

Antiparallel EmrE exports drugs by exchanging between asymmetric structures

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Small multidrug resistance transporters provide an ideal system to study the minimal requirements for active transport. EmrE is one such transporter in *Escherichia coli*. It exports a broad class of polyaromatic cation substrates, thus conferring resistance to drug compounds matching this chemical description. However, a great deal of controversy has surrounded the topology of the EmrE homodimer. Here we show that asymmetric antiparallel EmrE exchanges between inward- and outward-facing states that are identical except that they have opposite orientation in the membrane. We quantitatively measure the global conformational exchange between these two states for substrate-bound EmrE in bicelles using solution NMR dynamics experiments. Förster resonance energy transfer reveals that the monomers within each dimer are antiparallel, and paramagnetic relaxation enhancement NMR experiments demonstrate differential water accessibility of the two monomers within each dimer. Our experiments reveal a ‘dynamic symmetry’ that reconciles the asymmetric EmrE structure with the functional symmetry of residues in the active site.

EmrE is a secondary active antiporter, driving uphill transport of each polyaromatic cation substrate against its concentration gradient by coupling it to downhill import of two protons across the inner membrane. As one of the smallest known active transporters, with only 110 amino acids and four transmembrane helices per monomer, EmrE would seem to be an ideal model system^{1–3}. There is broad agreement that the minimal functional unit is a dimer^{4–7}, but its structure and topology remain controversial^{3,8–13}, perhaps because the membrane topology and oligomeric state are exquisitely sensitive to sequence alteration and environment^{14–17}.

EmrE is proposed to function through a single-site alternating access model^{18–20}, as shown in Fig. 1a. In this well-established model^{21,22}, transporters are inherently dynamic proteins, converting between inward- and outward-facing conformations to move substrates across a membrane barrier. To achieve coupled antiport, both substrates share a single binding site and conformational exchange only occurs when substrate (two protons or one polyaromatic cation) is bound. Thus, saturating EmrE with its polyaromatic cation substrate, tetraphenylphosphonium (TPP⁺), should drive EmrE into a two-state equilibrium (Fig. 1a, b) suitable for direct observation and quantitative analysis of conformational exchange with site-specific resolution by solution NMR spectroscopy.

To take advantage of well-developed high-resolution solution NMR dynamics methods, we solubilized EmrE in isotropic bicelles, which surround the protein with a more native-like lipid environment than detergents while preserving the fast tumbling needed for solution NMR^{23–25}. Several features of the ¹H-¹⁵N transverse relaxation-optimized spectroscopy (TROSY) spectrum of TPP⁺-bound ²H/¹⁵N-EmrE in isotropic bicelles are worth noting (Fig. 1c). First, the chemical shift dispersion is much greater than expected for a helical bundle membrane protein. This dispersion is probably enhanced by ring currents from the bound polyaromatic substrate

and the many aromatic residues in the binding site. Second, there are twice as many peaks (approximately 210 resolved peaks) as expected for a monomer of EmrE (105 non-proline residues). A solid-state NMR study of EmrE specifically labelled at Glu 14 (ref. 26) also noted such a peak doubling for this active-site residue in the TPP⁺-bound state.

There are two possible explanations for this peak doubling: (1) an asymmetric dimer, as observed by cryo-electron microscopy and X-ray crystallography^{4,7,27}, where each monomer has a distinct structure and thus different chemical shifts or (2) exchange between two symmetric (parallel) homodimers that is slow on the NMR timescale. Naively, we might expect four sets of peaks in the case of both structural asymmetry and slow conformational exchange (Supplementary Fig. 1). However, as has been previously noted, there is a pseudotwofold symmetry axis in EmrE, perpendicular to the membrane normal, such that reorientation of the helices in monomers A and B could effectively convert the structure of monomer A into that of monomer B and vice versa¹⁸ (Fig. 1b). This unique model results in inward- and outward-facing states that are identical except for their orientation. Such a mechanism is only possible in an antiparallel homodimer, and no previous experimental data have directly tested this hypothesis.

