The porphyrin ring rather than the metal ion dictates long-range electron transport across proteins suggesting coherence-assisted mechanism

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The fundamental biological process of electron transfer (ET) takes place across proteins with common ET pathways of several nanometers. Recent discoveries push this limit and show long-range extracellular ET over several micrometers. Here, we aim in deciphering how protein-bound intramolecular cofactors can facilitate such long-range ET. In contrast to natural systems, our protein-based platform enables us to modulate important factors associated with ET in a facile manner, such as the type of the cofactor and its quantity within the protein. We choose here the biologically relevant protoporphyrin molecule as the electron mediator. Unlike natural systems having only Fe-containing protoporphyrins, i.e., heme, as electron mediators, we use here porphyrins with different metal centers, or lacking a metal center. We show that the metal redox center has no role in ET and that ET is mediated solely by the conjugated backbone of the molecule. We further discuss several ET mechanisms, accounting to our observations with possible contribution of coherent processes. Our findings contribute to our understanding of the participation of heme molecules in long-range biological ET.

Electron transfer | conductive polymers | heme | porphyrin | biopolymers

Iron-containing protoporphyrin, also known as heme, is one of the most important molecules in nature, participating in numerous biological processes, including the binding of gas molecules (as O2) to its iron ion. The proper activity of heme molecules is dependent on the ability of specific proteins, which are referred to as heme-proteins, to bind them. The natural activity of heme is owed to its exceptional heme–iron coordination with gas molecules or specific amino acids. In fact, an iron-free porphyrin or a degraded product of heme, bilirubin, can result in toxicity of heme is owed to its exceptional heme–iron coordination with gas molecules or specific amino acids. We further discuss several ET mechanisms, accounting to our observations with possible contribution of coherent processes. Our findings contribute to our understanding of the participation of heme molecules in long-range biological ET.

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Significance

Electron transfer (ET) across proteins is fundamental in many biological processes, such as photosynthesis and aerobic respiration. Recent discoveries from the bacterial world have shown that proteins can support long-range ET pathways on the order of micrometers. Here, we introduce a protein platform that allows us to explore how proteins can support long-range ET and what are the important molecular features and ET mechanisms that enable it. We focus on the role of the metal ion within heme molecules in long-range ET and find that the molecular ring surrounding the metal ion is the ET mediator, and not the metal ion. We suggest a coherence-assisted ET mechanism as a possible explanation for our high measured ET efficiency.

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extracellular ET observed with OmcS nanowire, we chose here only protoporphyrin as the electron-mediating cofactors across our protein-based platform, although other macromolecules are known to participate in various biological pathways. Using our platform, we can now change the metal ion inside the porphyrin, remove the metal ion altogether, or even use bilirubin (the open form of the ring) as ET mediators. We used electrochemical impedance measurements, as well as three-terminal transistor measurements, to explore the role of the porphyrin variant in mediating ET. Our results allow us to discuss possible mechanisms of ET across our protein platform, involving either incoherent or coherence-assisted ET.

Results

The protein-based macroscopic platform that we use here is based on electrospun bovine serum albumin (BSA) mats, which were shown to be able to bind heme molecules, resulting in long-range ET across this artificial platform (27). As stated, here, we aim to understand how protoporphyrins mediate ET. Accordingly, we molecularly doped the BSA mats with different variants: metal-free protoporphyrin IX (PPIX), the common Fe-containing PPIX (heme), PPIX with three other transition metals (Co, Cu, and Zn), and bilirubin. The successful doping of the BSA mat is easily observed by a distinct color change of the mat in accordance with the porphyrin color (Fig. 1). Moreover, the doping level, i.e., the number of dopants per volume within the mat, can be easily modulated by the duration of the doping process and can be verified by using ultraviolet-visible (UV-Vis) spectroscopy.

