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# Charge transport at the protein-electrode interface in the emerging field of BioMolecular Electronics

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## Abstract

The emerging field of BioMolecular Electronics aims to map out in detail the electronic properties of biomolecules with two main outcomes envisioned. The first is to use nature's efficient electron transport mechanisms as an inspiration to build the next generation of hybrid bioelectronic interfaces towards a more sustainable, green, and efficient technology. The second is to understand this ubiquitous physico-chemical process in life, which is exploited in fundamental biological processes such as signalling, respiration, photosynthesis, enzymatic catalysis, and would lead us to a better understanding of many disease mechanisms. Extracting charge transport signatures from a biomolecule requires optimised methods for tethering the molecules to an electrode surface. It is desired to have precise electrochemical control over the energy levels of the hybrid biomolecule-electrode interface. Here, we review recent progress towards understanding the transport mechanisms at the protein-electrode interface, which involves the latest single-molecule and ensemble nanoscale electronic junction designs. While the field is rapidly evolving, a number of distinct electrical signatures of a protein-electrode interface have been observed, such as the essential role of the protein-electrode chemical contact, the highly efficient intramolecular charge transport, and the key role of the secondary protein structure. Moving forward, a key focus area will be to explore in more detail the role of the amino

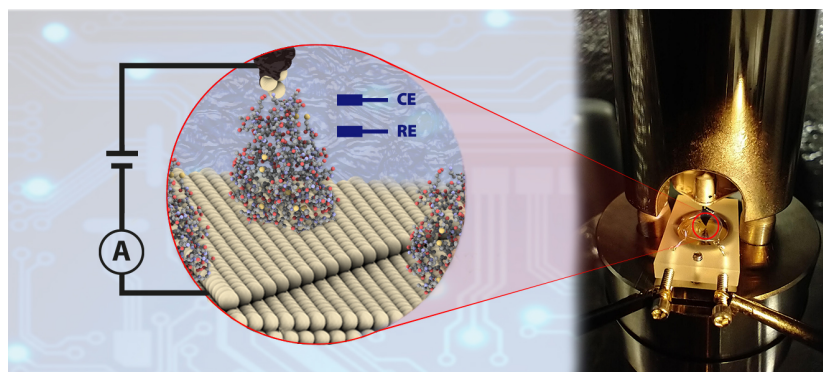
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acid matrix and how parameters like stiffness, intrinsic electrostatic charge, and solvent, influence the detailed transport pathways and mechanisms.

*Keywords:* BioMolecular Electronics, single-protein junctions, protein films, electron transport, electron transfer, electrode surface, bio-engineering, coupling, hybridisation, protein-electrode interface, contacts, tapping, blinking

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## 1. Introduction

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Proteins are vital electronic charge mediators in nature [1, 2]. Although  
5 the amino acid building blocks are mostly insulating, folded proteins have remarkable long range charge transfer properties [3]. Proteins fulfil many critical functions in cellular processes governed by electron movement, such as respiration [4], photosynthesis [5], and enzyme catalysis [6]. The field of BioMolecular Electronics studies the electrical properties of proteins and other biomolecules  
10 with two main objectives. The first is to design and build the next generation of hybrid bioelectronic interfaces towards more efficient and biocompatible electronic devices. The second is to enhance our understanding of medicinal chemistry, via understanding the chemical interactions leading to biological electron transport. Both aims involve utilising and manipulating nature's efficient  
15 bioelectricity, such as the outstanding bacterial bioelectrical circuitry based on multiheme cytochrome wires [7]. The field envisions the creation of more efficient bio-compatible and non-invasive biosensors for point of care detection of analytes in our bodies.

The majority of electronic studies on proteins to date have focused on  
20 solution-based electron transfer and catalytic properties [8, 9, 10, 11]. Early work on surface-bound proteins came from Ulstrup, Facci, Canters, Cannistraro et al. [12, 13, 14]. These pioneering studies used surface-sensitive spectroscopy and electrochemistry to probe the details of the protein-electrode interface, see [Figure 1A](#). However, such techniques provide a bulk picture averaging out over  
25 large numbers of proteins, often lacking information about surface interactions, surface orientation, structural changes, and reliable quantification of the surface coverage [15].

[1] Total word count is 2471

[2] Intro is 370 words

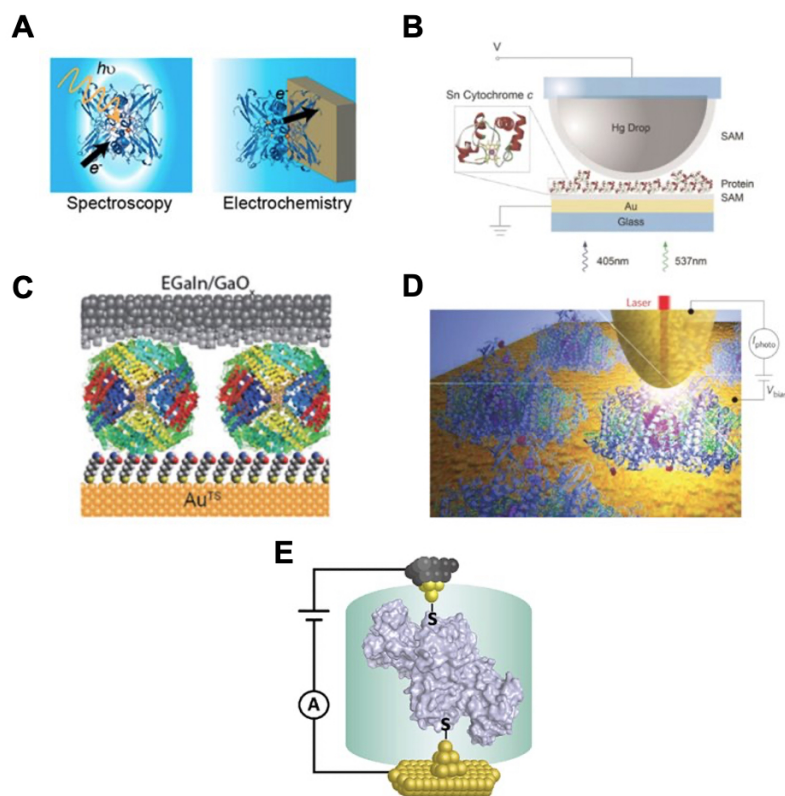


Figure 1: **Experimental techniques in the field of BioMolecular Electronics.** (A) Solution-based techniques to study electron transfer, using light (left), or a single surface working electrode (right). Reprinted with permission from reference [16], <https://pubs.acs.org/doi/10.1021/acs.jpcc.8b07431>. Contact ACS directly for permission requests related to this article. (B) Ensemble liquid metal junctions. Reprinted with permission from reference [17]. (C) Ensemble using EGaln junctions. Reprinted with permission from reference [18]. (D) Scanning near-field optical microscopy for the measurement of photo-currents. Reprinted with permission from reference [19]. (E) Single-molecule STM junction. Reprinted with permission from reference [20]\*\*.

