Skoltech
Skolkovo Institute of Science and Technology

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. Konstantin Lukyanov

I confirm the absence of any conflict of interest	**Signature:** **Date: 18-04-2019**

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

The dissertation work by Yulia Zhitnyuk aims to develop a method of mRNA delivery into mammalian cells using modified virus-like particles (VLP). A novel strategy was suggested: to use a specific RNA-binding domain fused with protein G of vesicular stomatitis virus (VSV-G) that should ensure efficient incorporation of the target mRNAs into VLP. After testing different RNA-binding domains and target RNAs, the author selected the most efficient one and demonstrated mRNA delivery into hard-to-transfect cells.

The suggested method appears to be a promising tool for transient transgene delivery of an mRNA of interest in different biological models, including delivery of genome editing complexes.

The topic of dissertation work is fully relevant to its actual content. A variety of methods were used, namely classical DNA cloning, mammalian cell cultivation and transfection, VLP and retroviral particles production, quantitative PCR, western blot, cross-linking immunoprecipitation assay, RNA-sequencing using the Illumina platform, LC-MS/MS protein analysis. Application of these methods made it possible to evaluate the performance of different steps of the suggested technique in detail.

The main results of dissertation work are published in a paper with the first authorship of Yulia Zhitnyuk in the Biochemical and Biophysical Research Communications (IF 2.559, Q2 according to Web of Sciences, and IF 2.591, Q1 according to Scopus Scimago Journal Rank). Yulia is also a coauthor of the paper in the mBio journal (IF 6.689, Q1 according to Web of Sciences).

Some minor criticism and questions:

1. Chapter 3 “Results” starts from technical details. A general experimental strategy is not described, that greatly complicates understanding of logics of further experiments. The readers can see an overall descripsion of the suggested experimental strategy only in Discussion (p. 76-77: “The schematic representation of the RNA delivery system we aimed to utilize is depicted at the Figure 4.1. We planned that fusing VSV-G with either MS2BP or L7Ae and cloning the cognate motifs in the mRNA structure would facilitate its incorporation into the VLPs and hence the delivery to the target cells. …”). I suggest to move this part from Discussion to Results.
2. Fig.4.1: The depicted pathway of mRNA entrance from internalized vesicle into cytosol of the target cell is unclear. Specifically, it seems for me that the shown intermediate step of “mRNA in a circle” (the upper part of the cell on the right) is incorrect – mRNA should go directly into cytoplasm after fusion of VLP and cellular membranes.
3. Fig. 3.1 legend: “n = 3, ± S.D”, but there is no SD in the plots.
4. Incorrect figure number (p. 62): “we proceeded with the qRT-PCR. EGFP mRNA was bound with VSVG-L7Ae and enriched more than 600-fold compared with the control VSVG-WT (Figure 2D). However, enrichment of the bound RNA was also observed with the endogenous mRNAs, such as GAPDH (~160-fold) (Figure 2E), β-actin (ACTB, ~110-fold) (Figure 2F), and 7SL lncRNA (RN7SL1, ~90-fold) (Figure 2G)” – in fact, it is Figure 3.13 and it contains a single histogram.
5. Page 15, section «1.2 Applications of mRNA delivery»: «The applications may be as following: to devise viral vaccines, to generate autologous dendritic cell vaccines for cancer immunotherapy, to replace disease-related defective protein in gene therapy, and to introduce genome-editing platforms to cells». An important direction was missed – delivery of mRNA for reprogramming of cells (induced pluripotent stem cells, iPSC) (e.g., see Bernal JA. RNA-based tools for nuclear reprogramming and lineage-conversion: towards clinical applications. J Cardiovasc Transl Res. 2013, 6:956-68). It was briefly mentioned in the Discussion (p. 75 “mRNA delivery has several exciting applications, such as mRNA vaccines, cellular reprogramming, and genome editing [86]” but without any details.

6. Sections 1.3 and 1.4 are in fact should be sub-sections within 1.2.
7. Figure 1.2 –This big scheme taken from a review looks too complex here to illustrate just single sentense. I would like to see some simplified scheme that focuses on points important for the present work (preferably, depicted by the author).
8. Pages 39, 42, Tables on recombination reaction: “Destination vector – 150 ng/µl”. How many microliters?
9. Page 48, Table on qRT-PCR does not show concentration of the primers, just “1 µl”.
10. Many references in the list have incorrect formatting. Mostly, it is absence of the journal title, but some other misprints can be found (e.g., “K. Institutet” (i.e. Karolinska Institutet) instead of the author name in Ref. 57). I found mistakes in the following references: 16, 17, 41, 54, 57, 58, 61, 68, 70-72, 77, 85, 86, 90, 91, 99, 106, 122, 123, 125, 127, 135-149.

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*