

cryo-EM Study of Mm-Cpn and human γ D-crystallin

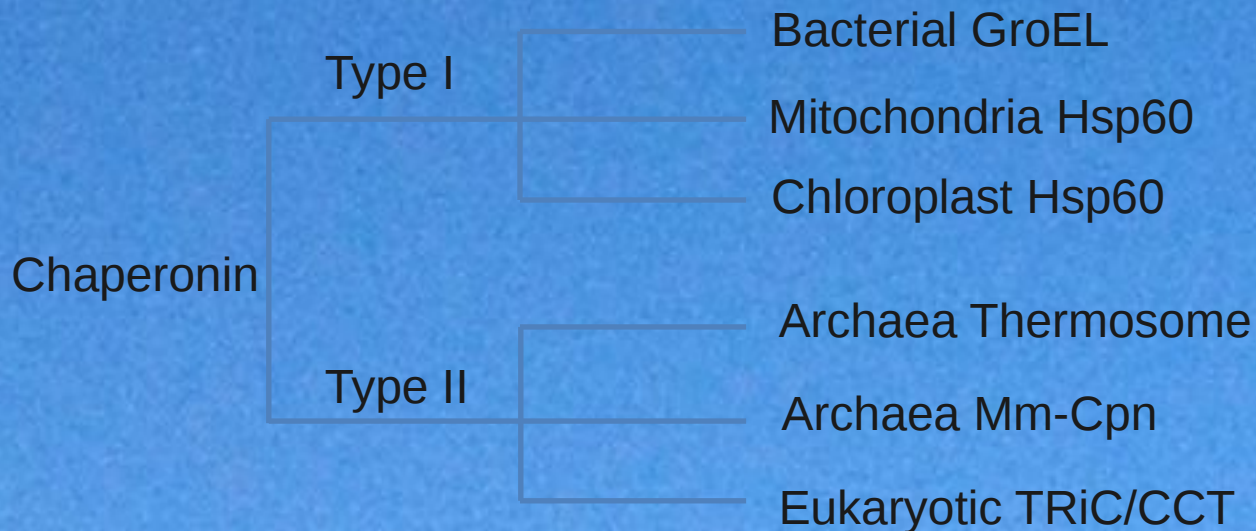
Bo Chen

Biochemistry and Molecular Biology Department
— Baylor College of Medicine

NRAMM Workshop
Nov. 15th, 2012

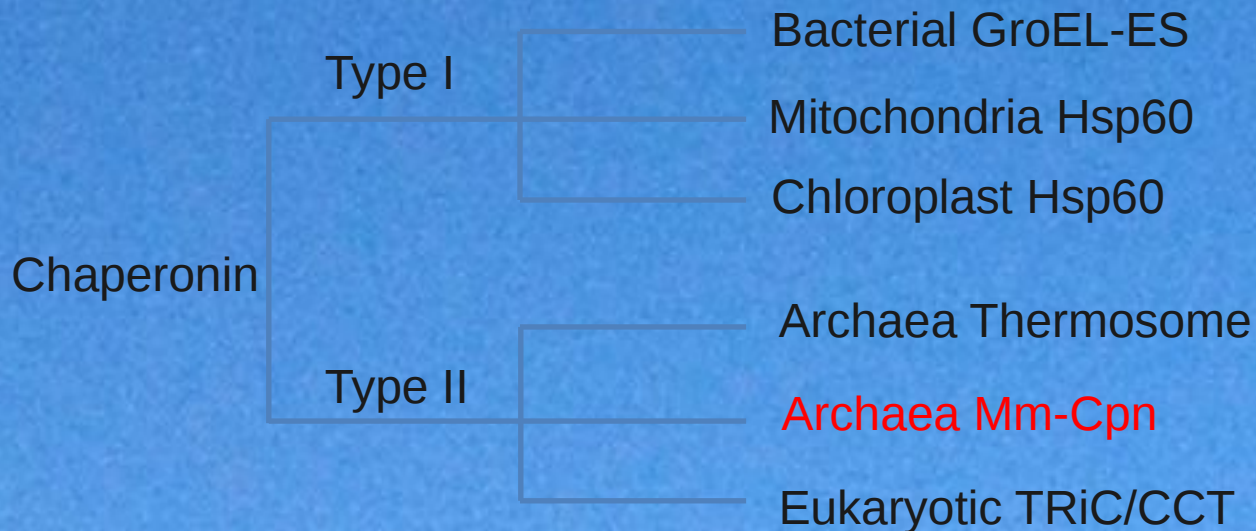
Protein Folding Machines

- Cells employ a cassette of chaperones to assist protein folding
Hsp40, Hsp60, Hsp70, Hsp90, Hsp100
- Hsp60 family is also called chaperonin.
- Chaperonin family is divided into type I and type II.



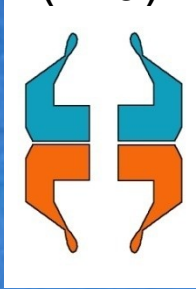
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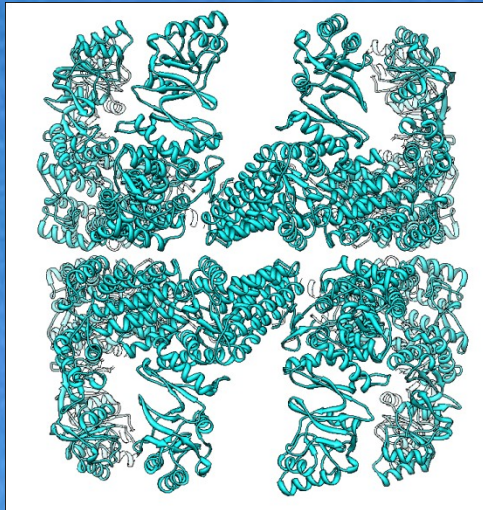


Cryo-EM Structures of Mm-cpn

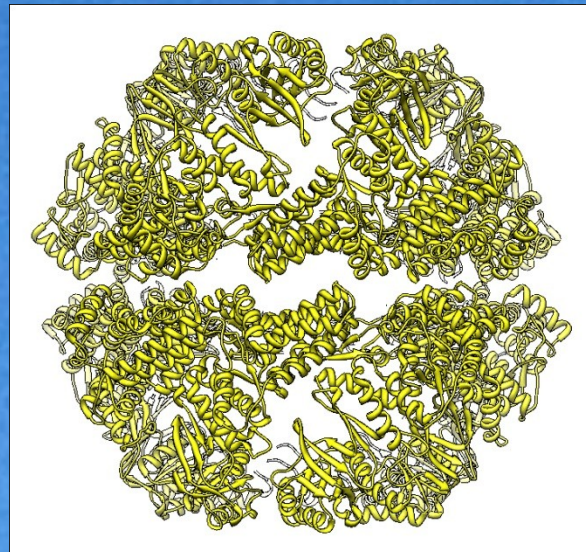
ATP-free
(Δlid)



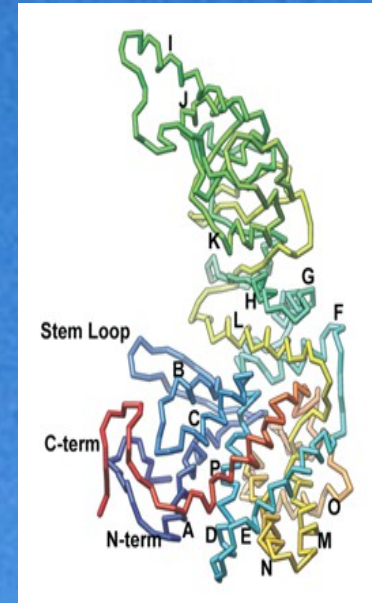
Post ATP-hydrolysis
Transition (Δlid)
(Using ATP/AlFx)



