Single Particle Refinement Strategies: Resolution, Heterogeneity and Dynamics

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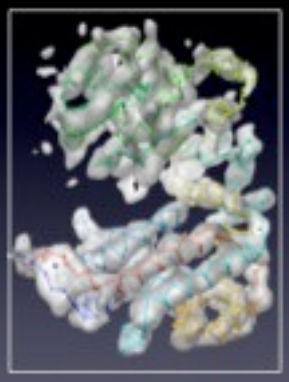
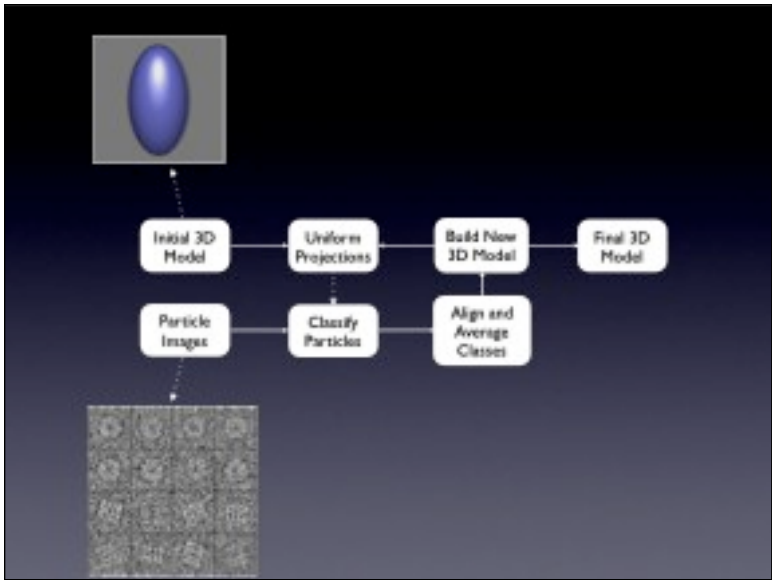
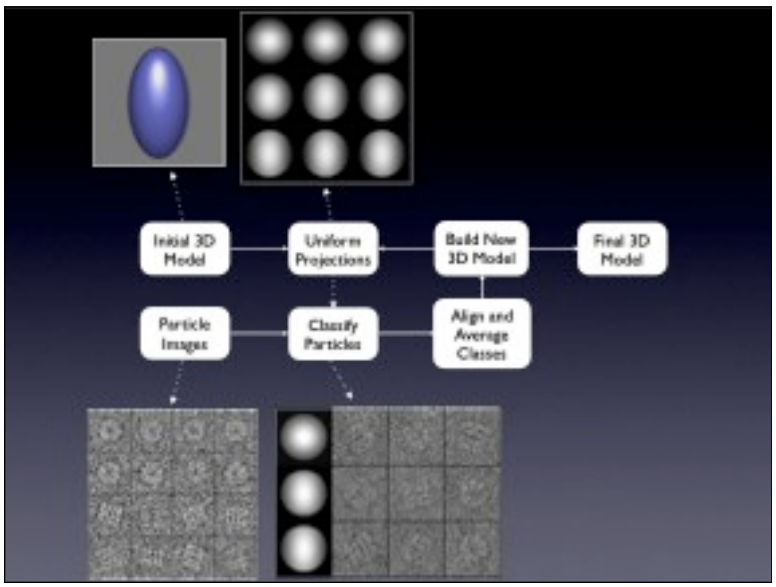
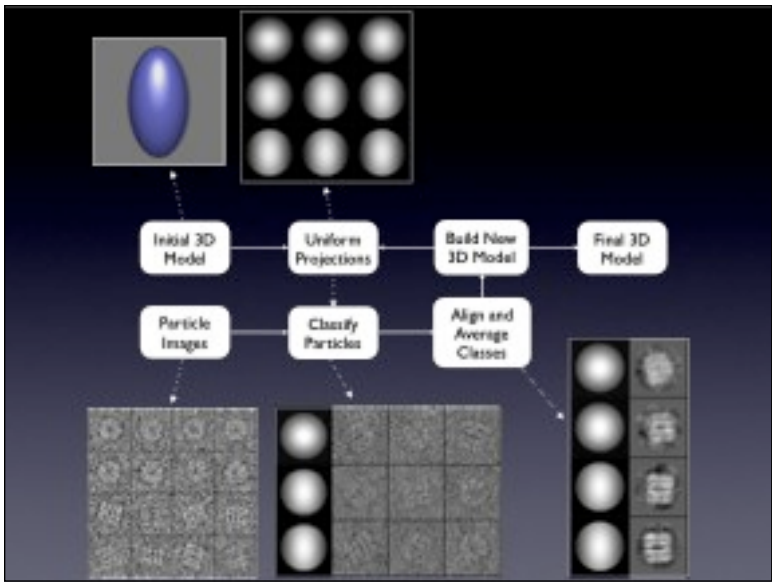
Baylor College of Medicine

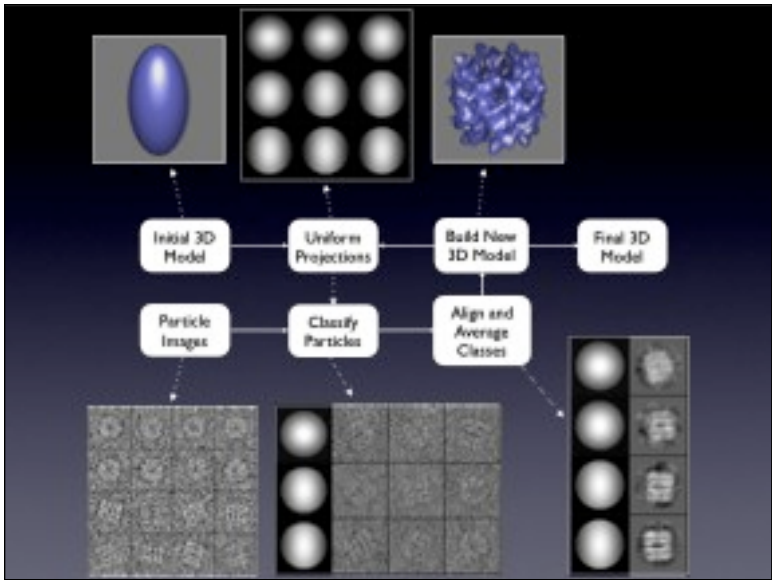


BCM NCRR NCMI NIH Roadmap






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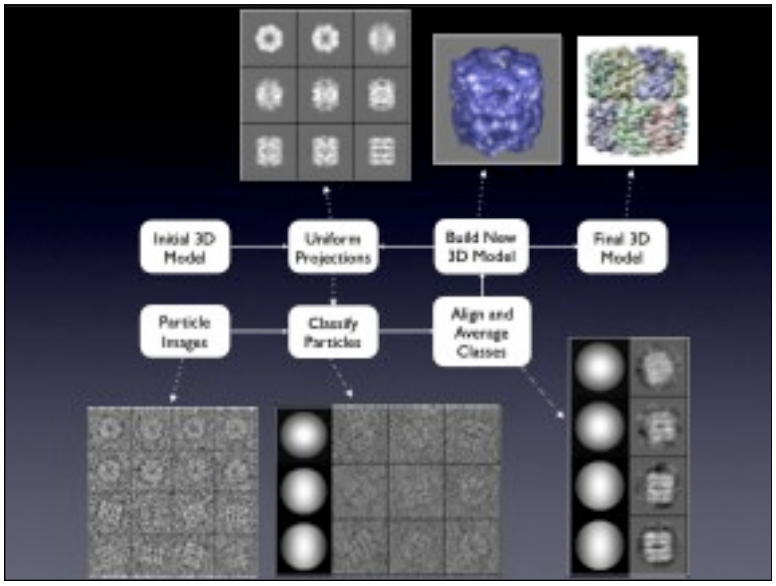
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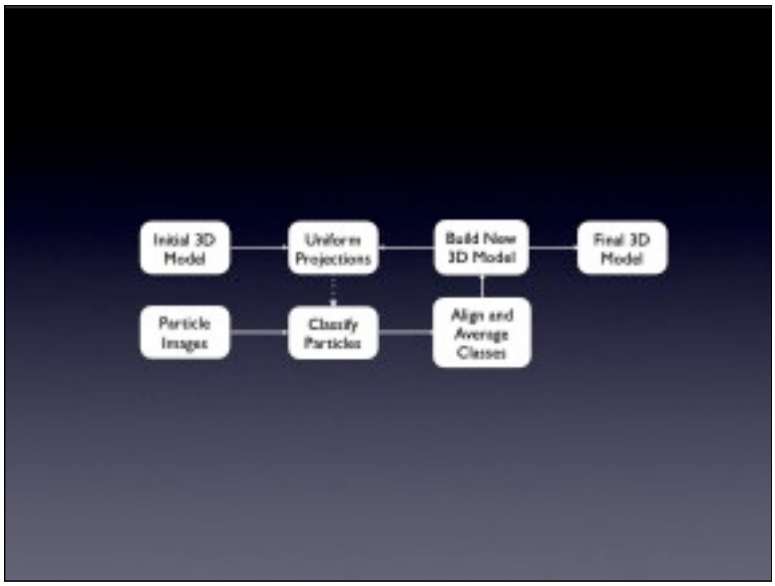
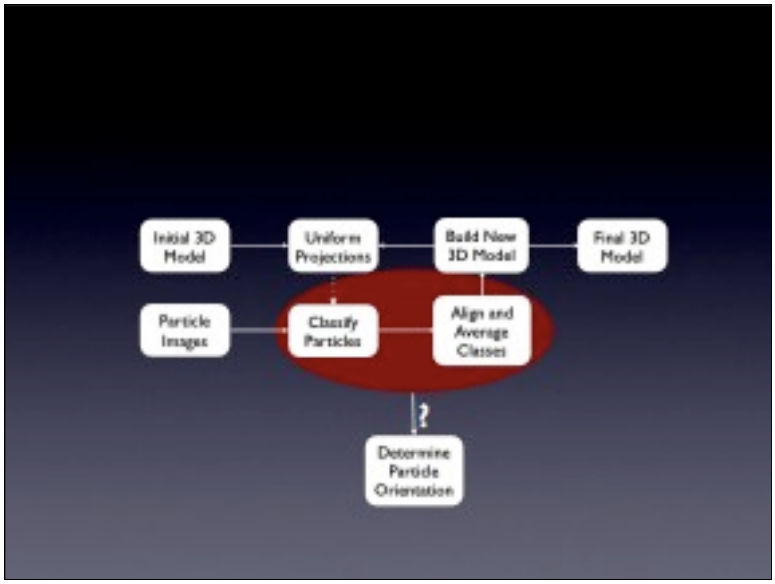
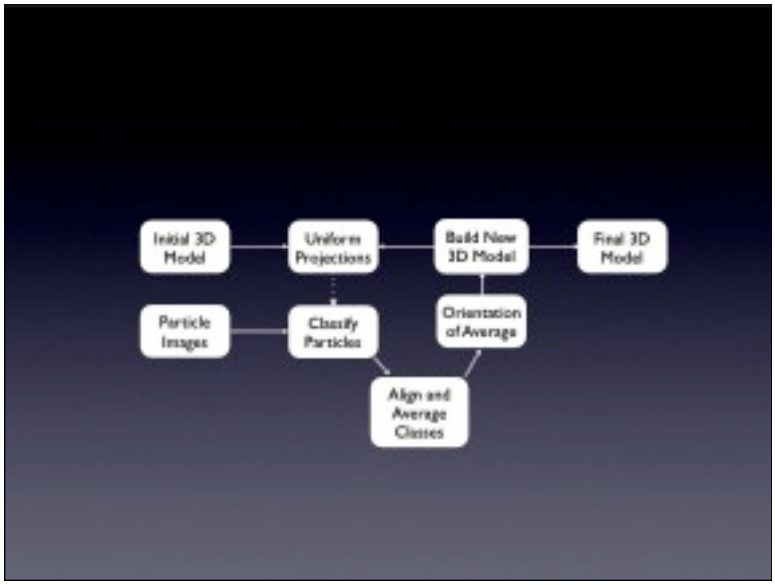
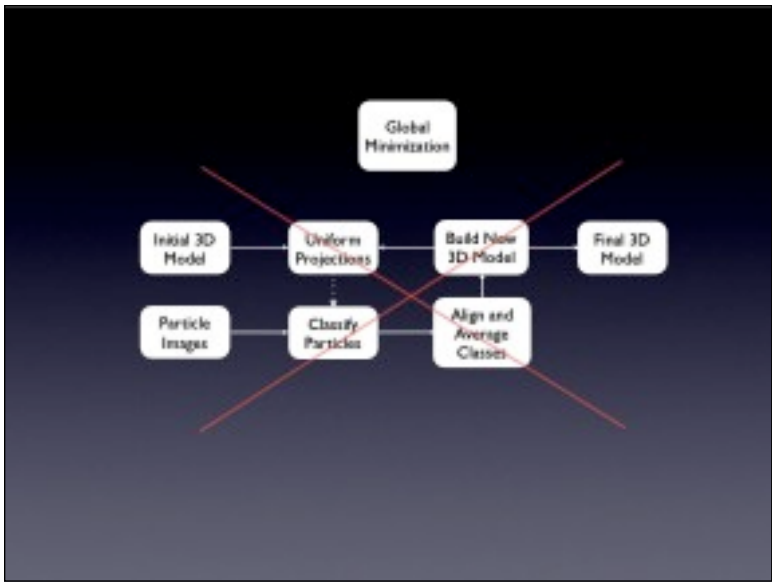
Align and Average Classes


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

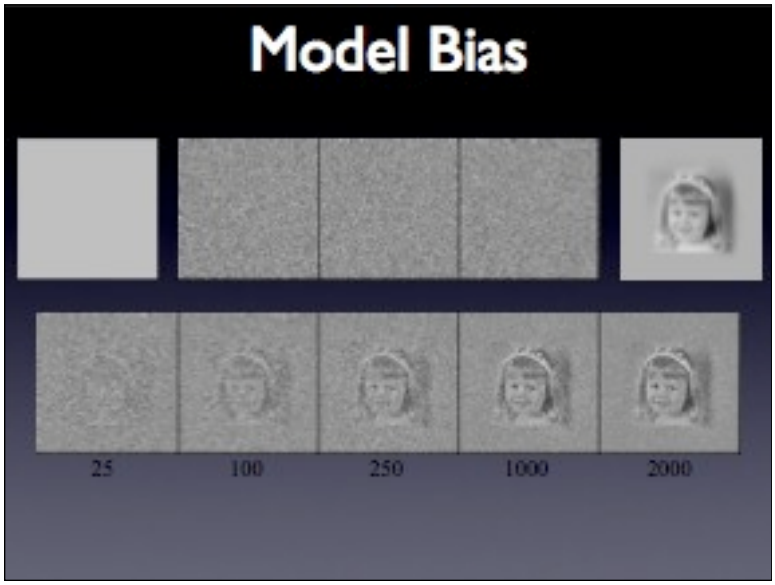

Initial 3D Model
Uniform Projections
Build New 3D Model
Final 3D Model
Particle Images
Classify Particles
Align and Average Classes
?
Determine Particle Orientation

