Supporting Information for Publication:
A structural study of ion permeation in OmpF porin from anomalous X-ray diffraction and molecular dynamics simulations

Balasundaresan Dhakshnamoorthy, Brigitte K. Ziervogel, Lydia Blachowicz and Benoît Roux

Table S1. Data collection and Refinement Statistics

	0.3MKBr-OmpF	0.2MKBr-OmpF	0.1MKBr-OmpF	0.2MNaBr-OmpF
Data collection				
Space group	C2	P3	P3	$P2_12_12_1$
Unit cell				
a (Å)	200.43	116.981	116.632	76.697
b (Å)	114.85	116.981	116.632	112.103
c (Å)	53.13	51.411	51.253	131.169
α (°)	90.00	90.0	90.00	90.0
β (°)	96.92	90.0	90.00	90.0
γ (°)	90.00	120.0	120.00	90.0
Solvent content (%, v/v)	54.97	53.70	53.28	49.97
No. molecules in ASU	3	2	2	3
Resolution range	50-2.09 (2.14-2.10)	50-2.24 (2.24-2.20)	50-1.90 (1.90-1.93)	50-2.10 (2.14-2.10)
No.independent reflections	220945	137909	285238	317931
Redundancy	3.1 (3.1)	3.4 (3.4)	4.6 (4.6)	5.0 (5.1)
Completeness (%)	98.7 (99.6)	99.9 (100)	100.0 (100.0)	97.5 (97.4)
R_{merge}	11.7 (65.4)	14.0 (79.2)	9.8 (78.7)	12.7 (49.2)
Refinement and model correction				
Resolution (Å)	2.09	2.2	1.96	2.10
No. reflections used for refinement	68077	40189	61336	61278
R factor (%)	18.9(26.8)	24.7(30.7)	26.7	22.86(28.0)
No. reflections used for R_{free}	3611	2779	6099	3269
R_{free} (%)	24.53(32.8)	29.85(33.0)	29.8	28.9(35.3)
Average B-factor ($Å^2$)	34.36	37.51	36.2	26.31
RMSD from ideal geometry				
Bond lengths (Å)	0.019	0.015	0.015	0.016
Bond angles (°)	2.117	1.740	1.503	1.681
No. of Br^- atoms	8	12	7	8
No. of Mg^{2+} atoms	8	1	-	6
No. of PEG molecules	11	-	-	4
No. of Glycerol molecules	4	-	2	-
No. of Detergent molecules	2		2	2

Table S2. Cl^--dominated interactions with OmpF residues from all-atom MD with 1 M KCl

OmpF Residue	OmpFRegion[a]	K^+ Contacts (% trajectory)	Cl^- Contacts (% trajectory)[b]
K25	EL	4.0	40.4
E71	EL	0.4	57.0
G72	EL	–	58.2
A73	EL	8.6	53.6
T165	EL	1.0	66.2
N198	EL	18.8	43.8
S248	EL	2.6	35.0
K279	EL	–	48.0
K281	EL	3.2	69.2
K323	EL	0.2	62.0
K80	EP	–	95.8
Y124	EP	2.2	26.8
S125	EP	0.2	101.6
K160	EP	0.8	22.8
R167	EP	–	159.4
R168	EP	–	237.6
K209	EP	1.0	97.8
K210	EP	0.8	40.8
R235	EP	–	52.6
K253	EP	0.6	67.4
R42	C	–	136.8
R82	C	–	111.0
R132	C	–	73.2
A1	PP	–	21.6
K16	PP	–	115.6
K46	PP	0.2	57.4
K89	PP	0.2	61.4
S142	PP	15.4	38.6
Y302	PP	1.2	38.6
Q339	PP	2.8	37.0
Y4	PT	0.6	41.2
K6	PT	0.8	110.8
K10	PT	1.0	56.0
K305	PT	2.4	110.2

[a]OmpF Region abbreviations: EL, extracellular loops; EP, extracellular pore vestibule; C, constriction zone; PP, periplasmic pore vestibule; PT, periplasmic turns

[b]Since residues can interact with more than one ion, ion contacts along the MD trajectory may be >100%.

Table S3. K^+-dominated interactions with OmpF residues from all-atom MD with 1 M KCl

OmpF Residue	OmpF Region[a]	K^+ Contacts (% trajectory)[b]	Cl^- Contacts (% trajectory)
S24	EL	26.0	–
E29	EL	62.0	21.2
N35	EL	46.8	1.6
E162	EL	51.0	7.6
D164	EL	71.2	7.8
D172	EL	144.2	0.2
D195	EL	144.4	0.8
E201	EL	66.6	3.8
Q203	EL	41.0	13.0
P204	EL	76.8	13.4
G206	EL	92.2	1.0
N207	EL	139.0	18.2
N246	EL	46.4	19.0
D282	EL	65.2	3.0
E284	EL	117.0	16.0
D288	EL	58.2	10.8
D319	EL	62.2	4.4
D321	EL	90.4	6.0
S328	EL	69.2	1.6
D329	EL	21.4	0.8
D330	EL	205.6	0.2
Y32	EP	27.2	–
N316	EP	92.0	–
D113	C	202.4	1.4
L115	C	26.2	0.4
E117	C	59.0	0.4
G119	C	96.4	0.4
G120	C	33.8	0.6
E48	PP	34.2	0.2
E181	PP	50.2	1.0
D221	PP	66.6	0.6
D54	PT	58.4	0.8
D92	PT	68.4	0.6
G146	PT	25.8	0.4
D149	PT	70.2	2.6
E183	PT	79.4	5.0
D266	PT	64.2	6.2

[a]OmpF Region abbreviations: EL, extracellular loops; EP, extracellular pore vestibule; C, constriction zone; PP, periplasmic pore vestibule; PT, periplasmic turns.
[b]Since residues can interact with more than one ion, ion contacts along the MD trajectory may be >100%.

