Electron flow in multiheme bacterial cytochromes is a balancing act between heme electronic interaction and redox potentials

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The naturally widespread process of electron transfer from metal reducing bacteria to extracellular solid metal oxides entails unique biomolecular machinery optimized for long-range electron transport. To perform this function efficiently, microorganisms have adapted multiheme c-type cytochromes to arrange heme cofactors into wires that cooperatively span the cellular envelope, transmitting electrons along distances greater than 100 Å. Implications and opportunities for bionanotechnological device design are self-evident. However, at the molecular level, how these proteins shuttle electrons along their heme wires, navigating intraprotein interactions and interprotein interfaces efficiently, remains a mystery thus far inaccessible to experiment. To shed light on this critical topic, we carried out extensive quantum mechanics/molecular mechanics simulations to calculate stepwise heme-to-heme electron transfer rates in the recently crystallized outer membrane deca-heme cytochrome MtrF. By solving a master equation for electron hopping, we estimate an intrinsic, maximum possible electron flux through solvated MtrF of 10⁷–10⁹ s⁻¹, consistent with recently measured rates for the related multiheme protein complex MtrCAB. Intriguingly, our calculations show that the rapid electron transport through MtrF is the result of a clear correlation between heme redox potential and the strength of electronic coupling along the wire: thermodynamically uphill steps occur only between electronically well-connected stacked heme pairs. This observation suggests that the protein evolved to harbor low-potential hemes without slowing down electron flow. These findings are particularly profound in light of the apparently well-conserved staggered cross-heme wire structural motif in functionally related outer membrane proteins.

R espiratory electron transfer (ET) is not restricted to the aqueous subunits and membranes inside cells but in specialized cases can also occur across the outer membrane to extracellular space. This possibility is heavily used by dissimilatory metal reducing bacteria (DMRB), which are capable of using extracellular solid metal oxides as terminal respiratory electron sinks, a process that has been suggested to proceed via direct cell-mineral contact (1), extracellular redox shuttles (2), and/or pilus-like appendages (3, 4). Although essential to the survival of the bacterium, extracellular ET also plays an important role in the biogeochemical cycling of transition metals (5–7). It is or could be exploited in a multitude of biotechnological applications ranging from mediator-less biofuel cells (8) to biological waste-to-electricity conversion (1), photocatalytic bioenergy generation (9), and even bioelectronic systems using directional electronic communication between living and nonliving systems (9).

The transport of electrons from the inner membrane, where they accumulate as a result of metabolic activity, across the periplasm and outer membrane to the extracellular space relies on an efficient network of ET proteins (10). It has been known for some time that multiheme c-type cytochromes play a central role in this process. Examples of such systems include the MtrCAB and MtrFDE transmembrane complexes of the bacterial strain Shewanella oneidensis MR-1 that form a biological nanowire of 20 c-type hemes, 10 from MtrC(F) and 10 from MtrA(D), wrapped in a β barrel porin MtrB(E) (10) (Fig. L4). MtrB(E) does not contain any hemes but is supposed to enable contact for ET between the periplasmic MtrA(D) and the outer membrane cytochrome MtrC(F). The latter is assumed to pass electrons on to extracellular substrates either directly or via redox mediators such as flavins (11).

The recently published crystal structure for MtrF (12) (and indeed the first one for any deca-heme cytochrome) reveals hemes arranged side by side in a sequence clearly intended for directional electron flow. However, the arrangement is not simply a linear chain of 10 cofactors; rather, it features a peculiar “staggered-cross” formation of the 10 hemes as shown in Fig. 1B, with a central tetra-heme chain between hemes 2 and 7 and two heme-triples branching off in orthogonal directions to yield an octaheme chain between hemes 5 and 10. The relative orientation of adjacent hemes also varies in three apparent types (see depictions in Fig. 1C), with coplanar pairs within the tetra-heme chain, stacked pairs within the two heme-triples, and T-shaped connections between tetra-heme chain and heme-triples. These motifs as such are not uncommon in biological ET: the stacked heme arrangement bears some similarity with the tightly packed chlorophylls in reaction center proteins (13), and a similar T-shaped connection is found for the heme a-a pair in cytochrome c oxidase (14) (Fig. S1). However, it is thus far an open question why all three heme-heme motifs are present in respiratory | density functional theory | Jan 14, 2014 | Vol. 111 | No. 2 | 611–616

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MtrF, and why the 10 hemes are arranged in a nonlinear cross-wired fashion.

Given the central role of multiheme cytochromes like MtrF in the extracellular ET processes of DMRB, elucidating their function on a molecular level is therefore at the heart of coming to understand and possibly adapt these astonishing capabilities. However, elementary aspects of electron transport through these proteins are difficult to assess in experiment. For example, although overall potential windows of operation for the whole protein can be established (12, 15), the cofactors’ almost identical chemical environment impedes deconvolution into redox potentials of single structurally assignable hemes (12). Similarly, although the analysis of tunneling spectroscopy-derived current-voltage curves for MtrC single molecules yielded two individual redox potentials consistent with the whole protein window (16), tunneling experiments do not necessarily involve the same heme-to-heme hopping mechanism of electron transmission as expected in the native protein function.

Computational methods fill this accessibility gap to provide molecular-level insight. They are not only able to elucidate properties of individual cofactors in these multiheme cytochromes but also allow for the analysis of structure-function relationships: Our recent previous study of the thermodynamics of electron transfer through MtrF and MtrC (20) allows us to generalize our findings to these transport rates through the multiheme protein complex MtrCAB (18). The structural similarity of MtrF with homologs UndA (19) and MtrC (20) allows us to generalize our findings to these cytochromes also, suggesting an important electron transfer strategy in nature well conserved because of its efficiency for long-range electron transport.

**Results**

**Heme-to-Heme Electronic Coupling.** As the separation distances between heme cofactors are short in MtrF (from van der Waals distance, 3.6 Å, to 8.3 Å), we assume that ET occurs via sequential through-space electron tunneling (hopping) between adjacent heme pairs \((i,j)\) as well as ET steps through an external electron acceptor/donor, \(k_{\text{out}}\) and \(k_{\text{in}}\), shown exemplary for hemes \(i = 10\) and 5. (C) Three different heme pair motifs found in MtrF.

\[
\begin{align*}
\langle \text{Fe}^{2+} - \text{h} \rangle_i + \langle \text{Fe}^{3+} - \text{h} \rangle_j & \xrightleftharpoons{k_{ji}} \langle \text{Fe}^{3+} - \text{h} \rangle_i + \langle \text{Fe}^{2+} - \text{h} \rangle_j. \end{align*}
\]

