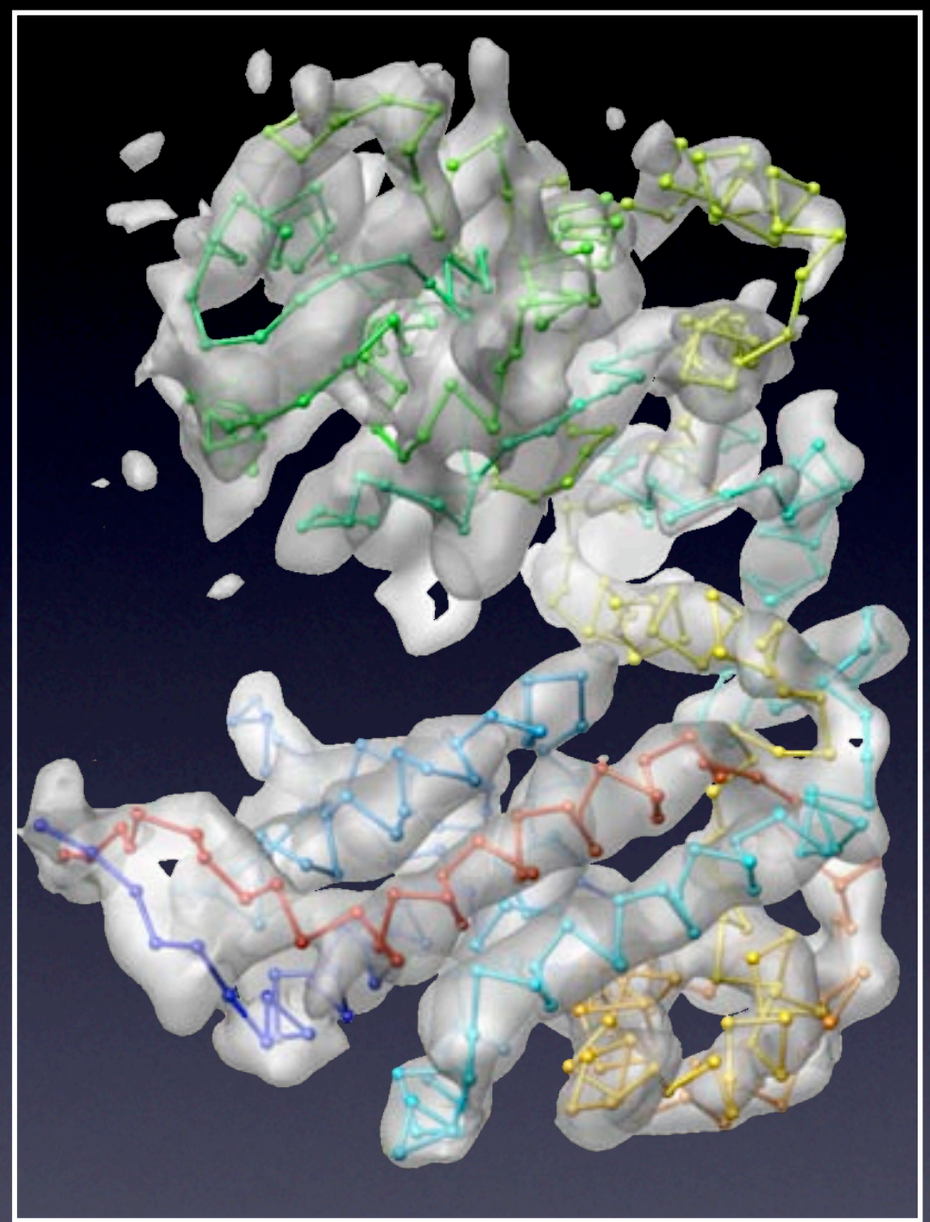
Single Particle Reconstruction: Resolution, Heterogeneity and Dynamics



Steve Ludtke

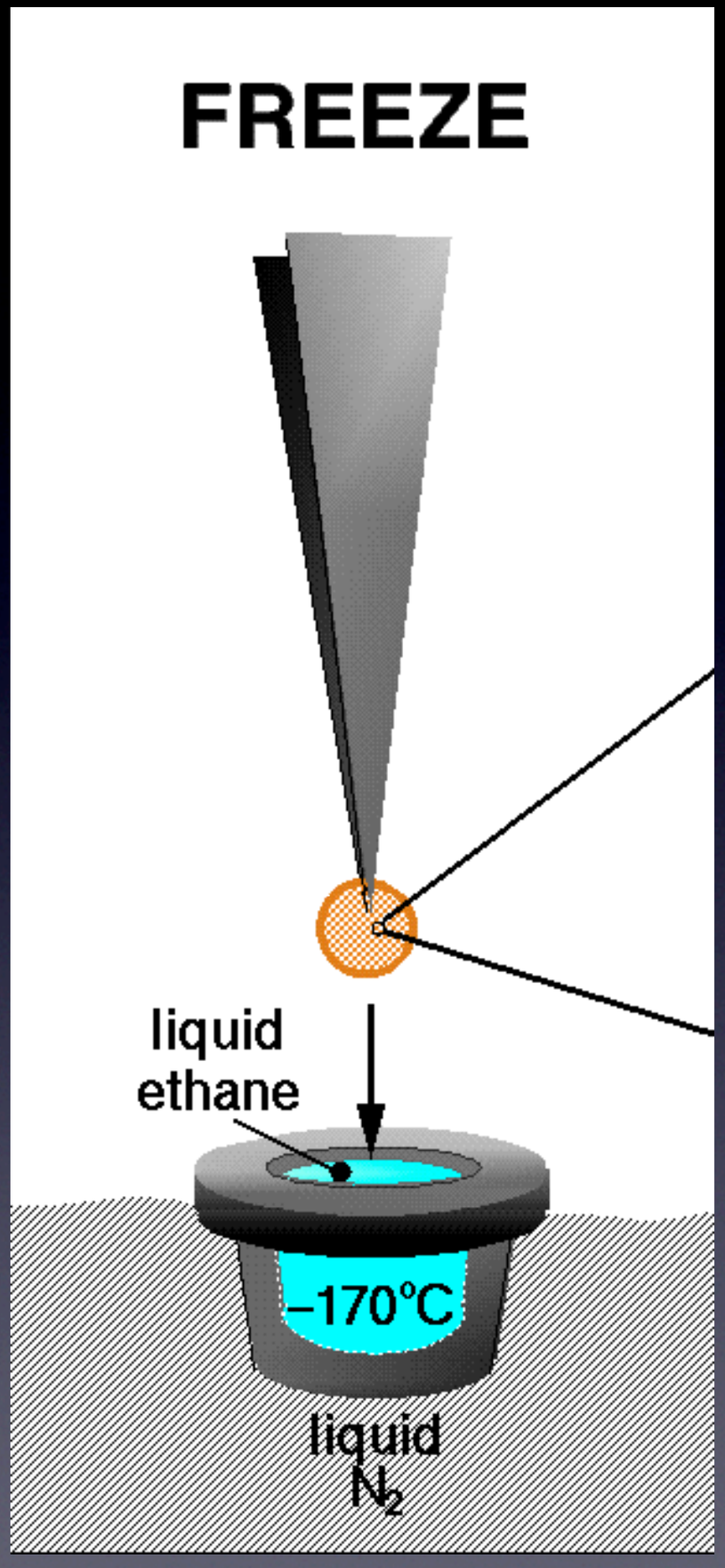
National Center for Macromolecular Imaging
Department of Biochemistry &
Molecular Biology
Baylor College of Medicine

