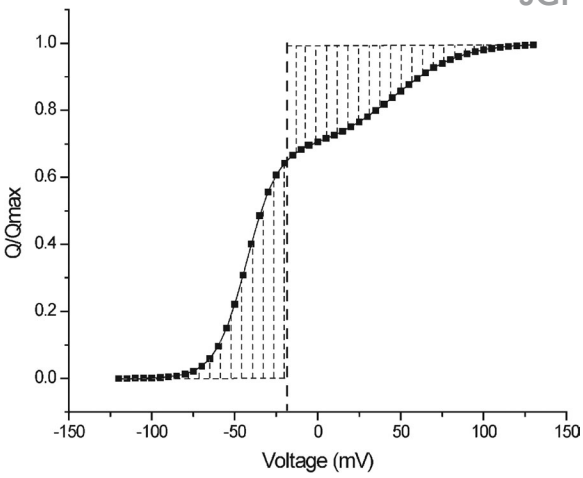
Taking a thermodynamic approach: A conversation with Baron Chanda

Transcript of a conversation between
Elizabeth M. Adler[1] and Baron Chanda[2]

[1]Executive Editor, *The Journal of General Physiology*
[2]Department of Neuroscience, University of Wisconsin, Madison, Madison, WI 53706



A Q-V curve with the median voltage of charge movement (Vm) marked by the vertical dashed lines (see Chowdhury and Chanda [2012]).

Transcript condensed and edited by EMA

***Elizabeth M. Adler: Hi, I'm Liz Adler, Executive Editor of* The Journal of General Physiology.**

I'm in San Francisco at the 58th Annual Meeting of the Biophysical Society, speaking with Baron Chanda, winner of the 2013 Paul F. Cranefield Award.

***The Cranefield Award, named in honor of long-time* JGP *Editor Paul Cranefield, is given by the Society of General Physiologists to an independent investigator early in his or her career who, in the preceding year, published an article of truly exceptional quality in the* JGP.**

Baron, who is an Associate Professor at the University of Wisconsin, received his award for a paper developing a method for using thermodynamic analysis to investigate ion channel gating.

Baron, congratulations on your award, and thank you for agreeing to be interviewed.

Baron Chanda: Thank you, Liz, this is a huge honor for me.

EMA: Before we get to the work for which you won the Cranefield Award, I'd like to talk a little about your prior work.

So, it looks like you've always been interested in voltage-dependent ion channels. Is that right?

BC: Yeah, it's true.

When I went to grad school in India, I was very interested in protein folding … in India there was really no ion channel field as such … but it did sound to me that ion channels were fascinating, so I did end up studying ion channels … but the community around me was a lot of protein-folding people, so I actually imbibed a lot of thermodynamics and protein design and folding.

EMA: So that gave you a slightly different take than the usual one.

BC: It gave me a very different take in that I was cut off in many respects from the ion channel community … I had a very different insight coming into the field because of my background.

EMA: So, for your thesis … you incorporated channels into proteoliposomes and did a functional assay?

BC: Yep.

Right, so we developed a functional assay to measure potassium channel activity; when we reconstitute proteins into bilayers, you know that some of the proteins are active, but that's not a measure of specific activity. And so I was interested in more macroscopic assays to get a sense of what fraction of total protein is active.

EMA: What was the functional assay? You didn't look at flux, did you?

BC: No, the idea was that potassium channels should clamp the membrane potential to the potassium reversal potential.

And the idea was that if you use a membrane potential–sensing dye to measure membrane potential and change concentrations across the membrane, they should follow the potassium equilibrium potential … and if you have a nonselective membrane, that would not be the case.

EMA: OK, so then, then you did a postdoc where you used voltage-clamp fluorimetry to look at sodium channel gating ….

BC: Yep.

EMA: So, I guess that was in the U.S. … you came to work with Bezanilla.

BC: That was in the U.S., right, so that's when I came to Pancho's laboratory.

Pancho had just published this paper on sodium channels, looking at inactivation with voltage-clamp fluorimetry [*editor's note*: reference is to Cha et al. (1999)], so then we decided to try to look at the activation of the voltage sensors and try to slow down the activation so that we could resolve the kinetics of each of them.

EMA: How does voltage-clamp fluorimetry work?

BC: Essentially in voltage-clamp fluorimetry what we do is, we have a cell or an oocyte which is expressing a channel of interest, and we try to label sites on this channel using fluorescent probes.