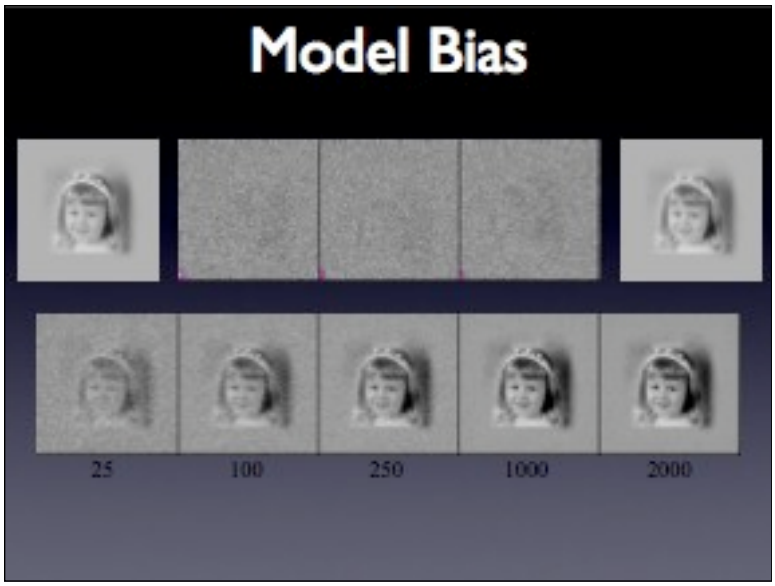

Initial 3D Model
Uniform Projections
Build New 3D Model
Final 3D Model
Particle Images
Classify Particles
Orientation of Average
Align and Average Classes

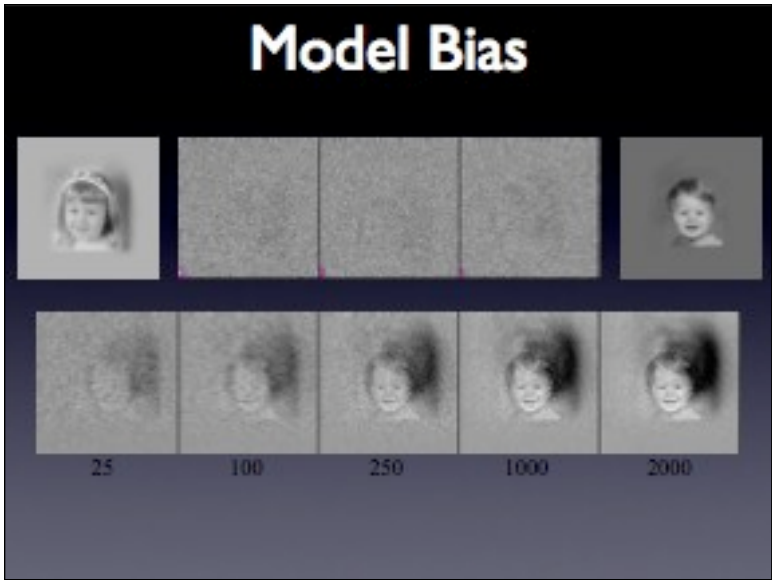


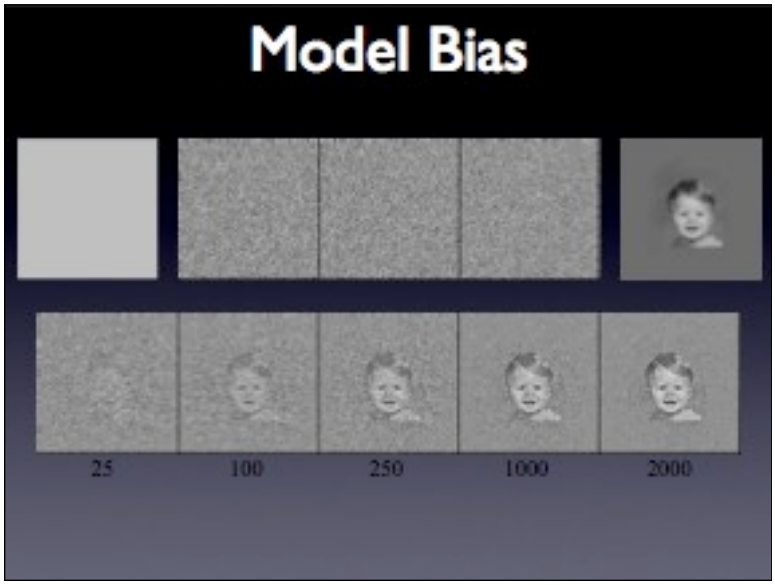
Model Bias ?

