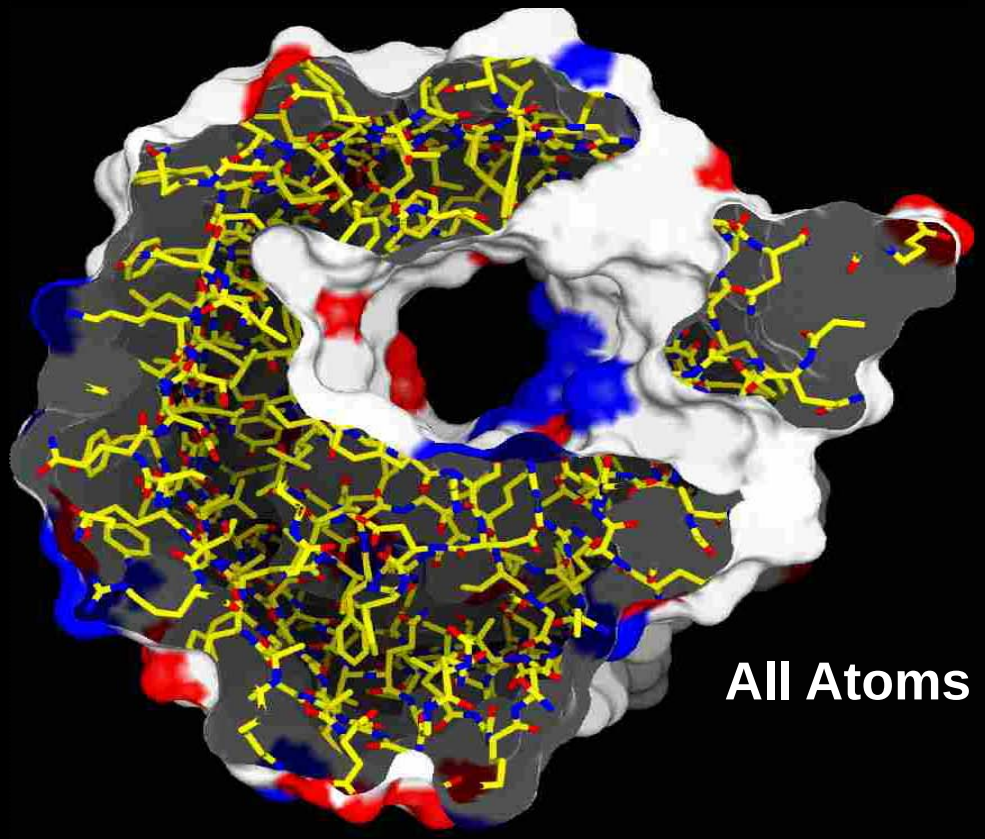
Ion Channels

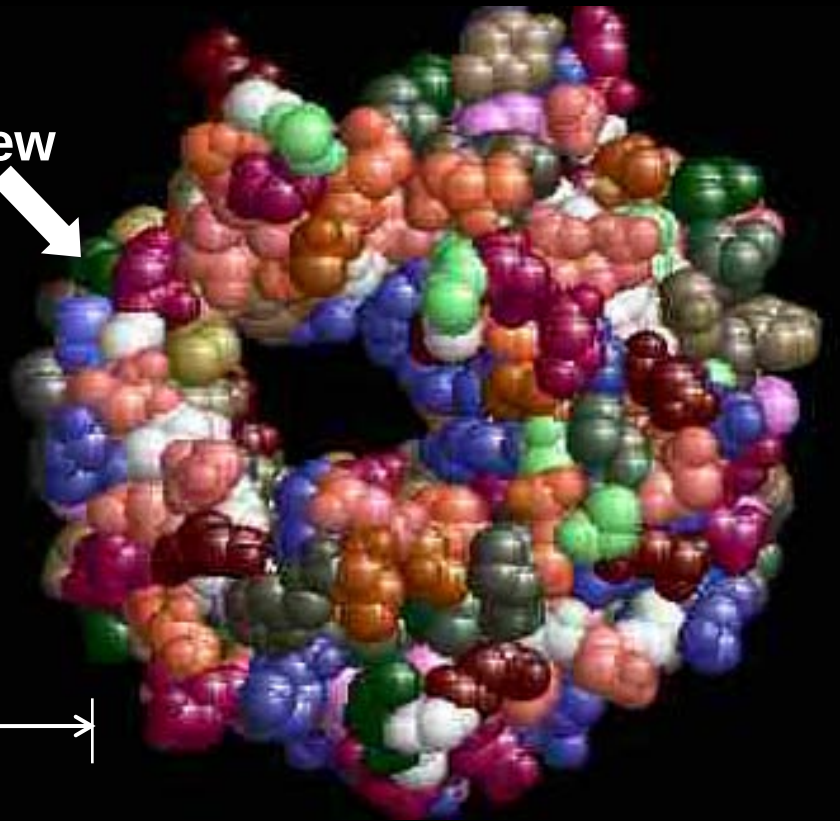
Proteins with a Hole

Figure by Raimund Dutzler

Chemist's View



All Atoms View

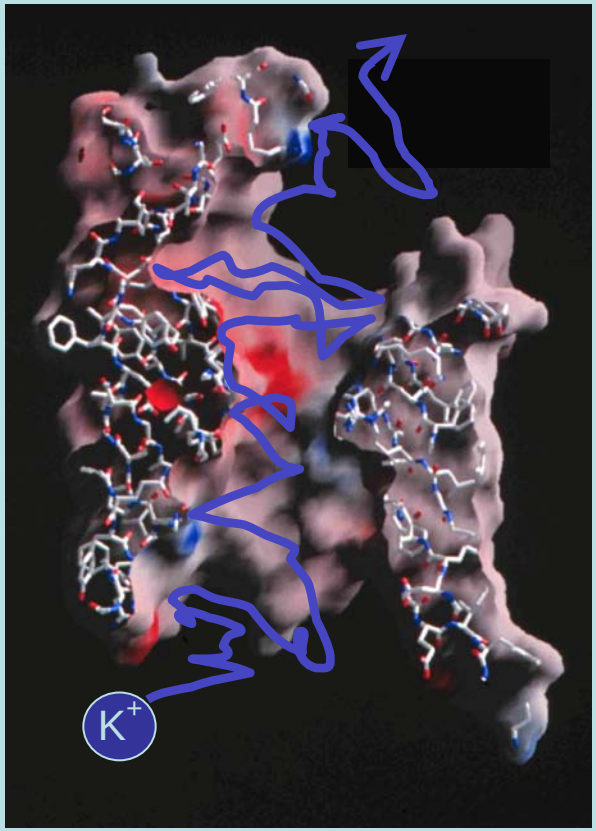


Chemical Bonds are lines
Surface is Electrical Potential
Red is negative = acid
Blue is positive = base

~30 Å

Ion Channels are Biological Devices, the Valves* of Cells

Main Controllers of Biological Function



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

Ions in Water

are the

Liquid of Life

Life Occurs in ~130 mM salt solutions

Ionic Solutions are NOT ideal.

Chemically Specific Properties of Ionic solution are their DEVIATION from IDEAL

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

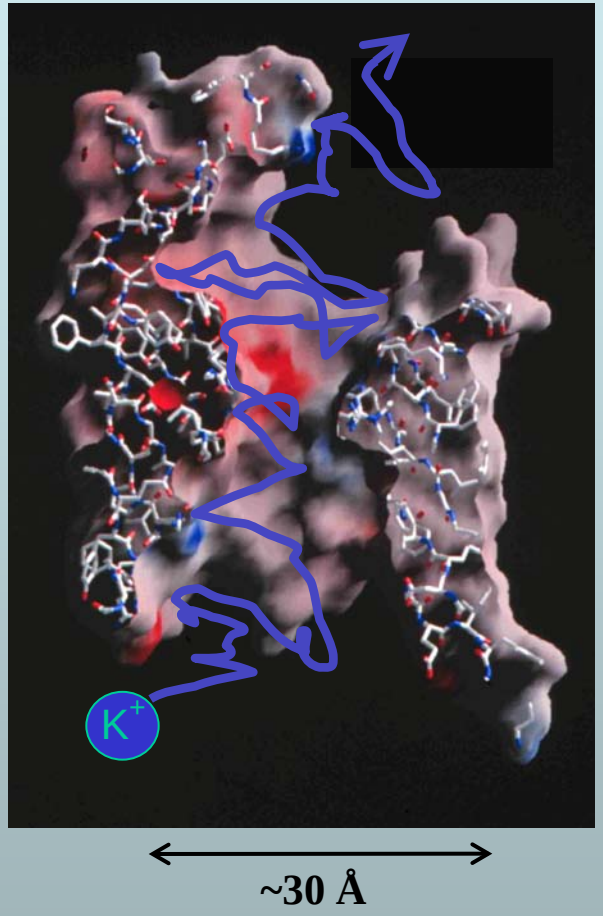
Ion channels produce signals of the nervous system

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

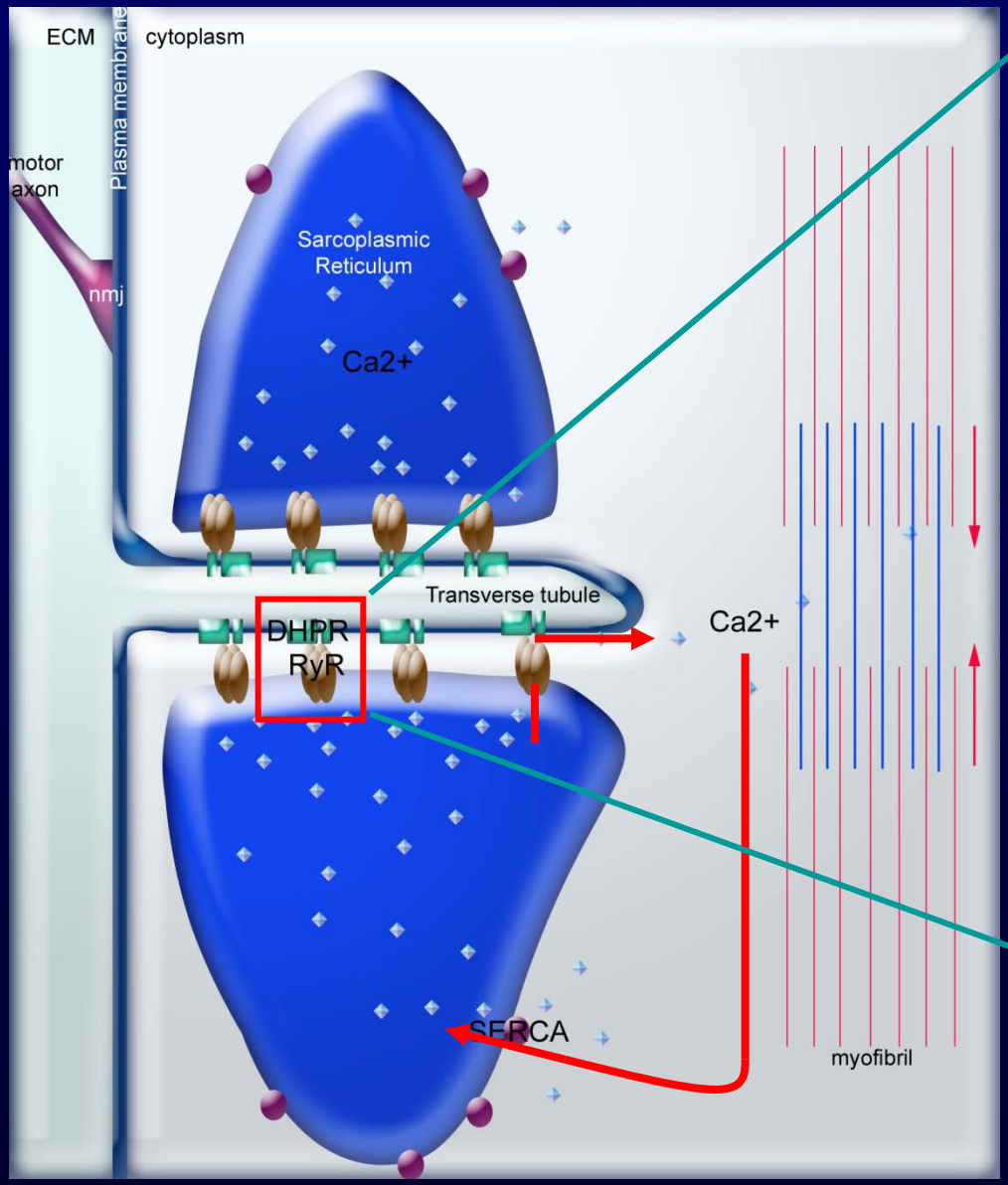
Ion channels have structures shown by x-ray crystallography in favorable cases.

