Coenzyme Q10 trapping in mitochondrial Complex I underlies Leber's Hereditary Optic Neuropathy

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Abstract

How does a single amino acid mutation occurring in the blinding disease, Leber's Hereditary Optic Neuropathy (LHON), impair electron shuttling in mitochondria? We investigated changes induced by the m.3460 G>A mutation in mitochondrial protein ND1 using the tools of Molecular Dynamics and Free Energy Perturbation simulations, with the goal of determining the mechanism by which this mutation affects mitochondrial function. Recent analysis suggested that the mutation's replacement of alanine A52 with a threonine perturbs the stability of a region where binding of the electron shuttling protein, Coenzyme Q10, occurs. We found two functionally opposing changes involving the role of Coenzyme Q10. The first showed that quantum electron transfer from the terminal Fe/S complex, N2, to the Coenzyme Q10 headgroup, docked in its binding pocket, is enhanced. However, this positive adjustment is overshadowed by our finding that that the mobility of Coenzyme Q10 in its oxidized and reduced states, entering and exiting its binding pocket, is disrupted by the mutation in a manner that leads to conditions promoting the generation of reactive oxygen species. An increase in reactive oxygen species caused by the LHON mutation has been proposed to be responsible for this optic neuropathy.

Significance Statement

In the genetic disease Leber's Hereditary Optic Neuropathy, inheritance of a specific, single mitochondrial DNA mutation can produce the sudden onset of permanent blindness in first one and then the other eye usually in young adulthood. The mutation affects proteins in

mitochondrial Complex I that produce energy for retinal neuron activity, but the mechanism by which the mutation causes mitochondrial dysfunction is unclear. Computational biochemistry tools were used to compare molecular interactions in the mutated protein and showed that electron transfer to Coenzyme Q10 is massively slowed, creating conditions favorable for the production of cell damaging reactive oxygen species, providing the first explanation of how the mutation disrupts mitochondrial function, initiating a cascade that may lead to blindness.

INTRODUCTION

Leber's hereditary optic neuropathy (LHON) is a maternally inherited genetic disorder that causes asynchronous, severe bilateral loss of vision especially in young adult males. Symptoms include rapid loss of visual acuity, dyschromatopsia, dense central scotoma, and optic atrophy (*1,2*). This is known to be caused by one of several different mutations in the Electron Transport Chain (ETC) of mitochondria (*3,4*). The mitochondrial DNA (mtDNA) mutation is necessary but not sufficient to cause vision loss through this optic neuropathy. Many carriers remain asymptomatic, with males more likely than females to lose sight, typically near the age of 20.

Aerobic ATP synthesis by mitochondria utilizes the energy from electron transfer including those from NADH to Coenzyme Q10 (CoQ10) to produce a proton gradient across the mitochondrial inner membrane. CoQ10 is extremely lipid soluble and relies on a binding channel within Complex 1 to enable its redox-active quinone headgroup to be stable at a point about 20 Å above the membrane surface, precisely positioned to receive electrons from the terminal Fe-S complex, N2, in Complex 1. CoQ10 reduction occurs where the electron and proton transfer assemblies of Complex 1 are most critical. This juxtaposition creates a crux for disease-causing mutations to interfere with electron transfer in a manner that promotes electron leakage, creating superoxide and other reactive oxygen species (*4*).

The LHON mutation known to produce the largest biochemical impairments evidenced in human patient cybrid cell models (*2,5*) is the one at mtDNA nucleotide position m.3460 of the ND1 protein of Complex I (6). The G>A mutation leads to the substitution of alanine with threonine at position 52. This results in a relatively modest loss of energy production (ATP), but a more severe increase in reactive oxygen species (ROS) production which appears to be the basis of injury to retinal ganglion cells (RGCs) that leads to blindness (*7*). ROS production occurring at Complex I is largely due to impaired transfer of electrons to CoQ10. A mouse

model of LHON caused by this mutation has demonstrated a decreased Complex I activity, increased ROS production, and little or no decrease of ATP production (*8*), very similar findings to those in patient-derived cybrid cells (*5*).

This tight-fitting binding channel in ND1 of Complex I accommodates most of the ~50 Å long CoQ10 molecular chain, supporting rapid ingress in its oxidized state (as a quinone) and its regress when reduced, bearing 1 or 2 electrons and protons (as a quinol) (*9*). The channel appears to optimize the binding affinity of oxidized CoQ10 while providing an environment in which reduced CoQ10 dissociation is not rate-determining. Reduction of bound CoQ10 is the rate limiting step for catalysis by Complex 1 (*9*), and in this report we show how the single-site, m.3460G>A mutation form of LHON naturally impairs egress, indicating that this aspect of Complex I efficiency is the cause of energetic dysfunction in mutation carriers.

