

CIC proteins

- “Chloride channels”
- Essential for chloride homeostasis in eukaryotic cells.

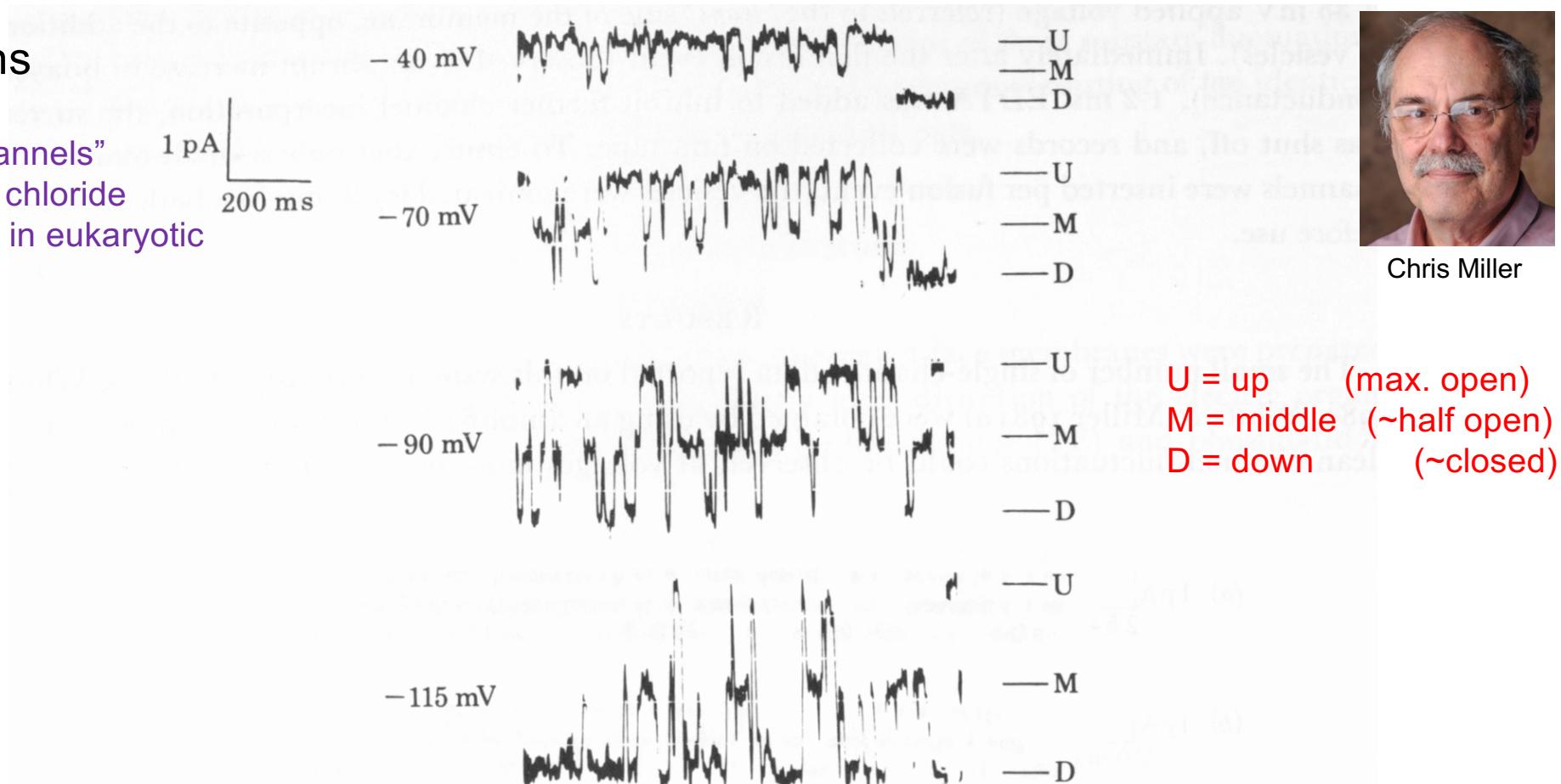
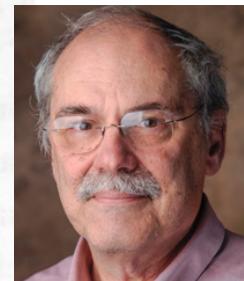


FIGURE 3. Voltage dependence of open channel substructure. A single Cl⁻ channel was inserted into a bilayer as in figure 2, and records were collected at the holding potentials indicated. Traces displayed are all taken from within an open-state duration, i.e. channel closing events are not shown. Lines mark the current levels corresponding to the U, M and D states of the open channel. Note the closing of the channel at the ends of the top two traces.



Chris Miller

U = up (max. open)
M = middle (~half open)
D = down (~closed)

CIC channels

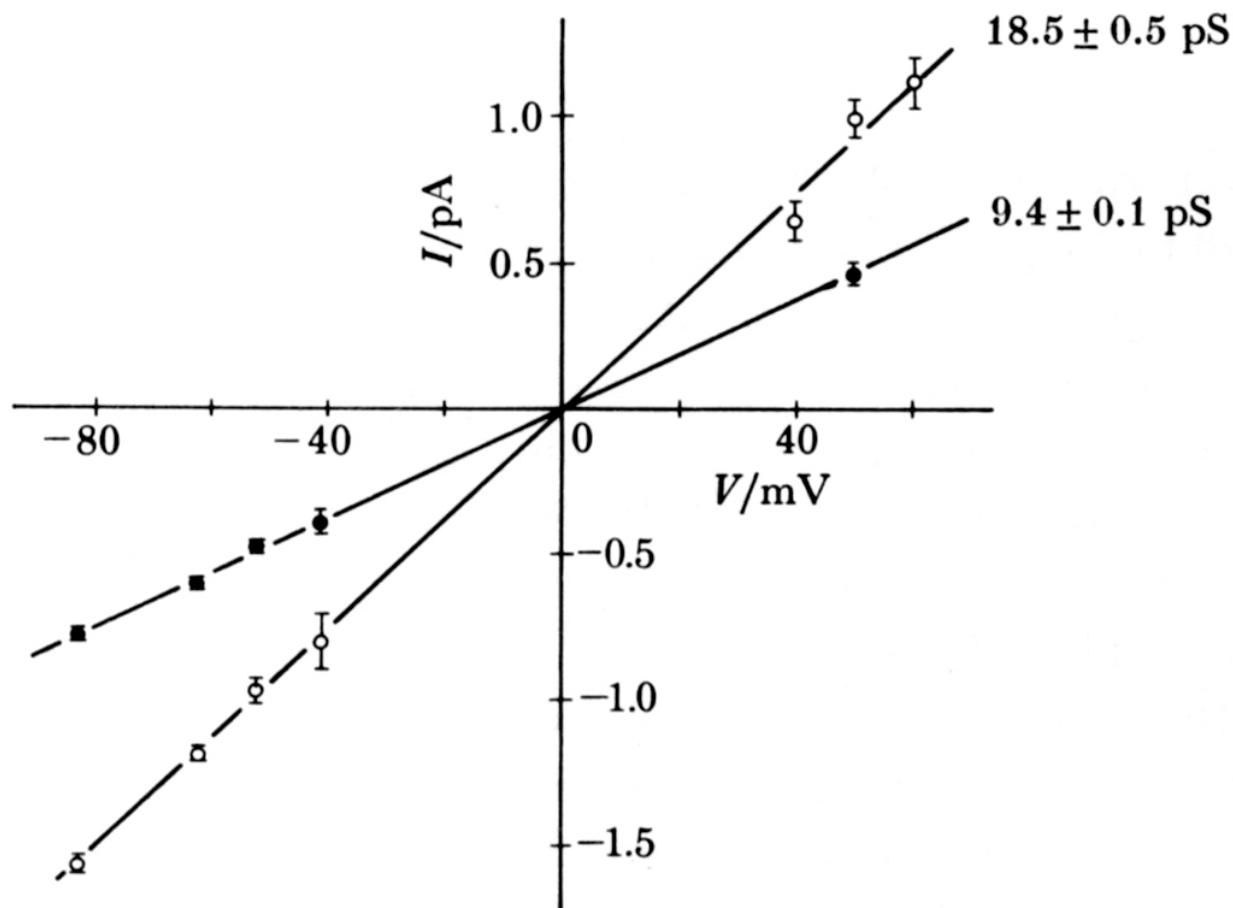
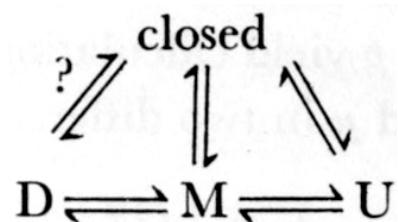


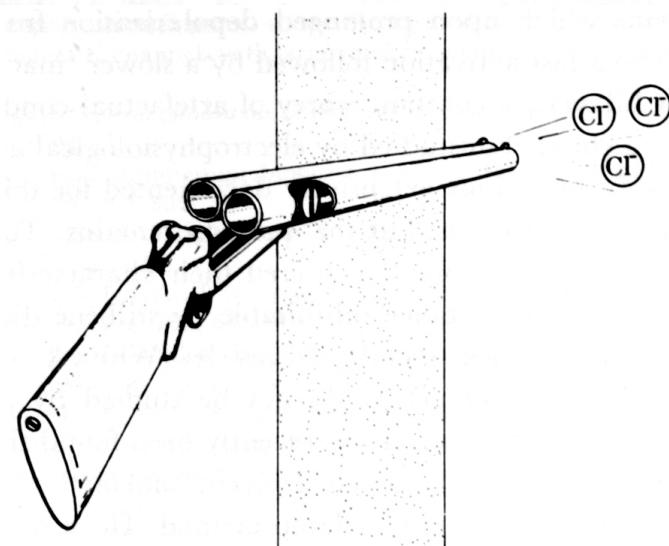
FIGURE 4. Current–voltage relation of open-channel substates. Single-channel substate records were collected at various voltages, and the currents corresponding to the U state (open points) and M state (filled points) were measured by hand. Each point represents the mean \pm s.e.m. of 5–20 determinations. Current was always measured with respect to the closed state of the channel. The D-state current was indistinguishable from zero at all voltages.

CIC channels

Mere inspection of the single-channel fluctuation records of the *Torpedo* Cl⁻ channel leads to a four-state model of the channel, containing one ‘closed’ and three ‘open’ states. Transitions between the closed and open states are slow, in the range of seconds, and have been studied previously (White & Miller 1979, 1981b; Miller & White 1980). Transitions between the three ‘open’ states, U, M and D, are much faster, in the 10 ms timescale (at pH 7.4). Furthermore, preliminary results (not shown) demonstrate that the channel can enter the closed state from either the U or the M state. (A transition from the D to the closed state would be electrically invisible.) Therefore, a minimal scheme to describe the Cl⁻ channel is as shown here:



→ CLC channels are dimers with two functionally independent (non-communicating, non-cooperative) subunits. Each monomer contains a single chloride conduction pathway



CIC channels

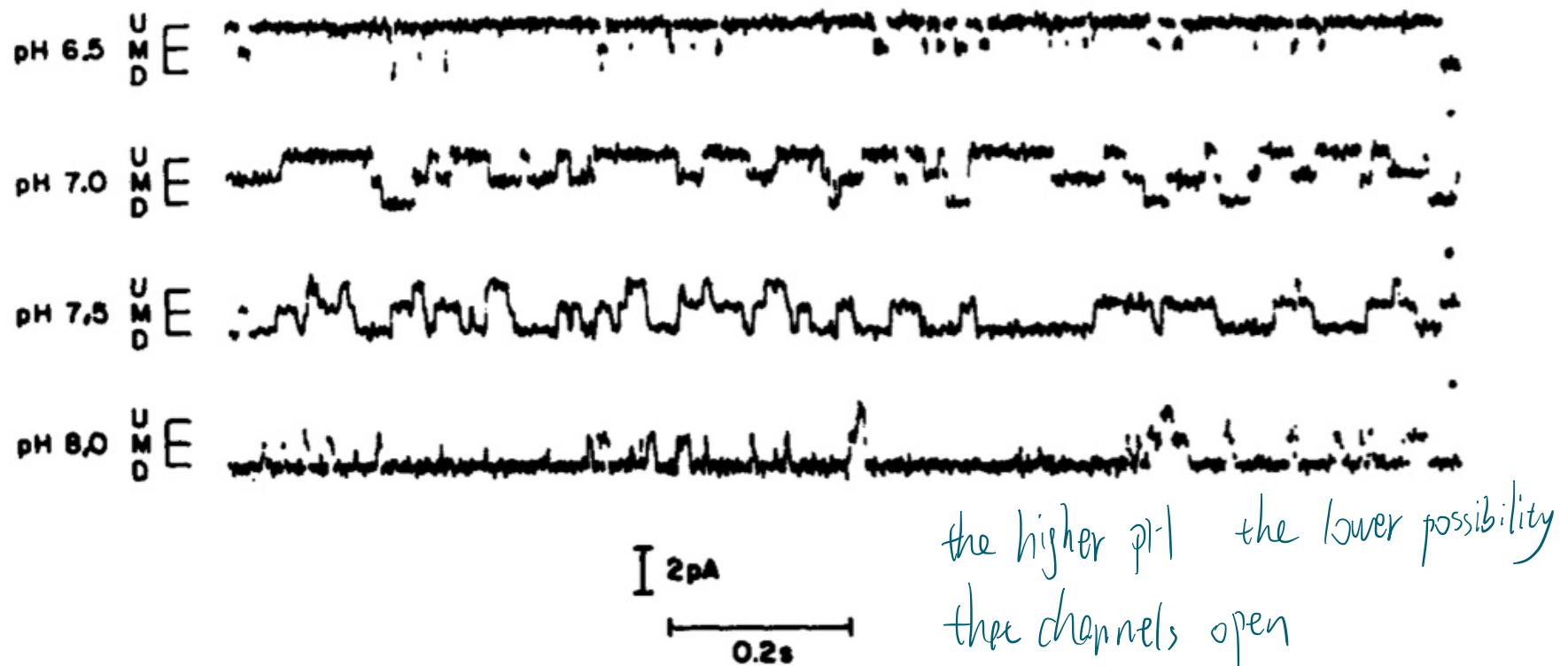
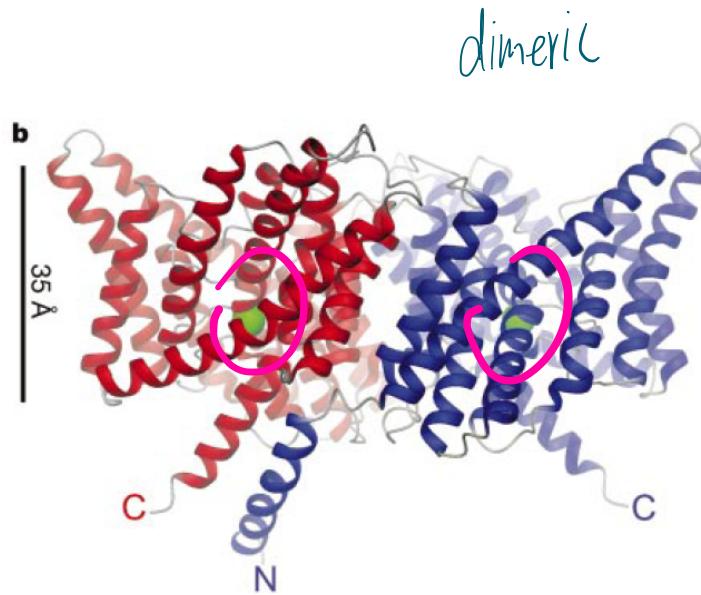


