Crystal structure of the entire respiratory complex I

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Complex I is the first and largest enzyme of the respiratory chain and has a central role in cellular energy production through the coupling of NADH:ubiquinone electron transfer to proton translocation. It is also implicated in many common human neurodegenerative diseases. Here, we report the first crystal structure of the entire, intact complex I (from Thermus thermophilus) at 3.3 Å resolution. The structure of the 536-kDa complex comprises 16 different subunits, with a total of 64 transmembrane helices and 9 iron–sulphur clusters. The core fold of subunit Nqo8 (ND1 in humans) is, unexpectedly, similar to a half-channel of the antiporter-like subunits. Small subunits nearby form a linked second half-channel, which completes the fourth proton-translocation pathway (present in addition to the channels in three antiporter-like subunits). The quinone-binding site is unusually long, narrow and enclosed. The quinone headgroup binds at the deep end of this chamber, near iron–sulphur cluster N2. Notably, the chamber is linked to the fourth channel by a ‘funnel’ of charged residues. The link continues over the entire membrane domain as a flexible central axis of charged and polar residues, and probably has a leading role in the propagation of conformational changes, aided by coupling elements. The structure suggests that a unique, out-of-the-membrane quinone-reaction chamber enables the redox energy to drive concerted long-range conformational changes in the four antiporter-like domains, resulting in translocation of four protons per cycle.

Complex I (also known as NADH:ubiquinone oxidoreductase) has a central role in the respiratory chain in mitochondria and many bacteria1–7. It catalyses the transfer of two electrons from NADH to ubiquinone (Q), coupled to the translocation of four protons (current consensus value8,9) across the bacterial or inner mitochondrial membrane:

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

The transfer of two electrons from NADH to oxygen, through complexes I, III (b,c) and IV (cytochrome c oxidase), results in the translocation of ten protons across the membrane10, creating the proton motive force (PMF) for the synthesis of ATP by ATP synthase11. Complex I is a reversible machine12 that is able to use PMF and ubiquinol to reduce NAD+.

Mutations in complex I subunits lead to the most common human neurodegenerative diseases8,13. The enzyme is also a key source of reactive oxygen species in mitochondria14, which can lead to mitochondrial DNA damage, and are implicated in Parkinson’s disease15 and ageing6.

Complex I is one of the largest known membrane proteins. The mitochondrial enzyme consists of 44 different subunits (≈980 kDa in total)7,16. The simpler prokaryotic version normally comprises 14 ‘core’ subunits (≈550 kDa total), highly conserved from bacteria to humans1,2,3,9,18, suggesting that the mechanism is also conserved. Both enzymes contain equivalent redox components and have a similar L-shaped structure, formed by the hydrophilic and membrane domains2,5,21. The ≈30 ‘accessory’ subunits of the mitochondrial enzyme mostly form a protective shell around the core1,22,23, although some may have a specialized functional role24–25.

We determined the first structures of the eight-subunit hydrophilic domain of T. thermophilus complex I at up to 3.1 Å resolution26,27. It contains all the redox centres of the enzyme: non-covalently bound flavin mononucleotide (FMN) and nine iron–sulphur (Fe–S) clusters. NADH transfers two electrons to FMN as a hydride ion and then electrons are transferred one by one, along the uniquely long (95 Å) chain of seven conserved Fe–S clusters, to the quinone-binding site (Q site) at the interface with the membrane domain.

We subsequently determined the architecture of the entire T. thermophilus complex I at 4.5 Å resolution, with the membrane domain resolved at the level of arrangement of subunits and α-helices27. X-ray analysis at 6.3 Å resolution of the Yarrowia lipolytica mitochondrial enzyme was published later28, but no subunits were identified and no models were deposited from this work owing to limited resolution. Currently all information on atomic structures of complex I is from our studies.

Recently we determined the 3.0 Å resolution structure of the membrane domain from Escherichia coli complex I19. The three largest subunits, NuoL, NuoM and NuoN (known as Nqo12, Nqo13 and Nqo14 in T. thermophilus, respectively; Supplementary Table 3), are homologous to each other and to Na+]H+ antiporter complex (Mrp) subunits30,31. Each contains 14 conserved transmembrane (TM) helices and a putative proton-translocation channel29. However, the crystals lacked NuoH (Nqo8 and ND1 in T. thermophilus and humans, respectively), the only core subunit of unknown structure.