One way to overcome some of these limitations is to decrease the number of proteins under investigation by introducing a second electrode, which serves as the top contact for ensembles of proteins all the way down to a single protein. These electrode-protein-electrode configurations have been reviewed great detail

[21]\* [11, 22]. Our focus here is to review the latest experimental developments using surface-bound proteins and the mechanism of electron transport through protein nanoelectronic junctions. We start with a brief overview of experimental techniques for the fabrication of electrode-protein-electrode junctions. We then discuss these junctions using an energy-level framework and how the crucial parameters of alignment (section 3) and the hybridisation (section 4) affect the junction properties. We then move on to discuss the still largely unanswered question of how electrons flow through biomolecules over long distances. Finally, we summarise our discussion and share some prospects for the next few years.

## 2. Tools to study protein charge transport

The metal-molecule-metal approach has been used extensively to study small organic molecules in the field of molecular electronics [23]. Experimental methods to create nanoscale electronic junctions have been discussed in great detail by Xiang et al. [24]. Depending on the technique, junctions consist of a (mono)layer of molecules, often referred to as ensemble junctions, or just a few and even single molecules. Here, we highlight a few techniques that have been successfully used to measure the charge transport properties of proteins.

### 2.1. Ensemble junctions

The liquid metal junction using a mercury drop has been around for a long time [25]. Recently, Nakamaru et al. used it to measure the light-dependent electrical properties of cytochrome c, see Figure 1B. They found that these protein junctions behave as efficient light-sensitive photoconductors and hence can act as photoelectrochemical switches. George Whitesides and coworkers later developed a more robust liquid metal technique, based on eutactic gallium-indium (EGaIn) [26]. An example is given in Figure 1C, where a layer of ferritin molecules is trapped between the gold substrate and the liquid metal drop, see section 5 for further details. David Cahen and coworkers use various other ensemble junction techniques, which have lead to various important findings in the field [11], see further below. Add in CP-AFM here?

[3] Techniques is 471 words

## 2.2. Single-protein junctions

Despite the prominence of single-molecule techniques in the field of molecular electronics, to date most single-protein charge transport studies have used scanning tunneling microscopy (STM) techniques. A notable exception is the pioneering report by Daniel Gerster and coworkers [19], who used scanning near-field optical microscopy to measure the photocurrent of a covalently bound single photosynthetic protein, illustrated in Figure 1D. Wenjing Hong and coworkers recently used the STM-based single-protein approach to register enzymatic catalysis [20]\*\*, see Figure 1E. Their results show that the presence of NAD co-enzyme responsible for the enzyme activity results in a decrease of the protein HOMO-LUMO gap, thus suggesting a connection between charge transport and enzymatic activity. Stuart Lindsay and coworkers also reported on enzymatic activity, which they monitored directly using the electrical current of the STM [27]\*\*. They showed that large current fluctuations are responsible for polymerase activity. These examples set the stage for integrating the enzyme-metal interface for the design of hybrid bioelectronics.

Furthermore, our group has used the STM extensively to study the charge transport properties of single azurin molecules [28]\*. Most notably, we reported the first single-protein junction characterised using the break-junction or tapping technique [29]\*\*. We then measured the current-voltage characteristics [30], followed by the demonstration of controlled redox switching using an electrochemical STM setup [31]\*\*. These electrochemical STM (EC-STM) studies highlight the importance of the energy levels of the electrode-protein-electrode junction, which we will explore in the following sections. First, we discuss the position [offset – gating] of the molecular [protein] energy levels with respect to the Fermi energy of the electrodes, followed by the degree of hybridisation [coupling – broadening] between the energy levels of the proteins and the electrodes.

## 3. Molecular energy levels

[4] Gating is  
334 words

The molecular energy levels of a (redox) protein strongly influence the electron transmission through a biomolecular junction. Particularly important, is the position of these energy levels with respect to the Fermi energy ( $E_F$ ) of the contacting electrodes. Precise control over the position of these energy levels  
95 using a gating electrode is a key tool for studying the details of the transport mechanisms in biomolecular junctions, see [Figure 2A](#) for an example. At gate potentials close to -0.4 V and +0.2 V, the junction current is low because the dominant energy level of the protein,  $D_{ox}$ , is not aligned with the Fermi energy of the electrodes. In contrast, the junction current is at a maximum when  
100 this energy level is aligned with  $E_F$ , at a gate potential close to -0.1 V in this example.

The EC-STM is a prominent tool for localising energy at the protein junction, see [Figure 2B](#). An electrochemical functionality is added to the standard STM setup in a bipotentiostat configuration. Using this setup, one has precise  
105 control over the electrochemical potential of the substrate and the tip independently. In turn, this control allows for probing the relevant (non)redox molecular energy levels, from which a picture can be drawn that highlights the chemical electron pathways in a protein supramolecular structure. Paolo Facci and coworkers first used this method to measure the charge transport behaviour  
110 of azurin [[32](#)].



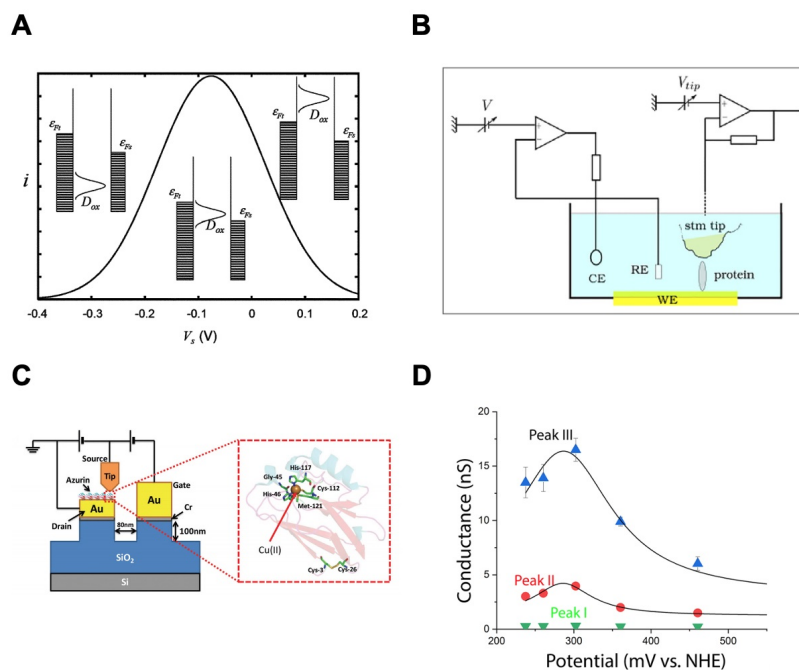


Figure 2: **The energy levels of the protein can be aligned to the Fermi level of the electrodes using an electrochemical gate.** (A) Energy-level framework of the gating effect, where the applied gate voltage shifts the energy levels of the protein into and out of resonance, enhancing the junction conductance at the resonant potential. Reprinted with permission from reference [32]. (B) Schematic setup of an electrochemical scanning tunnelling microscope. Reprinted with permission from reference [33]. (C) Side-gated conducting probe atomic force microscopy experiment. Reprinted with permission from reference [34]. (D) The conductance of streptavidin depends on the electrochemical cell potential. Reprinted with permission from reference [35]\*\*.

