We have been using a new approach to understanding the molecules involved in the pathology of LHON. In particular, we have looked at the effects on structure and chemistry and even physics that occur as a consequence of the LHON mtDNA mutation of 3460.

The LHON mutation known to produce the largest biochemical impairments evidenced in both cybrids and humans is at nucleotide position 3460. The G > A mutation leads to a substitution of threonine for alanine at position 52 of the ND1 protein of Complex I (Howell). The function of ND1 is to transfer the electrons from the terminal iron/sulfate cluster to ubiquinone resulting in the reduced form, ubiquinol (collectively referred to as CoQ10). Remarkably, as we've seen from several experiments, the LHON mutation results only in a relatively mild loss of energy production (ATP), less than 20%. Much of the problem is from ROS production that occurs at Complex I, probably due to the incomplete transfer of electrons to ubiquinone which is made worse by the ND1 mutation. This excessive reactive oxygen species (ROS) over production is likely to be the basis for injury to retinal ganglion cells (RGCs) and subsequent blindness. Our mouse model of LHON done in conjunction with Doug Wallace at CHOP confirms that there is a reduction of Complex I activity and increased levels of ROS in the face of no reduction of ATP production in the brain (Lin, Sharpley, Fan et al. and Wallace).

Our present experiments ask how this ND1 mutation affects the transfer of electrons to ubiquinone. Specifically, we used computational chemistry to obtain the structure of the normal and mutated ND1 subunit, and establish its delta G for the association with ubiquinone and its subsequent dissociation with the reduced form, ubiquinol. We find that the effect of the 3460 G>A mutation is primarily to prevent the unbinding of ubiquinol.

The mitochondrial DNA G > A mutation at nucleotide 3460, known to cause LHON, produces a change from the amino acid alanine to threonine at position 52. This change projects an OH group from the threonine directly and centrally into the channel through which ubiquinone must slide in to procure a pair of electrons from the terminal iron/sulfate Remarkably, this ubiquinone head and the last three iron/sulfate molecules line up at distances at about 11-12 A. The channel also must permit the now reduced ubiquinol, to slide out.

When we imposed a tugging force at the tail of ubiquinol to pull it from the channel, it took much more energy in the mutated ND1 as compared to the wild type channel. All the newly formed serrations of the rachet impose a new energy requirement of 5-6kT. This is very large and unlikely to be exceeded by the thermal energy of biological systems.

Hence, the ubiquinol (reduced CQ10), in the setting of the mutation is trapped or at least escapes much slower than from the wild type channel. This barrier makes it thermodynamically nearly impossible for the ubiquinol to move out of the channel. The channel remains blocked and electrons cannot be shuttled in the usual way. Electrons can only leave Complex I through the Fe/S clusters by moving backwards and spilling out from the first Fe/S cluster, thus producing the ROS that kill the RGCs.

Clinically, it's well known that patients with LHON (including the 3460 mutation) may develop sudden and profound loss of vision in each eye due to changes in the optic nerve (ref). Closer consideration shows that axonal damage begins with the smallest fibers that occupy the papillomacular bundle in the inferior temporal aspect of the optic nerve head. It makes sense that the retinal ganglion cell nerve fibers are the most vulnerable, because they do not enjoy the efficiency conferred by myelination until they leave the eye via the optic nerve head. In addition, the smallest fibers have the

