



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

## OPPONENT'S REVIEW ON BACHELOR THESIS

**Name of the student:** Isabella Guger

**Thesis title:** Crystallization and modelling studies of human oxidosqualene cyclase hOSC wild type and 580W mutant variant

**Supervisor:** Mgr. Tatyana Prudnikova, Ph.D.

**Referee:** Pavel Grinkevich, Ph.D.

**Referee's affiliation:** University of South Bohemia

	Point scale <sup>1</sup>	Points
<b>(1) FORMAL REQUIREMENTS</b>		
<b>Extent of the thesis</b> (for bachelor theses min. 18 pages, for master's theses min. 25 pages), <b>balanced length of the thesis parts</b> (recommended length of the theoretical part is max. 1/3 of the total length), <b>logical structure of the thesis</b>	0-3	3
<b>Quality of the theoretical part (review)</b> (number and relevancy of the references, recency of the references)	0-3	3
<b>Accuracy in citing of the references</b> (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	3
<b>Graphic layout of the text and of the figures/tables</b>	0-3	2
<b>Quality of the annotation</b>	0-3	3
<b>Language and stylistics, complying with the valid terminology</b>	0-3	2
<b>Accuracy and completeness of figures/tables legends</b> (clarity without reading the rest of the text, explanation of the symbols and labeling, indication of the units)	0-3	3
<b>Formal requirements – points in total</b>		<b>19</b>
<b>(2) PRACTICAL REQUIREMENTS</b>		
<b>Clarity and fulfillment of the aims</b>	0-3	3
<b>Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions</b>	0-3	3
<b>Discussion quality – interpretation of the results and their discussion with the literature</b> (absence of discussion with the literature is not acceptable)	0-3	2
<b>Logic in the course of the experimental work</b>	0-3	3
<b>Completeness of the description of the used techniques</b>	0-3	3

<sup>1</sup> 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	2
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

POINTS IN TOTAL (MAX/AWARDED)	44	(0-48) <sup>2</sup>
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### **Comments of the reviewer on the student and the thesis:**

The bachelor thesis presented by Isabella Guger deals with developing a crystallization strategy for human oxidosqualene cyclase and gaining a preliminary structural insight by means of homology modeling. Oxidosqualene cyclase is involved in sterol biosynthesis pathway and can serve as an attractive target for development of hypocholesterolemic drugs. With a limited amount of protein, Isabella was able to optimize crystallization conditions by changing protein concentration, precipitation composition and protein-to-precipitant ratios. The outcome was needle-shaped crystals that can be used for X-ray diffraction experiments. She also learned all common crystallization techniques using lysozyme as a model protein and applied a computational approach to create a homology model of oxidosqualene cyclase and its mutant form.

The thesis is written in a concise and easy-to-follow language with occasional blemishes that do not hinder the overall understanding.

### **Typos/mistakes and general notes:**

Some figures are rather low-res. Figure 12 is too small, not readily readable.

P. 14, more 'severe' effect? Does that mean that the temperature optimum for S580W mutant is 22 C vs 37 C for the wild type?

P. 16, table 1, Running/stacking gel and staining/destaining buffer composition is not provided, instead the components to prepare the gels are listed without giving the amounts/percentages.

P. 17, table 3, precipitant composition is not given accurately, some concentrations are missing.

P. 17, SDS-PAGE: gel composition is given here, not in the table 1, therefore a more appropriate name for table 1 would be 'components used for SDS-PAGE'.

P. 24, The title for 3.6.3 paragraph should read 'Theoretical pI and Molecular Weight Calculations'

P. 29 Figure 19: the gel is a composite image, it's not quite clear what the molecular weights of the hOSC samples are.

### **Questions:**

1. After analyzing the human oxidosqualene cyclase samples by SDS-PAGE you conclude that the samples contain impurities. There are three bands of molecular weight of ~70, ~50 and 14 kDa on the gel in Figure 19. What could be an alternative explanation for

<sup>2</sup> Enter the number of points awarded.

- this and given that the calculated molecular weight of hOSC is around 83 kDa?
2. Given that the closest relative of human oxidosqualene cyclase that is available from PDB has only 26% sequence identity, what is your opinion on the accuracy of the homology model you obtained? What are the mathematical measures of similarity commonly used to compare structural alignments of molecules?

**Conclusion:**

**In conclusion, I recommend the thesis for the defense and I suggest the grade 1 .**

Ceske Budejovice,  
12 June 2019



signature