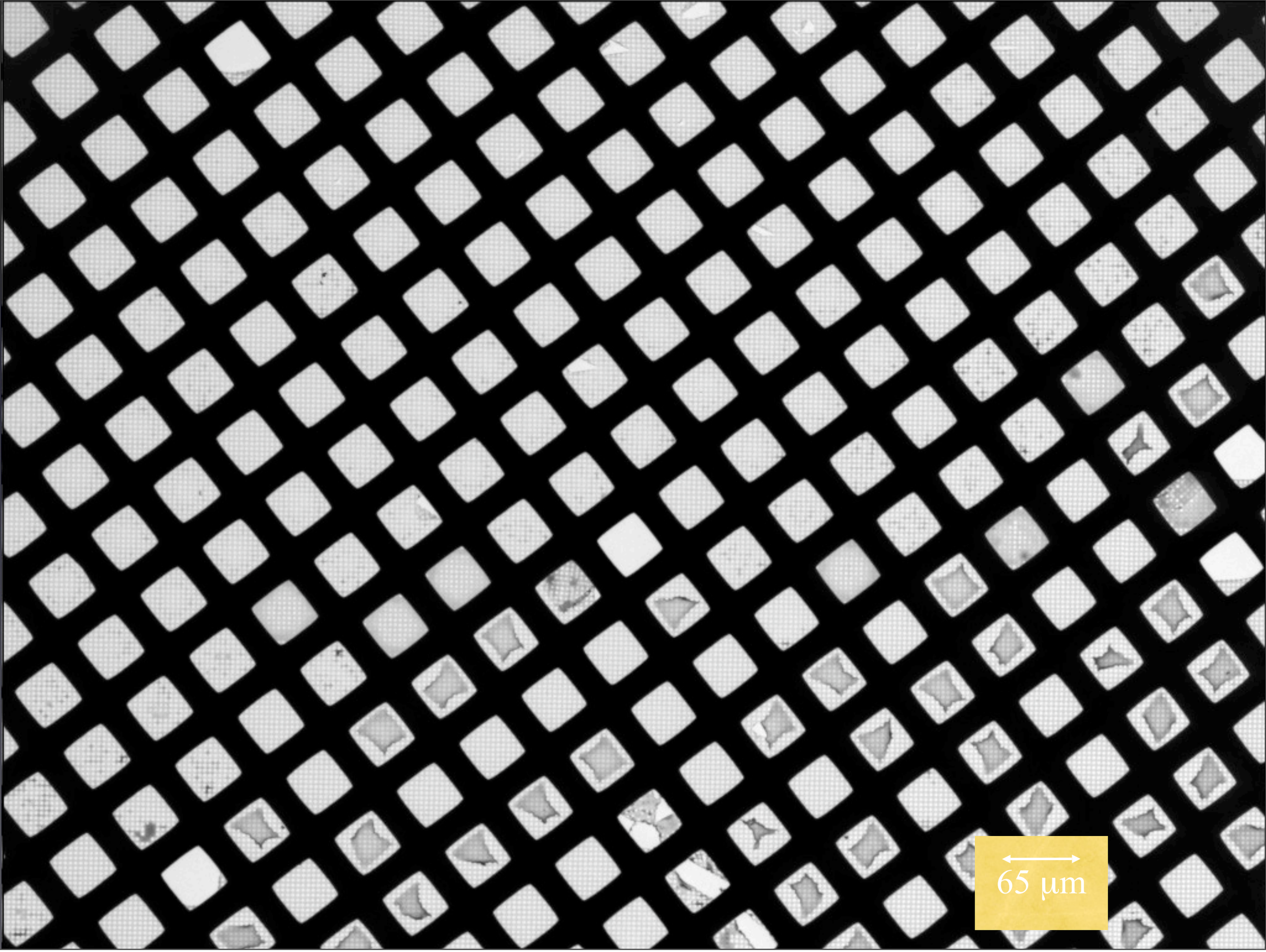
BCM Baylor College of Medicine

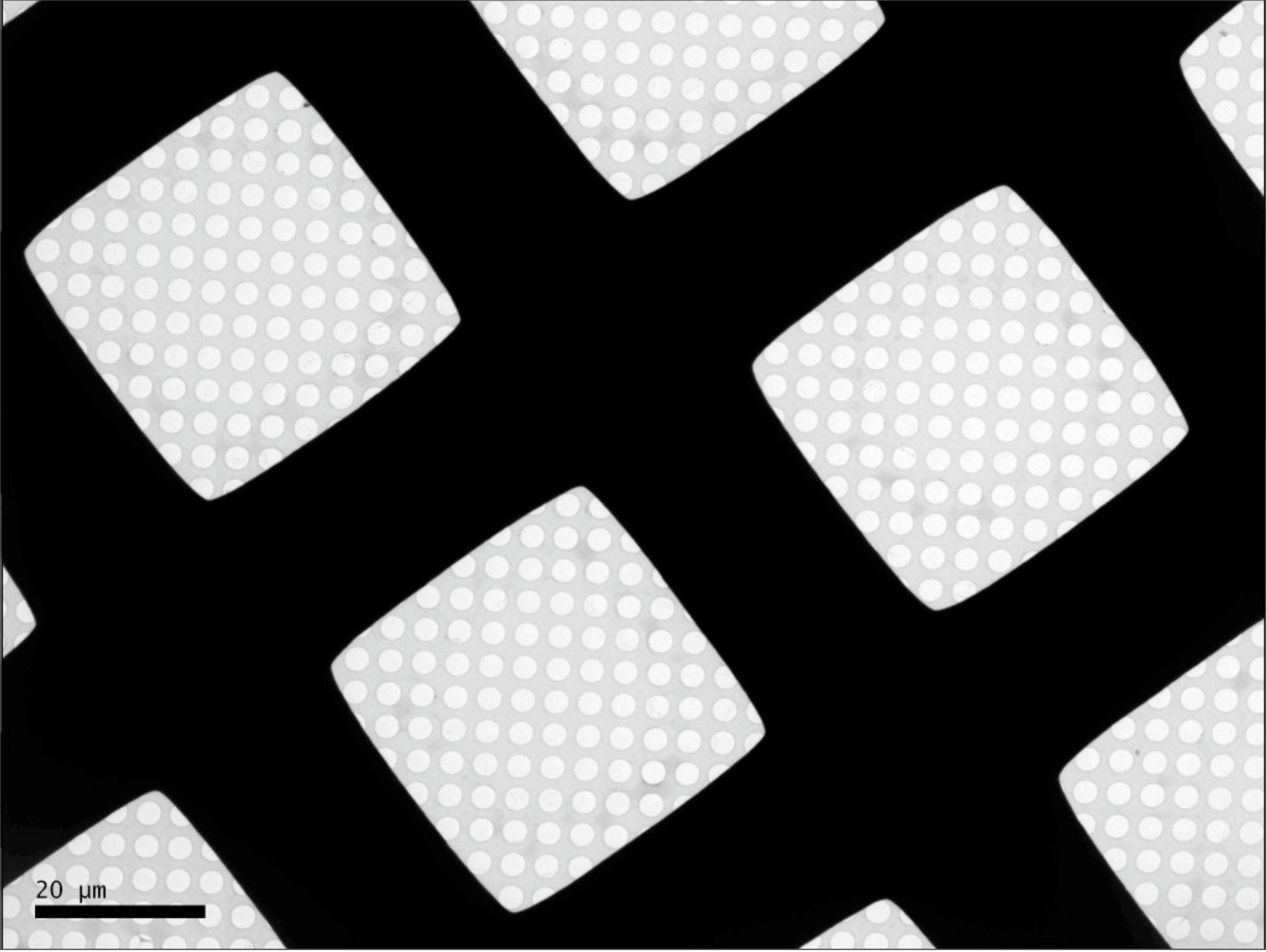
NCRR

NCMI National Center for Macromolecular Imaging

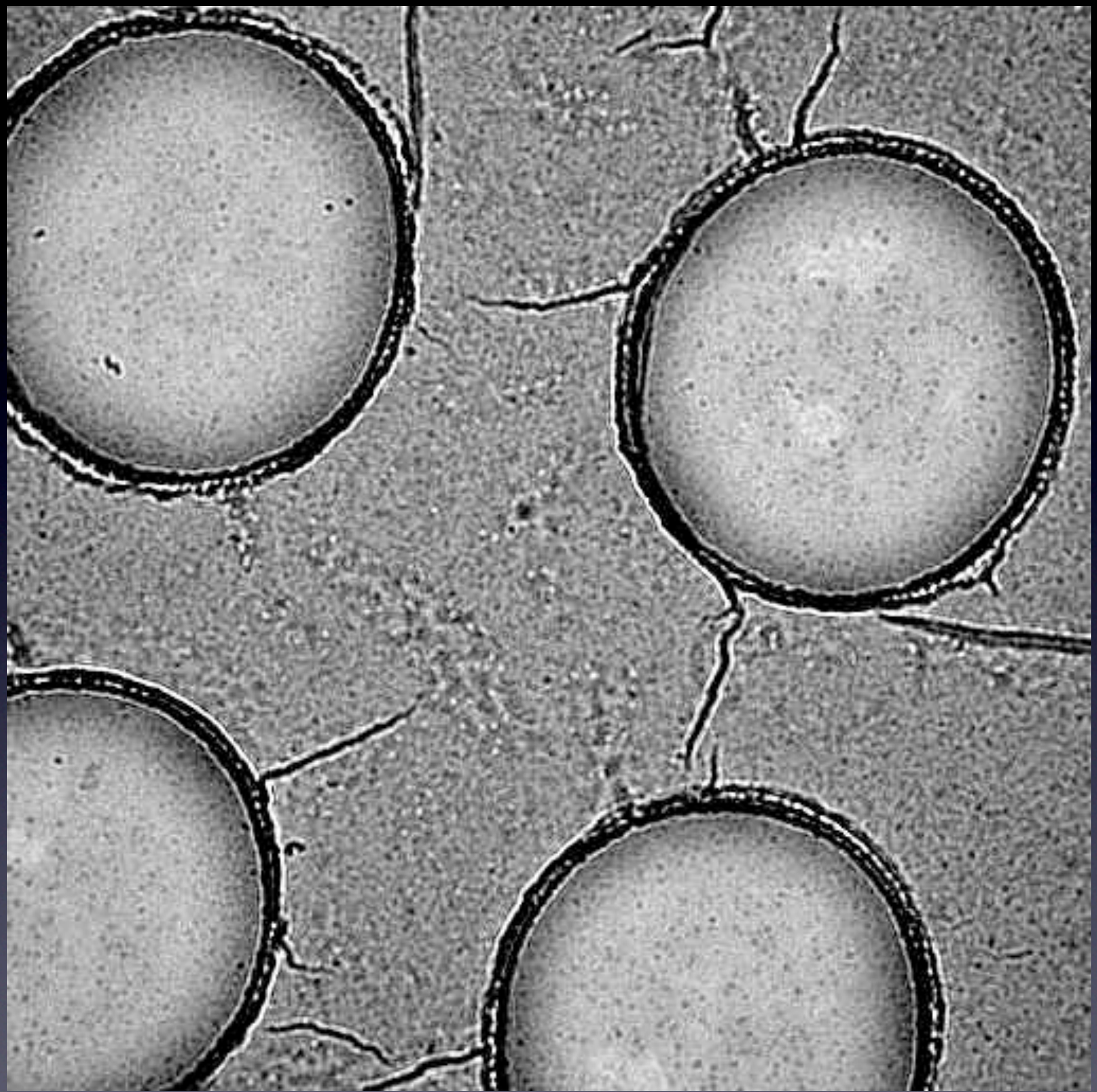
NIH Roadmap

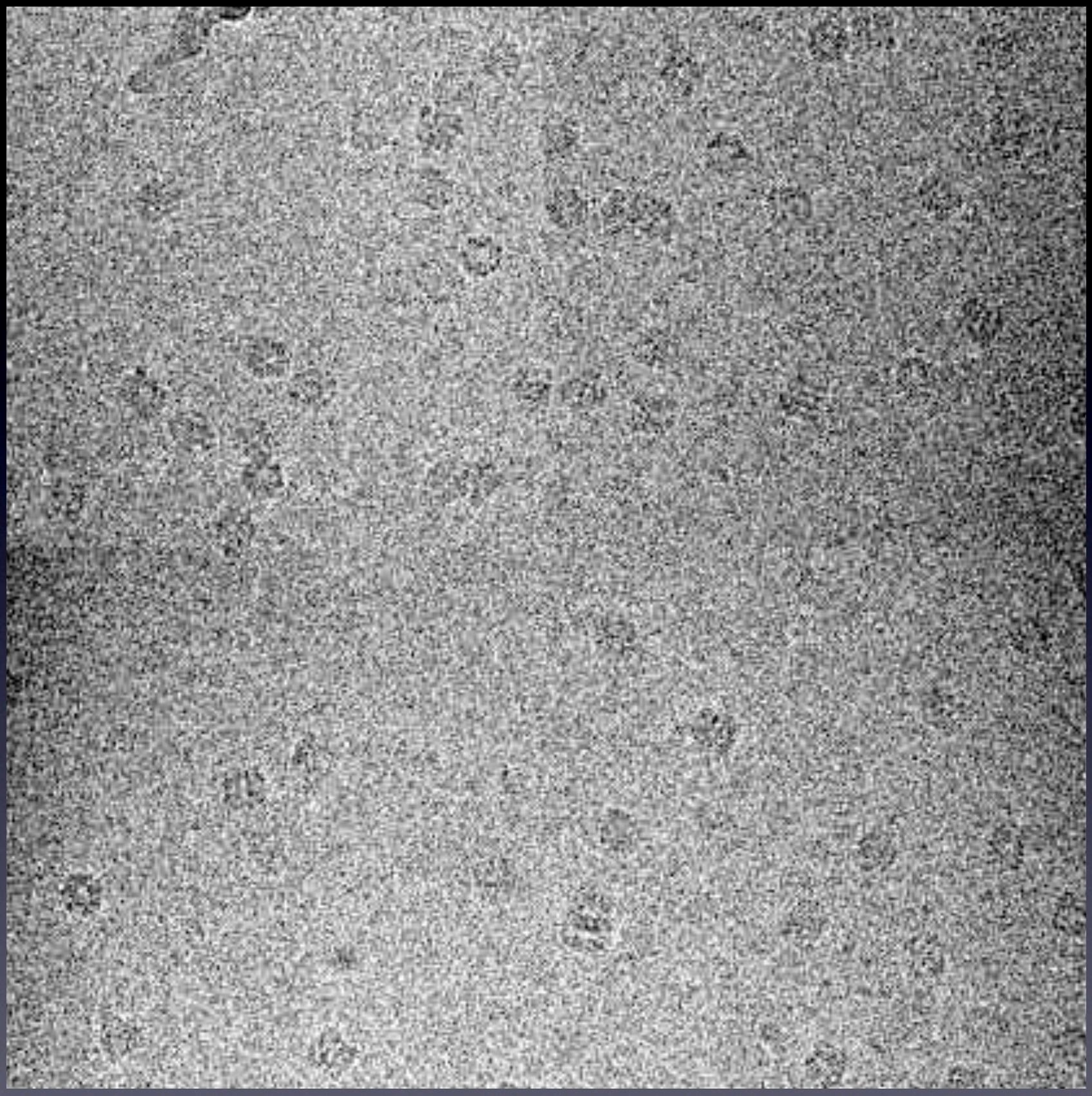

FREEZE
liquid
ethane
–170°C
liquid
N_2

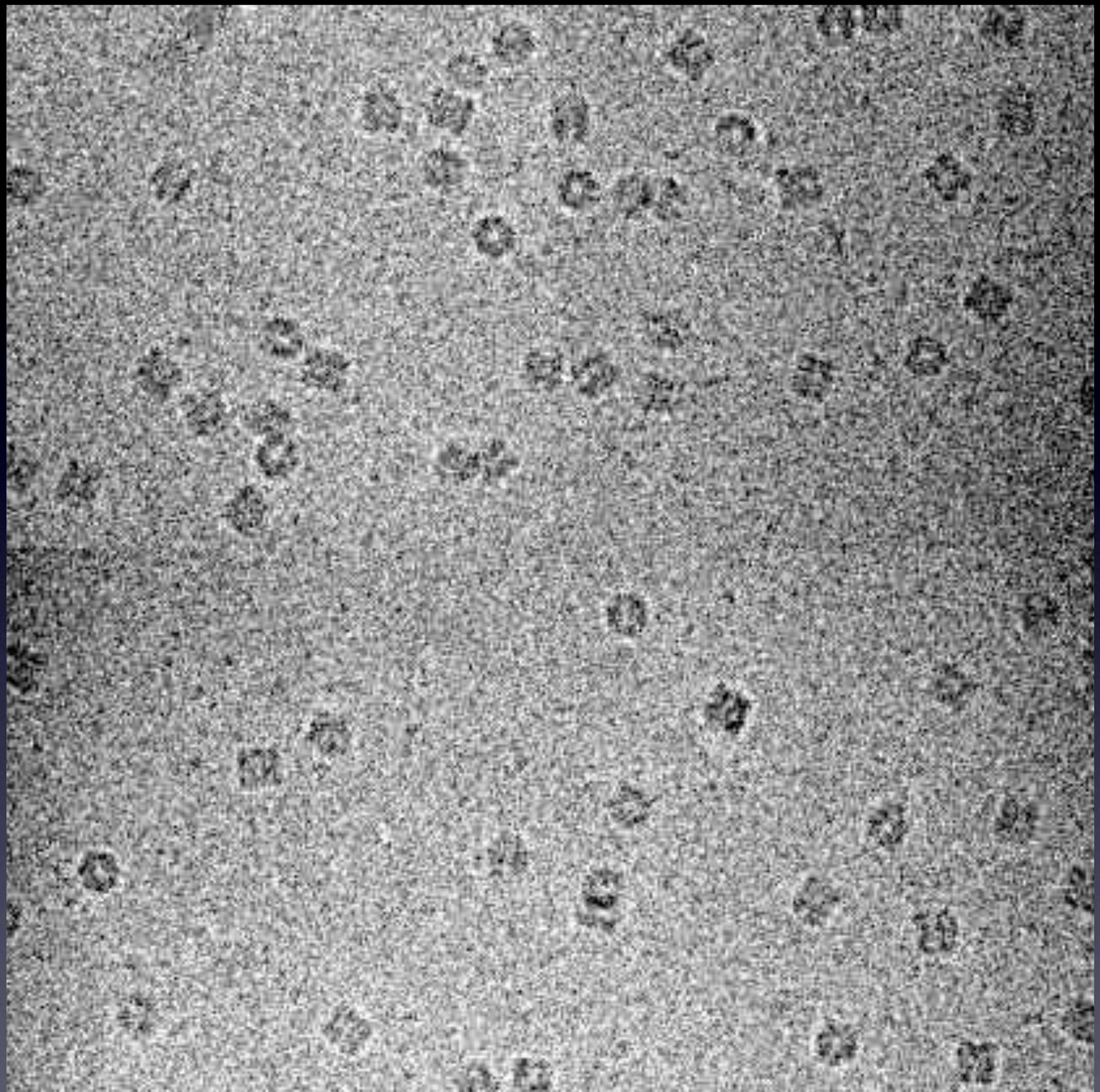

65 µm

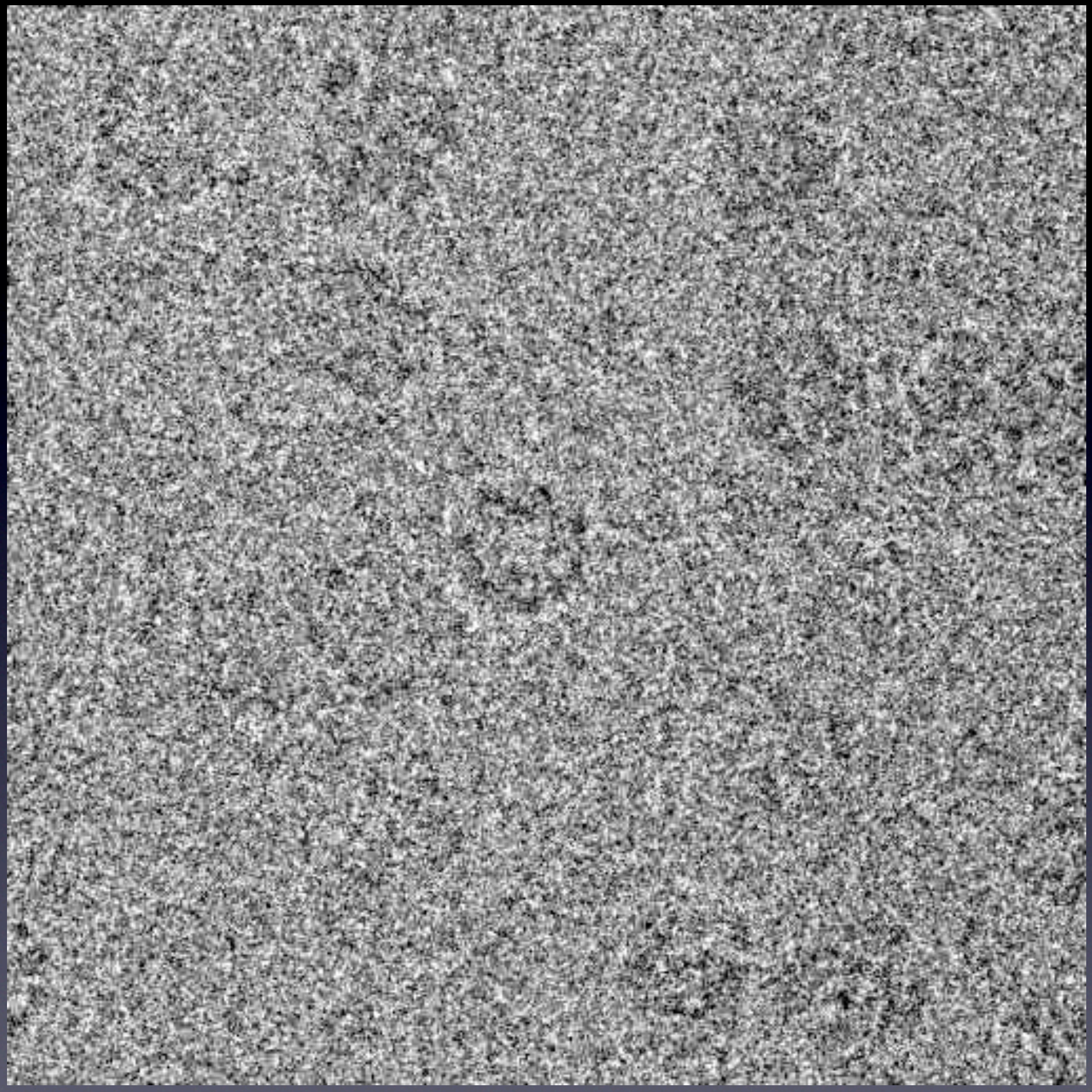


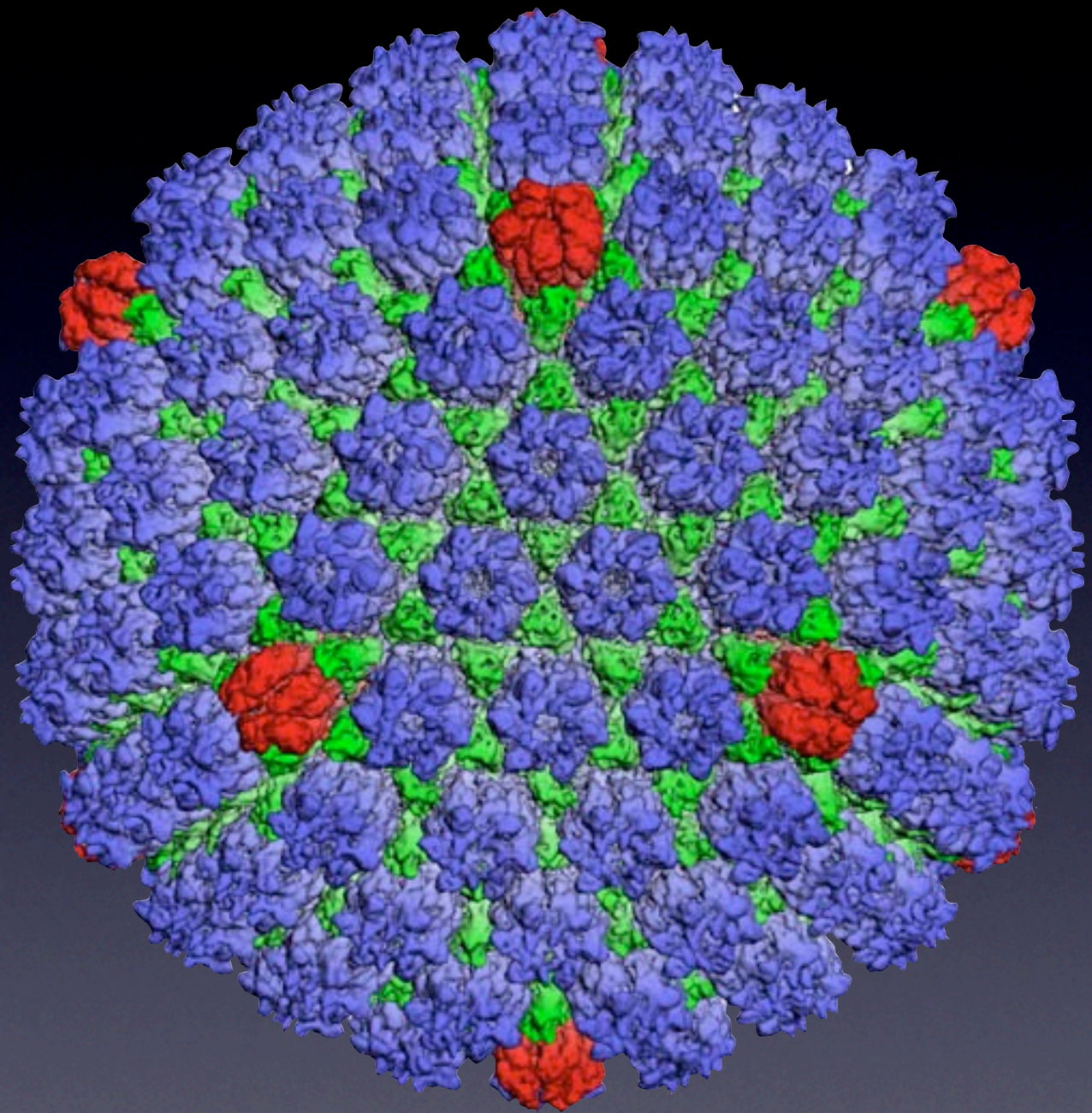
Tuesday, January 29, 2008


~1 μm

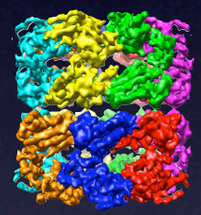

1000 Å


1000 Å

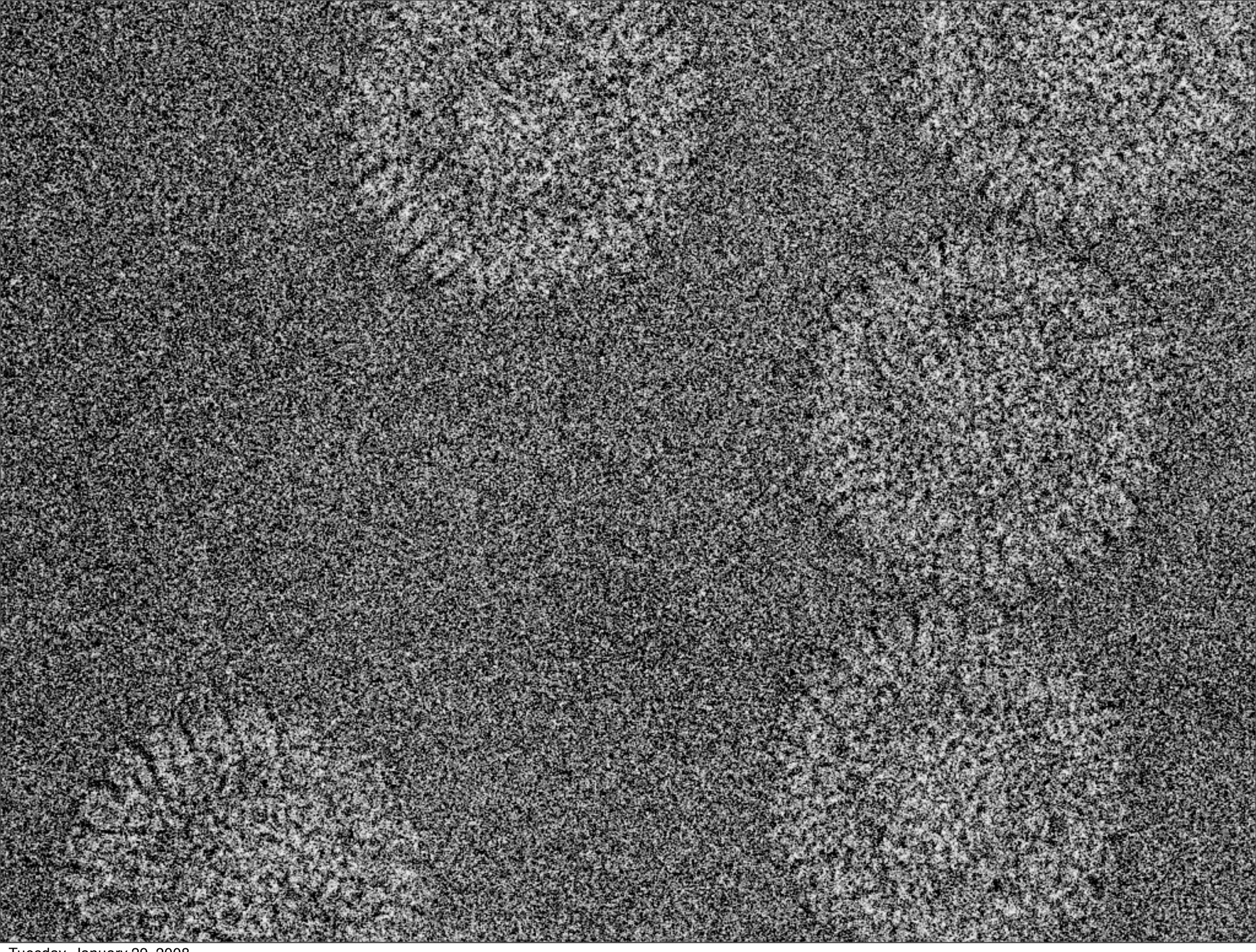

200 Å



200,000 kDa



800 kDa



Tuesday, January 29, 2008

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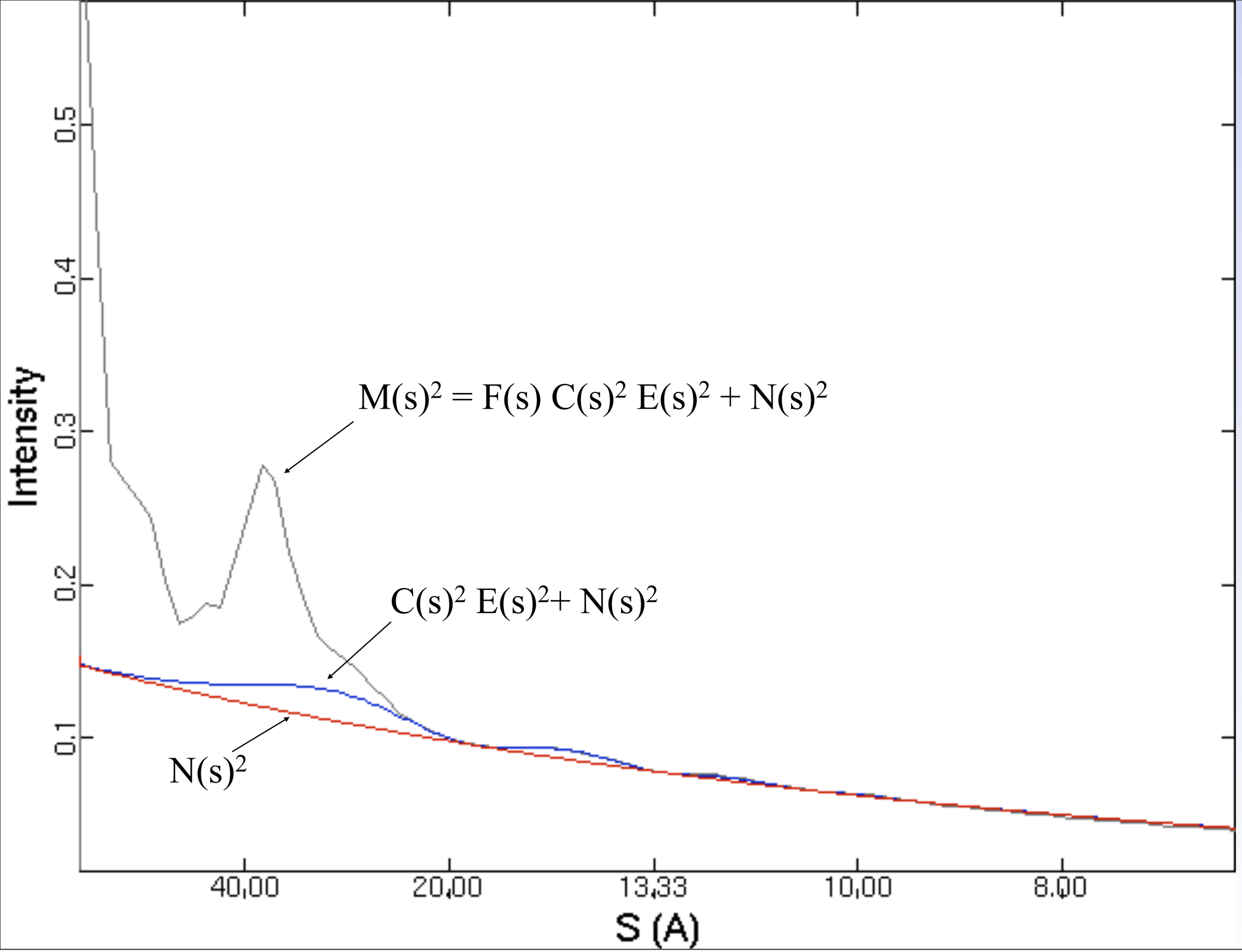
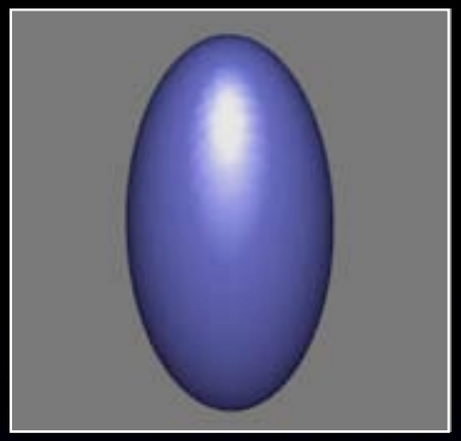

Intensity
0,5
0,4
0,3
0,2
0,1
M(s)² = F(s) C(s)² E(s)² + N(s)²
C(s)² E(s)²+ N(s)²
N(s)²
40,00
20,00
13,33
10,00
8,00
S (A)



Initial 3D Model

Uniform Projections

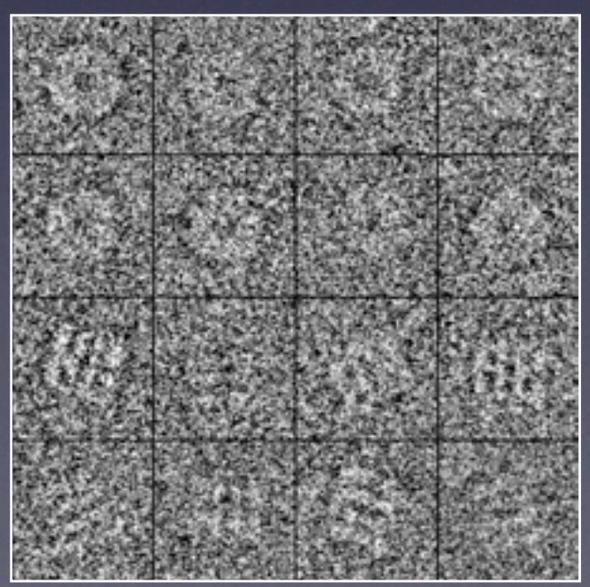
Build New 3D Model

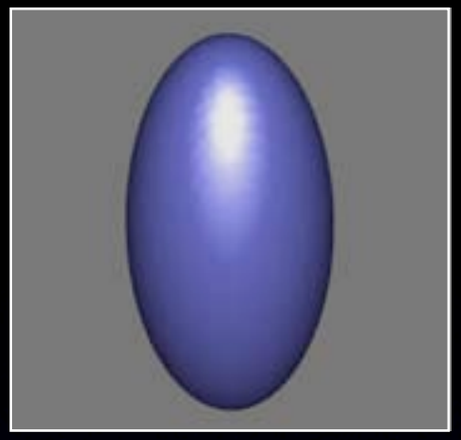
Final 3D Model

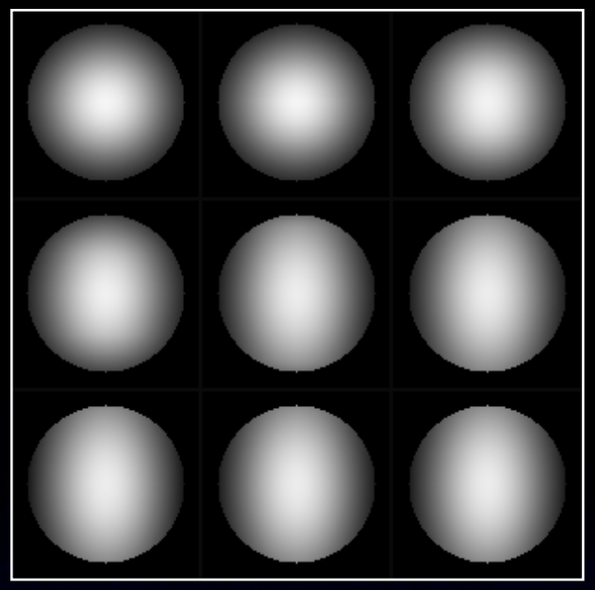
Particle Images

Classify Particles

Align and Average Classes







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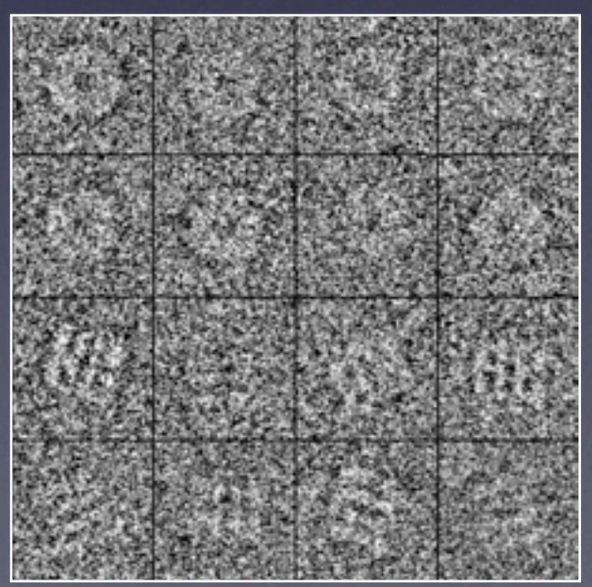

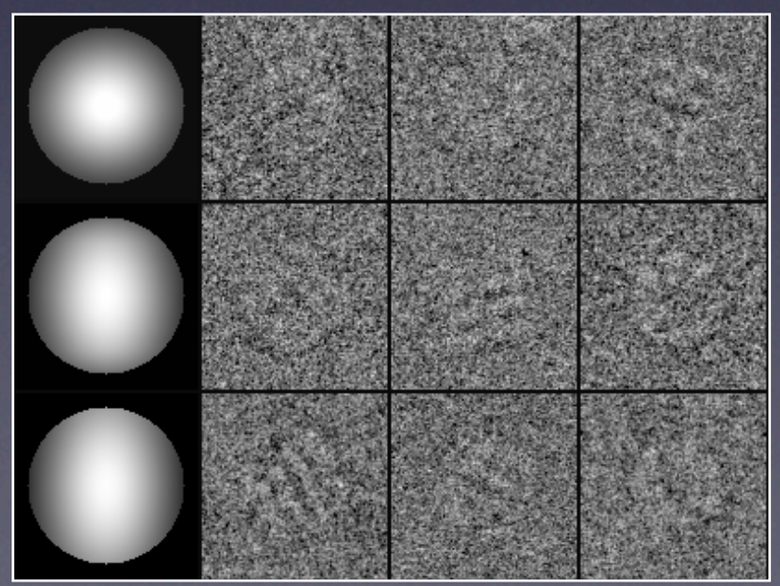

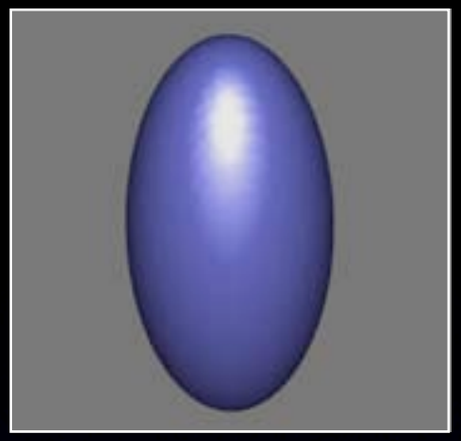

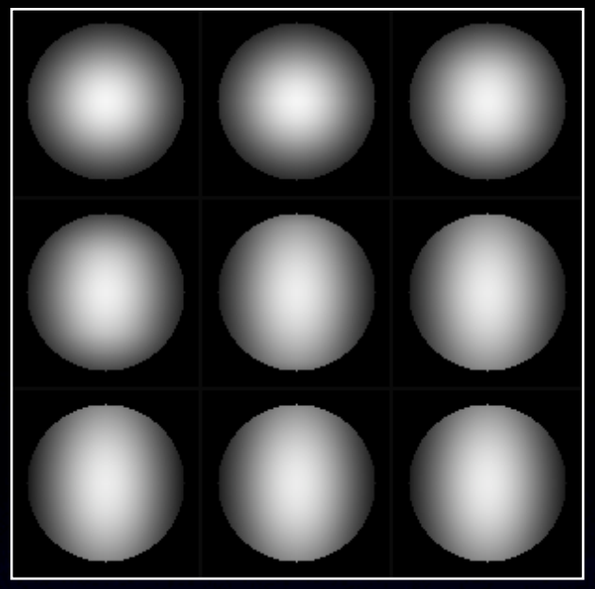

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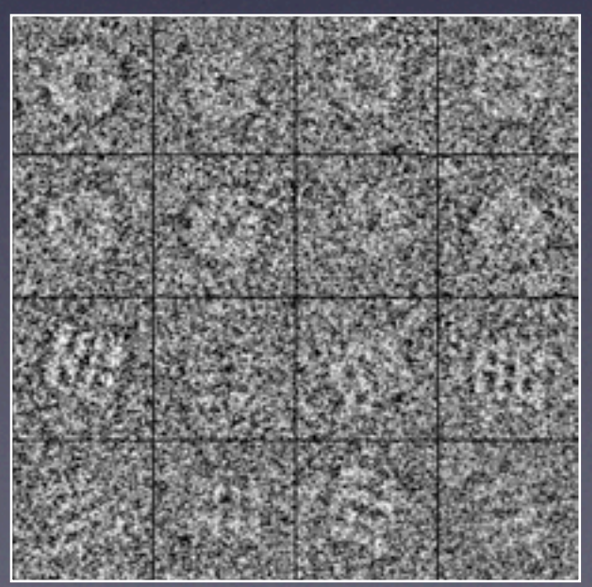

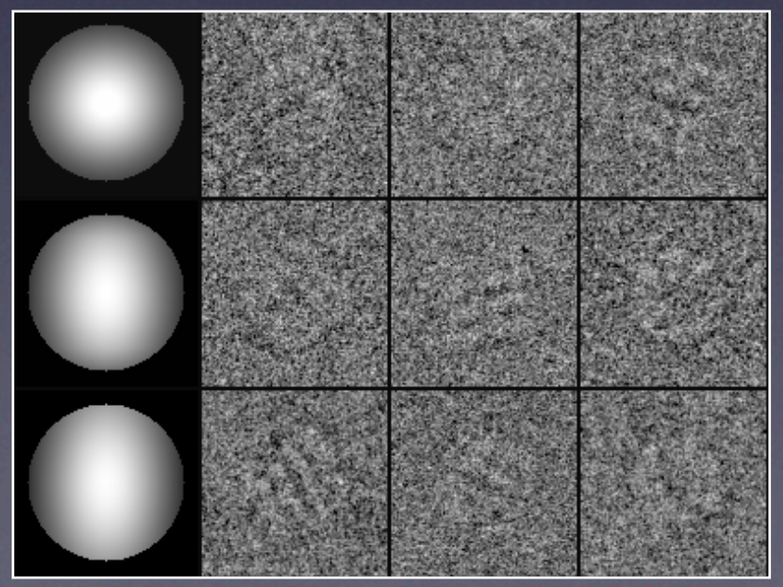

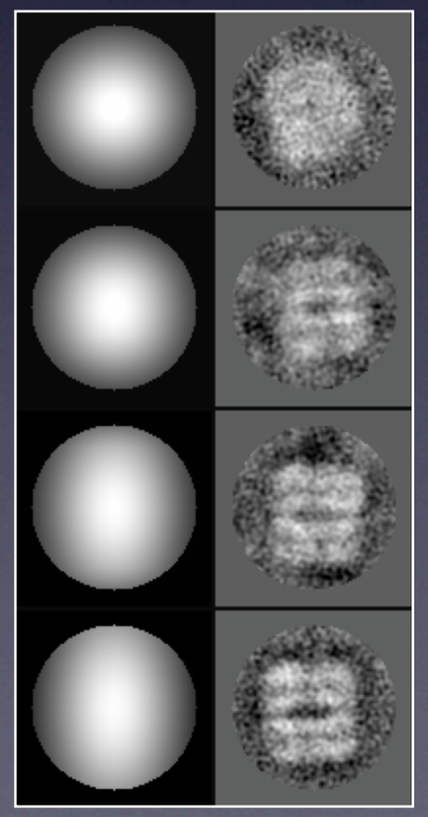

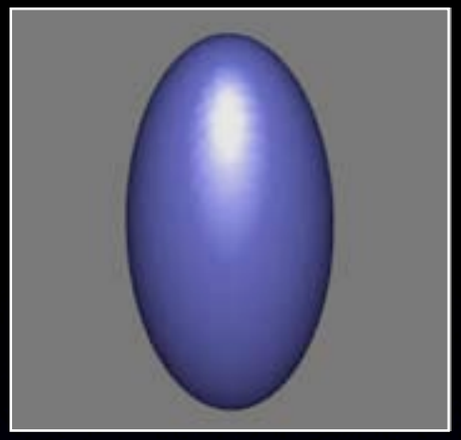

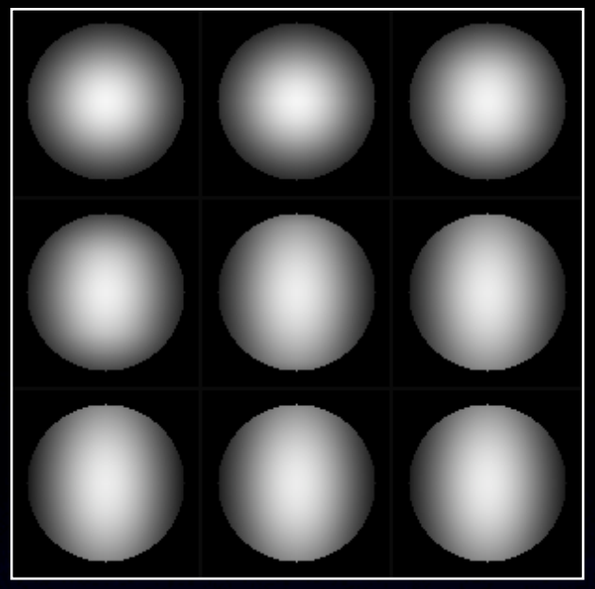

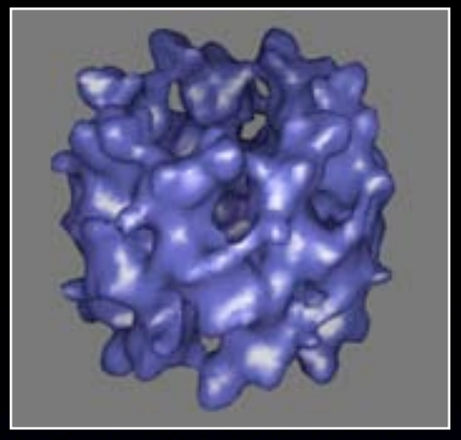

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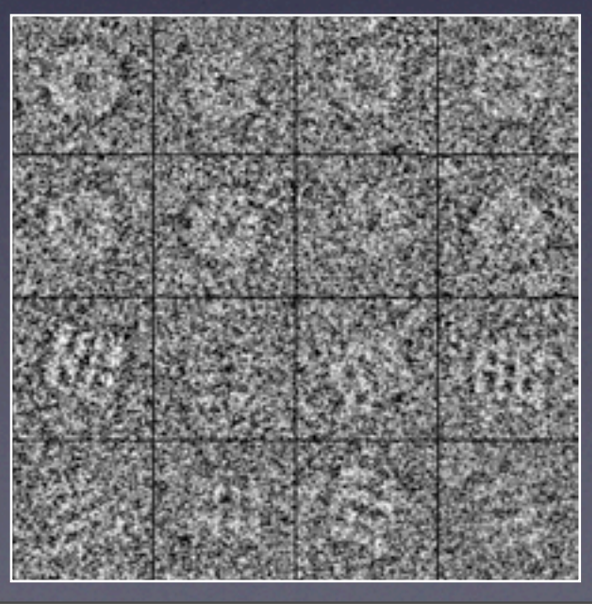

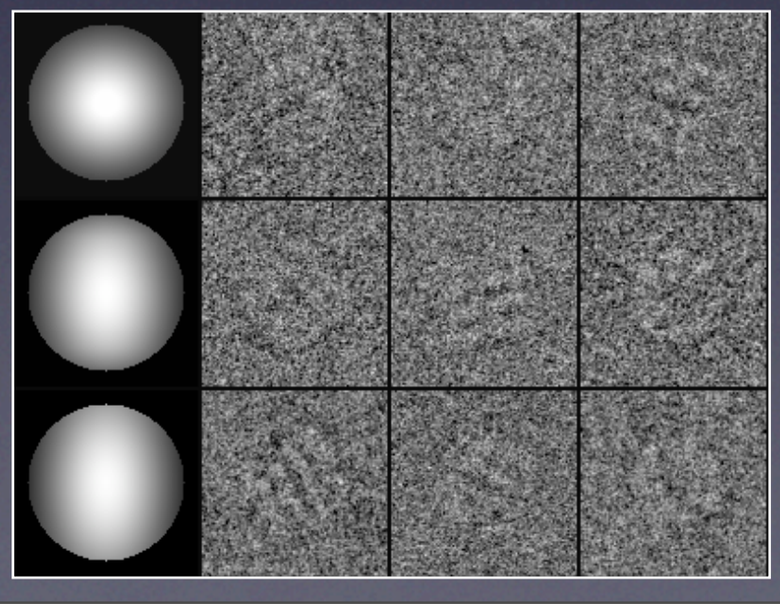

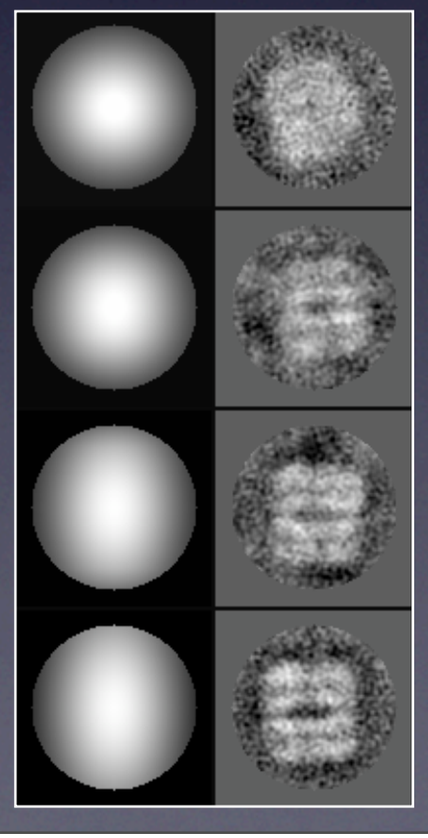

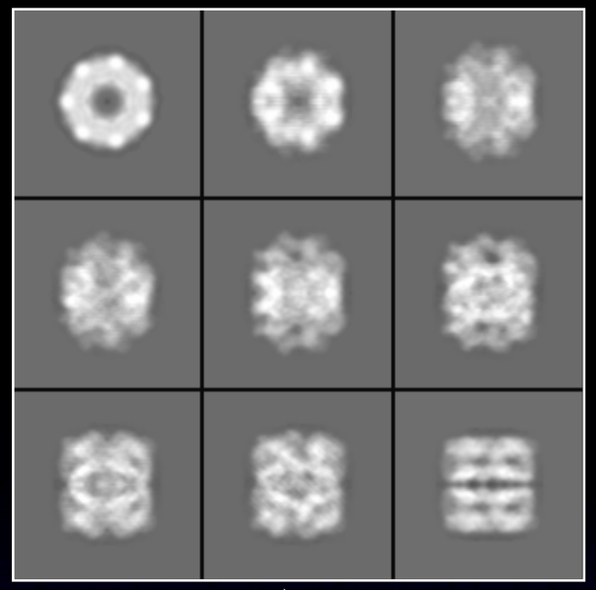

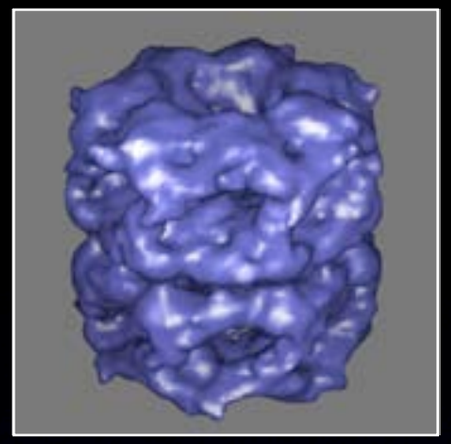

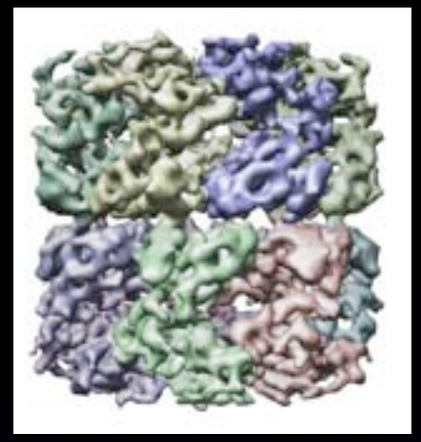

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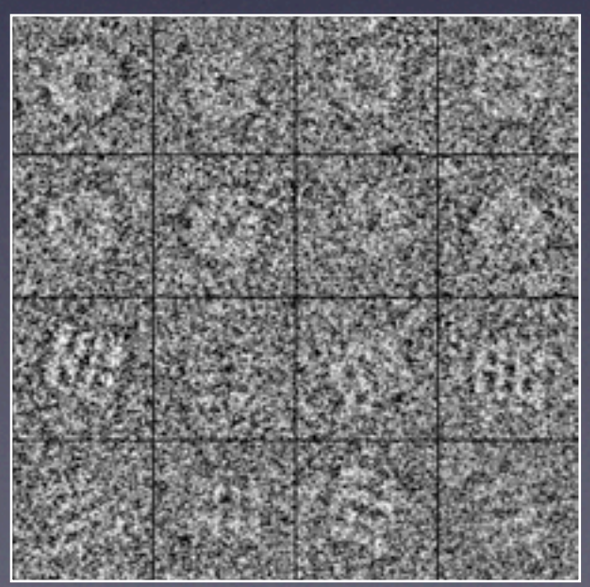
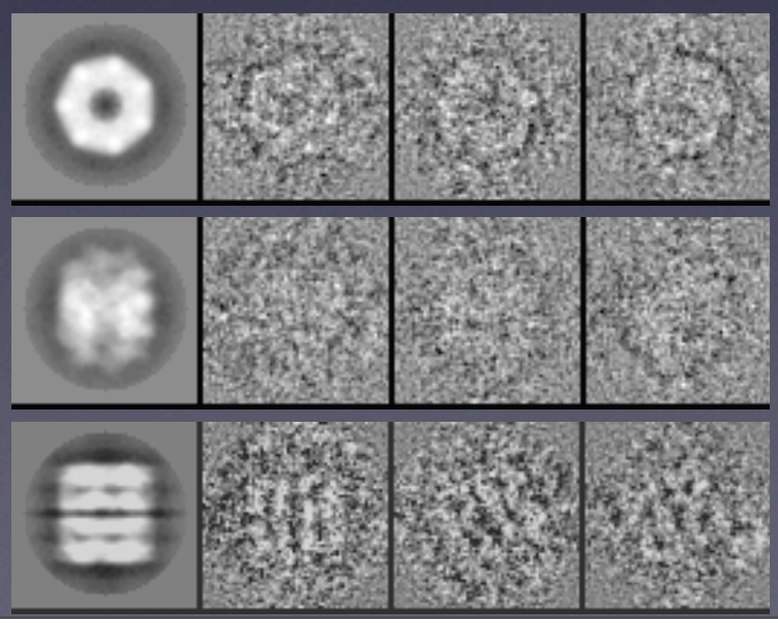


