Opponent review on BSc thesis

Student: Katharina Perfahl
Supervisor: RNDr. Daniel Sojka, PhD.
Reviewer: Mgr. Jan Kotál

The bachelor thesis of Katharina Perfahl focuses on characterizing an isoform of cathepsin L - a protease involved in blood digestion in the tick *Ixodes ricinus* midgut. The work is thematically attractive and brings new insight to the function of proteases in ticks. It is a part of a larger project of Dr. Daniel Sojka, whose team has significantly contributed to the understanding of tick blood digestion.
The thesis has a standard concept of chapters, covers 28 pages and the aims of the thesis are clearly stated. I have happily agreed to review this thesis and my comments are the following:

The thesis is written in good English with a minimum of grammar mistakes or typos. In some cases segmenting long sentences to shorter ones would ease reading and improve understanding.
Unfortunately, the thesis contains quite many formal mistakes that could have been avoided by spending more time and thorough checking during finalizing the work. Latin names were often fully spelled more than once or only used in the abbreviated form; not all figures have a link to them in the text (Fig. 1, Fig. 3A, B) or the link refers to a wrong figure (page 24, link to Fig. 12 instead of Fig. 2); references are not in uniform style and one of them does not mention the title of cited publication (Kane et al.). I strongly recommend using a citation manager in the future.
The introduction part is well written and describes ticks and blood digestion in a "general to specific" way, which I appreciate. Except for few minor complaints, I have one major – text under Fig. 3 is, without few shortened parts, identical to text in the cited publication.
The Materials and Methods section describes most of the experiments in sufficient detail. I miss more information about isolation of inclusion bodies and about protein activity measurement, which is only given under Fig. 7 in Results.
The chapter Results shows data from gene PCR amplification, cloning, protein expression, antibody production and antibodies testing by Western blot and IHC. Katharina shows a successful IrCL3 purification by affinity chromatography, confirmation of its proteolytic activity, three pictures of Western blots and a microscopy picture of tick midgut section with visualized IrCL1 and IrCL3. The text of this chapter should focus more on commenting the results and figures and less on repeating the experimental procedure (e.g. beginning of 4.2). I also find confusing using red color for IrCL1 in WB and green in IHC and the opposite coloring for IrCL3. It would help easier understanding Fig.10 if labels above gel lanes were not 1, 2, 3… but rather unf., 3d, 5d, …, as described under the figure.

As a conclusion, the presented thesis shows promising results and well-designed experiments. Yet, there is still room for time-consuming improvement by adding new methods, more results to present a coherent and well written story.

Despite my critical comments, I recommend the BSc thesis of Katharina Perfahl to be defended with a final mark depending on the oral presentation of her work.

Questions to the author:

1. In chapter 1.5 you refer to residues at P1 and P2 positions without any further explanation. What are these residues and what is their role?
2. In chapter 3.6 you describe method of IrCL3 protein expression. Are the expression conditions result of some optimization or did you use the same conditions as described for IrCL1 by Franta et al. 2011? Can you briefly describe the isolation of inclusion bodies?
3. In Fig. 8B and C you show Western blot of tick midgut using antibodies against two different CatL isoenzymes. Can you discuss, why IrCL1 antibodies detect one band at ca 45kDa, while antibodies against IrCL3 detect two bands at ca 30 and 60kDa?

In České Budějovice, January 22nd 2019, worked out by

Mgr. Jan Kotál