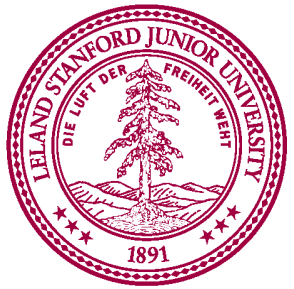
CENTER FOR
PROTEIN FOLDING
MACHINERY



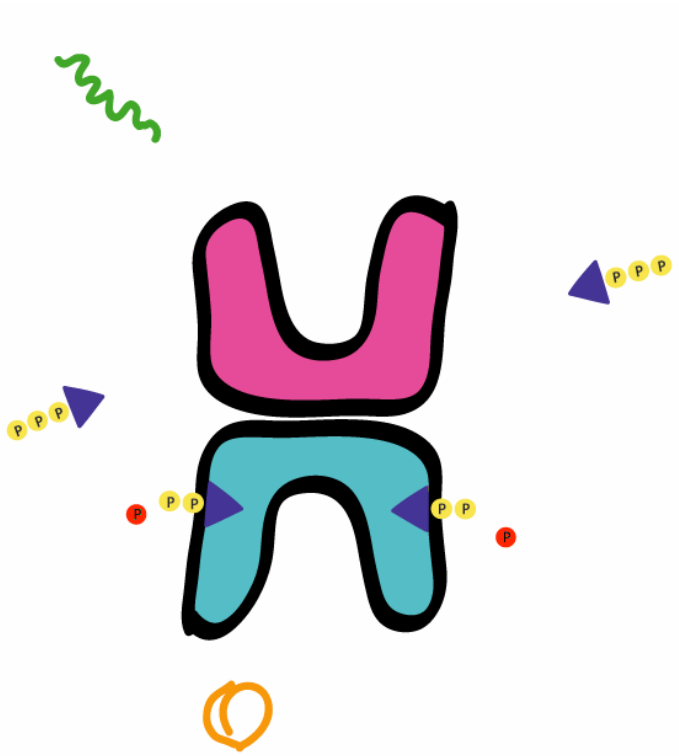
Multiple states of a chaperonin and a method for analyzing heterogeneous conformations in cryo-EM

Junjie Zhang
Department of Structural Biology, Stanford
EMAN Workshop, Mar. 2011

Major research goals

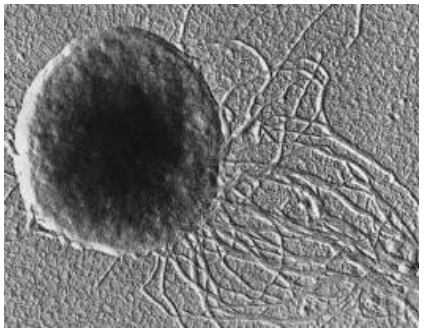
- Solve chaperonin structures at different nucleotide states

- Develop new algorithm to model all the dynamics of chaperonins with experimental data

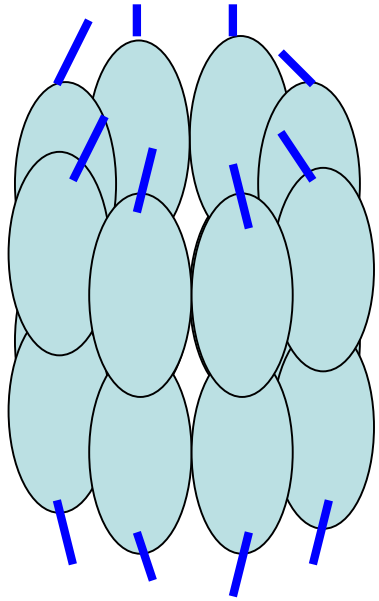


Mm-cpn Chaperonin

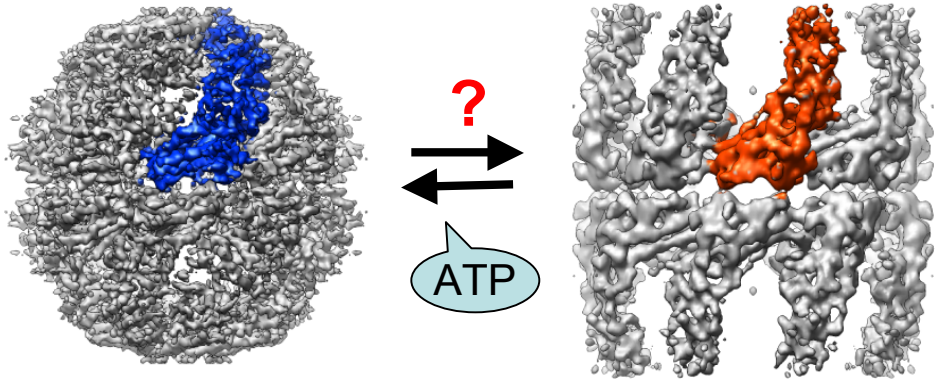
- From Methanococcus maripaludis
- Molecular mass ~1 MDa
- Consists of 16 identical subunits
- Arranged in 2 rings (8 subunits per ring)
- Open and close by a built-in lid (Group II)
- Folds proteins in an ATP-dependent manner
- Has similar allosteric regulation properties of mammalian chaperonins (TRiC)

