COMMENTARY

Multiple hops move electrons from bacteria to rocks

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The central dogma of bioenergetics is that photons energize electrons in molecules, those electrons push protons, and proton concentration gradients forge chemical bonds. An understanding of how this works—at the level of electrons and atoms—is elusive, especially in organisms that live life on the edge of survival. How do bacteria or bacterial cables move electrons between sources and sinks separated by micrometers to centimeters? In PNAS, van Wonderen et al. show that a heme-to-heme electron hopping transport mechanism enables the egress of electrons through the bacterial cell envelope in "rock-breathing" bacteria that use minerals as their terminal electron acceptors (1).

The stunning discovery that some biological redox reactions can proceed at cryogenic temperatures (2) motivated the development of vibronically coupled electron-tunneling theories (3). Theory predicted that electron transfer (ET) reactions could occur over several nanometers on time scales as short as milliseconds, supporting the notion that electron tunneling could undergird molecular bioenergetics. Experimental studies of metal-labeled proteins (4, 5) and tunneling pathway theories (6) brought atomistic detail to the description of protein-mediated ET. These studies indicate that electron tunneling is enabled by both through-bond and through-space interactions, dictated by the molecular structure of the folded protein and by its cofactors.

Electron transport through extracellular appendages (bacterial nanowires) on micrometer length scales is not reconciled by the theory of single-step electron tunneling (7). How can electrons exit the cell envelope and propagate for micrometers on biologically relevant timescales with little thermodynamic driving force? Modeling of nanowires requires that electron tunneling would have to occur between cofactors in near van der Waals (vdW) contact to explain the measured currents (7, 8). In PNAS, van Wonderen et al. report that transport in the decaheme MtrC protein, which enables electrons to flow across the bacterial cell envelope to the nanowires, also occurs by rapid through-space tunneling between cofactors jammed into vdW contact (1).

A particularly powerful approach to determine how proteins mediate electron tunneling is to attach ET active molecules that inject or remove an electron (4). Then, charge motion can be tracked through the protein using transient spectroscopy. Studies of this kind produced "timetables" for electron tunneling (9), validating the predictions of the tunneling pathway theory that identifies the key through-bond and through-space interactions that underpin protein-mediated electron tunneling (10). A tenet of the tunneling pathway theory is that chemical bonds present lower tunneling barriers than empty space (so tunneling rates are faster through bond than through space at a given distance). For example, tunneling a 5-Å gap through bond is predicted to be about 30 times faster than tunneling through space. Even with tunneling acceleration provided by bonded networks, electrons cannot tunnel across membranes and larger structures in a single step. Evolution has discovered a trick to accelerate electron transport over these longer distances.

Daisy chaining many short tunneling steps accelerates electron transport exponentially compared to proceeding in one long step, and living systems exploit this multistep hopping mechanism. Extracellular bacterial appendages—nanowires—use hopping to move electrons over micrometer distances, and cable bacteria seem to use the same strategy on the centimeter length scale (11). Multistep hopping allows some bacteria to dump low-energy waste electrons into extracellular charge sinks, like iron oxide, when oxygen is not available. The measured electron currents in nanowires can only be

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rationalized when long chains of cofactors are arrayed in near vdW contact (7). The three-dimensional (3D) structure of multiheme ET proteins, improving theoretical descriptions of heme-to-heme coupling interactions, and advances in kinetic modeling support the multistep hopping mechanism (12). While short distances produce faster tunneling, through-space tunneling between cofactors in contact is not usually assisted by amino acid residues, since mediating atoms cannot typically squeeze into the tiny gaps between cofactors to boost the couplings. As such, the barrier height for through-space tunneling is much larger than for bond-mediated tunneling. Popular exponential decay models for ET rates (13) are anchored at short distance by data taken from ultrafast photosynthetic reactions associated with electronic excited states, and uncertainties surrounding reaction free energies, state delocalization, and transport mechanisms complicate the analysis of the associated rates (14). The tunneling pathway model explicitly weights through-space and through-bond contributions to tunneling, as dictated by the 3D protein structure and the cofactors. Pathway theory therefore predicts slower-than-average ET rates across through-space gaps compared to covalent media (10, 15). The transition between through-space and through-bond tunneling mechanisms with distance is explained in Fig. 1. Additional complexity may arise from the orbital symmetry of the cofactors, their relative orientation, conformational dynamics, and nearby amino acids that may polarize or indirectly couple them.