8.0 Å



4.7 Å



HyD-crystallin : substrate of Mm-Cpn

- A 20 kDa protein with two domains
- Is essential for maintaining lens transparency
- Aggregation of misfolded HyD-crystallin is responsible for the onset of cataract
- Aggregation can be suppressed by Mm-Cpn
- Refolding can occur with Mm-Cpn and ATP

Experimental Conditions

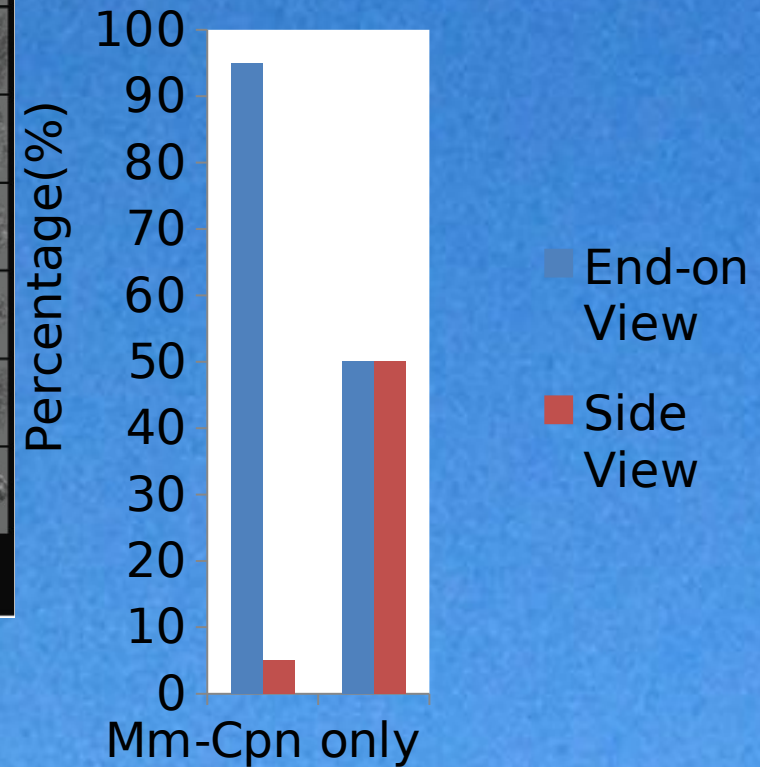
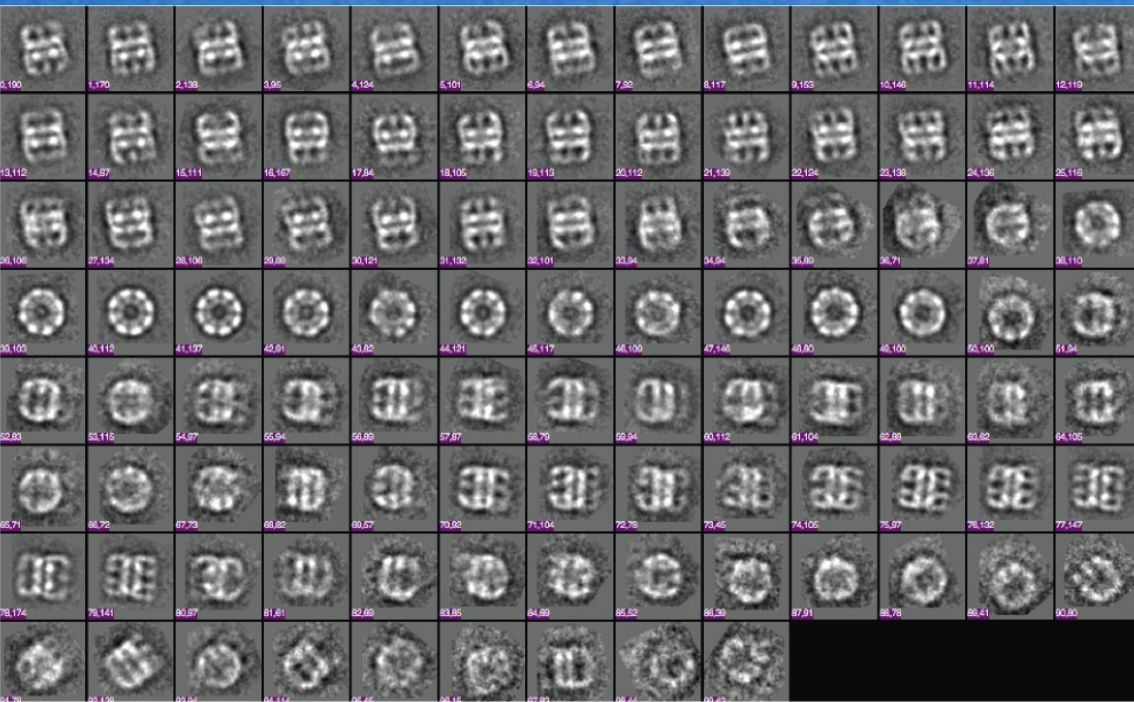
- Specimens
 - Apo Mm-Cpn+GuHCl
 - Mm-Cpn + GuHCl denatured HyD-crystallin
- Imaging of Mm-Cpn & HyD-crystallin Sample
 - 3200FSC electron microscope
 - 300 kV
 - Gatan 10k CCD camera
 - 2.0 Å/pixel (detector magnification of 89,000x)
 - Defocus range: 2.0um ~ 3.5um
- Data Processing
 - EMAN1

cryo-EM raw image of Cpn-hyD sample @ 3200FSC

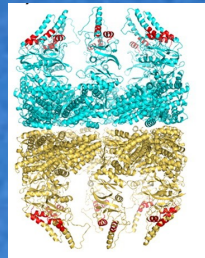
500 Å



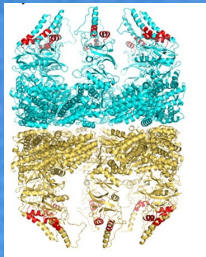
Reference free 2D class averages of Mm-Cpn & HyD-crystallin sample



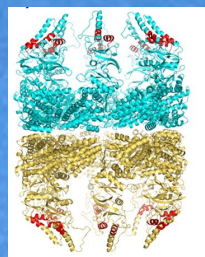
Multiple Reaction Products May Exist in Mm-Cpn & HyD-crystallin Sample



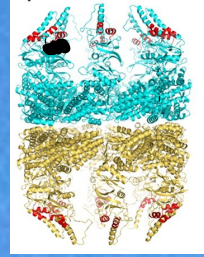
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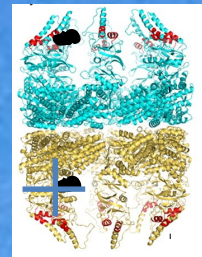
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+