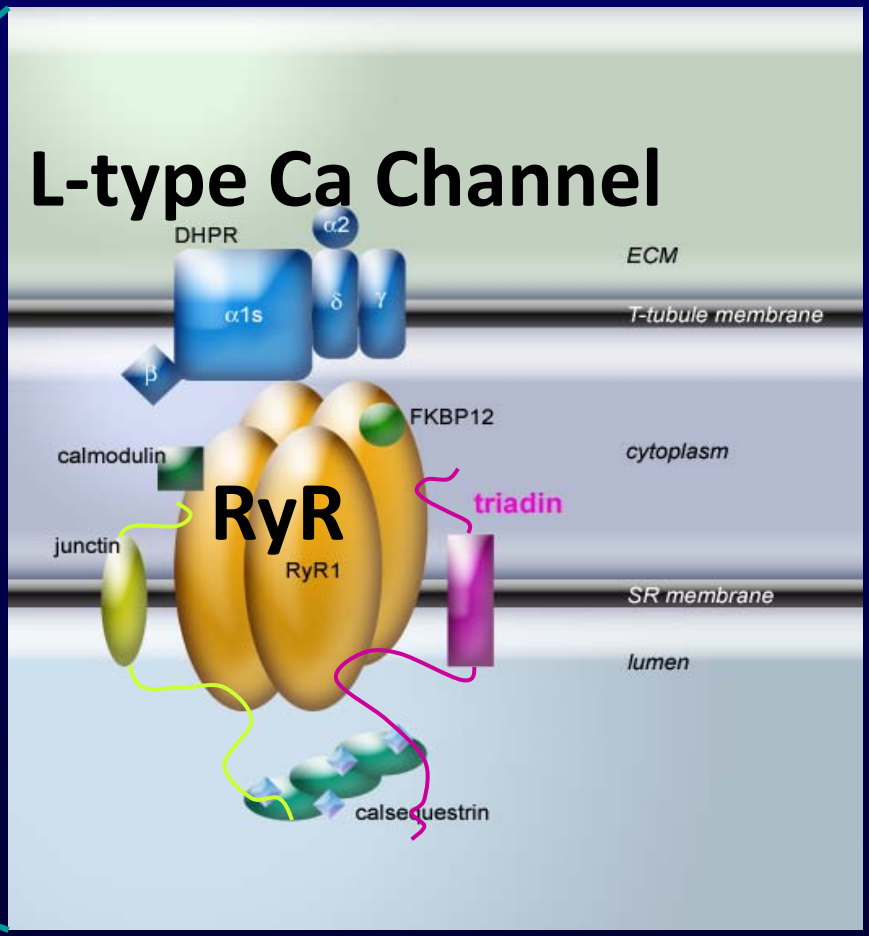


*nearly pico-valves: diameter is 400 - 900 pico-meters

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

L type Ca Channel RyR ryanodine receptor





László Csernoch, Debrecen, Hungary

Isabelle Marty, Grenoble, France

Goal:
Understand Selectivity

Selectivity Differs in Different Types of Channels

Wolfgang Nonner, Dirk Gillespie, Douglas Henderson, Dezső Boda

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

RyR model of Gillespie is best worked out for ~ 120 solutions

Selectivity of K Channel is studied in ~1 solution at infinite dilution

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

“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

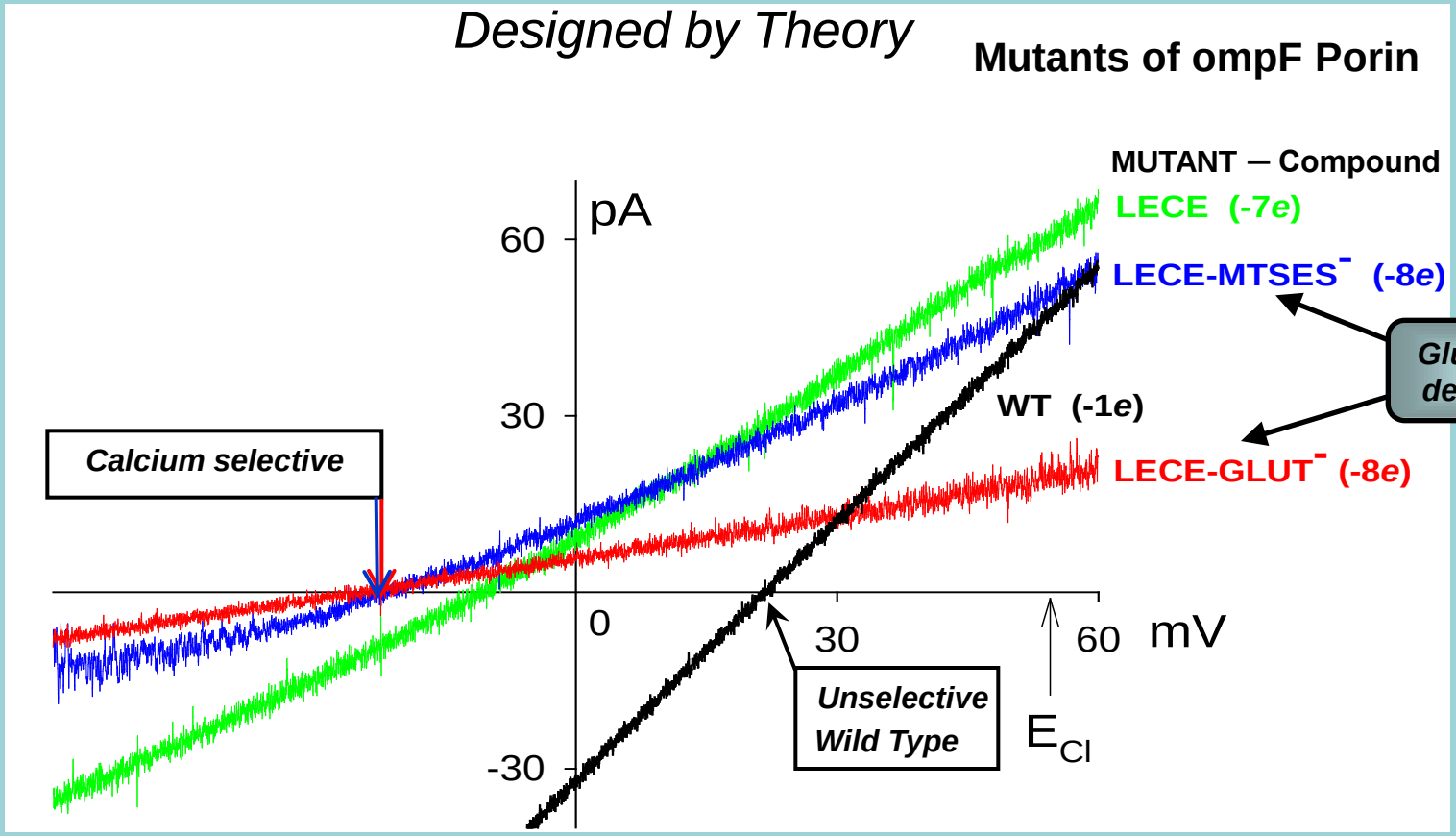
and to

Make a Calcium Channel

Experiment

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?

