Mathematics
describes only a tiny part of life,

But

Mathematics* Creates

our

Standard of Living

**e.g., Electricity, Computers, Fluid Dynamics, Optics, Structural Mechanics, ….*

How can we use mathematics to describe biological systems?

I believe some biology is

Physics 'as usual'

'Guess and **Check'**

But you have to know which biology!

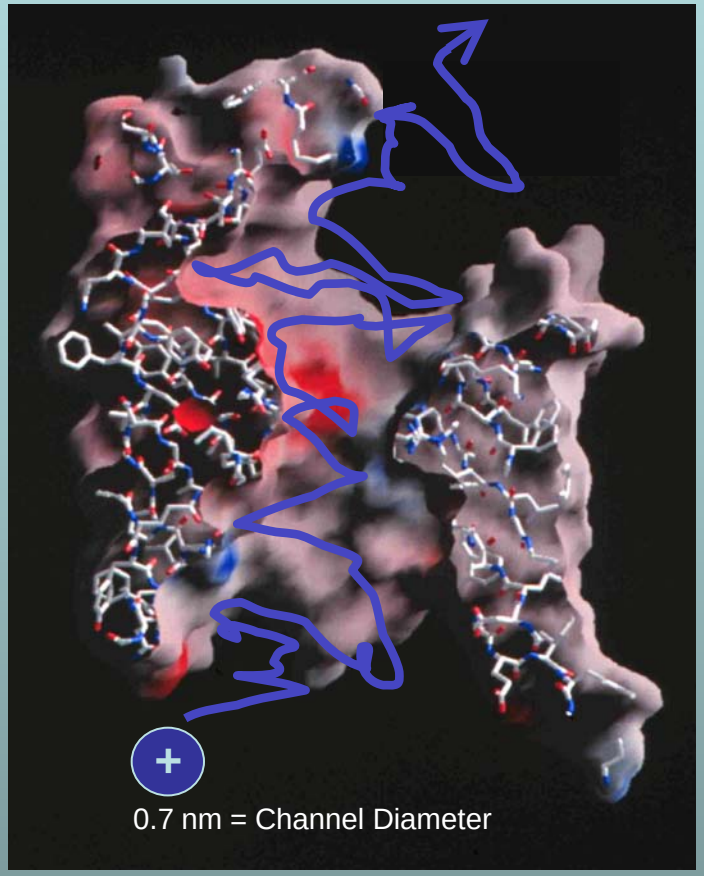
Ion Channels are the Valves of Cells

Ion Channels are the Main Controllers of Biological Function

Selectivity

Different Ions carry Different Signals

Chemical Bonds are lines
Surface is Electrical Potential
Red **is negative (acid)**
Blue **is positive (basic)**



~30 Å

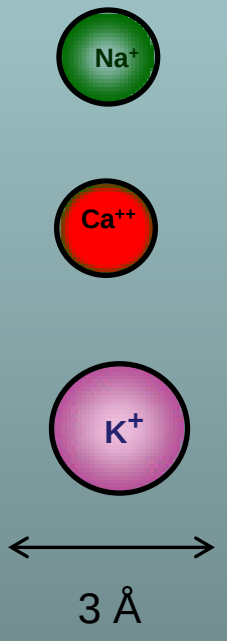
Figure of ompF porin by Raimund Dutzler

Ions in Water

are the

Liquid of Life

Hard Spheres



Ion Channels are Biological Devices

Natural nano-valves* for atomic control of biological function

Ion channels coordinate contraction of cardiac muscle, allowing the heart to function as a pump

Ion channels coordinate contraction in skeletal muscle

Ion channels control all electrical activity in cells

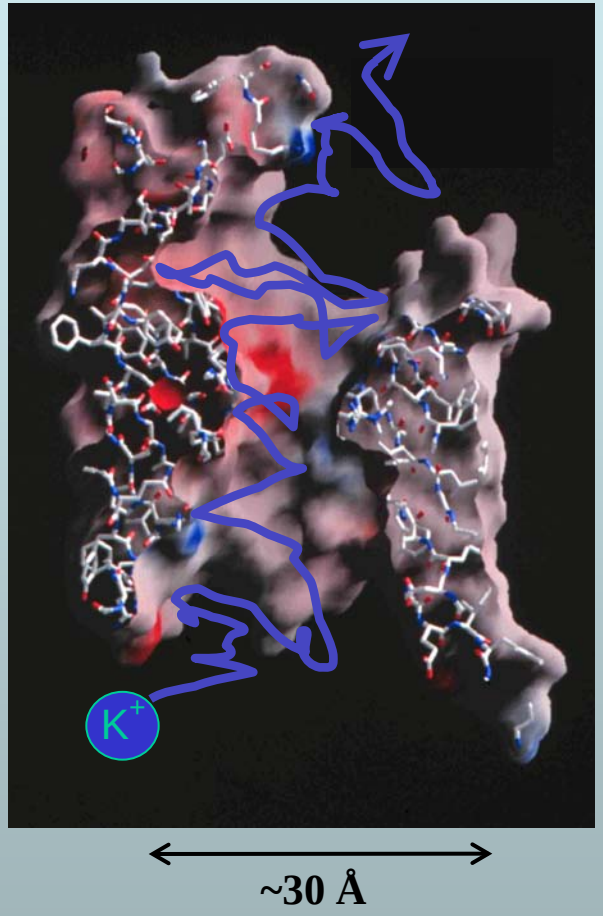
Ion channels produce signals of the nervous system

Ion channels are involved in secretion and absorption in all cells: kidney, intestine, liver, adrenal glands, etc.

Ion channels are involved in thousands of diseases and many drugs act on channels

Ion channels are proteins whose genes (blueprints) can be manipulated by molecular genetics

Ion channels have structures shown by x-ray crystallography in favorable cases

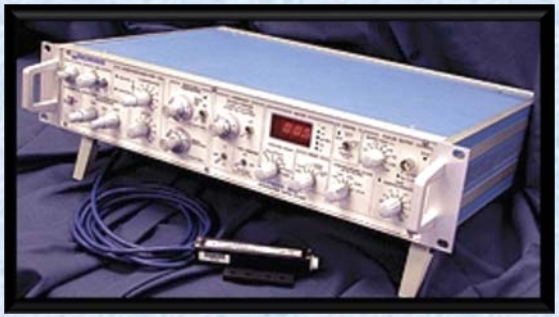


*nearly pico-valves: diameter is 400 – 900 picometers

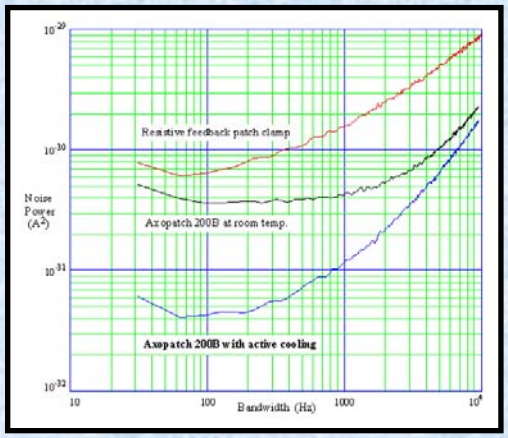
Thousands of Molecular Biologists Study Channels every day,

One protein molecule at a time

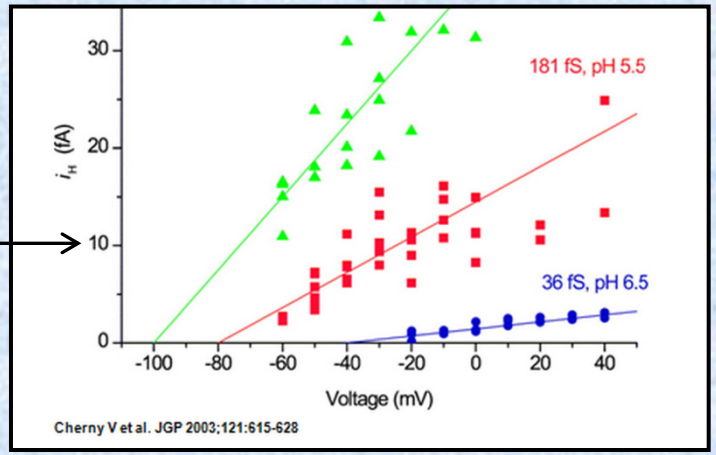
This number is not an exaggeration.
We have sold >10,000 AxoPatch amplifiers



AxoPatch 200B



Femto-amps (10^{-15} A)



Ion_channel_newsletter

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Popular publications for March (view most recent)

1. Molecular basis of infrared detection by snakes. *Nature*
2. The Angelman Syndrome Protein Ube3A Regulates Synapse Development by Ubiquitinating Arc. *Cell*
3. AMPA receptors--another twist? *Science*
4. Molecular Basis of Calcium Signaling in Lymphocytes: STIM and ORAI. *Annu Rev Immunol*
5. Neurological Channelopathies. *Annu Rev Neurosci*
6. New antiarrhythmic drugs for treatment of atrial fibrillation. *Lancet*
7. A Glial Signal Consisting of Gliomedin and NrCAM Clusters Axonal Na(+) Channels during the Formation of Nodes of Ranvier. *Neuron*
8. Small Molecule Activators of TRPML3. *Chem Biol*
9. Truncated {beta}-amyloid peptide channels provide an alternative mechanism for Alzheimer's Disease and Down syndrome. *Proc Natl Acad Sci U S A*
10. Modelling the molecular mechanisms of synaptic plasticity using systems biology approaches. *Nat Rev Neurosci*

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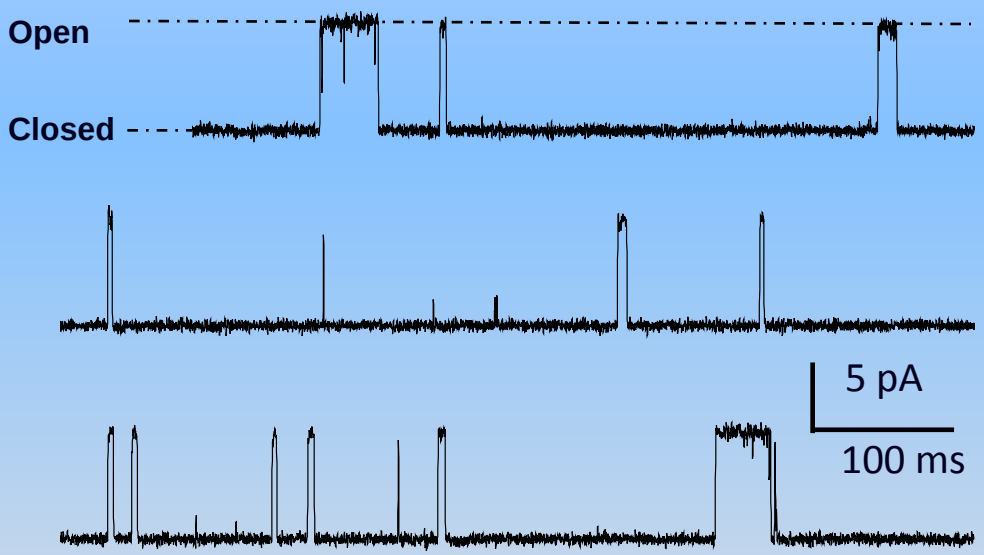
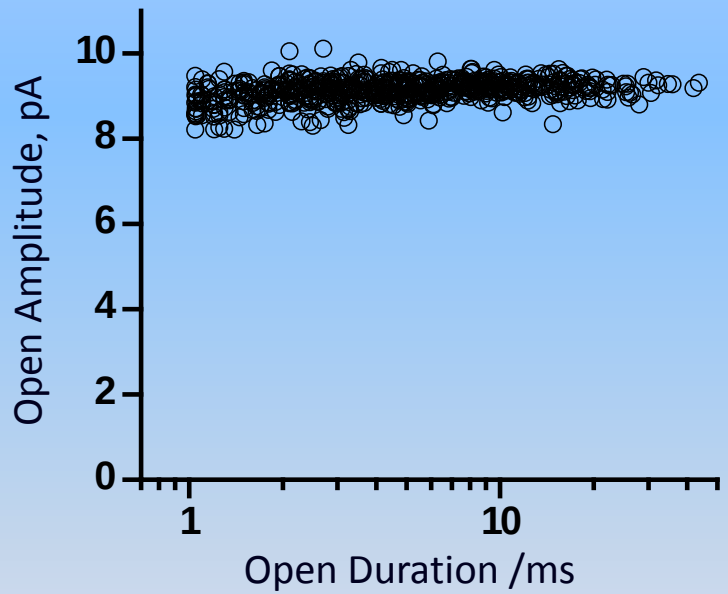
Channel Structure Does Not Change

once the channel is open

Current vs. time



Amplitude vs. Duration



Lowpass Filter = 1 kHz Sample Rate = 20 kHz

Typical Raw Single Channel Records

Ca^{2+} Release Channel of Inositol Trisphosphate Receptor: slide and data from Josefina Ramos-Franco. Thanks!

Channels are only Holes

Why can't we understand and build them?

Where to start?

Why not compute all the atoms?

Multiscale Issues

more later

Computational Scale	Biological Scale	Ratio
Time 10^{-15} sec	10^{-4} sec	10^{11}
Space 10^{-11} m	10^{-5} m	10^{6}
Spatial Resolution	*Three Dimensional* $(10^{6})^{3}$	10^{18}
Solute Concentration		10^{11}

Biological Scales Occur Together
so must be

Computed Together

This may be impossible in simulations

Physicists and Engineers rarely try

Multiscale Issues

It may not be possible to deal accurately

with

Ratios of Scales

of

10^{11} 10^{6} 10^{18} 10^{12}

All at Once

Physicists and Engineers rarely try!

Computational Biology is
NOT doing 'Physics as Usual'

Why can't we understand and build channels?

Uncalibrated Simulations will not make devices that actually work

Unpopular view because
Calibration is Hard Work
particularly for Non-Ideal systems
with
Correlations, Finite Size effects, and Flows

Where do we start?

Physics ‘As Usual’

‘Guess and **Check’**

Stochastic ‘Derivation’

Later

will include biological adaptation
of Correlations and Crowded Charge

We start with Langevin equations of charged particles

Opportunity and Need

Simplest stochastic trajectories
are
Brownian Motion of Charged Particles

Einstein, Smoluchowski, and Langevin ignored charge
and therefore
do not describe Brownian motion of ions in solutions

We use

Theory of Stochastic Processes

to go

from Trajectories to Probabilities

Once we learn to count Trajectories of Brownian Motion of Charge, we can count trajectories of Molecular Dynamics

Schuss, Nadler, Singer, Eisenberg

Langevin Equations

Positive ***cat*ion**,
e.g., p = Na^+

Negative ***an*ion**,
e.g., n = Cl^-

$$\ddot{x}_k^p - \frac{f_k^p(\tilde{x};q_k)}{m} = -\gamma \dot{x}_k^p + \sqrt{\frac{2\gamma kT}{m}}\dot{w}_k^p$$

$$\ddot{x}_k^n - \frac{f_k^n(\tilde{x};q_k)}{m} = -\gamma \dot{x}_k^n + \sqrt{\frac{2\gamma kT}{m}}\dot{w}_k^n$$

Newton's Law

Friction & Noise

Electric Force
from **all charges** including
Permanent charge of **Protein,**
Dielectric Boundary charges,
Boundary condition charge

Schuss, Nadler, Singer, Eisenberg

Electric Force from Poisson Equation

Electric Force
from **all charges** including
Permanent charge of **P**rotein,
Dielectric Boundary charges,
Boundary condition charge

Excess
'Chemical'
Force

$$f_k^{\,p}(\vec{\mathbf{x}}) = f_{xs} + q_k(\vec{\mathbf{x}})\,\mathbf{div}\left(\varepsilon(\vec{\mathbf{x}})\vec{\mathbf{E}}\right) = \frac{e}{\varepsilon_0}\mathrm{P}(\mathbf{x}) + \frac{e}{\varepsilon_0}\sum_i z_i\rho_i(\mathbf{x})$$

Implicit Solvent
'Primitive' Model
or
Primitive Solvent Model

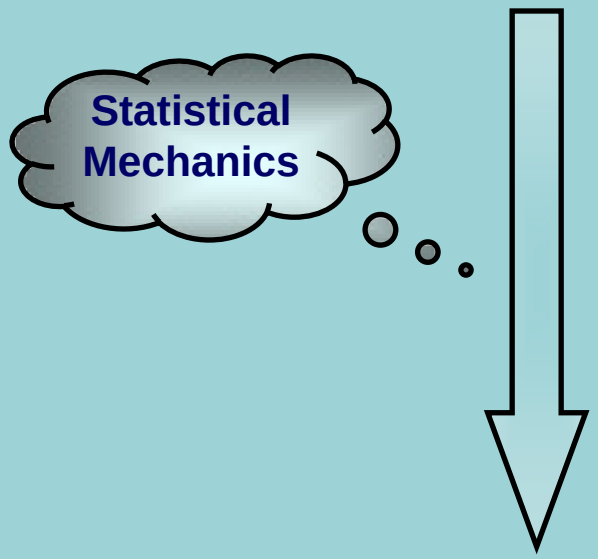
Schuss, Nadler, Singer, Eisenberg

Equilibrium Thermodynamics

Configurations

Boltzmann Distribution

$$\lim N,V \to \infty$$



Thermodynamics

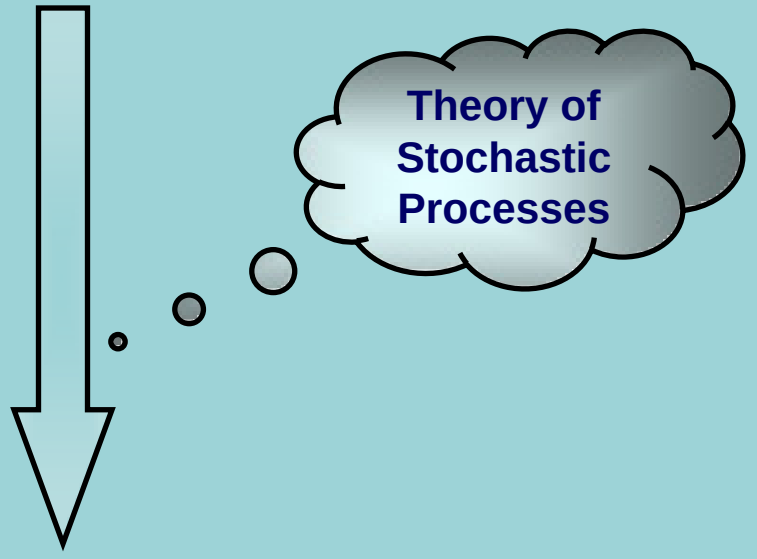
Nonequilibrium

Schuss, Nadler, Singer & Eisenberg

Trajectories

Fokker Planck Equation

Finite OPEN System



Device Equation

From Trajectories to Probabilities

Main Result of Theory of Stochastic Processes

Joint probability density of position and velocity

$$p\left(\tilde{x},\tilde{v}\right)=\Pr\left\{\left\{x_j,v_j\right\}_{j=1}^{2N}\right\};\quad N=\text{ Number of Particles}$$

satisfies a Fokker Planck equation

$$0=\sum_j \mathsf{L}_j^p\, p\left(\tilde{x},\tilde{v}\right)+\sum_j \mathsf{L}_j^n p\left(\tilde{x},\tilde{v}\right)$$

with Fokker Planck Operator

$$\mathsf{L}_j^c p=-v_j^c\cdot\nabla_{x_j^c}\; p+\nabla v_j^c\cdot\left(\gamma v_j^c-\frac{f_j^c}{m_j^c}\right)p+\nabla\cdot\nabla_{v_j^c}\;\frac{\gamma kT}{m_j^c}\,p$$

Coordinates are positions and velocities of N particles in 12N dimensional phase space

Schuss, Nadler, Singer, Eisenberg

Conditional PNP

Electric Force $\nabla\bar{\phi}$ depends on Conditional Density of Charge

$$\nabla_y \cdot \left[\frac{\varepsilon_0 \varepsilon(y)}{e} \nabla_y \bar{\phi}(y \mid x) \right] = \boldsymbol{P}(y) + \rho_+(y \mid x) - \rho_-(y \mid x)$$

Permittivity, Dielectric Coefficient, Charge on Electron

Channel Protein

Closures or **Approximations** Needed

Nernst-Planck gives UNconditional Density of Charge

$$\nabla_y \cdot \left[\frac{1}{m\gamma(x)} \rho_+(x) \left[e\nabla_y \bar{\phi}(y \mid x)\Big|_{y=x} - (\text{Other Forces}) \right] \right] = 0$$

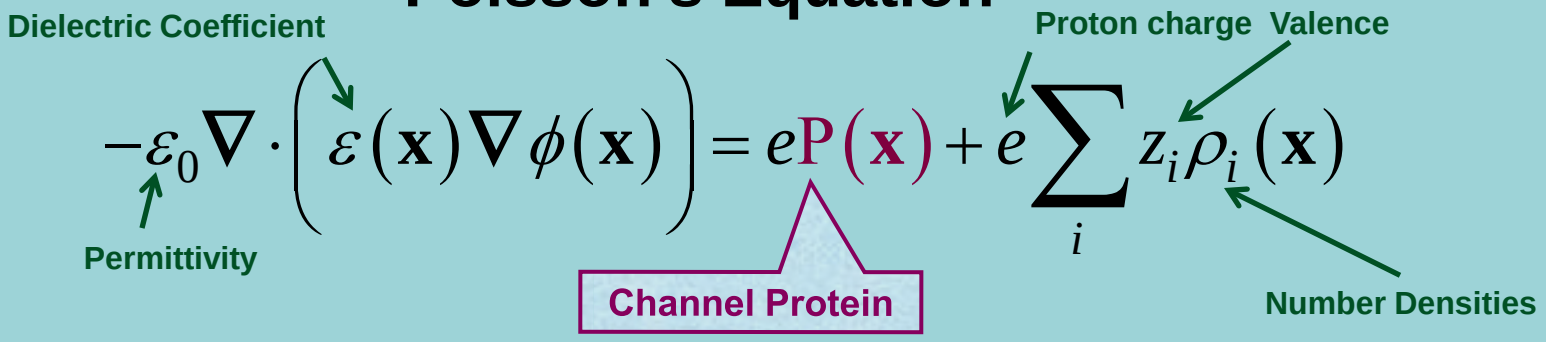
Mass

Friction

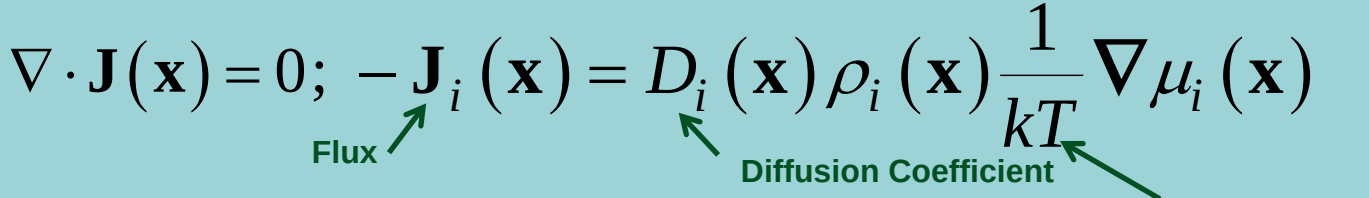
Schuss, Nadler, Singer, Eisenberg

Poisson-Nernst-Planck (PNP)

