Engineering channels: Atomic biology

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Ion channels are protein valves that control an enormous range of biological function. Atomic-scale properties of channel proteins control macroscopic flows across otherwise insulating membranes of cells and subcellular compartments, and so the study of channels is a study in atomic biology. Ion channels are intrinsically multiscale devices that allow the amino acid side chains of a protein to control nerve signaling and coordinate muscle contraction—including the contraction that allows the heart to pump blood—and a host of other biological activities. A Google search for "ion channels" or "channelopathy" (1) shows the enormous importance of channels in biology and medicine.

Atomic Biology

Ion channels are natural nanodevices that use atomic structures to control macroscopic flows. Ion channels depend on evolutionary engineering on the atomic scale. Picostructures—side chains only tens of picometers in dimension—control the specific chemical properties of ion channels by crowding ions and side chains in a tiny space (2). Simulations show that crowding of ions can explain the properties of different types of channels when using models that contain only two parameters (3). These parameters are not adjusted as solutions are changed or as the channel is mutated from one type to another. Some mutations produce different valves that specifically control the fluxes of different types of ions.

Nature has also shown how to build picovalves that can be turned on and off by a wide range of stimuli, whether chemical, physical, or biological. Nature has built many channel types that respond selectively (in milliseconds) to one or two molecules of an agonist or drug (4). Sakmann and Neher (5) along with others (6) have shown us how to record single channels, and single-channel recording can be done in thousands of laboratories today.

Biotechnology tries to take advantage of this knowledge and experience with biological channels to build systems that can exploit channels for our technological use. A substantial fraction of the work on the technology of channels has used the bacterial channel α-hemolysin αHL as its basic tool (7, 8) despite its complex molecular biology arising from its self-assembled heptameric structure. αHL passes huge currents, sometimes too large to be handled by patch-clamp amplifiers set up for biological use, and so has large signal-to-noise ratios. These large signal-to-noise ratios provide an impressive technological advantage when the αHL system is used as a detector of a single molecule. Hagan Bayley has shown in some 50 articles (www.chem.ox.ac.uk/bayleygroup/professorbayley.htm) how αHL can be used in a wide variety of clever and useful ways, including his recent development of a robust single-channel system usable outside of the laboratory (9).

Most channels, including αHL, under many conditions naturally open and close in a random process that can only be controlled partially—stochastically that is to say—by controlling mean and variance (etc.) of the random process. Gating is not under deterministic experimental control. This random natural gating interferes with technological use: The complex currents associated with natural stochastic opening and closing make it harder to recognize current signals useful for our technology. The channel might be closed when we want it open to do our bidding. An important step in the adaptation of a channel for technological use is learning to keep it open. Braha *et al.* (7) found conditions and preparations in which gating in αHL was not a problem.

Mutations that change flexibility of parts of the protein can greatly reduce natural gating.

ompG

Bayley's laboratory (10) has now developed another channel for technological use, ompG, and learned to keep it open. ompG is a monomer and so has a much less complex structure than αHL, and it resembles other porins in which selectivity can be designed to our purpose (11–15). ompG, however, has natural gating that interferes with its use. In this issue of PNAS, Chen *et al.* (10) address that issue with a clever combination of molecular dynamics and protein engineering. They show how mutations that change flexibility of parts of the protein can greatly reduce natural gating.

Just as importantly, Chen *et al.* (10) show that a cyclodextrin molecular adapter can be used with ompG to allow chemical engineering of its properties as a stochastic sensor. They use a specific cyclodextrin to make ompF respond to ADP, although they do not investigate (in this first article on the molecule) the specificity of that sensing. It would be interesting to know where the cyclodextrin binds in the ompG and how it confers selective behavior. It would also be interesting to see whether creation of hydrophobic regions in ompG could induce gating, and sensitivity to inert gas anesthetics like xenon (16), perhaps by a bubble gating mechanism (17), although admittedly such investigation might not lead to useful technological results.

The results of Chen *et al.* (10) are already an important advance in stochastic sensing and will become even more so once ompG and cyclodextrin adapters become widely used in many laboratories. Just as interesting to me are the implications of the clever tools used by Bayley's laboratory in this study, their limitations, and their possible extensions.

Chen *et al.* (10) use molecular dynamics in a most successful way, following the work of Sansom's group on porins (18) to motivate molecular design. Investigating the flexibility of the ompG molecule with principal component analysis, Chen *et al.* identify promising sites for mutations, make two distinct mutations, combine them, and achieve the desired result. Other mutations, including large scale deletions, will no doubt be tried, but the mutations presented are impressively useful as they stand.

