



## OPPONENT'S REVIEW ON BACHELOR THESIS

Name of the student: Lehmayr Leonie  
Thesis title: Localization of the F1-ATP Synthase Subunit alpha and its N' and C'  
Terminal Fragments in *Trypanosoma brucei* cells.  
Supervisor: RNDr. Alena Zíková, Ph.D.  
Bc. Brian Panicucci  
Referee: Sneha Kulkarni, MSc  
Referee's affiliation: Institute of Parasitology, Biology Centre CAS, České Budějovice, Czech Republic;  
& Faculty of Science, University of South Bohemia, České Budějovice,  
Czech Republic.

	Point scale	Points
<b>(1) FORMAL REQUIREMENTS</b>		
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	3
Quality of the annotation	0-3	3
Language and stylistics, complying with the valid terminology	0-3	2
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	3
Formal requirements – points in total		19
<b>(2) PRACTICAL REQUIREMENTS</b>		
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	2

Logic in the course of the experimental work	0-3	3
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	3
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
<b>POINTS IN TOTAL (MAX/AWARDED)</b>	<b>48</b>	<b>45</b>

Comments of the reviewer on the student and the thesis:

The thesis is of sufficient length, with a detailed Materials and Methods section. The Introduction covers all the relevant topics of the thesis; and the Discussion section includes interpretation of each result, along with the troubleshooting for some of the experiments, which shows Leonie's scientific aptitude. She has done sufficient work for a Bachelor thesis, fulfilled all the aims of her project, and has used many sophisticated molecular biology techniques, which is impressive given the limited time.

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

**I have a few comments about the overall writing format and style:**

1. I appreciate how Leonie has explained the logic behind most of the steps in the protocols, in the materials and methods section; which shows that she understands the experimental design.

2. However, she needs to pay more attention to the correct format of scientific terms. The scientific names of organisms, *T. brucei*, and *E. coli*, have not been italicized many times. Other Latin-derived phrases such as *in vitro*, *in vivo*, *in silico*, have not been italicized either, but it is understandable, as there is an increasing trend to dispense with italics of such words in scientific publishing now-a-days.

3. One instance of needing to be more careful while writing is, the abbreviation IMS, which has been incorrectly expanded as "inner" membrane space, on page IV. Throughout the thesis however, it has been used as either "inter" or "inner" membrane space. These two words are not inter-changeable, and convey completely different meanings.

4. A general observation: Leonie needs to improve her scientific sentence construction. For example, on page 3, "protons want to run down their gradient"; or on page 20, "cells were induced to take up foreign DNA by shocking them with heat"; could have been more precise.

**Questions about the content:**

1. In section 1.1.2.2, page 5, you mention that the ATP synthase works in reverse, by hydrolyzing ATP to ADP and inorganic phosphate, in the bloodstream form of *T. brucei*. Does it rotate in the opposite direction? Is there any other closely related organism (trypanosomatid), which employs the same strategy (reverse function of ATPase) throughout its life? Why does it

need to do that?

2. In section 4.6, page 45, you mention, “we wanted to verify that the proteins expressed in each cell line were indeed being synthesized from the expected DNA sequence.” You carried out PCR on the genomic DNA to verify this. Was there any special reason that you used bloodstream form genomic DNA as negative control, or was it simply because of availability? Will the genomic DNA PCR tell you if your FL construct is being transcribed with the expected size? If not, which technique/s could you use to test that? And for that technique, can you use procyclic and bloodstream form samples inter-changeably, like you did for gDNA PCR?

3. In section 5, page 50, you discuss the possibility of C-terminal  $\square$  subunit, having an internal targeting sequence for the mitochondria, referring to the example of *T. brucei* alternative oxidase, for which the internal targeting sequence was identified by *in silico* analysis. Did a similar *in silico* analysis of your protein predict any internal targeting sequence? Are the characteristics (eg. charge, hydrophobicity *etc.*) of these internal sequences known?

4. This question is more about the future directions of the project. In section 5, page 49, you discuss that in order to find out if the cleavage of  $\square$  subunit is essential for enzyme activity, you could deplete the responsible protease, and see if it affects the ATPase activity. But this approach requires you to know which protease is involved. In absence of that knowledge, is there any other strategy you could use based on the primary sequence of the  $\square$  subunit itself?

Apart from the minor suggestions mentioned previously, I am completely satisfied with the thesis.

Conclusion:

In conclusion, I

r e c o m m e n d

the thesis for the defense and I suggest the grade 1 .<sup>1</sup>

In České Budějovice

date 10/09/2018



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signature

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<sup>1</sup> 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. Grades: excellent (1). Very good (2), Good (3), Unsatisfactory/failed (4).