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POSTER ABSTRACT PRESENTATIONS

SESSION TITLE: STRUCTURE OF VIRUSES AND CHAPERONINS

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Abstract P-45: Structure of the Bacteriophage AR9 *Bacillus Subtilis* Chaperonin According to Cryo-Electron Microscopy

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Background: Chaperonins are a family of molecular chaperones Hsp60 (heat shock proteins 60). GroEL is a bacterial chaperonin. It ensures the correct folding of proteins, using the energy of ATP hydrolysis. Three-dimensional reconstructions of its predicted orthologs were obtained and biochemically characterized in free and nucleotide-bound states for bacteriophages EL *Pseudomonas aeruginosa*, OBP *Pseudomonas fluorescens* (Kurochkina, L. P. et al., *Journal of virology*, 2012; Semenyuk, P. I. et al., *Biochemical Journal*, 2016; Stanishneva-Konovalova, T. B. et al., *Journal of Structural Biology*, 2020). Physicochemical studies were carried out for the bacteriophage AR9 *Bacillus Subtilis* and confirmed that the protein has chaperone activity and does not require co-chaperonin to function (Semenyuk P. I. et al., *International Journal of Biological Macromolecules*, 2020).

Methods: The recombinant chaperonin of the *B. subtilis* bacterial phage AR9 (gp228) was isolated and purified in a free state and vitrified in Vitrobot Mark IV. Data were collected using a Titan Krios cryo-TEM and processed in Warp, RELION and cryoSPARC software.

Results: The final structures of the chaperonin were reconstructed with a C1 and C7 symmetry at the resolution of 4.5 Å and 4.0 Å respectively. Significant heterogeneity of the apical domains was addressed further using 3D classification and symmetry expansion in RELION resulting in a set of classes reflecting the conformational transition of the subunits between different states. At least four different conformational states of the subunit were clearly resolved.

Conclusion: Gp228 structure show similarities between bacteriophage chaperonin and also bacterial chaperonin GroEL. It is formed by a single ring

consisting of seven identical subunits, each has three domains: equatorial, intermediate, and apical. The subunits of the apo-form chaperonin Gp228 exhibit significant conformational flexibility in the apical and intermediate domains.

Key Words: chaperonin • cryo-EM • structure

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