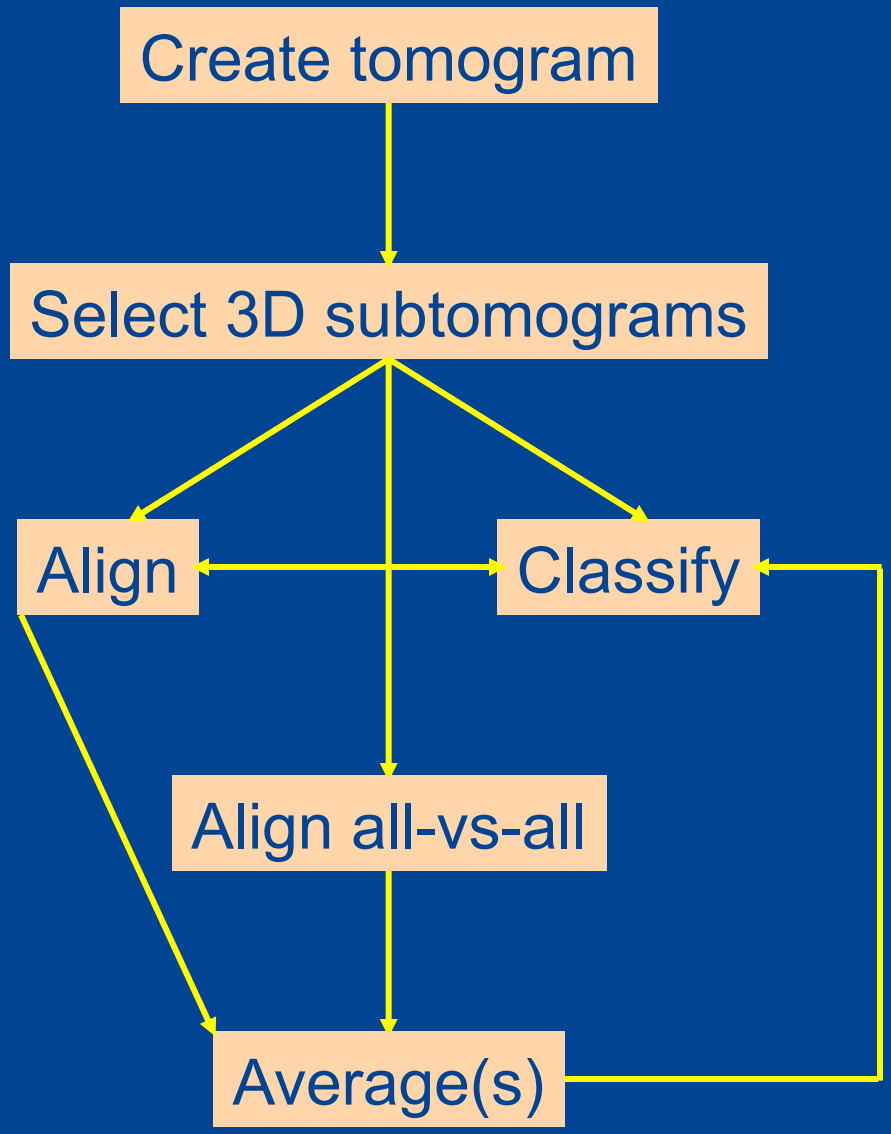
Single Particle Tomography

Michael F. Schmid

The National Center for Macromolecular Imaging

National Center for Research Resources

Flow Chart for processing subtomograms



Key Points/Concepts

Effect of the Missing Wedge

Image considerations

filtering, masking

All-vs-all alignment as an option

Optimizing search parameters for efficiency

Examples

carboxysome, herpes virion, trypanosome flagella, small RNA

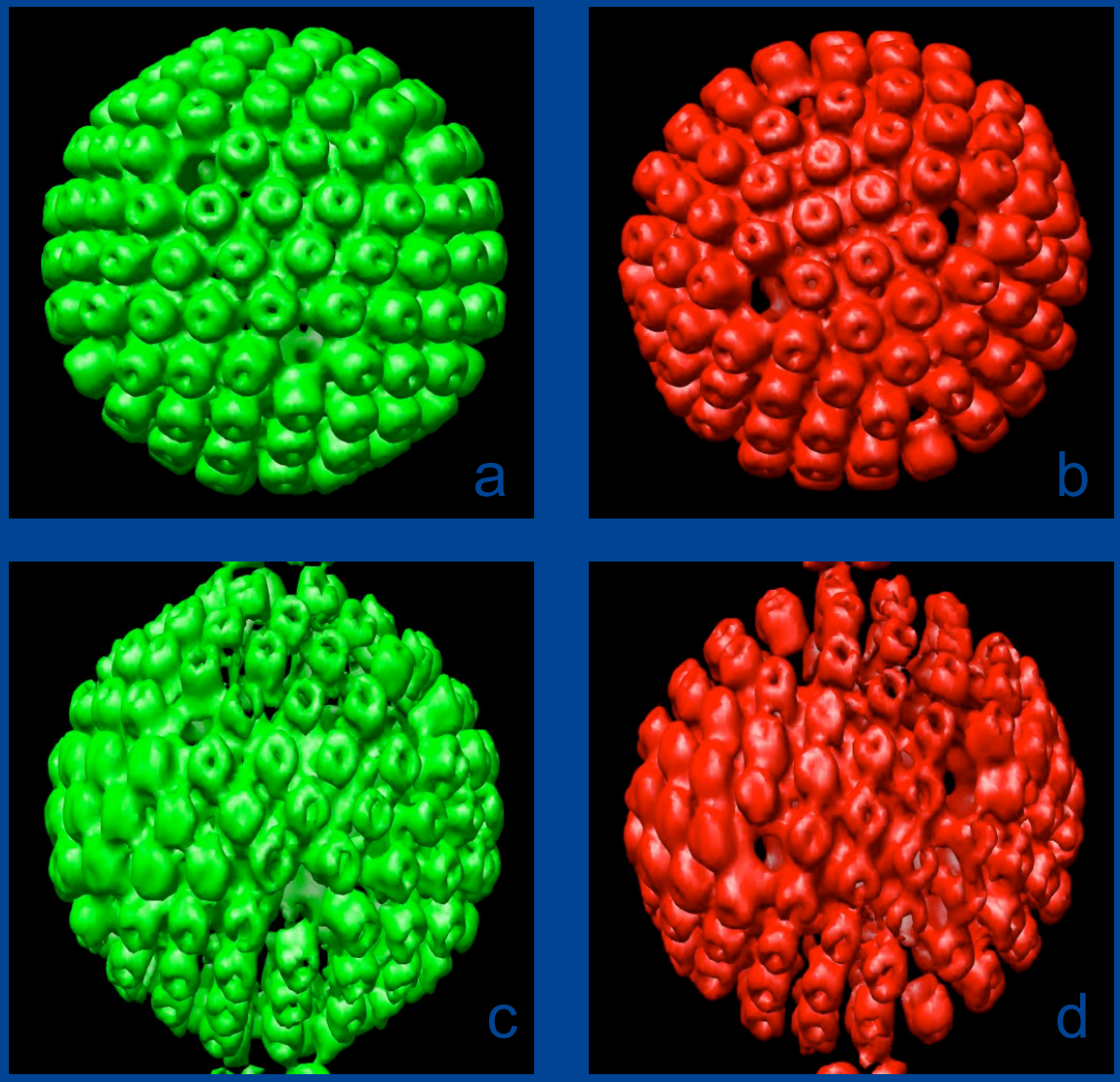
Effect of the missing wedge

- Tomographic data is limited to ±70˚ max tilts
- Distorts the reconstructions
- Makes mutual alignment difficult

Demonstration of the effect of the missing wedge

No missing wedge

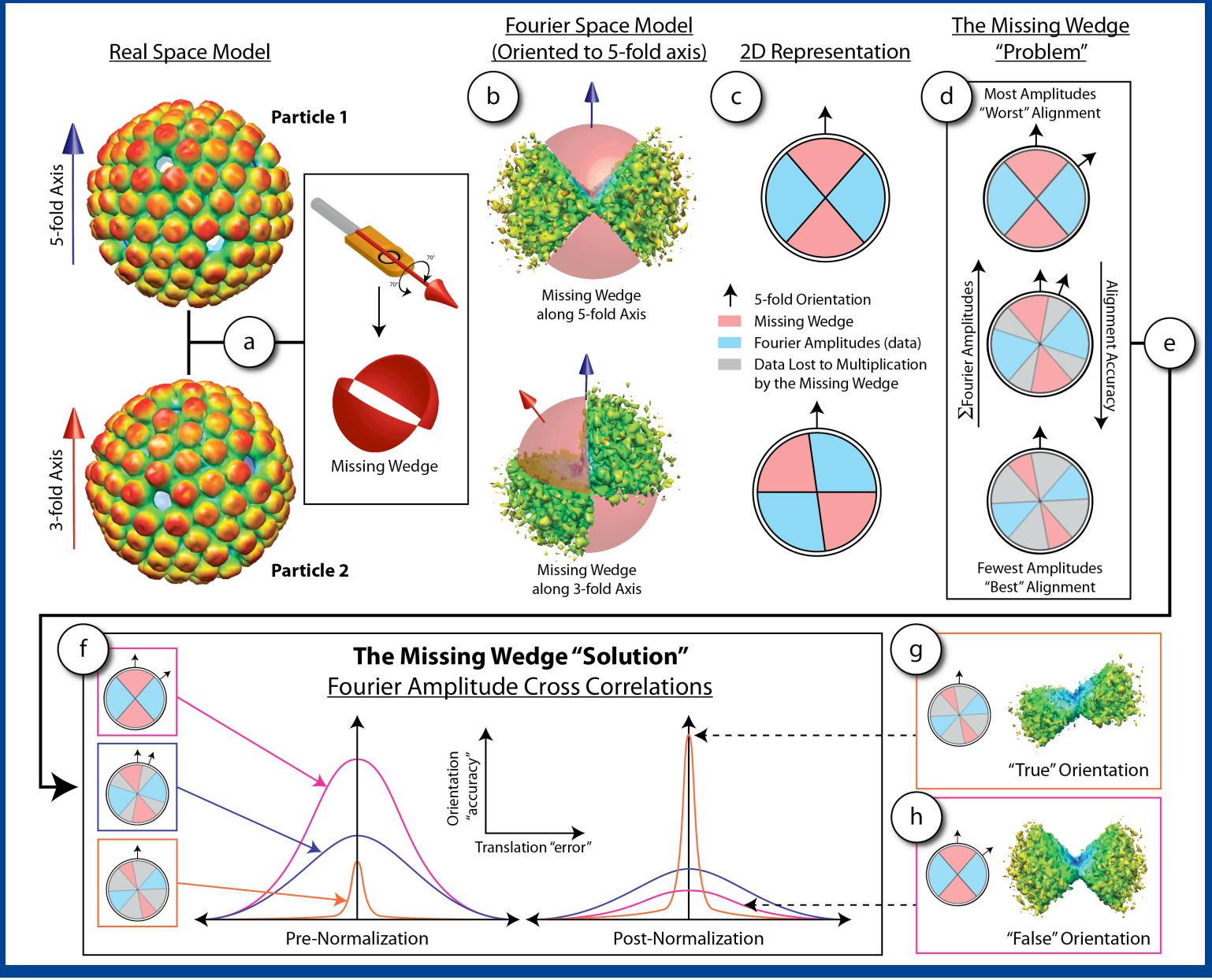
40% missing wedge (Equivalent to ±54˚ tilt)



