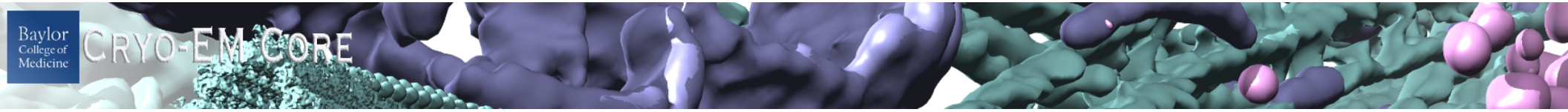


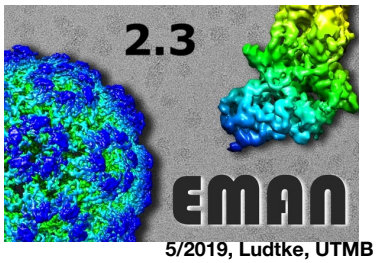
Variability Analysis

Steve Ludtke

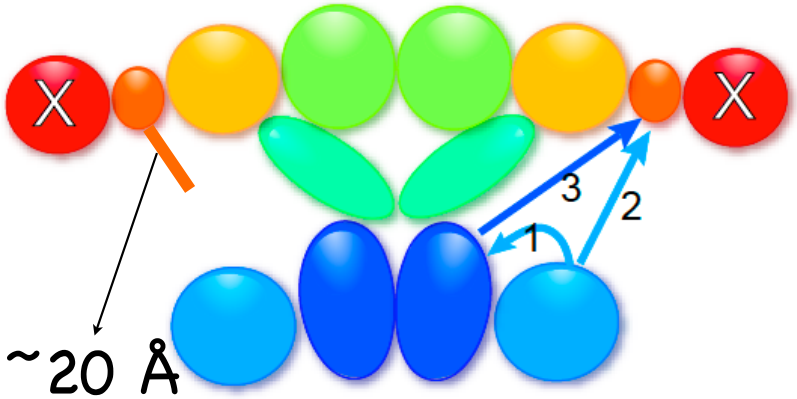
Charles C. Bell Professor
Biochemistry and Molecular Biology
Director, CryoEM/CryoET Core
Co-director CIBR Center
Baylor College of Medicine

Baylor College of Medicine

VERNA & MARRS McLEAN
DEPARTMENT OF
BIOCHEMISTRY AND
MOLECULAR BIOLOGY

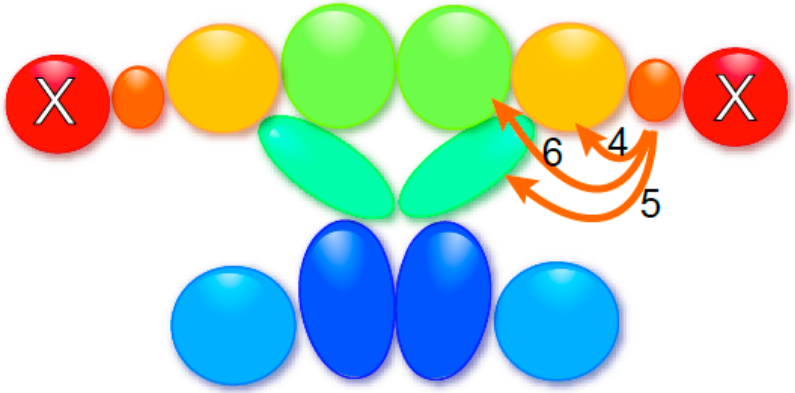


Methods in Enzymology
The Resolution Revolution: Recent Advances In cryoEM
Edited by R.A. Crowther
Volume 579, Pages 2-445 (2016)



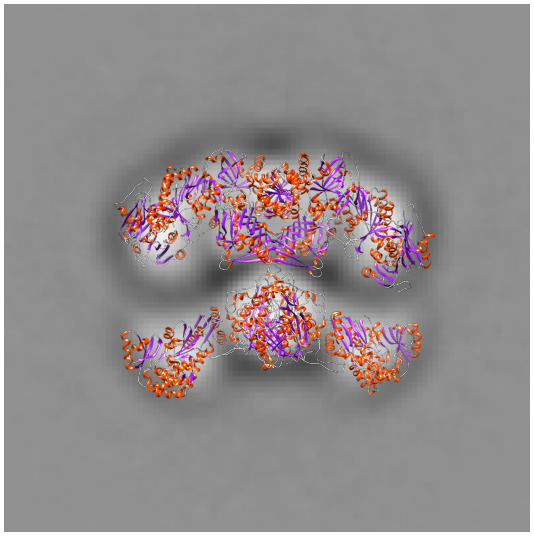
1. Loading Acetyl-CoA
2. Loading Malonyl-CoA
3. Condensation

~80 Å !?

