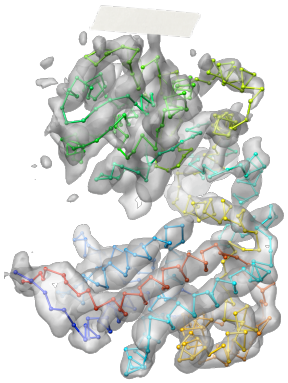
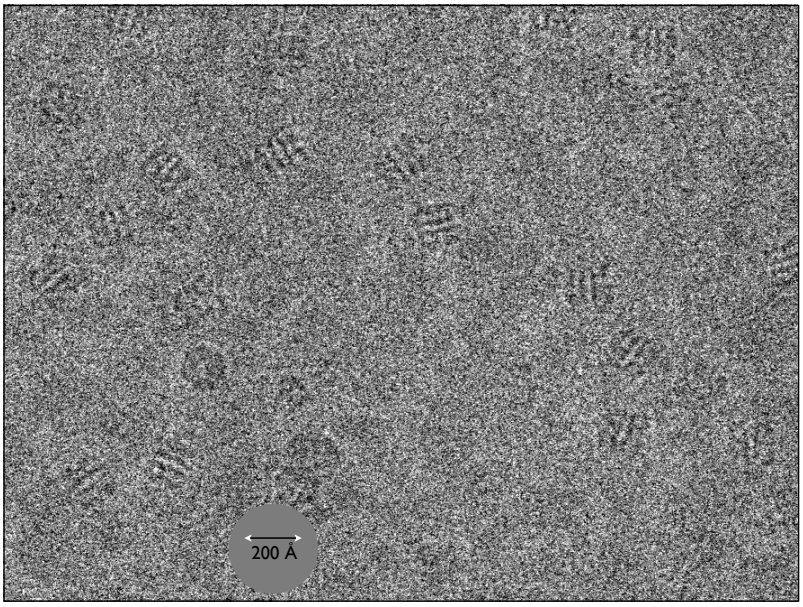
Single Particle Refinement Strategies & Introduction to EMAN2

Steve Ludtke
National Center for Macromolecular Imaging
Baylor College of Medicine



BCM Baylor College of Medicine NCRR NCMI National Center for Macromolecular Imaging NIH Roadmap

1



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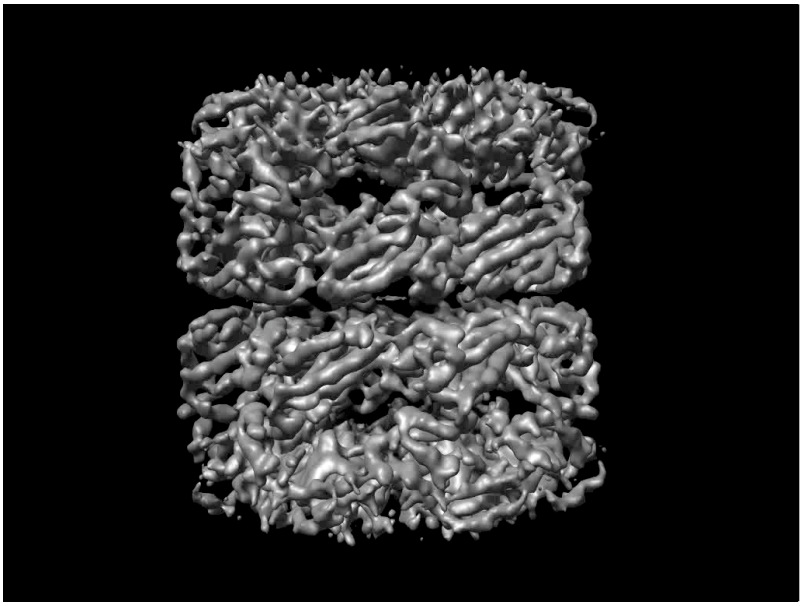

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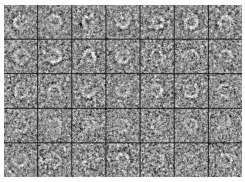
2D Analysis

4

2D Refinement

Particles



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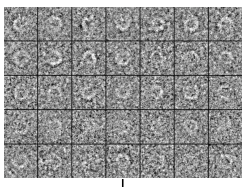
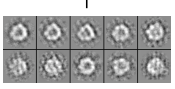
2D Refinement

Particles





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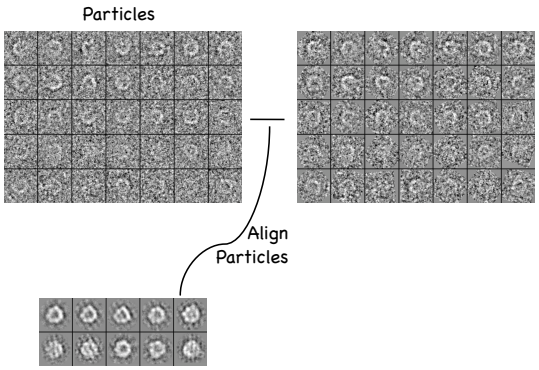
2D Refinement



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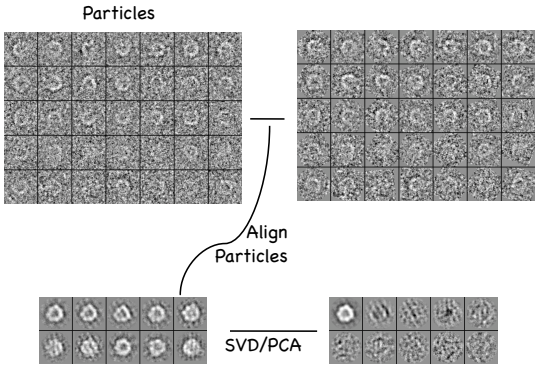
2D Refinement



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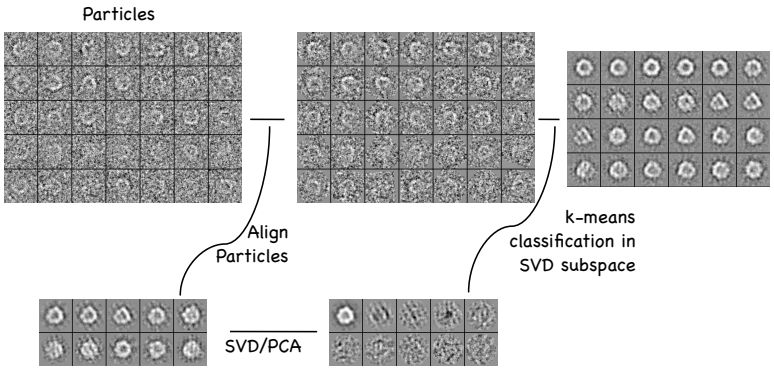
2D Refinement



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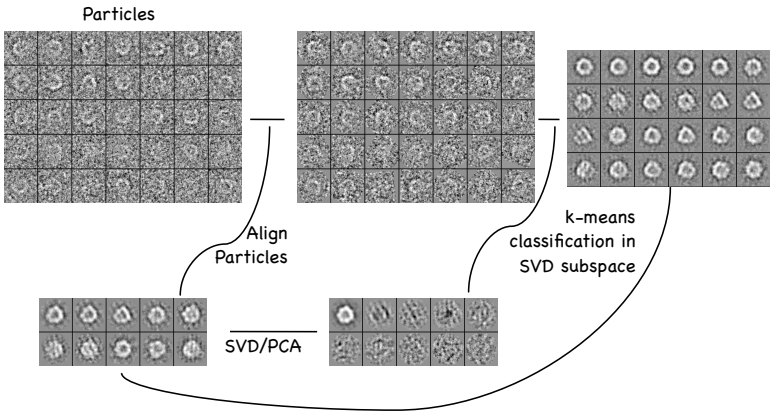
2D Refinement



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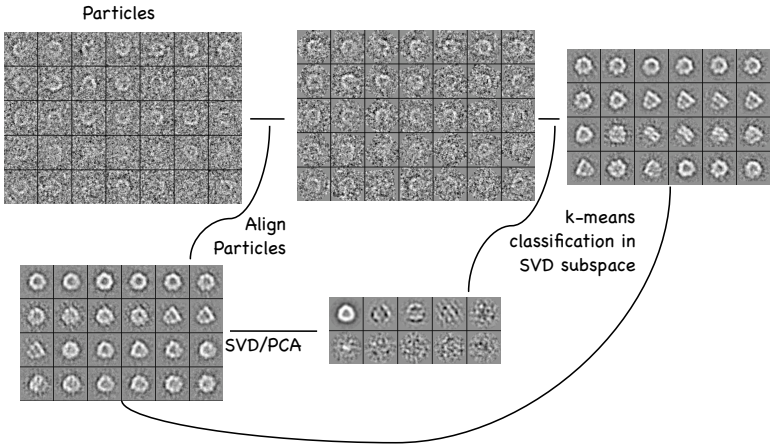
2D Refinement



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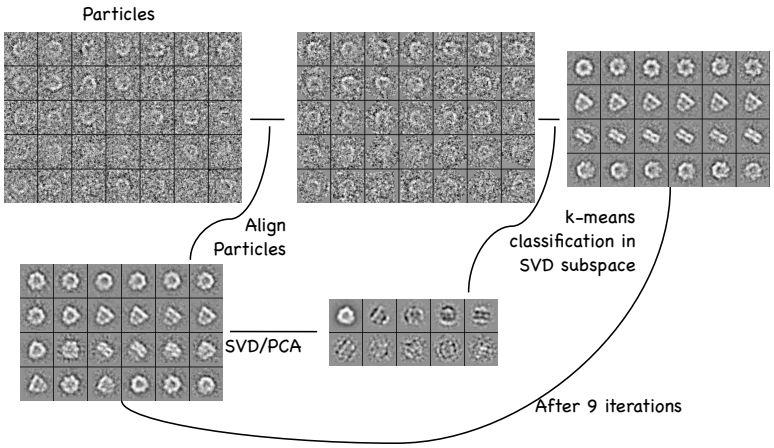
2D Refinement



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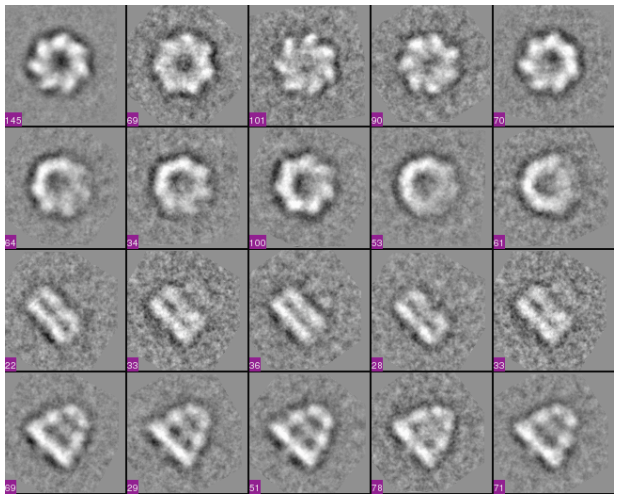

