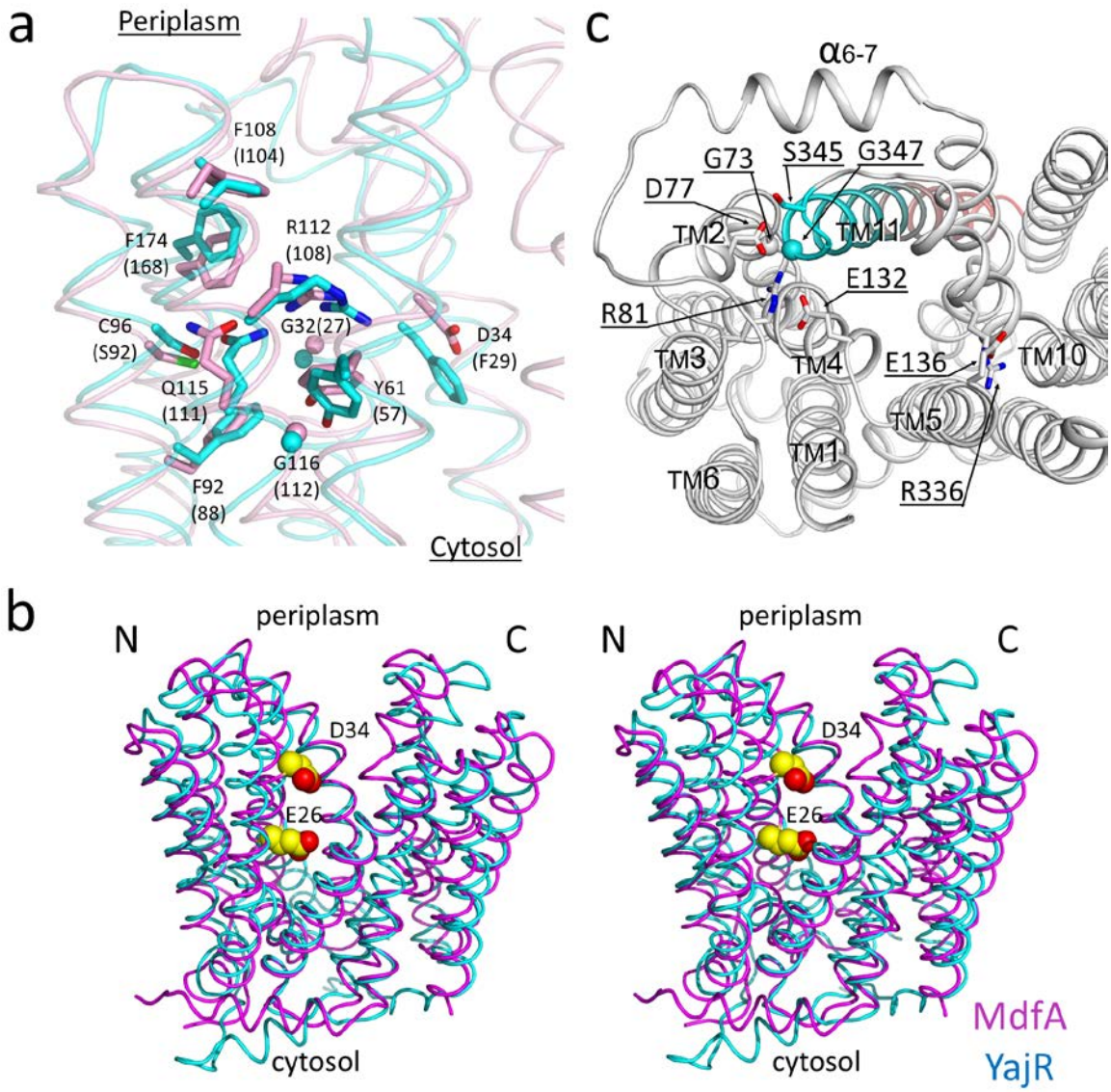

Figure S3. Structural comparison between MdfA and YajR.

(a) Superposition of motif-B from MdfA (magenta) and YajR (cyan; PDB ID: 3WDO). Side chains of the residues of the "3D motif-B" are shown as stick models and labelled. YajR numbers are in parentheses. *(b)* A putative model of the C_{Out} conformation of MdfA. Structure superposition of MdfA and YajR is shown in stereo view. The N- and C-domains of MdfA are individually superimposed on the corresponding domains of YajR in its C_{Out} conformation. The rmsd is 1.7 Å for 153 Cα-atom pairs between the N-domains of MdfA and YajR (using a 3.0-Å cut-off), and that between the C-domains is 2.0 for 132 Cα-

atom pairs. The titratable residues E26 and D34, both inside the cavity of MdfA, are shown as sphere models. *(c)* Modelling of the motif-A of MdfA in the C_{Out} state. D77 and the N-terminal of TM11 form a charge-dipole interaction, potentially stabilizing the C_{Out} state. The helix dipole of TM11 is shown in a colour gradient, from the N-terminal in cyan to the C-terminal in red. D77, R81, and E132 form a charge-relay triad, potentially regulating the charge-dipole interaction. Similar interactions were first reported in the crystal structure of the C_{Out} state of YajR [6].