Přírodovědecká fakulta
Faculty of Science

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

OPPONENT'S REVIEW ON BACHELOR/DIPLOMA* THESIS

Name of the student: **Bc., Bsc Hana Pechová**

Thesis title: Analysis of trypsin inhibitor-like cysteine-rich domain-containing peptides (TIL-domain inhibitors) from the tick *Ixodes ricinus*

Supervisor: Jan Kotál, PhD

Referee: **Jan Perner, PhD**
Referee`s affiliation: **Institute of Parasitology, Biology Centre, Ceske Budejovice**

	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	2
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	1
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	1
Formal requirements – points in total		16
(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	1

* Choose one

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

Completeness of the description of the used techniques	0-3	1
Experimental difficulty of the thesis, independence in experimental work	0-3	2
Quality of experimental data presentation	0-3	1
The use of up-to-date techniques	0-3	2
Contribution of the thesis to the knowledge in the field and possibility to publish the results (after eventual supplementary experiments)	0-3	1
Practical requirements – points in total		17
POINTS IN TOTAL (MAX/AWARDED)	**48**	**33**[2]

Comments of the reviewer on the student and the thesis:

It is a large master thesis with all its advantages and drawbacks. Certainly, the thesis served its purpose and Hanka proved the capacity and skills to plan and conduct a range of experimental methods.
I would like to highlight that some parts of the introductory section were perfectly meaningful and were interesting to read. Even though Hanka managed well to build the narrative, most of the rest of the thesis was difficult to enjoy reading due to numerous disturbances (not well-placed sentences, unnecessary duplications, inconsistencies, etc). I would recommend for Hanka´s thesis defence to spend a good time on Figure visualisation to convey a message clearly. Generally, the thesis would highly benefit from trimming of "excessive fat" and use data only as a support for your storytelling, rather than the effort to come near to "the-more-the-better" saying. The above criticism includes:

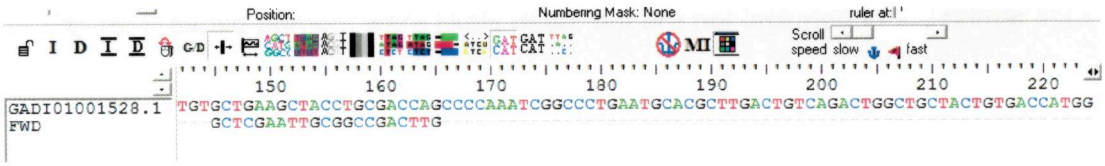
- Unnecessary dual use of the term "Figure" and "Graph" in Figure legends.
- Gel lane numbers vs name
- Gel images were generally of poor quality
- Results headers overlap too much with Methods headers
- Individual result sections should be larger
- Figures legends do not always make it clear what experimental analysis we look at
- Figure legends should not interpret results (Graph 1 legend)
- Figure legends should provide n of replicates
- Imperfect annotation text ! (molecular cloning of the representative protein-ENCODING TRANSCRIPT, representative TIL-domain ~~gene~~ PROTEIN is provided for their structure)
- Absence of WB marker (Fig 18, right); arrows do not overlap with those in SDS-PAGE
- etc.

[2] Enter the number of points awarded.

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

1. I severely miss a section, why TIL1 was selected for functional studies. As TIL-encoding transcripts (DOI: 10.1186/s13071-017-2312-4) are highly over-represented in males (over females) in *Rhipicephalus zambeziensis* ticks (though blood-feeding too), one must make good prioritisation of candidates before embarking on functional studies. The tissue expression profile of *til1* transcript does not support your narrative of tick-host interaction either. Instead, participation in midgut-related processes might be anticipated, such as anti-microbial activity. This assumption is strengthened by the fact that TIL-encoding transcripts are found in SG of the *Antricola delacruzi* (10.1016/j.ibmb.2012.01.003) tick, a non-blood-feeding tick species (guanophagous/mycophagous). Could TILs be used to limit fungi overgrowth, or assist in mycophagy? Why was Ir_TIL-1 selected in the first place, if there is no clear reason, why were haemostatic-related assays selected for a midgut protein? Would be any anti-microbial assessment more reasonable?

2. Absence of such abovementioned section has substantial implications for following parts. While you talk about choosing TIL-1 (without stating reasons why) on p21, you then pasted sequence on p. 22 of TIL that we annotated as TIL 9 (DOI: 10.1038/srep36695). And also clearly your primers (p 23) would not align well with *til-1* transcript. This needs to be made clear.



