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To whom it may concern

Evaluation of the PhD Thesis “Factors involved in Trypanosoma brucei mitochondrial morphology” submitted by Shaghayegh Sheikh

The topic of the dissertation deals in several studies with proteins that shape the morphology of the mitochondrial inner membrane. By tracing an arc across the ancestor of mitochondria, the alphaproteobacteria, *T. brucei* and giant viruses, the studies take on an interesting evolutionary biology aspect that raises questions about the origin, diversification and maintenance of the shape-giving proteins and leaves room for speculation about whether their original functions may have evolved during adaptations. Examples of such adaptations are those that arise within an organism, such as throughout the different life stages of *T. brucei*, or in evolutionary history through endosymbiotic transformation of a photosynthetic bacterium into an aerobic mitochondrion, or possibly through DNA transfer that allows a giant virus to shape the membranes of its host in its favor.
Focusing on mitochondrial cristae, the thesis manuscript contains results from different studies that were published in three articles, and unpublished results. A first article investigates the cristae rearrangements during the lifestyle of T*. brucei*. The focus of the candidate S. Sheikh, who shares first co-authorship, is the comparison of mitochondria and crista morphotypes in the bloodstream form (BSF)v ersus procyclic form (PCF) or *T. brucei*. Data provided by transmission electron microscopy and 3D reconstruction clearly demonstrate that the size of BSF cristae is reduced compared to PCF cristae, consistent with the notion that BSF rely on a glycolytic metabolism and PCF on an oxidative proline metabolism. Another central topic of the thesis is the crista developing function of the mitochondrial contact site and cristae organizing system (MICOS) complex, especially with Mic60. The second paper, to which the candidate contributed as a middle author, structurally and functionally analyses the alphaproteobacterial Mic60 homolog and the co-transcribed adjacent Orf53 in *R. sphaeroides* and *R.palustri.* The study reveals an interaction of Mic60 with BamA, which has been preserved throughout evolution in mitochondria. The finding importantly underlines that vital factors of photosynthetic intracytoplasmic membranes and contact site formations are the precursors of today’s crista architecture. The thesis of S. Sheikh further draws our attention on the crista developing and remodeling factors OPA1 and Mgm1, two conserved metazoan and fungi proteins, respectively,

that belong to the dynamin related protein (DRP) superfamily, and have been lost in *T. brucei*. At this occurrence the third published paper, first authored by S. Sheikh, reports on the novel and unknown DRP subgroup MidX, which appears on eukaryotes and a giant virus as revealed by phylogenic comparison of viral and eukaryotic DRPs. The undertaken phylogenetic analysis revealed a sister lineage with Mgm1. To investigate the function of MidX, which could similarly play a role in mitochondrial membrane remodeling, MidX was exogenously expressed in *T. brucei*. The phenotypical analyses of mitochondrial ultrastructure however indicate a dramatic organellar reorganization. The results are novel in many aspects including the unexpected impact of viral MidX on forming cytoplasmic protrusions into mitochondria and will require further investigation on the mechanisms of MidX in deforming the mitochondrial ultrastructure.
In her unpublished results, S. Sheikh follows up on a previous topic that questions the evolutionary conservation of Mic60 in *T. brucei* and considers the MICOS subunits Mic34 and Mic40 that carry the mitofilin signature that the putative *T. brucei* Mic60 has lost. Expression of Mic34 or Mic40 in *E. coli* remodels the cytoplasmic membranes and knocking down Mic34 or Mic40 in *T. brucei* alters crista and mitochondrial morphology.

The contribution of S. Sheikh in these different studies is remarkable. A large part of her contribution was the considerable amount of calculation of the surfaces and volume of mitochondria and the size, number, length and volume of cristae by electron microscopy and the acquisition of 3D techniques for the reconstruction of mitochondria and cristae shapes. In addition to the extensive morphological investigations using immunofluorescent and transmission electron microscopy and statistical analyses, she also carried cell cultivation and growth measurements, recorded the membrane potential and conducted detailed biochemical studies for the subcellular and submitochondrial localization of MidX and thereby also significantly contributed to the novel study on MidX. In her unpublished study on the cryptic mitofilin-domain proteins she takes a more independent role in designing and conducting her experiments.

