Communication: Microsecond dynamics of the protein and water affect electron transfer in a bacterial bc_1 complex

Daniel R. Martin and Dmitry V. Matyushov^{a)}

Department of Physics and Department of Chemistry and Biochemistry, Arizona State University, P.O. Box 871504, Tempe, Arizona 85287, USA

(Received 1 March 2015; accepted 15 April 2015; published online 24 April 2015)

Cross-membrane electron transport between cofactors localized in proteins of mitochondrial respiration and bacterial photosynthesis is the source of all biological energy. The statistics and dynamics of nuclear fluctuations in these protein/membrane/water heterogeneous systems are critical for their energetic efficiency. The results of 13 μ s of atomistic molecular dynamics simulations of the membrane-bound bc_1 bacterial complex are analyzed here. The reaction is affected by a broad spectrum of nuclear modes, with the slowest dynamics in the range of time-scales ~ 0.1 – 1.6 μ s contributing half of the reaction reorganization energy. Two reorganization energies are required to describe protein electron transfer due to dynamical arrest of protein conformations on the observation window. This mechanistic distinction allows significant lowering of activation barriers for reactions in proteins. © 2015 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4919222>]

Tunneling of electrons between electronic states localized on cofactors within proteins is a fundamental process basic to the energy production by all living organisms. The negative charge is moved in a sequence of tunneling electron hops from the outer to the inner side of the membrane. Electrons appearing at the inner side are neutralized by protons in the solution. The resulting cross-membrane electrostatic potential and the gradient of the proton concentration drive the synthesis of adenosine triphosphate and are ultimately responsible for all energy produced by living cells.¹

The condition for electron tunneling is the resonance of the donor and acceptor electronic energy levels, i.e., zeroing out of the donor-acceptor energy gap X .² This variable becomes a natural choice for the reaction coordinate to monitor the progress of the electron-transfer reaction.^{3,4} Fluctuations of X are driven by thermal nuclear motions of a highly heterogeneous protein/water/bilipid interface, also influenced by ions dissolved in water and by the ionization state of the surface protein residues. A significant mechanistic question relevant to protein electron transfer is what are the interaction mechanisms influencing the breadth of the fluctuations (statistics) and what are the relevant time-scales (dynamics).^{4–6}

Both questions are essential for the modeling of the electron-transfer rates. Clearly, the amplitude of the fluctuations affects the reaction activation barrier.² On the other hand, many electron-transfer reactions can proceed on time-scales significantly shorter than some of the protein fluctuations. Ergodicity breaks down for such reactions and the relative magnitudes of the rate and the relaxation time(s) of the bath affect the observable activation barrier. The nonergodic kinetics describing such activated processes requires both the statistics and the dynamics of the bath fluctuations affecting X to calculate the reaction rate.⁶

With the long-standing goal of understanding the overall performance of biology's energy chains, we have performed simulations of the rate-determining electron-transfer step in the bc_1 complex of *Rhodobacter sphaeroides* bacterium (PDB:2QJP, Fig. 1(a)). bc_1 complexes are membrane proteins found in bacteria and mitochondria, and they are central to mitochondrial respiration and photosynthesis. The rate-limiting reaction that we study here is electron transfer from the quinol substrate (QH_2) at the Q_0 active site of the complex on the outer side of the membrane to the Fe_2S_2 active site of the Rieske iron-sulfur protein (ISP), Fig. 1(b).

The reaction mechanism and intermediates involved in the initial bifurcated electron transfer are still highly debated.^{7,8} In addition to QH_2 , the de-protonated quinol QH^- , which donates its proton to histidine-152 of the ISP (Fig. 1(b)), was suggested as the initial state from which electron is transferred to Fe_2S_2 .^{7,9} We have previously studied this configuration by molecular dynamics (MD) simulations but found the length of trajectories (~ 200 ns) insufficient to converge the parameters needed to calculate the rate.¹⁰ Here, we present new 13 μ s simulation data produced on the Anton supercomputer.¹¹ We find that μ s fluctuations significantly affect the parameters essential for the reaction activation barrier.