Impedance Measurements

Following the successful doping of the mat, which we refer to as full doping (vide infra), we turned into electrical characterization. It is important to mention that even though all of our electrical measurements were performed in the solid-state (i.e., not in solution), the used protein mats retained a substantial amount of water, consisting of ~150 wt% of the mat; hence, the environmental conditions were more a like biological ET. At first, we used alternating current (AC) electrochemical impedance spectroscopy (EIS) over a wide frequency range (10 to 10⁷ Hz) to investigate the macroscopic electrical properties (across 2.5 mm) of the various doped mats. Our results are displayed in the form of a Bode plot, showing the absolute value of impedance as a function of frequency (Fig. 2A). We extracted the bulk conductivity of the doped mat using an equivalent circuit fitting (SI Appendix, Fig. S2). Before doping, the mat had a relatively low conductivity of 0.1 mS·cm⁻¹ due to proton conductance (28). To our surprise, following full doping with the various metal porphyrins and with metal-free PPIX, all of the mats exhibited a similar (~30-fold) increase in conductivity (to a value of ~3 ± 0.4 mS·cm⁻¹), which we attributed to ET, and not to a change in the ionic conductivity across the mat or any structural change due to the dopant solution (SI Appendix, Fig. S3). This result clearly implies that the observed long-range ET across our protein platform is not mediated by the metal ion within the PPIX and that charge-carrier mobility is enhanced by the conjugated π-system of the porphyrin rings themselves. To establish this assumption, we also examined bilirubin as a dopant, since its molecular structure has the same conjugated tetrapyrrole motif as PPIX, albeit linear rather than cyclic. We found that bilirubin indeed exhibited similar improvements in conductivity, as was observed for the other porphyrin dopants (Fig. 2A). To further explore the ET mechanism across the different mats, we measured the temperature dependence of the EIS response, displayed in the form of a Nyquist plot (the impedance imaginary part as a function of the impedance real part) (SI Appendix, Fig. S4). The plot shows a semicircle in the high-frequency area, corresponding to bulk conductivity, and a curved tail in the low-frequency area, attributed to double-layer capacitance at the electrolyte and blocking-electrode interface, interrupted by the ionic diffusion process. In these temperature-dependence studies, we found that all of the doped mats had a similar activation energy in the order of 0.2 ± 0.03 eV (SI Appendix, Table S1). The similar activation energy values imply that all of the mats we used, some of which contained a metal ion within the PPIX and some of which did not, share a similar ET mechanism (as discussed below).

After establishing that the conductivity of the doped mats is determined only by the amount of porphyrin bound to the BSA mat, regardless of the metallic element (in analogy to semiconductors, we refer to this property as “doping density”), we then determined how doping density influences conductivity. In this context, we need to consider that each of the different molecular dopants in this study has a different affinity to the BSA scaffold. This property determines the number of molecules that the mat will absorb in any time range and with any initial parameters. Hence, any comparison between doped mats must consider their doping density. Since absorbed molecule quantity is dependent on factors such as doping time and concentration of porphyrins in the doping solution, it is essential to determine these relationships for each specific sample. Accordingly, we prepared numerous different samples with different doping densities, which were determined by measuring the amount of porphyrin dopant that was absorbed into the mat from the dopant solution (SI Appendix, Table S2 and discussion within). As can be seen in Fig. 2B, which plots the change in extracted bulk resistivity (as determined by EIS measurements at 25 °C) as a function of doping density, the resistivity of each of the mats clearly depends on the porphyrin molecule density, irrespective of the particular porphyrin dopant. Even metal-free PPIX and bilirubin exhibited the same correlation. These findings support our claim that transport efficiency is not related to the metal redox potential, but solely to the similar conjugated π-system that is common to the different molecular dopants.

Transistor Measurements

Next, we used field-effect transistor (FET) measurements (29–31), in which our doped mats acted as the active layer, to determine the charge-carrier type (electrons vs. holes), their mobility, and the number of charges that contribute to the measured conductivity (charge-carrier density). FETs are three-terminal devices, consisting of a gold contact, which serves as the transistor’s source and drain, on a SiO₂/Si substrate with a bottom Si gate, in which the doped mat is placed on the gold contacts (Fig. 3A). Fig. 3B and SI Appendix, Fig. S5 show that the source-drain current (I_DS), as a function of the source-drain voltage (V_DS), decreased when the gate voltage (V_GS) was changed from negative bias to positive bias. This behavior is indicative of a p-type carrier behavior, as negative gate bias gives rise to an accumulation of positive charges in the FET channel, thus increasing the source-drain current. Accordingly, we can conclude that the porphyrin molecules in the doped mats facilitate hole transport (rather than electron transport), as reported.

