Outward open conformation of a Major Facilitator Superfamily multidrug/H\(^+\) antiporter provides insights into switching mechanism

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Multidrug resistance (MDR) poses a major challenge to medicine. A principle cause of MDR is through active efflux by MDR transporters situated in the bacterial membrane. Here we present the crystal structure of the major facilitator superfamily (MFS) drug/H\(^+\) antiporter MdfA from *Escherichia coli* in an outward open conformation. Comparison with the inward facing (drug binding) state shows that, in addition to the expected change in relative orientations of the N- and C-terminal lobes of the antiporter, the conformation of TM5 is kinked and twisted. In vitro reconstitution experiments demonstrate the importance of selected residues for transport and molecular dynamics simulations are used to gain insights into antiporter switching. With the availability of structures of alternative conformational states, we anticipate that MdfA will serve as a model system for understanding drug efflux in MFS MDR antiporters.
Eflux transport of antibiotics and other potentially harmful compounds from the bacterial cytoplasm by multidrug resistance (MDR) transporters represents an increasing challenge for the treatment of pathogenic bacterial infection\(^1\text{-}^3\). A large number of MDR transporters belong to the Major Facilitator Superfamily (MFS), found in both Gram-positive and -negative organisms\(^1\text{-}^2\). Typical MFS transporters possess 12 transmembrane helices (TMs) divided into two pseudo-symmetrical 6TM N- and C-terminal lobes. Changes in relative orientation of the two lobes within the plane of the bilayer (the rocker-switch mechanism\(^4\)) allow alternating access to the cytoplasmic and extracellular/periplasmic sides of the membrane, facilitating directed transport of substrates across the membrane, with the transporter cycling between outward open (O\(_o\)), inward open (I\(_o\)) and intermediary occluded states\(^5\text{-}^7\). Despite progress in structural determinations of these states for uniporter and symporter MFS transporters, few such data are available for antiporters.

MdfA, an MFS-MDR transporter from *E. coli* with homologs in many pathogenic bacteria, is an extensively characterized drug/H\(^+\) antiporter\(^8\). It transports lipophilic, cationic, and neutral substrates, in each case driven by the proton motive force\(^9\text{-}^10\). Two acidic residues within TM1, Glu\(_{26}\)TM1 and Asp\(_{34}\)TM1, have been implicated in proton (H\(^+\)) and substrate transport coupling\(^11\text{-}^13\). Asp\(_{77}\)TM2 (from conserved motif A) is in addition part of an electrostatic cluster involving Arg\(_{81}\)TM3 and Glu\(_{132}\)TM5, with an adjacent cluster including Arg\(_{78}\)TM2 and residues of the intermediate loop (Arg\(_{198}\)TM6) and helix (Asp\(_{211}\)TM6\textsuperscript{14-16}). In the ligand bound I\(_f\) state, in which the two lobes rotate largely as rigid bodies by 33.5° about an axis parallel to the plane of the membrane bilayer, these multiple interactions are replaced by predominantly hydrophobic contacts between the periplasmic halves of TMs 1, 2, and 5 of the N-terminal domain and TMs 7, 8, and 11 of the C-terminal domain. This is effected by a sliding of TM11 along TM2 and a significant rearrangement of TM5 (see below). The drug binding pocket observed in the I\(_f\) state is disrupted in the O\(_o\) state through displacement of Ala\(_{150}\)TM5 and Leu\(_{151}\)TM5 (see below) as well as lateral movement of C-terminal domain residues from TMs 7 and 8 (Supplementary Fig. 1).

**Results**

**Overall structure of MdfA in the outward open (O\(_o\)) state.** The crystal structure of Fab-bound MdfA presented here reveals the transporter in the outward open (O\(_o\)) state, with the N- and C-lobes approaching each other closely at the intracellular face of the transporter (Fig. 1). The N-terminus of TM5 juxtaposes the C-termini of TM8 and TM10 and the N-terminus of TM11 nests between the C-termini of TM2 and TM4. Access to the transporter cavity from the cytoplasmic face is sealed off by formation of a hydrophobic plug through intercalation of side-chains from each of these helices centered around Phe\(_{340}\)TM10 (Fig. 2). These contacts are supported by mutually favorable interactions between the side chain of Arg\(_{336}\)TM10 and the C-terminal dipole of TM4, and Asp\(_{77}\)TM2 and the N-terminal dipole of TM11. Asp\(_{77}\)TM2 (from conserved motif A) is in addition part of an electrostatic cluster involving Arg\(_{81}\)TM3 and Glu\(_{132}\)TM5, with an adjacent cluster including Arg\(_{78}\)TM2 and residues of the intermediate loop (Arg\(_{198}\)TM6) and helix (Asp\(_{211}\)TM6\textsuperscript{14-16}).

