BioMolecular Engineering

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Bioengineering
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Experimental Effort needed without theory is Enormous

Bio-Molecular Engineering

is the

Future of Molecular Biology,

whether biologists know it or not

How does it work?

How do a few atoms control

(macroscopic)

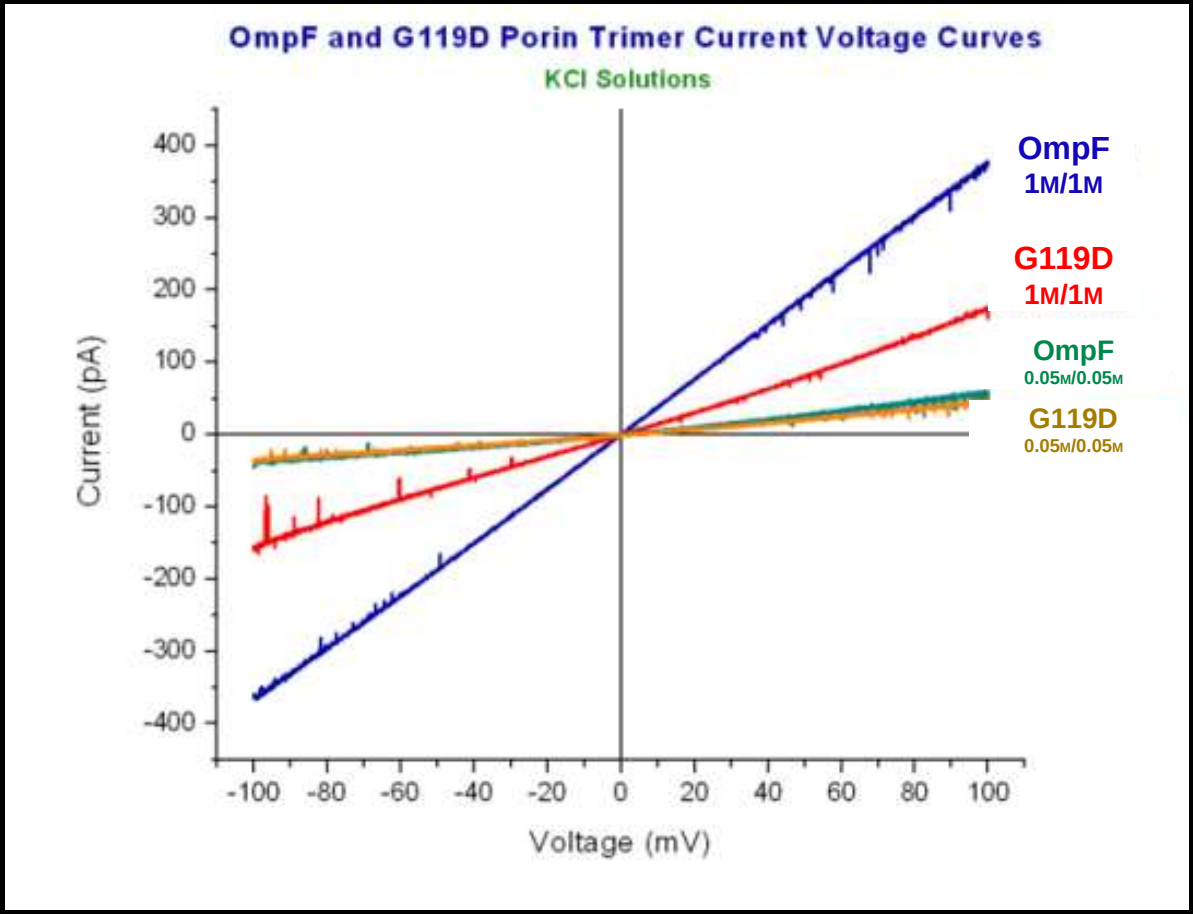
Biological Function?

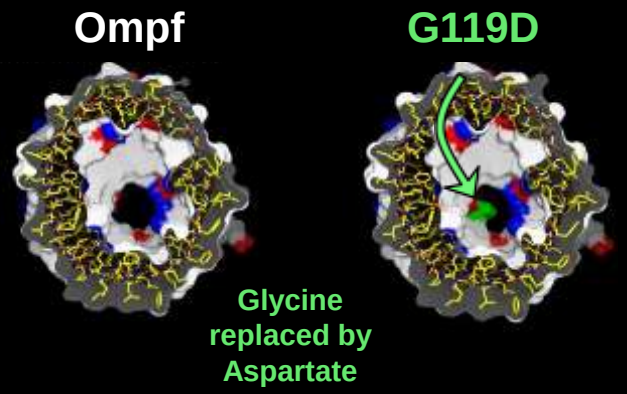
Much Molecular Biology

is

Reverse Engineering

A few atoms make a BIG Difference





Structure determined by
Raimund Dutzler
in Tilman Schirmer's lab

Current Voltage relation by
John Tang
in Bob Eisenberg's Lab

Trial-and-Error Biology
is very inefficient
but it is (almost)
All we have available,
today.

Experimental Effort needed without theory is Enormous

USA: NIH $3.1×10^10

devoted almost entirely to descriptive experimental work

Trial-and-Error Biology
is very inefficient
but it is (almost)
All we have available,
today.

Biology, Medicine. Engineering

are all about

Reduced Models

in which

SOME atomic details Control Function

If Devices are to Work

Engineers* must Grasp

and not just reach

Uncalibrated Devices do not Work!

“Ah, … a man's reach should exceed his grasp,
Or what's a heaven for?”
Robert Browning
"Andrea del Sarto", line 98

Poets hope we will never learn the difference between Dreams and Realities

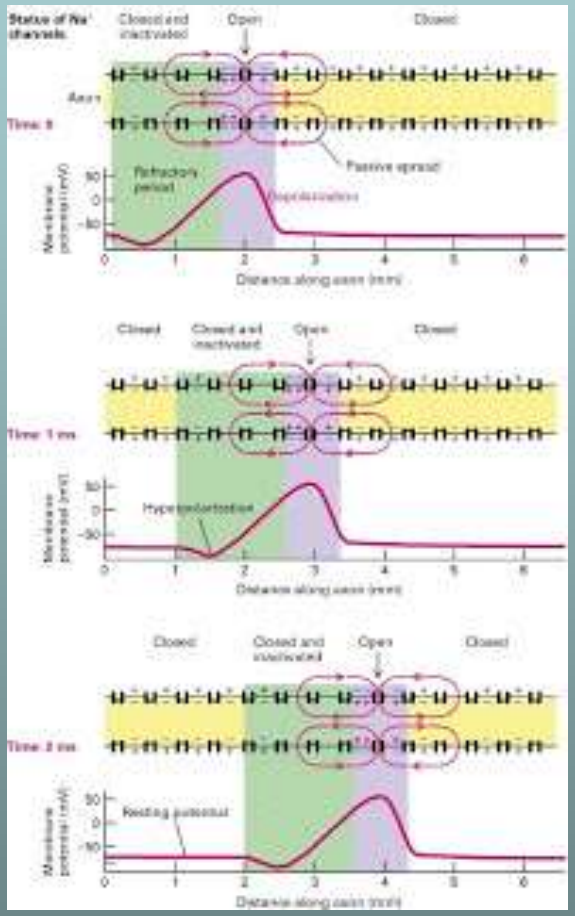
***Scientists and Poets can Reach**
but Engineers must Grasp

Biology is made of

Devices

and they are

MULTISCALE

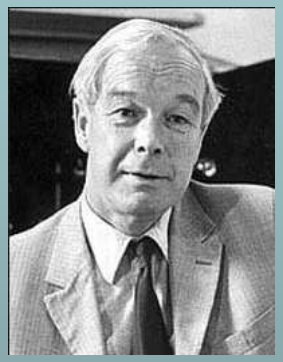


Hodgkin's Action Potential
is the
Ultimate Multiscale model
from
Atoms to Axons
Ångstroms to Meters

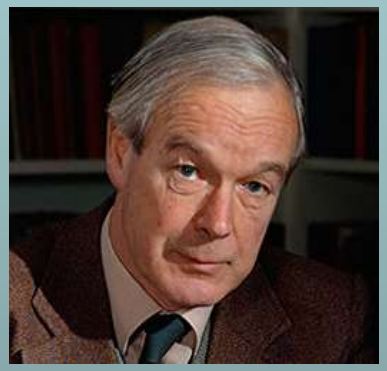
Device Approach to Biology

is a Great Success

widely unknown



Alan Hodgkin
friendly



Alan Hodgkin:
“Bob, I would not put it that way”