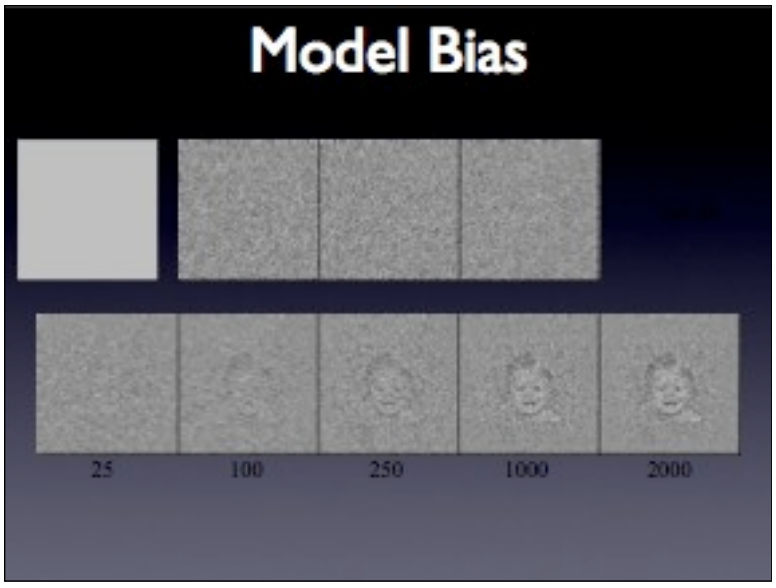
Model Bias

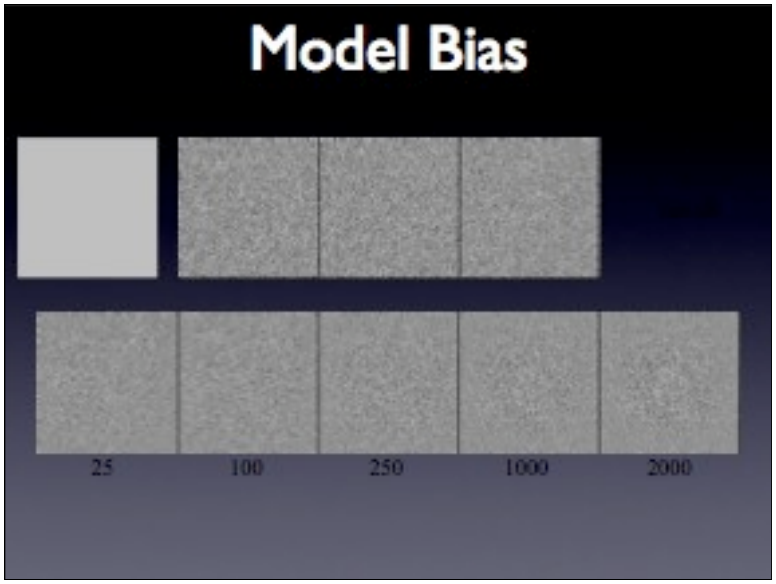


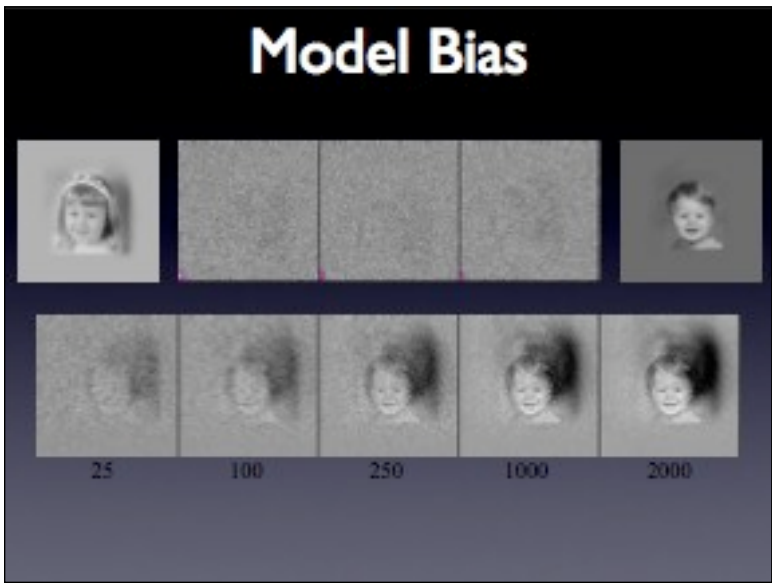

Model Bias
25
100
250
1000
2000

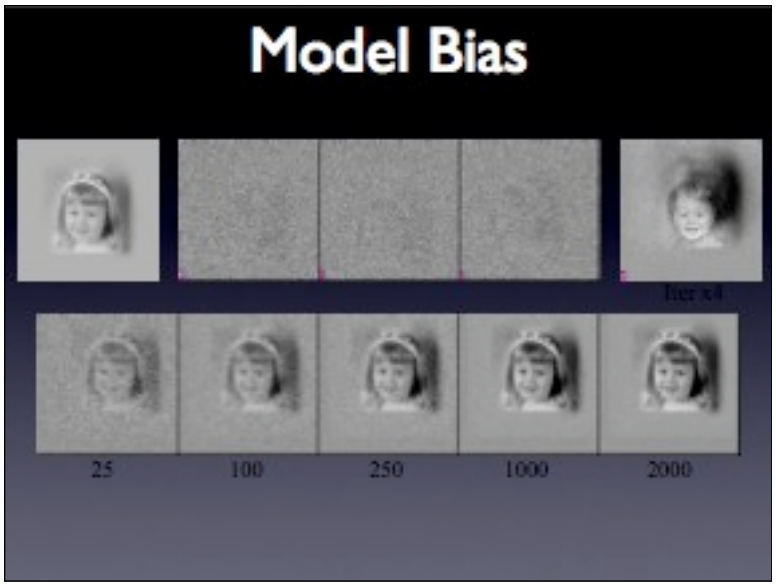

Model Bias
25
100
250
1000
2000

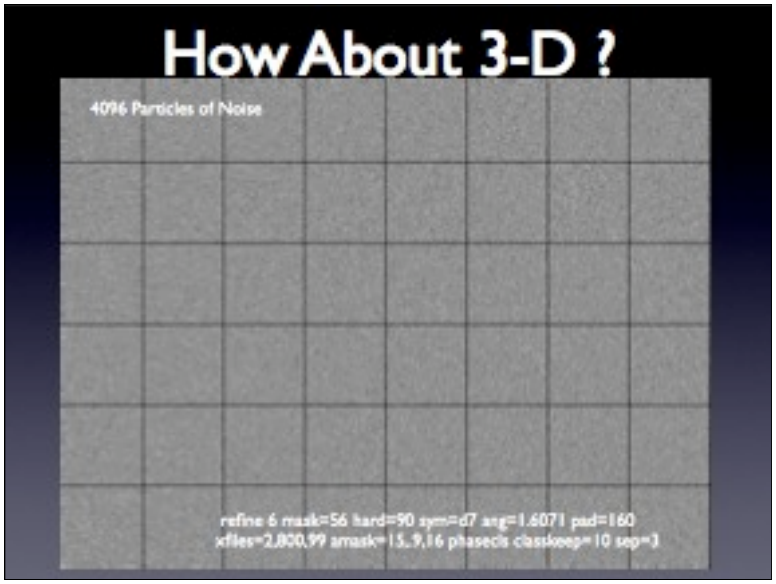

Model Bias
25
100
250
1000
2000

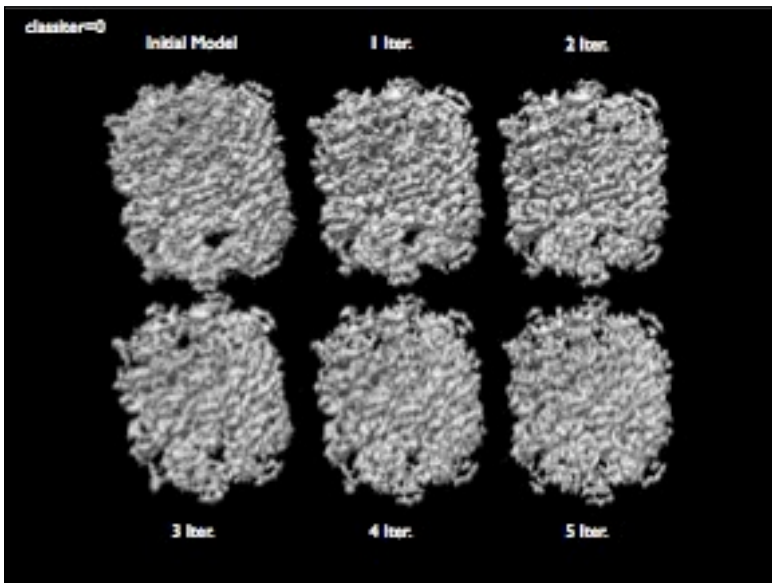

Model Bias
25
100
250
1000
2000

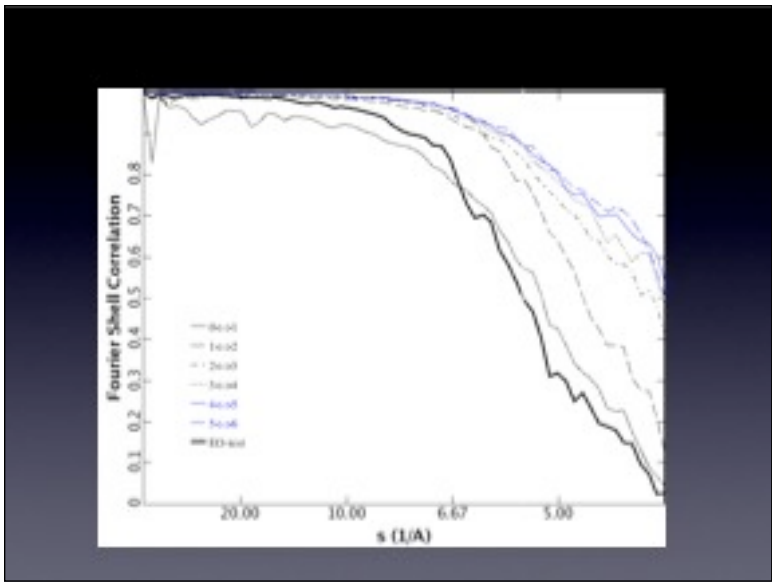

Model Bias
25
100
250
1000
2000

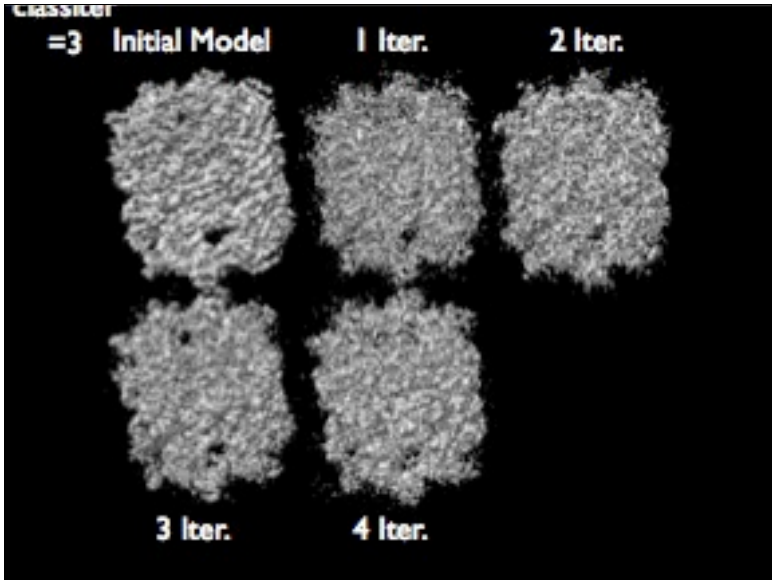

Model Bias
25
100
250
1000
2000

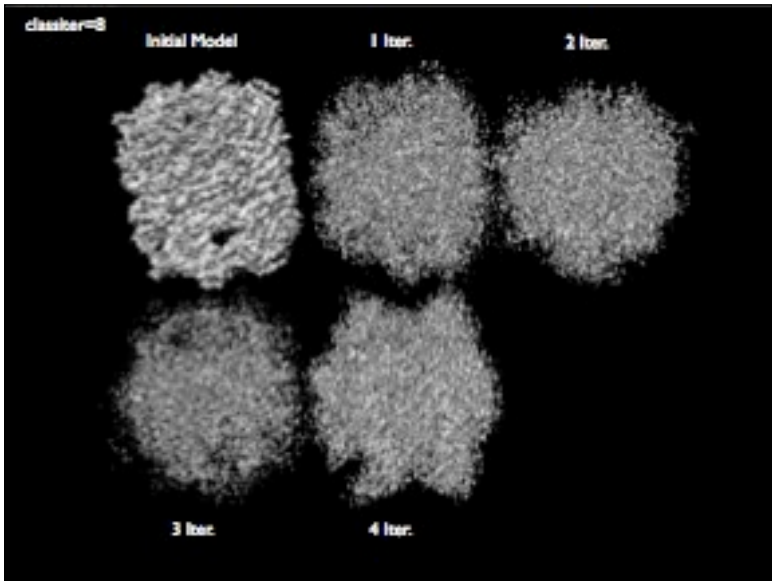

Model Bias
Iter x4
25
100
250
1000
2000

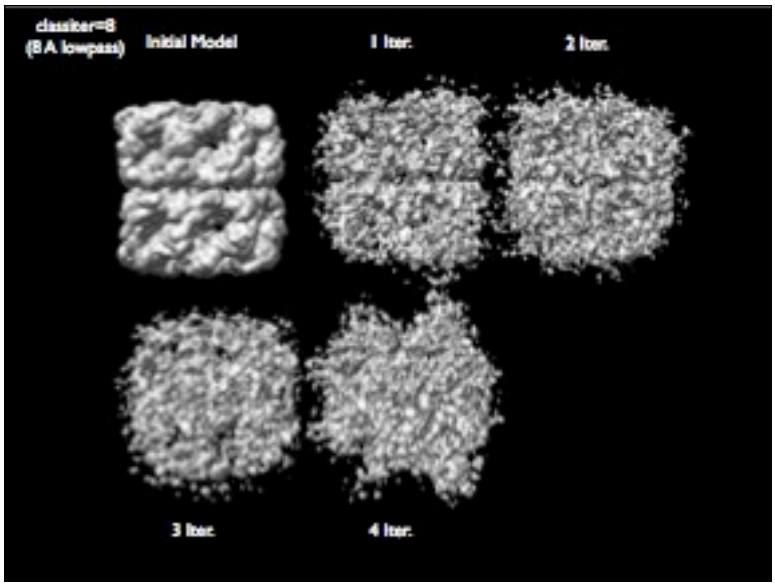

How About 3-D ?
4096 Particles of Noise
refine 6 mask=56 hard=90 sym=d7 ang=1.6071 pad=160
xfiles=2,800,99 amask=15,9,16 phasecls classkeep=10 sep=3

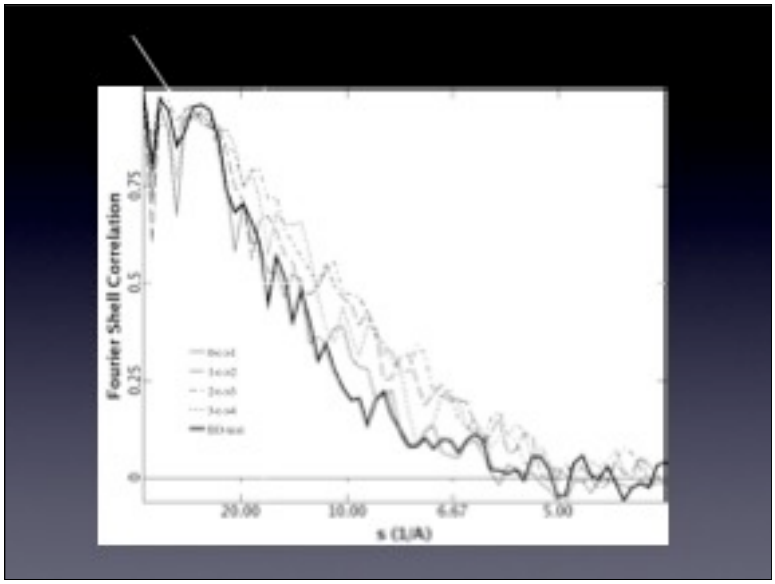

classiter=0
Initial Model
1 Iter.
2 Iter.
3 Iter.
4 Iter.
5 Iter.

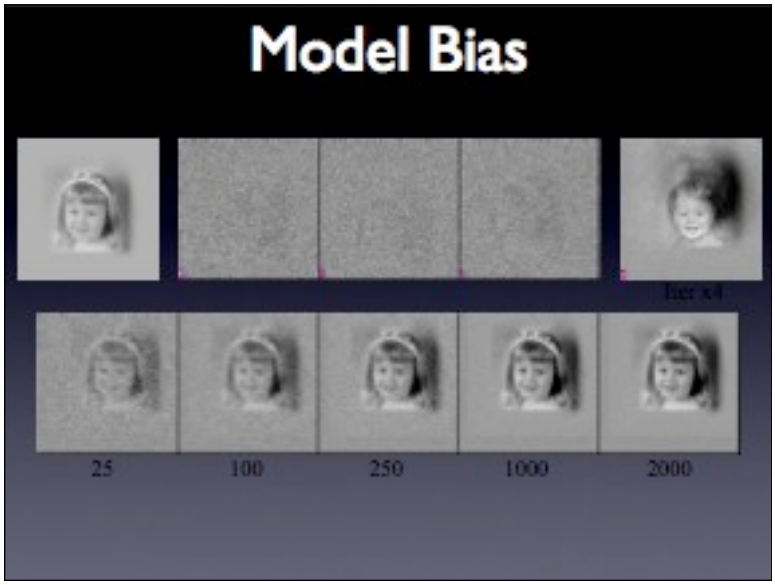

Fourier Shell Correlation
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
20.00
10.00
6.67
5.00
s (1/A)


=3
Initial Model
1 Iter.
2 Iter.
3 Iter.
4 Iter.


classker=8
Initial Model
1 Iter.
2 Iter.
3 Iter.
4 Iter.


classker=8
(8 A lowpass)
Initial Model
1 iter.
2 iter.
3 iter.
4 iter.


Fourier Shell Correlation
0.75
0.5
0.25
0
20.00
10.00
6.67
5.00
s (1/A)


Model Bias
Iter x4
25
100
250
1000
2000

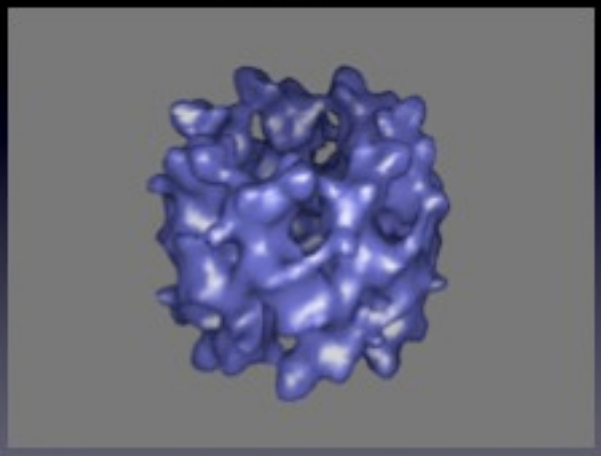
How Do we Stop This ?

- (In EMAN) use classiter>3 for a few rounds
- Always refine from multiple starting models

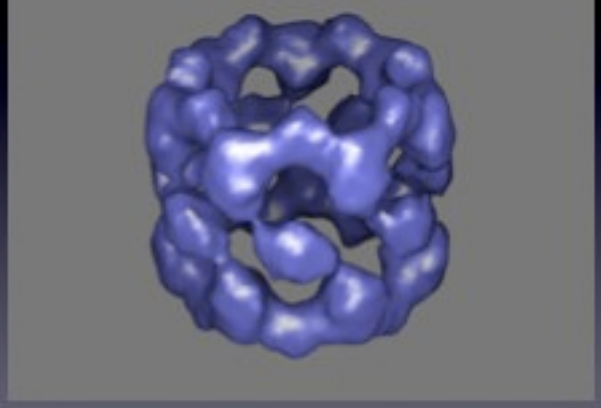
  (note - you can shrink the data first)
- If the results are not effectively the same, try to establish which one is correct by looking at self consistency of projections/class-averages
- Compare with results of 2D analysis

