Biology 5357: Chemistry & Physics of Biomolecules
Fall 2023

Lecture 8:
Membrane proteins: direct & secondary active transport

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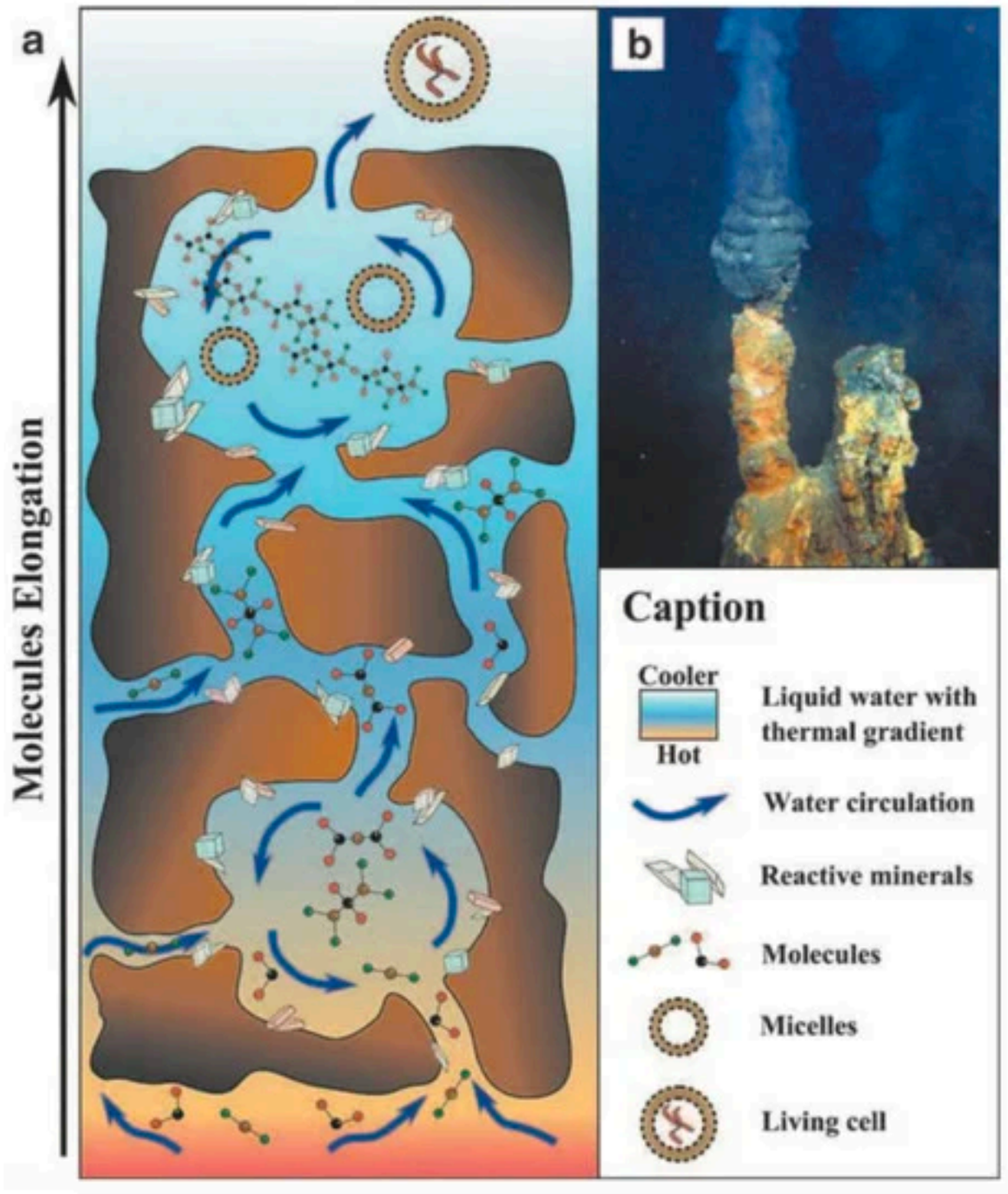
Background reading for this week:

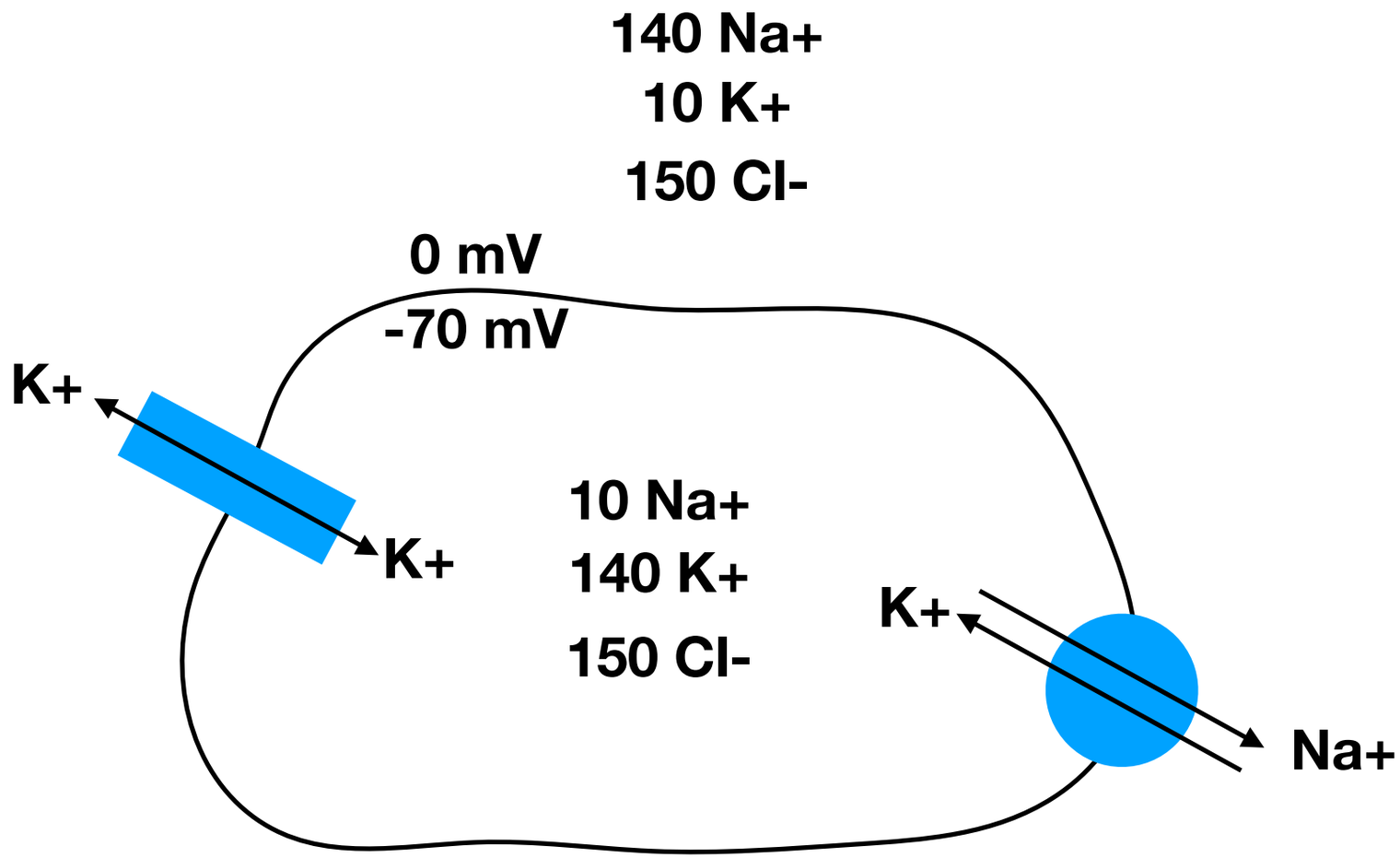
Cell boundaries: *How membranes and their proteins work*. CRC Press 2022, Stephen H. White, Gunnar von Heijne, Donald M. Engelman

Origins of cellular life



Longo, A. & Damer, B. Factoring Origin of Life Hypotheses into the Search for Life in the Solar System and Beyond. *Life* **10**, 52 (2020).

Biological function relies on gradients across membranes



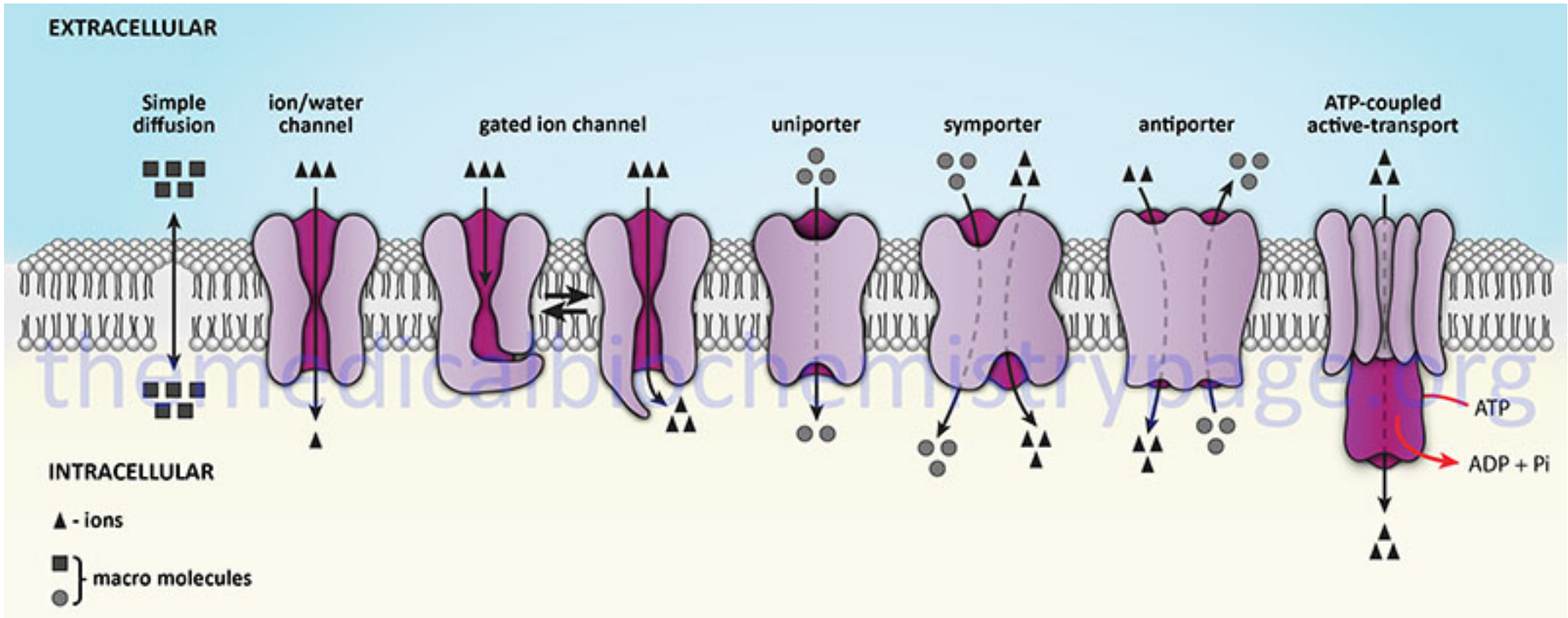
EK = RT/zF ln(Ko/Ki)

At 25C, RT/F ~ 25 mV

=> EK ~ -70 mV

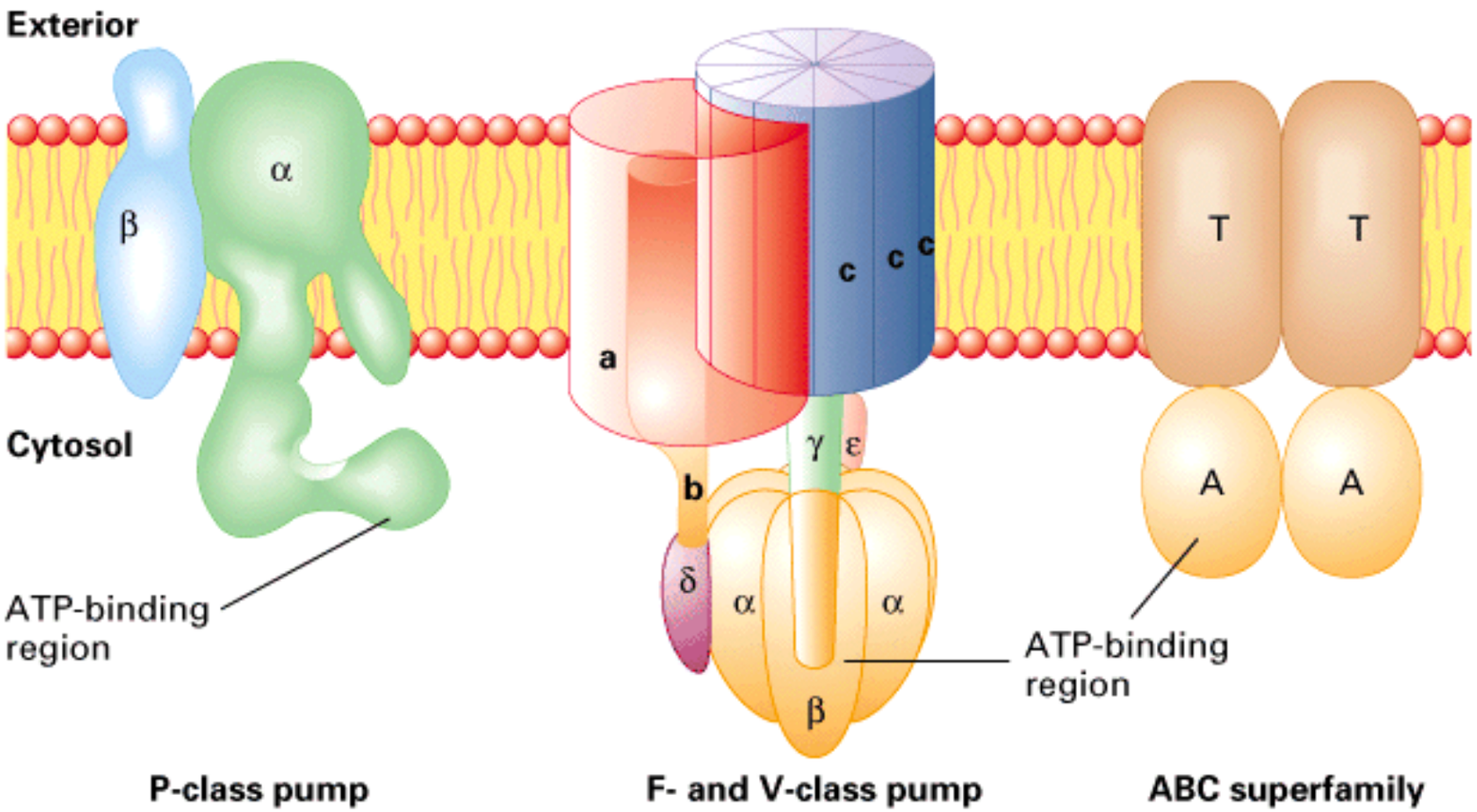
- How is the ionic concentration gradient set in the first place?
- How do we make ATP?
- How do cells obtain key nutrients, or expel unwanted substances?

