Přírodovědecká fakulta
Faculty of Science

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

OPPONENT'S REVIEW ON BACHELOR/~~DIPLOMA~~* THESIS

Name of the student: **Sebastian Deisenhammer**

Thesis title: Characterization of *Trypanosoma brucei* MICOS subunit Mic20.
Supervisor: **doc. Hassan Hashimi, Ph.D.**

Referee: RNDr. Tamara Smutná, Ph.D.
Referee`s affiliation: **Institute of Parasitology, Faculty of Science, Charles University, BIOCEV Vestec**

	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	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	1
Graphic layout of the text and of the figures/tables	0-3	1
Quality of the annotation	0-3	2
Language and stylistics, complying with the valid terminology	0-3	1
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	0
Formal requirements – points in total		9
(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	1
Discussion quality – interpretation of the results and their discussion with the literature (absence of discussion with the literature is not acceptable)	0-3	0
Logic in the course of the experimental work	0-3	3
Completeness of the description of the used techniques	0-3	2

* Choose one

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

Experimental difficulty of the thesis, independence in experimental work	0-3	2
Quality of experimental data presentation	0-3	0
The use of up-to-date techniques	0-3	2
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		15
POINTS IN TOTAL (MAX/AWARDED)	**48**	**(0-48)[2]** 24

Comments of the reviewer on the student and the thesis:

Bachelor thesis of Sebastian Deisenhammer with the *topic Characterization of Trypanosoma brucei MICOS subunit Mic20* describes potential role of this protein in MIA pathway.

The Aims of the Thesis were to optimize the expression of soluble protein and to determine the redox potential of purified protein. There were several molecular biology techniques used to achieve these goals. The scheme of experiments is very well designed to establish basic experimental skills in the field of molecular biology. It includes PCR techniques, expression of proteins in *E. coli* expression system, affinity purification, SDS-PAGE and immunoblot analysis as well as the determination of redox potential using glutathione redox system.

However, the thesis is written in a very sloppy way.

- There are paragraphs which contain the same word several times without using appropriate synonyms, I recommend to use such words as „supplemented with" for solutions containing low concentrations of the others chemicals (for example „was solubilized in PBS buffer and triton and A and B"- in PBS buffer supplemented with x% triton, xM A, yM B etc.).
- Wrong references to Figures (page 5 in main text, legend to figure 13)
- Incorrect text style in chapter 3.1
- *E. coli* is not written in italic (p. 8), *Escherischia Coli* (p. 29)
- Inconsistent alignment of paragraphs throughout the text (*Discussion*)
- Wrong format of references (3 times)
- Passive voice and the inconsistency in use (on page 11 author switched from passive voice to imperative and then continues with passive voice in present form...)
- Page 13: varying concentrations of reduced glutathione – at least the range should be written, ThermoFisher Scientific- Scientific is out of brackets.
- Page 14: Very short legend, in the equation n.5, F and R represent constants.

Figures

- Figure 6: It is very hard to believe that the protein has molecular mass 22kDa from this figure, it would be worth repeating this electrophoresis. (Or at least describe in the legend that the lower part of the gel is skewed)
- Figure 7: *"The band corresponding to the protein is marked with an arrow. An improvement of the condition can be observed in this Figure."* I don't see any arrow.
- Figure 9: W2 instead of W3 in label of ELFO lines.

---

[2] Enter the number of points awarded.

- Figure 10: There should be the band of AcTEV protease (27kDa) marked in the middle line R. Otherwise the line R has no additional information, because in line P you present protein without any tag after successful tag cleavage and in line C protein with His-tag as purified.
- Figure 11: Here is a lot of confusing information. The legend is incomprehensible. "Fluorescence emission spectra of 5uM rMic20 recorded of the reduced (..) and the oxidized (…)" It is not clear of what. If it is protein rMic20, so that doesn't fit with conditions described in the brackets. In the buffer supplemented with 20mM GSH protein must be fully reduced and if there is no GSH only GSSG, the protein is fully oxidized. So, the solid line represents fully oxidized rMic20 and the dashed line displays the fully reduced state (in 20mM GSH). Inset in figure is incorrect as well- 10mM and 20mM GSH are reversed. I doubt the author comprehends the experiment.
- Figure 12: wrong concentration range in the legend, in the end of the legend I would recommend to include the chapter in which reader can find the Eq.2.
- Figure 13: missing numbers on axis y
- Figure 14: arrows?
- Figure 15: The legend is very brief. The reader should understand what it is about without reading the main text…what is C80, C83…. And it would be nice to know what the plans with these mutated proteins are. Finishing like this it is like book with open ending.

Discussion

Discussion is not a discussion; it is more like conclusion chapter. You should discuss, why it is difficult to express rMic20, what about expression of Tb mitochondrial proteins at all, compare conditions with different thioredoxin-like proteins etc…while citing the literature. **There are only 2 references total!!!**

The bachelor thesis is the first publication of most of the students. However, the scientific writing is not a skill we are born with, the accurate presentation of own results is a requirement.
Brief legends, missing numbers on axes, incorrect information, these are mistakes which can't be overlooked. It is not only the students' reputation, but a message for future students as well, that it is necessary to pay attention and invest time to write a successful thesis.

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

- On the page 20, in chapter *Expression and extraction of rMic20 in E. coli* you mentioned protein rMic32, whose expression failed. You write that this protein appears in some figures as a band of molecular mass 34kDa.
I haven't found any information about the function or expression of this protein, neither in chapter *Introduction,* nor in *Material and Methods*. Could you elucidate, (1) which figure(s) contains this protein band, (2) why you consider this information important in the context of the results of your thesis, and (3) in what way does rMic32 relate to the rMic20 project?

- In figure 11 you present data from the determination of optimal wavelength for detection of the fluorescence emission. It was determined to be 333nm and at this wavelength you recorded fluorescence emission for several reaction set-ups varying in concentration of GSH. From the graph in figure 12 it appears that there were 7 different concentrations of GSH, ranging from (probably) 0,1mM to 20 mM (not 0,1M to 20 M as you write in the legend).
Could you please clarify (1) what were the real concentrations of all these 7 points? (2) how many scans (independent reactions) for each concentration of GSH were measured and (3) whether the values in the graph represent the average of these values?

- Data obtained from figure 12 are used in figure 13 for the calculation of standard redox potential of TbMic20 (main text).
Why did you use only 3 values to create a line for linear regression analysis? I assume that these 3 values represent the concentrations of GSH in figure 11 and represent limit concentrations of GSH used in detection of appropriate emission wavelength. (In figure legend you refer to data from figure 1...)

- What do you think are the reasons of the complicated expression of rMic20? (Please refer to similar complications with expression of others MICOS subunits or thioredoredoxin-like proteins described in literature, special regions of protein...)
I would have expected that this issue will be included in the discussion.

Conclusion:

In conclusion, I

~~**recommend**~~ **/ do not recommend***

the thesis for the defense and I suggest the grade 4 .[3]

In date **08.09.2021**

.....

signature

[3] 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).