Variability Analysis

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Baylor College of Medicine

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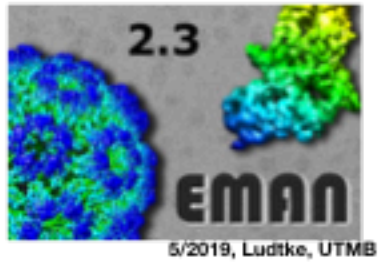
VERNA & MARRS McLEAN DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY



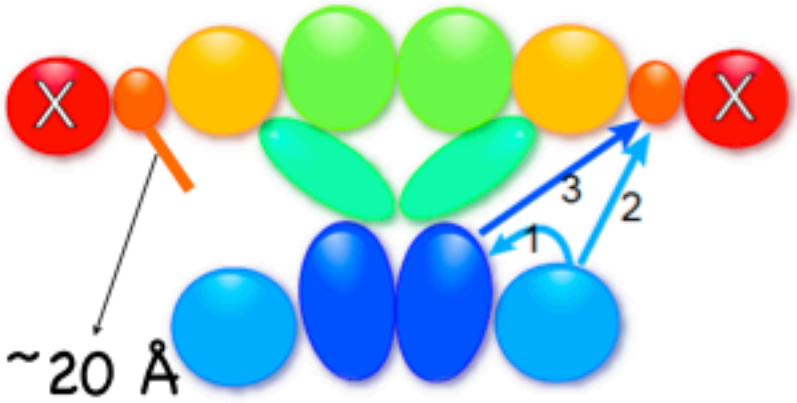
Methods in Enzymology
The Resolution Revolution: Recent Advances In cryoEM
Edited by R.A. Crowther
Volume 579, Pages 2-445 (2016)

Chapter Six - Processing of Structurally Heterogeneous Cryo-EM Data in RELION
S.H.W. Scheres
Pages 125-157

Chapter Seven - Single-Particle Refinement and Variability Analysis in EMAN2.1
S.J. Ludtke
Pages 159-189

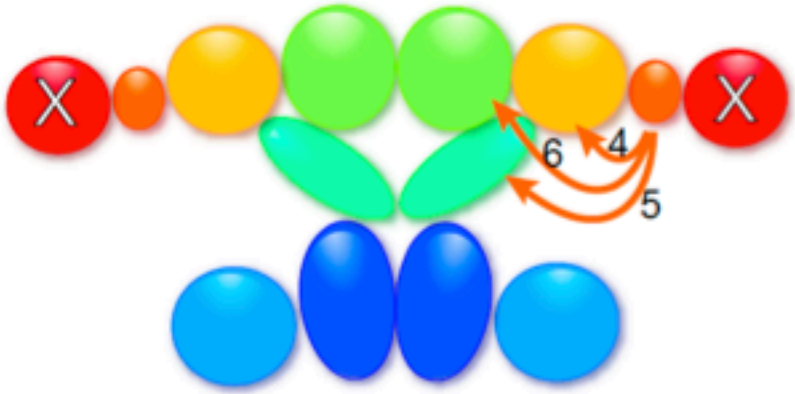
Chapter Eleven - Refinement of Atomic Structures Against cryo-EM Maps
G.N. Murshudov
Pages 277-305

PMC5101015



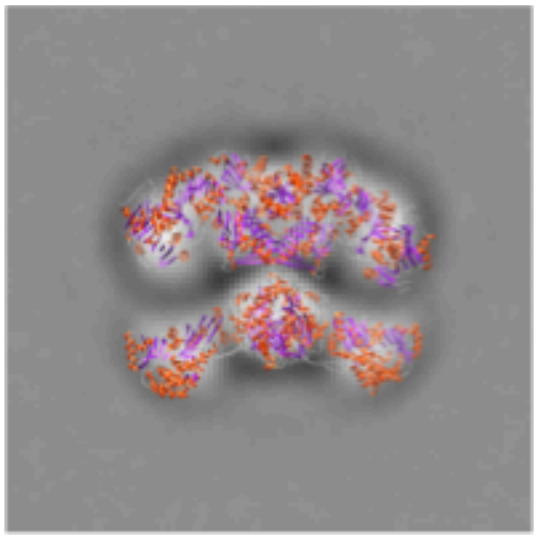
1. Loading Acetyl-CoA
2. Loading Malonyl-CoA
3. Condensation

~80 Å !?

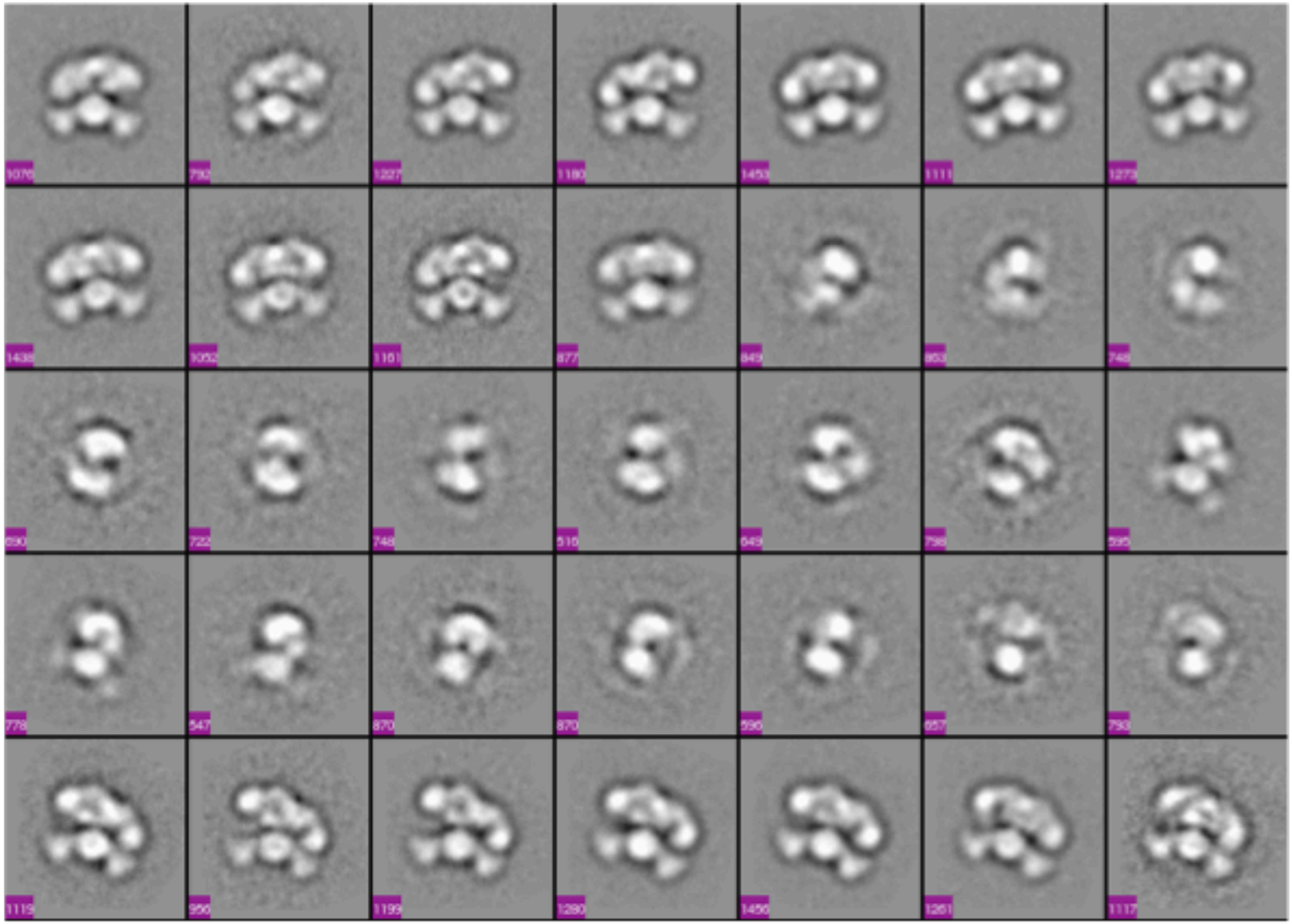


4. Keto reduction
5. Dehydration
6. Enoyl reduction

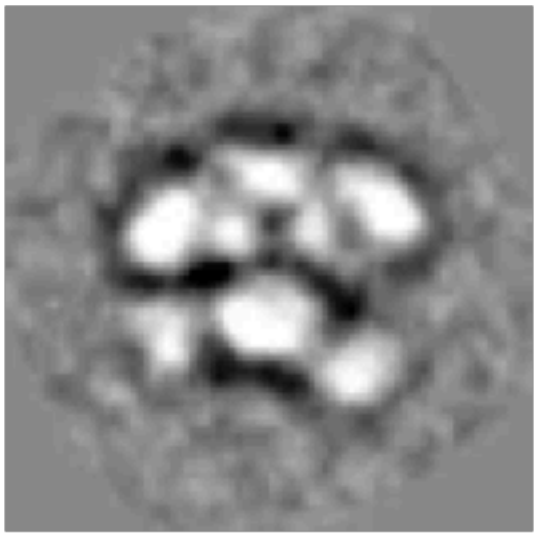
Single View CryoEM Average



150 Å



Fatty Acid Synthase, ~30 Å motion

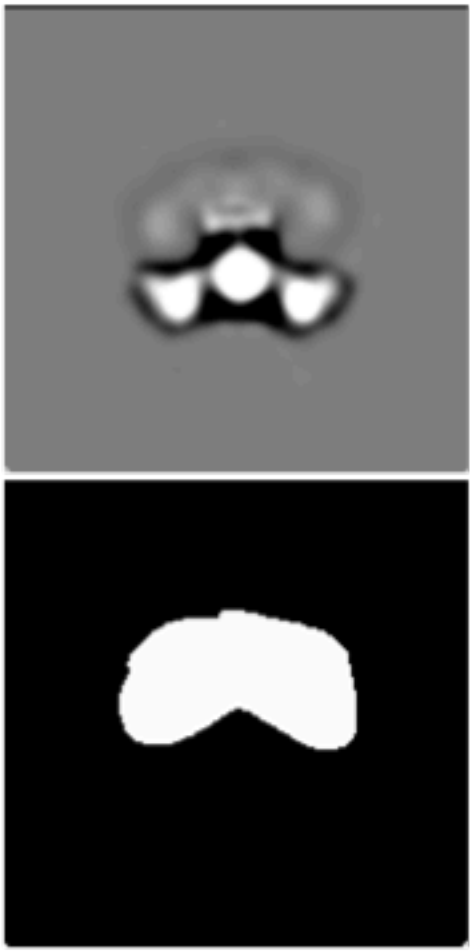


150 Å

S. Ludtke, Baylor College of Medicine

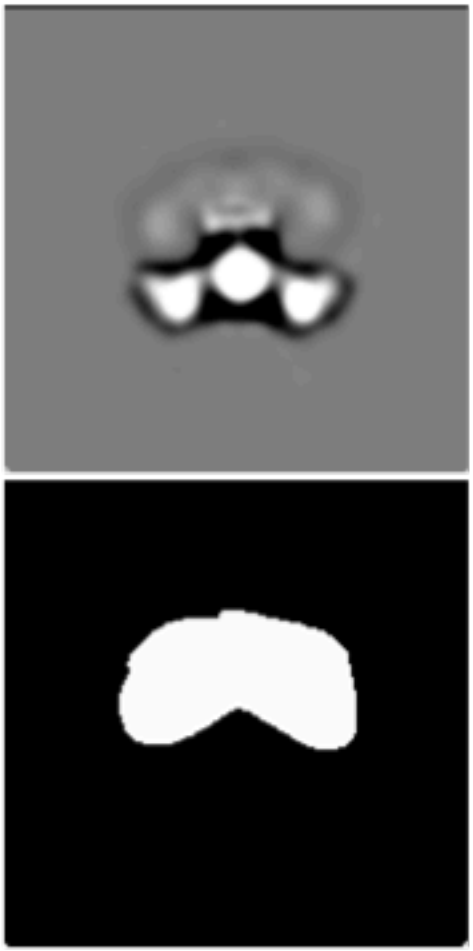
Align to

Align to



Variability Mask

Align to



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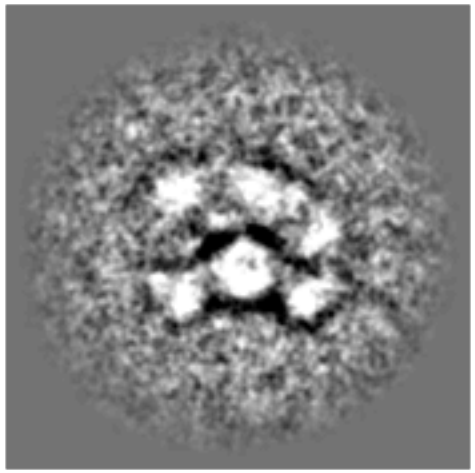

