Published in final edited form as:

J Phys Chem B. 2021 July 22; 125(28): 7763–7773. doi:10.1021/acs.jpcc.1c05064.

Why Do Most Aromatics Fail to Support Hole Hopping in the Cytochrome *c* Peroxidase - Cytochrome *c* Complex?

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Abstract

Electron transport through aromatic species (especially tryptophan and tyrosine) plays a central role in water splitting, redox signaling, oxidative damage protection, and bioenergetics. The cytochrome *c* peroxidase (CcP) - cytochrome *c* (Cc) complex (CcP:Cc) is used widely to study inter-protein electron transfer (ET) mechanisms. Tryptophan 191 (Trp191) of CcP supports hole hopping charge recombination in the CcP:Cc complex. Experimental studies find that when Trp191 is substituted by tyrosine, phenylalanine, or redox-active aniline derivatives bound in the W191G cavity, enzymatic activity and charge recombination rates both decrease. Theoretical analysis of these CcP:Cc complexes finds that the ET kinetics depend strongly on the chemistry of the modified Trp site. The computed electronic couplings in the W191F and W191G species are orders of magnitude smaller than in the native protein, due largely to the absence of a hopping intermediate and the large tunneling distance. Small molecules bound in the W191G cavity are weakly coupled electronically to the Cc heme, and the structural disorder of the guest molecule in the binding pocket may contribute further to the lack of enzymatic activity. The couplings in W191Y are not substantially weakened compared to the native species, but the redox potential difference for tyrosine vs. tryptophan oxidation accounts for the slower rate in the Tyr mutant. Thus, theoretical analysis explains why only the native Trp supports rapid hole hopping in the CcP:Cc complex. Favorable free energies and electronic couplings are essential for establishing an efficient hole hopping relay in this protein-protein complex.

TOC Graphic

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Supporting Information Available

Details of derivation of eq. 8, MD simulations equilibrium validation, ZINDO/S calculations, localization metric threshold test, squared electronic coupling convergence, and ET parameter calculations are given in the supporting information.

Introduction

Charge hopping through aromatic amino acids occurs in many biological redox systems, including photosystem II, ribonucleotide reductase, and photolyase.^{1–9} DNA charge transport, oxidative damage protection, redox signaling, and bioenergetic redox chains are also believed to exploit hole hopping through aromatic species.^{10–15} A challenge faced in describing multi-protein electron transfer structures is to understand the combination of factors that contribute to the kinetics. The time scales for molecular recognition and docking, conformational change, tunneling, and multi-step hopping can all influence what is observed experimentally.^{14,16–18} Rates may be limited by familiar electron transfer factors like activation free energies and tunneling distances, or by protein conformational change.^{19,20} Potential transitions between single-step tunneling and multi-step hopping, as well as transitions from activation control to conformational gating control can lead to controversies in interpreting experimental kinetic data. The interplay among the many factors that may underpin biological electron transfer kinetics makes these reactions rich and diverse.^{13,21–23}

Redox active tryptophan (Trp) and tyrosine (Tyr) are studied widely as hole hopping mediators.^{24–29} The yeast cytochrome *c* peroxidase (CcP) - cytochrome *c* (Cc) complex (CcP:Cc) is an important test bed to study inter-protein ET.^{30–36} The complex also enables exploration of heme-oxygen chemistry, and allows the examination of structural and energetic constraints on hole hopping transport. Zinc-porphyrin (ZnP) modified CcP:Cc structures enable the examination of photo-induced ET in the CcP:Cc complex.^{37–41} The photocycle for ET in ZnPCcP:Cc is shown schematically in Figure 1. ZnP is photo-excited and relaxes to its triplet excited state (³ZnP) on the nanosecond time scale. The triplet excited state reduces Cc, with the rate k_f . Back ET then regenerates the ground state ZnP species, and the recombination is believed to involve hole hopping via W191 of CcP, that is, $k_w \gg k_b$ (see Fig. 1).^{42–45}

Possible conformational gating of the CcP:Cc ET kinetics is of great interest.^{37,38} In order to understand how structure and dynamics may influence CcP:Cc ET across the protein-protein interface, site-directed mutagenesis studies have been done on interfacial residue (Phe82) of cytochrome *c* to change the structure of the complex, and they found that the charge recombination rate (from Cc to CcP) is insensitive to donor-acceptor distance changes caused by the mutations.⁴⁴ Theoretical analysis found that an interplay among changes in the reaction free energy, reorganization energy, and electronic couplings of the

protein complexes compensate, producing little net change in the observed rates.⁴² In the ZnPCcP:Cc complex, as in the native CcP:Cc complex, charge recombination between ZnP^+ and Cc(II) is mediated by Trp191, which is believed to serve as a hole hopping intermediate.^{42–45} Many experimental studies demonstrate that the protein environment influences the stability of the CcP Trp191 cation radical.^{46–50} These studies have found that the Trp191 radical stability depends on i) a hydrogen bonding network formed by Trp191, Asp235, and the proximal heme ligand His175^{46,47,51} and ii) the electronegative character to the binding pocket, influenced in part by the Met residues.^{47–49} Ascorbate peroxidase (APX) contains a Trp residue analogous to Trp191; however, APX Compound I forms a porphyrin rather than a Trp radical, owing to a closely situated K^+ binding site.⁵² Incorporation of a cation binding site into yeast CcP indeed destabilizes the Trp radical,⁵³ whereas incorporation of Met residues into APX stabilizes a Trp radical.⁴⁸ Leishmania CcP (LmP) shares with APX a neighboring site for binding a monovalent cation K^+ , yet still forms a Trp radical like yeast CcP.^{54,55} In LmP, local Met residues and an additional Cys residue appear to provide the electronegative character necessary to stabilize the Trp radical.⁵⁴

In order to examine the possibility of using exogenous aromatic compounds to support peroxidase activity and to understand the influence of hopping intermediate on the ET mechanism, Payne et al. mutated CcP Trp191 to tyrosine (W191Y), phenylalanine (W191F), and glycine (W191G), and they investigated the ET kinetics of the resulting ZnPCcP:Cc complexes. The W191G mutant can bind small aromatic species in the cavity produced by the mutation.^{56–58} Indeed, aromatic species with redox potentials similar to tryptophan were found to bind in this cavity (see Figure 2).⁵⁹ It is shown that only the native CcP:Cc complex supports peroxidase activity, and only the native ZnPCcP:Cc system supports rapid charge recombination compared to all of the mutant species.⁵⁹ These experimental studies indicate that redox-active aromatic species with similar redox-potentials, placed at the same site in a protein, can play very different roles in biological ET.

