Pathways of Trans-Membrane Electron Transfer in Cytochrome *bc* Complexes; Dielectric Heterogeneity and Inter-Heme Coulomb Interactions

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Supporting Information

The Supporting Information provides documentation underlying a new perspective, a mapping of internal dielectric constants between the transmembrane *b*-hemes in the dimeric hetero-oligomeric bacterial integral membrane protein, cytochrome bc_1 complex. In a framework describing the *b*-heme centers in the core of the cytochrome bc_1 complex, and electrophoretic and spectrophotometric characterization of the complex, the kinetics of *b*-heme reduction and concomitant formation of an excitonic CD spectrum were measured simultaneously. Simulations are presented of the time course of formation of the absorbance and excitonic circular dichroism changes arising from dithionite reduction of the hemes, which document preferential reduction of the n-side inter-monomer heme pair on the electro-negative side of the membrane.

Fig. S1:

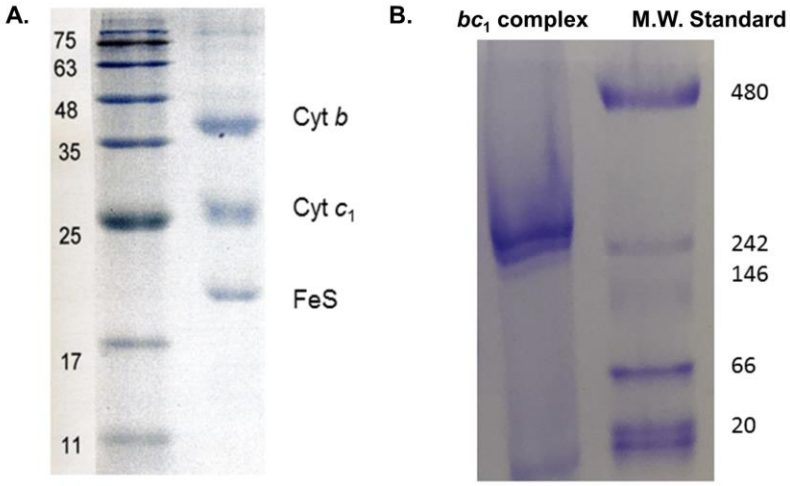

Fig.S1: (A) SDS-PAGE analysis and (B) Native gel analysis of the cytochrome bc_1 complex from *Rb.capsulatus*.