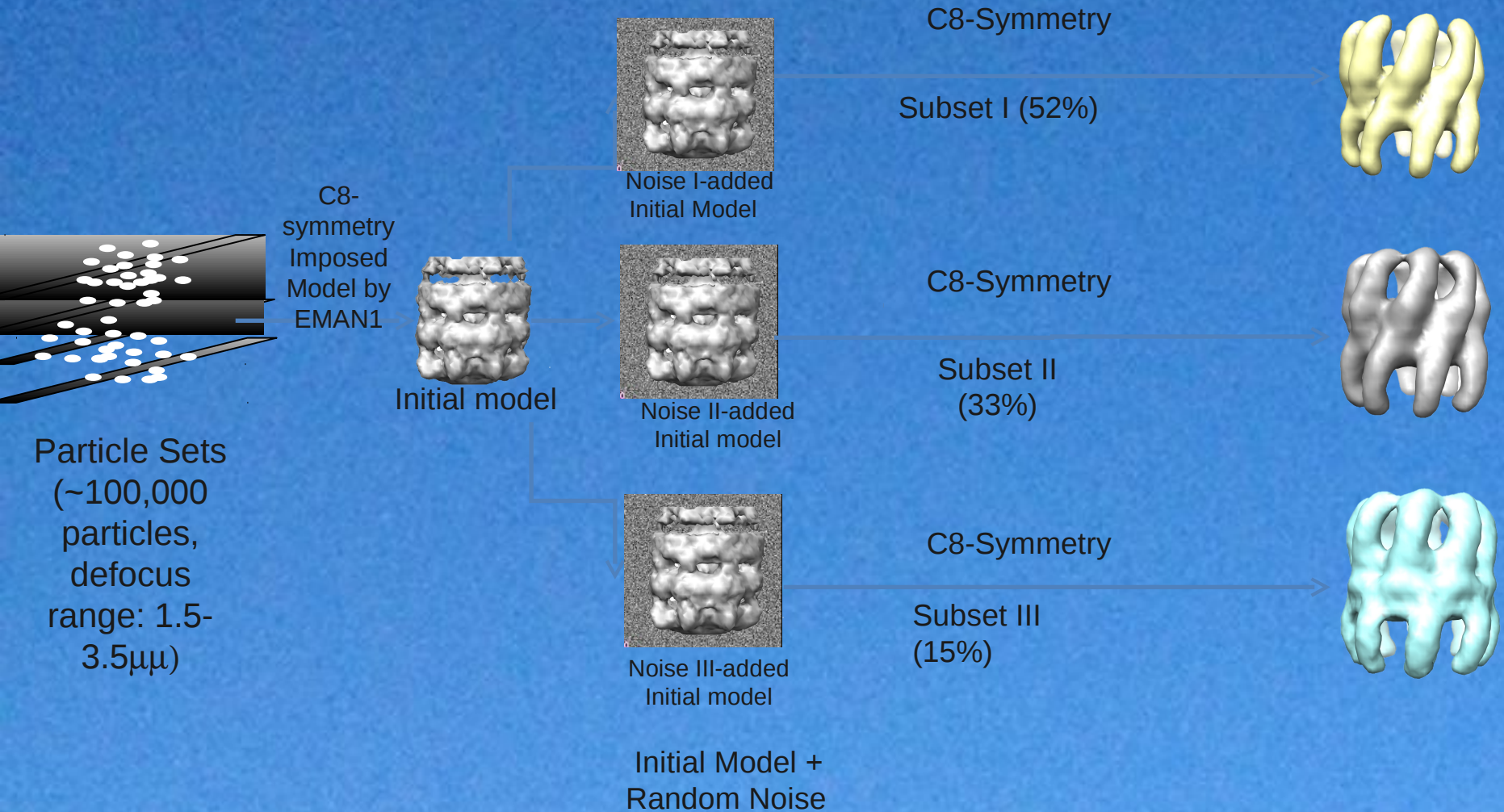
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Unsupervised Multiple Model Refinement Process

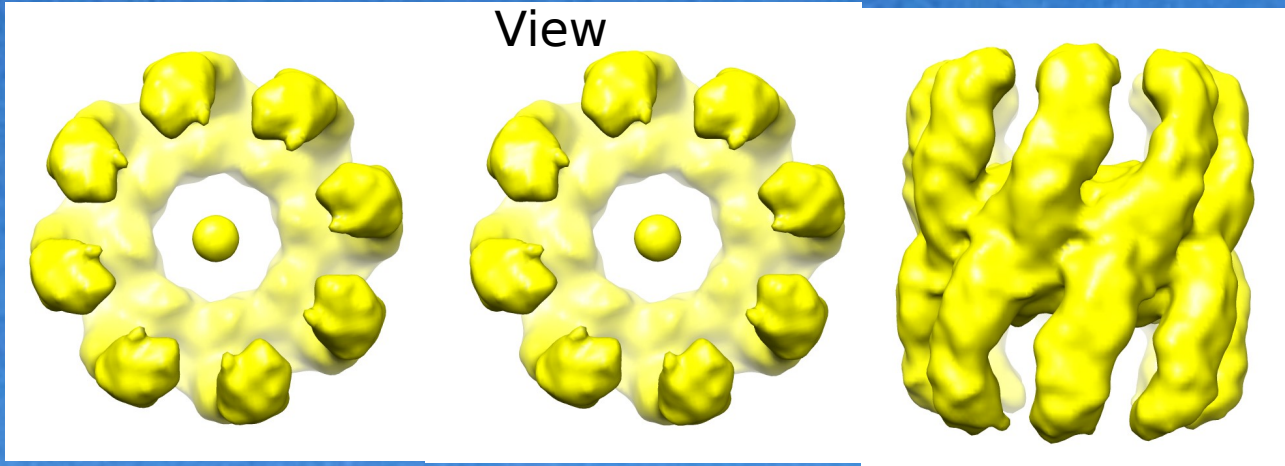


Subset I Model Resembles Apo State Mm-Cpn

Top View

Bottom View

Side View

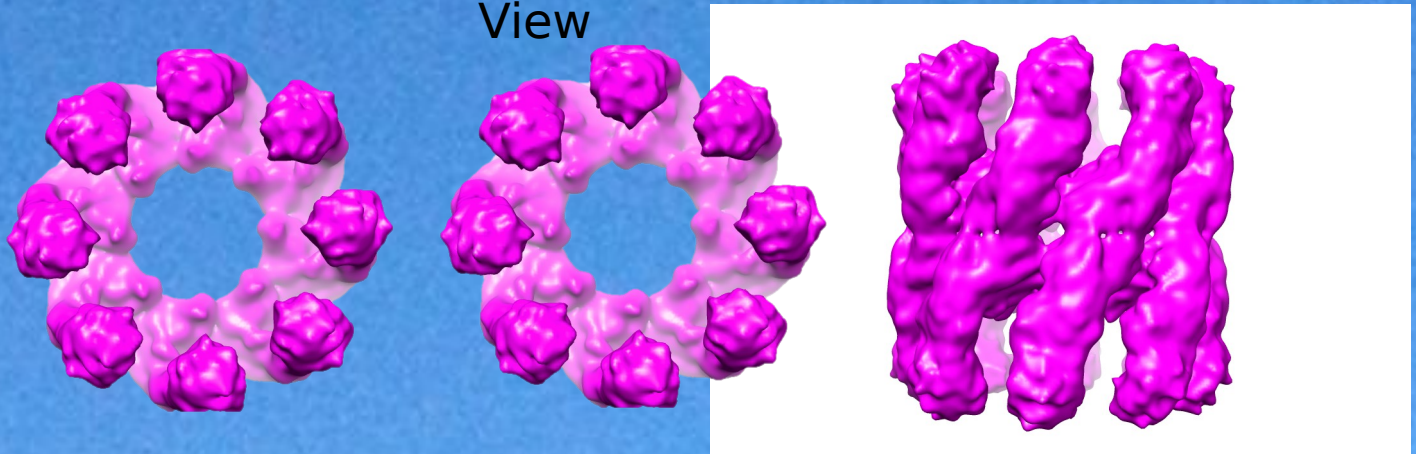


Subset I
(52%)

Top View

Bottom View

Side View



Lidless Mm-
Cpn (Zhang,
Nature, 2010)

