

Reviewer's Dissertation Thesis Report

Student: Mgr. Barbora Kaščáková

Dissertation Thesis Title: Structural insight into the salivary serpins of *Ixodes ricinus*.

Reviewer: Doc. Mgr. Lukas Trantírek, Ph.D.

Reviewer's Workplace: Central European Institute of Technology, Masaryk University, Kamenice
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Text of the report

Topic and timeliness of the work. Ph.D. thesis of Mgr. Barbora Kaščáková is a compilation of her published works and additional unpublished data. About 30 pages long introductory part of the thesis provides an overview of the structure, mechanism of action, and physiology of serpins, an important class of serine protease inhibitors, and X-ray crystallography, a principal technique used by the student to characterize the structure of four distinct serpins from hematophagous parasite *Ixodes Ricinus*. The thesis results section consists of the reprints of two published papers and two chapters corresponding to submitted manuscripts. Mgr. Kascakova is the first author of one published article and two submitted manuscripts.

Quality assessment of the thesis: The thesis has a logical structure. The subject and the methodology are discussed in sufficient detail. Relevant references support the text. The experimental design used in the published works and two additional manuscripts follows an established workflow in X-ray crystallography comprising overexpression of the protein under study in host *E. coli*, its purification, preparation of mono-crystals for X-ray analysis, and structure determination. Although there are no significant shortcomings in the thesis text, I ask the student to elaborate more on the following issues during the thesis defense:

- 1] Post-translational modifications (PTM) of the studied serpins: Bioinformatics analysis indicated two glycosylation sites in serpin-3. Does the glycosylation concern also other serpins investigated? Did the student consider the existence of other post-translational modifications, i.e., tyrosine-O-sulfation, which might be relevant to the tick's physiology (Ripoll-Rozada et al. *Biochem Soc Trans*, 2022 Feb 28;50(1):387-401)? Could the student discuss the potential impact of PTMs on serpins' structural equilibria and functions?
- 2] On the same note, could the student justify the choice of *E. coli* as an overexpression system regarding the lack of post-translational protein modification machinery in this host?
- 3] In the Introduction, the student recalls Anfinsen's theorem of protein folding and claims that serpins are an "exception" from it. Can the student justify this statement regarding the possibility of the existence of kinetically favored (which seems to correspond, based on the description provided, to the "native conformation") and thermodynamically stable conformation (which appears to conform to the "latent conformation")? Is X-ray

crystallography a suitable technique for the structural characterization of kinetically favored conformations?

Overall assessment: In the dissertation thesis, the student has demonstrated her creative abilities in the relevant research area and I am recommending the dissertation thesis for defense.

Date 2022-22-05

Doc. Mgr. Lukas Trantirek, Ph.D.