A long road towards the structure of respiratory complex I, a giant molecular proton pump

Leonid A. Sazanov1, Rozbeh Baradaran*, Rouslan G. Efremov2*, John M. Berrisford3* and Gurdeep Minhas*

1Medical Research Council Mitochondrial Biology Unit, Wellcome Trust/MRC Building, Hills Road, Cambridge CB2 0XY, U.K.

Abstract

Complex I (NADH:ubiquinone oxidoreductase, EC 1.6.5.3) is central to cellular energy production, being the first and largest enzyme of the respiratory chain in mitochondria. It couples electron transfer from NADH to ubiquinone with proton translocation across the inner mitochondrial membrane and is involved in a wide range of human neurodegenerative disorders. Mammalian complex I is composed of 44 different subunits, whereas the ‘minimal’ bacterial version contains 14 highly conserved ‘core’ subunits. The L-shaped assembly consists of hydrophilic and membrane domains. We have determined all known atomic structures of complex I, starting from the hydrophilic domain of Thermus thermophilus enzyme (eight subunits, nine Fe-S clusters), followed by the membrane domains of the Escherichia coli (six subunits, 55 transmembrane helices) and T. thermophilus (seven subunits, 64 transmembrane helices) enzymes, and finally culminating in a recent crystal structure of the entire intact complex I from T. thermophilus (536 kDa, 16 subunits, nine Fe-S clusters, 64 transmembrane helices). The structure suggests an unusual and unique coupling mechanism via long-range conformational changes. Determination of the structure of the entire complex was possible only through this step-by-step approach, building on from smaller subcomplexes towards the entire assembly. Large membrane proteins are notoriously difficult to crystallize, and so various non-standard and sometimes counterintuitive approaches were employed in order to achieve crystal diffraction to high resolution and solve the structures. These steps, as well as the implications from the final structure, are discussed in the present review.

Introduction

Complex I (NADH:ubiquinone oxidoreductase, EC 1.6.5.3) plays a central role in the respiratory chain in mitochondria and many bacteria [1–5]. It catalyses the exergonic transfer of two electrons from NADH to ubiquinone, coupled to the translocation of four protons (current consensus value [6–8]) across the bacterial or inner mitochondrial membrane, in the following reaction:

\[ \text{NADH} + H^+ + Q + 4H^+_m \rightarrow \text{NAD}^+ + QH_2 + 4H^+_\text{out} \]

In total, the transfer of two electrons from NADH to oxygen, through complexes I, III (bc1) and IV (cytochrome c oxidase), results in the translocation of ten protons across the membrane [9]. Complex I thus contributes approximately 40% of the proton flux which creates the pmf (protonmotive force) for the synthesis of ATP by ATP synthase [10]. Complex I is a reversible machine [11], able to utilize pmf and ubiquinol to reduce NAD⁺.

Mutations in complex I subunits lead to the most common human neurodegenerative diseases [4,12]. The enzyme is also a major source of reactive oxygen species in mitochondria [13], which can lead to mitochondrial DNA damage, implicated in Parkinson’s disease [14] and aging [15].

Complex I is one of the largest known membrane protein assemblies. Mammalian mitochondrial complex I consists of 44 different subunits (∼980 kDa in total) [16,17]. The simpler prokaryotic enzyme normally consists of 14 ‘core’ subunits (∼550 kDa total), conserved from bacteria to humans [1,2,4,18,19] with nearly identical arrangement and folds in both prokaryotic and eukaryotic enzymes [20]. Both enzymes contain equivalent redox components and have a similar L-shaped structure, formed by the hydrophilic (peripheral) and membrane domains, of roughly equal size with seven subunits in each [2,4,21]. The high conservation of core subunits suggests that the mechanism of complex I is conserved throughout all species, therefore we can use the bacterial enzyme as a ‘minimal’ model of human complex.

Towards the structure, building from blocks

We started our work on the structure of complex I approximately 15 years ago, long after some other research groups. Initially, we attempted crystallization of complex I from Escherichia coli. We have optimized for yield and activity the preparation from wild-type cells (i.e. no overexpression or affinity tags, as with all our preparations from other species so far). As with all our preparations intended for crystallization, we first check the stability of

Key words: bioenergetics, complex I, membrane protein structure, NADH:ubiquinone oxidoreductase, respiratory chain, X-ray crystallography.