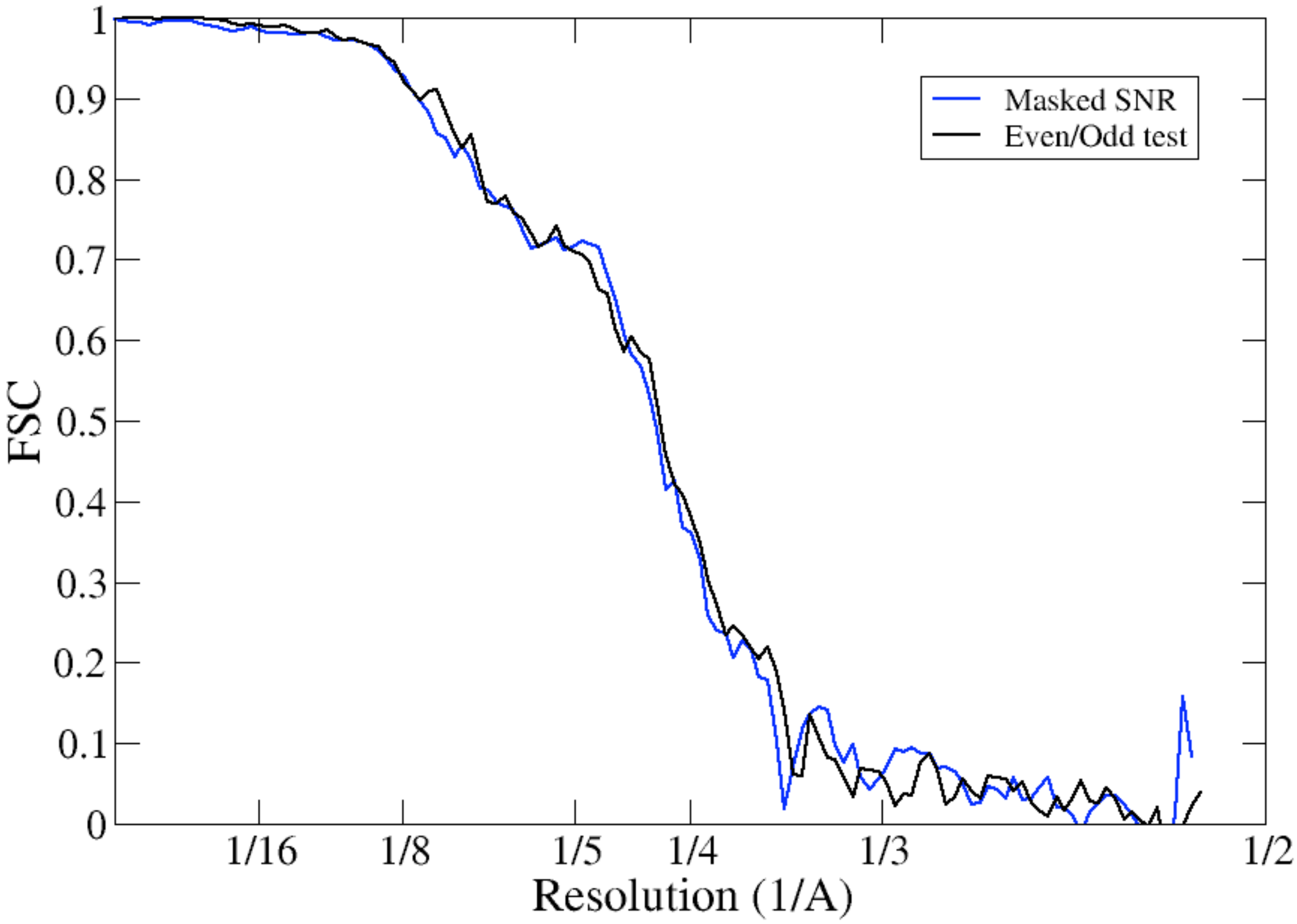


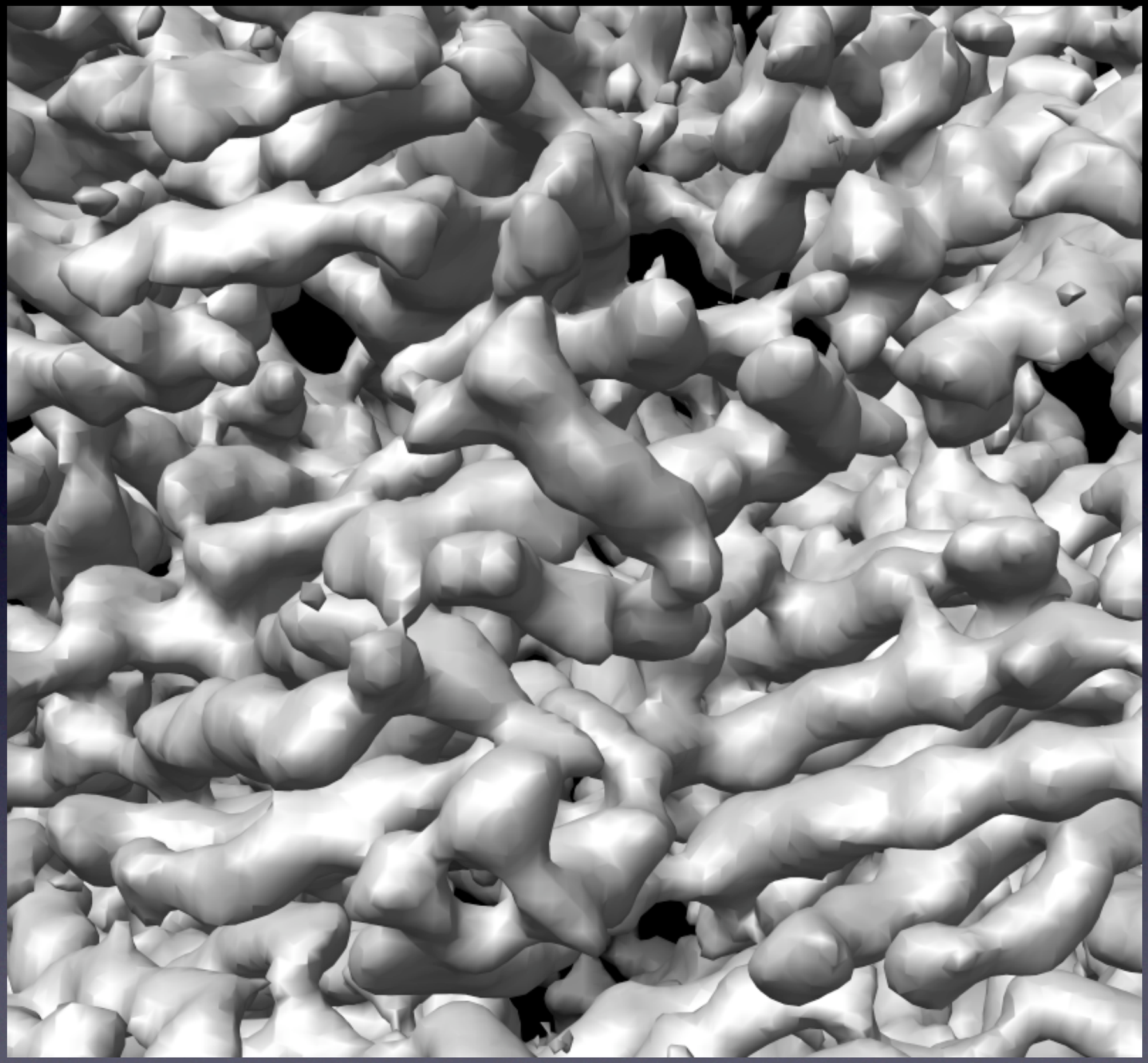
GroEL

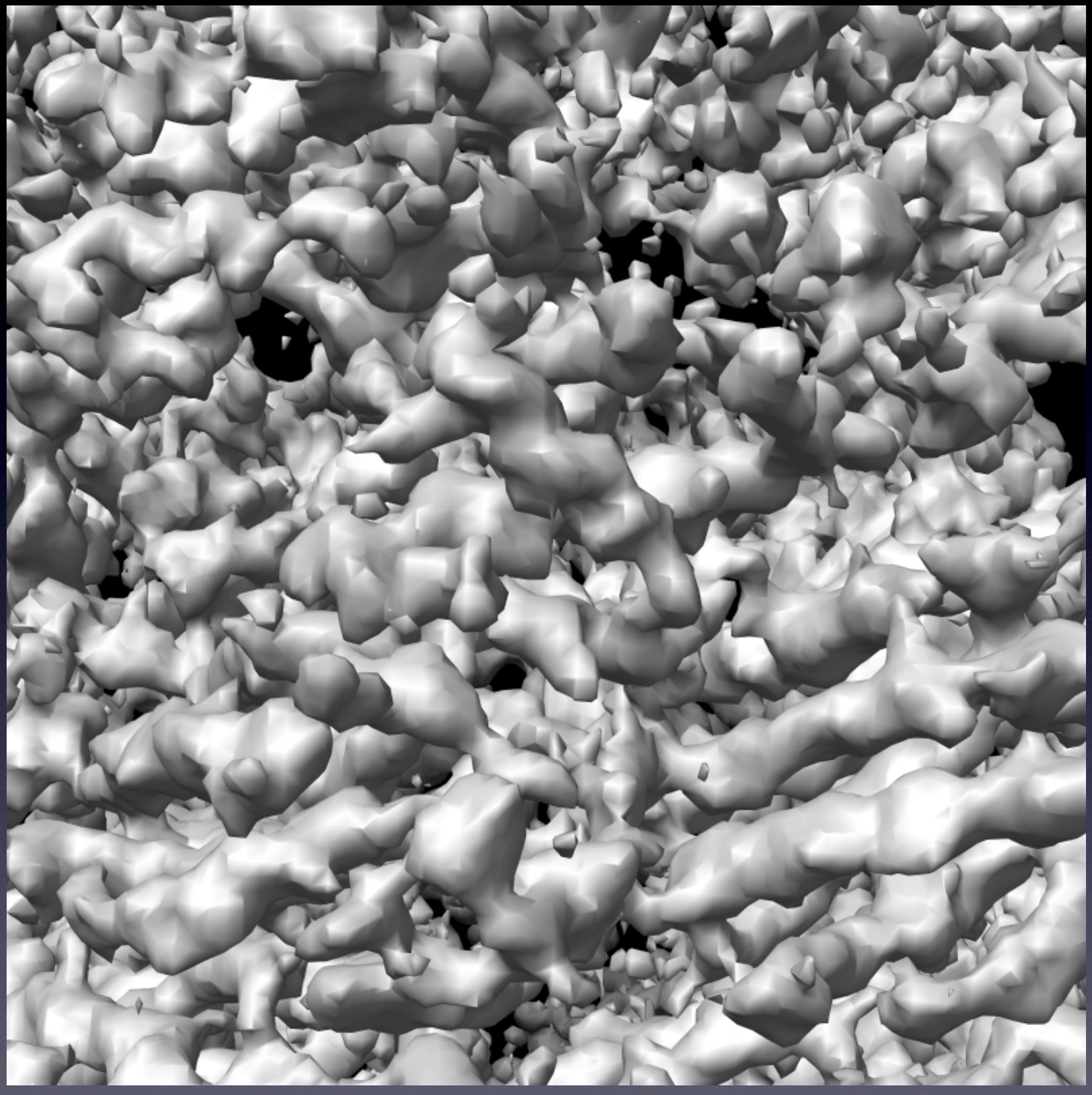
- Type I chaperonin → GroES co-chaperonin
- homo 14-mer
- 2 back to back 7 membered rings
- ~800 kDa, ~200 Å

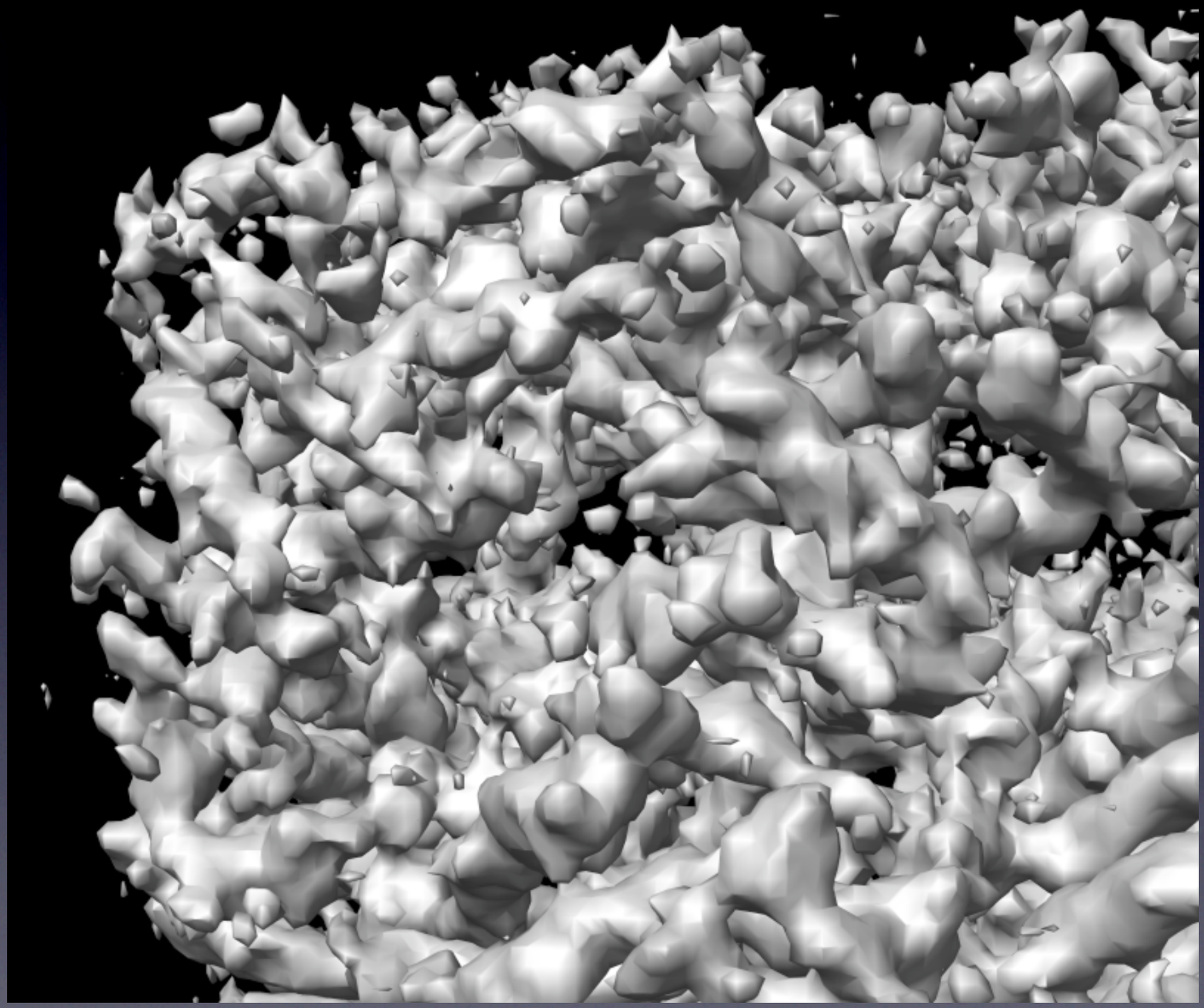
Data Collected 2005

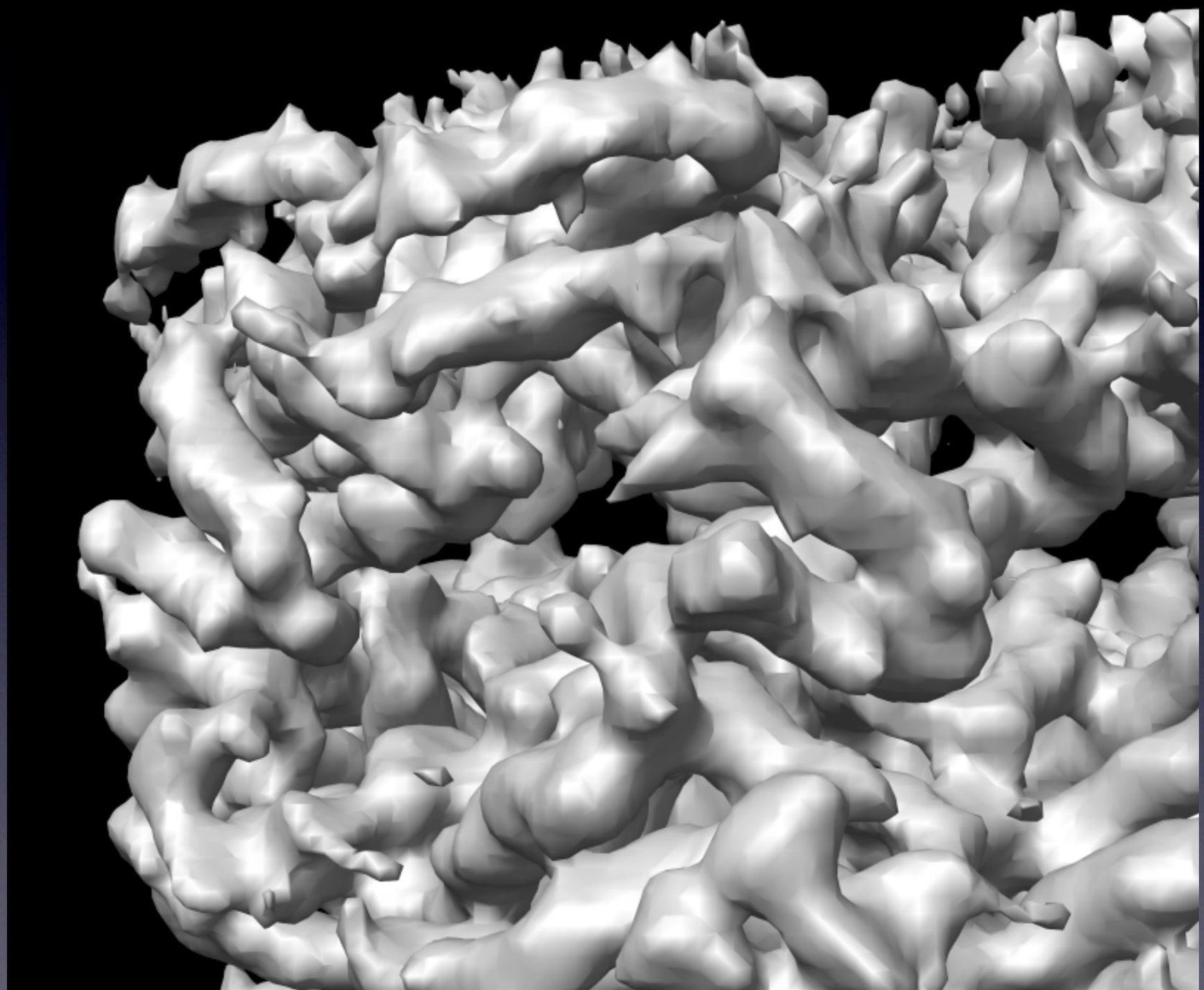
- Native, unliganded GroEL, no ATP/ADP (?)
- JEOL 3000SFF (Yoshi-style) at LHe temp
- 6 microscopy sessions, Film
- 825 micrographs, Nikon 9000 @ 6.35 µm scan step
- 60k mag → 1.06 Å/pix
- 135 micrographs used → 20,401 particles
- Defocus 1.2 – 2.2 µm

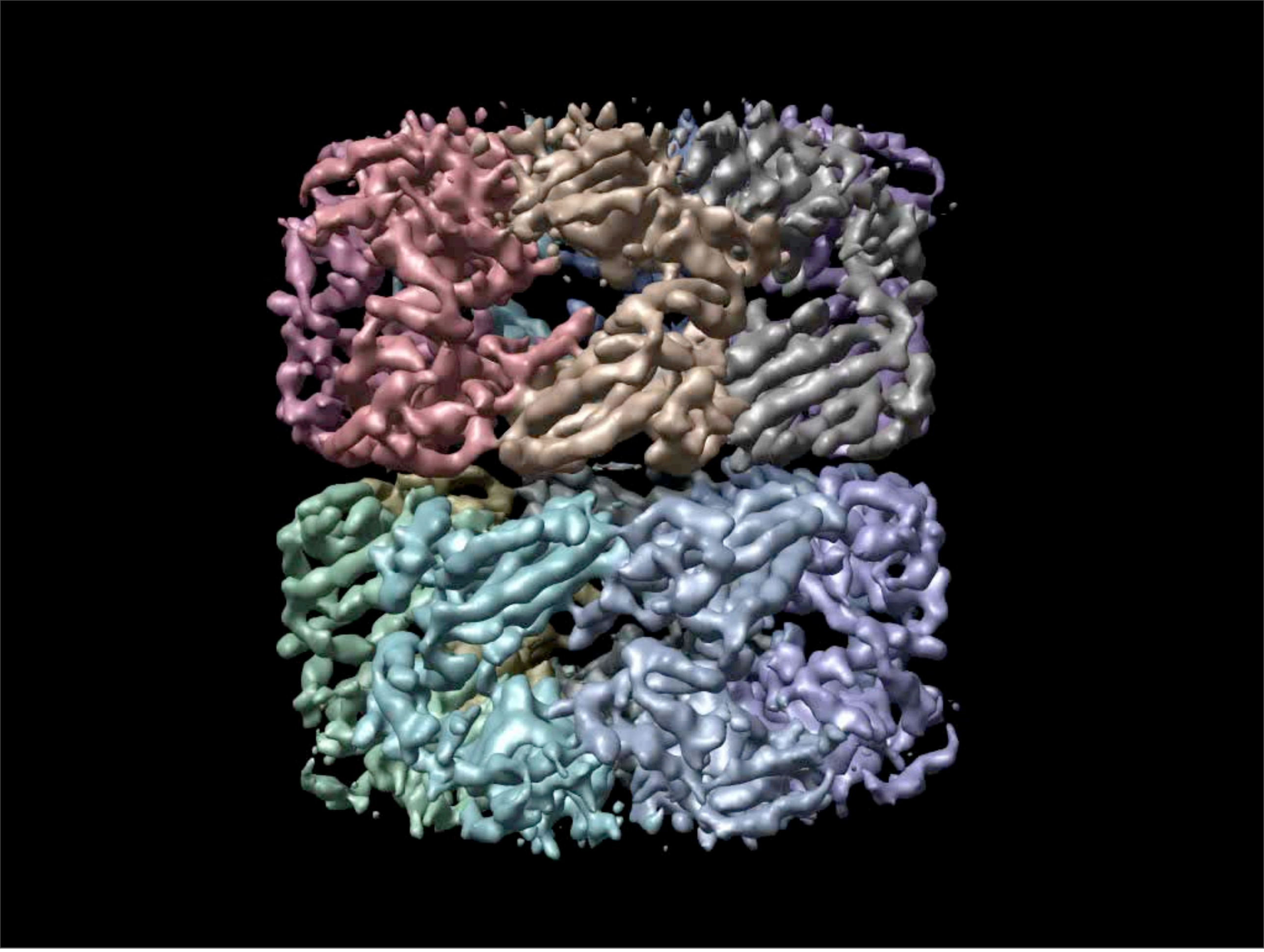

1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
FSC
Masked SNR
Even/Odd test
1/16
1/8
1/5
1/4
1/3
1/2
Resolution (1/A)