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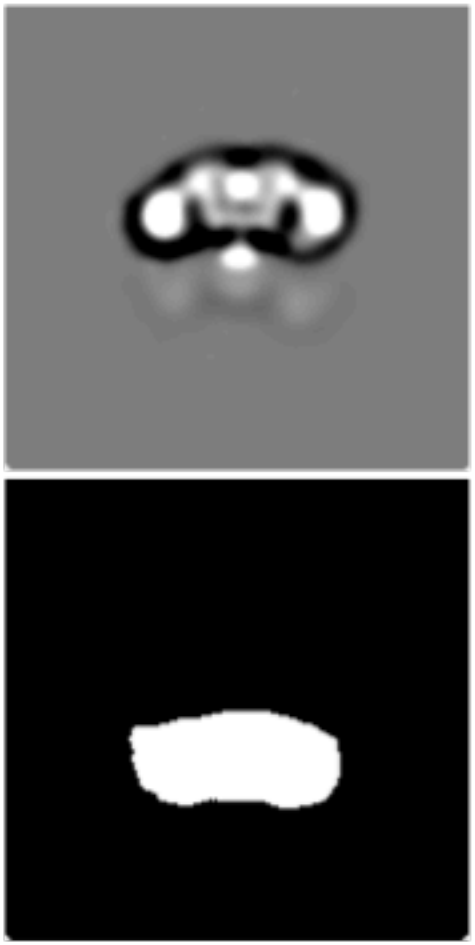

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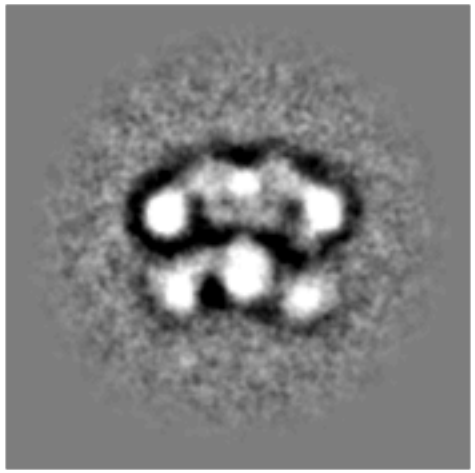
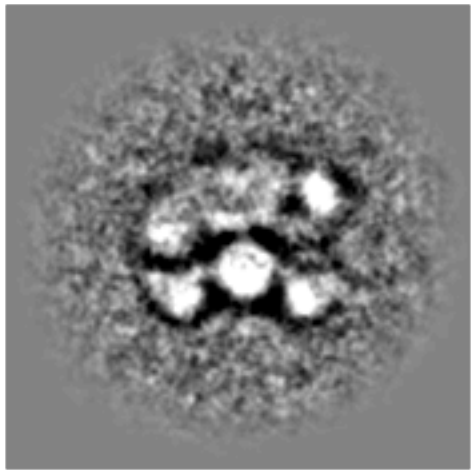


Align to

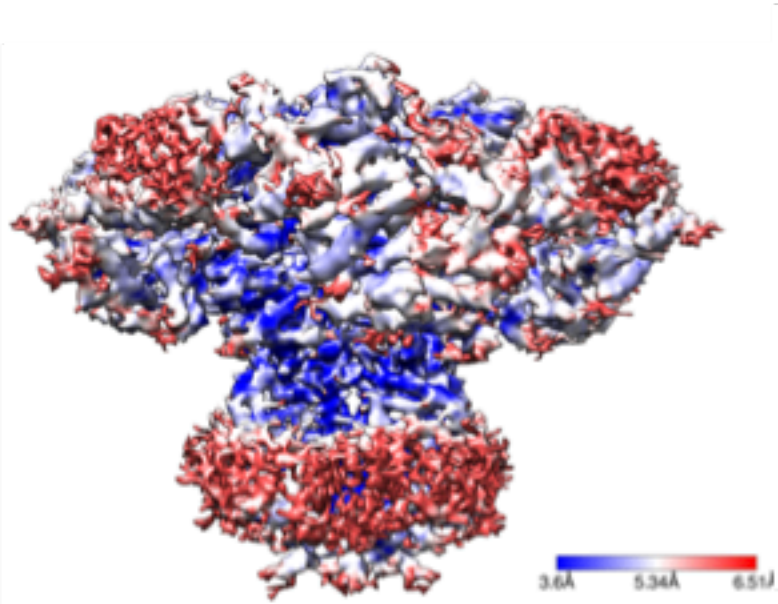
Variability Mask

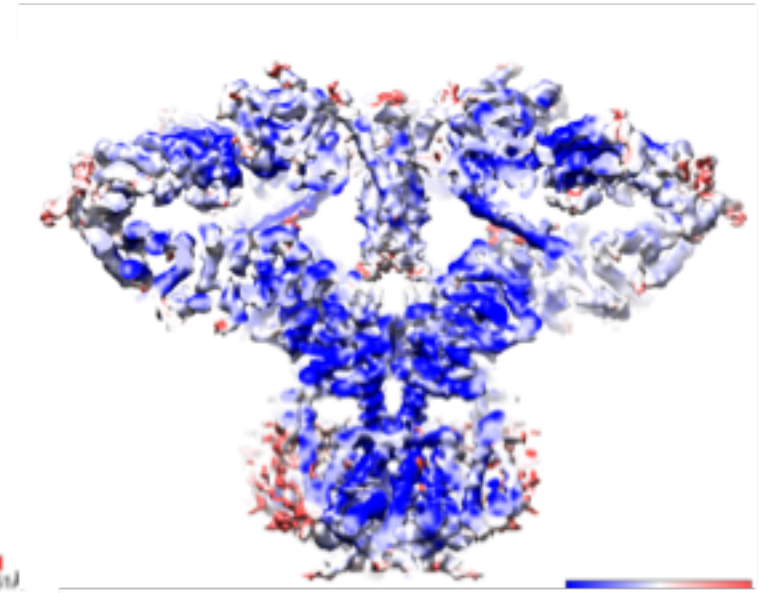


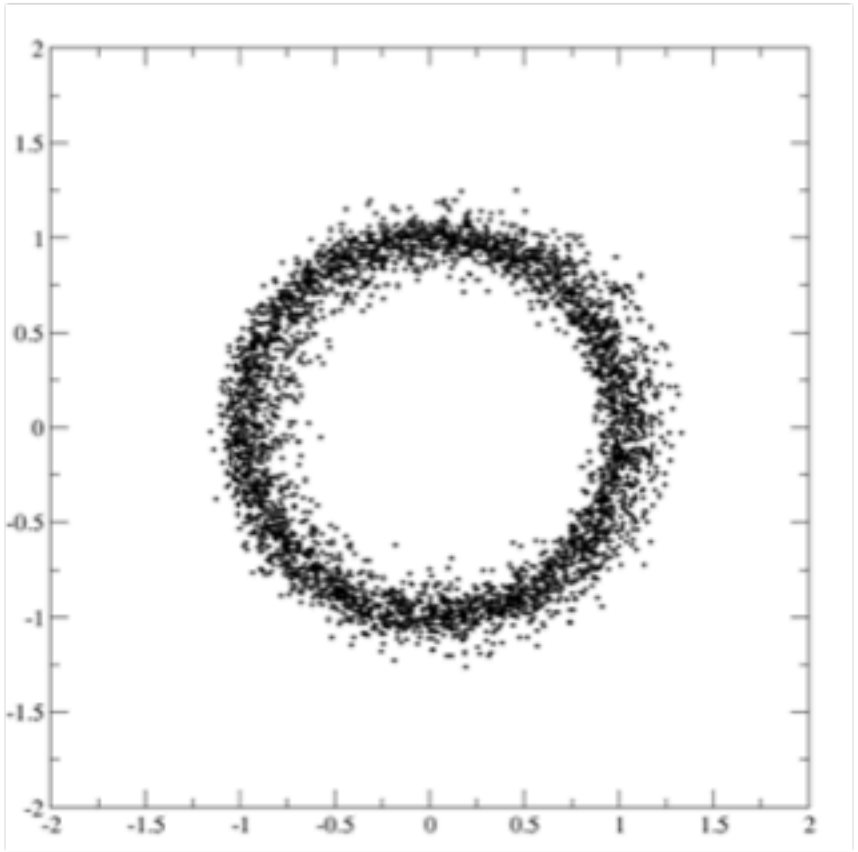
Local Variability

- Local resolution estimate using windowed FSC
  - e2fsc.py and others
- ResMap
  - Based on local features visible in the map (local SNR esimate)
- Variance map
  - Statistical bootstrapping, make N maps and compute local variance

ResMap



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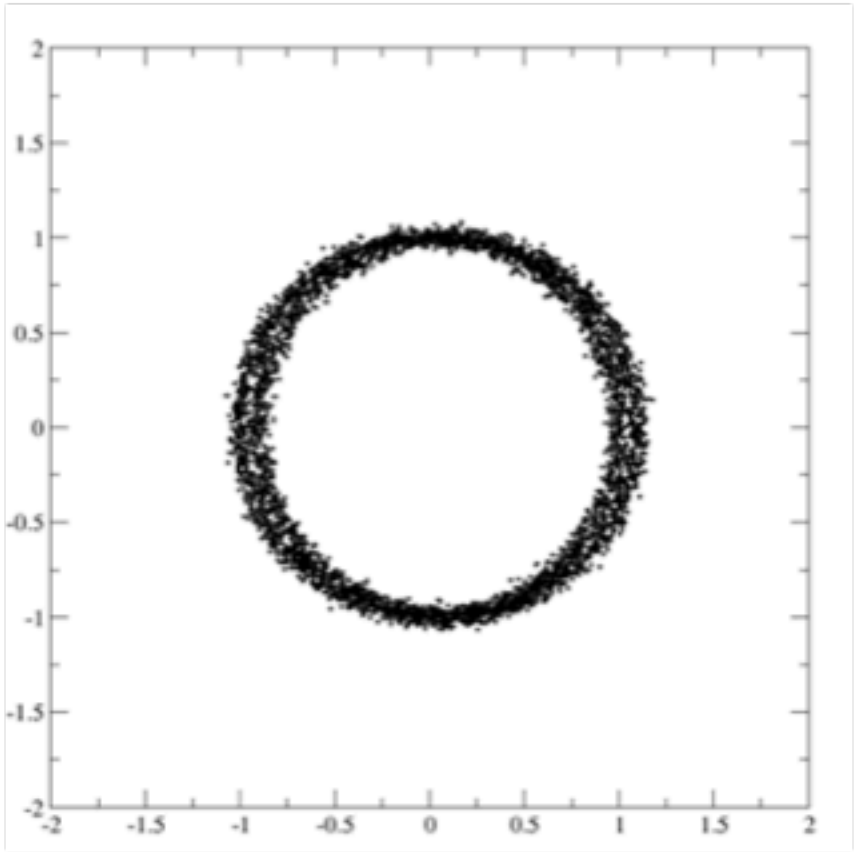
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2
-1.5
-1
-0.5
0
0.5
1
1.5
2