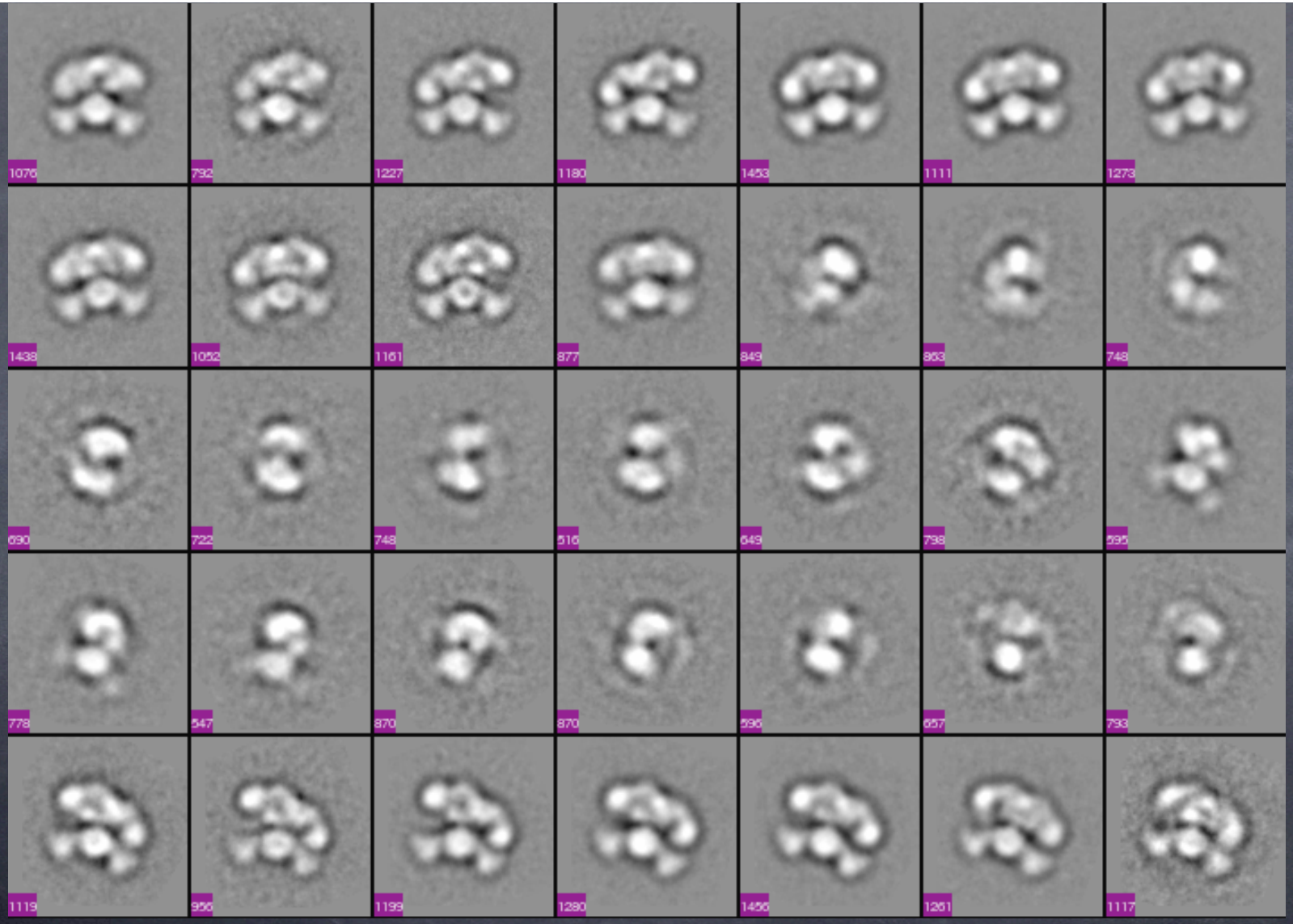


4. Keto reduction
5. Dehydration
6. Enoyl reduction

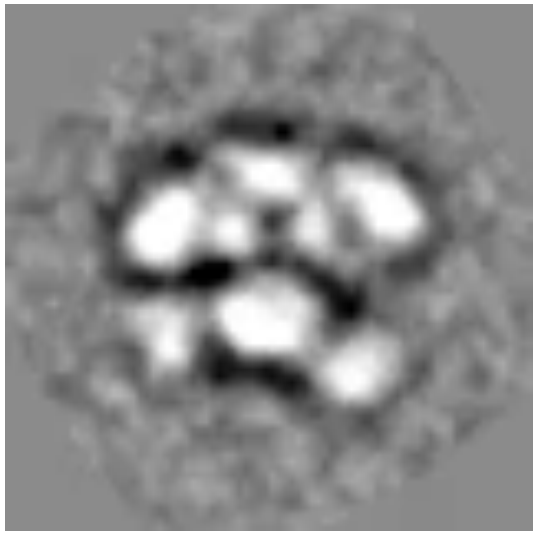
Single View CryoEM Average



150 Å

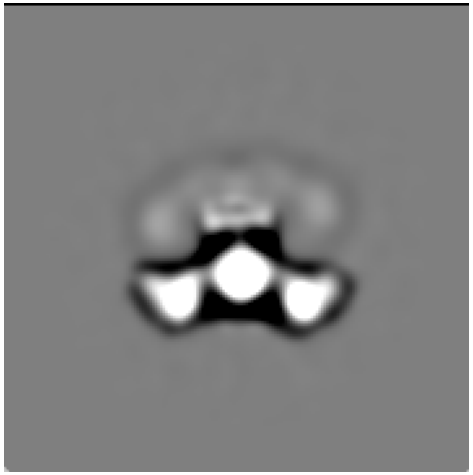

1076
732
1227
1180
1453
1111
1273
1438
1052
1161
877
849
853
748
690
722
748
516
649
798
595
778
547
870
870
596
657
793
1119
956
1199
1280
1456
1261
1117

Fatty Acid Synthase, ~30 Å motion

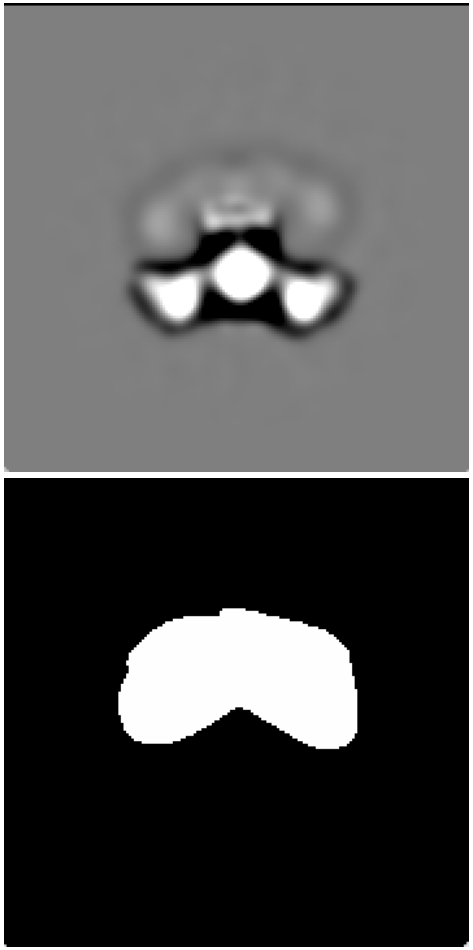


150 Å

Align to

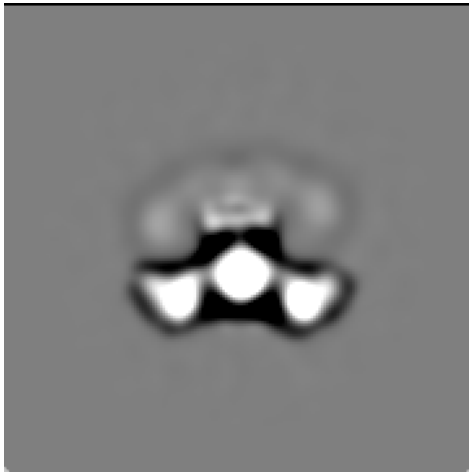


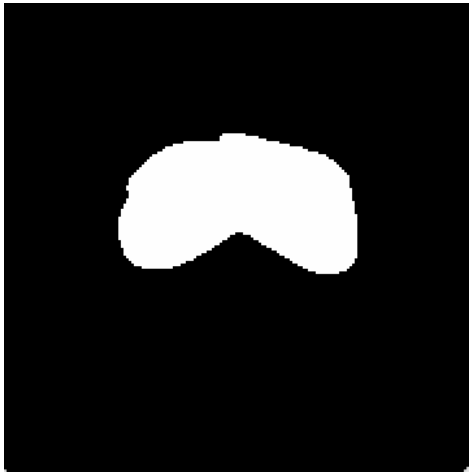
Align to



Variability Mask

Align to





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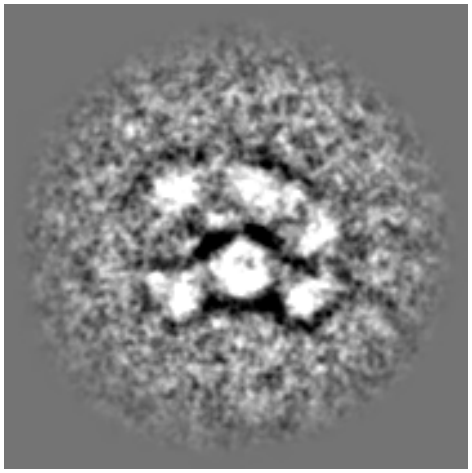
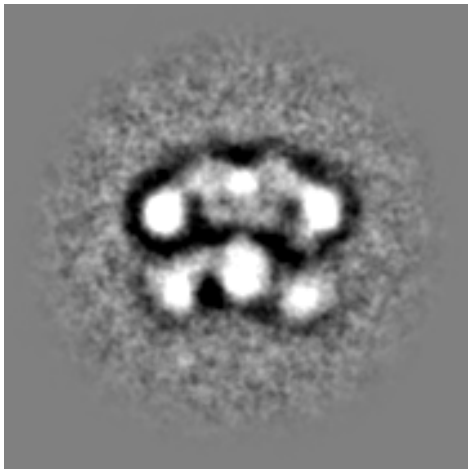
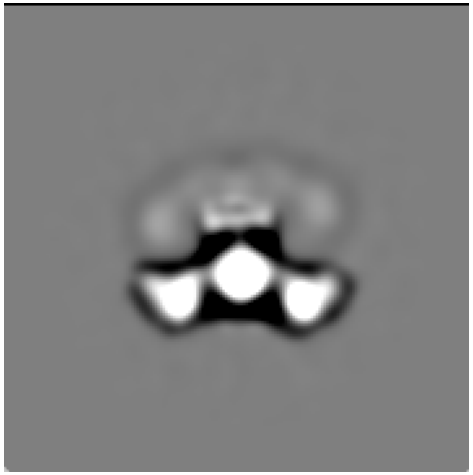


