



Methods for High Resolution Refinement in Single Particle Processing

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Single Particle Reconstruction with EMAN

GroEL

Donghua Chen

Joanita Jakana

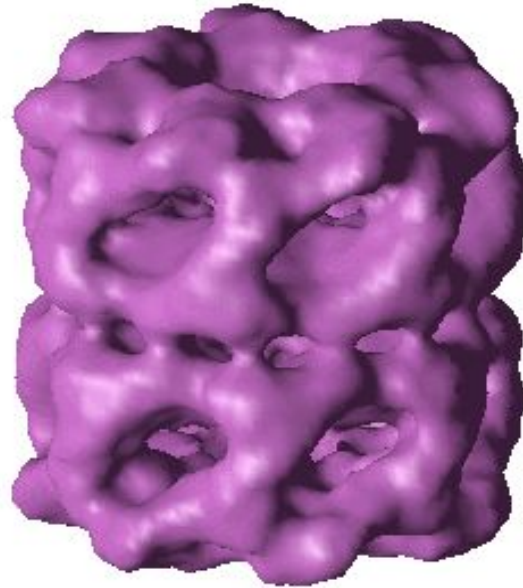
Wah Chiu

Jiu-Li Song (UT-SW Med)

David Chuang (UT-SW Med)

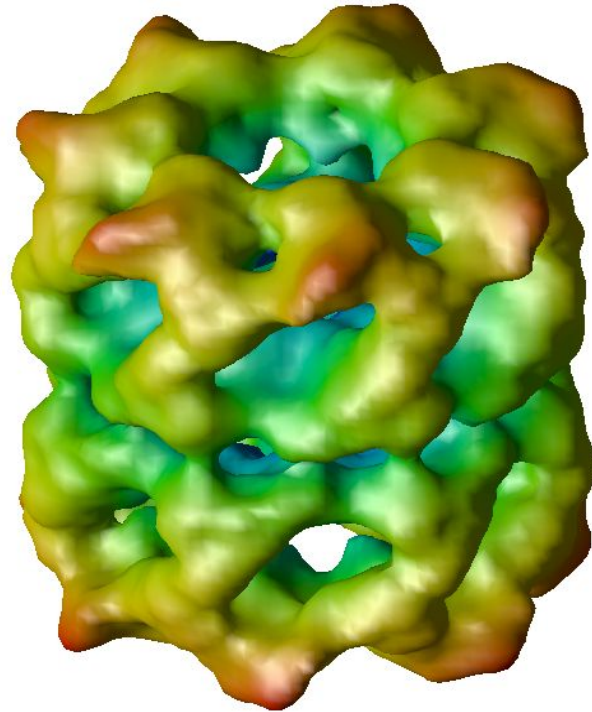
EMAN: <http://ncmi.bcm.tmc.edu/eman>

GroEL 2000 (15 Å)



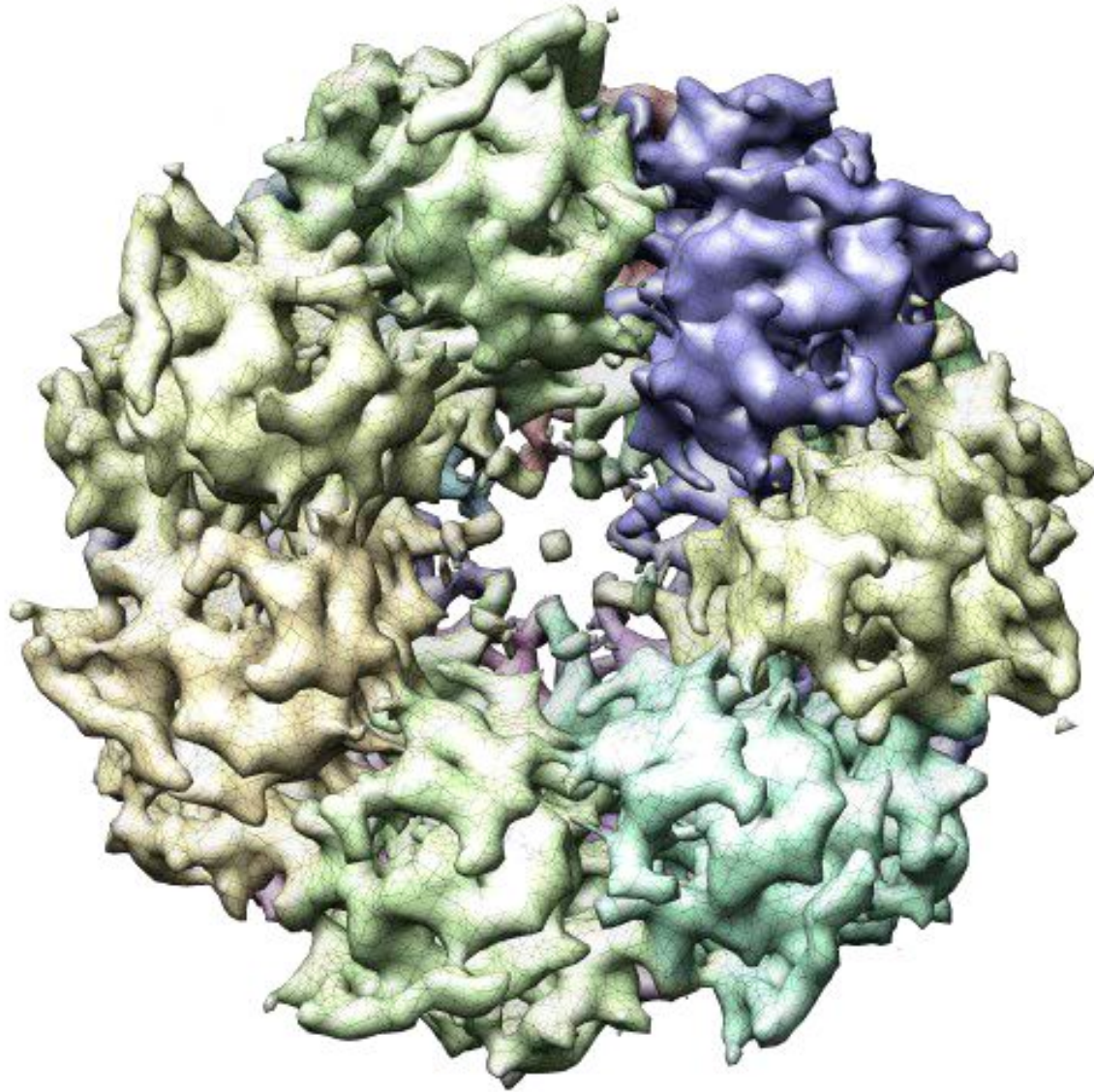
5000 particles, JEOL 4000

GroEL 2001 (11.5 Å)



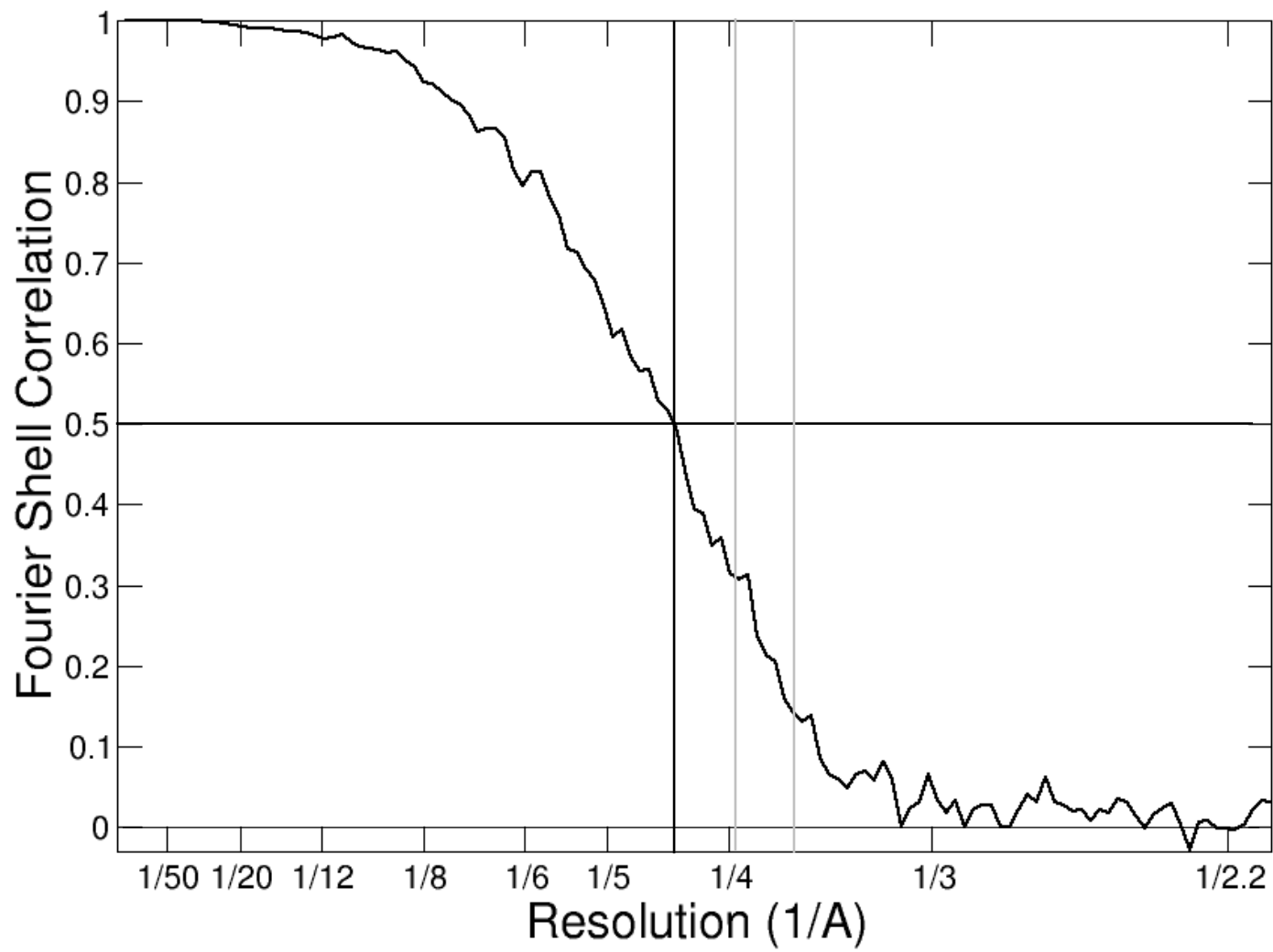
5000 particles, JEOL 4000

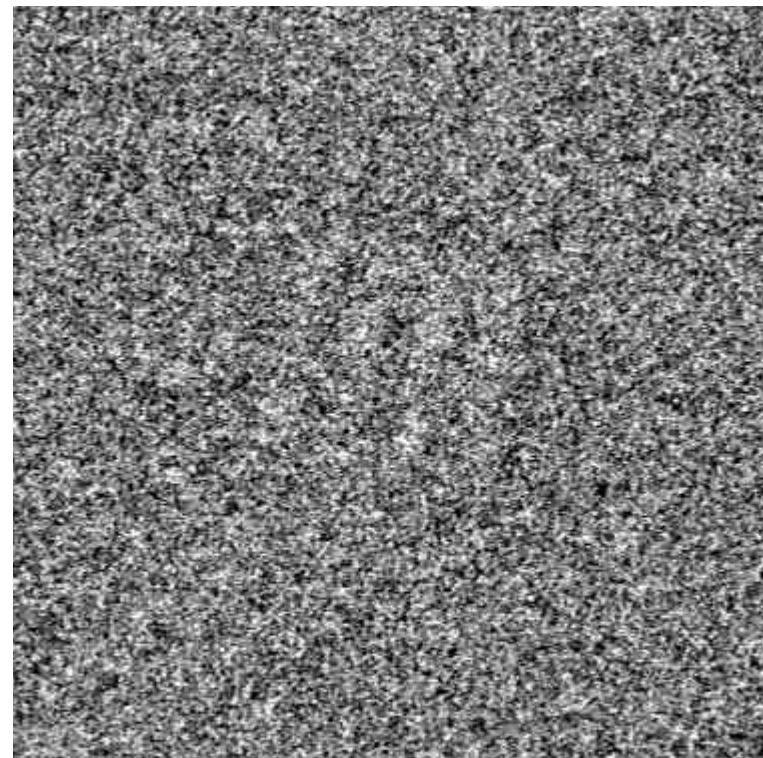
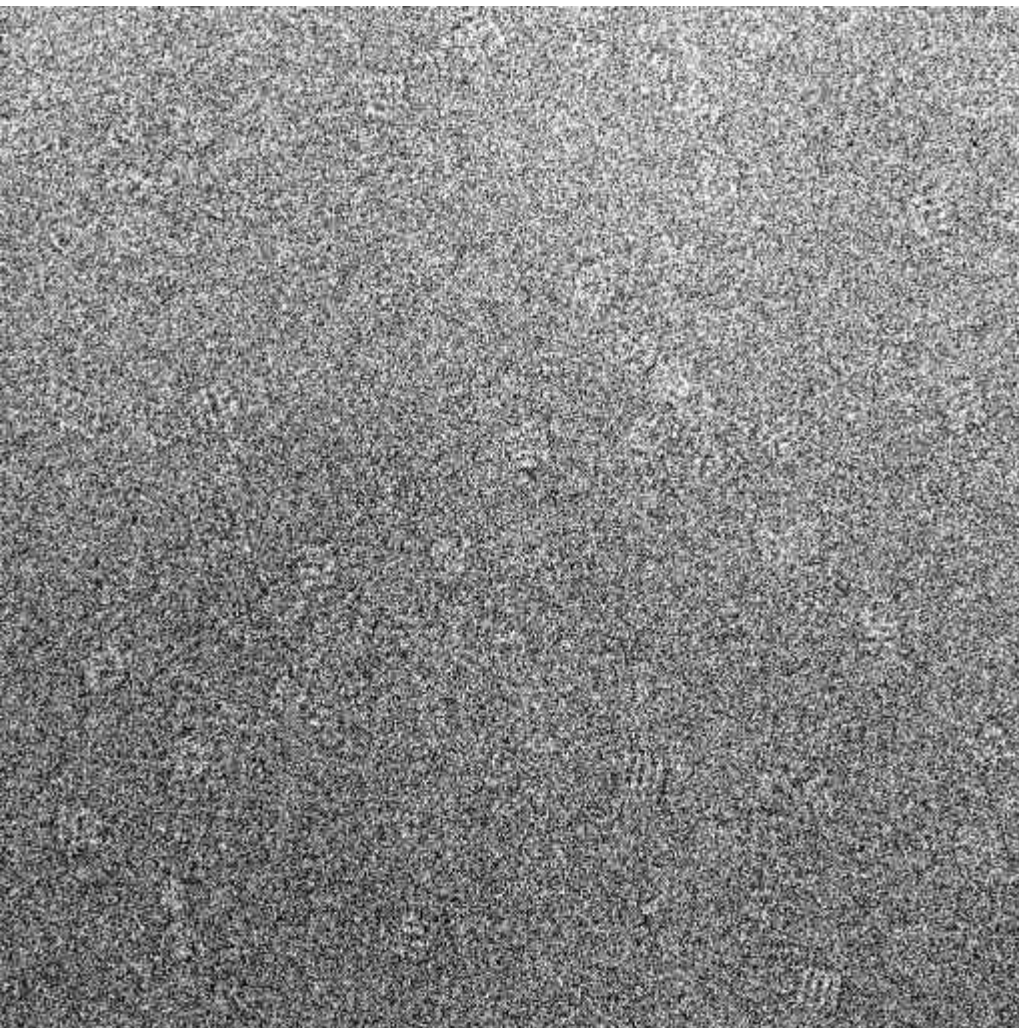
GroEL 2003 (6 Å)



30,000 particles, JEOL 2010F

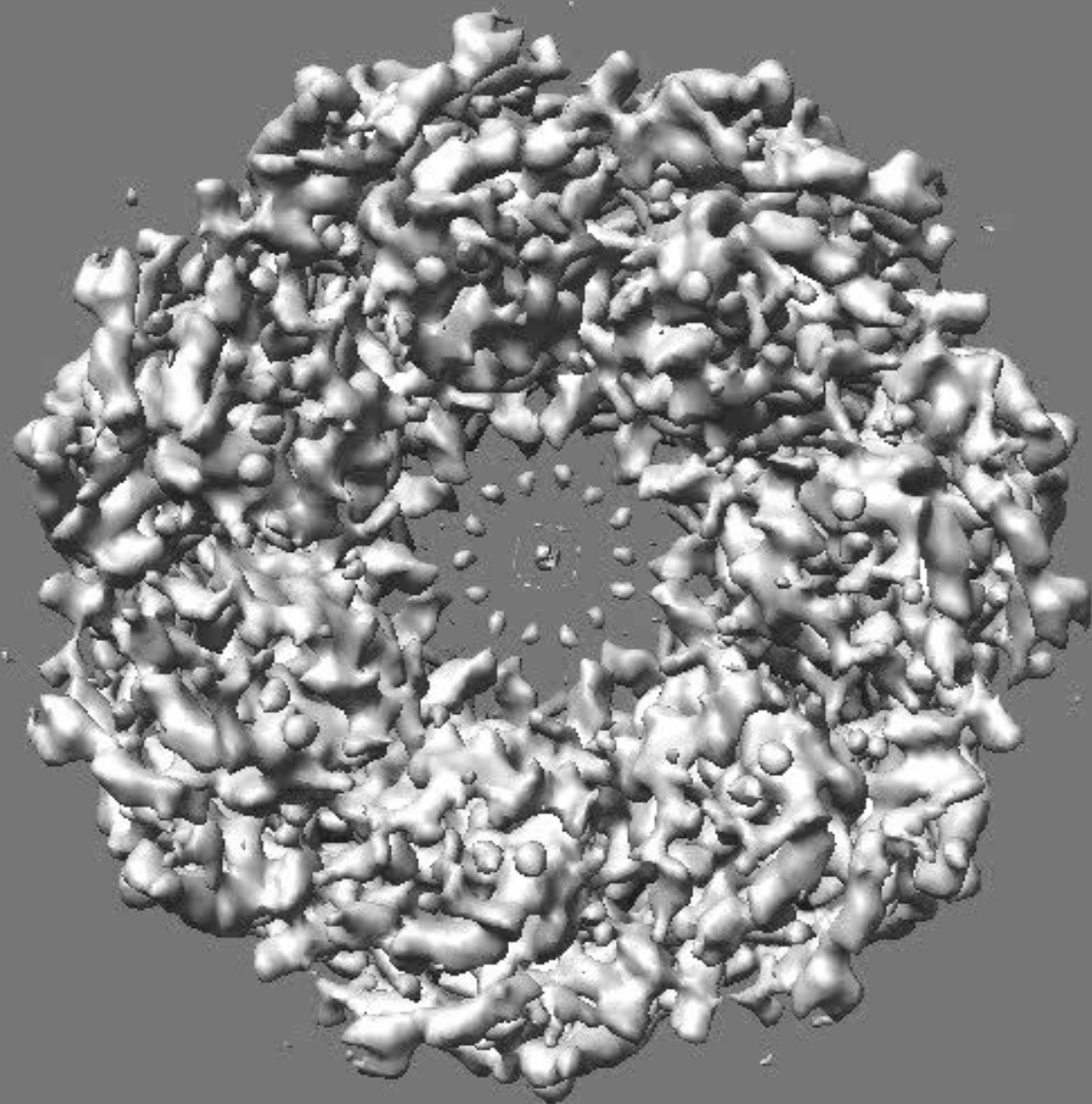
2005



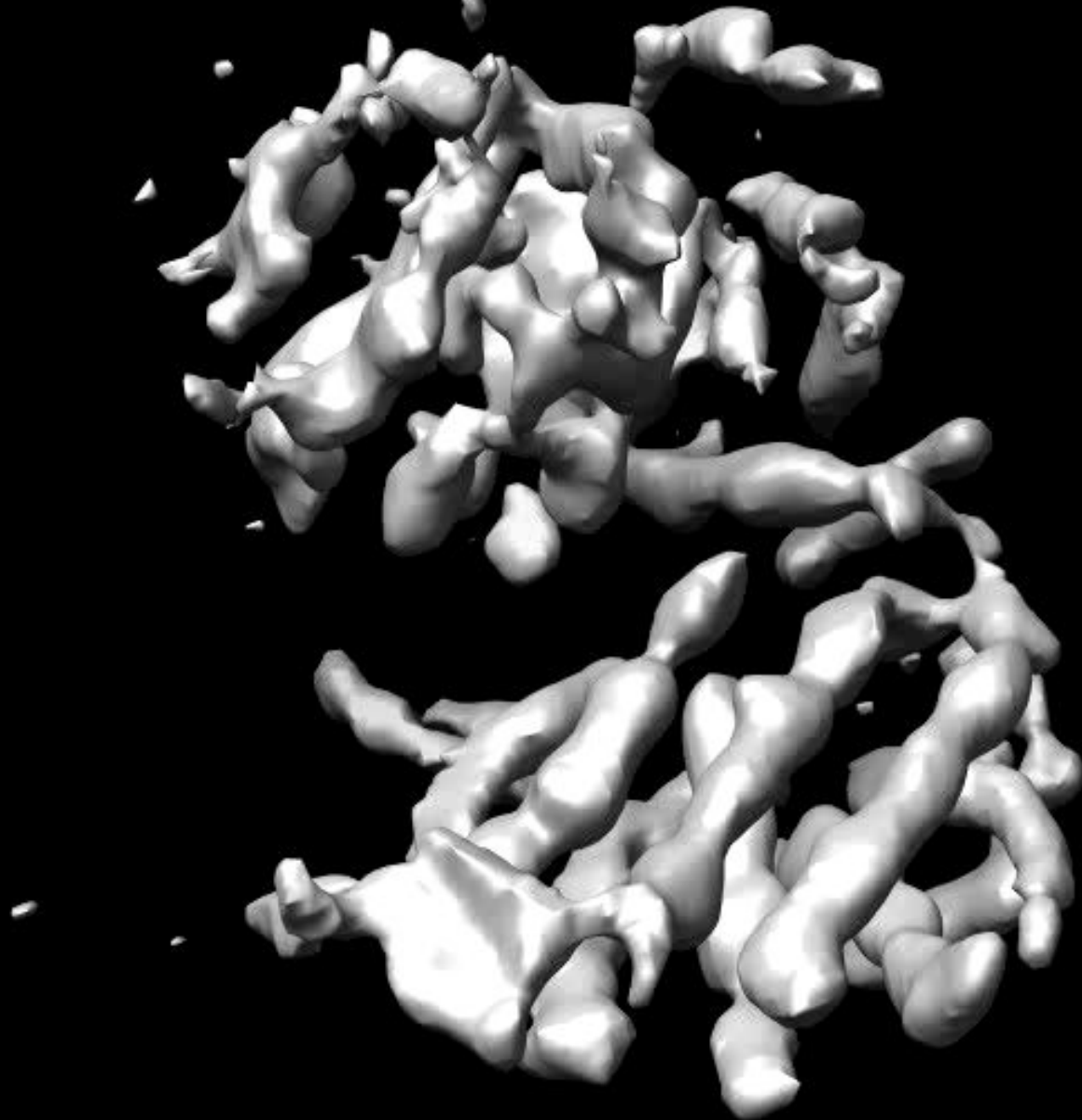


Jeol 3000
7 Days of imaging, 910 micrographs
1.06 Å/pix, Nikon 9000 scanner
135 used, 34,868 particles

