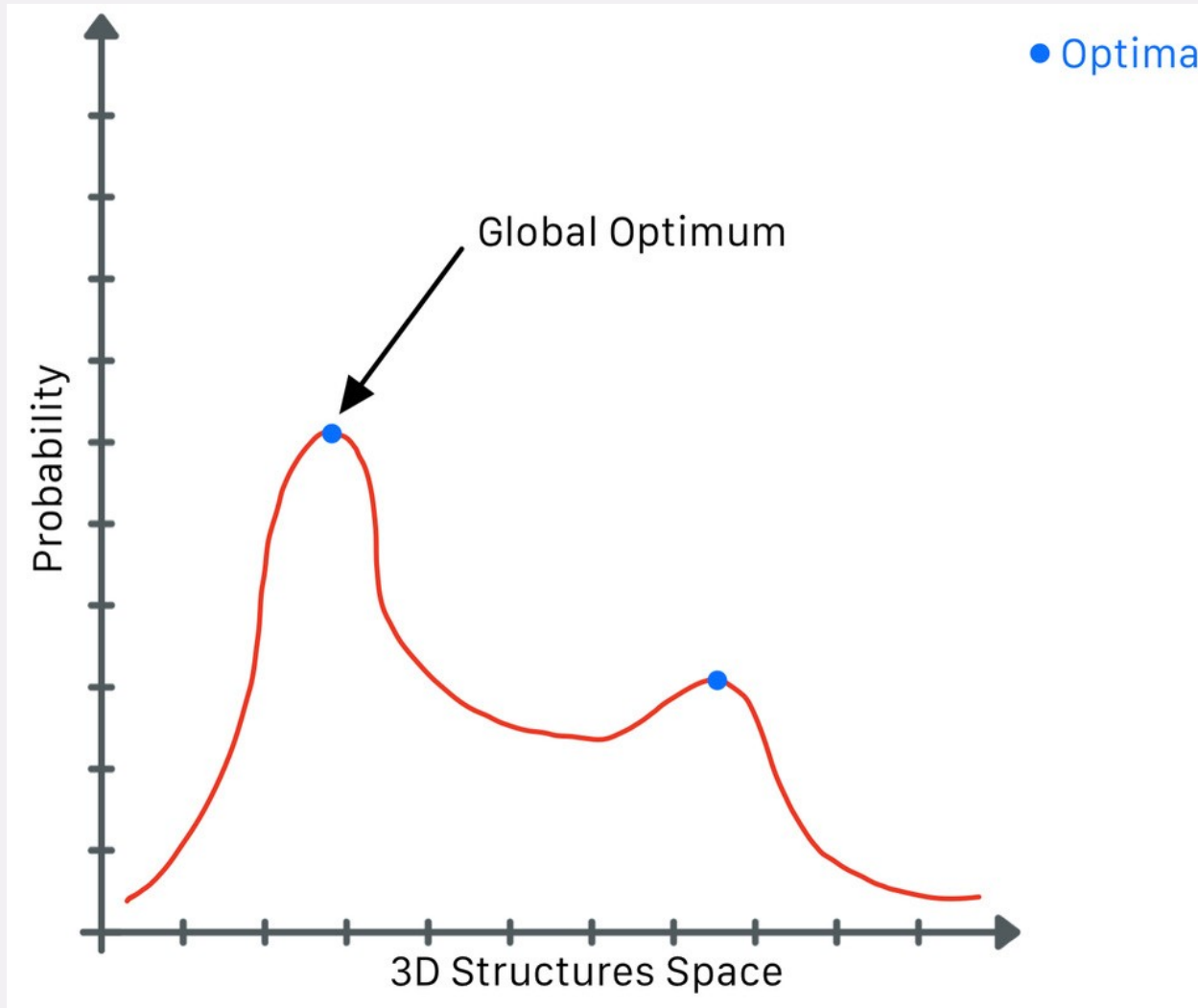
*p is the probability of generating a structure, , given the images, , taken
is the pose or 3D rotation and 2D translation*

