Supplementary Information for

Ion transfer mechanisms in Mrp-type antiporters from high resolution cryoEM and molecular dynamics simulations

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Supplementary Note

Coordination of internal water molecules by conserved polar and titratable residues

In MrpA, conserved polar and protonatable residues (Fig. 2, Supplementary Fig. 3, Supplementary Fig. 6, Supplementary Table 4) form a contiguous network extending from the putative proton entry site at Lys408MrpA/Glu409MrpA to His248MrpA in the A conformation. Water molecules W72 – W78 are arranged around the strictly conserved Lys408MrpA. The ligation of the water molecules indicates that Gln309MrpA and Tyr447MrpA and several serine and threonine residues play a crucial role, e.g. water molecule W72 is within hydrogen bonding distance of the highly conserved Ser308MrpA and Tyr447MrpA and moderately conserved Gln309MrpA and Thr444MrpA.

From His248MrpA a hydrophilic connection to the cytosolic side is formed by strictly conserved Tyr101MrpA, Ser244MrpA, Lys299MrpA and highly conserved residues Thr241MrpA and Asp297MrpA (Fig. 2). Tyr101MrpA and Thr241MrpA are within hydrogen bonding distance of W70 and W71. Ser244MrpA binds water W69. Note that residues corresponding to Ser244MrpA also bind a water molecule in respiratory complex I1,2.

His248 in the B conformation is associated with a network of polar residues that connects to the strictly conserved Glu140MrpA/Lys223MrpA pair of MrpA. Water W68 is coordinated by the strictly conserved residues Ser146MrpA, Thr170MrpA and non-conserved Ser147MrpA. The adjacent water molecules W66 and W67 are mainly bound by Glu140MrpA and the highly conserved residues Ser143MrpA and Thr222MrpA. Interestingly, the indole NH moiety of the strictly conserved Trp139MrpA coordinates both water molecules.

At the interface of MrpA and MrpD, water W65 is bound to Tyr136MrpA and the strictly conserved Lys392MrpD. In MrpD, we modelled six water molecules (W59 – W64) between Lys392MrpD and the highly conserved His332MrpD. The water molecules W59 – W64 are further coordinated by Gln307MrpD, highly conserved His303MrpD and several polar residues of which Tyr329MrpD is highly conserved. Following the hydrated region towards the core of MrpD, Lys250MrpD, His333MrpD and strictly conserved Lys337MrpD form an arrangement that is highly similar to the His349MrpA/Lys254MrpA/Lys353MrpA triad in MrpA described above. Interestingly, the antiporter from A. flavithermus shows a different set of residues in this region of the MrpD subunit. His303MrpD, Gln307MrpD and His333MrpD are replaced by Asn303MrpD, Ala307MrpD and Asp333MrpD, respectively. An extensive analysis of sequence alignments showed that the presence of a Gln or Asn at position 303 is strictly linked with the absence of a Gln at position 307 and the presence of an Asp at position 333 (Supplementary Fig. 7).
In contrast to MrpA, two water molecules (W56 and W57) are located in the center of MrpD, coordinated by the strictly conserved residues Lys250\textsuperscript{MrpD} and Tyr233\textsuperscript{MrpD} and highly conserved Thr249\textsuperscript{MrpD}. A pathway from the center of MrpD to the cytosol is expected but not obvious in the structure. We have proposed that in antiporter-like complex I subunits, conserved residues at the end of TMH10 mark the entrance of a proton channel that can be closed by a conserved phenylalanine residue in TMH11 \textsuperscript{2}. In Mrp, the corresponding residues at the putative channel entry, Asp295\textsuperscript{MrpD} and Lys297\textsuperscript{MrpD}, are also conserved and water molecule W58 is bound in close proximity. However, no other water molecules were found between W58 and water molecules in the central axis, which might indicate that the pathway is blocked by the strictly conserved Phe341 in TMH11 (Supplementary Fig. 3) as recently described for complex I \textsuperscript{2}. An overlay of ND2, ND4 and MrpD shows that the position of this residue agrees well with the “closed” conformation of the ND4 subunit in complex I (Supplementary Fig. 4).

A hydrated path runs from the center of MrpD to the neighboring MrpC subunit. Tyr233\textsuperscript{MrpD} bridges between the W56/W57 pair and a cluster of five water molecules (W51 – W55). This cluster is coordinated by Gln166\textsuperscript{MrpD} and the highly conserved Tyr162\textsuperscript{MrpD}. The latter residue engages in a hydrogen bond with the strictly conserved Ser143\textsuperscript{MrpD}. Water molecule W50 is bound between Lys219\textsuperscript{MrpD} and Glu137\textsuperscript{MrpD}, the strictly conserved Lys/Glu pair of MrpD, and is further ligated by Ser170\textsuperscript{MrpD}. We modelled Lys219\textsuperscript{MrpD} in two conformations, only one of which allows for a hydrogen bond to the water molecule.

In the neighboring MrpC subunit, TMH2 and 3 are at the center of a highly hydrated area. A cluster of water molecules W32 – W37 at the interface of MrpC and MrpD is coordinated by Glu137\textsuperscript{MrpD} and by moderately conserved Ser36\textsuperscript{MrpC}, His40\textsuperscript{MrpC} (see below), Ser80\textsuperscript{MrpC}, Thr84\textsuperscript{MrpC}. Towards the C-terminal domain of MrpA, a large water cluster (W21 – W31) is bound by polar residues of MrpC, His37\textsuperscript{MrpC} and His40\textsuperscript{MrpC}, as well as Thr690\textsuperscript{MrpA} and strictly conserved Gln683\textsuperscript{MrpA} and Glu687\textsuperscript{MrpA}. A polar residue at position 690 of MrpA is strictly conserved. Residues His37\textsuperscript{MrpC} and His40\textsuperscript{MrpC} in TMH2 were recently described as being critical for sodium binding \textsuperscript{3}. We note that neither residue is strictly conserved, but histidine or asparagine are the only residues allowed at position 40. A string of water molecules W16 – W20 connects TMH18 of MrpA with the highly conserved Asp38\textsuperscript{MrpF}. Residue Ser75\textsuperscript{MrpF} was modelled in two conformations one of which binds water W16. Two clusters of waters (W6 – W15) are located at the interface of MrpF and MrpG. The larger cluster is arranged around Asp38\textsuperscript{MrpF} and further binding interactions exist with the adjacent Thr39\textsuperscript{MrpG}, Ser68\textsuperscript{MrpF} and Thr40\textsuperscript{MrpG}, Thr44\textsuperscript{MrpG} and Thr79\textsuperscript{MrpG}. Polar residues at positions 40, 44 and 79 of MrpG are highly conserved. The smaller cluster is coordinated by residues from both subunits but none of them is conserved.
A previous study suggested that sodium entry from the cytoplasm occurs at the interface between MrpG and MrpE. We find water molecules W1 – W5 distributed around the proposed sodium entry site and several of the hydrogen-bonding residues including highly conserved His37MrpG, Thr116MrpE, and strictly conserved Thr113MrpE, His131MrpE. A sodium exit or proton entry site was proposed near the highly conserved residues Asp776MrpA and Glu780MrpA in TMH21 of MrpA. Water molecules W45 and W46 are close to the critical residues and are further coordinated by strictly conserved residues Thr777MrpA and Thr75MrpC. Water molecules W38 – W44 are found in a cavity that may form a pathway to the cytoplasm. They are coordinated by the strictly conserved residues Asp678MrpA, Asn766MrpA, and Asp121MrpB. Asn766MrpA was modelled in two different conformations oriented towards different waters in the hydrated path.