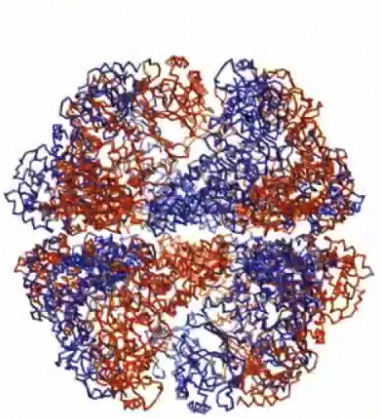


http://www.bact.wisc.edu/Bact303/Methanococcus.jpeg



Structures of Mm-cpn under two nucleotide states





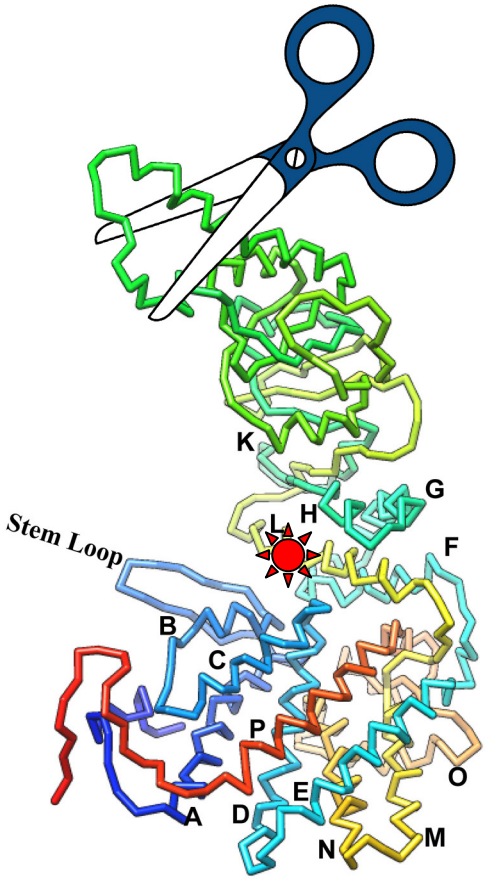
closed state
ATP/AlFx

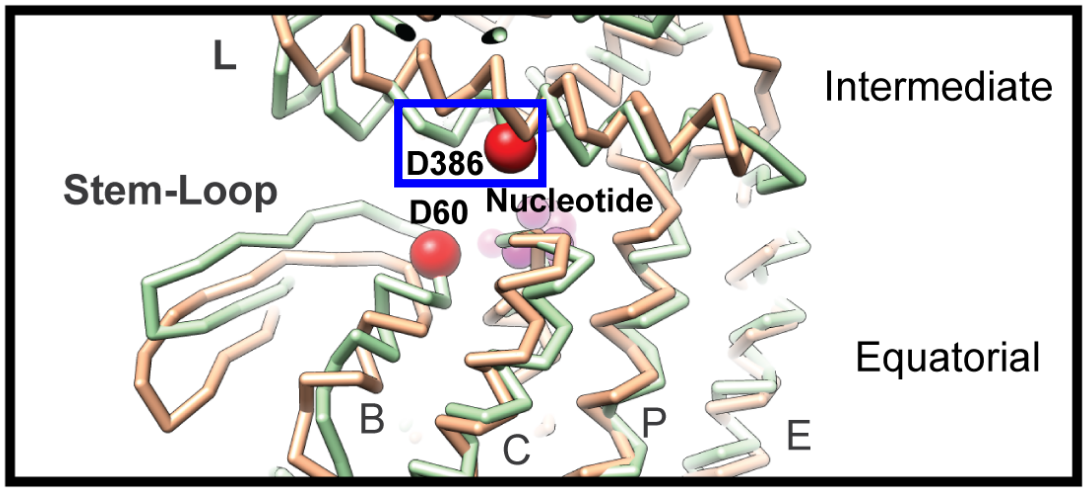
open state
ATP-free

Morphing
between them

Zhang *et. al.*, Nature 2010

Cryo-EM structures suggest possible engineering site for affecting ATP activity in Mm-cpn

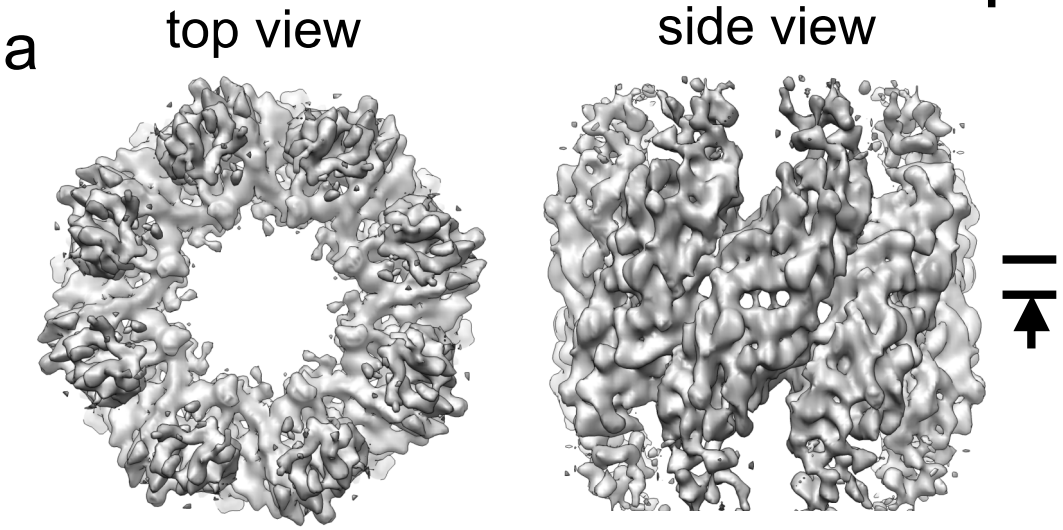


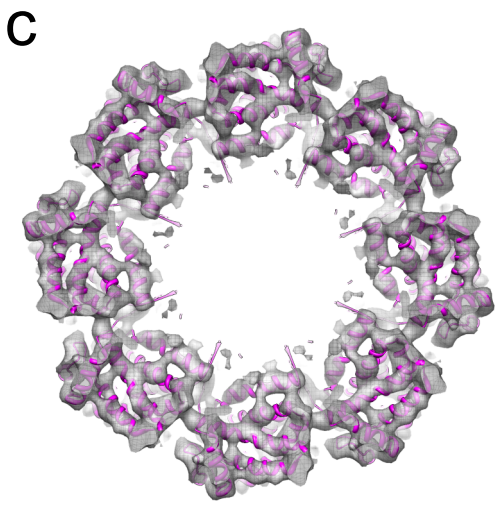


Zhang *et. al.*, Nature 2010

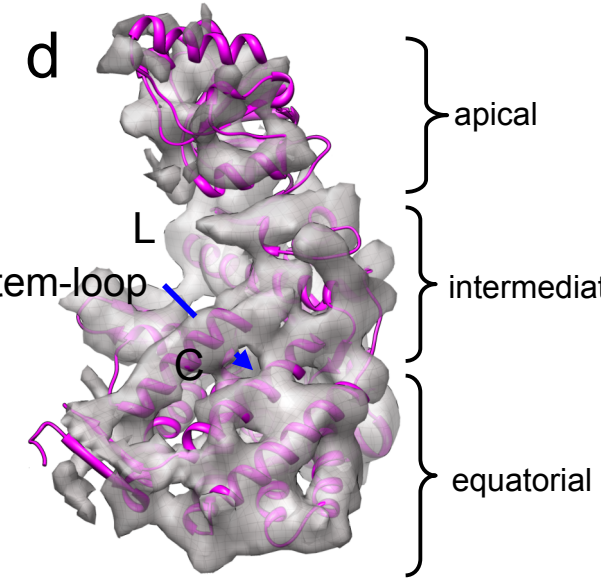
Lidless D386A Mm-cpn variant

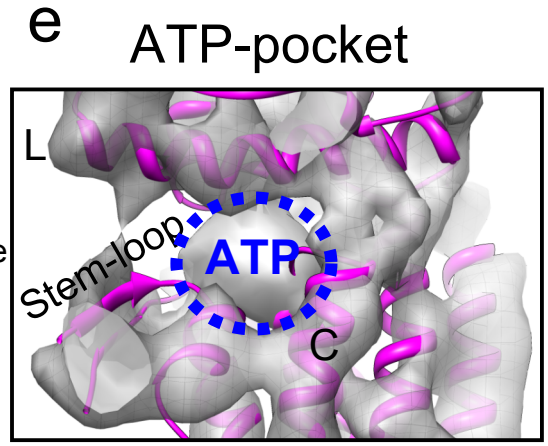
Capturing pre-hydrolysis ATP-bound state with this Mm-cpn variant





