

Structural Characterization of the Eukaryotic Chaperonin TRiC/CCT by cryo-EM

Yao Cong

Center For Protein Folding Machinery
National Center for Macromolecular Imaging
Baylor College of Medicine, Houston, TX USA

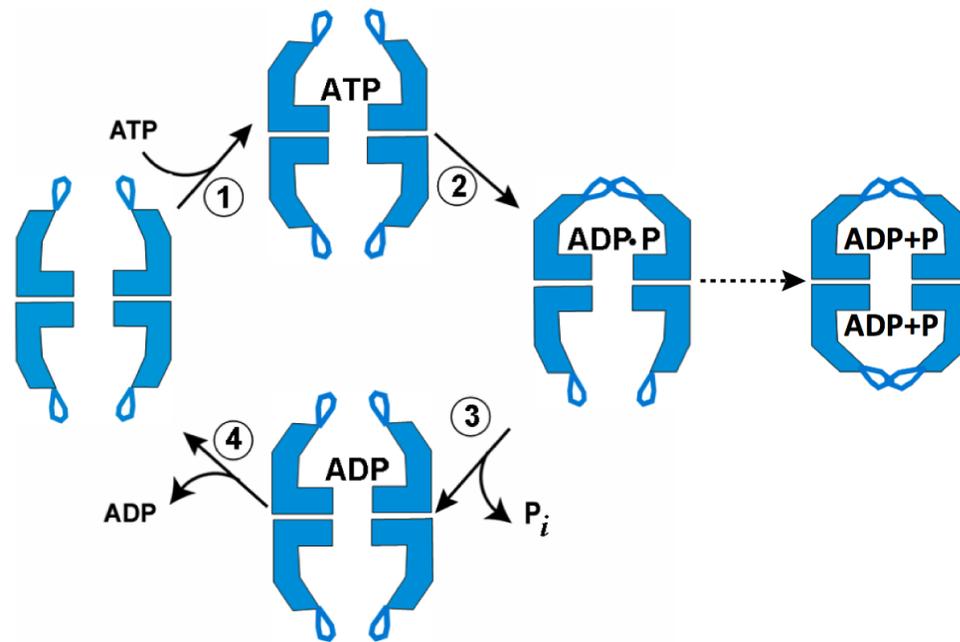


Introduction

- Defects in protein folding lead to many human diseases:
Alzheimer's, Huntington's, or Parkinson's disease
- Chaperonins can assist protein folding correctly in the cell
- Two groups of chaperonin:
 - Group I: in Bacteria (GroEL-GroES)
 - Group II: { in Archaea (Thermosome, Mm-cpn)
 { in Eukarya (TRiC/CCT)
- TRiC/CCT folds approximately 5-10% of cytosolic proteins
- Substrates of TRiC: many key structural and regulatory proteins, i.e. actin, tubulin, and VHL tumor suppressor.
- Many of TRiC substrates cannot be folded by any other chaperonin.

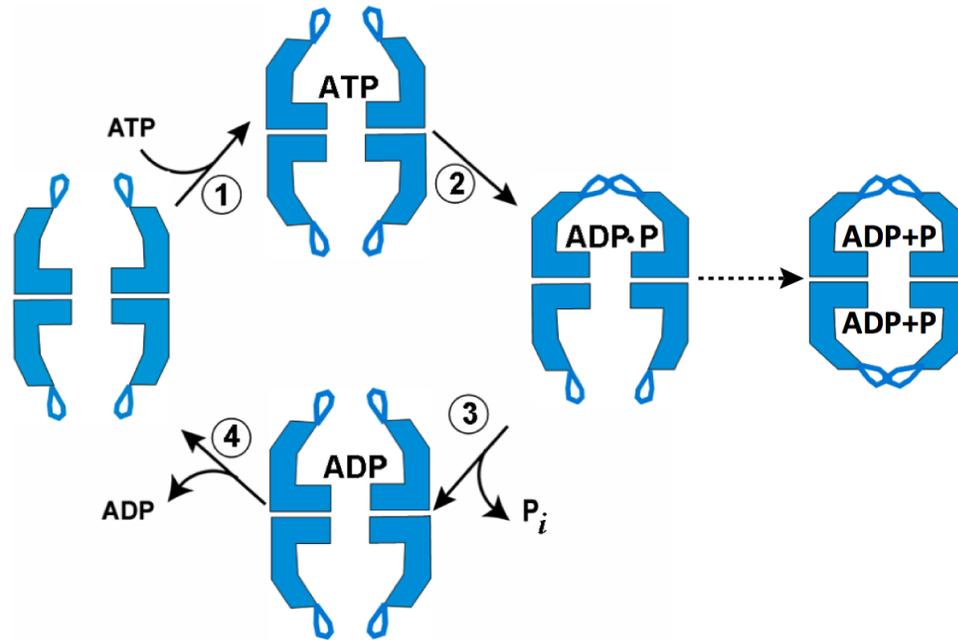


TRiC Conformational Changes in an ATP-driven Manner



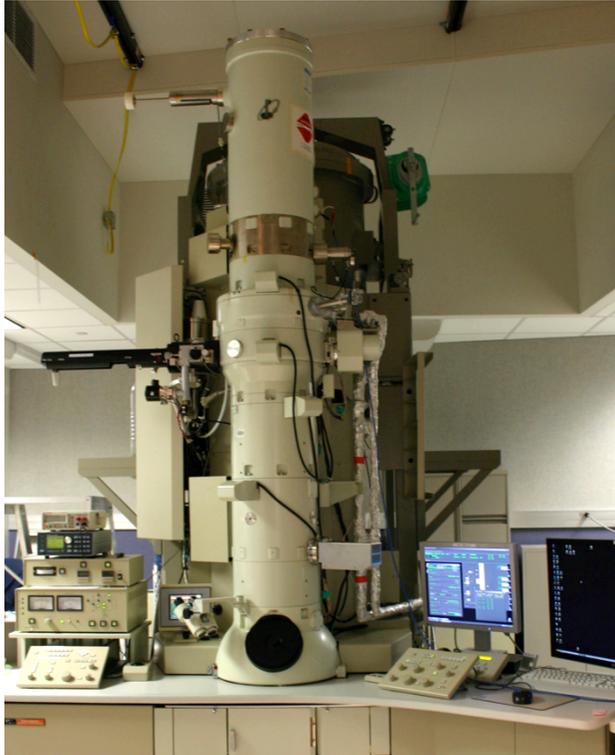
- TRiC is the most complicated chaperonin
- TRiC-assisted substrate folding is closely associated with its ATP-driven conformational changes.
- No high resolution structure of ATP-hydrolysis transition state yet.
- The structural effects of ATP-binding and hydrolysis on the two lids of TRiC remain uncertain.