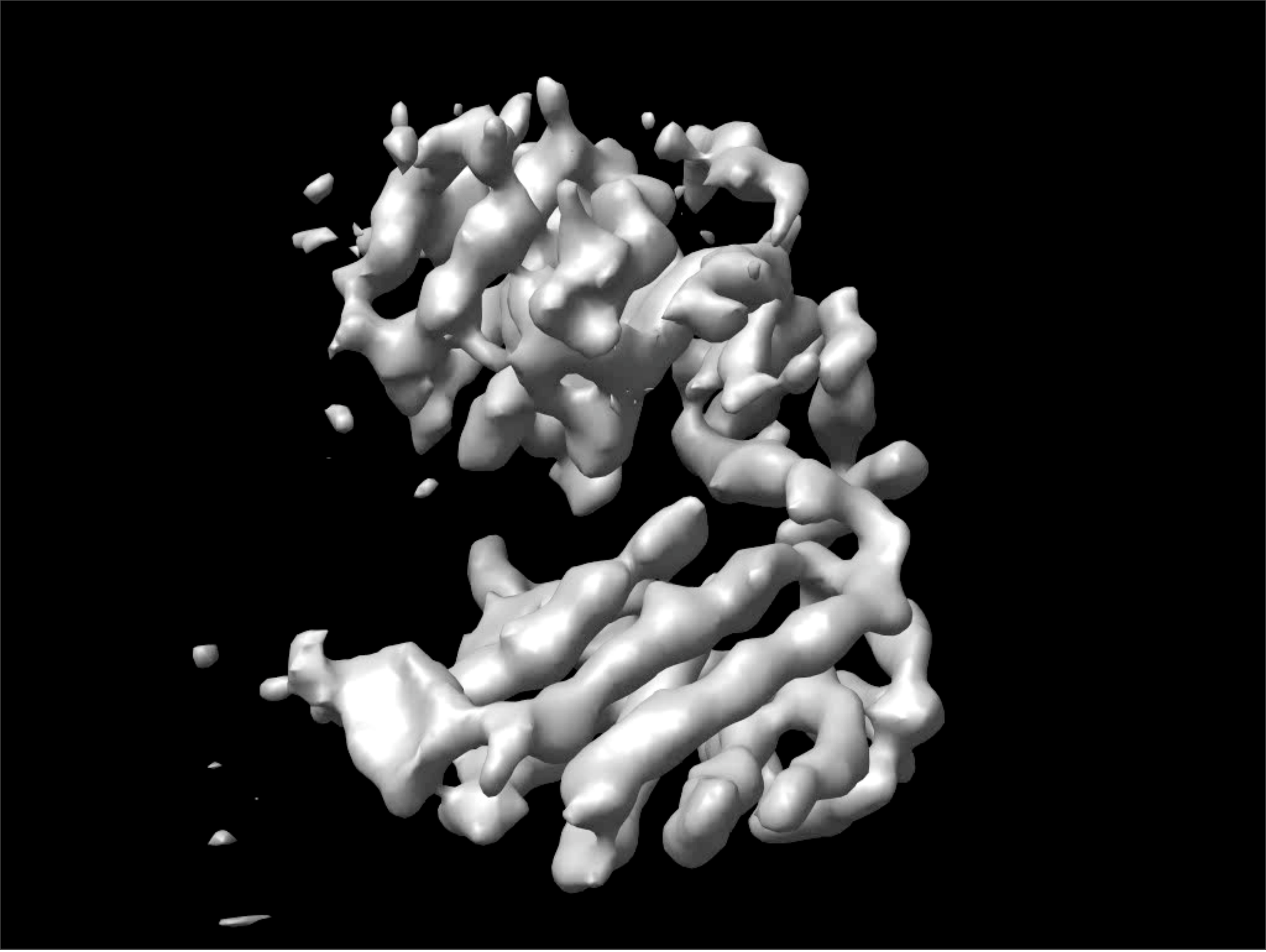


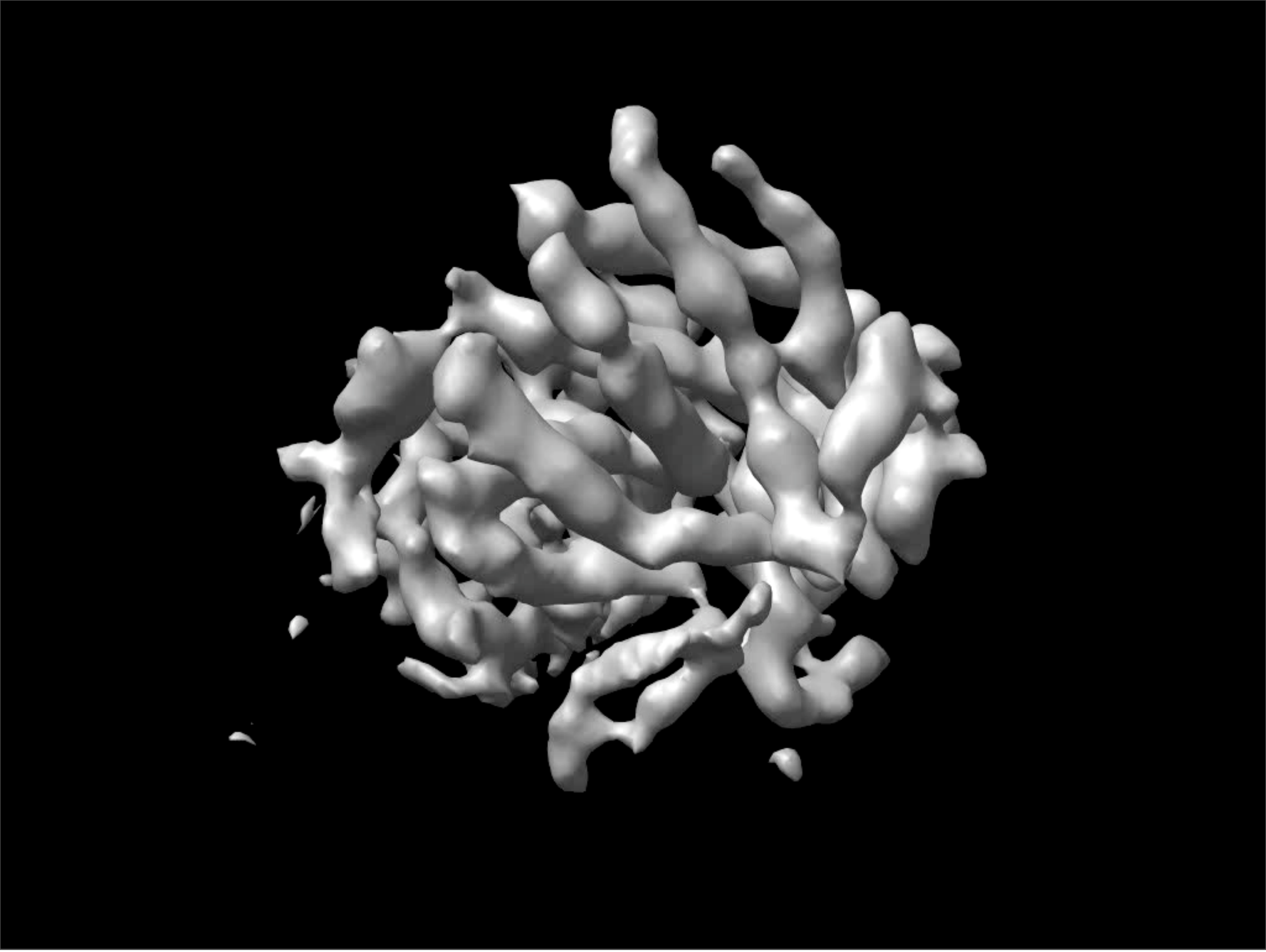






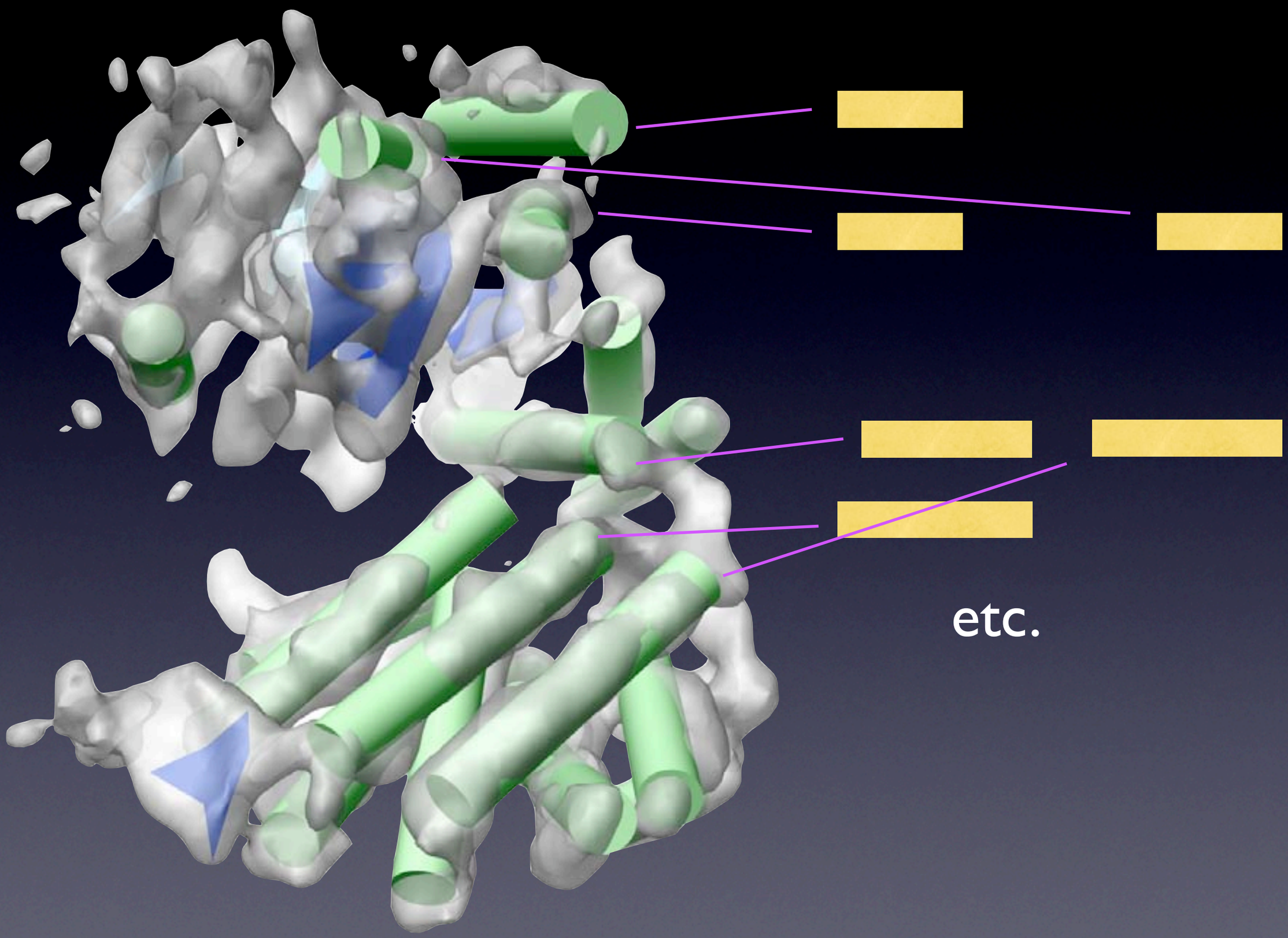




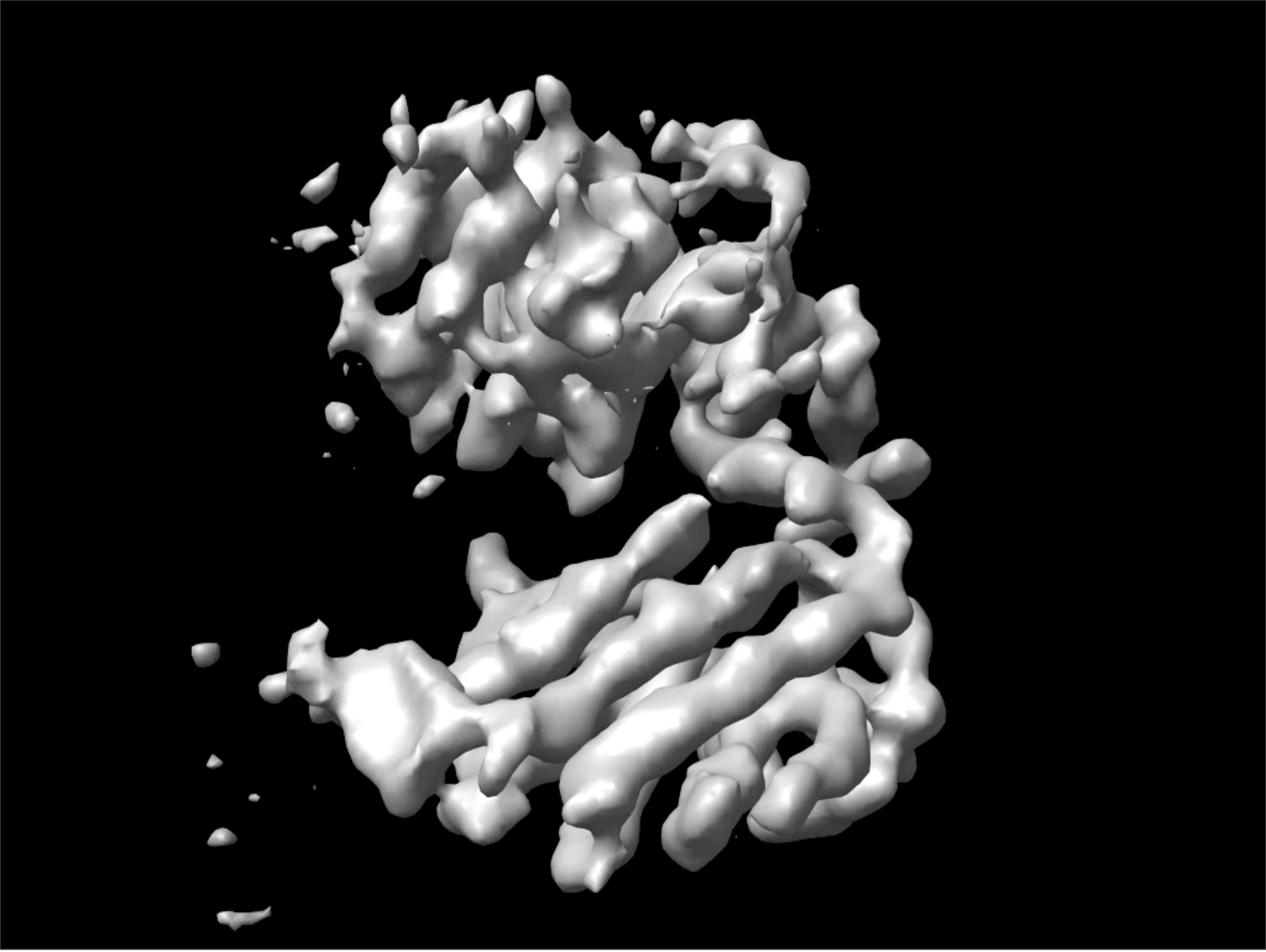


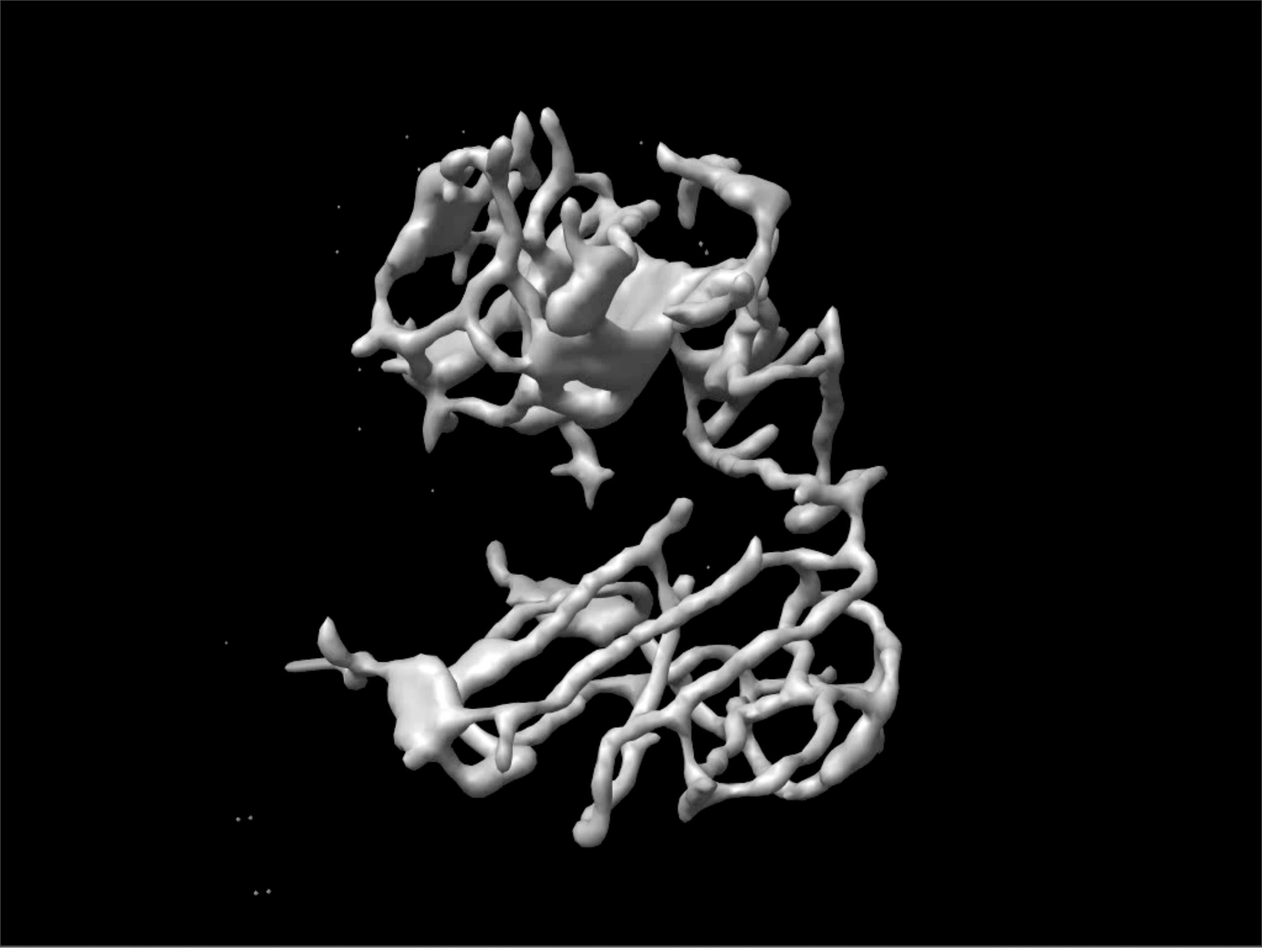
How much can we get at 4 Å ?

SSEHunter



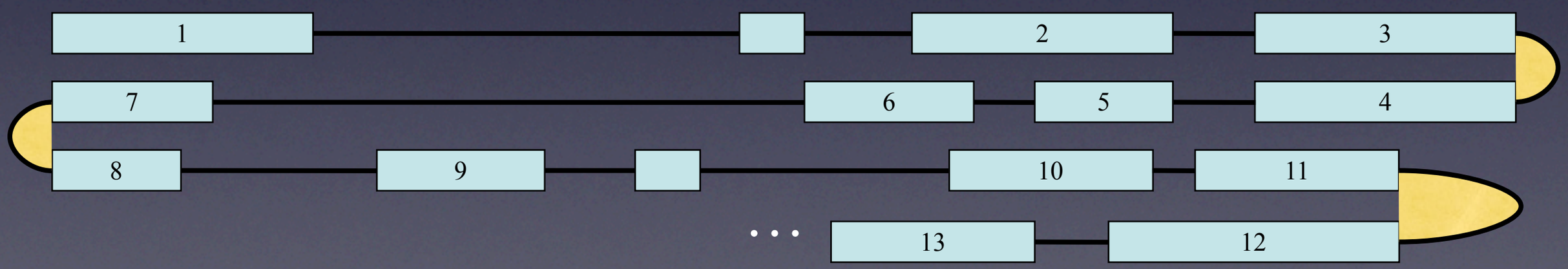
etc.





Sequence Based Secondary Structure Prediction

```
1    AAKDVKFGNDAGVKMLRGVNVLADAVKVTLGPKGRNVVLDKSFGAPTITKDGVSVAREIE

61   LEDKFENMGAQMVKEVASKANDAAGDGTTTATVLAQAIITEGLKAVAAGMNPMDLKRGID

121  KAVTVAVEELKALSVPCSDSKAIAQVGTISANSDETVGKLIAEAMDKVGKEGVITVEDGT

181  GLQDELDVVEGMQFDRGYLSPYFINKPETGAVELESPFILLADKKISNIREMLPVLEAVA

241  KAGKPLLIIAEDVEGEALATAVVNTIRGIVKVAAVKAPGFGDRRKAMLQDIATLTGGTVI

301  SEEIGMELEKATLEDLGQAKRVVINKDTTTIIDGVGEEAAIQGRVAQIRQQIEEATSDYD

361  REKLQERVAKLAGGVAVIKVGAATEVEMKEKKARVEDALHATRAAVEEGVVAGGGVALIR

421  VASKLADLRGQNEDQNVGIKVALRAMEAPLRQIVLNCGEEPSVVANTVKGGDGNYGYNAA

481  TEEYGNMIDMGILDPTKVTRSALQYAASVAGLMITTECMVTDLPKNDAADLGAAGGMGGM

541  GGMGGMM
```

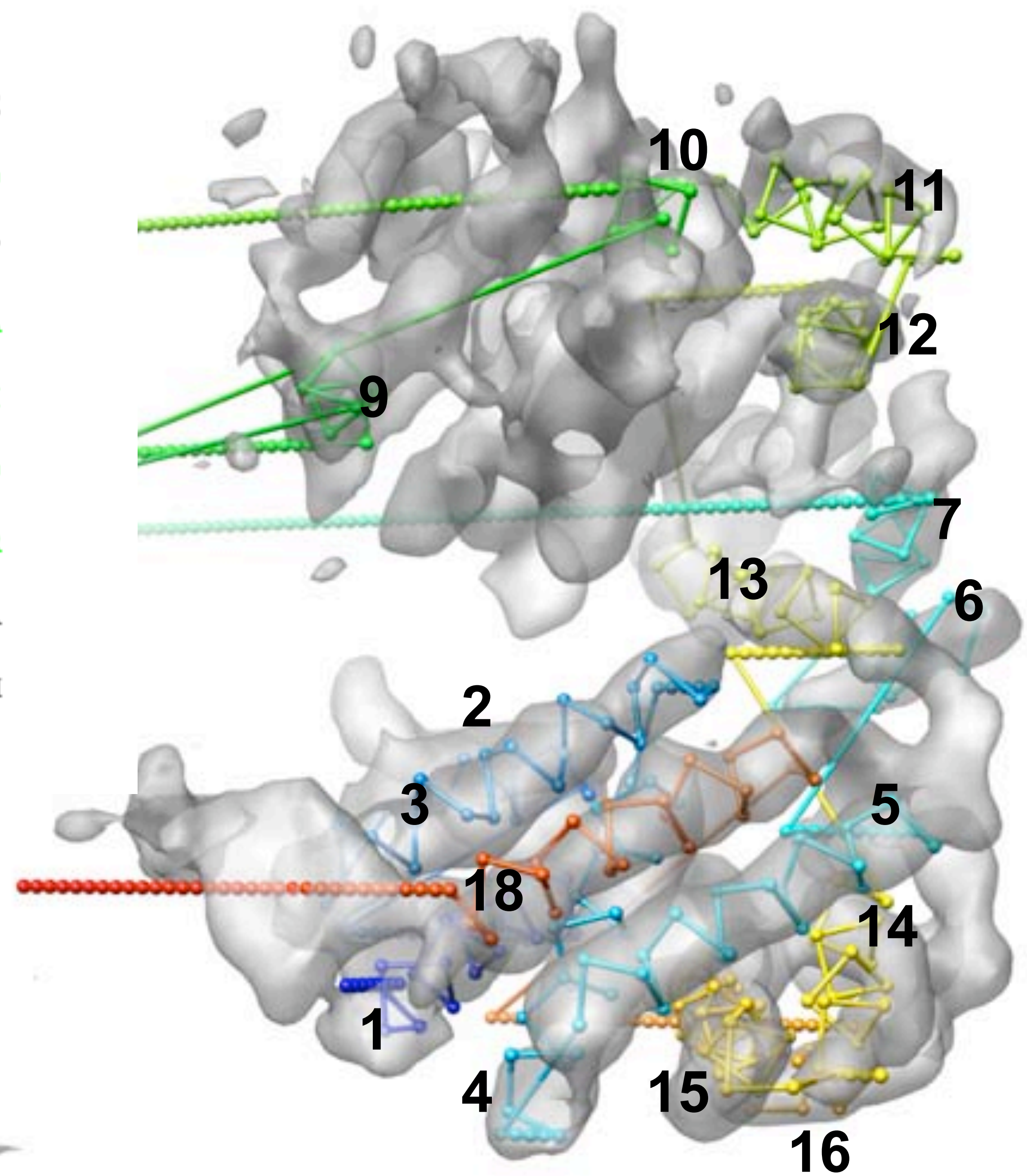


Put it all together

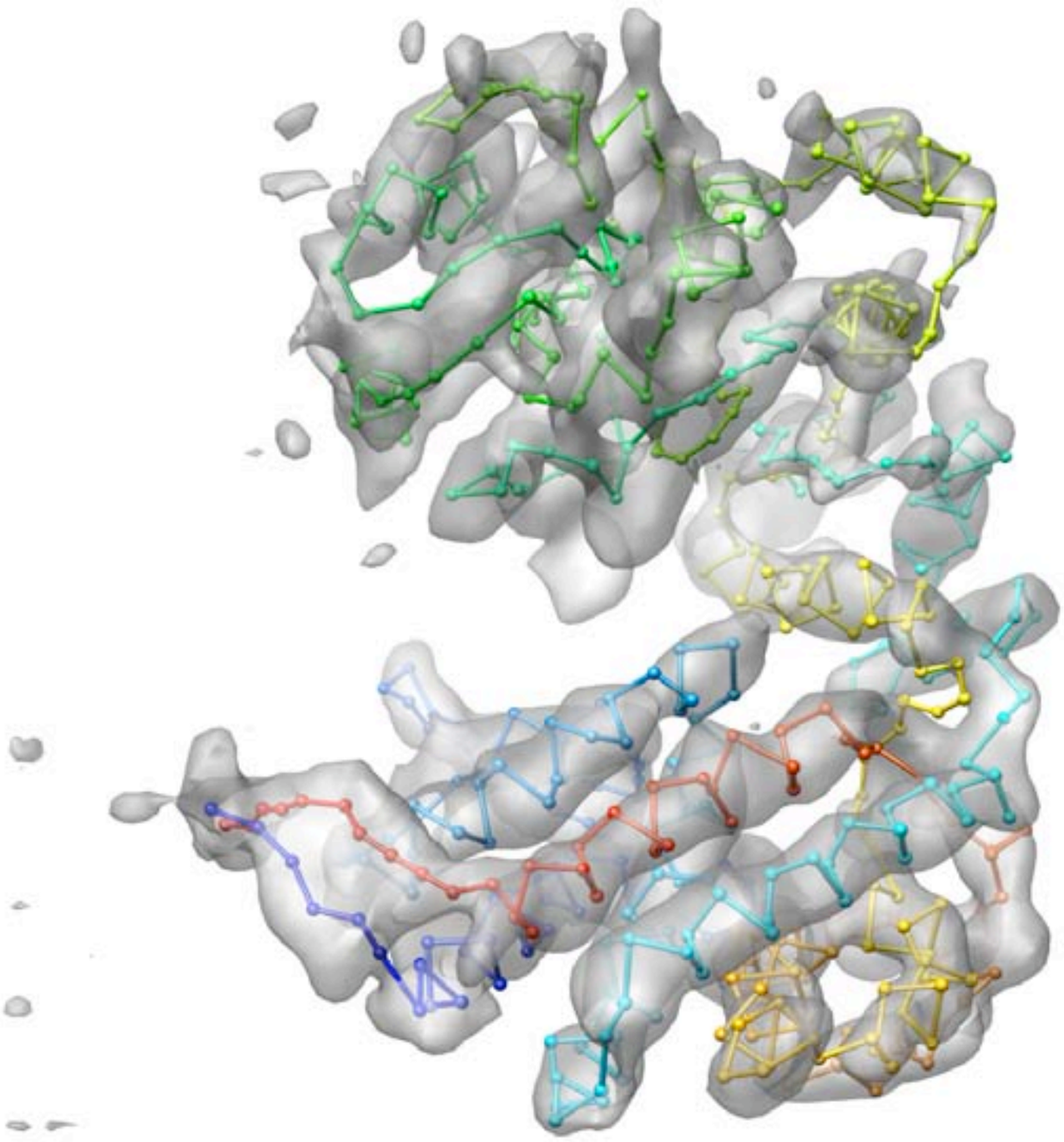
- SSEHunter
  - lengths of helices
- Skeleton
  - Pathlengths between helix endpoints, possible connectivity
- Sequence based Sec. Structure Pred.
  - Lengths of and maximum distances between helices. Relation to primary sequence. Errors ?
- MANY permutations,
  - eg. > 10^{16} possible helix assignments

Build the Model

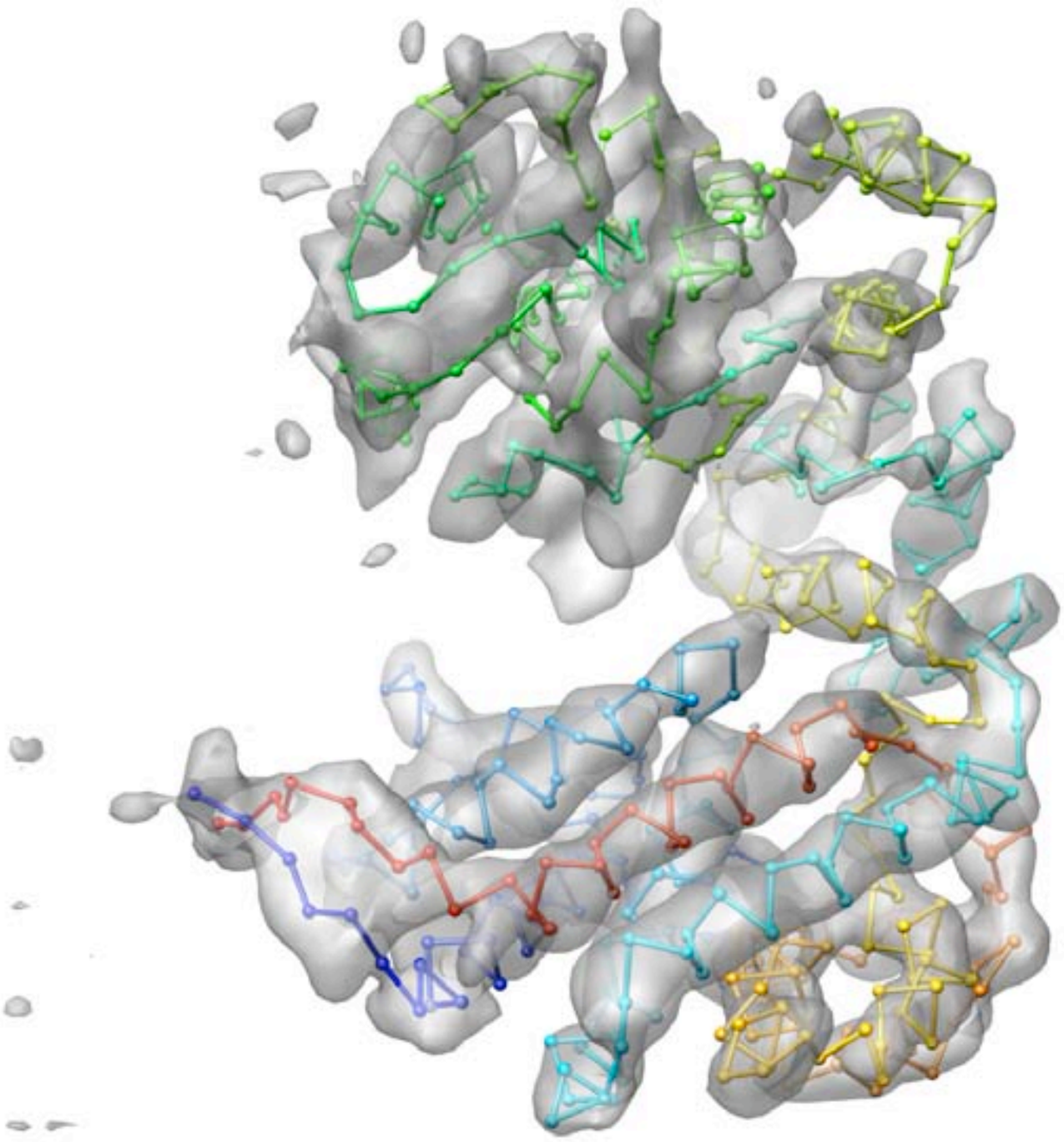
```
                1                                                      2
1      AAKDVKFGNDAGVKMLRGVNVLADAVKVTLGPKGRNVVLDKSFGAPTITKDGVSVAREIE
            3                     4                            5
61     LEDKFENMGAQMVKEVASKANDAAGDGTTTATVLAQAIITEGLKAVAAGMNPMDLKRGID
                           6             7
121    KAVTVAVEELKALSVPCSDSKAIAQVGTISANSDETVGKLIAEAMDKVGKEGVITVEDGT
                                                                8
181    GLQDELDVVEGMQFDRGYLSPYFINKPETGAVELESPFILLADKKISNIREMLPVLEAVA
                   9                                       10
241    KAGKPLLIIAEDVEGEALATAVVNTIRGIVKVAAVKAPGFGDRRKAMLQDIATLTGGTVI
                                                           11
301    SEEIGMELEKATLEDLGQAKRVVINKDTTTIIDGVGEEAAIQGRVAQIRQQIEEATSDYD
          12                         13                        14
361    REKLQERVAKLAGGVAVIKVGAATEVEMKEKKARVEDALHATRAAVEEGVVAGGGVALIR
                             15            16
421    VASKLADLRGQNEDQNVGIKVALRAMEAPLRQIVLNCGEEPSVVANTVKGGDGNYGYNAA
          17                    18
481    TEEYGNMIDMGILDPTKVTRSALQYAASVAGLMITTECMVTDLPKNDAADLGAAGGMGGM

541    GGMGGMM
```



