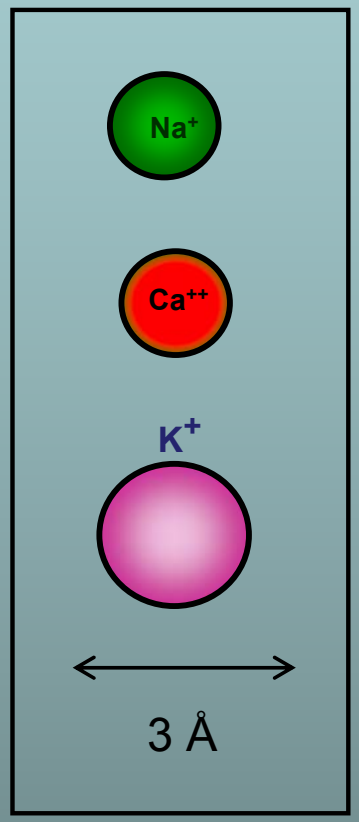
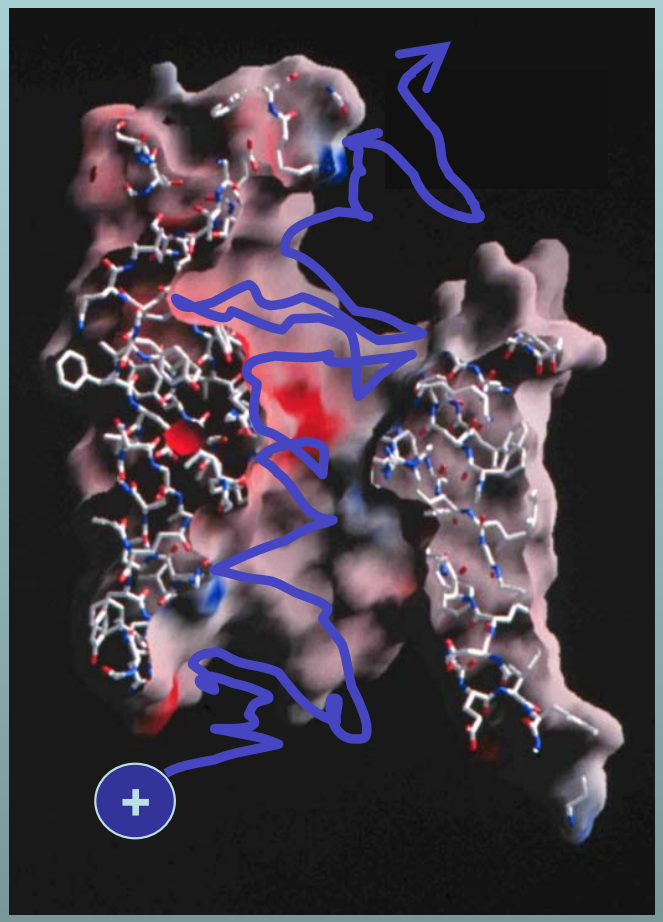
Robert Eisenberg
Rush Medical

Ionic Channels
are
Valves* of Cells

KITP Evo Cell
Jan 12, 2010

Main Controllers of Biological Function





~30 Å

Flow time scale is 0.1 msec to min

Figure of ompF porin by Raimund Dutzler

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

*Pun:
Valve = Vacuum Tube
≈ PNP Transistor
≈ FET Transistor

BritSpeak

Biology and Chemistry are about Chemically Specific Properties

<u>Chemically Specific</u> Properties are the same thing as their DEVIATION from IDEAL

Life Occurs in ~130 mM salt solutions

mostly Sodium Na^+, Potassium K^+, and Calcium Ca^{++} Chloride Cl^-

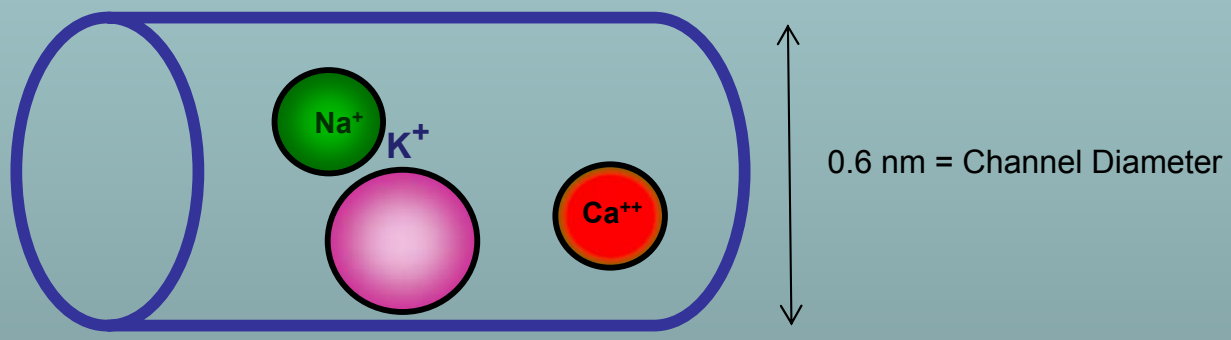
Ions in Water

are the

Liquid of Life

Life Occurs in ~130 mM salt solutions

mostly Na^+, K^+, and Ca^{++} Cl^-



Ions in water and channels are NOT ideal solutions because

<u>Chemically Specific</u> Properties of Ionic solution are their DEVIATION from IDEAL

Molecular Dynamics Force Fields are Calibrated *<u>in the ideal in Infinitely Dilute solutions</u>*, for the most part.

Biological Theory
and
Molecular Dynamics Simulations almost always assume ideal solutions

In my opinion

New Mathematics

is needed to deal

with the INTERACTIONS

that make ionic solutions non ideal

and that can create the

CHEMICAL SPECIFICITY

of life

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No theory is available for properties of mixtures of ions

Brownian Motion theory is for UNcharged particles.
Brownian Motion theory ignores Interactions

Ion Channels are a good Test Case

Simple Physics (Electrodiffusion)

Single Structure (once open)

Simple Theory is Possible
and Reasonably Robust
because Channels are Devices
with well defined
Inputs, Outputs and **Power Supplies**

Channels are also Biologically Very Important

Ion Channels are Biological Devices

Natural nano-valves* for atomic control of biological function

Ion channels coordinate contraction in the heart, allowing the heart to function as a pump

Ion channels coordinate contraction in skeletal muscle

Ion channels control all electrical activity in cells

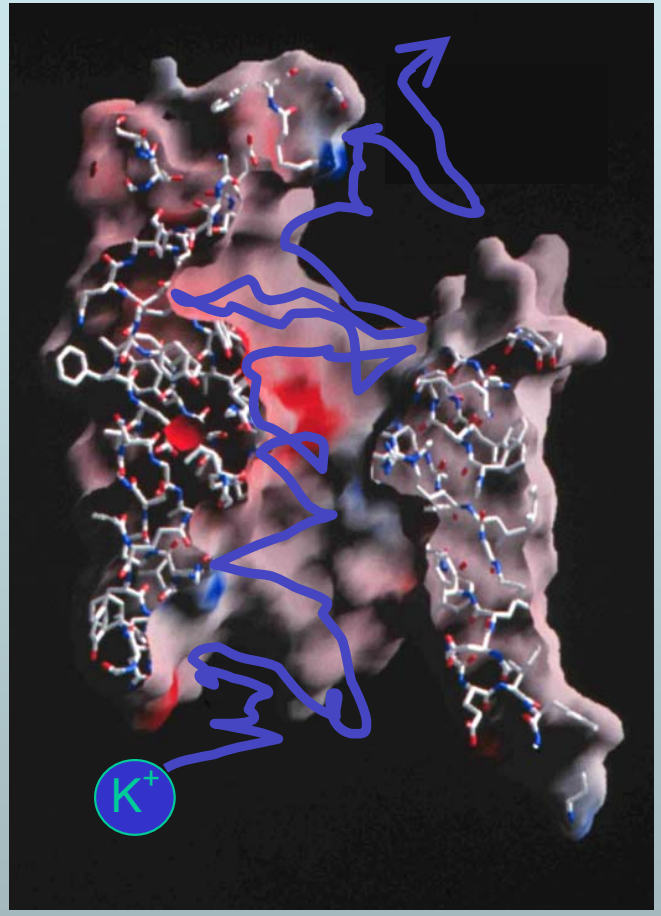
Ion channels produce signals of the nervous system

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.



~30 Å

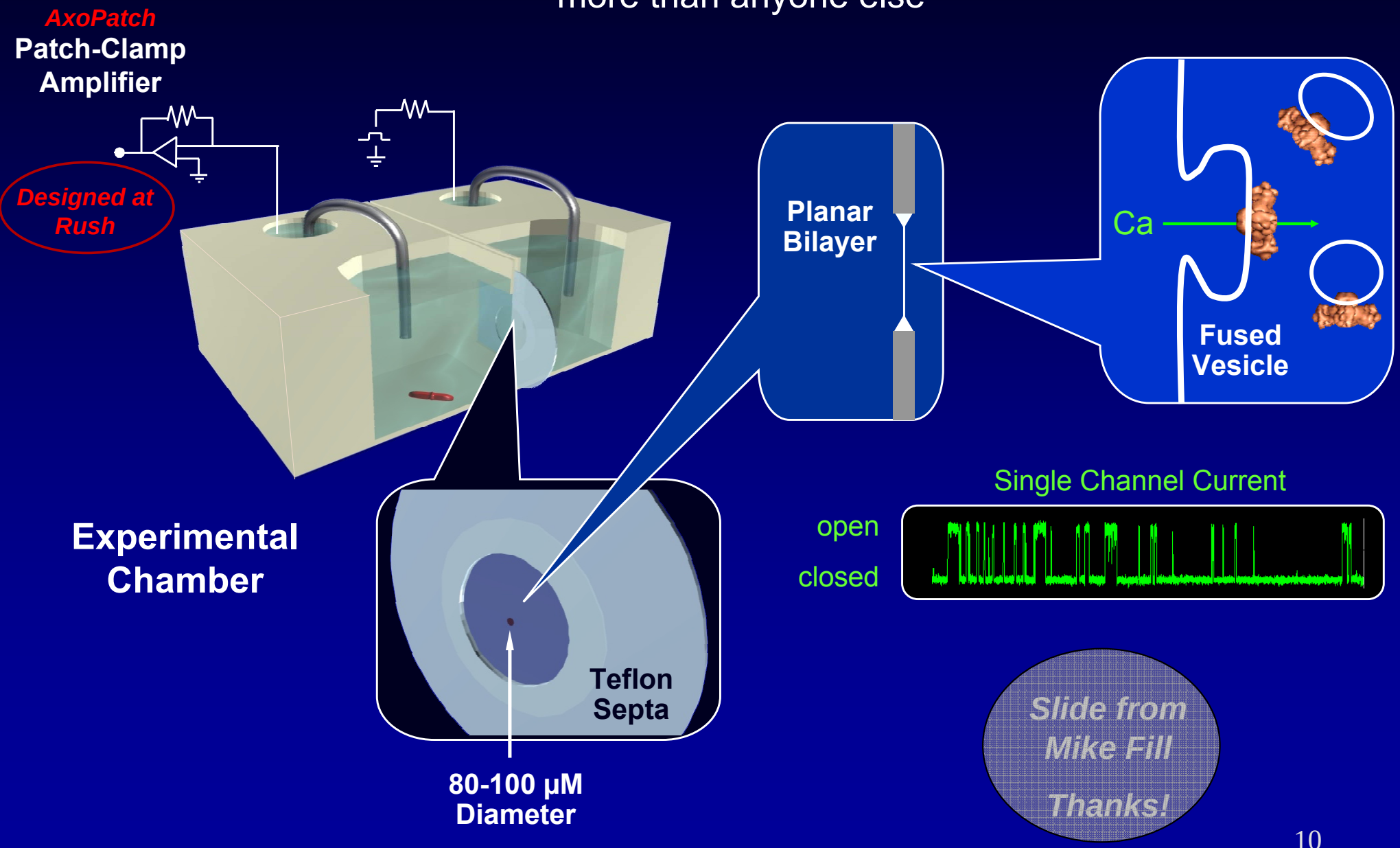
*nearly pico-valves: diameter is 400 - 900 pico-meters

Ion Channels are studied experimentally by thousands of molecular biologists every day because they are so important.

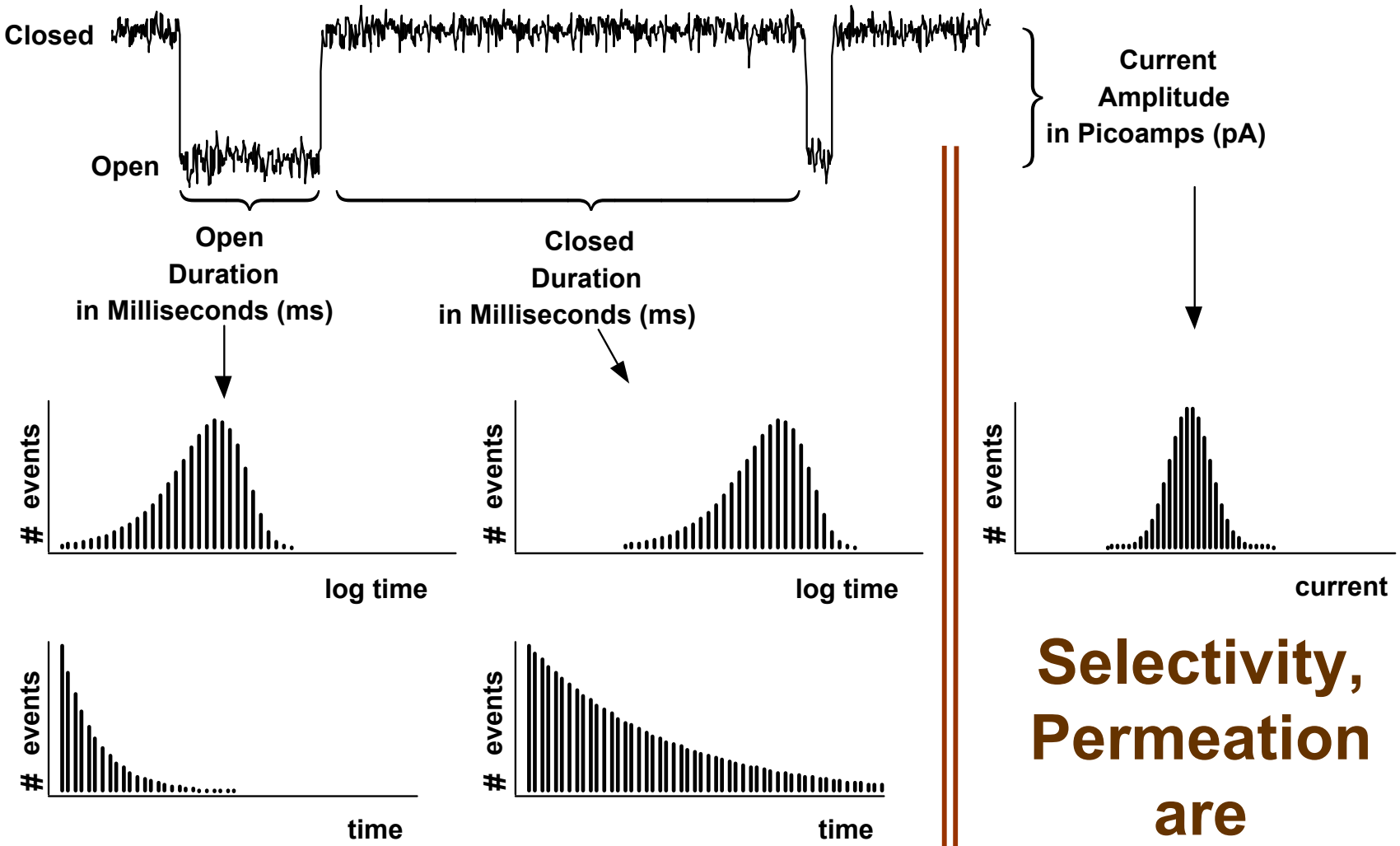
This number is not an exaggeration.
We have sold over 10,000 AxoPatch Amplfiers

Reconstitution of Single Channels….
Thanks to Chris Miller !!!

more than anyone else



Gating and Permeation



Gating is Time Behavior

Selectivity, Permeation are Amplitude

Goal:
Understand Selectivity

Selectivity Differs in Different Types of Channels

Wolfgang Nonner, Dirk Gillespie, Douglas Henderson, Dezső Boda

Goal:
Understand the Adaptation that makes Selectivity

Selectivity
is an Adaption
Necessary for Channels to Function

Selectivity of Different Channel Types Studied in Many Solutions

RyR Channel	Calcium Channel	Sodium Channel	Synthetic Ca Channel
Selectivity filter *DDDD* 4 − charges	Selectivity filter *EEEE* 4 − charges	Selectivity filter *DEKA* 2 −, 1+ charge	Selectivity filter *Various* many − charges
PNP/DFT	PNP/DFT Monte Carlo	Monte Carlo	PNP/DFT

<u>RyR model</u> of Gillespie is best worked out for ~ 120 solutions

<u>Selectivity of K Channel is studied in ~1 solution at infinite dilution</u>

K channel of Roux has atomic detail but is studied at infinite dilution

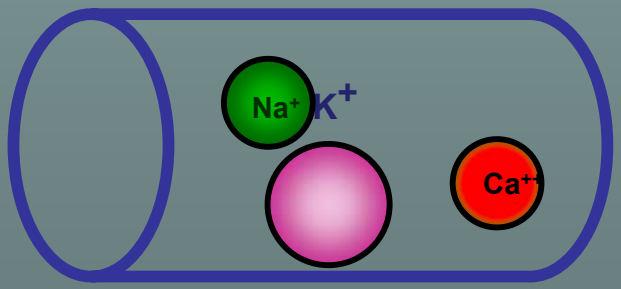
Quantum /K of Rempe has atomic detail but is studied at infinite dilution

Nonner, Gillespie, Henderson, Boda

“There is only one word that matters in biology and that is **Specificity** ....”

