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Chemical Science

EDGE ARTICLE

Direct evidence for heme-assisted solid-state electronic conduction in multi-heme *c*-type cytochromes

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Introduction

In extra-cellular respiration an organism oxidizes the organic matter inside the cell and exports the produced electrons outside the cells, either by communication with other cells¹ and/or to reduce extracellular oxidized minerals (mostly metal oxides).^{2,3} Although the exact mechanism of this electron transport is not resolved, multi-heme *c*-type cytochromes present in such cells' outer membranes are found to play a central role in electron transfer to outside the cell. Earlier studies pointed out that the heme cofactors of these

Multi-heme cytochrome *c* (CytC) proteins are key for transferring electrons out of cells, to enable intracellular oxidation to proceed, also in the absence of O₂. In these proteins most of the hemes are arranged in a linear array suggesting a facile path for electronic conduction. To test this, we studied solvent-free electron transport across two multi-heme CytC-type: MtrF (deca-heme CytC) and STC (tetra-heme CytC). Transport is measured across monolayers of these proteins in solid state configuration between Au electrodes. Both proteins showed 1,000x higher conductance than single heme, or heme-free proteins, but similar to monolayers of conjugated organics. Conductances are found to be temperature-independent (320–80K), suggesting tunneling as the transport mechanism based on present experimental data. This mechanism is consistent with modelling the I-V curves, results of which could be interpreted by having protein-electrode coupling as rate limiting, rather than transport within the proteins.

cytochromes are arranged in molecular wire-like fashion and that several such proteins span the cellular envelope, allowing electron transfer over long distances (>10 nm).^{4,5} Due to these remarkable electron transfer properties, such multi-heme cytochromes are of prime interest for, e.g., potential bioelectronics and bio-sensing. Integrating such proteins into electronic circuits is indeed an exciting prospect.⁶ Hence, it is important to understand the electron transport (ETp) properties of these fascinating proteins on a molecular level. In earlier work it was shown that these cytochromes are essential for efficient electrical transport.⁷

Several mechanisms for electron transfer in these multi-heme proteins (in solution / in the membrane) have been suggested and analyzed, including band-like transport,⁸ flickering resonance (FR),⁹ superexchange-mediated tunneling (SE),¹⁰ and charge hopping.⁵ In a recent review we have suggested that ET across the fully solvated decaheme protein MtrF occurs by stepwise (incoherent) transport, electron hopping, between neighboring Fe²⁺ / Fe³⁺ heme pairs.¹⁰ The relatively small electronic coupling between heme cofactors (compared to e.g. DNA bases) makes FR and SE unlikely as dominant ET mechanisms in multi-heme proteins. Here we address the question which mechanism(s) dominate in solid state electron transport (ETp) via dry multi-heme proteins, a process which has similarities with, *but also clear differences from ET in aqueous solution, as discussed in detail in references*^{11,12}. ETp has been studied in a variety of proteins, using “dry” junctions of monolayers,^{11,13,14} in which the proteins maintain only structural, tightly bound H₂O. In such junctions the donor and acceptor, involved in ET in solution,¹⁵ are replaced by metallic contacts of nm-s to mm size, and electron transport is

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measured as the current, I , as function of applied voltage, V (I - V characteristics). Such junctions also allow temperature-dependent I - V measurements.

STM-based solid state I - V measurements showed deca-heme proteins (MtrF, MtrC and OmcA) to be good electron conductors.^{6,16,17} As we noted in a 2014 summary of literature data, current values reported by STM measurements have a wide spread, which can be due to factors such as the presence of a vacuum, or air (as in refs.^{6,16,17}) gap contact geometry, and/or low S/N ratio (relative to larger-area junctions). Also, possible future devices are unlikely to use STM contacts. Thus here we use larger area contacts to help provide insights in multi-heme protein solid state ETp.¹³

To that end we study two multi-heme proteins, the 3-dimensional crystal structures of which have been determined, viz. a tetra-heme protein (STC)¹⁸ and a larger deca-heme protein, MtrF, one of the largest among the multi-heme cytochromes in extra-cellular electron transport.^{19,20}

Results and Discussion

We prepared MtrF and STC monolayers between Au electrodes and measured ETp across them. A self-assembled monolayer of MtrF or STC was covalently bonded by a S-Au bond to a polycrystalline Au substrate to one of the relatively exposed cysteine thiolates of these proteins. Since in native STC all the eight Cys residues form covalent bonds to heme porphyrins via their thiol residues, an additional Cys was introduced by replacing Ser87, which is proximal to Heme IV at the terminus of the approximately linear heme arrangement. In MtrF, cysteines of Domain I (111,115) and Domain III (428,437) are exposed; of these, Domain III cysteines are most likely to form a covalent bond with the Au substrate.²¹ It is possible that when Cys (428/437) forms Au-S bond with the substrate, heme 5 (Domain II) may also contact the substrate (Figure 1).

