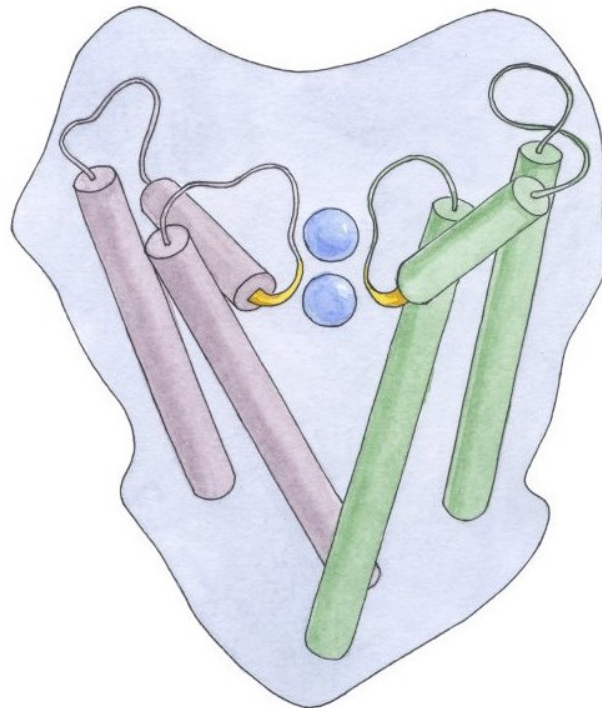
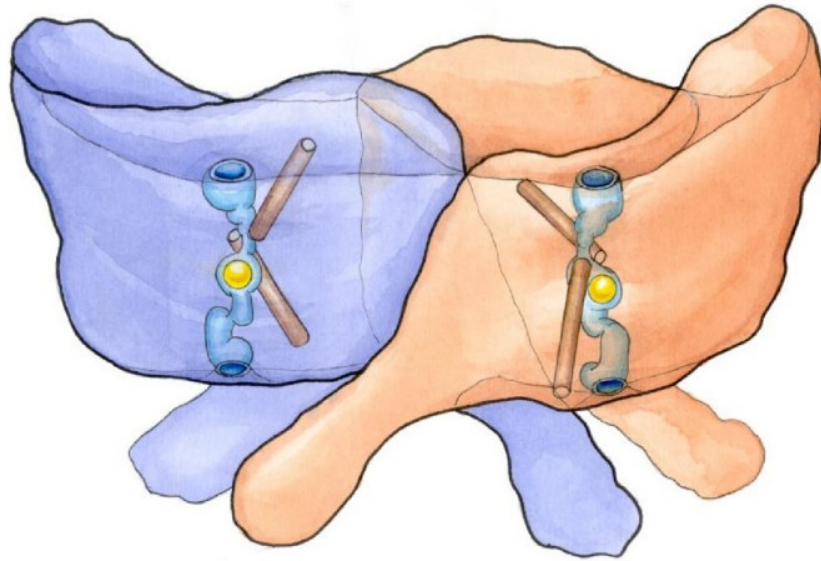
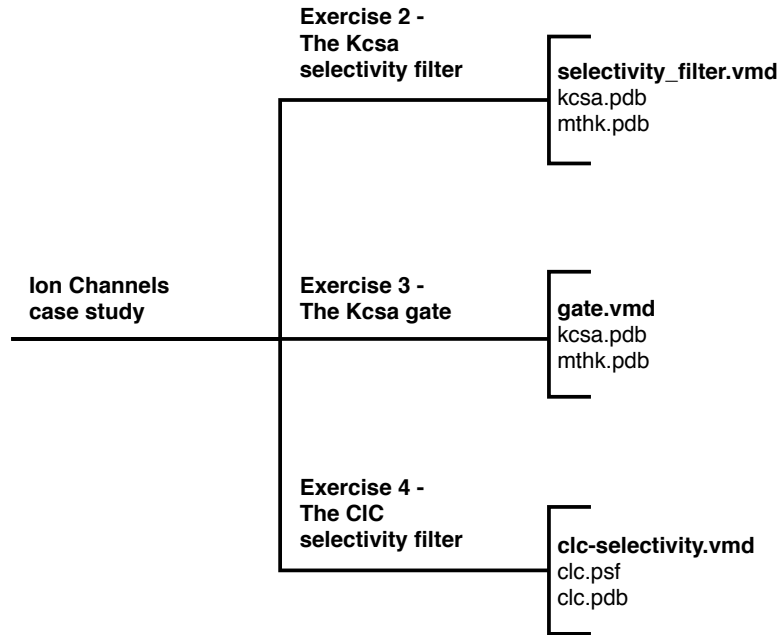


Case Study: Structure of Ion Channels

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These are the files you will need to complete the exercises in this case study.



Introduction

The physiological function of ion channels had been observed long before they were known to exist. Many cells, such as nerve and muscles cells, were known to have excitable cell membranes that would respond to variations in their electrical membrane potential through an all-or-nothing response. In the 50s and 60s, Hodgkin, Huxley and Katz performed a number of investigations of the excitable membrane of the squid giant axon, a gigantic elongated nerve cell reaching up to 6mm in length [1]. In studying the propagation of the electrical signal along the axon, they noticed that the membrane's permeability to ions such as Na^+ and K^+ would abruptly change when they altered the axon's transmembrane electrical potential beyond a certain threshold value. More surprisingly, it was later found that the currents for Na^+ and K^+ are independent of each other, implying that neural membranes contain channels specific for Na^+ and K^+ conduction. Something in the membrane was regulating which ion species could cross it, and when. It was soon discovered that the regulators of ion passage across biological membranes were specialized proteins called ion channels.

Ion channels are transmembrane proteins that selectively allow a given species of ion to pass through them. Ion channels, unlike ion transporter, are passive, which means that they do not require a source of energy in order to function. This means that the inside and outside of the cell must be out of equilibrium in order for a net flow of ions to occur. The force that drives the ions through an ion channel is a combination of the electrical transmembrane potential and of the ion concentration gradient across the membrane, and the combination of these two effects is called the electro-chemical gradient. The condition at which these two forces balance and equilibrium occurs is called the Nernst equation, and applies separately to every species of ion

present around the membrane:

$$\Delta V = V_{\text{in}} - V_{\text{out}} = -\frac{k_B T}{ze} \ln \left[\frac{c_{\text{in}}}{c_{\text{out}}} \right] \quad (1)$$

where ΔV is the transmembrane potential, k_B is the Boltzmann constant, e is the elementary charge, T is the temperature, z is the ion valence (1 for Na^+ , 2 for Ca^{2+} , -1 for Cl^- , *etc.*), and c_{in} and c_{out} are the inside and outside concentrations of ions.

