SUPPLEMENTAL MATERIAL

Lim et al., http://www.jgp.org/cgi/content/full/jgp.200810112/DC1

TABLE S1
Chemical Modification at E203C

Reagent	Number of cysteines/subunit	% modified
Untreated	1.12 ± 0.03	0
IAA	0.07 ± 0.03	94
FIAA	0.29 ± 0.09	74
MTSCE	0.01 ± 0.01	99
MTSES	−0.01 ± 0.03	100

The reagents used to modify E203C on an otherwise cysteine-free protein, the amount of free thiol remaining after at the end of the reaction, and the percent modification. In negative control experiments, protein incubated for 24 h in the absence of electrophile led to only 6–8% loss of thiol.

TABLE S2
Amino Acid Propensity at the Glu_{in} Position

Amino acid	Bacteria	Eukaryota	Archaea	TOTAL (%)
A	17	5	0	22 (3.6)
C	3	0	0	3 (<2)
D	6	3	0	9 (<2)
E	121	144	1	266 (44)
G	5	0	0	5 (<2)
L	19	3	1	23 (3.6)
M	7	0	0	7 (<2)
N	5	0	0	5 (<2)
P	4	6	0	10 (<2)
Q	3	6	0	9 (<2)
S	9	10	0	19 (3)
T	8	2	0	10 (<2)
V	112	63	10	185 (30)
I	26	0	9	35 (5.7)
SUM	345	242	21	608

Uniprot database was examined for CLC homologues. Frequency of occurrence of each residue at the 11th position of the conserved PIGG-pen sequence (Accardi et al., 2005), the equivalent of E203 in CLC-ec1, is reported here. Bolded entries represent ∼80% of all homologues.

TABLE S3

X-ray Crystallographic Statistics

	E203H	E203V
Accession no.	3EJY	3EJZ
Space group	C2 a = 231.5, b = 96.8, c = 172.8 $\alpha = 90.00, \beta = 132.8, \gamma = 90.00$	C2 a = 231.3, b = 96.4, c = 170.2 $\alpha = 90.00, \beta = 131.8, \gamma = 90.00$
Resolution	58.5–3.2 Å	58.8 – 2.9 Å
No. of reflections	43,956 (3,208)	58,605 (4,325)
Completeness (%)	99.6 (99.9)	99.3 (99.6)
R_{merge} (%)	9.0 (57)	7.2 (54)
$\langle I \rangle / \sigma$	19.6 (3.0)	19.8 (2.6)
R_{factor} (%)	24.6 (31.3)	25.3 (35.7)
R_{free} (%)	27.9 (36.6)	28.1 (38.5)
RMSD bond, Å	0.01	0.009
RMSD angle, deg	1.27	1.32

Crystals of CLC-ec1E203H and E203V were grown in sitting drops at 20°C by equilibrating a 1:1 mixture of protein (10 mg/ml in 100 mM NaBr, 10 mM Tris-Cl, 5 mM DM, pH 7.5) and well solutions against well solution. Well solutions were: 37% PEG 300 (w/v), 20 mM NaBr, 50 mM glycine-NaOH, pH 9.5, for E203H and 36% PEG 300, 20 mM NaBr, 50 mM tris-SO_4, pH 8.5, for E203V. Values in parentheses represent the highest resolution shell.