Objectives of this Study



- Reveal TRiC conformational changes throughout the entire ATPase cycle.
- Capture the intermediate ATP hydrolysis transition state, which is the physiological substrate folding state of TRiC.
- What's the mechanisms of TRiC negative inter-ring cooperativity and chamber closure?
- Why homologous chaperonins adopt different chamber closing mechanisms?

Imaging Condition of TRiC in the ATPase Cycle



- JEM-3200FSC
- 300 kV, liquid N₂ temperature
- 50,000 Magnification
- Energy filter with slit @ 15 eV
- 3D Reconstruction using EMAN1.8+
- 8-fold (C8) symmetry enforced
- Flexible fitting using DireX

4 Å Resolution Cryo-EM Map of TRiC Reveals its Unique Subunit Arrangement

TRiC Introduction

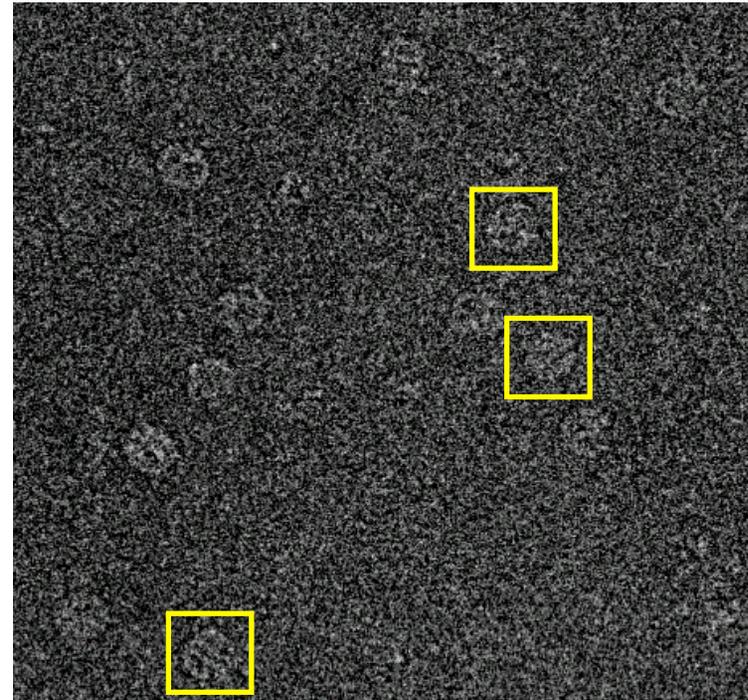
- TRiC: the most complicated chaperonin
 - 2 rings, each ring has 8 different subunits
 - They share 27~39% sequence identity
- Purposes of our study:
 - Spatial arrangement of the 8 distinct subunits in each of the two rings
 - Chemical properties of the inner chamber
- Model system:
 - TRiC-ATP-AIFx in the both ring closed conformation



Image of TRiC-ATP-AIFx



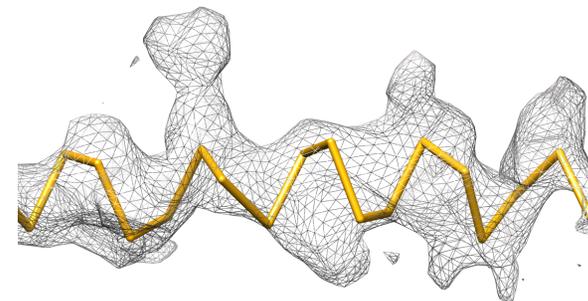
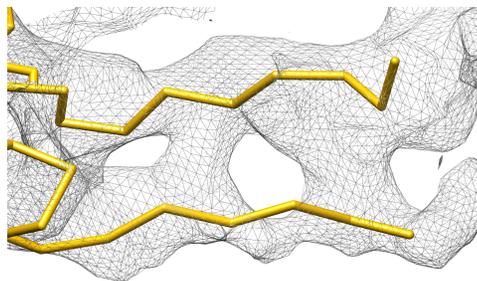
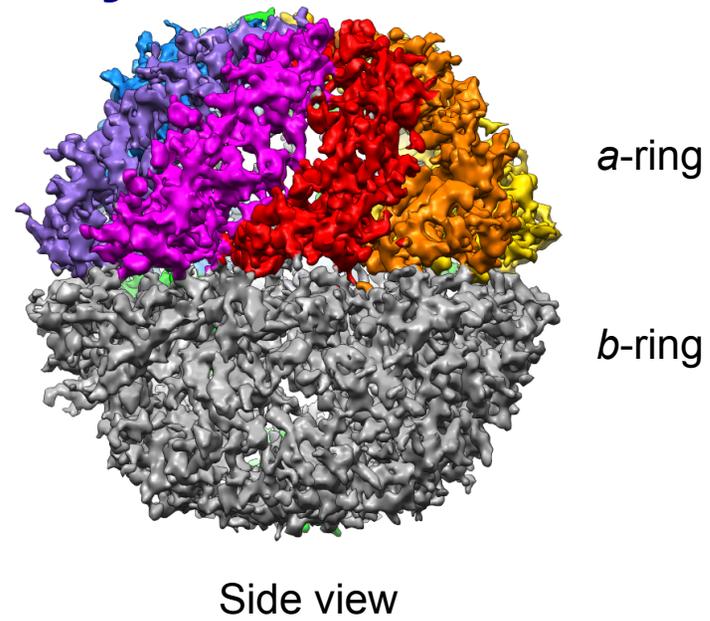
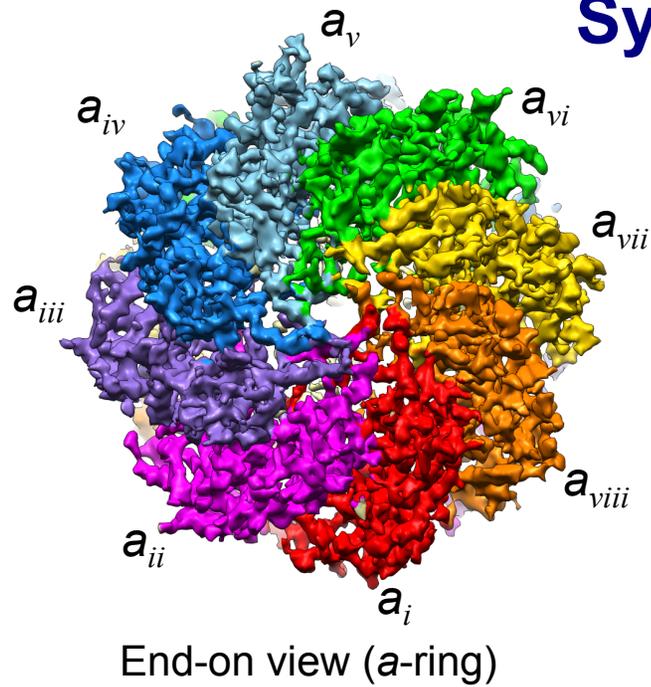
- JEOL 3200FSC EM
- 300 kV, LN₂ temperature



