Review report master thesis

Author: **Roland Malych**

Title: **Proteins interacting with oxygen and its reactive species in Naegleria gruberi**

The master thesis by Bc Malych is written clearly and to the point. Results and conclusions presented make sense and may lead to interesting findings in the future. By necessity, the work started by Bc Malych is not fully concluded and cannot give a definitive answers to all the issues at hand, yet forms a solid foundation on which Bc Malych (or other investigators) can build a more complete story. Indeed, to produce and characterize recombinant proteins with strange-sounding names (hemerythrin, protoglobin and rubrerythrin) and little known function from such a wonderfully weird organism (N. gruberi – this is from a person who mostly studies mice and humans) is a long distance run rather than a short track event.

While I find the work to be of a very good quality for such an early stage investigator (with a minor gripe that the discussion could be less of a repetition of results, which will undoubtedly improve with practice), I have a several additional questions that I would like to put to the author:

1. Recombinant proteins were produced in E.Coli, precluding post-translational modifications. Do you expect the proteins to be post-translationally-modified in N. gruberi and would you expect these modification to affect their function? Are postranslational modification prevalent in N gruberi?
2. Response to oxidative stress: proteins do not need to change their expression level in response to an oxidant and still be crucial for oxidative stress protection. Any thoughts about this with respect to the conclusions? Any plans to address this? Can you do knockouts/knockdowns?
3. Fig 17: Were the newly raised antibodies also tested on the produced recombinant proteins, or only on the cell lysates? Did they work?
4. Figs. 29, 30: What was the effect of the compounds/metal ions on viability? The observed response could simply be related to cells getting stressed and dying.
5. Figs. 19-21: Any insight from the modelling whether di-/oligomerization of the studied proteins could be important for their function?
6. Immunofluorescence images are shown only for rubrerythrin. Have you tried to do these stainings also with hemerythrin and protoglobin antibodies?
7. Where do you expect that N. gruberi (or fowleri) could encounter oxidative stress? Would it be during the normoxia/hypoxia switch? Is this relevant for pathology? What is the setup of the planed hypoxia experiment?
8. You speculate that hemerythrin isoform in N. fowleri could induce the virulence which is not observed for N. gruberi. Could differences in hemerythrin sequence from the two species shown in Fig. 10 account for this varied virulence? The other studied proteins seemed more similar (on a quick glance) between the two species.
9. The higher bands in gels shown in Figs. 14-16 are often twice the size of the monomeric proteins. Do you think these are dimers?
10. Practical: why did you do purification from inclusion bodies for hemerythrin, when you also did recombinant protein production under native conditions (that was sufficient for the other proteins)

In general, I am impressed with the effort of Bc. Malych and I wish him a good fortune in his further endeavors.

The master thesis fulfils all required criteria, I recommend admission to the defense. Suggested grade: 1

Předložená práce splňuje nároky na diplomovou práci, práci doporučuji k obhajobě a navrhuji známku 1.

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June 30, 2020