The overall architecture of complex I suggests that the coupling mechanism involves long-range conformational changes: there are no cofactors in the membrane and the antiporter-like subunits (hereafter termed ‘antiporters’ for brevity) are distant from the interface with the hydrophilic domain21,29. Exactly how these changes are coupled to redox reactions remains unclear, although mechanical coupling elements have been suggested21,28,29. Notably, Fe–S cluster N2, which donates electrons to the quinone, is ~25–30 Å away from the membrane surface21, suggesting that the quinone has to move out of the membrane to accept electrons. Nqo8 is the most conserved membrane subunit of complex I (Supplementary Fig. 1), forming the...
interface with the hydrophilic domain and contributing to the Q site\(^2\). This subunit emerged only once during evolution, joining hydrogenase and antiporter modules of complex I-related enzymes\(^2\). Clearly, the atomic structure of the entire complex, including Nqo8, holds the key to understanding the enigmatic coupling mechanism.

**Determination of structures**

The diffraction of crystals of the entire *T. thermophilus* complex has been improved to 3.3 Å resolution (Methods). Crystals are, however, twinned and so to overcome the problem of model bias we crystallized the isolated *T. thermophilus* membrane domain. These crystals were non-twinned and contained subunit Nqo8. The structure was solved at 3.3 Å resolution by molecular replacement with our *E. coli* model (Protein Data Bank (PDB) code, 3RKO) (Supplementary Tables 1, 2 and Supplementary Fig. 2). It contains seven subunits (Nqo12 (16), Nqo13 (14), Nqo14 (14), Nqo10 (5), Nqo11 (3), Nqo7 (3) and Nqo8 (9), in which the numbers in parentheses indicate the number of TM helices in each subunit). Antiporters Nqo12–14 show an arrangement of helices (Supplementary Fig. 3) and key residues similar to the corresponding helices in each subunit. Antiporters Nqo12–14 show an arrangement of helices (Supplementary Fig. 3) and key residues similar to the *E. coli* structure: each subunit contains two inserted-symmetry related half-channels. The cytoplasm-linked TM4–8 half-channel contains a central lysine on the discontinuous, thus flexible, TM7 (here termed Lys TM7) and its pK\(_a\)-modulating glutamate on TM5 (here termed Glu TM5), whereas the periplasm-linked TM9–13 half-channel contains a central lysine (Glu in Nqo13) on discontinuous TM12 (here termed Lys TM12) (Fig. 1b). The half-channels are linked into a single full proton-translocation channel by charged residues, including a lysine from the broken (partly unwound in the middle) TM8 (His in Nqo12). The long connecting helix HL, from the carboxyl-terminal extension of Nqo12, is straighter in *T. thermophilus* than in *E. coli* (Supplementary Fig. 3). On the opposite side of the domain, the β-hairpin–helix connecting element (βH) shows a very similar arrangement in both species. Thus, both previously proposed coupling elements\(^2\) seem to be common complex I features.

*T. thermophilus* Nqo8 contains eight conserved TM helices and an additional C-terminal TM helix. Unexpectedly, TM1 of Nqo7 (NuoA) is in a different position compared to the *E. coli* structure, forming a part of the Nqo8 helical bundle (Fig. 1a and Supplementary Fig. 3). Presumably, when NuoH dissociates in *E. coli*, this helix moves closer to the remaining subunits.

The *T. thermophilus* membrane domain structure was then used with the hydrophilic domain structure (PDB code, 3I9V) to solve the structure of the entire complex by molecular replacement. The 3.3 Å resolution structure (Fig. 1a) of the 536-kDa complex contains 9 hydrophilic subunits with 9 Fe–S clusters and 1 FMN molecule, as well as 7 membrane subunits with 64 TM helices (Supplementary Tables 1 and 2). This includes the novel assembly factor-like hydrophilic subunit Nqo16, essential for crystallization but not necessary for activity (Supplementary Discussion). Although many assembly factors for mitochondrial complex I are known\(^3\), this is, to our knowledge, the first example for bacterial complex I.