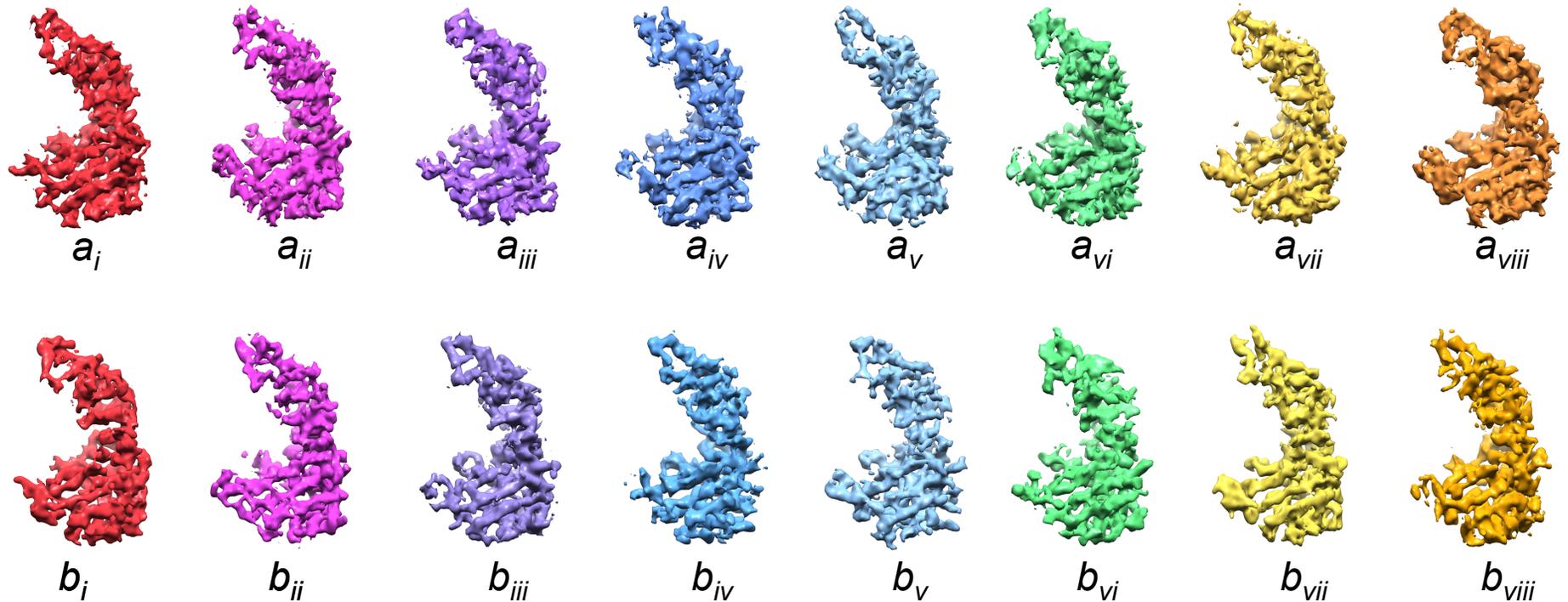
- ~101,000 particles for 3D reconstruction
- 3D reconstruction: EMAN1.8+
- 2D image alignment: FRM2D

No symmetry imposed in the 3D reconstruction!