FIGURE 3. Effect of pH on substrate fluctuations. Single channels were observed at a holding potential of -100 mV, in solutions adjusted symmetrically to the indicated pH values. Each trace is taken from a separate membrane.

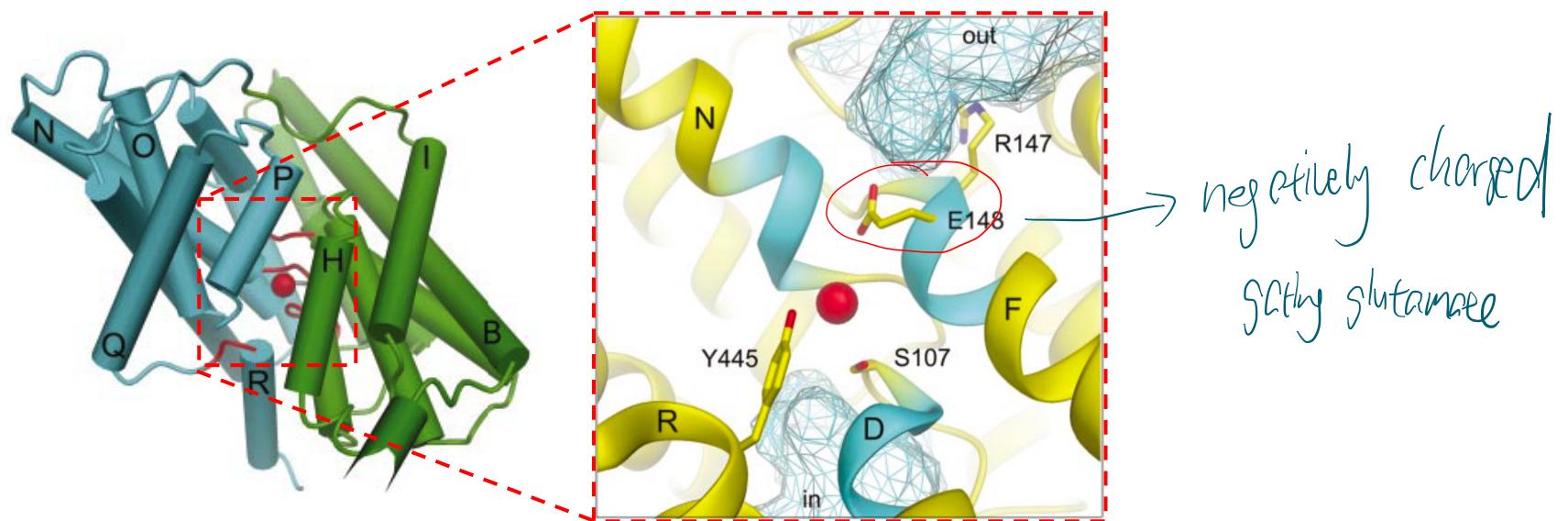
Hanke W and Miller C, *J Gen Physiol* **82**: 25-45 (1983)

CIC protein structure

- Structure: large tilting angles of TM helices, and several short helices that don't cross the membrane.
- A "gating" glutamate side chain is in the immediate vicinity of a bound Cl^- ion.



Raimund Dutzler



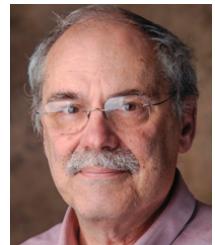
Dutzler R et al., *Nature* **415**: 287 (2002)

pymol CIC

PDB ID: 1OTS.pdb

- Pymol scene 1: Ribbon dimer
- Pymol scene 2: Electrostatic surface potential of CIC dimer, no passage visible
- Pymol scene 3: Sticks, 2 2 Cl⁻ ions in central pathway (anom. diffraction bromide)
- Pymol scene 4: Narrowest point: 2 Cl⁻ ions and a Glu side chain block passage
- Pymol scene 5: 2 Cl⁻ ions and a Glu side contained in one monomer, not at dimer interface

Secondary active transport mediated by a prokaryotic homologue of CIC Cl^- channels

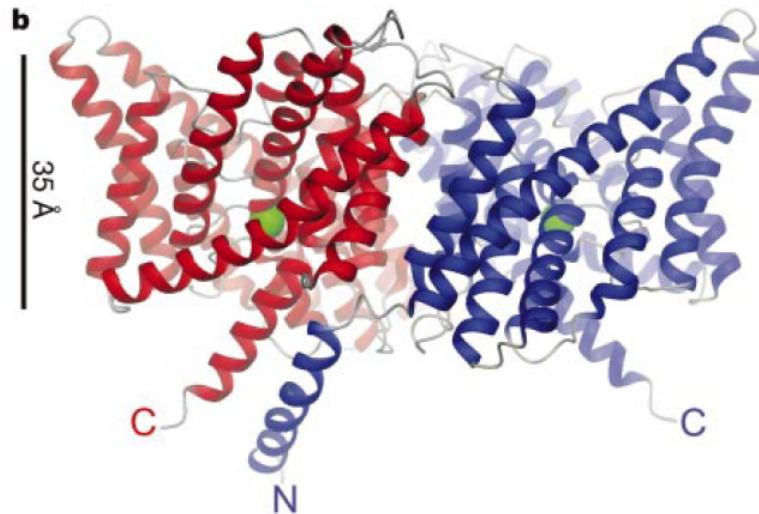


Chris Miller

Alessio Accardi & Christopher Miller

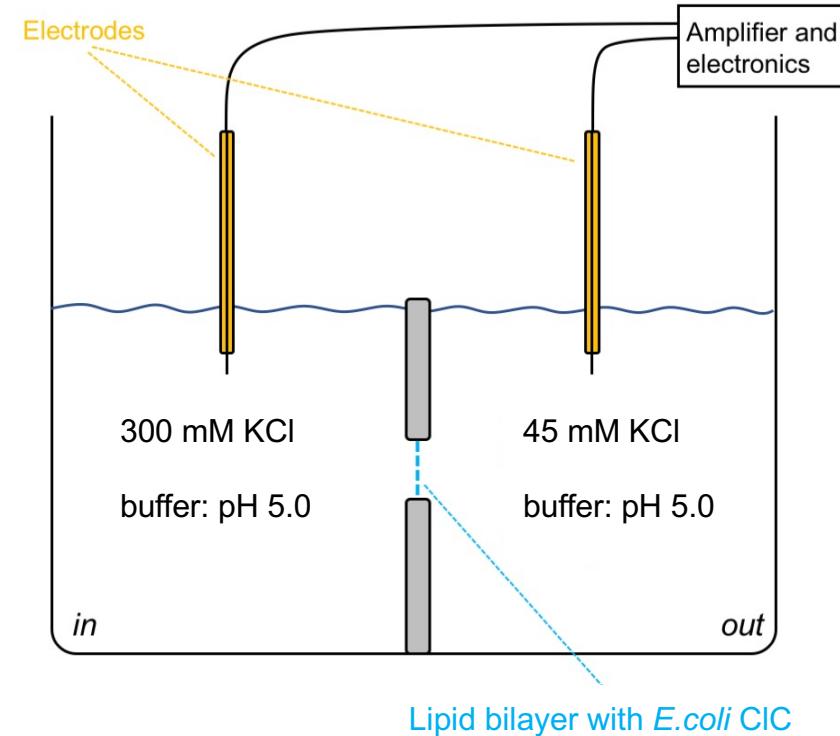
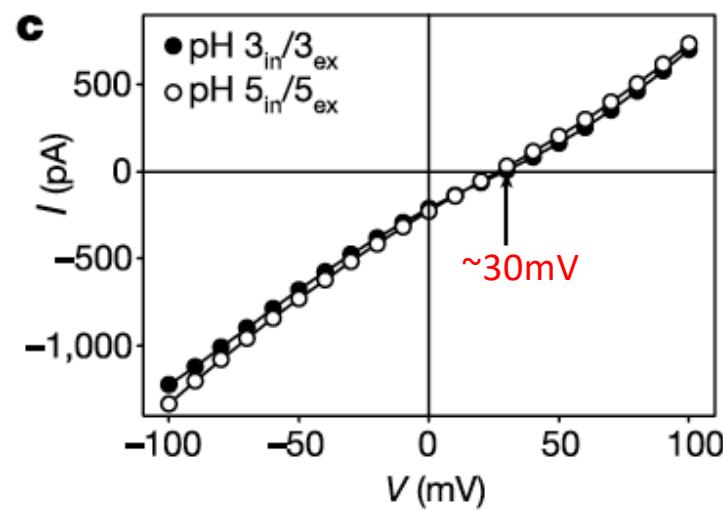
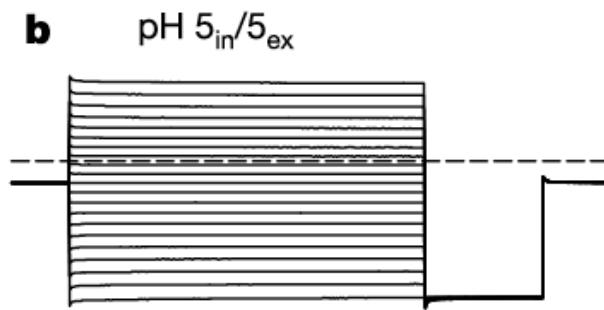


Alessio Accardi



Nature **427**: 803 (2004)

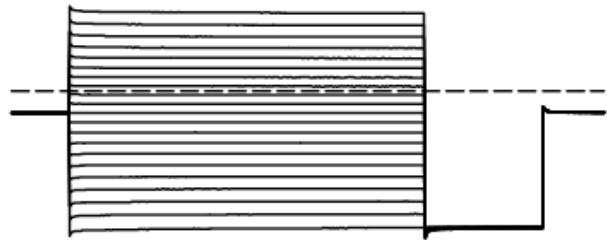
Electrophysiological study of *E. coli* CIC



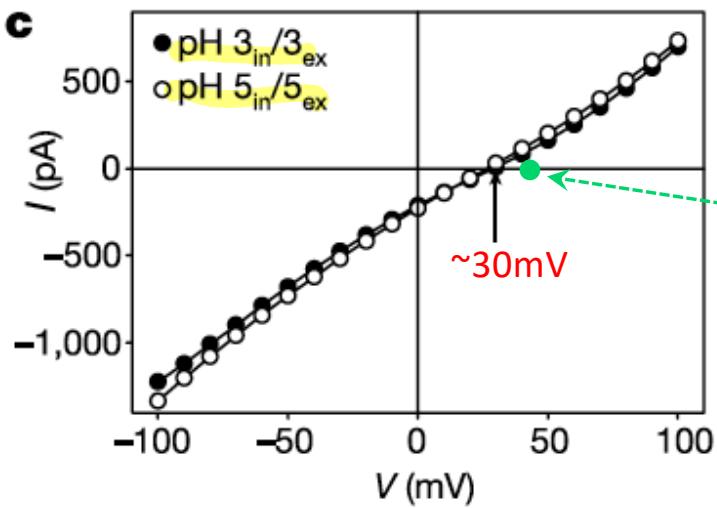
adapted from Accardi A et al., *Nature* **427**: 803 (2004)

