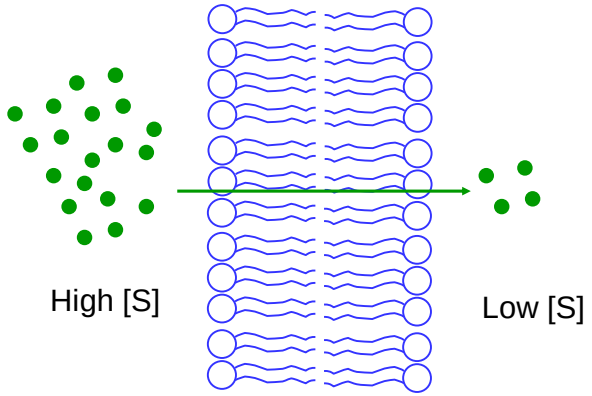
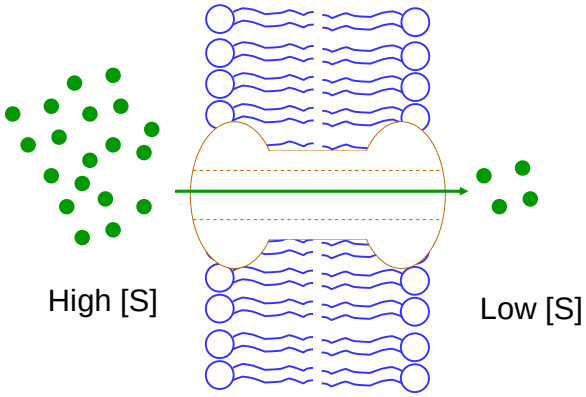
Transport of solutes across membranes

Simple diffusion: solutes move <u>down</u> a concentration gradient to reach equilibrium

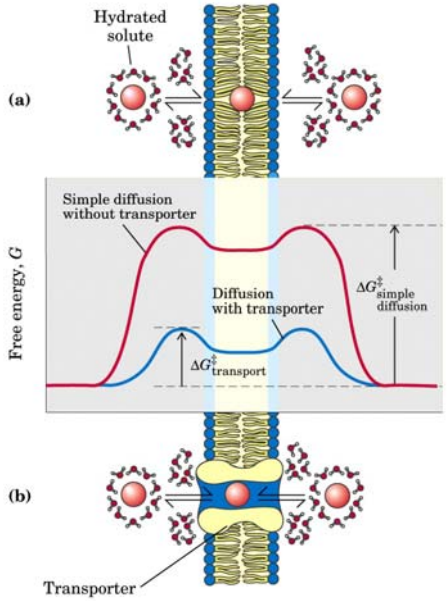


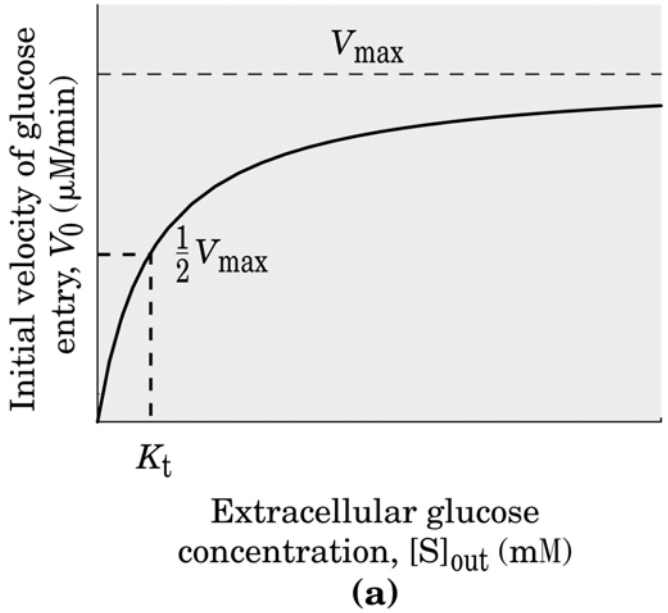
"downhill" movement

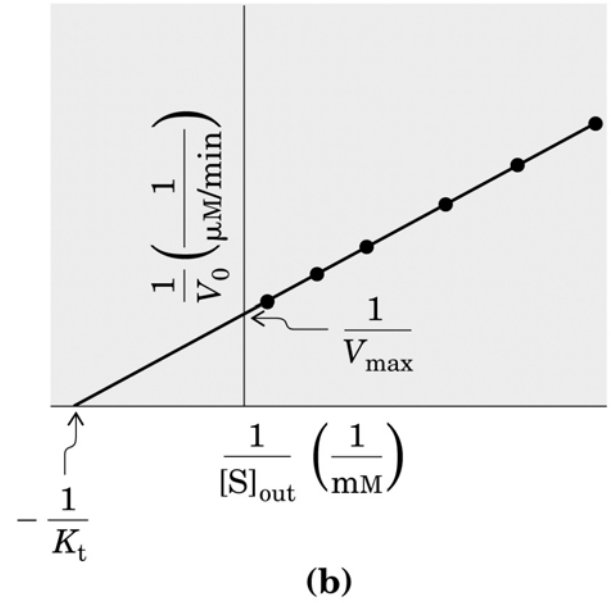
Passive transport: solutes move <u>down</u> a concentration gradient in a process facilitated by a transmembrane protein

