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: **Guy Wolf**

Thesis title: Encapsulation of bacterial photosynthetic reaction centers into viral capsids
Supervisor: Mgr. David Kaftan, Ph.D.; prof. Mgr. Roman Tůma, Ph.D.

Referee: Mgr. Lenka Malinovská, Ph.D.
Referee`s affiliation: National Centre for Biomolecular Research, Faculty of Science, Masaryk university

	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
Graphic layout of the text and of the figures/tables	0-3	2
Quality of the annotation	0-3	2
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	2
Formal requirements – points in total		16
(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	2
Logic in the course of the experimental work	0-3	3

* Choose one

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

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	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	3
Practical requirements – points in total		25
POINTS IN TOTAL (MAX/AWARDED)	**48**	**(41)[2]**

Comments of the reviewer on the student and the thesis:

The submitted bachelor's thesis is methodologically diverse, with the student employing methods of molecular biology, protein production, and purification. The amount of work carried out and the results obtained are above average. The set aims appeared ambitious, yet the student managed to accomplish them.

The theoretical part, of the appropriate scope, introduces the reader to the topic. However, a more organized description of the photosystem of *R. sphaeroides* would be welcomed. The combination of the description with Figure 3, which had an insufficiently explained color scheme, was somewhat confusing. Additionally, more information on the planned utilization and applications of the photosynthetic nanoreactor (mentioned in the thesis as the so-called "bigger project") would be appreciated. All the methods used are adequately described (with only minor omissions), and the Results section contains an impressive amount of data that would benefit from a more detailed description and deeper discussion (for example, I missed comments on the significance of the second cleavage site for thrombin in the SP141 protein).

Overall, I assess the thesis as successful and recommend it for defense.

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

1) Page 22: "The restoring peak caused by 1M imidazole was probably due to other bound proteins, smaller than the RC-LH1, and therefore interacting better with the column."
Was this assumption tested using SDS-PAGE? How many proteins were observed in the fraction?

2) Page 24: "Both expressed proteins forming the capsids can be found again in the insoluble fraction, confirming the assumption that the capsids can be found there."
This could also indicate that the proteins are produced in improperly folded form within the inclusion bodies. Is there any way to verify the correct production of capsids, other than their successful isolation?

3) Page 26, Figure 18: Why are there two prominent bands in the positive control? In Figure 17, there is only one band in the positive control corresponding to the expected product.

4) SP141 is cleaved by thrombin at two sites. How could this affect subsequent experiments? Do you plan to remove the unwanted protein fragment in the future?

5) Page 33: "However, handling of the scaffolding protein was challenging because of the lacking thrombin

[2] Enter the number of points awarded.

cleavage site preventing the removal of its HisTag, thereby exposing the cysteine."
Does this mean that NTA maleimide can only react with the terminal cysteine? What does the term "exposing the cysteine" mean?

Mistakes, which the students should avoid in the future:
References should be part of the sentence, not standalone fragments.

On the other hand, I definitely do not recommend making figures part of the main text, which frequently occurs in the thesis (e.g., "The chromatogram of that purification is depicted in the following Figure 20:"; the colon is then followed by the respective figure). Figure 25 should be in the Materials and Methods section, where it is even referenced, not in the Results section.

There should be spaces between values and their corresponding units. The work often presents values combined with units, but not consistently. In other words, the writing of values and units is additionally inconsistent, sometimes even within the same sentence ("The ratio between the peak at 870 nm and 510nm can be..."). Inconsistencies also occur with certain technical terms (*in vitro* vs. in vitro or *in silico* vs. in-silico, within the same paragraph).

Graphs (specifically Figure 10 and 20) lack scale labels on the axes, which makes orientation difficult. I also could not find the composition of "SDS-buffer" and the conditions for performing "colony PCR" in the methods section - the description of all used methods should be complete for potential experiment replication.

Conclusion:

In conclusion, I

recommend / ~~do not recommend~~*

the thesis for the defense and I suggest the grade Very good .[3]

In **Brno** date 9. 6. 2023

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signature

[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. Grades: excellent (1). Very good (2), Good (3), Unsatisfactory/failed (4).