Gram-negative bacteria, extensively reviewed in Li et al. (2015). A well-studied example is the role of MexXY-OprM efflux pump in the resistance of *P. aeruginosa* to AGAs (Morita et al. 2012). In addition, other AGA efflux pumps were identified in Gram-negative (VcmB in *Vibrio cholerae*) (Begum et al. 2005), Gram-positive (LmrA in *Lactobacillus lactis*) (Poelarends et al. 2002), and mycobacteria (Rv1258c in *M. tuberculosis*) (Balganesh et al. 2012). In a recent work, overexpression of MexXY in *P. aeruginosa* was associated with reduced aminoglycoside susceptibility and development of chronic lung infections in patients with multidrug-resistant cystic fibrosis (Singh et al. 2017). In Gram-negative bacteria, aminoglycosides are expelled mainly by an intrinsic 3-component AcrAD-TolC-type efflux pump (classified in the resistance-nodulation-division (RND) family of efflux pumps), which comprises the drug–proton membrane antiporter AcrD, the membrane fusion protein AcrA, and the outer membrane component TolC (Nikaido 2011). Although this is the main type of transporter, AGAs may be a substrate for efflux pumps of different families (extensively reviewed in Li et al. (2015)) The crystal structure of AcrD, shown to capture aminoglycosides from both the periplasm and the cytoplasm (Aires and Nikaido 2005), was reported (unpublished, PDB accession code: 4r86), however, in the absence of an aminoglycoside substrate. A cryo-electron microscopy structure of the AcrAB-TolC multidrug efflux pump from *E. coli*, in resting and drug transport states, has been recently reported, increasing the knowledge on the assembly and operation of the AcrAB-TolC pump (Wang et al. 2017). Comparison with the known structure of the RND multidrug transporter AcrB (e.g. PDB accession code: 5eno (Sjuts et al. 2016)) revealed that large periplasmic loops of AcrD seem to play a role in AcrD's selectivity for aminoglycosides (Elkins and Nikaido 2003). In a recent work (Ramaswamy et al. 2017), apart from the flexible protein loops, selectivity differences may be attributed to a higher lipophilicity of AcrB. Furthermore, as described by Nakashima and colleagues (2011) for the 3D structures of AcrB bound to the high-molecular-mass drugs rifampicin and erythromycin, these transporters enclose two substrate binding pockets: a proximal multisite binding pocket (accommodating high-molecular-mass drugs) and a phenylalanine cluster region (distal pocket, accommodating low-molecular-mass drugs), separated by a loop. According to the authors, the remarkably broad substrate recognition of AcrB is due to the existence of these two distinct high-volume multisite pockets. A mutagenesis study demonstrated that the MexY transporter from *P. aeruginosa* is capable of binding aminoglycosides (Lau et al. 2014), but comparison with a homology model for MexY produced from the 3D structures of AcrB and MexB (PDB accession code: 3w9j) explains that although similar, MexY cannot be inhibited by pyridopyrimidine derivatives. This is due to the presence of a tryptophan residue in MexY, instead of the phenylalanine in AcrB (Nakashima et al. 2013). Structural characterization of AcrD and MexY or many other efflux pumps known to bind aminoglycosides is needed. Current