EmrE is functional in isotropic bicelles

We first verified that EmrE is properly folded and functional in bicelles, because it has not been studied in this environment previously. To prepare bicelles, we purified EmrE in detergent, reconstituted it into liposomes and then added DHPC to form bicelles (see Methods). Extensive studies have confirmed that EmrE is a properly folded, functional dimer in dodecylmaltoside (DDM), and when reconstituted into liposomes^{4,6,13,28–31}. The TROSY NMR spectra (Fig. 1c) of TPP⁺-bound EmrE in dilauroylphosphatidylcholine/

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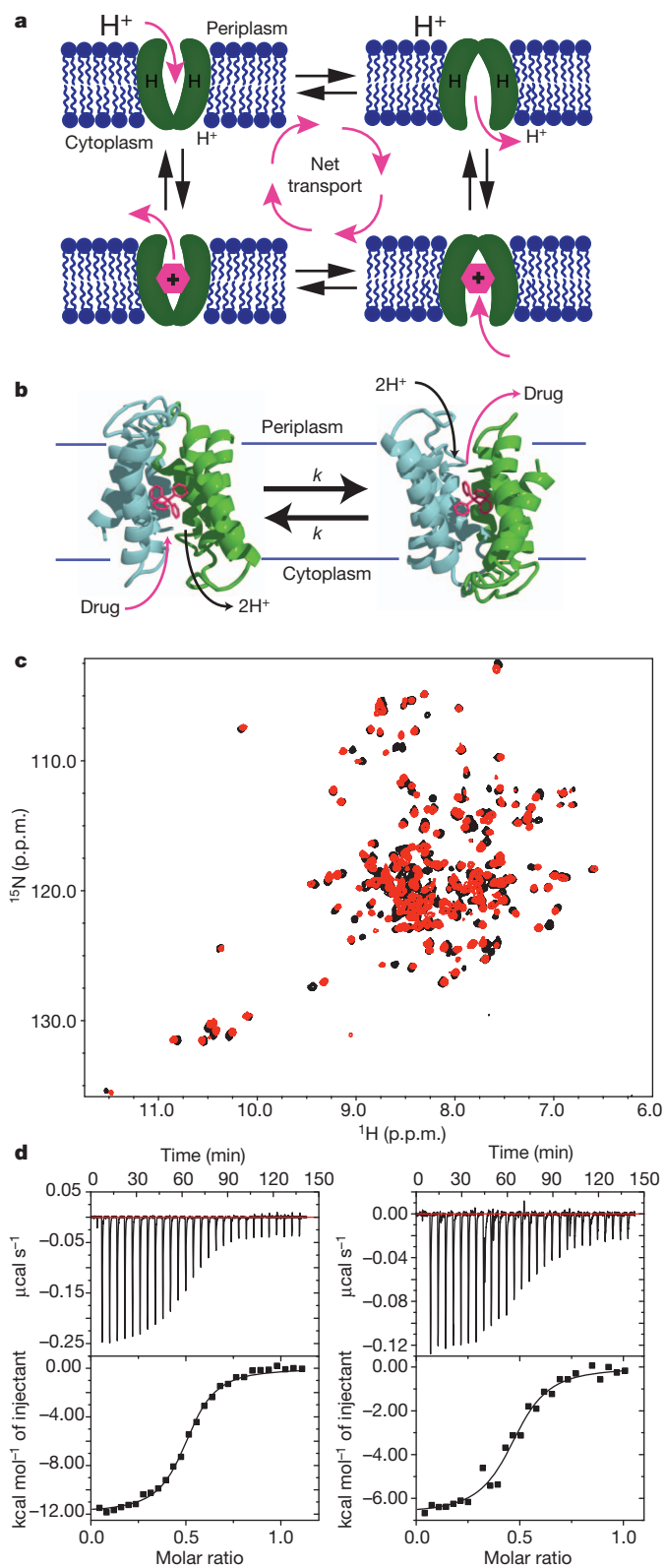


Figure 1 | Conformational interconversion and symmetry in the single-site alternating access model of EmrE transport. **a**, Each state in the transport cycle is only open to one side of the membrane, and the two states only interconvert when substrate (two protons or one polyaromatic cation) is bound. **b**, Conformational exchange as proposed for antiparallel, asymmetric EmrE¹⁸: the two monomers exchange conformations and the two states have identical structures. **c**, ¹H-¹⁵N TROSY spectra of TPP⁺-bound ²H/¹⁵N-EmrE at pH 7, 45 °C, are nearly identical for DMPC/DHPC (black) or DLPC/DHPC (red) bicelles and are well resolved with twice as many peaks as expected for a monomer. **d**, EmrE is functional in isotropic bicelles. The affinity of EmrE for TPP⁺ is nearly identical in DMPC/DHPC isotropic bicelles (right) and DDM (left), as measured by ITC at pH 7, 45 °C.

features of good chemical shift dispersion and peak doubling are preserved, confirming that the overall structure of EmrE is the same in both environments.

