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
In her work “Efficient in vivo synthesis of lasso peptide pseudomycoidin proceeds in the absence of leader and leader peptidase” Tatyana Zyubko discovered a new lasso peptide pseudomycoidin and explored in detail functional and structural characteristics of both pseudomycoidin and proteins maintaining its maturation and functionality.

Tatyana discovered pseudomycoidin by homology search performed by PSI-BLAST to the B2 protein from the already known lasso peptide cluster from Firmicutes phylum. Then she identified all the putative proteins forming the lasso peptide gene cluster.

Next, Tatyana used extensively MALDI mass spectrometry combined with mutational study, at the level of genes and of individual amino acids in proteins. Tatyana clearly demonstrated that, opposite to other lasso peptides, pseudomycoidin can be formed in vivo without B1/B2 proteins. Such a formation was shown for the first time. The principal consequence is that a number of similar lasso peptides could be overlooked because the homology search is made using B1/B2 (or similar) proteins. Next, Tatyana showed that the structure of pseudomycoidin can be unthreaded by heating but mutation L21F fixes the structure in a lasso conformation.

The results of the work are scientifically significant and comply with the international level and current state of the art. The work has clear perspectives both of practical application and for new research studies. The methods applied for the work were appropriate.

Tatyana has two papers in good journals (Chem. Sci. and MBio), in one paper being a first co-author. The quality of publications is good.

The thesis is clearly written with a lot of illustrations. The formatting is sometimes inaccurate but this minor issue does not influence the overall good impression from the thesis.

Minor questions and issues:

- p. 69: I see no evidence that trypsin cleaves exclusively between Lys⁵ and Arg⁶. I would say that trypsin cleaves additionally after Arg⁶, giving a mixture of the differently cleaved peptides. This does not influence the following analysis, so no reason to make a stronger unjustified statement.
- There is little details about the bioinformatics analysis given in the paper. I am not sure that a reference to the protocol from some paper is enough for PhD thesis. At least, Tatyana could give the parameters of PSI-BLAST.
- It is not explained why Tatyana chose E.coli BL21 strain, not E.coli K12 strain.
- P. 31: Are “Mccj25”, “Mclj25”, and “Mccj25” the designations of the same stuff?
- Some typos, the annotated pdf was sent to Tatyana.

Overall, I rate the PhD thesis of Tatyana Zyubko as very important, of high quality and scientifically significant.

Provisional Recommendation

- I recommend that the candidate should defend the thesis by means of a formal thesis defense
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<tr>
<th>Recommendation</th>
<th>Details</th>
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<td>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</td>
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<tr>
<td>The thesis is not acceptable and I recommend that the candidate be exempt from the formal thesis defense</td>
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