Ion transporters, on the other hand, couple ion translocation across the membrane to a source of energy such as a chemical compound or the transmembrane gradient of another ion. By using such an alternate source of energy, ion transporters can force a given species of ions in or out of the cell against its electro-chemical gradient.

Exercise 1: The Nernst Equation

- Derive the Nernst equation (Eq. 1) from first principles. You can do this by considering that the probability that an ion will find itself inside or outside of the cell depends on the energy difference on either side.
- The following table shows ionic concentrations inside and outside of a typical mammalian skeletal muscle cell in units of molar (1 M = 1 mol/L), using values from [2].

ion	c_{in}	c_{out}
Na^+	12 mM	145 mM
K^+	155 mM	4 mM
Ca^{2+}	0.1 μM	1.5 mM

Use the values in the table to derive the transmembrane potential for each ion species in units of mV.

- For each ion species there can be many different types of ion channels that regulate it, and each of these channels is designed to open and close under different conditions. When the membrane is at rest (*i.e.*, when it is not being electrically excited), some types of channels will be open and some will be closed. The resting potential of a typical mammalian skeletal muscle cell is about -90 mV (the negative sign means that the cell interior is at a lower potential than the exterior). By comparing this potential to those that you calculated earlier, what can you say about which types of channels are predominantly conducting when the cell membrane is at rest (also consider that there are other common ions, such as H^+ , Cl^- , which can contribute to the cell's electrical gradients)?
- In the previous question, you probably found that one ion species' equilibrium transmembrane potential, in particular, matches that of the cell membrane. It turns out that cell membranes at rest are often very permeable for that ion species. Since this ion type is free to flow in and out of the membrane (through permanently open channels), why is its concentration so different on both sides of the membrane? Briefly explain how this is possible.

Because under a cell's normal operation, the ions on both side of the cell membrane typically need to be kept in non-equilibrium conditions (*i.e.*, at different ion concentrations and electric potentials), ion channels sometimes need to be able to selectively block the flow of ions. Thus, most ion channels are gated. Ion channel gates, which are the parts of the channels that can move in order to allow or prevent ion currents across ion channels, are typically linked to a sensor. Sensors are the parts of the channel that can detect certain conditions or signal, such as a change in the transmembrane voltage, the pH, or even the presence of certain compounds. Such signals are used by ion channels to determine when they should activate or deactivate, and therefore allow or disallow ions to pass through them.

In this case study, we will examine the structural features of two families of ion channels: the potassium channels and the ClC chloride channels/transporters. We will then explain how these channels make use of their structures in order to accomplish their function, notably, permeation, selectivity and gating.

Structural features of the K⁺ channel

Here we focus on the structural properties of the KcsA K⁺ channel. KcsA, a bacterial K⁺ channel from *Streptomyces lividans*, was the first ion channel crystallized [3]. Discovering the structure of KcsA significantly contributed to the understanding of the ion conduction mechanism in ion channels and won the 2003 Nobel prize in chemistry [4].

Tetrameric structure of KcsA

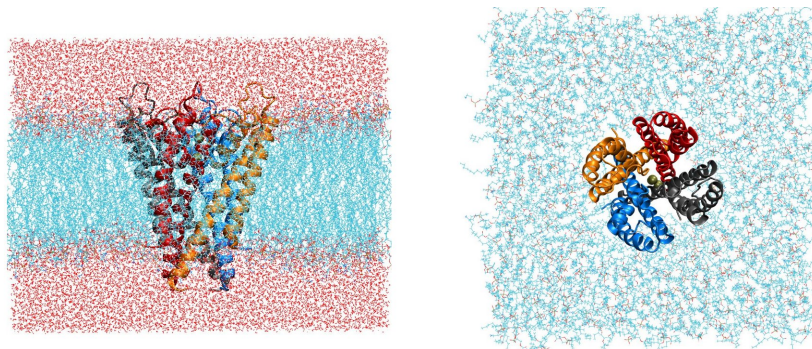


Figure 1: Side view (left) and top view (right) of the KcsA K⁺ channel after equilibration in a lipid bilayer. The protein is shown in cartoon representation, with each subunit colored differently. Lipid and water molecules are shown in line representation and colored by atom type. The figure can be reproduced using the provided VMD saved state `protein-lipids.vmd`.

Ion channels are membrane proteins, located in a lipid membrane. A snapshot of a simulation of KcsA embedded in a lipid membrane is shown in Figure ??, illustrating how the channel connects the solutions on both sides of the membrane.

Like all other K⁺ channels and most cation channels, KcsA is a tetramer composed of four identical subunits arranged symmetrically around a four-fold axis (Figure 2). Each of the four

subunits is composed of two transmembrane α -helices, called M1 (outer) and M2 (inner) and a tilted pore helix which runs only half way through the membrane pointing its C-terminal end toward the ion conduction pore. The inner hole surrounded by the four subunits defines the ion conduction pore, which is easily identifiable due to the K^+ ions revealed inside it in crystal structures of various K^+ channels.

The selectivity filter

The selectivity filter is the name for the narrowest region of an ion channel pore, which interacts with the permeating ions and ensures that ions pass through the channel selectively. In the case of KcsA, the selectivity filter is located at the extracellular mouth of the protein, as shown in Figure 2. At that point, the ion conduction pore narrows to about 3 Å in diameter, ensuring a tight interaction between protein and permeating ions. Specific K^+ -protein interactions in this region is what allows KcsA to select K^+ ions over other ions, in particular over Na^+ ions. Because the selectivity filter is what defines the nature of a given ion channel, this region is highly conserved both in sequence and in structure across a given type of ion channel and K^+ channels are no exception. The selectivity filter in KcsA is composed of a sequence of five amino acids: TVGYG. This stretch of amino acids, also known as the signature sequence of K^+ channels, is often used for identifying their functions. Figure 3 shows the conserved sequence of the selectivity filter in three different K^+ channels, KcsA and MthK [5], and Kv1.2 [6].