Electrophysiological study of *E. coli* CIC

b pH 5_{in}/5_{ex}



c

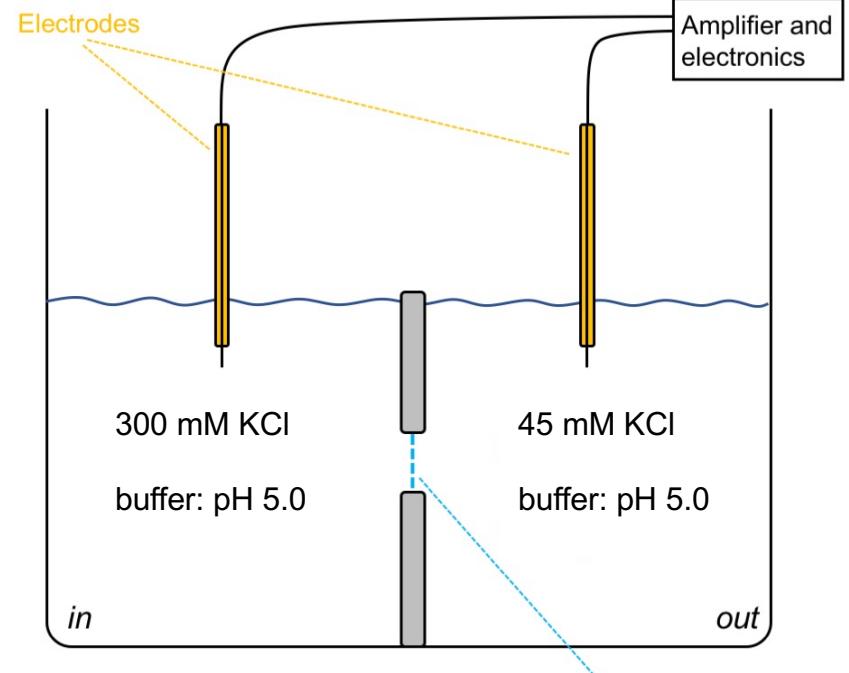


E_{rev} expected according to Nernst:

$$E_{Cl} = \frac{RT}{F} \ln \frac{[Cl]_{in}}{[Cl]_{out}}$$

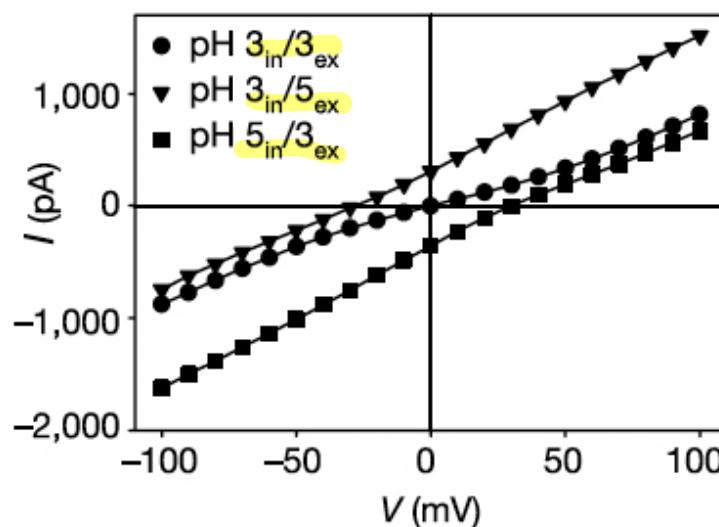
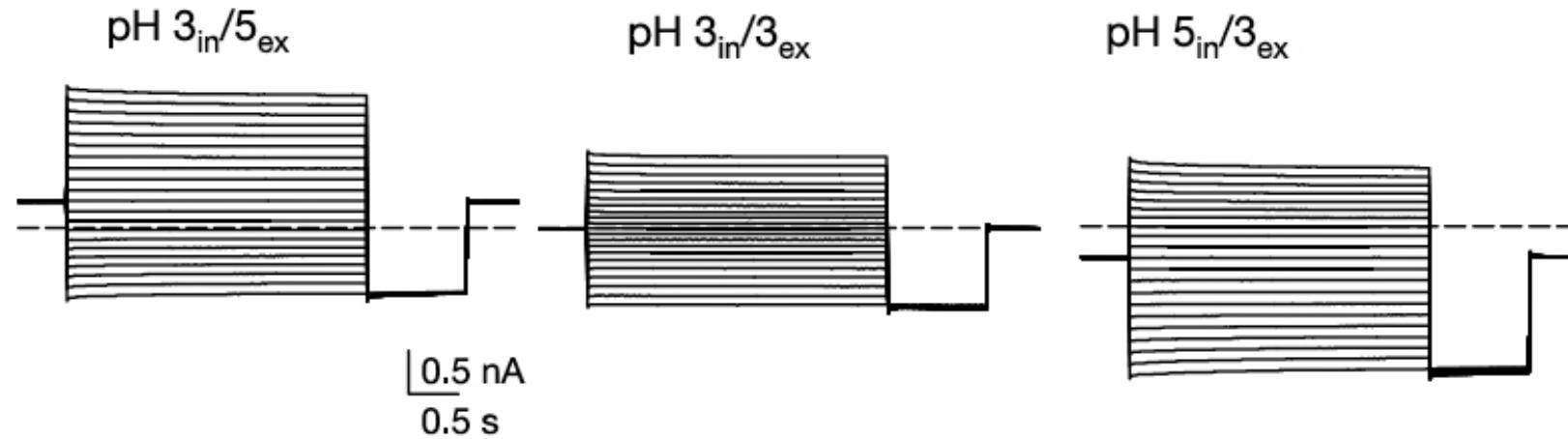
≈ 48 mV (at 298K)

adapted from Accardi A et al., Nature 427: 803 (2004)



they move coupled
instead of moving
independently since in
this case pH of two
sites is the same

Electrophysiological study of *E. coli* CIC



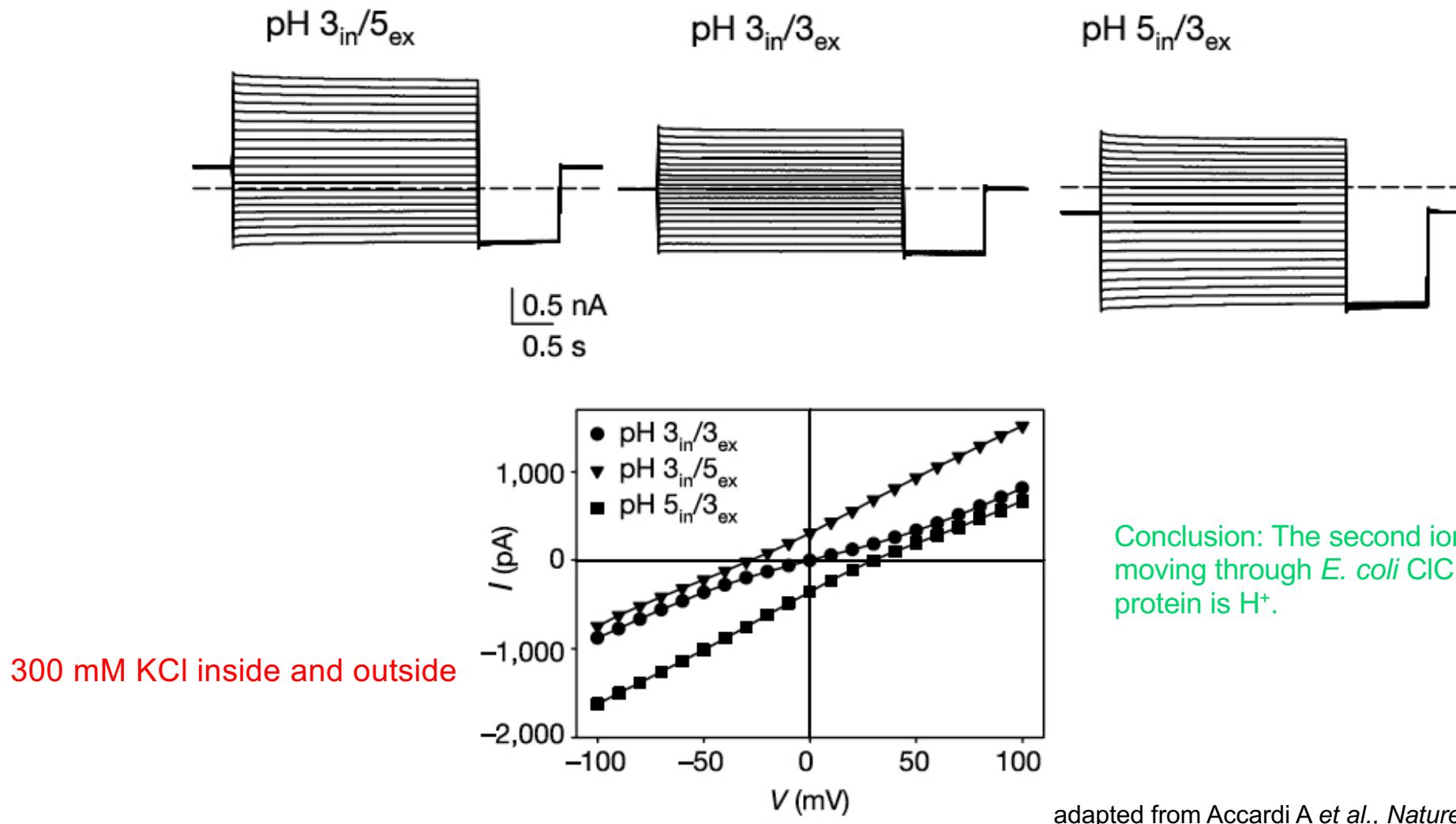
300 mM KCl inside and outside

equal concentration
(should be pass
through origin)

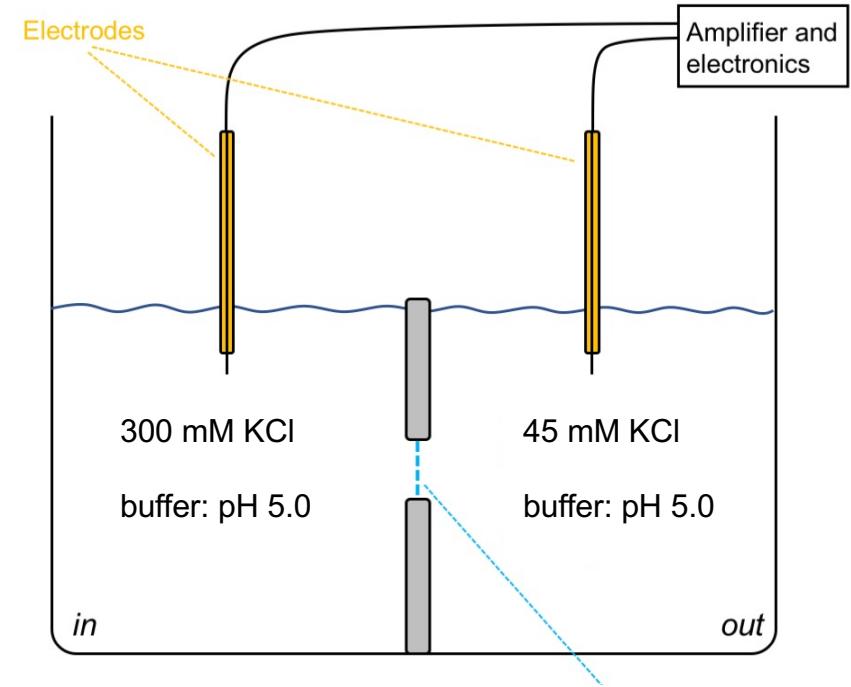
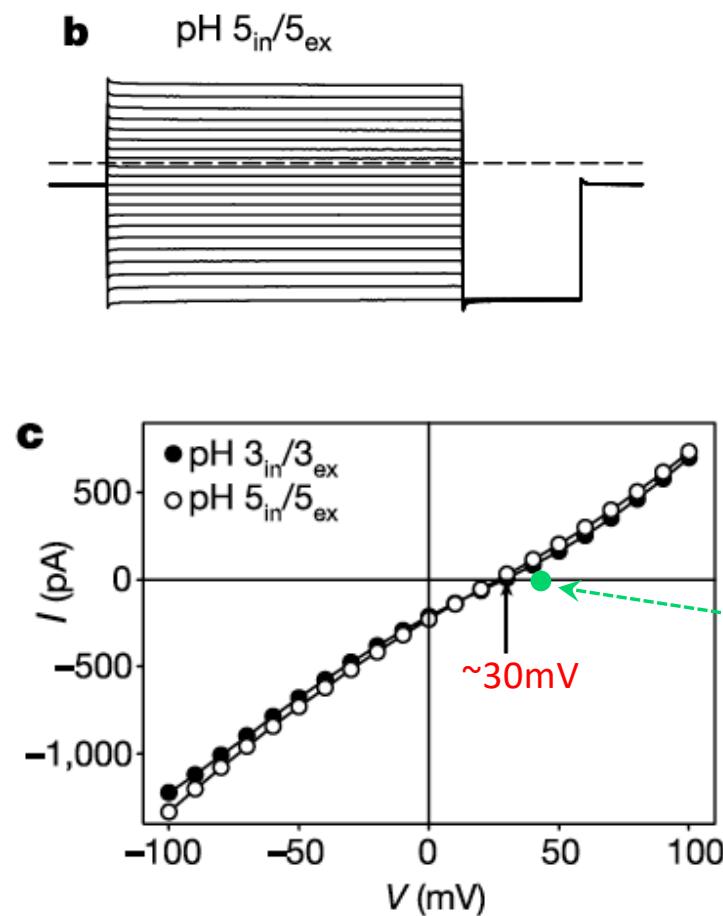
seems like this channel
selective also for protons

adapted from Accardi A et al., Nature 427: 803 (2004)

Electrophysiological study of *E. coli* CIC



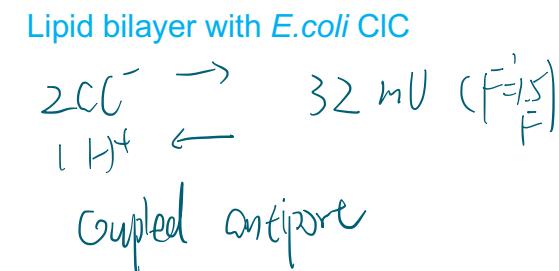
Electrophysiological study of *E. coli* CIC