Protein hydration and residue-water hydrogen bonding analysis

Our additional analyses reveal that a change in protonation state of an amino acid changes the hydrogen bonding patterns with the surrounding water molecules. As expected, the charged state of a residue forms a larger number of hydrogen bonds with water molecules, whereas fewer hydrogen bonds are observed with charge neutral states (Supplementary Fig. 10). This is also observed by plotting the radial distribution function of water molecules around the residue, where its charged state shows a clear sharp peak relative to its neutral state (Supplementary Fig. 10). We find that the internal hydration of the protein stabilizes rapidly (see also 5) in both cases when the protein is simulated with structural waters or without them (Fig. 4a and Supplementary Fig. 9e). Our data also reveal that the internal hydration of the protein resembles the structural water content more when simulations are performed in the P state (Fig. 4a and Supplementary Fig. 9, panels a-d, see methods). In the S state simulations, additional water molecules diffuse into the protein interior because of the charged states of amino acid residues. Even though we observe close agreement between the structural waters and the water occupancy map from simulations, some non-overlapping areas are observed (Fig. 4a and Supplementary Fig. 9). These are likely due to the differences in time scales of simulations and experiments (see also 6).

In the simulated time scales, most of the structural water molecules were found to be replaced by bulk water molecules (Supplementary Fig. 9f). However, the water occupancy in internal regions of the protein remain in agreement with the structural water content, when visualized both at 20% (Fig. 4a) and 50% (Supplementary Fig. 9, panels a-d) occupancy level. Scrutinizing hydrogen bonding between water molecules and selected titratable residues, as well as their lifetimes allowed us to estimate residence times of water molecules in their vicinity (Supplementary Table 8). This also led to the classification of amino acid residues (see Supplementary Table 8). Titratable residues that face
bulk or near-bulk like situations do not show a difference in hydrogen bonding lifetimes when the protonation state changes. Interestingly, a putative sodium binding site acidic residue (Glu780\textsuperscript{MrpA}) consistently show nanosecond long residence times of water molecules either in its charge neutral or deprotonated state. This is because in its anionic state, it strongly binds a sodium ion, which replaces the role of its protonated sidechain. In addition, we find that the buried residues with conserved structural water next to them show relatively longer hydrogen bonding residence times of water molecules (Supplementary Table 8).
Supplementary Fig. 1.

Workflow of single-particle data processing for *B. pseudofirmus* Mrp. (a) Initial stages of data processing before particles were subjected to different processing strategies. (b) Dimer refinement. In the final round of 3D classification, three meaningful classes were observed, showing different angles between the protomers. The one with an intermediate angle was selected for final refinement, (marked by blue rectangle) and yielded the dimer map with the highest quality. The micelle density, lowpass filtered to 12 Å, is overlaid on the final map to visualize membrane boundaries. (c) Monomer refinement.
Supplementary Fig. 2.

Local resolution and examples of cryoEM densities. (a) Local resolution map of the *B. pseudofirmus* Mrp monomer. (b) Two alternative conformations of residues 246–252 in MrpA. Overlaid models from two different views are outlined. Separated models of two conformations are shown below. (c) Alternative sidechain conformations of K408 and E409 in MrpA. (d) Alternative sidechain conformations of E687 in MrpA. (e) Alternative sidechain conformations of F41 in MrpB. (f) Alternative sidechain conformations of K219 in MrpD. (g) All solvent densities that were modelled as water. Maps are resampled to 0.536 Å/pixel for visualization.
Supplementary Fig. 3.

Sequence alignment of orthologous subunits in Mrp antiporters and respiratory complex I.

Alignments include the Mrp antiporter subunits A-G and A’-G from the species *Bacillus pseudofirmus* (BpMrp), *Anoxybacillus flavithermus* (AfMrp), *Bacillus subtilis* (BsMrp), *Staphylococcus aureus* (SaMrp), *Halomonas* sp. (HsMrp), *Lysinibacillus sphaericus* (LsMrp), *Marinobacter hydrocarbonoclasticus* (MhMrp), *Dietzia* sp. (DsMrp), *Bartonella henselae* (BhhMrp), *Pseudomonas aeruginosa* (PaMrp) and *Agrobacterium tumefaciens* (AtMrp), as well as the complex I subunits ND2, ND4, ND5, ND6 and ND4L from the species *Paracoccus denitrificans* (PdND), *Yarrowia lipolytica* (YlND), *Brassica oleracea* (BoND), *Thermus thermophilus* (TtND) and *Ovis aries* (OaND).

The alignments were created using Clustalo 2 with default values in Jalview 2.11.1.4 and are color-coded in the ClustalX preset filtered by 50% conservation. Selected residues are marked above the alignments with their sequence position in BpMrp. The secondary structure elements of BpMrp subunits are indicated as blue (transmembrane helices: TM), red (helices outside the membrane: α) or green (β-sheets: β) boxes. (a) Alignment of subunits MrpA, MrpA’ and ND5. (b) Alignment of the subunits MrpA, MrpA’ and ND6. (c) Alignment of subunit MrpB. (d) Alignment of subunits MrpC and ND4L. (e) Alignment of subunits MrpD, ND2 and ND4. The MrpD sequences are differentiated between group 1 (separate MrpA and MrpB) and group 2 (MrpA’ fusion protein) operons. A 68 residue insert between Asp447 and Pro515 in Ds MrpD is not displayed (position highlighted in black). (f) Alignment of subunit MrpE. (g) Alignment of subunit MrpF. (h) Alignment of subunit MrpG.
Supplementary Fig. 4.