Motions of many particles of the thermal bath add up to produce fluctuations of X . The statistics of X is, therefore, to a good approximation Gaussian.^{2,12} This implies a quadratic free energy penalty $F_i(X)$ for producing a deviation $X - \langle X \rangle_i$ of the energy gap from its average value $\langle X \rangle_i$ (Fig. 2),

$$F_i(X) = \frac{(X - \langle X \rangle_i)^2}{2\beta\sigma_X^2} + F_{0i}. \quad (1)$$

The subscript $i = 1, 2$ here specifies the state of the donor-acceptor complex: the electron is on the donor when $i = 1$ and on the acceptor when $i = 2$. Further, $\beta = 1/(k_B T)$ and F_{0i} is the free energy at the minimum such that $\Delta F_0 = F_{02} - F_{01}$ is

^{a)}Electronic mail: dmitrym@asu.edu

FIG. 1. Structure of the membrane-bound *bc*₁ complex from *Rhodospirillum rubrum* (PDB:2QJP) (a) and the magnified view of the Q₀ active site (b). (a) coloring shows the protein (purple), 6 hemes (c₁, b_L, and b_H, yellow), the Fe₂S₂ cluster of the ISP (green), and two quinols (cyan). The 1-palmitoyl, 2-oleoyl-sn-glycero-3-phosphocholine (POPC) membrane is shown with the carbons colored gray, hydrogens white, oxygens red, nitrogens blue, and phosphorus atoms gold. (b) The QH⁻ cofactor (light blue) and the iron-sulfur center of the ISP (green). Also shown is histidine-152 of ISP protonated by QH₂ in the initial configuration.⁷

the reaction free energy (vertical distance between the minima in Fig. 2).

The variance of the energy-gap $\sigma_X^2 = \langle (X - \langle X \rangle_i)^2 \rangle$ in Eq. (1) is proportional to temperature T according to the fluctuation-dissipation theorem (FDT).¹³ The proportionality coefficient defines the reorganization energy of electron transfer $\lambda = \beta\sigma_X^2/2$. An alternative definition is based on the difference of average energies $\langle X \rangle_i$:⁶ $2\lambda^{\text{St}} = \langle X \rangle_1 - \langle X \rangle_2$ (horizontal distance between the minima in Fig. 2). $\langle X \rangle_i$ represent maxima of vertical spectroscopic transitions and their difference is typically associated with the spectroscopic Stokes shift (“St” superscript). In the notation used below, we will drop the subscript $i = 1$ for brevity when the forward electronic transition from QH⁻ to Fe₂S₂ is considered.

The standard model (SM) of electron transfer relies on the Gaussian approximation in Eq. (1) and, additionally, on the Gibbs ensemble to calculate the statistical averages. The latter assumption leads to the connection between the parabolas’ minima and ΔF_0 : $\langle X \rangle_i = \Delta F_0 \pm \lambda$, where plus and minus correspond to $i = 1$ and $i = 2$, respectively. In this case, $\lambda^{\text{St}} = \lambda$ and only one reorganization energy is required.² The equality of two reorganization energies is a special case of the FDT linking, in its static limit, the first and second moments of an observable.¹⁴

When some subset of the nuclear degrees of freedom becomes inaccessible on the reaction time-scale, the ergodicity breaks down and the Gibbs ensemble becomes limited to a constrained part of the phase space known as a component in glass science.¹⁴ The averages taken over such component do not satisfy the FDT. The connection between the first and second moments is lost and two reorganization energies, λ^{St} and λ , are required.⁶ One still has the condition of crossing of two parabolas in Eq. (1) at $X = 0$, which results in the relation $\langle X \rangle_i = \kappa_G \Delta F_0 \pm \lambda$, where the same convention as above applies to the $\pm$ sign and $\kappa_G = \lambda/\lambda^{\text{St}}$. From this relation, one can derive the activation barrier of the forward reaction

$$F^{\text{act}} = \frac{\langle X \rangle_1^2}{4\lambda} = \frac{(\lambda^{\text{St}} + (\lambda/\lambda^{\text{St}})\Delta F_0)^2}{4\lambda}. \quad (2)$$

Both the lowering of the activation barrier and the reduction of the free energy lost in an activationless reaction ($F^{\text{act}} = 0$) are achieved when $\lambda^{\text{St}} < \lambda$ and $\kappa_G > 1$. This

FIG. 2. Free energy surfaces of electron transfer (Eq. (1)). The solid lines in show $F_i(X)$ based on $\lambda = 3.99$ eV from Anton simulations, $\lambda^{\text{St}} = 1.46$ eV from separate MD runs of 200 ns long, and $\Delta F_0 = 0.15$ eV from experiment.⁹ The dashed lines are the result of the standard model² assuming $\lambda = \lambda^{\text{St}} = 1.46$ eV. The points show distributions calculated from 200 ns trajectory (green) and from 13 μ s (blue).