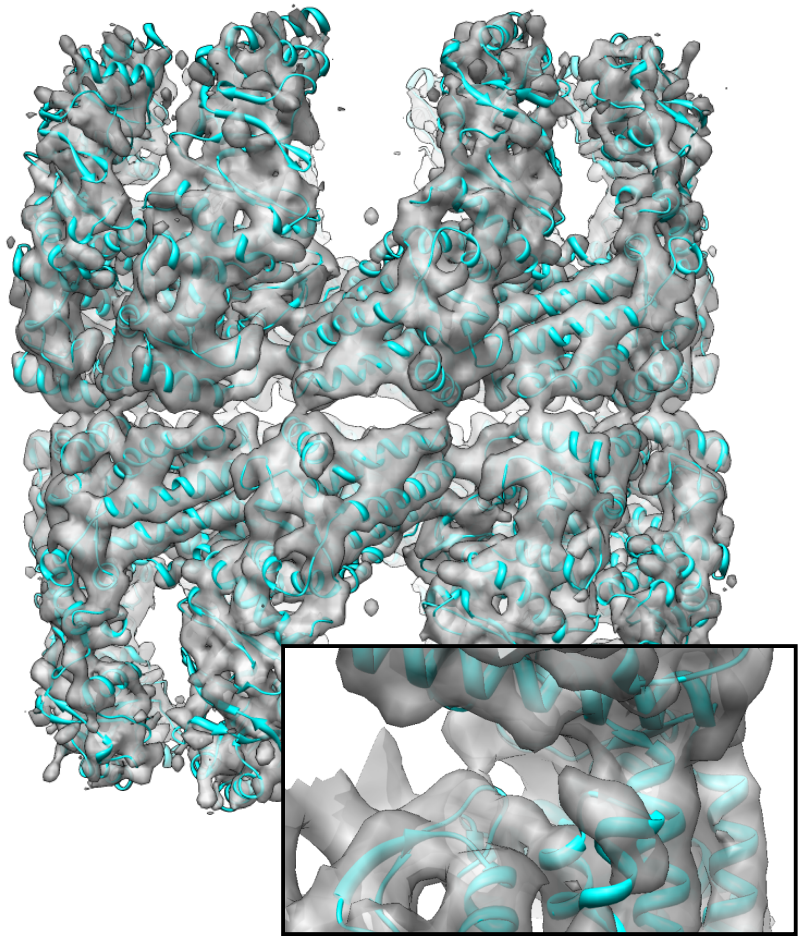
8Å resolution





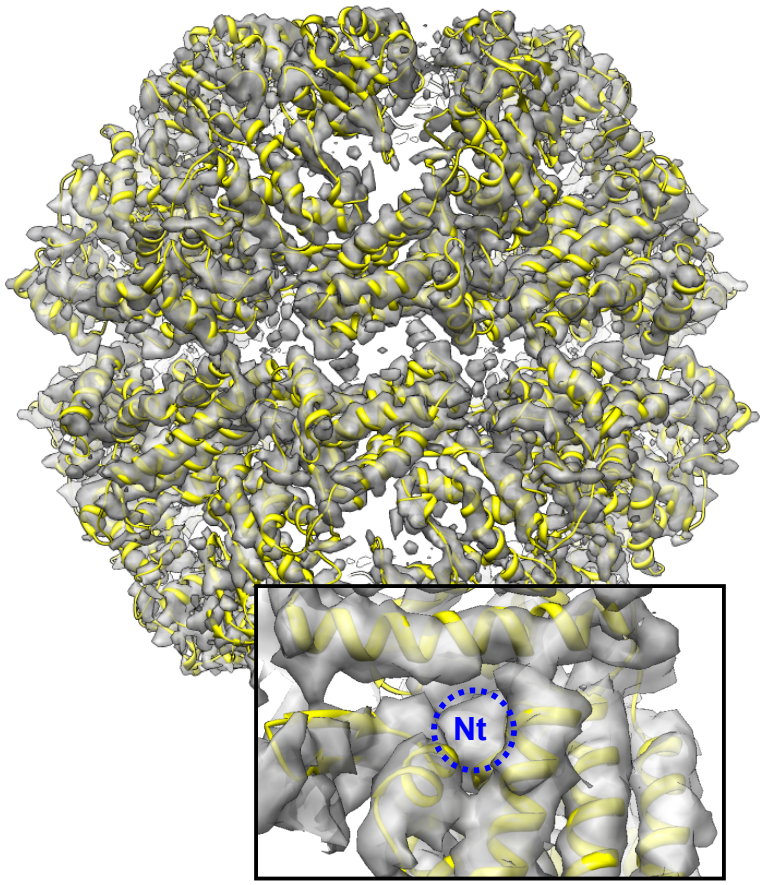
Zhang *et. al.*, Structure 2011, accepted

