See potassium run

Christopher Miller

Nearly all cells have membranes spanned by potassium-conducting channel proteins, without which your nerves (and much else) simply wouldn't work. Ion permeation through these channels can now be seen in dazzling detail.

Scientific discovery has its breakthroughs, and breakthroughs their aftermaths. Three years ago, Roderick MacKinnon's group at Rockefeller University in New York achieved a stunning advance in molecular neurobiology with the first atomic-resolution picture of a potassium (K^+) channel[1]. Three papers in this issue[2–4] now give us a mechanistic and dynamic view of how these pore proteins work.

Potassium channels are important membrane-spanning proteins that directly catalyse the ionic movements required to generate and shape electrical signals in neurons (and also in many other cells not so well endowed with brain-associated pizzazz). The original structure[1], though a bit fuzzy at 3.2 Å resolution, evoked gasps as it revealed the molecular design of these pores. It gave hope for an eventual understanding of the perplexing functional hallmark of K^+ channels — the combination of high selectivity and rapid throughput that allows K^+ to pass at such high rates that the protein seems to present no barrier at all, while simultaneously acting as a concrete wall to the smaller Na^+ ion.

The structure[1] sketched out the molecular basis of this specificity: a narrow 'selectivity filter' in the shape of an oxygen-lined electronegative tunnel in which dehydrated K^+ (but not Na^+) fits precisely. This structure rationalized why a K^+ ion is so willing to leave its thermodynamically comfortable home in aqueous solution to enter the pore in a largely dehydrated form; the channel interior mimics the embrace of the water molecules in the inner hydration shell surrounding the ion in solution.

And now, the aftermath. MacKinnon's group[2,3] (pages 37 and 43) presents an improved structure of the K^+ channel, while Bernèche and Roux[4] (page 73) carry out computer simulations of this same channel protein; together the results provide a deep understanding of how ions diffuse through this pore so rapidly. The new X-ray crystallographic structures, at a sharp 2 Å resolution, are dazzling. They offer three fundamental insights into the physical chemistry of ion conduction in biology: they show how ions are coordinated in the selectivity filter; they reveal the nature of K^+ ions in transit between the channel and the aqueous solvent; and they indicate the character of water in the cation's inner hydration shell.