- A stochastic gradient descent algorithm is utilised

This reconstruction is a non-convex optimisation problem



Mathematical Methods



- Stochastically “bounces around” the 3D space
- Runs multiple iterations using random subsets of the images taken (several)
- In doing this, the cryoSPARC can arrive at the global optimum (~the actual particle structure)
Other software may get “stuck” at another optimum, resolving an incorrect structure

S Protein Models

Comparing ~9.5 Angstrom resolution to ~8 Angstrom resolution

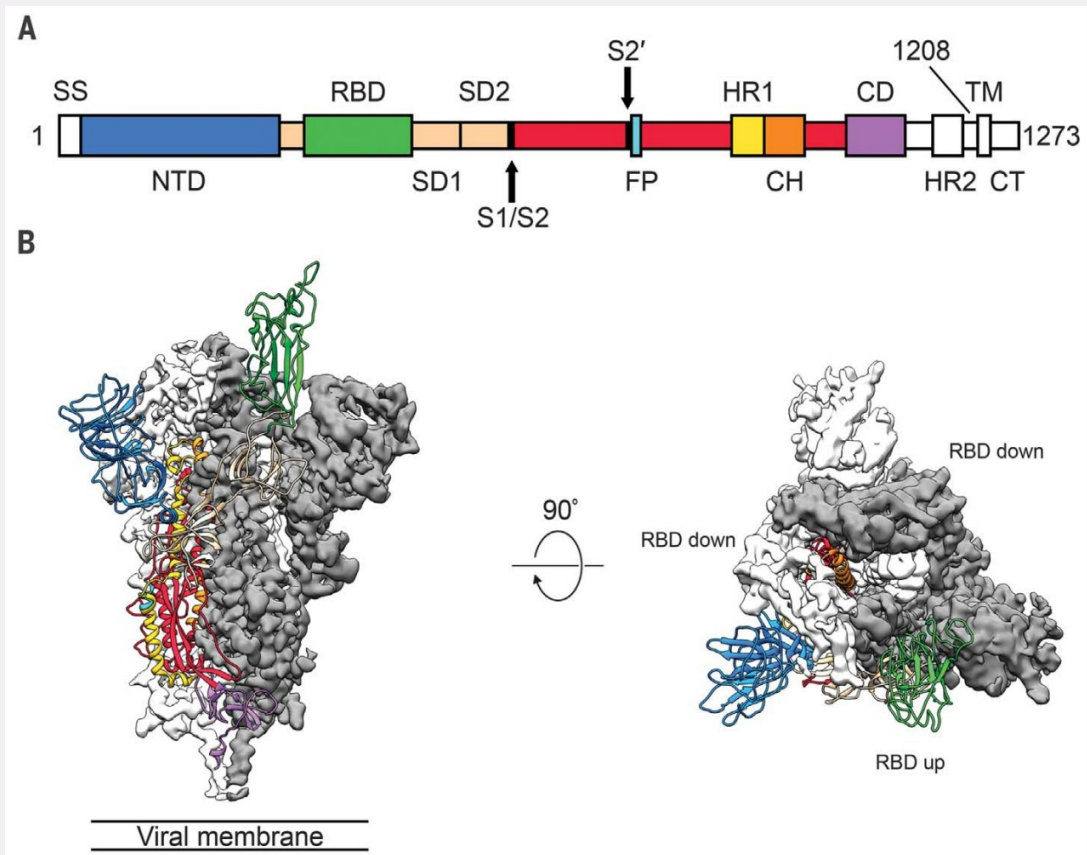
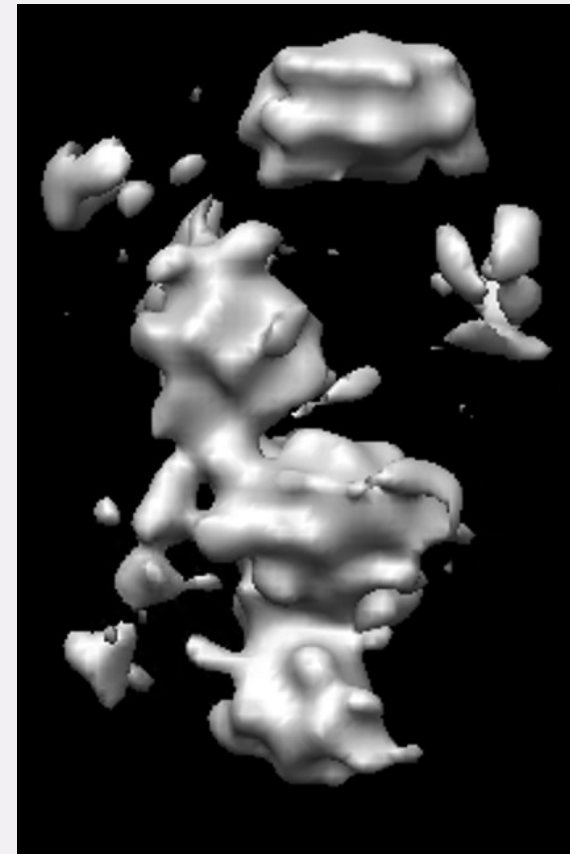
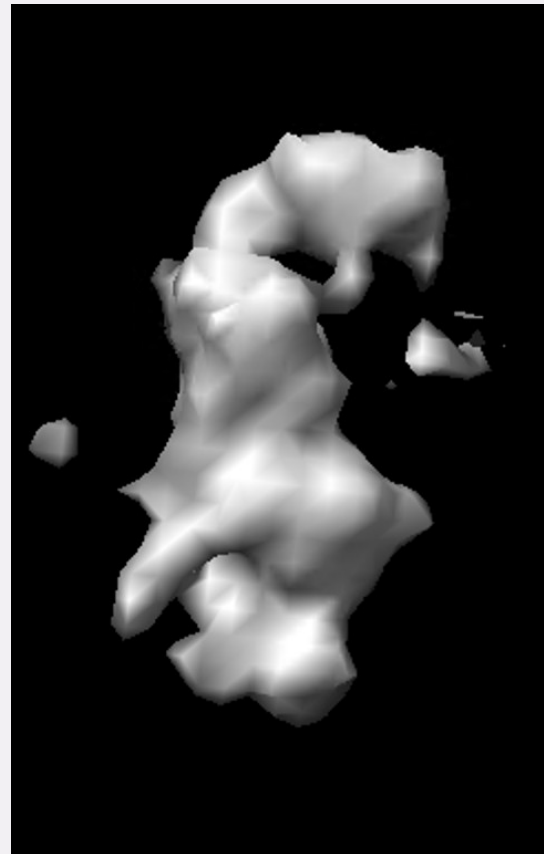