Fig. S2

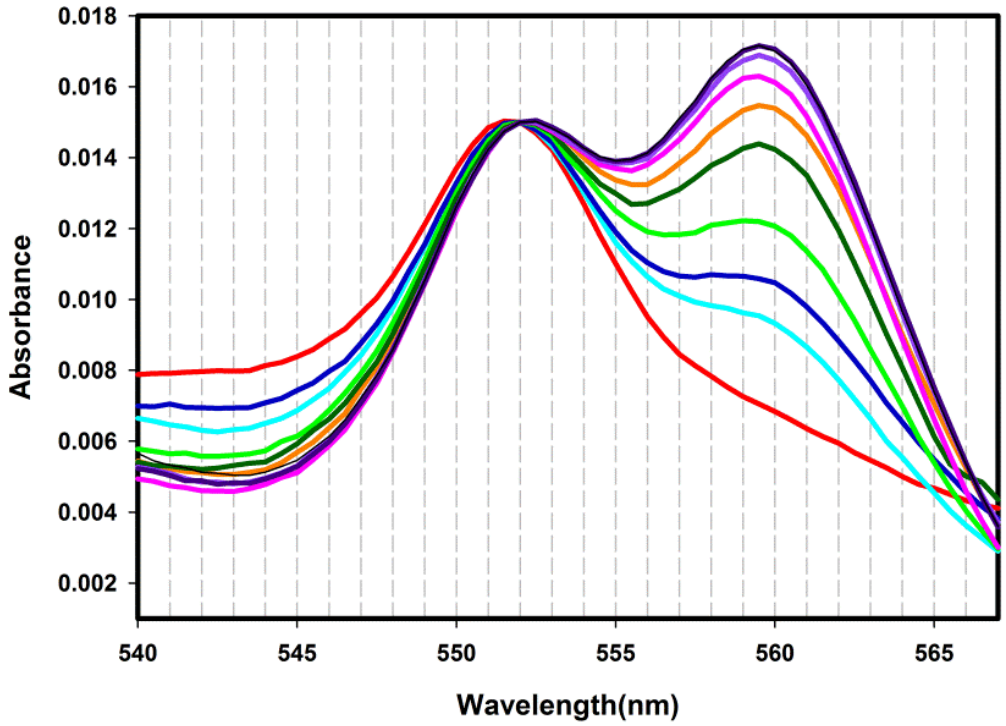

Fig S2. Reduced minus oxidized absorbance difference spectra of the *Rb. capsulatus* cytochrome bc_1 complex in 30 mM Tris-HCl, pH 7.5, 50 mM NaCl, 0.1mM EDTA, 0.04% DDM; α-band absorbance difference spectra, with maxima at 551 nm and 560 nm, respectively, initially oxidized by ferricyanide (FeCN, 30 µM), and subsequently reduced by sodium ascorbate (0.5 mM), yielding an absorbance difference spectrum (in red) with a peak at 551 nm. Subsequently, the sample was reduced with sodium dithionite (ca. 2 mM) yielding an absorbance maximum in the difference spectrum at 559 - 560 nm (green). Concentration of cytochrome complex, 30 µM. The difference spectra were taken at time points 12s (cyan), 48s (dark blue), 96s (light green),132s (dark green), 168s (orange), 216 s (pink), 276 s (purple), and 324s (black), after addition of dithionite.

S3. Simulation of kinetics of absorbance and circular dichroism changes resulting from addition of reductant.

Due to lack of knowledge regarding possible rate and other effects associated with efficiency of the protein reduction by the reductant, the proposed kinetic equation defines the simplest model that describes the reduction of the four heme cytochrome bc_1 complex. Presently, there is no conceptual basis for a more complicated model. Hence, first order linear kinetics have been chosen. It has been shown that the electron transfer in cytochrome-like complexes occurs on ultrafast-to-fast time scales[1, 2]. Hence each reduction state of the protein is in thermodynamic equilibrium. The kinetics of the reduction of the cytochrome bc_1 complex by a one electron donor are represented using the following scheme:

$$\boxed{N_0(t)} \xrightarrow[\bar{e}]{k} \boxed{N_1(t)} \xrightarrow[\bar{e}]{k} \boxed{N_2(t)} \xrightarrow[\bar{e}]{k} \boxed{N_3(t)} \xrightarrow[\bar{e}]{k} \boxed{N_4(t)} \qquad \text{(S3.1)}$$

where $N_i(t)$ corresponds to the population of the protein dimer complexes containing i electrons (i = 1…4), and k is the rate of electron transfer from the donor (dithionite) to the protein complex. The kinetics can be described with the system of first order differential equations:

$$\begin{cases} dN_0(t)/dt = -kN_0(t) \\ dN_1(t)/dt = kN_0(t) - kN_1(t) \\ dN_2(t)/dt = kN_1(t) - kN_2(t) \\ dN_3(t)/dt = kN_2(t) - kN_3(t) \\ dN_4(t)/dt = kN_3(t) \\ N_0(0) = 1, \quad N_1(0) = N_2(0) = N_3(0) = N_4(0) = 0 \end{cases} \qquad \text{(S3.2)}$$

The analytical solution of the system of equations, as it applies to the bc_1 complex, has been discussed previously.[3]

The kinetics of the CD signal for the two models ("*n-n"* and *"n-p*", Fig. 4A,B) are then expressed as:

$$CD_{"n-n"}(t) = d_{n1-n1}N_2(t) + (d_{n1-n1} + d_{n1-p1} + d_{n1-p2})N_3(t) + N_4(t) \qquad \text{(S3.3)}$$
$$CD_{"n-p"}(t) = d_{n1-n1}N_2(t) + (d_{n1-n1} + d_{n1-p1} + d_{n1-p2})N_3(t) + N_4(t)$$

The coefficients "d" represent the CD signal intensity originating from different reduced *b*-heme pairs within the bc_1 dimer, as denoted by the subscript (Table I).

The result of the simulations for k = 0.0053 s^{-1}, the rate constant of the slower component in the experimental data, was fit with the data shown in Fig. 3C. The CD signal is expected to be significantly delayed with respect to the absorbance signal in the "n-n" model, because it takes three electron transfer events to form the first reduced b_{n1}-b_{p1} pair that is the main source of the CD signal (Fig. 3C). Indeed, the OD reaches ~ 65% of its maximum value in 50 s, while the same increase in the CD signal occurs in ~200 s. The experimental profiles were fit (Fig. 3C) with the assumption that there are two pools of the complexes that differ only in the reduction rate.