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3D Reconstruction

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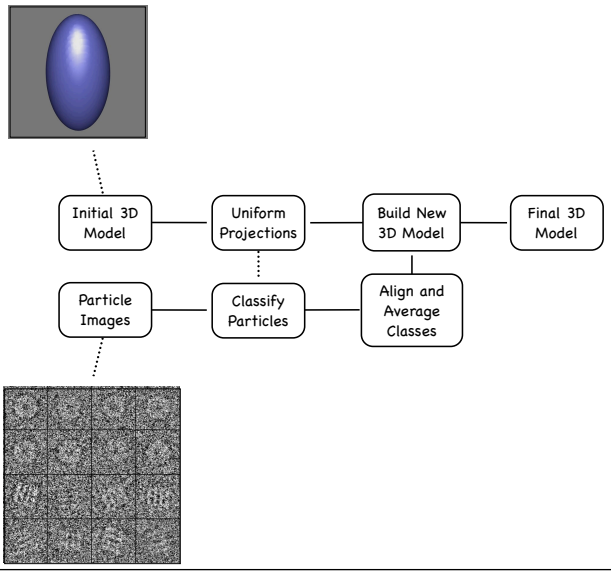

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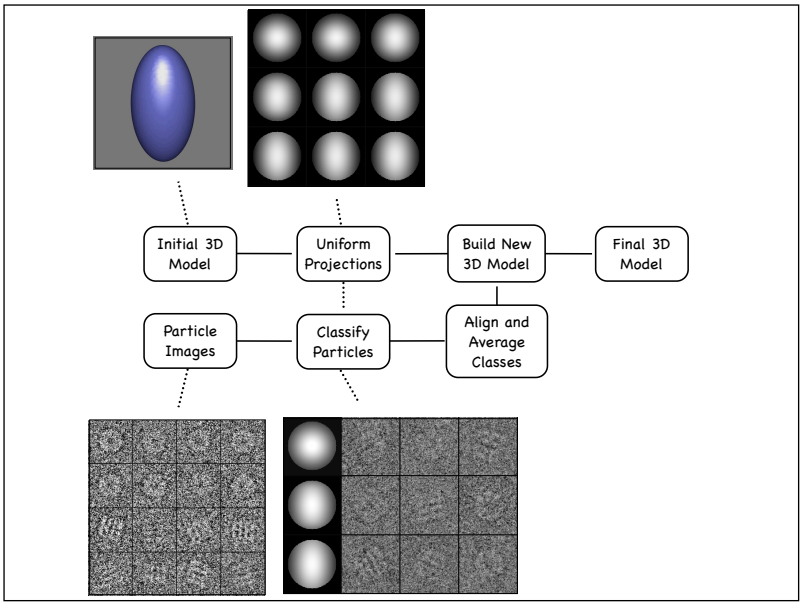
Initial 3D Model
Uniform Projections
Build New 3D Model
Final 3D Model
Particle Images
Classify Particles
Align and Average Classes

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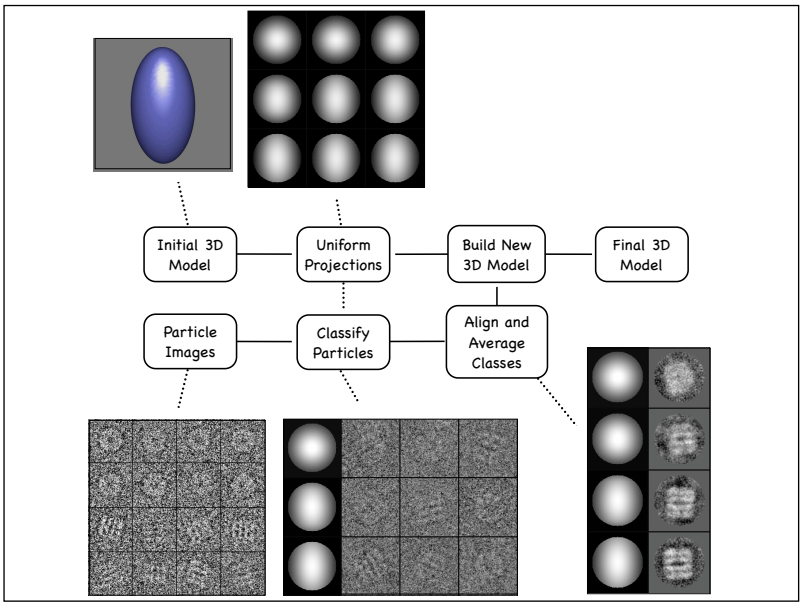
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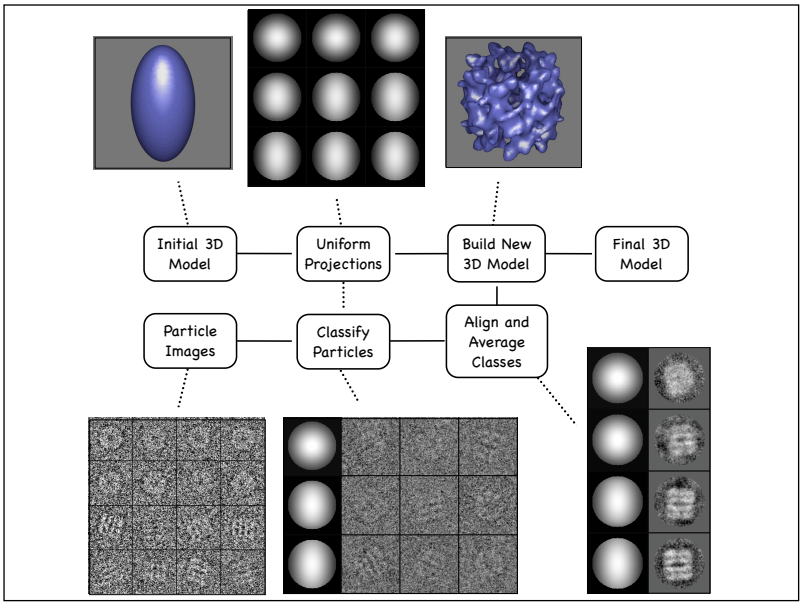
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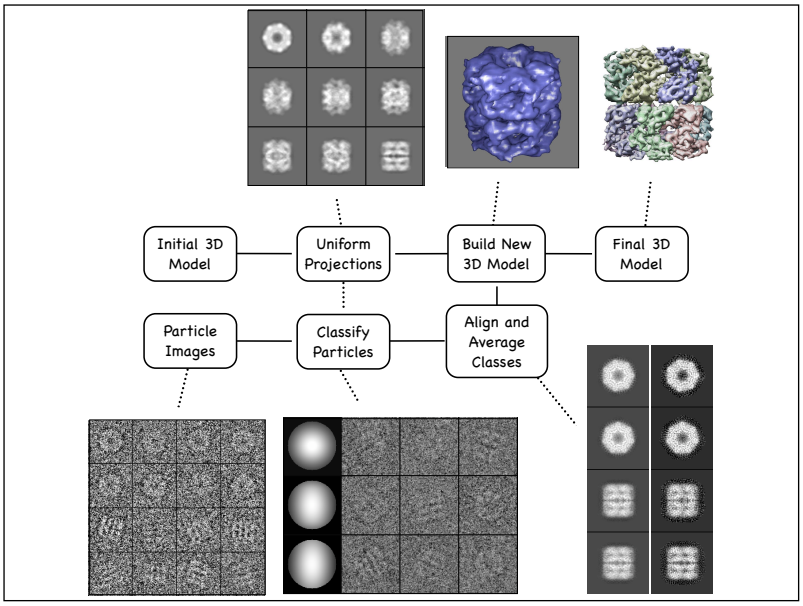
Initial 3D Model
Uniform Projections
Build New 3D Model
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Particle Images
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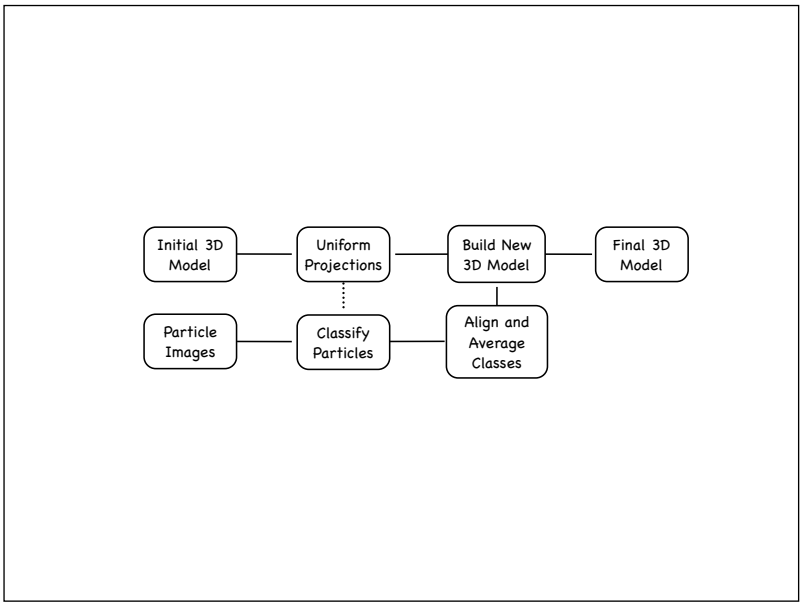
Initial 3D Model
Uniform Projections
Build New 3D Model
Final 3D Model
Particle Images
Classify Particles
Align and Average Classes

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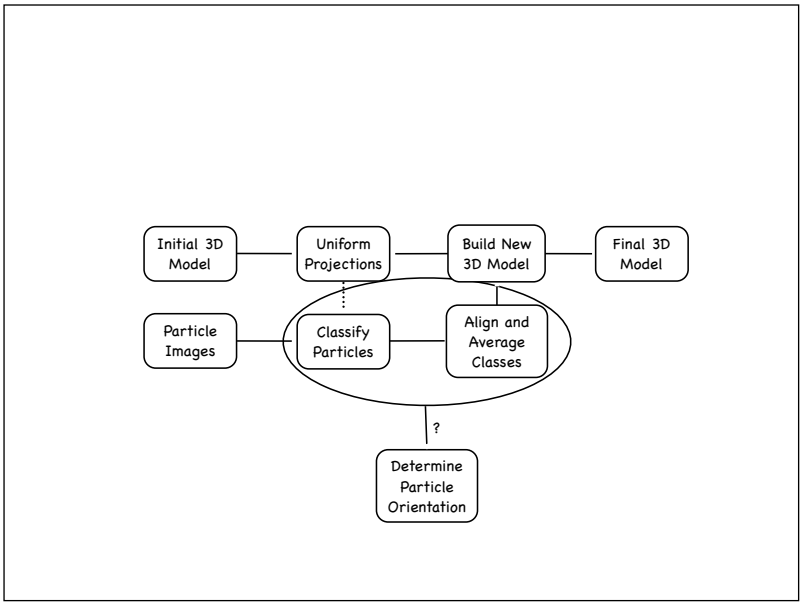
Initial 3D Model
Uniform Projections
Build New 3D Model
Final 3D Model
Particle Images
Classify Particles
Align and Average Classes
?
Determine Particle Orientation

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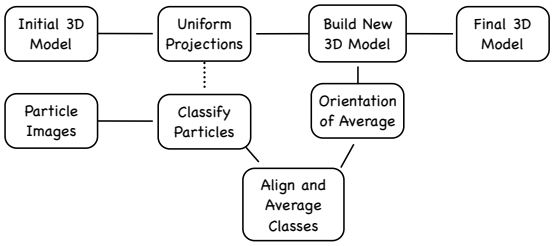