In order to gain a complete picture of the efflux mechanism, however, structural data for alternative states are required. Here we report the crystal structure of MdfA in the O\(_o\) state and identify conformational changes that accompany transitions between the I\(_f\) and O\(_o\) states. With the availability of structures of alternative conformational states, we anticipate that MdfA will serve as a model system for understanding drug efflux in MFS MDR antiporters.
found in TM5, which ends in the antiporter motif C153AlaProXaaXaaGlyPro158 that is absent in symporters and uniporters. Whereas TM5 in the I$_f$ structure adopts an $\alpha$-helical conformation of almost ideal geometry up to motif C, residues 136 to 153 in the O$_o$ structure exhibit a profound 15° kink, accompanied by a ca. 45° clockwise twist parallel to the helix axis that terminates with the two-proline-containing motif C (Fig. 3; Supplementary Fig. 3; Supplementary Movie). This results in a repositioning of the hydrophobic side chains Ile142$^{TM5}$, Leu145$^{TM5}$, Met146$^{TM5}$, and Val149$^{TM5}$ with respect to the N-terminal domain core. Leu145$^{TM5}$, which in the I$_f$ conformation associates with the N-terminal domain, engages instead with residues of the C-terminal domain in the O$_o$ state. The carboxamide of conserved Asn148$^{TM5}$ is removed from a (presumably hydrophobic) membrane exposed location in the I$_f$ state to form hydrogen bonds with the side chain of Asn272$^{TM8}$ and the main

**Fig. 2** Cytoplasmic and periplasmic faces of MdfA in the outward open conformation. **a** The cytoplasmic entrance to the ligand-binding pocket is closed in the O$_o$ conformation by numerous interactions between the N- and C-lobes (view obtained by rotating Fig. 1 90° about a horizontal x-axis). The N-terminus of TM5 juxtaposes the C-termini of TM8 and TM10, and the N-terminus of TM11 nestles between the C-termini of TM2 and TM4. Hydrophobic sidechains from each of these helices pack against each other to form a hydrophobic plug that seals off access to the transporter cavity from the cytoplasmic face, supported by additional mutually favorable electrostatic interactions. **b** View from the periplasmic face (following a 180° rotation about a horizontal x-axis), demonstrating the deep cavity between the two domains in the outward open conformation. Dotted line denotes approximate boundary delineated by the bacterial membrane outer leaflet head groups.
whose hydroxyl moiety is ca. 2.5 Å from the side chain carboxylate group of Glu26TM1.

C

initial (orange) models. See also Supplementary Fig. 3 and Supplementary Movie

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tions19, whereas transport proved unaffected by mutation of

of a hydrophobic cluster near the periplasmic face of MdfA that

chains of residues Val43TM1, Tyr47TM1, and Trp53TM2 form part

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Rearrangements in the N-terminal domain hydrophobic core.

Also of note are small yet significant changes in the region Leu41TM1–Val54TM2, which runs from the C-terminus of TM1 and the N-terminus of TM2 (Supplementary Fig. 4). The side chains of residues Val43TM1, Tyr47TM1, and Trp53TM2 form part of a hydrophobic cluster near the periplasmic face of MdfA that includes Met40TM1, Ile105TM4, and Phe108TM4 from TM4, and Trp170TM6 and Phe174TM6 at the N-terminus of TM6. This cluster, which juxtaposes the buried guanidinium moiety of Arg112TM4 that is absolutely conserved among MFS homologs (motif B), exhibits a small but significant expansion in the present structure compared to that in the If conformation. While the structural differences may appear to be small, we note that the transporter MdfA is stabilized by ligand binding14,16, so that the transporter in the unbound If state could well differ from the ligand bound MdfA If structures presented by Heng et al.14,17.