In mitochondrial Complex I (NADH ubiquinone oxidoreductase), two electrons are transferred from NADH to a lipid-soluble carrier, CoQ10. After leaving its binding channel, the reduced product, $CoQ10H_2$, (ubiquinol), freely diffuses within the membrane, delivering the electrons to Complex III where further contributions to the proton gradient across the inner mitochondrial membrane are made. By what mechanism(s) does the mutation perturb the function of ND1? We show here that the m.3460G>A mutation, a known cause of LHON, produces, among other actions, a change in ND1 that projects the OH and CH_3 side groups of the new threonine directly into the channel, dramatically slowing the rate at which CoQ10 can pass through the channel, thereby impairing the mechanism mediating electron transfer from the terminal Fe/S cluster, N2.

RESULTS AND DISCUSSION

We performed molecular dynamics simulations of the CoQ10-ND1 complex for the native protein and the mutant at 310 K and 1.01325 bar using stochastic velocity rescaling (*10*) and Nosé-Hoover Langevin piston pressure control (*11, 12*) (see Methods for details). We used a truncated model of human Complex I taken from the cryo-EM structure 5XTD (*13*). We included only chain B residues 58–210 (NDUFS8), chain C residues 58–213 (NDUFS7), chain Q residues 80–463 (NDUFS2), chain S residues 1–30 (NDUFA1), chain j residues 1–50 (ND3), and chain s residues 1–318 (ND1). We also included the last three 4Fe-4S clusters (N6a, N6b,

and N2) (**Fig. 1a**). CoQ10 in its oxidized or reduced (quinone or quinol) forms was docked manually in the binding channel and subjected to equilibration. CHARMM-GUI (*14-17*) was used to build a membrane around the truncated model composed of 130 palmitoyl-oleyl-phosphatidylcholine molecules, 91 palmitoyl-oleyl-phosphatidylethanolamine molecules, and 39 tetra-hexadecenoyl cardiolipin molecules (50:35:15 ratio) following the protocol of Parey et. al. (*18*). The model was solvated with 27,911 water molecules, 131 Na cations, and 50 Cl anions (100 mM plus neutralizing cations) (**Fig. 1b**). All amino acids were modeled in their usual protonation states, with histidine protonation states determined by inspection. Cardiolipin was modeled in its –2 protonation state.
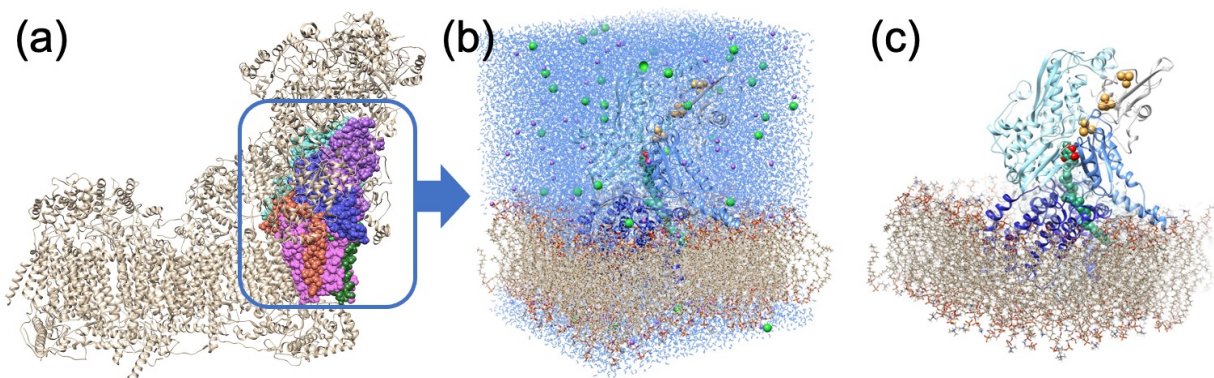

Fig. 1. (a) The full Complex I taken from the cryo-EM structure 5XTD, with the chains used in the simulations shown in color. (b) The periodic image of the fully solvated structure, including the colored part of the images in (a), the membrane, and the Fe/S clusters. (c) Image with the solvent and electrolyte ions removed for clarity.

These simulations show that the mutation neither distorts the protein nor significantly alters the relationship between CoQ10 and the binding channel (**Fig. 2a**). The series of Fe/S clusters remain aligned and equidistant at about 11 Å, to allow for the electron hopping toward CoQ10. However, the position of CoQ10 with respect to the nearest Fe/S cluster (N2) in the m.3460G>A mutant becomes slightly closer (**Fig. 2b**). Specifically, in the WT, the distance between the center of N2 and the center of the CoQ10 headgroup peaks at 14.1 Å, whereas in m.3460G>A, the distance distribution is bimodal with one peak still at 14.1 Å, and another, more prominent peak at 13.5 Å. The bimodal character describes the dynamic behavior in which the

CoQ10 substrate rattles in the binding pocket, and frequently visits conformations where it is more proximal to the N2 cluster than can be achieved in the WT. Since the tunneling rate is strongly affected by the tunneling barrier width, the tightened contact between N2 and CoQ10 is expected to enhance electron transfer by narrowing the tunneling barrier. Based on Marcus theory, the tunneling rate in the mutant is estimated to be ca. a factor of 3 greater than in WT (*SI Appendix*). The conformations placing the head group at 13.5 Å from N2 will dominate the electron transfer in the mutant. Electron transfer can go both ways: In its oxidized form, CoQ10 accepts electrons and protons upon reduction, constituting the normal function of the complex. In the reduced form, $CoQ10H_2$ may transfer electrons backwards up the series of Fe/S clusters, resulting in the production of ROS and failure to move the electrons to Complex III. We do not explicitly simulate the electron transfer in this work, as this effect appears secondary in the mechanism of mutation-induced change of the ND1 function (*vide infra*).