Building Models for ATP-free and ATP-hydrolysis States



ATP-free

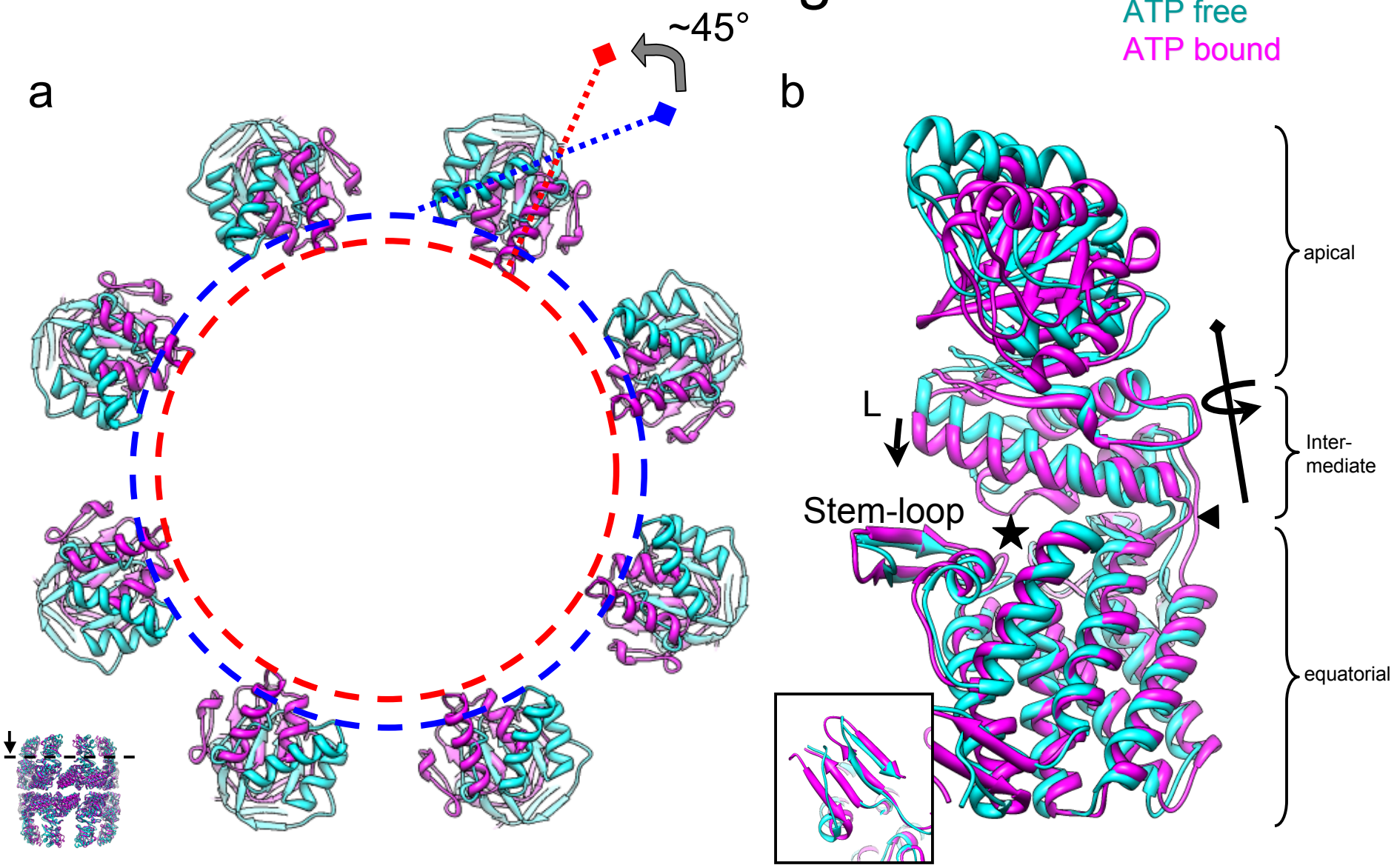
8Å resolution



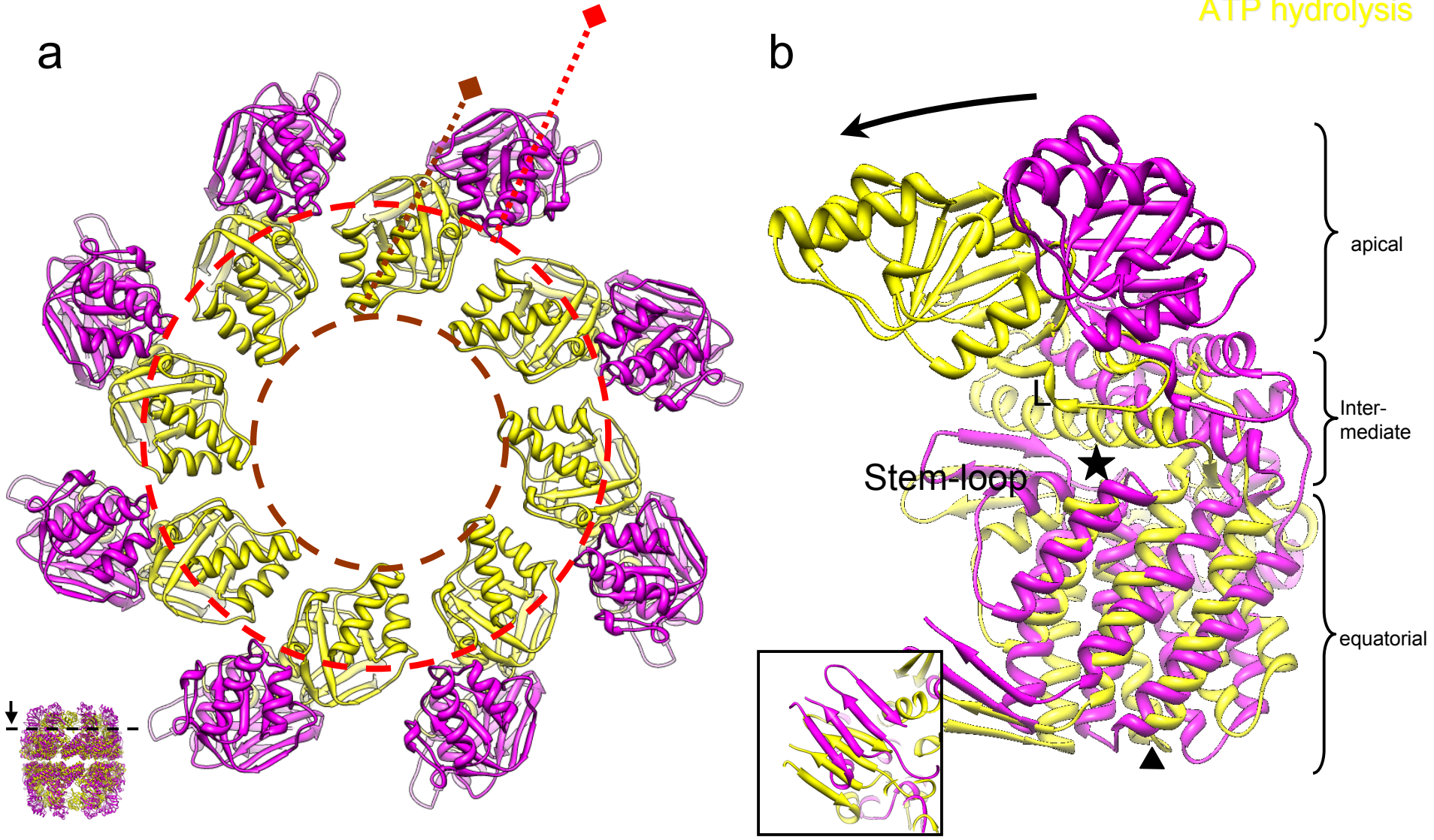
ATP-hydrolysis (ATP/AlFx)