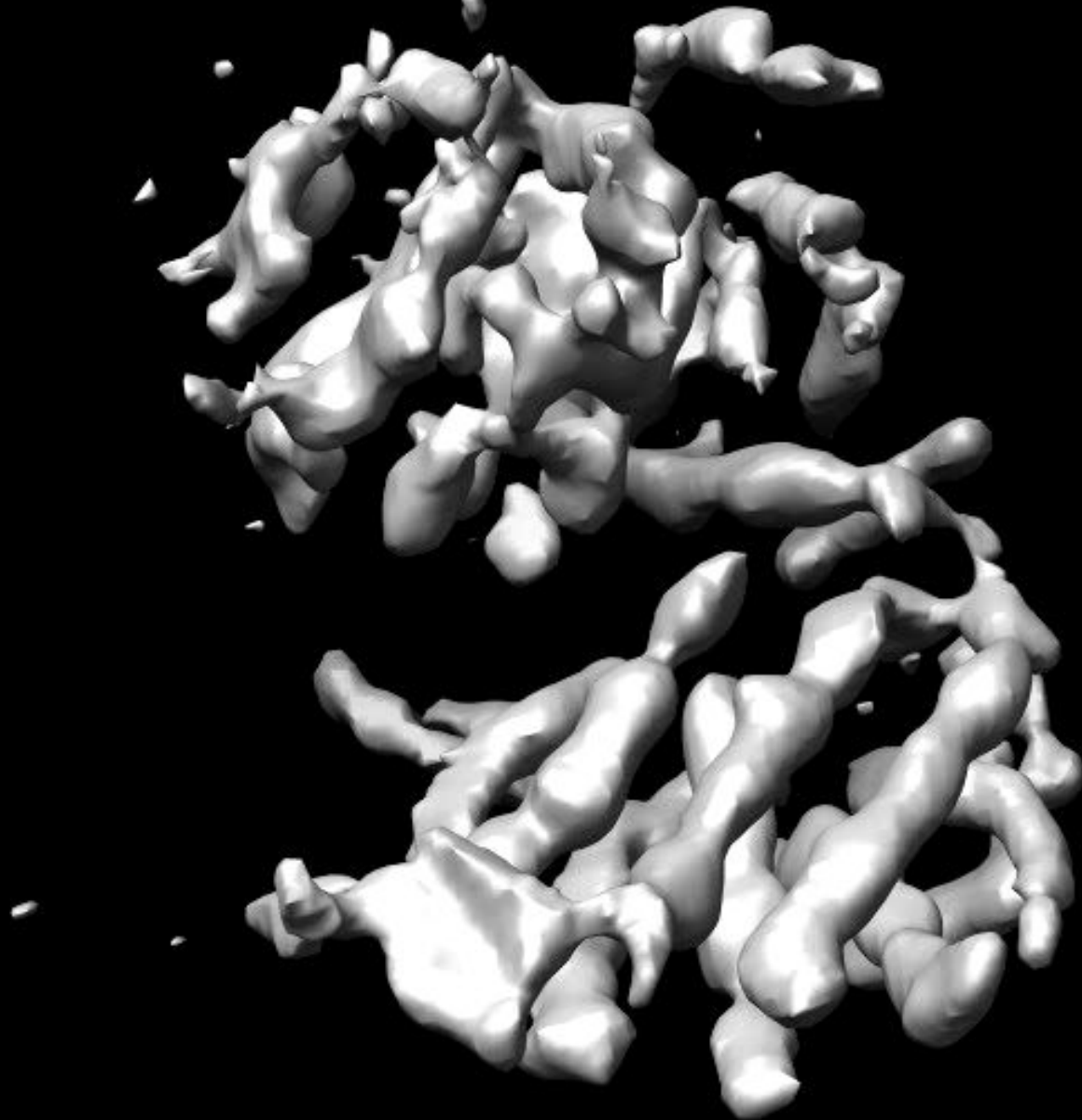
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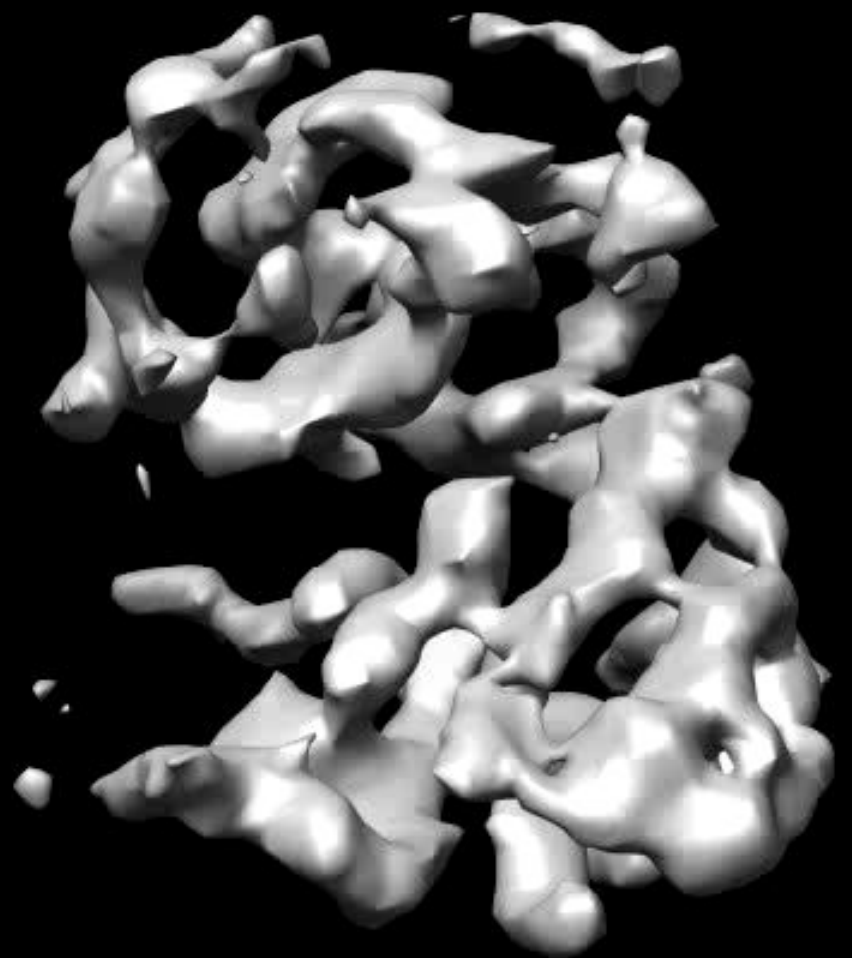
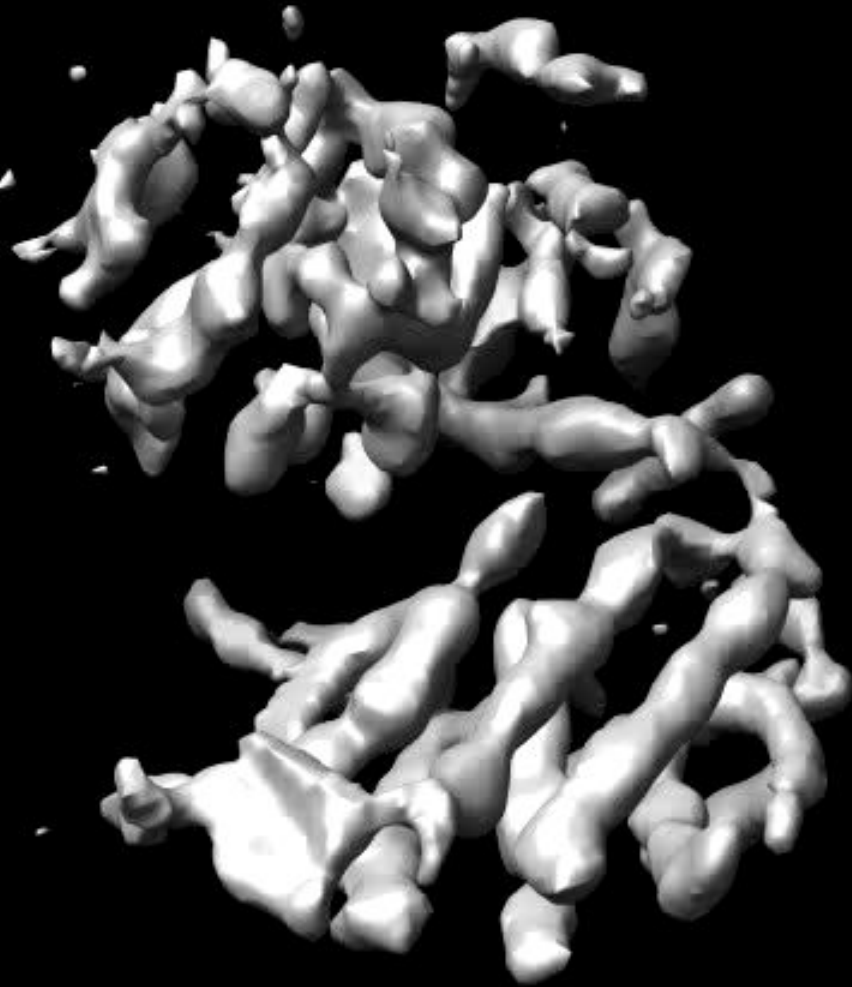
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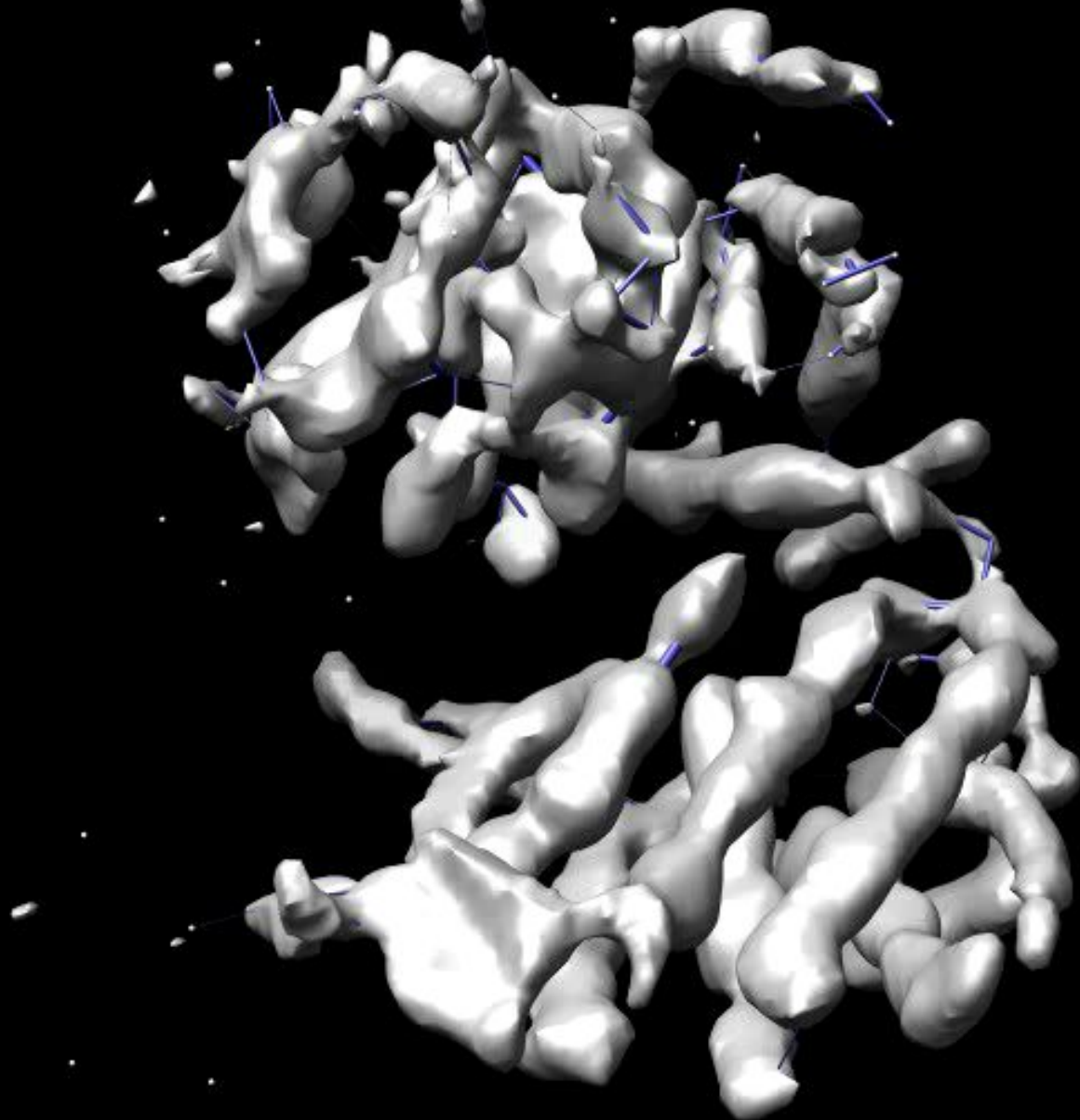
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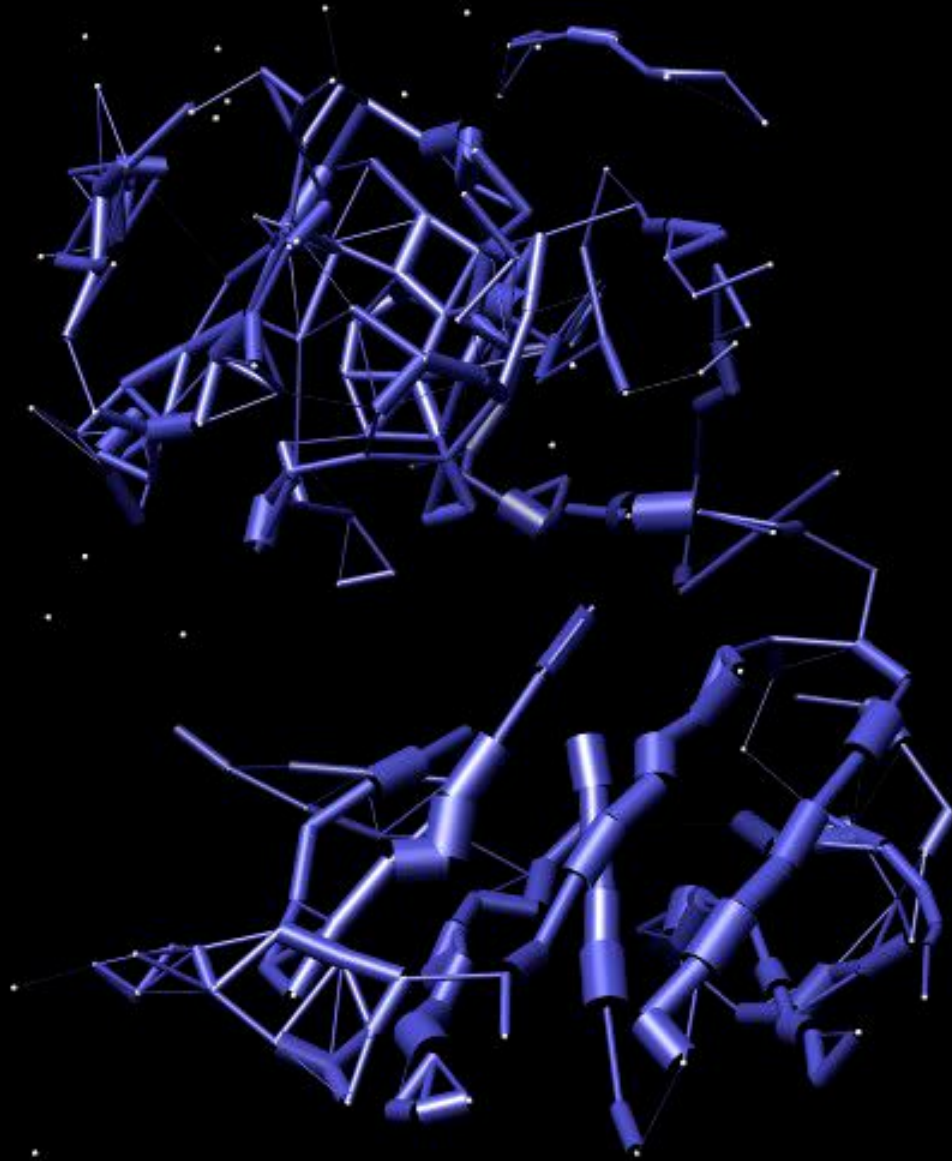
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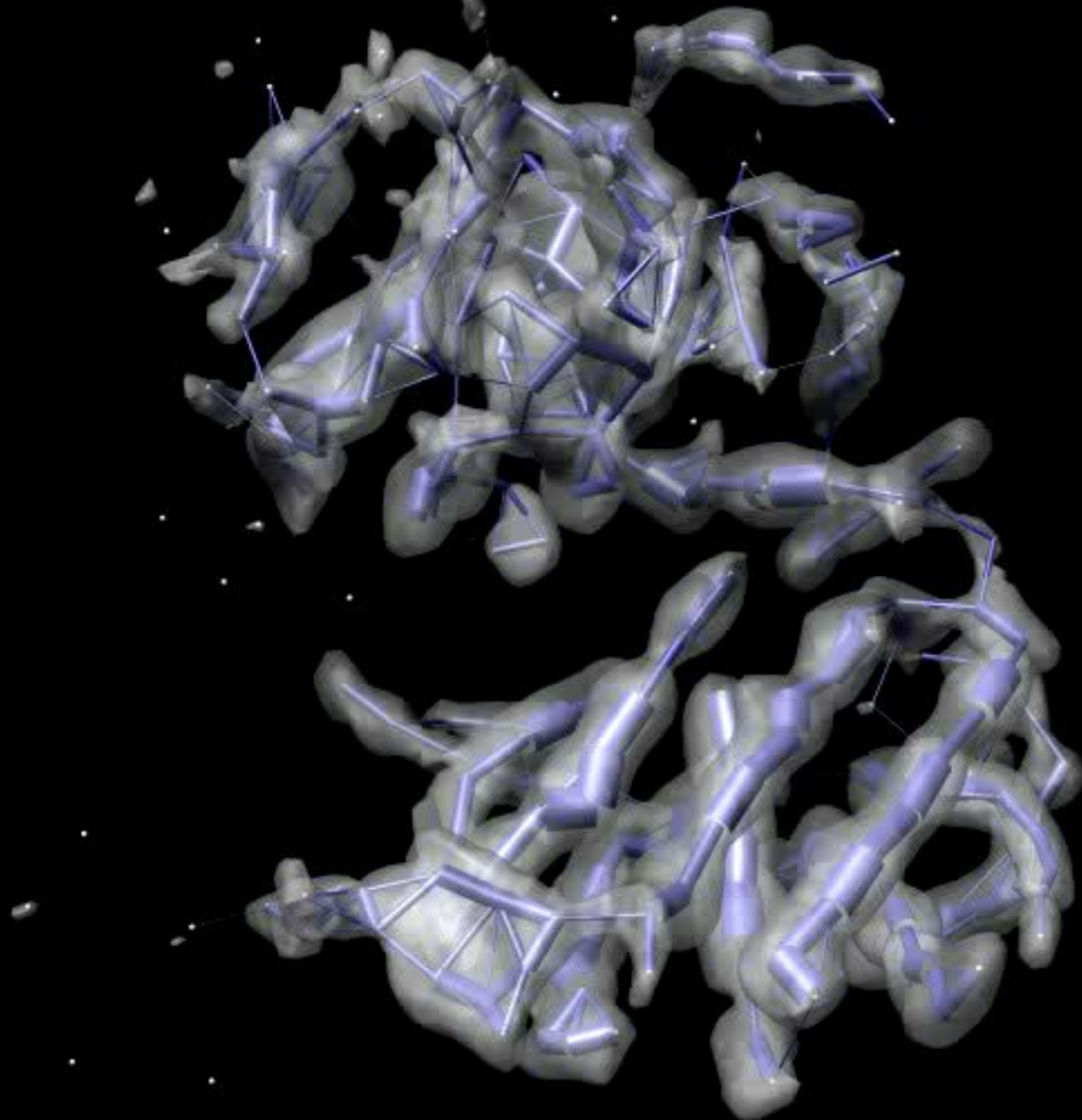
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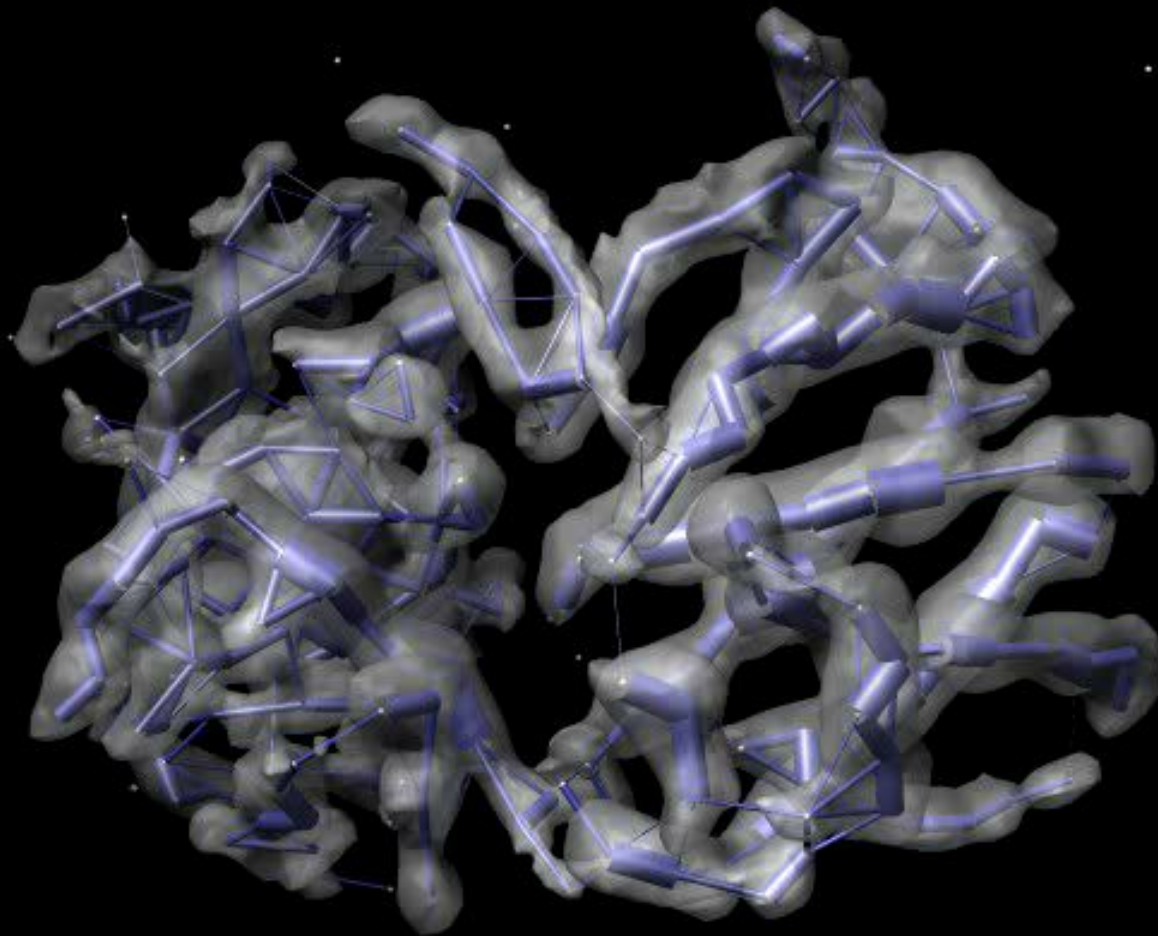
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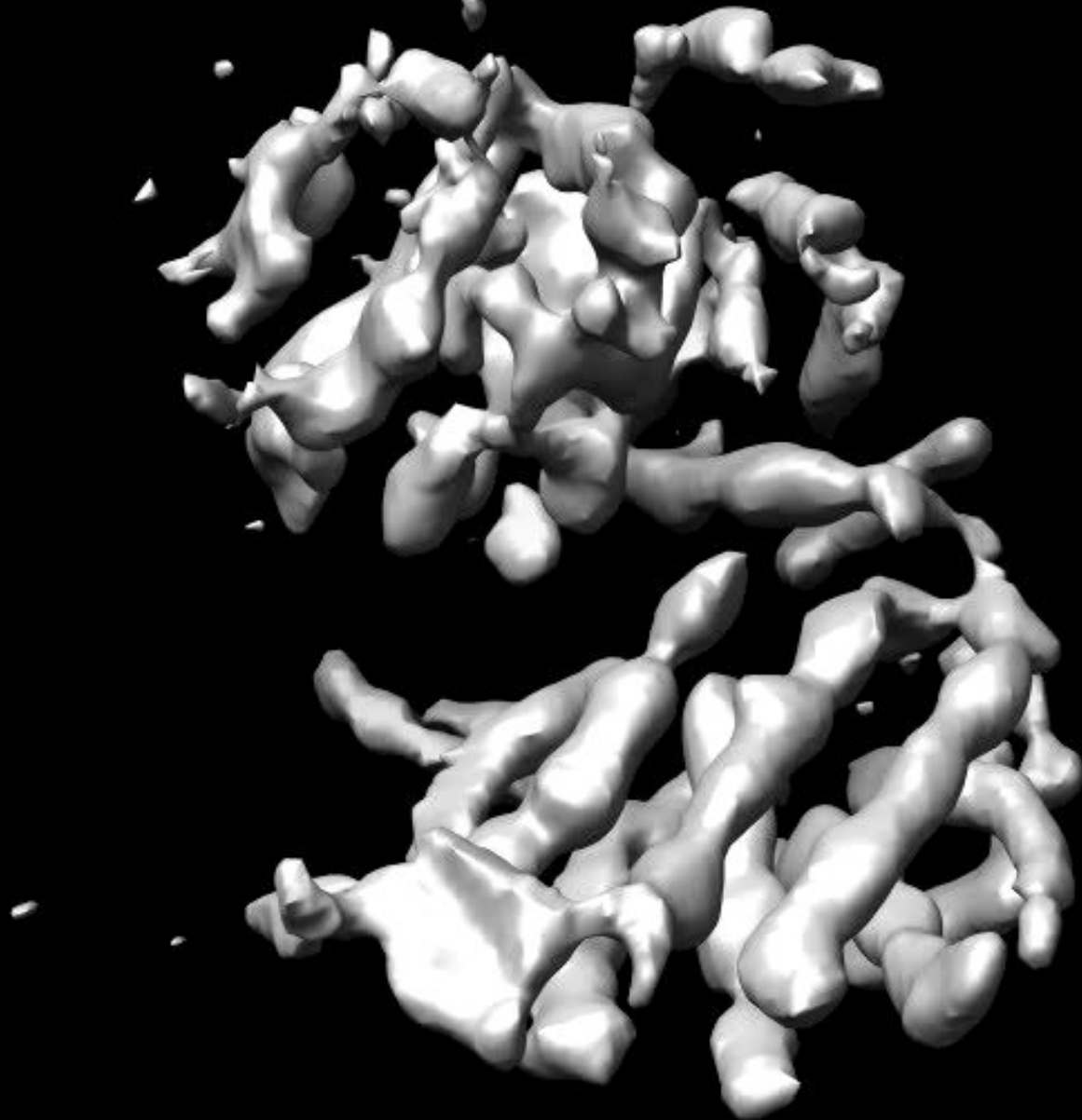
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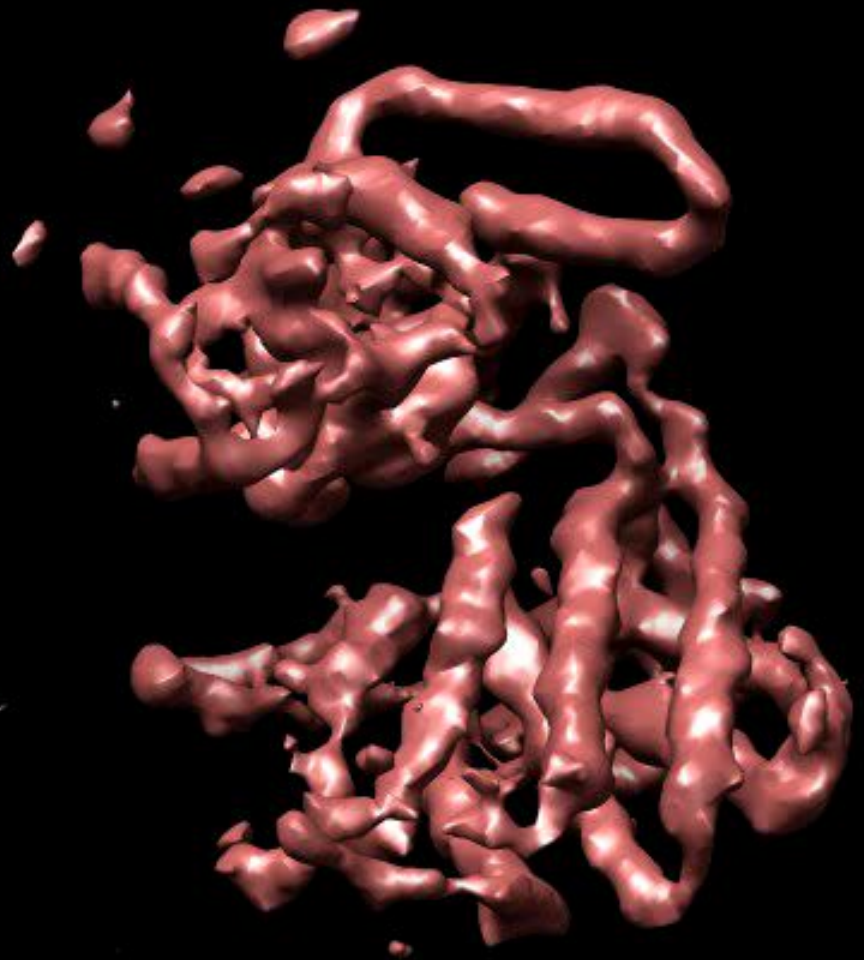
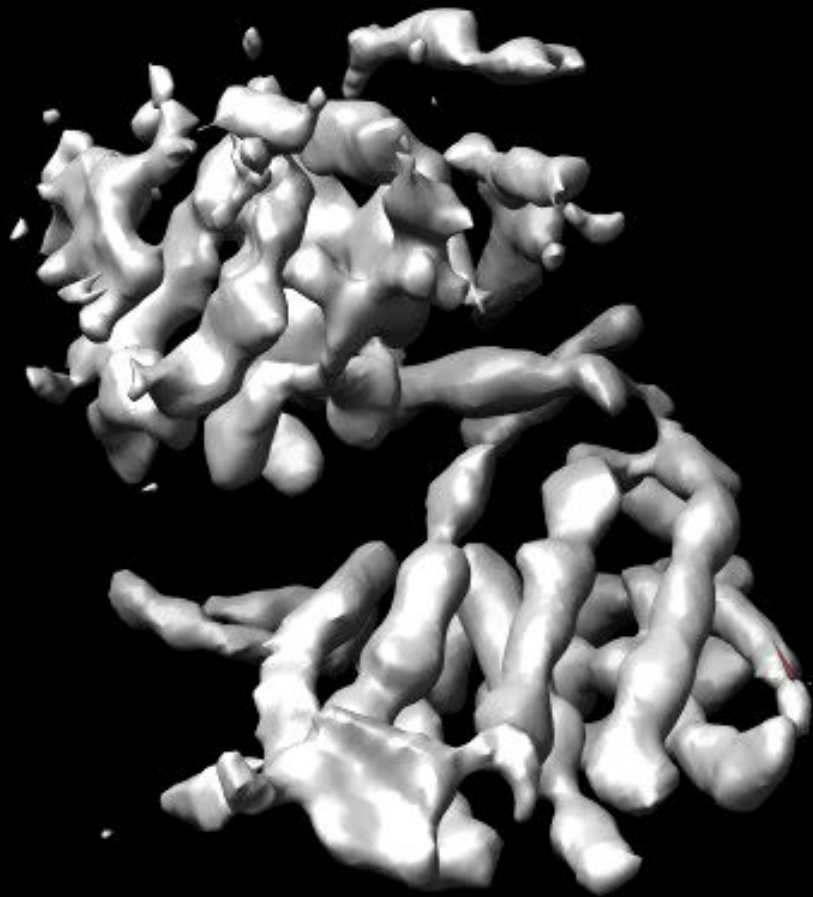
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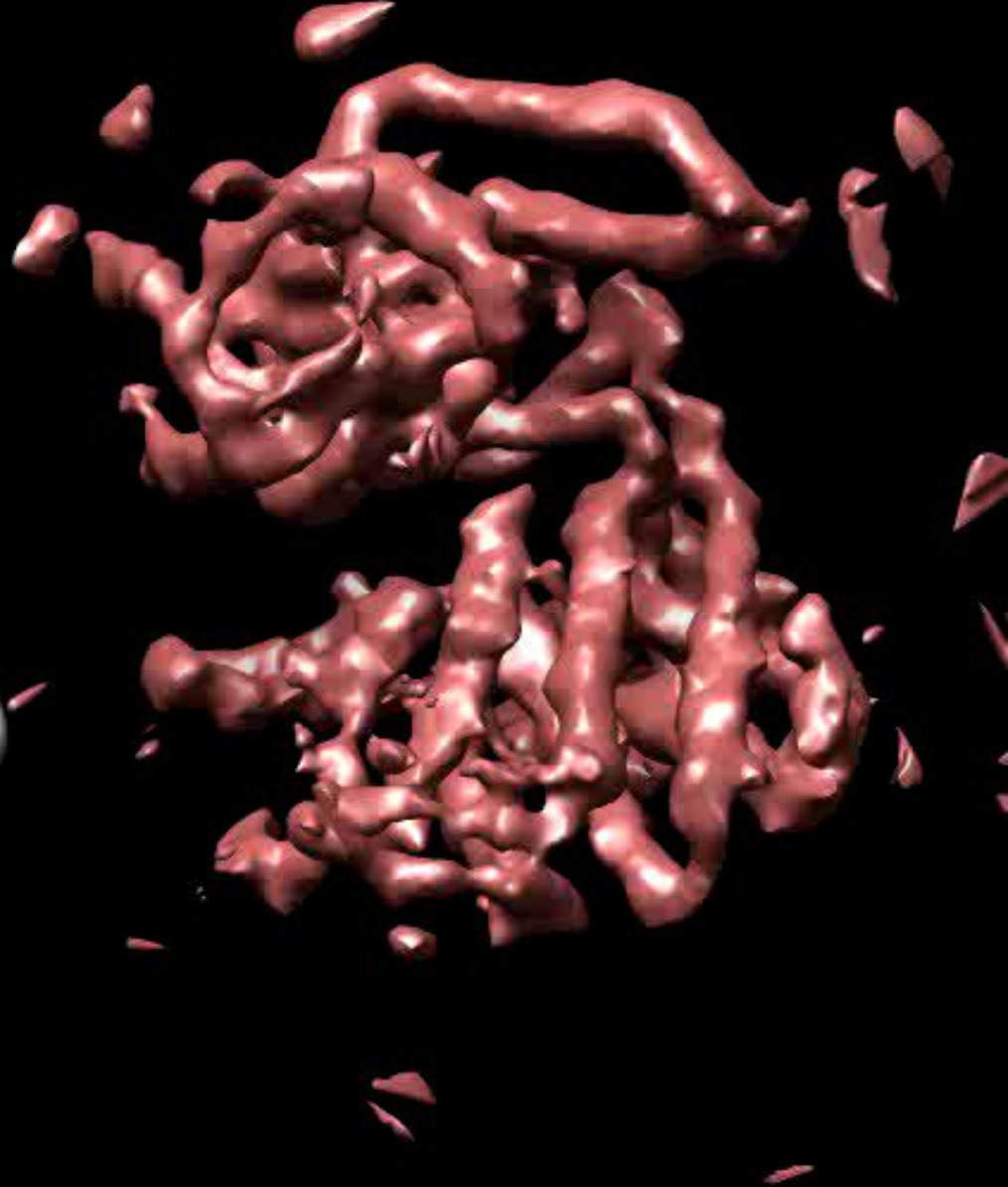
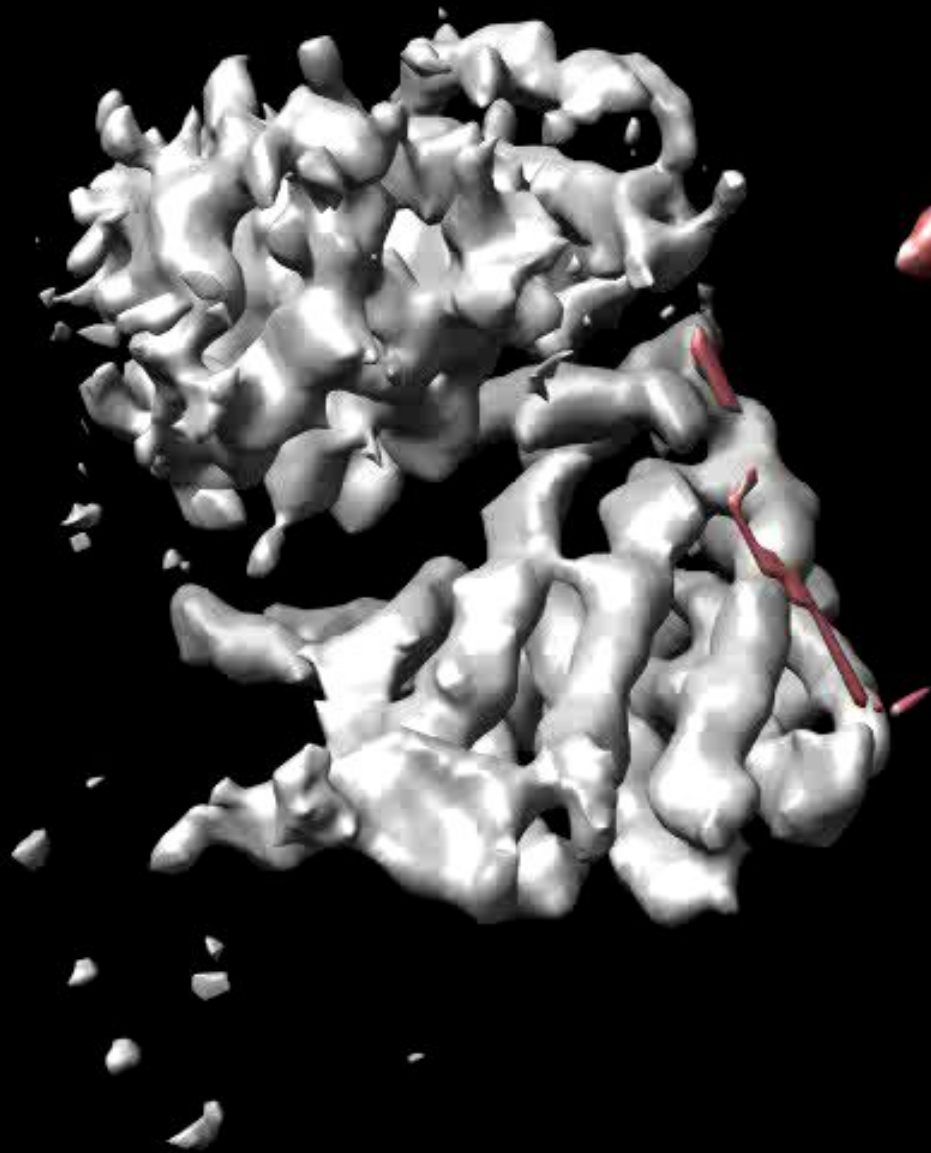
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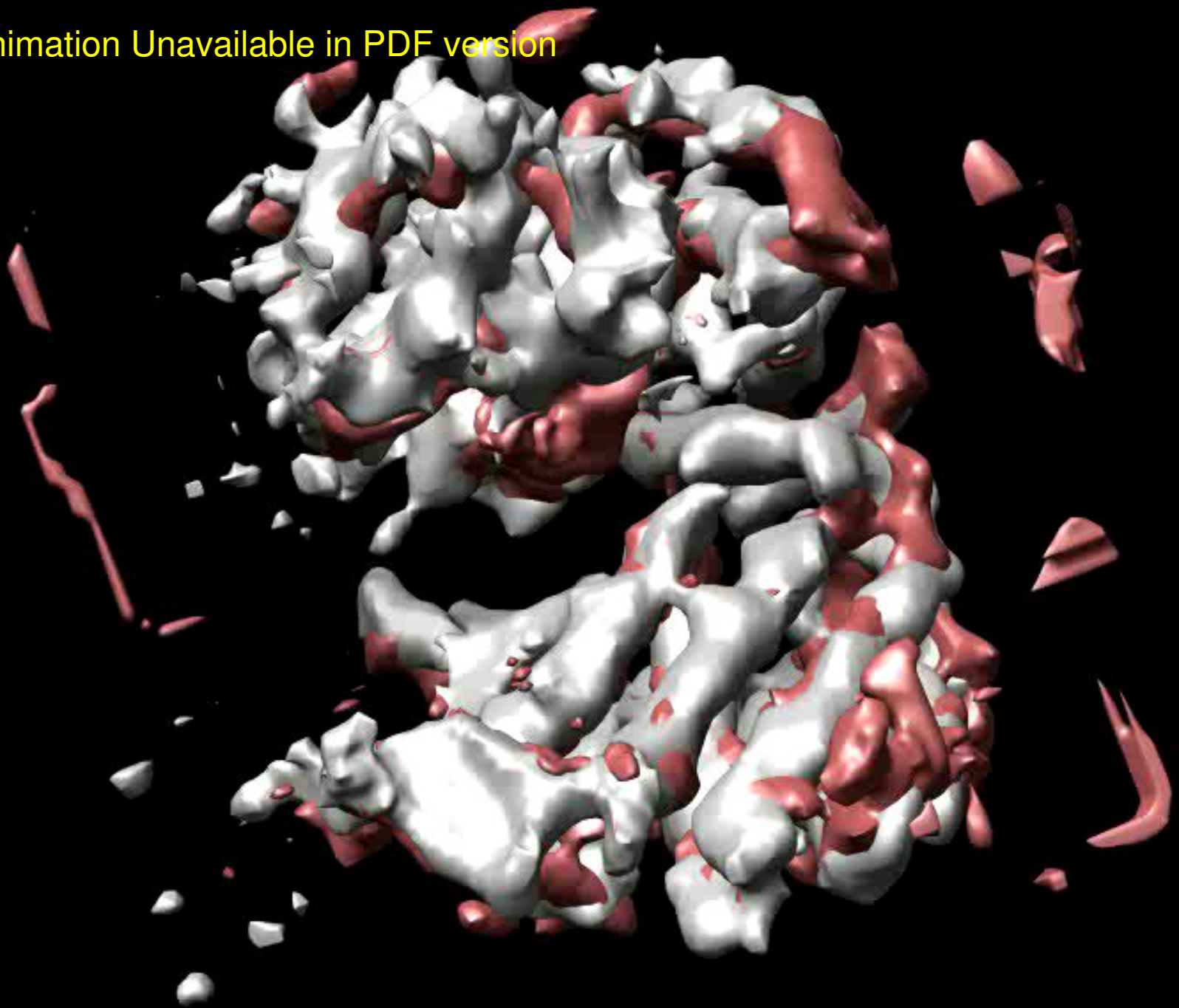
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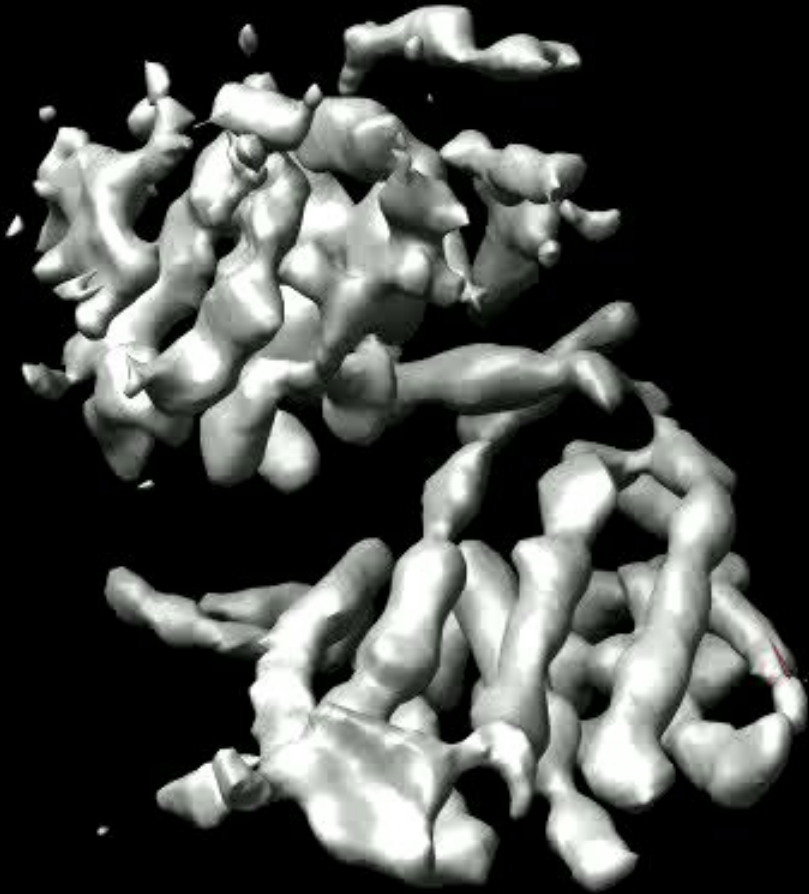
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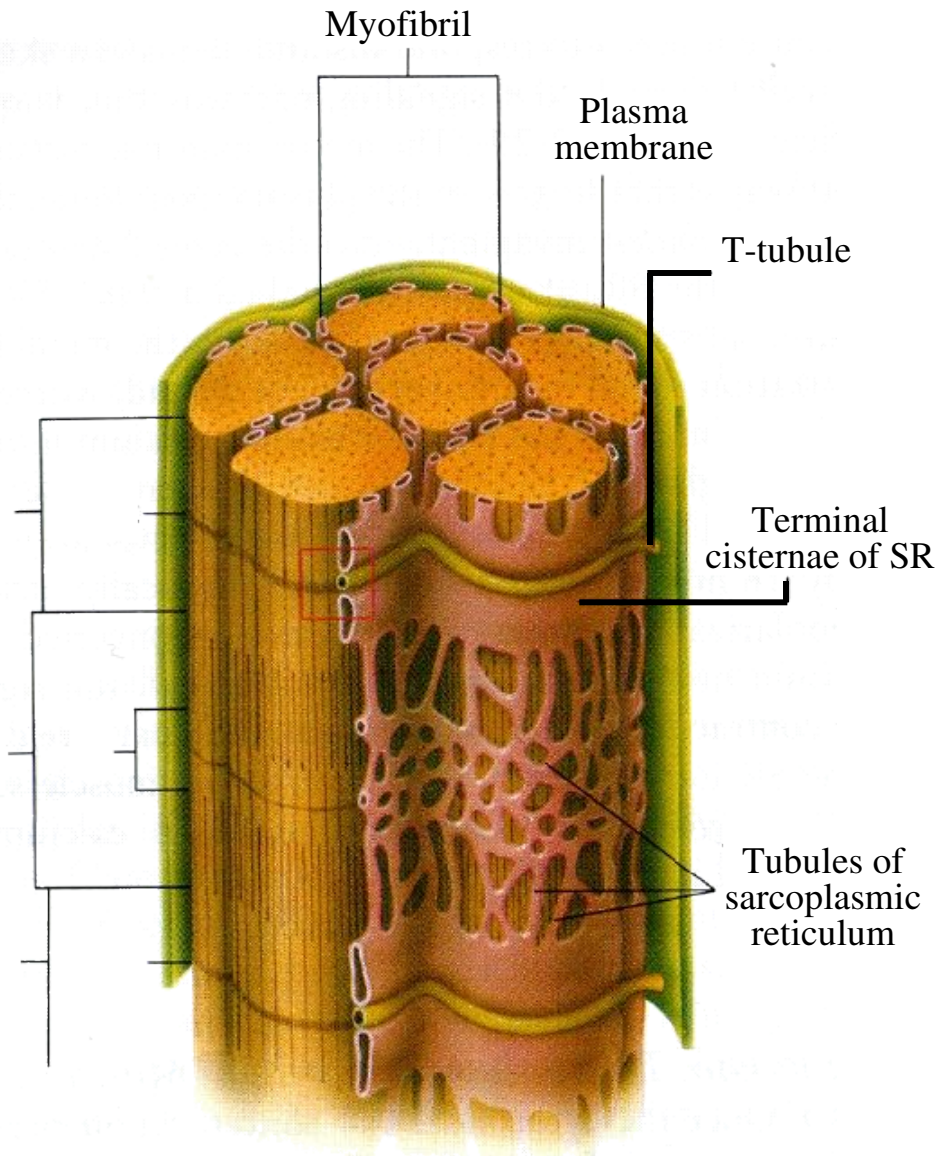
Ca²⁺ Release Channel

