GUEST COMMENTARY

Vacuuming the Periplasm

Olga Lomovskaya^{1*} and Maxim Totrov²

*Mpex Pharmaceuticals, Inc.,¹ and Molsoft,
LLC,² San Diego, California*

Ever since their discovery multidrug resistance (MDR) transporters have provided a plethora of fascinating and challenging topics for fundamental and applied research. Recognized first as a cause of resistance of tumor cells to anticancer agents (reviewed in reference 5), they were later discovered in all living organisms, from bacteria to humans. In bacteria the expression of MDR transporters can raise antibiotic resistance by several orders of magnitude, rendering some antibiotics clinically useless, especially against gram-negative pathogens (reviewed in reference 30). The structural diversity of substrates for some MDR pumps is striking: a single efflux system may accommodate positively or negatively charged compounds, as well as compounds that are neutral or zwitterionic (reviewed in reference 20). Establishing the basis for multidrug recognition and transport, which provides a single protein with both broad specificity and high selectivity, is a fundamentally intriguing challenge. Aside from being intellectually rewarding, unraveling the mechanistic details will greatly facilitate the fight to combat antibiotic resistance. The results of the study by Aires and Nikaido published in this issue of the *Journal of Bacteriology* (1a), demonstrating that MDR transporter AcrD could capture its substrates directly from the periplasm and extrude them into the external medium, bring us one step closer to elucidation of this fascinating biochemical process.

The AcrD transporter from *Escherichia coli* (32) is a typical member of the resistance-nodulation-division (RND) family (37), which includes the major agents of intrinsic and acquired antibiotic resistance in gram-negative bacteria—pathogens which pose a great clinical challenge owing to their decreasing susceptibility to the majority of current antibacterial agents. In fact, out of all approved antibiotics (some 160 antibiotics), representatives of only three classes (fluoroquinolones, beta-lactams, and aminoglycosides) have any clinical utility for the treatment of *Pseudomonas aeruginosa* infections. Even these antibiotics are under threat from multiple mechanisms of acquired resistance, with MDR efflux pumps playing a prominent role (reviewed in reference 31). The problem becomes even more serious because MDR efflux pumps can act on newer compounds in development that have been identified and optimized using all the powerful techniques of modern drug discovery such as comparative genomics, structure-guided drug design, and combinatorial chemistry.

It would be unfair to attribute the extent of intrinsic and

acquired resistance in gram-negative bacteria (which may reach several orders of magnitude) solely to the activity of MDR pumps; such pumps also exist in gram-positive bacteria. What is different is the so-called transenvelope efflux, whereby a toxic compound is extruded directly into the external medium, from which entry is slowed by the outer membrane, which retards both hydrophilic and hydrophobic compounds (reviewed in references 29 and 44).

Transenvelope multidrug efflux is performed by tripartite protein complexes comprised of an inner membrane transporter, an outer membrane channel (OMP), and a membrane fusion protein (MFP) located in the periplasm. While tripartite systems may contain MDR transporters from different families (33), it is the RND-containing ones that have been studied the most.

Progress in elucidating the structural biology of these tripartite pumps has been remarkable, yielding high-resolution three-dimensional (3D) structures of all three individual components. The structure of the *E. coli* OMP TolC was solved in 2000 (18). Quite recently, the structure of the *P. aeruginosa* OMP OprM became available (2). The structure of the *E. coli* RND protein AcrB was published in 2002 (26), followed by the structure of the same protein in combination with several ligands in 2003 (40), and the structure of the last player, the MFP component, MexA from *P. aeruginosa* (3, 17) was reported in 2004.

X-ray crystallography provides a strong structural basis to define the process of transenvelope efflux. Both AcrB and TolC are homotrimers and contain large periplasmic domains. There appears to be a perfect fit between the funnel-like opening of the headpiece of AcrB and the proximal end of the tunnel-like TolC. Thus the deep “crater” at the tip of the periplasmic segment of AcrB may serve as a portal connecting to the TolC subunit. The latter likely serves as an “exhaust pipe,” conducting the substrates expelled by the pump through the outer membrane and into the extracellular space. Indeed, the sum of the periplasmic lengths of AcrB and TolC (170 Å) is large enough to cross the entire periplasmic space. Recent studies have demonstrated that AcrB and TolC can be cross-linked in vivo, experimentally confirming the close proximity of their periplasmic domains (36). As for the MFP, based on its interactions with the other two components of the system (25, 42), it was concluded that this protein is required for stable association of the inner and outer membrane components of the pump. The 3D structure of the MFP MexA is compatible with this interpretation (3, 17). An unexpected finding by Aires

* Corresponding author. Mailing address: Mpex Pharmaceuticals, Inc., 5500 Campanile Dr., San Diego, CA 92182. Phone: (415) 254-0560. Fax: (619) 594-5378. E-mail: olomovskaya@mpexpharma.com.

and Nikaido suggests, however, an additional and perhaps more active role for the MFP (see below).