Fig. 1. Molecular doping of BSA mats. Photo and scanning electron microscopy image (see also SI Appendix, Fig. S1) of the BSA mat (Left) and photos of mats after doping with different PPIX-based molecular dopants (Right) are shown.
for most porphyrin-based materials (32–35), as well as for *S. oneidensis* nanowires (17). We further extracted the hole mobility using a modified equation for gate voltage-dependent mobility (31):

\[
\mu = \pm \frac{\partial I_{DS}}{\partial V_{GS}} \cdot \frac{L}{WCGSV_{DS}},
\]

where \(\partial I_{DS}/\partial V_{GS}\) is the gradient of \(I_{DS}\) vs. \(V_{GS}\) with a constant \(V_{DS}\); \(C_{GS}\) is the gate dielectric capacitance per unit area; and \(L\) and \(W\) are the transistor’s length and width, respectively. We found that all of our doped mats exhibited similar mobility values in the range of 0.008 to 0.01 cm\(^2\)·V\(^{-1}\)·s\(^{-1}\) (SI Appendix, Table S3), further supporting our notion that the transport is due solely to the conjugated porphyrin ring. Moreover, and very importantly, mobility values were found to be similar for different doping densities. If the molecular dopant had been spread randomly within the protein matrix, we would have expected much higher measured mobilities for larger dopant densities. Since this was not seen, and mobility values for different doping densities were similar, this suggests that there is a strong preference in the location of the molecular dopants in specific locations inside the protein. Doping density, therefore, mainly influences the charge-carrier density, which was calculated by using the common conductivity relation:

\[
\sigma = \mu ne,
\]

where \(\sigma\) is the mat conductivity, \(n\) is the charge density, and \(e\) is the elementary charge. As can be seen in Fig. 3B, the extracted charge-density values of the mats are indeed related to the calculated doping density. However, only a fraction (~3%) of the porphyrin dopants contribute to the observed hole-transport process. From a physical point of view, it is clear that not all of the porphyrin molecules within the mat can contribute to the charge density. The low calculated fraction of charge density, compared with the doping density, further supports our suggestion that electron conduction probably occurs via distinct pathways along the protein fibrils comprising the sample.

**Discussion**

In Results, we showed that the tetrapyrrole conjugated \(\pi\)-system of the porphyrin backbone is the electron mediator of long-range ET across proteins, regardless of the nature of the bound metal ion or even its presence. This conclusion supports several theoretical works that suggested electron delocalization across the conjugated macrocycle, from the early works of Hopfield to more recent works (36–40). The immediate question we would like to discuss in this section is: What ET mechanism appears to be the most plausible in our system?

While referring to solid-state measurements, the lack of the metal-center involvement within the porphyrin in mediating electrons has been observed in a molecular-junction configuration, meaning that an electron transport (ETp) process was followed (distinguishing itself from the common ET notion) for distances of ~3 nm (41, 42). Recently, Blumberger and co-workers (43) explored the ETp mechanism across a multiheme containing protein in a molecular-junction configuration using

![Fig. 2. Impedance measurements.](image)

![Fig. 3. FET measurements.](image)

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density functional theory (DFT + Σ) calculations and found that delocalized orbitals over several heme molecules dominate the transport in the off-resonant tunneling regime. However, the short distance of molecular junctions permits specific ETp mechanisms, such as cotunneling between the leads, which may not apply directly for long-range ET across numerous porphin molecules as discussed here (only one to four porphin units are present between the electrodes in the reported molecular junction experiments). Nevertheless, the irrelevance of the metal to the conductivity in our results implies that, regardless of the ET mechanism (discussed below), the electrons will not be transported via molecular orbitals involving the metal center within the molecular dopant, but, rather, across the molecular orbitals associated with the conjugated porphin backbone. While this is also confirmed by naive DFT calculations of porphyrins in the gas phase (SI Appendix, Computational Details), which show that the frontier molecular orbitals (FMOs) do not reside on the metal center of the porphin, we cannot rely on these calculations in our study, as the use of the BSA mat does not permit us to know the exact protein structure, the exact binding configuration of the porphyrins to the BSA, and, accordingly, the formation of any axial ligands to the metal center. The latter axial ligands, such as to histidine residues or water molecules, are crucial for the location of the molecular orbitals (39, 44). Instead, the mentioned DFT calculations of Blumberger and co-workers (43), based on a known crystal structure of the protein, do show a molecular-orbitals delocalization of the valence band across several heme molecules and argue that this valence-band delocalization is responsible to efficient ETp involving a coherent transport mechanism. Moreover, and what seems to be most relevant to our study, they show that, while molecular orbitals involving the metal center can be located within the conduction band, such orbitals do not contribute significantly to the ETp mechanism, and replacing the metal center did not result in a change in the calculated ETp efficiency across the junction.

