Advanced Biochemistry

MEMBRANE TRANSPORT

This is a Tutorial/Silent Lecture

What is a Tutorial/Silent Lecture?

a sequence of "slides" formatted to guide you through the exploration/study of the topic

you are the main actor in this active learning experience
think of it as working with a tutor without having to pay for it

as the slide sequence unfolds, you will get opportunities to engage with the material

- **by thinking about/answering questions**,
(my answer is always provided on the next slide).
- **by completing a "short assignment"**
(it never will take more than a few minutes, if at all that long),
- by **watching a short video/clip**
(the embedded links will take you to my YouTube@VsBCBMB channel;
key moments are captured as still and are shown in the slide-deck, in case you don't want to watch the videos)

of course, you can skip the active learning aspect and look at the answers right away.

Why Give This a Go?

- **benefits: you set the pace** taking as much or as little time as you need.
- you **can turn tutorials/silent lectures into fully immersive experiences** (eg playing your favourite music while working through the content),
- **or invite friends to over the Q&A structured/guided materials together,** discussing the questions before looking at answers.

each of these features help you to hold on to the material.

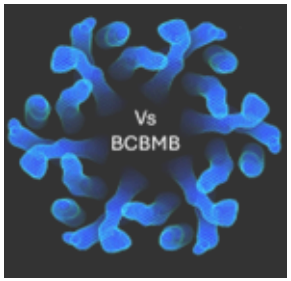
Advanced Biochemistry

This collection of handouts builds on the "Biochemistry Fundamentals" collection = the chapters assume that you know the basics covered in the "Fundamentals" handouts (free downloads), and have some basic knowledge of molecular/cell biology

you will get the **greatest benefits** from this particular "silent lecture" by

- **Reviewing the "Advanced Biochemistry – LIPIDS and MEMBRANES" chapter**
- Spending 5-10 minutes to **summarize for yourself what you already know/remember** about Membrane Transport Processes, and
- **Take advantage of the "interactive" elements**, be that by watching short videos that, at times, are linked to the pdf-files, or by playing along, trying to answer questions posed before looking at the answer on the following slide.

I welcome your thoughts and ideas for further improvements of the chapters. You can submit your comments by contacting me at pdf-comments@vsbcbmbstudy.com



Goals

by the end of this chapter you will know

- why distinctive transport processes are needed and what their functions are
- how basic thermodynamic principles constrain the design of transport proteins
- what the defining difference is that sets channels and transporters apart
- how transport of small molecules is accomplished
- how different subtypes of membrane transporters share universal mechanistic elements

The narrative of this chapter invites you on a journey that builds understanding of membrane transport processes by looking at increasingly complex membrane transport challenges.

In the context of K-channel gating, this chapter also takes a significant look at "membrane potentials" because understanding this subtopic really helps with understanding channel/transport related issues and cell function in general. If you are not interested in this "detour", please consult the Table of Content to see when and what to skip.

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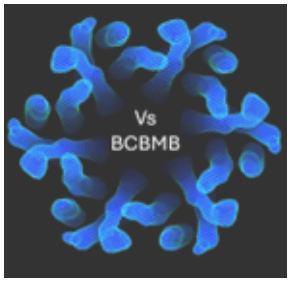
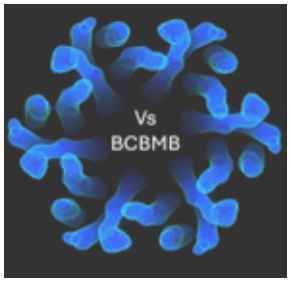
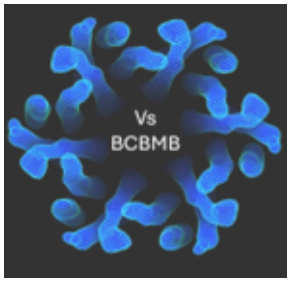


TABLE OF CONTENT



Transport – The Very Basics

Quick Reminder – How Did We Get Here = Why and Where Do We Need Transport?



Transport – The Very Basics

Quick Reminder – How Did We Get Here = Why and Where Do We Need Transport?

answer: impermeability of the bilayer makes it mandatory to create passageways for polar molecules (eg carbohydrates, amino acids, nucleotides, metabolites), ions, and - in some cases - larger macromolecules like proteins, or even nucleic acids

therefore

transport is needed at all membranes → each cell and organelle has its dedicated set of proteins/mechanisms facilitating solute shuttling across the bilayers

---

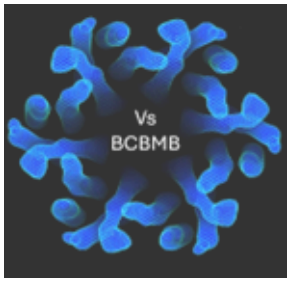
before going into any more detail, I want to invite you to think about ways how YOU would design membrane transport processes

what components? how would they work (in principle)?

draw a simple cartoonish picture illustrating the main ideas of your ideas.

….start by drawing a "membrane" … put a shape on one side … and then try to find questions whose answers would help you to design a mechanism to get the shape across the membrane.

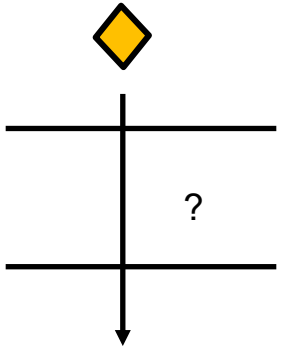
the benefit you get from doing this is potentially quite large because it will create insights/ideas/hypothesis/questions that later will be connected to the actual solutions Nature implemented



Transport – Design Challenge

Here is my peace-offer – how does it compare?

to get across = create a device that can embed in membrane and has a polar/aqueous interior to allow cargo to pass through membrane → **need integral membrane protein**

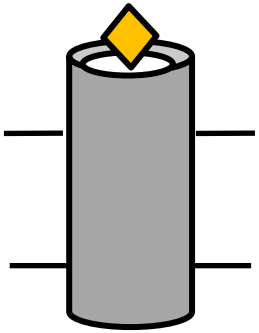


Does the cargo go just one way or both ways?

How much cargo it is already on the other side = do I need to "push" it against a concentration difference across the membrane?

Do I control this or just let it go?

Simplest Idea
just a "hole"
(…learned about β-barrels…in PROTEINS chapter, but could use helical bundles as well)



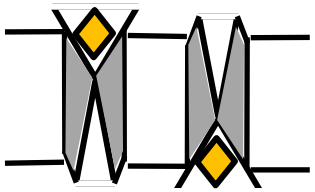
This would make it **go both ways.**

Does not allow for concentration differences

Intuitively this looks hard to control because nothing moves …it's just "open" …
→ should I invent a second "state" where, somehow, the hole cannot pass the cargo? That would give control and intuitively sounds like a good idea ….

"Seesaw"

(hole with obstruction
→ pivot about center to translocate)

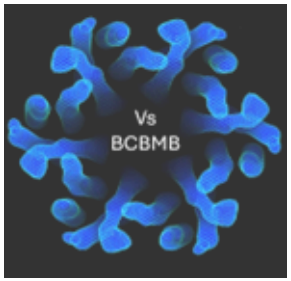


This would also make it **go both ways.**

This does allow for concentration differences, if needed, by coupling to an energy source

Intuitively this looks like one can built in some controls because it needs a "seesaw" movement of the protein

→ how do I trigger that movement?
→ how do I couple to energy source if needed?



Transport – The Very Basics

if you did the challenge … you may have noticed
that the basic ideas behind membrane transport are
not overly complicated ….

where things get "complicated" is in the "nitty gritty details " … eg: how do you make any of these transport devices specific for whatever they transport? How do you regulate or energize these devices? Much of the chapter will deal with exactly those questions

… but before going there let's add one more way how to transport things …. the way the challenge was put (asking you to think about "a" hypothetical molecule), makes it easy to overlook hat cells also employ a lot of

"bulk transport" through vesicular mechanisms

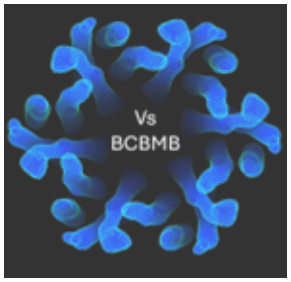
(eg, endocytosis, macropinocytosis, neurotransmitter release, protein export, cargo shuttling between organelles [eg ER to Golgi, Golgi to Lysosomes, plasmamembrane to endosome]).

this type of transport involves membrane remodeling, membrane fusion and fission, and

utilizes complex ligand-receptor systems for targeting.

in addition, cargo acquisition often requires more specialized transport mechanisms at the beginning of the process [eg loading of neurotransmitter into a vesicle]

this Chapter will not deal with vesicular transport (beyond of what was mentioned in the "Membrane Remodeling" Chapter)



Transport – The Very Basics

returning to the basic ideas that fell out of doing the initial "challenge" ….

trying to let sink in what "transporting across a membrane" does makes it easy to appreciate that cells pay close attention to and control what, when and how much crosses its membranes

all transport processes are regulated.

regulation of transport can be achieved **at four levels**:

- amount of any given transporter protein

(this first argument is quite intuitive)

- choice of transport mechanism

(=whether the cell let's it pass by simple diffusion or actively brings it in/throws it out)

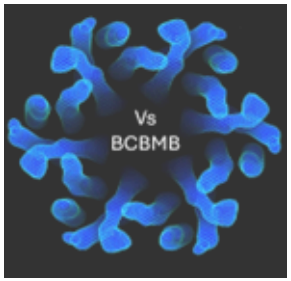
- posttranslational modifications of transporter proteins

(chemical modifications like adding/removing phosphate groups are a very powerful tool for changing the functional output of the transporter protein = increase or decrease the activity of the protein)

- through trapping of conduits in inactive conformations

(the most widely known example is desensitization of channels, which we will encounter when looking at how K^+ ions are transported across the membrane)

in the case of vesicular transport, more complex mechanisms are required to regulate the process: need to control targeting components, membrane remodeling, and - in many cases - cargo acquisition.

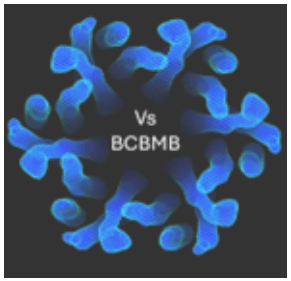


General Functions of Membrane Transport

hearing or reading the term "membrane transport" **typically conjures an image of small molecules/ions being acquired or disposed of** ….
true, such processes make up for a big part of membrane transport

….but

can you think of any other function that requires membrane transport? …try ….



General Functions of Membrane Transport

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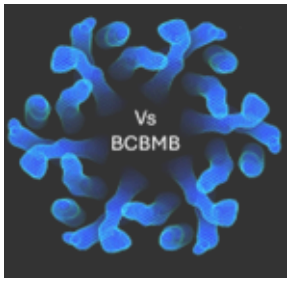
….but

equally important:

- global homeostatic purposes (eg membrane potential, osmotic regulation)
- energy generation
- signaling (eg action potential/neurotransmitters, Ca^{2+})
- differential processing of metabolites/proteins (eg, fatty acid degradation, sterol synthesis, some post translational modifications)

why stressing different purposes?

…what are your thoughts? ….



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- **differential processing of metabolites** (eg sterol synthesis, some posttranslational modifications)

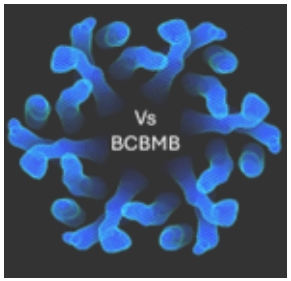
Why stressing different purposes?

Answer:
leads to the **realization that two fundamentally different types of transport** are needed**:**

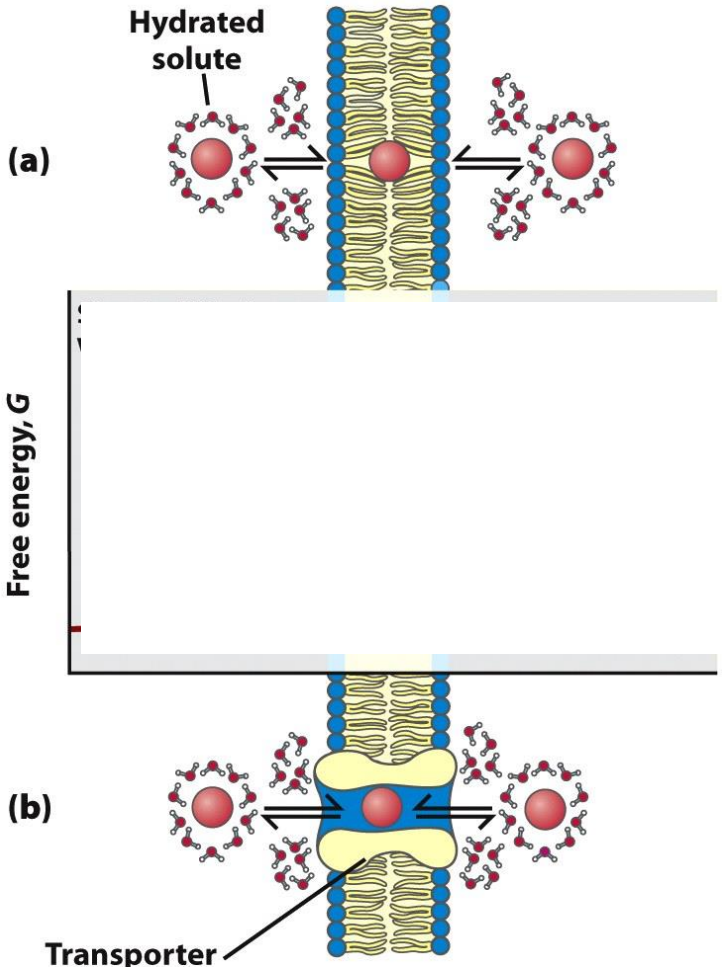
<u>active and passive</u>

why?
because in principle (and you likely came across this thought when engaging with the challenge at the beginning of the chapter), **transporters are nothing but "selective holes" that make an impermeable barrier semipermeable for a particular chemical species**

what does that mean and what is the consequence?



Biological Transporters Lower the Activation Energy for Transbilayer Diffusion



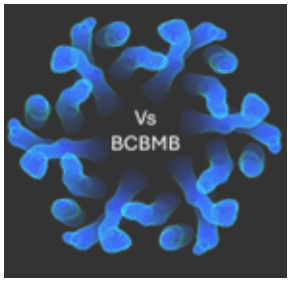
let's unpack what "selective hole" means

your turn to start this by thinking about how the the Free Energy Profiles Look for

- spontaneous diffusion across the bilayer of a small, uncharged & amphiphilic molecule (= a molecule that besides some hydrophilic aspect has enough hydrophobicity to allow unassisted passage)
- the same molecule but undergoing assisted diffusion through the bilayer.

….how do you think the two curves look like?

(hint: processes that require energy and/or do not happen spontaneously in the indicated direction will have $\Delta G > 0$)



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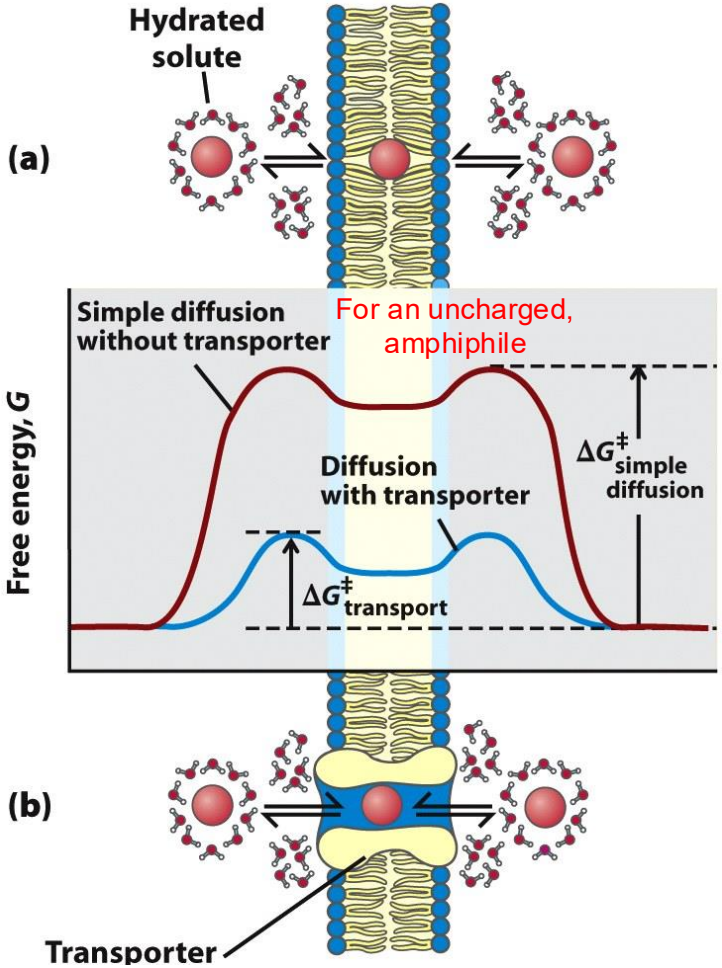
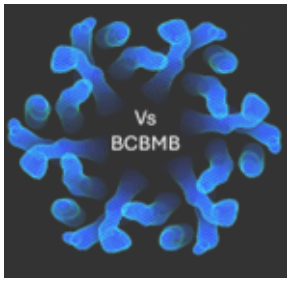


Answer

is that what you pictured?

if so … tell me: …. why does this look symmetric about the center of the bilayer?

Also
the figure in the textbook had no "annotation"
→ why would I tell you that this diagram is for an "*uncharged, amphiphile*"? = would this look any different for a molecule that has a charge? If so … how?



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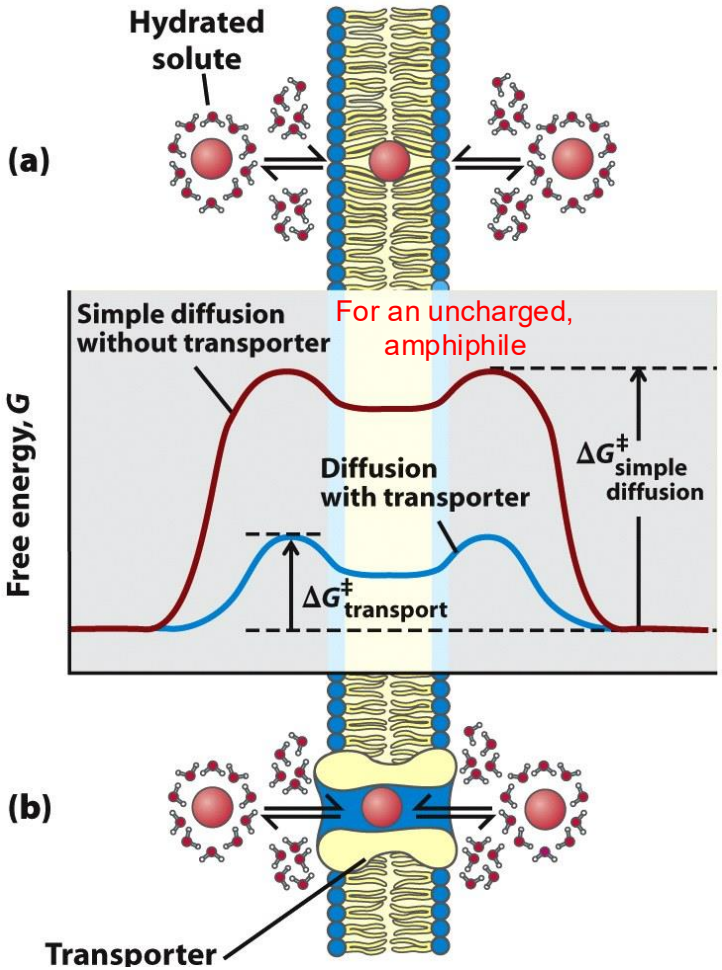


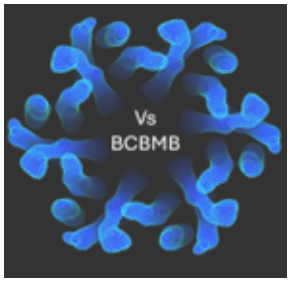
Answer

is that what you pictured?

if so … tell me: …. why does this look symmetric about the center of the bilayer?

it looks symmetric because the picture shows that the molecule can travel **both ways** = the curve you see is the sum of the profiles going from left → right and right → left.

the figure also assumes that, energetically, there is **no** preference for either direction (= no concentration difference on the two sides)



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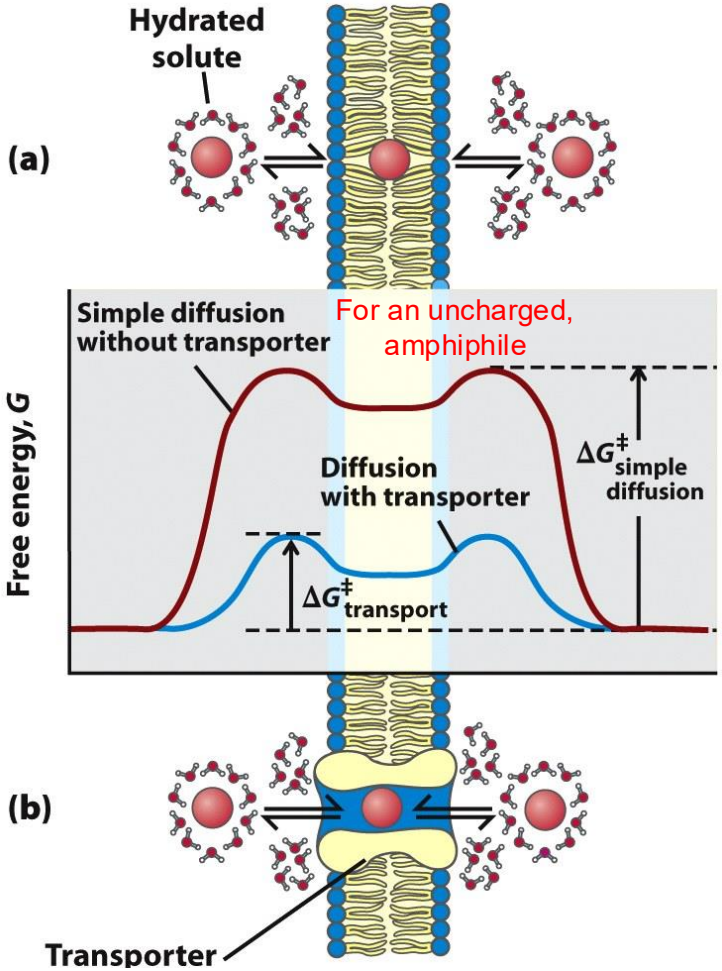


Answer

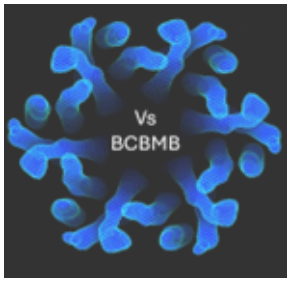
also: the figure in the textbook had no "annotation" → why would I tell you that this is for an "*uncharged, amphiphile*"? = would this look any different for a molecule that has a charge? If so … how?

the curve would look very different if this was not drawn for an amphiphile!

for starters: if the molecule had a charge, then ΔG would not plateau but display a maximum at the midpoint of the bilayer (= the ion would be the "least happy" there).

if furthermore this referred to a "real cell membrane" … then the curve would not be symmetric either because of the cell's membrane potential (slide 48 onwards)

other than that – the curves actually do make sense…..why?



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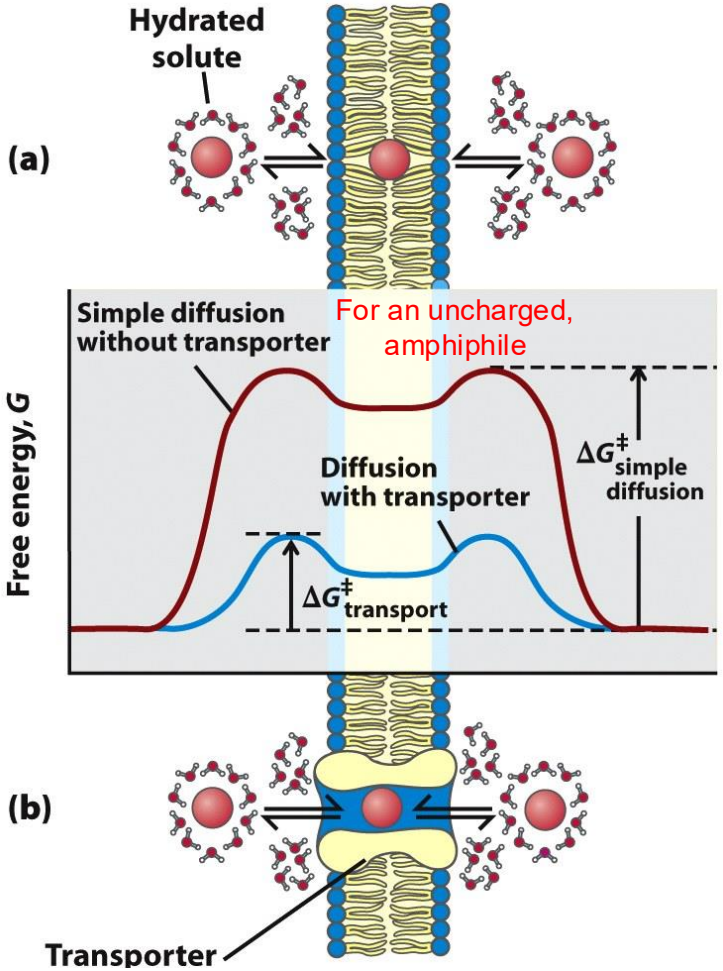
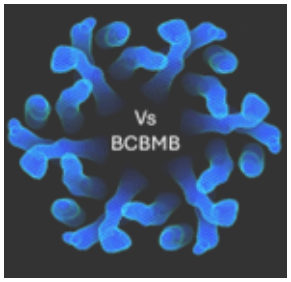


other than that – the curves actually do make sense.… why?

- the hydrated solute (terminology that refers to a molecule that is dissolved in solvent – water in this case) starts out by stripping off the water molecules that surround it
→ overall, the desolvation is endergonic = it does **not** happen spontaneously as long as water is present → ΔG goes up.

- once the molecule enters the bilayer, it gets "solvated" by the fatty acid chains of the lipids …. since the molecule is an amphiphile, this causes a small decrease in ΔG (because now the hydrophobic parts of the molecule are "happy", **but overall**, the molecule is "less happy" inside the membrane than outside)

- once the molecule reaches the far side of the membrane … water molecules can come back on ➔ this leads to a big decrease in ΔG because the molecule returns to the more comfortable solvent. Note, however, that this decrease is masked by the energy profile describing passage in the other direction … → that is why the ΔG profile looks symmetric everywhere.



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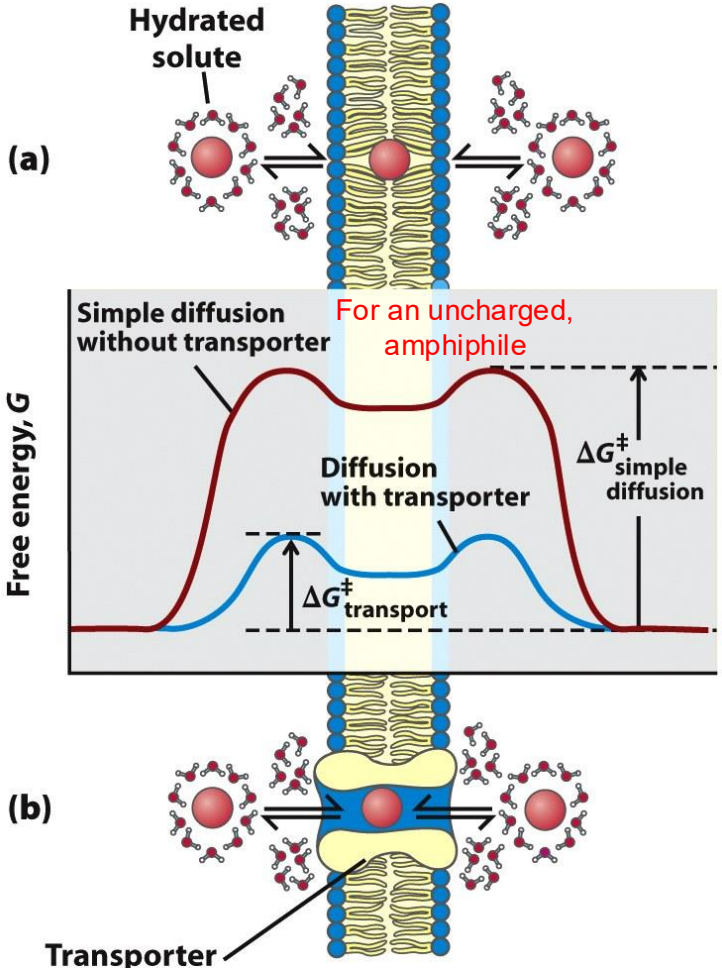


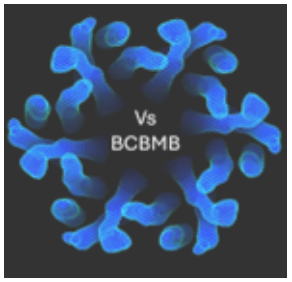
other than that – the curves actually do make sense…. why?

- looking at the second case – assisted diffusion – the profile looks very similar in general terms because the **"transporter" does not change the physics of the process** …

but it DOES lower the ΔG cost for the diffusion

by helping with "stripping off water molecules" and shielding the molecule from the very hydrophobic interior of the bilayer.

interestingly: the transporter DOES NOT create an environment that is more favorable than the aqueous phase on either side of the membrane …
…..can you guess the reason for this?



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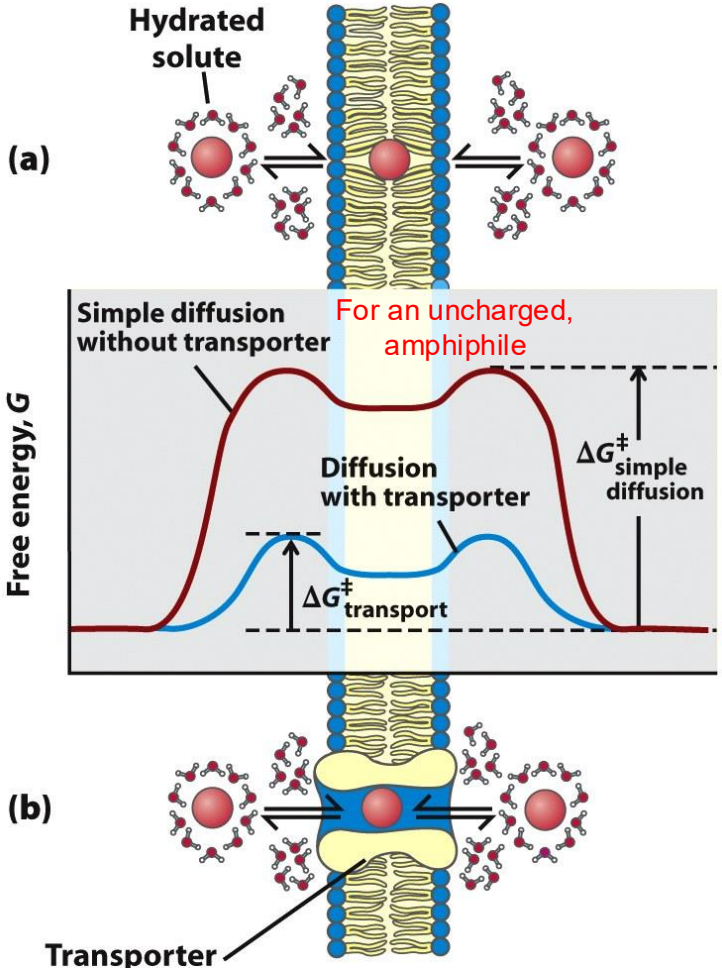

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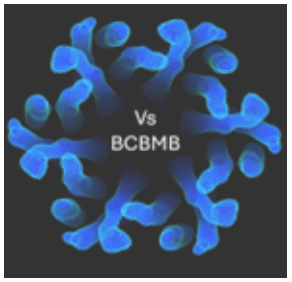
but it **DOES** lower the ΔG cost

for the diffusion by helping with "stripping off water molecules" and shielding the molecule from the very hydrophobic interior of the bilayer.

- interestingly: the transporter **DOES NOT** create an environment that is more favorable than the aqueous phase on either side of the membrane … can you guess the reason for it?

- creating an environment more favorable than that on either side of the membrane would cause molecules to get "stuck" inside the transporter … which defeats its purpose….

