Přírodovědecká fakulta
Faculty of Science

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

OPPONENT'S REVIEW ON BACHELOR THESIS

Name of the student: Anirudhan Ravi

Thesis title: Mass spectrometry in structural proteomics: study of viral protein complexes

Supervisor: Mgr. Dyčka Filip, Ph.D.

Referee: Mgr. Dmitry Loginov, Ph.D.
Referee`s affiliation: BIOCEV - Institute of Microbiology AV 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	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	3
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
Formal requirements – points in total		18
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	0
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	0
Discussion quality – interpretation of the results and their discussion with the literature (absence of discussion with the literature is not acceptable)	0-3	0
Logic in the course of the experimental work	0-3	1
Completeness of the description of the used techniques	0-3	2

[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	2
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	0
Practical requirements – points in total		11
POINTS IN TOTAL (MAX/AWARDED)	48	29

Comments of the reviewer on the student and the thesis:

The idea behind the thesis was a very complicated and interesting task, which required knowledge in the structural mass spectrometry. Unfortunately, this task was too ambitious for the bachelor student. Obviously, it was hard for him to understand both used methods (HDX MS and cross-linking) and analyze all obtained data. In my opinion, it would have been much better to focus only on one of them, but in more details. Overall, this work represents combination of two modern MS-based techniques, each implemented with mistakes, and obtained data were not fully understood.

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

1. Why do you have such a high error of MALDI measurement (>4000 ppm between panel A and B)? Also, why did the middle peak increased its mass (around 1000 m/z) while another one lost 200 m/z?
2. Do you have SDS-PAGE confirming cross-linking? Have you performed optimization of cross-linking?
3. How did you distinguish between intra- and inter- molecular cross-links when you have a homo complex?
4. You detected cross-links with distance above 24A. Does it mean that predicted structures are incorrect? If yes, is it correct to use? Is there a possibility to apply detected cross-links for a better structure prediction?
5. In the discussion part you wrote that cross-links linked to the SUMO part have no biological relevance. So, why did you use protein with SUMO, what is the role of SUMO part?
6. Why did you use 50 mM phosphate buffer, pH 2.6 for quenching reactions in the HDX experiments? Why did not you add reduction agents in it (like TCEP)? What impact unreduced Cys might have onto HDX data?
7. The sequence coverage in HDX MS experiment is rather low for a single protein. Have you tried to improve the digestion conditions (for example, adding urea to the quench buffer) or different proteases?
8. On the MALDI spectra there were at least 2 forms of the protein. How can you be sure that HDX data were not affected by this factor?
9. What is the error of the HDX measurement? The kinetics looks strange because it has

ups and downs. There should not been decreasing in the deuterium uptake. Have you performed data normalization using fully deuterated samples?

10. Have you noticed appearance of Ex1 kinetics in the deuterium uptake upon RNA binding?
11. The introduction part should be focused on the relevant things to the subject. In the context of the study. It was not necessary to describe in details basic principals of MS and mass spectrometers. The information of using cross-linking and HDX MS in the context of viral proteins studying is missing. Cross-linking and HDX MS might be written better, for example, types of cross-links and deuterium exchange kinetics are missing.
12. The mistake in the description of fig. 11.

Conclusion:

In conclusion, I

r e c o m m e n d

the thesis for the defense and I suggest the grade good .

In **Prague** date **12.01.2022**

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signature