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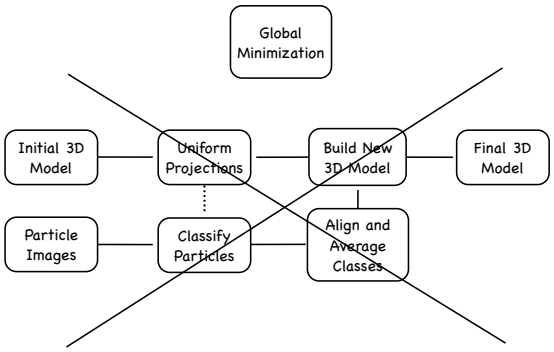

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GroEL 2005

- Native, unliganded GroEL, no ATP/ADP (?)
- JEOL 3000SFF (Yoshi-style) at LHe temp
- 6 microscopy sessions, Film
- 825 micrographs, Nikon 9000 @ 6.35 µm scan step
- 60k mag → 1.06 Å/pix
- 135 micrographs used → 20,401 particles
- Defocus 1.2 – 2.2 µm

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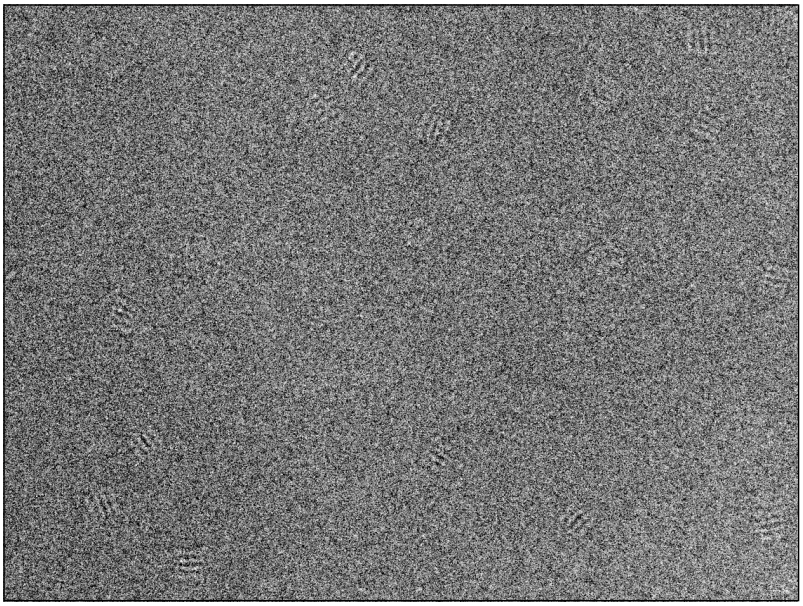

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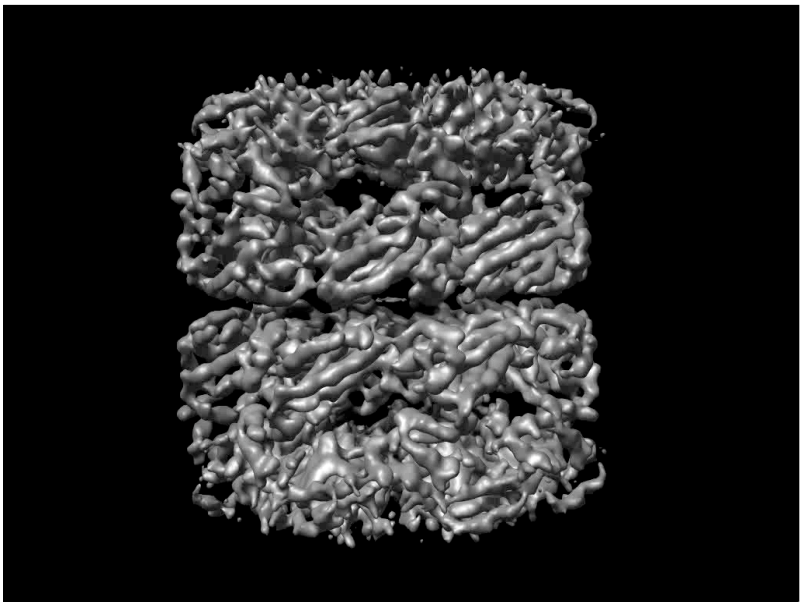

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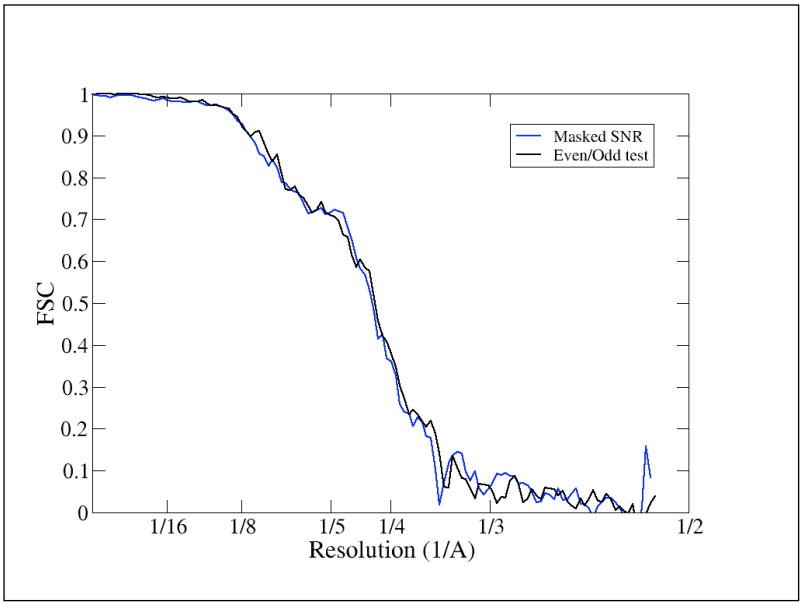
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
FSC
1/16
1/8
1/5
1/4
1/3
1/2
Resolution (1/A)
Masked SNR
Even/Odd test

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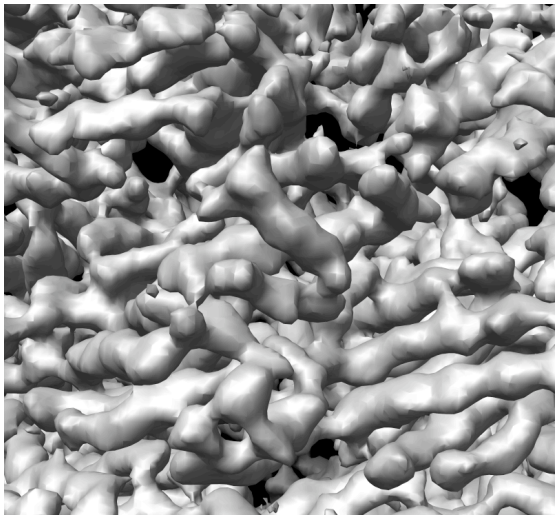

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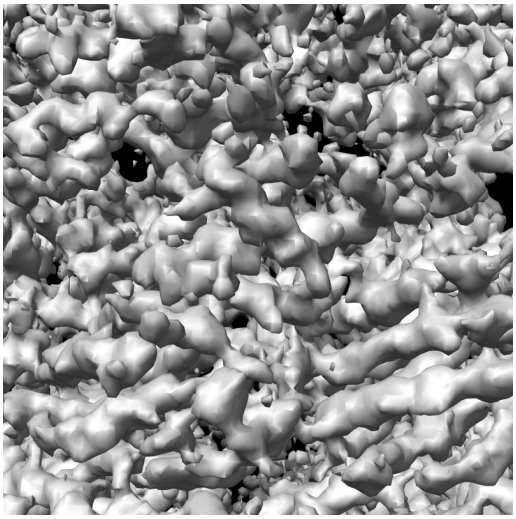

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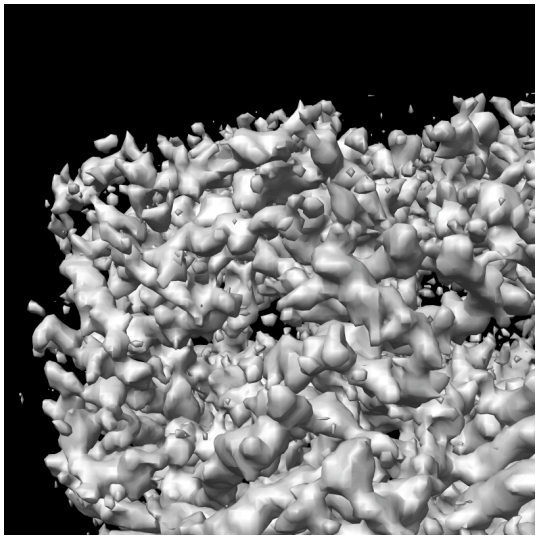

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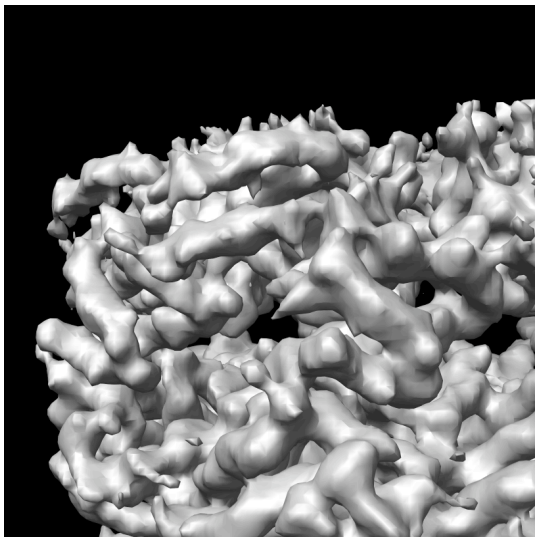

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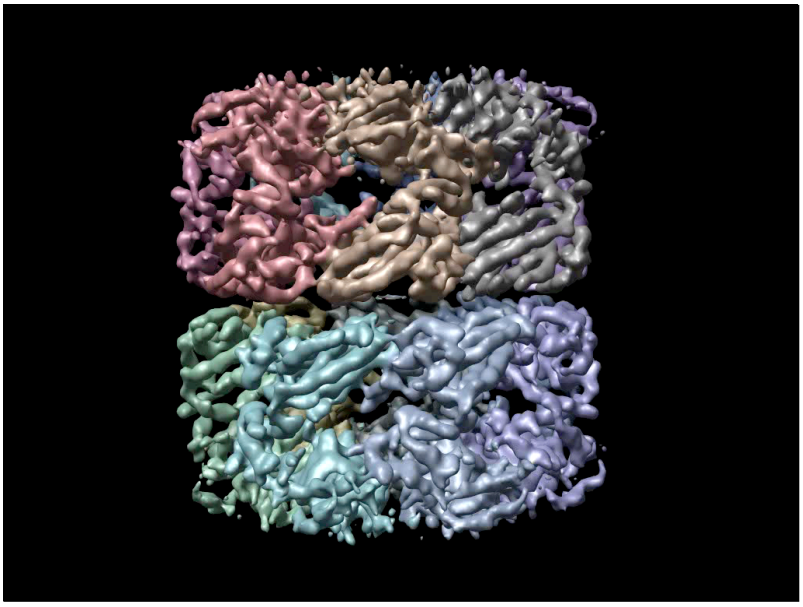