➔ OK … let's put this all into more formal "language"



Biological Transporters Lower the Activation Energy for Transbilayer Diffusion

two important conclusions here:

- **first, transporters/channels lower the activation energy for diffusion** (= they are catalysts).

to determine the total change in non-standard free energy ΔG, think of the transport process as a chemical reaction for which

$$\Delta G = \Delta G'^{0} + RTlnQ$$

$\Delta G'^{0}$ is the standard free energy change at physiological conditions, R is the gas constant, T the temperature [K], and Q is mass action ratio that reflects the concentration dependence of the free energy change.

in the case of transport, this relationship becomes simpler because the transported solute is **not** chemically altered, and hence

$$\Delta G'^{0} = 0$$

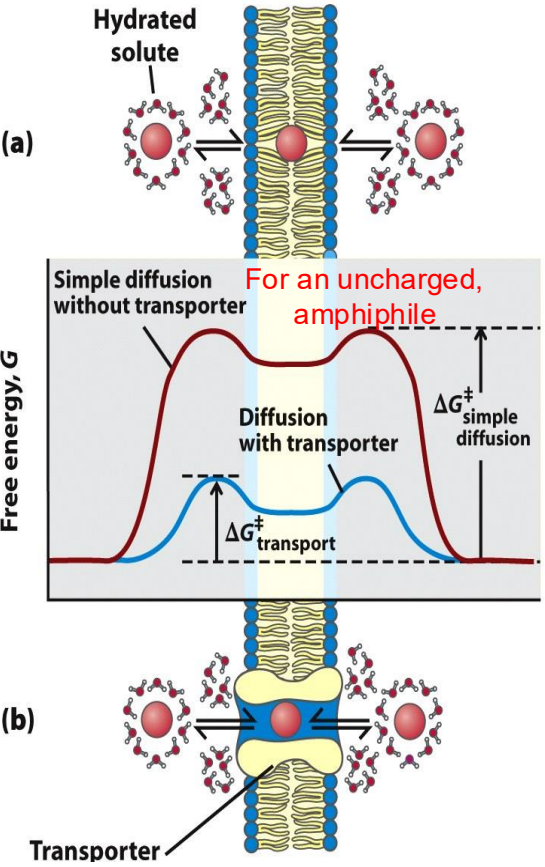
Which leaves you with

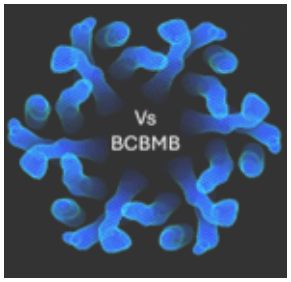
$$\Delta G = RT \ln \frac{C_2}{C_1} + ZJ\Delta\Psi$$

C: concentration; Z: charge of ion (if applicable), J: Faraday Constant, $\Delta\Psi$; membrane potential.

for an uncharged solute: the change in free energy for the transport process only depends on the concentration difference between the two compartments.

for charged species: need to add a term that accounts for the amount of charge, and the voltage gradient across the bilayer (see slide 48)





Biological Transporters Lower the Activation Energy for Transbilayer Diffusion

two important conclusions here:

- **transporters/channels lower the activation energy for diffusion** (= they are catalysts)

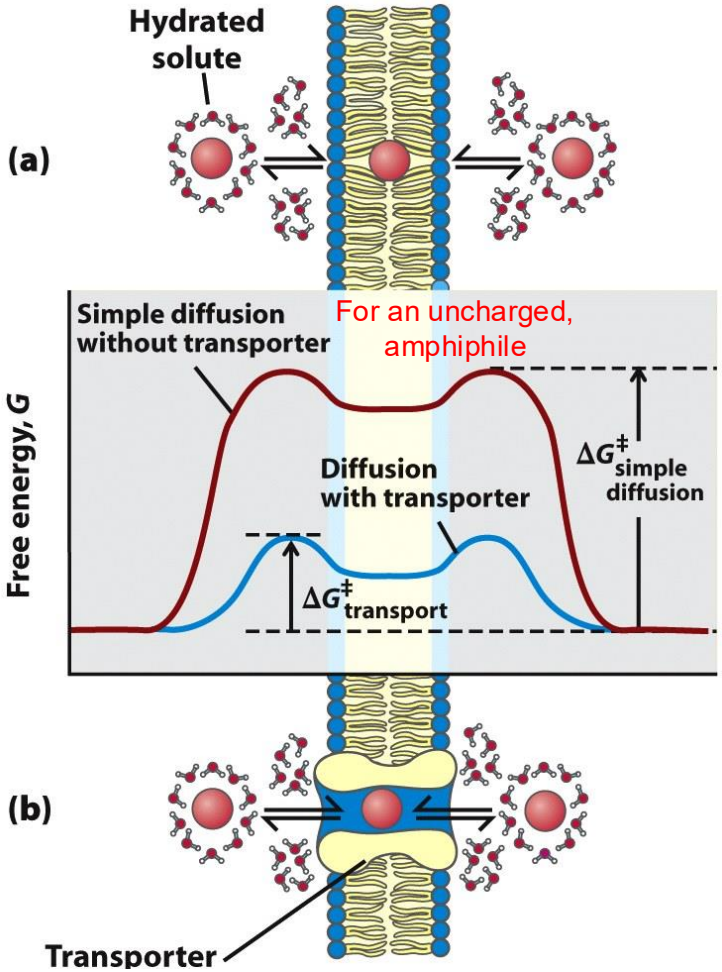
- **transport processes are reversible**

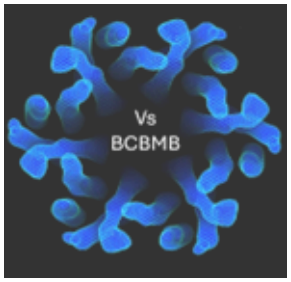
*(note: we already found this **reversibility in macromolecular interactions** ("How Do Molecules See? Pt2, slides 18-23); **protein folding** "Advanced Biochemistry – PROTEINS, slide 20) **and will find this again in catalysis of chemical reactions** later on)*

starting this whole section by thinking about "selective holes" and the implications of having them in membranes, you now realize that:

given the biological purposes of biological transport processes (slide 12), the general reversibility of transport processes is a catastrophe…..!

why?

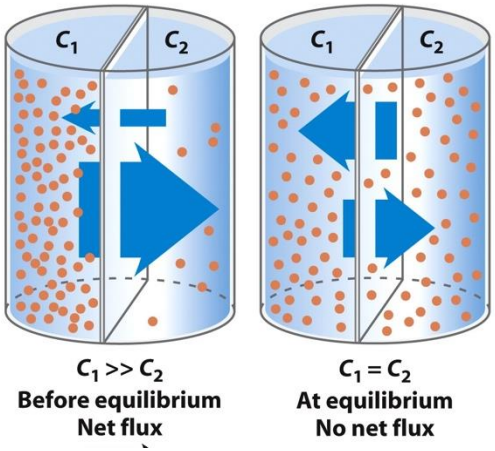




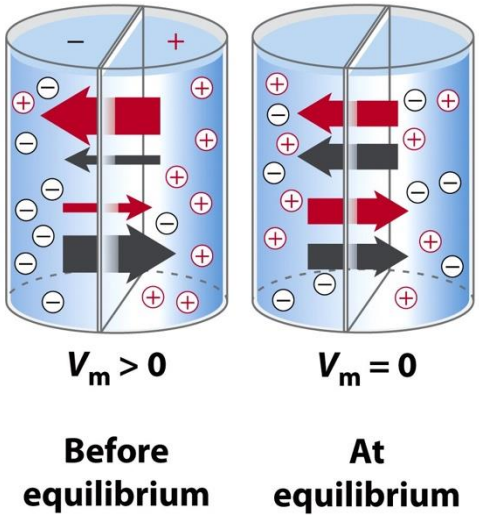
Active and Passive Transport

Solutions to a Thermodynamic Challenge

ANSWER: Generation of a semipermeable membrane will dissipate gradients.



passive diffusion along a concentration gradient, or an electrochemical gradient



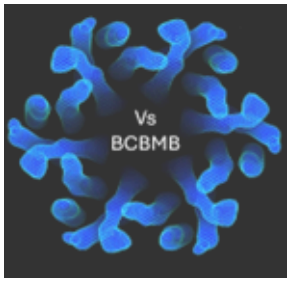
- **acceptable for some processes**

(eg osmotic regulation, uptake of certain nutrients like glucose into most tissues).

HOWEVER

- **overall life is a non-equilibrium process**. **Consequently, transport processes must contribute to keeping an organism from reaching equilibrium. This requires energy and is the rationale for why active transport processes exist.**

put into perspective: **25% (!) of the daily energy consumption goes into maintaining non-equilibrium conditions for Na^+ and K^+ across the membrane** (slide 124). This non-equilibrium forms the basis for the **membrane potential** (= a voltage across the membrane, slide 48), which is key for the generation of action potentials in neurons (slide 65 onwards)



INTERMISSION

**With the General Considerations and Arguments Out of the Way,
We Now Want To Turn Our Attention to the
Specifics of Membrane Transport**

for this, we will explore increasingly complex transport processes to illustrate how Nature tackled and solved various challenges to meet the needs of living systems

as a starting point, we will look at a simple passive diffusion event.

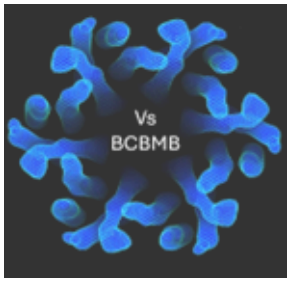
the solute: **water**

thinking about the hydrophobicity of membranes, water is THE most likely molecule many students think off when asked what substances cannot freely pass biological membranes (followed by ions) ….

….thinking of water is not bad at all in this context, though remember ….

when we looked at the structure of membranes, we discovered that computer simulations of membranes predict that water can cross bilayers spontaneously (Advanced Biochemistry - LIPIDS & MEMBRANES slide 75)

BUT …..



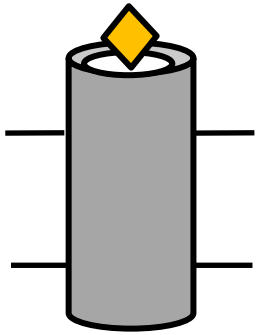
Transport Process: Water

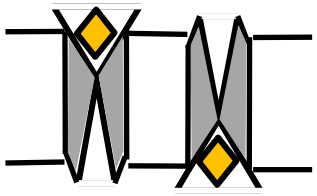
- spontaneous diffusion of water across the bilayer is not fast enough to allow cellular homeostasis.

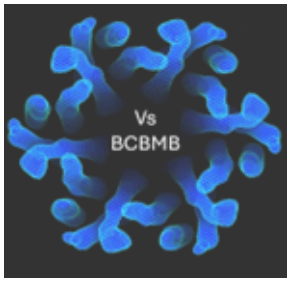
a good example are erythrocytes (red blood cells) - they have to respond to rapid changes in osmolarity as they travel through tissues/organs. Without a mechanism to take up/loose water quickly they would burst.

coming back to our original challenge ….
which solution would you use to implement water transport ….
Why?

"hole" …… or ……seesaw







Transport Process: Water

first off: water can traverse the bilayer unassisted, but not fast enough to allow cellular homeostasis.

a good example are erythrocytes (red blood cells) - they have to respond to rapid changes in osmolarity as they travel through tissues/organs. Without a mechanism to take up/loose water quickly they would burst.

coming back to our original challenge ….
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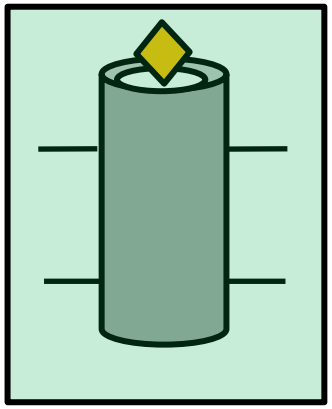
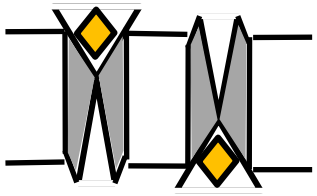
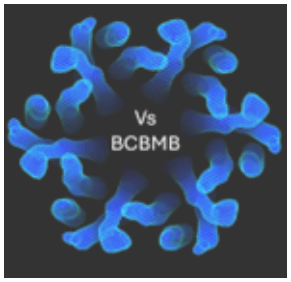




ANSWER:
Needs to be fast, so protein dynamics should be minimal → a **passive pore ("hole")** would do well and be fully sufficient (for most cases).

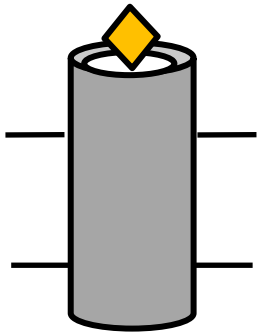


Transport Process: Water

Needs to be fast, so protein dynamics should be minimal → a passive pore ("hole") would do well and be fully sufficient (for most cases).

- **definition:** membrane proteins that provide a structurally "static" scaffold for solute diffusion to occur function as "**channels**".

this definition
does NOT imply that these pores are always open
(as we suspected during the initial "challenge")



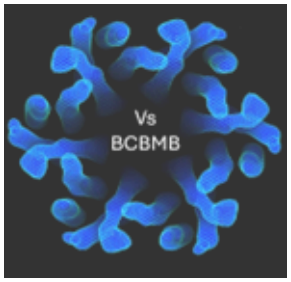
the pores can transition between open/closed states, but once that transition is made, flux of solute does not involve additional significant conformational changes and solely depends on the concentration gradient.

implementing this simple passageway **should be "pretty" straightforward** – shouldn't it?

you'd think so … **but** there is one **TRULY NASTY**

property of about water that makes design of a suitable passageway for passive diffusion **SUPER difficult**…..

do you happen to know what it is (no worries if you don't)**?**



Transport Process: Water

Answer

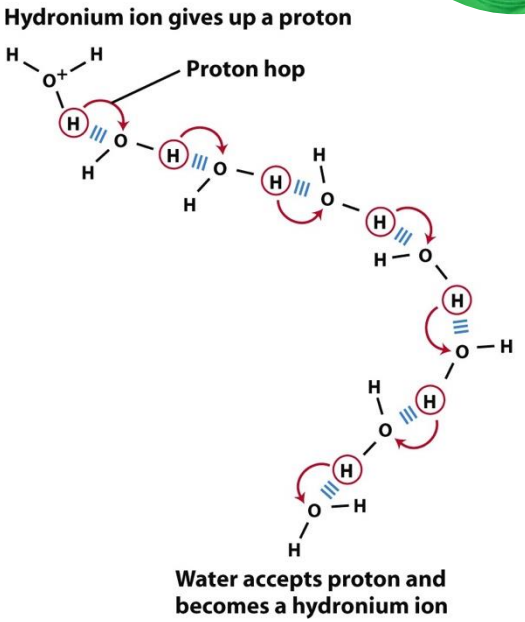
proton hopping

→however the channel is designed, it must prevent formation of a fully hydrogen bonded single file of water

let's "unpack" **proton hopping**

as shown in the diagram, a fully hydrogen bonded, single file chain of water molecules can transport a proton **WITHOUT physically moving** the proton at all.

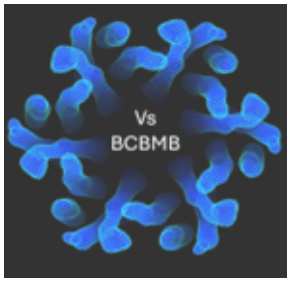
→ proton hopping refers to a quantum mechanical phenomenon where a proton added at one end of the single file will **instantaneously** be released at the other end



mechanistically think of it as "rebranding" of electron properties within the single file of water molecules. Specifically, the proton will be transferred to the chain by changing the hydrogen bond between a $[H_3O]^+$ and the terminal H_2O into a covalent bond. At the EXACT same moment ALL H-bonds along the water chain are rebranded into covalent bonds = the moment the proton binds at one end, it magically appears on the other where it is released by transfer to the next available H_2O molecule.

➔ no matter what the details are for the water transport the water transporting protein **MUST** prevent proton hopping because otherwise this protein will cause pH gradients across membranes to break down … which would have catastrophic consequences (simply put: you die)

based on all this … how would YOU design the protein to make sure it ONLY conducts water….?



Transport Process: Water

based on all this … how would YOU design the protein to make sure it ONLY conducts water….?

Answer

- you **cannot transport in bulk** because once the narrowest point of the passageway can let >1 water molecule through at the same time, you also will allow $[H_3O]^+$ to pass.

➔ if you must narrow it down to allow passage of single water molecules, one at a time, then the only way to prevent proton hopping is to **exploit the weaknesses of this mechanism**.

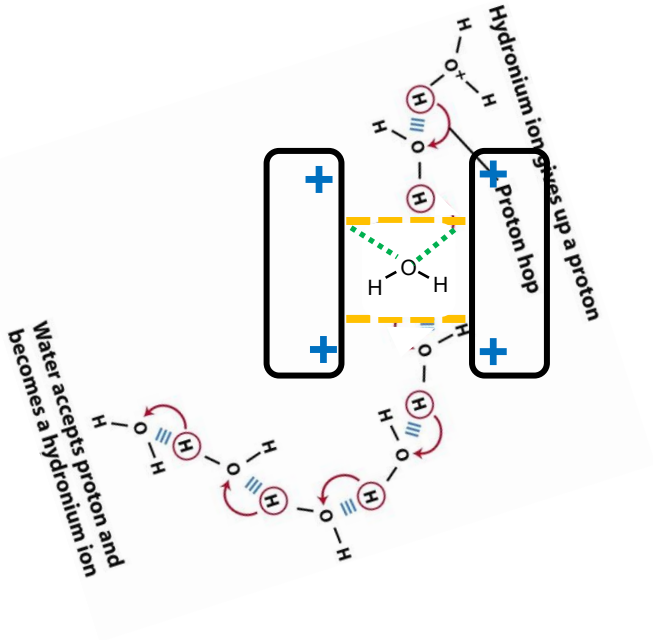
weakness 1: for proton hopping to occur, a $[H_3O]^+$ must come close enough to the constriction to transfer its proton to the terminal water molecule of the single file chain

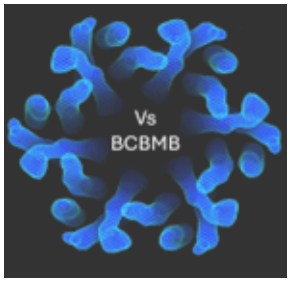
solution 1: Place "+" charged amino acids close to the constriction to "repel" $[H_3O]^+$

weakness 2: the single file water chain needs to be fully H-bonded along the chain

solution 2: disrupt the H-bonding along the single file by reorienting the very H_2O that is about to pass the narrowest part of the pore. This will break its H-bonds with the water above and below = shutting the door to proton hopping!

to accomplish this, the protein needs to offer alternate H-bonding partners that temporarily replace the H-bonds as the water molecule passes through the constriction





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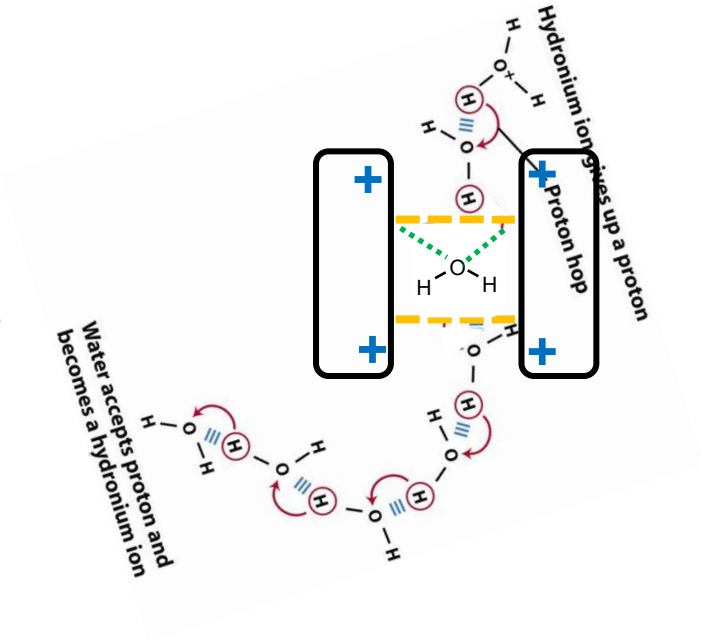
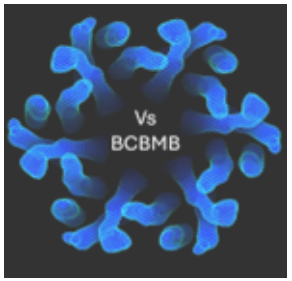


…and there you have it, this is **exactly** how Nature solved the problem – very intuitive, clever and amazing!

this design allows for very rapid transport of water that @ $3x10^9$ molecules per second is close to the diffusion limit of water molecules in bulk solution.

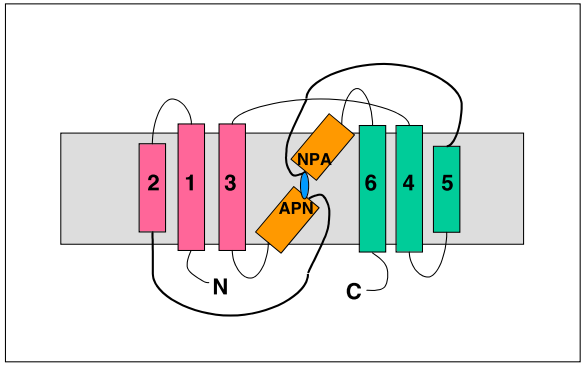
note this high speed is possible because the protein itself does not need to move at all … if the pore is in its open state, then passing water only requires diffusion and molecular rotation of the water molecules ….

….with all this in mind … I hope you are curious how this water channel actually looks like! ….



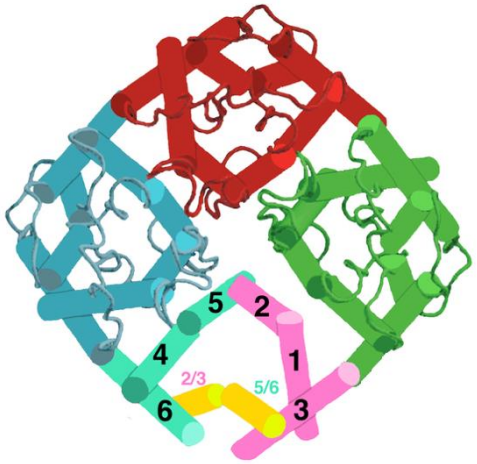
Aquaporins

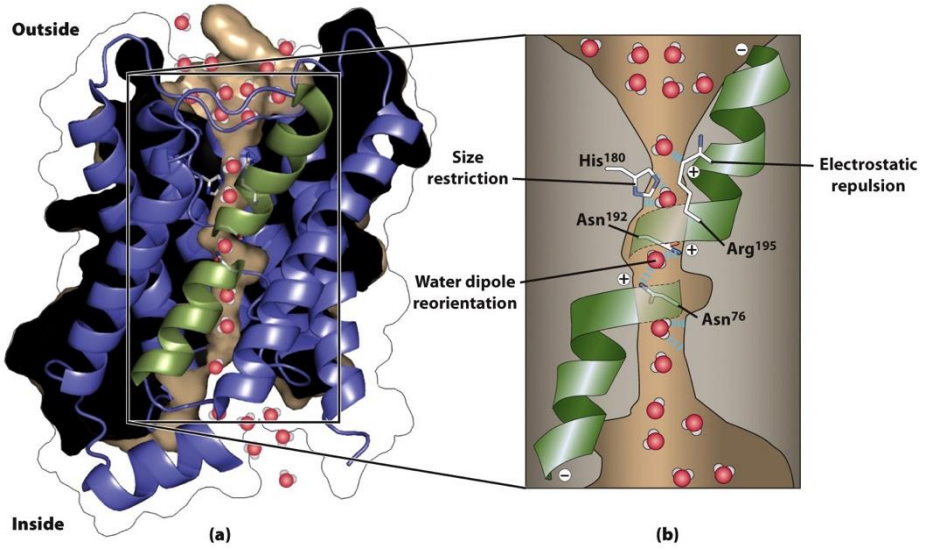
water channels are known as aquaporins; 10 different types, some facilitate diffusion of glycerol, urea, nitrate, chloride in addition to water.

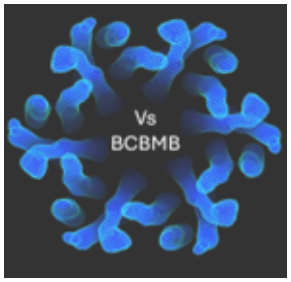


- 6 TM segments + two "half helices" that carry the hallmark "**NPA-motif**" (asparagine-proline-alanine). The asparagine of each of the two "half helices" are the H-bond donors that assist with reorientation of the water molecule
- aquaporins form quaternary structure; they are tetramers, but water transport occurs within each of the monomers

- at short time scales (millisecond): changes in pH, modifications like phosphorylation or interactions with a calcium sensing protein will close the channel = regulation



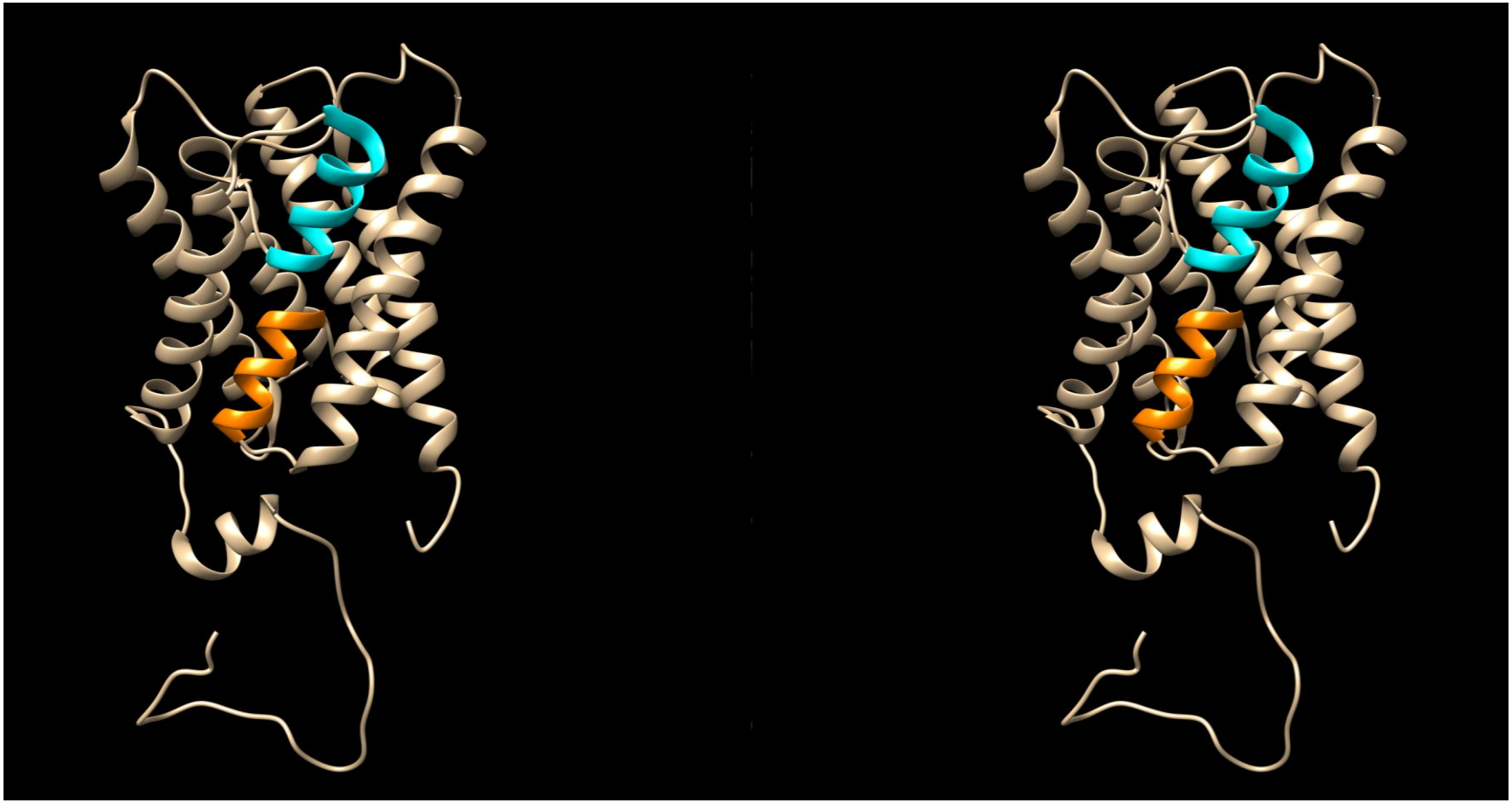




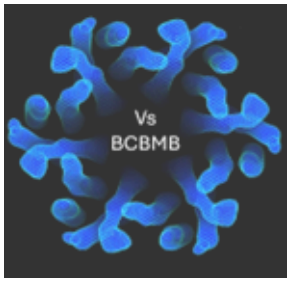
Aquaporins

this and the next slide are cross-eyed stereo views of an aquaporin 0 monomer, seen parallel to the membrane to let you get a better sense for the spatial relationships

to achieve the stereo effect – hold ~30cm away, look at the image with your eyes crossed and adjust eyes/head tilt until you get 3D perception.

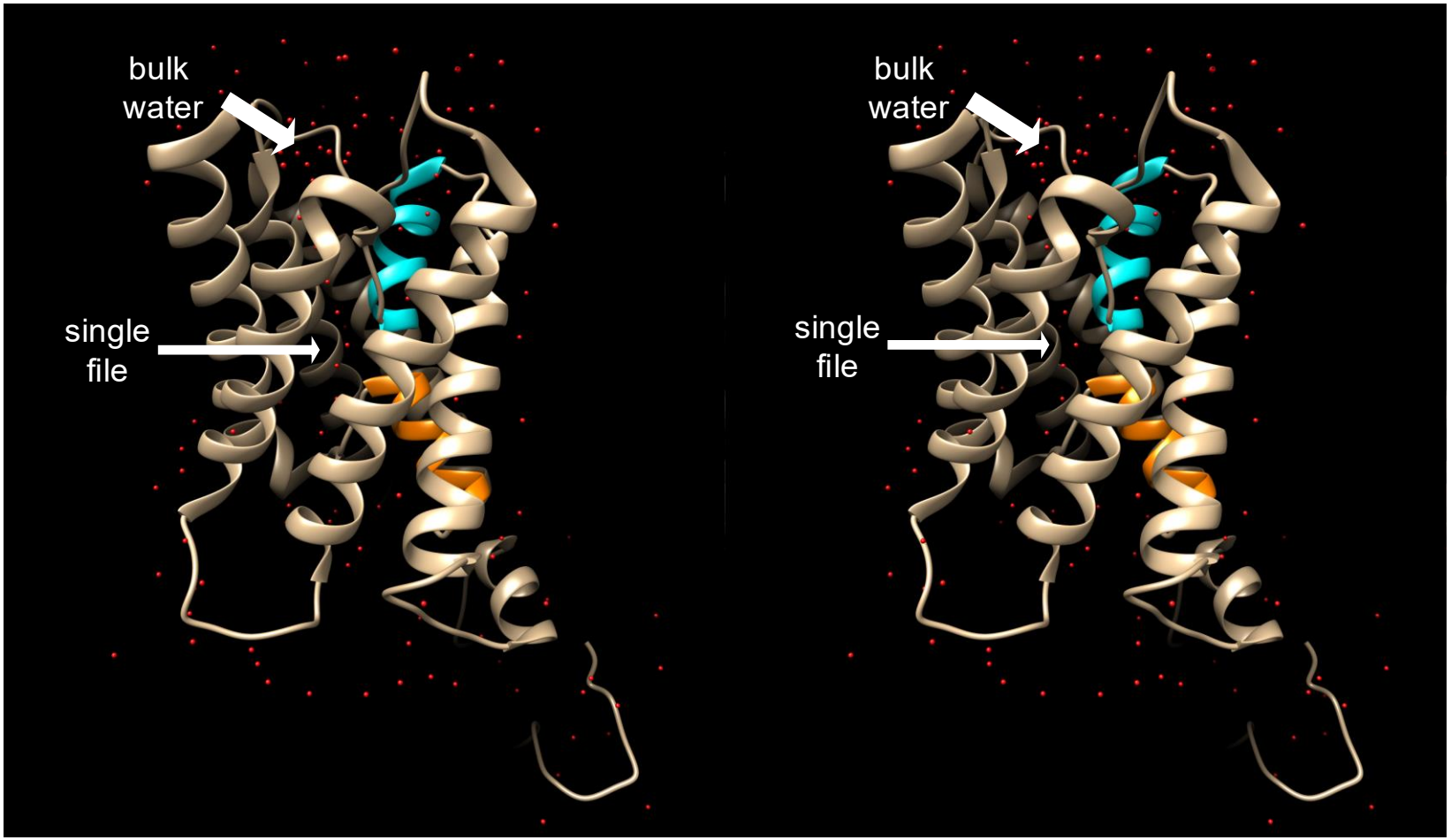


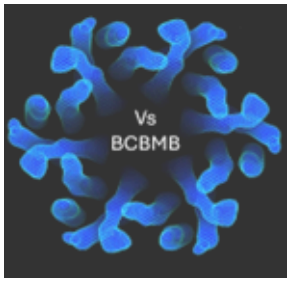
the two "half-helices" are colored orange and cyan



Aquaporins

in this view I also display the water molecules (each red dots corresponds to the oxygen atom of a water molecule).
if you zoom in a little, you can clearly see the single file of water molecules inside the pore. Note, how this single file emerges form the "bulk" water you can see close to the top entrance to the pore.