Pathway calculations (21) were carried out to confirm this assertion. Similar results were obtained previously for heme a to a3 ET in cytochrome c oxidase suggesting that through-space tunneling is indeed the main mechanism for such short heme-heme distances (22). In accord with nonadiabatic Marcus theory (23), the electronic coupling matrix element is calculated for the transition state where initial and final state are degenerate. This ensemble was sampled for each of the nine ET reactions in our previous work, this completes the set of quantities necessary to describe ET rates in the framework of nonadiabatic Marcus theory, an approach found to be appropriate to describe ET through MtrF. The simulations reveal that the rapid transport rate through MtrF is a consequence of a subtle balancing act: energetically uphill steps occur only between the tightest packed, electronically best-connected hemes. The potential slowing of ET rates by low potential hemes is thus compensated by high electronic coupling. In fact, the rates for the thermodynamically unfavorable ET steps do not fall below those for the thermodynamically reversible steps. As a result, the maximum intrinsic electron flux through the heme wire is maintained at \(10^4 - 10^5 \text{ s}^{-1}\), just slightly higher than recently measured acceptor-limited transport rates through the multiheme protein complex MtrCAB (18). The structural similarity of MtrF with homologs UndA (19) and MtrC (20) allows us to generalize our findings to these cytochromes also, suggesting an important electron transfer strategy in nature well conserved because of its efficiency for long-range electron transport.
Fig. 2. Modulus of electronic coupling matrix elements ($|H_{ab}|$) for ET between ferrous and ferric heme cofactors in MtrF as a function of the heme edge-to-edge distance. The scattered data points are obtained from configurations sampled with molecular dynamics simulation at room temperature. They are colored according to the heme-heme orientation: stacked in blue, T-shaped in hollow red, and coplanar in green. $|H_{ab}|$ values obtained for the crystal structure configuration are indicated by triangles (four for stacked, two for T-shaped, three for coplanar). Root-mean-square averages of the scattered data points, $\langle |H_{ab}|^2 \rangle$, were calculated for bins (denoted by black circles) of width 0.4 Å (left) and 0.6 Å (right) and fit to two separate exponentials, one for the stacked heme pairs and one for the T-shaped/ coplanar heme pairs (solid black lines). Corresponding free-energy-optimized ET-rates $k_{ET}^i$ ($\beta = \Delta A \beta_{Hab}$) are indicated on the axis to the right. The Moser-Dutton ruler is shown for the default packing density ($\rho = 0.76$, dotted lines) and for a reduced packing density accounting for through space tunneling [$\rho = 0.48$ (29), dashed lines]. For comparison, the electron fluxes from heme 10 to the three exit sites 2, 5, and 7 are $0.9 \times 10^6$, $1.5 \times 10^6$, and $2.6 \times 10^5 \text{s}^{-1}$, respectively.

Fig. 3. (A) Computed redox potentials of the heme cofactors in MtrF vs. standard hydrogen electrode, taken from ref. 17, and root-mean-square average couplings for each pair as indicated by circles with area proportional to $\langle |H_{ab}|^2 \rangle$. The $x$ axis refers to the heme labels defined in Fig. 1. Numerical values for $\langle |H_{ab}|^2 \rangle$ are indicated in units of meV, and the same color code as in Fig. 2 is used. (B) Rate constants $k_{ET}^i$ for ET between heme pairs in MtrF for the forward direction (heme $10 \rightarrow 5$, full colors) and for the backward direction (heme $5 \rightarrow 10$, shaded colors). Each bar indicates the rate constant between the two hemes denoted at the base to the left and to the right of the bar unless indicated otherwise. Note the symmetry between forward and backward rates for electron flow along the $10 \rightarrow 5$ and $5 \rightarrow 10$ directions.

The stacked pairs exhibit the smallest distances (3.6–5.0 Å), followed by the T-shaped (5.2–6.5 Å) and coplanar motif (5.3–8.3 Å). For a given donor-acceptor distance, the coupling matrix elements fluctuate strongly in particular for the T-shaped and coplanar motifs as a consequence of the orientational thermal motion of the donor and acceptor hemes. The distance dependence of the resultant thermal root-mean-square averages, $\langle |H_{ab}|^2 \rangle^{1/2}(r)$ (denoted by circles), is best described by two exponential decay constants: one for the stacked motif, $\beta = 2.25 \text{ Å}^{-1}$ and $\lambda_A = 5.55 \text{ meV}$ ($R^2 = 0.9997$), and one for the T-shaped/ coplanar motifs, $\beta = 0.8 \text{ Å}^{-1}$ and $\lambda_A = 0.8 \text{ meV}$ ($R^2 = 0.85$), where we used the fit function $\langle |H_{ab}|^2 \rangle^{1/2}(r) = A \exp(-\beta r - \lambda_A r_0)/2$. $r_0 = 3.6 \text{ Å}$. Although a fit of all data to a single exponential with parameters $\beta = 1.65 \text{ Å}^{-1}$ and $\lambda_A = 3.77 \text{ meV}$ yields a reasonable correlation of $R^2 = 0.91$, two separate fits clearly give a better description of the data. A somewhat smaller decay constant is obtained from a single exponential fit when the Fe-Fe distance metric is used ($\beta = 1.30 \text{ Å}^{-1}$, $R^2 = 0.90$; see Fig. S2 and SI Text for discussion).

The decay constants obtained for the different motifs are within the range of previously reported values for idealized model heme-heme motifs in the gas phase (24). The $\beta$ values obtained from a single exponential fit to all data are a little larger than experimental estimates for tunneling through proteins: $\beta = 1.4 \text{ Å}^{-1}$ using the edge-to-edge metric (25) and $\beta = 1.1 \text{ Å}^{-1}$ using the Fe-Fe distance (26, 27). The small deviation can be rationalized by the fact that our couplings are based on through-space tunneling between closely spaced cofactors (as justified above), whereas the experimental data are predominantly based on protein-mediated electron tunneling reactions over significantly longer distances. Interestingly, the free-energy optimized tunneling rates corresponding to the calculated couplings ($k_{ET}^i$) are one to two orders of magnitude below the empirical Moser-Dutton ruler (dashed and dotted black lines in Fig. 2) (25). It is very unlikely that this is due to inaccuracies of our calculations, as the same computational methodology was shown to give chemically accurate predictions for ET reaction rates in aqueous solution (28) and for coupling matrix elements in small π-conjugated molecules (29). For small distances, the Moser-Dutton ruler relies on a few known rates for bacterial reaction center and photosystem proteins. Visual inspection of crystal structures reveals that despite similar edge-to-edge distances, the porphyrin overlap in the special pair of the reaction center protein is much larger than in MtrF (Fig. S1), which may explain why the computed optimized tunneling rates are not well described by the empirical distance relationship. Our findings reinforce the importance of specific local molecular structure and charge distribution effects on understanding multiheme ET kinetics beyond empirical rules.

Correlation Between Electronic Coupling and Driving Force. The free energy landscape for single-electron flow through MtrF, as we recently reported (17), is shown in Fig. 3A and juxtaposed to the average electronic coupling ($\langle |H_{ab}|^2 \rangle^i$) for each individual heme-heme pair, depicted by circles using the same color code as in solvated MtrF using molecular dynamics simulation. Configurations from the respective MD trajectories were taken to calculate heme-heme electronic coupling matrix elements ($H_{ab}$) using a previously introduced fragment-orbital density functional theory (FODFT) method within a QM/MM scheme (see Materials and Methods for details). The combined set of coupling values obtained are plotted in Fig. 2 against the heme edge-to-edge distance ($r$). The data are color-coded according to the type of heme-heme mutual orientation: the stacked pairs at the entrance and exit of the octaheme chain are shown in blue (10–9, 9–8, 8–7, and 7–6), the T-shaped pairs connecting the octa- and tetra-heme chains in red (8–6 and 1–3), and the coplanar pairs of the tetra-heme chain in green (6–7, 7–6, and 1–2).
Fig. 2. Hemes are arranged following the octa-heme chain from heme 10 to heme 5, with hemes 7 and 2 branching off from heme 6 and 1, respectively. In this presentation, it becomes apparent that the couplings are virtually symmetric with respect to the center of the protein, correlating with the quasi-symmetry of the cofactor arrangement in MtrF (although the surrounding protein is not symmetric). Comparing this “coupling landscape” with the free energy landscape, a correlation between the two emerges: electronic couplings are particularly high for ET with large free energy changes (peaked topology) and significantly smaller for ET with small free energy changes (flat topology). Evidently, electronic coupling is greatest where it matters most: at the energetic up-hill steps 10 → 9 and 3 → 4 in the forward direction for conduction (10 → 5) and at hemes 5 → 4 and 8 → 9 in the reverse direction (5 → 10).