Altogether, the thesis manuscript is well written and well organized. The introduction gives a clear overview on general mitochondrial functions, origin and evolution and describes comprehensively what is known on mitochondrial morphology, cristae organization, dynamin related proteins, and the model system *T. brucei*. The result section is divided in published and unpublished results. Published results consist in the three published manuscripts where the candidate is co-first, middle and first author. The unpublished results are presented in a manuscript format. The results have been critically evaluated and placed in a larger framework with the discussion, and the techniques and their limitation have been discussed.

As a comment, I would like to point out the broad spectrum of the thesis and mention two associated risks.

By participating in several projects, where besides preparing figures and drafting the manuscript the candidate took on different tasks as first, co-first or middle author and certainly accumulated many skills. Not focusing on a defined, single and own study from the beginning could have been disadvantageous. However, on a very positive note, her unpublished results on the two cryptic mitofilin domain proteins belonging to MICOS and their presentation are evidence of being capable of planning of individual tasks and work steps and independent interpretation of the results and written presentation. These are signs of scientific maturity, autonomy and self-confidence.

A downside of a thesis with a too broad spectrum is the broad context and thus some aspects can easily be overlooked in the discussion. Though the evolutionary context is amply addressed, the metabolic regulation of mitochondrial morphology is an important factor that is mentioned but comes too short. For this reason, the defense is a good opportunity to follow up on that. To prepare this, I enclose a few questions below.

Based on the work presented in the manuscript thesis, I highly recommend the thesis for defense.

Karin Nowikovsky

Metabolism questions related to the paper
"Ultrastructural changes of the mitochondrion during the life cycle of *T brucei"*

The work on 3D reconstruction of mitochondria and cristae morphology in *T. brucei* across different life cycle stages is remarkable. My question in this context is aimed at seeing this work in a broader context. In this regard, I also miss more clarity in your thesis introduction about the different metabolic cycles associated with the changes in life cycle stages, which I would have prefered to see as a scheme rather than an illustration of the OXPHOS of a glioblastoma.

1a) The lifecycle of trypanosomes is very complex and divided into the two main stages as bloodstream form (BSF) and procycle form (PCF). Let's consider the two different BSFs: long slender and short stumpy and restrict the PCFs to mesocyclic trypomastigotes of the midgut and epimastigotes of the salivary gland, what are the mitochondrial i) metabolic, ii) morphological and ii) cristae characteristics of these 4 stages, and how are these characteristics interrelated?
1b) The BSFs and PCFs also traverse differentiation and proliferation states, is something known about mitochondrial metabolic and morphological rearrangements associated with the shifts between differentiation and division?
1c) Could you imagine an in vitro system allowing to induce the different lifecycle stages that can be studied for morphological changes using 3D reconstruction and analyzed in parallel for metabolic properties.
1d) You mentioned that the poor architecture of cristae in BSF is associated to the absence of respiratory CIII and IV. Could it be as well, or rather, associated with the reverse ATPase activity? Is there anything known about cristae remodeling under reverse ATPase activity in other organisms?

General metabolism questions

2a) NADH:ubiquinone oxidoreductases. You mention in your thesis that the NDH2 function is still obscure. Is it sensitive to rotenone? Does NDH2 works similarly to the NADH hydrogenase of yeast *S.cerevisiae* , is it a soluble or integral protein? Are respiratory complex subunits encoded by kinetoplast DNA, and which ones?
2b) Trypanosome alternative oxidase (TAO): integral protein? Why is it essential in BSF? How do *T. brucei* rely on alternative oxidase activity for ATP production via glycolysis? Could it be targeted to kill the pathogenic parasites?
2c) Is the proline catabolic metabolism unique to trypanosomes? Does it exist for example in cancer cells?

2d) Proline catabolism provides glutamine, how is it further metabolized in the TCA cycle of procyclic trypanosomes?