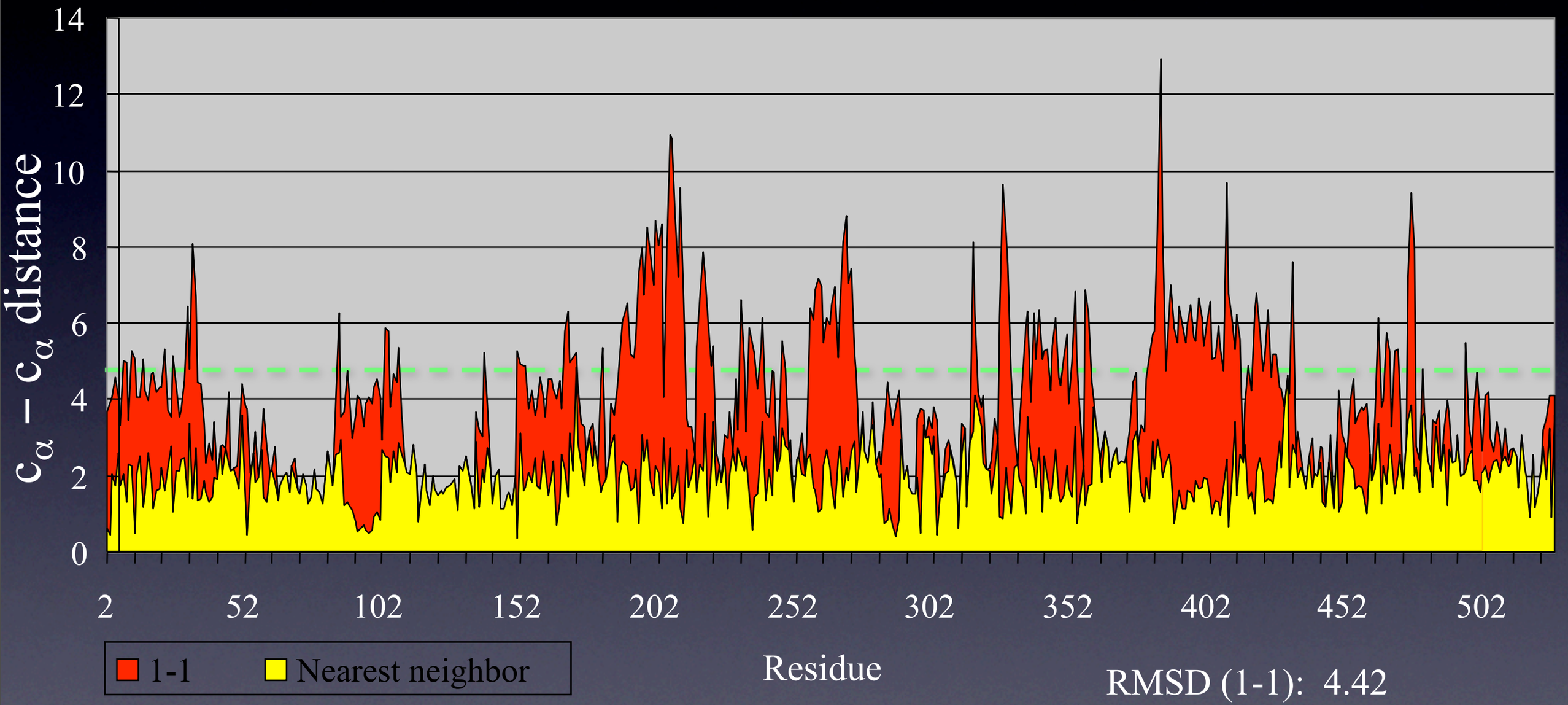
GroEL Chain Trace

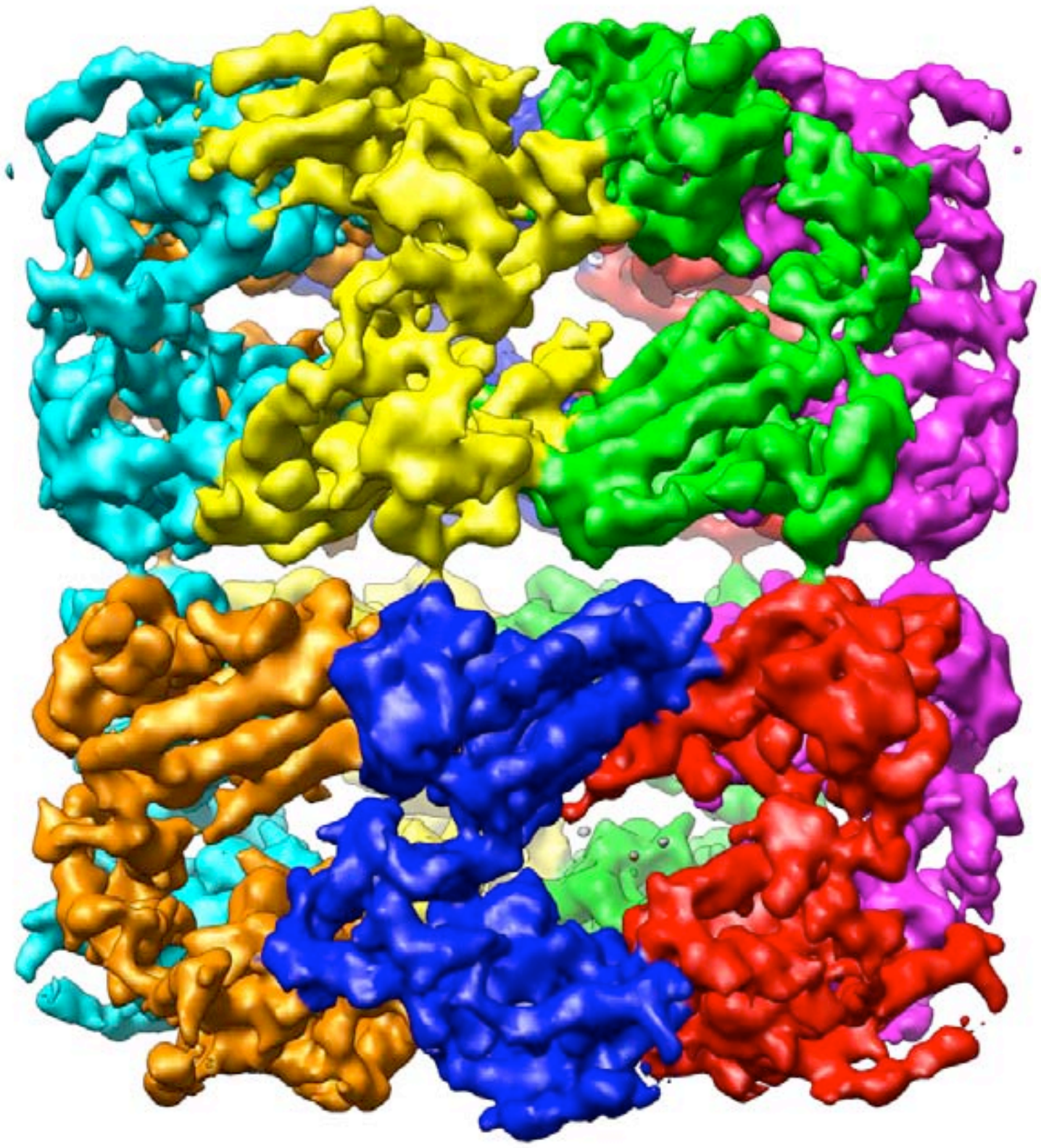


GroEL Chain Trace

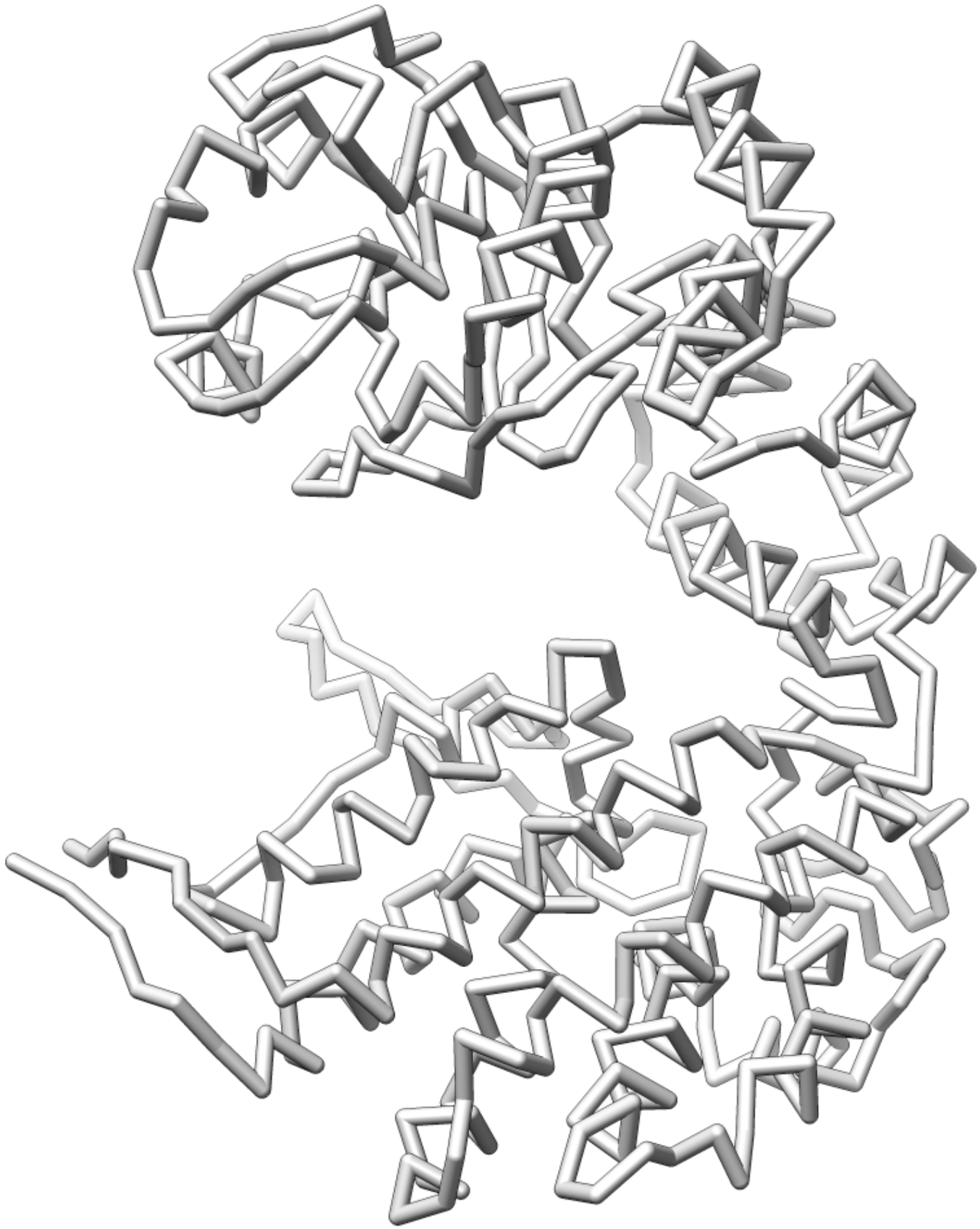


RMSD vs. PDB Structure



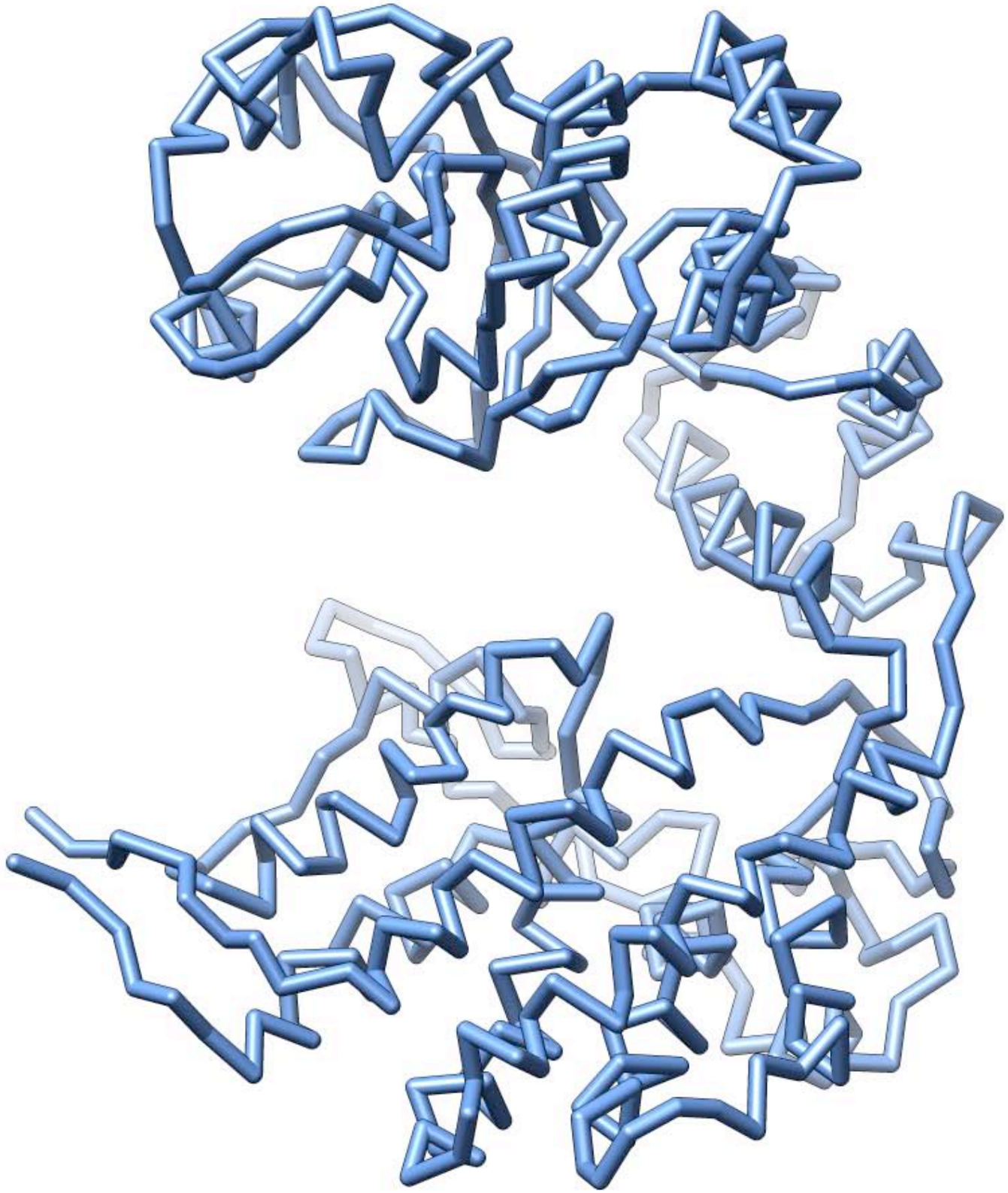


D7



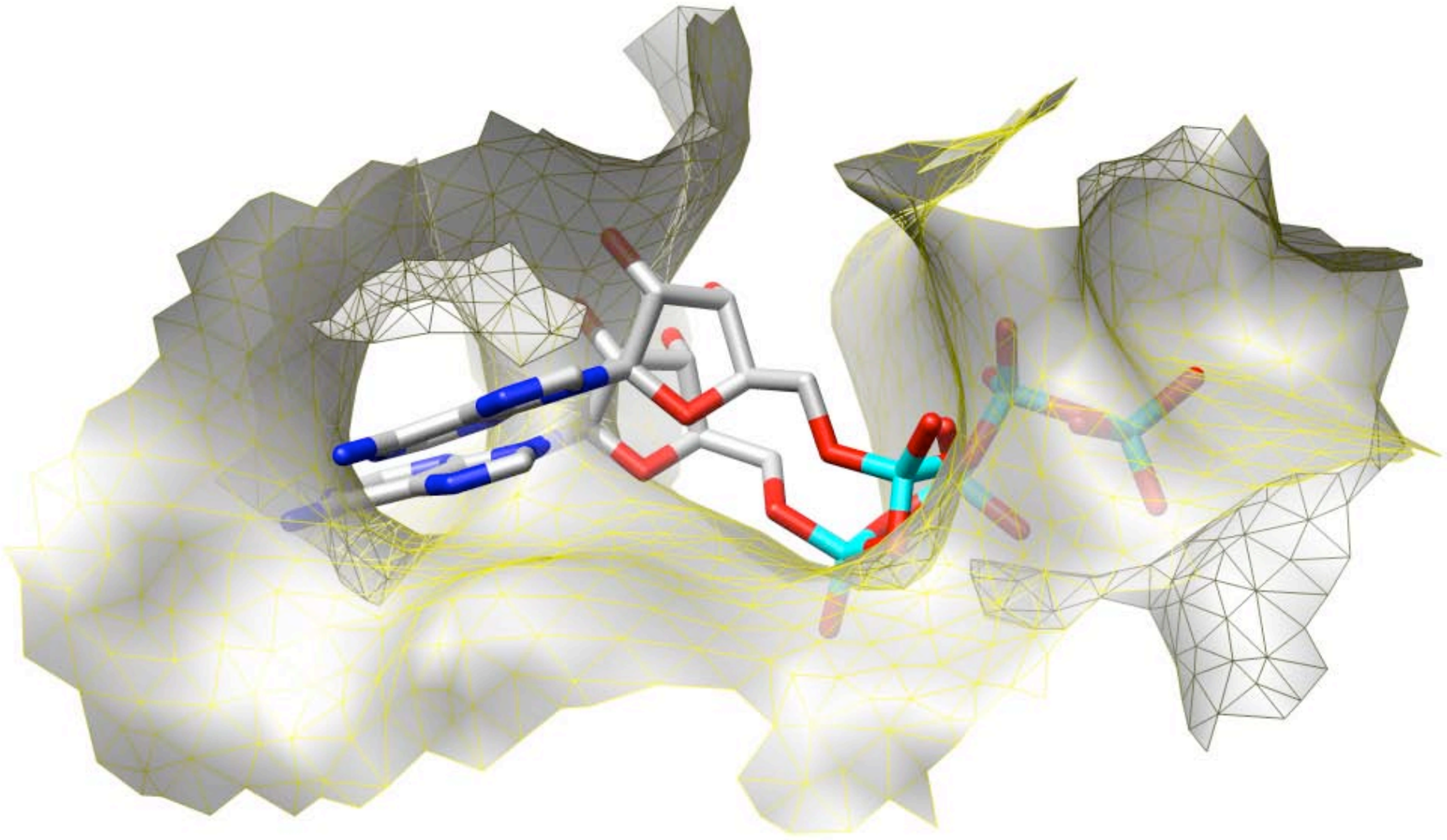
Bottom Ring C7

Top Ring C7



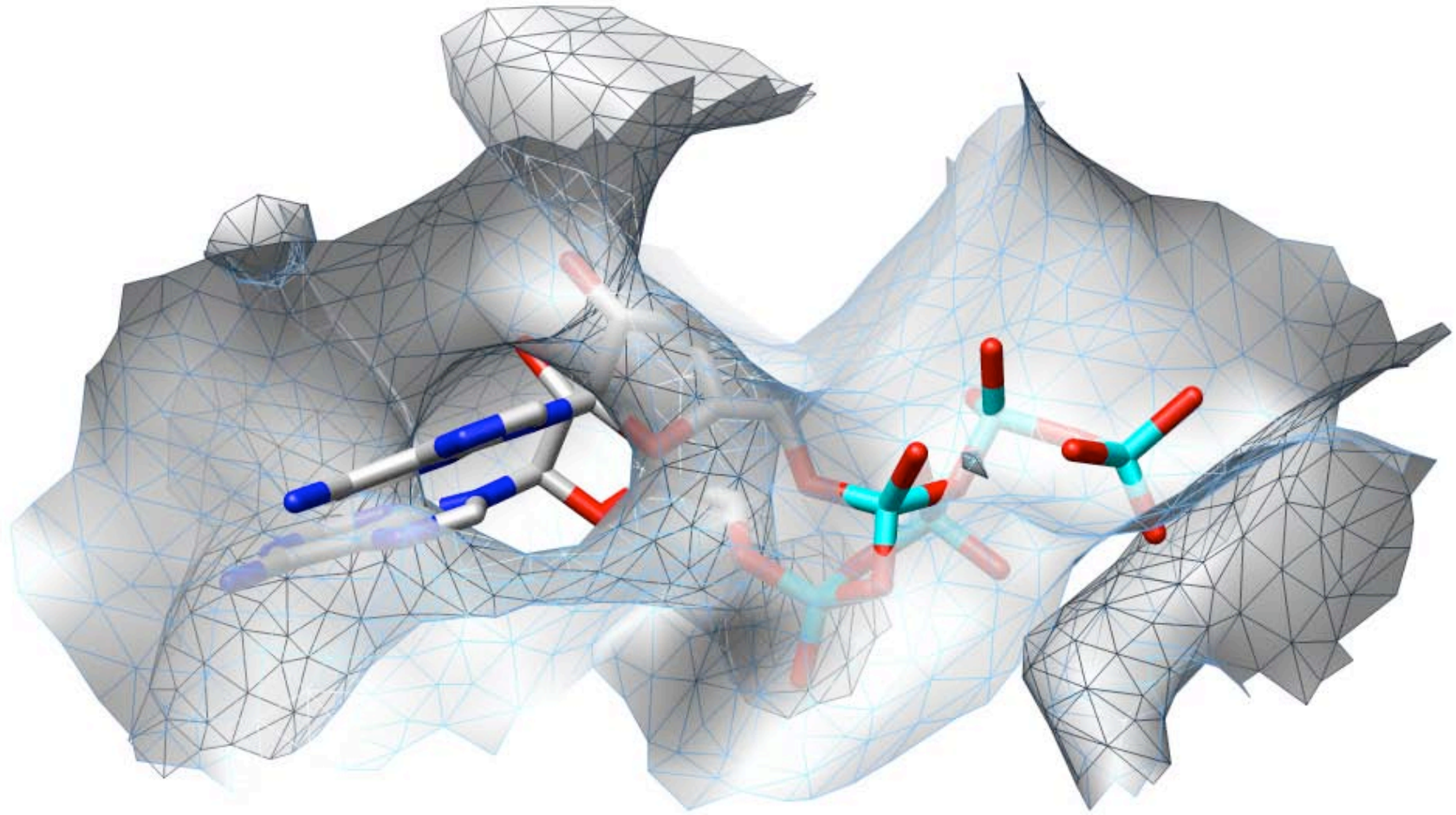
Bottom Ring

(ATP Binding Site)



Top Ring

(ATP Binding Site)