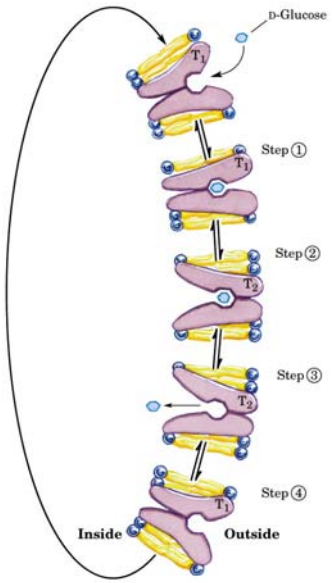


"downhill" movement

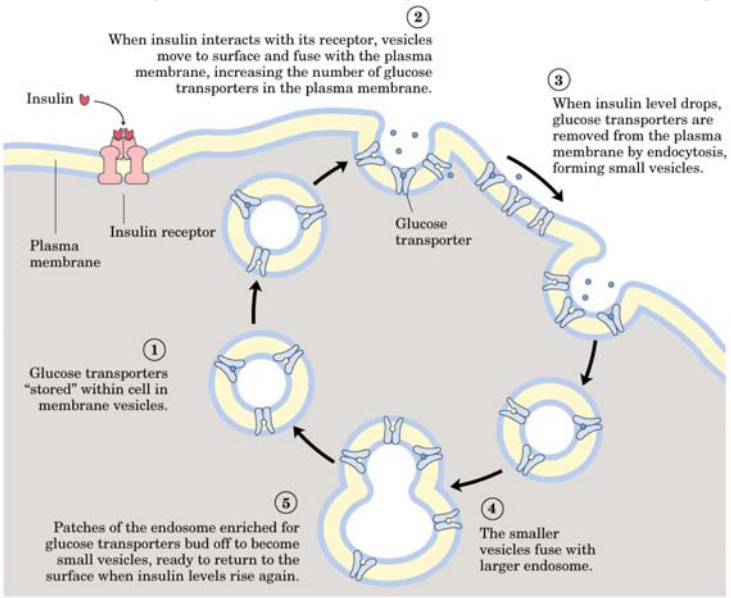


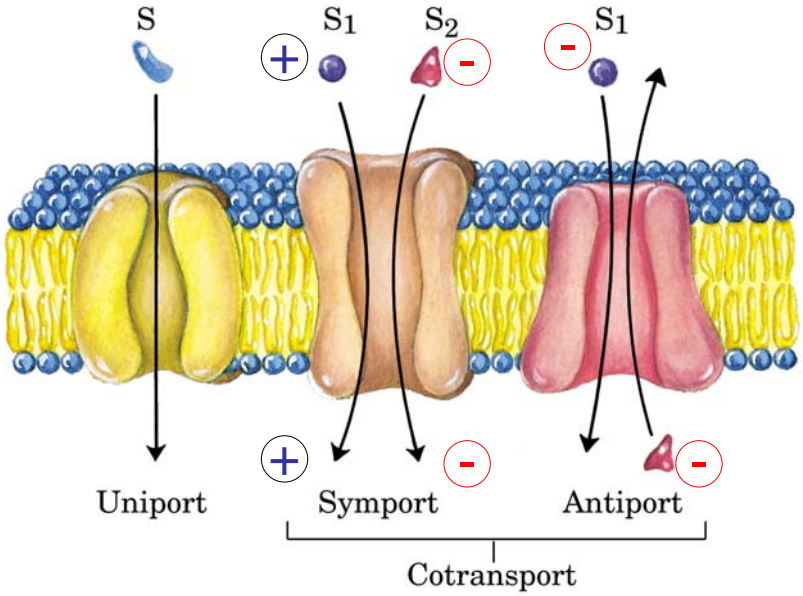




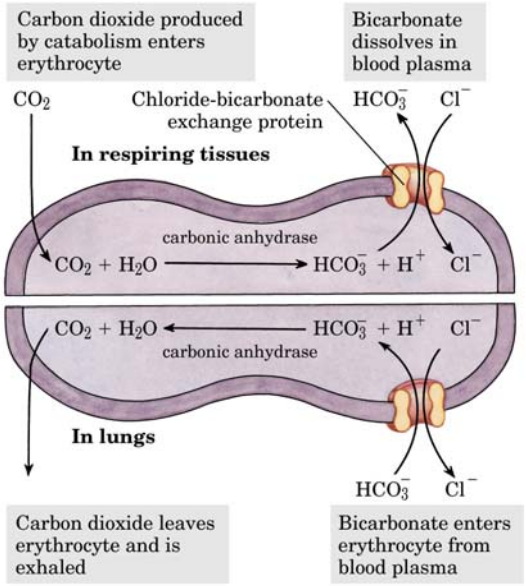


Insulin regulation of glucose transport

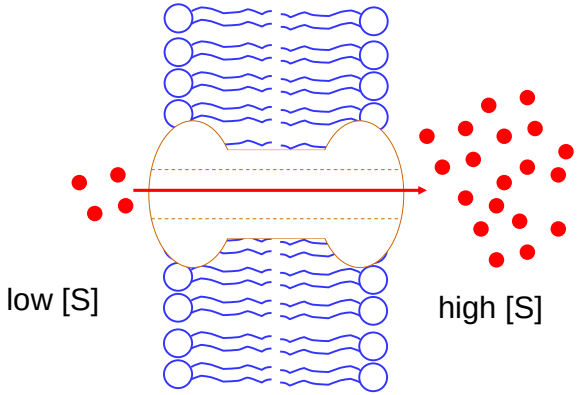




The chloride-bicarbonate exchanger



Active transport: solutes transported <u>against</u> a concentration gradient

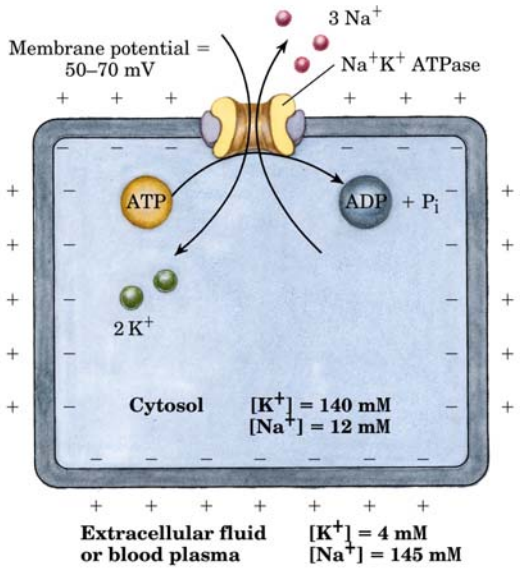


"uphill" movement

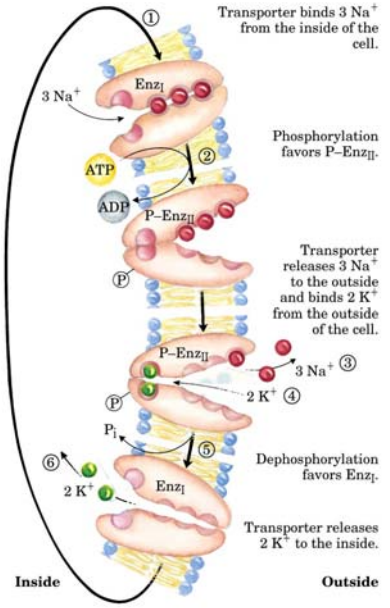
Active transport

- Primary: solute movement is coupled directly to an exergonic reaction
- Secondary: solute movement is coupled to exergonic movement of a different solute, that was earlier pumped uphill by primary active transport

Primary active transport: the Na+ K+ ATPase

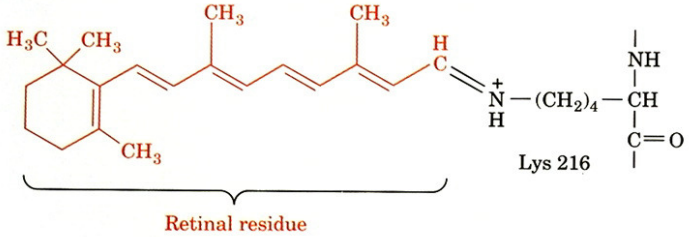


- Animal cells: higher [K^+] and lower [Na^+] than external environment
- Breakdown of ATP coupled to ion movement against concentration gradients
- $2K^+_{out} + 3Na^+_{in} + ATP \rightarrow 2K^+_{in} + 3Na^+_{out} + ADP + P_i$
- Generates a transmembrane electrical potential
- ~25% of human's energy yielding metabolism goes to support this reaction