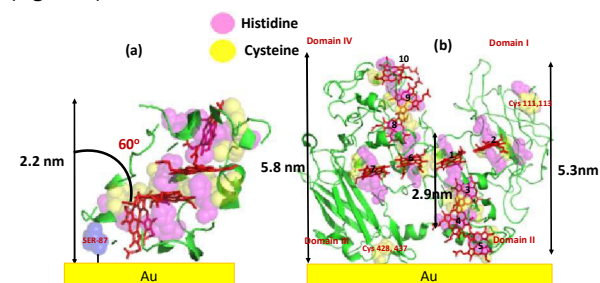


Figure 1. Schematic representation of (a) STC protein on Au using crystal structure (pdb: 1m1q). (b) MtrF protein on Au using crystal structure (pdb: 3pmq). Note, the actual adsorption structure may differ from the schematic shown here.

The monolayers were formed by incubating the proteins on freshly cleaned and activated Au substrates at 4 °C for 4h. The resulting protein monolayers were found by ellipsometry to be 4.0 ± 0.1 nm and 2.2 ± 0.1 nm thick, for MtrF and STC, respectively; nano-scratching with the tip of an atomic force microscope (AFM) gave 4.8 ± 0.5 nm and 2.4 ± 0.5 nm for MtrF and STC, respectively (Figure S3 and S4). Comparison of these values with the crystal structures of these proteins, indicates

that MtrF stands in a roughly upright position; Because this protein has a shape, somewhat akin to that of a staggered cross (figure 1(b)), we cannot define a unique height, but describe it by three lengths, 5.8 nm and 5.3 nm at the edge and 2.9 nm in the middle, which may be consistent with ~ 4.0 nm thickness, in agreement with the ellipsometry-derived value. In STC the theoretical length determined by crystal structure (pdb 1m1q) is 3.7 nm and the observed monolayer width is 2.2 nm (Figure 1(a)). To fit to the determined thickness, we assume that the protein is tilted at 60° from the normal. We note that for both the proteins other orientations are also possible with similar thicknesses. AFM measurements (in tapping mode), indicated that the monolayers were compactly packed with rms roughness of 0.9 nm and 2.1 nm for STC and MtrF, respectively (Figure 2 (a) and (b)). Amide I and Amide II peaks at 1664 and 1538 cm^{-1} , respectively, in the Polarization modulation-infrared reflection-absorption spectroscopy PMIRRAS, are evidence for the presence of the protein on the Au (Figure 2 (c)). The integrity (secondary structure) of the protein in the monolayers was confirmed by UV-Vis absorption spectroscopy. To that end monolayers were formed on quartz by S-S linkages using (3-Mercaptopropyl)-trimethoxysilane (MPTMS) as linker. The Soret band of MtrF at 412 nm and STC at 409 nm, respectively, were found to be the same in the monolayers as in solution (Figure 3(a)). This confirms that there is no significant change in the molecular environment of the heme groups upon monolayer formation, suggesting that there is no change in the protein conformation.

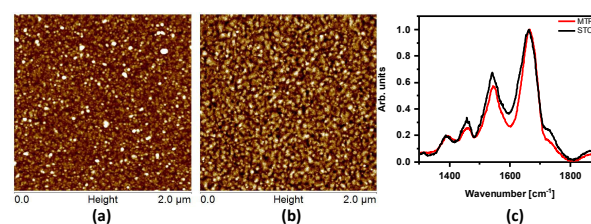


Figure 2. (a) AFM image of MtrF and (b) STC monolayers on gold using the tapping mode; (c) PMIRRAS of MtrF and STC monolayers on gold.