Image by Wrapp, et al



Conclusion & Future Work

- Cryo-EM is a powerful tool for imaging miniscule particles; it has the power to offer high resolutions (near-atomic distance)
- cryoSPARC is a unique software that applied mathematical algorithms to solve optimisation problems
- Given more time, I would further refine the S protein to achieve a higher resolution reconstruction (comparable to the Wrapp image)
- I would also like to compare various mutants to one another to see if there is a noticeable conformational difference
- Other highly-symmetric particles in our data set would be nice to analyse



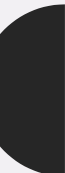
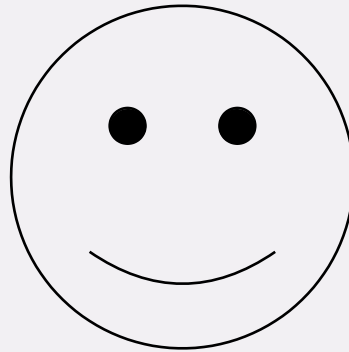
*Thank
You!*

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...and, of course, all of you!



References

- [1] Punjani, Ali, et al. “CryoSPARC: Algorithms for Rapid Unsupervised Cryo-Em Structure Determination.” *Nature Methods*, vol. 14, no. 3, 2017, pp. 290–296., doi:10.1038/nmeth.4169.