In order to estimate the relative binding free energies of CoQ10 to the WT and the mutant protein, we performed Free Energy Perturbation (FEP; *19*) simulations (*SI Appendix*), in which the mutation was alchemically introduced in the channel. The mutation is analyzed with the CoQ10 unbound and bound. By completing the thermodynamic cycle shown in **Fig. 2c**, the otherwise intractable difference in the binding free energies of the two protein variants can be calculated as follows:

$$\Delta\Delta G_{bind} = \Delta G_{bind}^A - \Delta G_{bind}^T = \Delta G_{mut}^{unbound} - \Delta G_{mut}^{bound}$$
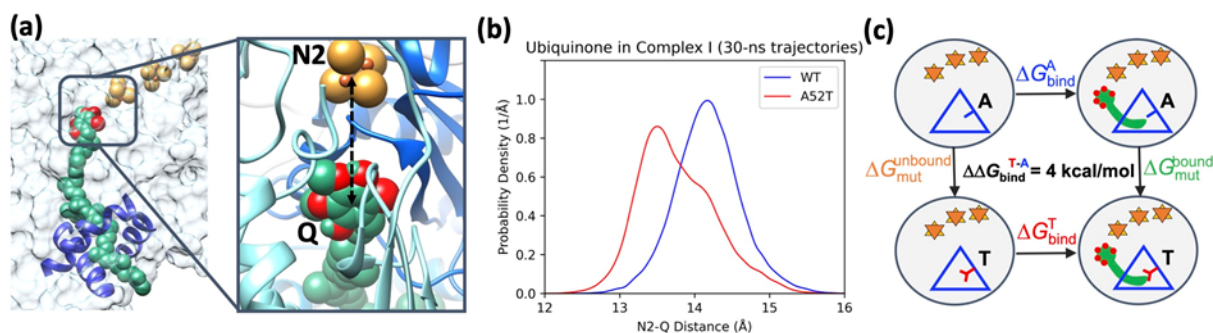

Fig. 2. Simulations show that the mutant binds CoQ10 less strongly by ~4 kcal/mol. (a) The distance between the center of the CoQ10 headgroup and the center of N2 (black dashed arrow) monitored throughout the molecular dynamics simulation showing **(b)** that shorter distances are accessible to CoQ10 in the mutated pocket (red curve), facilitating a higher rate of electron

transfer. (c) Thermodynamic cycle for the FEP simulations to compute the relative binding affinity of CoQ10 in WT and A52T.

This shows that the m.3460G>A mutant appears to bind CoQ10 ca. 4 kcal/mol less strongly than the native variant of ND1, suggesting that the binding of the oxidized CoQ10 to the mutant would be relatively disfavored. The difference in the binding affinity does not appear to result from any specific gained interaction, but from a collection of subtle structural changes. One likely explanation is the better solvation of threonine compared with alanine, when the pocket is void of CoQ10. The natural question is whether or not the mutant would bind CoQ10 at all. Likely it will, because the affinity of the WT is more than likely greater than 4 kcal/mol, and thus, the mutant also has some affinity. However, this result is overshadowed by the more important effect of the kinetics.

The mutation may alter the kinetics of binding of oxidized CoQ10, and unbinding of the reduced product, CoQ10H$_2$, (ubiquinol). This hypothesis is based on the fact that the mutated residue is larger in volume (**Fig. 3a**) and is located at the entry channel of the ND1 protein, where it can hinder the passing of the oxidized and/or reduced forms of the substrate. Starting from equilibrated, bound structures of CoQ10 and CoQ10H$_2$ with ND1 and its m.3460G>A mutant, we performed steered molecular dynamics simulations (*20-22*) to assess the difference in the kinetics (**Fig. 3b**). The distance between the α-carbon of residue 52 (52 CA) of ND1 and the final sp^2-hybridized carbon of the CoQ10 tail (CoQ C54) was the pulling coordinate (**Fig. 3a**). We equilibrated the systems with a harmonic restraint force constant of 2 kcal/mol/Å^2 centered at 10 Å (corresponding to the bound state with the tip of the CoQ10 tail extruding from the binding channel). We then tested the tugging force at the tail of CoQ10 required to pull it from the pocket. First, the simulations were performed at several different pulling velocities, and the results shown in **Fig. 3** correspond to the entire 48 Å pull over a 30 ns period, averaged over 10 trajectories for each system. It took much more work (approximately ~50 kT at room temperature) to pull CoQ10 out of the mutated channel compared with the wild type. This is a very large energy hurdle and unlikely to be exceeded by the thermal energy of biological systems (*SI Appendix*). The insets show the result of slower and therefore more accurate simulations, of the aliphatic group in the tail of CoQ10, and the larger head of CoQ10 passing the mutation site. These simulations cover 5 Å in 30 ns. We note that these simulations are very expensive and