Aaron Klug

quoted in the first sentence of H. Pearson, Nature (2008) 455:160-164



Goal:

Understand Selectivity

well enough to

Fit Large Amounts of Data*

and to

Make a Calcium Channel

*from many non-ideal solutions

Molecular Biologists need a framework
to interpret their results.
Evolutionary Biology, Physics, and Chemistry can provide that framework.

Reduced Models

form the framework for understanding experiments.

Reduced models describe the

Input, Output, and Power Supply.

They describe the essential physics and chemistry.

Reduced models are the adaptation

created by evolution

to perform the biological function of selectivity.

Reduced models are the Phenotype

of the channel

in my understanding of the meaning of that word

.

Reduced models are Device Equations

like Input Output Relations of Engineering Systems.

Finding the reduced model,
checking its validity,
estimating its parameters, and their effects,
are all part of the

Inverse Problem:

‘Reverse Engineering’ of Selectivity

In my view,

Finding the evolutionary adaptation,

Finding the underlying physics,

Finding the Device equation, and

'Reverse engineering' Selectivity

are nearly the same things as

Understanding Physiology

Enough Vague Stuff
Goal:

Understand Selectivity

well enough to

Fit Large Amounts of Data*

and to

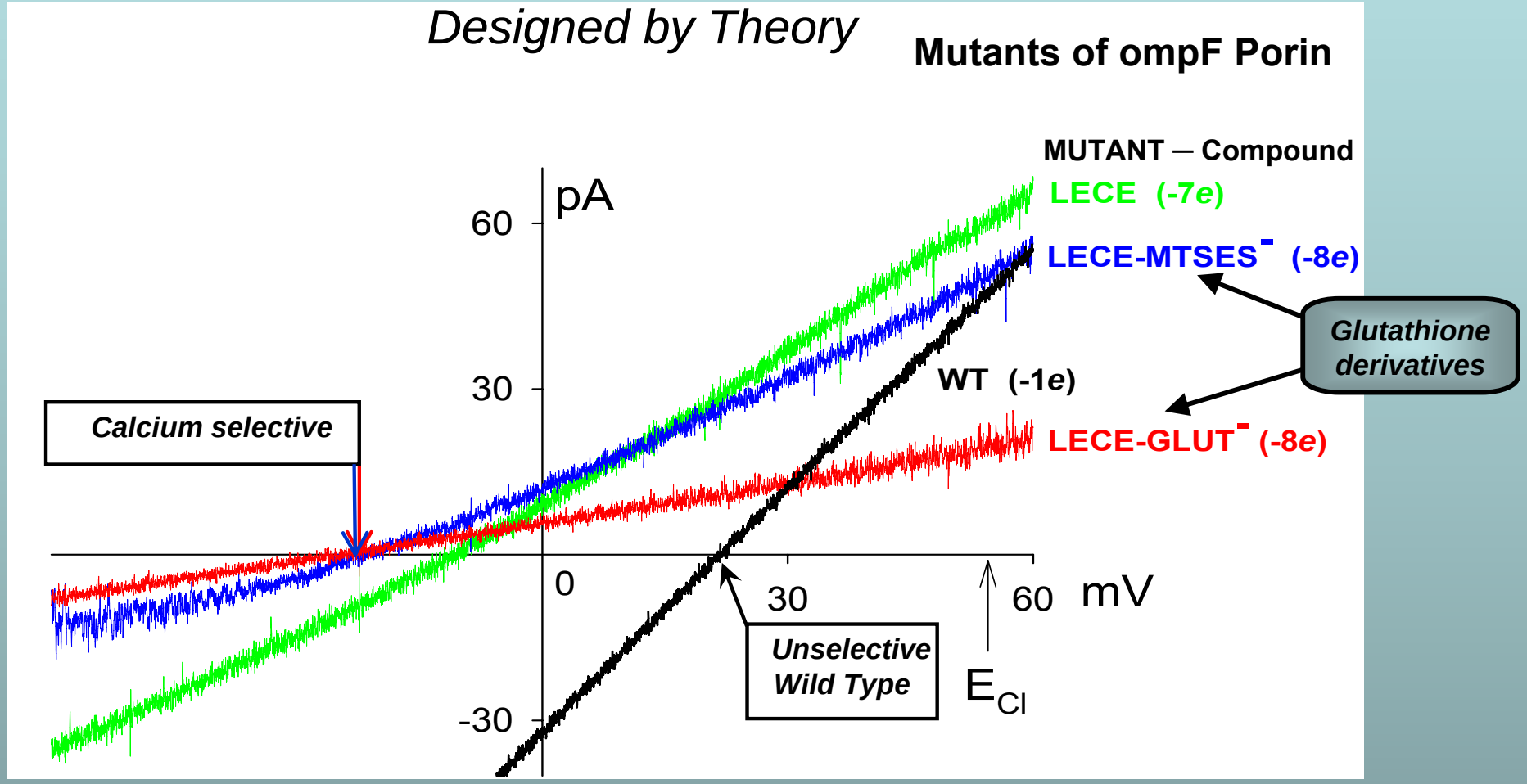
Make a Calcium Channel

*from many non-ideal solutions

Experiments have built

Two Synthetic Calcium Channels

Designed by Theory



As density of permanent charge increases, channel becomes calcium selective

$E_{rev} \rightarrow E_{Ca}$

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

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

Channels are only Holes

Why can't we understand and build them?

Must have high quality measurements

Must know physical basis of function

Where do we start?

Not with molecular mythology

Not with gas phase models of traditional channology

Liquids are not Gases

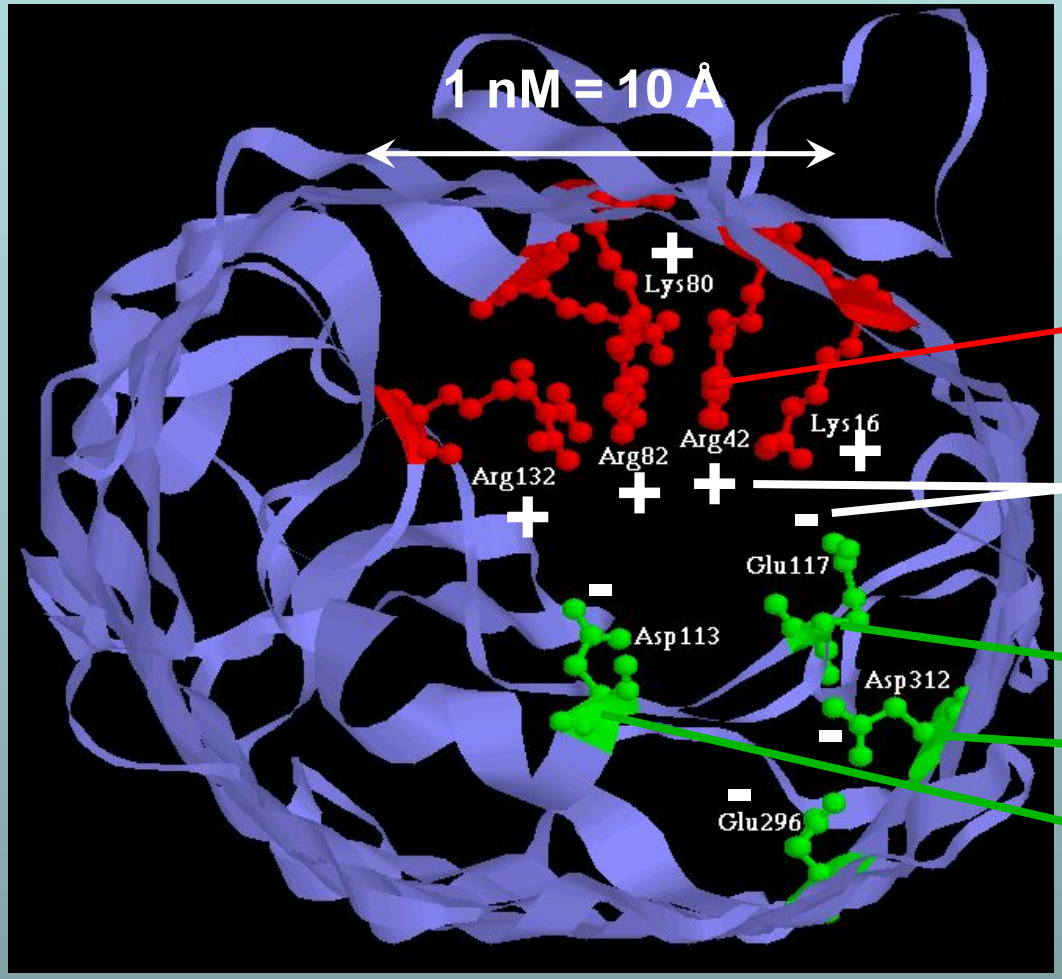
Not with guesses about trajectories of structural biologists

Counting and Statistics are essential when atomic trajectories are involved

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

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

Pure water is 55 M



Side Chains mix with

Ions are Crowded

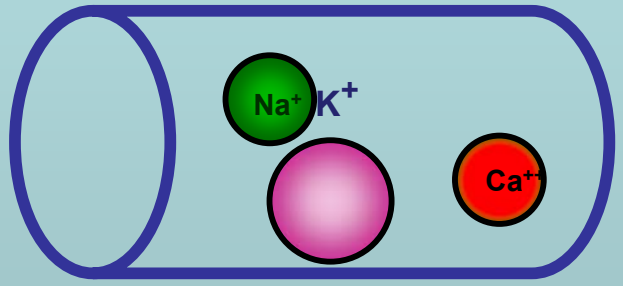
Side Chains MIX with Water, Ions

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

Figure adapted from Tilman Schirmer

Finite Size Effects

Working Hypothesis Implicit Solvent Model



Chemically Specific Properties

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

Diameter and Charge

Atomic Detail

and dielectric 'constant' of ionic solution

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

We need a **model of selectivity** and
method of calculation

to describe the **Reduced Model**,
which is also **the Evolutionary Adaptation**

that shows how
Selectivity arises from Crowding of ions

At equilibrium,
we use **Monte Carlo simulations**,
because they are calibrated.

At nonequilibrium, we have used
Density Functional Theory (of fluids)
combined with PNP
Poisson Nernst Planck theory of drift and diffusion

We now introduce the
<u>Energy Variational Method</u>
of the theory of complex fluids

Implicit Solvent ("Primitive") Model
of
Ionic Solutions

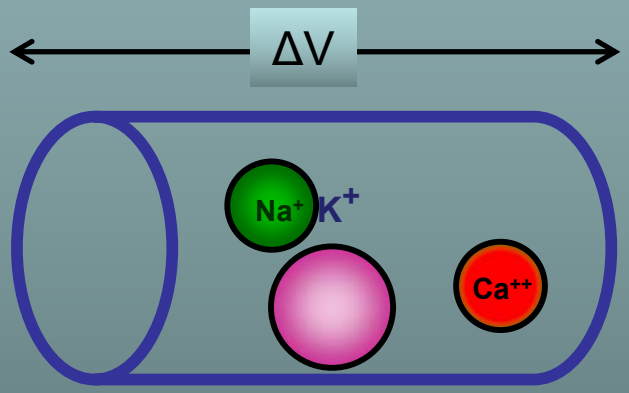
Almost always an equilibrium theory,

no flows of any kind

Usually in the 'Thermodynamic Limit'

No boundary conditions, even for the electric field.

Maxwell died too soon!



Energetic Variational Analysis

EnVarA

being developed by

Chun Liu,

Yunkyong Hyon, and Bob Eisenberg

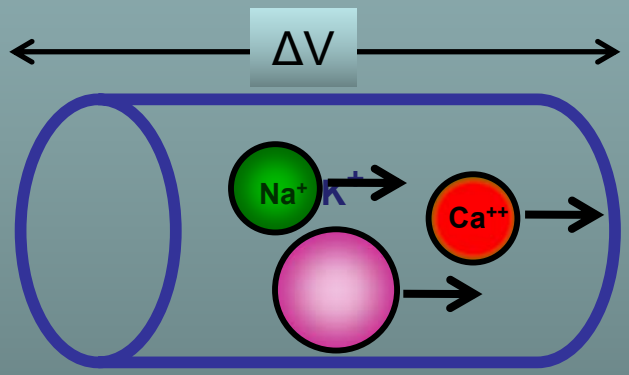
creates a

Field Theory of non-ideal Ionic Solutions

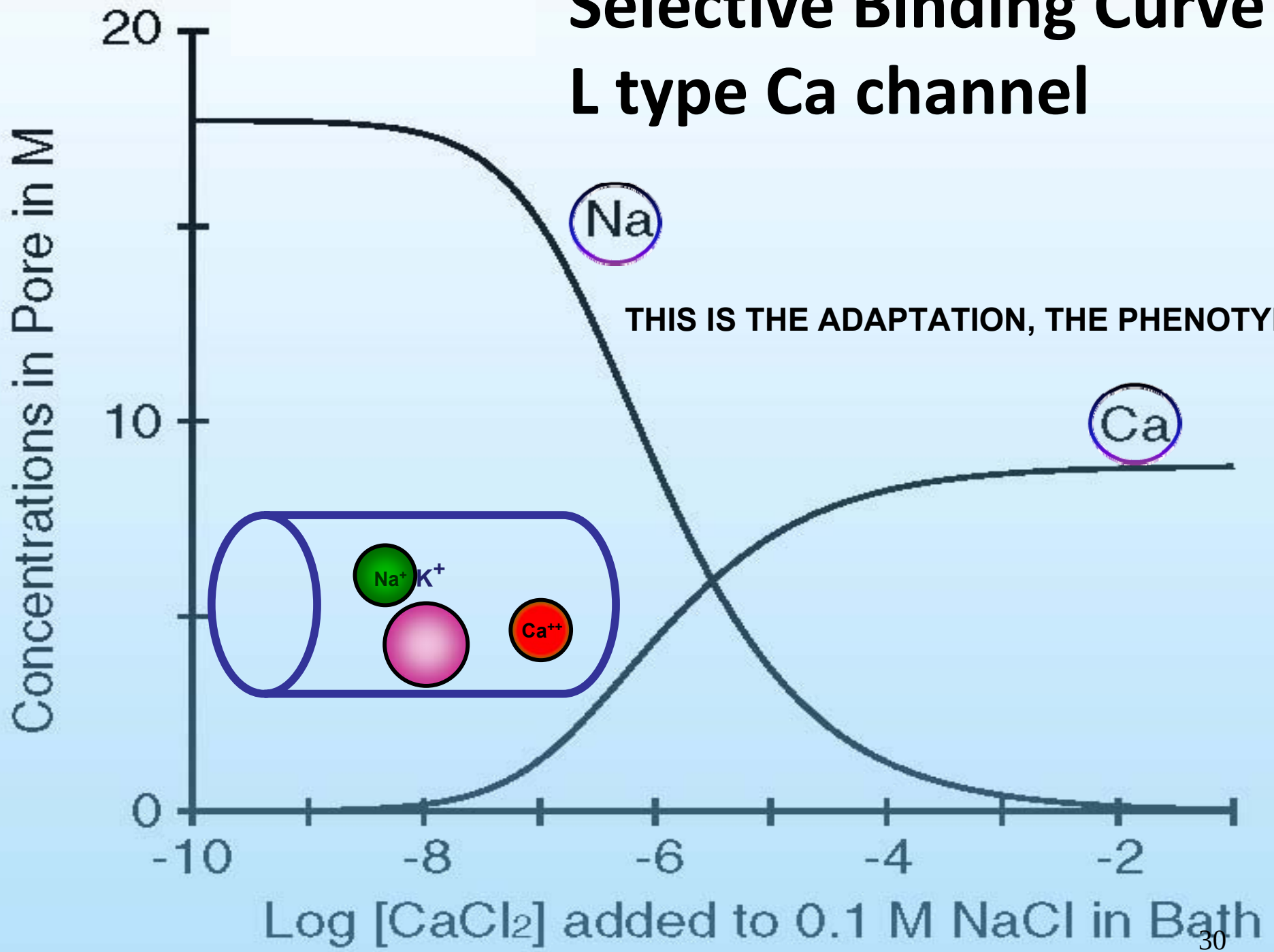
that allows boundary conditions and flow