<u>Not</u> with gas phase models of traditional channology

Liquids are not Gases

<u>Not</u> with guesses about trajectories of structural biologists

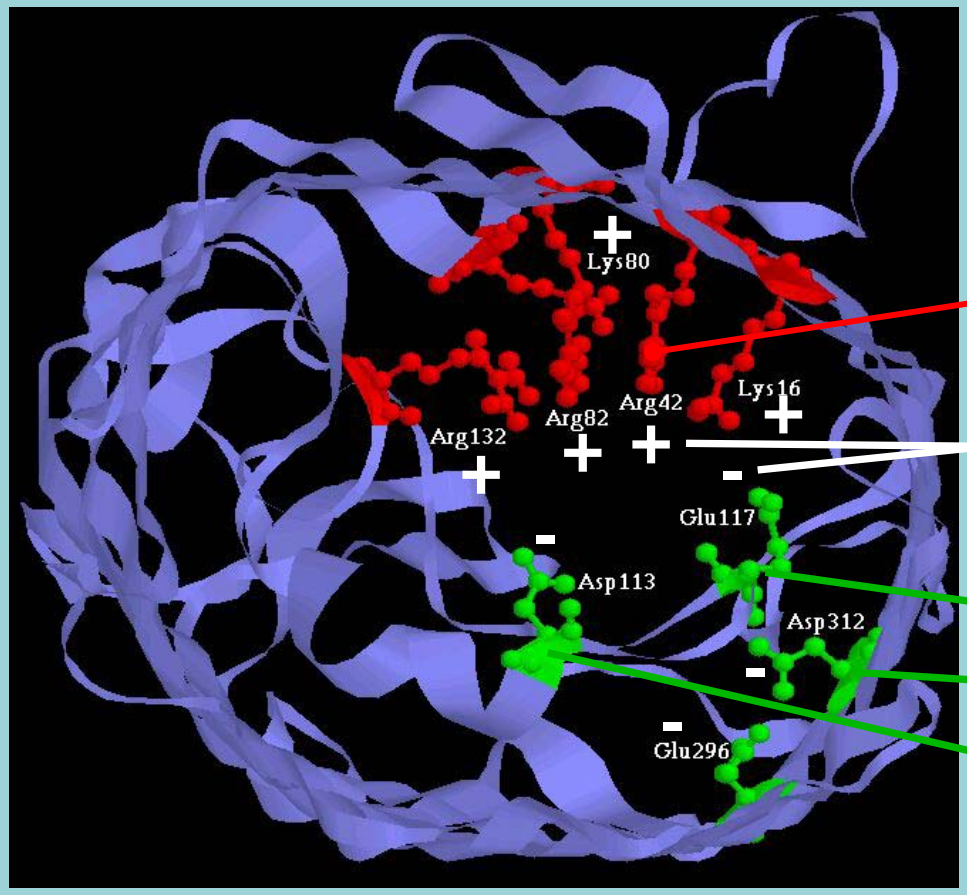
Counting and Statistics are essential

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

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

Pure water is 55 M

1 nM = 10 Å



Induced Fit of Side Chains

Ions are Crowded

Induced Fit of Side Chains

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

Figure adapted from Tilman Schirmer

Life Occurs in ~130 mM salt solutions

Ionic Solutions are NOT ideal

Chemically Specific Properties of Ionic solution are their DEVIATION from IDEAL

Finite Size Effects

Working Hypothesis

Chemically Specific Properties

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

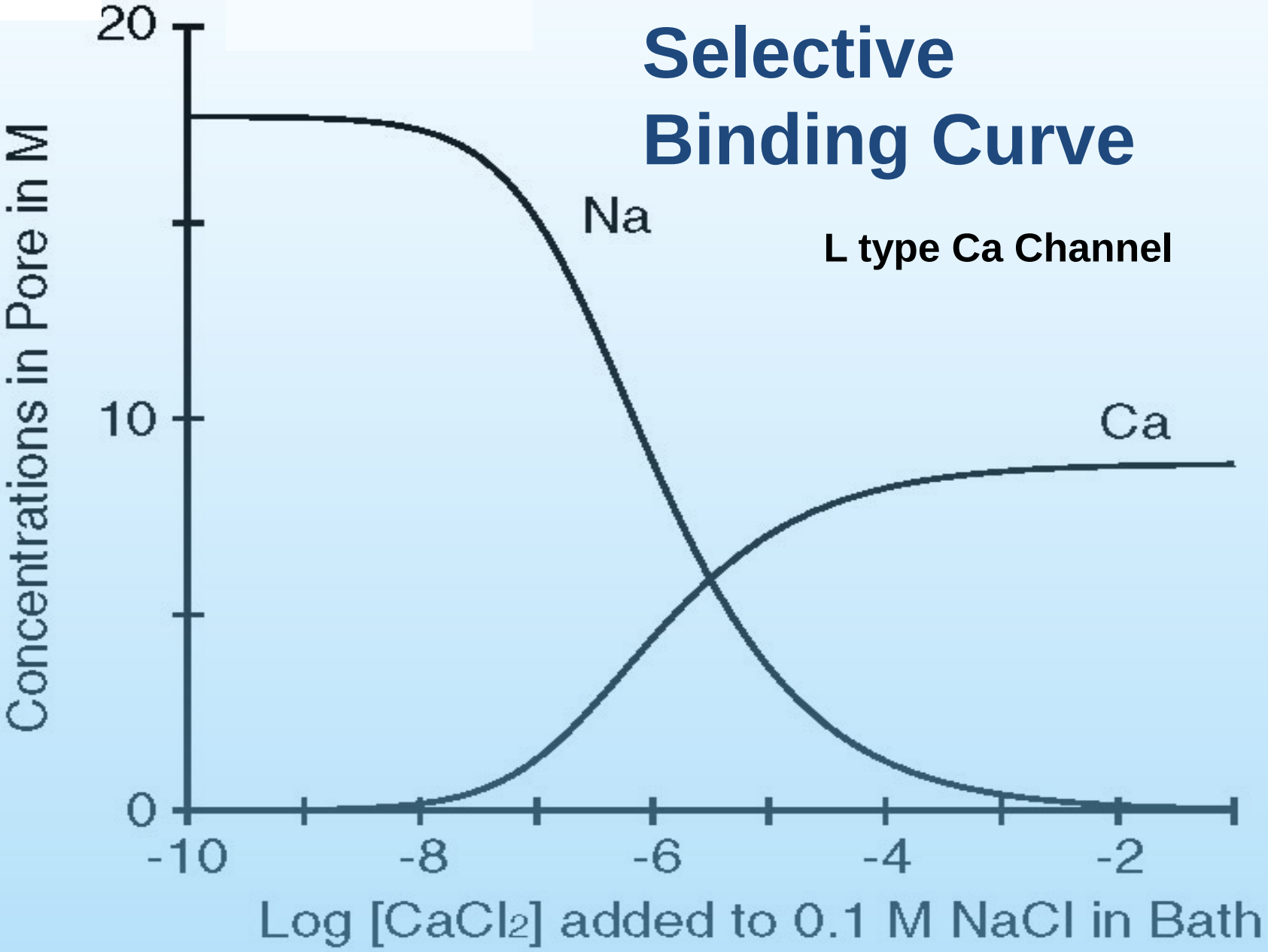
Diameter and Charge

and dielectric ‘constant’ of ionic solution