4.8Å resolution

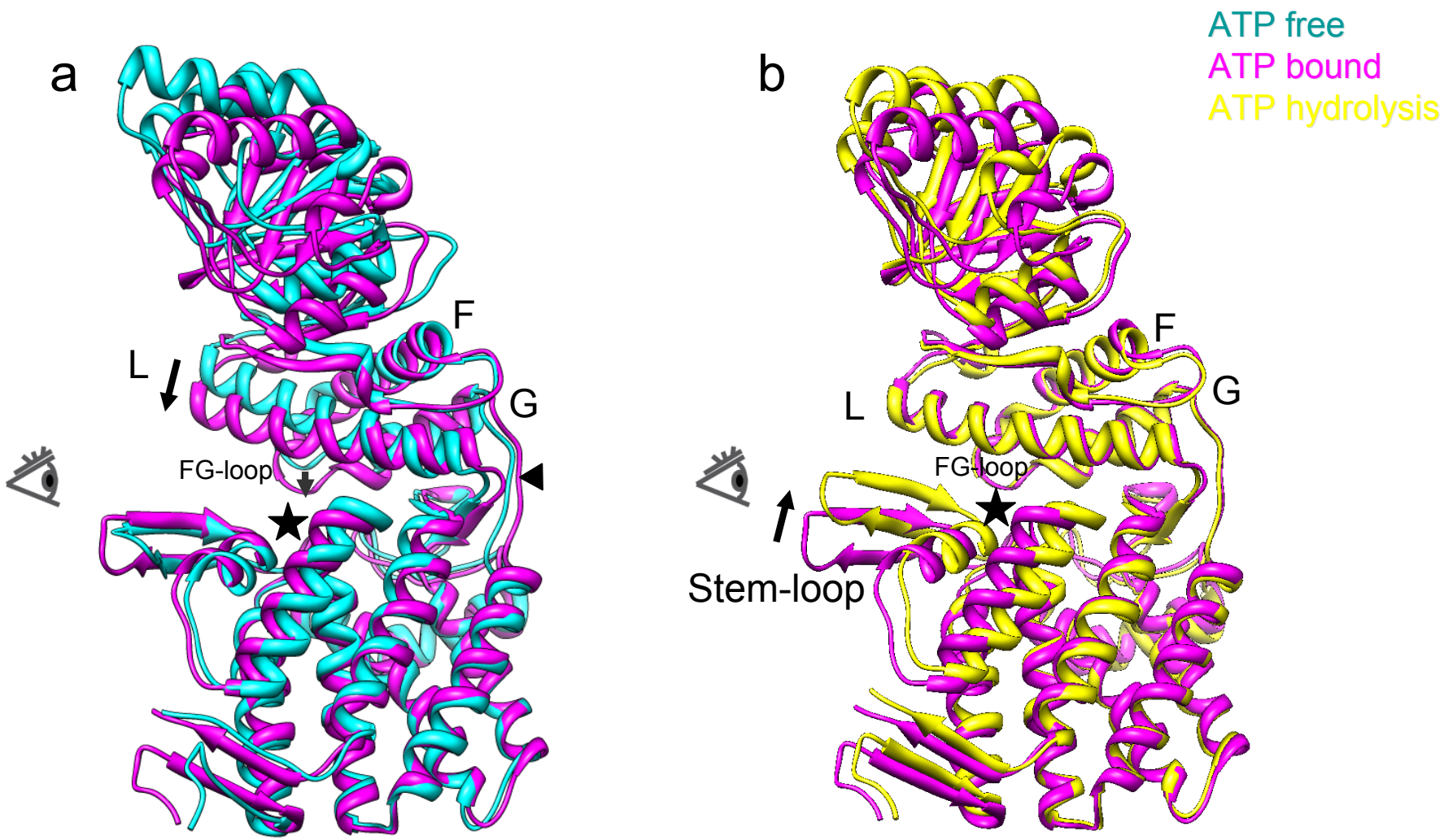
Global conformational changes



Global conformational changes



Local conformational changes

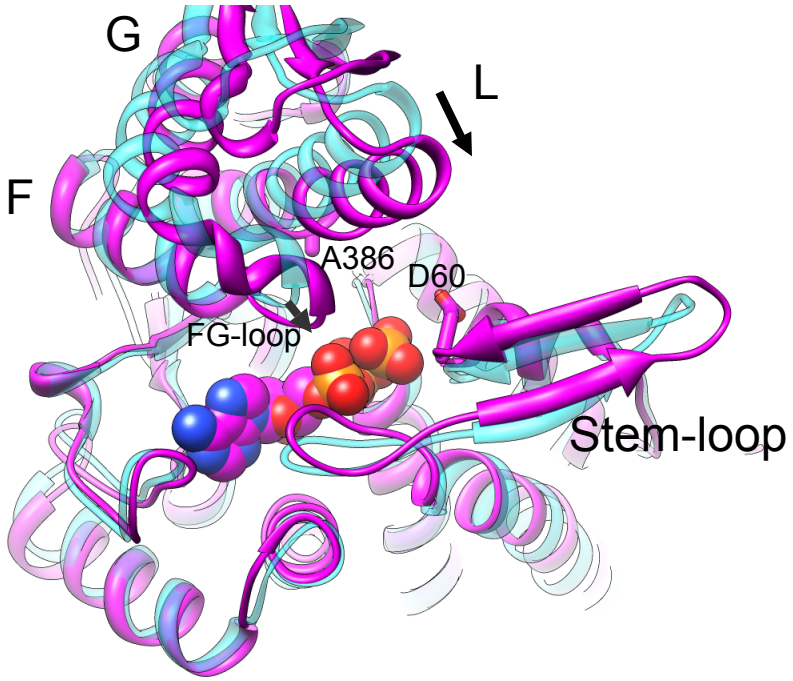


ATP-binding

ATP-hydrolysis

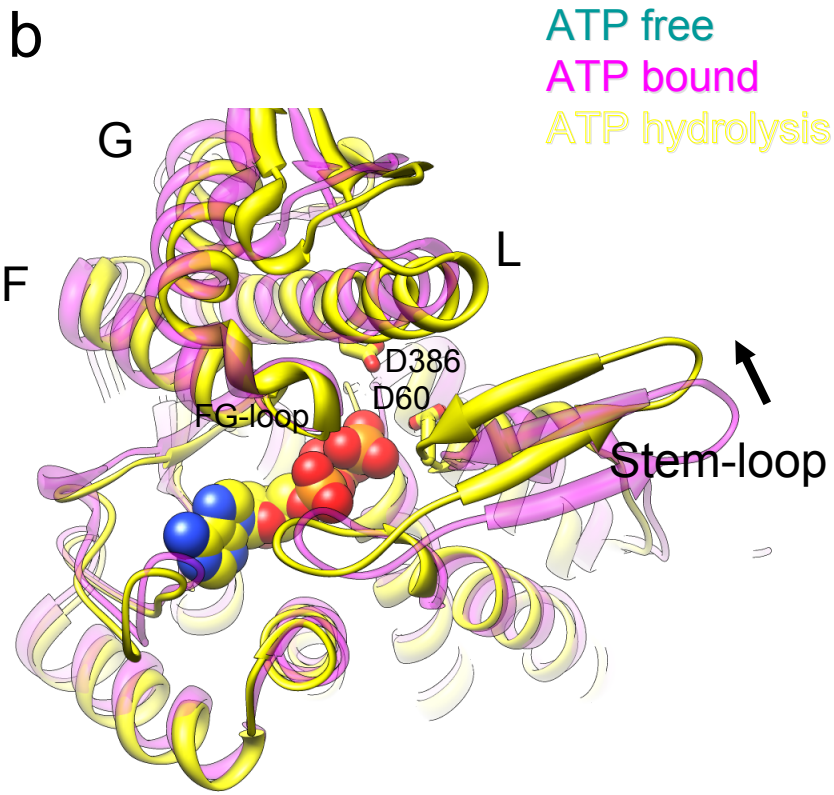
Changes around ATP-binding pocket

a



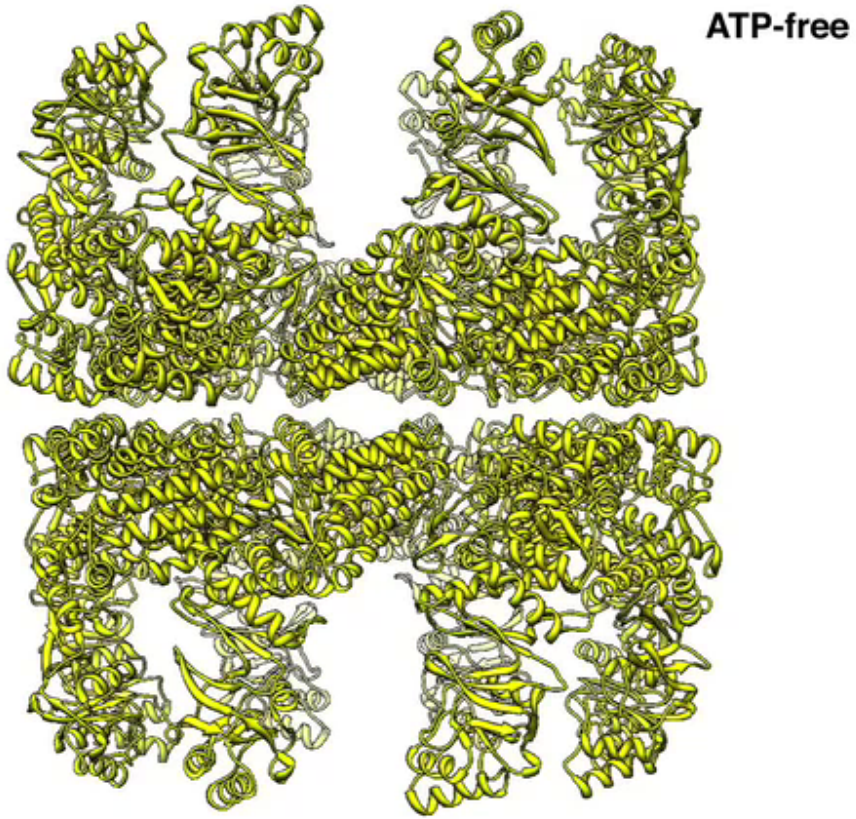
ATP-binding

b



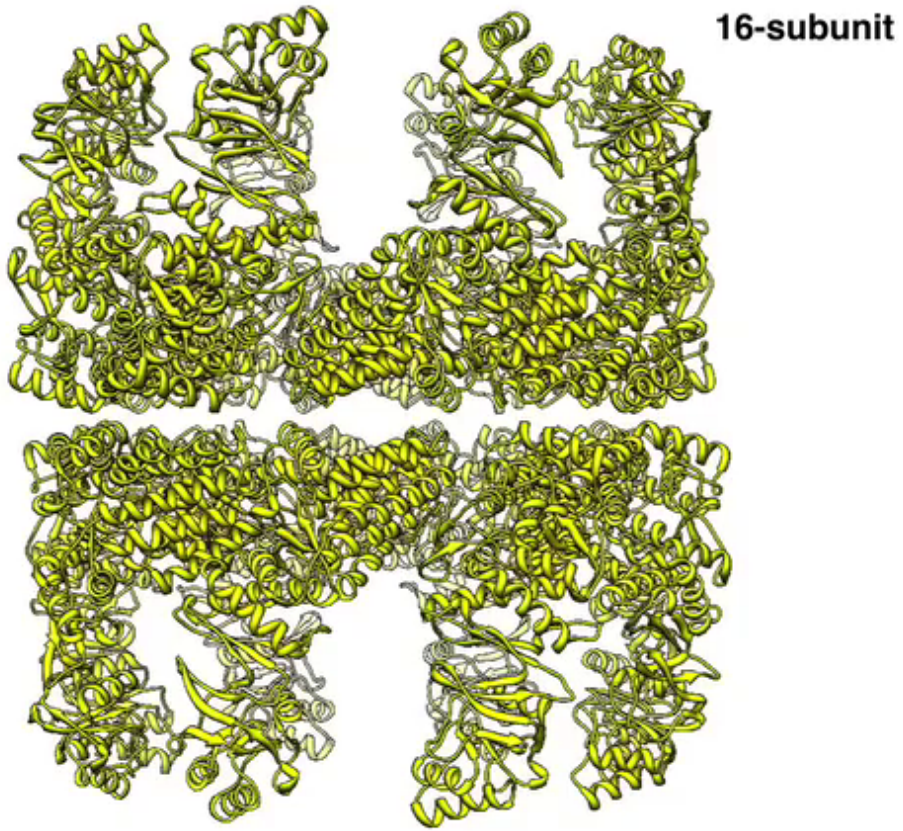
ATP-hydrolysis

Morphing between ATP-free, ATP-bound and ATP-hydrolysis states



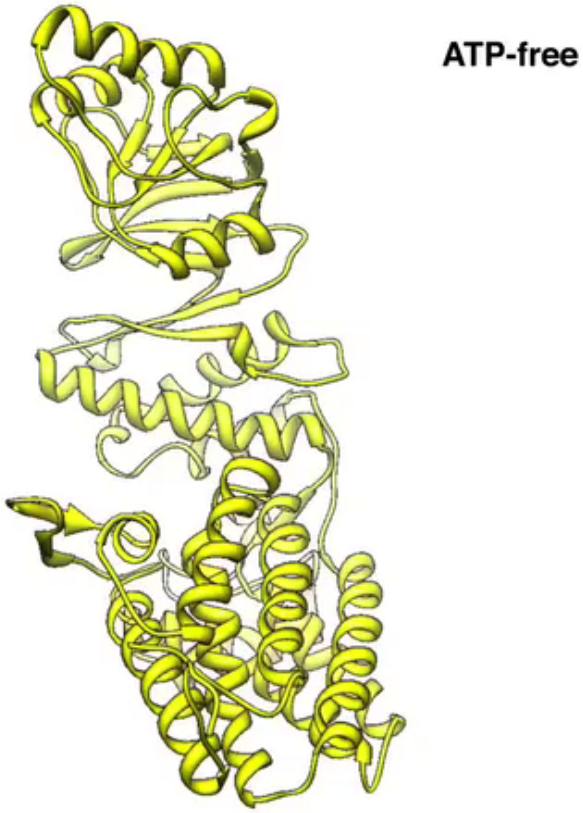
