All Spheres Model of the L type calcium channel

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I thought it would be useful to describe a canonical all spheres channel model that we have studied in something like 40 papers (peer reviewed journals, not counting invited reviews, etc.) with many different mathematical methods (Metropolis Monte Carlo, MSA (mean spherical approximation), solvent primitive model, DFT-PNP, EnVarA, steric PNP, asymptotic analysis and classical singular perturbation theory, geometric singular perturbation theory, inverse problem theory (Tikhanov regularization), Brownian-Poisson dynamics, several related methods of Gillespie and Boda extending MC, DFT to nonequilibrium systems, PNP-F (with Fermi distribution). References and PDF files are available on request and can be found in my CV. The CV contains live links which should allow easy download of whatever you wish to read.

https://ftp.rush.edu/users/molebio/Bob_Eisenberg/Reprints/Webpages/Full.CV.pdf

We think we have gone down many false paths and wasted much time learning to deal with the all spheres model of calcium channels. I write this so you (all) do not have to go down those paths, unless you want to. You may want to try paths we think are false ones because you think you can do better than we did. That is GOOD and I encourage you to do that. Just don't ignore our mistakes, lest you waste a lot of taking making and then correcting them.

The RyR receptor so well analyzed by Dirk Gillespie is an alternative system to study with an all sphere model. You are should contact Dirk directly at dirk_gillespie@rush.edu to get all the details you need. In my view, the key paper is the following. It is VERY important to read the supplementary material.

> *Gillespie, D. 2008. Energetics of divalent selectivity in a calcium channel: the ryanodine receptor case study. Biophys J 94:1169-1184.*

Geometry and Parameters

The "All Spheres Model" of the L type calcium channel is specified precisely (I think) in the Methods Section and Figure 1 **AND CAPTION** of the following paper. NOTE: the ionic radii are in the **CAPTION.** These are of course somewhat arbitrary so it is all the more important to stick exactly to these values, unless you have a specific reason to do otherwise. Comparisons of results of different methods are much more useful when exactly the same parameters are used. Note that in the very crowded confines of a calcium channel small changes in diameter can make large changes in binding.

Boda, D., M. Valisko, D. Henderson, B. Eisenberg, D. Gillespie, and W. Nonner. 2009. Ionic selectivity in L-type calcium channels by electrostatics and hard-core repulsion. Journal of General Physiology 133:497-509.

This paper should be available for download from PDF or by its PMCID: PMC2712969 or from the Journal of General Physiology doi: 10.1085/jgp.200910211

Side Chains are mobile!!

Note how we deal with the side chains of the protein EEEE (glutamate residues as they are often called for historical reasons: the amino acids of proteins were originally identified in biochemistry labs as residues of a chemical procedure that degraded, i.e., hydrolyzed and depolymerized, the protein they were in, leaving behind residues of the original protein).

The side chains of this calcium channel are **known experimentally** to mix with ions and to be accessible to reagents in the bathing solution—UNlike side chains in the famous KcsA channel for example—so we treat the side chains of calcium channels as MOBILE spheres (but of course the side chains are kept within the channel itself) that assume different locations when ionic solutions in the baths are changed, when transmembrane potentials (i.e., trans-channel, i.e., by changing the electrical potential specified by the Dirichlet boundary conditions) are changed, or 'most anything else' is changed either.

In the MC calculations these side chains find their own location. They are in the locations that minimize free energy. Thus, our MC model is **self organized** and the protein has an **induced fit** to the 'substrate', i.e., calcium ions. **Of course, if the EEEE channel is in bathing solutions made of sodium ions, the locations are very different from the locations when the EEEE channel is in bathing solutions made of calcium ions.**

How to implement these mobile side chains is an issue that each model may do differently. Side chains may be kept in one location (i.e., immobile) if the computational complexity introduced is not thought to be worthwhile but that is a distinctly different model from the model we used in the paper below.