In the Zn substituted native ZnPCcP:Cc complex, when Cc(III) oxidizes 3ZnP , the produced CcP radical product equilibrates between its ZnP^+ and W^+ forms, with an equilibrium constant (K_{eq}) that depends on the free energy difference ΔG_{eq}° between these two radical cation species ($[ZnP^0W^{\cdot+}]$ and $[ZnP^{\cdot+}W^0]$),

$$K_{eq} = \frac{k_f^{eq}}{k_r^{eq}} = \frac{[ZnP^0W^{\cdot+}]}{[ZnP^{\cdot+}W^0]} = e^{-\Delta G_{eq}^\circ/kT} \quad (1)$$

Charge recombination from Cc(II) to ZnP^+ could conceivably occur with or without the involvement of an oxidized Trp191,

If the electron exchange reaction between ZnP^+ and W^0 is much faster than the charge recombination (i.e., $k_f^{eq}, k_r^{eq} \gg k_{eb}^{indirect}, k_{eb}^{direct}$), the observed charge recombination reaction is dominated by charge recombination between W^+ and Cc(II) (eq. 2a), with the rate constant k_{obs} being the rate constant for the indirect hole transfer to W^+ ($k_{eb}^{indirect}$), effectively weighted by the equilibrium constant K_{eq} between the ZnP^+ and W^+ species when $K_{eq} \ll 1$ in the CcP:Cc complex (see eq. 3). ET rate measurements from early studies support a fast ZnP^+ to W^0 rate regime. ET from Cc(II) to W^+ is much faster ($k_{eb}^{indirect} = 2 \times 10^6 \text{ s}^{-1}$)⁶⁰ than direct ET to ZnP^+ ($k_{eb}^{direct} \sim 10^1 - 10^2 \text{ s}^{-1}$).⁴³

$$k_{obs} = k_{eb}^{indirect} K_{eq} \quad (3)$$

Note that eq. 3 holds for $K_{eq} < 1$. If the radical cation does not localize on the 191 residue of CcP , the observed charge recombination rate will be limited by direct transfer to ZnP:CcP (k_{eb}^{direct}) (see eq. 2b)

$$k_{obs} = k_{eb}^{direct} \quad (4)$$

Payne et al. found that for the wild type ZnP:CcP:Cc complex and the W191Y mutant, radical cation population is observed on tryptophan and tyrosine at site 191, indicating that the charge recombination reaction follows the mechanism in eq. 2a. In contrast, in the W191F and W191G mutants, the radical cation does not localize on phenylalanine and glycine at site 191, indicating that the redox potentials of phenylalanine and glycine are unfavorable for charge hopping, so the charge recombination reaction follows the mechanism in eq. 2b.

Understanding how different aromatic species influence biological ET is expected to shed light on the control of biological ET processes.

We used theoretical methods to explore why only the native Trp191 species supports rapid charge recombination in the CcP:Cc complex. Our analysis found that electron transfer pathways, electronic couplings, and reaction free energies conspire to enable Trp191 to serve as the most effective hole hopping recombination mediator, among the derivatives studied, in the CcP:Cc complex. Our computational analysis focuses on gaining mechanistic insight in the charge recombination reaction in the CcP:Cc complex, and we are interested in the trends of our quantitative analysis, not exact numbers.

Methods

We performed classical molecular dynamics (MD) simulations to sample structures of the Zn-PCcP:Cc complexes (see SI section S2 for further details). Ten *ns* of MD simulations were carried out for each protein complex, and the 2000 snapshots in the last 4 *ns* at equilibrium states were used for further ET pathway analysis and ET parameter calculations.

The charge recombination reaction rate is described using nonadiabatic ET theory with the Marcus high temperature approximation for the Franck-Condon factor

$$k_{et} = \frac{2\pi}{\hbar} |H_{DA}|^2 \frac{1}{\sqrt{4\pi\lambda kT}} e^{-\frac{(\Delta G^\circ + \lambda)^2}{4\lambda kT}} \quad (5)$$

The key ET parameters are the donor-acceptor electronic coupling (H_{DA}), the reorganization energy (λ), and the reaction free energy (ΔG°). ΔG° is estimated using the site energy (HOMO or highest occupied molecular orbital energy) gap for the donor-acceptor pair. The HOMO energy is obtained using density functional theory with the B3LYP functional and the 6-31++G** basis set (see SI section S3 for details). Below, we examine the different charge recombination reaction regimes (eq. 2a and eq. 2b) for the native ZnPCcP:Cc protein and its mutants, and we compute the corresponding ET parameters.

Tunneling pathway analysis

Electron transfer mechanisms in biological systems have been studied with a range of methods.^{45,63–66} We used Pathway analysis⁶⁷ to estimate the relative ET couplings and to determine the dominant ET pathway in the ZnPCcP:Cc complexes. The Pathways model estimates the relative couplings with the product of decay factors for the strongest coupling path (see eq. 6), where ϵ^C , ϵ^H , ϵ^S are tunneling decay parameters for covalently bonded, hydrogen bonded, and through-space contacts, respectively. The details of the Pathway analysis can be found in SI section S3.

$$H_{DA} \propto \prod_i \epsilon_i^C \prod_j \epsilon_j^H \prod_k \epsilon_k^S \quad (6)$$

2000 snapshots extracted from MD simulations were used to analyze the ET coupling pathways between the donor and acceptor for various ZnPCcP:Cc complexes. In the Pathways analysis, we use the Fe atom in the Cc heme as the electron donor (hole acceptor) for all ZnPCcP:Cc complexes. For the W191F and W191G mutants, experiments showed that the radical cation can only localize on the CcP ZnP,⁵⁹ indicating that the charge recombination reaction occurs following the mechanism of eq. 2b, and we use aromatic heavy atoms in the CcP ZnP as the electron acceptor (hole donor). For the native protein and the other mutants, the radical cation can localize on the CcP residue 191, so the charge recombination reaction occurs as indicated in eq. 2a, and we use the aromatic heavy atoms of CcP residue 191 as the electron acceptor (hole donor).

For every MD snapshot, the Pathways analysis ranks the relative donor-acceptor coupling strengths of the strongest tunneling pathways, and we selected the strongest 10 coupling pathways to define the residues that mediate charge recombination, with every coupling pathway coarse-grained by residue. For every ZnPCcP:Cc structure using 2000 MD snapshots, we analyzed the occurrence frequency of every coupling pathway coarse-grained by residue, and the coupling path with the highest occurrence frequency is defined as the strongest coupling pathway. The residues in the strongest coupling pathway are included in subsequent ZINDO/GMH analysis to characterize the molecular orbitals involved in the charge recombination reaction and to compute mean-squared electronic couplings to estimate charge recombination rates for each protein complex.

Electronic coupling analysis with ZINDO/GMH methods

We use the semiempirical quantum mechanical ZINDO/S method, together with generalized Mulliken-Hush (GMH) analysis, to estimate the donor-acceptor electronic coupling (see Figure 3).^{68,69} The semiempirical ZINDO/S method is used extensively to estimate the relative electronic couplings in biological donor-acceptor species, and has proven to be qualitatively accurate.^{70–74} More extensive electronic structure analysis could be used to provide more quantitative estimates of each coupling value, but calculations of that kind are well beyond the intended scope of our study.

In the one-electron approximation, the electron (hole) transfer interaction can be approximated by the interaction between the HOMO orbitals of the donor and acceptor species. The frontier molecular orbitals (MOs) were assigned to donor or acceptor, based on the magnitudes of the wave function coefficients localized on the donor or acceptor fragments. (see SI section S4 for details)

For each pair of donor and acceptor orbitals (e.g., $i \in D$ and $j \in A$), the two-state generalized Mulliken-Hush (GMH) method was used to calculate the electronic coupling H_{ij} (see eq. 7),

$$H_{ij} = \frac{\mu_{ij} \Delta E_{ij}}{\sqrt{(\mu_i - \mu_j)^2 + 4\mu_{ij}^2}} \quad (7)$$

E_{ij} is the energy difference between donor and acceptor MOs, μ_i and μ_j are the corresponding dipole moments of the MOs, and μ_{ij} is the transition dipole moment between donor and acceptor MOs.

