
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:

I confirm the absence of any conflict of interest

(Alternatively, Reviewer can formulate a possible conflict)

Signature: 

Date: 17-04-19

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 thesis of Yulia Zhitnyuk “DEVELOPMENT OF MESSENGER RNA DELIVERY SYSTEM VIA VIRUS-LIKE PARTICLES” is devoted to the development of mRNA delivery system. This is a very important and highly competitive field. The author applied original approach based on fusion protein G of Vesicular stomatitis virus (VSV-G) with a ribosomal protein L7Ae of Archeoglobus fulgidus. The efficiency of delivery of EGFP and SpCas9 mRNAs were evaluated. It was shown that mRNA delivery was independent of BoxC/D motif in the mRNA sequence. Endogenous kink-turn motif were shown to be essential for incorporation of messenger RNAs inside virus-like particles. VSVG-L7Ae VLPs effectively delivered mRNA to human iPS cells and monocytes, the cells notoriously difficult to transfect. Reported data is novel, original and potentially contains IP for industrial applications. The structure is well balanced with 35 pages concise literature review, 15 pages of methods, 25 pages results and 27 pages of discussion, total of 102 pages excluding references.

Experimental part contains 22 figures and tables of high quality.

- The relevance of the topic of dissertation work to its actual content.
The topic is relevant to the content. The dissertation is focused on the development of the transfection technique. For that several technical problems were solved. The idea of fusion L7Ae ribosomal protein of *A. fulgidus* with VSV-G was original and paid off with great result.

- The relevance of the methods used in the dissertation.
The methods used are adequate and allowed to achieve sought after results. The speciality of this dissertation methodological approach is based on combination of clever cloning strategy and elegant reconstruction of the virus particles. Moreover, mass spectrometric analysis was applied which provided very important structural data. The expertise gained by the candidate is unique and valuable not only for academic labs working on similar problems, but also for pharmaceutical companies working on gene therapy approach using mRNA vectors.

- The scientific significance of the results obtained and their compliance with the international level and current state of the art.
The author gives extensive and very detailed literature review supplied with graphic explanations of the platforms in use in other laboratories. The authenticity of the approach applied by the author is undoubtful. Significance and compliance with international level is supported by peer reviewed publication. It is very likely that the paper will attract lots of attention and will be cited at a very high level.

- The relevance of the obtained results to applications (if applicable).
There is immediate applicability of the technique to the pharma approaches aimed at gene therapy, particularly genome editing, where CRISPR/Cas system is used now based on AAV delivery, which has serious limitations.

- The quality of publications
Both publications are of high quality. The first publication describes the main results of the thesis. The second publication is more methodological, but fits well enough to the subject of the dissertation.

I have not found any major faults or mistakes in the dissertation.

**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 |