It is not possible to measure transport activity of solubilized protein, so substrate affinity is the best proxy for function. In a good environment EmrE is dimeric and binds TPP⁺ tightly^{4,19,29,30,33} with the affinity weakening from dissociation constant $K_d \approx 2$ nM at pH 8.5 to 50 nM at pH 7 (DDM micelles, 4 °C)^{19,20}. The pH-dependent TPP⁺ affinity is consistent with substrate competition between TPP⁺ and protons for the single binding site. In a poor environment, EmrE is monomeric and TPP⁺ binding weakens by three to four orders of magnitude^{33,34}. Using isothermal titration calorimetry we confirmed that EmrE in DDM micelles at 4 °C binds TPP⁺ tightly, as previously reported^{4,19,29,30,33} (Supplementary Fig. 3). At 45 °C, the temperature used for the NMR experiments, EmrE has the same affinity for TPP⁺ in DDM ($K_d = 120 \pm 12$ nM) or isotropic bicelles (170 ± 70 nM) at pH 7 (Fig. 1d and Supplementary Table 1). These values fall well within the variation observed for EmrE reconstituted into liposomes with different lipid composition³³. The binding stoichiometry is 0.5 (one TPP⁺ per dimer), as expected, indicating that all the EmrE is functional in isotropic bicelles.

TPP⁺-bound EmrE exchanges between two conformations

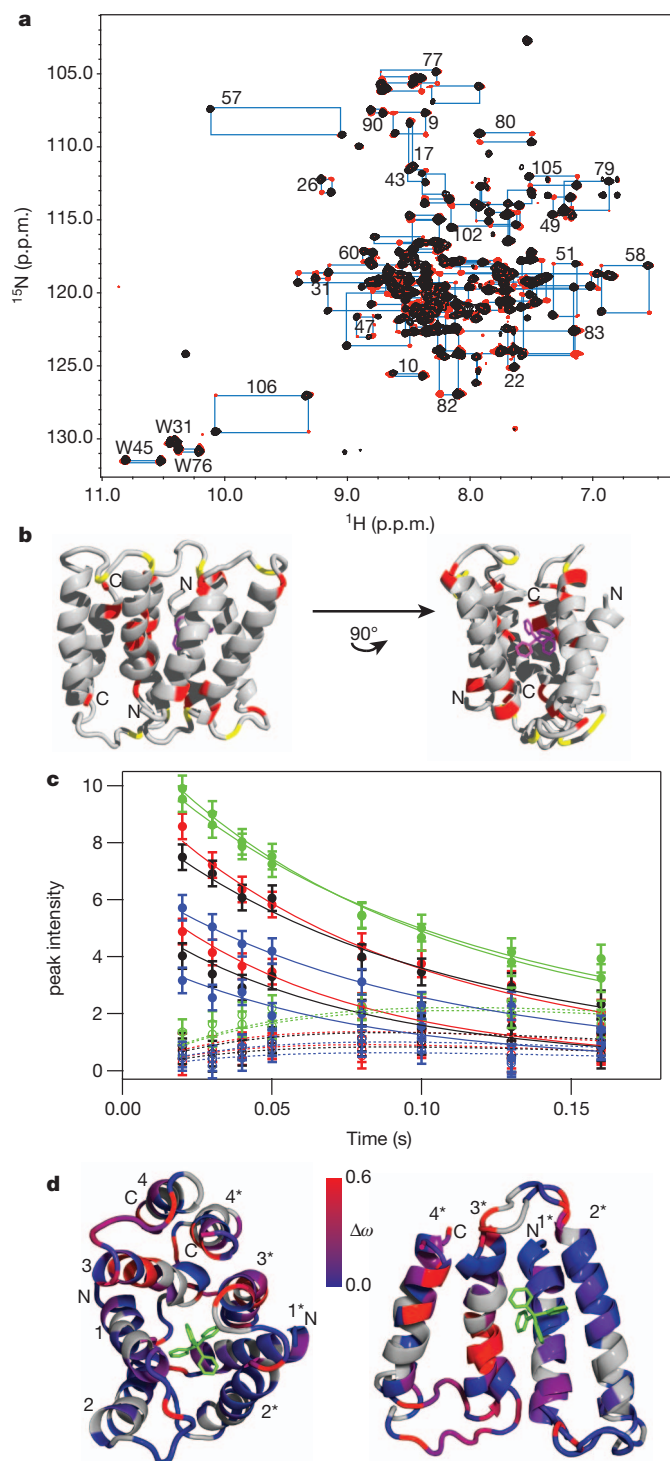
With the suitability of isotropic bicelles established, we turned to the key question: does TPP⁺-bound EmrE interconvert between two states as predicted by the single-site alternating access model? To test this hypothesis we performed a TROSY-selected ZZ-exchange NMR experiment³⁵, modified to measure the dynamics of the protein without interference from the high lipid concentration in our samples (see Supplementary Information). In these experiments, the ¹⁵N chemical shift of each amide is recorded, followed by a mixing period, and then the ¹H chemical shift is recorded. In the absence of conformational exchange, the spectrum observed will be identical to the TROSY spectrum. If conformational exchange between states A and B does occur during the mixing time, cross-peaks will appear in the ZZ-exchange spectrum at ¹⁵N_A/¹H_B and ¹⁵N_B/¹H_A, forming a 'box' connecting the TROSY peaks corresponding to the chemical shifts of a single amide in state A (¹⁵N_A/¹H_A) and state B (¹⁵N_B/¹H_B) (Fig. 2a). It is immediately apparent that there is widespread conformational exchange with nearly every resolved TROSY peak assigned to exchange pairs. Analysing the peak volumes of the well-resolved exchange pairs reveals equal populations for both states (Supplementary Fig. 4), consistent with a concerted global exchange process.

