Reviewer's report on the dissertation thesis entitled „ Structural insights into innate immune evasion mechanisms of African trypanosomes and type C adenoviruses"

PhD candidate: Hagen Sülzen, MSc
Scientific supervisor: Sebastian Zoll, Ph.D.

The PhD thesis focuses on the structural and functional characterization of surface proteins of human pathogens, namely African trypanosomes and type C-type adenoviruses. The thesis is written in English in a "long" format with a total of 186 pages and follows a conventional structure consisting of Introduction, Aims, Materials and Methods, Results, Discussion, and Conclusions along with a list of 268 references. Included are also three manuscript reprints and a BioRix preprint (already accepted for publication) underlying the thesis. It is pleasing to see that all manuscripts associated with the thesis are of very high-quality and ranking very well in their respective fields (Nat Commun, PLoS Pathogens, Sci Rep, J Virol). I really enjoyed reading the Introduction part with detailed information about both Trypanosomes and Adenoviruses.

In the first part of the Results and discussion section, the applicant summarized his findings that ISG65 serves as a receptor for human complement C3b and reports the first structures of TbgISG65 in complex with human C3 and C3b. The second part sheds light onto the structural and functional aspects of interactions between C adenoviruses and human lactoferrin that can serve as an entry receptor. Both publications and the thesis clearly document that during his PhD training the applicant became proficient in a host of experimental techniques, including heterologous protein expression and purification, X-ray crystallography, cryoEM, and biophysical measurements (SAXS, SPR, HDX), all of which should provide a solid foundation for his future scientific career. Additionally, well-written discussion points towards deep understanding of the studies topics, excellent analytical thinking and mastering the literature work. Overall, the thesis represents a nice piece of work and in addition to contributions to basic science advancements, reported findings can hold promise for the development of novel antivirals and antiparasitic drugs.

I would like the candidate to elaborate on the following issues/questions:

1. On page 20, you mentioned that "hydrodynamic forces created by the cell's motility can be responsible for marked increase in endocytosis and recycling rate of VSG endocytosis". Are there more examples of flow rates influencing endocytosis in living cells (e.g., immune cells or hairy epithelial cells) or is this phenomenon only described for Trypanosomes having the specialized endocytosis-proficient flagellar pocket?

2. In several parts of your Discussion section you discuss discrepancies between SPR data from various groups that might stem from "experimental errors" such as measurement design and data analysis/interpretation. Could you please sum up "a good practice in SPR

measurements" and point out common mistakes that scientists make when running SPR experiments?

3. *In silico* modeling was used in the thesis to create models of complexes as well as to help defining structured part of target proteins to facilitate expression/crystallization. How do you see the future of structural biology in the context of the advent of AI technologies? Will computational results form a mainstay of structural work with experimental data having only confirmatory roles?

In conclusion, the PhD thesis by Mr. Hagen Sülzen fulfills all criteria required for the successful defense of his work. Therefore, it is my pleasure to recommend the candidate to be awarded a PhD degree.

Prague, May 10, 2024

RNDr. Cyril Bařinka, PhD
Laboratoř strukturní biologie
Biotechnologický ústav AV ČR
Průmyslová 595, Vestec
telefon: +420-325-873-777
e-mail: http://lsb.avcr.cz/