the basic concept of scattering cross-section from an entity, as new concepts are invoked.

It seems that interesting debates are underway involving experimenters, instrument scientists and theoreticians about epi-thermal neutron spectroscopic data and their interpretation currently.

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ACKNOWLEDGEMENTS. Kind permission from Prof. Chatzidimitriou-Dreismann for reproducing the figures is gratefully acknowledged.

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A molecular voltmeter: Solution to a fifty-year-old puzzle?*

M. K. Mathew

These are the best of times for membrane biologists with a surge of interest in membrane proteins and processes, and an ever-increasing number of techniques coming to bear on all matters membranous. These are the worst of times for membrane biologists with the disparity between the number of structures of soluble and membrane proteins solved growing to three orders of magnitude and counting. The voltage-gated K^+ channel is now perhaps the best studied membrane protein, with a variety of techniques mapping aspects of function onto specific portions of the sequence. However, an understanding of how the protein senses transmembrane potential and transduces changes in potential into channel opening requires a three-dimensional structure. This has now been provided by the recent structures of the archaebacterial channel KvAP reported by Rod MacKinnon's group[1,2].

Electrical signalling in the nervous system is digital in nature, nerve cells generating an 'all-or-none' action potential that propagates down the nerve axon. The electrical events underlying the action potential were elucidated fifty years ago by Hodgkin and Huxley. The nerve cell, which is initially slightly permeable to potassium, becomes massively permeable first to sodium and then to potassium, before returning to its initial low conductance state. These changes in permeability have since been shown to be brought about by ion-channel proteins which mediate phenomenal fluxes of ions – up to 10^8 ions/second through a single channel – but are exquisitely selective for the ions they pass. Moreover, they are closed at resting membrane potentials but open in response to changes in membrane potential, i.e. they are gated by transmembrane voltage. The 'holy grail' of channel biophysicists over the past 50 years has been to explain the manner in which these apparently contradictory design requirements are met.

The very high flux through a single channel can only be explained by the protein enclosing a column of water traversing the membrane, through which the ions diffuse. The column has to be wide enough to permit free diffusion of the ions, but also have a region narrow enough to allow for discrimination. At this point, the 'selectivity filter', the column diameter would be comparable to that of the unsolvated ion to permit interaction with the protein. An hour-glass shaped aqueous column was imagined (Figure 1). High selectivity normally implies strong binding, which is incompatible with fluxes as high as 10^8 per second. Moreover, the issue of gating the channel had to be resolved.

The first major breakthrough in getting at the architecture of these proteins was the cloning of cDNAs encoding voltage-gated ion channels. Numa's group cloned a rat brain Na^+ channel in 1984 (ref. 3), but the molecule was huge – with a predicted amino acid sequence close to 2000

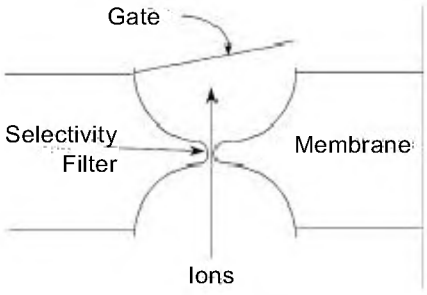

Figure 1. Schematic of an ion channel. The protein was presumed to stabilize a column of water spanning the membrane. The column was wide enough to allow free diffusion of the ion, but at its narrowest point was comparable in diameter to the unsolvated ion to facilitate interactions with the protein, ensuring selectivity. This cartoon presents an exaggerated view of the pore, the selectivity filter and a structure which physically 'gates' the channel.

*Dedicated to Prof. S. Ramaseshan on his 80th birthday.

residues long. Analysis of the cDNA revealed that the protein consisted of four repetitions of a basic unit, each of which had six transmembrane helices. The cloning of the Shaker gene from *Drosophila* in 1987, in a triumph of neurogenetics[4–6], revealed that the K^+ channel had separate polypeptides for each of its repeat units, resulting in cDNAs small enough to be subjected to the full power of the techniques of molecular biology. Over the following decade, a combination of mutagenesis, expression in systems such as the oocyte of the African frog *Xenopus laevis*, and electrophysiology resulted in mapping various aspects of function onto specific stretches of the sequence of the proteins. The voltage sensor was identified as the 4th transmembrane segment (S4) in each subunit, which is positively charged and has since been shown to move in response to changes in transmembrane potential. The elements of selectivity were narrowed down to a few residues in the loop connecting the 5th and 6th transmembrane segments (S5–S6 loop or pore-loop), which was also presumed to line the aqueous channel enclosed by the protein (Figure 2 *a*). However, an understanding of how these polypeptide stretches carried out their function required information on the three-dimensional structure of the protein.