4.7 Å cryo-EM Map of TRiC with No Imposed Symmetry



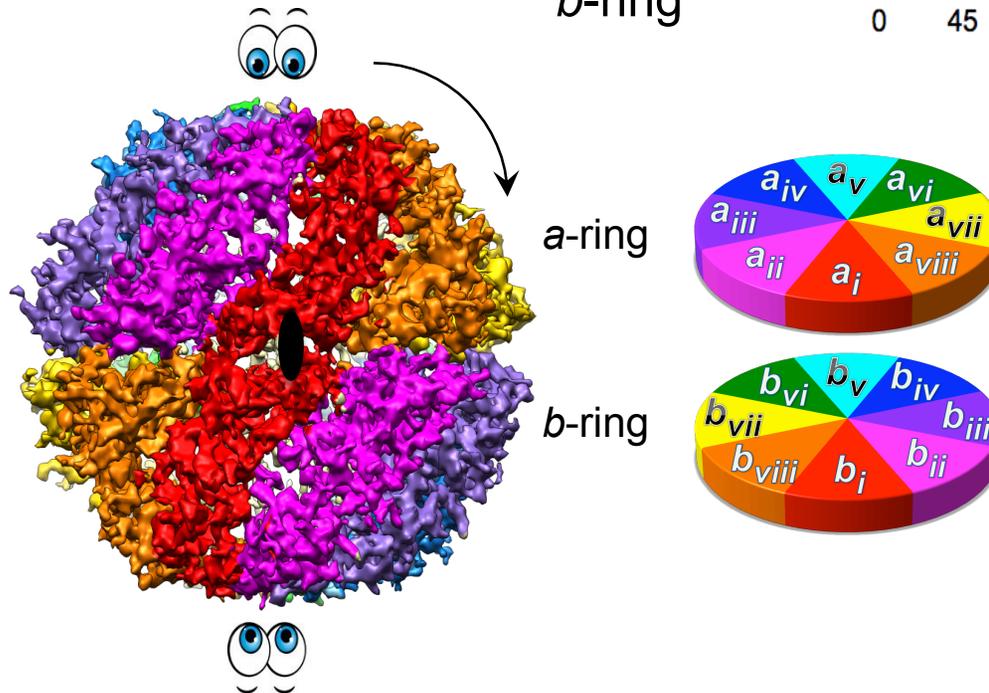
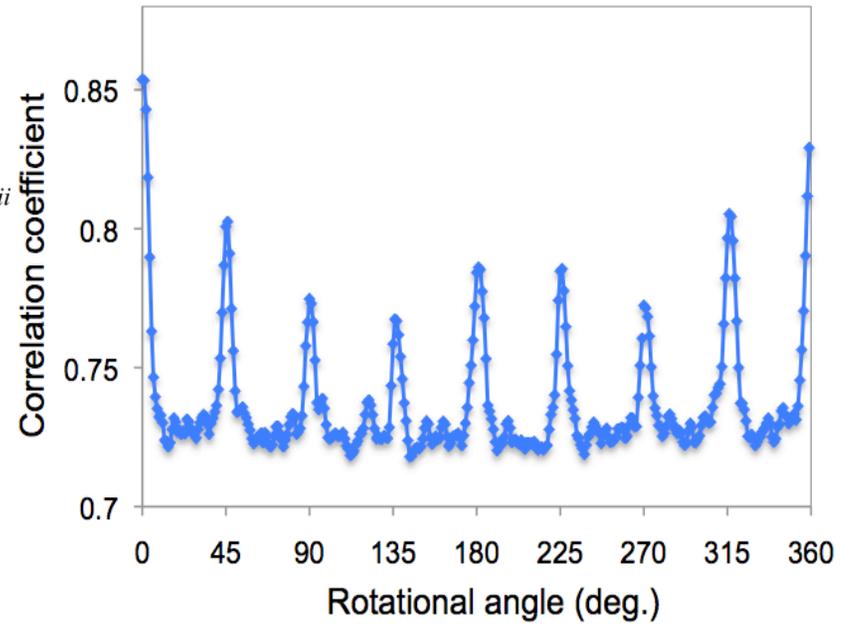
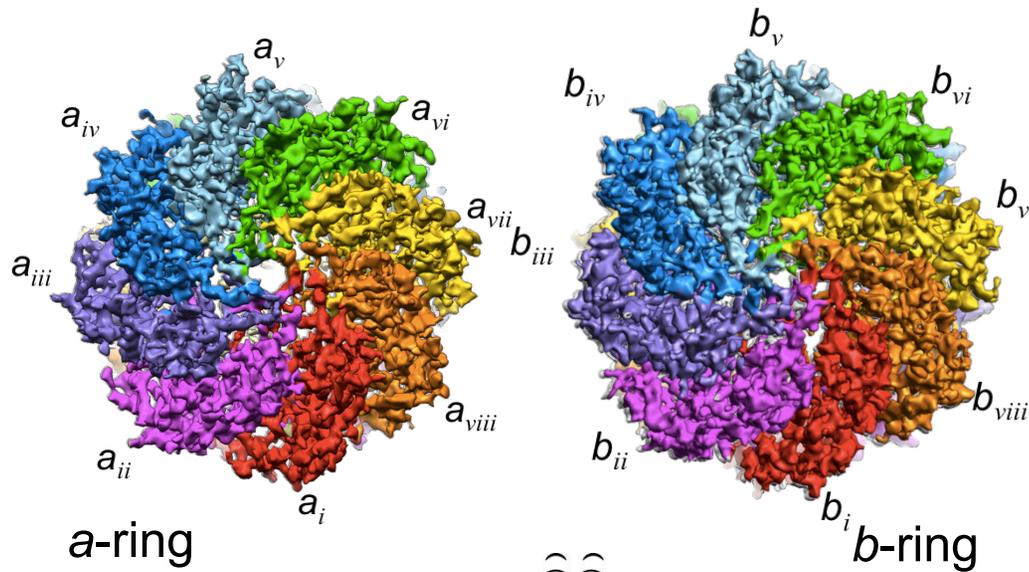
Subunits Ordering in the Two Rings



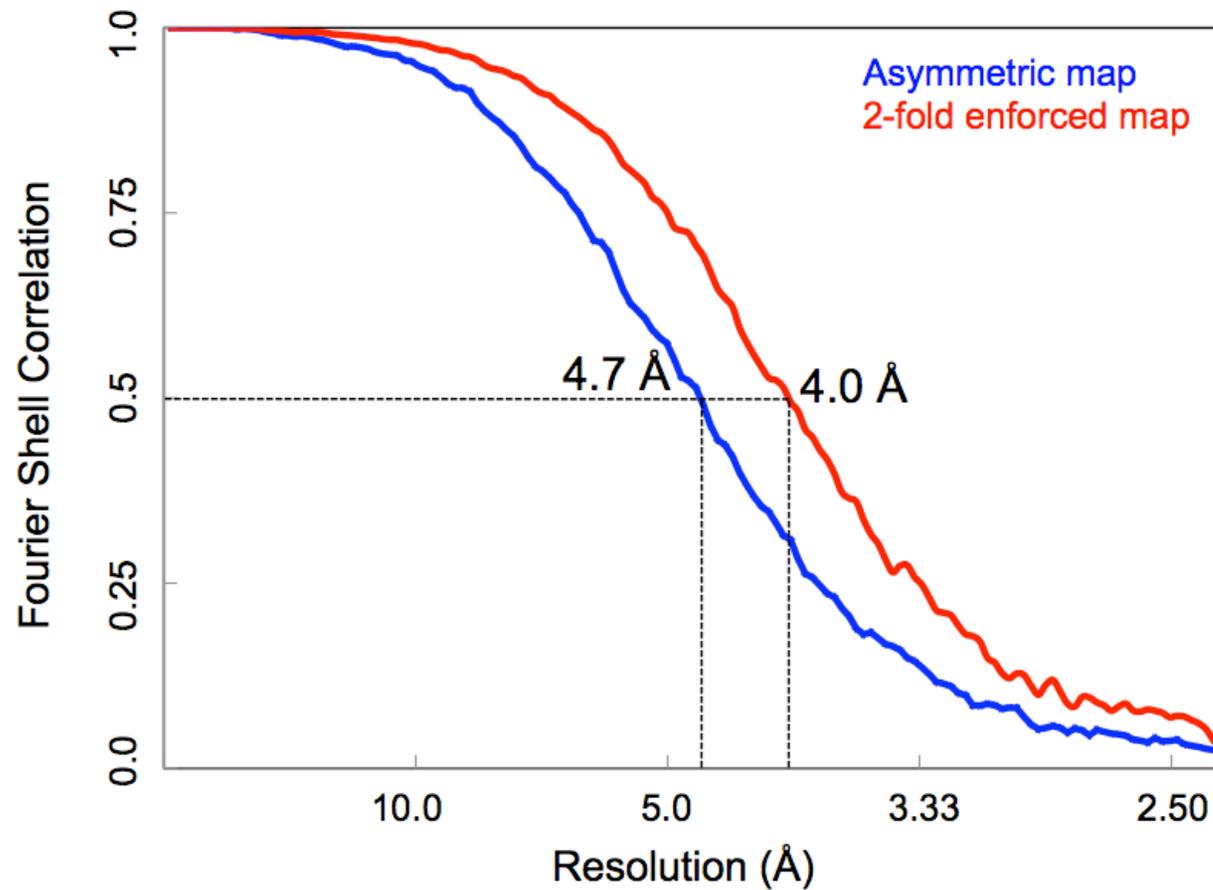
a-ring subunits	a_i	a_{ii}	a_{iii}	a_{iv}	a_v	a_{vi}	a_{vii}	a_{viii}
Identical subunits in b-ring	b_i	b_{ii}	b_{iii}	b_{iv}	b_v	b_{vi}	b_{vii}	b_{viii}