The exit of electrons from *Shewenella* to extracellular nanowires requires the arduous transit across the cell envelope, a distance of ~160 Å, via the MtrA and MtrC proteins. van Wonderen et al. (1) probed the heme-to-heme transfer steps in MtrC. The studies used the approach of Gray and coworkers (4) to inject an electron to heme 10 by photoexciting a ruthenium complex attached to the protein's surface. The charge then executes a few tunneling steps before being trapped on the chemically modified heme 8. Electron tunneling between hemes was found to occur on the microsecond timescale over about ~4-Å distances (the computed rate for free energy optimized tunneling between hemes is on the nanosecond time scale). The average decay model (13) overestimates these rates by several orders of magnitude—perhaps because of the short-distance anchoring of the simple exponential model, as described above, and the weakness of through-space tunneling between directly interacting hemes.

The tunneling pathway model predicts rapid tunneling decay across through-space contacts. Early experimental studies on metal-labeled heme proteins anticipated this behavior: ET rates in heme proteins extrapolated to contact distances are found to fall well below the "universal" rate predictions, consistent with substantial through-space tunneling penalties (5). Interestingly, direct through-space tunneling between hemes also mediates ET in heme copper oxidases, and pathway-style models are required to understand those redox reactions too (16).

Electron hopping networks appear throughout biochemistry. Multistep hopping is important in biosynthesis (17) and may underpin oxidative damage protection (18). Hopping chains have been engineered in metal-modified proteins (19), and biological signaling networks seem to engage multistep hopping networks (20). Cable bacteria (11) appear to use centimeter-scale hopping transport, and electron bifurcation reactions requires a two-electron donor to deliver electrons into two spatially separated hopping chains (21).

The MtrC studies reported in PNAS (1) are initiated by charge injection from a surface-tethered redox active species. As such, the measurements track the return to equilibrium following light-induced reduction. Most biological charge-hopping chains, like those found in electron bifurcation or

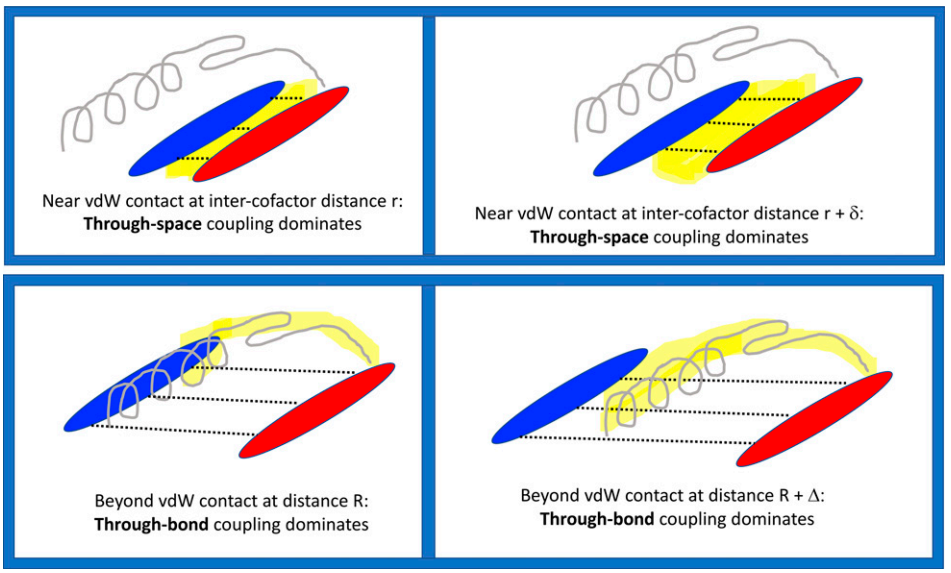

Fig. 1. Why might tunneling interactions between hemes (red and blue ovals) at near contact be dominated by though-space rather than through-bond interactions? Imagine a protein that binds two hemes and that the blue heme is shifted in position with other atomic positions fixed. (*Upper*) The upper left panel shows hemes at a relatively short distances r and the upper right panel indicates a larger distance of r + δ. Tunneling is mediated by through-space interactions (indicated by the dotted lines) at both of these distances, highlighted in yellow. (*Lower*) At larger distances, R (at lower left) and R + Δ (at lower right), the tunneling is mediated mostly by through-bond interactions via the (gray) protein. The dominant coupling pathways are highlighted in yellow, and the through-bound pathways are much stronger than the through-space routes (dotted lines) at these distances. As the distance grows, more bonds enter the dominant tunneling paths. Increasing the distance weakens the tunneling interactions in both the upper and lower panels, but the through-space tunneling (*Upper*) decreases more rapidly with distance than the bond-mediated tunneling (*Lower*) (10).

bacterial nanowires, operate near steady state. Many-body charge correlations (which may give rise to electronic traffic jams) are expected to occur under near-steady-state conditions. These many-electron effects are not readily accessed in most light-triggered redox experiments and could be significant in hopping networks. Exploring many-body effects on hopping transport will require new approaches of theory and experiment that allow moving beyond familiar return to equilibrium studies. In the case of electron bifurcation, for example, correlated many-particle motion engenders high efficiency by minimizing energy-dissipating short-circuiting reactions (21). Important insights into energy transduction, signaling, and sensing mechanisms will come from a deeper understanding of tunneling and many-body correlations in hopping chains with densely packed cofactors. The lessons yet to be learned promise to drive future discoveries in biogeochemistry, energy and environmental science, and biomedicine.