The 3D architecture of the AcrB trimer is extremely interesting (reviewed in references 9 and 28). It revealed three large vestibules, formed by neighboring protomers, wide open to the periplasm. These vestibules lead to a central cavity inside the pump, inviting the speculation that substrates gain access to the pump from the periplasmic space and that this spacious cavity serves as a binding site for multiple substrates. And indeed, in later cocrystallization studies several structurally unrelated substrates of AcrB were bound in the central cavity (39).

The structural studies indicate that RND transporters could capture their substrates directly from the periplasm or from the outer leaflet of the cytoplasmic membrane. This conclusion is in good agreement with results from several laboratories that indicate that residues in two large periplasmic loops of RND transporters determine their substrate specificity (8, 11, 12, 22, 24, 27, 35).

Missing from this logical picture is biochemical evidence of periplasmic capture of substrates. Such evidence is now provided by the elegant study of Aires and Nikaido (1a). To assess the location of substrate capture by the RND transporter, they used the well-defined system of reconstituted proteosomes with an artificially imposed proton gradient (in their case, inside acidic, defining the intravesicular space as the “periplasm”). Such a system should allow the determination of activity of a transporter depending on whether a substrate is added to the external medium (the “cytoplasm”) or is exclusively present in the intravesicular space (the periplasm). However, the construct can serve as a model for an efflux system only if the substrate cannot spontaneously cross the membrane. Highly charged aminoglycoside antibiotics satisfy this criterion, and this dictated the choice of the RND transporter AcrD from *E. coli*, which is known to extrude aminoglycosides as part of the AcrA-AcrD-TolC tripartite MDR. In this system cytoplasmic capture should result in (radiolabeled) substrate movement across the membrane and accumulation in the intravesicular space, where it can be measured directly. Conversely, periplasmic capture by AcrD would not move the substrate across the membrane, so an indirect way to detect the binding event was needed. The authors reasoned that since the activity of RND transporters is driven by proton gradients (13, 41), substrate capture and release should be accompanied by the transport of proton across the membrane, resulting in dissipation of the artificially imposed gradient. Using this proton transport assay, Aires and Nikaido provided the first evidence for the periplasmic capture of aminoglycosides by AcrD. Interestingly, their experiments also demonstrated that some, but not all, aminoglycosides could be captured from the cytoplasmic side of the membrane. What was not anticipated and came as a complete surprise was the absolute essentiality of AcrA for both periplasmic and cytoplasmic capturing: proton transport was recorded only when AcrA was present within proteoliposomes.

Thus, it appears that AcrA plays some important (but unknown) role in RND-mediated transport in addition to facilitating the assembly of the tripartite complex. Does an RND transporter need to be “activated” by the MFP protein in order to capture its substrates? Perhaps cocrystallization of the RND

and MFP components can provide an accurate picture of potential substrate binding pockets.

Interestingly, the presence of AcrA makes AcrB proteoliposomes somewhat more leaky for protons in the absence of substrates. Is this just an artifact of the system, or does the presence of AcrA stimulate substrate-independent AcrD-mediated proton transport? Perhaps this system contains substrates even in the absence of aminoglycosides—possibly lipids or the valinomycin that was used to create the proton gradient?

More data are needed to understand whether the MFP is required for the transport activity of other reconstituted RND transporters. An MFP stimulated the activity of AcrB, though these data were interpreted in the context of membrane fusion (41). Another reconstituted RND transporter, CzcA, had considerable transport activity in the absence of the corresponding MFP (13).

How well can periplasmic capturing and subsequent trans-envelope transport be described in terms of available concepts? Secondary transport in which the translocation of H^+ is coupled to the transport of substrate against its concentration gradient comes in two forms: either “symport” or “antiport.” In the former case, both proton(s) and substrate are transported in the same direction across the same membrane. In the latter case they cross the same membrane in opposite directions. Periplasmic capture followed by the release of a substrate into the external medium by a tripartite transporter complex does not fit either case: the H^+ is moved across the inner membrane, while the substrate proceeds across the outer membrane of gram-negative bacteria. This type of transport mechanism calls for a new definition, and we will refer to it as “periporport.” Periporport will qualify as a new mechanism of secondary transport only once it is shown to be fully reversible.