Table S4. Ion interactions with OmpF residues from all-atom MD with 150 mM KCl

OmpF Residue	OmpF Region*	K^+ Contacts (% trajectory)	Cl^- Contacts (% trajectory)
E71	EL	27.4	–
D172	EL	45.4	–
D195	EL	66.4	–
R196	EL	28.6	–
E201	EL	22.4	–
D290	EL	22.0	–
D319	EL	23.8	–
D330	EL	32.8	–
D113	C	27.0	–
E48	PP	26.6	–
E181	PP	48.0	–
K219	PP	34.0	–
D221	PP	22.8	–

*OmpF Region abbreviations: EL, extracellular loops; C, constriction zone; PP, periplasmic pore vestibule

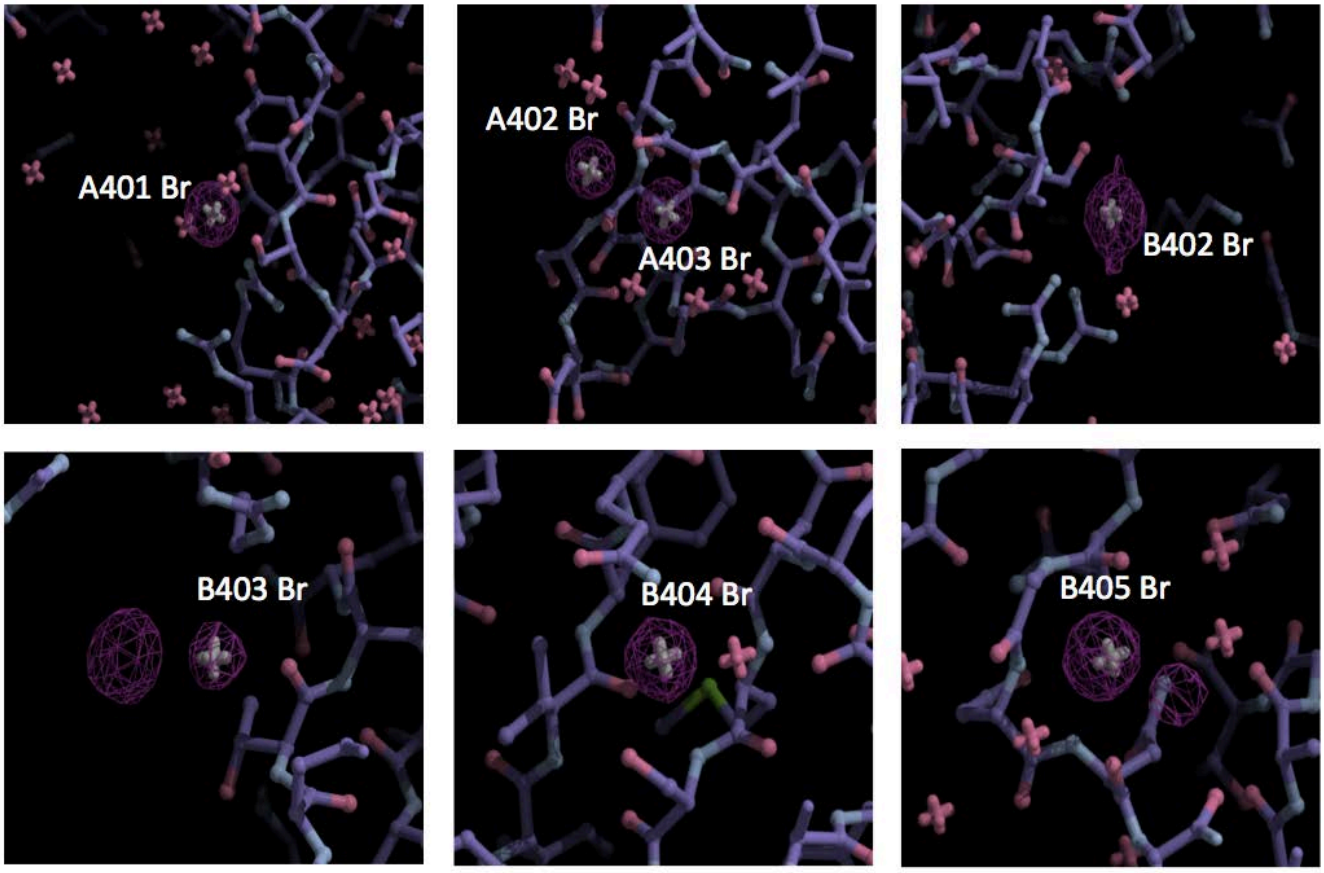
Crystallographic Information File (CIF)