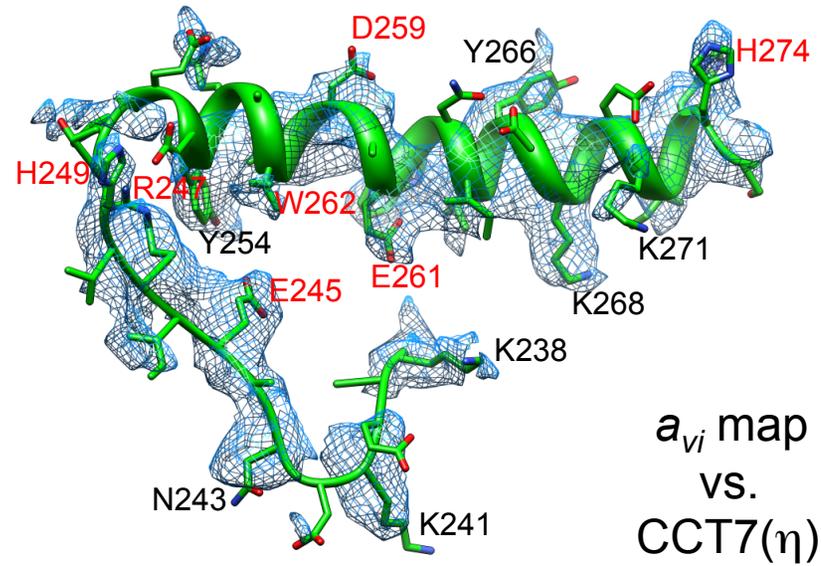
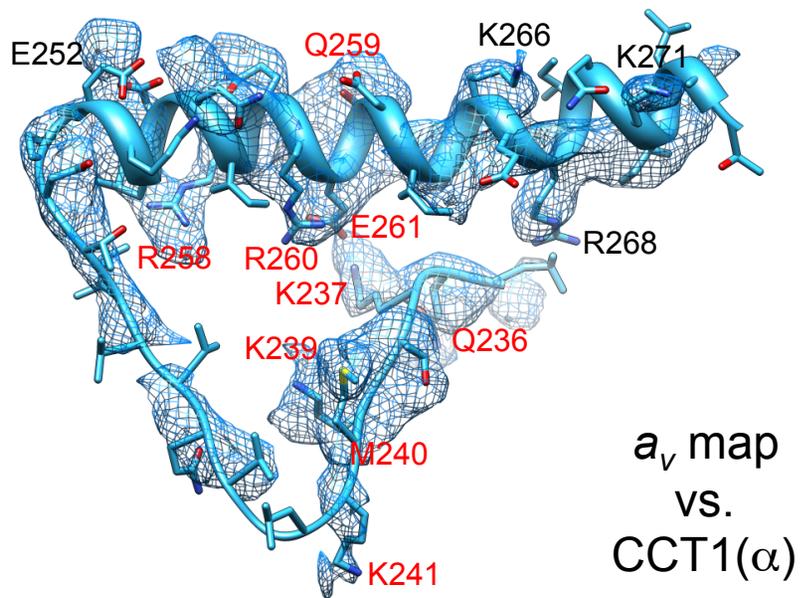
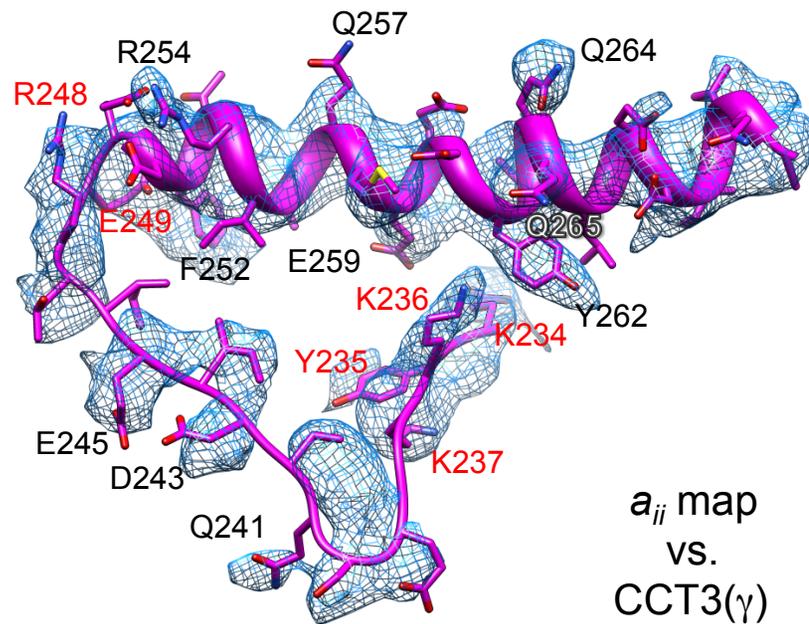
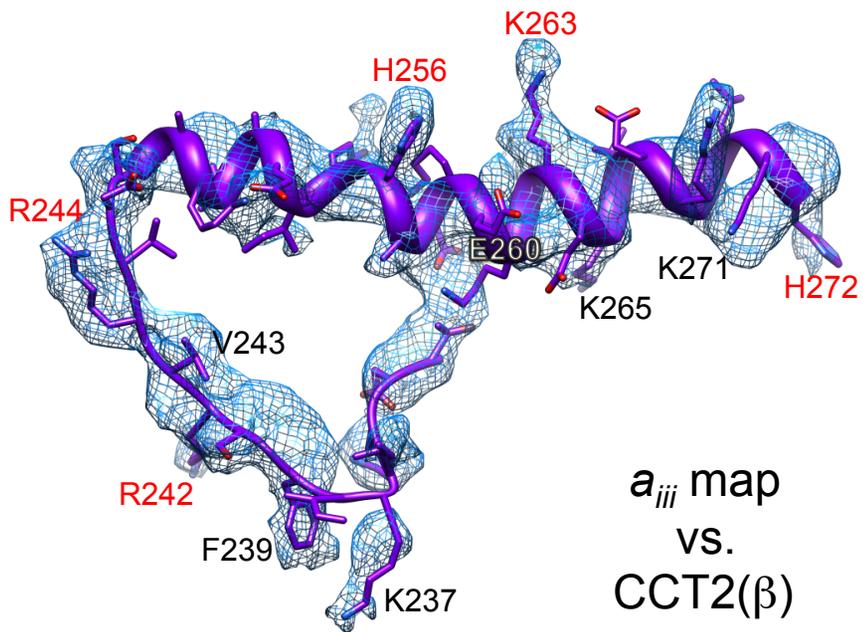
- Subunits follow the same ordering in the two rings
- 2-fold axis between the rings exists

