昆山杜克大学
DUKE KUNSHAN UNIVERSITY

Special Thanks

Shixin Xu

Jian-Guo Liu

Prof. Peter Pickl

and Yiewei Xiong for her hard work!

Zu Chongzhi Distinguished Lecture Series

Ion Channels and Transporters: how do they work? (1)

Sept. 11, Friday
9:00-10:30 AM Kunshan Time

Sept. 10, Thursday
8:00 - 9:30 PM Chicago Time

Zoom Meeting ID:
932 7767 0707

Ion Channels and Transporters: how do they work? (2)

Sept. 18, Friday
9:00-10:30 AM Kunshan Time

Sept. 17, Thursday
8:00 - 9:30 PM Chicago Time

SCAN for more details!

Stochastic Basis of Mean Field Theories

Sept. 27, Sunday
9:00-10:30 AM Kunshan Time

Sept. 26, Saturday
8:00 - 9:30 PM Chicago Time

DUKE KUNSHAN
Zu Chongzhi Center for Mathematics and Computational Sciences

Speaker: Prof. Robert Eisenberg,
Illinois Institute of Technology

Zu Chongzhi Distinguished Lecture Series

Open discussion

Zoom Meeting ID:
932 7767 0707

Ion Channels and Transporters: how do they work? (1)

Sept. 15, Tuesday

10:30 – 11:30PM
Kunshan Time,
9:30 – 10:30AM
Chicago Time

Ion Channels and Transporters: how do they work? (2)

Sept. 22, Tuesday

10:30 – 11:30PM
Kunshan Time,
9:30 – 10:30AM
Chicago Time

SCAN for more details!

Stochastic Basis of Mean Field Theories

Sept. 29, Tuesday

10:30 – 11:30PM
Kunshan Time,
9:30 – 10:30AM
Chicago Time

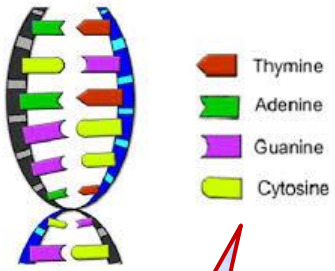
DUKE KUNSHAN
Zu Chongzhi Center for Mathematics and Computational Sciences

Speaker: Prof. Robert Eisenberg,
Illinois Institute of Technology

Life is special because it is inherited from a tiny number of atoms

First Day
September 4
2020

How can a few thousand atoms conceivably control 10^{25} atoms?



YouTube

How can a few thousand atoms conceivably control 10^{25} atoms?

ANSWER:
by forming a
HIERARCHY of DEVICES

Working hypothesis:

The Structures of Biology

make a

Hierarchy of Devices

that span the scales

And make biology

The ULTIMATE MULTISCALE MACHINE

From

Atoms to Axons to Meters

Vocabulary

Words are Needed at each level of Hierarchy of Scales
Extensive Vocabulary is a Barrier for Students

Best Source for Vocabulary are Video Clips, often from YouTube

or from educational websites like MIT

or from Wikipedia

Or from general Internet Search

and I will provide a Vocabulary List

YouTube

YouTube

Skeletal

A MOTOR UNIT

A motor neuron innervates one set of muscle fibers.

Axon

YouTube

Muscle

Spinal cord

Muscle fibers

YouTube

A pool consists of many motor neurons, each of which innervates a motor unit with the muscle.

