

Single Ion Channels

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Single ion channels are proteins that control an enormous range of biological function. Life as we know it depends on the ability of a few atoms of channel proteins to control biological functions throughout animals and plants.(Alberts *et al.*, 1994; Koeppen & Stanton, 2009) The atomic structure of channel proteins has been optimized by natural selection to perform these functions since the beginning of life (perhaps a billion years ago).

Ion channels selectively control the flow of ions—molecules with permanent electrical charge—that otherwise cannot enter cells, as has been apparent since the Nobel Prize experiments and calculations of Hodgkin & Huxley, described in delightful historical context in (Huxley, 1992, 2000). Different ions carry different signals through many different types of ion channels in every cell of animals and plants.

Ions are signals that control different biological functions in different ways. For example, the ‘bio-ions’ sodium Na^+ , potassium K^+ , and chloride Cl^- are single atoms that control the size of cells and the electrical potential (‘voltage’) across the membranes of cells.(Ashcroft, 2012) Death occurs when the resting electrical potentials across cell membranes disappear and cells swell and burst. These bio-ions are spheres of permanent charge and nearly unchanging diameter.

Calcium Ca^{2+} (with two permanent positive charges) is another particularly important bio-ion. Calcium ions Ca^{2+} are used in nearly every cell to control biological function (Alberts *et al.*, 1994; Koeppen & Stanton, 2009) in the same sense that a gas pedal controls the speed of a car. Many functions inside cells are accelerated dramatically when Ca^{2+} flows through calcium channels. The internal concentration of Ca^{2+} near channels is the signal analogous to the position of the gas pedal. Outside cells Ca^{2+} concentrations are nearly one million times larger than inside cells, around 5×10^{-3} M outside cells compared to . less than 10^{-8} M inside cells. Calcium is hardly present in resting cells. The flow of Ca^{2+} through channels raises the internal concentration (near the channel) to around 5×10^{-7} M or higher, sometimes much higher.

Ion channels can control these ionic concentrations and signals because ions cross membranes only through channels. Ions cannot cross lipid membranes. Their movement into cells is catalyzed by channels and transporters. Transporters are close cousins of ion channels, with very similar structures, that have historically been described very differently,(Eisenberg, 1990; Hille, 2001) despite their similar structures.

Most biological functions are controlled by channels: Information flow in the nervous system, the contraction of muscle, including the contraction of the heart that allows it to pump blood, and secretion of hormones are controlled by ionic signals

regulated by the flow of ions through channel proteins. A substantial fraction of the energy used by a human is determined by detailed properties of the voltage dependence of the sodium channels Na_V of nerve cells in the human brain. (Alle *et al.*, 2009; Magistretti, 2009)

The function of ion channels has been a central subject in physiology and medicine for more than a century. (Huxley, 1992, 2000) Many diseases are channelopathies produced by specific malfunctions of specific channels in particular cells and tissues (Ashcroft, 1999), as a google search for ‘channelopathy’ or ‘channel disease’ shows most convincingly.

Ion channels are remarkable proteins. A handful of atoms—often in a region smaller across than 5×10^{-10} meters, i.e., 5 Å—control biological functions and clinical symptoms that range through an entire animal, over distances sometimes larger than 2 meters, some 4×10^9 (four billion) times larger across (and 6×10^{28} larger in volume) than the handful of atoms themselves.

Ion channels are the ultimate multiscale device since their range of control extends nearly twenty-eight orders of magnitude in space (i.e., volume) and twenty five orders of magnitude in time, from the time scales of atomic motion 10^{-16} sec to the time scale of (biological) macroscopic life. Most biological function starts at 10^{-3} sec and sometimes extends 2.52×10^9 sec (80 years) before death. The failure of function in ion channels is one of the main causes of death. The irreversible swelling of tiny nerve terminals in the brain is often the immediate cause of death, and that swelling is produced by a failure of transporters to keep up with leaks through channels. The deadly leak is usually the excessive flow of Na^+ in and K^+ out of tiny nerve terminals which have huge surface to volume ratios because they are so small.

The recent discovery of the structures of ion channel proteins, (MacKinnon, 2004) and of their genes and mutant genes (that provide the blueprint for the structures) (Hille, 2001; Nadeau, 2011) provides an extraordinary opportunity for all of us. The atomic control of biological function can now be studied in hundreds of (different types of) channel proteins. Atomic manipulation of these proteins can and will allow treatment of disease and extension of productive lifespans. It will allow construction of remarkable technologies making our lives easier and more fun. We simply need to understand and control ion channels as well as we can already control the devices of our semiconductor technology.