ideally would need to be performed infinitely slowly and averaged over an infinite number of trajectories, which is clearly impossible. However, the trend persists as the pulling speed is gradually reduced by two orders of magnitude, providing confidence to our qualitative findings. In addition, the hindered kinetics makes chemical sense: The small aliphatic group passes with equal ease in WT and A52T, and the same is expected for all the repeating CH_2 units within CoQ10. However, the passing of the headgroup is significantly more hindered in the mutant, and we attribute the hindrance to the mechanical effect of the larger threonine residue and the less favorable electrostatic interactions with the threonine sidechain.
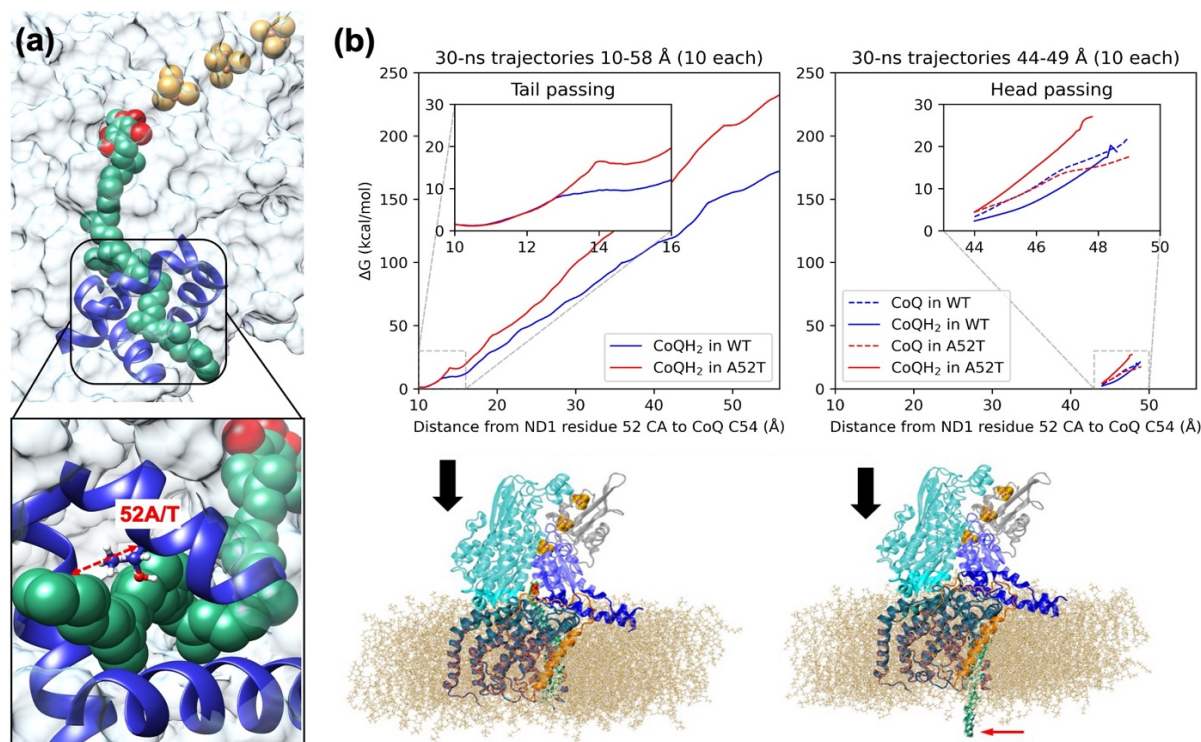

Fig. 3. **CoQ10 extraction from its ND1 binding pocket.** The ND1 protein forms part of the 38 Å long CoQ10 binding tunnel of respiratory complex I. (**a**) CoQ10 (shown in green) is fully inserted, with the electron receiving end (reducible oxygens shown in red) in close proximity to the terminal Fe-S complex in the Fe-S cluster chain (shown as yellow/orange group). The mutation site is labeled in the inset. The pulling reaction coordinate is shown as the red dashed arrow in the inset (52A/T). (**b**) The reduced CoQ10H$_2$ has been "pulled" out by the tail (red arrow at lower right)). Unbound CoQ10H$_2$ diffuses within the membrane bilayer to Complex 3 where it is oxidized. The full extraction simulation over 48 Å is done over the course of 30 ns (left panel). The inset corresponds to the tail of CoQ10 (15 Å) passing the mutation site. (Right panel) The head of CoQ10 (15 Å) passing the mutation site, performed over the course of 30 ns, showing a more pronounced hindrance caused by the mutation. The legends indicate whether the trajectory is WT or A52T mutant, whether CoQ10 is oxidized or reduced (*SI Appendix*).