Reversible or not, periporport as manifested by periplasmic substrate binding comes as very good news for those of us in the business of combating RND-efflux-mediated MDR with small-molecule inhibitors (21). If substrates can be captured in the periplasm, the same can be true for inhibitors. Consequently, to inhibit an RND transporter, it may not be necessary to design a compound that can cross both membranes, which is a daunting challenge.

It is worth noting that periplasmic substrate capture and trans-envelope export (periporport) might be the basis of the high, nonadditive levels of antibiotic resistance that are observed when a single-component efflux pump, located in the inner membrane and extruding its substrates into the periplasm, and a tripartite RND-containing pump, picking substrates from there and exporting them into the external medium, are present in the same cell (19, 43).

What is known about periplasmic binding sites? While the available structural data suggest that substrates bind in the central cavity between the transmembrane and periplasmic subunits (40), the ligand-protein interactions in these complex structures appear weak and insufficient to explain substrate specificity. In fact, the few amino acid residue side chains observed to be interacting with the ligands are highly conserved across various efflux pump sequences of broadly varying substrate specificity. Analysis of the X-ray structure unveils a number of large lateral pockets within the periplasmic segment of AcrB (Fig. 1). These lateral pockets stretch between the periplasm and the central cavity. Mutation data for several

FIG. 1. Cross section of AcrB. The surface of AcrB was calculated by the icmMacroShape method (M. Totrov and R. Abagyan, unpublished data) implemented as the macro in the Molsoft ICM package (MolSoft ICM 3.2 program manual; MolSoft LLC, San Diego, Calif., 2003 [http://www.molsoft.com/icm_pro.html [1]). The method generates a volume density map represented by the van der Waals potential of the molecule. Putative pathways of substrates and proton are indicated by arrows. See the text for details.

homologous RND transporters suggest the importance of a number of residues adjacent to these pockets for substrate transport and specificity (12, 22). These observations are suggestive of an alternative pathway (dashed blue arrows) involving substrate binding in the peripheral pockets. The proximity of these pockets to the upper portal might allow their connection to the portal upon a conformational change associated with proton transport. It has previously been postulated (27, 28) that substrates that accumulate in the central cavity are actively transported into the upper portal space via the channel that should open along the central axis of the structure (solid blue arrows). However, a very significant conformational change associated with channel opening would have to be coupled with proton transport (turquoise arrow) via the transmembrane domain in order to accommodate the passage of substrates. The existence of additional binding pockets implies that in order to reach the headpiece funnel (and eventually the OMP tunnel) substrates do not need to use this central pore channel as a major substrate translocation pathway.

Although the substrate translocation pathway of RND transporters remains to be solved, the very fact of periplasmic capture differentiates them from the other families of MDR pumps, including ABC transporters such as the extensively studied human P-gp (4) and LmrA (38) from *Lactococcus lactis*; major facilitators such as LmrP (23), QacA (7), and MdfA (6) from *L. lactis*, *Staphylococcus aureus*, and *E. coli*, respectively; and SMR transporters such as EmrE (34) from *E. coli*. In all these cases the amino acid residues that are responsible for substrate specificity have been mapped to transmembrane domains, and in several instances membrane capture of substrates has been confirmed in rigorous biochemical experiments, justifying their characterization as “membrane vacuum cleaners” (16). By the same token, capture of substrates by RND-type MDRs directly from the periplasm, coupled with extrusion across the outer membrane renders them periplasm vacuum cleaners (9).

Another important aspect of the study of Aires and Nikaido was that AcrD was apparently able to capture its substrates

from the cytoplasmic side as well. Not only that, but it also appeared to discriminate between different aminoglycosides: uptake from the cytoplasmic side was observed for gentamicin and tobramycin but not streptomycin. This result indicates a substrate-specific translocation pathway formed by the transmembrane domain. However, the finding that chimeric RND transporters containing periplasmic loops and transmembrane domains from different transporters (AcrB and AcrD [10] or MexY and MexB [8]) retained the substrate specificity of the transporter from which the periplasmic loops were taken leaves us with the conclusion that substrate-specific binding sites are located in the periplasmic domains. More data are needed to resolve this contradiction. We note that both facilitated diffusion and active transport following cytoplasmic capturing has been demonstrated for the metal RND transporter CzcA in very similar reconstitution experiments. However, site-directed mutagenesis, targeting the most likely (but still somewhat limited) amino acid residues, did not succeed in identifying a cytoplasmic substrate-specific binding site (13).

