MASARYKOVA UNIVERZITA

Annex No. 11 to the MU Directive on Habilitation Procedures and Professor Appointment Procedures

Habilitation Thesis Reviewer's Report

Masaryk University Faculty	Faculty of Science
Procedure field	Biomolecular Chemistry
Applicant	Mgr. Pavel Plevka, Ph.D.
Applicant's home unit, institution	Masaryk University
Habilitation thesis	Virion Structures and Genome Release Mechanisms of Picorna-like Viruses
Reviewer	Professor Félix A. Rey, PhD,
Reviewer's home unit, institution	Institut Pasteur Structural Virology Unit Paris, France

The thesis presented by Pavel Plevka is based on eleven publications on viruses of the *Picornaviridae* family, in which he signs as senior author. Reprints of these publications were provided in an appendix. The narrative of the thesis is a lucid explanation of the context of each of those publications, and an analysis of the crucial information extracted in each case. In these publications, Pavel Plevka provides important new insight about the organization of viruses belonging to several different genera in the *Picornaviridae* family. Dr. Plevka's work provides a comparative description of the organization of the viral particles of the various genera. Moreover, the structures described give useful insights into the way these viruses deliver the genomic RNA into the cell, showing that they use multiple strategies for this purpose. The data on particles in which a few pentamers pop out to allow genome release are highly significant. The information provided is unique to understand the entry process of non-enveloped viruses in general, which remains much less understood than the entry of enveloped viruses. Furthermore, the characterization of viruses that infect honeybees by Dr. Plevka constitute very important contributions from his laboratory, as these viruses are understudied in general. Overall, Dr. Plevka has demonstrated a real drive to carry out original research, and to have reached an indisputable international standing in the field of structural virology. His thesis document is well written and pleasant to read. I have attached a version with a few comments on the text, which Dr. Plevka may want to consider.

In summary, Pavel Plevka's work has had a substantial impact in virology, pushing the frontiers of our understanding of genome delivery by this category of viruses and thereby shifting the current paradigm. He totally deserves to be awarded this habilitation.

Reviewer's questions for the habilitation thesis defence (number of questions up to the reviewer)

I only have a few question concerning enteroviruses:

1. In section 2.1, the candidate writes that for picornaviruses, "it is unlikely that vaccination will ever be practical". Given that vaccination against poliovirus has been highly efficient. Leading to elimination of this picornavirus except for a few places in the world, perhaps he could expand on the meaning of his sentence?
2. In Section 2.3, page 9, where he explains that the canyon in enteroviruses contains the binding sites for receptors with the immunoglobulin fold, he also writes: "Receptors with other types of protein fold bind to protrusions at the virion surface". What is the reason why receptors with an immunoglobulin fold would not bind to those protrusions? I found the sentence intriguing.
3. In the same paragraph, it is explained that small molecules that bind to a hydrophobic pocket in VP1 in enteroviruses block particle activation and genome release. Such molecules could be used as leads to develop antivirals. Yet in section 2.1 it is stated that there are no approved antiviral drugs against this class of viruses. Could the candidate discuss what are the reasons why these compounds have not been folloed up?
4. At the top of page 10, it is explained that VP4 of most enteroviruses is myristoylated. Is the myristoyl group essential for virus entry, or is it dispensable?

Conclusion

The habilitation thesis entitled "Virion Structures and Genome Release Mechanisms of Picorna-like Viruses" by Mgr. Pavel Plevka, Ph.D. ***fulfils*** requirements expected of a habilitation thesis in the field of Biomolecular Chemistry.

05 -03- 2020

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Reviewer's questions for the habilitation thesis defense - Professor Félix A. Rey, PhD.

Reviewer's questions are in blue italics, my responses in normal black font.

1. In section 2.1, the candidate writes that for picornaviruses, "it is unlikely that vaccination will ever be practical". Given that vaccination against poliovirus has been highly efficient. Leading to elimination of this picornavirus except for a few places in the world, perhaps he could expand on the meaning of his sentence?

A: The statement was intended to signify that it is impractical to vaccinate against hundreds of strains of enteroviruses that are immunologically distinct. However, as pointed out by the reviewer, in case of highly pathogenic viruses such as polio the vaccination can be undertaken and is highly effective. It should be noted that there are only three strains of poliovirus.

2. In Section 2.3, page 9, where he explains that the canyon in enteroviruses contains the binding sites for receptors with the immunoglobulin fold, he also writes: "Receptors with other types of protein fold bind to protrusions at the virion surface". What is the reason why receptors with an immunoglobulin fold would not bind to those protrusions? I found the sentence intriguing.

A: Enteroviruses usually employ two types of receptors (1) attachment receptors that enable initial binding to a cell and (2) uncoating receptors that help to initiate transition of virions to activated particles and subsequent genome release. To trigger the genome release the uncoating receptors bind to a canyon at the capsid surface and induce conformational changes in the capsid proteins. Majority of the uncoating receptors have the immunoglobulin fold. However, if a protein with immunoglobulin fold serves as attachment receptor it may bind to surface protrusions of enterovirus capsid.

3. In the same paragraph, it is explained that small molecules that bind to a hydrophobic pocket in VP1 in enteroviruses block particle activation and genome release. Such molecules could be used as leads to develop antivirals. Yet in section 2.1 it is stated that there are no approved antiviral drugs against this class of viruses. Could the candidate discuss what are the reasons why these compounds have not been followed up?

A: Pharmaceutical companies as well as numerous academic research groups tried to develop anti-enterovirus drugs. One of the most promising candidates is the compound pleconaril, which targets the hydrophobic pocket in capsid protein VP1. The compound proceeded to phase III clinical trials and it was shown that it can shorten the duration of the common cold infection. However, pleconaril was not approved by FDA, because it shortened the sickness only by 1.4 days (average duration of recovery from common cold is 7.2 days). Furthermore, resistant variants of rhinoviruses appeared very quickly, and the compound interfered with birth control medicines. Pleconaril is now approved for "compassionate use" in life-threatening situations.

4. At the top of page 10, it is explained that VP4 of most enteroviruses is myristoylated. Is the myristoyl group essential for virus entry, or is it dispensable?

A: Myristoylation of VP4 is essential for replication of enteroviruses. Reverse-engineered mutants of polioviruses lacking the myristylation signal are non-viable. Furthermore, blocking of myristylation pathway in infected cells results in production of non-infectious particles.