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: **Nora Müller**

Thesis title: The novel kinetoplastid kinesin TbKIFx and its partner TbPH1 are associated with specific cytoskeletal structures of *Trypanosoma brucei*

Supervisor: doc. Hassan Hashimi, Ph.D.
Referee: **Mgr. Vladimír Varga**
Referee`s affiliation: **Institute of Molecular Genetics of AS CR**

	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	2
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	3
Formal requirements – points in total		19
(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

* Choose one

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

Completeness of the description of the used techniques	0-3	2
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	2
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		25
POINTS IN TOTAL (MAX/AWARDED)	48	44

Comments of the reviewer on the student and the thesis:

For a bachelor thesis the presented thesis is of a high quality. The thesis is relatively long, but not tedious. The extent of the thesis rather reflects the amount of experimental approaches used and results obtained. Individual parts of the thesis are well balanced and follow in a logical order. Overall, the thesis is well written with the author paying attention to detail. The text is neat with the frequency of errors and typos being low and not distracting a reader. Figures and tables support the text in both Introduction and Results sections. Figures with legend are mostly self-explanatory. Valid terminology is used throughout the thesis and the text is properly referenced.

The thesis has 6 clearly stated scientific aims. The relatively higher number of aims reflects the fact that this work represents a continuation of a larger project pursued by the laboratory. To fulfill the aims a number of standard molecular biology, cell biology and biochemistry approaches were employed (PCR, preparation of transgenic trypanosome cell lines, measurements of cell growth, immunofluorescence, western blotting, cell fractionation etc.). The overall quality of presented data is very good indicating that the student had mastered all those approaches. For my suggestions and questions regarding the methods and results see the section bellow. The obtained results, such as localization of the two proteins to the microtubule quartet, which makes them some of the first identified molecular markers of the structure, or the observed dependence of recruitment of TbPH1 to the FAZ/quartet on TbKIFx, are novel and interesting and could certainly be a part of a publication by this group on the function of the two proteins in *T. brucei*. The results are appropriately discussed. From my point of view more valuable than Discussion is the section 'Future perspectives', which shows that the author is able to critically assess her data, identify the results, which need to be further studied and propose sensible experiments to address them. This is one of essential elements of scientific work and it is positive that the student can manifest this already in her bachelor thesis.

In conclusion, this thesis fulfilled its aim- the author mastered a number of approaches standardly used in cell biology laboratories and produced data, some of which are of a publication quality (this is a bonus, which I would not necessarily expect from a bachelor thesis). Furthermore, the author demonstrated ability to assess a scientific question based on existing literature, and critically assess her own data.

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

Questions:

1/ Growth curve- Figure 22- the author claims that a significant effect was observed from day 5. I would argue there was a difference already at day 3. How could this be resolved?

3/ In my laboratory we noticed that doing cytosolic or flagellar preparations on cells settled on glass slides may be misleading; some proteins can bind directly to glass and are therefore not solubilized by detergent treatment. Could the student suggest an approach to truly assess solubility of trypanosome proteins?

4/ During fractionation the presence of tubulin was noticed in the detergent-soluble fraction, e.g. on page 35 and 36. This has been attributed to 'incomplete sub-fractionation of the cytoskeleton'. Could the author elaborate on that? Are there any other explanations for presence of tubulin in the soluble fraction? Can any of these be tested?

5/ In my opinion the only significant disagreement in experimental data is in results of biochemical fractionation (Fig. 12), which show that the two studied proteins mostly partition into the cytoskeletal fraction versus the immunofluorescence staining of whole cells showing a cytosolic signal (Fig. 13). While the author claims that 'lack of information led us to perform IFA on cytoskeletons' which subsequently led to localizing the proteins to the microtubule quartet, I would argue that the IFA result on whole cells is significant (given the negative control). Is there a way the results of fractionation and IFA on whole cells could be reconciled?

Suggestions:

1/ While the Material and Methods section provides sufficient information on most methods, some information is missing. For example used antibodies would require further specification, such as the catalog number. There may be several products provided by the same producer, yet they will significantly differ in their suitability for a particular application. Likewise, giving specification for some instruments is essential- while this may not be necessary for a PCR machine or an SDS gel tank, it is important for a sonicator (there are several types of sonicators, which significantly differ in the way they work) and for electroporators (there are various types, plus different methods can be applied on a particular instrument, which may to a large extent affect the outcome).

2/ While the language used throughout the thesis is good, informal expressions are occasionally present, e.g. on page 18- more or less identical; p. 38- expressing RNAi; the proper names of the used cell lines are SmOxP927 and SmOxB427 etc. These should be avoided in the future. However, as mentioned above, these errors are infrequent.

3/ While I find a majority of the immunofluorescence images presented in the thesis convincing and would agree with conclusions of the author on protein localization, plus bloodstream cells are known to be difficult to image, DIC images are often suboptimal. The author should either attempt to improve the DIC imaging or try alternatives, such as phase contrast.

Conclusion:

In conclusion, I

r e c o m m e n d

the thesis for the defense and I suggest the grade 1 .[2]

In **Prague** date **27.01.2020**

Vladimír .....

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