5-fold vertical

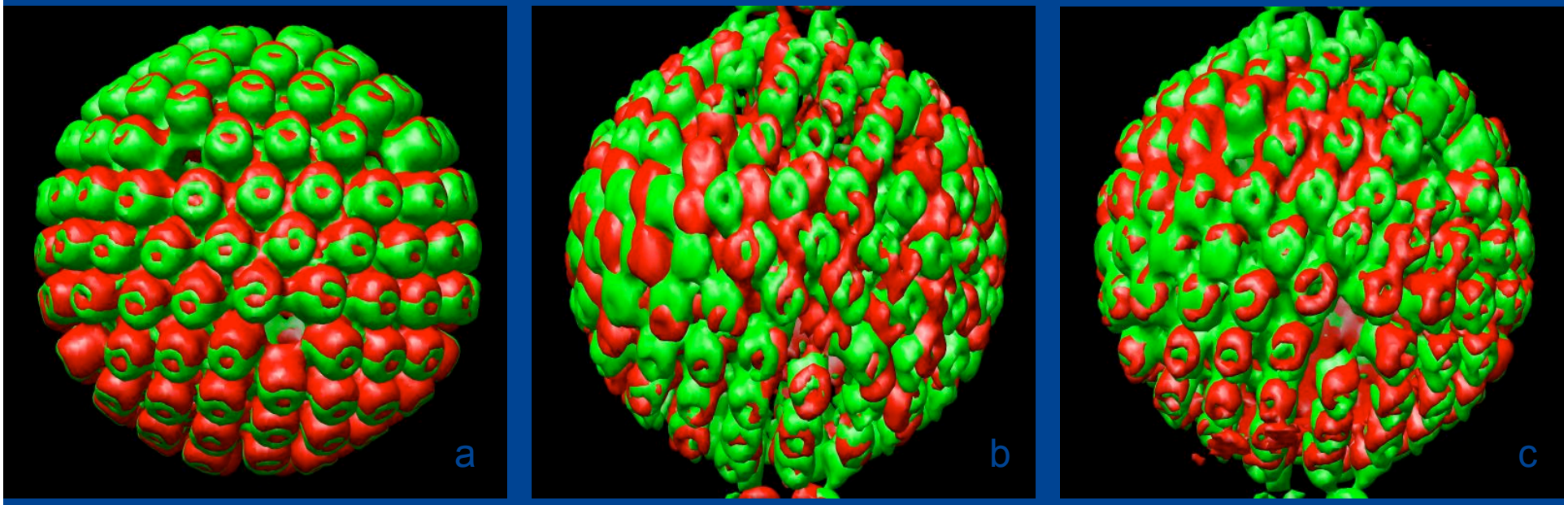
3- fold vertical

The Missing Wedge



Mutual alignment of a 3-fold oriented map to the 5-fold for a ±54˚ tilt series

(exact correct answer = 37.72˚, 18˚, -18˚)



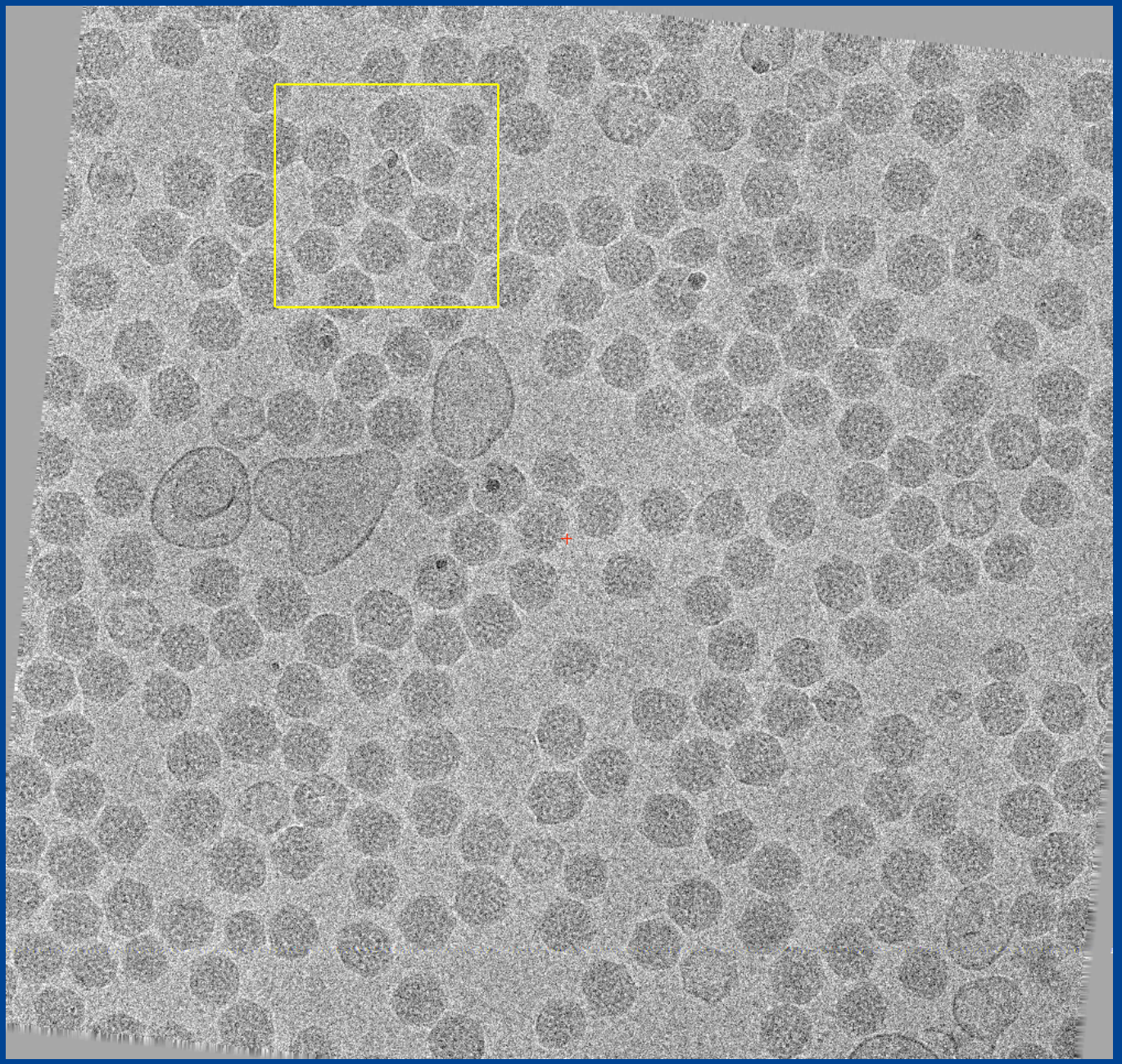
Alignment (5˚ step size) of 3-fold maps to 5-fold maps
a- No Missing wedge (40˚, 20˚, -20˚ - RIGHT (to within 5˚ step size))
b- Missing wedge without compensation (5˚, 15˚,-15˚ - WRONG)
c- Missing wedge with compensation (40˚, 20˚, -20˚ - RIGHT)

Image Preparation Considerations

1. Contrast-flipping, initial rotation and hand-choice
   - Reconstruction is usually contrast-reversed
   - Some reconstructions do not have missing wedge along z
   - Some reconstructions have hand-flip
2. Filtering
   - Subtomograms are inherently low-res and high-noise, so alignment should not be overly optimistic (~40Å lowpass is appropriate)
   - Large-scale features of the object may allow even more aggressive filtering for initial alignment
3. Normalization
   - Useful for classification based on cross-correlation peak values
4. Masking
   - Spherical Zero mask insures correct “fill-in” function for rotated volumes
5. Always carry out resulting operations (rotation and translation) on the ORIGINAL files, not the low-passed, etc. versions!
6. Ultimate goal is to keep track of all transformations to be able to re-insert the average into the tomogram at the orientation of each instance of the structure

Carboxysome

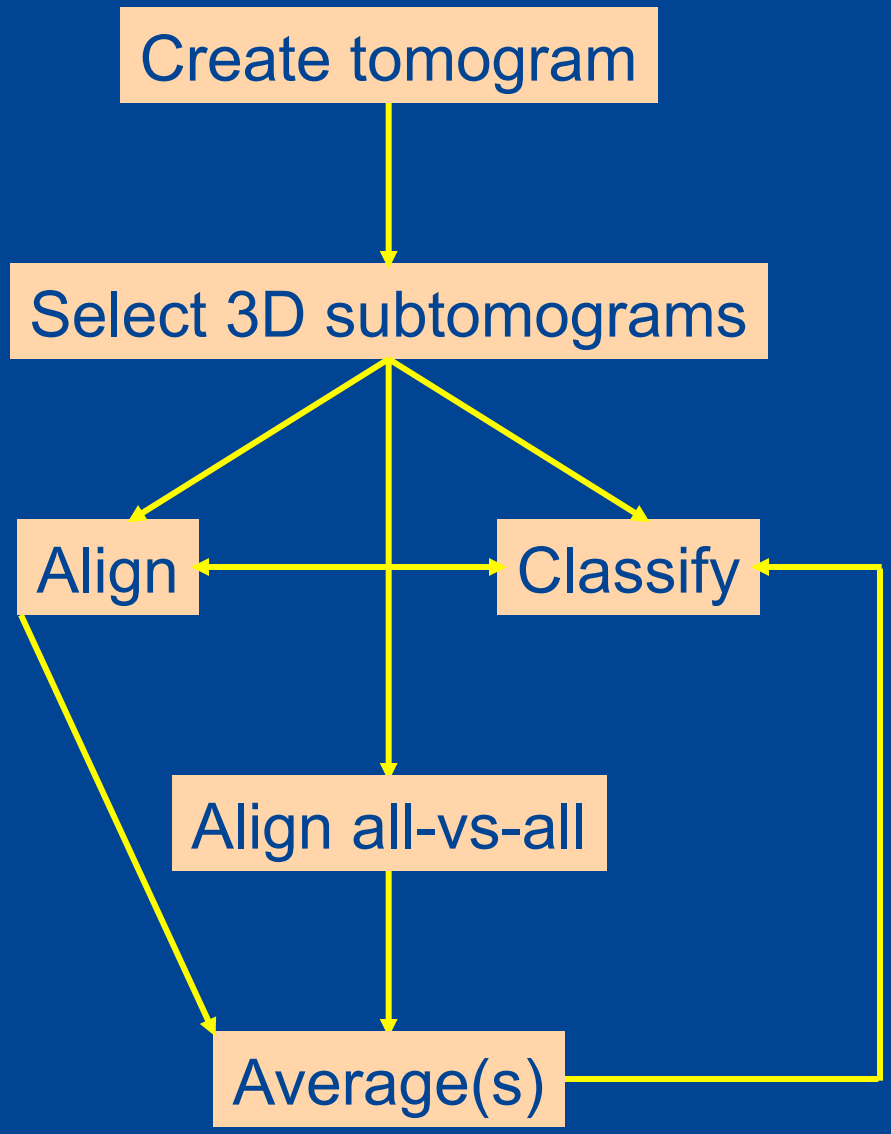
- Found in photosynthetic and chemoautotrophic bacteria
- "Polyhedral" bodies - ~100 nm diameter, thin angular shell, granular interior
- Contain RuBisCO - fixes CO_2
- Regulated
- Size, shape, symmetry of carboxysome and arrangement of RuBisCO unknown
- What if we want to inventory macromolecular machines in the cell if we don't know much about them *a priori*