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

Selective Binding Curve

L type Ca Channel

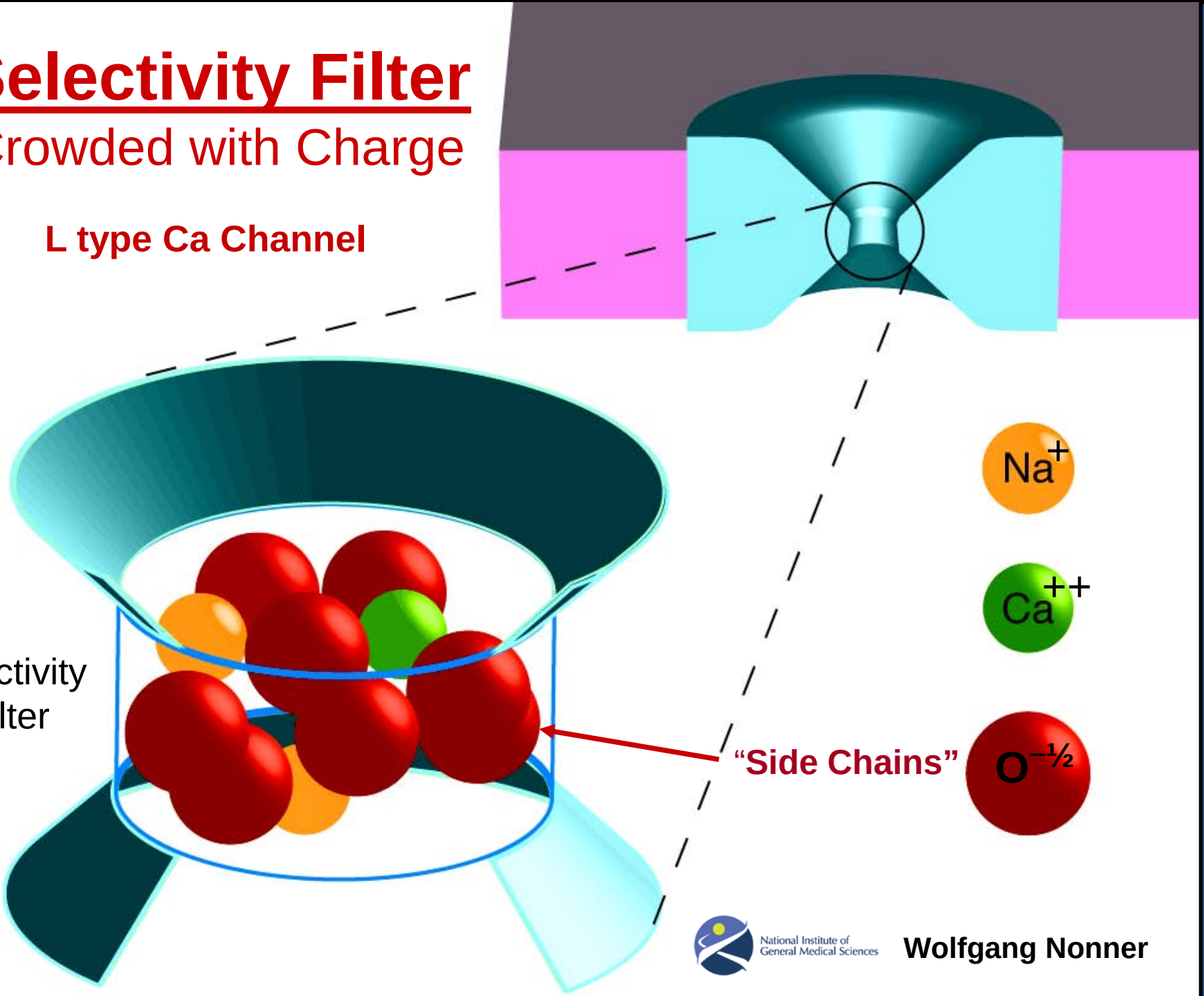


Wolfgang Nonner

Selectivity Filter

Crowded with Charge

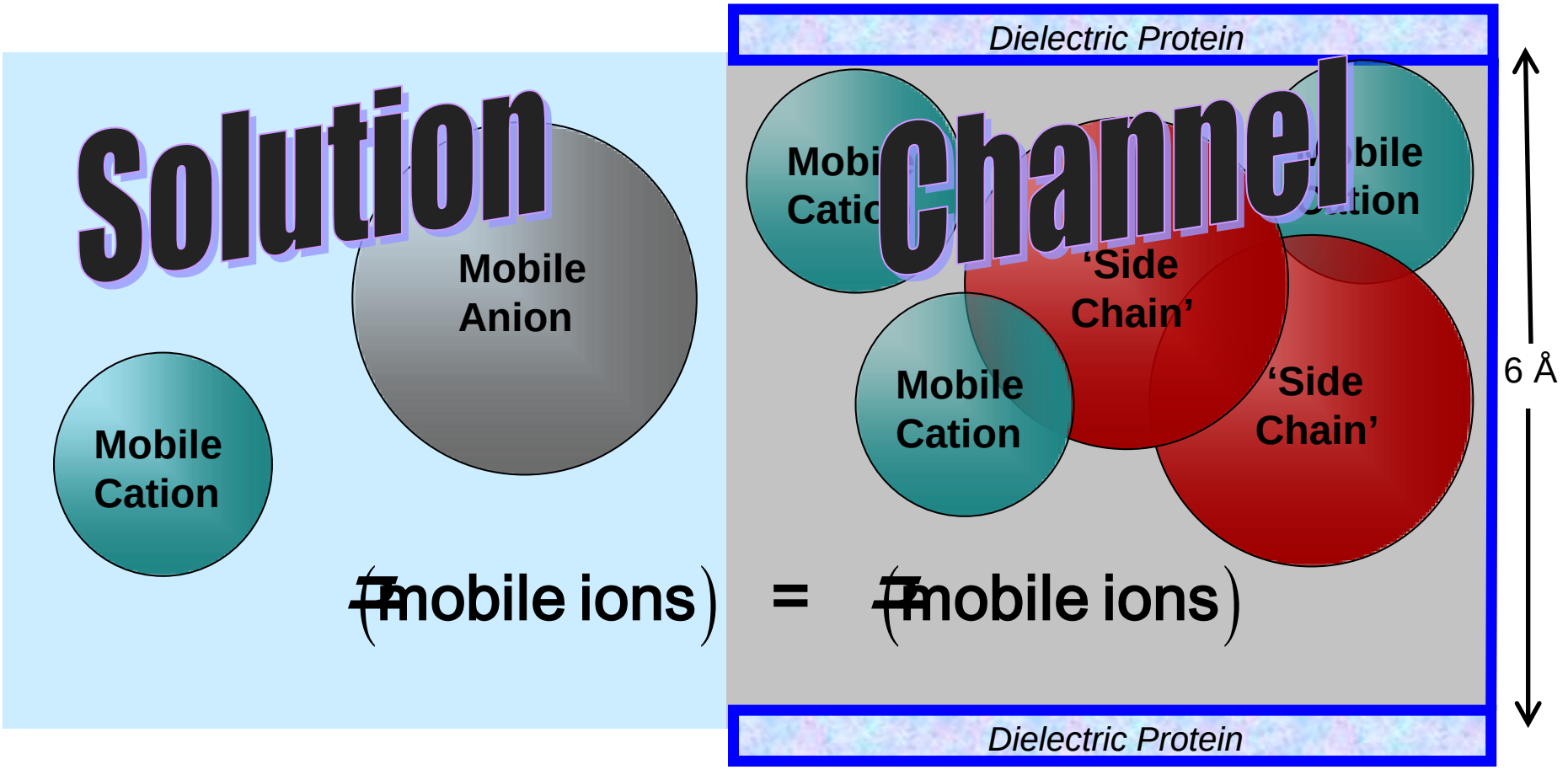
L type Ca Channel



National Institute of General Medical Sciences

Wolfgang Nonner

Ion 'Binding' in Crowded Channel



Classical Donnan Equilibrium of Ion Exchanger

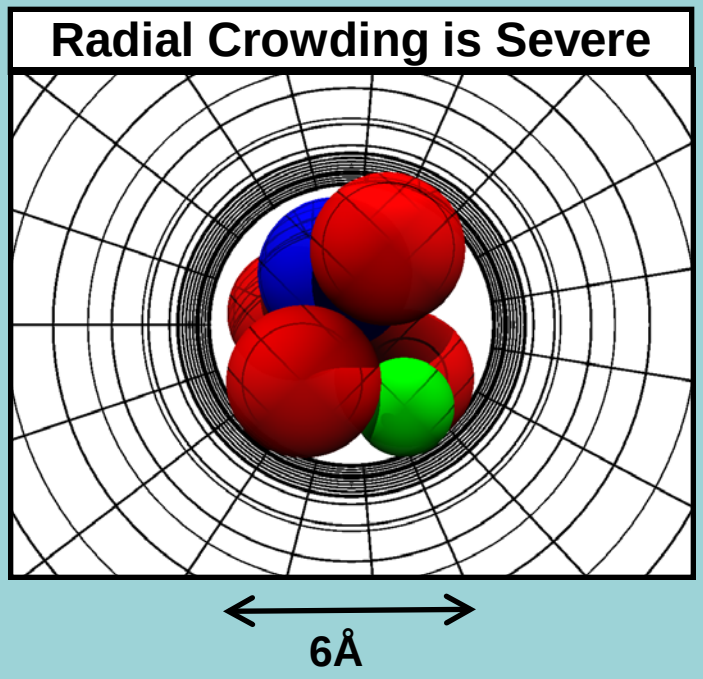
large mechanical forces

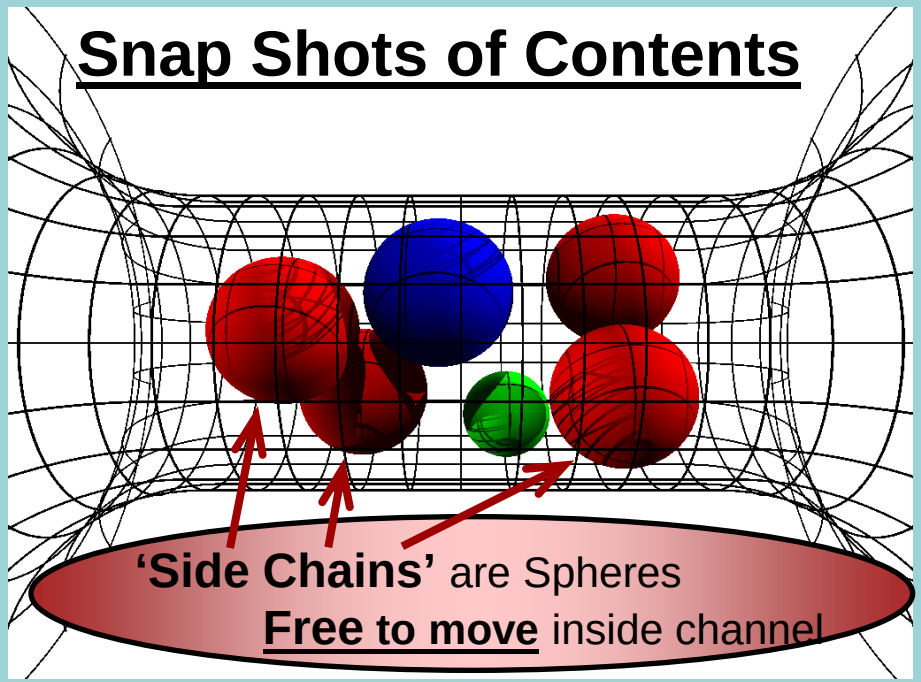
Side chains move within channel to their equilibrium position of minimal free energy.

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

Boda, Nonner, Valisko, Henderson, Eisenberg & Gillespie

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

Selectivity Filter

is a

Self-Organized Structure

with Side Chains at position of
Minimum Free Energy

The Protein Fits the Substrate

“Induced Fit Model of Selectivity”