E_{rev} expected according to Nernst:

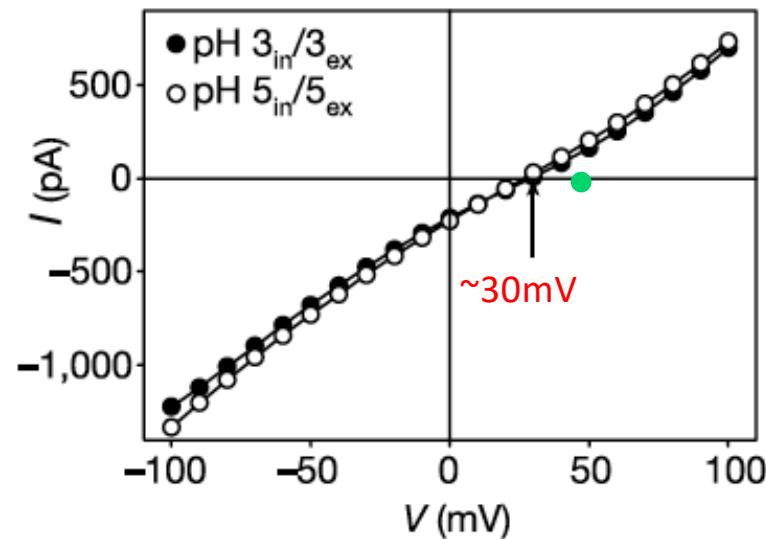
$$E_{Cl} = \frac{RT}{F} \ln \frac{[Cl]_{in}}{[Cl]_{out}}$$

≈ 48 mV (at 298K)



adapted from Accardi A et al., *Nature* 427: 803 (2004)

E. coli CIC: chloride - proton symport or antiport?



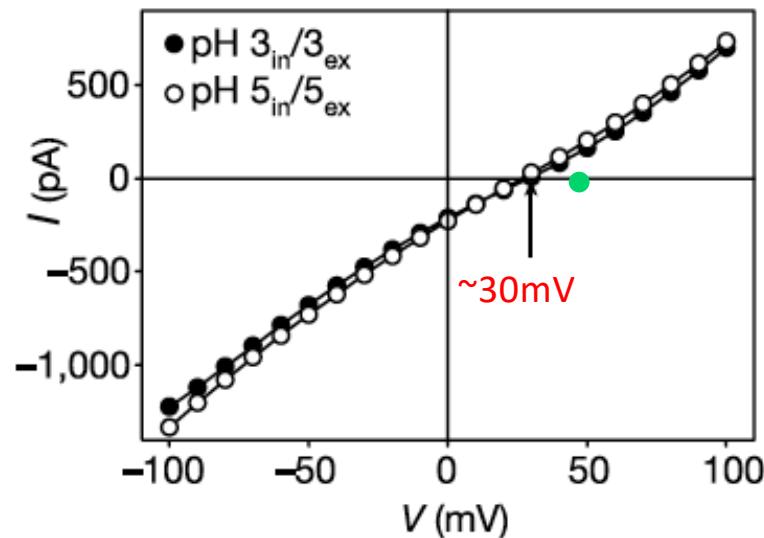
$$E_{\text{comb}} = \frac{n E_{\text{Cl}^-} + m E_{\text{H}^+}}{n + m}$$

$$E_{\text{comb}} = \frac{1}{1 + r} (E_{\text{Cl}^-} + r E_{\text{H}^+})$$

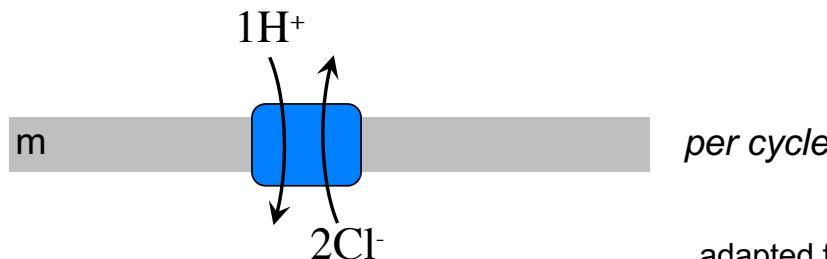
$$\text{with } r = \frac{m}{n}$$

adapted from Accardi A et al., *Nature* **427**: 803 (2004)

E. coli CIC: chloride - proton symport or antiport?



E. coli CIC
(and several, but not all,
human CIC proteins)



$$E_{\text{comb}} = \frac{n E_{\text{Cl}^-} + m E_{\text{H}^+}}{n + m}$$

$$E_{\text{comb}} = \frac{1}{1 + r} (E_{\text{Cl}^-} + r E_{\text{H}^+})$$

$$\text{with } r = \frac{m}{n}$$

adapted from Accardi A et al., *Nature* **427**: 803 (2004)

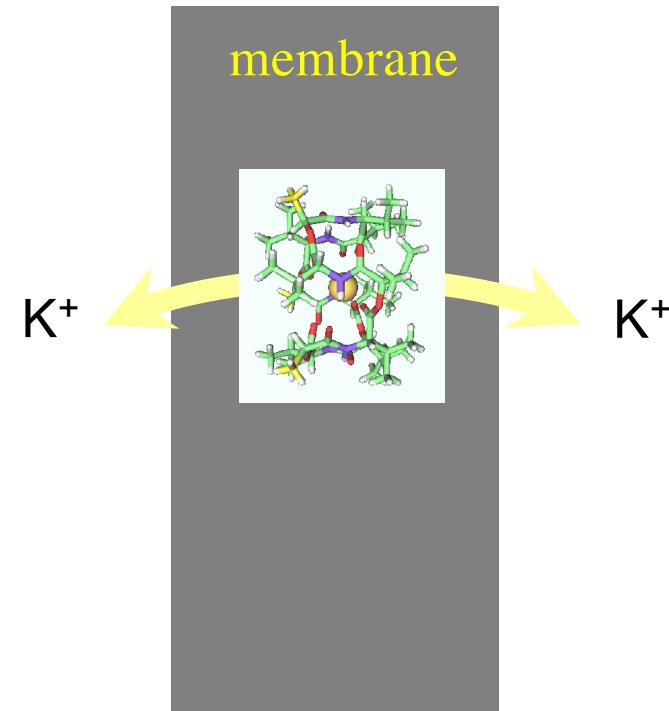
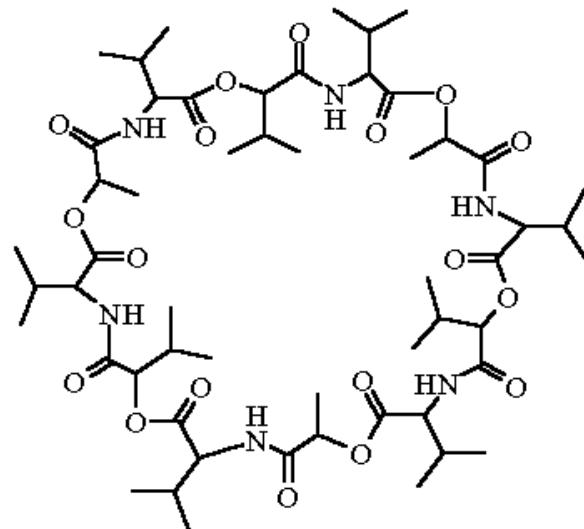
Active transport of CIC antiporter in proteoliposomes: blackboard

K^+ (add valinomycin) push H^+ and Cl^- moving

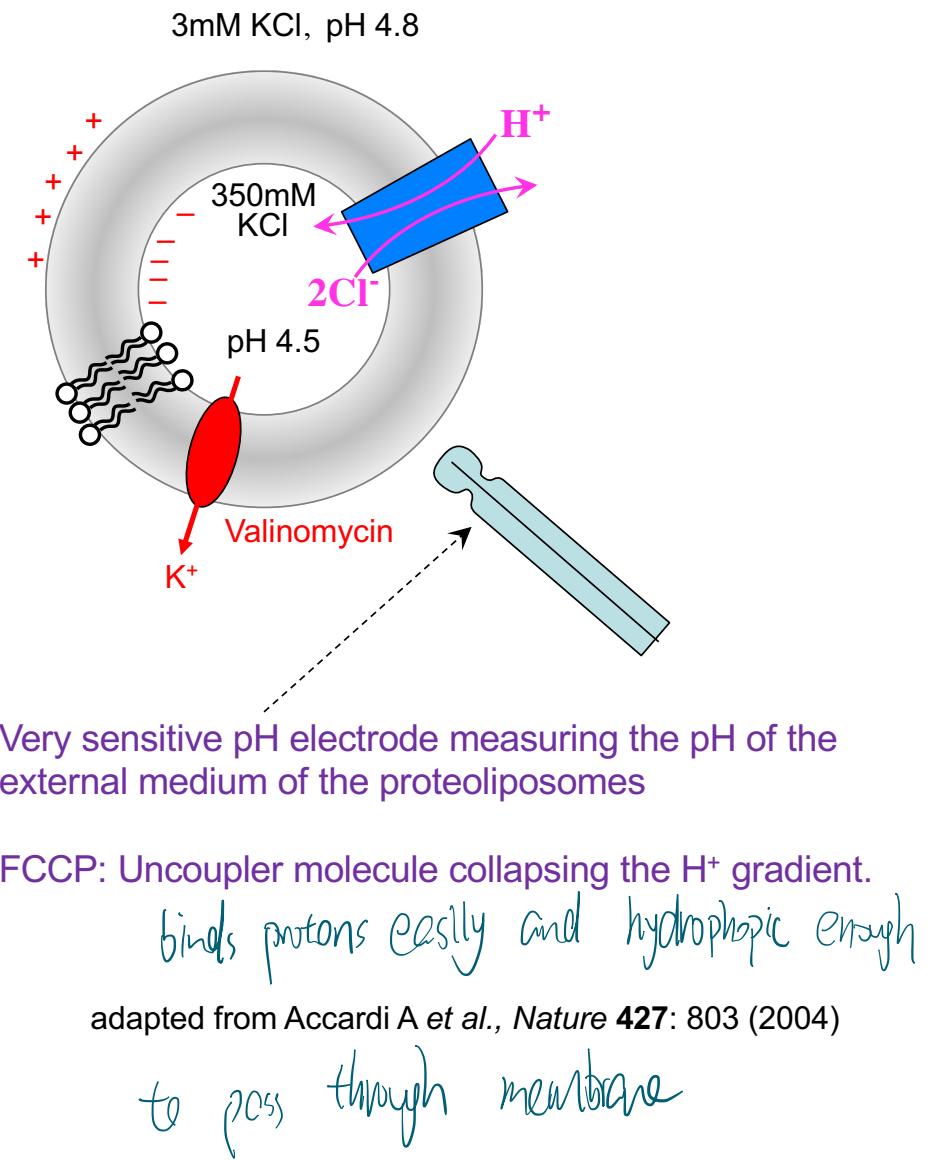
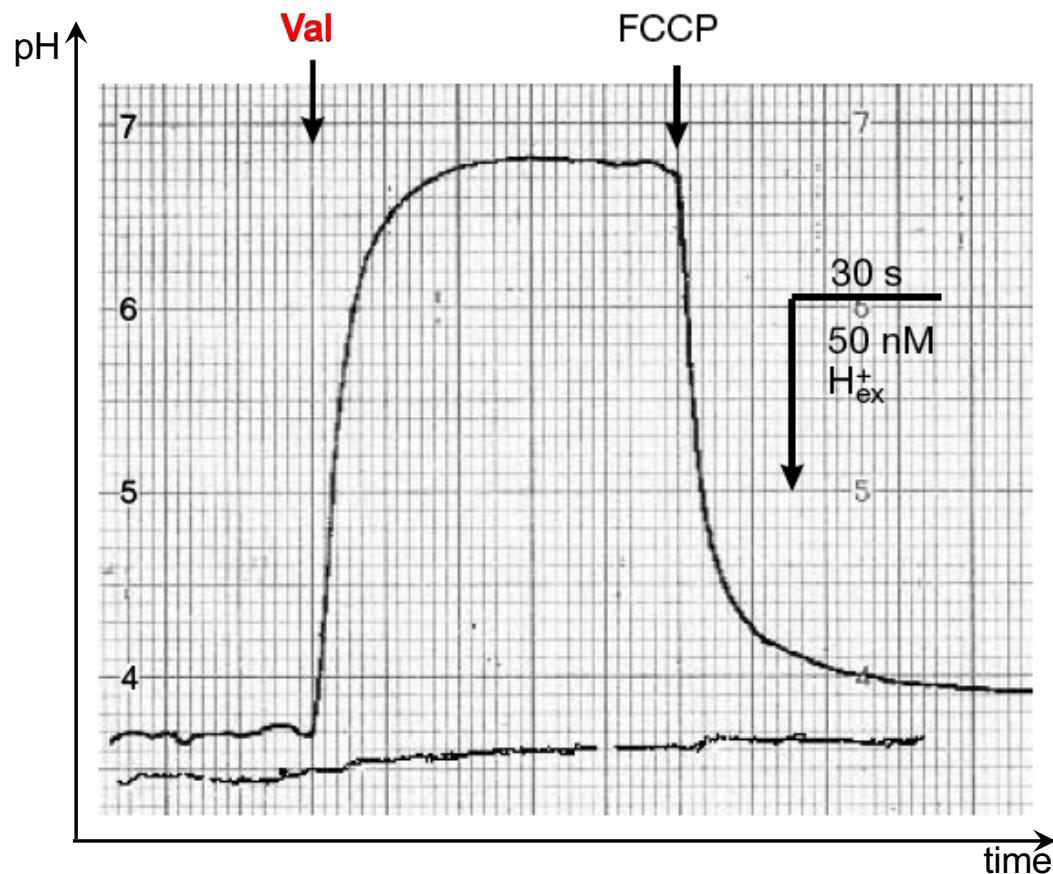
expect pH outside increases
but in fact it fails since
it runs in very few moments.
After one turnover, ± 3 charges.
It would be E inhibiting this transport
therefore, we need helper to study this active
transport (e.g. Valinomycin)
create K^+ gradient in favor of $Cl^- H^+$ moving

Valinomycin

- Valinomycin is a cyclic peptide obtained from the cells of several *Streptomyces* strains. It consists of L-lactate, L-valine, D-hydroxyisovalerate, and D-valine.
- Valinomycin acts as a potassium-selective ionophore that facilitates the translocation of K^+ ions across cell or membranes. This causes damage to bacterial cells, which is why valinomycin acts as an antibiotic. It is widely used to control electrochemical potentials across artificial membranes.