In this long-range inter-protein charge recombination reaction, the electron donor orbital is doubly occupied and the acceptor is singly occupied. As such, the HOMO-HOMO electron couplings, and the couplings between other low lying orbital pairs localized on donor and acceptor, contribute to the donor-acceptor interaction. We calculated the effective squared electronic coupling, $\langle H_{DA}^2 \rangle$, by considering possible tunneling paths that are represented by many donor-acceptor frontier MO combinations, and the contribution of each tunneling path to $\langle H_{DA}^2 \rangle$ is weighted by its orbital energetics. (see SI section S1 for details)

$$\langle H_{DA}^2 \rangle = \frac{1}{N_{total}} \sum_N \sum_{i \in D_{MO}}^{N_D} \sum_{j \in A_{MO}}^{N_A} \left(1 - \frac{1}{1 + e^{(E_{j,N} - E_{A,N})/kT}} \right) e^{-\frac{\delta_{ij,N}^2}{4\lambda kT}} e^{-\frac{2\delta_{ij,N}(\Delta G_{DA} + \lambda)}{4\lambda kT}} H_{ij,N}^2 \quad (8)$$

$H_{ij,N}$ is the electronic coupling between one electron donor MO(i) and one electron acceptor MO(j) of snapshot N . N_D and N_A are the number of frontier MOs localized on the donor and acceptor, respectively. $E_{j,N}$ is the energy of electron acceptor MO(j) of snapshot N , and $E_{A,N}$ is the highest electron acceptor MO(A) energy of snapshot N . $\delta_{ij,N}$ is the reaction free energy correction for any donor-acceptor (ij) orbital pair of snapshot N , $\delta_{ij,N} = \Delta G_{ij,N}^\circ - \Delta G_{DA}^\circ$, where ΔG_{DA}° is the reaction free energy between donor HOMO and acceptor HOMO.

We calculated $\langle H_{DA}^2 \rangle$ at equilibrium using the last 2000 snapshots from the MD simulations for all ZnPCcP:Cc complexes with eq. 7 and eq. 8. The calculated mean-squared electronic coupling converges with these 2000 equilibrium snapshots (see SI section S6 for further discussion).

Reorganization energy

The reorganization energy is divided into inner- and outer-sphere components. For charge recombination in the ZnPCcP:Cc complex, the inner-sphere reorganization energy is associated with structural changes in the coordination sphere of the heme iron, while the outer-sphere reorganization energy involves structural changes of the remaining protein and solvent. The inner-sphere reorganization energy was estimated by using either experimentally measured changes in metal-ligand bond distances or theoretical calculations.⁷⁵ These inner-sphere reorganization energies are typically less than 0.1 eV and are usually neglected compared to the outer-sphere contribution. The outer-sphere reorganization energy is estimated with the Marcus two-sphere model,⁷⁶

$$\lambda = e^2 \left(\frac{1}{\epsilon_{op}} - \frac{1}{\epsilon_s} \right) \left(\frac{1}{2a_1} + \frac{1}{2a_2} - \frac{1}{r_{DA}} \right). \quad (9)$$

e is the charge transferred from the donor to the acceptor, a_1 and a_2 are the radii of the donor and acceptor respectively, and r_{DA} is the center-to-center donor-acceptor distance. $\epsilon_{op} = 2$ and $\epsilon_s = 4$ are the optical and static dielectric constants of the medium, respectively. The static dielectric constant value $\epsilon_s = 4$ is commonly used for protein media.^{77,78} We also calculated the outer-sphere reorganization energy with a dielectric constant from 4 to 20, in order to test the robustness of our results.

To study the validity of neglecting the inner-sphere reorganization energy, we also treated the total reorganization energy as a free variable in the range of 0.2 to 1.0 eV and examined the corresponding charge recombination rates. The λ is calculated based on

ensemble-averaged distance from the last 2000 snapshots of the ZnPCcP:Cc complex MD simulations.

Results and discussion

Pathway analysis

We determined the strongest coupling pathways from donor to acceptor using the standard Pathways search approach^{7,64,79} and assessed the strengths of specific pathways by evaluating their electronic couplings with ZINDO/GMH methods.

We examine how altering the hole donor position for the charge recombination in the ZnPCcP:Cc complex (CcP ZnP or residue 191) may affect the mediating residue composition of the strongest tunneling pathway. For each ZnPCcP:Cc complex, the ET tunneling pathway was determined using the Pathways plugin,⁶⁷ where the electron donor (hole acceptor) is the Fe atom in the Cc heme, and the electron acceptor (hole donor) is set appropriately for the charge recombination reaction of interest (see eqs. 2a and 2b).

Table 1 shows the strongest tunneling pathways for different ZnPCcP:Cc complexes. The strongest tunneling pathway is sensitive to the spatial location of the hole donor. For the native protein and the W191Y species where the charge recombination occurs following eq. 2a, the mediating amino acids in the strongest coupling pathway are Ala193 and Gly192 in CcP. For the W191F and W191G mutants, the charge recombination follows eq. 2b, and the mediating amino acids are Ala194, Asn195, and Gly178 of CcP. The strongest coupling pathway is the same for both the native protein and the W191Y mutant, where residue 191 is set as the acceptor, for both the W191F and the W191G mutants, where the CcP ZnP is set as the acceptor. The same strongest coupling pathways for the protein complex with the same hole donors (native protein and W191Y, W191F and W191G) is consistent with crystallographic studies that show the binding modes of these four CcP:Cc complexes are very similar.⁵⁹ For the W191G mutant with four different redox active molecules bound, Table 1 shows that the strongest tunneling pathway is very similar to that found in the native protein, mediated by Ala193, Gly192, and Gly191.

Figure 4 shows the locations of mediating residues in CcP. As the hole donor (radical cation) localization changes from the CcP ZnP to CcP residue 191, the spatial separation between the hole donor and the hole acceptor (Cc heme) shrinks. The strongest tunneling pathway is also changed due to this hole donor position change (from the pathway in orange to the pathway in yellow). The tunneling pathway analysis shows the important role of the radical cation localization for determining the strongest pathway. Indeed, changing the hole donor from the CcP ZnP to the 191 residue changes the strongest coupling pathway (see Table 1), thus affecting the ET parameters and charge recombination kinetics.

In the following section, the residues in the strongest coupling pathway are included in the semiempirical ZINDO/GMH analysis to compute mean-squared electronic couplings and estimate charge recombination rates for each protein complex.

Charge recombination rates in species with different acceptor locations

Studies of Payne et al. compared enzymatic activities and/or charge recombination rates in the native CcP:Cc complex with the W191X mutants (X = Y, F, and G) and a set of redox active small molecules bound in the W191G cavity.⁵⁹ The data suggest that the positioning and the reduction potentials of the hole hopping intermediate must satisfy both geometric and energetic constraints in order to support charge hopping. We hypothesize that different radical cation localization positions change the key ET parameters, specifically the electronic couplings and the reaction free energies, thus altering the corresponding charge recombination pathways and the kinetics. We assume that the strongest tunneling pathway determines the mean squared electronic coupling value. Next, we calculate ET parameters and charge recombination rates for each protein complex with corresponding acceptor positions.

Semiempirical electronic coupling analysis—Donor-acceptor electronic couplings were calculated with eqs. 7 and 8, approximating the donor and acceptor with the corresponding frontier MOs. Figure 5 shows the distributions of $\log H_{DA}^2$ of the ZnPCcP:Cc complexes for MD sampled geometries (see SI section S5 for further discussion). The $\log H_{DA}^2$ values have an approximately normal distribution. The effective electronic couplings derived by including multiple frontier MO pairs (eq. 8) are one order of magnitude larger than that inferred by including only the HOMOs of the donor and acceptor species (eq. 7). This larger coupling indicates that multiple tunneling channels contribute to charge recombination and that they enhance the electronic coupling. The effective squared electronic couplings are sensitive to the choice of the energetics and reorganization energies, and the HOMO-HOMO coupling makes the most significant contribution. We included the 10 highest energy MOs in the effective electronic coupling calculations using eq. 8.