Mechanisms of membrane transport



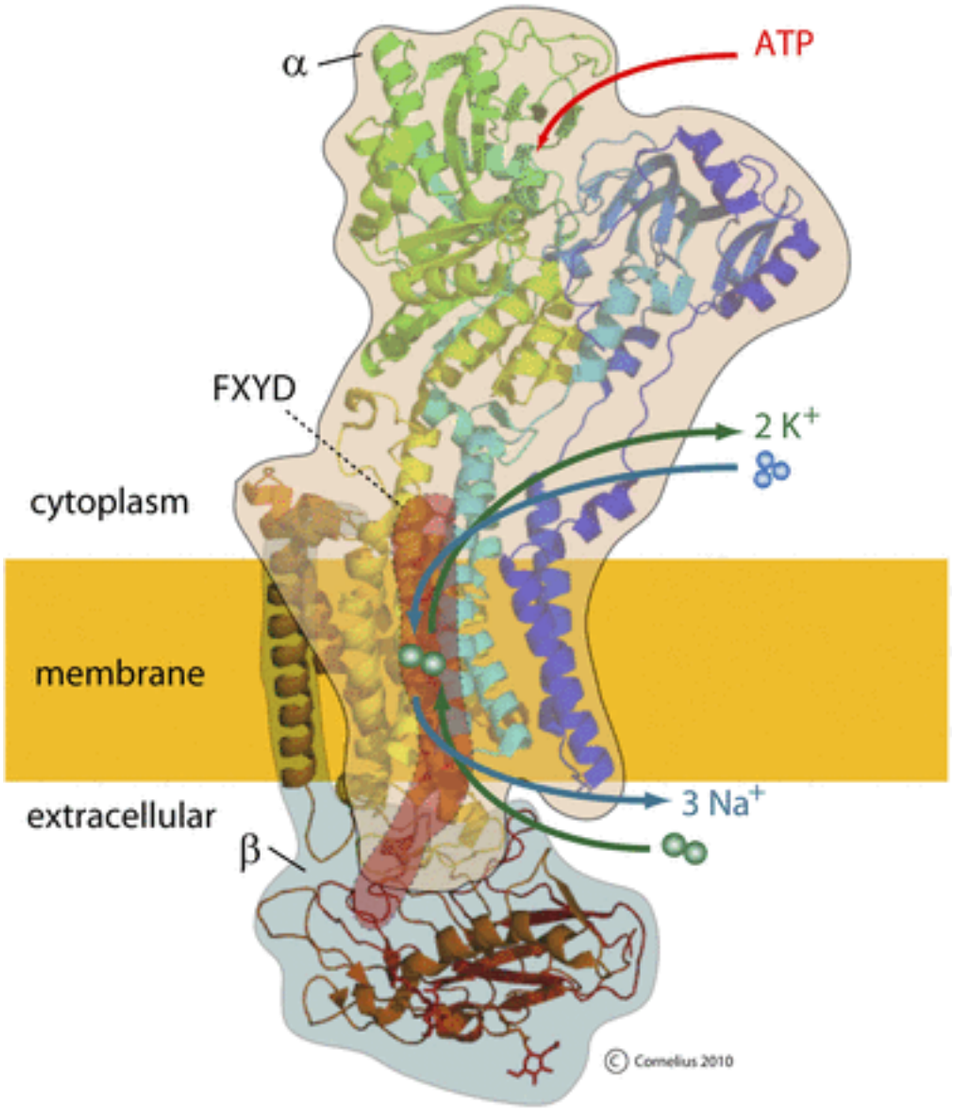
https://themedicalbiochemistrypage.org/biological-membranes-and-membrane-transport-processes/

Primary transporters



- Otherwise known as “pumps”
- Couples ATP hydrolysis directly to uphill movement of species (ions, substrates)
- 3 types of pumps - P, F, V
- Also ABC (ATP binding cassette) transporters

P-type ATPases: e.g., the Na^+/K^+ pump



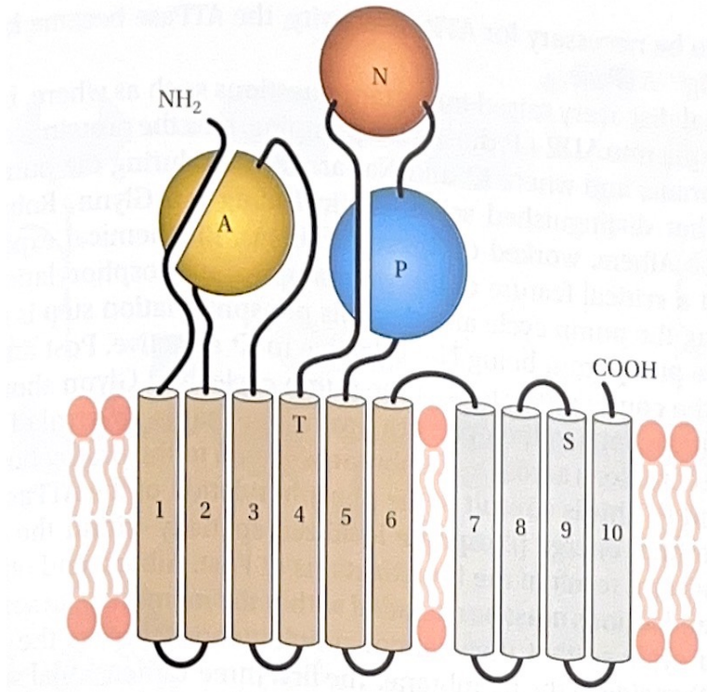


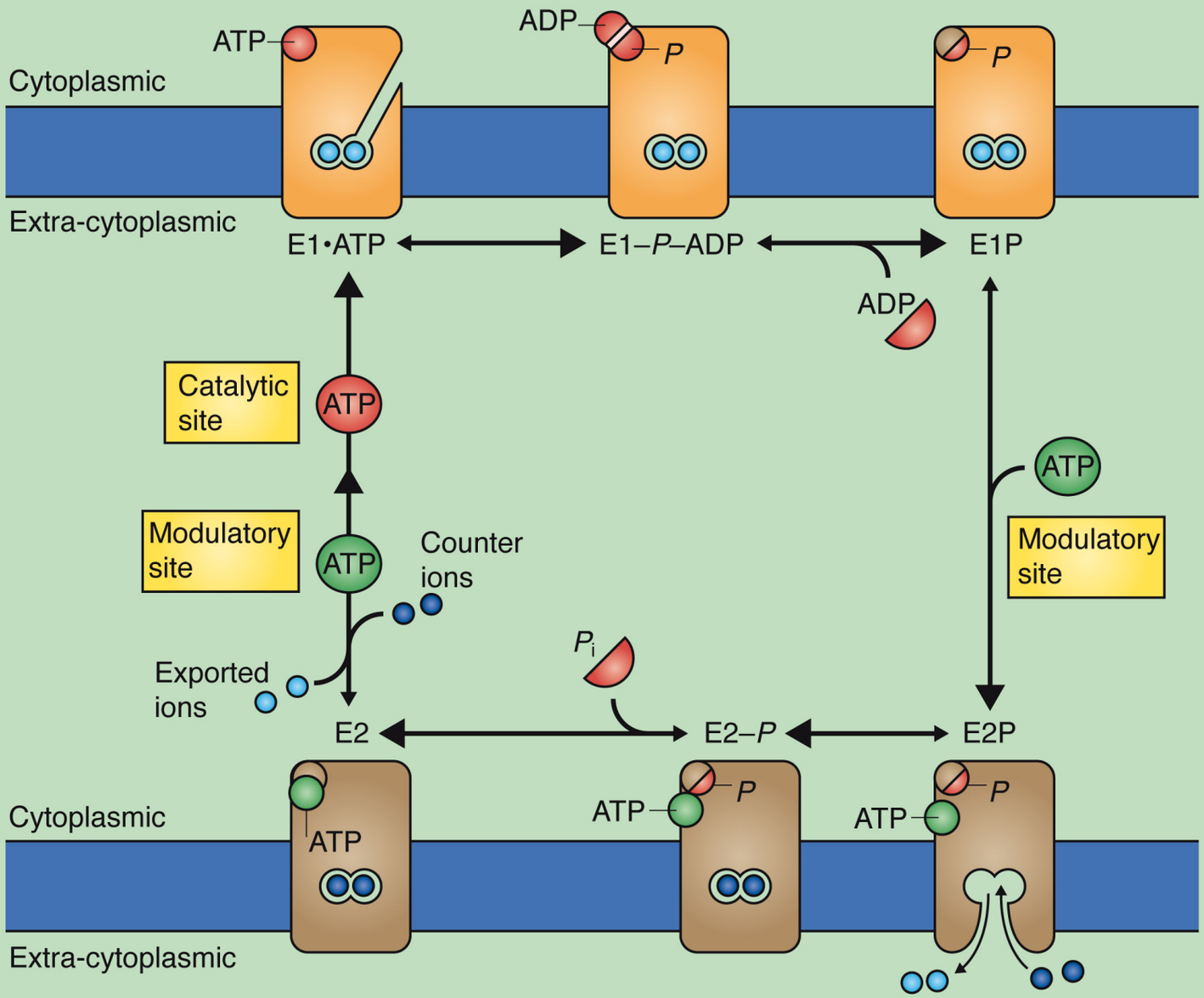
Figure 12.2 Schematic structure of P-type ATPases. The actuator (A) domain is comprised of the N-terminal tail and the first cytoplasmic loop. The nucleotide-binding (N) and phosphorylation (P) domains are inserted into the intracellular loop between TM4 and TM5. The first six TM helices form the transport (T) domain. The support (S) domain is comprised of the last four TM helices. (Palmgren MG, Nissel P [2011] *Annual Review of Biophysics* 40: 243–266. With permission from Maike Bublitz.)

Figure 1.
The molecular structure of Na^+,K^+-ATPase. The figure is based upon the crystal structure of the homologous Na^+,K^+-ATPase in the E_2P_2K conformation (Shinoda et al., 2009) and drawn by Flemming Cornelius (Aarhus University, Aarhus, Denmark). The Na^+,K^+ pump comprises an α and a β subunit, a glycoprotein that participates in the translocation of the molecule from the cell interior to its correct position in the lipid bilayer of the plasma membrane. A regulatory subunit (FXYD) is also shown. During each transport cycle of the Na^+,K^+ pump, one ATP molecule is bound to the cytoplasmic site of the α subunit; its hydrolysis provides energy for the active transport of Na^+ and K^+. The transmembrane domain consists of 10 transmembrane helices and contains the binding sites for three Na^+ or two K^+ ions, respectively, which pass sequentially through the same cavity in the molecule during each transport cycle.

P-type ATPases

3. The catalytic cycle

P-type ATPases cycle between two main conformational states E1 and E2. Hydrolysis of one ATP molecule fuels one transport cycle of ions against counter ions through the membrane.



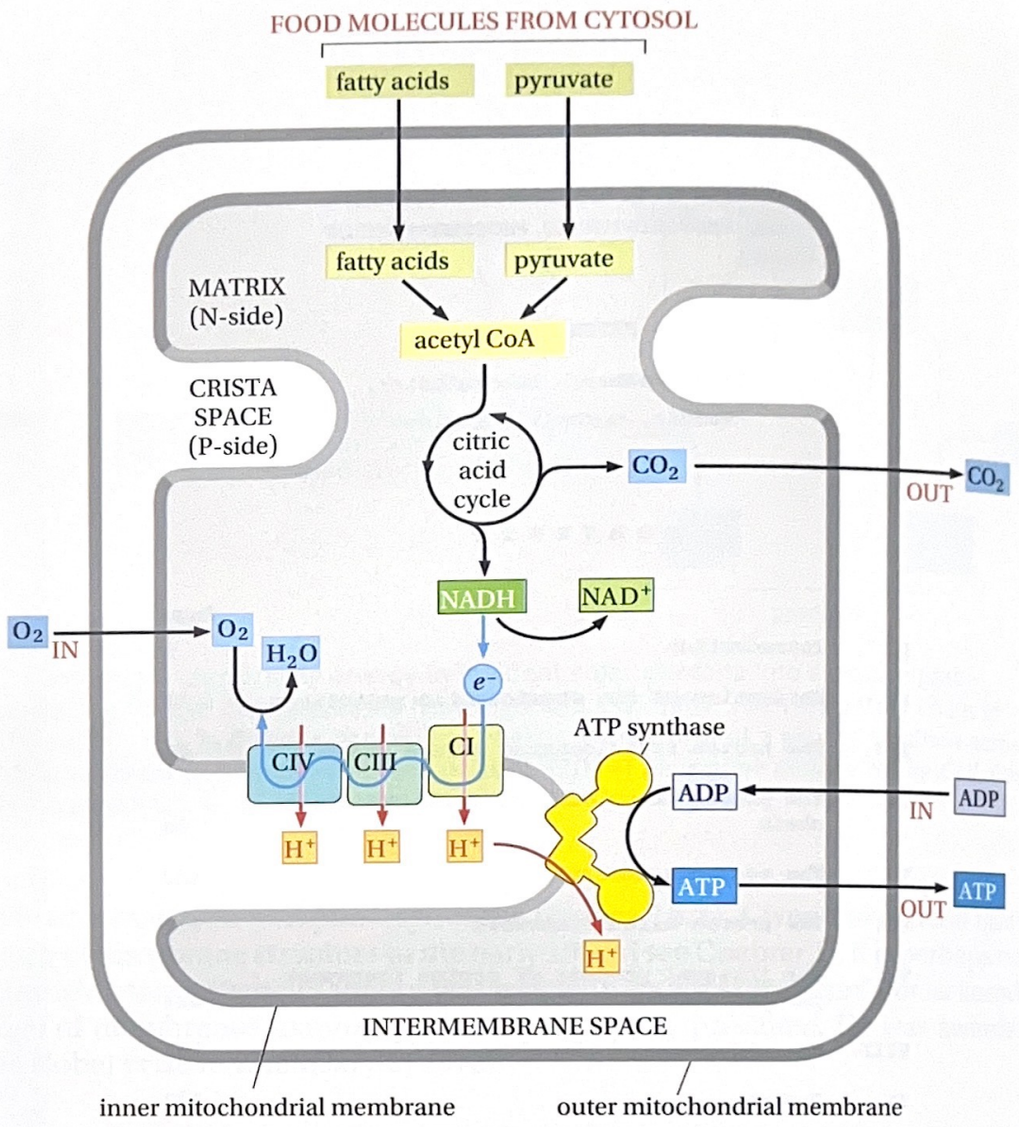
Generic cycle exemplified by a transport stoichiometry of 2:2 ions

J. Cell Sci. (2011) 124, 2515–2519

Chemiosmotic theory
How does ATP get made?

- Mitchell, 1961
- Electron transport chain builds up a proton motive force (electrochemical proton gradient)
- This drives ATP synthesis via ATP synthase, an Fo/F1 ATPase
- What else is needed?

Figure 14.3 Overview of mitochondrial energy conversion. CO_2 and reduced NADH are produced in the citric acid cycle. NADH feeds electrons into the respiratory chain, ultimately reducing O_2 to H_2O and maintaining the H^+ gradient across the inner membrane, which in turn drives ATP synthesis. (From Alberts B, Johnson A, Lewis JH et al. [2014] *Molecular Biology of the Cell*, 6th Edition. Garland Science, New York. With permission from W. W. Norton.)

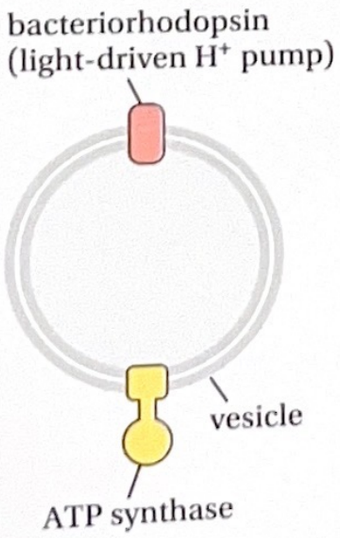


Reconstitution provides evidence that proton gradients drive ATP synthesis

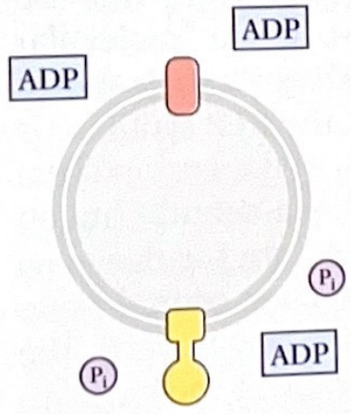
BOX 14.6 A DEMONSTRATION THAT A PROTON GRADIENT CAN DRIVE ATP SYNTHESIS

In 1974, Efiam Racker and Walther Stoeckenius conducted an experiment that remains the most important test of the Mitchell chemiosmotic hypothesis: that a proton gradient can cause a membrane-bound complex from mitochondria to synthesize ATP. Bacteriorhodopsin, discovered by Stoeckenius, uses light energy to move protons across a membrane (Box 14.8). As shown in Figure 1, molecules of oriented bacteriorhodopsin were put into lipid vesicles that contained the ATP synthase from mitochondria [1], and ADP and P_i were added to the solution [2]. When in the dark, no ATP was made [3]; however, in the light, the bacteriorhodopsin used light energy to pump protons into the vesicle, creating a proton gradient, and ATP was produced [4]! This remarkable experiment definitively settled the debates that had been raging for and against the Mitchell hypothesis.