2
1.5
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0.5
0
-0.5
-1
-1.5
-2
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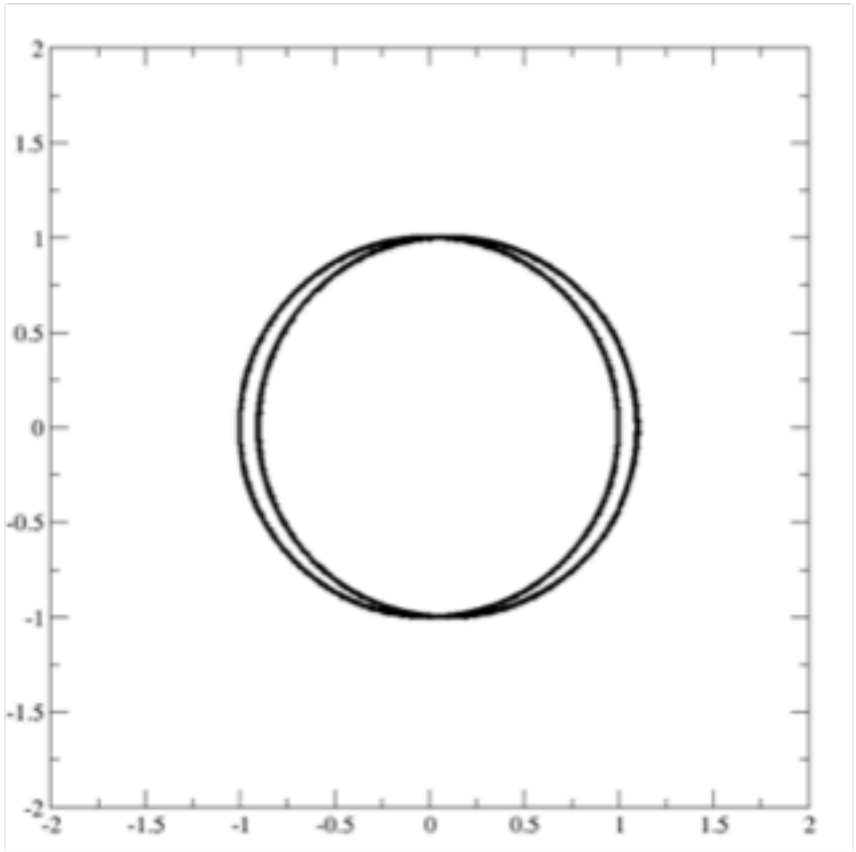
-1.5
-1
-0.5
0
0.5
1
1.5
2


2
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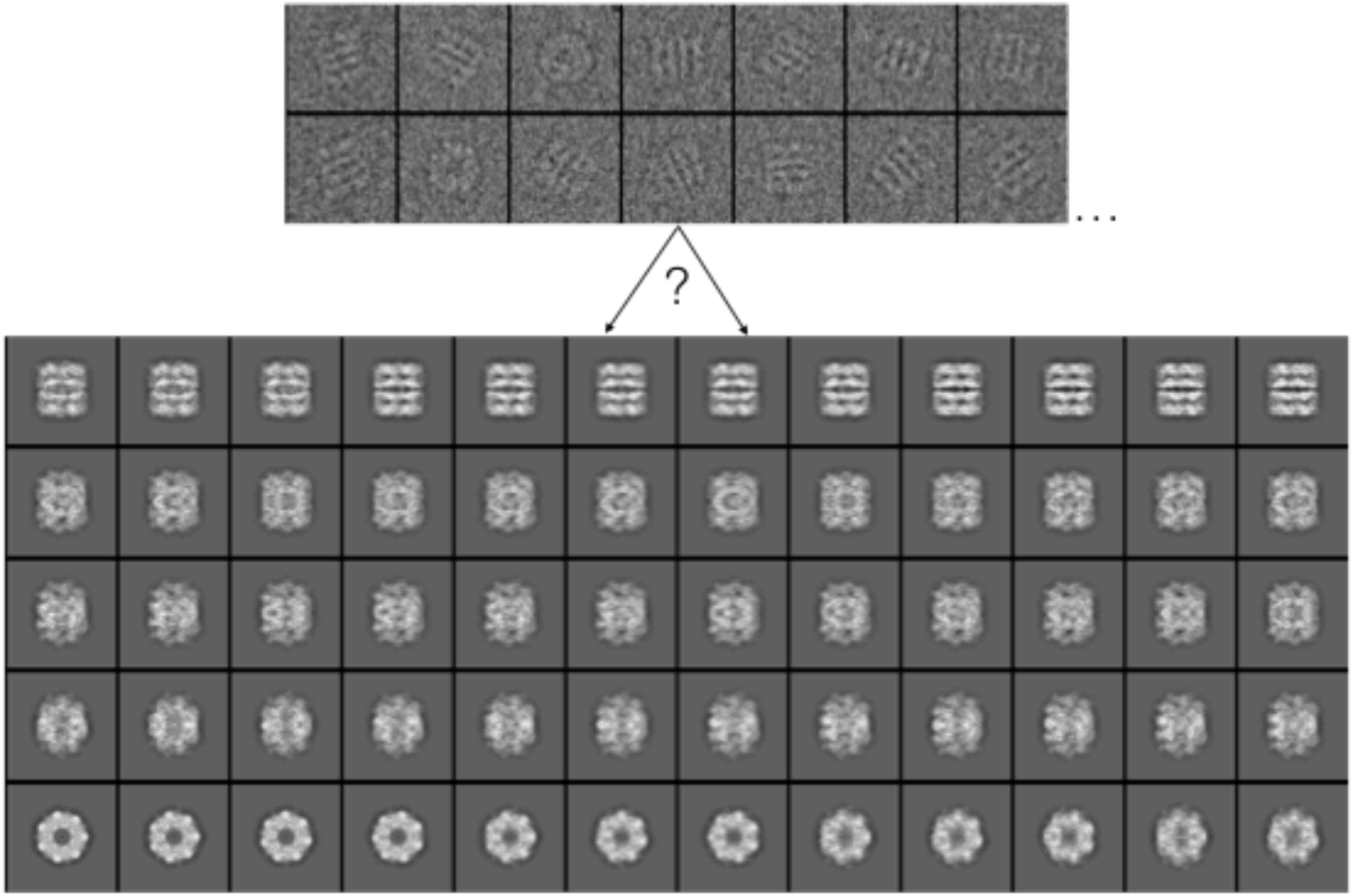
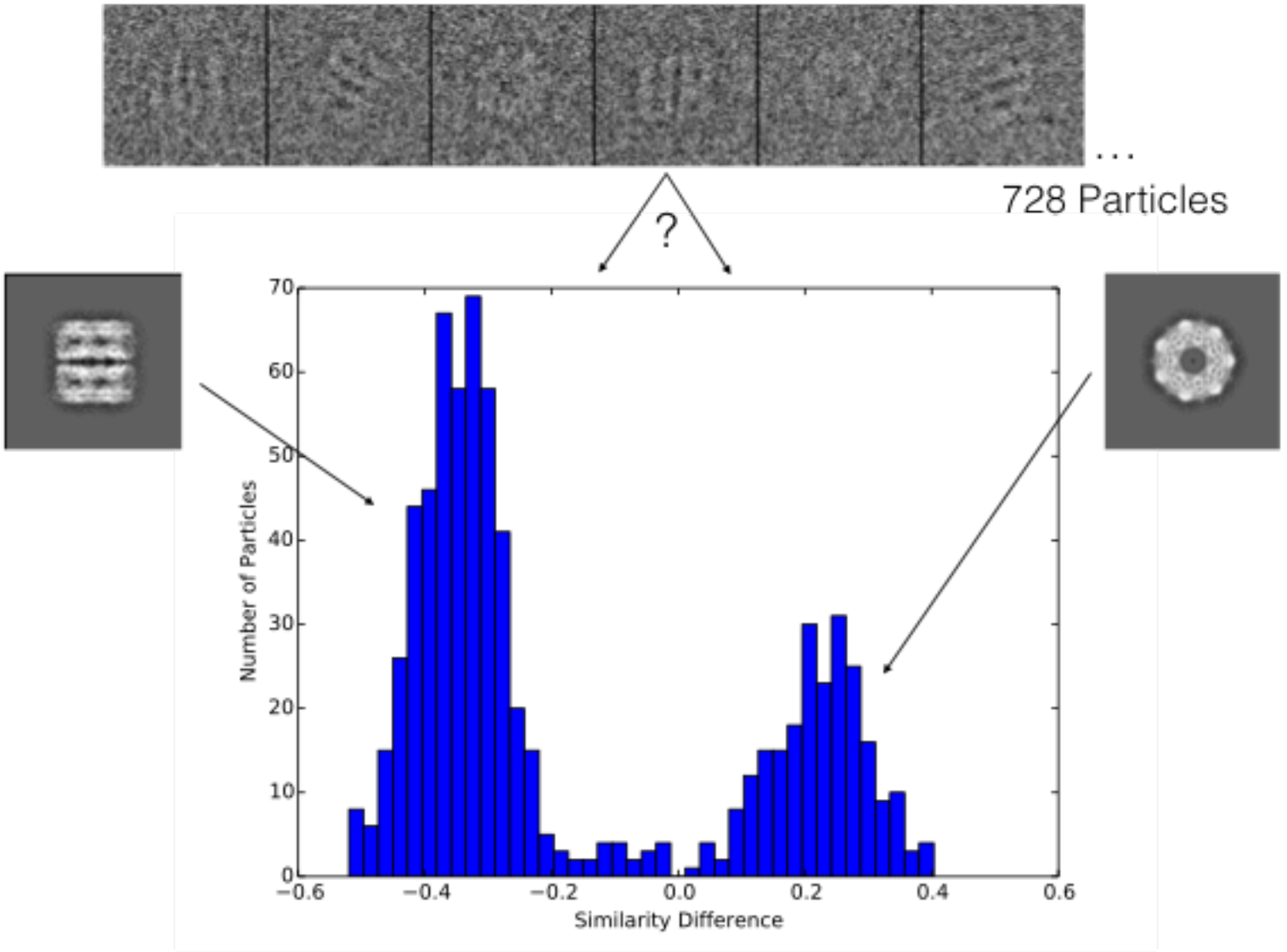
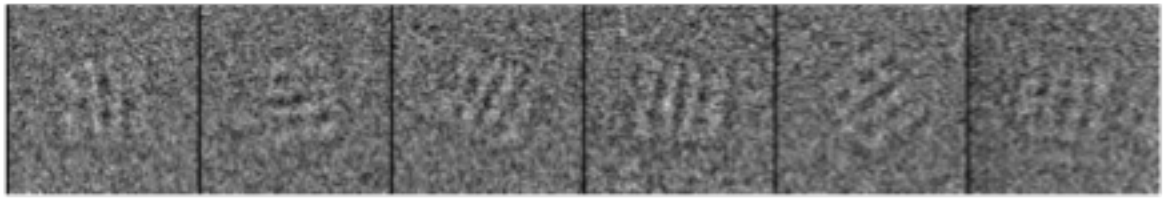
0
-0.5
-1
-1.5
-2
-2
-1.5
-1
-0.5
0
0.5
1
1.5
2

