Complex I or NADH:ubiquinone oxidoreductase functions as a redox-driven proton pump in aerobic respiratory chains. It catalyzes the electron transfer (eT) from NADH to quinone (Q) through a series of iron-sulfur (FeS) centers, and employs the free energy released to pump four protons across the *ca.* 200 Å long membrane domain, generating a proton-motive force (*pmf*) [1]. The catalytic process is initiated by a hydride transfer reaction between NADH and flavin mononucleotide (FMN) cofactors of the enzyme. The two electrons are subsequently transferred to the FeS centers chain, which are one-electron acceptors. Two of the FeS clusters, N1a and N3, are located in the proximity of the NADH/FMN binding site. However, only N3 is proposed to participate in the eT to quinone, whereas the actual role of N1a remains controversial. To understand the molecular mechanism, energetics, and dynamics of the proton-coupled electron transfer (PCET) reactions in the NADH/FMN binding site of complex I, we employ here multi-scale quantum and classical molecular simulations [2]. We present a mechanistic model for the NADH/FMN hydride transfer reaction and the subsequent eT to N1a and N3, and discuss their implications for the function of complex I.

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P1 a/17
Two-electron reduction of ubiquinone in respiratory complex I

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Respiratory Complex I performs two-electrons/two-protons reduction of a ubiquinone (Q) ligand bound at its Q-binding pocket that ultimately generates a ubiquinole (QH_2) molecule. We propose a mechanism in which two electrons are transferred together with two protons in a concerted fashion. A method to evaluate the coupling matrix element that corresponds to a concerted tunneling of two electrons was developed. On one side a coupled electron/proton transfer occurs from reduced N2 cluster and protonated His38 residue respectively, while on the other side a hydrogen radical transfer occurs from neutral Tyr87 residue. Study of N2 Fe4S4 cluster spin states shows energetically favorable electron localization at the lower two iron centers which is mainly due to the extra delocalization contributed by the tandem Cys45 and Cys46 residues. In addition, His38 residue is presumed to act as a rotating proton shuttle; this is supported by our molecular dynamics simulation which shows that His38 can assume hydrogen-bonded or edged-T conformations as related to Q-benzoquinone group. Overall, our calculations indicate that the concerted reaction is feasible, in which case a transient tyrosyl radical is formed during the catalytic cycle of the enzyme.

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P1 a/18
NAD^+ Binding Site-Independent Energy-Linked Reverse Electron Transfer in Respiratory Complex I

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The substrate (NAD^+/NADH) binding site of the respiratory complex I is specifically blocked by NADH-OH, a derivative of NADH [1,2]. This tightly binding inhibitor prevents the proton motive force (**pmf**)-generating NADH:quinone reductase and/or reverse **pmf**-utilizing quinol:NAD^+ (or :ferricyanide (**Ferri**)) reductase activities as well as non coupled FMN-mediated NADH:**Ferri** or :hexaammineruthenium III (**HAR**) reductase activities [3,4]. A simple procedure for measuring ATP-dependent reverse electron transfer (**RET**) from ubiquinol to **HAR** as catalyzed by coupled bovine heart submitochondrial particles or coupled *Paracoccus denitrificans* plasma membrane vesicles is introduced. ATP induces **HAR**-mediated oxygen consumption by the respiratory chain-inhibited particles supplemented with succinate. In contrast to **pmf**-dependent **RET** with NAD^+ or **Ferri**, the reaction with **HAR** is insensitive to NADH-OH. The results suggest that a site (or mechanism) of **HAR** reduction in **RET** catalyzed by complex I is different from that for NAD^+ or **Ferri**. Two possible explanations are discussed: (i) electron connection exists between reduced FMN and **HAR** different from that for NAD^+ and **Ferri**; (ii) during **RET HAR** accepts electrons from iron-sulfur N-2 cluster–quinone junction site.

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P1 a/19
Using molecular approaches to understand complex I deficiency in the Ndufs4 knockout mouse model

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Complex I (NADH:ubiquinone oxidoreductase) is the largest enzyme of the mitochondrial electron transport chain. Mammalian complex I contains 44 different subunits [1], encoded on both the nuclear and mitochondrial genomes, and genetic mutations in many of these proteins are associated with neuromuscular diseases [2]. The nuclear gene *Ndufs4* encodes an 18 kDa protein which is a supernumerary subunit of complex I. In humans, mutations of *Ndufs4* can cause complex I dysfunctions, and result in clinical presentations including Leigh syndrome [3]. Therefore, in this study, we used the *Ndufs4* knockout mouse model [4] to investigate the pathogenic mechanisms of