MASARYK UNIVERSITY

L-09
STRUCTURAL CHARACTERISATION OF VIRUS REPLICATION *IN SITU*

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Research performed by the group of Pavel Plevka within the framework of the National Institute of Virology and Bacteriology will be focused on structural characterization of the replication cycles of human enteroviruses and of phages infecting pathogenic bacteria.

Viruses from the genus *Enterovirus*, including coxsackieviruses and rhinoviruses, cause billions of human infections every year. Diseases caused by enteroviruses include upper and lower respiratory tract infections, gastroenteritis, hand-foot-and-mouth-disease, and life-threatening encephalitis. Rhinoviruses are responsible for 40% of common cold cases, which result in a yearly cost of tens of billions of US$ in treatments and lost working hours worldwide. Despite the societal and economic impact of enterovirus infections, understanding of enterovirus replication cycle is incomplete. We will characterize enterovirus replication *in situ* using cryo-EM and tomography (cryo-ET) to determine: (i) how enteroviruses deliver their genomes into the cell cytoplasm[1,2]; (ii) the organization of "replication factories" that enteroviruses form in infected cells; and (iii) whether enterovirus capsids assemble around genomes or if the genomes are packaged into capsids (Fig. 1A). Results obtained within this aim will provide insight into the mechanism of cell membrane penetration by non-enveloped viruses, which is generally poorly understood. Furthermore, observing assembly intermediates in infected cells will enable characterization of the formation of enterovirus particles for which the genome-packaging mechanism is currently unknown. Because of the fast mutation rates enteroviruses exist as swarms of mutants called quasi-species that can evolve resistance to a treatment much faster than cellular organisms. Therefore, effective anti-enterovirus treatment will require the simultaneous use of at least three compounds targeting different steps in the enterovirus life cycle. Characterization of enterovirus replication, as planned in this proposal, will identify new targets for future anti-enterovirus therapeutics.

In 2017, the World Health Organization declared *Staphylococcus aureus* to be an antibiotic-resistant pathogen for which new therapeutics are urgently needed. Upon infection, *S. aureus* forms biofilms that can only be treated by the long-term application of several antibiotics in high doses or the surgical removal of the infected tissues. An alternative approach, phage therapy, has not been approved for clinical use, because the effects of phage infection of a biofilm are not sufficiently characterized. We propose to study the dynamics of the propagation of *Herelleviridae* phage phi812 in a *S. aureus* biofilm and molecular details of phi812 replication in a cell (Fig. 1B)[3]. We will study if and how sub-populations of metabolically dormant or phage-resistant cells in a biofilm provide herd immunity against phi812 infection. Structures of previously uncharacterized phi812 replication and assembly intermediates in *S. aureus* cells will be determined. Results obtained within this project will enable characterization of the function of bacterial membranes and macromolecular complexes in the initiation and completion of phage genome delivery, the assembly of phage portal complexes and heads, and the mechanisms of genome packaging and head-tail attachment. This aims's biological significance lies in its focus on the as-yet uncharacterized interactions of phages and bacteria under biologically and clinically relevant conditions. Analyses of phage spread in a biofilm, herd immunity against phage infection, and phage replication in cells performed within this project may identify approaches for making phage therapy more effective.

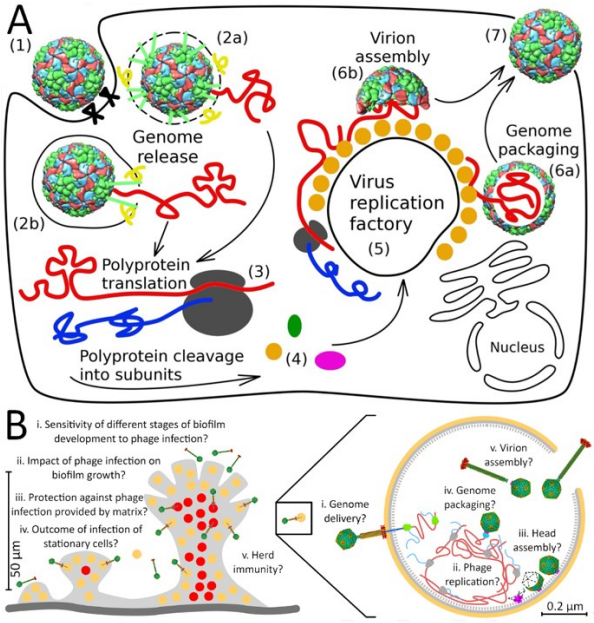

Fig. 1. **Overview of research questions directed towards characterizing enterovirus (A) and phage (B) replication cycles.**

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