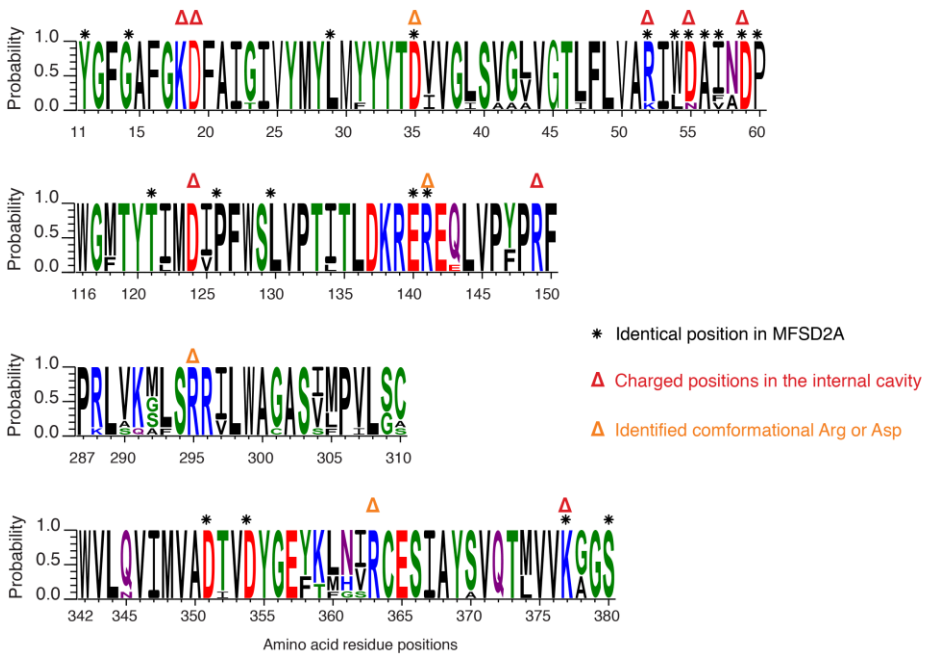
a

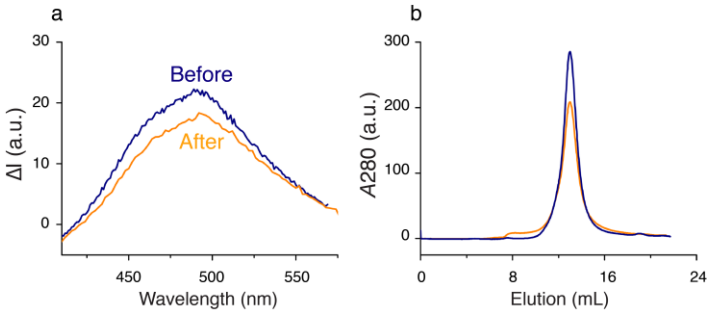


b

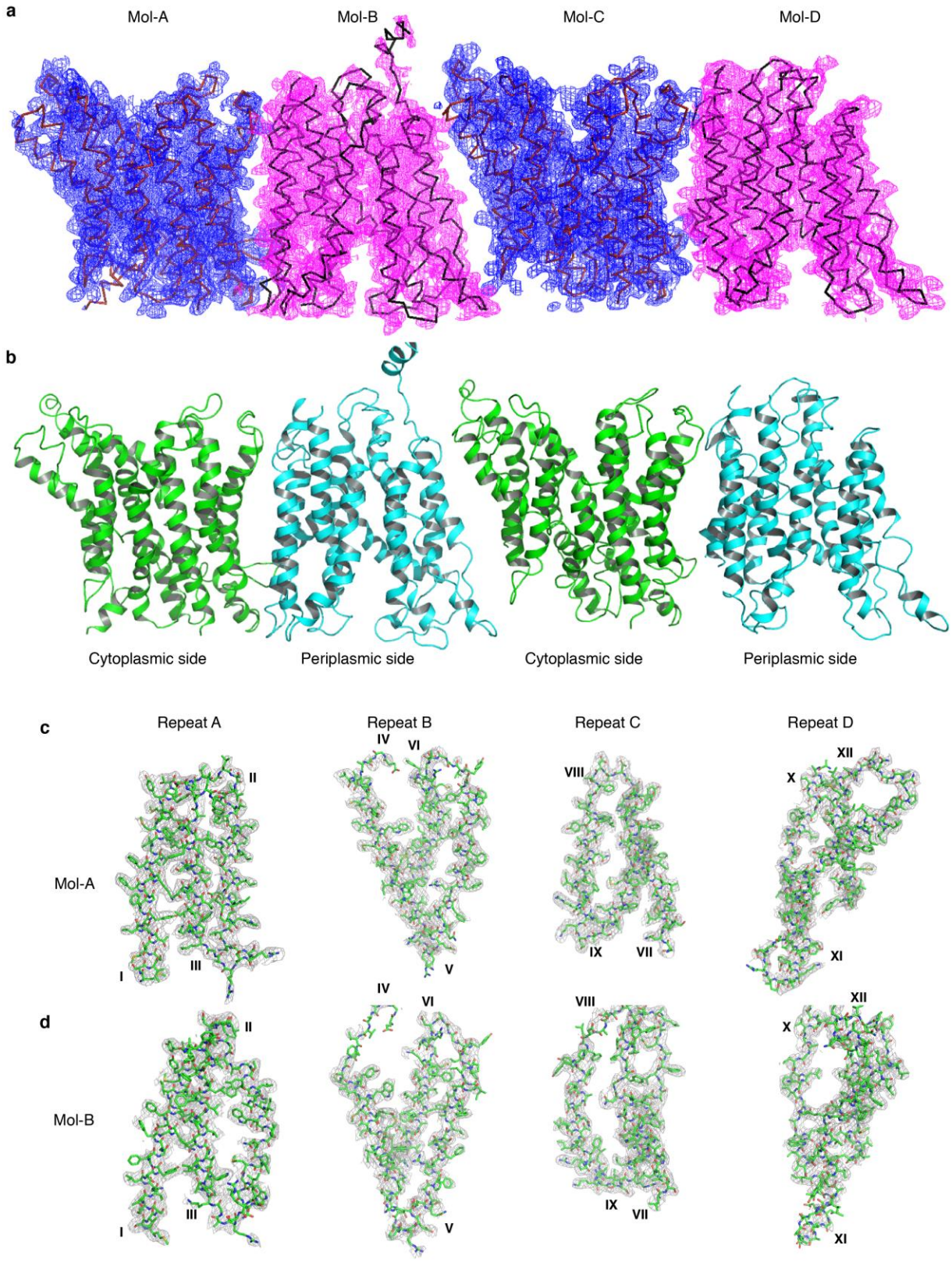
	Cation-binding site	L-2
MelB_*Salmonella typhimurium*	48-FLVARIWDAINDP-60	120-YTIMDIPFWSLVPTITLDKREREQLVPFPR-149
MelB_*Klebsiella pneumoniae*	48-FLVARILDAIADP-60	120-YTIMDIPFWSLVPTITLDKREQLVPYPRFF-149
MelB_*Enterobacter aerogenes*	48-FLVARILDAIADP-60	120-YTIMDIPFWSLVPTITLDKREREQLVPYPR-149
MelB_*E.coli*	48-FLVARIWDAINDP-60	120-YTIMDIPFWSLVPTITLDKREREQLVPYPR-149
MelB_*Shigella flexneri*	44-FLVARIWDAINDP-56	120-YTIMDIPFWSLVPTITLDKREREQLVPYPR-145
MelB_*Salmonella typhi*	48-FLVARIWDAINDP-60	120-YTIMDIPFWSLVPTITLDKREREQLVPFPR-149
MelB_*Salmonella paratyphi*	48-FLVARIWDAINDP-60	120-YTIMDIPFWSLVPTITLDKREREQLVPFPR-149
MelB_*Citrobacter freundii*	48-FLVAKIWNAFNDP-60	120-YTIMDIPFWSLVPTITLDKREREQLVPYPR-148
MelB_*Vibrio shilonii*	48-FLVARIWDAVNDP-60	120-YTLMDIPFWSLVPTLTLDKREREELVPYPR-149
LacS_*Streptococcus thermophilus*	60-ISILRILEVFIDP-72	135-YSIKDIGFWSMLPALSLDSHEREKMATFAR-164
GalP_*Lactocaccus lactis*	55-VVIIRLVEIIFDP-68	133-YSFKDIAFWSMIPALSEKNSERETLGTFAR-162