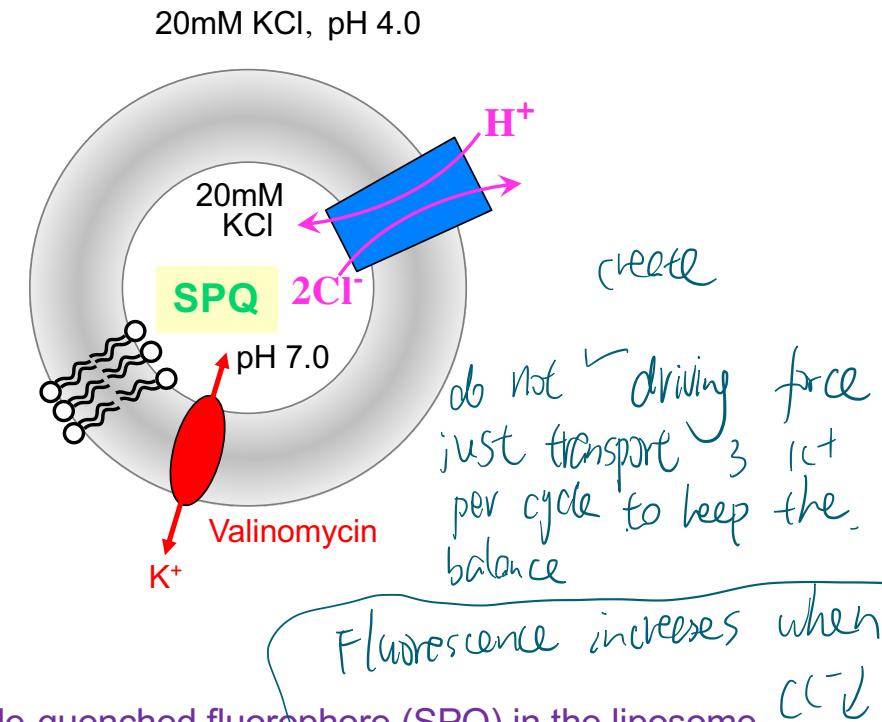
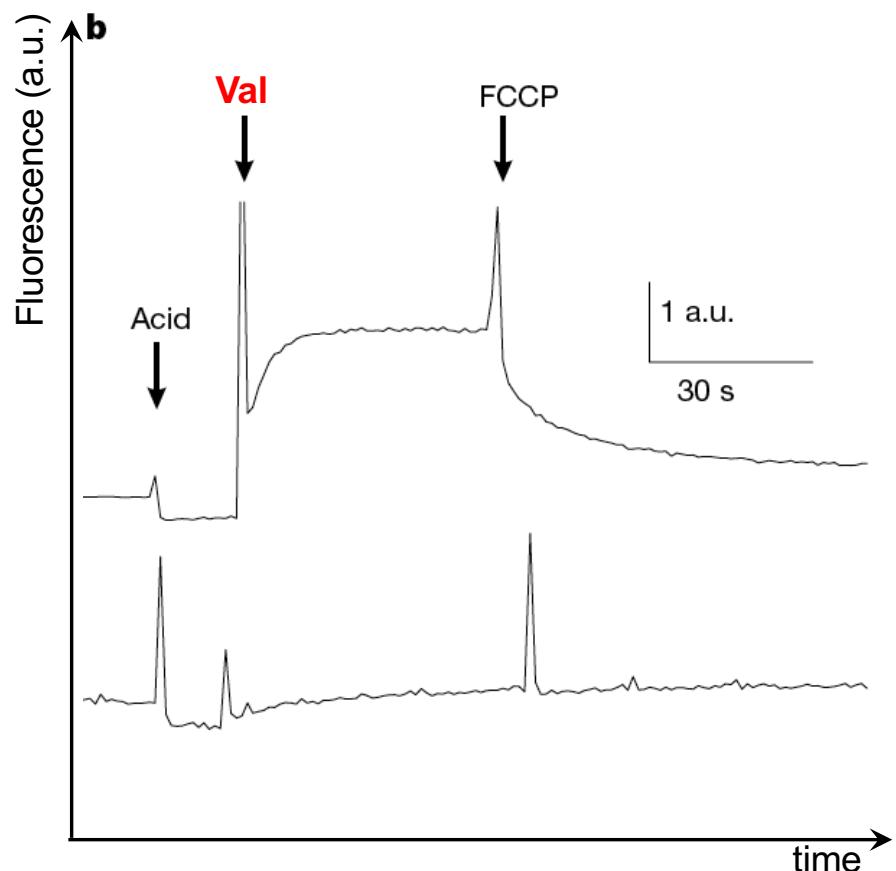


E. coli CIC: Chloride-driven proton transport



not channel but antiporters

E. coli CIC: Proton-driven chloride transport



A chloride-quenched fluorophore (SPQ) in the liposome lumen measures the internal chloride concentration.

FCCP again collapses the pH gradient, slowly reversing chloride flux.

adapted from Accardi A et al., Nature 427: 803 (2004)

Functions of human CIC proteins

The main distinction is the location in all

voltage-gated
anion channels

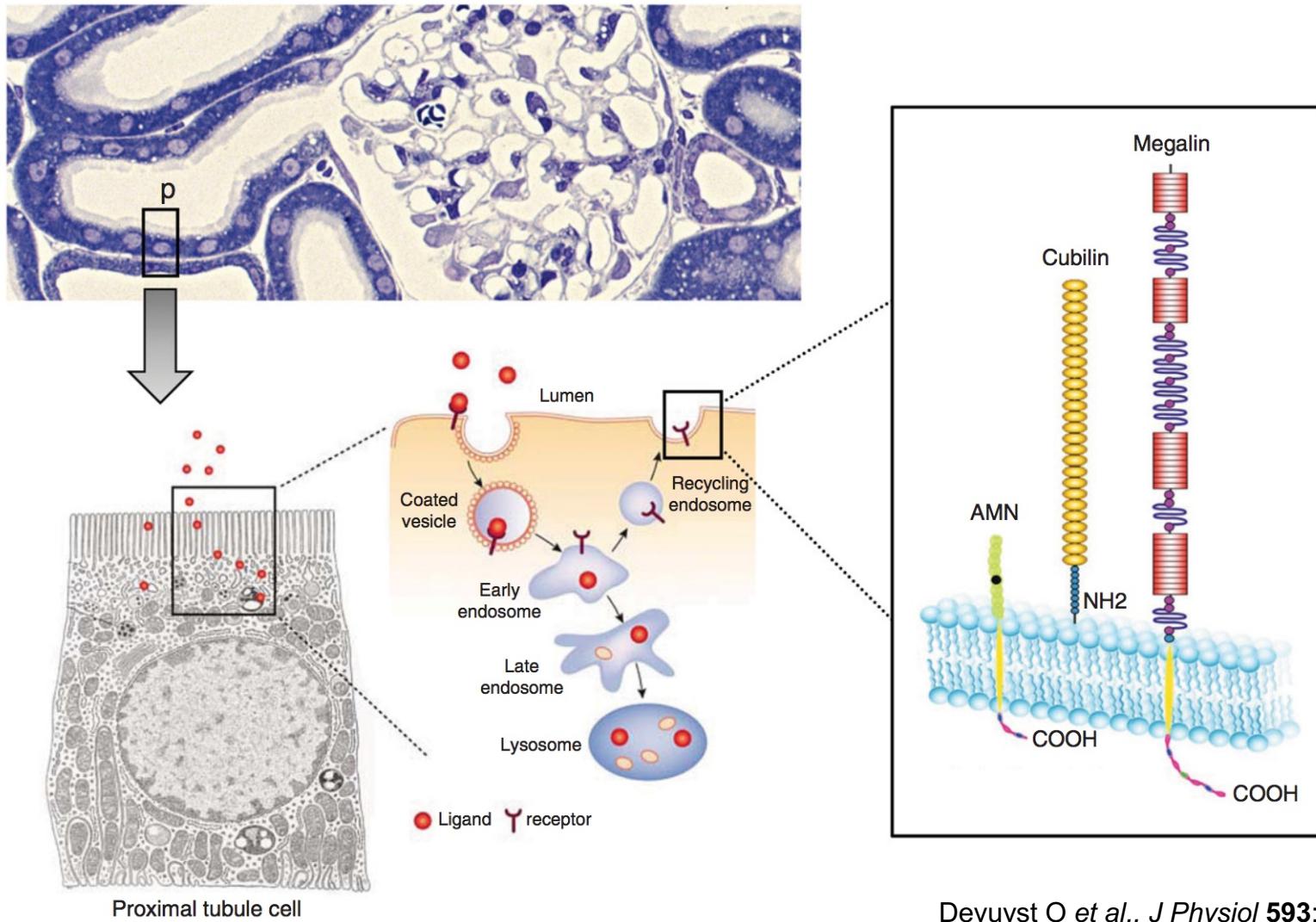
	β-subunit	expression	function	mouse model	human disease
	CIC-1	skeletal muscle	stabilization of membrane potential	myotonia congenita (adr mouse)	recessive & dominant myotonia
	CIC-2 <i>± glialCAM</i>	wide	transepithelial transport extracell. ion homeostasis regulation excitability	degener. retina & testes leukodystrophy	leukodystrophy (loss of function) aldosteronism (gain of function)
	CIC-Ka <i>/barttin</i>	kidney, inner ear	transepithelial transport	diabetes insipidus	?
	CIC-Kb	kidney, inner ear	transepithelial transport	renal salt loss	Bartter III (renal salt loss)
<hr/>					
	CIC-3	wide (brain, kidney, liver...)	acidification & ion homeostasis of late endosomes (and synaptic vesicles?)	degeneration of CNS & retina	?
	CIC-4	wide (brain, kidney, muscle...)	ion homeostasis of endosomes	no obvious phenotype	mental retardation epilepsy
	CIC-5	kidney (also: intestine...)	acidification & ion homeostasis of endosomes	impaired renal endocytosis	Dent's disease (proteinuria and kidney stones)
	CIC-6	neuronal	ion homeostasis of late endosomes	lysosomal storage in neurons	?
	CIC-7 <i>/Ostm1</i>	wide	lysosomal ion homeostasis & acidification osteoclast resorption lacuna	recessive osteopetrosis with CNS & retina degeneration, dominant osteopetrosis	recessive osteopetrosis assoc. with CNS & retina degeneration, or dominant osteopetrosis

chloride-proton
exchangers
(antiporters)

they are all
in organelles, never
insert into plasma
membrane

Jentsch TJ et al., Physiol Rev 98: 1493 (2018)