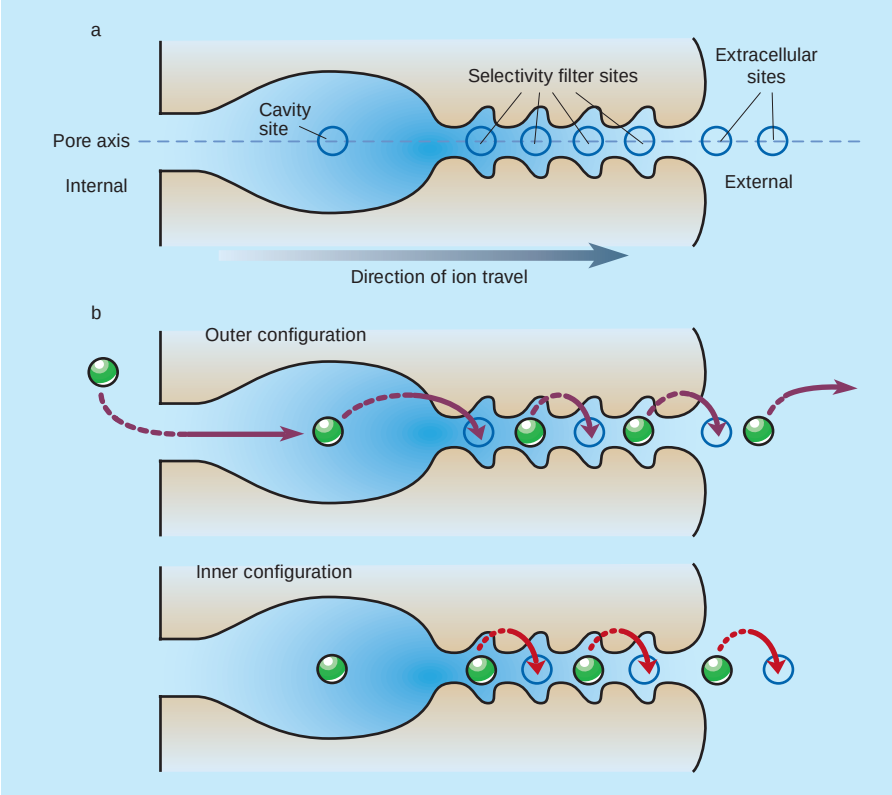

Figure 1 **Permeation of K^+ ions through the pore of a K^+ channel, as surmised from the new results[2–4] discussed here.** a, **There are seven main sites for ions along the pore axis: one in the pore cavity, four in the selectivity filter and two just beyond the external end of the pore. The cavity site is fully occupied, but (as indicated in** b**) only half of the remaining six are occupied at any one time.** b, **The two main ion configurations, known as outer and inner, that are postulated to exist within the pore. Purple arrows indicate ion shifts that are linked directly to concerted ion entry into and exit from the pore. Red arrows represent shifts within the pore without ion entry and exit. As shown here, then, ion passage through the selectivity filter and extracellular sites occurs in bucket-brigade fashion.**

According to the familiar cautionary riff, X-ray crystallography is a snapshot-based technique impoverished in dynamic information about protein function. But these new pictures let the viewer imagine K^+ ions actually traversing the pore, because they catch the ions at several stages of movement. This is an unusual situation, unknown, for example, in crystallographic enzymology, which seeks to freeze the substrate at different stages of chemical transformation, each requiring a separate crystal structure. With K^+ channels, a single image makes these multiple stages visible all at once because, in contrast to enzymes, channels process several substrate ions simultaneously in bucket-brigade fashion[5,6]. Potassium ions are now seen in seven distinct sites along the pore-axis (Fig. 1a). Four of these reside in the narrow selectivity filter, and one in the wider hydrated cavity, as described earlier[1,7]. By solving structures at varying ion concentrations, MacKinnon and colleagues argue that the four selectivity-filter sites are not all occupied simultaneously; rather, a pair of K^+ ions separated by a single water molecule shifts in a concerted fashion between two configurations within the filter — inner and outer — occupying each about half the time (Fig. 1b).

This ion-shifting between two pairs of positions, presumably on the conduction timescale of 10–100 nanoseconds, is at the heart of the permeation mechanism emerg-

ing from this work. As any long-distance cyclist knows, you make better time on level ground than in terrain with hills or valleys. It's the same for channel-dwelling K^+ ions, which permeate so rapidly because, as shown here by ion-occupancy measurements, the inner and outer ion configurations are precisely balanced in free energy, perfectly designed to eliminate barriers to high throughput. This point is buttressed by experiments with Rb^+ — a slightly larger, non-biological K^+ analogue indifferent to the demands of evolution — for which the two configurations are energetically out of balance; the resulting hilly landscape deduced from the X-ray data crisply explains the slower conduction of Rb^+ (refs 3, 8).

Two big surprises leap out of the results, and both enrich our comprehension of what life is like for ions diffusing through the channel. First, K^+ shows up at two additional on-axis positions just beyond the extracellular end of the pore, positions partially occupied by an ion shifting between the two. This ion is mainly in contact with aqueous solvent, so it is astonishing that it is localized enough to be visible in the electron-density maps used to pinpoint features by X-ray crystallography; strong electrostatic focusing by the channel surface is the likely explanation. Most dramatically, in the position closest to the pore entrance a K^+ ion is caught *in flagrante*, coordinated in front by four protein carbonyl groups reaching outwards, and behind by solvent; this must represent the long-postulated 'dehydration transition state' in which the ion sheds its water while entering the pore[9]. It is now seen not to be a high-energy transition-state at all, but rather a true intermediate, an integral part of the flat landscape.