Abbreviations used: ASU, asymmetric unit; CMC, critical micellar concentration; CYMAL-7, 7-cyclohexyl-1-heptyl-α-maltoside; DDM, dodecyl maltoside; MAD, multi-wavelength anomalous dispersion; MR, molecular replacement; NDSB, non-detergent sulfobetaine; OMF, octyl maltoside fluorinated; pmf, protonmotive force; SeMet, selenomethionine; TDM, tridecyl maltoside; TM, transmembrane; UDM, undecyl maltoside.

*Present address: IRB Department of Structural Biology, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussels, Belgium.

©The Authors Journal compilation ©2013 Biochemical Society
the complex in a range of detergents by incubating it over a period of several days at room temperature (preferable to 4°C), followed by analytical gel-filtration chromatography.

The eluted fractions are then analysed by SDS/PAGE for subunit content and checked for oxidoreductase activity. *E. coli* complex I fulfilled the stability conditions and produced 2D crystals after reconstitution with lipids [22], resulting in 8 Å (1 Å = 0.1 nm) resolution projection maps [23]. However, no well-diffracting 3D crystals were obtained.

**Thermus thermophilus** hydrophilic domain

We therefore turned to the enzyme from *T. thermophilus*, as it was expected to be more stable. It has not been purified previously, and our initial preparations using DDM (dodecyl maltoside) as a detergent resulted in preparation of only the hydrophilic domain, with the loss of the membrane domain early in the purification [24]. However, the hydrophilic domain on its own was extremely stable; it was necessary to boil it in SDS in order to separate subunits [24]. To be monodisperse, the domain still required the presence of detergent due to a hydrophobic patch at the interface with the membrane domain and so we exchanged the detergent into the small-micelle octyl glucoside, to maximize chances of crystallization. Initial trials using commercial screens (sitting drops in 96-well plates) led to several hits in high-molecular-mass PEGs. These were optimized in 24-well plates, leading to large plate-like crystals of up to 0.5 mm × 0.3 mm × 0.03 mm in size (Figure 1A). Crystals diffracted X-rays to 3.3 Å resolution and belonged to space group *P*₂₁₀. The ASU (asymmetric unit) contained four complexes, with a total molecular mass of 1120 kDa, including 36 Fe–S clusters. Anomalous signal from these intrinsic iron atoms was used for phasing. Because crystals were thin in one dimension, they were prone to radiation damage due to a small volume of the crystal exposed to the beam. This was especially the case at iron peak absorption wavelength, and so normally only one wavelength dataset could be collected from each crystal. Despite the large number of iron atoms, a very large protein mass in the ASU meant that anomalous signal was relatively weak, with maximal cross-correlation of approximately 0.35 at low resolution. Nevertheless, a combination of SHELXD/SOLVE/SHARP/DM steps in iterative manner [25] allowed us to locate all the clusters. Even with weak phases (due to large protein mass), it was possible to obtain good quality electron density after careful refinement of heavy atom parameters in SHARP and density modification. It was important to refine positions of heavy atoms to relatively low resolution (~6 Å), where the signal was still significant, and then use these positions for phasing to maximal resolution. Density modification was performed in DM (or DMmulti for some of our subsequent structures) with some extreme extension of resolution from approximately 6–8 Å to the resolution limit (3.3 Å) in 1000 cycles, using NCS.

The structure was solved using additional data from two heavy atom derivatives [26]. It shows (Figures 1A and 2A) how subunits related to various smaller redox proteins were put together by Nature to provide a uniquely long (~95 Å) electron transfer pathway from NADH to the primary electron acceptor FMN and through the seven conserved Fe–S clusters to the Q-site (quinone-binding site) at the interface with the membrane domain. Apart from seven conserved ‘core’ subunits, an additional subunit specific to thermophiles, with a frataxin-like fold, was present in the structure. It may stabilize the domain and/or help to regenerate nearby clusters. Later, we determined the structures of the hydrophilic domain reduced by NADH and/or dithionite under anaerobic conditions and improved the overall resolution to 3.1 Å, leading to the improved model in some surface-exposed areas [27]. We observed conformational changes at the interface with the membrane domain, which appear to be driven by the nearby Fe–S cluster N2, co-ordinated by the unusual tandem cysteine motif, and could represent a part of the catalytic cycle [27].

