I. MEMBRANE POTENTIALS

Background to Nerve Impulses

We have all heard that nerve impulses are electrical impulses. Stimuli at one end of a nerve cell are communicated to the far end of the nerve cell through a change in electrical potential that moves like a wave from one end of the nerve cell to the other. This is a pretty remarkable phenomenon, because some neurons are tens of centimeters long, so the pulse must travel a significant distance along the length of the cell. What follows is a basic description of the electrochemistry of the nerve impulse.

The Resting Potential

Nerve cells are not at electrochemical equilibrium. All along the length of the nerve cell are membrane proteins known as Na/K ATPases. These are enzymes that catalyze the translocation of sodium ions and potassium ions across the cell membrane at the expense of a single molecule of ATP (Eq. I.1 and Figure I.1)

$$ATP + H_2O + 3\ Na^+_{(inside)} + 2\ K^+_{(outside)} \rightarrow ADP + P_i + 3\ Na^+_{(outside)} + 2\ K^+_{(inside)} \qquad \text{(Eq. I.1)}$$

The action of this enzyme serves to create an imbalance in metal ion concentration on either side of the membrane (Table I.1). Note that a third important ion, chloride, has a concentration imbalance as well, but its concentration gradient is maintained by a different transport system.

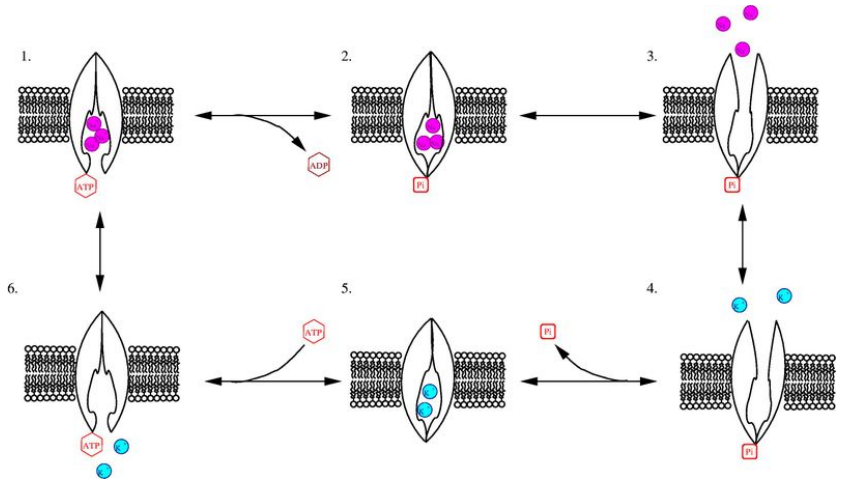

Figure I.1 Action of a Na/K ATPase (figure taken from http://en.wikipedia.org/wiki/Image:NaKpompe-cycle.jpg on 11/16/08).

Table I.1. Concentrations of electrochemically relevant ions in mammalian nerve cells.

Ion	Internal Conc. (mM)	External Conc. (mM)
Na^+	10	150
K^+	140	5
Cl^-	4	110

The imbalances in ion concentration lead to an electrochemical potential across the cell membrane (the **resting membrane potential**). Why? Given the following reversible process:

$$K^+_{(inside)} \Leftrightarrow K^+_{(outside)} \qquad \text{(Eq. I.2)}$$

one sees that the ΔG° for this process is zero. At the standard state, there are equal concentrations of potassium on product and reactant sides (1 M). However, at the concentrations relevant to the mammalian cell, ΔG must be calculated away from the standard state:

$$\Delta G = \Delta G^\circ + RT\ln Q = 0 + RT\ln([K^+]_o/[K^+]_i) = -8.6 \text{ kJ/mol} \qquad \text{(Eq. I.3)}$$

Not surprisingly, the transit from the inside of the cell to the outside is spontaneous for K^+ because the internal concentration is higher than the external concentration. Sadly, the electrochemical world does not use ΔG, but rather used electrochemical potentials, measured as $\boldsymbol{E}$, which can be calculated using equation I.4.