Creating molecular junctions for current-voltage measurements requires care, so as not to damage the protein, i.e., making electrical contacts to them has to be nondestructive. At the same time the junction has to be stable over a wide temperature range to allow low-noise, low-current measurements. To this end, we used two techniques, first is the “suspended-nanowire” technique,^{22,23} with which I - V can be measured from RT to 80K (to 10K, if needed) and the second uses InGa eutectic as top contact for the RT measurements.²⁴ For the suspended nanowire technique, the protein monolayer was coupled covalently, as described above, to pre-patterned Au microelectrodes (Figure 3(b)). Au nanowires, ~ 300 nm in diameter and ~ 4 μm long, were trapped di-electrophoretically onto the electrodes (Figure 3(b)), as reported previously.²⁵ In total we made ~ 325 Au-protein-Au junctions, of which 65 MtrF junctions and 80



junctions for STC had the desired configuration (nanowire aligned with short on one side, details in SI (Figure 3(b))), based on RT *I-V* measurements.

Among these groups of junctions, 10 were chosen that had the statistically most probable currents at 0.5 V, as deduced from a Gaussian fit of currents via all junctions, for measurements down to 80 K. Details of statistics of all junctions are given in SI (figure S1).

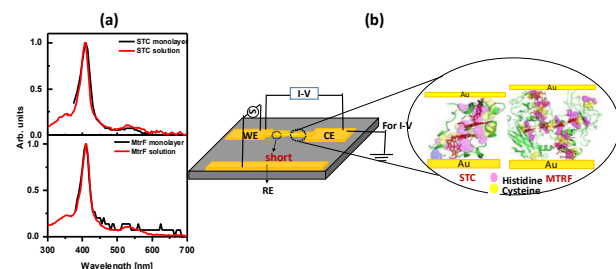


Figure 3. (a) UV-Vis spectra of MtrF and STC proteins in solution and of monolayers on quartz. (b) Schematics of di-electrode configuration, where AC bias is applied between the WE and RE electrode, and the *I-V* measurement setup.

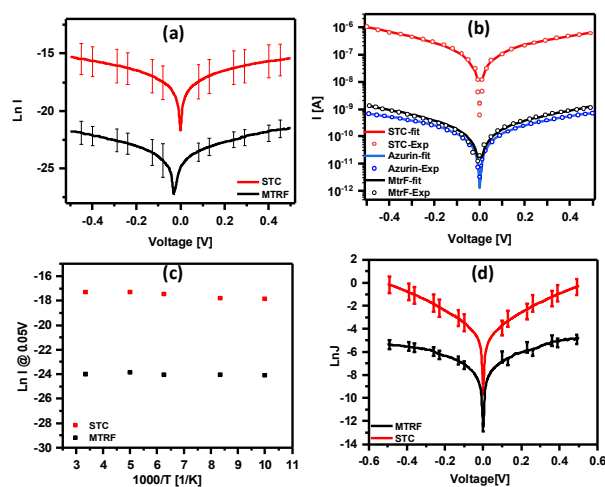


Figure 4. (a) Ln *I-V* curves obtained using suspended nanowire junctions; (b) Coherent tunneling fit (solid lines) of the experimental *I-V* curves (circles) of STC, MtrF and Azurin (for comparison) (Linear *I-V* curves are given in Fig. S2); (c) Ln *I* vs 1000/*T* curves obtained using suspended nanowire junctions; (d) Ln *J-V* curves obtained using Au substrate with InGa eutectic as top contact for STC and MtrF monolayers.

Three orders of magnitude higher conductance was observed via the STC protein junctions than via the blue Cu protein Azurin (Az) junctions,²⁸ which forms monolayer junctions of similar thickness (2 ± 0.2 nm), (figure 4(b)). The comparison measurements on Az were done using the same contacts and measurement method to exclude the effect of contact resistance. For MtrF, even though its monolayer thickness is double that of Az monolayers, i.e., the separation between electrodes is twice that of Az junctions, *I-V* curves, very similar to those of Az were observed (figure 4(b)). The observed higher conductance of STC than that of Az and the similar one of MtrF to that of the much smaller Az, is consistent with the idea that the multi-heme arrays in MtrF and STC can markedly enhance conductance. Similar *I-V* curves have been obtained

for MtrF and STC when, instead of a Au nanowire, an InGa top contact was employed (figure 4(d)). For those experiments monolayers were formed on freshly cleaned Au substrates (100 nm thick) with freshly made InGa as top contact (Scheme given in the SI).