02MNaBrrefine.cif

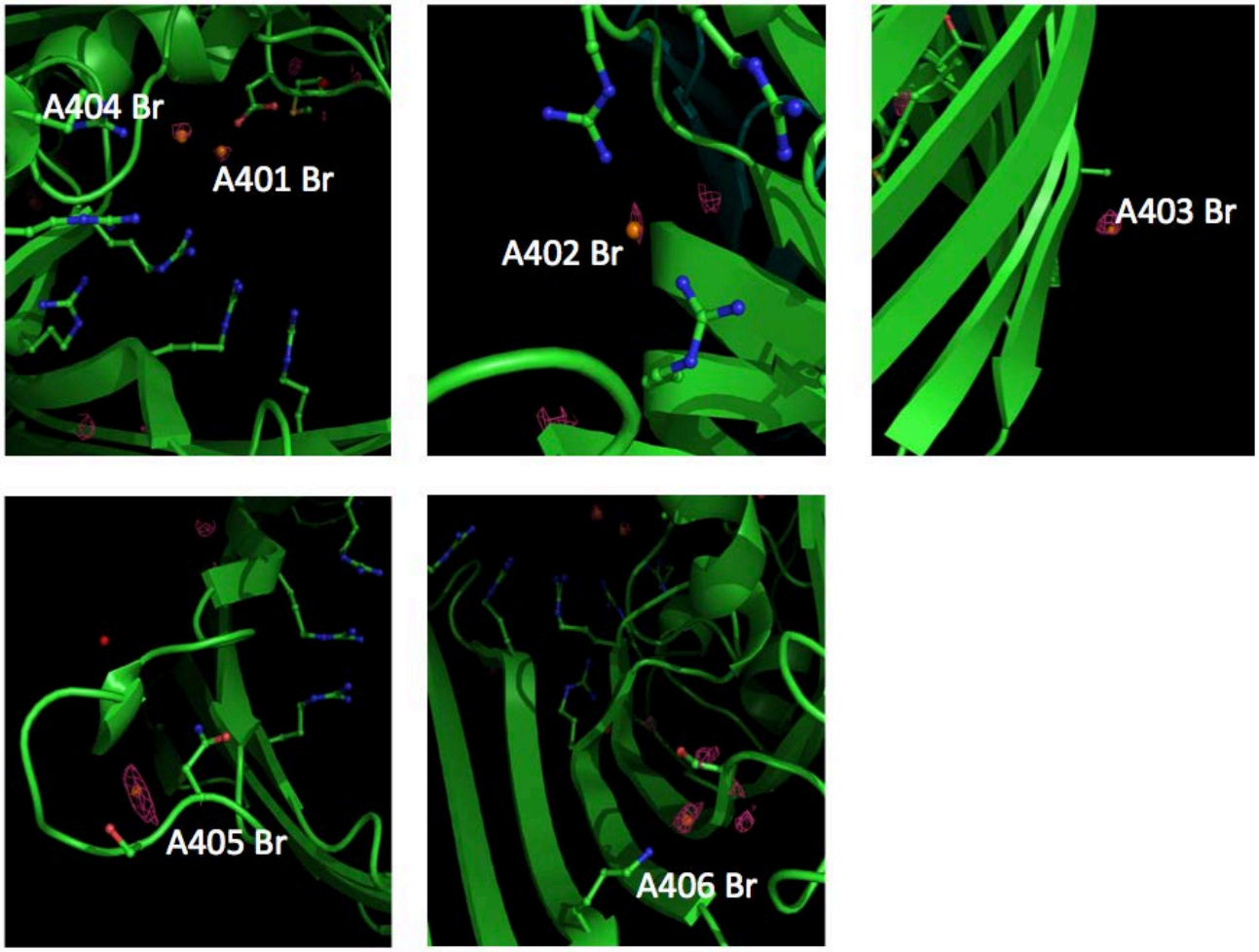
Kbr01mrefine.cif

Kbr02mrefine.cif

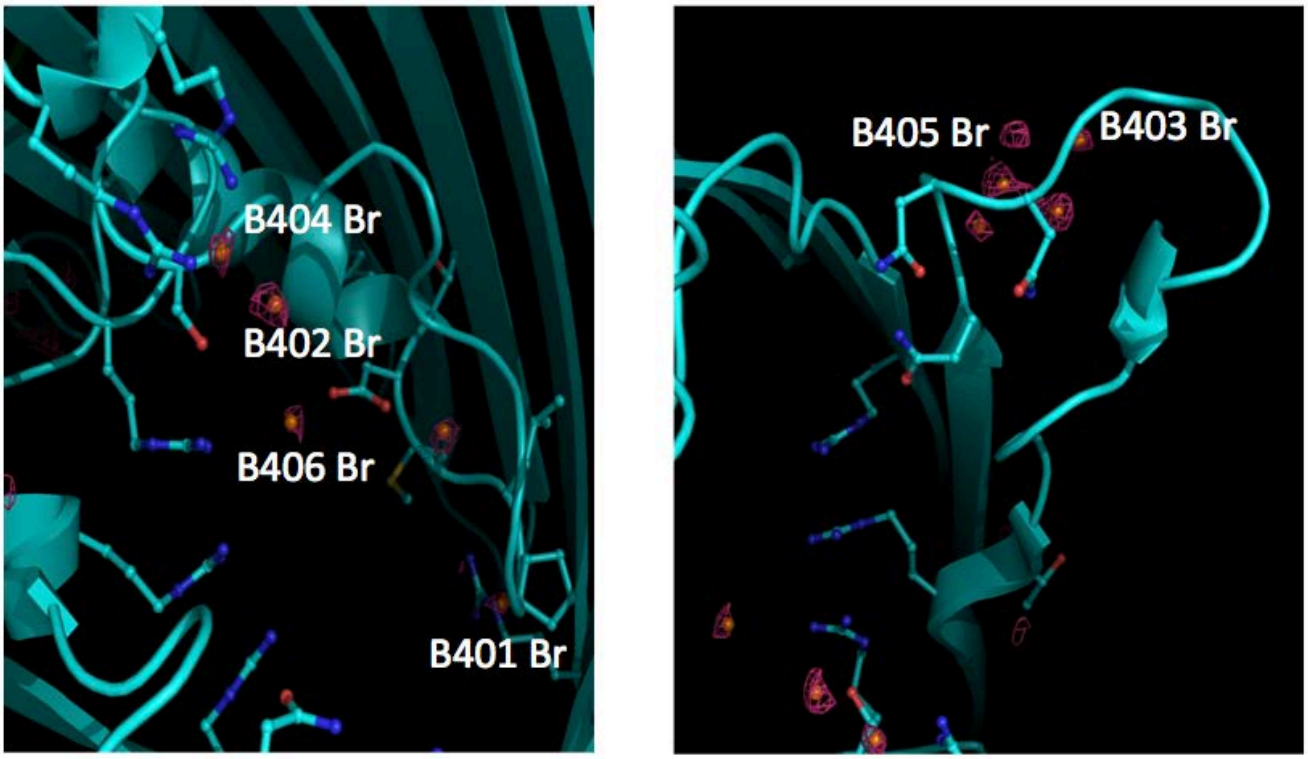
kbr03mrefine.cif



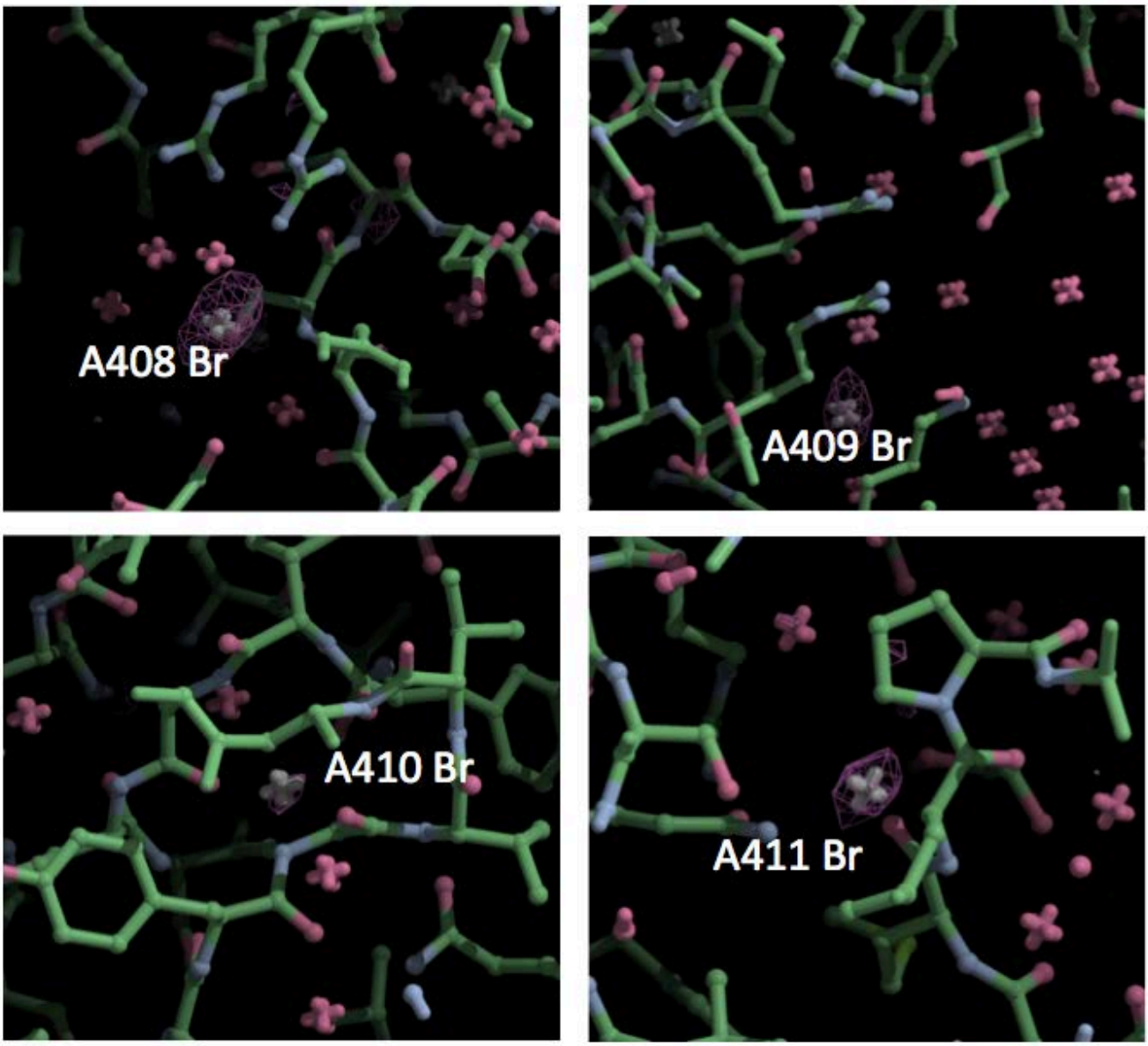
Supplemental Figure S1. A close-up view of the location of Br^- ions in 0.1MKBr OmpF structure for chains A and B. The residues close to the Br^- ions in the OmpF molecule are shown in ball-and-stick representation. The Br^- ions are represented by grey dots and the water molecules are represented by red dots. The Bromide anomalous map for the Br^- ions at 10.0σ contour level is shown by magenta. The figure was prepared using COOT.