Irina Serysheva

Wah Chiu

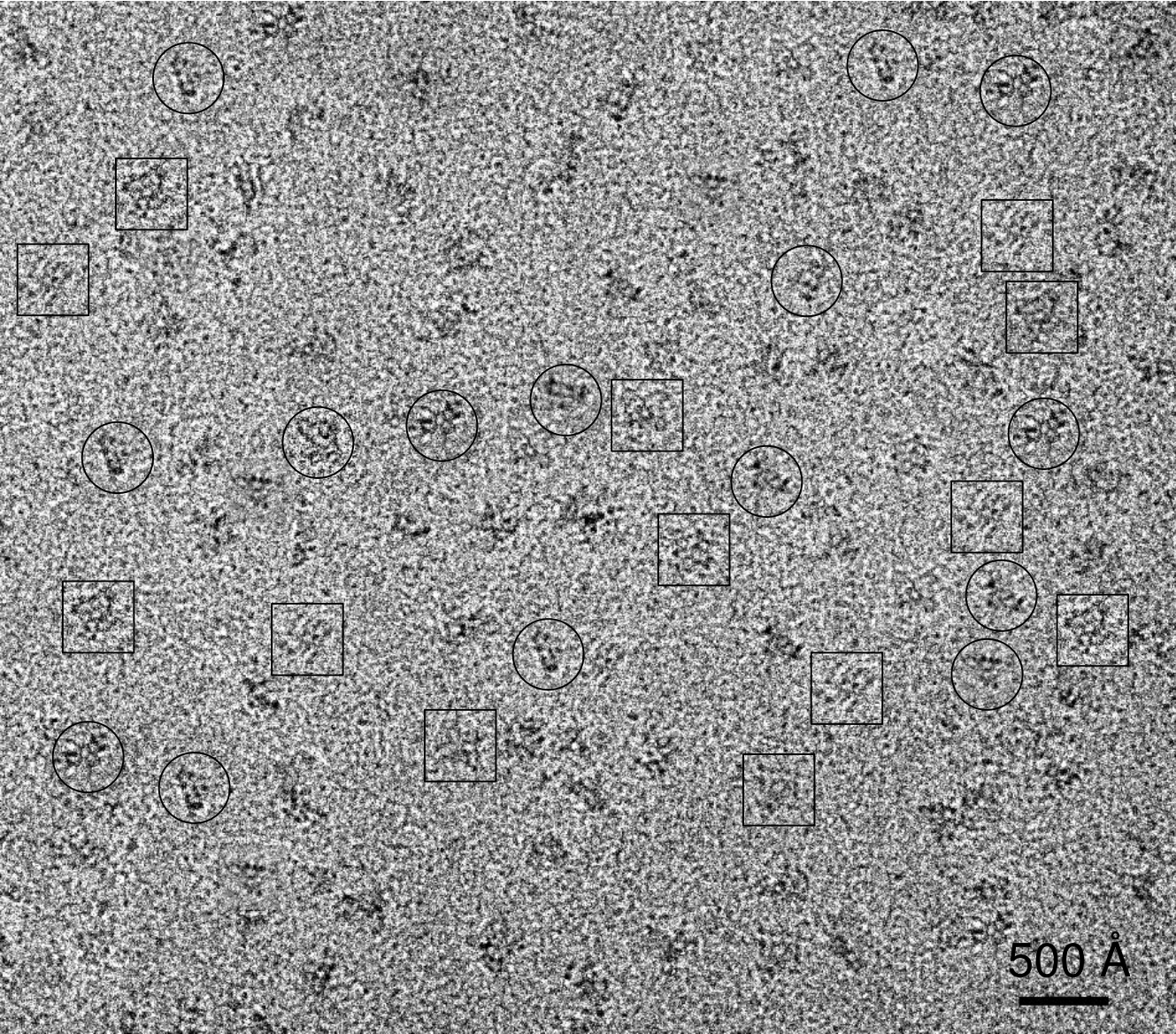
Susan Hamilton

Ca²⁺ Release Channel

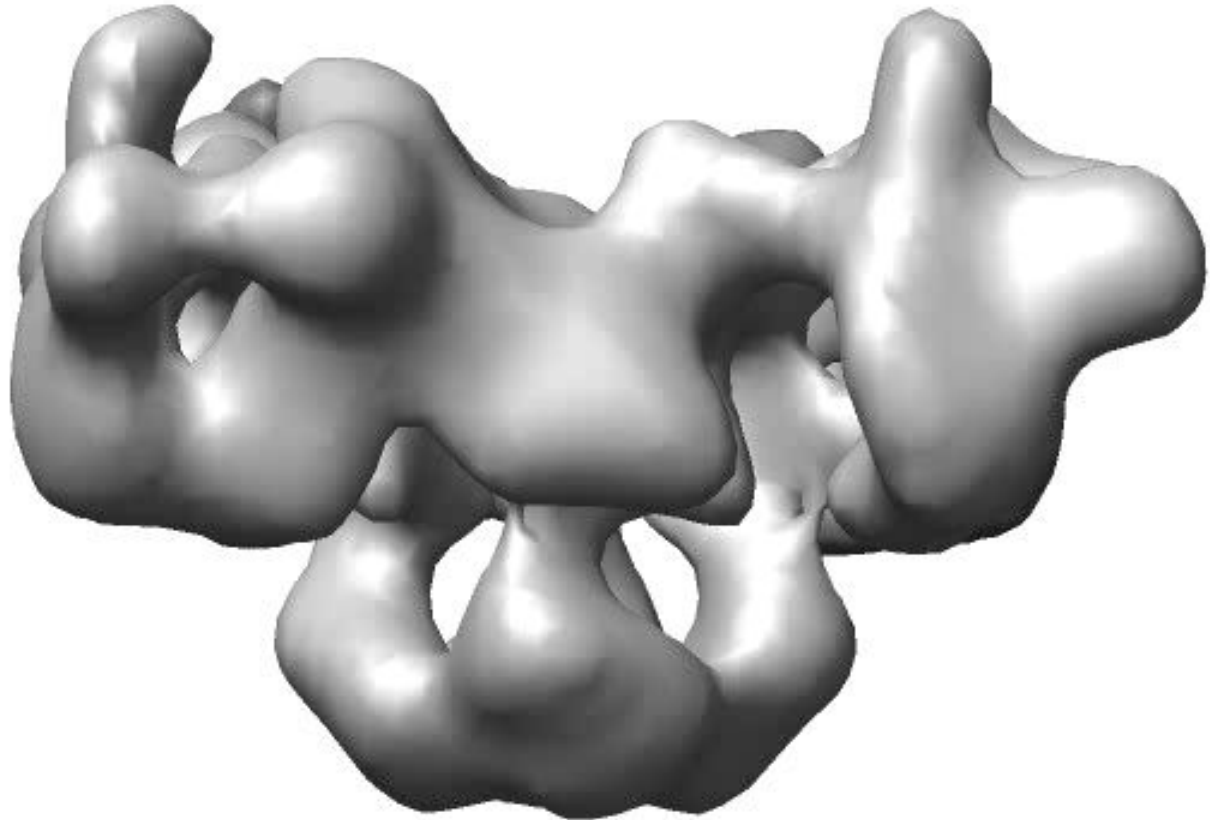


- **SR membrane, triggered by DHPR in T-tubule**
- **Homotetramer**
- **~2200 kDa**
- **Releases Ca⁺⁺ which initiates cross-bridge cycle**

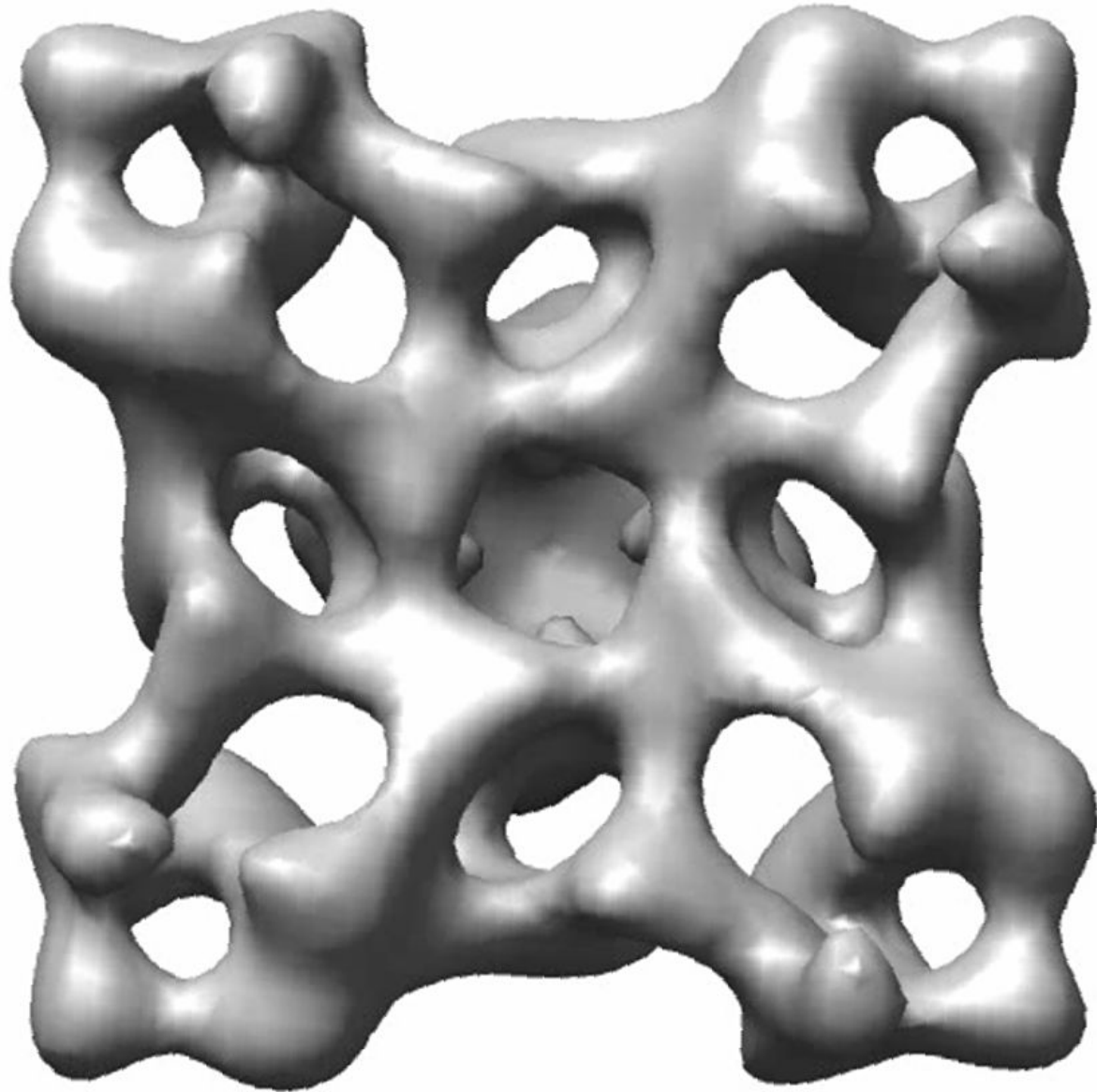
200 kV image of ice-embedded RyR1 (no continuous CF)



