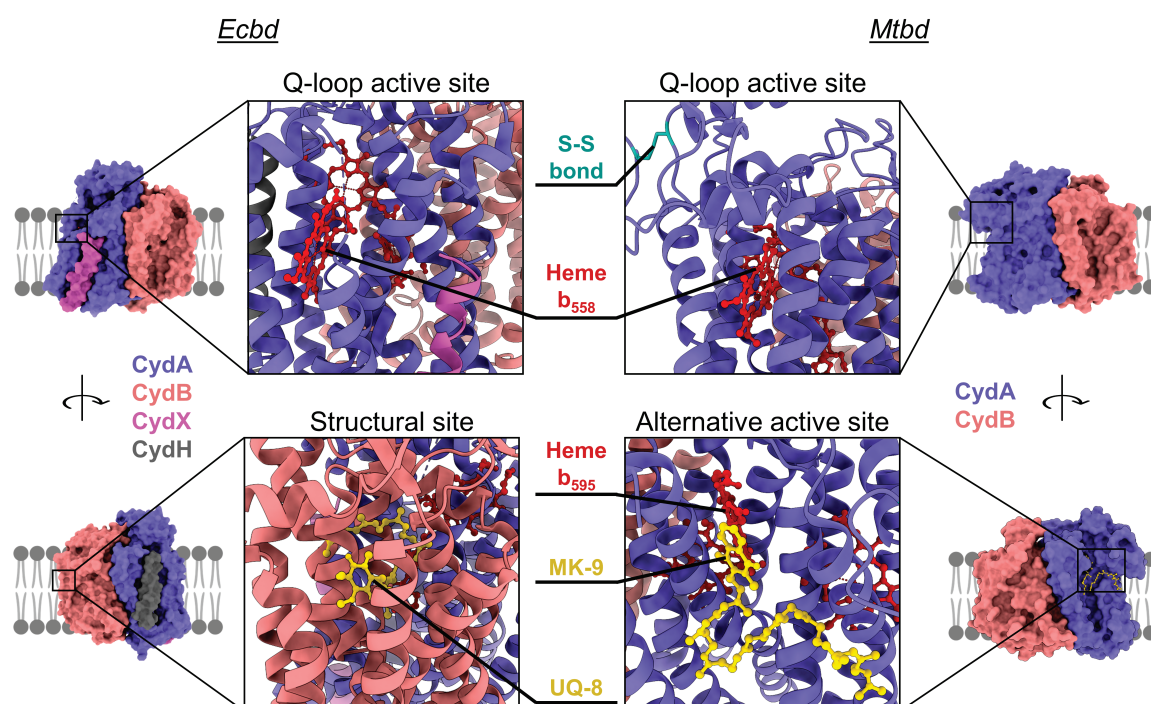


Supplementary information

Menaquinone-specific oxidation by *M. tuberculosis* cytochrome *bd* is redox regulated by the Q-loop disulfide bond

Tijn T. van der Velden¹, Caspar Y. J. Waterham¹, Lars J. C. Jeuken¹

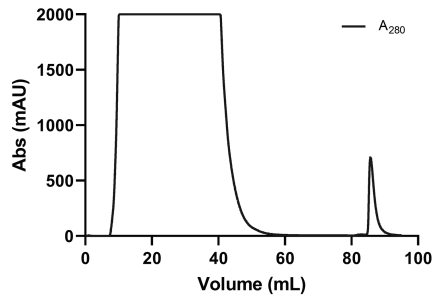
¹ Leiden Institute of Chemistry, Leiden University, PO Box 9502, 2300 RA, Leiden, The Netherlands



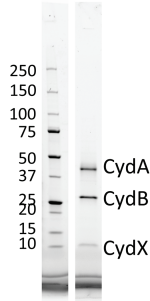
SI figure 1: Cryo EM structures of *Ecbd* (PDB: 6RKO) and *Mtbdb* (PDB: 7NKZ) with active and structural sites indicated.