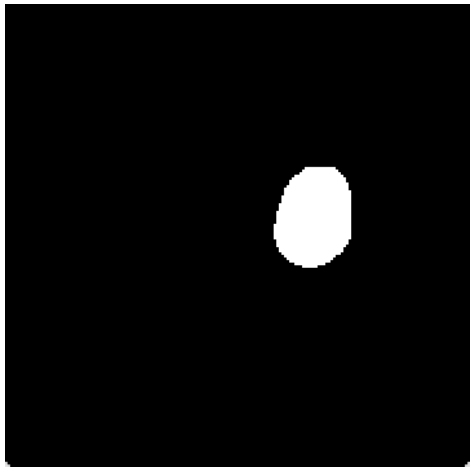
Align to

Variability Mask

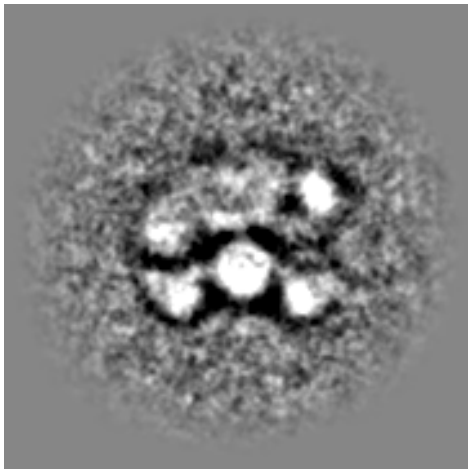


Align to





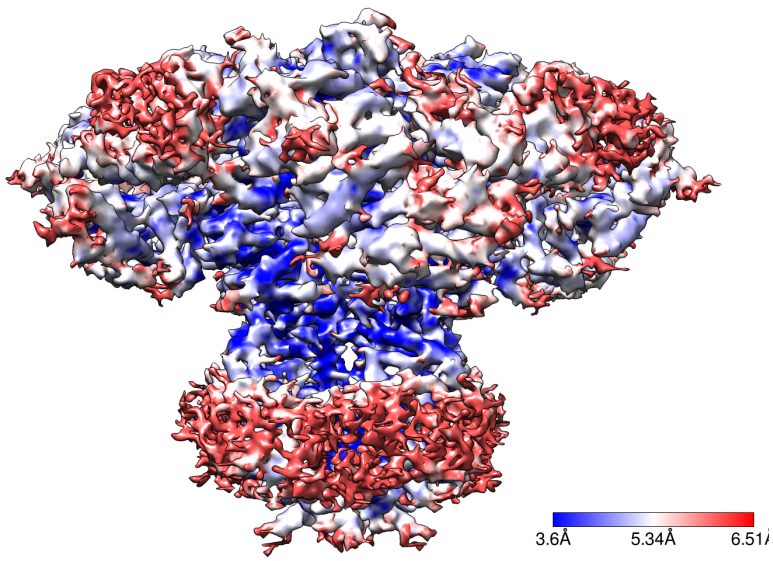
Variability Mask

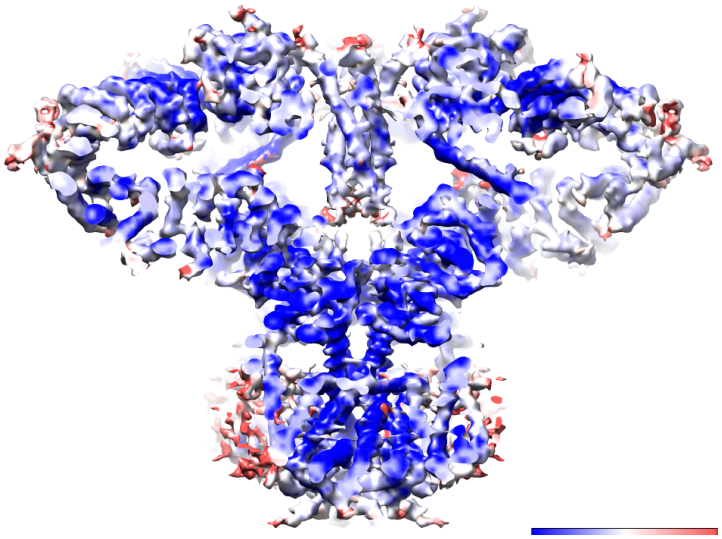


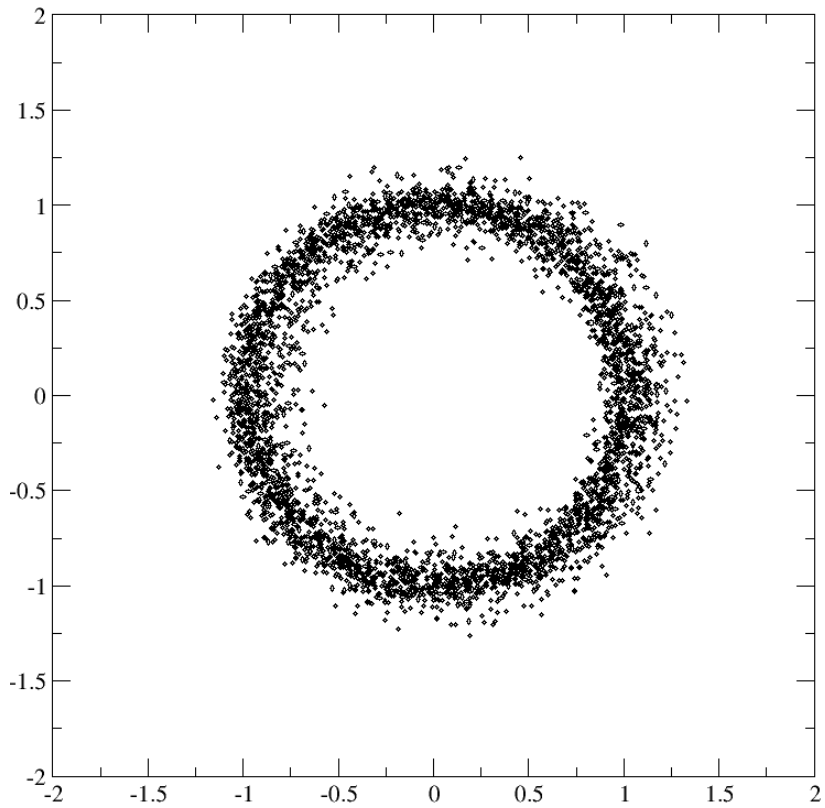
