



OPPONENT'S REVIEW ON MASTER THESIS

Name of the student: Bc. Anna Koutská

Thesis title: Molecular, biochemical, and structural characterization of secreted ferritin II from *Ixodes ricinus*

Supervisor: prof. Mgr. Ivana Kutá Smatanová, Ph.D.

Referee: RNDr. Jindřich Chmelař, Ph.D.

Referee's affiliation: Department of medical biology, Faculty of Science, 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	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	3
Graphic layout of the text and of the figures/tables	0-3	2,5
Quality of the annotation	0-3	3
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	2,5
Formal requirements – points in total		18
(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	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	2,5
Completeness of the description of the used techniques	0-3	2

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

Experimental difficulty of the thesis, independence in experimental work	0-3	2
Quality of experimental data presentation	0-3	2,5
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	2
Practical requirements – points in total		21
POINTS IN TOTAL (MAX/AWARDED)	48	39

Comments of the reviewer on the student and the thesis:

The aims of the thesis was to produce soluble recombinant ferritin II and analyze it with CryoEM, which were successfully fulfilled. Student used multiple expression vectors and bacteria to achieve the best results.

The thesis fulfils the formal requirements well. It has sufficient extent, balanced length of individual thesis parts and proper use of tables and figures. English language needs an improvement since I found quite a lot of grammar errors and it would need text double check for typos etc. In the **introduction**, there are some factual inaccuracies that are results of either inadvertence or misunderstanding of the issue. For example spirochetes are also bacteria (page 1), ferritin II probably doesn't have detrimental effect on tick development (page 8). Introduction delivers necessary information about ferritins and ticks, however, since the work is also about optimizing protein expression, it could include also some part about expression of recombinant proteins, how different vectors and bacterial strains can affect the outcome.

Methods are ok, although sometimes I did not understand, what author means. For example in section 2.4 there is: "Conventional cloning using restriction enzymes NEBuilder® HiFi DNA Assembly (New England Biolabs) was used..." . Is there a missing word "or" or it means that NEBuilder HiFi Assembly includes restriction enzymes? I miss the description of restriction enzymes (company, cat. No), without it, the times used for digestion cannot be checked. Table 1 lacks the information about the concentration of components. On page 23, units of molecular weight are missing. Some of these issues are only details, but altogether it shows that the work would deserve one more thorough check.

Results describe the process of cloning and expression. In 3.1, gradient PCR is described.? To me, the range seems to be rather narrow to show any difference and the result shows the same. Did author expect some difference between used annealing temperatures

Concerning the pilot expression, I would like to discuss the comparison between BL21 and Rosetta cells. To me it seems that petSUMO and pASK-IBA37+ vector and Rosetta cells produce more pure protein with similar concentrations as in BL21. There seem to be some higher bands in BL21 cells that are actually recognized by an antibody. What this band could be and couldn't it be a problem? Why did you continue with pET100, when both petSUMO and pASK-IBA37+ produced protein in insoluble fraction, moreover it looked much more pure than pET100. Please explain the selection process in more details.

Did you see some protein precipitation during refolding? What was the loss of the protein? At page 34 you write that 600 kDa is the proper size of refolded protein. It means that multimerization already occurred during refolding. Was HisTag removed before refolding? Could it interfere with multimerization?

In discussion, author compare her own results with other works. She discusses mostly the solubility and stability. Could the need of multimerization be the reason why the protein did not appear in soluble fraction? The discussion doesn't bring much novel insight, it seems only as a comparison with similar works and whether they had similar or different results. I know that the nature of the work is about the technique itself, but there could be for example more about the structural properties of ferritin and how it could influence the expression and refolding.

Overall, the thesis fulfils the requirements of the Faculty of Science on master theses and I recommend it for the defense. There are definitely issues that could be improved, therefore I recommend grade very good (2).

Conclusion:

In conclusion, I

r e c o m m e n d

the thesis for the defense and I suggest the grade Very good (2) .²

In **České Budějovice** date 12.5.2023

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signature

² You can suggest a grade, which can be modified during the defense based on the presentation. However, if the reviewer is not present at the defense, the grade will not be counted. Grades: excellent (1). Very good (2), Good (3), Unsatisfactory/failed (4).