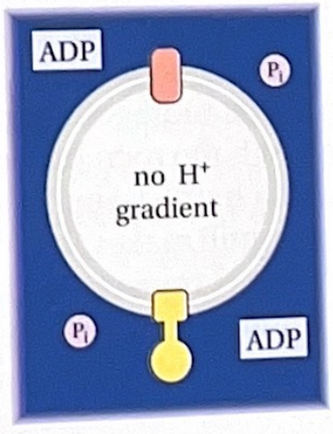
1 ATP synthase and bacteriorhodopsin were incorporated into membrane vesicles



2 ADP and P_i were added on the outside of the vesicles



3a one sample was kept in the dark: no ATP was made



3b one sample was exposed to light: ATP was made

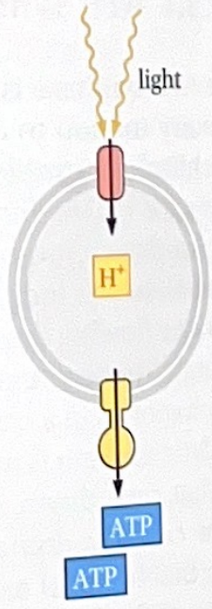
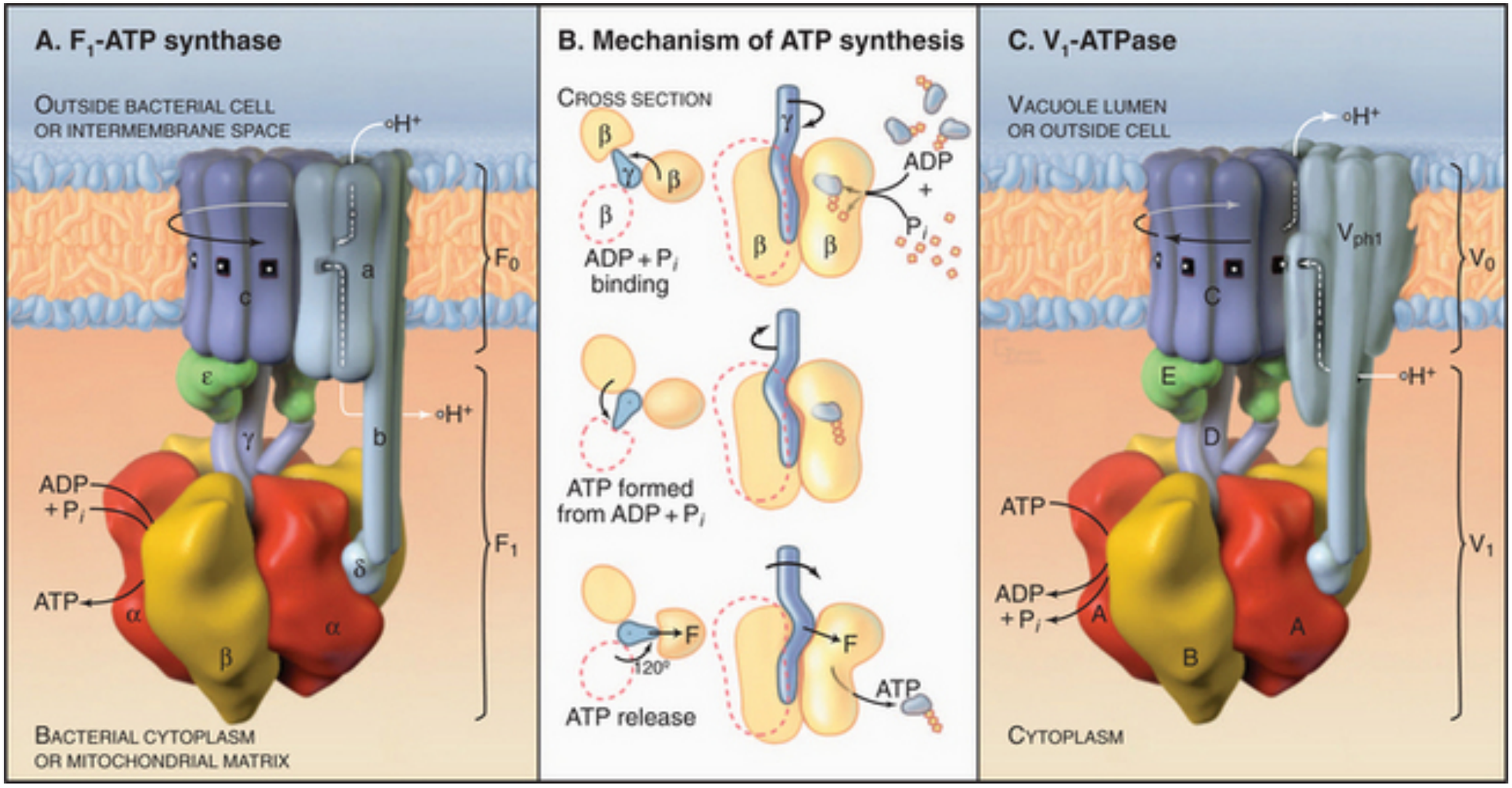
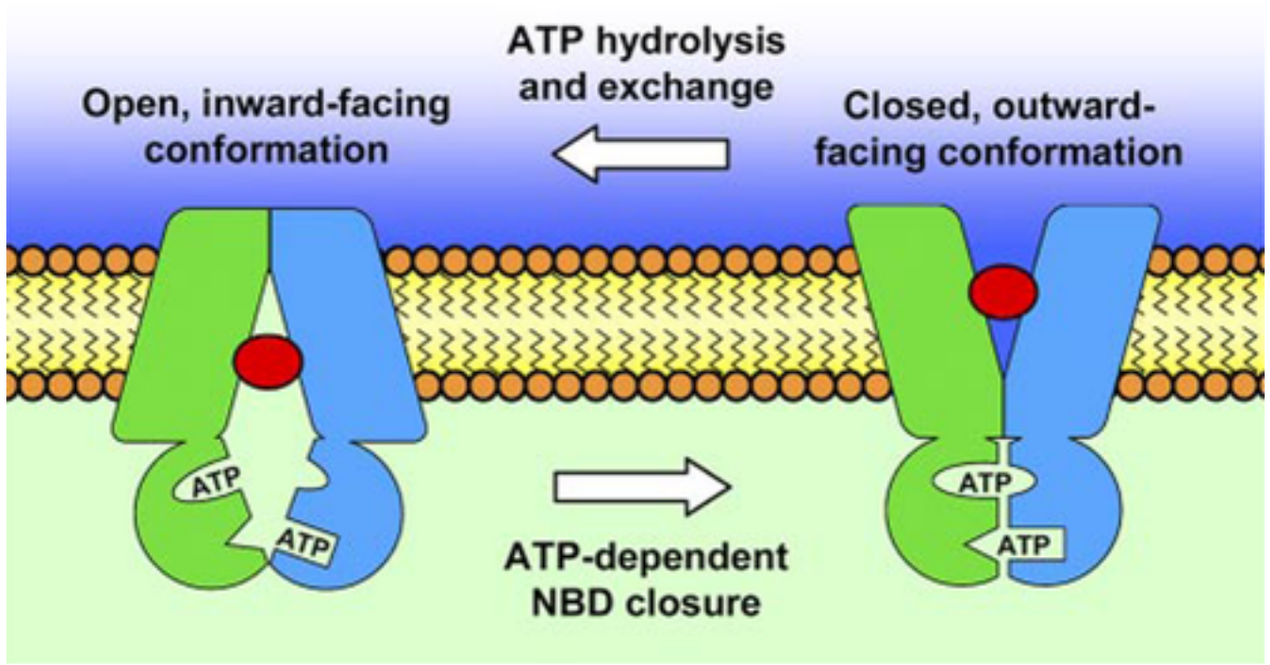


Figure 1

ATP Synthases

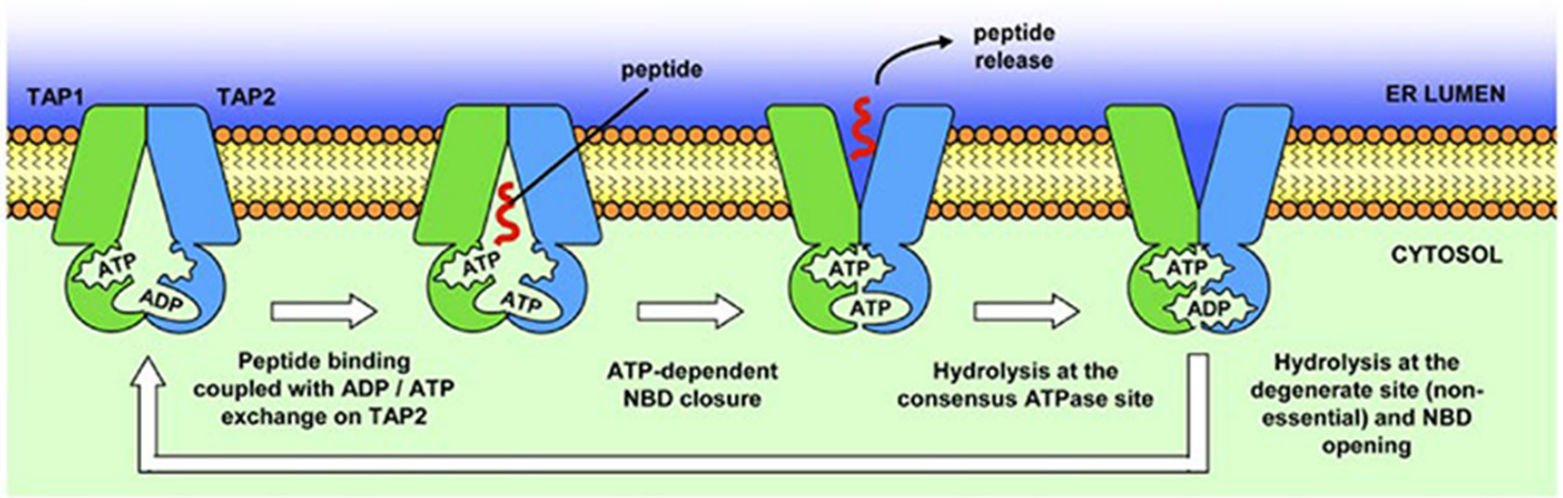


ABC transporters

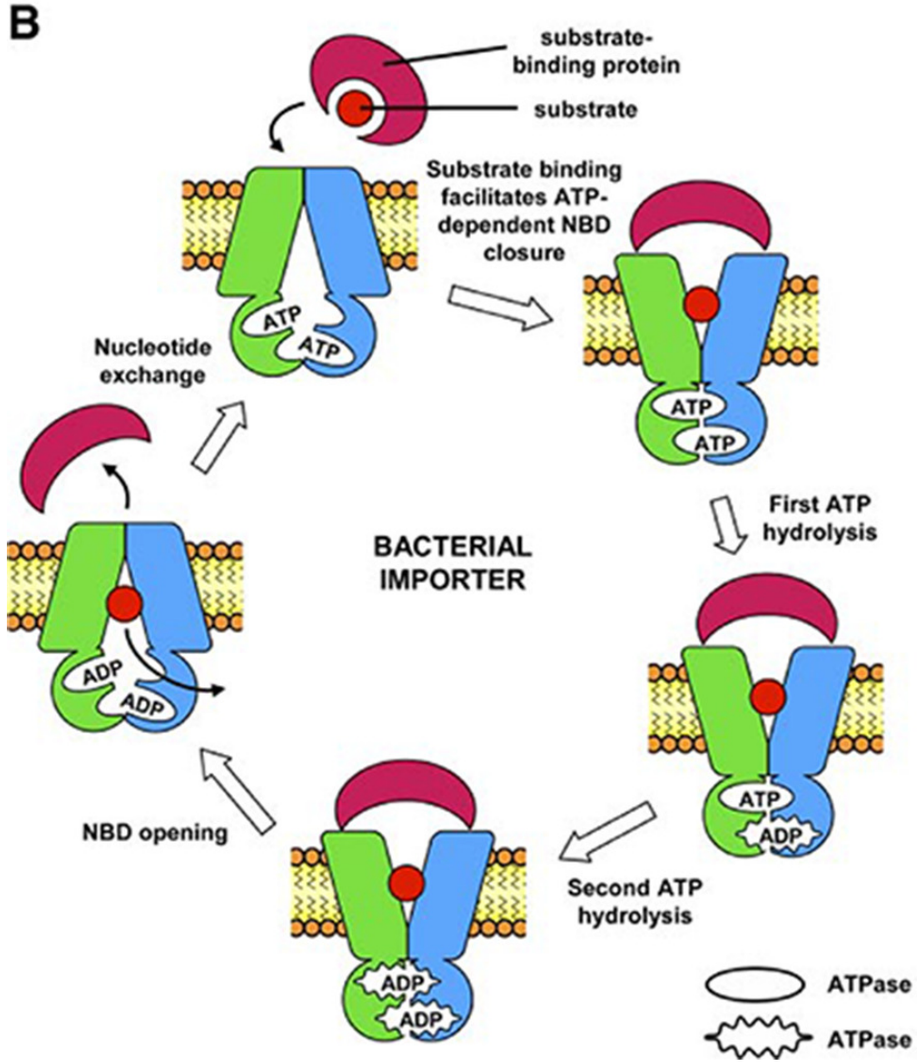


Procko et al., 2009

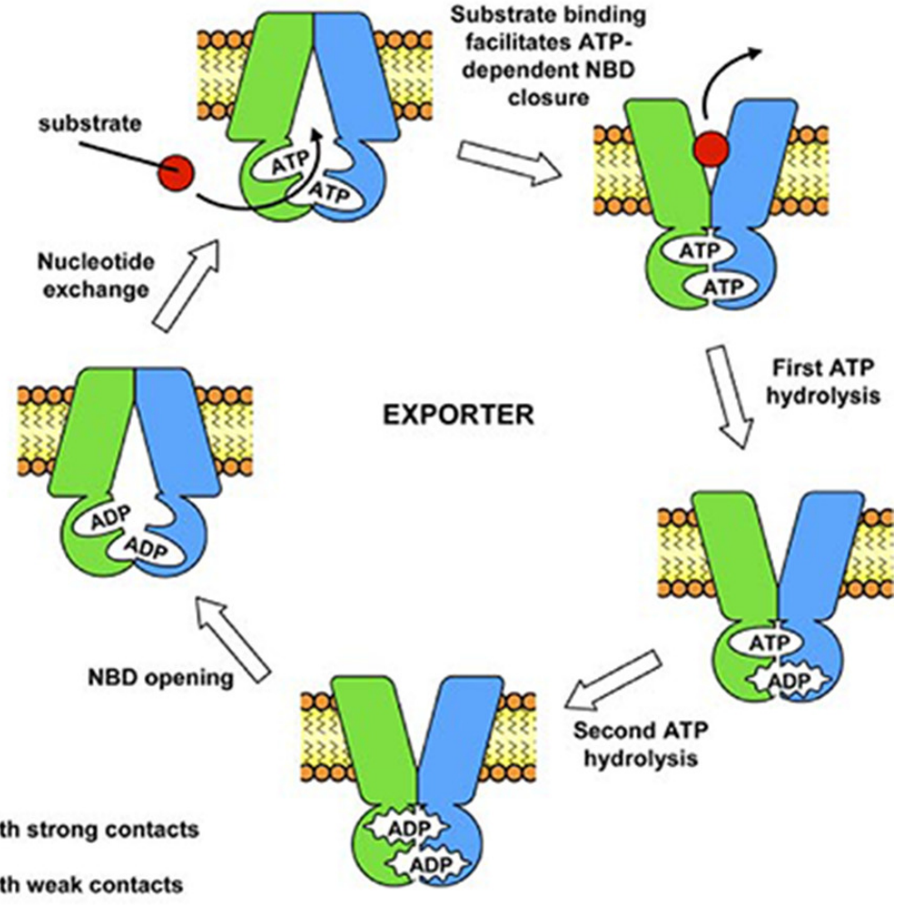
A

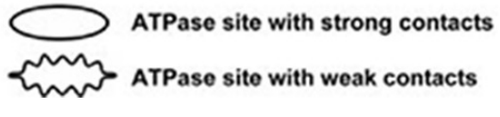

TAP1
TAP2
peptide
peptide release
ER LUMEN
CYTOSOL
ATP
ADP
Peptide binding coupled with ADP / ATP exchange on TAP2
ATP-dependent NBD closure
Hydrolysis at the consensus ATPase site
Hydrolysis at the degenerate site (non-essential) and NBD opening