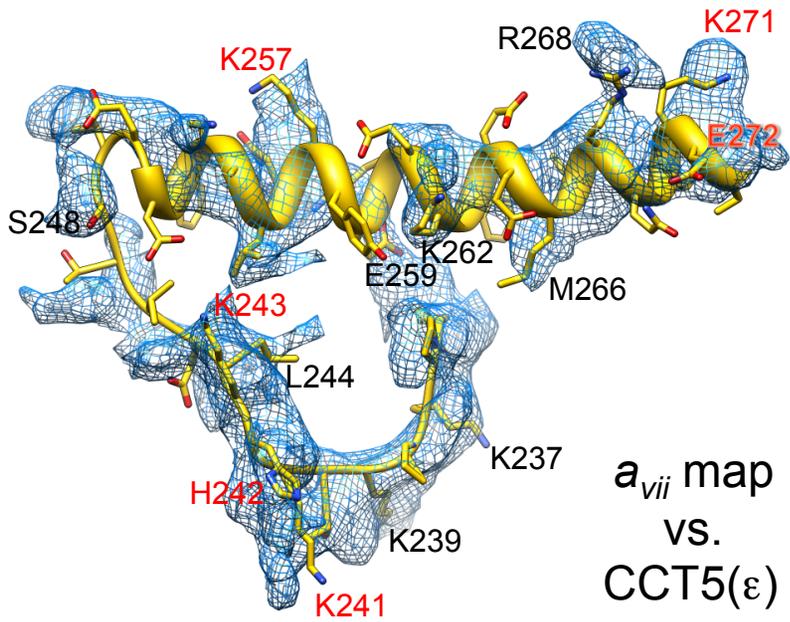
2-fold Axis Identification



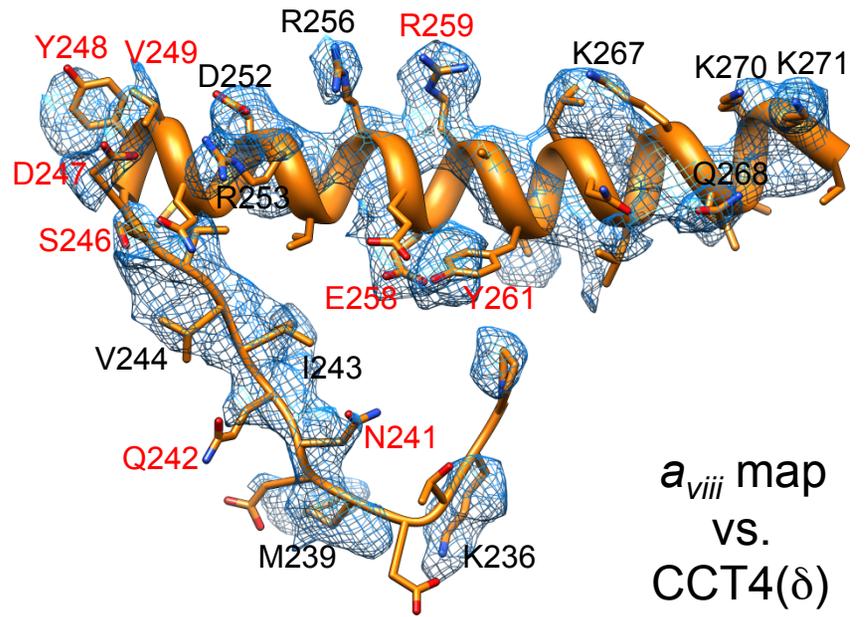
2-fold Enforced Reconstruction at 4.0 Å





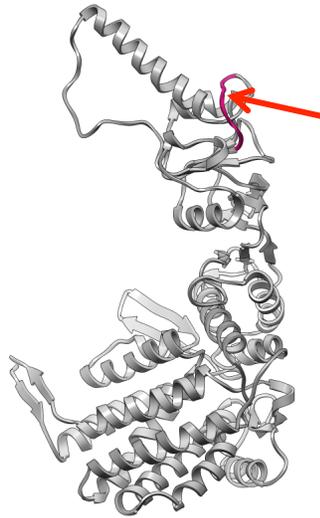


a_{vii} map
 vs.
 CCT5(ϵ)



a_{viii} map
 vs.
 CCT4(δ)

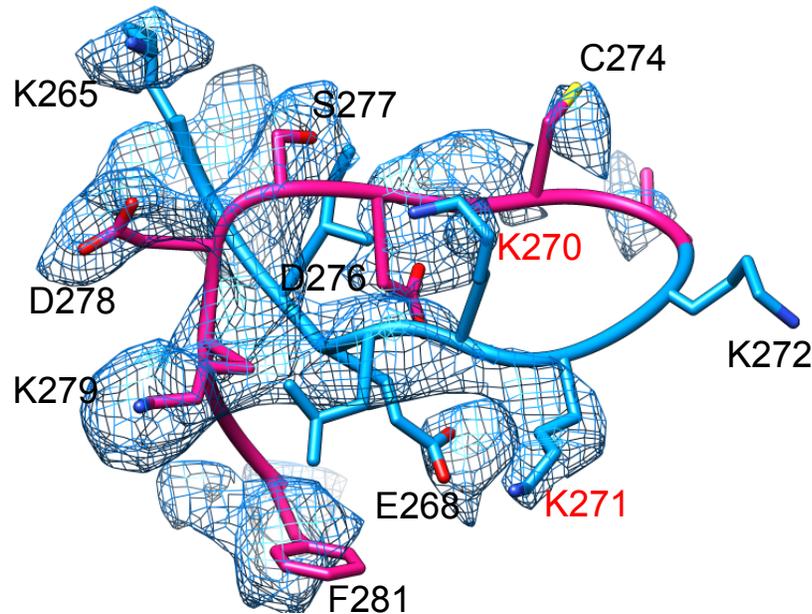
TRiC 8 Subunits Spatial Arrangement Identification



