

Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Tatyana Zyubko

PhD Program: Life Sciences

Title of Thesis: Efficient in vivo Synthesis of Lasso Peptide Pseudomycolidin Proceeds in the Absence of Leader and Leader Peptidase

Supervisor: Prof. Konstantin Severinov

Date of Thesis Defense: 19 December 2019

Name of the Reviewer: Timofei Zatsepin

<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: 17-11-2019</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

Tatiana Ziubko performed a comprehensive study on the structure-activity relationship for a new lasso peptide pseudomycolidin. She used a common pipeline for heterologous expression of the novel gene cluster in E.coli followed by mutational analysis to uncover the role of the proteins encoded in the cluster. Then she confirmed the role of each protein *in vitro* using both knockout strains and mutated proteins with an inactivated reactive center. As a result Tatiana found that this lasso peptide can be formed *in vivo* without B1/B2 enzymes. This outcome contributes a lot to the field as these enzymes were supposed to be obligatory in the synthesis of lasso peptides. Another interesting observation is pseudomycolidin glycosylation assisted by *psmN* protein. However the question on the nature of sugar moiety is still open. The PhD thesis is written in a classic way, literature overview is solid and up to date. Publications in high-impact journals fit all criteria and confirm a high level of this study. This study is rather far from creating a startup, but can contribute into the development of new antibiotics in a very indirect way.

Major concerns:

- Short peptides have multiple specific functions, while no activity was found/proposed for pseudomycolidin;
- The question on the native structure of pseudomycolidin *in vivo* (either lasso or just circularized N-end) is still open as lasso formation could happen during purification;

Minor points:

- I recommend intensive text proofreading to remove typos and improve the text – for example, slang like “collected elution fractions were confirmed by MALDI MS” (p. 46), “heightened stability” (p.12), “nanomolar affinity” is not true for KD 200 nM (p.27) should be corrected.
- Intensive proof-reading for the list of the cited literature is the must – now numerous references are filled only partially (for example, ref. 153, 156,158,161, 165, etc.);
- “semi-preparative C18 column” (p.46) – semi-preparative starts from diameter = 10 mm, please remove;

This research is novel, original and thesis fits the criteria for PhD thesis in Skoltech Life Science PhD program. Some minor improvements of PhD thesis should be done, however Tatiana is almost ready for the PhD defense in Skoltech.

Provisional Recommendation

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

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