Refine from Gaussian Ellipsoid

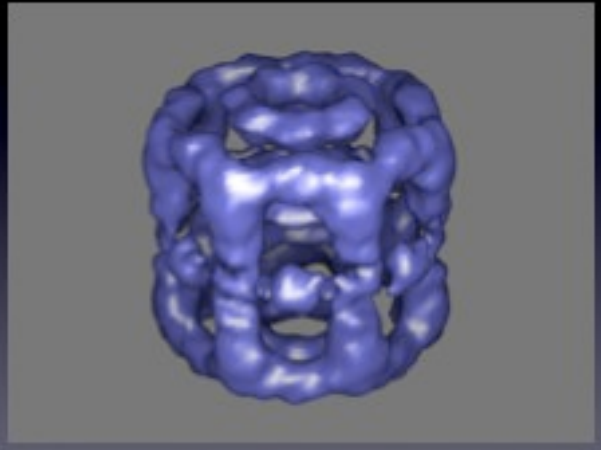
Iteration 1



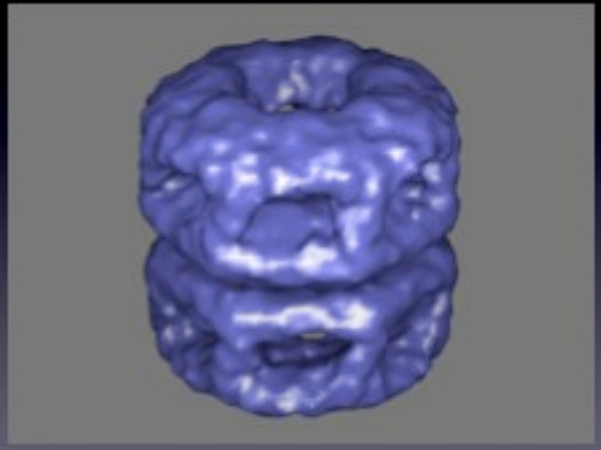
Iteration 2



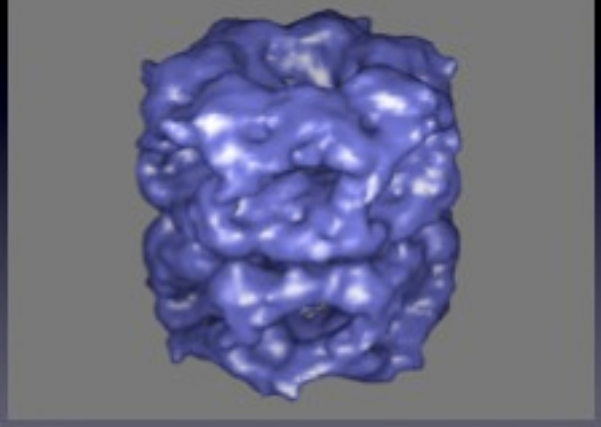
Iteration 3

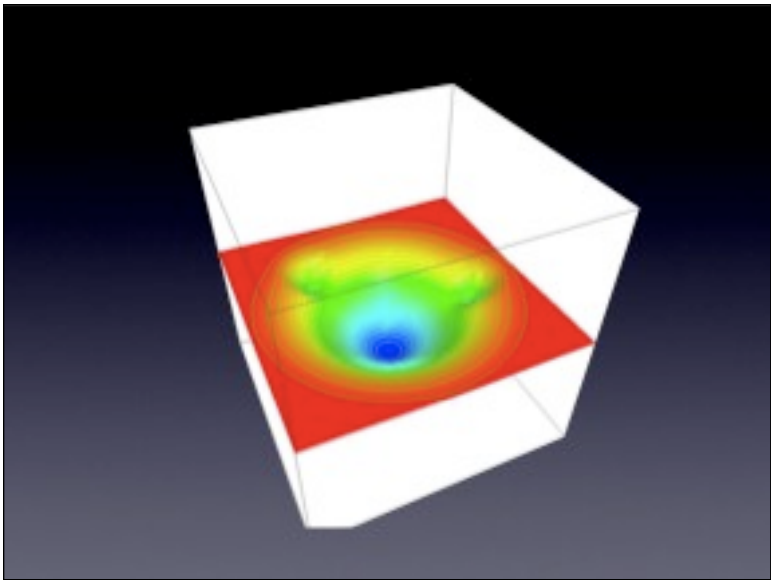


Iteration 4

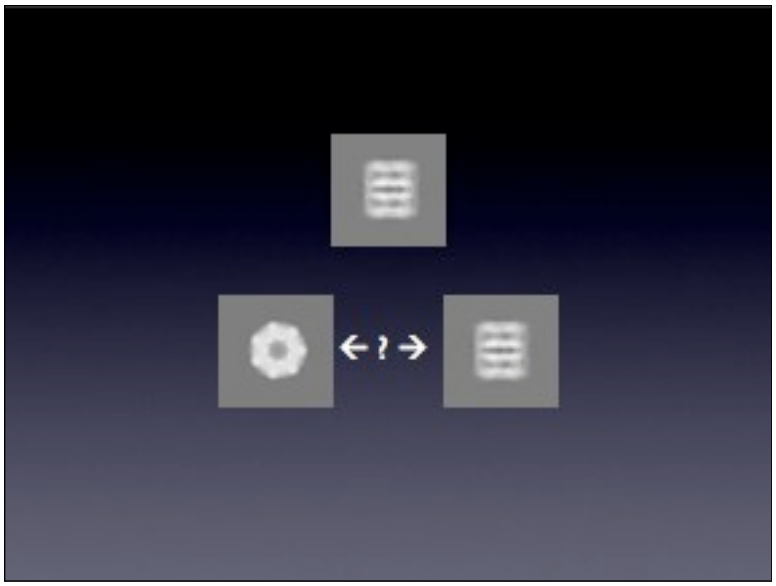


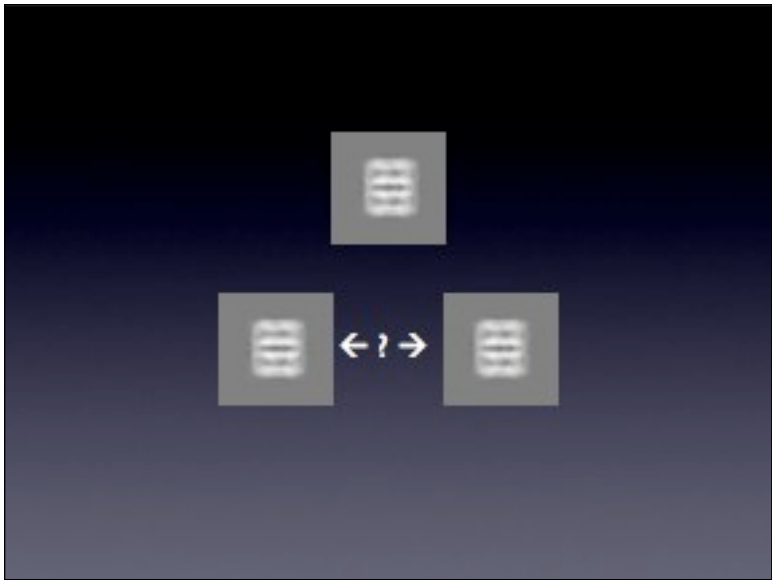
Iteration 5

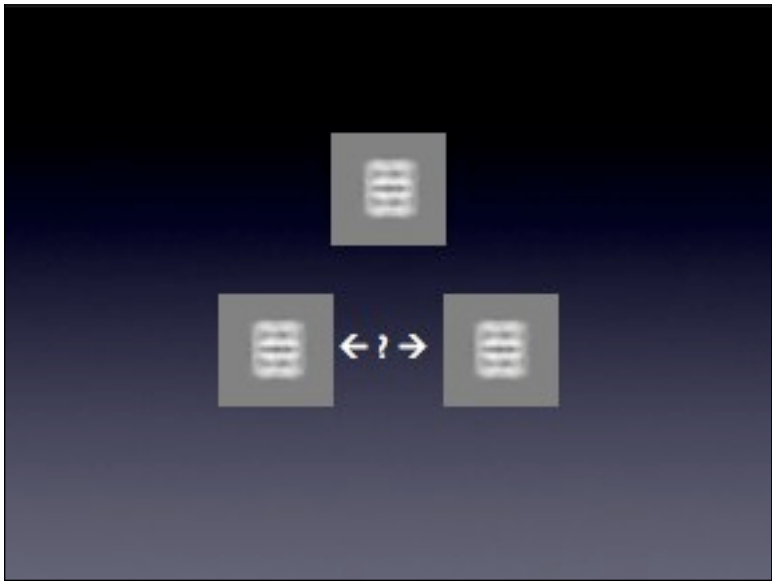




Measures of Similarity

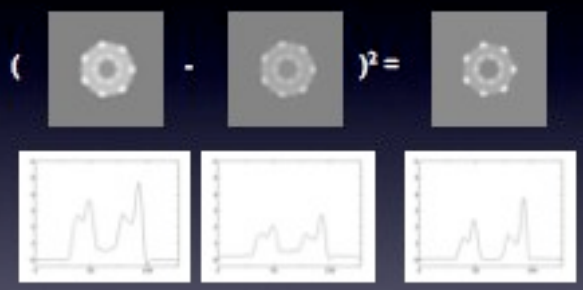


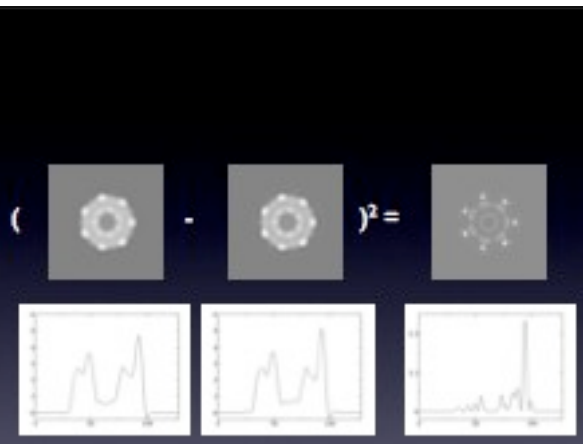


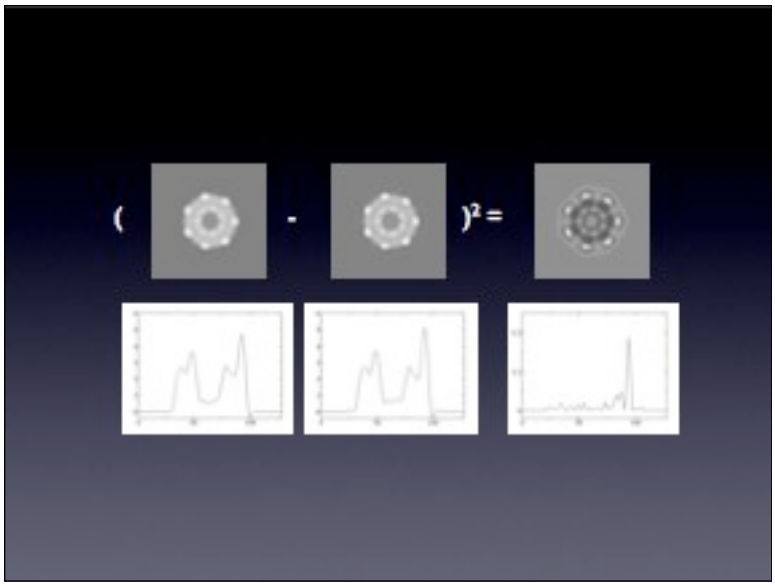


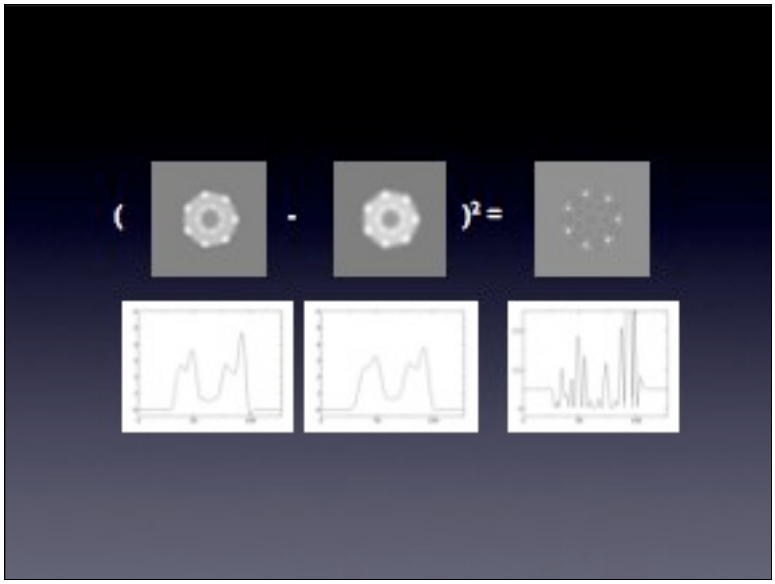
Measures of Similarity

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









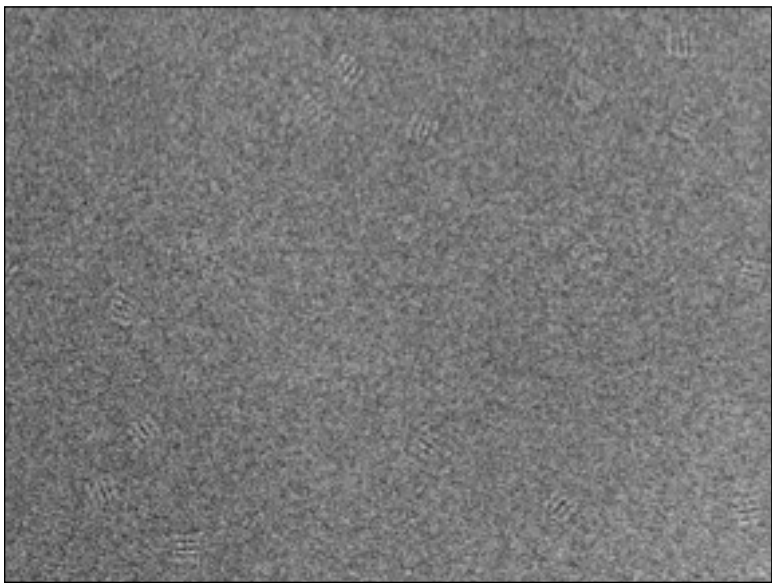
One Answer ...

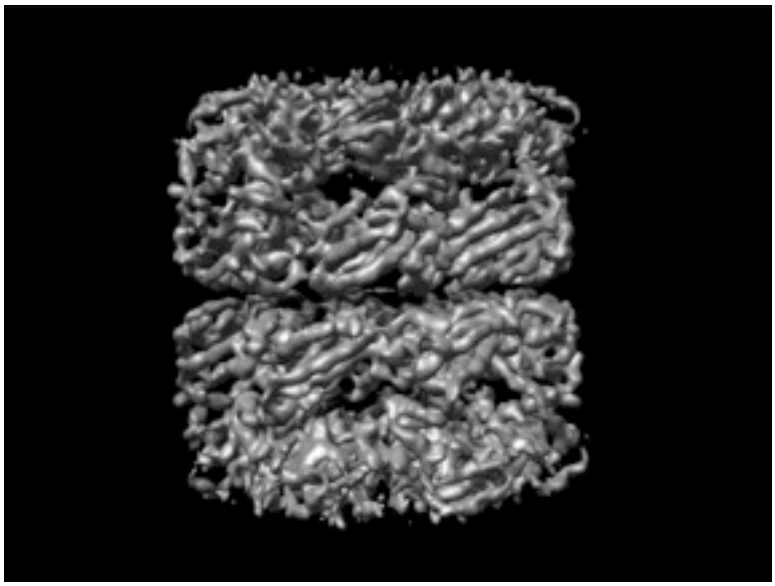
- Wiener filter particle
- Filter reference to match
- Normalize reference density to particle
- Calculate variance

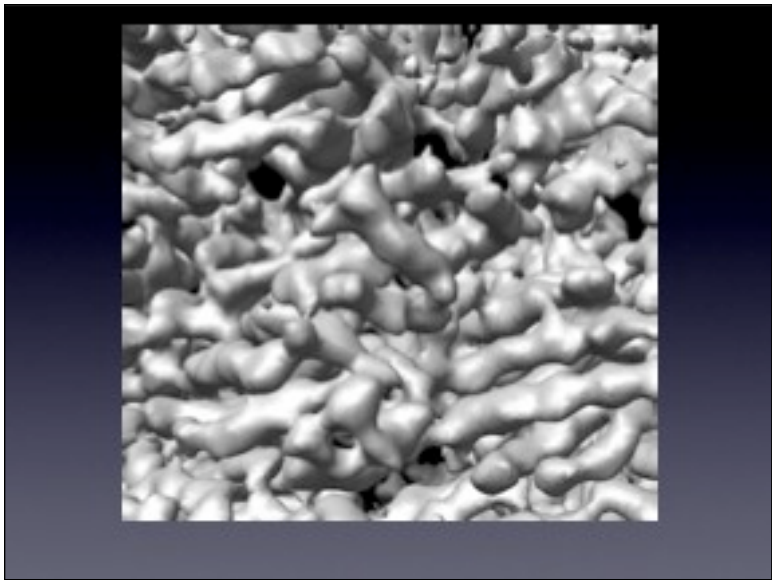
Resolution

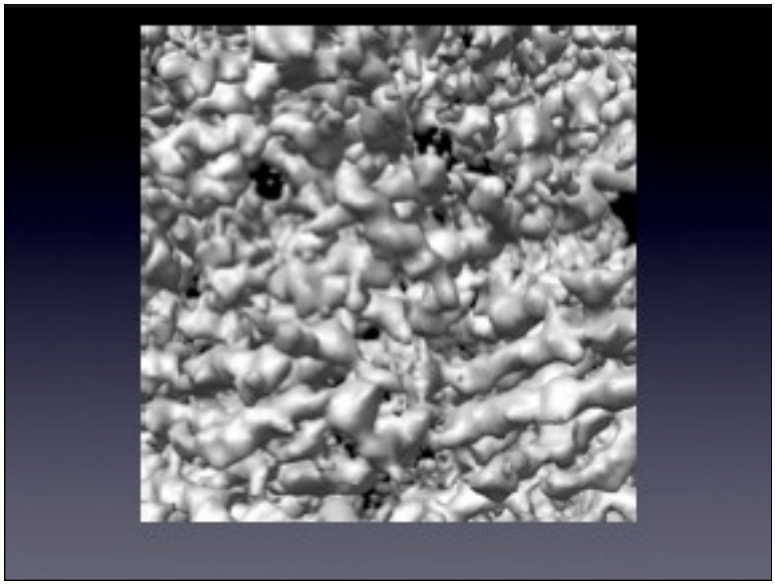
Data Collected 2005

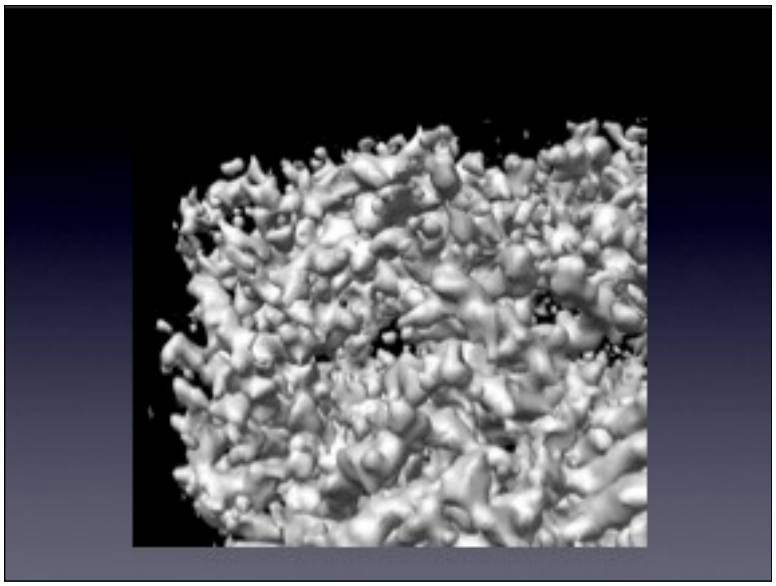
- Native, unliganded GroEL, no ATP/ADP (?)
- JEOL 3000SFF (Yoshi-style) at LHe temp
- 6 microscopy sessions, Film
- 825 micrographs, Nikon 9000 @ 6.35 µm scan step
- 60k mag → 1.06 Å/pix
- 135 micrographs used → 20,401 particles
- Defocus 1.2 – 2.2 µm