Hence, the ubiquinol (reduced $CoQ10H_2$), in the setting of the mutation is essentially trapped as it would diffuse out of the mutated channel at a rate of about 2×10^{-9} slower than from the WT channel. This makes it nearly impossible for the $CoQ10H_2$ to leave the channel, and the channel remains effectively blocked. Thus, the electrons cannot be shuttled in the usual manner, and will only leave Complex I via the more narrowly placed CoQ10 head to the series of Fe/S clusters by moving backwards and spilling out, and at an increased rate increased due to the narrowing of the tunneling barrier, thus producing ROS.

Where would this matter most? Clinically, it is well known that patients with LHON (including the m.3460 mutation) may develop sudden and profound loss of vision in both eyes due to loss of axons in the optic nerve (1). Closer consideration shows that this begins with the smallest axon fibers that occupy the papillomacular bundle in the inferior temporal aspect of the optic nerve head. This makes sense insofar as the metabolic needs are very high in neurons, especially those with long axons (23). Each axon potential results in depolarization that requires the Na^+/K^+ ATPase pumps to restore their polarization at a high cost of ATP. Indeed, the brain, weighing about 2% of the total body in humans, consumes about 20% of the oxygen and calories of the body. But biology has provided a partial solution through myelinization of axons which limits the area of transmembrane current flux to the nodes of Ranvier, reducing the metabolic needs of axons by about 3 orders of magnitude. However, RGC axons in the eye, before becoming the optic nerve, traverse several millimeters across the surface of the retina, where they must remain transparent to allow light to penetrate to the underlying rods and cones. Myelin is opaque to light so myelination begins posterior to the lamina cribrosa of the optic nerve head. This unmyelinated segment makes the RGC axons particularly vulnerable to energy impairments. This problem is made worse in the smallest fibers that suffer from adverse surface area to volume ratios, the former reflecting the metabolic cost of repolarization and the latter the number of mitochondria available (23). So, with LHON, these small fibers are impaired and die first (24).

There is a growing consensus that that in LHON, the cause of RGC death comes from an excess of ROS production, not lower bioenergetics. ATP depletion would impair axon function long before leading to a wave of apoptosis as is seen clinically in LHON (4). Furthermore, ATP depletion is relatively minor compared with increased ROS production as noted in cybrid and animal models of LHON (5,7,8).

CONCLUSIONS

We used molecular dynamics and free energy perturbation simulations to elucidate the mechanistic impact of the m.3460G>A mutation on the function of the ND1 protein, leading to Leber's Hereditary Optic Neuropathy. These simulations show how, in the case of the m.3460G>A mutation, the alanine to threonine substitution at position 52 leads to the extended CH_3 and an additional OH in the path of the CoQ10. This creates a kinetic hindrance for CoQ10 to pass through the binding channel. The thermal energy required to move the CoQ10 out of the channel is estimated about 50 kT (*SI Appendix*), far too much to allow diffusion to occur at appreciable rates. Additionally, we find that the CoQ10 bound to the mutant ND1 has dynamical access to conformations where its head group is placed ~0.6 Å closer to the nearest FeS cluster, N2, and such conformations are furthermore predominant for the mutant while inaccessible for the WT. The shorter distance indicates a narrower tunneling barrier, which will exponentially increase the electron tunneling probability between N2 and CoQ10. Since CoQ10 is blocked in the channel, this will effectively prevent the reduced form of CoQ10 from leaving the binding channel, and thus bring up the probability of electron transfer in the reverse the direction: from the reduced CoQ10 back to N2.

A biochemical analysis of transmitochondrial cybrids and lymphocytes harboring the m.3460G>A mutation showed that while mitochondrial maximal respiration rate was reduced by ~25%, the underlying proportion initiated specifically in Complex I dysfunction was a ~79% reduction, with none in Complex 2 (3), consistent with much reduced electron transfer via CoQ10. A more recent analysis of cybrids carrying the m.3460G>A mutation, showed defects in the assembly and activity of complex I including respiratory deficiency, reduced ATP production and increased ROS and apoptosis (5). Much earlier biochemical studies of the m.3460G>A mutation, including kinetic analysis using ubiquinone analogs, indicated a potential inability of complex I to interact with CoQ10 (25, 26).

Studies of the structure and kinetics of the electron transfer chain performed with X-ray diffraction and cryo-EM have confirmed that the ND1 channel is already a very tight fit for CoQ10 (27), as well as indicating two stable binding positions for CoQ10 within the tunnel, with perhaps only one close enough to N2 for effective e-transfer (28). Further, refined investigations with cryo-EM and X-ray crystallography performed on the mutant complex with CoQ10 will

possibly provide supporting evidence of the distance of the CoQ10 head group to the N2 FeS cluster. Failed electron transfer will lead to electron spillage and ROS production at the proximal end of the FeS cluster series. ROS are a known cause of the blindness from Leber's Hereditary Optic Neuropathy, and this work thus links the molecular origin of ROS overproduction to the disease process.