To further compare the present results with those obtained with other proteins, current densities for STC and MtrF are estimated assuming the maximum contact area for the nanowire method of $0.03 \times 0.1 \mu\text{m}^2$. The current density at 0.05 V was calculated to be $\sim 0.3 \text{ A/cm}^2$ and $2 \times 10^2 \text{ A/cm}^2$ for MtrF and STC, respectively. Earlier we have reported results of ETp measurements via monoheme cytochrome c (Cyt C) and Az, which impose a similar electrode separation (~ 2 nm), and also bind covalently via a cysteine thiolate to one of the electrodes. Those proteins, though, were measured in a different device configuration, namely Si / SiO₂ (1 nm) / linker (0.6 nm) / protein / Hg. The SiO₂ and (an organic molecule) linker add an insulating layer of $\sim 16 \text{ \AA}$, which lowers the currents by some 5 orders of magnitude, assuming a current decay factor, β , across SiO₂ and alkane chains in solid state junctions (with molecules sandwiched between electrodes) as 0.7 \AA^{-1} (for mostly saturated molecules β for transport across molecules in these junctions is $0.6\text{--}1.0 \text{ \AA}^{-1}$).^{29–32} The current density values after correction (Table 1) for two Cyt C mutants (E104C, V11C) that bind to the Au electrode via cysteines²⁶ and for Az²⁷ are $0.2\text{--}0.5 \text{ A/cm}^2$ at 0.05V. These values are similar to those we have now determined for MtrF (which has double the size of the former proteins; 4 nm). In contrast for STC, which has the same size (2 nm, namely yielding similar electrodes' separation as do Az and Cyt C) we observe a 10^3 higher conductance.

Table 1. *J-V* comparison of literature reported proteins in Si/SiO₂/Linker/Protein/Hg device configuration

Protein	Thickness	$J@0.05 \text{ V}$ [A/cm ²]	Corrected* J[A/cm ²]
Cyt c (E104C) ²⁶	2.1 nm	4.9×10^{-6}	0.49
Az ²⁷	2.1 nm	1.2×10^{-6}	0.1
Cyt c (V11C) ²⁶	1.8 nm	2.6×10^{-6}	0.26

* for insulating layer of SiO₂+linker

To compare the conductivity of these proteins with other proteins, saturated molecules and conjugated molecules, we update and present here an earlier summary of data (current density ($J[\text{A}/\text{nm}^2]$) at 0.1 V vs molecule length(\AA) in the junction, i.e., separation between the electrodes) of such molecules and of proteins.¹³ The bias of 0.1 V is used, because most data are from the literature and reliable data at lower bias are too scarce. By adding STC and MtrF to this plot, we clearly see that their data points are in the region of conjugated molecules (Figure 5). Thus, at this point we tentatively conclude that monolayers of MtrF and STC conduct like monolayers of conjugated molecules.



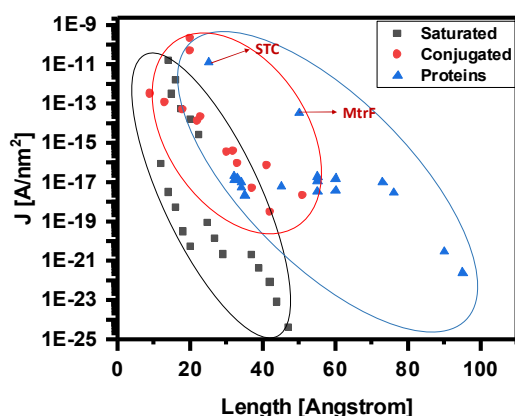


Figure 5. Current densities at 0.1 V [A/nm^2] as a function of junction width [\AA] (the shaded areas provide visual guides only). with extrapolated data corresponds to MtrF and STC is indicated by arrows. Where applicable, the data are corrected for the current attenuation by 1 nm Si oxide and 0.7 nm saturated organic linker (as described in text). Adapted with permission from reference 13, Amdursky et al., John Wiley and Sons.