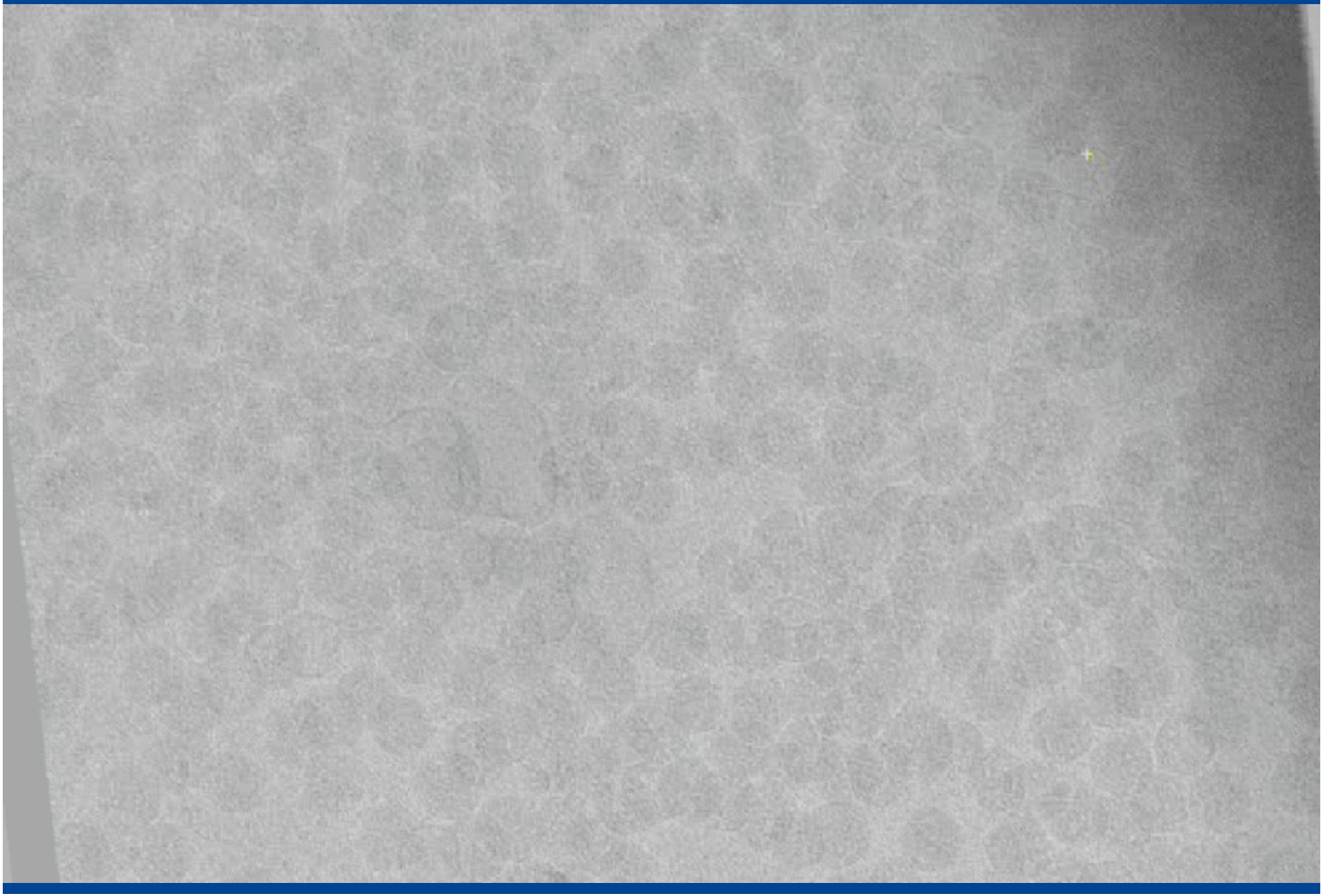
Approach

- Conventional single particle processing with icosahedral symmetry using common lines did not work
- Our approach - averaging 3D subvolumes extracted from tomograms (subtomograms)
  - but subtomograms have a missing wedge in Fourier space the same shape as the missing wedge of the entire tomogram
- In the literature of post-tomographic averaging, subtomograms have been aligned against a 3D model template which does not have a missing wedge
- Size heterogeneity and unknown symmetry make it difficult to choose a starting model
- Therefore we chose to mutually align subtomograms to each other