$$E = -\Delta G/nF \qquad \text{(Eq. I.4)}$$

F is Faraday's constant (96485 Coulombs)[1] and n is the charge transferred in the chemical/physical process. (Note that I calculated ΔG in Joules, above, because E is measured in Volts, and 1 V is 1 J/C). Thus, E for the above exchange of K^+ in a mammalian cell is:

$$E_K = -\frac{RT}{nF}\ln\left(\frac{[K^+]_o}{[K^+]_i}\right) = -0.089 \text{ V or } -89 \text{ mV} \qquad \text{(Eq. I.5)}$$

Similarly, the potentials associated with sodium and chloride are +68 mV and +88 mV. While these potentials would seem to cancel each other out, in fact the overall membrane potential E_m is determined not simply by the sum of the individual ion potentials, but by a weighted sum that takes into account the permeability of each ion through the membrane. It turns out that potassium is the most permeable ion under resting conditions because of the presence of "leak channels" that permit unregulated diffusion of K^+ out of the cell – roughly 95% of the ion diffusion taking place under resting condition. Given the following equation:

[1] Some units of interest: The Coulomb (C) is a unit of charge. One mole of e^- carries 96495 C. Current is measured in Amperes (A). 1 A = 1 C/s. Potential is measured in Volts (V). 1 V = 1 J/C.

$$E_m = E_K \frac{P_K}{P_{tot}} + E_{Na} \frac{P_{Na}}{P_{tot}} + E_{Cl} \frac{P_{Cl}}{P_{tot}} \qquad \text{(Eq. I.6)}$$

Where P_X/P_{tot} is the fraction of total permeability due to ion X, E_m is largely set by potassium to a **resting potential** of -70 mV. Note that, although K^+ is more permeable, it won't all exit the cell through the leak channels. Eventually the anions it leaves behind are dissatisfied by the absence of cations and the cations on the outside are dissatisfied by the absence of anions. *One way of thinking of this resting situation is that there is a driving force for anions to exit the cell (joining the departed K^+ cations) and for cations to enter the cell (to make up for the departed K^+ cations).*

Action Potential[2]

Nerve impulses may be initiated by a number of chemical or physical events, but once initiated, they are propagated in a similar fashion – as a traveling wave of a disequilibrium potential, in which a signal is transmitted as a slightly decreased negative potential, about -40 mV. All along the nerve cell there are two kinds of **voltage-gated ion channels** – gated Na^+ channels and gated K^+ channels. They open and close, in sequence, to this perturbation of the resting potential (-70 mV) to the action potential (-40 mV). First, the sodium channels (normally closed at -70 mV) open and allow Na^+ ions to flow into the cell, following the concentration gradient. That depolarizes the cell. Where the resting potential was maintained by the K^+ gradient, the sudden uptick in Na^+ permeability shifts the membrane potential to a much more positive value – about +40 mV, at which point the Na^+ channels become blocked to further transport in a process known as **inactivation**.

Note that, at this point, there is an excess of positive charge on the **inside** of the cell (hence the positive potential). In response to this positive potential (actually in response to any potential in excess of roughly -40 mV) the K^+ channels open as well, and remain open until the membrane potential returns to a strongly negative value, below -70 mV, reflecting the high permeability of K^+ in this phase. At this point the membrane is in a refractory period. The K^+ and the Na^+ channels are both inactivated, and the nerve cell only returns to its resting potential upon diffusion of external K^+ away from the membrane. Once the resting potential is re-established, a second nerve pulse may be transmitted.

Measuring ion flow in cells

Technically, it is quite demanding to measure ion flow through a cell membrane. It requires electrodes small enough to sample the interior of the cell – but folks have been doing it for years. One can measure both the current across a cell membrane or across an artificial bilayer containing the channel of interest, or one can even measure the current through a single channel! The plots arising from this work typically measure ion current (in Amperes, equivalent to Coulombs/s) vs. time. Since the cell potential is key to changing ion current through voltage-gate ion channels, one typically notes the applied potential on a bar above the current trace (see Figure I.2). Figure I.2 shows a current trace obtained from measuring current through voltage-gated K^+ channels. One sees that current is very low when the membrane potential is held at -50 mV, but increases rapidly

[2] For a good animation of the action potential, see: http://www.youtube.com/watch?v=SCasruJT-DU.

when the current is shifted to +100 mV. This corresponds to the opening of the voltage-gate, allowing ions to flow through the K^+ channel. The current decays exponentially following its peak due to the process of inactivation.