XylP-*Lactobacillus pentosus*	72-FLVARIVDAFDGP-84	144-YSAVNIPITSILPSLTSNPQERVTLSTIRQ-173
GusB_*E.coli*	50-LLLVRVFDAFADV-62	123-YSLVNIPYGSLATAMTQQPQSRARLGAARG-152
TogT_*Klbesiella pneumoniae*	45-FSVASIIDAISNP-57	116-YTSIMVPYETLATEMTDDFSLRSKLTGYKA-145
Sucrose transporter 1_*Zea mays*	76-WLCGPIAGLVVQP-88	161-DFSNNTVQGPARAMMADLCGHEGPSAANSI-190
MFSD2A_*Homosapiens*	99-LFVGRAWDAITDP-111	171-VTCFHVPYSALTMFISTEQTERDSATAYRM-200
MFSD2B_*Homosapiens*	84-LFGGKVSGAAADP-96	156-ATFFQVPYTALTMLLTPCPRERDSATAYRM-185

	L-1	L-2	L-3
MelB_*Salmonella typhimurium*	287-PRLVKMLSRRI-297	351-DTVDYGEFKLNIRCE-365	
MelB_*Klebsiella pneumoniae*	287-PRLVKGLSRRI-297	351-DTVDYGEYTMNIRCE-365	
MelB_*Enterobacter aerogenes*	287-PRLVKGLSRRI-297	351-DTVDYGEYTMNIRCE-365	
MelB_*E.coli*	287-PRLVKSLSRRI-297	351-DIVDYGEYKLHVRCE-365	
MelB_*Shigella flexneri*	283-PRLVKSLSRRI-293	347-DTVDYGEYKLHVRCE-361	
MelB_*Salmonella typhi*	287-PRLVKMLSRRI-297	351-DTVDYGEFKLNIRCE-365	
MelB_*Salmonella paratyphi*	287-PRLVKMLSRRI-297	351-DTVDYGEFKLNIRCE-365	
MelB_*Citrobacter freundii*	286-PRLSKALSRRV-296	350-DTVDYGEYKFNVRCE-364	
MelB_*Vibrio shilonii*	287-PKLAQMFSRRV-297	351-DTVDYGEYKLGSRCE-365	