**Heme-to-Heme ET Rates.** Considering the previously reported values for reorganization free energy in solvated MtrF $[\lambda = 0.7 – 1.1 \text{ eV}, \text{obtained from electronically polarizable force field and solvent models (30–32)}]$, we find that $|H_{ab}| \ll \lambda$. That is, the ET is well within the nonadiabatic regime, justifying the picture of a localized electron hopping along the heme wire. Therefore, the computed electronic couplings and previously determined values for a local driving force ($\Delta A = -0.2 \text{ to } +0.1 \text{ eV}$) (17) can be used to calculate microscopic heme-to-heme ET rate constants according to Eq. 3. The stepwise rate constants obtained for electron conduction along the forward (heme 10 → heme 5) and reverse (heme 5 → heme 10) directions are shown in Fig. 3B and summarized in Table S1. The rates span six orders of magnitude, ranging from $2.4 \times 10^9$ s$^{-1}$ for the slowest ET 7 → 6, to $3.1 \times 10^7$ s$^{-1}$ for the fastest ET 4 → 5. Interestingly, the rates decrease by a factor of at most three if the thermally averaged electronic couplings are replaced by the values obtained for the single crystal structure configuration in Eq. 3, indicating that thermal averaging, although important for a quantitative description, has only a moderate effect for this protein. The small influence of finite temperature might be a consequence of the tight binding of the cofactors to the protein via two covalent cystine linkages in addition to the coordinative ligand bonds. The average heme edge-to-edge distances obtained from MD simulation are indeed very similar to the distances in the crystal structure (Table S2). Notably, the stepwise rate constants are almost symmetric in forward and reverse directions, due to the symmetry in electronic couplings and driving force along the chain (effects due to the non-symmetric variations in $\lambda$ are minor). The only asymmetry is caused by pairs 6–7 and 1–2, as discussed elsewhere (17).

**Electron Flux Through MtrF.** In the following, we use the computed heme-to-heme ET rates to estimate the overall steady-state electron flux ($J$) through MtrF using a master equation formalism as detailed in Materials and Methods. We consider the case where the rate for ET from the electron donor to the electron entrance site (heme 10) is much larger than the smallest heme-to-heme ET rate in the protein and vary the rate for ET from the electron exit site (hemes 2, 5, or 7) to the electron accepting substrate ($k_{out}$). The results are shown in Fig. 4. We find that for small output rates $J$ increases linearly with $k_{out}$ to asymptotically reach a maximum flux ($J_{max}$) for large values of $k_{out}$. The relation is well described by the functional form $J = J_{max}/(1 + J_{max}/k_{out})$ as shown in SI Text and Fig. S3. The values obtained are $J_{max} = 0.9 \times 10^{16}$, 1.5 $\times$ 10$^5$, and $2.6 \times 10^4$ s$^{-1}$ for electron exit from hemes 2, 5, and 7, respectively. The corresponding rate-limiting single steps for these routes are $1.2 \times 10^7$ (at 1 → 2), $2.9 \times 10^7$ (at 1 → 3), and $3.0 \times 10^7$ (at 6 → 7). Hence, the order of magnitude difference in the respective rate-limiting steps translates into a corresponding difference in $J_{max}$. The flow out of heme 7 is particularly large as it avoids the slow ET steps between coplanar hemes, 6 → 1 ($k_{16} = 4.0 \times 10^9$).

**Discussion**

Our calculations have revealed a remarkable evolutionary design principle for long-range biological ET. The electronic coupling and driving force are correlated along the heme wires in MtrF; free energy uphill steps occur precisely for those heme pairs that have the largest electronic couplings. Hence, the low potential hemes are incorporated into the protein in a way that does not adversely affect through-protein transport. They may allow for a more efficient reduction of low potential substrates such as flavins. This explanation remains a hypothesis, however, because intermolecular reduction depends on other factors as well, such as the lifetime of the protein-flavin complex, which is unknown, and the residence time of the electron on the high potential hemes, the latter depending on the overall charge state of the protein. The master equation approach enables us to investigate possible scenarios where the subtle balance between electronic couplings and free energies is lost. For example, if we assume that the stacked hemes (high couplings) are replaced by coplanar hemes (low couplings) and that the free energy landscape remains the same, the maximum electron flux would decrease 17-fold from $1.5 \times 10^{10}$ to $9 \times 10^8$ s$^{-1}$. At the same time, the distance traveled by the electron would increase by only a fraction of the one for the original system. Alternatively, considering the couplings as fixed and changing the redox landscape such that the first 0.2-eV uphill step moves from 10 → 9 (stacked) to 6 → 1 (coplanar) would yield a 25-fold decrease to $6 \times 10^7$ s$^{-1}$. These estimates clearly demonstrate that a suitable match of cofactor alignment and redox potentials is key to sustain the inherent maximum possible electron flow to important environmental electron acceptors.

The flux calculations shown in Fig. 4 can be used to interpret recent experiments where MtrCAB was assembled into a proteoliposome and the rates for ET from an internal chemical electron donor (methyl viologen) across the lipid membrane via MtrCAB to solid phase Fe(III) oxides were measured (18). The overall rate constant reported was dependent on the type of Fe(III) mineral used, ranging from 1,133 to 8,500 s$^{-1}$. This observation indicates that transport kinetics was limited by ET from the terminal heme to the mineral. Hence, the highest value reported (8,500 s$^{-1}$) should be considered as a lower limit to the intrinsic flux calculated here for MtrF, a functional homolog of MtrC. Interestingly, this highest experimental rate is not much smaller than our calculated maximum flux through MtrF, ranging from $10^7$ to $10^8$ s$^{-1}$ depending on the heme exit site. A similar
intrinsic flux seems feasible for MtrCAB: modeling MtrA as a dimer of two NrfB (33) proteins, half-inserted into the membrane porin MtrB and docked to MtrF (in place of MtrC) (10), we obtain a steady-state current similar to the one for MtrF alone, provided the free energy landscape for ET through MtrA is not unfavorably shaped (see SI Text for details and Fig. S4). This prediction is based on the reasonable assumptions that heme edge-to-edge distances are not larger in MtrA than in MtrF (which is true for NrfB, used here as a model) and that reorganization free energies in the solvent exposed part of MtrA are also comparable to MtrF. As an aside, a similar flux calculation for electron transport through solvated MtrF in contact with two electrodes (or an approximate adaption to a dry environment) cannot reproduce the nA currents observed in recent current-voltage measurements on conductive bacterial pili (3) (Fig. S5). Although possible explanations are speculative at this point, we think that the high currents observed in experiments are due to the involvement of redox states inaccessible under solution conditions without external bias, as discussed earlier in Wigginton et al. (16, 34).