B

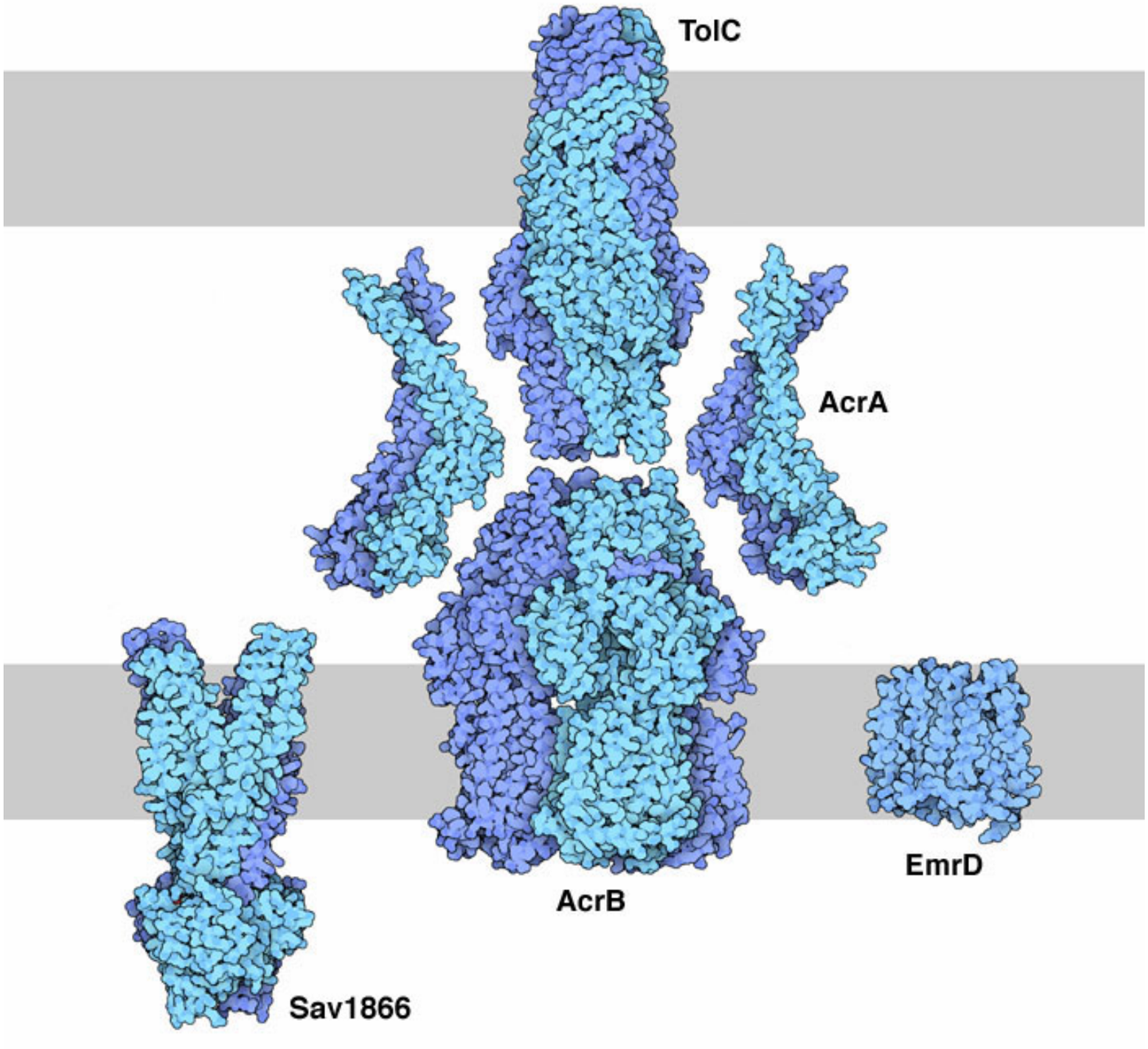

substrate-binding protein
substrate
Substrate binding facilitates ATP-dependent NBD closure
Nucleotide exchange
First ATP hydrolysis
BACTERIAL IMPORTER
NBD opening
Second ATP hydrolysis
ATP
ADP

C


substrate
Substrate binding facilitates ATP-dependent NBD closure
Nucleotide exchange
First ATP hydrolysis
EXPORTER
NBD opening
Second ATP hydrolysis
ATP
ADP

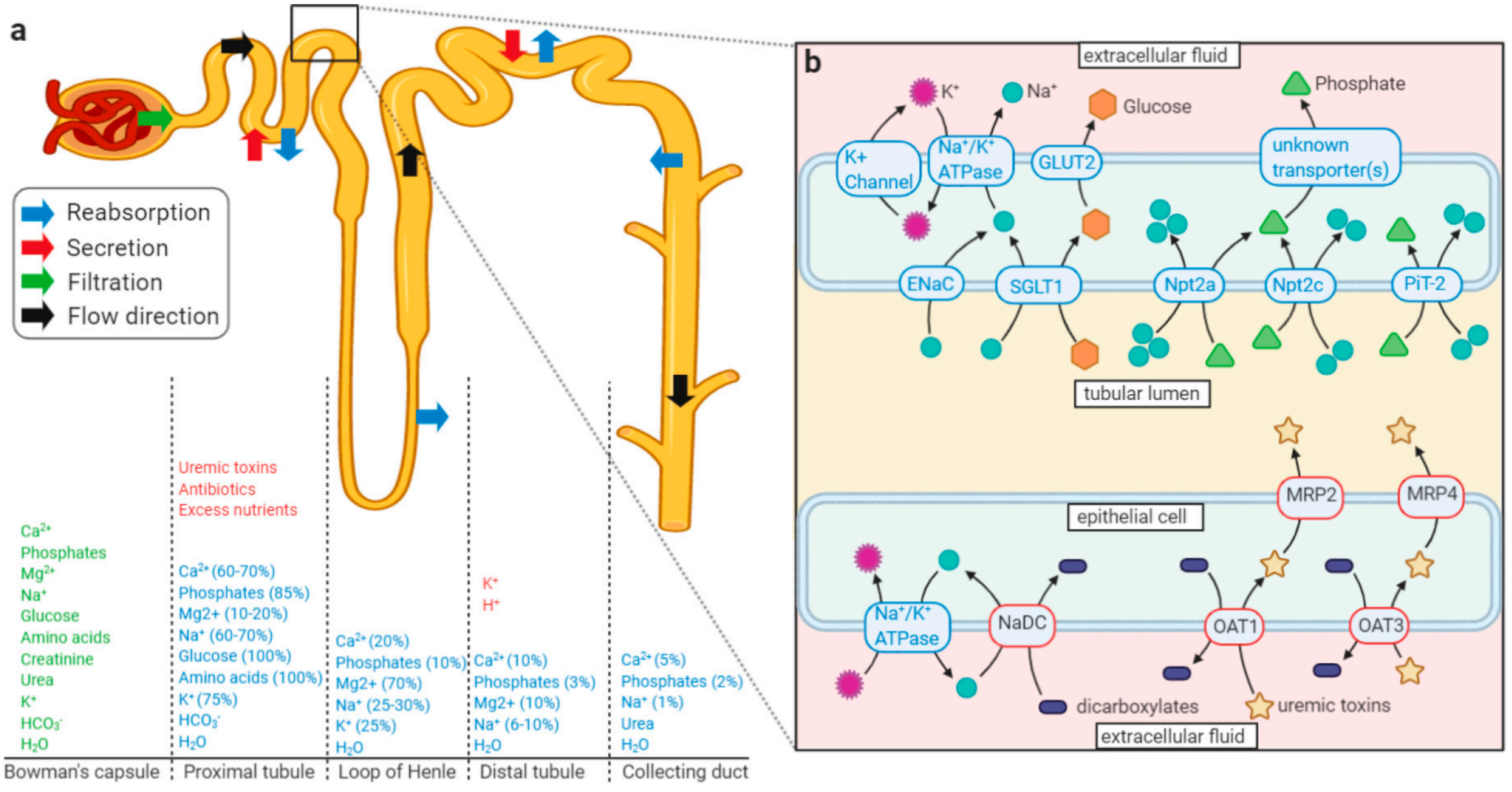

ATPase site with strong contacts
ATPase site with weak contacts

Multi-drug transporters



Bacteria have developed many different types of multidrug resistance transporters to protect themselves from natural and therapeutic antibiotics. Some use the ATP-powered scissoring motion like Sav1866, which is similar to transporters that move other molecules, such as lipids and vitamins, across cell membranes. Some are simpler, such as EmrD from *Escherichia coli* (PDB entry 2gfp) which forms a small membrane pump that is powered by the flow of hydrogen ions through the protein. Some are very much more complex. The AcrB transporter (PDB entry 1iwg) pumps drugs out of the inner membrane of *Escherichia coli* and into a tube formed by TolC (PDB entry 1ek9), which directs the drugs all the way out through the outer membrane of the cell. The protein AcrA (PDB entry 2f1m) is thought to form a ring that connects AcrB and TolC, linking the entire complex into a closed tube.

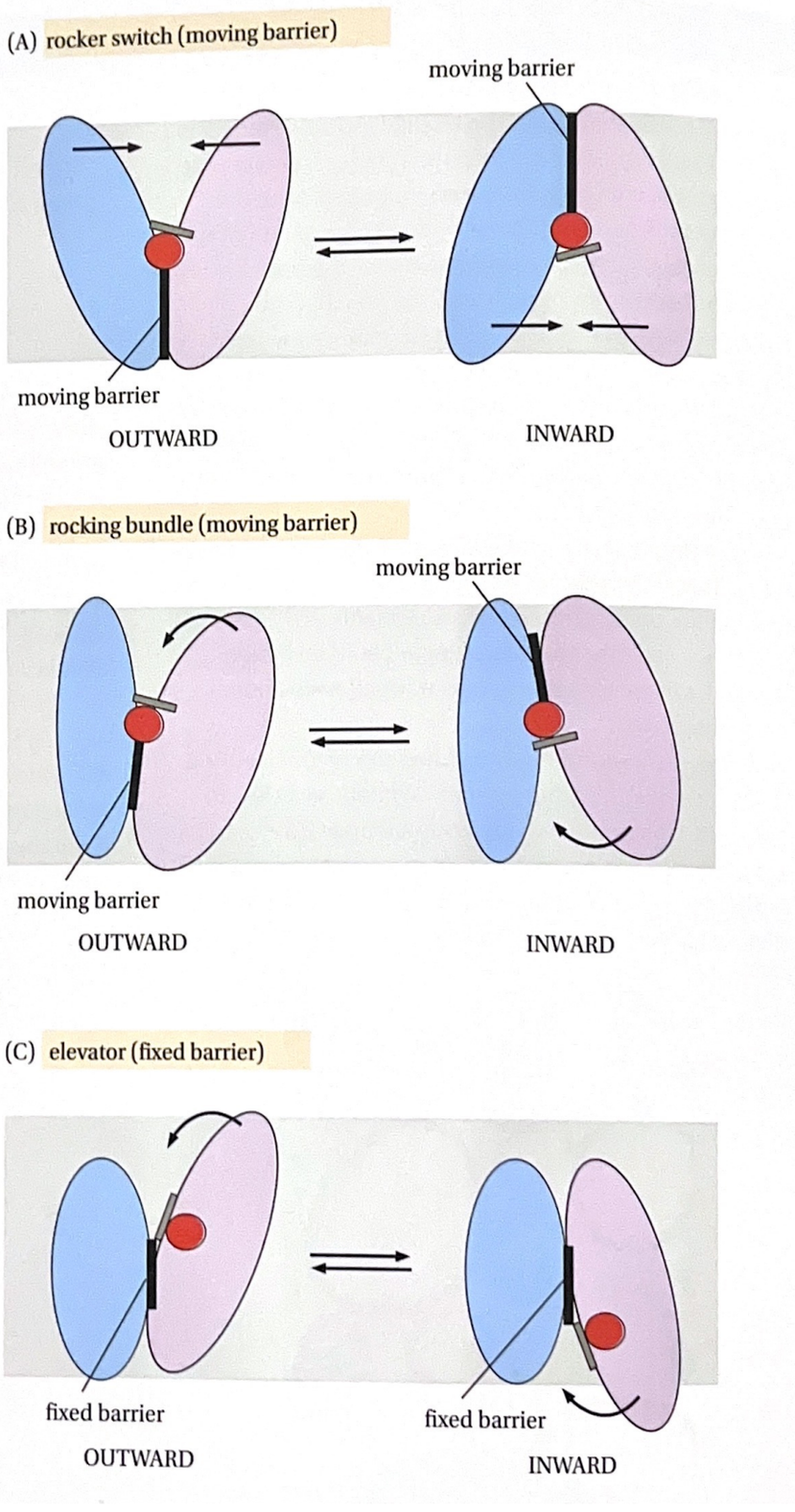
Transport is required to select rarer, biologically important molecules



10.1021/acsbiomaterials.1c00408

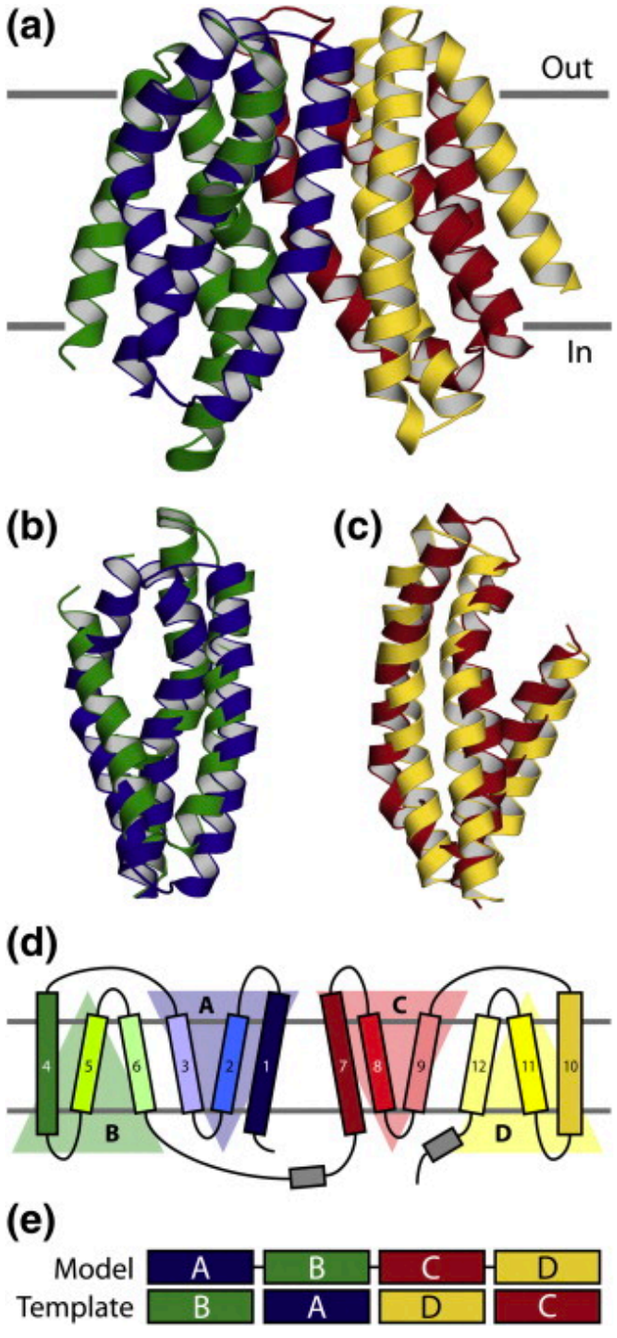
Mechanisms for alternating access across membranes

Figure 13.2 Cartoon schemes for alternating-access transport. The schemes recognize that the pumps have at least two domains that move with respect to one another to enable alternating access. The bold black lines indicate contacts between domains. The bold grey lines represent barriers to substrate movement. The transported substrate is represented by red spheres. The simplest pumps have two structurally distinct domains within the structure (e.g., sugar transporters such as lactose permease). The more complex ones are multi-subunit proteins in which the subunits move relative to one another (e.g., amino acid transporters). In some cases, there may be occluded states along the pathway, not open to either side (see Figure 13.3 and Box 13.1). (Figure reproduced with permission from Drew D, Boudker O [2016] *Annu Rev Biochem* 85: 543–572. Copyright 2016 Annual Reviews.)