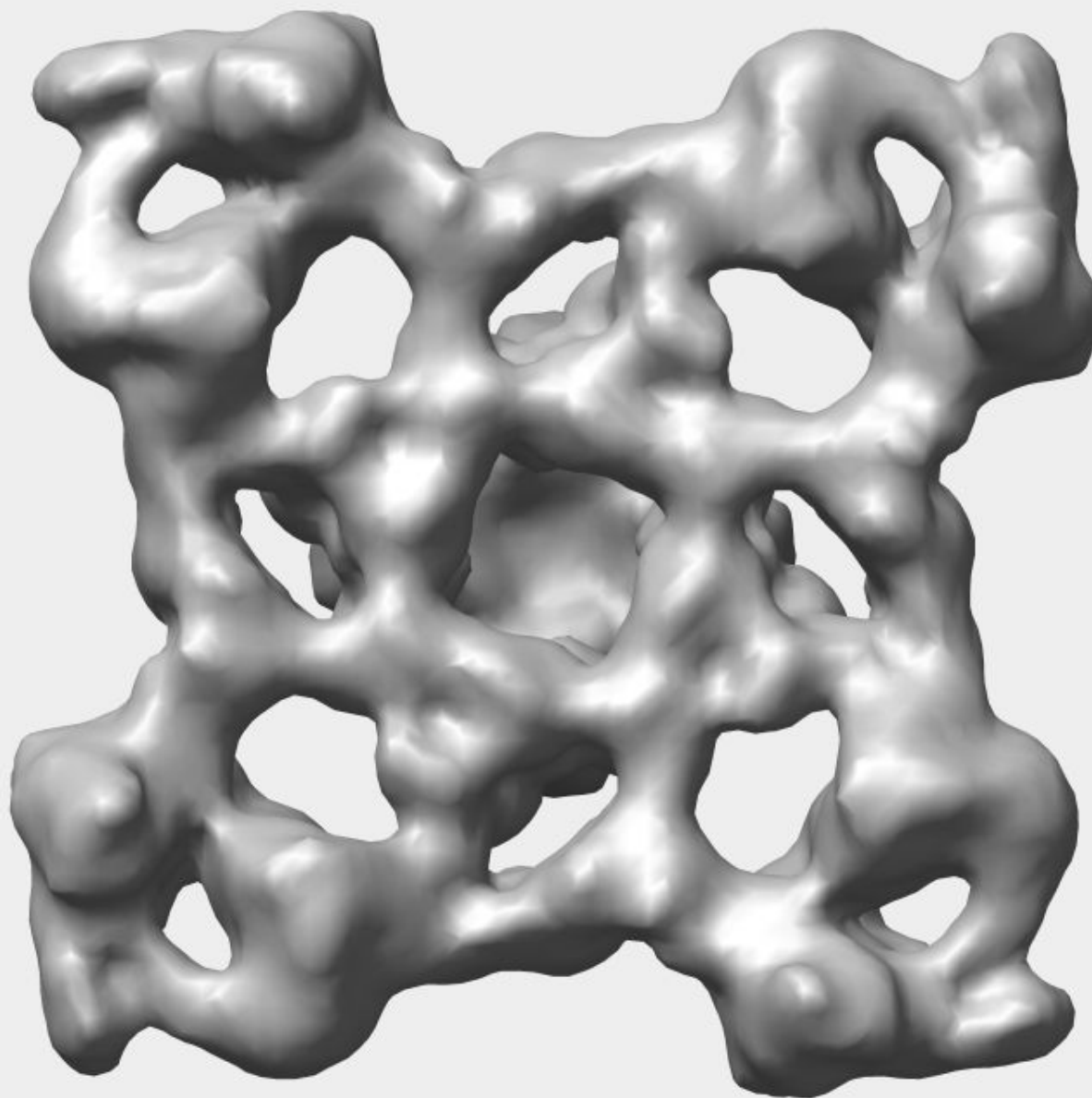
~30 Å Resolution



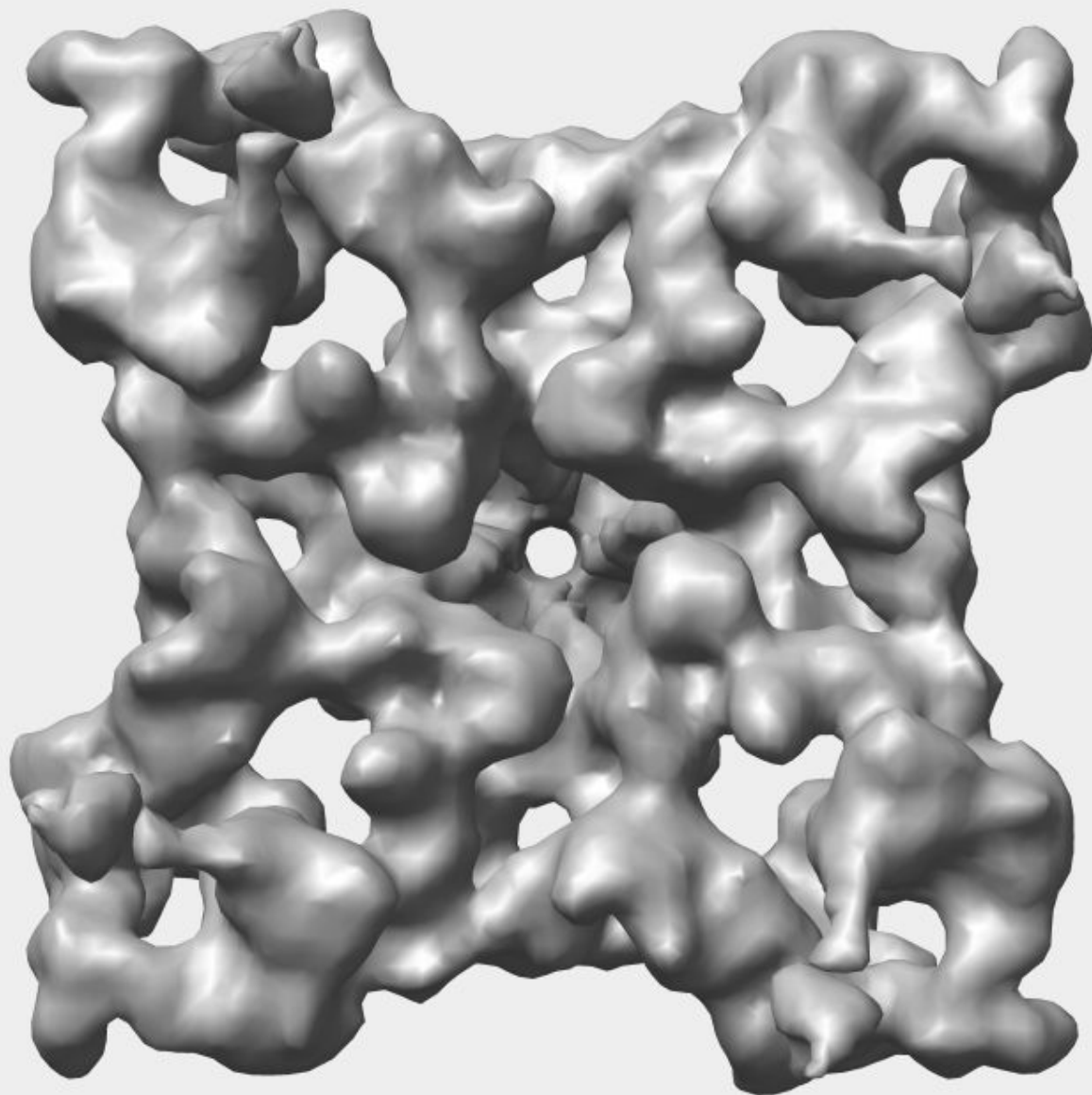
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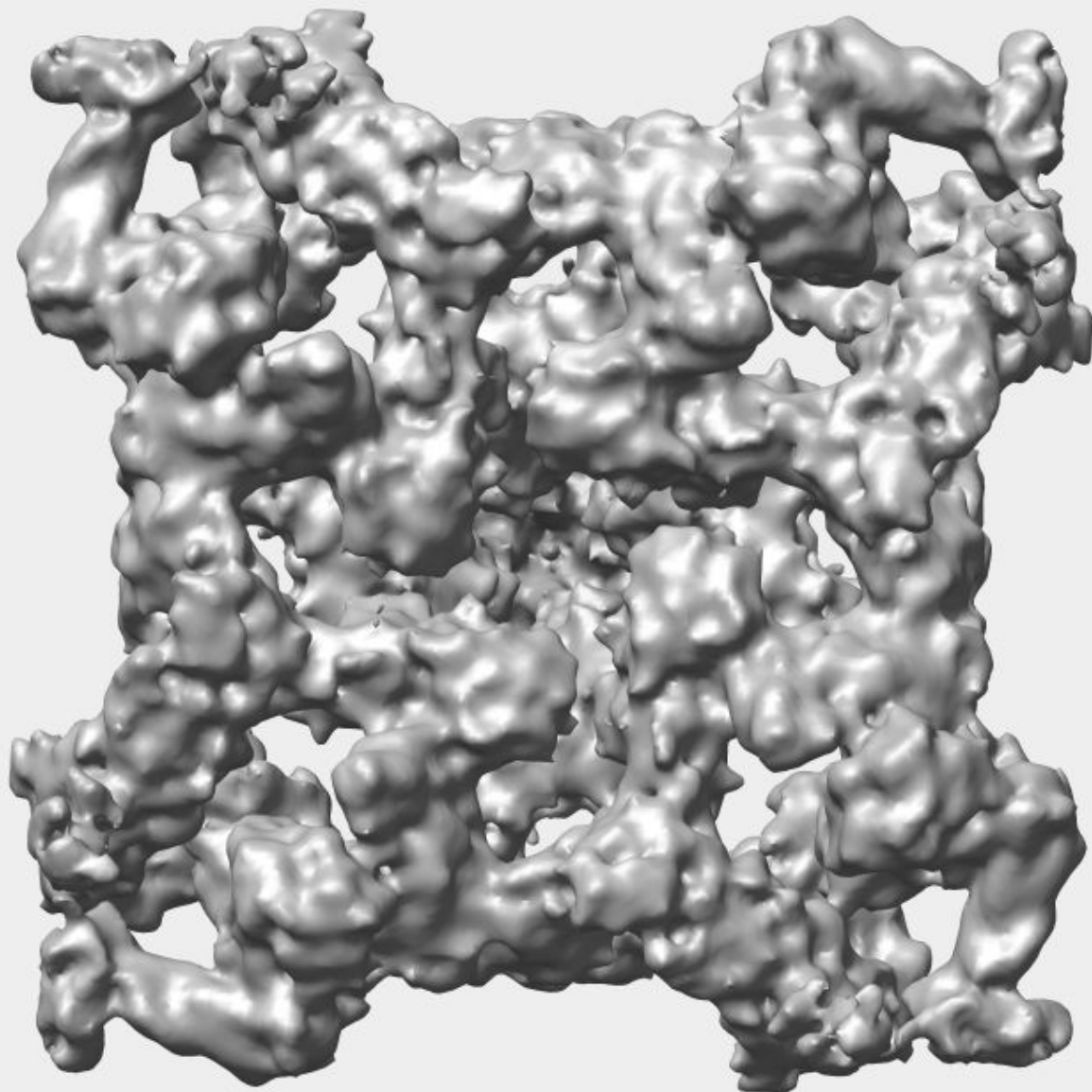
20 Å Resolution



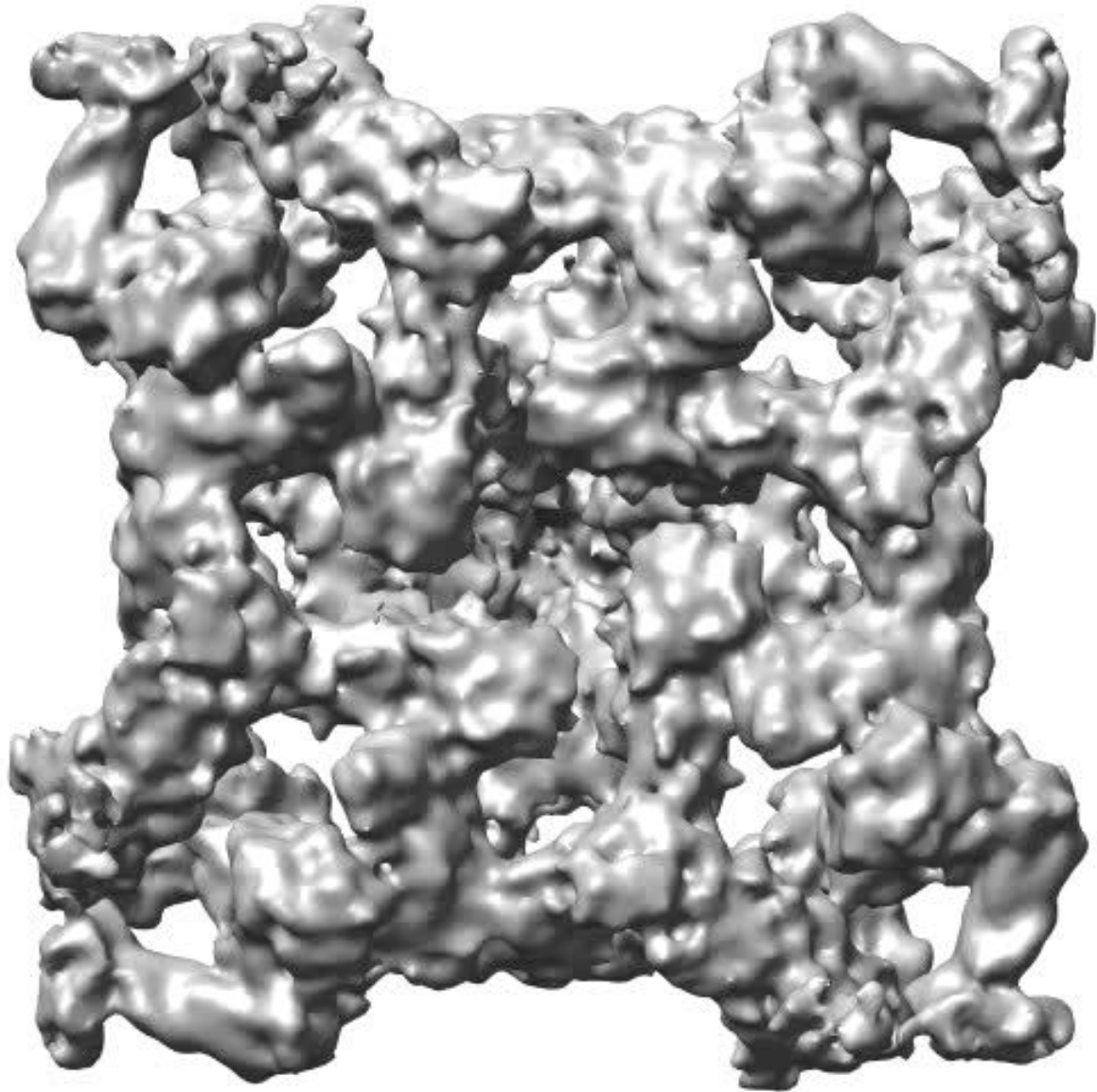
14 Å Resolution

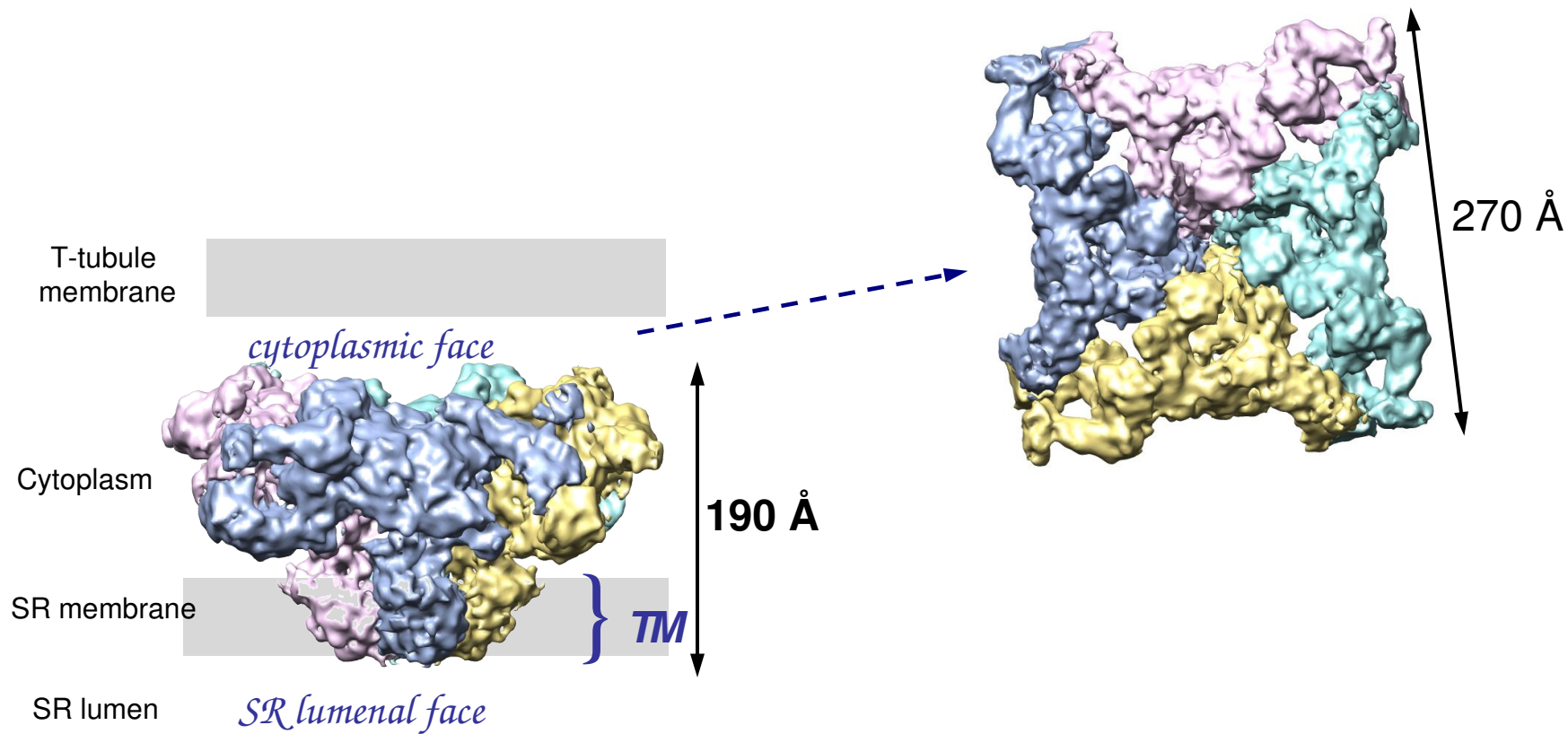


9.6 Å Resolution



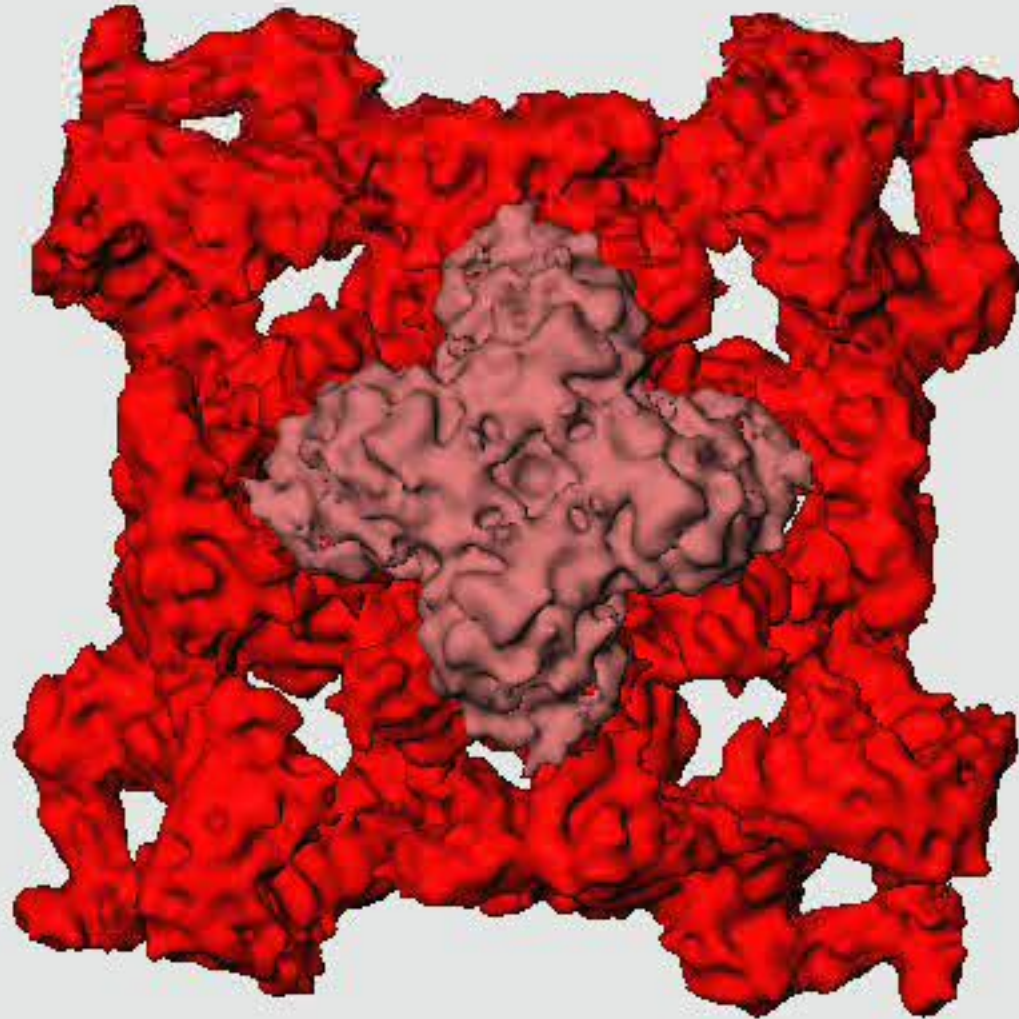
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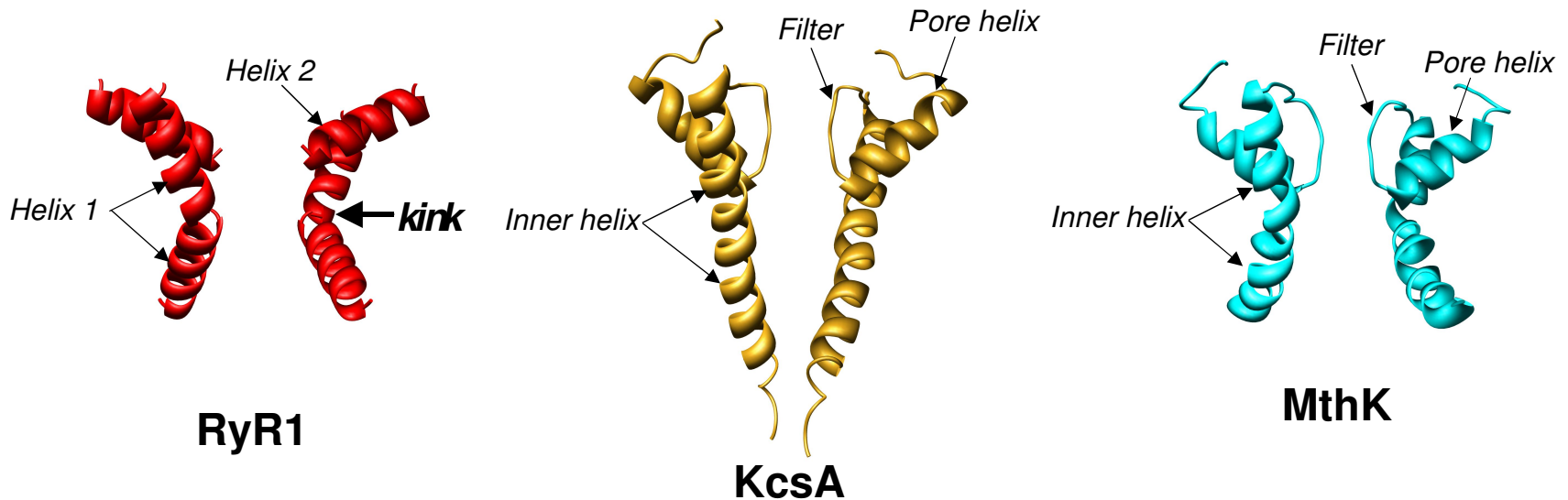
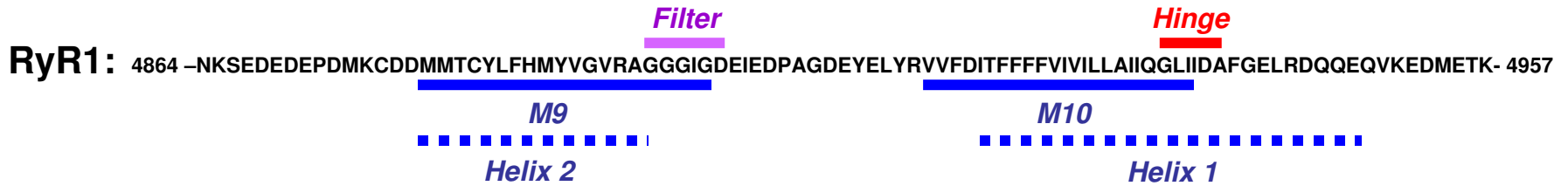