David Cahen and coworkers recently reported a different approach using a side-gated transistor configuration combined with conducting probe AFM, as shown in Figure 2C [34]. They combined their gating experiment with a variation in the work function of their substrates to map out the resonance positions of different azurin junctions. Stuart Lindsay and coworkers took a different approach and measured the charge transport characteristics of proteins that have no redox activity, see Figure 2D. They used a combination of rest potentials

and different electrode materials (also making use of differences in metal work functions), and found peculiar resonance peaks in streptavidin, immunoglobulin E, and  $\Phi$ 29 polymerase, despite their lack of redox centres [35]\*\*.

#### 4. Bioengineering the protein-electrode interface

We now turn our attention to the protein-electrode interface, and how the chemical interactions influence the charge transport properties. The efficiency of electron transport through molecular systems strongly depends on the coupling at the electrode-molecule interface and this problem has been studied thoroughly in the field [36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46]. The formation of the electrical contact in an integrated protein-electrode interface is fundamental in the electrical characteristics of the final protein junctions. One of the major issues in nano-biotechnology is the design and compatibility of this integration. Figure 3A illustrates a Lorentzian function of the HOMO and LUMO levels of a molecular junction relative to the band energies of the metal,  $E_F$ . The magnitude of electron transmission depends on the extent of hybridisation, or protein-electrode coupling, which broadens the Lorentzian curve ( $\Gamma$ ) and thus area under the curve. It also depends on the energetic alignment ( $\Delta E$ ) of the molecular orbitals with respect to  $E_F$ , as described in section 3. The hybridisation refers to the chemical mixing between energy levels of the proteins and of the metal electrode surface. Crucially, the hybridisation can be boosted by appropriate and specific chemical modifications to both the electrodes and the proteins under investigation.

Jens Ulstrup and coworkers contributed significantly towards protein-electrode coupling, and their early work has been crucial in the effort to optimise bio-interfaces. For example, they adsorbed azurin molecules to gold electrodes directly through surface cysteine residues [12] or via alkanethiol monolayers [47] to form a stable functional monolayer. Building on these pioneering reports, a number of other notable contributions towards the design of hybrid bio-interfaces include further electrode surface modification with monolayers

[5] Interface  
is 669 words

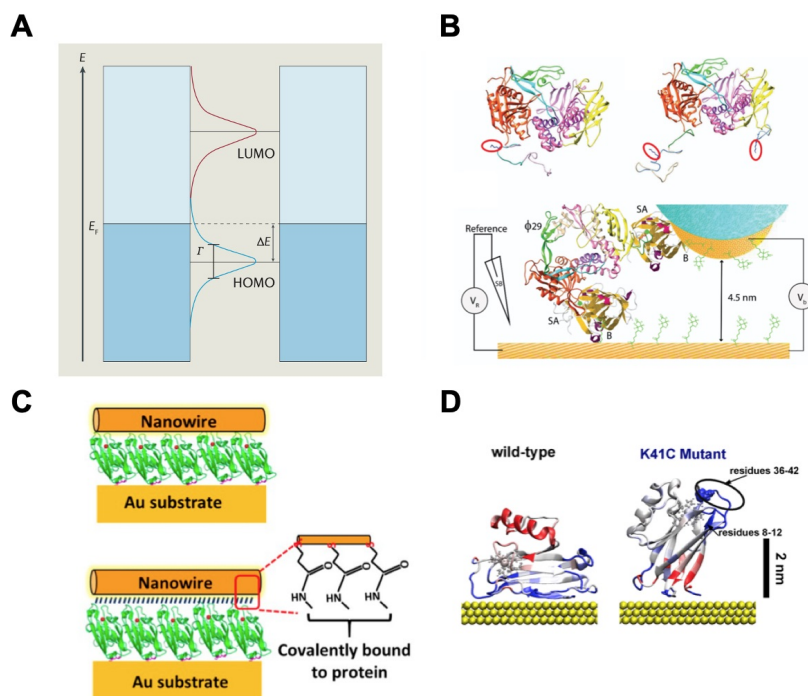


Figure 3: **The efficiency of charge transport is strongly influenced by the details of the molecule-electrode interfaces.** (A) Lorentzian plot of the transmission function plotted against energy. This shows the conductance of hybridised HOMO-LUMO orbitals of a single molecule relative to the band energies of a metal. Reprinted with permission from reference [44]. (B) Top:  $\Phi 29$  polymerase with one (left) or two (right) specific lysine contacts. Bottom: Streptavidin immobilised on electrodes via thiolated biotin anchoring groups, which encloses a  $\Phi 29$  polymerase. Reprinted with permission from reference [27]\*\*. (C) Ensemble of proteins trapped between a gold substrate and a gold nanowire. Reprinted with permission from reference [56]\*\*. (D) A molecular dynamic simulation to investigate the effect of point mutagenesis on the orientation of a protein. Reprinted with permission from reference [57]\*.

[48, 49, 50, 51, 52, 53], bioengineering contacts [48, 52, 54], and the use of chemically linked redox mediators and relays [51, 55]. All of these methods aim to improve orientation, stability, and establish electrical interactions between insulated enzyme redox centers and electrodes.

Stuart Lindsay and coworkers have used chemical modifications to bioengi-

neer specific contacts to a  $\Phi$ 29 polymerase protein [27]\*\*. Figure 3B shows the modified protein in the top, either with a single thiolated contact on or two of these specific anchoring units. In the bottom part, streptavidin is tethered to gold electrodes using these thiolated biotin groups. A higher junction conductance is measured when two specific contacts are used, which is attributed to the result of biotin binding into a deeper pocket of the streptavidin. In turn, this configuration provides a more efficient injection of charge carriers into the hydrophobic interior of the protein. Using contacts to modify electrodes also eliminates unpredictable orientations of proteins at the interface that yield protein junctions with a low electrical activity. Instead, proteins can bind to specific bioengineered contacts, resulting in a decrease of the energy barrier for electron injection, and thus more efficient charge transport.

David Cahen and coworkers have extensively used another technique to fabricate ensemble protein junctions, see Figure 3C. This gold-nanowire technique relies on the formation of self-assembled monolayers of proteins, before gold nanowires are deposited on top to function as the top electrode [56]\*\*. They show how the coupling strength of the chemical linkers can act as a switch between different electron transport mechanisms through the protein layer [56]\*\*. In another study, they reported the immobilisation of cytochrome c using the surface-exposed cysteine 104 residue that binds covalently to gold electrodes, forming an oriented, robust monolayer that allows for electron transport at room and cryogenic temperatures [58]\*. Mukhopadhyay et al. reports the reproducibility of current densities of three different proteins on SAM modified electrodes within six types of junction configurations from different laboratories across the world [46]. The study concludes that the protein-electrode interface can dominate the efficiency of electron transport without altering the mechanism.

