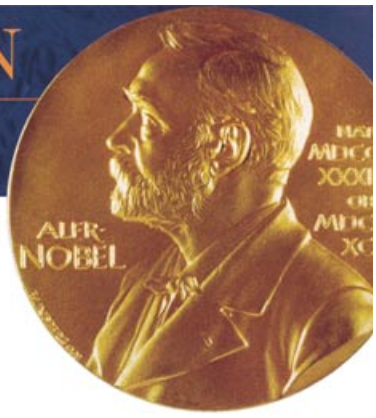
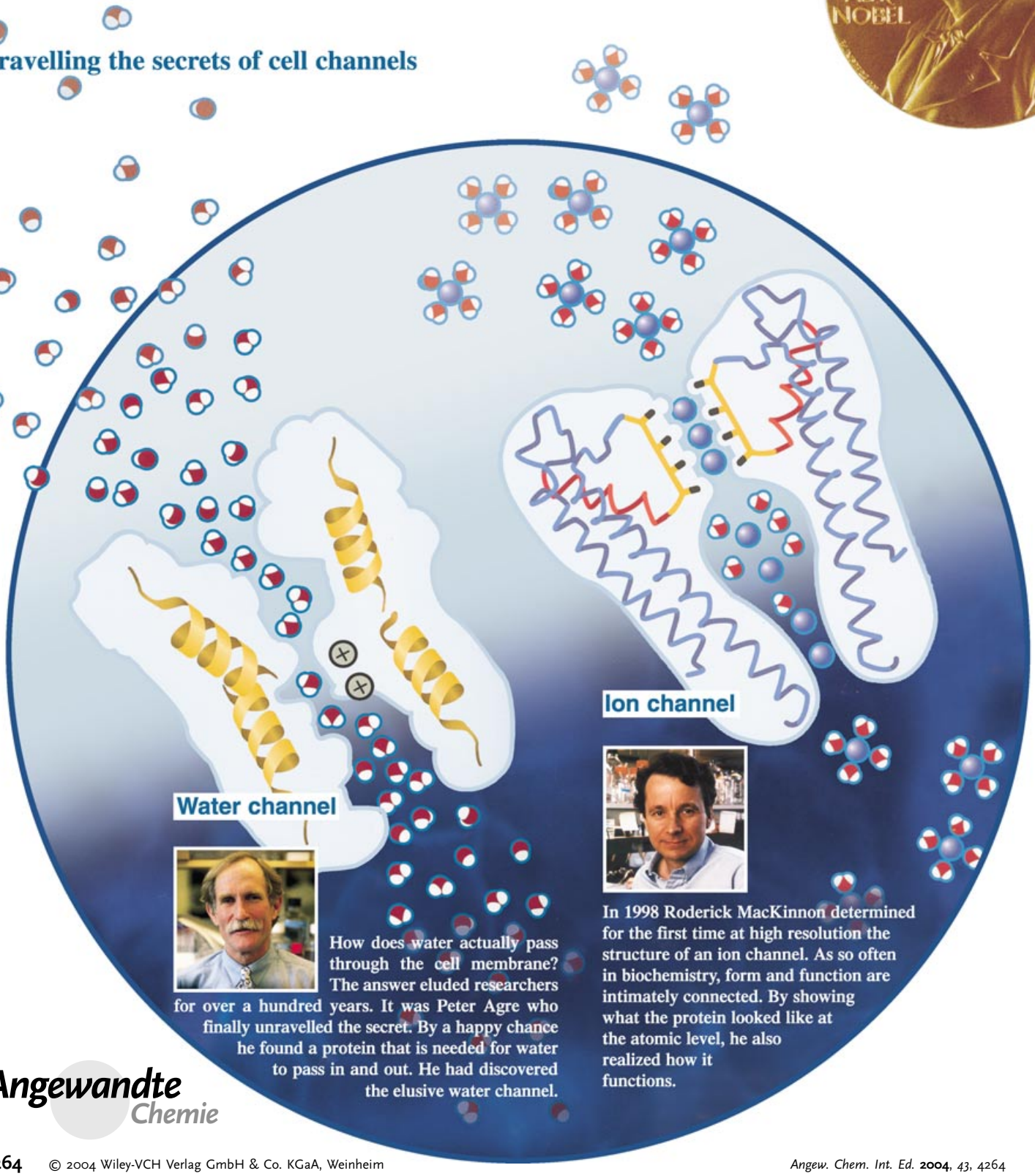
# THE NOBEL PRIZE IN CHEMISTRY 2003




## Unravelling the secrets of cell channels






How does water actually pass through the cell membrane? The answer eluded researchers for over a hundred years. It was Peter Agre who finally unravelled the secret. By a happy chance he found a protein that is needed for water to pass in and out. He had discovered the elusive water channel.



In 1998 Roderick MacKinnon determined for the first time at high resolution the structure of an ion channel. As so often in biochemistry, form and function are intimately connected. By showing what the protein looked like at the atomic level, he also realized how it functions.



Ion Channels

# Potassium Channels and the Atomic Basis of Selective Ion Conduction (Nobel Lecture)**

*Roderick MacKinnon**

**Keywords:**
ion channels · membranes · Nobel Lecture · protein structures · structure elucidation

## From the Contents



*I was born on February 19, 1956 in the middle of a snowstorm. It remains one of those humorous family stories that my mother likes to tell. My father, the planner, had rehearsed the way to the hospital but apparently things looked a lot different at night in a blizzard. Eventually they made it and so did I, the fourth of seven children. My father was a postal worker when I was very young but studied computers and became a programmer on the big IBM main frames. My mother worked as a part-time schoolteacher, but mostly took care of the children at home. Thinking back on it now, I know we did not have much money but I never knew that growing up. My parents provided a happy environment and made their expectations clear to us. Television is bad for you, reading is good for you, and you better get an A for effort in school. What you end up doing in life is up to you. Just make sure you enjoy what you do because then you will do it well. We all pursued completely different walks of life. I became the scientist.*

*I suppose there were some early indications of my tendency to a life of curiosity. Apparently from a very young age I had a habit of asking lots of questions: "what would happen if ...?" was a favorite. I also liked having facts straight and knowing how things worked, and did not hesitate to give explanations to those around me, apparently to an annoying degree sometimes. I remember one day my father, at the end of his patience, commenting that I was a "compendium of useless information". I certainly can understand his plight with one of the seven having way too many questions and answers all the time. On the positive side, I learned a new word that day when I looked up compendium in the dictionary.*

*There were probably even indications that my curiosity might be scientific. Burlington Massachusetts was rural when I was young and I loved to roam and explore. I had rock collections and read children's books on geology and the history of the Earth. I made little volcanoes out of plaster of paris and added baking soda and vinegar to the craters to simulate volcanic eruptions. I had an accident one day that made my mother laugh, to my utter frustration: at that young age I failed to appreciate the humor in a little boy telling his mother he had dropped a volcano on his toe! In the summer I collected butterflies, turtles, snakes, and other living things. One summer my mother enrolled me in a science-enrichment class for elementary school students and I was allowed to take home a microscope. I used it to look at everything I could find: microorganisms from the nearby pond, leaves, and blades of grass. I spent hour after hour alone, mesmerized by the tiny little things that I could see. My scientific curiosity took a back seat to athletics through junior-high and high school. Gymnastics was a good match to my small build and to my solitary nature. I was a member of a team, but gymnastics is an individual sport. You learn a technique, then a "move", and then a "routine". Then you perfect it through practice, working mostly alone. I had a very good no-nonsense teacher, coach Hayes, who really instilled in me the idea of perfection through practice. I was actually not all that bad, particularly at floor exercise and high bar. I even considered pursuing gymnastics in college, but during my final year of high school I began to wonder what I should pursue for a career. I attended the University of Massachusetts in Boston for one year and then transferred to Brandeis University. Brandeis was an eye-opening experience for me. For the first time in my life I was in a seriously intellectual environment. The classes tended to be small, intense, and stimulating. I discovered that I had a passion for science, and that I was very good at it. I chose Biochemistry as a major and a newly arrived assistant professor named Chris Miller for my honors thesis advisor. He had a little laboratory with big windows and*

