Structural characterization of E. coli complex I: an important mechanistic model

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Introduction

Electrons harvested mostly from the catabolic processes of tricarboxylic acid cycle (TCA), fatty acid oxidation and glycolysis enter the electron transport chain (ETC) on the inner mitochondrial membranes. ETC consists of four protein complexes: NADH:ubiquinone oxidoreductase (Complex I), succinate dehydrogenase (Complex II), cytochrome bc1 complex (Complex III) and cytochrome c oxidase (Complex IV). The electron transfer through the ETC is coupled to the proton translocation out of the mitochondrial matrix by the proton pumps: Complex I, III and IV. Complex II does not pump protons. The complexes create an electrochemical gradient across the membrane (proton motive force, pmf) which drives the ATP synthase (complex V) (**Fig. 1**) (Sazanov, 2015).

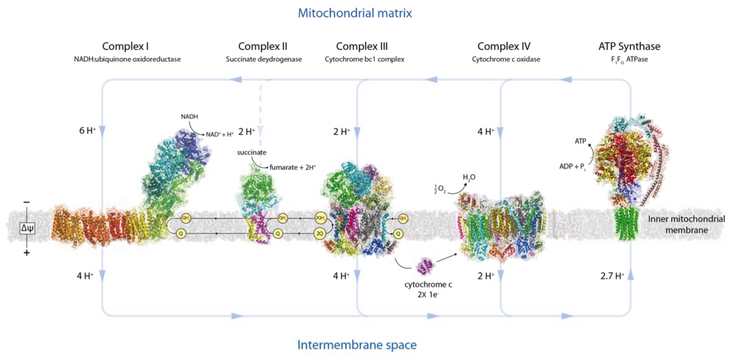

Figure 1. The mammalian mitochondrial electron transport chain (ETC) includes the proton-pumping enzymes complex I (NADH–ubiquinone oxidoreductase), complex III (cytochrome bc1) and complex IV (cytochrome c oxidase), which generate proton motive force that in turn drives F1 FO-ATP synthase. Figure adopted from (Sazanov, 2015).

The mitochondrial complex I is the first and the largest enzyme of the respiratory chain. In mammals it is composed of 45 subunits making its total molecular weight almost 1 MDa, while the bacterial form is about 550 kDa. Complex I (CxI) has a characteristic L-shape and contains 14 "core" subunits that are essential for its function and strictly conserved among species **(Fig. 2).** Hydrophilic (matrix) catalyzes electron transfer while the membrane arm of the complex pumps protons. Four protons are pumped through eight half-channels, which work together as four full channels and are connected via coupling elements. The overall coupling mechanism suggests a cascade of conformational changes but is still poorly understood (**Fig. 2, 3**).

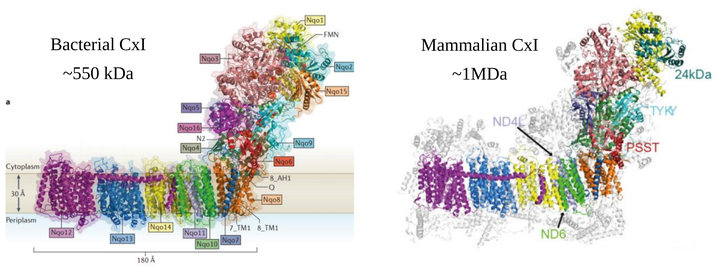

Figure 2. The characteristic CxI L-shape is conserved from bacteria (*Thermus thermophilus*, left) to mammals (*Ovis aries*, right). 14 core subunits are depicted in different colors, the same subunits have the same color. Additional subunits in the mammalian structure are in grey. Figures of bacterial and mammalian CxI are adopted from (Sazanov, 2015) and (Fiedorczuk et al., 2016), respectively.

Relevance and open questions

Complex I is an essential enzyme and its dysfunctions caused by genetic and environmental factors play significant roles in many diseases. Understanding the cause of the mutations is hindered by the lack of mechanistic models of complex I.

The coupling mechanism between electron transfer and proton translocation is still unknown and remains the biggest enigma in complex I research.

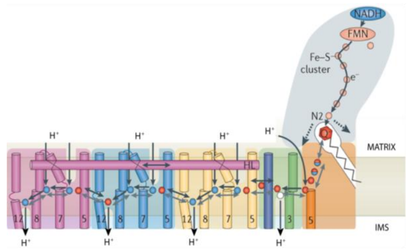

Figure 3. Schematic overview of the CxI key structural parts. Upon electron transfer from the last Fe–S cluster N2, negatively charged Q (or charged residues nearby) initiates a cascade of conformational changes, propagating from the E-channel (orange and green blocks) to the rest of membrane domain via the central axis (grey arrows) comprising charged and polar residues that are located around flexible breaks in key transmembrane helices (TMHs). Figure adopted from Sazanov, 2015.