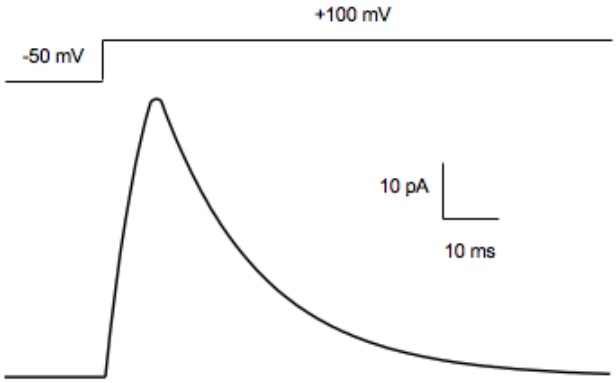

Figure I.2. Sample current trace for activation of a bulk sample of voltage-gated K^+ channel. Note that the y-axis is current and x-axis is time (noted by the small inset legend in pA and ms). The potential being applied to the sample is noted on the top of the figure, indicating the timing of the change in applied potential.

With this background, we are now able to discuss the structural biochemistry of transport. As may be clear from the above, we have two concerns to address. The first is specificity – how do Na^+ and K^+ channels distinguish their cognate ions from the obvious and closely related non-cognates, and the second is how is transport regulated by membrane potential. The latter process is referred to as voltage gating. Because my writing is not always characterized by suspense, you may not be surprised to learn that the proteins under discussion are referred to as voltage gated ion channels.

Specificity of Ion Transport

As should be obvious from the above, Na^+ and K^+ ions must be distinguished from each other with great care. Despite their obvious similarity, the structural chemistry and thermodynamics of the two ions are quite distinct. Of primary importance is the ionic radius of the ions. Na^+ is considerably smaller than K^+ (Table I.2). As a result, the two ions have differing preferences for coordination geometry and possess differing energetics for ligand binding.

Table I.2. Ionic radii of alkali metal cations and affinity for nonactin, an ionophore.

	Radius (Å)	K_d Nonactin
Lithium	0.60	
Sodium	0.95	1.9 mM
Potassium	1.33	0.033 mM
Rubidium	1.48	
Cesium	1.67	

The smaller sodium ion prefers a smaller number of ligands (six) than potassium (eight). Because ligands approach the Na^+ ion more closely, there is less space for them around the surface of the smaller ion. In solution, sodium exists as $Na(H_2O)_6^+$ and potassium as $K(H_2O)_8^+$. Binding these ions to alternate ligands (such as proteins) will require dehydration. That comes at an enthalpic expense due to the momentary loss of electrostatic stabilization by bound water ligands. It is harder to desolvate Na^+ than K^+ (97 vs. 79 kcal/mol).

From these differences, there are three tools that proteins can use to distinguish between the two ions: (1) Coordination number. More ligands favors K^+, fewer ligands favors Na^+. (2) Ligand strength. Sodium will be favored by more negatively charged ligands. Because of the greater enthalpic cost of desolvation, a bigger compensating benefit must be offered. Also, because ligands bond more closely to the smaller Na^+, there is greater electrostatic benefit to a negatively charged ligand. (3) Size. Simply – build a smaller binding site, you favor Na^+, a larger one – K^+.

There is a long history of studying selective binding of one ion from another. For example, there is a class of compounds named ionophores (ion carriers) that are natural bacterial agents excreted by some bugs to kill others. The biological membrane is not highly permeable to ions (hence the existence of membrane potentials), but if it were made to be, ion concentrations would rapidly equilibrate and lead to cell death (bacteria have their own reasons for retaining ion gradients). Nonactin, a commonly studied ionophore shows high selectivity for potassium over sodium (Figure I.3; Table I.2). Its octacoordinate geometry, preferred metal-ligand distances of 2.8 Å and lack of charged ligands creates an ideal binding site for potassium.