Upon switching from molecular junctions to natural biological systems, meaning in ambient (and usually surrounded by water) conditions, ET takes place usually for distances of <10 nm, and the most common ET mechanism used to describe such ET pathways involves a change in the redox state of the cofactor. This is a nonadiabatic ET mechanism, whose multiple steps are referred to as sequential hopping. However, unlike most biological short-range ET pathways that usually comprise different electron mediators, each of which has a different redox potential, in our system, the chemical identities of all of the mediators in a given sample are identical. This is in line with the probable long-range ET across the OmcS-based G. sulfurreducens bacterial wire, which is self-assembled from hexaheme OmcS cytochromes (25, 26).

The identical chemical identity of the electron mediators in our system can support several possible ET mechanisms (Fig. 4). We will start with the mentioned common incoherent nonadiabatic hopping mechanism, which is also the immediate suspect for our system as well (Fig. 4A). The electron (hole) hopping rate is commonly described by the Marcus–Hush formula:

$$k_{ET} = |H|^2 \sqrt{\frac{\pi}{h^2 k_B T}} \exp\left(-\frac{(\lambda + \Delta G^0)^2}{4k_B T}\right),$$

where $H$ is the electronic coupling energy between electron donor and acceptor, i.e., the two porphin moieties in our case; $\lambda$ is the reorganization energy; $k_B$ is the Boltzmann constant; $T$ is the absolute temperature; and $\Delta G^0$ is the driving force, i.e., the energy difference between electron donor and acceptor. Since the ET in our case is between two identical molecules (porphyrins), we assume that $\Delta G^0$ is close to zero. Under the latter assumption, the rate-limiting reorganization energy can be calculated from our measured activation energy (0.2 eV), resulting in $\lambda = 0.8$ eV.

Using our calculated charge-mobility values, we can calculate the electron-diffusion coefficient using the mobility definition:

$$D = \frac{\mu k_B T}{e},$$

reaching a diffusion coefficient value in the order of $2.3 (±0.3) \times 10^{-4}$ cm$^2$·s$^{-1}$ for all different molecularly doped mats. Since our system comprises a uniform chain of molecular dopants, the diffusion coefficient can be expressed as:

$$D = k_{ET} d^2,$$

where $d$ is the distance between adjacent molecular dopants. One of the main experimental difficulties in our system is our inability to resolve the exact structure of the protein matrix and, thus, also the exact location (i.e., binding sites) of the different porphyrins used here. We can, therefore, only estimate the distance between the porphyrins, $d$. The doping density cannot be used to calculate $d$, since the electrospun mat is porous, and only the protein fraction can bind the dopant. Indeed, the highest measured doping density (fully doped samples), which is around $3 \times 10^{19}$ cm$^{-3}$, corresponds to a $d$ value of 3.2 nm. This value is not realistic, as it is too large to support any ET across the film. A close inspection of several scanning electron microscopy images of the mat (such as the one in SI Appendix, Fig. S1) suggests that around 20 to 25% of the mat volume is composed of fibrils; hence, this latter value should be factorized. We can calculate the average numbers of porphyrins per BSA molecule in the mat, given the number of porphin molecules inside the mat (doping density) and the number of proteins in the mat, obtained by weighing the nondoped mat. The latter calculation results in an average of 10 porphyrin molecules per BSA molecule in the mat. Our upper estimation for the distance between porphyrins is, therefore, based on their alignment along the very long axis of the BSA protein, which is around 8 nm, resulting in an average distance of 0.8 nm between porphyrins, which is a realistic value. Moreover, in line with our claim that the FMO of the porphin is the ET mediator, the real distance should be the edge-to-edge distance between the porphin rings; hence, the latter value is probably an overestimation. Using this value and the calculated diffusion coefficient, we can calculate $k_{ET}$ (Eq. 5) to be in the order of $3.6 \times 10^{10}$ s$^{-1}$. Using Eq. 3, we can now estimate the average electronic coupling ($H$) between the porphyrins to be in the order of 65 meV. Since our distance ($d$) is most probably an overestimation, our estimated $k_{ET}$ and $H$ are probably underestimated values. Notice that our estimated $H$ values already seem too high to be realistic (4, 5). Indeed, these values are unrealistically high for two porphyrins that are 0.8 nm apart, or
even for much closer porphyrin rings (39). An important rationale for large electronic-coupling matrix elements can be attributed to an increase in the packing density \( \rho \) of protein atoms in the volume between donor and acceptor centers. This empirical approach was first introduced by Dutton and coworkers (45) to provide a rationale for the ET within proteins for distances up to 1.4 nm between redox centers. However, using the empirical formula for averaged packing density (as described in ref. 45), the packing density needed to explain that our estimated electronic coupling, \( \rho = 1.75 \), is unrealistically high, as all discussed proteins showed \( 0.5 < \rho < 1 \). It should be noted that since the detailed spatial arrangement of the porphyrins in the system cannot be revealed, we cannot exclude the possibility that, for specific intramolecular orientations, the overlap between nearby rings might increase the electronic coupling to such unusually high values.

Another possibility is that the seemingly too large coupling values are obtained within the assumption of a sequential hopping mechanism (Eq. 3), whereas a different transport mechanism may be consistent with smaller, more realistic, values. Indeed, it was recently suggested that an ET along a bacterial wire of *G. sulfurreducens*, which is self-assembled from the PilA protein, can involve a combination of coherent and incoherent transport across a chain of aromatic amino acids (Phe and Tyr) (24). We also recently suggested a mixed coherent-hopping mechanism for ET through the multiheme OmcsS-based bacterial nanowire (46). The chemical identity of the mediators in the present system may also permit unique charge-transfer mechanisms that involve some coherent ET, or, rather, a combination of coherent and incoherent transport, which most probably cannot take place in common short-range protein-based ET. The set of densely packed porphyrins with similar local molecular environments can be associated with a uniform energy landscape. This formally implies that differences between the vertical ionization energies of the different porphyrins can be smaller than the interporphyrin electronic couplings, namely, \( \Delta G_k^H < H \) (Eq. 3). In this case, delocalized charging states can be formed that extend over several neighboring porphyrins (46). Such delocalized charging states can promote charge transport over long distances only if the rate of dephasing between the different porphyrins is slow on the charge-transfer time scale \( 1/k_B T \). For this to hold, the electronic coupling to the molecular and solvent vibrations must be sufficiently weak. This implies a relatively rigid molecular environment, which seems plausible for the fibrillar protein backbone in the present case. It is important to note in this stage that the described conditions for this mechanism to hold are untraditional, while taking into consideration the floppy nature of biological molecules in natural conditions.

Nevertheless, the recent accumulation of evidence for the role of delocalized electronic states in coherent ET through multiheme proteins (43), as well as the unique features of our synthetic protein system, encourage us to discuss the relevance of this rather unconventional mechanism in some detail below.

Following the latter evidence for a charge delocalization over several porphyrin molecules, we turn now to a mixed coherent-hopping transport model for explaining the long-range nature of charge transport through the multiporphyrin protein scaffold. Nevertheless, detailed structural information is still needed in order to confirm the possibility for any charge delocalization over several porphyrin molecules in our system; hence, our mixed coherent-hopping transport model is of a speculative nature, though, as will be discussed below, it gives the best fit to our results in terms of realistic energetic landscape. The emerging physical picture (*SI Appendix, Mixed Coherent ET Model*) is that the ET mechanism involves porphyrin blocks, which are protected from coupling to molecular vibrations, and where each such block supports coherent superpositions of several locally charged porphyrins (Fig. 4B). Diffusion between these blocks is facilitated by protein vibrations (thermal fluctuations), via an incoherent (Marcus-like) hopping step. Considering two neighboring blocks (Fig. 4B), Eq. 3 is replaced by a set of hopping rates from the \( m \)-th superposition state on the \( m \)-th block, into the \( (m') \)-th state of the \( (n') \)-th block:

\[
k_{m,n-m,n} = H_{m,m}^{-1/2} \sqrt{\frac{\rho}{\hbar^2 \lambda_{n,m} k_B T}} \langle \left( e_n^{(m)} - e_n^{(m')} \right)^2 \rangle^{1/2}.
\]