Simulations: Success and Limitations

Long simulations (>3 ns) are used (and evidently needed) to achieve reliable results. The many nanoseconds required for computing such structures were beyond reach until recently, even though relaxation times of ionic atmospheres

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observed in experiments (19) strongly suggested that such long times would be needed. After all, ions are involved in almost all protein function, for a number of reasons. Binding site, active sites, and the walls of channels almost always contain acidic and basic (i.e., negatively and positively charged) groups that have very high concentrations (number densities) of counterions nearby. It is not clear how useful the results of simulations would be if simulations studied the properties of systems while they were still sensitive to the (usually arbitrary) initial conditions of the computation.

What is striking is how much can be done with simulations that do not come close to computing the biological or technological function of the system. Even these very long simulations used by Chen *et al.* (10) are some 10^7 times briefer in duration than biological function. The currents and fluxes that are the biological function shown in ref. 10 occur on time scales of nearly seconds whereas even the massive calculations shown last 10 ns. The remarkable fact is that a brief calculation can be used to design the biological or biomimetic system even though the calculation provides only a brief snapshot of the protein's behavior and does not compute function at all.

Just as striking, the calculations of molecular dynamics can be very useful even though they do not include the main driving forces for biological or chemical function. Functions of proteins, whether enzymes or channels (20), are controlled by thermodynamic variables like concentration, chemical potential, electrical potential, and electrochemical potential (4, 21, 22). Yet these thermodynamic variables are neither specified nor reported in most molecular dynamics calculations because their computation is most naturally done on the macroscopic scale, some 10^3 to 10^9 times longer than most molecular dynamics simulations.

It could be argued, as many structural biologists do, that atomic-scale structure is so important in determining the function of channel proteins that simulations of snapshots are enough. Such 10-ns snapshots are certainly enough to help design quiet channels, as Chen *et al.* (10) have shown.

My own view is that sweeping conclusions are not warranted given how little is actually predicted from the calculations of snapshots. The qualitative design is a triumph, but quantitative predictions are not made.

Perhaps we should avoid a vitalist bias that biological engineering is fundamentally different from other engineering: Engineering becomes far more successful and efficient when based on calculated properties of tested models. Perhaps we should say that prototypes can be built from qualitative analysis, but finished designs need more. In my view, snapshots can and need to be augmented by quantitative analysis involving estimates of behavior on the biological scale if specific engineering designs are to be optimized and refined in biology as they are in engineering.

Molecular Simulations and Biological Reality

Extending simulations to biological and technological reality will not be easy. It will not be easy to include the concentration of ions, analytes, and so on as well defined input variables in simulations including all atoms of the protein and solution. It will be even harder to include electrochemical potential as a well defined and calibrated variable. It may prove to be impossible to compute the electrochemical potential of analytes, drugs, transmitters, hormones, and cofactors that are the natural controllers of many protein functions. The control here often depends on trace concentrations, micro- or nanomolar, sometimes less. Simulations of all of the atoms of such systems requires enough molecules of the controller to produce a statistically significant estimate of number density (i.e., "concentration"). Each micromole of a controller (active at a micromolar concentration) is accompanied by 55 moles of water. An exceedingly large number of atoms are needed to simulate trace concentrations.

Reduced Models

Perhaps it will not be necessary to simulate all of the atoms of these systems in full detail. Recent simulations of reduced systems (3) have shown how a model with just two adjustable parameters (channel diameter and channel dielectric constant) can produce the main properties of calcium-selective channels (in many solutions over a wide range of concentrations), using crystal radii for ions. Calcium channels can be mutated into sodium-selective channels. Surprisingly, the same model that produces calcium selectivity also produces sodium selectivity when its side chains are changed from EEEA to DEKA, without changing any parameters whatsoever, thus reproducing experimental work. It would be interesting to see how well these reduced models describe ompG of Chen *et al.* (10). It would be wonderful if Bayley's lab could then build a calcium-selective channel as Miedema (11–14) has and construct a sodium-selective channel as Boda *et al.* (3) suggest should be possible. Of course, such construction will require an understanding of how the cyclodextrin adapter fits into QompG and how adapters confer selectivity, but those are just the kind of questions that an extraordinarily ingenious laboratory like Bayley's are likely to address and answer.

Protein engineering is well on its way without quantitative design or analysis. Imagine how it will do when it applies the quantitative approaches used in engineering in general.

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