Orientation Determination



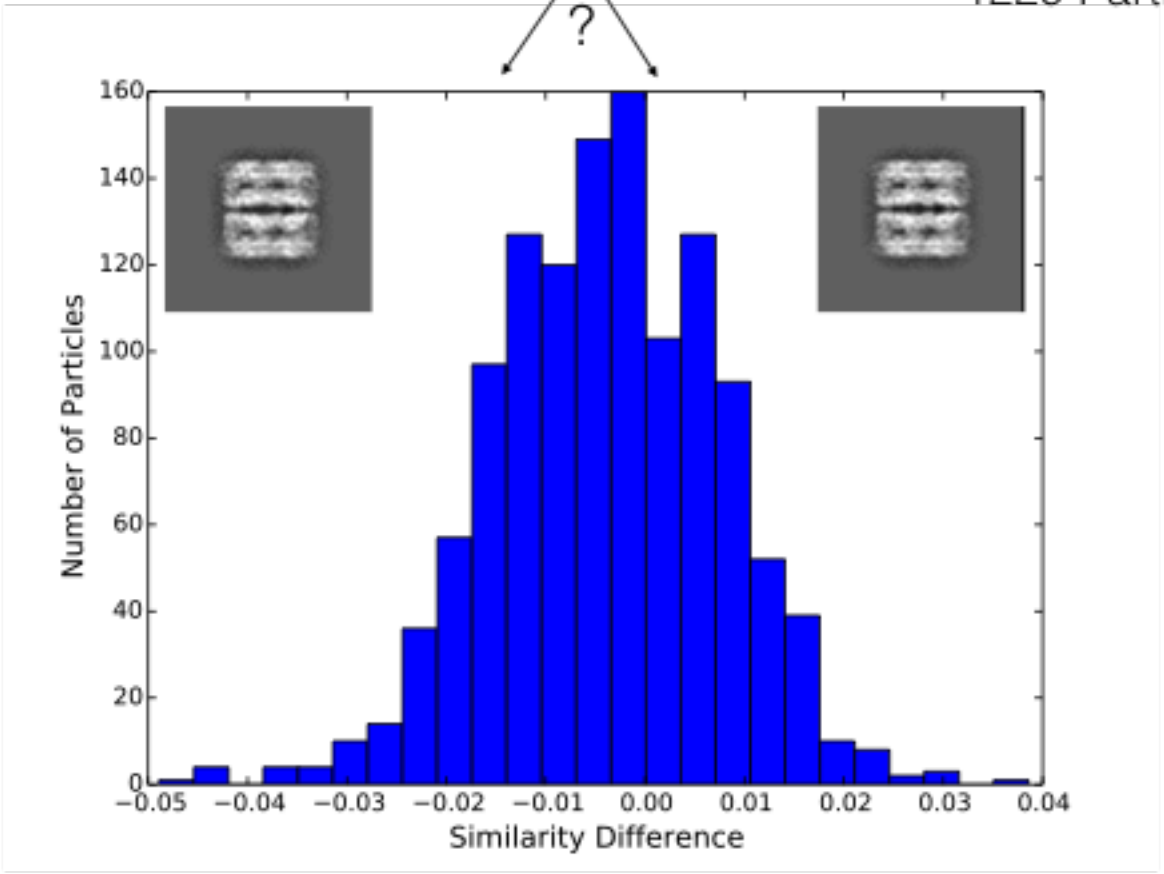

...
728 Particles
?
Number of Particles
Similarity Difference
0
10
20
30
40
50
60
70
−0.6
−0.4
−0.2
0.0
0.2
0.4
0.6



...

1229 Particles

?



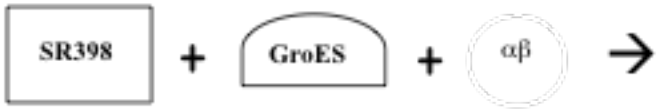
How can we improve?

- Higher contrast particle images
  - Thinner ice
  - Better buffer
  - Intelligent use of dose
- Smarter similarity metric
- Local mask

Multi-component Systems

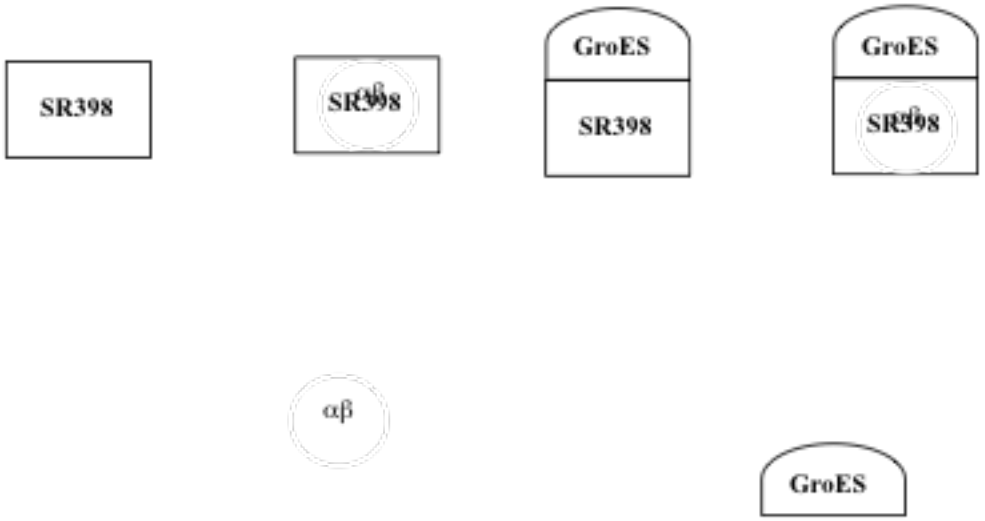
GroEL, GroES & Substrate

mitochondrial branched-chain α-ketoacid dehydrogenase

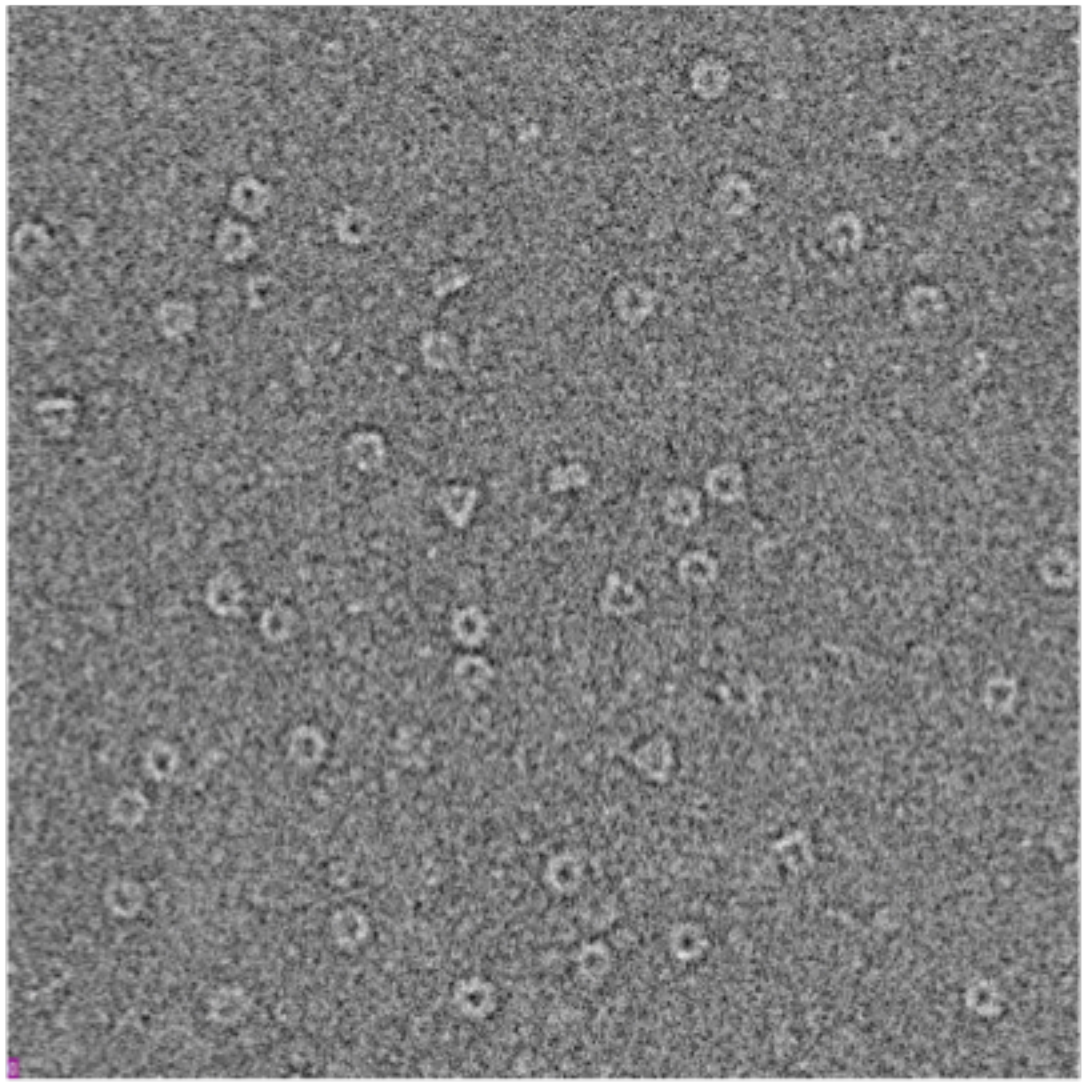


Chen D-H, Song J-L, Chuang DT, Chiu W, Ludtke SJ. An expanded conformation of single-ring GroEL-GroES complex encapsulates an 86 kDa substrate. Structure. 2006. 14(11):1711–22.