Potassium Channels

now that you understand how Nature solved the problem of moving water across bilayers at high speed, let's move on to another challenge …..

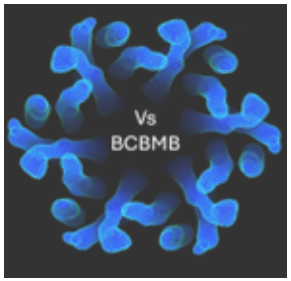
transporting something charged across the membrane.

….

take note: just like water, ions **can cross a membrane spontaneously, but @ only 1-10 ions per second (for potassium),**

the rate is **WAY TO SLOW to sustain life**
(perspective: a single electrical action potential in an average neuron with a total surface area of 2000μm^2 requires between ~12.5 million potassium ions to flow out of the cell ➔ **assume** spontaneous rate 5 ions/s & need 5 million for single nerve impulse ➔ would take ~30 days! ….if every ion that diffuses stays in place on the other side…. = would NEVER happen)

with that in mind … **what do you think are the major differences between the mechanism for transporting water and transporting ions, potassium ions in our example?**



Potassium Channels

what do you think are the major differences between the mechanism for transporting water and transporting ions, potassium ions in our example?

Answer

by far the biggest difference will be that in contrast to water molecules, potassium ions are surrounded by a **shell of water molecules**.

why is this problematic?

- the water layer increases the effective size of the ion
- the hydration shell blurs the "identity" of the ion

what are the consequences here?

for all practical purposes: the ion channel cannot transport the hydrated ion because that would increase the risk of letting other things (like protons for instance) slip through

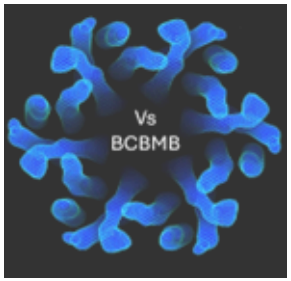
that is

the ion channel needs to have a "changing room" where the ion gets stripped of its water shell so that the channel can "see" what ion it is.

once the ion is stripped naked – the channel needs to provide a substitute for the hydration shell to allow the ion to pass through the channel

➔ that last aspect provides the means to make channels specific because **without the hydration shell, each ion has a very characteristic size, charge and chemical properties**

➔ "all" the channel has to do is to provide a **complementary** surface that provides **weak interactions** to make the ion feel "welcome" and allows passage



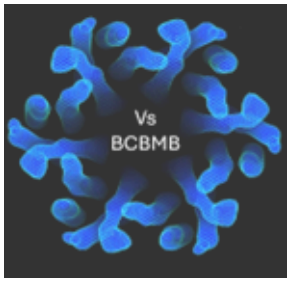
Potassium Channels

➔ "all" the channel has to do is to provide a **complementary** surface that provides **weak interactions** to make the ion feel "welcome" and allows passage

this is where you come in with some original thinking….

…**if you had to design a potassium channel that invites ions in and will let them pass at a high rate**

how would you do it?



Potassium Channels

This is where you come in with some original thinking….

…if you had to design a potassium channel that will let ions pass at a high rate … how would you do it?

Answer

the **first impulse is to put something "negatively charged" into the surface** that interacts with the ion (e.g. one of the negatively charged amino acid sidechains)….

….allowing for an ionic interaction, K^+ ions probably would love to "go there" ….

…and yeah… they sort of would ….
….too much actually…..

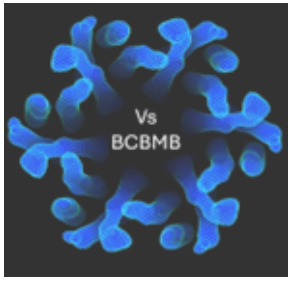
➔ putting outright "negative" charge there would turn the channel into a trap …..

if not that, what then?

…what did we learn from looking at aquaporins ….they replace H-bonds that form within a single file string of water molecules by H-bonds formed with the sidechain of the amino acid asparagine….

…replacing "like for like"

…let's look at what stabilizes the hydration shell in K^+ ions



Potassium Channels

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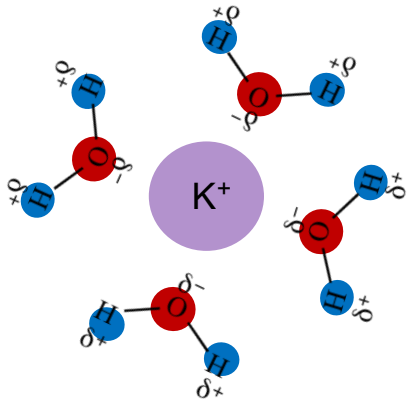
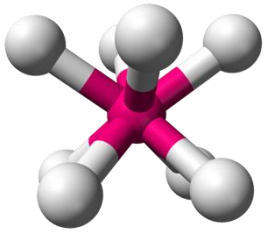


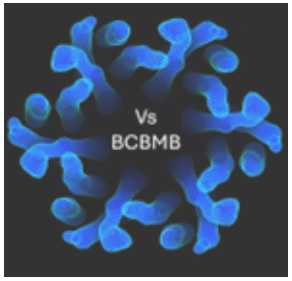
in water, potassium ions are stabilized by the negative end of the water dipoles

more specifically – potassium likes to surround itself with ~6-8 water molecules



➔ ideally – you want to replicate this using protein components ….

…**test your memory … what part of a protein would give easy access to "dipoles containing oxygen?"**



Potassium Channels

….so let's look at what stabilizes the hydration shell in K^+ ions

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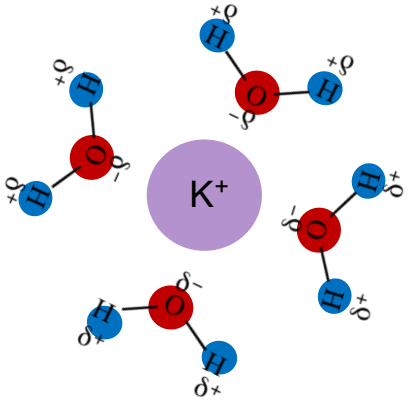

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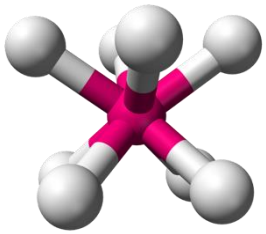

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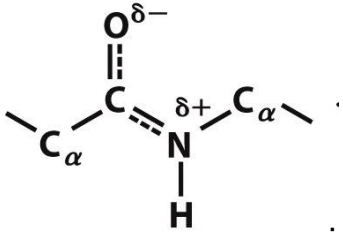


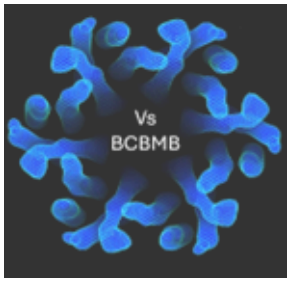
Answer

one prominent protein feature that fits the bill is the peptide bond!
Apart from that: sidechains that contain –OH (hydroxyl groups)
➔ serine, threonine, tyrosine (S, T, Y)

in aquaporins we saw that "NPA-motifs" at the end of two half-helices set up the alternate hydrogen bond donors …. so…

….what if potassium channels use the protein backbone and some of the -OH containing side chains to mimic water??

HOME RUN … meet KcsA…the first K-channel whose structure was solved



KcsA - the Best Known Ion Channel (K^+-Ions)

- like Aquaporins a tetramer

BUT

- passageway for potassium ions is at interface between subunits

the monomer is made of

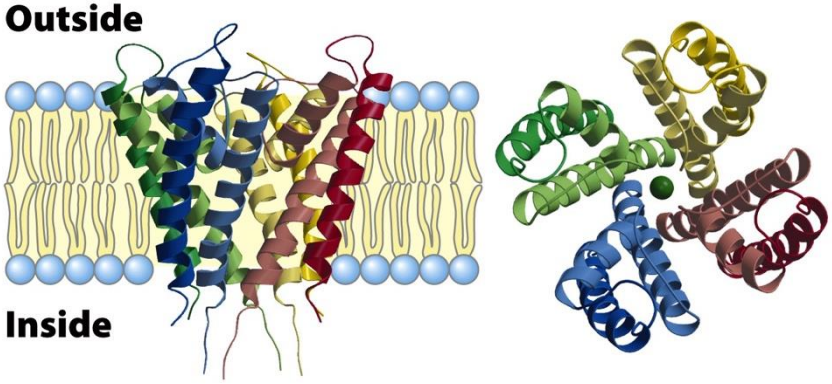
- 2 transmembrane helices (TM) +
- a short pore helix (akin to the "half helices in aquaporins) that in the context of the tetramer creates an extracellular vestibule +
- an extended stretch of peptide containing the hallmark sequence motif **"TVGYG" that in its generalized form (TxGYG) serves as** "selectivity filter" in all K-channels (the part of the channel that assures that the right ion is transported)

note: TVGYG = threonine-valine-glycine-tyrosine-glycine

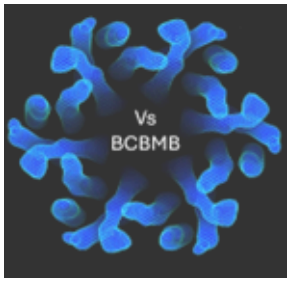
T, Y = two of the amino acids that have -OH groups in their sidechain!

G has no sidechain BUT is extremely flexible, allowing the polypeptide to adjust locally when ions are conducted

V (or **I** or **L** in some channels**)** has a hydrophobic sidechain that enforces a certain local structure of the selectivity filter, helping it to maintain a conformation that is optimal for accommodating potassium ions



how do these elements come together to form a potassium conductive pore?



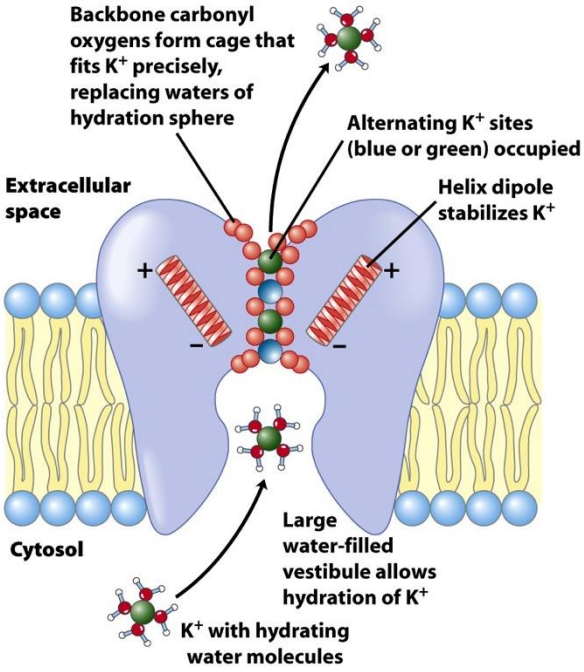
KcsA - the Best Known Ion Channel (K^+-Ions)

how do these elements come together to form a potassium conductive pore?

let's start with a cartoon summary ….

to understand this, you want to take for now that the
default direction for flow is inside → outside
(because the intracellular potassium ion concentration is very high @ 140mM, compare to ~5mM outside the cell)

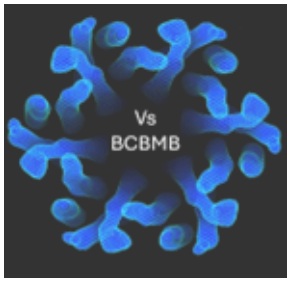
starting its journey on the inside of the cell … **transport** across the bilayer **starts** with a
BIG SURPRISE ….



the channel protein structure forms an **aqueous chamber that reaches all the way half across the membrane !!!**
this is HUGE …because it "**cuts in half " the thickness of the bilayer** that needs to be traversed" & **K^+-ions can get to the short selectivity filter/transport region simply by diffusion** without any issues…

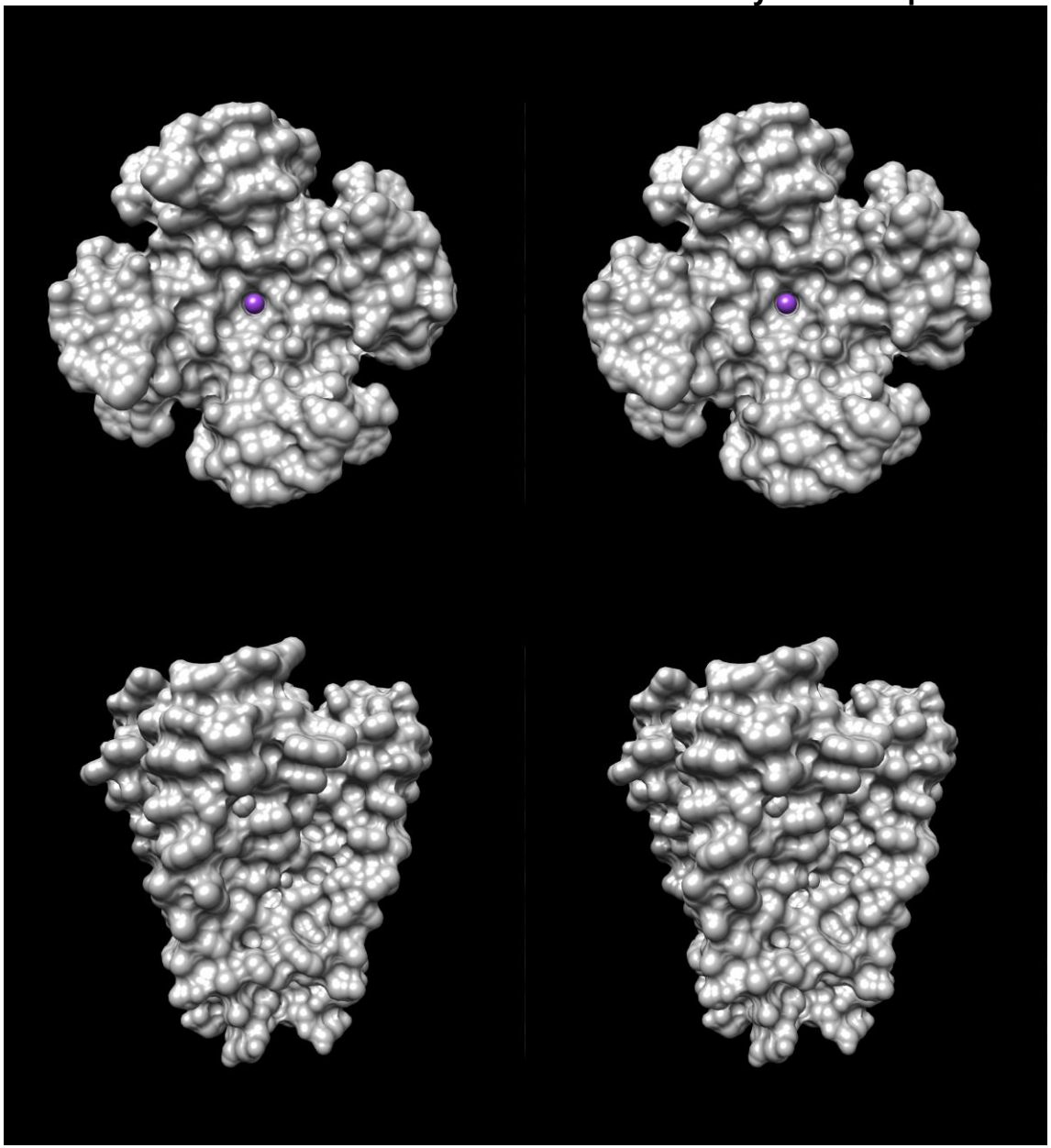
as the potassium **approaches the narrow selectivity filter, it feels a weak attraction by the negative end of the helix dipole** that is associated with the short pore lining helices (see PROTEIN chapter, slide 34)

it is at that entrance that the ion loses its hydration shell and enters the **selectivity filter where backbone carbonyl oxygens and –OH groups from T,Y sidechains create a suitable coordination environment that allows the ions to move** before exiting the channel on the extracellular end (all driven by the free energy difference that is stored in the concentration gradient across the membrane)



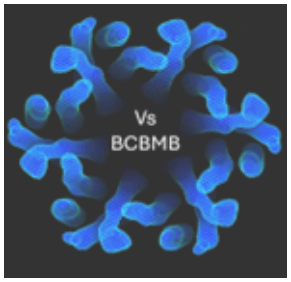
KcsA

now let's look at the **actual structure in cross-eyed stereo pictures.**



top view of surface from extracellular side
(purple sphere is potassium ion)

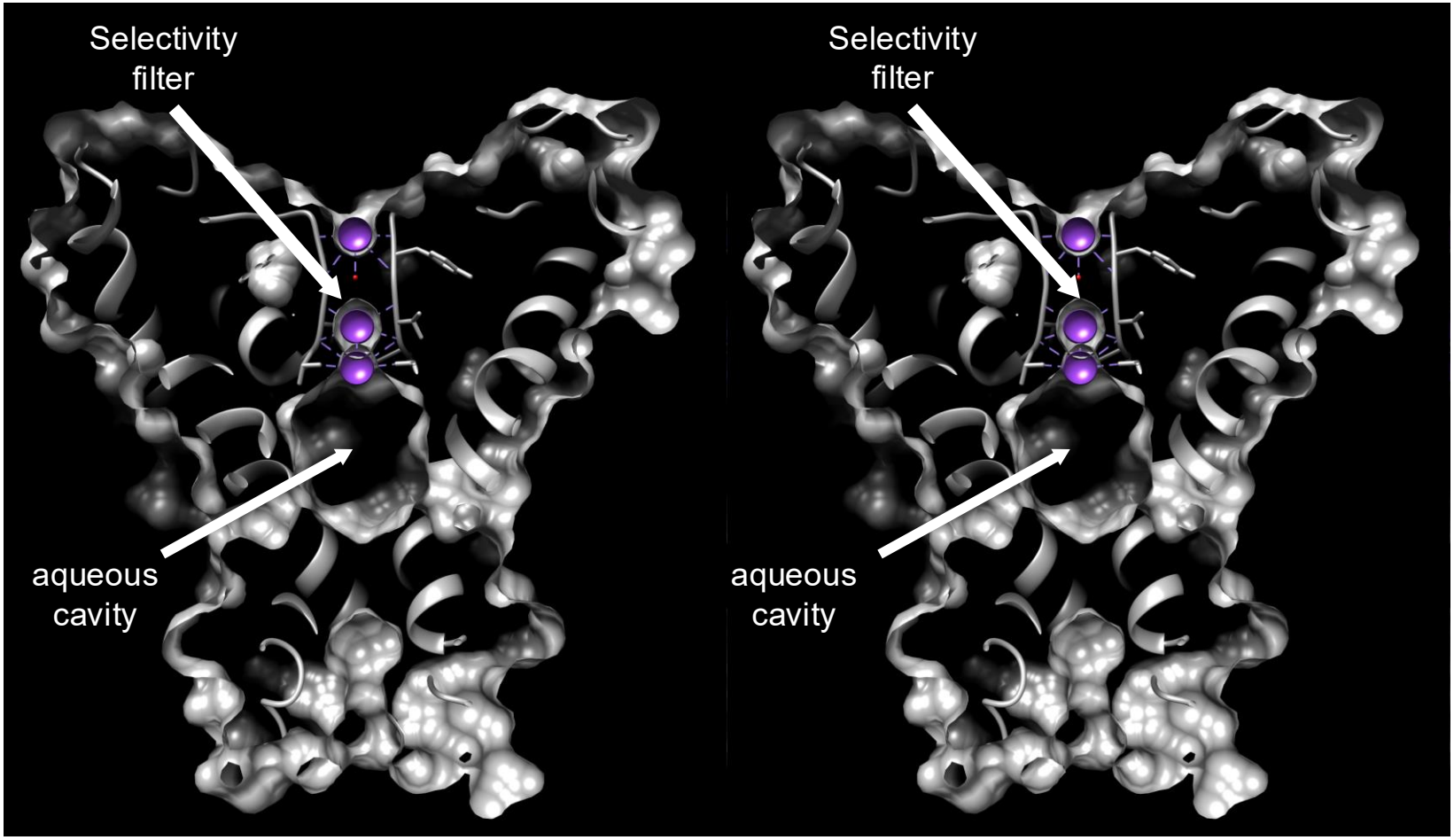
side view of surface
(parallel to membrane)

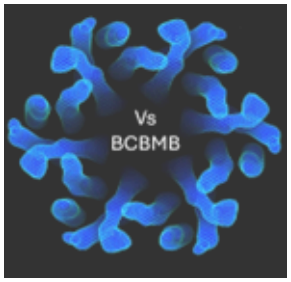


KcsA

cross-eyed stereo pictures.

side view of passageway
note: the structure resolved 3 potassium ions and 1 water molecule
(red dot) in the selectivity filter

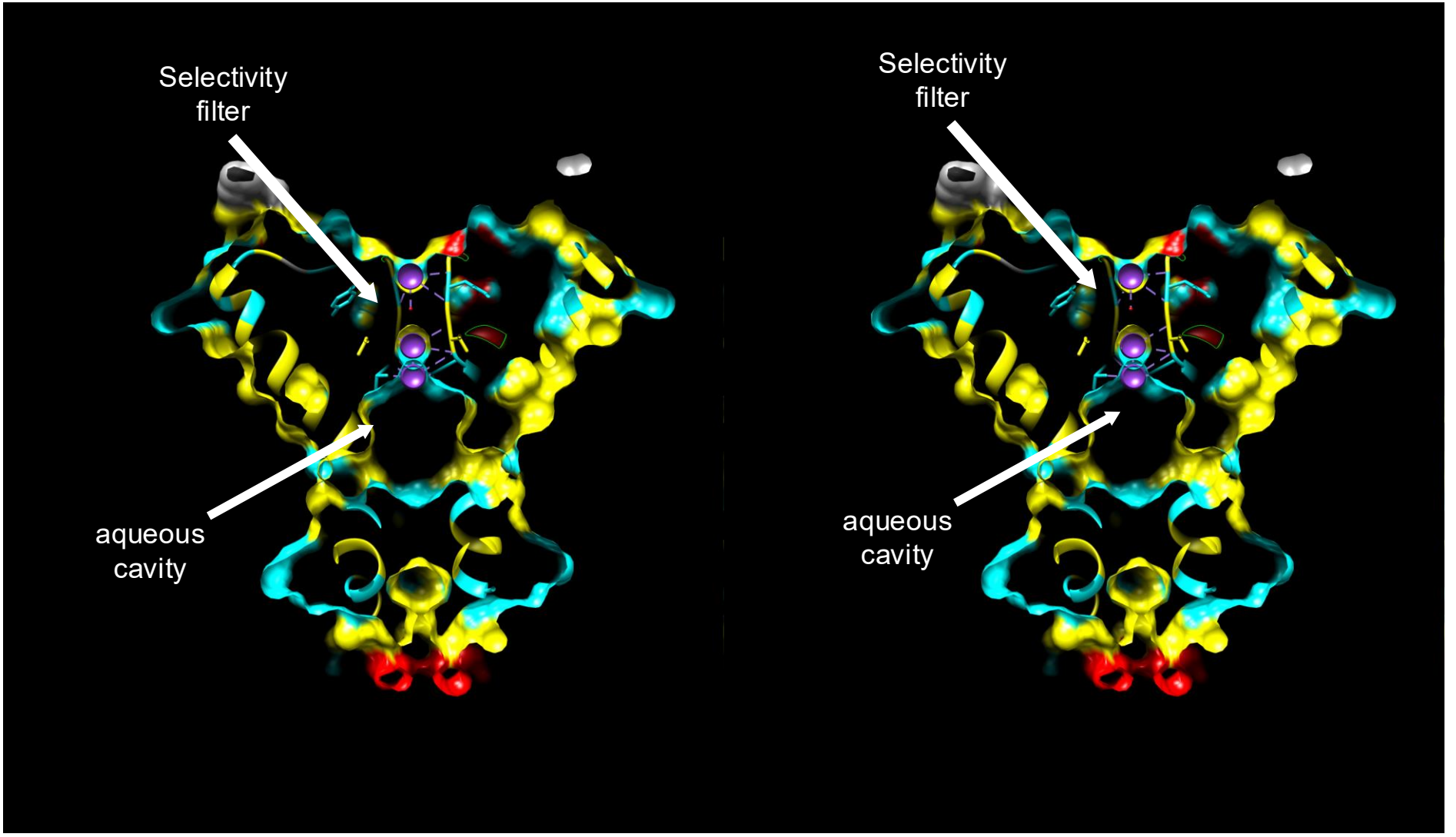


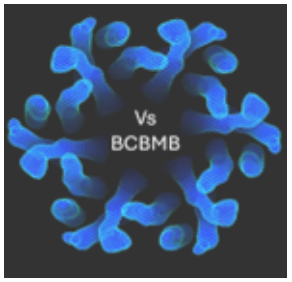


KcsA

cross-eyed stereo pictures.

side view of passageway
chemical Properties: red = negative, cyan = hydrophilic, yellow = hydrophobic





KcsA – Conduction Mechanism

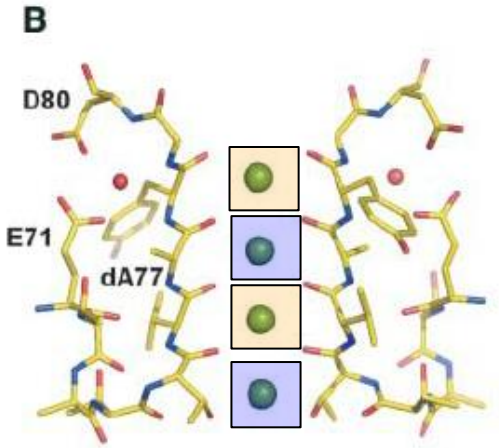
inspection of the experimentally determined structures revealed that the channel provides 4 distinct coordination sites with ~2 ½ of them occupied by potassium and one by water.

thinking about this outcome … non-integral number of ions in the selectivity filter makes no sense ….

to understand this peculiar observation, you need to consider that the experimental "protein density map" is the average of millions of molecules = if K^+-ions were in different positions across this population, then an "odd" overall average distribution of ions within the selectivity filter is not that "weird" …just needs a little more effort to interpret

one thing was clear though: there was one water molecule in the filter = you won't find a state where three or even all four consecutive sites are occupied by potassium, ions – why?

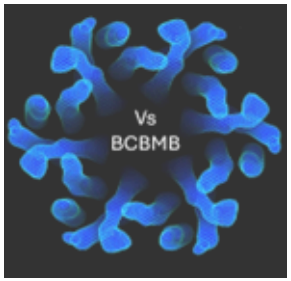
Answer: electrostatic repulsion between the residual positive charges of K^+ (= the peptide dipoles only partially compensate the charge of the cation)



Valiyaveetil, F et al (2006) Science 314:1004-7

putting all this together the following summarizes the key principles of potassium conduction by K-channels

- picture shows 4 solutes (green circles) in the filter occupying the four defined coordination sites.
- during conduction only 2 are occupied by ions (orange overlay)…these ions are separated by water molecules (blue overlay)
- conduction follows a "bumper-to-bumper" mechanism where the whole column of molecules moves "up or down" one position at a time



Speed and Specificity

with the structure and basic mechanism of potassium ion transport covered this leaves only three questions.

1. **what is the rate of K^+ conduction?**

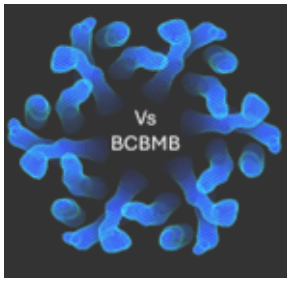
…pick a number …. (flashback: spontaneous rate is 1-10 ions/s)

2. **does the channel ever make a mistake transporting something else instead?**

….what do you think? …..

3. **how is the flux of ions regulated?**

…regulation is crucial (if you don't know, you will understand shortly why that is). Do you know how ion flux through KcsA and channels in more general is regulated?



Speed and Specificity

with the structure and basic mechanism of potassium ion transport covered, this leaves only three questions.

1. **what is the rate of K^+ conduction?**

~10,000,000 ions/s (10^8)!
that is near the diffusion limit (= cannot go much faster)

2. **does the channel ever make a mistake transporting something else instead?**

yes … like every biological (or mechanical, or behavioral process) … even an almost perfect device makes mistakes

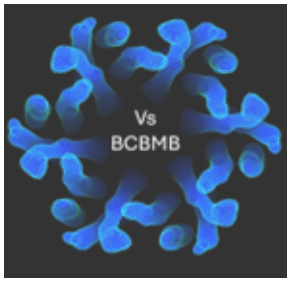
…in this case
for every 10,000 K^+, the channel will pass 1 Na^+

that is an astounding accuracy and **really curious** once you consider that @ r=0.95Å the **sodium ion is smaller than** the **potassium ion** (r=1.33Å)

→ how is that possible???? It can conduct the larger ion .. why is the smaller not just slipping through??

the short answer is: the selectivity filter is not flexible enough to compensate for the size difference
= it cannot bring the carbonyl oxygens close enough to accommodate the sodium … isn't that weird?

not really, and here is why: reaching something that is close to you … you can get to it if it is at arms length …beyond that ..you'll stretch and contort yourself …. but from a certain distance…no matter how hard you try … it is just too far for you to reach (often artistically exploited in movies …..) – same here – the polypeptide has a certain tolerance …but beyond that .,..it can't reach, even if it is only a fraction of an Å.



K-Channel Regulation …. Detour

how is the flux regulated?

for you to understand the answer, we need to make a detour, looking at something probably all of you have heard about …..

membrane potential

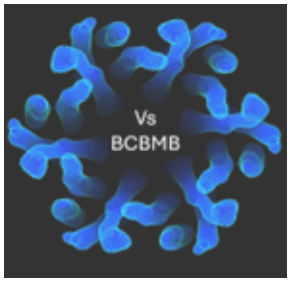
already mentioned earlier (slides 21 & 23), we want to fill in more detail because it will help big time with understanding how the function of certain channels is regulated, and how cells solve transport challenges that require to move molecules against their physiological concentration gradient

(if you would like to skip this and continue with the narrative about channel regulation without this interruption … go to slide 57)

what is Membrane Potential?

Answer: a voltage difference across cellular membranes with

or in other words: **membranes are biological "capacitors",** where the aqueous extra- and intracellular environments act as conductors that are separated by an insulator (the bilayer)



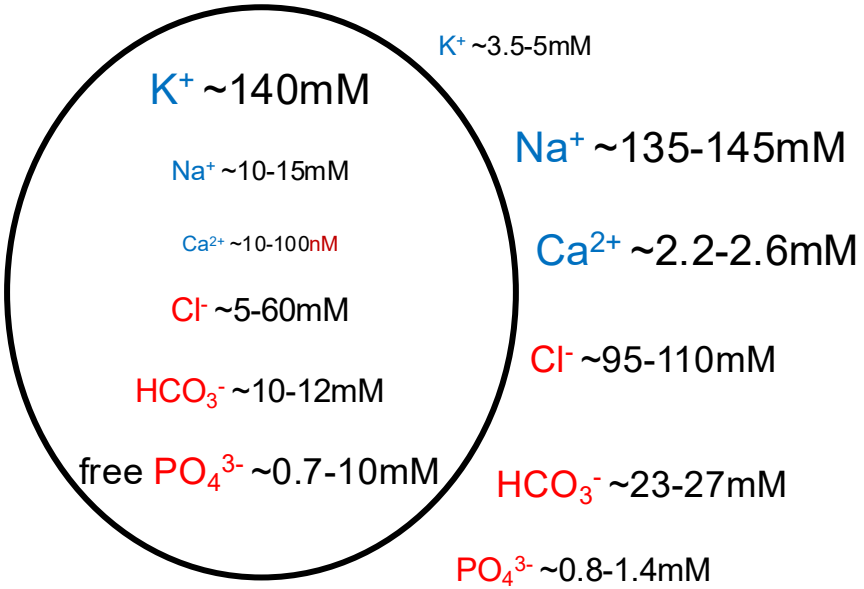
K-Channel Regulation …. Small Detour

before diving into regulation of (K)-channel activity, we want to take a very brief look at something probably all of you have heard about

…..

membrane potential

we already alluded to this earlier on (slides 21 & 23). We now want to fill in a little more detail because it will be important for understanding how the function of certain channels is regulated, and how cells solve transport challenges that require to move molecules against their physiological concentration gradient



what is Membrane Potential?