Solving major problems in biology often requires identifying the appropriate preparation. Obtaining large amounts of a mammalian membrane protein for crystallization proved limiting, the best efforts being limited to a few milligrams per litre of culture. The MacKinnon laboratory attacked the problem by choosing to work with bacterial channels which could be churned out in large quantities by *E. coli* and also turned out to be extremely stable. Even on being taken out of the membrane environment, they remained tetrameric and crystallized well in a detergent mixture. The laboratory started its *tour de force* through the realm of K^+-channel crystallography with the structure of a K^+ channel from *Streptococcus lividans*, KcsA[7]. This protein consists of just two helices and the included P-loop (Figure 2 *b*). The aqueous pore it encloses balloons in the middle of the membrane to form an antechamber in which ions can await their turn to transit the selectivity filter. Within this antechamber they are stabilized by the dipoles of the helices holding the selectivity filter. The selectivity filter, in turn, consists of backbone carbonyls pointing into the pore with a geometry optimized for the desolvated K^+ ion. A series of such rings of carbonyls ensures selectivity, with numerous interactions providing rigidity to the filter. Ions are thus stabilized in the antechamber and hop through the selectivity filter. The big surprise in the structure was that the aqueous pore is lined not by polar amino acids, but by hydrophobic residues – essentially greasing the passage of the transported ion and abrogating the possibility of it settling into energy minima along the way.

Subsequent work from the laboratories of Yellen and Perozo showed that the S6 helices crossed each other at the narrowest point in the pore, and that rotation of this helix was required to open the channel[8,9]. The MacKinnon laboratory then solved the structure of another two-helix bacterial K^+ channel, the MthK channel[10,11]. The MthK channel structure appears to be that of a channel which is open compared to the KcsA structure which is almost closed or the KirBac channel structure[12] which is completely closed. K^+ channels are gated by a variety of stimuli ranging from electrical potential to ligands to mechanical pressure. In principle, each channel would have a sensor for the appropriate stimulus and couple that to rotation of the S6 helix. The MthK channel is gated by Ca^{++} and the MacKinnon laboratory proposed a means for applying the torque for channel-opening[11]. Understanding the manner in which the voltage sensor moves in response to changes in transmembrane potential, however, requires a knowledge of the three-dimensional structure of the 6-transmembrane protein.

The MacKinnon laboratory argued that the difficulty in crystallizing the 6-transmembrane protein lay in the mobility of the voltage sensor. They succeeded in crystallizing an archaebacterial voltage-gated channel KvAP using antibody fragments to restrict motion of the sensor. Amazingly, they have been rewarded with not one but two high resolution structures – corresponding to the sensor being in the closed and open state[1,2]. The latter structure consists of just the first four helices of the protein – the voltage sensor *sans* the channel. The major surprise here is that the voltage-sensing S4 helix is not oriented close to the membrane normal, but instead is almost parallel to the cytoplasmic membrane surface in the closed state (Figure 3). The sensor structure moreover, appears to include a portion of the classical S3 helix as well, forming a structure the authors refer to as the 'voltage-sensing paddle', which sticks out into the lipid and away from the protein. The paddle moves almost 20 Å through