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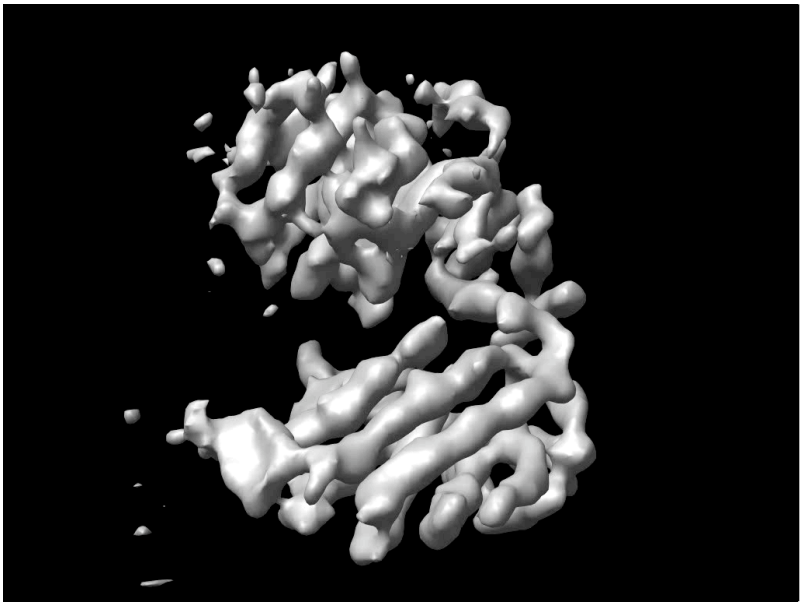

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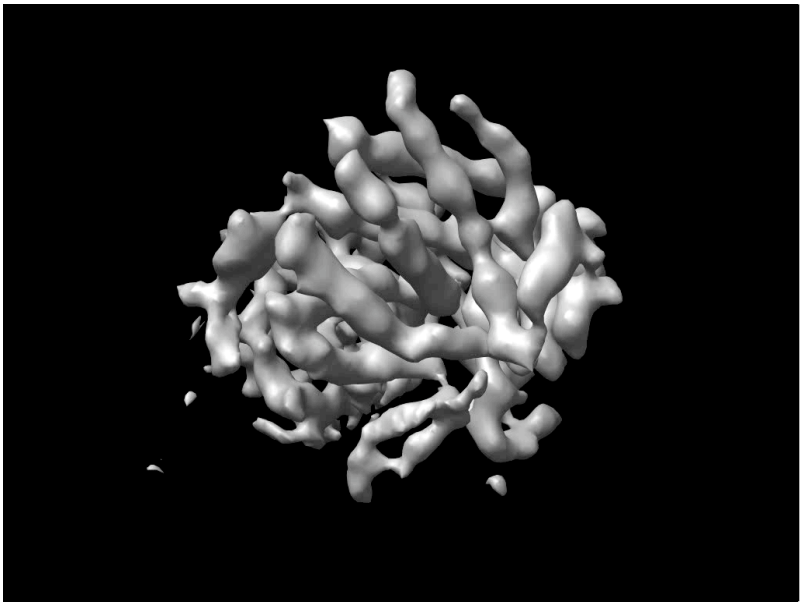

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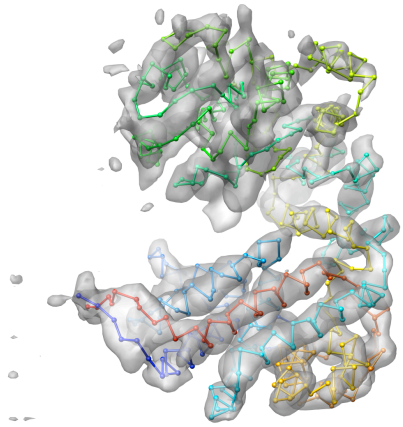
GroEL Chain Trace



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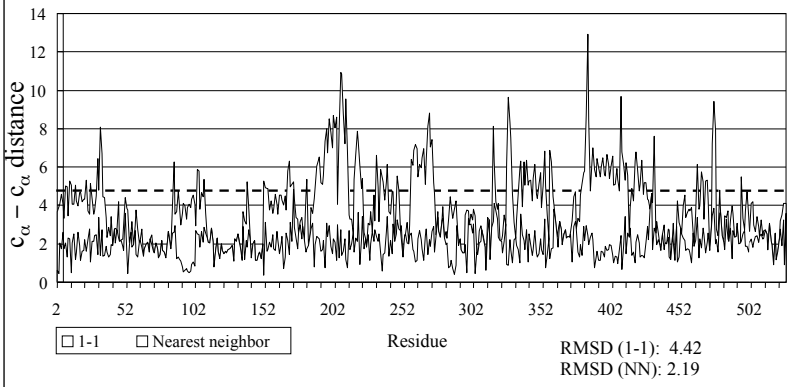
RMSD vs. PDB Structure



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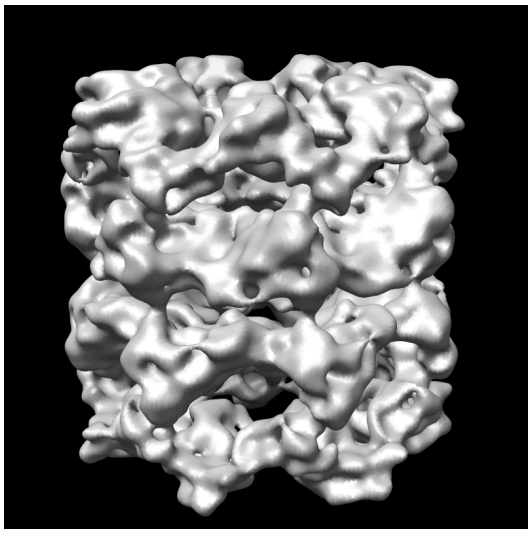
11.5 Å GroEL



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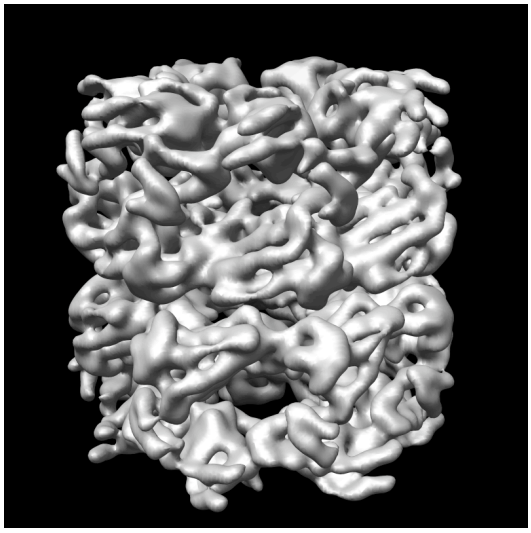
4 Å →11.5 Å GroEL



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CTF Correction

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4CAA

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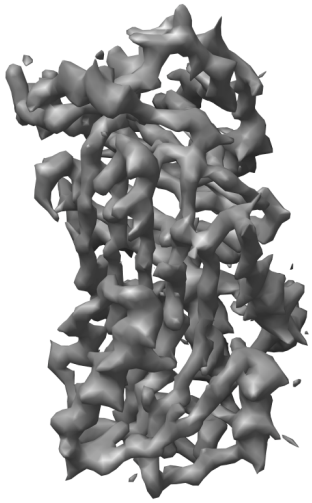
4CAA



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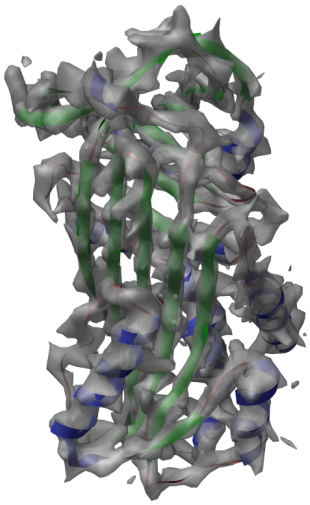
4CAA



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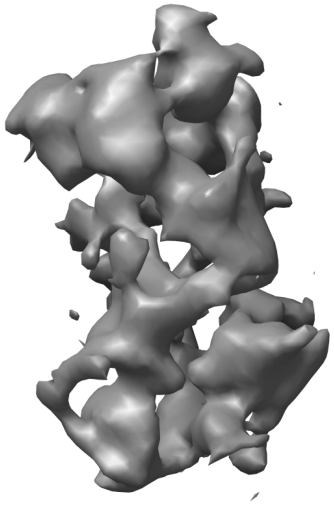
No CTF Corr (1 defocus)



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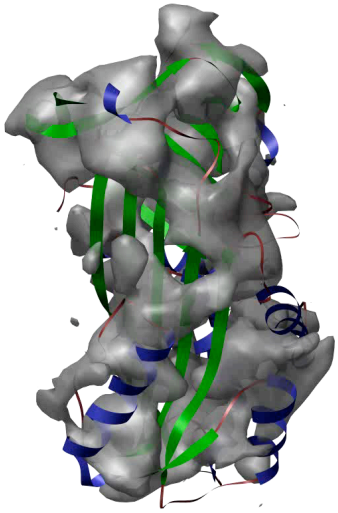

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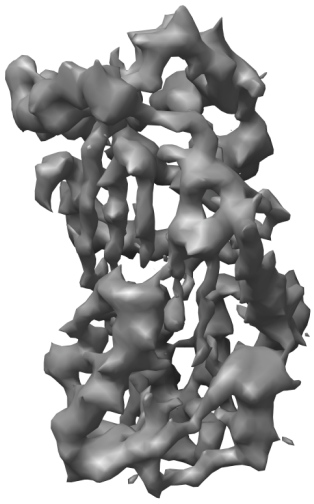
Phase Flipped (1 defocus)



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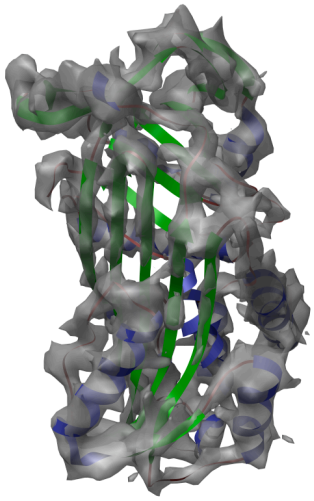
Phase Flipped (1 defocus)



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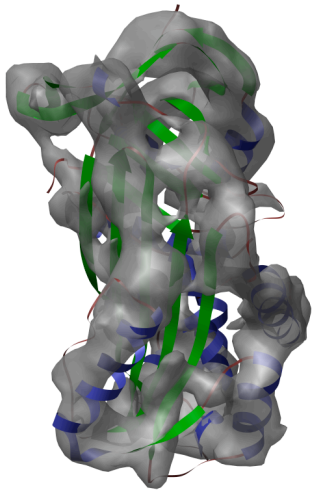
Phase Flipped (mult defocus)