Table 2 shows ensemble averaged DA couplings for the native ZnPCcP:Cc complex and three mutants. The mean squared DA effective couplings for both W191F and W191G species are on the order of 10^{-14} eV^2 , which is in accordance to the early semi-empirical study of CcP:Cc complex, without the involvement of Trp191,⁴² and are about 6 orders of magnitude smaller than the values found for the wild type and the W191Y mutant. These small mean squared couplings indicate that, without involving the tryptophan in the charge recombination process, the ZnP-heme pair in both the W191F and W191G mutants is weakly coupled. Varying the radical cation (hole donor) localization position changes the bridging residues in the strongest tunneling pathway, as shown in Table 1, thus altering the ET mediating amino acids and the donor-acceptor separation distance for both the W191F and the W191G species. Lengthening the tunneling pathway weakens the DA couplings for the W191F and the W191G species. This ZINDO/GMH coupling analysis is consistent with the more qualitative pathway analysis.

To investigate the difference between the exogenous redox active small molecules bound to the W191G mutant and the native CcP:Cc complex, we also calculated the DA couplings for these species. Table 3 shows the mean squared DA couplings for these four CcP:Cc complexes. The electronic couplings computed for these four W191G species are within 1 order of magnitude of the values found for the wild type species. The electronic coupling

decrease for the W191G derived species may stem from structural disorder and/or weak binding of the guest molecules.⁵⁹ We compared the edge-to-edge distance between donor and acceptor for these four mutants and for the W191Y species. Table 4 shows that the edge-to-edge distances for these W191G mutants are 1 to 2 Å longer than for the W191Y species. The weakly bound aromatic guests in the W191G cavity lengthen the tunneling pathways and decrease the corresponding electronic couplings.

Reorganization energy—The computed reorganization energies for different ZnPCcP:Cc complexes are shown in Table 5 using the Marcus two-sphere model with ensemble averaged distances. The reorganization energy differences are determined by both the choice of the hole donor and the size of the hole donor for each protein complex. For both the wild type complex and the W191Y mutant, the effective radius of the hole donor (Trp191 or Tyr191) is smaller than that of the W191F and W191G species (heme), producing larger reorganization energies, in spite of the fact that the shorter donor-acceptor distances decreases the reorganization energies.

We computed the reorganization energies for the W191G mutant with four different redox active guest molecules bound in the cavity. Table 6 compares these values to those of the wild type protein complex and the W191Y mutant, shown in Table 5. The weak binding of these four aromatic molecules in the W191G cavity presumably contributes to lengthening the center-to-center donor-acceptor separation distance, thus increasing the reorganization energy.

Charge recombination rate analysis—We evaluated charge recombination rates, assuming that the radical cation is localized either on CcP residue 191 or on the ZnP in the different ZnPCcP:Cc complexes (eqs. 3 and 4), using the electronic couplings and reorganization energies described above.

Table 7 summarizes the computed charge recombination rates. The indirect pathway with radical cation equilibrium in the native protein complex has the fastest computed charge recombination rate. The indirect charge recombination rates ($k_{eb}^{indirect}$) in eq. 2a for both the native protein complex and for the W191Y mutant are 6 and 9 orders of magnitude larger than the direct charge recombination rates (k_{eb}^{direct}) of the W191F and W191G mutants due to the orders of magnitude larger electronic coupling, supportive of the fast ZnP^+ to W^0 ET rate assumption ($k_{eb}^{indirect} \gg k_{eb}^{direct}$). For the native protein complex and the W191Y mutant, which follow the recombination mechanism of eq. 2a, the equilibrium constant (K_{eq}) for $[ZnP^0X^{\cdot+}]$ and $[ZnP^{\cdot+}X^0]$ ($X = W$ and Y , respectively) should be included and multiplied by the indirect charge recombination rate to obtain the charge recombination rate. With K_{eq} included for these species, the observed charge recombination rate k_{obs}^{calc} for the native protein complex is about 3 orders of magnitude larger than the rates for the W191F and W191G mutants. Although the direct hole transfer rate k_{eb} is about 3 order of magnitude smaller in the native protein complex than in the W191Y mutant, the redox potential difference between the tyrosine and tryptophan species makes the equilibrium constant for the formation of $[ZnP^0Y^{\cdot+}]$ in the W191Y mutant about 9 orders of magnitude smaller

than that of the native protein complex, resulting in an observed charge recombination rate that is about 5 orders of magnitude smaller than that found for the native protein complex and similar to those of W191F and W191G mutants. For the W191Y mutant, we only consider the charge recombination reaction between the Cc heme and the tyrosine radical cation, neglecting the fact that the neutral tyrosyl radical following deprotonation can also participate in the charge recombination reaction. The calculated SOMO (singly occupied molecular orbital) energy of neutral tyrosyl radical is 0.2 eV smaller than that of the tyrosine HOMO, resulting in a slower indirect charge recombination rate. Thus, even with the fast deprotonation rate, the predicted charge recombination rate with a neutral tyrosyl radical may be further diminished and smaller than that of the wild type protein complex. The redox potential difference between tryptophan and tyrosine determines the effective role of tryptophan as a hole intermediate in the CcP:Cc system; however, one should notice that in some other systems with different redox potential landscapes (e.g., ribonucleotide reductase),^{80,81} tyrosine serves as an effective hole hopping intermediate.

We also predicted the charge recombination rates for the W191G mutant with guest redox active molecules bound to the ZnPCcP:Cc complexes. We assume that the radical cation can localize on these redox active small molecules, so the indirect pathway is enabled and is the leading channel contributing to the observed charge recombination rate. The indirect hole transfer rates ($k_{eb}^{indirect}$) are computed and have the same order of magnitude compared to the wild type protein, so the equilibrium constant for the formation of $[ZnP^0X^+]$ plays a significant role. Of all four redox active guest molecules, the potentials of 24dma ($\Delta G_{eq}^\circ = 0.051$ eV) and 3abt ($\Delta G_{eq}^\circ = 0.826$ eV) cause the K_{eq} values to change by several orders of magnitude, leading either to fast charge recombination (5.46×10^6 s⁻¹ for 24dma) or slow charge recombination (1.04×10^{-6} s⁻¹ for 3abt). Structural disorder of these guest molecules may further diminish the charge recombination rates below the computed values. We calculated the RMSDs of these guest molecules to quantify how much the ligand binding pose changed over the course of the simulation. The RMSDs for the last 2000 snapshots are about 0.2 nm, indicating a small change in the ligands' position (see SI section S7 for details). Moreover, with the structural differences between the native tryptophan residue and the aniline derivatives considered, structural disorder of these guest molecules can be expected in the binding pocket. The charge recombination rate for the 24dma species is on par with that of the wild type protein, so the lack of activity in this species may result from the structural disorder of the corresponding ligand in the binding pocket. For the 3abt, otol, and an species, the calculated charge recombination rates are considerably lower than the wild type protein and thus they should not display significant activity. As such, we predict that the small molecules will not support charge recombination hopping in the CcP:Cc complex.

We scanned the static dielectric constant for the protein medium ranging from 4 to 20. Figure 6 shows that for the native ZnPCcP:Cc complex and the three residue 191 mutants, varying the static dielectric constant from 4 to 20 decreases the charge recombination rates by less than 1 order of magnitude, and the rate in the native protein complex is several orders of magnitude faster than in the W191Y, W191F, and W191G mutants.