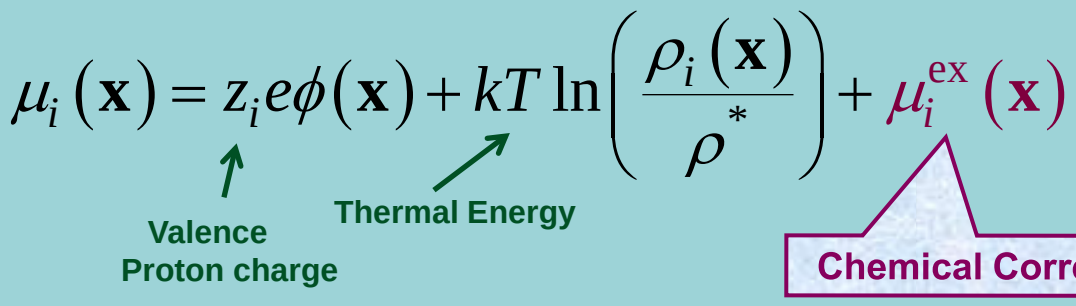
Poisson's Equation



Drift-diffusion & Continuity Equation

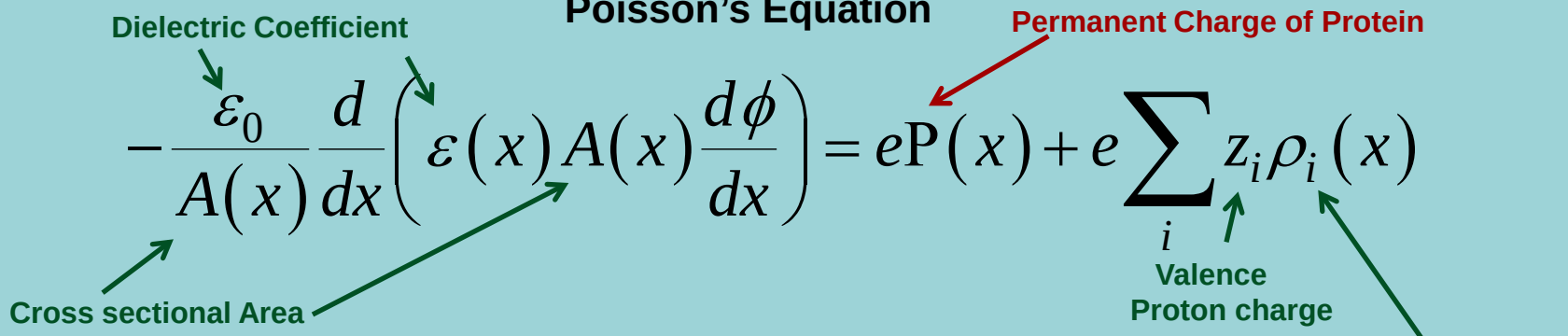


Chemical Potential $\mu_i(x)$

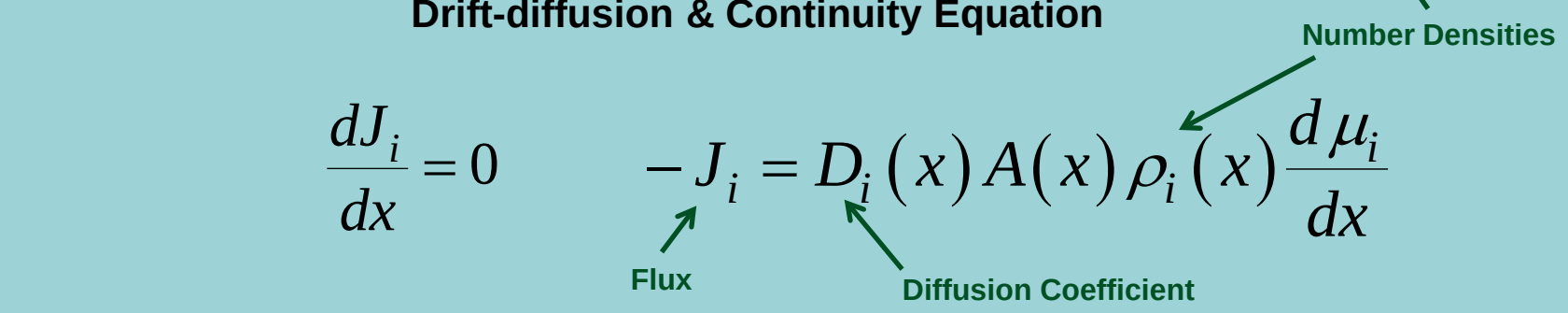


Semiconductor Equations:
One Dimensional PNP

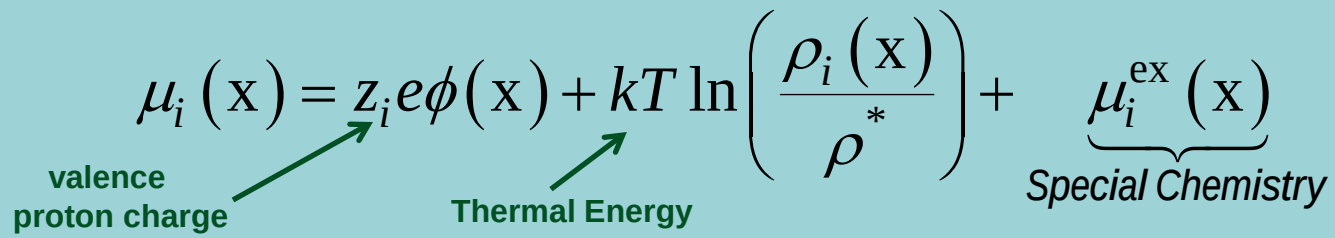
Poisson's Equation



Drift-diffusion & Continuity Equation



Chemical Potential $\mu_i(x)$



Counting at low resolution gives
‘Semiconductor Equations’

Poisson-Nernst-Planck (PNP)

Ions are Points in PNP
contains only the
Correlations of Means

Gouy-Chapman, (nonlinear) Poisson-Boltzmann,
Debye-Hückel,
are siblings with similar resolution
but at equilibrium, without current or flux of any species
Devices do not exist at equilibrium

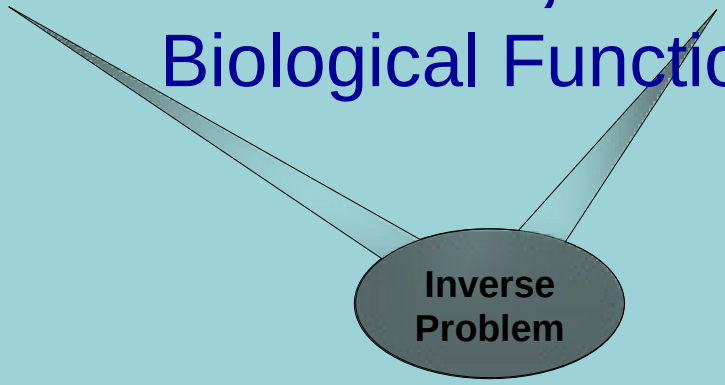
How do we check the theory?

Compare with Biological Function!

Our task is to

Discover & Understand, Control & Improve

Biological Function



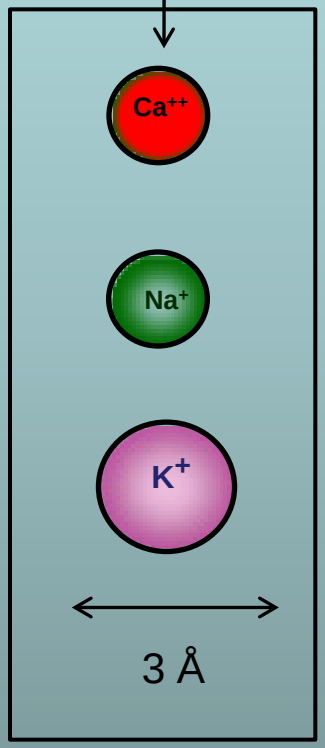
That means Selectivity

Ions are not Ideal

Potassium K^+ ≠ Na^+ Sodium

Channels are Selective

Different Ions Carry Different Signals through Different Channels

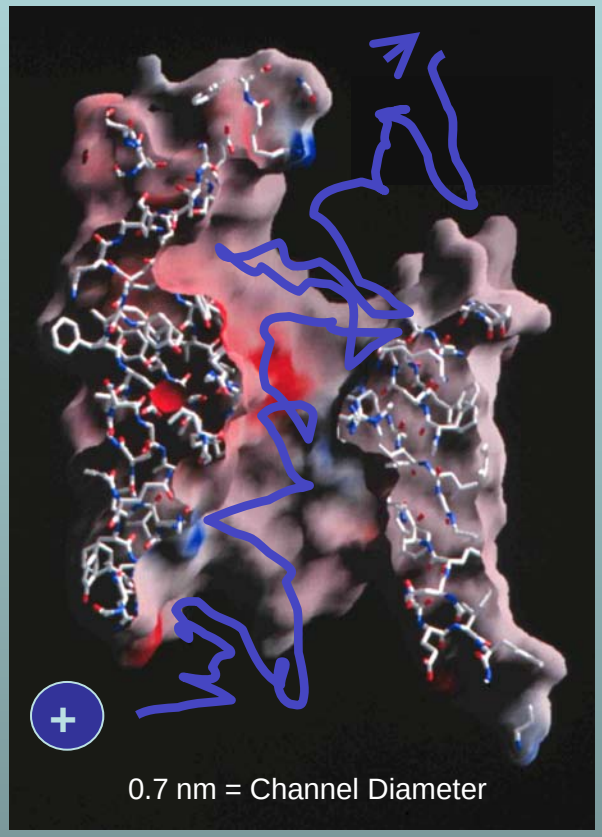


Diameter matters

Diameter is the Only Difference between K^+ and Na^+

In ideal solutions $K^+ = Na^+$

ompF porin



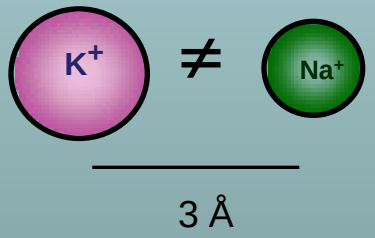
~30 Å

Flow time scale is 0.1 msec to 1 min

Figure of ompF porin by Raimund Dutzler

Channels are Selective
because
Ions are NOT Ideal

Potassium K^+ ≠ Na^+ Sodium



Ideal Electrolytes are Identical

if they have the same charge

Modelers and Mathematicians, Bioengineers: **this is reverse engineering**

How does the

Channel control Selectivity?

Inverse Problems

Many answers are possible

Central Issue

Which answer is right?

Core Math Problem has actually been solved
using methods for the

Inverse Problem of a Blast Furnace

Burger, Eisenberg and Engl (2007) SIAM J Applied Math 67: 960-989

How does the

Channel control Selectivity?

Inverse Problems: many answers possible

Central Issue

Which answer is right?

Key is

ALWAYS

Large Amount of Data

from

Many Different Conditions

Burger, Eisenberg and Engl (2007) SIAM J Applied Math 67: 960-989

Goal:

Understand Selectivity

well enough to

Fit Large Amounts of Data

from many solutions and concentrations

and to

Make a Calcium Channel

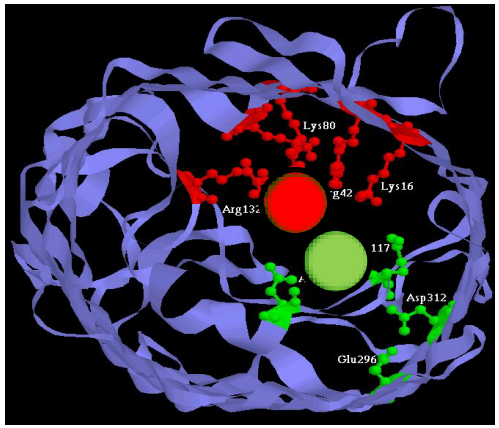
Macro Scale

Atomic Scale

Experiments have built

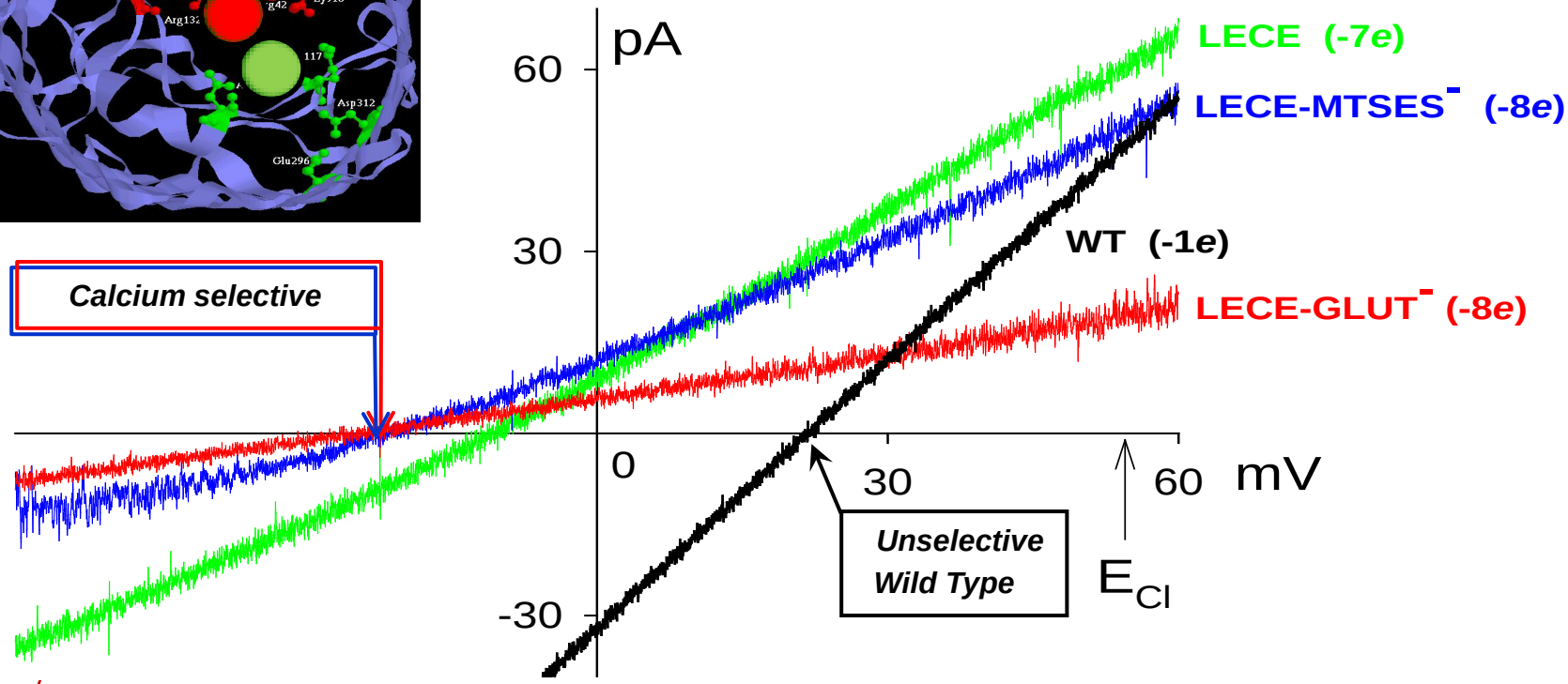
Two Synthetic Calcium Channels

Atomic Scale



Designed by Theory

Glutathione derivatives



As density of permanent charge increases, channel becomes calcium selective

$E_{rev} \rightarrow E_{Ca}$ in 0.1M || 1.0 M $CaCl_2$

Macro Scale

built by Henk Miedema, Wim Meijberg of BioMade Corp.,Groningen, Netherlands

Miedema** et al, **Biophys J 87: 3137–3147 (2004)

Comparison with Experiments shows

Potassium K^+ ≠ Sodium Na^+

Must include Biological Adaptation!

Working Hypothesis

Biological Adaptation is

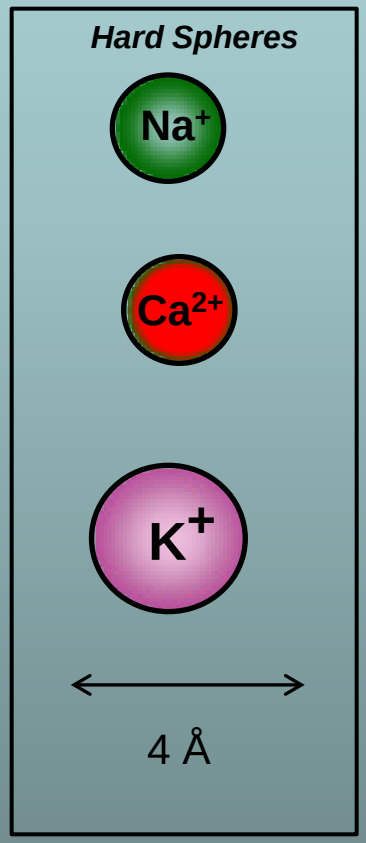
Crowded Ions *and* Side Chains

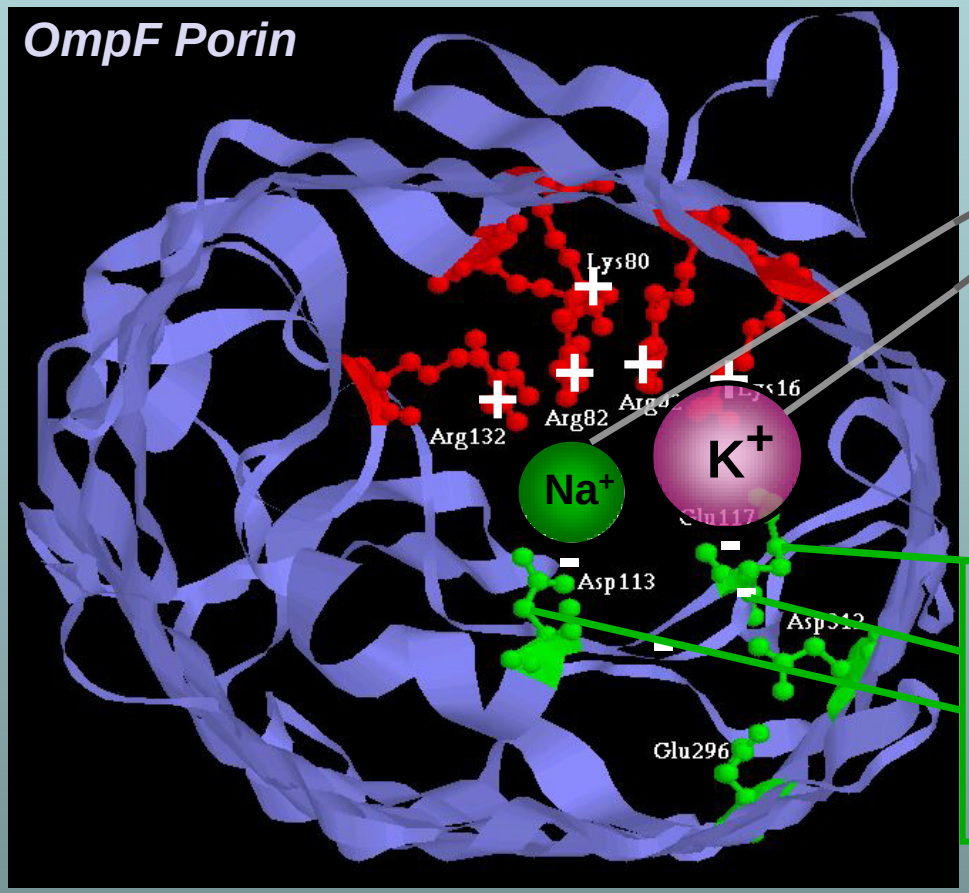
Physical basis of function

Active Sites of Proteins are ***Very*** **Charged**

7 charges ~ **20 M net charge** = 1.2×10^{22} cm^{-3}

liquid **Water** is **55 M**
solid **NaCl** is **37 M**



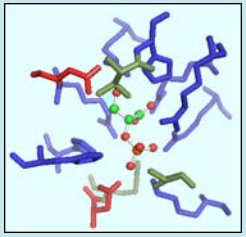


Ions are Crowded

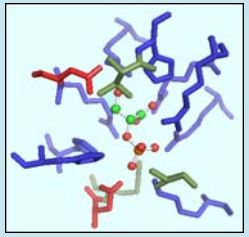
Induced Fit of Side Chains

Selectivity Filters and Gates of Ion Channels are **Active Sites**

Figure adapted from Tilman Schirmer



Charge Density 22 M



		#AA	MS_A^3	CD_MS+	CD_MS-	CD_MSt
EC1:Oxidoreductases	Average	47.2	1,664.74	7.58	2.82	10.41
	Median	45.0	1,445.26	6.12	2.49	8.70
EC2:Transferases	Average	33.8	990.42	13.20	6.63	19.83
	Median	32.0	842.43	8.18	6.71	14.91
EC3:Hydrolases	Average	24.3	682.88	13.14	13.48	26.62
	Median	20.0	404.48	11.59	12.78	23.64
EC4:Lyases	Average	38.2	1,301.89	13.16	6.60	19.76
	Median	28.0	822.73	10.81	4.88	16.56
EC5:Isomerases	Average	31.6	1,027.15	24.03	11.30	35.33
	Median	34.0	989.98	9.05	7.76	16.82
EC6:Ligases	Average	44.4	1,310.03	9.25	9.93	19.18
	Median	49.0	1,637.98	8.32	7.95	17.89

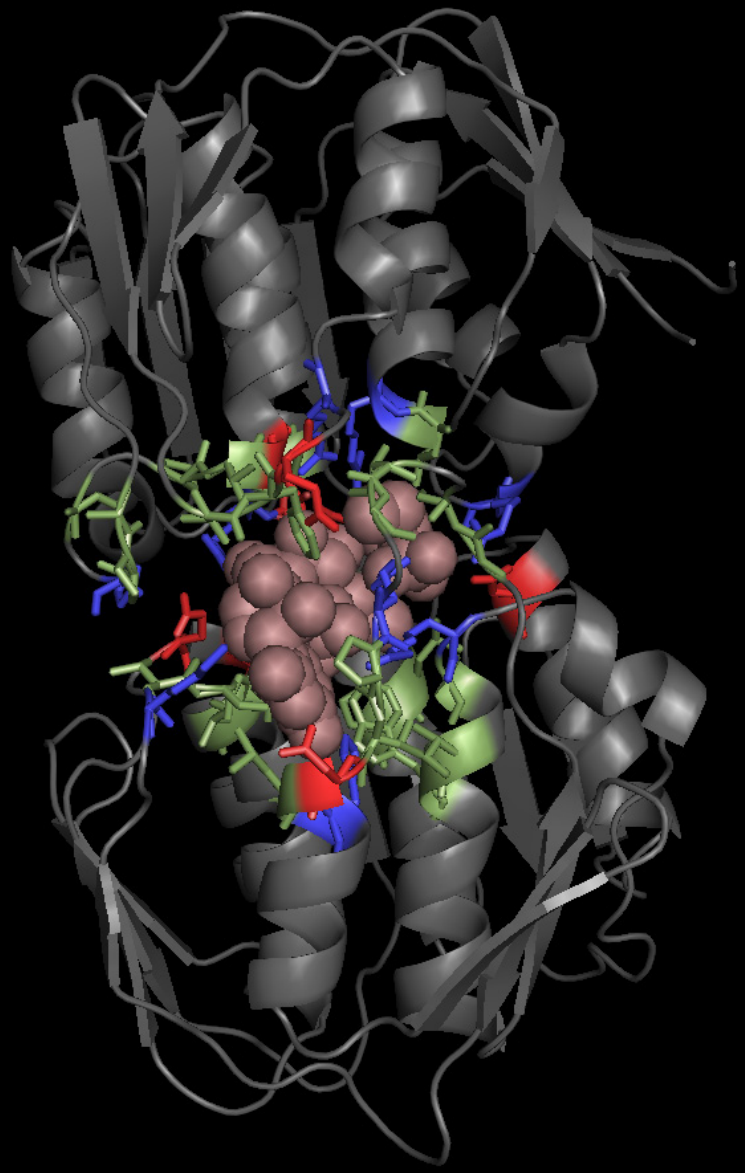
		#AA	MS_A^3	CD_MS+	CD_MS-	CD_MSt
Total *n= 150*	Average	36.6	1,162.85	13.39	8.46	21.86
	Median	33.0	916.21	8.69	7.23	16.69

EC#: Enzyme Commission Number based on chemical reaction catalyzed
#AA: Number of residues in the functional pocket
MS_A^3: Molecular Surface Area of the Functional Pocket **(Units Angstrom^3)**
CD_MS+: Charge Density **(positive)**
CD_MS-: Charge Density **(negative)**
CD_MSt: Total Charge density

Jimenez-Morales, Liang, Eisenberg

EC2: TRANSFERASES

Average Charged Density: 19.8 Molar



Example:
UDP-N-ACETYLGLUCOSAMINE ENOLPYRUVYL TRANSFERASE (PDB:1UAE)

Functional Pocket Molecular Surface Volume: 1462.40 A^3
Density Charge: 19.3 Molar (11.3 M+. 8 M-)

Green: Functional pocket residues

Blue: Basic = Positive charged = R+K+H

Red: Acid = Negative charged = E + Q

Brown URIDINE-DIPHOSPHATE-N-ACETYLGLUCOSAMINE

Jimenez-Morales, Liang, Eisenberg

Working Hypothesis

Biological Adaptation is

Crowded Ions *and* Side Chains

Everything interacts

Working Hypothesis

Interactions in Channels

come mostly from

Finite Size Effects

Chemically Specific Properties

come from

Diameter and Charge

learned from Doug Henderson, J.-P. Hansen, Stuart Rice, among others…
Thanks!

