Přírodovědecká fakulta
Faculty of Science

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

STATEMENT OF THE BACHELOR THESIS REVIEWER

Name of the student:	**Sabine Kaltenbrunner**
Thesis title:	The Localization of the Mitochondrial Proteins MRP1, KREL2 and LSU1 of *Trypanosoma brucei*
Supervisor:	Mgr. Ph.D. Hassan Hashimi
Reviewer:	**Corinna Benz, PhD**
Reviewer` affiliation:	**Institute of Parasitology, Biology Centre, Academy of Sciences of Czech Republic and Faculty of Science, University of South Bohemia**

	Point scale[1]	Points
(1) FORMAL REQUIREMENTS		
Extent of the thesis (for bachelor theses min. 18 pages, for masters theses min. 25 pages), **balanced extents of the thesis divisions** (recommended extent of the theoretical part is max. 1/3 of the total extent), **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	3
Graphic layout of the text and of the figures/tables	0-3	3
Adequacy and clarity of the results and conclusions	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 even without reading the rest of the text, explanation of the symbols and labeling, indicating the units)	0-3	3
Formal requirements – points in total		22
(2) PRACTICAL REQUIREMENTS		
Clarity of the aims	0-3	3
Fulfillment of the aims	0-3	3
Discussion quality – interpretation of results and their discussion with the literature	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

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

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	2
Formal requirements – points in total		25
POINTS IN TOTAL (MAX/AWARDED)	**51**	**47**

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

- What other ways can you think of to show that a protein is functional (in addition to its ability to form a complex)?
- You tagged you proteins at the endogenous locus (C-terminally) – why might expression levels of the tagged proteins still not correlate with endogenous expression levels?
- Why did you choose to work on these three proteins?
- You are talking about future FRET experiments – which proteins would you test for interaction with 'your' proteins?

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

Aim for a better balance of introduction, results and discussion.

Eventual additional comments of the reviewer on the student and 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 **1.3** .[2]

In **České Budějovice** date **18.12.2013**

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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.

(1) Formal Requirements

- Length requirements are more than fulfilled, I have the feeling that the balance is a bit off though. While you explain very nicely and in great detail all experimental procedures, I would have liked to see a bit more of an introduction and discussion. The logic is perfectly fine (2)
- Again, as I said earlier a little bit more background information would have been nice (2)
- I think you mixed up references 1 and 3, but everything else looks good (3)
- Perfectly fine (3)
- Your results are well presented and your conclusions are sound (3)
- Perfectly fine (3)
- I think your figure/table labels are very clear, accurate and easily understandable (3)
- That gives you a total of 22 points in this section

(2) Practical Requirements

- Your aims are perfectly clear to me (3)
- And you managed to tag and localise all three proteins and show that tagged MRP1 is in a complex with endogenous MRP2 (3)
- You don't really discuss your findings except to say that your proteins are as expected mitochondrial and tagged MRP1 forms a complex with endogenous MRP2. You could have for example suggested something like localised translation in the mitochondrion (since your ribosomal subunit is non-uniformly distributed)... (2)
- Again your work is perfectly logical (3)
- And your experimental procedures are extremely well described (3)
- While making tagging constructs and transfecting trypanosomes isn't exactly rocket science, getting an IP to work can require quite a lot of effort (3)
- Your figures are really clear and the results well described (3)
- GeneArt cloning, Dynabeads etc have arrived rather recently (3)
- Well, all these proteins were already known to be mitochondrial and while fluorescently tagged proteins can of course be used for things like FRET and FRAP, that would require a whole lot more work... (2)
- That gives you a total of 25 points in this section
- And 47/51 in total

Some more comments (just for future reference, I don't want to be this picky)

- There are more than three morphological states of trypanosomes (stumpy forms in the bloodstream, long and short epimastigotes in the tsetse)
- pBR322 origin on your plasmid is not for replication in tryps (your vector integrates at the endogenous locus and is thus multiplied in every cell division with the chromosome it's on)

- Did you really wash your DNA with cytomix buffer before transfection?
- We don't have CO_2 in the procyclic incubators
- You can strip nitrocellulose membranes just as well as PVDF ones
- $1.5x10^7$ cells/ml is not log phase anymore
- You could mention different exposure times used for your IFA – to me the intensities of the signals look very similar (unlike the band intensities on the Western)
- Maybe mention how many cells (or percentages of total) you are loading per lane in your IP Western figure. I know it's in your materials and methods, but just for a quick comparison (since it worked so perfectly).