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*lots of light shining in. I studied calcium transport and learned about the cell membrane as an electrode. I could see that Chris Miller was a man having lots of fun in his daily life and it was inspiring to me, and the memory of this stayed with me. But the biggest influence Brandeis had on my life happened in Physics class. There I met my future wife Alice Lee, whose sparkling eyes and sharp mind caught my attention. Against Chris Miller's advice I went to medical school after Brandeis. I studied at Tufts University School of Medicine and then at Beth Israel Hospital Boston for house-officer training in Internal Medicine. I learned a lot, but in the end I should have taken Chris' advice to pursue science. Medicine required a lot of memorization and little analytical problem solving. To keep a certain part of my brain active I began to study mathematics, and continue this even today, learning new methods and solving problems with the same disciplined approach I had learned in gymnastics. I started back to science near the end of house-officer training when I worked with Jim Morgan studying calcium in cardiac muscle contractility, which was very enjoyable and kept me connected to medicine. But I had a yearning to work on a very basic science problem, which meant I would have to break my medical ties. This was a difficult decision because I had invested so many years in medical education; to abandon it was to admit to myself that I had misspent a big piece of my life. There were practical considerations as well. It was time finally to get a permanent job; after all, my wife Alice had supported me through years of training. Not to mention I was nearly 30 years old with no real basic science training beyond my Brandeis undergraduate education: would I even be able to make it as a scientist?*

*Two factors had the greatest influence on my decision. Back in my first year of medical school I lost my sister Elley, an artist only two years my senior. Diagnosed with leukemia during my hematology clerkship as I learned about the dreaded disease, she lasted only two months. This horrifying event impressed upon me how fragile and precious life is, and how important it is to seize the moment and enjoy what you do while you can. I remember thinking when I look back upon my life at the age of seventy, thirty will seem young: just go for it. The second factor was Alice. She had complete faith in my ability to succeed. Never mind that postdoctoral studies meant a reduction of my already piddling house-officer salary. She simply said you have no choice; we will manage somehow.Memories of Chris Miller's laboratory beckoned so I returned for postdoctoral studies. Of course I will never out-live his reminding me that I should have listened to him in the first place. Feeling far behind in my knowledge, I approached my postdoctoral studies with intensity, learning techniques and theory. I felt I should be an expert in electrochemistry, stochastic processes, linear systems theory, and many more subjects. I read books, solved the problem sets, mastered the subjects, and carried out experiments. I had the very good fortune of a co-worker Jacques Neyton, a postdoctoral scientist from France. Jacques is a very critical thinker who would brood on a problem. We exchanged ideas often. When I would tell him one of my ideas he had*

Alice Lee, 1986, at the time of my decision to leave medicine for science.

*a tendency just to listen quietly. Then, after a while, if his response started with "Hey Roddy, there's something I don't understand" I knew I was in trouble—my idea was probably no good!After I completed a series of biophysical studies on $K^+$ channels, it came time to apply for an academic position. During the late 1980s physiology departments were more interested in hiring channel gene cloners than biophysicists. But Peter Hess convinced his colleagues at Harvard that my work showed promise and I was offered an assistant professorship there. My laboratory made good progress on $K^+$ channels. It was exciting for a while but in just a few years I began to feel that the return on what we could learn from studying the functional effects of mutations was diminishing. We had identified the signature sequence of the $K^+$ channel, but without knowing its structure we never would understand the chemical principles of ion selectivity in $K^+$ channels. I decided at that point to learn X-ray crystallography to someday see a $K^+$ channel.*

*I began to learn methods of protein purification and X-ray crystallography while still at Harvard, initially working with channel toxins and a small soluble protein called a PDZ domain. However, I thought it best to move away from my familiar environment at Harvard to pursue channel structure. There were really two reasons motivating me to move. First was the practical issue of obtaining funding to work in an area in which I had no background: start-up funds associated with moving to a new university would be useful for this purpose. The second, and far more important, reason was that moving would enable me to immerse myself completely in the new endeavor. A change of environment would remove the distractions of everyday life, isolate me from the temptation to fall back on channel physiology studies that I was already good at, and allow me to focus with singular purpose on the structural studies. I needed this to become an expert in membrane protein biochemistry and X-ray crystallography, and to develop a "feel" for protein structure. When the president of Rockefeller University Torsten Wiesel heard about my scientific plans he suggested that I move to Rockefeller University and I did. Rockefeller provided a wonderful environment for concentrating on a difficult problem.*

*It has been said that giving up my already successful laboratory at Harvard to pursue the structure of a $K^+$ channel was a risky thing to do. At the time I was told that my aspirations were altogether unrealistic. From my perspective I had little choice because I wanted to understand $K^+$ selectivity and I knew that the atomic structure provided the only path to understanding. I would rather fail trying than never try at all. It helped that I was accustomed to making transitions and had become good at teaching myself new subjects. I have to admit that few people working with me at the time wanted much to do with the new endeavor—only one new postdoctoral scientist Declan Doyle was enthusiastic. My wife Alice, an organic*

  

A recent photograph of Rod and Alice.

*chemist, saw that I was going to be pretty lonely and decided to join me in the lab. And to my good fortune she has worked with me since. I have learned that most people do not like change, but I do. For me change is challenging, good for creativity, and it definitely keeps life interesting. I think of the past eight years of my life in New York at Rockefeller University as a personal odyssey. The new laboratory started out very small, with only Declan, Alice, and me. But it grew in the first year with the addition of other enthusiastic postdoctoral scientists, including João Morais Cabral, and John Imredy. Working with membrane proteins was very difficult, as expected. We had our periods of despair, but every time we felt left without options something good happened and despair gave way to excitement. Persistence and dedication eventually paid off. The atomic structure of the $K^+$ selectivity filter was more informative and more beautiful than I ever could have imagined. My laboratory now is an incredible place, over-flowing with excitement and ideas sustained by the continual infusion of bright young scientists who come from around the world to work with me. It gives me great satisfaction to know that these young scientists who are sophisticated in their knowledge of protein chemistry and structure will lead the field of ion-channel research into the future. This has been a wonderful adventure.*

*I owe thanks for the life I have: to Alice, to all my loving family of MacKinnons and Lees, to my scientific family of students, postdoctoral researchers, and colleagues, to senior colleagues who have helped me along my way to pursue my passion, and to the Rockefeller University, the Howard Hughes Medical Institute, and the National Institutes of Health for their support. I am very thankful for my life as a scientist, for the opportunity to understand in some small way the world around me. I hope my best experiment and scientific ideas are yet to come. This hope keeps me going.*

## *Introduction*

All living cells are surrounded by a thin, approximately 40-Å-thick lipid bilayer called the cell membrane. The cell membrane holds the contents of a cell in one place so that the chemistry of life can occur, but it is a barrier to the movement of certain essential ingredients including the ions $Na^+$, $K^+$, $Ca^{2+}$, and $Cl^-$. The barrier to ion flow across the membrane—known as the dielectric barrier—can be understood at an intuitive level: the interior of the cell membrane comprises an oily substance and ions are more stable in water than in oil. The energetic preference of an ion for water arises from the electric field around the ion and its interaction with neighboring molecules. Water is an electrically polarizable substance, which means that its molecules rearrange in an ion's electric field so that negative oxygen atoms point in the direction of cations and positive hydrogen atoms point toward anions. These electrically stabilizing interactions are much weaker in a less-polarizable substance such as oil. Thus, an ion will tend to stay in the water on either side of a cell membrane rather than enter and cross the membrane. Yet numerous cellular processes, ranging from electrolyte transport across epithelia to electrical signal production in neurons, depend on the flow of ions across the membrane. To mediate the flow, specific protein catalysts known as ion channels exist in the cell membrane. Ion channels exhibit the following three essential properties: 1) they conduct ions rapidly, 2) many ion channels are highly selective, which means only certain ion species flow while others are excluded, 3) their function is regulated by processes known as gating, that is, ion conduction is turned on and off in response to specific environmental stimuli. Figure 1 summarizes these properties.