Structural features of the Mrp antiporter conserved in complex I. Overlay of complex I (Y. lipolytica, PDB 7O71, yellow) and Mrp antiporter subunits (color as in Fig. 1), (a) MrpA/ND5, (b) MrpD/ND4, (c) MrpA/ND6, (d) MrpD/ND2, (e) MrpC/ND4L. (f) The position of a highly conserved phenylalanine residue opens or closes a proton pathway in subunits ND2 (green, open) and ND4 (cyan, closed); the position of the corresponding residue Phe341 in MrpD (magenta) matches the closed conformation. (g) In complex I structures, residues corresponding to His248\textsuperscript{MrpA} (dark blue, compare Fig. 2) are either in the A (e.g. T. thermophilus PDB 6I1P (yellow), Y. lipolytica PDB 7O71 (magenta)) or B conformation (e.g. human PDB 5XTD (cyan), sheep PDB 6ZKA (green)); residues corresponding to Ser146\textsuperscript{MrpA}, Ser244\textsuperscript{MrpA}, and Thr306\textsuperscript{MrpA} (compare Fig. 2) are strictly conserved in complex I and form connections to three pathways for protons.
Supplementary Fig. 5.

Mrp antiporter from *B. pseudofirmus* compared with Mrp antiporters from *A. flavithermus* and *Dietzia* sp. (a) Side view and (b) top view of an overlay of Mrp antiporters from *B. pseudofirmus* (magenta) and *A. flavithermus* (yellow, PDB 6Z16); note that both structures show one protomer of a dimeric complex. (c) Side view and (d) top view of an overlay of Mrp antiporters from *B. pseudofirmus* (magenta) and *Dietzia* sp. (green, PDB 7D3U); note that the Mrp antiporter from *Dietzia* sp. was isolated as a monomeric complex and that MrpA and MrpB are fused.
Supplementary Fig. 6.
Water molecules and ligating residues in the B. pseudofirmus Mrp antiporter. (a) A total of 360 water molecules (red spheres) were modelled. More than 70 water molecules define putative ion translocation pathways in the complex interior (compare Fig. 2). (b) Detailed view including polar residues not shown in Fig. 2. (c) Glu409\textsuperscript{MrpA} interacts with strictly conserved His528\textsuperscript{MrpA} and engages in a hydrogen bonding network at the periplasmic proton entry site; in a second conformation it points towards Thr537\textsuperscript{MrpA} and the water chain leading to His248\textsuperscript{MrpA}. Lys408\textsuperscript{MrpA} changes between a position pointing towards highly conserved Ser407\textsuperscript{MrpA} and His345\textsuperscript{MrpA} and a position where it binds two water molecules. (d) Lys219\textsuperscript{MrpD} either orients towards waters in the center of MrpD or to a water molecule that is liganded by Ser170\textsuperscript{MrpD} and Glu137\textsuperscript{MrpD} of the MrpD K/E pair.
Supplementary Fig. 7.

Co-evolution of residues in MrpD of Group 1 Mrp antiporter operons. (a) Close-up view of the region around the strictly conserved Lys250 in TMH8 of MrpD of *B. pseudofirmus* (BpMrp) (compare Fig. 2). (b) Superposition of (a) and (c). (c) Close-up view on the region around the strictly conserved Lys250 in TMH8 of MrpD of *Anoxybacillus flavithermus* (AfMrp; PDB: 6Z16). (d) Consensus of 1200 sequences of Mrp group 1 operons for the residues shown in (b). Two clear subgroups can be identified. The Bp-Type (present in about 1/3 of the sequences) has His in position 2, Ser in 3, Gln in 4 and His or Asn in 5. The Af-Type (present in about 2/3 of the sequences) expresses Asn or Gln in position 2, varying residues in 3, small residues in 4 and Asp in 5.
Supplementary Fig. 8.

**MD simulation model systems and lipid bilayer arrangement.** (a) Model system of Mrp antiporter (cartoon representation) embedded in a hybrid lipid membrane (white and gray spheres) and solvent (turquoise surface representation, Na\(^+\) and Cl\(^-\) ions omitted for clarity). POPE lipids are shown as white and POPG as gray spheres. Protein is colored as in Figure 1. (b) RMSD of protein CA atoms in SA1 (black) and SB1 (pink) simulations. (c) RMSD of protein CA atoms in PA1 (black) and PB1 (pink) simulations. (d) Simulation snapshot reveals bending in the lipid membrane occurs closer to the periplasmic surface of the putative proton-uptake site. Protein is shown in ribbons representation colored as in Figure 1. Phosphorus atoms of lipids surrounding the protein are shown as yellow spheres. Lipid fatty acid side chains are omitted for clarity. (e) Membrane thickness observed from simulation replicas PB1 (combined 3 µs data) using VMD MEMBPLUGIN tool\(^{10}\). The thinner membrane regions are marked with white dashed circles. The protein is shown in a top view from the cytoplasmic side. The phosphorus atoms of the structural lipids are shown in olive green.
Supplementary Fig. 9.

Comparison of internal hydration volume maps at 20 and 50 % occupancies. (a) Using the same criterion as in Figure 4 (waters within 6 Å of residues displayed), the volume map of the internal hydration is shown from all PB1 simulation replicas with dark gray mesh at 50 % isovalue. Internal structural waters are shown in red spheres. (b) Comparison of the volume map in panel A (50%, left panel) and Figure 4a (20 %, right panel) at the interface of subunits MrpA and MrpD. (c) Same comparison as panel (b) but at the central axis region. (d) Same comparison as panel (b) but at the Glu780 region. (e) Number of internal water molecules in simulation states where structural water molecules were removed i.e. dry run. S refers to standard protonation state and P to propka based protonation state. The blue line indicates the number of structural internal water molecules. (f) Water molecules in PB1 simulation at 1000 ns. Red spheres show the internal structural waters and gray spheres the water molecules that have diffused into the interior from the bulk aqueous phase. A comparison of the hydration pattern to panel (a) and Figure 4a shows that the majority of the original internal structural waters exchange with waters from the bulk.
Supplementary Fig. 10.