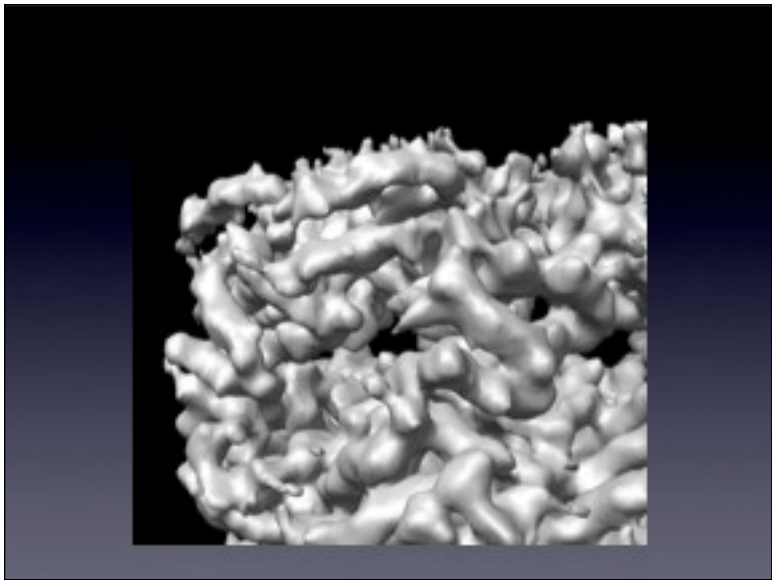


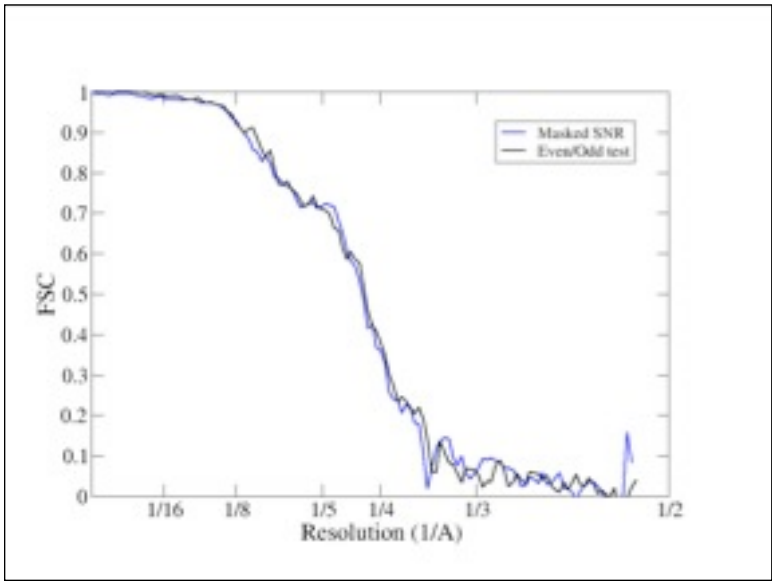


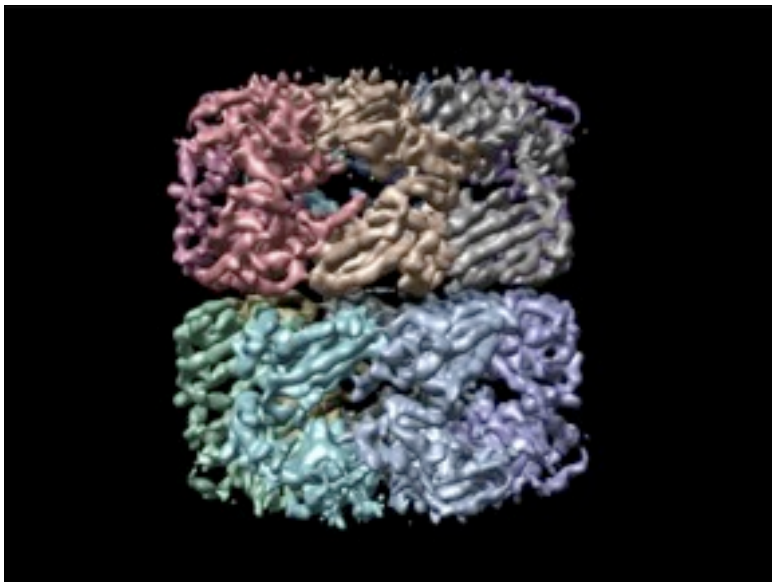


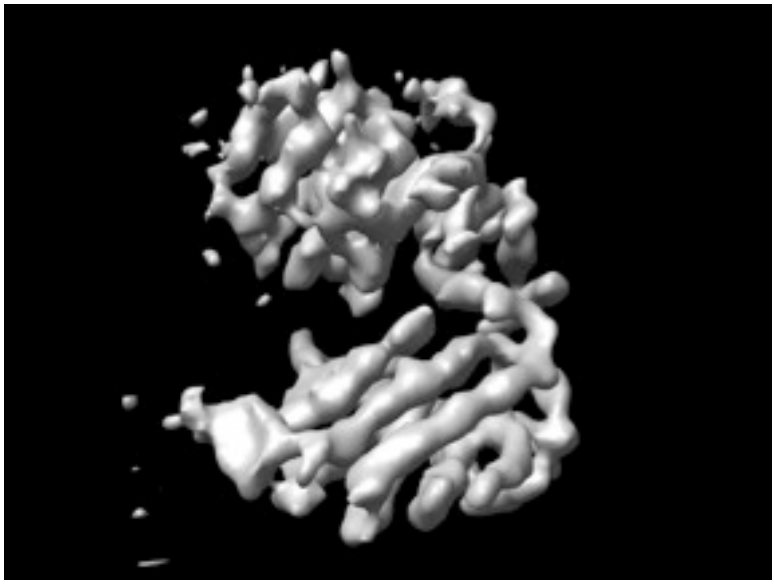


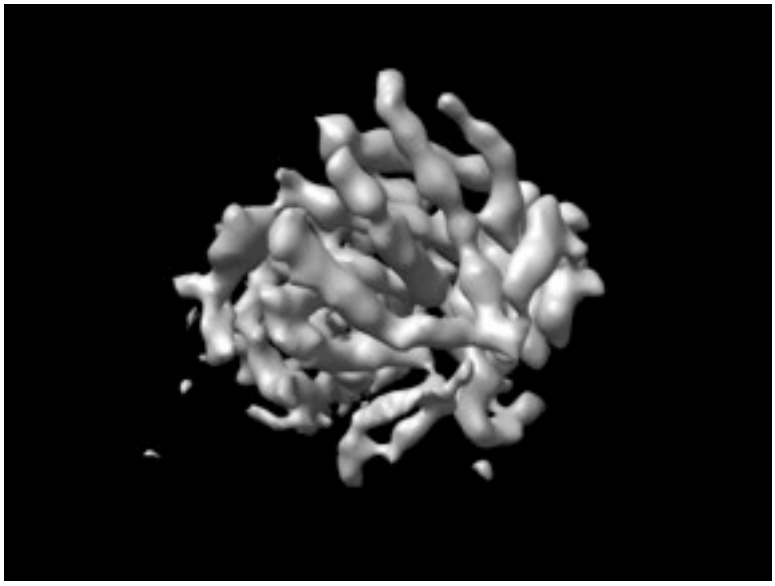




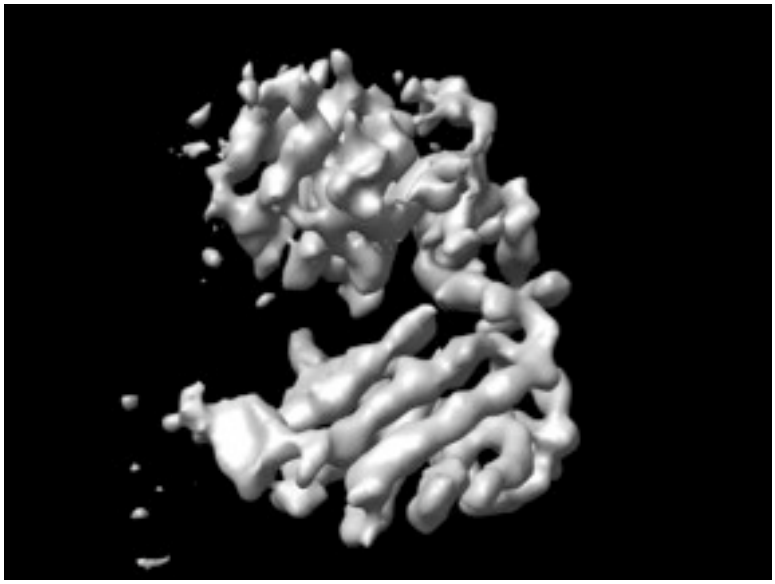

FSC
Resolution (1/A)
1/16
1/8
1/5
1/4
1/3
1/2
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Masked SNR
Even/Odd test

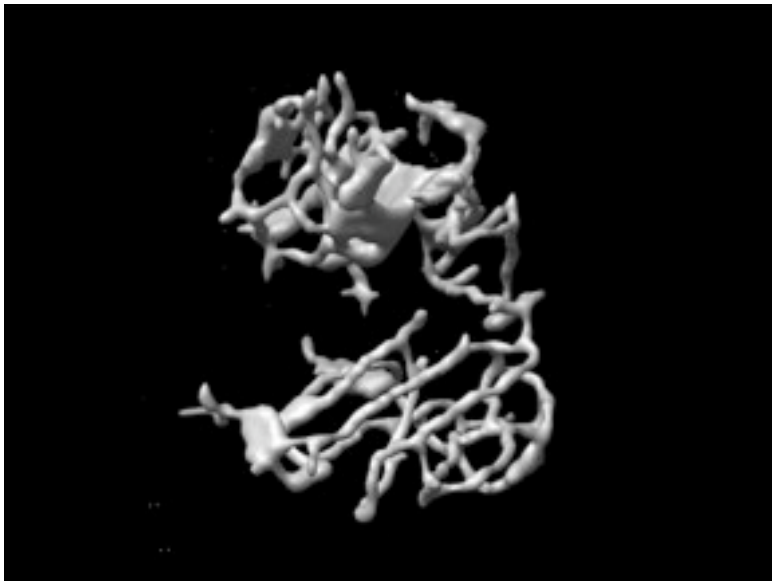






How much can we get at 4 Å ?



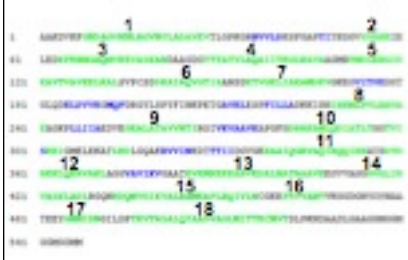


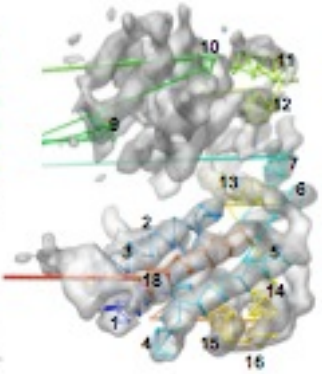
Sequence Based Secondary Structure Prediction

Put it all together

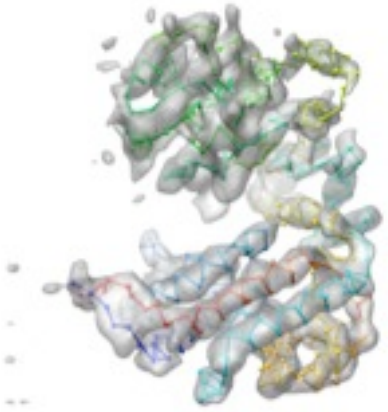
- SSEHunter
  - lengths of helices
- Skeleton
  - Pathlengths between helix endpoints, possible connectivity
- Sequence based Sec. Structure Pred.
  - Lengths of and maximum distances between helices. Relation to primary sequence. Errors ?
- MANY permutations,

Build the Model

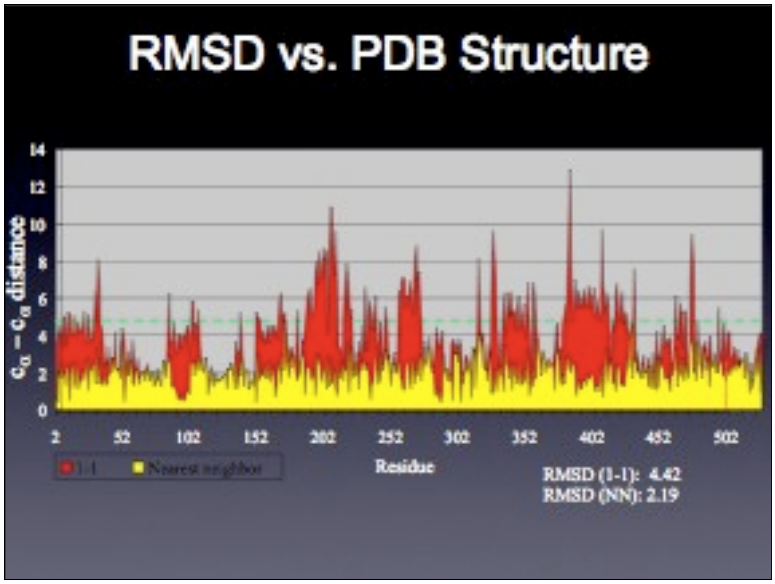


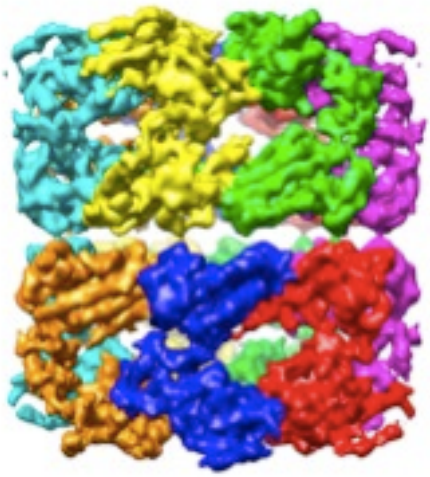


GroEL Chain Trace



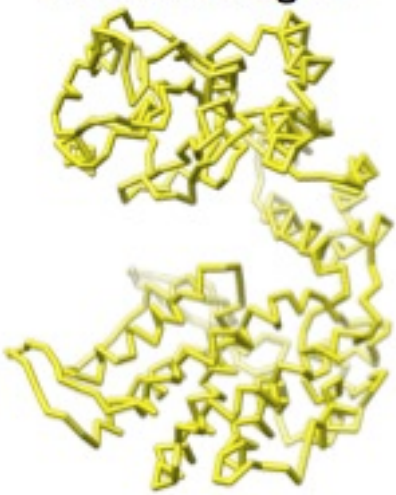
RMSD vs. PDB Structure



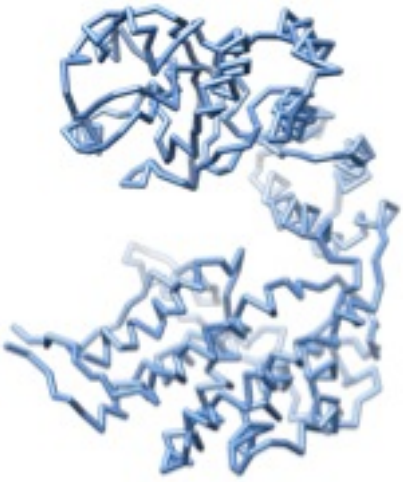


D7

Bottom Ring C7

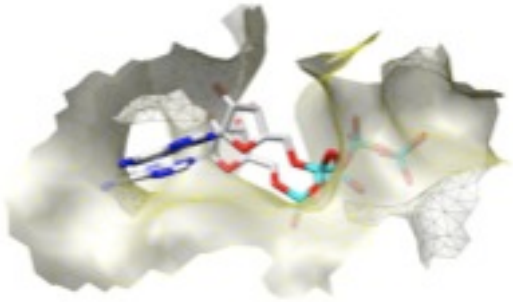


Top Ring C7



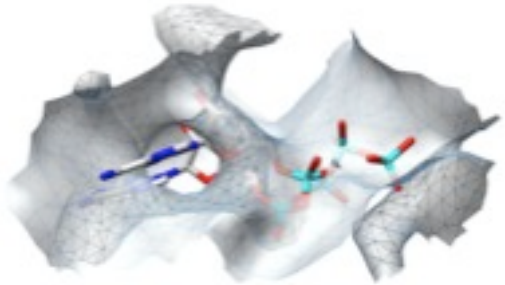
Bottom Ring

(ATP Binding Site)