In order to test the effect of amino acid substitutions on chloramphenicol transport experimentally, MdfA and its variants were reconstituted in proteoliposomes following procedures described for the chloroquine resistance transporter from Plasmodium falciparum18 (Fig. 4). Purified reconstituted wild-type MdfA was able to transport 50 pmol chloramphenicol (per mg protein per minute), which compares favorably with the 3 pmol mg⁻¹ min⁻¹ determined using crude membrane preparations19, whereas transport proved unaffected by mutation of Glu26TM1 to Gln, suggesting that the charge state of this residue is not crucial for chloramphenicol transport. The variants Tyr127TM4Phe, Met146TM5Ala, and Trp170TM6Ala of the hydrophobic core all showed significant reductions in transport in the presence of a pH gradient. As expected, chloramphenicol transport was low in the absence of ApH, arising from downhill transport due to the initial infinite substrate gradient.

Molecular dynamics simulations. To gain further insights into the transport cycle, molecular dynamics (MD) simulations were performed with all possible combinations of Glu26TM1 and Asp34TM4 protonation states, starting from either the Oo structure without Fab or the If structure without chloramphenicol. Initial trials with the present structure assuming both acidic residues to be deprotonated [Oo(E26p/D34p)] and Oo(E26p/D34p)

Fig. 3 The Oo and If conformations differ by local twisting of TM5. a In the Oo state, TM5 (green) in the N-terminal domain is partially distorted, resulting in Cα displacements compared to the If state of up to 2.9 Å (Met146TM5). The side chain of Met146TM5 rests against the phenolic side chain of Tyr127TM4, whose hydroxyl moiety is ca. 2.5 Å from the side chain carboxylate group of Glu26TM1. b TM5 adopts an almost ideal α-helical conformation in the If state through displacement of the Tyr127TM4 side chain by that of Met146TM5. TM5 straightens, rotating around its axis such that its hydrophobic side chains can engage/disengage the C-terminal domain. c Electron density for TM5 in the Oo conformation, superimposed with coordinates of the final (green) and initial (orange) models. See also Supplementary Fig. 3 and Supplementary Movie.
Fig. 4 Chloramphenicol transport by MdfA reconstituted in proteoliposomes. a Chloramphenicol transport into reconstituted proteoliposomes is dependent upon the presence of MdfA and a pH gradient. b Time course for uptake using reconstituted MdfA. In the absence of a pH gradient (open circles), downhill-like transport (with the substrate gradient) occurs rapidly due to the small volume of the proteoliposomes. In the presence of a pH gradient, however, chloramphenicol uptake (filled circles) involves at least three phases: following a rapid initial downhill transport phase (not visible), uphill accumulation of the substrate in the liposomal lumen against the concentration gradient takes place at the expense of proton export (II). Within a few minutes, the situation is reversed due to lumen acidification, leading to chloramphenicol efflux (phase III). Crucially, collapse of the pH gradient through administration of the H⁺-ionophore CCCP (open squares) results in rapid chloramphenicol efflux (downhill transport) until the luminal concentration reaches that observed in the absence of a pH gradient. c, d Schematic diagram illustrating the phases of chloramphenicol (CLM) uptake in the reconstituted system. e Uptake by proteoliposomes containing purified MdfA variants in the presence (closed bars) and absence (open bars) of a pH gradient at 1 min. Data are mean values ± s.d., n = 3.

Discussion

The structure of MdfA presented here reveals features of the Oo conformation and allows comparison with the previously determined ligand bound I₇ state. Going from the I₇ to the Oo state, the N- and C-terminal domains of the transporter reorient in the membrane largely as rigid bodies, with the exception of three regions: (i) transmembrane helix TM5 kinks and twists, (ii) the periplasm-proximal hydrophobic core of the N-terminal domain reorganizes, and (iii) a cytoplasmic loop of the C-terminal domain rearranges to accommodate closure of the cytoplasmic entrance (see Supplementary Movie). The twisting of the helix in the Oo conformation appears to be prevented from transiting to a straight-form as seen in the I₇ state by juxtaposition of the
Tyr127\textsuperscript{TM4} and Met146\textsuperscript{TM5} side chains, with the aromatic side chain hydroxyl of Tyr127\textsuperscript{TM4} held in place by a hydrogen bond to Glu26\textsuperscript{TM1}. Our reconstitution experiments demonstrate the importance of Tyr127\textsuperscript{TM4} and Met146\textsuperscript{TM5} for transport, and suggest that the charge state of Glu26\textsuperscript{TM1} is of little significance for chloramphenicol transport in the presence of a pH gradient, which is consistent with previous results\textsuperscript{19}. It should be noted that, strictly speaking, the conclusions presented here apply only for chloramphenicol transport (for which structural data of the If form are available); whereas we expect them to be generally valid for other neutral MdfA substrates, the situation may differ for other substrates.