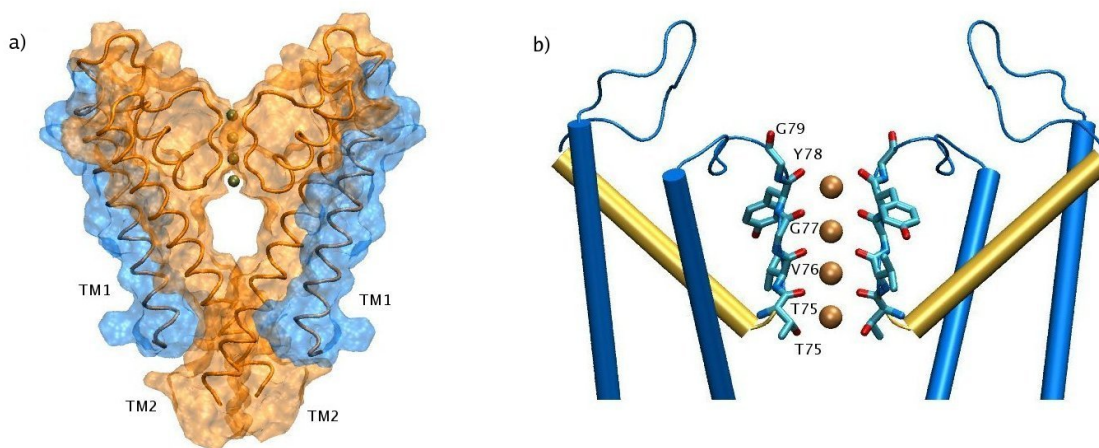


Figure 2: (a) Side view of the KcsA K^+ channel in tube and transparent surface representation of VMD. M1 and M2 transmembrane helices are colored in blue and orange, respectively, with four K^+ ions in the selectivity filter. (b) Selectivity filter of KcsA. Four K^+ binding sites inside the selectivity filter are shown. The figure can be reproduced in VMD, using the provided saved state file, `binding_sites.vmd`. For clarity only two of the four subunits are shown here.

A closer look at the selectivity filter (Figure 2) reveals five oxygen atoms per subunit, coming mainly from the channel's backbone carbonyl groups, which face the ion conduction pathway. Four binding sites for K^+ ions are also found in this region. In each of these binding sites, the K^+ ion is located at the center of a cage formed by 8 oxygen atoms, mainly from carbonyl groups (two from each subunit). This particular arrangement of the selectivity filter is designed such

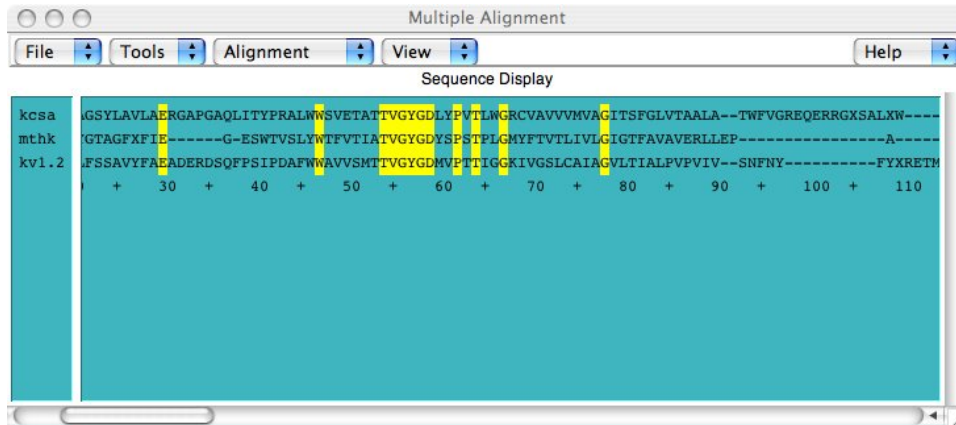


Figure 3: Sequence alignment of KcsA, MthK, and the pore domain of Kv1.2 potassium channels. Conserved residues are highlighted in each sequence. (The alignment can be reproduced using the multiseq extension of VMD [7], and the provided files `kcsa.pdb`, `mthk.pdb`, and `kv1.2.pdb`.)

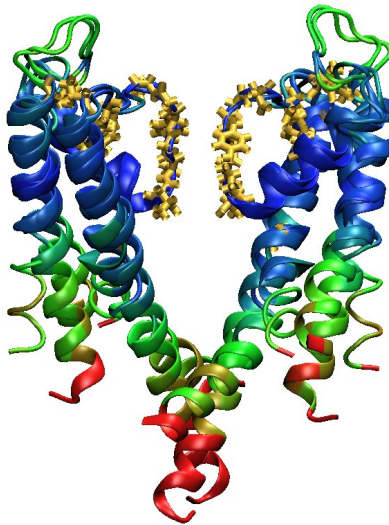


Figure 4: Structural alignment of KcsA, MthK, and the pore domain of Kv1.2 potassium channels, produced using VMD multiseq [7]. Alignment colored according to *structure* similarity. Blue and red regions correspond to high and low structural similarities, respectively. The conserved residues, as shown in Figure 3, are highlighted in yellow bond representation.

that the binding sites can accommodate a K^+ ion (with a radius of 1.33\AA) easily, while at the same time, the smaller Na^+ ions (with a radius of 0.95\AA) are too far from oxygen atoms at the same position to bind strongly to the pore.

For the selectivity filter to acquire such an unusual structure the peptide main chain has to adopt an unusual conformation, in which the dihedral angles of the TVGYG sequence alternate between left-handed and right-handed helical regions of the Ramachandran plot. Figure 5 shows the Ramachandran plot of the residues in the selectivity filter. One can see that the dihedral angles of the sequence TVGYG alternate between the left-handed (lower left) and right-handed (upper right) helical regions. Naturally occurring amino acids strongly prefer right-handed helical angles, which corresponds to the blue region at the lower left of the plot. However in the selectivity filter two of the glycine residues, Glycine 79, Glycine 77, and Threonine 75 all have ψ and ϕ values in the left-handed helical region. Glycines can adopt left-handed conformations more easily than other residues because they do not have sidechains. However, the high energy configuration of Threonine 75, has to be compensated for by some other interactions in the selectivity filter. This feature of the K^+ channels, allow the unusual secondary structure of the selectivity filter to be maintained and will be explored in exercise 2.

