# Case Studies in Resolution, Validation, and ...

Steve Ludtke
Baylor College of Medicine

# My Assignment

- "We hope Steve will get into the nitty gritty of his comparison… even more than his excellent talk last winter at Hybrid Methods meeting. Practical solutions."
- Maybe I could re-refine our recent structures with the Gold Standard refinement and compare...
- NCMI: 72 EMDB structures in last 5 years
- Ok, maybe not ALL of them...

# Gold Standard ?

- Refine all data together, split at the end, no refinement of halves before FSC
- Refine all data together, split at end, partial orientation redetermination of halves
- Split at the beginning, low-pass filtered same starting model, independent refinements with resolution-limited info
- Split at the beginning, randomized different starting models, independent refinements with arbitrary info

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- Split at the beginning, randomized perturbed starting models, independent refinements with arbitrary info

# Molluscan Hemocyanin


中山大學
SUN YAT-SEN UNIVERSITY

NCMI
National Center for Macromolecular Imaging

Xinghong Dai
Qinfen Zhang

South China Sea Fisheries Research Institute

Jiangyong Wang

Wah Chiu
Michael Schmid
Yao Cong
Junjie Zhang
Donghua Chen



SKLBC10B05, NSFC, China
Guangdong Province Agricultural Project, China

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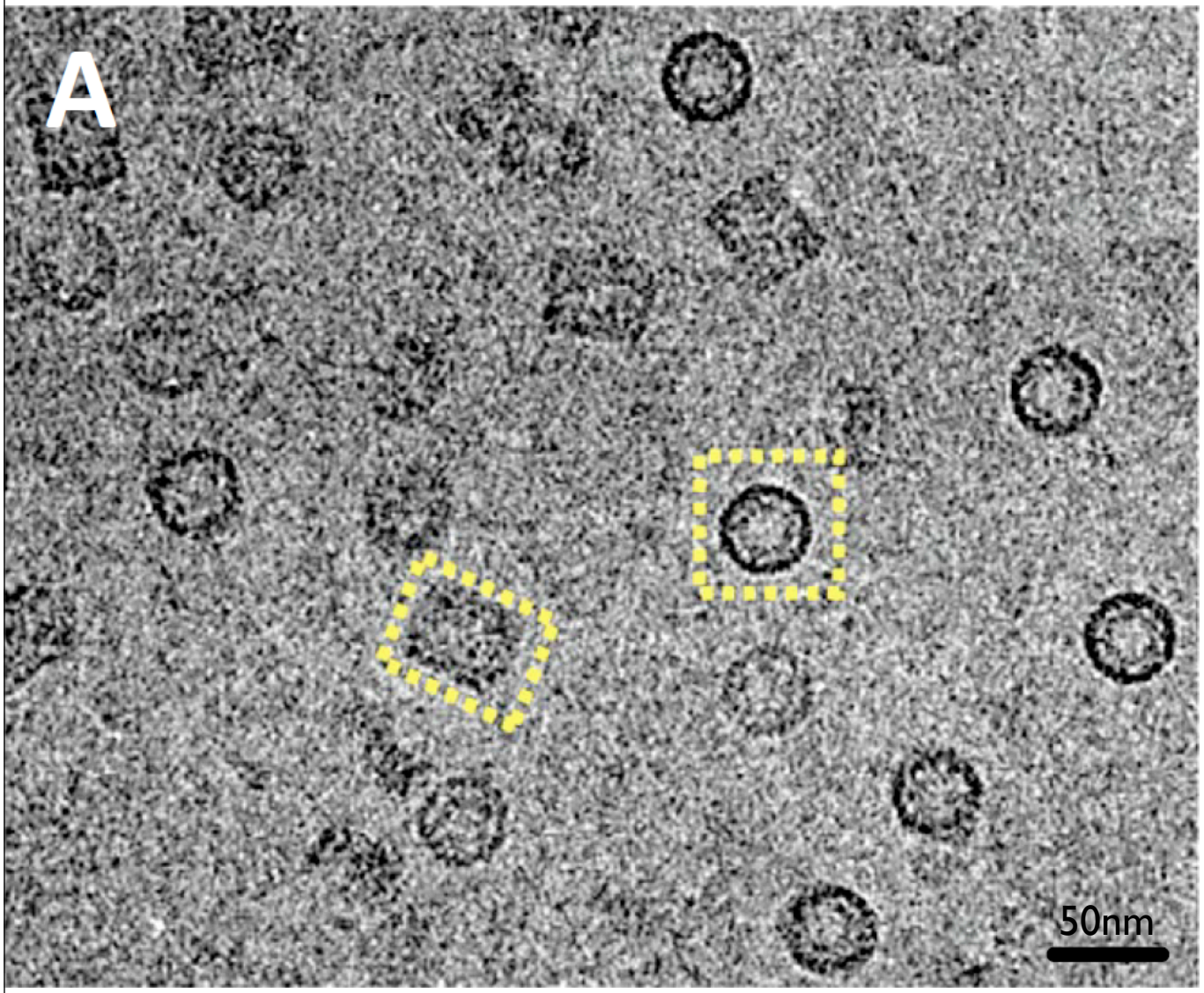
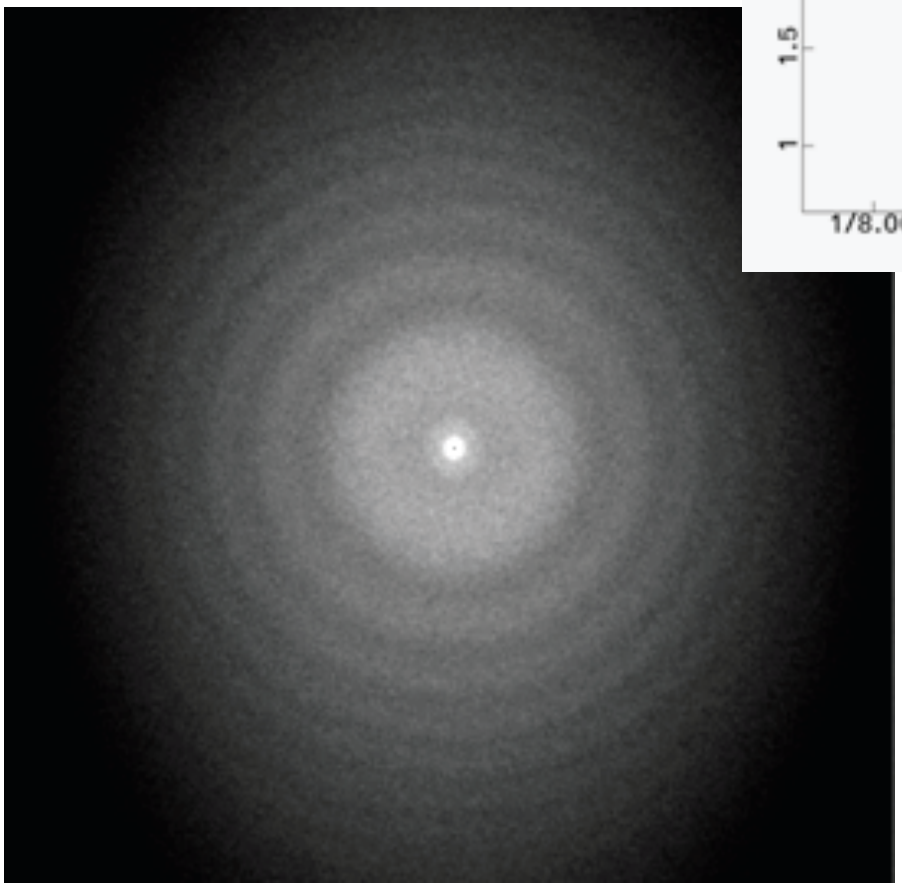
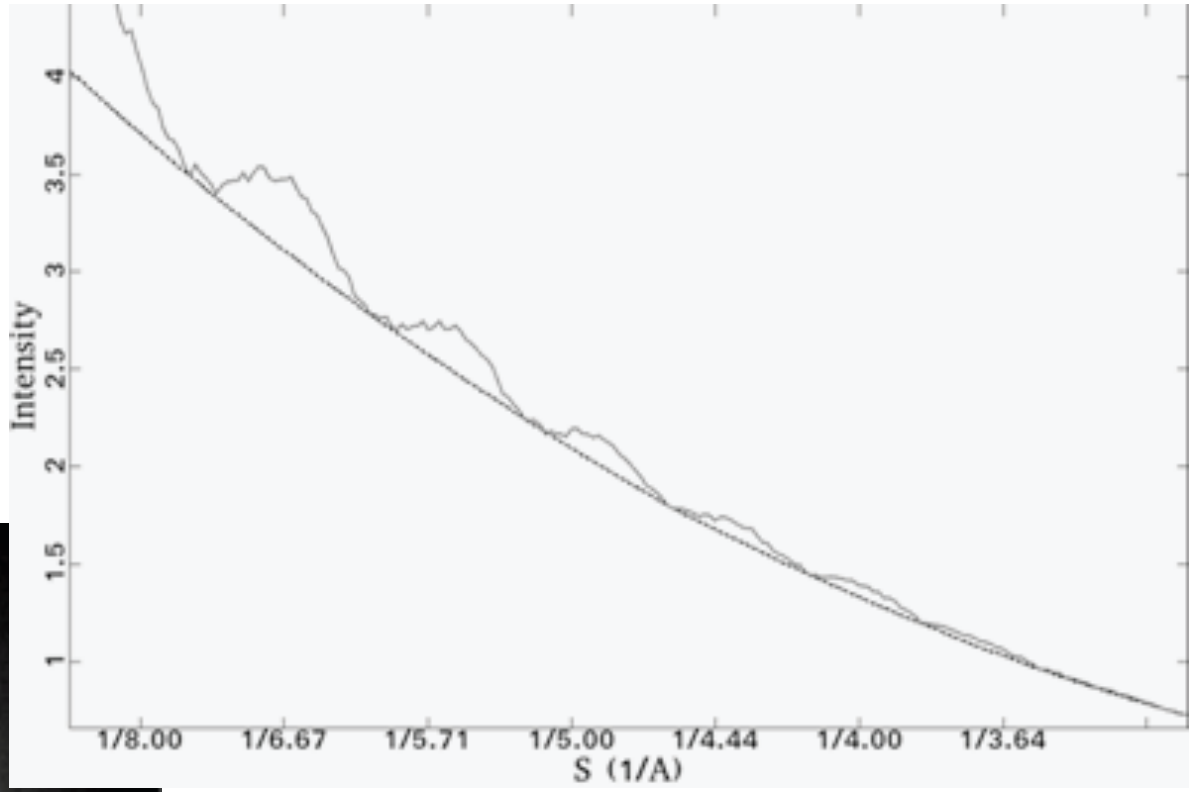
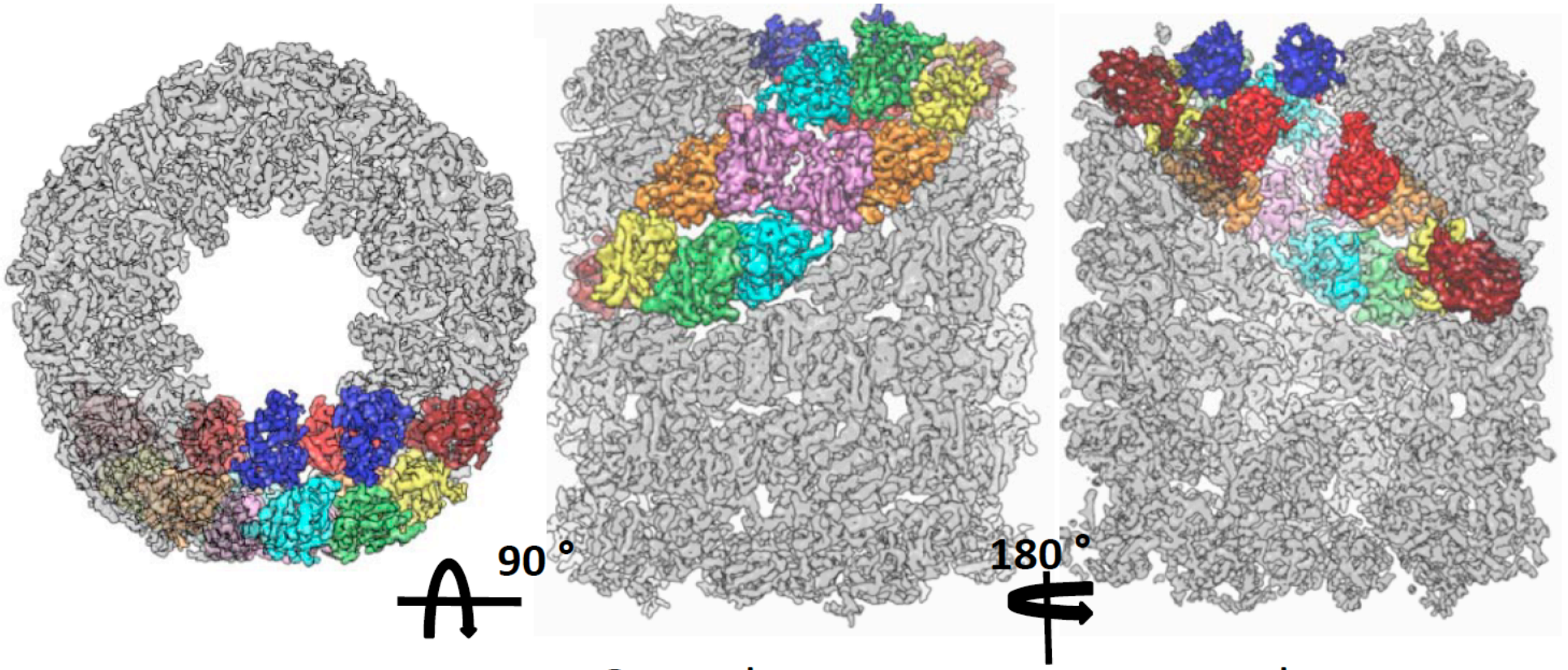
TACC (Computational Resouces)




- JEOL3200SFF
- 300 keV
- SO-163/Nikon
- 28,641 Particles used
- 1.06 Å/pix
- D5 Symmetry
- Homo-dimer in each asym unit
- 8 pseudo-equivalent FUs per monomer
- ~8 MDa

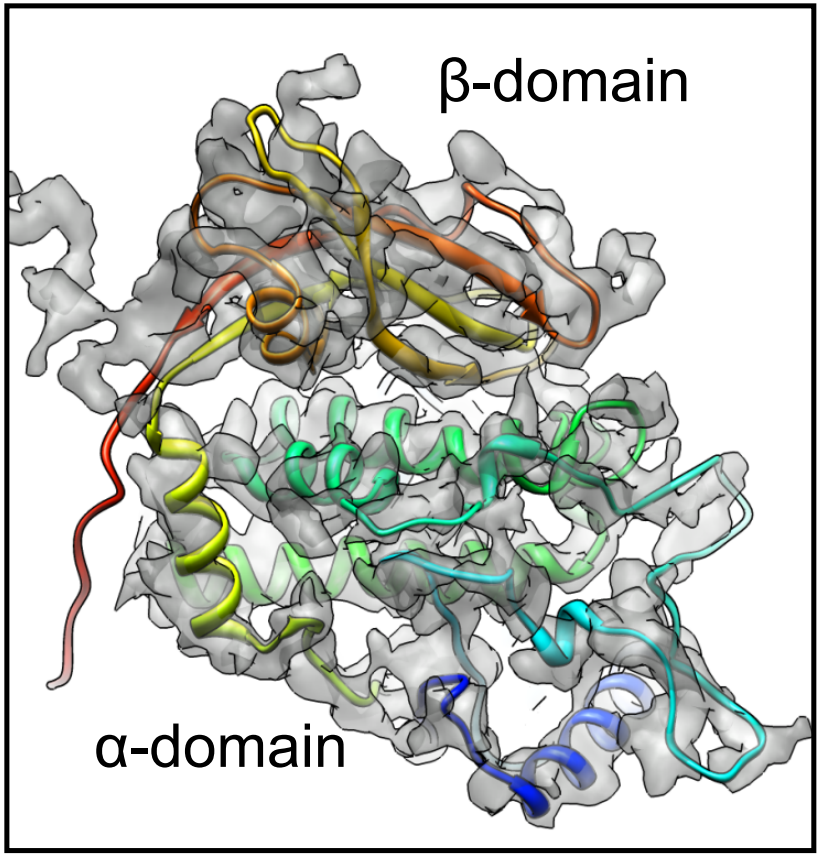


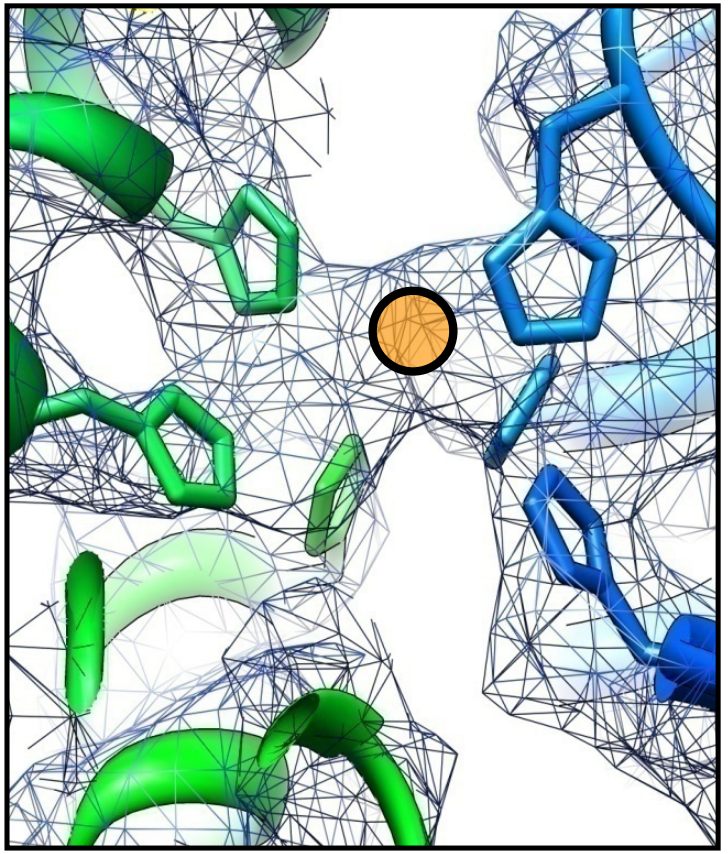

Intensity
4
3.5
3
2.5
2
1.5
1
1/8.00
1/6.67
1/5.71
1/5.00
1/4.44
1/4.00
1/3.64
S (1/A)

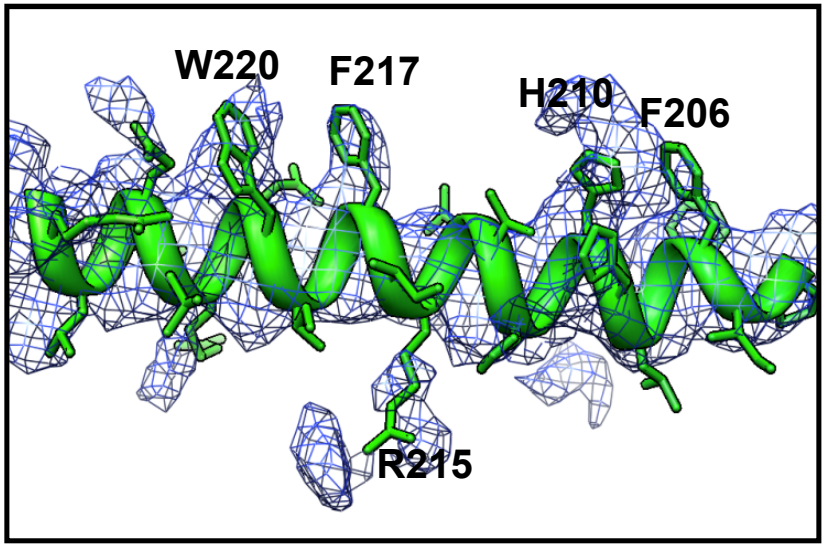


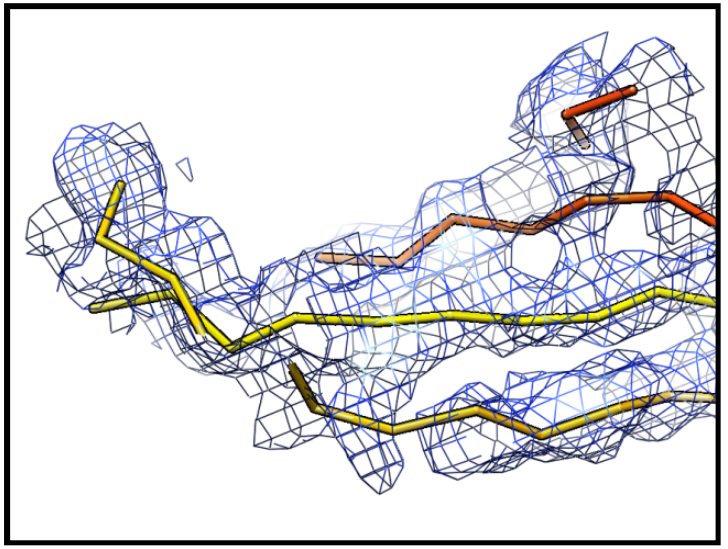

D5 Symmetry x 2 subunits x 8 FUs

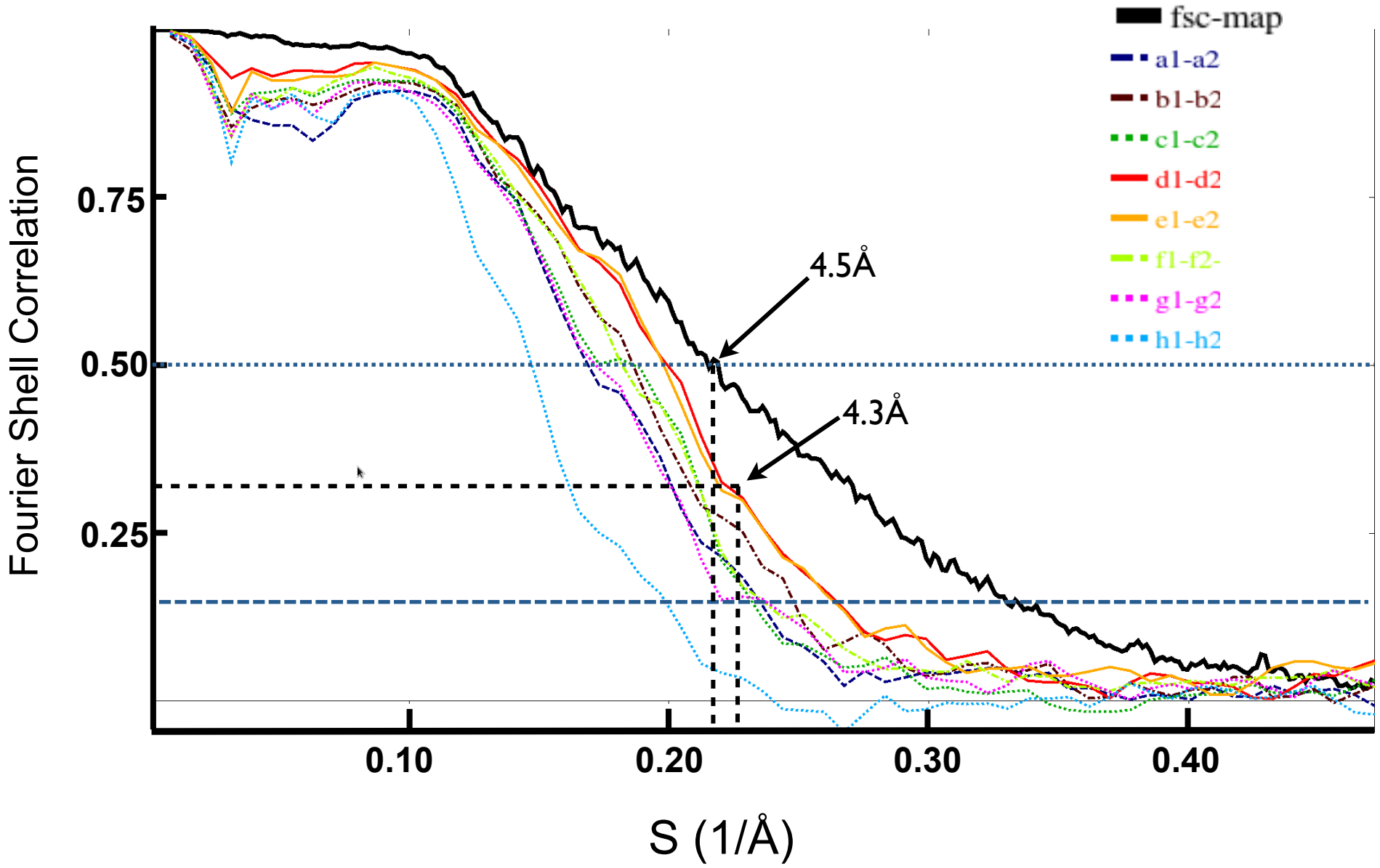
FUs have ~45% identity


β-domain
α-domain




W220
F217
H210
F206
R215



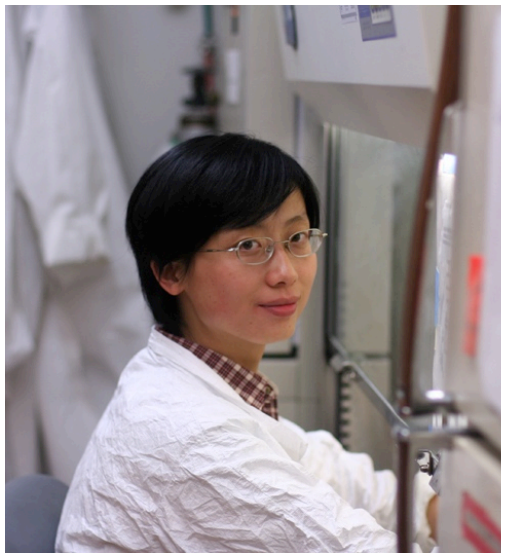

fsc-map
a1-a2
b1-b2
c1-c2
d1-d2
e1-e2
f1-f2-
g1-g2
h1-h2
4.5Å
4.3Å
Fourier Shell Correlation
0.75
0.50
0.25
0.10
0.20
0.30
0.40
S (1/Å)