The relevance of cytoplasmic capture per se in protection against compounds that attack bacteria from the outside is difficult to assess if the transporter is also able to extrude its substrates from the periplasm. Clearly it would be very useful for the cell if the transporter were able to catch even those molecules that had escaped periplasmic vacuuming. However, aminoglycosides appear to stimulate proton transport at concentrations (30 to 70 μ M) that are several orders of magnitude higher than those (20 to 30 nM) needed to inhibit protein synthesis (14).

While one can argue about the importance of cytoplasmic capturing in protection against environmental toxins, it may be very important in providing defense against toxic charged metabolites, which might otherwise accumulate inside cells. In fact, induction of the expression of AcrB has been demonstrated in several mutants with blocks in biosynthetic pathways. These mutants accumulate biosynthetic intermediates formed before the block, leading to the suggestion that "toxic waste disposal" might be an important physiological function of AcrB (15).

Thus, the study by Aires and Nikaido has provided many important insights into the function of the MDR RND pump, a versatile and sophisticated transporter. Many unanswered questions remain. What is the mechanism of coupling of the proton translocation across the inner membrane with the transenvelope export of substrates? How many protons are translocated across the inner membrane per molecule of substrate exported? How is the activity of individual protomers coordinated? Are different substrates extruded with the same or varying rates? Can the polarity of transport by tripartite RND-containing transporters be reversed? It is clear that prevention of efflux-mediated MDR requires a detailed understanding of the molecular mechanism of transport as well as more high-resolution structures of transporter complexes.