Our findings open up questions regarding the functionality of MtrF: given that stacked heme pairs exhibit the highest ET rates, why does the protein feature nonstacked heme pairs at all? Three issues seem relevant here. First, a higher total flux may not yield any metabolic benefit if the preceding metabolic reactions or the electron output to external substrates is rate limiting. In this case, there is no evolutionary pressure to increase the flux by further optimizing the mutual orientations of all heme pairs. Second, the coplanar pairs may serve a distinct function in addition to electron transport along the heme network. The coplanar tetra-heme chain 2–1–6–7 exhibits a large contiguous surface area, which could possibly form an effective multipoint contact site for solid substrates that would still be accessible if hemes 10 and 5 were already docking to substrates or partner cytochromes. Structural modeling indicates that it would be difficult to obtain the same contiguous area if hemes 1 and 6 were part of a continuous stacked octa-heme chain. Third, nonstacked hemes are necessary for the formation of the staggered cross heme motif. The latter could serve as a building block for a supramolecular 2D network as previously assumed in a modeling study of a conducting bacterial pili (35).

Conclusions

Our work unveils some evolutionary design principles for long-range biological electron transport. We show that long-range electron transport through the multiheme protein MtrF, and likely its complexes, is not a steady downhill process but one with (potentially many) ups and downs on the free energy landscape. The calculations uncover nature’s method of implementing a dual-purpose electron transport system: uphill processes, possibly necessary for reduction of soluble electron shuttles, are coupled to high electronic coupling matrix elements between closely spaced hemes to achieve metabolically required rates. Heme distance and orientation are suitably selected for this purpose. This picture is consistent with the tight coupling between ET and molecular redoxchemistry well known for oxidoreductases, whose primary function is the reduction/oxidation of soluble substrates within the cell, but expanding its significance in that the same principle carries over to the multiheme proteins that support extracellular respiration.

Materials and Methods

Classical MD. For simulation of the transition state for ET, the charges of the two heme cofactors in question were morphed to the half-reduced state by setting the averages of the charges for the reduced and oxidized state. For each of the nine heme-heme pairs, simulations were initiated from previously equilibrated models of solvated MtrF [specifically, from thermodynamic integration runs with coupling parameter for heme oxidation = 0.5, initiated from the crystal structure of MtrF, Protein Databank (PDB) ID 3PMQ (12); see ref. 17]. The protein was equilibrated for 6 ns at constant pressure and temperature and the following 100 ns used for the calculation of electronic coupling matrix elements. The classical MD simulations were carried out with the AMBER03 protein force field (36) together with the TIP3P water model (37). Force field parameters for the heme cofactors were taken from earlier work (32, 38, 39).

Hout from QM/MM. Twenty-five snapshots per pair were then selected from the corresponding MD trajectories in 4-ns intervals, and the electronic coupling matrix elements were computed using the FODFT method as implemented in the CPMD program (29, 40) on heme QM models interacting with the environment as implemented in the CPMD/Gromos QM/MM coupling scheme (41), and without interaction for comparison. The QM system was comprised of the porphyrin ring with all substituents saturated by a dummy hydrogen atom and the axial histidines replaced by imidazole ligands saturated with a hydrogen at the β-C atom. The Perediev-Burke-Erzerhof (PBE) functional (42) was used for the QM part together with Goedecker-Hutter pseudopotentials (43) (in a semicore version for Fe). The plane-wave cutoff was 130 Ry. Experimental evidence gives the 3d electron configuration of low-spin ferric hemes as d5^2 d7^2, with δ_j denoting a linear combination of the out-of-plane orbitals δ_j^1 and δ_j^2 (44), implying that the ET between two hemes is mediated by the two δ_j^1 states. We found that with PBE, the highest occupied molecular orbital for ferrous heme was consistently δ_j^1 followed by the two closely spaced (quasi-degenerate) δ_j orbitals, denoted here δ_j^1 and δ_j^2. Thus, we chose the latter two orbitals on the donor (D) and acceptor (A) for the coupling calculation

$$
\langle H_{\text{ab}} \rangle = c_{\text{corr}} \left[ \sum_{j \in \Omega_{\text{ab}}} \left( \delta_j^1 \langle \hat{\mu}^D_1 \hat{\mu}^A_1 \rangle \langle \hat{\mu}^A_2 \hat{\mu}^D_2 \rangle \right)^2 \right],
$$

where $c_{\text{corr}}$ is the Kohn–Sham Hamiltonian constructed from fragment orbitals of the reduced donor and acceptor hemes (29, 40) as obtained from QM/MM calculations. The final coupling matrix element is taken as the root mean square over all four possible couplings according to Eq. 2 (23). The additional correction factor $c_{\text{corr}}$ (estimated as 1.75) accounts for the lack of polarization due to the classical treatment of the partner heme and is derived as explained in Table S3. FODFT couplings did not change significantly if a hybrid functional (PBE0) was used (Table S4).

The thermal averages of the squared coupling matrix elements and the previously calculated values for driving forces $\Delta A$ and reorganization free energies $\lambda$ are then inserted in Eq. 3 (45) to obtain heme-to-heme ET rates.
The rates $k_{ij}$ for all heme-to-heme ET steps, together with the corresponding values for $(\Delta H_{i}^{\ddagger})$, $\lambda$, and $\Delta\alpha$ are summarized in Table S1.

Electron Flux from the Master Equation. We assume that the electron flux from heme $i$ to heme $j$, $j_{i} = j_{i,j}$, can be described by a master equation $j_{i} = k_{i}P_{i}(1-P_{j}) - k_{j}P_{j}(1-P_{i})$, with $k_{ij}$ being the ET rate constant according to Eq. 3 and $P_{i}$ being the electron population of heme $i$ (0 for oxidized heme, 1 for reduced) (46, 47). The terms $(1-P_{i})$ account for the fact that each heme can be occupied by only one electron. The external electron donor and acceptor are assumed to be in excess concentration. Thus, the fluxes into the protein entrance site (e.g., heme 10) and out of the protein exit site (e.g., heme 5) are given by $J_{10,in}=k_{10,in}P_{10} - k_{10,out}P_{10}$ and $J_{5,out}=k_{5,out}P_{5} - k_{5,in}P_{5}$, respectively. Requiring steady state, i.e., $J_{10,in}=J_{10,out}=0$ in $i \forall$ gives the following recursive relationship for the steady-state populations: $P_{i} = \left( \frac{k_{i}P_{i+1}}{k_{i+1}P_{i}} \right) + \left( \frac{k_{i}P_{i+1}}{k_{i+1}P_{i}} \right)$, which can be solved for all $P_{i}$, insertion of $P_{j}$ and $J_{i}$ in the above expression for $J_{i}$ gives the steady-state flux $J_{i,j}$. For the modeling of electron flux through MtrF (Fig. 4), we assumed that ET in and out of the protein is irreversible ($k_{10,out}=k_{5,in}=0$). This assertion should give a good description of the experimental conditions, where the reduced potential of the external electron donor (MV) is much lower than the ones for the hemes, and the external acceptor (reduced iron oxo) is removed from equilibrium through dissolution (18). Similar calculations are carried out for the exit sites of hemes 2 and 7.

