Supporting Information

The reorganization energy in cytochrome c is controlled by the accessibility of the heme to the solvent

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Materials and Methods

Electrochemical Measurements

A Potentiostat/Galvanostat mod. 273A (EG&G PAR, Oak Ridge, USA) was used to perform cyclic voltammetry (CV). Potentials were calibrated against the MV^{2+}/MV^{+} couple (MV = methylviologen).[1] All chemicals were of reagent grade. Nanopure water was used throughout. The working gold electrode was cleaned by flaming it in oxidizing conditions; afterwards, it was heated in concentrated KOH for 30 min then, after rinsing in water, in concentrated sulfuric acid for 30 min. To minimize residual adsorbed impurities, the electrode was subjected to 20 voltammetric cycles between +1.5 and -0.25 V at 0.1 V s^{-1} in 0.1 M H_2SO_4. Finally, the electrode was rinsed in water and anhydrous ethanol. The Vycor® set was treated in an ultrasonic pool for about 1 min. Protein solutions were freshly prepared before use in 10 mM phosphate buffer at pH 6 and their concentration (typically 0.2 mM) was spectrophotometrically checked with a mod. V-570 spectrophotometer (Jasco, Easton, USA). The formal reduction potentials $E^{0'}$ for the species were calculated from the average of the anodic and cathodic peak potentials[2] and found almost independent in the range of the scan rate used. For each species, the experiments were performed at least two times and the reduction potentials were found to be reproducible within ± 2 mV. k_s values were averaged over five measurements and found to be reproducible within 4% which was taken as the associate error. The CV experiments at different temperatures were carried out with a cell in a "nonisothermal" setting,[2] namely in which the reference electrode was kept at constant temperature (21 ± 0.1°C) whereas the half-cell containing the working electrode and the Vycor® junction to the reference electrode was under thermostatic control with a water bath. The nonisothermal behavior of the cell was carefully checked by determining the $\Delta H^{0'}_{rc}$ and $\Delta S^{0'}_{rc}$ values of the ferricyanide/ferrocyanide couple.[3,4]

Simulation Details

The GROMACS software package[5] was used to perform all the MD simulations. In the simulations twin range cut-off scheme (using cutoffs 1.0/1.0 nm and a pair list update frequency of once every 5 steps) including PME algorithm for long-range electrostatic interactions was used. The water was modeled as SPC (Simple Point Charge[6]). A time step of 2 fs was used. Bond lengths were constrained with LINCS algorithm.[7] The simulations were performed in the NPT ensemble using periodic boundary conditions. The pressure was weakly coupled[8] using an isotropic scheme in which the pressure equals 1 bar. The coupling time was 1 ps and compressibility $4.5 \cdot 10^{-5}$ bar^{-1}. The temperature was also weakly coupled with a Nosè-Hoover thermostat[9,10] (coupling time 0.1 ps) to T=300K.

Calculation of the outer reorganization energy λ_{out} To evaluate λ_{out} from the molecular dynamics simulations, the central quantity in our approach is the energy gap $\Delta E_{redox}(t)$ between the reduced and the oxidized protein state. $\Delta E_{redox}(t)$ is defined as:

$$\Delta E_{redox}(t) = E_{red}(t) - E_{ox}(t) \tag{1}$$

where $E_{red}(t)$ ($E_{ox}(t)$) is the total energy of the reduced (oxidized) system for a given instant t along the MD trajectory of either the reduced or the oxidized protein. Note that E_{red} and E_{ox} are calculated using the same atomic coordinates, i.e., that proper for the time t. They have been obtained in our calculations by changing the atomic charges from the reduced values to the oxidized ones. No other changes at the force field have been made. We calculated λ_{out} by using the following relation that connects it to the fluctuations of the energy gap $\Delta E_{redox}(t)$:

$$\lambda_{out} = \frac{1}{2kT} \langle (\Delta E_{redox} - \langle \Delta E_{redox} \rangle)^2 \rangle \tag{2}$$

This relation gave similar results to linear response methods for cytc.[11] In particular, we have calculated the energy gap $\Delta E_{redox}(t)$ each 0.5 ps along the trajectory of the reduced species, in the last 20 ns of each trajectory. Errors on λ_{out} has been estimated by the block averaging method applied to the $\langle(\Delta E_{redox} - \langle\Delta E_{redox}\rangle)^2\rangle$ function. λ_{out} calculated in this way corresponds to the hypothetical protein+solvent contribution for an ET to a distant redox partner.

