Oponent review – PhD thesis of

Hagen Sulzen

entitled

Structural insights into innate immune evasion mechanisms of African trypanosomes and type C adenoviruses

Candidate Hagen Sulzen's doctoral thesis spans 174 pages and is combination of four publications with short introduction, aligning with the regulations and guidelines of the Department of Physical and Macromolecular Chemistry at Charles University. Formally, this thesis embodies the requisite attributes of style, language, and scientific rigor as prescribed by the department.

Sulzen employs a rich array of expressive language, exemplified by phrases such as "solemnly declare" and "plethora," enhancing the readability of the thesis. The English usage is proficient, aptly encapsulating the subject matter. The overarching focus of the thesis delves into the intricate mechanisms underlying host-pathogen interactions, particularly examining surface proteins from two biologically distinct organisms: *Trypanosoma brucei gambiense* and type C adenovirus.

The candidate’s primary focus was on understanding the interaction between Trypanosoma's invariant surface glycoprotein 65 (ISG65) and the host factor human complement C3 and C3b, and it further explores the flexibility and dynamics of ISG64, ISG43, and ISG75 in solution. A notable breakthrough in this thesis was the elucidation of the precise biological function and mechanisms underlying ISG65-driven inhibition of the C3 cascade. This achievement was made possible through the utilization of surface plasmon resonance, complemented by structural insights from cryo electron microscopy (CryoEM), which revealed the molecular architecture and the mechanism and manner of the interactions between these proteins. Consequently, the model of inhibition of the C3 cascade by ISG64 was outlined.

At first glance, pursuing research on Trypanosoma, a pathogen primarily affecting tropical Africa, may seem inadequate for scientific pursuits in central Europe. However, the opposite holds true. Despite its limited prevalence in central Europe, Trypanosoma's devastating socioeconomic impact, including the deaths of millions of cattle, underscores the significance of studying host-pathogen interactions. Such investigations not only yield invaluable insights into the pathogen itself but often also serve as essential avenues for understanding human biology, with the potential to translate findings into therapeutic applications. This thesis, conducted by Hagen Sulzen under the supervision of Dr. Sebastian Zoll at the Institute of Organic Chemistry and Biochemistry in Prague, exemplifies this paradigm.

It is my firm recommendation that the committee bestows upon Hagen Sulzen the title of PhD.

13. 5 .2024 in Prague

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RNDr Jan Silhan PhD
Institute of Organic Chemistry and Biochemistry in Prague

List of questions or remarks for the candidate:

1. Could you provide further detail on the selection and rationale behind utilizing specific biophysical techniques such as SPR to investigate the interactions between ISG65 and human complement factors C3 and C3b? Were there any unexpected observations or sample behaviors that necessitated troubleshooting or altered the project's conceptual framework?

2. How did the preparation of CryoEM samples and grids contribute to the successful acquisition of data regarding the interactions between ISG65 and complement factors? Were there any innovative techniques or challenging circumstances encountered during this process?

3. In summary, could you elaborate on how the study of ISG65 in Trypanosoma brucei gambiense, despite its relatively low prevalence in central Europe, contributes to broader insights into host-pathogen interactions and potential therapeutic implications?

Remarks:

I need to emphasize not only the quality of the thesis but also the outstanding clarity in informing the reader about the author's contributions in each section. However I have one comment.
Personally, I would prefer the author to reference all the data discussed in the figures, for instance, in section 4.2.2, specifically showing or referencing the results associated, e.g. a particular figure in that section,

“SPR analysis revealed that native C3 has the lowest affinity for ISG65 (KD = 130 nM), followed by C3b (KD = 81 nM) and C3-methylamine (C3MA), a mimic for C3(H2 O) (KD = 18 nM)”.

No data points, curves, or fits are present within this part of the thesis and are only referenced to a final publication. However, this absence does not significantly diminish the high quality of this PhD thesis and the work it encompasses. It simply necessitates additional and unnecessary effort from the reader to locate specific data within the accompanied paper. Nonetheless, considering that this thesis is a condensed format comprising four publications bound together with a brief introduction, this critique is relatively minor.