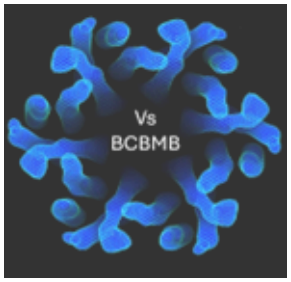
Answer: a voltage difference across cellular membranes with

or in other words: membranes are biological "capacitors", where the extra- and intracellular environments act as conductors that are separated by an insulator (the bilayer).

in order for a voltage to exist, there must be a charge imbalance between the two sides of the membrane.

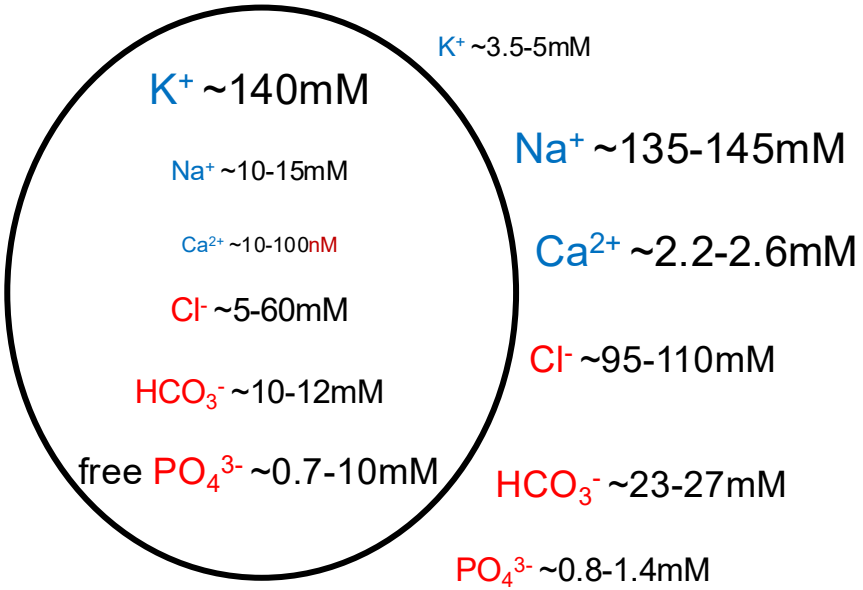
➔ a good starting point for understanding membrane potential is to just inspect the concentrations of ions on both sides of the membrane

conclusion: K^+ is high intracellular, while Na^+, Ca^{2+}, Cl^- are high extracellular.
look closely, and you will notice that the **concentrations of cations and anions inside the cell don't add up**.
is that the imbalance we are looking for? Unfortunately, it is not because inside cells, negatively charged protein surfaces balance the excess positive charges



Membrane Potential - Continued

if it is not the glaring **inorganic** cation:anion imbalance that causes a transmembrane voltage, then what else could be the reason for it?



Answer

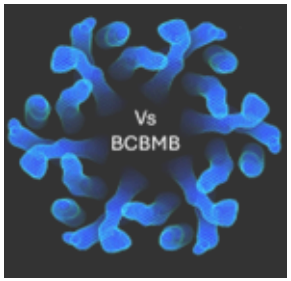
…the answer comes from cryptic comments that were embedded earlier (but not "pitched" in this context)

while membranes ARE largely impermeable to ions, there IS some residual permeability that allows ions to cross.

the membrane permeability differs for different ions because of the nature + amount of charge & their size

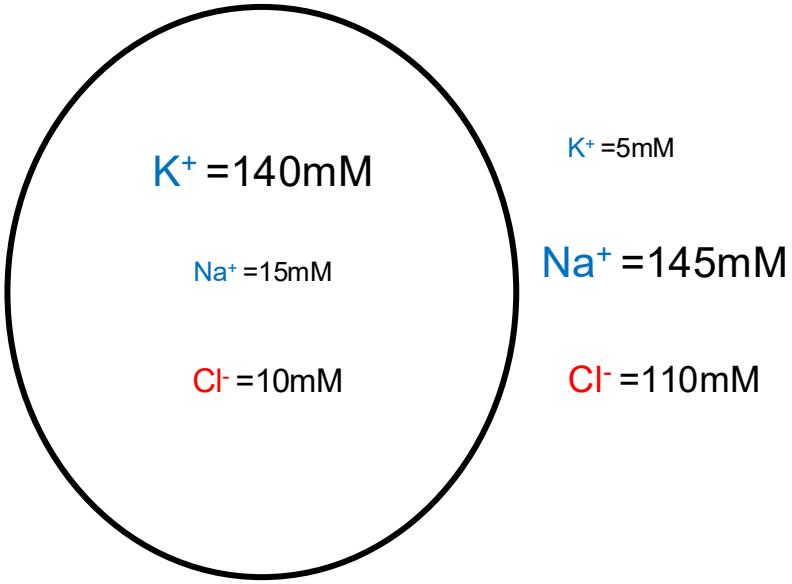
Ion	Permeability (P) in cm/s
Potassium (K^+)	1×10^{-12}
Sodium (Na^+)	1×10^{-14}
Chloride (Cl^-)	1×10^{-11}
Calcium (Ca^{2+})	1×10^{-14}

looking at the concentration gradients: Cl^- and Na^+ would want to go inside, while K^+ would like to leave the cell.
would this, over time, lead to a voltage gradient?



Membrane Potential - Continued

Would this, over time, lead to a voltage gradient?



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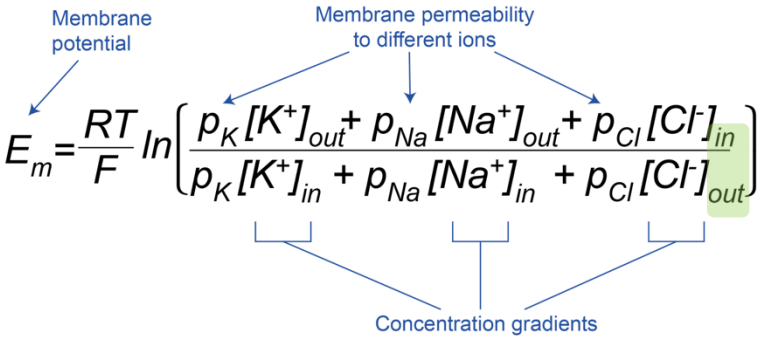
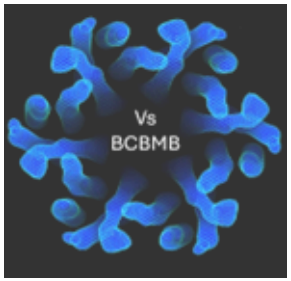
Answer
yes – it would.

to be more precise: you can calculate the membrane potential if you know the permeability coefficients and concentrations on both sides.

the equation to do that is called the Goldman-Hodgkin-Katz equation



Answer
using only the most significant players and the values shown ….the transmembrane voltage in an unassisted case is
V= -65.5mM => inside is negative
(note: this is because for the negative Cl^- the "in/out" ratio is reversed in the equation)



Membrane Potential - Continued

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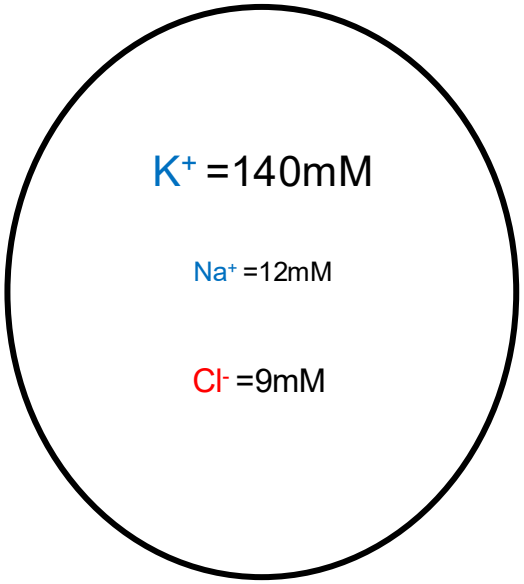

K^+ =5mM

Na^+ =145mM

Cl^- =110mM

let's compare this to the voltages that would be caused if we consider each ion separately

For K^+ only V= -88.3mV

For Na^+ only = +66mV

For Cl^- only = -66.3 mV

→ the voltage for the unassisted case basically equals the chloride equilibrium potential, which makes sense because the Cl^- permeability is higher than that for the other ions.

all good then?

sadly …. no … there is another twist to the story …

……

Ion	Permeability (P) in cm/s
Potassium (K^+)	1×10^{-12}
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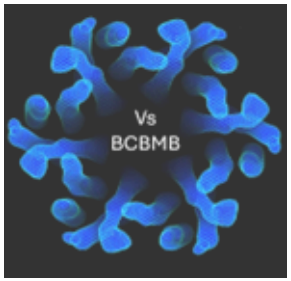

Membrane Potential - Continued

unassisted case is
V= -65.5mM => inside is negative

let's compare this to the voltages that would be caused
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For K^+ only V= -88.3mV
For Na^+ only = +66mV
For Cl^- only = -66.3 mV

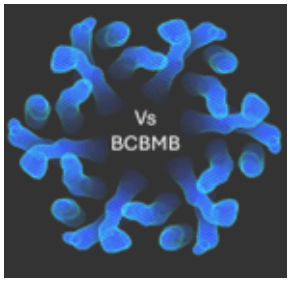
All good then?
Sadly …. no … there is another twist to the story …

Cell Type	Resting Membrane Potential (mV)
Skeletal muscle cell	-90
Neuron	-70
Smooth muscle cell	-60
Photoreceptor cell	-40
Erythrocyte	-8.4
Chondrocyte	-8.0

measuring the membrane potential in resting cells, you will find that **different cells have different potentials!**

moreover, none of those is "just the chloride"/"unassisted" potential.
skeletal muscle is closer to the K^+ potential, Neurons are inbetween the K^+ and the "unassisted" potential, while the rest are lower than the unassisted case …

…**something is missing here….**
…do you have any ideas what it could be? ….



Membrane Potential - Continued

unassisted case is
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For Cl^- only = -66.3 mV

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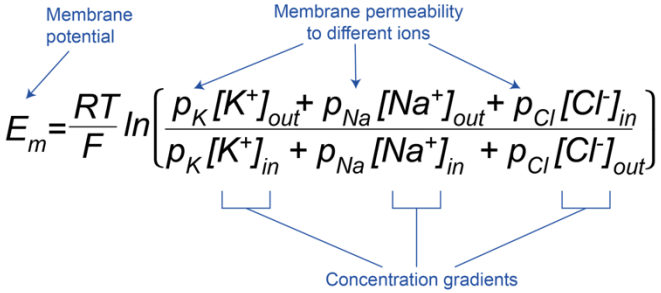


something is missing here

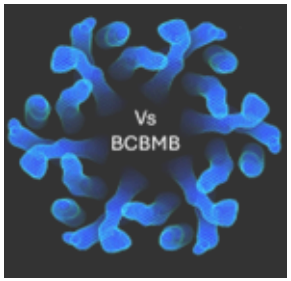
the equation for the potential gives you a clear hint … because it tells you what factors can cause potentials to be different from each other:

either the ion concentration differences are different in different cell types

or

something is "messing" with the permeability coefficients…

which one do you think it is?



Membrane Potential - Continued

unassisted case is
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Skeletal muscle cell	-90
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Chondrocyte	-8.0

Membrane potential

Membrane permeability to different ions

$$E_m = \frac{RT}{F} \ln\left(\frac{p_K[K^+]_{out} + p_{Na}[Na^+]_{out} + p_{Cl}[Cl^-]_{in}}{p_K[K^+]_{in} + p_{Na}[Na^+]_{in} + p_{Cl}[Cl^-]_{out}}\right)$$

Concentration gradients

either the concentration differences are different in different cell types
or
something is "messing" with the permeability coefficients…
which one do you think it is?

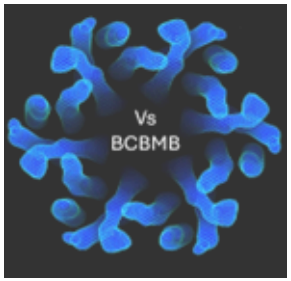
the second, hands down! why?

- **changing the ion concentrations** in different cell types/tissues **would change the ENTIRE chemistry of each cell type (**impossible).

changes in permeability coefficients, on the other hand, are easy to accomplish because

- **the permeability coefficient depends on the thickness of bilayer and the type of lipids used.** as we learned in the LIPID and MEMBRANES Chapter (slides 80 & 87), the lipid compositions of membranes are quite different for different organelles, and tissues/cell types so that is a match here…

- thinking about **membrane transport processes** …and K-channels in particular …that also would give us a great and **"easy" handle on manipulating permeability** (for any ion, in fact**)**!



Membrane Potential - Continued

- the permeability coefficient depends on the thickness of bilayer and the type of lipids used.

- thinking about **membrane transport processes** …

turns out: both these mechanisms are exploited to tune membrane potential to the cell's need.

most interesting to us here is a family of integral membrane proteins that function as
"leak channels" for potassium ions.

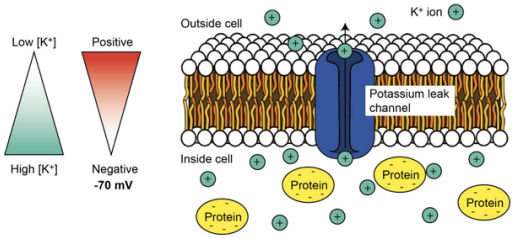


Fig 3.14. Positively charged potassium leaving the cell sets up an electrochemical gradient. The inside of the cell is negative with respect to the outside of the cell, stopping more potassium ions from leaving.

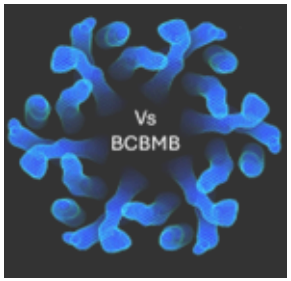
as the name suggests, these K^+-channels allow a "higher than natural" passage of K^+-ions across the membrane, which causes excess negative charge to build up inside the cell.

In terms of permeability coefficient: the **presence of leak channels increases the membrane permeability for potassium ions ~100,000- to 1,000,000 fold** (making it higher than Cl^- permeability)

➔ **explains how some cells can be at or close to the K^+ potential of -88mV**

moreover, **differences between cell types** can easily be explained by the fact that humans have **15 different potassium leak channels**, known as K2P-family. Each of these channels has **slightly different properties** in terms of how much leakage they allow and what factors (like mechanical stretch, pH, lipid composition of membrane, or temperature) affect their activity.
➔ different cells can establish different membrane potentials by using different "mixes" of leak channels.

the K2P-family is a great example for **redundancy** (illustrating how "tweaks to a common template structure" [in this case] allow for fine tuning of properties to need a cell's needs)



K-Channel Regulation

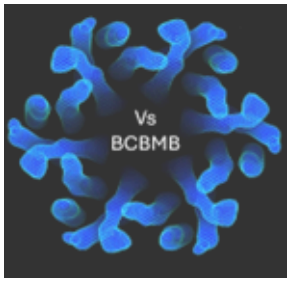
the existence of leak channels for potassium brings us back to the question we posed some 11 slides prior:

how is the flux of ions regulated? This time, the question comes back with real urgency …. why?

for starters: if left to themselves and without means to control them, potassium channels would **erase** the potassium ion gradient across cells (if that were to happen, you die)

beyond that, the fact that cells use different types of K-channels in support of different functions also is a **strong indication** that cells very carefully control how much K^+ can cross the membrane and when.

if you had to design this – how would you go about it?



K-Channel Regulation

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if you had to design this – how would you go about it?

Answers

- **the simplest (and most intuitive) way would be to equip the channel with a physical "gate" which is cued into a physiological trigger that causes the gate to "open" or "close"**

ironically: all channels, **except for leak channels and aquaporins**, have physical gates that control open/conductive – closed/non-conductive states of the channel

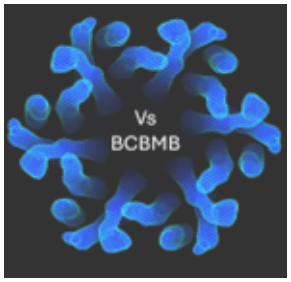
Leak channels, on the other hand, are **constitutively open**…

…since you are alive this **implies that "open" is not the same as conductive.…**

…how weird is that? Initially it is, but – yes, there is a second mechanism that controls ion flux

- this second mechanism is called **desensitization**

which in general terms refers to a state of a channel (or receptor) where the channel (or receptor) is no longer responsive to the stimulus = stops conducting ions for channels; stops signaling in case of receptors



K-Channel Regulation

desensitization
= the ability to stop doing what you should be doing despite the stimulus still being there is an **"auto shutoff" safety mechanism of the channel**

at first this does not really seem to make a lot of sense….

…..and this will **forever stay that way …UNTIL you start to think about all of it at the small scale of cellular environments**

point in case: if you think of an average neuron (nerve cell) … it has a total surface area of ~2,000μm^2

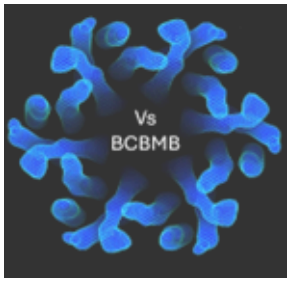
if this cell has a membrane potential of a typical neuron (-70mV), then **passing ~4,370 K$^+$-ions/μm^2** (that is less than 0.00001% of what is "available " inside the cell) **will COMPLETELY erase the membrane potential rendering the neuron non-functional**

given the flux rates they can achieve**, a SINGLE LEAK channel** in that 1 μm^2 area **would accomplish this in ~0.5ms** if there was no way of regulating the flux in absence of a physical "gate".

…. curious … how many leak channels are there in that area? … depends on the type of neuron and where you look **… ~0.5 to 3,000 per μm^2**

what???!!!?
…. if 1 leak channel per μm^2 is potentially lethal …having >1 per μm^2 is insane –
how does this help?!?

turns out, having **only one (or less) per μm^2 everywhere** on the neuron is what **would kill** you because single molecule behavior is "random" = this would "pop open" at some point, killing you before it closes
…. this is crazy, **what is going on here??**



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turns out, having only one (or less) per μm^2 is what would kill you because single molecule behavior is "random" = this would "pop open" at some point, killing you before it closes …. this is crazy, **what is going on here??**

this is one of these moments where you come to realize that life is possible only because of "ensembles" ("Not so typical introduction to cells" slide 17; "How Do Molecules See-Pt2, slide 23) = **while you cannot accurately control ONE channel, YOU CAN control the overall output from a POPULATION of channels ….**

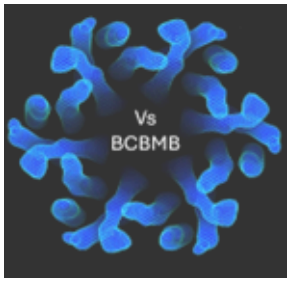
….IF regulating their function includes an "auto shutoff" (= desensitization) as control mechanism

why?
desensitization puts most channels into "deep sleep"

… occasionally they wake up and kick into action

….but even if they do, they may not conduct at full speed (like you taking some time to wake up, or cars going from 0–60mph) …for a myriad of reasons (molecular vibrations, tiny local changes in conformation in or at the selectivity filter, other molecules/ions blocking the entrance/exit … and on and on)

= conduction is **stochastic,** not deterministic
= you only will get a "smooth" and "predictable" response if you employ a population of channels and design other processes around that population output



K-Channel Regulation

let's put all of what we looked at for the "Leak Channels" into a summary ….

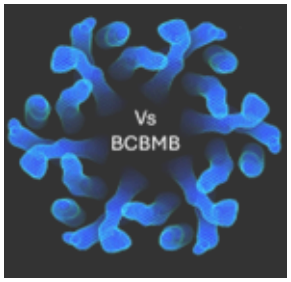
Comparison of Time Scales for Leak Channels (K2P)

Measurement	Time Scale	What it Represents	Biological Reality
Actual Open Time	**Microseconds (μs)**	The duration of a single, uninterrupted "flicker" of ion flow.	This is the "true" speed. The channel is constantly oscillating between "on" and "off" due to molecular vibrations.
Mean Open Time	**1 to 10 Milliseconds (ms)**	The statistical average of a "burst" of openings	This is what determines the resting potential. Even though it's made of tiny flickers, the cell "feels" it as a single open event.
Desensitisation Time	**100 ms to Seconds (s)**	The time it takes for the channel to enter a "deep sleep" (refractory) state.	This is the safety switch. It prevents the channel from staying open too long and draining the cell's ion battery.

OK – this looks more reasonable …an actual open time in the µs range is close to viable … but there is **still something missing to resolve the puzzle completely**.

there actually are several more layers here, but the most important is that **K^+ ions do not just leave cells … they are constantly brought back in too** by another transporter, the Na^+/K^+-ATPase (slide 124)

➔ **it's the balance between opposing processes that makes it all work out**



K-Channel Regulation

now that you understand the fundamental components of channel regulation

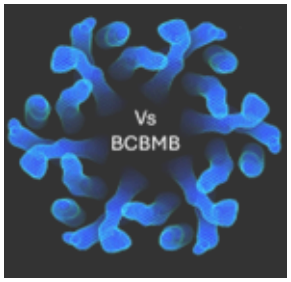
GATES
&
DESENSITIZATION

how does this all look for KcsA, the small bacterial K-channel that serves as prototype for the structural design principles of K-channels

KcsA exhibits **gating hysteresis**, meaning it "remembers" its prior state. The energy required to open the channel is different from the energy required to close it because of the stable "open-inactivated" state. National Institutes of Health (NIH) | (.g... +1

Kinetic State	Activation Gate	Selectivity Filter	Status
C/O	Closed	Conductive	Resting/Closed
O/O	Open	Conductive	Open & Conducting
O/I	Open	Inactive (Collapsed)	Inactivated
C/I	Closed	Inactive (Collapsed)	Deep Closed (Inhibited)

compared to leak channels, the **big difference here** is that in addition to desensitization, **KcsA also has a true gate that can open and close. What is the trigger?**



K-Channel Gating

in KcsA, the gate resides inside the cell and is closed by default. The gate opens if it senses a drop in intracellular pH

…but wait – isn't intracellular pH buffered and maintained?

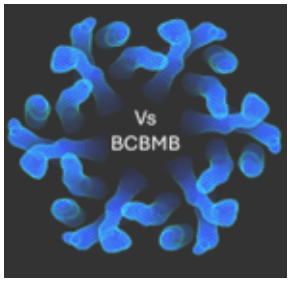
Answer:
yes….under normal circumstances….

however
there are a few reasons that can imbalance pH homeostasis

- **extracellular pH drops below 5.5** (at that point, many bacteria cannot properly maintain their internal pH any longer; letting K+-ions escape in this situation is part of a complex response that involves several processes as the cell tries to regain control)
- **The presence of weak organic acids outside the cells** can cause intracellular acidification because some of these acids can diffuse across the membrane in a neutral state and then release a proton once exposed to neutral pH inside.
- Lastly…**sudden high metabolic activity can throw off intracellular pH** because incomplete oxidation of fuels leads to accumulation of acidic end products

beyond gating in this one specific example, **KcsA,**
the combination of "gates" and desensitization creates powerful constraints on channel activity that allow the safe local clustering of channels (up to 20,000 channels per μm^2 = so close they physically touch each other!) in regions where cellular function requires it.

this leaves the question of what makes channels so extremely useful in supporting cellular functions?



K-Channel Gating – A Classic Example

what makes these channel molecules so useful in supporting cellular functions?

Answer
redundancy

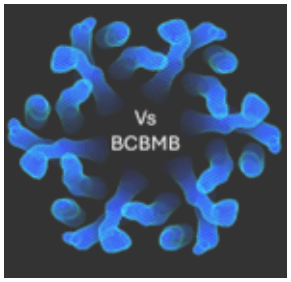
meaning?
once you designed a selectivity filter for an ion (K^+, Na^+, Ca^{2+}, or Cl^-), you can "functionalize it" by adding gates that respond to different triggers, and fine tune desensitization kinetics through small changes in the structure

to digest this and to generate a concrete understanding, we want to look at a "classic" example for a biological process that is build around tuning of channel function …..

action potential
you probably know this already …

action potential is a rapid and temporary local change in membrane potential
(here you see why we made an effort to cover it in some detail earlier on...it really is THE BEST illustration for how channel function, and how mechanisms are exploited to reproducibly generate an exceptionally precise event)

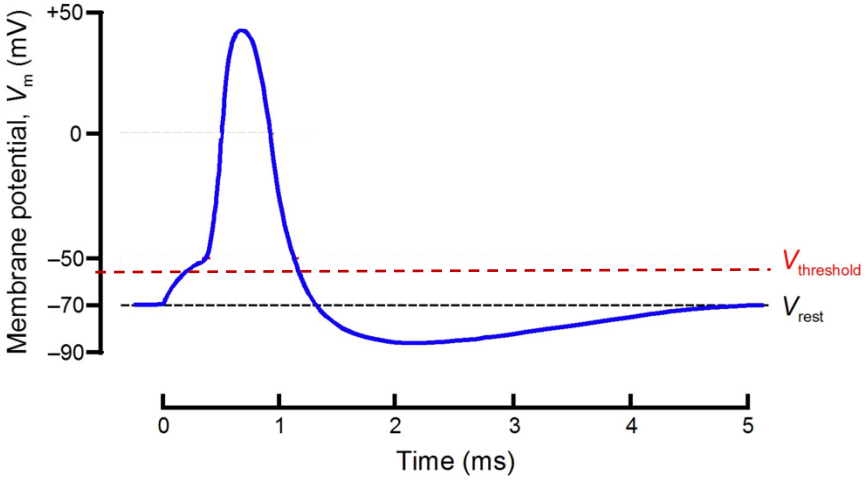
action potentials are key to the function of neurons and muscle fibers.
in neurons, action potentials are generated and propagated to carry electrical signals in the nervous system.
in muscle, action potentials trigger muscle fiber contraction in response to an electrical stimulus issued by the nervous system



K-Channel Gating – A Classic Example

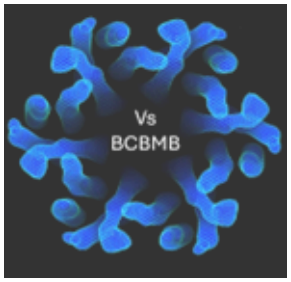
action potentials are key to the function
of neurons and muscle fibers.

Feature	Neuronal Action Potential	Muscle Action Potential
Primary Goal	Transmit information	Trigger calcium release for work
Duration	Very fast (1-2 ms)	Slightly longer (2-5 ms in skeletal)
Propagation	Along the axon to a synapse	Along the sarcolemma into T-tubules

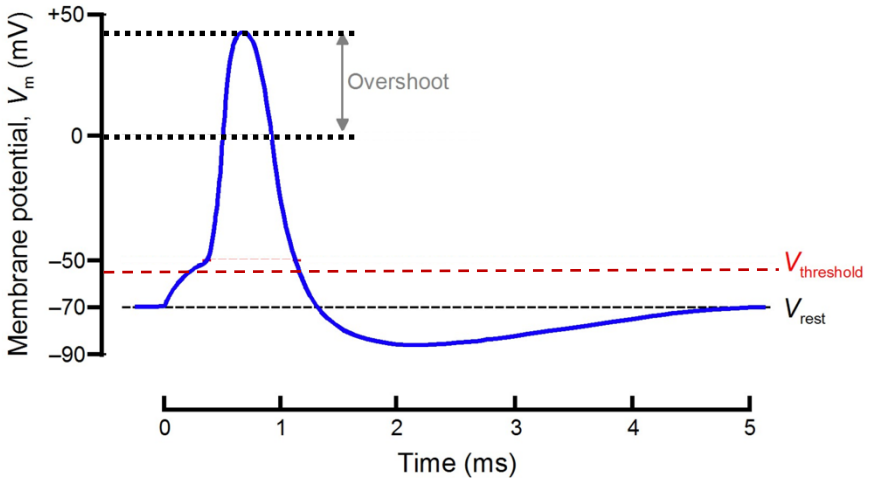


among students, this curve is –
perhaps – the best known curve
(hmmm …apart from Michaelis-Menten Kinetics)

even if this is hard
pretend you have never seen this
before …what do you see?…describe
this in your own words



Channel Gating – A Classic Example

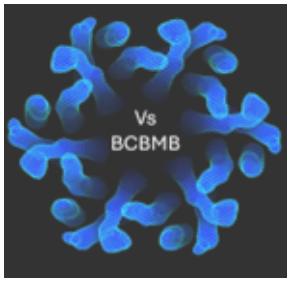


overall shape: an asymmetric pulse/"spike" of membrane potential (mV, linear) **vs time** (ms, linear)

- going from left to right … the cell membrane **sits at -70mV** … (V_{rest})
- it **gradually becomes more positive** (terminology: begins to **depolarize)**
  - **at -55mV** the **gradual depolarization slows before**
- depolarization **rapidly accelerates**, completely **erasing the membrane potential**,
- and **overshoots** (reaching about +40mV) effectively **reversing** the membrane potential
- after peaking, membrane potential promptly **begins to fall** (terminology: begins to "**repolarize**"), without any special events,
- until it **bottoms out at a potential that is more negative than -70mV** (terminology: **hyperpolarized**)
- lastly …the membrane potential slowly **returns to the -70mV baseline**.

- overall: this is an **"all-or-nothing"** event once **-55mV** is reached = there is a **threshold**

why make you look at this and put it into your own words (if you did engage)?



Channel Gating – A Classic Example

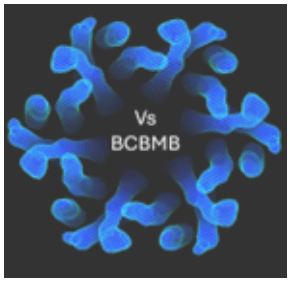
why make you look at this and put it into your own words?

to break the cycle of simply memorizing things …

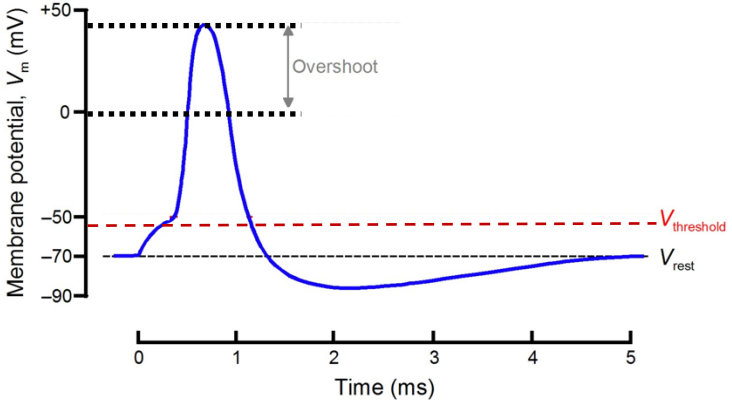
as you look at it, you realize that each of the statements poses a question…
finding initial answers to those questions is not difficult and creates a clear narrative where everything is causally connected
(apart from two "trivia" facts you need to know)

based on what we covered, you can answer all but one of these questions

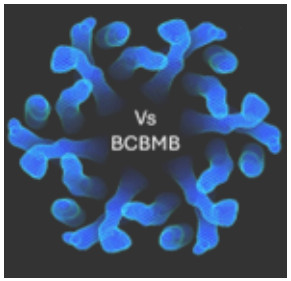
…give it a *try ….convert the "statements" into questions….*



A Classic Example – Action Potential



➢ **overall shape: an asymmetric pulse/"spike"** (mV, linear) **vs time** (ms, linear)	what does the "shape" tell me?
➢ going from left **sits at -70mV** …	what causes the resting potential?
➢ **gradually becomes more positive**	what causes the gradual depolarization?
➢ **at -55mV depolarization slows before it**	what causes the change in behavior?
➢ **rapidly accelerates**, **erasing the membrane potential**,	what causes the erasure of membrane potential
➢ **overshoots** - ~40mV – **reversing potential**	why does the potential overshoot
➢ **begins to fall** without any special events,	why does it reverse/what causes repolarization?
➢ **bottoms out more negative than -70mV**	why does it overshoot -70mV?
➢ **returns to the -70mV baseline**.	how does it return to -70mV & what is the significance of this phase?
➢ **"all-or-nothing"** once about **-55mV threshold is reached**	already answered above Q5



Action Potential – Reconstruction from Data

K^+ =140mM

Na^+ =12mM

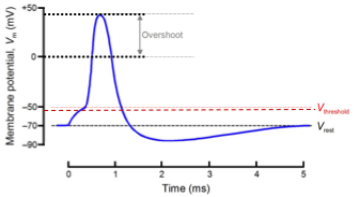
Cl^- =9mM

K^+ =5mM

Na^+ =145mM

Cl^- =110mM

Ion	Permeability (P) in cm/s
Potassium (K^+)	1×10^{-12}
Sodium (Na^+)	1×10^{-14}
Chloride (Cl^-)	1×10^{-11}
Calcium (Ca^{2+})	1×10^{-14}



For K^+ only V= -88.3mV
For Na^+ only = +66mV
For Cl^- only = -66.3 mV

K2P: potassium leak channels

what does the "shape" tell me?

the main "peak" says: "on-off" or "open-close" because the rise and fall are linear;
slightly different slopes for rise (typically "steeper") and fall (typically "less steep") tell you that they likely are caused by different "on-off" processes;
regions of interest preceding and following the peak need closer attention

what causes the resting potential?

this is one of two trivia aspects you need to know : -70mV is maintained by tuning the K^+ permeability (leak channels) – in theory it could also stem from chloride, but Nature chose K^+ instead.

what causes the gradual depolarization?

