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The aim of this study was to estimate the membrane potential of isolated mitochondria on the single-particle level. We used a fluorescence correlation spectroscopy setup with a 532-nm laser to detect fluorescence signals of single TMRE-doped mitochondria in suspension. The brightness of the fluorescent particles increased after the addition of a respiratory substrate (succinate) in the presence of rotenone and decreased after the addition of an uncoupler (dinitrophenol). Thus, the fluorescence signals of the particles correlated well with membrane potential magnitudes under our experimental conditions. Using an empirical formula of Gaussian–Lorentzian distribution of the brightness in the confocal volume, we found the fluorescence intensity of a single energized mitochondrion passing through the center of the observation volume. Given the fluorescence intensity of a single TMRE molecule, we estimated the number of TMRE molecules bound to a single mitochondrial particle. The number of mitochondrial particles per mg of protein (1.5×10^9) determined from the statistical distribution of fluorescence intensities and the magnitude of the membrane potential (190 mV) estimated by the Nernst equation were consistent with values of these parameters measured previously by other techniques.

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(S15) bc_1 complexes symposium lecture abstracts

S15/1 The Q_O site semiquinone state in isolated cytochrome bc_1 (complex III) from *Rhodobacter capsulatus*

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The Q_o site semiquinone of cytochrome bc_1 has been assigned pivotal roles in productive energy-conversion and destructive superoxide generation. After a 30 year search for direct evidence of this semiquinone state, a transient, Q_o site inhibitor sensitive semiquinone EPR radical has been revealed in a genetic heme b_H knockout, which effectively improves the energetics for semiquinone formation at the Q_o site. This first observation was performed in native membranes of the purple photosynthetic bacterium *Rhodobacter capsulatus*. To remove possibilities that the signal was a result of either an unforeseen semiquinone state in another redox protein of the native membranes, or damage resulting from knockout of heme b_H, we have examined for the Q_o site semiquinone state in isolated and purified cytochrome bc_1 equipped with a full complement of cofactors. Combined in a hybrid system with reaction centers (*Rba. sphaeroides*; thanks to Colin Wraight, Urbana-Champagne, IL), ubiquinone and cytochrome c_2 (*Rba. capsulatus* thanks to John Fitch and Michael Cusanovitch, Tucson, Az), light activation generates an EPR signal in a manner similar to that seen in native membranes and fully consistent with its identity as a key state of the cytochrome bc_1.

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S15/2 Domain conformational switch of the iron sulfur protein in cytochrome bc_1 complex is induced by the electron transfer from cytochrome b_L to b_H

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Intensive biochemical, biophysical and structural studies of the cytochrome (cyt) bc_1 complex in the past have led to the formulation of the "protonmotive Q-cycle" mechanism for electron and proton transfer in this vitally important complex. The key step of this mechanism is the separation of electrons during the oxidation of a substrate quinol at the Q_P site with both electrons transferred simultaneously to ISP and cyt b_L when the extrinsic domain of ISP (ISP-ED) is located at the *b*-position. Pre-steady state fast kinetic analysis of bc_1 demonstrates that the reduced ISP-ED moves to the c_1-position to reduce cyt c_1 only after the reduced cyt b_L is oxidized by cyt b_H. Structural analyses of Pm or Pf inhibitor loaded crystals revealed two ISP-ED binding positions on cyt *b*. However, the question of how the conformational switch of ISP-ED is initiated remains unanswered. The results obtained from analysis of inhibitory efficacy and binding affinity of Pm and Pf inhibitors, under various redox states of the bc_1 complex, suggest that the electron transfer from heme b_L to b_H is the driving force for the releasing of the reduced ISP-ED from the *b* position to c_1 position to reduce cyt c_1.

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S15/3 Regulatory interactions in the dimeric cytochrome bc_1 complex

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The dimeric cytochrome bc_1 complex catalyzes oxidation-reduction of quinol and quinone at sites located in opposite sides of the membrane in which it resides. The kinetics of electron transfer and inhibitor binding in the isolated yeast and *Pseudomonas denitrificans* bc_1 complexes reveal functional interactions between the quinol oxidation site at center P and quinone reduction site at center N in opposite monomers in conjunction with inter-monomer electron equilibration between the cytochrome *b* subunits of the dimer. The resilience of center P catalysis to inhibition caused by partial pre-reduction of the b_H hemes can be explained by inter-monomer electron transfer between the two cytochrome *b* subunits in the bc_1 dimer. A model for the mechanism of the bc_1 complex has emerged in which binding of ligands that mimic semiquinone at center N regulates half-of-the-sites reactivity at center P and binding of ligands that mimic binding of ubiquinol at center P regulates half-of-the-sites reactivity at center N. An additional feature of this model is that inhibition of quinol oxidation at the quinone reduction site is avoided by allowing catalysis in only one monomer at a time, which maximizes the number of redox acceptor centers available in cytochrome *b* for electrons coming from quinol oxidation reactions at center P and minimizes the leakage of electrons that would result in the generation of damaging oxygen radicals.

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S15/4 The loneliness of the electrons in the bc_1 complex

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