Exercise 2: Selectivity filter of the KcsA K^+ channel. In this exercise you will explore the structural characteristics of the selectivity filter in KcsA channel. Start a new VMD session and load the VMD saved state file `selectivity_filter.vmd`. You should see the backbone of the protein in cartoon representation along with two crystallographic K^+ ions.

- Create a new representation for the selectivity filter amino acids, by selecting "noh protein and resid 75 to 80", and the Licorice drawing method. You should see the oxygen atoms from each monomer surrounding the K^+ ions in the filter.
- Make a Ramachandran plot of the residues located in the selectivity filter. From the VMD main menu, choose **Extension**→ **Analysis**→ **Ramachandran Plot**. In the selection field, type "resid 75 to 80". Clicking on each point on the plot shows you the corresponding residue in the protein. Identify each residue in the left-handed and right-handed helical regions of the Ramachandran plot. Which residues are located in the left-handed region?
- It is common to have left-handed dihedral angles in the active sites of the enzymes. Usually the binding energy of the substrate helps to maintain the secondary structure of the protein. In the case of K^+ channels, what are the possible interactions necessary to maintain the secondary structure of the filter? What would you expect to happen if there are not enough K^+ ions in the selectivity filter?

The vestibule

One feature of the K^+ channel is the presence of a water-filled cavity in the middle of the ion conduction pathway. Several ion channels possess such water vestibules on either or both ends

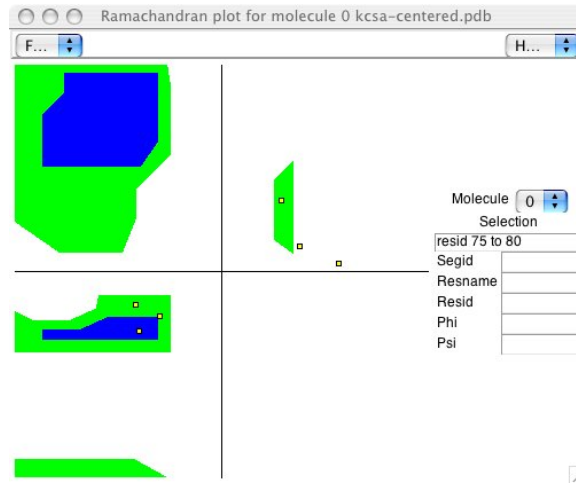


Figure 5: Ramachandran Plot of the residues in the selectivity filter of KcsA. Thr75, Gly77, and Gly79 are located in the left-handed helical region. Details of how to generate this plot are given in Exercise 2.

of the conduction pore. The vestibules serve as recesses inside the protein that effectively reduce the width of the pore that needs to be crossed by the permeating ions.

KcsA possesses a large vestibule half-way through the membrane, right below the selectivity filter, where the ion conduction pore is widened to provide a cavity of 5 Å radius. The cavity is lined by non-polar residues, and is wide enough for about 50 water molecules to reside there. Two tilted pore helices, located behind the selectivity filter, also point their C-terminal negative charges toward the center of the cavity. The dipole-moment of these half helices helps to stabilize K⁺ ions inside the vestibule, thus lowering the energy barrier for entrance of the K⁺ ions inside the cavity.

The conduction gate

Most ion channels conduct when called upon by a specific stimulus, such as the binding of a ligand or a change in the membrane potential. The process by which ion conduction is turned on and off is called gating, and usually involves a conformational changes of the protein that occludes or opens the ion conduction pathway.

The crystal structures of KcsA and MthK [5], shown in Figure 6 are two examples of K⁺ channels in their closed and open states, respectively. Examining the difference between these two structures provides valuable insight into the conformational changes that happen upon opening of the channel. In the KcsA structure (closed state) the pore is obstructed at the intracellular mouth of the protein, where the inner transmembrane helices (M2) come together and form a helix bundle as shown in Figure 6. The ion conduction pathway narrows from a diameter of 12 Å in the central cavity to 3.5 Å at the helix bundle crossing. However, in the structure of MthK, that is seen in Figure 6 in the open state, the inner helices tilt and bend

away from the pore axis. The ion conduction pathway is about 20 Å wide in diameter, thus allowing diffusion of the K⁺ ions to the selectivity filter. This mobile region defines the gate of the channel.

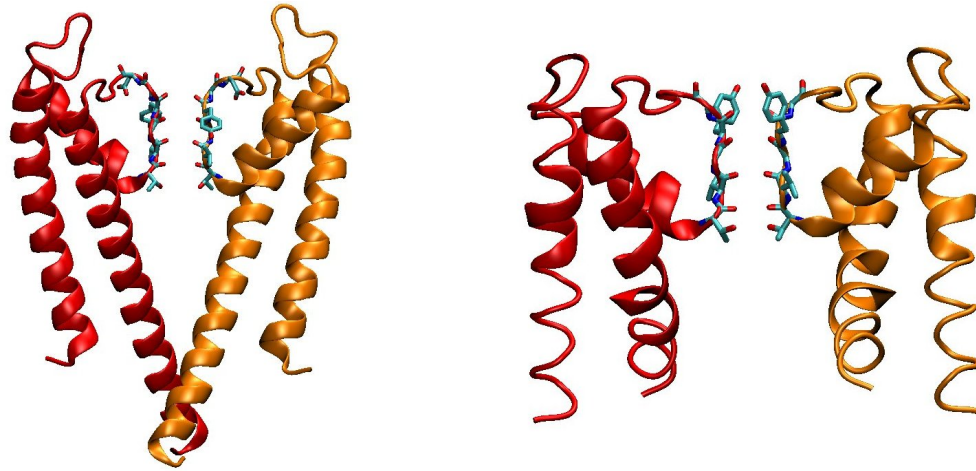


Figure 6: KcsA (left) is crystallized with its gate closed, while MthK (right) is crystallized with its gate open. For clarity only two of the four subunits are shown. The figures can be reproduced in VMD using the included pdb files `kcsa.pdb` and `mthk.pdb`.

Exercise 3: Hydrophobic Gate in the KcsA channel. In this exercise, you will explore the gate of the KcsA K^+ channel. Start a new VMD session and open the VMD saved state file `gate.vmd`. You should get a cartoon representation of the KcsA backbone, along with two K^+ ions in the filter.