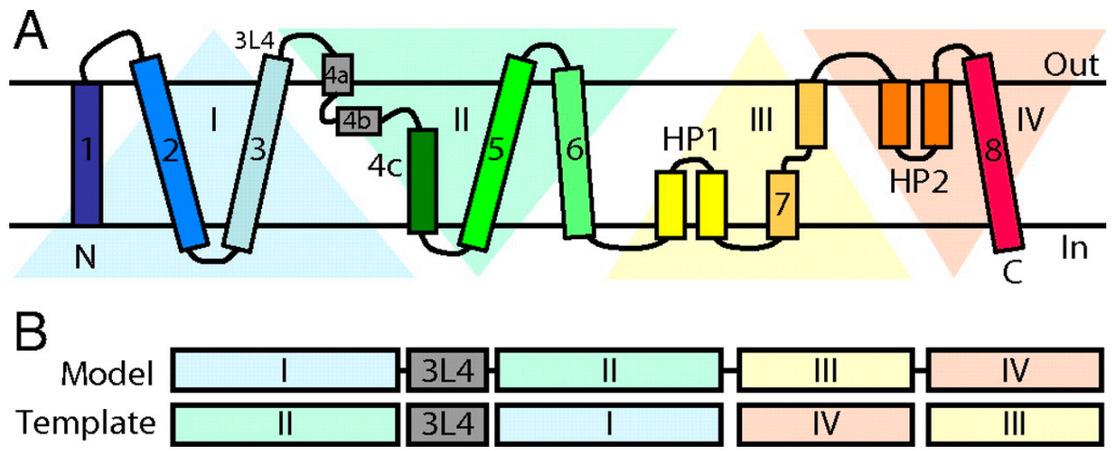
Inverted topology repeats in transporters encode for alternate access

MFS transporters

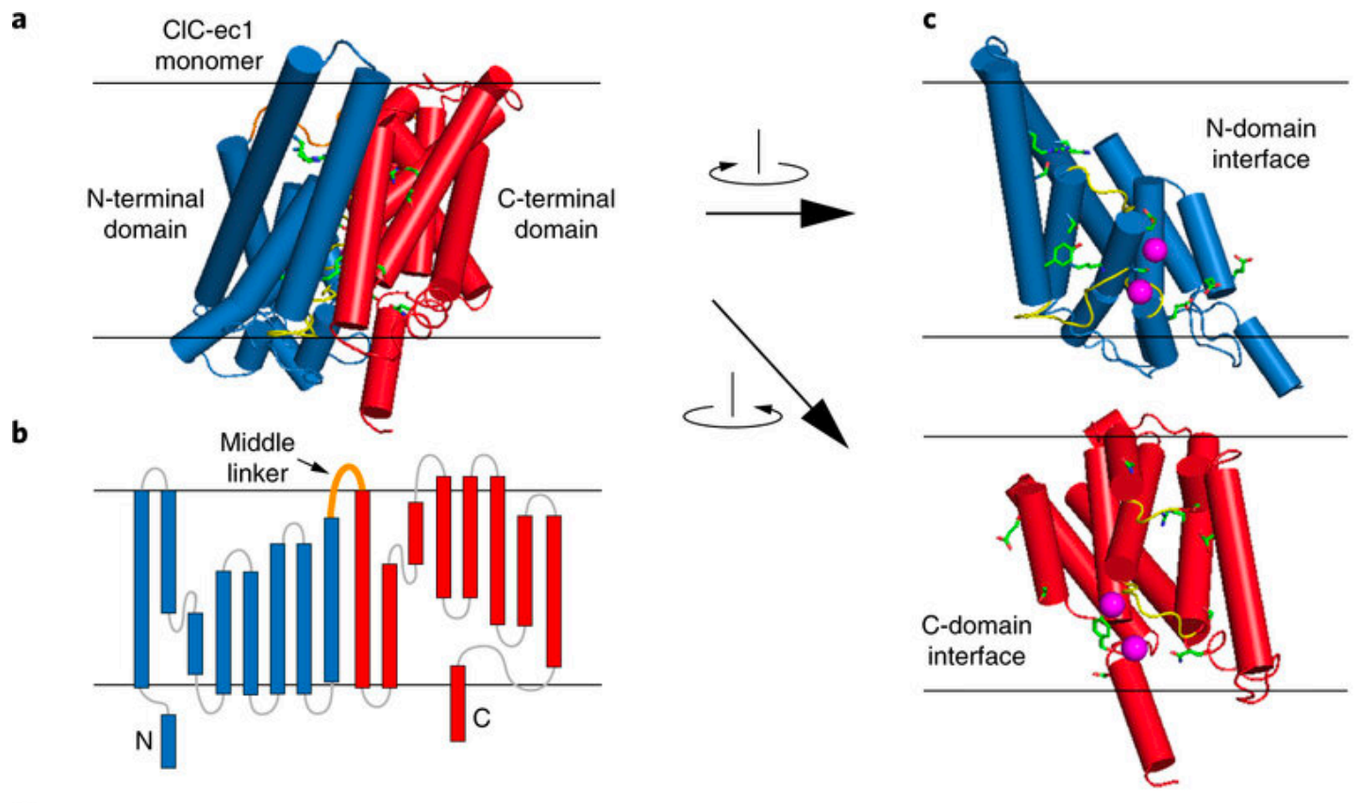


https://doi.org/10.1016/j.jmb.2011.02.008

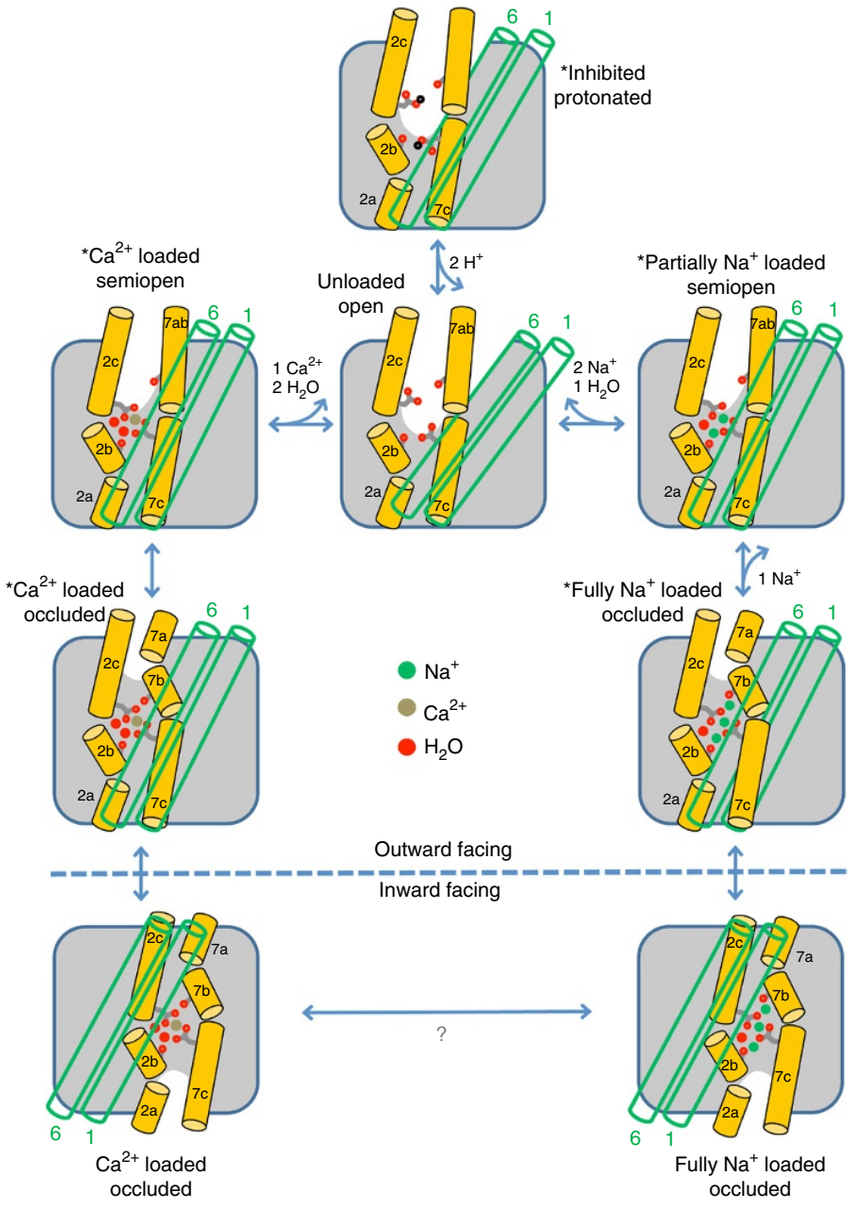
Elevator model transporters

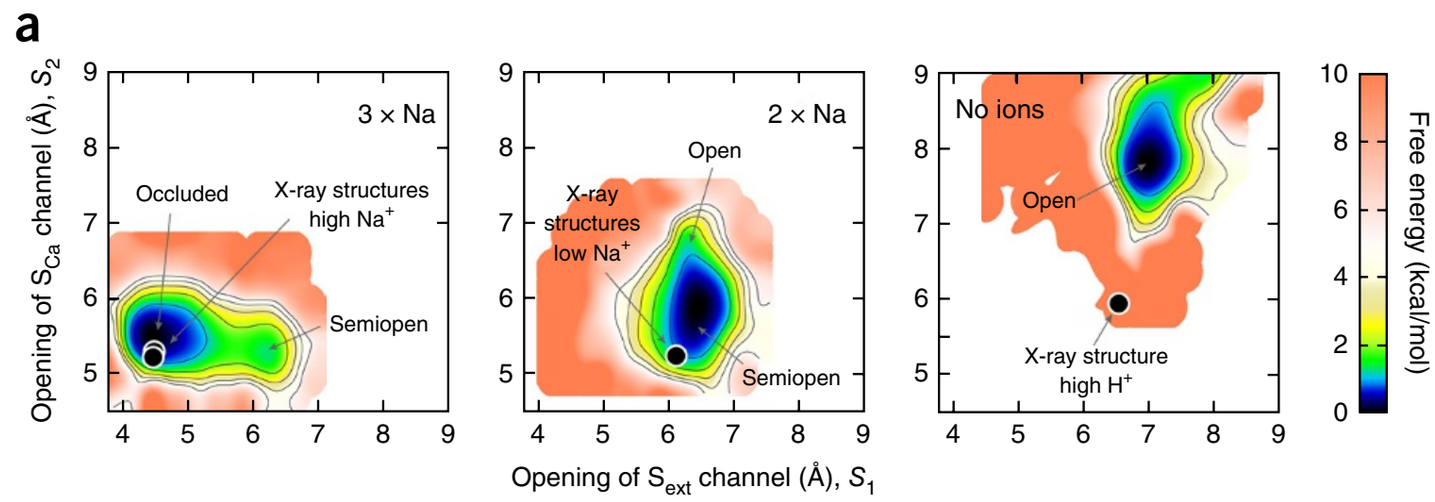


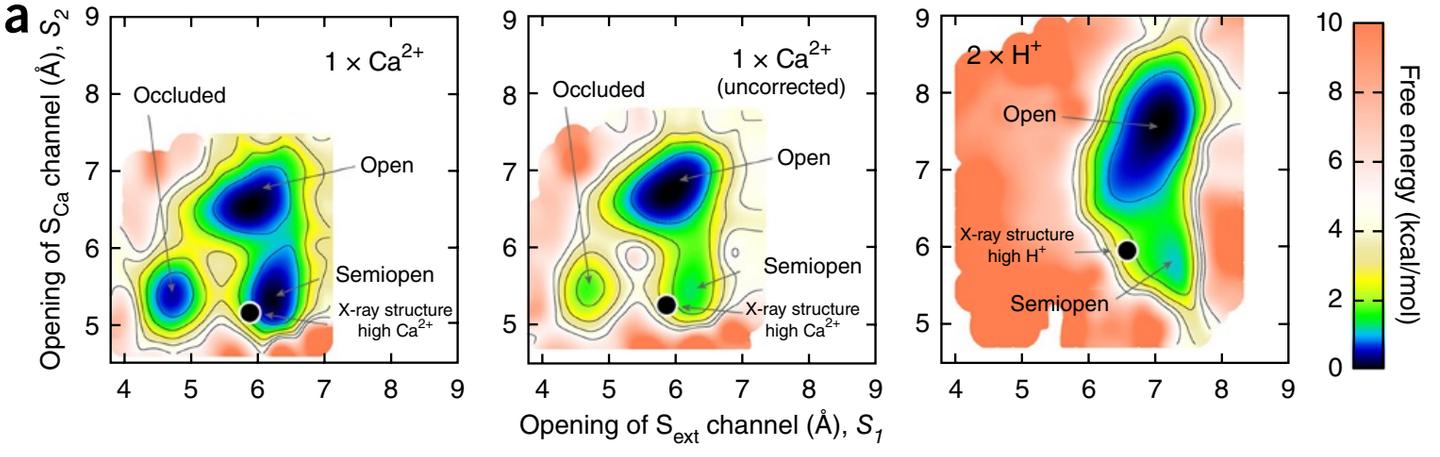
CLC transporters



Conformational changes providing alternate access tightly linked to substrate binding

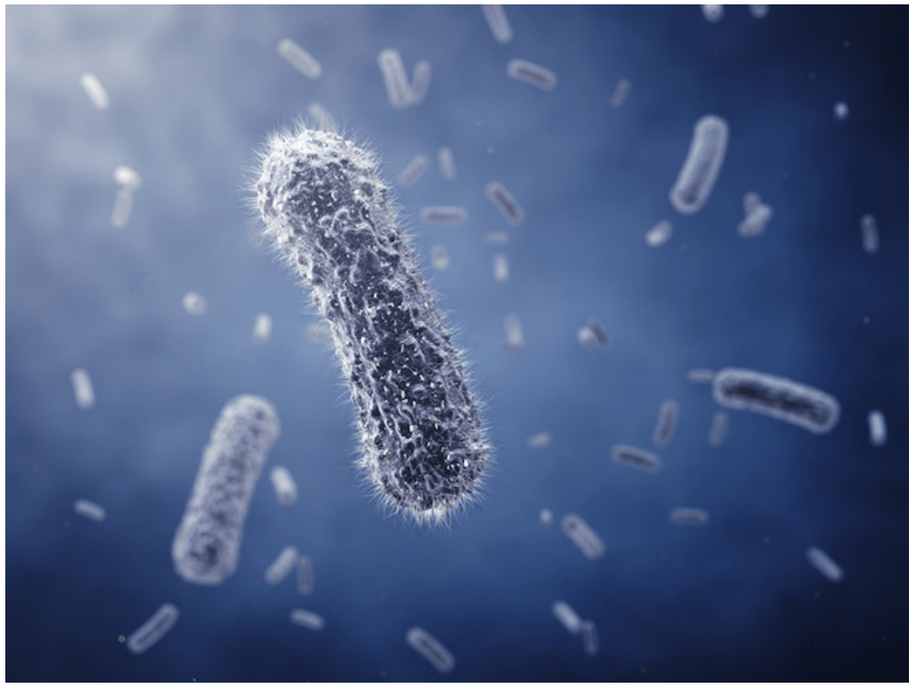


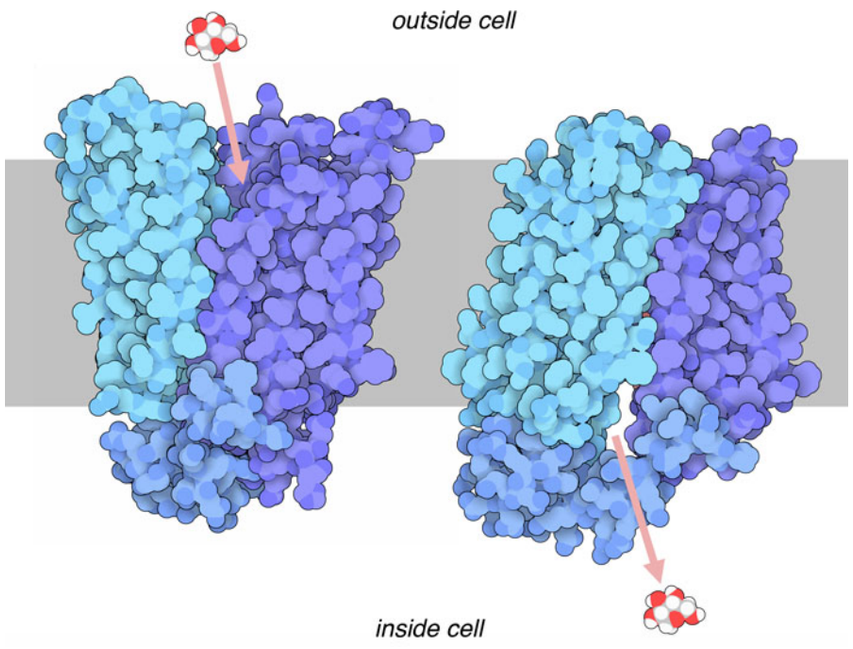




The Na+/Ca2+ exchanger

Passive transport/Facilitative diffusion

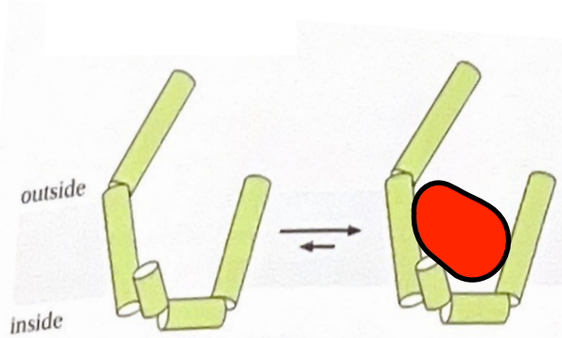




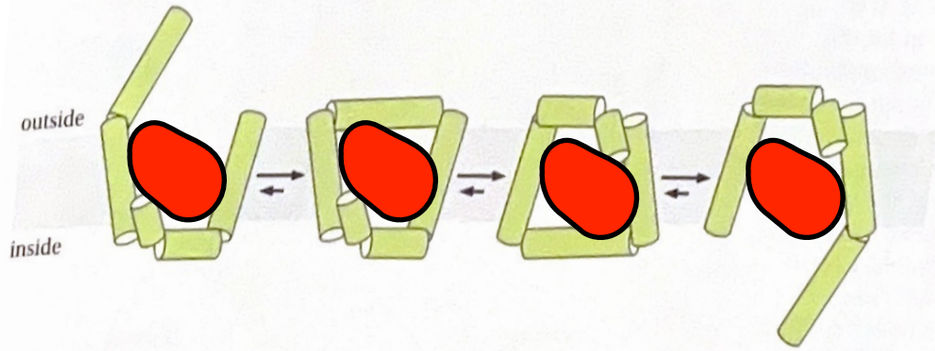
Glucose transporters manage the traffic of glucose across the cell's outer membrane. They act by alternating between two states. First, the transporter has an opening facing the outside of the cell, and it picks up a molecule of glucose. Then it shifts shape, and opens towards the inside, releasing glucose into the cell. Glucose transporters generally act passively: since glucose is rapidly phosphorylated by hexokinase, the concentration of free glucose in the cytoplasm is generally very low, and the higher concentration of glucose in the blood drives transport of glucose into the cell.