Ca Channel

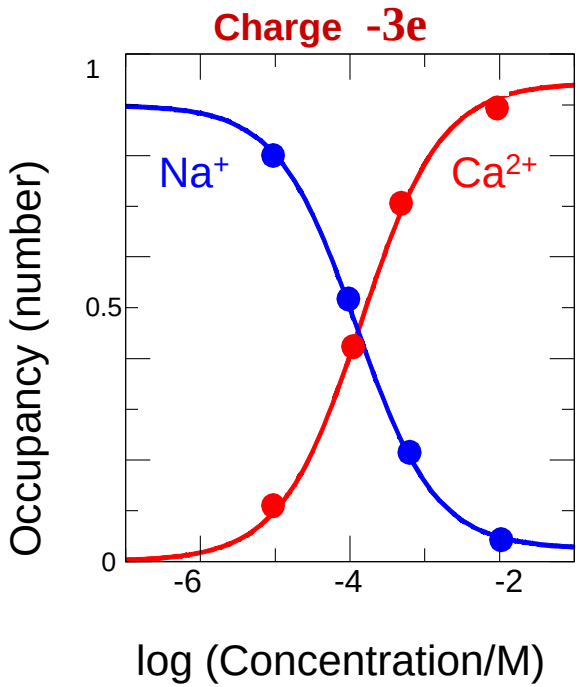
Mutation

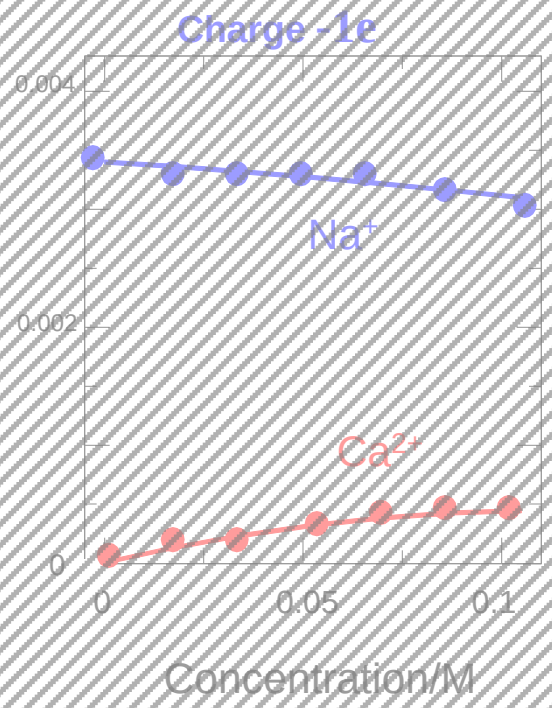
Na Channel

Same Parameters

E
E
E
A

D
E
K
A





EEEE has full biological selectivity in similar simulations

Boda, et al

Calcium Channel

has been examined in ~32 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

Now, 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

Na^+ vs K^+ selectivity

Ca Channel

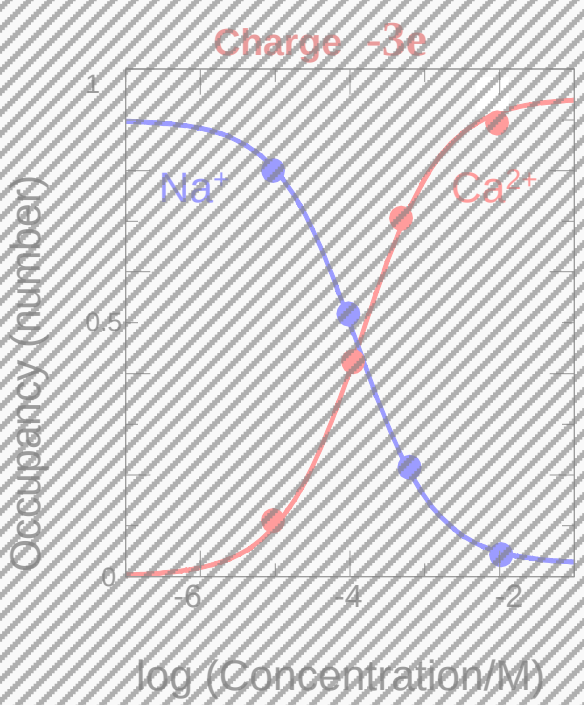
Mutation

Same Parameters

Na Channel

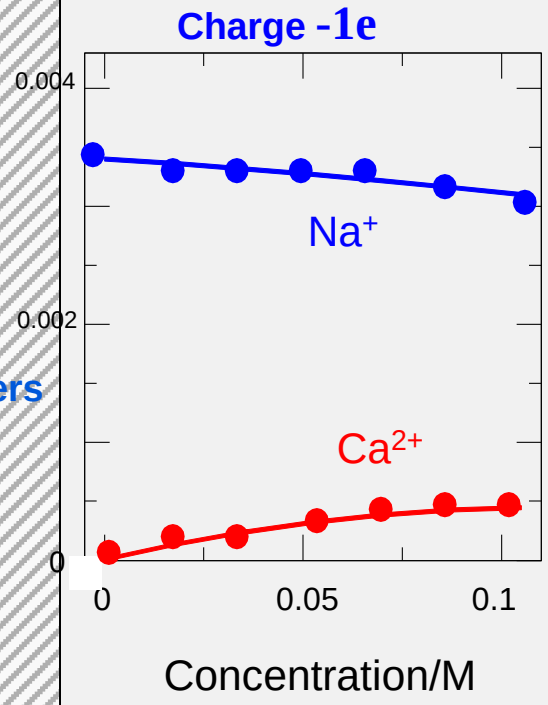
E
E
E
A

D
E
K
A



Mutation

Same Parameters



EEEE has full biological selectivity in similar simulations

Boda, et al

DEKA Na Channel Selects Na^+ *vs.* K^+

Nothing was changed

from the

EEEA Ca channel

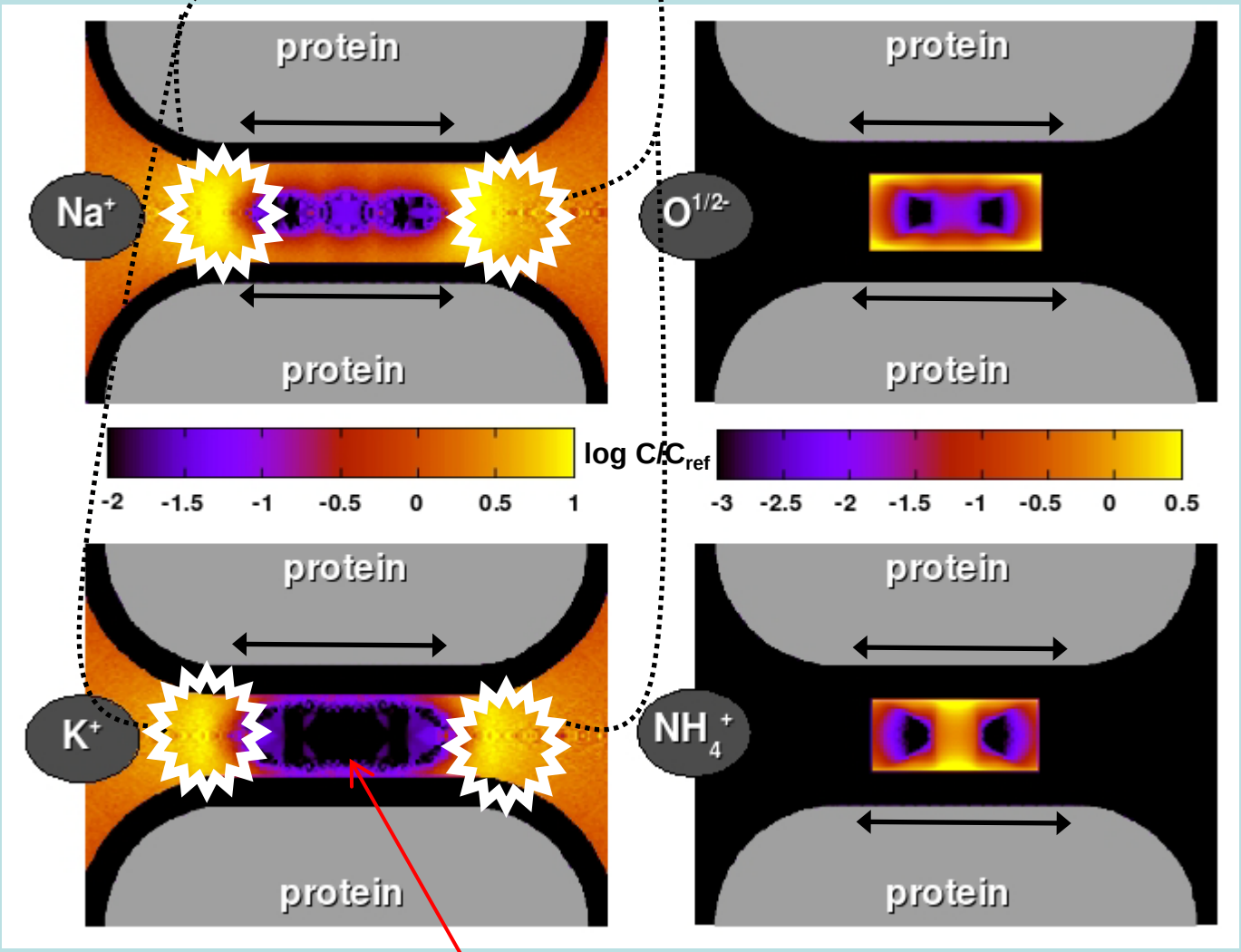
except the amino acids

Calculations and experiments done at pH 8

Size Selectivity

Binding Sites

NOT selective



Na vs K Size Selectivity is in

Depletion Zone

BLACK = Depletion=0

Boda, et al

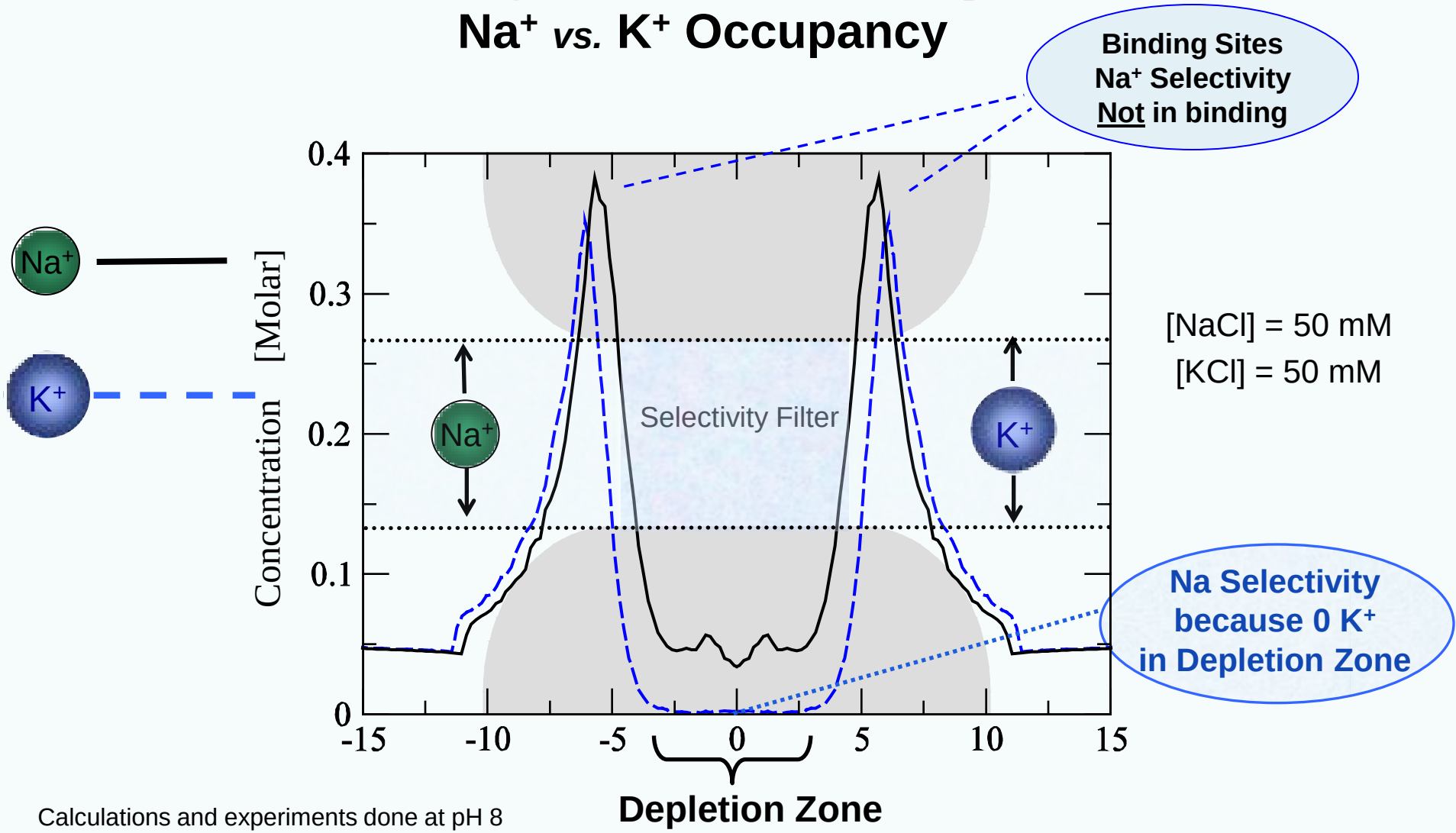
*Binding Sites are outputs of our INDUCED FIT Model of Selectivity, ***not*** *structural inputs*

[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
$O^{1/2-}$ D or E	2.80 Å
Na Channel DEKA *6 Å*	

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

Control Variables

are obvious in simulations of the Na channel,
but not the Ca channel

Control Variables

in DEKA Na channel

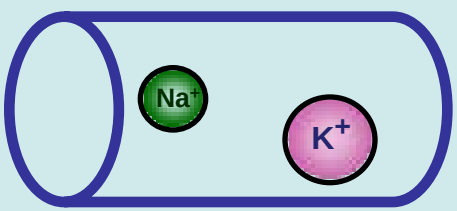
- **Selectivity Na^+ *vs* K^+**
  depends only on pore diameter