CCT2(β)	KMKEKVERILKHGIN-----CFIN
CCT7(η)	ILYDKLEKIHHSYAK-----VVLS
CCT1(α)	ITKERIQKILATGAN-----VILT
CCT5(ϵ)	KFEEMIRQIKETGAN-----LAVC
CCT4(δ)	YILNLVKQIKKTGCN-----VLLI
CCT3(γ)	YIQQLCEDIIQLKPD-----VVIT
→ CCT6(ζ)	FIEDRVK KIIE LKK KVCGDSDKGFVVIN
CCT8(θ)	LMDAQVKAIAADTGAN-----VVVT

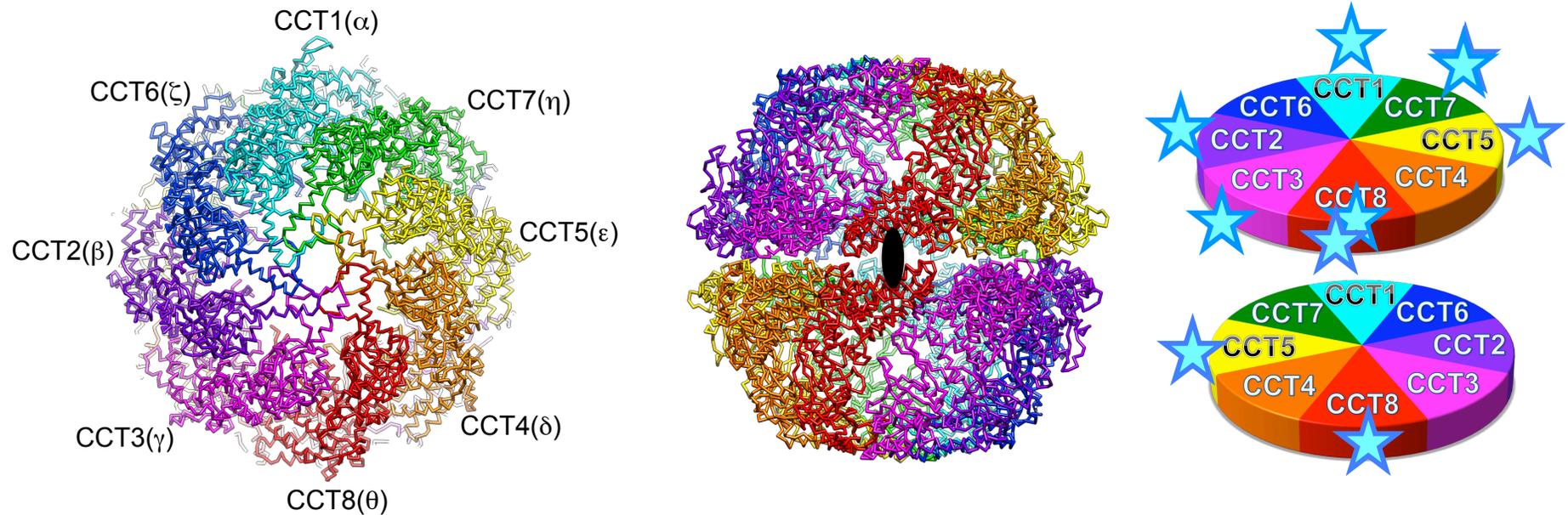
a_{iv} map vs. CCT6(ζ)

a_{iv} map



²⁶⁵KIIE**LKK**KVCGDSDKGF²⁸¹

Refined C α Backbone Model and Subunits Spatial Arrangement of TRiC

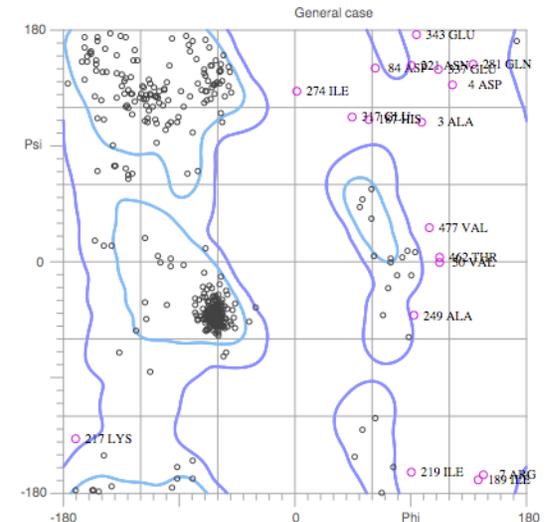
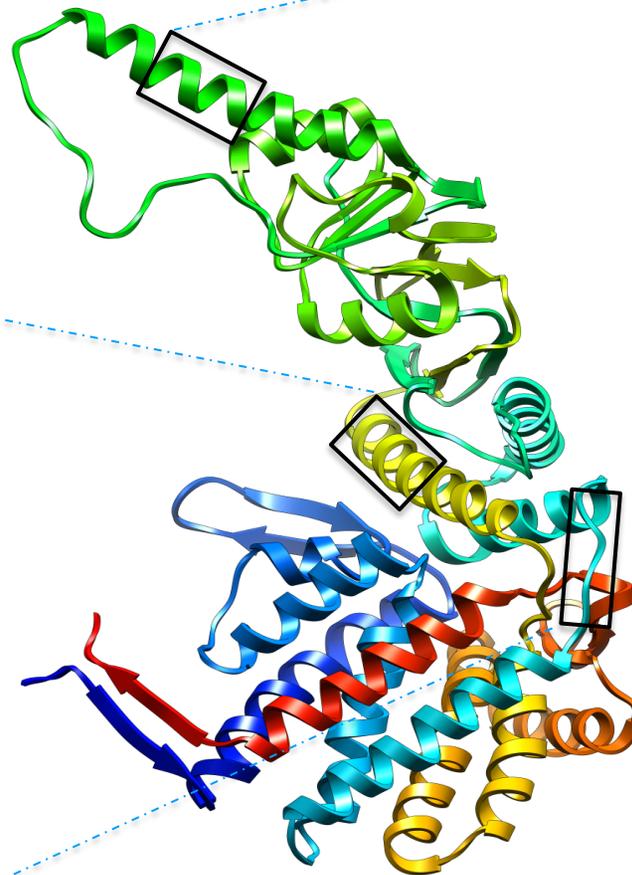
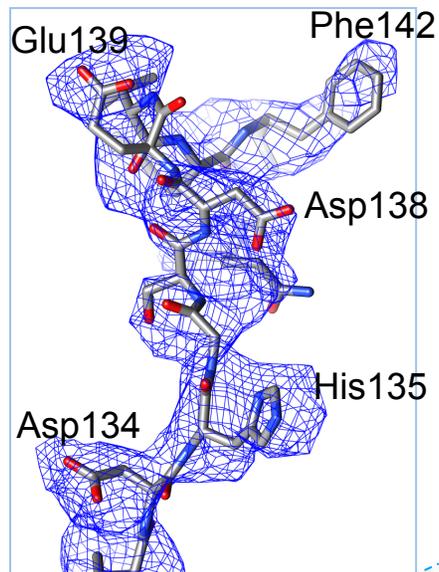
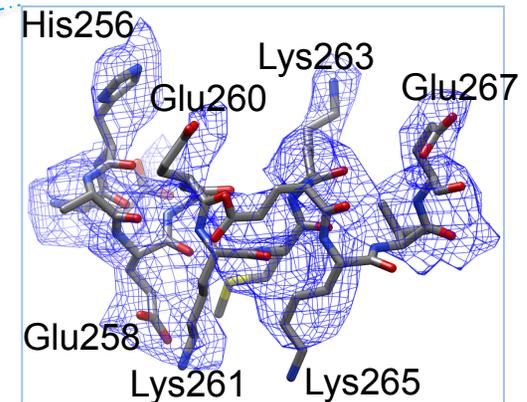
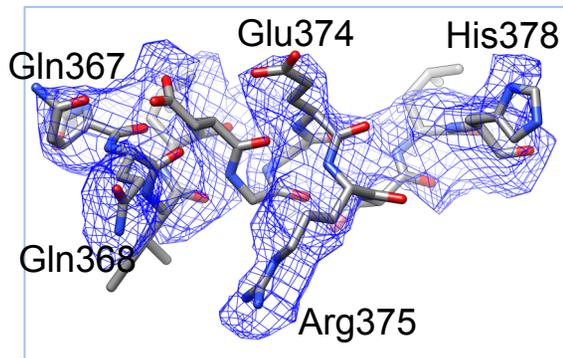