Hydrogen bonds and radial distribution function of water molecules around selected residues in different protonation states. The histograms display the occupancy of hydrogen bond between a residue and water molecules in different protonation states (PA1, PB1, SA1 and SB1, refers to data from setups PA1, PB1, SA1 and SB1, respectively, see also Supplementary Table 6). The hydrogen bonding criteria used are Donor-Acceptor distance 3.5 Å and Donor-Hydrogen-Acceptor angle 30 degrees. The radial distribution function $g(r)$ is calculated based on distance between sidechain nitrogen/oxygen atom and oxygen of water molecules. Overall, the trend is that the neutralization of amino acid sidechain destabilizes hydrogen bonding with water molecules.
Supplementary Fig. 11.

His248^{MrpA} dynamics and mutation. (a) Variation in sidechain of neutral His248^{MrpA} (with δ nitrogen protonated) in S state simulations where all amino acids are in their standard states. The scatter plot shows distances between NE2 atom of His248^{MrpA} and OG atoms of Ser146/Thr306^{MrpA} from SA (pink dots) or SB (black dots) simulations. Solid yellow and orange spheres indicate the structural distances seen in alternative A and B conformations. Note that in the S state simulations, conformations of His248^{MrpA} are not populated (no overlap with solid orange and yellow circles), whereas they are clearly observed in P state simulations, where protonation states of sidechains are determined by pKa calculations (Fig. 5a). (b) Complementation of E. coli strain KNabc without intrinsic antiport activity (red curve) with an expression vector carrying the wild-type Mrp operon of B. pseudofirmus sustains salt tolerant growth (green curve) in contrast to complementation with the His248^{MrpA} mutant (blue curve); each data point represents the averaged measurements of at least three biological replicates with the standard deviation given as error bar. (c) SDS-PAGE (n=2) of purified His248^{MrpA} mutant (left) and wild-type BpMrp (middle) and molecular mass standard (right). Source data for panel (b) and (c) are provided as a Source Data file.
Supplementary Fig. 12.

**Phe41^{MrpB} dynamics and sodium gating.** (a) Dihedral angle (C-CA-CB-CD) of Phe41^{MrpB} from SA1 (3 x black) and SB1 (3 x pink) simulations. (b) Snapshot at 200 ns from SA1 simulation. Carbon atoms in the MrpB subunit are green; in MrpA they are lilac. In the simulations, two sodium atoms bind to residues in their charged residues spontaneously. Phe41^{MrpB} adopts the A conformation regardless of the initial structure as is also seen in panel (a). (c) Dihedral angle (C-CA-CB-CD) of Phe41^{MrpB} from PA1 (3 x black) and PB1 (3 x pink) simulations. (d) Snapshot at 200 ns from one PB1 simulation. Colors as in (b). Since Glu780^{MrpA} and Asp121^{MrpB} are uncharged in the PB1 simulation, no sodium ions interact with them, allowing Phe41 to remain in the B conformation.
**Supplementary Table 1. Cryo-EM data and model statistics**

*B. pseudofirmus Mrp*

### Data collection

|                          |                  |
|--------------------------|------------------|
| Microscope               | Titan Krios 2    |
| Camera                   | K3               |
| Magnification            | 105,000          |
| Voltage (kV)             | 300              |
| Energy filter slit width (eV) | 20               |
| Electron flux rate (e⁻/pixel/sec) | 15.0          |
| Number of frames         | 45               |
| Electron exposure (e⁻/Å²) | 50.0             |
| Defocus range (μm)       | −0.8 to −2.0     |
| Calibrated pixel size (Å) | 0.837            |
| Number of movies         | 7,332            |

### Data processing

|                          | Monomer | Dimer  |
|--------------------------|---------|--------|
| Final particle images (no.) | 513,743 | 96,337 |
| Final pixel size (Å)      | 1.07136 | 1.07136|
| Symmetry imposed          | C1      | C2     |
| Map resolution (Å)        | 2.24    | 2.96   |
| Half map FSC = 0.143      |         |        |
| Map sharpening B factor (Å²) | −16.7  | −34.5  |
| Local resolution range (Å) | 2.2–2.8 |        |

### Refinement

|                          |                  |
|--------------------------|------------------|
| Initial model (PDB codes) | 6Z16             |
| Refinement resolution (Å) | 2.20             |
| Model resolution (Å)      |                  |
| Map-model FSC = 0.5       | 2.24             |
| Model composition         |                  |
| Non-hydrogen atoms        | 16,100            |
| Protein residues          | 1,957             |
| Water                     | 360               |
| Other ligands             | 3 (POPE)          |
| Average B factors (Å²)    |                  |
| Protein                   | 50.1              |
| Water                     | 54.6              |
| Other ligands             | 112.6             |
| R.m.s. deviation          |                  |
| Bond lengths (Å)          | 0.019             |
| Bond angles (°)           | 2.312             |

### Validation

|                  |                  |
|------------------|------------------|
| MolProbity score | 1.79             |
| Clashscore       | 6.52             |
| Rotamer outliers (%) | 2.50             |
| CB outliers (%)  | 0.49             |
| CaBLAM outliers (%) | 0.83             |
| Ramachandran plot |                |
| Favored (%)      | 97.37            |
| Allowed (%)      | 2.58             |
| Outliers (%)     | 0.05             |
Supplementary Table 2. Identifier for water molecules in the hydrophobic transmembrane region.

| water | water in PDB file | water | water in PDB file |
|-------|------------------|-------|------------------|
|       | Chain ID | Residue Nr. |       | Chain ID | Residue Nr. |
| 1     | G       | 204       | 40    | B       | 213       |
| 2     | G       | 201       | 41    | B       | 201       |
| 3     | E       | 309       | 42    | A       | 1016      |
| 4     | G       | 208       | 43    | B       | 218       |
| 5     | E       | 307       | 44    | B       | 209       |
| 6     | G       | 210       | 45    | A       | 1055      |
| 7     | F       | 115       | 46    | C       | 220       |
| 8     | F       | 108       | 47    | A       | 1123      |
| 9     | G       | 202       | 48    | D       | 601       |
| 10    | F       | 104       | 49    | D       | 576       |
| 11    | G       | 207       | 50    | D       | 594       |
| 12    | G       | 214       | 51    | D       | 593       |
| 13    | G       | 212       | 52    | D       | 554       |
| 14    | F       | 111       | 53    | D       | 604       |
| 15    | F       | 105       | 54    | D       | 527       |
| 16    | F       | 110       | 55    | D       | 512       |
| 17    | F       | 112       | 56    | D       | 550       |
| 18    | F       | 119       | 57    | D       | 502       |
| 19    | A       | 1135      | 58    | D       | 552       |
| 20    | A       | 1108      | 59    | D       | 546       |
| 21    | A       | 1018      | 60    | D       | 605       |
| 22    | A       | 1044      | 61    | D       | 511       |
| 23    | C       | 231       | 62    | D       | 578       |
| 24    | C       | 235       | 63    | D       | 609       |
| 25    | C       | 203       | 64    | D       | 508       |
| 26    | A       | 1121      | 65    | A       | 1061      |
| 27    | C       | 213       | 66    | A       | 1063      |
| 28    | C       | 233       | 67    | A       | 1088      |
| 29    | C       | 208       | 68    | A       | 1039      |
| 30    | C       | 224       | 69    | A       | 1004      |
| 31    | C       | 228       | 70    | A       | 1117      |
| 32    | C       | 212       | 71    | A       | 1096      |
| 33    | D       | 537       | 72    | A       | 1012      |
| 34    | D       | 563       | 73    | A       | 1007      |
| 35    | C       | 229       | 74    | A       | 1086      |
| 36    | C       | 216       | 75    | A       | 1109      |
| 37    | D       | 597       | 76    | A       | 1110      |
| 38    | A       | 1009      | 77    | A       | 1136      |
| 39    | B       | 217       | 78    | A       | 1122      |
Supplementary Table 3. Orthologous subunits in Mrp antiporters, MBH, MBS and respiratory complex I