Bulk Solutions:

Interactions come mostly from

Finite Size Effects

Chemically Specific Properties

of ions (e.g. activity = free energy per mole)
are known to come from interactions of their

Diameter and Charge

and dielectric 'constant' of ionic solution

Atomic Detail

'Primitive **Implicit Solvent Model'**
learned from Doug Henderson, J.-P. Hansen, Stuart Rice, among others…
Thanks!

Ions in Water are the Liquid of Life

They are not ideal solutions

Everything Interacts with Everything

For Modelers and Mathematicians

Tremendous Opportunity for Applied Mathematics

Chun Liu's Energetic Variational Principle

EnVarA

Variational Principles Deal with Interactions Consistently and Automatically

Chun Liu,
with Yunkyong Hyon, and Bob Eisenberg

EnVarA

$$\overbrace{\frac{\delta E}{\delta \vec{x}}}^{\textit{Conservative 'Force'}} - \overbrace{\frac{1}{2}\frac{\delta \Delta}{\delta \vec{u}}}^{\textit{Dissipative 'Force'}} = 0$$

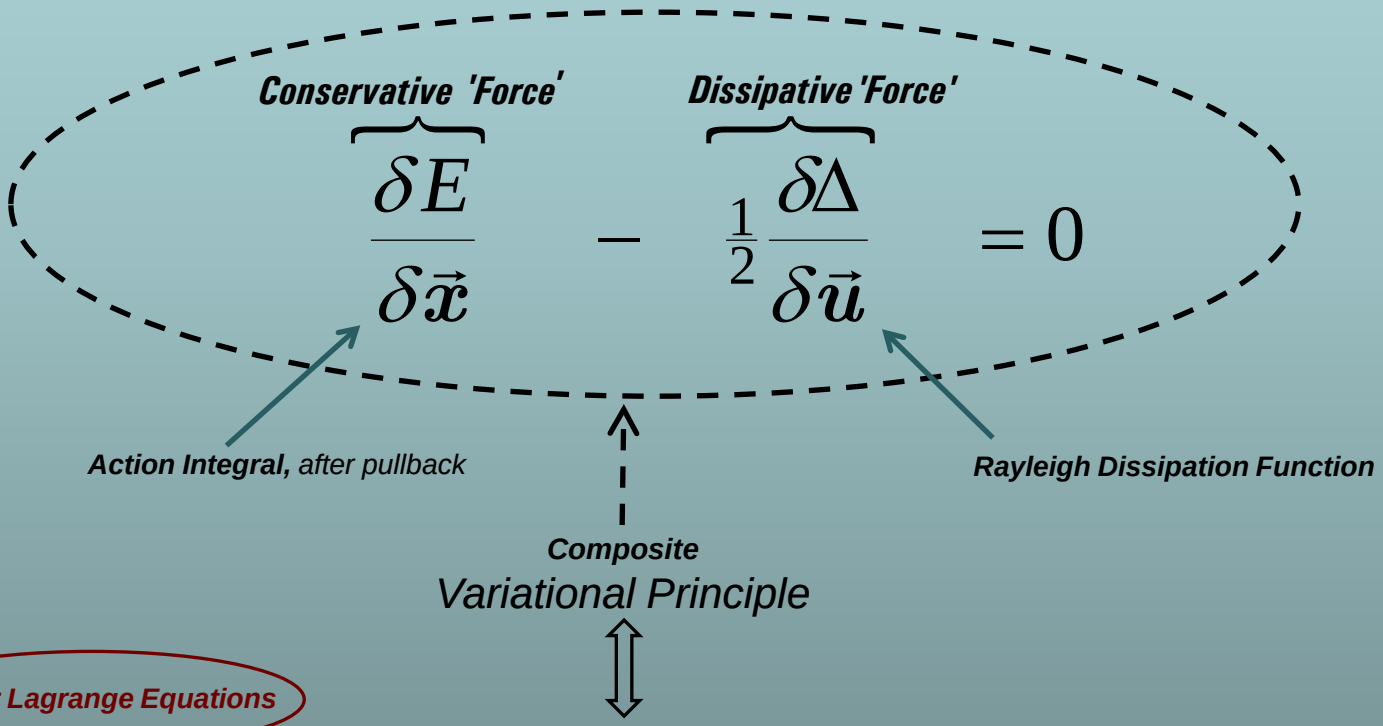
New Component (or Scale)
implies
New Field Equations (Euler Lagrange)
by
Algebra Alone
No new Assumptions

Energetic Variational Approach

EnVarA

Chun Liu, Rolf Ryham, Yunkyong Hyon, and Bob Eisenberg

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



that allows boundary conditions and flow and deals with Interactions of Components self-consistently

Variational Analysis of Ionic Solution

EnVarA

Generalization of Chemical Free Energy

$$E(\text{Primitive Phase};t)=\int\Bigg[\underbrace{\underbrace{\tfrac{1}{2}\rho\left|\vec{u}_{IP}\right|^2}_{\text{Hydrodynamic Kinetic Energy}}+\underbrace{w(\rho)}_{\text{Hydrodynamc Potential Energy Equation of State}}}_{\textbf{Macroscopic (hydrodynamic)}}+\lambda\Big[\underbrace{\tfrac{1}{2}\varepsilon\left|\nabla\phi\right|^2}_{\text{Electrostatic}}+\underbrace{k_BT(c_n\log c_n+c_p\log c_p)}_{\text{Entropy}}+\underbrace{E(\textit{Solid Spheres})}_{\text{Finite Size Effect}}\Big]\Bigg]d\vec{x}$$

Dielectric Coefficient

from Poisson Eq.

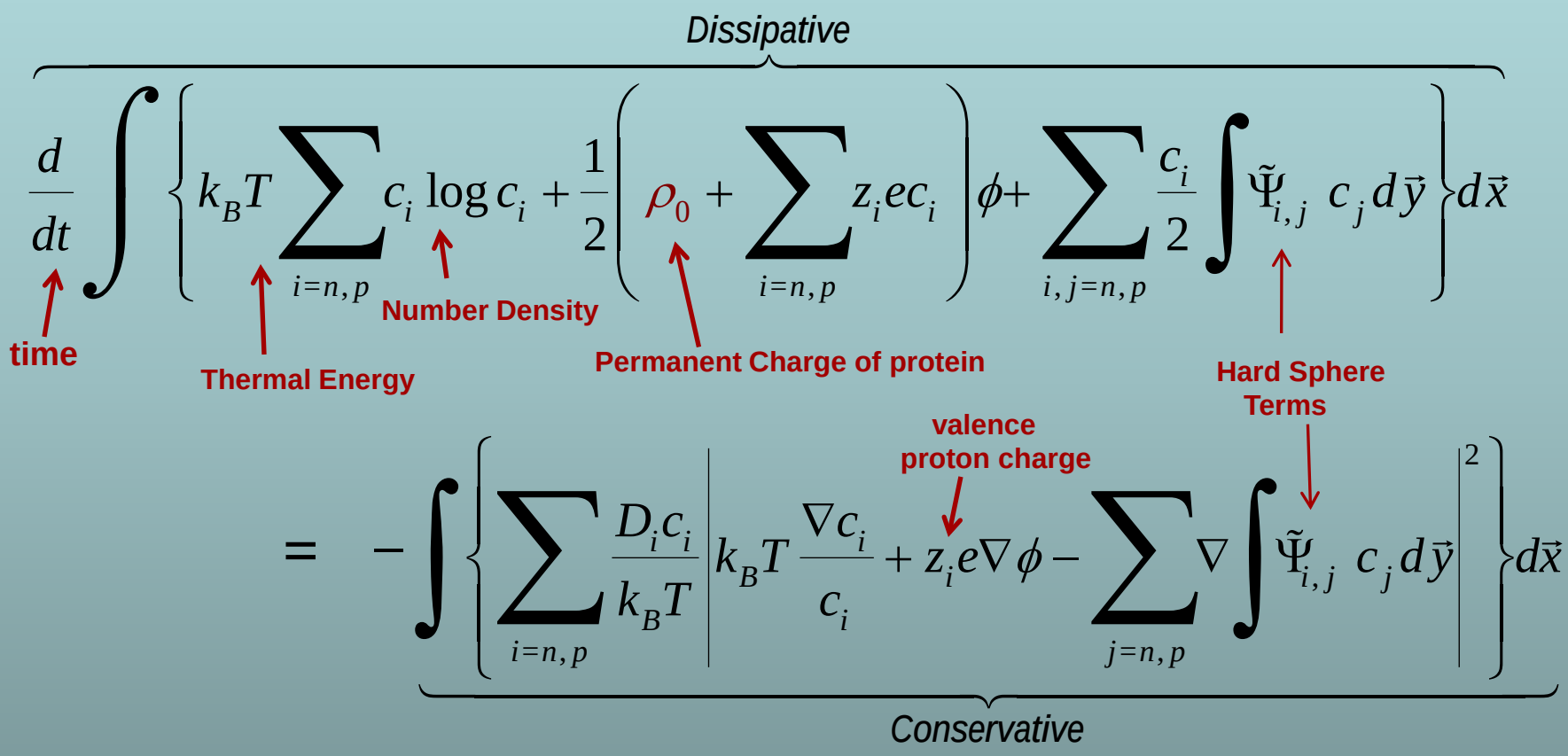
Number Densities

Lennard Jones

Lagrange Multiplier

Microscopic (atomic)

Dissipation Principle for Ions



$$\frac{d}{dt}\int\left\{k_BT\sum_{i=n,p}c_i\log c_i+\frac{1}{2}\left(\rho_0+\sum_{i=n,p}z_iec_i\right)\phi+\sum_{i,j=n,p}\frac{c_i}{2}\int\tilde{\Psi}_{i,j}\,c_j\,d\vec{y}\right\}d\vec{x}$$

$$=-\int\left\{\sum_{i=n,p}\frac{D_ic_i}{k_BT}\left|k_BT\frac{\nabla c_i}{c_i}+z_ie\nabla\phi-\sum_{j=n,p}\nabla\int\tilde{\Psi}_{i,j}\,c_j\,d\vec{y}\right|^2\right\}d\vec{x}$$

c_i *number density;* k_BT *thermal energy;* D_i *diffusion coefficient;* n *negative;* p *positive;* z_i *valence*

Field Equations with Lennard Jones Spheres

Non-equilibriium 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

valence proton charge

Permanent Charge of Protein

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

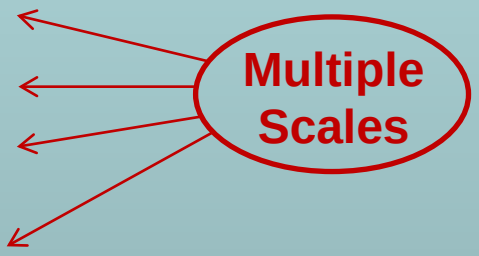
Energetic Variational Approach

EnVarA across biological scales: molecules, cells, tissues

developed by Chun Liu

with

(1) Hyon, Eisenberg	**Ions in**	**Channels**
(2) Bezanilla, Hyon, Eisenberg	**Conformation Change of**	**Voltage Sensor**
(3) Ryham, Eisenberg, Cohen	**Virus fusion to**	**Cells**
(4) Mori, Eisenberg	**Water flow in**	**Tissues**



creates a new

Multiscale Field Theory of Interacting Components

that allows boundary conditions and flow

and deals with

Ions in solutions self-consistently

Energetic Variational Approach

developed by Chun Liu

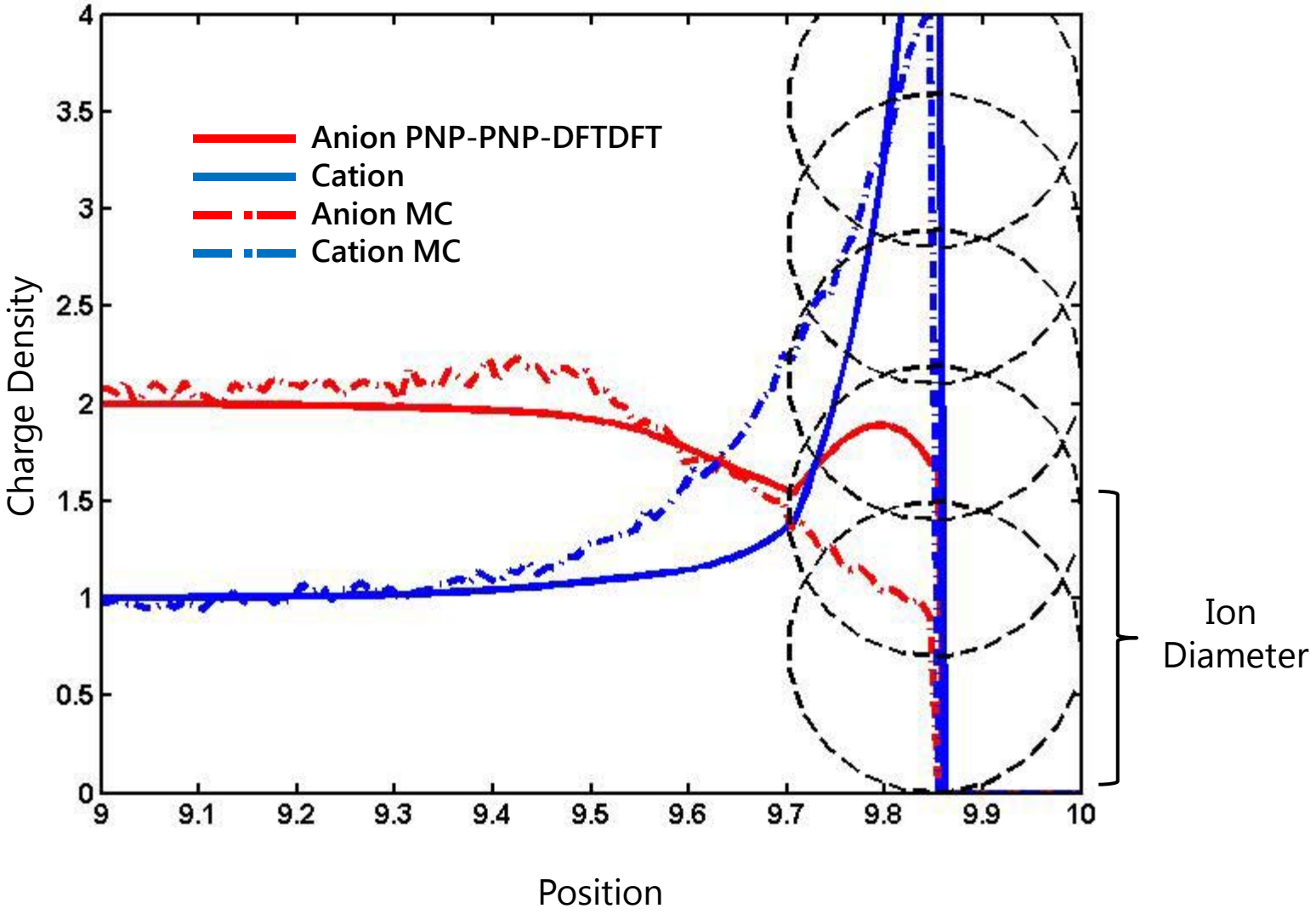
Preliminary Results

and

Provocations

Layering: Classical Interaction Effect

Comparison between PNP-DFT and MC



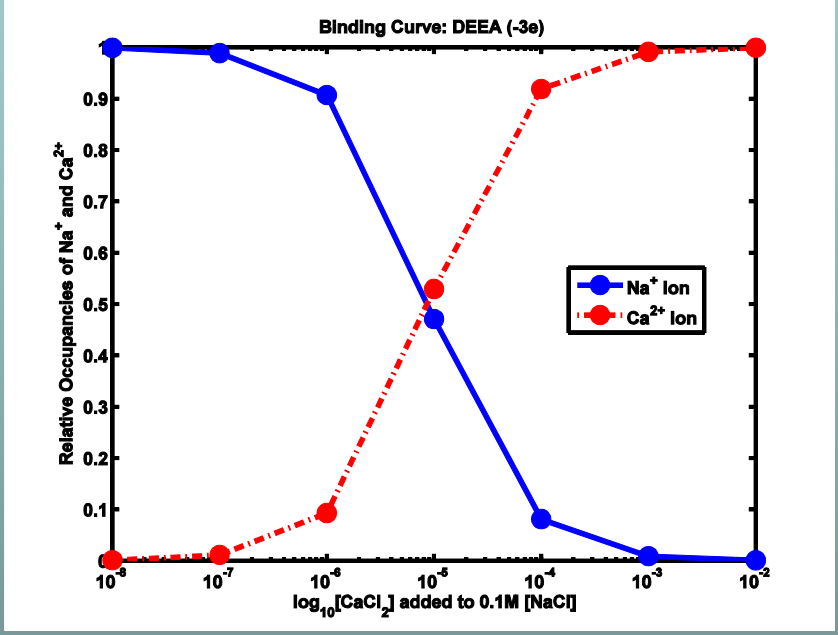
Eisenberg, Hyon, and Liu

Nonequilibrium Computations

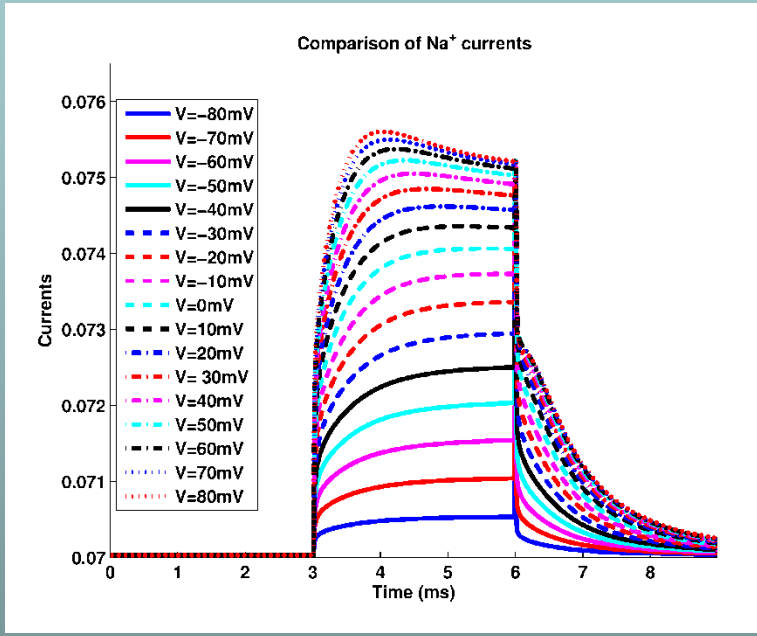
with Variational Field Theory

EnVarA

Binding Curves



Current Voltage **Time** Curves



Energetic Variational Approach

EnVarA

New mechanisms*
(e.g., active transport)
can be added

***if they define an energy and its variation**

Energy defined by simulations or theories or experiments is OK

Full micro/macro treatment is needed for an Atomic Model, with closure, as in liquid crystals

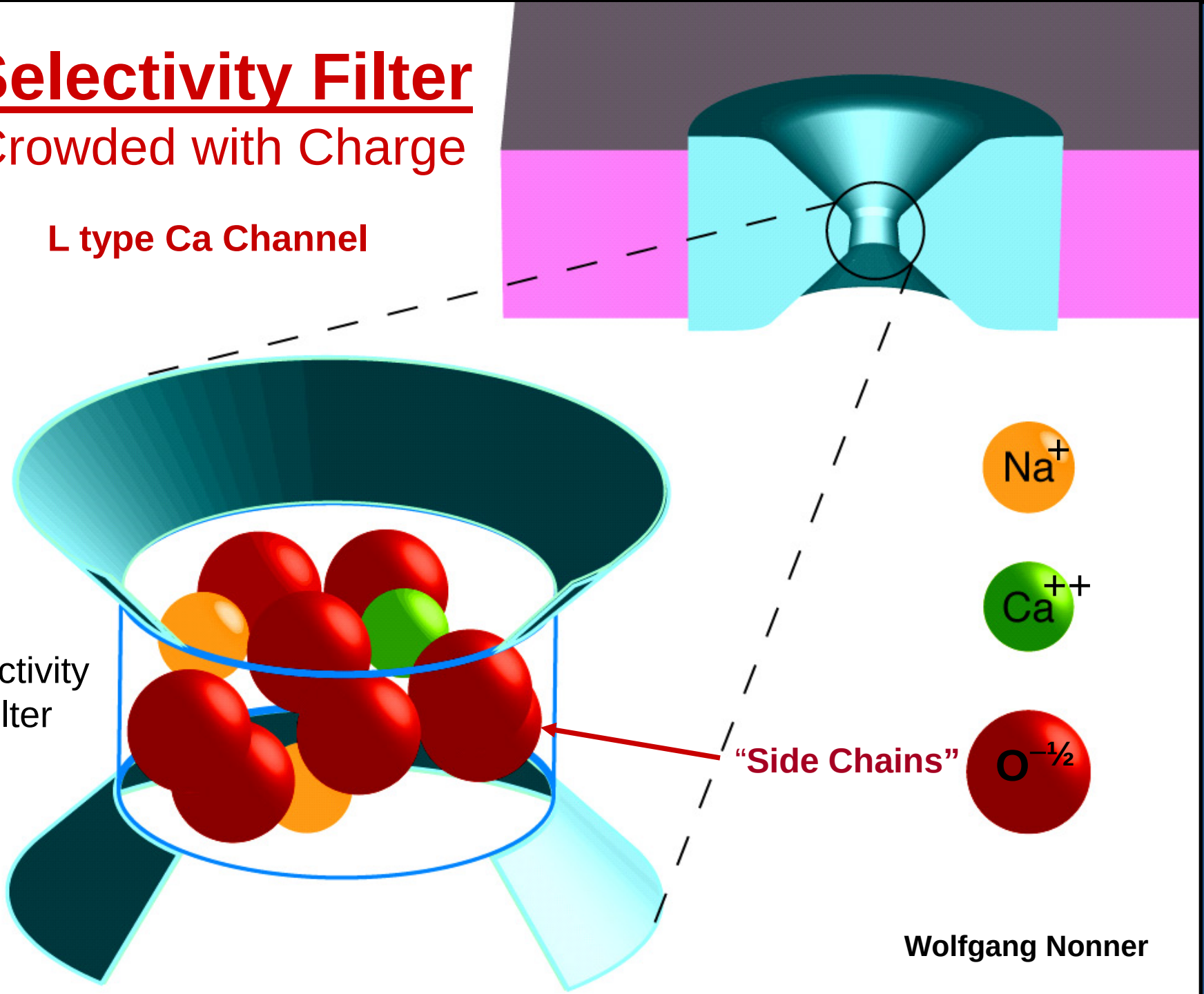
Back to the Calcium Channel

Then, the Sodium Channel

Selectivity Filter

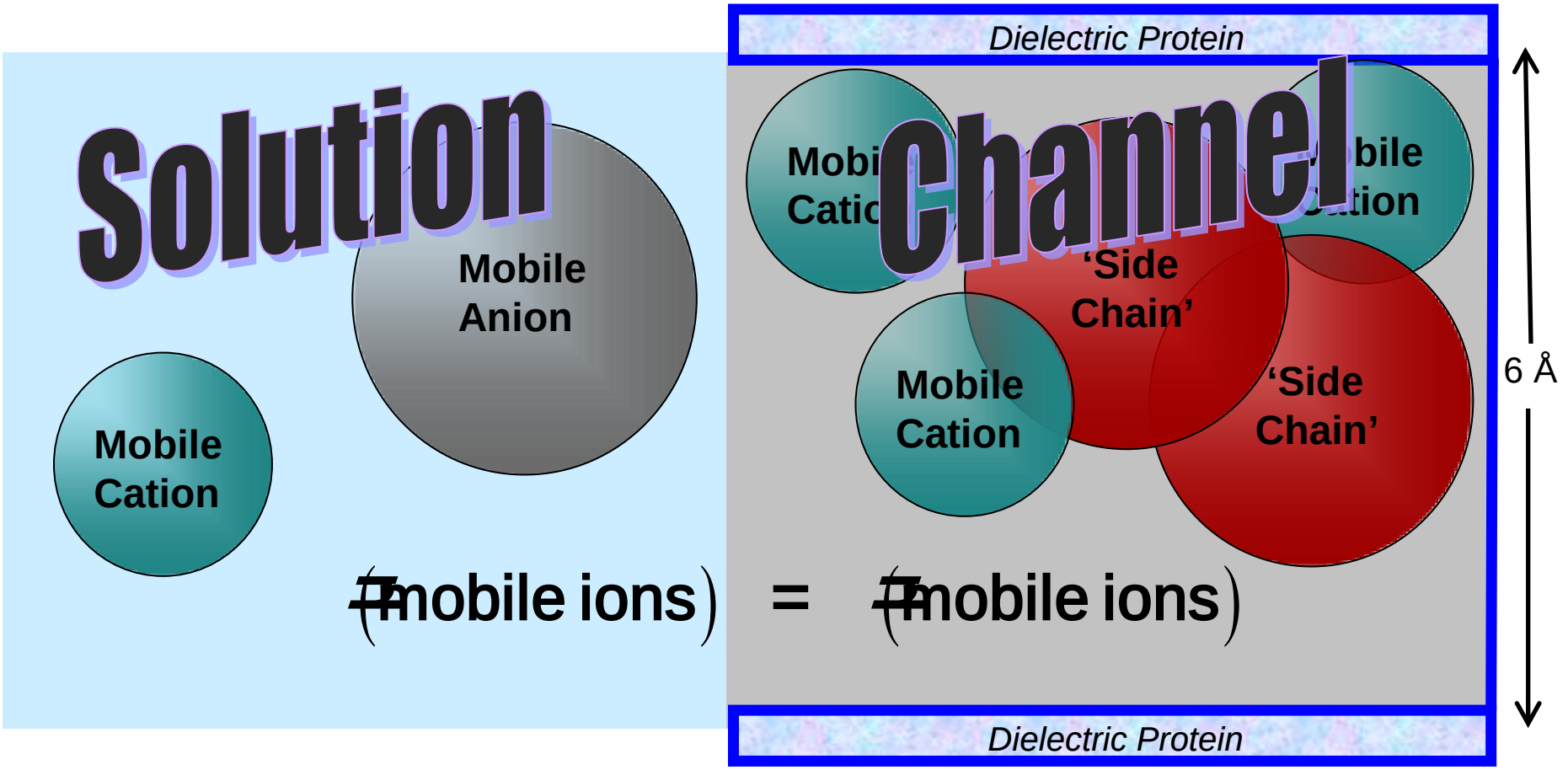
Crowded with Charge

L type Ca Channel



Wolfgang Nonner

Ion 'Binding' in Crowded Channel



Classical Donnan Equilibrium of Ion Exchanger

large mechanical forces

Side chains move within channel to their equilibrium position of minimal free energy.