The kinetics of conformational exchange can be directly determined by varying the mixing time and analysing cross-peak build-up and auto-peak decay as a function of time in the TROSY-selected ZZ-exchange experiment. Cross-peaks grow in and auto-peaks decay owing to exchange between states A and B, and all peaks decay because of the intrinsic relaxation (R^*) of the $S_Z I_\beta$ spin state active during the mixing time (see Supplementary Information)^{35,36} according to

$$\frac{d}{dt} \begin{bmatrix} A \\ B \end{bmatrix} = \begin{bmatrix} -R^{*A} - k & k \\ k & -R^{*B} - k \end{bmatrix} \begin{bmatrix} A \\ B \end{bmatrix} \quad (1)$$

Because the populations are the same, the forward and reverse exchange rate constants are identical (k). Inspection of the data shows

dihexanoylphosphatidylcholine (DLPC/DHPC) or dimyristoylphosphatidylcholine/dihexanoylphosphatidylcholine (DMPC/DHPC) isotropic bicelles are nearly identical and characteristic of a folded protein. Spectra collected on TPP⁺-bound EmrE solubilized in DDM micelles required longer acquisition time due to poor signal to noise, a pattern previously noted for other integral membrane proteins in DDM³². Nevertheless, the TROSY spectra (Supplementary Fig. 2) are remarkably similar in DDM and bicelles, and the key



quite clearly that the common assumption of equal intrinsic relaxation in both states ($R^{*A} = R^{*B}$) is not true for many residues (Fig. 2c and Supplementary Figs 5, 7 and 8). The Palmer group has developed an alternative method for analysing data that eliminates any effects of different R^* (ref. 37). The composite ratio of peak intensities (\mathcal{E}) calculated according to their method has a simple quadratic dependence on the mixing time (t), and in the case of equal populations $\mathcal{E} = k^2 t^2$ (see Supplementary Information). For EmrE, analysis of \mathcal{E} shows that all residues collapse onto a single curve (Supplementary Fig. 6), confirming that this is a global conformational exchange process with a single timescale. Fitting the individual peak intensities as a function of time requires a more complex equation (Supplementary equation (3)), but results in the same global rate constant (Fig. 2c, Supplementary

Figure 2 | **TPP⁺-bound EmrE interconverts between two conformations.** **a**, Overlay of TROSY ZZ-exchange (red, 100 ms mixing time) and ^1H - ^{15}N TROSY heteronuclear single-quantum coherence (HSQC) spectra (black) of TPP⁺-bound $^2\text{H}/^{15}\text{N}$ -EmrE in isotropic bicelles. Cross-peaks demonstrate conformational exchange and blue boxes connect peaks corresponding to a single amide. All residues experience exchange. Assignments are shown for several well-resolved residues. **b**, Residues used for quantitative analysis are coloured based on whether the intrinsic relaxation rate is the same (red) or different (yellow) in the two states. **c**, Best fit of ZZ-exchange auto- (solid circles) and cross-peak (open circles) intensities as a function of mixing time (Supplementary equation (3)) yields a single global conformational exchange rate of $k = 4.9 \pm 0.5 \text{ s}^{-1}$. Each residue is a different colour (additional data in Supplementary Fig. 5). Error bars are estimated from the noise in each spectrum. **d**, Chemical shift difference ($\Delta\omega$) between states A and B (Supplementary equation (4)) are plotted onto the antiparallel dimer model (left) or an overlay of the two monomers with transmembrane helices 1–3 aligned to compare the two monomers within the antiparallel dimer (right).

Fig. 5). This global conformational exchange rate is also detected in analysis of the tryptophan side chain dynamics (Supplementary Information and Supplementary Fig. 9).