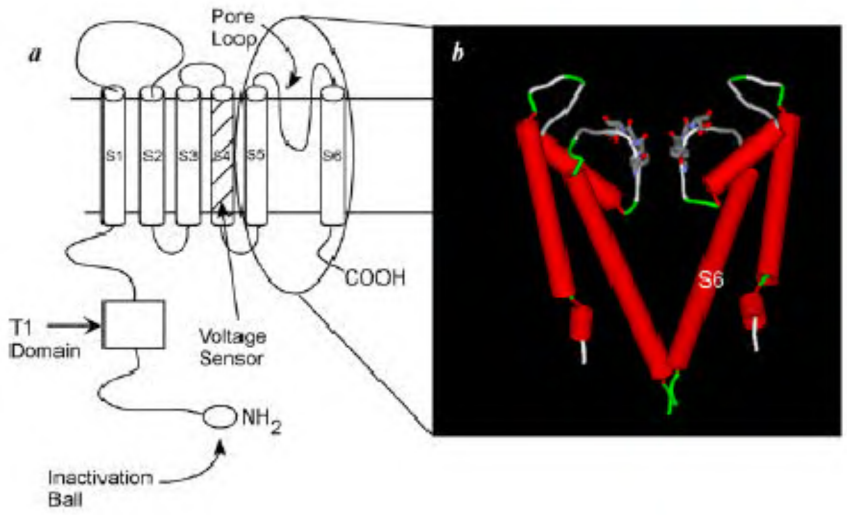

Figure 2. Potassium channel. ***a***, Schematic of the 6-transmembrane K^+ channel indicating functional elements such as voltage sensor, pore-lining loop, inactivating ball. ***b***, Structure of KcsA, a bacterial channel consisting of just two helices and the included P-loop corresponding to S5 and S6 in the 6-transmembrane channel. Only two subunits in the tetramer are shown for clarity, the residues comprising the selectivity filter being highlighted. The pore-lining helix corresponds to the S6 helix in (***a***) and is also marked.

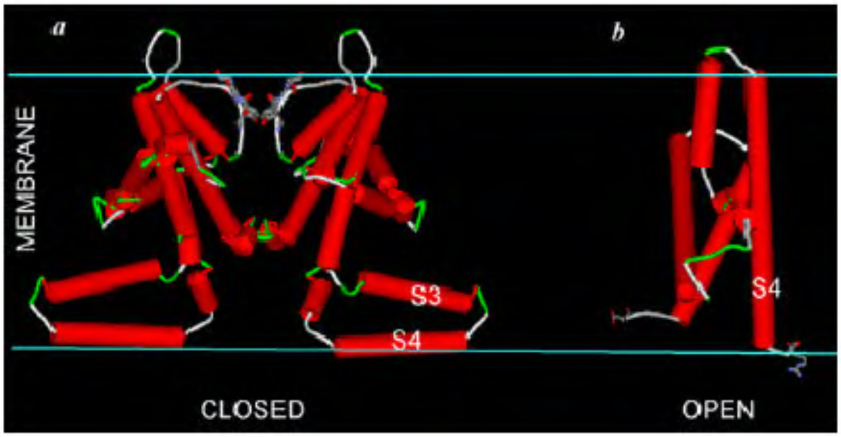

Figure 3. KvAP. ***a***, Structure of KvAP protein corresponding to the closed channel. Only two subunits of the tetramer are shown for clarity, the selectivity filter being highlighted as for the KcsA structure in Figure 2. Note the voltage-sensing segments of S3 and S4 which form the 'voltage-sensing paddle' lying parallel to the membrane surface. Blue lines approximate the limits of the membrane. ***b***, Structure of just the first four helices. This structure is presumed to mimic the open channel inasmuch as the voltage sensor has moved towards the extracellular surface. Note that the S4 helix is longer in this structure as it now includes residues that formed the S4–S5 loop in the full-length protein.

the membrane in response to changes in the transmembrane potential. This is a radical departure from the standard model of the sensor and its motion. The sensor was originally envisaged as primarily the S4 helix, which was presumed to be located within the interior of the protein, as opposed to KvAP where the paddle is located on the periphery.

The KvAP structure has the S3–S4 linker close to the cytoplasmic face of the membrane in its resting state and this may be inconsistent with results on accessibility of these residues based on chemical-labelling experiments from numerous laboratories[13]. Moreover, cross-linking experiments in the fly Shaker channel indicate that S4 is in close proximity to the pore[14]. In addition, while the MacKinnon group proposes a mechanism for coupling movement of the paddle to opening of the channel in outward rectifiers (those that open when the membrane becomes less negative inside), it is not clear that this mechanism will operate for inward rectifiers (those that open when the membrane becomes more negative inside). Furthermore, the structure appears to have significant unstructured expanses within the bilayer, a feature not seen in the structures of other membrane proteins.

Such inconsistencies with established viewpoints may imply that the structures reported represent non-physiological states of the protein. It is conceivable that the archaebacterial channel has a different architecture from animal channels. It is also possible that the antibody fragments used to facilitate crystallization could have imposed some constraints on the final structure, a problem compounded by the fact that constraints normally imposed by the bilayer would be absent in the crystallization medium. On the other hand, it could well be that the established viewpoints rely on too small a database, and that the KvAP structures will expand these horizons. The flurry of experiments set off by KvAP will surely generate much heat and dust before clarity is achieved. It is a far more exciting period that membrane biologists look forward to than anything we have seen so far.

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