REFERENCES

1. Abagyan, R. A., M. M. Totrov, and D. N. Kuznetsov. 1994. ICM—a new method for protein modelling and design. Applications to docking and structure prediction from the distorted native conformation. *J. Comp. Chem.* **15**:488–506.
- 1a. Aires, J. R., and H. Nikaido. 2005. Aminoglycosides are captured from both periplasm and cytoplasm by the AcrD multidrug efflux transporter of *Escherichia coli*. *J. Bacteriol.* **187**:1923–1929.
2. Akama, H., M. Kanemaki, M. Yoshimura, T. Tsukihara, T. Kashiwagi, H. Yoneyama, S. I. Narita, A. Nakagawa, and T. Nakae. 2004. Crystal structure of the drug-discharge outer membrane protein, OprM, of *Pseudomonas aeruginosa*: dual modes of membrane anchoring and occluded cavity end. *J. Biol. Chem.* **279**:52816–52819.
3. Akama, H., T. Matsuura, S. Kashiwagi, H. Yoneyama, S. Narita, T. Tsukihara, A. Nakagawa, and T. Nakae. 2004. Crystal structure of the membrane fusion protein, MexA, of the multidrug transporter in *Pseudomonas aeruginosa*. *J. Biol. Chem.* **279**:25939–25942.
4. Ambudkar, S. V., S. Dey, C. A. Hrycyna, M. Ramachandra, I. Pastan, and M. M. Gottesman. 1999. Biochemical, cellular, and pharmacological aspects of the multidrug transporter. *Annu. Rev. Pharmacol. Toxicol.* **39**:361–398.
5. Ambudkar, S. V., C. Kimchi-Sarfaty, Z. E. Sauna, and M. M. Gottesman. 2003. P-glycoprotein: from genomics to mechanism. *Oncogene* **22**:7468–7485.
6. Bibi, E., J. Adler, O. Lewinson, and R. Edgar. 2001. MdfA, an interesting model protein for studying multidrug transport. *J. Mol. Microbiol. Biotechnol.* **3**:171–177.
7. Brown, M. H., and R. A. Skurray. 2001. Staphylococcal multidrug efflux protein QacA. *J. Mol. Microbiol. Biotechnol.* **3**:163–170.
8. Eda, S., H. Maseda, and T. Nakae. 2003. An elegant means of self-protection in gram-negative bacteria by recognizing and extruding xenobiotics from the periplasmic space. *J. Biol. Chem.* **278**:2085–2088.
9. Elkins, C. A., and H. Nikaido. 2003. 3D structure of AcrB: the archetypal multidrug efflux transporter of *Escherichia coli* likely captures substrates from periplasm. *Drug Resist. Updates* **6**:9–13.
10. Elkins, C. A., and H. Nikaido. 2003. Chimeric analysis of AcrA function reveals the importance of its C-terminal domain in its interaction with the AcrB multidrug efflux pump. *J. Bacteriol.* **185**:5349–5356.
11. Elkins, C. A., and H. Nikaido. 2002. Substrate specificity of the RND-type multidrug efflux pumps AcrB and AcrD of *Escherichia coli* is determined predominantly by two large periplasmic loops. *J. Bacteriol.* **184**:6490–6498.
12. Franke, S., G. Grass, C. Rensing, and D. H. Nies. 2003. Molecular analysis of the copper-transporting efflux system CusCFBA of *Escherichia coli*. *J. Bacteriol.* **185**:3804–3812.
13. Goldberg, M., T. Pribyl, S. Juhnke, and D. H. Nies. 1999. Energetics and topology of CzcA, a cation/proton antiporter of the resistance-nodulation-cell division protein family. *J. Biol. Chem.* **274**:26065–26070.
14. Greenber, W. A., E. S. Priestley, P. S. Sears, and P. B. Alper. 1999. Design and synthesis of new aminoglycoside antibiotics containing neomine as an optimal core structure: correlation of antibiotic activity with in vitro inhibition of translation. *J. Am. Chem. Soc.* **121**:6527–6541.
15. Helling, R. B., B. K. Janes, H. Kimball, T. Tran, M. Bundesmann, P. Check, D. Phelan, and C. Miller. 2002. Toxic waste disposal in *Escherichia coli*. *J. Bacteriol.* **184**:3699–3703.
16. Higgins, C. F., and M. M. Gottesman. 1992. Is the multidrug transporter a flippase? *Trends Biochem. Sci.* **17**:18–21.
17. Higgins, M. K., E. Bokma, E. Koronakis, C. Hughes, and V. Koronakis. 2004. Structure of the periplasmic component of a bacterial drug efflux pump. *Proc. Natl. Acad. Sci. USA* **101**:9994–9999.
18. Koronakis, V., A. Sharff, E. Koronakis, B. Luisi, and C. Hughes. 2000. Crystal structure of the bacterial membrane protein TolC central to multidrug efflux and protein export. *Nature* **405**:914–919.
19. Lee, A., W. Mao, M. S. Warren, A. Mistry, K. Hoshino, R. Okumura, H. Ishida, and O. Lomovskaya. 2000. Interplay between efflux pumps may provide either additive or multiplicative effects on drug resistance. *J. Bacteriol.* **182**:3142–3150.
20. Li, X. Z., and H. Nikaido. 2004. Efflux-mediated drug resistance in bacteria. *Drugs* **64**:159–204.
21. Lomovskaya, O., and W. Watkins. 2001. Inhibition of efflux pumps as a novel approach to combat drug resistance in bacteria. *J. Mol. Microbiol. Biotechnol.* **3**:225–236.
22. Mao, W., M. S. Warren, D. S. Black, T. Satou, T. Murata, T. Nishino, N. Gotoh, and O. Lomovskaya. 2002. On the mechanism of substrate specificity by resistance nodulation division (RND)-type multidrug resistance pumps: the large periplasmic loops of MexD from *Pseudomonas aeruginosa* are involved in substrate recognition. *Mol. Microbiol.* **46**:889–901.
23. Mazurkiewicz, P., W. N. Konings, and G. J. Poelarends. 2002. Acidic residues in the lactococcal multidrug efflux pump LmrP play critical roles in transport of lipophilic cationic compounds. *J. Biol. Chem.* **277**:66–71.
24. Middlemiss, J. K., and K. Poole. 2004. Differential impact of MexB mutations on substrate selectivity of the MexAB-OprM multidrug efflux pump of *Pseudomonas aeruginosa*. *J. Bacteriol.* **186**:1258–1269.
25. Mokhonov, V. V., E. I. Mokhonova, H. Akama, and T. Nakae. 2004. Role of the membrane fusion protein in the assembly of resistance-nodulation-cell division multidrug efflux pump in *Pseudomonas aeruginosa*. *Biochem. Biophys. Res. Commun.* **322**:483–489.
26. Murakami, S., R. Nakashima, E. Yamashita, and A. Yamaguchi. 2002. Crystal structure of bacterial multidrug efflux transporter AcrB. *Nature* **419**:587–593.
27. Murakami, S., N. Tamura, A. Saito, T. Hirata, and A. Yamaguchi. 2004.

