Virion structures and genome release mechanisms of picornalike viruses

Commentary on the habilitation thesis

Picornaviruses are the causative agents of human diseases ranging from the common cold to life-threatening encephalitis. In contrast, several dicistroviruses and iflaviruses are economically important pathogens of honeybees causing their decline in North America and Europe. Despite their societal and economic impact, many of these viruses had not been structurally characterized, and there was limited information about their genome delivery.

This thesis summarizes X-ray crystallography and cryo-electron microscopy studies of virions of picorna-like viruses and changes in their capsids required for genome release. Picornaviruses have capsids with pseudo T = 3 icosahedral symmetries built from proteins with jellyroll folds. Receptor binding or acidic pH in endosomes trigger the genome release of these viruses. Nevertheless, viruses from the order Picornavirales differ in the behavior of their particles before genome release: the capsids of picornaviruses and iflaviruses expand, whereas those of dicistroviruses remain compact. In contrast to previous speculations, our results indicate that particles of picorna-like viruses crack open to release their genomes in microsecond time scales. The speed of the process prevents the genomic RNA from being degraded by ribonucleases. Furthermore, viruses from the order Picornavirales differ in the mechanisms that enable delivery of their genomes across cell membranes into the cytoplasm. Whereas picornaviruses and dicistroviruses use VP4 subunits for membrane disruption, virions of iflaviruses probably lack VP4 subunits and employ protruding domains of VP3 subunits or minor capsid proteins attached to the particle surface to penetrate membranes.

This thesis is based on eleven research papers published in the years 2016 to 2019 that describe studies of virus infection by X-ray crystallography, cryo-electron microscopy (cryo-EM), and biochemical methods. I contributed to the publications as the corresponding author by conceiving the research questions, designing experiments, measuring and analyzing data, and writing the manuscripts. Reprints of the papers are included in the appendix of the thesis.

The introduction explains my motivation to study human and honeybee viruses from the order Picornavirales. It also summarizes aspects of virion architecture, replication cycles, and protein-naming conventions of these viruses. A general description of virus structure determination by X-ray crystallography and cryo-EM is given. The results presented in this thesis describe the structures of virions of viruses from the order Picornavirales and provide information on how the particles release and deliver their genomes into the cell cytoplasm. The combined discussion and results section focuses on identifying the general mechanisms that enable viruses from the order Picornavirales to infect cells.

Contribution of Pavel Plevka to publications that form the basis of habilitation thesis:

I. Kalynych S, Pálková L, **Plevka P**. *The structure of Human Parechovirus-1 reveals an association of the RNA genome with the capsid.* J Virol. 2016; 90(3):1377-86

I conceptualized the study, contributed to the production and purification of the virus, collected the diffraction data and solved the crystal structure. I supervised building of the molecular structure, wrote 50% of the first draft of the paper and directed the work on paper revisions.

II. Sabin C, Füzik T, Škubník K, Pálková L, Lindberg AM, **Plevka P**. *Structure of Aichi virus 1 and its empty particle: clues to kobuvirus genome release mechanism.* J Virol. 2016; 90(23):10800-10810.

I conceptualized the study, contributed to the production and purification of the virus, collected the diffraction data, solved the crystal structure, collected cryo-EM data and supervised determination of the structure of the empty particle. I supervised building of the molecular structures, wrote 80% of the first draft of the paper and directed the work on paper revisions.

III. Mullapudi E, Nováček J, Pálková L, Kulich P, Lindberg AM, van Kuppeveld FJ, **Plevka P**. *Structure and genome release mechanism of human cardiovirus Saffold virus-3.* J Virol. 2016; 90(17):7628-39.

I conceptualized the study, contributed to the production and purification of the virus, collected the diffraction data, solved the crystal structures, collected cryo-EM data and supervised determination of the structure of activated particle. I supervised building of the molecular structures, wrote 90% of the first draft of the paper and directed the work on paper revisions.

IV. Mullapudi E, Přidal A, Pálková L, de Miranda JR, **Plevka P**. *Virion structure of Israeli acute bee paralysis virus.* J Virol. 2016; 90(18):8150-9.

I conceptualized the study, collected the diffraction data, and solved the crystal structures. I supervised building of the molecular structures, wrote 90% of the first draft of the paper and directed the work on paper revisions.

V. Mullapudi E, Füzik T, Přidal A, **Plevka P**. *Cryo-EM study of genome release of dicistrovirus Israeli acute bee paralysis virus.* J Virol. 2017; 91(4):2060-16.

I conceptualized the study, supervised collection of cryo-EM data, and supervised building of the molecular structures. I wrote 90% of the first draft of the paper and directed the work on paper revisions.

VI. Spurny R, Přidal A, Pálková L, Tran Kiem HK, de Miranda JR, **Plevka P**. *Virion structure of black queen cell virus, a common honeybee pathogen.* J Virol. 2017; 91(6):2100-16.

I conceptualized the study, solved the crystal structure, and supervised determination of the structure of the virus. I supervised building of the molecular structure, wrote 75% of the first draft of the paper and directed the work on paper revisions.

VII. Kalynych S, Přidal A, Pálková L, Levdansky Y, de Miranda JR, **Plevka P**. *Virion structure*

of iflavirus slow bee paralysis virus at 2.6Å resolution. J Virol. 2016; 90(16):7444-55.

I conceptualized the study, solved the crystal structures, and supervised determination of the structures of the two forms of the virus. I supervised building of the molecular structures, wrote 50% of the first draft of the paper and directed the work on paper revisions.

VIII. Kalynych S, Füzik T, Přidal A, de Miranda J, **Plevka P**. *Cryo-EM study of slow bee paralysis virus at low pH reveals iflavirus genome release mechanism.* PNAS. 2017; 114(3):598-603.

I conceptualized the study and supervised the determination of cryo-EM structures. I supervised building of the molecular structures, wrote 75% of the first draft of the paper and directed the work on paper revisions.

IX. Škubník K, Nováček J, Füzik T, Přidal A, Paxton RJ, **Plevka P**. *Structure of deformed wing virus, a major honey bee pathogen. PNAS.* 2017; 114(12):3210-3215.

I conceptualized the study and supervised building of the molecular structures. I wrote 75% of the first draft of the paper and directed the work on paper revisions.

X. Procházková M, Füzik T, Škubník K, Moravcová J, Ubiparip Z, Přidal A, **Plevka P**. *Virion structure and genome delivery mechanism of sacbrood honeybee virus.* PNAS. 2018; 115(30):7759-7764.

I conceptualized the study and supervised building of the molecular structures. I wrote 80% of the first draft of the paper and directed the work on paper revisions.

XI. Buchta D, Füzik T, Hrebík D, Levdansky Y, Sukeník L, Mukhamedova L, Moravcová J, Vácha R, **Plevka P**. *Enterovirus particles expel capsid pentamers to enable genome release.* Nat Commun. 2019; 10(1):1138.

I conceptualized the study and wrote 80% of the first draft of the paper and directed the work on paper revisions.