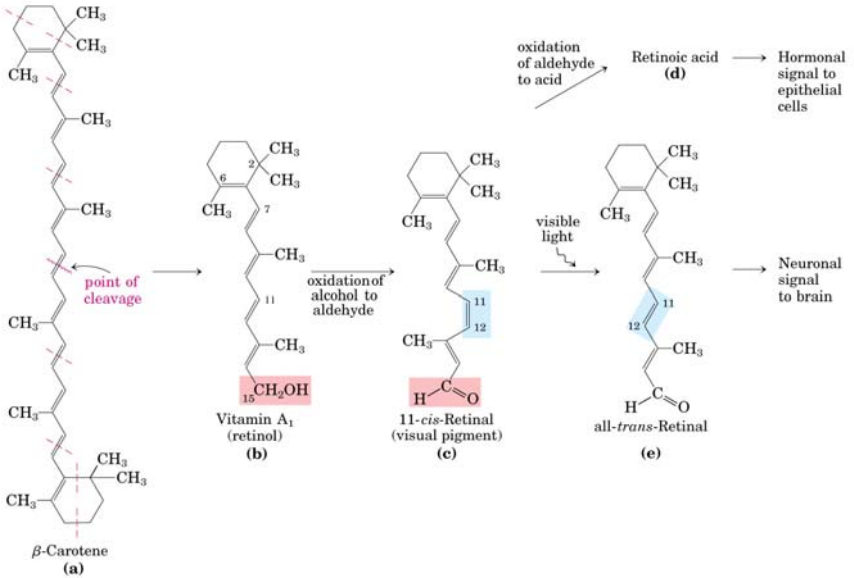


Bacteriorhodopsin: a light-driven proton pump

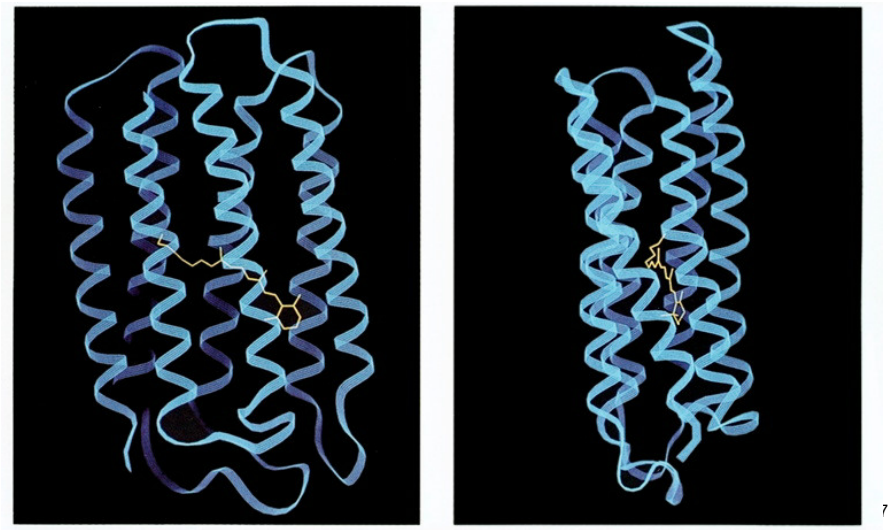
- Requires retinal, covalently linked to a lys
- Where have we seen this coenzyme before?



Vitamin A and vision

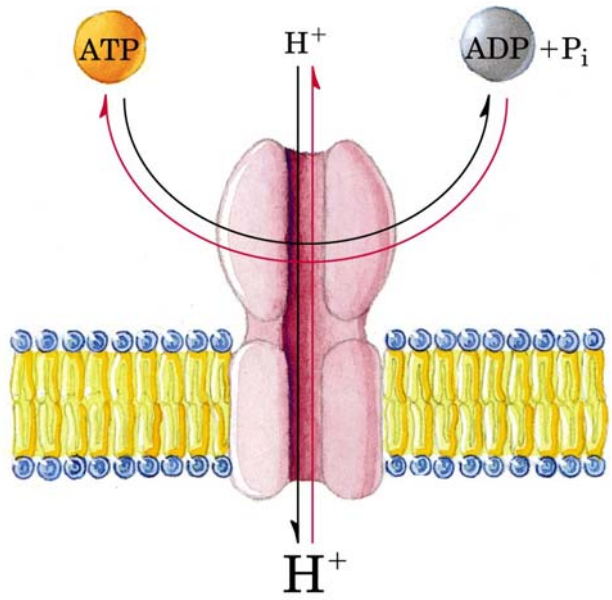


The two conformations of bacteriorhodopsin (see kinemage EMS 8a)

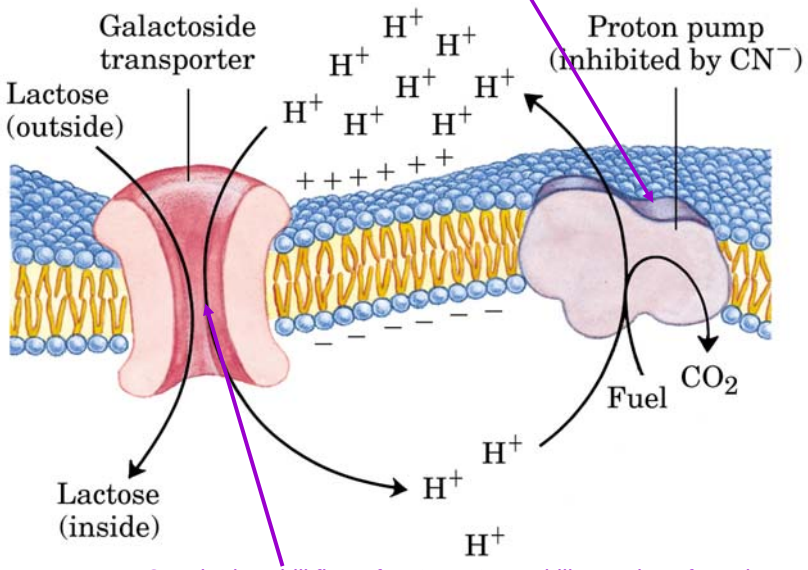


Secondary Active transport

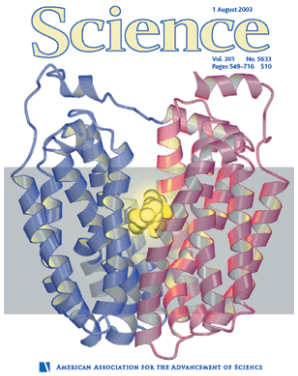
1. Form an ion gradient by primary transport of Na^+ or H^+ against a concentration gradient
2. Couple downhill flow of Na^+ or H^+ to uphill pumping of another metabolite



Form an ion gradient by primary transport of Na^+ or H^+ against a concentration gradient