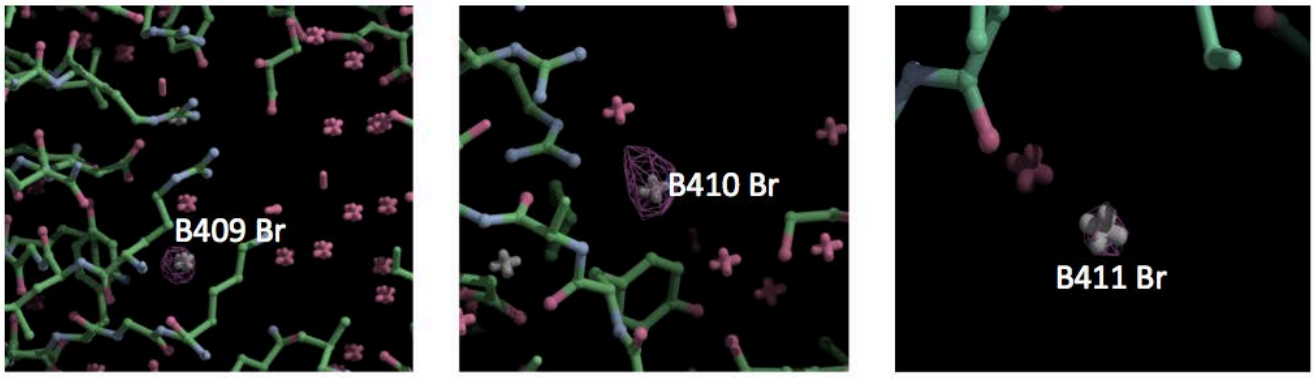
Supplemental Figure S2a. A close-up view of the location of Br^- ions in 0.2MKBr OmpF structure for chain A. The residues close to the Br^- ions in the OmpF molecule are shown in ball-and-stick representation. The Br^- ions are represented by orange spheres. The Bromide anomalous map for the Br^- ions at 4.0σ contour level is shown by magenta. The OmpF molecule for chain A (green) is shown by cartoon representation. The figure was prepared using Pymol.



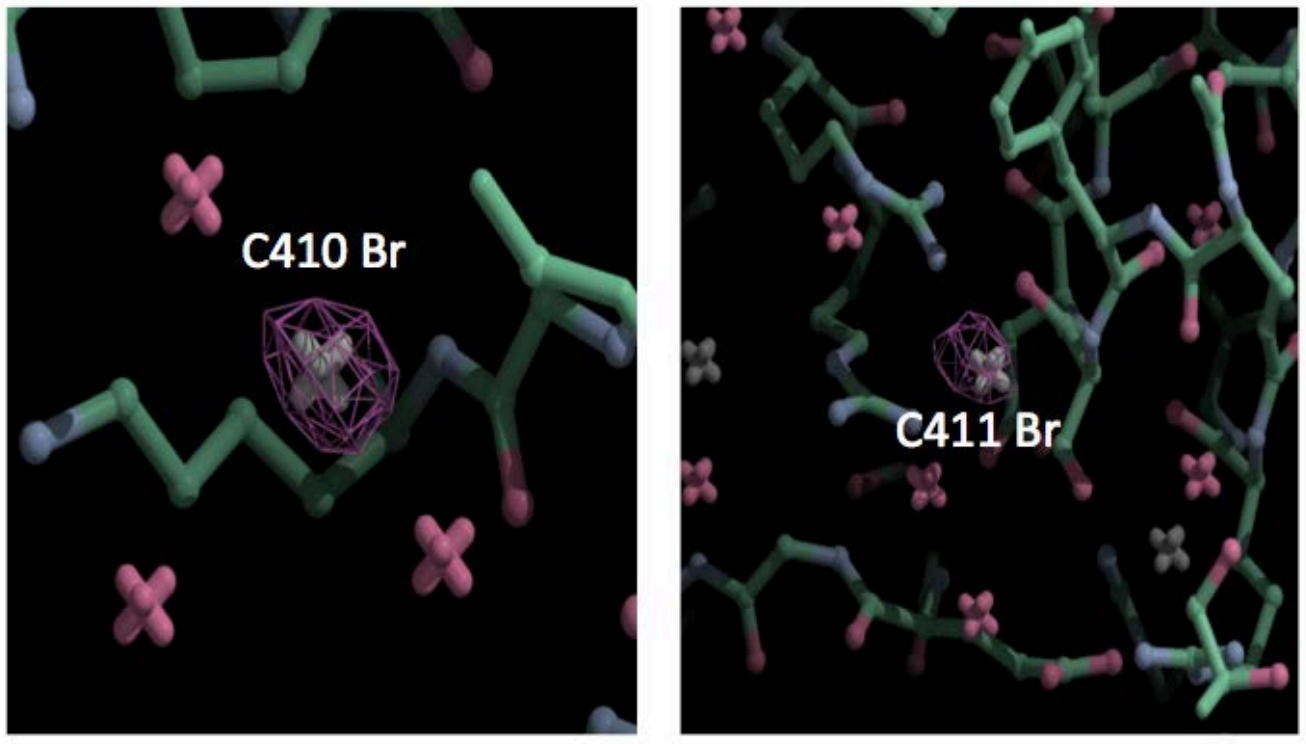
Supplemental Figure S2b. A close-up view of the location of Br^- ions in 0.2MKBr OmpF structure for chain B. The residues close to the Br^- ions in the OmpF molecule are shown in ball-and-stick representation. The Br^- ions are represented by orange spheres. The Bromide anomalous map for the Br^- ions at 4.0σ contour level is shown by magenta. The OmpF molecule for chain B (cyan) is shown by cartoon representation. The figure was prepared using Pymol.