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Supporting Information

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SI Text

Calculation of Electronic Coupling Matrix Elements. Derivation of correction factor \( c_{\text{corr}} \). In the standard fragment-orbital density functional theory (FODFT) method for calculation of \( H_{ab} \), the diabatic states are constructed by combining Kohn–Sham orbitals optimized for the two isolated monomer fragments in the gas phase (1, 2). Thus, spurious delocalization of the excess electron hole due to the electron delocalization error of standard exchange correlation functionals is avoided, but possible electronic polarization effects between the two monomers are missing. To estimate this contribution, coupling matrix elements obtained with FODFT were compared with values obtained with constrained density functional theory (CDDF) (3) as implemented in CPMD (1), where the diabatic states are constructed by optimizing the density for the dimer in the gas phase subject to a constraint on the charge difference between donor and acceptor.

We found that CDDFT(PBE) optimizations (i.e., using the Perdew–Burke–Ernzerhof, or PBE, density functional) on the heme dimers taken from the protein structure resulted in relatively large spin leakage from the ferric to the ferrous heme. Although we have not investigated this problem further, it is likely that it can be cured by using CDDFT with exact exchange functionals (4). Instead, we found that CDDFT(PBE) calculations on a set of idealized dimer structures as used by Smith et al. (5) were straightforward and did not yield spurious spin delocalization. Hence, we used the five heme dimer structures of Smith et al. (5) at an Fe–Fe distance of 16.0 Å for the comparison between CDDFT and FODFT calculations (Table S3). All calculations used the same electronic structure method as for the calculations on the heme pairs in the protein, i.e., PBE functional, a 130 Ryd plane wave cutoff, and Goedecker–Teter–Hutter pseudopotentials (semicore for Fe). We found that for each configuration, the ratio \( H_{ab}(\text{CDDF})/H_{ab}(\text{FODFT}) \) lies close to their average of 1.75. The latter can be regarded as a correction factor for the missing electronic polarization between donor and acceptor in the FODFT calculations.

In the FODFT quantum mechanics/molecular mechanics (QM/MM) method, the calculation of \( H_{ab} \) is carried out similarly as above for the gas phase. The only difference is that the density of one monomer fragment is optimized in the static electrostatic field created by the force field charges of the other monomer fragment, the protein, and the solvent (the MM part). As mentioned in the main text, the effect of the MM part on the coupling values is negligibly small, not more than about 10%. This small effect probably means that the electronic polarization between donor and acceptor is not effectively mimicked by the charges of the MM fragment (a compensation between the latter and the protein/water seems unlikely). Thus, to account for the missing electronic polarization effects between the two fragments, we opted to apply the same correction factor as obtained from the gas-phase calculations above, i.e., the \( H_{ab}(\text{FODFT}) \) values obtained from QM/MM are multiplied by \( c_{\text{corr}} = (H_{ab}(\text{CDDF})/H_{ab}(\text{FODFT})) = 1.75 \) as indicated in Eq. 2. Table S1 summarizes the corrected FODFT QM/MM \( H_{ab} \) values for the heme pairs in the crystal structure and averaged over molecular dynamics (MD) trajectories. The latter were used for the calculation of electron transfer (ET) rates.

Impact of functional on FODFT couplings. To check whether the particular choice of the generalized approximation functional had any impact on the coupling matrix elements, we also calculated FODFT couplings for the five test dimers above using the BLYP functional (i.e., Becke exchange part, Lee–Yang–Parr correlation part) instead of PBE. The numbers obtained were virtually identical. To check whether exact exchange was relevant to FODFT couplings between the protein cofactors, PBE and PBE0 were compared for the nine heme pairs in the crystal structure of MtrF (at a lower plane wave cutoff of 90 Ryd to keep the exact exchange calculations feasible and in the gas phase). As shown in Table S4, for the majority of pairs, the difference between PBE and PBE0 is relatively small. The average change from PBE to PBE0 is around 10%; although individual changes are larger, if they average out to a small global change here then something similar could be expected for the ensemble averages to be calculated from the snapshots extracted from MD. It was hence concluded that exact exchange effects could be neglected in calculating the FODFT ensemble averages \( \langle |H_{ab}|^2 \rangle \).

Electronic Coupling Decay: Edge-to-Edge vs. Fe-Fe Distance Metric. In addition to the edge-to-edge distance metric used in Fig. 2, we also determined the coupling decay with respect to the Fe-Fe distance (Fig. S2). For the T-shaped and coplanar pairs, this yields a decay constant \( \beta = 0.6 \, \text{Å}^{-1} \) \((R^2 = 0.79)\), similar to the 0.8 Å\(^{-1}\) \((R^2 = 0.85)\) for edge-to-edge distance owing to the fact that both distances increase in the same way (the Fe-Fe distance increases by about 1 Å for every 1 Å increase in the edge-to-edge distance). For the stacked pairs, however, the Fe-Fe distance yields a lower decay constant: \( \beta = 1.05 \, \text{Å}^{-1} \) \((R^2 = 0.96)\) compared with 2.25 Å\(^{-1}\) \((R^2 = 0.9997)\) for edge-to-edge distance. This decrease in \( \beta \) can be rationalized by the fact that for the stacked pairs, the Fe-Fe distance increases about twice as fast as the edge-to-edge distance (i.e., for an edge-to-edge distance increase of 1 Å the Fe-Fe distance increases by around 2 Å) so that the distance decay is just about half as strong. A global fit would yield a decay constant of \( \beta = 1.30 \, \text{Å}^{-1} \) \((R^2 = 0.90)\) compared with \( \beta = 1.65 \, \text{Å}^{-1} \) \((R^2 = 0.91)\) for edge-to-edge, but the two individual fits describe the individual bin points better as measured by average absolute differences between bin points and regression. Thus, the two metrics give a similar description for coplanar and the T-shaped motifs, but the edge-to-edge distance metric is clearly superior for stacked pairs \((R^2 \text{ very close to } 1)\). The latter metric’s better performance stems from the fact that, according to DFT calculations, the atoms of the macrocycles contribute to the electron mediating molecular orbitals with their \( p_z \) atomic orbitals (Fig. 1C) and one can expect that the overlap between these \( p_z \) atomic orbitals determines to a large extent the total electronic coupling.