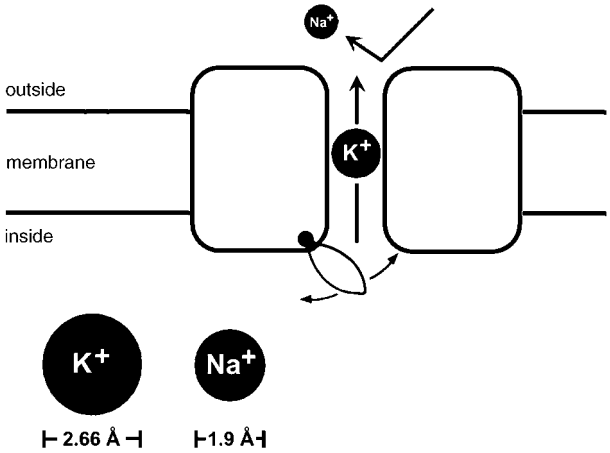


***Figure 1.*** Ion channels exhibit three basic properties: 1) They conduct specific ions at high rates: for example, $10^7$–$10^8$ $K^+$ ions per second flow through a $K^+$ channel, 2) they are selective (a $K^+$ channel essentially excludes $Na^+$ ions), and 3) conduction is turned on and off by opening and closing a gate, which can be regulated by an external stimulus such as ligand binding or membrane voltage. The relative size of $K^+$ and $Na^+$ ions is shown.

The modern history of ion channels began in 1952 when Hodgkin and Huxley published their seminal papers on the theory of the action potential in the squid giant axon.[1–4] A fundamental element of their theory was that the axon membrane undergoes changes in its permeability to $Na^+$ and $K^+$ ions. The Hodgkin–Huxley theory did not address the mechanism by which changes in the membrane permeability occur: ions could potentially cross the membrane through

  

channels or by a carrier-mediated mechanism. In their words "*Details of the mechanism will probably not be settled for some time*".[1] It is fair to say that the pursuit of this statement has accounted for much research on ion channels over the past fifty years.

As early as 1955 experimental evidence for channel-mediated ion flow was obtained when Hodgkin and Keynes measured the directional flow of $K^+$ ions across axon membranes using the isotope $^{42}K^+$.[5] They observed that the flow of $K^+$ ions in one direction across the membrane depends on the flow in the opposite direction, and suggested that "*the ions should be constrained to move in single file and that there should, on average, be several ions in a channel at any moment*". Over the following two decades Armstrong and Hille used electrophysiological methods to demonstrate that $Na^+$ and $K^+$ ions cross cell membranes through unique protein pores—$Na^+$ channels and $K^+$ channels—and developed the concepts of selectivity filters for ion discrimination and gates for regulating ion flow.[6–12] The patch recording technique invented by Neher and Sakmann then revealed the electrical signals from individual ion channels, as well as the extraordinary diversity of ion channels in living cells throughout nature.[13]

The past twenty years have been the era of molecular biology for ion channels. The ability to manipulate amino acid sequences and express ion channels at high levels opened up entirely new possibilities for analysis. The advancement of techniques for protein-structure determination and the development of synchrotron facilities also created new possibilities. For me, a scientist who became fascinated with understanding the atomic basis of life's electrical system, there could not have been a more opportune time to enter the field.

## Early Studies: The Signature Sequence of the $K^+$ Channel

The cloning of the Shaker $K^+$ channel gene from *Drosophila melanogaster* by Jan, Tanouye, and Pongs revealed for the first time the amino acid sequence of a $K^+$ channel and stimulated efforts in many laboratories to discover which of these amino acids form the pore, selectivity filter, and gate.[14–16] In Chris Miller's laboratory at Brandeis University I developed an approach to find the pore amino acids. Chris and I had just completed a study showing that charybdotoxin, a small protein from scorpion venom, inhibits a $K^+$ channel isolated from skeletal muscle cells by plugging the pore and obstructing the flow of ions.[17] In one of those late night "let's see what happens if" experiments while taking a molecular biology course at Cold Spring Harbor I found that the toxin—or what turned out to be a variant of it present in the charybdotoxin preparation—inhibited the Shaker $K^+$ channel.[18,19] This observation meant I could use the toxin to find the pore, and it did not take very long to identify the first site-directed mutants of the Shaker $K^+$ channel with altered binding of the toxin.[20] I continued these experiments at Harvard Medical School where I had become assistant professor in 1989. Working with my small group at Harvard, including Tatiana Abramson, Lise Heginbotham, and Zhe Lu, and sometimes with Gary Yellen at Johns Hopkins University, we reached several interesting conclusions concerning the architecture of $K^+$ channels. They had to be tetramers in which four subunits encircle a central ion pathway.[21] This conclusion was not terribly surprising but the experiments and analysis to reach it gave me great pleasure since they required only simple measurements and clear reasoning with binomial statistics. We also deduced that each subunit presents a "pore loop" to the central ion pathway (Figure 2).[22] This "loop" formed the binding sites for scorpion toxins[20,23,24] as well as

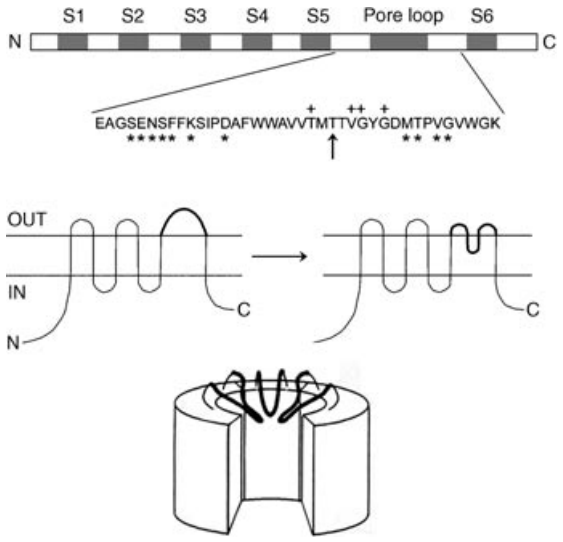


***Figure 2.*** One of the first pictures of a tetrameric $K^+$ channel with a selectivity filter made of pore loops. A linear representation of a Shaker $K^+$ channel subunit on top shows shaded hydrophobic segments S1 to S6 and a region designated the pore loop. A partial amino acid sequence from the Shaker $K^+$ channel pore loop highlights amino acids shown to interact with extracellular scorpion toxins (*), intracellular tetraethylammonium (↑), and $K^+$ ions (+). The pore loop was proposed to reach into the membrane (middle) and form a selectivity filter at the center of four subunits (bottom).

the small-molecule inhibitor the tetraethylammonium ion,[25,26] which had been used by Armstrong and Hille decades earlier in their pioneering analysis of $K^+$ channels.[9,27] Most important to my thinking was that mutations of certain amino acids within the "loop" affected the channel's ability to discriminate between $K^+$ and $Na^+$ ions, the selectivity hallmark of $K^+$ channels.[28,29] Meanwhile, new $K^+$ channel genes were discovered and they all had one clear feature in common: the very amino acids that we had found to be important for $K^+$ selectivity were conserved (Figure 3). We called these amino acids the signature sequence of the $K^+$ channel, and imagined four pore loops somehow forming a selectivity filter with the signature sequence amino acids inside the pore.[22,29]

When you consider the single-channel conductance of many $K^+$ channels found in cells you realize just how incredible these molecular devices are. With typical cellular electrochemical gradients, $K^+$ ions conduct at a rate of $10^7$ to $10^8$ ions per second. That rate approaches the expected collision frequency of $K^+$ ions from solution with the entrance

  

| | | |
|---|---|---|
| Bacteria | 2TM : | TATTVGYG |
| Archea | 6TM : | TATTVGYG |
| Plant | 6TM : | TLTTVGYG |
| Fruitfly | 6TM : | TMTTVGYG |
| Worm | 6TM : | TMTTVGYG |
| Mouse | 6TM : | SMTTVGYG |
| Human | 2TM : | TQTTIGYG |
| Human | 6TM : | TMTTVGYG |

***Figure 3.*** The signature sequence of the $K^+$ channel (shown as single letter amino acid code) is highly conserved in organisms throughout the tree of life. Some $K^+$ channels contain six membrane-spanning segments per subunit (6TM) while others contain only two (2TM). The 2TM $K^+$ channels correspond to 6TM $K^+$ channels without the first four membrane-spanning segments (S1–S4 in Figure 2).

to the pore. This means that $K^+$ ions flow through the pore almost as fast as they diffuse up to it. For this to occur the energetic barriers in the channel have to be very low, something like those encountered by $K^+$ ions diffusing through water. All the more remarkable is that the high rates are achieved in the setting of exquisite selectivity: the $K^+$ channel conducts $K^+$ ions, a monovalent cation of Pauling radius 1.33 Å, while essentially excluding $Na^+$ ions, a monovalent cation of Pauling radius 0.95 Å—this ion selectivity is critical to the survival of a cell. How does nature accomplish high conduction rates and high selectivity at the same time? The answer to this question would require knowing the atomic structure formed by the amino acids forming the signature sequence, that much was clear. The conservation of the signature sequence amino acids in $K^+$ channels throughout the tree of life, from bacteria[30] to higher eukaryotic cells, implied that nature had settled upon a very special solution to achieve rapid, selective $K^+$ conduction across the cell membrane. For me, this realization provided inspiration to want to directly visualize a $K^+$ channel and its selectivity filter.