and deals with

Interactions of Components Self-consistently



Selective Binding Curve
L type Ca channel



THIS IS THE ADAPTATION, THE PHENOTYPE

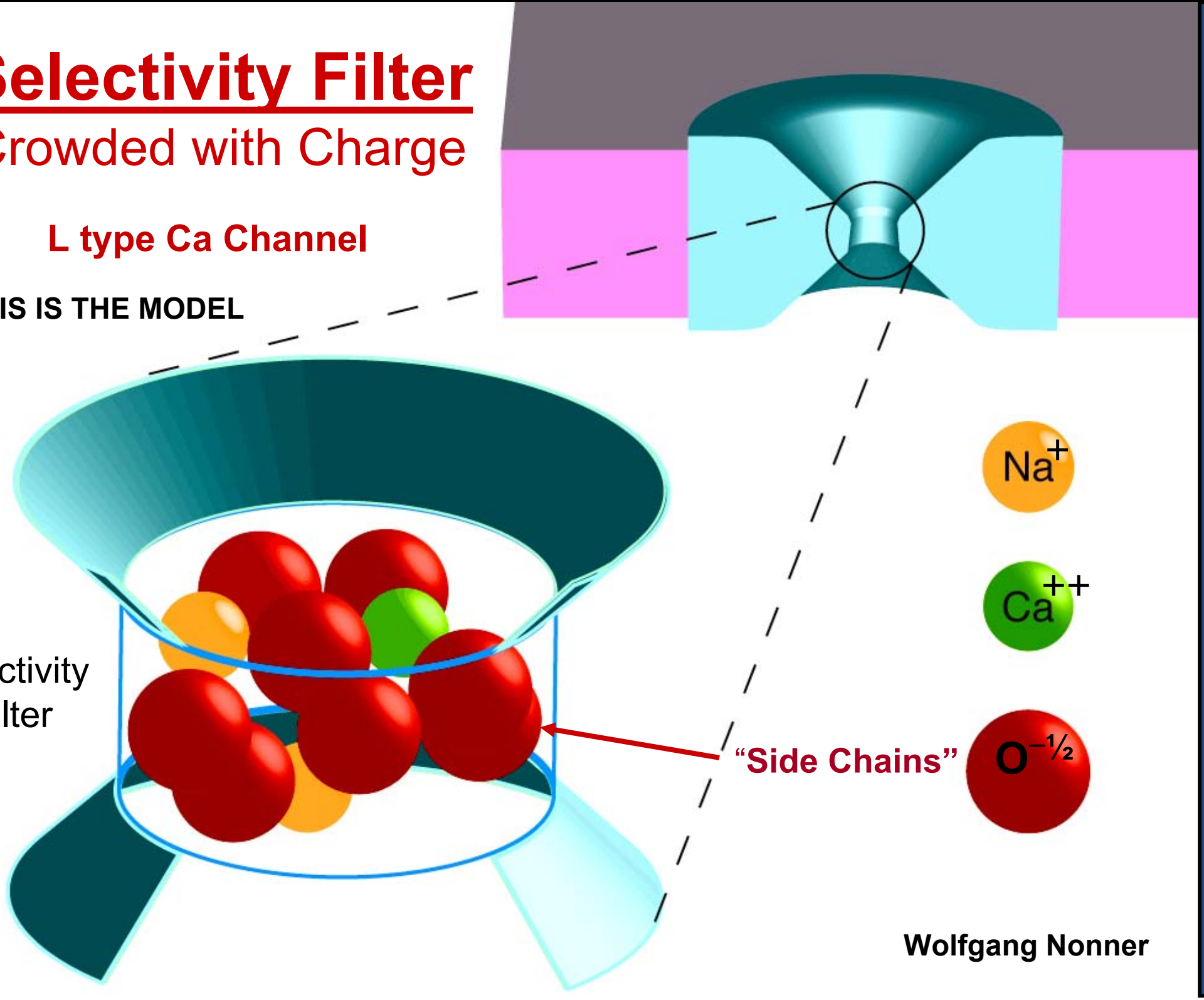
Wolfgang Nonner

Selectivity Filter

Crowded with Charge

L type Ca Channel

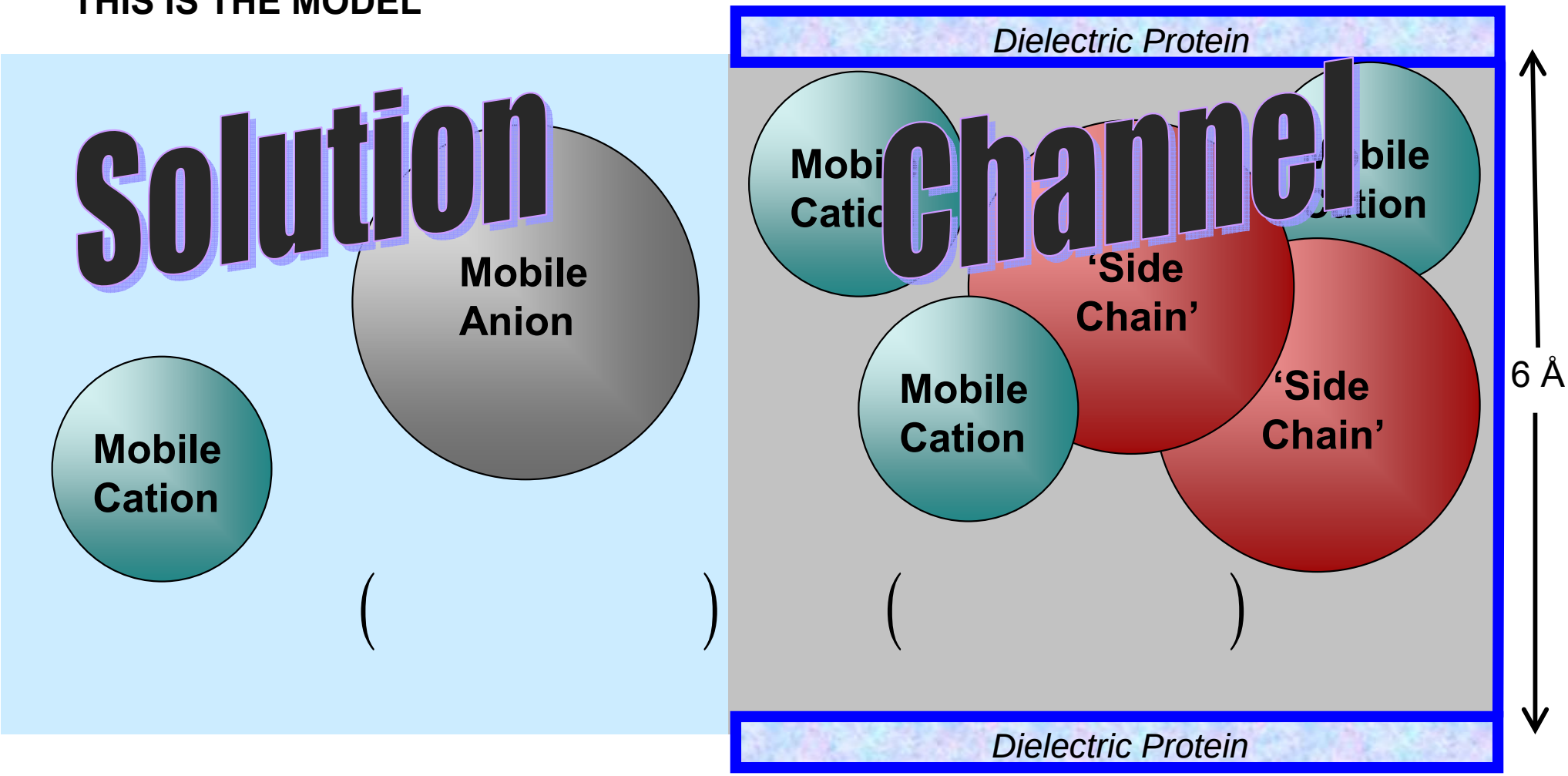
THIS IS THE MODEL



Wolfgang Nonner

Ion 'Binding' in Crowded Channel

THIS IS THE MODEL



Classical Donnan Equilibrium of Ion Exchanger

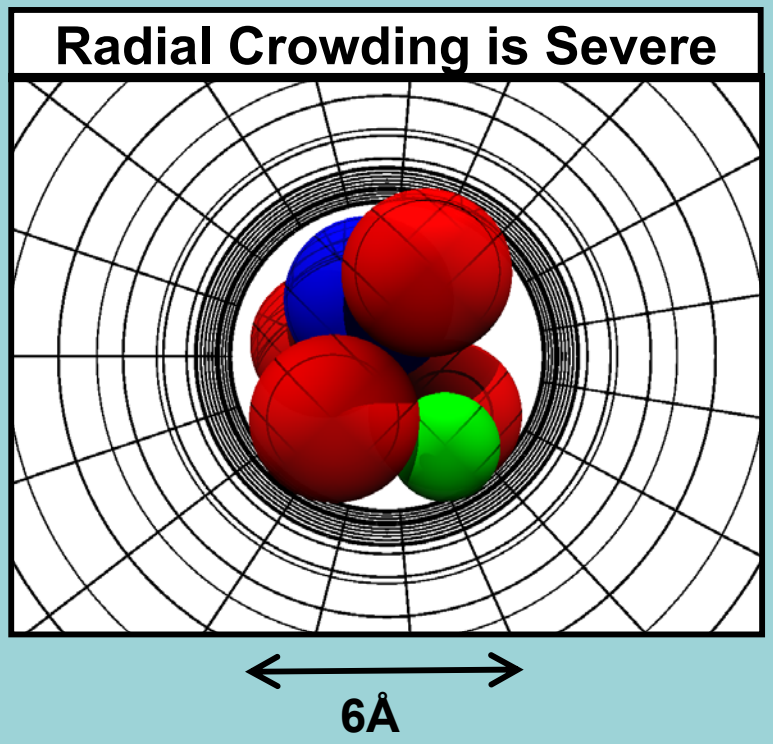
large mechanical forces

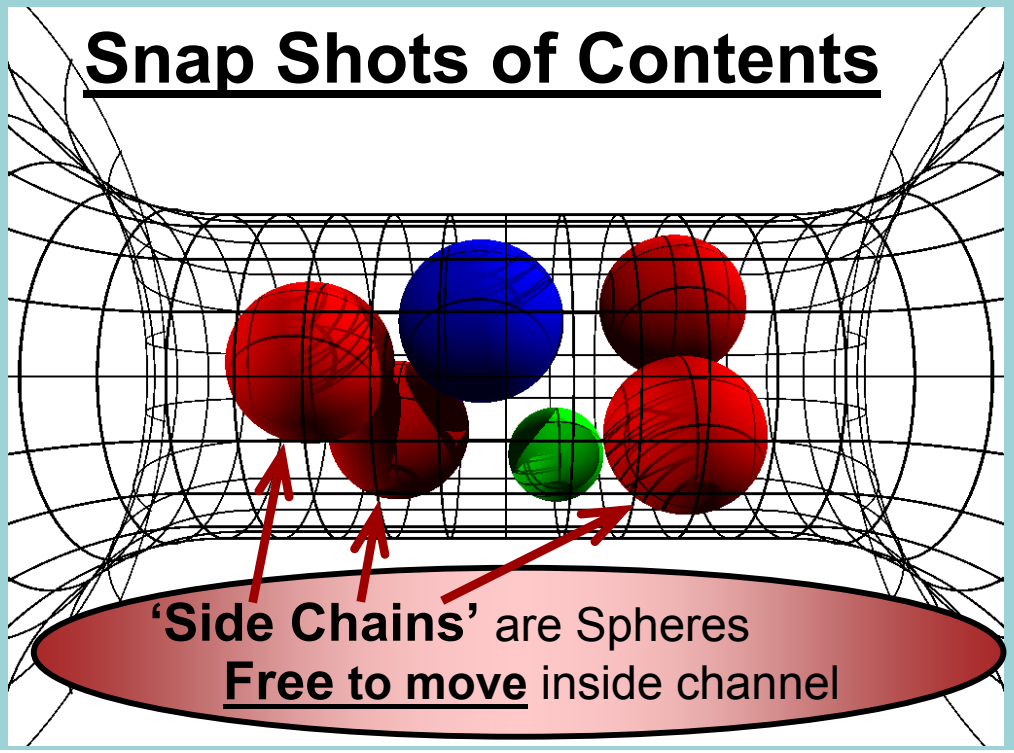
Side chains move within channel to their equilibrium position of minimal free energy.

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

Boda, Nonner, Valisko, Henderson, Eisenberg & Gillespie

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

Central Problem*

How does the channel control selectivity

How does the protein produce the phenotype?

***an example of "*Reverse Engineering*"**

Most of biology is an <u>Inverse Problem</u>

How does a biological <u>device</u> work?

How can we make it work better?

The device equation is the mathematical statement of the function.

It is the 'slow' variable in the language of mathematics of complex system.

Inverse Problems

Badly posed,
simultaneously over and under determined.

<u>Exact choice of question and data are crucial</u>

Inverse Problems learned from Heinz Engl and Martin Burger with thanks!

PNP inverse problem (with DFT, noise, systematic error) solved in
Burger, Eisenberg and Engl (2007) SIAM J Applied Math 67: 960-989

Inverse Problem for Selectivity

Badly posed,
simultaneously over and under determined
with noise and systematic error

has <u>actually been solved</u>

using methods for the

Inverse Problem of a Blast Furnace.

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

Ca Channel

Mutation

Same Parameters

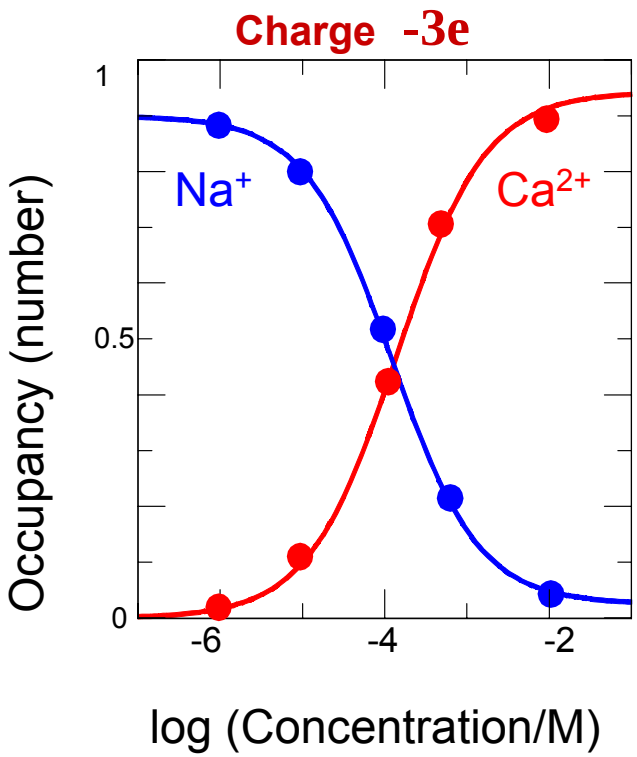
Na Channel

E → D

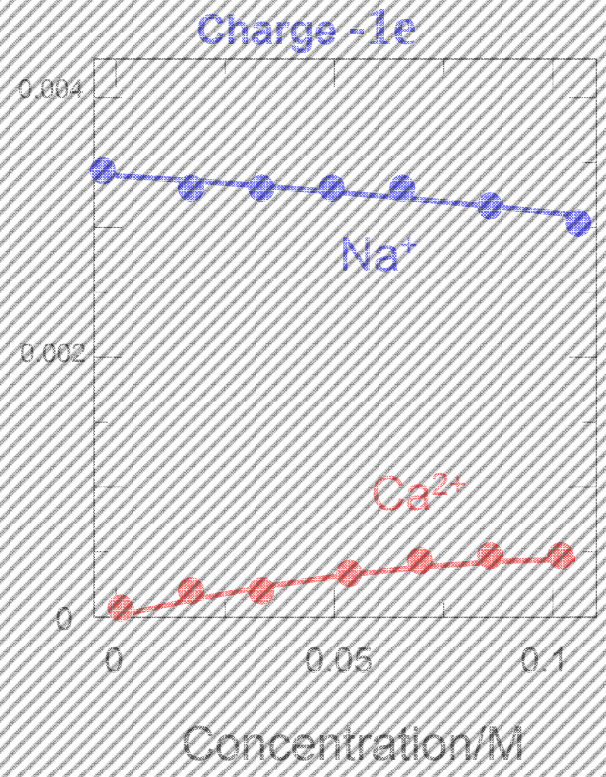
E → E

E → K

A → A

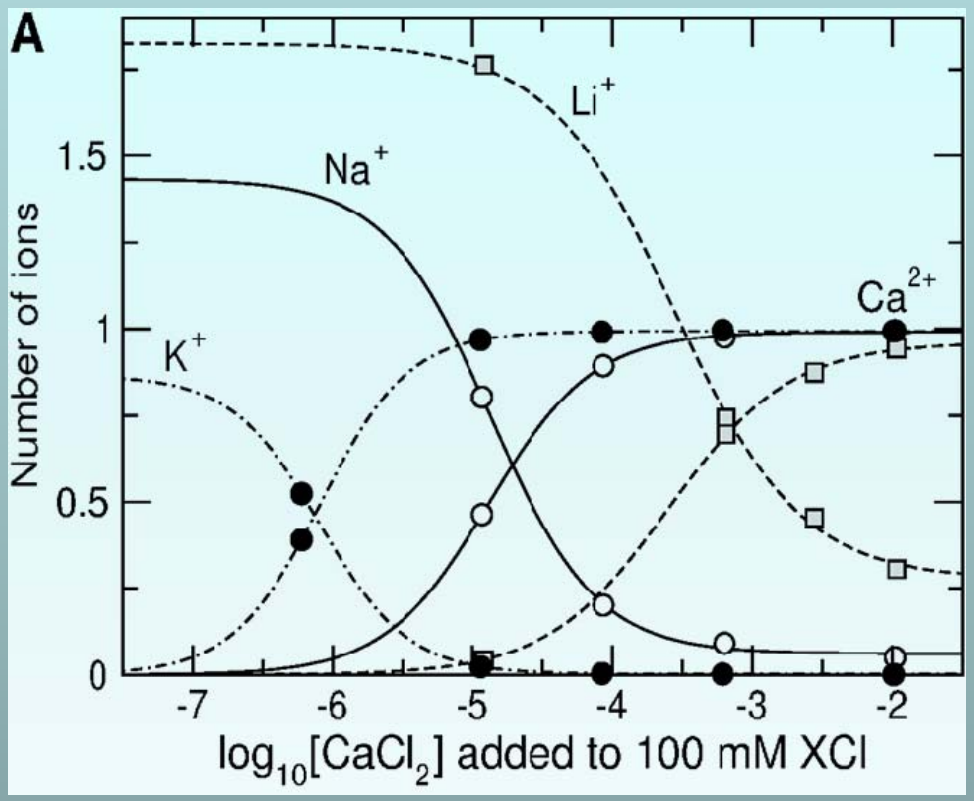


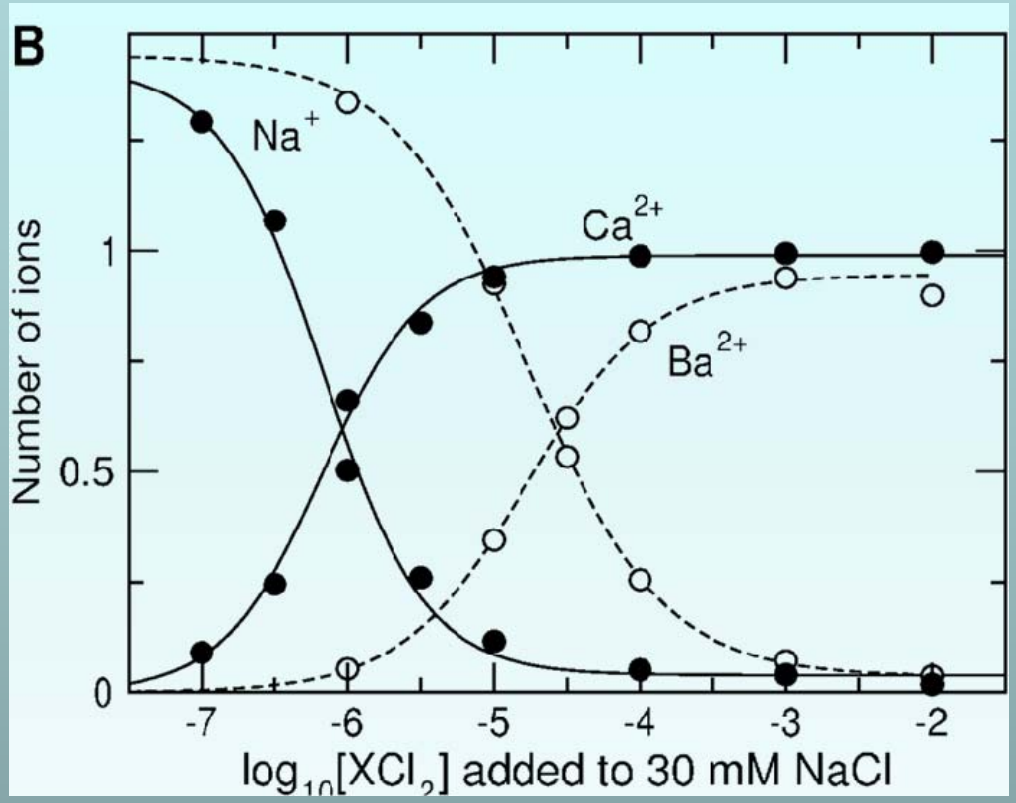
EEEE has full biological selectivity in similar simulations