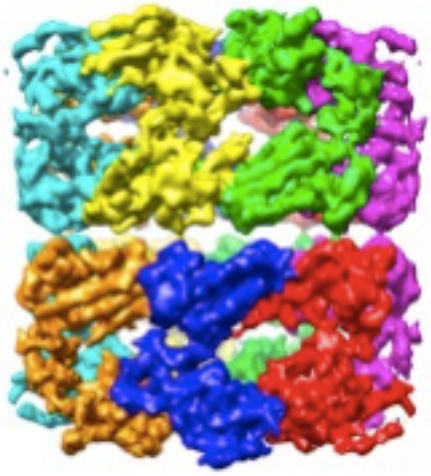


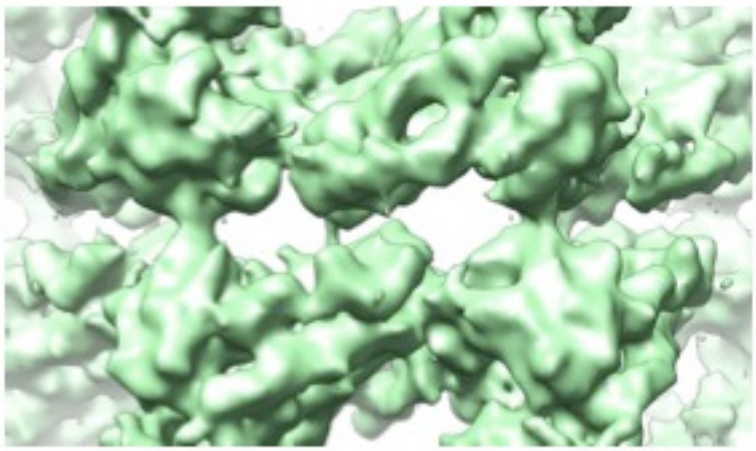
Top Ring

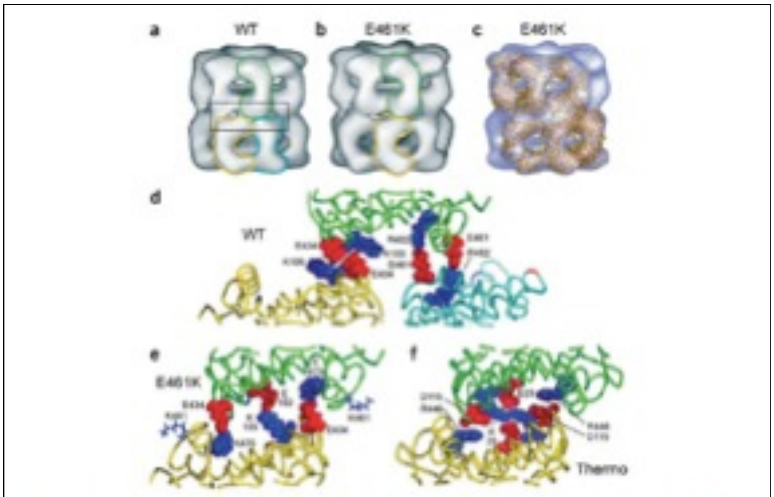
(ATP Binding Site)



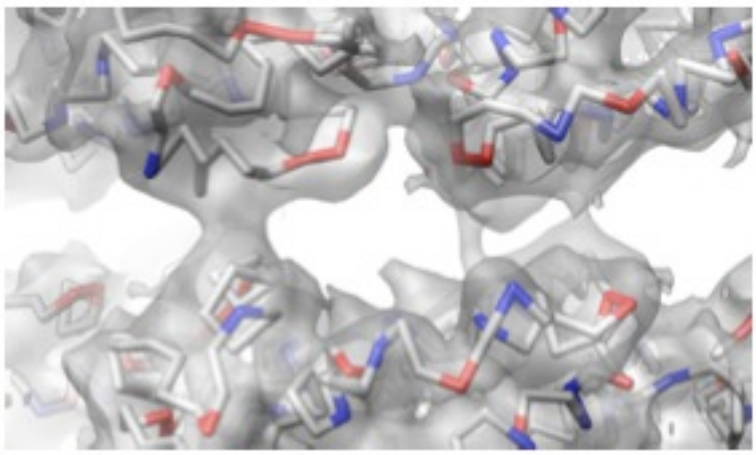
Inter-ring Salt Bridges ?

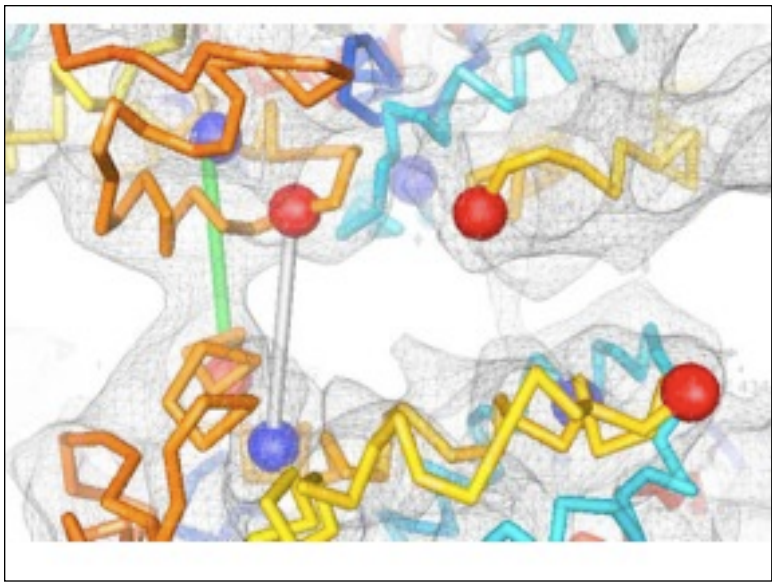






B Trevor Sewell, Robert B Best, Shaoxia Chen, Alan M Roseman, George W Farr, Arthur L Horwich & Helen R Saibil. (2004) **A mutant chaperonin with rearranged inter-ring electrostatic contacts and temperature-sensitive dissociation.** *Nature Structural & Molecular Biology* 11, 1128 – 1133.

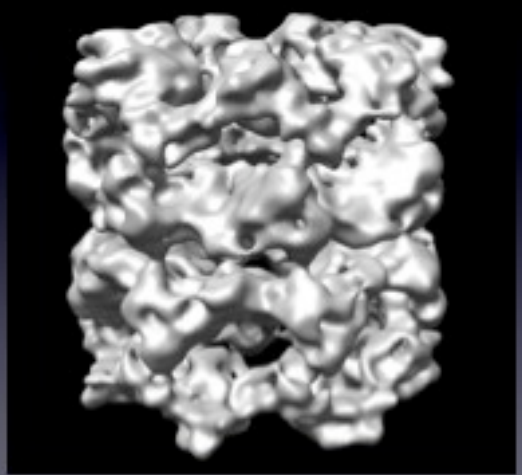




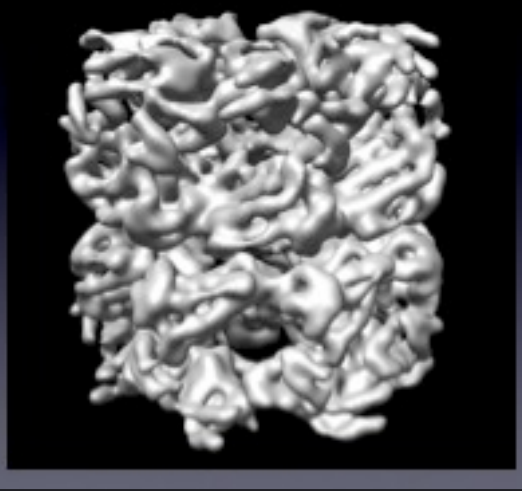
GroEL results

- ~4 Å resolution
- Single particle based Cα trace
- Asymmetric in solution
  - 1 Ring like apo crystal structure
  - Other ring similar to nucleotide bound state

11.5 Å GroEL



4 Å → 11.5 Å GroEL



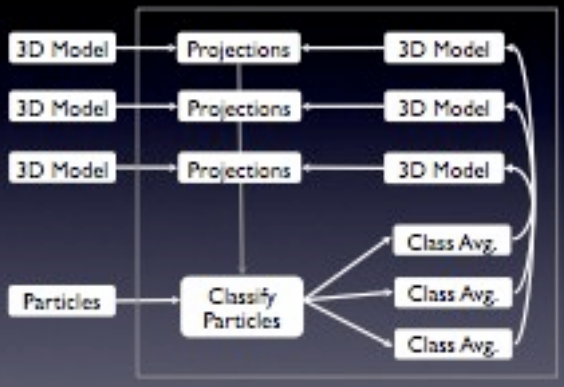
Heterogeneity/ Dynamics

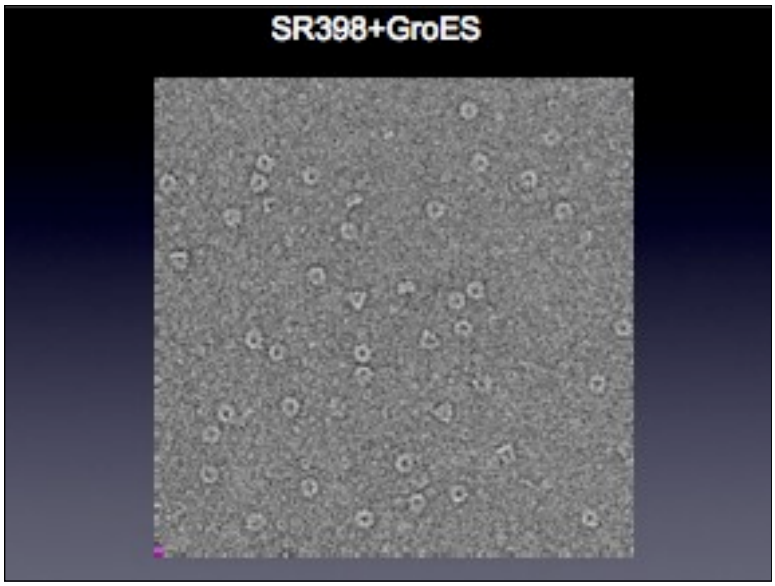
Start with an (active) GroEL Mutant:

