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:Sabrina KALTENBRUNNER

Thesis title	**Characterization of TbPH1, a kinetoplastid specific pleckstrin homology domain containing kinesin-like protein**
Supervisor:	**Hassan Hashimi**
Reviewer:	**Linda KOHL**
Reviewer`s affiliation:	**National Museum of Natural History, Paris, France**

	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	2,5
Graphic layout of the text and of the figures/tables	0-3	2,5
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		18
(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	2
Discussion quality – interpretation of 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	2,5
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	2,5
Quality of experimental data presentation	0-3	2
The use of up-to-date techniques	0-3	2,5
Contribution of the thesis to the knowledge in the field and possibility to publish the results (after eventual supplementary experiments)	0-3	2,5
Formal requirements – points in total		21
POINTS IN TOTAL (MAX/AWARDED)	48	39[2]

Suggestions and questions, to which the student has to answer during the defense:

1. What are the characteristics of the cell line SmOxP9 ? Why was this line chosen ?
2. *In silico* studies :
    - Do you know other kinesins which have with these domains, i.e. kinesin with homeo-domain and/or pleckstrin domain ? What are their functions ?
    - You speculate that the modification in the Walker A domain renders the protein non functional.
        - How could you test this hypothesis ?
        - From a structural point of view, what is the difference between a Glycine and a proline ?
        - If the protein is not functional as a kinesin, what could its role be ? It seems essential – at least in BSF.
4. Are the RNAi mutant clonal lines ? How many transfectants did you analyse?
5. What is a trypanosome 'octopus' ? Is it one cell or a multitude of cells, in a rosette ? These cells appear very late (at Day 6 of induction), where division seems almost absent. What percentage of cells are 'octopus' ?
6. How do you explain the difference between BSF and PCF RNAi cells? If the problem is the same, involving a cell cycle progression deficit, why is the effect so much stronger in BSF than PCF ?
7. Concerning the RNAi mutants, could you discuss the difference between your results on fitness in PCF and BSF and those of the genome wide screen ?
8. In the RNAi mutants, what cell types are the 'other' ? are they multinucleated, or zoids ?
9. FACS-measurements:
    - what type of cells do you think are visible in the induced population left of the P3 gate ?
10. Localisation experiments: is the labelling the same in all cell cycle stages (1K1N, 2K1N, 2K2N)?
11. KIF11: what family does it belong to ? Do you have any idea what its role could be ? Is it essential ?
12. How do you think the two kinesins interact ?

Eventual mistakes, which the students should avoid in the future:

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2 Enter the number of points awarded.

A few small comments regarding the manuscript:

1. The figures are well chosen, but they could be a little bigger, especially as there seems to be no limitation in the number of pages.
2. There is a large bibliography, however the papers are not always the most adequate or the more recent: For example, p2, references for the FAZ are Vickerman (1969), Woods (1989), this structure has been studied in detail over the last years and there are recent references that could have been used (ex. Sunter & Gull, 2016). Similarly, the kinesin family reference is Wickstead 2006, the same author published a more recent, more comprehensive study in 2010.
3. p24. You don't induce RNAi, you induce the production of dsRNA – this is a common error
4. P26. It's a shame that you didn't continue the BSF RNAi growth curve to 48H, fig11 indicated that the problem might be visible just after 24H. The differences in fig12 seem slight to me. You have to be very critical in your results, especially if it's 1 transfectant, 1 experiment.
5. Fig16. Use the same colour for the same timepoint: left 12H control=blue, right panel, it is green; and 24H is green on the left and red on the right. The same colour scheme makes it easier to compare things for the reader
6. Subcellular localisation: show phase pictures. It shows what shape the cells are in, especially in fig 19 & 23. In fig 19, the phase would allow to see the difference between FAZ and flagellum

Eventual additional comments of the reviewer on the student and the thesis:

The thesis by Sabine Kaltenbrunner is generally well written and interesting . It is obvious that the student has done a lot of work and manged successfully to learn a lot of very different techniques. Many results are still preliminary, but this is to be expected. I am convinced that Sabine will defend her work well and that she will go on to a good PhD.

Conclusion:

In conclusion, I r e c o m m e n d the thesis for the defense and I suggest the grade very good (could go to excellent if defense is very good).[3]

In **Paris** , May 17th, 2017

Kohl

Linda KOHL

[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.

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STATEMENT OF THE DIPLOMA THESIS REVIEWER

Name of the student: Sabine Kaltenbrunner

Thesis title: Characterization of TbPH1, a kinetoplastid-specific pleckstrin homology domain containing kinesin-like protein

Supervisor: Hassan Hashimi, Mag. Ph.D.