To study the influence of neglecting the inner-sphere reorganization energy and to probe the robustness of our conclusions, we varied the total reorganization energy from 0.4 to 1.4 eV, and computed the corresponding charge recombination rates for the four ZnPCcP:Cc complexes. Figure 7 shows that charge recombination rates are affected by 2 orders of magnitude in this range, and the rate in the wild type protein complex remains several orders of magnitude faster than those for the W191Y, W191F, and W191G mutants. Thus, the native species are predicted to be enzymatically active, while the others are predicted to be inactive.

An important criterion to select guest redox-active small molecule, in order to replace the role of tryptophan as a hole hopping intermediate in this charge recombination reaction, is its similar redox potential compared to tryptophan. In ET parameters, both the squared electronic coupling and the reorganization energy are strongly distance dependent, so if the binding mode of the ZnPCcP:Cc complex does not change with modification of site 191, the reaction free energy will control the charge-recombination kinetics. We treat the HOMO energy of TRP as a free variable and the other ET parameters ($\langle H_{DA}^2 \rangle$ and λ) as fixed, and Figure 8 shows how fine tuning the HOMO energy of the site 191 redox active molecules (comparing to TRP HOMO) affects the equilibrium constant K_{eq} , the indirect back ET rate k_{eb} , and the charge recombination rate k_{obs}^{calc} . As the radical cation of the redox species becomes less oxidizing compared to TRP ($[HOMO(X)-HOMO(Trp)] > 0$; $[HOMO(ZnP)-HOMO(X)] > 0$), the k_{eb} between the redox species and the Cc heme will decrease (Fig. 8, red trace), but K_{eq} will increase (Fig. 8, green trace), resulting in an overall increase in the k_{obs}^{calc} (Fig. 8, blue trace). A HOMO difference of > 0.15 eV results in $K_{eq} > 1$, indicating that the radical cation of the redox species is now less oxidizing compared to the π -cation radical of ZnP. With $K_{eq} > 1$, eq. 3 no longer holds and this situation is beyond the scope of our study. This analysis shows that by varying the reduction potential of the radical species at site 191, making it less oxidizing (in a limited range), the charge recombination rate can be accelerated by up to 2 orders of magnitude.

Conclusions

We used ET theory to analyze the charge recombination kinetics in native and mutant CcP:Cc species to understand why only Trp191 serves as a viable hole hopping station for charge recombination in CcP:Cc. The mutants fail to support charge recombination for several reasons. The large values of the redox potentials for phenylalanine and glycine cause the charge recombination reaction for the ZnP-heme electron transfer to occur over a longer distance in a single step. The computed donor-acceptor electronic couplings in the W191F and W191G mutants are orders of magnitude smaller than the couplings in the native protein complex, since the native complex supports hole hopping via tryptophan with a corresponding ~ 6 Å shorter tunneling distance. Redox active aromatic molecules bound to the W191G cavity have couplings similar to those of the native species. The computed charge recombination rates for the 24dma species is comparable to that of the wild type protein. The lack of activity may result from the structural disorder of the ligand in the binding pocket. For the 3abt, otol, and an species, the computed charge recombination rates are considerably lower than in the wild type protein, indicating poor activities. The redox

potential difference between tyrosine and tryptophan apparently slows the Tyr-mediated recombination rate by orders of magnitude through the decreased equilibrium constant (K_{eq}) for W191Y. Our analysis thus indicates that only the native Trp191 is expected to serve as a functional hole hopping charge recombination intermediate. These findings explain the recent experimental kinetic findings that identified only the native CcP:Cc protein complex as having peroxidase activity and fast recombination rates, and highlight the critical role played by both structure and energetics in biological hole transport.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement

X.R., P.Z., and D.N.B. acknowledge support from the National Institute of Health (Grant No. GM-48043). B.C. acknowledges support from the National Science Foundation (Grant No. MCB-1715233)

References

- (1). Cordes M; Giese B Electron transfer in peptides and proteins. *Chem. Soc. Rev* 2009, 38, 892–901. [PubMed: 19421569]
- (2). Cordes M; Köttgen A; Jasper C; Jacques O; Boudebous H; Giese B Influence of Amino Acid Side Chains on Long-Distance Electron Transfer in Peptides: Electron Hopping via “Stepping Stones”. *Angew. Chem. Int. Ed* 2008, 47, 3461–3463.
- (3). Warren JJ; Ener ME; Vl ek A Jr; Winkler JR; Gray HB Electron hopping through proteins. *Coord. Chem. Rev* 2012, 256, 2478–2487. [PubMed: 23420049]
- (4). Warren JJ; Winkler JR; Gray HB Hopping maps for photosynthetic reaction centers. *Coord. Chem. Rev* 2013, 257, 165–170. [PubMed: 23275678]
- (5). Lukacs A; Eker AP; Byrdin M; Brettel K; Vos M H Electron hopping through the 15 Å triple tryptophan molecular wire in DNA photolyase occurs within 30 ps. *J. Am. Chem. Soc* 2008, 130, 14394–14395. [PubMed: 18850708]
- (6). Reece SY; Seyedsayamdost MR; Stubbe J; Nocera DG Direct observation of a transient tyrosine radical competent for initiating turnover in a photochemical ribonucleotide reductase. *J. Am. Chem. Soc* 2007, 129, 13828–13830. [PubMed: 17944464]
- (7). Winkler JR; Gray HB Long-range electron tunneling. *J. Am. Chem. Soc* 2014, 136, 2930–2939. [PubMed: 24499470]
- (8). Ru X; Zhang P; Beratan DN Assessing Possible Mechanisms of Micrometer-Scale Electron Transfer in Heme-Free *Geobacter sulfurreducens* Pili. *J. Phys. Chem. B* 2019, 123, 5035–5047. [PubMed: 31095388]
- (9). Beratan DN; Liu C; Migliore A; Polizzi NF; Skourtis SS; Zhang P; Zhang Y Charge transfer in dynamical biosystems, or the treachery of (static) images. *Acc. Chem. Res* 2015, 48, 474–481. [PubMed: 25307316]
- (10). Arnold AR; Grodick MA; Barton JK DNA Charge Transport: From Chemical Principles to the Cell. *Cell Chem. Biol* 2016, 23, 183–197. [PubMed: 26933744]
- (11). Boal AK; Genereux JC; Sontz PA; Gralnick JA; Newman DK; Barton JK Redox Signaling between DNA Repair Proteins for Efficient Lesion Detection. *Proc. Natl. Acad. Sci. U.S.A* 2009, 106, 15237–15242. [PubMed: 19720997]
- (12). Boon E; Livingston A; Chmiel N; David S; Barton J DNA-Mediated Charge Transport for DNA Repair. *Proc. Natl. Acad. Sci. U.S.A* 2003, 100, 12543–12547. [PubMed: 14559969]
- (13). Teo RD; Rousseau BJG; Smithwick ER; Di Felice R; Beratan DN; Migliore A Charge Transfer between [4Fe4S] Proteins and DNA Is Unidirectional: Implications for Biomolecular Signaling. *Chem* 2019, 5, 122–137. [PubMed: 30714018]