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Calcium Release Channel @9.6Å



Sequence assignment of observed helices

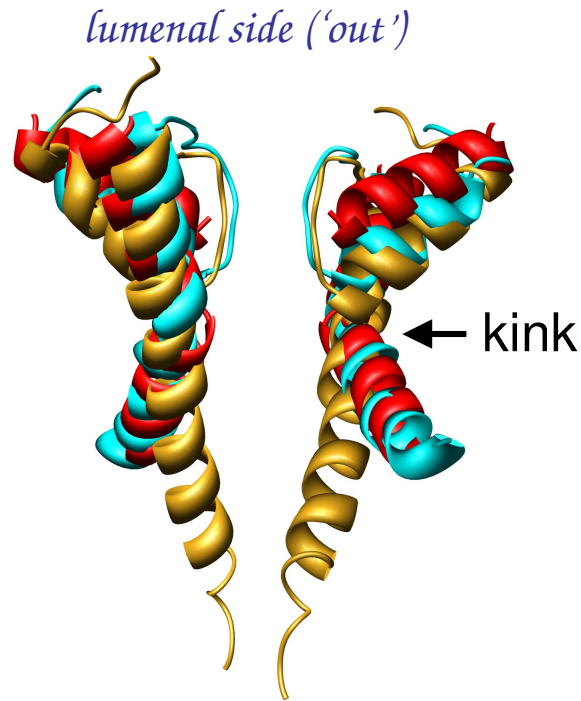


Sequence assignment of observed helices

RyR1: 4864 -NKSEDEDEPDMKCDDMMTCYLFHMYVGVRRAGGGIGDEIEDPAGDEYELRVVFDITFFFFVIVILLAIQGLIIDAFGELRDQQEQVKEDMETK- 4957

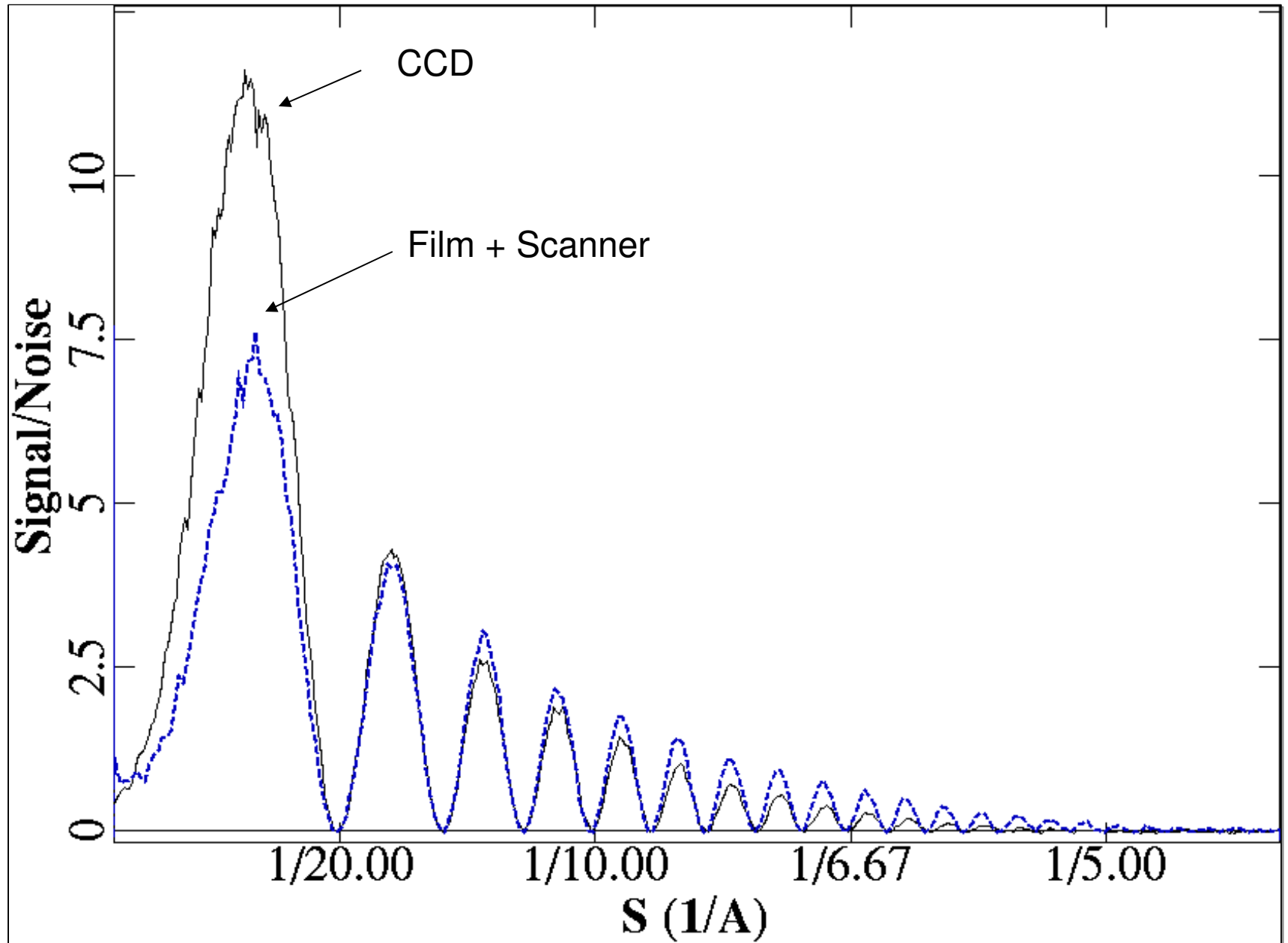
Filter *Hinge*

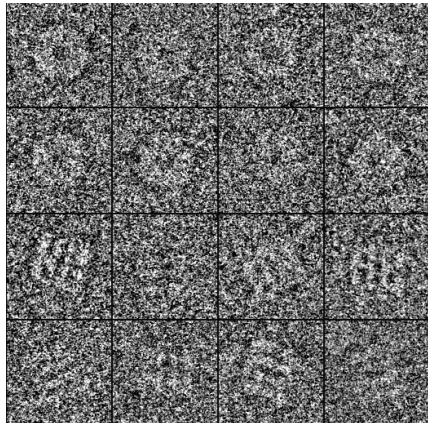
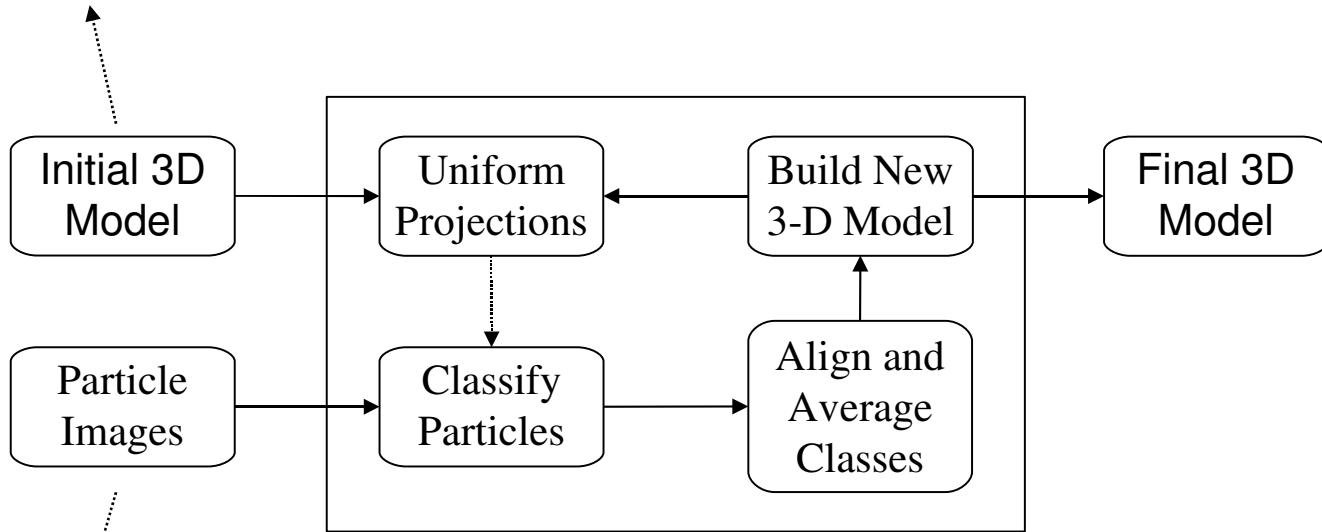
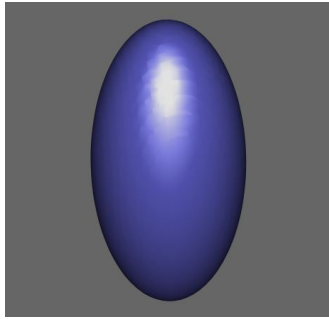
M9 *M10*

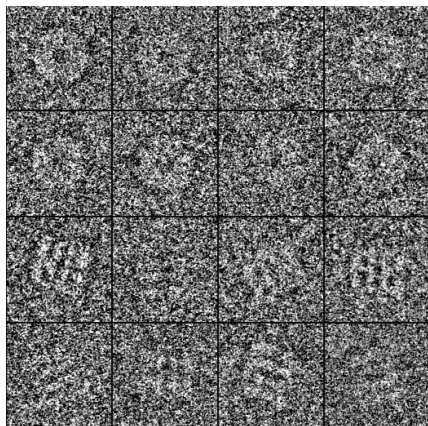
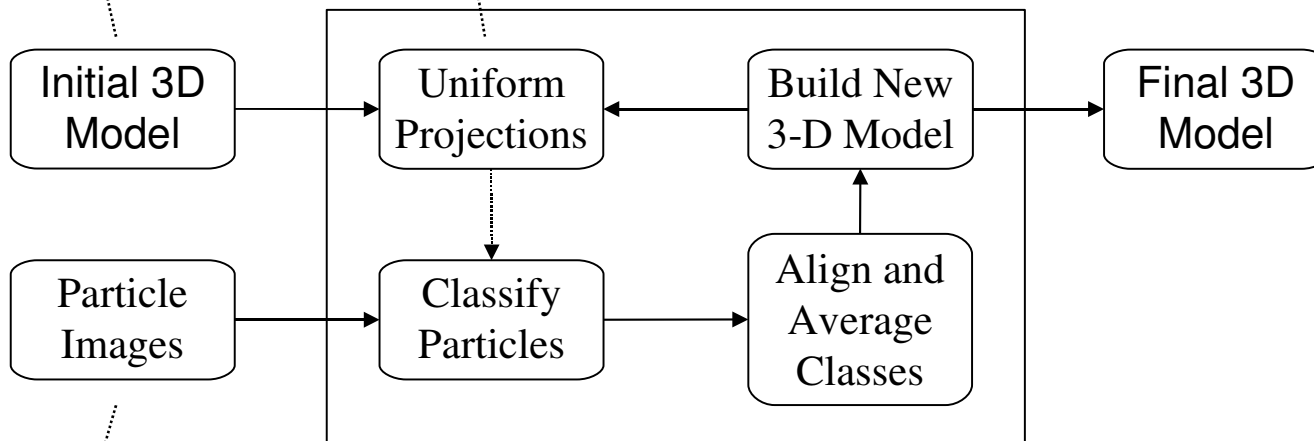
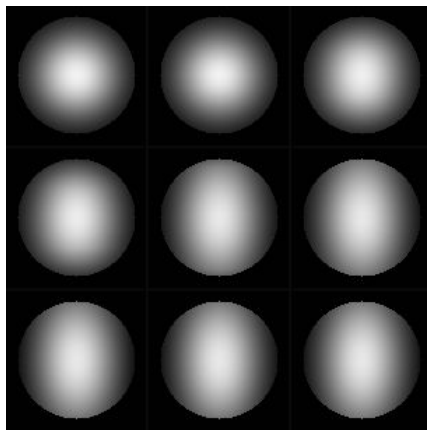
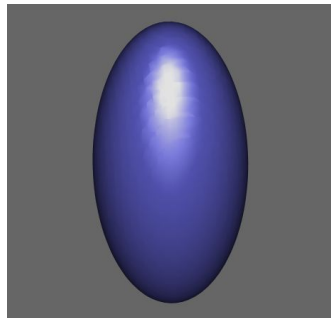


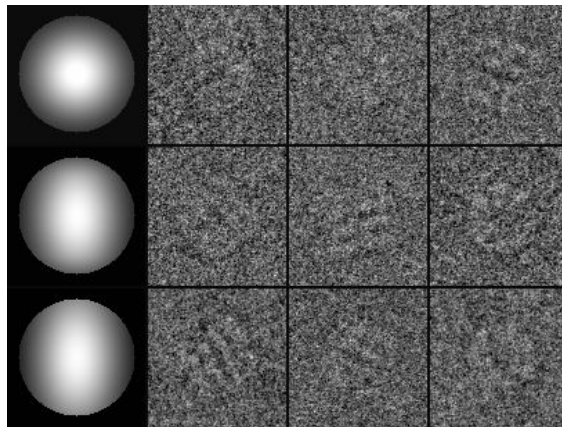
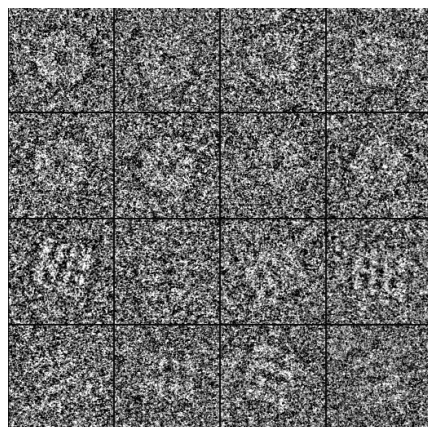
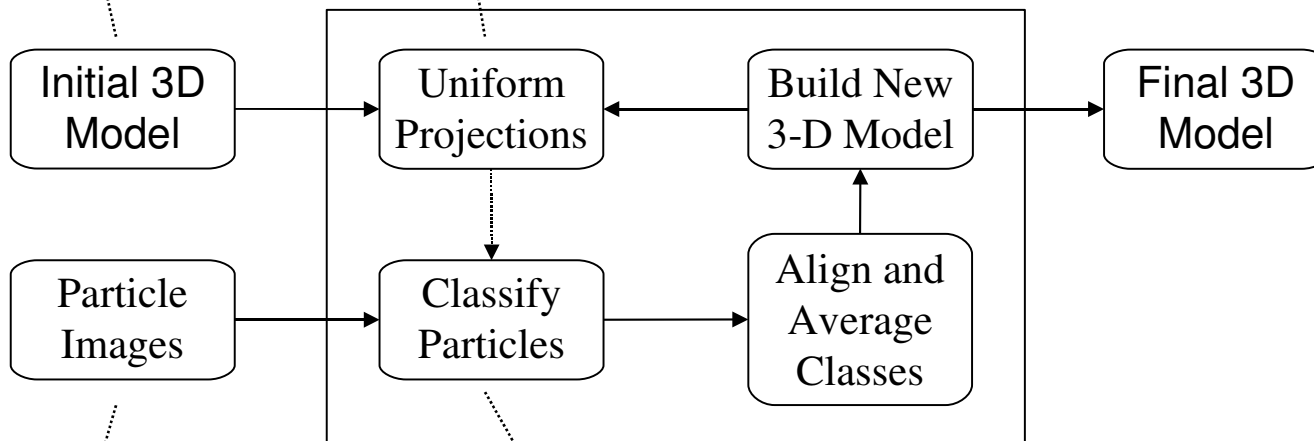
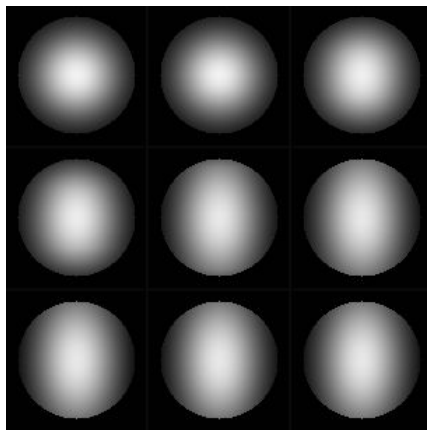
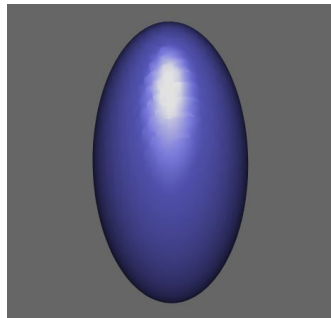
RyR1/KcsA/MthK