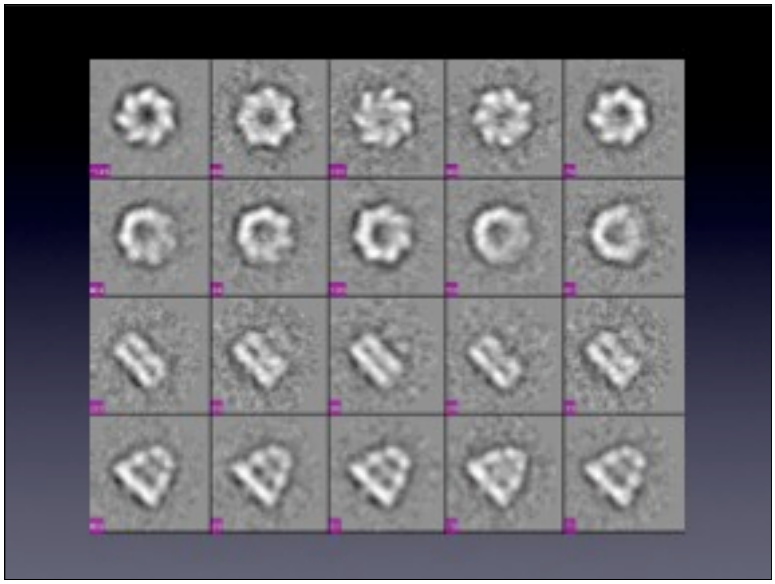
SR398 + GroES + ATP →

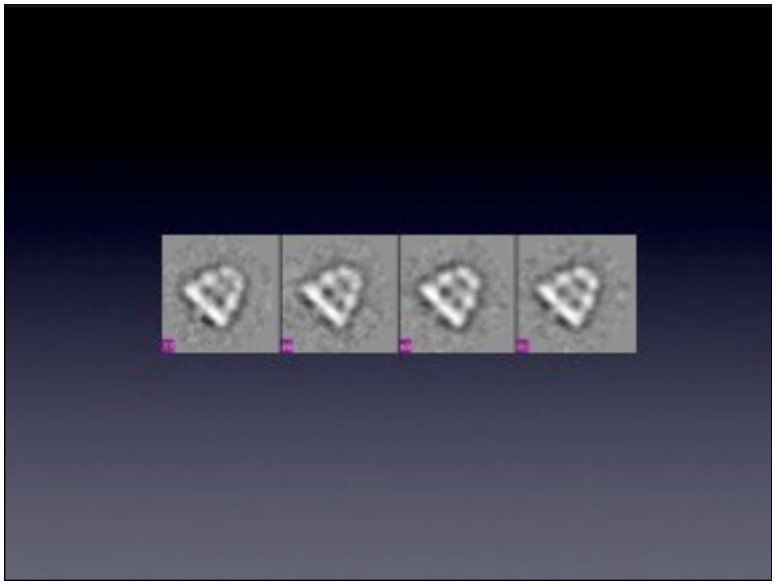
Produces a Mixed Population

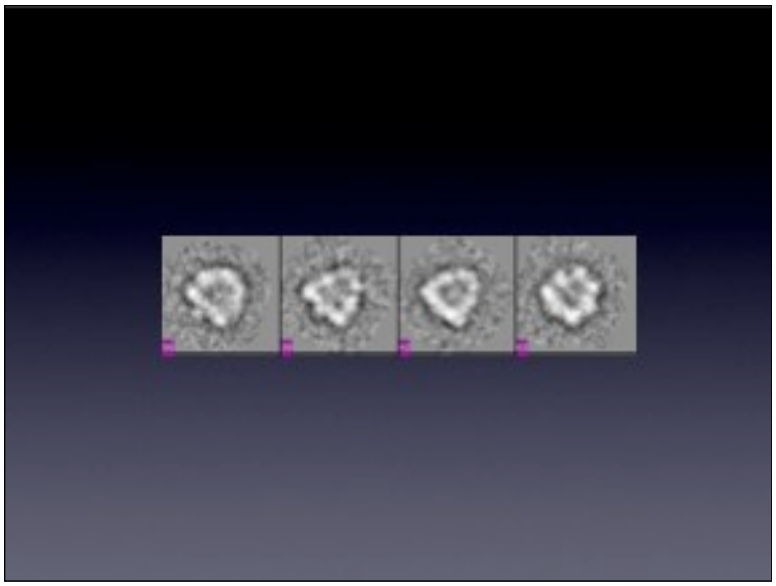
Control Expt. – No Substrate

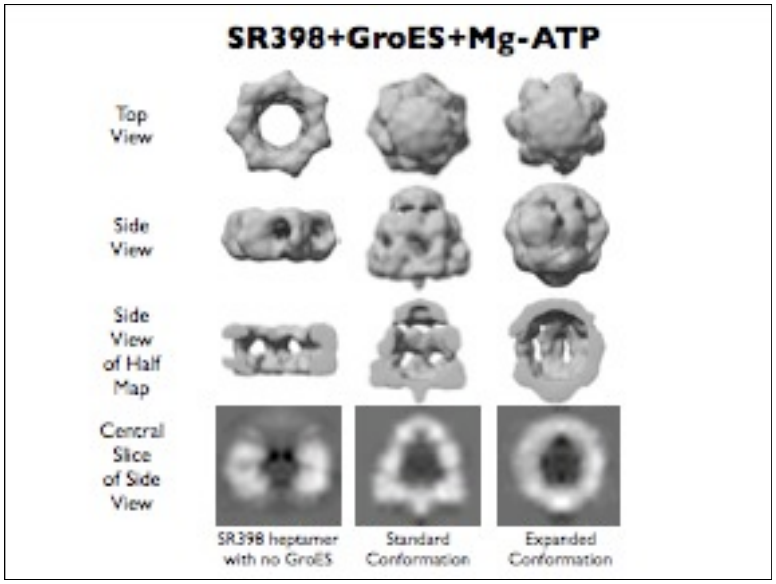


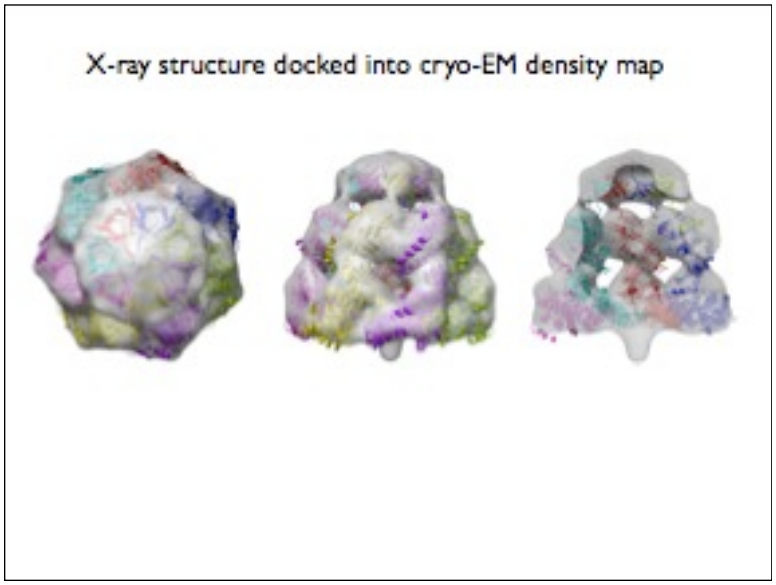

SR398+GroES



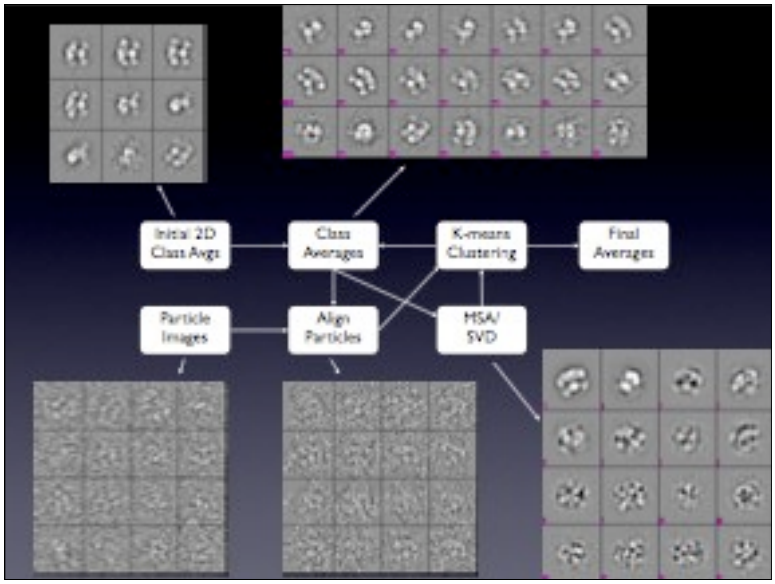




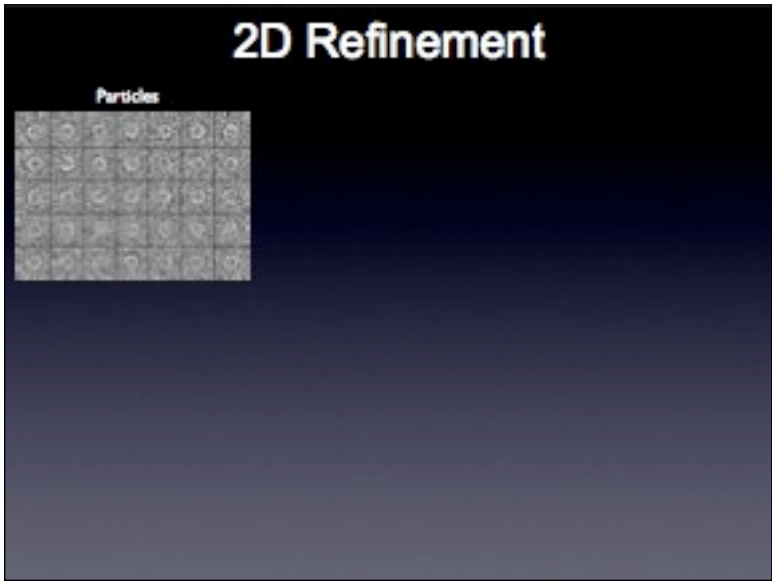

SR398+GroES+Mg-ATP
Top View
Side View
Side View of Half Map
Central Slice of Side View
SR398 heptamer with no GroES
Standard Conformation
Expanded Conformation

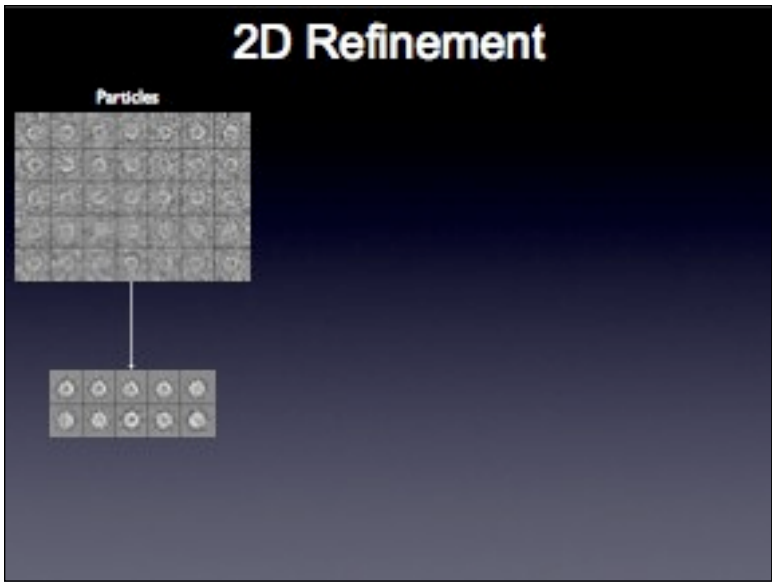

X-ray structure docked into cryo-EM density map

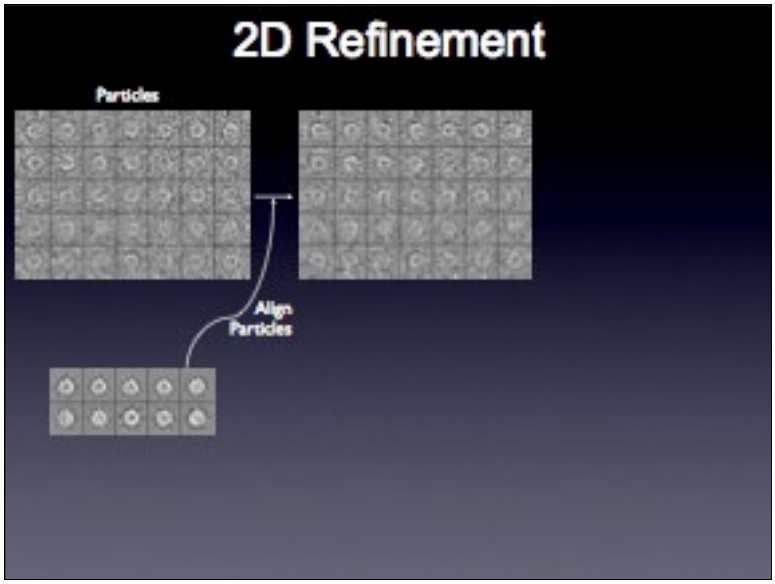
2D Analysis

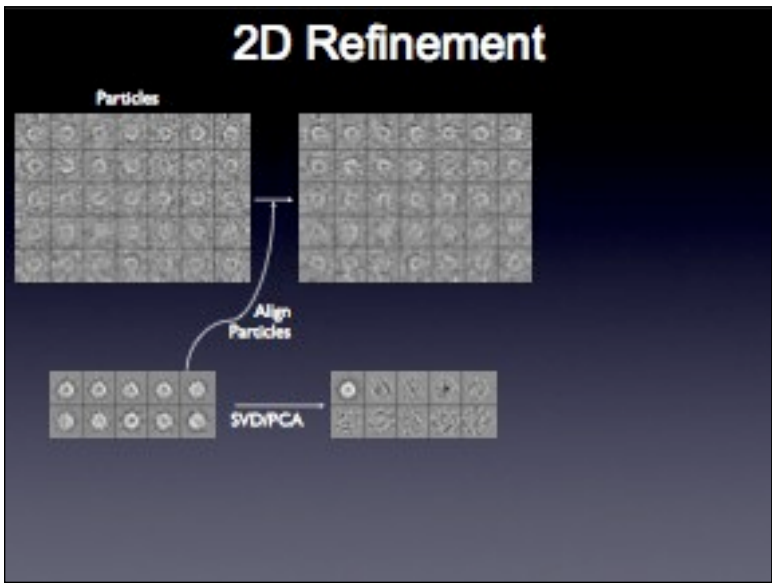


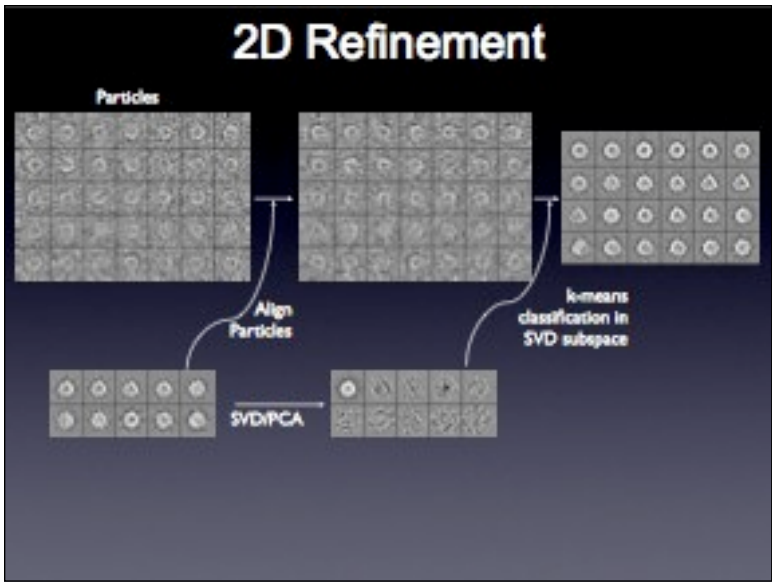
2D Refinement

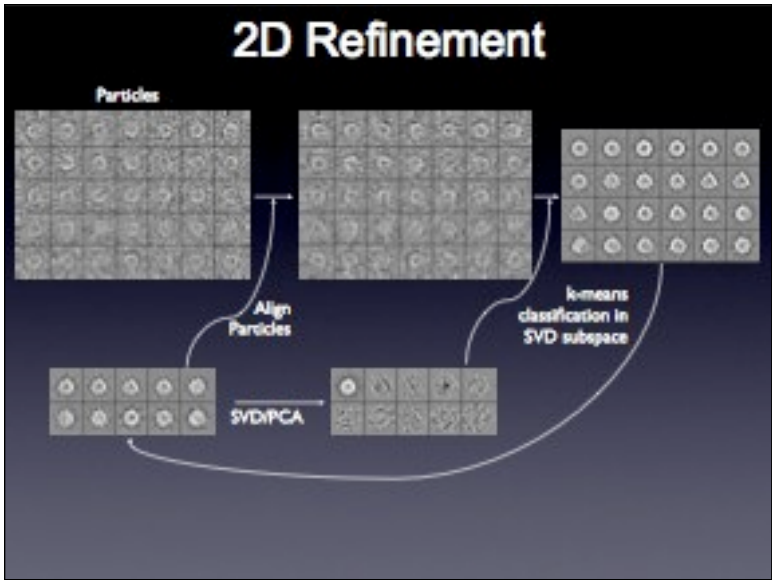


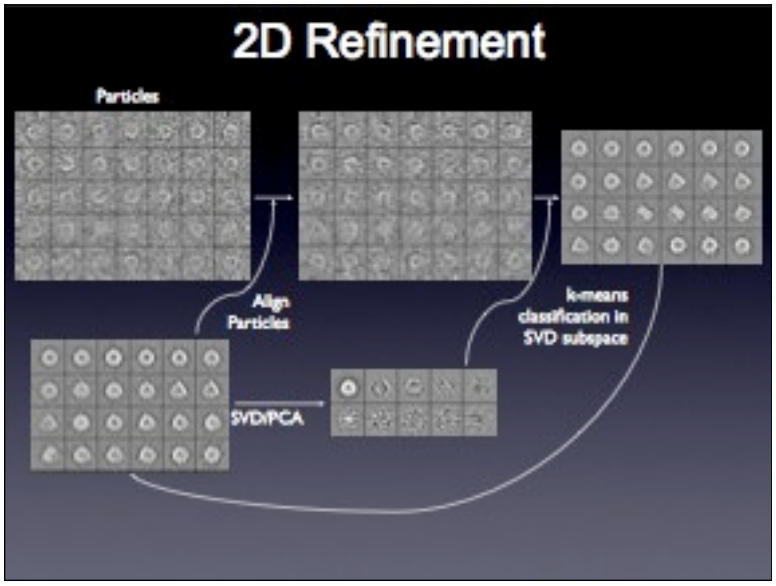

2D Refinement
Particles

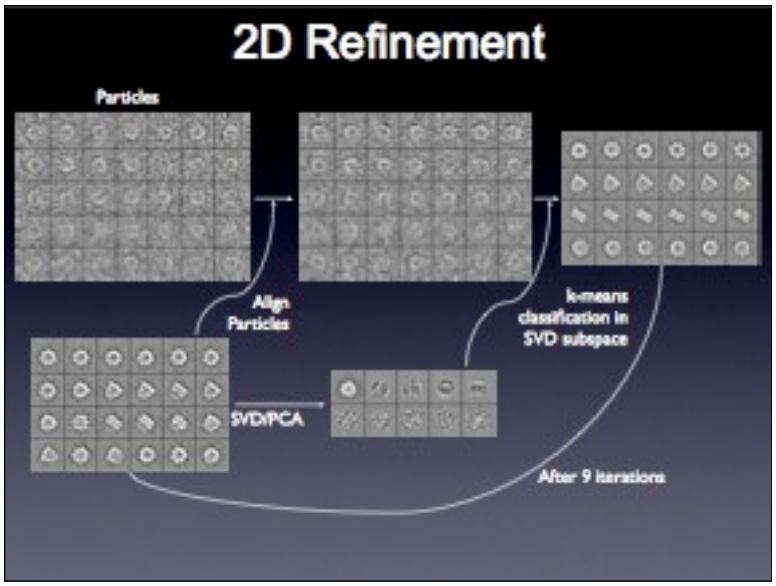

2D Refinement
Particles
Align
Particles


2D Refinement
Particles
Align
Particles
SVD/PCA


2D Refinement
Particles
Align Particles
k-means classification in SVD subspace
SVD/PCA


