

Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Yulia Zhitnyuk

PhD Program: Life Sciences

Title of Thesis: Development of Messenger RNA Delivery System via Virus-Like Particles


Supervisor: Prof. Konstantin Severinov

Chair of PhD defense Jury: Prof. Yuri Kotelevtsev

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Date of Thesis Defense: 17 May 2019

Name of the Reviewer: Kaspars Tars

<p>I confirm the absence of any conflict of interest</p> <p>(Alternatively, Reviewer can formulate a possible conflict)</p>	<p>Signature:</p>  <p>Date: 20-04-2019</p>
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The purpose of this report is to obtain an independent review from the members of PhD defense Jury before the thesis defense. The members of PhD defense Jury are asked to submit signed copy of the report at least 30 days prior the thesis defense. The Reviewers are asked to bring a copy of the completed report to the thesis defense and to discuss the contents of each report with each other before the thesis defense.

If the reviewers have any queries about the thesis which they wish to raise in advance, please contact the Chair of the Jury.

Reviewer's Report

Reviewers report should contain the following items:

- Brief evaluation of the thesis quality and overall structure of the dissertation.
- The relevance of the topic of dissertation work to its actual content
- The relevance of the methods used in the dissertation
- The scientific significance of the results obtained and their compliance with the international level and current state of the art
- The relevance of the obtained results to applications (if applicable)
- The quality of publications

The summary of issues to be addressed before/during the thesis defense

Doctoral thesis of Yulia Zhitnyk are devoted to the establishment and characterization of mRNA delivery system by modified virus-like particles (VLPs) of vesicular stomatitis virus (VSV). VLPs of various kinds lately have found a number of applications, including construction of vaccines and development of nanocontainers for delivery of various cargo. In general, Yulias thesis are scientifically sound, timely and could lead to further development of modified VSV VLPs as delivery tools.

Thesis are written on 114 pages and contain all four classical parts – Literature review, Materials and methods, Results and Discussion. Thesis are written in good English, have a logical structure and the text is easy to follow.

The Literature Review part is written on 24 pages and includes chapters about mRNA delivery, gene therapy, genome editing, and very extensive review about virus-like particles (VLPs) as delivery tools, including utilization of paramyxoviral, retroviral, ssRNA phage, plant virus and targeted VLP delivery systems. In general, Literature review part is extensive, appropriate and timely, clearly demonstrating that pretendant has a deep knowledge and understanding of the research field.

Materials ad Methods part (14 pages) is pretty detailed, covering most of the methods used in investigations. To my understanding, some of methods are described in too much detail – I doubt that it is actually necessary to describe pretty basic protocols, such as DNA restriction or PCR in PhD thesis, which might be more appropriate for lower-level bachelors or masters thesis. Also, in some protocols the mathematical precision is somewhat exceeding the physical reality – for instance, I do not think that it is actually possible or necessary to take exactly 16.375 μ l of water to be added to the PCR reaction mixture...

Results section is written on 19 pages and is dealing with development of mRNA delivery system, based on modified VLPs of VSV. Two different RNA binding proteins were attached to G protein of VSV and the RNA-binding properties of resulting VLPs suggested that L7A protein containing particles have better RNA-binding potential, compared to MS2 coat protein containing particles. Furthermore, it was discovered that packaging of mRNA is largely independent on the presence of specific RNA-binding sequence. The obtained modified VLPs were successfully further used as transfection agents for various hard-to-transfect cell lines, demonstrating usefulness of the developed system. Although in general the results are convincing, one thing which I missed was a clear demonstration that VLPs have actually been obtained – preferably by electron microscopy. In the absence of EM or comparable data it remains possible that VLPs are actually not formed and mRNA is delivered by some sort of monomeric or aggregated fusion proteins. Yet another problem is that the obtained VLPs did not seem to be quantified or assessed for purity.

Discussion part is written on 17 pages and is devoted to re-evaluation of results and description of L7A and similar protein interactions with RNA-kink motifs.

The results are published in two articles, main results in medium impact journal BBRC, where Yulia is a first author and some of the results also in a high impact journal MBio, where Yulia is in a middle of authors list. Although I am not familiar with requirements for number of publications for PhD thesis in Skoltech (if such requirements exist...), to my understanding, two papers might be an average performance.

In general, to my opinion, thesis of Yulia Zhithyuk meets the required criteria and I recommend awarding her a doctoral degree.

Riga, 19th April 2019



Prof. Kaspars Tars

Leading researcher & study director

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Provisional Recommendation

I recommend that the candidate should defend the thesis by means of a formal thesis defense

I recommend that the candidate should defend the thesis by means of a formal thesis defense only after appropriate changes would be introduced in candidate's thesis according to the recommendations of the present report

The thesis is not acceptable and I recommend that the candidate be exempt from the formal thesis defense