METHODS

Simulations were performed in NAMD (*29*) with the CHARMM36 force field from July 2020 (*30–36*). Parameters for 4Fe-4S clusters were taken from (*37*). Parameters for CoQ10 were taken from (*38*). Water was modeled as TIP3P (*39*). All bonds to hydrogen were kept fixed to allow for a 2 fs timestep. The system with oxidized CoQ10 was minimized in four stages of 5,000 steps each. Membrane molecules were minimized first to correct pathological initial geometries. Water and ions were added for the second minimization, and all amino acids were added for the third. Finally, all restraints were removed, releasing 4Fe-4S and CoQ10. All further simulations were performed at 310 K and 1.01325 bar using stochastic velocity rescaling (*10*) and Nosé-Hoover Langevin piston pressure control (*11, 12*). The minimized system was equilibrated for 2 ns, after which it was used to create three additional systems: WT with reduced CoQ10 and A52T with both oxidized and reduced CoQ10. These four systems were equilibrated for an additional 10 ns. Two more systems without CoQ10, both WT and A52T versions, were constructed from these simulations and equilibrated for an additional 10 ns.

To analyze the effect of the A52T mutation, we used steered molecular dynamics, wherein a time-dependent external force is applied to investigate CoQ10 unbinding (20), and Jarzynski's identity (21, 22). We chose the distance between the alpha-carbon of residue 52 of ND1 and the final sp2-hybridized carbon of the CoQ10 tail as the pulling coordinate. We equilibrated the systems with a harmonic restraint force constant of 2 kcal/mol/Å2 centered at 10 Å (corresponding to the bound state with the tip of the CoQ10 tail extruding from the binding channel) for 10 ns each. We then began extracting structures, one every 100 ps, from which we started pulling trajectories. We ran trajectories at three different constant pulling velocities for each system. The fastest speed moved the harmonic restraint to 58 Å over 3 ns, the intermediate speed moved it to 58 Å over 30 ns, and the slowest speed moved it to 15 Å over 30 ns. We ran additional trajectories equilibrated at 44 Å and pulled to 49 Å over 30 ns. The values for the

harmonic restraint center, the measured coordinate, and the accumulated work were output every 500 fs. We used 0.1 Å bins in the analysis.

We also performed binding free energy calculations using free energy perturbation (FEP; 19), where the perturbation corresponded to the mutation, with the goal of assessing the free energy differences in the binding affinity of CoQ10 between the two variants. We constructed dual-topology structures from equilibrated A52T systems with reduced CoQ10, with oxidized CoQ10, and without CoQ10. Each topology included the sidechains of both alanine and threonine for residue 52 of ND1. We split each FEP simulation into 10 windows, using the values of perturbation parameter l of 0, 0.03125, 0.0625, 0.125, 0.25, 0.5, 0.75, 0.875, 0.9375, 0.96875 and 1. For each window, we performed simulations for both forward and reverse alchemical transformations, with 500 ps of equilibration and 2 ns of data acquisition every 500 fs. The free energy change for each window was calculated using Bennett's acceptance ratio (40). The transformed sidechains were frozen during the FEP calculations, so probability density corrections were calculated using the k-Nearest Neighbor approach (41). For each of the six endpoints, 2.5 ns of additional simulations were performed with the distance from the respective frozen structure measured every 500 fs. The fourth nearest neighbor was used for final corrections. Visualization was done using UCSF Chimera (42), and Visual Molecular Dynamics, VMD (43).

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Investigation: ANA, JTF, PA

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Supporting Information Appendix

Limitations of the computational methodology:

We calculated the equilibrium probabilities of observing various states of the CoQ10 system, where a "state" is defined by the value of a specific interatomic distance. In general, the least likely state along a series of states should dominantly affect the distribution of times (and therefore the average time) it takes for a system at equilibrium to move from the first state to the last state in the series. In our system, this corresponds to the rate at which CoQ10 exits the binding channel of the enzyme. Following conventions in the field of chemistry, we call the least likely state the transition state and quantify the probability difference as the free energy barrier.

The results of this paper are based on the Jarzynski Identity (*1*). This identity states that equilibrium (time-independent) properties can be calculated from non-equilibrium (finite time) trajectories. The practical difficulty in applying this identity is that it requires the population average of a specific quantity over the trajectories. The distribution of the population of that quantity is so skewed that, without extensive sampling to sufficiently observe rare events, the sample average is not quantitatively correct.

According to the Jarzynski Identity, the timescale of the simulations does not affect the final result or the calculated free energy barrier at the transition state. However, the Jarzynski Identity only applies to the population average. In practice, the timescale of the simulations affects the convergence of the sample average to the population average. Slower simulations require fewer trajectories for the quantity of interest to converge. The distribution resulting from faster trajectories is more skewed, with most trajectories requiring extra energy and a few outliers that require much less. With high probability, the sample average describes typical

trajectories at a given timescale. The extra energy required by typical trajectories can then be understood as timescale-dependent "friction".

This can be seen in our results, as values from our slowest trajectories show the least friction and presumably give the best approximations to the true energy barriers, although we doubt that they are sufficiently converged for the values to be absolutely precise. However, we do believe that all the results are qualitatively accurate when comparing wild-type and mutant enzymes. We could rephrase our method as averaging the work performed during a process in a way that converges to the free energy difference in the limit of infinite trajectories but drawing qualitative conclusions as approximations before we reach that limit.