Calculation of SASA Solvent Accessible Surface Area (SASA) has been calculated by using the Connely algorithm as implemented in the software VMD.[12] SASA for the entire protein was defined by a spherical probe with radius 1.4 Å, then only the contribution pertaining to the heme group was used to calculate SASA. This has been done for snapshots extracted every 0.5 ps within the last 20 ns of the molecular dynamics. The statistical error on this quantity was estimated by block averaging. We have also checked that the SASA trend did not change using the SASA definition implemented in the g_sas tool of GROMACS 4.5.3.

Electrochemical results

The cathodic and anodic peak separation varies between 60 and 110 mV over the range of the scan rate investigated. The peak current ratio $i_{anodic}/i_{cathodic}$ is approximatively 1 for all temperatures investigated and their intensity increase linearly with respect to $v^{1/2}$ (v=scan rate), which indicates that the electron transfer process is under diffusion control condition. The $E^{0'}$ values and the reduction thermodynamics for all the investigated species were reported by us in a previous work and are reported in Table 1 of the manuscript.[2] The diffusion coefficient was determined using the Randles-Sevcik equation plotting the peak current intensity versus $v^{1/2}$, the value obtained was equal to 5.15 ± 0.35 cm^2 s^{-1}. This value was used to determine the heterogeneous electron transfer constant k_s, as previously described by Nicholson,[13] for the electron exchange between the heme and the electrode.

Analysis of the solvent accessibility of heme: further data

In the main text we have reported an analysis of the solvent accessibility to the heme based on Solvent Accessible Surface Area (Fig. 4 main text). Since the MD simulations performed explicitly include water molecules, we have complemented that analysis by calculating the average number of water molecules within a given cut-off distance from the heme group. In particular, the average has been taken on the last 20 ns of each MD trajectory, coherently with SASA and λ_{out} calculations, and we have chosen a cut-off distance of 4 Å (but qualitatively similar results were found for 3, 5 and 6 Å). The experimental λ values plotted versus this calculated number of water molecules is shown in Figure 1:

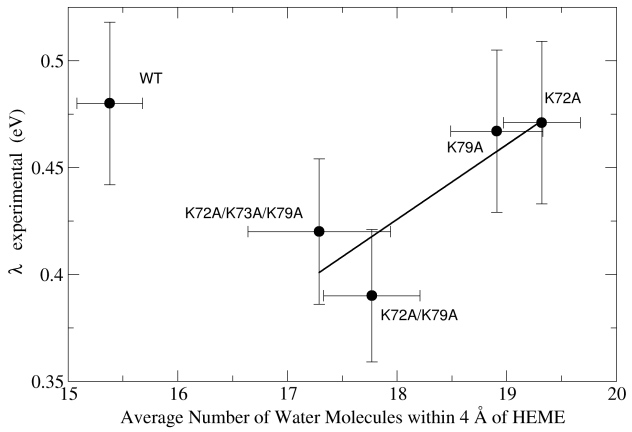

Figure 1: **Experimental λ vs. calculated average number of water molecules within 4 Å from the heme.**

It is apparent that the degree of correlation between λ and the actual solvent accessibility of the heme is very similar to what shown in the main text for λ and SASA, taking into account the statistical errors. The outlier is still the native cyt c, as for SASA. This correlation strengthens the conclusion that λ sensibly depends on the solvent accessibility of the heme.

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