Analytical Model Describing Electron Flux Through MtrF. We derive an analytical function for the electron flux \( J \) as a function of \( k_{\text{out}} \) (Fig. 4) by coarse graining the states of the system. We assume that electron transport in MtrF can be modeled by four distinct sites A to D: site A corresponds to an external donor injecting electrons into the protein; site B denotes the protein electron entrance site; site C represents the protein electron egress site; and site D denotes an external electron acceptor. The kinetics is then described by the following scheme:

\[
A \xrightarrow{k_a} B \xrightarrow{k_{\text{in}}} C \xrightarrow{k_{\text{out}}} D.
\]

where electron injection into the protein (A to B) and ejection from the protein (C to D) is considered irreversible as can be assumed to be the case in the experiments of White et al. (6). The complicated kinetics of electron flux through the protein is condensed into one effective forward and backward rate constant \( k_t \)
and $k_b$, respectively. Under steady-state conditions, the net flux $J$ between any two adjacent sites is equal

$$J = k_{in}(1 - P_B) = k_iP_B(1 - P_C) - k_b(1 - P_B)P_C = k_{out}P_C.$$  \[S2\]

where $P_B$ and $P_C$ denote populations in the interval [0, 1]. The populations of A and D are assumed to be 1 and 0 at all times, i.e., the electron donor is in excess concentrations and the electron on the acceptor is immediately removed from equilibrium. Hence these populations do not appear in the expression for the flux. The factors $(1 - P_B)$ and $(1 - P_C)$ account for the fact that sites B and C can only be occupied by at most one electron. We thus have two equations for the two unknowns $P_B$ and $P_C$. Solving for $P_B$ in terms of $P_C$ and reinserting to solve for $P_C$ yields the equation

$$P_C = \left(\frac{k_i}{k_i + k_{in}}\right)P_C^2 - \left(\frac{k_i}{k_i + k_{out}}\right)P_C + \frac{k_i}{k_{out}}.$$  \[S3\]

In the limit of high electron injection rates, $k_{in} \gg k_i, k_b$, Eq. 3 reduces to the simple expression

$$P_C = \frac{k_i}{k_i + k_{out}}.$$  \[S4\]

Insertion of Eq. S4 in the last identity of Eq. S2 gives

$$J = \frac{k_i}{1 + \frac{k_i}{k_{out}}}.$$  \[S5\]

Thus, for high injection rates, the flux through the protein depends only on the effective forward rate $k_i$ and the ejection rate $k_{out}$. Furthermore, if we take the limit $k_{out} \rightarrow \infty$, $J$ becomes equal to $k_i$: The latter in fact represents the maximal possible flux $J_{\text{max}}$ through the protein. We can therefore rewrite Eq. S5 as

$$J = \frac{J_{\text{max}}}{1 + \frac{J_{\text{max}}}{k_{out}}}.$$  \[S6\]

Fig. S3 shows the curves for $J$ vs. $k_{out}$ along the octa-heme chain of MtrF in the 10 → 5 and 5 → 10 direction as obtained by solving the master equation for the full problem (solid lines). Fits to Eq. S6 (dash-dotted lines) match the respective data very well, with values $J_{\text{max}} = 1.57 \times 10^4$ for the forward and $J_{\text{max}} = 1.06 \times 10^4$ for the backward direction. Thus, the full kinetic problem can be successfully mapped on the coarse four-state model in Eq. S1.

**Modeling of Electron Flux Through MtrCAB.** In the following, we try to model electron flux through the multiheme protein complex MtrCAB, i.e., the transmembrane complex assembled in a proteoliposome by White et al. (6), where electrons are transported from liposome-contained methyl viologen via MtrCAB to external iron oxides. MtrA is known to be a deca-heme cytochrome (7), whereas MtrB is a membrane pore protein proposed to enable close contact between MtrA and MtrC (8). We thus need a model and ET parameters for MtrC, MtrA, and the contact between them. For MtrC, we use our ET parameters for MtrF motivated by the homology model of ref. 9, and we assume electron transport along the octa-heme chain from heme 10 to heme 5 (egress site). The structure of MtrA is not known; however, the heme-binding motifs in its N-terminal half can be sequence-aligned with the penta-heme cytochrome NrfB (10), and MtrA has been found to be of a rod-like shape of around 100 Å length (11). We therefore decided to model MtrA as a NrfB head-to-tail homodimer. We can then use our regressions for the two coupling regimes in Fig. 2 to estimate approximate couplings for the heme pairs in the crystal structure of NrfB (12), as well as for the contact between the two NrfB subunits on the one hand and between NrfB and MtrC/F on the other hand (by some crude manual docking of protein structures that should suffice for this modeling). In regard to reorganization free energies, the most significant difference should occur between heme pairs located in the solvent-exposed part of MtrA and those located within the membrane-buried part making contact to MtrC. With a membrane thickness of around 40–50 Å (6), MtrA should be roughly half-buried into the membrane, with the other half exposed into the periplasm. Thus, for the first five heme pairs (as well as the final MtrA-MtrC contact), we assume a reorganization free energy of 0.9 eV (i.e., a typical number for MtrF), whereas for the four remaining membrane-buried pairs, we assume 0.57 eV, the reorganization energy previously obtained (13) for heme a to heme a$_2$ ET in membrane-embedded cytochrome c oxidase. The final set of parameters to be estimated are then the driving forces of each ET step. Although the overall electrochemical response of MtrA has been studied (8), redox potentials of individual cofactors are not known. A crude fit to the voltammogram in Hartshorne et al. (8) yields a set of 10 distinct redox potentials, however, that enable to estimate minimal and maximal flux through MtrCAB within the model described thus far by assigning redox potentials to the 10 cofactors of MtrA to either yield the smallest possible or highest possible rate-limiting single ET rate. We thereby obtain the two curves in Fig. S4, delimiting upper and lower limits for the flux through MtrCAB based on our model. As can be seen, depending on the combination of parameters the flux through MtrCAB could reach the same level as for MtrF itself (maximal flux for flux-maximizing parameters, black curve: 14,300 s$^{-1}$); it could also be one order of magnitude smaller than for MtrF (maximal flux for flux-minimizing parameters, blue curve: 800 s$^{-1}$), but this is rather unlikely as it requires the steepest possible free energy uphill step to have a small electronic coupling and a high reorganization energy.

**Current-Voltage Response of MtrF in Solution and in Air.** Modeling of current-voltage response. Pioneering measurements using an atomic force microscopy tip and a gold electrode revealed that bacterial pili can support very high currents of several nano-Ampere at moderate voltages (14, 15). It is generally thought that the conduction along pili is facilitated by multiheme proteins, and that when a multiheme protein is sandwiched between two electrodes, the conduction occurs via electron hopping along the heme groups (16, 17). Here we would like to investigate if the hopping mechanism can account for the observed nano-Ampere currents when the ET parameters are used that are reported in our current and previous works. To this end, we model the current-voltage response of a single MtrF protein placed between two electrodes with potential difference $V$ by solving a master equation similarly as described in the main text (see below for details), but with the vital difference that the driving force for heme-heme ET is gradually decreased by $eV/(n + 1)$ as the electrode potential difference is stepped up ($e$ is the unit charge and $n$ the number of hemes between the left and the right electrodes).

The current-voltage characteristic obtained for the calculated ET parameters in solution is illustrated in Fig. S5 (black lines). Two different regimes are shown depending on the ratio of heterogeneous input (=output) rate, $k_{10,\text{in}} = \left(k_{out} \right)$, and the smallest heme-heme ET rate, $k_{\text{min}}$, evaluated at zero potential bias, $r = k_{10,\text{in}}/k_{\text{min}}$. The current shown in solid lines ($r = 100$) is limited by ET through MtrF, and the current shown in dash dotted lines ($r = 1$) is, where different from the solid line, limited by heterogeneous ET. We find that the increase in current is approximately linear at low voltages but sharply increases to a maximum at $V \approx |n + 1|/e$ to decrease for higher voltages. The
existence of a maximum is a consequence of the parabolic relationship between \( \log k_{\text{ET}} \) and \( \Delta A \) (Eq. 3). The maximum current we obtain at protein-limiting conditions is 36 picoampere (pA) at a voltage of 8.2 V.

