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ORAL ABSTRACT PRESENTATIONS
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Abstract OR-11: Conformations of a Viral Chaperonin in Different Nucleotide-Bound States

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Background: Chaperonins are protein complexes which assist nascent or misfolded proteins in attaining their native conformation. Several viral chaperonins have recently been found in the genomes of bacteriophages. In this study cryo-EM structures of a viral chaperonin from the phage OBP in different nucleotide-bound states were obtained.

Methods: The recombinant OBP chaperonin was expressed in *E.coli* and purified using chromatography on Q-sepharose. Chaperonin complexes with nucleotides were vitrified in Vitrobot Mark IV (FEI). Images were collected in cryo-TEM Titan Krios (Thermo-Fisher) equipped with Schottky FEG electron source and direct electron detector Falcon II. Image processing and model generation was performed using Warp, CryoSPARC and Relion.

Results: 4.5-Å-resolution structure of the apo-form of the OBP chaperonin is a single ring composed of 7 identical subunits with a surprising degree of asymmetry at the upper part of the complex. Because of two sets of nucleotide-binding sites in chaperonin revealed by isothermal titration calorimetry, there are two structurally different subunit conformations. Therefore, subunits are arranged in the ring into three pairs stabilized by a number of salt bridges. The unpaired subunit lacks these contacts, which leads to a higher mobility and the fact that its intermediate and apical domains were not resolved in our reconstruction.

To study the effects of nucleotide binding on the OBP conformations, we obtained reconstructions at 4-6 Å of the OBP with ADP, ATPγS (a non-hydrolysable ATP analogue) and ATP. In the case of ATPγS, the conformation was the same as for the ADP-bound chaperonin. In all these structures only one or two pairs of subunits were resolved above the intermediate domains, which indicates that nucleotide binding results in the partial breakage of inter-subunit salt bridges and a higher structural mobility. Importantly, none of the tested nucleotides cause rotation of the apical domains typical for GroEL. The rotation, which provides cis/trans switching in GroEL, is enabled by the "hinge" residues, Gly192 and Gly375, between the intermediate and apical domains. In the OBP chaperonin they are replaced by the

large polar residues Glu191 and Asn376 thus fixing the position of the apical domains relative to intermediate.

Conclusion: Phage OBP chaperonin possesses a unique asymmetric single-ring structure and a different ATP-dependent protein folding cycle compared to its presumable ancestor GroEL.

Key Words: chaperonin • phage OBP • cryo-EM

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