Local Variability

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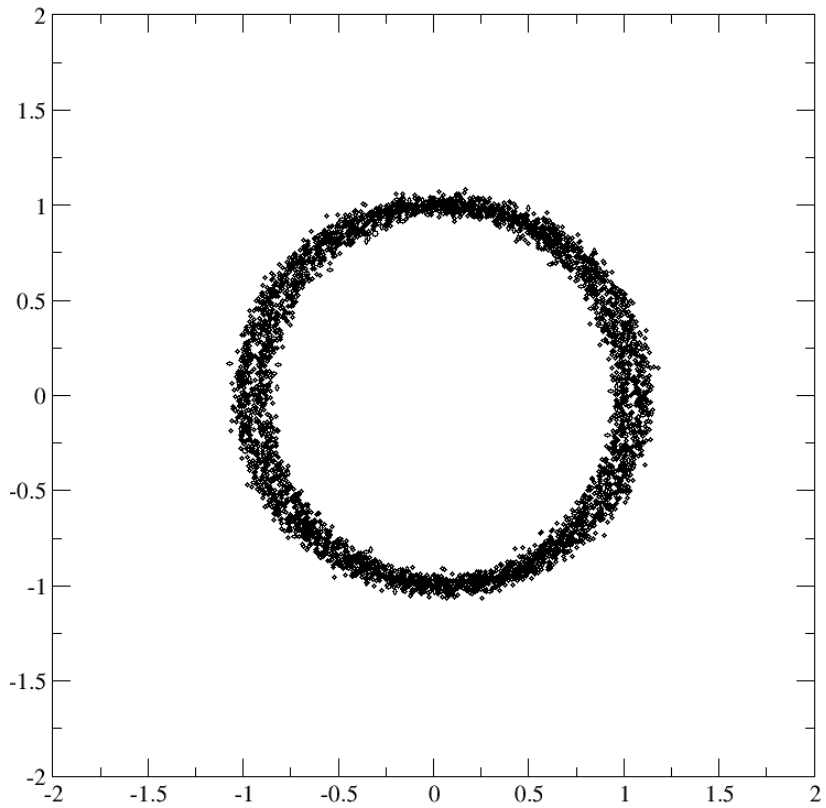
1
1.5
2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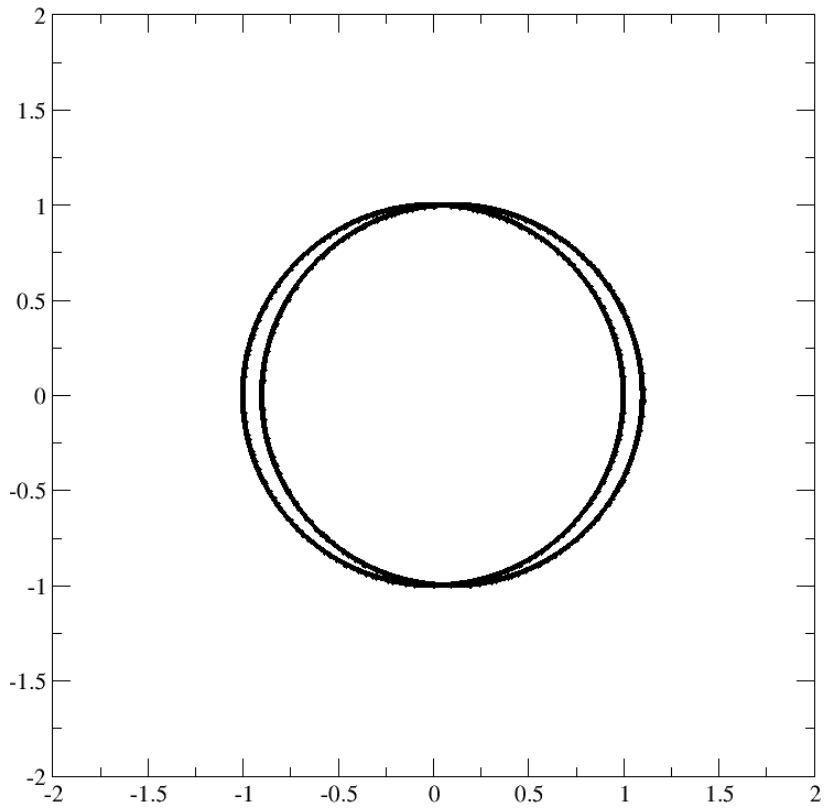
1
1.5
2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0