These NMR dynamics experiments directly detect global conformational exchange between two states with equal populations and a rate constant of $k_{\text{open-in to open-out}} = k_{\text{open-out to open-in}} = k = 4.8 \pm 0.5 \text{ s}^{-1}$ for TPP⁺-bound EmrE in isotropic bicelles at 45 °C. The atomic resolution provided by NMR demonstrates that the entire protein is involved in the conformational exchange process, as shown by the complete peak doubling. Importantly, the well-resolved residues used for quantitative analysis of the dynamics are distributed across the protein (Fig. 2b), indicating that the whole protein undergoes exchange on the same timescale. Second, residues with different intrinsic relaxation rates, R^* , map to the loop regions that alternate between a tightly packed protein environment and a loosely packed solvent-exposed environment as EmrE interconverts between inward- and outward-facing states, providing an explanation for the differential R^* (Supplementary Information). These results demonstrate the power of NMR methods to characterize quantitatively the kinetics (rate constants) and thermodynamics (populations) of global conformational exchange in a substrate-bound transporter, the key step for substrate transport across the membrane.

A previous study²⁰ monitoring intrinsic tryptophan fluorescence to measure substrate binding to EmrE detected an additional slow process with a rate constant of 1.5 s^{-1} that was attributed to a conformational change after TPP⁺ binding. Considering that the experiments were performed in different environments and at different temperatures, there is good agreement between the measured rates. This suggests both techniques are observing the same process and provides additional evidence that conformational exchange on this timescale in TPP⁺-bound EmrE is an intrinsic property of the protein.

However, the topology of the exchanging EmrE is also needed to understand the molecular mechanism. Two possibilities are consistent with the NMR data (Supplementary Fig. 1): (1) parallel, symmetric EmrE dimers interconverting between inward- and outward-facing states (AA to BB exchange) and (2) the unique model of asymmetric antiparallel EmrE dimers interconverting between two states that are identical but open to opposite sides of the membrane (AB to BA exchange). With equal populations (1) requires two distinct EmrE conformations that happen to have exactly equal free energies, whereas (2) entails inherently equal populations because each dimer consists of one monomer in each conformation. To distinguish these two possibilities experimentally, we turned to Förster resonance energy transfer (FRET) experiments.

Antiparallel topology within EmrE dimers

There is still significant controversy about the topology of EmrE dimers. Several accessibility studies have shown that EmrE exists in both orientations in its native *E. coli* membrane^{17,38}. Most recently,

Schuldiner and colleagues¹⁷ have demonstrated equal populations of both monomer orientations in the native inner membrane of *E. coli* based on the accessibility of single cysteine residues to the outside of the cell. However, these studies do not directly address the relative orientation of monomers within the dimer. Structural studies consistently show an asymmetric antiparallel dimer^{4,6,26,27,39,40}. Cross-linking of single-cysteine mutants and tandem genetic fusions have been used to enforce a parallel topology between monomers and these constructs are capable of transport^{11,17}. However, an antiparallel genetic fusion is also capable of transport¹⁷. Charge bias mutants and fusion of fluorescent reporters provide more conflicting results, most probably because the topology is exquisitely sensitive to sequence alteration^{16,17}.

We performed bulk and single-molecule FRET and cross-linking experiments to determine the relative topology of EmrE monomers within a dimer. All the single-cysteine mutants used in these experiments are known to be functional^{13,38,41}. In addition, two-dimensional ¹H-¹⁵N TROSY spectra of TPP⁺-bound mutants confirm that these single-cysteine mutants have the same structure as wild type (Supplementary Fig. 10). For bulk FRET measurements, we reconstituted single-cysteine EmrE mutants into liposomes to label specifically a single face of the protein (see Methods). To test for antiparallel topology, we labelled with donor and acceptor from opposite sides of the membrane (Fig. 3a). Significant acceptor fluorescence was observed upon