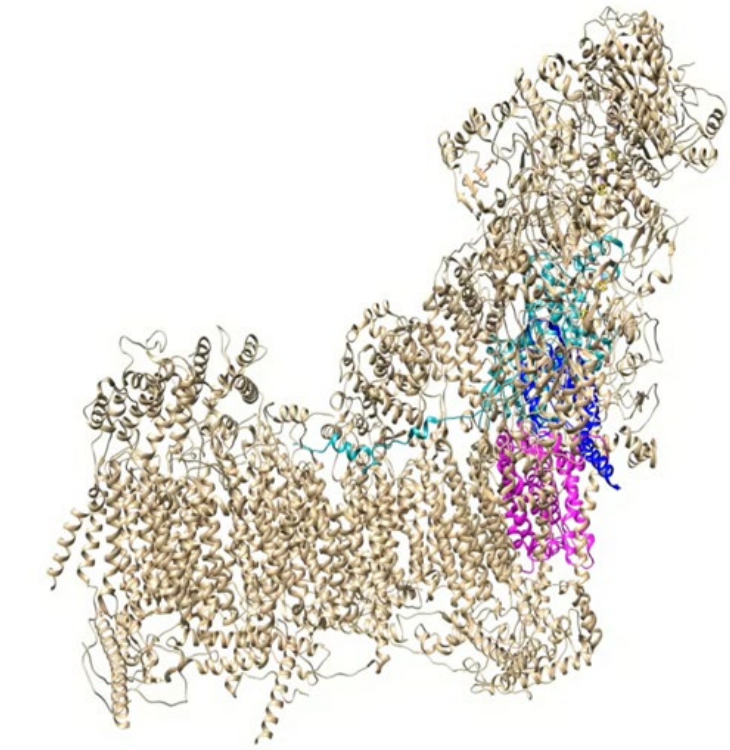
larges surface to volume ratio and are thus metabolically most stressed. In general, neurons have very high metabolic needs. Each axon potential results in depolarization that requires the Na+/K+ pumps to restore their polarization at a high cost of ATP. Indeed, the brain, about 2% of body weight in 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 depolarization to the Nodes of Ranvier reducing the metabolic needs of axons by 2-3 orders of magnitude. However, the axons of the optic nerve leave their somas and traverse several millimeters across the anterior 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 can only begin posterior to the lamina cribrosa of the optic nerve head. This unmyelinated area makes the RGC axons that form the optic nerve particularly vulnerable to metabolic 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. So, with LHON, these fibers are impaired and die first.

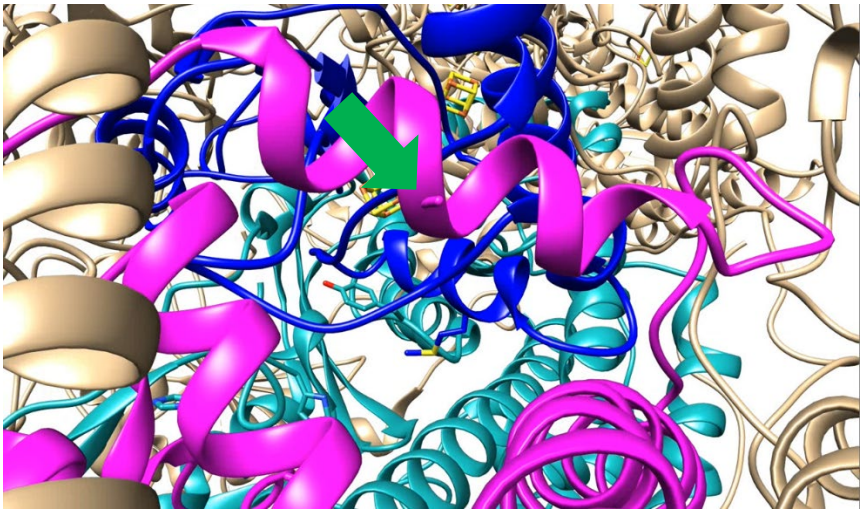
The current work demonstrates why, in the case of the 3460 G>A mutation, the alanine to threonine substitution at position 52 leads to an additional OH right in the path of the moving ubiquinol. Further, the thermal energy required to move the ubiquinol out of the channel is far too much to allow diffusion to occur at appreciable rates. As a consequence, electrons, blocked from exiting from N2 to ubiquinone reverse their direction leading to spillage and ROS production at the other end.

We are now further testing how the 3460 G>A mutation causes an OH from threonine to intrude into the ubiquinone/ubiquinol channel in ND1 at different temperatures. Quantum electron tunneling is not susceptible to temperature changes and we are measuring all of this in cybrids using an Oroborus as well as Seahorse system. These fundamental studies may eventually lead to new approaches in the treatment of the blindness that comes from LHON.