Decisive Role of the Electric Field

in Semiconductors and Ionic Solutions

Everything Interacts with Everything Else

Semiconductor *PNP* Equations

For Point Charges

Poisson's Equation

Dielectric Coefficient

Permanent Charge of Protein

$$-\frac{\varepsilon_0}{A(x)}\frac{d}{dx}\left(\varepsilon(x)A(x)\frac{d\phi}{dx}\right)=e\mathrm{P}(x)+e\sum_i z_i\rho_i(x)$$

Cross sectional Area

Valence Proton charge

Number Densities

Drift-diffusion & Continuity Equation

$$\frac{dJ_i}{dx}=0 \qquad -J_i=D_i(x)A(x)\rho_i(x)\frac{d\mu_i}{dx}$$

Flux

Diffusion Coefficient

Chemical Potential $\mu_i(x)$

$$\mu_i(\mathrm{x})=z_i e\phi(\mathrm{x})+kT\ln\left(\frac{\rho_i(\mathrm{x})}{\rho^*}\right)+\mu_i^{\mathrm{ex}}(\mathrm{x})$$

valence proton charge

Thermal Energy

1 2 3

Finite Size Special Chemistry

All we have to do is

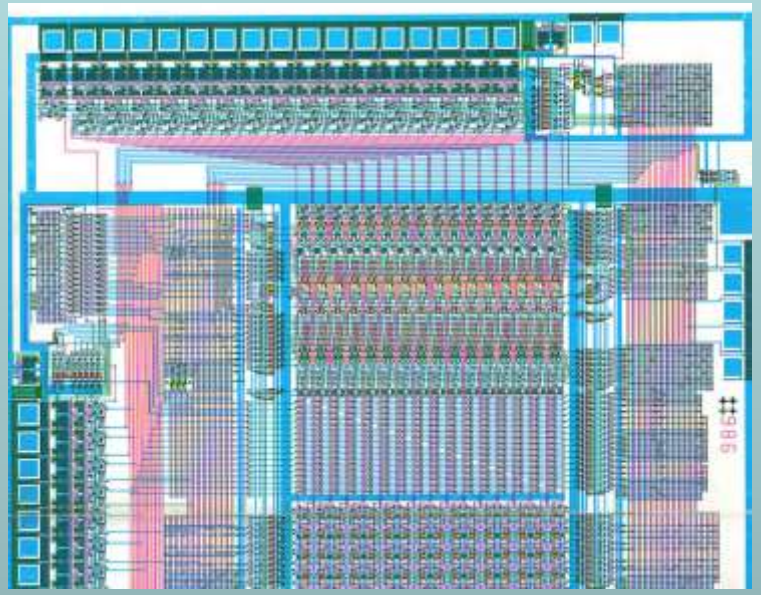
Solve it / them!

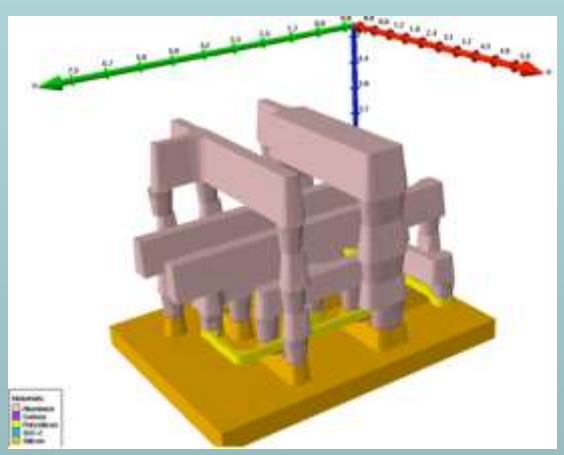
Boundary conditions:

STRUCTURES of Ion Channels

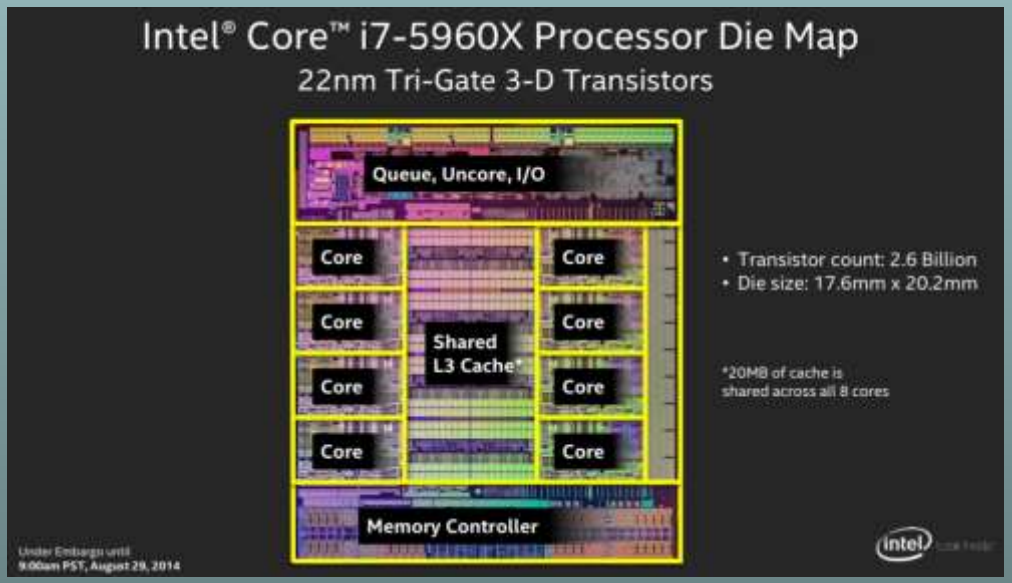
STRUCTURES of semiconductor devices and integrated circuits

Integrated Circuit





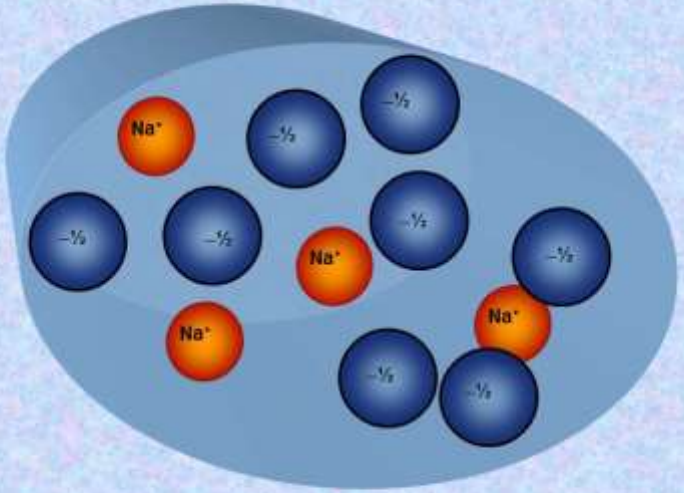
Too small to see!