Before scientists can understand and control, biologists have to describe. Engineering is not possible without a list of parts. Biology is not possible until we know what parts there are in our biological systems and what those parts do.

Biology remains mostly a descriptive science because the parts list is so large and so hard to construct. But the parts list of a human—not including the nervous

system—is not beyond our reach. The number of parts in a nuclear submarine that functions in isolation for six months (1.6×10^7 sec) is comparable to the number of parts in a human, outside the nervous system. We can look forward to a quite complete catalog of the parts of a human, including a remarkable precise description of their structure, within the lifetime of our grandchildren, with all that implies.

Ion channels are studied in hundreds of laboratories with remarkable resolution as biologists seek to identify, classify, and describe these crucial parts of our cells and tissues.

The genes of many channels are now known—so many that I have lost track—and the regulation of the expression of these genes is a topic attracting the attention of large numbers of descriptive biologists. (Hille, 2001; Nadeau, 2011) The different expression of different channel genes in different places governs a large fraction of biology. Different versions of sodium channels Na_V for example are found in different parts of the muscle of the heart (e.g., in different locations across the wall of the left ventricle). The slightly different properties of these channels allow the heart to pump blood efficiently. Clearly, putting the right channel in the right place in the right amount is essential to building a cell or tissue that works well. We can expect that many pathologies of biological function arise from mistakes in the expression of channel genes and mistakes in the localization of their products, the ion channels themselves.

Ion channels are studied one molecule at a time as they perform their natural function thanks to two technologies, the patch clamp (Sakmann & Neher, 1995) of channels in cells and reconstitution of channels into lipid bilayers. (Mueller *et al.*, 1962) Both techniques demonstrate that the classical membrane phenomena of physiology arise in channels. (Hille, 2001) Measurements from single channels give the same results (in detail) whether the single channel is recorded in a biological cell in an animal or isolated in a bilayer setup. Single channels have the same properties (in detail) whether synthesized by the wonderful methods of molecular genetics and biology or by the cell itself. Single channel recording avoids the horrible confusion produced by measurements all at once of the (sum of the) currents produced by the complex mixtures of channel proteins found in cell membranes in their natural state. Progress in understanding the protein enzymes of life required measurements on homogeneous ('clean') systems ("Don't waste clean thoughts on dirty enzymes" was the guiding rule in the heroic age of enzymology); rapid progress in channology required the isolation of the currents flow through single channel proteins. Otherwise scientists must spend most of their time trying to clean up studies of 'dirty' mixtures of many channel types.

Single channel recording is possible because of the contrasting electrical

properties of the channel molecule and the surrounding lipid membrane. Lipid membranes are insulators that prevent ion movement; channels are conductors that catalyze the movement of ions. (Eisenberg, 1990) When recording single channels, significant current flows only through the channels. (The analogy with the channels of transistors in our semiconductor technology is striking. (Eisenberg, 2012) In transistors, channels of silicon with permanent charge ('doping') conduct current through surrounding insulating SiO₂ glass.) Current through one ion channel can be isolated by the gigaseal, and then collected and studied by specialized picoammeters ('patch clamp amplifiers') widely available commercially: indeed, many thousands of our version of the amplifier (the Axopatch) are in laboratory use every day. The gigaseal is the wonderful name given to describe the gigaohms of shunt resistance needed to seal off a single channel so its single channel current can be measured in isolation from irrelevant currents, that flow through other pathways. Gigaseals make dirty mixtures clean and allow quite unambiguous measurements from individual channels.

Because of the ease of single channel recording, and the importance of the results, descriptive experimental work on ion channels has far exceeded our physical understanding of how channels work. Thousands of papers on ion channels are published every year. Thousands of presentations are given at international meetings, most notably the annual meeting of the American Biophysical Society. Hundreds of clinicians study channelopathies every day that are responsible for many of the maladies of men and women (and animals and plants for that matter).

Much of the data on ion channels concerns systems of clinical importance with immense financial significance to the pharmaceutical industry. Nearly every drug of interest to the pharmaceutical industry must be applied to channels of the heart to look for side effects. Many organic compounds have dangerous effects on the Kv11.1 channel—encoded by the hERG (KCNH2hRg) gene—that can lead to QT prolongation in the EKG (electrocardiogram) and to an often fatal arrhythmia called 'torsade de pointes'. Measurements of dangerous effects should be made on single channels of Kv11.1, to avoid ambiguous results, if financially possible.