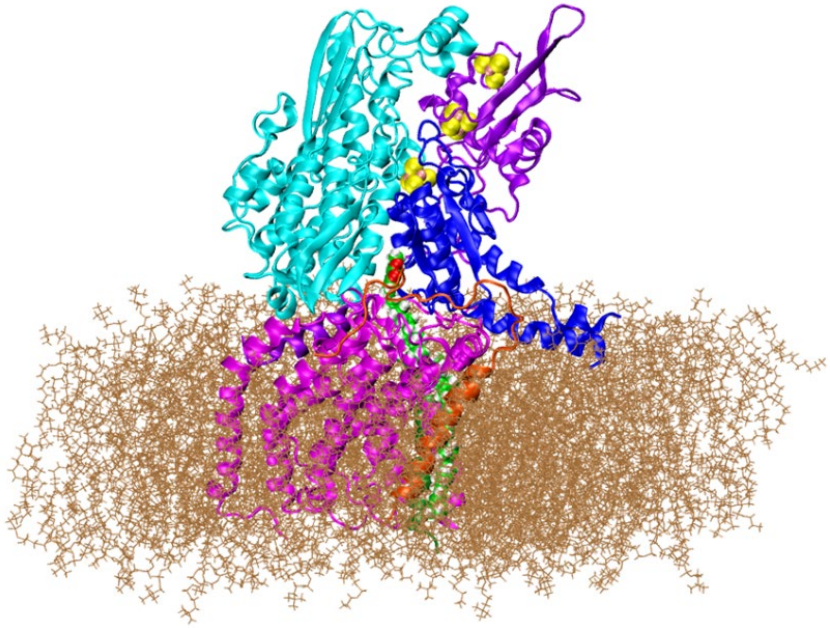
Figures:

1 A and B. Low and High magnification views of the computational chemistry figure of Complex I after the 3460 mutation. Magenta is ND1 channel for CoQ10 at the heel of the boot. Green arrow points to the OH protruding into the channel by dint of the threonine now at position 52. The green and blue are ND4 and ND6? Mention the closeness of all the LHON mutations to the channel in results, and maybe even in discussion.





Figures 2 A and B. Magenda is the ND1 forming a channel for CoQ10 in green. In A the CoQ10 is fully inserted. In B the CoQ10 has been "pulled" out by the tail (blue arrow).



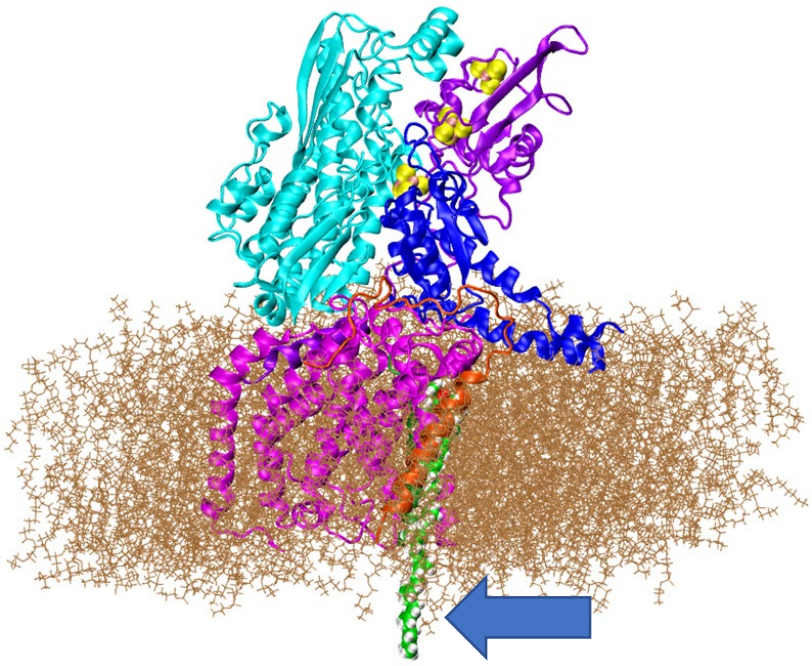


Figure 3. The thermal energy required to move CoQ10 changes after the 3460 mutation. More than 50kT more is required because of the electrostatic forces created by the newly position OH.