| Mrp                  | MBH                      | MBS  | Complex I (mitochondrial) | Complex I (bacterial) |
|----------------------|--------------------------|------|---------------------------|-----------------------|
| MrpA (TM 1-14)       | -                        | MbsH' | ND5                       | Nqo12                 |
| MrpA (TM 15,16 + lateral helix) | MbhI (C-terminal) |       |                           |                       |
| MrpA (TM 17-19)      | MbhD                     | MbsD | ND6                       | Nqo10                 |
| MrpA (TM 20-21)      | MbhE                     | MbsE |                           |                       |
| MrpB                 | MbhF                     |      |                           |                       |
| MrpC                 | MbhG                     | MbsG | ND4L                      | Nqo11                 |
| MrpD                 | MbhH                     | MbsH | ND2 / ND4                 | Nqo13 / Nqo14         |
| MrpE                 | MbhA                     | MbsA |                           |                       |
| MrpF                 | MbhB                     | MbsB |                           |                       |
| MrpG                 | MbhC                     | MbsC |                           |                       |
Supplementary Table 4. Conservation of residues in putative ion translocation pathways

| Mrp-Subunit | Residue | Conservation in Mrp | Conservation in Complex I (Substitutions) | Mutants with no or minor impact on activity | Mutants with negative impact on activity |
|-------------|---------|---------------------|-------------------------------------------|---------------------------------------------|------------------------------------------|
|             |         |                     | ND2 | ND4 |                    |                                         |                                         |
| MrpD        | Glu137  | strictly            |     |     | E137D<sup>2,5</sup> |                                         | E137A<sup>2,5,6</sup>, E137Q<sup>2,5</sup> |
| MrpD        | Met139  | moderately (Met or Leu) | No | No |                    |                                         |                                         |
| MrpD        | Ser143  | strictly            | No  | No  |                    |                                         |                                         |
| MrpD        | Tyr162  | highly              | No  | No  |                    |                                         |                                         |
| MrpD        | Glu166  | moderately          | No  | No (strictly Thr) |                    |                                         |                                         |
| MrpD        | Ser170  | moderately          | moderately | highly |                    |                                         |                                         |
| MrpD        | Leu172  | No                  | No  | No  |                    |                                         |                                         |
| MrpD        | Met218  | No                  | No (moderately Val or Leu) | No (moderately Phe or Met) |                    |                                         |                                         |
| MrpD        | Lys219  | strictly            | strictly | strictly |                    |                                         | K219A;<sup>2</sup> K220A<sup>2</sup> |
| MrpD        | Tyr233  | strictly            | moderately | No (highly His) |                    |                                         |                                         |
| MrpD        | Thr249  | highly              | No  | No (highly Leu) |                    |                                         |                                         |
| MrpD        | Lys250  | strictly            | strictly | strictly |                    |                                         | K251A<sup>3</sup>                       |
| MrpD        | Asp295  | No                  | No | No |                    |                                         |                                         |
| MrpD        | Lys297  | moderately (Lys or Arg) | highly | strictly |                    |                                         |                                         |
| MrpD        | His303  | highly* (Asn or His) | No (highly Ser) | No (highly Ser) |                    |                                         |                                         |
| MrpD        | Ser306  | moderately* (Ser or No) | No | moderately (Ala or Ser) |                    |                                         |                                         |
| MrpD        | Gln307  | moderately* (Gln or small) | No (highly His or Asn) | No (highly His) |                    |                                         |                                         |
| MrpD        | Tyr328  | moderately (Phe or Tyr) | No | No |                    |                                         |                                         |
| MrpD        | Tyr329  | highly              | moderately | highly |                    |                                         |                                         |
| MrpD        | His332  | highly              | No | No |                    |                                         |                                         |
| MrpD        | His333  | highly* (Asp or His) | No (highly Tyr) | highly |                    |                                         |                                         |
| MrpD        | Lys337  | highly* | No (moderately Asn or Thr) | No (moderately Ser or Thr) |                    |                                         |                                         |
| MrpD        | Phe341  | highly (Phe or Tyr) | highly | highly |                    |                                         | F341A<sup>2</sup>                       |
| MrpD        | Lys392  | strictly            | strictly | No (strictly Glu) |                    |                                         | K392A<sup>3</sup>                       |
| MrpD        | Phe393  | No                  | No | No |                    |                                         |                                         |
| MrpD        | Met423  | No                  | No (moderately Ile, Val or Leu) | No (moderately Leu or Ile) |                    |                                         |                                         |
| Mrp-Subunit | Residue<sup>a</sup> | Conservation in Mrp<sup>b</sup> | Conservation in Complex I<sup>c</sup> (Substitutions) | Mutants with no or minor impact on activity<sup>c</sup> | Mutants with negative impact on activity<sup>c</sup> |
|-------------|---------------------|-----------------------------|---------------------------------|-----------------------------|---------------------------------|
| MrpA        | Tyr101              | strictly                     | highly                          | -                           | -                               |
| MrpA        | Met118              | No                           | No                              | -                           | -                               |
| MrpA        | Phe119              | strictly                     | highly                          | -                           | -                               |
| MrpA        | Tyr136              | No                           | No (highly Phe)                 | Y136A<sup>4</sup>          | E113Q<sup>6</sup>; E132A<sup>9</sup>; E140A<sup>2</sup> |
| MrpA        | Trp139              | strictly                     | strictly                        | -                           | -                               |
| MrpA        | Glu140              | strictly                     | strictly                        | E113Q<sup>6</sup>          | -                               |
| MrpA        | Ser143              | highly                       | No (highly Gly)                 | -                           | -                               |
| MrpA        | Ser146              | strictly                     | highly                          | -                           | -                               |
| MrpA        | Ser147              | No                           | No                              | -                           | -                               |
| MrpA        | Met167              | No                           | No                              | -                           | -                               |
| MrpA        | Thr170              | strictly                     | No (strictly Asn)               | -                           | -                               |
| MrpA        | Thr222              | highly                       | No (highly Gly)                 | -                           | -                               |
| MrpA        | Lys223              | strictly                     | strictly                        | K196A<sup>6</sup>          | K223A<sup>2</sup>; E213A<sup>9</sup> |
| MrpA        | Pro240              | highly                       | strictly                        | -                           | -                               |
| MrpA        | Thr241              | highly                       | strictly                        | -                           | -                               |
| MrpA        | Pro242              | strictly                     | strictly                        | -                           | -                               |
| MrpA        | Val243              | highly                       | highly                          | -                           | -                               |
| MrpA        | Ser244              | strictly                     | strictly                        | -                           | -                               |
| MrpA        | Ala245              | highly                       | highly                          | -                           | -                               |
| MrpA        | Tyr246              | highly                       | No (highly Leu)                 | -                           | -                               |
| MrpA        | Leu247              | strictly                     | moderately                      | -                           | -                               |
| MrpA        | His248              | strictly                     | strictly                        | H221A<sup>6</sup>          | H248A<sup>10</sup>             |
| MrpA        | Ser249              | highly                       | No (highly Ala)                 | -                           | -                               |
| MrpA        | Ala250              | strictly                     | moderately                      | -                           | ΔA240<sup>9</sup>              |
| MrpA        | Thr251              | highly                       | highly                          | T224A<sup>6</sup>          | -                               |
| MrpA        | Met252              | highly                       | highly                          | M225I<sup>6</sup>          | -                               |
| MrpA        | Lys254              | strictly                     | No (moderately Thr)             | K244A<sup>9</sup>          | -                               |
| MrpA        | Asp297              | highly                       | strictly                        | -                           | -                               |
| MrpA        | Lys299              | strictly                     | strictly                        | K299A<sup>4</sup>          | -                               |
| MrpA        | Thr306              | strictly                     | strictly                        | -                           | -                               |
| MrpA        | Ser308              | highly                       | strictly                        | -                           | -                               |
| MrpA        | Gln309              | moderately                   | high (Gln or His)               | -                           | -                               |
