



OPPONENT'S REVIEW ON BACHELOR THESIS

Name of the student: Ondřej Kuchynka

Thesis title: Studies of expression and localization of endogenous G-protein subunits using bioluminescence imaging

Supervisor: Mgr. Alexey Bondar, Ph.D.

Referee: RNDr. Zdeněk Franta, Ph.D.

Referee's affiliation: UCH, USB

	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	3
Quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0-3	3
Accuracy in citing of the references (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	3
Graphic layout of the text and of the figures/tables	0-3	3
Quality of the annotation	0-3	3
Language and stylistics, complying with the valid terminology	0-3	3
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	3
Formal requirements – points in total		21
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	3
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	3
Discussion quality – interpretation of the results and their discussion with the literature (absence of discussion with the literature is not acceptable)	0-3	3
Logic in the course of the experimental work	0-3	3
Completeness of the description of the used techniques	0-3	1,5

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

Experimental difficulty of the thesis, independence in experimental work	0-3	3
Quality of experimental data presentation	0-3	3
The use of up-to-date techniques	0-3	3
Contribution of the thesis to the knowledge in the field and possibility to publish the results (after eventual supplementary experiments)	0-3	3
Practical requirements – points in total		25,5
POINTS IN TOTAL (MAX/AWARDED)	48	(46,5) 2

Comments of the reviewer on the student and the thesis:

Ondřej Kuchynka in his bachelor thesis entitled *Studies of expression and localization of endogenous G-protein subunits using bioluminescence imaging* describes the optimization imaging process to study G-proteins using bioluminescence. The thesis has 35 pages in total and is divided into 7 chapters (introduction, aims, materials and methods, results, discussion, conclusion, and references). The introduction is quite nicely written and contains enough information about the G proteins and their receptors, which are important to get the theoretical overview for the experimental work. What I am missing a bit here is the linkage between text and figures presented in the introduction. I am also missing here any 3D structure of G protein subunits. Their organization is described in the text, but it would be easier for the reader to see them. The thesis contains several typos and a few discrepancies (e.g., the figure numbering at the end of results does not correspond. The author states figure 8, but it is figure 9 (page 25) or calls the chapter 3.2.5 Image analysis, but it is Image acquisition), which could be avoided by better proofreading. The discussion is quite well written, and deals with major findings presented in the thesis.

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

Mistakes, which the students should avoid in the future:

1: During your work you focus on G-protein subunits Gβ-1, GY-5, GY-11, GY-12. It is not clear to me why exactly these four out of many others have been chosen.

2: can you show, which part of G protein was modified by CRISPR-Cas9?

3: chapter M and M is not written in the standard format. Besides the methodological part it contains description of chosen bioluminescence over fluorescence, or the description of used cameras. Not sure if this should be part of M and M, I think it is more suitable for discussion. What I am missing in this chapter is better description experimental setup, because it is not sometimes clear how exactly was the experiment done (e.g. optimization of sample imaging using either sCMOS or EMCCD cameras using NanoGlo is not described anywhere). The missing data are the major drawback of this chapter as I am not sure if any reader would be able to repeat the experiment. I would also recommend stating the number of cells you used for the experiment. This could help the reader with his/her initial setup.

4: Why did you transfect with pcDNA 3.1 vector. What was the purpose of it?

² Enter the number of points awarded.

5: To measure the luciferase activity, you need to add the substrate. I did not find any information about this in your thesis. When and how did you add it?

6: have you tried to transfect non CRISPR-Cas-9 modified cells? Would this work as a negative control? (Based on the literature the LgBiT alone can have some activity)

7: in figure 8 you show the membrane/cytoplasm mean pixel intensity ratio. How would you explain such a broad data distribution for GY-12?

Besides all my comments and questions, the presented thesis of Ondřej Kuchynka is of high quality and fulfils all the criteria of Faculty of Sciences, University of South Bohemia.

Conclusion:

In conclusion, I

r e c o m m e n d

the thesis for the defense and suggest the grade excellent.

In České Budějovice

date 10.05.2023

.....

signature