electron transport chain, the second electron is stored in N1a to shorten the lifetime of the FMN semiquinone radical that might react with abundant dioxygen to generate the hazardous superoxide anion. To prove this hypothesis, a variant of the module missing cluster N1a was produced.

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1L.4 Progress towards the molecular mechanism of mitochondrial complex I
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Complex I (NADH:ubiquinone oxidoreductase) is crucial to respiration in many aerobic organisms. In mitochondria it oxidises NADH (regenerating NAD+ for the tricarboxylic acid cycle and fatty-acid oxidation), reduces ubiquinone (the electrons are then used to reduce oxygen to water), and transports protons across the mitochondrial inner membrane (contributing to the proton motive force that supports ATP synthesis and transport processes). Complex I is also a major contributor to cellular reactive oxygen species production. Our approach to determining the reaction mechanism of complex I is to consider it in several simpler parts that can be tackled and defined individually, before being recombined to produce the complete picture. Thus, the mechanism of complex I comprises four sequential steps. Two steps, NADH oxidation by the flavin mononucleotide, and intramolecular electron transfer from the flavin to bound quinone (along a chain of iron–sulphur clusters), are increasingly well understood. Conversely, the mechanisms of quinone reduction and proton translocation (including the possible involvement of semiquinone species in reactive oxygen species production) are very poorly understood. This talk will present and discuss recent data that address the mechanisms of quinone reduction and proton translocation by complex I.

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1L.5 Mitochondrial respiratory chain super-complex I–III in physiology and pathology
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Recent investigations by native gel electrophoresis showed the existence of supramolecular associations of the respiratory complexes, confirmed by electron microscopy analysis and single particle image processing. Flux control analysis in our laboratory demonstrated that Complex I and Complex III in mammalian mitochondria kinetically behave as a single unit with control coefficients approaching unity for each component, suggesting the existence of substrate channeling within the super-complex. On the other hand Complex II and Complex IV appear kinetically independent in mammalian mitochondria. Reconstitution studies demonstrate that the formation of the supramolecular unit comprising Complex I and Complex III (super-complex I–III) largely depends on the lipid content and composition of the inner mitochondrial membrane: at high lipid content or with peroxidized lipids the super-complex association is impaired, as demonstrated by electrophoretic and kinetic analysis. The function of the super-complexes appears not to be restricted to kinetic advantages in electron transfer: we discuss evidence on their role in the stability and assembly of the individual complexes, particularly Complex I, and in preventing excess oxygen radical formation or anyway in changing the sites of superoxide generation. There is increasing evidence that disruption of the super-complex organization leads to functional derangements responsible for pathological changes, as we have found in K-ras-transformed fibroblasts, where loss of the highest molecular weight super-complexes is associated with enhanced formation of reactive oxygen species and strongly diminished Complex I activity.

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