We compute the Tertiary Structure as the structure of minimal free energy.

Boda, Nonner, Valisko, Henderson, Eisenberg & Gillespie

Multiscale Analysis at Equilibrium

Solved with Metropolis Monte Carlo

MMC Simulates Location of Ions

both the mean and the variance

Produces Equilibrium Distribution

of location

of Ions and 'Side Chains'

MMC yields Boltzmann Distribution with correct Energy, Entropy and Free Energy

Other methods

give nearly identical results:

Equilibrium Multiscale

MSA (mean spherical approximation

SPM (primitive solvent model)

DFT (density functional theory of fluids),

Non-equilibrium Multiscale

DFT-PNP (Poisson Nernst Planck)

EnVarA…. (Energy Variational Approach)

etc

Metropolis Monte Carlo Simulates Location of Ions

both the mean and the variance

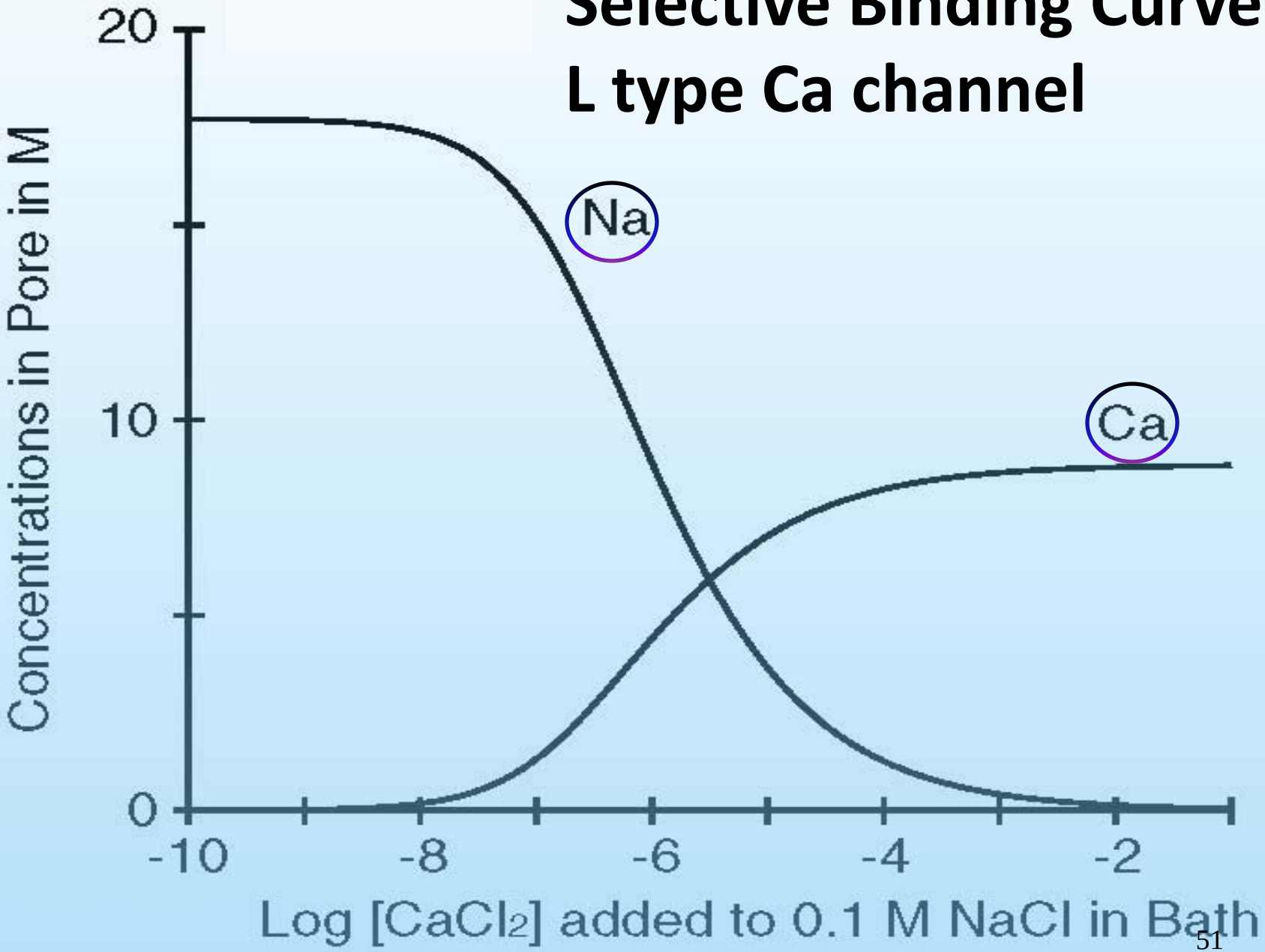
Details:

1) Start with Configuration *A*, with computed energy E_A
2) Move an ion to location *B*, with computed energy E_B
3) If spheres overlap, $E_B \rightarrow \infty$ and configuration is rejected
4) If spheres do not overlap, $E_B \rightarrow 0$ and configuration is accepted
5) If $E_B < E_A$: accept new configuration.
6) If $E_B > E_A$: accept new configuration with probability $\exp\left[-\left(E_A - E_B\right)/k_B T\right]$

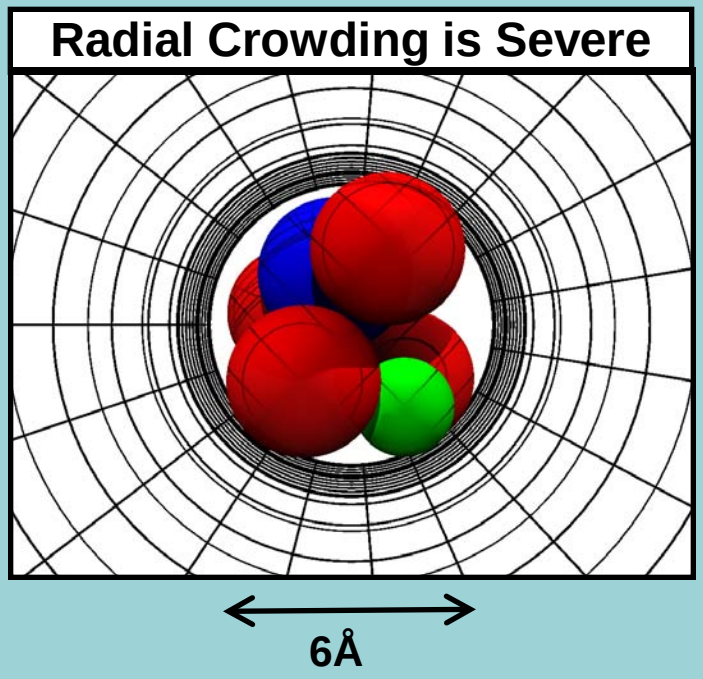
Key idea

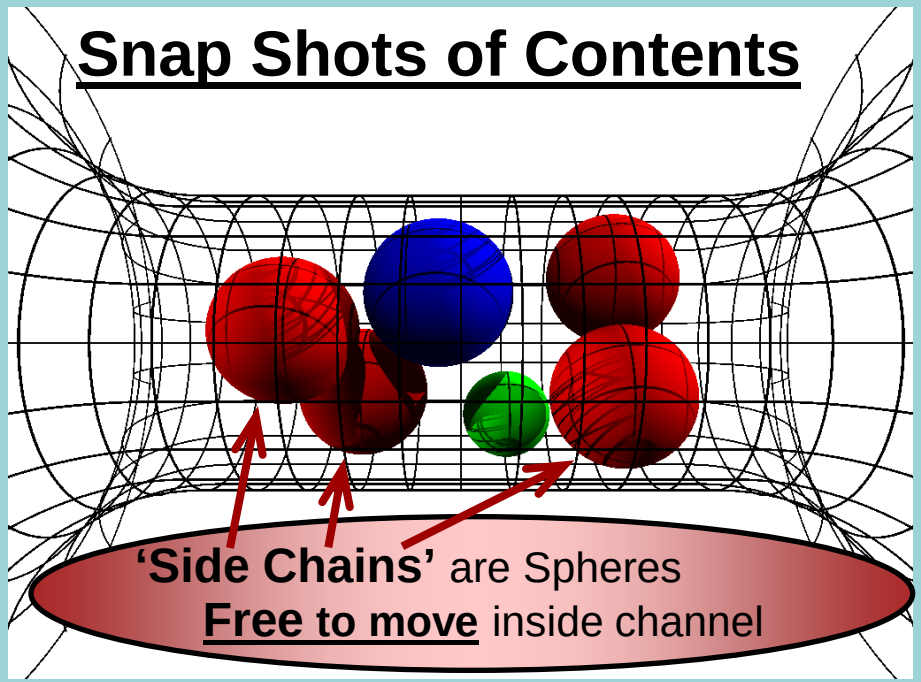
MMC chooses configurations with a Boltzmann probability and weights them evenly instead of
choosing them from uniform distribution and then weighting them with $\exp(-E/k_B T)$

Selective Binding Curve
L type Ca channel



Crowded Ions





Ion Diameters *'Pauling' Diameters*	
Ca^{++}	1.98 Å
Na^{+}	2.00 Å
K^{+}	2.66 Å
'Side Chain' Diameter	
Lysine K	3.00 Å
D or E	2.80 Å
Channel Diameter 6 Å	

Parameters are Fixed in all calculations in all solutions for all mutants

Experiments and Calculations done at pH 8

Boda, Nonner, Valisko, Henderson, Eisenberg & Gillespie

Ca Channel

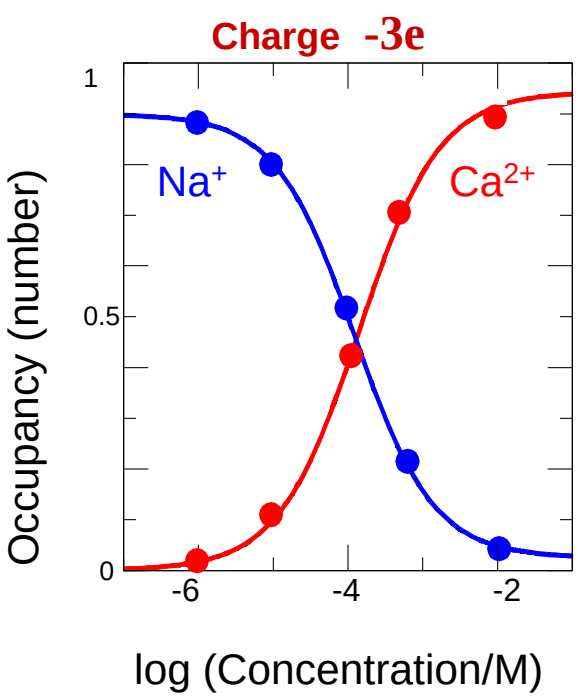
Mutation

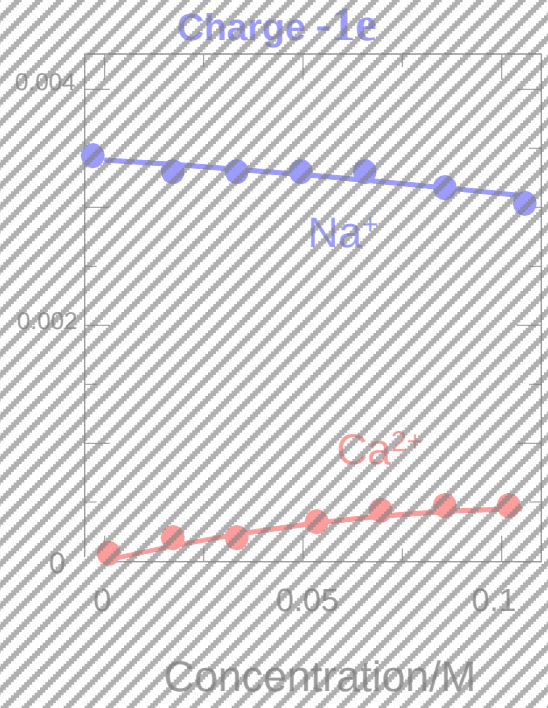
Na Channel

Same Parameters

E
E
E
A

D
E
K
A

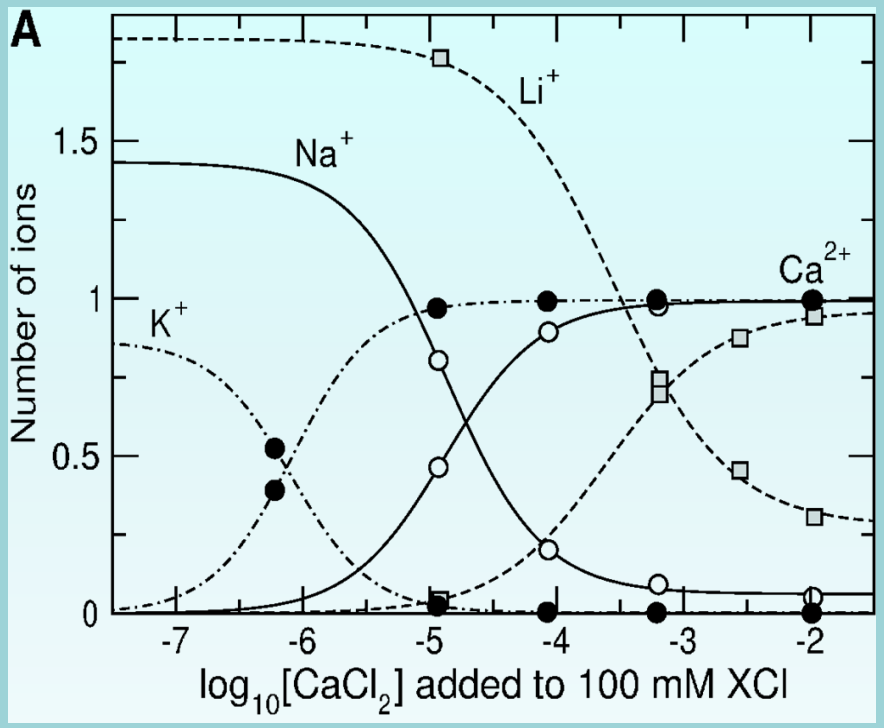


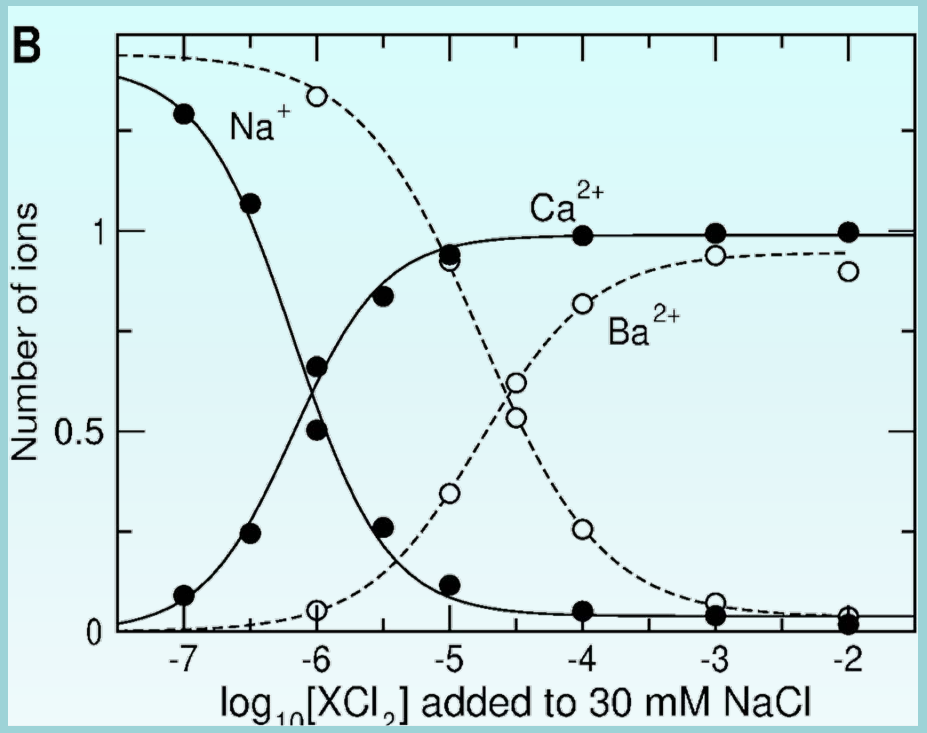


EEEE has full biological selectivity in similar simulations

Boda, et al

Na, K, Li, Ca, Ba Binding in Calcium Channel





Calcium Channel

has been examined in ~35 papers, e.g.,

Nonner, W., D. P. Chen, and B. Eisenberg. 1998. Anomalous Mole Fraction Effect, Electrostatics, and Binding in Ionic Channels. Biophysical Journal 74:2327-2334.

Nonner, W., L. Catacuzzeno, and B. Eisenberg. 2000. Binding and Selectivity in L-type Ca Channels: a Mean Spherical Approximation. Biophysical Journal 79:1976-1992.

Nonner, W., D. Gillespie, D. Henderson, and B. Eisenberg. 2001. Ion accumulation in a biological calcium channel: effects of solvent and confining pressure. J Physical Chemistry B 105:6427-6436.

Boda, D., W. Nonner, D. Henderson, B. Eisenberg, and D. Gillespie. 2008. Volume exclusion in calcium selective channels. Biophys. J.:biophysj.107.122796.

Boda, D., M. Valisko, B. Eisenberg, W. Nonner, D. Henderson, and D. Gillespie. 2006. Effect of Protein Dielectric Coefficient on the Ionic Selectivity of a Calcium Channel. Journal of Chemical Physics 125:034901.

Boda, D., T. Varga, D. Henderson, D. Busath, W. Nonner, D. Gillespie, and B. Eisenberg. 2004. Monte Carlo simulation study of a system with a dielectric boundary: application to calcium channel selectivity. Molecular Simulation 30:89-96.

Boda, D., M. Valisko, B. Eisenberg, W. Nonner, D. Henderson, and D. Gillespie. 2007. The combined effect of pore radius and protein dielectric coefficient on the selectivity of a calcium channel. Physical Review Letters 98:168102.

Most of the papers are available at

ftp://ftp.rush.edu/users/molebio/Bob_Eisenberg/Reprints

http://www.phys.rush.edu/RSEisenberg/physioeis.html

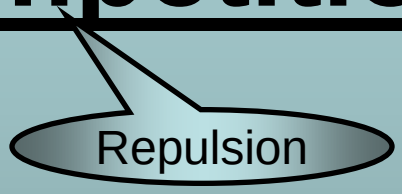
Selectivity

comes from

Electrostatic Interaction

and

Steric Competition for Space



Location and Strength of Binding Sites
Depend on Ionic Concentration and
Temperature, etc

Rate Constants are Variables

for

Biologists:
a Word Picture

How does Calcium Selectivity Work?
qualitatively

How does it work qualitatively?

(for biologists)

2 Ca^{++}

are

LESS CROWDED

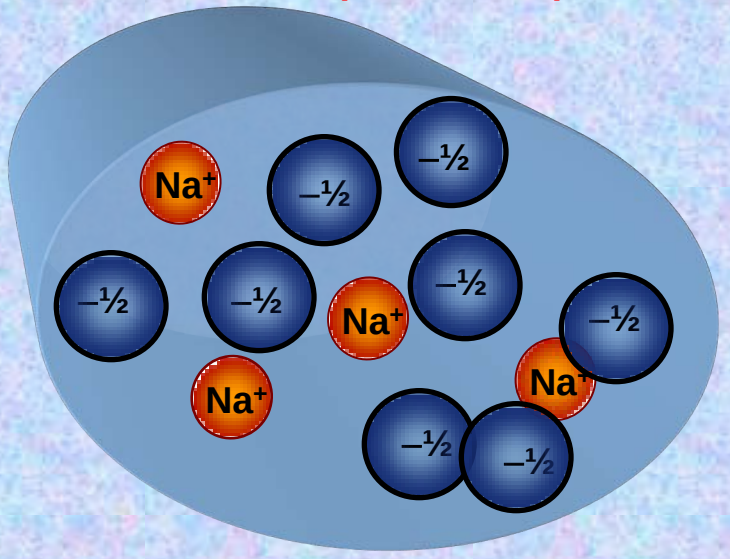
than 4 Na^{+}

Selectivity from Crowded Charges

2 Ca^{++} are less crowded than 4 Na^{+}

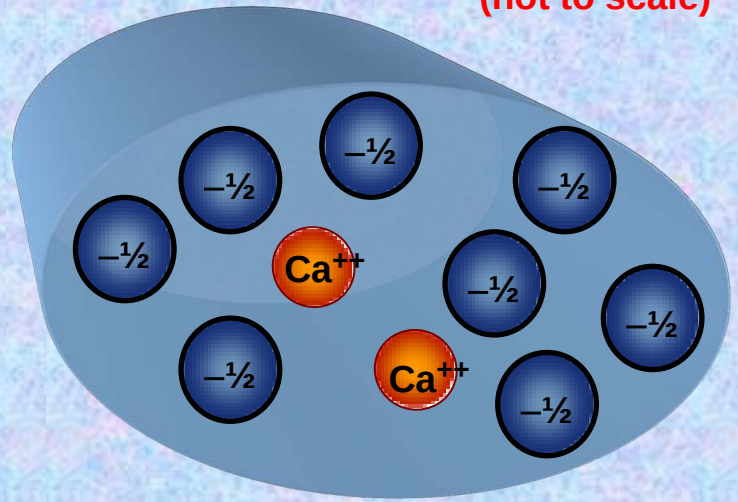
Ca Channel Filled with Na^{+}

(not to scale)



Ca Channel Filled with Ca^{++}

(not to scale)



Channel Protein

Glutamate Oxygens = 4e

8 of –½ charge each

Volume 0.38 nm^3

Dielectric Constant 64

Outside the Filter

Bulk Solution

NaCl and $CaCl_2$

4 Negative Charges of glutamates of protein

DEMAND 4 Positive Charges nearby

either 4 Na^{+} or 2 Ca^{++}

Nonner and Eisenberg

Ionic Selectivity in Protein Channels
Crowded Charge Mechanism
Simplest Version: MSA

2 Ca^{++} are **LESS CROWDED** than 4 Na^{+},

Ca^{++} **SHIELDS BETTER** than Na^{+}, so

Protein Prefers Ca^{++}

because

Ca^{++} is less crowded

Nonner and Eisenberg

What does the protein do?

