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Skolkovo Institute of Science and Technology

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 Pseudomycoidin Proceeds in the Absence of Leader and Leader Peptidase

Supervisor: Prof. Konstantin Severinov

Date of Thesis Defense: 19 December 2019

Name of the Reviewer: Douglas A Mitchell

I confirm the absence of any conflict of interest	**Signature:**  **Date:** 03-11-2019

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

This thesis provided by Tatyana Zyubko is of sufficient quality for her to undergo her defense next month. For the most part, the thesis is well organized, easy to follow, informative, and most importantly, the science itself is interesting. Her data advance the field in more than an incremental way. While some aspects of the work remain speculative and incomplete, what has been firmly established is exciting and actionable by others. For transparency, I wish to disclose that I first became aware of this work on pseudomycoidin at a conference in Spain earlier this year where Prof. Severinov presented many of the findings that are detailed in Ms. Zyunko's thesis. As the committee is likely aware, the majority of what is found in this thesis has been published in a recent Chemical Sciences article (30 Aug 2019). I enjoyed reading that paper as well as the current thesis. I have a few comments/corrections that would strengthen the thesis. Mostly, these corrections are cosmetic (and grammar needs to be improved in several spots), but others are factual inaccuracies that must be changed. These required changes are provided below in chronological order:

Page 9: there are font size inconsistencies in the abbreviations listing. MBP, “maltose” typo.

Page 11: self-immunity is only needed if there is self-toxicity. Many metabolites could be toxic to other organisms but not the producer itself. Consider rephrasing.

Page 11 (and throughout the thesis): abbreviations are defined and then not used. Numerous instances of BGC not being used after being defined. PTM on page 12, etc. etc.

Figure 1: need NH2 group for the N-terminus, not NH.

Page 12: bottromycin has a follower, not a leader.

Figure 2: Cyanobactin misspelled. Also, inconsistent hyphenation in “lasso peptide”. Recommend not using hyphenation

Figure 3: Low quality. Use standardized drawing settings so all chemical structures in the thesis are consistent.

Page 13: Gram-negative is a phenotype and not useful for taxonomic classification. For instance, eukaryotes are “gram-negative” but microcin B17 does not kill them.

Page 14: TOMM is a term that is no longer in usage. Just use LAP.

Figure 4 (and elsewhere): If figures are being reproduced from others’ publications, it should be clearly stated as such in the caption.

Page 15: there is a square character in place of a the Greek symbol, I presume, in paragraph 2. The same is true for page 29.

Page 16: by my count, there are 70 reported lasso peptides, so if table 1 is meant to be comprehensive, it is not. This page also has grammar issues in the first and last paragraphs. Lastly, to say lasso peptides “normally” are threaded is misleading. There is no reason to believe any are unthreaded. The lassomycin report really muddied the water on this topic. Regarding the top of page 18: the NMR solution structure of lassomycin was obviously incorrectly assigned by the authors. There is no reason to believe lassomycin unthreaded in that solvent. However, this is what was observed by Link (fuscanodin, 2019 JACS).

Figure 5: not particularly pleasing on the eye. If you wish to show the topological chirality, there are better ways to depict these structures are non-superimposable mirror images of one another.

Page 18: what is so “unusual” about a macrolactam bond being formed? This type of PTM is very common.

Table 1: recommend Courier font for the core sequence column so the character spacing is consistent.

Figure 8: I think some discussion of the pre-folded state is in order. As is, it would be hard for a non-expert to know what is happening here.

Page 27: I am fairly certain that StrA leader peptide binds to StrB as a beta strand, not alpha helix. Also, does it make physical sense that LarB1 could interact with the core of LarA?

Page 28: Most B2 proteins do not have a conserved Asp residue, so calling this a catalytic dyad would be more accurate

Page 29: Although it hasn’t been definitely proven, the prevailing view is that the lasso cyclase would emulate the AsnB reaction. Thus, the N-terminus displaces AMP (not ADP) from the carboxylate sidechain.

Figure 9: ATP is not required for leader peptide cleavage, thus this figure should be corrected. The text on page 31 should be corrected to so as to not propagate incorrect information. Also, Figure 10. It's pretty clear that the ATPase activity is attributed to co-purification of an ATP-dependent chaperone.

Page 30: What is the direct evidence that McjC can recognize and cyclize a linear lasso peptide (to yield a branched cyclic structure, I presume)? How can you be sure it didn't recognize a pre-folded substrate, then it forms the macrolactam, which immediately unfolds after dissociation from the enzyme since the steric locks where insufficient? This seems more plausible to me, given what is known in the current literature.

Page 36: The first paper on RODEO found 1400. The more recent publication catalogued ~3000 lasso BGCs. Other corrections needed are: capistruin and mcj25 bind to enzymes while siamycin does not. It binds lipid II!

Page 37: portions of the middle paragraph feel repetitive and thus unnecessary

Page 38: several inaccuracies on this page. 1. The epitope that was grafted into the mcj25 loop was to engender integrin binding, not angiotensin. The reference to Bode here is inappropriate, as he didn't even make a "lasso peptide" in that study. Also, to my knowledge, there have not been any other chemical syntheses of lasso peptides except for the BI-32169 case. What are the other reports (reference 166 does not make a lasso peptide, as previously stated)

The rest of the thesis (the actual science part, as opposed to background) is considerably stronger and much freer of errors.

Figure 20: appears a bit fuzzy, and thus harder to interpret

Figure 23: Polymyxa is misspelled. Also, the length of B1 should be shorter than B2. The ORF arrows should be drawn to scale.

Figure 25 (and anywhere high-resolution MS data are shown): provide calc mass values and give error in ppm.

Figure 28: Was the Y-shaped (tryptic product) ever characterized by MS/MS to ensure the authors are making conclusions based on the right information?

Page 73: The evidence that pseudomycoidin isolated from heterologous expression had undergone unthreading would be more compelling if H/D exchange was performed. If unthreaded, literally all amide NH's would exchange immediately. If threaded, several residues in the ring and tail would very slowly exchange. This can be done on very small amounts of material by using MS.

Figure 33/35: Curious as to why H23F and L21F variants were prepared as opposed to Trp. Larger is better if one was suspecting unthreading.

Page 82: Bioinformatics surveys have been published using both the cyclase and the leader peptidase as the "foothold". They yield very similar BGCs thus it is highly improbable that "many" lasso BGCs were overlooked. Surely there are a few but the data would suggest they are rare overall.

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*