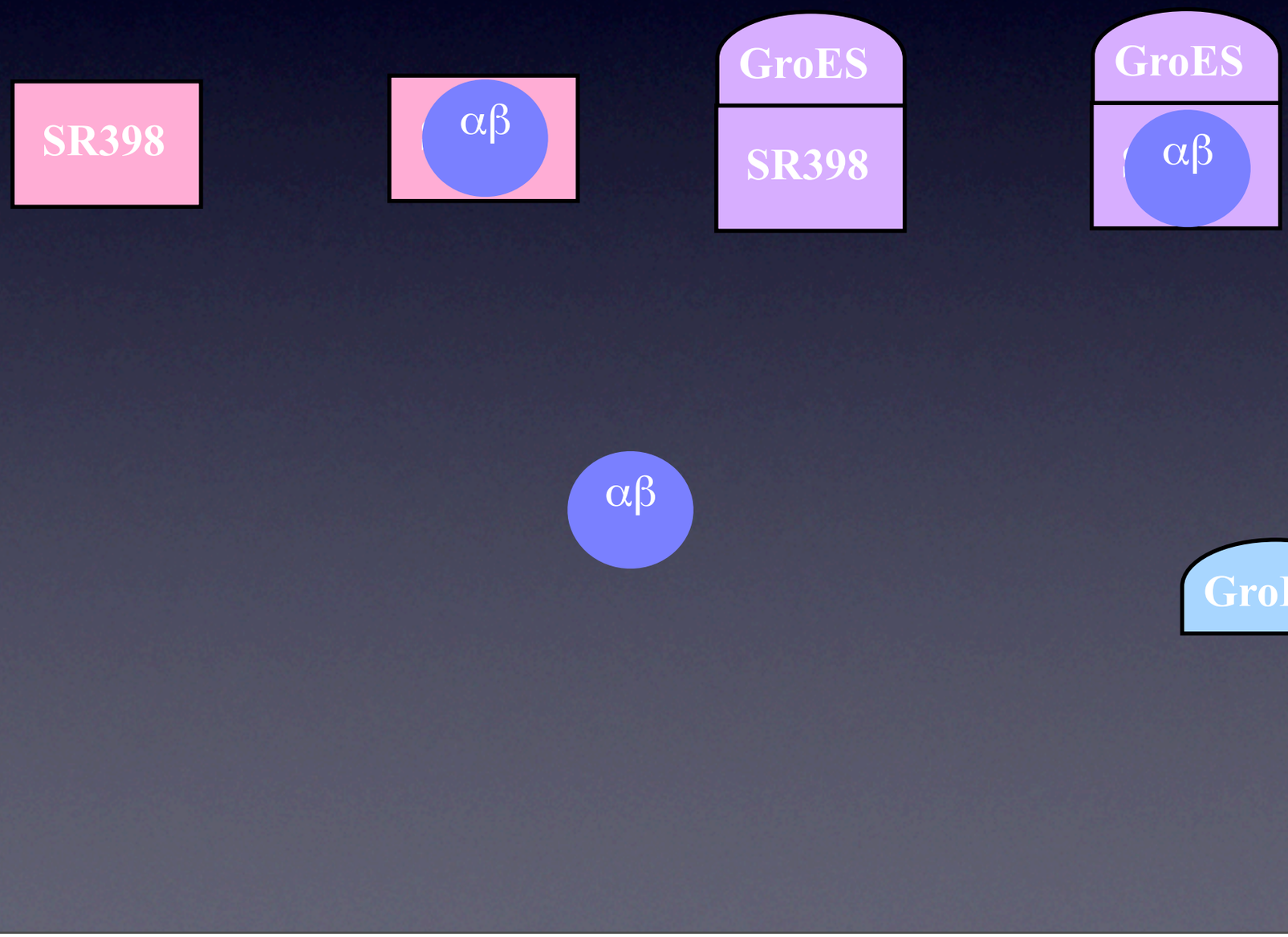
GroEL results

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

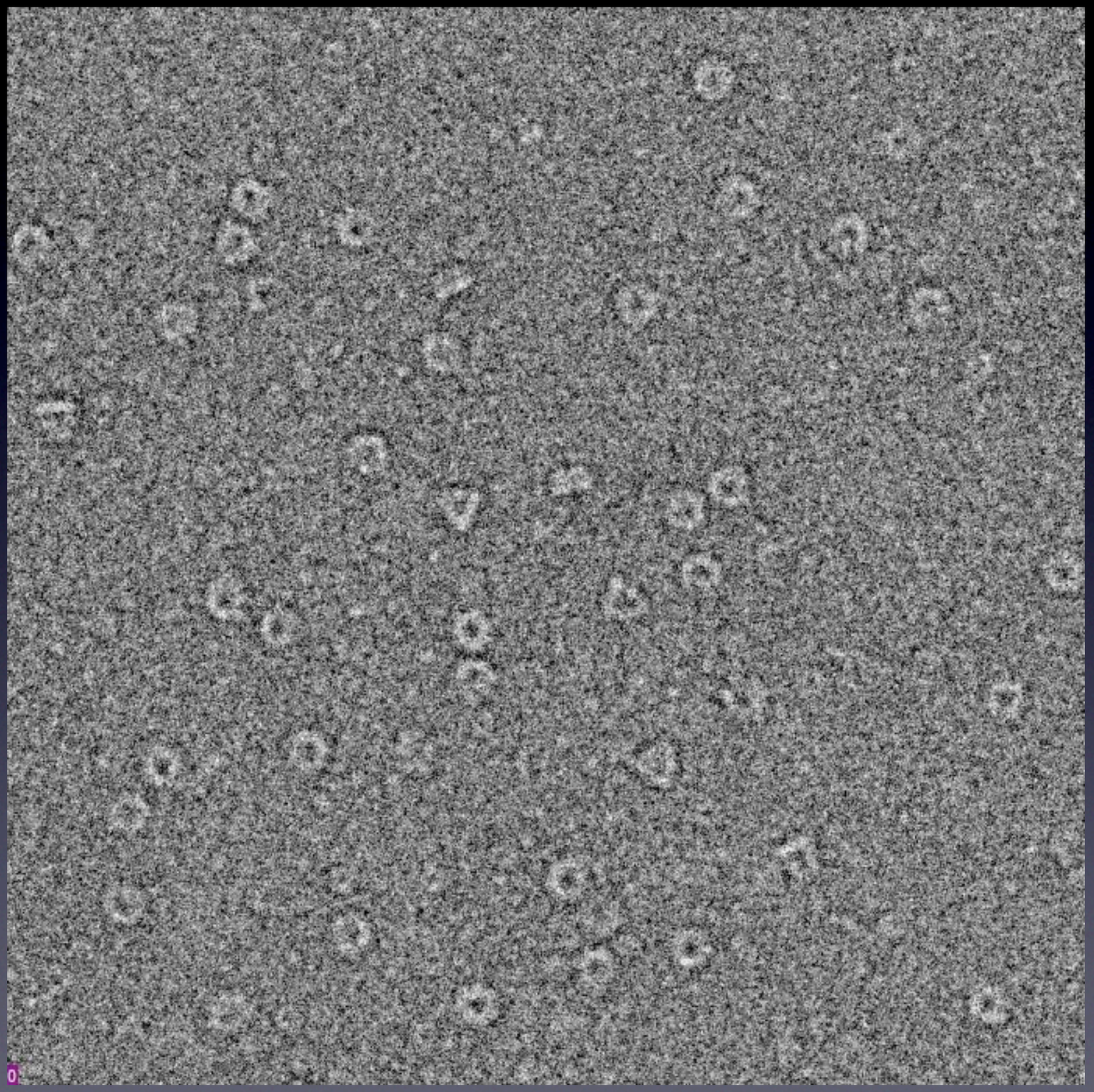
Multi-component Systems

GroEL, GroES & Substrate

Produces a Mixed Population

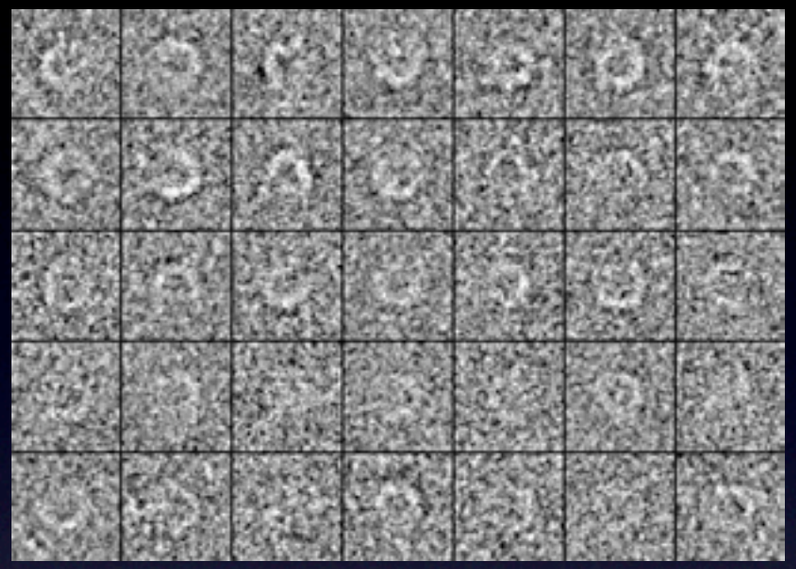


SR398+GroES



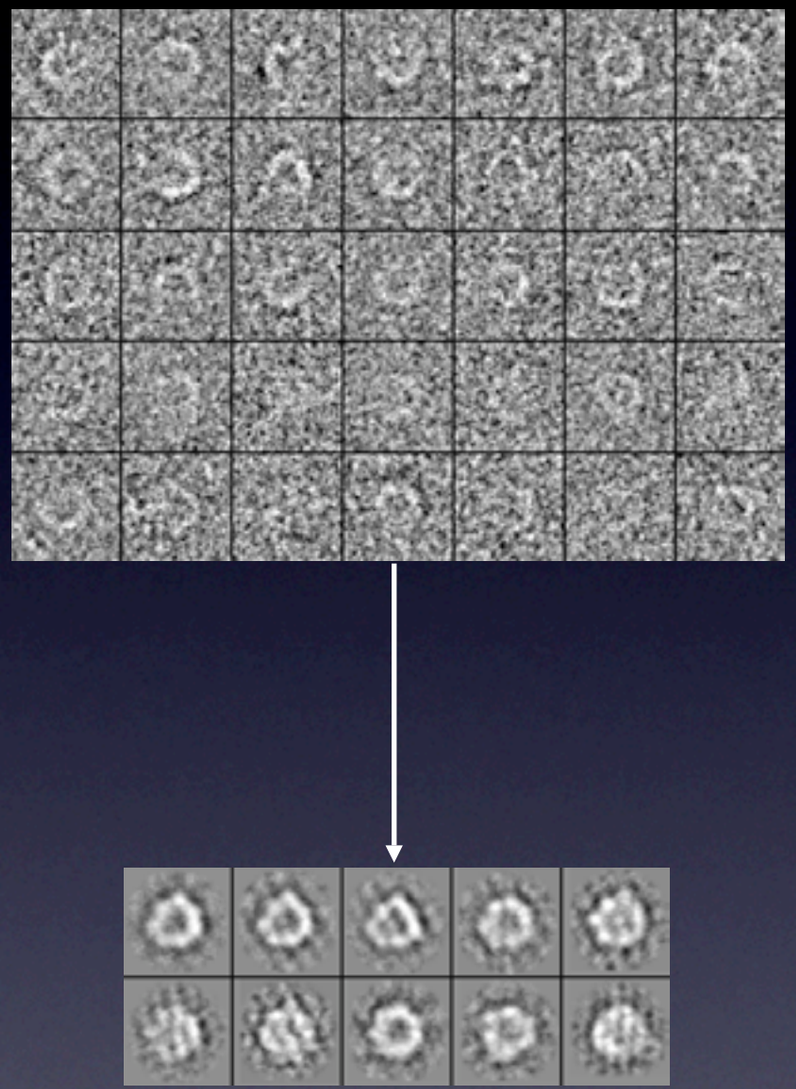
2D Refinement

Particles



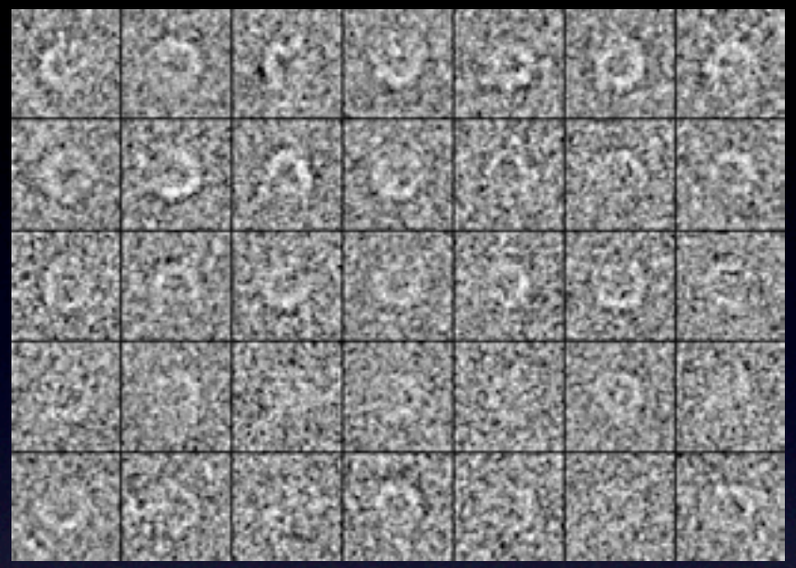
2D Refinement

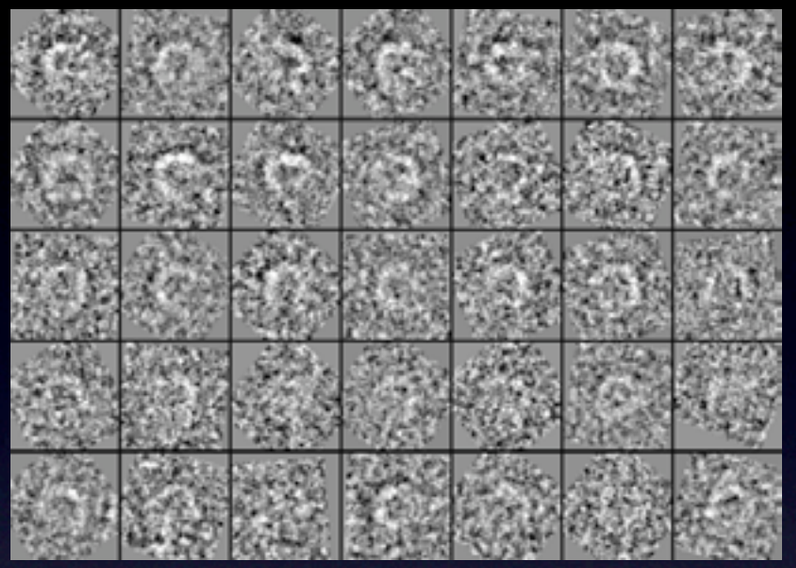
Particles



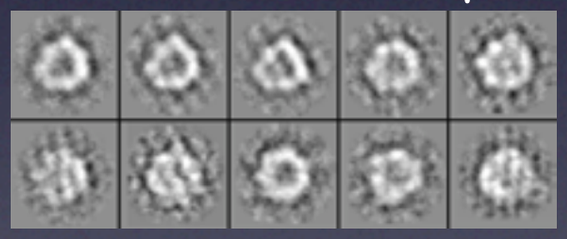
2D Refinement

Particles



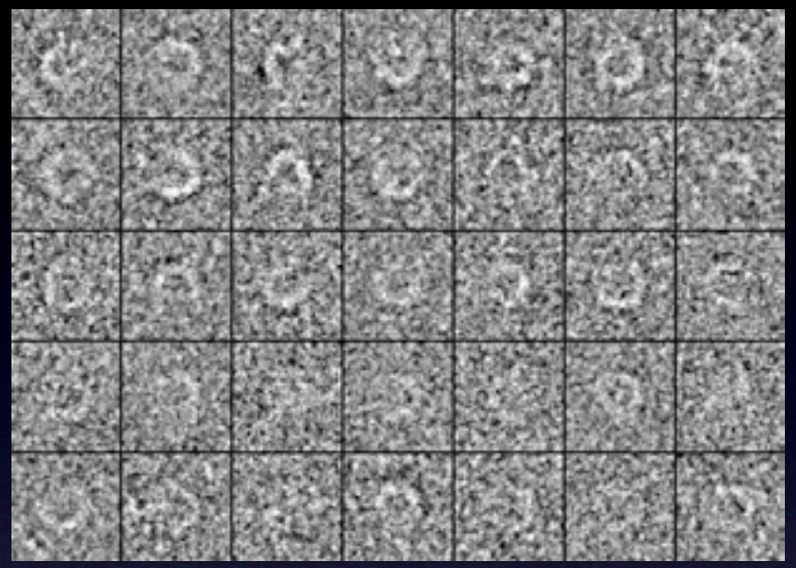


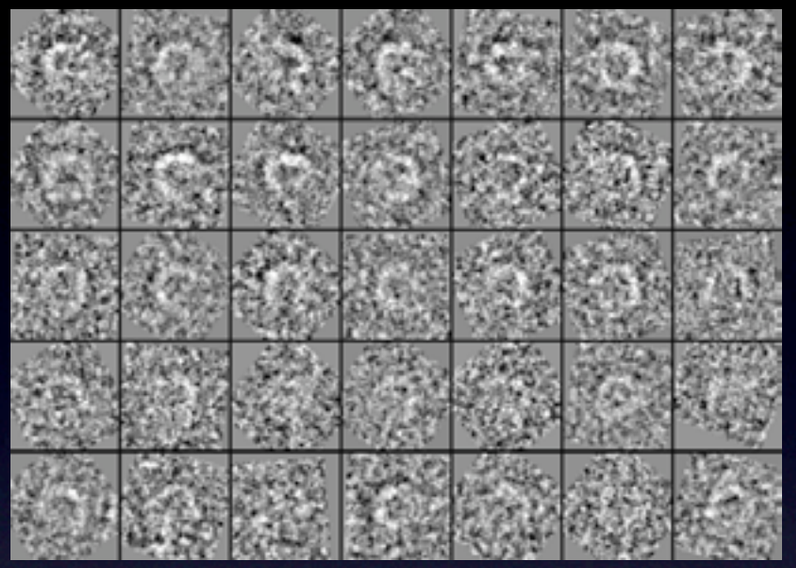
Align Particles



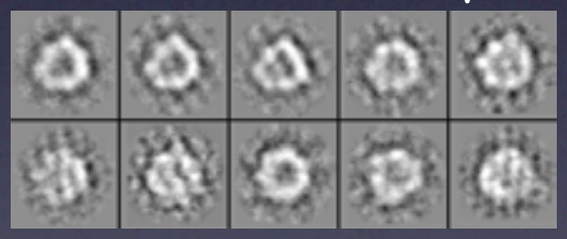
2D Refinement

Particles

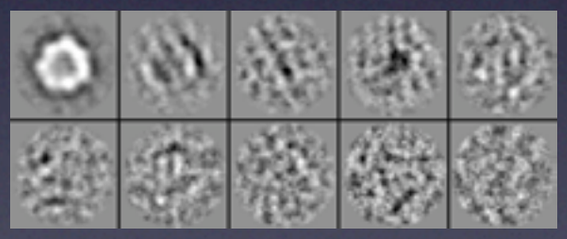




Align Particles

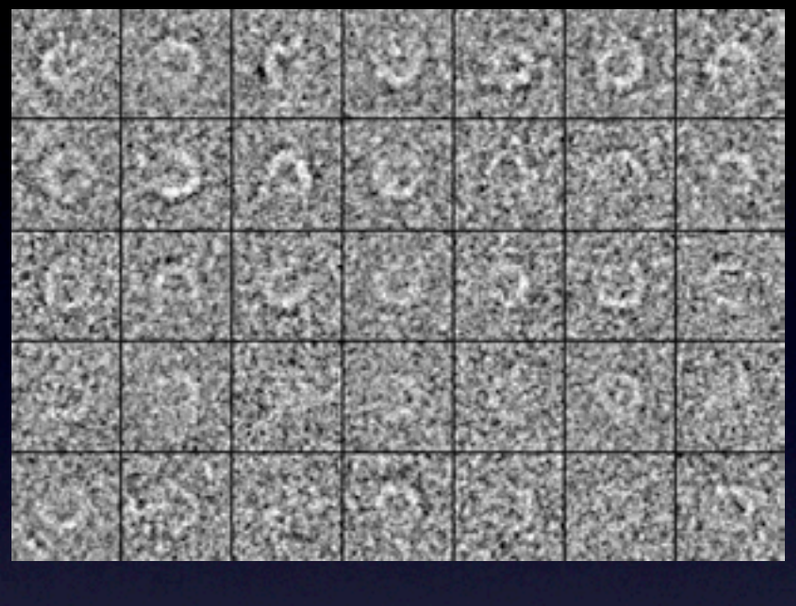


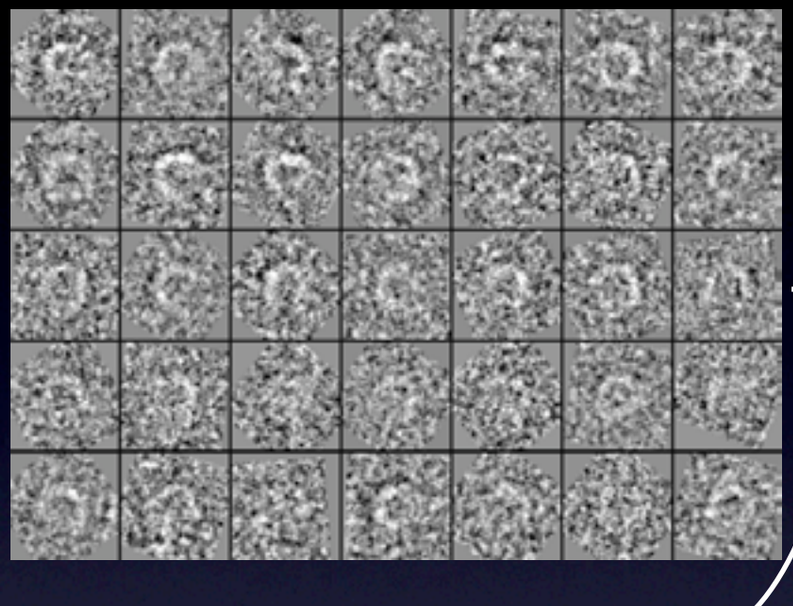
SVD/PCA

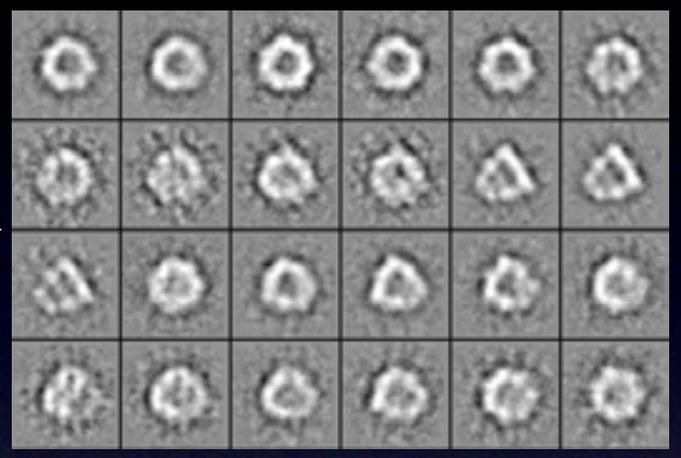


2D Refinement

Particles

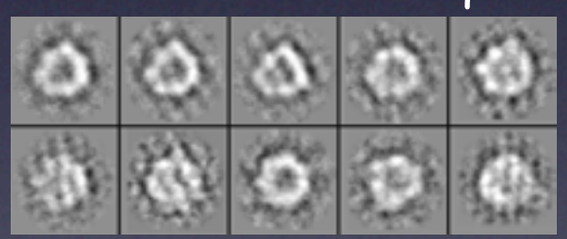




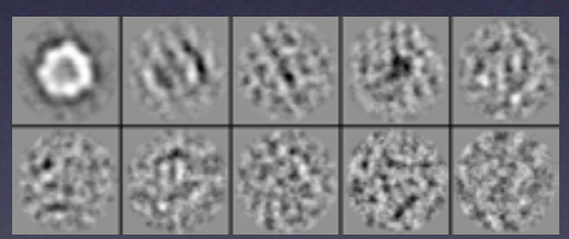


Align Particles

k-means classification in SVD subspace

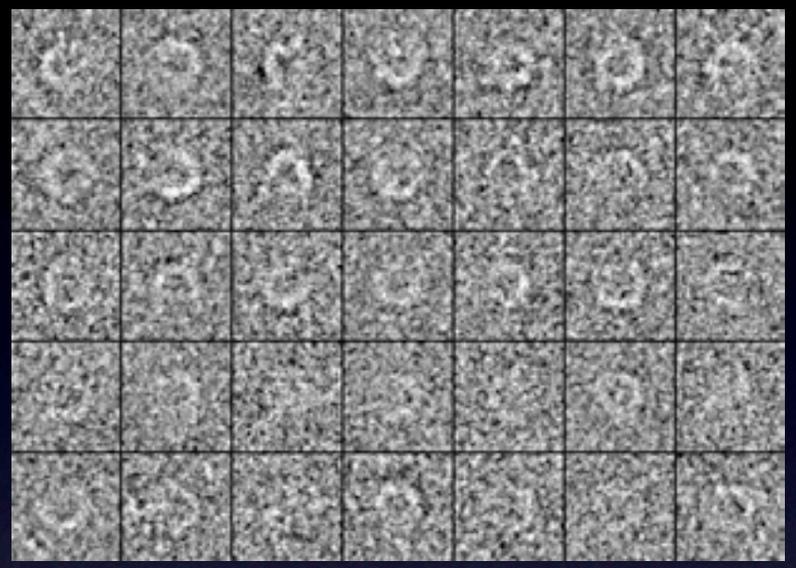


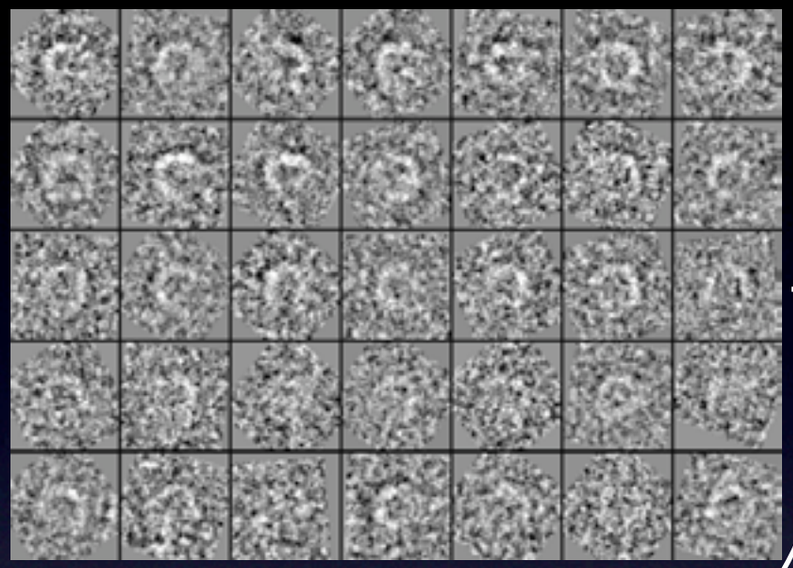
SVD/PCA

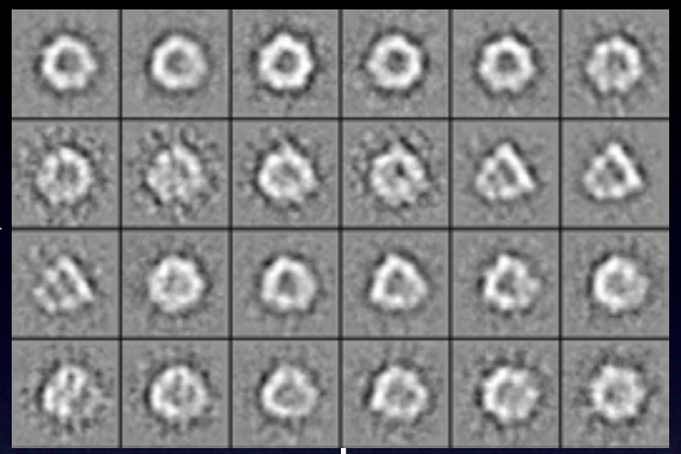


2D Refinement

Particles

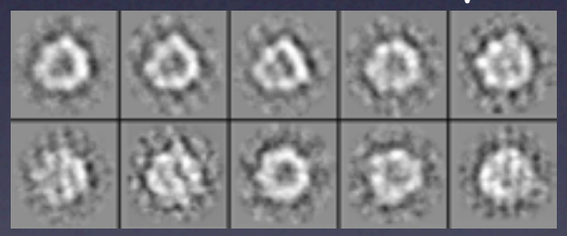




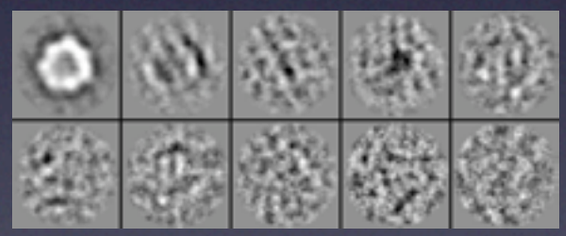


k-means classification in SVD subspace

Align Particles

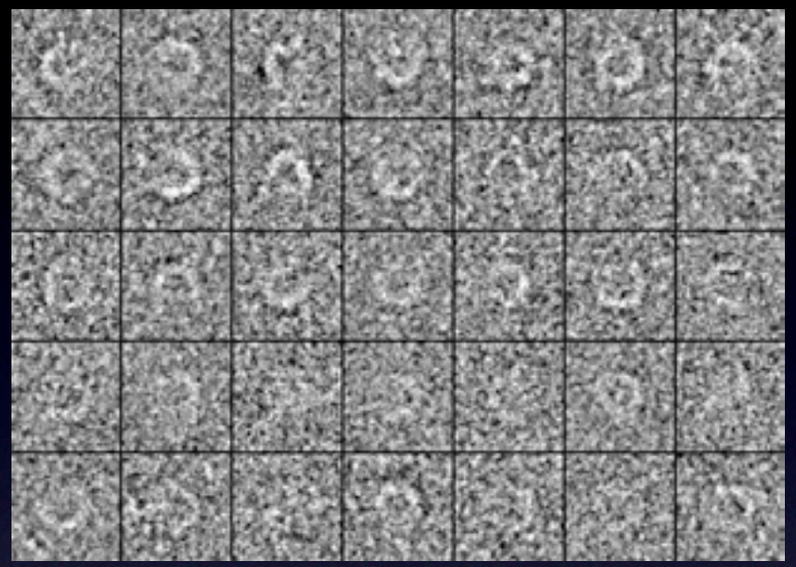


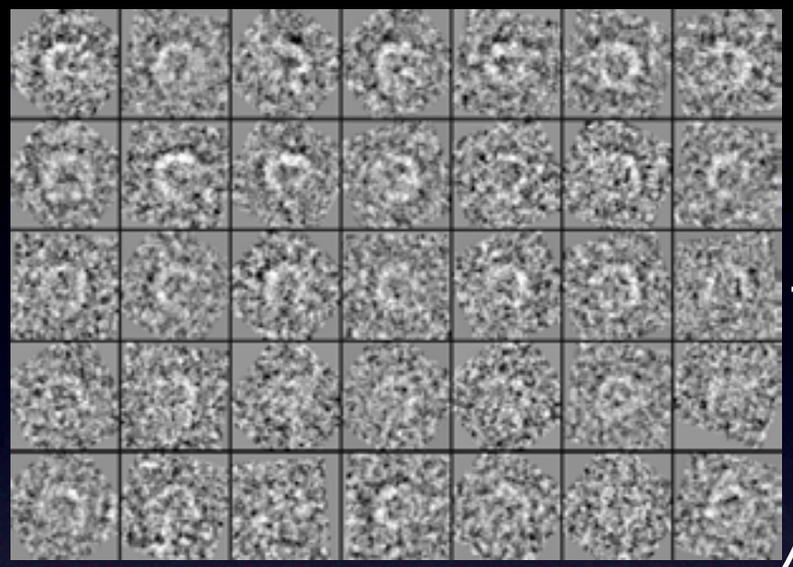
SVD/PCA

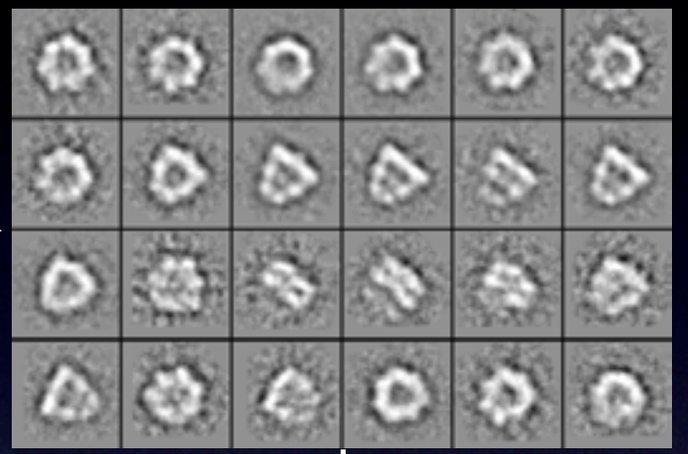


2D Refinement

Particles

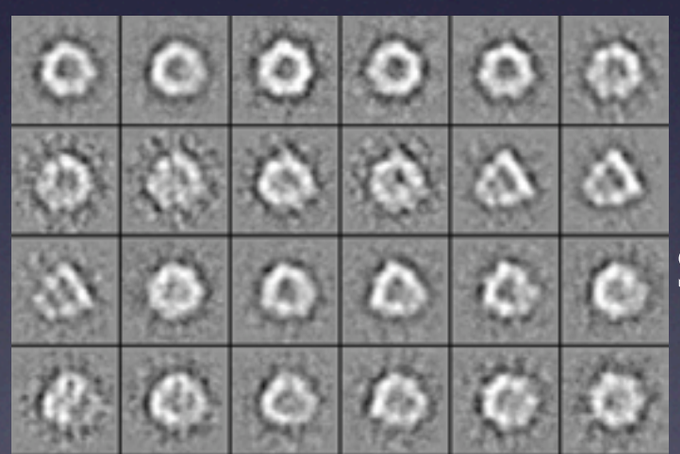




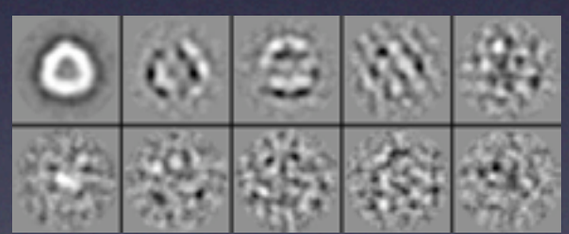


k-means classification in SVD subspace