Channel and Contents
form a
Self-Organized Structure

with Side Chains at position of
Minimum Free Energy

Protein Fits the Substrate

“Induced Fit Model of Selectivity”

What does the protein do?

(for biologists)

Certain MEASURES of structure are

Powerful DETERMINANTS of Function

e.g., Volume, Dielectric Coefficient, etc.

Induced Fit Model of Selectivity

Atomic Structure is not pre-formed

Atomic Structure is an important output of the simulation

What does the protein do?

Protein maintains

Mechanical Forces*

Volume of Pore

Dielectric Coefficient/Boundary

Permanent Charge

* ***Driving force for conformation changes ??***

Binding Sites* are **outputs**
of our Calculations

Induced Fit Model of Selectivity

Our model has no preformed structural binding sites

but

Selectivity is very Specific

*Selectivity is in the Depletion Zone,
NOT IN THE BINDING SITE
of the DEKA Na Channel

Challenge

from leading biophysicists

Walter Stühmer and Stefan Heinemann

Max Planck Institutes, Göttingen, Leipzig

Can a physical theory explain the mutation Calcium Channel into Sodium Channel?

DEEA ⟺ ***DEKA***

Calcium Channel — ***Sodium Channel***

DEKA Sodium Channel

has very different properties from Ca channel,

e.g., ‘binding’ curve,

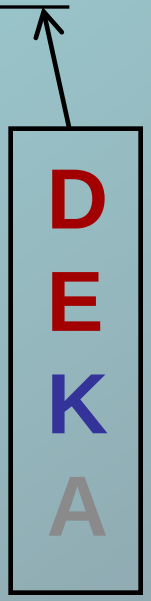
Na^+ vs Ca^{++} selectivity

Na^+ vs K^+ selectivity

Sodium Channel

specifically, the

DEKA Sodium Channel 6 Å



Aspartate	**D**	**Acid**	**Negative**
Glutamate	**E**	**Acid**	**Negative**
Lysine	**K**	**Basic**	**Positive**
Alanine	**A**	**Aliphatic**	**Neutral**

QUALITATIVELY DIFFERENT Properties from the Calcium Channel

Ca Channel

Mutation

Same Parameters

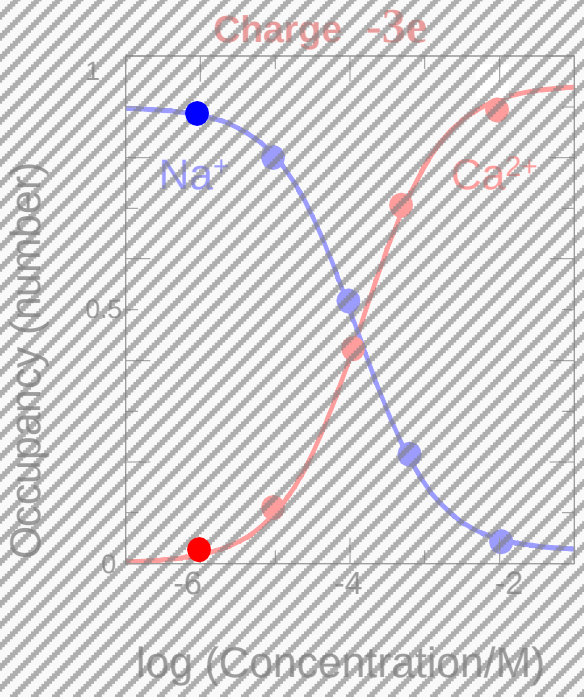
Na Channel

E → D

E → E

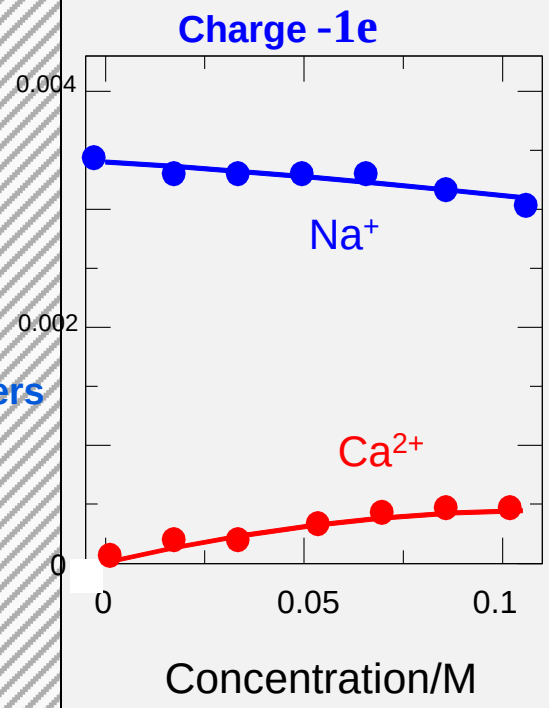
E → K

A → A



Mutation

Same Parameters



EEEE has full biological selectivity in similar simulations

Boda, et al

Nothing was changed

from the

EEEA Ca channel

except the amino acids

Calculated DEKA Na Channel
Selects
Ca^{2+} ***vs.*** **Na^{+}** *and also* **K^{+}** ***vs.*** **Na^{+}**

Calculations and experiments done at pH 8

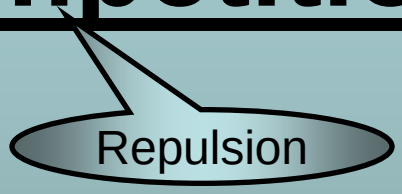
Selectivity

comes from

Electrostatic Interaction

and

Steric Competition for Space



Location and Strength of Binding Sites
Depend on Ionic Concentration and
Temperature, etc

Rate Constants are Variables

We can actually compute the Structures that determine Selectivity

New Miracle??

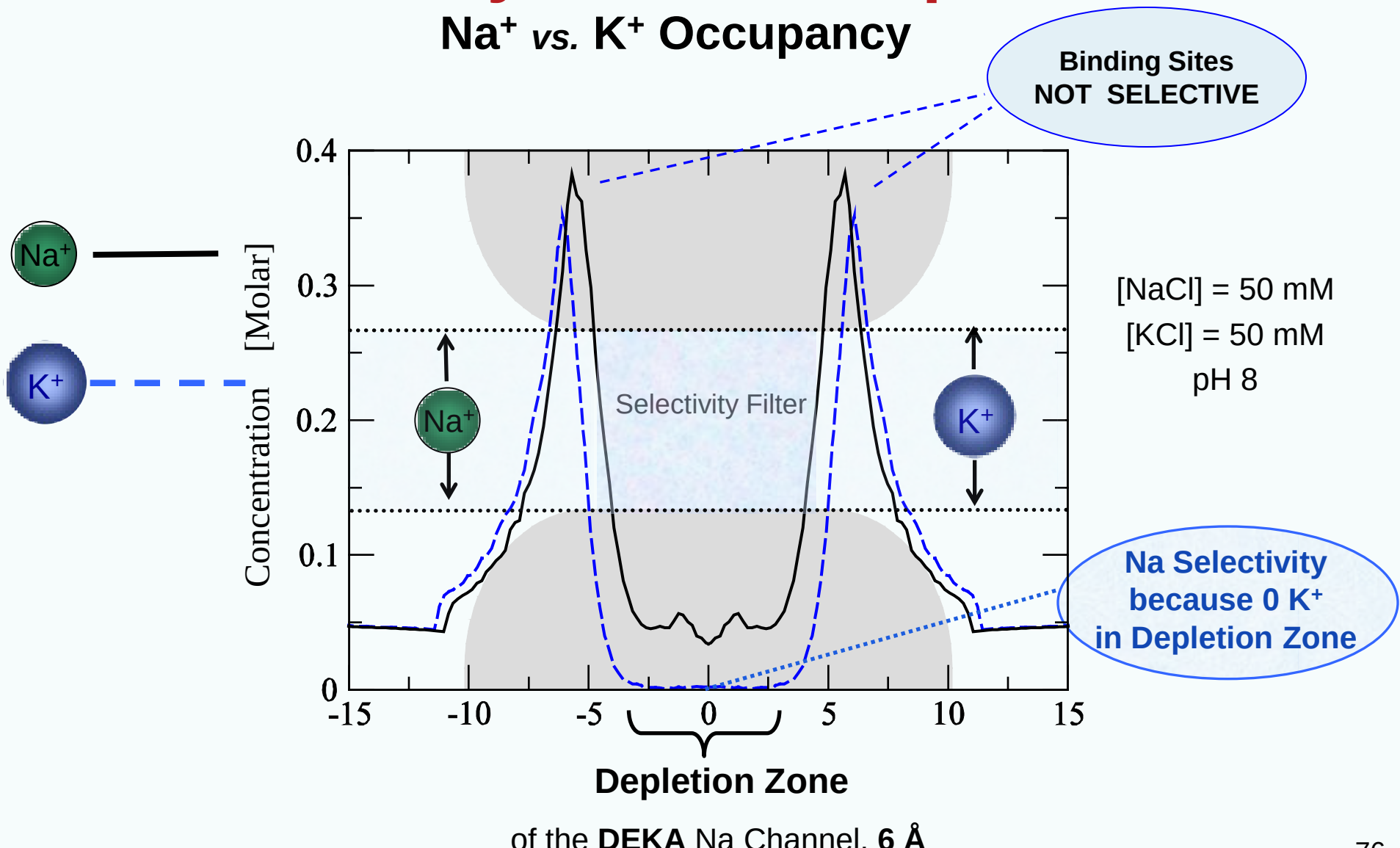
Can *EnVarA* actually compute the Function of these systems?

How?

How does the DEKA Na Channel Select Na^+ *vs.* K^+ ?

Calculations and experiments done at pH 8

Size Selectivity is in the Depletion Zone



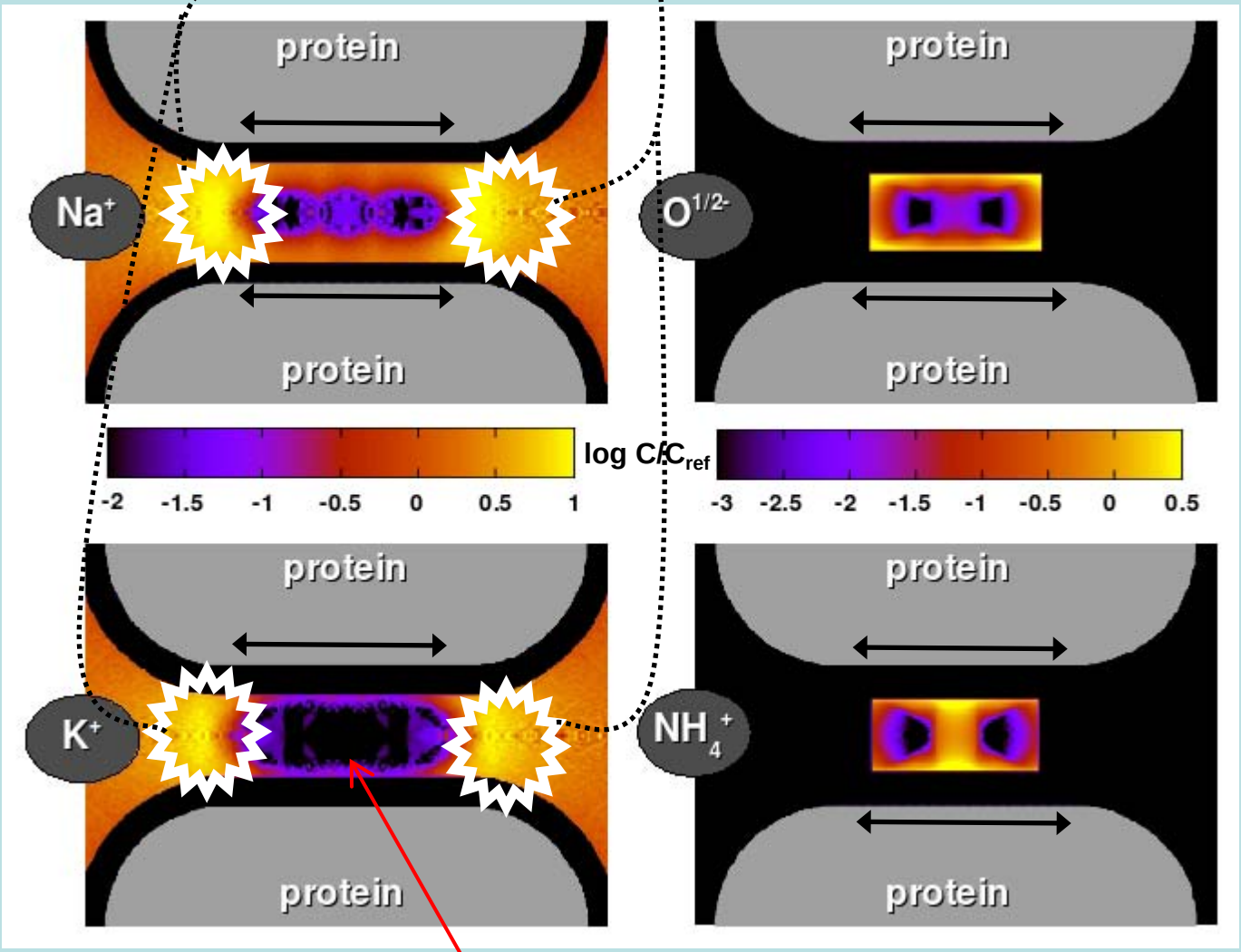
of the **DEKA** Na Channel, **6 Å**

Boda, et al

Size Selectivity

Binding Sites

NOT selective



Na vs K Size Selectivity is in

Depletion Zone

BLACK = Depletion=0

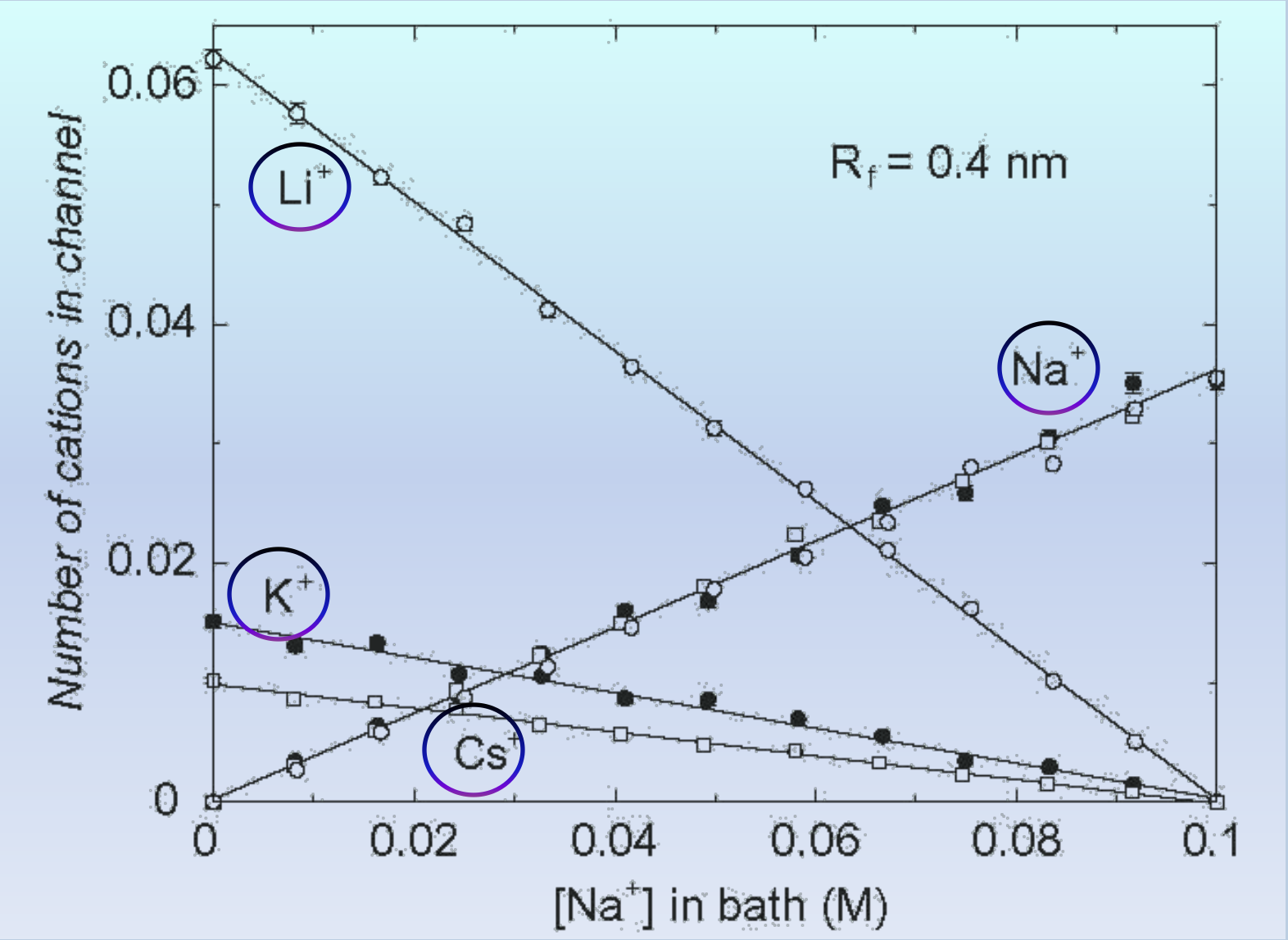
Boda, et al

*Binding Sites are outputs of our INDUCED FIT Model of Selectivity, ***not*** *structural inputs*

[NaCl] = [KCl] = 50 mM

Ion Diameter	
Ca^{++}	**1.98 Å**
Na^{+}	**2.00 Å**
K^{+}	**2.66 Å**
'Side Chain' Diameter	
NH_4^+ Lys or K	**3.00 Å** *pH 8*
$O^{1/2-}$ D or E	**2.80 Å** *pH 8*
Na Channel **DEKA** ***6 Å***	

Na, K, Li, Cs Binding in Sodium channel



What do the Variables do?

What happens

if we

Vary Diameter

and

Vary Dielectric Coefficient?

Inverse Problem

We discover

Orthogonal Control Variables*

in simulations of the Na channel,
but not the Ca channel.

**These emerge as outputs. They are not inputs.*

Control Variables

Selectivity *Na^+ vs K^+*

Selectivity Depends on Structure

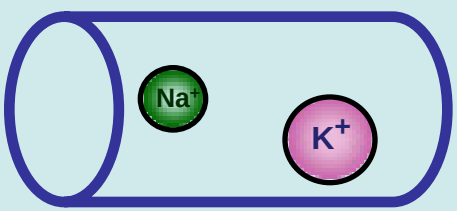
Depends STEEPLY on channel diameter

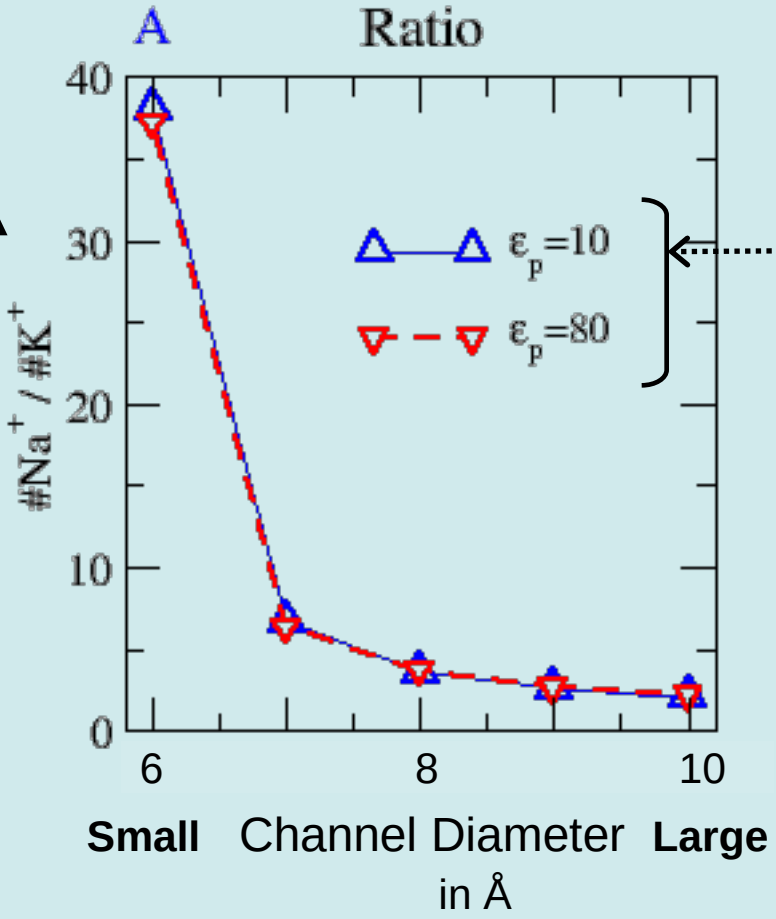
Depends only on channel diameter

Na^+ vs K^+ (size) **Selectivity** *(ratio)* **Depends on Channel Size,**

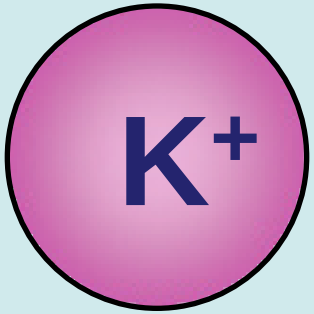
not Protein Dielectric Coefficient*

Selectivity
for small ion





2.00 Å



2.66 Å

Boda, et al

*****_in_ **DEKA** Na Channel

Control Variables

Conductance of DEKA Na^+ channel

Conductance Depends Steeply on Dielectric

Contents of Channel depend only on dielectric

but

Selectivity does not depend on Dielectric

Selectivity depends ***only*** on Structure

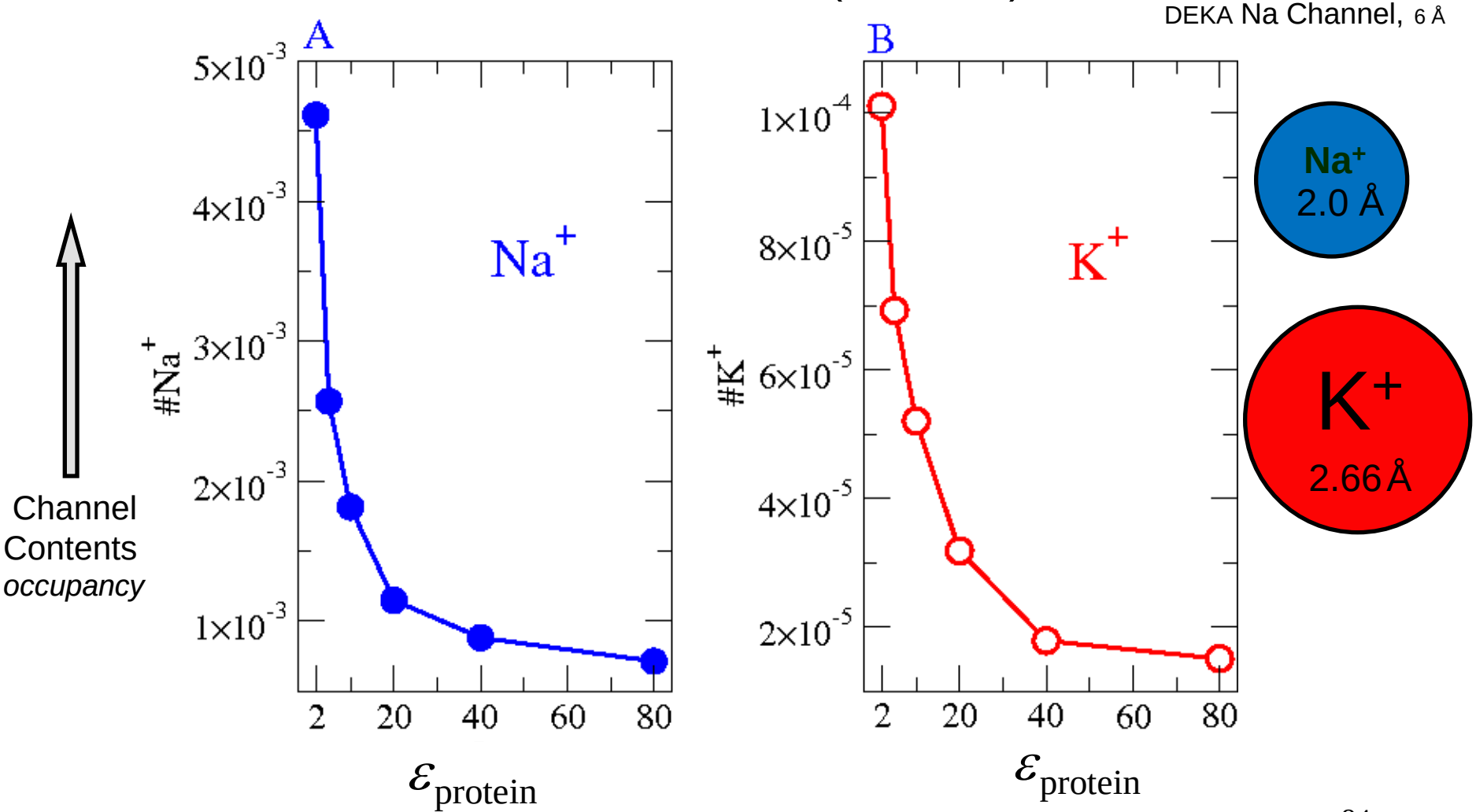
Control Variable

Channel Contents (occupancy)

depends on

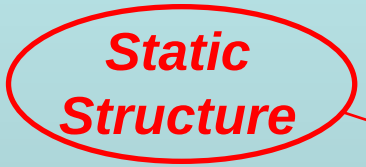
Protein Polarization (dielectric)

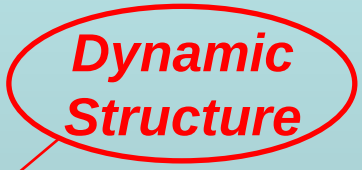
DEKA Na Channel, 6 Å



Boda, et al

$\varepsilon_{solvent} = 80$





Channel Diameter

and

Dielectric Coefficient

emerge as

Orthogonal Control Variables*

in simulations of the Na channel,
but not the Ca channel.

