Refree´s review of master thesis by Mr. Michal Kamenicky, (Bc; BSc)

Mr. **Michal Kamenicky,** (Bc; BSc) submitted the master thesis entitled ***"Recombinant expression, refolding and initial NMR studies of PsbO"*** which he worked out in the Institute of Organic Chemistry at Johannes Kepler University in Linz under guidance of Prof. Rudiger Ettrich and Dr. Jaroslava Kohoutova, from the University of South Bohemia and Czech Academy of Sciences. I am pleased to state the master thesis is niece piece of work from both points of view; a formal side - clearly proves the author of master thesis is highly dedicated for the research experimental work, the thesis has been elaborated with required carefulness and responsibility; an aspect of the content has brought results of high importance for further research on the subject especially due to having explored/scrutinized demanding approaches of refolding of the recombinant PsbO protein. Properly refolded recombinant protein is a *"conditio sine qua non"* for further research on any recombinant protein, especially those they have more complicated tertiary or quaternary structure, recombinant proteins which structure is dependent on properly made intramolecular or intermolecular disulfide bridges. This is exactly the case of PsbO protein.

Michal´s master thesis has individual chapters very well outweighed, having provided enough deep introduction to the PsbO protein, one of the key protein of the PSII in cyanobacteria, another part has covered very well a broad array of microbiological, molecular biological, biochemical and physico-chemical methods including electrophoreses, chromatography techniques Raman spectroscopy (RS), Fourier transform infrared spectroscopy (FTIS), circular dichroism (CD), intrinsic fluorescence of proteins, and last not least nuclear magnetic resonance (NMR), which is the final destination of the project to use it for structural biology study of PsBO.

The inclusion bodies made by recombinant proteins during its over-expression in E. coli were the main problem associated with experimental performance of the thesis which the author of master thesis had to face. PsbO protein as many others has disulfide bridges participated on correctly established tertiary structure. However, disulfide bridges are not the only factor of properly folded proteins, and thus my question for the author is:

- how much the disulfide bridge (there is just one?) affects the tertiary structure of PsbO, can be answered this question based on two refolding procedures (gylcerol; glutathion) that were carried out;

- which parameter of inner environment of the cell (including prokaryotic cells of cyanobacteria, E. coli, etc.) plays an essential role in protein with disulfide bridges during folding posttranslational modification;

- why protein like PsbO of cynobacteria origin is not properly folded (making inclusion bodies) in E. coli even though it is a prokaryotic organism too;

- what about a time course experiments on PsbO refolding by glutathion (red/ox); why PDI (protein disulfide isomerase) was not use as a catalyst in the glutathion redox experimental PsbO refolding;

- is there any option to have a functional assay (besides spectroscopy techniques) to measure/evaluated a degree (or better say an extend) of correctly folded PsbO molecules;

In conclusion: Hereby I am so pleased to state that I have considered the master thesis worked out and submitted by Mr. Michal Kamenický (Bc; BSc) of high quality, having met all requirements on master theses (at least some of results achieved by the author could be published as a research paper), and therefore Mr. Michal Kamenicky can receive an academic degree of Master Of Science in Biological Chemistry provided a successful defence of the thesis by its other, and provided all legal circumstances are recognized and met.

Prof. RNDr. Libor Grubhoffer, CSc.

In Ceske Budejovice, July 30, 2015