**Architecture of the intact *T. thermophilus* complex**

The next step in our quest was to crystallize the intact *T. thermophilus* complex. Two major modifications to the purification procedure allowed us to obtain the intact enzyme. First, we performed all steps at room temperature. Previously, similarly to our preparations of complex I from other species, we used 4°C to try to keep the complex stable. However, we reasoned that, for a thermophile, this may be too low; hydrophobic interactions will be stronger at the elevated temperature and perhaps keep the two domains together. Secondly, DDM was replaced by a milder detergent, TDM (tridecyl maltoside). This led to improved stability of the intact complex, so that it could be prepared in sufficient amounts. Trials against commercial and custom screens identified several crystallization conditions with protein in TDM or exchanged on small anion-exchange columns into DDM, UDM (undecyl maltoside), OMF (octyl maltoside fluorinated) or CYMAL-7 (7-cyclohexyl-1-heptyl-β-D-maltoside). Most conditions involved high-molecular-mass PEG (~4000 kDa) as a precipitant and resulted in similarly-looking thin rod-like crystals in *P*₂₁₀ space group (Figure 1D). Crystallization in the presence of *E. coli* polar lipids led to bulky cuboid crystals in *P*₂₁₂₁ space group, but despite many attempts of optimization, including different lipid mixtures, the resolution was limited to approximately 8 Å [21]. Plate-like crystals were observed with PEG 400 as precipitate, but it was not possible to improve their diffraction beyond ~7 Å resolution. Many rounds of optimization involved protein exchange into new primary detergent and addition of different secondary detergent, to modify micelle properties. A wide range of small-molecule additives was explored, including different NDSBs (non-detergent sulfobetaines). In the end, TDM was found to be the best primary detergent, whereas several detergents could be used as a secondary [OMF, DDM, CYMAL-4 (4-cyclohexyl-1-heptyl-β-D-maltoside), Fos-Choline-8 (n-octylphosphocholine) fluorinated, etc.], at approximately 1× CMC (critical micellar concentration). They were always used in subsequent set-ups [28] as a secondary detergent.
screen along with various PEG concentrations. Only rod-like crystals could be optimized to diffract to relatively high resolution (initially 4.5 Å [21]). However, they were always pseudo-merohedrally twinned [28], which hampered structure solution by experimental phasing and even by molecular replacement due to model bias. Therefore an additional route towards the structure was necessary.

In parallel with this work, we pursued crystallization of the membrane domain of *E. coli* complex I. It was separated from the intact complex by incubation in 0.4 M MgCl₂. Optimization of initial hits again involved selection of primary and secondary detergent (initially DDM plus heptyl glucoside [21]) and optimization of additives, with PEG 4000 as the main precipitant. Additional dimension of complexity in this search was due to necessity of lipids for crystallization, which were optimized as a 3:1 mixture of DMPC (dimyristoyl phosphatidylcholine) and *E. coli* polar lipids. Crystals (*P*2₁2₁2₁ space group) were relatively chunky of a brick-like shape up to approximately 50 μm × 70 μm × 400 μm (Figure 1B), but initially diffracted to only ~7 Å resolution. Attempts at optimizing the diffraction by dehydration in the drop or using the dehydration machine...
**Figure 2 | Mechanisms of electron transfer and proton translocation**

(A) Electron transfer in the hydrophilic domain. The main pathway is indicated by blue arrows, a diversion to cluster N1a is shown by a green arrow. The distances between the centres given in Å were calculated both centre-to-centre and edge-to-edge (shown in parentheses). FMN accepts electrons from NADH, cluster N2 donates electrons to the quinone. From Sazanov, L.A. and Hinchliffe, P. (2006) Structure of the hydrophilic domain of respiratory complex I from *Thermus thermophilus*. *Science* 311(5766): 1430–1436. Reproduced with permission from AAAS.

(B) Proposed mechanism of proton translocation. Schematic drawing of conformational changes between the two main (low-energy) conformations. In the oxidized state (as crystallized) C-terminal half-channels are proposed to be open to the periplasm, releasing four protons. Upon delivery of two electrons from NADH, negatively charged quinone (or charged residues nearby) initiates a cascade of conformational changes, propagating from the nearest proton-translocation channel to the three antiporters via the central axis (red arrows) of charged and polar residues located around flexible breaks in key TM helices. Cluster N2-driven shifts of nearby helices (blue arrows) probably assist overall conformational changes. Helix HL and the ¶H element help to co-ordinate conformational changes by linking discontinuous TM helices between the antiporters. As a result of conformational changes, in the alternative (reduced) state, C-terminal half-channels close and N-terminal half-channels open to the cytoplasm, taking up four protons. It is possible that the order is reversed, i.e. in the oxidized state N-terminal half-channels open to the cytoplasm and in the reduced state C-terminal half-channels open to the periplasm. In any case, the overall cycle results in the translocation of four protons, in agreement with experiments. Residues shown in red and blue indicate key negatively and positively charged residues, whereas black circles indicate conserved proline residues from the break in TM12. Reproduced with permission from Baradaran, R., Berrisford, J.M., Minhas, G.S. and Sazanov, L.A. (2013) Crystal structure of the entire respiratory complex I. *Nature* 476, 414–420.