Similar to the functionalisation of small molecules with chemical anchoring groups, chemical modifications can also be introduced to the protein amino acid matrix. An example is given in Figure 3D, where such modifications are used to control the orientation at the protein-interface is [57]\*. This theoretical study

showed that the wild-type azurin adsorbs along two favoured conformations and  
185 undergoes major reorientation, whereas the modified azurin proteins gives rise  
to additional vibrations resulting in the adsorption using new conformations,  
see further details in [section 5](#).

## 5. The enigma of charge transport mechanisms in proteins

190 Understanding the mechanisms of charge transport at the nanoscale is an  
essential step towards the aims of BioMolecular Electronics, as outlined in the  
[Introduction](#). In electronic junctions of relatively simple organic molecules, the  
transport mechanisms are mainly influenced by the length of the tunnelling bar-  
rier and the temperature [59]. Shorter molecules mostly display off-resonance  
195 tunnelling, where the transport is not affected by the surrounding temperature,  
and longer molecules are dominated by a thermally-activated hopping mecha-  
nism. This effect is illustrated in [Figure 4A](#), where the effective distance of the  
tunnelling barrier is proportional to the molecular length. At a certain threshold  
value, indicated by the dashed line, the mechanism changes from tunnelling to  
200 hopping. However, in biomolecular junctions, the transport mechanisms do not  
align with either of the two scenarios above. Instead, an anomalous long-range  
transport mechanism prevails in most junctions consisting of wild-type proteins.  
This mechanism often depends on complex factors, such as solvent, pH, and the  
supramolecular structure and conformation of the protein amino acid matrix.  
205 For example, Christian Nijhuis and coworkers showed how the charge transport  
mechanism in monolayers of ferritin molecules depends on the iron content of  
the molecules [18]. Similarly, Garg et al. found that with two or more heme  
centres present in c-type cytochromes the conduction efficiency is increased by  
several orders of magnitude [60].

[6] Mecha-  
nisms is 481  
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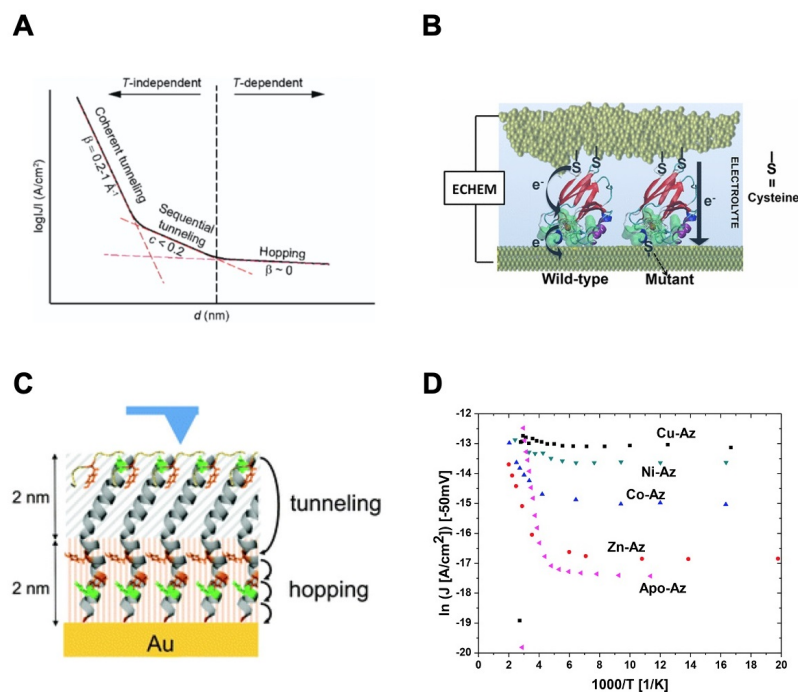


Figure 4: **Anomalous thermally-independent long-range electron charge transport is the dominant mechanism in biomolecular junctions.** (A) Long-range electron transport is temperature independent in iron-containing ferritin junctions. Reprinted with permission from reference [18]. (B) Mutagenesis of azurin molecules changes the transport mechanisms from sequential tunnelling to fully coherent tunnelling. Reprinted with permission from reference [61]\*\*. (C) Self-assembled monolayers of thiolated protein nanowires from *Geobacter sulfurreducens* demonstrate a hybrid mechanism along different parts of the amino acid chain. Reprinted with permission from reference [62]. (D) Theoretical models reveal that the mechanism strongly depends on the redox-active metal centre in azurin junctions. Reprinted with permission from reference [63].

210 When point-site mutations are introduced as chemical modifications, the charge transport channels in redox proteins can be altered significantly. Our group recently reported charge transport properties of a modified azurin protein using an EC-STM configuration [64][61]\*\*. By exchanging the natural lysine at the 41 position for an additional cysteine, the mechanism of transport changed

215 from a two-step sequential model to complete coherent tunnelling, see Figure 4B.

Similar outer protein mutations have been used to control the orientation of the protein junction which, in turn, dictates the final electron pathway [65]. Gemma Reguera and coworkers took this approach to functionalise protein nanowires from *Geobacter sulfurreducens* and used these to form self-assembled monolayers on gold substrates, see Figure 4C. They showed that tunnelling prevails in the top part of the layer, where no aromatic residues are present, whereas hopping takes over in the presence of such aromatic rings in the bottom part of the layer [62].

Finally, despite significant challenges in theoretical modelling of charge transport through large molecules such as proteins, Spiros Skourtis and colleagues reported fitted trends through several azurin junctions, see Figure 4D. They confirmed the vital role of copper in azurin as previously reported by David Cahen and coworkers, as well as by other groups. Another exciting development is the recent report by Gábor Vattay and colleagues, who reported a method to model the charge transport through protein junctions using the Landauer approach using clever approximations [66]. In addition, Ioan Bâldea highlighted that the measurement of the electrical current could be too slow to register conformational fluctuations in the protein and that noise power experiments could give us more insight [67].

## 6. Conclusions and outlook

In this review, we have summarised recent progress in the field of BioMolecular Electronics, with a specific focus on the electron transport mechanisms across protein nanoelectronic junctions. In the energy-level framework of the metal-protein-metal junction, the position and hybridisation dominate the charge transport efficiency. Therefore, the two key areas that will continue to receive significant attention are energy-level gating of the junctions and the chemical functionalisation of the protein-electrode interface. An anomalous thermally-independent long-range charge transport mechanism is prevalent in most wild-type proteins. When specific point-site mutations are introduced to the amino

[7] Conclusion is 192 words

acid matrix, this efficient mechanism often breaks down, indicating the crucial role of the supramolecular protein structure. However, the exact nature of this interaction, and how it allows for the efficient long-range charge transport mechanism remains an open question. Over the next few years, we expect to see  
250 a focus on the role of the supramolecular protein structure for efficient charge transport. Breakthrough findings in this area will be a crucial step towards our goal of building hybrid BioMolecular sensors and transistors while at the same time setting the stage for discovering mechanisms in medicinal chemistry for enhanced drug delivery.