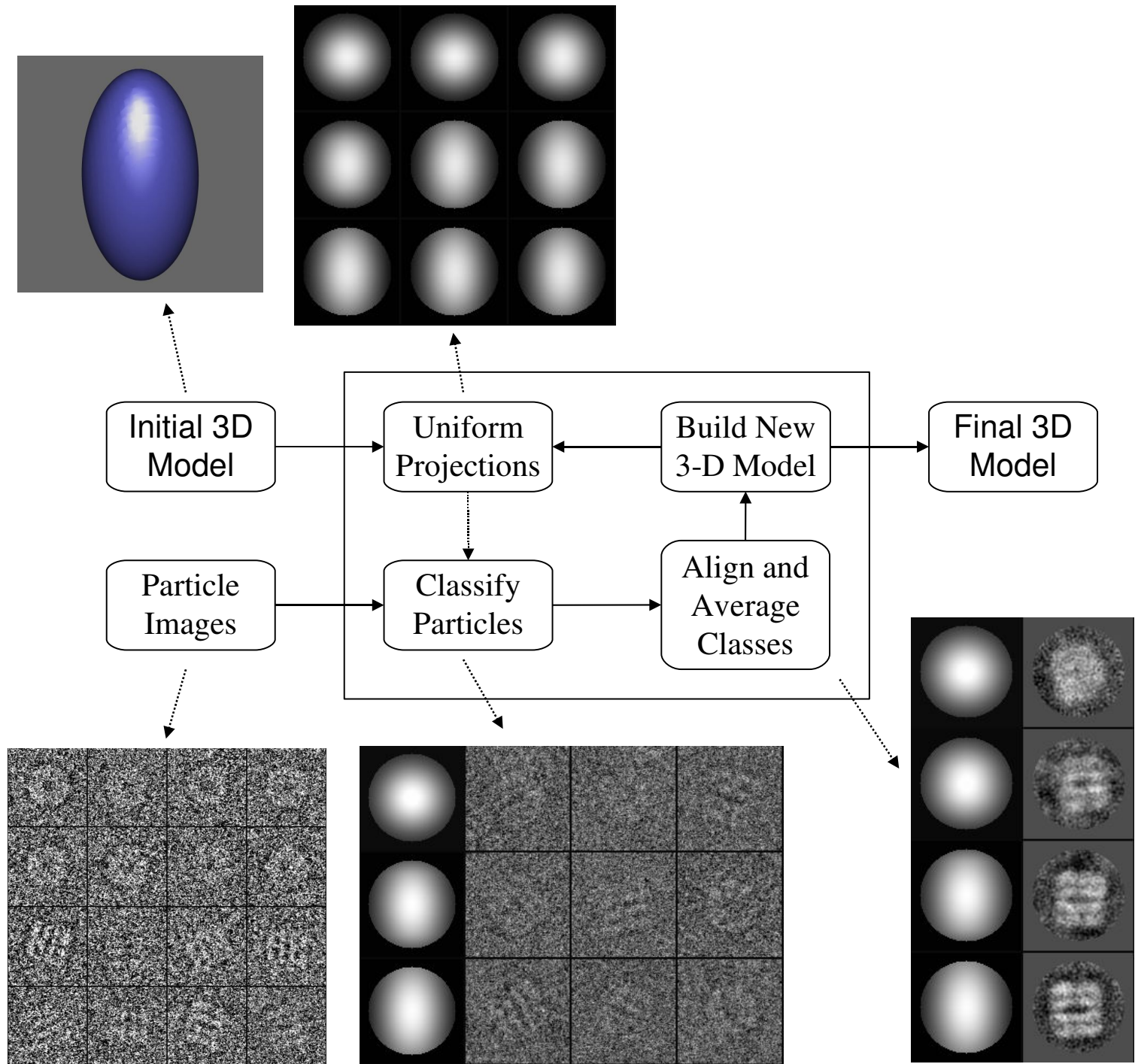
CCD vs. Film

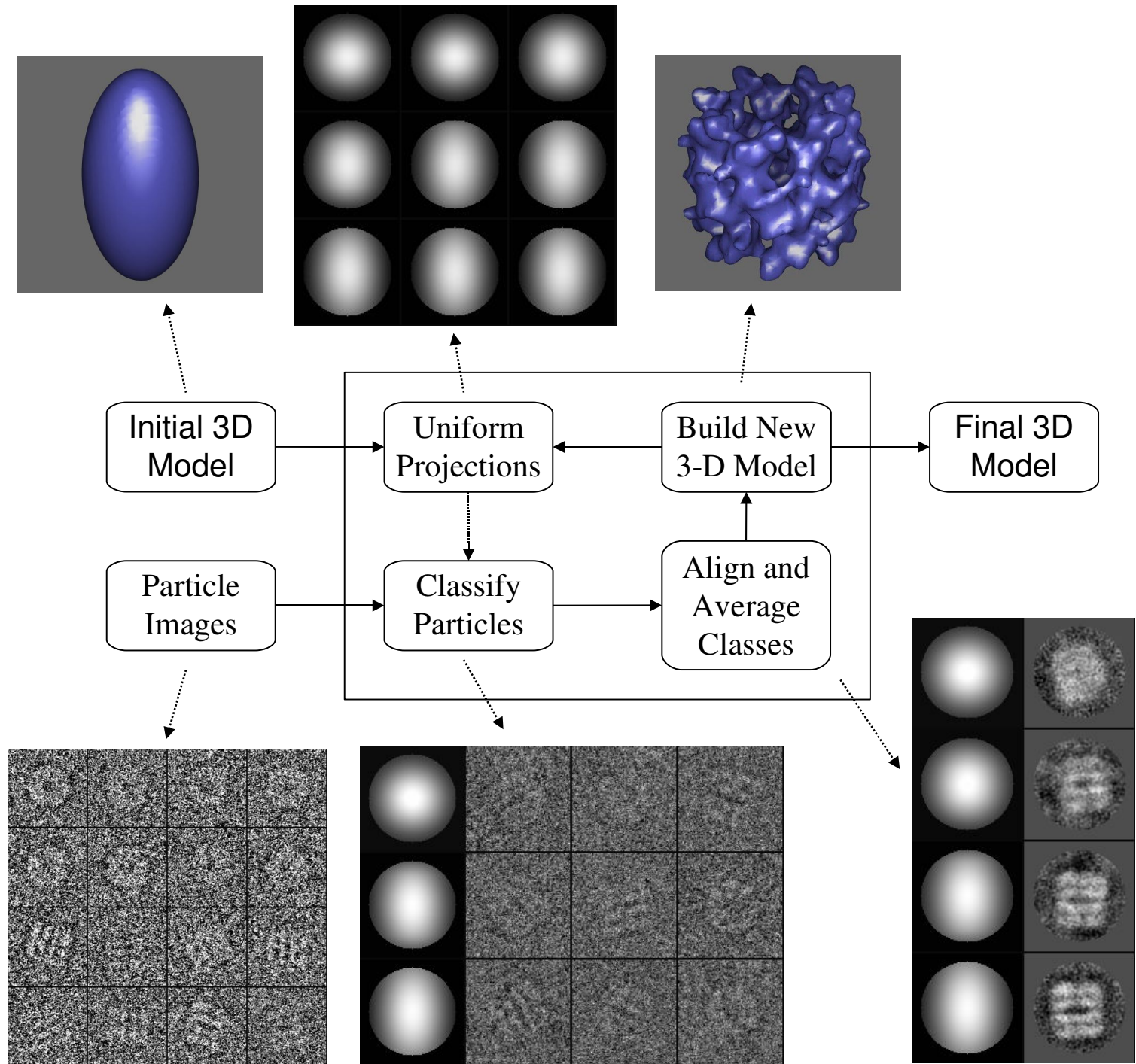


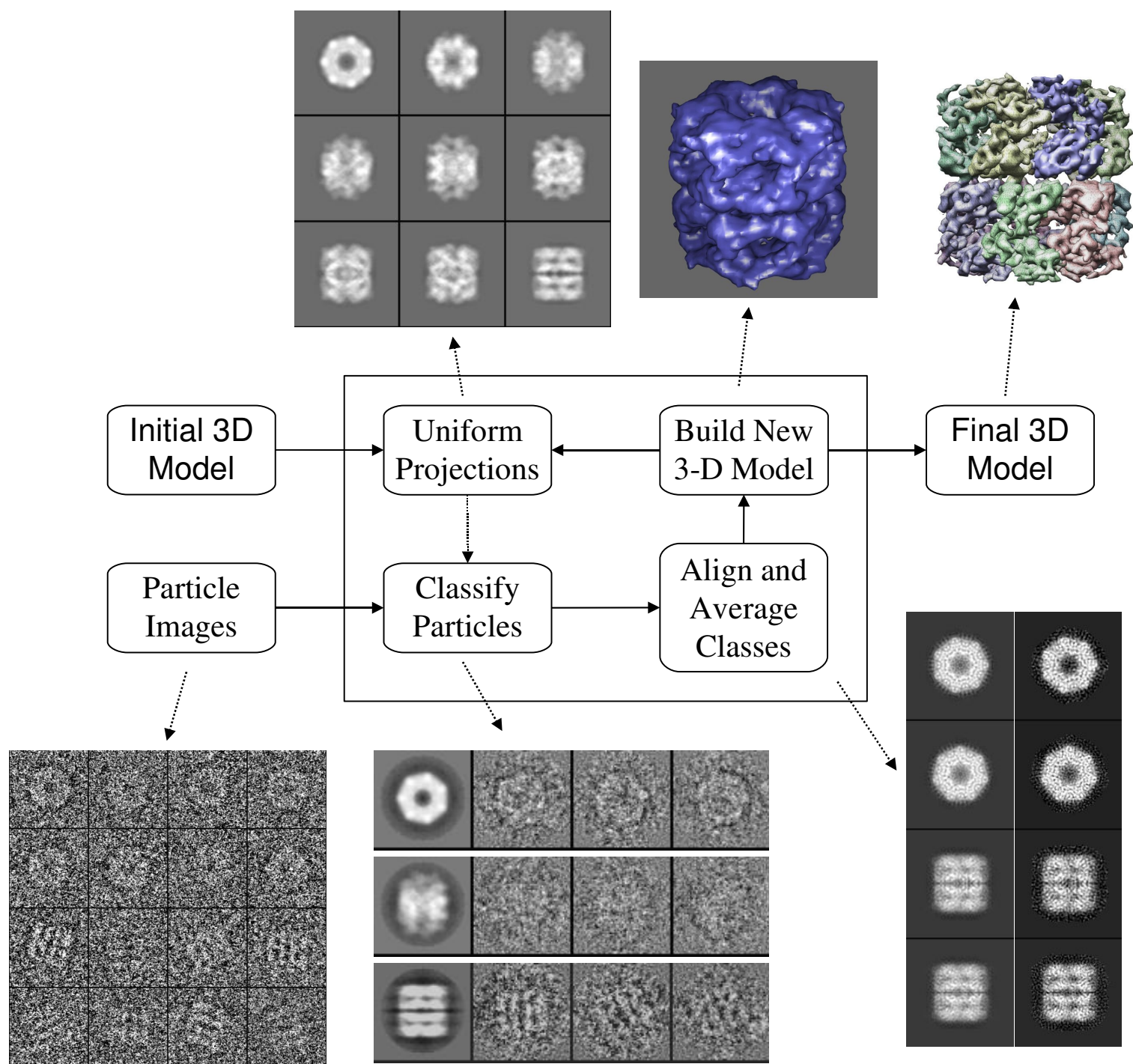


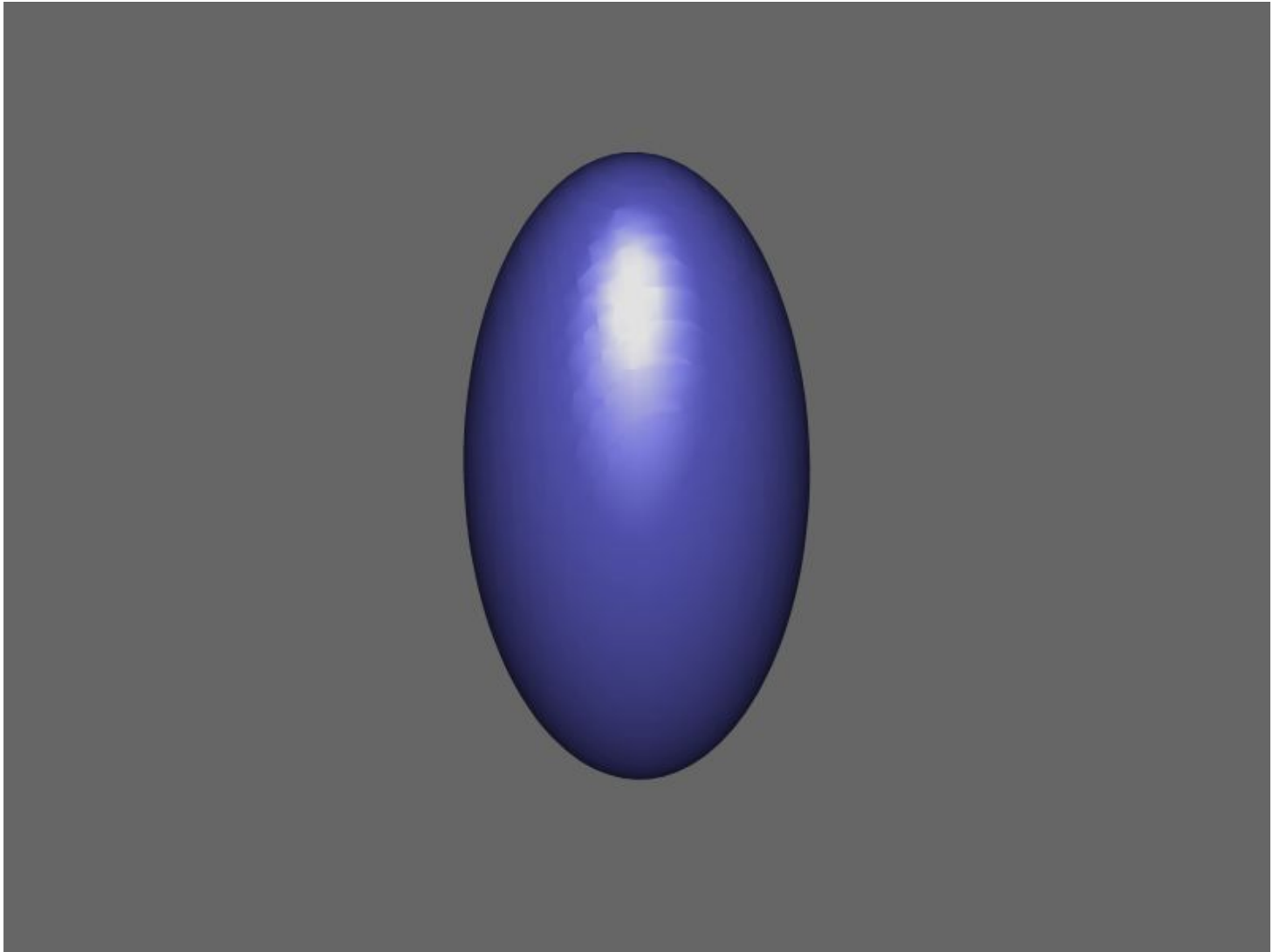


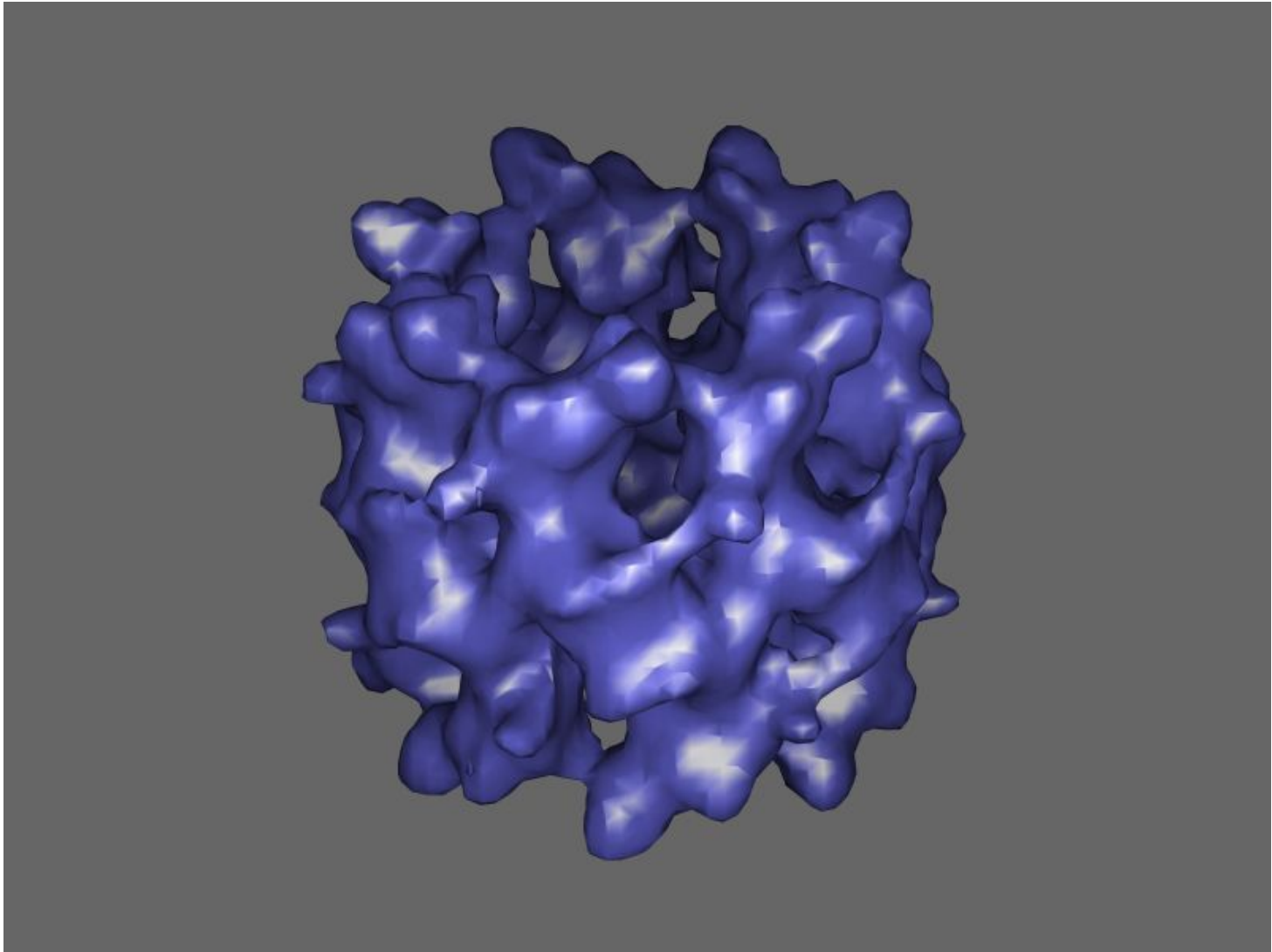


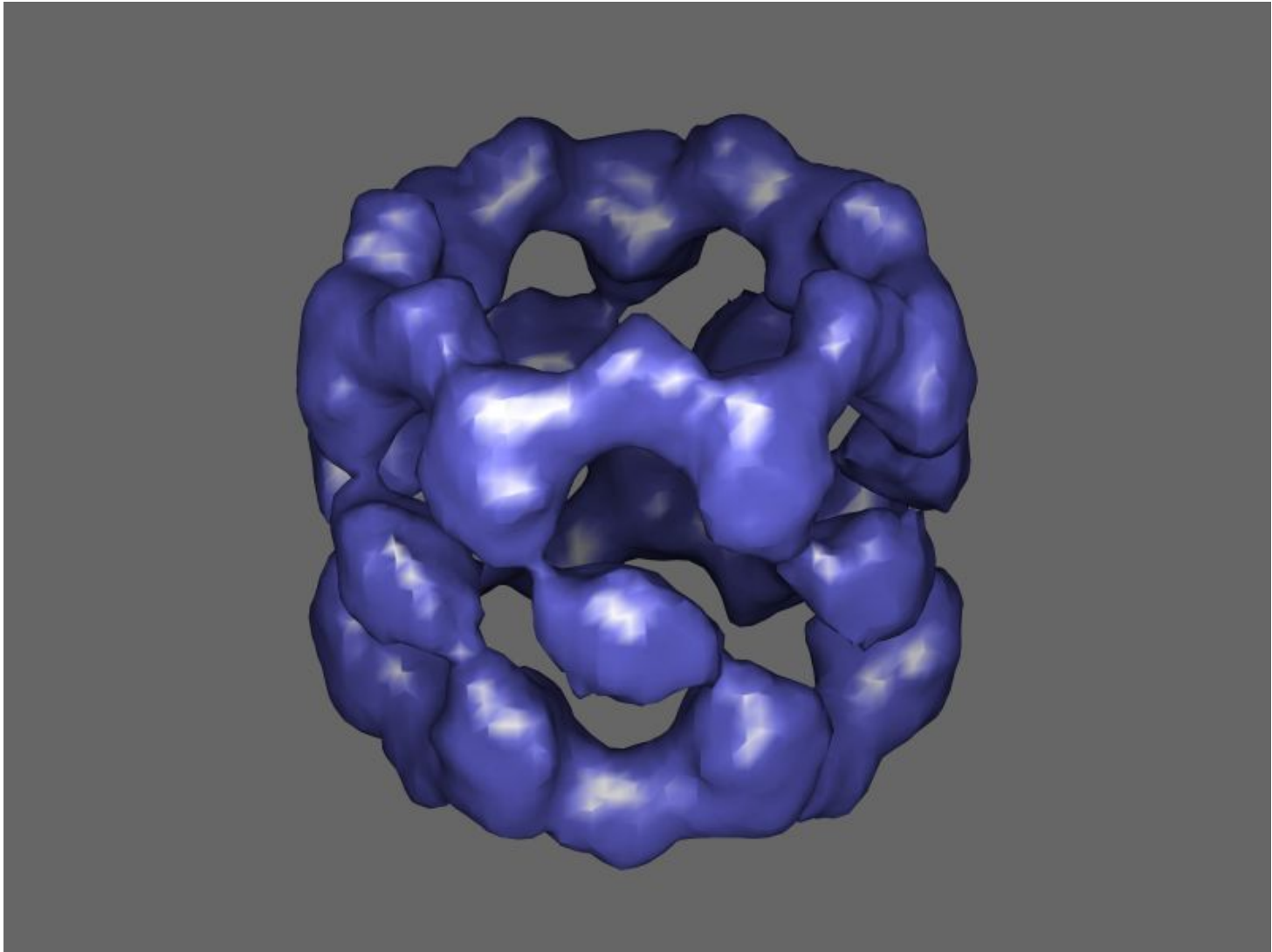


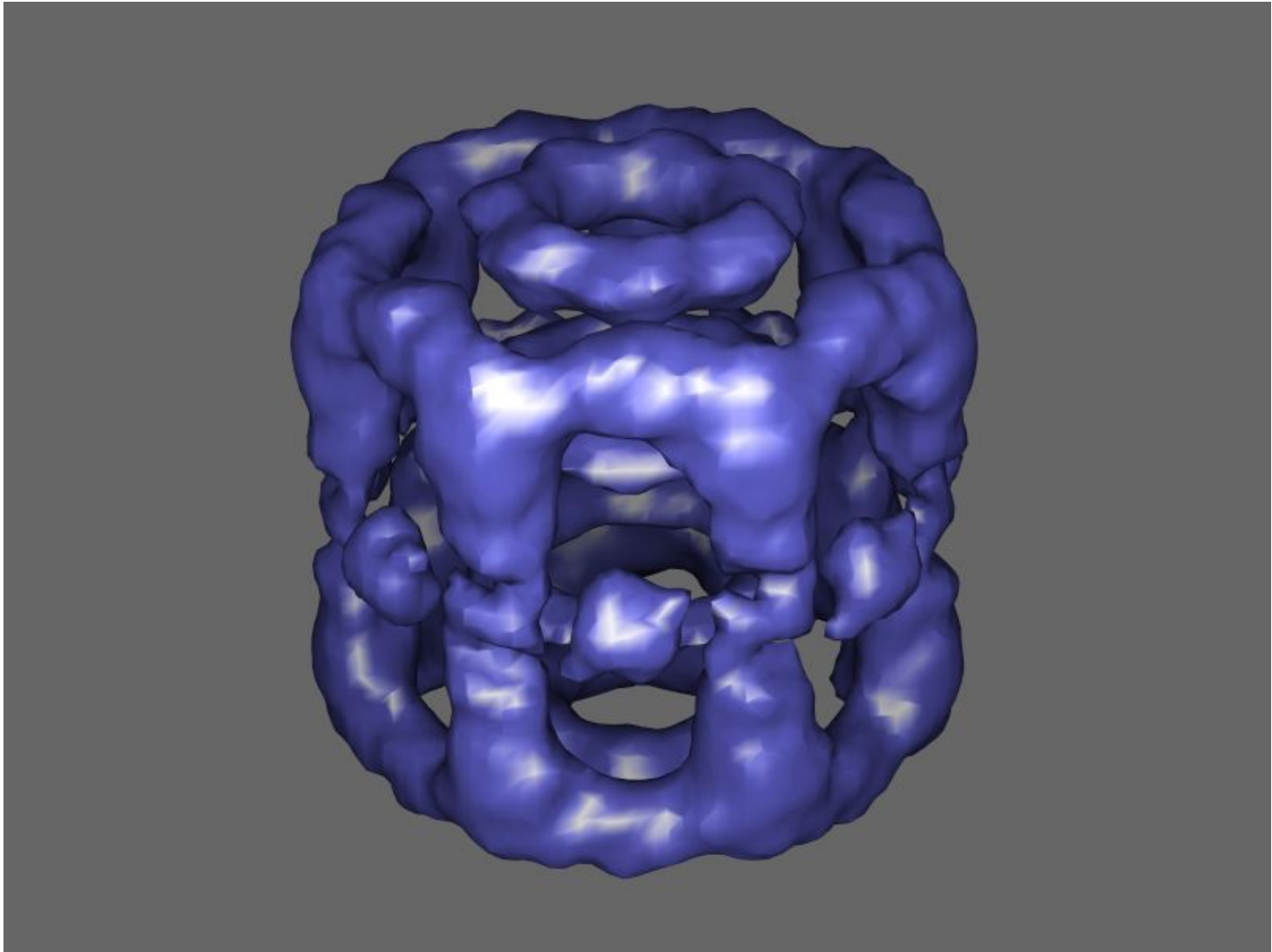


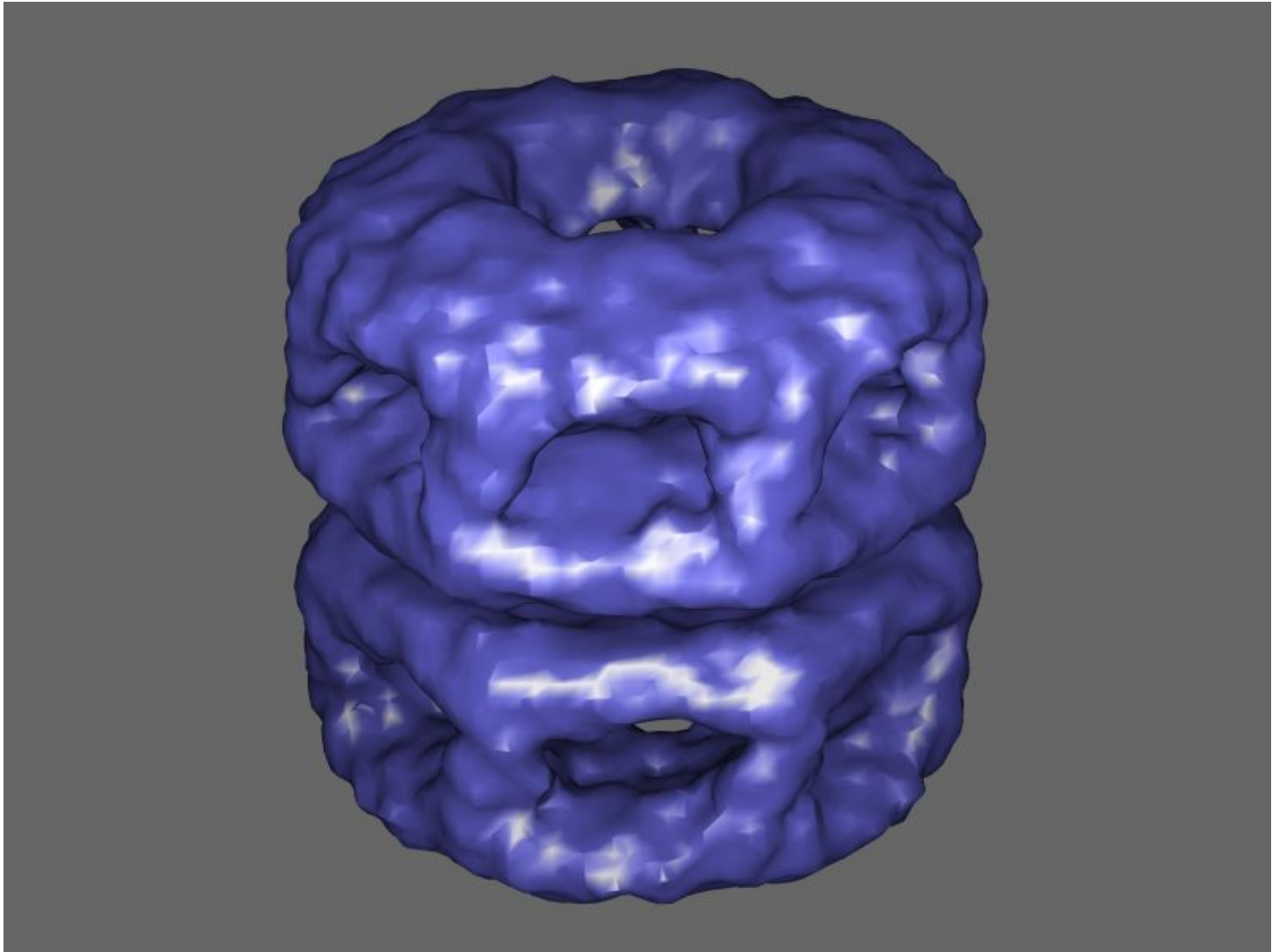


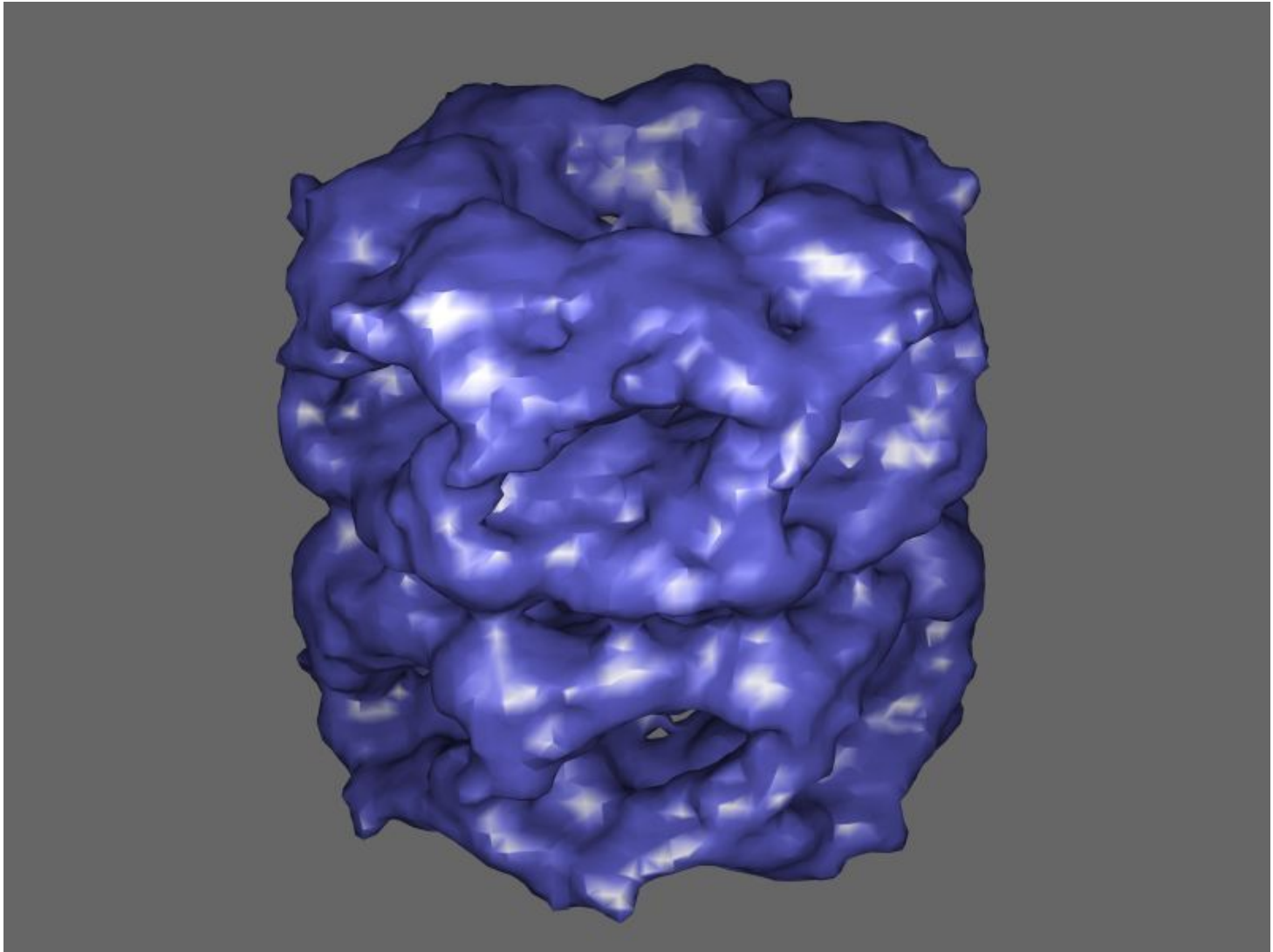












How do we get to Higher Resolutions?