>JAA72280.1 Putative til domain protein_**your_TIL1**
MLKFLLTALLAYLVVAAADHHEHEHHADVTTPACGENEVAKTCVSSSCAEATCDQPQIGPE
CTLDCQTGC
YCDHGFYRNAEHVCVTKEACPAGSAAHTYVETPHVH
>IrSigP-112907_**til_1**
MLKIVVIALLGYLALASARPEQYDGGLYIPTRRPHRCGENEVYKSCVSSSCAEATCERPRV
GPACTMDCVYGCFCQRGFF
RDEEKNCVTRRECPEDSPAYTYKANDYALQ
>Ir-112121_**til_9**
GFLVLLAASVASARIAADLPWVCGPREVFKDWVSSCCAELKCGMEGMPIGCTKDCVSGC
FCAPGFYRRGHRECVPRSECQ
IEPLKPMPKA

3. Clearly, your recombinant protein migrates differently to its expected theoretical mass. However, please do not place 24kDa mark halfway between 15 kDa and 25 kDa mark (Figure 17). Rather suggest some reasoning of the observation/literature reference, why you think this might be happening.

4. Even though I appreciate the effort to cleave off the protein tag, I am ok with the use of N-terminally tagged protein, if the tag is used in the control group, and provided your TIL-inhibitory loop is not N-terminally placed and, therefore, unlikely to interfere. Having said that, a smaller tag might be preferred, as it is unlucky to have bigger tag than the protein of interest itself. So, even though I am ok with the tag as a negative control, I severely miss

positive controls in most experiments (coagulation assay, thrombin assay, trypsin assay). Why are these not included?

5. Description of trypsin (the same applies to thrombin assay) assay (p 31) does not specify your dilution of 6 uM stock. Let´s suppose you diluted your TIL 30× (i.e. 1.6 ul of TIL into 50 ul rxn buffer), you woud then end up with 10 000× molar excess of your inhibitor in the reaction over trypsin (20pM). Even though you did not observe any effect, should not a good inhibitor work well even at better stoichiometry? In other words, even if you did observe an inhibition, would you find it meaningful? Please compare with other (more potent) trypsin inhibitors.

Small things:

1. p1: *"The binding of the substrate towards the active site of the protease is highly specific"*, it implies as if the catalitically active residues were also critical for binding. Is it true?
2. SOC = SOB + 20mM glucose
2. Animal family is not written in italics, only a species name is (p 5, 21)
3. Indicate what % you mean (w x v / w x v; p 19/20)
4. State a full buffer composition, esp. if it is called Buffer. I assume Tris was titrated for the desired pH with HCl, correct (p. 19/20)?
5. Last paragraph on p 27 is not clear. "*Cells were broken by freezing supernatant*" is confusing, with very good chance of being even wrong. I assume you meant the cell pellet was resuspended in 20mM Tris-Cl + 0.5M NaCl. And this suspension was subjected to the freeze/thaw?
6. Fig 16: Plasmid primers were likely used, but are not included in the primer list

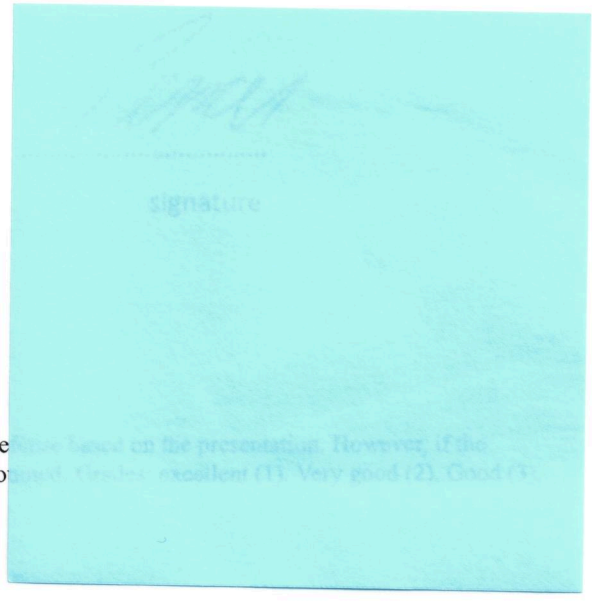
Conclusion:

In conclusion, I

recommend ~~**/ do not recommend***~~

the thesis for the defense and I suggest the grade **2-.**[3]

In **České Budějovice** date **18.1.2021**



[3] You can suggest a grade, which can be modified during the de… reviewer is not present at the defense, the grade will not be co… Unsatisfactory/failed (4).