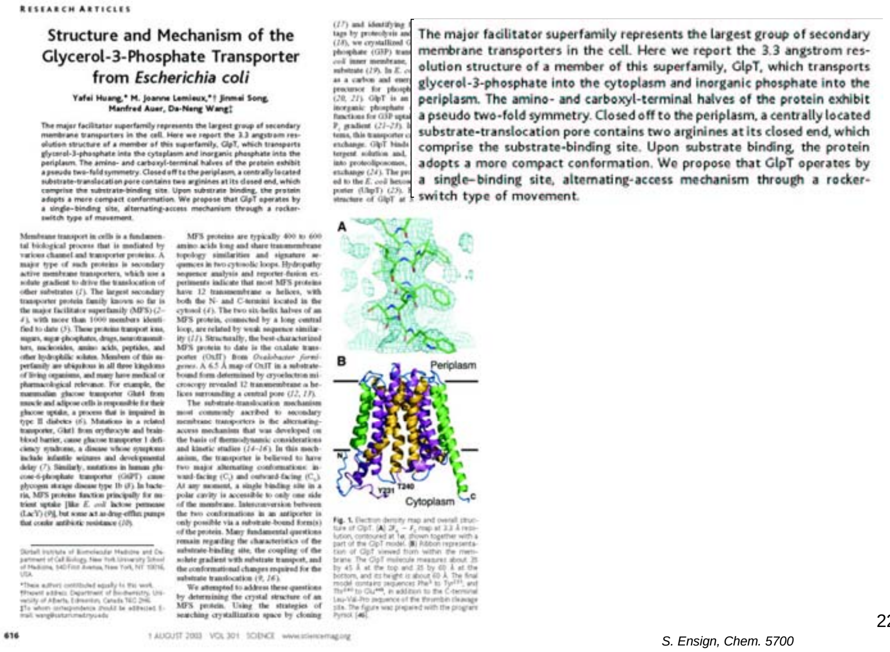
Couple downhill flow of Na^+ or H^+ to uphill pumping of another metabolite



RESEARCH ARTICLES

Structure and Mechanism of the Lactose Permease of *Escherichia coli*

Jeff Abramson, Irina Smirnova, Vladimir Kasho, Gillian Verner, H. Ronald Kaback, So Iwata



RESEARCH ARTICLES

Structure and Mechanism of the Glycerol-3-Phosphate Transporter from *Escherichia coli*

Yafei Huang, M. Joanne Lemieux, Jinmei Song, Manfred Auer, Da-Neng Wang

The major facilitator superfamily represents the largest group of secondary membrane transporters in the cell. Here we report the 3.3 angstrom resolution structure of a member of this superfamily, GlpT, which transports glycerol-3-phosphate into the cytoplasm and inorganic phosphate into the periplasm. The amino- and carboxyl-terminal halves of the protein exhibit a pseudo two-fold symmetry. Closed off to the periplasm, a centrally located substrate-translocation pore contains two arginines at its closed end, which comprise the substrate-binding site. Upon substrate binding, the protein adopts a more compact conformation. We propose that GlpT operates by a single-binding site, alternating-access mechanism through a rocker-switch type of movement.

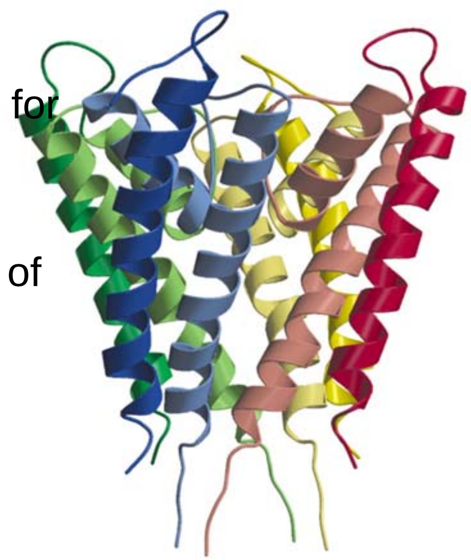
table 12–5

Cotransport Systems Driven by Gradients of Na^+ or H^+

Organism or tissue	Transported solute (moving against its gradient)	Cotransported solute (moving down its gradient)	Type of transport
E. coli	*Lactose*	H^+	*Symport*
	Proline	H^+	*Symport*
	Dicarboxylic acids	H^+	*Symport*
Intestine, kidney of vertebrates	*Glucose*	Na^+	*Symport*
	Amino acids	Na^+	*Symport*
Vertebrate cells (many types)	Ca^{2+}	Na^+	*Antiport*
Higher plants	K^+	H^+	*Antiport*
Fungi (Neurospora)	K^+	H^+	*Antiport*