Align Particles

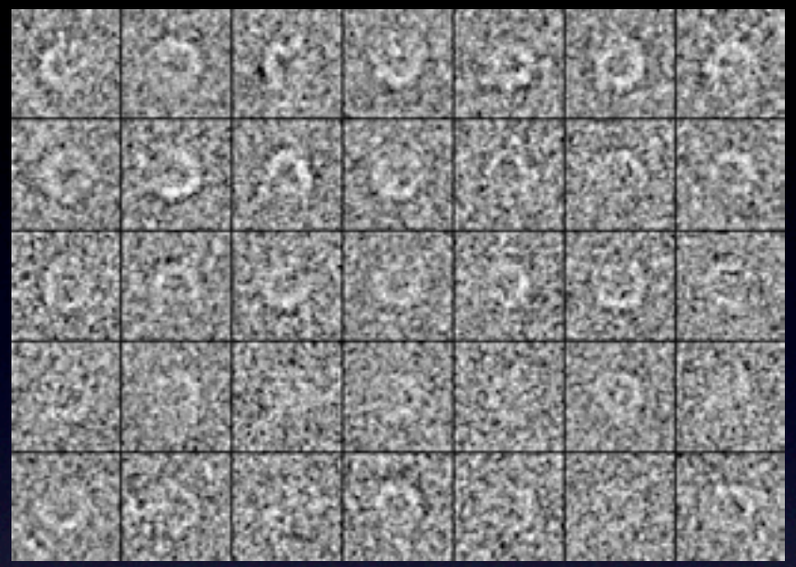


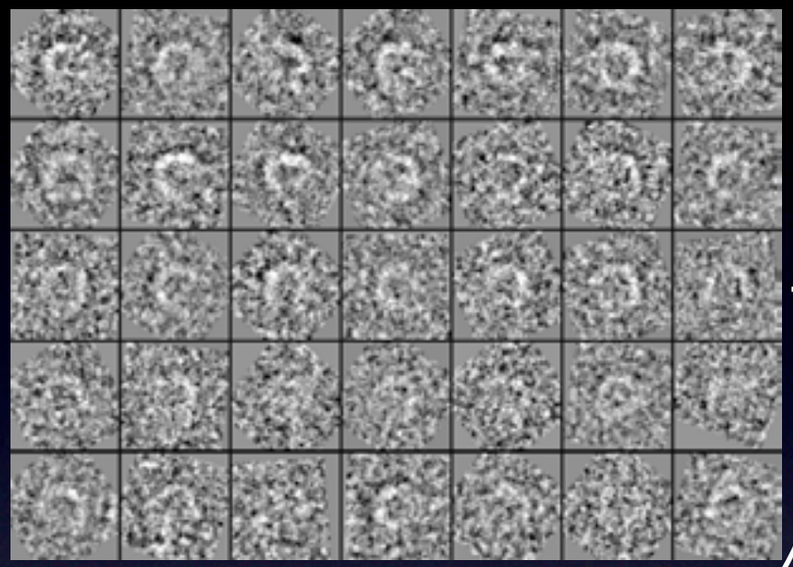
SVD/PCA

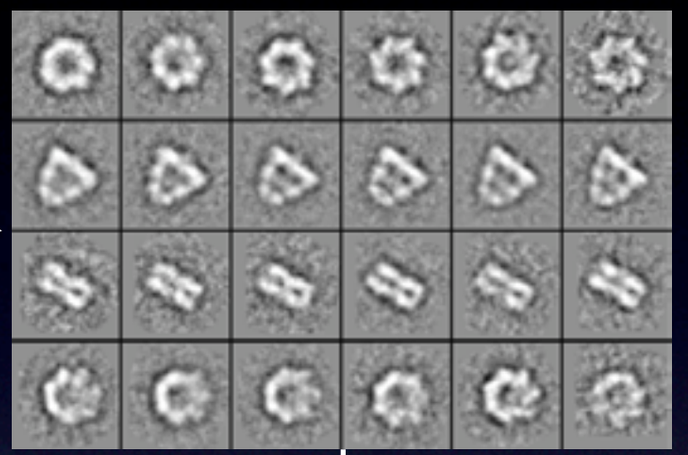


2D Refinement

Particles

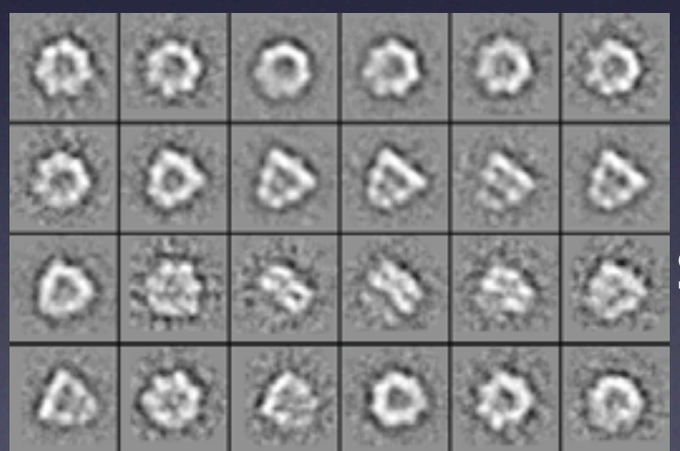




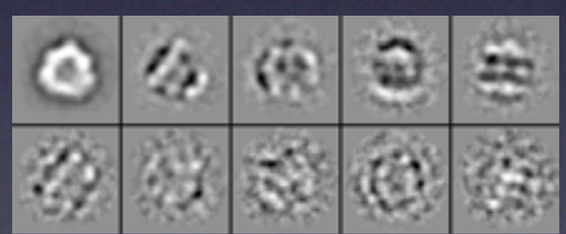


Align Particles

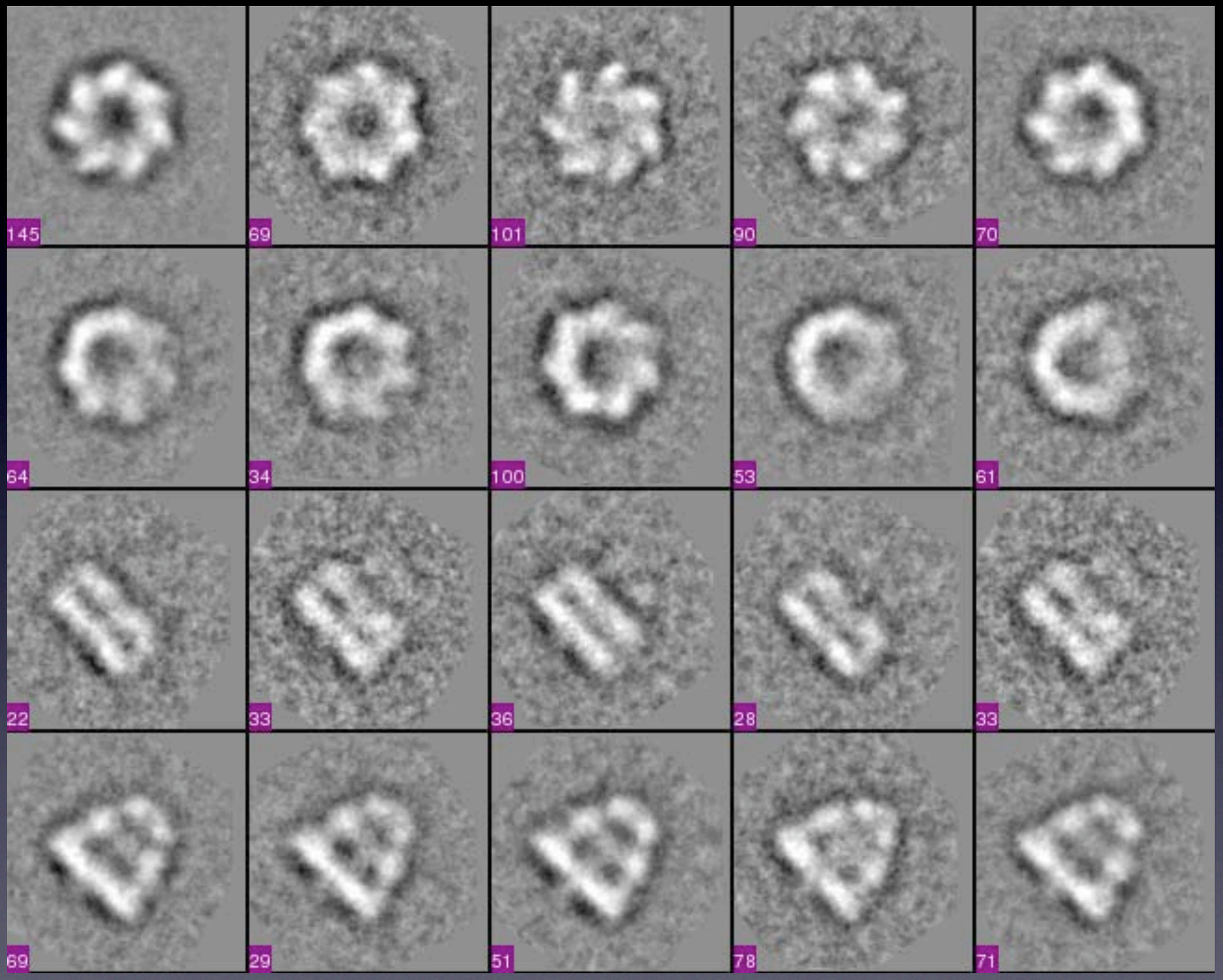
k-means classification in SVD subspace



SVD/PCA



After 9 iterations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100
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61
22
33
36
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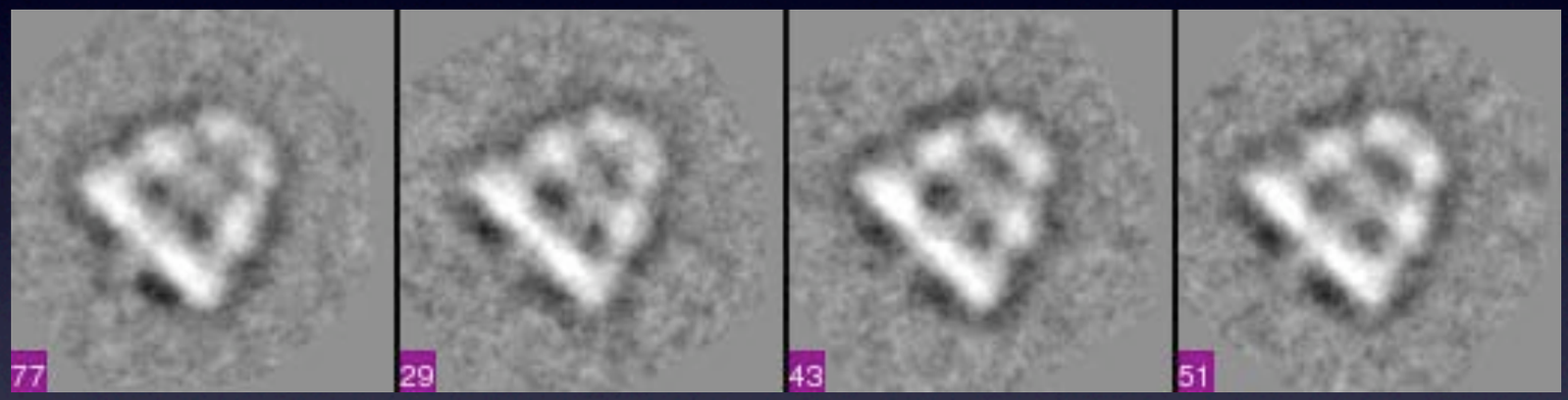
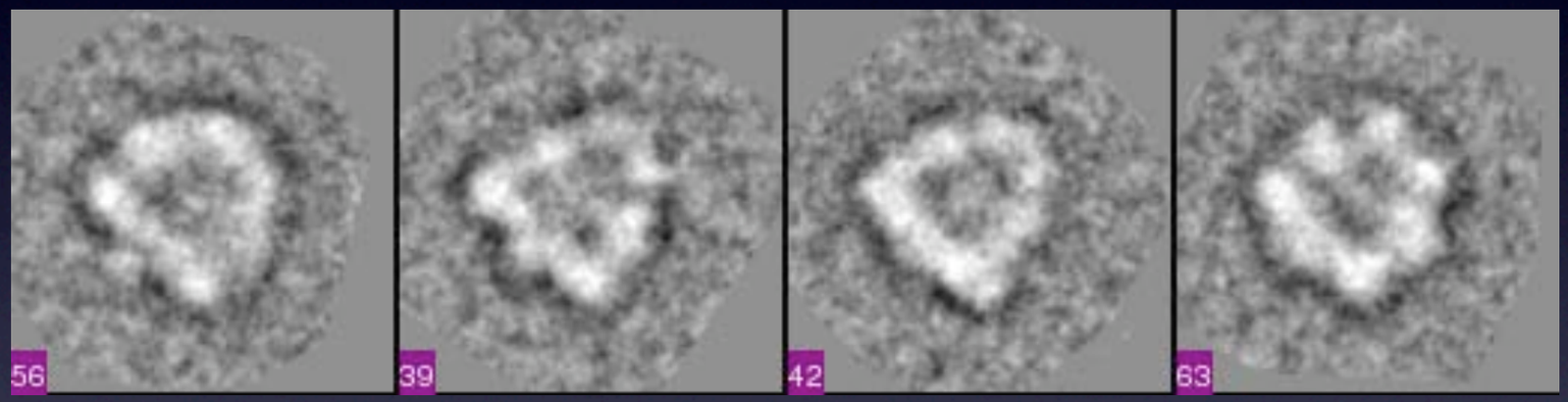
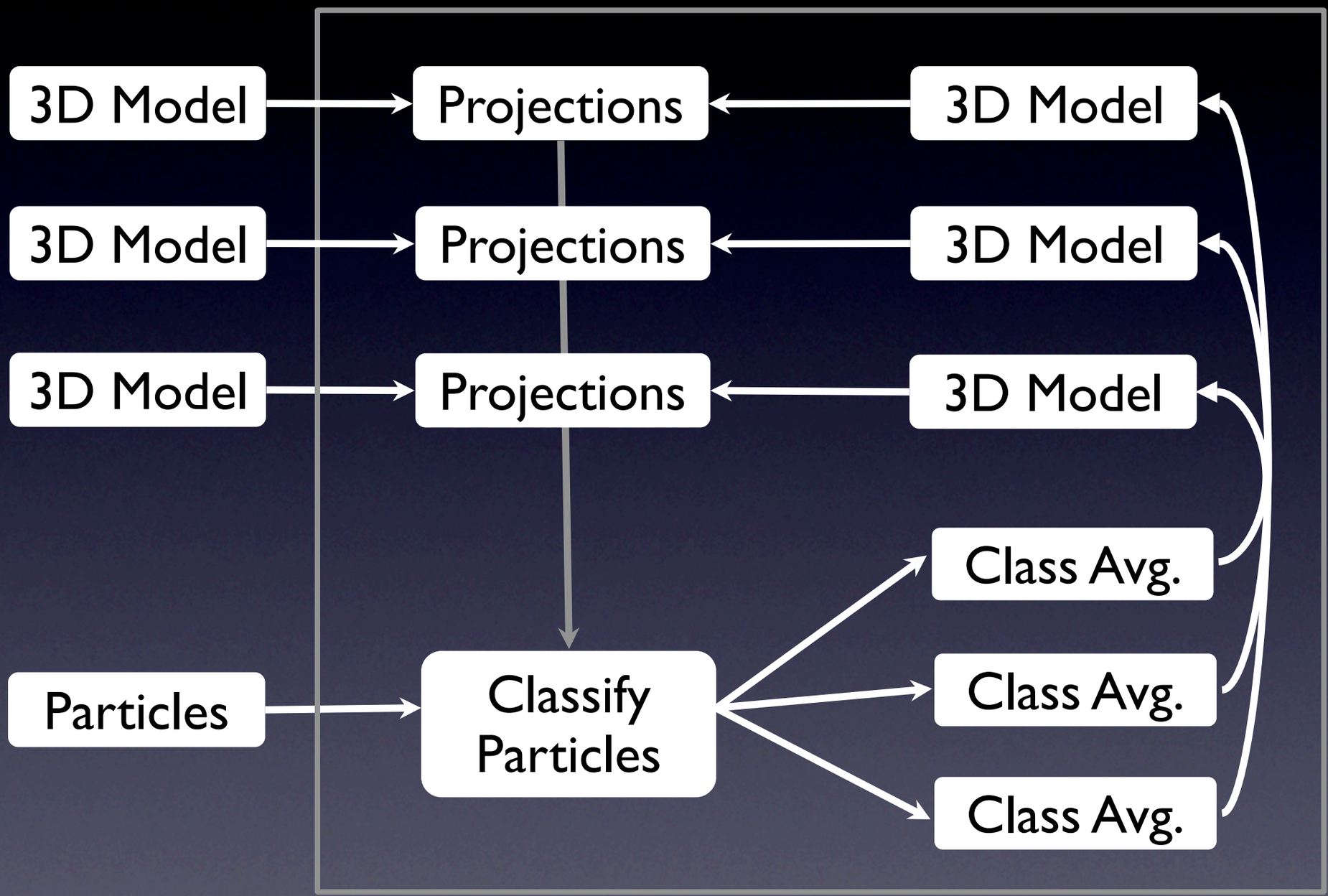
33
69
29
51
78
71


77
29
43
51


56
39
42
63

Multireference Refinement



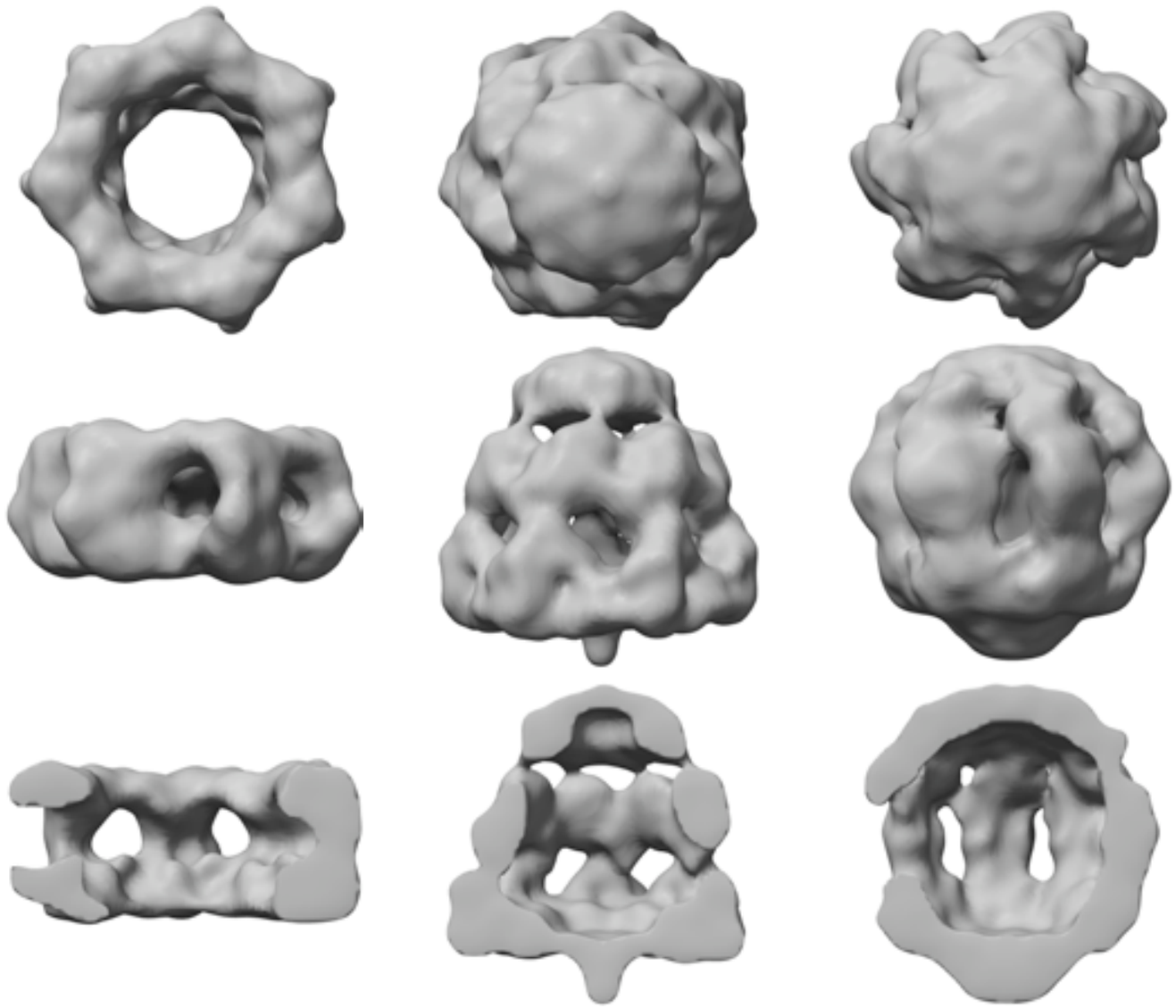
SR398+GroES+Mg-ATP

Top View

Side View

Side View of Half Map

Central Slice of Side View



SR398 heptamer with no GroES

Standard Conformation

Expanded Conformation

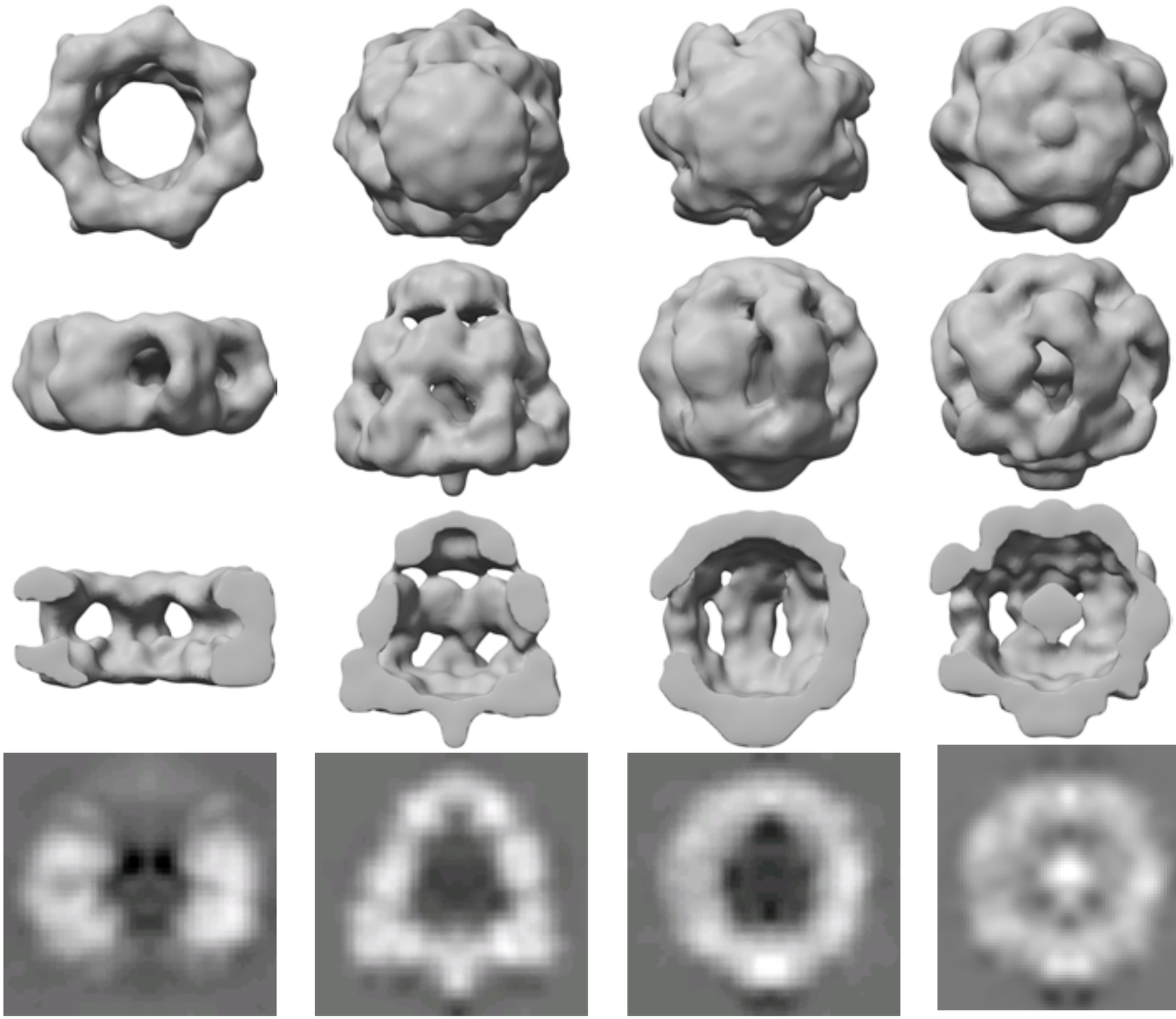
SR398+GroES+Mg-ATP

Top View

Side View

Side View of Half Map

Central Slice of Side View



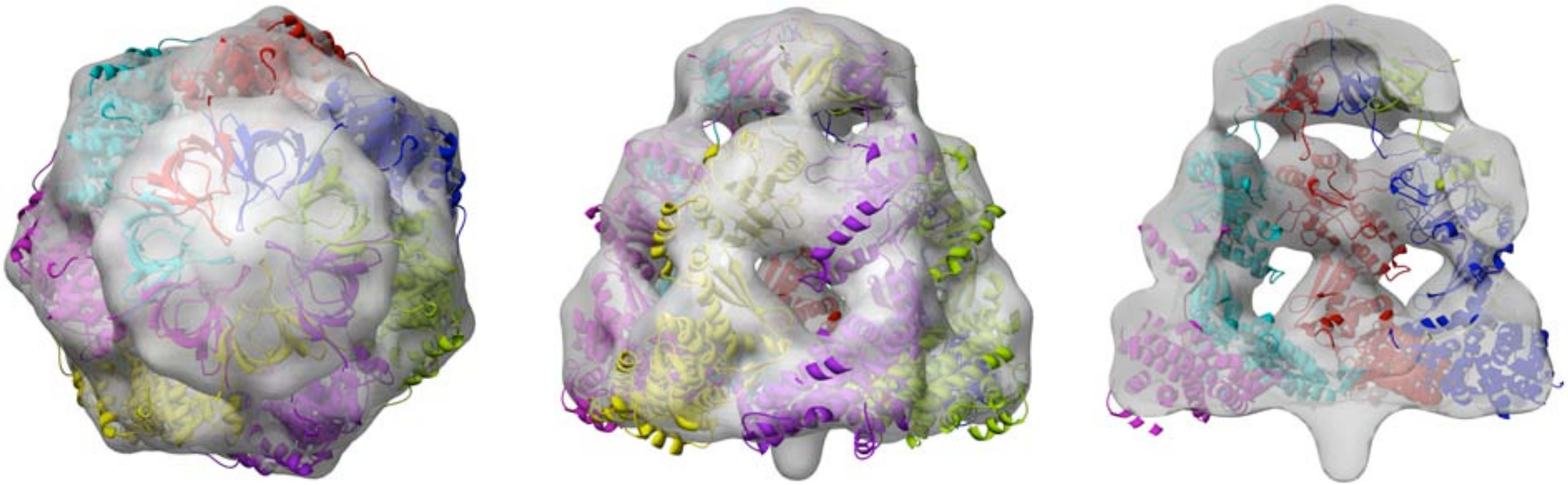
SR398 heptamer with no GroES

Standard Conformation

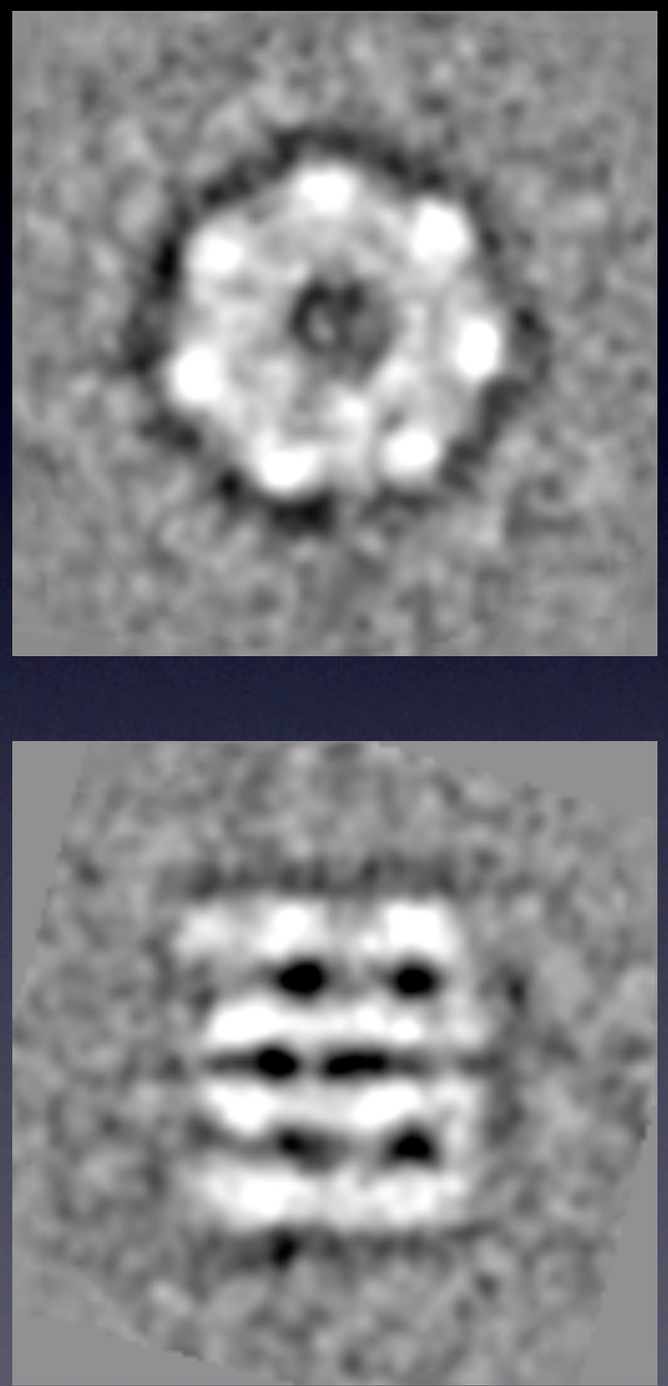
Expanded Conformation

With Substrate

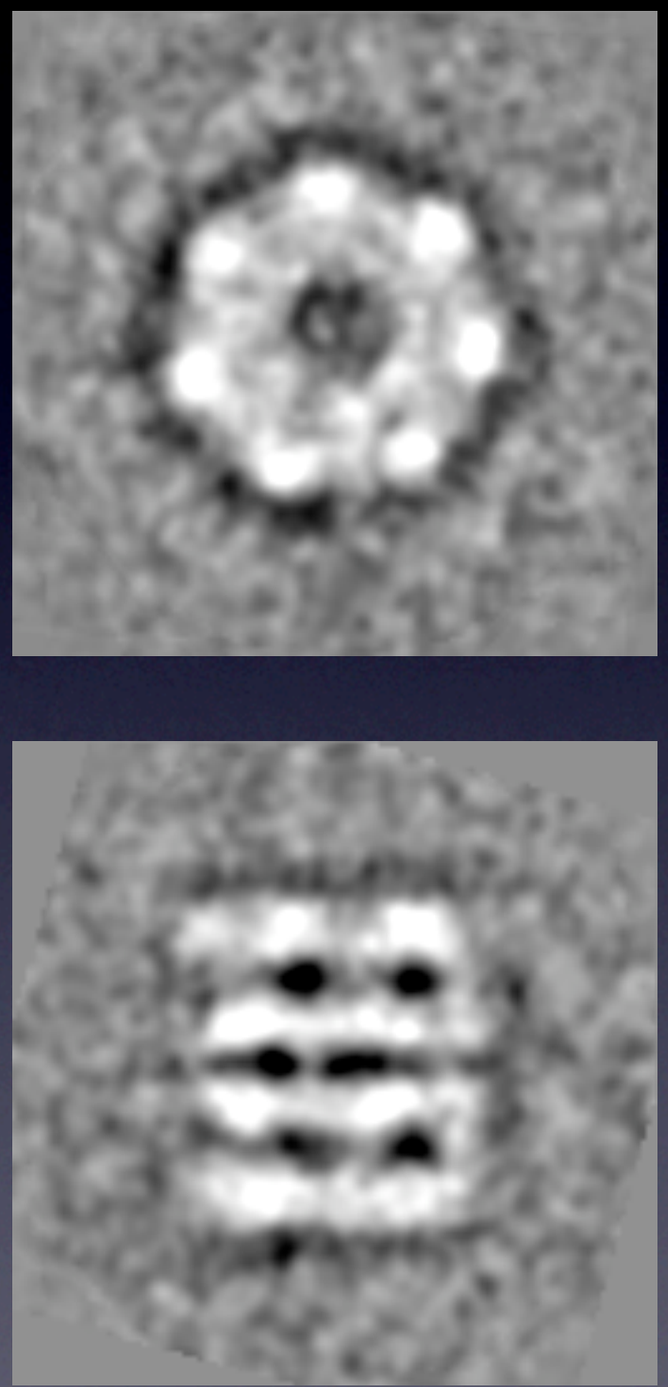
X-ray structure docked into cryo-EM density map



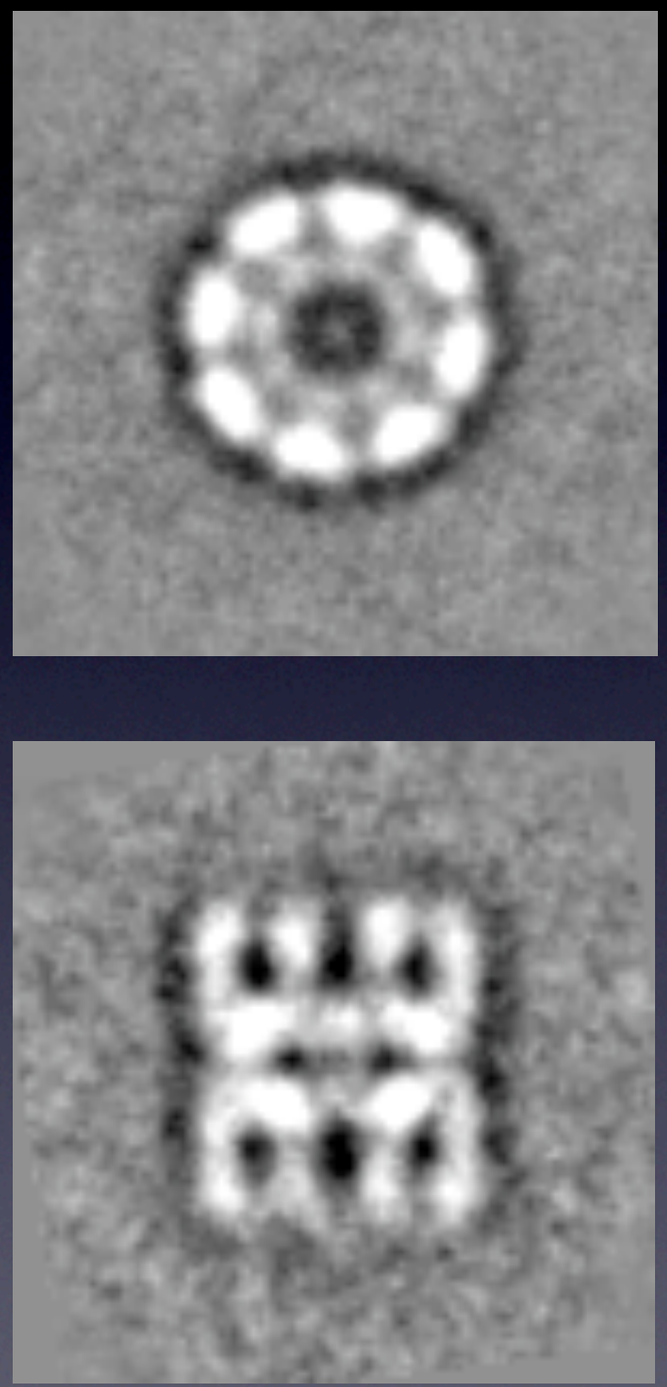
GroEL



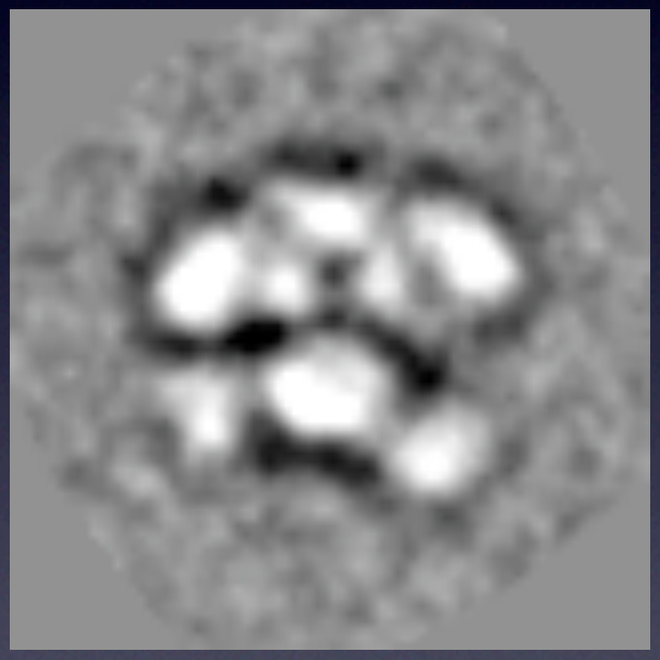
GroEL



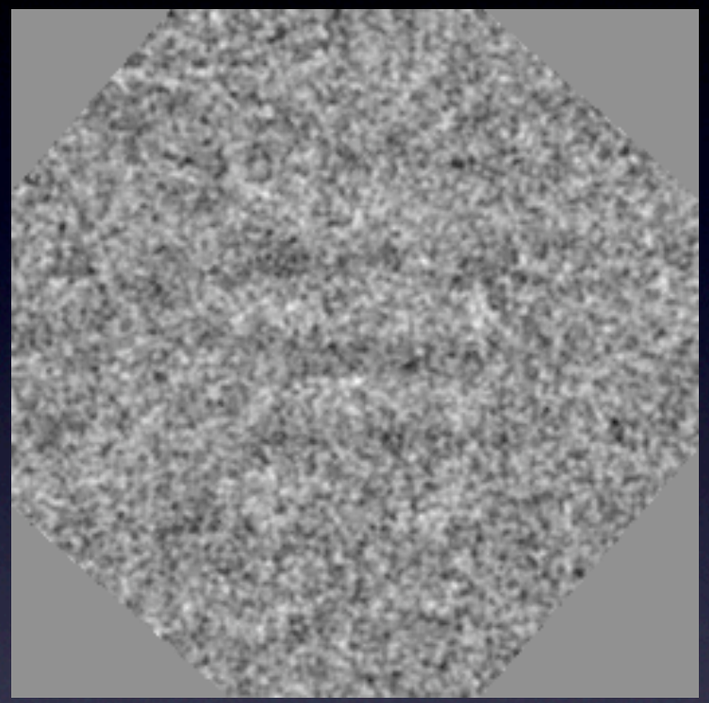
mmcpn



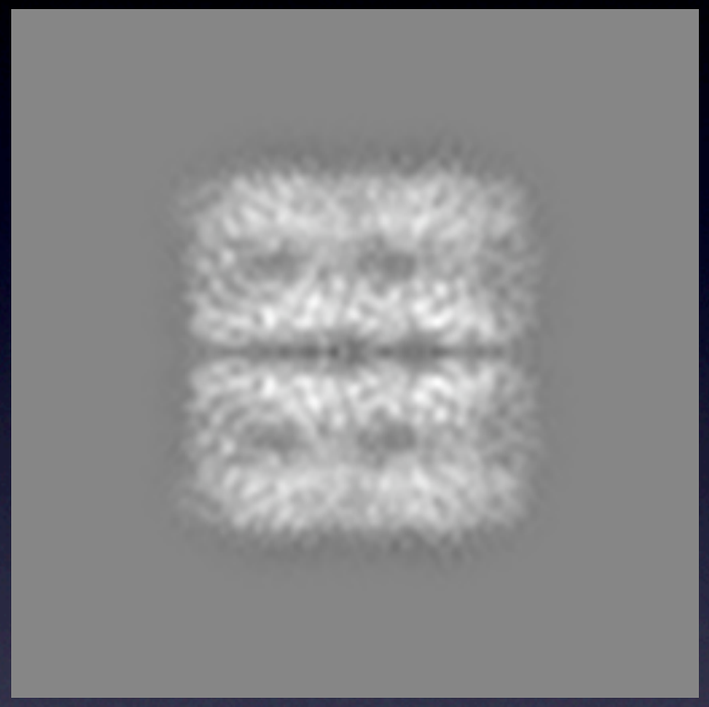
Fatty Acid Synthase



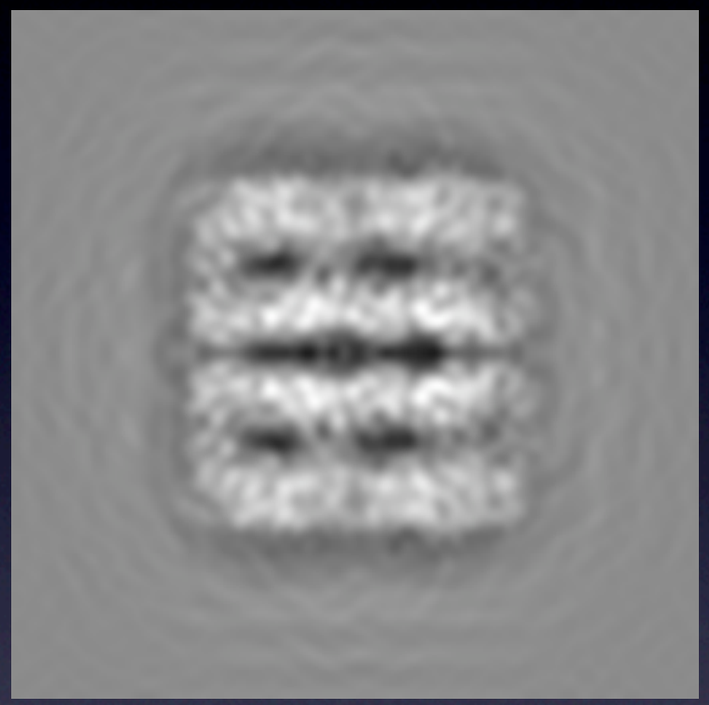
Can we subtract what we don't want ?