# DARK

## (Drosophila Apaf-1 related killer)

BOSTON UNIVERSITY
School of Medicine

## Acknowledgements

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University of London

Christopher W. Akey
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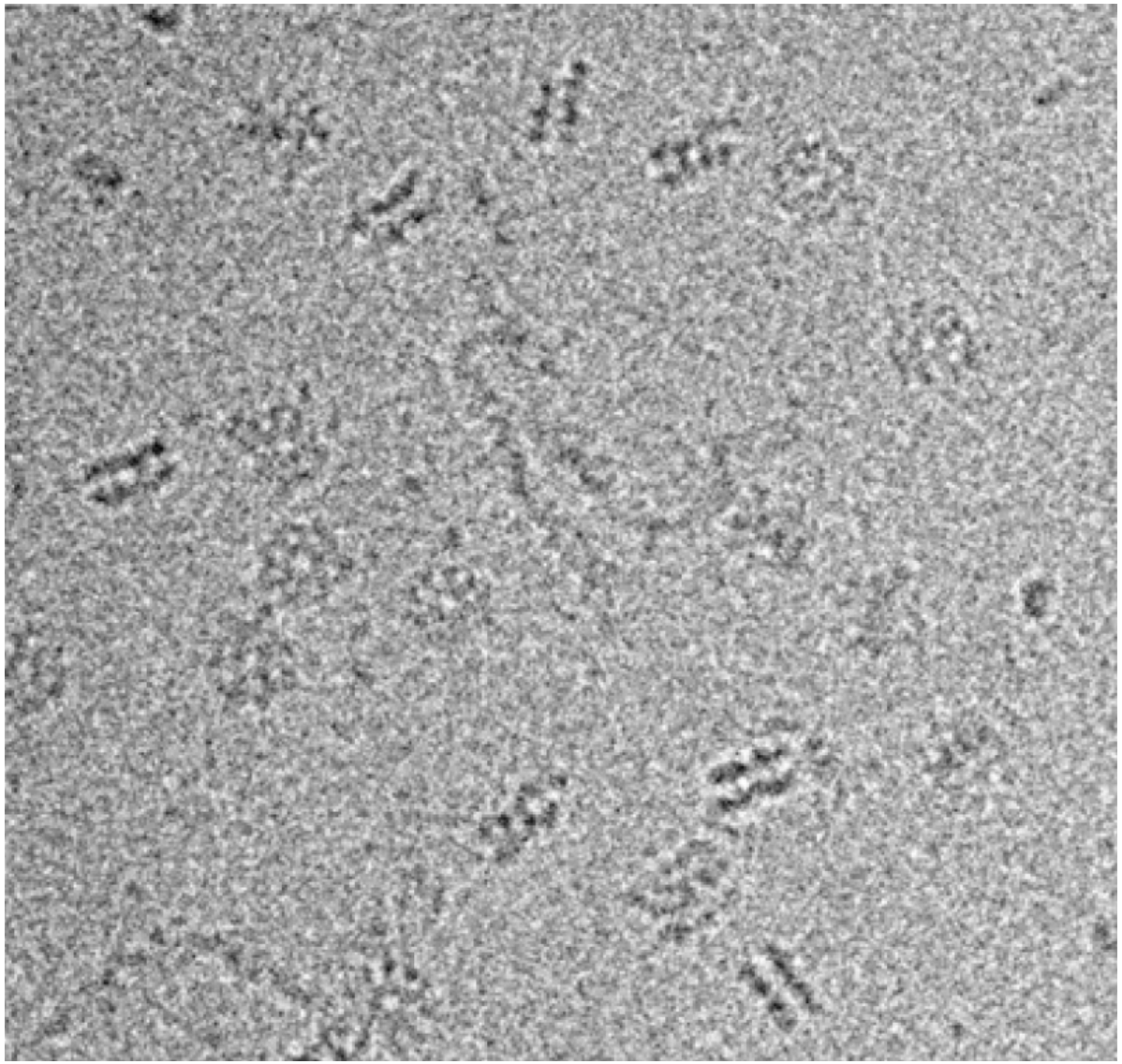
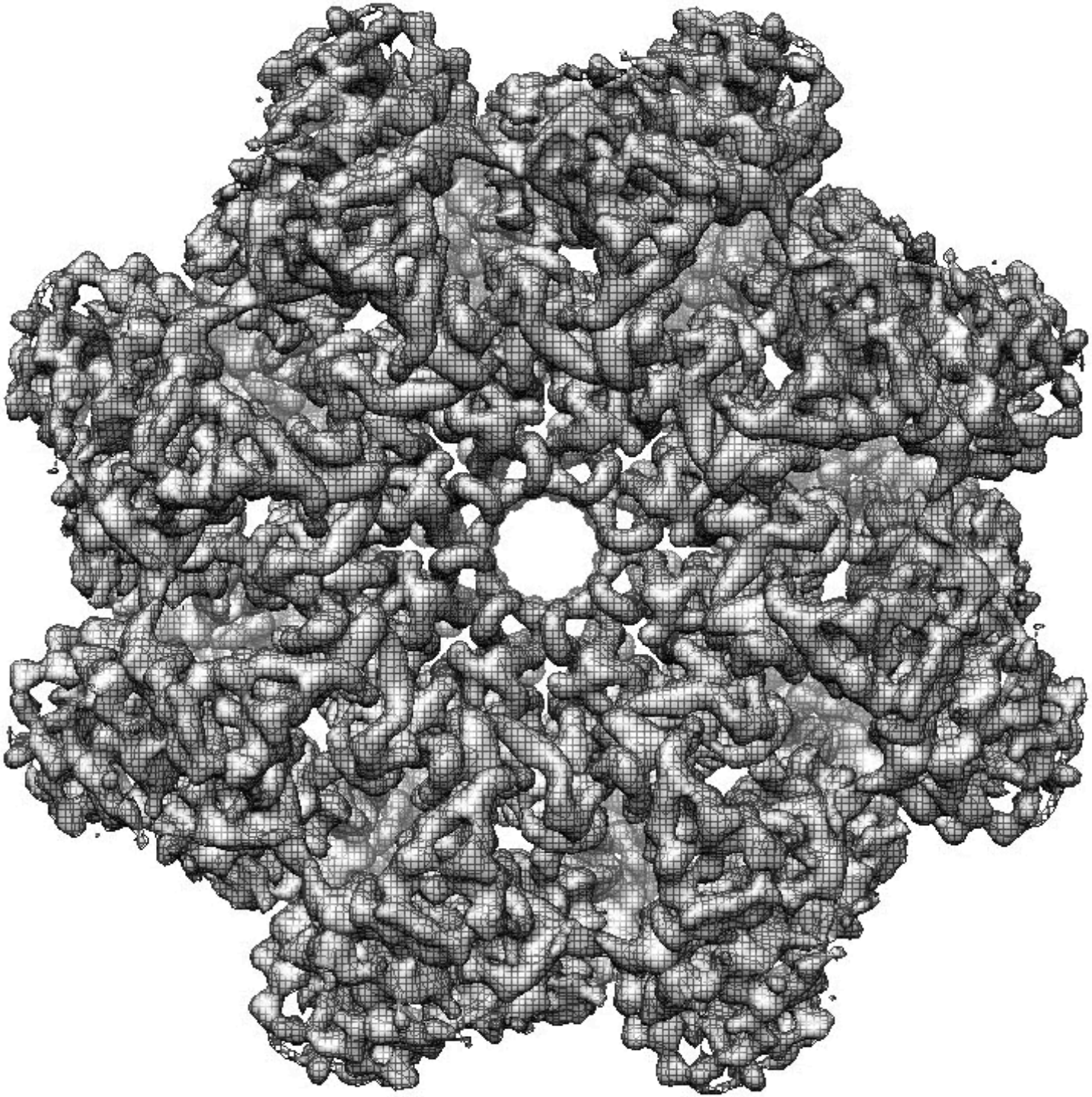
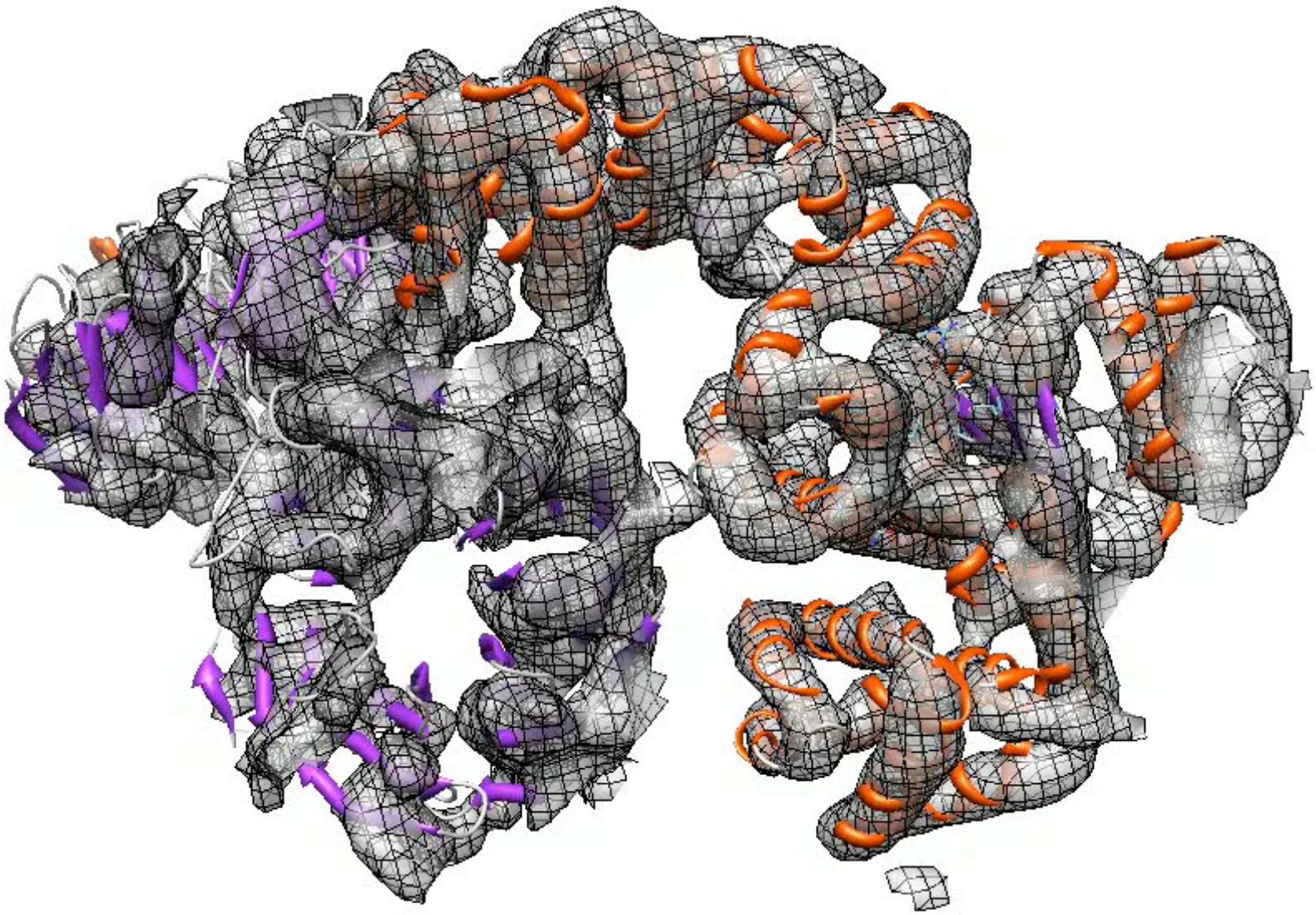
of Medicine

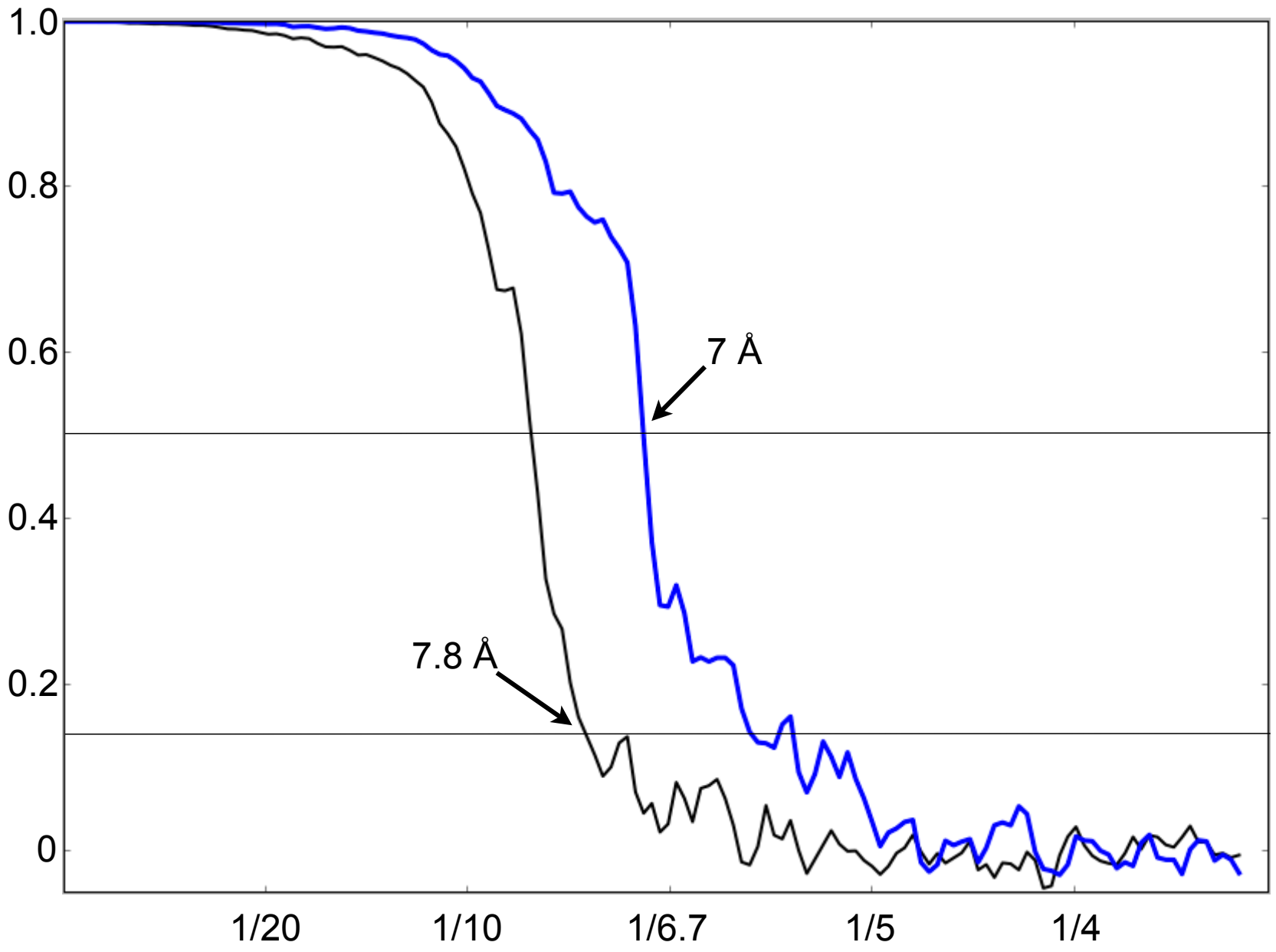
Research supported by NIHGMS



- Tecnai F20
- 160 kV
- 4k Tietz CCD
- 1.7 Å/pix
- D8 Symmetry
- ~48,000 particles
- ~2.5 MDa
- Homology Modeling with MODELLER