- Rotate the molecule and identify the selectivity filter region. Below the selectivity filter there is a cavity. The intracellular gate of the channel is located right below the cavity. The narrowest region of the ion conduction pathway is where the M2 (inner helices) come together and form a helix bundle crossing. Identify the helix bundle crossing in the KcsA structure.
- How wide is the ion conduction pathway at the gate? Turn off the cartoon representation of the protein, and create a new representation. Select “protein and chain A C”, and choose `ResType` coloring method. Draw the molecule in `VDW` representation. How wide is the conduction pathway at the helix bundle crossing? How does it compare to the radius of the K^+ ion of 1.33\AA ? Render a snapshot of your figure, in VMD, by selecting `File`→ `Render` from the VMD main menu.
- What are the residues at the helix bundle crossing? Identify the hydrophobic residues that face the ion conduction pathway at the intracellular gate. For this purpose, change the `VDW` representation of the protein to `new cartoon`, and create a new `VDW` representation for “hydrophobic and resid > 100 and chain A C”. Color these residues by `ResID`. You should be able to identify three residues in each chain, that face the narrowest region of the pore.
- Describe the possible role of the hydrophobic residues at the intracellular gate in preventing the K^+ conduction to the selectivity filter.

Conduction and selectivity in the K^+ channels

In this section, we will look in more detail at how the K^+ channel’s structure is coupled to its function. K^+ channels must operate under two constraints: they must allow only passage of K^+ ions, but despite the rather strict selectivity K^+ ions must conduct quickly. K^+ channels use several strategies to satisfy these two constraints. We will investigate three such strategies in particular, the knock-on ion conduction, the dehydration barrier, and the “two conduction states” mechanisms.

The KcsA knock-on mechanism

The selectivity filter of the K^+ channels, as shown in Figure ??, contains four potential binding sites for K^+ ions. When the K^+ ion concentration exceeds 20 mM in the external solution, then the selectivity filter of KcsA will typically be occupied by two K^+ ions separated by a single water molecule [8–10]. The electrostatic repulsion between any two K^+ ions in adjacent sites is very large, about 40 kcal/mol, making it energetically very unfavorable for two K^+ ions to

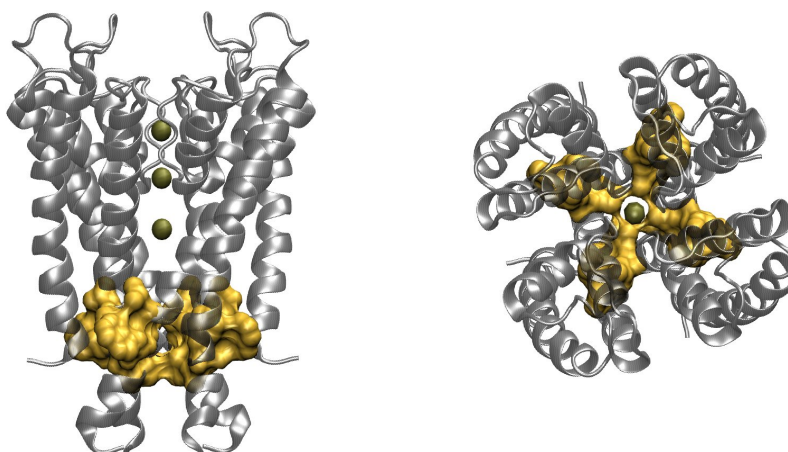


Figure 7: Hydrophobic gate in the KcsA K^+ channel. Side (left) and top (right) view of the KcsA backbone in cartoon representation. The hydrophobic amino acids at the intracellular gate are highlighted in gold. Details of how to reproduce these figures in VMD are given in Exercise 3.

occupy neighbouring sites in the KcsA selectivity filter, and this is why K^+ ions permeating through the filter are mostly found at least two sites apart.

The single file of water and K^+ ions move in a concerted fashion between two states: a K^+ -water- K^+ -water configuration, which is referred to as (1,3) using ionic binding site numbering (Figure 8), and a water- K^+ -water- K^+ configuration in which ions bind at positions (2,4). The (2,4) and (1,3) configurations share a similar energy, and the energy barrier between these two states is less than 5 kcal/mol. At room temperature, the ions alternate between these states until a third ion from the intracellular vestibular cavity approaches the selectivity filter at position (4). The incoming K^+ ion, then initiates a concerted transition to a state in which the selectivity filter is occupied by three ions.

The transient state, in which the outermost ion is coordinated by only four oxygen atoms, has a higher energy than the (1,3) and (2,4) configurations. This high energy transient state is followed by rapid dissociation of the outermost ion, from the extracellular side, reaching the more stable (2,4) state. The result is the conduction of one K^+ ion from the intracellular to the extracellular solution. In the ion conduction mechanism as described above each ion moves a fraction of the total distance, but the result is transfer of one charge from one side of the membrane to the other. This mechanism, known as the knock-on-mechanism was proposed by Hodgkin and Keynes [11] long before the structure of the first K^+ channel became known.

The high conductance of the channel for K^+ ions (10^7 ions/second), along with its selectivity is the result of a balance between the high affinity of selectivity filter for K^+ ions and the electrostatic forces between K^+ ions. The high affinity for K^+ ions is necessary for the channel to discriminate between K^+ and other cations; however, the repulsion between the K^+ ions in the selectivity filter, lowers the binding affinity of K^+ ions and allows the K^+ ions to permeate through the channel with a rate close to the diffusion of K^+ ions in the bulk water.

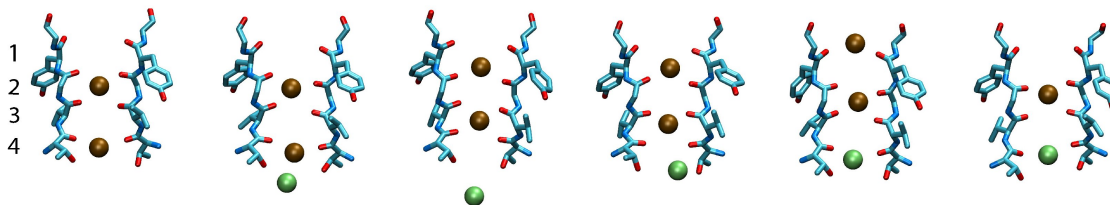


Figure 8: Snapshots of the selectivity filter of the Kv1.2 channel [6] during a molecular dynamics simulation, showing conduction of one K^+ ion through the filter. Four K^+ binding sites, S1-S4 are shown in the figure. A movie of the simulation trajectory is provided in `pot_conduction.mpg`.