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-1
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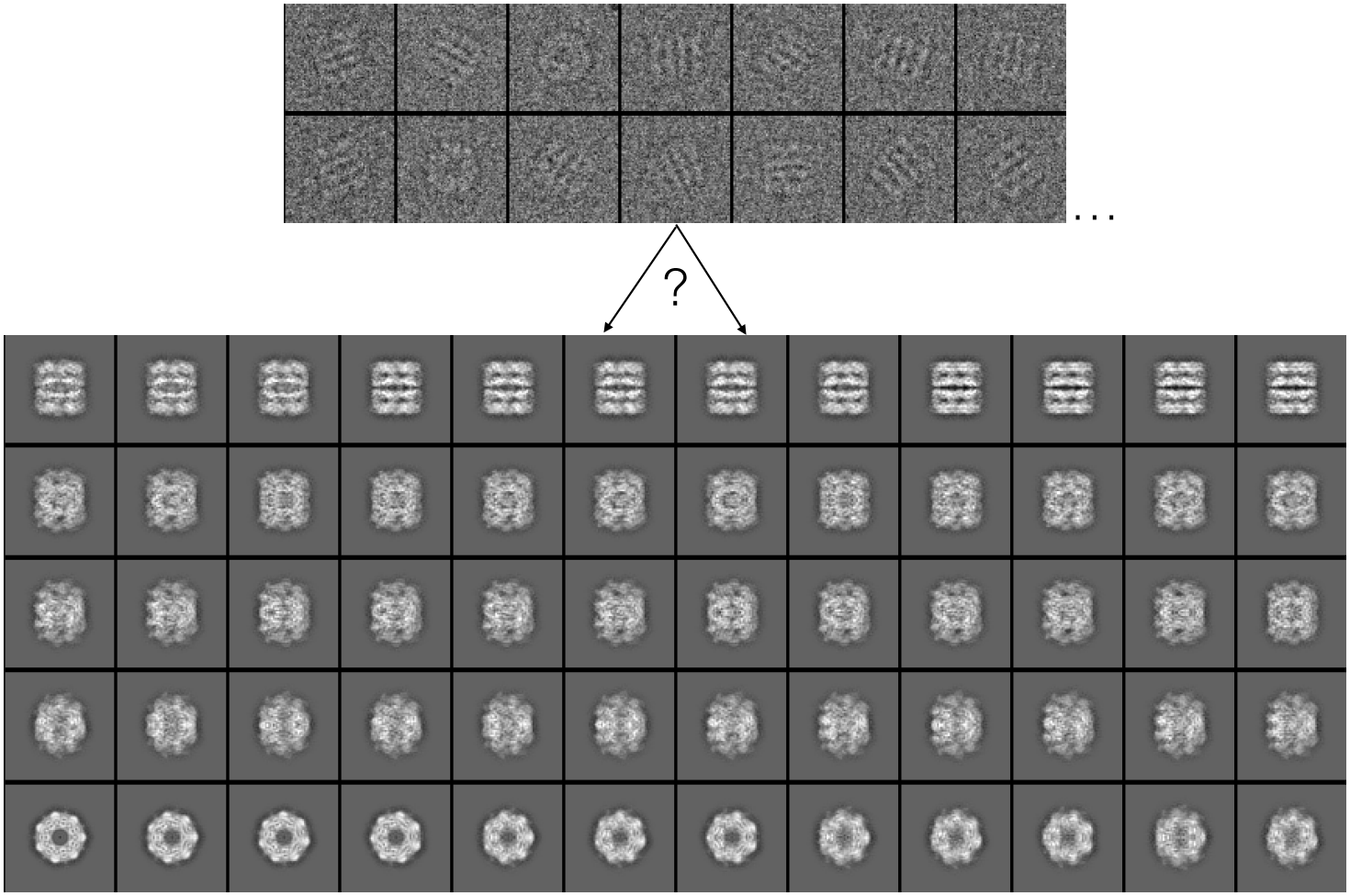
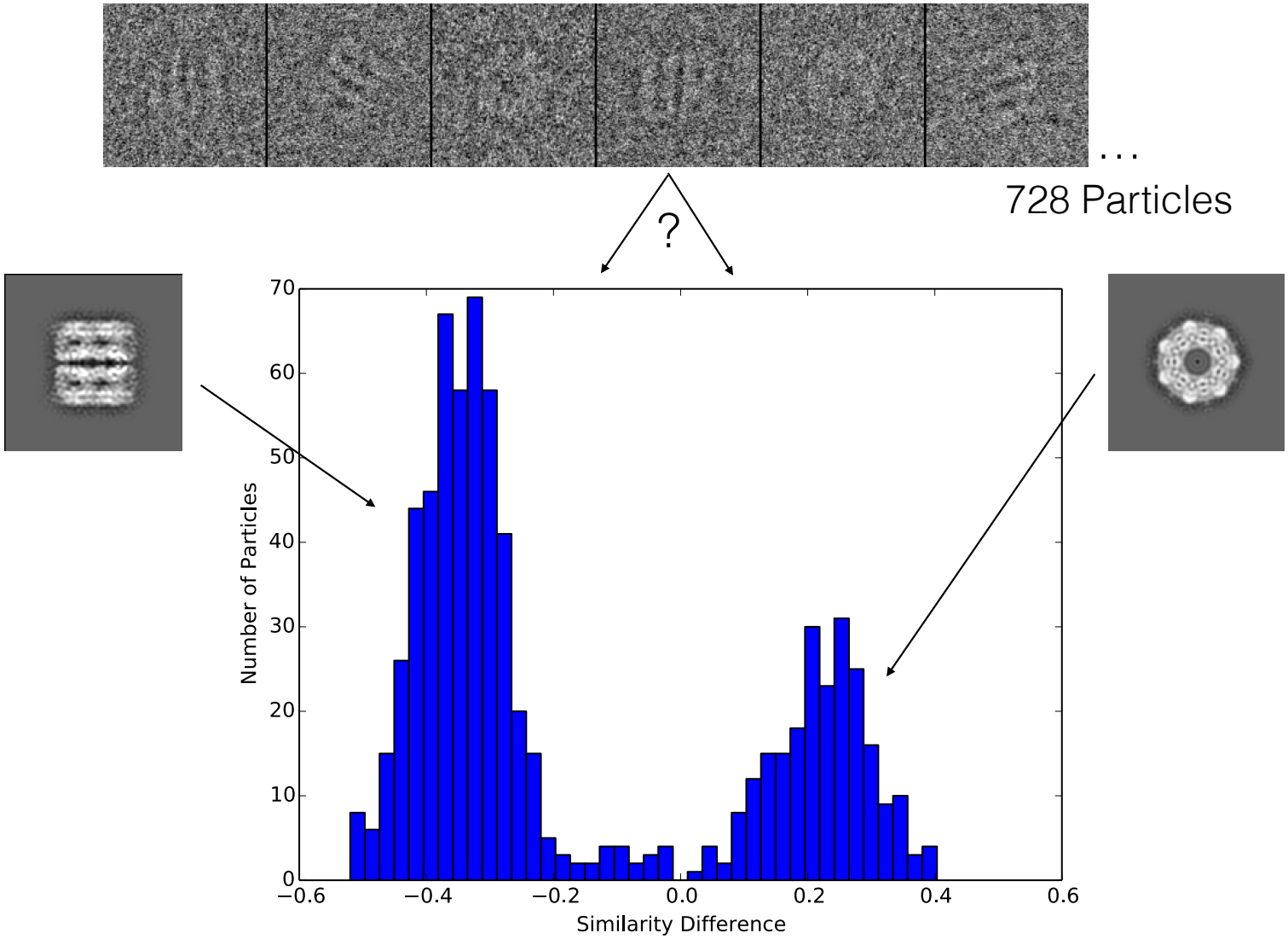
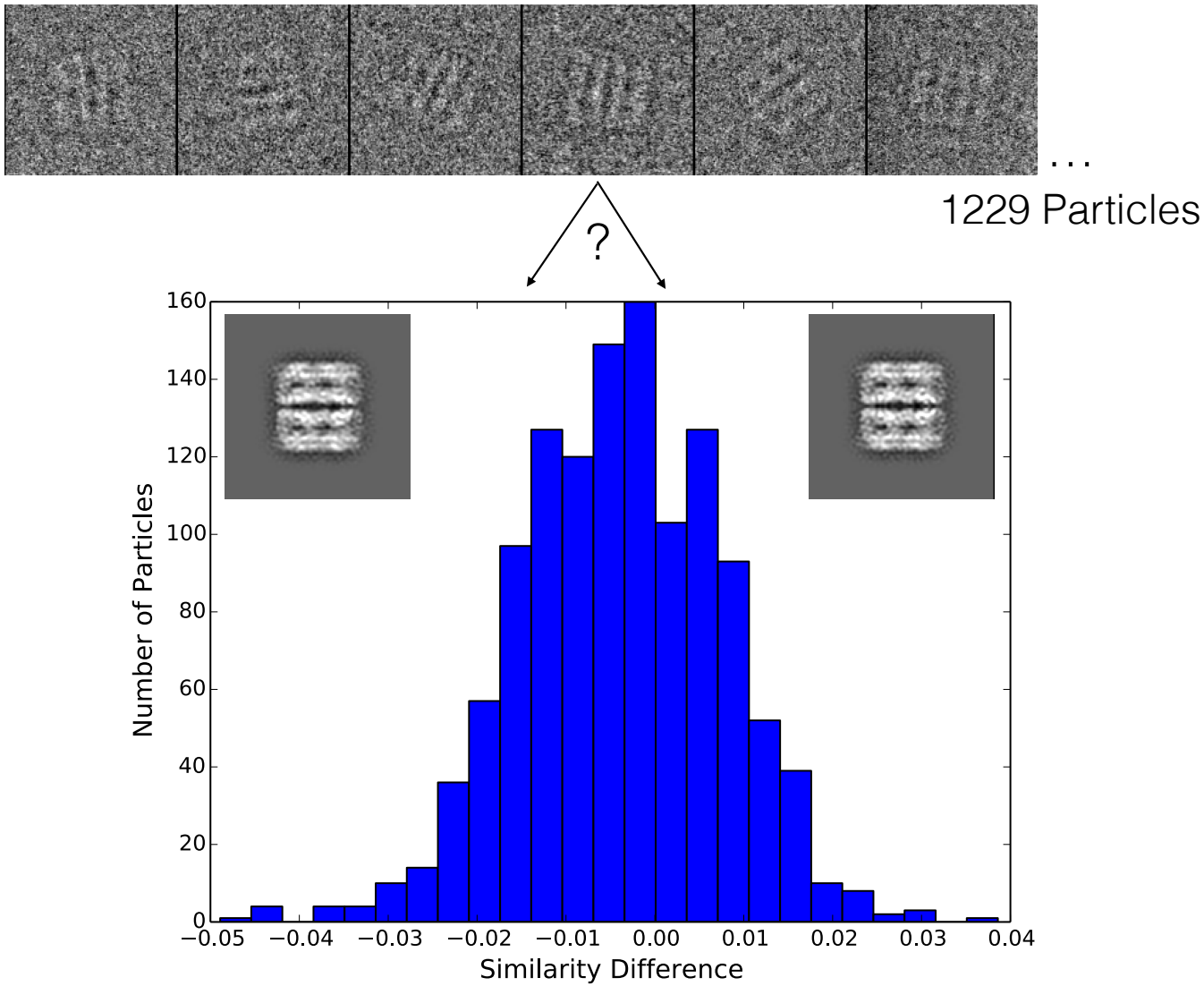
0
0.5
1
1.5
2