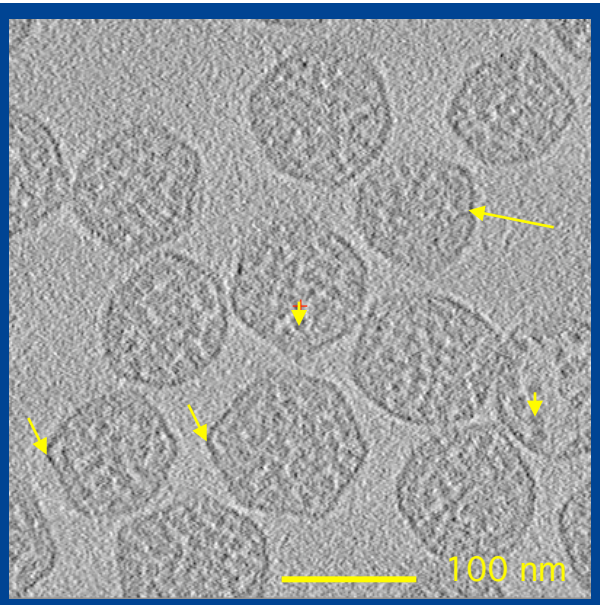
Flow Chart for processing subtomograms

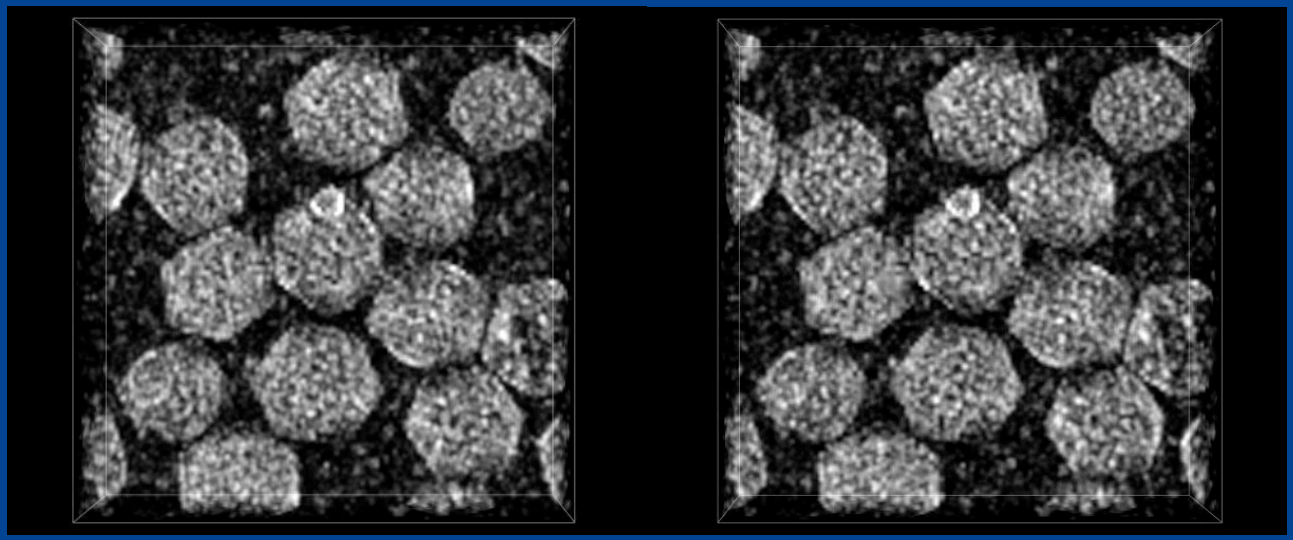


Halothiobacillus carboxysome tilt series

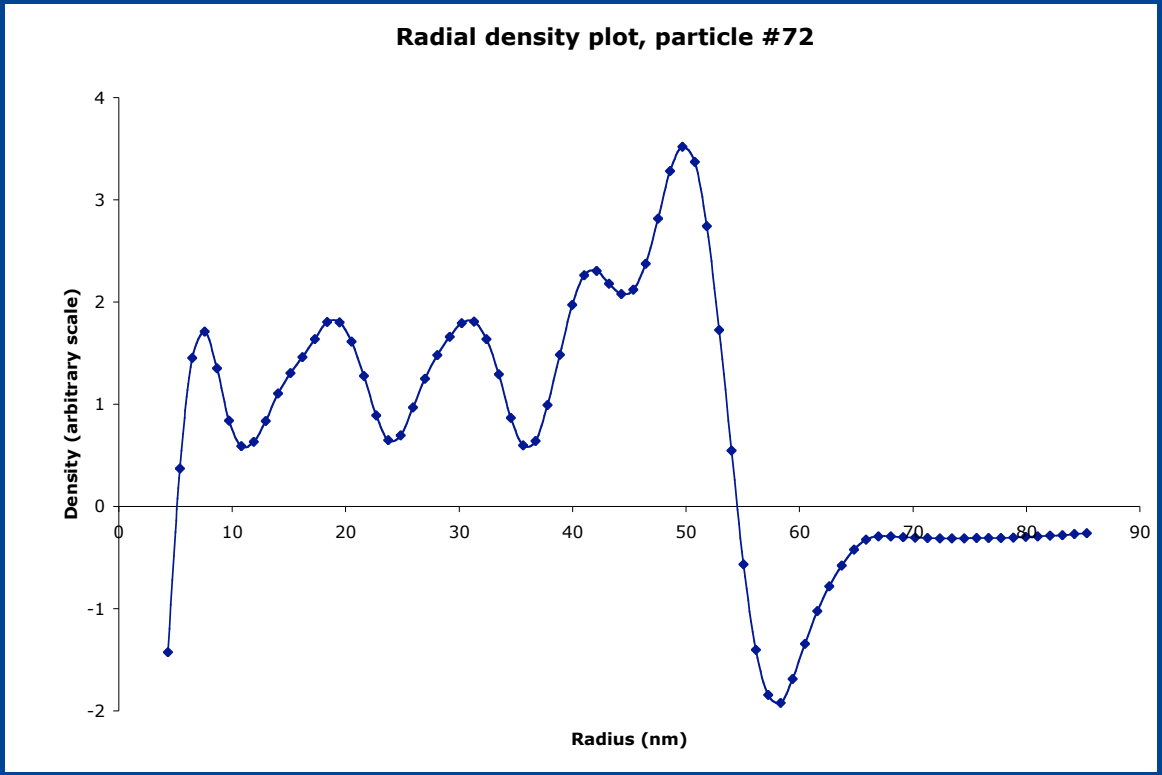


Tomographic reconstruction

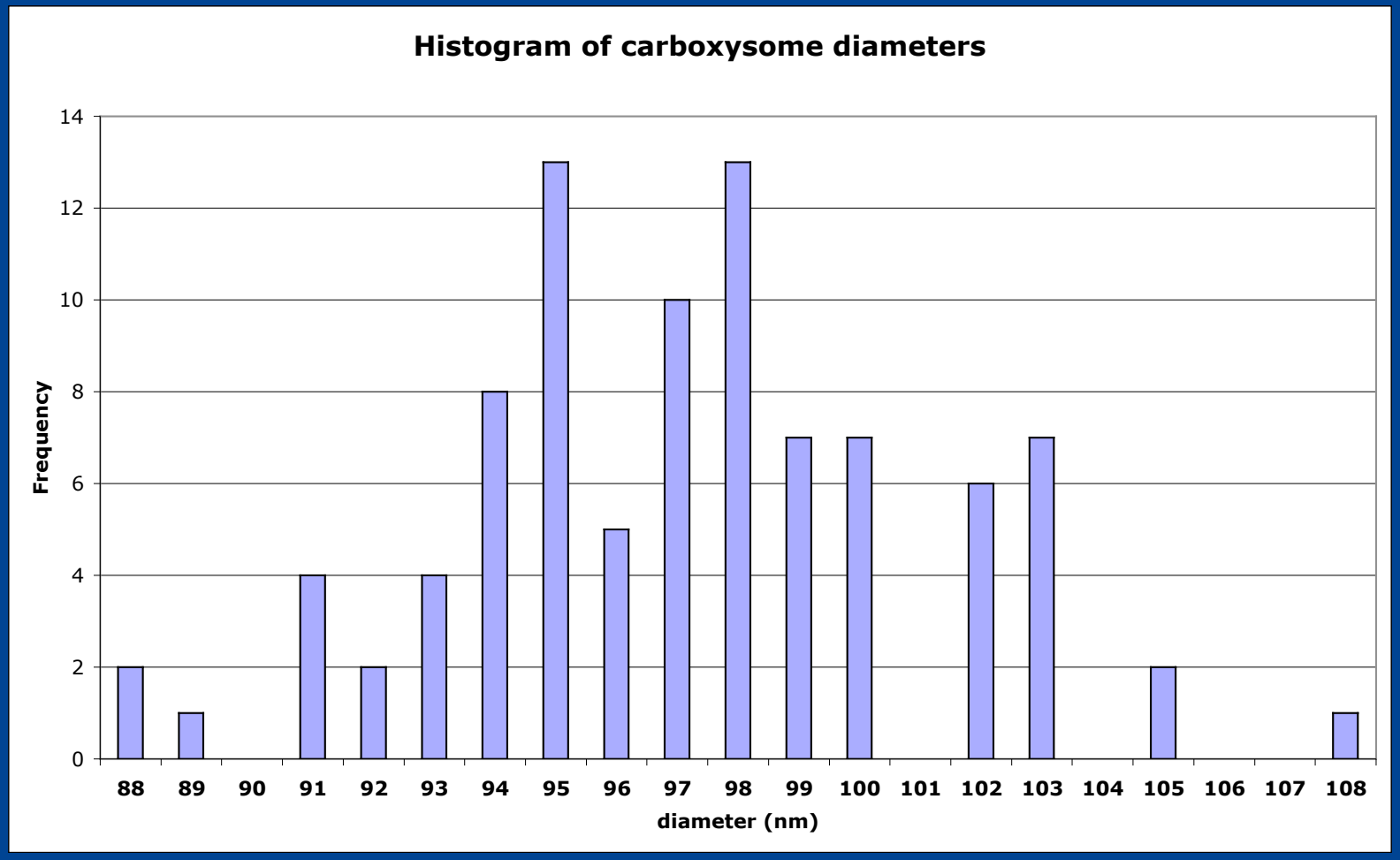

100 nm



Plot of density for 1 of 92 3-D Volumes



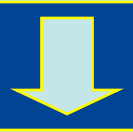
Carboxysomes have size heterogeneity



Reference-free 3-D alignment and averaging

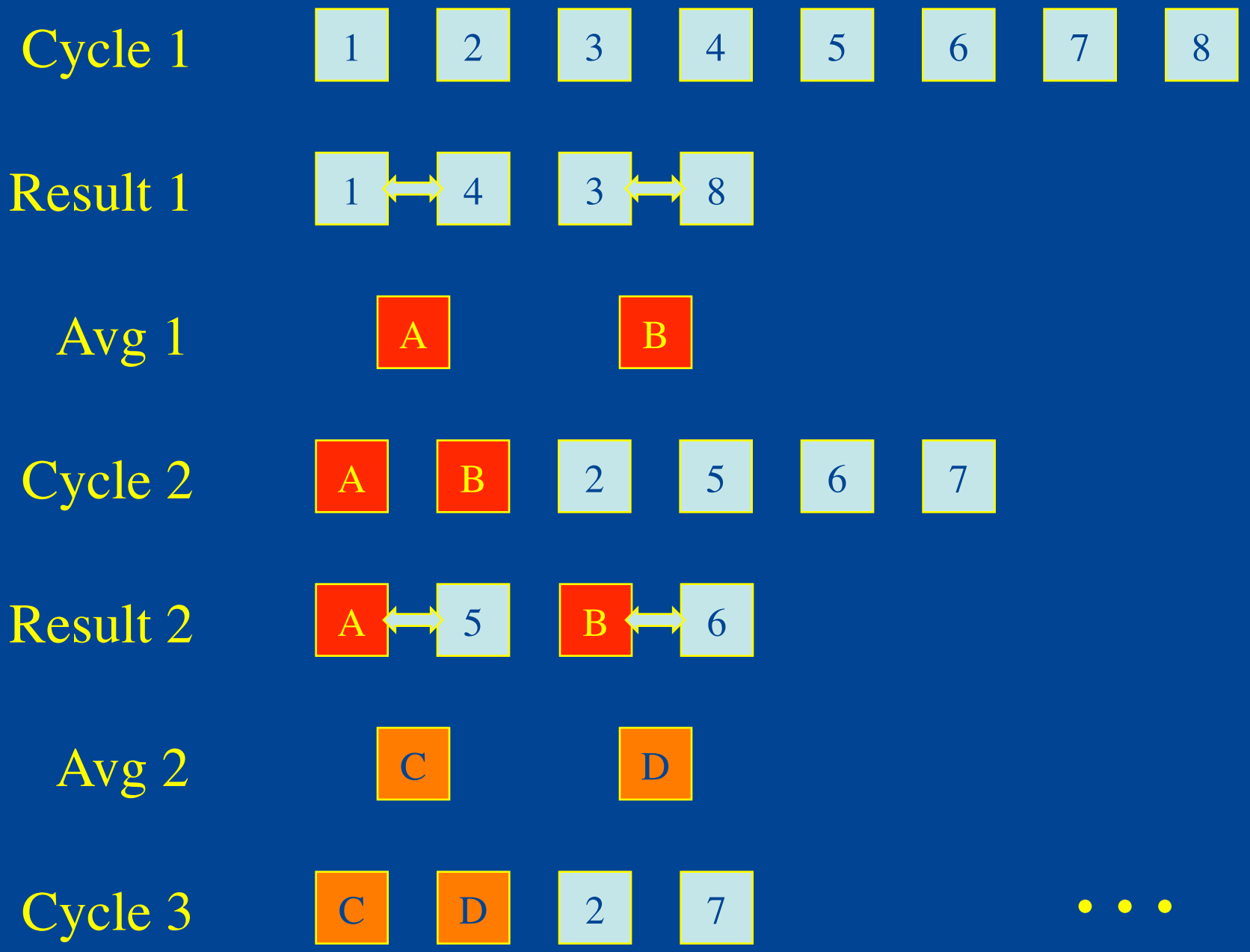
Roughly split 3-D subvolumes into 9 diameter classes

All-vs.-all mutual cross-correlation orientation alignment within each class, and also with the next larger and smaller diameter classes, shifting if necessary