Ecbd

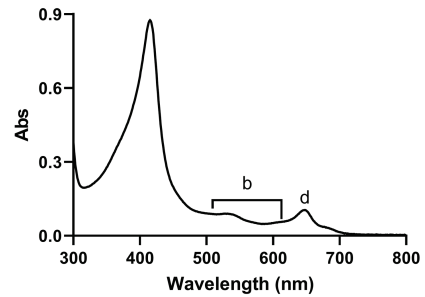
Affinity purification



SDS-page

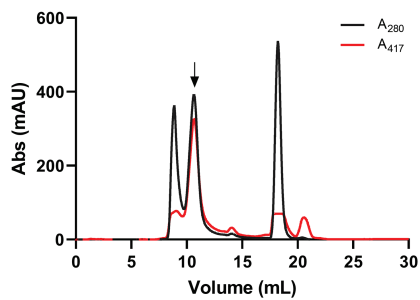


UV-Vis

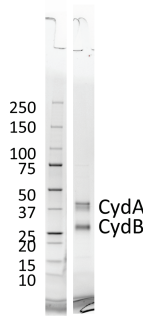


Mtbd

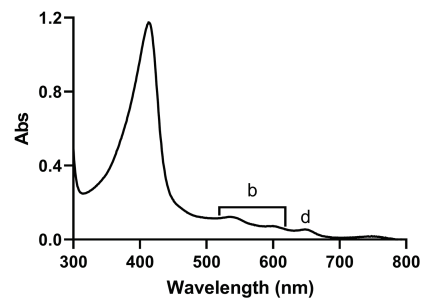
Size exclusion chromatography



SDS-page

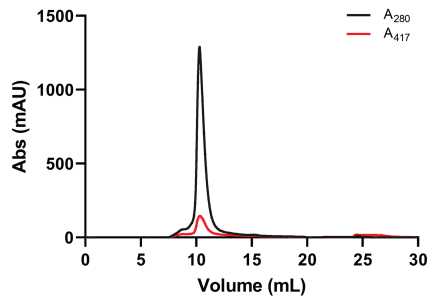


UV-Vis

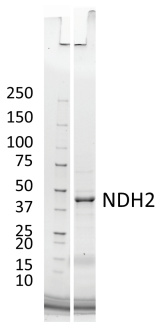


C. thermarum NDH2

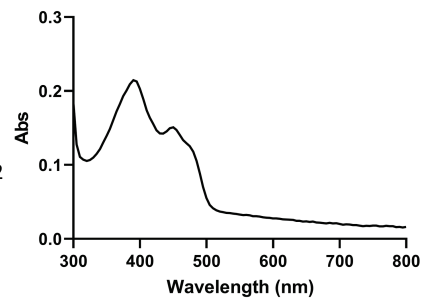
Size exclusion chromatography



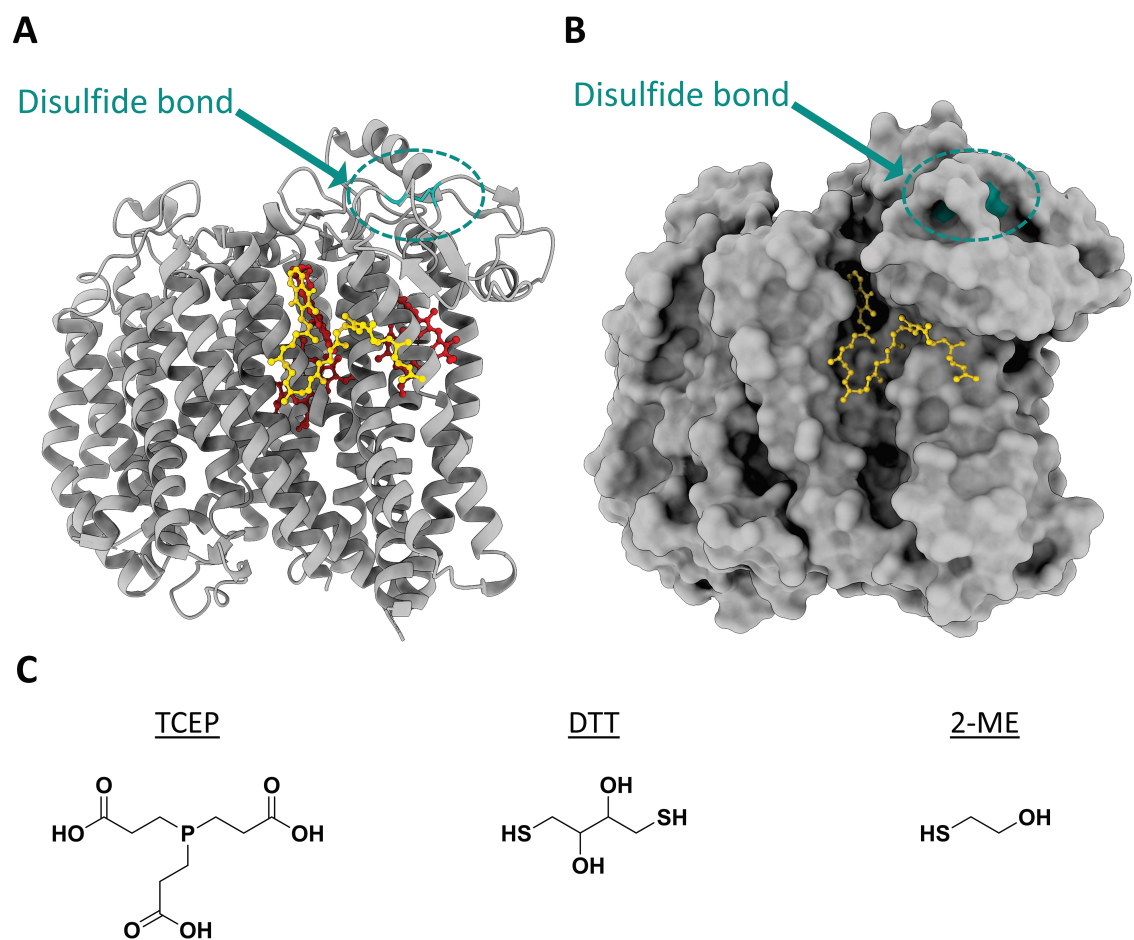
SDS-page



UV-Vis



SI figure 2. Purification, SDS-Page, and UV-Vis spectra of the isolated *Ecbd*, *Mtbd* and *C. thermarum* NDH2.



SI figure 3. Accessibility of the *Mtbd* disulfide bond. Cartoon (**A**) and surface (**B**) representation of *Mtbd* with hemes (Red), MK-9 (Blue) and disulfide bond (Purple). (**C**) chemical structure of the reductants used in this study.

Calculation of *E. coli* membrane quinone content

Variables	Value	Ref
UQ content <i>E. coli</i>	1.5×10^3 nmol/g dry cell weight	1
	3.6×10^2 nmol/g dry cell weight	2
<i>E. coli</i> dry weight	3.0×10^{-13} gram per cell	3
Number of lipids per <i>E. coli</i> cell	2.2×10^7 lipids	3
Avogadro's number	6.022×10^{23}	

Calculation

Number of lipid per cell: $2.2 \times 10^7 / 6.02 \times 10^{23} = 3.6 \times 10^{-17}$ mol/cell

Number of lipids in 1 gram cells: 3.6×10^{-17} mol/cell $\times (1/3.0 \times 10^{-13}$ g/cell) = 1.2×10^5 nmol/g

% UQ in membrane¹: $= 1.5 \times 10^3$ nmol/g / 1.2×10^5 nmol/g $\times 100\%$ = 1.23%