(Maatel HC-1b) at the home X-ray source led to only limited improvements, possibly due to relatively short times involved. We observed poorly reproducible improvement of diffraction to ∼3.5 Å along one crystallographic axis upon increasing the PEG concentration in mother liquor from 10 to 40%. We therefore exploited a rather unusual approach of transferring the crystals into microdialysis buttons followed by gradual exchange of the outside buffer. This permitted a fully controlled slow dehydration process, allowing the crystal lattice time to adjust and the unit cell to shrink. Furthermore, dialysis allowed for not only the PEG concentration increase, but also a simultaneous decrease in salt (sodium/potassium formate) concentration, which was essential in order to achieve isotropic improvement in diffraction (Figure 3). Daily exchange of outside buffer with PEG concentration increasing in 5% increments to 30–40% over 4–5 days led to diffraction improving to ∼3.9 Å resolution under initial conditions [21] and to 2.7 Å resolution in the final set-up [29]. It is possible that such slow and controlled dehydration process will benefit many other protein crystals, especially of large macromolecular complexes.

The initial α-helical structure was solved at 3.9 Å resolution using four native and three TaBr derivative crystals. Multi-crystal electron density averaging and modification in DMMulti resulted in excellent quality electron density, allowing mostly automatic building of an α-helical model [21]. It has revealed for the first time the arrangement of 14 TM (transmembrane) helices in antiporter-like subunits. Complex I contains three such subunits, homologous with each other and with the Mrp antiporter family, probably each pumping one proton per cycle. Unexpectedly, a long
amphipathic helix was found to straddle nearly the entire domain, joining the antiporter subunits together. It was tempting to suggest that it may also drive conformational changes in analogy to a coupling rod from a steam engine, although accumulating structural and functional evidence indicates that the main driving force may be the central hydrophilic axis ([28] and see below). A similar helix was observed in the low-resolution (∼6 Å) X-ray analysis of complex I from Yarrowia lipolytica [30]. We then used the E. coli backbone structure for molecular replacement to determine the architecture of the entire T. thermophilus enzyme, at 4.5 Å resolution [21]. One of the major surprises was the location of Fe–S cluster N2, which donates electrons to quinone: it is approximately 25–30 Å away from the membrane surface, suggesting that, unprecedentedly, quinone has to come out of the membrane in order to be reduced.

**Structure of E. coli membrane domain**

The next step was to determine the atomic structure of the membrane domain of the E. coli complex I [29]. Here, the crucial improvement came from identifying a different detergent mixture: CYMAL-7 as primary detergent with cholate and NDSB-256 or nonyl glucoside as the secondary/tertiary detergents. In this case, crystals formed in space group P1 with two molecules per ASU and diffracted strongly, up to 2.7 Å resolution (anisotropically, with the complete dataset collected to 3.0 Å). This was achieved only after dehydration in microdialysis buttons as described above. In order to solve the structure an additional phasing from SeMet (selenomethionine) derivaties was essential. The positions of selenium atoms were also essential markers in deriving the fold, as there were no homologues of any of the subunits in the PDB. For SeMet labelling, a feedback-inhibition method [31] was applied during wild-type cell growth on minimal medium with 0.6% malate, optimized for maximal complex I yield. Despite the challenges presented by anomalous data collection in the P1 space group, several selenium MAD (multi-wavelength anomalous dispersion) datasets were obtained using a low intensity beam and 360–720° rotations of the crystal. Positions of 160 selenium atoms in the ASU were determined using phases calculated with a previously built polyalanine backbone model. Data from several native, selenium and TaBr P1 and P2;2;2, datasets were then used in DMmulti to produce excellent quality electron density. A solvent-flipped map calculated in CNS (also with extreme phase extension) using only the selenium MAD dataset was also of good quality, with better side-chain density for some residues. The whole process from initial purification attempts to structure solution was a major undertaking: ∼80 000 crystallization conditions tested, ∼1000 crystals frozen and tested at the synchrotrons, and ∼80 datasets collected.