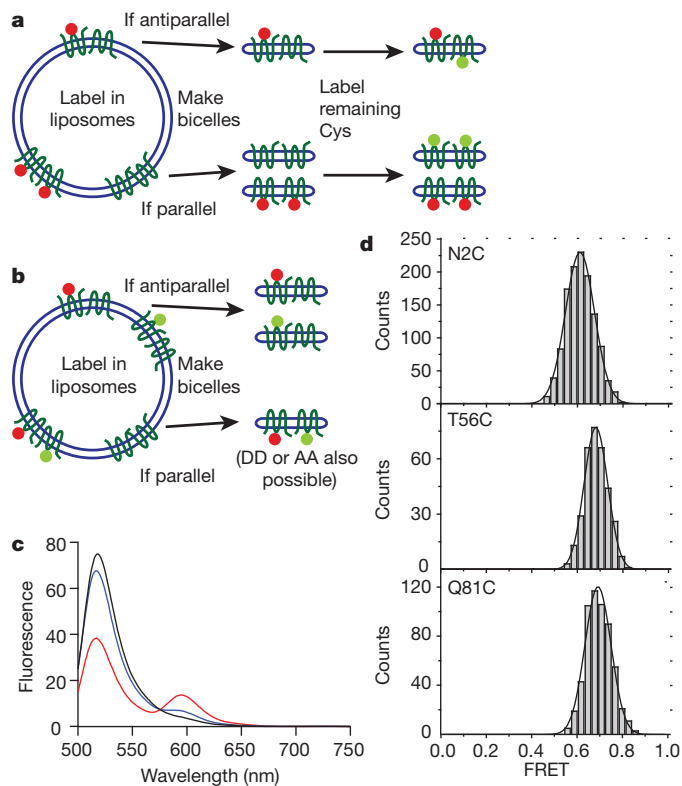


Figure 3 | EmrE is an antiparallel homodimer. T56C-EmrE was labelled in liposomes with two different schemes. **a**, Labelling with a single dye on each side of the membrane will produce FRET only if EmrE is antiparallel. **b**, Labelling with both donor and acceptor on the same side of the membrane will produce FRET only if EmrE is parallel. **c**, Observation of both donor (Alexa Fluor 488) and acceptor (Alexa Fluor 568) fluorescence upon donor excitation in sample **a** indicates FRET and antiparallel topology (red line). In contrast, only minimal FRET was observed in sample **b** (blue line). The control (black line, see Methods) excludes monomer swapping and direct excitation of acceptor. All fluorescence spectra are normalized to total donor fluorescence in SDS, which monomerizes EmrE⁴⁹. **d**, For single-molecule FRET experiments, N2C-, T56C- or Q81C-EmrE was labelled with Cy3/Cy5 in micelles and then reconstituted into bicelles. Particles containing one donor and one acceptor were selected based on photobleaching events. A single, narrow FRET distribution is observed in each case, with a FRET efficiency consistent with an antiparallel topology.

donor excitation (Fig. 3c, red line), indicating FRET occurs and antiparallel dimers are present. To test for parallel topology, EmrE was labelled with both dyes from only one side of the membrane (Fig. 3b). Only minimal FRET was observed (Fig. 3c, blue line), which could be due to dye leakage into liposomes during labelling or a small population of higher-order oligomers (Supplementary Information and Supplementary Fig. 13). No FRET is observed in the control experiment (Fig. 3c, black line), demonstrating that monomers do not swap between dimers under these conditions.

To simplify labelling and avoid leakage issues, we used single-molecule FRET⁴². Single-cysteine EmrE was stoichiometrically labelled with Cy3/Cy5 in micelles at one of three different positions, N2C, T56C or Q81C (Supplementary Fig. 11), followed by incorporation into bicelles. These bicelles contained 0.1% biotinylated lipid to tether specifically Cy3/Cy5-EmrE-containing bicelles to neutravidine molecules on the polymer-coated slide surface (Supplementary Fig. 11). The single-molecule FRET experiments were performed with a wide-field total internal reflection fluorescence microscope set up⁴³. Observation of photobleaching events during single molecule time traces allows dimers to be selected that contain one donor and one acceptor fluorophore. A single FRET distribution is observed for single-site labelled EmrE (Fig. 3d). The FRET efficiency of 0.6–0.7, depending on labelling site, corresponds to a distance of approximately 50–55 Å, consistent with transmembrane labelling. Based on the dimensions of the cryo-electron microscopy structure, donor and acceptor would not be more than about 35 Å apart, corresponding to a FRET efficiency greater than 0.9, if they were on the same side of the membrane as required for a parallel topology with this labelling scheme. These results confirm the antiparallel arrangement of monomers within an EmrE dimer.

To test this model further, we cross-linked S107C-EmrE with the heterobifunctional cross-linker sulpho-*N*-(γ -maleimidobutyryloxy) succinimide) (s-GMBS). Nearly complete cross-linking is observed (Supplementary Fig. 14), demonstrating that residues K22 and S107C are in close proximity in the dimer. Because these positions are on opposite sides of the membrane in each monomer, heterobifunctional cross-linking is only possible with an antiparallel topology (Supplementary Information and Supplementary Fig. 14). Together with the NMR results, this means that TPP⁺-bound EmrE must exist in an antiparallel asymmetric dimer that interconverts between two identical, oppositely oriented states (AB–BA dimer exchange) when bound to TPP⁺ (Supplementary Fig. 1).

According to this model, no conformational exchange should be observed in the single molecule FRET time traces, because the donor–acceptor distance will be identical in both states. Inspection of the time traces confirms this prediction (Supplementary Fig. 12), even though the rate of conformational exchange determined by NMR is within the range observable in these experiments. Our NMR spectra are very clean, with the double set of peaks fully accounted for by a single population of antiparallel EmrE. We do not observe additional minor peaks (Supplementary Fig. 1), so parallel EmrE is not present in our samples in measurable amounts (less than 5%).