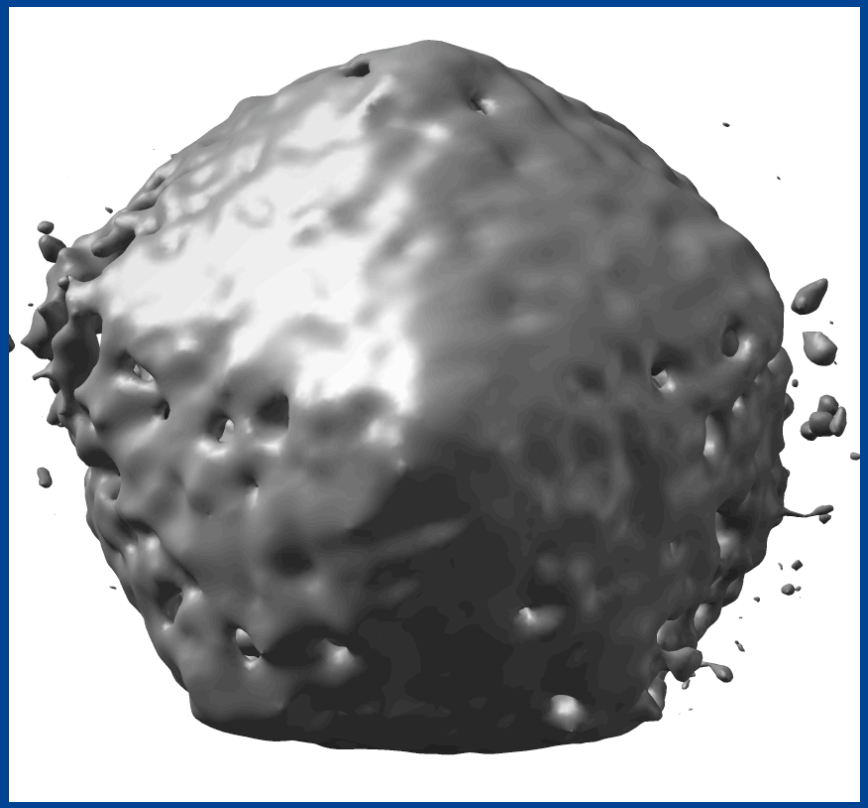


Average best pairs of alignments
These replace the original data pairs in new all-vs-all round

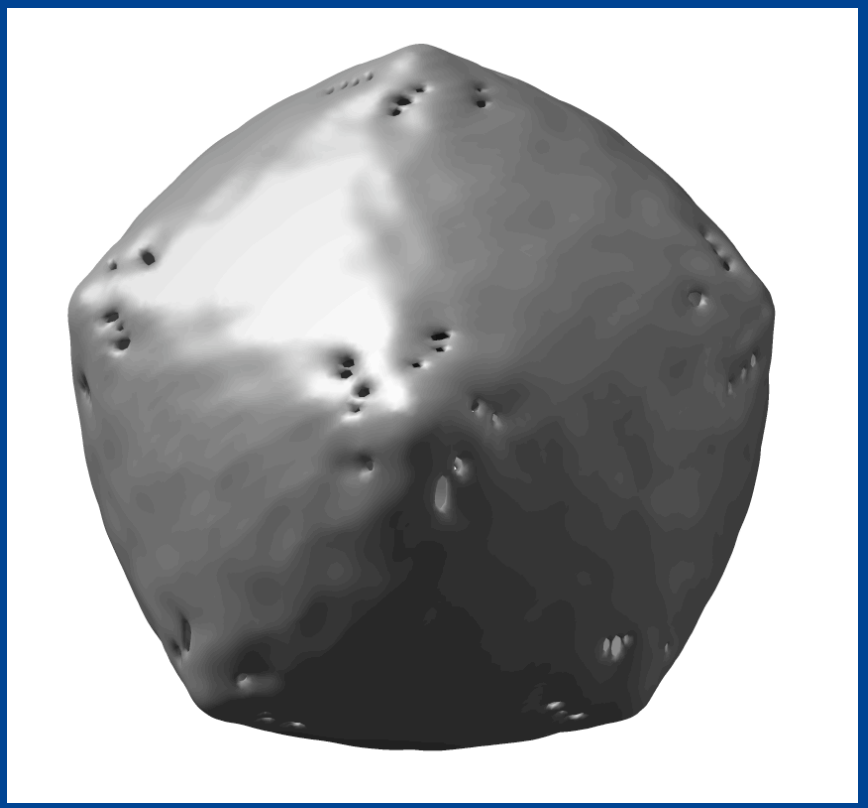
Schematic


Cycle 1
1
2
3
4
5
6
7
8
Result 1
1
4
3
8
Avg 1
A
B
Cycle 2
A
B
2
5
6
7
Result 2
A
5
B
6
Avg 2
C
D
Cycle 3
C
D
2
7
• • •

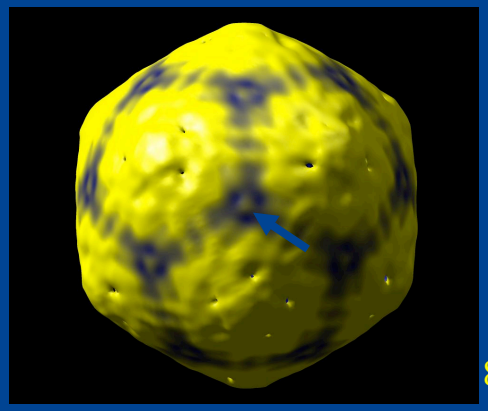
Result of all-vs.-all mutual cross-correlation searching and averaging in size classes