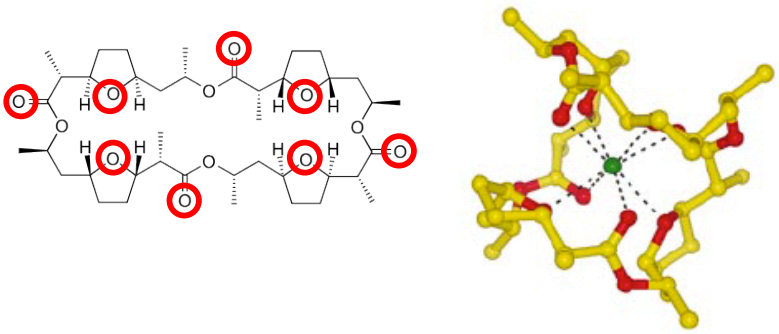

Figure I.3 Structure of nonactin, a potassium specific ionophore.

Structure of KscA – A Potassium Ion Channel

Rod Mackinnon, working at the Rockefeller Institute, scored one of the major triumphs in protein crystallography (and a Nobel Prize) by solving the first structure of a potassium-selective voltage gate ion channel.[3] KscA was purified from *Streptococcus lividans*, a bacterium, but found to be structurally and functionally homologous to mammalian ion channels. Like mammalian potassium

[3] Read a paper that won a Nobel Prize: Doyle et al. (1999) *Science* **280**, 69-77. Well actually, there's a lot of supporting work that merited that prize, but among other things this paper is significant for having been one of the early structures of a membrane protein.

channels, it exhibits strong selectivity for K^+ over Na^+. Ion channels generally operate under non-equilibrium conditions, and rather than express selectivity as a ratio of equilibrium constants, it is more commonly reflected in a ratio of rate constants for transport. A single molecule of KscA, under saturating conditions can transport 10^8 K^+ ions/s, while Na^+ is transported at 10^4 ions/s, reflecting a 10,000-fold difference, or a 5.6 kcal/mol difference in free energy of activation.

However, it is also possible to study ion channels in equilibrium concentrations. Using isothermal titration calorimetry, which uses the heat that is released or absorbed during a binding event, as a signal to be monitored, MacKinnon and colleagues found that KscA has a dissociation constant of 0.43 mM for K^+, but no apparent affinity for Na^+ (Table I.3). Larger alkali metal cations, Rb^+ and Cs^+, likewise bind exothermically to the channel, with an unexpected increase in entropy. The increase in entropy is likely due to the dehydration of the metal ion as it binds to the protein. The mildly exothermic binding of the larger ions suggests that they the costs of dehydration are compensated by the ligand environment of the protein and the freeing of bound water molecules. One might argue that sodium is not adequately compensated enthalpically by the ligand environment, but the result with Ba^{2+} suggests otherwise. Barium has an ionic radius of 1.35 Å (compared to 1.33 Å for K^+). It binds endothermically reflecting a ligand environment that does not handle the additional charge well, but bound waters gain sufficient entropy to compensate. The argument was made that size is as important as ligand environment in determining selectivity. Na^+ (0.95 Å radius) would make poor contacts to the protein ligands, which are likely fixed in position, and it would release fewer water molecules leading to a poor gain in entropy.

Table I.3. Binding data for various metal cations to KcsA, obtained via isothermal titration calorimetry.[4] Mg^{2+} and Ca^{2+}, like Na^+, were not observed to bind.