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
?
Number of Particles
Similarity Difference
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20
40
60
80
100
120
140
160
−0.05
−0.04
−0.03
−0.02
−0.01
0.00
0.01
0.02
0.03
0.04

How can we improve?

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

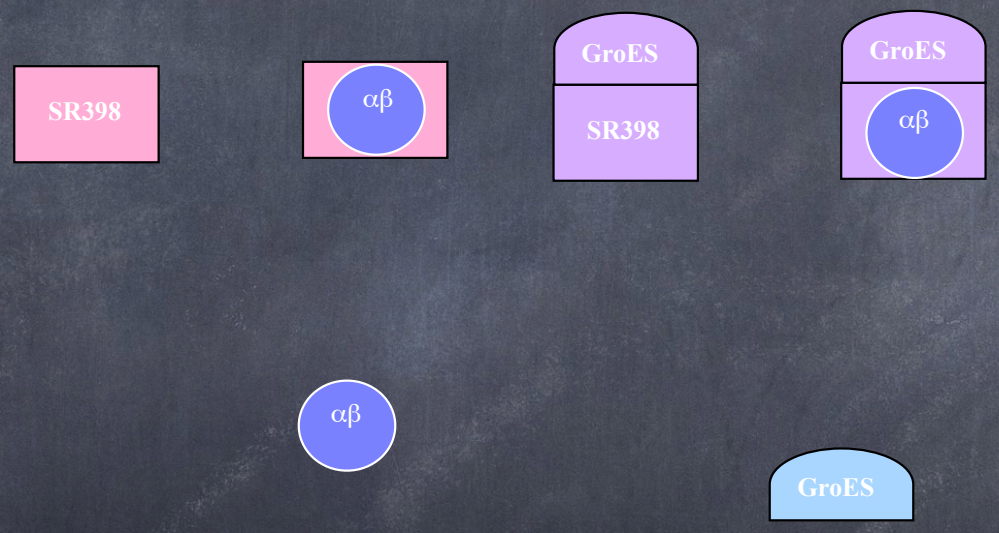
Multi-component Systems

GroEL, GroES & Substrate

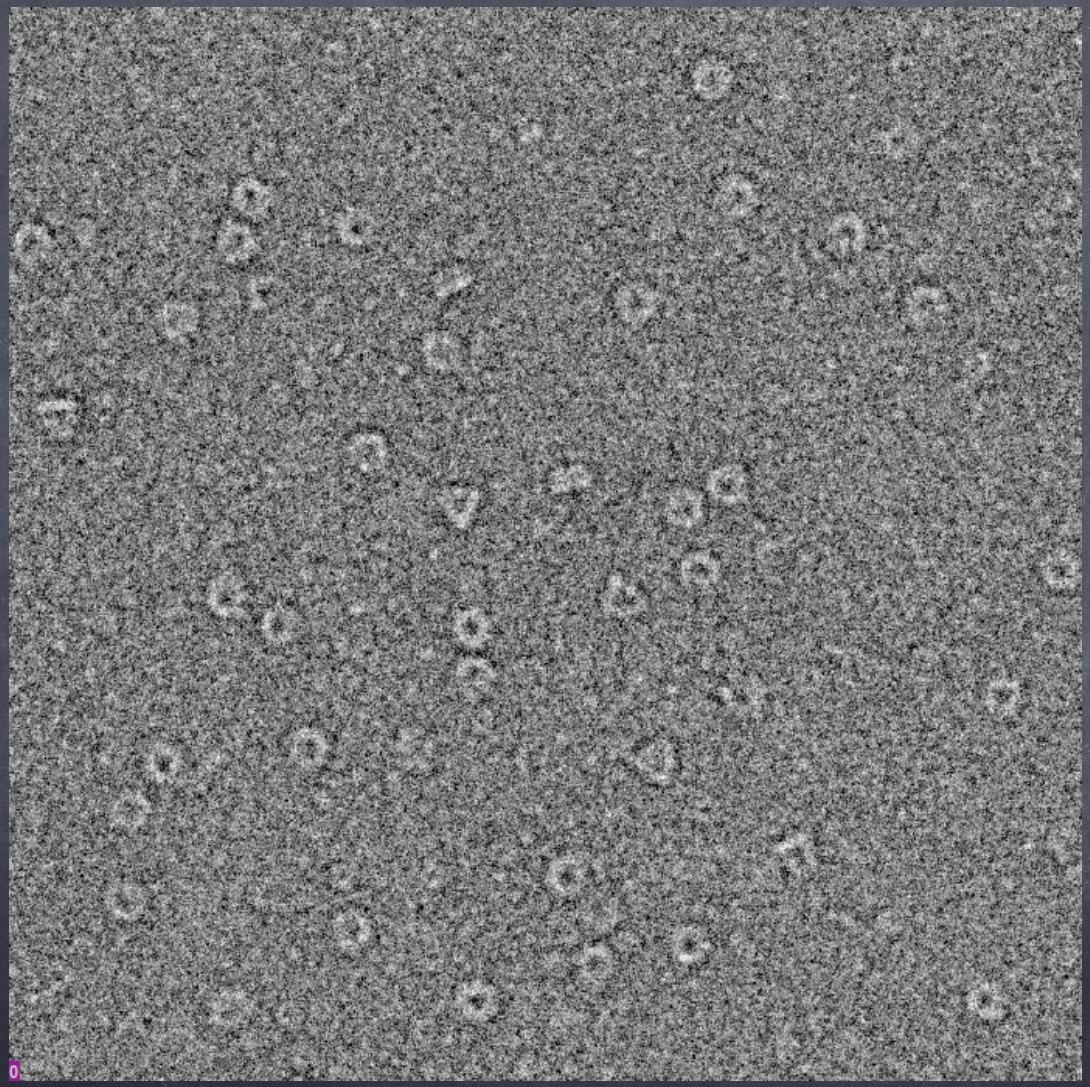
mitochondrial branched-chain α-ketoacid dehydrogenase

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.