LacS_*Streptococcus thermophilus*	305-PSLAGKFNRKR-315	365-DSVEYGQWKTGHRDE-379	
GalP_*Lactocaccus lactis*	300-PTLAKKFGRRK-310	359-DSVEYGQWKNGVRNE-373	
XylP_*Lactobacillus pentosus*	308-PWTAKRIGKRN-318	370-DSVDYGEWKNGVRAE-384	
GusB_*E.coli*	281-APLVPGMVARI-291	346-DTVEYGEYLTGVRIE-360	
TogT_*Klbesiella pneumoniae*	289-GLCVKKGFSKP-299	358-DVDEIYTGRREGIY-372	
Sucrose Transporter 1_*Zea mays*	332-QISAFDEGVRV-342	398-DYHGYVQDAITASTS-412	
MFSD2A_*Homosapiens*	361-QWFLTRFGKKT-371	421-DVIDDFHLKQPEFHG-435	
MFSD2B_*Homosapiens*	337-EWVLQRFGKKT-346	395-DVVDDFQLQHRHGPG-409	

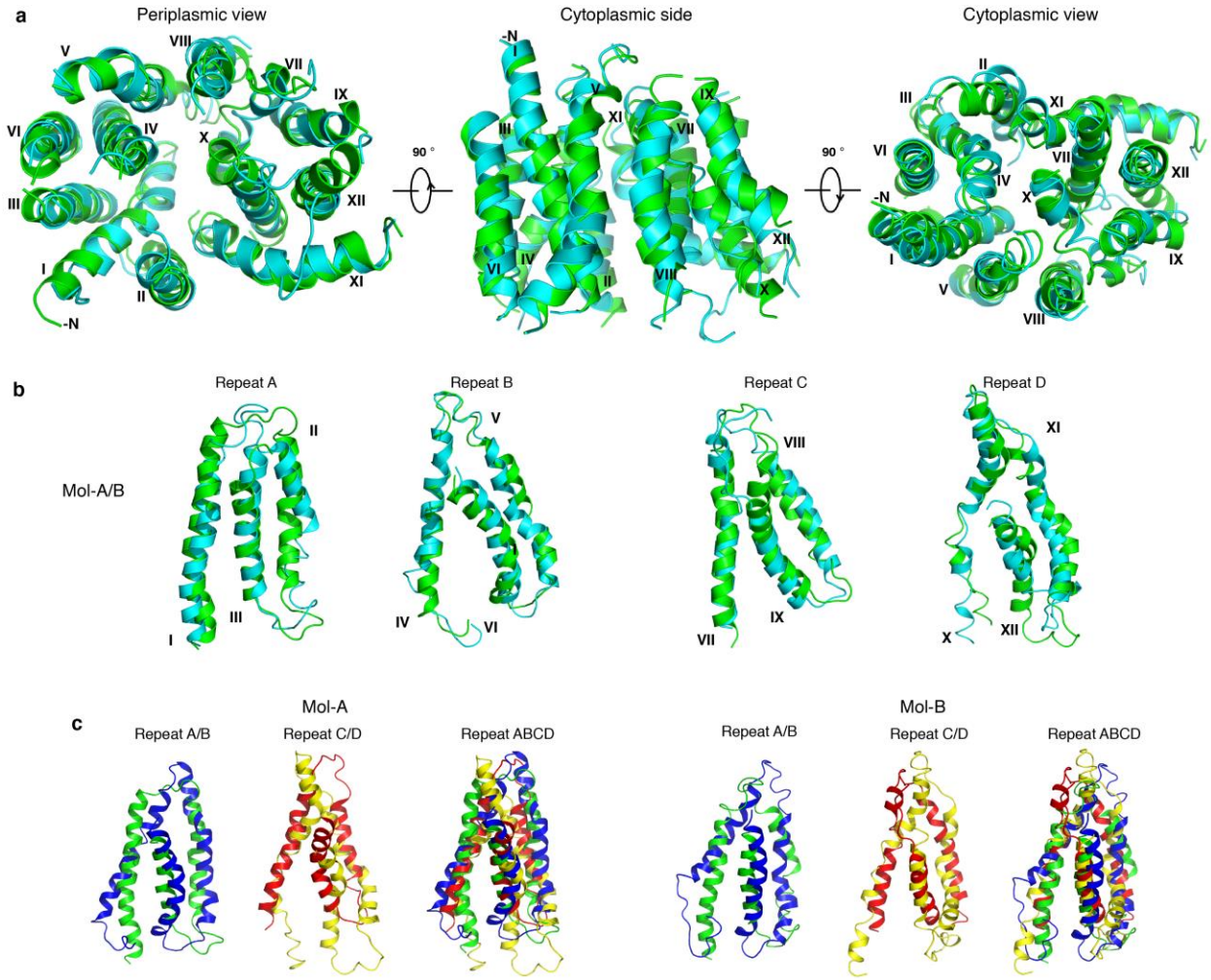
Supplementary Figure S1 | Sequence alignment. **a**, MelB orthologues with MFSD2A. Four stretches (11-60, 116-150, 287-310, 342-360) of primary-sequence alignments of $MelB_{St}$ (Gene ID: 16767549) with other MelB orthologues from *Klebsiella pneumoniae,* GI:150957831; *Enterobacter aerogenes*, GI:3914018; *E. coli*, GI:353526256; *Salmonella typhi,* GI:20141536; *Shigella flexneri,* GI:81723953; *Salmonella paratyphi*, GI:81360816; *Citrobacter freundii*, GI:75416057; *Vibrio shilonii,* Uniprot:A6CV97; MFSD2A, GI: 