tion is offered for (a) the 1 e^-/2 H^+ stoichiometry of the overall reaction ($4\,c^{2+}+O_2+8\,H^+_{in}\rightarrow 2\,H_2O+4\,H^+_{out}$); (b) extraction of oxidative potential from O_2 in two 2-electron transfer steps, the second of which creates two transient oxidants (Fe(IV)=O and Tyr237.) as O_2^{2-} (bridging peroxide) is reduced to two O(−II)-atoms; (c) an overall "oxidative acidification" that leads to (d) the loss of a proton from a histidine residue that faces into an extensive water pool between subunits I and II − namely, we identify a common step for proton pumping that is coupled to redox chemistry.

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C2/4 Electrons, protons, and low-energy transitions in heme-copper oxidases

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Studies of electron, proton, and ligand transfer reactions in heme-copper oxidases by spectroscopic and kinetics measurements as a function of temperature reveal low-energy conformational transitions and their effects, both on active sites and distant structures. These transitions may in turn influence functional processes in the enzymes. For example, heme–heme electron transfer rates may be regulated *via* conformational control of electron transfer probability. Intraprotein proton transfers are observed that are "cryptic" in the sense that no kinetic isotope effects or pH dependences are observed — in other words, the rate determining steps for proton transfer are other than proton transfer itself. Limitations on proton transfer rates may constrain allowable proton transport mechanisms in the oxidases, thus mechanisms of proton-coupled electron transfer and proton translocation.

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C2/5 Evidence for the H-channel proton pump in bovine cytochrome *c* oxidase

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The H-channel, detectable in X-ray structure of bovine cytochrome *c* oxidase, is composed of a hydrogen bond network and a water channel in tandem. The former including Asp51 at one end near the positive side surface, Arg38 at the other end and a peptide bond in the middle is hydrogen-bonded with the formyl and propionate groups of heme *a*. The structure suggests that protons are taken up by Arg38 from water molecules (or hydronium ions) transferred through the water channel from the negative side space and that the proton-transfer from Arg38 to Asp51 are driven electrostatically upon oxidation of heme *a*, to release protons to the positive side space. The peptide bond included in the network provides unidirectional proton transfer. The redox-coupled conformational changes in Asp51 and the water channel in the X-ray structure are consistent with the proton pump function of H-pathway. All three mutations for the key amino acids in H-pathway, Asp51Asn, Ser441Pro (for blocking proton transfer through the peptide) and Val380Leu/Met390Trp (for blocking the water transfer) completely abolished proton pump without affecting O_2-reduction activity. A D-pathway mutation which decouples the proton pump of bacterial enzymes did not affect the proton pump of bovine cytochrome *c* oxidase. These results strongly suggest that proton pump of the bovine enzyme is driven by H-pathway, not by D-pathway.

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(C2) Controversial issues in cytochrome oxidase colloquium abstracts (poster and raised abstracts)

C2.6 Theoretical and computational analysis of the membrane potential generated by cytochrome *c* oxidase upon single electron injection into the enzyme and the identity of its proton pumping site

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We have developed theory and the computational scheme for the analysis of the kinetics of the membrane potential generated by cytochrome *c* oxidase upon single electron injection into the enzyme. The theory allows one to connect the charge motions inside the enzyme to the membrane potential observed in the experiments. The developed theory is applied for the analysis of the potentiometric data recently reported by the Wikström group (Belevich et al., PNAS 104, 2685, 2007) on the O to E transition in *Paracoccus* oxidase. Our analysis suggests, that the electron transfer to the binuclear center is coupled to a proton transfer (proton loading) to a group just "above" the binuclear center of the enzyme, from which the pumped proton is subsequently expelled by the chemical proton arriving to the binuclear center. The identity of the pump site could not be determined with certainty, but could be localized to the group of residues His326 (His291 in bovine), propionates of heme a_3, Arg 473, and Trp164. The analysis also suggests that the dielectric distance from Cu_A to Fe *a* is 0.4 or larger. The difficulties and pitfalls of quantitative interpretation of potentiometric data will be discussed.

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C2.7 Ca^{2+} binding to cytochrome *c* oxidase affects redox properties of heme a

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Subunit I of cytochrome *c* oxidase (COX) from mitochondria and many bacteria contains a cation binding site located near heme a and facing the P-phase. Mitochondrial COX binds reversibly Ca^{2+} or Na^+. In the bacterial oxidase of wild type (WT) the site is occupied by tightly bound Ca^{2+} but replacement of "critical" aspartate (D477 in *Paracoccus denitrificans* COX) converts Ca^{2+}binding to the reversible. Recently we have found that Ca^{2+} binding to bovine COX stabilized reduced state of heme a by rising its midpoint potential for 15 mV. Here we have studied the effect of Ca^{2+} on the D477A mutant COX from *P. denitrificans*. Redox titrations of heme a in cyanide complex of D477A COX carried out with Ca^{2+}-loaded (0.2 mM Ca^{2+}/0.1 mM EGTA)enzyme were compared to that with Ca^{2+} depleted (0.1 mM EGTA) enzyme. It was found that Ca^{2+} binding to D477A COX increased heme a midpoint potentials for about 40 mV. Roughly the same values have been

obtained from theoretical simulations of the electrostatic perturbations modulated in heme a and copper redox centers by positively charged Ca^{2+} and replacement of D477A. Energies of electrostatic interactions were calculated according to DelPhi Program. It should be empathized that no effect has been found upon addition of Ca^{2+} or chelators to the WT COX in which 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 (ΔP) and when ΔP and oxygen consumption (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 Cyta 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±SD (n=6).

The cells contained 33.8±1.8 pmol of CytOx per 2×10^7 cells, baseline VO_2 was 16.7±1.6 O_2/CytOx/s and were 8.7±1.4% and 6.0±3.0 occupied respectively. Under anoxic conditions, and were 69.0±9.8 and 28.7±6.3% occupied respectively when ΔP was maintained by glycolytic ATP reversing the ATP synthase, and 13.7±6.9 and 86.1±7.1% occupied respectively when the membrane potential was collapsed with FCCP or oligomycin. At normal oxygenation, VO_2 fell to 4.8±4.2O_2/CytOx/s after inhibition by oligomycin, increased to a maximum of 38.2±4.2O_2/CytOx/s with increasing FCCP and then declined upon further addition of FCCP. and occupancy increased linearly with VO_2 from 6.0±1.3% to 12.6±1.6% and 4.1±2.1% to 5.1±2.2% occupied respectively as VO_2 varied from minimum to maximum. These results are consistent with VO_2 being regulated by ΔP varying the entry of electrons into the binuclear center.

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