

OPPONENT'S REVIEW OF THE BACHELOR'S THESIS

„Identifying Protein-Protein Interaction of *Trypanosoma brucei* F₀F₁-ATP Synthase Subunits Using the Yeast Two Hybrid System“

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The bachelor's thesis of Michaela Kunzová offers not only theoretical overview of selected topic, which is typical of most bachelor theses but also contains the practical part. The thesis is distributed into 56 pages, illustrated with the several pictures. The topic is of current interest, because ATP hydrolysis by F₀F₁-ATPase is a potential drug target in the infectious stage of *T. brucei*. The main aim of the author was to identify the interacting partners of the F₀F₁-ATP synthase subunits in *T. brucei*, utilizing the yeast two-hybrid system. In the introduction, the author briefly describes the life cycle of the parasite *T. brucei*, its metabolisms during differentiation, structure and function of ATP synthase and classical method for determining protein-protein interactions. In this part of the thesis, I expected more detailed discription of the selected topics. In contrast, the methods section forms 40% of whole thesis, where the author describes in detail not only the experimental techniques used, but also their principles. The results of individual experiments are described in a logical sequence, documented reasonably with pictures and tables. The author prepared number of constructs for Y2H system and also validated the expression of several proteins. Subsequently, in the discussion the author evaluates their achievements, description of possible errors and shortcomings, and proposes ways forward. The used terminology is correct and consistent throughout the whole text. Except for a few formatting errors (e.g. mistakes in a numbering table) the thesis is well processed. Despite the fact that the main aim of this thesis was not reached, I believe that the methodology used and the partial results of the young scientist serve as a good basis for further experiments.

I have following observations/questions:

- The bibliography includes references that are not cited in the text, e.g. [Bringaud *et al.*, (2006); Stock (1999); Laemmli (1970)].
- Some of the results of western blot are not documented by figure, e.g. 2930AD/BD, 3320AD/BD or α , α N, OSCP BD.
- On page 19 and page 30 you describe that DNA/proteins coated with SDS, which are negatively charged run to the cathode. Is this sentence correct?

- What are the differences between construct α -mls cds pGADT7 AD and α -mls N-term pGADT7 AD?
- Could you explain in more detail and compare the differences of F_0F_1 -ATP synthase subunits in *T. brucei* and the well known eukaryotic organisms (yeast or human)?
- What alternative methods could you have used to determine protein-protein interactions?
- Could you propose explanation, why the protein interaction in Y2H system does not occur?
- Did you try to co-transform both the plasmids into one yeast?

Finally, I would like to emphasize that this feedback does not reduce the quality of the thesis. Therefore I recommend this thesis for defense and evaluation.

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Mgr. Eva Hegedúsová, PhD.