inequality was reported in previous simulations⁶ and is also found in this study, thus indicating ergodicity breaking. Ergodicity can be broken either continuously, when the reaction rate limits the phase space within each phase-space component (Eq. (4) below), or discontinuously, when some phase-space components become not reachable on the reaction time-scale. The condition found here is related to the latter scenario, in which different components correspond to distinct conformational states of the protein.⁶ The breakdown of the FDT does not specify whether the Gaussian width σ_X^2 in Eq. (1) should change with electron transfer; σ_X^2 and λ are the same for $i = 1$ and $i = 2$ in the standard model.² The same situation is empirically found in MD simulations of proteins⁶ (the subscript i is dropped from σ_X^2).

The free energy surfaces $F_i(X)$ (Eq. (1)) based on the parameters found from our MD simulations are shown by the solid lines in Fig. 2; the points in the plot show the distributions of X found in long (13 μ s, $F_1(X)$) and short (200 ns, $F_2(X)$) simulations. In contrast to the standard two-parameter model,² one needs three parameters to produce $F_i(X)$: λ^{St} , λ , and ΔF_0 . λ is provided by our Anton simulations (Table I) and $\Delta F_0 = 0.15$ eV is taken from experiment.⁹ In addition, since $\langle X \rangle_i$ converge much faster than λ , we have calculated $\lambda^{\text{St}} = 1.46$ eV ($\kappa_G = 2.7$) from two separate 200 ns trajectories. More detail on the simulation protocol is provided in the supplementary material,¹⁵ and we now turn to the mechanistic question of what are the nuclear modes and interactions affecting X .

The main contribution to the donor-acceptor energy gap is the Coulomb interactions of the charges of the donor

TABLE I. Contribution to the average energy gap $\langle X^{\text{C}} + X^{\text{ind}} \rangle$ and the reorganization energy, λ from components of the thermal bath (missing entries are too small to affect the results). The results refer to 13 μ s MD simulations of the forward, $1 \rightarrow 2$, reaction.

eV	All	Protein	Water	Lipid	Ions
$\langle X^{\text{C}} \rangle$	1.39	1.06	1.14	-0.64	-0.18
$\langle X^{\text{ind}} \rangle$	-0.50	-0.57	0.07	0.01	...
$\langle X^{\text{C}} + X^{\text{ind}} \rangle$	0.89	0.49	1.21	-0.63	-0.18
λ^{C}	2.56	2.16	3.57	0.52	1.59
λ^{ind}	0.32	0.17	0.14	...	...
λ	3.99	2.45	4.59	0.51	1.60

FIG. 3. Loss function of the energy gap, $\nu = \omega/(2\pi)$. The total loss spectrum is given by the solid black line and the blue and green shaded curves specify the water and protein contributions, respectively.

and acceptor with the atomic charges of the thermal bath.^{2,3} For protein electron transfer, the thermal bath includes protein residues, lipids, water molecules, and the ions of the electrolyte. Correspondingly, the Coulomb component of the energy gap is $X^C = \sum_j \Delta q_j \phi_j$, where the sum runs over the partial charges of the donor and acceptor atoms, which are altered by electron transfer to produce difference charges $\Delta q_j = q_j^{(2)} - q_j^{(1)}$ between the final (2) and initial (1) states. Further, ϕ_j is the instantaneous electrostatic potential of the thermal bath at site j of the donor-acceptor complex.

While Coulomb interactions are by far strongest intermolecular interactions, they are also significantly compensated between opposite charges. In contrast, interactions with the induced electronic dipoles are additive and often contribute to X comparably to X^C .¹⁶ The solvent-induced part of X is then the sum of the Coulomb and induction components, $X^C + X^{\text{ind}}$, with averages comparable in magnitude (Table I). The induction shift from simulations is also consistent with a simple estimate assuming uniform distribution of polarizability in a medium characterized by the refractive index n . In case of the charge-shift reaction considered here, $X^{\text{ind}} = -(3e^2/2)(n^2 - 1)/(n^2 + 2)(R_A^{-1} - R_D^{-1})$, where $R_{D,A}$ are the donor and acceptor effective radii, respectively (see supplementary material¹⁵).

In contrast to $\langle X \rangle$, λ is not strongly affected by induction forces and is mostly produced by fluctuations of the Coulomb interactions. The reason is that the density fluctuations required for the fluctuation of induction interactions are significantly constrained in the dense protein/lipid/water medium. On the contrary, electrostatic fluctuations are mostly caused by orientational motions of the medium multipoles, which are less hindered. We also note that λ^C and λ^{ind} do not add up to yield λ because of the induction-Coulomb cross term in the variance σ_X^2 (see supplementary material¹⁵).