## *The KcsA Structure And Selective $K^+$ Conduction*

I began to study crystallography, and although I had no idea how I would obtain funding for this endeavor, I have always believed that if you really want to do something then you will find a way.

By happenstance I explained my plan to Torsten Wiesel, then president of Rockefeller University. He suggested that I come to Rockefeller where I would be able to concentrate on the problem. I accepted his offer and moved there in 1996. In the beginning I was joined by Declan Doyle and my wife Alice Lee MacKinnon and within a year others joined, including João Morais Cabral, John Imredy, Sabine Mann, and Richard Pfuetzner. We had to learn as we went along, and what we may have lacked in size and skill we more than compensated for with enthusiasm. It was a very special time. At first I did not know how we would ever reach the point of obtaining enough $K^+$-channel protein to attempt crystallization, but the signature sequence of the $K^+$ channel continued to appear in a growing number of prokaryotic genes, thus making expression in *Escherichia coli* possible. We focused our effort on a bacterial $K^+$ channel called KcsA from *Streptomyces lividans*, discovered by Schrempf et al.[31] The KcsA channel has a simple topology with only two membrane-spanning segments per subunit that corresponds to the Shaker $K^+$ channel without segments S1–S4 (Figure 2). Despite its prokaryotic origin, KcsA closely resembled the amino acid sequence of the Shaker $K^+$ channel's pore, and even exhibited many of its pharmacological properties, including inhibition by scorpion toxins.[32] This surprised us from an evolutionary standpoint, because why should a scorpion want to inhibit a bacterial $K^+$ channel? However, from the utilitarian point of view of protein biophysicists we knew exactly what the scorpion toxin sensitivity meant, KcsA had to be very similar in structure to the Shaker $K^+$ channel.

The KcsA channel produced crystals but they were poorly ordered and not very useful in the X-ray beam. After we struggled for quite a while I began to wonder whether some part of the channel was intrinsically disordered and interfering with crystallization. Fortunately my neighbor Brian Chait and his postdoctoral colleague Steve Cohen were experts in the analysis of soluble proteins by limited proteolysis and mass spectrometry, and their techniques applied beautifully to a membrane protein. We found that KcsA was as solid as a rock, except for its C-terminus. After removing the disordered amino acids from the C-terminus with chymotrypsin, the crystals improved dramatically and we were able to solve an initial structure at a resolution of 3.2 Å.[33] We could not clearly see $K^+$ ions in the pore at this resolution, but my years of work on $K^+$-channel function told me that $Rb^+$ and $Cs^+$ ions should be valuable electron-dense substitutes for $K^+$ ions, and they were. Difference Fourier maps with $Rb^+$ and $Cs^+$ ions showed these ions lined up in the pore—as Hodgkin and Keynes might have imagined in 1955.[5]

The KcsA structure was altogether illuminating, but before I describe it, I will depart from chronology to explain the next important technical step. A very accurate description of the ion-coordination chemistry inside the selectivity filter would require a higher-resolution structure. From the results of the 3.2-Å data we could infer the positions of the main-chain carbonyl oxygen atoms by applying our knowledge of small-molecule structures, that is, our chemical intuition, but we needed to see the selectivity filter atoms in detail. A high-resolution structure was actually quite difficult to obtain. After more than three additional years of work by João and then Yufeng (Fenny) Zhou, we finally managed to produce high-quality crystals by attaching monoclonal Fab fragments to KcsA. These crystals provided the information we needed, a structure at a resolution of 2.0 Å in which $K^+$ ions could be visualized in the grasp of selectivity filter protein atoms (Figure 4).[34] What did the structure of the $K^+$ channel tell us and why did nature conserve the signature sequence of amino acids in the $K^+$ channel?

Not all protein structures speak to you in an understandable language, but the KcsA $K^+$ channel does. Four subunits surround a central ion pathway that crosses the membrane (Figure 5a). Two of the four subunits are shown in Figure 5b together with electron density from $K^+$ ions and water along the pore. Near the center of the membrane the ion pathway is very wide, forming a cavity about 10 Å in diameter with a hydrated $K^+$ ion at its center. Each subunit directs the C-terminal end of a "pore helix" (shown in red)

  

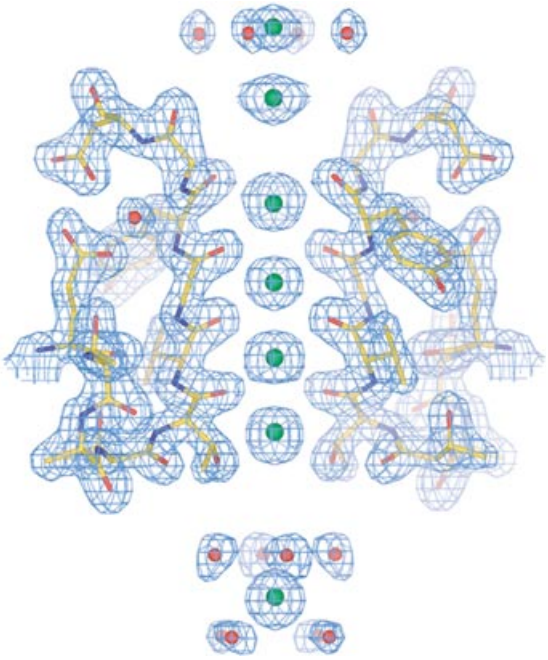

***Figure 4.*** Electron density ($2F_o-F_c$ contoured at $2\sigma$) derived from a high-resolution structure of the KcsA $K^+$ channel. This region of the channel features the selectivity filter with $K^+$ ions and water molecules along the ion pathway. The refined atomic model is shown in the electron-density representation. Adapted from ref. [34].

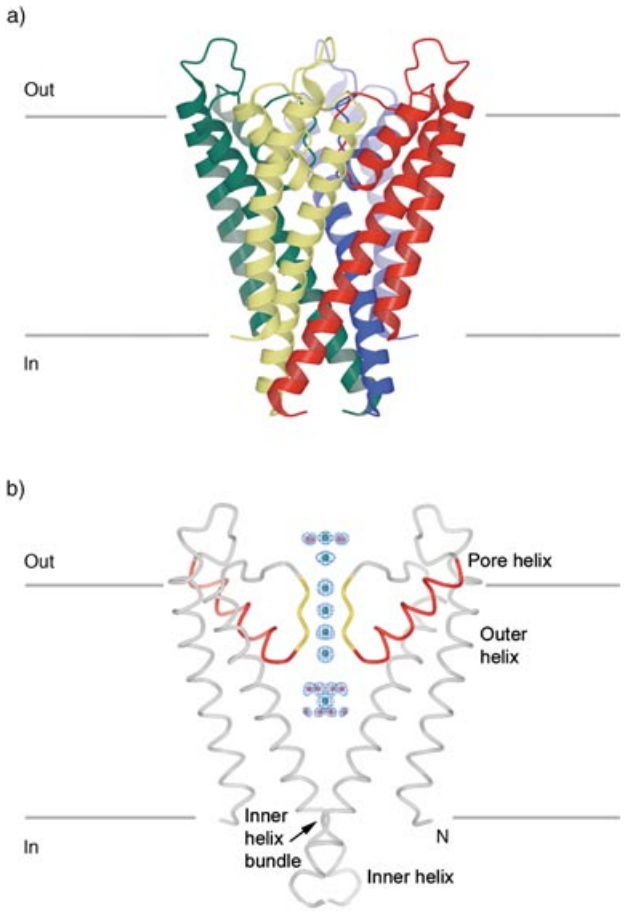


***Figure 5.*** a) A ribbon representation of the KcsA $K^+$ channel with its four subunits colored differently. The channel is oriented with the extracellular solution on top. b) The KcsA $K^+$ channel with front and back subunits removed. The pore helices are shown in red and selectivity filter in yellow; the electron density along the ion pathway is shown in a blue mesh. The outer and inner helices correspond to S5 and S6 in Figure 2.

toward the ion. The C-terminal end of an α-helix is associated with a negative "end charge" as a result of carbonyl oxygen atoms that do not participate in secondary-structure hydrogen bonding, so the pore helices are directed as if to stabilize the $K^+$ ion in the cavity. At the beginning of this lecture I raised the fundamental issue of the cell membrane being an energetic barrier to ion flow because of its oily interior. KcsA allows us to intuit a simple logic encoded in its structure, and electrostatic calculations support the intuition:[35] the $K^+$ channel lowers the membrane dielectric barrier by hydrating a $K^+$ ion deep inside the membrane, and by stabilizing it with charges at the ends of the α-helix.