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

http://www2.phys.rush.edu/RSEisenberg/physioeis.html

Calcium Channel

Summary Simulation Paper (and target for new experiments!) in Experimental Journal

Next, the Sodium Channel

Next, the

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

DEKA Sodium Channel

has very different properties from Ca channel,

e.g., ‘binding’ curve,

Na^+ vs Ca^{++} selectivity

<u>Na^+ vs K^+ selectivity</u>

Challenge

from leading biophysicists

Walter Stühmer and **Stefan Heinemann**

Max Planck Institutes, Göttingen, Leipzig

Can a physical theory explain the mutation DEEA into DEKA?

Ca Channel

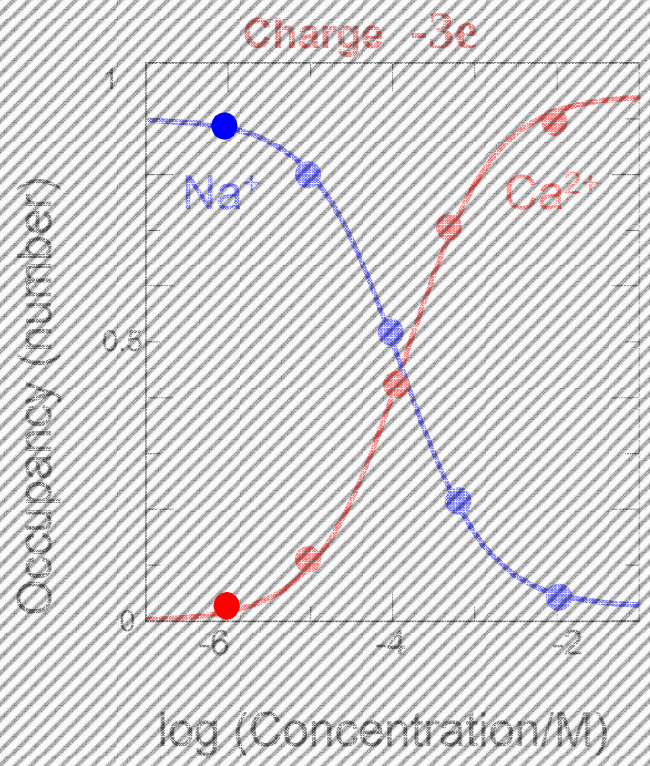
Mutation

Same Parameters

Na Channel

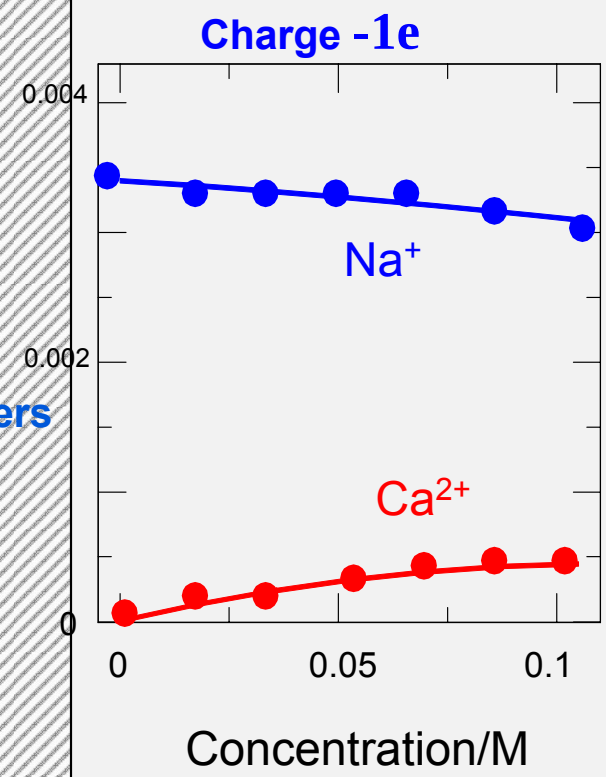
E
E
E
A

D
E
K
A



Mutation

Same Parameters



EEEE has full biological selectivity

in similar simulations

Boda, et al

<u>Nothing was changed</u>

from the

EEEA Ca channel

except the amino acids

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

Calculations and experiments done at pH 8

How?

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

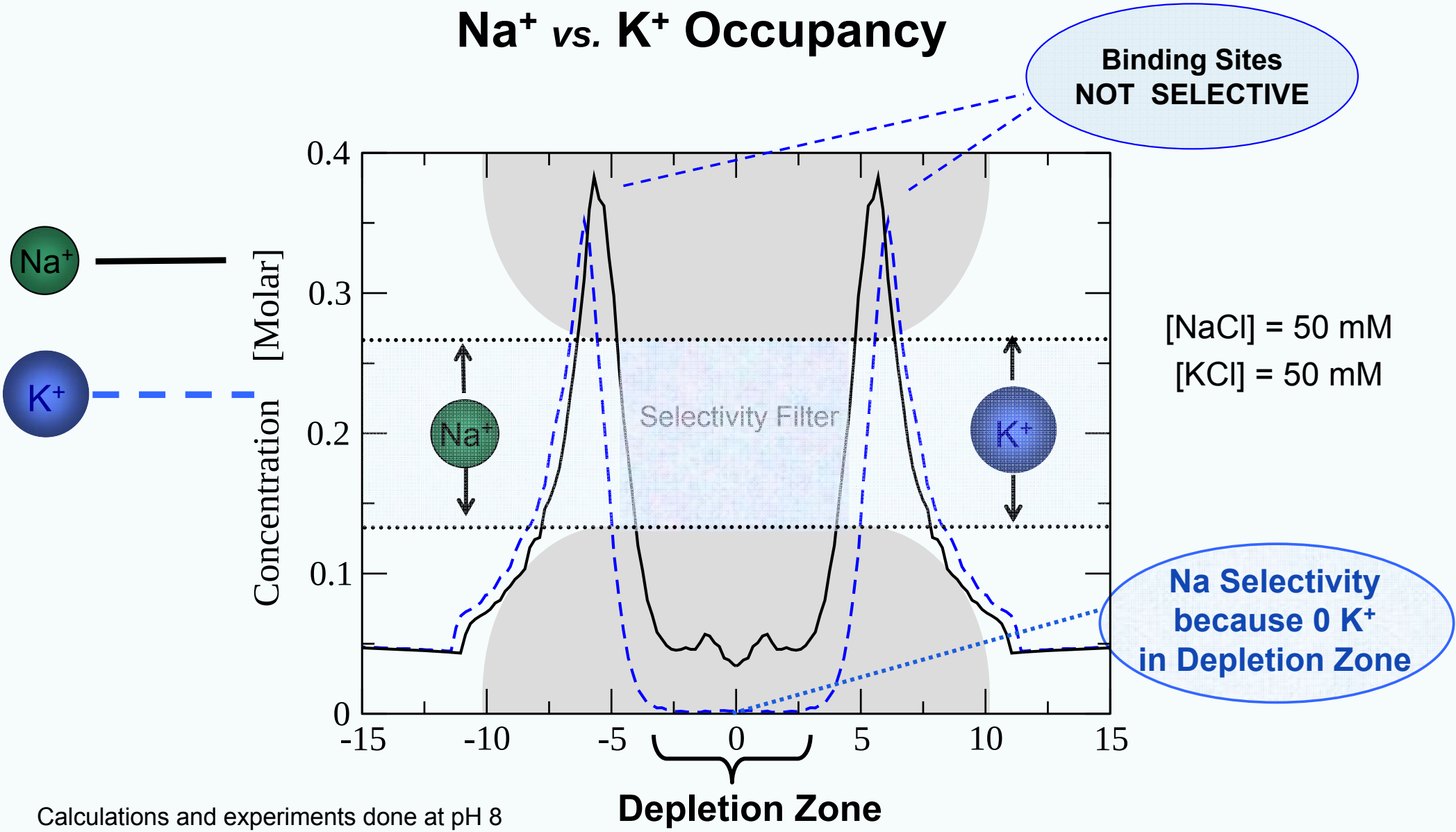
Inverse Problem

“Reverse Engineering”

Calculations and experiments done at pH 8

Size Selectivity is in the Depletion Zone

Na^+ *vs.* K^+ Occupancy



Calculations and experiments done at pH 8

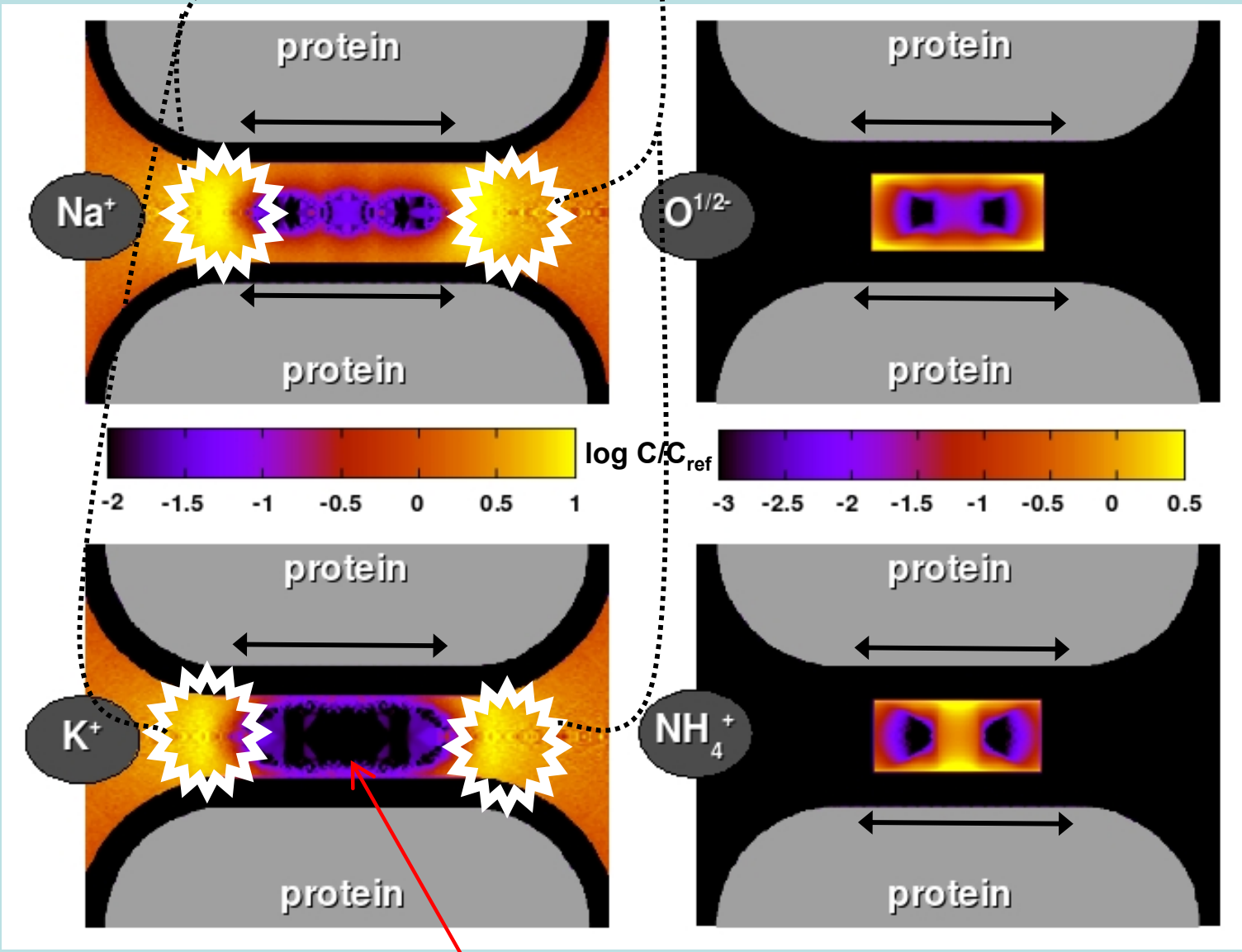
of the **DEKA** Na Channel, **6 Å**

Boda, et al

Size Selectivity

Binding Sites

NOT selective



Na vs K Size Selectivity is in Depletion Zone

BLACK = Depletion=0

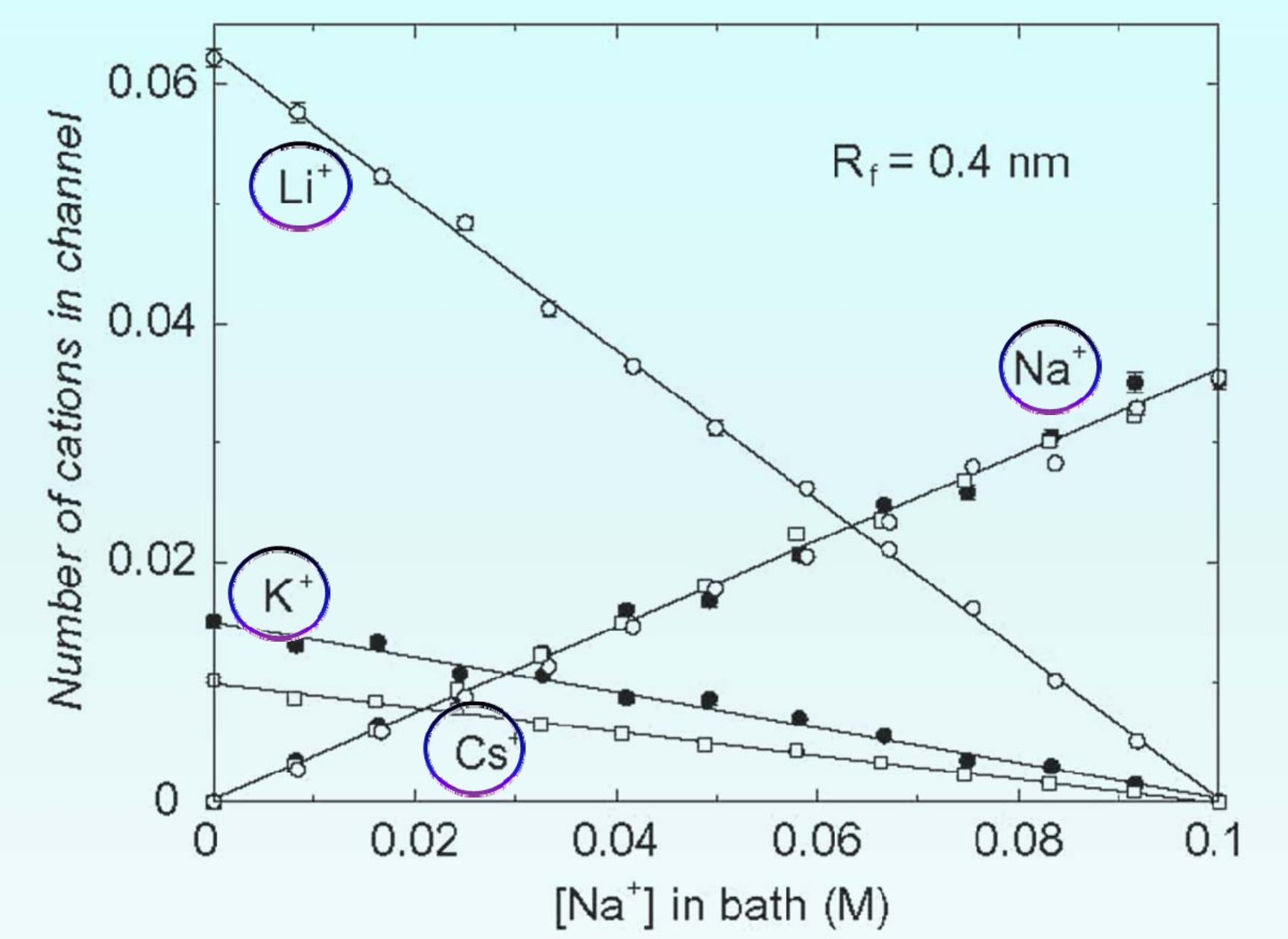
Boda, et al

*Binding Sites are <u>outputs</u> 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
$O^{1/2-}$ D or E	2.80 Å
Na Channel **DEKA** ***6 Å***	