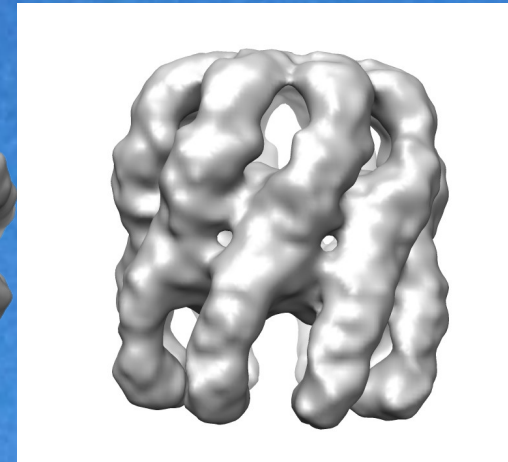
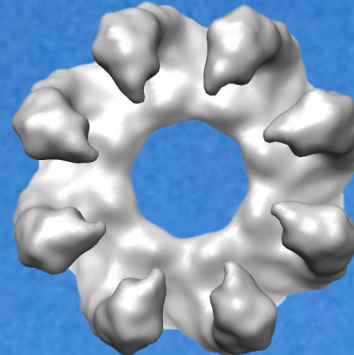
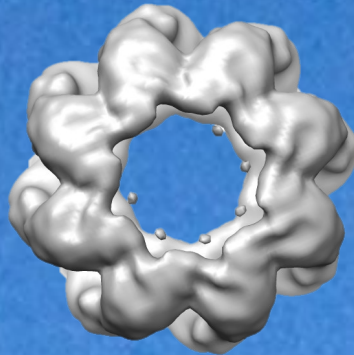
Refined Map of Subset II Shows One-ring Less Open and One-ring Open Conformation

Top View

Bottom View

Side View

Subset II
(33%)

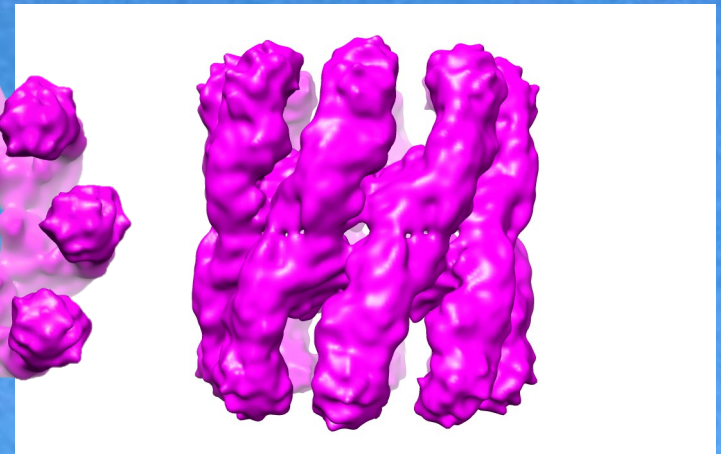
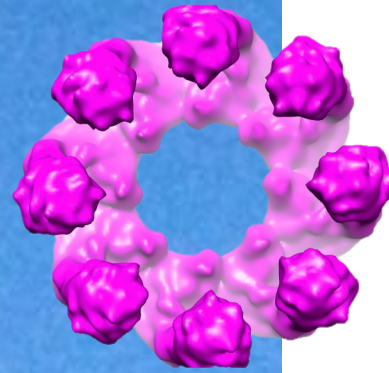
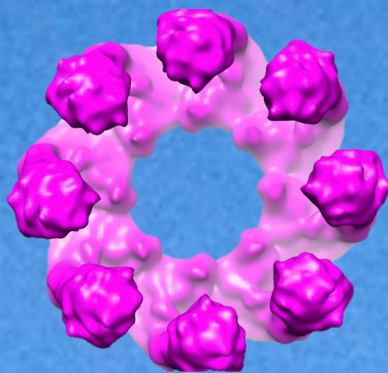


Top View

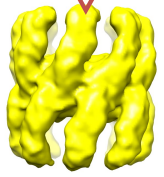
Bottom View

Side View

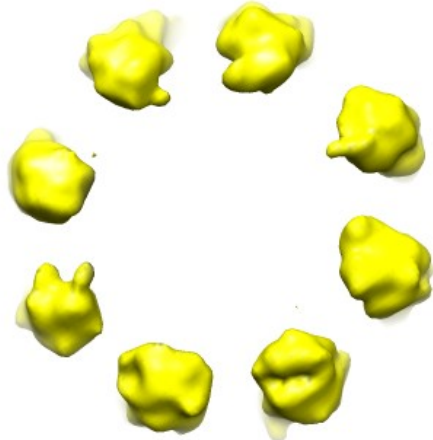
Lidless Mm-
Cpn (Zhang,
Nature, 2010)



Symmetry-Free Reconstructions of 2 Subsets

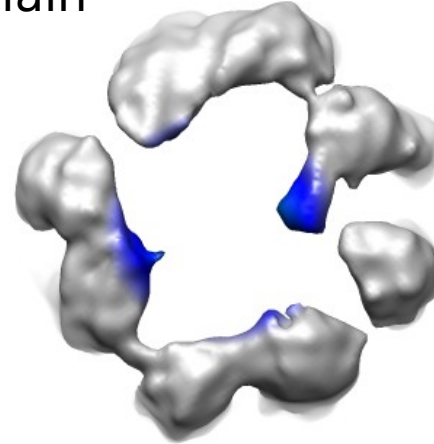


Top View of Apical Domain

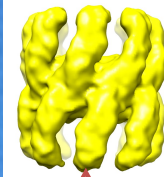


Subset I
(30,000 particles)

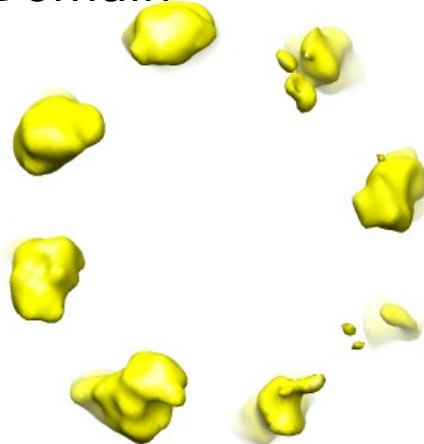
Top View of Apical Domain



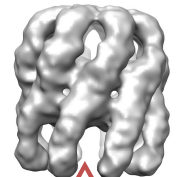
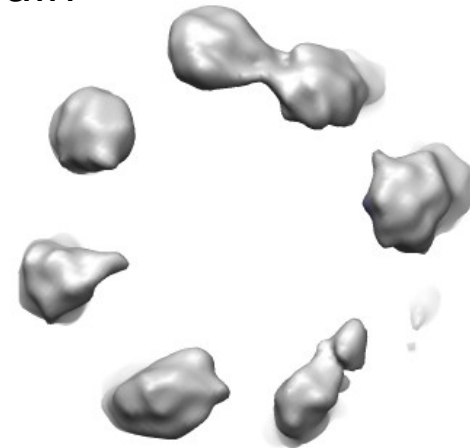
Subset II
(23,421 ptcls)



Bottom View of Apical Domain



Bottom View of Apical Domain



Conclusions

- ~50% of particles of Mm-cpn + denatured HyD-crystallin (Subset I) correspond to Apo state Mm-Cpn.
- ~33% of complex in the Mm-Cpn + denatured HyD-crystallin (Subset II) has one ring entirely open and another ring less open.
- Symmetry free reconstruction shows additional density in the apical region in one subunit of one ring with a break-down of 8 fold symmetry (subset II).
- The observed protruding density may correspond to part of the HyD-crystallin

Future Directions

- Cryo-EM Biochemical gold labeling of HyD-crystallin

Acknowledgement

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Oksana Sergeeva, MIT
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Previous NCMI Members:

Junjie Zhang
Yao Cong
Ge (Jerry) Zhang
Guang (Grant) Tang

Current NCMI Members:

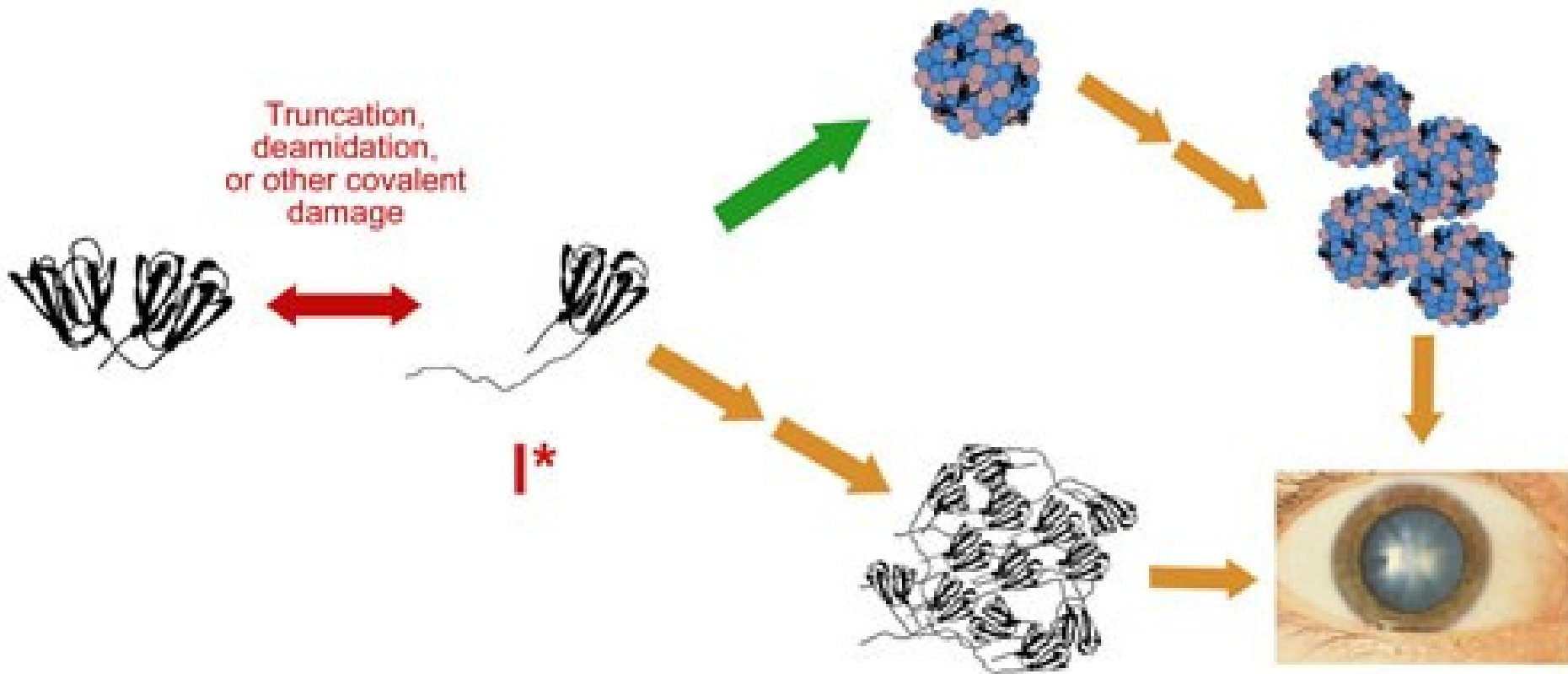
Joanita Jakana
Jesus Galaz Montoya
Boxue Ma
Ming Cheng
Htet Khant
Caroline Fu
All the NCMI personals

Funding Resources

Center for Protein Folding Machinery and National Institutes of Health Roadmap-supported Nanomedicine Development Center.

Backup Slides

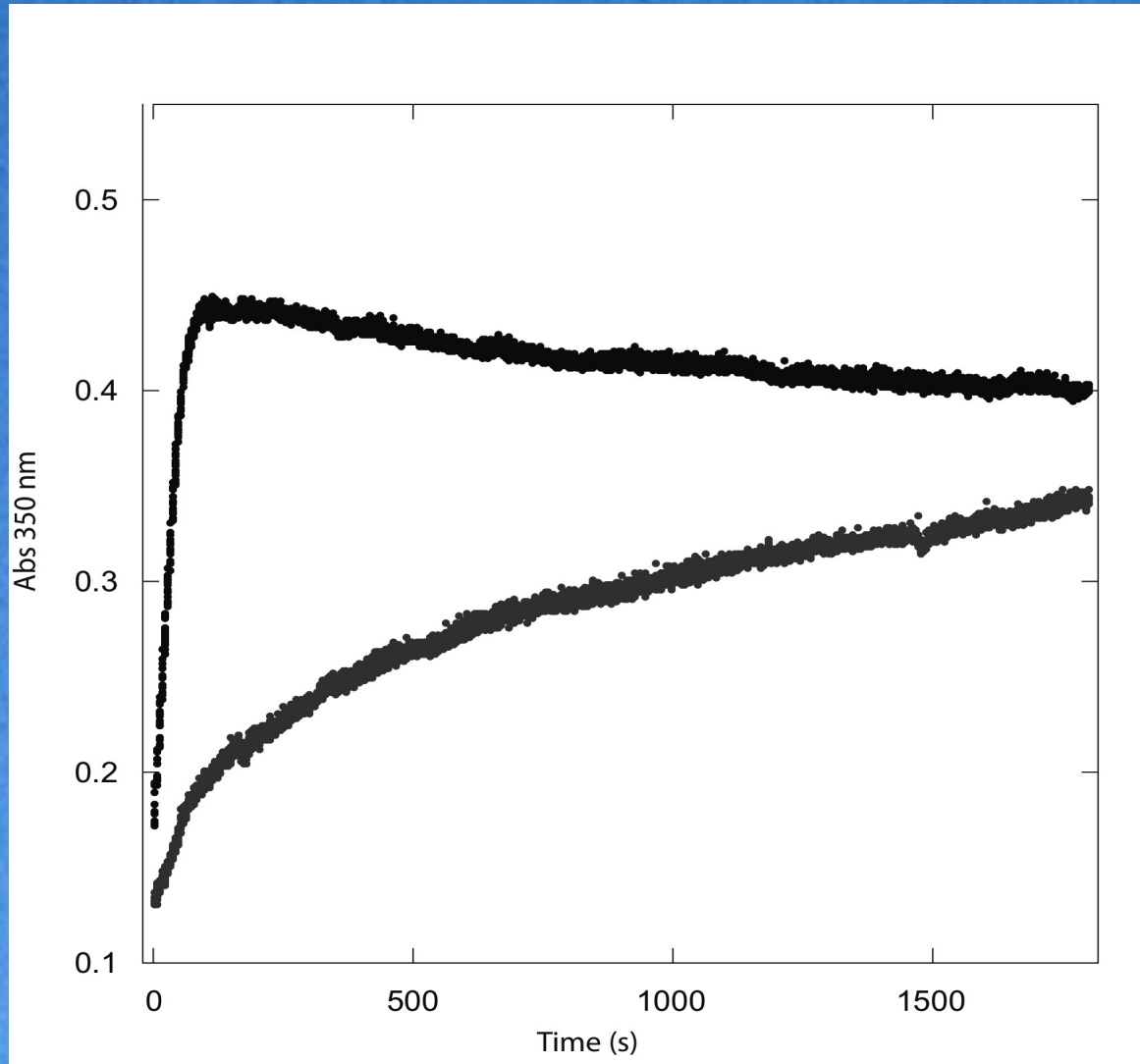
Unfolding/Misfolding of HyD-crystallin Causes Onset of Cataracts