Cross-linking and nearest neighbor analysis result

Crosslink	CCT1-CCT7	CCT7-CCT5	CCT8-CCT3	CCT2-CCT5	CCT8-CCT8
Our model	☺	☺	☺	☺	☺

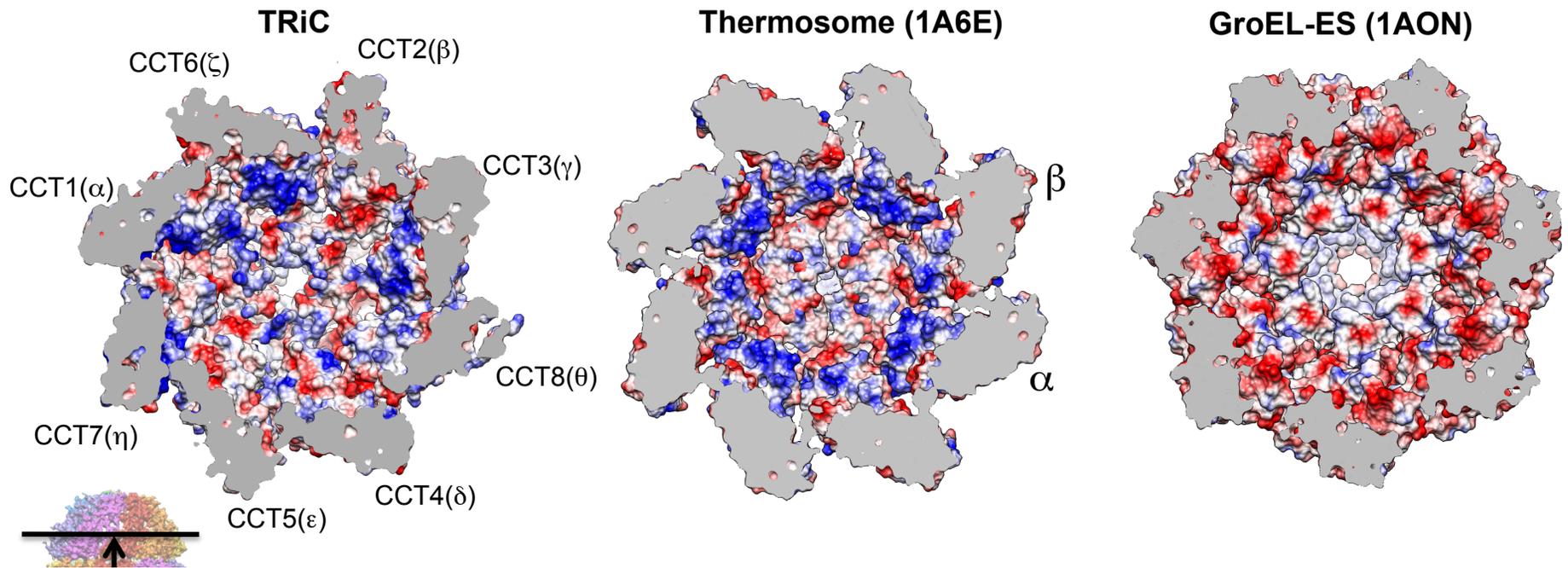
- Cross-linking data support our model of subunits arrangement

Refined All-Atom Model and Quality Evaluation



~96% of the dihedral angle in the allowable region

Surface Property Comparison of Chaperonin



- An unevenly distributed positively charged wall lining the closed folding chamber of TRiC, strikingly different from other chaperonins.
- This might be related to TRiC's differential ability to fold some substrates that can not be folded by any other chaperonins.

Summary

- 4.7 Å resolution TRiC asymmetric cryo-EM structure, determined the location of the 2-fold axis between the two rings.
- Based on the 4.0 Å resolution 2-fold imposed map, we identified the TRiC 8 distinct subunits arrangement.
- The subunit arrangement was supposed by independent cross-linking and near neighbor analysis
- An unevenly distributed positively charged wall lining the closed folding chamber TRiC, strikingly different from other chaperonins.
- This might be related to TRiC's substrates specificity.

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