Overall Structural Change

Morphing between ATP-free, ATP-bound and ATP-hydrolysis states



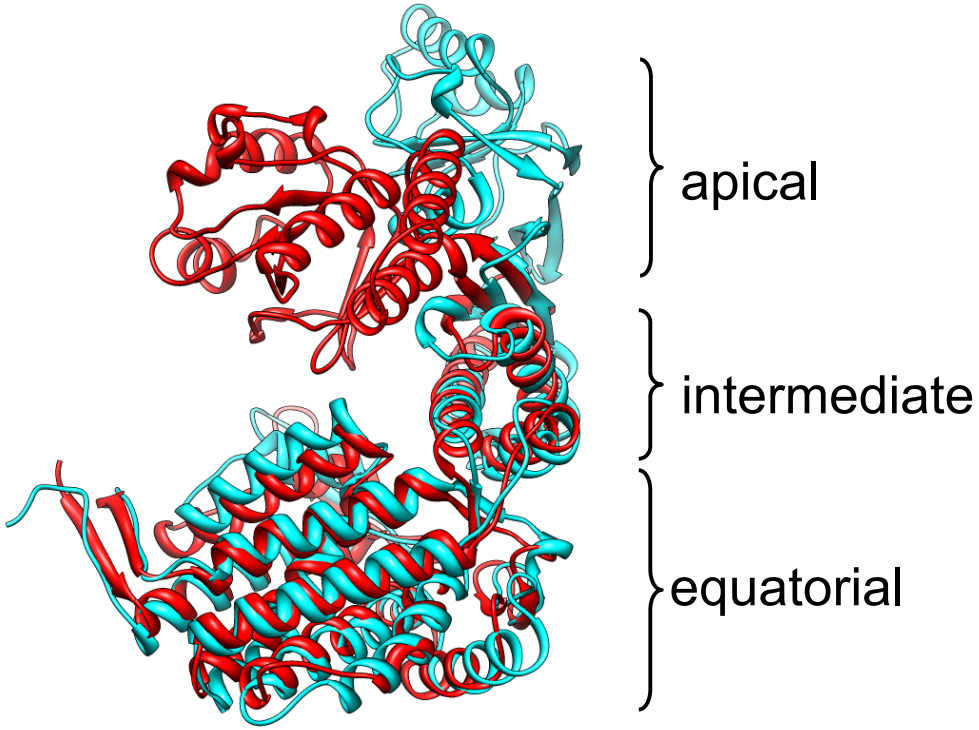
Subunit Movement and Interactions

Morphing between ATP-free, ATP-bound and ATP-hydrolysis states

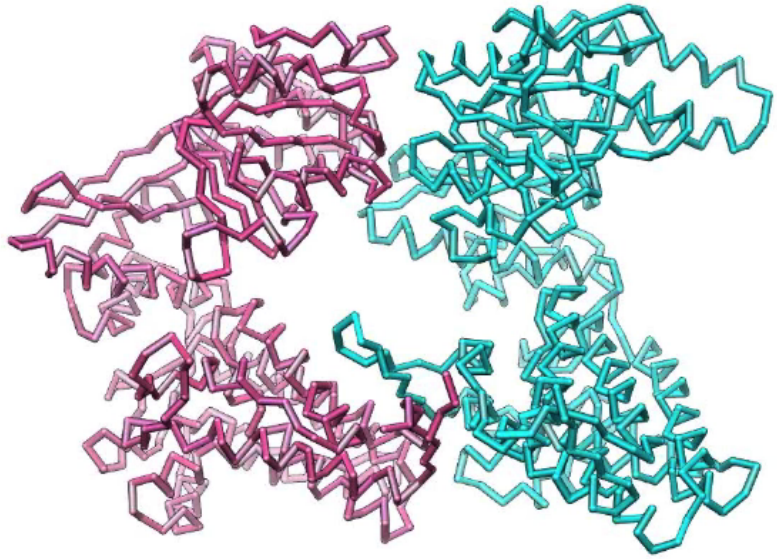


Nucleotide Interactions

Different Behavior in Group I Chaperonins

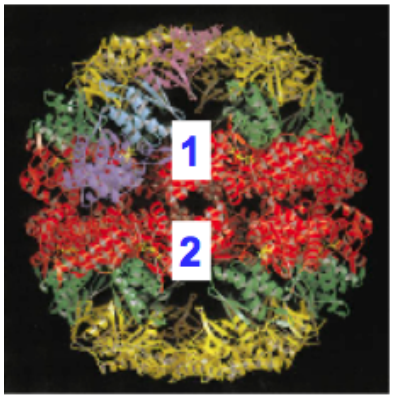


Overlay of Mm-cpn (cyan) and GroEL(red) subunits in the ATP-free state

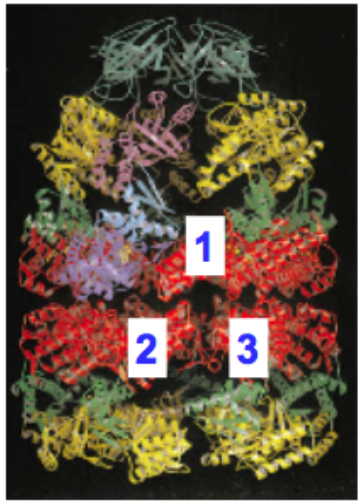