| MrpA        | His345              | highly                       | strictly                        | H345A<sup>2</sup>          | -                               |
| MrpA        | Leu346              | moderately                   | moderately                      | (Leu or Ile)               | (Leu or Val)                    |
| MrpA        | His349              | strictly                     | strictly                        | -                           | -                               |
| MrpA        | Lys353              | strictly                     | strictly                        | K329A<sup>9</sup>          | -                               |
| MrpA        | Ser407              | highly                       | highly                          | -                           | -                               |
| MrpA        | Lys408              | strictly                     | strictly                        | -                           | K384A<sup>9</sup>              |
| MrpA        | Glu409              | strictly                     | strictly                        | -                           | E385A<sup>9</sup>              |
| MrpA        | Thr413              | No                           | No                              | -                           | -                               |
| MrpA        | Thr444              | moderately                   | highly                          | -                           | -                               |
| MrpA        | Tyr447              | highly                       | strictly                        | -                           | -                               |
| MrpA        | Tyr525              | No                           | No                              | -                           | -                               |
| Mrp-Subunit | Residue | Conservation in Mrp \(^b\) | Conservation in Complex I \(^b\) (Substitutions) | Mutants with no or minor impact on activity \(^c\) | Mutants with negative impact on activity \(^c\) |
|-------------|---------|----------------|---------------------------------|---------------------------------|---------------------------------|
| MrpA        | His528  | highly         | No                             | -                               | -                               |
| MrpA        | Asn531  | No             | No                             | -                               | -                               |
| MrpA        | Glu533  | No             | No                             | -                               | -                               |
| MrpA        | Thr537  | moderately     | No (moderately (Thr or Ser) Lys or Glu) | -                               | -                               |
| MrpA        | Ile650  | No             | No                             | -                               | -                               |
| MrpA        | Ala656  | No             | No                             | -                               | -                               |
| MrpA        | Val657  | No             | No                             | -                               | -                               |
| MrpA        | Val660  | No             | No                             | -                               | -                               |
| MrpA        | Asp678  | strictly       | No                             | -                               | -                               |
| MrpA        | Thr682  | strictly       | No                             | -                               | -                               |
| MrpA        | Gln683  | strictly       | No                             | -                               | -                               |
| MrpA        | Val686  | moderately     | No                             | -                               | -                               |
| MrpA        | Glu687  | strictly       | No                             | -                               | -                               |
| MrpA        | Thr688  | No             | No                             | -                               | -                               |
| MrpA        | Thr690  | moderately     | No                             | -                               | -                               |
| MrpA        | Val691  | No             | No                             | -                               | -                               |
| MrpA        | Leu694  | No             | No                             | -                               | -                               |
| MrpA        | Leu704  | No             | No                             | -                               | -                               |
| MrpA        | Glu707  | No             | No                             | -                               | -                               |
| MrpA        | Asn766  | strictly       | No                             | -                               | -                               |
| MrpA        | Asp771  | highly         | No                             | -                               | -                               |
| MrpA        | Asp776  | strictly       | No                             | -                               | -                               |
| MrpA        | Thr777  | strictly       | No                             | -                               | -                               |
| MrpA        | Glu780  | strictly       | No                             | -                               | -                               |
| MrpB        | His34   | strictly       | -                             | H34A\(^2\)                      | -                               |
| MrpB        | Pro37   | strictly       | -                             | -                               | P37G\(^2\)                     |
| MrpB        | Gly38   | strictly       | -                             | -                               | -                               |
| MrpB        | Gly39   | strictly       | -                             | -                               | -                               |
| MrpB        | Gly40   | strictly       | -                             | -                               | -                               |
| MrpB        | Phe41   | strictly       | -                             | -                               | F41A\(^2\)                     |
| MrpB        | Glu111  | No             | No                             | -                               | -                               |
| MrpB        | Glu113  | No             | No                             | -                               | -                               |
| MrpB        | Asp121  | highly         | -                             | D121E\(^5\)                    | D121A\(^5\); D121N\(^5\)       |
| MrpC        | Leu26   | No             | No                             | -                               | -                               |
| MrpC        | Arg27   | moderately     | No                             | -                               | -                               |
| MrpC        | Val30   | No             | No                             | -                               | -                               |
| MrpC        | Gly31   | highly         | No                             | -                               | -                               |
| MrpC        | Ser36   | moderately     | No                             | -                               | -                               |
| MrpC        | His37   | moderately     | No                             | -                               | -                               |
| MrpC        | His40   | moderately     | (His, Tyr or Asn) No           | -                               | -                               |
| Mrp-Subunit | Residue^a | Conservation in Mrp | Conservation in Complex I | Mutants with no or minor impact on activity | Mutants with negative impact on activity |
|-------------|-----------|---------------------|--------------------------|--------------------------------|----------------------------------|
| MrpC        | Leu74     | strictly            | No                       | -                              | T75A^2                           |
| MrpC        | Thr75     | strictly            | No (highly Ala)          | -                              | I76F^7                           |
| MrpC        | Ala76     | moderately (Ala or Ser) | moderately               | -                              |                                  |
| MrpC        | Ile77     | strictly            | No                       | -                              |                                  |
| MrpC        | Val78     | strictly            | No (highly Glu)          | -                              |                                  |
| MrpC        | Ile79     | strictly            | No                       | -                              |                                  |
| MrpC        | Ser80     | No                  | No                       | -                              |                                  |
| MrpC        | Thr84     | moderately          | No                       | -                              |                                  |
| MrpC        | Ser85     | No                  | No                       | -                              |                                  |
| MrpC        | Thr113    | highly              | -                        | -                              | T113A^1,2; T113Y^2               |
| MrpC        | Thr116    | moderately (Thr or Ser) | -                        | -                              |                                  |
| MrpC        | Met119    | No                  | -                        | -                              |                                  |
| MrpC        | Thr116    | moderately (Thr or Ser) | -                        | -                              |                                  |
| MrpC        | His131    | strictly            | -                        | H131A^1                        |                                  |
| MrpF        | Met13     | No                  | -                        | -                              |                                  |
| MrpF        | Ile34     | Moderately (Val or Ile) | -                        | -                              |                                  |
| MrpF        | Asp38     | highly              | -                        | D36A/F40D^9; D38A^5; D36A^9; D38N^3; D36A/I33D^9; D35L^3; D36L^9; D36N^3; D38E^5 |
| MrpF        | Thr39     | No                  | -                        | -                              |                                  |
| MrpF        | Asn43     | No                  | -                        | -                              |                                  |
| MrpF        | Ser68     | No                  | -                        | -                              |                                  |
| MrpF        | Ser75     | Moderately (Thr or Ser) | -                        | -                              |                                  |
| MrpG        | Ser19     | No                  | -                        | -                              |                                  |
| MrpG        | Ser23     | No                  | -                        | -                              |                                  |
| MrpG        | Ser23     | No                  | -                        | -                              |                                  |
| MrpG        | His37     | Moderately          | -                        | -                              |                                  |
| MrpG        | Thr40     | moderately (Thr or Ser) | -                        | -                              |                                  |
| MrpG        | Thr44     | moderately (Thr or Ser) | -                        | -                              |                                  |
| MrpG        | Phe75     | highly              | -                        | -                              |                                  |
| MrpG        | Thr79     | highly              | -                        | -                              |                                  |

