

Structures of the capsid and the tail of Myoviridae bacteriophage TaPaz, revealed by cryo-EM

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Introduction

The bacteriophages (phages) are viruses that infect bacteria and use their resources to reproduce themselves. Phages are common in all-natural environments infecting bacteria specific for their habitats.

Acinetobacter baumannii - one of the most infectious pathogens in hospitals. These nosocomial bacteria are able to form biofilms on various biotic and abiotic surfaces. Many strains of *A. baumannii* are resistant to most antibiotics known today, as well as to disinfectants and antiseptics. Also, pathogenic *A. baumannii* are tolerant to detergents, ultraviolet irradiation, and drying. Therefore, the search for antibacterial agents to combat *Acinetobacter* infections is an important pharmacological challenge. The use of specific bacteriophages is a promising strategy. Lytic bacteriophages were reported to be effective to treat the pan-resistant strain of *A. baumannii* (Schooley et al, 2017). Bacteriophages prospective for phage therapy should be comprehensively studied, including their genomics, proteomics, stability, receptor interactions etc (Luong et al, 2020). In this regard, the structural investigation of a new phage TaPaz isolated on the bacterial lawn of *A. baumannii* NIPH601 with a K47 capsular polysaccharide structure has both fundamental and applied significance. The TaPaz linear double-stranded DNA genome of 93,703 bp contains 178 open reading frames. The phage was identified as a member of the family *Myoviridae* by transmission electron microscopy.

Results

We used helical reconstruction in RELION (Scheres, 2012) to obtain the reconstruction of phage TaPaz extended tail at 3.1 Å resolution. A total of 118 raw frame stacks were processed for the extended tails. Motion correction was performed with Relion implementation of MotionCor2 with frames grouped in pairs. CTF was estimated with gctf software. Extended tails helical segments were automatically picked with cryolo using 400×400 pixels box size and 360 pixels overlap. The subsets were subjected to 2d classification in Relion. After 2d classification we selected total of 8700 helical segments of the extended tails.

The extended tail's initial helix parameters were estimated using the 2d class average power spectrum. Estimated parameters were measured as follows: rise 36.4 Å, twist 25.7 deg, 6-start helix, repeat ~255 Å. Several rounds of CTF refinement and Bayesian polishing were performed on the extended tails dataset. The final helical reconstruction yielded map (Fig. 1A) with 3.1 Å resolution according to FSC 0.143 criterion (Fig. 1B). The refined helix parameters: twist 25.5 deg, rise 36.5 Å.

To obtain the 3D reconstruction of the capsid we used 1500 raw frame stacks and applied an icosahedral symmetry (Fig. 2A). The resulting resolution was 4.3 Å, according to FSC.

Acknowledgments

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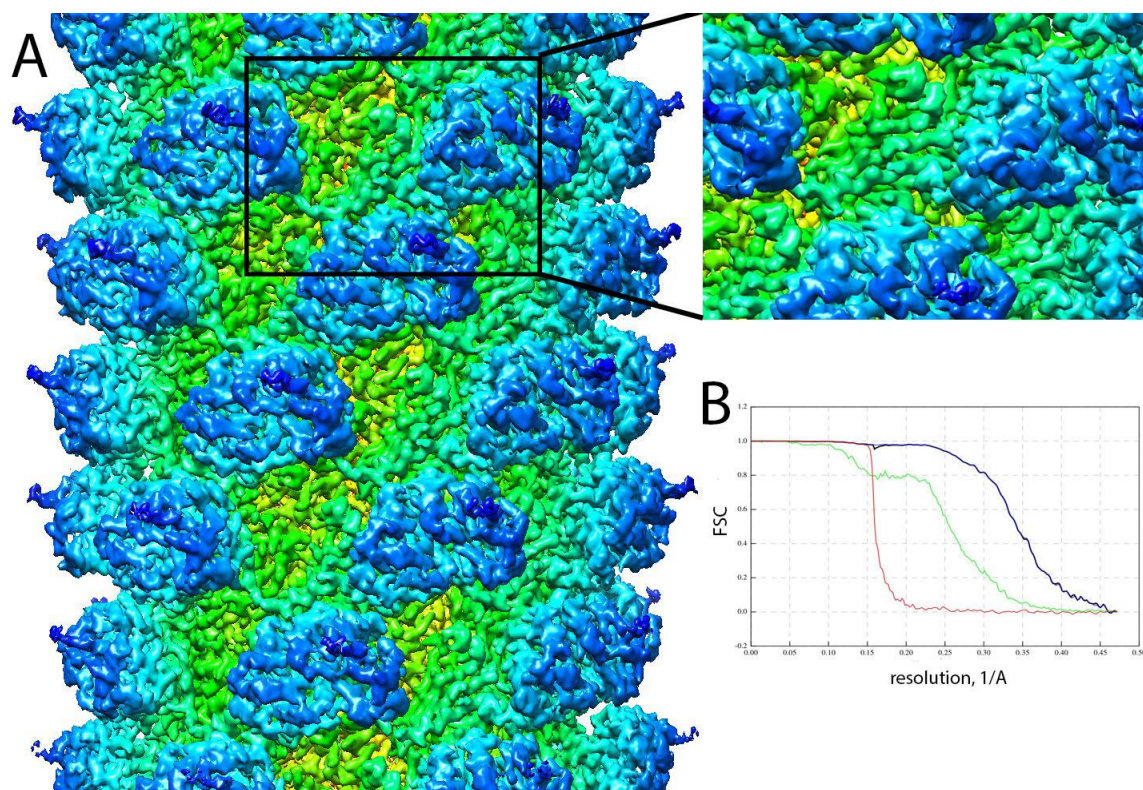