Fig S3:

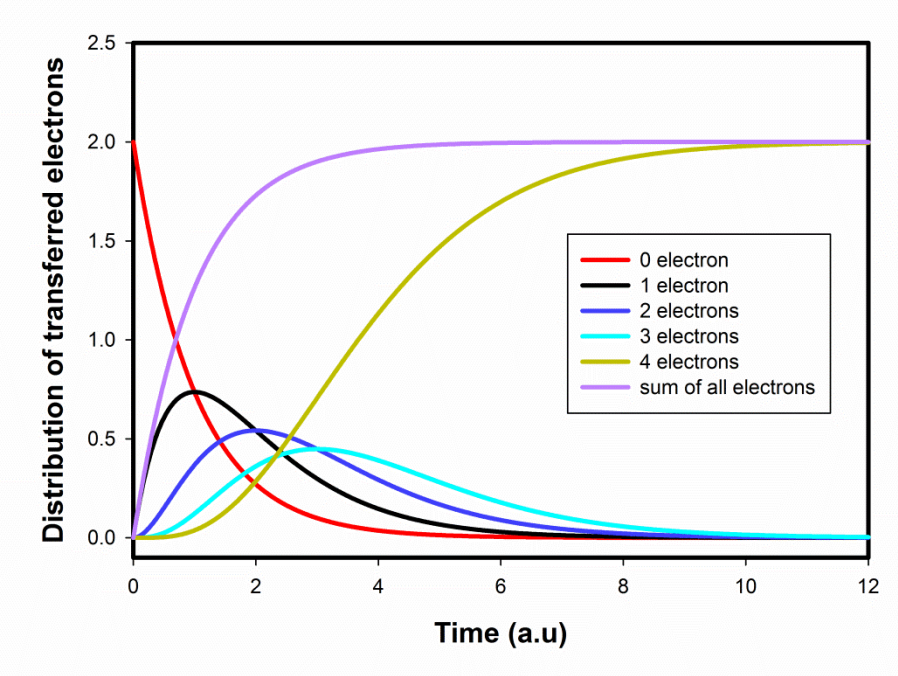

Fig. S3: Distribution of the populations corresponding to the transfer of 0,1,2,3,4 electrons to each monomer of the dimeric complex and a sum of all 4 states (purple).

Fig.S4

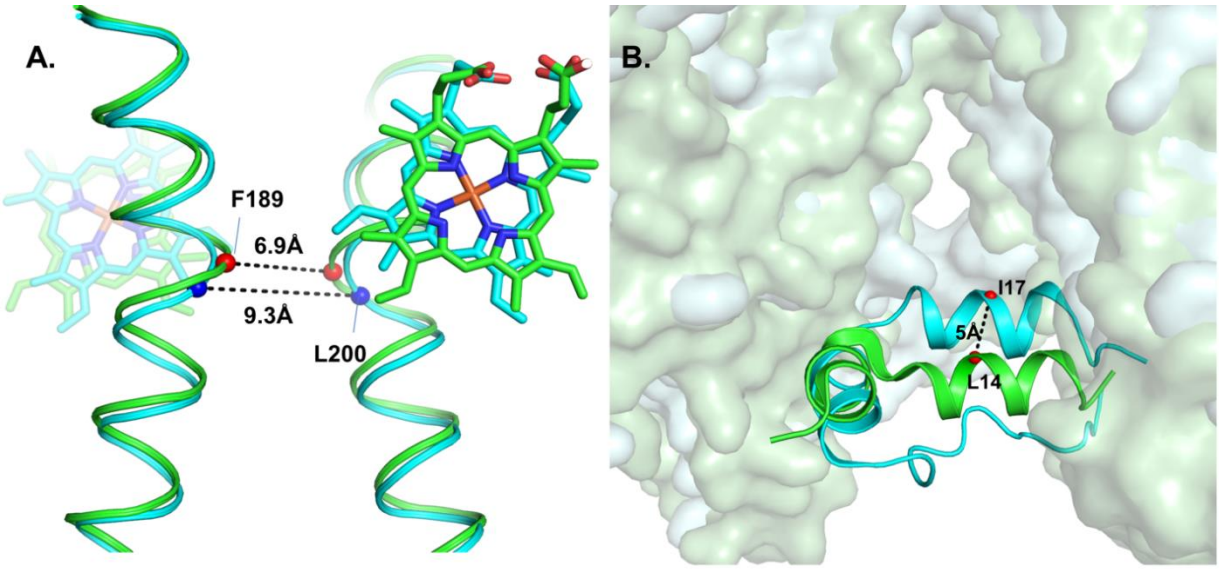

Fig. S4: (A) Structural alignment of D-helices lining the inter-monomer cavity in the cyt bc_1 (cyan,PDB 1ZRT) and b_6f complex (green, 4OGQ). The distance between F189 and L200 in the corresponding turn of the D-helix of the b_6f and bc_1 complexes, differs by 2.4Å, which indicates the extent by which the distance between the two monomers is diminished in b_6f compared to bc_1. (B) Cartoon structures show that the N- terminal n-side surface helix of b_6f (green), is displaced further from the n-side surface than the corresponding helix in bc_1 (cyan) by a distance of 5 Å, as indicated by the dotted line that bridges the two helices.

References

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