Morphing between ATP-free and ATP-bound Gro-EL two subunits

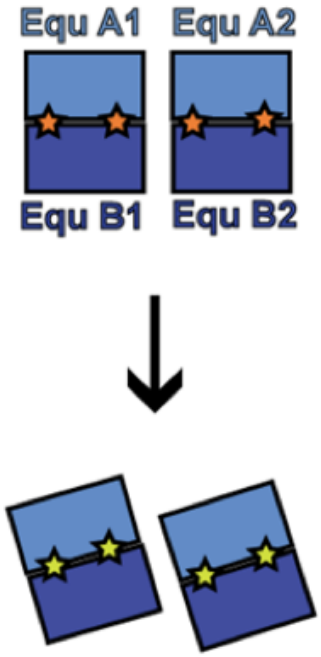
Quaternary structural difference leads to different mechanisms



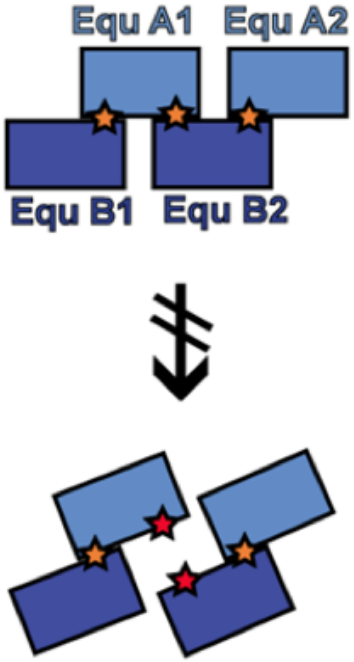
Group II



Group I



Group II



Group I

Summary

- ATP binding causes counter-clockwise rotation of the Mm-cpn apical domains and shrinkage of the folding-chamber entrance
- ATP hydrolysis causes the rocking of the Mm-cpn subunit and completely close the folding chamber
- Different ways of assembly of similar parts lead to different mechanisms