**These emerge as outputs. They are not inputs.*

Supplementary Material

RyR Channel:
Current Voltage Curves

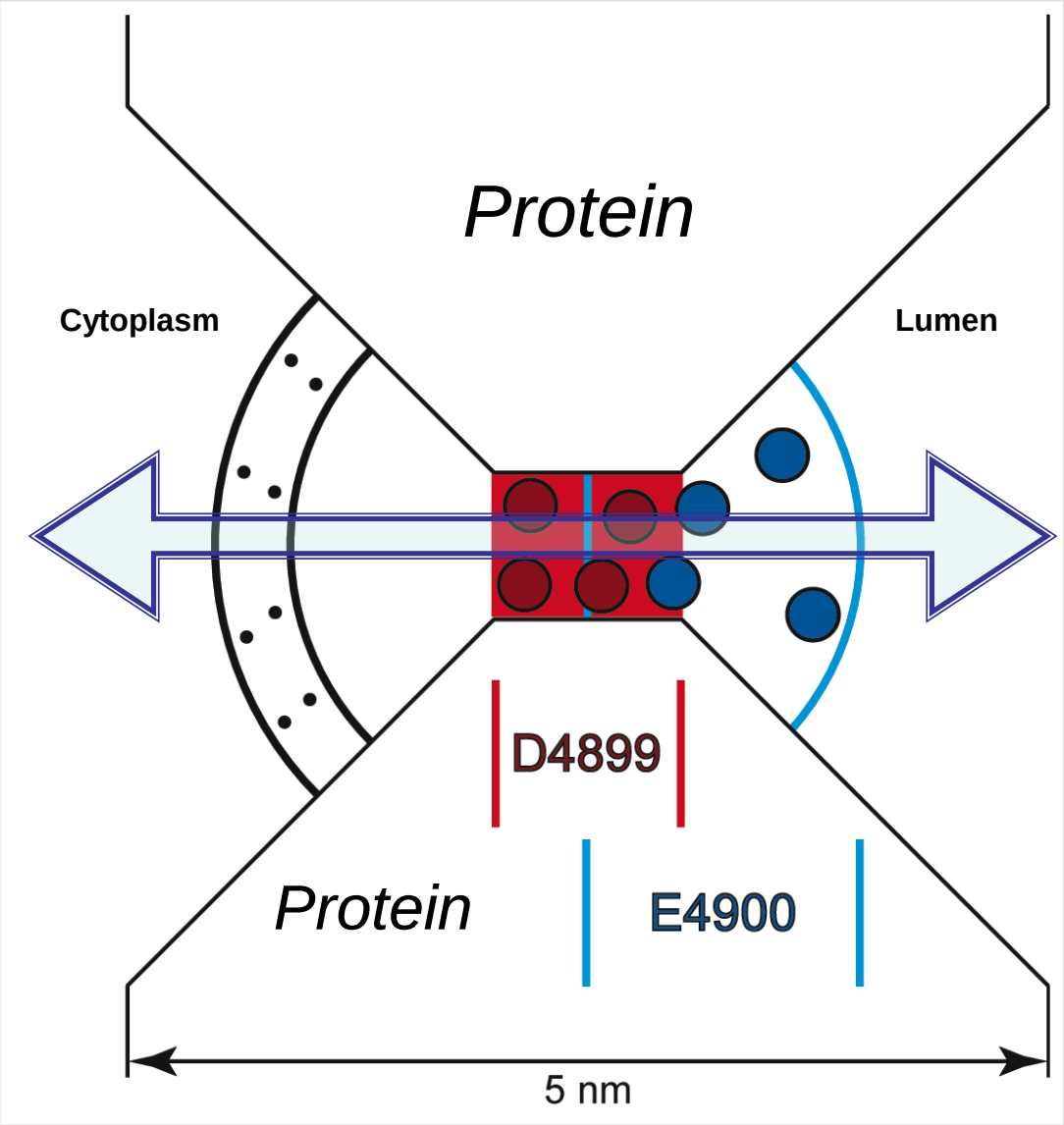
Best Evidence is from the

RyR Receptor

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

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

The Geometry



Selectivity Filter

- is 10 Å long and 8 Å in diameter
- confines four **D4899** negative amino acids.

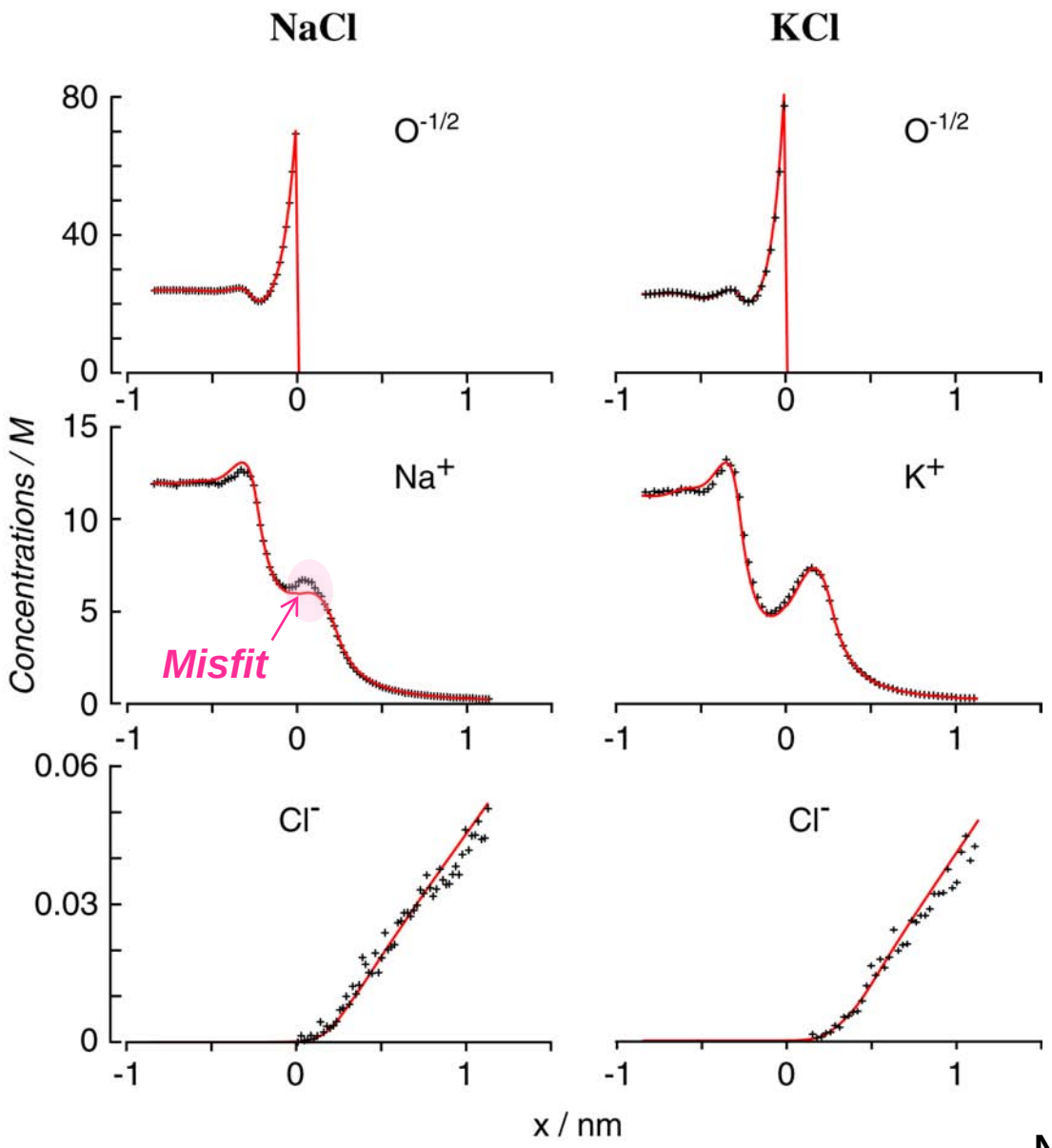
Four **E4900** positive amino acids are on lumenal side, overlapping D4899.

Cytosolic distributed charge

D. Gillespie *et al.*, *J. Phys. Chem.* 109, 15598 (2005).

DFT/PNP *vs* Monte Carlo Simulations

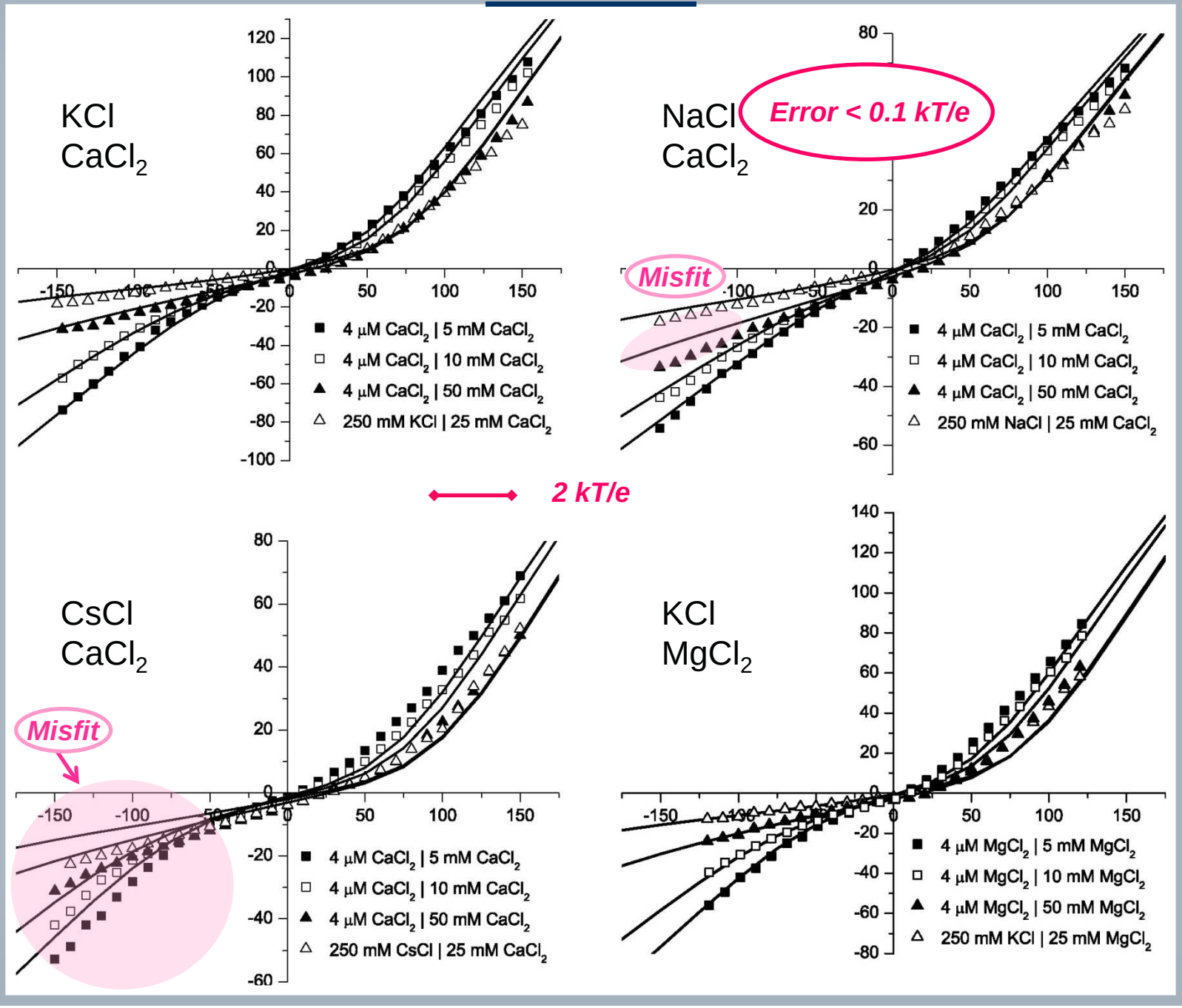
Concentration Profiles



Nonner, Gillespie, Eisenberg

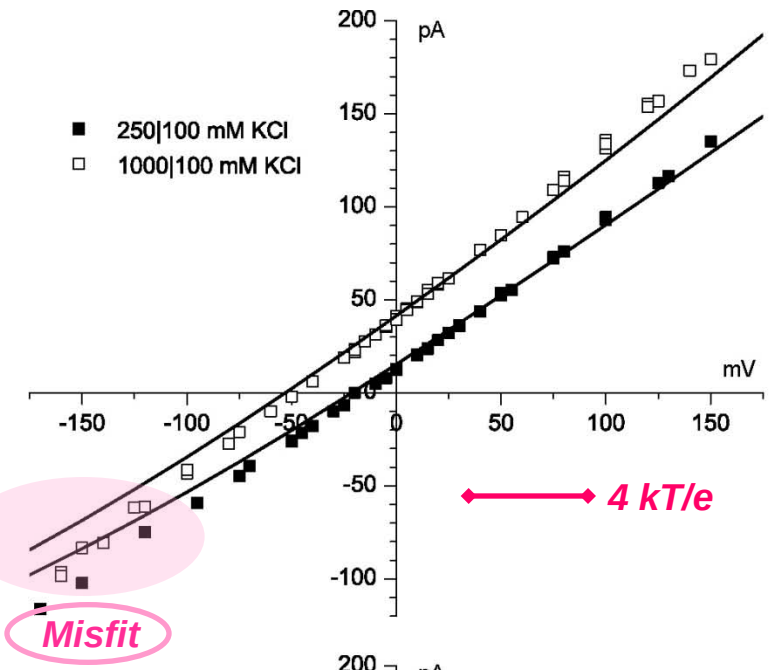
Divalents

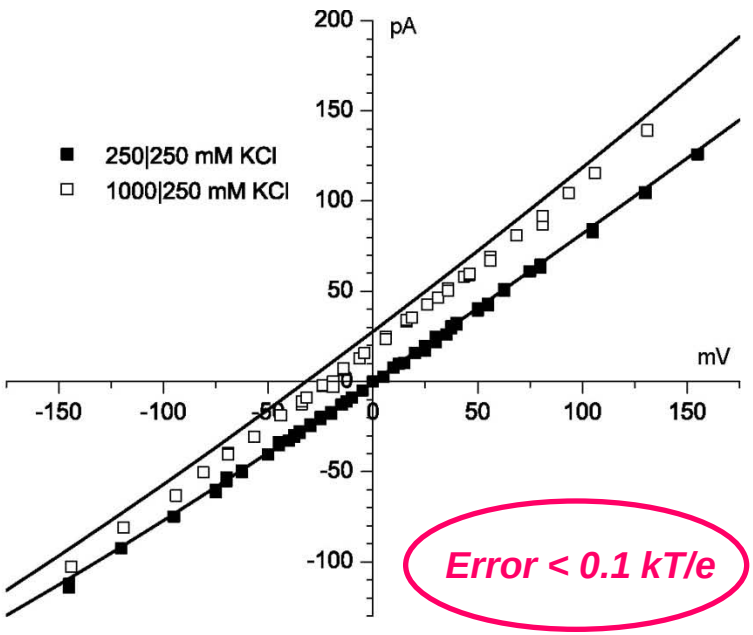
Gillespie, Meissner, Le Xu, et al

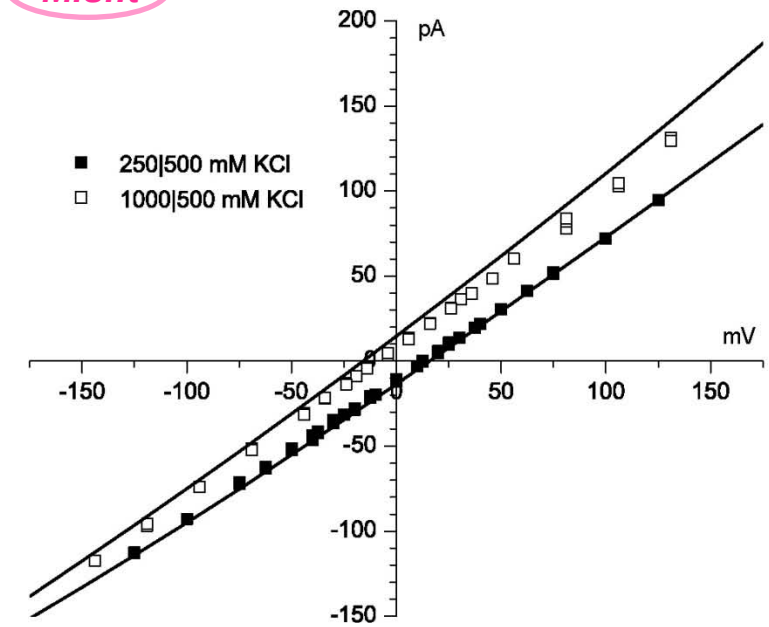


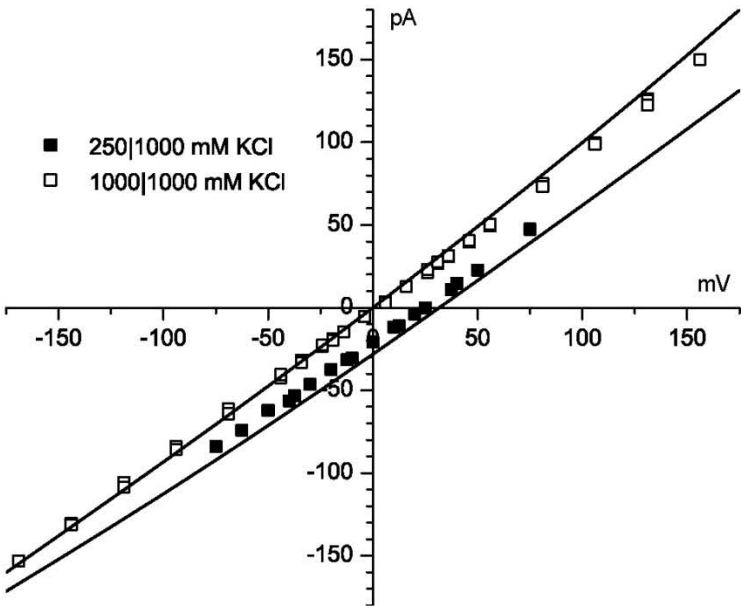
KCl

Gillespie, Meissner, Le Xu, et al





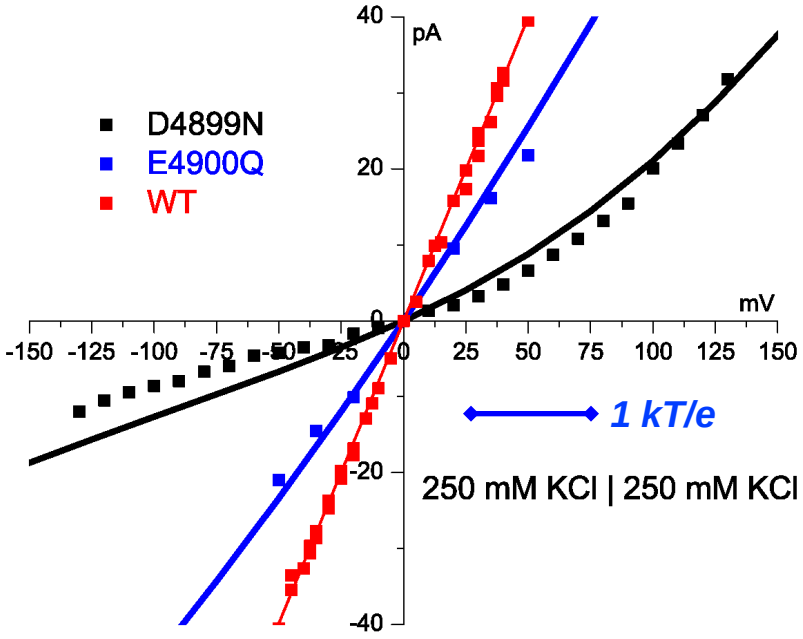




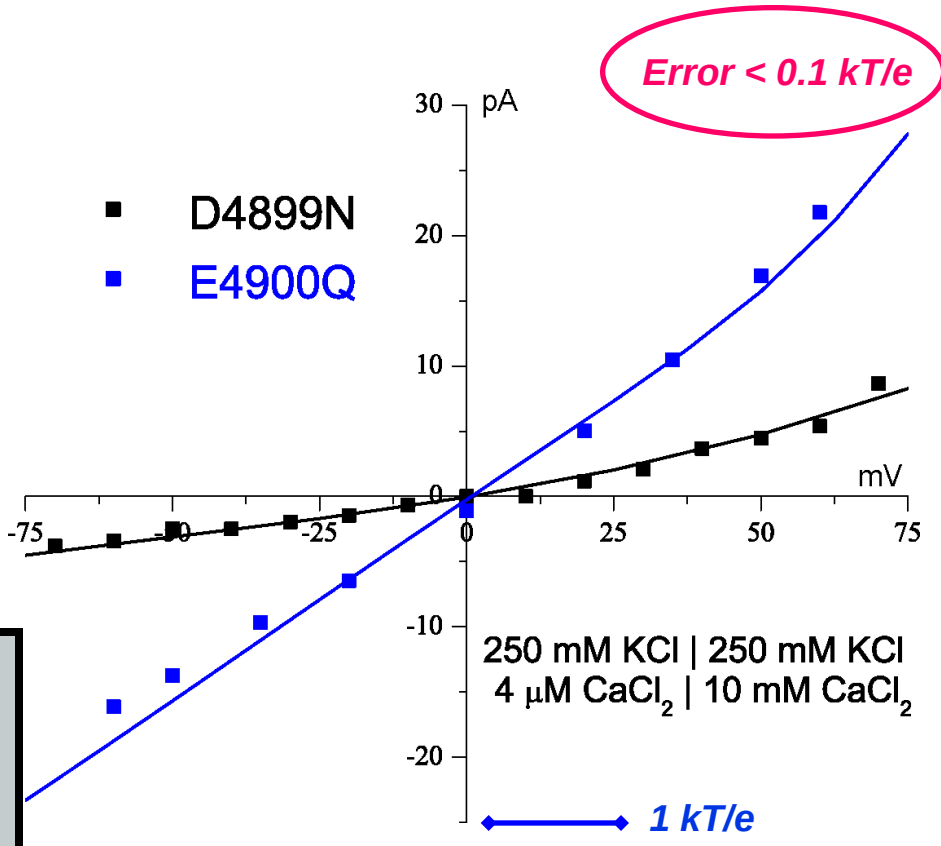
Theory fits Mutation with Zero Charge

No parameters adjusted

Theory Fits Mutant in K



Theory Fits Mutant in K + Ca



Protein charge density

wild type* 13 M ⇨ 0 M *in* D4899

Water is 55 M

some wild type curves not shown, 'off the graph'

Gillespie *et al*
J Phys Chem 109 15598 (2005)

Vaccination against Traditional Models

Traditional Biochemistry
and
Traditional Molecular Dynamics
Assume
Ideal Solutions

Ions in Water and Life are NOT ideal

Life Occurs in ~130 mM salt solutions

Ions in Water are the Liquid of Life

No gas phase models of traditional channel biochemistry

Liquids are not Gases

Rate Constants are Variables

No discussions of individual trajectories of Structural Biologists

Counting and Statistics are essential

Computation Starts From Crystal Structure

when available

but

Crystal Structures cannot determine Selectivity

because

1) **Crystal Structures are measured in only one unphysiological solution**
2) **Crystal Structures are not accurate enough**
3) **Crystal Structures do not give entropy**

Selectivity

Depends Sensitively on Self-organized Structure and their Flexibility

Induced Structure is Different in Different Solutions

so

Structure must be Computed!

