

## Crystal Structure of Group II Chaperonin in the Open State

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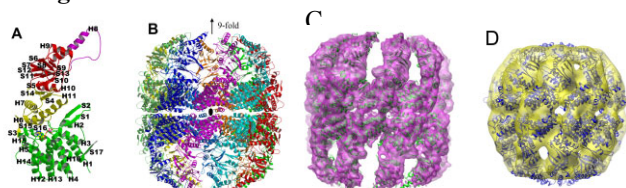
### Abstract

Thermosomes are group II chaperonins responsible for protein refolding in an ATP-dependent manner. Little is known regarding the conformational changes of thermosomes during their functional cycle due to a lack of high resolution structure in the open state. Here we report the first complete crystal structure of thermosome (rATcpn $\beta$ ) in the open state from *Acidianus tengchongensis*. There is a  $\sim 30^\circ$  rotation of the apical and lid domains compared with the previous closed structure. Besides, the structure reveals a conspicuous hydrophobic patch in the lid domain, and residues locating in this patch are conserved across species. Both the closed and open forms of rATcpn $\beta$  were also reconstructed by electron microscopy (EM). Structural fitting revealed the detailed conformational change from the open to the closed state. Structural comparison as well as protease K digestion indicated only ATP binding without hydrolysis does not induce chamber closure of thermosome.

### Crystal structure of rATcpn $\beta$ in the open state

The first complete structure of group II chaperonin in its open state was solved at 3.7Å high-resolution. The subunit shares a common fold with other group II chaperonins in closed state, but the first beta strand was absent compared to other closed structure (Fig1 A). This crystal structure is also the first reported 9-fold chaperonin, thus supplying us useful information to understand the diverse assembly of chaperonins (Fig1 B).

Fig 1.



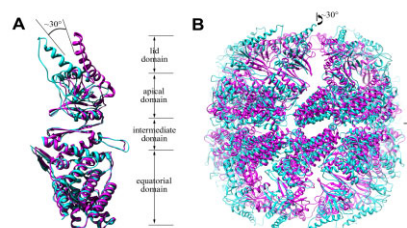
### Electron microscopy study of rATcpn $\beta$ and structural fitting

Three-dimensional cryoEM structures of rATcpn $\beta$ \_apo and rATcpn $\beta$ \_ATP with imposed 9-fold symmetry were reconstructed with resolution 8.8Å and 8.4Å. Docking the crystal structure of the rATcpn $\beta$  subunit into the cryoEM density, all equatorial, intermediate and apical domains exhibit an excellent fit (Fig 1 C). Docking the thermosome TKcpn subunit structure (PDB 1Q3R) en bloc into the cryoEM map of rATcpn $\beta$ \_ATP doesn't yield good fit. Three-dimensional negative stained EM structures of rATcpn $\beta$  were reconstructed at 14Å. Docking the crystal structure of the rATcpn $\beta$  subunit into the density doesn't yield good fit. But the thermosome TKcpn subunit can fit very well (Fig 1 D).

### Structure comparison between open and closed state

The rATcpn $\beta$  thermosome models in open and closed form were aligned after superimposing their 9-fold axes and equatorial planes, exhibiting large conformational changes. Both a  $\sim 30^\circ$  counterclockwise rotation of the apical and lid domains (Fig2 A) and an inward movement of the entire subunit were found to be indispensable for transition from open to closed state (Fig2 B).

Fig 2.



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