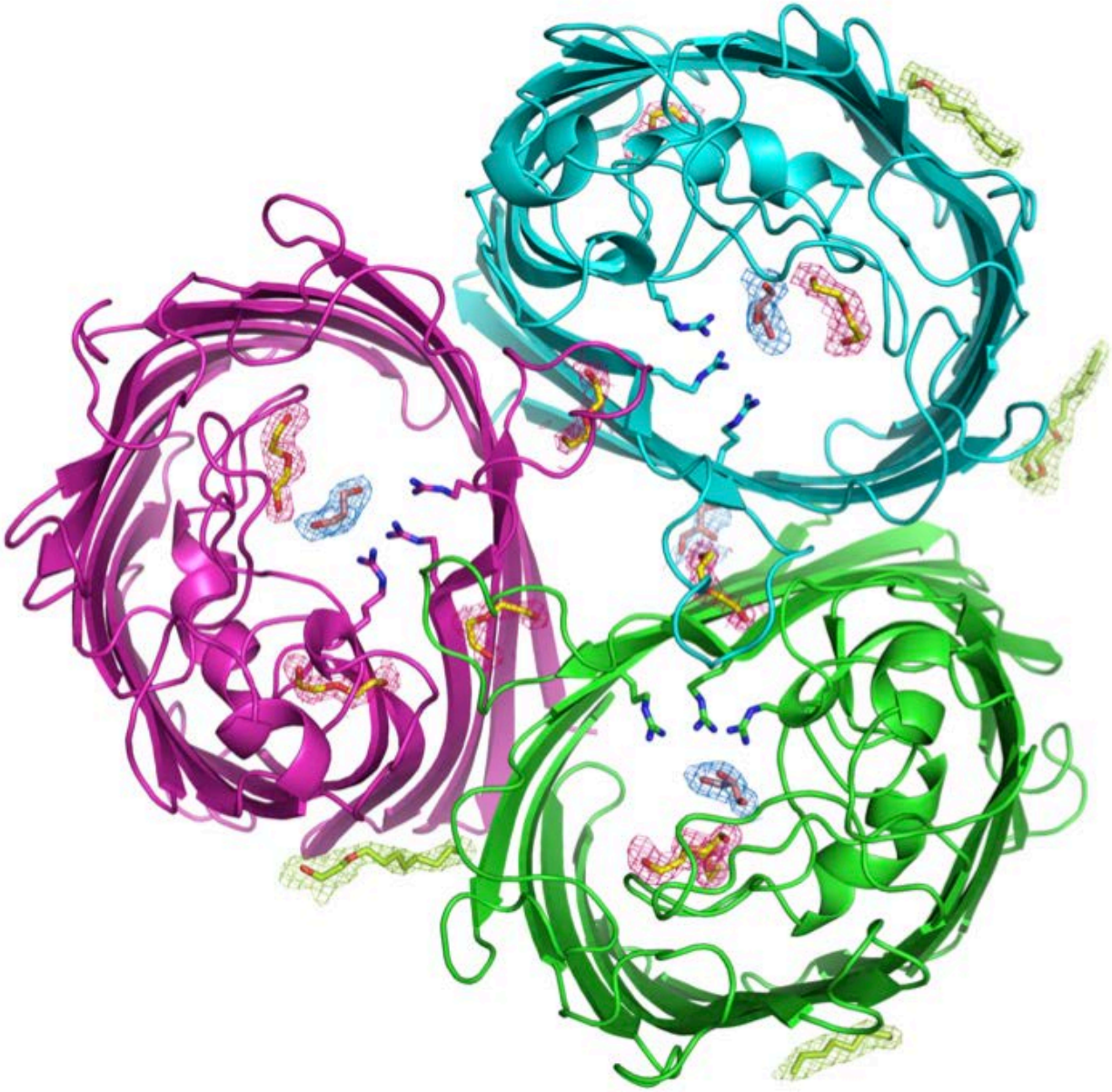
Supplemental Figure S3a. A close-up view of the location of Br^- ions in 0.3MKBr OmpF structure for chain A. The residues close to the Br^- ions in the OmpF molecule are shown in ball-and-stick representation. The Br^- ions are represented by grey dots and the water molecules are represented by red dots. The Bromide anomalous map for the Br^- ions at 6.0σ contour level is shown by magenta. The figure was prepared using COOT.