Modeling I - V curves can (help to) identify the current-limiting transport mechanism. In the coherent tunneling model (Simmons Model) the protein matrix is approximated by a single effective energy barrier with height ϕ , length L and symmetry factor α as fitting parameters, $I \propto \exp[-(\phi - \alpha V)^{1/2} L]$.^{33,34} Remarkably, this model yields excellent fits (Fig. 4(b) and Fig. S2 solid lines, correlation coefficients = 0.999), but with tunneling lengths ($L=1.22$, 1.56 and 1.21 nm for STC, MtrF and Azurin, respectively) that are much shorter than the measured widths of the respective monolayers. For STC and MtrF such lengths are more characteristic of tunneling from an electrode to one of the protein's hemes. We speculate that subsequent intra-protein conduction, possibly facilitated by heme electronic energy levels, is fast and not resolved in experimental measurements. In this regard, we note that recent calculations predicted that cysteine linkages inserted between the two terminal 1-2 and 3-4 heme pairs of STC significantly enhance overall electron flow through the solvated protein, due to weak mixing of the S 3p orbital with the Fe-heme d orbitals.³⁵ A similar effect may operate for the dry proteins studied here. In solution-phase ET there can be additional electrostatic effects, due to redox-linked structural modifications, but such effects should be minimal in solid state electron transfer.³⁶

To gain further insight into the possible transport mechanisms, temperature-dependence of the ETp via the proteins was measured. No temperature dependence of the current at 50 mV was observed from 80 to 300K (averaged data shown in Figure 4(c)). Such behavior is consistent with a coherent tunneling mechanism, the model now used to fit the experimental I - V curves. Since the ETp is temperature-independent, the possibility of flickering resonance, as the mechanism for conduction, is unlikely.¹⁰ Such temperature-independent ETp behavior is also inconsistent with a hopping mechanism.¹¹ Though theoretically, for electron transfer, a

mechanism involving delocalization of orbitals of conjugated molecules,³⁷ hopping could be temperature-independent.

It was suggested earlier that Super-exchange-mediated tunneling could be the dominant but not exclusive coupling mechanism for long-range ET.³⁸ Since the mediating states and energy gaps are rarely identified for this mechanism, it is difficult to define exactly whether it is tunneling or super-exchange-mediated-tunneling. Thus, tunneling, is the most plausible mechanism as validated by the theoretical fitting of the experimental I - V curve. This tunneling behavior can be assumed to be intrinsic to the protein, because if transport across the protein monolayers was temperature-dependent, tunnelling into and out of the proteins from/ to the electrode, would not be sufficient to yield temperature-independent transport.

This can be related to having ET electron tunneling *within* folded peptides or proteins, occur through covalently linked or hydrogen-bonded pathways between donor and acceptor moieties, redox centers in our case.^{38,39} Thus, at this point we tentatively conclude that MtrF and STC monolayers conduct somewhat like conjugated molecules, via tunneling.

Experimental

MtrF protein preparation:

Culture of the MR-1 mtr operon mutant (LS623) with the plasmid containing the gene encoding his-tagged MtrF were grown aerobically in Luria-Bertani medium (containing 25 $\mu\text{g}/\text{ml}$ kanamycin) at 303 K overnight. For scale-up, each initial overnight culture (5 mL) was used to inoculate 1 L of fresh Luria-Bertani medium (containing 25 $\mu\text{g}/\text{ml}$ kanamycin). For standard MtrF preparations, 8 x 1 L cultures were grown to aerobically at 303 K until the OD_{600} of the culture reaches 0.6 (usually needs 4-5 h). L (+)-Arabinose was added up to final concentration of 1 mM and induced for 17 h. Cells were harvested by centrifugation (6000 g, 277 K, 15 min), washed and resuspended in 277 K in 50 ml ice cold buffer B (buffer B: buffer A, lysozyme 0.2mg/ml, DNase 0.01mg/ml, protease inhibitor and 1% CHAPS [3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate]) (buffer A: 20 mM HEPES [N-(2-hydroxyethyl)piperazine-N'-ethanesulfonic acid], pH 7.8 and 150 mM NaCl) and kept for stirring overnight at 277 K. Unsolubilized proteins were removed by centrifugation at 12000 rpm for 30 min. The solubilized protein supernatant was loaded onto 10 ml of Ni^{2+} -NTA histidine-tagged agarose column (flow rate of 1.4 ml/min) that has been pre-equilibrated with buffer A in 277 K. The column was washed with 40 ml of each of the following ice-cold buffers in sequential order: buffer D (20 mM HEPES, pH 7.8, 300 mM NaCl, 0.5% CHAPS, 10% glycerol and protease inhibitor), buffer E (buffer D and 10 mM imidazole), and buffer F (buffer D and 40 mM imidazole) in 277K. The final elution of the protein was done with ice cold buffer G (buffer D, 250 mM imidazole and 10% glycerol) and collected 1.5 ml/fraction in 277 K. Eluted protein was washed and concentrated using 20 mM HEPES, pH