To gain further insights into the transport process, we performed MD simulations involving different protonation states of the two acidic residues identified previously as being important in vivo studies\textsuperscript{11,16,21}, Asp34\textsuperscript{TM1} and Glu26\textsuperscript{TM1}. Within the timescale of our simulations, protonation of Asp34\textsuperscript{TM1} through exposure to the low pH periplasmic space leads to an occluded state in which the acidic side chain becomes enclosed in an internal cavity that recapitulates its environment in the I\textsubscript{r} conformation. TM5 continues to be twisted in this occluded state and the Glu26\textsuperscript{TM1}–Tyr127\textsuperscript{TM4} hydrogen bond remains intact, although the charge state of Glu26\textsuperscript{TM1} does not appear to play a role in this. Nevertheless, previous in vivo studies have shown that Glu26\textsuperscript{TM1} is critical for the transport of cationic substrates\textsuperscript{11,16}, so that the situation may be different for cationic and lipophilic substrates, in which the initial I\textsubscript{r} state assumed here might not apply.

MD simulations also demonstrate that an occluded state and a twisted TM5 conformation can be obtained starting from the I\textsubscript{r} state. The fact that the transporter is stabilized by ligand binding\textsuperscript{14,16} however means that the I\textsubscript{r} structures presented by Heng et al.\textsuperscript{14,16} might not provide an accurate representation of MdfA in the unbound I\textsubscript{r} state (moreover, these ligand-bound structures were obtained using a mutated variant Gln131\textsuperscript{TM4}Arg, which has recently been reported to be transport inactive\textsuperscript{22,23}). Thus TM5 untwisting might occur on going from the occluded to the I\textsubscript{r} state, or upon ligand binding to form the I\textsubscript{r} state.

Recent structure determinations of other transporters\textsuperscript{5–7} indicate that individual helices within each domain can exhibit significant variability upon conformation switching. For MdfA, the existence of structurally underdetermined \( I_r \) and intermediate occluded states (whereby ligand bound and unbound occluded states are likely to be different) thus precludes a detailed description of the complete transport cycle. Nevertheless, the combination of data presented here suggests an important role for the interaction between Glu26\textsuperscript{TM1} and Tyr127\textsuperscript{TM4}. We note that through their common location on helix TM4, the orientation of the Tyr127\textsuperscript{TM4} side chain could couple with the environment of the motif B Arg112\textsuperscript{TM4} side chain. The buried guanidinium moiety is involved in an elaborate hydrogen bonding network involving the even more buried Gln115\textsuperscript{TM4}, the carbonyl carbon of Gly32\textsuperscript{TM1}, and (via a solvent molecule identified in the high-resolution structure\textsuperscript{14}) Asn33\textsuperscript{TM1} and Asp34\textsuperscript{TM1}. Residues Arg112\textsuperscript{TM4}, Asp34\textsuperscript{TM1}, Gln115\textsuperscript{TM4}, and Gly32\textsuperscript{TM1} have all been shown to be important for MdfA action\textsuperscript{11,14,16,19,24}, and a role of the surrounding hydrophobic cluster is confirmed by the deleterious effect on chloramphenicol transport of the Trp170\textsuperscript{TM6}Ala mutation. Changes in the chemical environment of Asp34\textsuperscript{TM1} (e.g. by ligand binding or changes in protonation) could therefore lead to the observed reorganization of the hydrophobic cluster immediately adjacent to Arg112\textsuperscript{TM4}. In turn, communication of this change through TM4 could influence the orientation of the Tyr127\textsuperscript{TM4} side chain, dictating the position of that of Met146\textsuperscript{TM5} and thereby the degree of TM5 twist. Releasing the twist of TM5 from the O\textsubscript{o} to the I\textsubscript{r} conformation would result in a repositioning of the hydrophobic side chains Ile142\textsuperscript{TM5}, Leu145\textsuperscript{TM5}, Met146\textsuperscript{TM5}, and Val149\textsuperscript{TM5} with respect to the N-terminal domain core, allowing Leu145\textsuperscript{TM5} to dissociate from the N-terminal domain to engage the C-terminal domain.