% UQ in membrane²: $= 3.6 \times 10^2$ nmol/g / 1.2×10^5 nmol/g $\times 100\%$ = 0.30%

Assuming a surface area of POPC to be $65 \text{ \AA}^2$ (MW = 760 g/mol) and a lipid bilayer thickness of 4 nm, 1% (w/w) UQ-10 (MW = 865 g/mol) is equivalent to 11 mM

Volume of 1 mol POPC bilayer:

$(6.022 \times 10^{23} \times 0.65 \text{ nm}^2 \times 4 \text{ nm}) / 2 = 7.8 \times 10^{23} \text{ nm}^3 = 0.8 \text{ L}$

1% (w/w) UQ-10 of 1 mol POPC is $= 0.01 \times 760 \text{ g} = 7.6 \text{ g}$ or $(7.6 \text{ g} / 865 \text{ g/mol}) = 8.8 \text{ mmol}$

$8.8 \text{ mmol} / 0.8 \text{ L} = 11 \text{ mM}$

Supplementary references

- (1) Sharma, P.; Teixeira De Mattos, M. J.; Hellingwerf, K. J.; Bekker, M. On the Function of the Various Quinone Species in Escherichia Coli. In *FEBS Journal*; 2012; Vol. 279, pp 3364–3373. <https://doi.org/10.1111/j.1742-4658.2012.08608.x>.
- (2) Uden, G.; Bongaerts, J. Alternative Respiratory Pathways of Escherichia Coli: Energetics and Transcriptional Regulation in Response to Electron Acceptors. *Biochim Biophys Acta* **1997**, *1320*, 217–234. [https://doi.org/10.1016/s0005-2728\(97\)00034-0](https://doi.org/10.1016/s0005-2728(97)00034-0).
- (3) Sajed, T.; Marcu, A.; Ramirez, M.; Pon, A.; Guo, A. C.; Knox, C.; Wilson, M.; Grant, J. R.; Djoumbou, Y.; Wishart, D. S. ECMDDB 2.0: A Richer Resource for Understanding the Biochemistry of E. Coli. *Nucleic Acids Res* **2016**, *44* (D1), D495–D501. <https://doi.org/10.1093/nar/gkv1060>.