Rate constants are variables that change dramatically with conditions

Supplementary Material

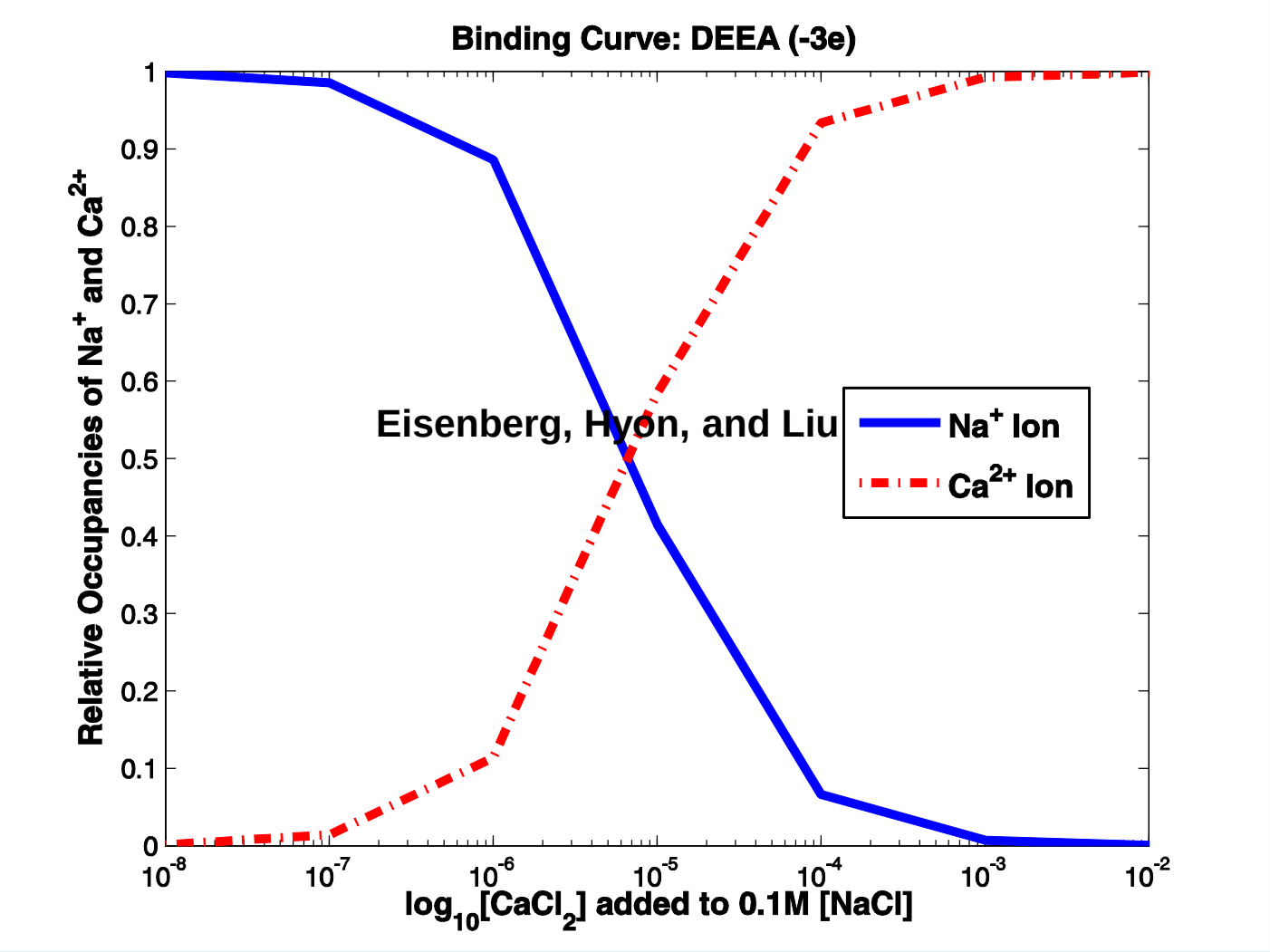
Chemically Specific Properties
of Ionic Solutions come from
Interactions

Molecular Dynamics Force Fields are Calibrated assuming no interactions with concentrations

Force Fields must be REcalibrated in each Biological Solution

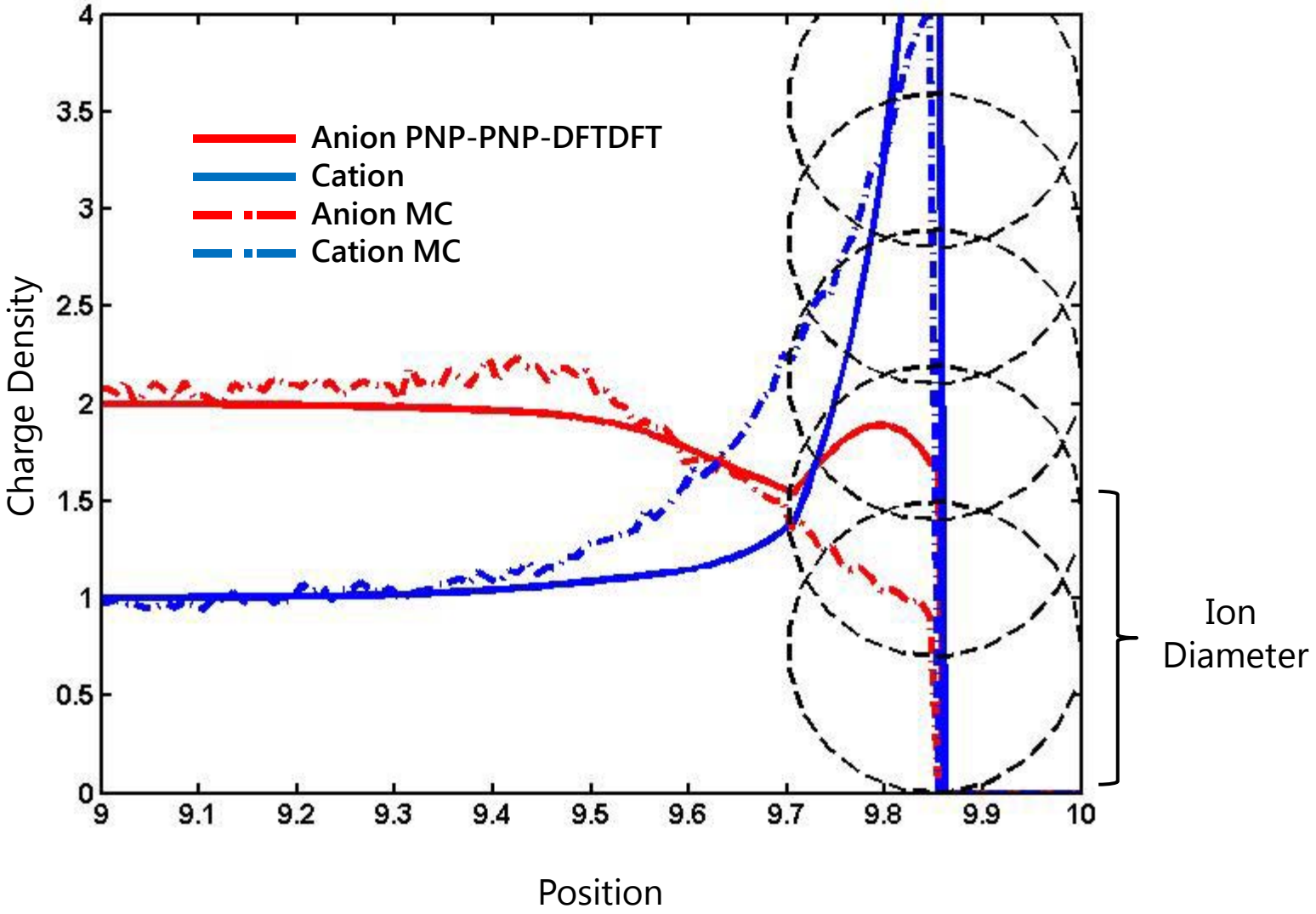
Ca^{2+} and Na^{+} Binding Curves

DEEA Calcium Channel



Layering: Classical Interaction Effect

Comparison between PNP-DFT and MC



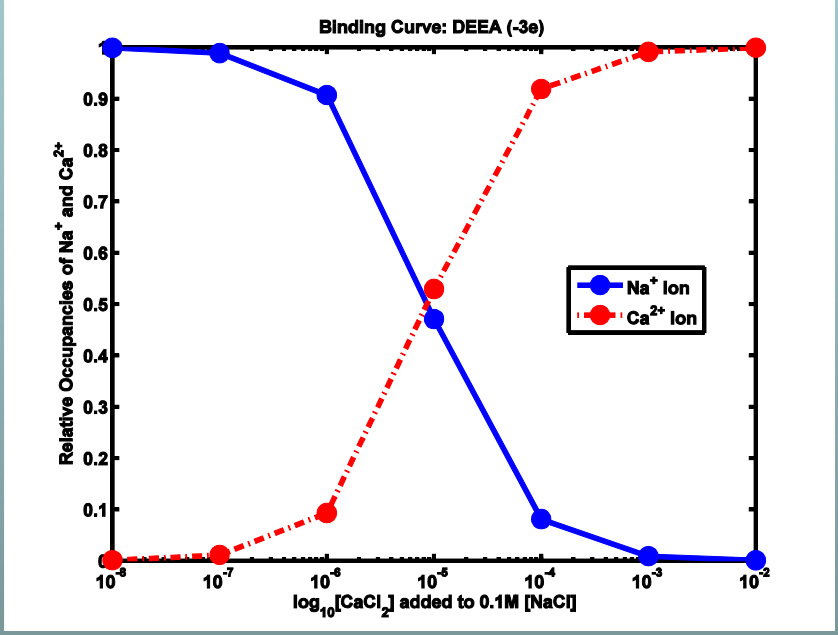
Eisenberg, Hyon, and Liu

Nonequilibrium Computations

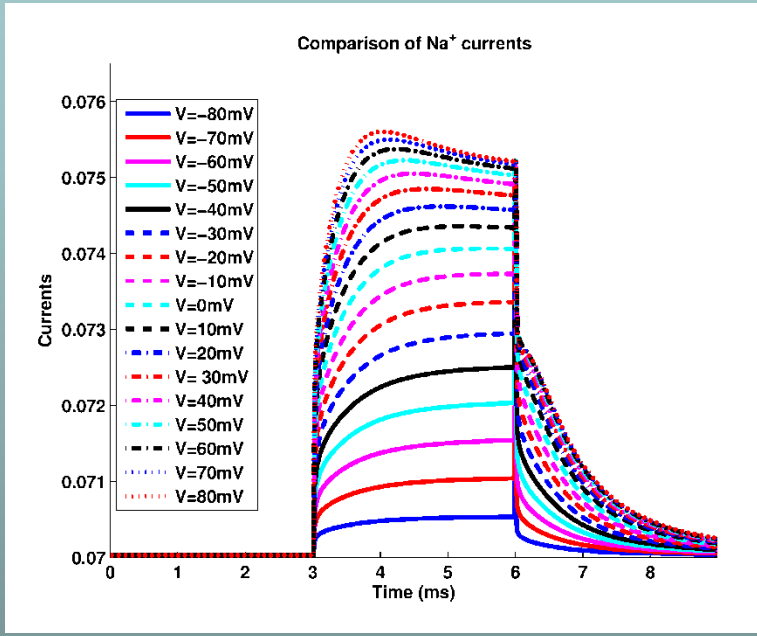
with Variational Field Theory

EnVarA

Binding Curves

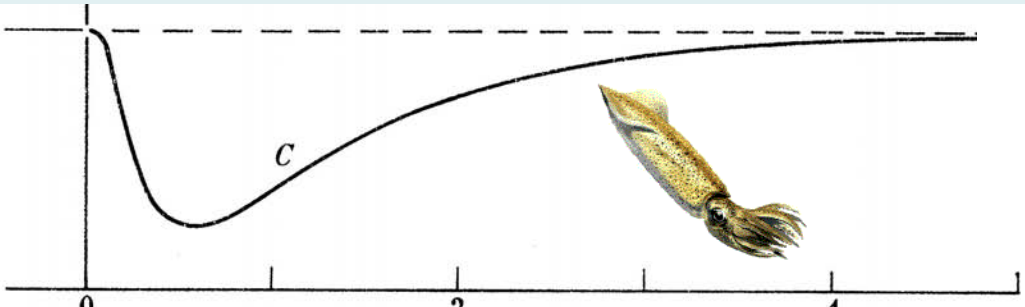


Current Voltage Time Curves

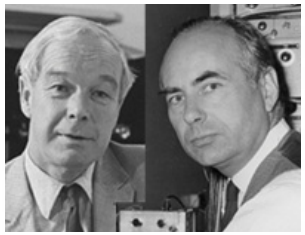


Sodium Conductance and **Inactivation**

in Squid Axon (nerve fiber)



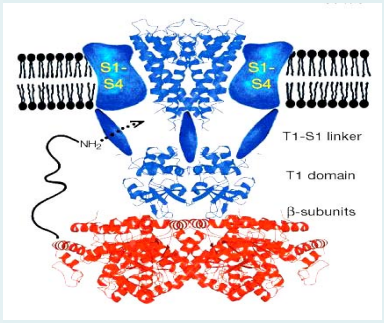
Hodgkin Huxley

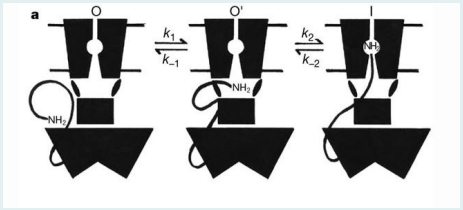


J. Physiol (1952)
116:497

FIGURE 9. Separation of current into components carried by Na and K, from Hodgkin & Huxley (1952*a*, figure 5). A depolarization of 56 mV was applied at $t = 0$; the temperature was 8·5°C. Outward current is shown upwards.

Conventional Explanation: Elaborate <u>Structural Change</u>





Inactivation is Important

Many diseases produced by changes in details of inactivation.

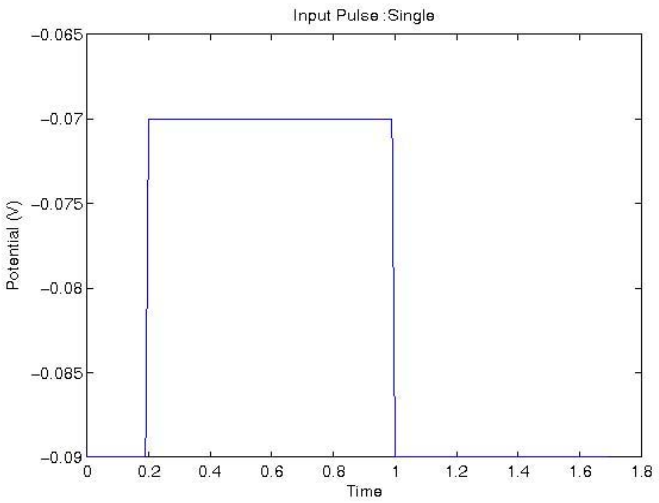
Energetics of Brain determined by details of inactivation*

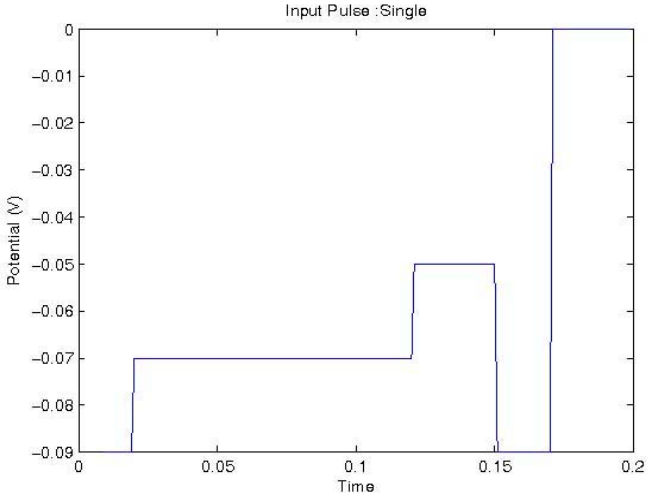
Energetics determined by time overlap of Na and K currents

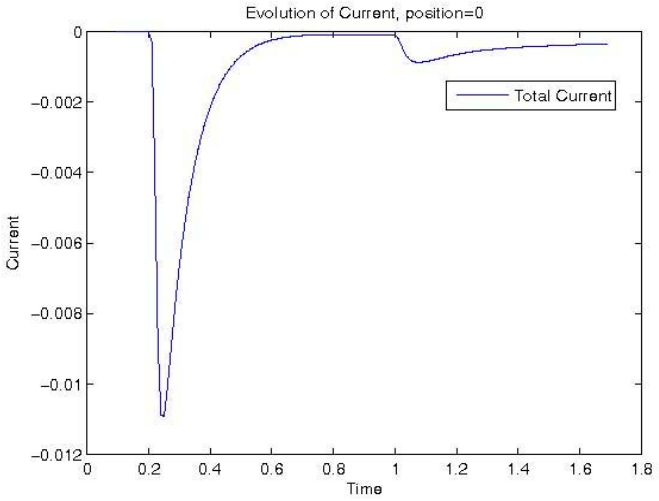
***Alle, Roth, and Geiger. Science (2009) 325:1405-8.**