but

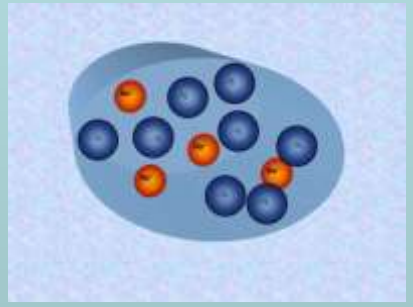
Ions are Spheres

Crowded in Channels



Nonner & Eisenberg

Ions in Channels



Ions in Bulk Solutions

are

Complex Fluids

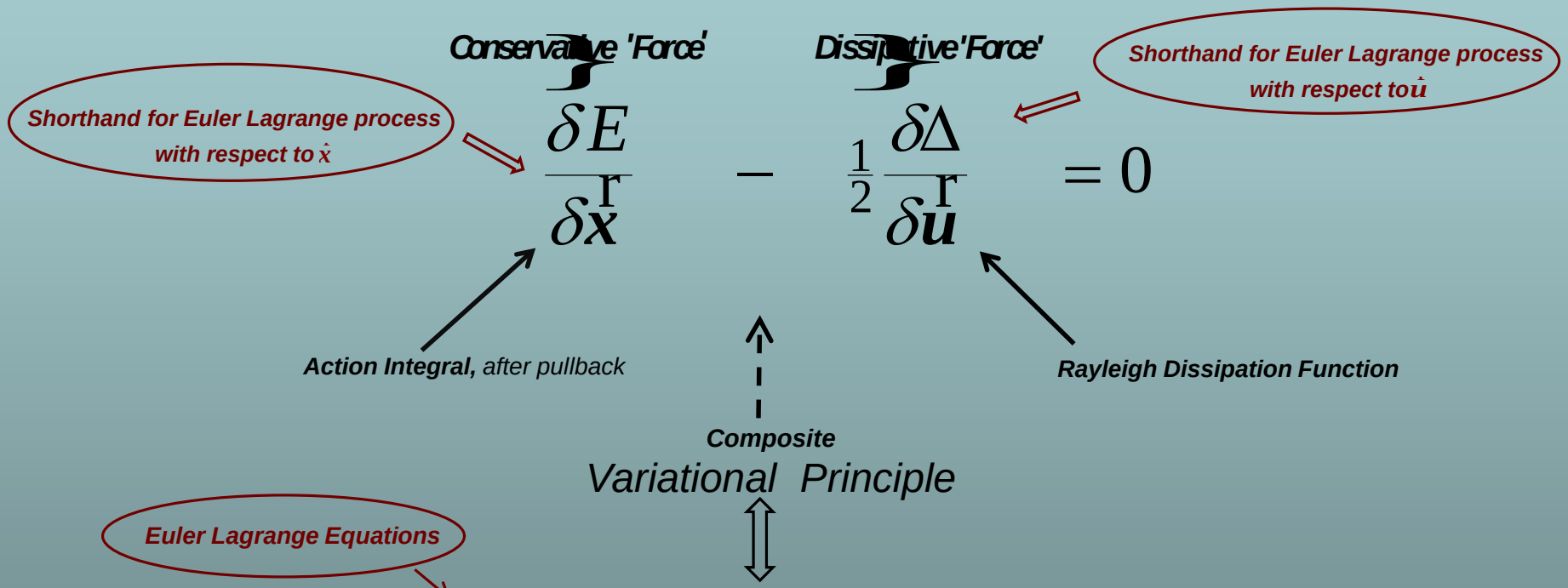
like liquid crystals of LCD displays

Energetic Variational Approach

EnVarA

Chun Liu, Rolf Ryham, and Yunkyong Hyon

Mathematicians and Modelers: two different 'partial' variations written in one framework, using a 'pullback' of the action integral



Field Theory of Ionic Solutions: Liu, Ryham, Hyon, Eisenberg

Allows boundary conditions and flow
Deals Consistently with Interactions of Components

PNP (Poisson Nernst Planck) **for Spheres**

Non-equilibrium variational field theory *EnVarA*

Nernst Planck Diffusion Equation

*for **number density c_n** of negative n ions; positive ions are analogous*

Diffusion Coefficient

Thermal Energy

Coupling Parameters

Ion Radii

Number Densities

$$\frac{\partial c_n}{\partial t} = \nabla \cdot \left[D_n \left\{ \nabla c_n + \frac{c_n}{k_B T} \left(z_n e \nabla \phi - \int \frac{12 \varepsilon_{n,n} (a_n + a_n)^{12} (\vec{x} - \vec{y})}{|\vec{x} - \vec{y}|^{14}} c_n(\vec{y}) d\vec{y} - \int \frac{6 \varepsilon_{n,p} (a_n + a_p)^{12} (\vec{x} - \vec{y})}{|\vec{x} - \vec{y}|^{14}} c_p(\vec{y}) d\vec{y} \right) \right\} \right],$$

Poisson Equation

Dielectric Coefficient

Permanent Charge of Protein

valence proton charge

$$\nabla \cdot (\varepsilon \nabla \phi) = -\left(\rho_0 + \sum_{i=1}^{N} z_i e c_i \right) \qquad i = n \text{ or } p$$

All we have to do is

Solve the
Partial Differential Equations
with Boundary Conditions

Fermi Poisson

Largest Effect
of
Crowded Ions
is
Saturation

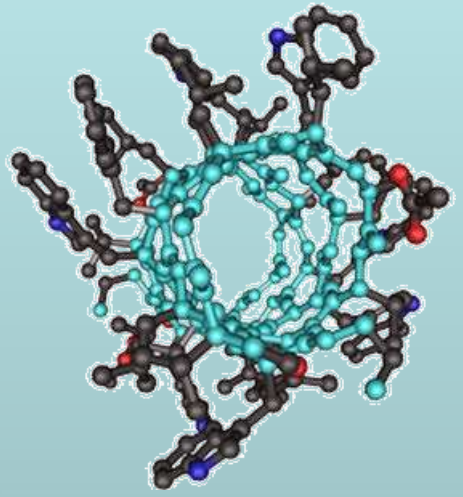
*Saturation cannot be described at all by classical Poisson Boltzmann approach and is described in a uncalibrated way by **present day** Molecular Dynamics when Mixtures and Divalents are Biologically Important in Concentrations of 10^{-8} to 10^{1} M*

Fermi Description

is designed to deal with

Saturation of Concentration

Simulating saturation by interatomic repulsion (Lennard Jones)
is a significant mathematical challenge
to be side-stepped if possible
Eisenberg, Hyon and Liu (2010). JChemPhys 133: 104104



Gramicidin A

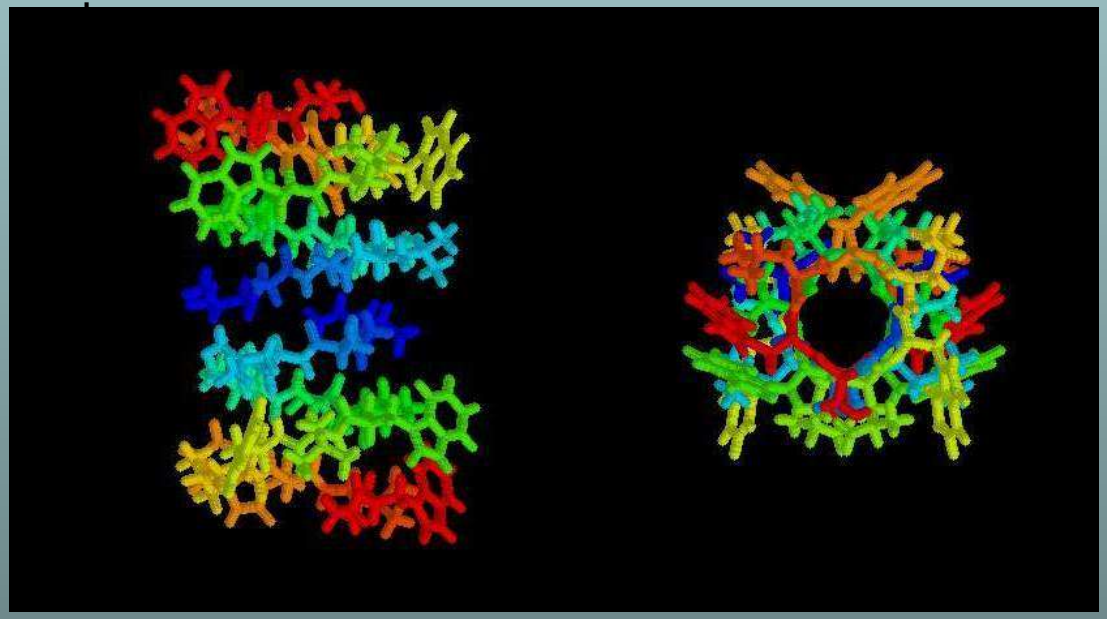
Unusual SMALL Bacterial Channel

often simulated and studied

Margaret Thatcher,
student of Nobelist Dorothy Hodgkin
Bonnie Wallace leading worker

Validation of PNP Solvers with Exact Solution

following the lead of
Zheng, Chen & Wei
J. Comp. Phys. (2011) **230**: 5239



PNPF

Poisson-Nernst-Planck-Fermi

Implemented fully in 3D Code to accommodate 3D Protein Structures

Flow

$$\nabla \cdot \mathbf{J} = 0$$

$$\mathbf{J}_i = -D_i \left[\nabla C_i + (z_i \boldsymbol{e} / k_b T)\ \nabla \phi - C_i \nabla S^{teric} \right]$$

Force

$$\nabla^2 \phi = \psi$$

$$\varepsilon_{water} \left(l_c \nabla^2 - 1 \right) \nabla^2 \phi(\mathbf{r}) \psi = \rho(\mathbf{r})$$

$\varepsilon_{water} \left(l_c \nabla^2 - 1 \right)$ approximates dielectric of entire bulk solution including correlated motions of ions, following **Santangelo** 2006[1] used by Kornyshev 2011[2] with Liu's corrected and consistent Fermi treatment of spheres