Metal Ion	ΔG° (kcal/mol)	ΔH° (kcal/mol)	ΔS° (cal/mol•K)	K_d (mM)
Na^+	-	-	-	-
K^+	-4.54	-1.24	11.2	0.43
Rb^+	-5.29	-1.93	11.4	0.12
Cs^+	-4.53	-1.81	9.2	0.44
Ba^{2+}	-5.03	+5.51	35.7	0.19

But I'm telling the story a little backwards – the structure of the KcsA channel had been solved 10 years earlier, and supports those thermodynamic data. KcsA is a tetramer of a 160 residue subunit, but only the transmembrane region of the protein was crystallized/resolved, comprising residues 23-119. Each subunit of the tetramer contains two transmembrane helices and they pack together to give the structure of a cone, with the wide end facing the extracellular space (Figure I.3). The two transmembrane helices bracket a "pore helix" and a "filter" with the sequence TVGYG. It should be noted that the structure was solved in the open state, with free access from one side to the other, though physiologically, ion flow is from the inside of the cell (100 mM K^+) to the outside (5 mM K^+).

[4] Lockless et al. (2007) *PLOS Biology*, **5**, 1079-1088.

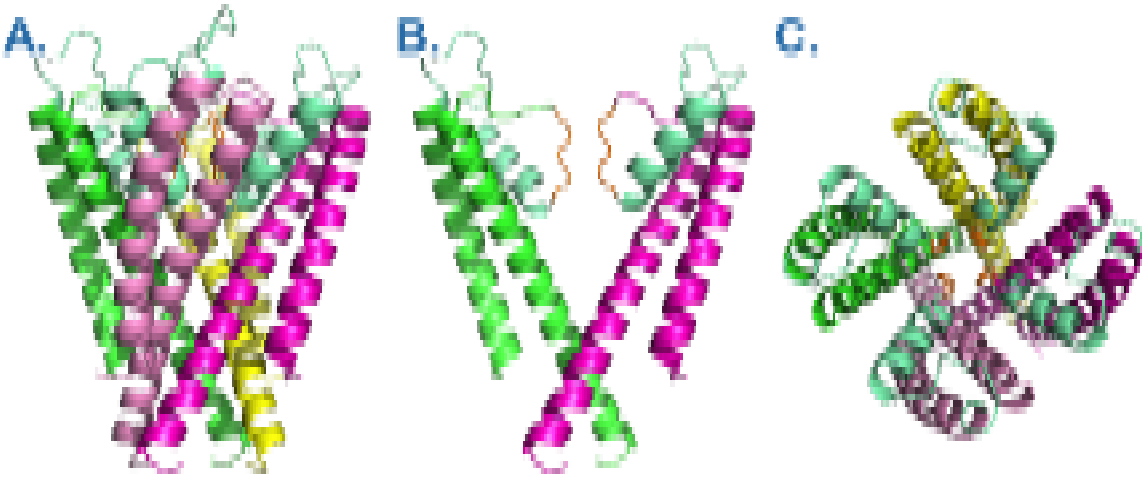

Figure I.3 Structure of KcsA. (A) View of tetramer, colored by subunit, in the plane of the bilayer. The extracellular medium is at the top of this view. (B) Same view as "A" except that the forward and back subunits are removed for clarity. The pore helix of the two remaining subunits is in cyan and the filter region is in orange. (C) View of the tetramer from above the plane of the bilayer, looking from the outside of the cell inwards.

The protein contains a 45 Å pore through which K^+ ions are obviously able to pass – they're even present in the crystal structure (solved under equilibrium conditions, with 150 mM KCl present). The pore can be separated into two regions (Figure I.4). As the K^+ ion travels from the cellular side to the extracellular side it first enters a vestibule surrounded by hydrophobic residues. This chamber is sufficiently large to bind the fully hydrated $K(H_2O)_8^+$ complex, which is observed in the electron density of a 2 Å structure obtained in 2001.[5] From there, the ion enters the selectivity filter that is lined with 16 backbone carbonyl oxygens and four hydroxyl groups arranged in five layers – with each layer obtaining four ligating oxygen atoms from the four subunits. Those oxygens replace the water ligands as the potassium ion leaves the ante chamber and enters the filter. Electron density for potassium exists at four positions within the filter, though, based on the strength of that density, it is likely that only two ions are present in any given filter, but there are two different arrangements that those ions can take.

[5] Zhou et al. (2001) *Nature* **414**, 43-48.