Typically, we would introduce a mutation in a particular site in the channel where we want to study its conformational change. And, once these channels are expressed in oocytes, then we can label them with a cysteine-reactive dye.

And, once labeled, because fluorescence is sensitive to environment, when we voltage clamp these cells, and, if the dye is in a site which undergoes a conformational change in response to, say, voltage jumps, etc., then we will see a fluorescence signal.

EMA: Uh huh.

BC: So, in a way, this allows us to look at the conformational change in somewhat of a local neighborhood of the dye.

EMA: So, what did you find?

BC: So, what we found was something really interesting … that, consistently, that domain 4, the kinetics—or the time course of activation—of domain 4 fluorescence, was slower than those that we measured from other domains.

Just to step back a little, sodium channels have four domains, so it's one big protein but it has four repeats, which come together to form a functional channel. And we found that the fourth repeat, the last one, was moving slower than the first three.

And that was very interesting because that goes back to an idea that Hodgkin and Huxley proposed at the time they were developing their models, saying that there are three activating particles and one inactivating particle, and the inactivating particle moves slowly, and that's why you have the channel opening first and then inactivating.

EMA: And that's the one in the fourth domain?

BC: And that's what it seems that we found—that the inactivation seems to correlate with the movement of the fourth domain.

And then we looked back and saw that, in the literature, there were ideas that the fourth domain is somewhat unique. But I think in terms of a direct functional measurement, or a direct structural parameter, I think we were the first to demonstrate that the fourth domain is different from other domains of the sodium channel.

EMA: So, when you said there were some hints in the literature that it was unique, you mean in terms of the sequence?

BC: No, Dottie Hanck had shown toxins, which they had reasonable evidence to suggest bind specifically to domain 4, would remove or slow down inactivation as if the activation of domain 4 was somehow responsible for inactivation [*editor's note*: reference is to Sheets and Hanck (1995) and Sheets et al. (1999)].

EMA: So, you have a sodium channel and let's say you reorganize the domains, so that rather than 1-2-3-4, you had 1-4-2-3, has anyone done that?

BC: Nobody has done that specific experiment, and I don't think it will work … the paddle motif swap experiments that Kenton [*editor's note*: Kenton Swartz] did with Frank Bosmans [*editor's note*: reference is to Bosmans et al. (2008)] where they took the S3–S4 region, which they defined as the paddle region, and they swapped it around, they see that domain 4 consistently confers a different property … and from multiple sequence alignment and direct coupling analysis [*editor's note*: reference is to Palovcak et al. (2014)] … when you look at the phylogenetic tree of the voltage-sensing domain, domains 1–3 fall into one clade and domain 4 in sodium and calcium channels falls into another clade as if they have evolved differently.

So, I think there is something intrinsic about the sequence. Whether that holds for calcium channels or not remains to be seen.

EMA: Let's move on to the paper that you won the Cranefield Award for.

In that one, you developed a thermodynamic approach to investigating gating in ion channels—is that right?

BC: Yep.

EMA: Specifically channels that are activated by both voltage and ligand?

BC: The way I think about this is that, when we talk about proteins as a biophysicist, what we are trying to do is to understand the interrelationship between structure, function, and forces. And once the structure is solved, it is [necessary] to figure out what are the forces that drive the structure that allow the protein to carry out a particular function—what are the interactions, etc., right?

So, a lot of our analysis is somewhat model dependent, and, in a way, if I were to just stretch this a bit, it's like saying that the temperature that you measure outside in San Francisco is not dependent on the quality of your thermometer, but on whether you believe in climate change or not.

So, if you think about it, we need parameters, or measures, which we can trust independently: where the model and the measures are not dependent on each other.

So, the idea is that we need to develop good ways to measure interactions, interaction energies, and energetics.

EMA: So, when you're talking about models, it's the number of different closed and activated states?

BC: Yeah.

EMA: That sort of a model?

BC: That sort of a model, yeah, because in many cases, when we are describing the forces that drive a channel, we need to characterize the occupancies of individual states in the gating process, right? And those occupancies come from various studies, mainly single-channel kinetics and things like that.

Then the next level, which would be, I think, one of the big challenges, is maybe to measure occupancies using some other method, which will measure the structural occupancies in each of the states, and from there we can derive the energetic of the channel gating.

This is a very laborious process; for every mutant, if you were to do this, it takes a lot of time. But then there are the short-cut ways of doing some of these things, which are more amenable to high-throughput methods … use the conductance/voltage

curves and derive from there a free energy measure of activation, and that's very widely used because it's a very straightforward measure. But that can be problematic in understanding the mechanism because those make certain assumptions, which are unlikely to be true.

