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: **Andreea-Adriana Avram**

Thesis title: Novel Photonic Materials: Isolation, purification and Imaging of native chlorine based antenna from *Chloroflexus aurantiacus'*
Supervisor: **Dr. David Kaftan**

Referee: David Bína
Referee`s affiliation: Institute of Chemistry, USB/BC CAS

	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
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	1
Graphic layout of the text and of the figures/tables	0-3	2
Quality of the annotation	0-3	3
Language and stylistics, complying with the valid terminology	0-3	1
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
Formal requirements – points in total		10
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	1
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	1
Logic in the course of the experimental work	0-3	1

* Choose one

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

Completeness of the description of the used techniques	0-3	2
Experimental difficulty of the thesis, independence in experimental work	0-3	2
Quality of experimental data presentation	0-3	0
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	1
Practical requirements – points in total		11

POINTS IN TOTAL (MAX/AWARDED)	48	(21)[2]

Comments of the reviewer on the student and the thesis:

The submitted experimental thesis describes isolation of chlorosomes, light-harvesting complexes from green phototrophic bacteria. The work performed by the student comprised maintenance of bacterial culture, pigment analysis, purification of chlorosomes by combination of sucrose density gradient ultracentrifugation and size-exclusion chromatography as well as imaging of prepared chlorosomes by electron microscopy and atomic force microscopy. As such, the work performed is clearly sufficient for a bachelor thesis, indeed it would be sufficient for a master thesis in my opinion.

That said, I do not consider the quality of presentation of the obtained results very high. The purpose of the thesis is not to simply give an overview of what was done, this could be solved by simply writing a list of methods that were used. On the contrary, the thesis is an exercise in the ability to present the results as a coherent body of knowledge. That is, it is not enough to obtain some data but one is expected to stop and think what they mean, it they conform to expectations, or not (and why). And, this should be made clear to someone not directly involved in the work. Unfortunately, the present thesis is not very successful in this respect and gives an appearance of a draft rather than a finished text. I suppose this is because it was completed under significant time pressure.

Several examples to illustrate the point:

Figure 5. The title: Chloroflexus pigments under different conditions, the caption says "In vivo absorption spectrum of cells...". If yes, the culture would be very, very unhappy. BChl c in vivo is found in aggregates absorbing well above 730 nm, BChla is bound to proteins and absorbing above 800 nm. This figure looks like spectra of extracts, except for the presence of scattering (but why scattering in the extract?). So either title or legend is wrong. This is also clear by comparison to Fig. 6.

Sucrose gradient. The 1st step (Figure 7, not 6) may show 4 different bands but only one is labeled in the figure ('I'). And, frankly, I don't see the other three very well. The spectra of the bands from sucrose gradient centrifugation are shown in Fig. 8 and 9. It is not clear how the fractions correspond to the bands shown in pictures of gradients (Fig.7). Also, there are two intense bands in gradient following step 2. Are their spectra in Fig 9? Did the spectra of the bands II and III differ at all? What are these bands? Perhaps the gradients were swapped? There is no attempt to explain the observation.

The last paragraph in the section 4.3.1. states "second step ... showed overall a better

[2] Enter the number of points awarded.

absorbance level...indicating an efficient purification." "a better absorbance level" is not a meaningful expression. If this simply indicates higher/larger absorbance it can hardly be an indication of a more efficient purification. Just of higher concentration. Apparently, all the spectra shown in fig 8 and 9 are quite similar to spectra of cells as shown in Fig. 6 and differ only in intensity. The author does not comment on the spectral feature at 870 nm. Moreover, in section 4.2., page 19, last sentence, it is stated that BChl a was found at "higher wavelengths of 780-820 nm". This suggests the author is not aware of the origin of the absorption band at 870 nm, which is also due to BChl a. In fact this feature is the most straightforward indicator of purity, or the lack of, of the chlorosome preparation since it originates in the integral antenna complex. This should not be present in pure chlorosomes. There is literature about this, for instance a Journal of Bacteriology paper by Pšenčík et al. (2009) which has a nice chlorosome spectra with different amount of membrane attached. This paper was, surprisingly, omitted in spite of the co-supervisor being one of the co-authors of this publication.