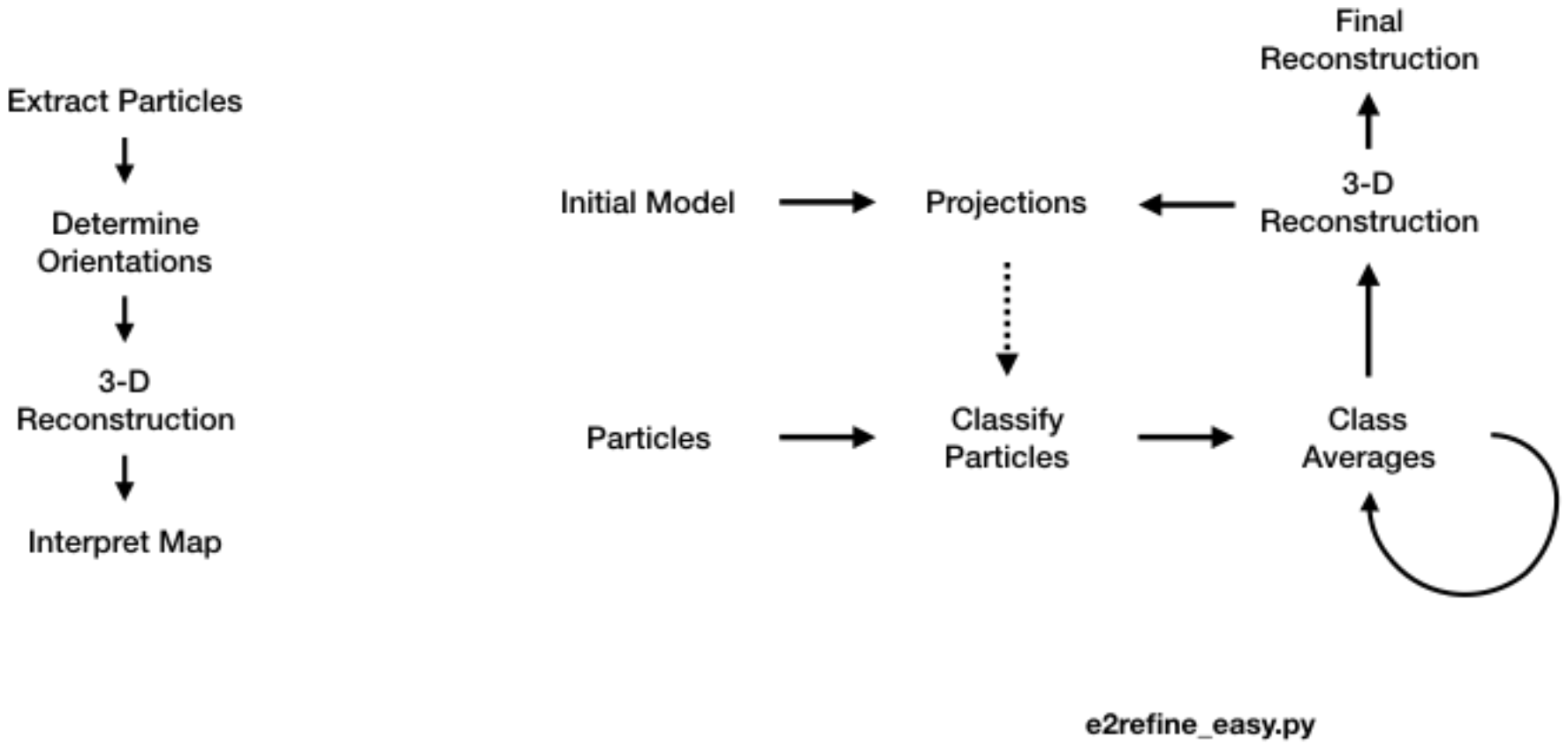
Produces a Mixed Population



SR398+GroES+ATP

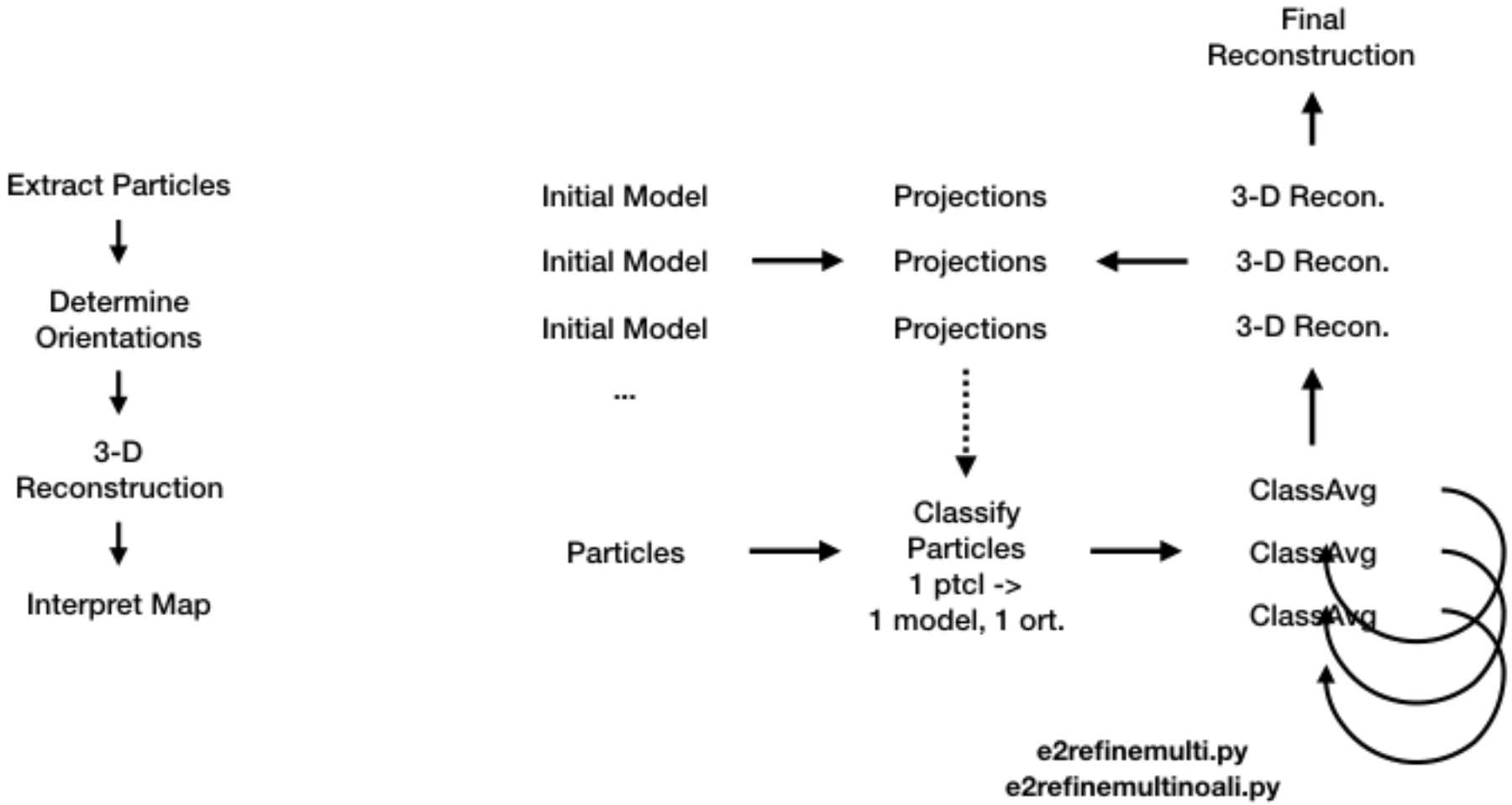


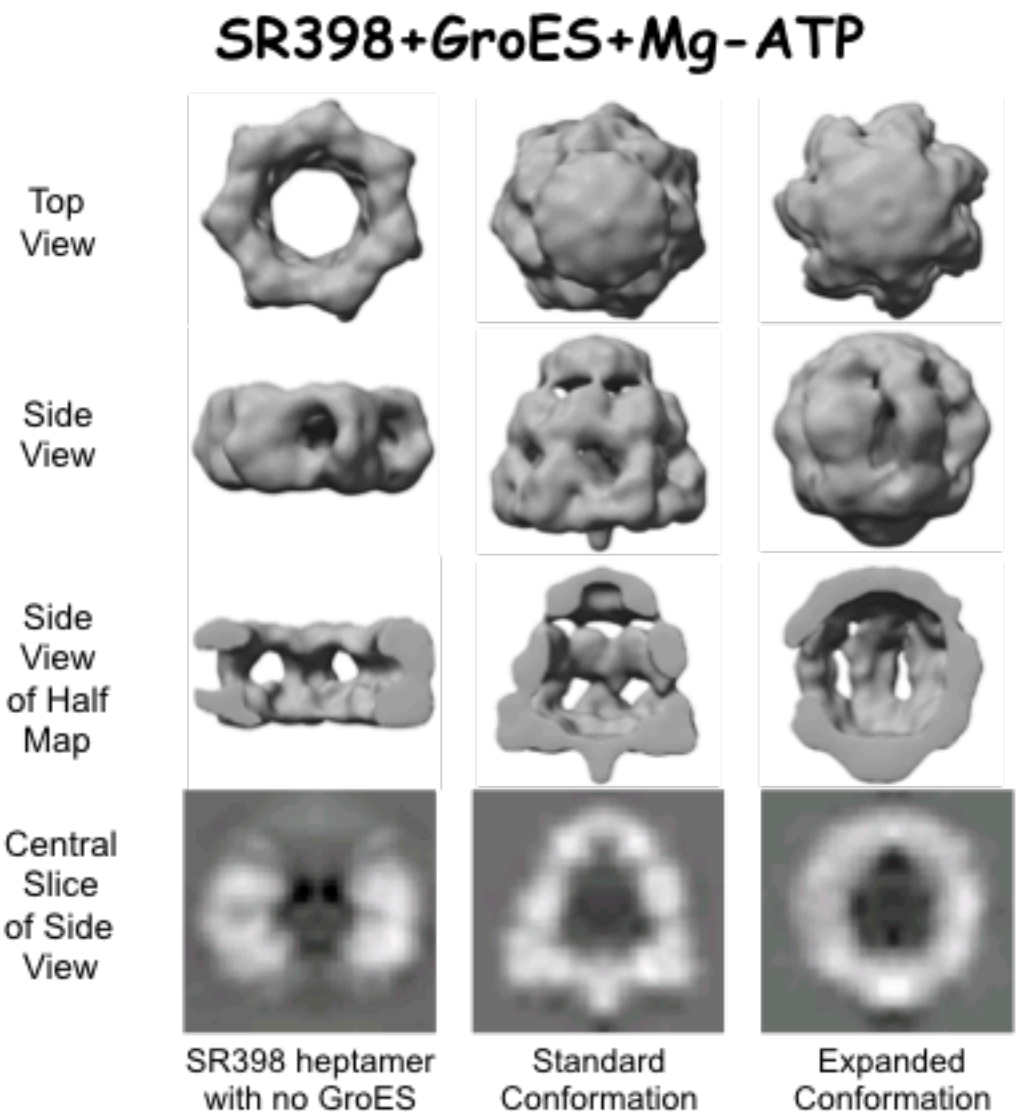
EMAN2

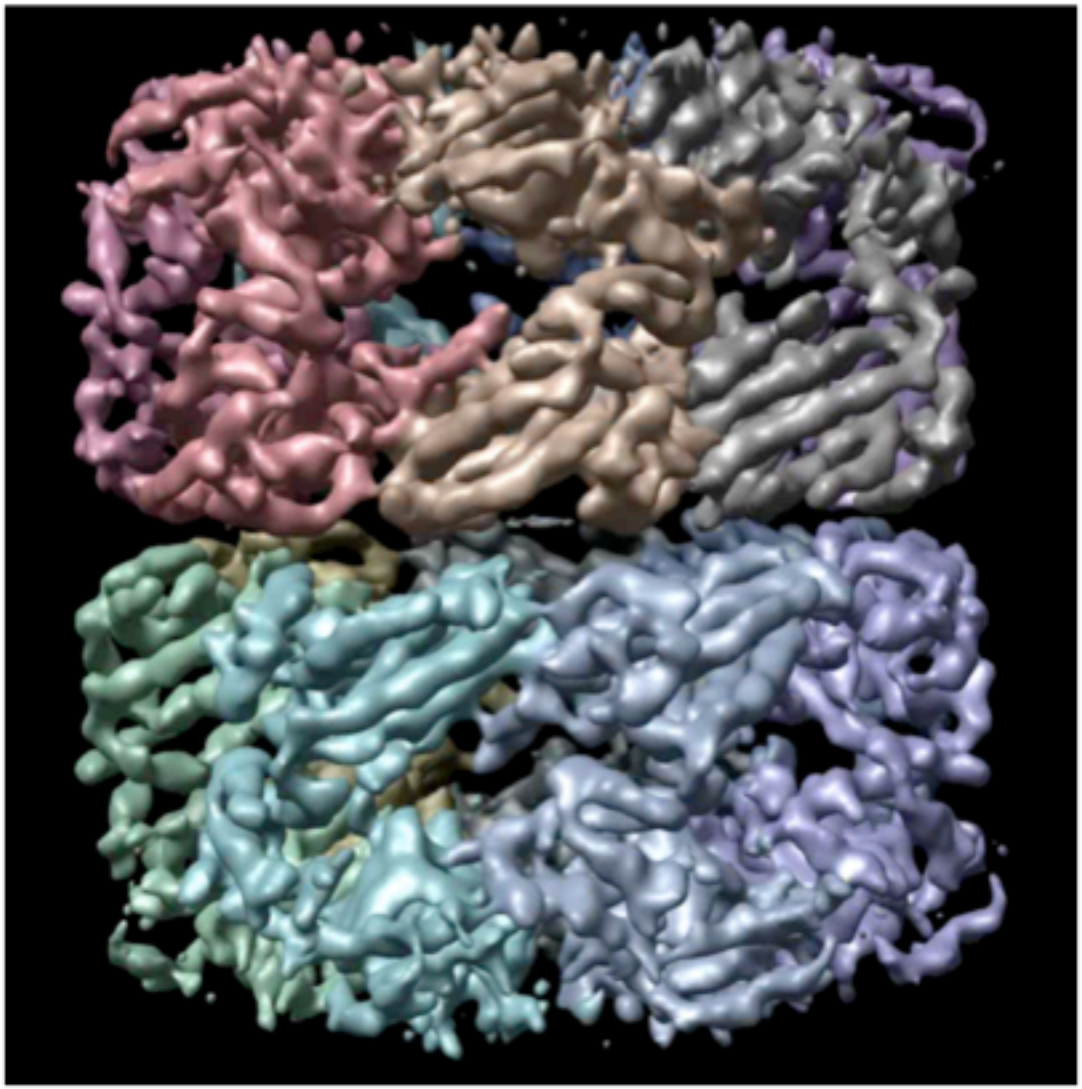


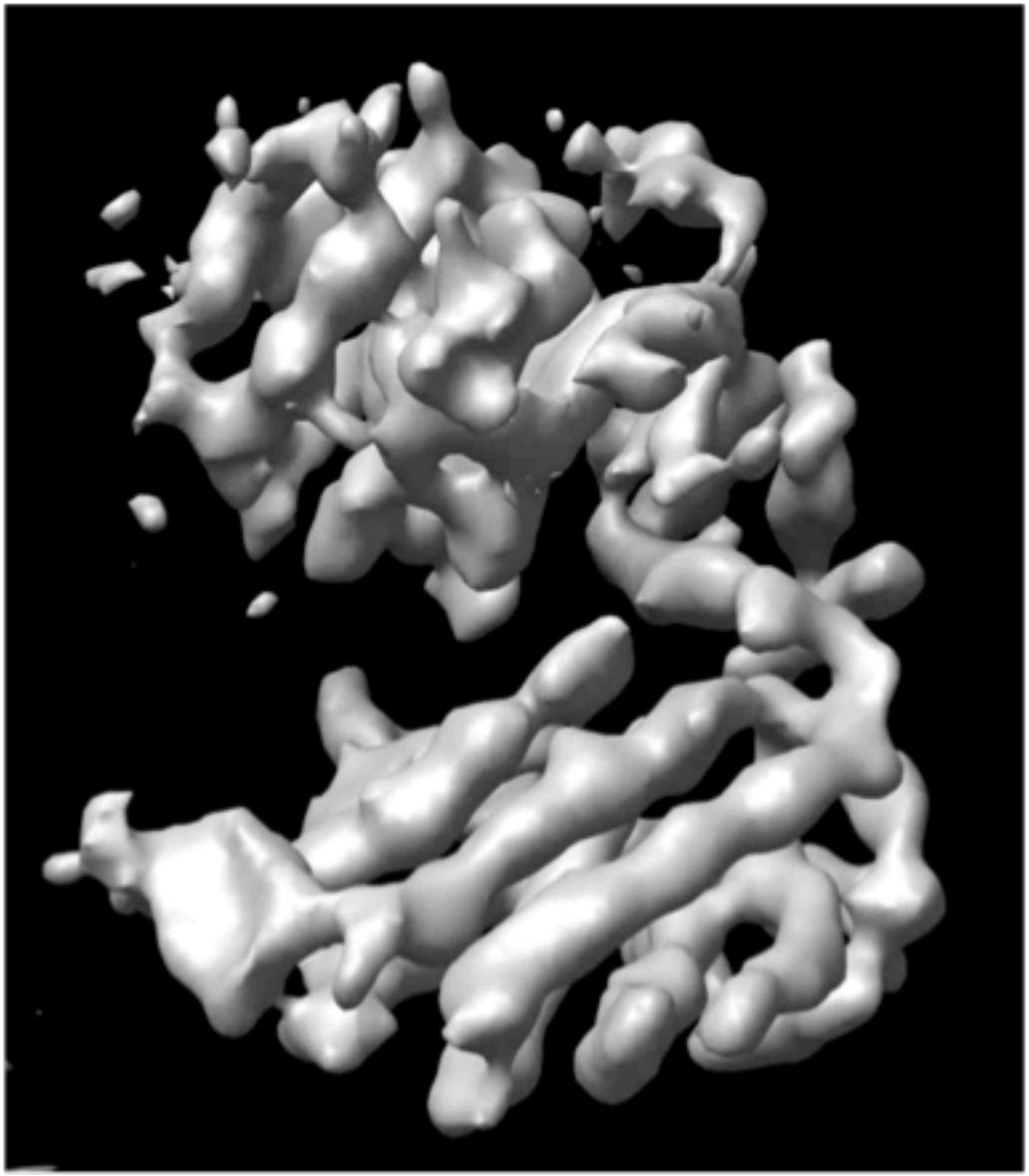
e2refine_easy.py

Multireference Refinement

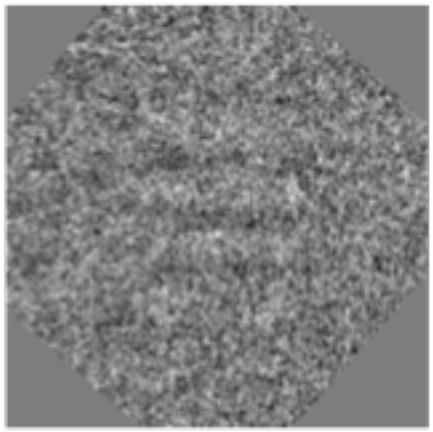



SR398+GroES+Mg-ATP
Top View
Side View
Side View of Half Map
Central Slice of Side View
SR398 heptamer with no GroES
Standard Conformation