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### **CRedit author statement**

Tracy Q. Ha performed writing - original draft, conceptualisation, and editing.  
260 ing. Inco J. Planje performed writing - original draft, conceptualisation, reviewing, and editing. Jhanelle White performed writing - editing. Albert C. Aragonès performed writing - reviewing, editing, and graphical abstract. Ismael Díez-Pérez acquired funding, carried out supervision, and performed writing - reviewing and editing.

### 265 **Declaration of interest**

None

### **References**

- [1] D. Beratan, J. Onuchic, Winkler, H. Gray, [Electron-tunneling pathways in proteins](#), *Science* 258 (5089) (1992) 1740–1741.  
270 [doi:10.1126/science.1334572](https://doi.org/10.1126/science.1334572).



URL <https://www.sciencemag.org/lookup/doi/10.1126/science.1334572>

[2] C. C. Page, C. C. Moser, X. Chen, P. L. Dutton, [Natural engineering principles of electron tunnelling in biological oxidation–reduction](#), *Nature* 402 (6757) (1999) 47–52. doi:10.1038/46972.

URL <http://www.nature.com/articles/46972>

[3] M. Cordes, B. Giese, [Electron transfer in peptides and proteins](#), *Chemical Society Reviews* 38 (4) (2009) 892. doi:10.1039/b805743p.

URL <http://xlink.rsc.org/?DOI=b805743p>

[4] G. T. Babcock, M. Wikström, [Oxygen activation and the conservation of energy in cell respiration](#), *Nature* 356 (6367) (1992) 301–309. doi:10.1038/356301a0.

URL <http://www.nature.com/articles/356301a0>

[5] D. J. Vinyard, G. M. Ananyev, G. Charles Dismukes, [Photosystem II: The Reaction Center of Oxygenic Photosynthesis](#), *Annual Review of Biochemistry* 82 (1) (2013) 577–606. doi:10.1146/annurev-biochem-070511-100425.

URL <http://www.annualreviews.org/doi/10.1146/annurev-biochem-070511-100425>

[6] J. Stubbe, W. A. van der Donk, [Protein Radicals in Enzyme Catalysis](#), *Chemical Reviews* 98 (2) (1998) 705–762. doi:10.1021/cr9400875.

URL <https://pubs.acs.org/doi/10.1021/cr9400875>

[7] S. Xu, A. Barrozo, L. M. Tender, A. I. Krylov, M. Y. El-Naggar, [Multiheme Cytochrome Mediated Redox Conduction through Shewanella oneidensis MR-1 Cells](#), *Journal of the American Chemical Society* 140 (32) (2018) 10085–10089. doi:10.1021/jacs.8b05104.

URL <https://pubs.acs.org/doi/10.1021/jacs.8b05104>

- [8] R. A. Marcus, [Chemical and Electrochemical Electron-Transfer Theory](#), Annual Review of Physical Chemistry 15 (1) (1964) 155–196.  
300 [doi:10.1146/annurev.pc.15.100164.001103](https://doi.org/10.1146/annurev.pc.15.100164.001103).  
URL <http://www.annualreviews.org/doi/10.1146/annurev.pc.15.100164.001103>
- [9] C. C. Moser, J. M. Keske, K. Warncke, R. S. Farid, P. L. Dutton, [Nature of biological electron transfer](#), Nature 355 (6363) (1992) 796–802. [doi:10.1038/355796a0](https://doi.org/10.1038/355796a0).  
305 URL <http://www.nature.com/articles/355796a0>
- [10] H. B. Gray, J. R. Winkler, [Long-range electron transfer](#), Proceedings of the National Academy of Sciences of the United States of America 102 (10) (2005) 3534–3539. [doi:10.1073/pnas.0408029102](https://doi.org/10.1073/pnas.0408029102).  
310 URL <http://www.pnas.org/cgi/doi/10.1073/pnas.0408029102>
- [11] C. D. Bostick, S. Mukhopadhyay, I. Pecht, M. Sheves, D. Cahen, D. Lederman, [Protein bioelectronics: a review of what we do and do not know](#), Reports on Progress in Physics 81 (2) (2018) 026601. [doi:10.1088/1361-6633/aa85f2](https://doi.org/10.1088/1361-6633/aa85f2).  
315 URL <http://stacks.iop.org/0034-4885/81/i=2/a=026601?key=crossref.ec73b13b9a6ad598d883add951b9746ahttps://iopscience.iop.org/article/10.1088/1361-6633/aa85f2>
- [12] Q. Chi, J. Zhang, J. U. Nielsen, E. P. Friis, I. Chorkendorff, G. W. Canters, J. E. T. Andersen, J. Ulstrup, [Molecular Monolayers and Interfacial Electron Transfer of Pseudomonas aeruginosa Azurin on Au\(111\)](#), Journal of the American Chemical Society 122 (17) (2000) 4047–4055. [doi:10.1021/ja993174t](https://doi.org/10.1021/ja993174t).  
320 URL <https://pubs.acs.org/doi/10.1021/ja993174t>
- [13] P. Facci, D. Alliata, S. Cannistraro, [Potential-induced resonant tunneling through a redox metalloprotein investigated by electrochemical scanning probe microscopy](#), Ultramicroscopy 89 (4) (2001) 291–298.  
325

[doi:10.1016/S0304-3991\(01\)00093-6](https://doi.org/10.1016/S0304-3991(01)00093-6).

URL <https://linkinghub.elsevier.com/retrieve/pii/S0304399101000936>

- 330 [14] R. Rinaldi, A. Biasco, G. Maruccio, V. Arima, P. Visconti, R. Cingolani, P. Facci, F. De Rienzo, R. Di Felice, E. Molinari, M. Ph. Verbeet, G. W. Canters, [Electronic rectification in protein devices](#), *Applied Physics Letters* 82 (3) (2003) 472–474. [doi:10.1063/1.1530748](https://doi.org/10.1063/1.1530748).

URL <http://aip.scitation.org/doi/10.1063/1.1530748>

- 335 [15] N. Kornienko, K. H. Ly, W. E. Robinson, N. Heidary, J. Z. Zhang, E. Reiser, [Advancing Techniques for Investigating the Enzyme–Electrode Interface](#), *Accounts of Chemical Research* 52 (5) (2019) acs.accounts.9b00087. [doi:10.1021/acs.accounts.9b00087](https://doi.org/10.1021/acs.accounts.9b00087).

URL <https://pubs.acs.org/doi/10.1021/acs.accounts.9b00087>

- 340 [16] N. L. Ing, M. Y. El-Naggar, A. I. Hochbaum, [Going the Distance: Long-Range Conductivity in Protein and Peptide Bioelectronic Materials](#), *The Journal of Physical Chemistry B* 122 (46) (2018) 10403–10423. [doi:10.1021/acs.jpcc.8b07431](https://doi.org/10.1021/acs.jpcc.8b07431).