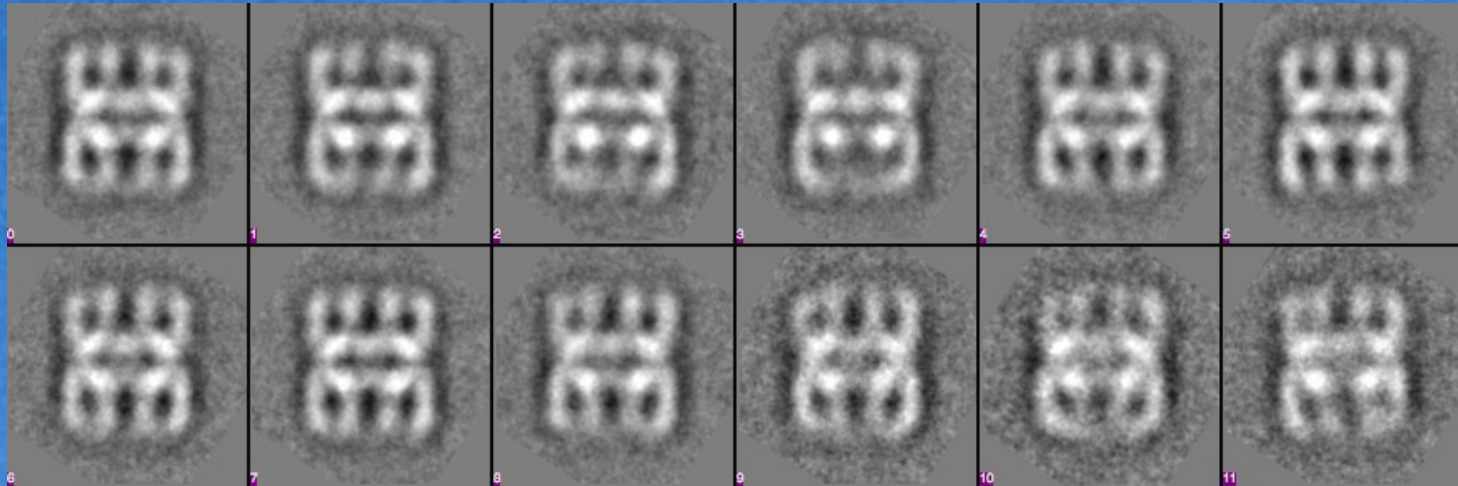
Jonathan King lab homepage, <http://web.mit.edu/king-lab/www/>

