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Review of the doctoral thesis of Rafal Bialek entitled

“Purple bacteria reaction centers in photovoltaic applications”

Opinion on the scientific value of the Author’s results

In his doctoral thesis Rafal Bialek describes his study of the electron transfer chain in photosynthetic proteins from purple bacteria and in biohybrid systems based on these photosynthetic proteins for converting light energy into electrical energy. The emerging field of biohybrid photosynthetic systems has been very rapidly expanding in recent years because it is seen as a possible blueprint for artificial light energy conversion systems or to be used directly as low cost renewable biophotovoltaic systems. The thesis of Rafal Bialek is very valuable in this context because it opens the path toward a truly systematic approach for understanding bottlenecks in photosynthetic biohybrid systems and thus guide their conceptual design and optimization.

The thesis describes four interconnected studies based on spectroscopy, electrochemistry and modelling providing kinetic and thermodynamic insights into reaction center from purple bacteria in various environments. The first chapter studies the charge recombination within the reaction center as a function of mutations in key positions. The second chapter focuses on the kinetic modelling of the reaction centers interfaced with semi-conductor electrodes. The last two chapters quantify the thermodynamic and kinetic properties of the electron transfer chain of the photosynthetic reaction center interfaced with electrodes via redox hydrogels.

Rafal Bialek published the results from the four chapter described in the thesis in four papers, as second author for the first chapter and as first author for the other three chapters. Rafal Bialek also published 6 other papers, including one as first author, that are not directly mentioned in the thesis.

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In my opinion the strongest points of the thesis are

(1) The determination of the kinetic constants of mutants of purple bacteria reaction centers at various temperatures. This chapter, in particular in terms of methodology and expertise is foundational for the subsequent chapters on the biohybrid systems based on the same photosynthetic protein.

(2) The establishment of a kinetic model that explains semi-quantitatively the photocurrent response of semiconductor electrodes modified with the purple bacteria reaction center. The main highlight is that the treatment of the electrode material with $TiCl_4$, and thus its surface chemistry correlates with the shape of the photocurrents as a function of the heterogeneous electron transfer rate. This is fundamental for the general understanding of charge recombination in biohybrid photosynthetic systems because it is currently one of the main hurdle preventing high energy conversion yields in these systems.

(3) Determination of thermodynamic properties of the natural electron transfer chain within the photosynthetic protein when integrated in a redox hydrogel matrix. This is a breakthrough because modelling and quantitative analysis of photocurrents often assumes that the properties of the photosynthetic proteins are unchanged upon immobilization. This often leads to misinterpretation and erroneous conclusion in the field. The demonstration from Rafal Bialek that the reduction potential of the redox site in the protein changes upon integration on electrodes will trigger more rigorous and systematic approach in the community.

(4) The determination of the intermolecular electron transfer between the protein and electron mediators in biohybrid systems is also highly important and often inaccessible. This is well illustrated in the last chapter where such measurements were not successful. Nevertheless, Rafal Bialek showed an indirect and elegant methodology to obtain such kinetic constants for the electron transfer between the mediator and protein in solution.

(5) The overall effort toward these highly valuable results were built through productive cooperation with biologists from the Bristol and with spectroscopists from Amsterdam. This cooperation cluster will serve as stepping stone toward future breakthrough which are much needed in the field of biohybrid systems.

(6) the thesis also contains an interesting description of the methods used in the different chapter. This will be highly valuable for future scientist pursuing similar research directions.

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Points to be discussed during the defense:

(1) The kinetic model of the biophotoelectrode seem to have been elaborated without scaling. While this is not a requirement for predicting photocurrent for a particular set of experimental parameters, it could have been useful for predicting trends regardless of specific parameter values. This reflects the statement on page 53: *"5. There is no one distinct parameter, whose change could improve the photocurrent in the studied design.".* But are there specific group of parameters that could be naturally associated to better visualize the trends in net photocurrent generation? For example, would the effect of heterogeneous electron transfer vs the rate of recombination at the protein be a useful parameter group? Or in other words, have any specific combination of reaction and/or diffusion processes been identified as playing a central role in charge recombination or in the amplitude of the photocurrent?