74751132 (Weblogos3.0). **b**, MelB with other GPH members. $MelB_{St}$ (GI: 16767549) orthologues include *Klebsiella pneumoniae*, GI:150957831; *Enterobacter aerogenes,* GI:3914018; *E. coli,* GI:353526256; *Shigella flexneri*, GI:81723953; *Salmonella typhi,* GI:20141536; *Salmonella paratyphi*, GI:81360816; *Citrobacter freundii,* GI:75416057; *and Vibrio shilonii,* Uniprot:A6CV97. MelB homologues of GPH family include LacS of *Streptococcus thermophiles,* GI:386345083; GalP of *Lactococcus lactis*, GI:4995688; XylP of *Lactobacillus pentosus,* GI:3688059; GusB of *E. coli*, GI:292630909; Oligogalacturonide transporter (TogT) of *Klebsiella pneumonia*, GI:206580788; Sucrose transporter 1 of *Zea mays*, GI:162463612; human MFSD2A, GI:74751132; and human MFSD2B, GI:298286913. Paired alignment of four stretches of primary sequences between $MelB_{St}$ and each protein were done manually. The three Asp residues (55, 59 and 124) important for Na^+ binding and three Arg (295, 141 and 363) and two Asp residues (351 and 354) important for lock interactions in $MelB_{St}$ are highlighted.