Features at the interface between the two main domains, missing in the individual structures, were resolved in that of the entire complex, including loops from Nqo4, Nqo7 and Nqo8 (Supplementary Figs 4 and 5). The Q site is found, as expected\(^2\), at the interface of Nqo4, Nqo6 and Nqo8, and is described below. Most of the interactions between the two domains involve Nqo8 (Supplementary Discussion and Supplementary Table 4), including the highly conserved first cytoplasmic loop. Another highly conserved Nqo8 loop (third cytoplasmic loop) lines the quinone cavity. The first cytoplasmic loop from Nqo7 (NuoA) wraps around Nqo8, stabilizing the interface between the domains.

**Subunit Nqo8 (ND1) forms part of a proton channel**

The fold of Nqo8 is unusual, with some TM helices very short (TM5, 14 residues), others very long (TM1, 35 residues), and with nearly all helices highly tilted relative to membrane normal (Fig. 2). Unexpectedly, TM helices 2–6 can be superimposed (root mean squared deviation 2.1–2.6 Å over ~140 residues, PDBeFOLD) on to the antiporters’ half-channel TM helices 4–8 or 9–13 (Fig. 2c). This similarity is not apparent from the sequence (~11–18% identity). Although in the antiporters most helices are roughly normal to the membrane, TM helices 2–6 from Nqo8 are tilted considerably, up to 45°. By contrast, TM1 is tilted in the opposite direction, so that it crosses TM6 at nearly 90°. TM9 is peripheral, consistent with its absence in most species.

In Nqo8, charged residues are found in similar positions to key antiporter residues: Glu 130 and Glu 163 in the Glu TM5 position and Glu 213 and Glu 248 near Lys TM7 (Fig. 2c). Overall, Nqo8 contains many more charged residues in the membrane (Fig. 3a) compared to the antiporters, and many of these residues (including Glu 163 and Glu 213) are conserved in complex I and in membrane-bound hydrogenases (Supplementary Figs 1 and 6). They form an unusual chain (or ‘funnel’) of charged residues leading from the Q site to a remarkable network of four interacting carboxylates deep in the membrane (Glu 130, Glu 163 and Glu 213 from Nqo8, plus conserved 7 (Asp 72) (in which prefix indicates subunit)). The charged network congregates around the highly conserved broken 10(TM3), a hotspot for human disease mutations\(^2\). On the other side of the break, conserved 10(Tyr 59) interacts with essential 11(Glu 32), part of a fourth proton-translocation channel proposed previously\(^2\). Nqo11 superimposes with helices 4–6 from the antiporter half-channels, overlaying Glu 32 with Glu TM5 (ref. 29).

**Figure 1 | Structure of the entire complex I from *T. thermophilus*.** a. An overview. FMN and Fe–S clusters are shown as magenta and red–orange spheres, respectively, with cluster N2 labelled. Key helices around the entry point (Q) into the quinone-reaction chamber, and approximate membrane position, are indicated. b. Putative proton-translocation channels in the antiporter-like subunits. Polar residues lining the channels are shown as sticks with carbon in dark blue for the first (N-terminal) half-channel, in green for the second (C-terminal) half-channel and in orange for connecting residues. Key residues—Glu TM5 and Lys TM7 from the first half-channel, Lys/His TM8 from the connection and Lys/Glu TM12 from the second half-channel—are labelled. Approximate proton-translocation paths are indicated by blue arrows.
Thus, the first half-channel formed by Nqo8 is linked to the second half-channel in Nqo10 and Nqo11, with 8(TM5) and 10(TM3) in the roles of the key discontinuous antiporter helices TM7 and TM12. This strongly suggests that input from the cytoplasm into the fourth channel is not, as previously proposed\(^\text{29}\), at the interface between Nqo14 and Nqo11 (which in \textit{T. thermophilus} is more clearly closed from the cytoplasm than in \textit{E. coli}), but is through Nqo8 instead. As with the amino-terminal half-channel of the antiporters, the Nqo8 half-channel is closed from the periplasm by large hydrophobic residues but is connected through an extensive network of polar residues both to the cytoplasm and to the Q site. Furthermore, similarly to the C-terminal half-channel of the antiporters, the Nqo10/Nqo11 half-channel is blocked from the cytoplasm, but is connected to the periplasm by polar residues. The two half-channels are linked into a single channel by the Glu/Asp quartet and putative water molecules (Fig. 3). We refer to it here as the ‘E-channel’ owing to abundance of glutamates in its centre. Many residues in the E-channel are conserved and essential for activity (Supplementary Table 7). Therefore it is, unexpectedly, arranged similarly to the three channels within the antiporters. This implies that proton-pumping stoichiometry in complex I is indeed 4 (not 3, as proposed recently\(^\text{35}\)).

