



OPPONENT'S REVIEW ON BACHELOR THESIS

Name of the student: Anna Kruglhuber

Thesis title: Mechanism, of retrograde transport in contact-dependent inhibition (CDI) toxins through the bacterial translocon

Supervisor: Mgr. Tomáš Fessler, Ph.D.

Referee: RNDr. Zdeněk Franta, Ph.D.

Referee's affiliation: UCH, USB

	Point scale ¹	Points
(1) FORMAL REQUIREMENTS		
Extent of the thesis (for bachelor theses min. 18 pages, for masters theses min. 25 pages), balanced length of the thesis parts (recommended length of the theoretical part is max. 1/3 of the total length), logical structure of the thesis	0-3	3
Quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0-3	2
Accuracy in citing of the references (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	3
Graphic layout of the text and of the figures/tables	0-3	2
Quality of the annotation	0-3	3
Language and stylistics, complying with the valid terminology	0-3	1.5
Accuracy and completeness of figures/tables legends (clarity without reading the rest of the text, explanation of the symbols and labeling, indication of the units)	0-3	2
Formal requirements – points in total		16.5
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	3
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	3
Discussion quality – interpretation of the results and their discussion with the literature (absence of discussion with the literature is not acceptable)	0-3	3
Logic in the course of the experimental work	0-3	3
Completeness of the description of the used techniques	0-3	3

¹ Mark as: 0-unsatisfactory, 1-satisfactory, 2-average, 3-excellent.

Experimental difficulty of the thesis, independence in experimental work	0-3	3
Quality of experimental data presentation	0-3	2
The use of up-to-date techniques	0-3	3
Contribution of the thesis to the knowledge in the field and possibility to publish the results (after eventual supplementary experiments)	0-3	3
Practical requirements – points in total		26
POINTS IN TOTAL (MAX/AWARDED)	48	(42.5) 2

Comments of the reviewer on the student and the thesis:

The bachelor thesis of Anna Kruglhuber entitled *Mechanism, of retrograde transport in contact-dependent inhibition (CDI) toxins through the bacterial translocon* covers the interesting topic of bacterial warfare. The thesis has 51 pages in total and is divided into 8 chapters (Introduction, Aims of the Thesis, Materials & Methods, Results, Discussion, Conclusion, References, and Appendix). The introduction has 8 pages and contains the basic information reader needs to get the overview about the topic. What I am missing here is better graphical support of the introduction. Author for example describes the structure of SecYEG as well as the translocation itself within the text, however adequate figures would strongly help the reader to understand the mechanism of the transport. Also the structure of CDI and collicin is just mentioned here, but figure would be beneficial (although it is shown in M and M section). The results with 22 pages represent the biggest part of the thesis and logically guide the reader through all the finding. The short discussion (2 pages) covers all the major results presented within the thesis and is followed by conclusion. The thesis contains 31 figures, however, some of them, minimally in my eyes, may be merged together (e. g. the pulling repeats), or placed into the supplement (Fig 31). The thesis is written in a bit heavy English and some expressions are harder to understand.

Suggestions and questions, to which the student has to answer during the defense.

Mistakes, which the students should avoid in the future:

1: You mention bacterial warfare in the introduction, do you know some other ways except the contact dependent one, how bacteria fight each other?

2: Is there anything know about target cell receptors for RBP?

3: On figure 1 you show the immune protein (CdiI) bound to CDI toxin after crossing the OM. Is it know, when and where is the CdiI removed (dissociated) from CDI toxin? Do they really recreate the complex upon unfolding on OM?

4: Figure 6 shows the translocation of CDI through SecYEG translocon. I like the figure, however the readability of the figure is rather low and I would also appreciate better description with arrows or so (e. g. the lateral gate is almost invisible for me). Have you tried to do the modelling with CdiA-I complex?

5: The pulling experiments (e.g. Fig7-9) nicely show the unfolding forces, however I would

² Enter the number of points awarded.

appreciate to get larger view on the protein structure. The individual parts are too small and larger structures would improve the figure clarity. I would also recommend merging the repeats into single figure, as the legend is triplicated within the text.

6: How did you create the permutants? Did you use some software or did it by hand? From figure 17 it seems that some loops connecting the individual secondary structures are much longer than others. Can you comment on that?

7: Figure 20 shows the structure of one of the permutants. When looking at colored schematic of alpha helices and beta sheets, it does not correspond with predicted structure (e.g. blue $\beta 1$ in scheme correspond to blue loop in the structure). Can you comment on this? I would also appreciate, if the color-coding will also be introduced to the sequences of individual permutants in the attachment.

8: The presentation of protein alignment in the attachment is not well chosen. Usually the alignment is done the way that the sequences of individual protein/gene are aligned together and colored based on the chosen threshold. The presented form is rather a list of individual protein sequences than the alignment.

9: Is there anything known about unfolding of CDI in periplasm? Are there any other protein or cofactors helping this?

10: All you work is *in-silico*, would you think about any wet lab experiment to confirm your results?

Finally, despite all my comments and question, the bachelor thesis presented by Anna Kruglhuber is of high quality and fulfils all the criteria of Faculty of Sciences, University of South Bohemia.

Conclusion:

In conclusion, I

r e c o m m e n d

the thesis for the defense and will suggest the grade based on the defense performance.

In České Budějovice

date 11.09.2023

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signature