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POSTER ABSTRACT PRESENTATIONS

SESSION TITLE: STRUCTURE OF VIRUSES AND CHAPERONINS

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Abstract P-46: Structure of *A. Baumannii* Phage Tapaz, Revealed with Cryo-Electron Microscopy

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Background: *Acinetobacter baumannii* is an opportunistic pathogen and one of the six most important multidrug resistant microorganisms in hospitals worldwide. Some of its strains are resistant to most of the antibiotics, *A. baumannii* is included into the Priority 1 part of Global Priority List of Antibiotic-resistant Bacteria. Phage therapy is considered to be an alternative strategy to antibiotic treatments.

Methods: *A. baumannii* strain NIPH601 cells were grown till OD_{600}0.4 and infected with the phage at MOI 10:1. After complete lysis took place cell debris was spined down and phage particles were precipitated with the PEG6000 (final concentration 10% PEG 6000, 0.5 NaCl). Virus particles were collected by centrifugation, resuspended at SM buffer and applied on CsCl step gradient. Gradient was spinned down for 2 hours at 40000g and the fraction containing phage particles was collected and dialyzed against SM buffer.

Purified phage particles were applied to Quantifoil 1.2/1.3 grids and plunge-froze in Vitrobot Mark IV (TFS) Micrographs were collected in HKU, Shenzhen campus with Titan Krios cryoelectron microscope (TFS), equipped with Gatan K3 direct electron detector. The micrographs were acquired with 1.06 Å pixel size and 1.5 um average defocus value in counting mode with 50 frames and 1.2 e/Å^2/frame dose rate. All image processing was performed with Relion3.0 software, except for the particle picking step performed with cryolo.

Results: Lytic *A. baumannii* phage TaPaz belongs to the family *Myoviridae*. BLAST search over NCBI "nr" (non-redundant) database revealed close homology with previously published sequences of *Acinetobacter* phage vB_AbaM_B9 and *Acinetobacter* phage BS46. However, no structural information about any homologous proteins was found among the PDB structures.

The cryo-EM map was reconstructed with single particle analysis independently for the capsid, tail and baseplate regions. The capsid was reconstructed at 3.9 Å resolution with I3 symmetry applied (Fig. 1A). The baseplate region of the phage was reconstructed at 3.5 Å resolution with C3 symmetry (Fig. 1B). The tail region was reconstructed at 2.6 Å resolution with helical symmetry (Rise 36.4 Å, Twist 25.7 deg). Initial atomic model for the tail region was built from sequence with Deeptracer and was further refined in coot (Fig. 1C).

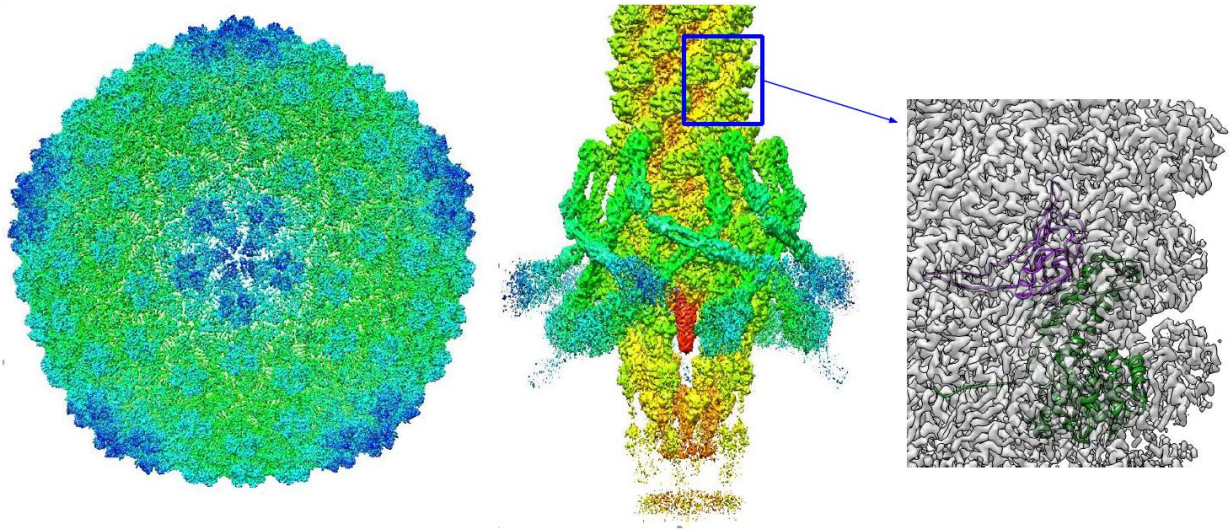

Fig. 1. *(A) – Capsid and (B) baseplate cryo-EM maps, (C) – Tail sheath and tube asymmetric subunit cryoem map with atomic model*

Conclusion: We successfully obtained the near-atomic resolution structural map of phage TaPaz. The data obtained contribute to enhancing knowledge of structural diversity of bacterial viruses infecting *A. baumannii*.

Key Words: cryo-EM •*A. baumannii* • phage TaPaz

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