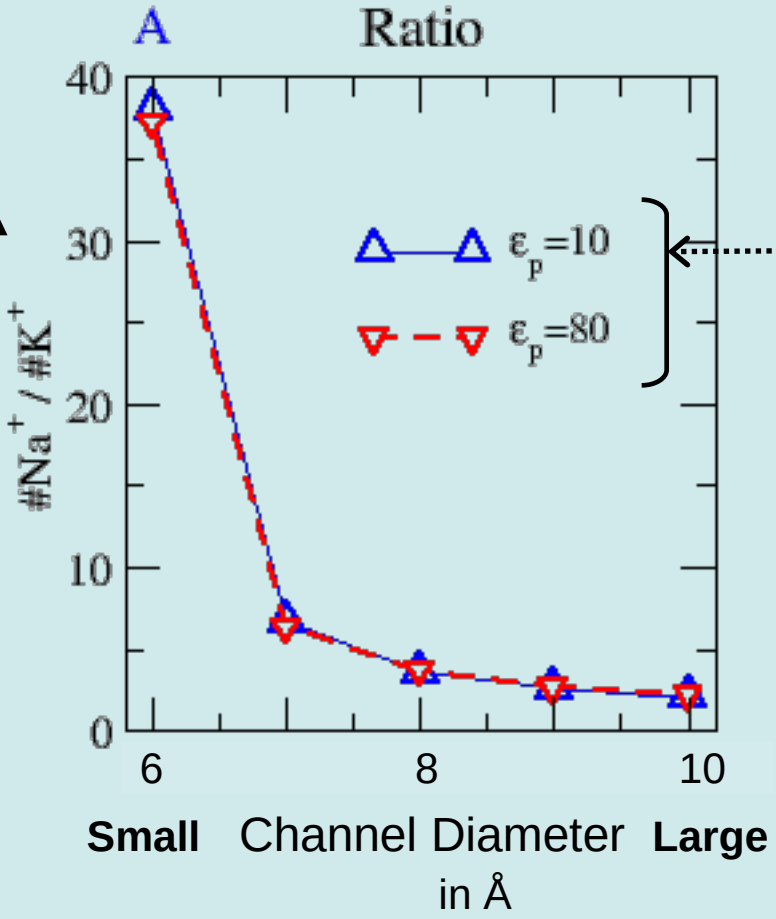
Diameter controls Selectivity

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

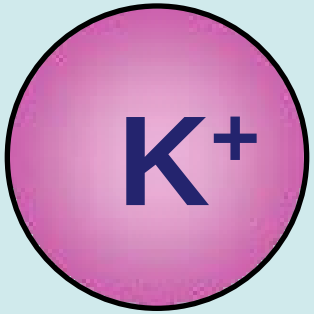
not Protein Dielectric Coefficient*

Selectivity
for small ion





2.00 Å



2.66 Å

Boda, et al

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

Control Variables

in DEKA Na channel

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

- **Conductance* depends on protein polarization**

Protein Dielectric Coefficient

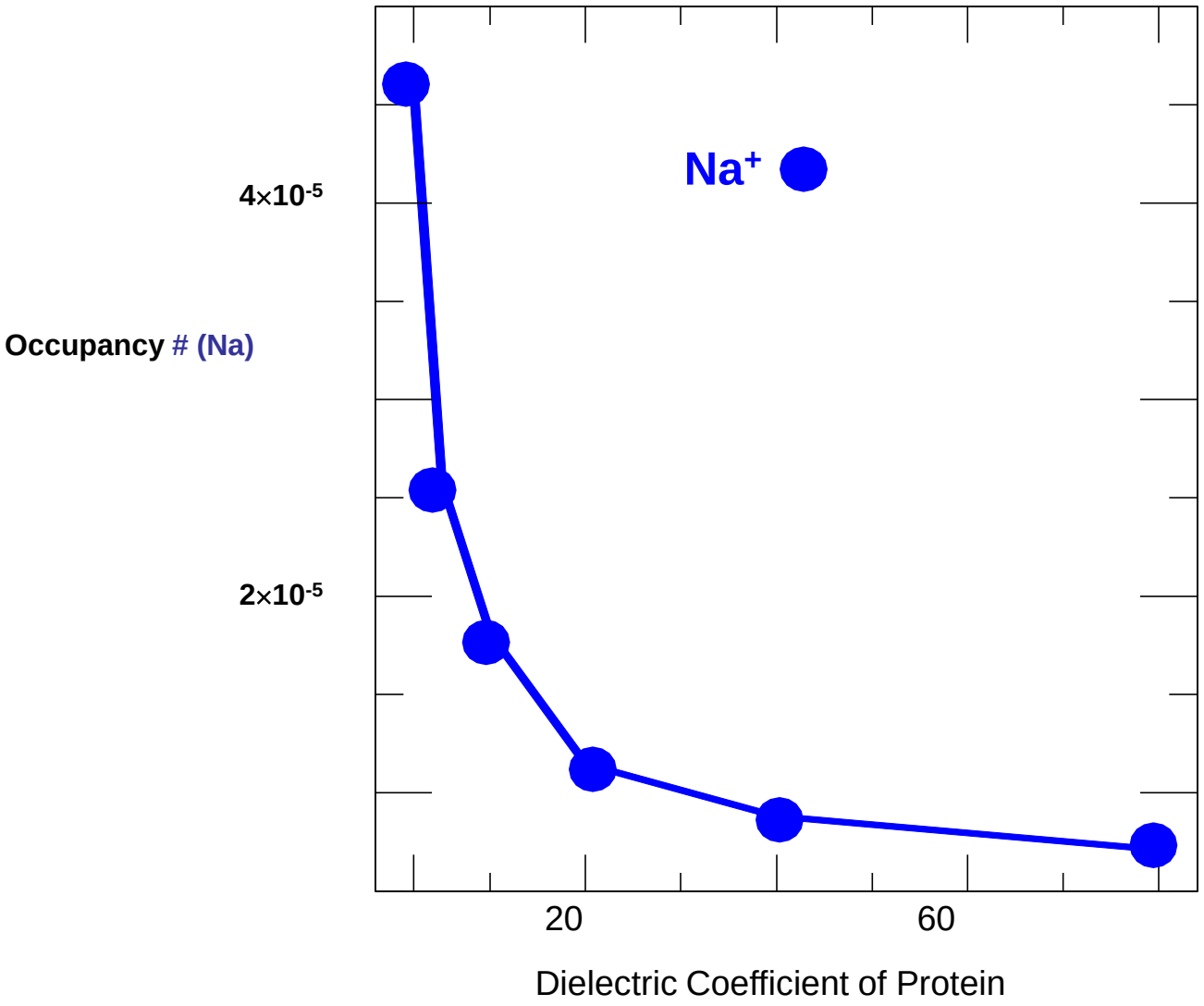
controls

Conductance

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

Control Variable

Occupancy depends on **Protein Dielectric**



Boda, et al,

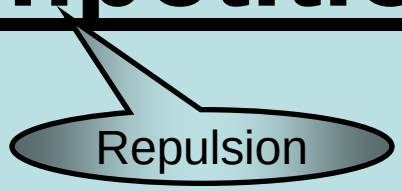
Selectivity

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

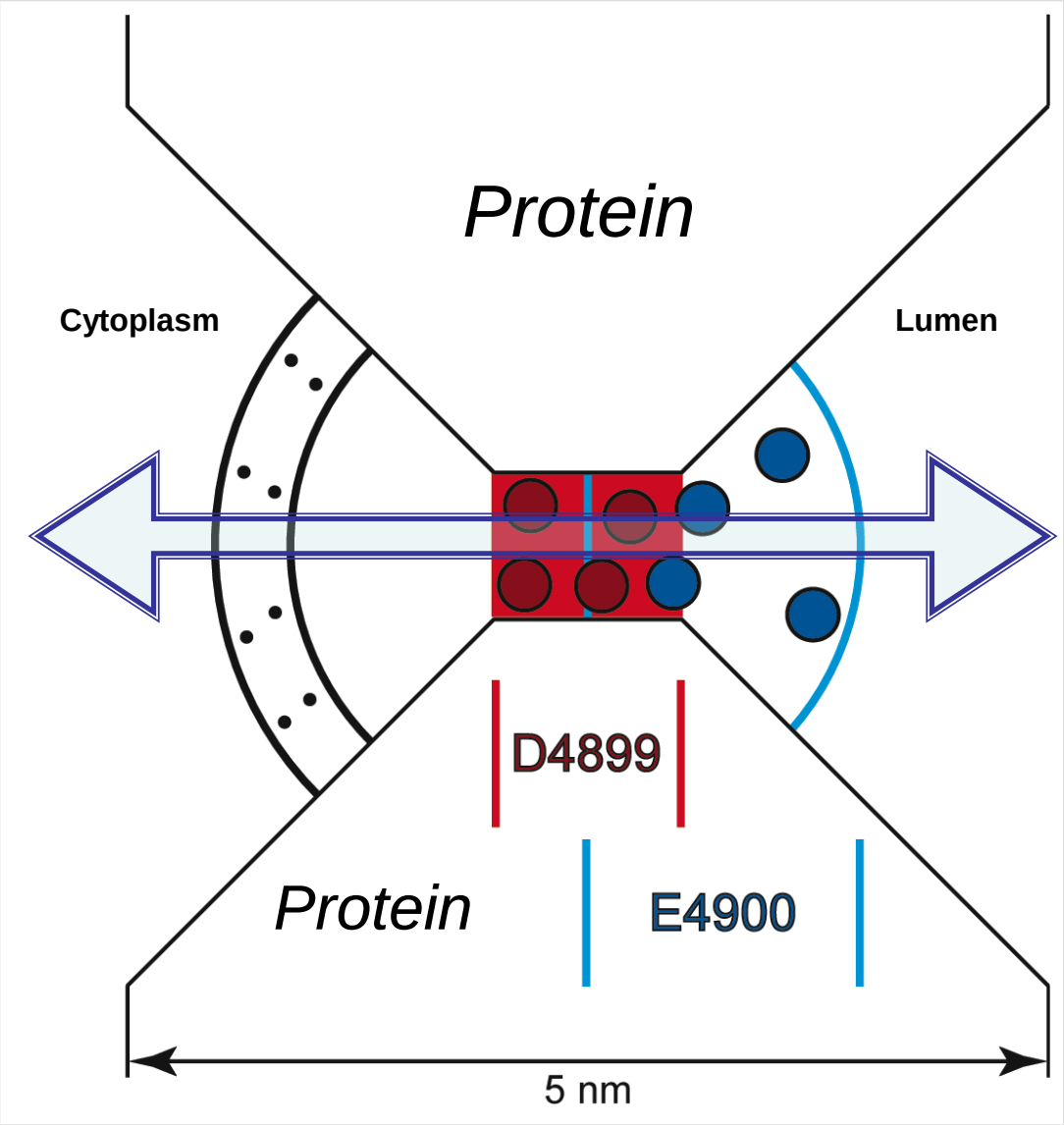
Best Evidence is from the

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.

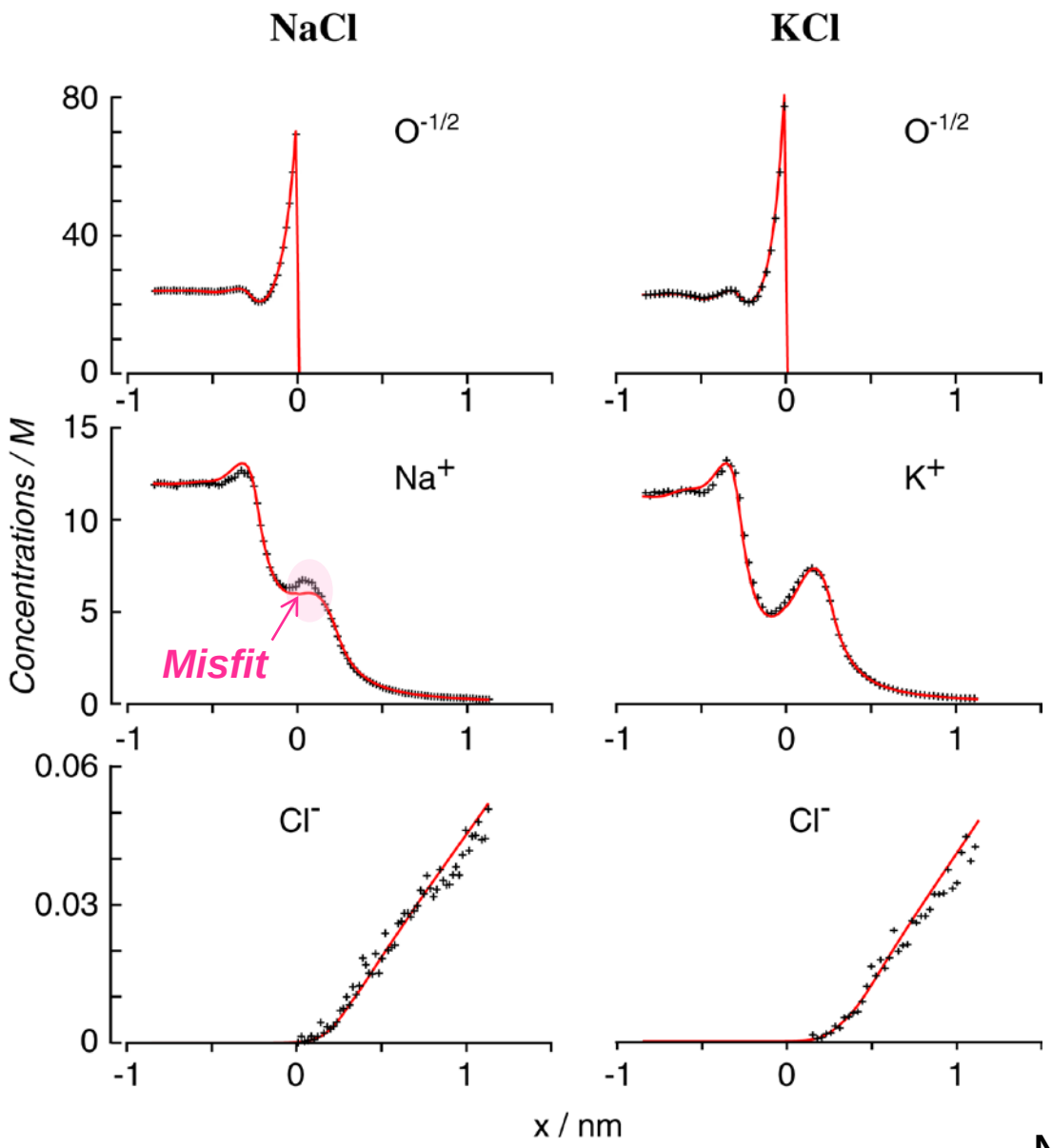
Four **E4900** positive amino acids are on lumenal side, overlapping D4899.

