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: **Ricarda Marko**

Thesis title: Counteracting constitutive activity of Orai1 with an H171Y mutation
Supervisor: **Mag.[a] Dr.[in] Irene Frischauf MLBT**

Referee: RNDr. Zdeněk Frantam PhD
Referee`s affiliation: **Institute of chemistry, USB**

	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	2,5
Quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0-3	1
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	1,5
Quality of the annotation	0-3	2
Language and stylistics, complying with the valid terminology	0-3	1,5
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	0,5
Formal requirements – points in total		
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	1,5
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	1,5
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	1,5
Completeness of the description of the used techniques	0-3	0,5

* Choose one

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

Experimental difficulty of the thesis, independence in experimental work	0-3	1,5
Quality of experimental data presentation	0-3	1
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
Practical requirements – points in total		
POINTS IN TOTAL (MAX/AWARDED)	48	(25)[2]

Comments of the reviewer on the student and the thesis:

Presented work of Ricarda Marko deals with characterization of pore forming subunit (ORAi1) of calcium activated (CRAC) membrane channel. The main goal of the thesis was to mutate chosen AA and evaluate the mutation effect. The thesis has 29 pages in total and is divided into six parts (introduction, Task, Materials and methods, Results, Discussion, and Literature). Thesis contains several typos and improper statements (e.g. Wrong volume for PCR mixture, *E. coli* shall be in italic, "Eisessig" instead of acetic acid, or "50ul of Ampicillin resistance are added"!). Despite the interesting topic, the thesis is rather weak and requires further improvement.

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

Minor points:

1: Thesis contains list of abbreviations, however these are not in alphabetical order and it is hard to search for specific one. Moreover not all abbreviations used in the thesis are mentioned here (e.g. SDS, HBSS, EDTA)

2: it will be good if you mark the mutation within your primers, I would also change the column name in Table 1 from "gene" to "mutation"

3: why did you make no polymerase control? What it will tell you?

4: what was your template DNA you use for PCR reaction?

5: what did you use kananycin for? Based on your MM section you work with pcDNA 3,1 , which has Amp resistance.

6: It is not clear which *E. coli* genotype did you use

7: There are only 17 papers used within the thesis. AS I am not much in the topic I did search for the available literature and found several papers, which shall be included in the thesis, as these are tightly associated with the topic of the thesis (e.g. Yeung, 2018: Mapping the functional anatomy of Orai1 transmembrane domains for CRAC channel gating, PNAS)

[2] Enter the number of points awarded.

Major points:

1: weak or no picture legends, all picture legends shall be edited to make the picture self-explanatory and giving the reader necessary information to understand the picture.

2: I am missing better introduction to the topic. The introduction in the thesis is rather broad, but lacks the part focused on Orai1 in more details. This goes hand to hand with the racionale of the thesis, why did author chose specific AA (why did you pick E106, F99, R91 and not V102 or L95) to mutate? Why shall H171Y mutation abolish Orai1 activity?

3: It is really mot clear to me, which mutation were performed by author and which not. You state show primers for H171Y, R91C, F99C, E106C, ANSGA (page 1), but protein aligment in results (page 19) show only F99C, H171Y, ANSGA. Moreover you also show other mutation (C126, 143, 195V, N223A), which are not further in the text. Why did you remove these endogenous cysteines? Can you make a scheme of constructs, you made and comment on this.

4: methods described in the thesis lacks many crucial informations and I do have the feeling that the author did not really master all the methods used within her thesis.

5: can you describe the complete procedure for cloning. It is not clear from your MM section how you performed it (e.g. You show pcDNA3.1 TOPO backbone, which uses AT overhands to insert gene, but you use PFU polymerase, which does not add A overhands! Moreover, when doing SDM, you amplify the whole plasmid with the mutated gene and there is no need to insert it into another vector.

6: how can you be sure, that you detect the protein of interest on WB (figures 11, -14). There are many other bands presents on the membrane. Have you try to identify these bands? I am also missing the control on native protein, without any mutation as well as proper negative control (cells without any overexpression).

7: I am missing more information about your primary antibody. Where did you get it and how did you test its specificity, as this is crucial for your results.

8: Why did you perform MW calculation just based on average AA weight, if you know the whole sequence? Can you calculate the exact predicted MW of your protein.

9: I am a bit skeptical about the results statements. How you can be sure only from the WB that the channel is deactivated? Did you perform any activity measurements? It is also not clear to me, how you can say from the WB results that "the H171Y mutation does not significantly change the structure of the pore".

Conclusion:

In conclusion, I

r e c o m m e n d

the thesis for the defense but I will suggest the grade based on candidate's performance during presentation and answers to my questions.[3]

In **České budějovice** date **19.01.2022**

Zdeněk Frank

signature