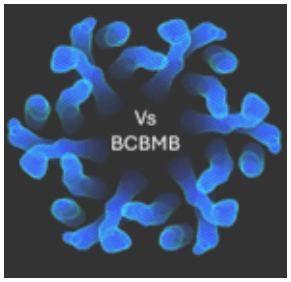
this is the other trivia aspect you need to accept/know (until you understand the whole process): the gradual depolarization can have different reasons ("integrating incoming inputs" or autonomous "pacemaker" activity that stems from sodium leak currents)

what causes the change in behavior?

"trigger" has been pulled that enables rapid depolarization.

what causes the erasure of membrane potential

depolarization erases the membrane potential → "+" charge is coming into the cell = must be Na^+
➔ Na^+-channel must have a "voltage activated gate" that flips open @ -55mV



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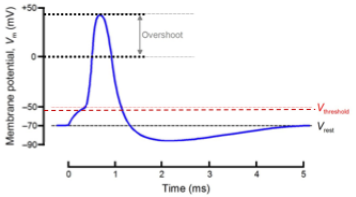

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For Cl^- only = -66.3 mV

K2P: potassium leak channels

why does the potential overshoot/reverse?

overshoots "0" because channel closing is a stochastic event = not all channels close at same time
(same as during a race, a sprinter will not abruptly come to full stop at finish line)
becomes positive because process is driven by Na^+-ions (could go to +66mV)

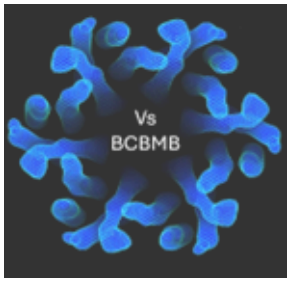
some stats for perspective: need to pass 4,375 Na^+-ions per μm^2 of membrane to bring potential to "0" and 2,500 more to go to +40mV = **total 6,875 Na^+ per μm^2**
each channel passes **>10,000 ions per milli**second (~2x the time to get from -55mV to the maximum!)
with channel densities being 100-200 per μm^2 at beginning of axon where the action potential is initiated & 1,000-2,000 at nodes of Ranvier (the regions where action potential is boosted in myelinated axons)
overshooting by only ~30-40mV is amazingly accurate.

what causes the repolarization?

repolarization = increasing negative charge inside, could be either Cl^- flowing inwards or K^+ flowing outwards … winner is …. K^+ outwards (steeper gradient than Cl^- = more driving force)
→ can't be leak channels because most are desensitized (slide 59), reversing this is too slow (slide 60)
➔ need another **K-channel that is voltage gated, opening the gate when the membrane potential gets depolarized.**

why does it overshoot -70mV?

already answered above … **stochastic nature of channels closing;**
In this case – however - there are fewer channels per μm^2 than for sodium and their flux rate is slower compared to the sodium channels (causing repolarization to be slower than depolarization)



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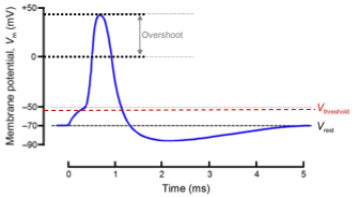

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For Na^+ only = +66mV
For Cl^- only = -66.3 mV

K2P: potassium leak channels

how does it return to -70mV

"clean up" ….meaning: after each "firing", the cell restores the original ion balance

this is necessary because if the cell just kept "firing" over and over again without restoring the original ion balances, the process would quickly run out of "steam" as the "battery gets drained, hitting "empty" eventually

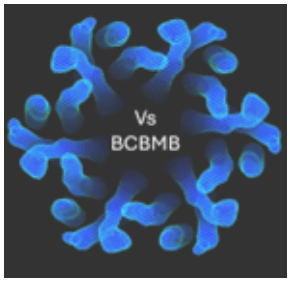
we will look at the specific transport mechanism that restores the balance later on because it is absolutely essential for life. (slide 124)

at some point **(-70mV), the "clean up" and "K leak channel" activity balance each other**, allowing the cell to settle at the resting potential.

what is the significance of this phase?

during the "hyperpolarization" phase, the neuron cannot generate another action potential (unless a MUCH larger stimulus is provided) because the more negative membrane potential throws a "wrench" into the finely tuned action of the powerful voltage gated channels that orchestrate the action potential.

This phase state of the neuron has a **special name: refractory period**.



Action Potential – Putting Structures in Place

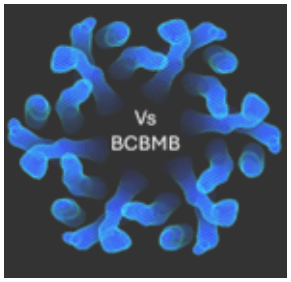
thinking about action potential through the lens of questions let you realize that the observable shape of the action potential requires that the major channels involved in this process

- **must** be able to "sense/measure" transmembrane voltage and
- **must** be able to use this measurement to trigger the gating mechanism when appropriate

➔**both the sodium channel and potassium channel are voltage gated, meaning: both must have a voltage sensor & (at least one physical) gate**

let's not spend too much time thinking about the gate(s) yet … but focus on the voltage sensing ….

…how would you design this?....try to come up with bold ideas!



Action Potential – Putting Structures in Place

voltage sensor & (at least one physical) gate

focus on the voltage sensing ….

…how would you design this?....

Reasoning
changing membrane potential = changing the distribution of "+" charge across the membrane

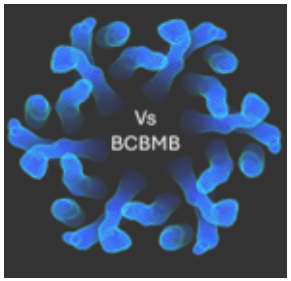
→ **how can you detect electrical charge?**
→ *"like with like"* = you can detect a charge **by strategically placing/displaying either "like" or "opposite" charge(s) on the protein surface**
➔ changing the charge density in the vicinity of this "sensing charge" will result in repulsion (if you use "like") or attraction if you use "opposite"

➔ **problem solved!**
why?
from the Proteins chapter (slides 44-47) you may remember that **native protein folds are** only marginally stabilized = proteins are **VERY TICKLISH**

meaning: change the delicate balance of weak interactions that stabilize the native structure and the protein's structure will change in turn

➔ putting this to work …. if you use "+" charges as sensor, then repulsion will cause the sensor to move if it detects an increase in "+" charge (=decrease in "-" charge) in the vicinity

➔ since the protein is a covalent polymer: movement of the gating charge is felt by the gate, causing it to open/close as the structure tries to adopt the most stable "new" state

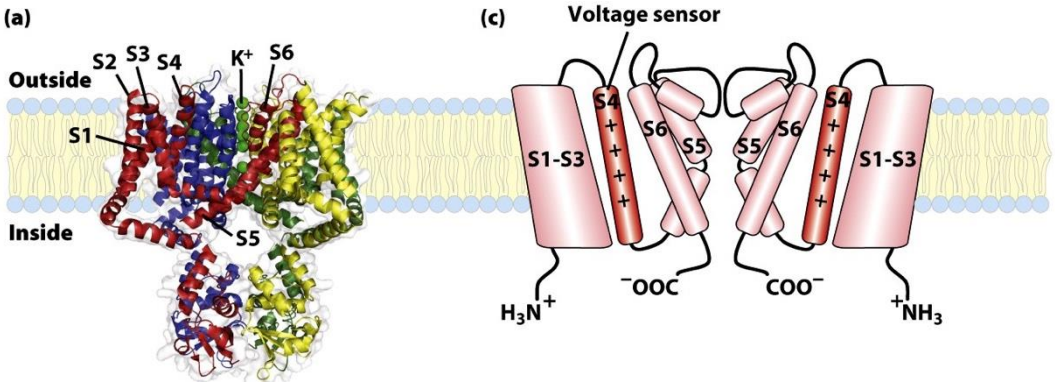


Voltage Gating

a positively charged part of the molecule senses changes in the membrane voltage.

- the sensor is in a structural element called “S4” - it carries several positively charged amino acids
  (a typical voltage sensor sequence is: …**R**-L-I-**R**-L-F-**R**-L-**K**-**R**...)

- movement of the voltage sensor triggers opening of the gate



AI Overview

Recent research (2024-2026) in Kv channel voltage sensor movement emphasizes that the positively charged **S4 helix** acts as a dynamic "sliding helix" that moves ~10–15 Å vertically and rotates outward upon depolarization. Advanced cryo-EM studies now visualize these movements in lipid membranes, showing that S4 translocates towards the cytoplasm and rearranges its interactions with phospholipid headgroups to "lock" the pore in a closed state under hyperpolarization (=before the action potential)

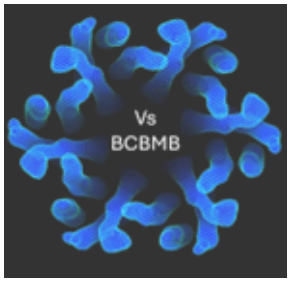
Key Aspects of Current Thinking

•**Sliding/Helical Screw Movement:** The dominant model holds that S4 moves inward (down) and rotates during hyperpolarization (resting state) and moves upward (out) upon depolarization (activated state).

•**Interplay with Lipid Bilayer:** The "down" conformation allows arginine residues on S4 to interact directly with anionic phospholipids in the inner leaflet, which stabilizes the resting state.

•**Membrane Deformation:** The movement of the sensor, particularly in the down conformation, is associated with a localized thinning of the lipid membrane, which acts to intensify the electrostatic force on the sensor.

•**"Down" Conformation Structure:** The down state involves the S4 helix tilting and sliding further away from S1 and S2, with a two-turn displacement toward the cytoplasm.



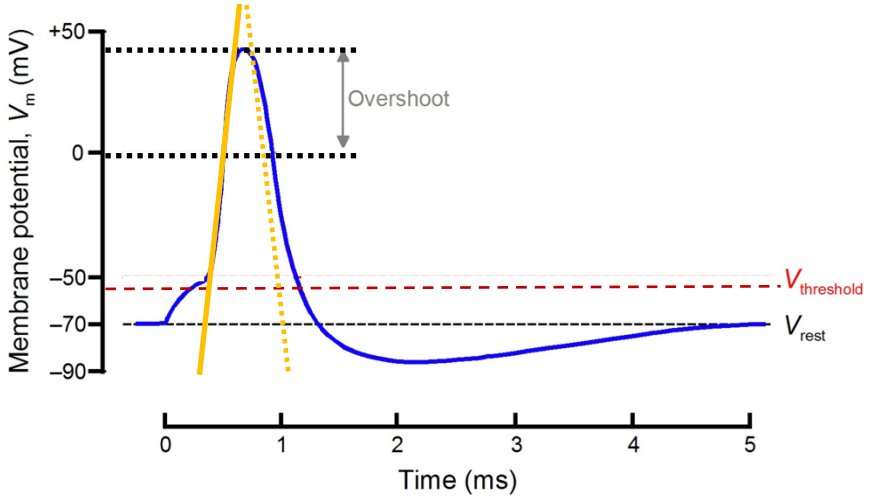
Voltage Gating Potassium-vs-Sodium Channels

the **design principles of voltage sensing are conserved** between K-channels and Na-channels

= **Na-channels use positive gating charges as well**

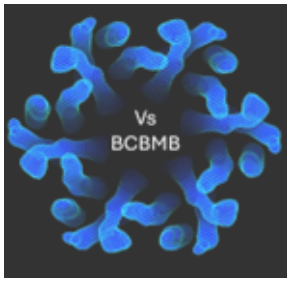
however, the Na-channel voltage sensor is generally more hydrophilic around the positive charges. This makes moving the gating charges easier = faster response compared to K-channels

why mentioning this seemingly arbitrary detail?

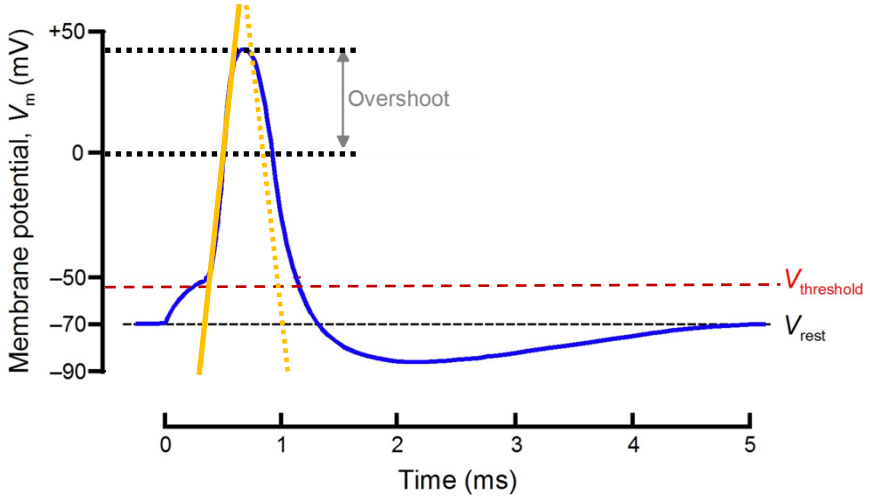


looking again at the shape of the action potential …the depolarization is linear and (typically) steeper than the repolarization….(the orange lines show how it would look if they were the same)

➔ if the gates of **both** channels are triggered at -55mV and **only** differ in their kinetics (Na^+-channel immediately, K^+-channel slower), then the upshoot reflects just the Na^+-current ….and that creates a puzzle once you remind yourself of the "stats", looking at them side-by-side



Voltage Gating Potassium-vs-Sodium Channels



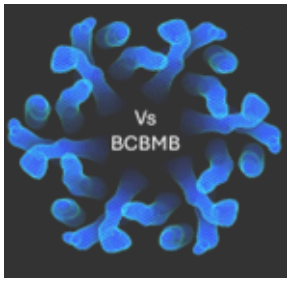
looking again at the shape of the action potential …the depolarization is linear and (typically) steeper than the repolarization….

➔ if the gates of **both** channels are triggered at -55mV and **only** differ in their kinetics (Na^+-channel immediately, K^+-channel slower), then the upshoot reflects just the Na^+-current ….and that creates a puzzle once you remind yourself of the "stats", looking at them side-by-side

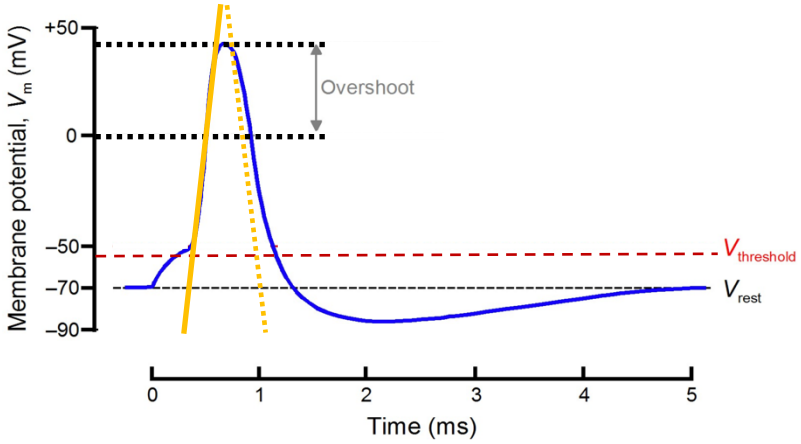
	number/μm²	**ions per channel per ms**
Na^+-channel	up to 2,000	>10,000
K^+-channel	up to 100	1,000-10,000

to change the membrane potential from **-55mV to 66mV requires only ~7,500 Na^+-ions per μm²** to cross the membrane

…do you see what the question/puzzle is



Voltage Gating Potassium-vs-Sodium Channels



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…do you see what the question/puzzle is

Puzzling Question?

given the numbers, **how does the cell avoid overshooting by more than it does?**

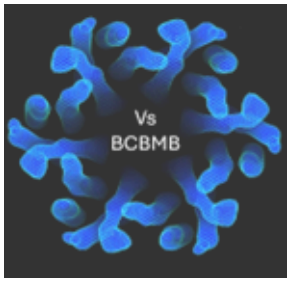
(potentially reaching the Na^+-ion potential of ~66mV)

or, put differently: **what acts as a "brake" here** before the K^+-channels/current catches up to turn the potential change around?

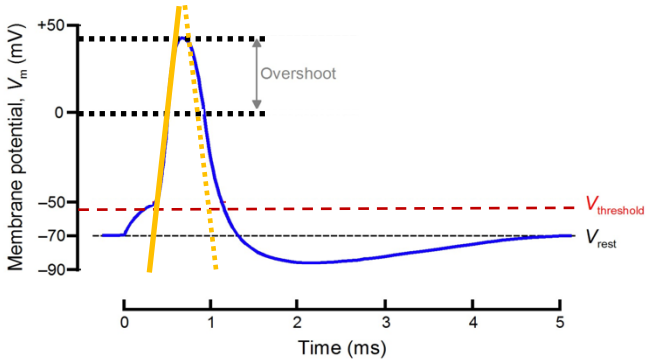
(the defined, narrow maximum tells you that the K-channels are very powerful too, and once they catch up, they really change the game)

Whatever is acting as a brake needs to "deploy" almost immediately after the Na^+-channels open!

any thoughts what it could be? *…Try! Be Bold!!*



Voltage Gating Potassium-vs-Sodium Channels



	number/μm²	ions per channel per ms
Na^+-channel	up to 2,000	>10,000
K^+-channel	up to 100	1,000-10,000

to change the membrane potential from **-55mV to 66mV requires only ~7,500 Na^+-ions per μm²** to cross the membrane

…do you see what the question/puzzle is

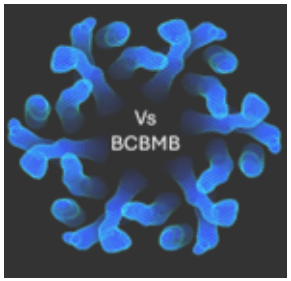
any thoughts what it could be? *…Try! Be Bold!!*

first thought may be "desensitization" (which is a valid auto-shutoff mechanism) - not bad, but not "**bold**"

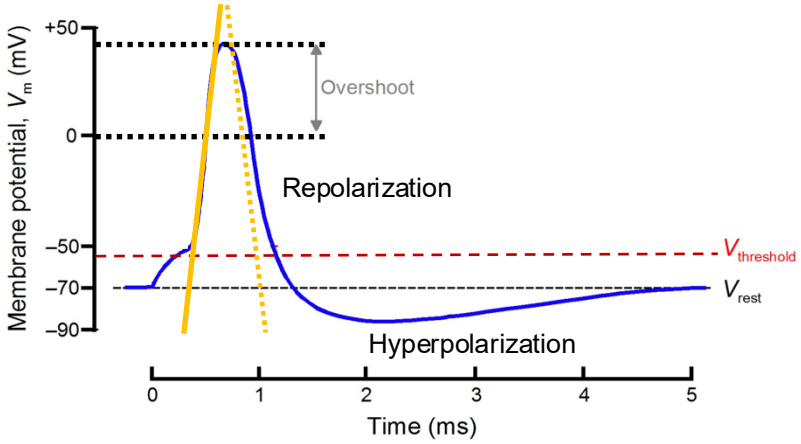
what **is** bold is to hypothesize that there must be a **second gate** in Na^+-channels that somehow gets triggered at almost the same time as the first one but acts **to close the channel** …..
(think of it as "oooopps….moment", saying ….maybe not?!?)

if that is what you thought – you "scare me" because that idea is
EXACTLY right!

we won't dwell on the details of how that second gate gets triggered, but we want to put it all together in one summary table before a visual summary of it all…..



Voltage Gating Potassium-vs-Sodium Channels



State	Sodium (Na^+) Activation Gate (Outside)	Sodium (Na^+) Inactivation Gate (Inside)	Potassium (K^+) Gate (Inside/ Pore)	Resulting Ion Flow
Resting	Closed	Open	**Closed**	No flow (Membrane at -70mV)
Depolarization	**Open** (Fast)	**Open** but starting to stochastically close across the ensemble of channel molecules	**Closed** (Slow to react)	Na^+ rushes **IN** But flow begins to slow as inactivation gates close
Peak / Repolarization	Open	**Completely Closed** across entire ensemble	**Open** (Finally opens)	K^+ rushes **OUT**
Hyperpolarization	**Closed** (Resets)	**Open** (Resets)	**Open** (Slow to close)	K^+ continues leaking out

Action Potential – Graphic Summary

if you are brave – then you will use the cartoon to tell the story in your own words

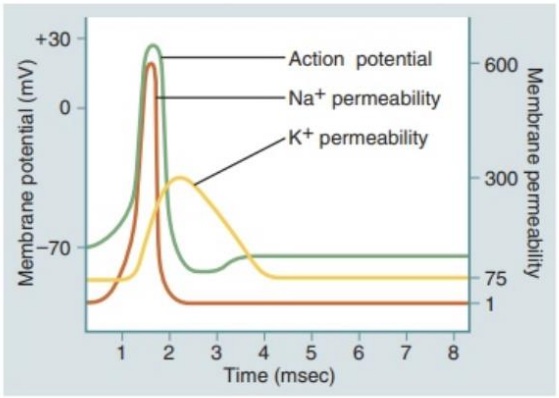
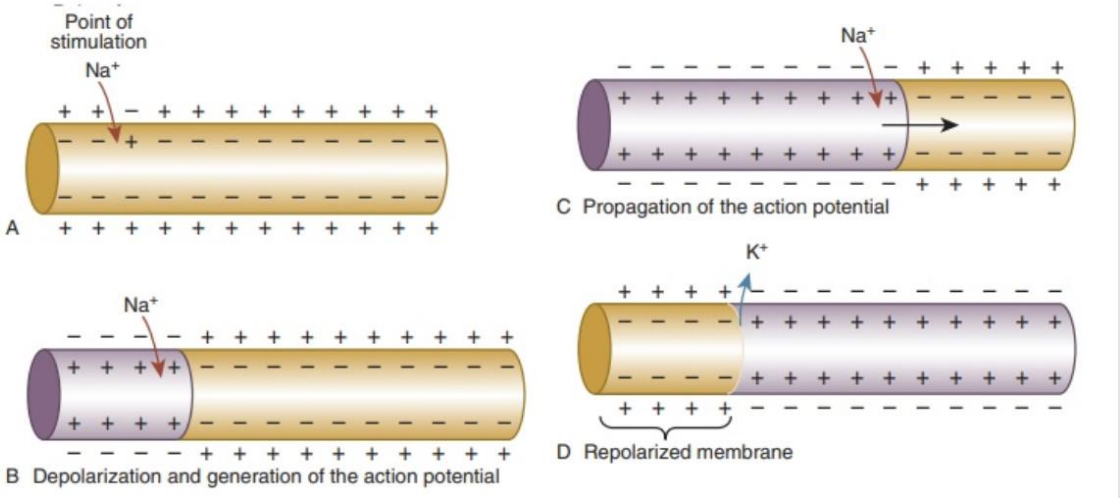


FIGURE 11-9 Nerve impulses.



this was a "tour de force" … but at this level only **leaves one question**…

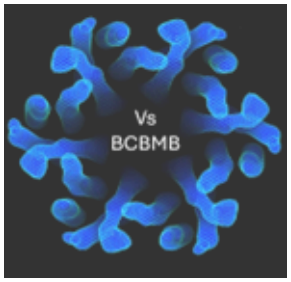
"point of stimulation" ….

=

how does all of it get started in the first place??

Answer

mostly through … yes …another class of channels whose gates are triggered by chemical ligands



Ligand Gated Ion Channels

some channels, involved in neurotransmission, are gated by ligands
binding of the ligand induces conformational change that leads to opening

most important examples

- **Nicotinic Acetylcholine Receptor** [neuromuscular junction, non-selective, conducting: K^+, Na^+, or Ca^{2+}; with Na^+ having the largest driving force due to its gradient across the membrane]
- **AMPA** (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid)-**receptor** [physiological ligand: amino acid glutamate; conducts: Na^+],
- **NMDA** (N-methyl-D-aspartate)-**receptor** (physiological ligand: glutamate; channel non-selective, conducting Na^+ and Ca^{2+})

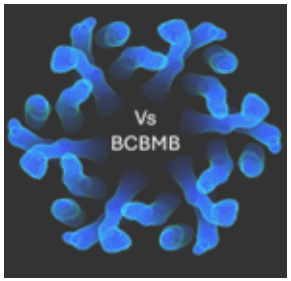
AMPA and NMDA receptors are named after the pharmacological substances that allowed initial discovery of these channels; note that the physiological ligand is "glutamate" in both cases

since these receptors function as **cation channels, they initiate depolarization** of the membrane **which can trigger action potentials if enough receptors get activated**, producing a sustained depolarization that builds up over time
AMPARs and NMDAs **are key for fast excitatory transmission, memory and learning** and are among the most complex ion channels known.

- glycine receptors (ligand: glycine; channel for: Cl^-)
- GABA receptors (ligand: γ-aminobutyric acid; channel for: Cl^-)

GABA and glycine receptors are found at inhibitory synapses
the influx of Cl^- hyperpolarizes the membrane (=makes the inside more negative)
which **prevents** action potentials from forming

(you will learn a LOT more about all this if you take a neurobiology course)



CLOSING POINT ABOUT CHANNELS

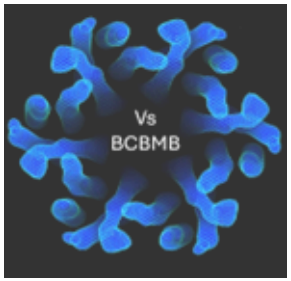
regardless of differences in gating (spontaneous, voltage, ligand)
ALL channels **share the following three properties**

- **ions can (and do) travel in both directions**
  (sometimes there is a preference for one direction, a behavior called = rectification)

- the **direction of net flux** is **only determined by the electrochemical gradient**
  (from higher → lower)

- **regardless of the number of physical gates, once opened channels allow very high flux rates for solutes**

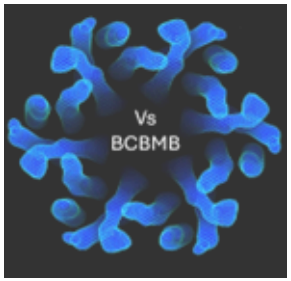
TAKE
A
BREAK



let's Move to “Solutes” Like Glucose that are Significantly Larger than Ions or Water

first off
what - if anything - do you expect to change in the design?

…any thoughts?...



let's Move to "Solutes" Like Glucose that are Significantly Larger than Ions or Water

first off
what - if anything - do you expect to change in the design?

Answer

as transported molecules get larger and chemically more complex, it will become very difficult – if not impossible - to use "selectivity" filters like the ones we saw in water and ion channels.

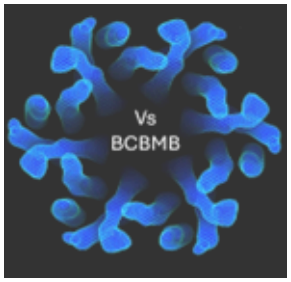
this is because the more complex structure of the molecule that gets transported requires an equally complex complementary surface.
this surface will be asymmetric

➔ designs like the K^+-channel selectivity filter become untenable

➔ you would expect the transporter to have a specific binding site for the transported solute

➔ if you carefully think ahead … you also may expect that this binding site must not be open to both sides of the membrane at the same time because if it were, then it's size would allow all sorts of things to pass in an uncontrolled manner.

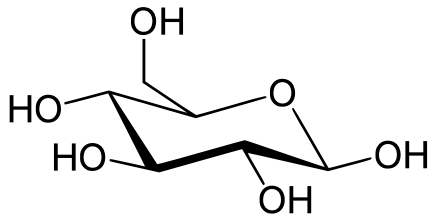
and just like that …you already understand what comes next



Example: Glucose Transport

glucose is exceptionally important

though best known as source of "energy", glucose has many other uses, which we will explore in a later chapter



looking at the structure, glucose is quite polar because of its –OH groups = it cannot pass the membrane spontaneously = it needs to be taken up by a transport mechanism

14 members (GLUT1-12) form a **redundant** **family** of glucose transporters

functions of only 6 (shown below) are well characterized, including fructose transport by GLUT5

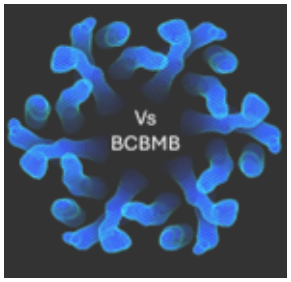
.