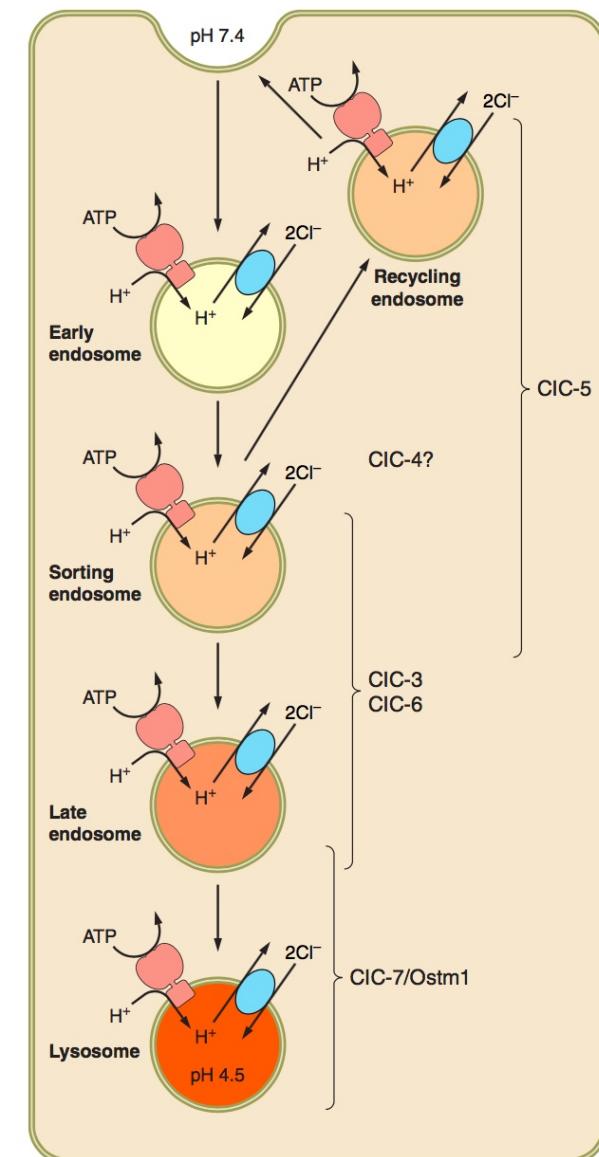
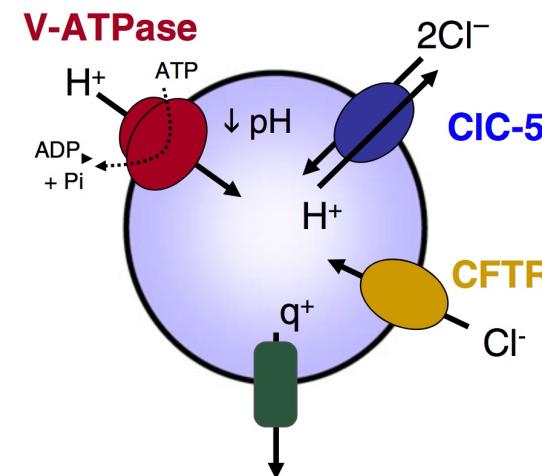
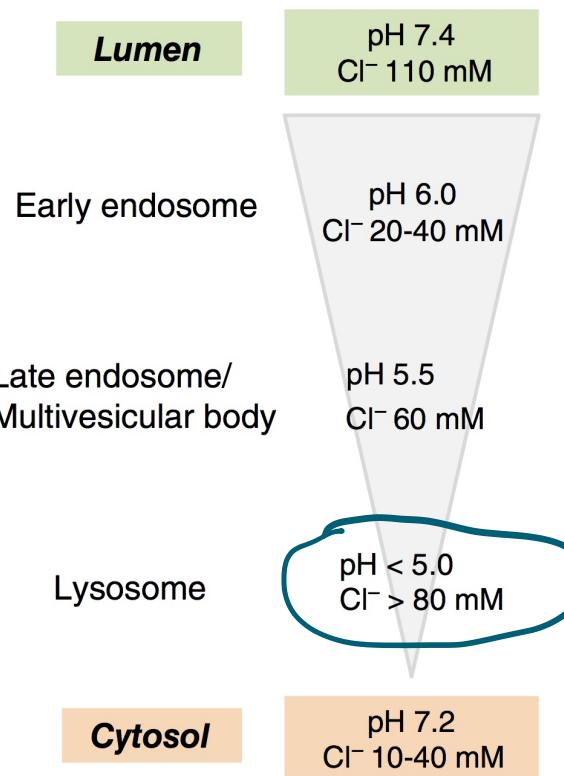
Functions of vesicular CIC proteins



Devuyst O et al., *J Physiol* **593**: 4151 (2015)

Functions of vesicular CIC proteins

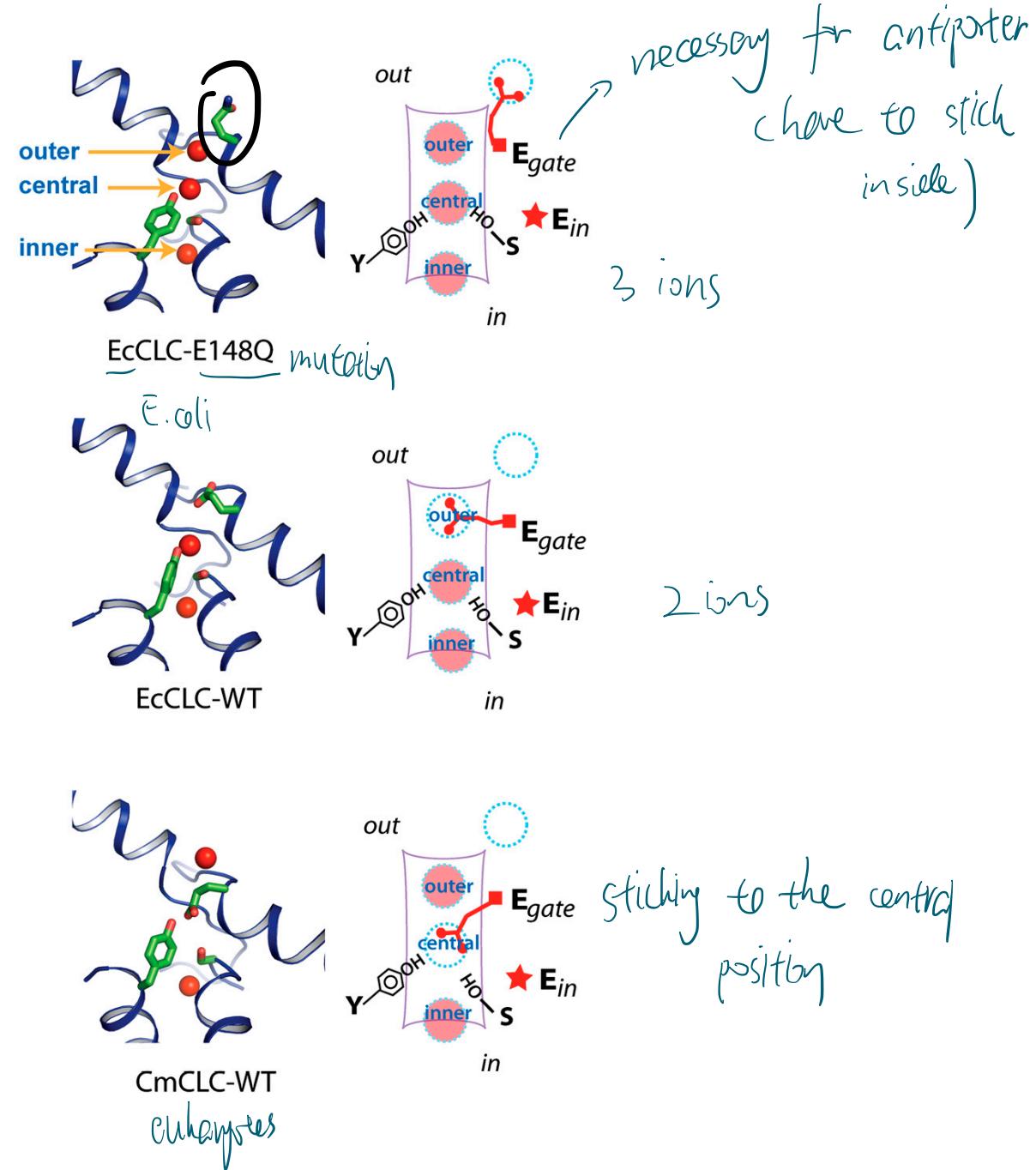
⇒ Control pH



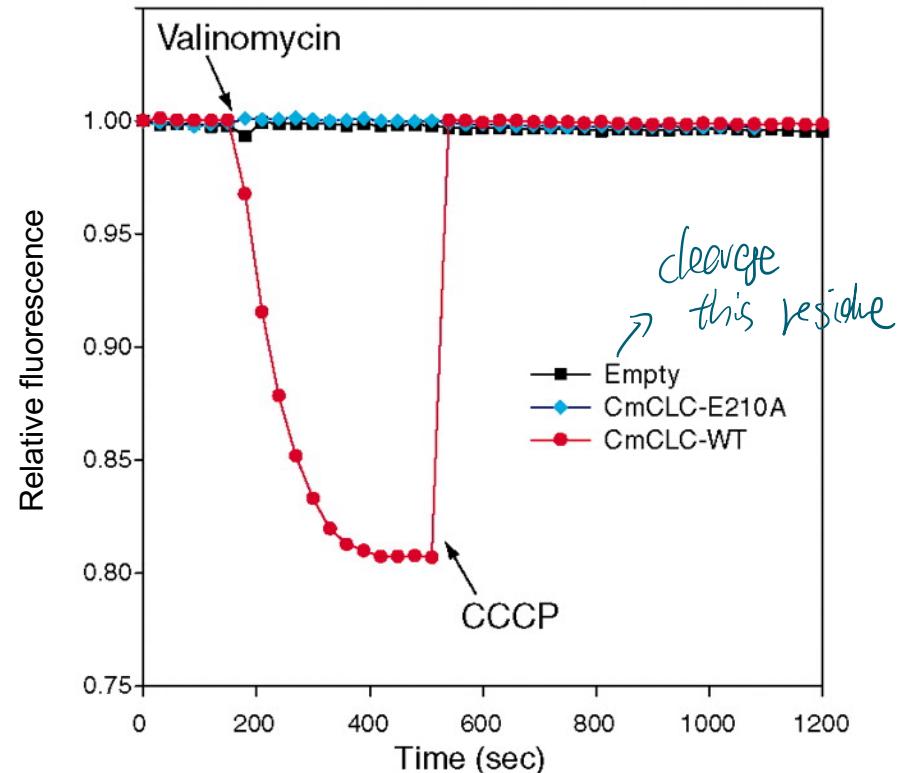
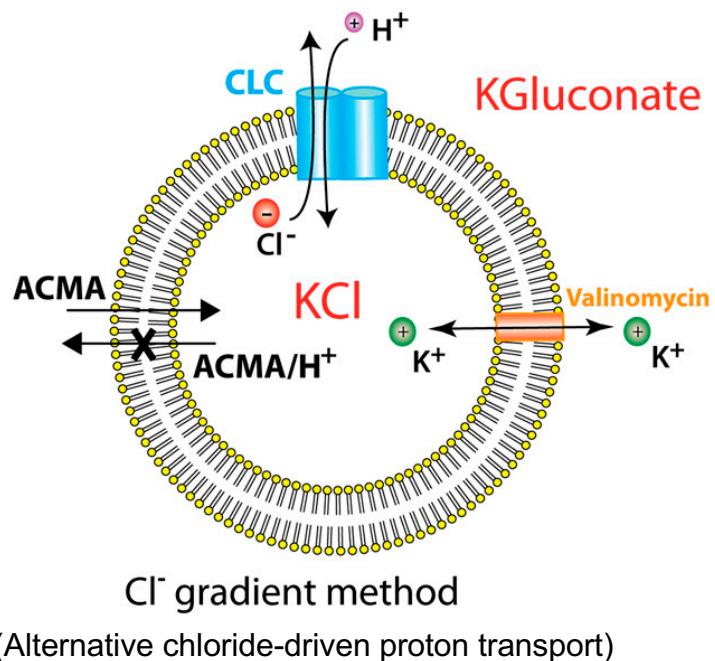
Devuyst O et al., *J Physiol* **593**: 4151 (2015)

Jentsch TJ et al., *Physiol Rev* **98**: 1493 (2018)

Comparison of CIC structures: Chloride positions and critical residues



CIC mechanism: Insight from eukaryotic *CmCIC* Cl⁻/H⁺ antiporter



Inside: 450 mM KCl, pH 7.4; Outside: 427.5 mM K-gluconate, 22.5 mM KCl, pH 7.5 (achieved by 20x dilution)

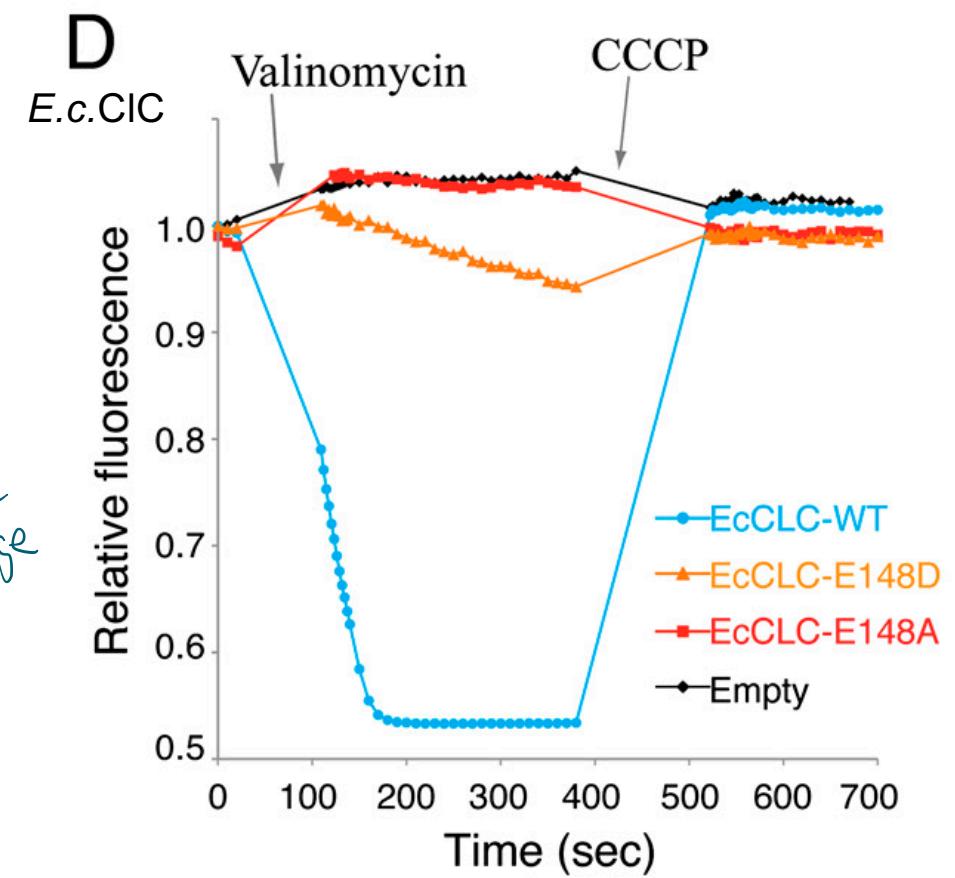
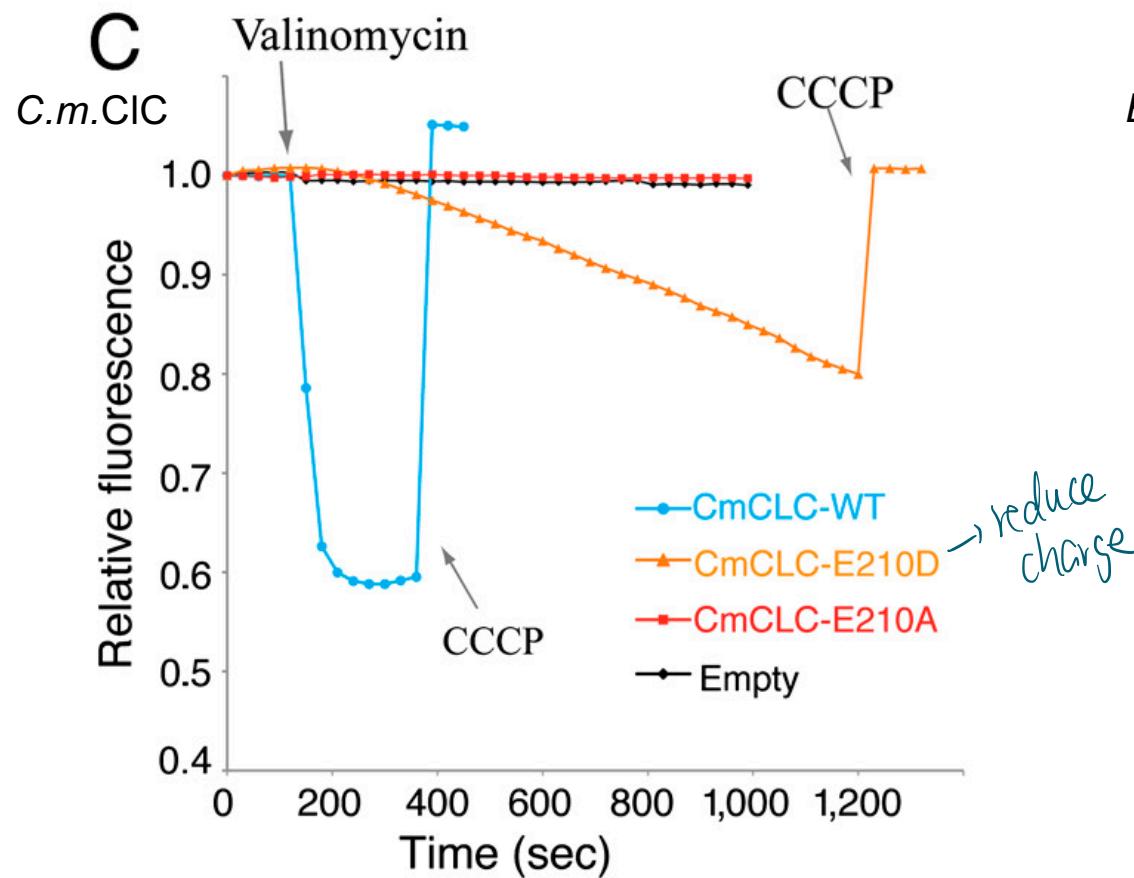
ACMA: Fluorescence dye 9-amino-6-chloro-2-methoxyacridine, quenched by ΔpH across membrane.