- (14). Teo RD; Wang R; Smithwick ER; Migliore A; Therien MJ; Beratan DN Mapping Hole Hopping Escape Routes in Proteins. *Proc. Natl. Acad. Sci. U.S.A*2019, 116, 15811–15816. [PubMed: 31341081]
- (15). Winkler JR; Gray HB Electron Flow through Biological Molecules: Does Hole Hopping Protect Proteins from Oxidative Damage? *Q. Rev. Biophys*2015, 48, 411–420. [PubMed: 26537399]
- (16). Yuly JL; Zhang P; Lubner CE; Peters JW; Beratan DN Universal free-energy landscape produces efficient and reversible electron bifurcation. *Proc. Natl. Acad. Sci. U.S.A*2020, 117, 21045–21051. [PubMed: 32801212]
- (17). Teo RD; Smithwick ER; Migliore A; Beratan DN A single AT–GC exchange can modulate charge transfer-induced p53–DNA dissociation. *ChemComm*2019, 55, 206–209.
- (18). Teo RD; Migliore A; Beratan DN Mutation effects on charge transport through the p58c iron–sulfur protein. *Chem. Sci*2020, 11, 7076–7085. [PubMed: 33250976]
- (19). Hoffman BM; Ratner MA Gated electron transfer: when are observed rates controlled by conformational interconversion? *J. Am. Chem. Soc*1987, 109, 6237–6243.
- (20). Davidson VL Protein control of true, gated, and coupled electron transfer reactions. *Acc. Chem. Res*2008, 41, 730–738. [PubMed: 18442271]
- (21). Beratan DN Why are DNA and protein electron transfer so different? *Annu. Rev. Phys. Chem*2019, 70, 71–97. [PubMed: 30726186]
- (22). Seefeldt LC; Hoffman BM; Peters JW; Raugei S; Beratan DN; Antony E; Dean DR Energy transduction in nitrogenase. *Acc. Chem. Res*2018, 51, 2179–2186. [PubMed: 30095253]
- (23). Polizzi NF; Skourtis SS; Beratan DN Physical constraints on charge transport through bacterial nanowires. *Faraday Discuss.* 2012, 155, 43–61. [PubMed: 22470966]
- (24). Gray HB; Winkler JR Hole hopping through tyrosine/tryptophan chains protects proteins from oxidative damage. *Proc. Natl. Acad. Sci. U.S.A*2015, 112, 10920–10925. [PubMed: 26195784]
- (25). Migliore A; Polizzi NF; Therien MJ; Beratan DN Biochemistry and theory of proton-coupled electron transfer. *Chem. Rev*2014, 114, 3381–3465. [PubMed: 24684625]
- (26). Winkler JR; Gray HB Could tyrosine and tryptophan serve multiple roles in biological redox processes? *Phil. Trans. R. Soc. A*2015, 373, 20140178. [PubMed: 25666062]
- (27). Plato M; Michel-Beyerle M; Bixon M; Jortner J On the role of tryptophan as a superexchange mediator for quinone reduction in photosynthetic reaction centers. *FEBS Lett.* 1989, 249, 70–74.
- (28). Aubert C; Mathis P; Eker AP; Brettel K Intraprotein electron transfer between tyrosine and tryptophan in DNA photolyase from *Anacystis nidulans*. *Proc. Natl. Acad. Sci. U.S.A*1999, 96, 5423–5427. [PubMed: 10318899]
- (29). Shih C; Museth AK; Abrahamsson M; Blanco-Rodriguez AM; Di Bilio AJ; Sudhamsu J; Crane BR; Ronayne KL; Towrie M; Vilek A et al. Tryptophan-accelerated electron flow through proteins. *Science*2008, 320, 1760–1762. [PubMed: 18583608]
- (30). Miller MA; Han GW; Kraut JA cation binding motif stabilizes the compound I radical of cytochrome c peroxidase. *Proc. Natl. Acad. Sci. U.S.A*1994, 91, 11118–11122. [PubMed: 7972020]
- (31). Nocek JM; Zhou JS; De Forest S; Priyadarshy S; Beratan DN; Onuchic JN; Hoffman BM Theory and practice of electron transfer within protein-protein complexes: application to the multidomain binding of cytochrome c by cytochrome c peroxidase. *Chem. Rev*1996, 96, 2459–2490. [PubMed: 11848833]
- (32). Lippard SJ; Berg JM *Principles of Bioinorganic Chemistry*; University Science Books, Mill Valley, CA, 1994.
- (33). Bertini I; Gray HB; Lippard SJ; Valentine JS *Bioinorganic Chemistry*; University Science Books, Mill Valley, CA, 1994.
- (34). Gray HB; Winkler JR Electron tunneling through proteins. *Q. Rev. Biophys*2003, 36, 341–372. [PubMed: 15029828]
- (35). Beratan D; Skourtis S Electron transfer mechanisms. *Curr. Opin. Chem. Biol*1998, 2, 235–243. [PubMed: 9667934]
- (36). Balzani V *Electron transfer in chemistry*; Wiley-VCH: Weinheim; New York, 2001.

- (37). Wallin SA; Stemp ED; Everest AM; Nocek JM; Netzel TL; Hoffman BM Multiphasic intracomplex electron transfer from cytochrome c to zinc cytochrome c peroxidase: conformational control of reactivity. *J. Am. Chem. Soc* 1991, 113, 1842–1844.
- (38). Nocek JM; Stemp ED; Finnegan MG; Koshy TI; Johnson MK; Margoliash E; Mauk AG; Smith M; Hoffman BM Low-temperature, cooperative conformational transition within [Zn-cytochrome c peroxidase, cytochrome c] complexes: variation with cytochrome. *J. Am. Chem. Soc* 1991, 113, 6822–6831.
- (39). Liang N; Kang CH; Margoliash E; Ho PS; Hoffman BM Long-range electron transfer from iron (II)-cytochrome c to (zinc-cytochrome c peroxidase)(+) within the 1: 1 complex. *J. Am. Chem. Soc* 1986, 108, 4665–4666.
- (40). Ho PS; Sutoris C; Liang N; Margoliash E; Hoffman BM Species specificity of long-range electron transfer within the complex between zinc-substituted cytochrome c peroxidase and cytochrome c. *J. Am. Chem. Soc* 1985, 107, 1070–1071.
- (41). Ho PS; Hoffman BM; Solomon N; Kang CH; Margoliash E Kinetics and energetics of intramolecular electron transfer in yeast cytochrome c peroxidase. *Biochemistry* 1984, 23, 4122–4128. [PubMed: 6091738]
- (42). Jiang N; Kuznetsov A; Nocek JM; Hoffman BM; Crane BR; Hu X; Beratan DN Distance-independent charge recombination kinetics in cytochrome c–cytochrome c peroxidase complexes: compensating changes in the electronic coupling and reorganization energies. *J. Phys. Chem. B* 2013, 117, 9129–9141. [PubMed: 23895339]
- (43). Seifert JL; Pfister TD; Nocek JM; Lu Y; Hoffman BM Hopping in the Electron-Transfer Photocycle of the 1: 1 Complex of Zn- Cytochrome c Peroxidase with Cytochrome c. *J. Am. Chem. Soc* 2005, 127, 5750–5751. [PubMed: 15839648]
- (44). Kang SA; Crane BR Effects of interface mutations on association modes and electron-transfer rates between proteins. *Proc. Natl. Acad. Sci. U.S.A* 2005, 102, 15465–15470. [PubMed: 16227441]
- (45). Wallrapp FH; Voityuk AA; Guallar V In-silico assessment of protein-protein electron transfer. A case study: cytochrome c peroxidase–cytochrome c. *PLOS Comput. Biol* 2013, 9, e1002990. [PubMed: 23555224]
- (46). Goodin DB; McRee DE The Asp-His-iron triad of cytochrome c peroxidase controls the reduction potential electronic structure, and coupling of the tryptophan free radical to the heme. *Biochemistry* 1993, 32, 3313–3324. [PubMed: 8384877]
- (47). Fishel LA; Farnum MF; Mauro JM; Miller MA; Kraut J; Liu Y; Tan XL; Scholes CP Compound I radical in site-directed mutants of cytochrome c peroxidase as probed by electron paramagnetic resonance and electron-nuclear double resonance. *Biochemistry* 1991, 30, 1986–1996. [PubMed: 1847080]
- (48). Barrows TP; Poulos TL Role of electrostatics and salt bridges in stabilizing the compound I radical in ascorbate peroxidase. *Biochemistry* 2005, 44, 14062–14068. [PubMed: 16245922]
- (49). Barrows TP; Bhaskar B; Poulos TL Electrostatic control of the tryptophan radical in cytochrome c peroxidase. *Biochemistry* 2004, 43, 8826–8834. [PubMed: 15236591]
- (50). Fitzgerald MM; Trester ML; Jensen GM; Mcree DE; Goodin DB The role of aspartate-235 in the binding of cations to an artificial cavity at the radical site of cytochrome c peroxidase. *Protein Sci.* 1995, 4, 1844–1850. [PubMed: 8528082]
- (51). Huyett JE; Doan PE; Gurbel R; Houseman AL; Sivaraja M; Goodin DB; Hoffman BM Compound ES of Cytochrome c Peroxidase Contains a Trp. π -Cation Radical: Characterization by Continuous Wave and Pulsed Q-Band External Nuclear Double Resonance Spectroscopy. *J. Am. Chem. Soc* 1995, 117, 9033–9041.
- (52). Patterson WR; Poulos TL; Goodin DB Identification of a Porphyrin. π . Cation Radical in Ascorbate Peroxidase Compound I. *Biochemistry* 1995, 34, 4342–4345. [PubMed: 7703248]
- (53). Bonagura CA; Sundaramoorthy M; Bhaskar B; Poulos TL The effects of an engineered cation site on the structure, activity, and EPR properties of cytochrome c peroxidase. *Biochemistry* 1999, 38, 5538–5545. [PubMed: 10220341]