**Unique quinone–reaction chamber**

To determine exactly where quinone binds, intact complex I was co-crystallized, or crystals soaked with the quinone analogues piericidin A (a complex I inhibitor) and decylubiquinone. Although \textit{in vivo} \textit{T. thermophilus} complex I uses menaquinone-8, it is also fully active with decylubiquinone\(^\text{31}\), and, in contrast to \textit{E. coli} complex I\(^\text{16}\), the \textit{T. thermophilus} enzyme does not contain any bound endogenous quinone after purification. X-ray data (Supplementary Table 1) clearly show (Fig. 4a, b) that piericidin A and decylubiquinone bind in a very similar manner, ~15 Å away from the membrane surface, at the deep end of a long narrow cavity. In this position, the quinone headgroup is ~12 Å (centre-to-centre) from the Fe–S cluster N2, appropriate for efficient electron transfer\(^\text{37}\). One of the decylubiquinone ketone groups is, as predicted\(^\text{26,34}\), hydrogen bonded to 4(Tyr 87), whereas another interacts, unexpectedly, with 4(His 38). Both residues are invariant and essential for activity\(^\text{34,38}\).

One of the most surprising structural features is that this 30-Å long chamber is completely enclosed from the solvent, with only a narrow (approximately 2–3 × 4–5 Å) apparent entry point for the quinone, framed by helices TM1, TM6 and amphipathic AH1 from Nqo8 (Figs 1a and 4), as well as TM1 from Nqo7. All residues facing the lipid bilayer here are hydrophobic, but the inside of the chamber is lined, unexpectedly, mostly by hydrophilic residues, especially in the area (‘front’) facing the tip of the membrane domain (to the left in Fig. 4d). However, at the opposite side (‘back’), a hydrophobic patch formed, surprisingly, mainly by residues from hydrophilic subunits...
Nqo4 and Nqo6, extends towards the entrance, sufficient to accommodate the quinone tail. The cavity front is mostly negatively charged, whereas the back is neutral and the ‘top’ (near cluster N2) is positively charged (Fig. 4d). The ionizable residues lining the chamber are all highly conserved (Supplementary Fig. 6 and Supplementary Table 8) whereas the back is neutral and the ‘top’ (near cluster N2) is positively charged (Supplementary Discussion). It is possible that sealing off the quinone-reaction chamber is well suited for this purpose: owing to tight protein packing near the bound headgroup, the quinone can be protonated only through the coordinating 4(Tyr 87) and 4(His 38). Notably, the charged species can exist in the chamber because it is relatively hydrophilic and distal from the membrane. The Q site is linked to the Glu/Asp quartet in the centre of the E-channel by a hydrophilic funnel, so these negatively charged species can interact electrostatically, driving conformational changes in this channel. Additional driving force is probably provided by moving upon N2 reduction Nqo4 and Nqo6 helices through the coordinating 4(Tyr 87) and 4(His 38). Notably, the charged species can exist in the chamber because it is relatively hydrophilic and distal from the membrane. The Q site is linked to the Glu/Asp quartet in the centre of the E-channel by a hydrophilic funnel, so these negatively charged species can interact electrostatically, driving conformational changes in this channel. Additional driving force is probably provided by moving upon N2 reduction Nqo4 and Nqo6 helices, which directly interact with flexible parts of Nqo8.

One of the most fascinating features of the structure is that the hydrophilic funnel is then continued, through a series of conserved residues, all the way to the tip of Nqo12. Interacting charged and polar residues, surrounded by a ‘river’ of water molecules, form a charged entrance to the cavity.
The concerted conformational changes currently unclear, but they probably contribute at least to the coordination between the three antiporters. The concerted conformational changes first in the neighbouring antiporter Nqo14 likely has a prominent role in the cycle. The most plausible scenario is that a sealed Q chamber, an antipporter-like Nqo8 fold and a hydrophilic funnel connecting quinone site to the E-channel—combine to suggest that redox-driven conformational changes propagate to four proton channels through the unique central hydrophilic axis, aided by coupling elements. Mutations in any core complex I subunit, whether known to cause human disease or introduced in model studies, can now be understood on a structural basis: observed effects are consistent with the proposed mechanism (Supplementary Discussion, Supplementary Fig. 7 and Supplementary Tables 5 and 6). Further details of conformational changes and how exactly they lead to proton translocation will need clarification from structures of different redox states of the enzyme and time-resolved studies.