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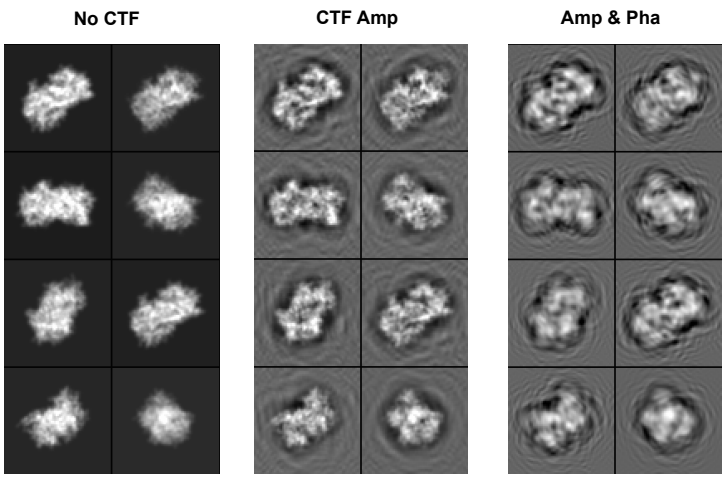
4CAA in 2D



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CTF Correction

Measured Image Ideal Particle Random Noise

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

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

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

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

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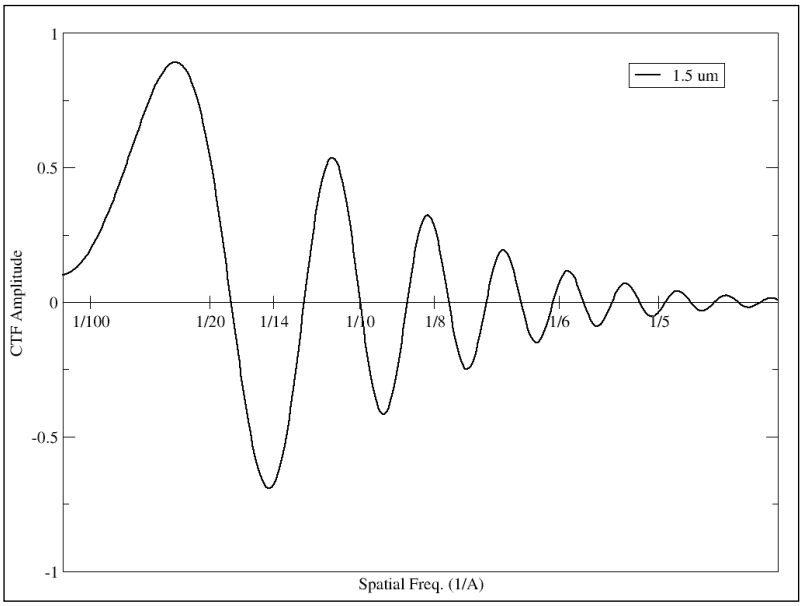

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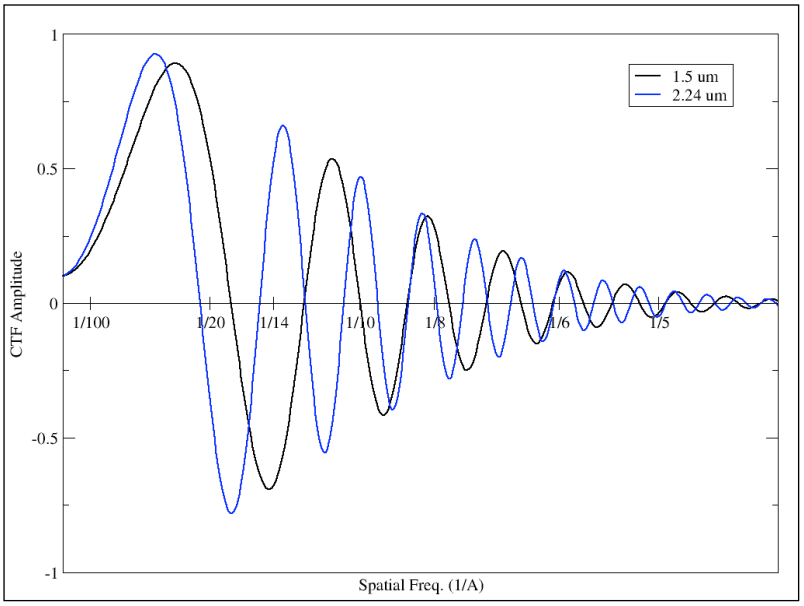

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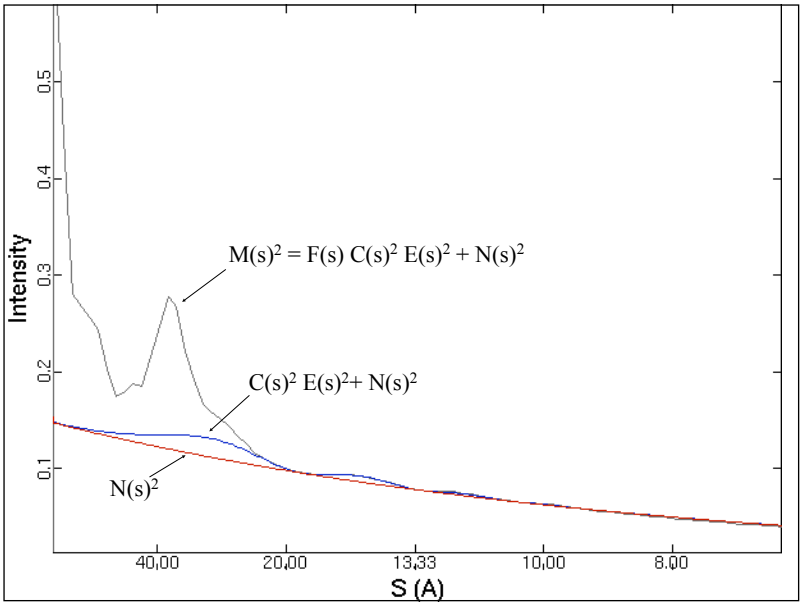

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CTF Correction

Reconstruction Weight Measured image

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

$$k_i = ?$$

- Maximize SNR of *T(s,θ)*
- Minimize RMSD between T and F

$$\sqrt{\sum_{x,y}(t(x,y)-f(x,y))^2}$$

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CTF Correction

Wiener Filter | CTF Correction | SNR Weight

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

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

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Model Bias ?

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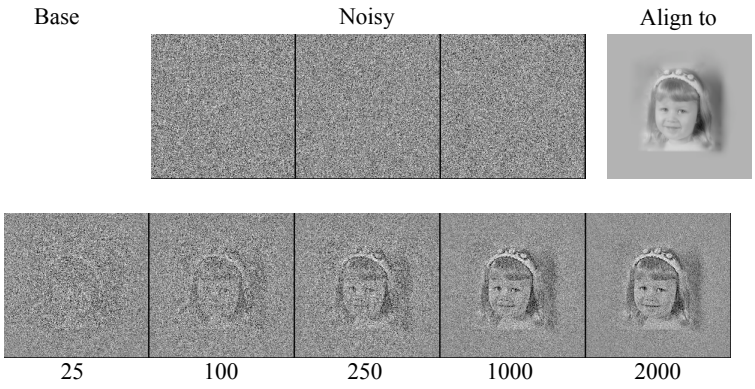
Model Bias



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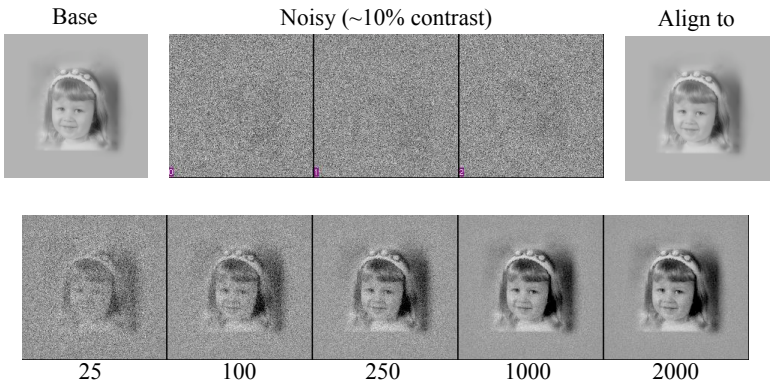
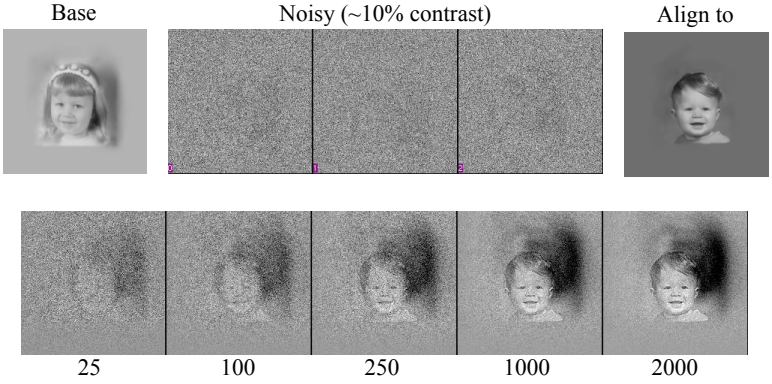
Model Bias