Single channels are open or closed. Single channel recordings show that single channels are either open or closed (to a good approximation). The current through a single channel is independent of the duration of the channel opening, whether the channel is open a few microseconds or many seconds. The current through any type of single channel is a sensitive function of the structure of the channel and of the position of the charges in the channel protein because the channel is so small and its charges are so close to the ions moving through the channel. We conclude then that the channel does not change structure significantly once it is open, on a time scale of

microseconds or longer.

The current through a single channel switches stochastically and forms a random telegraph signal. The biologically important current through membranes is the sum of huge numbers of these stochastically open and closed single channel currents. In classical cases, channels operate independently, coupled only by the electric field they have in common, so the single channel currents just sum to make the biologically important macroscopic current.

The biologically important current depends on the total number of channels, the fraction of the time a single channel is open ('open probability'), and the current through a single channel. The time course of the biologically important current flow depends on time course of the open probability, and the delay with which it responds to stimuli, more than anything else.

Different types of channels open in response to different stimuli because different channel proteins have different structural modules to sense the stimuli. Some channels have modules that respond to voltage. Others have modules that respond to temperature. Still others have modules that respond to specific chemicals, e.g., capsaicin, the ingredient that makes chili peppers taste hot. New channels with new types of sensitivity are constantly being discovered and the diversity is amazing.

Many of the important properties of channels are determined by the time course of the open probability and so there are many measurements of the kinetics of channel opening in response to stimuli. Although elaborate 'Markov' models have been developed to describe the opening of ion channels (Sakmann & Neher, 1995; Hille, 2001), these have little use in engineering design because they have little predictive value. These models rarely predict what will happen when ion type or concentrations are changed, or the potential or other variables vary.

The widely held view of channel opening as a 'conformational change' is more metaphor than model. It is not a physical model that yields predictions. Physical models of channel opening are not yet available that allow such predictions. In my view, a physical model must deal with the central fact that the open channel has only one conformation (on the biological time scale) independent of the duration of the opening.

Selectivity of the open channel. The open channel has been analyzed successfully with physical models in a few cases, the EEEE (glu glu glu glu) Ca_v or L-type calcium channel, the DEKA (asp glu lys ala) voltage activated sodium channel, and the ryanodine receptor, all reviewed in (Eisenberg, 2011).

The successful model of the selectivity of an open channel must deal with the variables important for macroscopic function, for example, the concentration of ions in the mixtures in which channels function in animals and plants. (The solutions inside

and outside cells are derived roughly speaking from seawater. They are always mixtures of bio-ions in which calcium plays an important role.) A successful model must actually predict the current through a channel under a variety of conditions, on the biological time scale.

Simulations including all the atoms of the channel are not yet successful models. All atom simulations have grave difficulties (Eisenberg, 2011) dealing with the enormous gap between the time scale of atomic motion and biological function. They cannot yet deal in a calibrated way the properties of ionic mixtures, or solutions containing calcium. We await the maturation of all atom simulations into calibrated and thus useful models of biological function.

The successful models of open channels (Eisenberg, 2011) have used an ‘all spheres’ approach introduced by Nonner and Eisenberg which grossly oversimplifies the structure of the channel. In these models relevant side chains as charged spheres, kept within the channel, but otherwise free to move to the position of lowest free energy. Such models, much to the surprise of their originators, using crystal radii of the bio-ions and just one or two parameters (never adjusted once they are set) can account for the selective binding properties of both the EEEE calcium channel of the heart (Ca_V) and the (very different) selective binding properties of the DEKA sodium channel over five orders of magnitude of concentration of calcium in many mixtures of ions. The diameter of the channel is mostly responsible for the selectivity of these models. The solvation properties (i.e., dehydrating desolvation from water and re-solvation by side chains of the protein) are handled by the dielectric coefficients as in implicit solvent models of ionic solutions, that are primitive used successfully in much of physical chemistry.(Fawcett, 1999) Calcium selective channels have been built and have the properties expected.(Vrouenraets *et al.*, 2006) Models of the ryanodine receptor in the same spirit produce current voltage curves that match experiments and predict detailed properties of those curves before the measurements were made.(Gillespie, 2008) Indeed, engineering these channels are possible.

Future of ion channels as nanodevices. As engineering reaches to make nanodevices, it is useful to remember that we have the ultimate multiscale nanodevice already (literally) in our hands: our fingers like the rest of us contain billions of ion channels. The description of ion channels has been and will be the work of generations of biologists. Engineers know that building things requires numbers and equations. The development of useful models of ion channels will require the knowledgeable interactions of those who know what to describe with those who know how to analyze the descriptions with numbers.

[2720 words]

In the press

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