The dehydration barrier

The most basic function of an ion channel is to regulate a membrane’s permeability for a given species of ion. At the simplest level, channels achieve this by forming a narrow transmembrane pore with an affinity for ions of a given charge. Given this very basic scenario, one might wonder how an ion channel can manage to discriminate between different ion species. After all, will a channel that has an affinity for a positively-charged ion such as K^+ not automatically attract Na^+ , Li^+ , or even Ca^{2+} along with any other positive ion (including larger organic ones), due to their similar charges?

One obvious candidate for regulating ionic selectivity is the channel’s pore diameter, which could be used to discriminate ions based on their size. However, adjusting only the pore diameter does not give the channel enough freedom to be able to select for just one ion over all others. For example, a “potassium” channel would obviously be wide enough to allow smaller ions such as Na^+ to pass through just as easily, yet it does not. In order to discriminate between ions of similar charges, a selective channel will need to select for ionic properties which differs significantly from one ion species to another. Aside from the ionic radius, the likely candidate for such a property is the ion’s solvation energy in water. Table 1 lists the solvation energies and radii for various atomic ions and shows that many ions of similar charge can be distinguished quite easily based on the combination of these two properties.

ion	$\Delta F_{solvation}$ (kcal/mol)	R (\AA)
Li^+	-131	0.60
Na^+	-105	0.95
K^+	-85	1.33
Rb^+	-79	1.48
Ca^{2+}	-397	0.99

Table 1: Solvation energies and approximate van der Waals radii for various atomic ions. Data taken from [12].

The solvation energy of an ion is the free energy of interaction between the ion and all neighboring water molecules. Since water is highly polar and mobile and ions have very strong charges, ions interact with water extremely favorably such that it is in general very hard to pull an ion out of water. An ion dissolved in water strongly influences the orientation of many

spherical layers of water around it (often called hydration shells). To be able to pull ions out of a water solution and into an ion channel. The ion channel needs to provide the ion with an environment that is similar to what the ion is experiencing when it is surrounded by water. So even though, hypothetically, a channel could be electrostatically attractive to both a K^+ and a Na^+ ion, these two ions have different solvation energies as well as different channel binding energies, (*e.g.* Na^+ is harder to dissociate from water than K^+ because it is smaller and has closer contacts with water molecules). Since the solvation energies and channel binding energies, in general, are quite substantive, small variations in these two energies can significantly affect the affinity of a channel for a given ion species. For example, if we assume that the pore-ion interaction energy for K^+ and Na^+ are both about -85 kcal/mol inside a given channel, then the channel would only allow K^+ to penetrate since that is enough to strip this ion from its hydration shell, but Na^+ with the same interaction will prefer to remain in the solution because of its stronger solvation energy. The interplay between ion-pore interactions inside the channel and solvation energies is what allows a channel to selectively allow the passage of one ion species over another.

For the case of KcsA, the channel recruits K^+ ions by emulating the water environment seen by K^+ . In solution, K^+ is surrounded by water molecules that have their negatively-charged oxygen atoms pointing toward it. As they enter or exit KcsA's selectivity filter, the K^+ ions are laterally coordinated by four water molecules (with oxygen pointing toward K^+). The selectivity filter itself consists of four layers each containing rings of four negatively-charged oxygen atoms from the protein backbone's carbonyl groups, as shown in Figure 9. Each of these rings emulates the spatial arrangement and charges of water oxygen atoms seen by K^+ when it is located in the vestibule right below the selectivity filter.

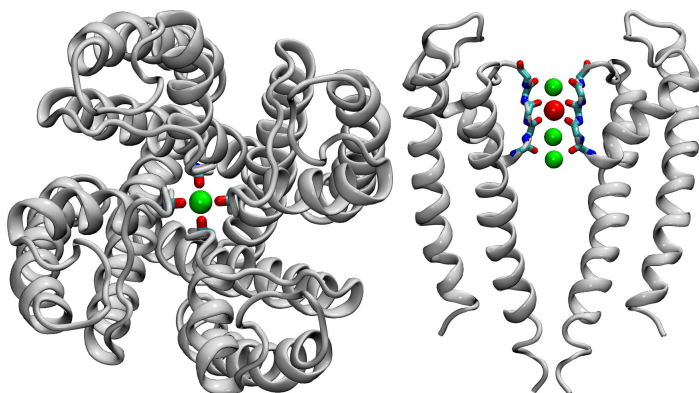


Figure 9: Top and side views of backbone carbonyl coordination of K^+ inside the selectivity filter. This figure can be reproduced by loading the file `kcsa.pdb` into VMD. The pore residues can be isolated using the selection "`protein and resid 74 to 80`" (optionally adding "`and chain A C`" to only show two of the monomers), and the ions can be shown using the atom selection "`ions`".

KcsA exhibits two conduction modes

KcsA has two distinct K^+ conduction regimes. At physiological conditions, KcsA allows ions through with a conductance of 500 pS. At concentrations of K^+ below about 20 mM, KcsA

switches to a low conduction regime of only 88 pS, which is almost six times lower than the high conductance state. Crystal structures of KcsA obtained with different concentrations of potassium ions reveal that these two conduction states might differ structurally [8]. The two states have different pore conformations. The high-conduction state is the one described above, in which the selectivity filter is populated at all times by two K^+ ions and requires a third ion for conduction using the knock-on mechanism, whereas the low-conduction state contains only one permanent K^+ ion in the selectivity filter and requires a second ion in the pore during conduction events. The low conduction state is differentiated from the normal state by the fact that the middle of the selectivity filter appears to be slightly collapsed, as shown in Figure 10.

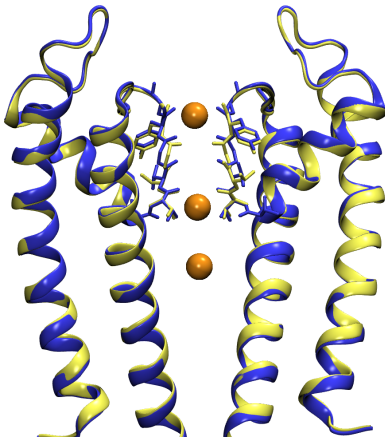


Figure 10: High conductance state (blue) and low conductance state with a collapsed selectivity filter (yellow) of KcsA. This figure can be reproduced by loading the `kcsa-conductance.vmd` VMD saved state.