The implementer should be warned that we have a number of MC papers that say it is important to allow the side chains to be in different positions in different conditions, e.g.,

Mobility of Side Chains

Be sure to look at Fig. 7 and p. 3493-3494 of

Boda, Dezső, Nonner, Wolfgang, Henderson, Douglas, Eisenberg, Bob, and Dirk Gillespie. (2008) Volume exclusion in calcium selective channels. Biophys. J., 94: 3486–3496 BioFAST: January 16, 2008. doi: 10.1529/biophysj.107.122796 PMCID: PMC2292364 [PDF]

Also relevant are

Giri, Janhavi, Fonseca, James. E., Boda, Dezső, Henderson, Douglas, and Eisenberg, Bob. (2011) Self-organized Models of Selectivity in Calcium Channels. Physical Biology 8 026004. doi: 10.1088/1478-3975/8/2/026004 PMID: 21263167 [PDF]

Boda, Dezső, Giri, Janhavi, Henderson, Douglas Eisenberg, Robert and Gillespie, Dirk. (2011) Analyzing the components of the free energy landscape in a calcium selective ion channel by Widom's particle insertion method. Journal of Chemical Physics. 134, 055102. doi: 10.1063/1.3532937 PMCID: PMC3045419 [PDF]

Dimensionality

It is obviously best to compute the model in full three dimensions. First of all that is the only way to specify the structure of the channel correctly. BUT EVEN MORE IMPORTANTLY the shape of the ions is different in different dimensions. One dimensional spheres, and two dimensional spheres, whatever they are, do NOT crowd against charged walls the same way three dimensional spheres do. This is a well documented fact in the MC world. It is also obviously true. Thus one expects that one and two dimensional spheres, whatever they are, will not pack into the confined space of a calcium channel the same way real spheres do. Thus reduction in dimensionality can be expected to have substantial effects because details of packing matter in highly crowded systems! What is amazing is that the one dimensional models do as well as they do even though they contain one dimensional spheres!!

Despite all these warnings, it has been and will be necessary to compute one dimensional models because they are likely to always be very much faster to compute than three dimensional models, and thus much more practical when for solving inverse problems. They are also much easier to program. A much wider parameter space can be investigated with one dimensional models.

One dimensional models and baths.

If you use one dimensional models, you might think that one could simply put Dirichlet boundary conditions at the ends of the channel *but that is a catastrophe*. The boundary layers inevitably associated with the boundary conditions then get into the channel and "all hell breaks loose". Experimentalists (and evolution) go to GREAT lengths to avoid such effects. IT IS ESSENTIAL TO HAVE BATHS AS BUFFER REGIONS.

It is CRUCIAL that baths be represented reasonably in a one dimensional model. As far as I know, this is impossible if the channel is one dimensional with constant radius. The reason is that the one dimensional cylinder outside the membrane has the same cross section as the channel itself. Thus its effect on current flow is roughly the same. In electrical terms, the resistance (per unit length) of the cylinder outside the channel is roughly the same as the resistance per unit length of the channel itself. The total resistance (ohms = resistance per unit length times length in the bath region) in the bath can then be much larger than the total resistance (ohms = resistance per unit length times length of

channel) of the channel. **THIS IS ENTIRELY DIFFERENT FROM THE REAL SITUATION**. In the real situation, the resistance of the bath is FANTASTICALLY SMALLER than the resistance of the channel. The bath has almost no effect on current flow. The channel is the valve that has the big effect on current flow. That is why it is a valve. The ratio of resistances is typically 10^9 or larger for a single channel.

Variable cross section.

One way to deal with baths in a one dimensional model, introduced by Nonner and myself (and used even earlier by Duan-ping Chen and myself) is to use a channel with VARIABLE cross section. The region outside the channel is made to have MUCH larger cross section (it is "infinitely" wider) than the channel itself. The channel diameter is a realistic figure, something like 0.7 nanometers (*but use the number in the Boda reference above unless you have a specific reason to use something different!!*) Thus the potential drops in the bath of large diameter outside the channel are insignificant. The modified one dimensional model has a VERY LARGE cross sectional area outside the channel so the resistance of the bath is very very much less than the resistance of the channel, i.e., the potential drops in the bath are entirely negligible compared to the potential drop across the channel itself.

The question then comes how does one increase the diameter of this one dimensional channel as one leaves the channel and moves into the bath.

The connection between the real channel, with diameter of say 0.7 nanometers and the bath with "diameter" of say 10 CENTImeters is of course arbitrary. It is a property of our model but not a property of the real channel and surrounding baths. Thus, one must carefully check that the main conclusions of the analysis are NOT sensitive to the details of this connection. That is to say "the convergence" of current flow into the channel (think of lines of flow or field lines of Faraday) will not be dealt with realistically in this one dimensional model and the effects of that error must be tested empirically (by varying the parameters of the region connecting the "one dimensional bath" with the channel itself).

