and proposed the involvement of the SQ_{Nf} site in energy transduction (1). SQ_{Nf} is extremely sensitive to transmembrane proton electrochemical potential. SQ_{Ns} may function as a $1/2e^-$ transfer converter, and might be a binding site to pool UQ. We determined N2 ⟺ SQ_{Nf} and N2 ⟺ Q_{Ns} distances as 12 Å and 30 Å, respectively, based on analysis of their spin coupling. The current consensus total H^+-pumping stoichiometry for complex I is ($4H^+/2e^-$). In our proposal (1), ($2H^+/2e^-$) stoichiometry is directly coupled with redox reaction and ($2H^+/2e^-$) stoichiometry is simultaneously via indirect proton pump, adopting suggestions by several groups (2,3).Sazanov and colleagues published a long-awaited structure of nearly complete complex I in 2010, missing only the NuoH subunit at the junction of the membrane and hydrophilic arm. In 2013, the same group published the structure of the entire complex I, including NuoH, which defined a "unique quinone reaction chamber" (3). Good evidence was obtained for a Q-binding site located at the top of the chamber, exactly the same distance from N2 to Q_{Nf}. The position for Q entry is consistent with the distance we measured between Q_{Ns} and N2. They proposed a 'piston-like' structure driving proton pumping via a long-range conformational change. In Sazanov's model, ($3H^+/2e^-$) stoichiometry is supplied by antiporter homologs (2), leaving ($1H^+/2e^-$) for quinone-linked translocation. We present an alternative proton pumping model consistent with homology, diffraction, and spectroscopic information.

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S4.P18

Membrane supported electrochemical characterization of respiratory complex I from *Rhodothermus marinus*

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Respiratory complex I (E.C.1.6.5.3) is a membrane-bound enzyme that has an essential function in cellular energy production. It couples NADH: quinone oxidoreduction to translocation of ions across the cellular (in prokaryotes) or mitochondrial membranes. Therefore, complex I contributes to the establishment and maintenance of the transmembrane difference of electrochemical potential required for ATP synthesis, transport and motility.

We have developed a strategy for reconstituting the bacterial complex I from *Rhodothermus marinus*, keeping its structural and functional properties, onto a biomimetic membrane supported on gold electrodes modified with a thiol self-assembled monolayer (SAM). Atomic force microscopy and faradaic impedance measurements give evidence of the biomimetic construction, whereas electrochemical measurements show its functionality. Both electron transfer and proton translocation by respiratory complex I were monitored, simulating in vivo conditions. Reconstitution of the respiratory complex I, in its native form on supported biomimetic membranes allows performing many fundamental studies about its function in cellular energy production.

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S4.P19

Characterization of the piericidin binding site of *Escherichia coli* NADH:ubiquinone oxidoreductase

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The NADH:ubiquinone oxidoreductase, complex I, is the first and largest enzyme of the respiratory chains. It couples the electron transfer from NADH to ubiquinone with a proton translocation across the membrane. The mechanism of ubiquinone (Q) binding and reduction is still unknown. Recently, the structure of the *Thermus thermophilus* complex I with co-crystallized/soaked decyl-ubiquinone and piericidin, a specific Q-site inhibitor, gave a first impression on the binding of ubiquinone. Tyr273 and His224 on subunit NuoCD of the *Escherichia coli* complex I are supposed to interact with the carbonyl groups of Q, while piericidin only interacts with Y273. It was shown by site-directed mutagenesis that Tyr273 is essential for the activity in mitochondrial complex I from *Yarrowia lipolytica*. We exchanged the tyrosine with several amino acids and the histidine to methionine, isolated the variants and determined their activity and the inhibition by piericidin. The mutation $Y273F^{CD}$ resulted in a more than two-fold higher IC_{50} to piericidin, while that of $Y273H^{CD}$ variant did not change. Thus, the hydroxyl group of $Y273^{CD}$ participates in piericidin binding in *E. coli* complex I. The IC_{50} of the $H224M^{CD}$ variant was significantly reduced, implying that $H224^{CD}$ involved in piericidin binding via a hydrogen network within the active site.

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S4.P20

Insights into the antiporter-like subunits of respiratory complex I

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Complex I (NADH:ubiquinone oxidoreductase) is the first and largest enzyme in the respiratory chain. This is the major contributor to the establishment and maintenance of the electrochemical potential required for ATP synthesis. Although the high resolution structure of complex I has been determined, the mechanism behind its catalytic activity is still not completely understood.