

## 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

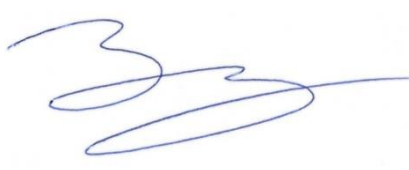
**Supervisor:** Prof. Konstantin Severinov

**Chair of PhD defense Jury:** Prof. Yuri Kotelevtsev

**Email:** Y.Kotelevtsev@skoltech.ru

**Date of Thesis Defense:** 17 May 2019

**Name of the Reviewer:** Prof. Timofei Zatsepin

I confirm the absence of any conflict of interest	<b>Signature:</b>  <b>Date: 17-04-2019</b>
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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

PhD thesis by Yulia Zhitnyuk is devoted to the development of mRNA delivery system based on virus-like particles. The thesis is written in a classic way. Literature overview is solid and up to date. After significant improvements in the review of literature, the logic of the study became clear and a reader can understand the goal of the study from the beginning. The main result of the study is a new fusion – VSVG-L7Ae that can deliver mRNA with kink-turn motif in mammalian cells with high efficacy. Initial hypothesis on Box C/D mediated enhancement failed, but Yulia was able to confirm that the process is driven by L7Ae domain. Confirmation of the mechanism was done by CLIP assay and use of K→A L7Ae mutants with abolished RNA binding. Transcriptome and proteome analysis allowed to provide a general view on mRNA binding to L7Ae domain and to determine components of VLP. This study is rather far from creating a startup, but can lead to a development of the tool in molecular biology.

Major concerns:

- There is no discussion/explanation on the unexpected loss of VSVG-L7Ae activity in comparison to WT for EGFP delivery by retroviruses (p. 68);
- RNA-seq data was not confirmed by qPCR or alternative methods
- Most of Western Blot data is presented as photos of a membrane, without calculations of the protein changes

Minor points:

- Experimental procedure for protein transfer to a membrane (p. 49) should be added;
- “Gene therapy” (p.17) – described examples of mRNA applications do not deal with gene therapy – all genes remain intact. This is a substitutional therapy;
- Sleng like “annealed oligonucleotides were run on the gel for 30 min” (p. 41) should be changed;
- I recommend intensive text proofreading to remove typos and improve the text – for example, “5'-methylation of cytosine” (p. 14); also figure legends (Fig. 1.6 (p. 23), Fig. 2 (p.45)) should be corrected.

Some minor improvements of PhD thesis should be done, however the study is good enough for defense in Skoltech.

**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*