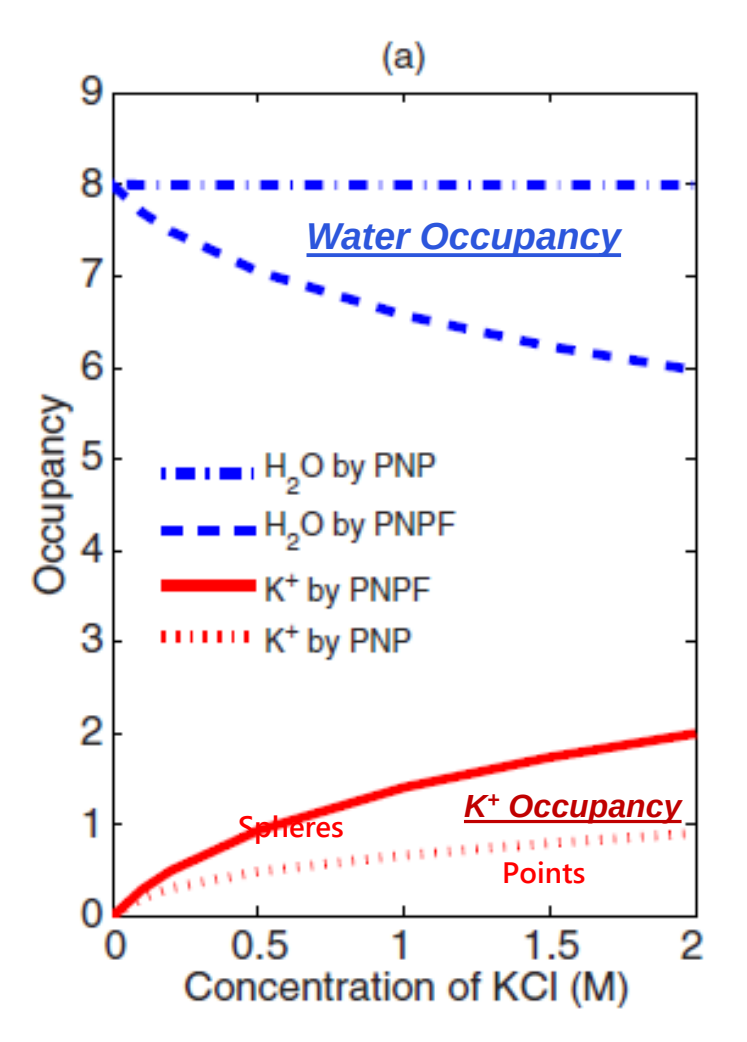
We introduce[3,4] **two second order equations** and **boundary conditions**

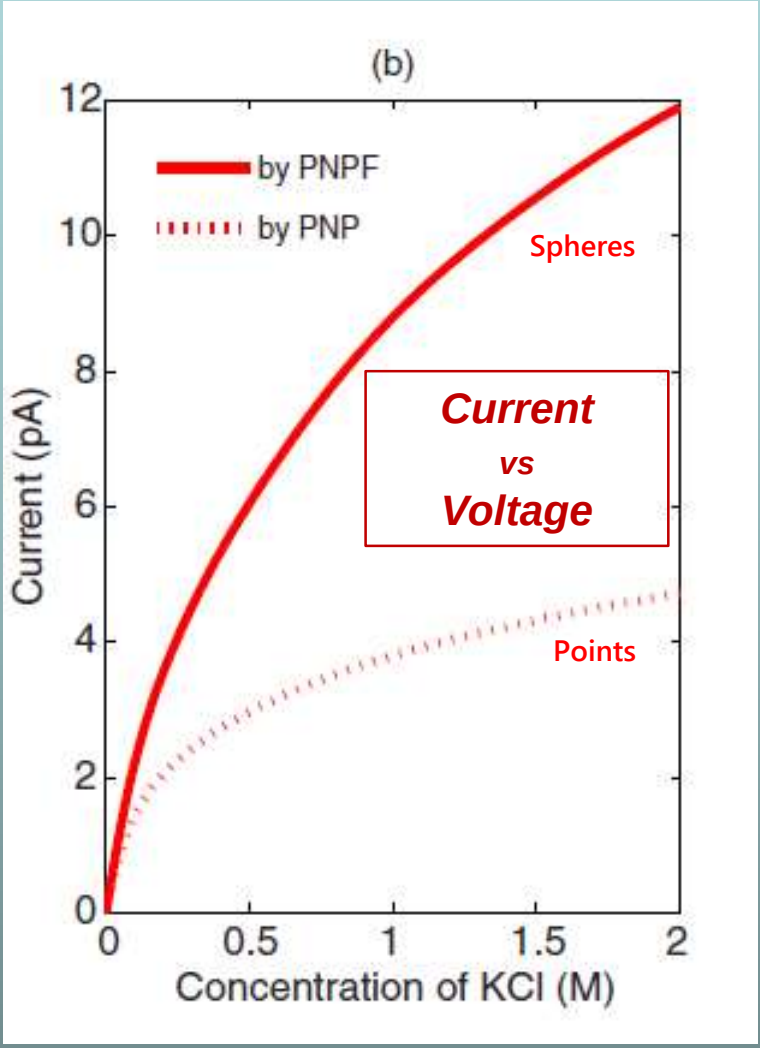
That give the polarization charge density $-\varepsilon_{water} \psi = \rho_{pol}$

3D computation is facilitated by using 2nd order equations

[1]PhysRev E (2006) 73:041512 [2]PhysRev Ltrs (2011) 106:046102 [3]JCompPhys (2013) 247:88 [4]J PhysChem B (2013) 117:12051

Steric Effect is Large in *(crowded)* Gramicidin
PNPF spheres *vs* PNP points

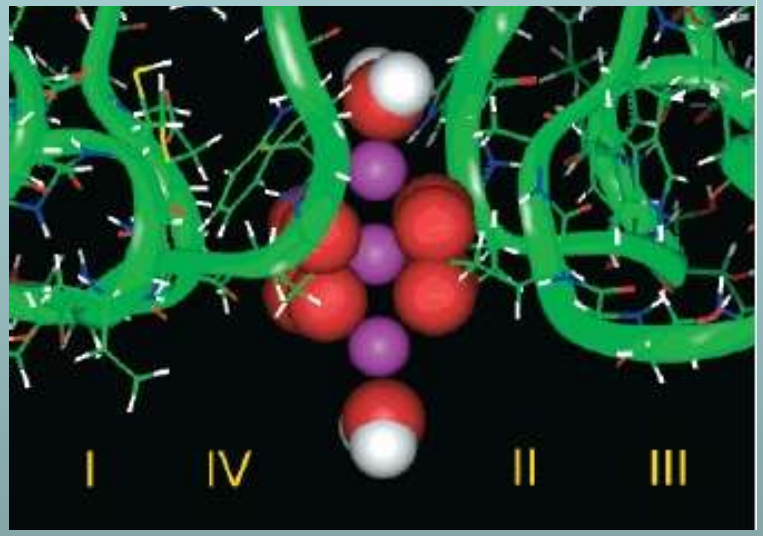




Three Dimensional Calculation Starting with Actual Structure

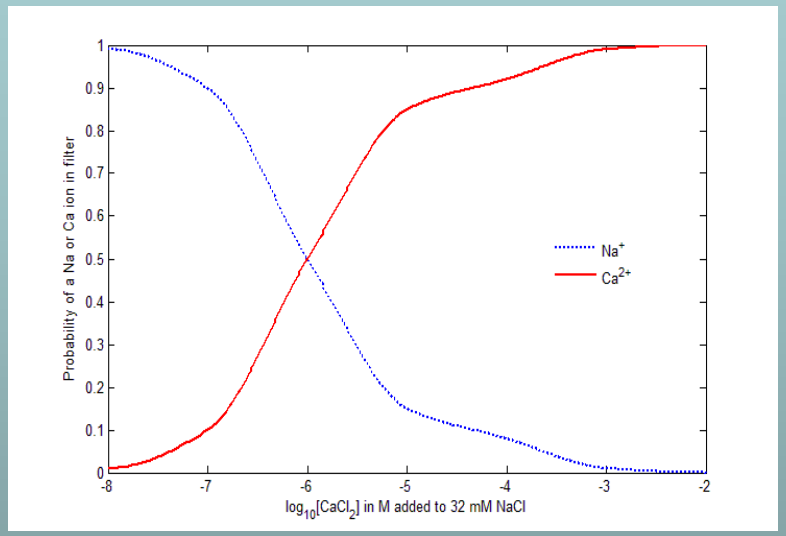
Cardiac Calcium Channel $Ca_V.n$

Lipkind-Fozzard Model



Ca^{2+} are shown in **violet**,
8 $O^{0.5-}$ in **red**, H_2O in **white** and **red**
Lipkind & Fozzard, Biochem (2001) **40** 6786