Figure 1. (A) Cryo-EM reconstruction of the TaPaz phage tail with helical symmetry applied; insert: zoomed part of the reconstruction reveals clearly resolved side chains' densities; (B) FSC curve indicates 3.1 Å resolution. Green – unmasked half-maps, blue – masked half-maps, red – phase randomized masked half-maps, black – corrected.

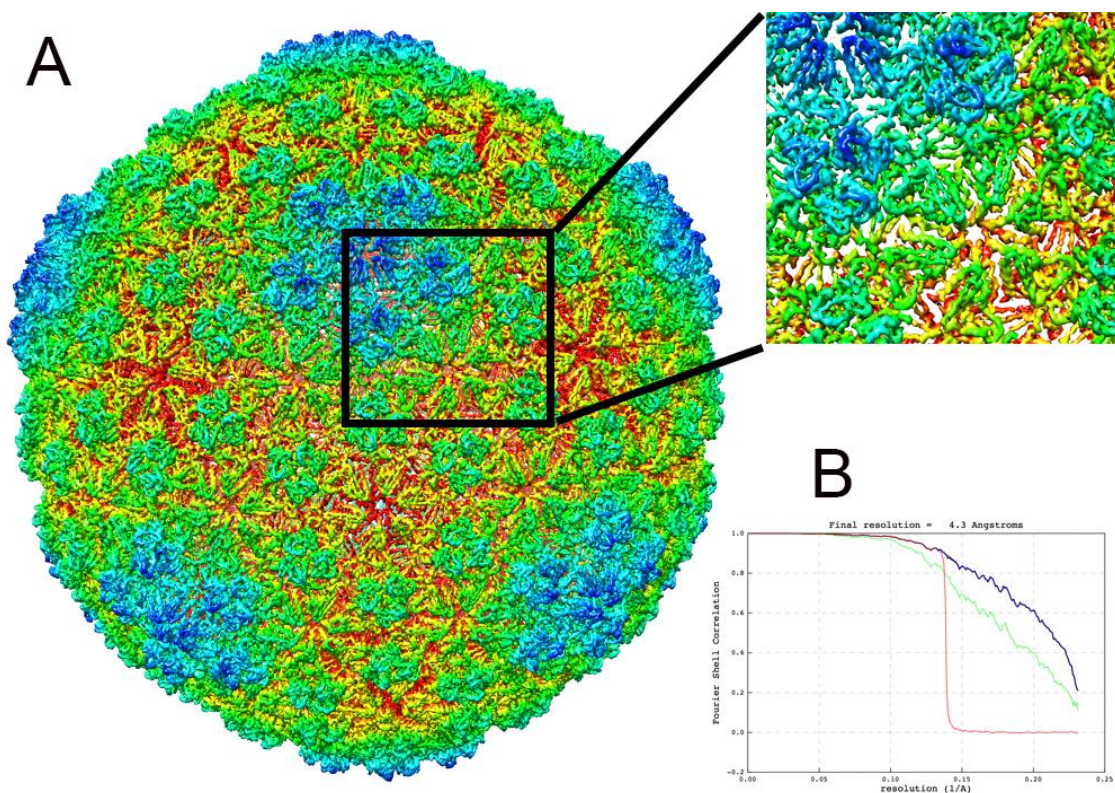


Figure 2. Cryo-EM reconstruction of the TaPaz phage capsid with icosahedral symmetry applied; insert: zoomed part of the reconstruction reveals clearly resolved helices; (B) FSC curve indicates 4.3 Å resolution; Bar = 50 nm. Curves colours same as in Fig. 1B.

References

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