Uniporters - couple binding to conformational changes

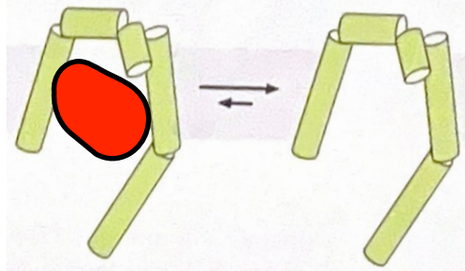
- Passive transport
- Called facilitated diffusion
- Must include an apo (empty transporter) transition to reset the cycle
- Conformations may have different binding affinities
- Each step is an equilibrium conformational change
- Driven due to the concentration gradient, can be reversed.



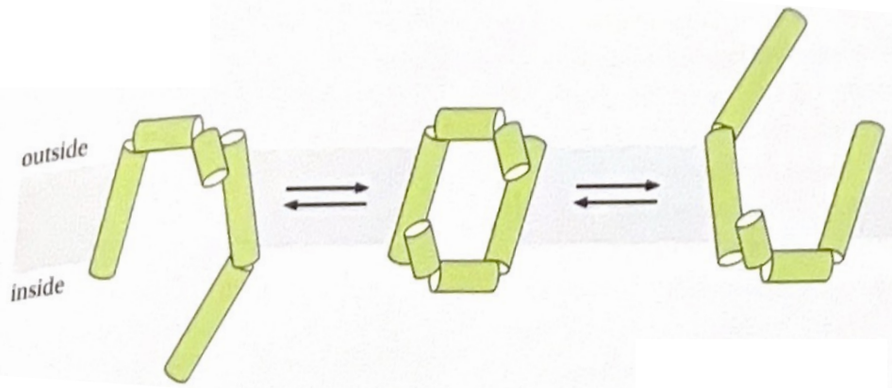
1. Substrate binding/ dissociation to the outside-open (OO) conformation



2. Outside-inside transition



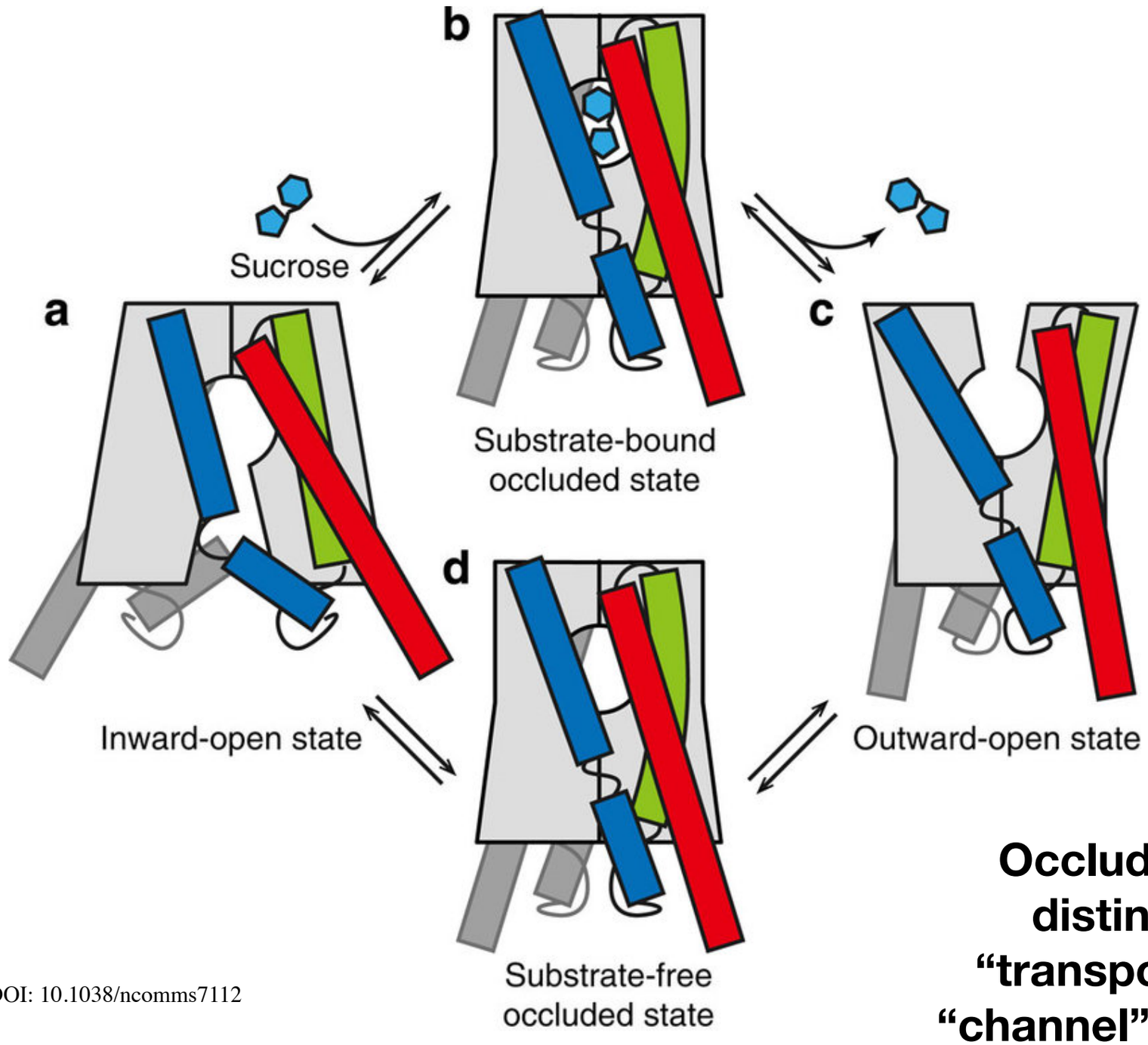
3. Substrate binding/ dissociation to the inside-open (IO) conformation



4. Resetting of the cycle (Apo)

Passive transport/Facilitative diffusion

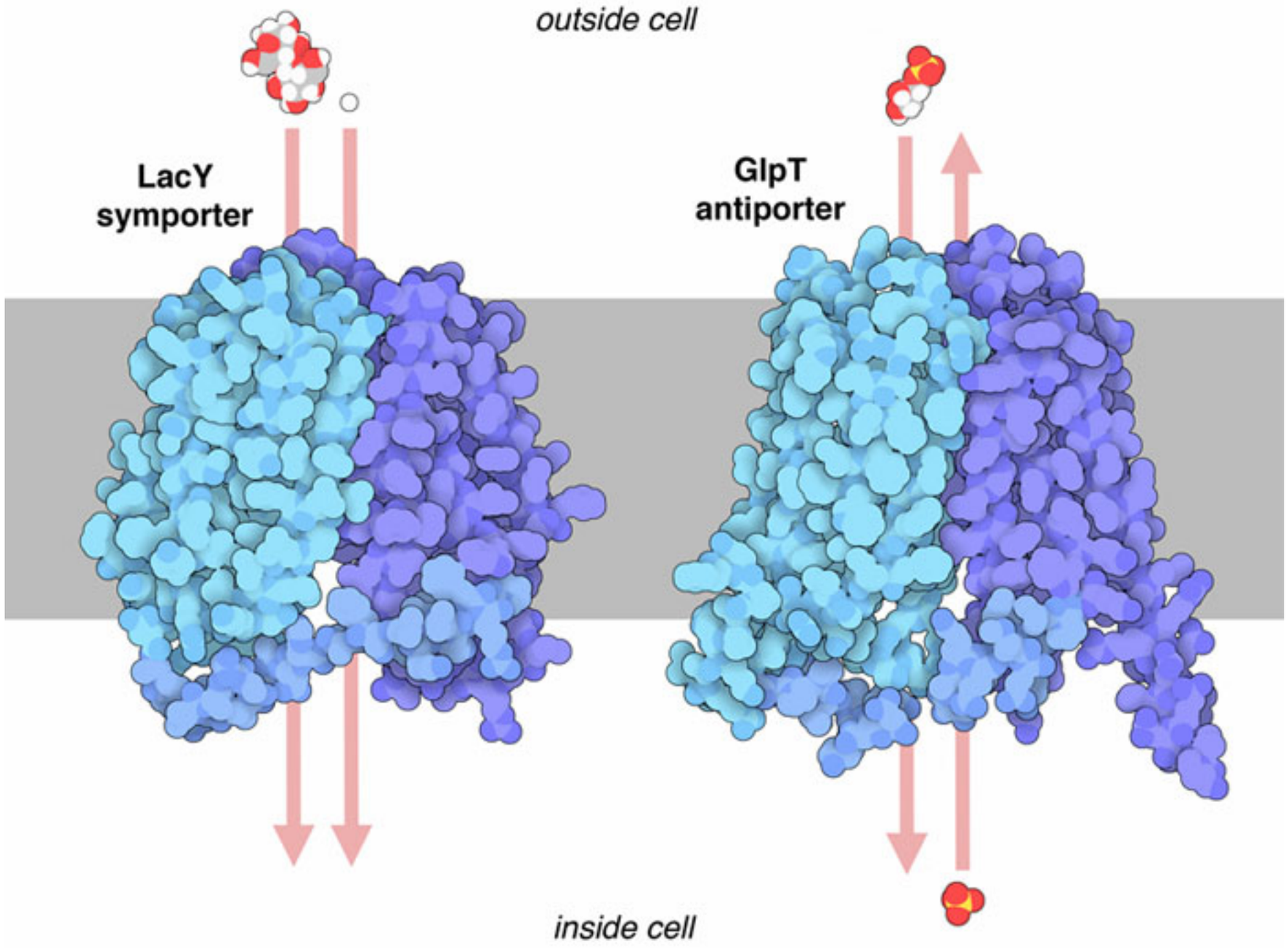
e.g. semi-sweet sugar transporter



Lee et al., 2015. DOI: 10.1038/ncomms7112

Occluded states distinguishes "transporter" from "channel" mechanism

Secondary active transport



Major Facilitators

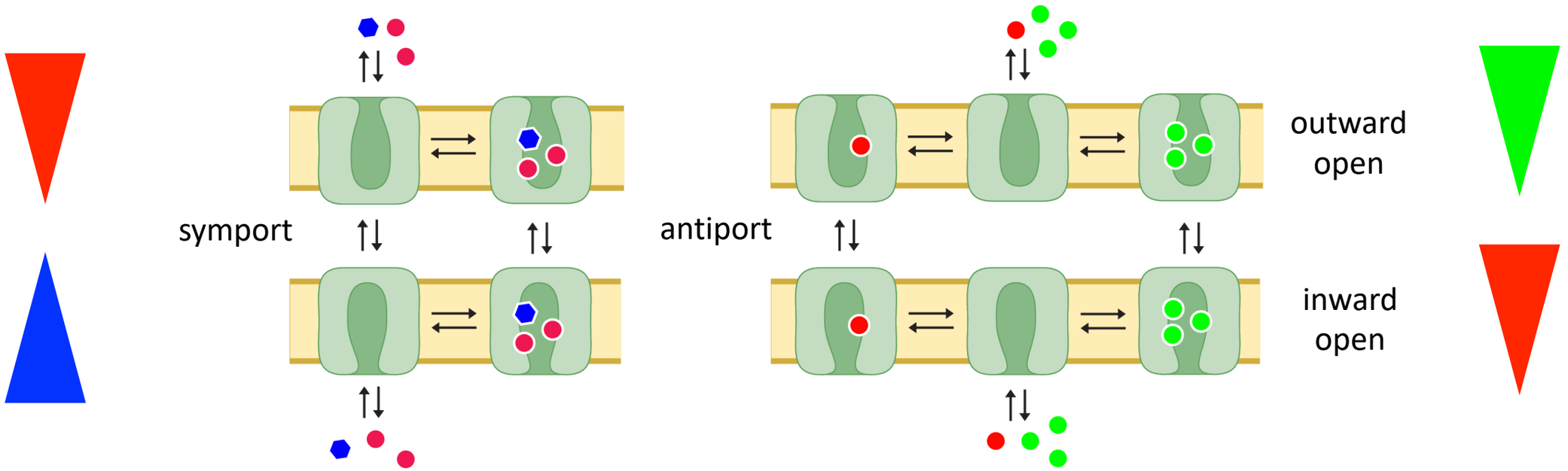
The GLUT family is part of a larger group of transporters, collectively termed the *major facilitator superfamily*. They share a similar mechanism, with two domains that rock back and forth to transport molecules across membranes. Many, however, link transport of two different molecules, harnessing a large concentration gradient of one to power transport of the other. For instance, the bacterial lactose permease LacY (PDB entry 1pv6) uses a hydrogen ion gradient to power the transport of lactose. Both molecules move in the same direction into the cell, so LacY is named a *symporter*. On the other hand, GlpT (PDB entry 1pw4) is an *antiporter* that uses a gradient of phosphate ions to pump glycerol-3-phosphate in the opposite direction.

Secondary active transport

Active membrane transport without ATP: the alternating-access hypothesis

Mitchell, Nature '57; Jardetsky, Nature '66

Explains how uphill transport of one substrate can be powered by a transmembrane gradient in the electrochemical potential of another

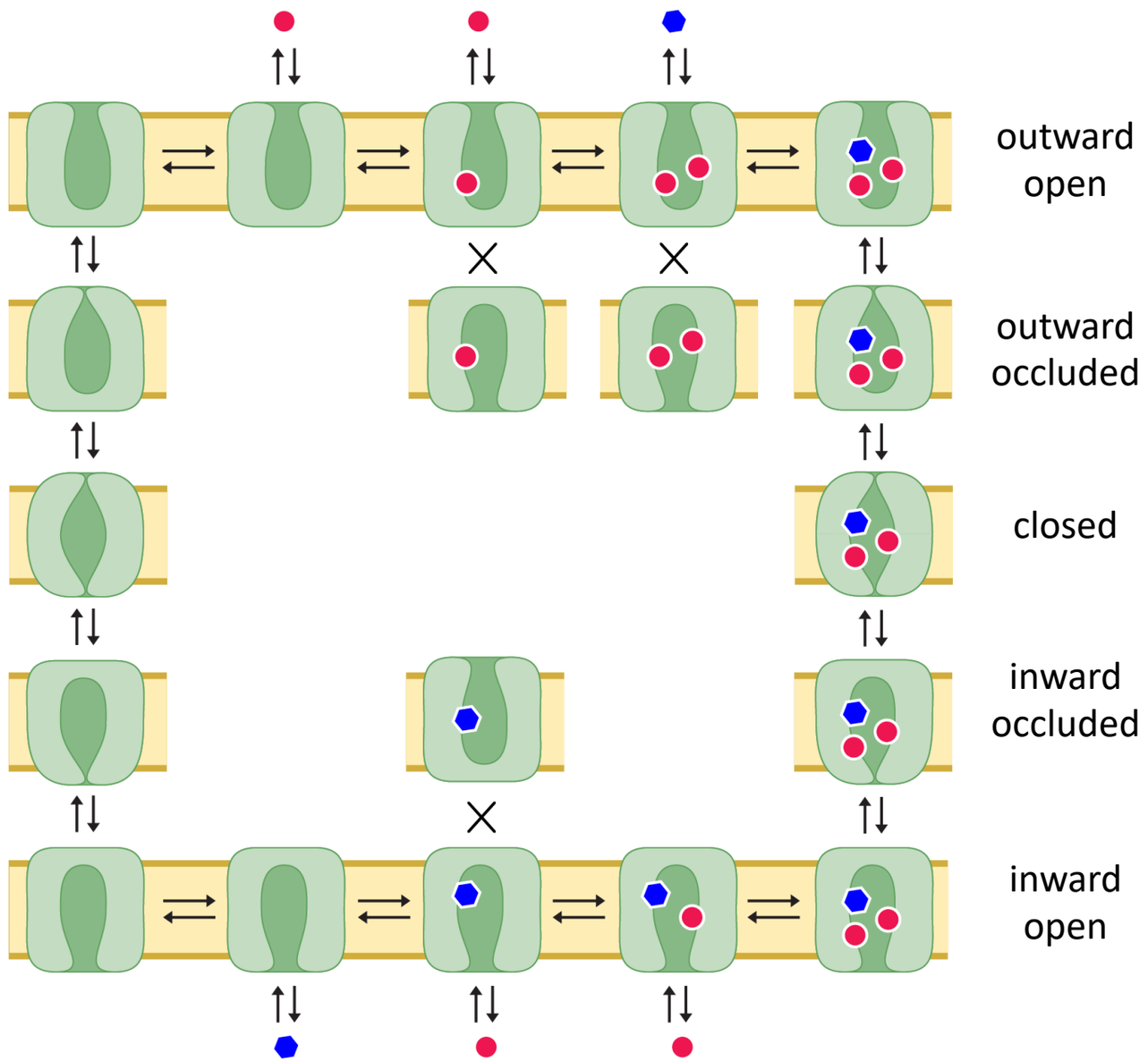


Conformational transitions are spontaneous and reversible
Preferred cycle directionality results from imbalance in electrochemical gradients

(From J. Faraldo-Gomez http://www.faraldolab.org/)