Expanded Conformation



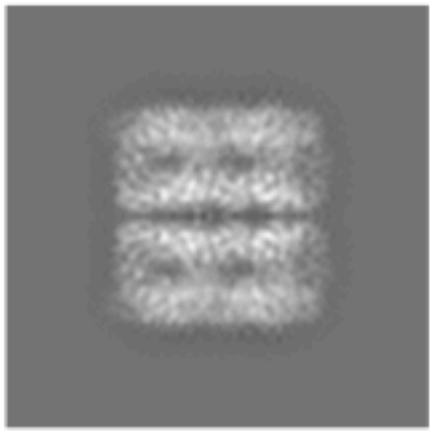


Can we subtract what we don't want ?



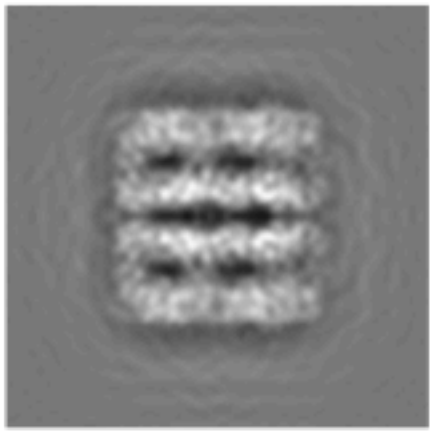
2007

Can we subtract what we don’t want ?



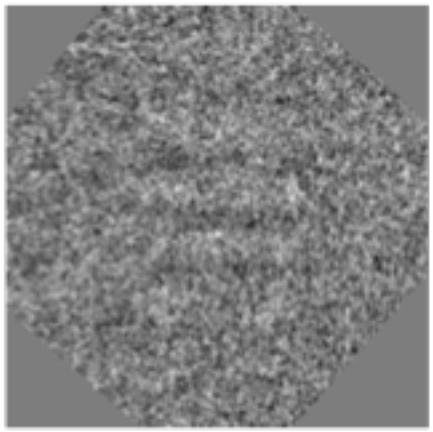
Projections

A lot of math →



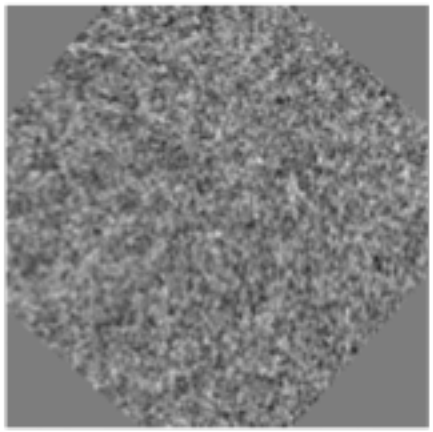
Spectrally matched projection
(CTF, etc)

Can we subtract what we don’t want ?



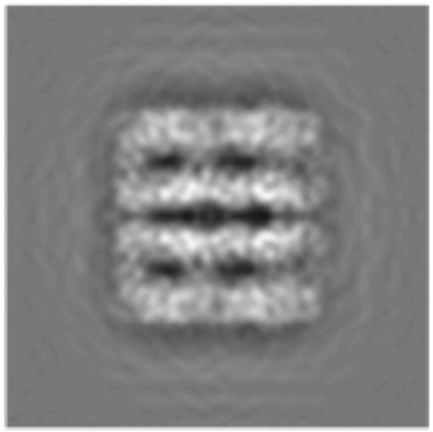
One Particle

Can we subtract what we don’t want ?