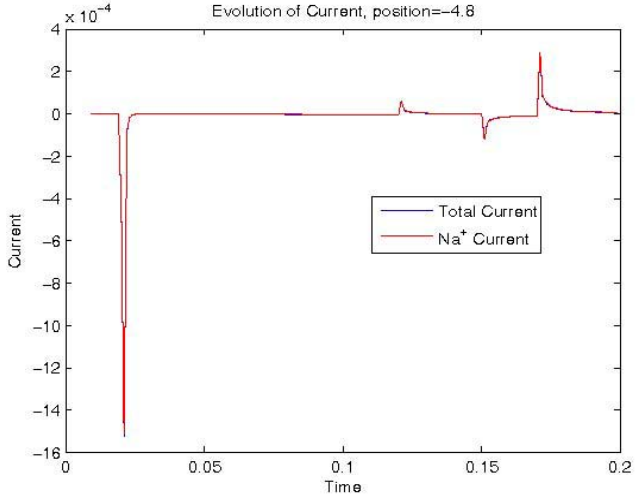
Sodium Conductance and Inactivation

Variational Computation in Fixed Structure









Eisenberg, Hyon, and Liu

Energetic Variational Analysis

EnVarA

Chun Liu, Yunkyong Hyon and Bob Eisenberg

New Interpretations

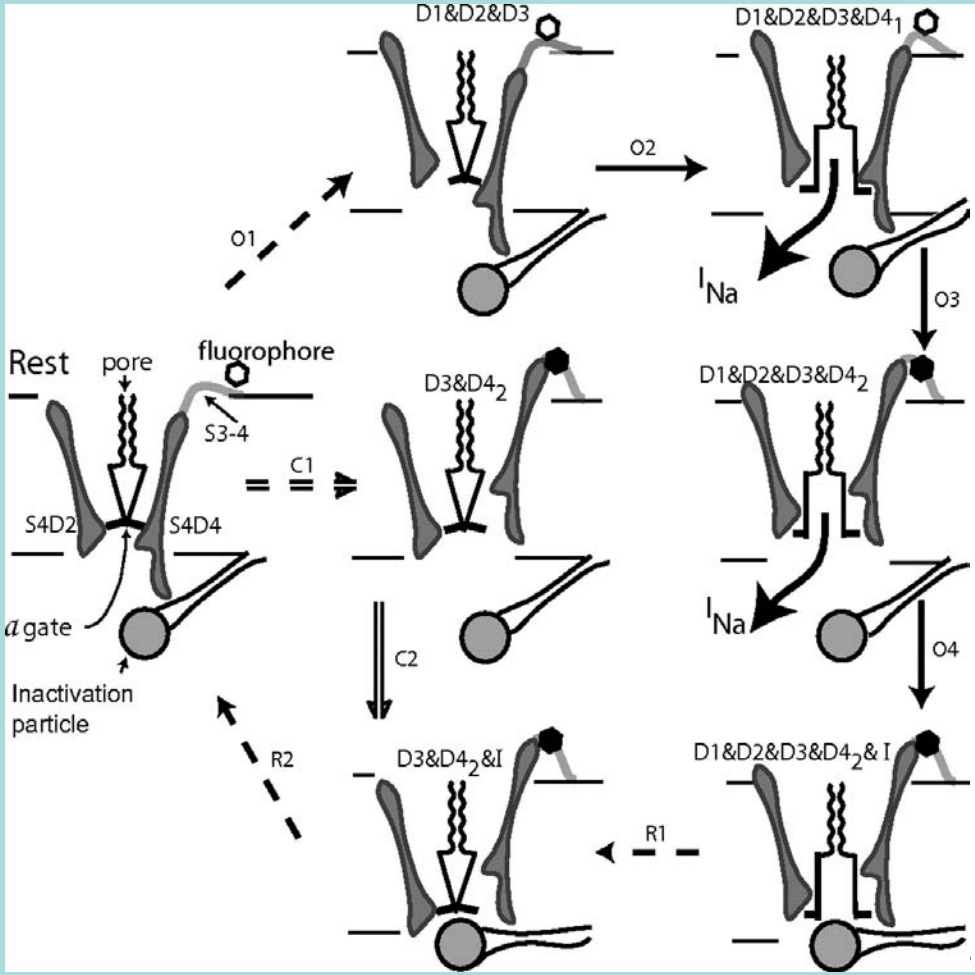
likely to be

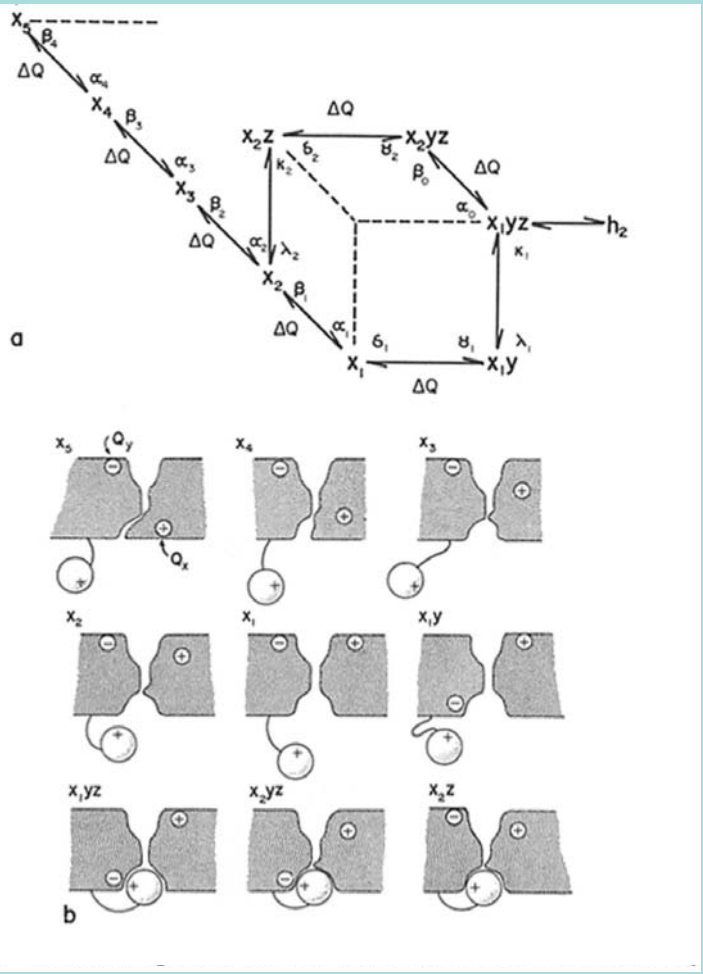
Controversial

but

Quantitative and Testable

Channel Activation and Inactivation
'Ball and Chain' Model





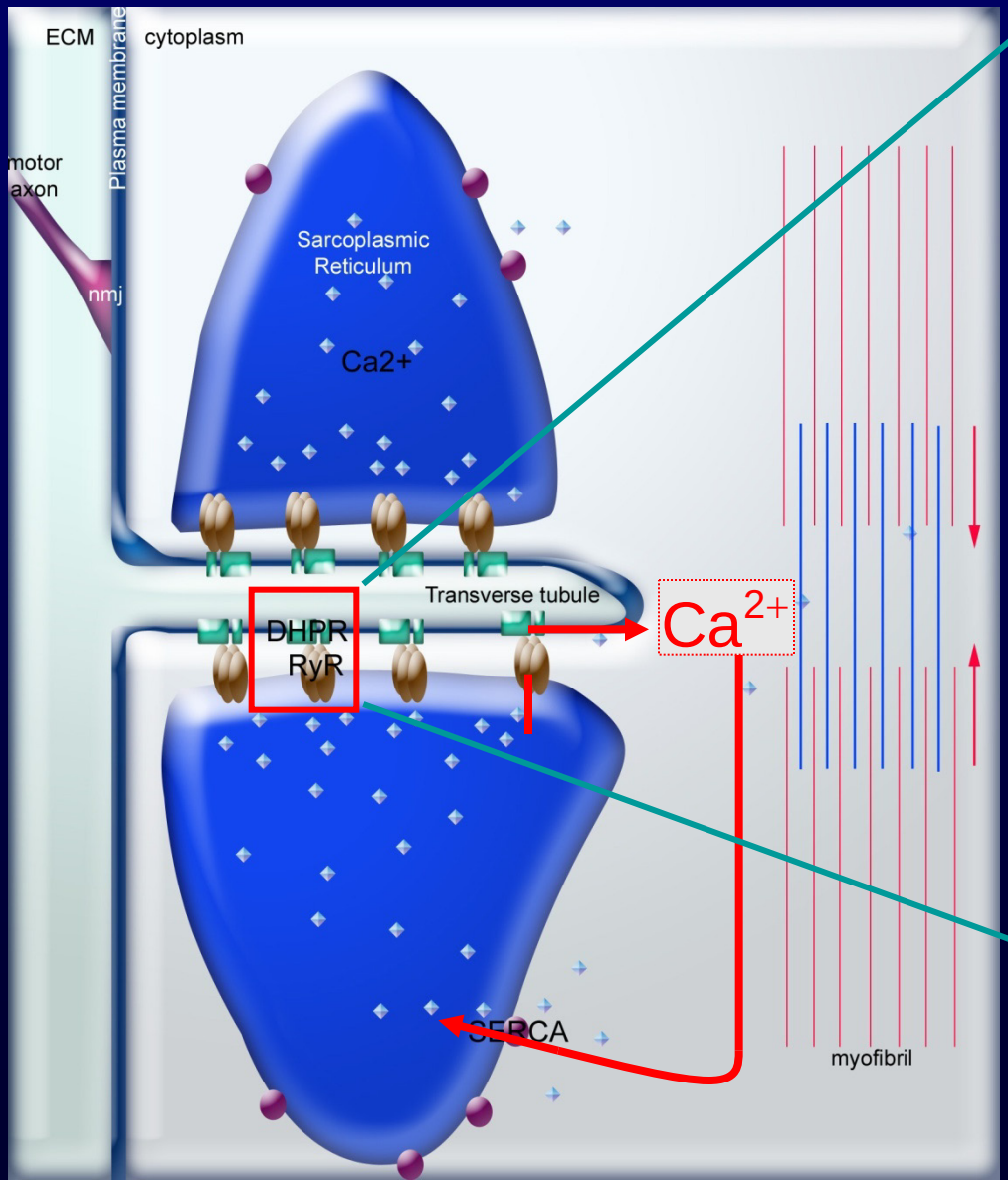
Armstrong PNAS 2006 103:17991

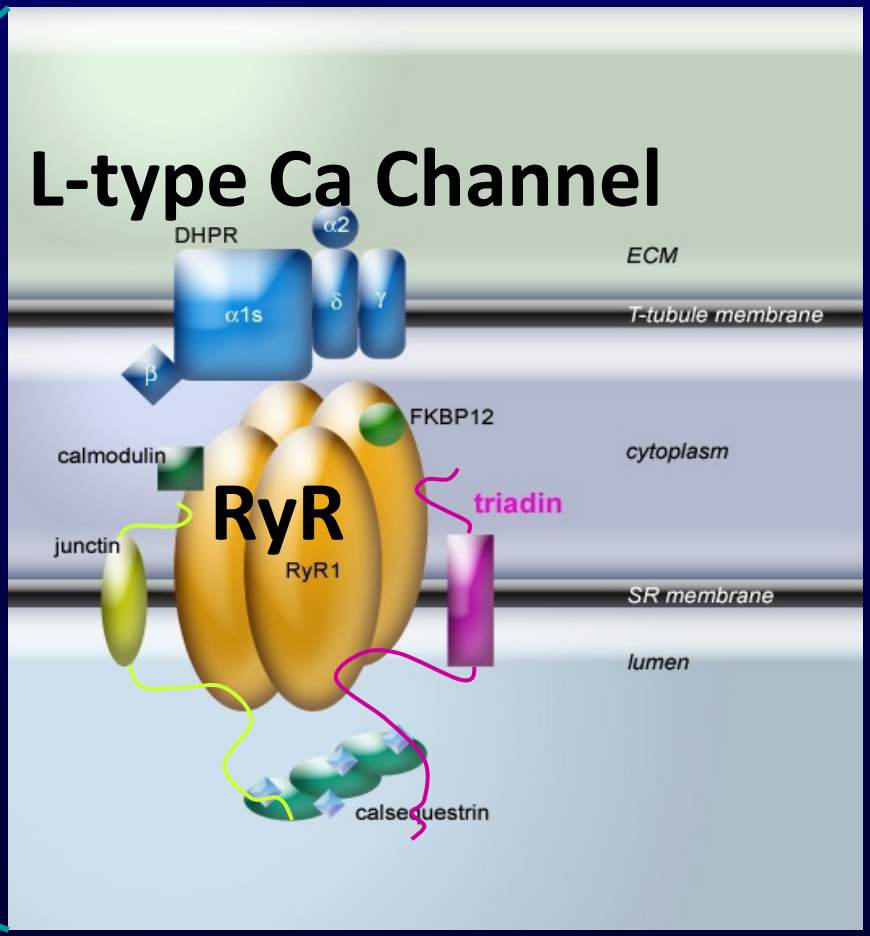
Armstrong & Bezanilla J Gen Physiol 1977 70:567

Existing Models are Structural and Mechanical
with no quantitative results

Channels are parts of Machines, e.g., Excitation-Contraction Coupling

L type Ca Channel RyR ryanodine receptor

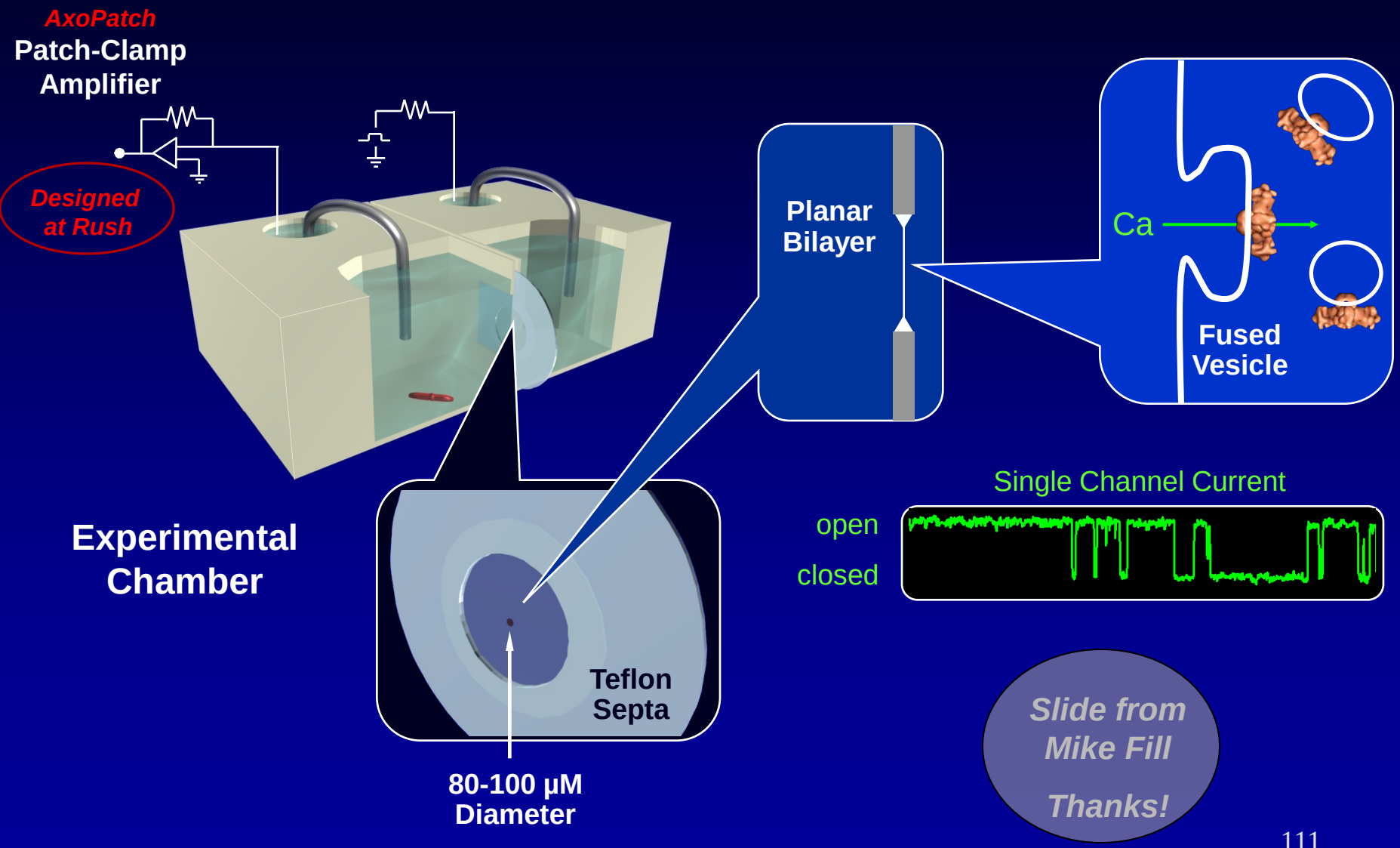




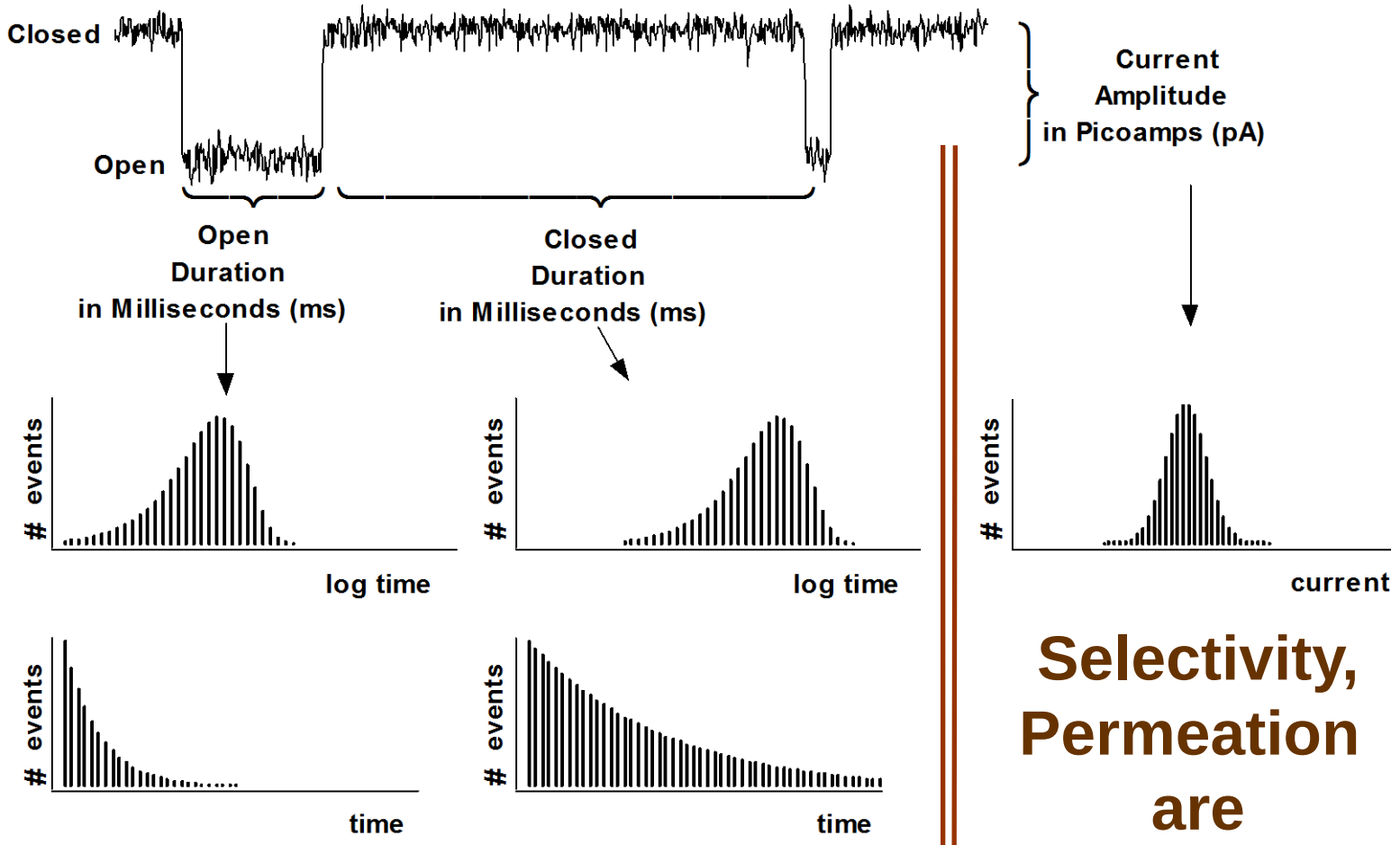
Thanks for the figure to
László Csernoch, Debrecen, Hungary
Isabelle Marty, Grenoble, France

Function of **SINGLE isolated** RyR Channels

in Artificial Planar Lipid Bilayers



Gating and Permeation



Gating is Time Behavior

Selectivity, Permeation are Amplitude

For Modelers and Mathematicians: **This is reverse engineering!**

Central Problem

How does the channel control Selectivity?

Inverse Problem for Selectivity

Badly posed, many answers are possible,
simultaneously over and under determined

with noise and systematic error

Core Math Problem has actually been solved

using methods for the
Inverse Problem of a Blast Furnace

Burger, Eisenberg and Engl (2007) SIAM J Applied Math 67: 960-989

Channels are Selective

Different Types of Channels

use

Different Types of Ions

for

Different Information

New Interpretations

likely to be

Controversial

but

Quantitative and Testable

Time Dependence

is

Important

Many diseases produced by inactivation

Energetics of Brain

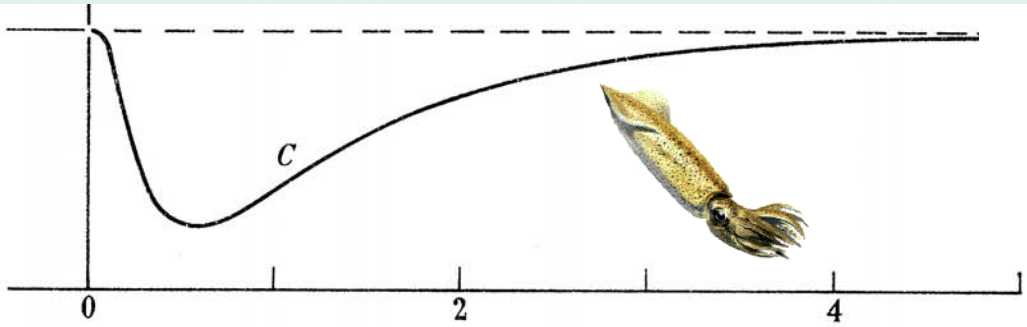
determined by inactivation*

***Energetics determined by time overlap of Na and K currents**

Alle, Roth, and Geiger. Science (2009) 325:1405-8.

Time Dependent Sodium Conductance

Inactivation in Squid Axon (nerve fiber)



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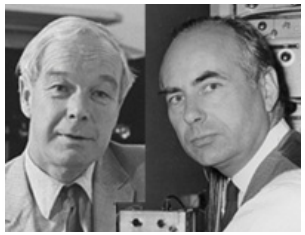

J. Physiol (1952)
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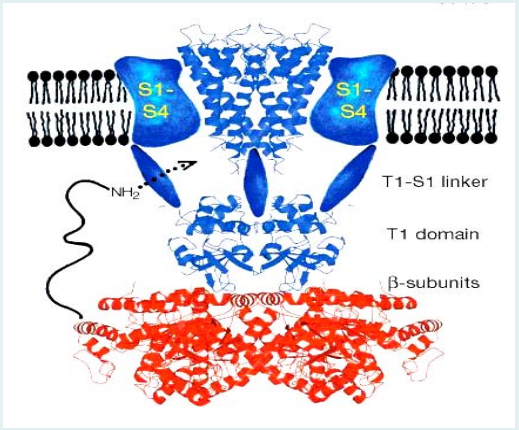
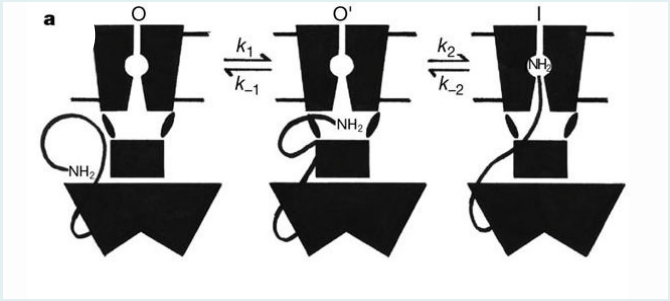
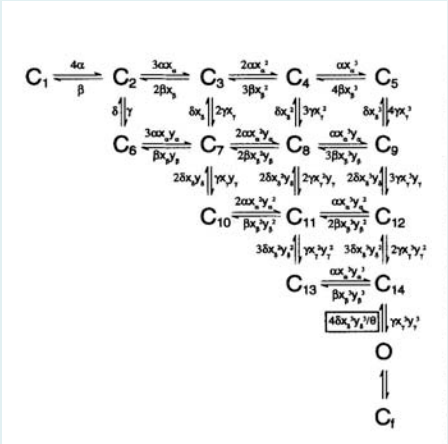
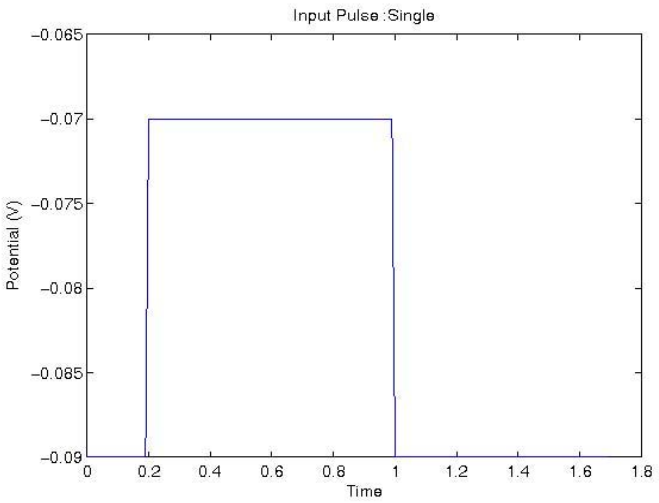


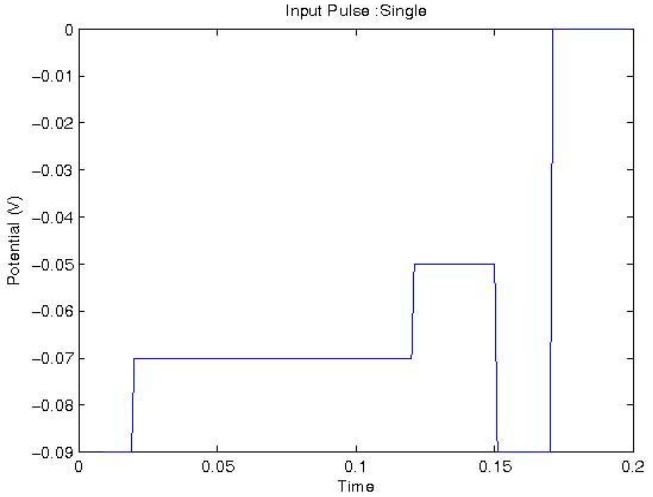


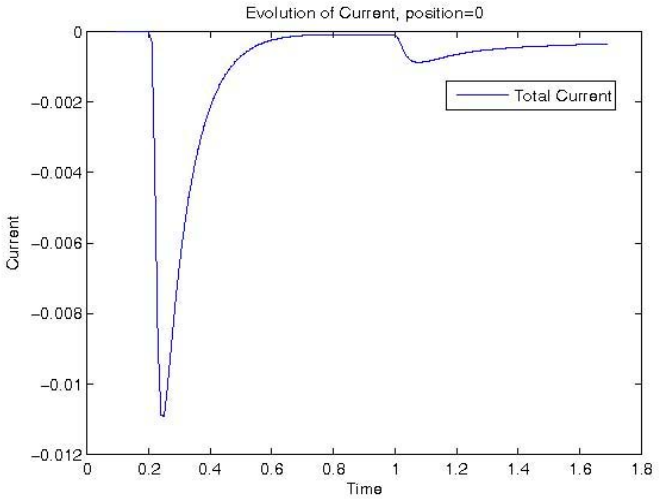


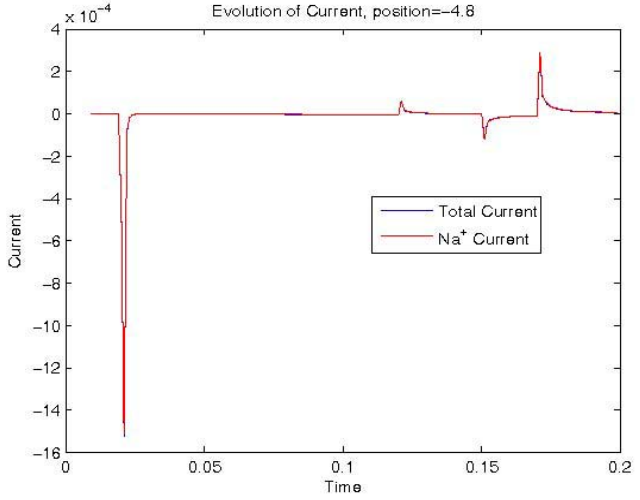
Sodium Conductance and Inactivation in Fixed Structure

Variational Computation









Eisenberg, Hyon, and Liu

Multiscale Issues are the key if we want to actually build channels that work

Computational Scale	Biological Scale	Ratio
Time 10^{-15} sec	10^{-4} sec *Action Potential*	10^{11}
Space 10^{-11} m	10^{-5} m *Side Chains of Proteins*	10^{6}
Spatial Resolution	*Three Dimensional* $(10^{6})^{3}$	10^{18}
Solute Concentration	10^{-11} to 20 Molar	10^{12}