Supplemental Online Material

Essential function of the built-in lid in the allosteric regulation of eukaryotic and archaeal chaperonins

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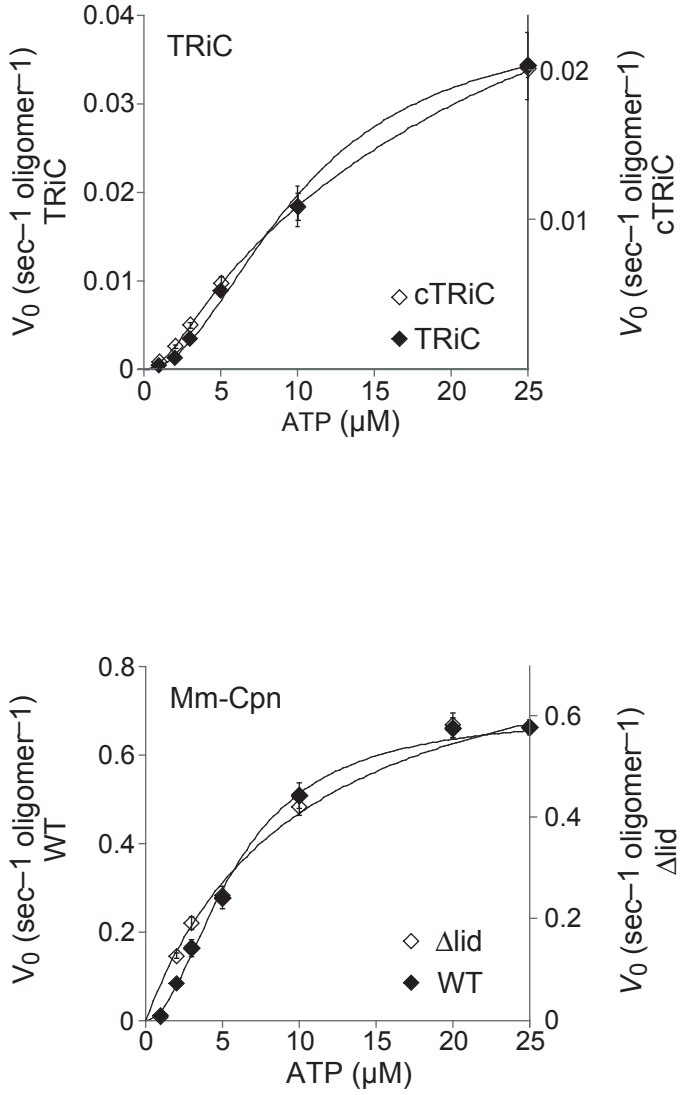
Supplementary Figure S1: Cryo-EM analysis reveals preservation of the chaperonin-like oligomeric assembly in cTRiC. Two representative reference free class averages of TRiC (*left*) and cTRiC (*right*) in top and a side view are shown.



Supplementary Figure S2: Direct comparison of the first allosteric transition in wild type and lid-less chaperonins. The kinetics obtained for intact and lid-less chaperonin from 0 to 25 μM ATP are overlaid here for both TRiC (top panel) and Mm-Cpn (bottom panel).

Supplemental Methods

Sample preparation for cryo EM analysis. cTRiC samples were prepared as described above but starting from 1 μM TRiC in buffer A without glycerol and PEG 8000. Samples were embedded in vitreous ice as follows: 3 μl of TRiC and cTRiC sample respectively were placed onto a washed, glow-discharged 200 mesh R2-1 Quantifoil continuous carbon grid (Quantifoil Micro Tools GmbH, Jena Germany). The grid was blotted and flash frozen in liquid ethane using a Vitrobot (FEI, Hillsboro, Oregon, USA). Grids were stored in liquid nitrogen until imaging.

Image Collection. Ice-embedded images were acquired at an effective magnification of 83,100x on a Gatan 4Kx4K CCD camera (Gatan, Pleasanton CA) using a JEOL2010F electron microscope (JEOL Inc, Tokyo Japan) with a field emission gun operated at 200 kV. The microscope was equipped with the JEOL telemicroscopy software package (FasTEM) (JEOL USA Inc, Peabody MA) and a Gatan Model 626 cryostage (Gatan, Pleasanton CA). In addition, a custom software package developed in-house, JEOL Automated Microscopy Expert System (JAMES), was used to acquire focal pairs of images semi-automatically, at a range of 0.8-2.5 nm underfocus for the first image and a range of 2-5 nm underfocus for the second image.

Generating Reference Free Class Averages. Particle images were selected from the micrographs by hand using the EMAN software program 'boxer'[44]. Using this procedure, 2,875 TRiC particles and 4,522 particles of clipped TRiC were identified for further image processing. Reference free class-averages were generated using the 'refine2d.py' program in EMAN[45]. Therefore 5 iterations of the SVD-based procedure were used to generate 100 reference free class averages from the ice embedded particle images. In the side and top views shown in the figure 1 between 50 and 80 particles from the same orientation were averaged to form the class average.