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Dear Colleagues,

I am writing this letter to fully support the dissertation of Lubos Voleman as evidence of completion of his Ph.D.

Based on my review of candidate Voleman's thesis, I find that he has made significant and original contributions to the field of *Giardia* research, and this work demonstrates his mastery of the topic and experimental expertise to merit its acceptance. I support that Lubos Voleman's dissertation is sufficient to merit the awarding of a Ph.D.

I also attach more general and specific critical comments in the form of questions to discuss below to help guide the discussion at his defense.

Sincerely,

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Review of Lubos Voleman Dissertation

I found the introductory chapter to be of sufficient quality for the dissertation. This chapter included an updated discussion of current research in model and non-model organisms of relevance to the experimental work present in Giardia. My overall review of the dissertation is organized into discussion points by topic with associated "thought" questions. I do not expect to discuss every point in detail but hope the questions provide sufficient detail for a collegial and broader discussion of the dissertation.

1. **How to interpret the imaging of candidate mitosomal proteins? What are the challenges?**
   - What are some experimental challenges in defining an organelle structure or dynamics based solely on light microscopy localization (using immunostaining or tagging)?
   - Does localization always imply that a protein is a functional component of an organelle? Part of a that organelle structure? A regulatory component? A transported component? Does the lack of localization always imply that a protein is not associated with an organelle's functioning?
   - What does protein localization tell you about the dynamics or turnover of a protein at the site of localization? How would you determine this?
   - How does light-based imaging agree with ultrastructural level (EM) used to verify the localization of candidate mitosomal proteins with a known mitosomal structure (central or peripheral inner membrane or outer membranes, etc).
   - How to evaluate viability of cells during live imaging (HaloTags, GFP, etc)? What are some ways to know that *Giardia* (or other cells) are viable and behaving normally during live imaging?

2. **What are the primary challenges of proteomic experiments and interpretations of mitosomes?**
   - How can you evaluate the efficacy of the BirA tagging approach given the diversity of candidate mitosomal proteins that are not co-localized, or localize to other organelles? What are caveats (or important controls) needed for the BirA based tagging approaches? How could the choice of promoter for expression of BirA impact the proteomic results? What would be other useful controls or alternative proximity-based tagging approaches you might use?
   - Alternatively, what are some other cell biological explanations why you would see a diversity of putative mitosomal proteins interacting with the BirA approach that do not localize with known mitosomal proteins?

3. **Given your understanding how mitosome are closely associated with the ER, how do you interpret prior functional characterizations of IscS or IscU that relied on fractionation?**

4. **What types of is future research needed to resolve questions of fission/fusion of mitosomes?**
   - How would a lack of fusion of Giardia mitosomes impact Giardia's overall cell biology?
   - What is speed/rate of fusion of mitochondria in known model organisms? What would be the prediction for Giardia? Would knowing the rate of fusion impact the design of your live imaging experiments?
   - How would you test the hypothesis that lipids were transferred between the contact sites of mitosomes and ER?

- Given more time, what are some additional experimental you would propose to confirm your hypothesis of a lack of mitosomal fusion?
- Other that providing support for mitosome "heterogeneity", what are other some alternative cell biological explanations for a lack of colocation of proteins to mitosomes (or really whenever proteins do not co-localize to an organelle)?

5. **What are more strategies to determine the cellular functions of mitosomal-localizing proteins?**
   - Briefly compare and contrast your evidence in support of the cellular role of the *Giardia* dynamin homolog with evidence in Rout et al. using a dominant negative. Are there significant methodological differences in constructs?
   - What is your expectation of the phenotype of an IscS or IscU knockout (or knockdown?) What would you expect the phenotype of a ferredoxin knockout to be? Lethal?