



Figure S2. The WebLogo representation of MFS transporters.

It was generated by the PFAM web-server [16] using 195 “seed” sequences from the MFS_1 subfamily (PF07690). The larger the letter is at a given position, the more conservative the position is. Pink regions indicate potential insertions. The residue numbers at the bottom are from ecMdfA. Blue labels indicate that the corresponding region is associated with certain motifs in the 3D structure. The cytosolic and periplasmic ends of each TM helix are in red and cyan, respectively. For each TM helix, the TM region is divided into five regions with short white lines. The following points may be noteworthy:

- 1) Insertions (pink regions) occur more often in regions that connect secondary structures.
- 2) The N-domain has more conserved features than the C-domain.
- 3) The most conserved residue is W170 in the TM6.1 region, and another conserved Trp (W) is located at TM7.5. Their roles may include anchoring the terminal position of the TM helices at the solvent-membrane interface.

- 4) Motif-A exists in both L2-3 and L8-9 regions. Motif-A₂₋₃ may be accompanied by D/E132^{TM4.5}, R/K346, G347^{TM11.5}, and G/A351^{TM11.5}; and motif-A₈₋₉ does not appear to have a charge-relay, but is possibly accompanied by R(139C) in MdfA, G(140I), and G(144A) in the TM5.5 region. For example, in YajR (containing motif-A₈₋₉) [6], R133 (139C) is located in the domain interface in the C_{Out} state.
- 5) Of cavity-helices (*i.e.* TMs 1, 4, 7, and 10), TM4 is the most conserved and contains the motif-B. The motif-B appears co-existing with Y61^{TM2.3} and F174^{TM6.2}.
- 6) Motif-C may exist in both TMs 5 and 11.
- 7) Among conserved residues, G/A324^{TM10.2}-G328^{TM10.3} packs against A267^{TM8.3}-G271^{TM8.3}, and P243^{TM7.2} packs with P38^{TM1.2} in the C_{In} state.