The single-site alternating access model predicts that the two lowest energy states in the TPP⁺-bound conformation are the inward- and outward-facing states. If EmrE is open to one side of the membrane in our NMR samples, then we should observe different water accessibility for residues at the loops and ends of helices upon exchange from state A to state B because these regions pack together to close off the active site of EmrE at one end and open up to allow access to the active site at the other. To test this, we recorded ¹H-¹⁵N TROSY spectra with increasing concentrations of a soluble, chelated gadolinium compound. This paramagnetic compound causes line broadening of nearby amides. The results (Fig. 4a) show that some amides are similarly affected in both states whereas others are differentially broadened. The amides with differential accessibility to water map exactly to the regions expected for interconversion of an antiparallel dimer between ‘symmetric’ states (Fig. 1b and Supplementary Fig. 1), confirming that the conformation

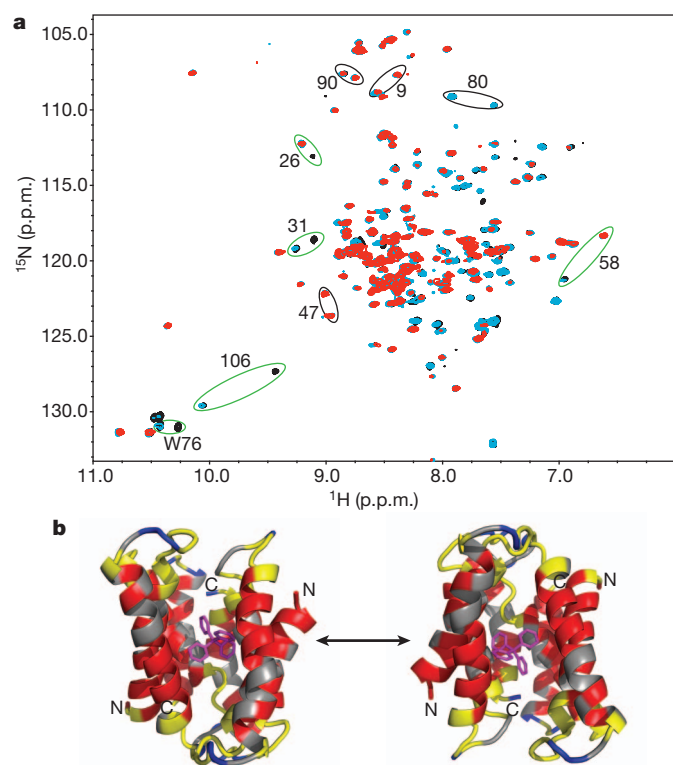


Figure 4 | EmrE has asymmetric water accessibility. **a**, ^1H - ^{15}N TROSY spectra of TPP^+ -bound EmrE in bicelles in the absence (black) and presence of 1 mM (blue) and 5 mM (red) paramagnetic gadobenate dimeglumine. Residues with different PRE effects (green circles) or the same PRE effect (black circles) in the two states are highlighted. **b**, Residues with equal accessibility (dark blue) or protection (red) from water in both states are plotted on the structure. Grey residues are not assigned in both states. Residues with differential accessibility to water (yellow) highlight the pore and loop regions, exactly as expected for inward- to outward-facing conformational exchange. This is consistent with an antiparallel dimer open only to one side of the membrane, as in the crystal structure (Protein Data Bank accession number 3B5D).

we observe is open to one side of the membrane and is consistent with the crystal structure (Fig. 4b).

The ZZ-exchange data assign pairs of peaks as exchange partners, representing the chemical shift of a single residue in each state. Chemical shift is quite sensitive to local structural changes. In the conformational exchange model proposed for EmrE, the two monomers swap conformations during the transition from inward- to outward-facing states (AB–BA). Thus, overlay of the two monomers (Fig. 2d) highlights regions where local structure changes upon conformational exchange in this model. The overlay reveals kinking of transmembrane helix 3 and movement of transmembrane helix 4 relative to the substrate-binding domain as the largest structural changes. This is exactly where the largest chemical shift differences between the two states (Supplementary equation (4)) are observed. The transmembrane helices lining the binding pocket move as a generally rigid body, with significant chemical shift differences only in the regions that close off or open up access to the transport pore (Fig. 2d). These chemical shift differences are entirely consistent with the structural differences between the two monomers, supporting the AB–BA exchange model. The two sets of NMR peaks correspond to the two distinct monomer conformations within an exchanging antiparallel asymmetric dimer.