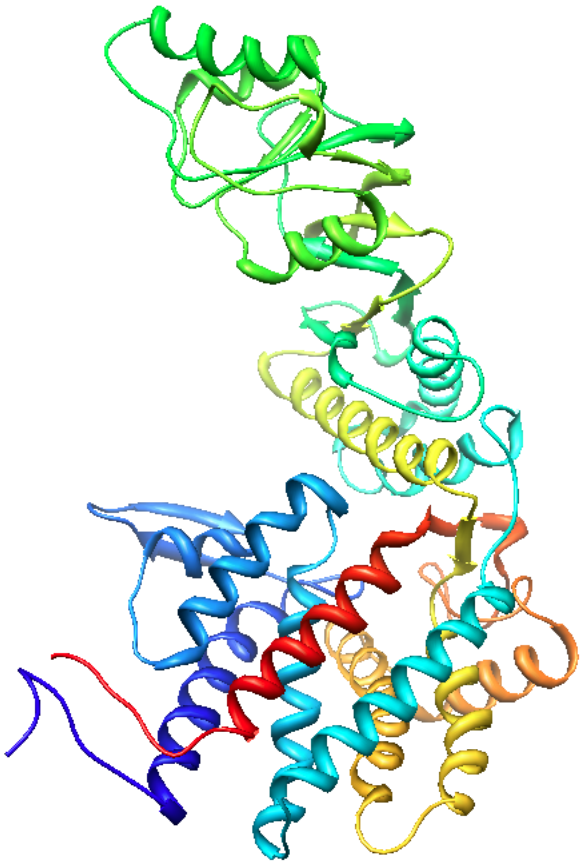
Model Validation for Lidless Mm-cpn in the open state at 8Å resolution

Overal Cα RMSD: 2.8Å

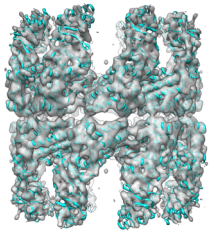
API Cα RMSD: 4.3Å

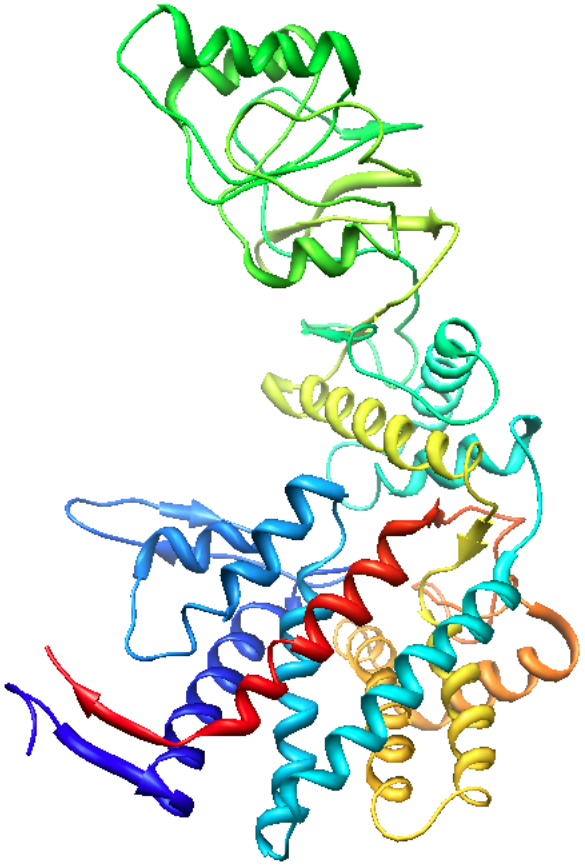
INT Cα RMSD: 2.3Å

EQU Cα RMSD:1.7Å



Rosetta-built Model

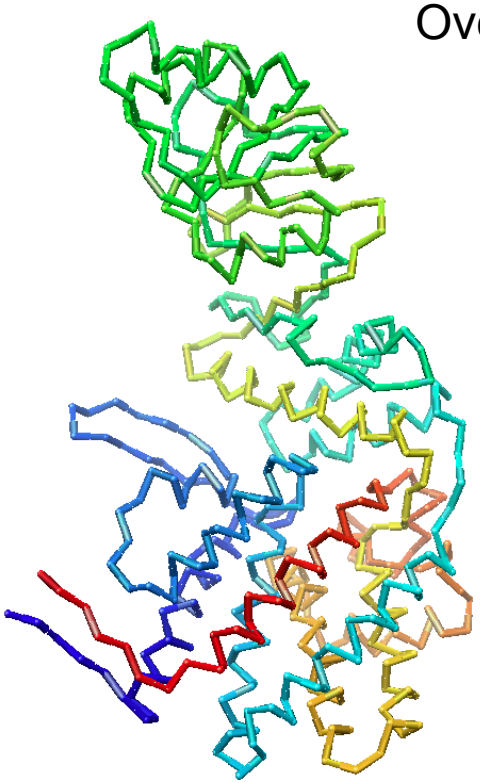


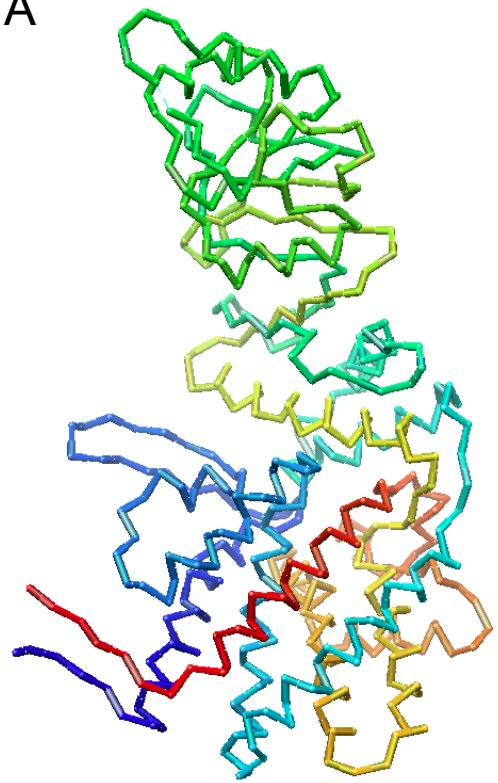


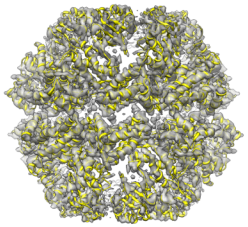
Direx-built Model

Model Validation for Lidless Mm-cpn in the closed state at 4.8Å resolution

Overal Cα RMSD: 1.7Å







EM model
PDB: 3J03

X-ray structure
PDB: 3KFE