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

Date of Thesis Defense: 17 May 2019

Email: Y.Kotelevtsev@skoltech.ru

Name of the Reviewer:

I confirm the absence of any conflict of interest

Signature: [Signature]

Date: 19-04-2019

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 PhD candidate Yulia Zhitnyuk, as part of her dissertation has developed a novel RNA delivery system using virus like particles (VLPs) and demonstrated its utility in delivery of eGFP and spCas9 RNA and subsequent protein expression in variety of cell systems including hard to transfect iPS cells in addition to HEK293 and THP-1 cells. To achieve this she used gesicle-VLP platform developed by Mangeot and co-workers using Vesicular Stomatitis Virus Protein G (VSV G) to deliver proteins and adapted it to pack and deliver mRNA by intelligently using RNA binding proteins (RBPs) to pack and demonstrate the utility in expressing the target proteins in different cells. There was significant effort in optimizing RBPs to deliver and express the proteins settling on archaeal L7Ae ribosomal protein from Archeoglobus fulgidus. This study demonstrates the utility of VLPs to potently delivery mRNA of interest and express them transiently in different types of mammalian cells. this platform has several applications ranging from gene editing to immunology and therefore is of tremendous interest to scientific community.

The introduction to the dissertation is very comprehensive and provides several details on different components necessary for building of VLPs, their advantages and drawbacks, their potential applications including a summary of clinical trials based on mRNA delivery. This forms a good basis for the work performed during the dissertation. The VLP system is relatively new and offers an exciting platform for the delivery of several biologically important molecules including RNA by overcoming limitations posed by currently available methods. Yulia’s work adds a new tool to the armamentarium of methods available to manipulate biological systems for academic research as well as clinical applications. The work is particularly interesting and novel because it exploits RBPs to pack and deliver mRNA. The results obtained by Yulia, clearly demonstrate the efficacy of the system to transiently express gene of interest in target cells using VLPs (that can be produced in mammalian cells as well). This demonstration forms the basis for optimization studies (to maximize delivery and transfection), packaging studies (multiple RNA species, length optimization), temporal control, conditional expression, alternative delivery methods, etc. which are beyond the scope of current PhD but can be pursued as individual projects. Yulia has two peer reviewed publications in respected journal with one as first author and other as middle author.

Overall, I would rate the PhD work done by Yulia Zhitnyuk as very interesting, important, explorative and novel, that can form the basis of several different lines of research.

** Corrections **

Page 5: Abstract: Change.. Applying messenger RNA (mRNA) has become a promising therapeutic modality in various spheres ranging from biotechnology to basic science to Applying messenger RNA (mRNA) has become a promising therapeutic modality in various spheres ranging from basic science to biotechnology.

Page 10: tRNA – transport RNA correct it to transfer RNA
Page 12:... making it useful for gene therapy and vaccine development. Please provide appropriate references

Page 12:... Change... Moreover, mRNA enables the defected protein... to Moreover, mRNA enables the defective protein...

Page 16:... figure 1.2 ... Provide a brief statement on the concept of dendritic cell vaccination after the figure legend.

Pages 20-21:... figure 1.4 ... This is a table, so list it as table 1.1 or so and please reference it accordingly.

Page 39:.. Materials and methods Section: Source of chemicals/plasmids missing. Please include them.

Page 45: Fig 2: Cells were plated at a density of 1.9-2.2 × 10^5; the units are missing. Please add (cells/mL?)

Page 49:.. The membrane was washed 3 x 10 min ... volumes are missing.

Page 53:.. Fig 3.2: Change the Y-axis legend to relative mRNA incorporation.

Page 59: Fig 3.8. Time course experiment. How long does the EGFP positivity in cells increase and at what time point it starts to decay? If the information on the time course is available even with one of the constructs, please include them. This will establish that the production of target protein is transient.

Page 69: Proteomics of VLP’s and Table 3.21. What does the number indicates? Is it representing relative intensity or ion count? If yes, please provide a brief description on how the protein content was quantified.

It is recommended that author provides a summary schematic figure similar to figure 1.8 to explain the different components assembled as part of the PhD thesis.
For figures throughout the document if the author is using the figure from the reference, state that the figure is adapted from the references quoted at the end of the figure legend. For e.g. Figure 4.3 [8], state figure adapted from reference 8.

The delivery of spCas9 to the target cells has been demonstrated using qRT-PCR as well as western blot. However functional activity of the enzyme spCas9 still needs to be validated. If any data (however preliminary) regarding functional efficacy of the spCas9 enzyme expressed using VLPs is available, please include.

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