Cytosolic distributed charge

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

DFT/PNP *vs* Monte Carlo Simulations

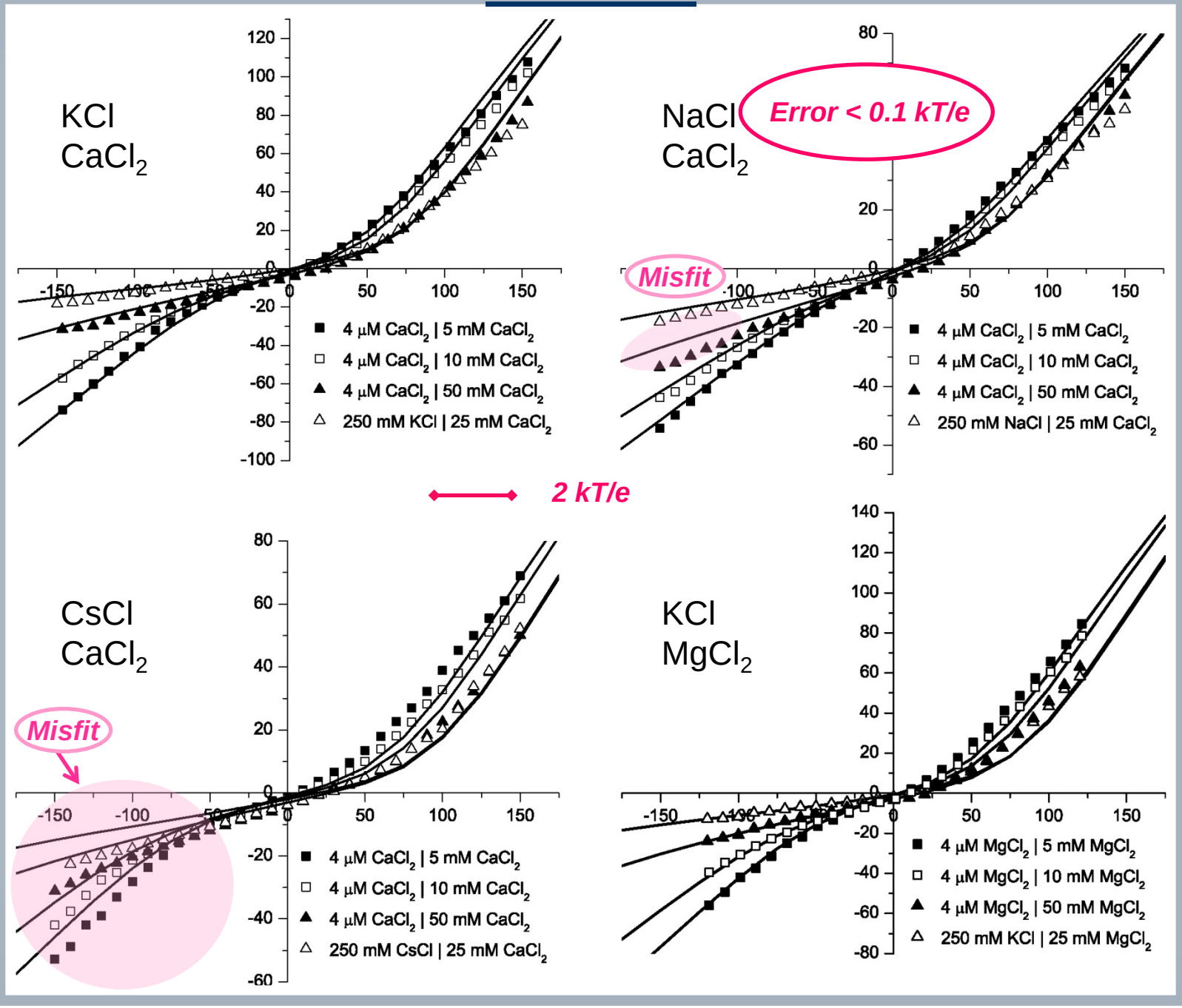
Concentration Profiles



Nonner, Gillespie, Eisenberg

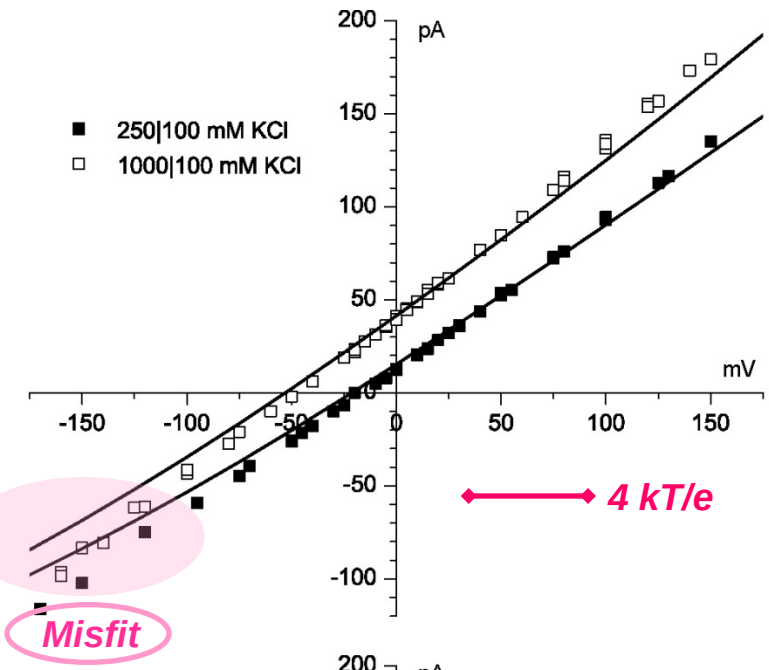
Divalents

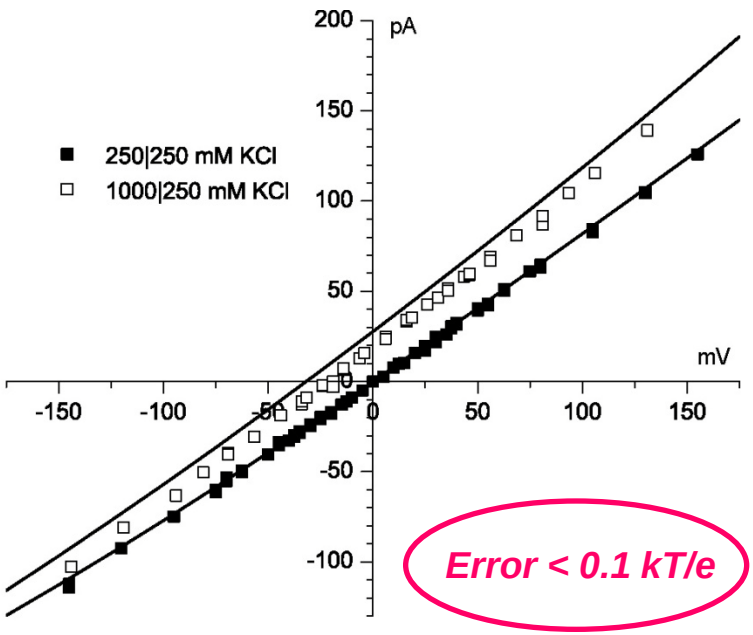
Gillespie, Meissner, Le Xu, et al

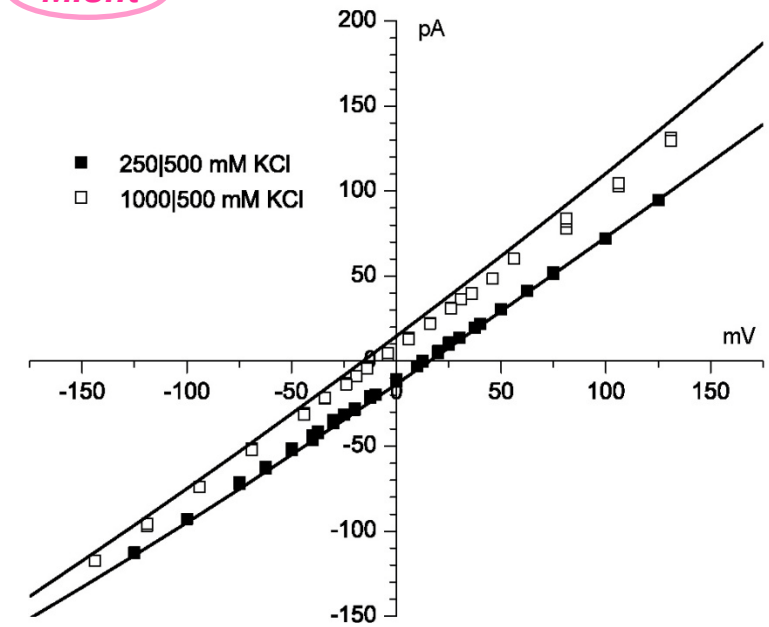


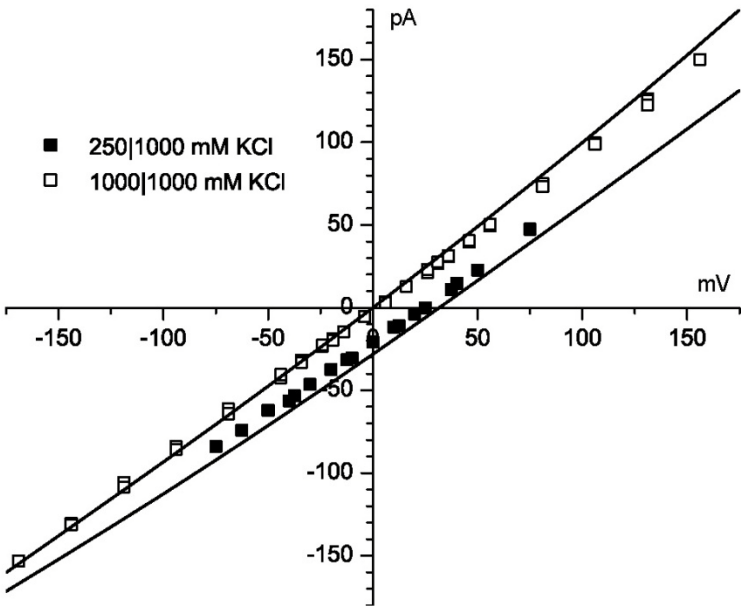
KCl

Gillespie, Meissner, Le Xu, et al





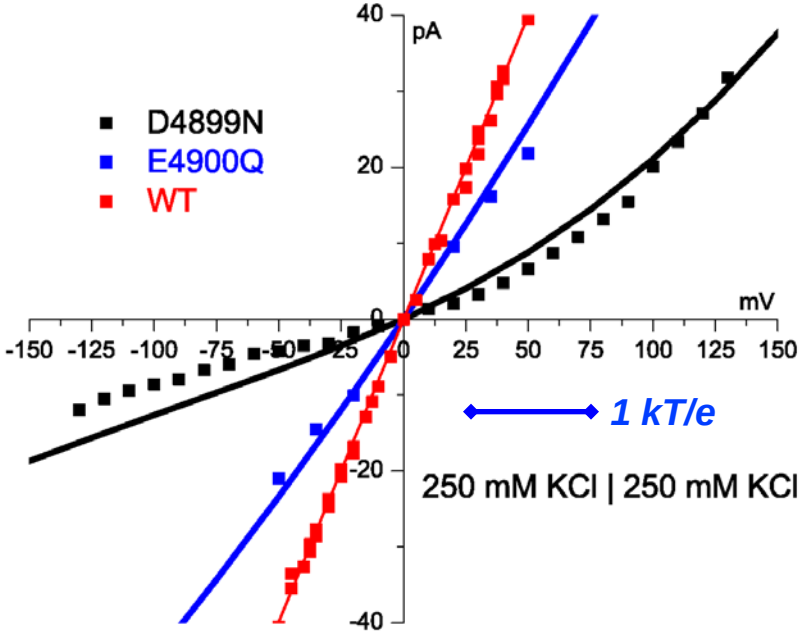




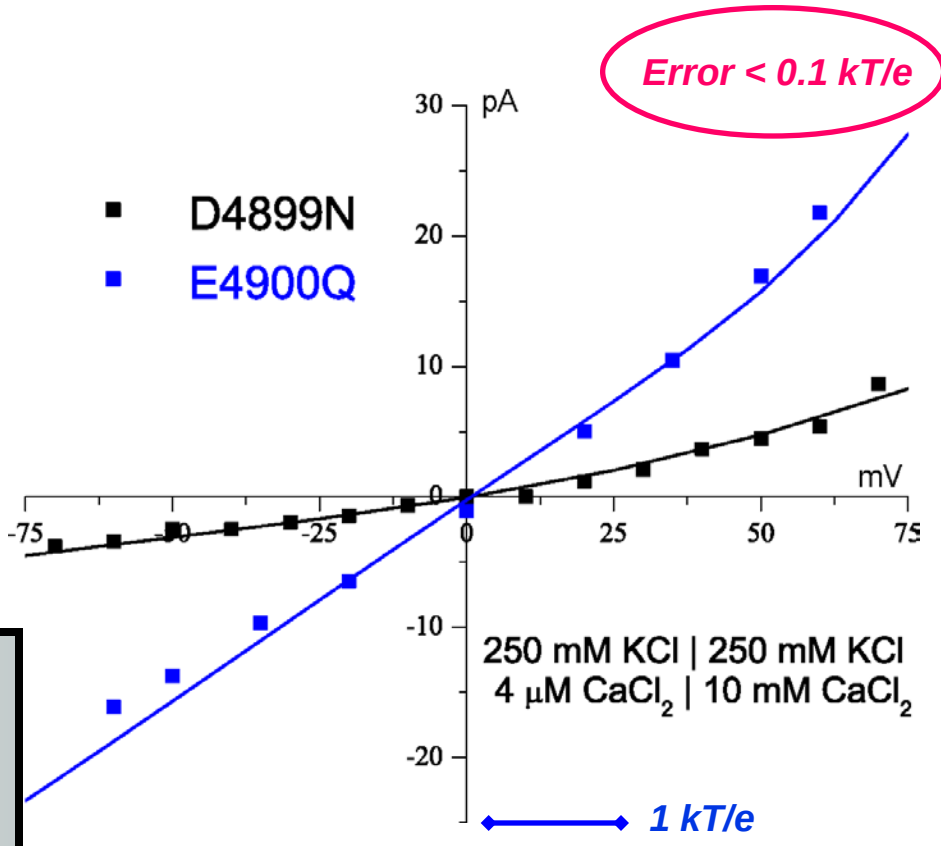
Theory fits Mutation with Zero Charge

No parameters adjusted

Theory Fits Mutant in K



Theory Fits Mutant in K + Ca



Protein charge density

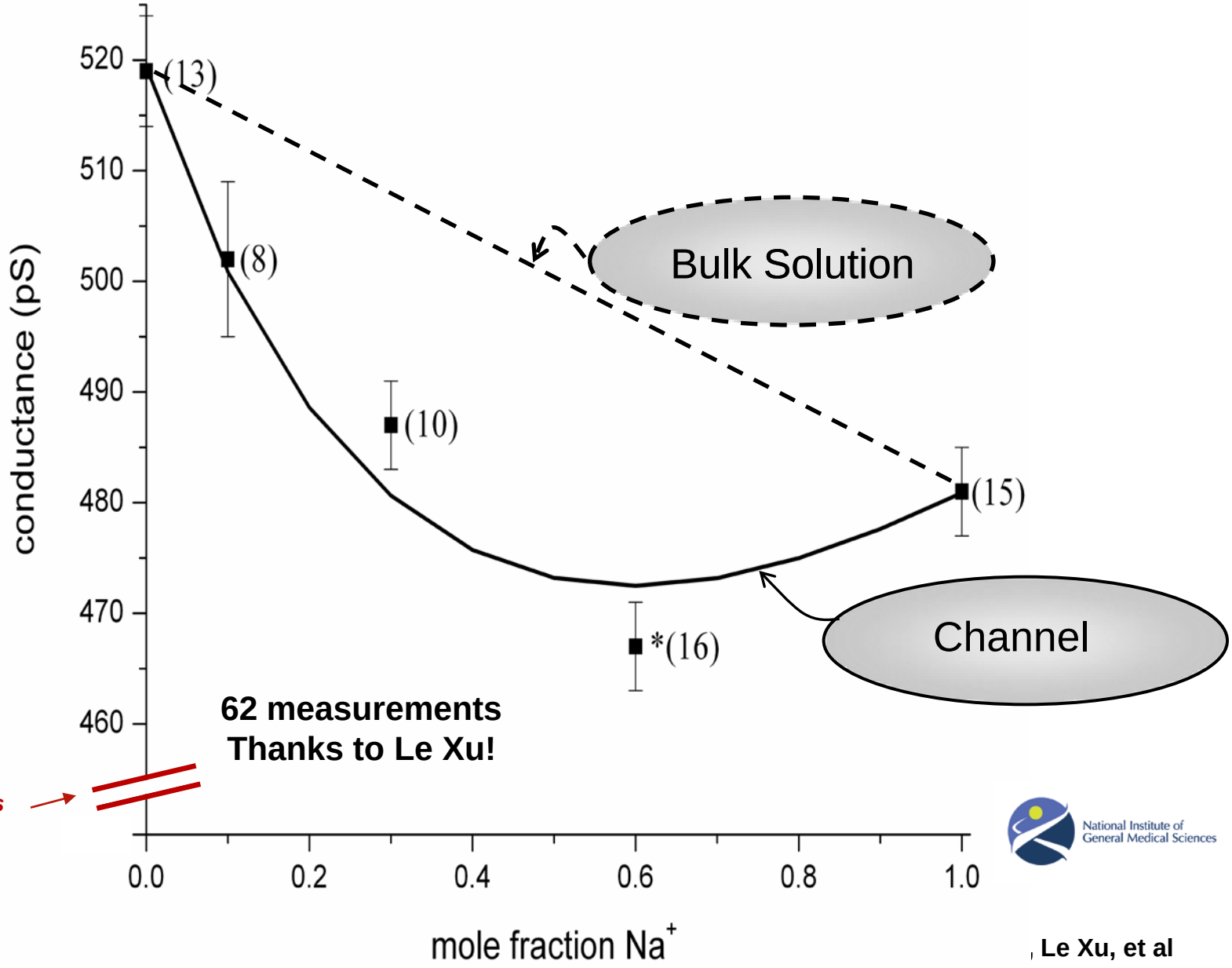
wild type* 13 M ⇨ 0 M *in* D4899

Water is 55 M

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

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

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



National Institute of General Medical Sciences

, Le Xu, et al

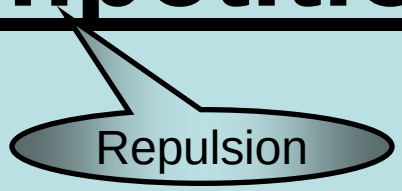
Selectivity

comes from

Electrostatic Attraction

and

Steric Competition for Space



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

Rate Constants are Variables

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

What does the protein do?

Protein maintains

Mechanical Forces*

Volume of Pore

Dielectric Coefficient/Boundary

Permanent Charge

Induced Fit Model of Selectivity

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

Nonner and Eisenberg

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

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

Induced Fit Model

- **Selectivity depends on**
  Induced Fit of Side Chains and Ions

- **Induced Fit is the**
  Self-Organized Structure