TABLE 11–3 Glucose Transporters in the Human Genome

Transporter	Tissue(s) where expressed	Gene	Role*
GLUT1	Ubiquitous	*SLC2A1*	Basal glucose uptake
GLUT2	Liver, pancreatic islets, intestine	*SLC2A2*	In liver, removal of excess glucose from blood; in pancreas, regulation of insulin release
GLUT3	Brain (neuronal)	*SLC2A3*	Basal glucose uptake
GLUT4	Muscle, fat, heart	*SLC2A4*	Activity increased by insulin
GLUT5	Intestine, testis, kidney, sperm	*SLC2A5*	Primarily fructose transport
GLUT6	Spleen, leukocytes, brain	*SLC2A6*	Lysosomal Glucose Transport

take note: GLUT1 is universal and always active, GLUT4 is under hormonal control

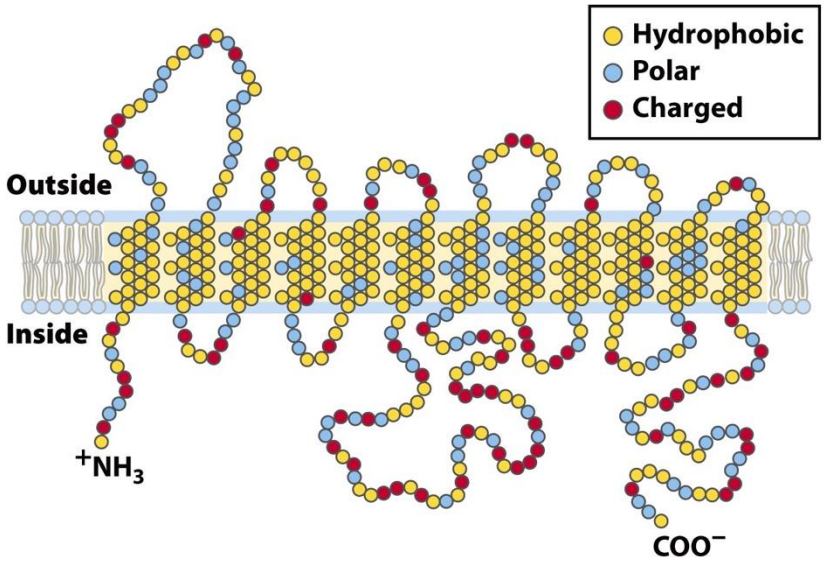
refer to slides 100&101 for more details on this

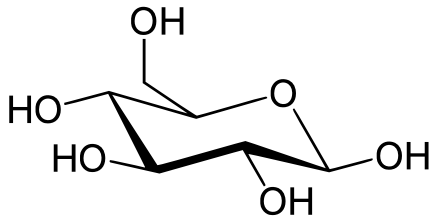


Glucose Transporter Structure

topology diagram for GLUT1

Diagram that shows the number of transmembrane helices and the disposition of N-/C-terminus with respect to the membrane





as expected

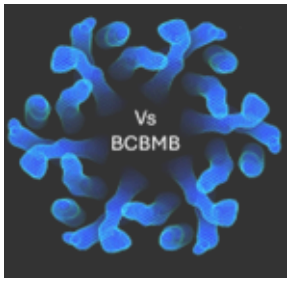
- the glucose transporter is much larger than KcsA (2 TM segments per monomer), or the subunits the voltage gated K^+-channels (tetramer, each has 6 TM segments)

- the TM segments are made mostly of hydrophobic amino acids (necessary to stably accommodate the protein in the membrane) but a few polar amino acids are also observed

→ makes sense because the glucose binding site needs to match the chemical properties of glucose

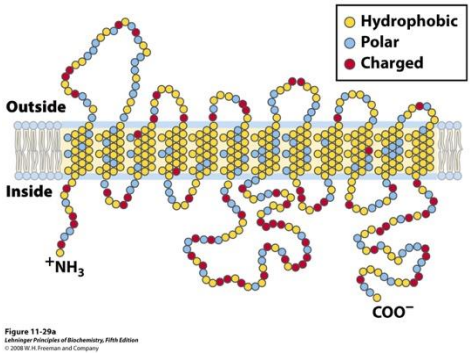
- the glucose transporter is active as a monomer

➔ the glucose binding site + transport mechanism all reside within the bundle that is made of 12 TM segments



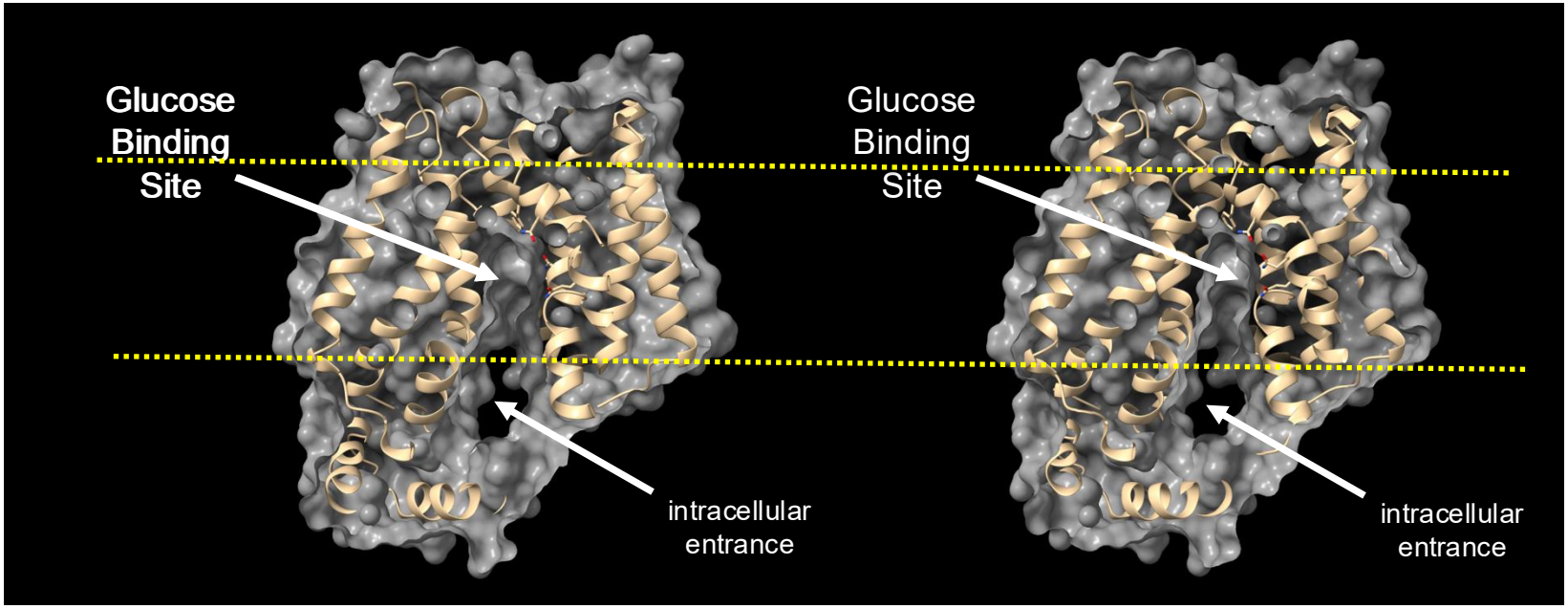
Glucose Transporter Structure

cartoon

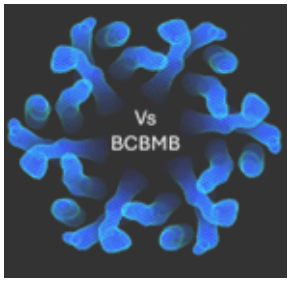


reality

cross-eye stereo view (cropped to view glucose binding pocket



as expected: there is a binding site inside the transporter that shields glucose from the hydrophobic membrane interior, and the path to the extracellular space is closed



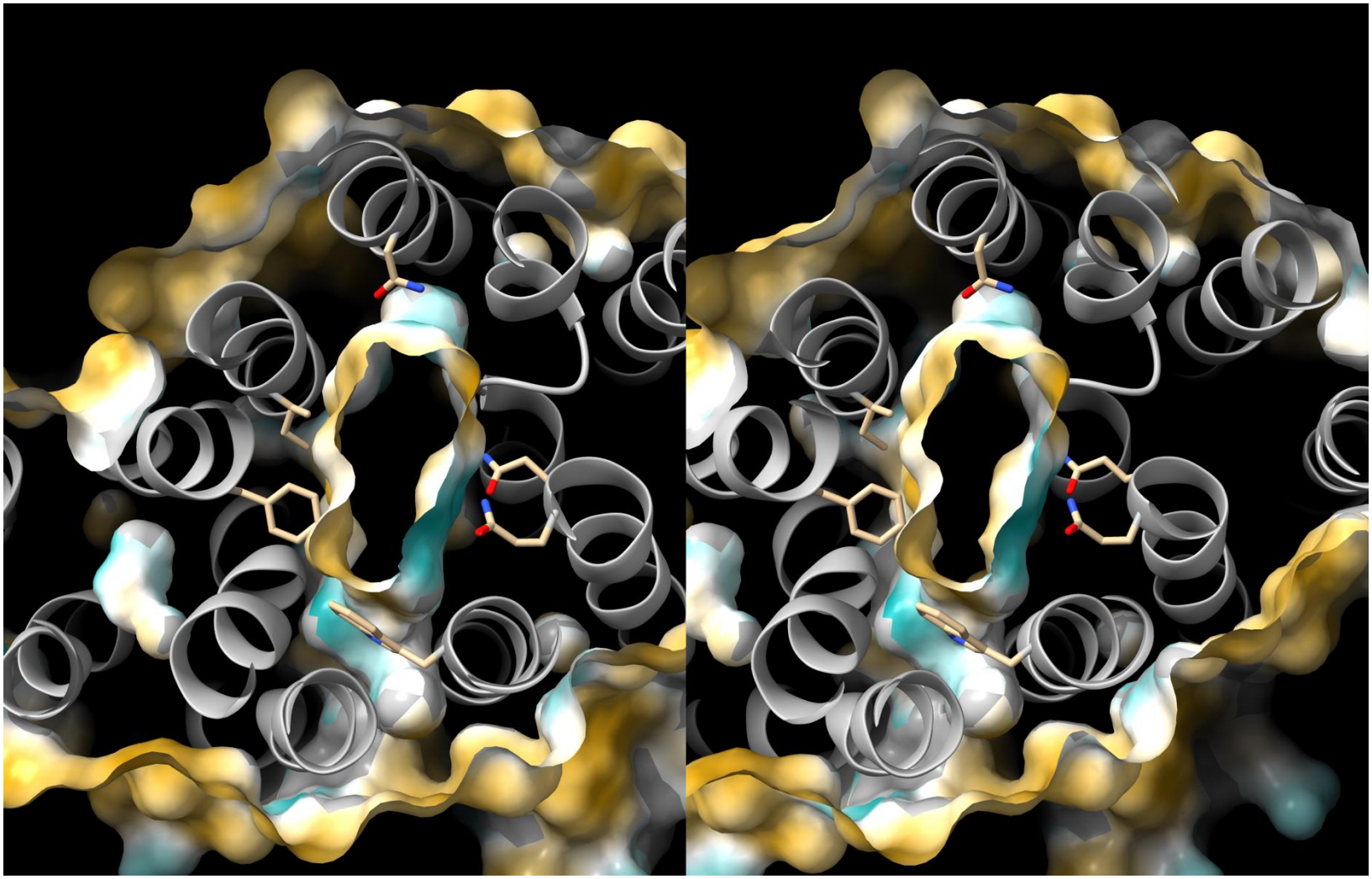
Glucose Transporter Structure

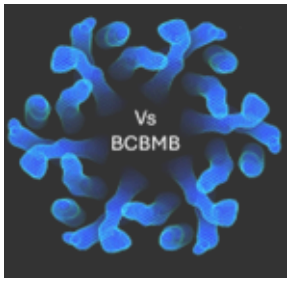
taking a closer look at the **glucose binding site**

(cross-eye stereo view, looking perpendicular to the membrane plane = along the the path of glucose transport)

we find a **surprise**

do you notice what that surprise is?

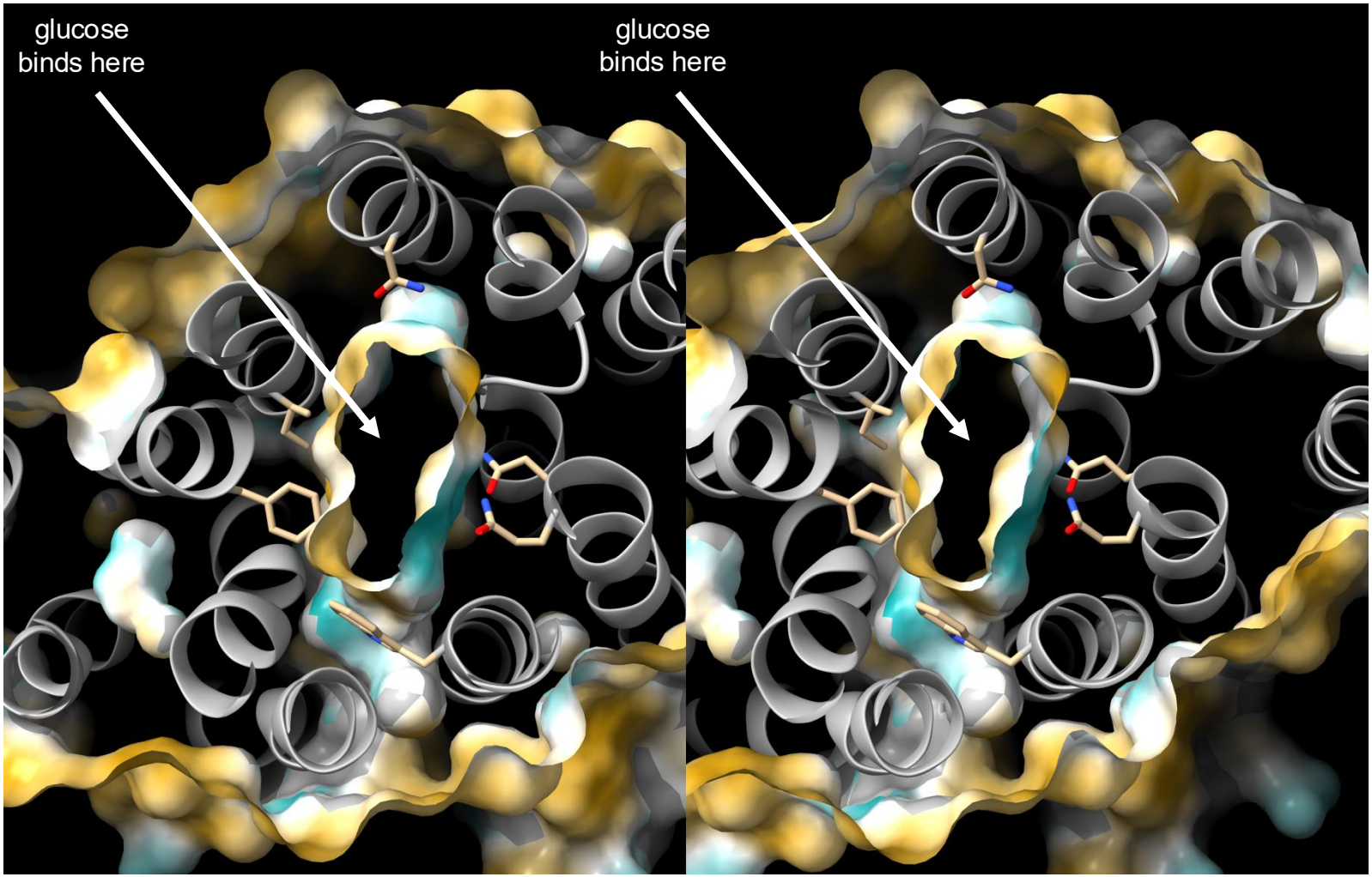


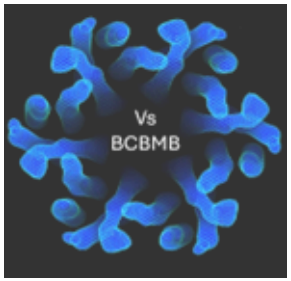


Glucose Transporter Structure

Do you notice what that surprise is?

the right wall of the binding pocket is hydrophilic (cyan color)
but
the **left wall is hydrophobic** (orange color)

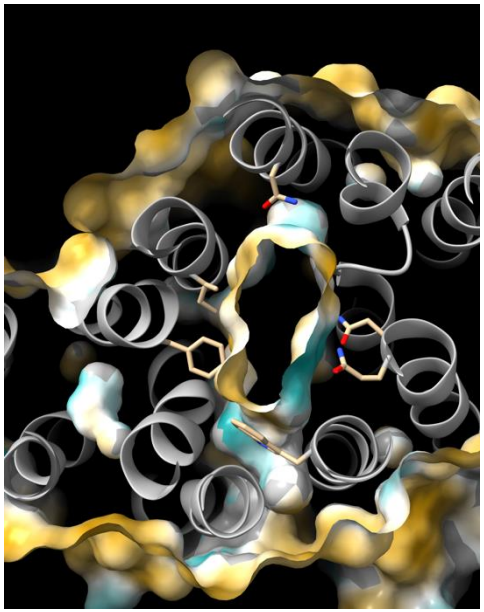




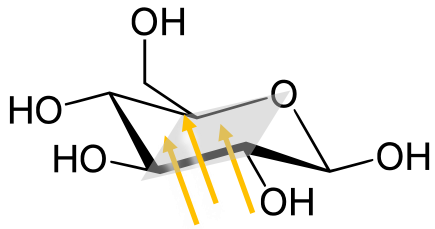
Glucose Transporter Structure

do you notice what that surprise is?

the **left wall is hydrophobic** (orange color)



though you may not have expected it, the hydrophobicity in the glucose binding pocket does make sense if you look at the glucose structure carefully



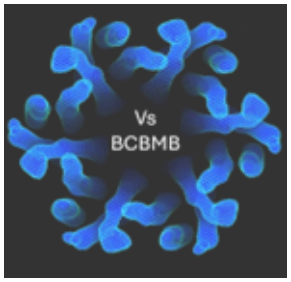
if you do, you notice that glucose has a small hydrophobic patch
you see it
if you look from "below and in the direction of the arrows"
from that vantage point you see "-C-H"

yes, I know …..why bother paying attention to such a minute detail?

Two reasons

- for Nature, paying attention to this detail is key to distinguish glucose from other, very similar sugars
and
- minimizing the number of hydrophilic contact points also will make the surface less "sticky" = transport will become easier because you don't give glucose too many reasons for "wanting to stick around"

= the transporter gives **just** enough hydrophilic support to make it happen, but not anything beyond that
(just like you often may only do what is necessary to accomplish an immediate goal)



Glucose Transporter
From Reality Back To Cartoons

now that we have some sense for the structure of glucose transporters,
let's move on to ask …
how does glucose get across the membrane?

this type of question goes after the **molecular mechanism of transport**
and in this context, you **always want to answer the same basic questions**

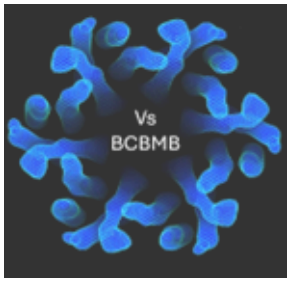
- Q1: what drives the transport process (does it happen spontaneously, or do you need to invest energy)?
- Q2: does the transport involve just the one solute you are interested in, or is there more to it?
- Q3: does transport require molecular motions to allow passage?

looking at ion channels we found:

Question 1: net ion flux **always is down an existing concentration gradient** = the energy comes from the gradient itself → the **transport process is passive** (= it happens without doing anything else once you open the gate(s))

Question 2: in most cases – it's just one ion, though some ion channels are non-selective (examples are some of the ligand-gated channels), and in some cases water molecules come along for the ride.

Question 3: **no – the part of the channel that conducts the ions does not move while they are flowing.** There are moveable parts (gates, sensors) … but the conduction happens within a "static" scaffold/conduit



Glucose Transporter
From Reality Back To Cartoons

Coming back to glucose …..

- **Q1: what drives the transport process?**
(does it happen spontaneously, or do you need to invest energy)?

➔ want to compare extracellular and intracellular **concentrations of glucose**….

extracellular: 4-6mM range (fasting)
intracellular: µM range (typically)

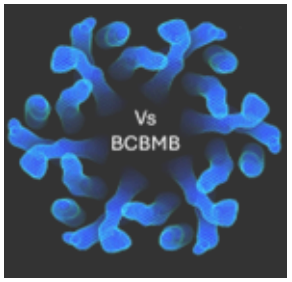
➔ cellular uptake of glucose is "downhill"
= no additional energy required
= passive transporter will suffice

important exception: gut epithelial cells …this is where glucose enters the body … inside these cells, glucose is 10-30mM…something we need to take a look at later.

- **Q2: does the transport involve just the one solute you are interested in?**

- Q2: yes – GLUT family glucose transporters transport only glucose (except for the few "black sheep" in the family that changed to move molecules other than glucose)

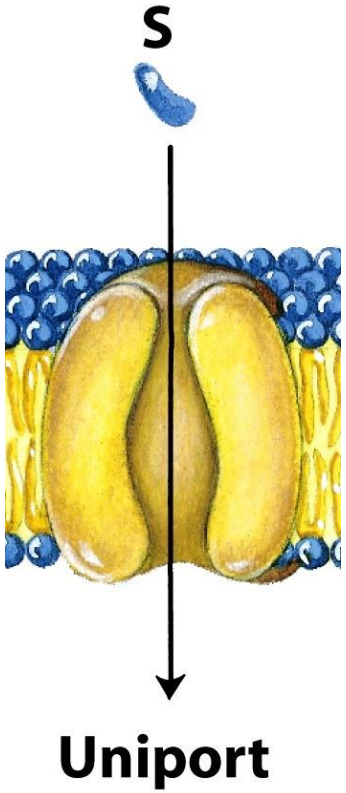
➔ **GLUT family** of glucose transporters are **"uniporters"** (just one solute) that **function as passive facilitators** (passive, downhill diffusion of glucose)



Glucose Transporter
From Reality Back To Cartoons

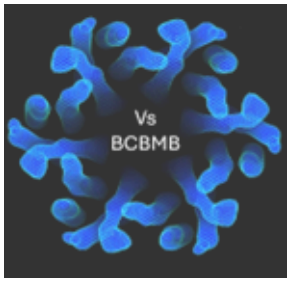
➔ **GLUT family** glucose transporters are **"uniporters"** (just one solute) that **function as passive facilitators** (passive, downhill diffusion of glucose)

which in many cartoons that illustrate "uniport" looks like in this one here



if you were to review the textbook that contains this illustration
what – if anything – would you tell the graphic designers that make these figures?

…try …. tell them!...



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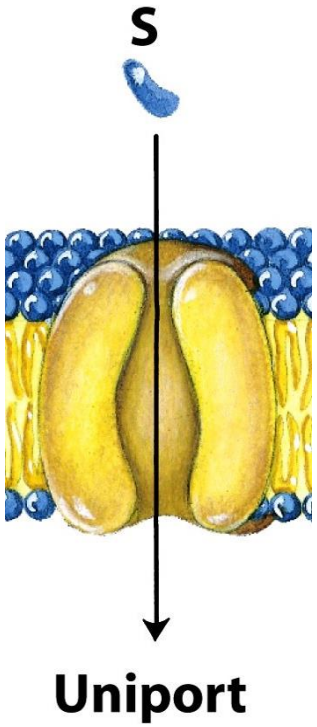


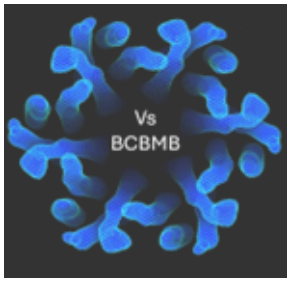
if you were to review the textbook that contains this illustration **what – if anything – would be your comments to the graphic designers that make these figures?**

hopefully you'd tell them that this cartoon can easily be misunderstood….

while the "idea" of uniport is certainly captured ..if the main features of the uniporter really were this way…it would be a disaster because "glucose" is a fairly large molecule …

meaning … if there just was a passive "hole" – like this cartoon suggest– it would be very unselective, allow ion gradients to collapse, and do all sorts of other mischief as well.

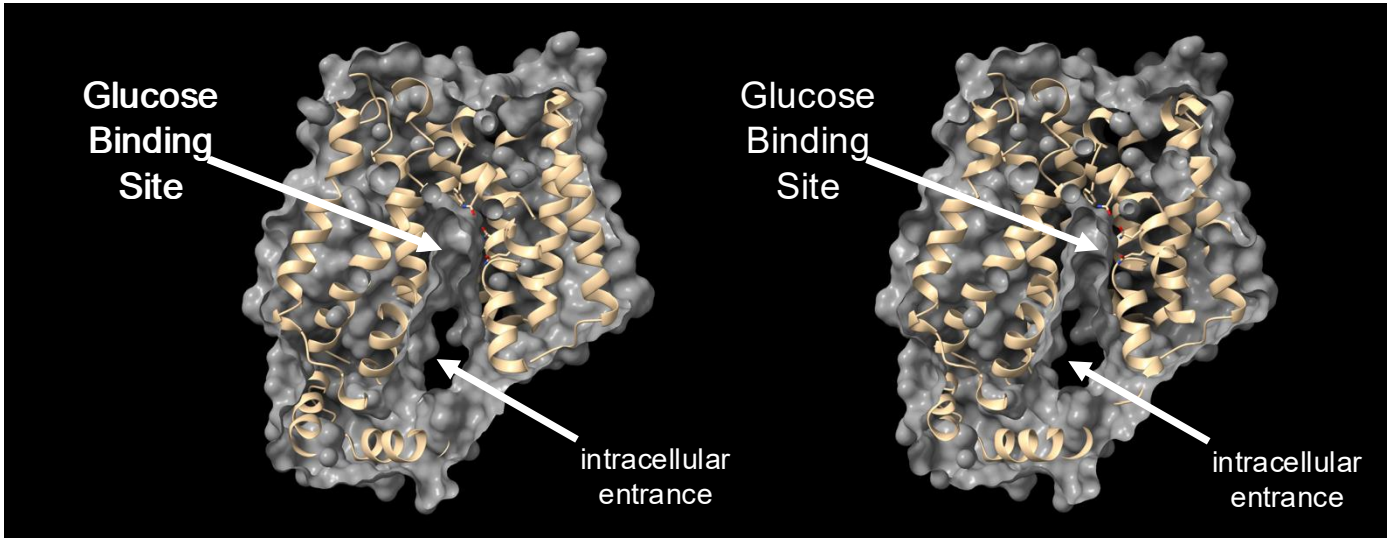
fair enough …. but instead of "yelling" … be constructive … **how can this be saved to more accurately reflect realities?**

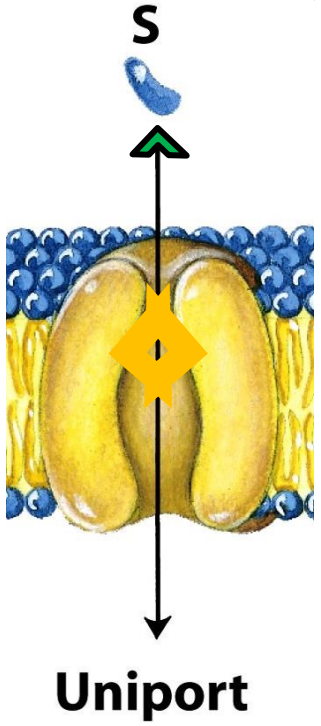


Glucose Transporter
From Reality Back To Cartoons

fair enough …. but instead of just "yelling" … be constructive …
how can this be saved to more accurately reflect realities?

by taking into account what you observed when looking at the structure

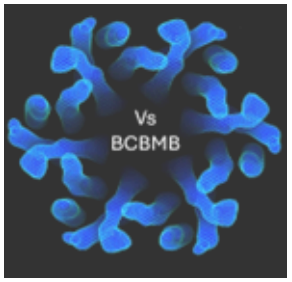




looking at the structure – you **clearly see** that the passage is obstructed … so just obstruct it in the cartoons as well, please …and make the arrow double headed since "S" can go both ways!...

- **Q3: does transport require molecular motions to allow passage?**

with the "obstruction" in place – this is easy to answer:
yes, transport of glucose will require motion ….
ideally, the transporter will pivot about the solute

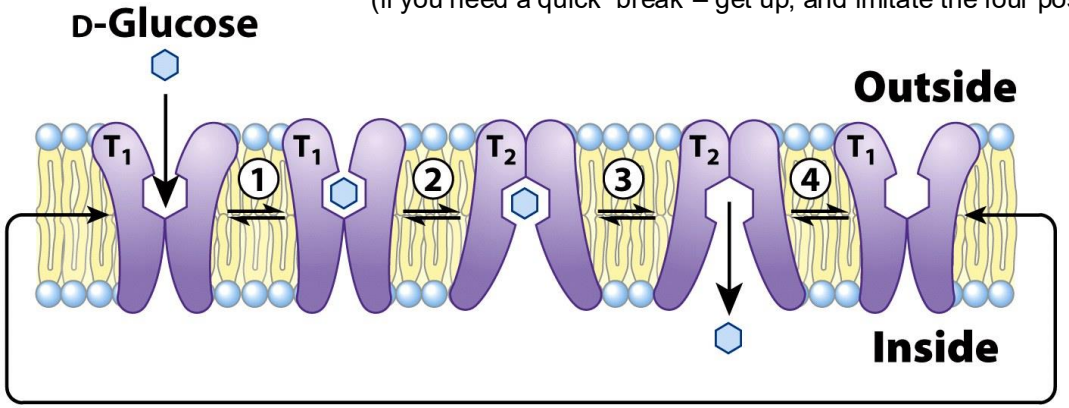


A Simple Mechanistic Model For Membrane Transporters

taking the lead from the structure, the **simplest model** to allow for specific transport without leakage **holds that the transporter can adopt two major conformations**

Outside Open → Inside Open

(if you need a quick "break"– get up, and imitate the four positions with your arms & legs)



$$S_{out} + T_1 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} S_{out} \bullet T_1$$

$$k_{-4} \downarrow\uparrow k_4 \qquad k_{-2} \downarrow\uparrow k_2$$

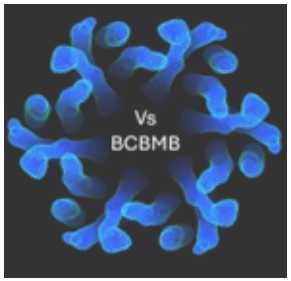
$$S_{in} + T_2 \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} S_{in} \bullet T_2$$

this model gives rise to a cyclic **kinetic scheme** where the **transporter visits a defined set of conformations <u>for each</u> solute molecule that gets transported**

outside open (empty) – outside open (engaged) - inside open (engaged) – inside open (empty) - reset to outside open (empty) without carrying cargo

EACH of these transitions is reversible = the molecule can (and does) move in either direction ….so just like in channels, it is about **net movement** that **follows the direction of the gradient**

note: step 2; goes through a **conformational state that is completely closed off to both sides of the membrane.** This state is called **the occluded state** (not explicitly shown in the cartoon)



A **Mechanistic Divide** between **Transporters and Channels**

the simple mechanistic model with two conformations holds for many other transporters as well (e.g. major facilitator superfamily - MFS)
and
is not limited to transporters that participate in passive diffusion

in fact, GLUTs are a rare example of transporters that act by passive diffusion.

however: GLUTs allow us to make an important point:

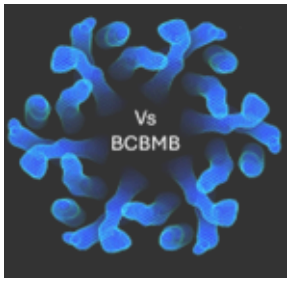
being a channel or (passive) transporter is uniquely defined by mechanism

- **channels operate non-stoichiometrically**!
  (one the channel is open, ion flux occurs **without** additional conformational changes)

- **transporters always involve stoichiometric turnovers**
  regardless of whether they are active or passive, electroneutral or electrogenic (explained later)
  = the protein must go through the entire sequence of conformational changes for each solute molecule(s) that is (are) transported

this has a really important implication - what is it?

…what do you think? ….



A **Mechanistic Divide** between **Transporters and Channels**

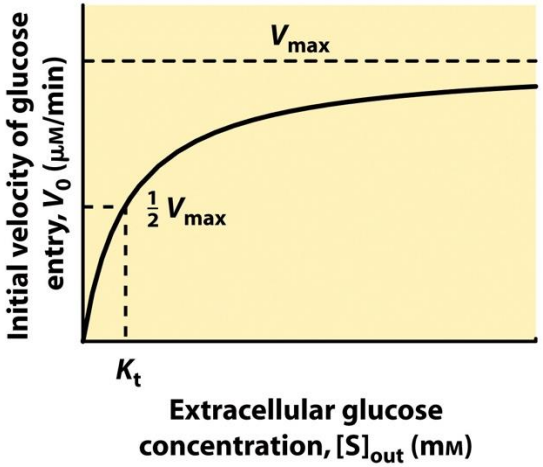
➢ **transporters always involve stoichiometric turnovers**
this has a really important implication - what is it?

transporters are saturable - channels are not!

meaning: each transporter has a **characteristic maximal uptake velocity** for its solute(s). Once reached, increasing the concentration of the transported solute(s) will not make the transport go faster because **the transporter needs a fixed amount of time to complete its cycle.**

measuring this for a single transporter is not easily done → researchers use ensembles (= many transporters) for each measurement → once the concentration of the transported solute(s) exceeds the concentration needed to engage **every** transporter in that ensemble, adding more solute will not increase the macroscopic rate up transport

==> this is essentially the same as waiting in line to be served at a fastfood restaurant … once the number of customers exceeds the number of staff taking orders … you have to wait until it's your turn



We will encounter this type of dependency again when we take a look at enzymes

$$V_0 = \frac{V_{max}[S]_{out}}{K_t + [S]_{in}}$$

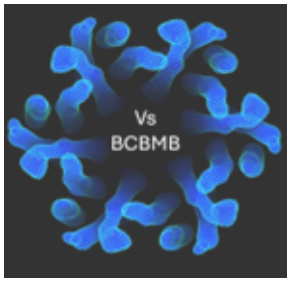
K_t = transport constant (it integrates the contributions of the rate constants for the individual steps along the transport cycle

1.5mM for D-Glucose
3 M for L-Glucose (stereochemistry matters!!)

20mM for D-Galactose (differs in position of one –OH group)
20mM for D-Mannose (also differs in position of one –OH group)

apart from this mechanistic difference in their function, transporters and channels are similar to enzymes in that:

- they lower the activation energy for translocation across the bilayer (= accelerate the rate of translocation)
- they are specific for their solutes
- they function in both directions



Exploiting Redundancy, Compartmentalization, and Recycling to Regulate Cellular Glucose Uptake

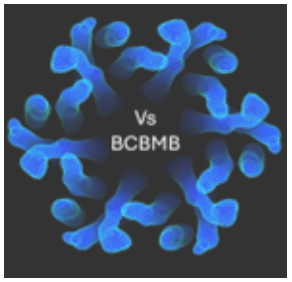
since glucose is such an essential molecule for cellular function, its supply and usage are carefully regulated to achieve proper homeostasis

this raises the question: **how you assure that each cell gets the appropriate amount of glucose?**

evaluating the equation for uptake velocity gives you the essential clues here.

