Statement of the bachelor thesis reviewer

Name of the student: Matin Kazemi

Thesis title: Isolation, purification and characterization of bacteriochlorophyll c for engineering of novel photonic materials

Supervisor: Mgr. David Kaftan, Ph.D.

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Review of the bachelor thesis "**Isolation, purification and characterization of bacteriochlorophyll c for engineering of novel photonic materials**" by **Matin Kazemi**.

The submitted thesis by Matin Kazemi aims to prepare bacteriochlorophyll aggregates and image these with advanced microscopy method AFM. The work aims include growing of microbial source material and purification of source pigment. The author had to learn all processes involved, including microbiological techniques, pigment purification by HPLC, purity analysis by mass spectrometry and operation of the AFM instrument. The thesis has 31 numbered pages. There are 25 pages of text and 62 references. The structure of the work appears results-heavy with 11 pages of *Results* versus 5 pages of *Introduction*. However, 8 of the 11 pages in *Results* are mostly covered with figures. Overall, considering the amount of methods and results, the structure of the thesis is acceptable though light in *Introduction*.

The thesis features a reasonable graphical layout. The weakest point in this regard is probably the quality of supplied graphs which are sufficient but could have been much better. The results of mass spectrometry analyses are provided in very rough format, probably as screenshots of the original machine software. The included page with a list of abbreviations has no title and is not listed in *Table of Contents*.

While the text of the thesis is mostly understandable, the writing leaves a lot to be desired. There are many omissions and awkward expressions. The use of English articles is also often not good. The overall quality of the text decreases strongly in *Discussion*. The work as a whole feels very rushed, a week or two of writing would improve the quality considerably.

The *Introduction* section covers basic information regarding the subject of bacteriochlorophyll aggregates, the source organism and intended use of the aggregates. The first section on photonic materials reads a bit like a strange marketing material rather than a professional text. The relatively small extent of the text shows up in many unexplained information throughout the text. The section 1.2.1. on BChl *c* is a good example and contains a big pile of information without much effort to organize it logically. As an illustration of the *Introduction* section, the 43 references (70% of the total

cited in the thesis) are crammed into effectively 3 pages of text. While this is obviously a very impressive effort by the author, the text is not better because of it.

The section on *Materials and Methods* covers all used methods in adequate depth. Regardless, some details needed for replication of the described work are missing. I could not find how much of the vitamin and trace element stock solutions need to be added to the growth medium. From the writing it appears that the cell pellets were homogenized without any addition of liquid which sounds very difficult and perhaps a bit of a waste of time, is that a correct description? Details of the centrifugation after pigment extraction are not mentioned (bottom p.7). On p.8, "hexane was added ... by ratio (10:1)" – is that a volumetric ratio? The description of HPLC experiments on p.9 contains four different columns that were used but size information, needed for understanding or replicating the work, is only given for two of those. It is not clear why experiments with each column used different wavelengths for detection of the same analyte in the same solvent (671, 674, 672 and 668 nm). While not wrong, the gradient as in Table V is described in strangely many lines. The very first sentence on MS, on p.10, "Mass spectrometry is one of the vacuum techniques, ..." feels odd, as if it was lifted from some other, longer, text on MS without any editing. A bit below, "Efficiency of this purification step was evaluated using LC-PDA-APCI-MS/MS technique ...", this could be correct but the unexplained abbreviation does not help in understanding what the author means here. The section on UV-VIS method, while likely correct, does not cite other references than "Nanowizard AFM Handbook" which does not contain anything about absorbance. I believe that this list suffices to illustrate my point here.

The *Results* section covers the work as it progressed from cell cultivation to other experiments. There is no text covering aim 4 of the thesis – preparation of BChl *c* aggregates. This can be well documented with absorption spectroscopy and is a necessary part to include before imaging of said aggregates. A weak part is also the graphics quality. Graphs presented here have very thin lines, no ticks on axes so data cannot be read and chromatograms do not show which wavelength is plotted (probably BChl *c* Qy maximum). Data could be described better, for example in Fig. 6b there is a big contribution of carotenoid absorption but it is only mentioned briefly later and then in *Discussion*. Here the lack of good *Introduction* shows up as well. On p.23 (AFM results), it is not mentioned which buffer was used for imaging. Considering that AFM can produce visually nice results, I'd welcome more images, perhaps of those polyhedral aggregates.

The *Discussion* is clearly the weakest part of the thesis and had this been good, the whole thesis would come out as much better. The first two paragraphs just summarize results, the third paragraph provides fragmentary information without apparent connection to the rest of the text. The rest of the discussion is often confused. No effort is made to explain observed phenomena. The best text that can be found just states that some other published results are similar. Only 9 references are included in the whole section in contrast to 43 in *Introduction*. The part on MS mentions that an ion with m/z of 841.6 is "the same" as an ion with m/z of 840 in a cited reference. The *Discussion* does not contain any text whatsoever on aggregates and AFM results, i.e. aims 4 and 5 of the thesis.

The *Conclusion* again suffers from poor writing and is mostly a repeat of the text at the beginning of *Discussion*. Only here on last line of p.25, for the first time in the whole thesis, it is mentioned that the aggregates were prepared by "the method of alkane facilitated aggregation". Clearly this information needs to first appear in *Introduction*.

In conclusion, it is my opinion that the submitted thesis by Matin Kazemi does not fulfill the criteria required of such work and **I do not recommend the thesis for defense**.

Should the defense proceed, these questions should be answered:

Q1) On p.18, in discussion of Fig.9, you mention that the peak at 342.5 nm is the Soret band of BChl *a*. This peak is present in similar extent also in the methanol extract which does not contain the BChl *a* Qy band around 770 nm. The UV peak then cannot come from BChl *a*. What is the origin of this peak?

Q2) In your HPLC results, the same peak pattern is present in all analyses. When using C18 column, the retention times are significantly shorter (9 min vs. ~30-33 min) than in other columns. Why is that?

Q3) Also to HPLC, given that your source material for using C18 column did not contain BChl *a*, would you say that the separation as presented in Fig. 10 was better than your earlier attempts?

Q4) You do not show any results of your work towards aim #4. Please include and discuss them in the presentation.

Q5) You comment on p.23 that the AFM results did not show fine details which could help understand the spatial arrangement of the BChl *c* molecules. The very last sentence of your *Conclusion* also mentions this. Why is that and what is needed to be able to analyze this?

RNDr. Radek Litvín, Ph.D.

In České Budějovice, 22 January 2020