

Editors' comments and author responses

Manuscript: The protonmotive force – not merely membrane potential

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Editors: Erich Gnaiger, Sabine Schmitt

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Manuscript edited 2022-11-20: *Only major points included.*

Editors

A 'difference' must be distinguished from a 'gradient'. In this manuscript, there is no measurement of a gradient, nor is there a conceptual link to gradients.

Authors

I agree with your concern. I have modified it to "difference".

Editors

Page 3: Does this really affect mtMP or only the detected fluorescence signal? Mitochondrial protein content or concentration? And isn't it the ratio of mt protein concentration and dye concentration.

Authors

Mitochondrial protein and dye concentration affect the fluorescence intensity (Scaduto Jr, Gotyohann 1999). A linear relationship between fluorescence intensity and $\Delta\Psi_{mt}$ can be observed only within limited concentration ranges and ratios of safranin and mitochondria due to mechanism of fluorescence quenching.

Editors

".. which is deviated" reads like a deviation from the Nernst equation - a different equation? This is probably not intended, please clarify.

Authors

I agree with you. We have modified already this part as follows: Mitochondrial protein and dye concentration affect the fluorescence intensity (Scaduto Jr, Gotyohann 1999). A linear relationship between fluorescence intensity and $\Delta\Psi_{mt}$ can be observed only within limited concentration ranges and ratios of safranin and mitochondria due to mechanism of fluorescence quenching (Figueira et al 2012). Additionally, fluorescence quenching could affect the $\Delta\Psi_{mt}$ calculated from the logarithmic Nernst equation which is used to describe the distribution of the

fluorescence dyes in the mitochondria (Scaduto Jr, Gotyohann 1999). Calibration of the fluorescence signal of safranin can be carried out with known-amount of KCl titration in the presence of valinomycin as discussed in details by Figueira et al 2012. Of note, owing to the complexity of the fluorometric measurement and calibration, we have used TPP⁺ to calculate $\Delta\Psi_{mt}$ expressed in mV (Kömłódi et al 2018; Tretter et al 2007).

Editors

Binding to the mtIM (and mtOM) is known as 'unspecific binding', which may occur independent of the membrane potential, and hence is not based on the Nernst equation. The necessity of corrections for unspecific binding must be clarified (see methods section).

Authors

I have accepted your suggestion.

Editors

“Decrease of pH_{in} is attributed to decrease of ROS generation, whereas alkalization is ascribed to elevated ROS release.” - Should this rather read: “A decrease of ROS generation is attributed to a decrease of pH_{in}.”? - What is cause and effect?

Authors

You are right. I have modified this as follows: “A decrease of ROS generation is attributed to a decrease of pH_{in},..”