57

Model Bias

Base | Noisy (~10% contrast) | Align to

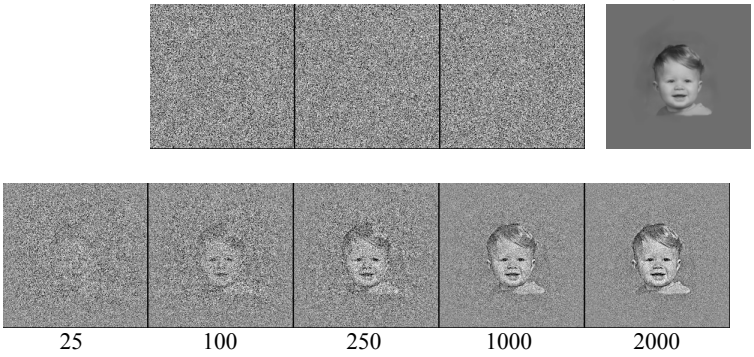


25 | 100 | 250 | 1000 | 2000

58

Model Bias

Base | Noisy | Align to



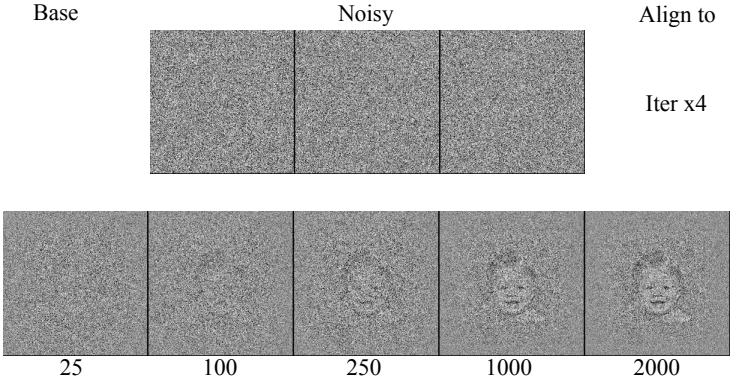
25 | 100 | 250 | 1000 | 2000

59

Model Bias

Base | Noisy | Align to

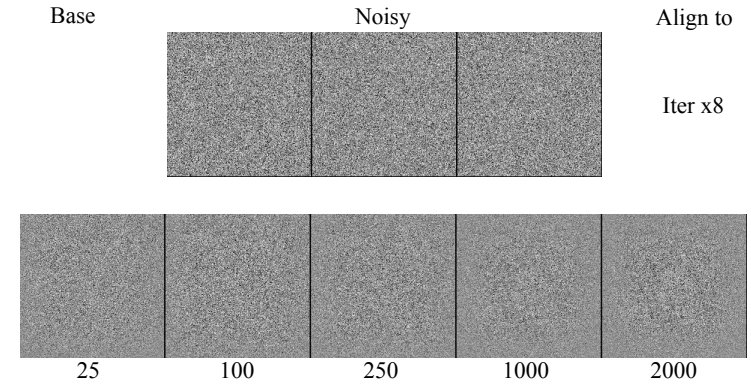
Iter x4



25 | 100 | 250 | 1000 | 2000

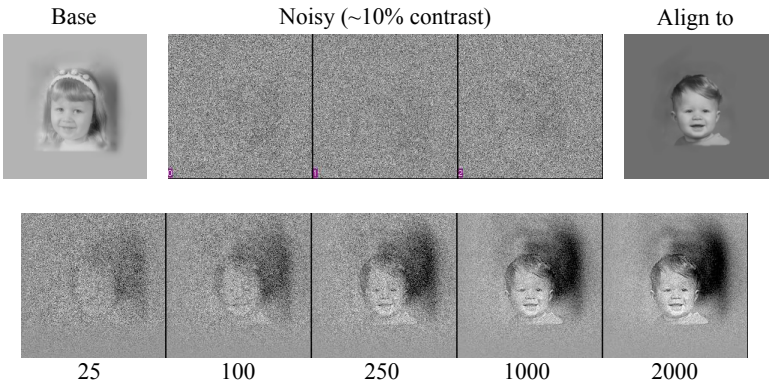
60

Model Bias



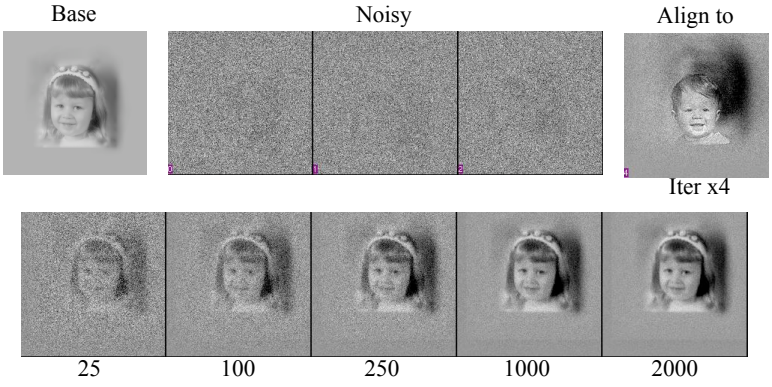
61

Model Bias



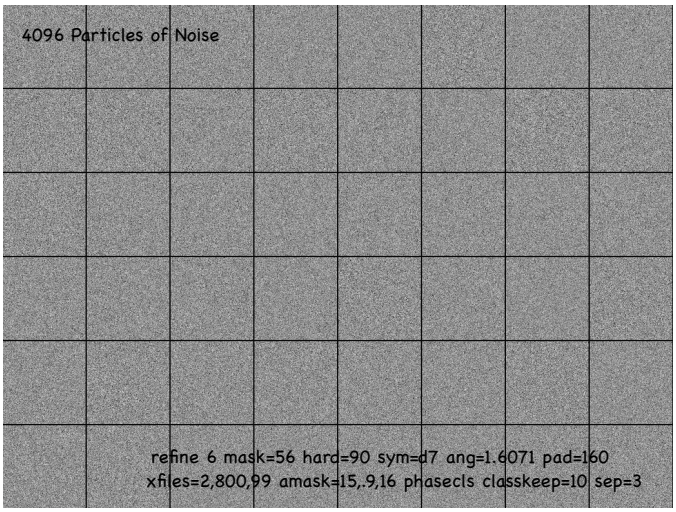
62

Model Bias



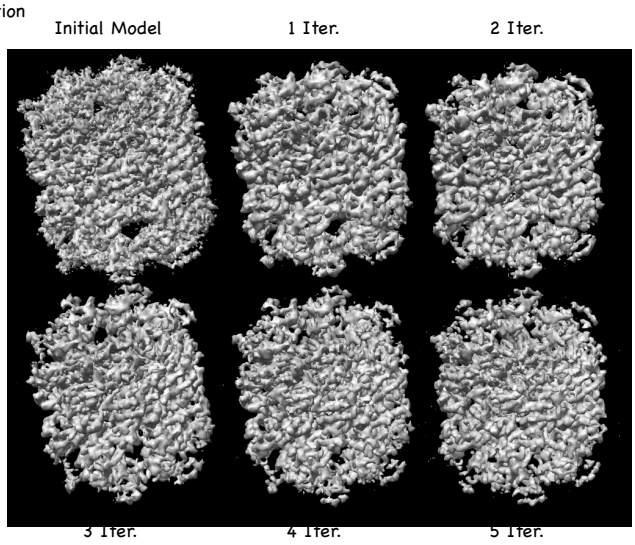
63

How About 3-D ?

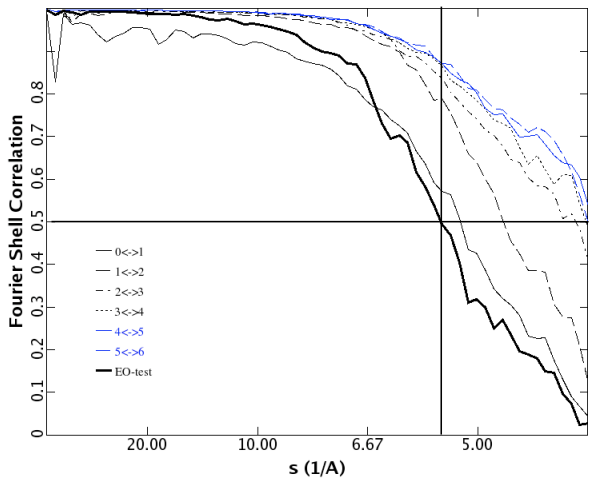


64

no iteration

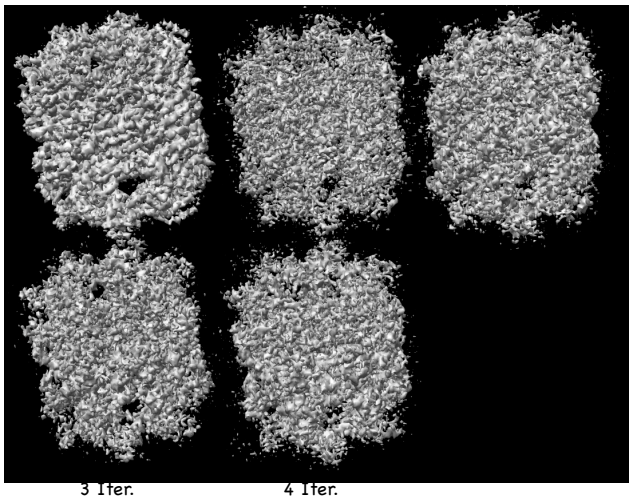


65



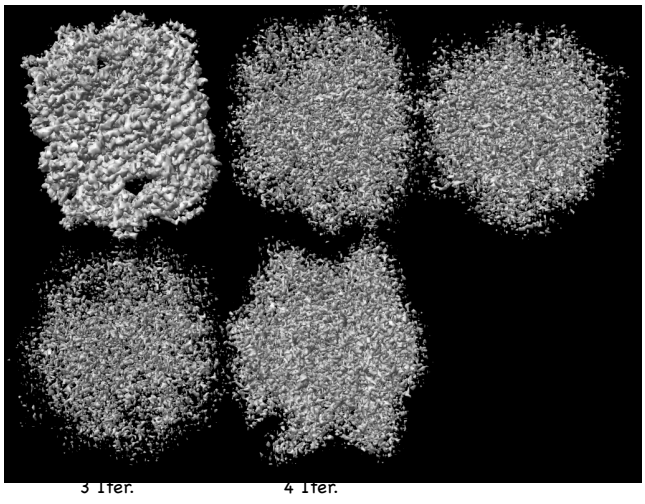
66

1 iteration



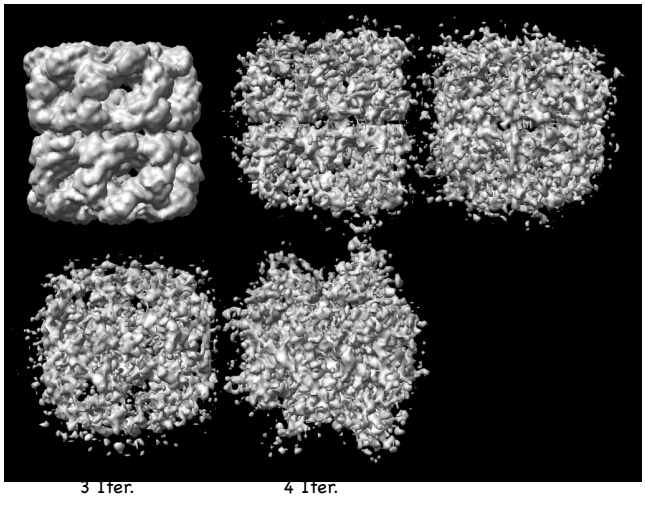
67

6 iterations

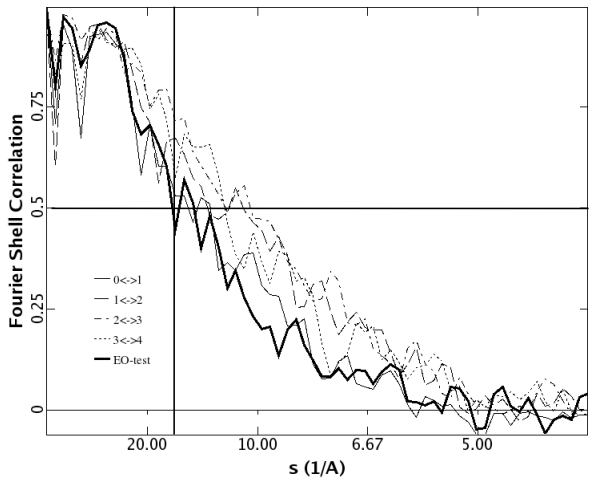


68

6 iterations
(8 A lowpass)



69



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How Do we Stop This ?

