that alone justifies the huge cost of maintaining a small quasi-autonomous genetic system in the chloroplast.

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C1/4 Energy and informational fluxes in evolution — The key to complexity in the biosphere

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Two great fluxes control the biosphere, — energy and information. Evolution, behavior, cellular recognition and signaling, and human communication are examples of the latter. Shannon recognized that information transmission required two components, engineering aspects and the semantic content or 'meaning' of the message. This semantic component cannot be quantified within the same thermodynamic framework as bioenergetic aspects. The message needs a thermodynamic carrier, but the meaning has value only in context and after translation and interpretation. These characteristics lead to some interesting conclusions about the role of semantic transmission in the development of complexity through evolution, and in human culture. Translation is essential, both mechanistically, and because it allows an increase in combinatorial potential through dimensionality. Exploitation of combinatorial potential is constrained by the evolutionary heritage, so that the biosphere shares a highly restricted informational base, which increases in complexity with time. In humans, complexity is extended to the cultural domain. Genetic and cultural channels for inheritance have different components, but they share a common feature in their need for a semantic component, and matching translational machineries, and this justifies a Universal Darwinism. This leads to a view of the evolutionary process in terms of success in exploitation of thermodynamic potential through an exploration of the nature of reality; — a molecular epistemology encapsulated in the commonality of life.

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(C2) Controversial issues in cytochrome oxidase colloquium lecture abstracts

C2/1 Controversial issues and conformational changes in cytochrome *c* oxidase

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Despite high resolution structures and powerful spectral and computational methods for analysis of terminal oxidases, the molecular mechanism remains contentious. Areas at issue include the pathways and key residues involved in proton movement, the timing of proton uptake and release with respect to electron transfer, the role of water clusters as proton acceptors, regulatory mechanisms and the nature of catalytic intermediates. Another subject of conflicting views and data is whether significant conformational changes are involved in the coupling mechanism. Small, localized changes, or no changes in structure upon complete reduction have been reported previously. We have solved the structures of resting, reduced and re-oxidized forms of *Rhodobacter sphaeroides* *Cc*O at 2.0–2.2 Å. The reduced form shows little change in overall structure, but the entire porphyrin ring of heme a_3 and its hydroxyl-farnesyl tail are shifted 1–3 Å, opening the top of the K proton channel and impacting critical residues in the K path. Also in the K-channel region, a high occupancy binding site for the bile acid deoxycholate is resolved. Bile acids are strong inhibitors of the bovine *Cc*O and a binding site is found in the same location as in *Rs*CcO. The results suggest that redox state-induced conformational change, and a conserved steroid binding site, could regulate proton uptake in the K path. (NIH GM26916; MSU REF03-016).

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C2/2 Rapid kinetic studies of electron transfer in cytochrome oxidase

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The 1-electron reduction of pulsed, oxidized cytochrome oxidase was investigated using a new photoactive binuclear ruthenium complex, $[Ru(bipyrazine)_2]_2$(quaterpyridine), (Ru_2Z). The photoexcited state Ru(II*) of Ru_2Z is reduced by aniline to Ru(I), which then reduces Cu_A with yields up to 60%. The pulsed O_H state of cytochrome oxidase was prepared by a stopped-flow-flash technique. Mixing fully reduced anaerobic enzyme with oxygenated buffer containing Ru_2Z resulted in formation of the oxidized O_H state within 5 ms. Ru_2Z was then excited with a laser flash to inject 1 electron into Cu_A. Electron transfer from Cu_A to heme a occurred with a rate constant of 20,000 s^{-1} in the bovine oxidase, followed by electron transfer from heme a to Cu_B with rate constants of 750 s^{-1} and 110 s^{-1} and 63% completion. The extent of electron transfer from heme a to Cu_B was only 30% in the non-pulsed O form, indicated a significant difference between the pulsed O_H and non-pulsed O form of bovine oxidase. In contrast, pulsing did not have a significant effect on electron transfer in *Rhodobacter sphaeroides* cytochrome oxidase. The role of electrostatics in controlling electron transfer in cytochrome oxidase has also been explored.

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C2/3 A redox coupled proton pumping mechanism for the B-type cytochrome *c* oxidases: Density functional studies of the *ba*$_3$-oxidase from *Thermus thermophilus*

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The aim of our work is to derive a mechanism of proton pumping by the heme-copper oxidases. After a brief introduction to structural details of *ba*$_3$-oxidase concerning the oxygen-uptake channel, the oxygen-in to water-out event, and the nature of the active-site, Fe_{a3}–Cu_B pair, evidence will be presented to support only one proton uptake path from the inside, namely a modified K-path. The bulk of the talk will focus on Density Functional Theory calculations of an ~200-atom active-site model, and thermochemical deductions therefrom, that support a novel, fourteen-step, chemically-detailed, redox-coupled mechanism for proton-pumping by the B-type oxidases. An explana-

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