Support is provided by MdfA rescue mutants. Selection for drug transport rescue in cells harboring the otherwise inactive TM1 variants Glu26\textsuperscript{TM1}Thr/Asp34\textsuperscript{TM1}Met and Glu26\textsuperscript{TM1}Thr resulted in the detection of mutants containing the acidic side chains Ala150\textsuperscript{TM5}Glu and Val335\textsuperscript{TM10}Glu\textsuperscript{11,25}. These residues would be well positioned to make hydrogen bonds to Tyr127\textsuperscript{TM4} in the outward open structure (Supplementary Fig. 8). Recent thermodynamic calculations and molecular dynamic simulations have led in principle to similar conclusions for the L-fucose/H\textsuperscript{+} symporter FucP\textsuperscript{28}. Using computational methods, it was
proposed that protonation of FucP Glu135 in TM4 allows surmounting of a ca. 2 kcal mol⁻¹ energy barrier between the inward and outward open states. An intermediate state in which TM11 is distorted is postulated, although a causative link between Glu135 in TM4 (de)protonation and TM11 distortion has not been described. Inspection of the FucP structure suggests that Glu135 could form a hydrogen bond with Tyr365 of C-terminal domain TM10 (Supplementary Fig. 8). Interestingly, C-terminal domain TM11 is the counterpart of TM5 in the (inverted topology) N-terminal domain, reflecting the pseudo-symmetry of the two domains, so that the antiporter MdfA and symporter FucP might be thought of as examples of repeat swapping to yield similar transport mechanisms.

The presence of the MFS-antiporter motif C would appear to be central to transporter switching—restricting the twist of TM5 to a small localized helical segment to facilitate relative rotation of the two domains, and transmitting these perturbations to an adjacent pliable hydrophobic cluster. As other structurally well-characterized antiporter families (such as the amino acid/polyamine/or ganocation (APC) transporter superfamilies and cation/H⁺ antiporter family) have been shown to utilize other mechanisms, this may be a property specific to MFS-antiporters. The O₆ structure presented here could serve as a template for the design of novel MFS inhibitors that are able to access their target directly from the bacterial exterior.

**Methods**

**Crystal structure solution.** Isolation of Fab fragments that recognize native conformations of MdfA in proteoliposomes has been described elsewhere. Crystals of MdfA in complex with Fab fragments grew in the lipidic cubic phase and diffracted to 3.4 Å resolution. Diffraction data were collected at 100 K at a wavelength of 1.0 Å on the SLS beamline PXI (X06SA) using the 16M Eiger detector. In order to resolve the very long c-axis (929 Å), the crystal was mounted so that the rotation axis was ca. 30° to the crystallographic c axis. The synchrotron beam (with beam size increased from 10 to 100 μm) was defocused from the crystal to the detector. Data sets (180° in 0.1° steps) were processed using the program XDS. The crystal belongs to the space group P6₁22 with one complex in the asymmetric unit. Phases were determined by molecular replacement with Phaser MR using the separated N- and C-lobes of MdfA in the inward open conformation (PDBID: 4ZP0) and an Fab fragment (PDBID:1BGI) as individual search models. The replacement model was rebuilt manually using COOT and refined using PHENIX with TLS refinement (three groups per polyepptide chain) to an Rmerge value of 28.3%. The final model consists of MdfA residues Glu14–Lys400, Fab heavy chain residues Leu4–Pro216, and Fab light chain residues Asp1–Arg211, as well as one sulfate ion. Ramachandran analysis demonstrates that 94.4% of the residues are in the favorable regions and 5.5% in the allowed regions. One residue (Ser65 in the Fab fragment heavy chain) was in the outlier region. Atoms for difference density correction were not added to the model in accordance with the low resolution of the data. The Fab binds to the cytoplasmic side of MdfA, where it may stabilize the outward open state and enhances crystal contacts. Data collection and refinement statistics are given in Table 1. RMSD Ca position differences between O₆ and I₆ states (Supplementary Fig. 2) were calculated as a function of residue number after separate superposition of the N- and C-terminal domains using the program LSQKAB from the CCP4 suite. The kink of a-helix TM5 was calculated using Kink Finder. Figures and movies were prepared using PyMOL (Schrodinger, LLC).

**Reconstitution of MdfA.** MdfA mutants were generated using a PCR site-directed mutagenesis kit (Agilent) with the primers listed in the Supplementary Table 2, and purified as for wild-type MdfA. Forty micrograms of purified wild type or mutant MdfA was mixed with 500 μg of azolexins liposomes (Sigma type II), frozen at 193 K for at least 10 min. The mixture was thawed quickly by holding the sample tube in the hand and dilated 60-fold with reconstitution buffer containing 20 mM MES-NaOH (pH 6.0), 0.1 M sodium chloride, 5 mM magnesium chloride. Reconstituted proteoliposomes were pelletted by centrifugation at 200,000 × g for 1 h, and suspended in 0.2 ml of same buffer.