How does the $K^+$ channel distinguish $K^+$ from $Na^+$ ions? Our earlier mutagenesis studies had indicated that the amino acids of the signature sequence would be responsible for this most basic function of a $K^+$ channel. Figure 6 shows the structure formed by the signature sequence—the selectivity filter—located in the extracellular third of the ion pathway. The glycine amino acids in the sequence TVGYG have dihedral angles in or near the left-handed helical region of the Ramachandran plot, as does the threonine residue, thus allowing the main-chain carbonyl oxygen atoms to point in one direction, toward the ions along the pore. It is easy to understand why this sequence is so conserved among $K^+$ channels: the alternating glycine amino acids permit the required dihedral angles, the threonine hydroxy oxygen atom coordinates to a $K^+$ ion, and the side-chains of valine and tyrosine are directed into the protein core surrounding the filter to impose geometric constraint. The end result when the subunits come together is a narrow tube consisting of four

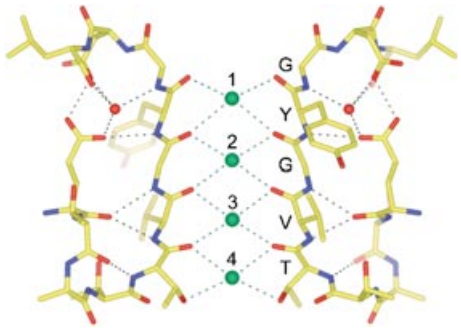


***Figure 6.*** Detailed structure of the $K^+$-selectivity filter (two subunits). Oxygen atoms (red) coordinate $K^+$ ions (green spheres) at positions 1 to 4 from the extracellular side. Single letter amino acid code identifies select amino acids of the signature sequence (yellow: carbon, blue: nitrogen, and red: oxygen). Green and gray dashed lines show O···$K^+$ and hydrogen-bonding interactions, respectively.

equally spaced $K^+$-binding sites, labeled 1 to 4 from the extracellular side. Each binding site is a cage formed by eight oxygen atoms on the vertices of a cube, or a twisted cube

  

called a square antiprism (Figure 7). The binding sites are very similar to the single alkali-metal site in nonactin, a $K^+$-selective antibiotic with nearly identical $K^+\cdots O$ distances.[36,37] The principle of $K^+$ selectivity is implied in a subtle feature of the KcsA crystal structure. The oxygen atoms surrounding the $K^+$ ions in the selectivity filter are arranged in a similar manner to the water molecules surrounding the hydrated $K^+$ ion in the cavity. This comparison conveys a visual impression of binding sites in the filter paying for the energetic cost of the dehydration of the $K^+$ ions. The $Na^+$ ion is apparently too small for these $K^+$-sized binding sites, so its dehydration energy is not compensated.

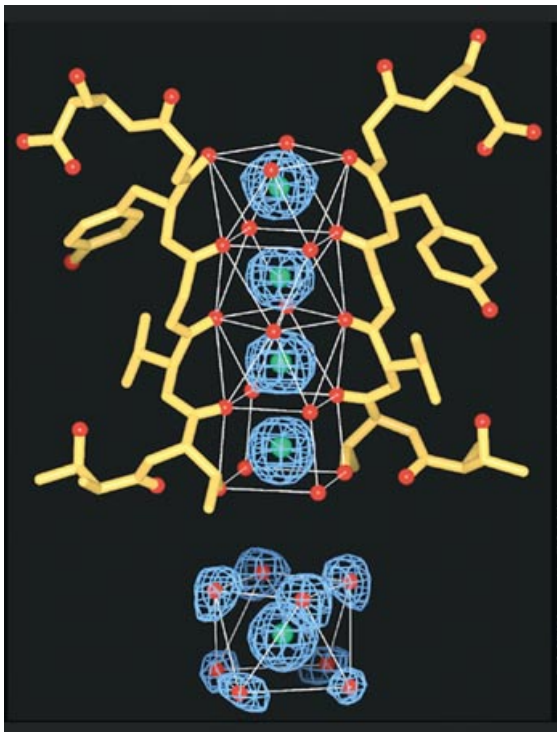

***Figure 7.*** A $K^+$ channel mimics the hydration shell surrounding a $K^+$ ion. The electron density (blue mesh) for $K^+$ ions in the filter and for a $K^+$ ion and water molecules in the central cavity are shown. White lines highlight the coordination geometry of $K^+$ ions in the filter and in water. Adapted from ref. [34].

The question that compelled us most after seeing the structure was exactly how many ions are in the selectivity filter at a given time? To begin to understand how ions move through the filter we needed to know the stoichiometry of the ion-conduction reaction, and that meant knowing how many ions can occupy the filter. Four binding sites were apparent, but are they all occupied at once? Four $K^+$ ions in a row separated by an average center-to-center distance of 3.3 Å seemed unlikely for electrostatic reasons. From an early stage we suspected that the correct number would be closer to two, because two ions more easily explained the electron density we observed for the larger alkali-metal ions $Rb^+$ and $Cs^+$.[33,38] Quantitative evidence for the precise number of ions came with the high-resolution structure and with the analysis of $Tl^+$ ions.[40] Thallium is the most ideally suited "$K^+$ analogue" because it flows through $K^+$ channels, has a radius and dehydration energy very close to $K^+$, and has the favorable crystallographic attributes of high electron density and an anomalous signal. The one serious difficulty in working with $Tl^+$ ions is the formation of the insoluble TlCl in the presence of $Cl^-$. Fenny meticulously worked out the experimental conditions and determined that on average there are between two and two and a half conducting ions in the filter at one time, with an occupancy at each position of around one half.

We also observed that if the concentration of $K^+$ (or $Tl^+$) ions bathing the crystals is lowered sufficiently (below normal intracellular levels), then a reduction in the number of ions from two to one occurs and is associated with a structural change to a "collapsed" filter conformation, which is pinched closed in the middle.[34,40] At concentrations above 20 mM the entry of a second $K^+$ ion drives the filter to a "conductive" conformation, as shown in Figure 8. Sodium, on the other hand, does not drive the filter to a "conductive" conformation, even at concentrations up to 500 mM.

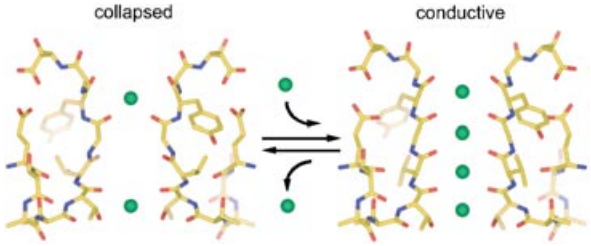


***Figure 8.*** The selectivity filter can adopt two conformations. At low concentrations of $K^+$ ions, one $K^+$ ion resides on average at either of two sites near the ends of the filter, which is collapsed in the middle. At high concentrations of $K^+$ ions, a second ion enters the filter as it changes to a conductive conformation. On average, two $K^+$ ions in the conductive filter reside at four sites, each with about half occupancy.