Antiparallel EmrE is competent for transport

We have used solution NMR spectroscopy to observe directly conformational exchange between inward- and outward-facing states of the small multidrug resistance transporter, EmrE. This is the key step in the transport cycle that moves the substrate across the membrane

from one aqueous compartment to another. Measurement of bulk transport is not possible in a solubilized system. However, solution NMR provides a powerful tool to follow directly the protein conformational changes that effectively ‘transport’ TPP^+ , and we have quantitatively measured this exchange process in TPP^+ -bound EmrE.

Our FRET experiments show that the monomers within each dimer are antiparallel. Thus, antiparallel substrate-bound EmrE is able to undergo the key conformational exchange step in the single-site alternating access model of antiport. Together with our chemical shift mapping and water accessibility data, this suggests that the low-resolution crystal and cryo-electron microscopy structures are essentially correct, and we are working to refine and improve the resolution of this structure using NMR restraints.

Our results provide experimental evidence for the unusual model proposed by Fleishman *et al.* that the inward- and outward-facing states are identical¹⁸ (Supplementary Fig. 1). This model reconciles the asymmetric antiparallel structural data^{4,6,26,27,39,40} with biochemical studies indicating functional symmetry of active site residues^{3,44,45} and single distances measured for most residues by electron paramagnetic resonance (EPR)¹³. These results demonstrate that equal insertion of EmrE in both orientations in the *E. coli* inner membrane¹⁷ is functionally relevant and they enhance the importance of EmrE as a potential model for evolution of dual topology membrane proteins⁹.

METHODS SUMMARY

EmrE was expressed and purified using a 6× His-tag, which was then removed with thrombin. Purification was performed in decylmaltoside or DDM. EmrE was reconstituted into DMPC or DLPC liposomes using standard methods and then formed into isotropic bicelles by addition of DHPC and several freeze–thaw cycles. Protein concentration was determined using absorbance at 280 nm, with an extinction coefficient determined by amino-acid analysis. Isothermal titration calorimetry (ITC) was performed by titrating 54 μM TPP^+ stock solutions into 10 μM EmrE, with matching concentrations of detergent or lipid in both solutions.

Bulk FRET labelling was performed in liposomes to label residues separately on either side of the membrane. An ‘antiparallel’ sample was labelled with donor and acceptor on opposite sides, and a ‘parallel’ sample was labelled with donor and acceptor on the same side. Donor-only and acceptor-only controls were labelled with dye only on the exterior of the liposome, reconstituted into bicelles and then mixed. Single-molecule FRET samples were labelled in micelles and experiments were performed using a wide-field total internal reflection fluorescence microscope set up⁴³.

NMR experiments were performed using a 700 MHz Varian NMR spectrometer or 800 MHz Bruker spectrometer equipped with a cryoprobe. All NMR samples contained 0.5–1.0 mM $^2\text{H}/^{15}\text{N}$ -EmrE in buffer conditions of 2 mM TPP^+ , 20 mM NaCl, 20 mM potassium phosphate, 2 mM TCEP, pH 7.0, 45 °C. The membrane mimetic in each sample (DDM micelles or isotropic bicelles) was as listed. The TROSY-selected ZZ-exchange experiment³⁵ was modified to include a lipid ‘flipback’ pulse. Data were processed and analysed with NMRPipe⁴⁶, NMRView⁴⁷, Sparky⁴⁸ and IgorPro (Wavemetrics). All EmrE structure figures were created in PyMOL using Protein Data Bank 3B5D with the backbone rebuilt to render the cartoons. Full-page versions of the spectra in the main figures are included in the Supplementary Information.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 20 January; accepted 7 November 2011.

Published online 18 December 2011.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank Y. Liu for assistance replicating the ITC data. We thank J. Villali for assistance growing isotopically labelled EmrE. We are grateful to Y. Li and A. Palmer for providing pulse programs and G. Chang for providing the EmrE expression plasmid. This work was supported by the National Institutes of Health (1R01GM095839) and the Searle Scholars Program (K.H.W.), the US Department of Energy, Office of Basic Energy Sciences (D.K.), the Howard Hughes Medical Institute (D.K. and T.H.), and an NSF graduate research fellowship to E.M. (DGE-1143954).

Author Contributions E.M. and K.H.W. optimized EmrE sample preparation, performed ITC experiments and recorded two-dimensional NMR spectra under different conditions. E.M. and G.D. performed the bulk FRET experiments. S.D. and R.V. performed the single molecule FRET experiments with guidance from T.H. G.D. and M.C. optimized the modified ZZ-exchange NMR experiment. M.C. collected the ZZ-exchange data and K.H.W. analysed it. G.D. performed the paramagnetic relaxation enhancement NMR experiments. A.B., E.M., G.D. and K.H.W. contributed to assignments. D.K. mentored initial project development. K.H.W. conceived the project and wrote the manuscript.

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