Quinone-transporting filaments extend the respiratory chain of Gram-positive bacteria

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Respiratory chains are essential for energy generation in all life, coupling redox chemistry to proton translocation and ATP synthesis. Central to this process are hydrophobic quinones, which shuttle electrons within the membrane. However, the mechanisms that enable soluble enzymes to interact with these membrane-bound carriers remain poorly understood. We recently uncovered a novel mechanism of **long-range quinone transport**, where specialised protein complexes extract quinones from the membrane and shuttle them to soluble redox enzymes[1,2]. Here, we present the structural and functional characterisation of the Ndh-Ncp complex from *Bacillus subtilis*, composed of the NADH dehydrogenase Ndh and a membrane coupling partner from the DUF1641 family, which we term the NDH-2 Coupling Protein (Ncp). Using negative stain and high-resolution cryo-electron microscopy, we show that Ncp forms a tetrameric scaffold for four Ndh subunits, structurally reminiscent of the hydrogenase Huc complex[2]. These complexes assemble into extended filaments containing a central, lipid-lined hydrophobic chamber that sequesters respiratory quinones. Biochemical assays, lipidomics, electrochemistry, and molecular dynamics simulations demonstrate that this internal chamber supports quinone reduction outside the membrane. Electrons are transferred from NADH to FAD in Ndh, then passed to quinone within the chamber, with reduced quinone returning to the membrane to fuel the respiratory chain. This architecture enables NADH oxidation without the need for a membrane-integrated electron relay or permanent membrane association. Our findings establish the Ncp/DUF1641 family as a widespread solution for respiratory chain coupling in *Bacillota* and reveal a structurally elegant strategy for bridging the membrane–soluble phase divide via protein-mediated quinone transport.

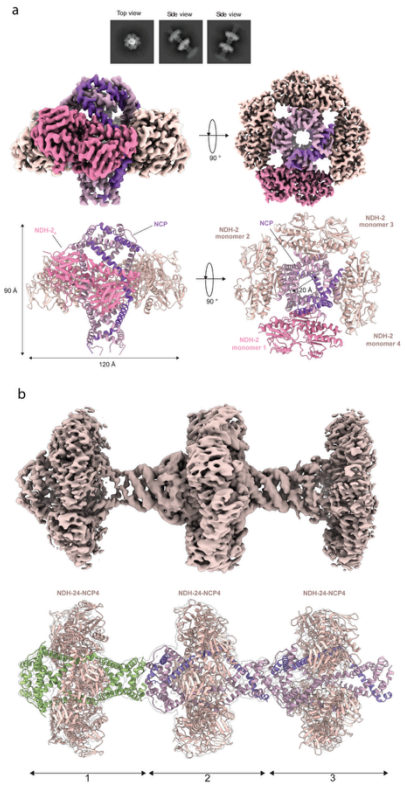

Figure 1. The Cryo-EM structure of the NDH2-NCP complex from *B. subtilis*. (a) The cryoEM structure of NDH-2-NCP complex shows that it has a 4:4 stoichiometry with the NDH-2 subunits attaching to a central NCP scaffold. (b) Helical reconstruction of the NDH-2-NCP shows head-to-tail interactions drive filament formation.

1 Kropp, A. *et al.* Quinone extraction drives atmospheric carbon monoxide oxidation in bacteria. *Nature Chemical Biology* (2025).
2 Grinter, R. *et al.* Structural basis for bacterial energy extraction from atmospheric hydrogen. *Nature* **615**, 541-547 (2023).