^a Residues that show multiple conformations in our structure are underlined.

^b The conservation is given in four categories based on alignments of 1200-2000 non-redundant sequences: strict (>99.5%); high (>90%); moderate (>80% or >90% in case residues with similar properties are conserved at this position) or not conserved (deviating from BpMrp).

^c Mutations are given in short form with the sequence numbering from the original source and the source given in superscript: 1 ref 11; 2 ref 12; 3 ref 13; 4 ref 14; 5 ref 15; 6 ref 16; 7 ref 17; 8 ref 18; 9 ref 19; 10 this work

* Refers to group 1 operons (separate MrpA and MrpB) only. See Supplementary Fig. 7
### Supplementary Table 5. Occupancies of alternative $A$ and $B$ conformations

| chain | residue type | residue number | alt$_A$ | alt$_B$ |
|-------|--------------|----------------|---------|---------|
| A     | MET          | 118            | 0.45    | 0.55    |
| A     | PHE          | 119            | 0.45    | 0.55    |
| A     | MET          | 167            | 0.45    | 0.55    |
| A     | TYR          | 246            | 0.50    | 0.50    |
| A     | LEU          | 247            | 0.43    | 0.57    |
| A     | HIS          | 248            | 0.43    | 0.57    |
| A     | SER          | 249            | 0.43    | 0.57    |
| A     | ALA          | 250            | 0.43    | 0.57    |
| A     | THR          | 251            | 0.43    | 0.57    |
| A     | MET          | 252            | 0.43    | 0.57    |
| A     | LEU          | 346            | 0.43    | 0.57    |
| A     | LYS          | 408            | 0.67    | 0.33    |
| A     | GLU          | 409            | 0.69    | 0.31    |
| A     | LEU          | 681            | 0.69    | 0.31    |
| A     | GLU          | 687            | 0.58    | 0.42    |
| A     | ASN          | 766            | 0.48    | 0.52    |
| B     | PHE          | 41             | 0.64    | 0.36    |
| D     | MET          | 139            | 0.47    | 0.53    |
| D     | LEU          | 172            | 0.47    | 0.53    |
| D     | MET          | 218            | 0.43    | 0.57    |
| D     | LYS          | 219            | 0.45    | 0.55    |
| D     | GLN          | 307            | 0.45    | 0.55    |
| D     | TYR          | 328            | 0.69    | 0.31    |
| D     | MET          | 423            | 0.54    | 0.46    |
| E     | MET          | 119            | 0.54    | 0.46    |
| F     | MET          | 13             | 0.58    | 0.42    |
| F     | ILE          | 34             | 0.76    | 0.24    |
| F     | SER          | 75             | 0.42    | 0.58    |
| G     | PHE          | 75             | 0.52    | 0.48    |
## Supplementary Table 6. Model systems and simulation time scales.