…take a look and propose mechanism(s) for regulating glucose uptake into different tissues and under different conditions ….

$$V_0 = \frac{V_{max}[S]_{out}}{K_t + [S]_{in}}$$



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evaluating the equation for uptake velocity gives you the essential clues here.

$$V_0 = \frac{V_{max}[S]_{out}}{K_t + [S]_{in}}$$

V_0: is the outcome = can't "use" this

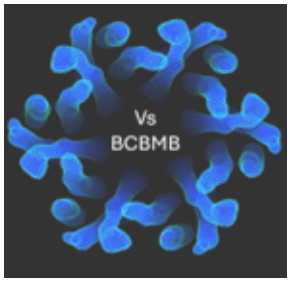
[S]:→ also no good because the goal is to keep these within very narrow ranges **through** the action of the transporters

leaving you with
K_t and V_{max}

well … both of those are great for regulating glucose transport because

- **K_t** is determined by the rate constants that **determine glucose binding, glucose release and the ease with which the transporter cycles through the different conformations**
  → changing anyone of these aspects will have a big impact on V_0

V_{max} is directly related to the number of transporters that are available
➔ you either physically present more/less transporters as needed, or act on a given population by switching transporters "on" or "off" as needed (eg through chemical modification)



Exploiting Redundancy, Compartmentalization and Recycling To Regulate Cellular Glucose Uptake

Quality

(all GLUTs)

$$V_0 = \frac{V_{max}[S]_{out}}{K_t + [S]_{in}}$$

different GLUT isoforms have **different K_t**

GLUT1: 1.5mM
(ubiquitous, always works at good speed ~80% of V_{max} for typical blood glucose concentration of ~5mM between meals)

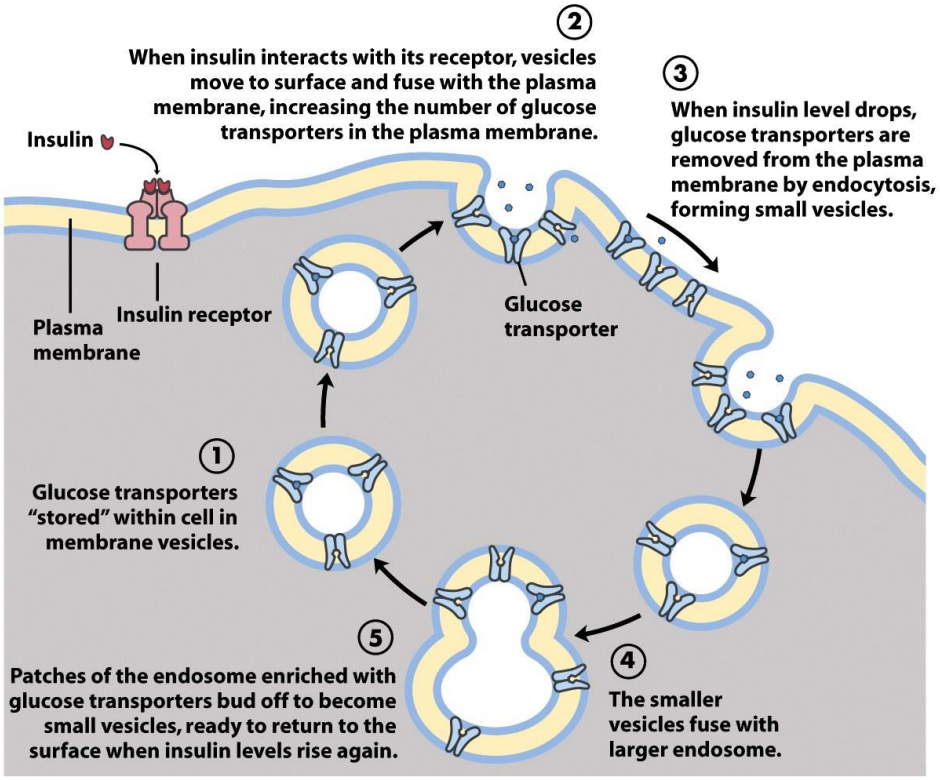
GLUT2: ~66mM
(liver, pancreas islet cells, intestine, **only works at significant rate** after meals when blood glucose is very high)

GLUT4: 5mM
(muscle, fat, heart; perfectly positioned for typical blood glucose concentrations between meals = very responsive to even small changes in glucose levels)

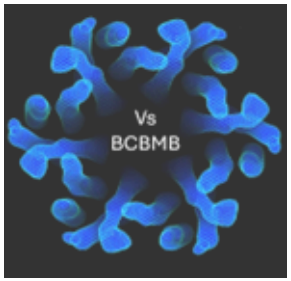
➔ molecular basis for ability to change functional output to meet different biological needs
(applicable concept here: **redundancy**)

Quantity

(GLUT 4)



incidentally, this also uses vesicular bulk transport!
relevant concept: **compartmentalization and recycling**



Uniporters are Nice but Limited

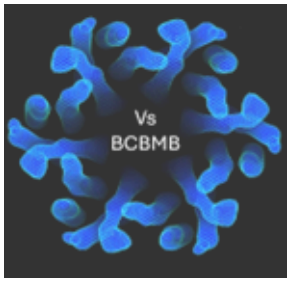
while uniporters are well suited for the passive uptake of glucose (and a few other nutrients) into cells, many physiological situations cannot be dealt with using uniporters.

a simple example is transport of CO_2 from tissues to the lungs.

carbon dioxide, CO_2 and H_2O are key endproducts in the metabolic breakdown of organic molecules. constant formation of these endproducts necessitates mechanisms for their removal from the body because they are "waste" products

water: you can loose as sweat, through breathing, or in urine

what about CO_2?



Uniporters are Nice but Limited

water: you can loose as sweat, through breathing, or in urine

what about CO_2?

Answer
CO_2 is a gas!

→ great! get rid of it by breathing it out!

yep … that will be easiest … but … to breathe it out … **how does CO_2 formed in peripheral tissues (eg your pinky or little toe) get to the lung?**

can't be as "gas" because your blood is not like a "fizzy"/carbonated drink

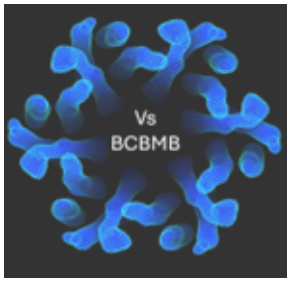
fortunately for us
carbon dioxide reacts spontaneously with water to form carbonic acid (H_2CO_3)
which at physiological pH dissociates to form

an inorganic "bicarbonate" ion and a proton
(take note: this is an EQUILBRIUM)

$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$$

this equilibrium will be on the side of bicarbonate if the CO_2 concentration is high, and on the CO_2 side when its concentration is low

really important to note here: there always is some CO_2 and this is significant because CO_2 can diffuse freely through membranes ➔ easy for cells to "get rid" of this waste product …. but what do you do once it gets into the blood … you know it is not "bubbly" … **so what happens?**



Uniporters are Nice but Limited

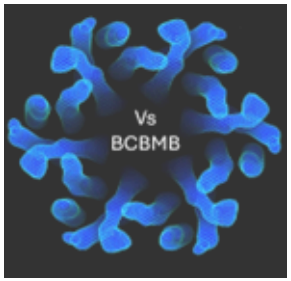
red blood cells have an enzyme, **carbonic anhydrase,** that makes the reaction much more efficient

$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$$

nice - but what problem will arise quickly?

….try to answer….

(***hint:*** *enzymes can catalyze both forward and backward reaction….)*



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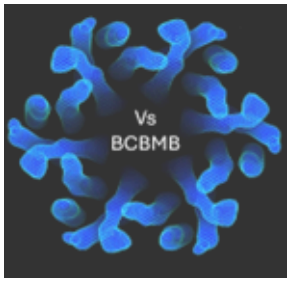
nice -but what problem will arise quickly?

Answer

the reaction will quickly come to a stop because the concentrations of products (bicarbonate and protons) will shoot up → forcing the reaction to go in reverse.

how could you avoid this problem?

…again … try to answer….



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Answer

the reaction will quickly come to a stop because the concentrations of products (bicarbonate and protons) will shoot up → forcing the reaction to go in reverse.

how could you avoid this problem?

Answer

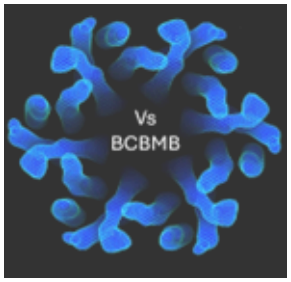
by "removing" either bicarbonate or protons

→ **Le Chatelier's Principle!** (hello … GenChem…!)

disturbing the equilibrium by removing at least one of the two reaction products will let the reaction proceed in the forward direction.

➔ **what would you remove? bicarbonate or protons? And How??**

….go again … try … and justify your answer….



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red blood cells have an enzyme, **carbonic anhydrase,** that makes the reaction much more efficient

$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$$

➔ **what would you remove bicarbonate or protons? And How??**

Answer

considering both options:
removing protons
possible, but difficult because **cytosol inside a cell** is a **very good buffer** – it will absorb protons if need be and hold on to them as long as bicarbonate is kept low….

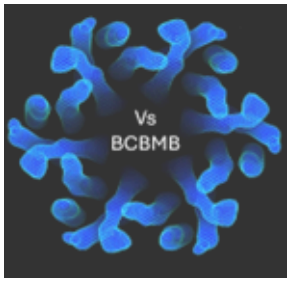
so looks like removing bicarbonate then!

yes indeed … (after all this is a chapter about membrane transport)… you move it out of the cells ….

…very cool … only ONE PROBLEM with this plan … do you see what that might be?

….try….what is the problem? ….and how could you solve it?

(hint: look at the title slide that has been riding along for some while now….)



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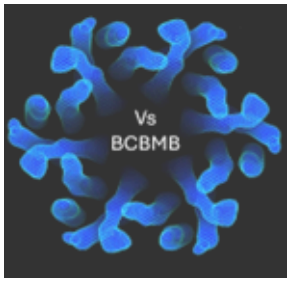
…very cool … only ONE PROBLEM with this plan … do you see what that might be?

Answer

transporting bicarbonate outwards would leave the "+" charge of the proton behind …. = as you keep doing this you would get a buildup of "+" charge inside which – in turn - would make transporting the bicarbonate anion (negative charge) harder and harder ……

what can you do to work around this?



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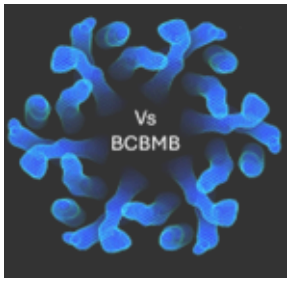
Answer

transporting bicarbonate outwards would leave the "+" charge of the proton behind …. = as you keep doing this you would get a big buildup of "+" charge inside, which – in turn – would make transporting the bicarbonate anion (negative charge) harder and harder ……

what can you do to work around this?

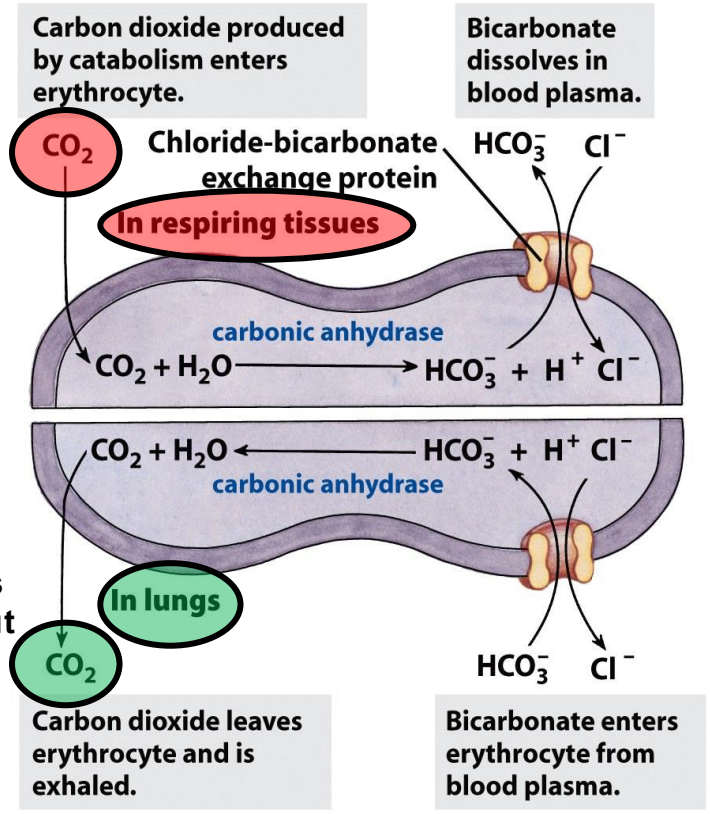
you build a transporter that **exchanges** bicarbonate for another anion that happens to be abundant outside ….. and which one would that be??

exactly … **chloride**



Antiporters – A Tale of Two Solutes

high CO_2



low CO_2 because it leaves as you breathe out

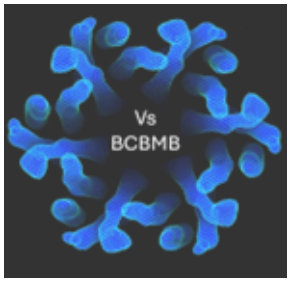
Calculating the "cost" of this exchange shows that it is at equilibrium! No "payment" either way (see slide 21)

➔ **bicarbonate/chloride exchange is passive**

$$\Delta G = RT \ln \frac{C_2}{C_1} + ZJ\Delta\Psi$$

Free Energy Components (kcal/mol)

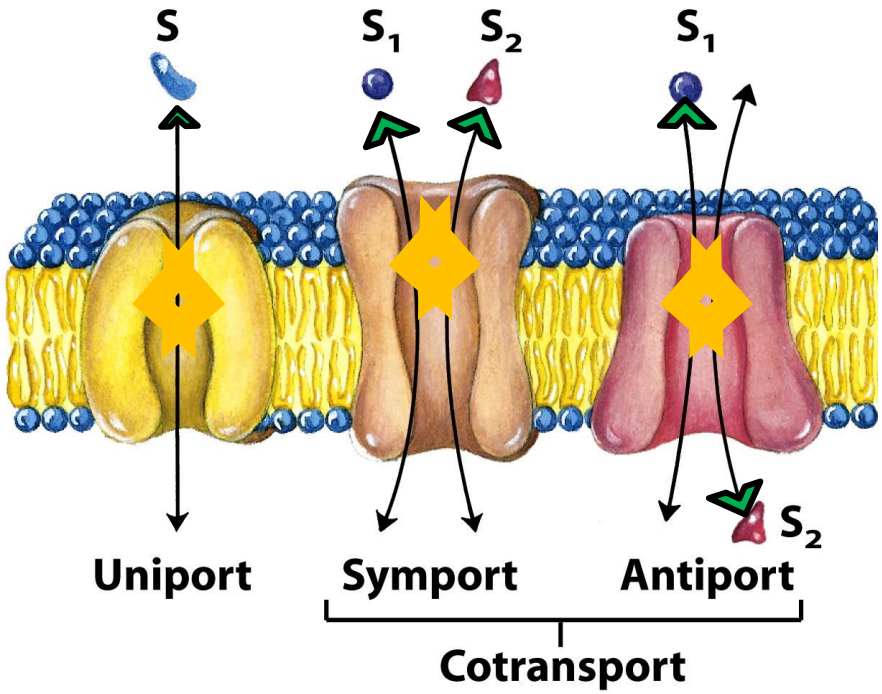
Ion	Direction	Chemical Work	Electrical Work	Net ΔG
Bicarbonate (HCO_3^-)	Efflux (Out)	+0.238	−0.231	+0.007
Chloride (Cl^-)	Influx (In)	−0.228	+0.231	+0.002



Diversifying the Repertoire of Transport Mechanisms

transporters that can shuttle more than one substrate greatly increase the versatility of transport processes.

there still is one problem here - what is it?



Cotransport Possibilities:
both passive (= downhill) → OK
one passive (= downhill), one active (uphill) → can't do yet

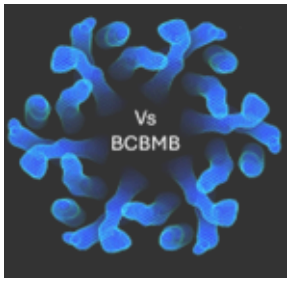
Answer

without yet another type of transporter, these engines

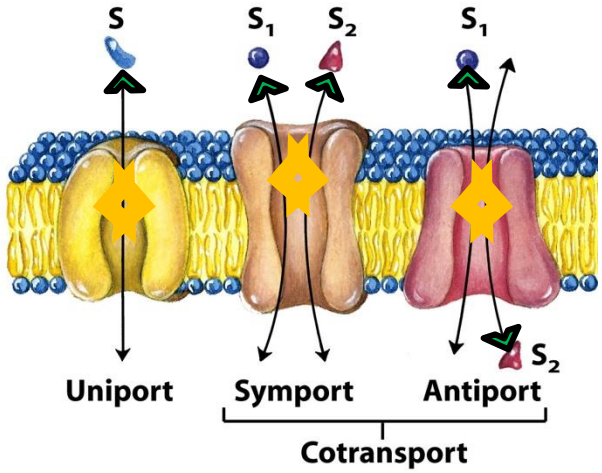
cannot establish non-equilibrium conditions de novo

obvious question: what is that missing link?

….what do you think? ….



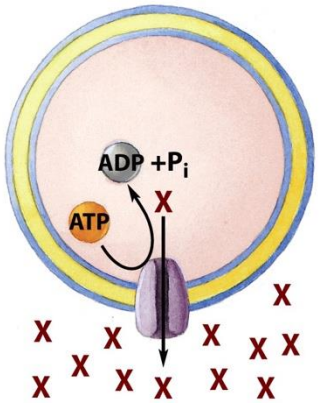
Diversifying the Repertoire of Transport Mechanisms



Cotransport Possibilities:

- both passive (= downhill)
- one passive (= downhill), one active (uphill)

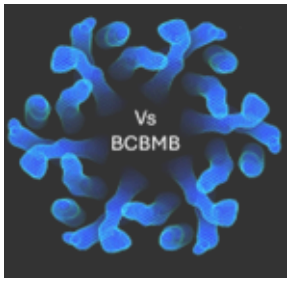
obvious question:
what is that missing link?



Answer
ATP (or light) driven pumps that use the respective energy source to move solutes against their concentration gradients ("uphill")

this mode of transport is referred to as **primary active transport**

and accordingly: cotransport that uses the downhill movement of one solute to power uphill movement of the second solute is called**:**
secondary active transport



Transport ATPases - Overview

membrane transport proteins that utilize energy from hydrolyzing ATP to move solutes against their concentration gradient are called Transport ATPases

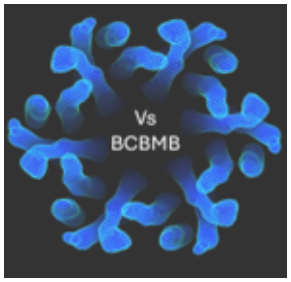
Four different types:

- **P-type** ATPases are **cation transporters**: best established are **Ca^{2+}-ATPase** and **Na^{+}/K^{+}-ATPase;**
  - P-type ATPases are single polypeptides,
  - 4 domains: M-domain (transmembrane, 8 or 10TM segments), N-domain (nucleotide binding), P-domain (phosphorylation domain), and A-domain (actuator domain)

- **F-type** ATPases/ATP-Synthases are **proton pumps**;
  - multiprotein complexes where the proton passageway and ATP-binding domains are on separate polypeptides
  - under certain circumstances (eg in mitochondria), this transporter operates in reverse = using a proton gradient to synthesize ATP

- **V-type** ATPases are **proton pumps** that acidify intracellular compartments (**v**acuoles [name giving], lysosomes, endosomes, transport vesicles),
  - structurally related to F-type ATPases, but the mechanism is unknown.

- **ABC Transporters** (from ATP-binding cassette)
  - diverse family of ATP-dependent transporters that pump amino acids, peptides, proteins, metal ions, lipids, bile salts and many hydrophobic compounds
  - two transmembrane domains and two nucleotide binding domains either all on one polypeptide or as two separate units (2* TM+NBD); arose by gene-duplication
  - mostly found on plasma membrane
  - responsible for drug resistance in all forms of life;



Transport ATPases - Examples

over the next few slides, you will meet three exceptionally important transport ATPases

FoF1 the dynamo that sustains life

Ca-ATPase the engine that drives muscle contraction

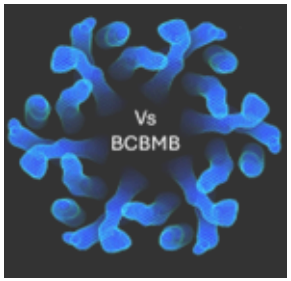
and

Na^+/K^+-ATPase the "cleanup" engine that maintains membrane potentials and enables most of secondary active transport

making separate lecture on each would not be difficult

however
within the framework of this introductory chapter on membrane transport processes we only will summarize the most important details

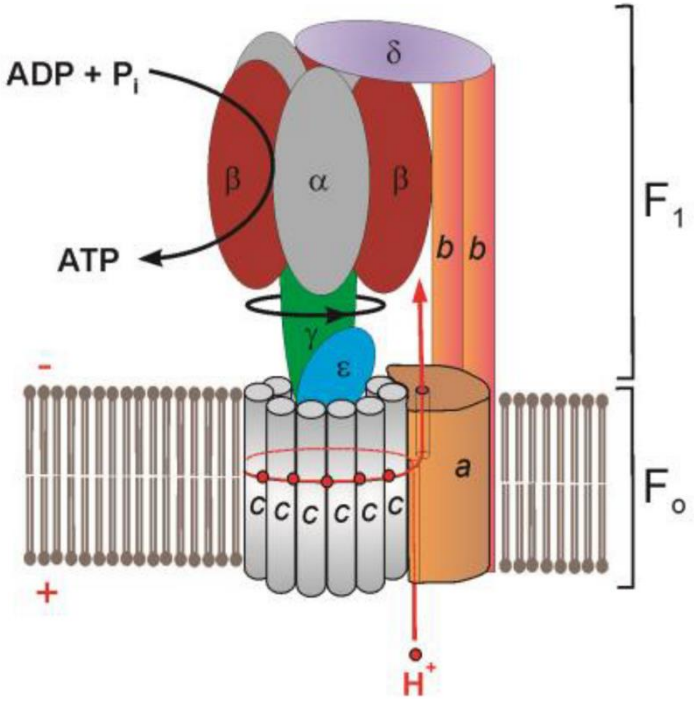
[if you feel that a more detailed treatment of these would be beneficial for you or general audiences, let me know; if I get enough requests I would be willing to develop more "case study" silent lectures]



F_oF_1 … Nothing Goes Without It

- mitochondrial F_oF_1-ATPase/Synthase is a complex molecular machine in the inner mitochondrial membrane.

mitochondrial matrix (= inside)

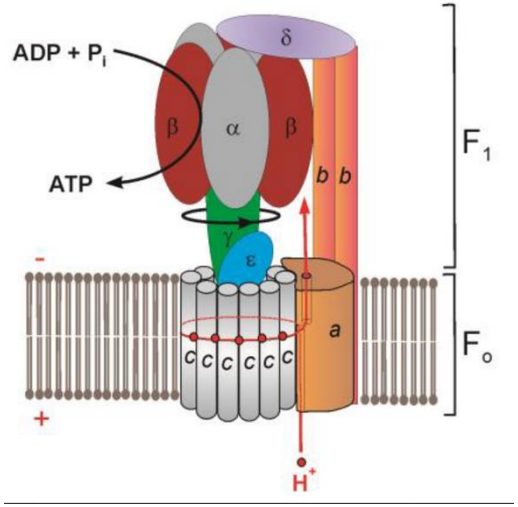


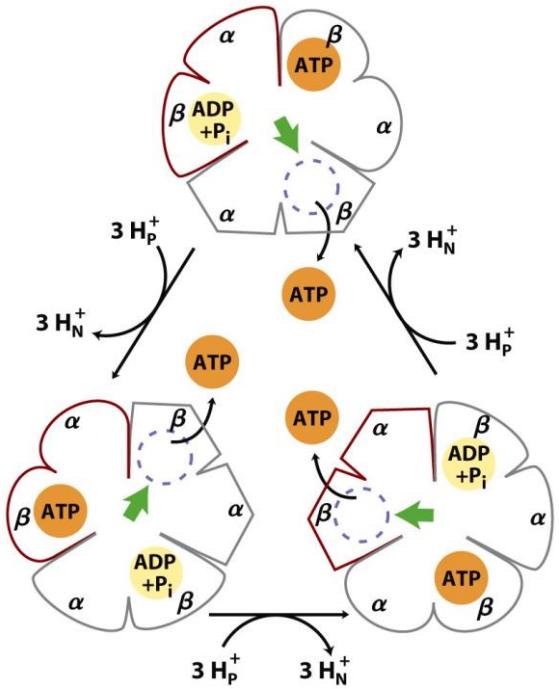
intermembrane space between outer and inner mitochondrial membrane

Int. J. Mol. Sci. **2023**, *24*(6), 5417;
https://doi.org/10.3390/ijms24065417

- **under normal conditions, F_oF_1-ATPase/Synthase synthesizes ATP** utilizing the proton gradient that is formed by the respiratory chain complexes
  (key to remember that transporters can work in both directions!)
- peripheral F_1 has the subunit composition $\alpha_3\beta_3\gamma\delta\varepsilon$
  (this is a complex quaternary structure!)
- the transmembrane part F_o (o for oligomycin sensitive) has the subunit composition $ab_2c_{10\text{-}12}$
  (also a complex quaternary structure)
- The ε subunit tethers the peripheral part of the enzyme to the c-ring and anchors the γ-subunit (stalk) which enters the trimeric set of $\alpha\beta$-pairs through a central cavity
- The ab_2/δ interaction holds the $\alpha_3\beta_3\gamma\varepsilon$ in place, which is necessary because during proton flux, the C-ring rotates
  (each of the C-ring subunits has a single acidic–carboxylate group that is positioned to face the membrane hydrophobic core ➔ only stable if a proton is bound, neutralizing the charge ➔ the protonation/deprotonation provides the driving force for the rotor to spin)
- because the stalk is tethered to the c-ring, rotation of the ring causes the stalk to rotate too
- this setup gives rise to a “rotational” catalytic mechanism
  (next slide)

F_oF_1-Mechanism





- note: the three αβ-subunit pairs are asymmetric - one is empty, one holds ADP+P_i, one holds ATP.
- the **empty β-subunit is the one in contact with the stalk** (γ-subunit, green arrow),

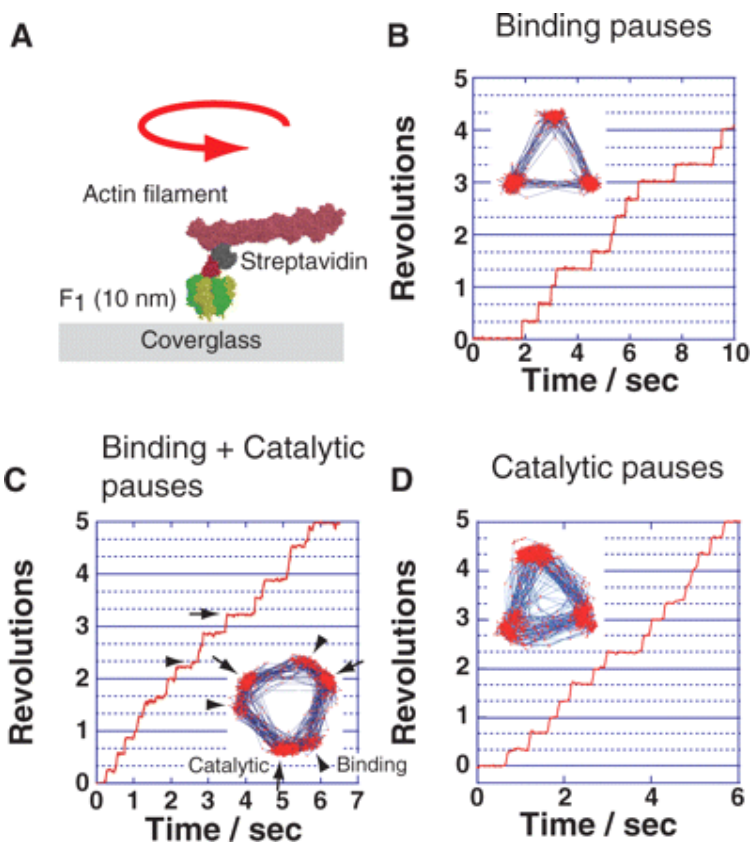
  ➔as the stalk rotates counterclockwise it will touch the next β-subunit along the path which still holds ATP. As the stalk arrives, ATP will be released, ADP+P_i will be loaded into to the β-subunit that the stalk departed from, and ATP synthesis takes place in the β-subunit that lies ahead on the rotational path.

- all three processes (release, reloading, synthesis) are **strictly coupled!** That is, an empty β-subunit is always flanked by one bound to ATP and the other one bound to ADP +P_i
- this process repeats itself each time the stalk rotates one "click" → after full rotation of γ, each β-subunit has gone through all three conformations (= reset itself)
- Each cycle is coupled to the movement of ~9 protons from the intermembrane space (H_P^+) to the matrix (H_N^+)

A **REALLY** helpful animation of this can be found here
(all the points made in this video have since been confirmed)

https://www.youtube.com/watch?v=PjdPTY1wHdQ

A Clever Experiment that Proved the Rotation Mechanism



The Journal of Biochemistry, Volume 149, Issue 6, June 2011, Pages 655–664, https://doi.org/10.1093/jb/mvr049

Fig. 3 single-molecule rotation assay of F1.

(A) a schematic image of the experimental setup. The α3β3-ring is fixed on the glass surface to suppress translational and rotational Brownian motion of the F1 molecule. A rotation probe (fluorescently-labelled actin filament) is attached to the γ-subunit to visualize the rotary motion under an optical microscope.

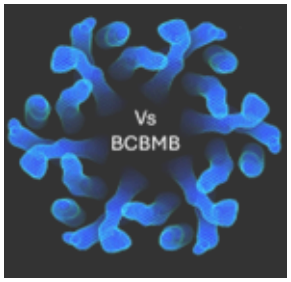
(B) rotation of F1-ATPase under ATP-limiting conditions (60 nM ATP). Inset shows the trajectory of the centroid of the probe.

(C) rotation of mutant F1-ATPase, β(E190D), at 2 μM ATP. Under this condition, 120° step is divided into 0° and 80° dwelling positions. Each pause corresponds to ATP binding and ATP catalytic dwelling positions, respectively. Arrow heads and arrows indicate the positions of ATP binding and catalytic dwell, respectively.

(D) rotation of a mutant F1-ATPase, β(E190D), at saturating ATP (2 mM). Hydrolysis rate is slowed by the mutation so that three pauses to wait for the hydrolysis reaction are observed.

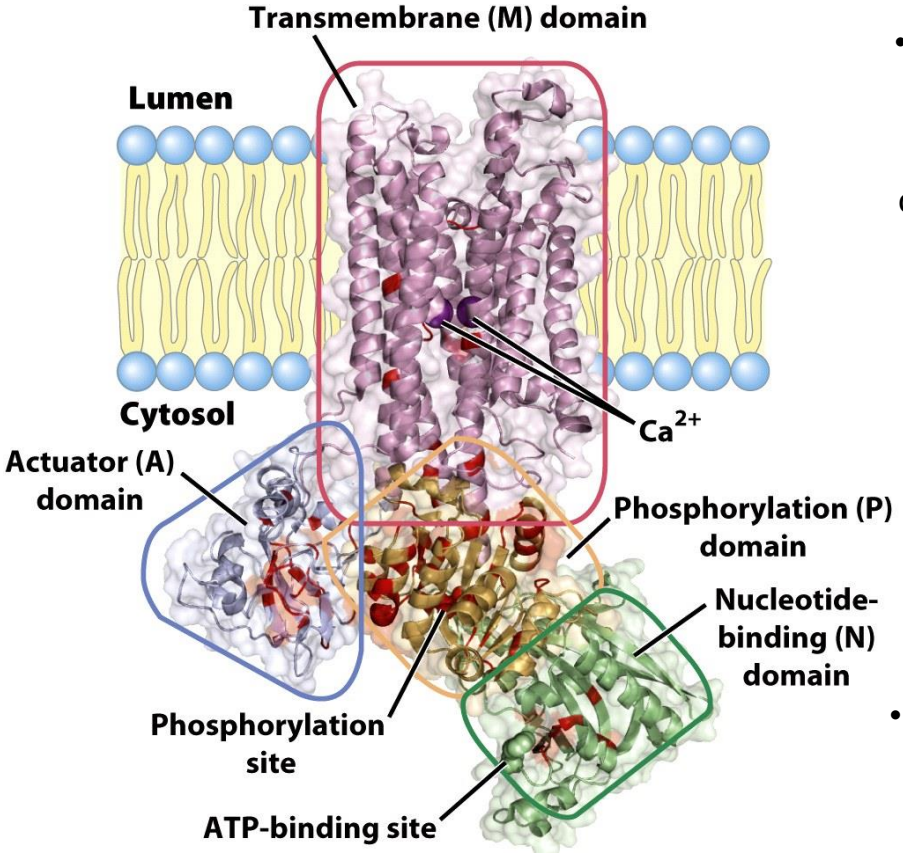
note: *in vivo* turnover of whole F_oF_1 is ~300 s^{-1}

(compare that with 10,000,000 K^+-ions per second → that is the difference between needing conformational change, and a static scaffold)



Transport ATPases - Example #2

Sarcoplasmatic Ca^{2+}ATPase



SERCA (sarcoplasmatic reticulum Ca^{2+}-ATPase)

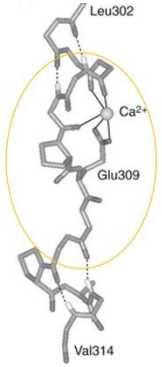
- **in muscle**; pumps Ca^{2+} into a specialized form of the endoplasmatic reticulum (sarcoplasmatic reticulum) to terminate muscle contraction.

other family members are in the plasmamembrane, pumping Ca-ions out of the cell

- **the Ca^{2+}-binding site is inside the membrane,** and accepts 2 Ca^{2+}, they are bound by acidic aspartate/glutamate sidechains

on two **partially unfolded helices**

(PROTEINS Chapter, Slide 93; excerpt shown here)



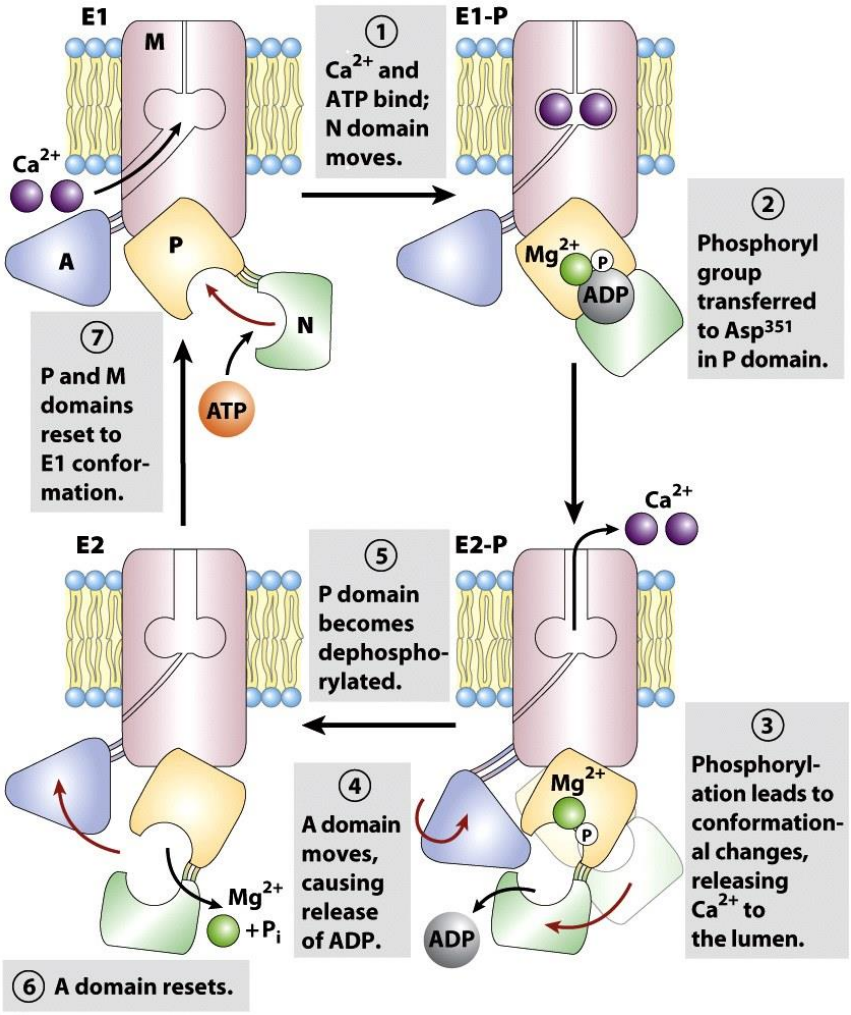
- the actuator domain communicates movements of the N and P domains to the two calcium-binding sites (~40-50Å away), lowering their affinity and eliciting calcium release
- the pump operates against a large gradient (µM in cytosol → mM in SR)

Tansport ATPases - SERCA continued

the pump cycles between two different conformations, referred to as E1 and E2

does that remind you of something?