1.0
0.8
0.6
0.4
0.2
0
7 Å
7.8 Å
1/20
1/10
1/6.7
1/5
1/4

# IP3R Acknowledgements


**UTHSC**
**Medical School**

**Serysheva Lab**

**Olga Popova**

**Thao Tran**

**Tri Le**

**Que Ngo**

**Penczek Lab**

**Zhengfan Yang**

**NCMI**
**Baylor College of Medicine**

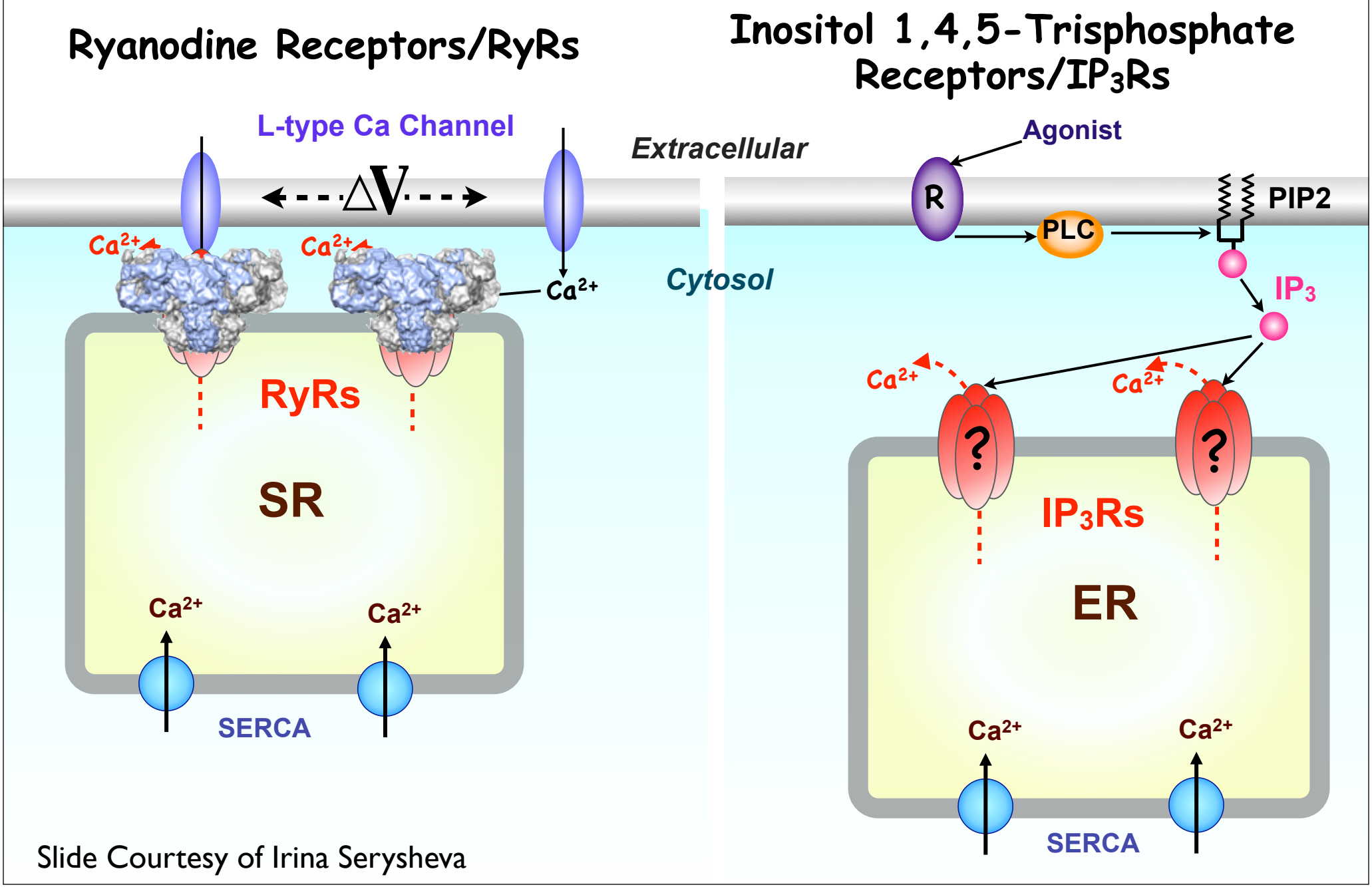
**Ludtke Lab**

**John Flanagan**

**Chiu Lab**

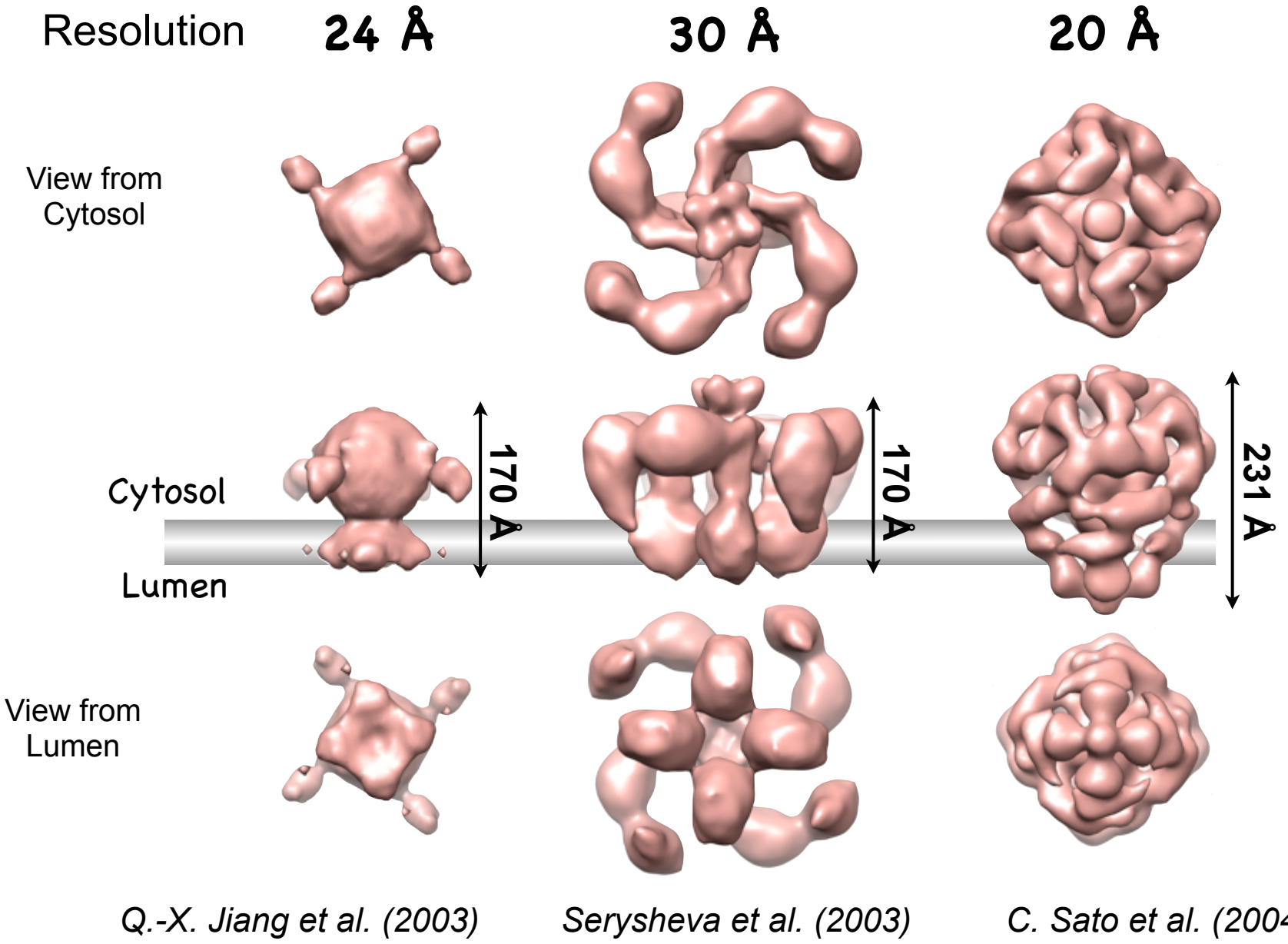
**Matthew Baker**

# Two Families of $Ca^{2+}$ Release Channels

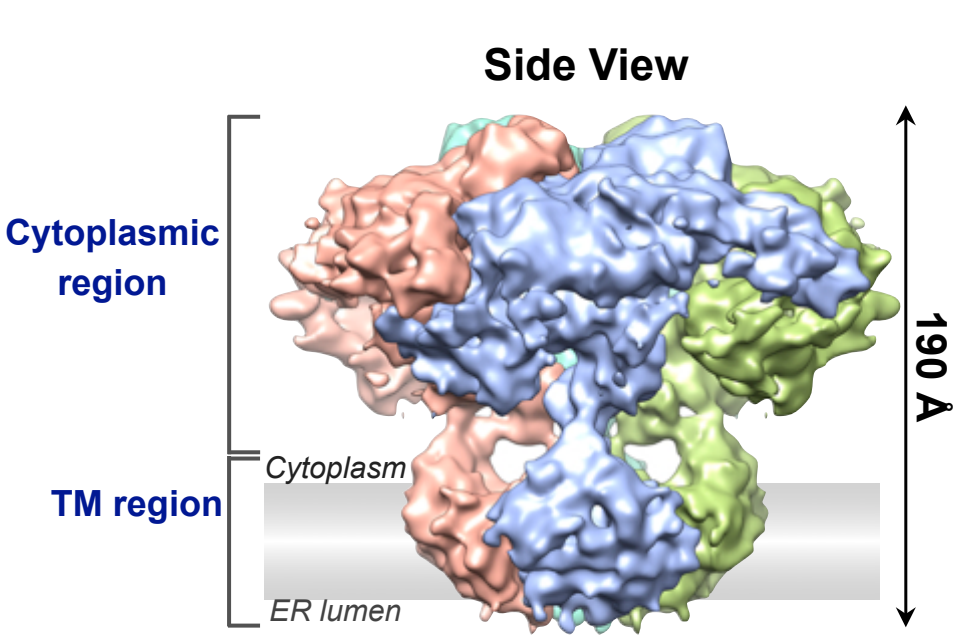


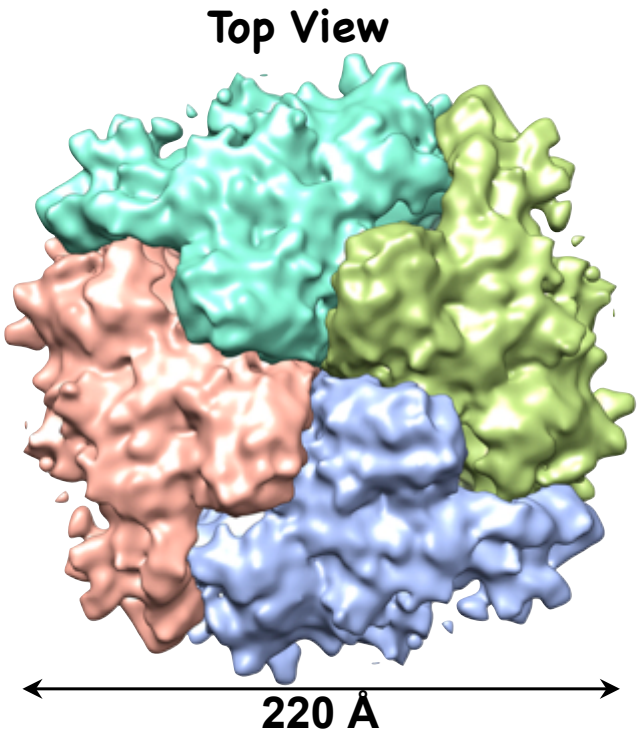

Slide Courtesy of Irina Serysheva

# Cryo-EM Maps of $IP_3R$

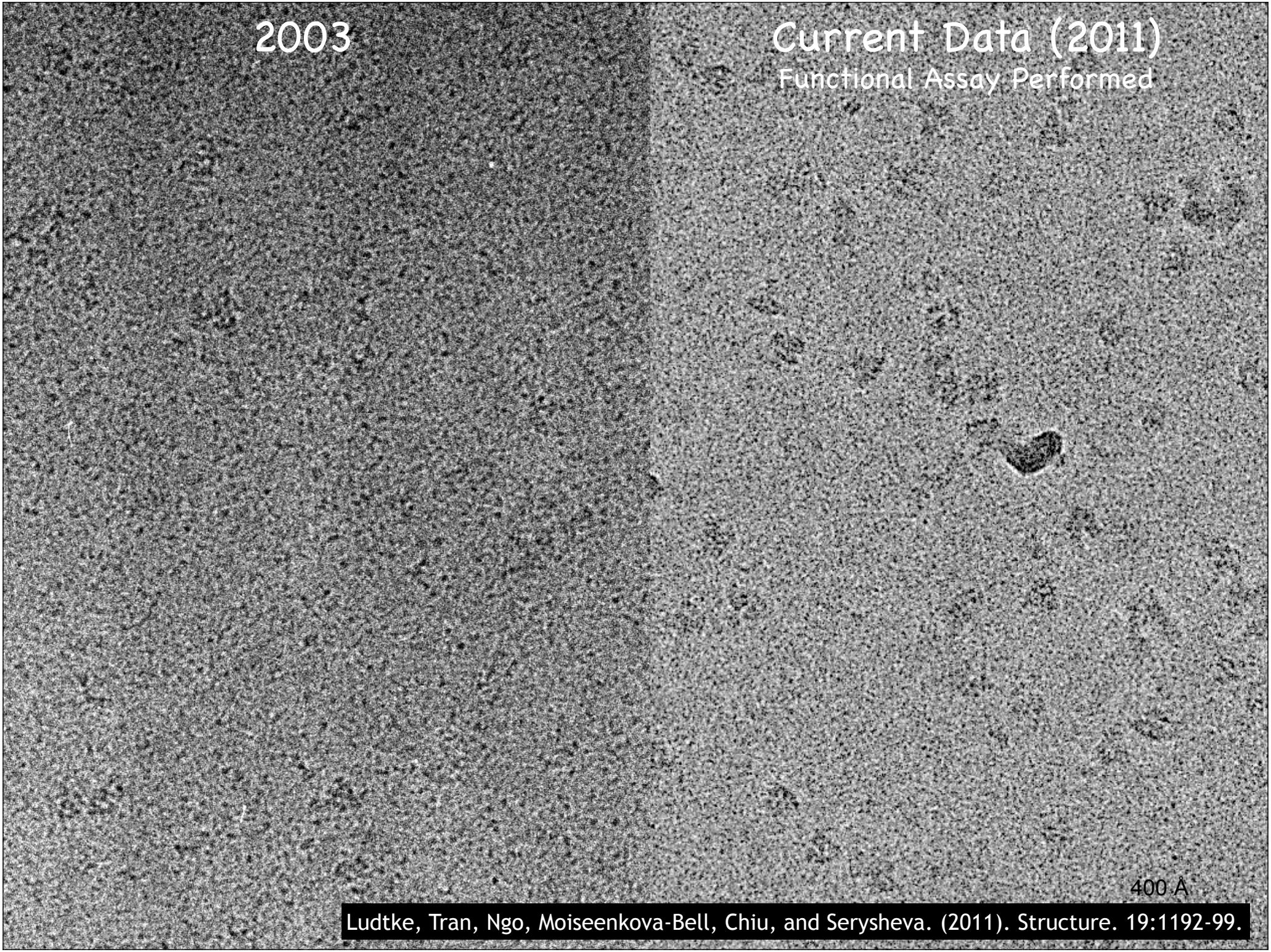


# The New Closed State $IP_3R$ Map

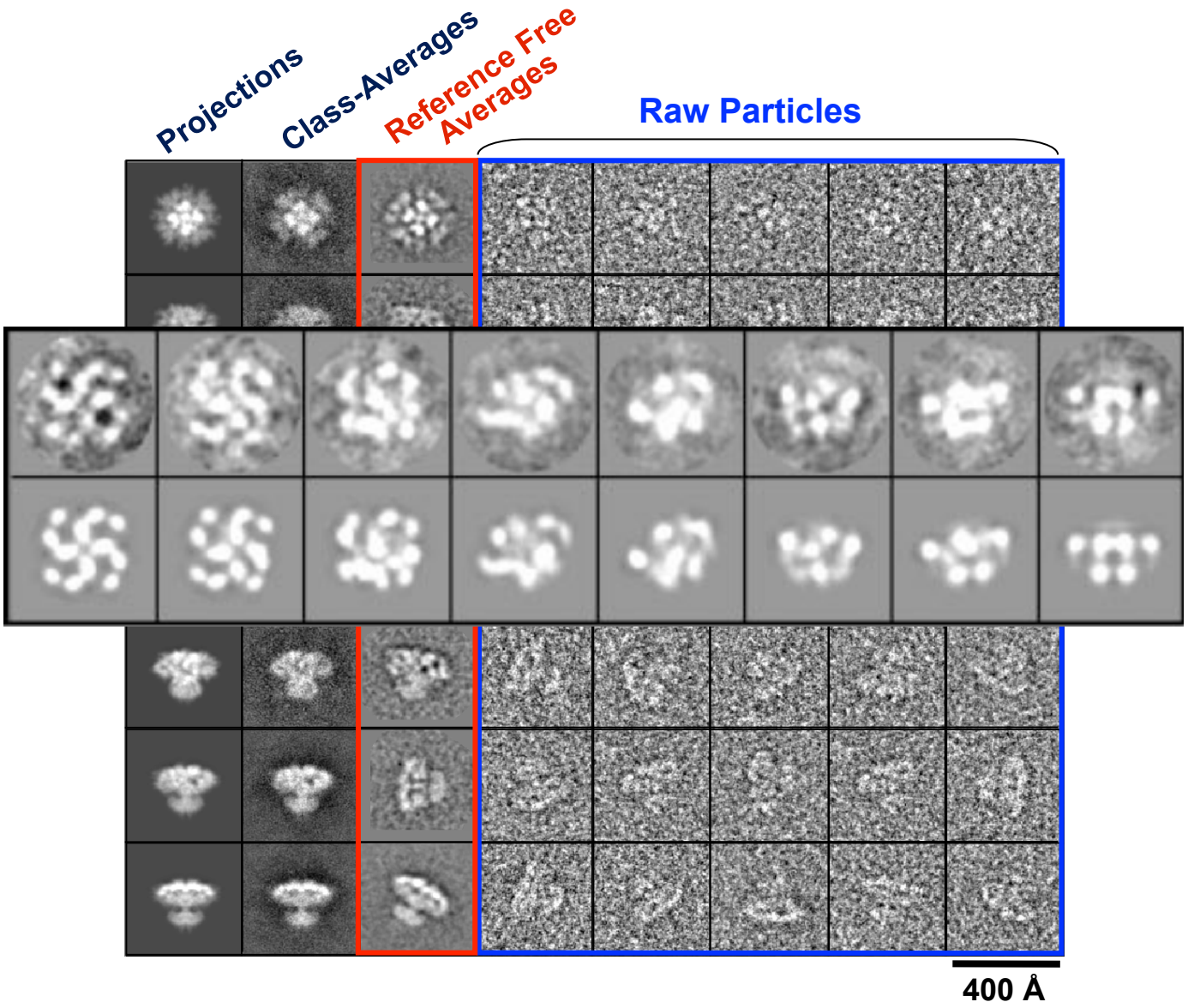



Ludtke, Tran, Ngo, Moiseenkova-Bell, Chiu, and Serysheva. (2011). Structure. 19:1192-99.


2003
Current Data (2011)
Functional Assay Performed
400 Å
Ludtke, Tran, Ngo, Moiseenkova-Bell, Chiu, and Serysheva. (2011). Structure. 19:1192-99.

# Self-consistency

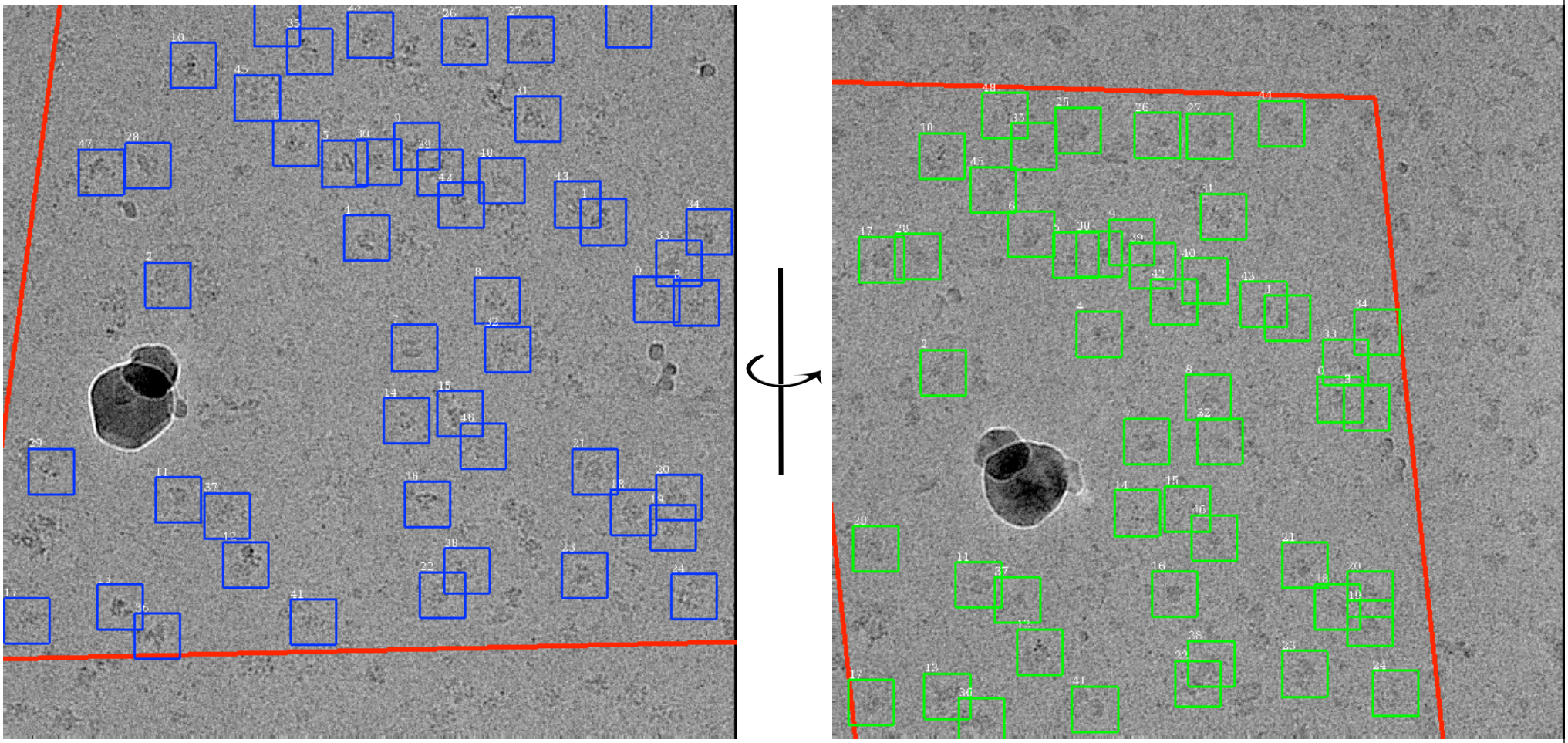



2003

# Validation

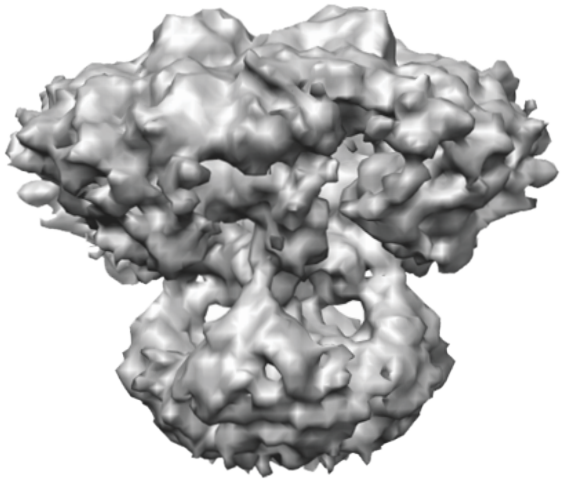
1. Random Conical Tilt
2. Tilt Validation
3. Different Software Packages
4. "Gold Standard" Resolution

# Method 1: Random-Conical Tilt



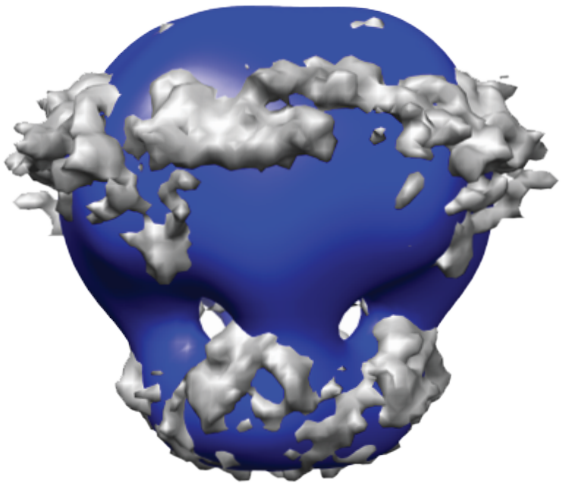
Radermacher, M., et.al. (1987). J Microsc.

