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Open peer review and authors' responses Mitochondrial respiratory function in living cells

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Reviewer 2: Steven Hand
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Manuscript reviewed 2025-04-10: *Only major points included.*

Reviewer 2

The manuscript succeeds in its dual goal of accuracy and clarity, and specific edits for smoother understanding are provided in the annotated file.

Authors

Thank you very much for your positive review and helpful edits to enhance readability.

Edits in the text:

- oxygen is essential for maintaining the 'fire of life' during the combustion of substrates used as fuel substrates.
- Section 1. Your comment: 'I agree that delaying a more detailed explanation of the *pmF* is reasonable for the sake of clarity and maintaining the focus on respiration. But you might consider adding here, parenthetically, something like '(relative contributions of ΔpH and ΔΨ to the *pmF* will be addressed in a later communication [5])' – something indicating to the reader that more is to come on this topic. – This is the added sentence: Yet, the *pmF* is not only made up of an electric potential across the mt-inner membrane; it also includes a diffusive component, which arises from the pH difference between the two sides of the membrane [5].
- Section 1.3. Your comment: 'Regarding this statement, it is of course accurate. But the reader should not infer that *non-idling* mitochondria do not produce heat. Is there an easy way to clarify this point, or would it be distracting here? Just something to consider.' – This is the added sentence: Heat dissipation, however, is associated with oxygen consumption in any respiratory state and mainly regulated by respiratory rate.
- Section 1.4. Your comment: 'Depending on the background of the reader in ROS and antioxidants, this sentence may not add much insight without more explanation. Perhaps consider deleting?' - I agree, and merely added the following: .. , but a functional interpretation of *rox* is difficult.

- Section 2: Your comment: 'Conversely, isolated mitochondria in some cases provide bioenergetic information not easily obtained with living cells.' – I added: Conversely, studies of isolated mitochondria provide bioenergetic information not easily obtained with living cells.
- Notes to Section 2: Your comment: 'For context, it might be useful to precede this sentence with a comment along the lines of: Measurement of oxygen kinetics with isolated mitochondria was reported 70 years ago for example by Chance and Williams [X].' – I added: Manometric techniques for measuring mitochondrial and cell respiration were replaced by electrochemical methods 70 years ago [13].