The dynamics of the donor-acceptor energy gap are represented by the Stokes-shift correlation function $C_X(t) = \langle \delta X(t) \delta X(0) \rangle$, where $\delta X(t) = X(t) - \langle X \rangle$. Its frequency Fourier transform $\tilde{C}_X(\omega)$ yields the loss function:⁶ $\chi''_X(\omega) = (\beta\omega/2) \tilde{C}_X(\omega)$ (Fig. 3). The peaks of $\chi''_X(\omega)$ provide frequencies of the nuclear modes affecting the energy gap and their heights represent relative contributions to $C_X(t)$ and λ . For the latter, the low-frequency relaxation components are enhanced by the ω^{-1} factor in the frequency integral producing the reorganization energy,

$$\lambda = \int_0^\infty \chi''_X(\omega) (d\omega/\pi\omega). \quad (3)$$

FIG. 4. The reorganization energy of electron transfer depending on the observation window allowed by the reaction rate constant k_{ET} (Eq. (4)).

Figure 3 presents the main novel result of this study. It shows the loss spectrum $\chi''_X(\omega)$ obtained from 13 μ s of MD simulations. With this simulation length, we were able to access a new window of time-scales and nuclear modes affecting the dynamics and statistics of electron transfer. The time correlation function $C_X(t)$ fits well to four exponential decay functions $C_X(t)/C_X(0) = \sum_{m=1}^4 A_m \exp[-t/\tau_m]$ (Table S3 in supplementary material¹⁵). We find a surprisingly long component in the decay with the relaxation time of $\approx 1.6 \mu$ s. It contributes 22% to $C_X(t)$ and λ . The next slowest component of ≈ 100 ns contributes 32%. The very slow relaxation of water in Fig. 3 might look surprising. The slow relaxation of water is due to its strong coupling to slow parts of the system. The slowest water peak roughly matches protein's peak while the second slowest peak matches the relaxation of the electrolyte (Fig. S1 in supplementary material¹⁵).

The existence of slow nuclear modes affecting electron transfer implies ergodicity breaking for reactions faster than those modes.⁶ While electron transfer in bc_1 , $\tau_{ET} = k_{ET}^{-1} \approx 1$ ms, is slower than all the modes sampled by simulations, the issue of dynamical freezing of the thermal bath on the reaction time-scale is of fundamental importance: this mechanism allows dynamical control of enzymatic reactions by affecting the reaction barrier through the medium dynamics. We therefore present in Fig. 4 the dependence of the reorganization energy on the “observation window” setup by the reaction time-scale τ_{ET} (see also the distributions shown in Fig. 2, which produce different curvatures on different simulation time-scales). By limiting the integration range by the rate constant k_{ET} from below in Eq. (3), one can apply the multi-exponential fit of the Stokes-shift dynamics to produce the non-ergodic reorganization energy⁶

$$\lambda(k_{ET}) = \lambda \sum_m (2A_m/\pi) \text{arccot}(k_{ET}\tau_m), \quad (4)$$

where $\sum_m A_m = 1$. A nearly continuous increase of $\lambda(k_{ET})$ with slowing the reaction illustrates that the activation barrier can be efficiently controlled by adjusting the rate to the bath dynamics.

The rate constant of (non-adiabatic²) electron transfer is given by the Arrhenius equation, which is derived by averaging the Golden-Rule tunneling probability over the nuclear configurations of the thermal bath. The equation for the rate, $k_{ET} \propto V^2 \exp[-\beta F^{\text{act}}]$, is proportional to the electron-transfer coupling V and has the activation free energy F^{act} taken from Eq. (2).

FIG. 5. Change of the electron transfer rate relative to the WT as a function of the reaction free energy. Each point corresponds to a mutant of *Rb. sphaeroides* as reported in Ref. 9: S154C (1), WT (2), S154 T (3), Y156F (4), Y156W (5), and S154A (6). The dashed line shows the result of the standard model (SM) assuming $\lambda^{\text{St}} = \lambda = 1.46$ eV.