- (In EMAN) use classiter>3 for a few rounds
- Use several different (random) starting models and insure that you get a good answer
- Compare 3D models with results of 2D analysis

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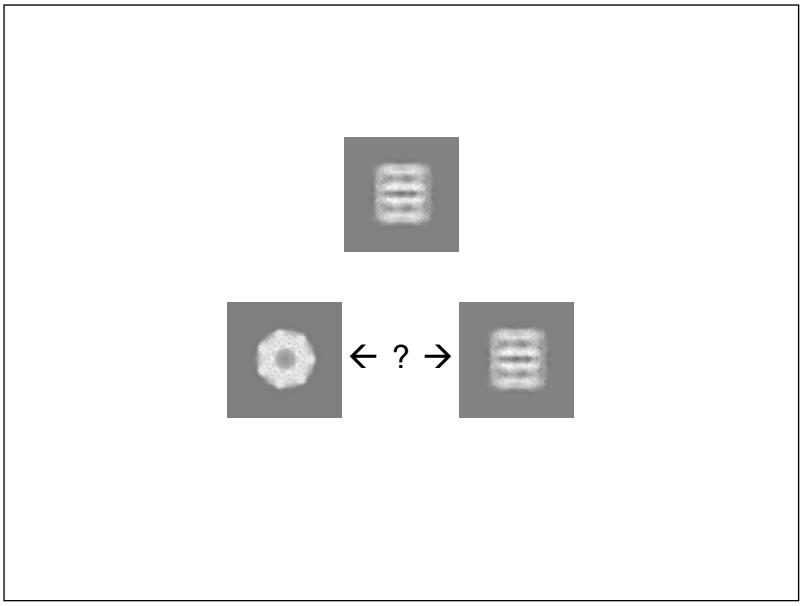
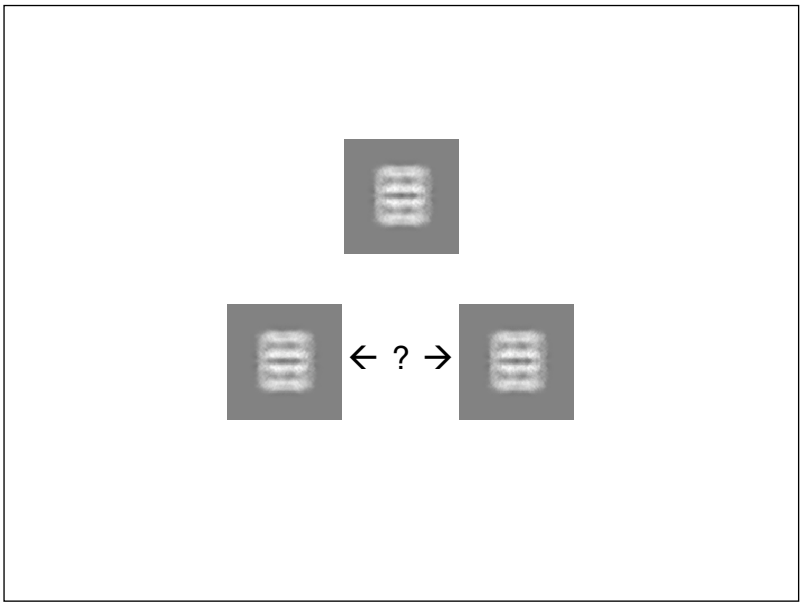
Measures of Similarity

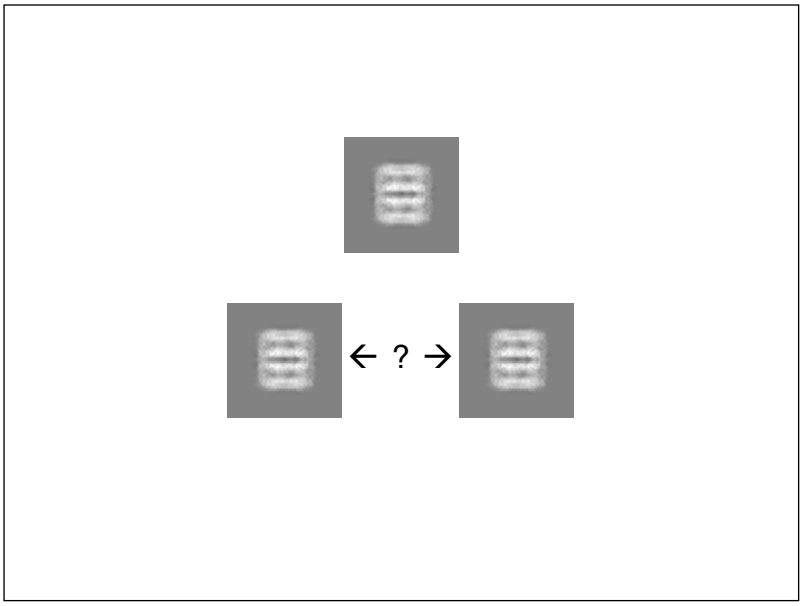
72



73



74



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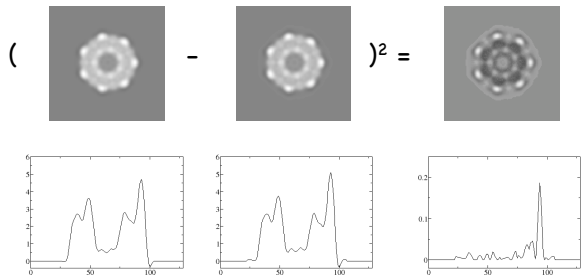
Measures of Similarity

- Correlation coefficient
- Variance (equivalent)
- Phase Residual
- FSC
- Mutual Information
- etc…

76



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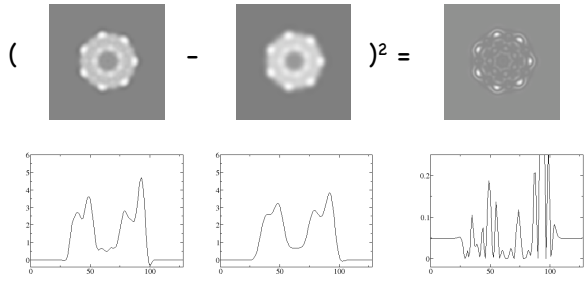

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EMAN2

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EMAN2 vs. EMAN1

- Improved CTF model
  - Automatic fitting, Astigmatism*, Energy filtered data
- New openGL based GUI
- Workflow infrastructure
- Embedded database for data storage and metadata archival
- EMEN2 Integration *
- Easily extensible image processing infrastructure
- New parallelism strategy ~
- CUDA support *

* - not yet ready for use

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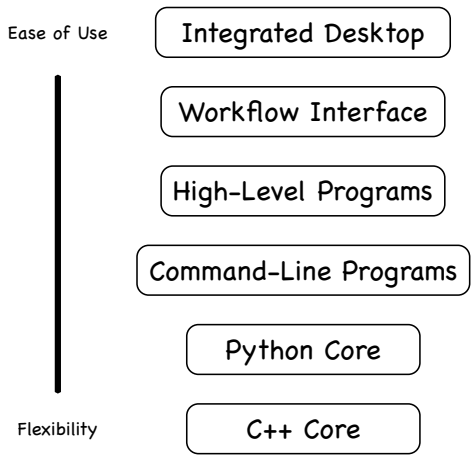
EMAN2 Architecture



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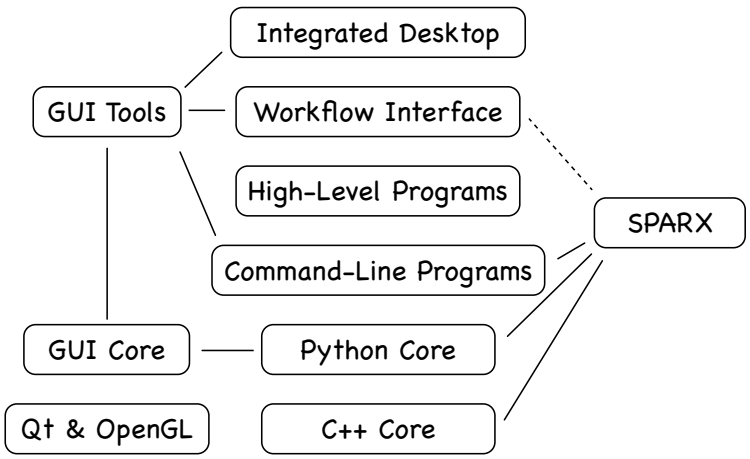
EMAN2 Architecture



Extensible Core

Type	Description	#
Processor	Generic image processing algorithms, filters, masks, thresholds, etc.	157
Aligner	Algorithms used to align 2 images or volumes to each other	11
Projector	Routines to generate 2-D projections of 3-D objects	7
Reconstructor	Routines to reconstruct 3-D objects from 2-D projections	11
Cmp	Similarity metrics used to compare two images or volumes	9
Averager	Average together stacks of images in various ways	9
Analyzer	Perform various operations on sets of images, such as classification or PCA	6
Orientgen	Routines describing how projections cover the asymmetric triangle	6

File Formats

BDB +

MRC	R/W	IMAGIC	R/W
SPIDER	R/W	HDF5	R/W
PIF	R/W	ICOS	R/W
VTK	R/W	PGM	R/W
Amira	R/W	Xplor	W
Gatan DM2	R	Gatan DM3	R
TIFF	R/W	Scans-a-lot	R
LST	R/W	PNG	R/W
Video-4-Linux	R	JPEG	W

Processors

(categories & examples)

- filter
  - filter.lowpass.gauss
  - filter.homomorphic.tophat
- mask
  - mask.sharp
  - mask.gaussian
- math
  - math.sqrt
  - math.laplacian
- misc
  - misc.localnorm
- normalize
  - normalize
  - normalize.edgemean
- testimage
  - testimage.scurve
- threshold
  - threshold.binary
  - threshold.clampminmax
- xform
  - xform.centerofmass
  - xform.fourierorigin.tocenter

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Similarity Metrics

(cmp)

With Default options, SMALLER -> more similar

- dot - dot product (negative by default)
- frc - Fourier ring correlation (weighted)
- optvariance - 'optimized variance' (EMAN1)
- phase - mean phase error
- quadmindot - Worst of quadrant dot products
- sqeuclidean - sum $(a-b)^2/n$

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Programs

- 49 Command-Line Programs (EMAN2)

syntax:

e2<name>.py --help

e2<name>.py <file> [--option=value] [--option] [-O]

<> - required parameter

[] - optional parameter

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GUI

- e2desktop.py (may not be stable yet)
- e2workflow.py
- e2display.py
- and other programs with the --gui option

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EMEN2

Electron Microscopy Electronic Notebook

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The Database Problem

- Encourage good recordkeeping (automation)
- Find something after a person leaves the lab
- Reconstruct an experimental protocol refined over years
- Mine experimental data for new information
- Minimize db overhead/administration

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Film/Frame Id	Defocus	Mag	Total Dose	ExpTime
ccd_2004031-#1	0.8 um	138.44k	20.0	1.0 s
ccd_2004031-#2	2.5 um	138.44k	20.0	1.0 s
ccd_2004031-#1	0.8 um	138.44k	20.0	1.0 s
ccd_2004031-#2	2.5 um	138.44k	20.0	1.0 s
ccd_2004031-#1	0.8 um	138.44k	20.0	1.0 s
ccd_2004031-#2	2.5 um	138.44k	20.0	1.0 s
ccd_2004031-#1	0.8 um	138.44k	20.0	1.0 s
ccd_2004031-#2	2.5 um	138.44k	20.0	1.0 s
ccd_search1 #1	0.8 um	138.44k	20.0	1.0 s
ccd_search2 #2	2.5 um	138.44k	20.0	1.0 s
ccd_search3 #1	0.8 um	138.44k	20.0	1.0 s
ccd_search4 #2	2.5 um	138.44k	20.0	1.0 s
ccd_search5 #1	0.8 um	138.44k	20.0	1.0 s
ccd_search6 #2	2.5 um	138.44k	20.0	1.0 s

Scientific Database

- Excellent mineability
- Good for centralized databases
- Limited flexibility
- Substantial overhead (design & maintenance)