7.8, 30 mM NaCl, 0.17% (wt/vol) CHAPS in 277 K. Aliquots of purified MtrF were stored using 20 mM HEPES, pH 7.5, 100 mM NaCl, 0.5% (wt/vol) CHAPS and 10% glycerol in 193K. CD spectrum of the protein was measured to check the protein secondary structure (Figure S6).

STC protein preparation:

S87C STC was purified from *Shewanella oneidensis* MR-1 after expression from the corresponding gene inserted into a pBAD202/D-TOPO vector. An N-terminal Strep II-tag was introduced to facilitate protein purification, full details will be provided elsewhere (van Wonderen *et al.*, ms. in preparation). Protein purity was confirmed by SDS-PAGE, Fig. S7. LC-MS analysis revealed a single-peak corresponding to a mass of 13 561 Da in excellent agreement with that predicted (13 558 Da) for the mature protein with four covalently bound hemes. Aliquots of purified S87C STC (200 μ M) in 20 mM TRIS, 100 mM NaCl, pH 8.5 were stored frozen at 193K. CD spectrum of the protein was measured to check the protein secondary structure (Figure S6)

Monolayer formation: Au-coated (50 nm) P++ doped Si wafers were cleaned by sonicating for 5 min each in acetone and ethanol, followed by UV/ozone treatment for 15 min. Cleaned Au slides/patterned chips were activated by treatment with hot ethanol for 30 min, dried with N₂ and immediately transferred to the protein solution and incubated at 4°C for 4h. After 4h, the slides on which protein was deposited were gently cleaned with H₂O and dried with N₂.

Ellipsometry:

Ellipsometry measurements were performed using a Woollam M-2000 V multiple-wavelength ellipsometer at a 70° angle of incidence. The Cauchy model was used to estimate the protein monolayer thickness.

AFM Imaging:

The topography of the self-assembled monolayer of proteins was characterized by AFM in the Scanasyt mode. A Bruker multimode-A and pyrex Nitride probes-Si₃N₄ SPM sensor with frequency 67 Hz and force constant 0.32N/m were used.

The scratching procedure was performed in contact mode a 1x1 μ m² square area was scanned with a large tip force (60 nN). The applied force is sufficiently large to scratch away the monolayer, but not sufficient to scratch the gold surface. After the scratching procedure, we switched back to Scan asyst mode to re-scan over a larger area, centered around the resulting hollow space after scratching (Figure S1).

UV-Vis optical absorption:

In solution these measurements were taken using a nanodrop-2000 spectrophotometer, where the path length is corrected for 1 cm. Protein monolayers were measured using a Quantaurus-QY (absolute PL quantum yield spectrometer), Hamamatsu C11437.

Circular Dichroism (CD):

The CD spectra were measured on a Chirascan spectrometer. The measurements were made using a 1 mm optical-path quartz cuvette. Respective buffers were used as a baseline (Figure S6).

PMIRRAS measurements:

Polarization modulation-infrared reflection-absorption spectroscopy (PM-IRRAS) was done, using a Nicolet 6700 FTIR, at an 80° incidence angle, equipped with PEM-90 photoelastic modulator (Hinds Instruments, Hillsboro, OR) with modulation wavelengths of 1600 cm⁻¹ for the amide I and II regions. Raw spectra were smoothed and baseline-corrected by a spline algorithm.

