Two rings for folding
Crystal structure of the mammalian chaperonin CCT in complex with tubulin

Inés G. Muñoz[1], Hugo Yébenes[2], José Maria Valpuesta[2] and Guillermo Montoya[1]

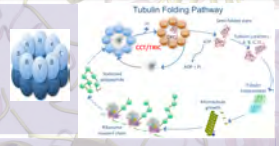
[1]Macromolecular Crystallography Group. Structural Biology and Biocomputing Programme. Spanish National Cancer Research Centre (CNIO). Melchor Fernández Almagro 3. 28029 Madrid. SPAIN.
[2]Centro Nacional de Biotecnología (CNB-CSIC). Campus de la Universidad Autónoma de Madrid. Darwin 3. 28049 Madrid, SPAIN.
E-mail: imunoz@cnio.es

What's CCT?

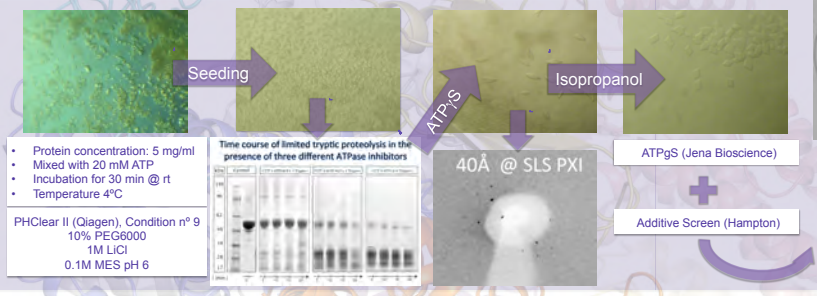
Protein folding is assisted by molecular chaperones. Among them, chaperonins are one of the most important families, and CCT (chaperonin containing TCP-1, or TRiC) its major representative. This 1 MDa nanomachine regulates the folding of important proteins including actin, α-tubulin and β-tubulin. We used an electron density map at 5.5 Å resolution to reconstruct CCT, which showed the structure in an open conformation with tubulin bound to the inner cavities of both rings.

This has provided information about its mechanism and the basis for understanding its function.

- It is found in the eukaryotic cytosol
- Composed by two rings (stacked back-to-back)
- Each ring consists on 8 different subunits of ~60 KDa, each divided in three domains: equatorial (ATP-binding site), apical (substrate binding), and intermediate domain
- The unfolded proteins bind to the central cavity of the rings
- ATP is the energy driving force



Crystallization



- Protein concentration: 5 mg/ml
- Mixed with 20 mM ATP
- Incubation for 30 min @ rt
- Temperature 4ºC

PHClear II (Qiagen), Condition nº 9
10% PEG6000
1M LiCl
0.1M MES pH 6

Time course of limited tryptic proteolysis in the presence of three different ATPase inhibitors

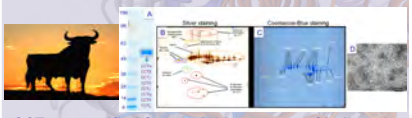
ATPgS (Jena Bioscience)

Additive Screen (Hampton)

Opti-Salts (Qiagen)

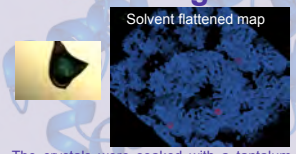
- Drop ratio screen
- Evaporation control

Protein Production



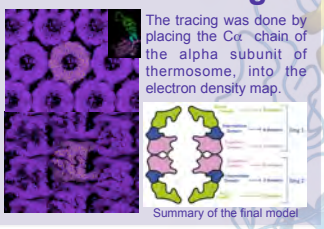
CCT was purified from soluble extracts of bull testis
(A) SDS-Page analysis of the CCT stock after gel filtration.
(B) & (C) 2D-electrophoresis gel.
Study of the posttranslational modifications.
(D) Electron microscopy image with negative staining.

Phasing



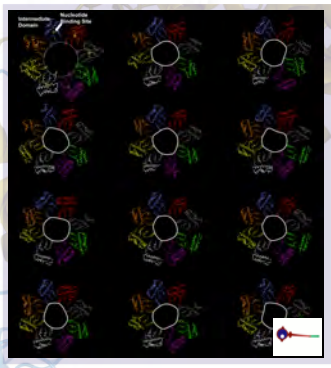
The crystals were soaked with a tantalum bromide cluster (2mM / Ta_6Br_{12} / 4h). SAD data was collected at PSI (SLS), from which we calculated the initial phases.

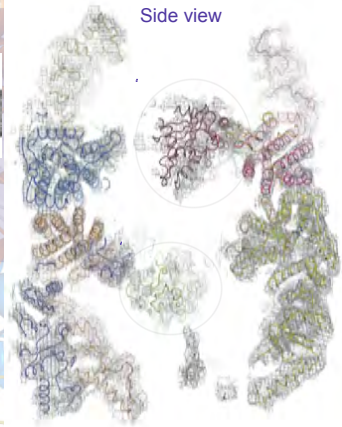
Model Building



The tracing was done by placing the Cα chain of the alpha subunit of thermosome, into the electron density map.

Summary of the final model





The initial map showed **two large internal extra densities**, one bound to each ring, corresponding to a trapped substrate that co-purified during the CCT protein preparation.

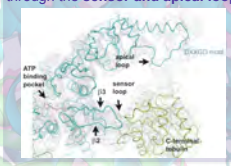
Substrate recognition and folding mechanism

Is the substrate interaction with the sensor loop inducing nucleotide binding, or, the nucleotide binding the cause of the loop extension towards the substrate? Different conformations adopted by all the subunits in one of the CCT rings are represented in the pictures. The white line joins the relative positions of the tip from the sensor loops, showing how this mechanical process could provide the necessary mechanical energy during the folding process, exerting force on the substrate (like a lever).

Results

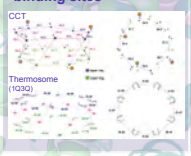
CCT Substrate characterization

CCT recognizes the substrate through the **sensor and apical loops**

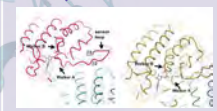


The different conformations adopted by the **apical domains** in the ring

Comparison of the ATP binding sites

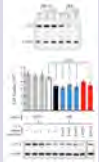


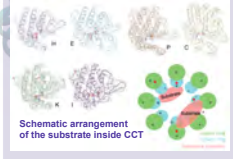
The equatorial domains **closer to the substrate, present clear electron density to position the ATPγS molecule**.



To confirm nucleotide presence along the ring.

To prove the relevance of the sensor loop we performed silencing analysis against the CCTβ subunit.





In both rings, **one of the subunits in contact with the substrate showed the presence of Se**. The second signal comes from a subunit that sits in an opposite place with no contact to the substrate.

References

1. Crystal structure of the open conformation of the mammalian chaperonin CCT in complex with tubulin. Muñoz, I.G., Yébenes, H., Zhou, M., Mesa, P., Serna, M., Park, A.Y., Bragado-Nilsson, E., Beloso, A., de Cárcer, G., Malumbres, M., Robinson, C.V., Valpuesta, J.M. and Montoya, G. (2011) *Nat. Struct. Mol. Biol.* **18**, 14-9.
2. Chaperonins: two rings for folding. Yébenes, H., Mesa, P., Muñoz, I.G., Montoya, G. and Valpuesta, J.M. (2011) *Trends Biochem. Sci.* **36**, 424-32. Review.