C.m.: Red algae *Cyanidioschyzon merolae*

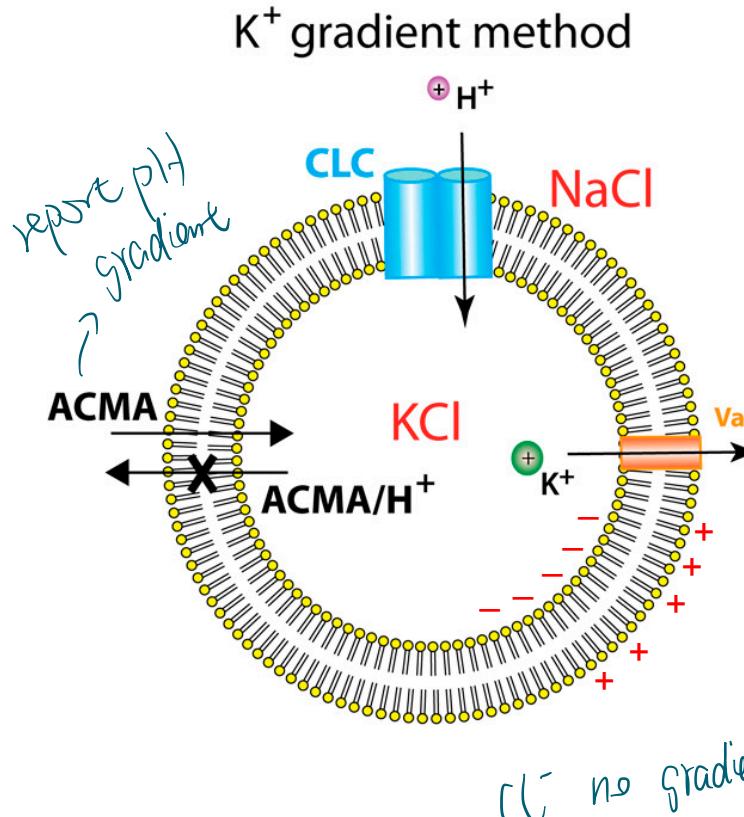
E210 is equivalent to E_{gate} and corresponds to E148 in *E. coli* protein)

Feng L et al.,
Science 330: 635 (2010)
and PNAS 109: 11699 (2012)

CIC mechanism: Similar mechanisms of *C.m.* and *E.c.* CIC antiporters



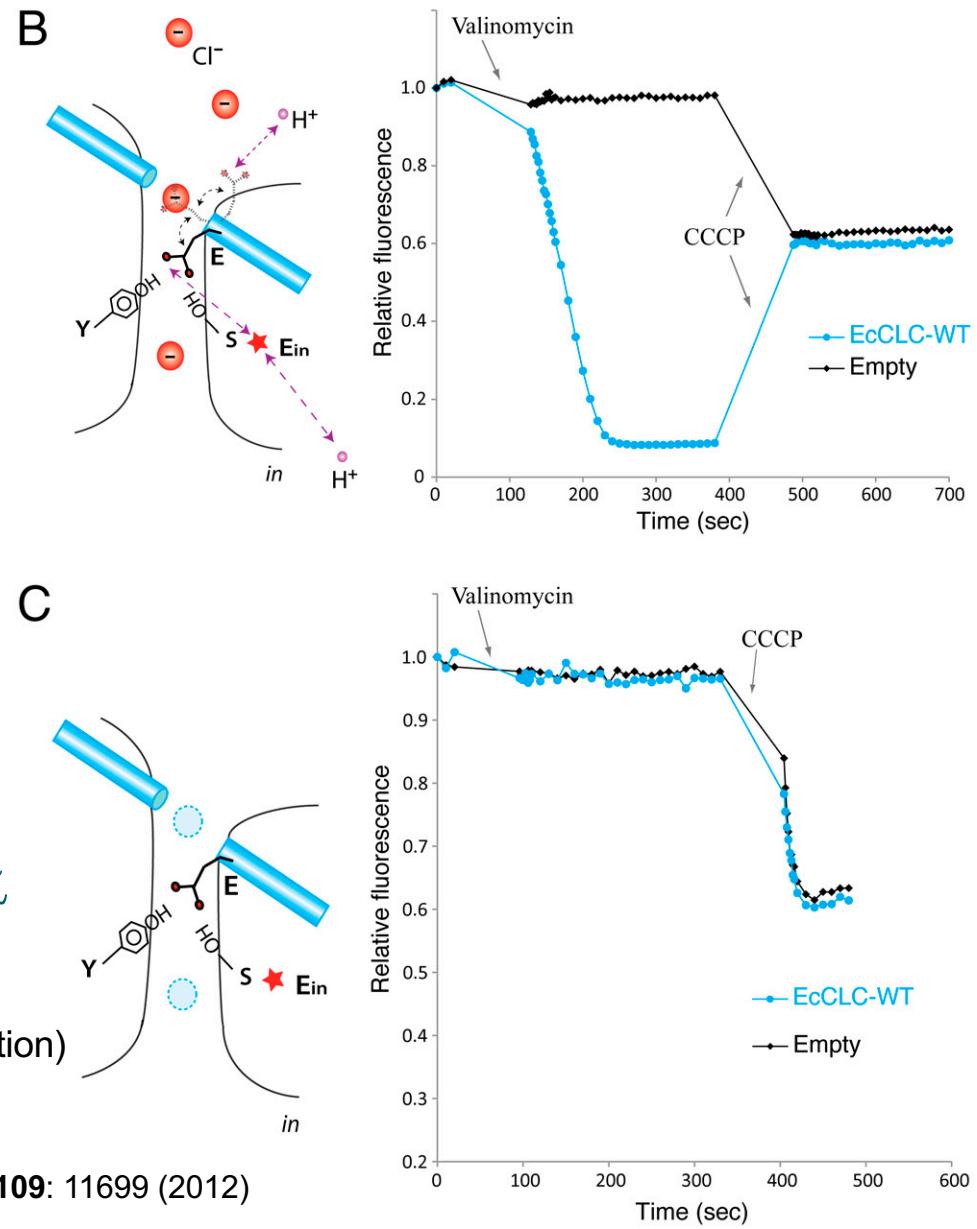
CIC mechanism: Isolating H⁺ flux 1



B: Inside: 450 mM KCl, pH 7.4

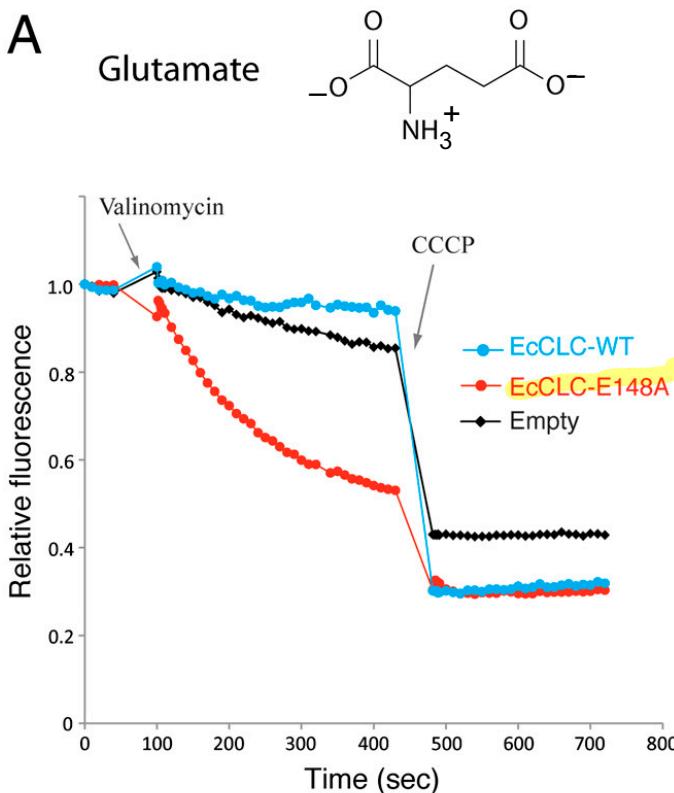
Outside: 427.5 mM NaCl, 22.5 mM KCl, pH 7.5 (achieved by 20x dilution)

