Posudek práce

předložené na Matematicko-fyzikální fakultě
Univerzity Karlovy

❑ posudek vedoucího ☒posudek oponenta
❑ bakalářské práce ☒ diplomové práce

Autor/ka: Bc. Tomáš Malina
Název práce: Artificial light-harvesting antenna based on an aggregation of bacteriochlorophyll c with selected pigments
Studijní program a obor: Fyzika, Biofyzika a chemická fyzika
Rok odevzdání: 2020

Jméno a tituly vedoucího/oponenta: RNDr. Radek Litvín, Ph.D.
Pracoviště: Ústav chemie, Přírodovědecká fakulta, Jihočeská univerzita v Českých Budějovicích
Kontaktní e-mail: rlitvin@prf.jcu.cz

Odborná úroveň práce:
☒ vynikající ❑ velmi dobrá ❑ průměrná ❑ podprůměrná ❑ nevyhovující

Věcné chyby:
☒ téměř žádné ❑ vzhledem k rozsahu přiměřený počet ❑ méně podstatné četné ❑ závažné

Výsledky:
☒ originální ❑ původní i převzaté ❑ netriviální kompilace ❑ citované z literatury ❑ opsané

Rozsah práce:
☒ veliký ❑ standardní ❑ dostatečný ❑ nedostatečný

Grafická, jazyková a formální úroveň:
☒ vynikající ❑ velmi dobrá ❑ průměrná ❑ podprůměrná ❑ nevyhovující

Tiskové chyby:
☒ téměř žádné ❑ vzhledem k rozsahu a tématu přiměřený počet ❑ četné

Celková úroveň práce:
☒ vynikající ❑ velmi dobrá ❑ průměrná ❑ podprůměrná ❑ nevyhovující

The evaluated master thesis “Artificial light-harvesting antenna based on an aggregation of bacteriochlorophyll c with selected pigments” by Bc. Tomáš Malina investigates several biophysical properties of artificial aggregates of bacteriochlorophylls and carotenoids. The thesis is of experimental nature, describing the results of several steady-state and time-resolved optical spectroscopy methods and of the AFM imaging method. The author mostly learned the required methods and acquired the results himself. The author also prepared the samples for experiments and thus acquired a good grasp of all aspects of the thesis work. The used method set and amount of obtained experimental data are very impressive and, in my opinion, above the amount expected from master theses.

The thesis is written in reasonably good English and consists of 107 numbered pages of which there are 10 pages of appendices. The thesis is of high technical quality with clean format, well prepared figures and tables and an extensive set of high quality references. The amount of small errors and mistakes is minimal. Of the total length of the thesis, there are 19 pages of Introduction, 17 pages of Material and Methods, 35 pages of Results and Discussion and 3 pages of Conclusions. The structure of the thesis appears to be well balanced between the chapters. There are 99 numbered references and lists of figures, tables and abbreviations are also included. Already at first glance it is obvious that the author carried out an impressive effort in preparing the thesis.

In the Introduction section the author describes in good language all important topics on which the thesis is built. The process of photosynthesis and energy sources for the biosphere are well covered and there is a nice introduction into the current state and benefits and drawbacks of organic solar technology. The last section on Goals of the thesis is a bit prolonged, at 5 pages of text and I’d rather like to see most of the text in a separate part of introduction, leaving only the aims without major explanations or discussion in this section. Personally, I’d also include more text on the method principles which are later introduced on an as-needed basis, for example the very first page of results (p. 40) describes the theory of how excitation and 1-T spectra are used to assess efficiency of excitation energy transfer. These issues are however frequently a matter of taste than strict requirement. My specific remarks are mostly minor and I will not list all of them. I’ll only mention that in the description of photosynthesis on p.9, the text could use a bit more introduction than jumping right into the details of “*First part in the chain of reactions is the light phase ...*”.

Like the rest of the thesis, also the Methods section is well-prepared and understandable though the text gets comparatively shorter as the author progresses from pigment purification and aggregate preparation to AFM and time-resolved spectroscopies. I have two specific comments here. First, although the HPLC principle is well described, no specific solvent method is mentioned. It is referenced to a publication but it should be at least briefly described as space was not an issue here. Second, the author uses “*10 mM*” as a description of optical path length two times (pp.32 and 34). One can make an error but here it appears that the author intentionally uses units of concentration instead of units of length.