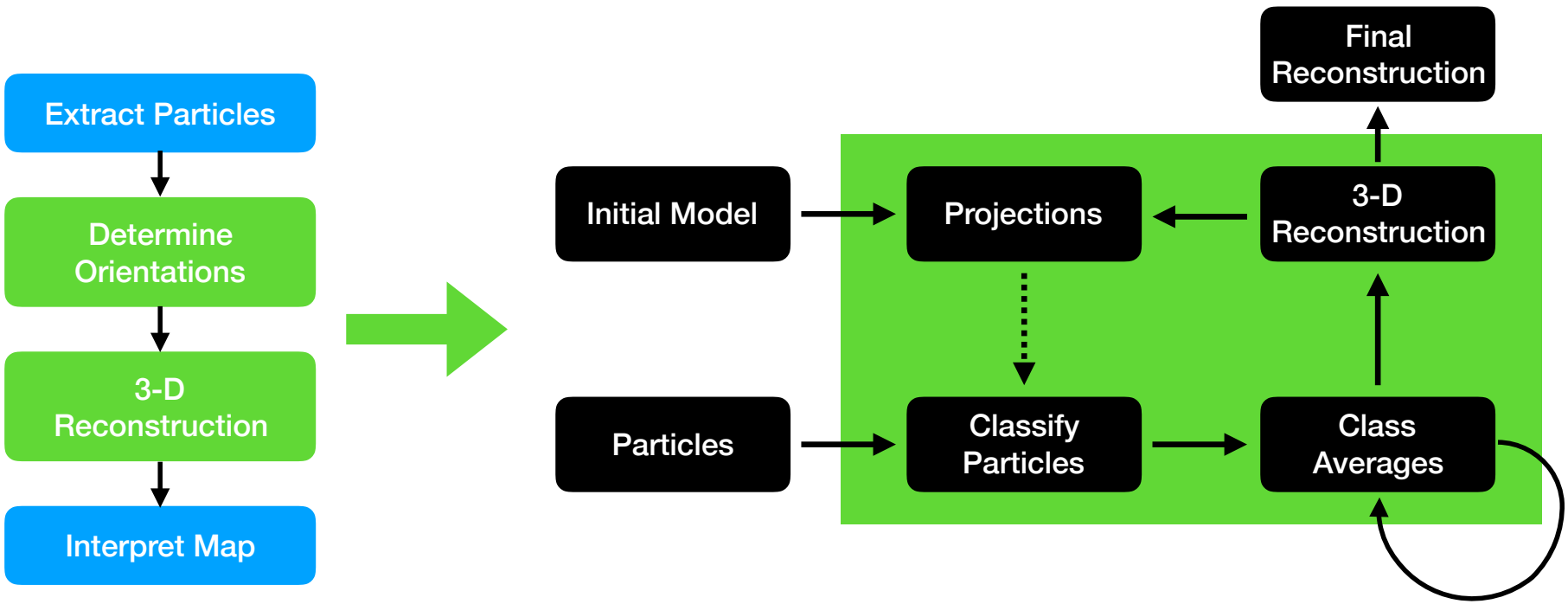
Produces a Mixed Population



SR398+GroES+ATP

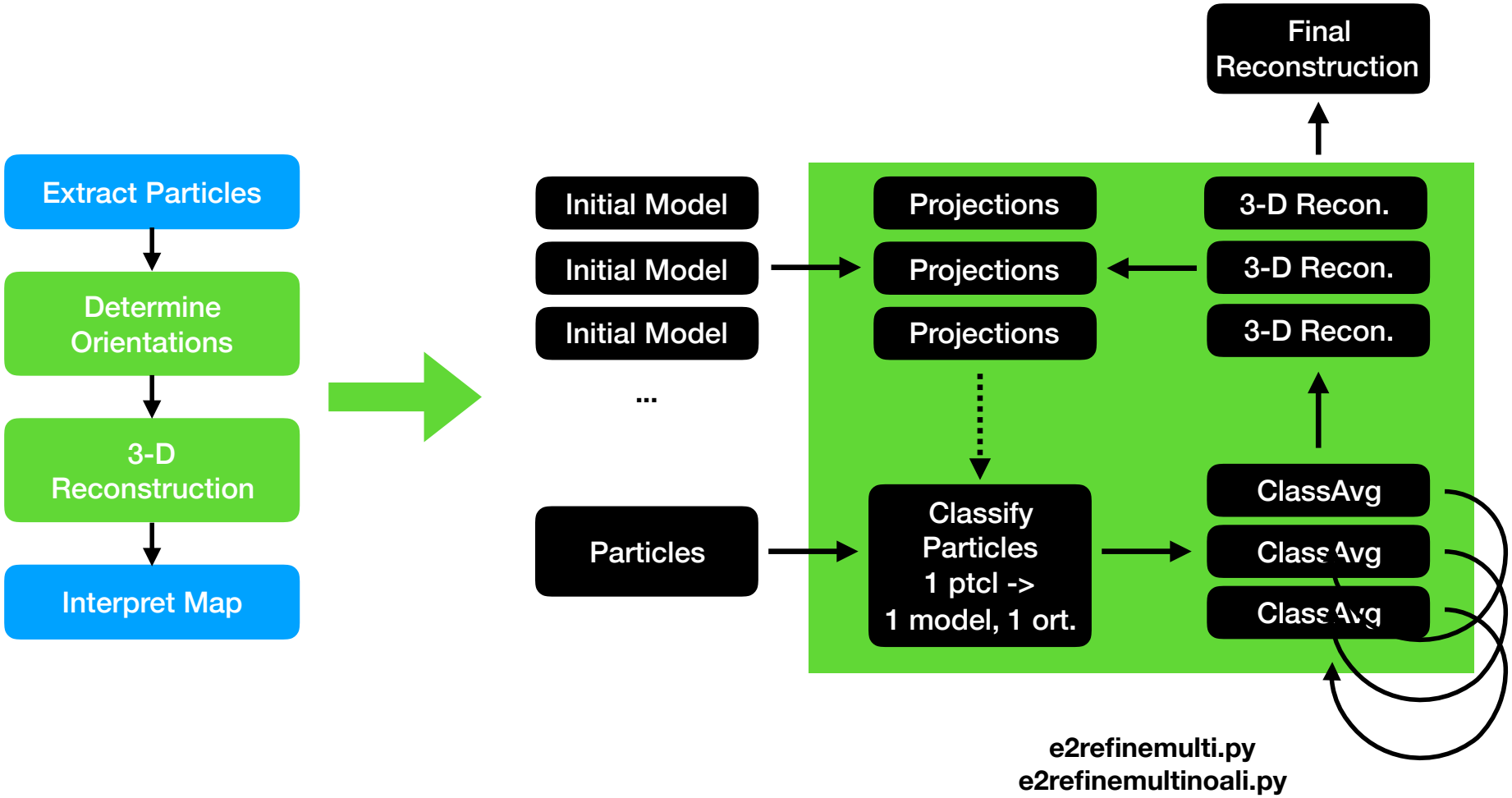


EMAN2

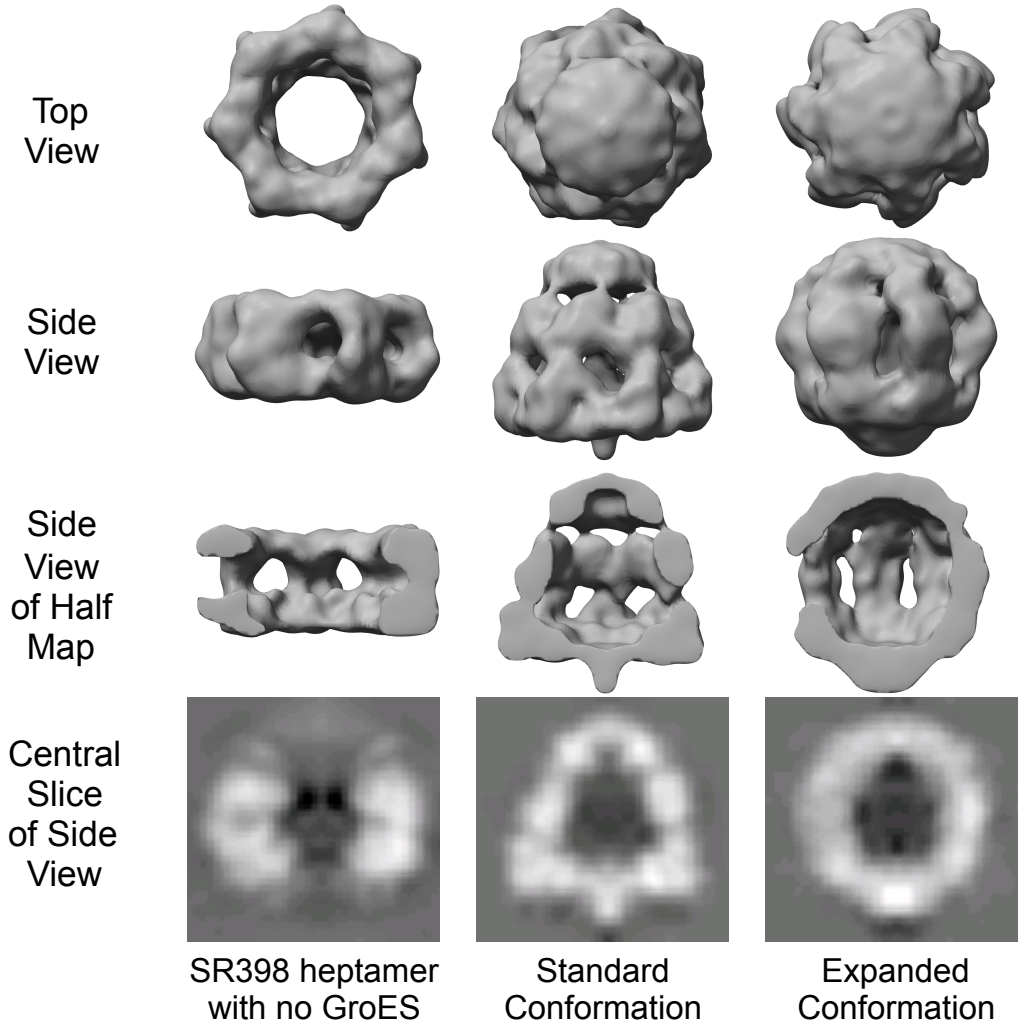


e2refine_easy.py

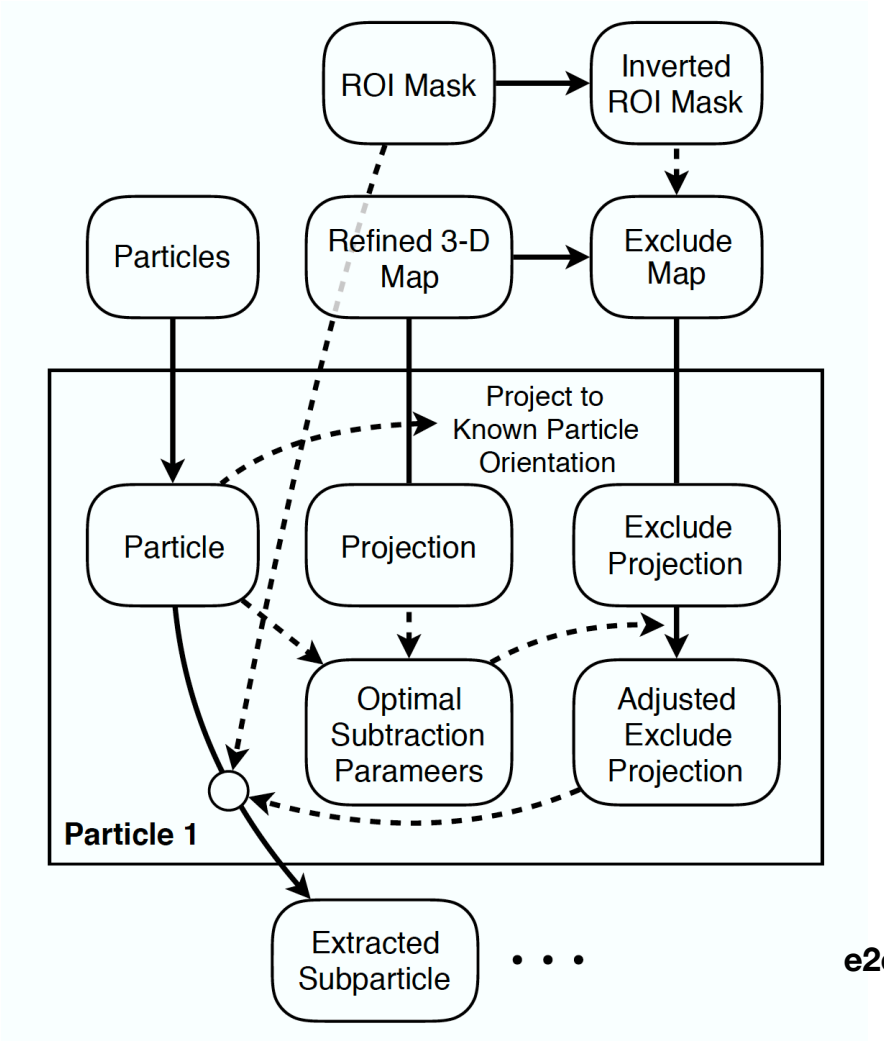
Multireference Refinement



SR398+GroES+Mg-ATP



Subparticles



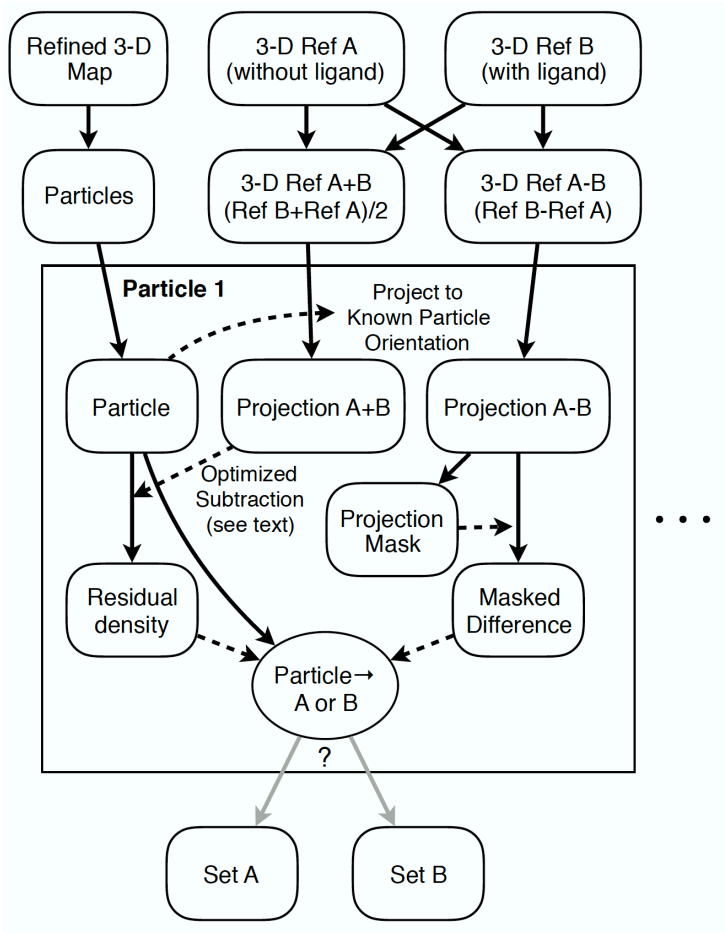
e2extractsubparticles.py

Alternative?

