

obtained from theoretical simulations of the electrostatic perturbations modulated in heme a and copper redox centers by positively charged  $\text{Ca}^{2+}$  and replacement of D477A. Energies of electrostatic interactions were calculated according to DelPhi Program. It should be emphasized that no effect has been found upon addition of  $\text{Ca}^{2+}$  or chelators to the WT COX in which  $\text{Ca}^{2+}$  is not removed by complexons. D477A COX was kindly provided by the Laboratory of Prof. M. Wikstrom from University of Helsinki.

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## **C2.8 The relationship between cellular oxygen consumption and cytochrome oxidase oxidation state**

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The aim of this study was to examine oxidation changes in Cytochrome Oxidase (CytOx) at physiological proton motive force ( $\Delta P$ ) and when  $\Delta P$  and oxygen consumption ( $\text{VO}_2$ ) were varied using oligomycin followed by an FCCP titration. Measurements were made on

RAW cells in a custom-built respirometer. Analysis of the CytOx spectral region revealed two components, one fitted well by the fully reduced minus oxidized spectrum of isolated CytOx and another with a peak at 601.3 nm. Within the framework of the neoclassical model, we assign these components to and respectively. Results are mean  $\pm$  SD ( $n=6$ ).

The cells contained  $33.8 \pm 1.8$  pmol of CytOx per  $2 \times 10^7$  cells, baseline  $\text{VO}_2$  was  $16.7 \pm 1.6$   $\text{O}_2/\text{CytOx/s}$  and were  $8.7 \pm 1.4\%$  and  $6.0 \pm 3.0$  occupied respectively. Under anoxic conditions, and were  $69.0 \pm 9.8$  and  $28.7 \pm 6.3\%$  occupied respectively when  $\Delta P$  was maintained by glycolytic ATP reversing the ATP synthase, and  $13.7 \pm 6.9$  and  $86.1 \pm 7.1\%$  occupied respectively when the membrane potential was collapsed with FCCP or oligomycin. At normal oxygenation,  $\text{VO}_2$  fell to  $4.8 \pm 4.2$   $\text{O}_2/\text{CytOx/s}$  after inhibition by oligomycin, increased to a maximum of  $38.2 \pm 4.2$   $\text{O}_2/\text{CytOx/s}$  with increasing FCCP and then declined upon further addition of FCCP. and occupancy increased linearly with  $\text{VO}_2$  from  $6.0 \pm 1.3\%$  to  $12.6 \pm 1.6\%$  and  $4.1 \pm 2.1\%$  to  $5.1 \pm 2.2\%$  occupied respectively as  $\text{VO}_2$  varied from minimum to maximum. These results are consistent with  $\text{VO}_2$  being regulated by  $\Delta P$  varying the entry of electrons into the binuclear center.

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