A fluoride interloper in a CLC-type Cl^-/H^+ antiporter

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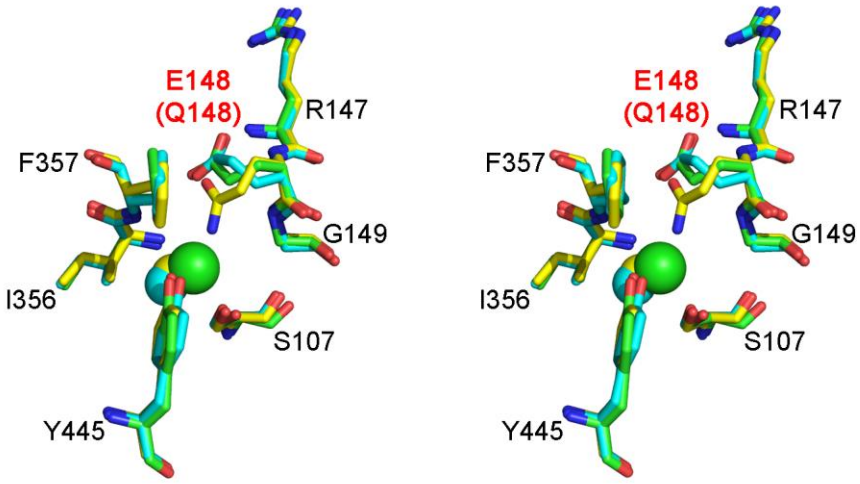
Supplementary Information

Supplementary Results



Supplementary Figure 1. Stereo view of merged structures near central anion binding site.
Crystal structures of wildtype CLC-ec1 with Cl^- (pdb #4ENE, *green*), with F^- (#4KJQ, *cyan*) and a mutant, E148Q with F^- (#4KK8, *yellow*) are displayed. Spheres indicate ions in each structure and are colored corresponding to the proteins.

Supplementary Table 1. CLC-ec1 constructs: Metrics for ion transport[a].

Protein	Cl^- transport (Cl^-/sec)	F^- transport (F^-/sec)
Wildtype	410 ± 9	4 ± 1
E148A	350 ± 13	680 ± 50
E148Q	200 ± 8	6 ± 1
E203Q	80 ± 4	5 ± 1
Y445A	190 ± 10	1.3 ± 0.2
EA/YA (E148A/Y445A)	20200 ± 1000	6200 ± 300

[a] Cl^- or F^- transport was performed in 300-fold Cl^- or F^- gradient in symmetrical pH (inside – 300 KX, 25 Mes-NaOH, pH 6.0; outside – 300 K-isethionate, 1 KX, 25 Mes-NaOH, pH 6.0, where X indicates Cl^- or F^-) with proteoliposomes (0.5 – 5 μg protein / mg lipid). Each transport value represents mean ± s.e.m. of 3-4 separate experiments, using 1-3 protein preps.

Supplementary Table 2. Thermodynamic parameters for anion binding.

Protein	pH	Anion	K_D (mM)[a]	ΔG^o (kcal / mol)	ΔH^o (kcal / mol)	No. of binding sites
Wildtype	6.0	Cl^-	0.48 ± 0.05	-4.51 ± 0.05	-6.6 ± 0.4	1
		F^-	0.96 ± 0.07	-4.11 ± 0.05	-5.2 ± 0.2	1
	7.5	Cl^- [b]	0.74 ± 0.01	-4.30 ± 0.01	-5.0 ± 0.1	1
		F^-	1.04 ± 0.05	-4.06 ± 0.03	-1.6 ± 0.2	1
E148A	7.5	Cl^- [c]	0.015 ± 0.002	-6.7 ± 0.1	-6.0 ± 0.6	1.8
		F^-	0.21 ± 0.02	-5.02 ± 0.05	-1.1 ± 0.2	1 (0.97)[d]
E148Q	7.5	Cl^- [c]	0.07 ± 0.01	-5.7 ± 0.1	-0.5 ± 0.1	2
		F^-	0.20 ± 0.01	-5.03 ± 0.04	-3.6 ± 0.2	1 (0.77)[d]

[a] obtained from 3-4 independent observations, each with 2-3 independent protein preps.
[b] adopted from Lim et al., *PLoS Biol*. **10**, e1001441 (2012).
[c] adopted from Picollo et al., *Nat. Struct. Mol. Biol*. **16**, 1294-1301 (2009).
[d] the number in parentheses were obtained from fitting the binding curve without pre-determination.

Supplementary Table 3. Statistics for x-ray crystallography.