**METHODS SUMMARY**

Intact complex I from *T. thermophilus* was purified as described previously, except that the DEAE column was replaced by a Mono-S column. The membrane and hydrophilic domains were separated on a gel-filtration column after treatment of intact complex I with 300 mM sodium acetate, pH 4.0, 100 mM NaCl and 50 mM EDTA.

Crystals were grown using either hanging drop (membrane domain in n-undecyl-β-maltoside) or sitting drop (intact complex in n-tridecyl-β-maltoside) crystal-

Figure 5 | Proposed coupling mechanism of complex I. a, Overview showing key helices and residues. Upon electron transfer from cluster N2, negatively charged quinone initiates a cascade of conformational changes, propagating from the E-channel (Nqo8, Nqo10, Nqo11) to the antiporters through the central axis (red arrows) of charged and polar residues located around flexible breaks in key TM helices. Cluster-N2-driven shifts of Nqo4 and Nqo6 helices (blue arrows) probably assist overall conformational changes. Helix HL and the βH element help to coordinate conformational changes by linking discontinuous TM helices between the antiporters. In the antiporters, Lys TM7 from the first half-channel is assumed to be protonated (through the link to cytoplasm) in the oxidized state. Upon reduction of quinone and subsequent conformational change, the first half-channel closes to the cytoplasm, Glu TM5 moves out and Lys TM7 donates its proton to the connecting Lys/His TM8 and then onto Lys/Glu TM12 from the second half-channel. Lys/Glu TM12 ejects its proton into the periplasm upon return from reduced to oxidized state. A fourth proton per cycle is translocated in the E-channel in a similar manner. TM helices are numbered and key charged residues (Glu TM5, Lys TM7, Lys/ Gln TM12, Lys/His TM8 from Nqo12–14, Glu (Glu 67), Glu (Glu 32), Glu (Glu 213) and some residues from the connection to O cavity) are indicated by red circles for Glu and blue circles for Lys/His. 10(Tyr 59), interacting with 11(Glu 32), is indicated by the empty circle. E. coli and human subunit names are also indicated. b, Schematic drawing illustrating conformational changes between the two main (low-energy) conformations. Analysis of networks of polar residues and modelled waters in the structure suggests that in the oxidized state (as crystallized) periplasmic half-channels are likely to be open. Residues shown as black circles indicate conserved prolines from the break in TM12.

Figure 5 | Proposed coupling mechanism of complex I.
hydrophilic domain model (PDB code, 319V). Anomalous data from the Fe edge (using the intrinsic Fe–S clusters) allowed modelling of novel features at the interface of the domains. Both structures were refined to excellent statistics (Supplementary Table 1) in cycles of manual re-building and refinement in PHENIX with secondary structure/NCS restraints and using TLS.

**Full Methods** and any associated references are available in the online version of the paper.

**Received 4 September; accepted 21 December 2012.

Published online 17 February 2013.**

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METHODS

Protein purification. Intact complex I from T. thermophilus was purified as described previously, except that the DEAE column was replaced by a Mono S cation-exchange column. Fractions from the ANX column were diluted to ~5 mM NaCl with buffer A (20 mM Bis-Tris, pH 6.0, 0.002% phenylmethylsulfonyl fluoride (PMSF), 2 mM CaCl₂, 10% (v/v) glycerol and 0.05% n-tridecyl-β-maltoside (TDM)) and applied to a Mono S HR 16/10 column, equilibrated with buffer A. The protein was eluted with a linear gradient of buffer B (buffer A with 100 mM NaCl, 10% ethylene glycol and 0.03% UDM). Fractions containing the membrane domain were pooled on the basis of purity (assessed by SDS–PAGE), concentrated to about 1 ml using 100-kDa molecular-weight cut-off (MWCO) concentrators and each applied to a HiLoad 16/60 Superdex 200 gel-filtration column, equilibrated with GF buffer (buffer A with 100 mM NaCl). Fractions from each run were pooled separately, concentrated to about 25 mg ml⁻¹ and an additional 15% glycerol added for storage in liquid nitrogen. Only the protein containing Nqo16 was crystallizable.