| System | Conformation | Charge state | Simulation length |
|--------|--------------|--------------|-------------------|
| SA1    | A            | Standard     | 3 x 1000 ns       |
| SB1    | B            | Standard     | 3 x 1000 ns       |
| PA1    | A            | Propka-based | 3 x 1000 ns       |
| PB1    | B            | Propka-based | 3 x 1000 ns       |
| PBE    | B            | Propka-based except H248<sup>MrpA</sup> HSE* | 3 x 500 ns |
| PB2    | B            | Propka-based except H248<sup>MrpA</sup> HSP** | 3 x 500 ns |
| SMA1   | A            | Standard, H248A | 500 ns          |
| SMB1   | B            | Standard, H248A | 500 ns          |
| PMA1   | A            | Propka, H248A | 500 ns          |
| PMB1   | B            | Propka, H248A | 500 ns          |
| SNA1   | A            | Standard, sodium ion modelled near anionic D38<sup>MrpF</sup> | 800 ns        |
| SNA2   | A            | Standard, snapshot from SNA1, but D38<sup>MrpF</sup> neutral | 160 ns         |
| SNA3   | A            | Standard, snapshot from SNA2, but E687<sup>MrpA</sup> neutral | 800 ns         |
| SNA4   | A            | Standard, snapshot from SNA1 but sodium ion modelled in the hydrated path towards E687<sup>MrpA</sup> | 100 ns, 13 ns  |
| SNA5   | A            | Standard, snapshot from SNA1, except E687<sup>MrpA</sup>, D771<sup>MrpA</sup>, D678<sup>MrpA</sup>, D121<sup>MrpB</sup>, E113<sup>MrpB</sup> and D38<sup>MrpF</sup> neutral. Sodium ion modelled in between E687<sup>MrpA</sup> and H37<sup>MrpC</sup>. | 110 ns         |
| SNA6   | A            | Standard, snapshot from SNA5 but H37<sup>MrpC</sup> and H40<sup>MrpC</sup> doubly-protonated. | 280 ns, 110 ns |
| SNA7   | A            | Snapshot from SNA6, but E137<sup>MrpD</sup> neutral. | 2 x 110 ns     |

# Alternative location A and B, as defined in PDB file.

* HSE means neutral histidine with ε nitrogen protonated

** HSP means that histidine is doubly-protonated
Supplementary Table 7. Protonation states of amino acids in A and B conformations based on pKa calculations (see methods)

| Subunit | Residue | Conformation A | Conformation B |
|---------|---------|----------------|----------------|
|         | pKa     | Charge state   | pKa            | Charge state   |
| MrpA    | Asp678  | 7.24           | 0              | 7.36           | 0              |
| MrpA    | Asp771  | 7.65           | 0              | 7.65           | 0              |
| MrpA    | Glu409  | 7.20           | 0              | 6.79           | -1             |
| MrpA    | Glu687  | 8.42           | 0              | 7.75           | 0              |
| MrpA    | Glu780  | 8.54           | 0              | 9.52           | 0              |
| MrpA    | His470  | 7.17           | +1             | 7.17           | +1             |
| MrpA    | Lys223  | 6.94           | 0              | 6.65           | 0              |
| MrpA    | Lys254  | 5.85           | 0              | 5.90           | 0              |
| MrpA    | Lys299  | 6.86           | 0              | 6.84           | 0              |
| MrpA    | Lys353  | 6.40           | 0              | 6.43           | 0              |
| MrpA    | Lys408  | 7.94           | +1             | 6.37           | 0              |
| MrpB    | Asp121  | 7.34           | 0              | 7.35           | 0              |
| MrpD    | Lys250  | 6.31           | 0              | 6.31           | 0              |
| MrpD    | Lys337  | 5.61           | 0              | 5.61           | 0              |
| MrpF    | Asp38   | 6.70           | -1             | 7.03           | 0              |
**Supplementary Table 8. Residence times of water molecules around key titratable residues based on hydrogen bond dynamics and lifetimes.** Blue – refers to those bulk-facing residues around which the residence of water molecules does not differ much with respect to changes in conformational/protonation states. Green – change in protonation state of amino acid residue changes the residence times of water molecules around it. Dark orange – putative sodium binding site, where no major differences in residence times of water molecules are observed either when sodium ion binds or when residue is protonated. NA – residence time not available because autocorrelation did not approach to zero in the given simulation time scales or higher standard deviation of the tail (see ref. 20). >ns – residence time is expected to be much longer than nanoseconds or tens of nanoseconds (lower bound value given). This may occur due to stable hydrogen bonding between structural water molecule and amino acid (red color). R1-R3 refer to three simulation replicas. The hydrogen bonding criteria used in this analysis is as follows: donor-acceptor distance 3.5 Å and donor-hydrogen-acceptor angle 35°, and forward hydrogen bonding lifetimes are reported using GROMACS software. ps – picoseconds; q – charge of the sidechain; S – standard and P – Propka-based charged state (see methods); A and B are alternative conformations.
| Protein Chain | Residue id | Residue type | S, A       | P, A        | S, B       | P, B        |
|--------------|------------|--------------|------------|-------------|------------|-------------|
| A            | 771        | ASP          | q          | -1          | 0          | -1          | 0           | 408.642 ps | 392.166 ps | 183.7 ps   |
| A            | 299        | LYS          | q          | +1          | 0          | +1          | 0           | 389.926 ps | 632.899 ps | 304.9 ps   |
| B            | 121        | ASP          | q          | -1          | 0          | -1          | 0           | 775.316 ps | 819.972 ps | 647.4 ps   |
| A            | 470        | HIS          | q          | 0           | +1         | 0           | +1          | 35.948 ps  | 82.866 ps  | 34.69 ps   |
| A            | 409        | GLU          | q          | -1          | 0          | -1          | 0           | 235.188 ps | 338.500 ps | 272.5 ps   |
| A            | 408        | LYS          | q          | +1          | +1         | +1          | 0           | NA (>ns)   | NA (>ns)   | NA (>ns)   |
| A            | 780        | GLU          | q          | -1          | 0          | -1          | 0           | 1.331 ns   | 1.655 ns   | 1.49 ns    |
| A            | 223        | LYS          | q          | +1          | 0          | +1          | 0           | NA (>ns)   | NA (>ns)   | NA (>ns)   |
| A            | 254        | LYS          | q          | +1          | 0          | +1          | 0           | NA (>ns)   | NA (>ns)   | NA (>ns)   |
| A            | 678        | ASP          | q          | -1          | 0          | -1          | 0           | NA (>ns)   | NA (>ns)   | NA (>ns)   |
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