The fact that KcsA can adopt two different pore conformations is important for its ability to conduct K^+ rapidly. As noted before, if K^+ binds too strongly to the pore of KcsA, it will not conduct efficiently because the K^+ inside the pore will have trouble dissociating and will simply block the pore for long periods of time. Now, in order to keep KcsA in its non-collapsed state so that it can accommodate two or more ions, the K^+ ions inside the channel must actually do work on the selectivity filter in order to fully open it (see Figure 11). Because of this, whenever two ions are bound inside the selectivity filter, part of their interaction energy with the pore is stored as tension inside KcsA itself, maintaining it in the higher energy high-conductance state. As such, compared to a pore with only one K^+ occupying it, a pore with two K^+ ions in it is still at a lower energy because of the electrostatic pore-ion interaction, however some of that energy gain is offset by maintaining the pore in a new conformation, thus reducing the effective binding energy between the second K^+ ion and the pore and making the K^+ ions inside the pore more mobile (since the two-ion state is at a higher energy as compared to other states as it would be if the channel didn't absorb any of the tension).

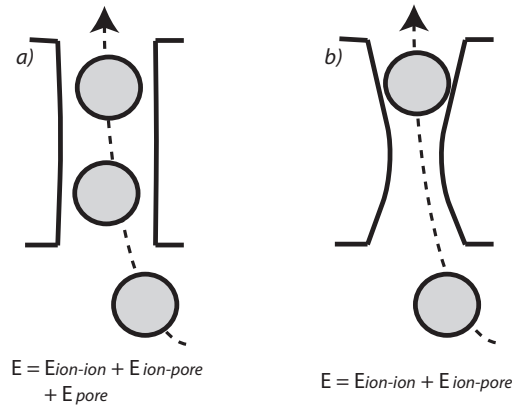


Figure 11: Diagram of (a) the high-conduction and (b) low-conduction states of KcsA. In the high-conduction case, energy is expended by the K⁺ ions in order to keep the selectivity filter open; this weakens the binding of a pair of K⁺ ions inside the selectivity filter, which would otherwise be stronger. This mechanism prevents K⁺ from being stuck in the pore due to an overly strong binding energy.

ClC Proteins

ClC are gated membrane proteins that selectively allow anions to permeate lipid membranes. They are a very ancient protein family that is found in all branches of life, including animals, plants, bacteria, and many protozoa. In humans, nine different ClCs are distributed in almost every cell of the body and play a wide variety of roles, such as the regulation of blood pressure, muscle tone, cell volume, acidification of organelles, *etc.*. ClC is a peculiar protein family in which various ClC variants have been found to be either passive Cl⁻ channels or Cl⁻-H⁺ antiporters (transporters which couple the transport of Cl⁻ in one direction to the transport of H⁺ in the other direction). This is the first example of a protein family which encompasses both ion channels and transporters, and this discovery has blurred the previously clear-cut line between channels and transporters. Unlike the various cation channels such as the potassium channels described above, ClCs do not discriminate specifically for Cl⁻, but allow a large variety of small inorganic anions to permeate. This is mainly because Cl⁻ is the only anion that is significantly present at physiological conditions, obviating the need for ClCs to be very selective. In this section, we will examine a prokaryotic ClC protein: the *E. coli* ClC Cl⁻-H⁺ antiporter [13, 14].

Structure of the *E. coli* ClC channel

While the *E. coli* ClC is really a Cl⁻ transporter, in many ways, it provides us with many clues on how its purely “channel” homologs function. In the following section, we will thus be focusing on *E. coli* ClC’s “channel-like” aspects, rather than on its transporter-like properties, which are still poorly understood.

The structure of *E. coli* ClC is shown in Fig. 12. Upon first observation, one can see that the structure of ClC appears to be very different from those of the K⁺ channels. For one, each ClC protein consists of a dimer with two independent pores. ClC’s transmembrane α -helices are also

spatially very jumbled, in comparison to the vertically ordered arrangement of helices observed in KcsA and in most other membrane proteins. Furthermore, unlike KcsA, the locations of ClC's conduction pores are not readily apparent. Fortunately, the location of ClC's selectivity filter can be easily identified by comparing the sequences of various ClCs and finding the region with the most conserved residues. It has also been found through numerous experiments that mutating the residues that constitute the selectivity filter often drastically alter the conduction properties of the entire ClC transporter. The *E. coli* ClC structures have been crystallized with one or more Cl⁻ ions bound inside each selectivity filter, providing additional evidence as to their location in the pores (shown in Fig. 12).

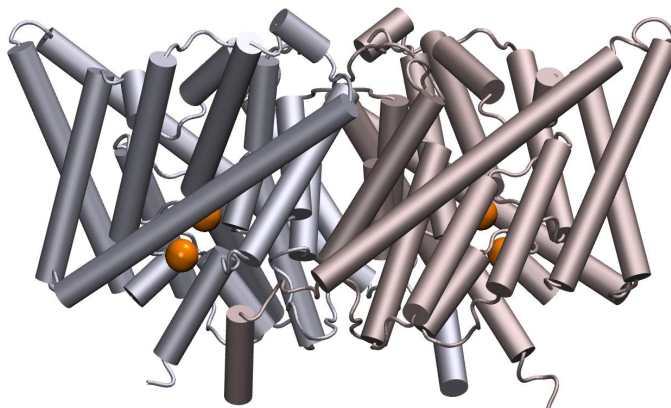


Figure 12: Dimeric structure of *E. coli* ClC with crystal ions at the location of the two selectivity filters. You can reproduce this figure using the file `clc-membrane.pdb`, which contains the *E. coli* ClC structure embedded in a membrane and an aqueous solution.

Despite their very different overall architecture, ClC and the K⁺ channels both conduct ions, and in this respect they do share some important and unique features. Just like the potassium channels, ClC has large vestibules of water. When we look at the cross-section of ClC, we can see that each pore in ClC has an hourglass shape which extends the reach of the external solution far into the membrane. The width of protein that Cl⁻ has to cross is slightly less than 20 Å, as compared to about 40 Å for the lipid membrane. This shortened conduction pathway which strongly facilitates Cl⁻ conduction is illustrated in Fig. 13. The water vestibules in ClC serves the same purpose as the ones in KcsA, and on one hand, speeds up transport by reducing the amount of travel that the ions have to do in a narrow protein channel, and on the other hand they provide a gradual transition for the ion going from the aqueous phase to the selectivity filter and vice-versa.

