



Přírodovědecká
fakulta
Faculty
of Science

Jihočeská univerzita
v Českých Budějovicích
University of South Bohemia
in České Budějovice

OPPONENT'S REVIEW ON BACHELOR/DIPLOMA* THESIS

Name of the student: Edrin Sulejmani

Thesis title: Mechanism of energy conversion in ATP driven molecular machines: Development of single-molecule techniques for observation of conformational changes in sec translocon during protein transport.

Supervisor: Joel Crossley, Ph.D.

Referee: M.Sc. Babak Minofar, Ph.D

Referee's affiliation: University of South Bohemia

	Point scale ¹	Points
(1) FORMAL REQUIREMENTS		
Extent of the thesis (for bachelor theses min. 18 pages, for masters theses min. 25 pages), balanced length of the thesis parts (recommended length of the theoretical part is max. 1/3 of the total length), logical structure of the thesis	0-3	2
Quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0-3	2
Accuracy in citing of the references (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	2
Graphic layout of the text and of the figures/tables	0-3	2
Quality of the annotation	0-3	2
Language and stylistics, complying with the valid terminology	0-3	2
Accuracy and completeness of figures/tables legends (clarity without reading the rest of the text, explanation of the symbols and labeling, indication of the units)	0-3	2
Formal requirements – points in total		14
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	2
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	2
Discussion quality – interpretation of the results and their discussion with the literature (absence of discussion with the literature is not acceptable)	0-3	2
Logic in the course of the experimental work	0-3	2

* Choose one

¹ Mark as: 0-unsatisfactory, 1-satisfactory, 2-average, 3-excellent.

Completeness of the description of the used techniques	0-3	2
Experimental difficulty of the thesis, independence in experimental work	0-3	2
Quality of experimental data presentation	0-3	2
The use of up-to-date techniques	0-3	2
Contribution of the thesis to the knowledge in the field and possibility to publish the results (after eventual supplementary experiments)	0-3	2
Practical requirements – points in total		18
POINTS IN TOTAL (MAX/AWARDED)	48	32

Comments of the reviewer on the student and the thesis:

The author studied the translocation of the unfolded proteins across the inner cell membrane in bacteria which is mediated by SecA, an ATPase protein in complex with the membrane channel of SecYEG. The work has been done experimentally by performing ensemble and single-molecule fluorescence resonance energy transfer (sm-FRET) setups. The work was targeted to optimize the application of single-molecule detection techniques for the investigation of translocation of trans-membrane proteins process in the SecYEG complex. The author explained the structure and Function of the SecA and the SecYEG Complex followed by the introduction of experimental methods such as Single-molecule Detection Techniques, and light Microscopy based methods such as fluorescence correlation spectroscopy (FCS), and single-molecule fluorescence resonance energy transfer (sm-FRET) methods where the author did not mention the abbreviation in the introduction just mentioned in the abstract which is not enough.

Both the introduction and experimental chapters are fulfilling the expectations for a bachelor thesis while the author could use higher-quality pictures. The results and discussion part could be expanded but in my opinion, it fulfills the requirements. In addition, the author could explain the results in more detail and describe the reason for higher or lower FRET measurements on the stability of the complex. The general graphical layout of the thesis is acceptable. The length of the thesis is also acceptable and fulfills the guidelines of the faculty.

Suggestions and questions, to which the student has to answer during the defense.

Mistakes, which the students should avoid in the future:

- 1) What is SecYEG abbreviated form as it is not explained in the text.
- 2) In the conclusions, the authors stated that “results show the best applicable focal depth is 10 μm , as this distance shows the best compromise between the highest molecular brightness and SNR”. From where such conclusion is derived?
- 3) In the “Summary of EPR of the investigated samples. APO denotes the sample with only SecA protein present. The addition of ATP on the SecA mutated at positions 330 and 714, decreased EPR from 0.32 to 0.33; whereas the sample mutated at positions 280 and 604 decreased it from 0.46 to 0.47.” What is the reason for having EPR for PPXD-HWD has a smaller absolute value than PPXD-NBD2.

Conclusion:

In conclusion, I

r e c o m m e n d

the thesis for the defense and I suggest the grade 2.

In **Ceske Budejovice** date **17/01/2023**

MINOEAR

signature