URL <https://pubs.acs.org/doi/10.1021/acs.jpcc.8b07431>

- 345 [17] S. Nakamaru, F. Scholz, W. E. Ford, Y. Goto, F. von Wrochem, [Photo-switchable Sn-Cyt c Solid-State Devices](#), *Advanced Materials* 29 (22) (2017) 1605924. [doi:10.1002/adma.201605924](https://doi.org/10.1002/adma.201605924).

URL <http://doi.wiley.com/10.1002/adma.201605924>

- [18] K. S. Kumar, R. R. Pasula, S. Lim, C. A. Nijhuis, [Long-Range Tunneling Processes across Ferritin-Based Junctions](#), *Advanced Materials* 28 (9) (2016) 1824–1830. [doi:10.1002/adma.201504402](https://doi.org/10.1002/adma.201504402).

350 URL <http://doi.wiley.com/10.1002/adma.201504402>

- [19] D. Gerster, J. Reichert, H. Bi, J. V. Barth, S. M. Kaniber, A. W. Holleitner, I. Visoly-Fisher, S. Sergani, I. Carmeli, [Photocurrent of a single](#)

- 355 [photosynthetic protein](#), *Nature Nanotechnology* 7 (10) (2012) 673–676.  
[doi:10.1038/nnano.2012.165](#).  
URL <http://www.nature.com/articles/nnano.2012.165>
- [20] X. Zhuang, A. Zhang, S. Qiu, C. Tang, S. Zhao, H. Li, Y. Zhang, Y. Wang,  
B. Wang, B. Fang, W. Hong, [Coenzyme coupling boosts charge transport](#)  
360 [through single bioactive enzyme junctions](#), *ISCIENCE* (2020) 101001 [doi:](#)  
[10.1016/j.isci.2020.101001](#).  
URL <https://doi.org/10.1016/j.isci.2020.101001>
- [21] J. M. A. Vivancos, J. Hihath, I. Diez-Perez, *Biomolecular electronics*, in:  
I. Báldea (Ed.), *Molecular Electronics*, Pan Stanford Publishing, 2016, Ch.  
365 *Biomolecul*, pp. 281–313.
- [22] S. Lindsay, [Ubiquitous Electron Transport in Non-Electron Transfer Pro-](#)  
[teins](#), *Life* 10 (5) (2020) 72. [doi:10.3390/life10050072](#).  
URL <https://www.mdpi.com/2075-1729/10/5/72>
- [23] J. C. Cuevas, E. Scheer, [Molecular Electronics](#), Vol. 1 of World Scientific  
370 *Series in Nanoscience and Nanotechnology*, WORLD SCIENTIFIC, 2010.  
[doi:10.1142/7434](#).  
URL <https://www.worldscientific.com/worldscibooks/10.1142/7434>
- [24] D. Xiang, X. Wang, C. Jia, T. Lee, X. Guo, [Molecular-Scale Electronics:](#)  
375 [From Concept to Function](#), *Chemical Reviews* 116 (7) (2016) 4318–4440.  
[doi:10.1021/acs.chemrev.5b00680](#).  
URL <http://dx.doi.org/10.1021/acs.chemrev.5b00680><http://pubs.acs.org/doi/10.1021/acs.chemrev.5b00680>
- [25] K. Slowinski, H. K. Fong, M. Majda, [Mercury-mercury tunneling junctions.](#)  
380 [1. Electron tunneling across symmetric and asymmetric alkanethiolate bi-](#)  
[layers](#), *Journal of the American Chemical Society* 121 (31) (1999) 7257–  
7261. [doi:10.1021/ja991613i](#).  
URL <https://pubs.acs.org/doi/10.1021/ja991613i>

- [26] R. C. Chiechi, E. A. Weiss, M. D. Dickey, G. M. Whitesides, [Eutectic Gallium–Indium \(EGaIn\): A Moldable Liquid Metal for Electrical Characterization of Self-Assembled Monolayers](#), *Angewandte Chemie International Edition* 47 (1) (2008) 142–144. doi:10.1002/anie.200703642.  
URL <http://doi.wiley.com/10.1002/anie.200703642>
- [27] B. Zhang, H. Deng, S. Mukherjee, W. Song, X. Wang, S. Lindsay, [Engineering an Enzyme for Direct Electrical Monitoring of Activity](#), *ACS Nano* 14 (2) (2020) 1360–1368. doi:10.1021/acsnano.9b06875.
- [28] J. M. Artés, I. Díez-Pérez, F. Sanz, P. Gorostiza, [Direct Measurement of Electron Transfer Distance Decay Constants of Single Redox Proteins by Electrochemical Tunneling Spectroscopy](#), *ACS Nano* 5 (3) (2011) 2060–2066. doi:10.1021/nn103236e.  
URL <https://pubs.acs.org/doi/10.1021/nn103236e>
- [29] J. M. Artés, I. Díez-Pérez, P. Gorostiza, [Transistor-like Behavior of Single Metalloprotein Junctions](#), *Nano Letters* 12 (6) (2012) 2679–2684. doi:10.1021/nl2028969.  
URL <https://pubs.acs.org/doi/10.1021/nl2028969>
- [30] J. M. Artés, M. López-Martínez, A. Giraudet, I. Díez-Pérez, F. Sanz, P. Gorostiza, [Current–Voltage Characteristics and Transition Voltage Spectroscopy of Individual Redox Proteins](#), *Journal of the American Chemical Society* 134 (50) (2012) 20218–20221. doi:10.1021/ja3080242.  
URL <https://pubs.acs.org/doi/10.1021/ja3080242>
- [31] J. M. Artés, M. López-Martínez, I. Díez-Pérez, F. Sanz, P. Gorostiza, [Conductance Switching in Single Wired Redox Proteins](#), *Small* 10 (13) (2014) 2537–2541. doi:10.1002/sml1.201303753.  
URL <http://doi.wiley.com/10.1002/sml1.201303753>
- [32] A. Alessandrini, S. Corni, P. Facci, [Unravelling single metalloprotein electron transfer by scanning probe techniques](#), *Physical Chemistry Chemical*

Physics 8 (38) (2006) 4383. doi:10.1039/b607021c.

URL <http://xlink.rsc.org/?DOI=b607021c>

[33] M. Elliott, High-resolution electrochemical STM of redox metallo-  
415 proteins, Current Opinion in Electrochemistry 4 (1) (2017) 152–158.  
doi:10.1016/j.coelec.2017.08.007.

URL <https://linkinghub.elsevier.com/retrieve/pii/S2451910317301199>

[34] B. Kayser, J. A. Fereiro, C. Guo, S. R. Cohen, M. Sheves, I. Pecht,  
420 D. Cahen, Transistor configuration yields energy level control in protein-  
based junctions, Nanoscale 10 (46) (2018) 21712–21720. doi:10.1039/  
c8nr06627b.

[35] B. Zhang, W. Song, J. Brown, R. Nemanich, S. Lindsay, Electronic Conduc-  
tance Resonance in Non-Redox-Active Proteins, Journal of the American  
425 Chemical Society 142 (13) (2020) 6432–6438. doi:10.1021/jacs.0c01805.