Reviewer: Vladimír Varga, Ph.D.
Reviewer`s affiliation: Institute of Molecular Genetics of ASCR, Prague

	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	2
Quality of the annotation	0-3	*
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		
***not sure what this means**		
(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	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
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	2

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

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		
**** for publication more experiments are needed**		
POINTS IN TOTAL (MAX/AWARDED)	48	(0-48)[2]

Comments of the reviewer on the student and the thesis:

The work provides new and interesting results. The most interesting ones are the observed strong growth phenotype in TbPH1 depleted cells and the biochemically proven interaction of the protein with the kinesin TbKIF11. A broad spectrum of molecular biology, cell biology and biochemistry approaches were employed for the purpose to characterize TbPH1. The experiments appear carefully performed. Results of different experiments do not always lead to the same conclusion and additional experiments will be needed to clarify these issues. In general, the student is apparently well qualified to perform laboratory experiments, mastered a range of techniques, and is able to pursue an independent project. The major weakness of the work is its "formal aspect" - some of the figures/tables are incorrectly referred to in the text, reference are not always numbered consecutively, there are numerous typos. Although clearly less important than the experimental results, greater care should be taken in the future to avoid those, as they tend to spoil the overall impression.

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

Questions:

Why did you decide to study TbPH1 in the first place? Was it based on some preliminary observations, bioinformatics or something else? It would be very useful for readers to include a statement on your initial motivation.

Two different tags on TbPH1 differ in their localization. What could be the reasons? How would you approach this to definitely prove the localization?

How do you reconcile your data on TbPH1 detergent solubility- your immunofluorescence (IF), digitonin and NP-40 fractionations lead to opposing conclusions.

I was intrigued by the observation of the "octopus" phenotype caused by depletion of TbPH1 in BSF trypanosomes. It would be useful to include an image of such a cell. Are these cells similar to the described phenotype caused by an absence of flagellar motility (e.g. Broadhead et al., Nature 2006)? Unexpectedly, this phenotype develops only at day six of RNAi induction, despite the strong reduction in the growth rate observed already on day two. How do the cells look between day two – six?

Related to the previous point, your DAPI counts show only rather moderate changes in proportions of cells in individual cell cycle stages following RNAi induction in BSFs, even after 48 hours (Fig. 13). Yet, there is a very strong growth phenotype (Fig. 11).

[2] Enter the number of points awarded.

How do you reconcile these two pieces of data?

Depletion of TbPH1 causes an increase in proportion of 1N2K cells. However, this is a rather broad category and the cells could be classified further, for example based on their morphology, such as the new flagellum length, which may provide indications for the function of TbKIF11. Furthermore and importantly, it is easy to distinguish whether the cells are prior to mitosis or in mitosis. You suggest that the delay in the cell cycle is caused by a potential delay in the progression of mitosis- so is it really the case?

Regarding the previous points can you speculate what could be the function of TbPH1 and its binding partner TbKIF11 in a trypanosome cell?

Questions regarding methodology:

In your microtubule-sieving experiments you resuspended the pellet by passing it through a 26G syringe and vortexing. From my own experience these detergent extracted cells (cellular cytoskeletons) are rather fragile and easy to break by shearing, e.g. by passing through a syringe. The broken cytoskeletons do not completely depolymerize and their fragments can be pelleted. Have you checked this is not the case, for example by phase contrast imaging or IF with anti-tubulin antibodies?

You use IF to colocalize TbPH1 with microtubules (Fig. 23). Yet the microtubules form a tightly bound corset underneath the entire trypanosome cell surface. My understanding is that any trypanosome protein is bound to appear as to "colocalize" at least with a fraction of microtubules in a 2D image. What do you mean by the protein being "overlapping or directly adjacent to microtubules"? Given how closely are the microtubules spaced in the corset, there is surely no chance to determine the relative position of TbPH1 to microtubules by light microscopy.

I am surprised by the need for the very long incubation with Dynabeads (chapter 3.7.2). Do you have any explanation why such a long incubation is required?

How would you address the question whether TbPH1 is an active kinesin (ATP-hydrolysis competent, actively moving on microtubules)?

Mistakes to avoid:

I strongly recommend the student to perform more extensive proofreading next time.

Some experiments are not sufficiently described in materials and methods, e.g. bioinformatics, proteomics (I understand this was done by some facility, but a brief description of the procedure should be nevertheless provided).

Vague statements- e.g. "the band looks bigger"-should be "migrates at a higher MW"

For future I suggest phase contrast images to be included in figures showing fluorescence signals.

Conclusion:

In conclusion, I

r e c o m m e n d

the thesis for the defense.

In **České Budějovice** date **23.5.2017**

signature