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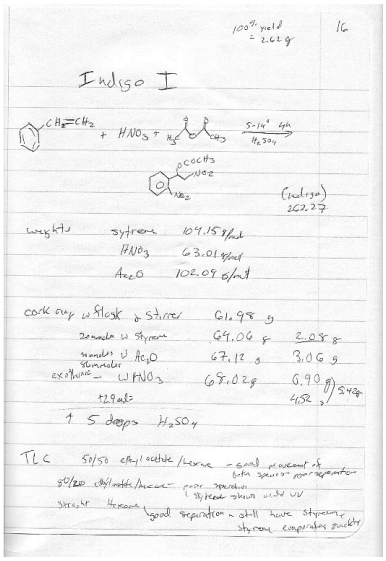

Electronic Lab Notebook

- Excellent flexibility
- Rich information content
- Limited mineability

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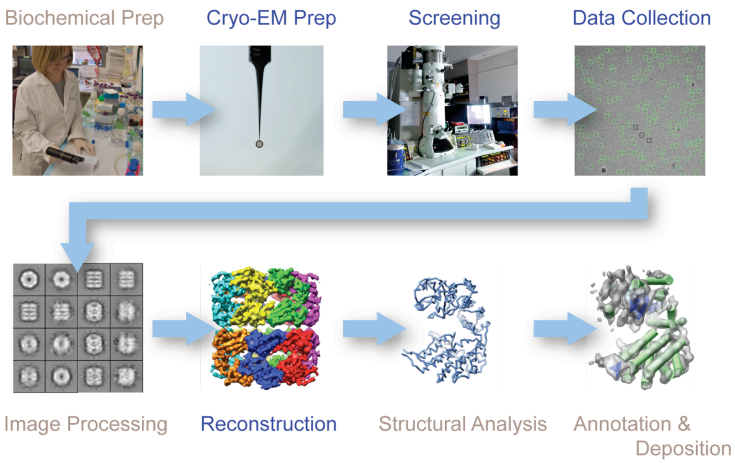
Archive Entire Cryo-EM Pipeline



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Object Oriented Database

- Flexible records not tied to rigid definitions
- Connectivity between arbitrary records
- Queries not easily posed in a traditional database
- Per-record security
- Security and queries aware of record hierarchy

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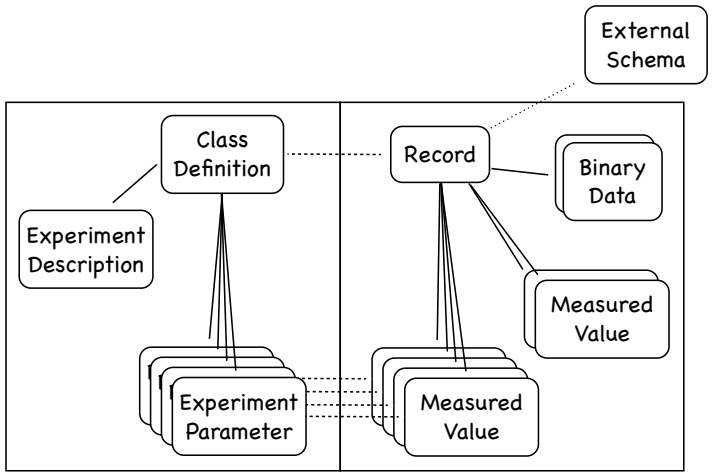

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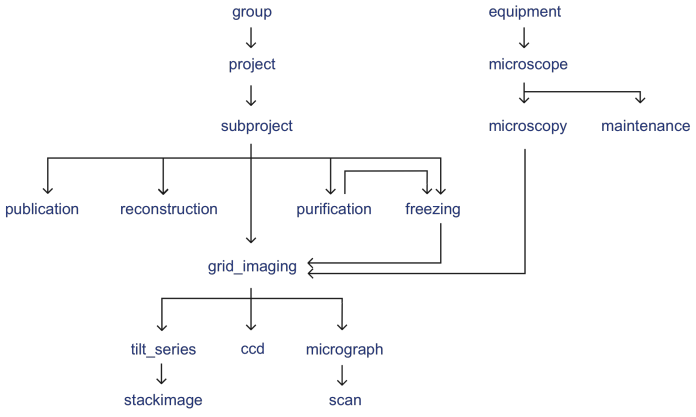
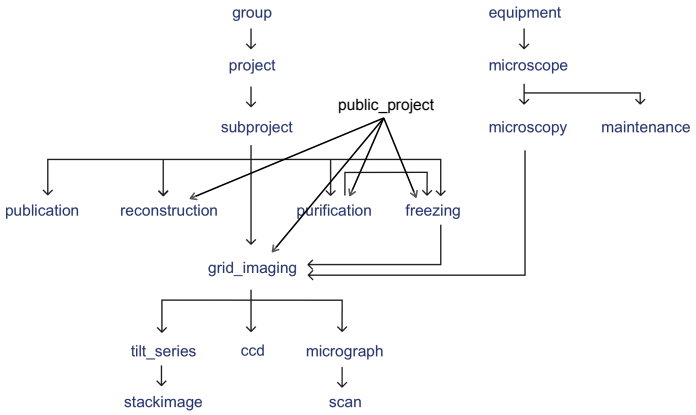
Record Organization



96

Record Organization



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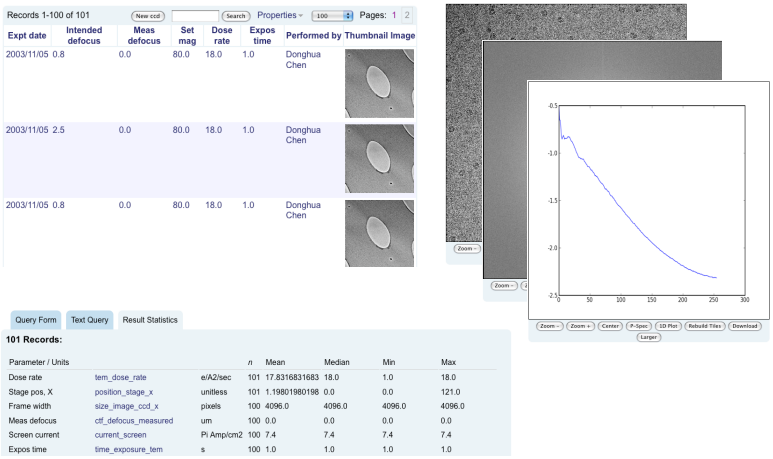
Analysis Tools



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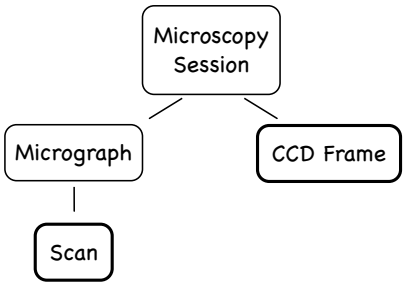
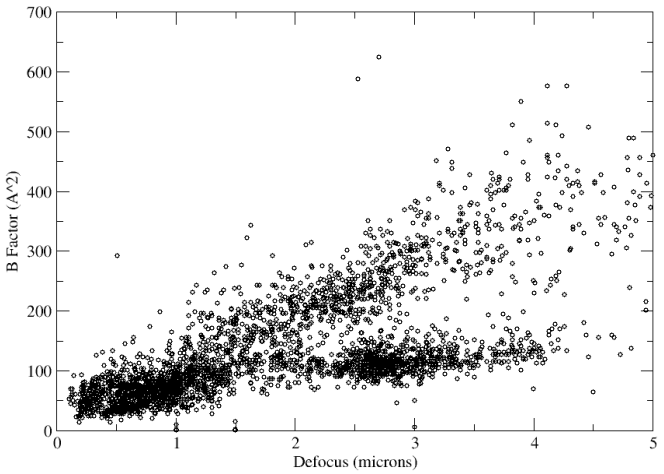
plot bfactor vs truedefocus where truedefocus is between 0.1 and 5.0 and bfactor is between 1 and 1000

99



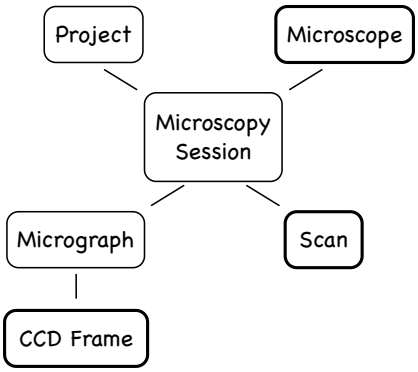
100



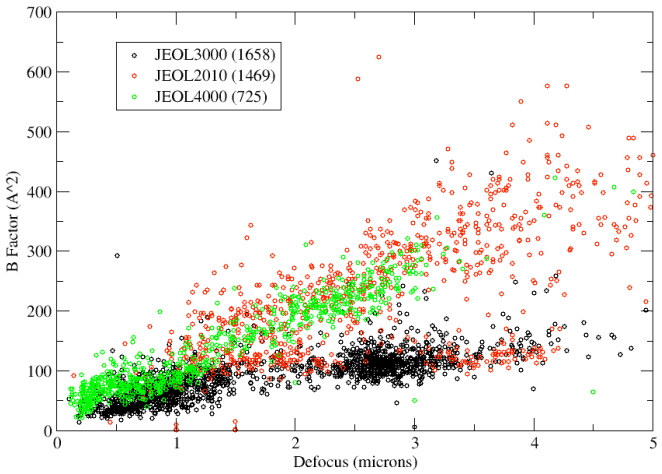
101

plot bfactor vs truedefocus where truedefocus is between 0.1 and 5.0 and bfactor is between 1 and 1000split by microscope

102



103



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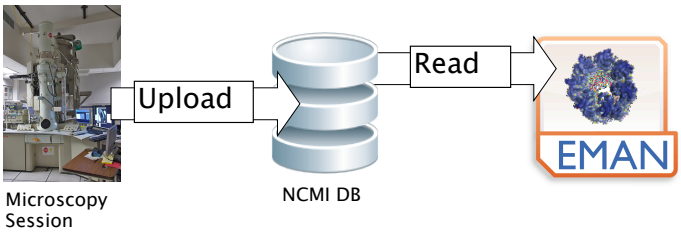
GOAL: Integrate with EMAN2



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GOAL: Integrate with EMAN2

Microscopy Session

Upload

NCMI DB

Read

Write

EMAN

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GOAL: Harvest Data for Export

NCMI DB

XML

WORLDWIDE PDB PROTEIN DATA BANK

EMDB EM DATA BANK

Value	EMEN2 Parameter	EMDB Name
	microscope: 76	
JEOL 3000SFF	tem_name	microscope
	microscopy: 204173	
kodak SO163 film	film_type	detector
	grid_imaging: 204174	
4.2 K	temperature_specimen	temperature
	micrograph: 204280, 204281, etc.	
25 e/A^2	tem_dose	electronDose
500 nM	ctf_defocus_set	(range) nominalDefocusMin
1200 nM		nominalDefocusMax

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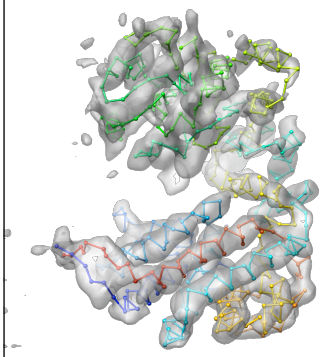
Acknowledgements



EMEN2

- Ian Rees
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- Wah Chiu

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- Guang (Grant) Tang
- Liwei Peng
- Ian Rees
- Phil Baldwin
- Deepy Mann
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- Julien Bert (UTH)
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- Justus Loerke (Charité)
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