EMA: What kind of assumptions?

BC: That the channel basically exists only in two states.

EMA: You mean closed and open?

BC: Closed and open. And that's true functionally, but that's not true energetically, and that's basically what it boils down to. So, functionally, yes, this is what we measure, but, energetically, that's not true and what we need is a better measure of energies that are associated with channel gating and which is something that is more robust and model independent, which, in a very simple way, you can apply this for any system as long as you measure the right parameters.

So what did we do?

Well, my graduate student, Sandipan Choudhury, he and I were very interested in trying to develop ways to figure out what is the role of perturbation … when you make a perturbation, what does it do to the protein?

EMA: So "perturbation," like a voltage change, or a ligand binding, or …?

BC: No. What I mean here is a perturbation like a mutation.

EMA: OK.

BC: And there is a bit of a history to it. When I started my own laboratory, I was interested in trying to figure out how voltage sensor movement leads to pore opening in the sodium channel.

And two of my postdocs did this work where they systematically mutated residues in the linker—the S4–S5 linker, which connects the voltage sensor to the pore—and then we did two sorts of measurements; one, we measured the conductance/voltage curves, which is a probe for pore gating, and then we measured the fluorescence/voltage curve—the fluorescence was measured from a probe which was attached to the voltage sensor—so the idea was that we would measure both the conductance as well as fluorescence at the same time in the background of these mutations—that will tell us what this mutation is doing. And it turned out that we could say that the mutation was having an effect—that was for sure—but, mechanistically, I don't think we could say more than that.

And part of it was because, even with the very simple models, the parameters of a model tend to be tied to each other. And this actually goes to the recent paper by Rick Aldrich…

EMA: Uh huh.

BC: on parameter identifiability and the idea was that you can have a curve and you can fit it with completely different set of parameters…

EMA: Right.

BC: and you can generate the same curve [*editor's note*: reference is to Hines et al. (2014)].

And essentially, that's what we landed [*sic*] up getting into.

EMA: So you saw changes when you made these mutations…

BC: Right.

EMA: in conductance and in movement, but when you tried to figure out what it meant in terms of actual movement of the channel and how that led to its opening, you could come up with different answers depending on the specific parameters of your model.

BC: Exactly.

So, at that time, Sandipan had just come to my laboratory, and then we started developing ideas of how we could come up with methods that will allow us to analyze the effects of mutation in a model-independent way. And that got us thinking about this idea of measuring free energies and how we can measure interaction energies in a model-independent way.

EMA: Uh huh.

BC: So, that's the way we got into this.

EMA: So, your model is generally applicable to any kind of channel?

BC: Yeah, the idea is, it's applicable to any system which is driven by a stimulus and, basically, by a force, whether it is a voltage-gated ion channel or a ligand-activated channel, mechanosensitive channel, heat sensitive, or any system that is driven by a force.

And it simply boils down to measuring what we define as the conjugate displacement, and so, just backing up a little, the idea is that when you drive a channel, say, a voltage-gated ion channel, from a closed to an open state, you do work on that system … right? And the idea is that the amount of work that you do on that protein depends upon the energy difference between the closed and the open state.

EMA: Yes.

BC: OK? So that, basically, in a reversible system, if you want to measure the energy difference between the closed and the open state, you measure the amount of work that you are doing to drive them.

And work is force times distance: this is classic physics. And, the question is, we have to measure force and displacement, and what is the right displacement? And that's what we have to focus on. So, for every system, for electrical work, it's the voltage which is the force, and the displacement is the gating charge.

And so what we then said was that we have to measure the gating charge versus voltage curve to essentially figure out what is the free energy difference between the closed and the open state in a potassium channel. And, in principle, this is applicable to all systems, whether it is a ligand-activated channel, stretch activated, or any of them. So, we have to find the conjugate displacement.

EMA: So, anything that undergoes a conformational change in response to force as part of its activation, or inactivation, I guess…

BC: Right.

EMA: you could apply your method to.

BC: You could apply this method to.

When Sandipan and I were talking about this, we were saying "Well this is kind of obvious, and maybe this is there somewhere in the literature." And, of course, Wyman had used this graphical method to actually get a single parameter … and we basically then said, "Well, we could do the same thing."

And, he did it for hemoglobin, which is an oxygen-binding protein

[*editor's note*: reference is to Wyman (1964 and 1967) and Wyman and Gill (1990)].