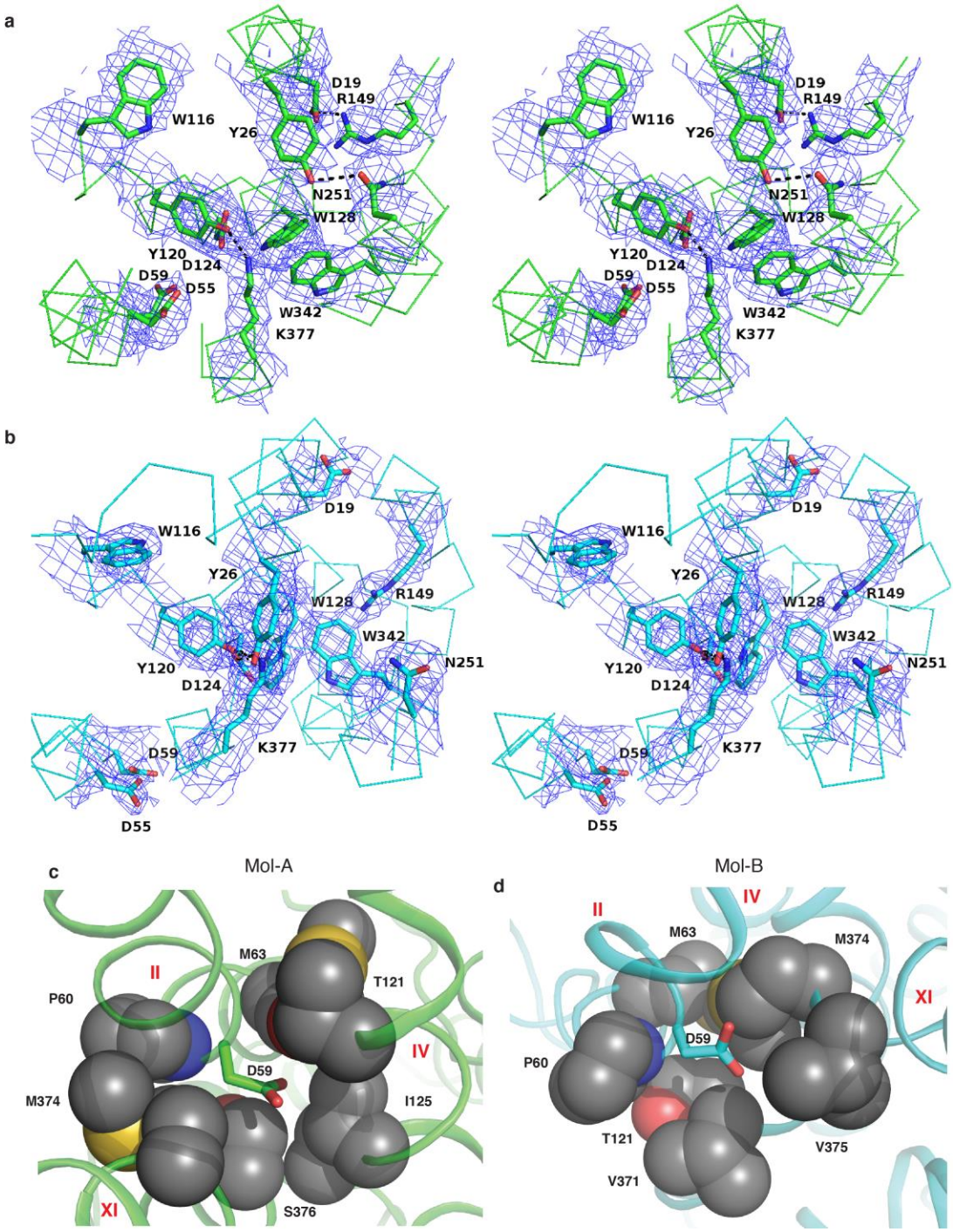
Supplementary Figure S2 | Stability of MelB$_{St}$. Purified MelB$_{St}$ proteins were incubated at 23°C for five days, and the samples before (blue) and after (orange) incubation were tested for stability. **a**, D^2G binding. D^2G FRET emission spectra (ΔI) obtained by displacement of D^2G with 50 mM melibiose. **b**, Gel filtration chromatography profiles (100 μg MelB$_{St}$).