- (54). Jasion VS; Polanco JA; Meharena YT; Li H; Poulos TL Crystal structure of Leishmania major peroxidase and characterization of the compound i tryptophan radical. *J. Biol. Chem* 2011, 286, 24608–24615. [PubMed: 21566139]
- (55). Jasion VS; Doukov T; Pineda SH; Li H; Poulos TL Crystal structure of the Leishmania major peroxidase–cytochrome c complex. *Proc. Natl. Acad. Sci. U.S.A* 2012, 109, 18390–18394. [PubMed: 23100535]
- (56). Fitzgerald MM; Churchill MJ; McRee DE; Goodin DB Small molecule binding to an artificially created cavity at the active site of cytochrome c peroxidase. *Biochemistry* 1994, 33, 3807–3818. [PubMed: 8142383]
- (57). Musah RA; Jensen GM; Bunte SW; Rosenfeld RJ; Goodin DB Artificial protein cavities as specific ligand-binding templates: Characterization of an engineered heterocyclic cation-binding site that preserves the evolved specificity of the parent protein 1. *J. Mol. Biol* 2002, 315, 845–857. [PubMed: 11812152]
- (58). Musah RA; Goodin DB Introduction of novel substrate oxidation into cytochrome c peroxidase by cavity complementation: oxidation of 2-aminothiazole and covalent modification of the enzyme. *Biochemistry* 1997, 36, 11665–11674. [PubMed: 9305956]
- (59). Payne TM; Yee EF; Dzikovski B; Crane BR Constraints on the radical cation center of cytochrome c peroxidase for electron transfer from cytochrome c. *Biochemistry* 2016, 55, 4807–4822. [PubMed: 27499202]
- (60). Wang K; Mei H; Geren L; Miller MA; Saunders A; Wang X; Waldner JL; Pielak GJ; Durham B; Millett F Design of a Ruthenium-Cytochrome c Derivative To Measure Electron Transfer to the Radical Cation and Oxyferryl Heme in Cytochrome c Peroxidase. *Biochemistry* 1996, 35, 15107–15119. [PubMed: 8942678]
- (61). Wardman P Reduction potentials of one-electron couples involving free radicals in aqueous solution. *J. Phys. Chem. Ref. Data* 1989, 18, 1637–1755.
- (62). DeFelippis MR; Murthy C; Broitman F; Weinraub D; Faraggi M; Klapper MH Electrochemical properties of tyrosine phenoxy and tryptophan indolyl radicals in peptides and amino acid analogs. *J. Phys. Chem* 1991, 95, 3416–3419.
- (63). Beratan D; Betts J; Onuchic J Protein electron transfer rates set by the bridging secondary and tertiary structure. *Science* 1991, 252, 1285–1288. [PubMed: 1656523]
- (64). Balabin IA; Beratan DN; Skourtis SS Persistence of structure over fluctuations in biological electron-transfer reactions. *Phys. Rev. Lett* 2008, 101, 158102. [PubMed: 18999647]
- (65). Guallar V; Wallrapp F Mapping protein electron transfer pathways with QM/MM methods. *J. Royal Soc. Interface* 2008, 5, 233–239.
- (66). Wallrapp F; Masone D; Guallar V Electron transfer in the P450cam/PDX complex. The QM/MM e-pathway. *J. Phys. Chem. A* 2008, 112, 12989–12994. [PubMed: 18823106]
- (67). Balabin IA; Hu X; Beratan DN Exploring biological electron transfer pathway dynamics with the Pathways plugin for VMD. *J. Comput. Chem* 2012, 33, 906–910. [PubMed: 22298319]
- (68). Ridley J; Zerner M An intermediate neglect of differential overlap technique for spectroscopy: pyrrole and the azines. *Theor. Chem. Acc* 1973, 32, 111–134.
- (69). Cave RJ; Newton MD Generalization of the Mulliken-Hush treatment for the calculation of electron transfer matrix elements. *Chem. Phys. Lett* 1996, 249, 15–19.
- (70). Voityuk AA Conformational dependence of the electronic coupling for singlet excitation energy transfer in DNA. An INDO/S study. *Phys. Chem. Chem. Phys* 2010, 12, 7403–7408. [PubMed: 20532402]
- (71). Voityuk AA Electronic couplings and on-site energies for hole transfer in DNA: Systematic quantum mechanical/molecular dynamic study. *J. Chem. Phys* 2008, 128, 03B608.
- (72). Voityuk AA Assessment of semiempirical methods for the computation of charge transfer in DNA π -stacks. *Chem. Phys. Lett* 2006, 427, 177–180.
- (73). Zheng J; Kang YK; Therien MJ; Beratan DN Generalized Mulliken-Hush analysis of electronic coupling interactions in compressed π -stacked Porphyrin- Bridge- Quinone systems. *J. Am. Chem. Soc* 2005, 127, 11303–11310. [PubMed: 16089459]