Device fabrication, I-V measurements and statistics:

To fabricate the protein junctions for transport measurements Au electrodes were deposited on a Si wafer by photolithography, yielding a substrate that contains 260 electrode pairs. The proteins were immobilized on the wafer as described above, for monolayer formation. After monolayer formation, gold nanorods were di-electrophoretically trapped to close the circuit, by applying an AC bias between working and reference electrodes, using water as dielectric medium.^{22,23} The final architecture of all measured junctions is similar to the configuration shown in Figures 4(a), with only a single Au nanorod as a top contact. Since the yield of trapping was only ~25%, only rarely two or more Au nanorods bridged between two contact pads and this was easily detected by optical microscopy, prior to electronic transport measurements. Next, the samples were loaded on an electrically floating sample stage and placed in a cryogenic Lakeshore probe station (TTPX). Current-voltage (I-V) measurements were performed to assess the transport efficiency across peptide monolayers, using a Keithley 6430 Sub-Femto amp Source-Meter, with a voltage scan rate of 20 mV/s at a vacuum of 10⁻⁵ mbar. For all measurements, a specific side of the junction was grounded, while the other one was biased, in a consistent manner (in order to ensure that the bias polarity was in the same direction for all measurements). In each set of experiments, scans were acquired that started and ended at 0 V (i.e., voltage sweep was 0 \rightarrow -0.5 V, -0.5 V \rightarrow 0.5 V, 0.5 V \rightarrow 0 V), to check if features in the I-V behavior originate from the polarity of the initial voltage that is applied and from the scan direction (hysteresis check). All the aligned nanowire junctions (~25% of 1300 junctions (5 chips with 260 junctions / chip) were measured; ~40% of the remaining 325 junctions showed no currents (possibly these were double junctions, rather than the desired single junction, with one nanowire/substrate contact shorted), ~35% (98) junctions were short circuited, and ~25% (~ 80) of the junctions showed single junctions with currents that fit that. The most probable current range for the protein was determined by statistics, for all currents, frequency of occurrence at 0.5V is checked and frequency count histogram was fitted to Gaussian, from the FWHM, the desired range of current is calculated (Figure S2). In case of MTRF, current range at 0.5V was found to be 0.1nA-5nA and for STC it was found to be 0.01 μ A to 5 μ A. To measure down to 80K, we have chosen 10 junctions each with current values at peak maxima, for MTRF 0.5-1 nA for STC 0.1-0.5 μ A.

Coherent tunneling model:

The experimental I-V data shown in Figure 4b (main text) and Figure S2 were fit to the following tunneling expression.^{33,34}

$$I(V) = A i(V) \quad \text{Eq. 1}$$



EDGE ARTICLE

Chemical Science

$$i(V) = \frac{e^2}{2\pi h L^2} [(\phi - \alpha V) \exp(-K\sqrt{(\phi - \alpha V)}) - (\phi + (1 - \alpha)V) \exp(-K\sqrt{(\phi + (1 - \alpha)V)})]$$
 Eq. 2

$$K = 4\pi L/h \sqrt{2me}$$
 Eq. 3

where I is the current, i the current density, A the contact area, e the elementary charge, h Planck's constant and m the electron mass. The fit parameters are the barrier height ϕ barrier length L and symmetry factor α . The contact area was obtained from the measured current and the estimated current density at 0.05 V (see main text), $A = I(0.05 \text{ V})/i(0.05 \text{ V})$, $i(0.05 \text{ V}) = 6.0 \times 10^{-8} \text{ A}$ and $9.9 \times 10^{-11} \text{ A}$ for STC and MtrF, respectively, and $i(0.05 \text{ V}) = 200 \text{ A/cm}^2$ and 0.3 A/cm^2 for STC and MtrF, respectively. Numerical values of the fit parameters are summarized in Table S1.

Conclusion

We conclude that the multi-heme proteins, MtrF and STC are significantly better conducting than non- or mono heme proteins. These multi-heme proteins conduct, somewhat like conjugated organic molecules in the dry phase. The electron transport process, being temperature independent and examined for coherent tunneling fit with the experimental I-V results, reveals that the transport is indeed by tunneling mechanism. Modeling of the I-V curves suggests that the tunneling process is from the electrode to one of the nearest protein heme, followed by intra-protein conduction that is so efficient that it is not resolved in the experimental measurements. Because we have no indication for any resolvable structural changes in the proteins, we assume that the electron migration rate within the proteins, whether by ET or ETp is comparable. If so, then these results present a significant challenge to our current understanding of electron transfer and transport via proteins, and as such may stimulate re-evaluation of existing models. In addition, these results follow other indications that solid-state conduction across proteins is limited by the coupling to the electrodes.

Conflicts of interest

There are no conflicts to declare.

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