**Mitochondrial respiratory states and rates:**

**Building blocks of mitochondrial physiology Part 1**

Comments from A.P. Sowton and Dr K.A. O’Brien

Line 108: *(and throughout the paper)* “protonmotive” perhaps as two words (personal preference?)

Line 128: The first point of the executive summary is more of an introduction. It may be better to have this as its own paragraph, ending with “the aspects of mitochondrial function we are focussing on are:”

Line 142: The word “vectorial” may confuse readers who aren’t familiar with this terminology

Line 160: ETS should be defined on first use

Line 283:“Only lately it is possible” could be better written as “Only recently has it been possible”

Lines 290-292: Here you consider mitochondrial homeostasis being controlled at the transcriptional and post-translational stages; is there epigenetic control of mitostasis too?

Line 294: Define LON

Line 298: This final sentence would be better situated nearer the beginning of the paragraph as it does not link well to the previous sentence

Line 302: “maternally inherited” **in humans**

Line 314: An example of a nuclear encoded protein, e.g. TFAM, may be useful here

Line 377: Adenosine is one word

Line 379: The ‘of’ following despite should be removed

Line 383: References: Korn, E. Cell membranes: structure and synthesis. Annu. Rev. Biochem. 38,

263–288 (1969). Comte, J., Maisterrena, B. & Gautheron, D.C. Lipid composition and protein

profiles of outer and inner membranes from pig heart mitochondria comparison

with microsomes. Biochim. Biophys. Acta 419, 271–284 (1976).

Line 387: Reference: Saks, V.A. et al. Permeabilized cell and skinned fiber techniques in studies of mitochondrial function in vivo. Mol. Cell. Biochem. 184, 81–100 (1998).

Line 402: An appropriate reference for the recovery ranges should be included

Line 404: An appropriate reference for this methodology should be included

Line 471: Figure 2 is a little difficult to read. This could be improved by limiting the number of arrows used, and (line 488) defining what colour the dashed arrows or (line 503) dotted arrows are to clarify descriptions.

Line 505: A clearer definition of H+pos and H+neg would be helpful, perhaps included in a caption for equation 1 (line 524)

Line 528: Define catabolic-ET pathway

Line 543: A short definition of dyscoupling at this first use should be include, or alternatively, a reference to its definition later in the text

Line 563: “transcription factor HIF1” seems grammatically incorrect, it would be more correct to say “transcription factors such as HIF1”

Line 569: An example of a hormone affecting mitochondria might be nice e.g. progesterone and glucacorticoids

Line 597-598: Replacing dashes with commas will make this sentence clearer

Line 611: Short circuit should be two words

Line 621: *(related to line 142)* the use of the words vectorial and scalar, without an obvious definition, may increase confusion, and don’t add much to the description of the states

Line 632: The use of the terminology “irreversibly lost” when talking about exergy is confusing given that early in the paragraph it is stated that “energy cannot be lost”; it may be clearer to just say “irreversibly dissipated” here

Line 638: An appropriate reference for the statement about cation cycling would be useful

Line 649: Figure 4: red. and ox. should be defined in the figure legend

Line 744: “Proton leak is a leak current of protons” does not add to the definition that is included the rest of the paragraph and therefore could be removed

Line 846: A mention about the fact that antimycin A can also lead to increased ROS production is pertinent (ref: Turrens et al. 1985; Cadenas et al. 1977)

Line 874: This paragraph doesn’t consider situations where E=P; for example, in the rat heart we find that addition of FCCP leads to inhibition and the apparent arrival of E<P

Line 902: This section is highlighting the flaws of existing terminology; might it be better to include this earlier in the article to help justify the need for new terminology

Line 1166: Is there a preference with using wet or dry weight, and how does this confound results in cases where there is possible tissue oedema?

Line 1248: The included quotation may not be necessary in this paragraph, as the point which it is relating to is made earlier on in the paper

Line 1327: If citrate synthase activity (CS) is modified acutely by a physiological challenge such as exercise, does this limit the use of CS as a marker?

Line 1475: Where possible the subpopulation of mitochondria utilised in studies should also be defined

Line 1502: Table 8 is useful summary of the terminology; its presence should be highlighted earlier in the paper.