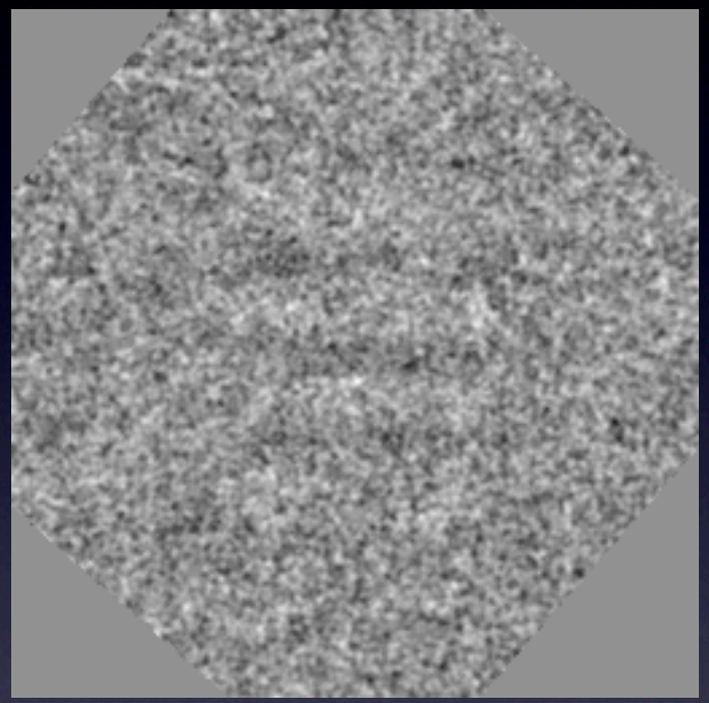
Can we subtract what we don't want ?



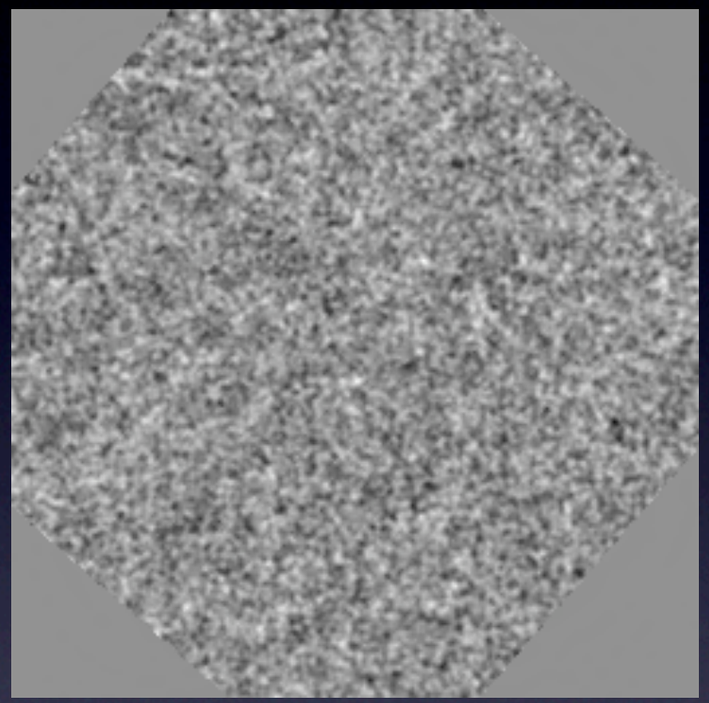
A lot of math →



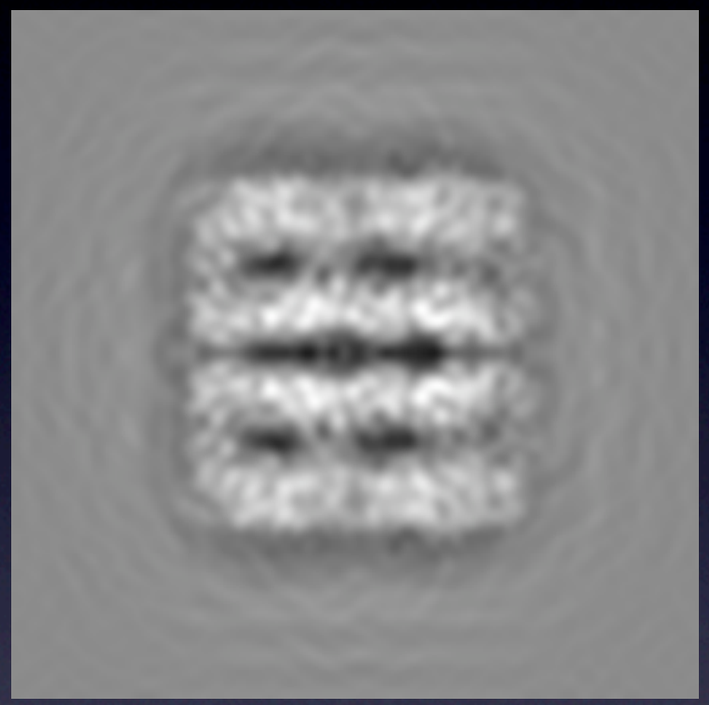
Can we subtract what we don't want ?



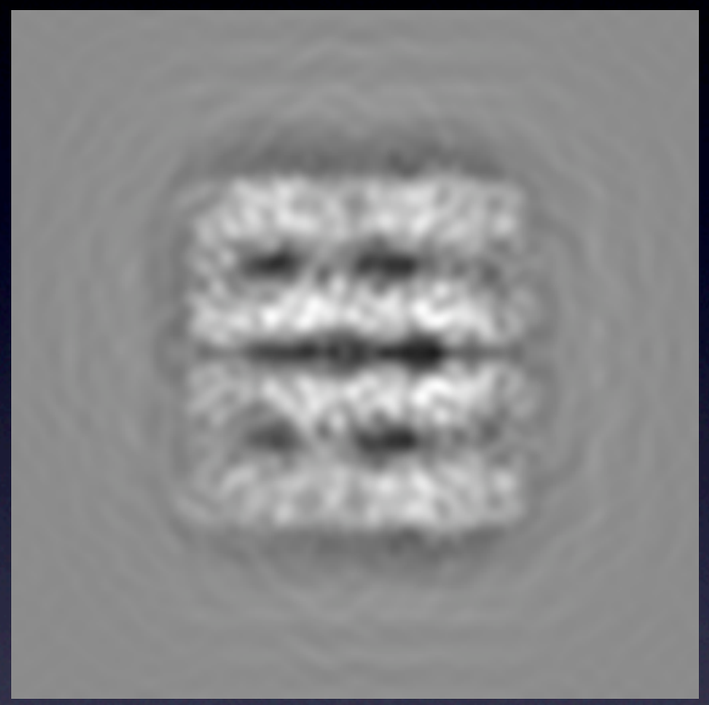
Can we subtract what we don't want ?



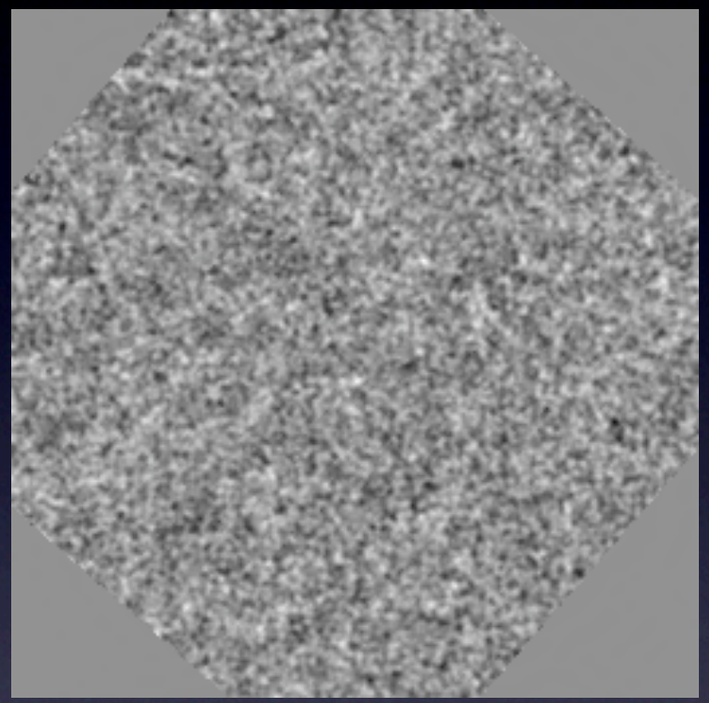
Remove One Monomer



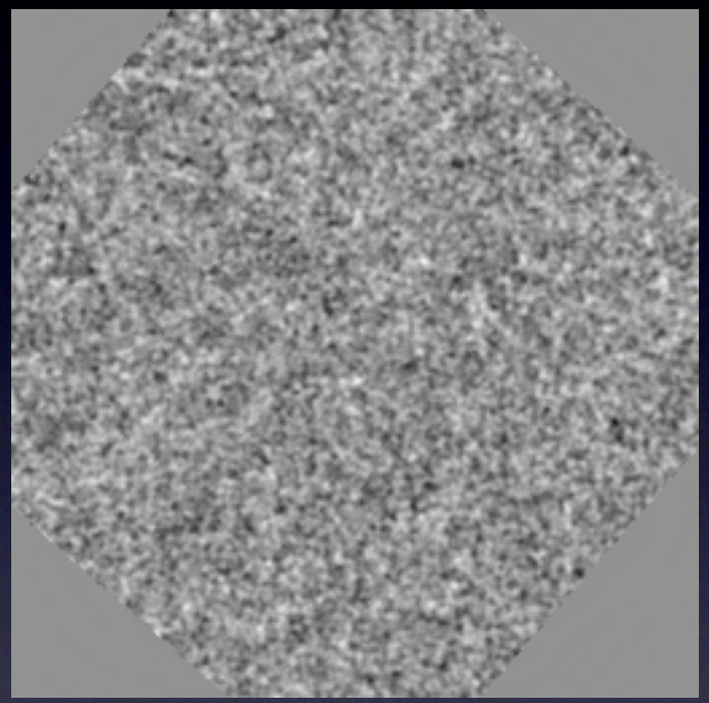
Remove One Monomer



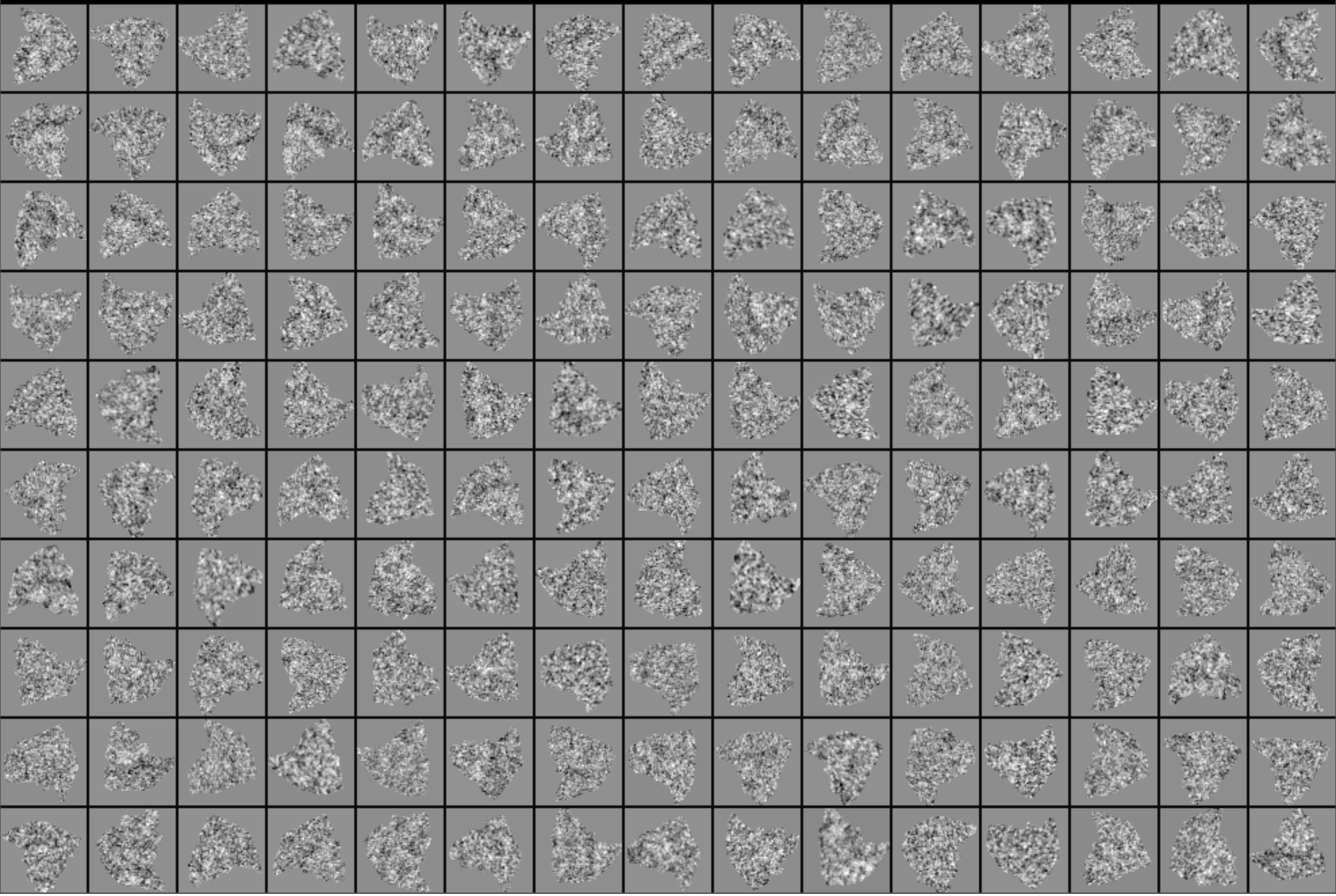
Remove One Monomer



Remove One Monomer



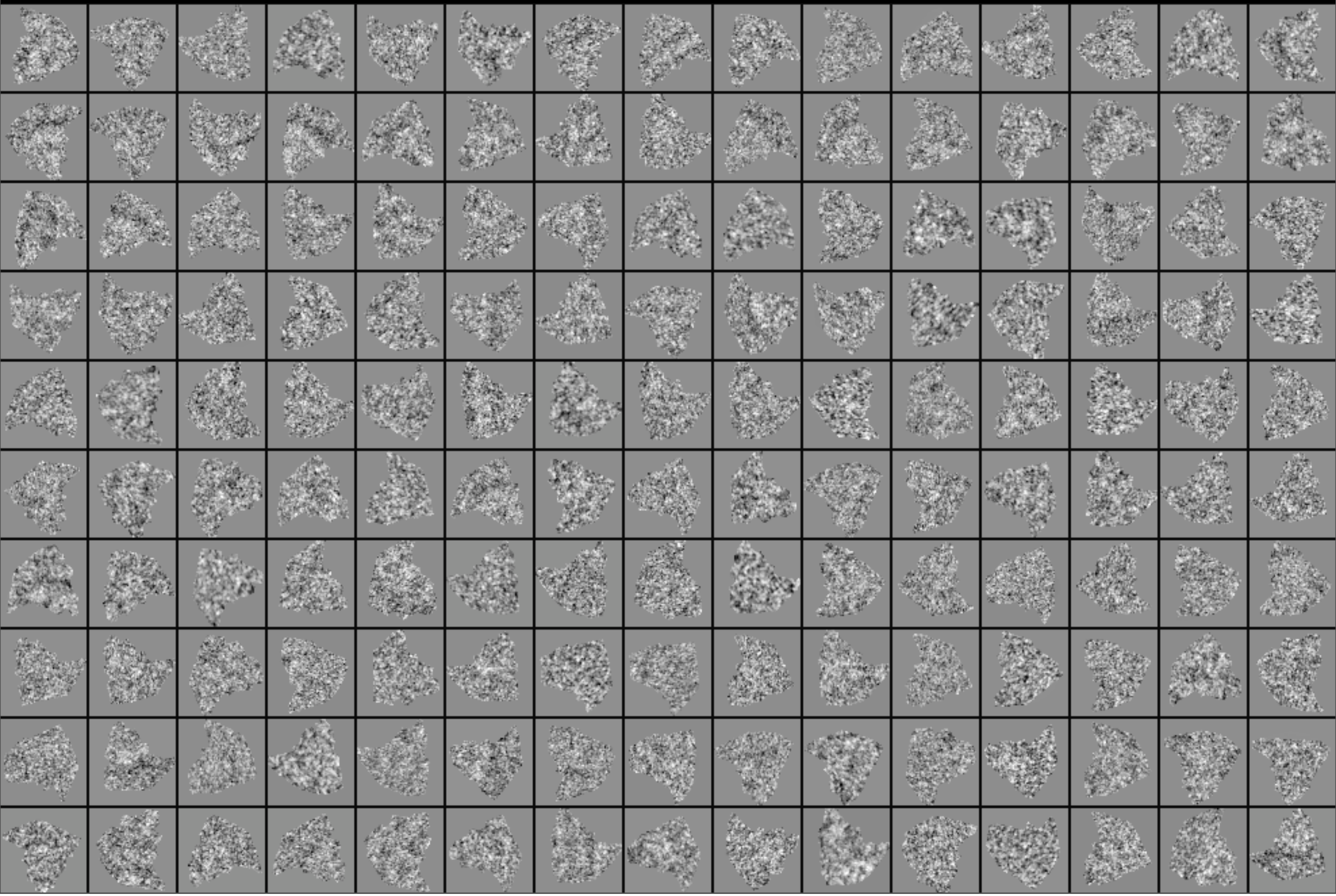
Many particles

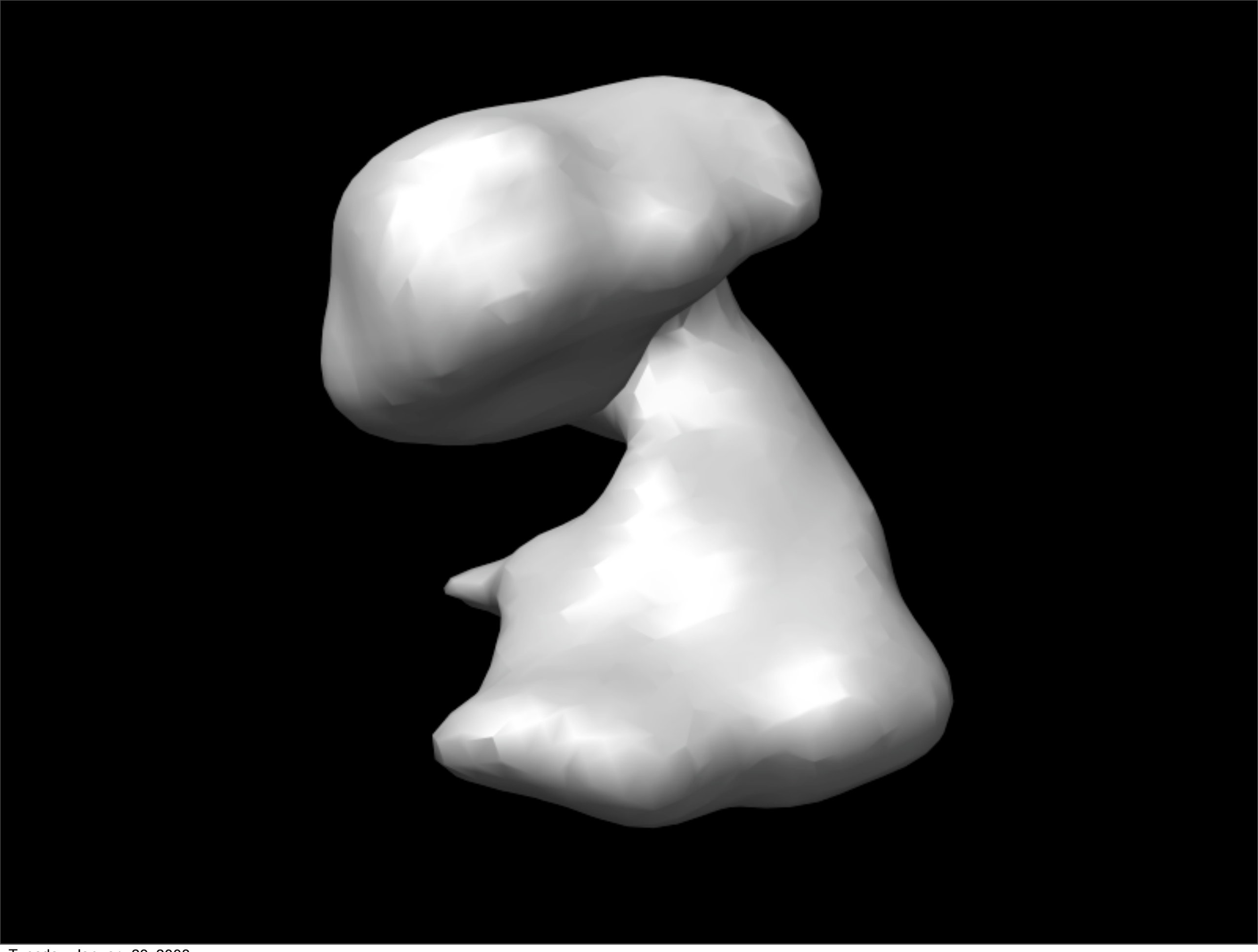


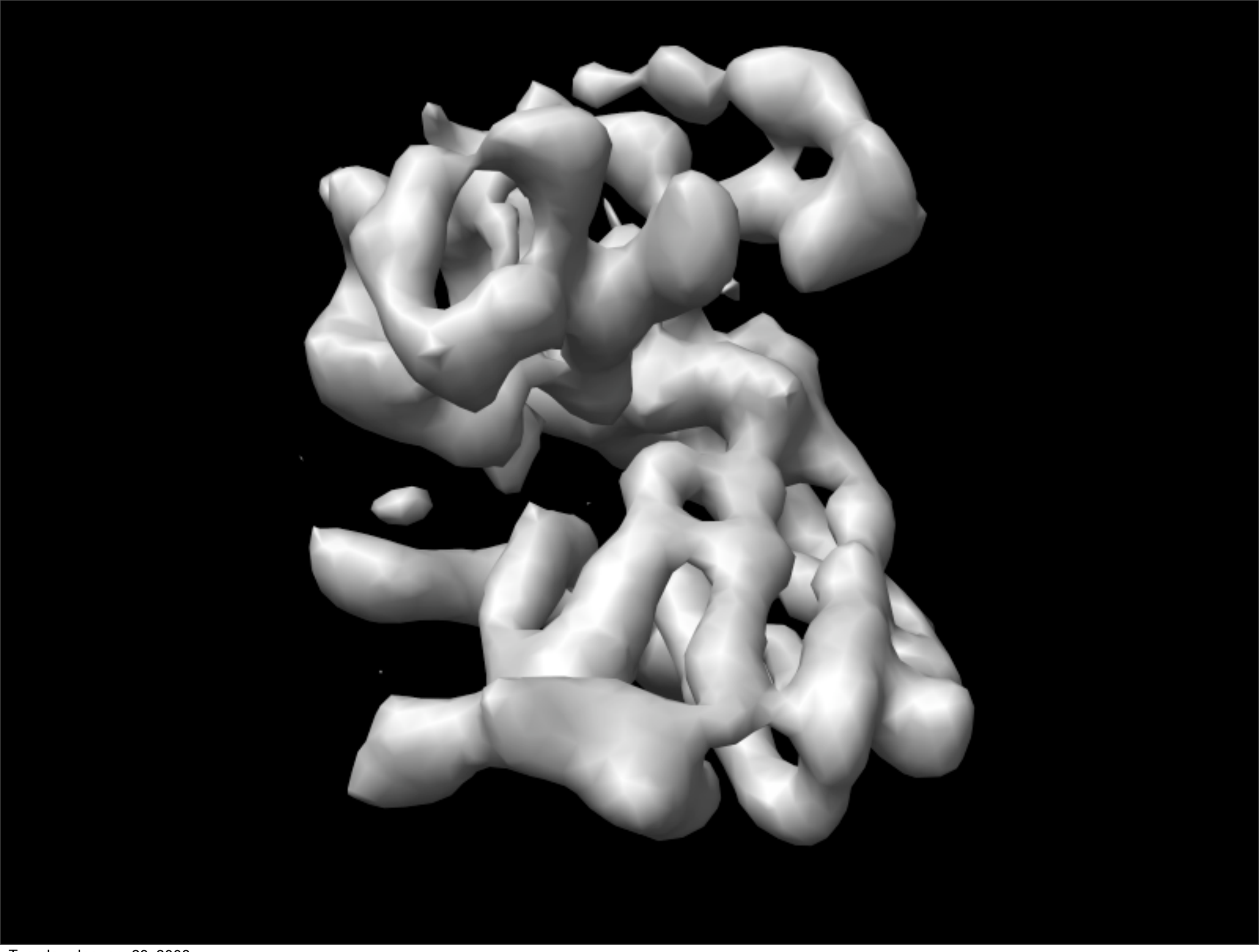
Many Particles

- 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

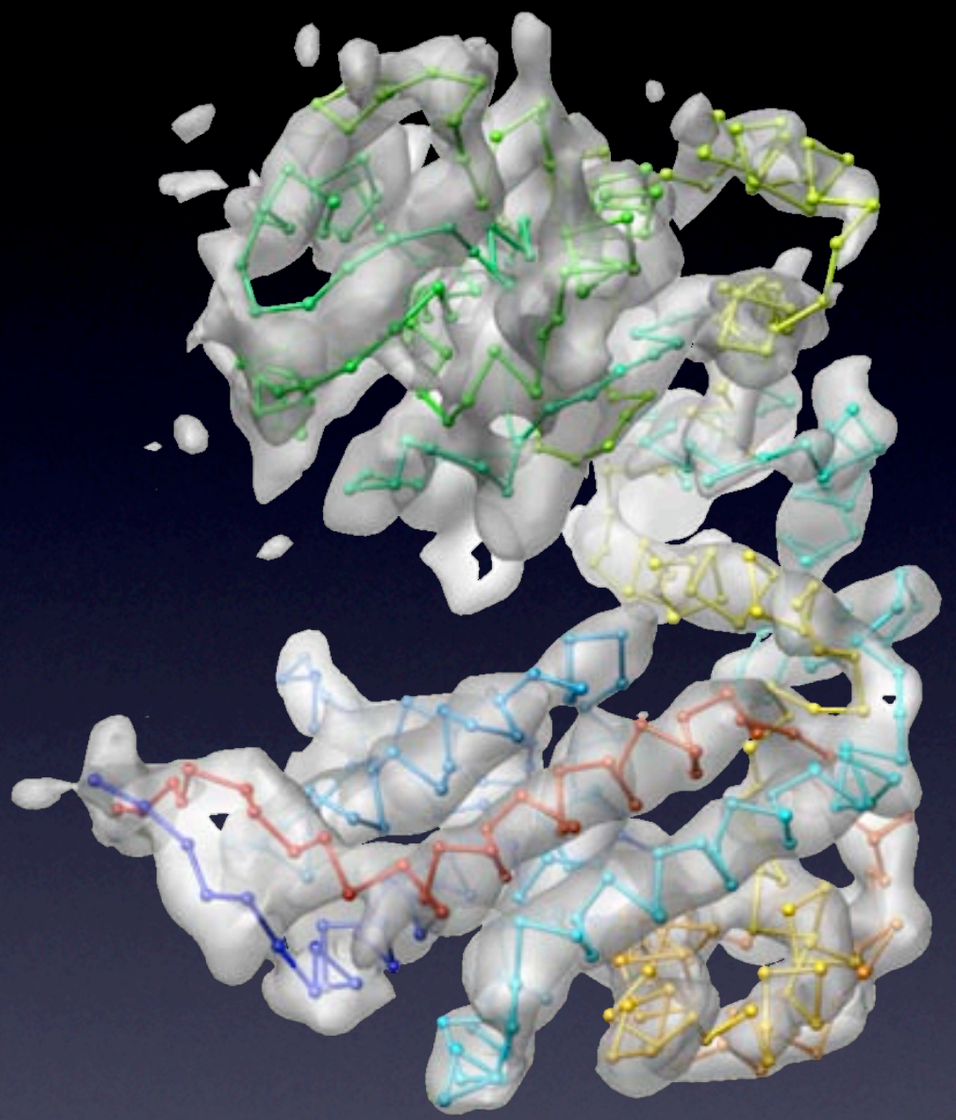
Many particles







Acknowledgements



GroEL

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

FAS

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

Mmcpn

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