- Get a better microscope
- Find a better microscopist
- Algorithm Improvements

Contrast Transfer Function

$$\overline{M}(s, \theta) = \overline{F}(s, \theta) C(s) E(s) + \overline{N}(s, \theta)$$

$$C(s) = \sqrt{1 - Q^2} \sin \gamma + Q \cos \gamma$$

$$\gamma = -\pi \left(\frac{1}{2} C_s \lambda^3 s^4 - \Delta Z \lambda s^2 \right)$$

$$E(s) = e^{-B s^2}$$

$$|N^2| = n_1 e^{n_2 s + n_3 s^2 + n_4 \sqrt{s}}$$

$$M(s)^2 = F(s)^2 C(s)^2 E(s)^2 + N(s)^2$$

8 Parameters

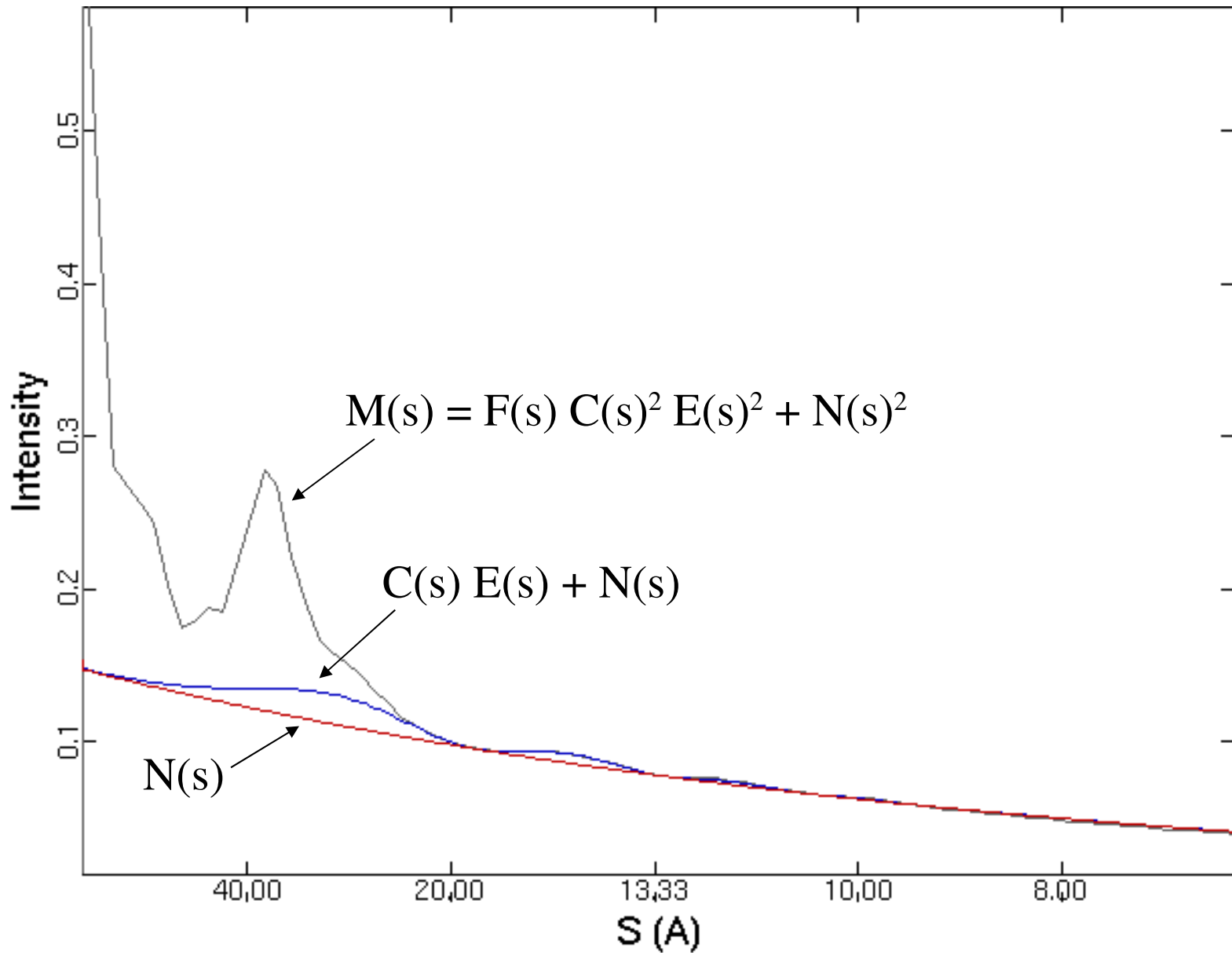
ΔZ - Defocus

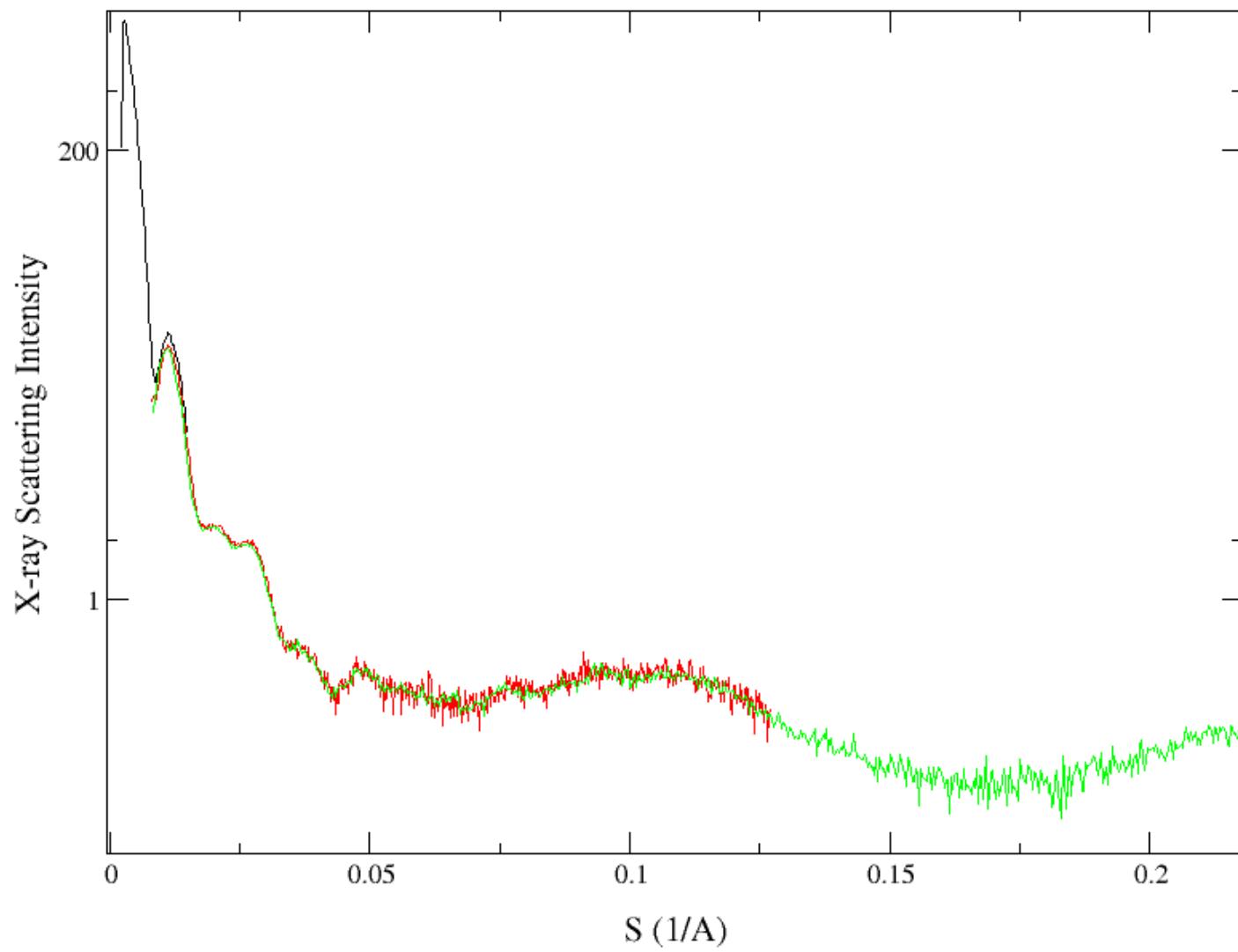
Q - Amplitude Contrast

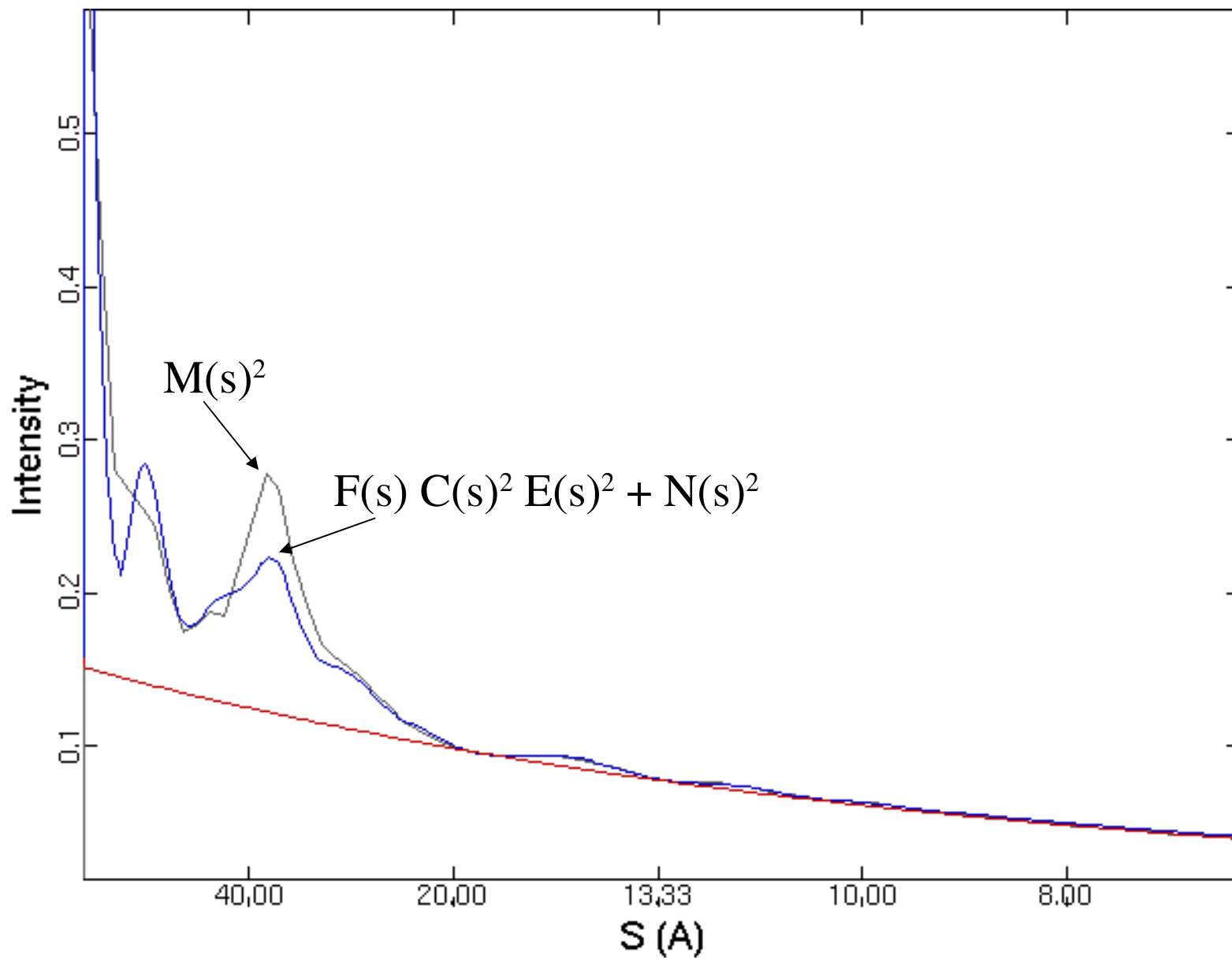
B - Gaussian Envelope Width

k - Signal Amplitude

n_{1-4} - Noise Parameters







CTF Correction

$$\bar{T}(s, \theta) = \sum_i k_i \bar{M}_i(s, \theta)$$

$$k_i = ?$$

- Maximize SNR of $T(s, q)$
- Minimize variance between $T(s, q)$ and $F(s, q)$

CTF Correction

Wiener
Filter

CTF
Correction

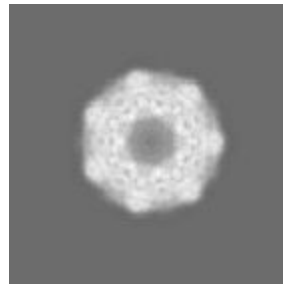
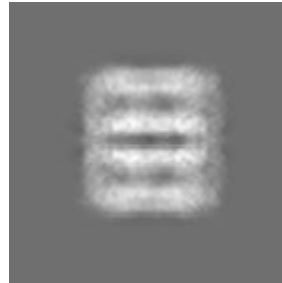
SNR
Weight

$$\bar{T}(s, \theta) = \frac{F^2(s) R(s)}{1 + F^2(s) R(s)} \sum_i \frac{1}{C_i(s) E_i(s)} \frac{R_i(s)}{R(s)} \bar{M}_i(s, \theta)$$

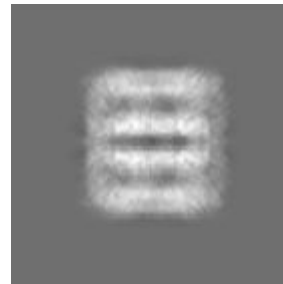
$$R_i(s) = \frac{C_i^2(s) E_i^2(s)}{N_i^2(s)}$$

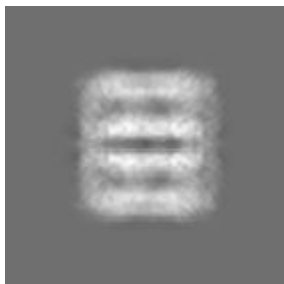
$$R(s) = \sum_i \frac{C_i^2(s) E_i^2(s)}{N_i^2(s)}$$

Image Classification

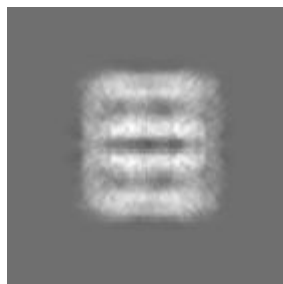


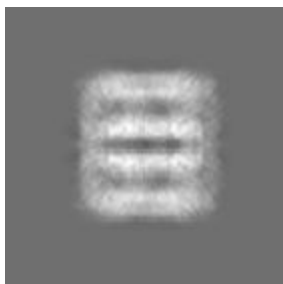
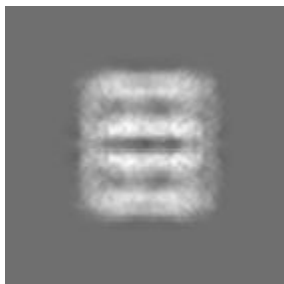
← ? →



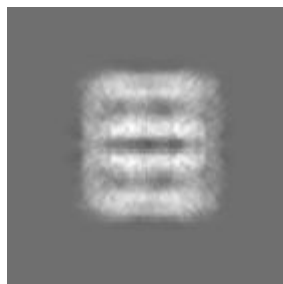


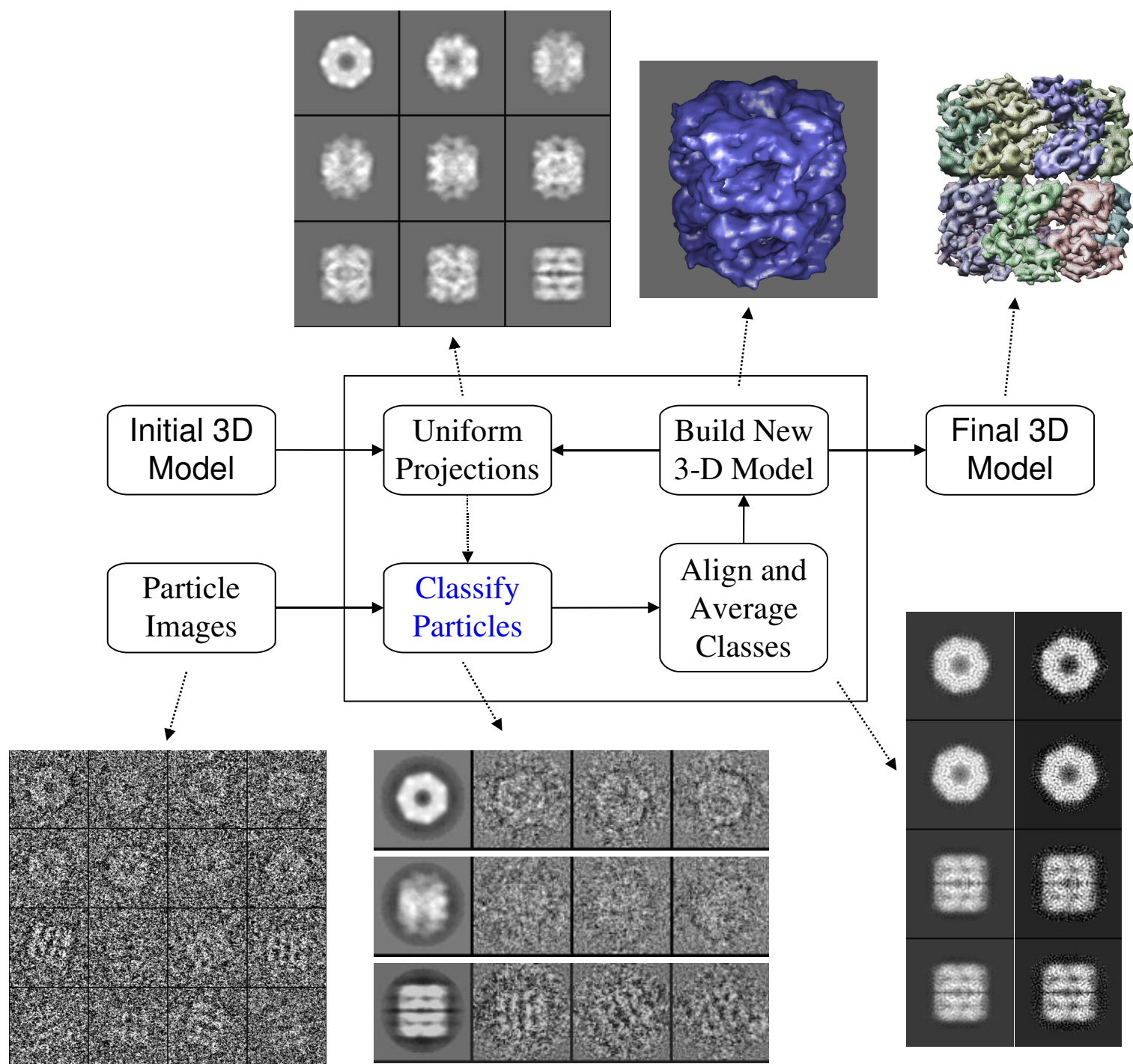
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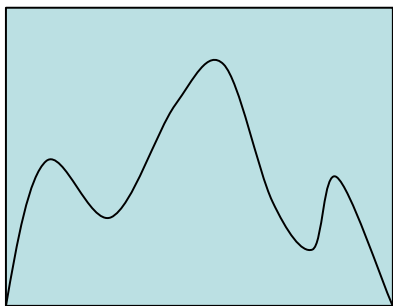


← ? →

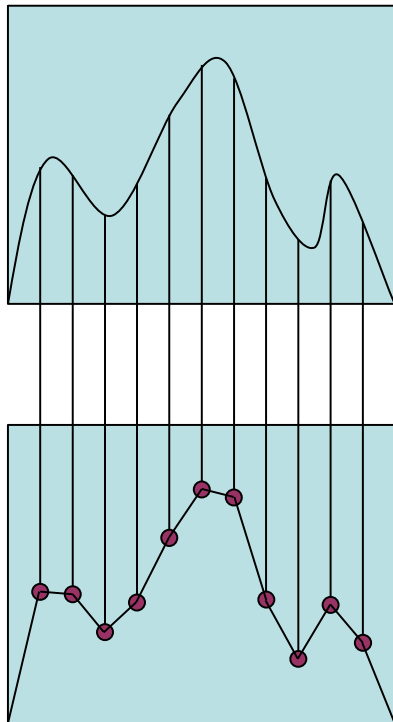




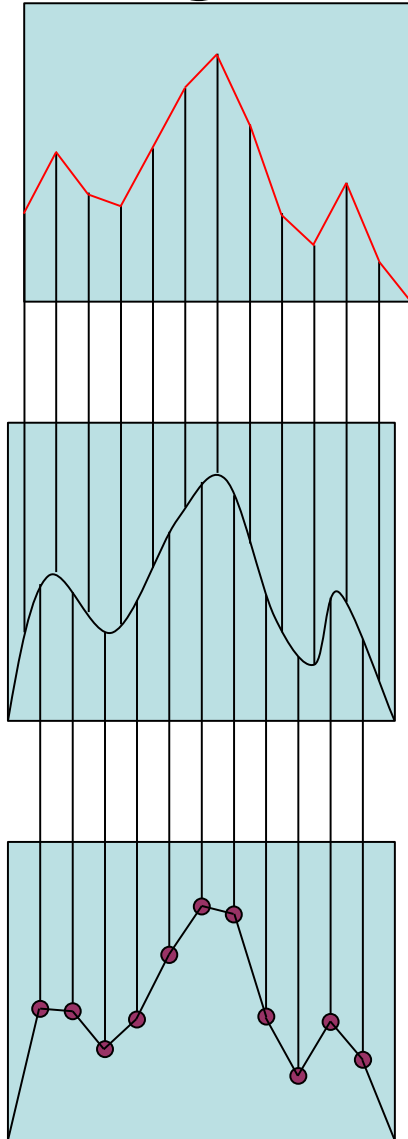
Alignment/Registration



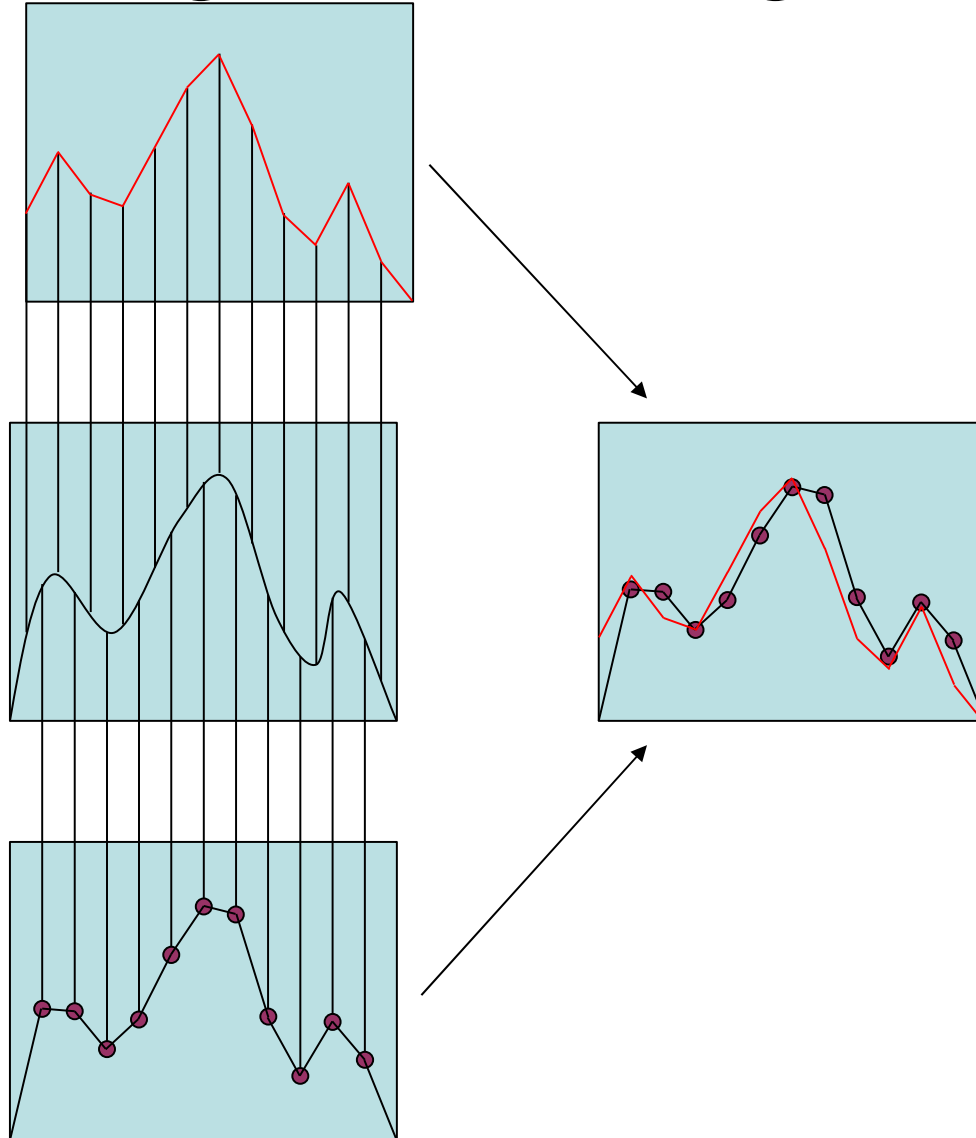
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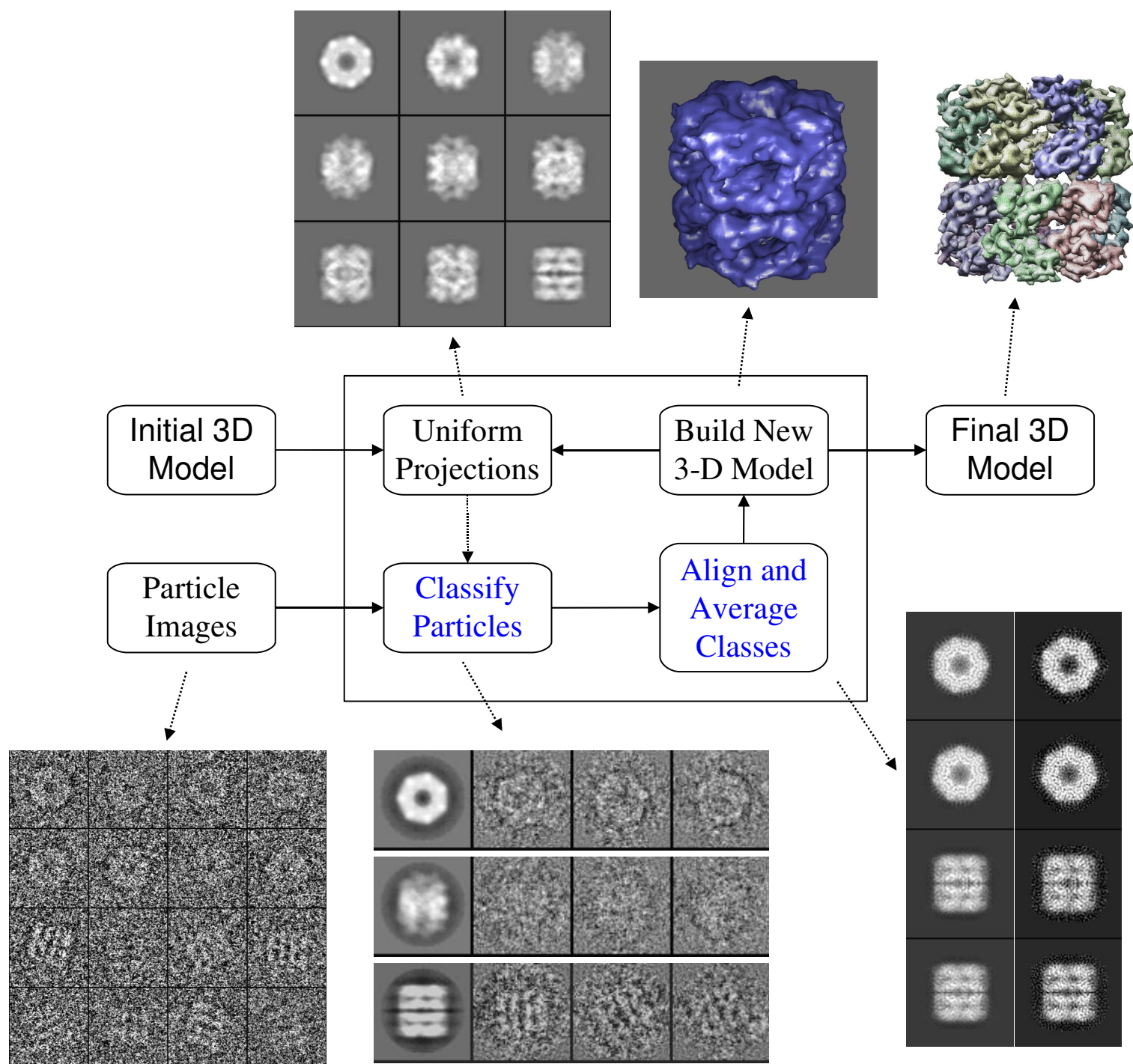


Alignment/Registration



Alignment/Registration

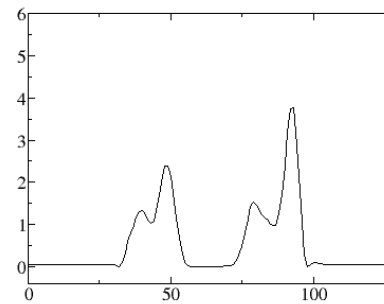
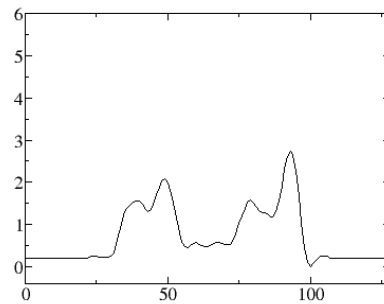
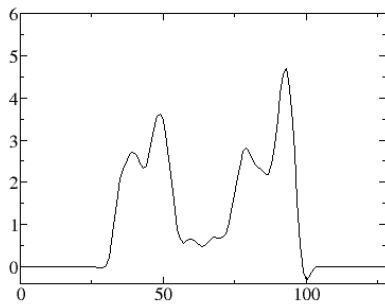