Previous experimental I-V measurements on conductive pili were carried out in air rather than solution. To account for the different environmental conditions, we reduce all reorganization free energies by around 50% (18–20) and set the heterogeneous redox potential differences, i.e., driving forces, all equal to zero (see details below for a justification). The resultant I-V curves are shown in red for \( r = 10 \) (solid line) and \( r = 1 \) (dash dotted). We observe again a linear increase in the current for low voltages (see inset), but a significant shift of the position of the maximum to smaller voltages, as is expected from the above relation between \( \lambda \) and \( V \). The maximum current at protein-limiting conditions is 58 pA at 3.6 V. Thus, our calculations suggest that the surrounding medium (air/solution) has a large effect on the voltage range but only a relatively small effect on the maximum current.

**Discussion.** Although our calculations are consistent with the kinetic measurements of White et al. (6) on MtrCAB, they do not reproduce the nano-Ampere currents observed in current-voltage measurements on bacterial pili (14, 15). Two factors could contribute to this discrepancy. (i) The measurements were directly conducted on the pili, and it is unknown how many proteins mediate the current. In ref. 16, it was estimated that the single protein current should be multiplied by at most a factor of \( 10^2 \), which would indeed shift the calculated pA currents into the nano-Ampere regime. However, we believe that a more realistic upper estimate would be one to two orders of magnitude lower as the factor of \( 10^2 \) was based on a hypothetical closest packing of hemes without any protein matrix. Hence, it is unlikely that the discrepancy can be explained by the protein number density alone. (ii) We assumed that the current in MtrF is mediated by electron hopping between neighboring \( Fe^{2+-3+} \)-hemes according to Eq. 1. Although this is almost certainly the dominating ET mechanism in the kinetic experiments on MtrCAB (6), it may not be the dominating conduction channel in the I-V measurements. A possible alternative could be that conduction is predominantly mediated by higher-lying, nonoccupied electronic states (21), such as the \( \epsilon_g \) manifold of the low-spin hemes or more delocalized states of the porphyrine ring. In this case, all hemes are likely to be in their reduced (neutral) state and temporarily adopt a negative charge when the conducting electron passes by.

**Modeling details.** The curves in Fig. S5 are calculated using the convention \( I = -J \). For calculation of the heme-to-heme ET rates in solution, the same \( H_{ab} \) and \( \lambda \) values are used as in the main text (summarized in Table S1), but with suitably modified driving forces: \( \Delta A \rightarrow \Delta A - e V / (n+1) \). The heterogeneous ET steps between heme 10 and the left electrode and heme 5 and the right electrode are treated as reversible (i.e., \( k_{10,\text{out}} = k_{5,\text{in}} \neq 0 \)) and symmetric (i.e., \( k_{10,\text{in}} = k_{5,\text{out}} \) and \( k_{10,\text{out}} = k_{5,\text{in}} \)) and are obtained via the electrochemical form of the nonadiabatic ET rate equation (16, 22)

\[
k_{10,\text{in}} = k_{\text{elec}} \int_{-\infty}^{+\infty} \exp \left[ -\left( \frac{\lambda + e(E - E_{10})}{k_B T} \right)^2 \frac{k_B T}{4\lambda} \right] dx, \tag{S7}
\]

\[
k_{10,\text{out}} = k_{\text{elec}} \int_{-\infty}^{+\infty} \exp \left[ -\left( \frac{\lambda - e(E - E_{10})}{k_B T} \right)^2 \frac{k_B T}{4\lambda} \right] dx, \tag{S8}
\]

where \( \lambda \) is the reorganization energy of the heterogeneous ET step, \( E \) is the potential level at the electrode, \( E_{10} \) is the potential at heme 10, \( k_{\text{elec}} \) is a constant denoting the average coupling between heme and electrode, and \( k_B \) and \( T \) are Boltzmann constant and temperature, respectively. Hence, to estimate the heterogeneous ET rates at the electrodes via this pair of equations, estimates are needed for the reorganization free energy \( \lambda \), the local potential drop \( E - E_{10} \), and the coupling constant \( k_{\text{elec}} \). Although no values are available for the reorganization free energy of MtrF in contact with an electrode, electrochemical measurements of WT and mutated cytochrome c on a gold electrode (23) yielded reorganization free energies of around 0.45 eV, which we chose to use in our model of current-voltage response in solution. For the potential drop at the electrode, we made the assumption that it is comparable to the voltage drop between adjacent hemes, which we also assumed to be equal (ignoring minor differences due to the nonlinear arrangement of hemes along the octa-heme chain): i.e., all potential drops were set to \( V/(n+1) \) with \( n \) as the number of redox sites between the two electrodes (eight for electron transport along the octa-heme chain), i.e., \( V/(n+1) = 0.11 \), which is similar to experimental estimates (14). For the electrode contacts, \( 1/2 \times \Delta E^0_{10,\text{in}} \), half the redox potential difference between the terminal hemes 10 and 5 was added to correct for the unequal potential levels of the terminal hemes at zero bias potential (yielding a nonzero current at zero potential otherwise).

The remaining parameter is then \( k_{\text{elec}} \), the constant summarizing the overall coupling between terminal redox site and electrode. Rather than just making one assumption for this electrode-protein coupling, we tried different values to obtain the current-voltage response in different regimes. For \( r = 100 \) (solution) and \( r = 10 \) (air), respectively (see above for the definition of \( r \)), we observe that the heterogeneous transfer rate at the electrodes is larger than the smallest intraprotein rate, indicating that for this value (and higher values) of \( r \) the current-voltage response is protein limited, giving rise to a clear maximum in \( I \) in Fig. S5 (solid lines), indicating the transition between normal and inverted Marcus regime for nonadiabatic ET. Decreasing \( r \) to 1 yields the broken lines that for dry conditions at first show an electrode-limited response, reaching a constant current indicating the maximal overlap between Gaussian redox peak and Fermi distribution in Eq. S7, until the slowest protein rate becomes slower than the heterogeneous rate on which the curve matches the corresponding curve for higher \( r \) again. For solution conditions, only the electrode-limited regime is visible in the potential range studied.

Under dry conditions, i.e., for a measurement in ambient air, we assume the couplings to stay the same given that these depend only on the heme cofactor arrangement that should not change significantly. In regard to the reorganization free energies, a significant change can be expected given that they are known to be highly affected by the solvent environment. Specifically, Tipmanne et al. (20) found for a set of model ET proteins that the solvent contributed one-half to three-quarters of the total reorganization free energy. Without more precise information on the corresponding solvent contributions in MtrF, we chose a \( \lambda \) of 0.4 eV for intraprotein ET and 0.2 eV at the protein-electrode interface for our ambient air model. In regard to driving forces, no information is available on these under dry conditions, but it can be assumed that without a solvation shell, ionizable groups should be either unionized or binding a counter ion so that the electrostatic potential in ambient air should be much more homogeneous than in water. We therefore decided to set all intrinsic driving forces equal to zero so that the total driving forces are equal to the contribution from the external potential. Obviously this crude set of parameters does not allow for detailed predictions and is only used to get an idea what the current-voltage behavior might be under dry conditions.
Fig. S1. Comparison of heme dimer motifs in MtrF with metal-containing porphyrin dimers in other ET-related proteins. (A) T-shaped pair 6–8 from MtrF (red/orange) vs. heme a-heme \(a_3\) from cytochrome \(c\) oxidase \(\text{blue/cyan} \); Protein Databank (PDB) ID 1V54. (B) Stacked pair 4–5 from MtrF (red/orange) vs. the chlorophyll-special pair from the photosynthetic reaction center from \(\text{Rh. sphaeroides}\) \(\text{blue/cyan}\); PDB ID 1M3X.
Fig. S2. Modulus of electronic coupling matrix elements ($|H_{ab}|$) for ET between ferrous and ferric heme cofactors in MtrF as a function of the heme Fe-Fe distance. Data points and fits are obtained as explained in Fig. 2, and the same color code is used. Bin width for $|H_{ab}|^2$ (circles) is 0.5625 (Left) and 0.7 Å (Right).