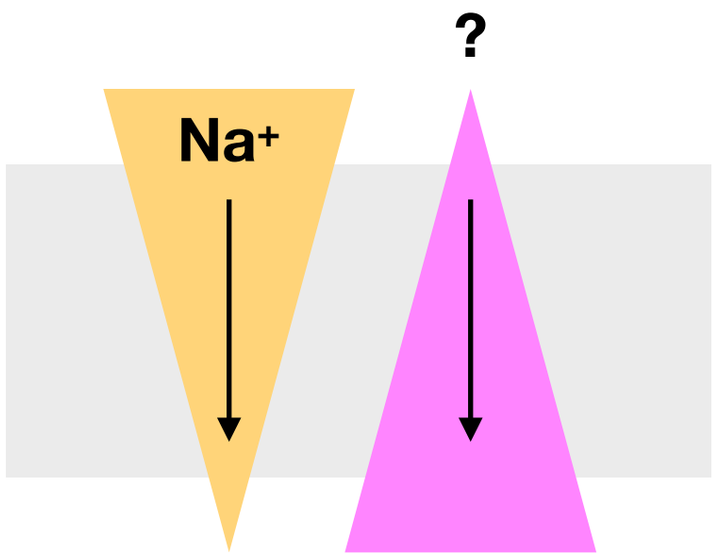
Symport mechanisms

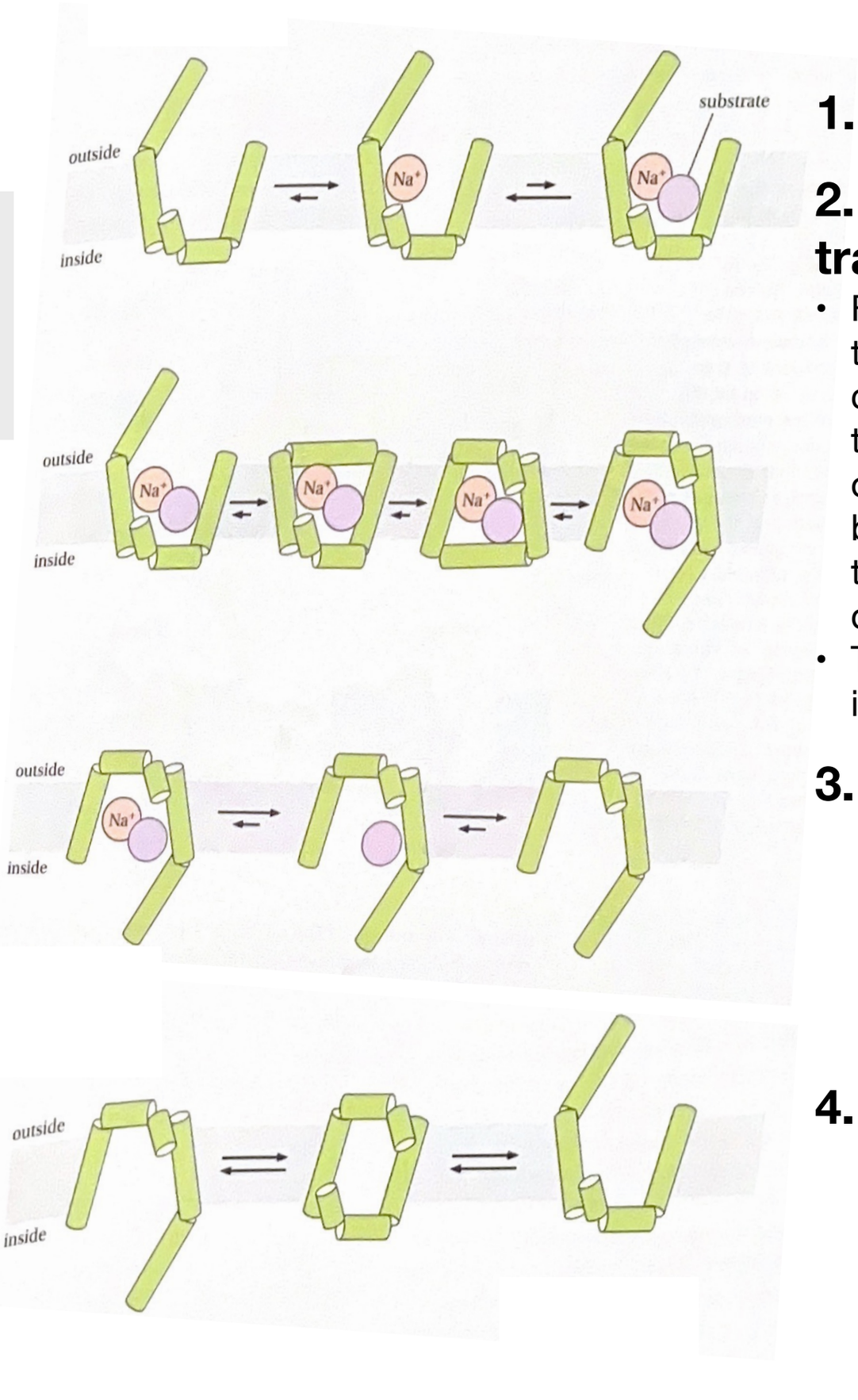
The DOs and DON'Ts of a symporter



(From J. Faraldo-Gomez http://www.faraldolab.org/)

Secondary active transport - cotransport





1. Outside-open (OO)

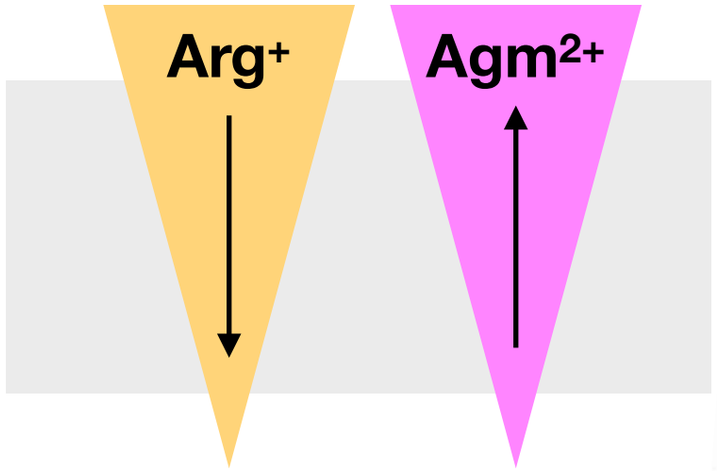
2. Outside-inside transition

- For uphill, secondary active transport to occur, this conformational change is only thermodynamically possible if and only if there are both substrates bound. Otherwise, transitions with the single substrate would dissipate the gradient.
- This is an occluded state, i.e. there is not pathway for dissipative flux

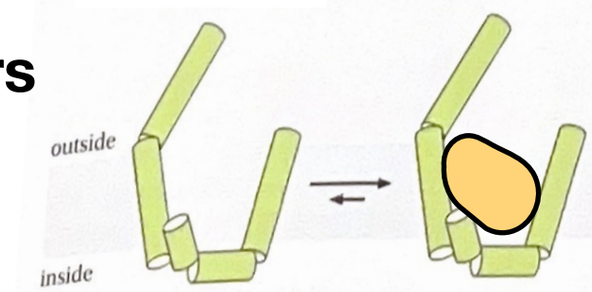
3. Inside-open (IO)

4. Resetting (Apo)

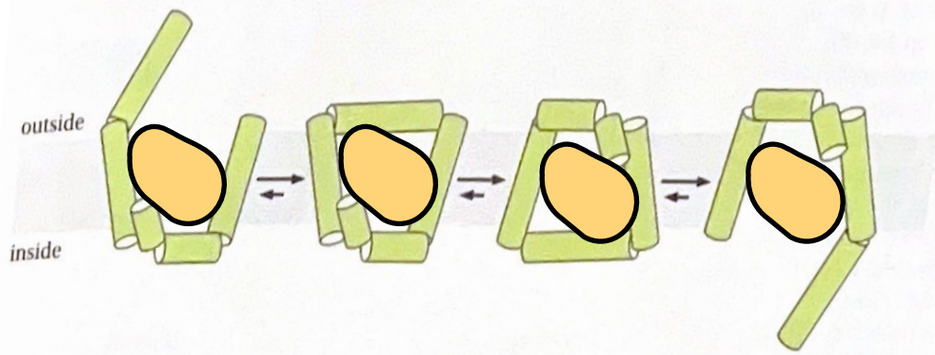
Antiporters or exchangers



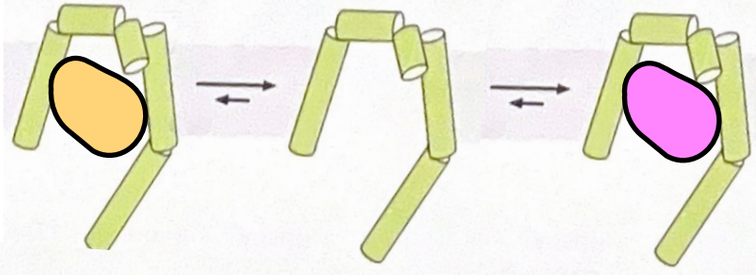
- No apo state transition



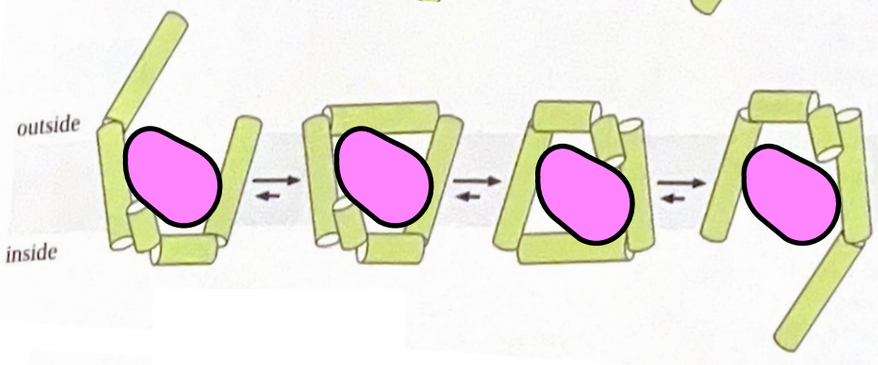
1. Outside-open (OO)