EMA: Yes.

BC: And, we basically then said that we could use this for a voltage-activated channel, and we defined this parameter, the median voltage of activation.

I remember, when we were talking about this, we were like, "OK, are we deluding ourselves into thinking that this is something interesting; I mean, maybe everybody knows about this." So, we said that we have to make sure that this works.

And the thing that actually convinced us—or convinced me, rather—was not the derivation that Sandipan did, but, also, he took the Zagotta, Hoshi, and Aldrich model, which is one of the most detailed models for an ion channel, the Shaker potassium channel [*editor's note*: reference is to Zagotta et al. (1994)].

EMA: Mmmhmmm.

BC: And you can calculate the free energy difference between the closed an open state from that model and then you do our method, with the Q-V curve, and you measure the free energy difference … that is almost identical.

And then we took the Horrigan and Aldrich model also, for the BK channel [*editor's note*: reference is to Horrigan and Aldrich (2002)]…

EMA: Uh huh.

BC: and that also works.

In principle, it works with every model that we have gotten our hands on, as long as we can measure a good Q-V curve.

And it's one of those things that, as a biologist, there's a lot of uncertainty in what we do, but this feels like it's the closest I have come to being exact about things, and that's what feels really nice. I think it's going to be downhill from this point onwards.

PAPER FOR WHICH THE CRANEFIELD AWARD WAS GIVEN

Chowdhury, S., and B. Chanda. 2012. Estimating the voltage-dependent free energy change of ion channels using the median voltage for activation. *J. Gen. Physiol.* 139:3–17. http://dx.doi.org/10.1085/jgp.201110722

REFERENCES

Bosmans, F., M.F. Martin-Eauclaire, and K.J. Swartz. 2008. Deconstructing voltage sensor function and pharmacology in sodium channels. *Nature.* 456:202–208. http://dx.doi.org/10.1038/nature07473

Cha, A., P.C. Ruben, A.L. George Jr., E. Fujimoto, and F. Bezanilla. 1999. Voltage sensors in domains III and IV, but not I and II, are immobilized by Na^+ channel fast inactivation. *Neuron.* 22:73–87. http://dx.doi.org/10.1016/S0896-6273(00)80680-7

Hines, K.E., T.R. Middendorf, and R.W. Aldrich. 2014. Determination of parameter identifiability in nonlinear biophysical models: A Bayesian approach. *J. Gen. Physiol.* 143:401–416. http://dx.doi.org/10.1085/jgp.201311116

Horrigan, F.T., and R.W. Aldrich. 2002. Coupling between voltage sensor activation, Ca^{2+} binding and channel opening in large conductance (BK) potassium channels. *J. Gen. Physiol.* 120:267–305. http://dx.doi.org/10.1085/jgp.20028605

Palovcak, E., L. Delemotte, M.L. Klein, and V. Carnevale. 2014. Evolutionary imprint of activation: The design principles of VSDs. *J. Gen. Physiol.* 143:145–156. http://dx.doi.org/10.1085/jgp.201311103

Sheets, M.F., and D.A. Hanck. 1995. Voltage-dependent open-state inactivation of cardiac sodium channels: gating current studies with Anthopleurin-A toxin. *J. Gen. Physiol.* 106:617–640. http://dx.doi.org/10.1085/jgp.106.4.617

Sheets, M.F., J.W. Kyle, R.G. Kallen, and D.A. Hanck. 1999. The Na channel voltage sensor associated with inactivation is localized to the external charged residues of domain IV, S4. *Biophys. J.* 77:747–757. http://dx.doi.org/10.1016/S0006-3495(99)76929-8

Wyman, J. Jr. 1964. Linked functions and reciprocal effects in hemoglobin: a second look. *Adv. Protein Chem.* 19:223–286. http://dx.doi.org/10.1016/S0065-3233(08)60190-4

Wyman, J. 1967. Allosteric linkage. *J. Am. Chem. Soc.* 89:2202–2218. http://dx.doi.org/10.1021/ja00985a037

Wyman, J., and S. Gill. 1990. Binding and Linkage: Functional Chemistry of Biological Macromolecules. University Science Books, Mill Valley, CA. 330 pp.

Zagotta, W.N., T. Hoshi, and R.W. Aldrich. 1994. Shaker potassium channel gating. III: Evaluation of kinetic models for activation. *J. Gen. Physiol.* 103:321–362. http://dx.doi.org/10.1085/jgp.103.2.321