Fig. S3. $J$ vs. $k_{out}$ for electron flux along the octa-heme chain in MtrF in both directions (solid lines; black: forward/10 → 5, blue: backward/5 → 10), together with analytic fits to the numeric curves using Eq. S6 (broken lines).

Fig. S4. $J$ vs. $k_{out}$ for electron flux through the protein complex MtrCAB (see text for model applied). Black curve: redox potentials in MtrA chosen to maximize flux; blue curve: redox potentials chosen to minimize flux.
Fig. S5. Current (I)-voltage (V) response of a single MtrF molecule in solution (black lines) and in air (red lines) assuming electron hopping as the conduction channel. Two different regimes are shown, protein limiting \( r = 100 \) (black solid line), \( r = 10 \) (red solid line) and electrode-protein limiting \( r = 1 \) (black and red dash dotted lines). See text for definition of \( r \).

Table S1. Coupling matrix elements \( |H_{ab}| \) obtained from QM/MM FODFT calculations according to Eq. 2 (\( c_{corr} = 1.75 \)), driving forces \( \Delta A_{ji} \) (1), reorganization free energies \( \lambda \) (2), and heme-heme ET rates for solvated MtrF, \( k_{ji} \) and \( k_{ij} \).

| Pair \( i-j \) | \( |H_{ab}| \) (crystal structure) (meV) | \( \langle |H_{ab}| \rangle^2 \) (MD) (meV) | \( \sigma(|H_{ab}|) \) (MD) (meV) | \( \Delta A_{ji} \) (eV) | \( \lambda \) (eV) | \( k_{ji} \) (s\(^{-1}\)) | \( k_{ij} \) (s\(^{-1}\)) |
|-----------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| 1–2       | 0.27            | 0.24            | 0.09            | 0.02            | 1.13            | \( 1.18 \times 10^4 \) | \( 2.19 \times 10^4 \) |
| 1–3       | 0.31            | 0.49            | 0.21            | 0.12            | 0.96            | \( 2.89 \times 10^4 \) | \( 3.37 \times 10^4 \) |
| 1–6       | 0.08            | 0.13            | 0.05            | 0.01            | 0.94            | \( 2.68 \times 10^4 \) | \( 3.95 \times 10^4 \) |
| 3–4       | 1.71            | 2.21            | 0.67            | 0.10            | 0.75            | \( 8.10 \times 10^5 \) | \( 4.19 \times 10^5 \) |
| 4–5       | 2.34            | 3.63            | 1.26            | -0.22           | 0.84            | \( 3.12 \times 10^5 \) | \( 5.81 \times 10^5 \) |
| 6–7       | 0.28            | 0.23            | 0.11            | -0.13           | 1.06            | \( 2.99 \times 10^5 \) | \( 2.38 \times 10^5 \) |
| 6–8       | 0.29            | 0.31            | 0.16            | 0.10            | 0.87            | \( 4.47 \times 10^5 \) | \( 2.31 \times 10^5 \) |
| 8–9       | 2.64            | 2.31            | 1.08            | 0.13            | 0.93            | \( 8.46 \times 10^5 \) | \( 1.11 \times 10^6 \) |
| 9–10      | 4.59            | 4.52            | 1.52            | -0.19           | 0.99            | \( 6.14 \times 10^8 \) | \( 4.43 \times 10^5 \) |

Electronic coupling for the single crystal structure configuration is compared with the root-mean-square average obtained from MD simulation, \( \langle |H_{ab}|^2 \rangle^\frac{1}{2}, \sigma = \langle |H_{ab}| - \langle |H_{ab}| \rangle \rangle^\frac{1}{2} \). The notation \( \Delta A_{ji} \) and \( k_{ji} \) refers to ET from heme \( i \) to \( j \) and \( k_{ij} \) is for ET from heme \( j \) to \( i \). Rate constants are calculated according to the nonadiabatic expression Eq. 3. See Fig. 1 for definition of heme labels.


Table S2. Heme edge-to-edge distance \( R \) in the crystal structure of MtrF [PDB ID 3PMQ (1)] and the average and root-mean-square fluctuation obtained from 100-ns MD simulation.

<table>
<thead>
<tr>
<th>Pair ( i-j )</th>
<th>( R ) (crystal structure) (Å)</th>
<th>( \langle R \rangle ) (MD) (Å)</th>
<th>( \sigma(R) ) (MD) (Å)</th>
</tr>
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<tr>
<td>1–2</td>
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<td>0.54</td>
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<td>1–3</td>
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<td>4.42</td>
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</tr>
<tr>
<td>9–10</td>
<td>3.94</td>
<td>3.82</td>
<td>0.17</td>
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</table>

Table S3. Coupling matrix elements $|H_{ab}|$ for five model heme dimers in the gas phase (1) as obtained with FODFT and CDFT

<table>
<thead>
<tr>
<th>Structure</th>
<th>FODFT (meV)</th>
<th>CDFT (meV)</th>
<th>CDFT/FODFT</th>
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<tbody>
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</tr>
<tr>
<td>B</td>
<td>0.48</td>
<td>0.79</td>
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</tr>
<tr>
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</tr>
<tr>
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<td>0.16</td>
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</tbody>
</table>


Table S4. Coupling matrix elements $|H_{ab}|$ for the nine heme pairs in the crystal structure of MtrF [PDB ID 3PMQ (1)] as obtained from gas phase FODFT calculations according to Eq. 2 ($c_{corr} = 1.75$) using the PBE and PBE0 functional

<table>
<thead>
<tr>
<th>Pair</th>
<th>PBE (meV)</th>
<th>PBE0 (meV)</th>
<th>PBE0/PBE</th>
</tr>
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<tr>
<td>1–2</td>
<td>0.59</td>
<td>0.64</td>
<td>1.09</td>
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<td>1–3</td>
<td>0.85</td>
<td>0.79</td>
<td>0.93</td>
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<tr>
<td>1–6</td>
<td>0.68</td>
<td>0.81</td>
<td>1.20</td>
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<tr>
<td>3–4</td>
<td>3.00</td>
<td>2.39</td>
<td>0.80</td>
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<tr>
<td>4–5</td>
<td>3.93</td>
<td>3.34</td>
<td>0.85</td>
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<tr>
<td>6–7</td>
<td>0.60</td>
<td>0.40</td>
<td>0.67</td>
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<tr>
<td>6–8</td>
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<td>1.20</td>
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<td>3.36</td>
<td>3.76</td>
<td>1.12</td>
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<tr>
<td>9–10</td>
<td>3.22</td>
<td>1.92</td>
<td>0.60</td>
</tr>
</tbody>
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