The membrane and hydrophilic subunits were separated by treatment of intact complex I at pH 4.0. Purified complex I (1 mg) was incubated in 300 mM sodium acetate, pH 4.0, 100 mM NaCl, 50 mM EDTA buffer, 25% (v/v) ethylene glycol and 0.03% (w/v) n-undecyl-β-maltoside (UDM) for 3 h at ~22 °C. The protein solution was loaded into a HiLoad 16/60 Superdex 200 gel-filtration column equilibrated with buffer M (20 mM Bis-Tris, pH 6.0, 2 mM CaCl₂, 100 mM NaCl, 10% ethylene glycol and 0.03 UDM). Fractions containing the membrane domain were pooled on the basis of purity (assessed by SDS–PAGE), concentrated to about 10 mg ml⁻¹ using 100-kDa MWCO concentrators and used immediately for crystallization trials. In addition to membrane domain subunits, the preparation contained the hydrophilic subunits Nqo4, Nqo5, Nqo6 and Nqo9, which dissociated upon crystallization.

Nqo9, which dissociated upon crystallization.

The membrane domain of T. thermophilus complex I were grown at 23 °C using sitting drop crystallization. First, purified complex I (18 mg ml⁻¹ in 20 mM Bis-Tris, pH 6.0, 2 mM CaCl₂, 100 mM NaCl, 0.002% PMSF, 25% glycerol and about 2% TDM) was incubated with additional TDM detergent (final concentration of about 4% (w/v)) at ~22 °C, and then mixed at 2:1 (v/v) ratio with crystallization reagent comprising 100 mM Bis-Tris, pH 6.0, 19–24% (w/v) polyethylene glycol (PEG) 4000, 100 mM KCl, 100 mM glutaric acid, pH 6.0, and 2.2 mM fos-choline-8, fluorinated. The addition of extra TDM before crystallization trials improved the size of the rod crystals (to about 50 × 50 × 700–700 μm). Crystals used for the iron peak data collection were grown in similar conditions, except that the fos-choline-8 additive in the crystallization reagent was replaced by either 0.6% (v/v) n-dodecyl-β-maltoside or 7.6 mM 4-cyclohexyl-1-butyl-β-n-maltoside (CYMAL-4). Different detergent additives promoted crystallization growth to a variable extent but they did not affect diffraction properties. Crystals were fully grown within 1 week. They did not tolerate dehydration and were cryo-protected in 100 mM Bis-Tris, pH 6.0, 9% PEG 4000, 50 mM KCl, 50 mM glutaric acid, pH 6.0, 25% ethylene glycol and 0.01% TDM before plunging into liquid nitrogen.

Coot. Crystals of intact complex I with piericidin A were obtained by mixing protein and inhibitor at a 1:1 molar ratio before crystallization. The co-crystal was further soaked in 100 μM piericidin A (added from 10 mM stock in 100% dimethylsulphoxide) for approximately 4 h, before cryo-cooling. Decylubiquinone soaks were performed by soaking native complex I crystals overnight in solution containing 500 μM decylubiquione (added from 50 mM stock in 100% ethanol). Crystals were processed by using CrystalClear software and data processed as for native crystals. Molecular replacement with the intact complex structure in Phaser was followed by refinement in REFMAC for 40 cycles with Jelle body restraints (sigma 0.02). This resulted in a significant drop of Rwork, however, the models were not re-built manually and should be considered preliminary. A strong positive difference electron density (shown in green in Fig. 4a, b) identified the position of the aromatic ring of the compounds; they were modelled into the electron density, added to the initial structure and REFMAC refinement repeated, resulting in the models shown in Fig. 4a, b.

Bioinformatics. Structure-based multiple sequence alignment was performed in CLUSTALW v1.83 (ref. 56) with the profile alignment option. Water molecules were modelled using Dowser software. When applied to the E. coli membrane domain structure (PDB code, 3RKO), a similar ‘river’ of waters was predicted, with crystallographic waters, where observed, coinciding with those modelled. Good agreement was observed for Supplementary Table 1 and 2 when the ConSurf server, with sequences for alignment selected to be between 90% and 30% similar, resulting in about 300–400 sequences per subunit. Figures were prepared in PyMOL. Surface charges were calculated with APBS plug-in in PyMOL.
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