However, it must be emphasized that achieving complete separation of the membrane from chlorosome in Chloroflexus is indeed difficult.

In relation to this, the fact that the structure of the photosynthetic apparatus of Chloroflexi, with respect to pigment-binding components is not discussed in the introduction is a shortcoming. Unfortunately, the stated aims no. 2 and 3 of the thesis are given as "Optimization". But the criteria used to evaluate the performance of purification are not at all clear.

Size exclusion chromatography. It is not apparent which fraction from the gradient was chosen for size-exclusion chromatography step, and how exactly it was chosen. It is said that this method was used to separate intact chlorosomes from fragments etc. The Fig. 10 just shows some chromatogram with two peaks. But according to the text it appears to be two injections of... of what, actually? It is not said if there were any chlorosome fragments or free pigments observed. Hence, it is not obvious whether the size exclusion step represented an improvement over the simple sucrose gradient centrifugation and thus whether the method met the expectations. Especially since the electron microscopy image shows something looking like debris. And the author herself says that chlorosomes were of "variable size". Not what would be expected from size-exclusion. Moreover, the spectra of the fractions from gel filtration are not shown.

Imaging. The author states in caption of Fig. 12, that panel b is an individual chlorosome. But it is also said that chlorosome dimensions were about 180×35×15 nm. However, the object in the panel b, Fig. 12 is apparently more than 300 nm long. My guess would be that is consists of about four chlorosomes sticking together, not a single chlorosome. Although the image is scaled so that vertical axis is different from horizontal, making size estimation more difficult.

Minor issues.

Page 1, section 1.1: "...light usually relates to radiation in the entire electromagnetic spectrum..."

What does it mean, relates? Also, what source this comes from? It would be much easier to find support for the statement that light refers specifically to the visible part of the spectrum of electromagnetic radiation.

P2, section 1.2. The reference [14] is hardly the proper source for the information regarding the carbon metabolism in Chloroflexi. Also, reference [15], which is appropriately used here, reappears as reference [30] later.

P4, section 1.3.1. quinines: I assume this is likely the work of the MSWord spellchecker, should be quinones

Similarly, chlorine/bacteriochlorine should be 'chlorin'. Given that the student aims for a degree in something which has "Chemistry" in name, such errors should be avoided.

P5, section 1.3.2. BChl d in chlorosomes of Chloroflexi. This is exactly the type of information that requires a proper reference. Was any BChl d observed in the present work?

P7, section 1.3.4. do chlorosomes indeed convert solar power to chemical energy as the sentence implies?

P15, section 3.5.4. the author states: "...molecules...cannot pass through the gel and therefore will pass right through the column." This does not seem to make much sense as a description of the operation of a size-exclusion column.

P18. Negative staining is not explained at all. I think it should be.

Although I do recommend the thesis for defense, suggested grade is 3. In summary, I would not complain if the experiments described were not successful, the problem of the present thesis is that the data seem to be presented with little consideration of what they actually mean.

Nevertheless, I am sure that the above stated objections can be addressed in the presentation and the final grade can be improved.

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

1. Could the author suggest some quantitative measure of chlorosome purity, based e.g. on absorption spectra?
2. In the size-exclusion chromatogram, where would the author expect fragments of chlorosomes, free pigments? How would she decide what is what, based e.g. on absorption spectrum:
3. It is said in the text that the chromatography setup is equipped with a light scattering detector, was it used in the present work?
4. On page 7, section 1.3.3. it is stated that absorption bands red-shifts by up to 80 nm, or 1600 cm^{-1}. What quantity is expressed in cm^{-1}? Does 80 nm shift always correspond to 1600 cm^{-1}?

Conclusion:

In conclusion, I

recommend / ~~do not recommend~~*

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

In České Budějovice, 21 January 2020

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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).