Mm-Cpn Suppresses HyD-crystallin Aggregation

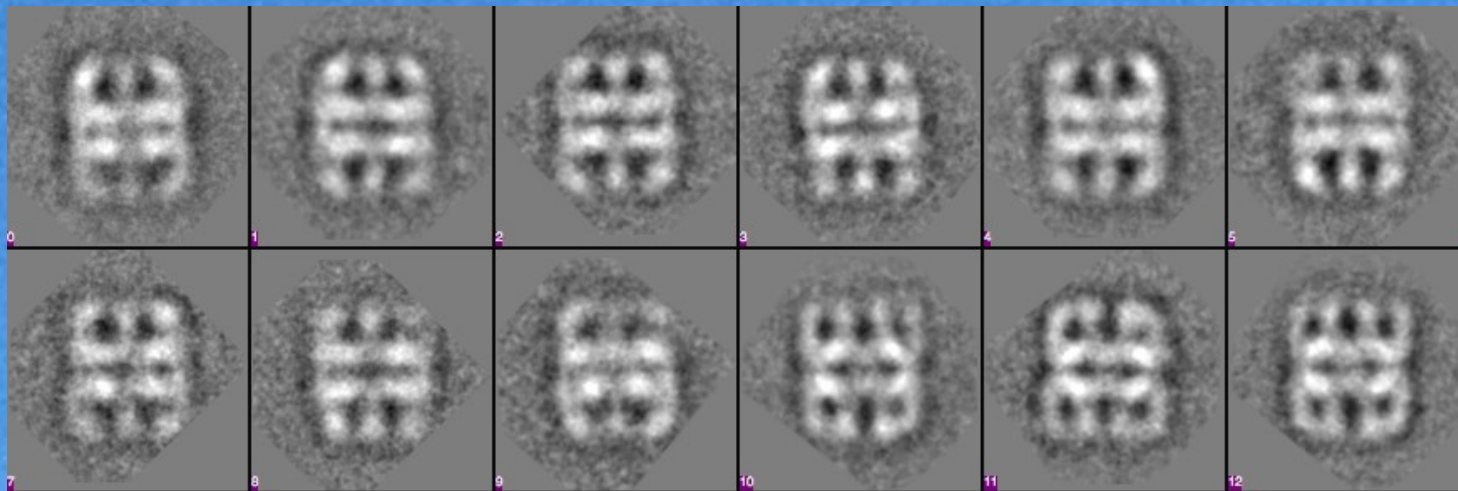
Aggregation Level



Differences is Observed Between Subclass I & II 2D Side Views

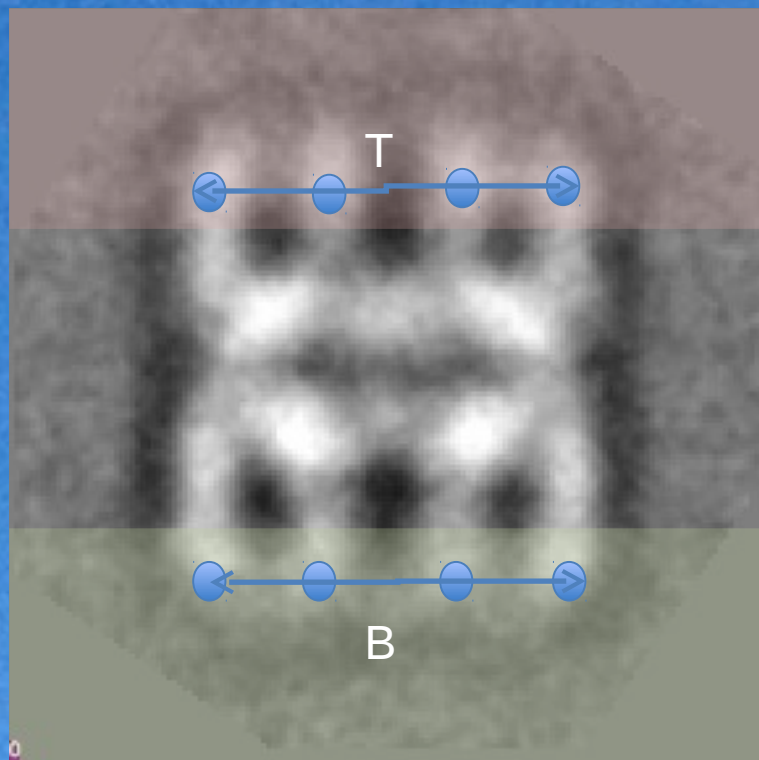


2D class averages of subset I



2D class averages of subset II

Statistical Analysis of 2D Class Averages in Subset I and II

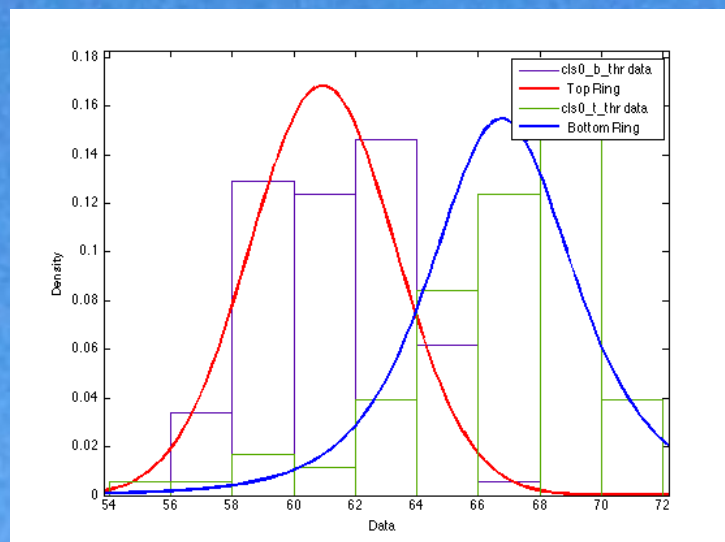
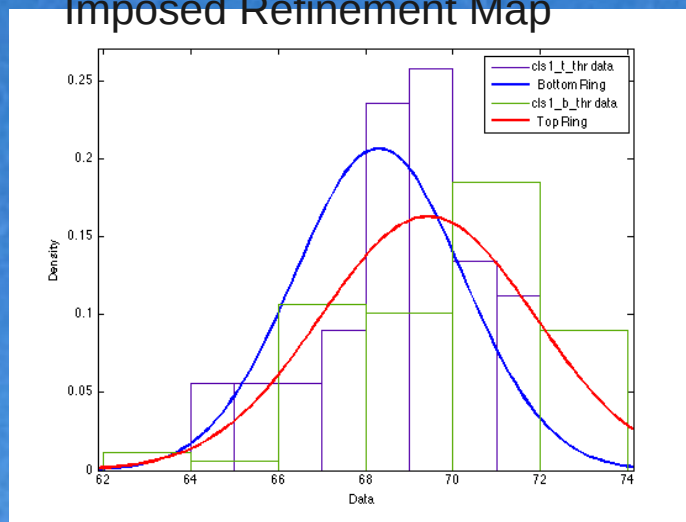


2D class Averages

Subset I

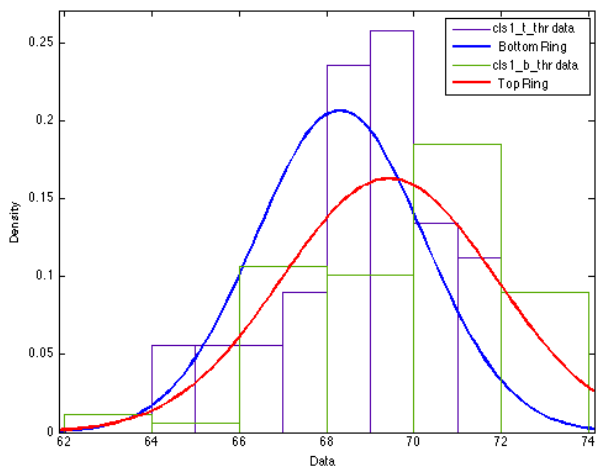
Subset II

2D Class Averages Associated with Angular Projections of C8-symmetry Imposed Refinement Map



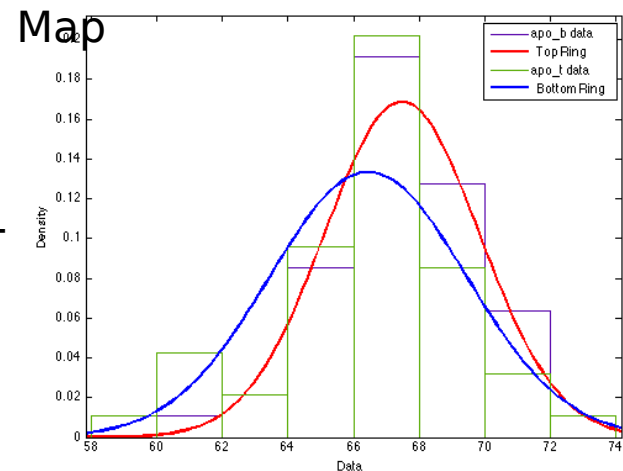
2D Class Averages Statistical Analysis Results

2D Class Averages Associated with Angular Projections of C8-symmetry Imposed Refinement Map

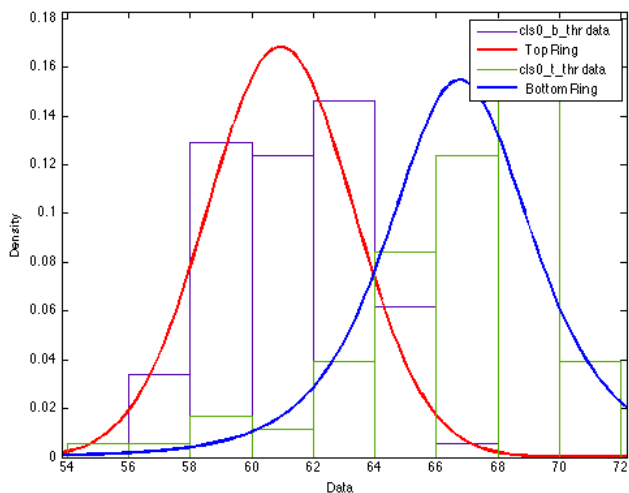


Subset I

2D Class Averages Associated with Angular Projections of C8-symmetry Imposed Refinement Map

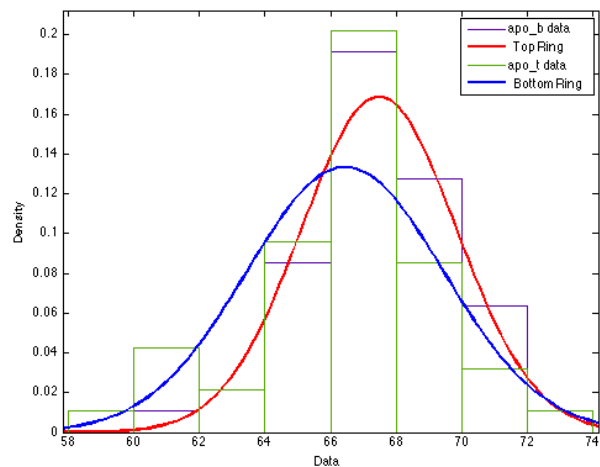


Apo Mm-Cpn control



Subset II

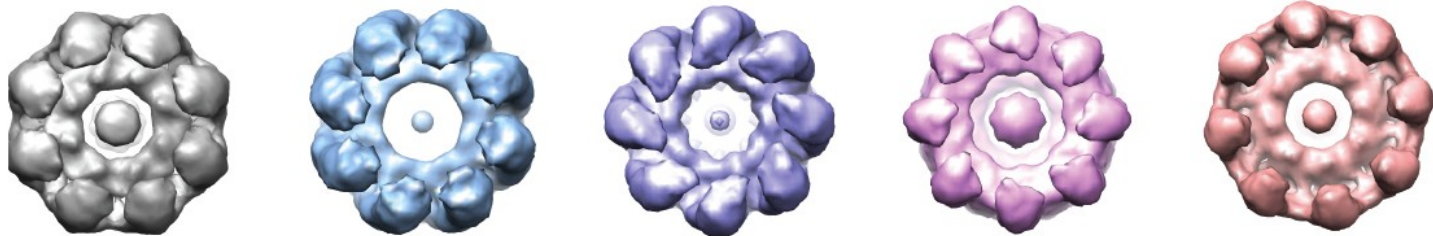
Apo Mm-Cpn control



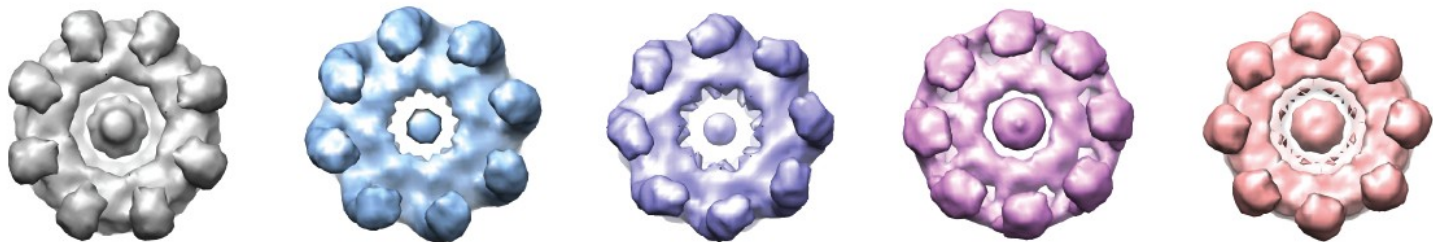
5/10 Subclasses (49%) Particles Show Double-ring Open Conformation