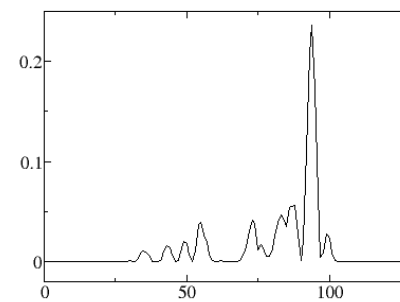
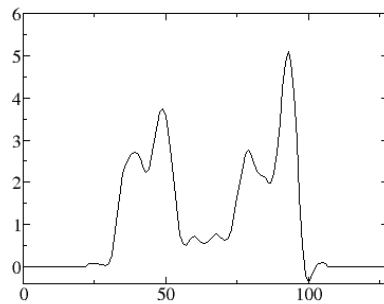
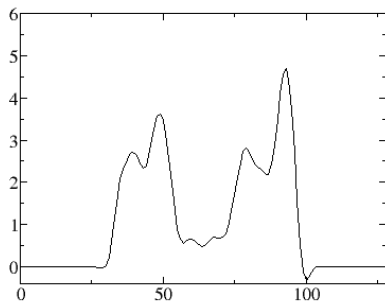
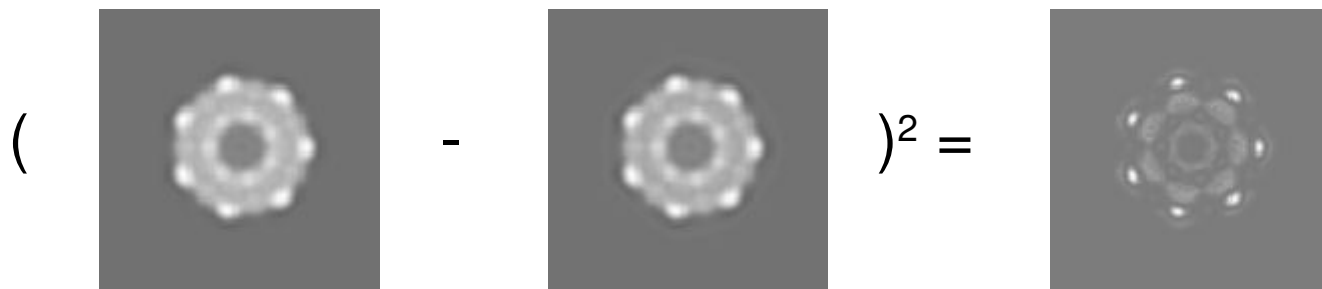


Measures of Similarity

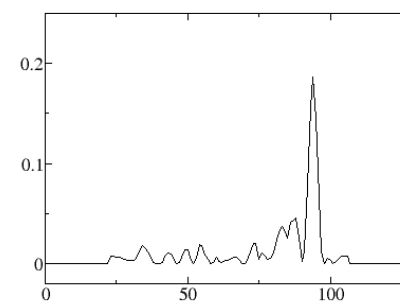
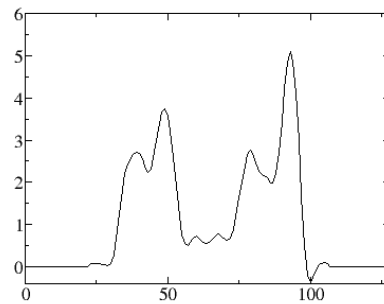
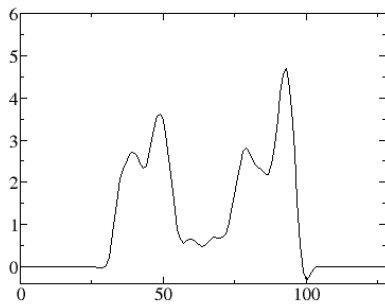
- Correlation Coefficient
- Variance (transformed density)
- Variance (matched filter)
- Phase Residual
- Mutual Information
- etc.

$$\left(\text{Image 1} - \text{Image 2} \right)^2 = \text{Image 3}$$

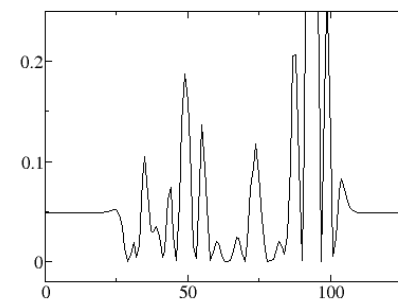
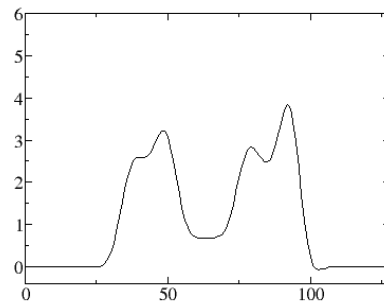
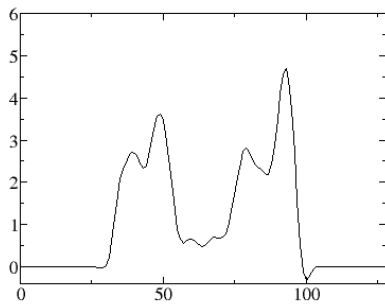




$$\left(\text{Image 1} - \text{Image 2} \right)^2 = \text{Image 3}$$

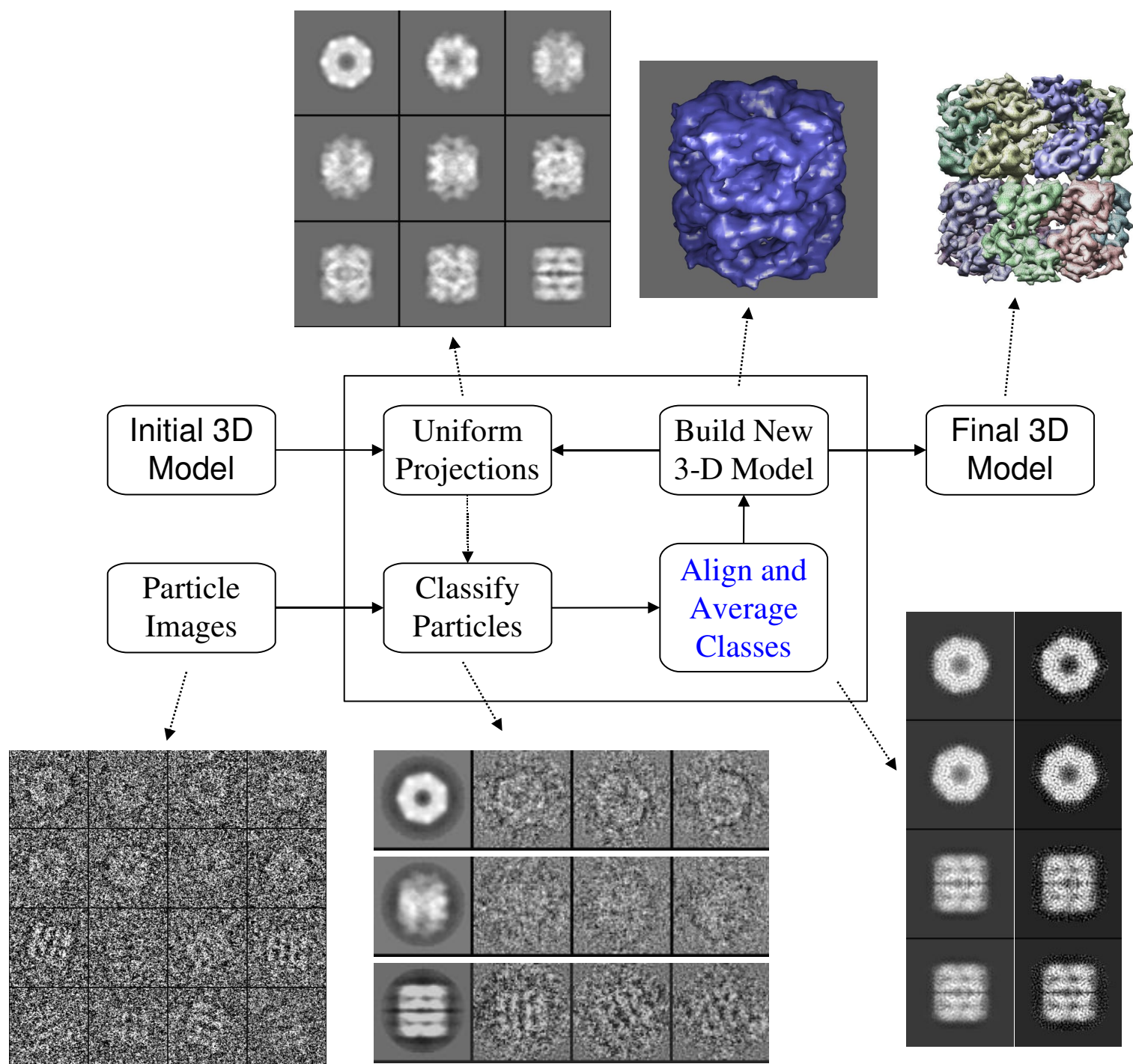


$$\left(\text{Image 1} - \text{Image 2} \right)^2 = \text{Image 3}$$



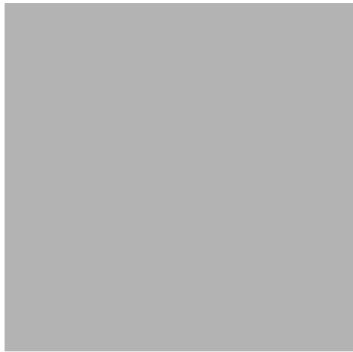
And the Answer is...

- Wiener filter particle
- Filter reference to match
- Normalize reference density to particle
- Calculate variance

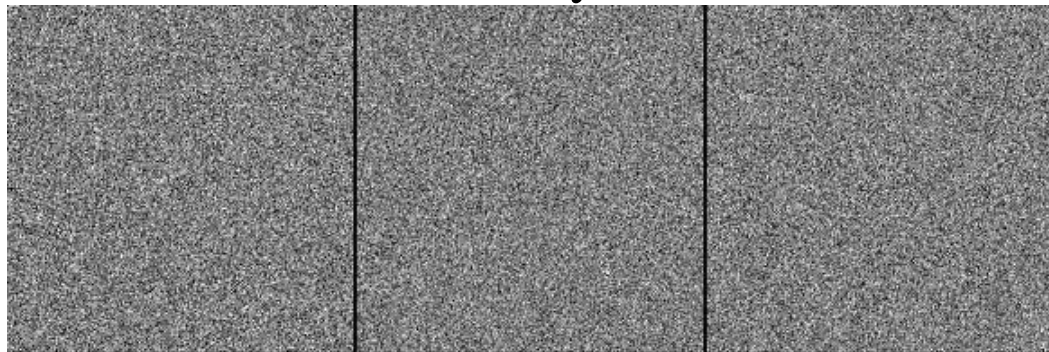


Model Bias

Base



Noisy



Align to



25

100

250

1000

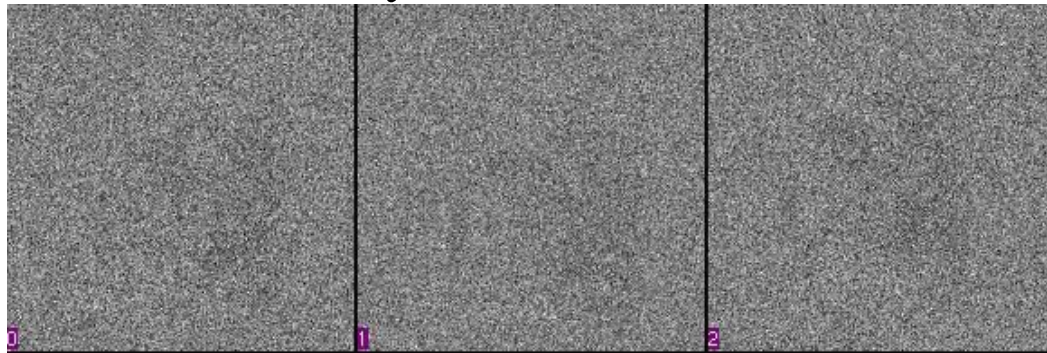
2000

Model Bias

Base



Noisy (~10% contrast)



Align to



25

100

250

1000

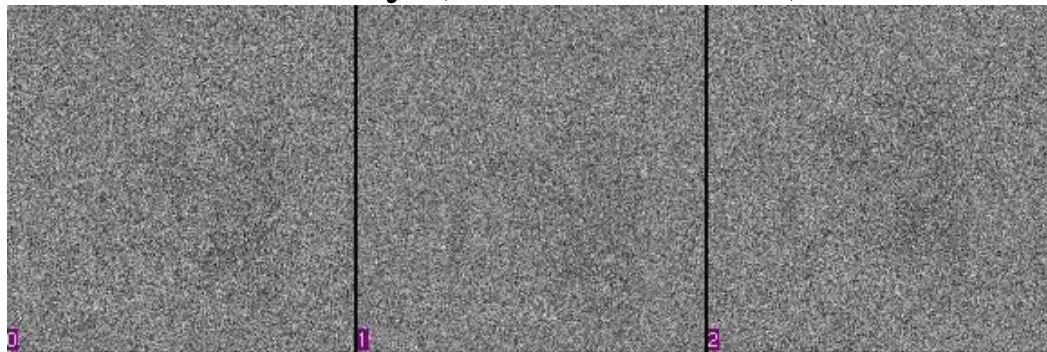
2000

Model Bias

Base



Noisy (~10% contrast)



Align to



25

100

250

1000

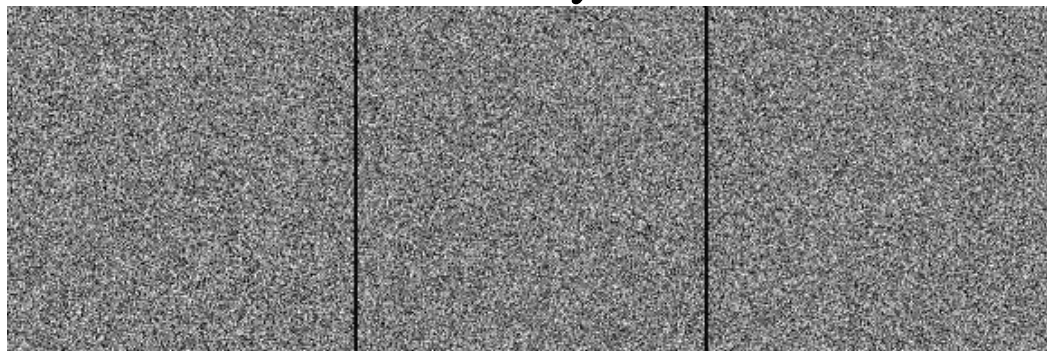
2000

Model Bias

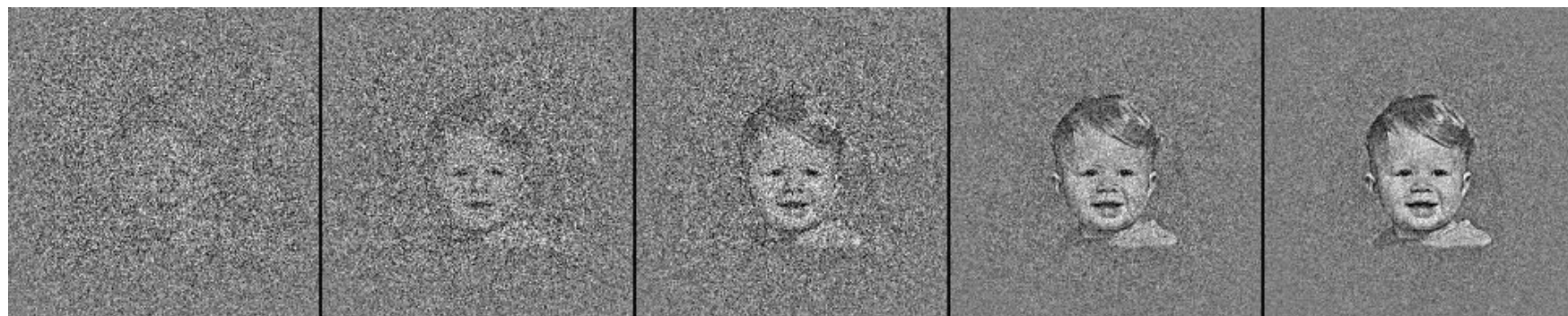
Base



Noisy



Align to



25

100

250

1000

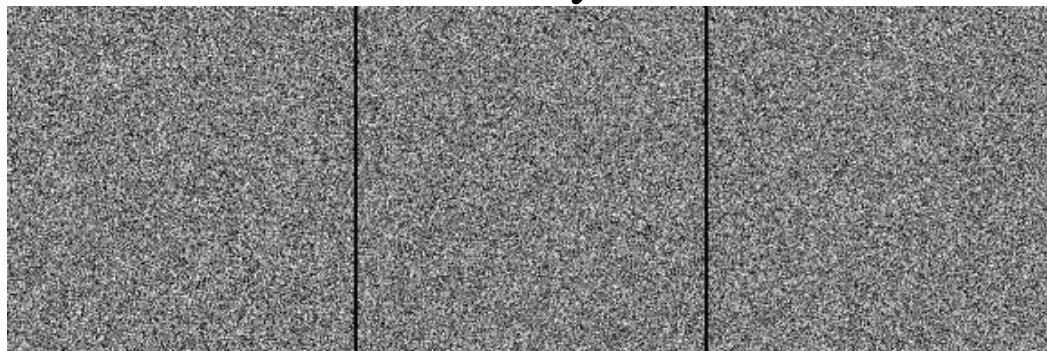
2000

Model Bias

Base

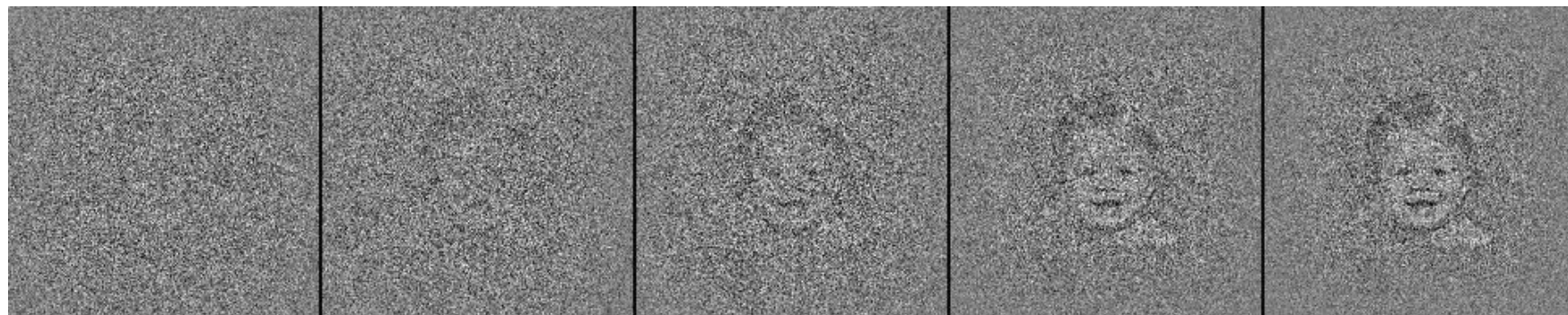


Noisy



Align to

Iter x4



25

100

250

1000

2000

Model Bias

Base

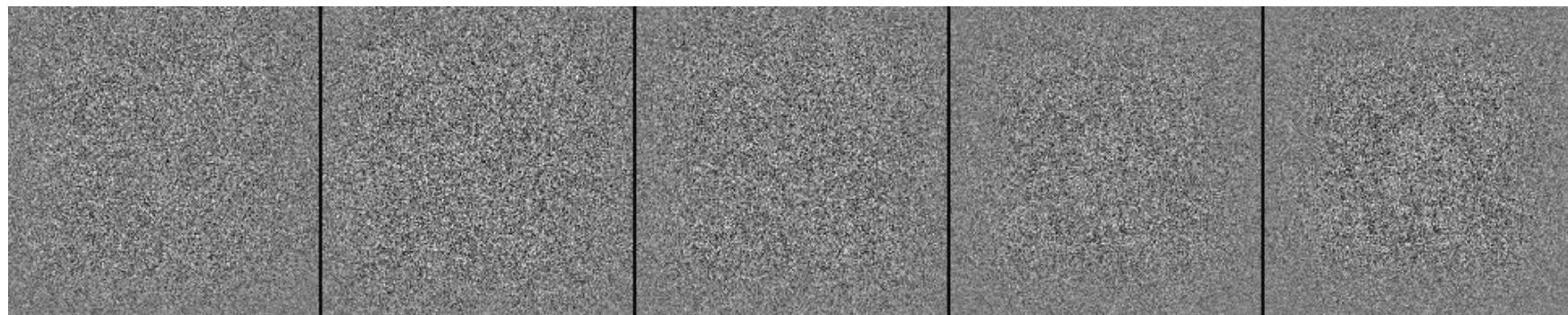


Noisy



Align to

Iter x8



25

100

250

1000

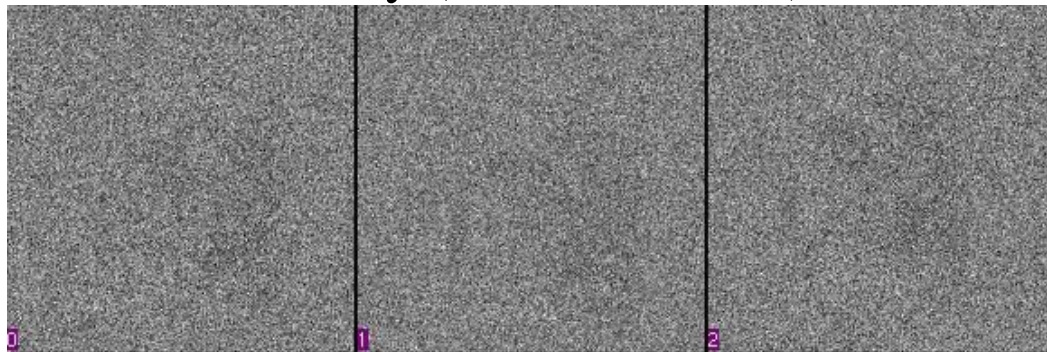
2000

Model Bias

Base



Noisy (~10% contrast)



Align to



25

100

250

1000

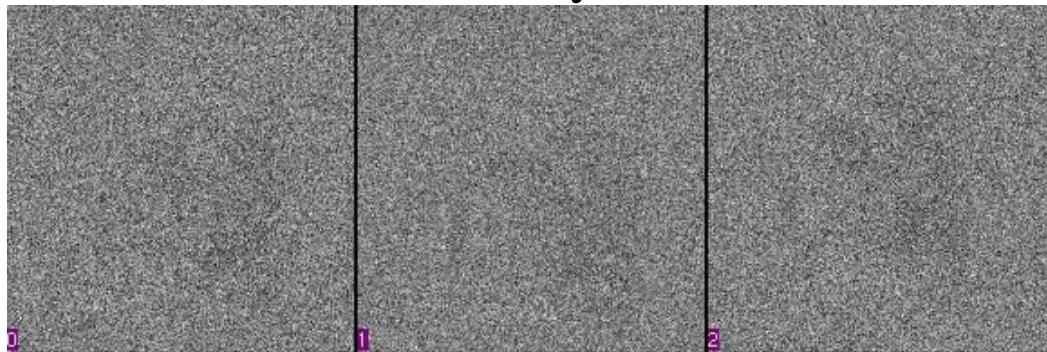
2000

Model Bias

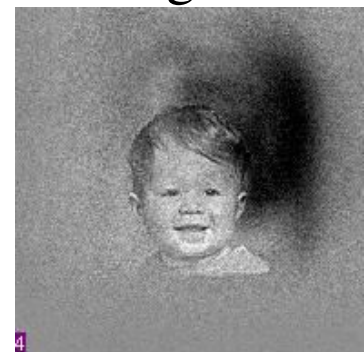
Base



Noisy



Align to



Iter x4



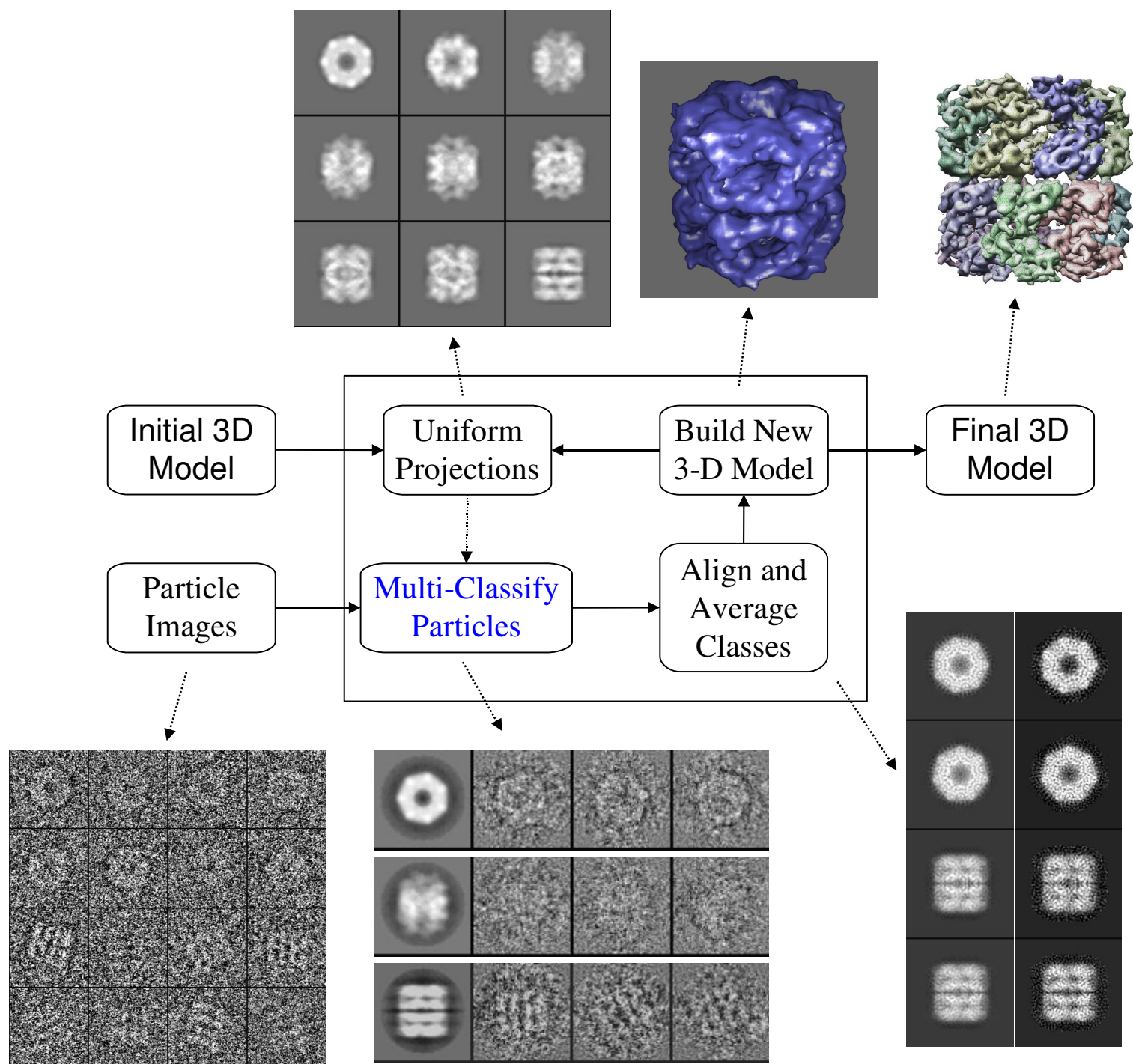
25

100

250

1000

2000



- Each particle -> best n classes
- More restrictive exclusion from class-avg

The Future

- Better similarity criteria
- Improved CTF model
- Per-particle CTF (at least defocus)
- Beam tilt
- Better 3-D reconstruction
- New refinement methodologies