



Přírodovědecká  
fakulta  
Faculty  
of Science

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

## OPPONENT'S REVIEW ON BACHELOR THESIS

Name of the student: Solomia Pylypchuk

Thesis title: Molecular cloning, expression, and purification of the tick salivary serpin IRS-7

Supervisor: Jan Kotál, Ph.D

Referee: Mohamed Amine Jmel, PhD

Referee's affiliation: Laboratory of genomics and proteomics of disease vectors, Institute of Parasitology, Biology centre CAS

|                                                                                                                                                                                                                                               | Point scale <sup>1</sup> | Points |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------|--------|
| <b>(1) FORMAL REQUIREMENTS</b>                                                                                                                                                                                                                |                          |        |
| 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                      | 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                      | 3      |
| Formal requirements – points in total                                                                                                                                                                                                         |                          | 20     |
| <b>(2) PRACTICAL REQUIREMENTS</b>                                                                                                                                                                                                             |                          |        |
| 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                      | 2      |
| Logic in the course of the experimental work                                                                                                                                                                                                  | 0-3                      | 3      |
| Completeness of the description of the used techniques                                                                                                                                                                                        | 0-3                      | 2      |

<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<br>(after eventual supplementary experiments) | 0-3 | 3     |
| Practical requirements – points in total                                                                                                      |     | 24    |
| POINTS IN TOTAL (MAX/AWARDED)                                                                                                                 |     | 48 44 |

### **Comments of the reviewer on the student and the thesis:**

Mrs Pylypchuk presented a very organized manuscript including a clear introduction stating the main subjects in relation with her thesis. She used up to date references and introduced her work in a very clear way which is understandable for scientific and non-scientific audiences.

The subject is of high interest and relevance as tick salivary protease inhibitors are increasingly highlighted as promising therapeutics for several diseases.

The candidate successfully cloned the IRS-7 gene into a pET-SUMO vector, expressed the protein of interest using BL21(DE3) pLysS E. coli strain, purified the protein and performed functional tests to assess the potential effects of the tick salivary Serpin on blood coagulation.

Although there was no observed effects of the protein, the candidate performed state of the art techniques and discussed well her results. I believe she satisfies all the requirements for the obtention of her Bachelor degree. In addition, she was supervised by two experts in the field of Serpins, namely Jan Kotál, Ph.D and Jindřich Chmelař Ph.D which allows her to discuss her experiments in an advanced way and reach scientific maturity through her work.

### **Suggestions and questions, to which the student has to answer during the defense.** **Mistakes, which the students should avoid in the future:**

- How many cysteines are contained in the expressed Serpin? And why the candidate chose to work with the soluble fraction and didn't try using the inclusion bodies?
- The candidate used BL21(DE3) pLysS E. coli strain but didn't mention using Chloramphenicol during the expression, is there a specific reason?
- Did the candidate perform an MS analysis of western blot for her protein?
- Did the candidate measure the activity of different proteases using a substrate in presence/absence of the Serpin?
- Was there any alignment of the amino acid sequence with other serpins? On which basis she speculates that there is no Arginine in the RCL of her Serpin.

**Conclusion:**

In conclusion, I recommend the thesis for the defense and I suggest the grade Excellent

In Mainz date 13/09/2022