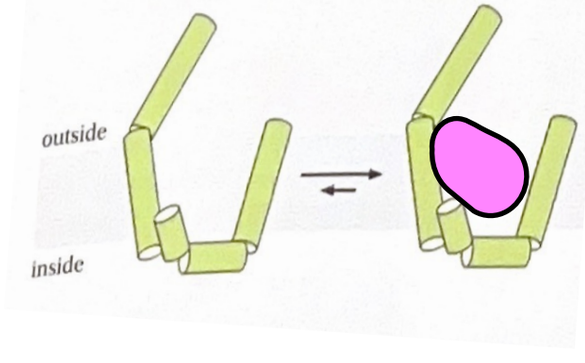
2. Outside-inside transition



3. Inside-open (IO)

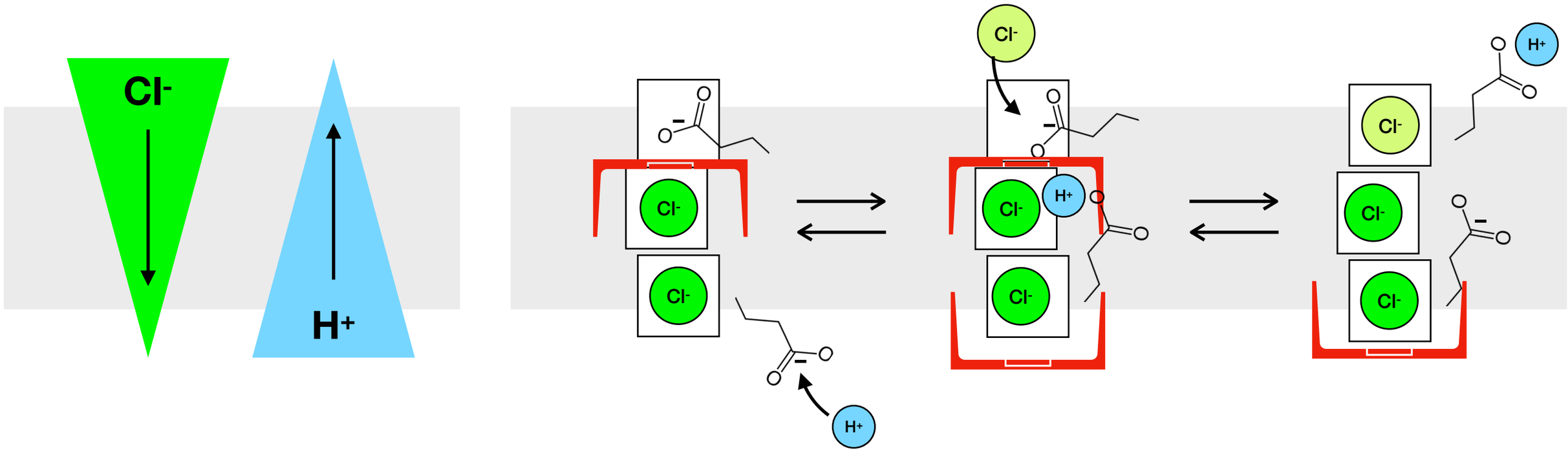


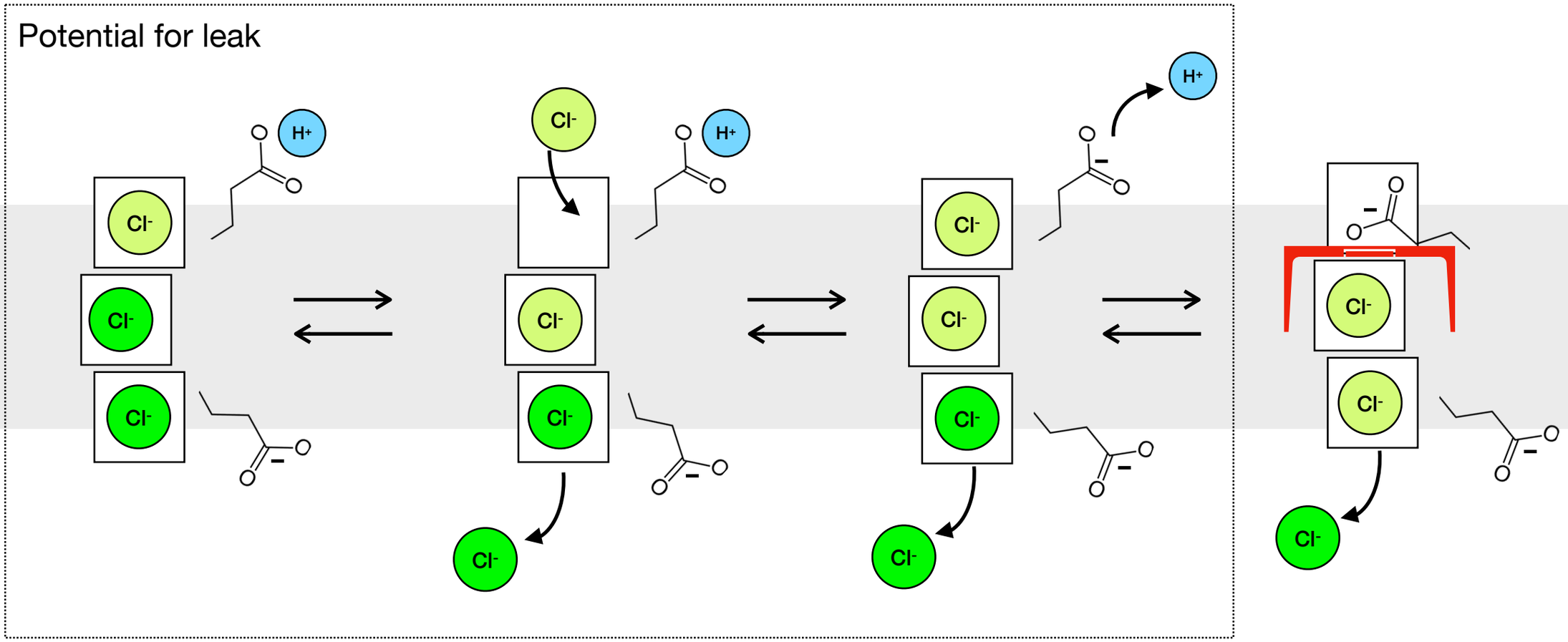
4. Inside-outside transition



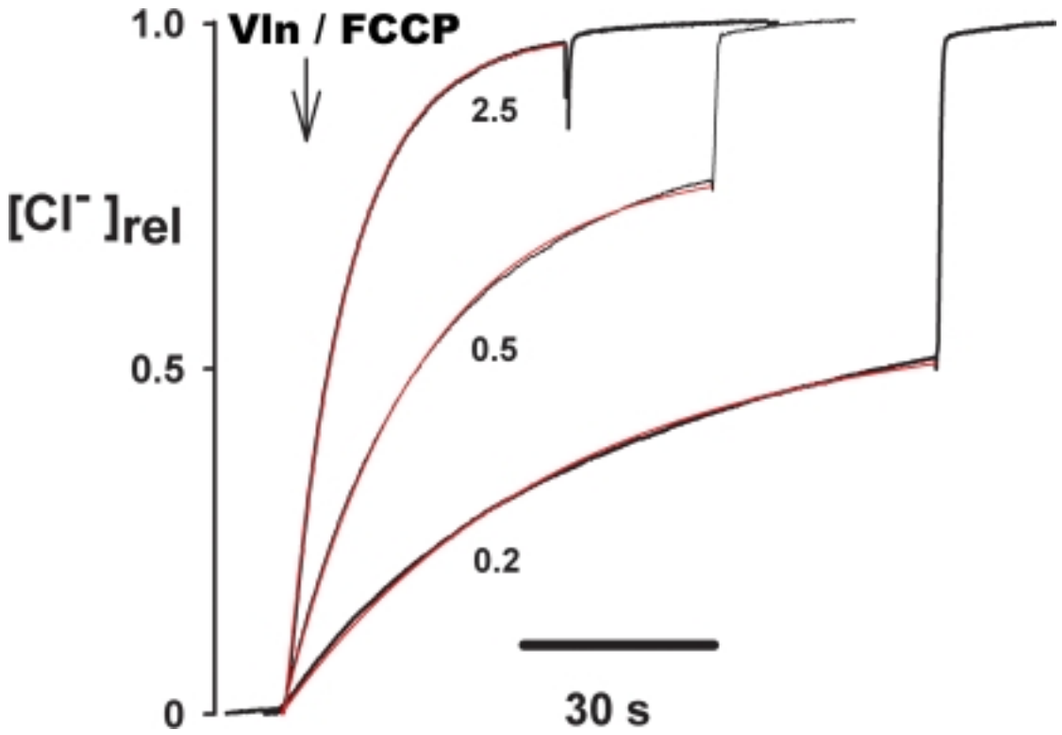
5. Outside open

Antiport - The special case of CLC antiporters vs. channels

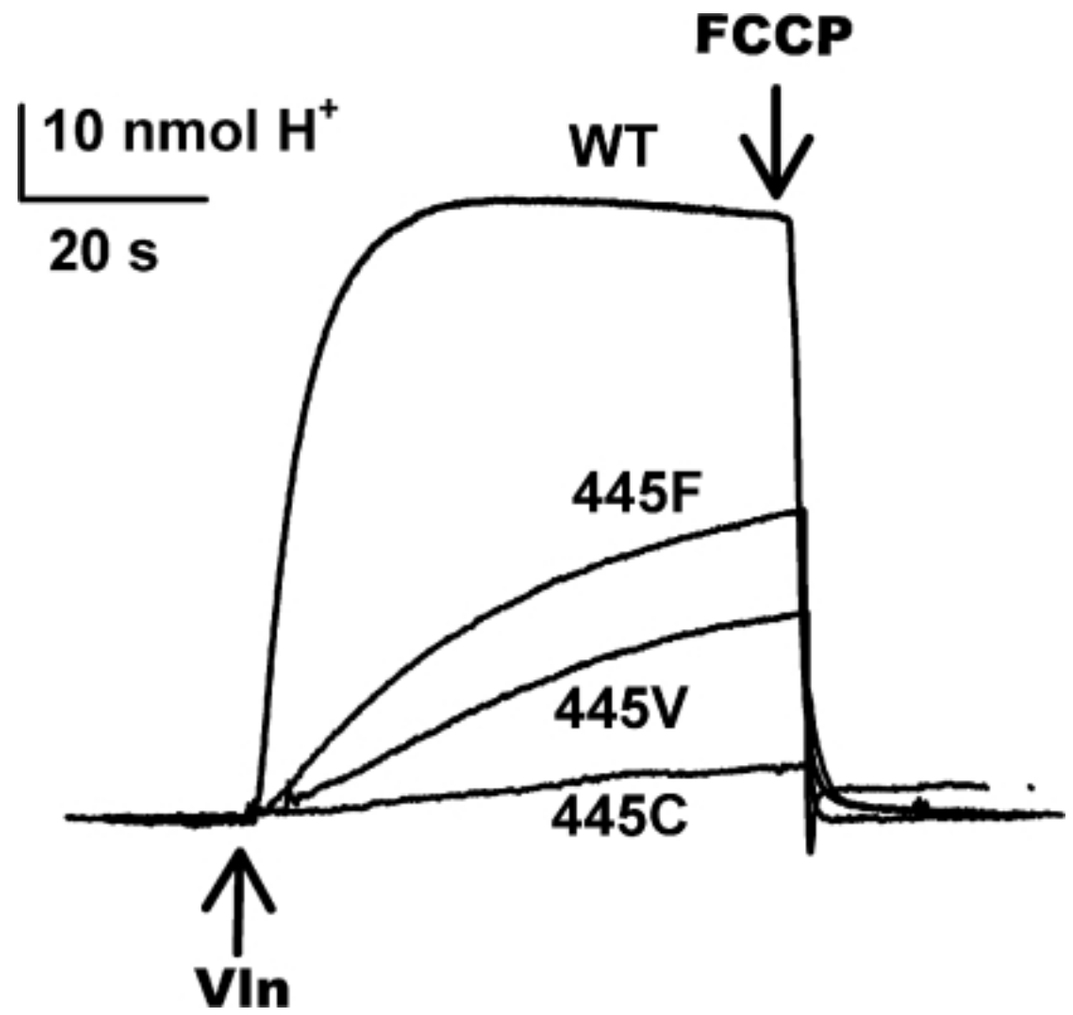




Transport assays



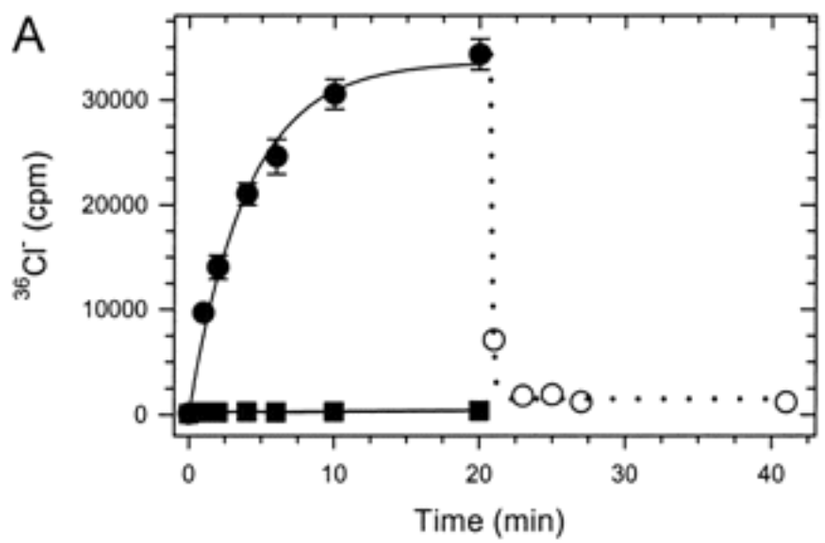
The “Cl⁻-dump” experiment: raw traces. Liposomes reconstituted with CLC-ec1 at the indicated protein density (μg/mg) and loaded with 300 mM Cl⁻ were suspended in 1 mM Cl⁻ medium, and external Cl⁻ concentration was monitored. Efflux was initiated by addition of Vln + FCCP . After most of the transporting liposomes had dumped their Cl⁻, detergent was added to release Cl⁻ from the entire population of liposomes. Released Cl⁻ is shown normalized to the fully dumped value. Experimental time courses (black traces) are fit with exponentials (red) as described in Materials and methods.

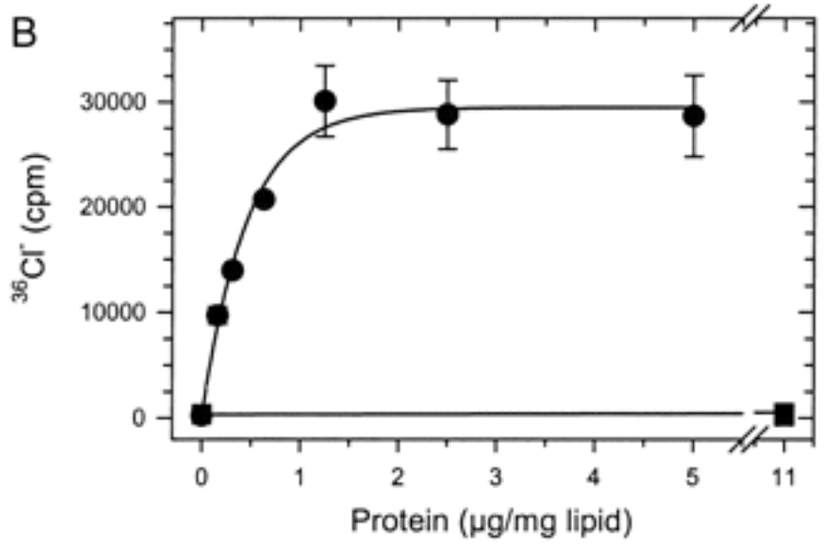


Cl⁻-driven H+ pumping by Y445 mutants. Proton uptake against a pH gradient, driven by outwardly directed Cl– gradient was assayed in liposomes reconstituted with the indicated CLC-ec1 variants. Traces of external pH are shown. Uptake was initiated by Vln addition and reversed by FCCP.

Radioactive concentrative uptake

Maduke et al., 1999. DOI: 10.1085/jgp.114.5.713



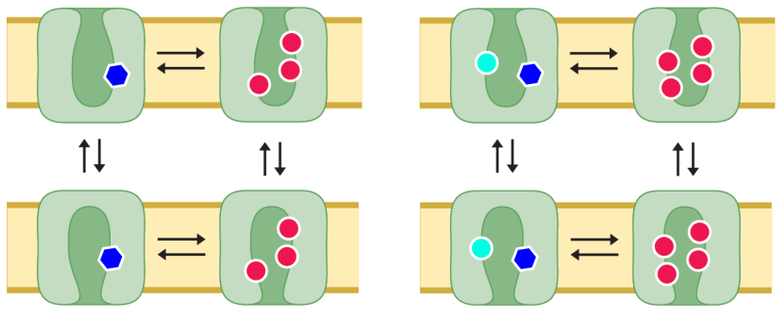


$^{36}Cl^-$ flux through reconstituted EriC. Concentrative uptake of $^{36}Cl^-$ was followed as in materials and methods. (A) Time course of accumulation of $^{36}Cl^-$ in vesicles reconstituted with 4.5 µg EriC/mg lipid (•, mean ± SEM, $n = 4$) or without protein (▪, $n = 1$). $^{36}Cl^-$ release was measured after addition of valinomycin at 21 min (○, $n = 1$). (B) Protein concentration-dependent accumulation of $^{36}Cl^-$ into vesicles reconstituted with EriC (○, •) or KcsA (▪). Uptake was measured at 20 min (mean ± SEM, $n = 3$).

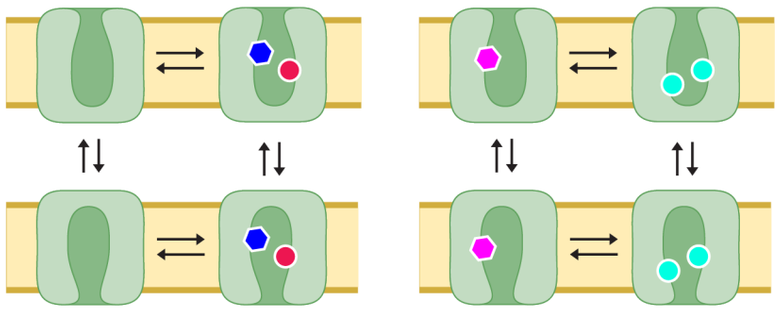
Secondary active transport

Alternating-access mechanism: what we don't know

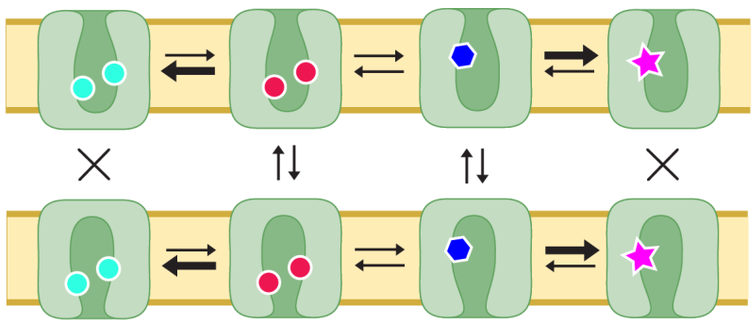
Same fold & function, different stoichiometry



Same fold, different function



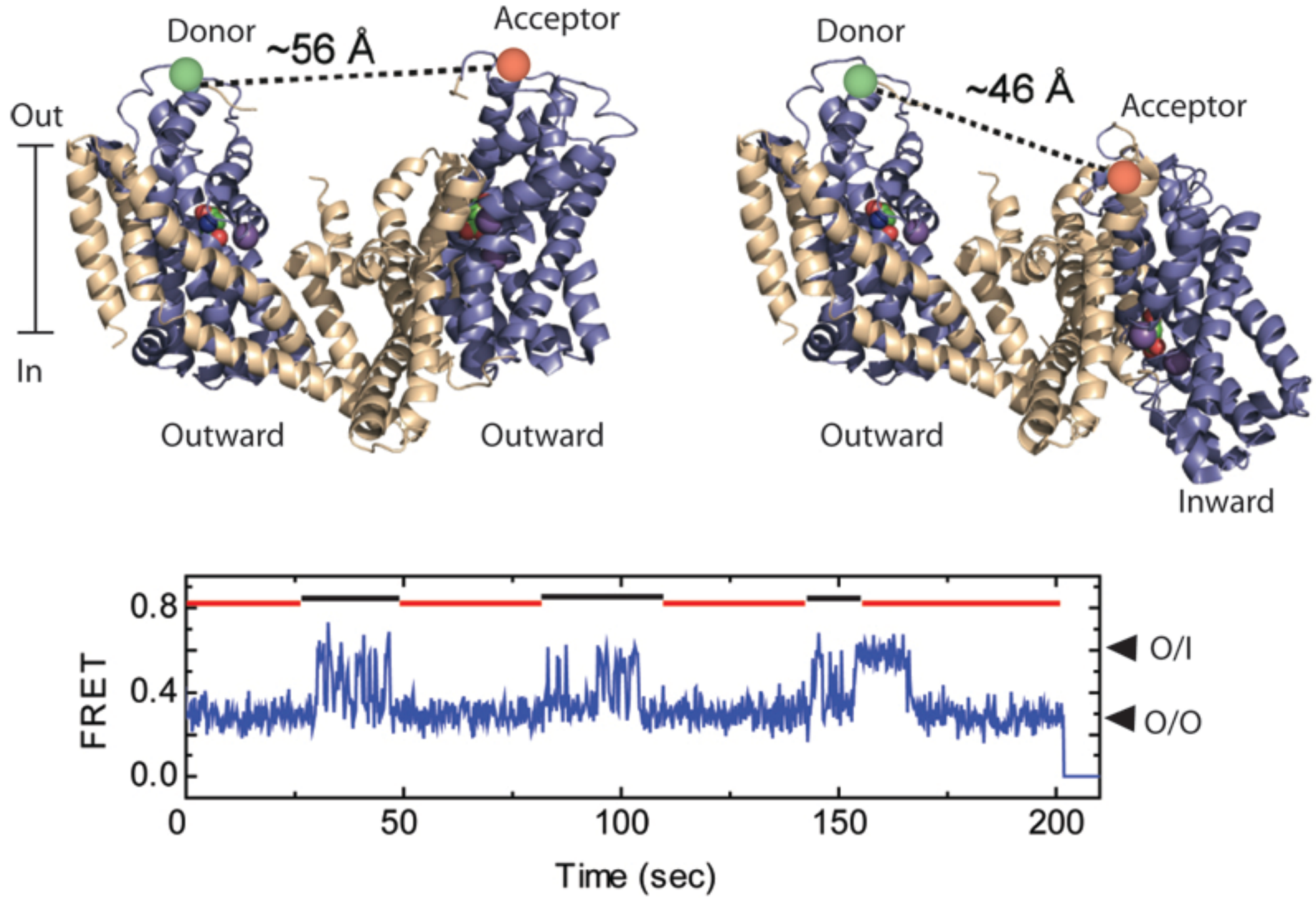
High-affinity ligands often inhibit,
are not transported



The functional specificity of a transporter,
i.e. its biological activity
cannot be trivially inferred from
static structures or binding properties

(From J. Faraldo-Gomez http://www.faraldolab.org/)

Single-molecule dynamics of transporters



Shown are two Glt_{Ph} protomers (top), labeled with the donor and acceptor fluorescent dyes, when both are in the outward-facing orientations (left), and when one of them is in an inward-facing orientation (right). The relative motions of the protomers are detected by smFRET (bottom). Such recordings reveal that protomers alternate between periods of rapid transitions and periods of quiescence, marked by black and red lines, respectively.