Nonner and I originally used a taper, i.e., a diameter that very rapidly increased as soon as the coordinate was in the bath. This is a "horn" problem (meaning the horn of an orchestra like a trumpet or trombone) and was extensively studied in applied math with a variety of analytical treatments and approximations. Nonner and I just used an easy to compute numerical procedure. But our original description seems to confuse people so I recommend the more precise descriptions in Amit Singer and John Norbury's papers (they are professional mathematicians of great talent and accomplishment) and Carl Gardner's paper (a professional physicist from Arizona State).

> Singer, A. Gillespie, D., Norbury J., and Eisenberg, R.S. (2008) Singular perturbation analysis of the steady state Poisson-Nernst-Planck system: applications to ion channels. European Journal of Applied Mathematics vol. 19, pp. 541–560. doi: 10.1017/S0956792508007596 PMCID: PMC2756831 [PDF]

> Singer, A. and J. Norbury. 2009. A Poisson-Nernst-Planck Model for Biological Ion Channels---An Asymptotic Analysis in a Three-dimensional Narrow Funnel. SIAM J Appl Math 70:949-968.

and

Gardner, Carl, Nonner, Wolfgang, and Eisenberg, Robert S. (2004) Electrodiffusion Model Simulation of Ionic Channels: 1D Simulations. Journal of Computational Electronics 3: 25–31. PMCID not available [PDF]

Note that I am citing these papers ONLY ONLY ONLY for the way they deal with the baths. The rest of these papers address other issues!!!! The channel itself should be described as in the Boda paper (J General Physiology) cited above.

Weishi Liu and I have in fact dealt with these issues using geometric singular perturbation theory and I attach a document Weishi has written explaining the relevance of the powerful analysis in

Eisenberg, Bob, Liu, Weishi (2007) Poisson-Nernst-Planck systems for ion channels with permanent charges. SIAM Journal on Mathematical Analysis 38, No. 6, pp. 1932–1966. PMCID not available [PDF]

Our experience is that a substantial but not dominating fraction of the potential drop (i.e., of the effective resistance, unit ohms) of the system is in the access regions on either side of the channel. The figure might be 15%. The role of the potential drop and perhaps concentration changes in these regions is not known or clear so in each one dimensional simulation, parameters of this access region (which is the bath within say 10 nanometers of the ends of the channel) have to be varied to be sure interesting results are not sensitive to the crude approximation in this region. Note that results should be entirely insensitive to what goes on in the baths far (>10 nanometers) from the ends of the channel because results in experiments are insensitive to the properties of the bath. That insensitivity is carefully check in experiments. If sensitivity is in fact found in calculations, the only remedy that is convincing to me is to use full three dimensional calculations.

Four electrode methods

In my experience most mathematicians and physicists seek to study the potential on the Dirichlet boundaries (i.e., on the electrodes) and the current that flows through those boundaries. EXPERIMENTALISTS NEVER DO THIS (if they can possibly avoid it) because there are all sorts of complexities/artifacts (e.g. boundary layers of concentration and charge) near the electrode (i.e., near the Dirichlet boundary condition). These complex boundary layers near the boundaries are ENTIRELY IRRELEVANT TO BIOLOGICAL FUNCTION and so EXPERIMENTALISTS WORK VERY HARD TO AVOID these complexities and in fact view them as artifacts.

Experimentalists use a four electrode method. That is to say they use two pairs of electrodes. One pair of electrodes measure potential. They have zero current through them. The other pair of electrodes implement the Dirichlet boundary conditions. These electrodes are further from the channel than the potential measurement electrodes. The more distant electrodes supply current and flux. Experiments use the two other (closer to the channel) electrodes to measure potential and there is no current of any kind across these other electrodes. These closer electrodes are voltage recording electrodes that simply report the potential at these locations. **They are not boundary conditions. They are observation points.** These electrodes are typically 10 nm away from the end of the channel or more. They can be modelled easily simply by evaluating the potential at those points. So current voltage curves would use the

current at the electrodes (i.e., at the Dirichlet boundary conditions) but the 'voltage' in the graph would be

potential DIFFERENCE **V(end of channel + 10 nm) — V(other end of channel -10 nm**).

Experimentalists routinely use the four electrode methods for other reasons, because of phenomena that are NOT included in PNP: when large-ish currents flow across real electrodes VERY strange things happen not accounted for at all by PNP. For large currents, or electrode potentials about say 3 volts (which is not a large voltage, from a technological point of view) gas bubbles can even form (made by the breakdown of water H_2O into hydrogen and oxygen gas and that can have exciting results, even explosions in some applications. Most of electrochemistry and battery technology occurs in these regions where electrode behavior is really strange and so it is essential to exclude that behavior from studies of channels or membranes. The four electrode methods does that exclusion quite well and is universally used for that reason.