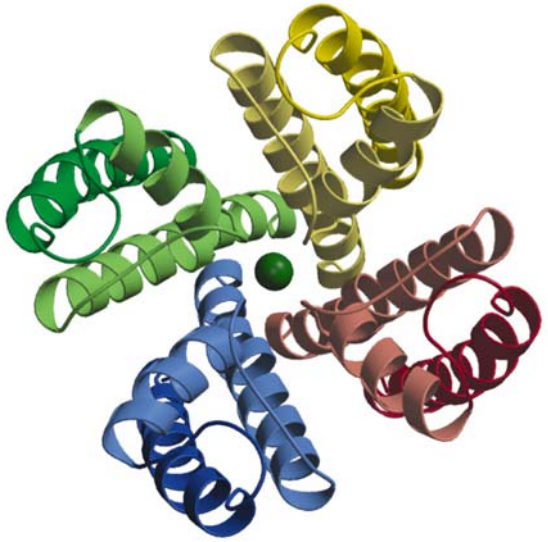
Ion channels

- Formed by proteins
- Extremely high affinity for substrate
- Not saturable
- Downward movement of ions
- Gated (regulated)
  - Ligand-gated
  - Voltage-gated

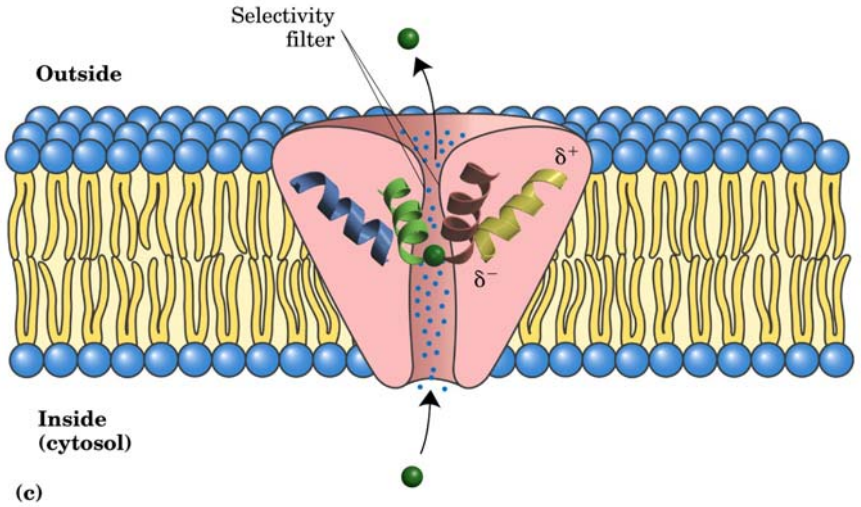


(a)

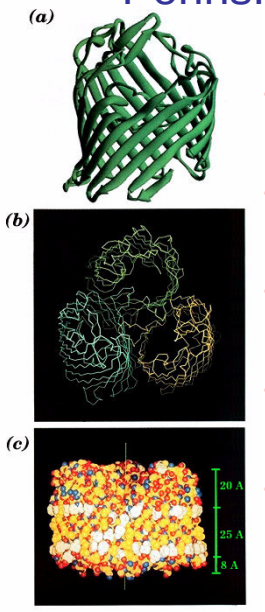
Looking down the K+ channel from the cytosolic side



(b)