Here, \( e_n^{(m)} = e_n^{(m')} \) and \( \lambda_{n,m} \) are, respectively, the corresponding driving force and reorganization energy. The electronic tunneling matrix element, \( H_{m,m} \), depends on the proximity and relative orientation of the terminal porphyrin units from the two different blocks, as well as on the projections of the coherent superpositions within each block, on the terminal units. In most cases (46), the mixed coherent-hopping mechanism promotes long-range ET, where the larger the block size, the smaller the interporphyrin electronic coupling needed to obtain the same diffusion coefficient. In *SI Appendix*, we demonstrate this effect for the relevant parameters associated with the present system, assuming a quasi-1D local diffusion pathway (*SI Appendix, Fig. S6*). The latter calculations show that our extrapolated diffusion coefficient is resulting in electronic coupling values of below 20 meV for block chains of above 15 porphyrin molecules. This electronic coupling value is much more reasonable compared to the one obtained by using the hopping model. While these numbers for the interporphyrin couplings seem to be in accordance with the data, the conclusion that the transport is facilitated by delocalization over 15 porphyrin molecules appears to stretch the currently believed delocalization limit, associated with a small number of molecules, and, thus, to be in conflict with the common view of suppressed delocalization in the presence of thermal fluctuations. Indeed, the proposed model assumes that an exceptionally rigid molecular environment within the fibrillar protein amounts to exceptionally low local-reorganization energies. Consequently, thermal fluctuations within such islands are suppressed, which facilitates persistent electronic delocalization over several porphyrins on the time scale of ET between islands.

**Concluding Remarks**

In conclusion, we have shown here that long-range (2.5 mm) ET across a protein-based matrix containing various protoporphyrin cofactors is mediated by the conjugated macrocycle ring and is not related to the metal center. Hole-mobility values of \( \sim 0.01 \) cm\(^2\) V\(^{-1}\) s\(^{-1}\) were obtained, corresponding to a charge-diffusion coefficient of \( \sim 2 \times 10^{-5} \) cm\(^2\) s\(^{-1}\). We discussed the possibility of several ET mechanisms from purely incoherent processes (electron hopping) to coherence-assisted mechanisms. Using our experimental values for molecular doping density, charge mobility, and activation energy, we showed that if the common sequential hopping mechanisms between metal redox centers were responsible for the observed ET, it would have required an unrealistically high electron-coupling element (~65 meV) between localized redox centers. We proposed that, due to the involvement of identical molecular dopants in each experiment and the suggested rigidity of our system, the charging states of a group of dopants can be delocalized over a larger area and form a local band-like structure, which merits a mixed coherent-hopping transport model.

In this study, we explored the role of protoporphyrin in ET. Although many macrocyclic nonprotoporphyrins containing different metal centers, such as Mg-containing chlorophylls or bacteriochlorophylls, Co-containing cobalamines (vitamin B12), and Ni-containing coenzyme F430, as well as metal-free phophyrins, have been shown to be involved in several ET pathways in nature, the only macrocyclic compound shown, to date, to
participate in the very-long-range bacterial extracellular ET is the Fe-containing heme. It is interesting to hypothesize why nature exclusively chose heme for this purpose. Our first speculation concerns the availability of the heme molecules. Due to the ubiquity of heme in nature, the enzymatic machinery required in order to produce it is well established in various biological systems; hence, it is easier to use heme than any other metal-containing porphyrin, even though we showed here that the metal center is not actually important for ET. Our second speculation relates to the use of the Fe ion for structural reasons rather than an ET mediator. As shown in the recently published structure of the pili of hexaheme cytochrome OmecS (25, 26), the protein subunits self-assemble by coordination to the Fe ion, i.e., two His residues from two different proteins are coordinated to one heme molecule.

