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Faculty
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Jihočeská univerzita
v Českých Budějovicích
University of South Bohemia
in České Budějovice

OPPONENT'S REVIEW ON BACHELOR THESIS

Name of the student: Laura Zellner

Thesis title: Preparation of antibodies to determine the association of mitoribosomal complexes with mitochondrial membrane

Supervisor: Mgr. Ondřej Gahura, PhD.

Referee: Mgr. Karolína Šubrtová, PhD.

Referee's affiliation: PŘF UK

	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	3
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	3
Quality of the annotation	0-3	3
Language and stylistics, complying with the valid terminology	0-3	3
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	1
Formal requirements – points in total		19
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	2
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	2
Discussion quality – interpretation of the results and their discussion with the literature (absence of discussion with the literature is not acceptable)	0-3	2
Logic in the course of the experimental work	0-3	2

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

Completeness of the description of the used techniques	0-3	3
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	2
Practical requirements – points in total		21
POINTS IN TOTAL (MAX/AWARDED)	48	(0-48)²

Comments of the reviewer on the student and the thesis. Suggestions and questions, to which the student has to answer during the defense. Mistakes, which the students should avoid in the future:

In her thesis, Laura Zellner's goal was to add to what is already known about mitoribosomal biogenesis in *Trypanosoma brucei*. Her challenging task was to express recombinant antigens, obtain and verify specific polyclonal antibodies against two assembly factors (mtSAF24 and mtSAF18) and two ribosomal proteins (bS6m and bS21m). These antibodies would have been used to determine whether mitoribosomes and their assembly precursors are associated with the inner mitochondrial membrane. High-titer and high-affinity antibodies can be an invaluable tool in biological research. Therefore, if the antibodies had been working well, they could have been used and appreciated by her lab members as well as the larger *Trypanosoma* community for years to come. Importantly, Laura's results from localization experiments would contribute to general understanding of the protein synthesis in mitochondria.

Overall, the thesis is very well written and organized with up-to-date references and presents a large amount of experimental work exceeding a typical bachelor thesis. The student convincingly showed that she understands the chosen topic in context and learned wide range of methods that will be useful for her future work. Unfortunately, parts of the experimental work lack important controls and therefore need to be repeated before any conclusions can be drawn. I have a few comments and questions that I would like to address during Laura's presentation (especially the ones in bold).

The Introduction and Aims sections are succinct and clear with only minor typos. The Methods section needed proof-reading – I found more typos and errors that disturb the flow of the text. More importantly, there is missing information about following:

- Source and details about DNA and AA sequences (Which genome database and which strain of *T.brucei* was used to plan the cloning? How did you calculate molecular weights of antigens? Did you consider added His tag?).
- Details of *T.brucei* culturing.
- Information about anti-HIS antibody.

² Enter the number of points awarded.

- Detailed BCA method (Was it measured in 1ml cuvettes or in microtitre plate?).
- Table 17 shows wrong molecular weights of some antigens and this error is then repeated in Results section - Figure 28. Please double-check the expected molecular weights.

The Results section suffers from one major issue – lack of labelling of DNA a protein ladders in many figures. This makes the results difficult or even impossible to interpret. For SDS-Page and Immunoblots I would recommend to add detailed information about how much volume or percentage/equivalent from a sample was loaded so a reader could compare protein yields in respective fractions (i.e. soluble/insoluble or wcl/mito).

I would like to ask following: Did you analyze AA sequences in order to find the best antigenic regions? Can you clarify if mtSAF24 (cloned by a colleague) was expressed as full length protein or only its N-terminal domain? There is a discrepancy in Figure 24 where it is labelled as 34.6kDa protein but in other figures we are expected to see the 12.2kDa domain. Please can you explain what is “planned truncation” (page 29)?

Figure 11- Can you explain what was used as positive control? The labelled bands run higher than you would expect with insert specific oligos - can you please clarify which primers were used for the colony PCR? Moreover, whenever you show DNA or RNA concentration please include purity as well (Table 51). Table 52 – Can you explain the discrepancy between the amount of bS6m antigen (0.6mg) – when the David’s Biotech company required minimum of 1mg?

Figures 25-27 (plus Figure 37 in Discussion) – Verification of antibodies: Unfortunately, this experiment and the Immunoblots (figures 25, 26, 27) are missing essential controls. Can you suggest proper controls for the digitonin fractionation and for the blots?

The Discussion is well written, provides explanation to questions and issues from the Results section and suggests solutions to experimental challenges and difficulties experienced by the student. Although, I strongly disagree with the sentence “Western blots showed that all the raised antibodies recognized the purified recombinant proteins.” - This was not presented in the thesis. In your future work, please include a short paragraph at the end to briefly summarize results. The last section – References is well organized and I found only minor inconsistencies in using abbreviations or full name of journals.

My last questions are following: What would you do to improve the quality of the antibodies? Is there any alternative method how to investigate the putative association of *T.brucei* mitoribosomes with the inner mitochondrial membrane?

Conclusion:

In conclusion, I

r e c o m m e n d

the thesis for the defense and I suggest the grade - between Excellent to Very good depending on the presentation.

In Prague date 20/01/2021