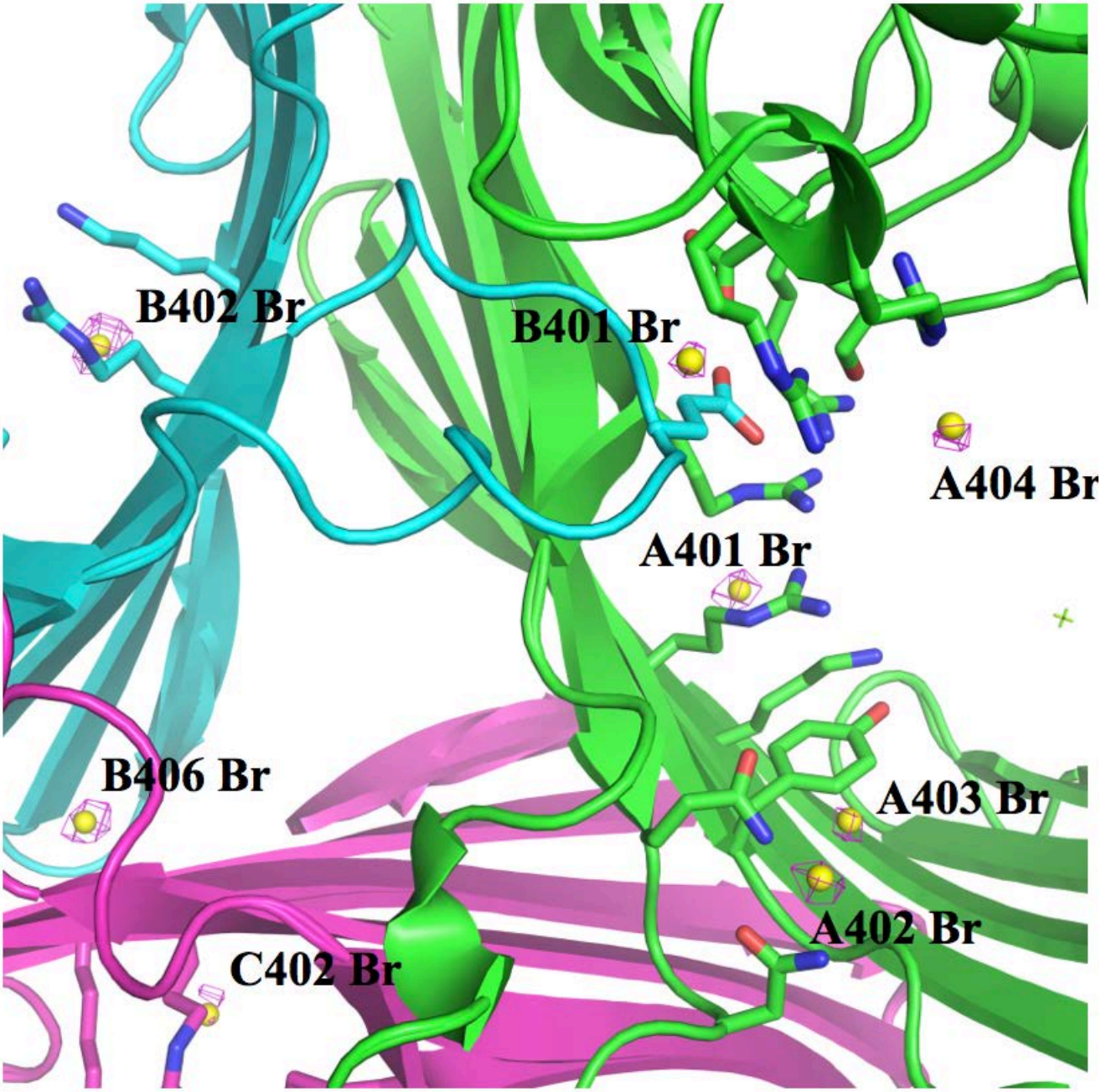
Supplemental Figure S3b. A close-up view of the location of Br^- ions in 0.3MKBr OmpF structure for chain B. The residues close to the Br^- ions in the OmpF molecule are shown in ball-and-stick representation. The Br^- ions are represented by grey dots and the water molecules are represented by red dots. The Bromide anomalous map for the Br^- ions at 6.0σ contour level is shown by magenta. The figure was prepared using COOT.