a
Mol-A
Mol-B
Mol-C
Mol-D
b
Cytoplasmic side
Periplasmic side
Cytoplasmic side
Periplasmic side
c
Repeat A
Repeat B
Repeat C
Repeat D
Mol-A
I
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII
d
Mol-B
I
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII

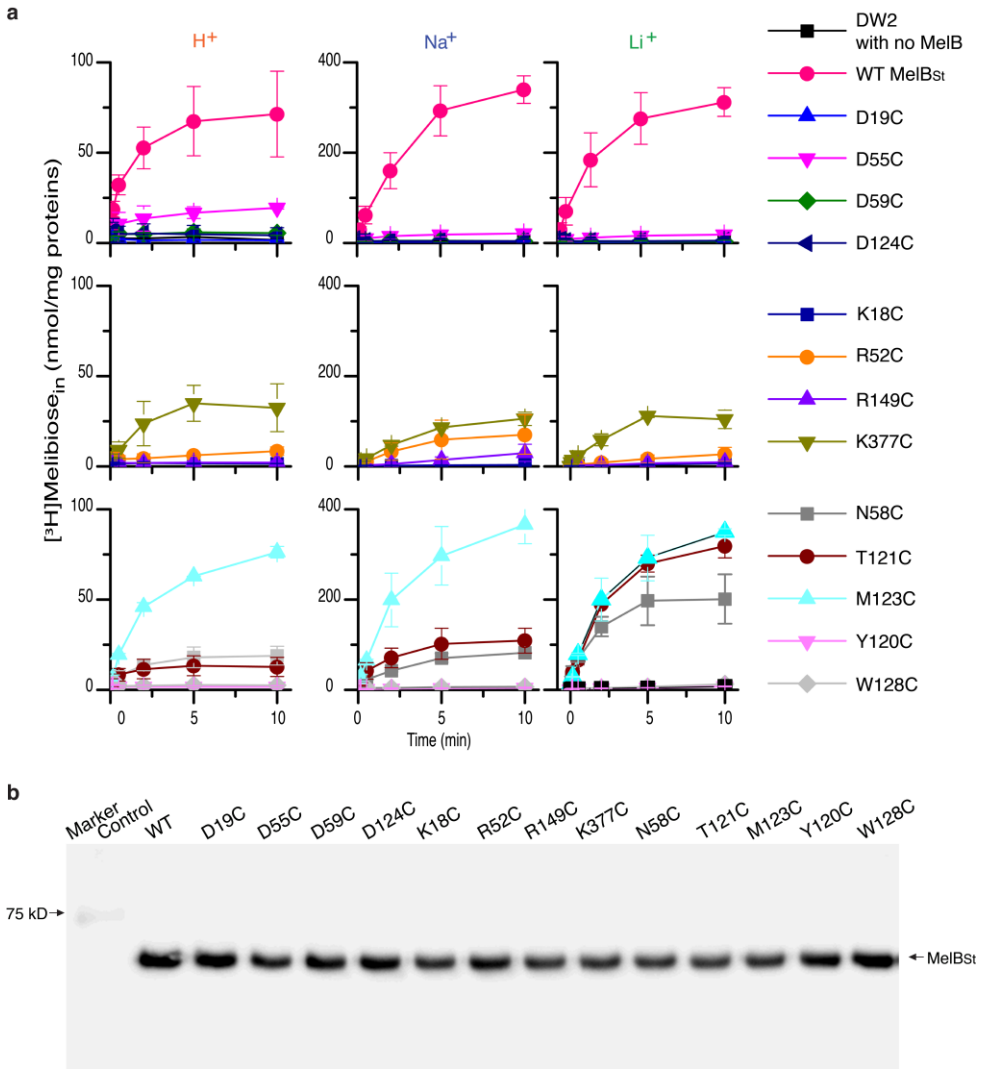
Supplementary Figure S3 | Electron density maps of $MelB_{St}$. a, The 2Fo-Fc maps of four molecules of $MelB_{st}$ contoured at 1.0 σ. Mol-A and Mol-C (blue mesh) and Mol-B and Mol-D (pink mesh) are related by 0.5 pseudo-translational symmetry along the *c* axis. **b**, The overall fold of Mol-A and Mol-C (green), Mol-B and Mol-D (cyan) in the asymmetric unit are shown as cartoon models. The N-terminal domain is on the left side of each molecule. **c and d,** The 2Fo-Fc electron density maps contoured at 1.0 σ for helices in each repeats. The helices are oriented for clear views of the density maps.



