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The protonmotive force – not merely membrane potential

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Reviewer 1: Alicia Kowaltowski

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Manuscript reviewed 2022-04-10: *Only major points included.*

Reviewer 1

Experiments shown were conducted with animal samples (guinea pig brain mitochondria), but ethics approval and extensive methodology or a reference to an experimental paper with detailed methods were not included.

Authors

In the legend of figure 1, a reference to an experimental paper with detailed methods is included. To avoid any distraction from the core message of the review, the ethics approval and the detailed methodology are added as Supplementary material.

Reviewer 1

The last sentence on Page 2 states that "A linear relationship between fluorescence intensity and $\Delta\Psi m$ can be observed for certain concentration ranges and ratios of safranin and mitochondria (Figueira et al 2012)." While this is true, it is not necessarily the norm, and most authors probably do not take the time to determine if such a relationship exists under their experimental conditions. Indeed, since fluorescent probe measurements in isolated mitochondrial experiments involve complex quenching properties, fluorescence intensity is not expected to exhibit a linear relationship with membrane potentials under most conditions. Adding to this complexity, membrane potentials obviously do not have a linear relationship with with detected probe concentrations, as probe distribution is predicted by the Nernst equation (logarithmic). Despite this, it is not uncommon to see literature papers in which a percent change in uncalibrated probe fluorescence is wrongfully interpreted as the same percent change in membrane potentials. I believe it would thus be useful to add a more comprehensive discussion on quantitative membrane potential measurements in this paper, perhaps referring to a few papers in which this was shown in detail. I believe this would add nicely to the experimental descriptions on how to quantitatively determine DpH.

Authors

Thank you for the useful comment. We have addressed it in the paper with red.