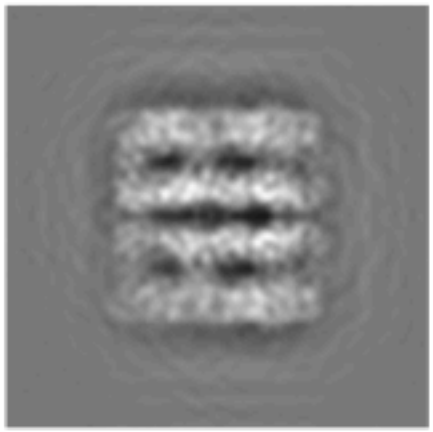


Subtracted

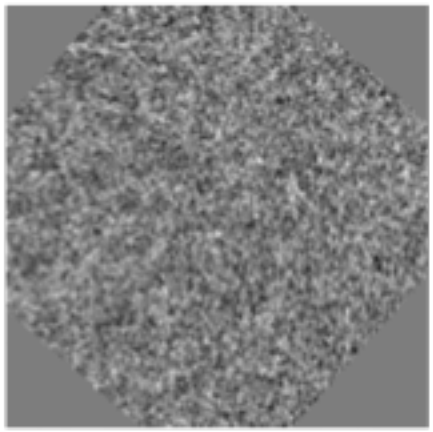
Remove One Monomer



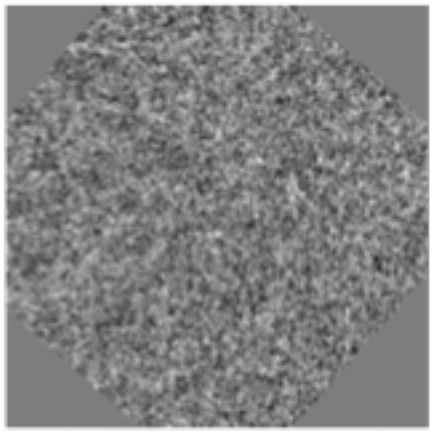
Remove One Monomer



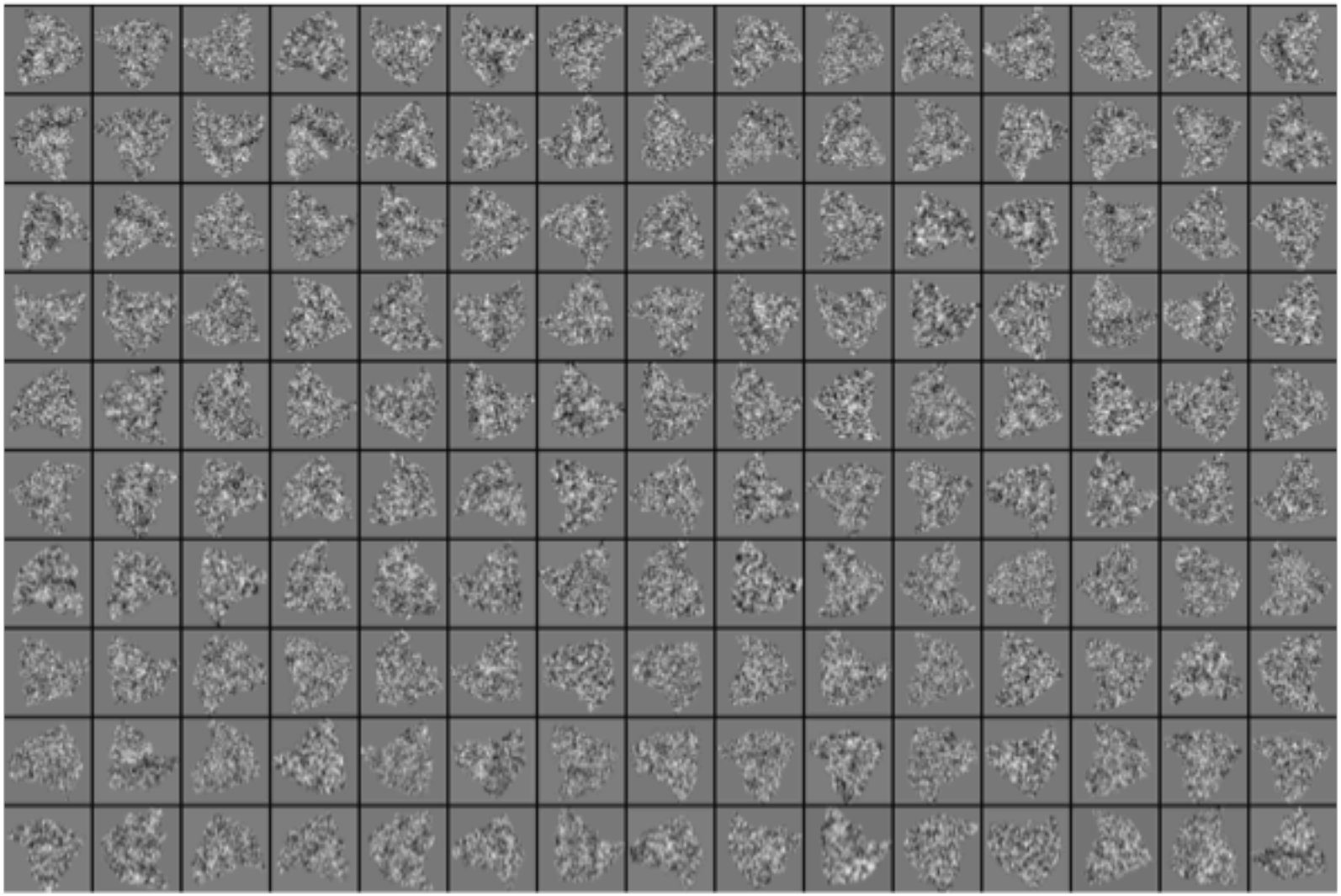
Remove One Monomer

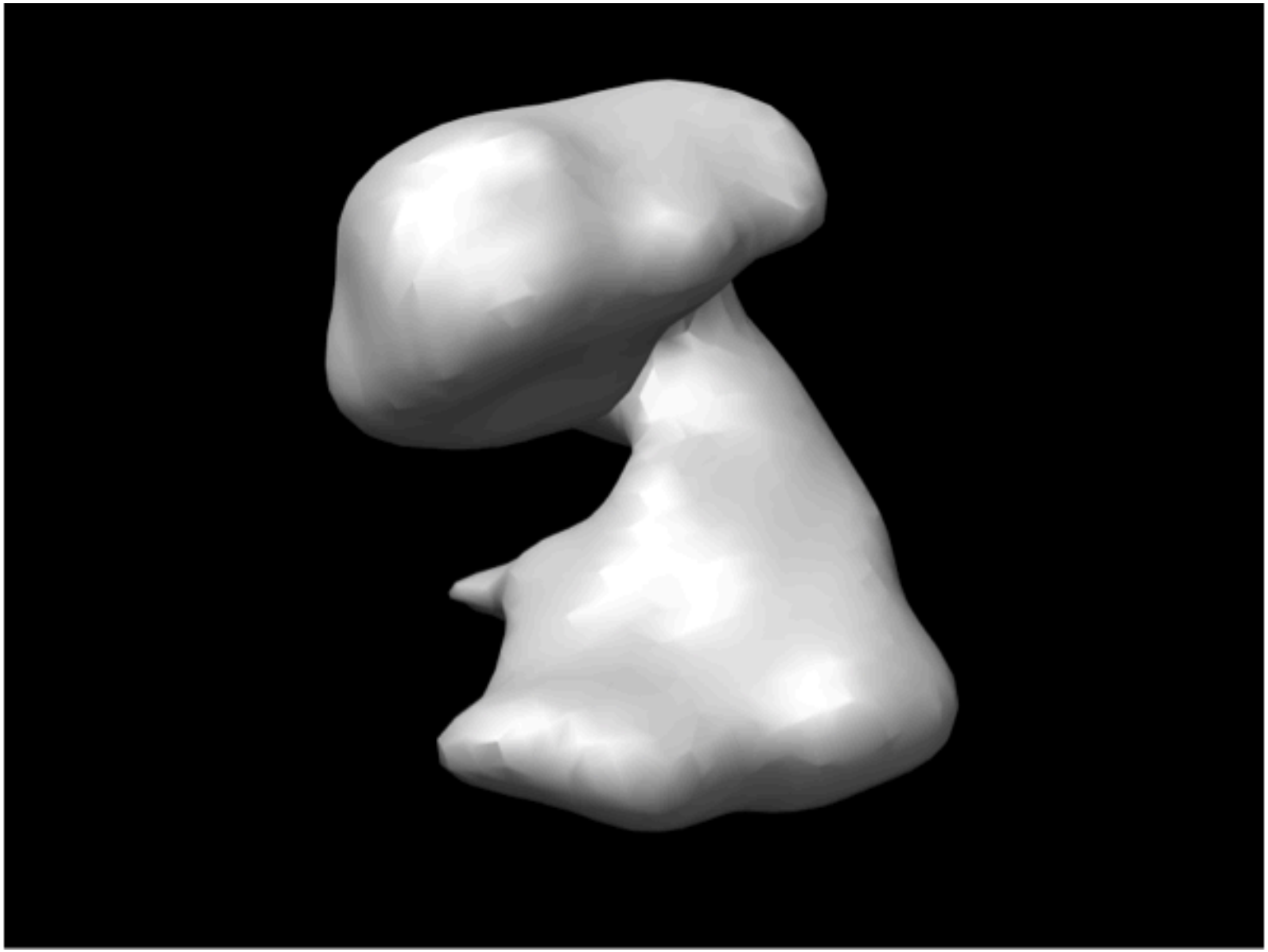


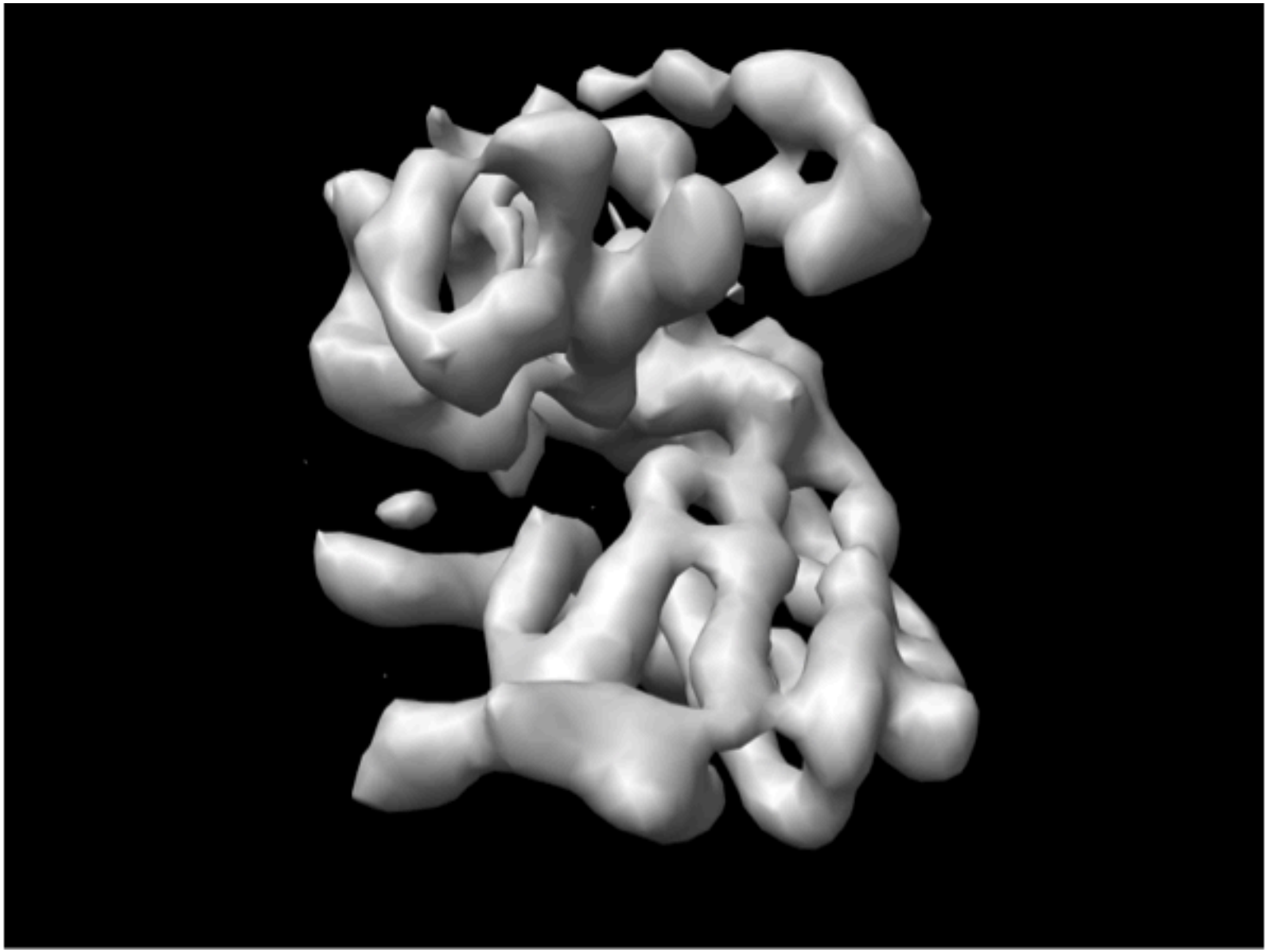
Remove One Monomer



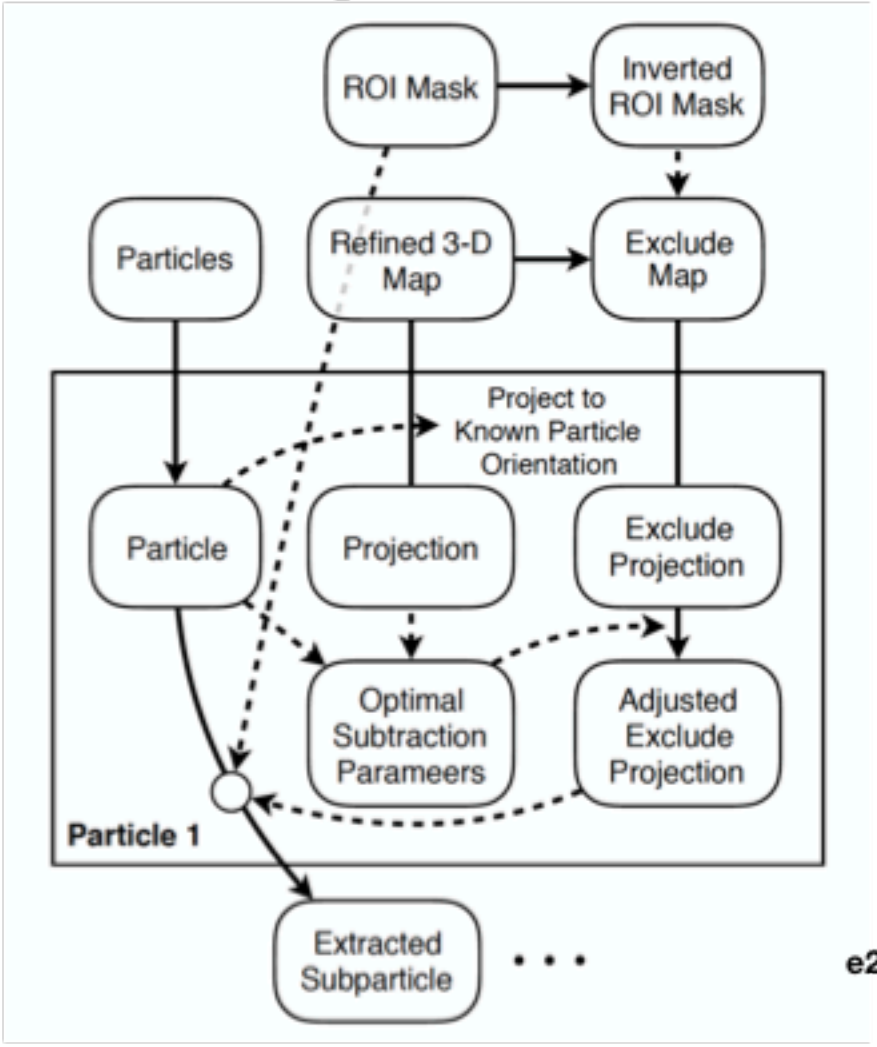
Many particles







Subparticles



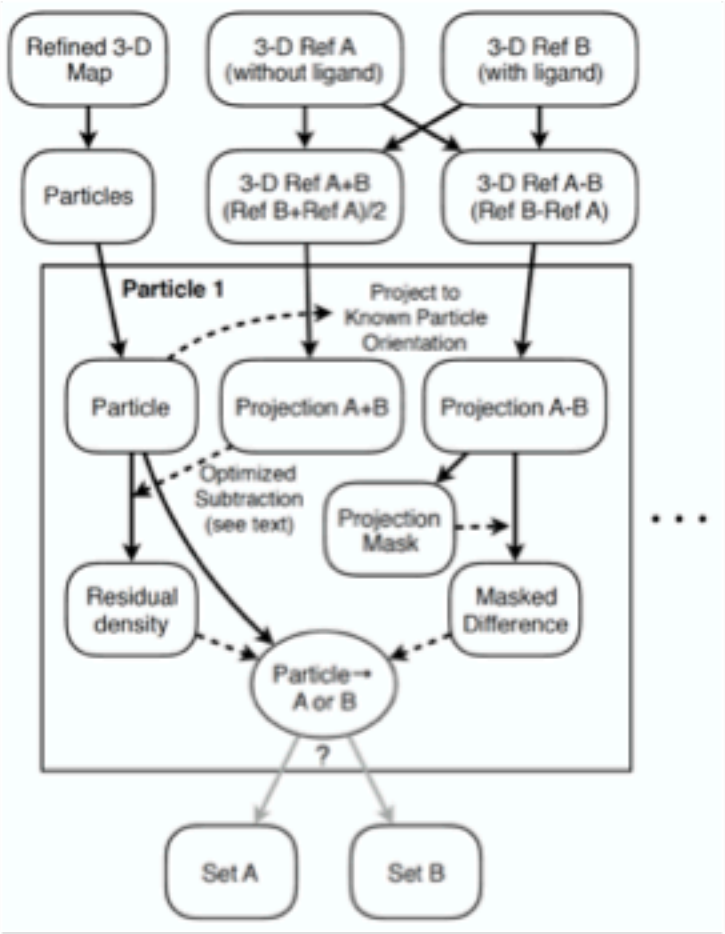