**Methods**

**Electrospinning of BSA Mats.** Electrospinning solution was prepared by dissolving BSA (MP Biomedicals) in 90% 2,2,2-trifluoroethanol (Apollo Scientific) for a final BSA concentration of 14% (weight [wt]%volume [vol]). After 12 h, 5% (vol/vol) 1-mercaptoethanol (Alfa Aesar) was added. Electrospinning was performed in a custom-built system with grounded collector. A 15-kV bias was applied on a 24-gauge blunt needle with injection rate of 1.5 mL/h. The needle was fixed 12 cm above the collector. Final mat thickness was 60 μm.

**Synthesis of Metal Protoporphyrins.** Metal protoporphyrins were synthesized following a procedure described in the literature (47, 48) with modification. Two milligrams of PPIX (STREM Chemicals) was dissolved with the respective metal salt (zinc chloride, copper chloride, and cobalt acetate) at a molar ratio of 1:10 in 1 mL of dimethyl sulfoxide (DMSO; Carlo-Erba). The solutions were then heated to 60 °C for 12 h and, after cooling, were centrifuged for 30 min. DMSO was used as a solvent due to the high solubility of PPIX in DMSO.

**Doping process.** Stock solutions were prepared by dissolving hemin (Sigma-Aldrich), PPIX (STREM Chemicals), and blubirin (AK Scientific) in 1 mL of DMSO (Carlo-Erba) for a final concentration of 3.2 mM. These stock solutions, as well as synthesized Co-, Zn-, Cu-PPIX stock solutions, were then diluted with 1 mL of DMSO and 5 mL of phosphate-buffered saline buffer to yield doping solutions of the desired concentration. The mats were then placed in the doping solutions on a stirring plate without light exposure for at least 24 h.

**UV/Vi absorption.** UV/Vi absorption of synthesized metal-protoporphyrins in DMSO was measured with an Agilent Cary 60 spectrophotometer using a quartz cuvette with a 1-cm path length. Doping-density evaluations were carried out by using the same spectrometer, by measuring the absorption spectra before and after placing the mats in the dopant solutions.

**Metal-Finger Electrode and Three-Terminal Device Fabrication.** Heavily p-doped silicon wafers with SiO2 dielectric layer (110 nm) and glass microscope slides were used as substrates for the devices used in the transistor and impedance measurements, respectively. The substrate was sonicated for 5 min in the following series of solvents: acetone followed by methanol, isopropanol, and, finally, ethanol. After the last sonication, silicon substrates were washed with distilled water and dried using hot air. Using an e-beam evaporator at a deposition rate of 2 Å/s under 5 × 10−7 Torr at room temperature, 100 nm of Au on 10 nm of Cr was evaporated through shadow masks. Both finger electrodes and the transistor’s source-drain electrodes were deposited simultaneously on the glass and silicon substrates, respectively. Mats were placed on finger electrodes or three-terminal devices and gently dried with filter paper to remove excess water. The water weight percentage within the BSA mat in all electrical experiment was around 150 wt%.

**Impedance measurements.** Impedance measurements were performed by using an MTZ-35 impedancemergean-phase analyzer (Bio-Lig). Mats on finger electrodes with large electrode distance of 2.5 mm were contacted by using a probe station micromanipulator. A 50-mV AC bias was applied during the measurements without direct-current bias. A frequency range of 10 MHz to 10 Hz was used. Temperature dependence was studied by using a Peltier-containing probe station (NSTEC) in the range of 5 °C to 25 °C.

**FET measurements.** FET measurements were carried out by using two source–measurement units (B2901A, Keysight) connected together via GPIB cables. Mats were placed on a bottom-contact, three-terminal device with 100-μm interelectrode distance and 1.35 or 2.7 cm width. Source-drain and gate electrodes were contacted by using a probe-station micromanipulator. Source-drain current were measured in the range of 0 to 1.5 V, while an alternating gate voltage was applied between −8 and 8 V.

**Data Availability.** All relevant data, materials, protocols, computational details, and theoretical analysis are included in the manuscript and SI Appendix.

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**Geobacter sulfurreducens**.