The structure revealed a novel fold for the antiporter subunits, with two inverted pseudo-screw symmetry-related 5-TM repeats containing half of a proton channel each. The two halves are connected in the middle of the membrane, forming a single channel. Uniquely, lysine residues rather than the usual carboxy groups appear to be the central protonable residues of the proton pumps.

**Structure of the entire T. thermophilus complex**

In the meantime, crystals of the intact T. thermophilus complex were optimized to diffract up to 3.2 Å resolution. Two key developments were necessary for this improvement, both rather counterintuitive. First, we increased the primary detergent (TDM) concentration in protein solution to approximately 4% (w/v). This is very high compared with the common notion of using detergent for crystallization of membrane proteins at approximately 1–2× CMC. It has to be said that for all of our crystallizations of the membrane domain or the entire complex, we have a high starting concentration of detergent as a result of concentrating the protein after the final gel-filtration column. Because the detergent concentrates even on a 100 kDa cut-off concentrator, usually the final concentration of detergent in ∼20 mg/ml protein solution is, as we determined, approximately 1–1.5%. Colorimetric assays show that the ratio of complex I to bound detergent is ∼4:1 (w/w), which still leaves at least 0.5% free detergent; much higher than the CMC. However, when we tried to reduce this free detergent concentration in the protein solutions, crystallization was negatively affected or crystals did not appear. One possibility is that, to ensure full solubility of such large hydrophobic proteins, an excess of free detergent is needed (that is also the case on gel-filtration columns, which we run at up to 0.5% detergent in the buffer). When we increased detergent concentration in the intact complex I solutions in steps to even higher values, crystals were bigger (rods became thicker) with the optimum at approximately 4% TDM. At these concentrations, we start to approach the phase-separation boundary, and so it is possible that increased local protein concentrations lead to bigger crystals. The second important
development was to decrease PEG 4000 concentration in harvest solutions to ∼9% (w/v), even though the crystals grew against 19–24% PEG. Unlike E. coli membrane domain crystals, these crystals do not tolerate dehydation by any of the methods. When we used 20% or higher PEG concentrations in earlier harvesting, crystals often started to bend and diffraction was hit-and-miss. The reason is that protein solution contains 25% glycerol, and so after equilibration against the reservoir, the drop becomes bigger than expected due to the hygroscopic nature of glycerol. As a result, the final PEG concentration in the drop is much lower than expected. We found that 9% PEG in the harvest solution was optimal to mimic the conditions in the drop, preserving the shape and diffraction properties of the crystals. (As a side note, attempts to match glycerol in the protein/drop and in the reservoir solutions did not lead to any crystal growth.)

Since these crystals are nearly perfectly twinned, due to model bias it was problematic to use the relatively distal E. coli membrane domain model (∼30–35% sequence similarity) for the structure solution. We therefore crystallized the isolated membrane domain from T. thermophilus complex I. The intact complex was split at low pH (4.0), removing subunits Nqo1–Nqo3 and Nqo15. The remaining hydrophilic subunits were lost upon crystallization. Conditions, identified after many rounds of optimization, included ∼1% UDM as the primary detergent and 5 mM CHAPS as the secondary detergent, with PEG 300 as precipitant. Crystals were rectangular in shape (∼100 μm × 100 μm × 500–700 μm) and diffracted to ∼3.3 Å resolution (Figure 1C), with relatively minor improvements upon dehydation by addition of 30% PEG 3350 into the harvest solution. Other dehydration approaches did not improve the diffraction further. These crystals were not twinned and formed in P1 space group with packing resembling that of the E. coli membrane domain. They contained subunit Nqo8, in contrast with E. coli, where the corresponding subunit (NuoH) found at the interface of the two main domains was lost upon crystallization. Thus the T. thermophilus membrane domain structure contained the full complement of seven subunits and 64 TM helices. Initial attempts at the structure solution by MR (molecular replacement) with the E. coli model were problematic, mostly due to significant shifts in the positions of some TM helices, especially in the distal antiporter subunit Nqo12. We therefore used the Rosetta MR method in PHENIX for one cycle, which shifted most helices into their positions (further cycles in Rosetta resulted in too divergent models, possibly due to limited resolution of the data). This brought 〈R_free〉 down from the initial 50% to 46% and allowed further successful refinement in CNS using DEN (Deformable Elastic Network) restraints (or Refmac with Jelly body restraints) to 〈R_free〉 of 36–37%. This (and density modification in DM) led to improved electron density so that subunit Nqo8 could be modelled and the structure rebuilt/refined.