Tomographic averaging - 100nm class - 20 particles

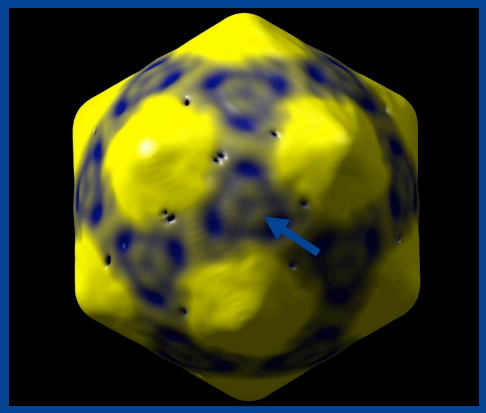


Tomographic plus icosahedral averaging



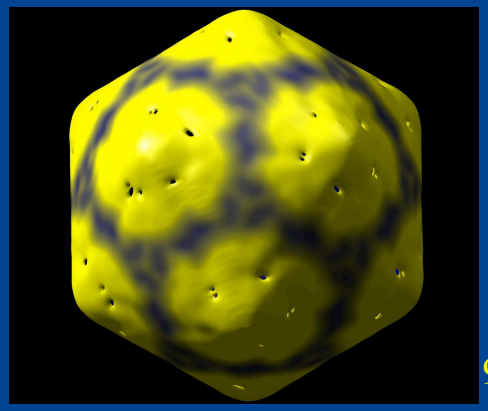
a

88nm



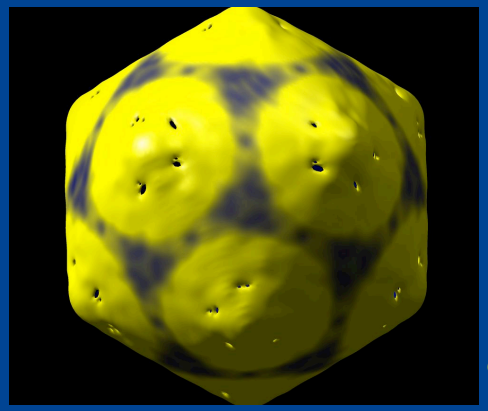
b

91nm



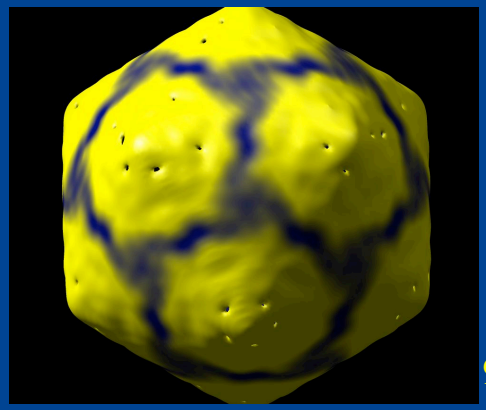
c

93 nm



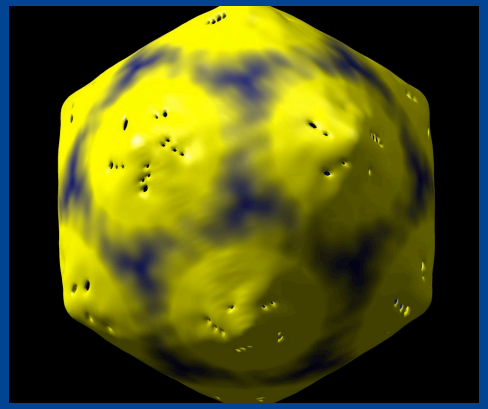
d

95 nm



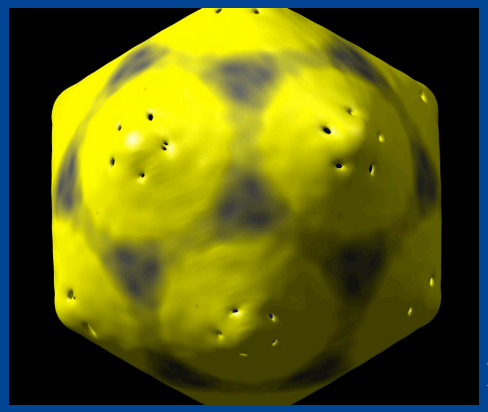
e

97 nm



f

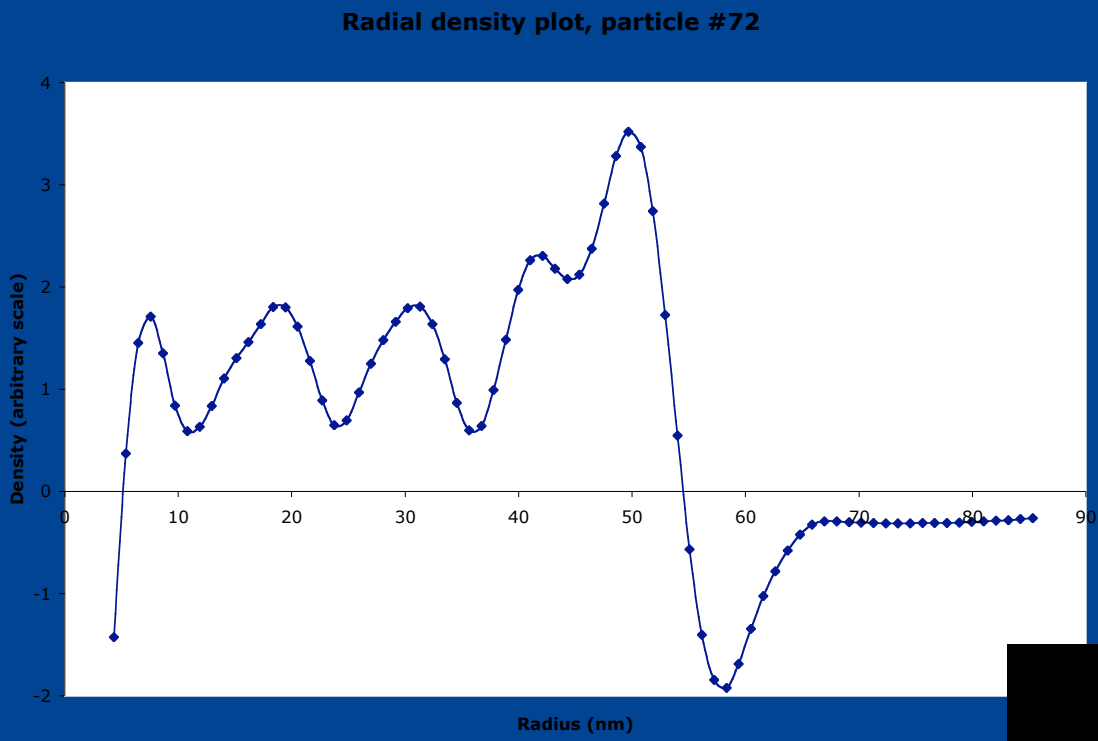
100 nm

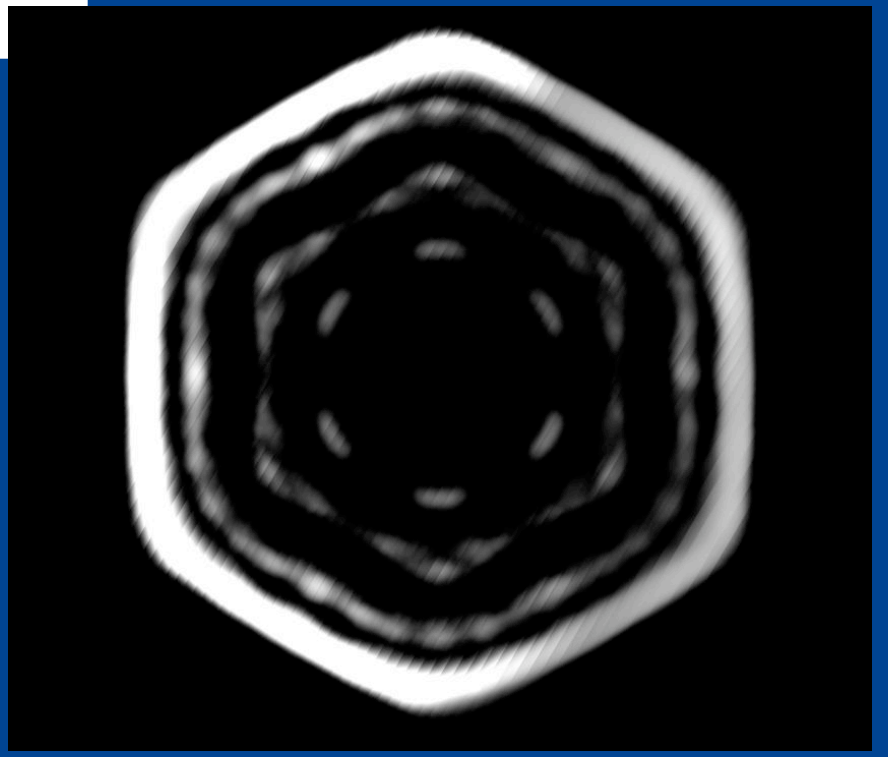


g

103 nm

Size classes





Central slice of averaged particle - 100nm class shell of average is higher density, interior densities do not have icosahedral symmetry.

Conclusions

- Shell symmetry is icosahedral
- Size of carboxysome varies from 88 to 103nm - unusual for an icosahedral particle
- Shell protein arrangement varies with size
- RuBisCO organization in layers inside, but not regular, nor constant amount per particle

- Specialized processing needed for determining mutual orientation and for averaging of particles with missing wedge

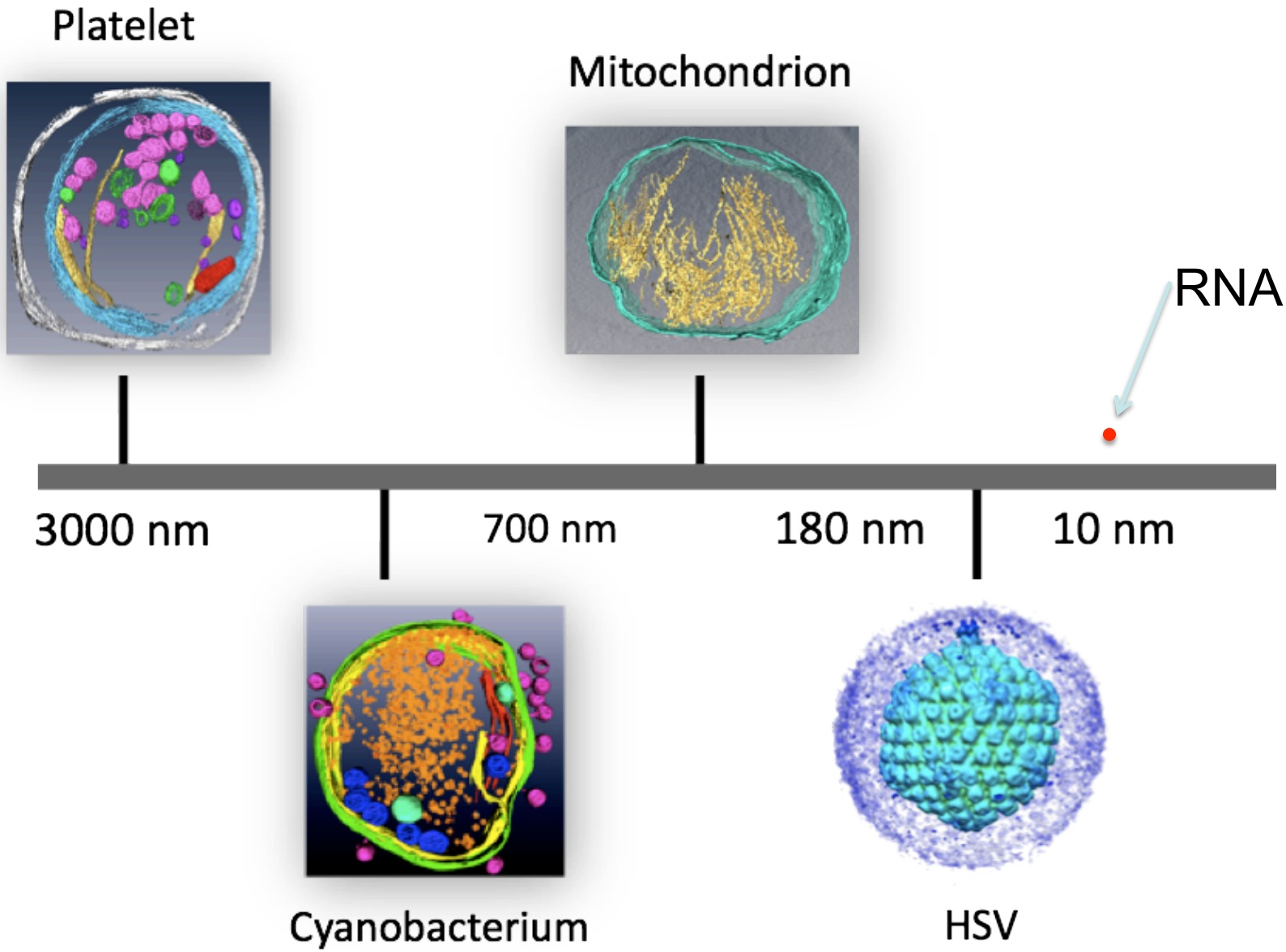
- Schmid et al. (2006) J. Mol. Biol. (Angel Paredes, J. Shively)