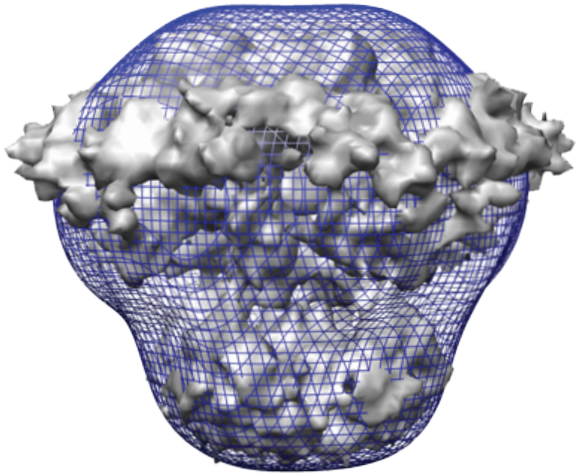
# Random-Conical Tilt



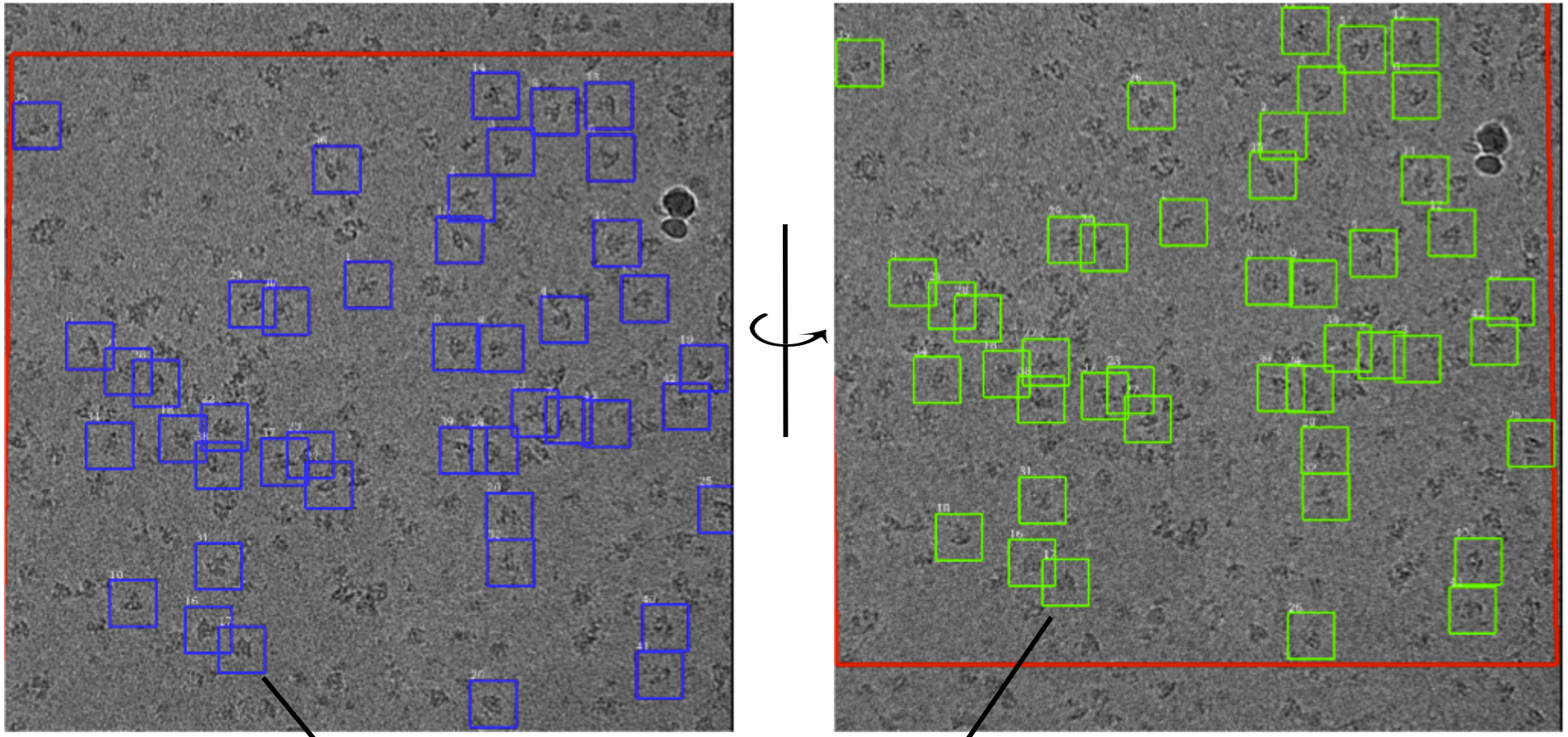
**RCT Reconstruction**

**50° tilt**





# Tilt Validation

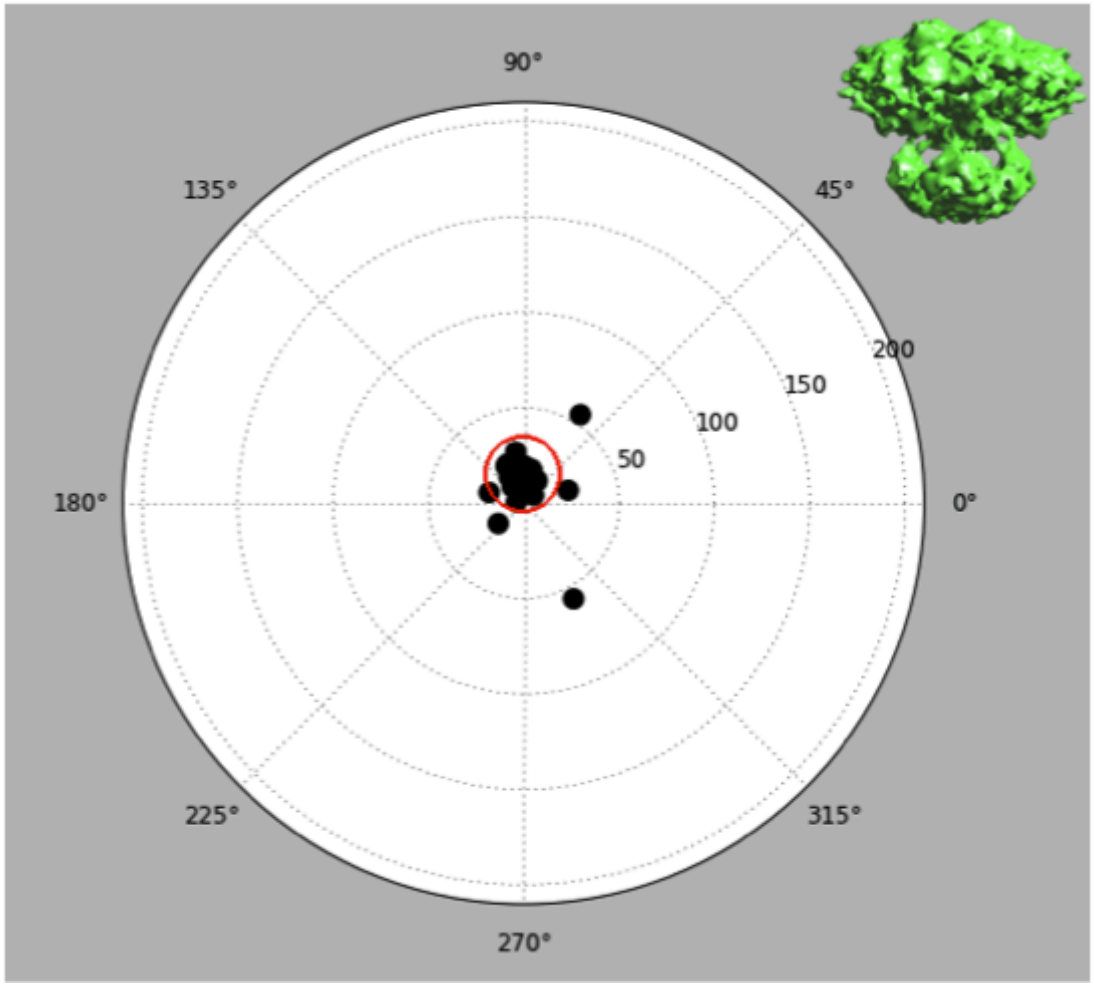


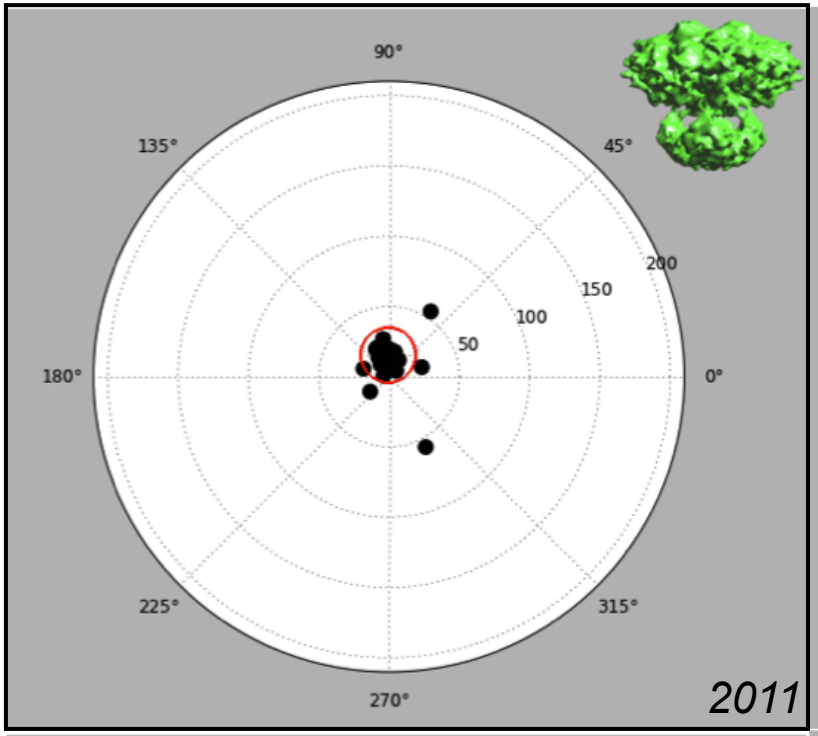
Find Orientation

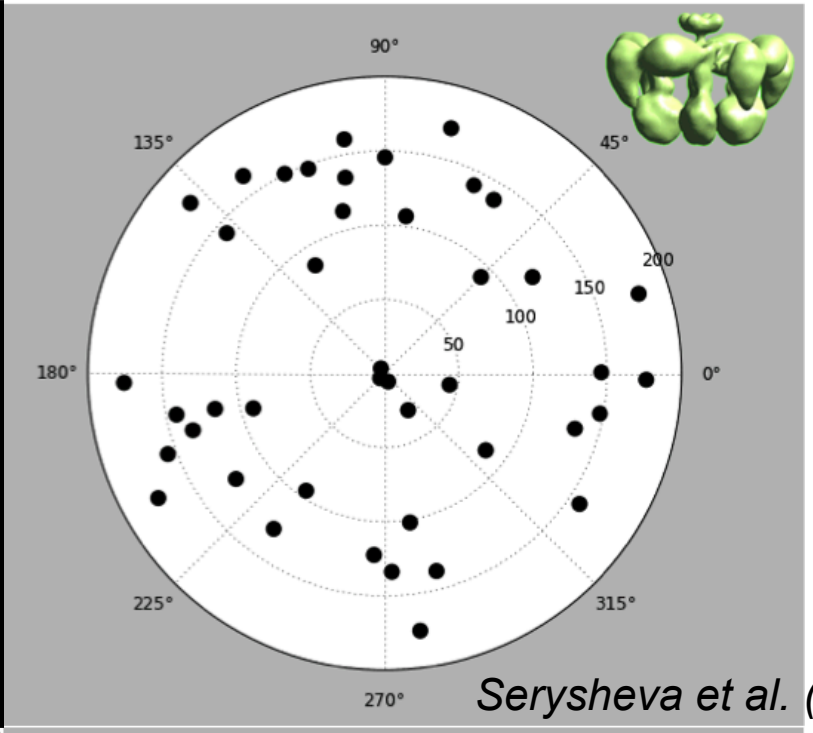
Find Orientation

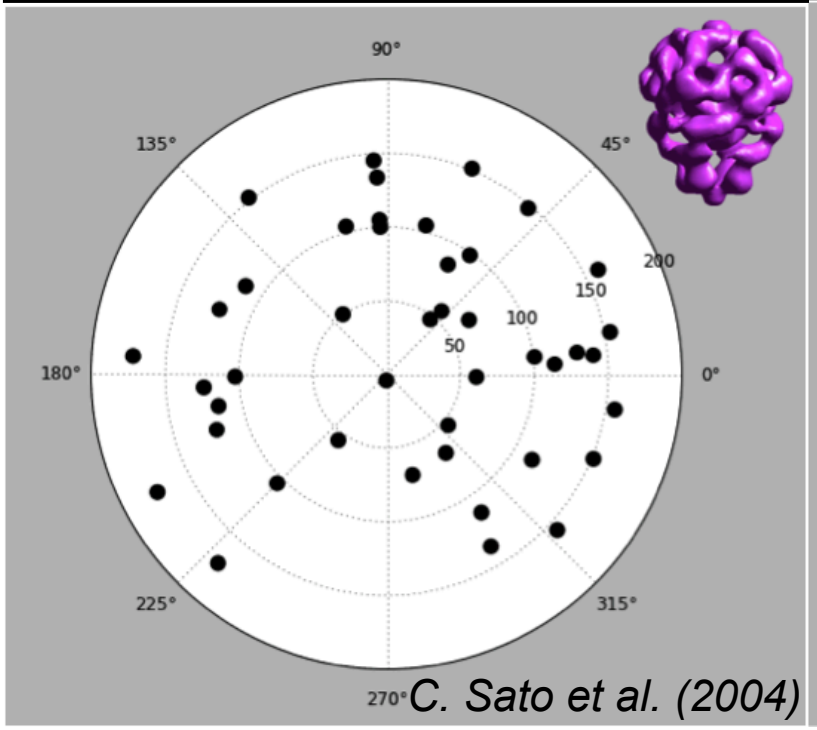
Compare to Known Tilt

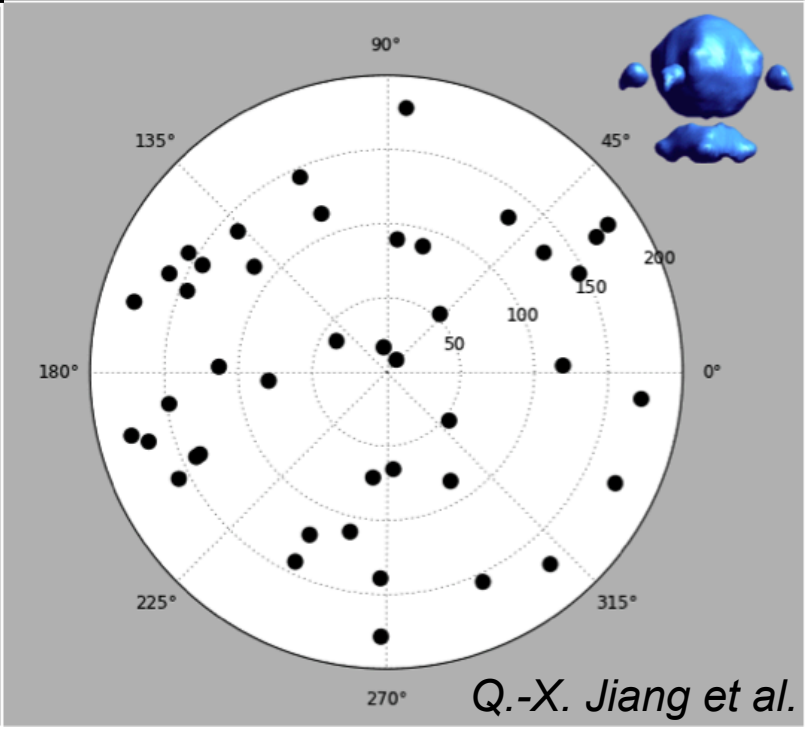
Method described in Rosenthal and Henderson, 2003 (JMB)


90°
135°
45°
180°
0°
225°
315°
270°
50
100
150
200


90°
135°
45°
180°
0°
225°
315°
270°
50
100
150
200
2011


90°
135°
45°
180°
0°
225°
315°
270°
50
100
150
200
Serysheva et al. (2003)

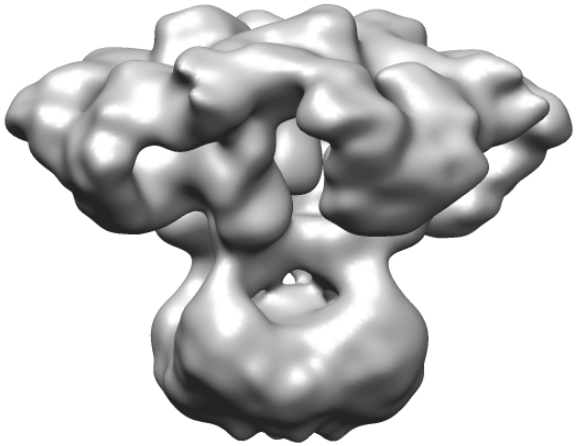

90°
135°
45°
180°
0°
225°
315°
270°
50
100
150
200
C. Sato et al. (2004)


90°
135°
45°
180°
0°
225°
315°
270°
50
100
150
200
Q.-X. Jiang et al. (2003)

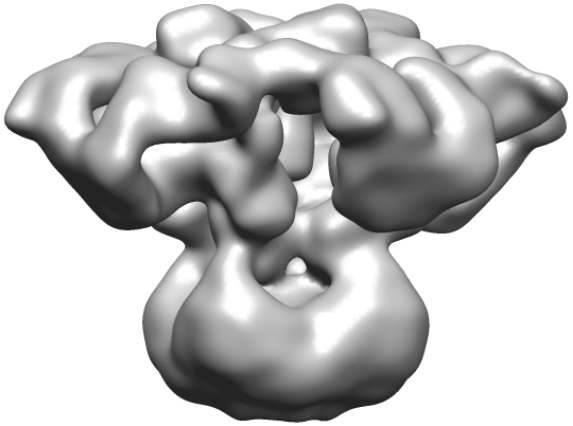
# Tilt Validation

- Confirms quaternary structure
- Rough assessment of particle orientation uncertainty
- Could be used as a tool for eliminating bad particles