[36] Y. Selzer, A. Salomon, D. Cahen, Effect of Molecule-Metal Electronic Cou-  
pling on Through-Bond Hole Tunneling across Metal-Organic Monolayer-  
Semiconductor Junctions, Journal of the American Chemical Society  
124 (12) (2002) 2886–2887. doi:10.1021/ja0177511.

430 URL <https://pubs.acs.org/doi/10.1021/ja0177511>

[37] J. Taylor, M. Brandbyge, K. Stokbro, Theory of Rectification in Tour  
Wires: The Role of Electrode Coupling, Physical Review Letters 89 (13)  
(2002) 138301. doi:10.1103/PhysRevLett.89.138301.

URL <https://link.aps.org/doi/10.1103/PhysRevLett.89.138301>

435 [38] J. M. Beebe, V. B. Engelkes, L. L. Miller, C. D. Frisbie, Contact resistance  
in metal-molecule-metal junctions based on aliphatic SAMs: Effects of sur-  
face linker and metal work function, Journal of the American Chemical  
Society 124 (38) (2002) 11268–11269. doi:10.1021/ja0268332.

URL <https://pubs.acs.org/doi/10.1021/ja0268332>

- 440 [39] X. Li, J. He, J. Hihath, B. Xu, S. M. Lindsay, N. Tao, [Conductance of Single Alkanedithiols: Conduction Mechanism and Effect of Molecule-Electrode Contacts](#), *Journal of the American Chemical Society* 128 (6) (2006) 2135–2141. doi:10.1021/ja057316x.  
URL <https://pubs.acs.org/doi/10.1021/ja057316x>
- 445 [40] Y. S. Park, A. C. Whalley, M. Kamenetska, M. L. Steigerwald, M. S. Hybertsen, C. Nuckolls, L. Venkataraman, [Contact Chemistry and Single-Molecule Conductance: A Comparison of Phosphines, Methyl Sulfides, and Amines](#), *Journal of the American Chemical Society* 129 (51) (2007) 15768–15769. doi:10.1021/ja0773857.  
450 URL <https://pubs.acs.org/doi/10.1021/ja0773857>
- [41] J. A. Malen, P. Doak, K. Baheti, T. Don Tilley, R. A. Segalman, A. Majumdar, [Identifying the length dependence of orbital alignment and contact coupling in molecular heterojunctions](#), *Nano Letters* 9 (3) (2009) 1164–1169. doi:10.1021/nl803814f.  
455 URL <https://pubs.acs.org/doi/10.1021/nl803814f>
- [42] Z.-L. Cheng, R. Skouta, H. Vazquez, J. R. Widawsky, S. Schneebeli, W. Chen, M. S. Hybertsen, R. Breslow, L. Venkataraman, [In situ formation of highly conducting covalent Au-C contacts for single-molecule junctions.](#), *Nature nanotechnology* 6 (6) (2011) 353–357. doi:10.1038/nnano.2011.66.  
460 URL <http://dx.doi.org/10.1038/nnano.2011.66>
- [43] J. S. Meisner, S. Ahn, S. V. Aradhya, M. Krikorian, R. Parameswaran, M. Steigerwald, L. Venkataraman, C. Nuckolls, [Importance of Direct Metal- \$\pi\$  Coupling in Electronic Transport Through Conjugated Single-Molecule Junctions](#), *Journal of the American Chemical Society* 134 (50) (2012) 20440–20445. doi:10.1021/ja308626m.  
465 URL <https://pubs.acs.org/doi/10.1021/ja308626m>

- [44] T. A. Su, M. Neupane, M. L. Steigerwald, L. Venkataraman, C. Nuckolls, [Chemical principles of single-molecule electronics](#), *Nature Reviews Materials* 1 (3) (2016) 16002. doi:10.1038/natrevmats.2016.2.  
470 URL <http://www.nature.com/articles/natrevmats20162>
- [45] B. Zhang, W. Song, P. Pang, H. Lai, Q. Chen, P. Zhang, S. Lindsay, Role of contacts in long-range protein conductance, *Proceedings of the National Academy of Sciences of the United States of America* 116 (13) (2019) 5886–  
475 5891. doi:10.1073/pnas.1819674116.
- [46] S. Mukhopadhyay, S. K. Karuppanan, C. Guo, J. A. Fereiro, A. Bergren, V. Mukundan, X. Qiu, O. E. Castañeda Ocampo, X. Chen, R. C. Chiechi, R. McCreery, I. Pecht, M. Sheves, R. R. Pasula, S. Lim, C. A. Nijhuis, A. Vilan, D. Cahen, Solid-State Protein Junctions: Cross-Laboratory  
480 Study Shows Preservation of Mechanism at Varying Electronic Coupling, *iScience* 23 (5). doi:10.1016/j.isci.2020.101099.
- [47] Q. Chi, J. Zhang, J. E. T. Andersen, J. Ulstrup, [Ordered Assembly and Controlled Electron Transfer of the Blue Copper Protein Azurin at Gold \(111\) Single-Crystal Substrates](#), *The Journal of Physical Chemistry B*  
485 105 (20) (2001) 4669–4679. doi:10.1021/jp0105589.  
URL <https://pubs.acs.org/doi/10.1021/jp0105589>
- [48] K. Ataka, B. Richter, J. Heberle, [Orientational Control of the Physiological Reaction of Cytochrome c Oxidase Tethered to a Gold Electrode](#), *The Journal of Physical Chemistry B* 110 (18) (2006) 9339–9347. doi:10.1021/  
490 jp0534131.  
URL <https://pubs.acs.org/doi/10.1021/jp0534131>
- [49] C. F. Blanford, R. S. Heath, F. A. Armstrong, [A stable electrode for high-potential, electrocatalytic O<sub>2</sub> reduction based on rational attachment of a blue copper oxidase to a graphite surface](#), *Chemical Communications* (17)  
495 (2007) 1710. doi:10.1039/b703114a.  
URL <http://xlink.rsc.org/?DOI=b703114a>