The section on Results and Discussion provides the core of the thesis. Here I'd personally prefer the traditional separation into two sections instead of the joint text. Especially in the first section on the effect of scattering (pp. 40 - 48) the text is quite chatty and could use some more aggressive editing. Coupled with the mixing of experimental results and fairly complex discussion it makes the text difficult to follow. The author spends most of the time in sections 3.1 (efficiency of energy transfer from beta-carotene to Bchl c, 9 pages) and in the last section on superradiance measurements (18 pages). On the contrary, the sections on quenching of Bchl c triplets and on AFM results are very short (5 pages together). Moreover the author jumps right in the middle, starting the section 3.3 with the sentence *"Slow-method aggregates showed two lifetime components, 9.5 ns and 1.2 µs (Figure 23)."* I'd welcome a rather more delicate introduction to the issue which could possibly only prolong this section by one paragraph. The section describing imaging effort by the AFM method is fairly short which reflects the minimal amount of data obtained. On the other hand it obviously required some extended effort to prepare the samples and obtain at least the results presented in the thesis.

The Conclusions section is 3 pages long which can be viewed as too much, in a similar way to the 'Goals of the thesis'. Here I'd also welcome a shorter version to provide concise conclusions instead of the long text present in the thesis.

Overall, my minor criticism shouldn't be taken as indicative of serious shortcomings of the thesis. I believe that the author fulfilled all requirements demanded from such a work and even exceed the expected scope in many ways. Therefore, **I recommend the thesis for the defense** with the grade of **excellent**.

In the following I have a few questions to the author, to be answered during the defense.

Questions:

Q1) To section 3.2, aggregates of Bchl c and Bchl a – If the energy transfer within the large aggregate of BChl c is extremely fast as mentioned in the introduction, the presence of secluded domains of Bchl a should not significantly decrease the EET efficiency to BChl a. Only in the case that the co-polymer or other molecules surrounds the BChl a domains and in effect insulate them from the BChl c aggregate would this be a suitable explanation. I'd welcome an absorption spectrum of the resulting aggregates. Is the absorption peak of BChl a red-shifted due to interaction with other pigments or does the molecule behave as if it was a monomer? Can something be said about the potential of proper incorporation of Bchl a into the Bchl c aggregate on the basis of the absorption spectra?

Q2) To the same section as above, p.52, beta-carotene in aggregates and EET efficiency – Can you really view the beta-carotene as a "physical obstacle" in a situation where you have a pool of pigment molecules in contact (and therefore likely strongly coupled, see also the large red-shift vs. monomeric BChl c)? Perhaps the efficient nonradiative deexcitation in the carotenoid removes part of the excitons from the aggregate?

Q3) p.70 and elsewhere - "*A delocalisation of emission over a minimum of 2 molecules*" - how is the number of molecules calculated?

Q4) p.74 - you quote efficiencies of energy transfer from beta-carotene to BChl c in chlorosomes as 50-80% (from literature). Given your difficulties of accurately determining the efficiency due to scattering effects, how were the literature numbers obtained and corrected? In chlorosomes the scattering effect must be comparable to your aggregates, no? Please comment on your opinion on the reliability of these published numbers.

Q5) p.74 - EET efficiency from BChl c to BChl a. You write that "*Intriguingly high EET efficiencies were found*" of about 95 %. Considering the expected distribution of excitons given by Boltzmann equilibrium in these molecules in (assumed) close contact, isn't it more surprising when the EET efficiency is low as in the slow-method aggregates? To me this is one of the reasons to believe that in the slow-method aggregates the BChl a molecules are at least partly insulated from the large BChl c aggregates.

Q6) p.75 - In AFM, is it possible to remove the leftover buffer by washing with water or is it just easier to use the submerged tip method?

Q7) In Introduction, you write that the Soret band corresponds to the S0-S3 and S0-S4 transitions. I'm a bit surprised by this information because I always thought that these are S0-S1 (Qx+Qy) and S0-S2 (Soret) transitions. I understand that the situation is not quite so simple and both notations can be used in certain circumstances. Can you please find some other sources and provide a conclusion to this issue during your defense? An additional question here, Jacques-Louis Soret originally described the band named after him in hemes (in blood actually, if I'm not mistaken) where Soret band is essentially the major peak and Qy, Qx are much weaker. I.e. the S0-S2 (or your S0-S3, S0-S4) transition(s) are much stronger than the S0-S1 transition. What is the reason for this difference of hemes and (Bacterio)chlorophylls?

In České Budějovice, 22 June 2020

RNDr. Radek Litvín, Ph.D.