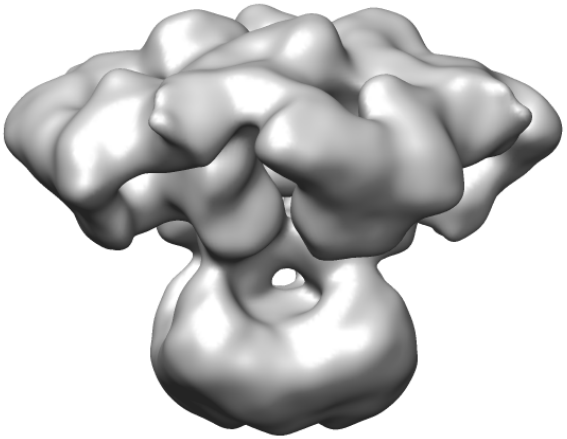
EMAN2



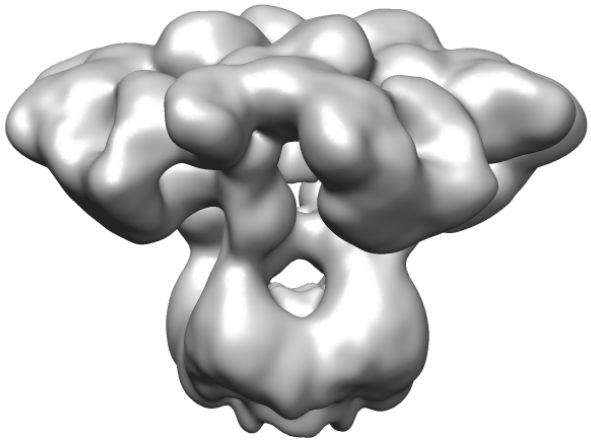
RELION

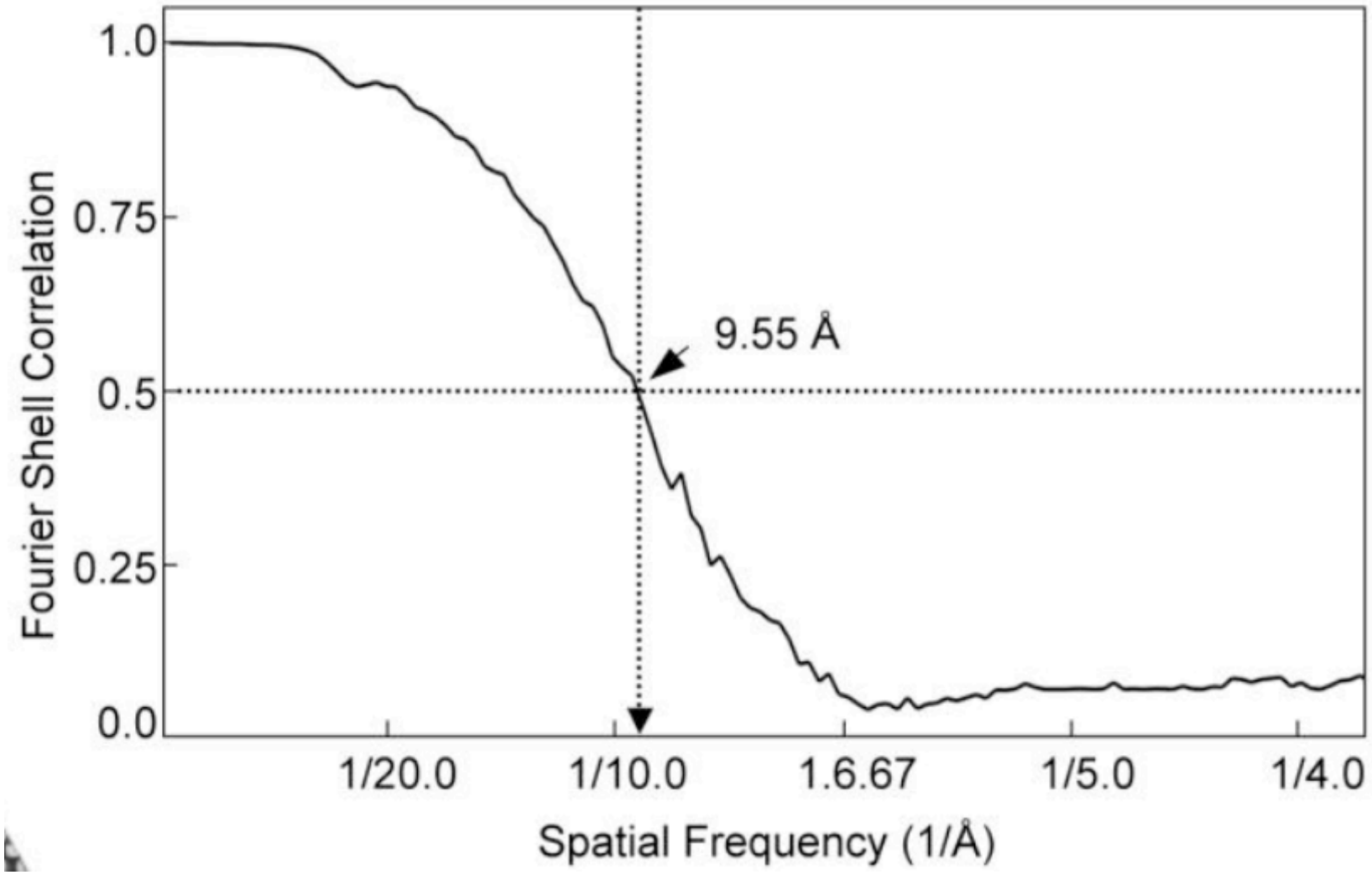


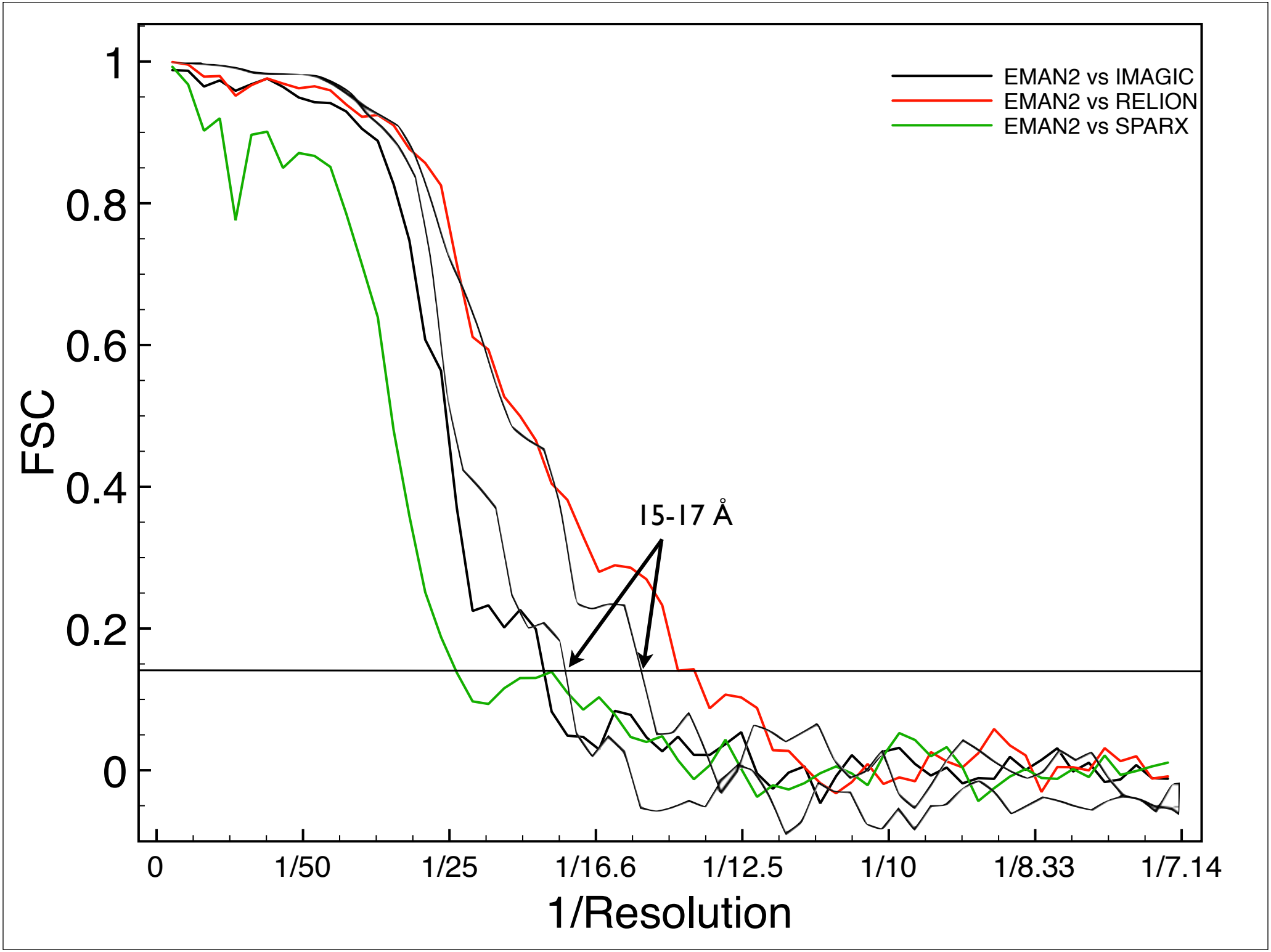
IMAGIC



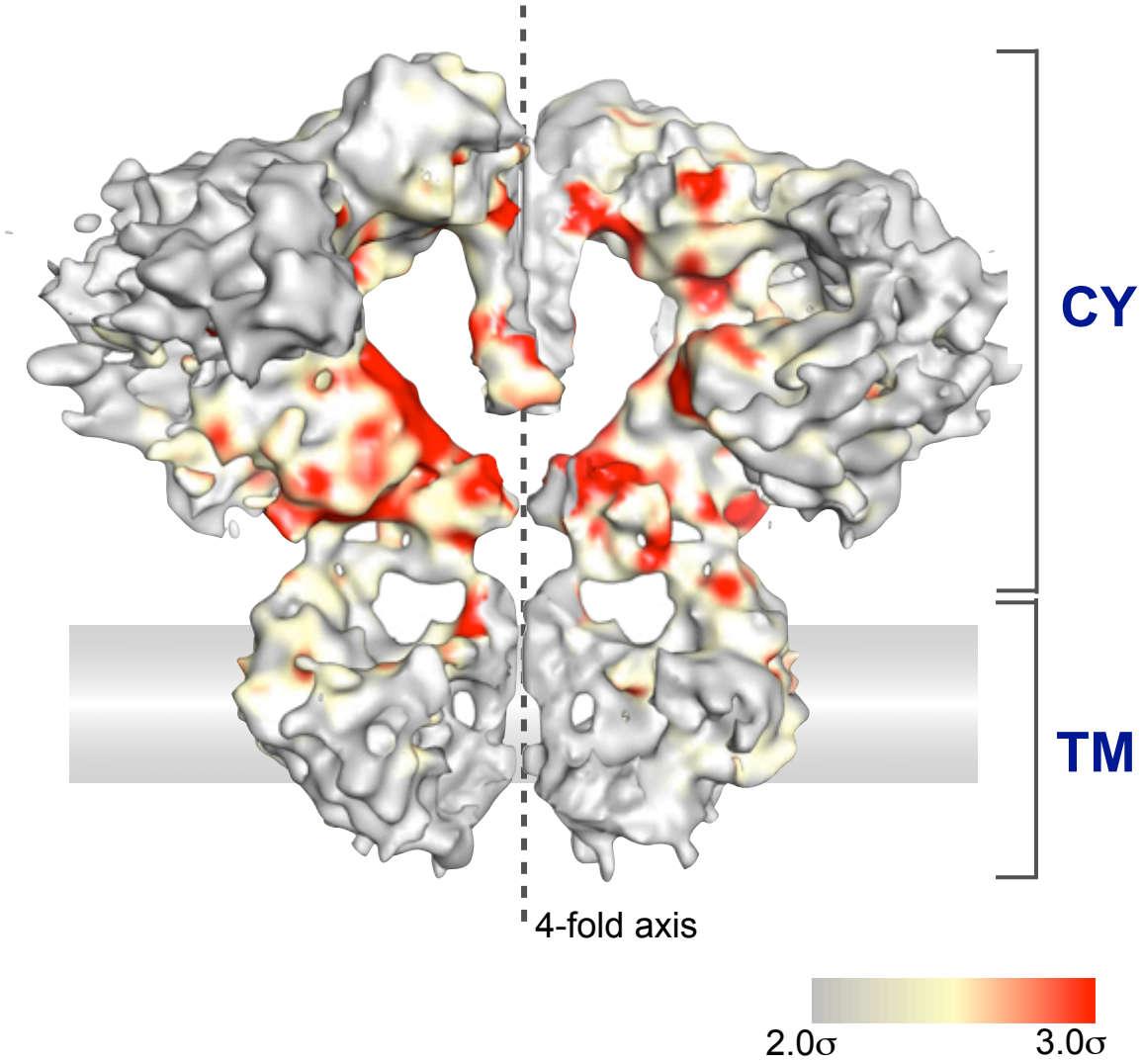
SPARX



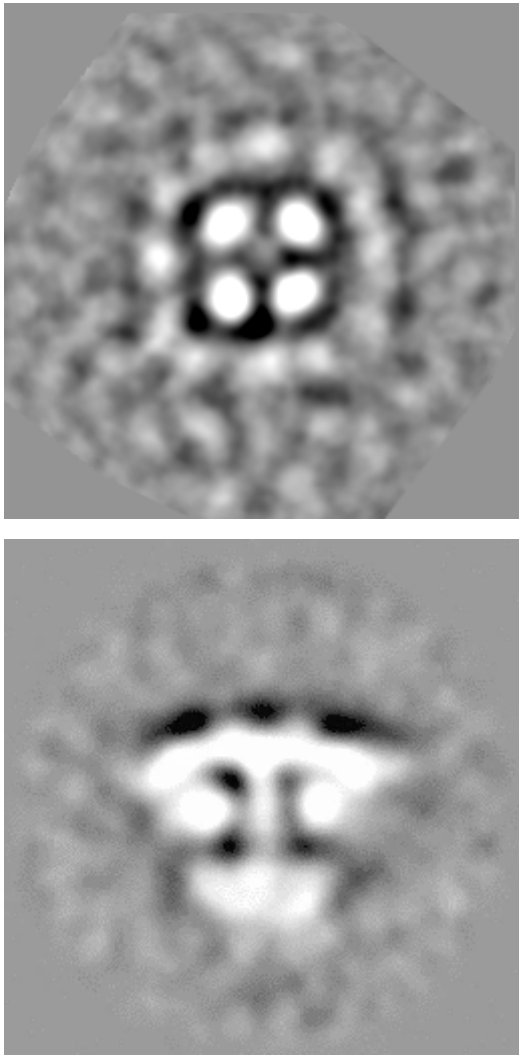

1.0
0.75
0.5
0.25
0.0
Fourier Shell Correlation
9.55 Å
1/20.0
1/10.0
1.6.67
1/5.0
1/4.0
Spatial Frequency (1/Å)


EMAN2 vs IMAGIC
EMAN2 vs RELION
EMAN2 vs SPARX
15-17 Å
FSC
1
0.8
0.6
0.4
0.2
0
0
1/50
1/25
1/16.6
1/12.5
1/10
1/8.33
1/7.14
1/Resolution