- Extramembrane central pore of multidrug exporter AcrB in *Escherichia coli* plays an important role in drug transport. *J. Biol. Chem.* **279**:3743–3748.
28. Murakami, S., and A. Yamaguchi. 2003. Multidrug-exporting secondary transporters. *Curr. Opin. Struct. Biol.* **13**:443–452.
 29. Nikaido, H. 2001. Preventing drug access to targets: cell surface permeability barriers and active efflux in bacteria. *Semin. Cell Dev. Biol.* **12**:215–223.
 30. Poole, K. 2004. Efflux-mediated multiresistance in Gram-negative bacteria. *Clin. Microbiol. Infect.* **10**:12–26.
 31. Poole, K., and R. Srikumar. 2001. Multidrug efflux in *Pseudomonas aeruginosa*: components, mechanisms and clinical significance. *Curr. Top. Med. Chem.* **1**:59–71.
 32. Rosenberg, E. Y., D. Ma, and H. Nikaido. 2000. AcrD of *Escherichia coli* is an aminoglycoside efflux pump. *J. Bacteriol.* **182**:1754–1756.
 33. Saier, M. H., Jr., and I. T. Paulsen. 2001. Phylogeny of multidrug transporters. *Semin. Cell Dev. Biol.* **12**:205–213.
 34. Schuldiner, S., D. Granot, S. S. Mordoch, S. Ninio, D. Rotem, M. Soskin, C. G. Tate, and H. Yerushalmi. 2001. Small is mighty: EmrE, a multidrug transporter as an experimental paradigm. *News Physiol. Sci.* **16**:130–134.
 35. Tikhonova, E. B., Q. Wang, and H. I. Zgurskaya. 2002. Chimeric analysis of the multicomponent multidrug efflux transporters from gram-negative bacteria. *J. Bacteriol.* **184**:6499–6507.
 36. Touze, T., J. Eswaran, E. Bokma, E. Koronakis, C. Hughes, and V. Koronakis. 2004. Interactions underlying assembly of the *Escherichia coli* AcrAB-TolC multidrug efflux system. *Mol. Microbiol.* **53**:697–706.
 37. Tseng, T. T., K. S. Gratwick, J. Kollman, D. Park, D. H. Nies, A. Goffeau, and M. H. Saier, Jr. 1999. The RND permease superfamily: an ancient, ubiquitous and diverse family that includes human disease and development proteins. *J. Mol. Microbiol. Biotechnol.* **1**:107–125.
 38. van Veen, H. W., A. Margolles, M. Muller, C. F. Higgins, and W. N. Konings. 2000. The homodimeric ATP-binding cassette transporter LmrA mediates multidrug transport by an alternating two-site (two-cylinder engine) mechanism. *EMBO J.* **19**:2503–2514.
 39. Yu, E. W., J. R. Aires, and H. Nikaido. 2003. AcrB multidrug efflux pump of *Escherichia coli*: composite substrate-binding cavity of exceptional flexibility generates its extremely wide substrate specificity. *J. Bacteriol.* **185**:5657–5664.
 40. Yu, E. W., G. McDermott, H. I. Zgurskaya, H. Nikaido, and D. E. Koshland, Jr. 2003. Structural basis of multiple drug-binding capacity of the AcrB multidrug efflux pump. *Science* **300**:976–980.
 41. Zgurskaya, H. I., and H. Nikaido. 1999. Bypassing the periplasm: reconstitution of the AcrAB multidrug efflux pump of *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **96**:7190–7195.
 42. Zgurskaya, H. I., and H. Nikaido. 2000. Cross-linked complex between oligomeric periplasmic lipoprotein AcrA and the inner-membrane-associated multidrug efflux pump AcrB from *Escherichia coli*. *J. Bacteriol.* **182**:4264–4267.
 43. Zgurskaya, H. I., and H. Nikaido. 2002. Mechanistic parallels in bacterial and human multidrug efflux transporters. *Curr. Prot. Pept. Sci.* **3**:531–540.
 44. Zgurskaya, H. I., and H. Nikaido. 2000. Multidrug resistance mechanisms: drug efflux across two membranes. *Mol. Microbiol.* **37**:219–225.

The views expressed in this Commentary do not necessarily reflect the views of the journal or of ASM.