Table S3-A. ***Wildtype CLC-ec1***

Halide (mM)	**No halide**	**100 F^-**	**100 F^- + 20 Br^-**	**20 F^- + 20Br^-**	**20 Br^-**
PDB #	**4KJP**	**4KJQ**	**4KJW**	**4KK5**	**4KK6**
Data collection					
Space group	C2	C2	C2	C2	C2
a, b, c (Å)	232.4, 98.2, 171.0	231.3, 100.2, 170.0	231.7, 98.9, 172.9	229.1, 98.2, 169.8	231.4, 100.9, 171.7
α, β, γ (^{o})	90.0, 132.1, 90.0	90.0, 132.0, 90.0	90.0, 132.9, 90.0	90.0, 131.7, 90.0	90.0, 132.1, 90.0
Resolution (Å)[a]	25 – 3.2 (3.26 – 3.2)	40 – 2.88 (2.92 – 2.88)	40 – 3.03 (3.1 – 3.03)	40 – 3.17 (3.26 – 3.17)	25 – 3.18 (3.26 – 3.18)
R_{merge}	0.127 (0.781)	0.108 (0.947)	0.101 (0.612)	0.112 (0.542)	0.093 (0.612)
$I / \sigma I$	17.3 (1.5)	31.7 (1.5)	23.3 (1.7)	17.9 (1.7)	20.8 (1.6)
Completeness (%)	99.5 (94.2)	100 (99.9)	100 (99.6)	98.8 (94.2)	99.6 (98.6)
Redundancy	7.4 (6.3)	7.5 (6.9)	3.9 (3.4)	3.8 (3.4)	3.9 (3.6)
Mosaicity (^{o})	0.66 – 1.07	0.53 – 0.83	0.51 – 1.27	0.65 – 1.65	0.51 – 0.90
Refinement					
Resolution (Å)	25 – 3.2	40 – 2.88	40 – 3.03	40 – 3.17	25 – 3.18
No. reflections	47156	65389	108408	91572	95417
R_{work} / R_{free}	22.2 / 26.5	22.0 / 26.3	23.0 / 26.9	23.2 / 27.1	23.5 / 26.9
No. atoms					
Protein	13214	13223	13223	13223	13214
Ligand/ion	-	2	-	-	4
Water	-	-	-	-	-
B-factor					
Protein	60.6	59.8	65.8	69.8	71.9
Ligand/ion	-	58.2	-	-	115.5
Water	-	-	-	-	-
R.m.s. deviations					
Bond lengths (Å)	0.006	0.009	0.005	0.006	0.006
Bond angles (^{o})	0.83	1.29	0.80	0.81	0.76
Ramachandran plot[b]	94.2 / 5.3 / 0.5	92.0 / 7.1 / 0.9	94.5 / 5.2 / 0.3	93.1 / 6.3 / 0.6	92.3 / 7.0 / 0.7
Molprobity (rank, %)[c]	2.34 (98^{th})	2.77 (83^{rd})	2.34 (97^{th})	2.39 (98^{th})	2.41 (97^{th})

(continued)