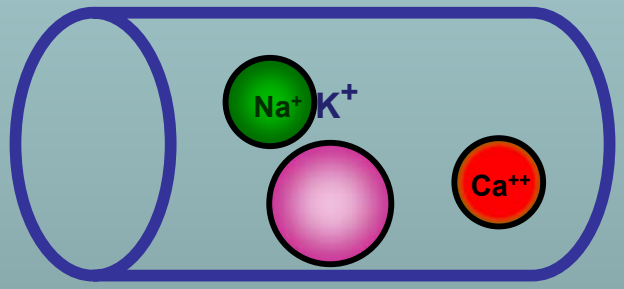
Na, K, Li, Cs Binding in Sodium channel



Control Variables

are obvious in simulations of the Na channel,

but not of the Ca channel*



Control Variables

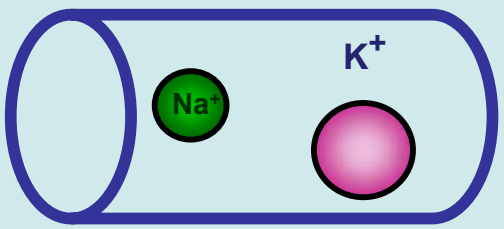
in DEKA Na channel

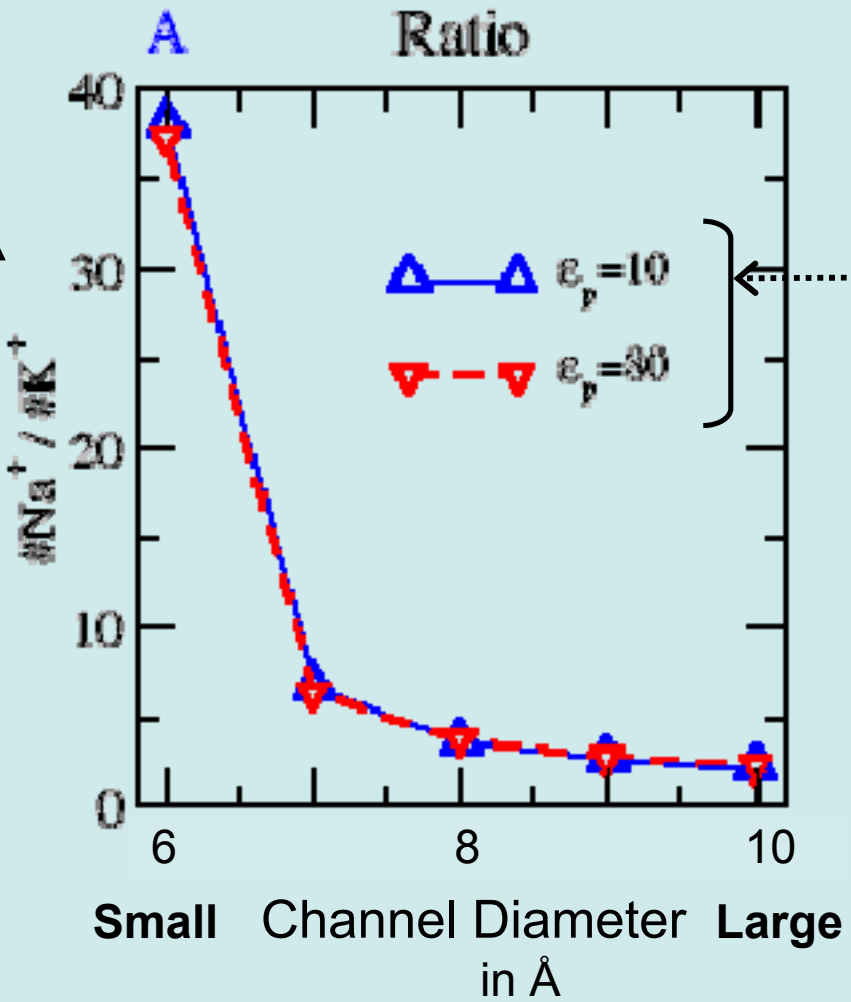
Selectivity *Na^+ vs K^+*
depends only on channel diameter

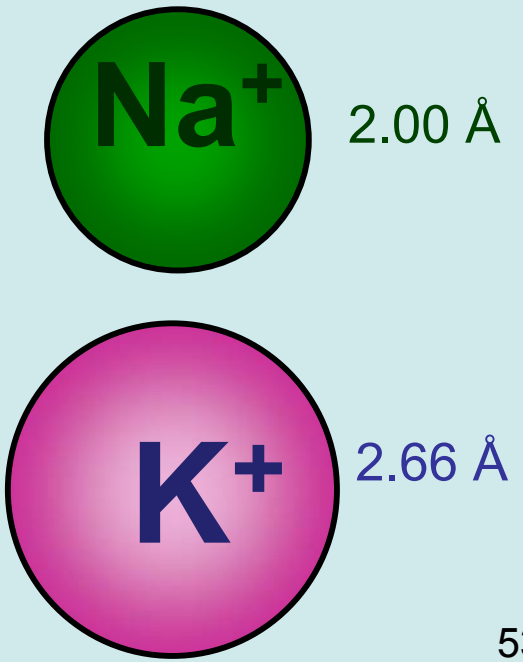
Channel Diameter
controls
Selectivity

Na^+ vs K^+ (size) Selectivity *(ratio)* Depends on Channel Size, *not* Protein Dielectric Coefficient*

Selectivity
for small ion







Boda, et al

*in DEKA Na Channel

Control Variables

in DEKA Na channel

- Selectivity *Na^+ vs K^+*
  depends only on pore diameter

- Conductance* depends on protein polarization

Protein Dielectric Coefficient

controls

Conductance

*Gillespie & Boda (2008) Biophysical Journal 95:2658

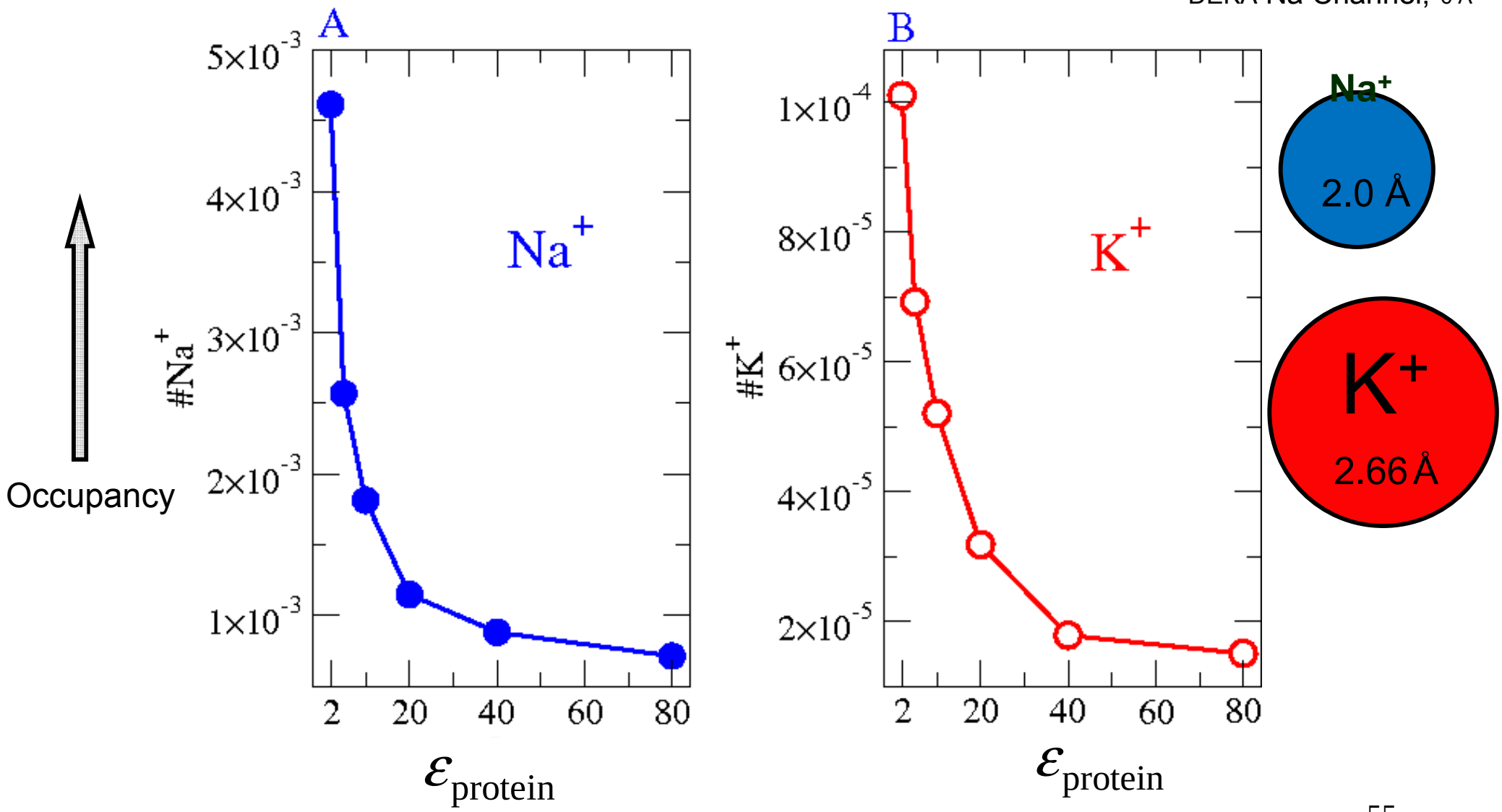
Control Variable

Occupancy

depends on

Protein Dielectric

DEKA Na Channel, 6 Å



Boda, et al

$\varepsilon_{solvent} = 80$

Selectivity

comes from

Electrostatic Interaction

and

Steric Competition for Space

Repulsion

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

Rate Constants are Variables

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?

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

Nonner and Eisenberg

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 <u>no</u> 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

Specificity

"There is only one word that matters in biology and that is specificity.

The truth is in the details, not the broad sweeps."*

The detail can be computed from the correct broadly applicable physics
sometimes†

†Bob Eisenberg's opinion
*Aaron Klug *quoted in the first sentence of Pearson Nature (2008) 455:160–164*

Miracle

We can actually compute the Structures that determine Selectivity

Working Hypothesis

Selectivity Filter Works because

it is a

Self-Organized Structure

with Side Chains at position of Minimum Free Energy

Protein Fits the Substrate

"Induced Fit Model of Selectivity"

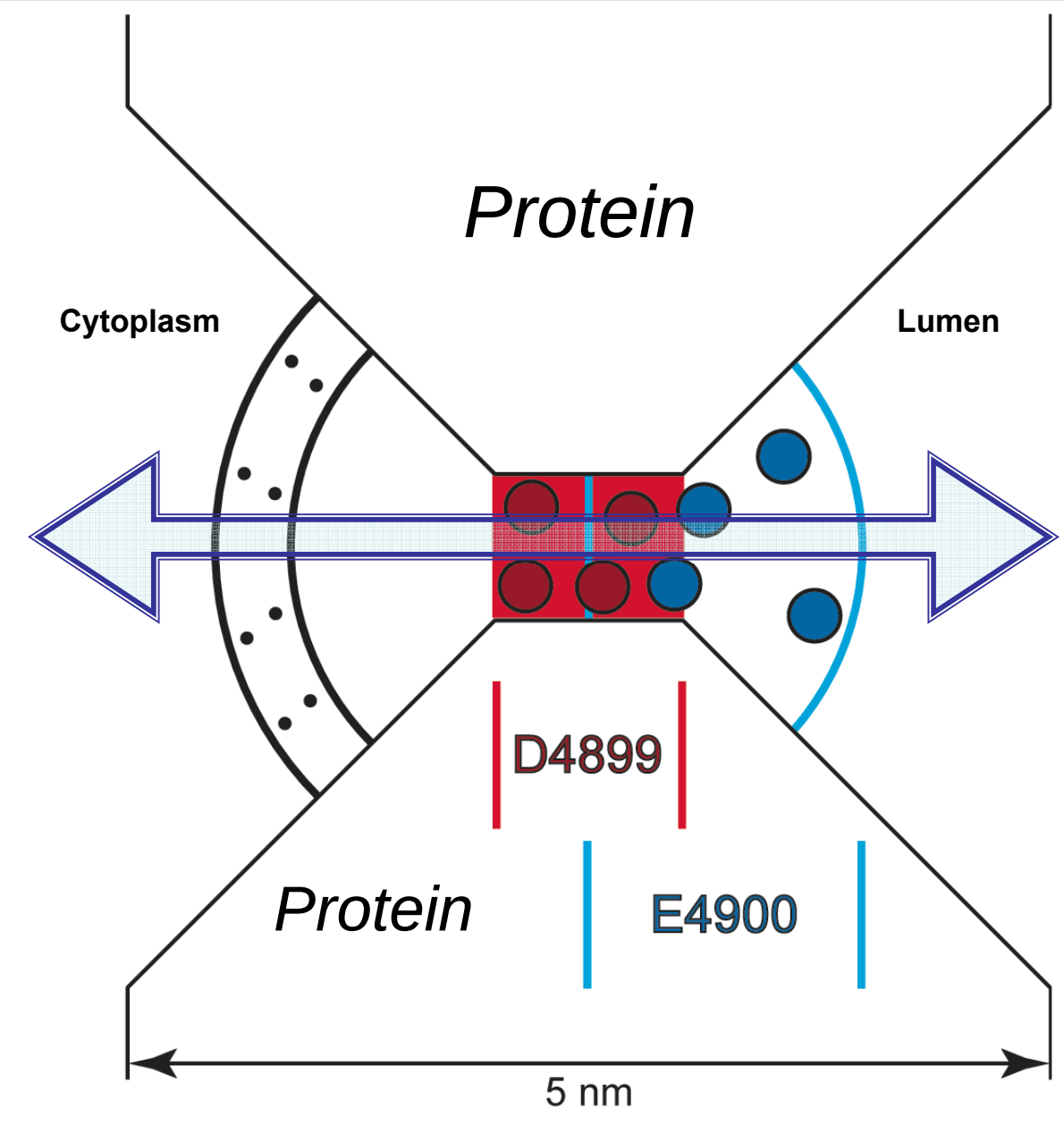
Best Evidence is from the

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.

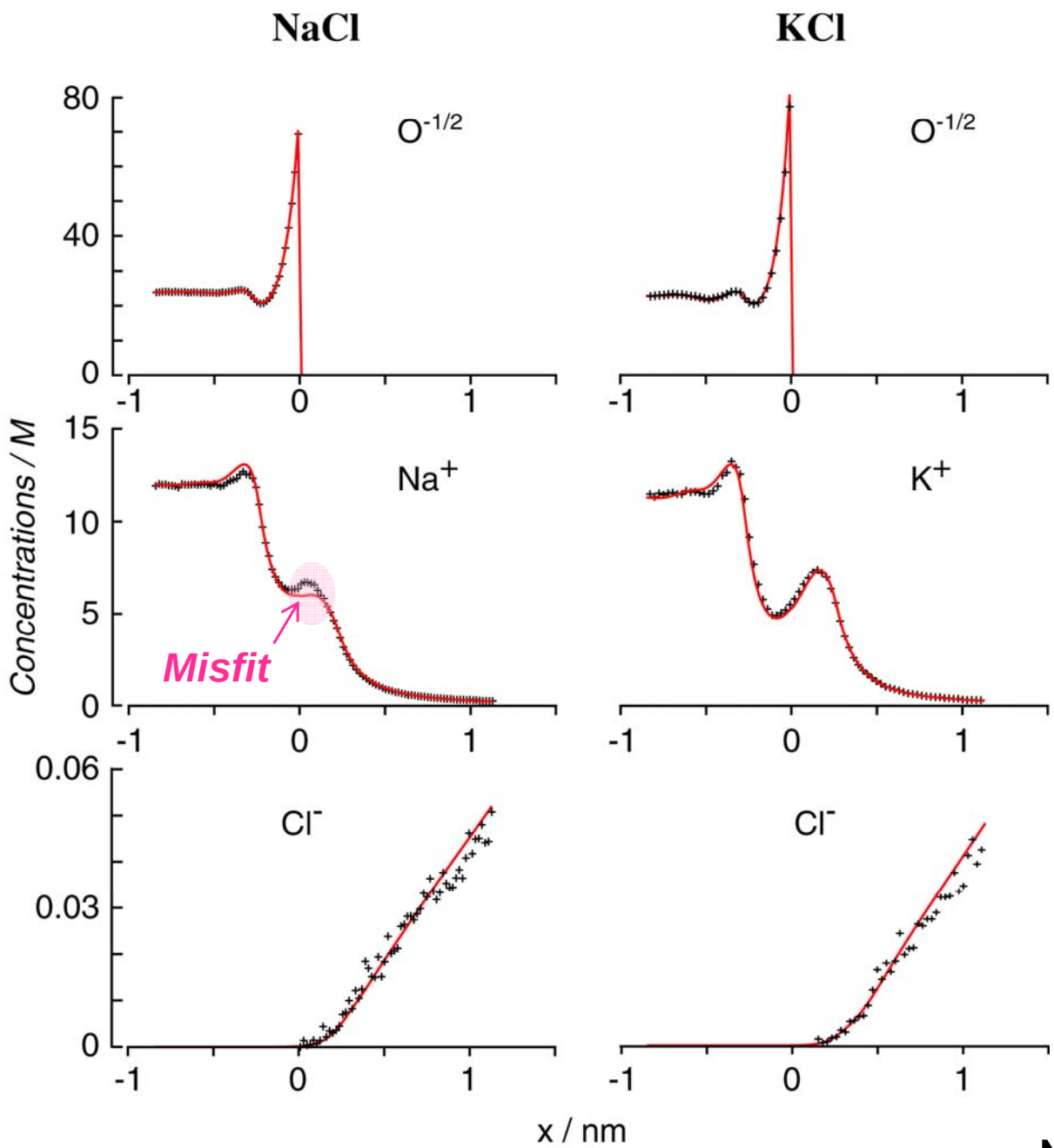
Four **E4900** positive amino acids are on lumenal side, overlapping D4899.