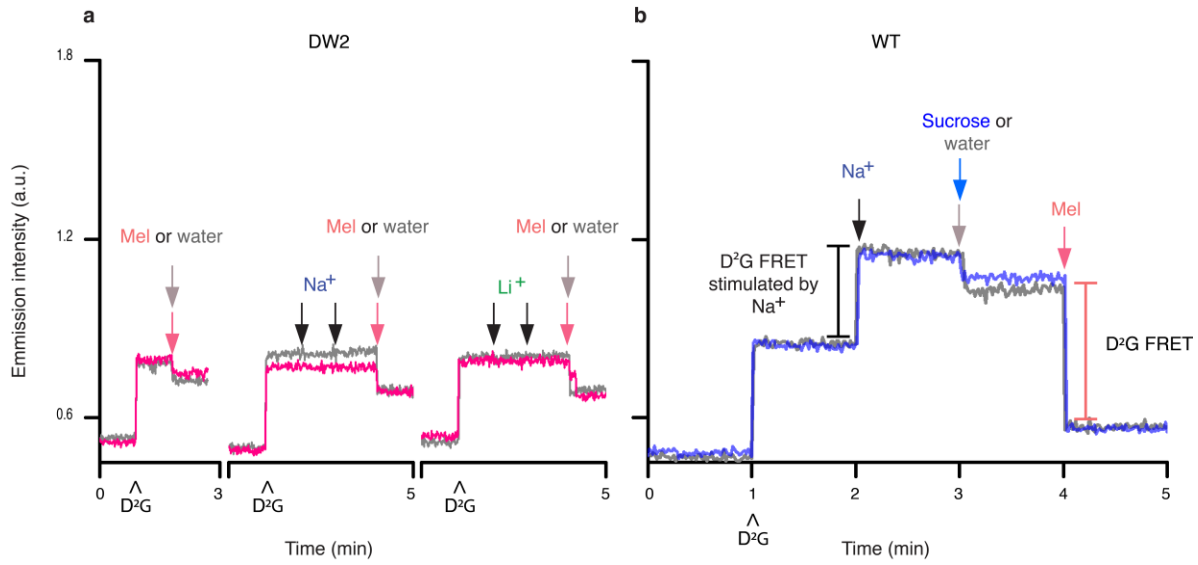
Supplementary Figure S4 | Repeats of $MelB_{St}$. **a**, Superposition of the overall structure of Mol-B (cyan) on Mol-A (green) viewed parallel or perpendicular to the membrane plane. **b**, Superposition of the overall structure of Mol-B (cyan) on Mol-A (green) is presented by four separate repeats. **c**, Superposition of repeats.



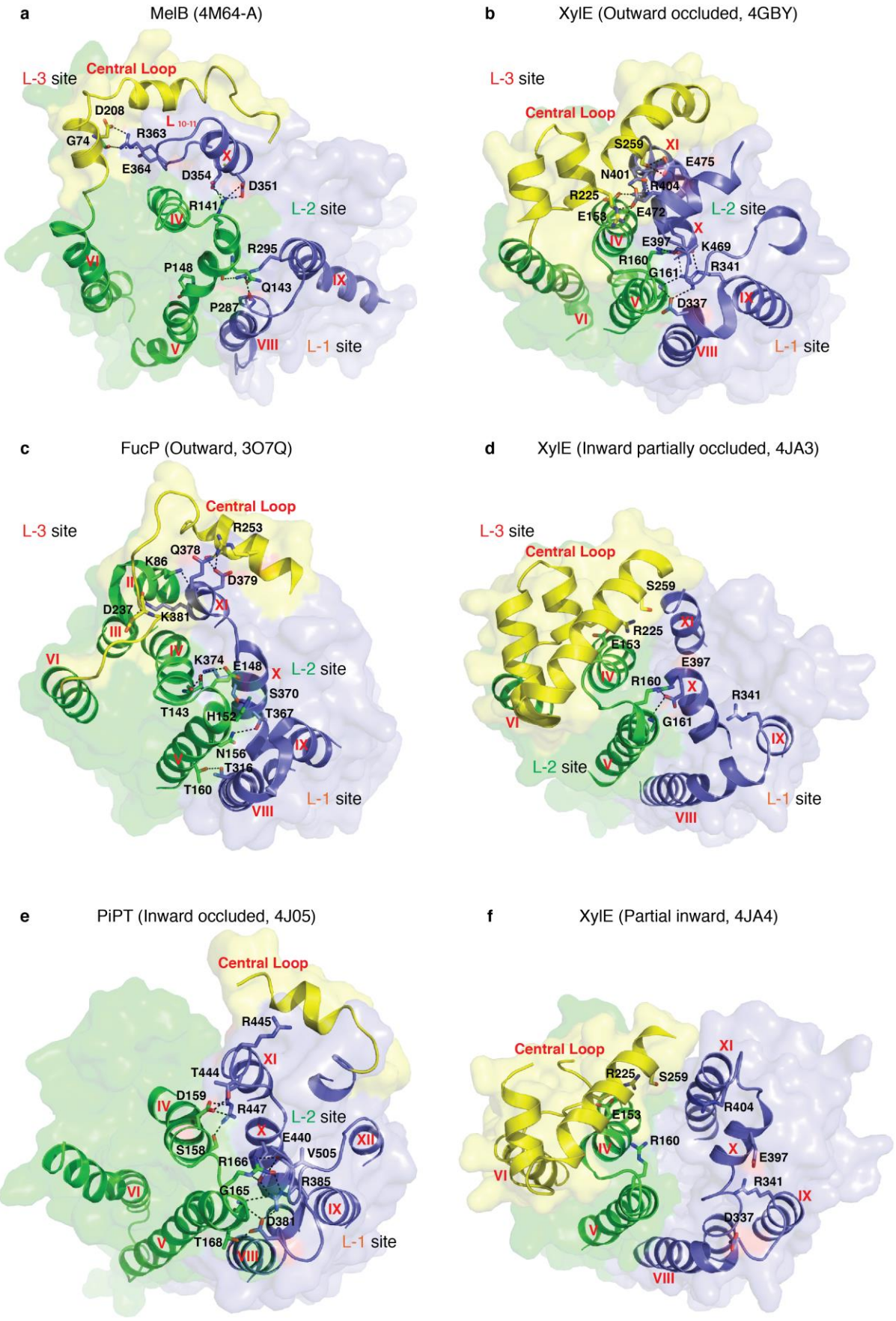
Supplementary Figure S5 | The internal cavity. Wall-eyed stereo view of the 2Fo-Fc maps of the cation- and sugar-binding residues in Mol-A (**a**) and Mol-B (**b**) contoured at 1.0 σ. Asp^{59} is surrounded by hydrophobic residues in Mol-A (**c**) and in Mol-B (**d**).



Supplementary Figure S6 | Mutational analysis. a, Melibiose transport time course. Intact *E. coli* DW2 cells (*mel*A$^+$, Δ*mel*B, Δ*lac*ZY), over-expressing WT $MelB_{St}$ or a given single-site Cys mutant, were assayed with [^{3}H]melibiose transport (0.4 mM) in the absence or presence of 20 mM NaCl or LiCl. Error bar, s.e.m., n = 2 for mutants and n = 10 for the WT and DW2. **b, Western blotting**. 25 μg of RSO membranes were loaded onto each well of SDS-16% PAGE. $MelB_{St}$ proteins were detected with anti-His tag antibody, and imaged by the *ImageQuant LAS 4000 Biomolecular Imager*. Protein marker with 75 kD is loaded on the same well with the negative control sample that contains the RSO vesicles prepared from DW2 cells without MelB.