A supplement to Bob's article: All spheres model of the L type calcium channel

Weishi Liu

August 23, 2014

This supplement explains briefly analytical results related to several parts of Bob's article. The same headings are used.

One dimensional models and baths.

Bob made it clear that one dimensional models with *constant radius* would be too simple to model channels and baths. This statement is also supported from a different angle – discussed below – by showing how *variable cross-section* could address important issues that *constant radius* fails to.

Variable cross section.

The one-dimensional models with *variable cross section* do have the ability to address issues concerning one-dimensional models that Bob raised in his article. This can be seen *easily* and *clearly* from, for example, the 2007 SIAM paper of Eisenberg and Liu cited in Bob's document where two ion species are considered. Specifically, the outer or slow system, which is valid for flows in the baths, is given by

$$\frac{d\phi}{dx} = \frac{\beta J_2 - \alpha J_1}{\alpha(\alpha + \beta)h(\tau)c_1}, \quad \frac{dc_1}{dx} = -\frac{\beta(J_1 + J_2)}{(\alpha + \beta)h(\tau)}, \quad \frac{dJ_1}{dx} = \frac{dJ_2}{dx} = 0, \quad \frac{d\tau}{dx} = 1,$$

together with $\alpha c_1(x) - \beta c_2(x) = 0$, where x is the one-dimensional spatial variable, ϕ is the electric potential, c_1 and c_2 are the concentrations of the two ion species, J_1 and J_2 are the fluxes of the two ion species, $\alpha > 0$ and $-\beta < 0$ are the valences of the two ion species, $h(\tau)$ is the cross-section area over $x = \tau$. This is the system (19) in Eisenberg and Liu's paper.

The system clearly says that: the **RATES** of changes of electric potential ϕ and concentrations c_1 and c_2 are INVERSELY proportional to the **cross-section area** $h(\tau)$. That is, if one chooses *large* radius for bath portions, then the changes of electric potential and concentrations over bath portions would be *small*; in particular, if the radius for bath portions is "infinity",

then the changes of electric potential and concentrations over bath portions could be ignored. **There is more**. Consider the following expressions for fluxes (it is system (23) in Eisenberg and Liu's paper)

$$J_1 = \frac{(c_1^L - c_1^{a,l})}{\int_0^a h^{-1}(s)ds} \left(1 + \frac{\alpha(\phi^L - \phi^{a,l})}{\ln c_1^L - \ln c_1^{a,l}}\right),$$
$$J_2 = \frac{(c_2^L - c_2^{a,l})}{\int_0^a h^{-1}(s)ds} \left(1 - \frac{\beta(\phi^L - \phi^{a,l})}{\ln c_2^L - \ln c_2^{a,l}}\right),$$

where the bath to the left of the channel can be viewed as a subinterval of $[0, a]$ from $x = 0$, and ϕ^L, $\phi^{a,l}$, c_j^L and $c_j^{a,l}$ are some intermediate variables whose specifics are not relevant for the purpose of present discussion (see Eisenberg and Liu's paper for details). What is important is the denominator $\int_0^a h^{-1}(s)ds$. The integral implies that the bath portion, over which the cross-section area $h(s)$ is *large*, has *small* contributions to the integral and hence to fluxes. Again, if the radius for the bath portion is "infinity", then the contribution of the bath portion to the fluxes can be ignored; that is, the fluxes are mainly characters of ionic flows in the channel portion.

Four electrode methods.

The experimental design using *four electrode methods* is perfectly consistent with the analysis from one-dimensional models with *variable cross-sections*. More precisely, if the outer pair of electrodes were able to implement Dirichlet boundary conditions with *electroneutrality*, then there will be NO boundary layers. Based on the above discussion, if the radius over the bath potion (say, between the outer pair of electrodes and the inner pair of electrodes) is *large*, then the potential difference between the inner pair of electrodes (the 'voltage' for the current-voltage curve in experiments) would be *essentially the same* as that between the outer pair of electrodes (the 'voltage' for the current-voltage curve in models) .

One cannot expect, for experiments, a perfect electroneutrality boundary condition at the outer electrodes. In this case, the analysis shows that there will be a boundary layer correction. The end point of the layer will provide a REDUCED boundary condition that satisfies electroneutrality. If the radius over the bath potion is *large*, then the potential difference between the inner pair of electrodes would be *essentially the same* as that of REDUCED potential – so the boundary layer, if any, near the outer pair of electrodes becomes IRRELEVANT.

The design of four electrode methods is SO brilliant !!!