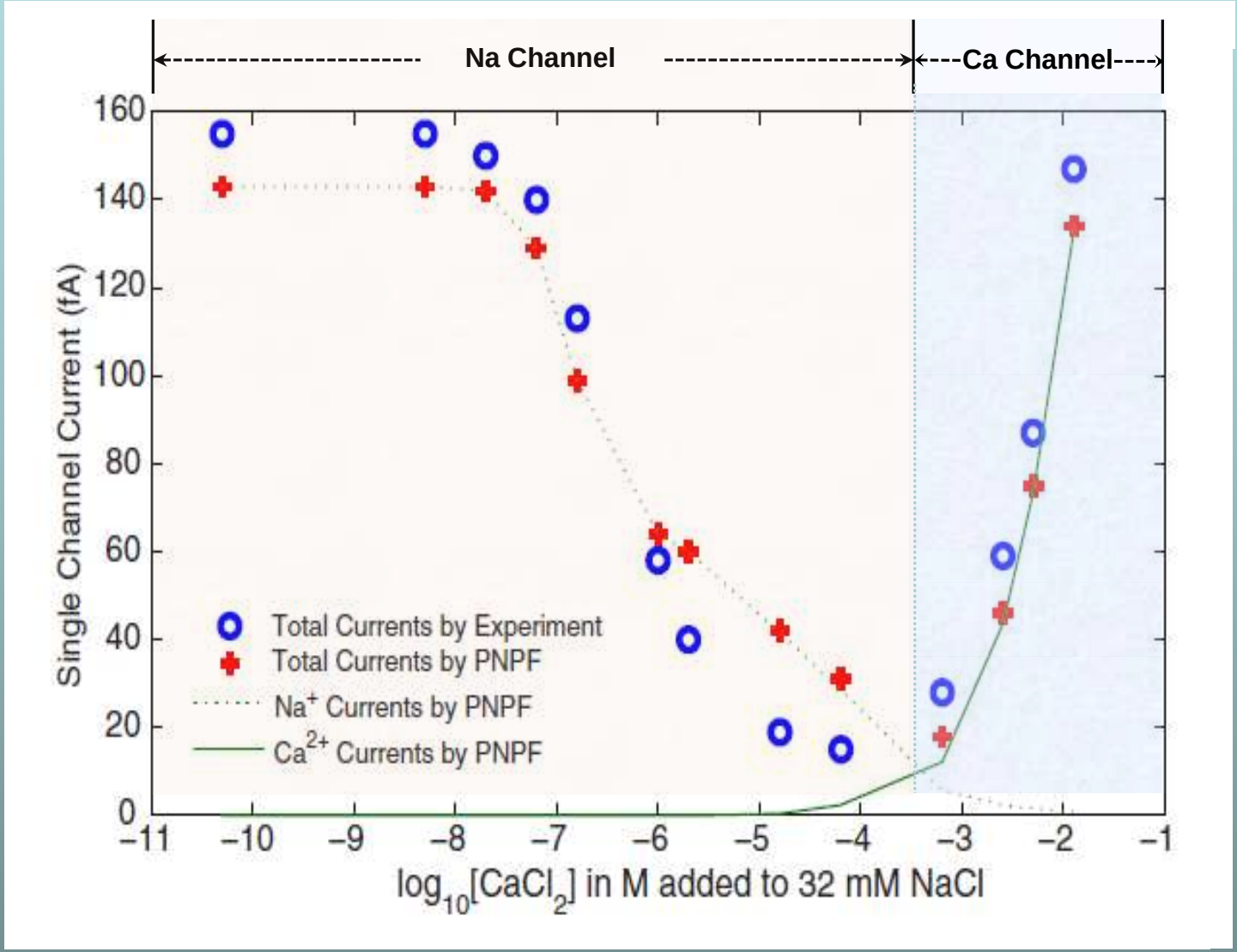
Binding Curve



Liu & Eisenberg J Chem Phys 141(22): 22D532

Cardiac Calcium Channel $Ca_V1.n$

Experimental Signature *Anomalous** Mole Fraction



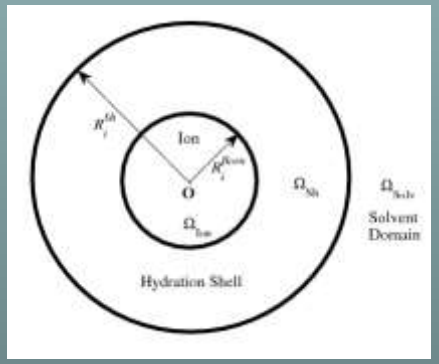
***Anomalous* because CALCIUM CHANNEL IS A SODIUM CHANNEL at $[CaCl_2] \cong 10^{-3.4}$**

Ca^{2+} is conducted for $[Ca^{2+}] > 10^{-3.4}$, but Na^+ is conducted for $[Ca^{2+}] < 10^{-3.}$

Liu & Eisenberg (2015) Physical Review E 92: 012711

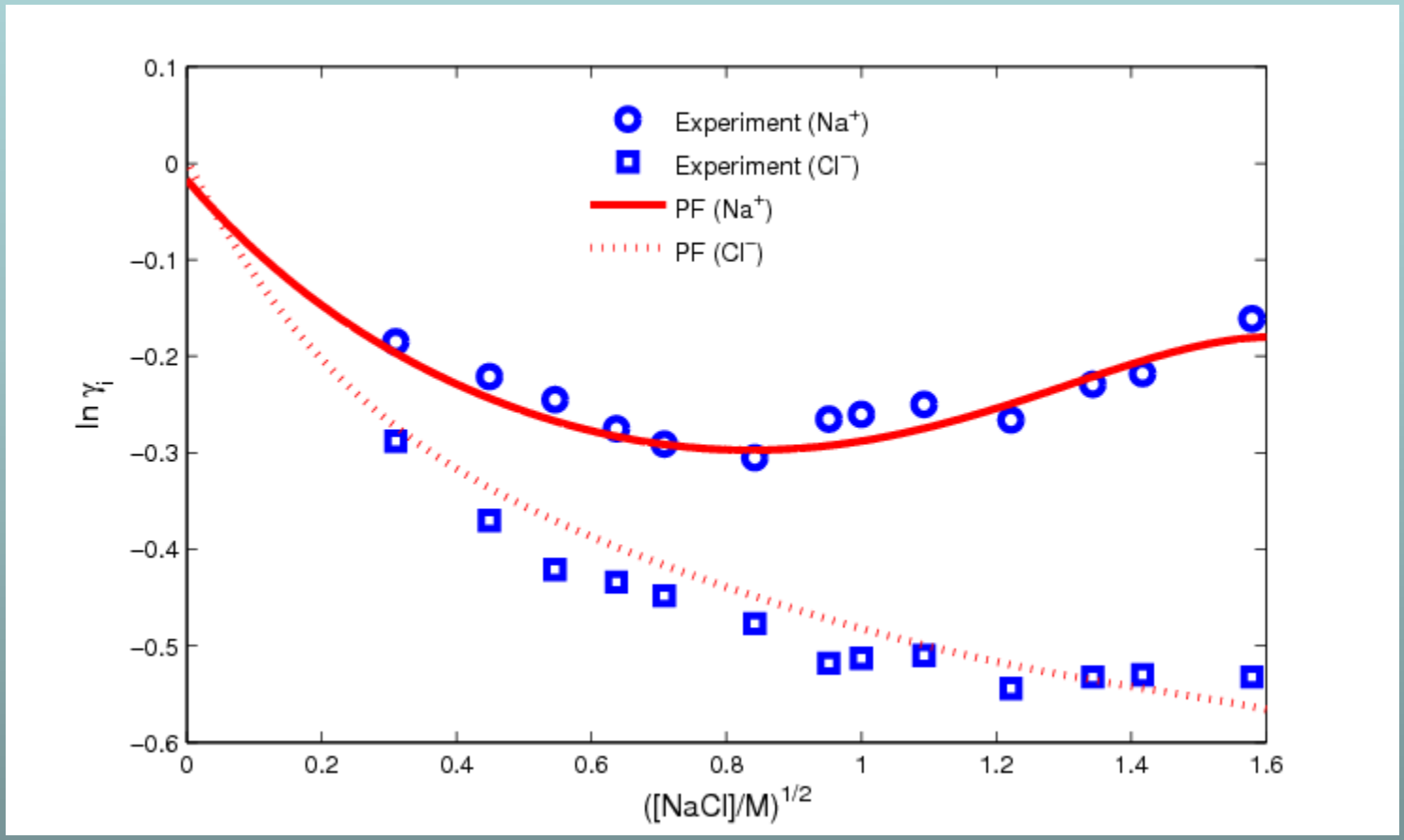
Poisson Fermi Approach to
Bulk Solutions