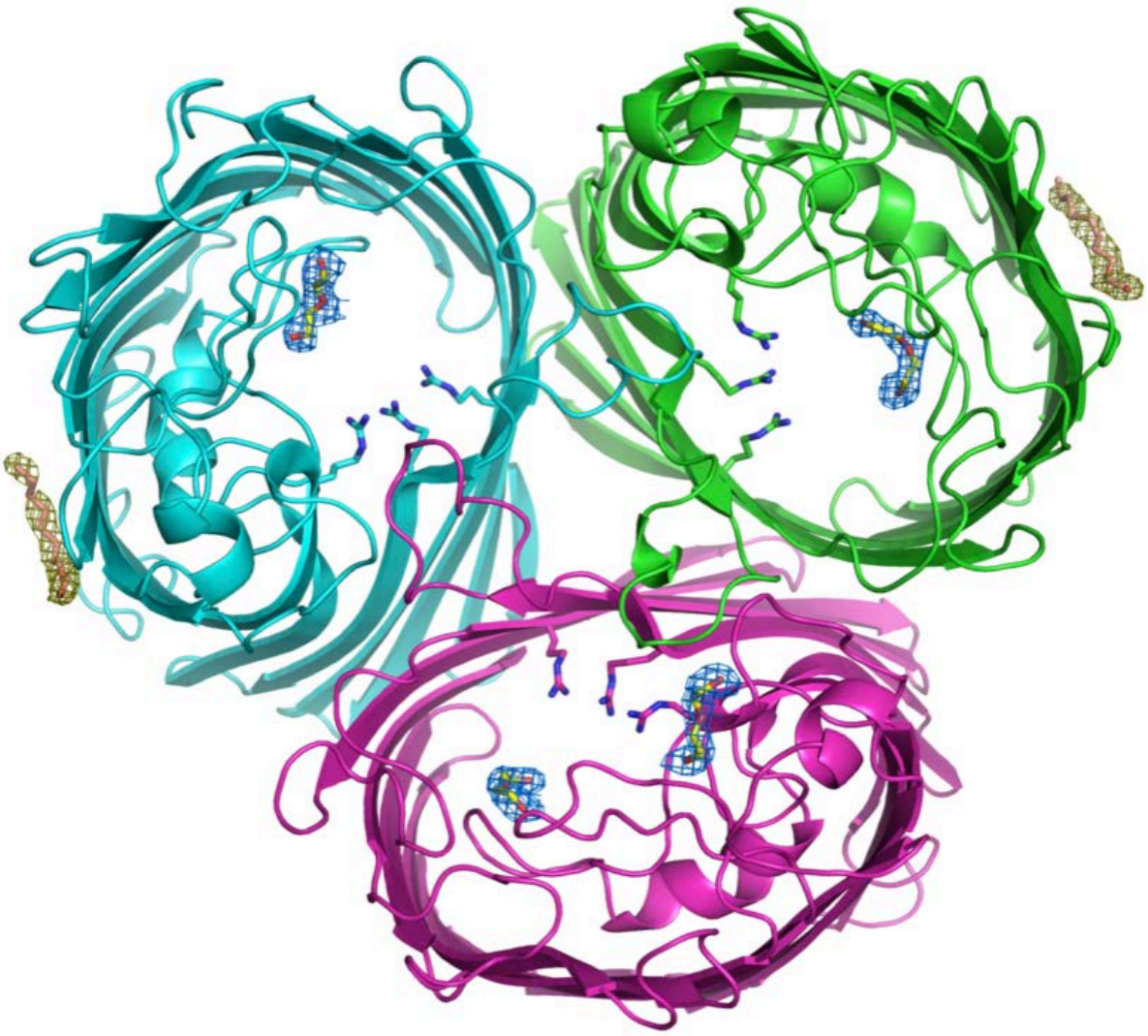
Supplemental Figure S3c. A close-up view of the location of Br^- ions in 0.3MKBr OmpF structure for chain C. The residues close to the Br^- ions in the OmpF molecule are shown in ball-and-stick representation. The Br^- ions are represented by grey dots and the water molecules are represented by red dots. The Bromide anomalous map for the Br^- ions at 6.0σ contour level is shown by magenta. The figure was prepared using COOT.



Supplemental Figure S3d. A view from the extracellular surface and the locations of the PEG (yellow sticks), glycerol (pink sticks) and detergent molecules (green sticks) are shown in 0.3MKBr-OmpF structure. The OmpF molecules, chain A (green), chain B (cyan) and chain C (magenta) are shown by cartoon representation. The $2F_o$-F_c electron densities at 1.0σ contour level for the PEG molecules are indicated by magenta, the glycerol molecules are indicated by aquamarine and the detergent molecules are indicated by green. The Arg clusters (Arg42, Arg82 and Arg132) in the constriction zone are represented by sticks.



Supplemental Figure S4a. A close-up view from the extracellular surface and the location of Br^- ions for chains A, B and C in 0.2MNaBr OmpF structure. The residues close to the Br^- ions in the OmpF molecule are shown in ball-and-stick representation. The Br^- ions are represented by yellow spheres. The Bromide anomalous map for the Br^- ions at 4.0σ contour level is shown by magenta. The OmpF molecules, chain A (green), chain B(cyan) and chain C(magenta) are shown by cartoon representation. The figure was prepared using Pymol.



Supplemental Figure S4b. A view from the extracellular surface and the locations of the PEG (yellow sticks), and detergent molecules (pink sticks) are shown in 0.2MNaBr-OmpF structure. The OmpF molecules, chain A (green), chain B (cyan) and chain C (magenta) are shown by cartoon representation. The $2F_o$-F_c electron densities at 1.0σ contour level for the PEG molecules are indicated by blue and the detergent molecules are indicated by green. The Arg clusters (Arg42, Arg82 and Arg132) in the constriction zone are represented by sticks.