The $K^+$-induced conformational change has thermodynamic consequences for the affinity of two $K^+$ ions in the "conductive" filter. It implies that a fraction of the second ion's binding energy must be expended as work to bring about the filter's conformational change, and as a result the two ions will bind with reduced affinity. To understand this statement at an intuitive level, it must be recognized that for two ions to reside in the filter they must oppose its tendency to collapse and force one of them out, that is, the two-ion "conductive" conformation is under some tension, which will tend to lower the affinity for $K^+$ ions. This is a desirable property for an ion channel because weak binding favors high conduction rates. The same principle, referred to as the "induced fit" hypothesis, had been proposed decades earlier by enzymologists to explain high specificity with low substrate affinity in enzyme catalysis.[39]

If two $K^+$ ions were randomly distributed in the "conductive" filter then they would occupy four sites in six possible ways. But several lines of evidence hinted to us that the ion positions are not random. For example, $Rb^+$ and $Cs^+$ ions exhibit preferred positions with clearly low occupancy at position 2.[38,40] With $K^+$, we observed an unusual

  

doublet peak of electron density at the extracellular entrance to the selectivity filter (see Figure 9).[34] We could explain this density if the $K^+$ ion is attracted from solution by the negative protein surface charge near the entrance and at the same time repelled by $K^+$ ions inside the filter. Two discrete peaks implied two distributions of ions in the filter. If $K^+$ ions are separated by a water molecule for electrostatic reasons then the two dominant configurations would be 1,3 ($K^+$ ions in positions 1 and 3 with a water molecule in between) and 2,4 ($K^+$ ions in positions 2 and 4 with a water molecule in between). A mutation at position 4 (threonine to cysteine) was recently shown to influence $K^+$ occupancy at positions 2 and 4 but not at 1 and 3, thus providing strong evidence for specific 1,3 and 2,4 configurations of $K^+$ ions inside the selectivity filter.[69]

Discrete configurations of an ion pair suggested a mechanism for ion conduction (Figure 10a).[38] The $K^+$ ion pair could diffuse back and forth between 1,3 and 2,4 configurations (bottom pathway), or alternatively an ion could enter the filter from one side of the membrane as the ion–water queue moves and a $K^+$ ion exits at the opposite side (the top pathway). Movements would have to be concerted because the filter is no wider than a $K^+$ ion or water molecule. The two paths complete a cycle: in one complete cycle each ion moves only a fraction of the total distance through the filter, but the overall electrical effect is to move one charge all the way. As two $K^+$ ions are present in the filter throughout the cycle, we expect there should be electrostatic repulsion between them. Together with the filter conformational change that is required to achieve a "conductive" filter with two $K^+$ ions in it, electrostatic repulsion should favor high conduction rates by lowering $K^+$ affinity.

The absolute rates from $10^7$ to $10^8$ ions per second are truly impressive for a highly selective ion channel. One aspect of the crystallographic data suggests that very high conductance $K^+$ channels such as KcsA might operate near the maximum rate that the conduction mechanism will allow. All four positions in the filter have a $K^+$ occupancy close to one half, which implies that the 1,3 and 2,4 configurations are equally probable, or energetically equivalent, but there is no evident reason why this should be. A simulation of ions diffusing around the cycle offers a possible explanation: maximum flux is achieved when the energy difference between the 1,3 and 2,4 configurations is zero because that is the condition under which the "energy landscape" for the conduction cycle is smoothest (Figure 10b). The energetic balance between the configurations therefore might reflect the optimization of the conduction rate by natural selection.[38] It is not so easy to demonstrate this point experimentally, but it is certainly fascinating to ponder.

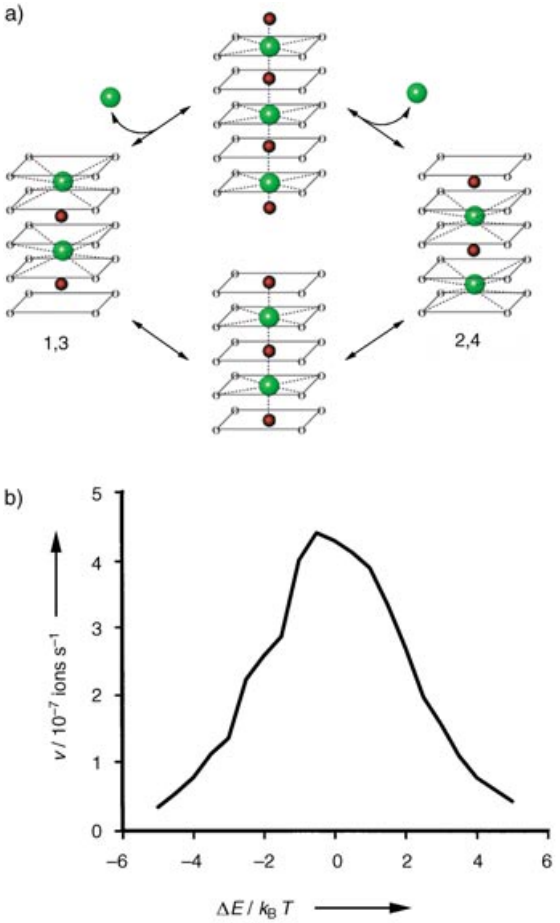


***Figure 10.*** a) Throughput cycle for $K^+$ conduction invoking 1,3 and 2,4 configurations. The selectivity filter is represented as five square planes of oxygen atoms. $K^+$ ions and water are shown as green and red spheres, respectively. b) Simulated $K^+$ flux around the cycle is shown as a function of the energy difference between the 1,3 and 2,4 configurations. Adapted from ref. [38].

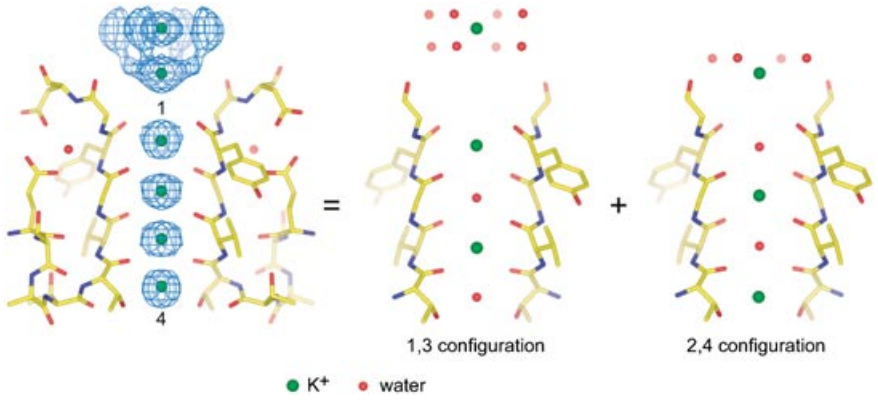


***Figure 9.*** Two $K^+$ ions in the selectivity filter are hypothesized to exist predominantly in the two specific configurations 1,3 and 2,4. Adapted from ref. [34].

## *Common Structural Principles Underlie $K^+$ And $Cl^-$ Selectivity*

The focus of this lecture is $K^+$ channels, but for a brief inter-

lude I would like to show you a $Cl^-$-selective transport protein. By comparing a $K^+$ channel and a $Cl^-$ "channel" we can begin to appreciate familiar themes in nature's solutions to different problems: getting cations and anions across the cell membrane. ClC chloride channels are found in many different cell types and are associated with a number of physiological processes that require flow of $Cl^-$ ions across lipid membranes.[41,42] As is the case for $K^+$ channels, genes of the ClC family are abundant in prokaryotes, which is a fortunate circumstance for protein expression and structural analysis. When Raimund Dutzler joined my laboratory he, Ernest Campbell, and I set out to address the structural basis of $Cl^-$-ion selectivity. We determined crystal structures of two bacterial members of the ClC $Cl^-$ channel family, one from *Escherichia coli* (EcClC) and another from *Salmonella typhimurium* (StClC).[43] Recent studies by Accardi and Miller on the function of EcClC have shown that it is actually a $Cl^-$–proton exchanger.[44] We do not yet know why certain members of this family of $Cl^-$-transport proteins function as channels and others as exchangers, but the crystal structures are fascinating and give us a view of $Cl^-$ selectivity. Architecturally the ClC proteins are unrelated to $K^+$ channels, but if we focus on the ion pathway certain features are similar (Figure 11). As we saw with the $K^+$ channels, the ClC proteins