Same Fermi Poisson Equations,
different model of nearby atoms in hydration shells

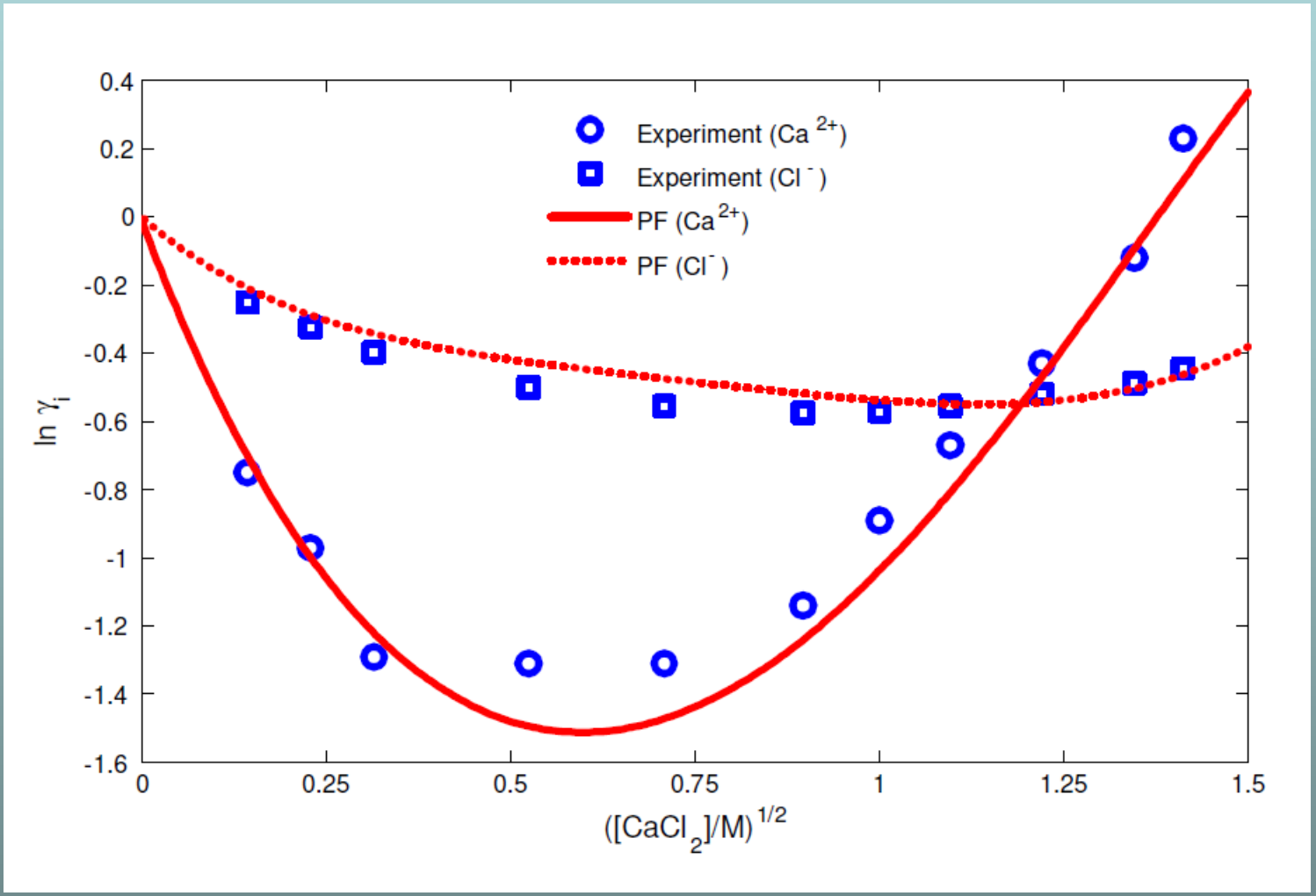


Activity Coefficients
Na^+ Cl^-

'normalized' free energy per mole



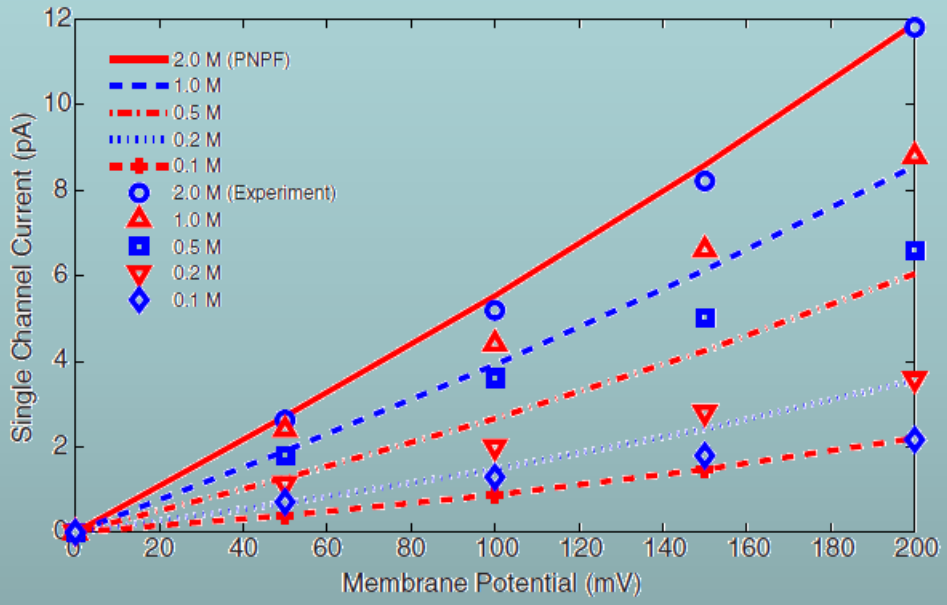
Activity Coefficients
$Ca^{2+}Cl_2^-$
'normalized' free energy per mole



The End

Any Questions?

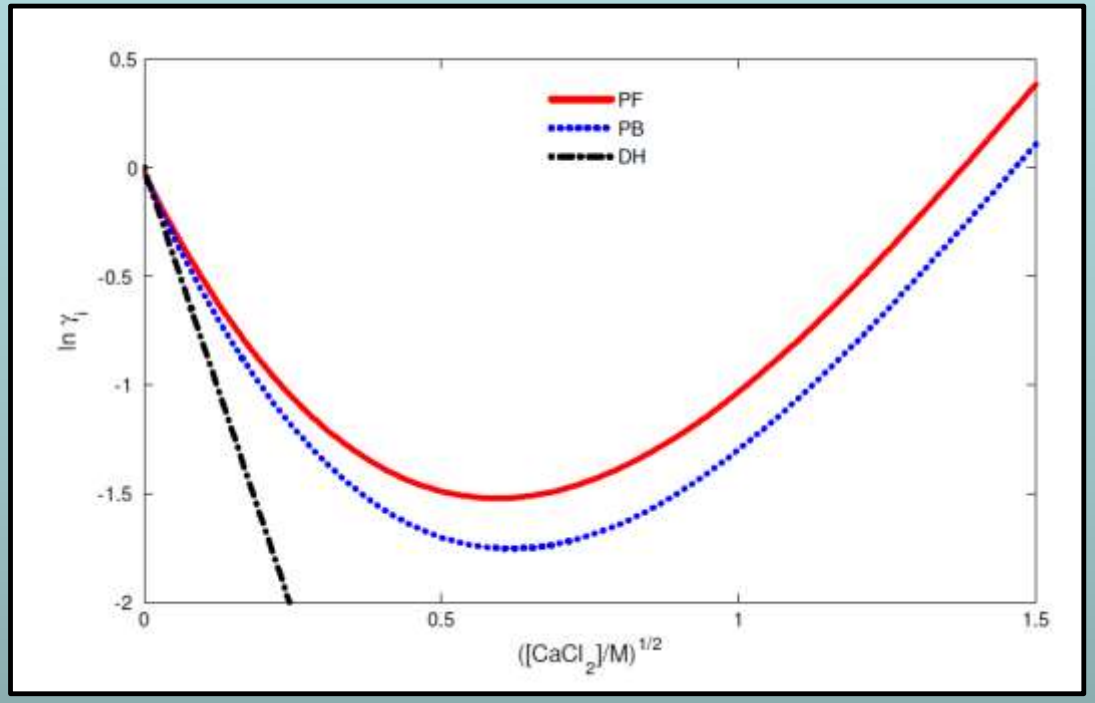
Three Dimensional Theory
Comparison with Experiments
Gramicidin A