MMLV RNA NMR & CryoET

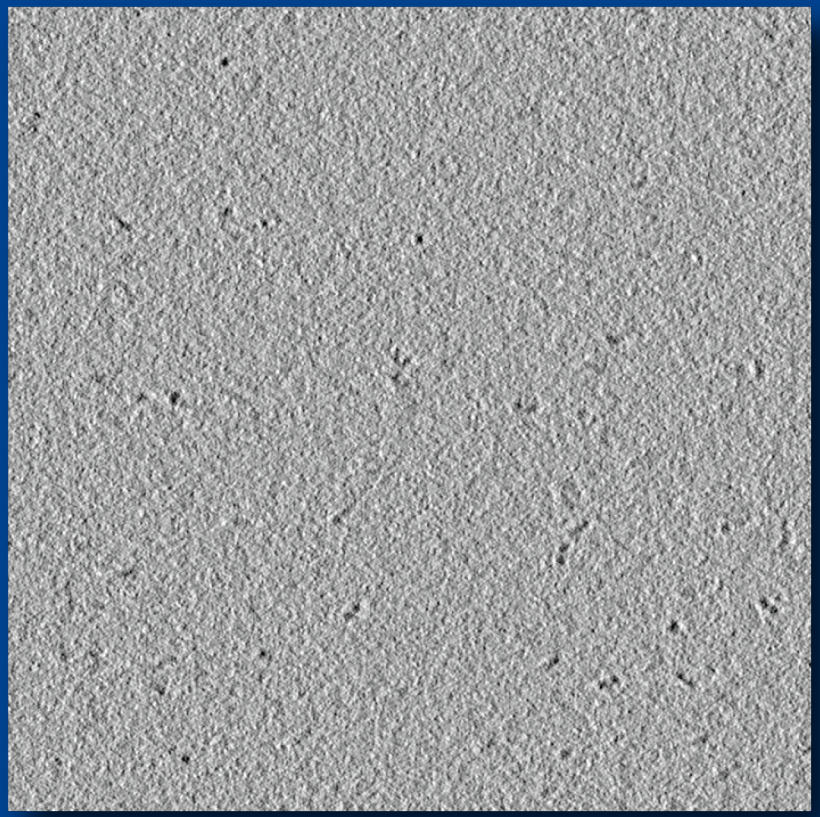
Rossi Irobalieva, NCMI

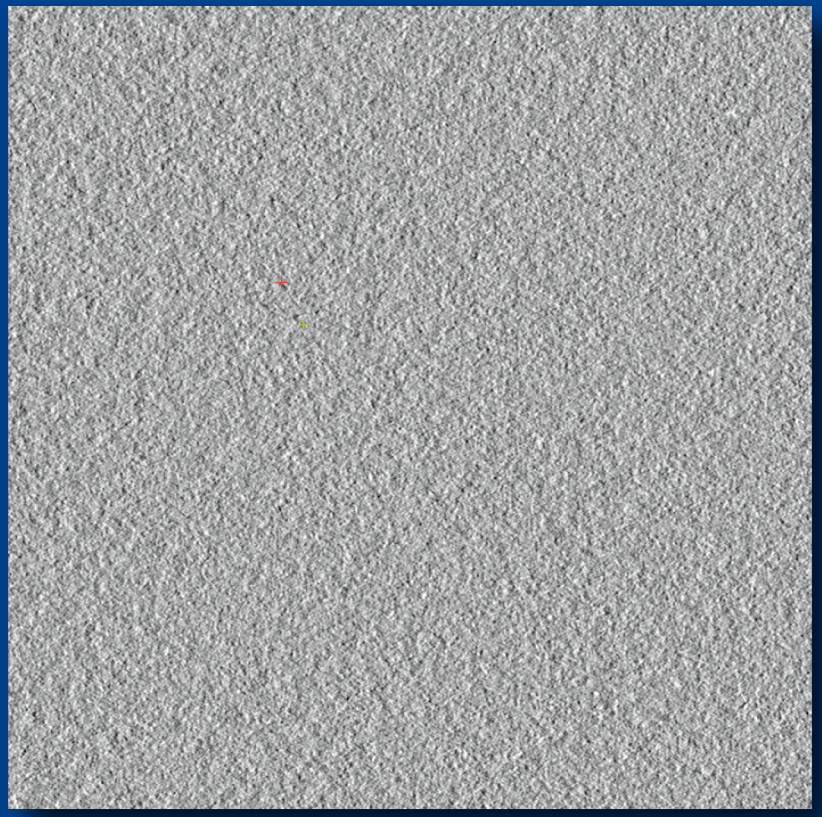
Michael Summers, UMBC

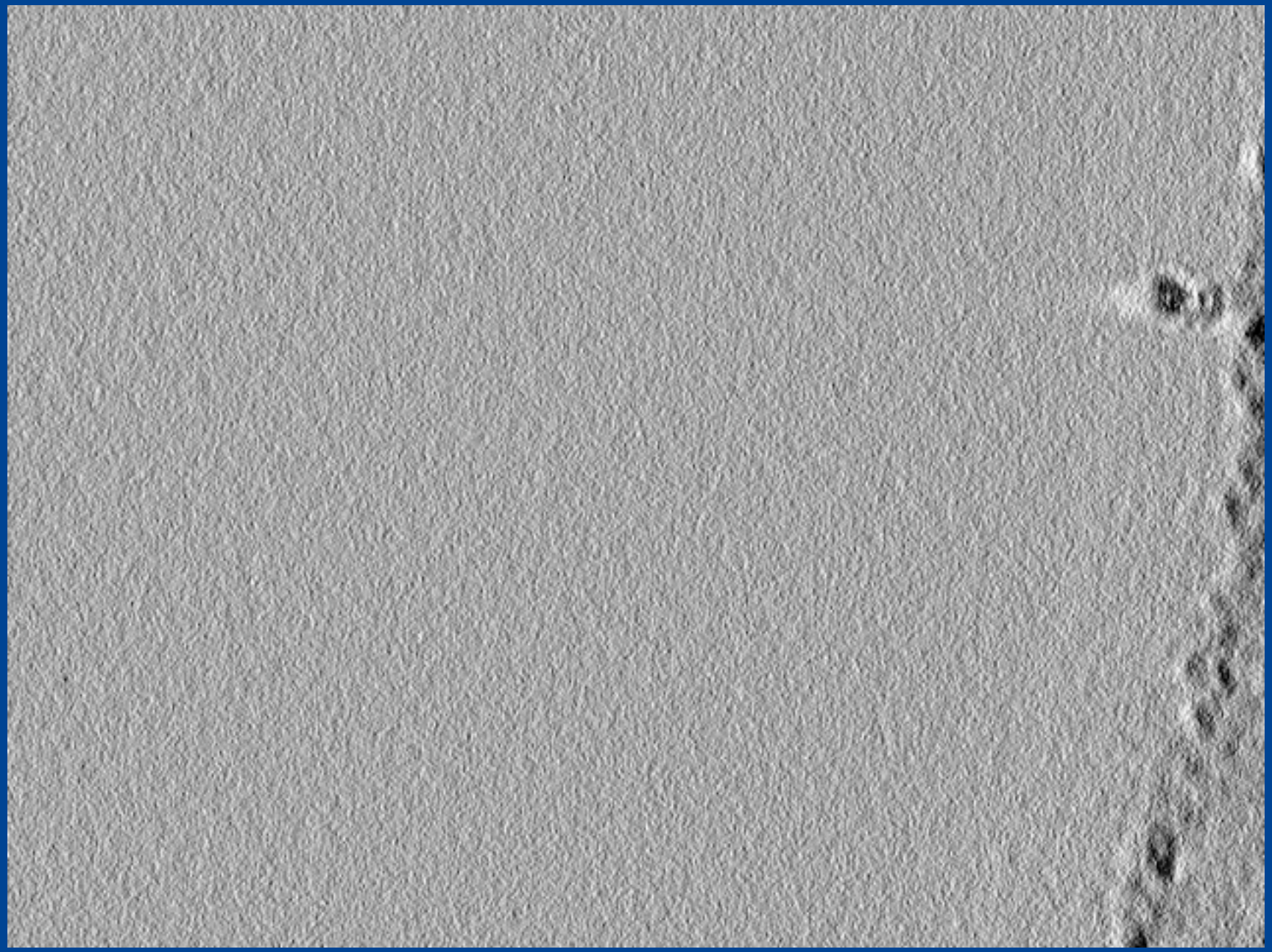
Small ssRNA segment
Each segment forms 2 hairpin loops.
Dimerizes.
Total 132nt, 42.8kDa, essential and conserved in retroviuses, eg. HIV

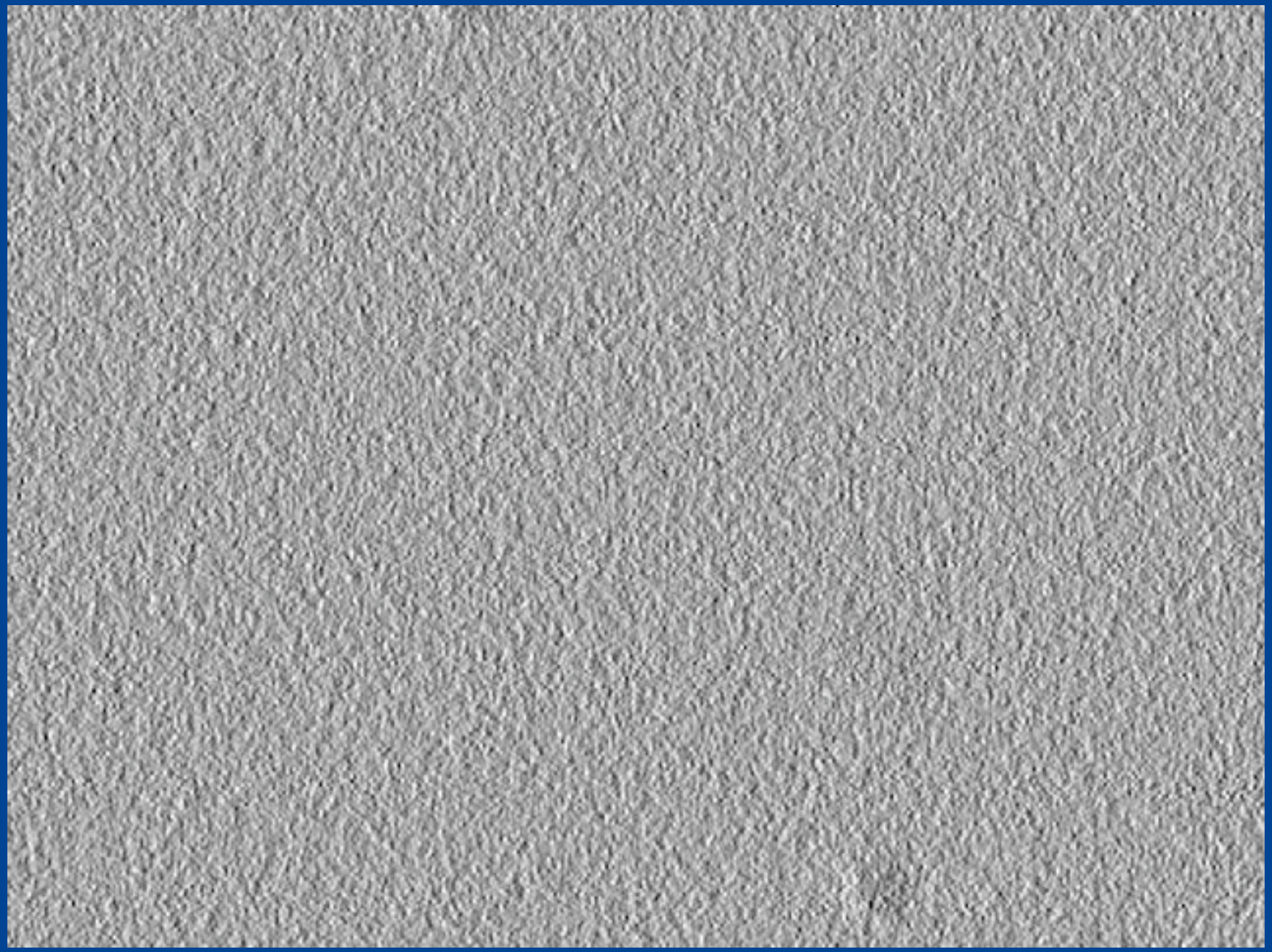

Platelet
Mitochondrion
RNA
3000 nm
700 nm
180 nm
10 nm
Cyanobacterium
HSV

Stack of Slices through tomograms of RNA (left) and buffer (right)