Cytosolic distributed charge

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

DFT/PNP *vs* Monte Carlo Simulations

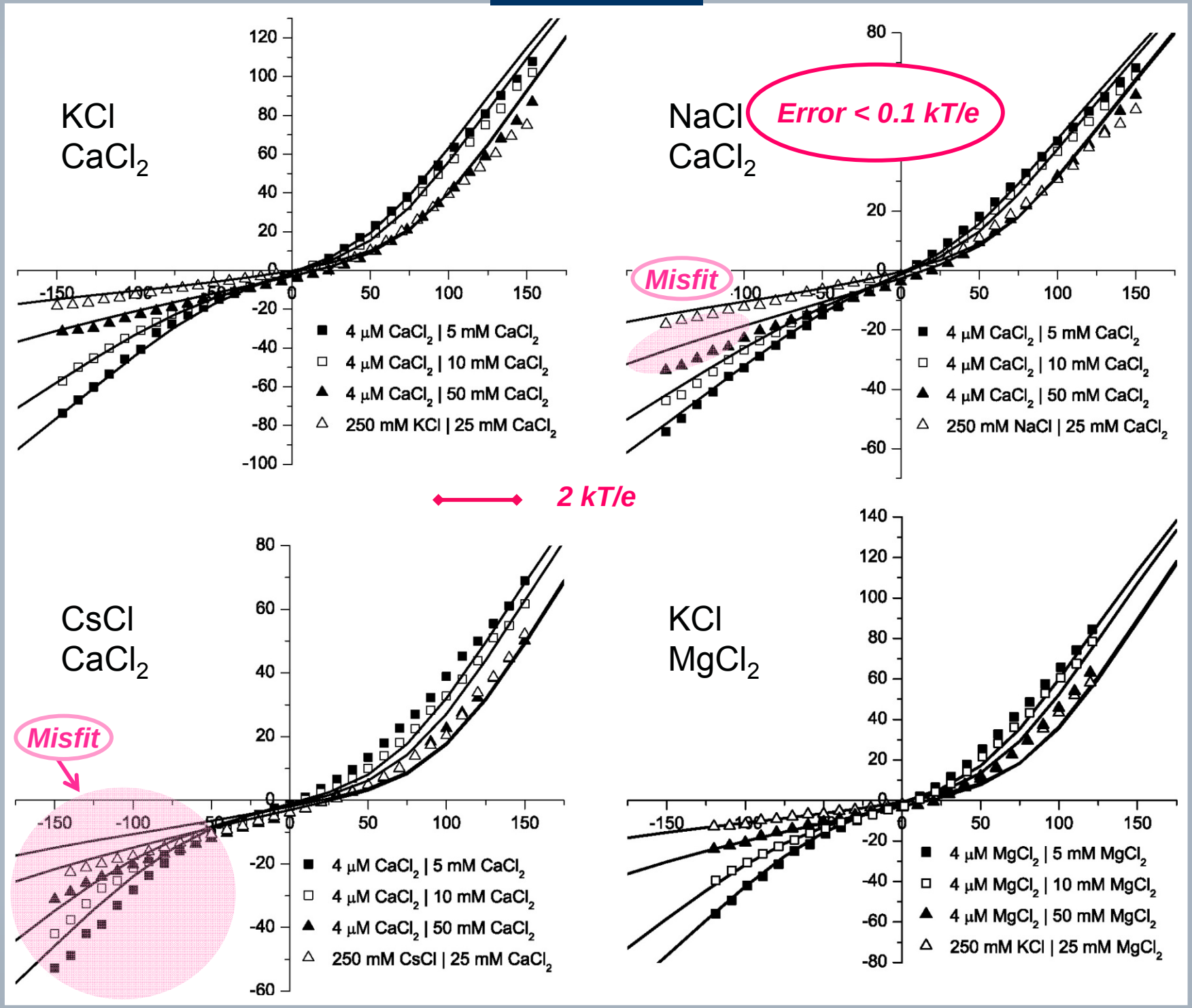
Concentration Profiles



Nonner, Gillespie, Eisenberg

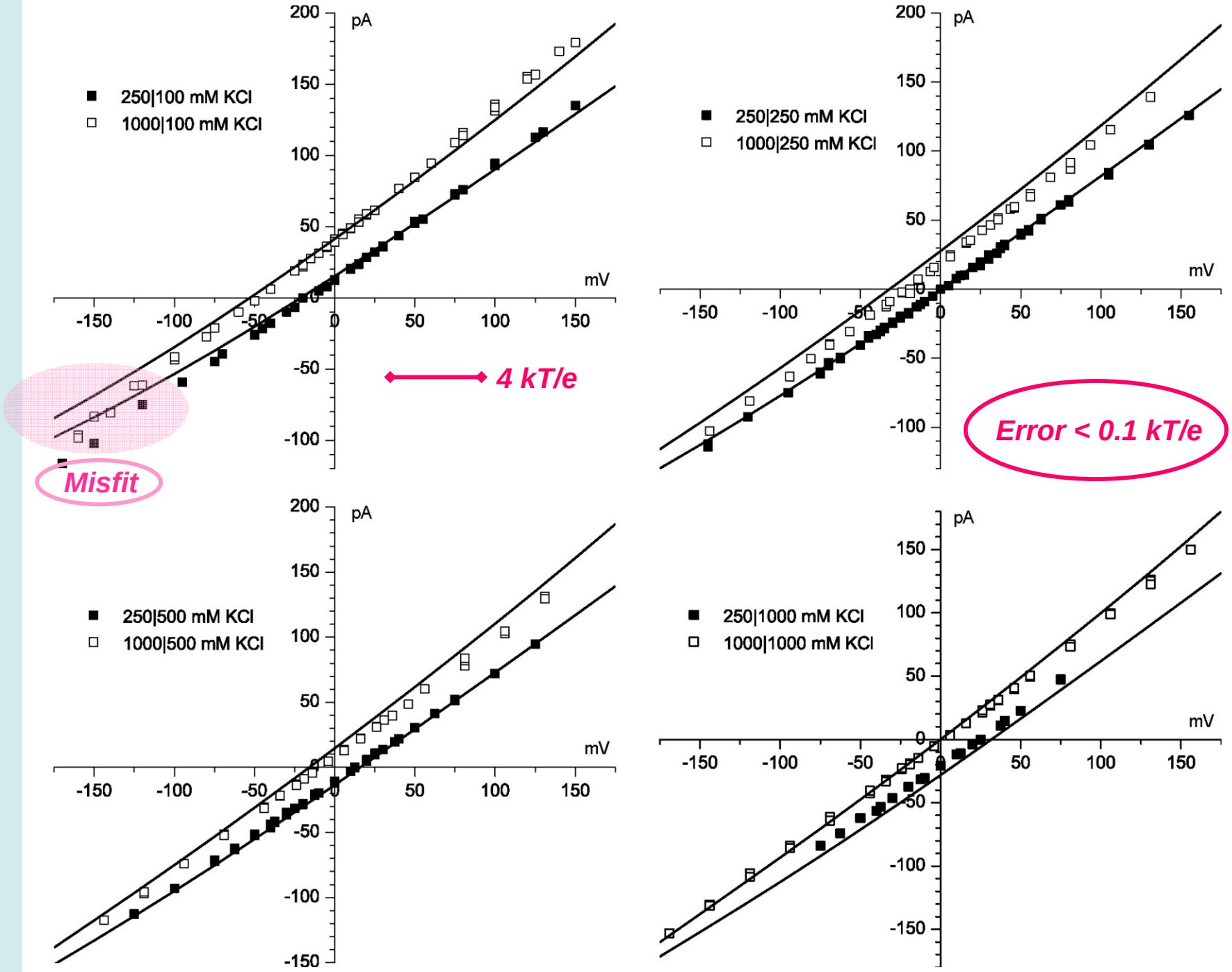
Divalents

Gillespie, Meissner, Le Xu, et al



KCl

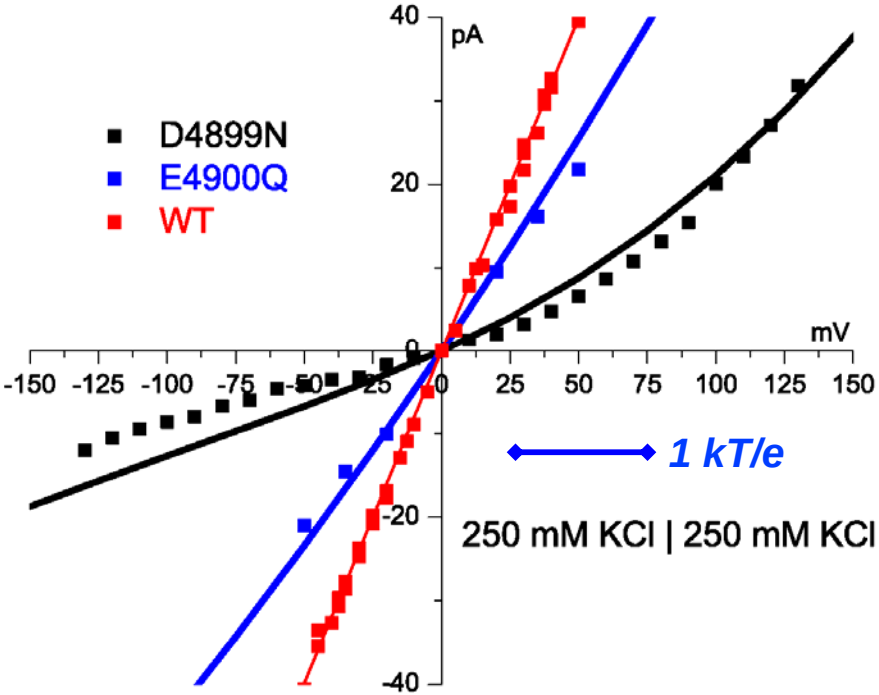
Gillespie, Meissner, Le Xu, et al



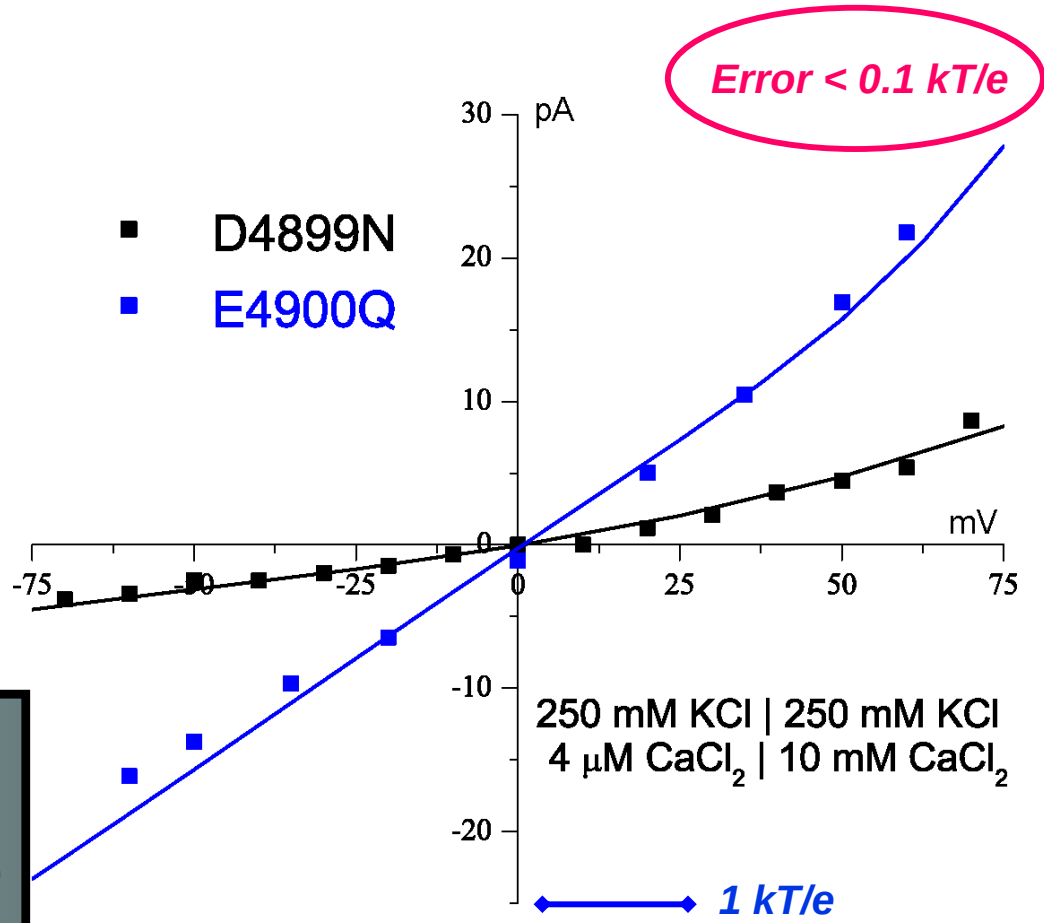
Theory fits Mutation with Zero Charge

No parameters adjusted

Theory Fits Mutant in K



Theory Fits Mutant in K + Ca



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

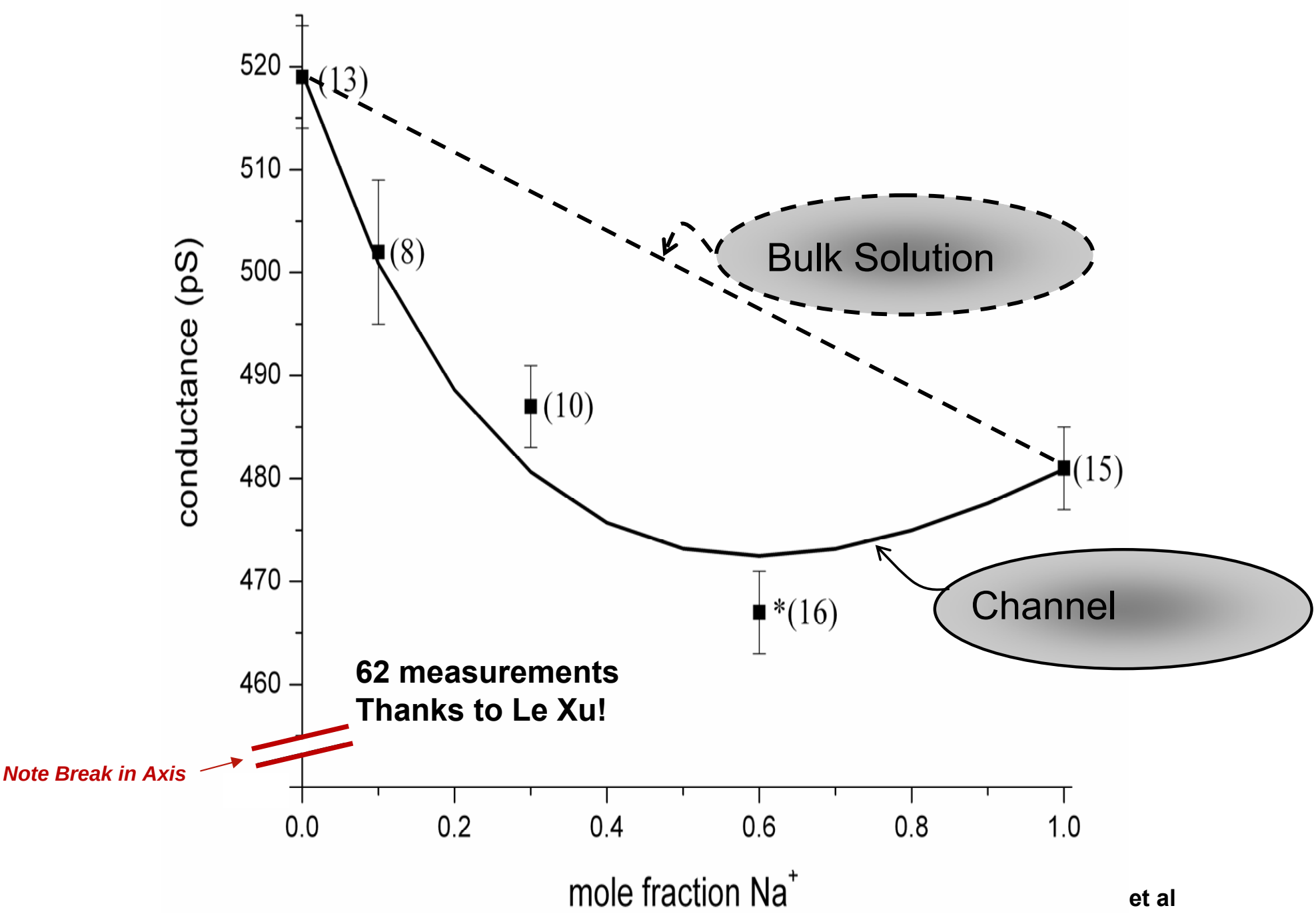
Protein charge density

wild type* 13 M ⇨ 0 M *in* D4899

Water is 55 M

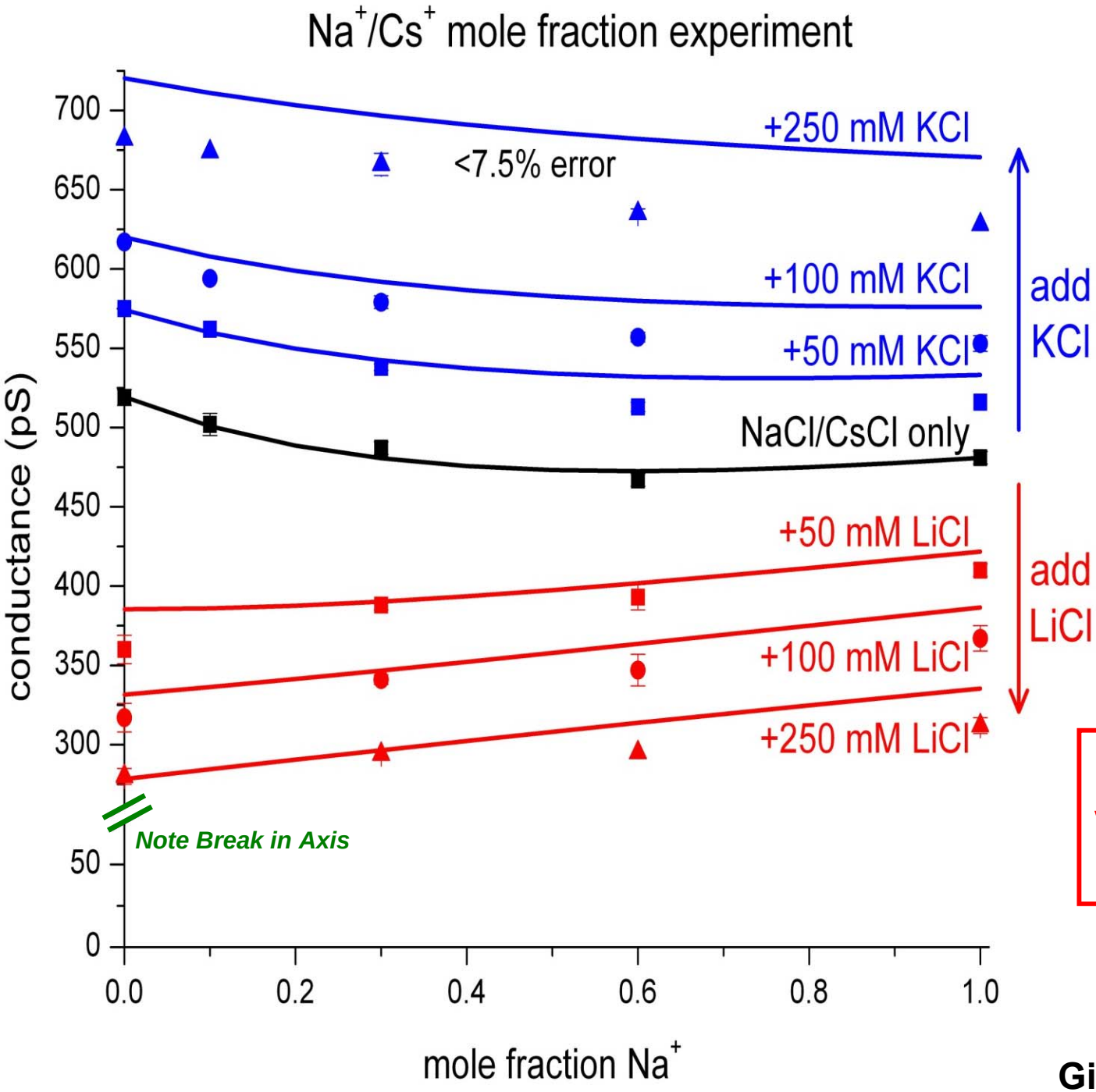
some wild type curves not shown, 'off the graph'

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



et al

The model predicted that AMFE disappears



The Na^+/Cs^+ mole fraction experiment is repeated with varying amounts of KCl and LiCl present in addition to the NaCl and CsCl. The model predicted that the AMFE disappears when other cations are present. This was later confirmed by experiment.

Prediction made without <u>any</u> adjustable parameters.

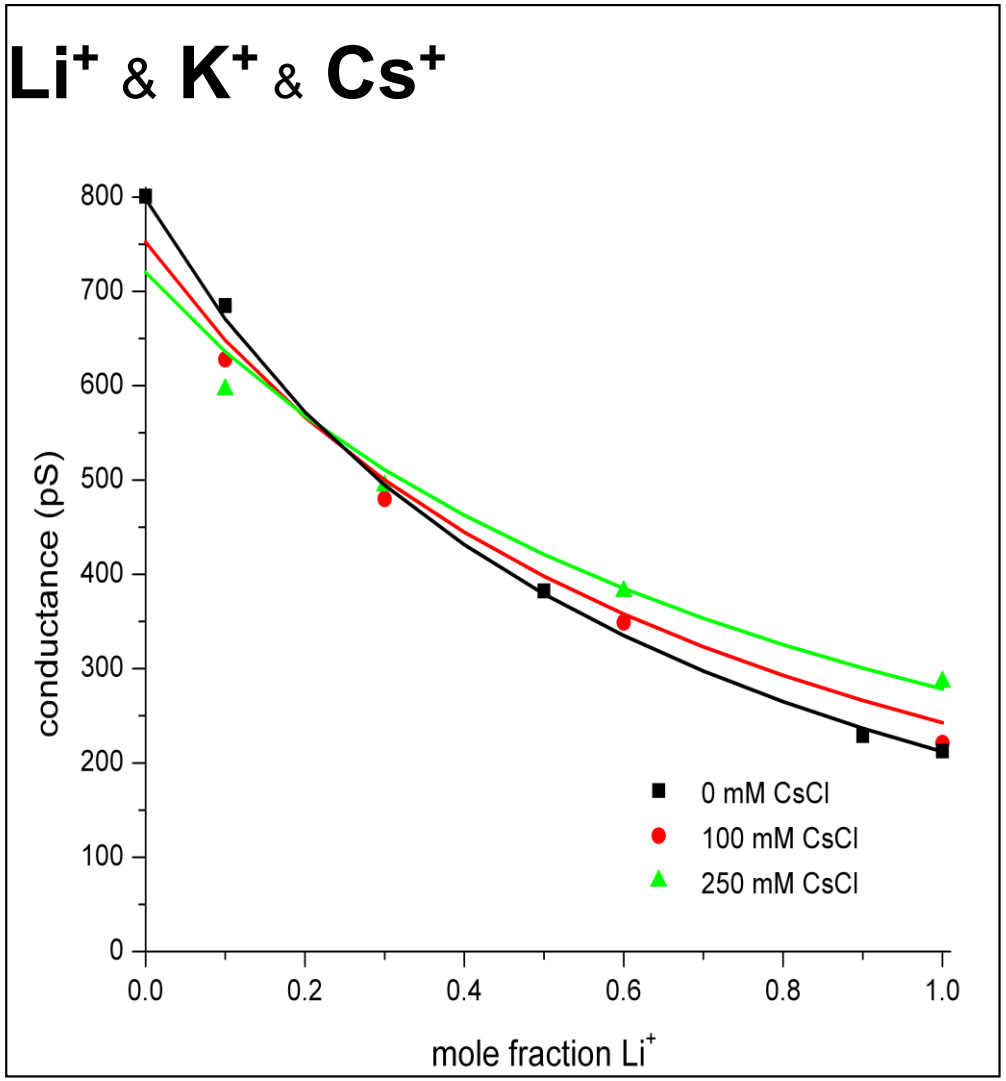
Error < 0.1 kT/e

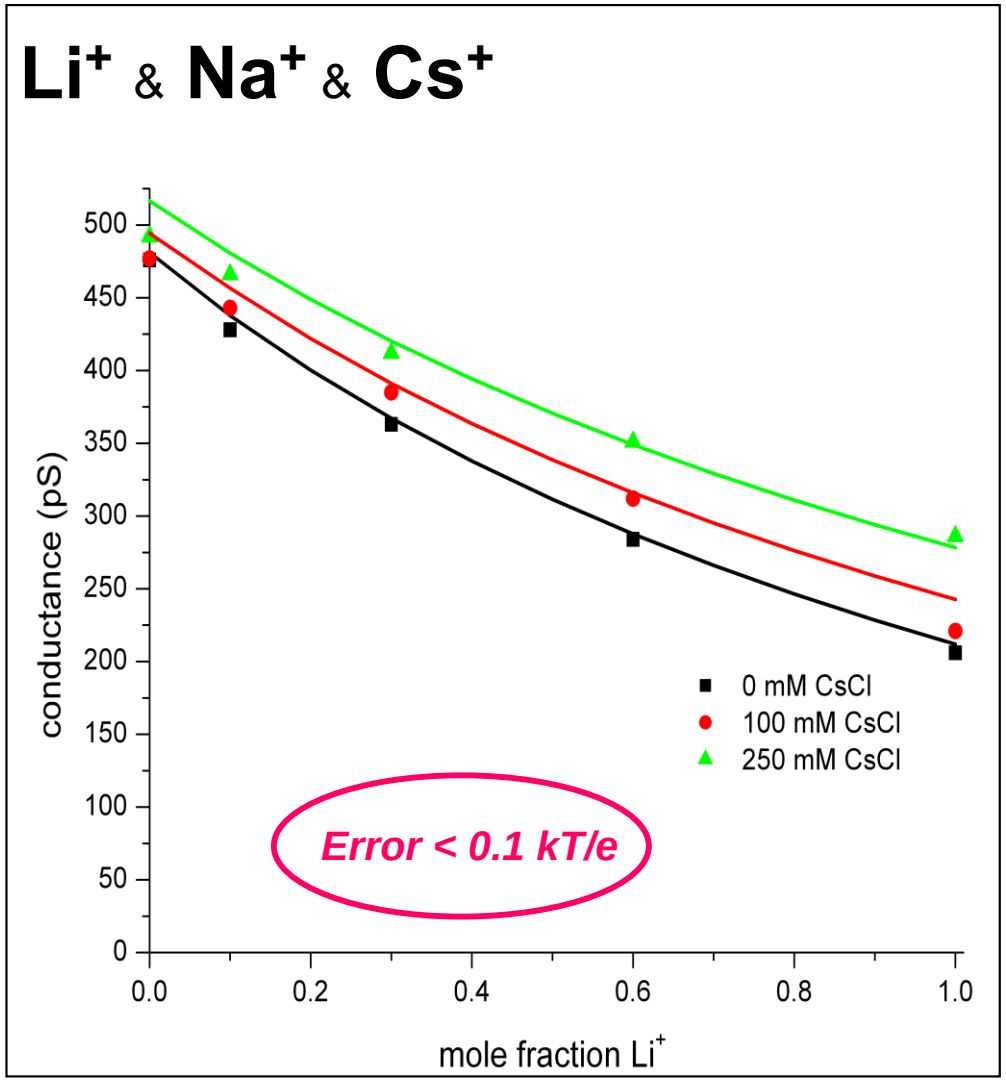
Gillespie, Meissner, Le Xu, et al

Mixtures of THREE Ions

The model reproduced the competition of cations for the pore without <u>any</u> adjustable parameters.

Gillespie, Meissner, Le Xu, et al





Energetic Variational Analysis

EnVarA

being developed by

Chun Liu

Yunkyong Hyon and Bob Eisenberg

creates a

Field Theory of Ionic Solutions

that allows boundary conditions and flow

and deals with

Interactions of Components Self-consistently

Energetic Variational Analysis

EnVarA

Chun Liu, Yunkyong Hyon, and Bob Eisenberg

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

Variational Principle

EnVarA

Generalization of Chemical Free Energy

$$E(\text{Primitive Phase}; t) = \int \Bigg(\underbrace{\{ \overbrace{\tfrac{1}{2}\rho |\vec{u}_{IP}|^2}^{\text{Hydrodynamic Kinetic Energy}} + \overbrace{w(\rho)}^{\text{Hydrodynamc Potential Energy Equation of State}}}_{\textbf{Macroscopic}\text{(hydrodynamic)}}$$

