Příloha 1

Referee´s review on master thesis of Bc. Petr Rathner, BSc.

Bc. Petr Rathner, BSc., a student of Biological Chemistry in the master joint degree program at the Johannes Kepler University in Linz and the University of South Bohemia in České Budějovice, submitted his master thesis entitled "NMR Solution Structure of the Protein PsbQ from Phostosystem II". Regarding formal features Petr´s master thesis consists of Introduction, Methods, Experimental part, Results and Discussion, Conclusion and References on 50 pages altogether, as well as 26 pages of Appandix containing resonance assignments of PsbQ.

Introductory part is well-done overview of the subject focused on both extrinsic proteins of Photosystem II in general emphasizing PsbQ and a few others being in a functional compound association within the "water splitting complex" as well on theoretical background of NMR spectrometry as currently topic methodology of structural biology.

The only deficiency in the first part of the master thesis I have found is a lack of clearly listed aims of the master thesis, and therefore reading the thesis is not clear from which organism PsbQ comes, either from *Spinacia oleracea* or *Synechocystis sp.*

Chapter of methods as well as of experimental part contains a broad array of techniques of preparatory and analytical biochemistry of recombinant proteins including over-expression of recombinant proteins in bacterial systems, isotopic labelling proteins for NMR spectrometry, isolation and purification of recombinant proteins with means of ion exchange chromatography columns, multi-dimensional NMR experiments, computer aided resonance assignment, methods of structure calculation and visualization of molecular structures.
In Results and Discussion chapter Petr Rathner has shown all results he achieved in the first preparatory part producing double isotope labelled recombinant proteins PsbQ as well in multidimensional MNR experiments to get data for calculation and visualization of the PsbQ structure based on exact identifications of secondary structure contributions – the precise structure share of alpha/beta structures.

My question for the author of the master thesis is as follows:
Is there any reasonable explanation of discrepancy for the secondary structure within residues 37-40 identified as alpha helix in NMR analysis and parallel beta-sheet published based on X-Ray of the PsbQ crystal structure. Did you find such discrepancy in any other already published polypeptide structures, and having possibility to compare NMR and X-ray analyses?
In conclusion, I would like to highlight the fact that Petr Rathner succeeded with such demanding subject as polypeptide structure analysis, calculation and visualization is. He approached and faced the problem in experimental and computational parts with using of exemplary effort, having done such great piece of work, and putting such an important small stone to the mosaic of understanding such mysterious part of photosynthesis as oxygen evolving within water splitting complex at the Photosystem II is. I am pleased to state that Bc. Petr Rathner, BSc. fulfilled successfully assignment of his master thesis, and thus I can recommend the thesis to defence, and evaluate it with Excellent mark.

Prof. Dr. Libor Grubhoffer

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