In Figure 3b, the ΔG axis shows the amount of work needed to overcome friction for CoQ10H₂ egress within the specified time period and should not be confused as showing the relative free energy between the two configurations, the former being a quantity that is addressed in Figure 2. The difference in the energy required for egress between the WT and A52T-mutant proteins is extremely high (~50 kT), suggesting that at the speed of this simulation, the egress of CoQ10H₂ from the A52T mutant is far less likely to occur than its egress from the WT. However, within the modelled time scale, the barrier for retracting CoQ10H₂ in WT is insurmountable, meaning that egress over a period of 3 or 30 ns is non-physiological. Unfortunately, what happens at lower speeds of egress cannot be simulated computationally given the time required from available resources.

The biophysics of this process requires a sufficiently rapid turnover of CoQ10 so as to allow delivery of electrons to Complex III and to prevent ROS-producing spillage. The biological timescale of CoQ10 mobility in Complex I is not known but considering the energetics encountered in this simulation, it is much slower that the 30 ns timescales used in these computations. Thus, the data in Fig 2b are considered qualitative, not quantitative. We can confidently say that there is much more friction in the A52T channel than in the WT channel, even if we cannot precisely quantify that difference. But the qualitative observation is sufficient to show that egress of CoQ10H₂ from the mutant channel is a much slower process than egress from the WT channel, a situation that leads to more electron spillage and ROS production, thus increasing the risk of blindness.

Our results also include other approximations, including classical force-fields, a truncated enzyme model, and periodic boundary conditions. These approximations lead to inaccuracies but in ways identical for the two systems that we compared, and thus they do not override the qualitative conclusions.

Marcus Theory of Electron Tunneling Kinetics:

Since the edge-to-edge distance between the donor (CoQ10) and acceptor (N2) is greater than 10 Å, we can consider the electronic states of each to be weakly coupled. Further, electron transfer timescales relative to nuclear motion follow the Frank-Condon principle, we can use the following Marcus Theory expression for the rate constant of electron tunneling (*2,3*):

$$k_{et} = \frac{1}{h}\left(\frac{\pi^3}{RT\Delta E_R}\right)^{\frac{1}{2}} (H_{et}^0)^2 e^{-\beta L} e^{-\Delta^{\ddagger}G/(RT)}$$

Here, H_{et}^0 is the electronic coupling matrix element at 0 distance between donor and acceptor, ΔE_R is the energy required to arrange the donor and acceptor into a conformation

favorable for charge transfer, and $\Delta^{\ddagger}G$ is the free energy required to activate charge transfer, and is dependent on the reorganization energy ΔE_R. The parameter β depends on the environment of the electron transfer, and for electron transfer in vacuum, takes on a value of ca. 30 nm^{-1}. Since the electron is not tunneling through a molecule from CoQ10 to N2, we can use this value for β in vacuum. Lastly, the parameter L is the edge-to-edge donor-acceptor distance, which can be determined by the peaks in Figure 2b.

Due to the wild-type and mutant structures of ND1 not affecting the donor and acceptor molecular structure, we can treat the electronic coupling H_{et}^0 to be the same between both wild-type and mutant ND1. Assuming that there is no further rearrangement cost of CoQ10 for ND1 wild type and mutant, we can estimate that the ratio of k_{et} for wild-type (WT) and mutant (MUT) using the dominant peak only is:

$$\frac{k_{et}(WT)}{k_{et}(MUT)} \approx 0.16$$

More accurately, if we consider a mean donor-acceptor distance for the mutant that is a weighted sum of both 13.5 Å and 14 Å peaks in Figure 2b, the ratio is:

$$\frac{k_{et}(WT)}{k_{et}(MUT)} \approx 0.32$$

This provides a strong implication that the reaction rate of electron tunneling, with simple assumptions, is higher for the mutant ND1, and given that the mutant ND1 causes CoQ10 to get kinetically trapped in the binding pocket, the probability of back-tunneling of the electron through the Fe-S cluster increases markedly.

FEP data

ubiquinol	ΔG_0 (kcal/mol)	ΔG_1 (kcal/mol)
mutant	17.87	-17.87
window1	1.79204	-1.72406
window2	1.83894	-1.80761
window3	4.07474	-3.97149
window4	8.75676	-8.32546
window5	19.2667	-19.3373
window6	0.632057	-0.48262
window7	0.7987	-0.713948
window8	0.576878	-0.569321
window9	0.324491	-0.343547
window10	0.445338	-0.389718