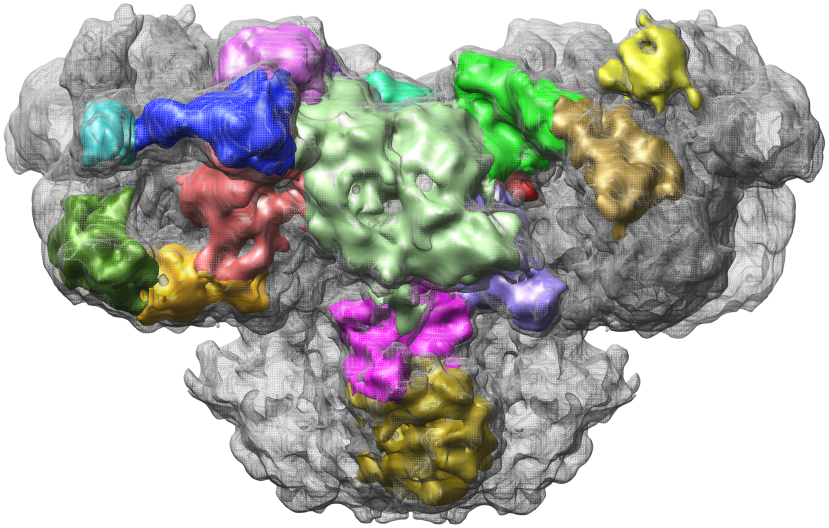
# 3-D Variance Map

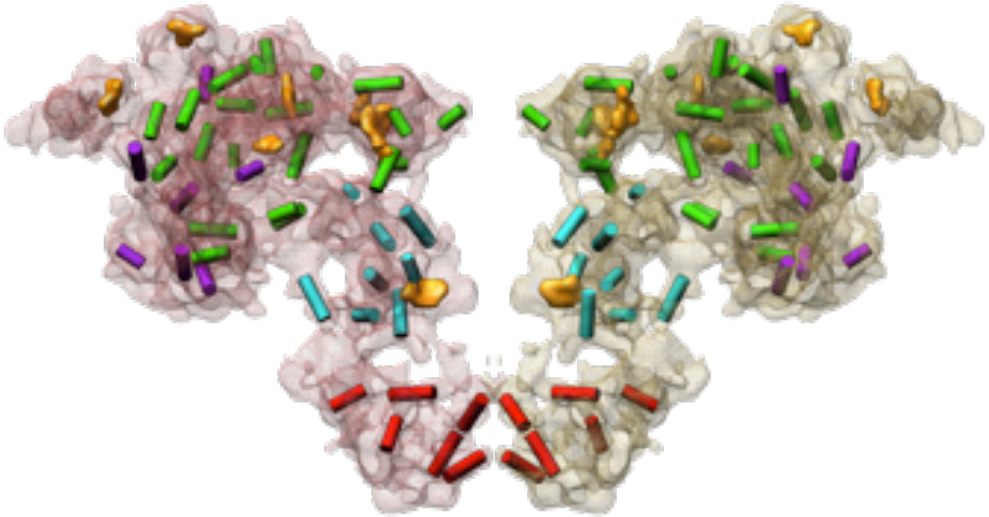


# $IP_3R$



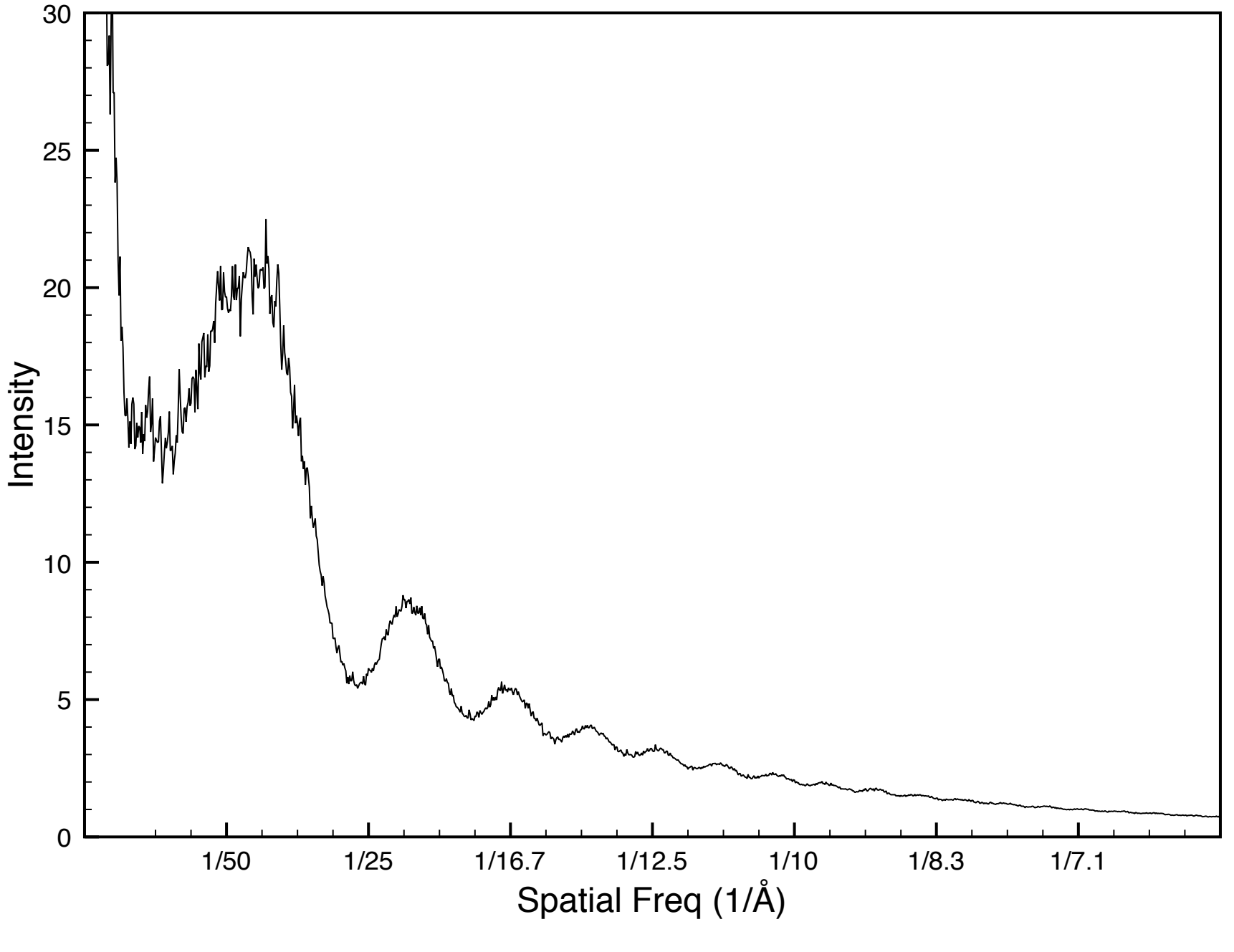
# $Ca^{2+}$ Release Channel



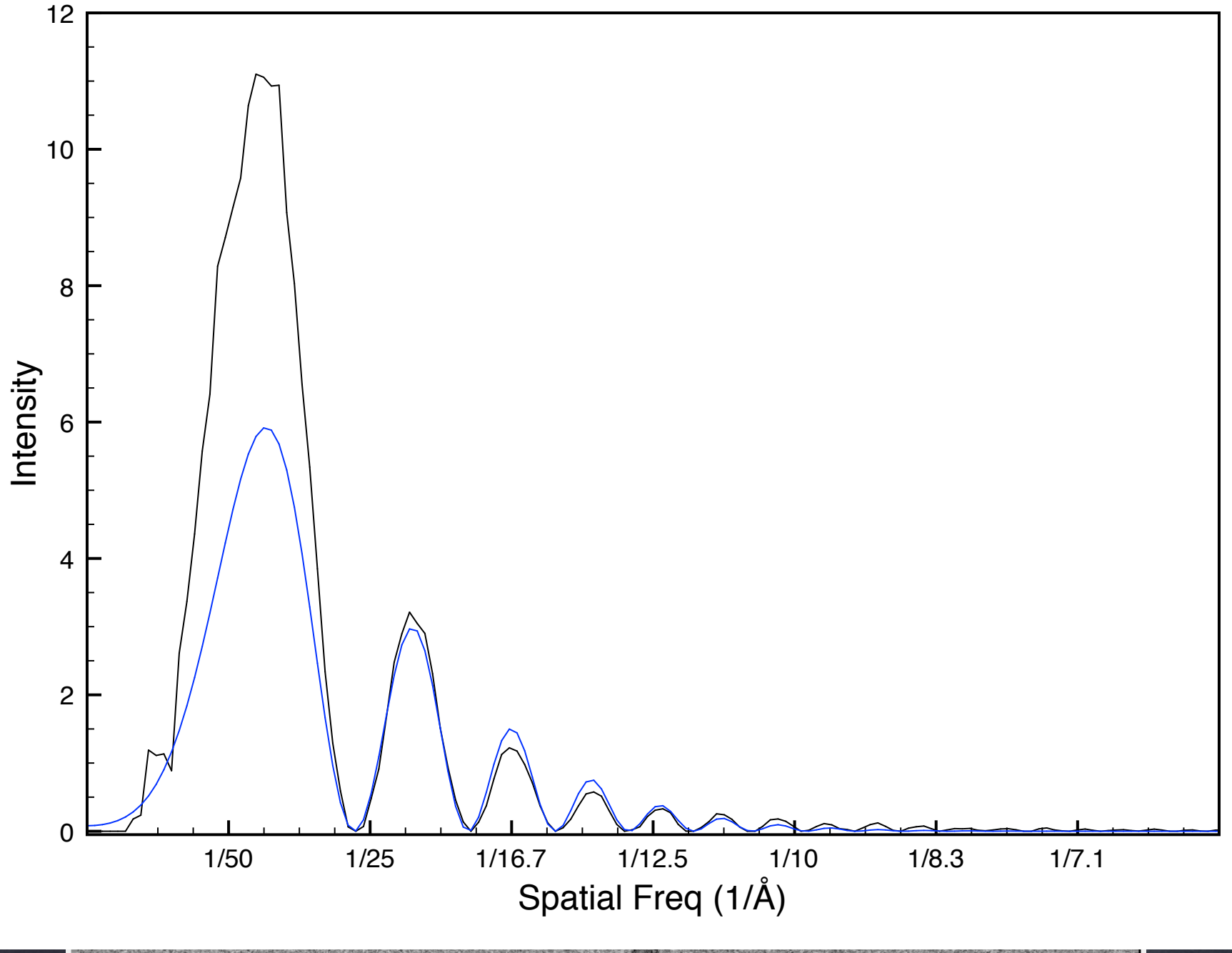


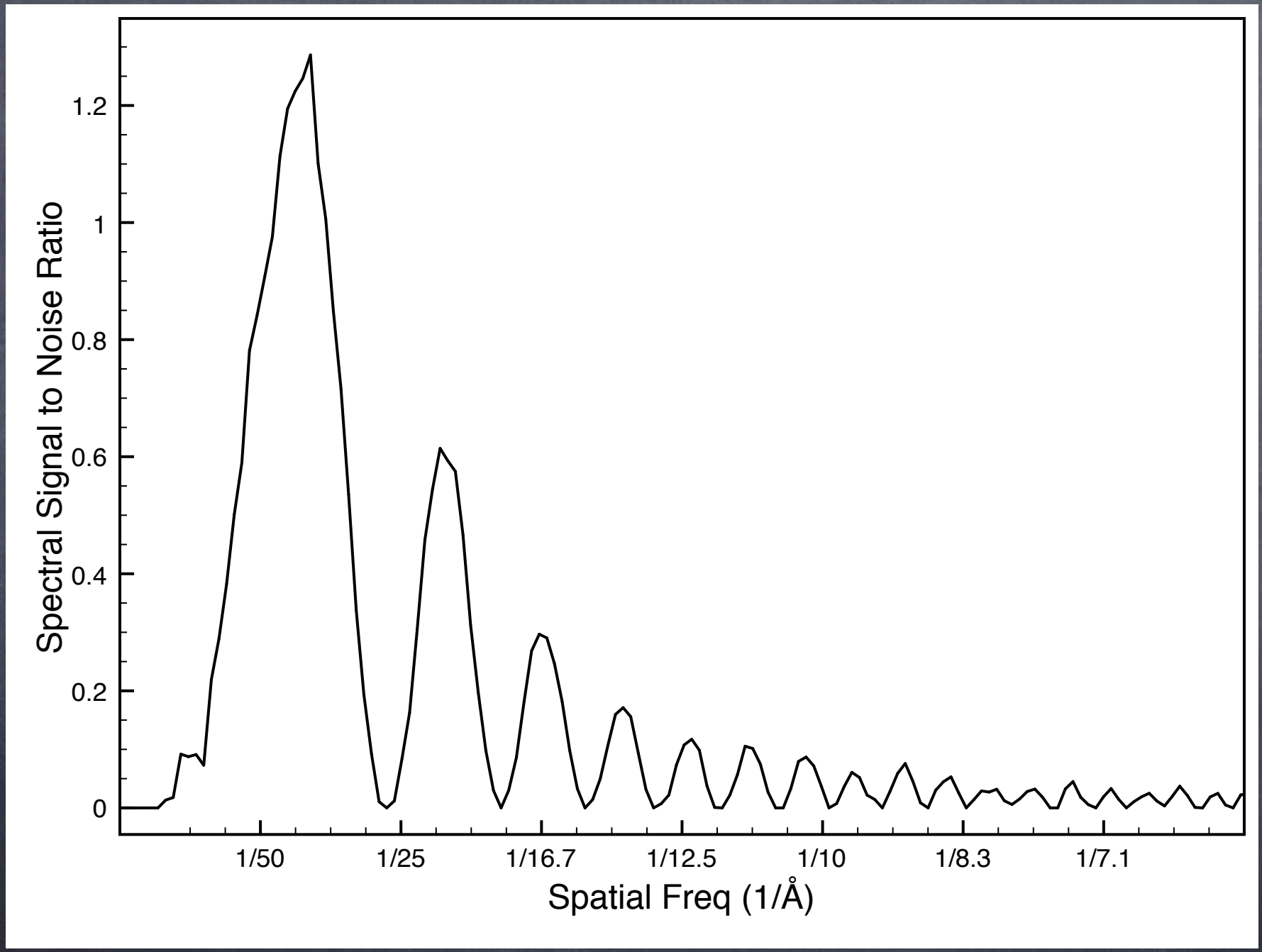
# Per-Particle SSNR

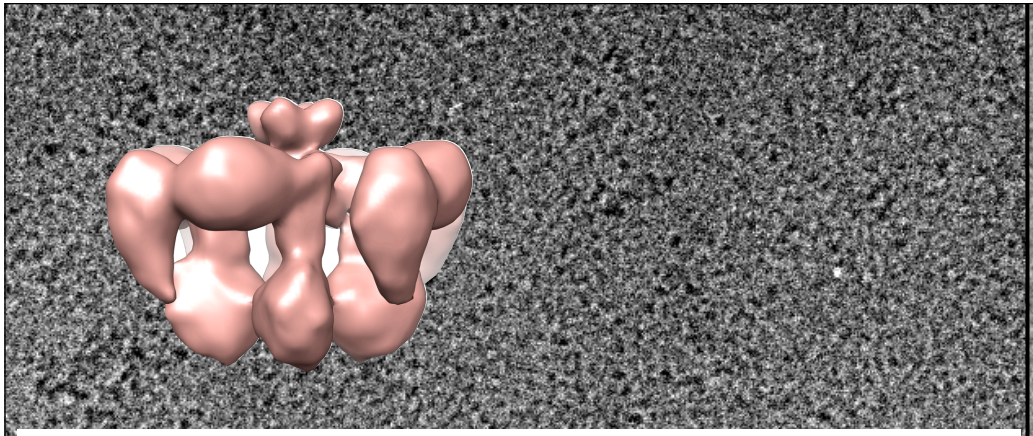
# Image Evaluation

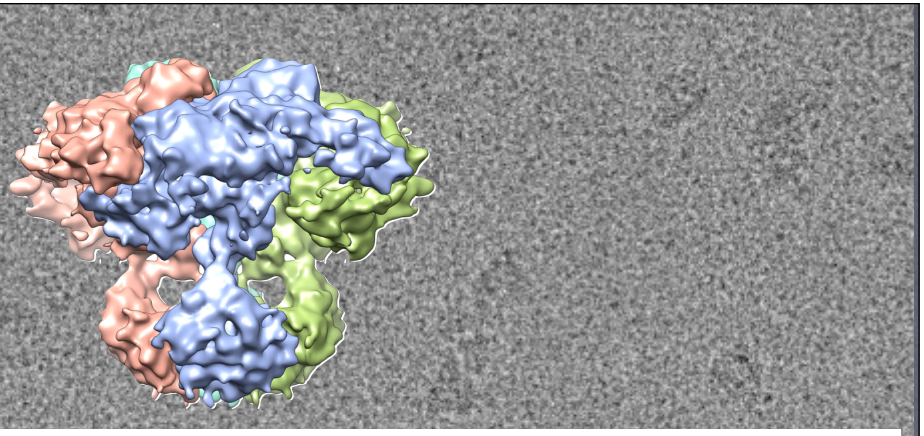


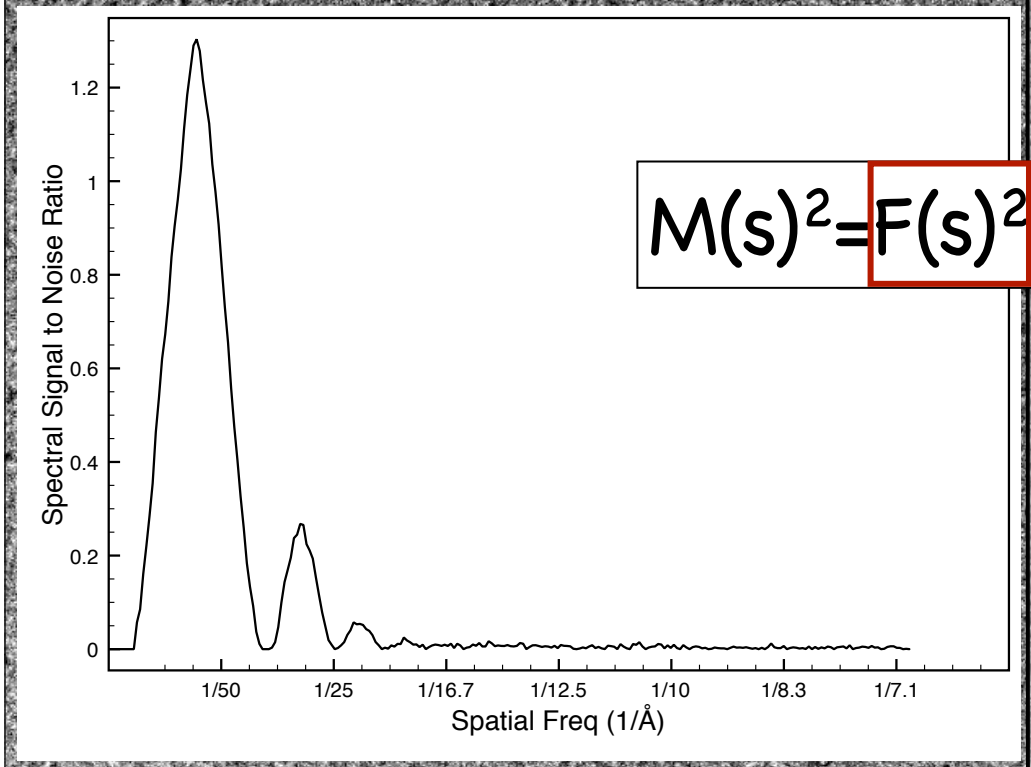
# Image Evaluation



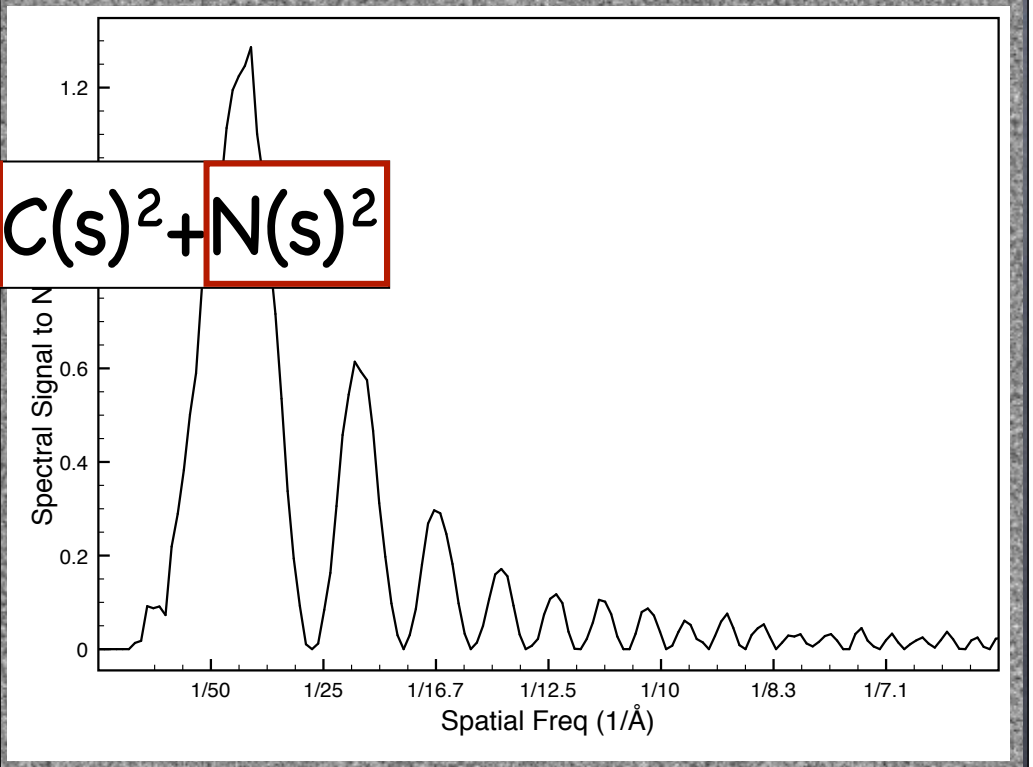

Spectral Signal to Noise Ratio
1.2
1
0.8
0.6
0.4
0.2
0
1/50
1/25
1/16.7
1/12.5
1/10
1/8.3
1/7.1
Spatial Freq (1/Å)