C: Cl⁻ replaced by gluconate inside and outside

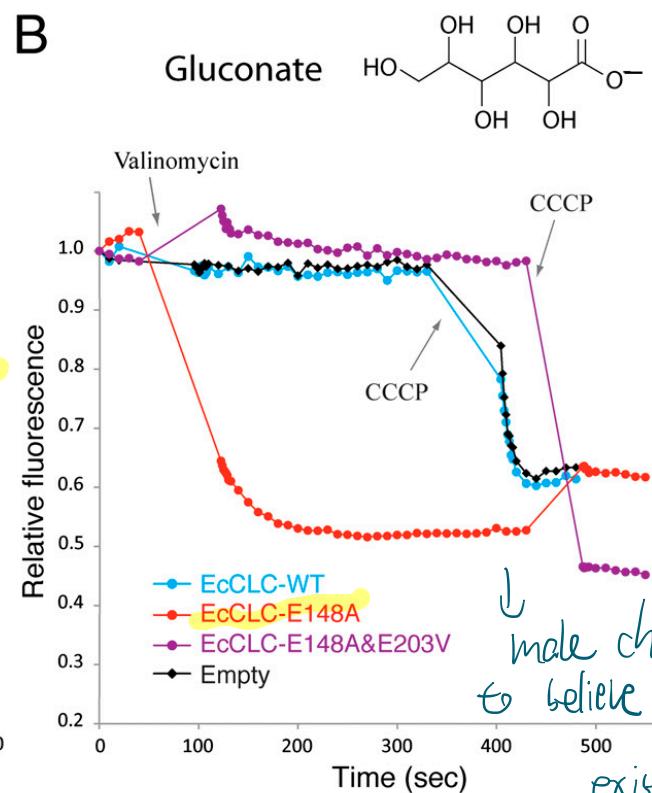


Isolating H⁺ flux 2, Identification of H⁺ pathway using K⁺ gradient method

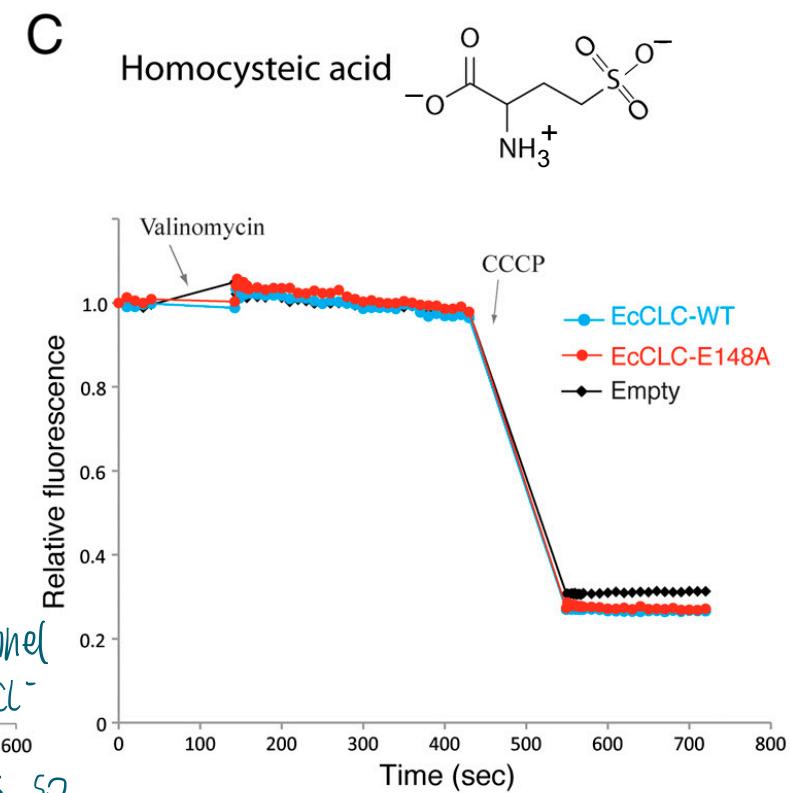
A



B

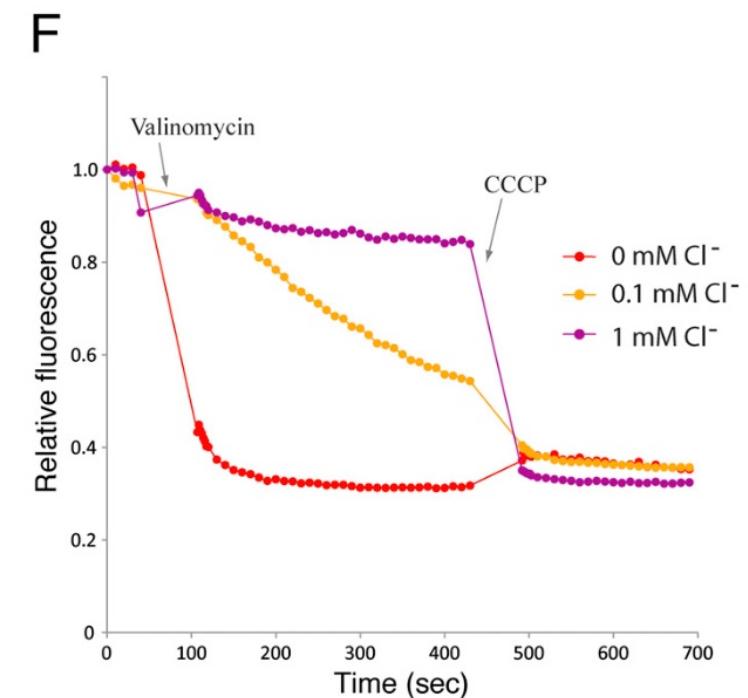
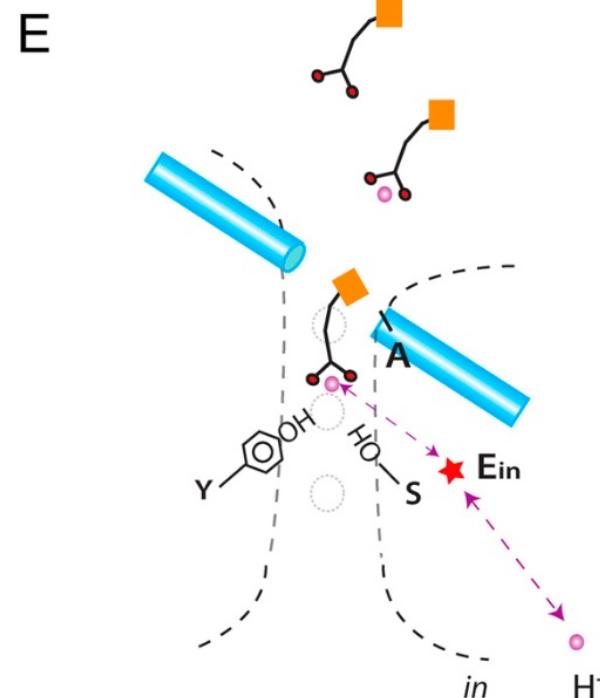
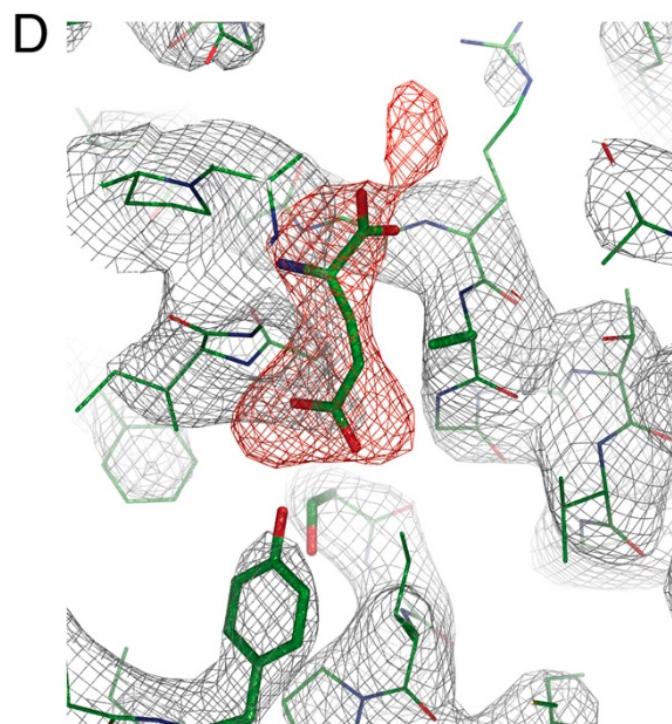


C

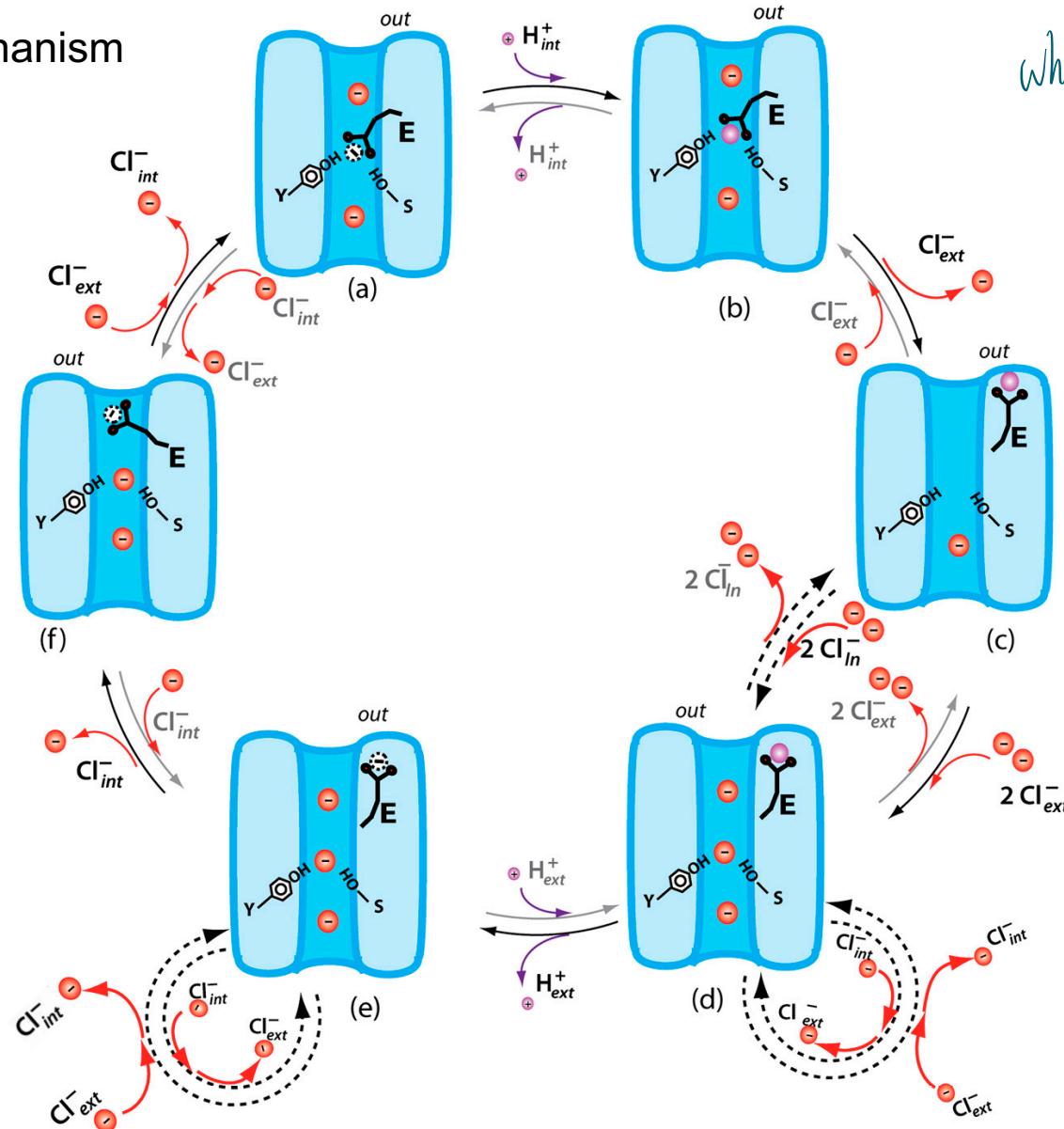


adapted from Feng L et al., PNAS 109: 11699 (2012)

CIC mechanism: H⁺ pathway and Cl⁻ competition in EcCIC-E148A mutant

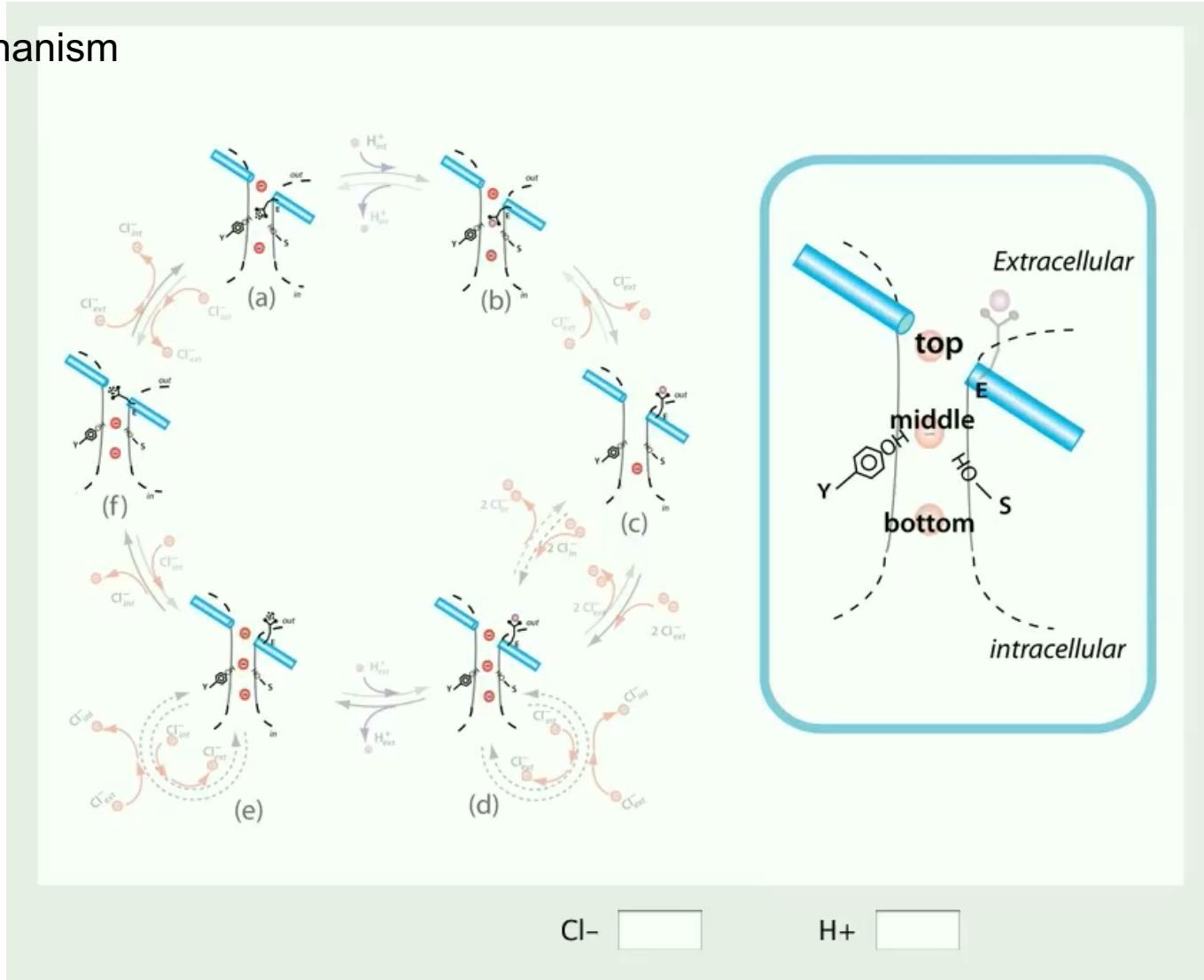


CIC antiporter mechanism



Feng L et al., PNAS 109: 11699 (2012)

CIC antiporter mechanism



Feng L et al., *Science* **330**: 635 (2012)