- (74). Hatcher E; Balaeff A; Keinan S; Venkatramani R; Beratan DNPNA versus DNA: effects of structural fluctuations on electronic structure and hole-transport mechanisms. *J. Am. Chem. Soc*2008, 130, 11752–11761. [PubMed: 18693722]
- (75). Sigfridsson E; Olsson MH; Ryde UA Comparison of the Inner-Sphere Reorganization Energies of Cytochromes, Iron-Sulfur Clusters, and Blue Copper Proteins. *J. Phys. Chem. B*2001, 105, 5546–5552.
- (76). Marcus RATutorial on rate constants and reorganization energies. *J. Electroanal. Chem*2000, 483, 2–6.
- (77). Pitera JW; Falta M; van Gunsteren WFDielectric properties of proteins from simulation: the effects of solvent, ligands, pH, and temperature. *Biophys. J*2001, 80, 2546–2555. [PubMed: 11371433]
- (78). Nakamura H; Sakamoto T; Wada AA theoretical study of the dielectric constant of protein. *Protein Eng. Des. Sel*1988, 2, 177–183.
- (79). Beratan DN; Onuchic JN; Winkler JR; Gray HBElectron-tunneling pathways in proteins. *Science*1992, 258, 1740–1742. [PubMed: 1334572]
- (80). Pagba CV; McCaslin TG; Veglia G; Porcelli F; Yohannan J; Guo Z; McDaniel M; Barry BAA tyrosine–tryptophan dyad and radical-based charge transfer in a ribonucleotide reductase-inspired maquette. *Nat. Commun*2015, 6, 1–8.
- (81). Minnihan EC; Nocera DG; Stubbe JReversible, long-range radical transfer in *E. coli* class Ia ribonucleotide reductase. *Acc. Chem. Res*2013, 46, 2524–2535. [PubMed: 23730940]

Figure 1:

Photoinduced ET in the ZnPCcP:Cc complex. The triplet state of zinc-porphyrin (ZnP) bound to CcP (^3ZnP) is produced by a 8 ns pulse of 532 – 560 nm light. ZnPCcP then either decays to the ground state (with a decay rate of k_D , the sum of both radiative and non-radiative decay rates), or undergoes an intermolecular, forward ET reaction with a rate k_f to yield a charge-separated intermediate. The latter returns to the ground state via a back ET from either the bound Cc(Fe^{2+}) to the ZnP-based π -cation radical ($\text{ZnP}^{+\bullet}$) with a rate constant k_b or from the W191 $^{+\bullet}$ species with the rate constant k_w . K_{eq} is the equilibrium constant between two radical cation species [ZnP^0W^+] and [ZnP^+W^0].

Figure 2:
Redox-active aniline derivatives that bind to the W191G cavity of CcP.^{61,62} Reduction potentials vs SHE are indicated.

Figure 3:

Schematic diagram indicating the two state GMH approach to analyze couplings. Molecular orbitals (MO) are divided into two sets according to their localization on donor and acceptor fragments. The red lines represent the occupied orbitals localized on the acceptor site, whereas the blue lines are the occupied orbitals localized on the donor site. For each MO donor and acceptor pair, the two state generalized Mulliken-Hush approach is used to compute H_{ij} in eq. 7

(b) Two charge recombination pathways

Charge recombination pathways in the ZnPCcP:Cc complex. (a) ZnPCcP:Cc complex with ZnP in CcP (left) and heme in Cc (right) highlighted in red. (b) Two charge recombination pathways with different radical cation localizations: site 191 (pathway highlighted in blue and Trp191 in purple) and ZnP (pathway highlighted in yellow).

(a) Wild type

(b) W191Y mutant

(c) W191F mutant

(d) W191G mutant

Figure 5:

Histogram of $\log H_{DA}^2$ with highest occupied donor orbital/ highest occupied acceptor orbital squared coupling (blue) and effective squared coupling (yellow). The X axis denotes $\log|H_{DA}^2|$, and Y axis denotes the number of occurrence in the 4 ns trajectory.

Figure 6:
Calculated charge recombination rates with various static dielectric constants for the protein.

Figure 7:
Calculated charge recombination rate as a function of reorganization energy for four different CcP:Cc complexes.

Figure 8:
Calculated equilibrium constant (K_{eq}), indirect back ET rate (k_{eb}), and charge recombination rate (k_{obs}^{calc}) as a function of HOMO energy compared to the Trp HOMO.

Table 1:

Strongest coupling pathways found with Pathways analysis.⁶⁷ The spatial localization of the radical cation determines the strongest charge recombination pathway.

Protein	Hole donor (radical cation localization)	Strongest coupling pathway
Wild type	Trp191	Heme → Ala193 → Gly192 → Trp191
W191Y	Tyr191	Heme → Ala193 → Gly192 → Tyr191
W191F	ZnP (CcP)	Heme → Ala194 → Asn195 → Gly178 → ZnP (CcP)
W191G	ZnP (CcP)	Heme → Ala194 → Asn195 → Gly178 → ZnP (CcP)
W191G+24dma	24dma	Heme → Ala193 → Gly192 → Gly191 → 24dma
W191G+3abt	3abt	Heme → Ala193 → Gly192 → Gly191 → 3abt
W191G+an	an	Heme → Ala193 → Gly192 → Gly191 → an
W191G+otol	otol	Heme → Ala193 → Gly192 → Gly191 → otol

Table 2:

Mean squared average effective electronic couplings for native ZnPCcP:Cc complex and three mutants. Couplings of Wild type protein and W191Y mutant are the couplings between Cc heme and 191 site of CcP; couplings of W191F and W191G mutants are the couplings between Cc heme and CcP ZnP.

Protein	Wild type	W191Y	W191F	W191G
$\langle H_{DA}^2 \rangle (eV^2)$	8.4×10^{-8}	1.2×10^{-5}	7.1×10^{-14}	4.2×10^{-14}

Table 3:

Mean squared electronic couplings for W191G with four guest redox active species bound.

Protein	W191G+24dma	W191G+3abt	W191G+an	W191G+otol
$\langle H_{DA}^2 \rangle (eV^2)$	4.4×10^{-7}	6.2×10^{-9}	1.1×10^{-6}	8.2×10^{-8}

Table 4:

Edge-to-edge distances for the W191Y species and the W191G mutant with four guest redox active molecules bound.

Protein	W191Y	W191G+24dma	W191G+3abt	W191G+an	W191G+otol
Distance (Å)	17.0	17.9	18.1	18.2	19.9

Table 5:

Averaged center-to-center donor-acceptor distances and reorganization energies for the ZnPCcP:Cc complexes with $\epsilon_s = 4$.

Protein	Wild type	W191Y	W191F	W191G
Distance ($\text{\AA}$)	21.1	21.6	28.0	27.4
λ (eV)	0.73	0.80	0.50	0.44

Table 6:

Averaged center-to-center donor-acceptor distances and reorganization energies for the W191G mutant with four different redox-active aromatic molecules bounded in the cavity with $\epsilon_s = 4$.

Protein	W191G+24dma	W191G+3abt	W191G+an	W191G+otol
Distance ($\text{\AA}$)	23.1	23.7	23.5	25.6
λ (eV)	0.85	0.96	1.08	0.98

Table 7:

Calculated (k_{obs}^{calc}) and experiment (k_{obs}^{exp}) observed charge recombination rates for different ZnPCcP:Cc complexes with $\epsilon_s = 4$.

Protein:	Wild type	W191Y	W191F	W191G	24dma	3abt	an	otol
Recombination regime	eq. 3	eq. 3	eq. 4	eq. 4	eq. 3	eq. 3	eq. 3	eq. 3
Equilibrium constant (K_{eq})	1.7×10^{-3}	4.5×10^{-12}	-	-	1.4×10^{-1}	9.7×10^{-15}	4.9×10^{-6}	1.1×10^{-4}
$k_{cb} (s^{-1})$	1.1×10^8	2.2×10^{11}	1.0×10^2	1.0×10^2	4.0×10^7	1.1×10^8	4.0×10^8	2.7×10^7
$k_{obs}^{calc} (s^{-1})$	1.9×10^5	9.7×10^{-1}	1.0×10^2	1.0×10^2	5.5×10^6	1.0×10^{-6}	2.0×10^3	3.0×10^3
$k_{obs}^{exp} (s^{-1})$	$10^4 - 10^6$	5.0×10^1	4.0×10^1	1.6×10^1	-	-	-	-