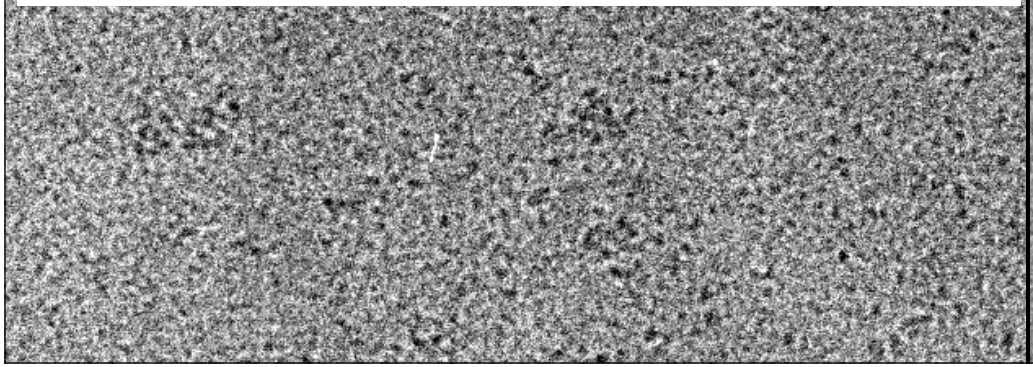


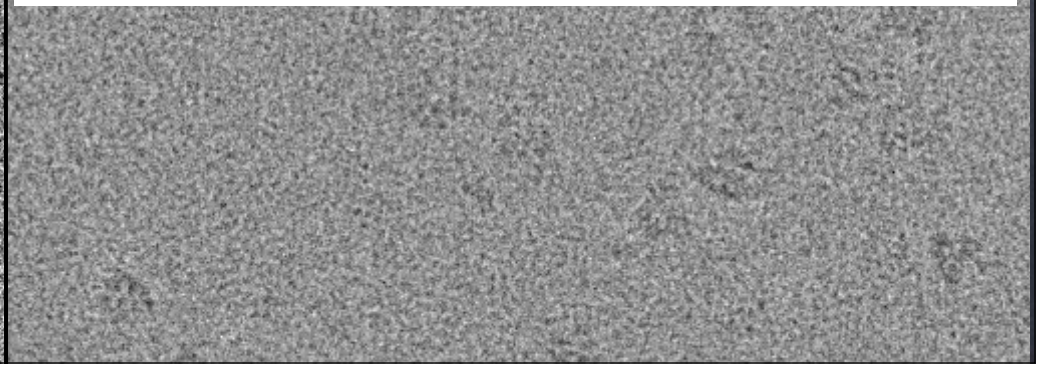




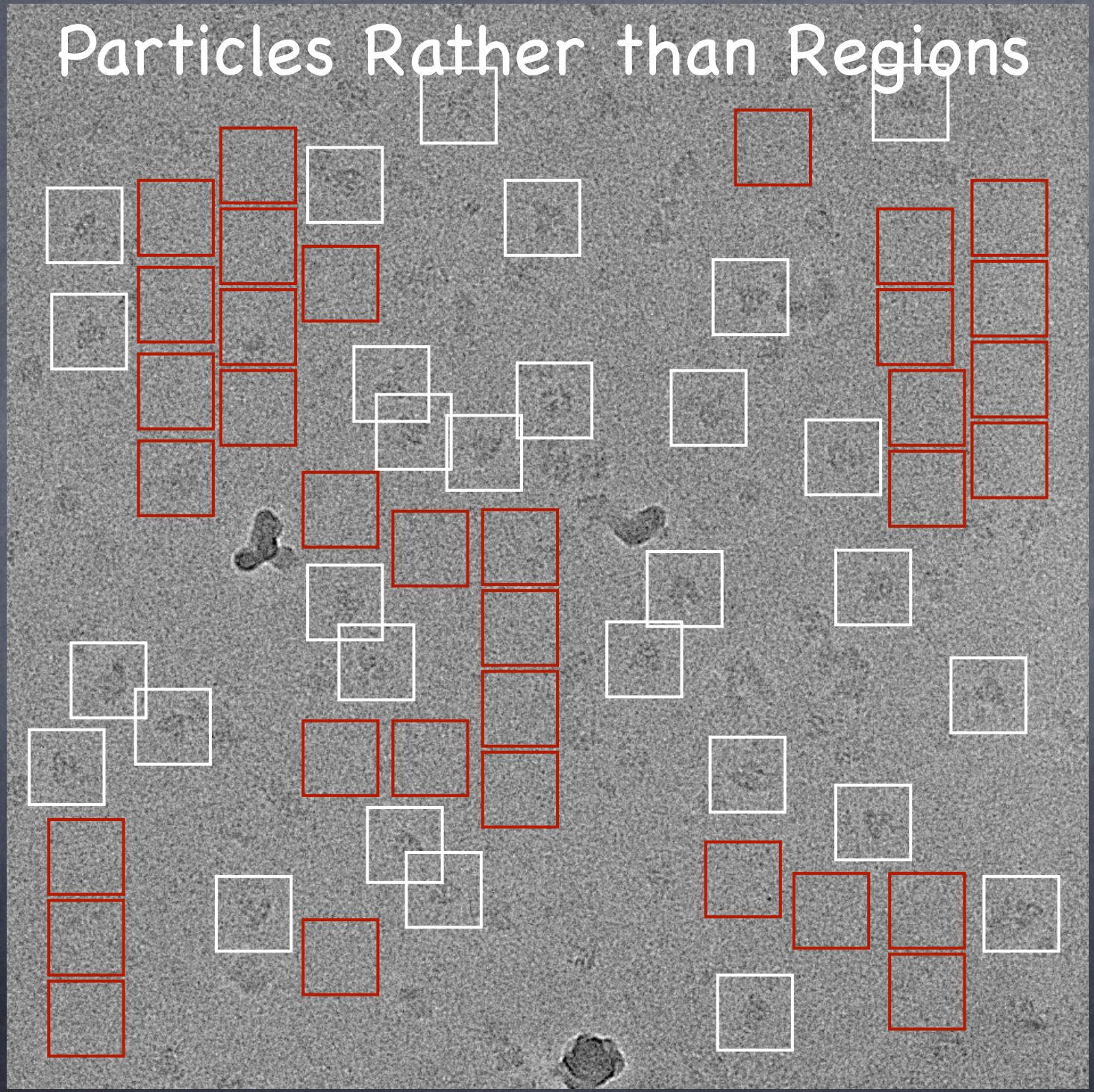

$$M(s)^2 = F(s)^2 C(s)^2 + N(s)^2$$



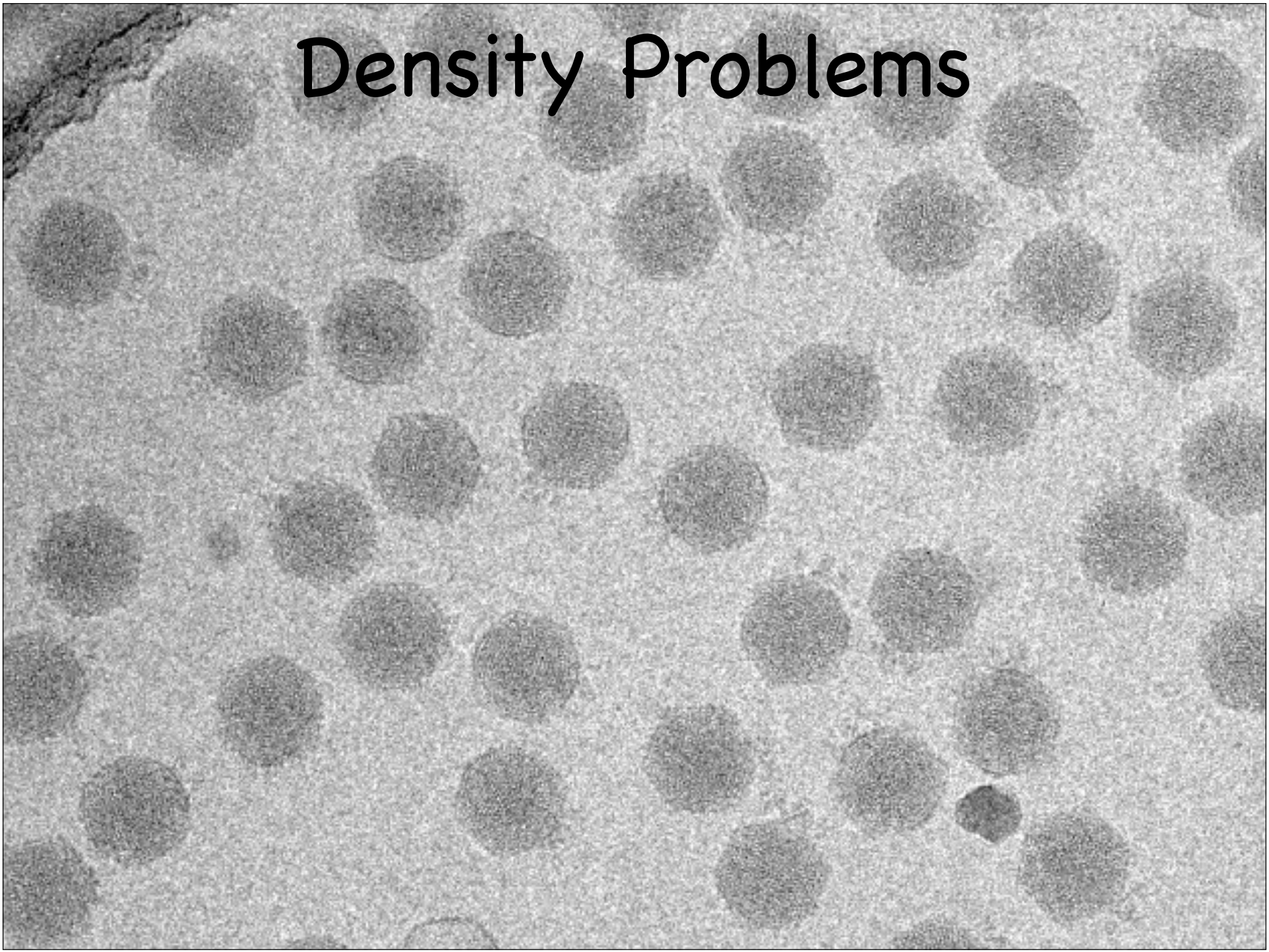





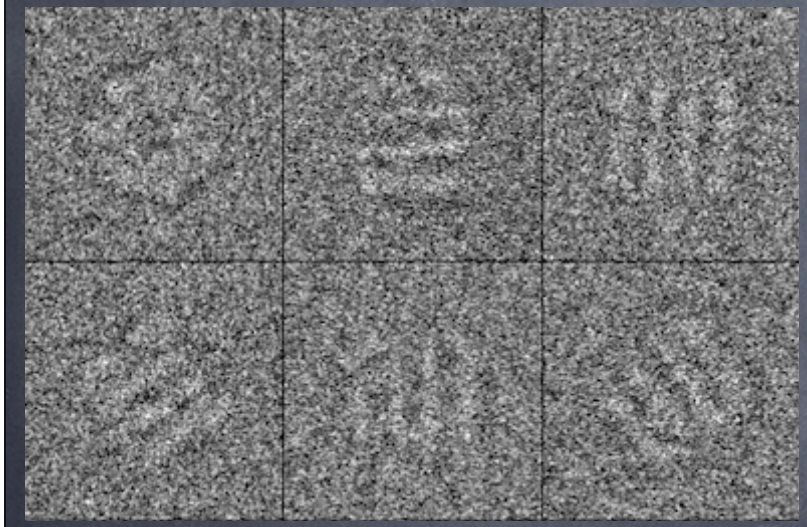
# Particles Rather than Regions

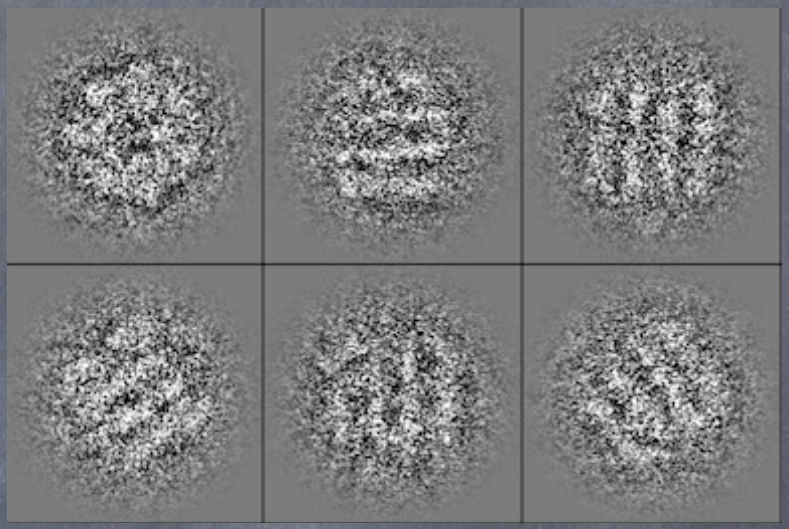


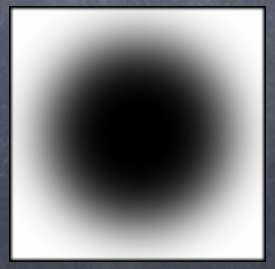
# Density Problems

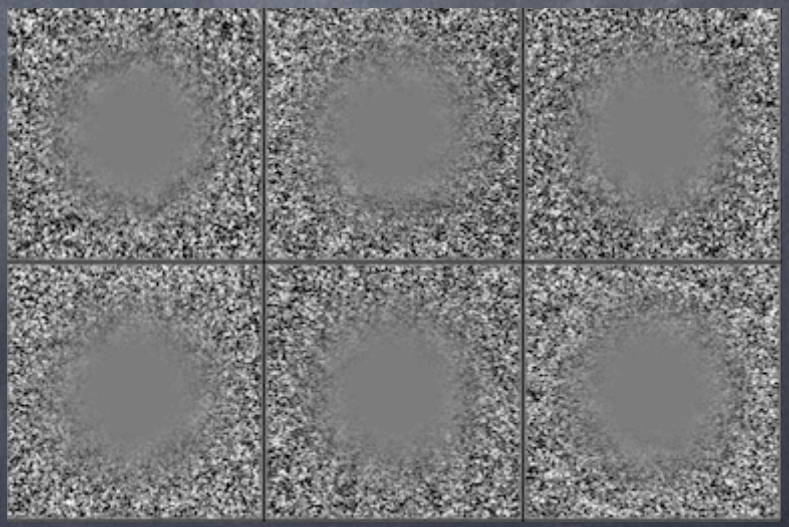


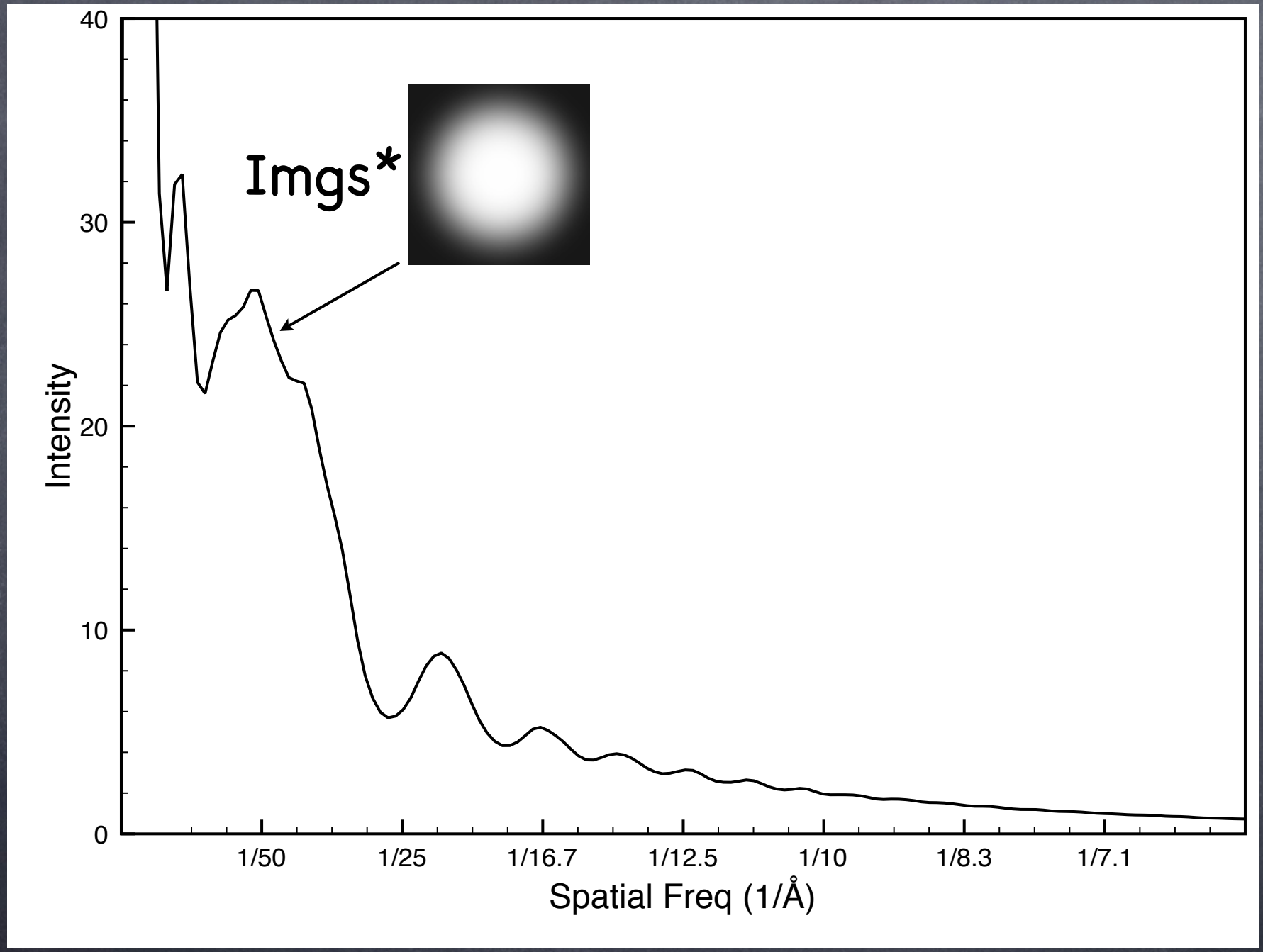
# Image Evaluation

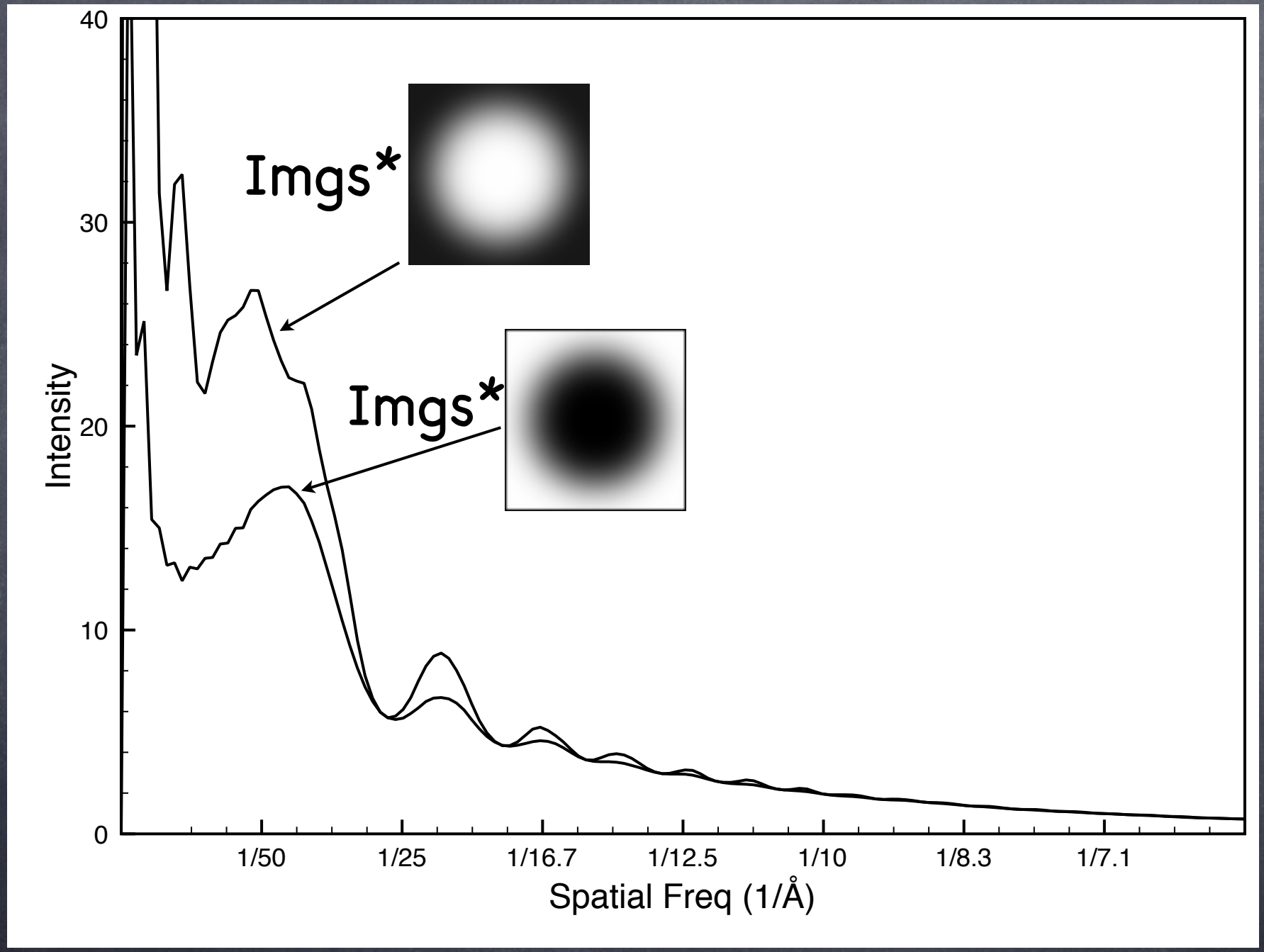


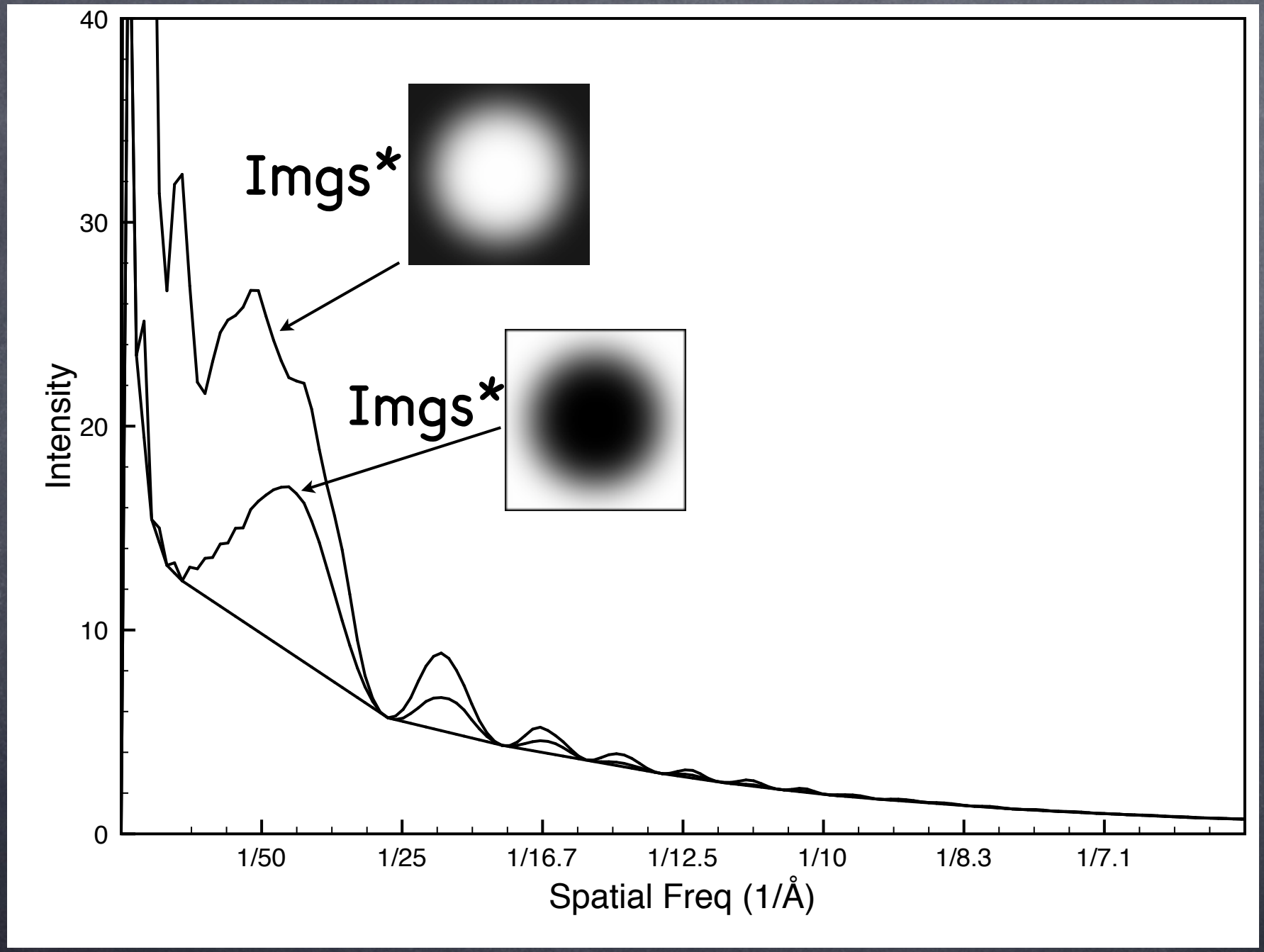


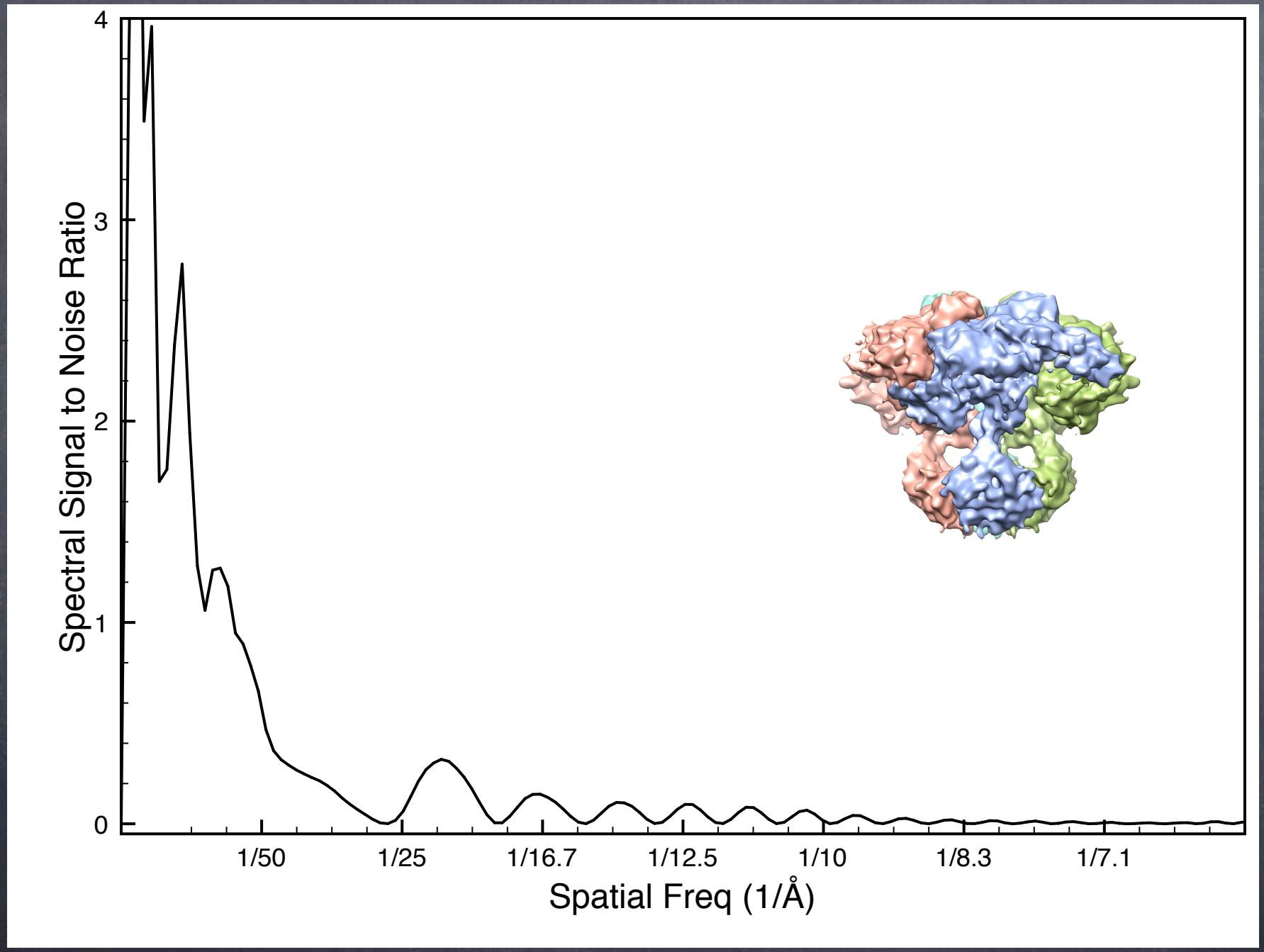


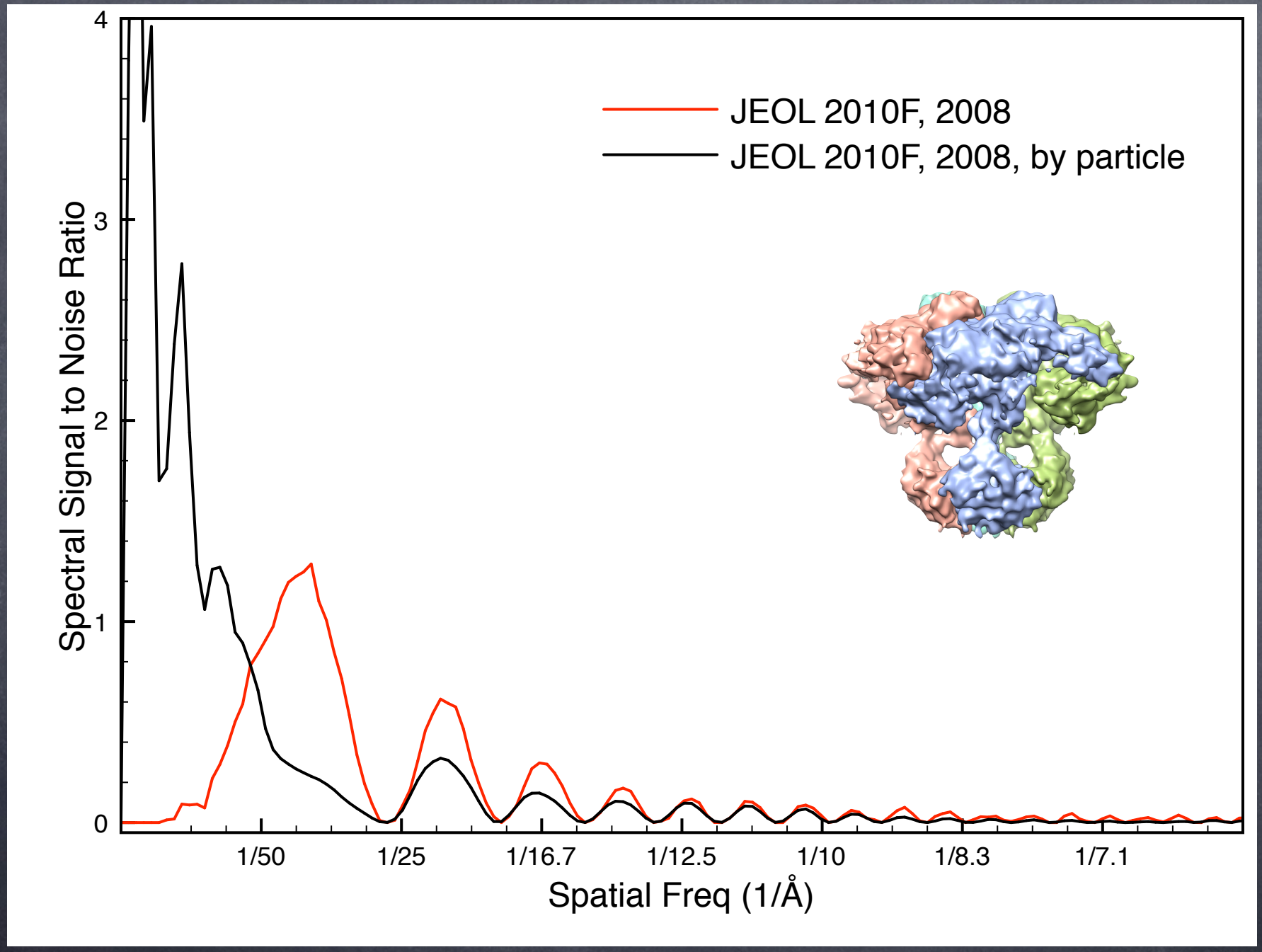


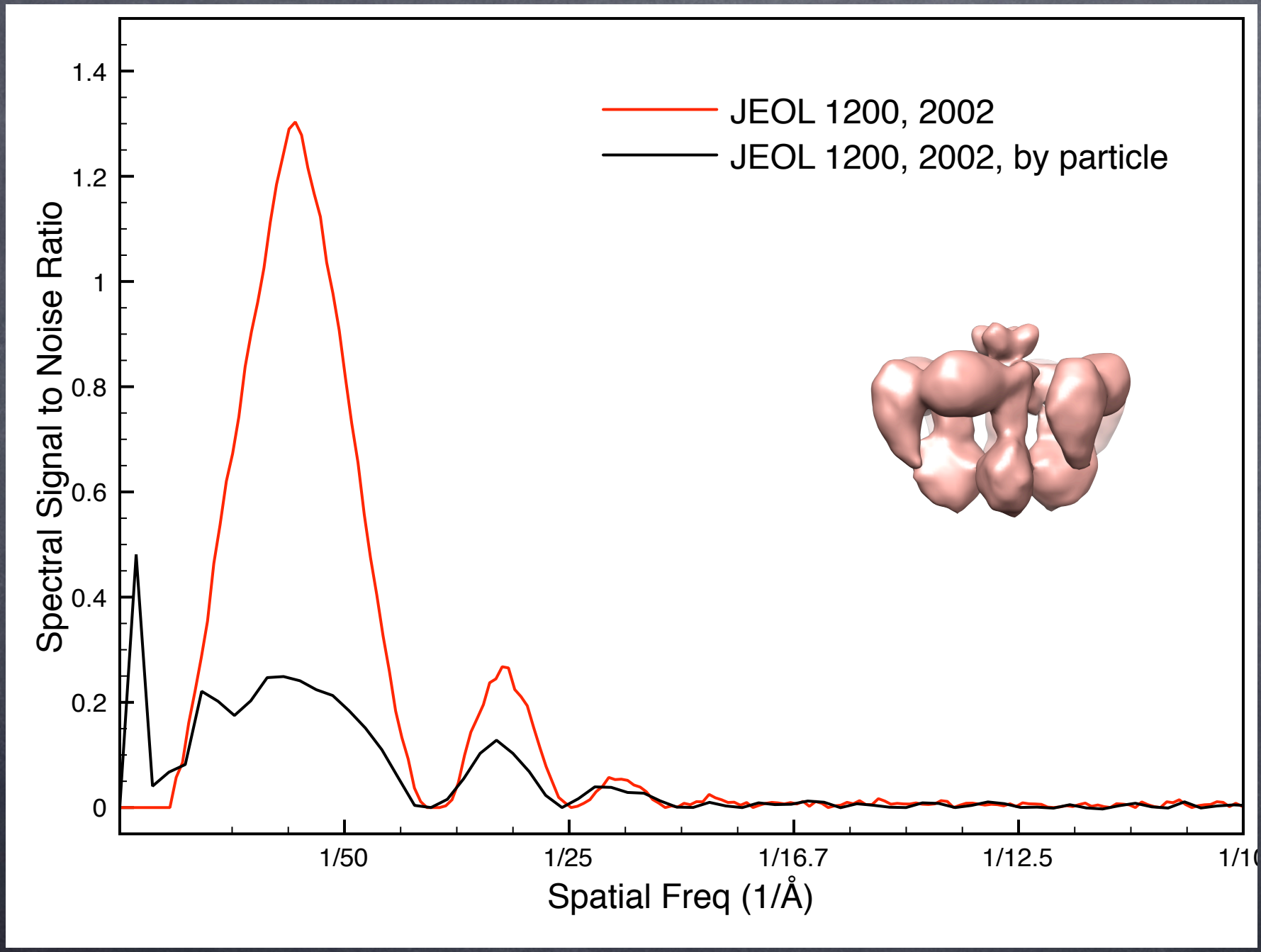

Imgs*
Intensity
40
30
20
10
0
1/50
1/25
1/16.7
1/12.5
1/10
1/8.3
1/7.1
Spatial Freq (1/Å)

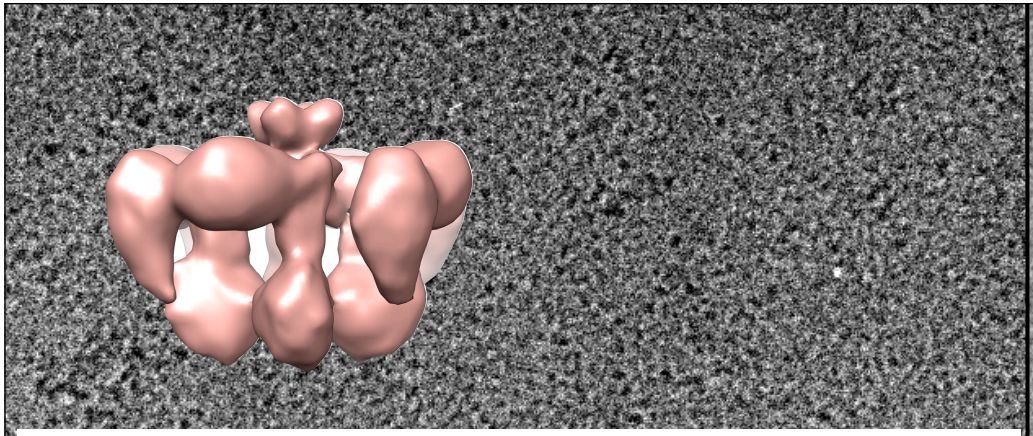

Imgs*
Imgs*
Intensity
40
30
20
10
0
1/50
1/25
1/16.7
1/12.5
1/10
1/8.3
1/7.1
Spatial Freq (1/Å)

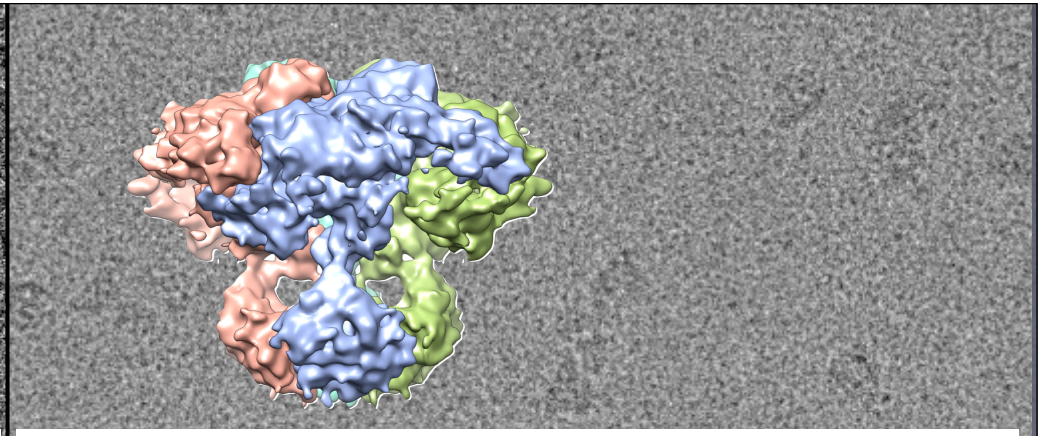

Imgs*
Imgs*
Intensity
40
30
20
10
0
1/50
1/25
1/16.7
1/12.5
1/10
1/8.3
1/7.1
Spatial Freq (1/Å)