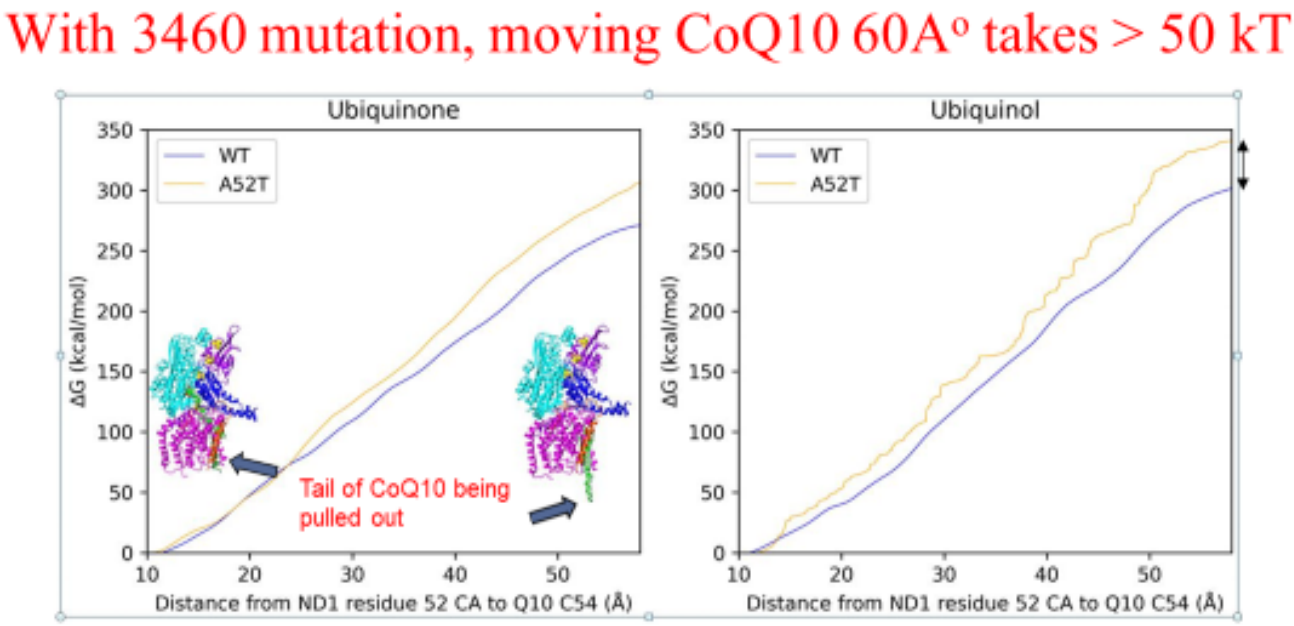
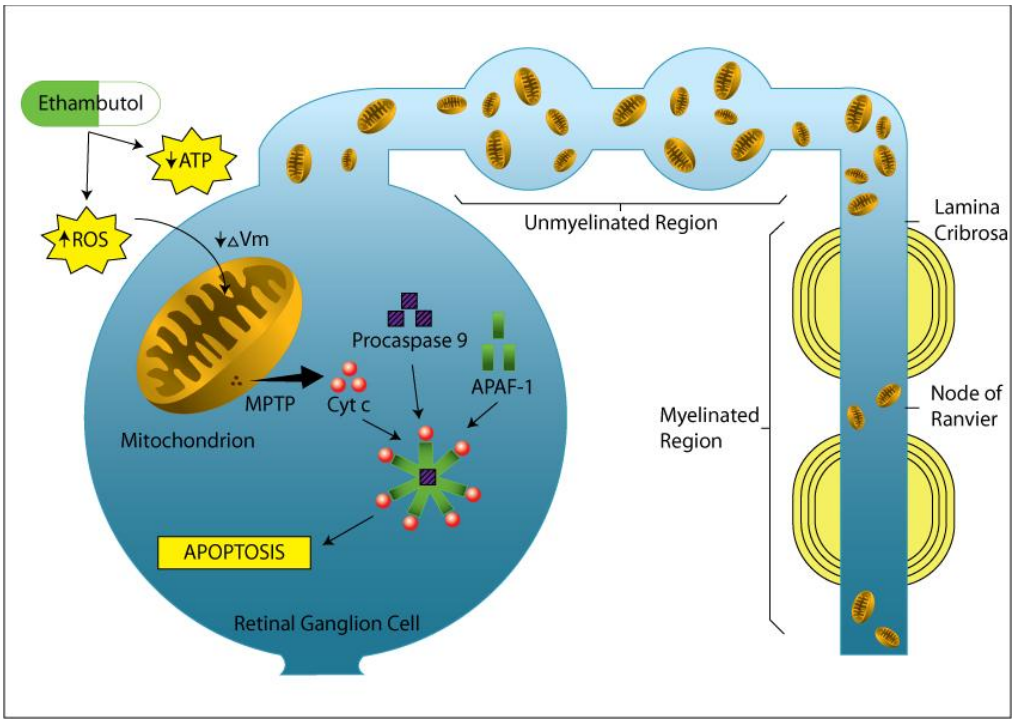


Figure 4



Modify this by adding in the top right hand corner a sketch of Complex I spilling ROS at the top, then a line to the mitochondrion shown.

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Some already pulled. I will gather the others.

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Leber hereditary optic neuropathy: identification of the same mitochondrial ND1 mutation in six pedigrees

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Bioenergetics of mitochondrial diseases associated with mtDNA mutations

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