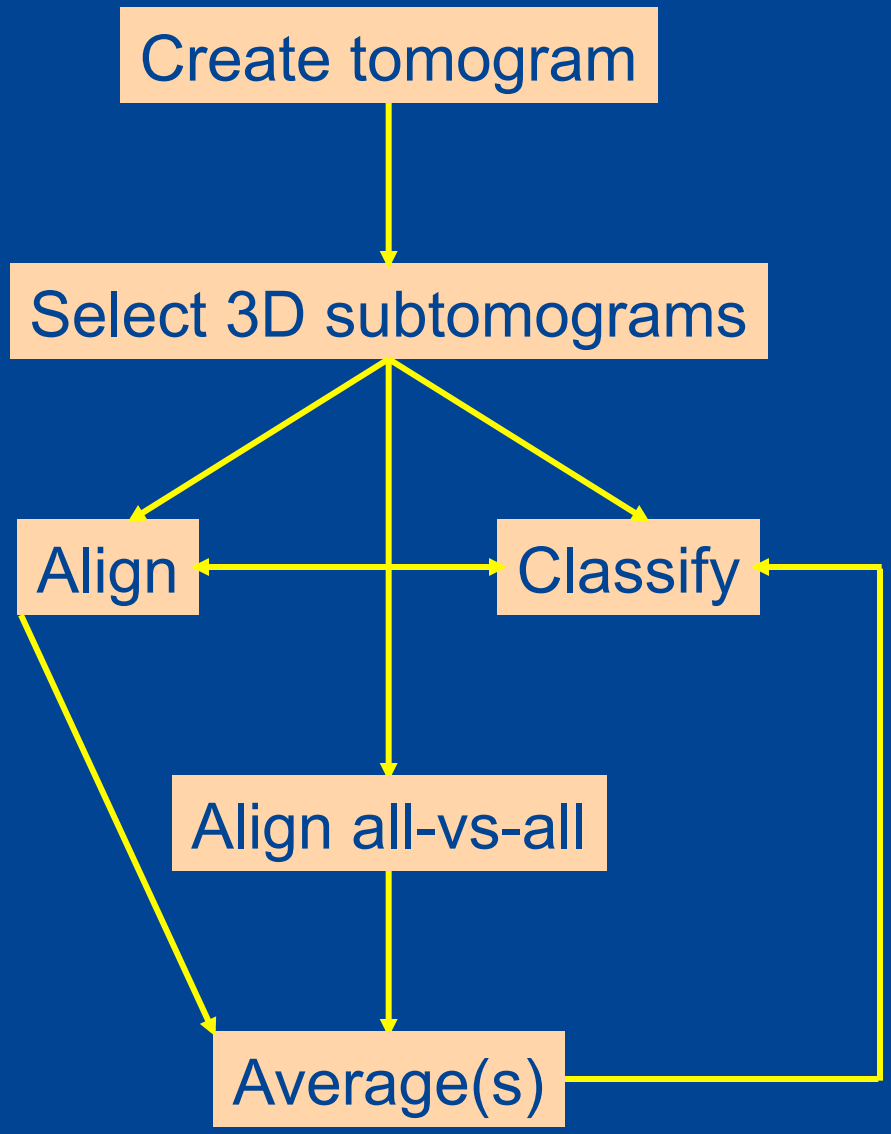


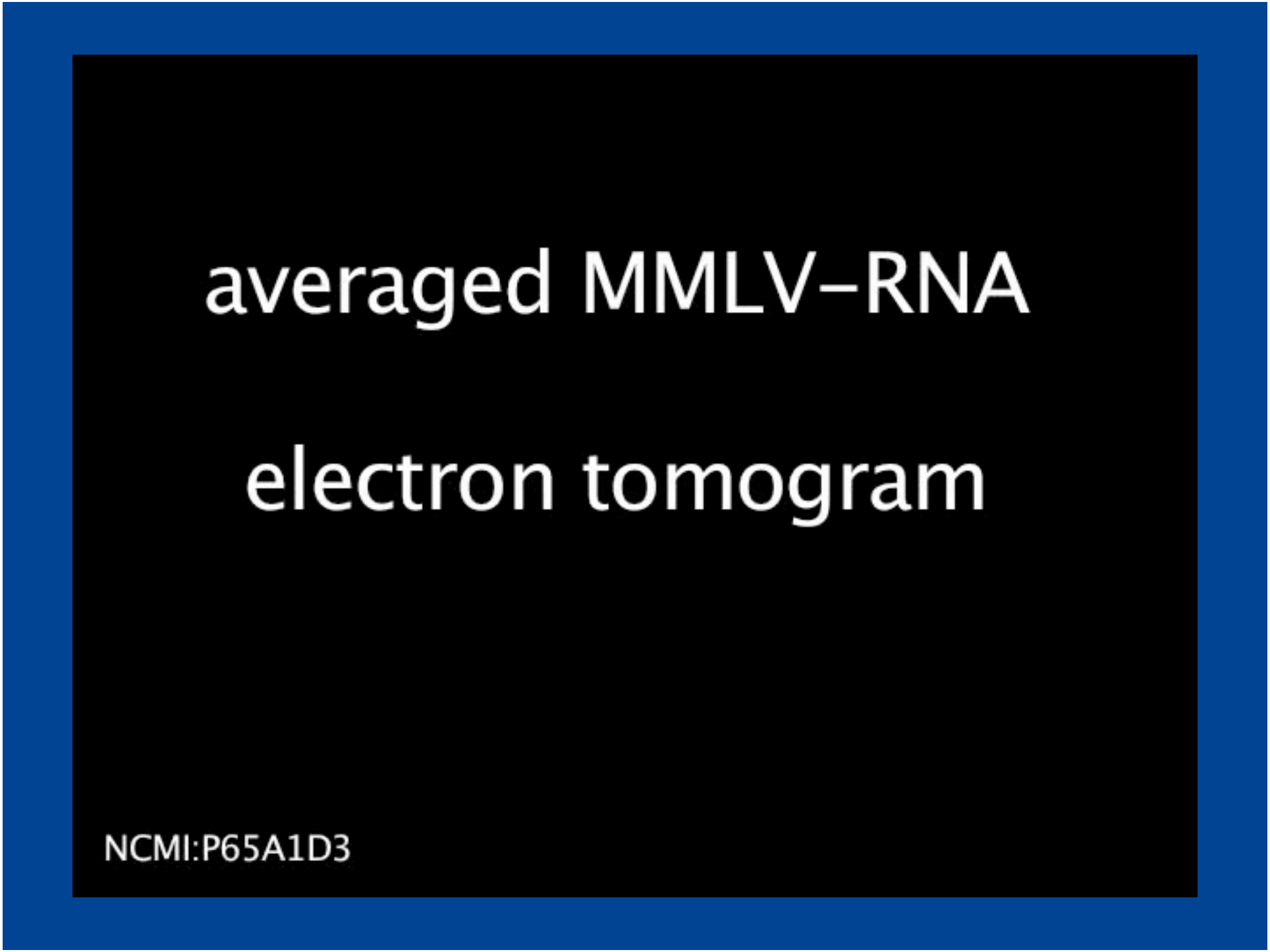




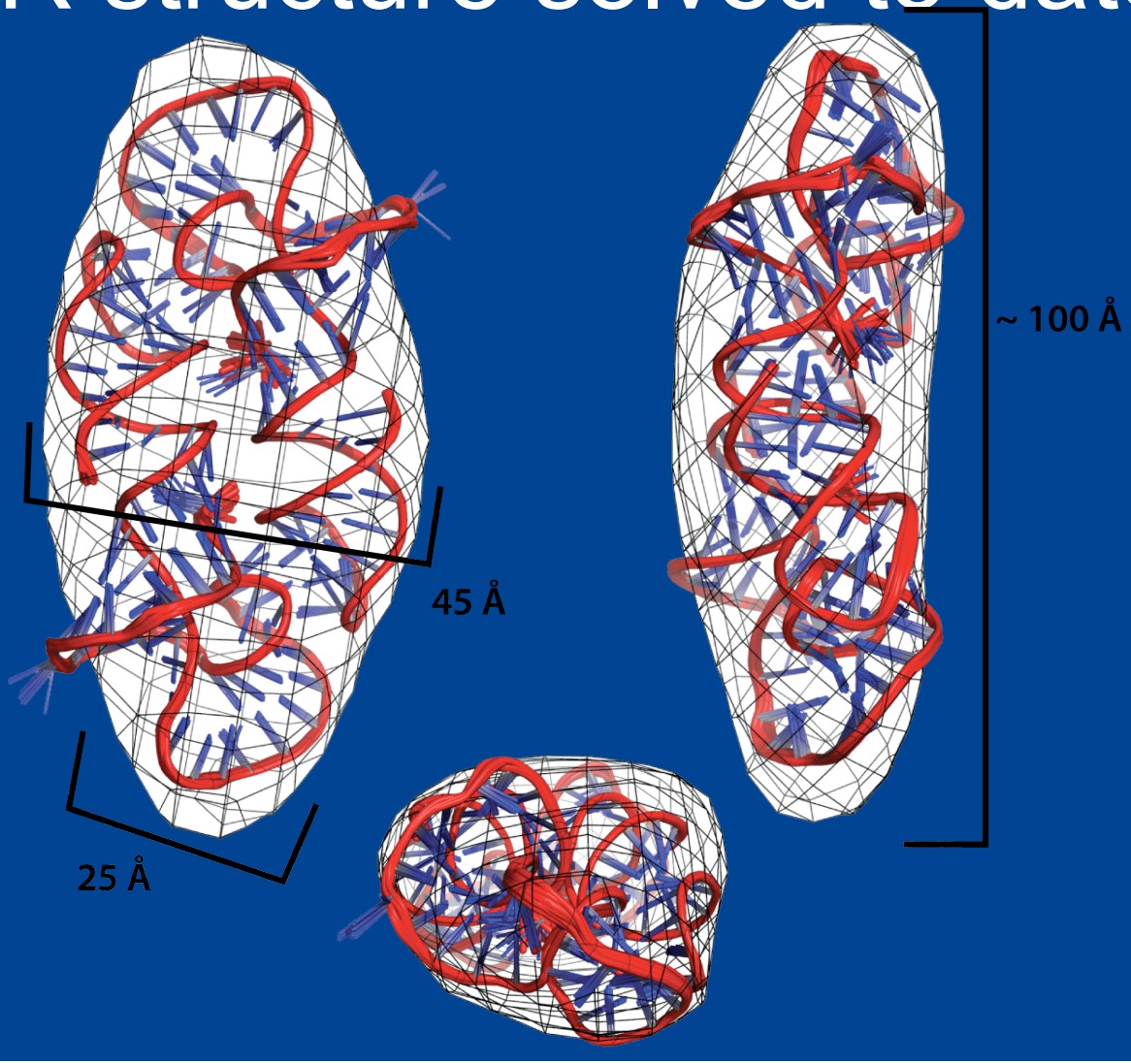


Flow Chart for processing subtomograms




averaged MMLV-RNA
electron tomogram
NCMI:P65A1D3

Smallest cryo-EM and largest NMR structure solved to date



Combine tomographic and single-particle approaches

Low resolution tomographic reconstruction provides 3D model(s) for alignment, classification, etc. of single particle projection images

Conclusion

Classification, alignment and averaging of tomographic subvolumes has led to biological insights into macromolecular complexes and machines