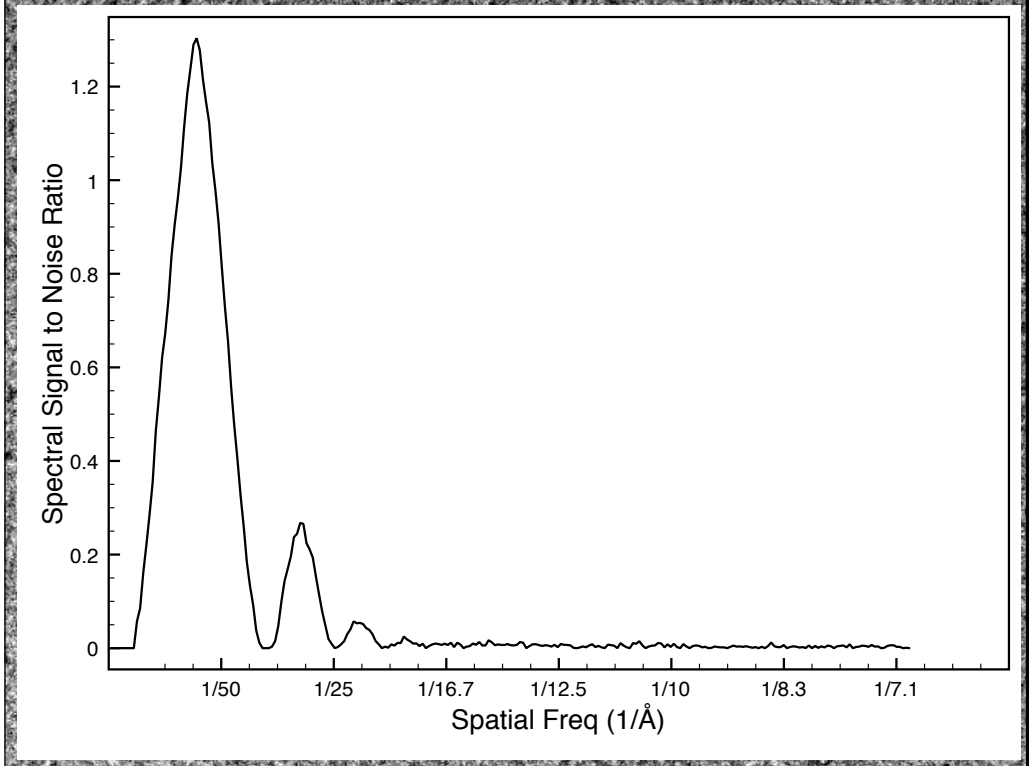

Spectral Signal to Noise Ratio
4
3
2
1
0
1/50
1/25
1/16.7
1/12.5
1/10
1/8.3
1/7.1
Spatial Freq (1/Å)

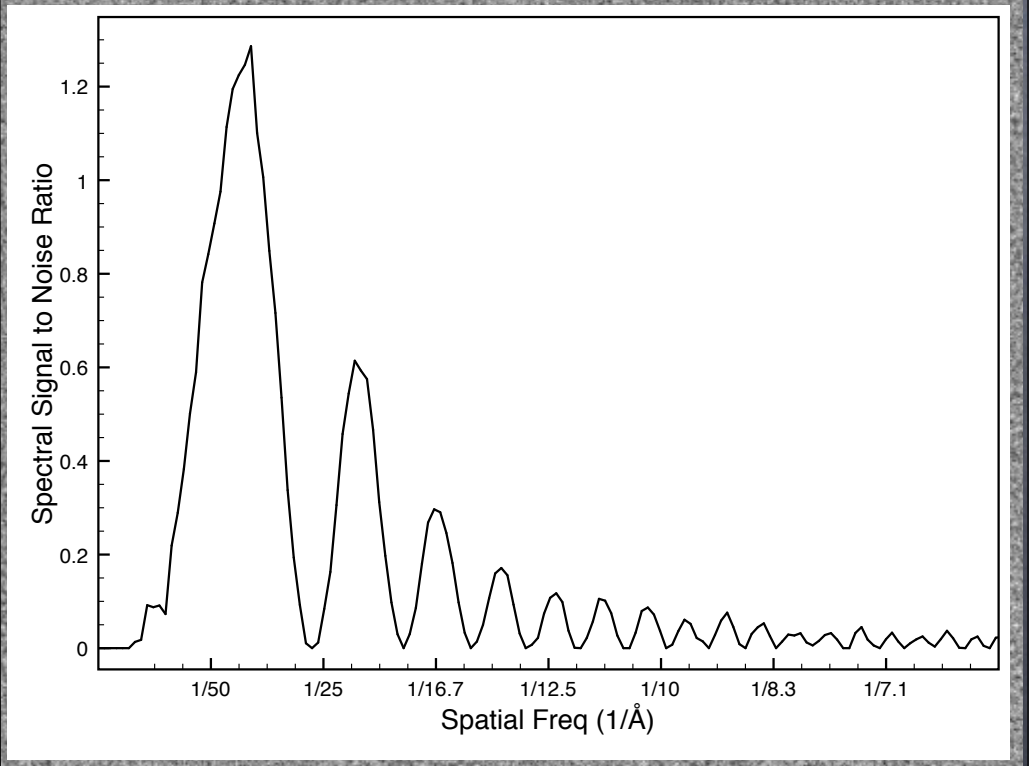

JEOL 2010F, 2008
JEOL 2010F, 2008, by particle
Spectral Signal to Noise Ratio
0
1
2
3
4
1/50
1/25
1/16.7
1/12.5
1/10
1/8.3
1/7.1
Spatial Freq (1/Å)

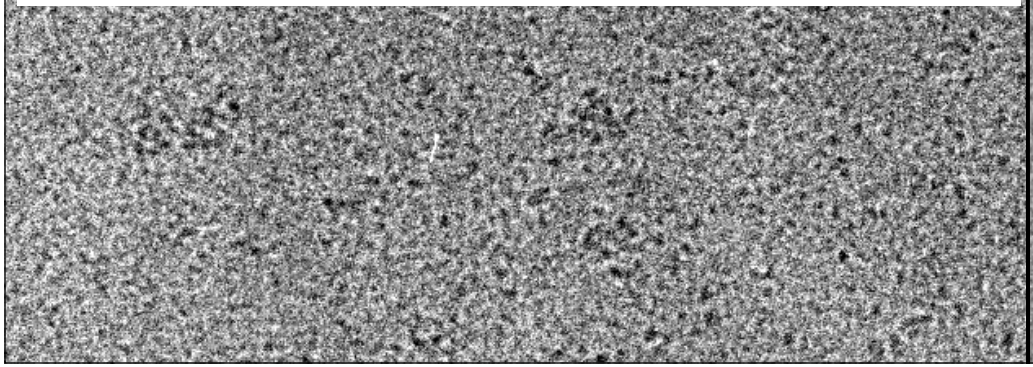

JEOL 1200, 2002
JEOL 1200, 2002, by particle
Spectral Signal to Noise Ratio
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1/50
1/25
1/16.7
1/12.5
Spatial Freq (1/Å)

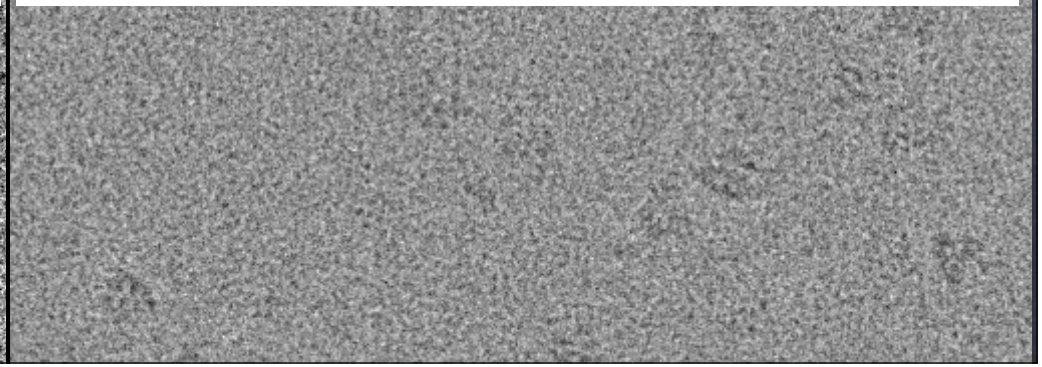


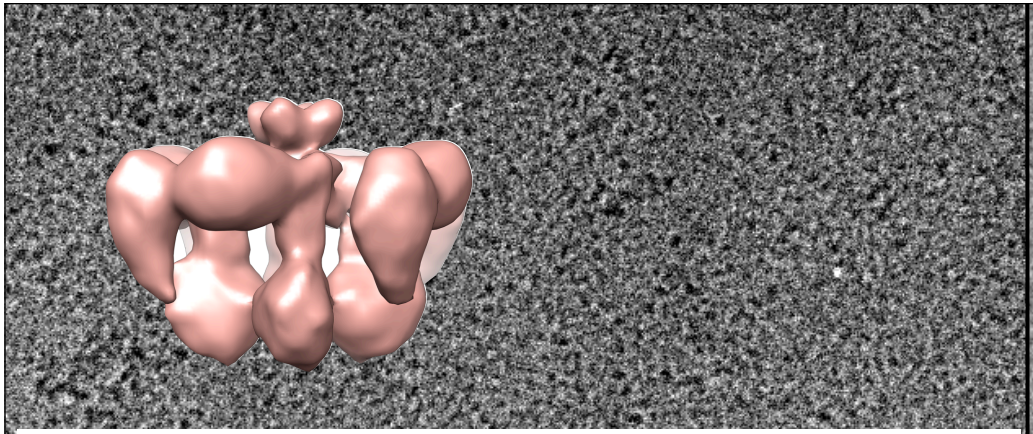


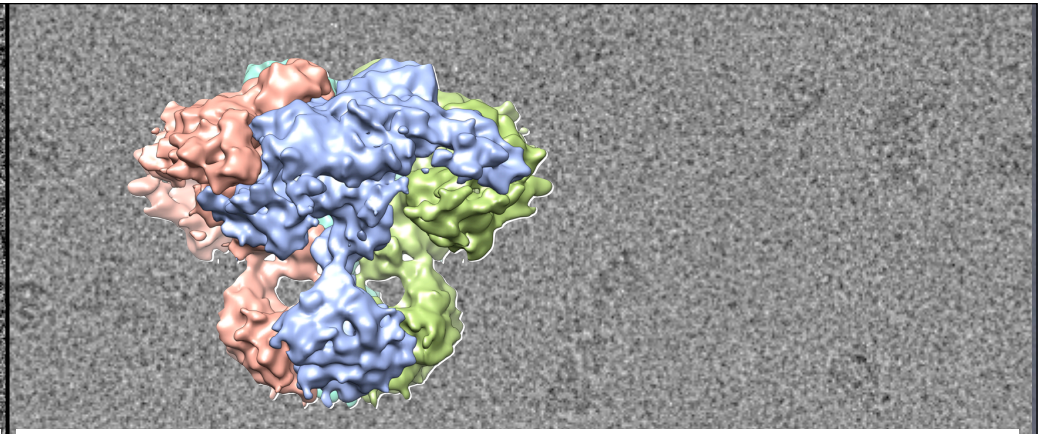

Spectral Signal to Noise Ratio
1.2
1
0.8
0.6
0.4
0.2
0
1/50
1/25
1/16.7
1/12.5
1/10
1/8.3
1/7.1
Spatial Freq (1/Å)

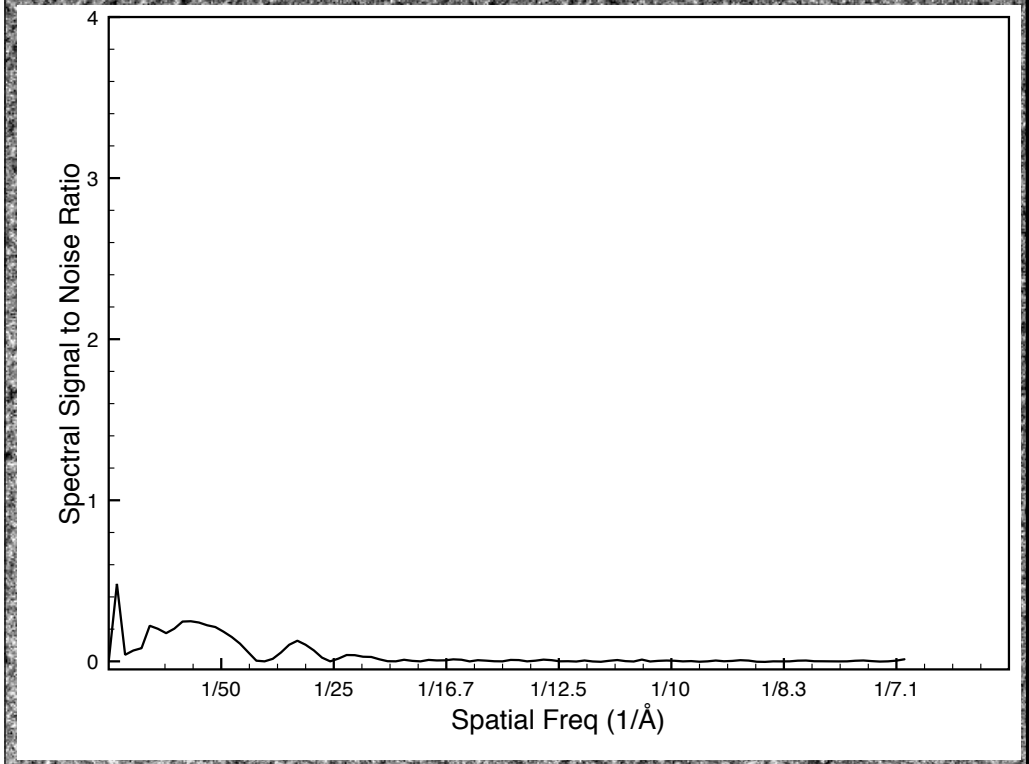

Spectral Signal to Noise Ratio
1.2
1
0.8
0.6
0.4
0.2
0
1/50
1/25
1/16.7
1/12.5
1/10
1/8.3
1/7.1
Spatial Freq (1/Å)

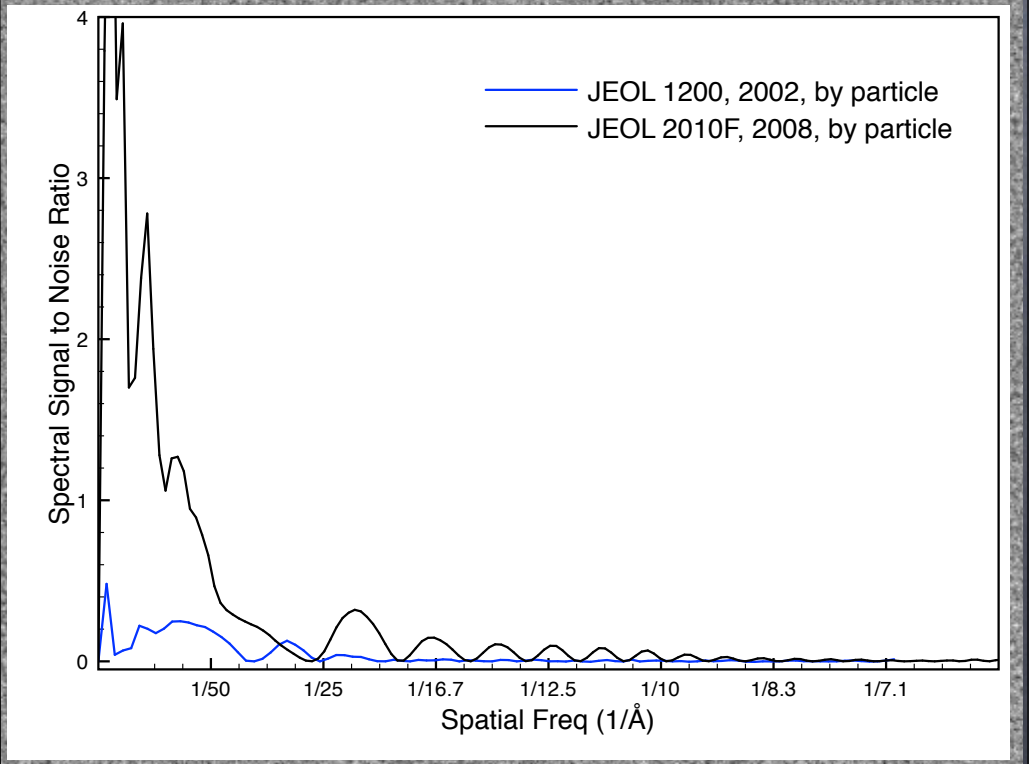


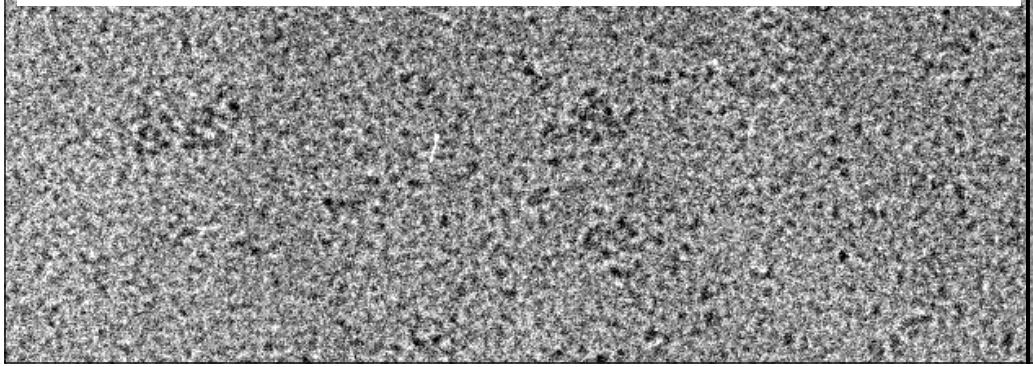


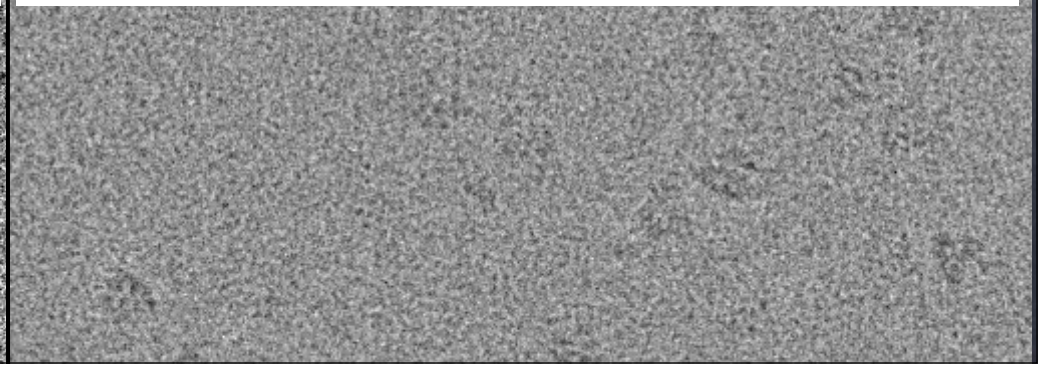


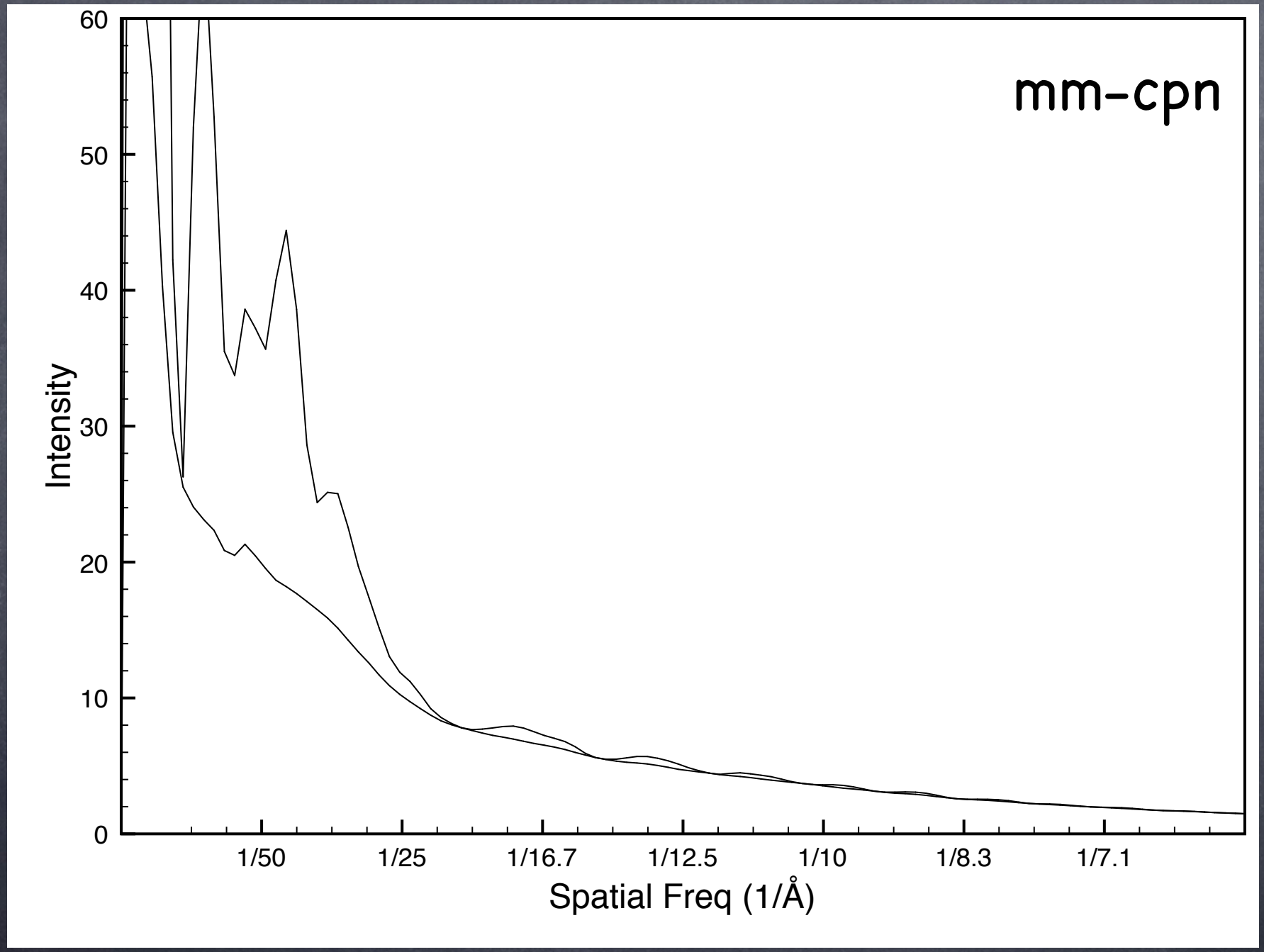



Spectral Signal to Noise Ratio
0
1
2
3
4
1/50
1/25
1/16.7
1/12.5
1/10
1/8.3
1/7.1
Spatial Freq (1/Å)


JEOL 1200, 2002, by particle
JEOL 2010F, 2008, by particle
Spectral Signal to Noise Ratio
0
1
2
3
4
1/50
1/25
1/16.7
1/12.5
1/10
1/8.3
1/7.1
Spatial Freq (1/Å)






mm-cpn
Intensity
60
50
40
30
20
10
0
1/50
1/25
1/16.7
1/12.5
1/10
1/8.3
1/7.1
Spatial Freq (1/Å)

# Conclusions

- Gold Standard FSC
  - Less overinterpretation of data
  - It only makes resolutions worse when they are overestimated
- Tilt validation for low resolution/low symmetry
- SSNR analysis of particles for data evaluation

# Questions ?