Data from
*Cole, Frost, Thompson, Cotten, **Cross**, & **Busath**, Biophys J (2002) **83**:1974*

Theory from *Liu & Eisenberg J ChemPhys 141: 22D532*
with one adjustable parameter never changed

Debye-Hückel Fails Disastrously
Poisson Boltzmann is quite inaccurate
Poisson Fermi does Surprisingly Well



Parameters, NOT further adjusted

$l_c = 2a_i$	correlation length	$i = Na^+, Ca^{2+}, Cl^-$	Å
a_{Na^+}, $a_{Ca^{2+}}$	radii	0.95, 0.99	Å
a_{Cl^-}, a_{H_2O}	radii	1.81, 1.4	Å
$R^0_{Na^+}$, $R^0_{Ca^{2+}}$, $R^0_{Cl^-}$	Born radii in Eq. (12)	1.617, 1.706, 2.263	Å
δ_{Na^+}, $\delta_{Ca^{2+}}$, δ_{Cl^-}	in Eq. (11)	4.2, 5.1, 3.8	
O_i^w	in Eq. (10)	18	

Evidence

(start)

Best Evidence is from the

RyR Receptor

Dirk Gillespie

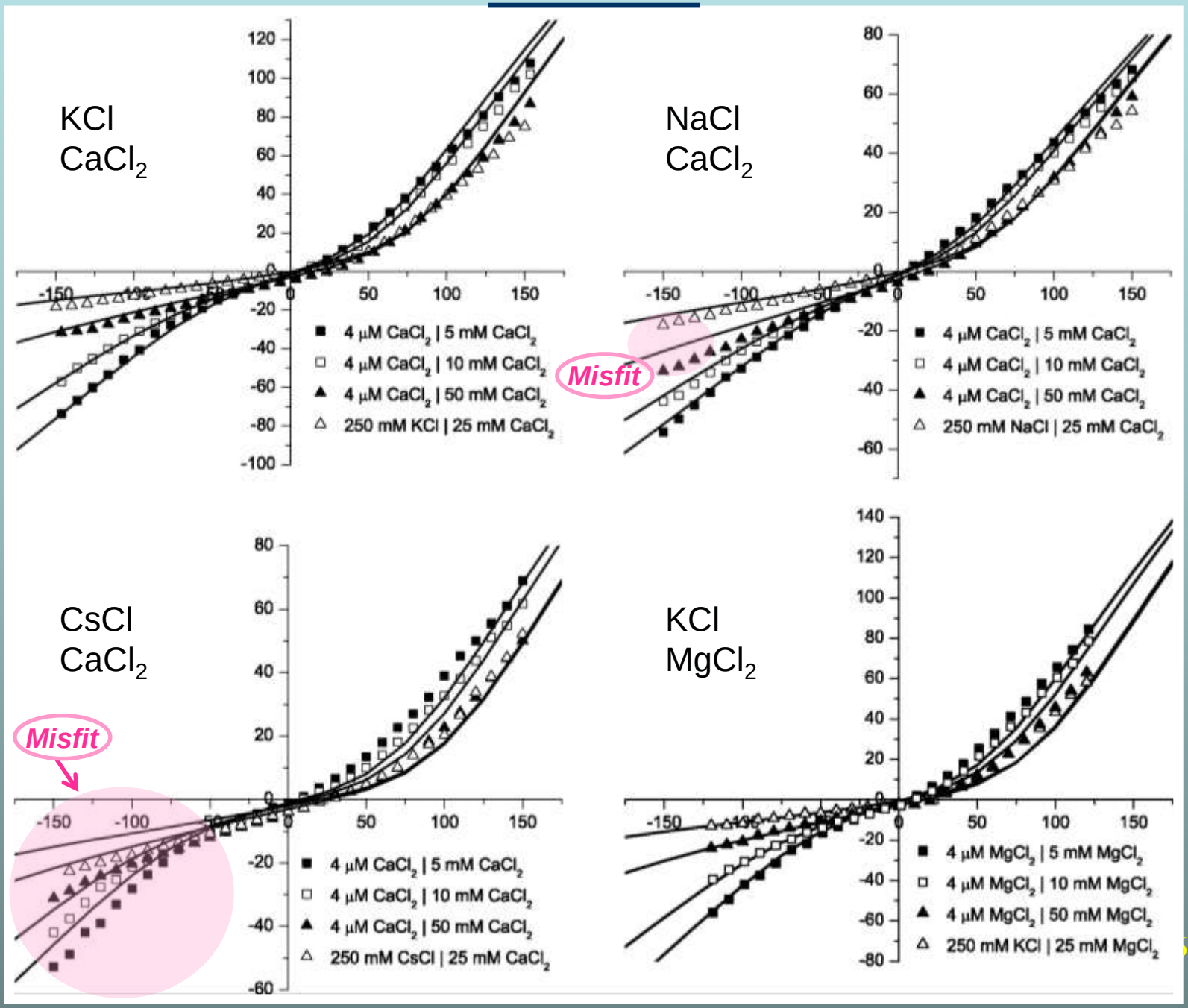
Dirk_Gillespie@rush.edu

Gerhard Meissner, Le Xu, et al,
not *Bob Eisenberg*

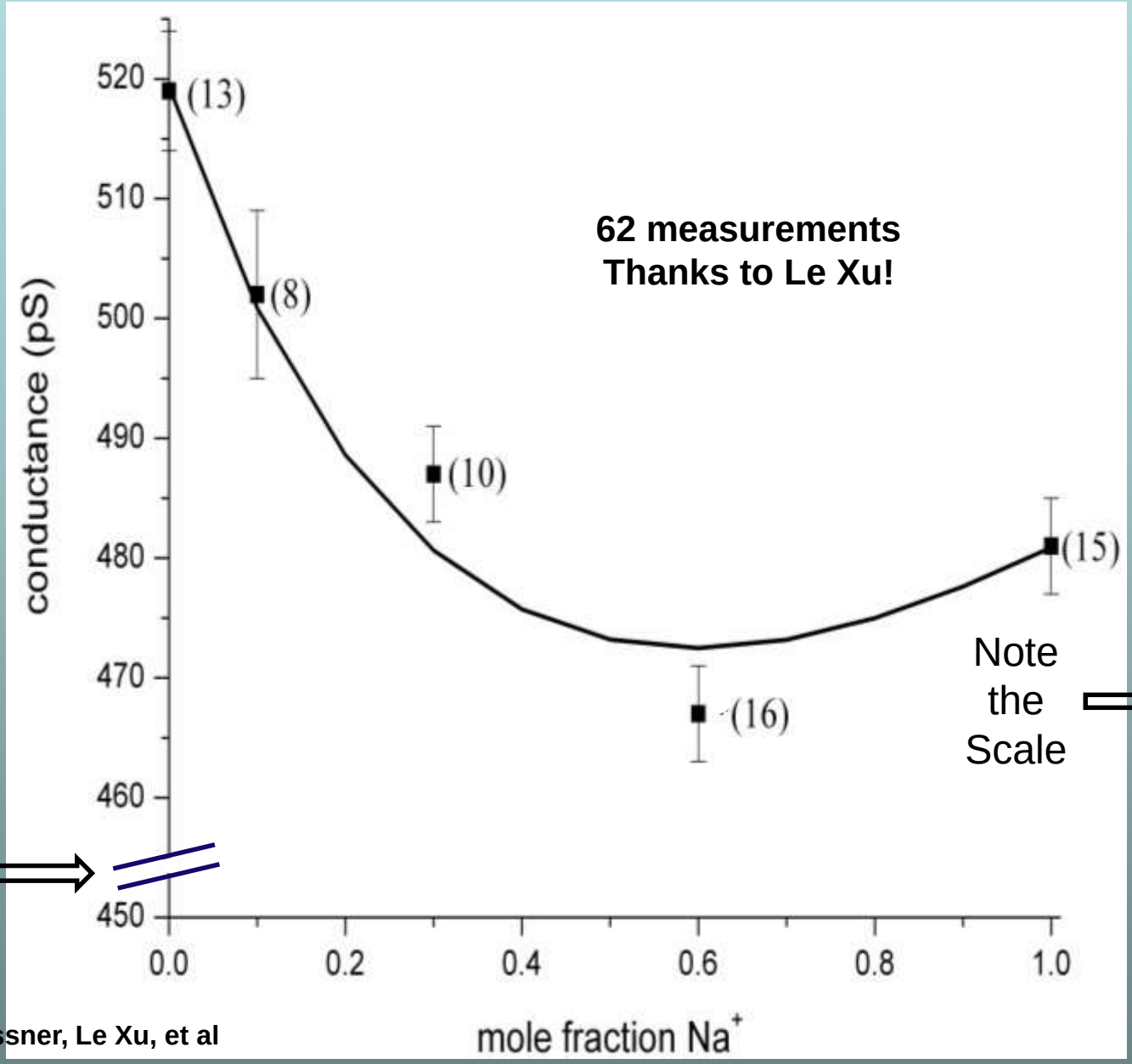
- **More than 120 combinations of solutions & mutants**
- **7 mutants with significant effects fit successfully**