Supplementary Table 3-B. ***E148A***

Halide (mM)	**100 F^-**	**100 F^- + 2 Br^-**	**100 F^- + 20 Br^-**	**20 F^- + 20Br^-**	**20 Br^-**
PDB #	**4KKL**	**4KK9**	**4KKA**	**4KKB**	**4KKC**
Data collection					
Space group	C2	C2	C2	C2	C2
a, b, c (Å)	231.4, 100.1, 170.2	231.6, 98.9, 170.4	232.2, 99.2, 170.2	231.7, 97.5, 170.6	231.2, 99.2, 170.5
α, β, γ (^{o})	90.0, 131.8, 90.0	90.0, 131.9, 90.9	90.0, 131.9, 90.0	90.0, 131.7, 90.0	90.0, 132.0, 90.0
Resolution (Å)[a]	25 – 2.79 (2.85 – 2.79)	40 – 3.0 (3.05 – 3.0)	40 – 3.0 (3.05 – 3.0)	30 – 3.02 (3.07 – 3.02)	30 – 3.18 (3.26 – 3.18)
R_{merge}	0.112 (0.866)	0.088 (0.625)	0.094 (0.727)	0.113 (0.680)	0.134 (0.694)
$I / \sigma I$	42.7 (2.2)	19.6 (1.6)	18.8 (1.5)	22.0 (1.4)	15.9 (1.5)
Completeness (%)	99.8 (99.7)	100 (99.7)	99.9 (99.5)	99.9 (99.1)	99.8 (98.2)
Redundancy	7.5 (7.3)	3.9 (3.7)	3.9 (3.7)	3.8 (3.6)	3.7 (3.2)
Mosaicity (^{o})	0.51 – 0.96	0.39 – 0.56	0.32 – 0.63	0.58 – 1.44	0.57 – 1.02
Refinement					
Resolution (Å)	25 – 2.79	40 – 3.0	40 – 3.0	30 – 3.02	30 – 3.18
No. reflections	71781	112618	112322	107647	93236
R_{work} / R_{free}	23.5 / 28.9	21.7 / 27.0	22.0 / 26.5	23.6 / 26.8	21.3 / 26.3
No. atoms					
Protein	13215	13206	13206	13215	13215
Ligand/ion	4	-	-	-	6
Water	-	-	-	-	-
B-factor					
Protein	59.7	56.6	61.2	76.4	56.3
Ligand/ion	52.5	-	-	-	85.7
Water	-	-	-	-	-
R.m.s. deviations					
Bond lengths (Å)	0.01	0.009	0.01	0.005	0.009
Bond angles (^{o})	1.32	1.30	1.28	0.81	1.26
Ramachandran plot[b]	91.2 / 8.2 / 0.6	90.4 / 8.9 / 0.7	92.0 / 7.2 / 0.8	93.8 / 5.7 / 0.5	90.6 / 8.5 / 0.9
Molprobity (rank, %)[c]	2.85 (74^{th})	2.94 (78^{th})	2.83 (84^{th})	2.37 (97^{th})	2.82 (90^{th})

(continued)

Supplementary Table 3-C. ***E148Q***

Halide (mM)	**No halide**	**100 F^-**
PDB #	**4LOU**	**4KK8**
Data collection		
Space group	C2	C2
a, b, c (Å)	231.6, 100.3, 171.0	231.2, 98.9, 170.0
α, β, γ (^{o})	90.0, 131.7, 90.0	90.0, 131.8, 90.0
Resolution (Å)[a]	40 – 2.98 (3.05 – 2.98)	25 – 2.86 (2.92 – 2.86)
R_{merge}	0.121 (0.744)	0.130 (0.654)
$I / \sigma I$	26.1 (1.4)	33.8 (1.8)
Completeness (%)	99.4 (91.6)	99.2 (88.5)
Redundancy	7.3 (4.8)	7.3 (5.0)
Mosaicity (^{o})	0.37 – 0.71	0.38 – 0.87
Refinement		
Resolution (Å)	40 – 2.98	25 – 2.86
No. reflections	58841	65721
R_{work} / R_{free}	21.0 / 25.3	21.0 / 25.6
No. atoms		
Protein	13243	13225
Ligand/ion	-	2
Water	-	-
B-factor		
Protein	44.1	64.4
Ligand/ion	-	58.4
Water	-	-
R.m.s. deviations		
Bond lengths (Å)	0.01	0.009
Bond angles (^{o})	1.27	1.23
Ramachandran plot[b]	91.5 / 8.0 / 0.5	92.9 / 6.5 / 0.6
Molprobity (rank, %)[c]	2.63 (91st)	2.64 (88th)

[a] numbers in parentheses represent values for the highest resolution shell.
[b] percentage of residues in the favored / allowed / disallowed region.
[c] MolProbity score was calculated as described (Chen VB et al. MolProbity: all-atom structure validation for macromolecular crystallography. *Acta Crystallographica D* **66**, 12-21, 2010). The rank indicates that 100th-percentile is the best among the structures of comparable resolutions.

Supplementary Table 4. Statistics for anomalous signals[a]

Protein	Halide (mM)	ρ_{ano}[b]		
		external	*central*	*internal*
WT	20 Br^-	–	17.8 ± 2.1	12.4 ± 1.8
	20 F^- + 20 Br^-	–	9.9 ± 0.8	9.7 ± 0.6
	100 F^- + 20 Br^-	–	–	6.0 ± 0.6
E148A	20 Br^-	19.0 ± 2.0	17.8 ± 1.6	6.0 ± 0.5
	20 F^- + 20 Br^-	30.2 ± 1.9	8.5 ± 0.8	4.8 ± 1.1
	100 F^- + 20 Br^-	22.9 ± 1.2	–	–
	100 F^- + 2 Br^-	15.6 ± 0.9	–	–

[a] obtained from 6 observations (two subunits in the asymmetric unit were considered as independent observation)

[b] peak heights of anomalous densities were normalized to σ. Since anomalous signals appear less than 4.0 σ were not discernible from noise, only peaks >4.0σ were considered.