



OPPONENT'S REVIEW ON BACHELOR THESIS

Name of the student: Katharina Wiener

Thesis title: Creating an in-vitro model system for studying phase separation in virus assembly

Supervisor: doc. Mgr. Roman Tůma, Ph.D.

Co-supervisor: Mgr. Barbora Kašćáková

Referee: RNDr. Martin Selinger, Ph.D.

Referee's affiliation: Department of Chemistry, Faculty of Science, 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	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 labelling, indication of the units)	0-3	2
Formal requirements – points in total		20
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfilment 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	2.5
Logic in the course of the experimental work	0-3	3

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

Completeness of the description of the used techniques	0-3	3
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	2.5
Practical requirements – points in total		26
POINTS IN TOTAL (MAX/AWARDED)	48	46

Comments of the reviewer on the student and the thesis:

The bachelor thesis of Katharina Wiener aimed to produce and purify reoviral μ NS protein (the main factor involved in the formation of viroplasm in infected cells) and subsequently establish an *in vitro* method for analysis of viroplasm formation using the purified μ NS protein. The protein was produced and purified in a fused form (μ NS-mNeon) and successful formation of globular bodies was described using various conditions. In addition, the attempt to prepare crystals of μ NS-mNeon was done. However, due to low stability of the protein as well as the limited time, the attempt was unsuccessful.

Overall, the thesis is very well written and I greatly appreciate its coherence and an excellent English. The length of 52 pages (including the list of references and appendix) is more than satisfactory. The thesis is standardly divided into eight chapters: Introduction, Aims, Materials and Methods, Results, Discussion, Conclusions, References and Appendix. Following text will comment each of the chapters with questions asked after the comments.

Introduction

Introduction part (13 pages) is very well written with almost no spelling mistakes. The author gives a thorough but coherent background about the avian reovirus and its structure, genome, and replication cycle with a detailed description of the μ NS protein, which is the protein of interest in this study. Figures included in this chapter appropriately illustrate the information in text.

Aims

Aims are clearly stated, I have no comments here.

Materials and Methods

This chapter covers 12 pages and is generally well written. I also highly appreciate the wide spectrum of methods, which was used in the thesis. I have no comments to this section either.

Results

The results are described on 17 pages. The chapter is well written with a good coherence and syntax. Considerable amount of experimental data is presented in 21 figures and 2 tables. I have few comments on this section:

- I am missing the percentage of separation gels in Figures 8-14 (since there are two values described in Methods part – 10% and 12.5%).
- I am missing the meaning of arrows in Figures 8-10.
- I am missing either figure or just a mention of unsuccessful transformation attempt with

E.cloni® cells, since the transformation method is mentioned in the Materials and Methods chapter. Therefore, I would move this information from the Discussion to the Results chapter.

- I am not sure about the origin (soluble/insoluble fraction) of μ NS-mNeon for Circular dichroism, formation of viroplasms, and crystallization experiments. As far as I have understood, the origin of μ NS-mNeon for all mentioned experiments was from the insoluble fraction? If so, I would state this information in the beginning of each chapter.

Discussion

The discussion includes 3 pages and author clearly summarizes her results and compares them with known scientific literature. Again, I have nothing to comment here.

Questions:

- 1) As I am not experienced with Circular dichroism method, could you please describe the basic principle of this method?
- 2) Based on the Figures 8 and 9, the band of ~101 kDa is nicely visible in the insoluble fraction. Why did you not directly focus on the purification of μ NS-mNeon from this fraction?
- 3) What could be the reason behind the different outcome of affinity chromatography in Figures 10 and 12?
- 4) Is there any information available regarding the size of viroplasms in infected cells in order to compare the size distribution of your *in vitro* results with *in vivo* ones?
- 5) Regarding future experiments – may I ask you about what additional factors would you use in combination with μ NS-mNeon NaCl/PEG buffers in order to further study the *in vitro* formation of viroplasms?

Conclusion:

The bachelor thesis of Katharina Wiener is an example of excellent work combining an interesting topic with a nice data acquired from a wide variety of methods, all being summarized in a very well written form. Therefore, I **recommend** the thesis for the defense and the final grade will depend on the performance of the candidate during the defense and her answers to my questions.

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