(2) The prediction on the photocurrent in chapter 2 matches perfectly the experimental current. This is evidently partly due to the proper selection of the kinetic scheme and thus a suitable hypothesis regarding the electron transfer mechanism. However, the excellent fit may also be partly due to large freedom for the choice of values for the numerous parameters involved in the model. In particular prediction of the photocurrent for the $TiCl_4$ treated materials vs the photocurrent from the non-treated materials seems to involve differences in values for multiple parameter (*see table 1 page 94 and table 2 page 96*). Such differences, even significant, are expected for the heterogeneous electron transfer since it takes places at the electrode interface, but how are these difference accounted for the parameters that may seem less influenced by the surface chemistry?

(3) In Chapter 3, regarding the shift of the reduction potential of the special pair of the purple bacteria reaction center when integrated in redox hydrogel, Rafal Bialek comments that the potential shift is "*likely due to interaction with the polymer matrix.*" Is there any direct experimental evidence or indication supporting this statement? Alternatively, which experimental strategy could be applied to identify the reason for this shift? Additionally, could the conditions in the hydrogel be specifically modulated to control or adjust the redox potential of the protein?

(4) The chapter 4 on the determination of the kinetic constants for the intermolecular electron transfer between the electron mediator and the protein sets the most challenging objectives which could only be reached for the system in solution. Several questions related to the technical challenges remain open. In particular,

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the authors state that "*in the conditions studied in this paper, P-Os entities exist most probably as particles with a hydrodynamic radius of no bigger than 16 nm, or loosely bound agglomerates of such particles, and are positively charged. This would be consistent with net anionic RCs and cationic P-Os particles forming complexes through electrostatic interactions*." These conclusions are rather speculative since they are based on measurements reported in other papers that used different experimental conditions. Besides the pH, other parameters such as the electrolyte composition, concentration of proteins and polymers or the temperature will also define the electrostatic interactions. The authors may comment why particle size could not be measured under the particular conditions used in the present study.

(5) The main objective of chapter 4 is the determination of the kinetic constants of the intermolecular electron transfer between the protein and the mediator which would be valuable to identify bottlenecks in particular by mean of electrochemical modelling. Would the values obtained in solution be useful for such a purpose? In particular, when considering the hydrogel film formation, entropy effects as well as crosslinking through further coordination of Os-complex limits the resolvation and swelling of the hydrogel film after drying in comparison to the hydrogel in solution that did not experience a drying step. This may lead to very significant change in the ET rates. Could the author comment on alternative approach for directly determining this kinetic constant in hydrogel films?

Selected minor points:

(1) In chapter 4, the redox silent polymer has a different backbone from the P-Os polymer. It is not clear why this was used for the control experiment.

Conclusion

In my opinion, in light of the comments written above, the doctoral thesis of Rafal Bialek is highly interesting and valuable due to its systematic and in-depth quantitative character to explain by means of spectroscopy, electrochemistry and modeling the general mechanism and bottleneck in biohybrid photosynthetic systems. This work will serve as a stepping stone for future research focused on quantitative characterization and optimization of biophotoelectrodes which is much needed to enhance the energy conversion performances of such electrodes.

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In my opinion the doctoral thesis by Rafal Bialek fulfills the formal requirements (Ustawa o stopniach i tytule naukowym z dnia 14 marca 2003 r., art. 13). In particular, it contains numerous original results obtained by the Author and demonstrates broad theoretical knowledge of the Author in the field of biophysics. Overall the thesis is among the best I have seen in this field, and as such I believe that the thesis qualifies for a distinction. I recommend continuation of doctoral degree conferral procedure for Rafal Bialek.

With best wishes,

Prof. Dr. Nicolas Plumeré

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