The parameter V in the expression for the rate is unknown for most but a few biological reactions.⁵ A phenomenological estimate of this parameter suggested by Dutton and co-workers¹⁷ leads to the rate constant estimate of $k_{\text{ET}} \approx 3 \times 10^5 \text{ s}^{-1}$ at the donor-acceptor distance of 9.7 Å (see supplementary material¹⁵). This value is higher than the experimental rate, $\approx 10^3 \text{ s}^{-1}$, and is required to be higher to account for the equilibrium fractions of QH_2 and QH^- forms unknown to us. However, the main difficulty in explaining experimental data⁹ was too low rates given by the standard model.^{2,17} This is clearly seen in Fig. 2 where the activation barrier is indeed much higher for the standard model. An additional insight can be gained from the energy gap law,² i.e., to the dependence of the rate on the reaction free energy ΔF_0 experimentally achieved by mutagenesis.⁹

These data are shown in Fig. 5 where the rates of electron transfer k_{ET} in different mutants relative to the rate in the wild type (WT) protein k_{WT} are plotted against experimental ΔF_0 . The relative rate $\log(k_{\text{ET}}/k_{\text{WT}})$ eliminates the unknown V from the rate expression. The plot shows that the use of λ^{St} and λ directly from simulations produces the energy gap law generally consistent with observations. However, these types of plots have little sensitivity to the details of the model. The dashed line in Fig. 5 refers the standard model ($\lambda^{\text{St}} = \lambda$), which follows the experimental points even better while predicting 5×10^3 slower absolute rates.

The analysis presented here supports the notion that two distinct reorganization energies $\lambda^{\text{St}} < \lambda$ are required to

characterize protein electron transfer. The distinction between them arises from a nearly universal ergodicity breaking, affecting protein electron transfer when some subsets of degrees of freedom are dynamically frozen on the reaction time-scale (protein conformations here). We find here a dramatic evidence of this general rule suggesting that even μs dynamics, never observed in simulations of electron transfer before, significantly contribute to the reorganization energy and the activation barrier of electronic transitions within proteins.

This research was supported by the National Science Foundation (Nos. MCB-1157788 and CHE-1213288). CPU time was provided through XSEDE (No. TG-MCB080116N). Anton computer time was provided through Grant No. P41GM103712-S1 from the National Institutes of Health and the Pittsburgh Supercomputing Center (No. PSCA13051P). The authors are grateful to David LeBard for his help with the system setup.

¹D. G. Nicholls and S. J. Ferguson, *Bioenergetics 3* (Academic Press, London, 2002).

²R. A. Marcus, *Rev. Mod. Phys.* **65**, 599 (1993).

³A. Warshel, *J. Phys. Chem.* **86**, 2218 (1982).

⁴A. Warshel and W. W. Parson, *Annu. Rev. Phys. Chem.* **42**, 279 (1991).

⁵S. S. Skourtis, D. H. Waldeck, and D. N. Beratan, *Annu. Rev. Phys. Chem.* **61**, 461 (2010).

⁶D. V. Matyushov, *J. Chem. Phys.* **139**, 025102 (2013).

⁷D. Victoria, R. Burton, and A. R. Crofts, *Biochim. Biophys. Acta, Bioenerg.* **1827**, 365 (2013).

⁸A. M. Barragan, A. R. Crofts, K. Schulten, and I. A. Solov'yov, *J. Phys. Chem. B* **119**, 433 (2015).

⁹S. Lhee, D. Kolling, S. Nair, S. Dikanov, and A. Crofts, *J. Biol. Chem.* **285**, 9233 (2010).

¹⁰D. R. Martin, D. N. LeBard, and D. V. Matyushov, *J. Phys. Chem. Lett.* **4**, 3602 (2013).

¹¹J. L. Klepeis, K. Lindorff-Larsen, R. O. Dror, and D. E. Shaw, *Curr. Opin. Struct. Biol.* **19**, 120 (2009).

¹²R. A. Kuharski, J. S. Bader, D. Chandler, M. Sprik, M. L. Klein, and R. W. Impey, *J. Chem. Phys.* **89**, 3248 (1988).

¹³R. Kubo, *Rep. Prog. Phys.* **29**, 255 (1966).

¹⁴A. Crisanti and F. Ritort, *J. Phys. A: Math. Gen.* **36**, R181 (2003).

¹⁵See supplementary material at <http://dx.doi.org/10.1063/1.4919222> for details of the simulation protocol.

¹⁶D. N. LeBard and D. V. Matyushov, *J. Phys. Chem. B* **113**, 12424 (2009).

¹⁷C. C. Moser, J. M. Keske, K. Warncke, R. S. Farid, and P. L. Dutton, *Nature* **355**, 796 (1992).