Přírodovědecká fakulta
Faculty of Science

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

STATEMENT OF THE BACHELOR/DIPLOMA* THESIS REVIEWER

Name of the student: Christian Resl

Thesis title: Characterization of Leucine aminopeptidase 1 in *Trypanosoma brucei*
Supervisor: Priscila Peña-Diaz

Reviewer: Ondřej Gahura
Reviewer`s affiliation: Institute of Parasitology, BC CAS, České Budějovice

	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	2
Quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0-3	1.5
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	1.5
Quality of the annotation	0-3	1.5
Language and stylistics, complying with the valid terminology	0-3	2.5
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	2.5
Formal requirements – points in total		14.5
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	2.5
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	1.5
Discussion quality – interpretation of the results and their discussion with the literature (absence of discussion with the literature is not acceptable)	0-3	1.5
Logic in the course of the experimental work	0-3	3
Completeness of the description of the used techniques	0-3	2.5
Experimental difficulty of the thesis, independence in experimental work	0-3	3

* Choose one

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

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		22.0
POINTS IN TOTAL (MAX/AWARDED)	48	36.5

Comments of the reviewer on the student and the thesis:

The thesis presents generation and phenotypic characterization of *T. brucei* cell lines with overexpressed, suppressed, or endogenously tagged TbLAP1. The methodology is diverse and together with the data documents the practical skills of the candidate obtained during the work. The key observation of the localization of TbLAP1 to the kinetoplast, and specifically to the "nabelschnur", is convincing and lead eventually to a publication in PLOS Pathogens. Some other experiments required further elaboration (e.g. suitable controls, replicates etc.) to be considered finalized. I find the presentation of incomplete data perfectly fine at the level of the bachelor thesis. However, the absence of any discussion of required additional experiments is rather unsatisfactory.

The thesis reads well and is generally comprehensible, the language and terminology is appropriate, only with minor problems. However, some parts of Results are too concise, reminding rather a paper in Science than a bachelor thesis. Also, the background on LAPs could be more extensive. Especially, I completely miss an explanation, why the protein became the subject of study.

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

Major points:

- The endogenous v5-tagging should be documented by a WB showing the expected size of TbLAP1-v5.
- All growth curves should be shown in the same format (compare Fig. 7 vs Figs. 9 and 11).
- Confirmation of TbLAP downregulation in the SMOX cell lines is missing. How do you know the observed defect was specific?
- The growth phenotype presented in the Fig. 11 is not "significant" (as stated). In fact, it is not convincing at all. The time points beyond 72 h would be needed.
- Fig. 15 needs controls, e.g. more time points and cells with different N/K content should be shown to document a potential link between the loss of kDNA and the mitotracker staining.
- The mt membrane potential measurement lacks an uncoupler (e.g. FCCP) as a control. The observed 72 h phenotype is not a "collapse", but a (perhaps significant) decrease. Given the minor changes, the experiment needs replicates.

Minor points:

- The first chapter in the thesis is called *Trypanosoma brucei*. The title "Literature Review", "Background", of "Introduction" would be more appropriate. In addition, this chapter should be divided into subsections.
- The origin of all antibodies should be provided.
- The resolution and quality of most figures in the Introduction is low. There are certainly much better schemes available.
- Avoid capitalization of common names and terms (Tsetse fly, Trypanosomiasis,...).
- *Mitochondria* is plural, *mitochondrion* singular.
- Be careful with statements concerning evolution. E.g. the statement "*...trypanosomes... ... being at the root of the eukaryotic tree of life and therefore sharing features common in most eukaryotes*" is wrong.
- The clone of the SMOX RNAi cell line used for the subsequent analyses should be specified after the growth curves are presented.

Questions:

1) How do you explain differences in proportions of DAPI cell counts at time point 0 between Fig. 10 and 12?
2) You stated that overexpressed TbLAP1-HA localizes to the kinetoplast. Where does it localize in cells, which have already lost the kinetoplast?
3) The presence of 1N0K cells 2 hr after induction of ectopic TbLAP1-HA suggests that the interval between the kinetoplast division and cytokinesis is less than 2 h. Can you comment on it in the context of the time frame shown in Fig. 3?
4) The decrease of mt membrane potential at 72 hr after TbLAP-HA induction could be a downstream effect of the kDNA loss. However, a significant proportion of cells still contains the kinetoplast, which would imply a presence of two populations of cells in the FACS profile. Could you comment on this?
5) The transfection has been done with 10 ug of linearized vector or PCR product. Is as much as 10 ug of the PCR product really needed?
6) Why did you use the LDS sample buffer and Bis-Tris PAA gels for SDS-PAGE?

Conclusion:

In conclusion, I

r e c o m m e n d

the thesis for the defense and I suggest the grade 2 .[2]

In **České Budějovice** date **15.9.2017**

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signature

[2] You can suggest a grade, which can be modified during the defense based on the presentation. However, if the reviewer is not present at the defense, the grade will not be counted.