Double-ring Open Conformation
(19,833/40,212, 49%)

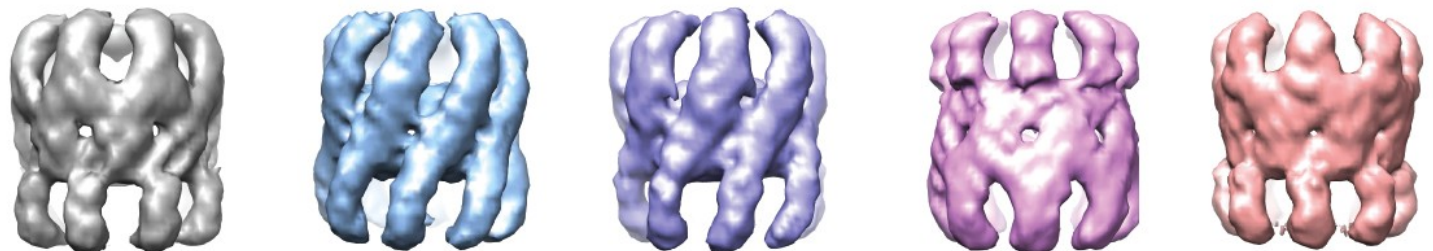
Top



Bottom

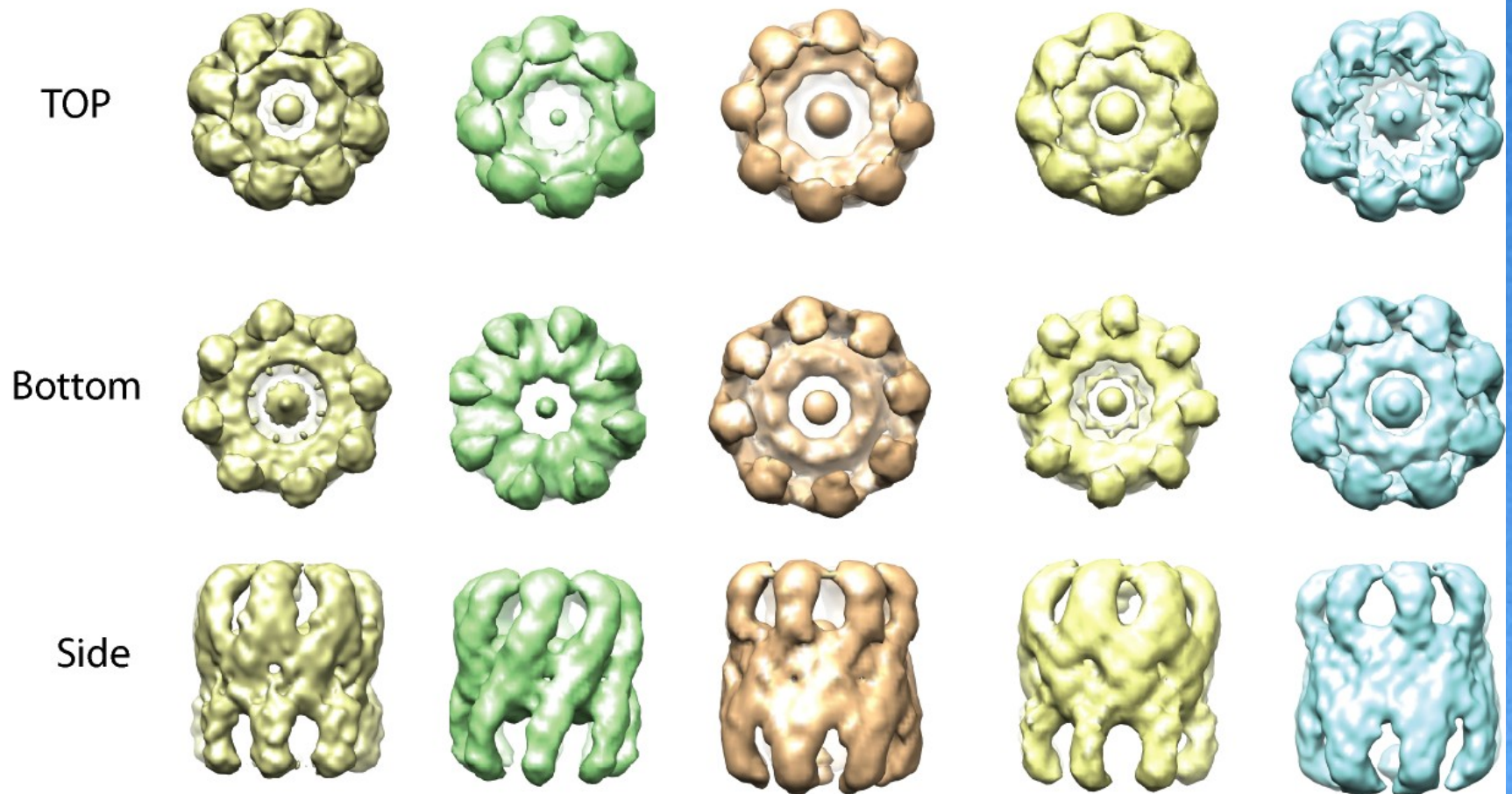


Side



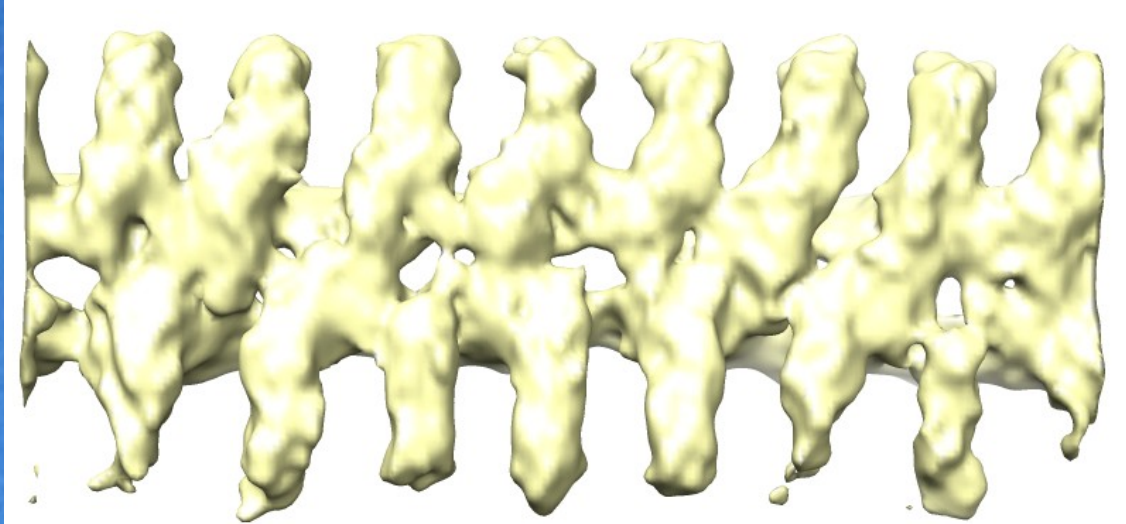
5/10 Subclasses (51%) Particles Show One-ring Open One-ring Closed Conformation

One-ring Less Open, One-ring Open Conformation
(20,379/40,212, 51%)



Continuous Density is Observed on the Apical Region of Top Ring in Subset II but not Subset I

Subset I



Subset II