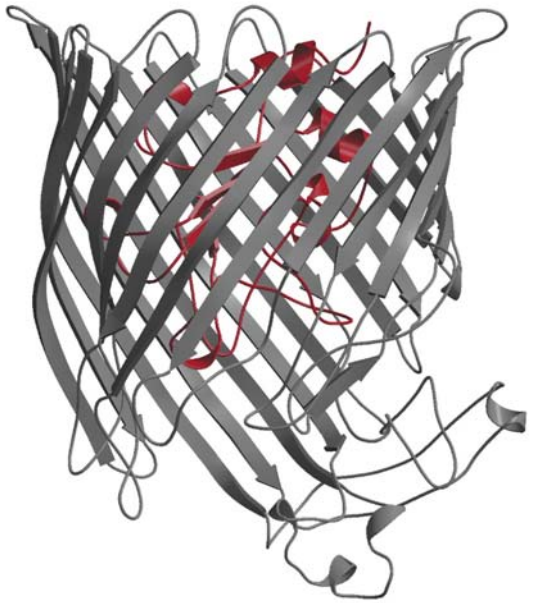


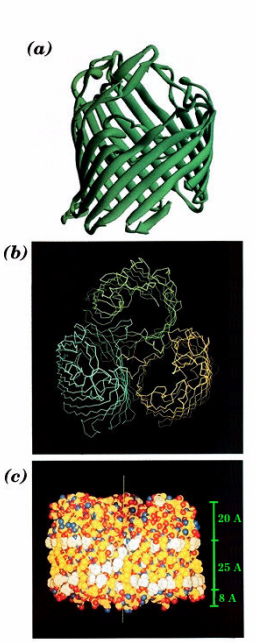
Porins: channel-forming proteins

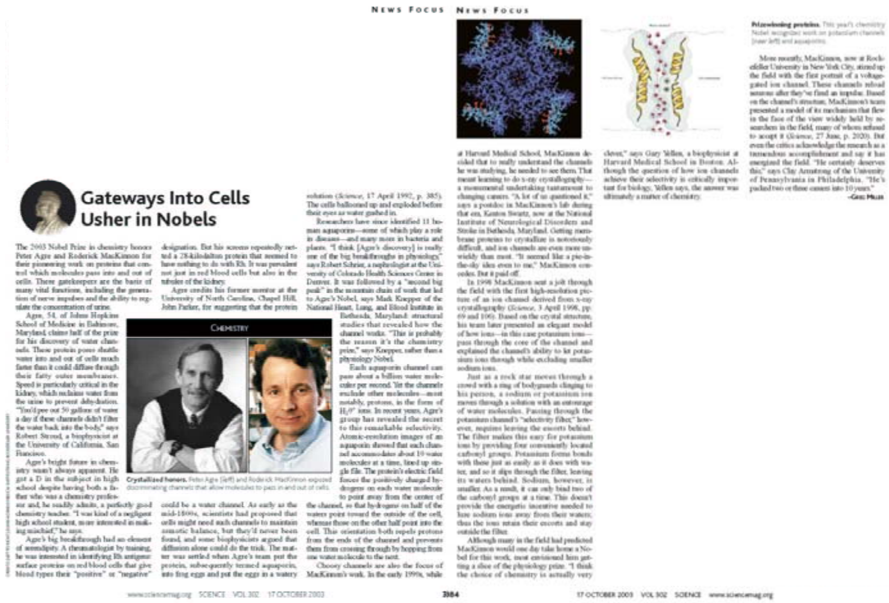


- Trimers, identical 30-50 kDa subunits
- Permit passage of small solutes (e.g. nutrients)
- Each monomer made of a 16 stranded anti II β barrel
- Pore length = 55 Å,inner diameter ~7 Å
- Membrane-exposed side chains are hydrophobic

Porin structure







NEWS FOCUS

Gateways Into Cells Usher in Nobels

The 2003 Nobel Prize in chemistry honors Peter Agre and Roderick MacKinnon for their pioneering work on proteins that control which molecules pass into and out of cells. These gatekeepers are the basis of many vital functions, including the generation of nerve impulses and the ability to regulate the concentration of urine.

Agre, 54, of Johns Hopkins School of Medicine in Baltimore, Maryland, claims half of the prize for his discovery of water channels. These protein pores shuttle water into and out of cells much faster than it could diffuse through their fatty outer membranes. Speed is particularly critical in the kidney, which reclaims water from the urine to prevent dehydration. "You'd pee out 50 gallons of water a day if these channels didn't filter the water back into the body," says Robert Stroud, a biophysicist at the University of California, San Francisco.

Agre's bright future in chemistry wasn't always apparent. He got a D in the subject in high school despite having both a father who was a chemistry professor and, he readily admits, a perfectly good chemistry teacher. "I was kind of a negligent high school student, more interested in making mischief," he says.

Agre's big breakthrough had an element of serendipity. A rheumatologist by training, he was interested in identifying Rh antigens: surface proteins on red blood cells that give blood types their "positive" or "negative" designation. But his screens repeatedly netted a 28-kilodalton protein that seemed to have nothing to do with Rh. It was prevalent not just in red blood cells but also in the tubules of the kidney.

Agre credits his former mentor at the University of North Carolina, Chapel Hill, John Parker, for suggesting that the protein could be a water channel. As early as the mid-1800s, scientists had proposed that cells might need such channels to maintain osmotic balance, but they'd never been found, and some biophysicists argued that diffusion alone could do the trick. The matter was settled when Agre's team put the protein, subsequently termed aquaporin, into frog eggs and put the eggs in a watery solution (*Science*, 17 April 1992, p. 385). The cells ballooned up and exploded before their eyes as water gushed in.

Researchers have since identified 11 human aquaporins—some of which play a role in diseases—and many more in bacteria and plants. "I think [Agre's discovery] is really one of the big breakthroughs in physiology," says Robert Schrier, a nephrologist at the University of Colorado Health Sciences Center in Denver. It was followed by a "second big peak" in the mountain chain of work that led to Agre's Nobel, says Mark Knepper of the National Heart, Lung, and Blood Institute in Bethesda, Maryland: structural studies that revealed how the channel works. "This is probably the reason it's the chemistry prize," says Knepper, rather than a physiology Nobel.

Each aquaporin channel can pass about a billion water molecules per second. Yet the channels exclude other molecules—most notably, protons, in the form of H_3O^+ ions. In recent years, Agre's group has revealed the secret to this remarkable selectivity. Atomic-resolution images of an aquaporin showed that each channel accommodates about 10 water molecules at a time, lined up single file. The protein's electric field forces the positively charged hydrogens on each water molecule to point away from the center of the channel, so that hydrogens on half of the waters point toward the outside of the cell, whereas those on the other half point into the cell. This orientation both repels protons from the ends of the channel and prevents them from crossing through by hopping from one water molecule to the next.

Choosy channels are also the focus of MacKinnon's work. In the early 1990s, while at Harvard Medical School, MacKinnon decided that to really understand the channels he was studying, he needed to see them. That meant learning to do x-ray crystallography—a monumental undertaking tantamount to changing careers. "A lot of us questioned it," says a postdoc in MacKinnon's lab during that era, Kenton Swartz, now at the National Institute of Neurological Disorders and Stroke in Bethesda, Maryland. Getting membrane proteins to crystallize is notoriously difficult, and ion channels are even more unwieldy than most. "It seemed like a pie-in-the-sky idea even to me," MacKinnon concedes. But it paid off.