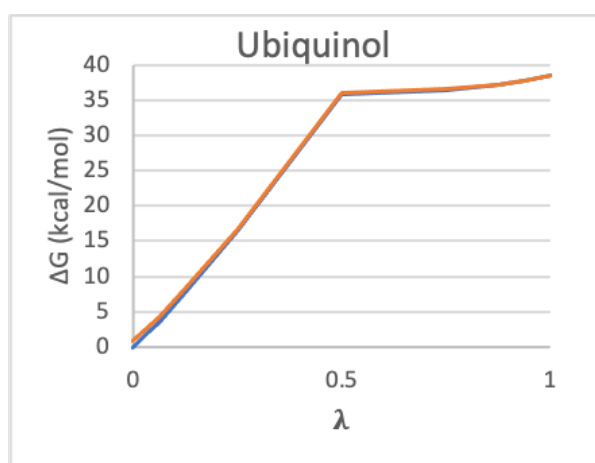|		
WT	-3.23	3.23
total	17.406644	-16.565074
lambda		
0	0	0.84157
0.03125	1.79204	2.56563
0.0625	3.63098	4.37324
0.125	7.70572	8.34473
0.25	16.46248	16.67019
0.5	35.72918	36.00749
0.75	36.361237	36.49011
0.875	37.159937	37.204058
0.9375	37.736815	37.773379
0.96875	38.061306	38.116926
1	38.506644	38.506644

Figure S1. FEP simulations results for the alchemical mutation on the protein in the presence of bound ubiquinol (forward and backward simulations are shown – the close agreement between the two indicates convergence of the result).

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ubiquinone	ΔG_0 (kcal/mol)	ΔG_1 (kcal/mol)
mutant	16.01	-16.01
window1	1.62679	-1.70242
window2	1.6834	-1.76937
window3	3.77133	-3.73895

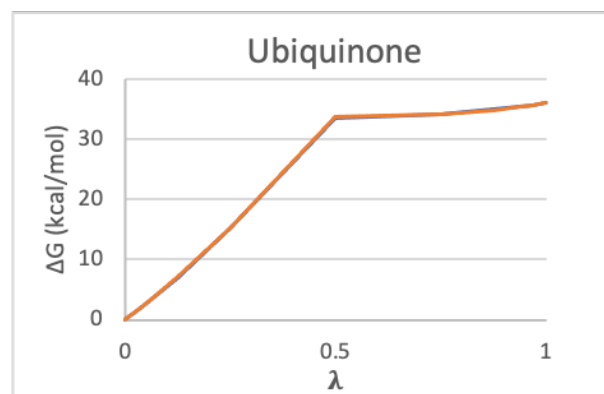|		
window4	8.30379	-8.05508
window5	18.12	-18.4254
window6	0.666215	-0.437323
window7	0.735912	-0.697356
window8	0.468739	-0.563217
window9	0.32482	-0.334724
window10	0.377544	-0.372715
WT	-3.19	3.19
total	16.87854	-16.896555
lambda		
0	0	-0.018015
0.03125	1.62679	1.684405
0.0625	3.31019	3.453775
0.125	7.08152	7.192725
0.25	15.38531	15.247805
0.5	33.50531	33.673205
0.75	34.171525	34.110528
0.875	34.907437	34.807884
0.9375	35.376176	35.371101
0.96875	35.700996	35.705825
1	36.07854	36.07854

Figure S2. FEP simulations results for the alchemical mutation on the protein in the presence of bound ubiquinone (forward and backward simulations are shown – the close agreement between the two indicates convergence of the result).

5

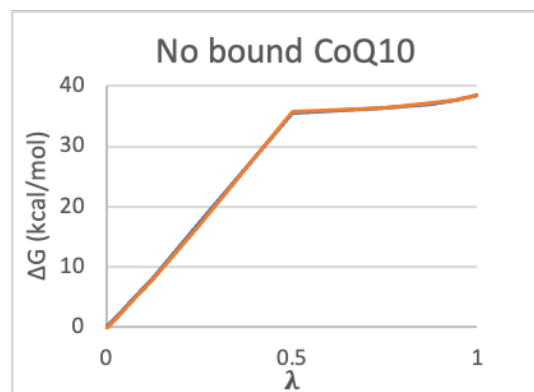| no bound CoQ10	ΔG_0 (kcal/mol)	ΔG_1 (kcal/mol)
mutant	13.93	-13.93
window1	1.88399	-1.92151
window2	1.99829	-2.07025
window3	4.30317	-4.1739
window4	9.04364	-9.01448
window5	18.3254	-18.7763
window6	0.769292	-0.571749
window7	0.701562	-0.881355
window8	0.635573	-0.565265
window9	0.386933	-0.310988
window10	0.376999	-0.379543
WT	-3.21	3.21
total	21.284849	-21.52534
lambda		
0	0	-0.240491
0.03125	1.88399	1.681019
0.0625	3.88228	3.751269
0.125	8.18545	7.925169
0.25	17.22909	16.939649
0.5	35.55449	35.715949
0.75	36.323782	36.287698
0.875	37.025344	37.169053
0.9375	37.660917	37.734318
0.96875	38.04785	38.045306
1	38.424849	38.424849

No bound CoQ10

Figure S3. FEP simulations results for the alchemical mutation on the protein in the absence of the bound CoQ10 (forward and backward simulations are shown – the close agreement between the two indicates convergence of the result).

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