…try to recall …..



Tansport ATPases - SERCA continued

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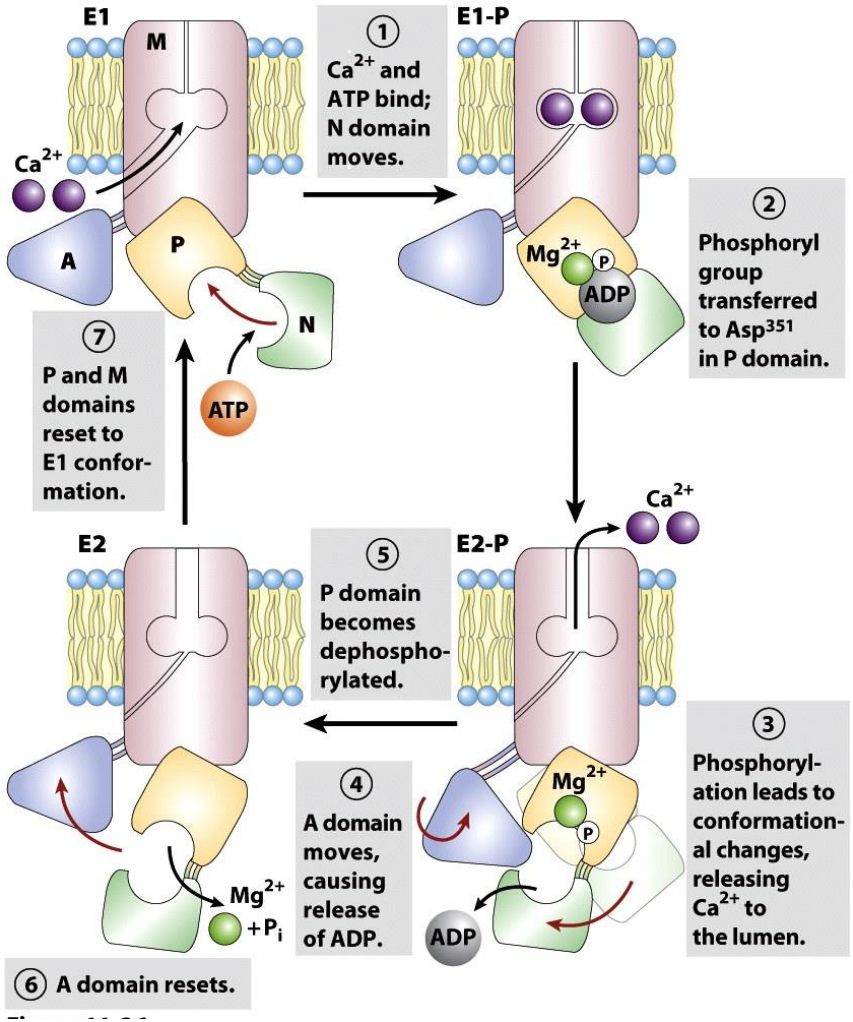


Answer

the principal aspects that define this pumping cycle resemble the mechanistic sequence of glucose transporters - it just got coupled to ATP-consumption

$$S_{out} + T_1 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} S_{out} \bullet T_1$$

$$k_{-4} \downarrow\uparrow k_4 \qquad k_{-2} \downarrow\uparrow k_2$$

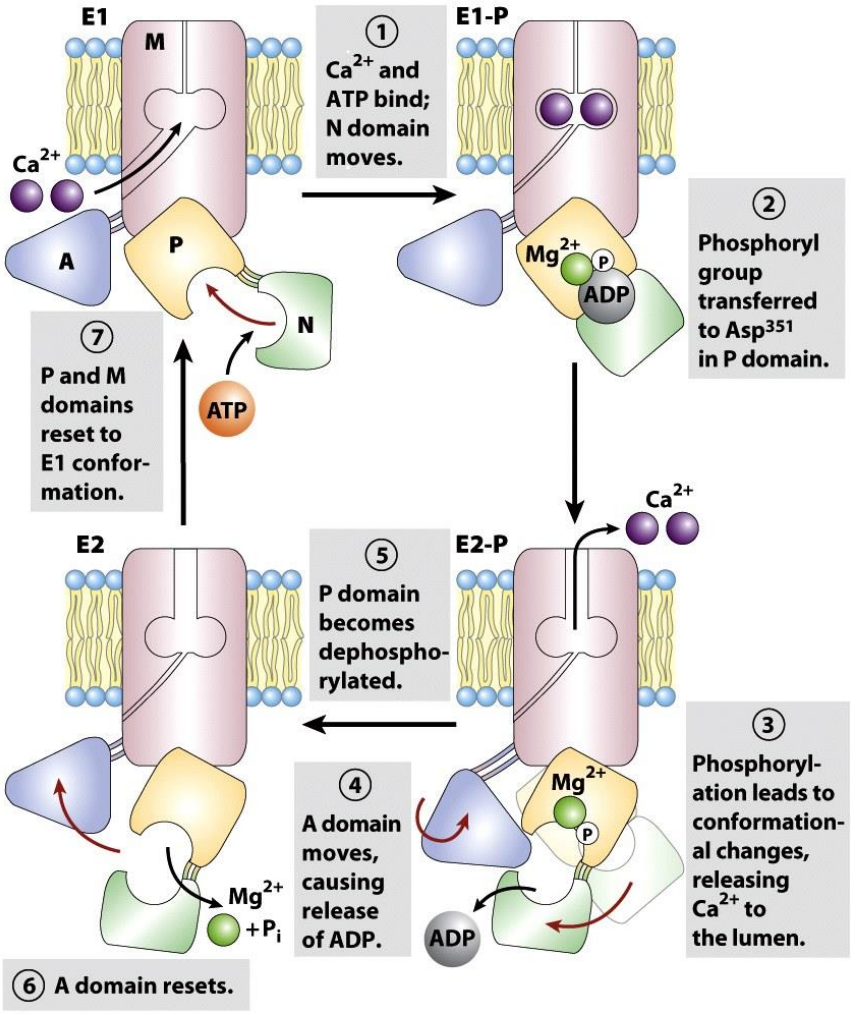
$$S_{in} + T_2 \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} S_{in} \bullet T_2$$

Turnover rate

200 cycles per second

(compare: GLUTs 3,000/s)

Tansport ATPases - SERCA continued



take note
the energy for the transport does not come from simply hydrolyzing ATP.

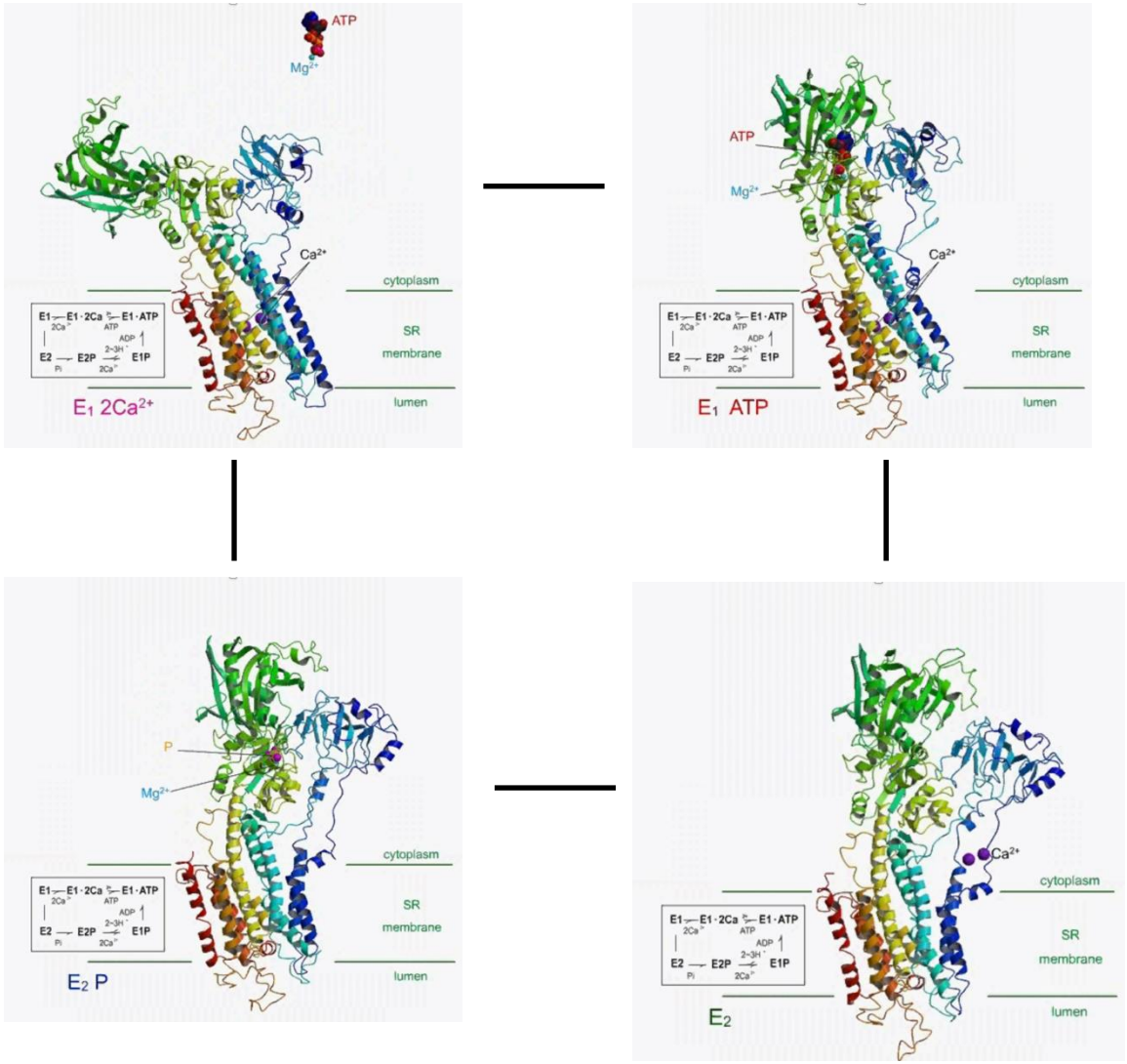
instead: the terminal phosphate of ATP is transferred to a specific amino acid sidechain of the pump!

this disturbance to the marginally stabilized native fold
(PROTEINS Chapter, slide 45)
will trigger molecular movements as the protein searches for a new global Free Energy minimum

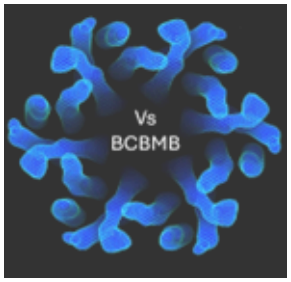
…and those changes trigger more changes …. and so on, until the phosphate is removed from the protein, allowing it to reset.

SERCA - The Movie

https://youtube.com/shorts/M_Y1w1x52rE (highly recommend watching this!)

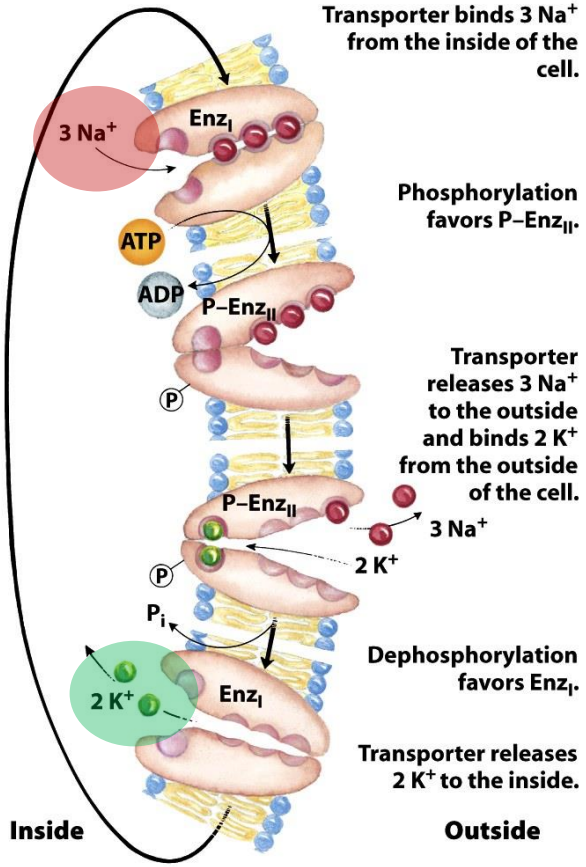


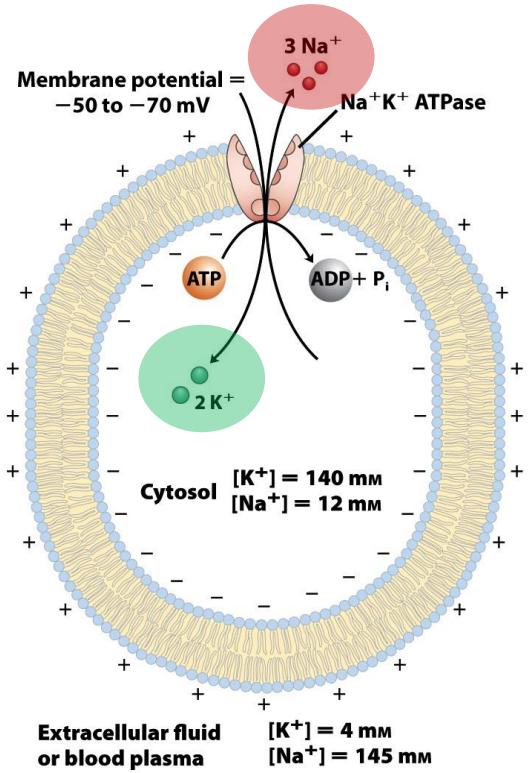
even in the still screenshots, you can appreciate just how large and significant the conformational changes are during the pumping cycle. Imagining that all this happens **200x per second** inspires awe



Transport ATPases – Example #3 Na^+/K^+-ATPase

this carrier is universal and required in all cells because it maintains the fundamentally important asymmetry of sodium and potassium ions across membranes





IMPORTANT NOTE

3 Na^+ out
2 K^+ in

→ this transport is

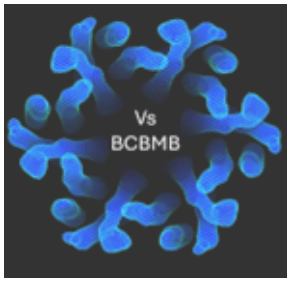
not electroneutral

it is

electrogenic

contributing to the buildup of negative charge inside the cell

key to formation of membrane potential which drives action potentials and - as we see next - secondary active transport

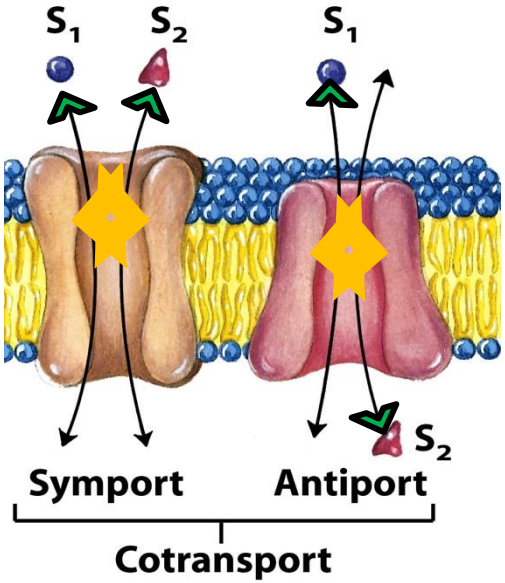


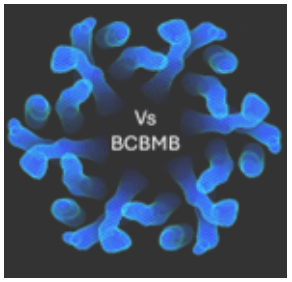
Transporter Types Revisited

having learned how gradients can be established by primary active transport, let's revisit symport and antiport

what is different this time we look at this?

…what are your thougths?....





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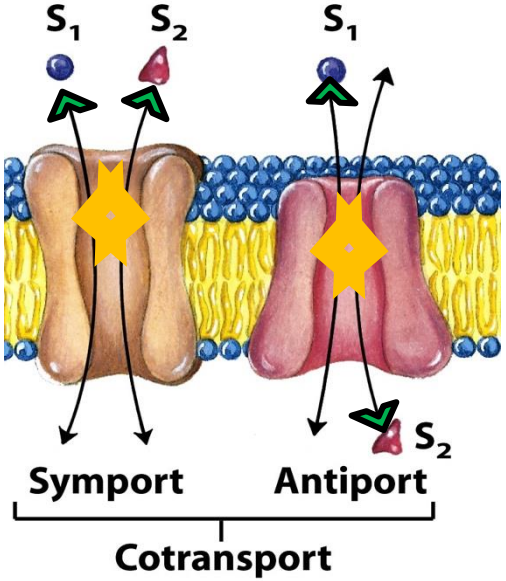


Answer

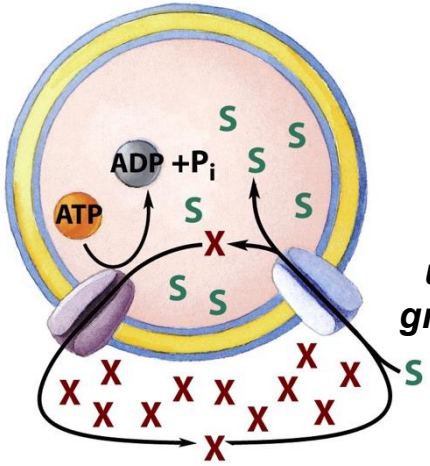
existing gradients can be exploited by symporters and antiporters to establish gradients too!

that is:

symport and antiport refer to a mechanistic/directional aspect, NOT

the **energetics** of it!

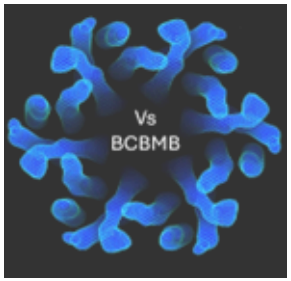
primary active
to establish a gradient
independent of *pre-existing gradient of something else*



secondary active
to establish any gradient
using pre-existing gradient *of something else*

what co-solutes would you use to turn symporters/antiporters into secondary active transporters?

…guess ….!



Transporter Types Revisited

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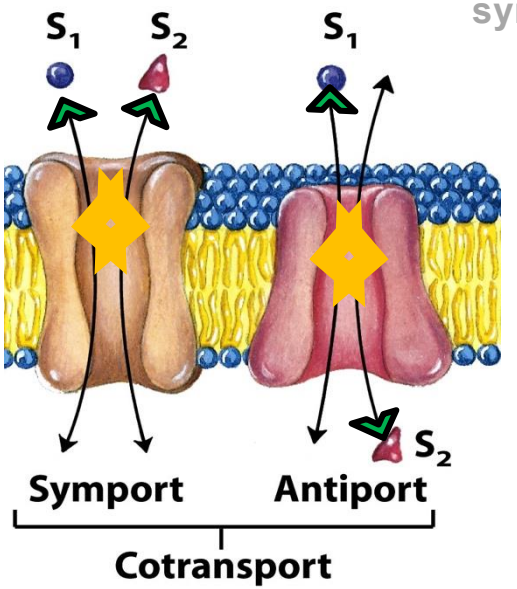


Answer

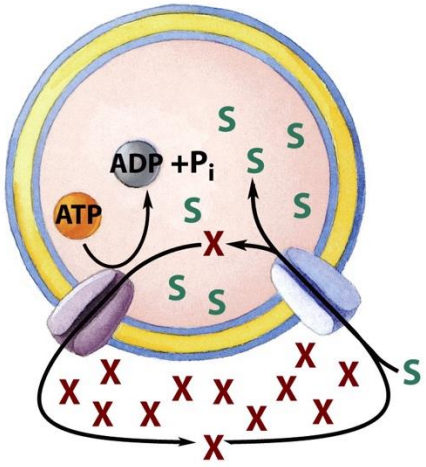
most common

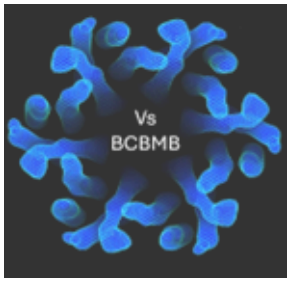
H^+

(easily available everywhere)

Na^+

(naturally abundant outside of cell)





Putting Pieces Together

when discussing cellular **glucose uptake**, we found it to be **passive in most cells** because intracellular glucose concentration typically is 2-3 orders of magnitude lower inside cells than in the blood stream.

however, on slide 92 it was already pointed out that a really important exception is found in the gut, where the glucose enters the body by passing into gut epithelial cells.

gut epithelial cells have **intracellular glucose** concentrations of 10-30mM – which his **higher than the concentration in the lumen of the gut**

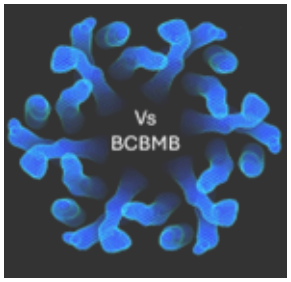
the **same is true for other sugars and nutrients like amino acids as well**

when exploring GLUT transporters that function as passive facilitators for glucose, we simply had to accept this exception in the gut and leave it at that.

now – however – we can understand how Nature solved the problem of having to transport glucose and nutrients against their concentration gradients at the point of initial uptake into body….

continuing along the line of what was just introduced …how would you solve the problem?

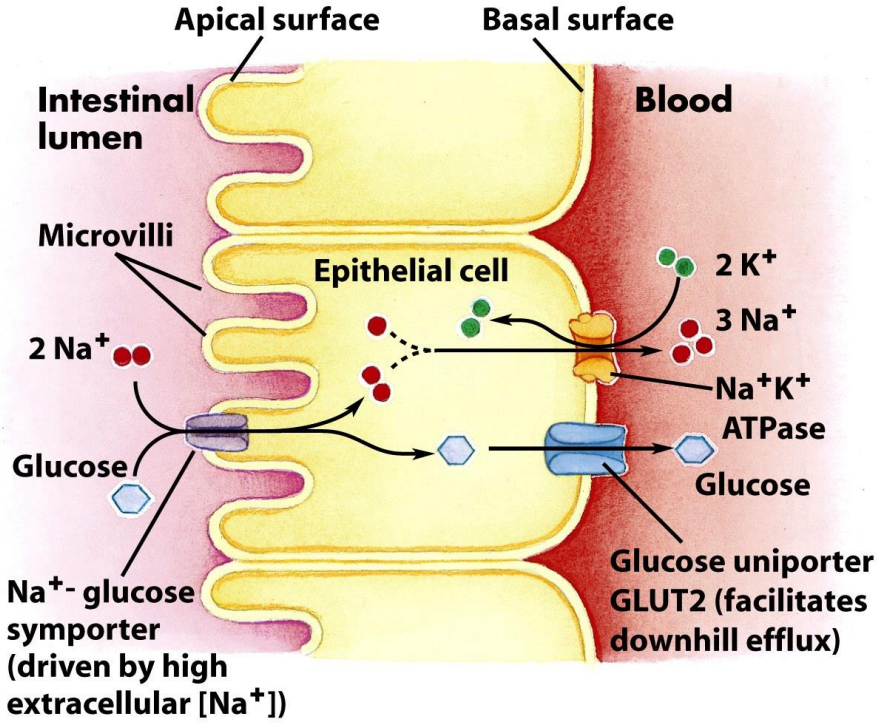
Primary Active or Secondary Active? Why?



Answer

Ion Gradients Provide Energy For Uphill Transport in Secondary Active Transport Processes

- epithelial cells exploit the existence of ion gradients to **push molecules uphill by symport**
- Na-ions are the most common co-solute



Ironically
strict low sodium diets are still recommended …

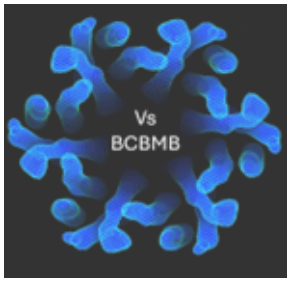
..and for people with high blood pressure, being conscious about sodium content of foods is a good thing

…but let's assume you are a healthy, high performing athlete …

…cutting back too much on sodium is actually doing harm

…especially if you sweat a lot or lift weights …
(gobbling down protein shakes does no good if you don't consume enough sodium and potassium along with it)

in fact: you may have experienced that your body and mind feel **really tired after an intense workout**/exercise **but for some reason you just cannot fall asleep**
if that is familiar: try eating a small amount of salty food …it likely will fix your troubles because the restlessness **can be caused by low sodium levels (hyponatremia)** …not being able to fall asleep is your body's way of telling you: "eat salt" … (sometimes, knowing some basic biochem is really useful :))



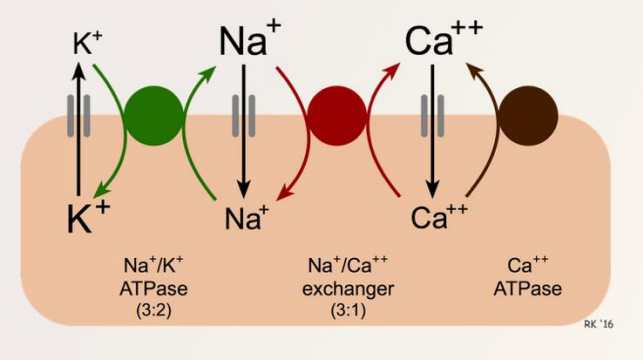
Answer

Ion Gradients Provide Energy For Uphill Transport in Secondary Active Transport Processes

gradients can also be exploited by antiporters to couple uphill transport to downhill transport of a second substrate in the opposite direction.

an important example is the non-ATPase dependent expulsion of Ca^{2+} coupled to Na^+-influx in cardiac and neuronal cells.

why would one need that?



Answer

Ca^{2+}-ions enter these cells during normal function (excitation-contraction coupling; synaptic signal transmission)

but

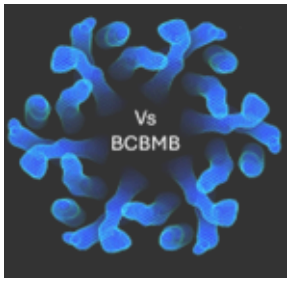
intracellular buildup of Ca^{2+}-ions toxic and interferes with intracellular, Ca-dependent signaling processes.

➔ calcium ions need to be pumped back out.

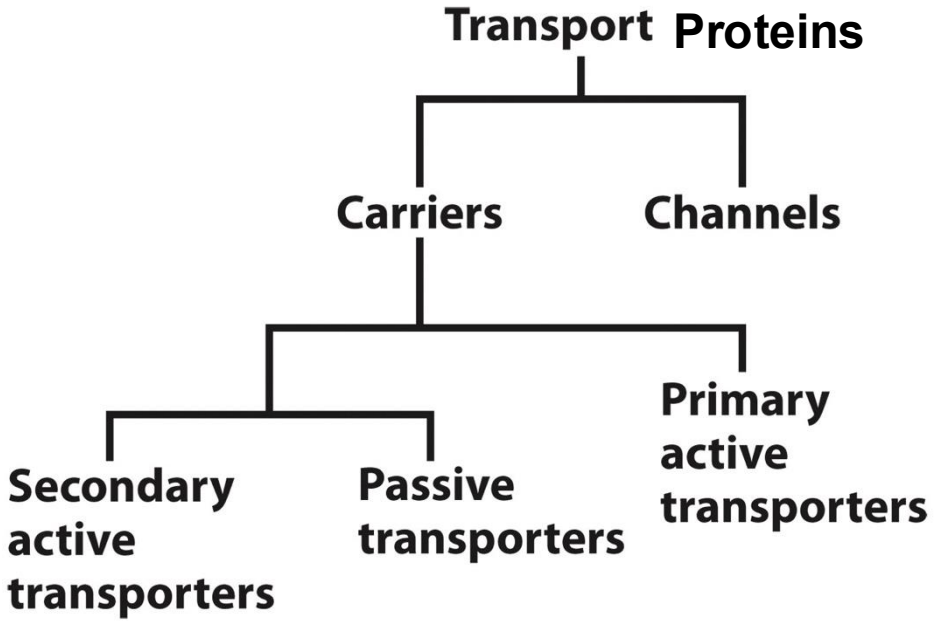
using a sodium dependent antiporter **and** a transport ATPase is very energy efficient because

- **Na^+/Ca^{2+}-exchangers have low affinity for Ca^{2+}-but turn over very rapidly**.
- **Ca^{2+}-ATPases have high affinity but are slower than the exchange**r

➔division of labor: exchanger extrude large quantities quickly at the beginning,
ATPases finish the job to restore and maintain very low intracellular Ca^{2+}-concentrations

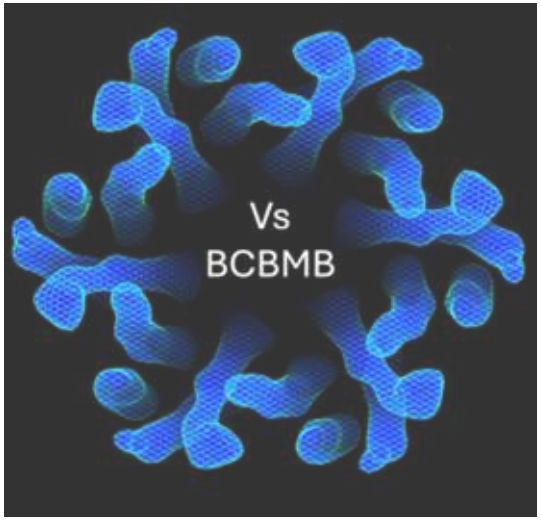


The Big Picture and Summary



- **general:** transport proteins facilitate diffusion of solutes across bilayers
- two fundamentally different types: passive (dissipation of gradients) and active (generation of gradients)
- channels can ONLY dissipate gradients (= the change in (electro)chemical potential provides all the required energy)
- channels and carriers/transporters are defined by their mechanism (non-stoichiometric vs stoichiometric) ➔ Transporters are saturable, channels are not

- active transport can be achieved by coupling to ATP-hydrolysis (primary active transport) or existing gradients of other solutes (secondary active transport)
- secondary active transport most commonly coupled to Na^+-gradients
- secondary active transport can be symport or antiport
- symporters and antiporters need not necessarily be active transporters



Thank You for Working Through This
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