Remarkably, in an independent computer simulation[4], Bernèche and Roux anticipated that a K^+ ion would be localized in solvent, right at the two sites where it is actually seen in the X-ray work. This successful prediction in advance of the facts — a rarity in computational biochemistry — enhances the confidence of sceptical experimentalists in the methods and parameters used in this theoretical work. This tends to fortify an additional conclusion emerging from the work — that not only are the K^+-binding configurations energetically similar, but so are the transitions between them. This means that the entire conduction process is energetically barrierless. So, overall, K^+ permeation looks rather like the concerted action of those steel pendulum toys known as Newton's balls. An ion entering the pore on the left expels an ion on the right into solution, while the four ions within and just outside the pore all shift one position to the right (Fig. 1b); if this occurs on energetically level ground, the process will be rapid.

The second surprise in the X-ray results is a structure that has often been imagined but never before glimpsed: the complete inner hydration shell of an aqueous cation. The channel's central cavity has room for about 50 water molecules, along with the single hydrated K^+ ion suspended there. Here, eight of these waters are individually visible, with their oxygens packed directly against the K^+ ion at the corners of a twisted cube. This inner-shell geometry closely matches the arrangement of the K^+-coordinating oxygens in the selectivity filter, and the authors wonder whether it might also mirror K^+ hydration in bulk liquid water. If so, they intimate, perhaps the four-fold architecture of K^+ channels evolved around the structure of hydrated K^+ to create an energetically welcoming home — the ultimate flat landscape — for ions forced to strip on their swift journey from one side of the membrane to the other. ■

Christopher Miller is in the Department of Biochemistry, Howard Hughes Medical Institute, Brandeis University, Waltham, Massachusetts 02468, USA.
e-mail: cmiller@brandeis.edu

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Quantum optics

The atomic nanoscope

Andrew Steane

An ideal probe should be as small as possible so it doesn't interfere with the observation. When measuring the distribution of a light field, it seems that a single atom is up to the job.

The quest for the small has led to much ingenuity in fabricating tiny devices, and each step from micrometre to 100-nanometre to smaller scales is celebrated. One goal of such work is to reach the scale of the atom — the most sharply pointed needle one can readily conceive would have at its tip a narrow pyramid finishing with a single atom. But a sharp needle is still far from ideal as a probe: at the atomic scale it looks like a great bulky mass of material, and introduces a large disturbance into its three-dimensional environment. A radical alternative is to throw the body of the needle away altogether, and just keep the last atom. This probe would be a just a fraction of a nanometre in size in all directions — if only we could make use of it.

Guthöhrlein and co-workers[1] from the Max Planck Institute in Garching, Germany, have just taken such a step, by controlling and using a single atom to probe the structure of a standing wave of light. On page 49 of this issue they reveal the most precise measurement yet of the three-dimensional structure of a light-wave field. But this is only the first indication of what can be done with this system. The ability to control simultaneously both atomic and optical aspects of the system opens up a range of possibilities.

The Garching experiment combines the techniques of ion trapping and laser fluorescence, together with a high-quality optical cavity. The authors used a set of electrodes with oscillating voltages (known as a radio-frequency trap) to confine a single atom of calcium that has a single positive charge. They then surrounded the trap with a pair of highly reflective concave mirrors to form an optical cavity — that is, a structure inside which light will reflect to and fro forever (except for small losses), just as sound does inside the body of a reverberating musical instrument such as a violin. In this situation the light forms a standing wave between the mirrors, so there is a series of regions of brightness and darkness, separated from each other by a quarter of a wavelength (Fig. 1). Because the wavelength in these experiments was 397 nm (this is just visible to the human eye as violet light), the light field inside the cavity goes from dark to bright to dark again every 198 nm.

The aim of the experiment was to measure this structure, and also the structure in the light field perpendicular to the standing wave, which consists of the Hermite–Gauss functions known to students of optics and quantum mechanics (see Fig. 2 on page 49). To do this, several features of ion-trap experiments come to the fore. First, ion traps are tight traps: even though the electrode structure providing the trapping is on the order of 1 mm in size, the ion is confined tightly enough to restrict its movements to a region a few tens of nanometres across. This is like confining a charged coin in a region the size of a wine glass by using charged metal plates situated a mile away.

Second, to achieve this sort of confinement the ion also needs to be cold, because otherwise its natural thermal vibrations would make it explore a larger region inside