E. coli complex I as an important mechanistic model

E. coli complex I (*Ec*CxI) has been used for decades to reveal key features of complex I in general. However, the structure of the entire complex is still unknown. So far, the gold standard model structure for interpreting experimental data was *Thermus thermophilus* complex I (*Tt*CxI), which enabled remarkable progress for enzyme understanding (Baradaran et al., 2013). However, this model system has some disadvantages (compared to *E. coli*) which hinders further research.

Any mechanistic predictions should be ideally verified by site-directed mutagenesis. The availability of public libraries of mutants, as well as mutants purified in our lab (unpublished), make *E. coli* the perfect model system (**Fig. 4**). Solving structure of wild type *Ec*CxI would be the key to interpret already published mutations. Visualizing those "snapshots" would be crucial for understanding the general complex I mechanism.

In summary, solving the *Ec*CxI structure and complementing the functional results with structural studies will provide a high-impact contribution to the field of bioenergetics, in particular, for complex I research.

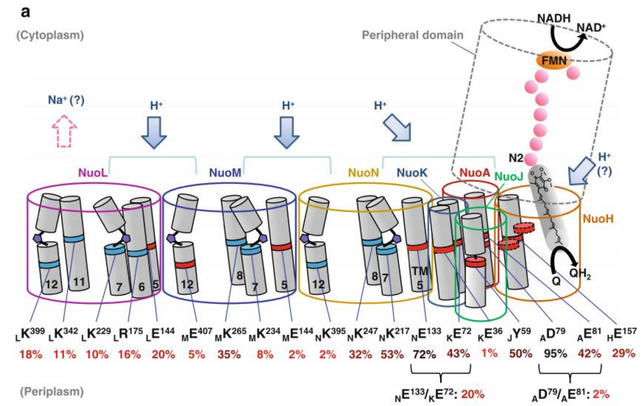

Figure 4. Schematic representation of *Ec*CxI membrane domain mutational map. Percent values indicate mutant enzyme's activity comparing to wild type. Figure adopted from Sato et al, 2014

Preliminary results

The protocol for highly pure, active and monodisperse protein has been already developed in our lab (Morgan & Sazanov, 2008; Sazanov et al., 2003). Those purifications were used to optimize grid conditions and collect preliminary dataset (**Fig. 5**). At the present moment, processing results in cryo-EM maps of the entire *Ec*CxI at better than 6 Å resolution.

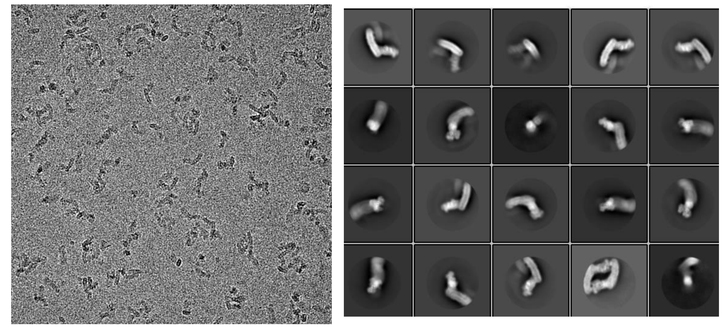

Figure 5. Example of micrograph and 2D classes from the collected dataset of *Ec*CxI. The classes are sorted based on distribution (from high to low). The data was collected on Titan Krios 300kV microscope, Falcon III linear mode, pixel size 1.061 Å. The particles are well defined and adopt different orientations. 2D classes reveal characteristic L-shape for complex I.

Currently, the main bottleneck and challenge is the conformational heterogeneity of the complex (**Fig. 6**). EcCxI has continuous heterogeneity which makes classification complicated. Roughly, the conformational states can be clustered into two: open and closed. As a rule, closed states go to higher resolution comparing to the open ones.

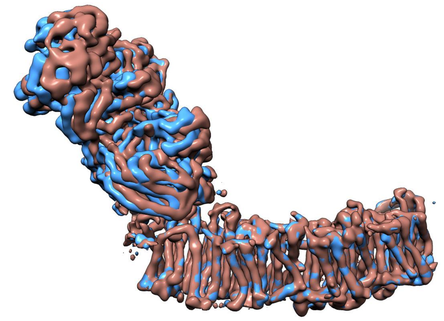

Figure 6. Cryo-EM density maps of the best *Ec*CxI classes. Complex I adopts different conformational states which can be classified into relatively closed and open.

Future prospective

In future, we plan to collect multiple datasets with various ligands to study the conformational dynamics of the complex in different states.

In the second stage of the global project, we plan to solve structures of different mutants to verify mechanistic hypotheses. Possibly we will be also able to observe enzyme, locked in an conformational states which correspond to intermediate steps of the reaction.