A second similarity between K⁺ channels and ClCs is that the residues lining the selectivity filter of ClC also have their backbone pointing toward the inside of the pore. However, instead of having the carbonyl groups sticking towards the channel, as is the case in KcsA, ClC, sticks its backbone's amide groups into the channel (see Fig. 14). This results in a backbone dipole moment opposite orientation (relative the the channel's interior) as compared to the K⁺ channels, such that the pore now favors the presence of negatively-charged particles inside it rather than positively-charged ones. Despite the reversed dipole, the strategy employed by ClC and KcsA is the same: by using many backbone dipoles the channel can attract ions of a given charge without

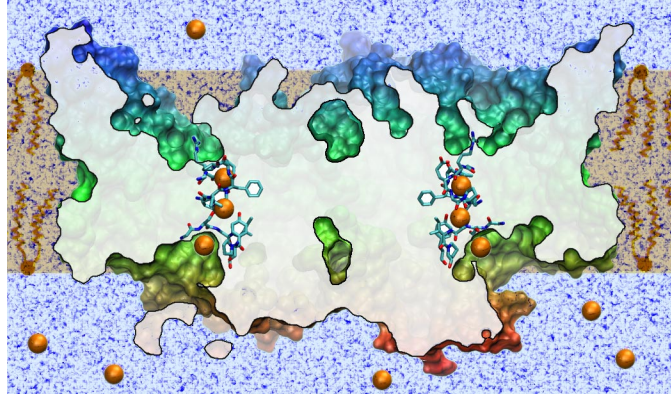


Figure 13: A slice through *E. coli* ClC showing the vestibules and the location of the selectivity filters as well as the Cl^- binding sites.

resorting to charged residues which would bind too tightly to the permeating ions. Also, both channels avoid using side chain dipoles which could bind to the conducting ions too strongly, and in addition, could flip and recruit ions of the opposite charge than what is desired.

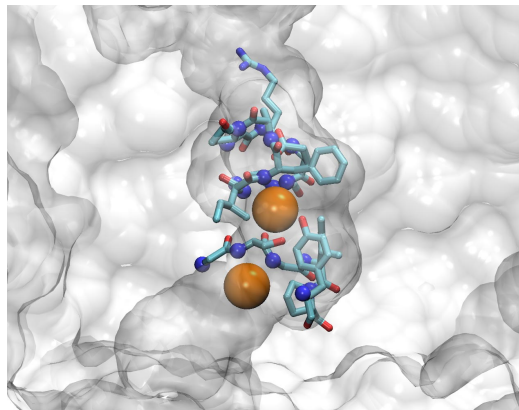


Figure 14: Selectivity filter of ClC highlighting the pore-lining residues and crystal ions. The backbone nitrogen atoms (which are part of amide groups) are displayed as spheres. One can readily see how the backbone is twisted so that its amid groups point toward the permeating Cl^- ions. You can examine this structure by following Exercise 4.

As can be seen from the crystal structures, as well as corroborated by other experiments, ClC, just like the potassium channels, uses multiple occupancy of ions in the pore during conduction. Indeed, ClC conducts two or more ions in tandem through the channel in order to decrease energy barriers and counter-balance the strong pore-ion attraction with the strong ion-ion repulsion so that Cl^- ions do not remain stuck in the protein.

Exercise 4: Inspecting the ClC selectivity filter. In this exercise, you will inspect the ClC selectivity filter of *E. coli*. To load this protein, start a new VMD session and open the VMD state file `clc-selectivity.vmd`. You should get a representation of the ClC dimer showing its irregular α -helix architecture, with two crystal Cl^- ions in each of its two pores.

- Every amino acid has an amide group, which is the -N-H functional group (identified by the VMD atom selection "`name N HN`"), protruding from their backbone. Amide groups (along with carbonyl groups) usually play a very important structural role in proteins, which you will determine. Do this by creating a new Licorice representation with the atom selection "`(backbone or name HN) and resid 150 to 190`". By inspecting the location and interactions of the backbone amide groups with their environment, can you determine what is the usual role that amide groups play in α -helical proteins such as ClC. You may find it helpful to also create a new HBonds representation with the same atom selection to highlight the amides' hydrogen bonds interactions.
- This time, examine the role of amide groups inside the conduction pore. Show the pore by creating a Licorice representation for the selection "`pore and noh`". Now, highlight all the hydrogen atoms in the pore that have a significant positive charge, by creating a VDW representation for the selection "`pore and hydrogen and charge > 0.3`". Of the charged hydrogen atoms lining the pore, what fraction of them come from amide group? Can you suggest a physical role for pore-lining amide groups? How does this compare to the pore-lining backbone carboxyl groups found in the selectivity filter of the KcsA potassium channel?
- In the above two bullets, you have found two different roles for the backbone amide groups. Are these two roles compatible or mutually-exclusive, or in other words, can a given amide group perform both the functions stated above simultaneously? And could ClC coordinate Cl^- ion conduction in its pore through the use of backbone amide groups if it had a structure that was 100% α -helical? Knowing that the structure of ClC (and of most membrane proteins) is predominantly α -helical, the amide groups' two roles must be reconciled. Can you then relate the unusual presence of broken helices in ClC to its biological function?

Conclusion

In this case study, we have looked at a few of the ways in which ion channels perform their function, notably to only allow a specific species of ion to cross the cell membrane efficiently. To achieve this, a fine balance must be established between, on the one hand, trying to attract a specific ion into the pore, and on the other hand, not binding to conducting ions too tightly so that ions do not stay stuck in the channel to block it. Because of this, even though different families of ion channels, such as ClCs and K⁺ channels, have very different structures and ancestries, they end up sharing many common strategies for accomplishing their similar tasks. Both ClCs and potassium channels use vestibules of water to reduced the length of the membrane that needs to be crossed. They both expose their backbones to the permeating ions in order to create a favorable electrostatic environment for the ion without resorting to large binding energies. And they both need multiple ions present in their pore in order to conduct rapidly. Thus, despite their strikingly different structures, ClCs and K⁺ channels share many similarities.

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