1. Gillespie, D., Energetics of divalent selectivity in a calcium channel: the ryanodine receptor case study. *Biophys J, 2008. 94(4): p. 1169-1184.*
2. Gillespie, D. and D. Boda, Anomalous Mole Fraction Effect in Calcium Channels: A Measure of Preferential Selectivity. *Biophys. J., 2008. 95(6): p. 2658-2672.*
3. Gillespie, D. and M. Fill, Intracellular Calcium Release Channels Mediate Their Own Countercurrent: Ryanodine Receptor. *Biophys. J., 2008. 95(8): p. 3706-3714.*
4. Gillespie, D., W. Nonner, and R.S. Eisenberg, Coupling Poisson-Nernst-Planck and Density Functional Theory to Calculate Ion Flux. *Journal of Physics (Condensed Matter), 2002. 14: p. 12129-12145.*
5. Gillespie, D., W. Nonner, and R.S. Eisenberg, Density functional theory of charged, hard-sphere fluids. Physical Review E, 2003. 68: p. 0313503.
6. Gillespie, D., Valisko, and Boda, Density functional theory of electrical double layer: the RFD functional. *Journal of Physics: Condensed Matter, 2005. 17: p. 6609-6626.*
7. Gillespie, D., J. Giri, and M. Fill, Reinterpreting the Anomalous Mole Fraction Effect. The ryanodine receptor case study. Biophysical Journal, 2009. 97: p. pp. 2212 - 2221
8. Gillespie, D., L. Xu, Y. Wang, and G. Meissner, (De)constructing the Ryanodine Receptor: modeling ion permeation and selectivity of the calcium release channel. *Journal of Physical Chemistry, 2005. 109: p. 15598-15610.*
9. Gillespie, D., D. Boda, Y. He, P. Apel, and Z.S. Siwy, Synthetic Nanopores as a Test Case for Ion Channel Theories: The Anomalous Mole Fraction Effect without Single Filing. *Biophys. J., 2008. 95(2): p. 609-619.*
10. Malasics, A., D. Boda, M. Valisko, D. Henderson, and D. Gillespie, Simulations of calcium channel block by trivalent cations: Gd(3+) competes with permeant ions for the selectivity filter. *Biochim Biophys Acta, 2010. 1798(11): p. 2013-2021.*
11. Roth, R. and D. Gillespie, Physics of Size Selectivity. *Physical Review Letters, 2005. 95: p. 247801.*
12. Valisko, M., D. Boda, and D. Gillespie, Selective Adsorption of Ions with Different Diameter and Valence at Highly Charged Interfaces. *Journal of Physical Chemistry C, 2007. 111: p. 15575-15585.*
13. Wang, Y., L. Xu, D. Pasek, D. Gillespie, and G. Meissner, Probing the Role of Negatively Charged Amino Acid Residues in Ion Permeation of Skeletal Muscle Ryanodine Receptor. *Biophysical Journal, 2005. 89: p. 256-265.*
14. Xu, L., Y. Wang, D. Gillespie, and G. Meissner, Two Rings of Negative Charges in the Cytosolic Vestibule of T Ryanodine Receptor Modulate Ion Fluxes. *Biophysical Journal, 2006. 90: p. 443-453.*

Divalents


KCl
CaCl2
4 μM CaCl2 | 5 mM CaCl2
4 μM CaCl2 | 10 mM CaCl2
4 μM CaCl2 | 50 mM CaCl2
250 mM KCl | 25 mM CaCl2
NaCl
CaCl2
Misfit
4 μM CaCl2 | 5 mM CaCl2
4 μM CaCl2 | 10 mM CaCl2
4 μM CaCl2 | 50 mM CaCl2
250 mM NaCl | 25 mM CaCl2
CsCl
CaCl2
Misfit
4 μM CaCl2 | 5 mM CaCl2
4 μM CaCl2 | 10 mM CaCl2
4 μM CaCl2 | 50 mM CaCl2
250 mM CsCl | 25 mM CaCl2
KCl
MgCl2
4 μM MgCl2 | 5 mM MgCl2
4 μM MgCl2 | 10 mM MgCl2
4 μM MgCl2 | 50 mM MgCl2
250 mM KCl | 25 mM MgCl2

The model predicted an AMFE for Na^+/Cs^+ mixtures before it had been measured

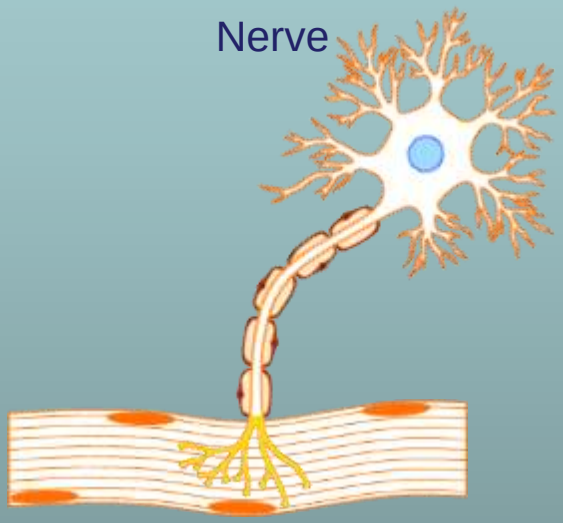


Gillespie, Meissner, Le Xu, et al

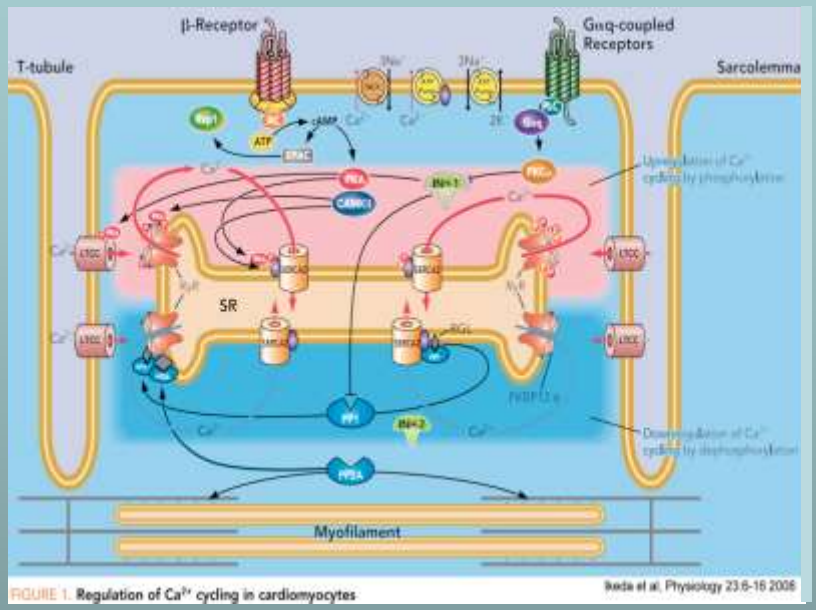
Evidence

(end)

All Spheres Models
work well for
Calcium and Sodium Channels



Skeletal muscle



Heart Muscle Cell

Fermi (like) Distribution

$$C_i(\mathbf{r}) = C_i^{bath} \exp\left(-\beta_i \phi(\mathbf{r}) + S^{teric}(\mathbf{r})\right)$$

$$S^{teric}(\mathbf{r}) = \ln\left(\Gamma(\mathbf{r}) / \Gamma(bath)\right)$$

$\Gamma(bath)$ = bulk void concentration; $\Gamma(\mathbf{r})$ = channel void concentration

Fermi (like) Distribution

is a general

Quantitative Statement of Charge-Space Competition

Simulated and compared to experiments in
> 35 papers of *Boda, Henderson, et al,*
and >10 papers of Gillespie, et al,

also gives

Gibbs Fermi Functional

J Comp Phys, 2013 247:88; J Phys Chem B, 2013 117:12051

so the Fermi approach
Can be embedded in the ***E***nergy ***V***ariational ***F***ormulation
EnVarA developed by ***Chun Liu***, more than anyone
Eisenberg, Hyon and Liu (2010). JChemPhys 133: 104104