In 1998 MacKinnon sent a jolt through the field with the first high-resolution picture of an ion channel derived from x-ray crystallography (*Science*, 3 April 1998, pp. 69 and 106). Based on the crystal structure, his team later presented an elegant model of how ions—in this case potassium ions—pass through the core of the channel and explained the channel's ability to let potassium ions through while excluding smaller sodium ions.

Just as a rock star moves through a crowd with a ring of bodyguards clinging to his person, a sodium or potassium ion moves through a solution with an entourage of water molecules. Passing through the potassium channel's "selectivity filter," however, requires leaving the escorts behind. The filter makes this easy for potassium ions by providing four conveniently located carbonyl groups. Potassium forms bonds with these just as easily as it does with water, and so it slips through the filter, leaving its waters behind. Sodium, however, is smaller. As a result, it can only bind two of the carbonyl groups at a time. This doesn't provide the energetic incentive needed to lure sodium ions away from their waters; thus the ions retain their escorts and stay outside the filter.

Although many in the field had predicted MacKinnon would one day take home a Nobel for this work, most envisioned him getting a slice of the physiology prize. "I think the choice of chemistry is actually very clever," says Gary Yellen, a biophysicist at Harvard Medical School in Boston. Although the question of how ion channels achieve their selectivity is critically important for biology, Yellen says, the answer was ultimately a matter of chemistry.

More recently, MacKinnon, now at Rockefeller University in New York City, stirred up the field with the first portrait of a voltage-gated ion channel. These channels reload neurons after they've fired an impulse. Based on the channel's structure, MacKinnon's team presented a model of its mechanism that flew in the face of the view widely held by researchers in the field, many of whom refused to accept it (*Science*, 27 June, p. 2020). But even the critics acknowledge the research as a tremendous accomplishment and say it has energized the field. "He certainly deserves this," says Clay Armstrong of the University of Pennsylvania in Philadelphia. "He's packed two or three careers into 10 years."

—GREG MILLER

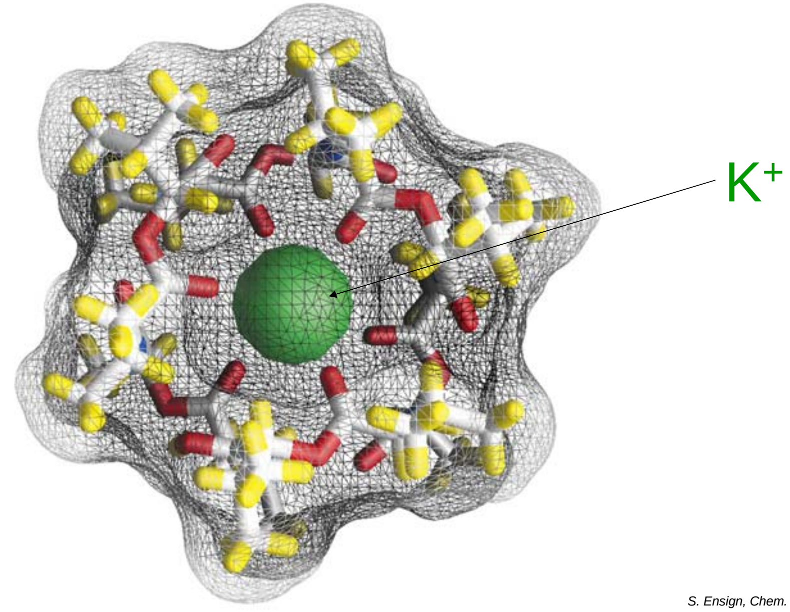
CHEMISTRY

Crystallized honors. Peter Agre (left) and Roderick MacKinnon exposed discriminating channels that allow molecules to pass in and out of cells.

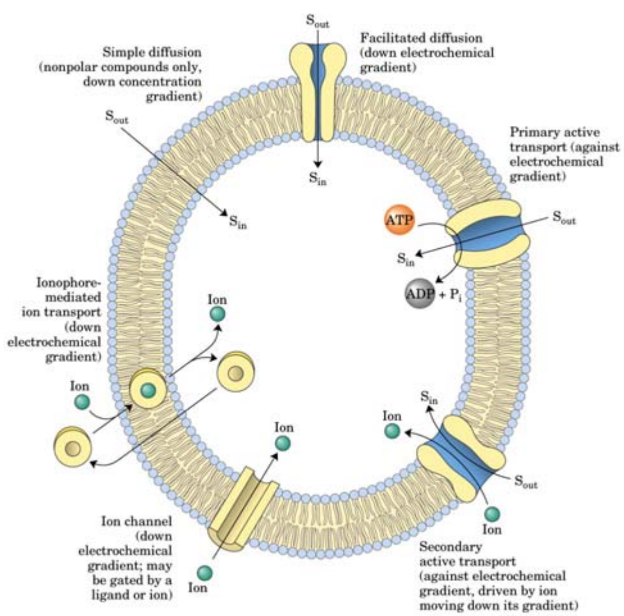
Prizewinning proteins. This year's chemistry Nobel recognized work on potassium channels (near left) and aquaporins.

www.sciencemag.org SCIENCE VOL 302 17 OCTOBER 2003 3384 17 OCTOBER 2003 VOL 302 SCIENCE www.sciencemag.org

Valinomycin: a peptide ionophore



Summary of transport strategies/mechanisms



The bacterial reaction center structure (Nobel prize)