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1 J. H. van Wonderen *et al.*, Nanosecond heme-to-heme electron transfer rates in a multiheme cytochrome nanowire reported by a spectrally unique His/Met-ligated heme. *Proc. Natl. Acad. Sci. U.S.A.* **118**, e2107939118 (2021).
2 D. DeVault, B. Chance, Studies of photosynthesis using a pulsed laser. I. Temperature dependence of cytochrome oxidation rate in chromatium. Evidence for tunneling. *Biophys. J.* **6**, 825–847 (1966).
3 J. J. Hopfield, Electron transfer between biological molecules by thermally activated tunneling. *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3640–3644 (1974).
4 J. R. Winkler, D. G. Nocera, K. M. Yocom, E. Bordignon, H. B. Gray, Electron-transfer kinetics of pentaammineruthenium(III)(histidine-33)-ferricytochrome c. Measurement of the rate of intramolecular electron transfer between redox centers separated by 15 Å in a protein. *J. Am. Chem. Soc.* **104**, 5798–5800 (1982).
5 S. L. Mayo, W. R. Ellis, Jr, R. J. Crutchley, H. B. Gray, Long-range electron transfer in heme proteins. *Science* **233**, 948–952 (1986).
6 D. N. Beratan, J. N. Onuchic, J. J. Hopfield, Electron tunneling through covalent and non-covalent pathways in proteins. *J. Chem. Phys.* **86**, 4488–4498 (1987).
7 N. F. Polizzi, S. S. Skourtis, D. N. Beratan, Physical constraints on charge transport through bacterial nanowires. *Faraday Discuss.* **155**, 43–62, discussion 103–114 (2012).
8 M. Y. El-Naggar *et al.*, Electrical transport along bacterial nanowires from Shewanella oneidensis MR-1. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 18127–18131 (2010).
9 H. B. Gray, J. R. Winkler, Long-range electron transfer. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 3534–3539 (2005).
10 D. N. Beratan, J. N. Betts, J. N. Onuchic, Protein electron transfer rates set by the bridging secondary and tertiary structure. *Science* **252**, 1285–1288 (1991).
11 J. T. Bjerg *et al.*, Long-distance electron transport in individual, living cable bacteria. *Proc. Natl. Acad. Sci. U.S.A.* **115**, 5786–5791 (2018).
12 J. Blumberger, Recent advances in the theory and molecular simulation of biological electron transfer reactions. *Chem. Rev.* **115**, 11191–11238 (2015).
13 C. C. Page, C. C. Moser, X. Chen, P. L. Dutton, Natural engineering principles of electron tunnelling in biological oxidation-reduction. *Nature* **402**, 47–52 (1999).
14 R. E. Blankenship, *Molecular Mechanisms of Photosynthesis* (John Wiley & Sons, New York, 2014).
15 D. N. Beratan *et al.*, Charge transfer in dynamical biosystems, or the treachery of (static) images. *Acc. Chem. Res.* **48**, 474–481 (2015).
16 D. N. Beratan, I. A. Balabin, Heme-copper oxidases use tunneling pathways. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 403–404 (2008).
17 E. C. Minnihan, D. G. Nocera, J. Stubbe, Reversible, long-range radical transfer in E. coli class Ia ribonucleotide reductase. *Acc. Chem. Res.* **46**, 2524–2535 (2013).
18 J. R. Winkler, H. B. Gray, Electron flow through biological molecules: Does hole hopping protect proteins from oxidative damage? *Q. Rev. Biophys.* **48**, 411–420 (2015).
19 C. Shih *et al.*, Tryptophan-accelerated electron flow through proteins. *Science* **320**, 1760–1762 (2008).
20 R. D. Teo *et al.*, Charge transfer between [4Fe4S] proteins and DNA is unidirectional. Implications for biomolecular signaling. *Chem* **5**, 122–137 (2019).
21 J. L. Yuly, P. Zhang, C. E. Lubner, J. W. Peters, D. N. Beratan, Universal free-energy landscape produces efficient and reversible electron bifurcation. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 21045–21051 (2020).