Přírodovědecká fakulta
Faculty of Science

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

OPPONENT'S REVIEW ON DIPLOMA* THESIS

Name of the student: **Bc. Kateřina Jaklová**

Thesis title: Characterization of nuclear and cytoplasmic interactome of tick-borne encephalitis virus capsid protein
Supervisor: **RNDr. Martin Selinger, Ph.D.**
Co-Supervisor: **RNDr. Pavlína Věchtová, Ph.D.**

Referee: **RNDr. Zdeněk Franta, Ph.D.**
Referee`s affiliation: **UCH**

	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	3
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	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	2,5
Formal requirements – points in total		20
(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

* Choose one

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

Logic in the course of the experimental work	0-3	3
Completeness of the description of the used techniques	0-3	3
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
Practical requirements – points in total		26
POINTS IN TOTAL (MAX/AWARDED)	**48**	**46**

Comments of the reviewer on the student and the thesis:

Diploma thesis of Bc. Kateřina Jaklová named *Characterization of nuclear and cytoplasmic interactome of tick-borne encephalitis virus capsid protein* focuses on the role of TBEV C protein in the host cells and exploits its interacting partners. The thesis is written on 59 pages and has a classical outline divided into nine chapters (Abstract, Literature review, Aims and objectives, Materials and methods, Results, Discussion, Conclusion, List of abbreviations, and References). The thesis also contains supplementary material for the MS experiments. I appreciate that author decided to write the thesis in English. The language is quite good and contains only a few typos. The main topic is very interesting, and the used approaches are up to date. It is clear from the thesis that the author had to establish various approaches in the lab, which clearly influenced gained results. Presented data are clear, however rather preliminary and require further experimental support.

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

Introduction:

The introduction is in general good written, however the first part (flavivirus taxonomy, subtypes and epidemiology) is not directly associated with the experimental work and out of topic. There are also a few inaccurate statements, which could be eliminated e.g.: on p7 saying all tick life stages (larvae, nymphs, adults), but not stating eggs, or stressing 3´Stem-loop structures, but omitting 5´stem-loop, which plays crucial role in replication initiation. But as said it is generally well written and I have only a few minor points here:

1 – on page 10 author states that NS1 exists in variety of shapes. I am not sure what do you think by this. Can you explain it in more details?

2 – Do you know about any other roles of NS4A and NS4B except the one you state here?

3 – How would you use the capsid protein as a drug candidate? What would you target?

M&N is nicely written and contains almost all information the reader needs.

1 – It is not clear to me; how did you lyse the cells in chapter 4.2 Rapid isolation of nuclei from cells in vitro?

2 – I am missing any washing steps upon WB transfer to the membrane and blocking with

milk. Did you forget to state it here or you really did not perform them?

3 – can you comment more on the immunoprecipitation experiments (chapter 4.9) what was the origin of Ab used? Did you somehow purify the Ab prior binding? I would be worried to use polyclonal Ab as all Immunoglobulins from the animal (not C specific) would bind. Did you perform any negative experiments (e.g. using preimmunize sera) to see the unspecific binding?

Results summarize all the major findings and guide the reader through the whole experimental process; I really appreciate the graphical scheme for individual experiments.

1 – can you show the SDS PAGE gels for your WB data? This would give a better overview of the protein profile.

2 – on figure 13 you show the WB signal for C protein in nuclear fraction only. This is an interesting result, as I would expect much more signal in cytosolic or membrane fraction. Do you have any explanation for this?

3 – From figures 14 and 15 it is clear that the signal for C protein is quite low. I would recommend optimizing the Ab concentration using whole cell lysate. This could save you some time and effort.

4 – another interesting result I found in figure 16. Did you really detect the NS3 and NS5 proteins in nucleus only? Which domain from these two proteins did you detect? How would you explain missing detection in cytosolic/membrane fraction?

Discussion is following the main findings and author supports the data by intensive literature search. I have only one question regarding this part. Could author comment on differences between her work and work published by Martin Selinger? Could there be a reason you detected different proteins?

Conclusion: Despite all my comments and questions, diploma thesis of Bc. Kateřina Jaklová is well written, and the author proved that she mastered various techniques during her master studies. I am convinced that the presented thesis fulfills all the criteria of Faculty of Science at University of South Bohemia and I have no hesitation in recommending the thesis for the defense

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 České Budějovice date **10.05.2023**

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[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).