2D Refinement
Particles
Align Particles
k-means classification in SVD subspace
SVD/PCA

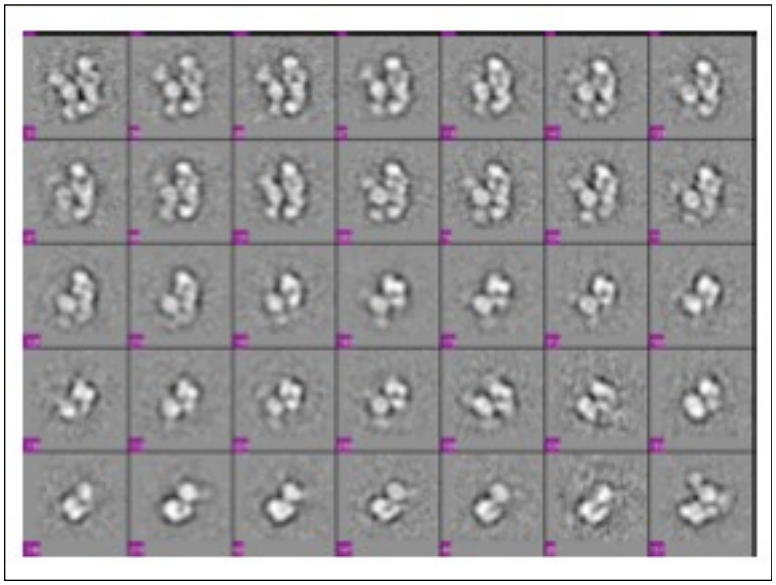

2D Refinement
Particles
Align Particles
k-means classification in SVD subspace
SVD/PCA

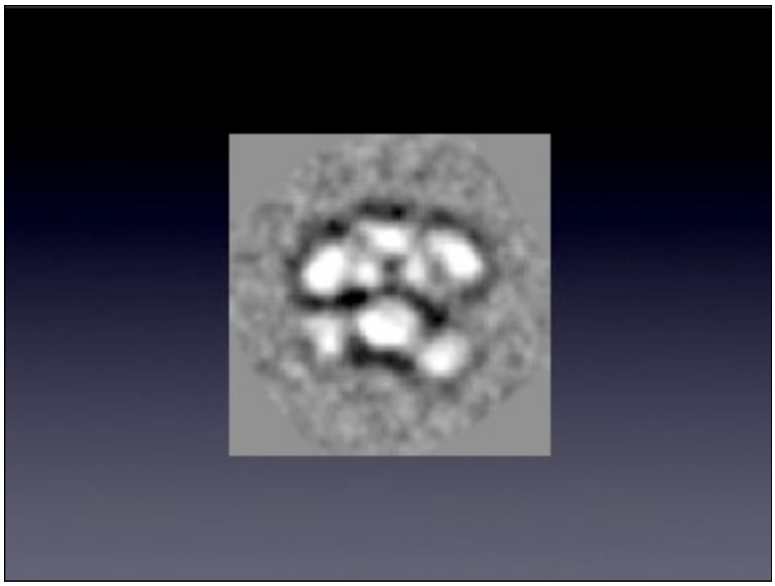


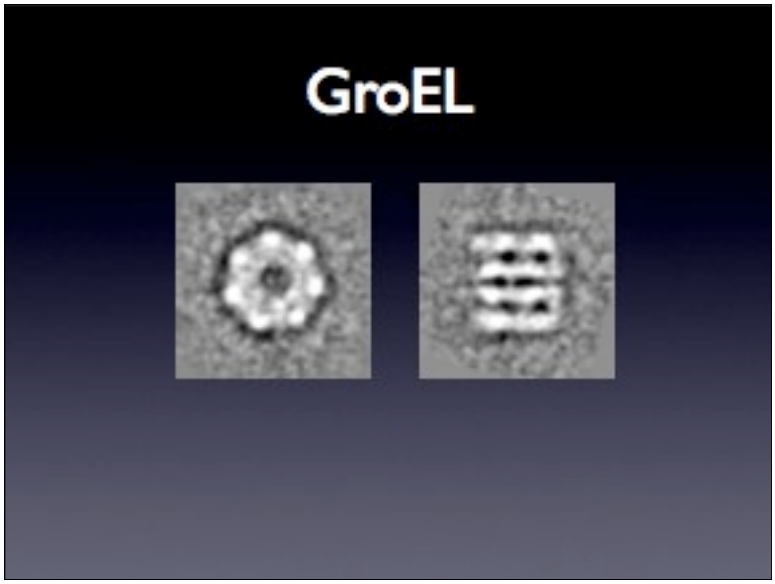
Fatty Acid Synthase

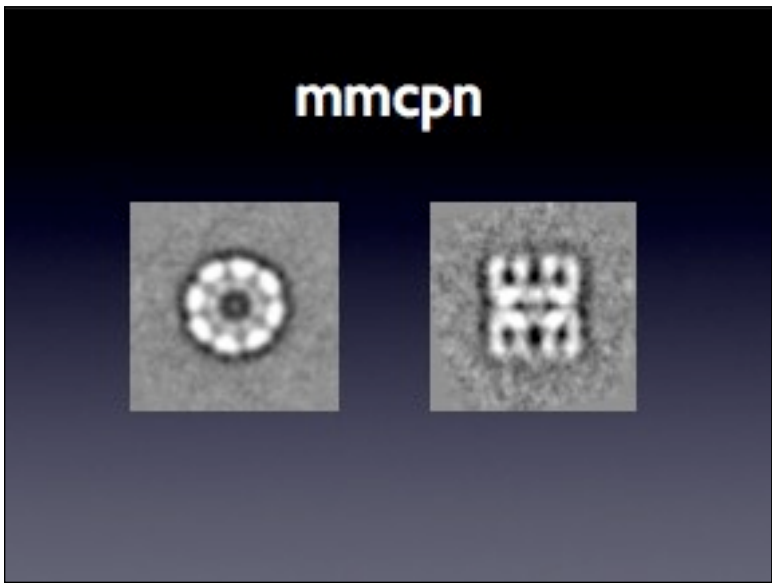
- Wei Zhang, Sub Chirala, Ziwei Gu,
- Salih Wakil, Flo Quiocho, Wah Chiu

- Synthesizes saturated hydrocarbon chains
- Homo-dimer, ~500 kDa
- 7 Enzymatic activities
- ~50 reactions to make palmitate
- Highly expressed in most tumors
- Particularly important in breast and prostate cancer
- FAS inhibitors can prevent tumor growth
- Obesity

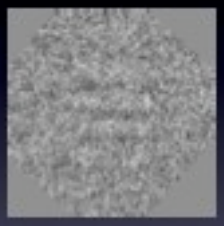





GroEL


mmcpn

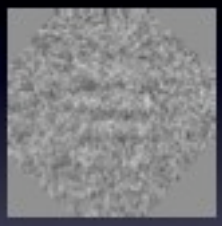
Can we subtract what we don't want ?



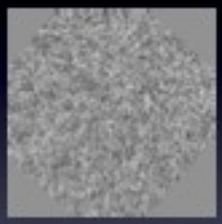
Can we subtract what we don't want ?

A lot of math →

Can we subtract what we don't want ?



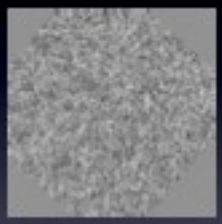
Can we subtract what we don't want ?



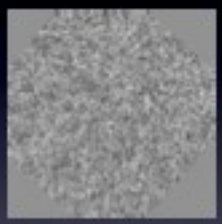
Remove One Monomer

Remove One Monomer

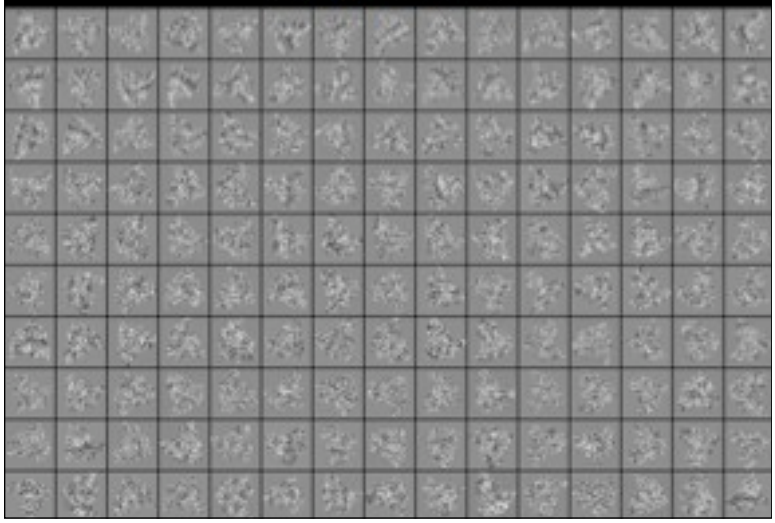
Remove One Monomer



Remove One Monomer



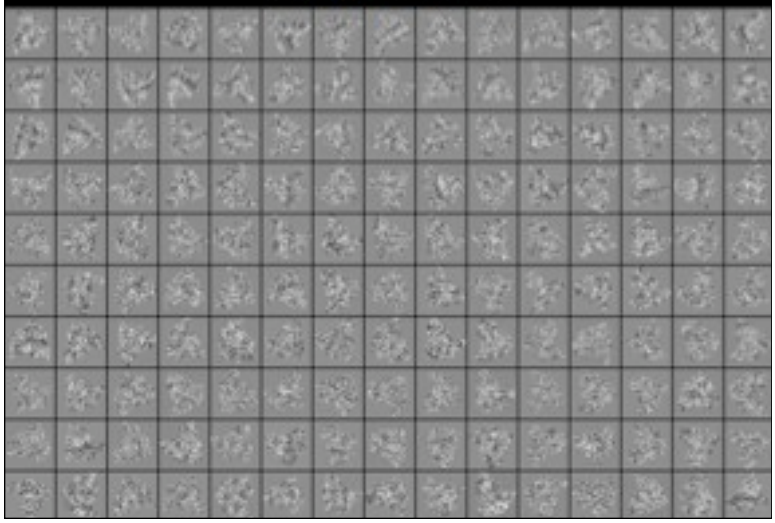
Many particles

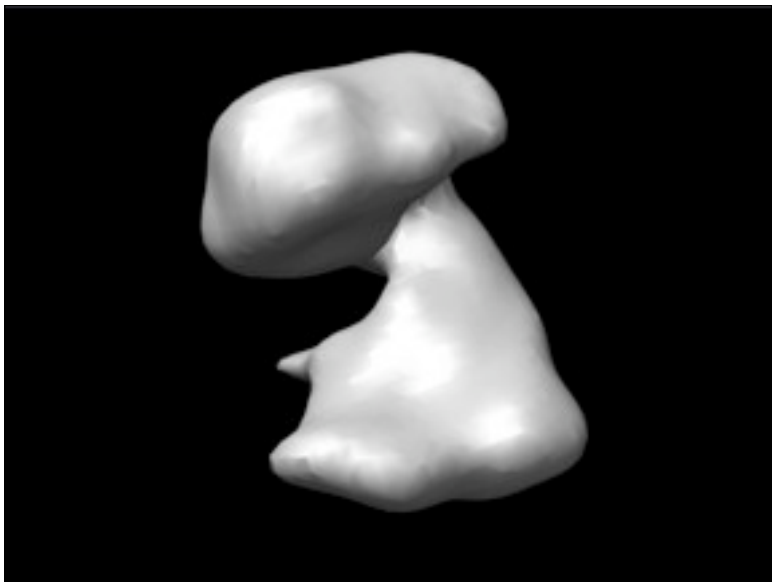


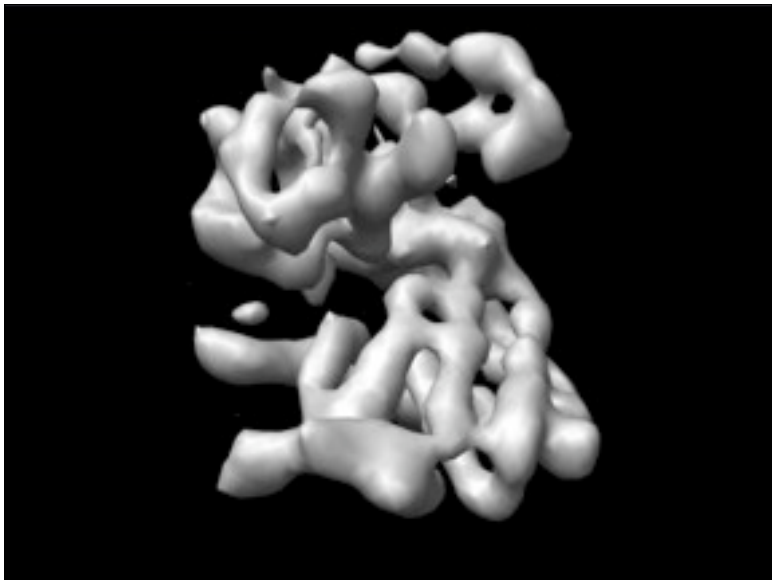
Many Particles

- 40,000 GroEL -> 560,000 monomers
- 128x128 with mask @ 1 Å/pixel
- ~250,000 CPU-hr for a full refinement
- Multi-model → millions of CPU-hr
- So,
- 64x64, 100,000 particles

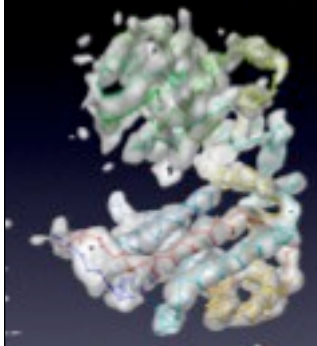
Many particles







Acknowledgements



GroEL
- Matt Baker
- Donghua Chen
- Jiu-Li Song (UTSW)
- David Chuang (UTSW)
- Wah Chiu

Mmcpn
- Wah Chiu
- Junjie Zhang
- Donghua Chen
- Judith Frydman (Stanford)
- Stefanie Reissmann (Stanford)

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