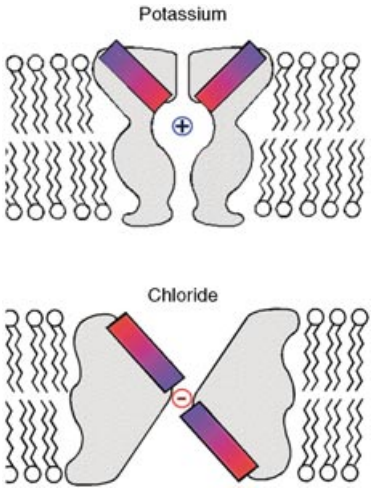


***Figure 11.*** The overall architecture of $K^+$ channels and ClC $Cl^-$-transport proteins is very different, but certain general features are similar. One similarity shown here is the direction of α-helix end charges toward the ion pathway. The negative C-terminal end charge (red) points to the $K^+$ ion. The positive N-terminal end charge (blue) points to the $Cl^-$ ion.

have α-helices pointed at the ion pathway, but the direction is reversed, with the positive charge of the N-terminus close to the $Cl^-$ ions. This makes perfect sense for lowering the dielectric barrier for a $Cl^-$ ion. In ClC we see that ions in its selectivity filter tend to be coordinated by main-chain protein atoms, with amide nitrogen atoms surrounding the $Cl^-$ ion instead of carbonyl oxygen atoms surrounding the $K^+$ ion (Figure 12). We also see that both the $K^+$ and $Cl^-$ selectivity filters contain multiple close-spaced binding sites and appear to contain more than one ion, perhaps to exploit electrostatic

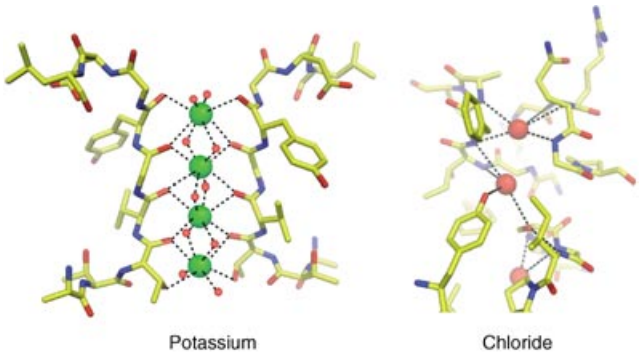


***Figure 12.*** $K^+$- and $Cl^-$-selectivity filters make use of main-chain atoms to coordinate ions: carbonyl oxygen atoms for $K^+$ ions (green) and amide nitrogen atoms for $Cl^-$ ions (red). Both filters contain multiple close-spaced ion-binding sites. The $Cl^-$-selectivity filter is that of a mutant ClC in which a glutamate amino acid was changed to glutamine.[64]

repulsion between ions in the pore. I find these similarities fascinating. They tell us that certain basic physical principles are important, such as the use of α-helix end charges to lower the dielectric barrier when ions cross the lipid membrane.

## *Trying to See a $K^+$ Channel Open and Close*

Most ion channels conduct when called upon by a specific stimulus such as the binding of a ligand or a change in membrane voltage.[45] The processes by which ion conduction is turned on are called gating. The conduction of ions occurs on a time scale that is far too rapid to involve very large conformational changes of the proteins. That is undoubtedly one of the reasons why a single KcsA structure could tell us so much about ion selectivity and conduction. Gating on the other hand occurs on a much slower time scale and can involve large conformational changes of the proteins. The challenge for a structural description of gating is to capture a channel in both opened (on) and closed (off) conformations so that they can be compared.

Gating in the KcsA $K^+$ channel is controlled by intracellular pH values and the composition of the lipid membrane, but unfortunately the probability of the KcsA channel being open reaches a maximum value of only a few percent in functional assays.[46,47] At first we had no definitive way to know whether a gate was open or closed in the crystal structures. In the 1970s Armstrong had proposed the existence of a gate near the intracellular side of the membrane in voltage-dependent $K^+$ channels because he could "trap" large organic cations inside the pore between a selectivity filter near the extracellular side and a gate near the intracellular side.[9,48] Following these ideas, we crystallized KcsA with tetrabutylantimony (TBA), a heavy-atom version of one of his organic cations, and found that it binds inside the central cavity of KcsA.[49] This was very interesting because the approximately 10-Å diameter of TBA far exceeds the pore diameter leading up to the cavity: in KcsA the intracellular pore entrance is constricted to about 3.5 Å by the inner helix bundle (Figure 5b). Seeing TBA "trapped" in the cavity

  

behind the inner helix bundle evoked Armstrong's classical view of $K^+$-channel gating, and implied that the inner helix bundle serves as a gate and is closed in KcsA. Mutational and spectroscopic studies in other laboratories also pointed to the inner helix bundle as a possible gate-forming structural element.[50,51]

Youxing Jiang and I hoped we could learn more about $K^+$-channel gating by determining the structures of new $K^+$ channels. From gene-sequence analysis we noticed that many prokaryotic $K^+$ channels contain a large C-terminus that encodes what we called RCK domains, and we suspected that these domains controlled pore opening, perhaps through binding of an ion or a small molecule. We determined the structure of isolated RCK domains from an *Escherichia coli* $K^+$ channel, but by themselves they were not very informative beyond hinting that a similar structure exists on the C-terminus of eukaryotic $Ca^{2+}$-dependent "BK" channels.[52] We subsequently determined the crystal structure of MthK, a complete $K^+$ channel containing RCK domains from *Methanobacterium thermoautotrophicus* (Figure 13).[53] This structure was extremely informative. The RCK domains form a "gating ring" on the intracellular side of the pore. In clefts between domains we could see what appeared to be divalent cation binding sites and the crystals had been grown in the presence of $Ca^{2+}$ ions. In functional assays we discovered that the open probability of the MthK channel increased as the concentration of the $Ca^{2+}$ or $Mg^{2+}$ ions was raised, thus giving us good reason to believe that the crystal structure should represent the open conformation of a $K^+$ channel.

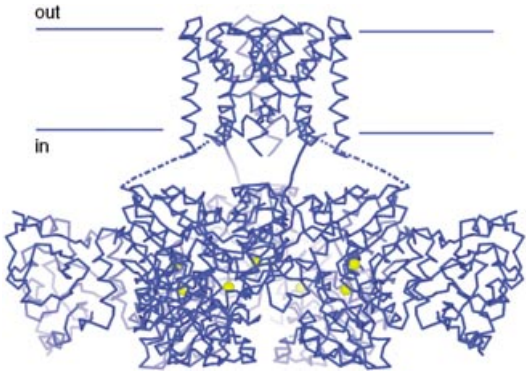


***Figure 13.*** The MthK $K^+$ channel contains an intracellular gating ring (bottom) attached to its ion-conduction pore (top). $Ca^{2+}$ ions (yellow) are bound to the gating ring in clefts in between domains. The connections between the gating ring and the pore, which were poorly ordered in the crystal, are shown as dashed lines.