e2extractsubparticles.py

Alternative?

Subtraction may not be mathematically useful, so:

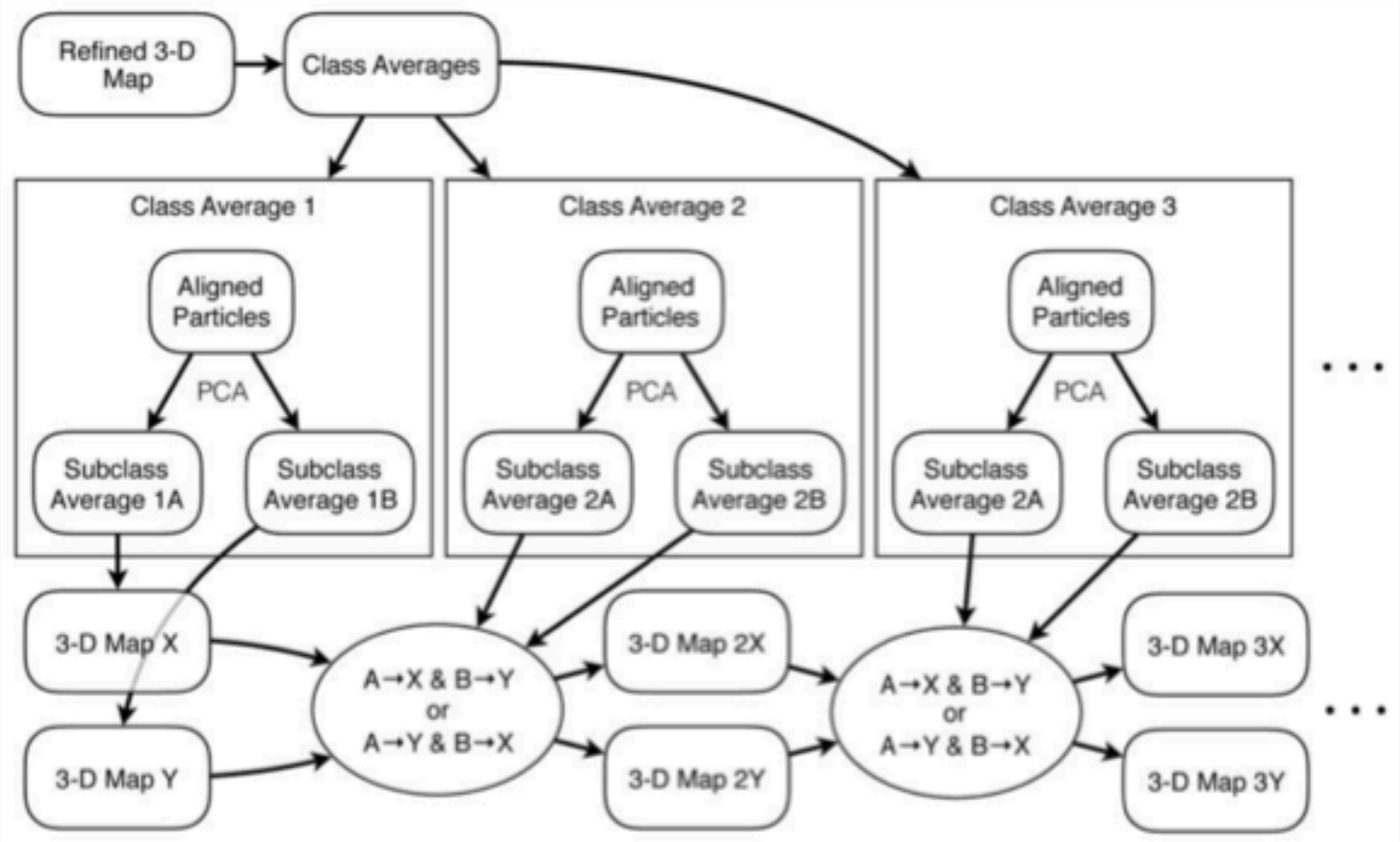
- Make n copies of each particle
- Force each one to be in a specific asymmetric unit
- Do a refinement under a 3-D mask for one subunit
  - Align without mask, classify with?
  - Align and classify with mask?

Examine Binary Separability



e2classifyligand.py

Split into Two Classes



e2refine_split.py