

# Biochemical portrait of the inner-membrane associated cytochrome CbcA: insights into its role in extracellular electron transfer

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Extracellular electron transfer (EET) is a respiratory mechanism that allows electrogenic bacteria to sustain their growth by using exterior electron acceptors, including electrode surfaces. This key metabolic feature involves the transfer of electrons through consecutive redox partners connecting the cell's interior to its exterior and can be explored in several biotechnological fields, namely bioremediation, bioenergy production and microbial electrosynthesis [1]. CbcBA is an inner-membrane oxidoreductase from *Geobacter sulfurreducens* that was shown to be essential for the reduction of extracellular metal oxides and electrodes with a redox potential below  $-210$  mV [2]. The complex is formed by CbcA that binds 7 *c*-type heme groups and is anchored to the membrane by a C-terminal  $\alpha$ -helix, and CbcB, an integral membrane di-heme *b*-type cytochrome. The periplasmic domain of CbcA (37 kDa) was heterologously produced in *E. coli* and different spectroscopic techniques were used to characterize it at the structural and functional level. The crystal structure of CbcA was determined by the multi-wavelength anomalous dispersion (MAD) technique, and the crystals diffracted up to 1.9 Å resolution. The apparent midpoint reduction potential value of CbcA was determined by potentiometric redox titrations and is the most negative ever reported for *G. sulfurreducens*' inner-membrane oxidoreductases. Finally, Nuclear Magnetic Resonance was used to monitor electron transfer reactions and probe biomolecular interactions with putative redox partners in the periplasm, shedding light on the complex networks for EET in *G. sulfurreducens*.

[1] Kumar R et al. (2015) Int J Energy Res 39, 1048–1067

[2] Joshi K et al.(2021) Mol Microbiol 116, 1124–1139

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