We then used this new structure of the membrane domain to solve the structure of the entire complex by MR, together with the previous hydrophilic domain structure (PDB code 3I9V). Some of the features at the interface of the two main domains were not well resolved since the conformation here changes upon connection/disconnection of the domains. It was important therefore to obtain the iron peak wavelength datasets from two rare crystals with relatively low twin fraction (∼0.4). Jelly body refinement with iron SAD (single-wavelength anomalous dispersion) target function in Refmac resulted in a much improved electron density for missing features, including a novel hydrophilic subunit Nqo16. This protein is essential for crystal contacts, but is not needed for activity and may play the role of an assembly factor. Understandably, arriving at the structure of entire complex took even more effort than for individual subcomplexes, with ∼3000 crystals tested at the synchrotrons and ∼200 datasets collected. The final structure contains 4780 residues, nine Fe–S clusters and one FMN molecule, and, with 64 TM helices, is the largest (asymmetric) membrane protein complex solved to date.

Conclusions and implications for the mechanism of complex I

Several common themes emerge from our experience with crystallization of large membrane protein complexes. (i) It is usualy necessary to use a mixture of a primary and a secondary detergent as an additive, presumably to optimize properties of the micelle and make it compatible with crystal contacts. The primary detergent must be relatively mild so that it preserves the fragile protein complex intact (this should be tested by gel-filtration chromatography). The concentration of this detergent (free) in the protein solution does not have to be near its CMC; on the contrary, it can be beneficial to have it at approximately 1–2%. The secondary/tertiary detergent can be quite harsh (but not necessarily) and can be used at approximately 1 × CMC (although higher and lower concentrations can and should be tested). (ii) The addition of native or artificial lipids to the protein can be beneficial in some cases, but not in others, and has to be evaluated on a case-by-case basis, including the optimization of the lipid mixture. (iii) Dehydration of crystals either by a common method (in the drop by increasing precipitant concentrations or by using a free mounting system on a beamline) or by the microdialysis method that we described can significantly improve the diffraction properties. This appears to work best if the initial diffraction is poor (∼6–8 Å resolution) and may not help if it is already reasonable (by the membrane protein standards, i.e. 3–4 Å resolution). (iv) For crystal harvesting (if not dehydrating), it is important to match exactly the conditions in the drop; they may not be as expected due to glycerol and other compounds from the protein solution. When cryoprotectant needed to be added, with our samples 25% ethylene glycol was consistently a better performer than glycerol. (v) Modern software and careful refinement of heavy atom parameters, combined with aggressive density modification/extension, can lead to high-quality electron-density maps even from weak anomalous/isomorphous signal. It is important to use NCS and all available sources of
phasing information, which can be combined by multi-crystal density averaging/modification.

The structures revealed several remarkable features unique to complex I, likely to be related to the unusual mechanism of coupling between electron transfer and proton translocation. These include: a long, enclosed and out-of-membrane quinone reaction chamber; central flexible hydrophilic axis of charged and polar residues (sitting on the breaks in TM helices) extending in the middle of the membrane from the quinone cavity all the way to the tip of the membrane domain; four copies of proton channels each formed from two connected half-channels (three in antiporters and one at the interface with the hydrophilic domain); lysine residues rather than carboxy groups as central residues in antiporters; connecting elements on the both sides of the membrane domain, etc. The mechanism which we propose (Figure 2B) involves long-range conformational changes in the membrane domain, driven, through the central hydrophilic axis, by the redox reactions around cluster N2 and electrostatic interactions with a negatively charged area around the quinone headgroup. In this two-state (one stroke) mechanism, the half-channels would be open to the opposite sides of the membrane in the oxidized and reduced states of the enzyme. Thus the next challenge is to crystallize the reduced form of the complex, in order to verify these proposals.

Acknowledgements

We thank the European Synchrotron Radiation Facility (Grenoble), the Swiss Light Source (Villigen) and the Diamond Light Source (Didcot) for the provision of synchrotron radiation facilities.

Funding

This work was funded by the Medical Research Council.

References

8 Ripple, M. O., Kim, N. and Sprenger, R. (2013) Mammalian complex I pumps 4 protons per 2 electrons at high and physiological proton motive force in living cells. J. Biol. Chem. 288, 5374–5380

Received 12 August 2013
doi:10.1042/BST20130193