- [50] M. Kondo, Y. Nakamura, K. Fujii, M. Nagata, Y. Suemori, T. Dewa, K. Iida, A. T. Gardiner, R. J. Cogdell, M. Nango, [Self-Assembled Monolayer of Light-Harvesting Core Complexes from Photosynthetic Bacteria on a Gold Electrode Modified with Alkanethiols](#), *Biomacromolecules* 8 (8) (2007) 2457–2463. doi:10.1021/bm070352z.  
URL <https://pubs.acs.org/doi/10.1021/bm070352z>
- [51] I. Willner, E. Katz, A. Riklin, R. Kasher, [Mediated Electron-Transfer in Glutathione-Reductase Organized in Self-Assembled Monolayers on Au Electrodes](#), *Journal of the American Chemical Society* 114 (27) (1992) 10965–10966. doi:DOI10.1021/ja00053a045.  
URL <https://pubs.acs.org/doi/pdfplus/10.1021/ja00053a045>
- [52] M. Kondo, K. Iida, T. Dewa, H. Tanaka, T. Ogawa, S. Nagashima, K. V. P. Nagashima, K. Shimada, H. Hashimoto, A. T. Gardiner, R. J. Cogdell, M. Nango, [Photocurrent and Electronic Activities of Oriented-His-Tagged Photosynthetic Light-Harvesting/Reaction Center Core Complexes Assembled onto a Gold Electrode](#), *Biomacromolecules* 13 (2) (2012) 432–438. doi:10.1021/bm201457s.  
URL <https://pubs.acs.org/doi/10.1021/bm201457s>
- [53] R. Das, P. J. Kiley, M. Segal, J. Norville, A. A. Yu, L. Wang, S. A. Trammell, L. E. Reddick, R. Kumar, F. Stellacci, N. Lebedev, J. Schnur, B. D. Bruce, S. Zhang, M. Baldo, [Integration of Photosynthetic Protein Molecular Complexes in Solid-State Electronic Devices](#), *Nano Letters* 4 (6) (2004) 1079–1083. doi:10.1021/nl049579f.  
URL <https://pubs.acs.org/doi/10.1021/nl049579f>
- [54] S. A. Trammell, A. Spano, R. Price, N. Lebedev, [Effect of protein orientation on electron transfer between photosynthetic reaction centers and carbon electrodes](#), *Biosensors and Bioelectronics* 21 (7) (2006) 1023–1028. doi:10.1016/j.bios.2005.03.015.

- 525 URL <https://linkinghub.elsevier.com/retrieve/pii/S0956566305000990>
- [55] M. Zayats, E. Katz, R. Baron, I. Willner, Reconstitution of apo-glucose dehydrogenase on pyrroloquinoline quinone-functionalized Au nanoparticles yields an electrically contacted biocatalyst, *Journal of the American Chemical Society* 127 (35) (2005) 12400–12406. doi:10.1021/ja052841h.  
530
- [56] J. A. Fereiro, G. Porat, T. Bendikov, I. Pecht, M. Sheves, D. Cahen, *Protein Electronics: Chemical Modulation of Contacts Control Energy Level Alignment in Gold-Azurin-Gold Junctions*, *Journal of the American Chemical Society* 140 (41) (2018) 13317–13326. doi:10.1021/jacs.8b07742.  
535 URL <https://pubs.acs.org/doi/10.1021/jacs.8b07742>
- [57] Ortega, Vilhena, Zotti, Díez-Pérez, Cuevas, Pérez, *Tuning Structure and Dynamics of Blue Copper Azurin Junctions via Single Amino-Acid Mutations*, *Biomolecules* 9 (10) (2019) 611. doi:10.3390/biom9100611.  
URL <https://www.mdpi.com/2218-273X/9/10/611>
- 540 [58] J. A. Fereiro, B. Kayser, C. Romero-Muñiz, A. Vilan, D. A. Dolgikh, R. V. Chertkova, J. C. Cuevas, L. A. Zotti, I. Pecht, M. Sheves, D. Cahen, *A Solid-State Protein Junction Serves as a Bias-Induced Current Switch*, *Angewandte Chemie International Edition* 58 (34) (2019) 11852–11859. doi:10.1002/anie.201906032.  
545 URL <https://onlinelibrary.wiley.com/doi/abs/10.1002/anie.201906032>
- [59] S. H. Choi, B. S. Kim, C. D. Frisbie, *Electrical resistance of long conjugated molecular wires*, *Science* 320 (5882) (2008) 1482–1486. doi:10.1126/science.1156538.  
550 URL <http://www.sciencemag.org/cgi/doi/10.1126/science.1156538>
- [60] K. Garg, M. Ghosh, T. Eliash, J. H. van Wonderen, J. N. Butt, L. Shi, X. Jiang, F. Zdenek, J. Blumberger, I. Pecht, M. Sheves, D. Cahen, *Direct*

- evidence for heme-assisted solid-state electronic conduction in multi-heme  
c -type cytochromes, *Chemical Science* 9 (37) (2018) 7304–7310. doi:  
555 10.1039/C8SC01716F.  
URL <http://xlink.rsc.org/?DOI=C8SC01716F>
- [61] M. P. Ruiz, A. C. Aragonès, N. Camarero, J. G. Vilhena, M. Ortega, L. A.  
Zotti, R. Pérez, J. C. Cuevas, P. Gorostiza, I. Díez-Pérez, *Bioengineering a  
Single-Protein Junction*, *Journal of the American Chemical Society* 139 (43)  
560 (2017) 15337–15346. doi:10.1021/jacs.7b06130.  
URL <https://pubs.acs.org/doi/10.1021/jacs.7b06130>
- [62] K. M. Cosert, R. J. Steidl, A. Castro-Forero, R. M. Worden, G. Reguera,  
*Electronic characterization of Geobacter sulfurreducens pilins in self-  
assembled monolayers unmasks tunnelling and hopping conduction path-  
ways*, *Physical Chemistry Chemical Physics* 19 (18) (2017) 11163–11172.  
565 doi:10.1039/C7CP00885F.  
URL <http://xlink.rsc.org/?DOI=C7CP00885F>
- [63] S. Valianti, J.-C. Cuevas, S. S. Skourtis, *Charge-Transport Mechanisms in  
Azurin-Based Monolayer Junctions*, *The Journal of Physical Chemistry C*  
570 123 (10) (2019) 5907–5922. doi:10.1021/acs.jpcc.9b00135.  
URL <https://pubs.acs.org/doi/10.1021/acs.jpcc.9b00135>
- [64] E. A. Della Pia, Q. Chi, D. D. Jones, J. E. MacDonald, J. Ulstrup,  
M. Elliott, *Single-molecule mapping of long-range electron transport for  
a cytochrome b562 variant*, *Nano Letters* 11 (1) (2011) 176–182. doi:  
575 10.1021/nl103334q.  
URL <https://pubs.acs.org/doi/10.1021/nl103334q>
- [65] E. A. Della Pia, J. E. Macdonald, M. Elliott, D. D. Jones, *Direct Binding  
of a Redox Protein for Single-Molecule Electron Transfer Measurements*,  
*Small* 8 (15) (2012) 2341–2344. doi:10.1002/sml1.201102416.  
580 URL <http://doi.wiley.com/10.1002/sml1.201102416>

- [66] E. Papp, D. P. Jelenfi, M. T. Veszeli, G. Vattay, [A Landauer Formula for Bioelectronic Applications](#), *Biomolecules* 9 (10) (2019) 599. doi:10.3390/biom9100599.  
URL <https://www.mdpi.com/2218-273X/9/10/599>
- 585 [67] I. Bâldea, [Impact of molecular conformation on transport and transport-related properties at the nanoscale](#), *Applied Surface Science* 487 (May) (2019) 593–600. doi:10.1016/j.apsusc.2019.05.112.  
URL <https://doi.org/10.1016/j.apsusc.2019.05.112>