Supplementary Figure S7 | Control experiments for Trp→D^{2}G FRET. Experimental setup was identical as described in Fig. 3d. **a, The negative control experiments.** RSO vesicles prepared with DW2 cells in the absence of MelB were used as the negative control. The assay was carried out under identical protocol as described in Fig. 3d. **b, Specificity**. RSO vesicles prepared with DW2 cells in the presence of $MelB_{St}$ were used as the positive control. After the addition of 10 μM D^{2}G, 20 mM NaCl, 120 mM sucrose or the same volume of water were added step by step. For both traces, 120 mM melibiose was added at the last step. The Na^+ stimulation of D^{2}G FRET and melibiose displacement of D^{2}G bound with $MelB_{St}$ are illustrated by black and pink blunt-ended bars, respectively.


a
MelB (4M64-A)
L-3 site
Central Loop
D208
L 10-11
R363
G74
E364
D354
D351
R141
L-2 site
R295
P148
Q143
P287
L-1 site
b
XylE (Outward occluded, 4GBY)
L-3 site
Central Loop
S259
E475
N401
R404
R225
E158
E472
L-2 site
E397
K469
R160
R341
G161
D337
L-1 site
c
FucP (Outward, 3O7Q)
L-3 site
Central Loop
R253
Q378
K86
D379
D237
K881
K374
E148
L-2 site
S370
T143
H152
T367
N156
T316
T160
L-1 site
d
XylE (Inward partially occluded, 4JA3)
L-3 site
Central Loop
S259
R225
E153
E397
R160
R341
G161
L-2 site
L-1 site
e
PiPT (Inward occluded, 4J05)
Central Loop
R445
T444
D159
R447
L-2 site
E440
S158
V505
R166
R385
G165
D381
T168
L-1 site
f
XylE (Partial inward, 4JA4)
Central Loop
R225
S259
E153
R404
R160
E397
R341
D337

Supplementary Figure S8 | Locks in MFS crystal structures. Surface presentation of all structures with an aligned origin: the N- and C-terminal domains are shown in green and blue, respectively, with the central loop in yellow. The helices are labeled with Roman numerals. Lock interactions are shown as broken lines.