Subtraction may not be mathematically useful, so:

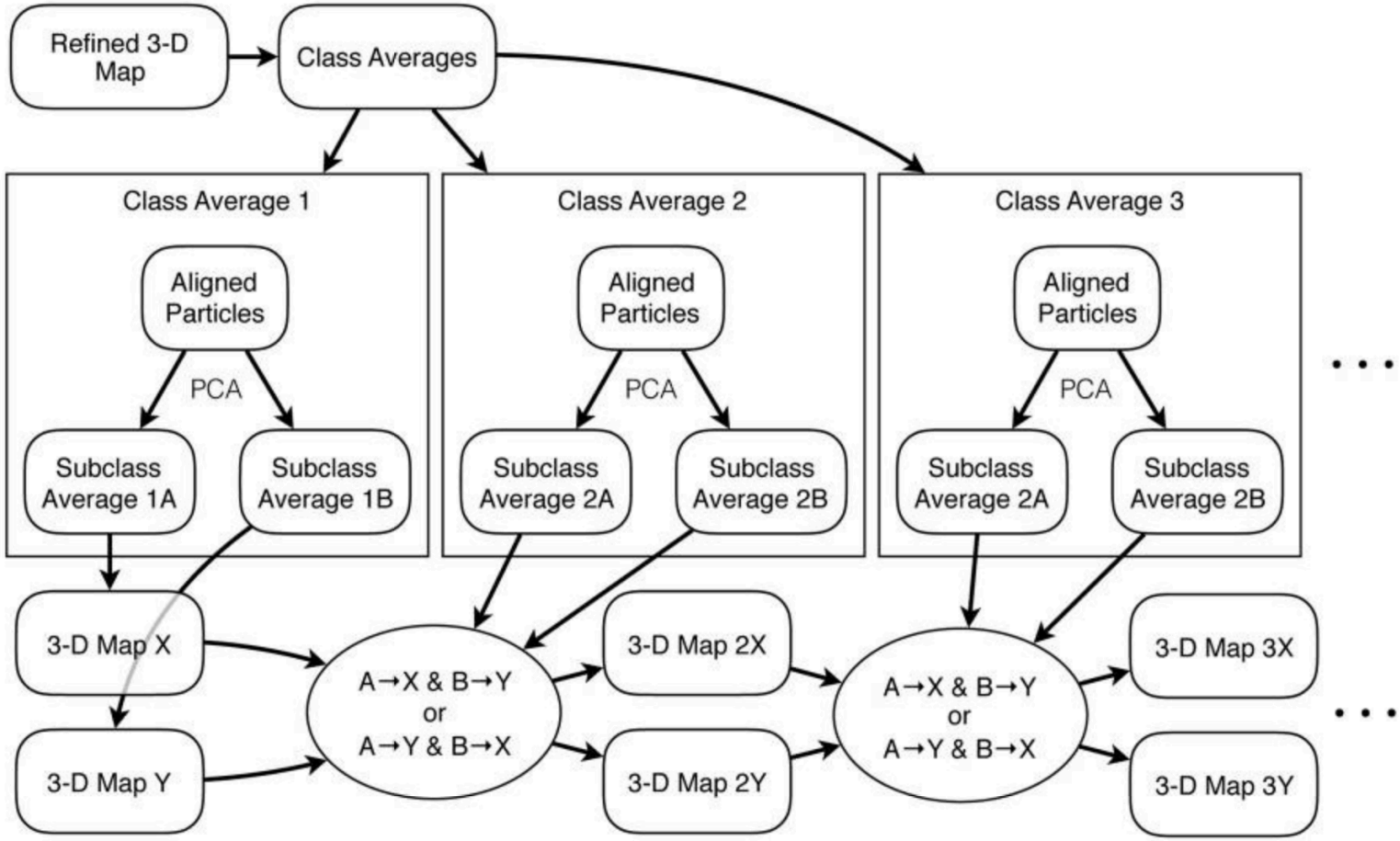
- Make n copies of each particle
- Force each one to be in a specific asymmetric unit
- Do a refinement under a 3-D mask for one subunit
  - Align without mask, classify with?
  - Align and classify with mask?

Examine Binary Separability



e2classifyligand.py

Split into Two Classes



e2refine_split.py