identified by EPR[7], which in turn activates the G-protein transducin. The tilting of a transmembrane α-helix in the proton pump bacteriorhodopsin that opens the proton pathway — characterized by electron microscopy (EM)[8] — is another classic example of rigid body movements in membrane proteins. Additionally, a concerted twisting motion of pore lining helices (also detected using EM) opens the acetylcholine receptor channel[9]. Likewise, the pore opening in the KcsA K^+ channel is highly likely to be the consequence of one — or the combination of several — rigid body movements.

For channels, receptors and pumps, high resolution structures both in the resting state and in the activated state are perhaps an optimal solution for the elucidation of molecular mechanisms. However, crystallization of these membrane proteins is extremely difficult, and static crystal structures can provide only limited data on protein dynamics. In contrast, whereas obtaining three-dimensional structures of proteins by EPR is difficult, the technique can readily provide data on the dynamic motions of a protein. Current EPR technologies also allow quantitative measurements of protein motions[10]. Small ~1 Å movements of signal transmitting transmembrane helices in the aspartate receptor have been recently characterized using these quantitative approaches[11]. The current success of Perozo and coworkers[1] with the K^+ channel and the advances being made in our understanding of receptor proteins[7,11] are excellent examples of the unique advantages of spin labeling EPR for investigating specific biological problems.

Since the structure of the KcsA channel was unknown at the time of these EPR experiments, Perozo and coworkers attempted to build a model for the membrane spanning helices, TM1 and TM2, of the tetrameric channel based on their EPR measurements[1]. In fact, it has been long suggested that, using spin labeling EPR, it is in principle possible to determine protein structure at the resolution of the peptide backbone. The attempt by Perozo *et al.* with their data on the KcsA channel has put this ambitious suggestion to a serious test for the first time. The nitroxide scanning ex-periments that were the major EPR strategy in their study rely heavily on the periodic behaviors of the spectral lineshapes as well as the relaxation times. This EPR approach for determining secondary structure works exceptionally well for long α-helices such as membrane-spanning α-helices. The quality of the secondary structure for TM1 and TM2 determined in the study by Perozo *et al.*[1] is excellent and is in good agreement with that seen in the crystal structure[2]. However, what the nitroxide scanning experiments cannot provide is distant constraints, which are absolutely necessary to position the secondary structural elements relative to one another in three-dimensional space. Due to the lack of such distance constraints, the α-helix packing and relative angles between helices are rather coarse in the structural model. With the new X-band EPR technique, the distance between two spin labels can be measured quite accuartly in the range of 7–25 Å[10]. Full implemantation of this advanced EPR method to obtain distance constraints will provide the next step in the attempt to derive the first EPR determined structure.

Yeon-Kyun Shin is in the Department of Chemistry, University of California at Berkeley, Berkeley, California 94720, USA.

Correspondence should be addressed to Y.-K. S. email: shin@cchem.berkeley.edu

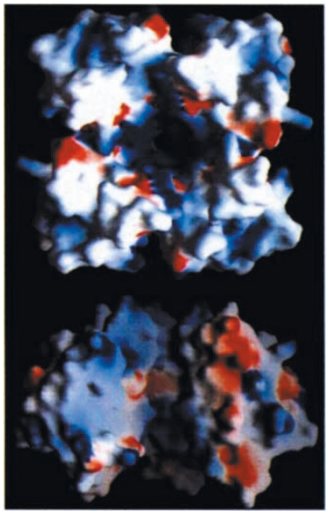
1. Perozo, E., Cortes, D.M. & Cuello, L.G. *Nature Struct. Biol.* **5**, 459–469 (1998).
2. Doyle, D. A. *et al. Science* **280**, 69–76 (1998).
3. Cuello, L. G. *et al. Biochemistry* **37**, 3229–3236 (1998).
4. Ogawa, S.& McConnell, H. M. *Proc. Natl. Acad. Sci. USA* **58**, 19–26 (1967).
5. Todd, A. P. *et al. Proteins Struct. Funct. Genet.* **6**, 294–305 (1989).
6. Armstrong, C. M. *et al. J. Gen. Physiol.* **80**, 663–682 (1982).
7. Farrens, D. L. *et al. Science* **274**, 768–770 (1996).
8. Subramanian, S., Gerstein, M. Oesthelt, D. & Henderson, R. *EMBO J.* **12**, 1–8 (1993).
9. Unwin, N. *Nature* **373**, 37–43 (1995).
10. Rabenstein, M. D. & Shin, Y.-K. *Proc. Natl. Acad. Sci. USA* **92**, 8239–8243 (1995).
11. Ottemann, K. M. *et al. Biochemistry* **37**, 7062–7069 (1998).

picture story

Channels, channels everywhere

The recent report of the bacterial KcsA potassium channel structure shows the most essential part of an ion channel — its selectivity pore. However, KcsA lacks other regions commonly found in related channels. KcsA has only two transmembrane domains per monomer, unlike many of its relatives, such as members of the Shaker family of K^+ channels which have six transmembrane regions per monomer. Also, the cytoplasmic N- and C-terminal regions in KcsA are shorter than those in Shaker channels. The ~130 amino acids immediately preceding the first transmembrane helix in the Shaker K^+ channel (a region known as the T1 domain) are known to play an important role; they stabilize the active tetrameric form of the channel to facilitate both homo- and hetero-multimerization, one of the mechanisms responsible for generating the functional and pharmacological diversity of K^+ channels.

A recent structure of the Shaker T1 domain shows that the monomers form a tetramer surrounding a water-filled hole that may possibly form the cytoplasmic entrance to the transmembrane region of the pore (see top image; Kreusch, A. *et al. Nature* **392**, 945–948). In this structure, interactions among 15 polar residues, which are conserved within the Shaker subfamily, stabilize the tetramer. In a side view of the molecular surface (bottom image; positive in blue, negative in red), the front subunit of the tetramer has been removed, and the two polar interaction surfaces of the monomers are shown. Because of the symmetry in the tetramer, the charged surface of the monomer on the left is identical to the charged surface of the removed monomer that would interact with the subunit on the right. The tendency of Shaker monomers to oligomerize only with other Shaker subfamily members and not with non-Shaker monomers is readily



explained: the 15 interface residues that are conserved in the Shaker subfamily have only ~7% conservation among non-Shaker channels, a much lower number than the ~39% conservation between Shaker and non-Shaker channel sequences throughout the whole protein. TLS