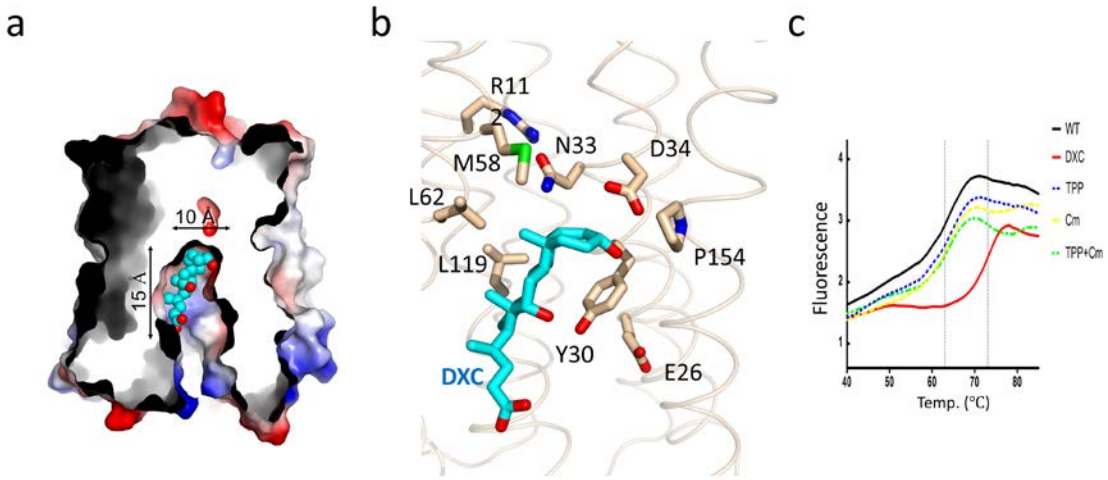

Figure S6. Binding of deoxycholate with MdfA

(a) Binding of the deoxycholate (DXC) in the central cavity observed in the 2.0-Å crystal structure. MdfA is shown in a cross-cut surface model, and deoxycholate is shown in a cyan sphere model. (b) Stick model of the deoxycholate binding site. (c) Changes in MdfA thermal stability upon ligand binding. An increase in fluorescent signal is associated with thermal denaturation of the MdfA transporter. The experiment was repeated three times, and representative curves are shown.

Notes: The negatively charged deoxycholate was used as an additive in the initial crystallization condition, and improved crystal quality. It was observed inside the cavity of the C_{In} crystal structure, sealing the inter-domain interface between TMs 2 and 11. To verify the effects of deoxycholate in solution, we performed the thermal denaturation analysis with MdfA in both the presence and absence of deoxycholate. The results showed that 100 μM sodium deoxycholate increased the melting temperature (Tm) from 63°C to 73°C at pH 6.0. In contrast, two other known substrates of MdfA, cationic tetraphenyl-porphyrin (TPP^+) and electroneutral chloramphenicol (both at 200 μM), did not shown any significant effect (individually or combined) on the thermal stability of MdfA. Together, these data confirmed that deoxycholate stabilizes MdfA against thermal denaturation.