$$+ \lambda \underbrace{\Bigg[\underbrace{\tfrac{1}{2}\varepsilon |\nabla \phi|^2}_{\text{Electrostatic}} + \underbrace{k_B T (c_n \log c_n + c_p \log c_p)}_{\text{Entropy}} + \underbrace{E(\text{Solid Spheres})}_{\text{Finite Size Effect}} \Bigg]}_{\textbf{Microscopic}\text{(atomic)}} \Bigg) d\vec{x}$$

Ionic Solution

Composite of Water and Ionic Fluids

Primitive Model Part 1

Solvent Water Phase

treated as incompressible conductive dielectric

$$\frac{\partial \rho_f}{\partial t} + \nabla \cdot \left(\rho_f \vec{\boldsymbol{u}}_f \right) = 0 \qquad \nabla \cdot \vec{\boldsymbol{u}}_f = 0$$

$$\underbrace{\rho_f \frac{\partial \vec{\boldsymbol{u}}_f}{\partial t}}_{\text{Acceleration}} + \underbrace{\rho_f \vec{\boldsymbol{u}}_f \cdot \nabla \vec{u}}_{\text{Convective Acceleration}} + \underbrace{\nabla p_f}_{\text{Pressure Gradient}} = \underbrace{M_f \nabla^2 \vec{\boldsymbol{u}}_f}_{\text{Viscosity}} + \underbrace{k(\vec{\boldsymbol{u}} - \vec{\boldsymbol{u}}_f)}_{\text{Coupling Drag}}$$

$$\frac{\partial \rho}{\partial t} + \nabla \cdot (\vec{\boldsymbol{u}} \rho) = 0$$

Eisenberg, Hyon, and Liu

Ionic Solution

Primitive Model Part 2

Macroscopic and atomic scale combined.

Ions in incompressible conductive dielectric

Acceleration ***Convective Acceleration*** ***Pressure Gradient***

$$\rho\left(\frac{\partial \vec{\boldsymbol{u}}}{\partial t}+\vec{\boldsymbol{u}}\cdot\nabla\vec{\boldsymbol{u}}\right)+\nabla p$$

$$=M\nabla^2\vec{\boldsymbol{u}}+\overbrace{k(\vec{\boldsymbol{u}}-\vec{\boldsymbol{u}}_f)}^{\text{Coupling Drag}}+\overbrace{(c_n(\vec{\boldsymbol{x}})-c_p(\vec{\boldsymbol{x}}))\nabla\phi}^{\text{Coulomb Force}}$$

Lennard Jones Solid Sphere

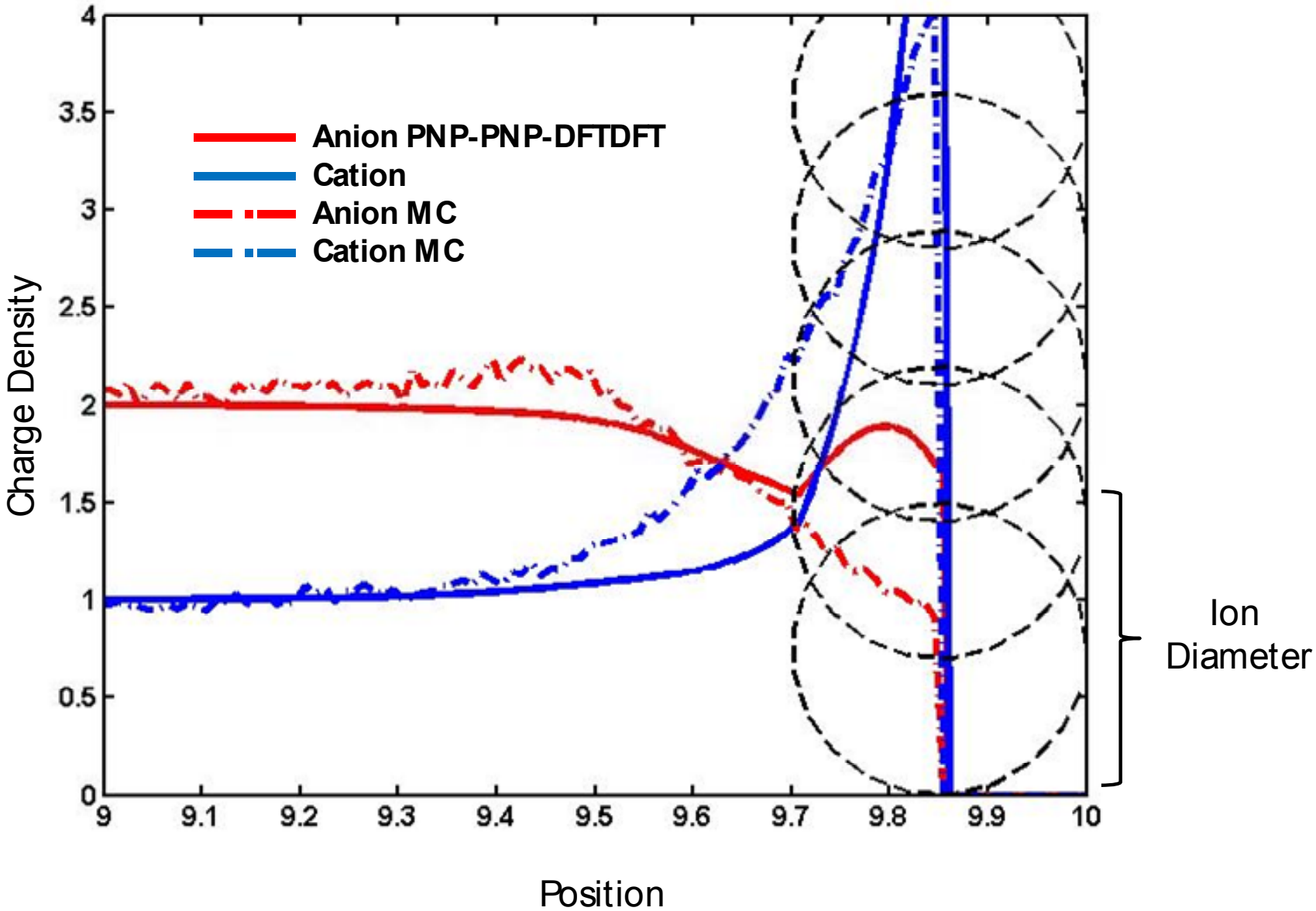
$$\left\{\begin{array}{l} -c_n(\vec{\boldsymbol{x}})\nabla\cdot\int\chi\left(|\vec{\boldsymbol{x}}-\vec{\boldsymbol{y}}|\right)\left(c_n(\vec{\boldsymbol{y}})+\tfrac{1}{2}c_p(\vec{\boldsymbol{y}})\right)d\vec{\boldsymbol{y}} \\ -c_p(\vec{\boldsymbol{x}})\nabla\cdot\int\chi\left(|\vec{\boldsymbol{x}}-\vec{\boldsymbol{y}}|\right)\left(\tfrac{1}{2}c_n(\vec{\boldsymbol{y}})+c_p(\vec{\boldsymbol{y}})\right)d\vec{\boldsymbol{y}} \end{array}\right.$$

$$c(|\vec{\boldsymbol{x}}-\vec{\boldsymbol{y}}|)=e_{i,j}\left(\frac{a_i+a_j}{|\vec{\boldsymbol{x}}-\vec{\boldsymbol{y}}|}\right)^{12}$$