  with
  Minimal Free Energy

  Energy and Entropy

Induced Fit Model

- **Monte Carlo computes the Structure of Minimal Free Energy**

  Energy and Entropy

- **Monte Carlo computes the Induced Structure 'perfectly'***

  but the model itself is far from perfect

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

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**

We can actually compute the Structures that determine Selectivity

Specificity

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

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

if the detail is computed with the correct broad sweeps of physics†

†Bob Eisenberg's opinion

***Aaron Klug** *quoted in the first sentence of Pearson Nature (2008) 455:160–164*

Conclusion

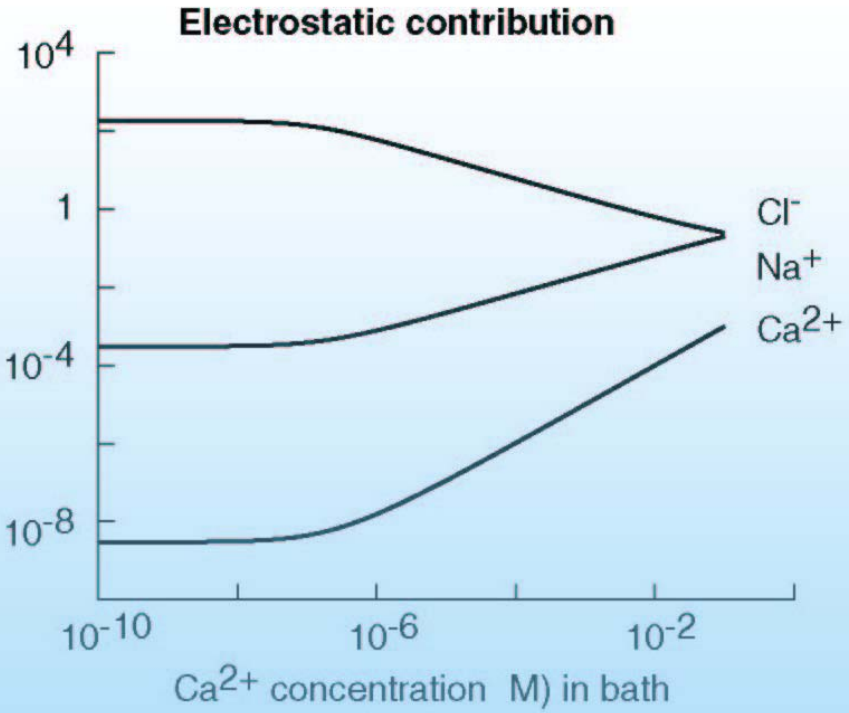
Selectivity can be understood by Reduced Models

K channels — Benoît Roux, Susan Rempe

Na & Ca channels — Nonner, *et al,*

Best Evidence

RyR channels — Gillespie & Meissner



Electrostatic Contribution

to 'Dissociation Constant' is large

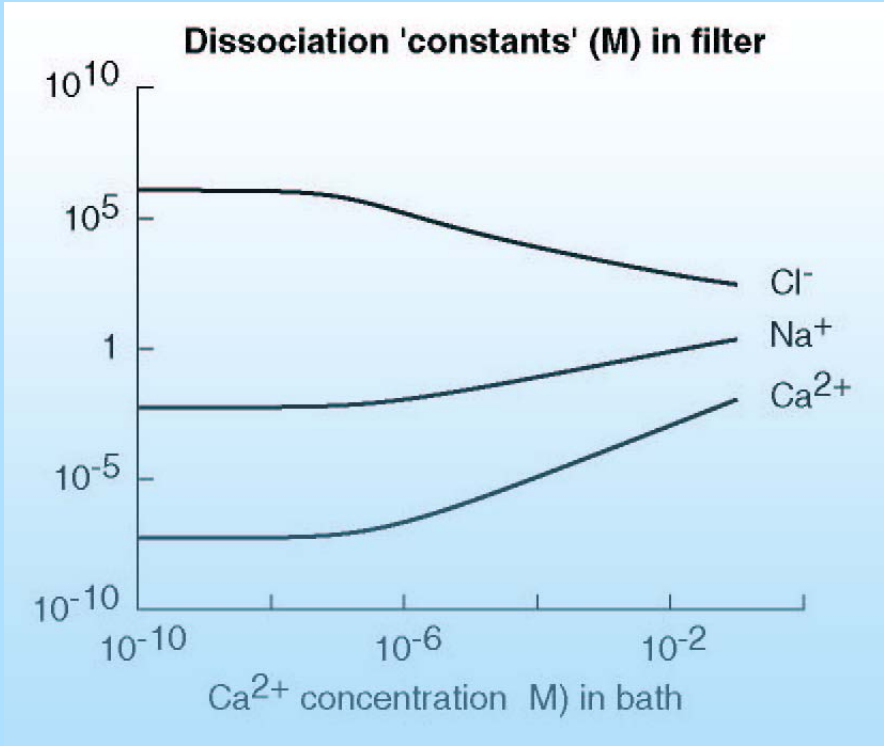
and is an

Important Determinant of Biological Properties

Change of Dissociation 'Constant'

with concentration is large and is an

Important Determinant of Biological Properties



Comparison with Traditional Approach

Traditional Biochemistry

(more or less)

Ignores the Electric Field

Ignores Crowding Effects

But

Rate Constants depend steeply

on

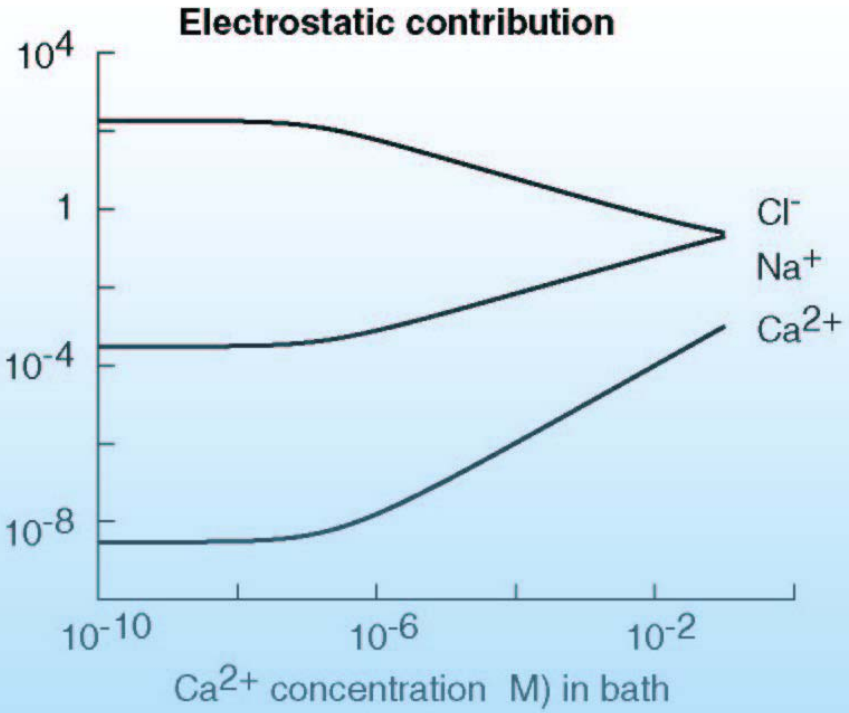
Electrical Properties

and

Concentration*

because of shielding and crowding, fundamental properties of matter.

*nearly always



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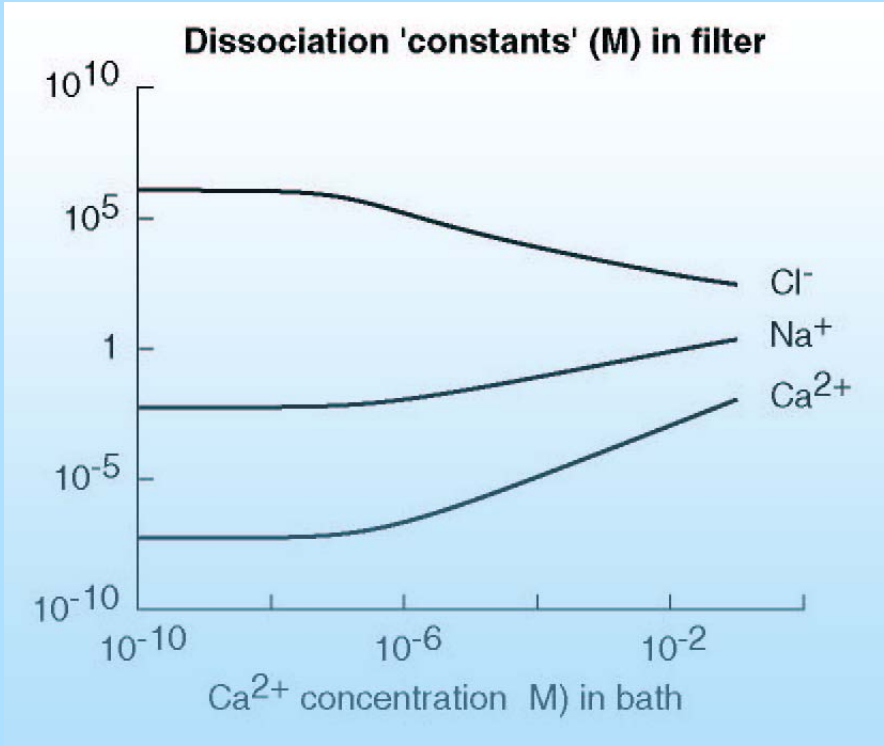

Conclusion about Ca channels

Remember

We can build them

(reasonably well)