In our MthK structure the inner helix bundle is opened like the aperture of a camera (Figure 14).[54] As a result, the pathway leading up to the selectivity filter from the intracellular side is about 10 Å wide, which explains how Armstrong's large organic cations can enter the cavity to block a $K^+$ channel, and how $K^+$ ions gain free access to the selectivity filter through aqueous diffusion. By comparing the KcsA and MthK channel structures it seemed that we were looking at examples of closed and opened $K^+$ channels, and could easily imagine the pore undergoing a conformational

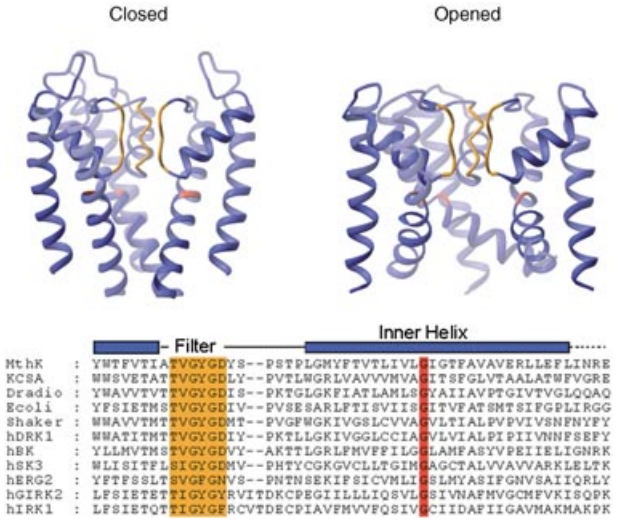


***Figure 14.*** KcsA and MthK represent closed and opened $K^+$ channels. Three subunits of the closed KcsA $K^+$ channel (left) and opened MthK $K^+$ channel (right) are shown. The inner helices of MthK are bent at a glycine hinge (red), thus allowing the inner helix bundle to open. Partial amino acid sequences from a variety of $K^+$ channels with different gating domains are compared. Colors highlighting the selectivity filter sequence (orange) and inner helix glycine hinge (red) match colors used in the structures. Adapted from ref. [54].

change from closed to open. To open, the inner helices would have to bend at a point halfway across the membrane as their C-terminus is displaced laterally away from the pore axis by conformational changes in the gating ring. A glycine amino acid facilitates the bending in MthK by introducing a hinge point in the middle of the inner helix. Like MthK, KcsA and many other $K^+$ channels contain a glycine at the very same location; its conservation suggests that the inner helices move in a somewhat similar manner in many different $K^+$ channels (Figure 14).

Gating domains convert a stimulus into pore opening. Further studies are needed to understand how the free energy of $Ca^{2+}$ binding is converted into pore opening in the MthK channel. The mechanistic details of ligand gating will vary from one channel type to the next because nature is very modular with ion channels, just like with other proteins. Gene sequences show us that a multitude of different domains can be found attached to the inner helices of different $K^+$ channels, thus allowing ions such as $Ca^{2+}$ or $Na^+$, small organic molecules, and even regulatory proteins to control the conformational state of the pore and so gate the ion channel (http://www.ncbi.nlm.nih.gov/BLAST/).[55–58]

A fundamentally different kind of gating domain allows certain $K^+$, $Na^+$, $Ca^{2+}$, and nonselective cation channels to open in response to changes in the membrane voltage. Referred to as voltage sensors, these domains are connected to the outer helices of the pore and form a structural unit within the membrane. The basic principle of operation for a voltage sensor is the movement of protein charges through the membrane electric field coupled to pore opening.[59–61] Like transistors in an electronic device, voltage-dependent channels are electrical switches. They are a serious challenge for crystallographic analysis because of their conformational

  

flexibility. Youxing Jiang and I working with Alice Lee and Jiayun Chen solved the structure of the voltage-dependent $K^+$ channel KvAP from the thermophilic Archea *Aeropyrum pernix* (Figure 15).[62,63] In the crystal of KvAP the voltage sensors, held by monoclonal Fab fragments, adopted a non-native conformation. This observation in itself is meaningful as it underscores the intrinsic flexibility of voltage sensors: in contrast Fab fragments had little effect on the more rigid KcsA $K^+$ channel and ClC $Cl^-$ channel homologue, both of which we determined in the presence and absence of Fab fragments.[33,34,43,64] KvAP's voltage sensors contain a hydrophobic helix-turn-helix element with arginine residues beside the pore,[63] and functional experiments using tethered biotin and avidin show that this element moves relative to the plane of the membrane.[70] Additional structures revealing different channel conformations will be needed to better understand the mechanistic details of voltage-dependent gating. However, the KvAP structure and associated functional studies have provided a conceptual model for voltage-dependent gating processes—one in which the voltage sensors move at the protein–lipid interface in response to a balance between hydrophobic and electrostatic forces. Rees and colleagues at the California Institute of Technology determined the structure of a voltage-regulated mechanosensitive channel called MscS, and although it is unrelated to traditional voltage-dependent channels, it too contains hydrophobic helix-turn-helix elements with arginine residues apparently against the lipid membrane.[65] MscS and KvAP are fascinating membrane protein structures. They do not fit into the standard category of membrane proteins with rigid hydrophobic walls against the lipid membrane core. I find such proteins intruiging.

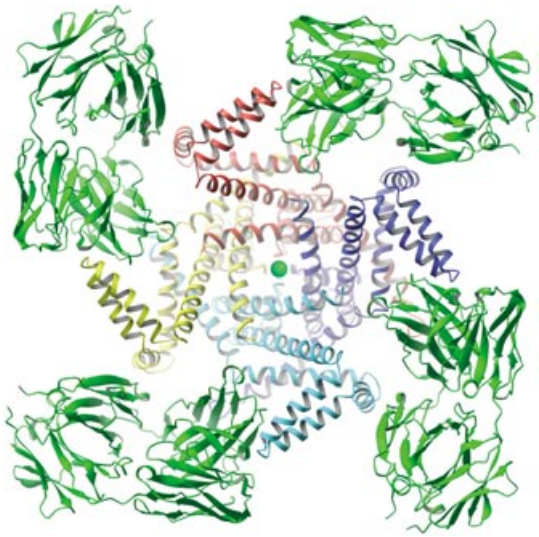

***Figure 15.*** Crystal structure of the KvAP $K^+$ channel in a complex with monoclonal antibody Fab fragments. The channel is viewed along the pore axis from the intracellular side of the membrane, with $\alpha$-helical subunits colored in blue, yellow, cyan, and red. One Fab fragment (green) is bound to the helix-turn-helix element of the voltage sensor on each subunit. Adapted from ref. [63].

We are only just beginning to understand the structural principles of ion-channel gating and regulation. Electrophysiological studies have uncovered a multitude of connections between cellular biochemical pathways and ion-channel function.[45] New protein structures are now beginning to do the same. The $\beta$ subunits of certain eukaryotic voltage-dependent channels are structurally related to oxidoreductase enzymes.[66,67] PAS domains on other $K^+$ channels belong to a family of sensory molecules,[68] and a specialized structure on G-protein-gated channels forms a binding site for regulatory G-protein subunits.[71] The interconnectedness of ion-channel function with many aspects of cell function is beginning to reveal itself as complex and fascinating.

## Concluding Remarks

I think the most exciting time in ion-channel studies is just beginning. So many of the important questions are waiting to be answered and we have the tools in hand to answer them. I am very optimistic about the future, and for the great possibilities awaiting young scientists who are now setting out to study ion channels and other membrane proteins. I consider myself very fortunate to have contributed to some small part of the knowledge we have today. Of course, my contributions would never have been possible without the

***Table 1:*** MacKinnon laboratory from 1989 to 2003.

| Postdoctoral researchers | | Students | Staff scientists | Collaborators |
|---|---|---|---|---|
| Laura Escobar | Francis Valiyaveetil | Lise Heginbotham | Tatiana Abramson | Gary Yellen |
| Zhe Lu | Xiao-Dan Pfenninger-Li | Michael Root | John Lewis | Maria Garcia |
| Adrian Gross | Ming Zhou | Patricia Hidalgo | Alice Lee MacKinnon | Gerhard Wagner |
| Kenton Swartz | Ofer Yifrach | Sanjay Aggarwal | Sabine Mann | Andrzej Krezel |
| Chul-Seung Park | Yufeng Zhou | James Morrell | Richard Pfuetzner | Brian Chait |
| Rama Ranganathan | Sebastien Poget | Alexander Pico | Anling Kuo | Steve Cohen |
| Chinfei Chen | Motohiko Nishida | Vanessa Ruta | Minhui Long | Martine Cadene |
| Declan Doyle | Uta-Maria Ohndorf | Ian Berke | Amelia Kaufman | Benoit Roux |
| John Imredy | Steve Lockless | | Ernest Campbell | Tom Muir |
| João Morais Cabral | Qiu-Xing Jiang | | Jiayun Chen | |
| Youxing Jiang | Seok-Yong Lee | | | |
| Jacqueline Gulbis | Stephen Long | | | |
| Raimund Dutzler | | | | |

efforts and enthusiasm of the young scientists who have come from around the world to study ion channels with me (Table 1). I also owe thanks to the Rockefeller University, the Howard Hughes Medical Institute, and the National Institutes of Health for supporting my scientific research. I also thank the synchrotrons CHESS, NSLS, ALS, APS, and ESRF as well as my assistant Wendell Chin.

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