**Transport assay.** The transport assay mixture (0.2 ml) containing 20 mM MOPS-Tris (pH 7.5) or 20 mM MES-NaOH (pH 6.0), 0.1 M sodium chloride, 5 mM magnesium chloride, and 2 μM [ring-3,5,2-H] chloramphenicol (0.5 MBq μmol⁻¹, PerkinElmer) was incubated at 300 K for 3 h. Proteoliposomes containing MdfA (0.5 μg protein per assay) (or liposomes as control) were added to the mixture to initiate transport, and incubated for a further 1 min. Aliquots (130 μl) were taken and centrifuged through a Sephacry G-50 (fine) spin column at 760 × g for 2 min. Radioactivity in the eluate was counted using a liquid scintillation counter (PerkinElmer). As a control, the ionophore carbonyl cyanide m-chlorophenyl hydrazone (CCCP) was added to the assay (final concentration 1 μM) after 1 min to reduce the pH gradient. Bovine serum albumin was used as a protein concentration standard.

**MD simulations.** Initial coordinates of MdfA in the O₆ conformation were taken from those of the crystal structure of the MdfA–Fab complex. N- and C-terminal MdfA residues were capped with acetyl and N-methyl groups, respectively. All histidine residues were protonated on the N6 atom. All acidic residues (excluding Glu26 and Asp34) and all basic residues were deprotonated and protonated, respectively (see Supplementary Table 3). Glu129 was protonated in the initial structure of the O₆/E26D/D34p MD run and Asp34 was protonated for the O₆/E26D/D34p and O₆/E26D/D34p simulation runs. After filling the large cavity on the periplasmic side of the protein with water molecules to prevent placement of lipid molecules there, the structure was solvated with water and ions using CHARMM-GUI. The orientation of MdfA relative to the lipid bilayer was determined analogously to that of MdfA in the I₆ state (PDB ID: 4ZP0) as deposited in the Orientations of Proteins in Membranes (OPM) database by alignment of the Ca atoms of residues 203–400. The rectangular simulation system generated by CHARMM-GUI (90.76 Å × 90.76 Å × 90.98 Å) was subjected to periodic boundary conditions. The system was composed of one protein, 223 1-palmitoyl-2-oleoyl-sn-phosphatidylethanolamine (POPE), 40 or 41 K lipids, and/or detergents were not added to the model in accordance with the low resolution of the data. The Fab binds to the cytoplasmic side of MdfA, where it may stabilize the outward open state and enhances crystal contacts. Data collection and refinement statistics are given in Table 1. RMSD Ca position differences between O₆ and I₆ states (Supplementary Fig. 2) were calculated as a function of residue number after separate superposition of the N- and C-terminal domains using the program LSQKAB from the CCP4 suite. The kink of a-helix TM5 was calculated using Kink Finder. Figures and movies were prepared using PyMOL (Schrodinger, LLC).
of the O(2)E(26)/(D34P) and O(2)E(26)/(D34P) runs calculated for the last 2.7 ps trajectory of the O(2)E(26)/(D34P) MD run were 1.24 ± 0.14 and 1.21 ± 0.08 Å, respectively. Corresponding values calculated for the last 0.5 ps trajectory of the I(2)E(26)/(D34P) MD run were 1.48 ± 0.16 and 1.23 ± 0.26 Å, respectively. Thus the two simulations converged to similar states.

Data availability

Data supporting the findings of this manuscript are available from the corresponding authors upon reasonable request. Coordinates of the MdfA Fab complex have been deposited in the Protein Data Bank under the accession number 6GV1 [https://doi.org/10.2210/pdb6GV1/pdb].
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Author contributions

K.N., F.J. and M.T. purified and crystallized MdfA and collected diffraction data. FabYN1074 was produced by Y.N.-N., K.L., Y.H. and N.N. using the antibody production platform for membrane proteins developed by S.I. and N.N. The structure was solved by K.N. and C.P. and analyzed together with M.T.S. and M.T. Reconstitution experiments were performed by N.J., T.M., H.O. and M.T. MD simulations were carried out by T.T. The project was conceived and designed by M.T. and supervised together with M.T.S. All authors participated in analysis and discussion of the results and contributed to the preparation of the manuscript.

Additional information

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