Eisenberg, Hyon, and Liu

Layering: Classical Interaction Effect

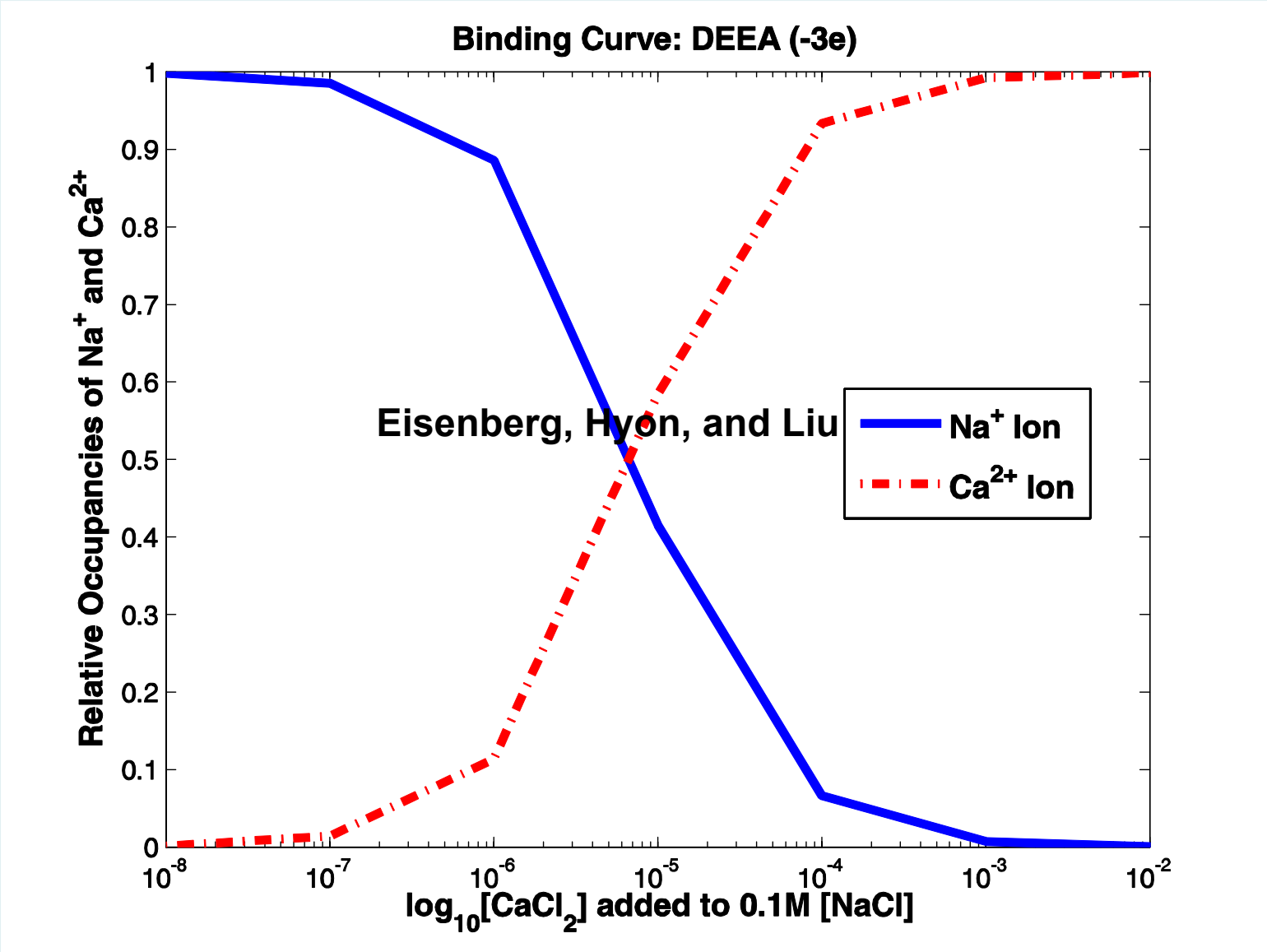
Comparison between PNP-DFT and MC



Eisenberg, Hyon, and Liu

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

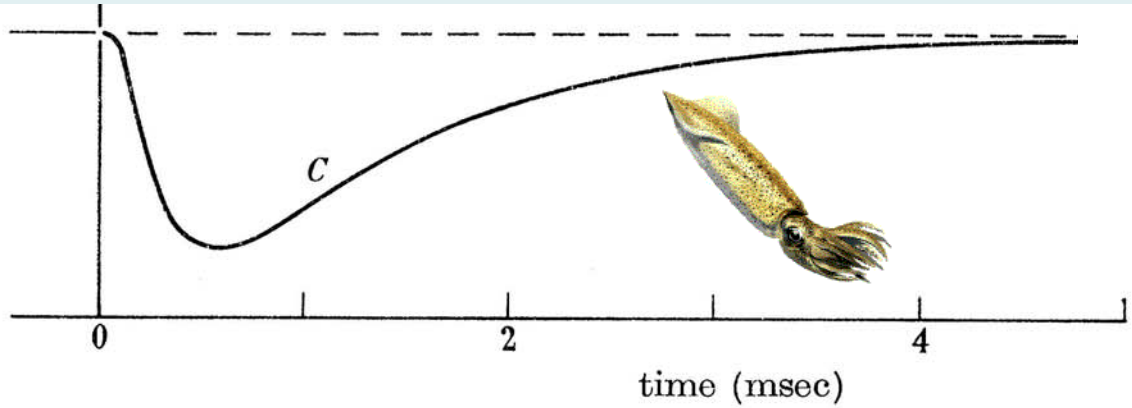
DEEA Calcium Channel



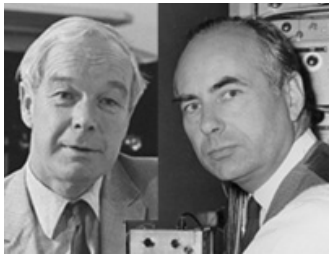
Eisenberg, Hyon, and Liu

Sodium Conductance and **Inactivation**

in Squid Axon (nerve fiber)



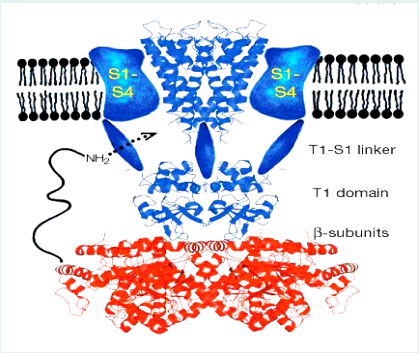
Hodgkin Huxley

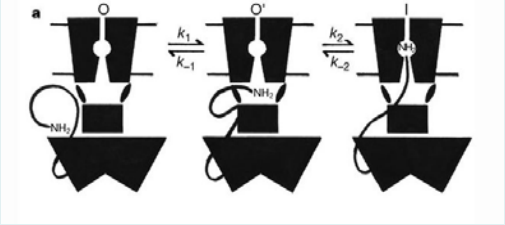


J. Physiol (1952)
116:497

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.

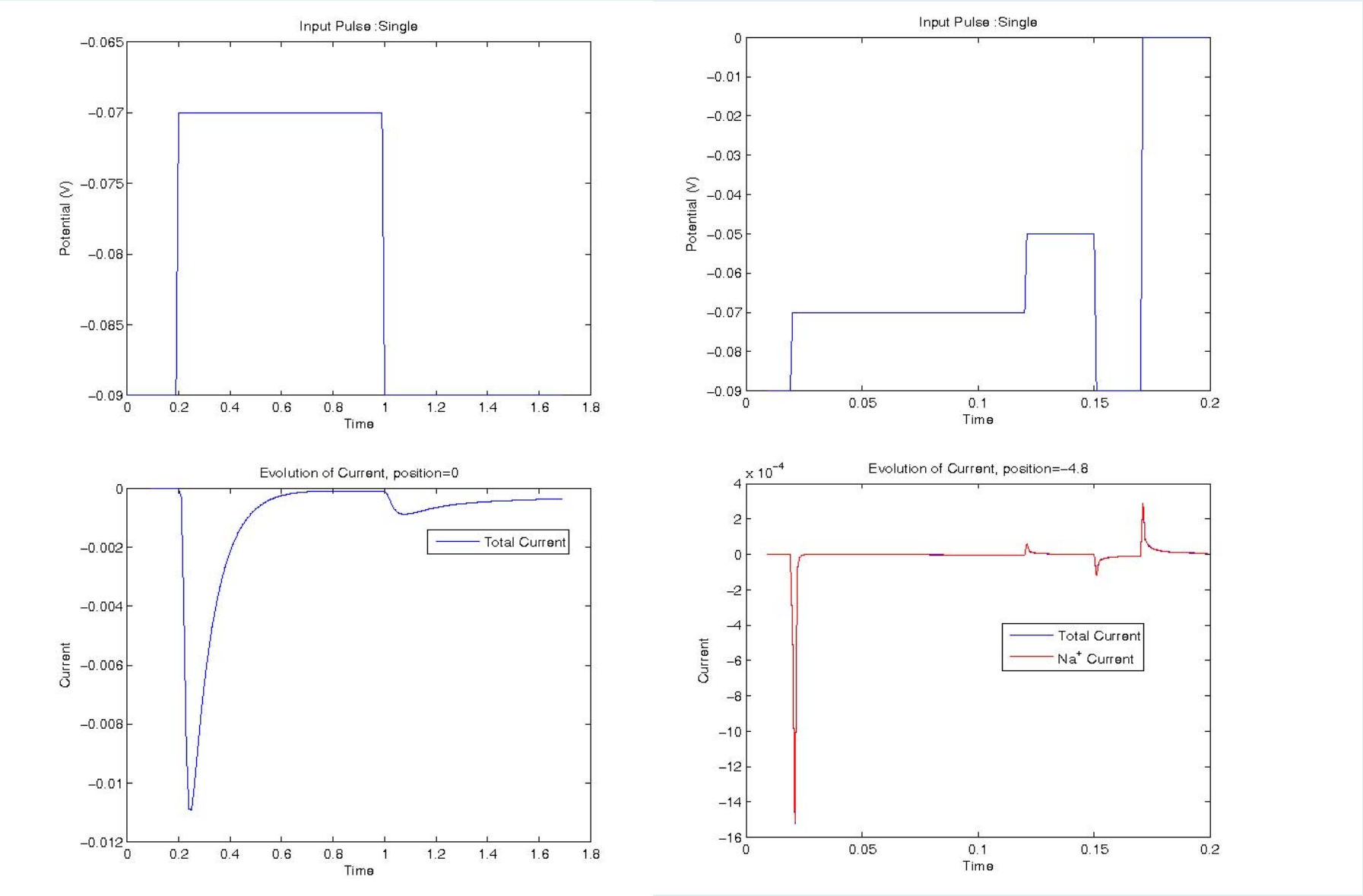
Energetics of Brain determined by details of inactivation*

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 <u>Fixed Structure</u>



Eisenberg, Hyon, and Liu

Energetic Variational Analysis

EnVarA

Chun Liu, Yunkyong Hyon and Bob Eisenberg

New Interpretations

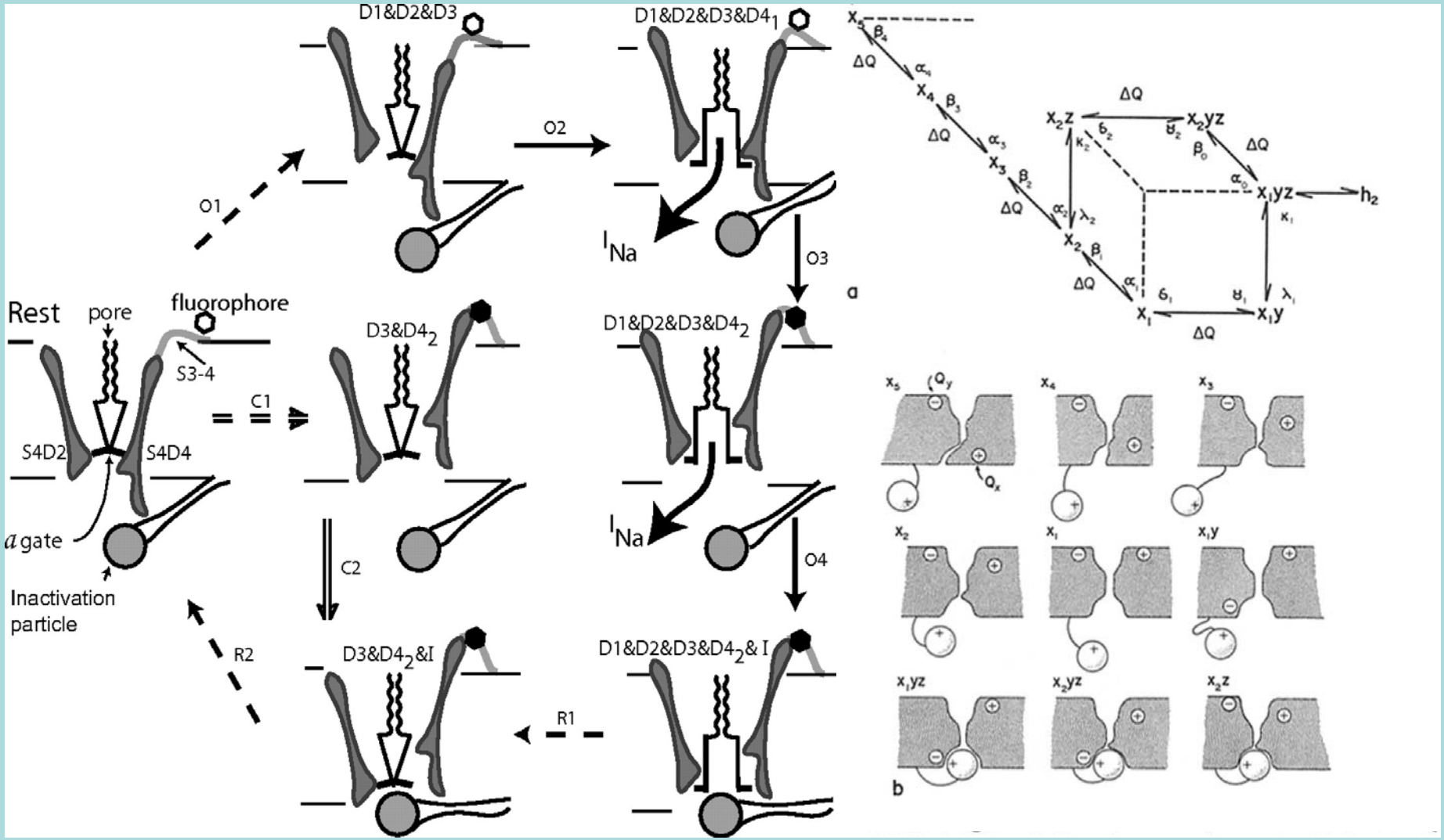
likely to be

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

Energetic Variational

Approach

EnVarA

New mechanisms* 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.**

Eisenberg, Hyon, and Liu

Can Variational Approach actually calculate Complex* Biology?

Rolf Ryham and Chun Liu have actually calculated

***Movies of Vesicle Fusion**

in preliminary work

with Fred Cohen and Bob Eisenberg

Vaccinations against Traditional Models

Vaccination 1:

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

Vaccination 2:

No gas phase models of traditional channel biochemistry

Liquids are not Gases

No discussions of individual trajectories of Structural Biologists

Counting and Statistics are essential

Vaccination 3:

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

Vaccination 4:

Computation Starts
From Crystal Structure
when available

but

Crystal Structures can<u>not</u> 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**

Vaccination 5:

Simulations must be calibrated against experimental data.

Simulations are not mathematics.

Molecular Dynamics is science not mathematics.

Vaccination 6:

Law of Mass Action is usually misused.

<u>Rate Constants are almost never constant</u>
as conditions are changed.
They often change by factor of 10^4 !!

$$A \underset{k_b}{\overset{k_f}{\rightleftharpoons}} B$$

$$-\frac{d}{dt}[A] = k_f\,[A] \qquad J_{A\to B} = k_f[A]$$

$$-\frac{d}{dt}[B] = k_b\,[B] \qquad J_{B\to A} = k_b[B]$$

Differential equations are almost always solved assuming k_f and k_b are constants.

[A] means the concentration of species A, i.e., the number density of A