Biology is made of Hierarchy of

Devices

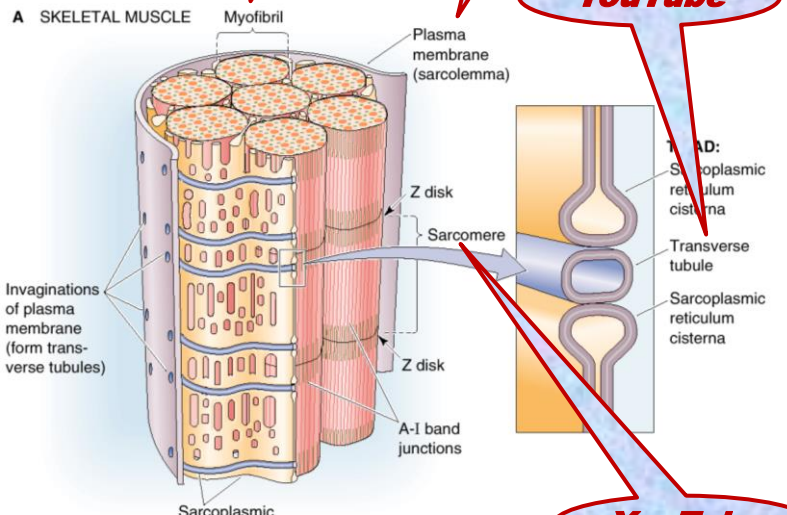
and they are Multiscale

YouTube

YouTube

YouTube

YouTube



YouTube

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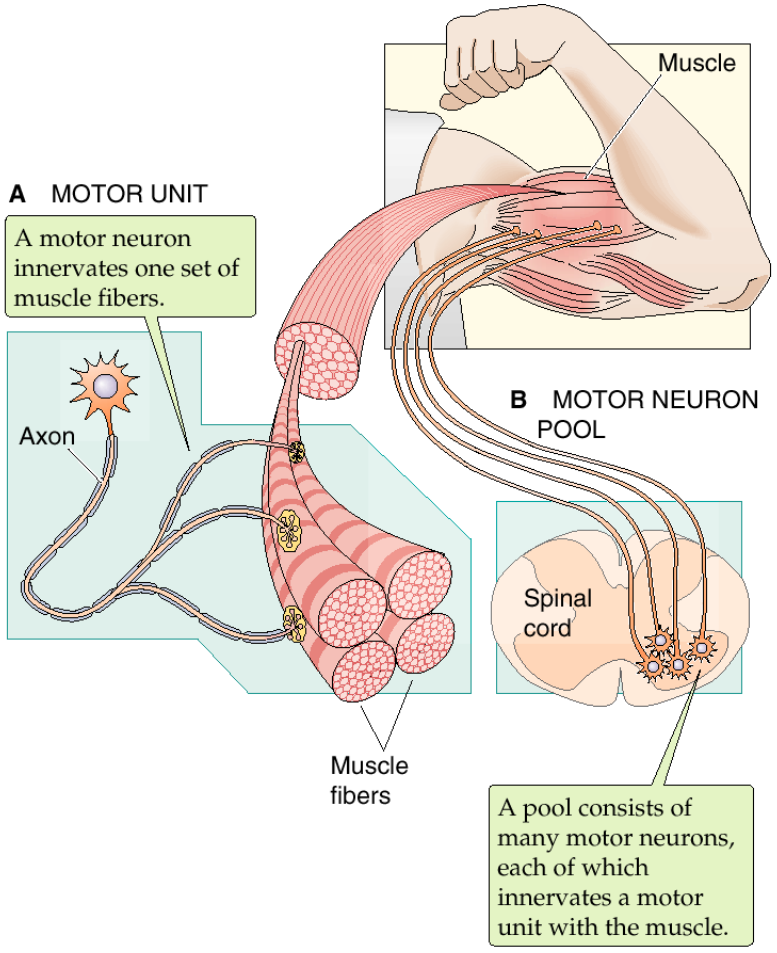
Biology is made of Hierarchy of **Devices**

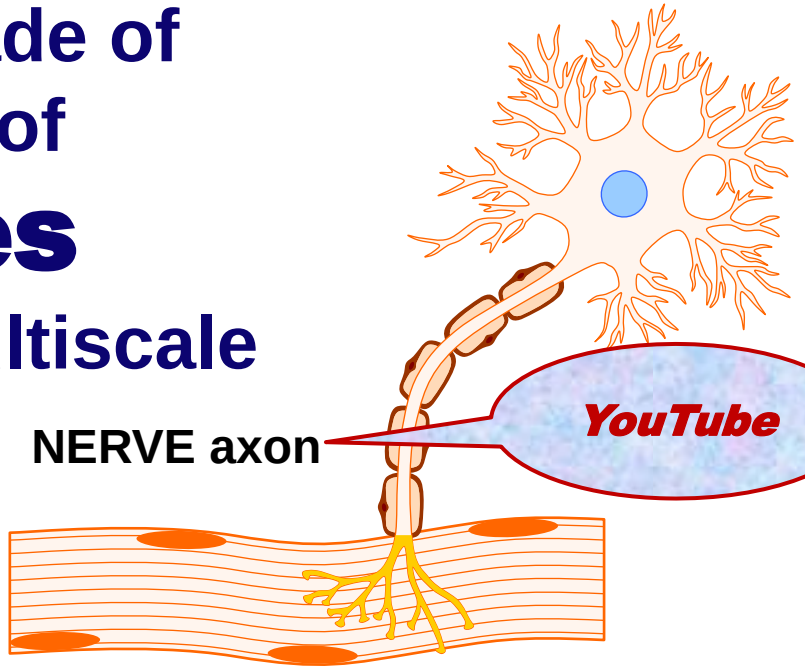
YouTube

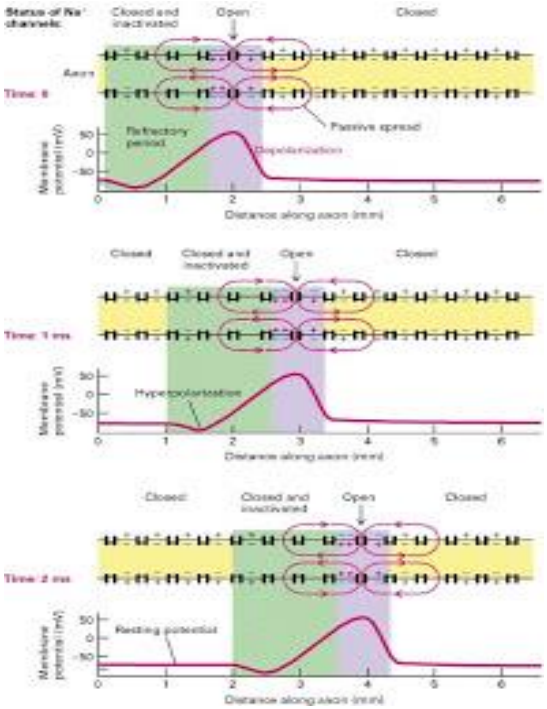
Nerve MUSCLE and they are Multiscale



NERVE axon

YouTube



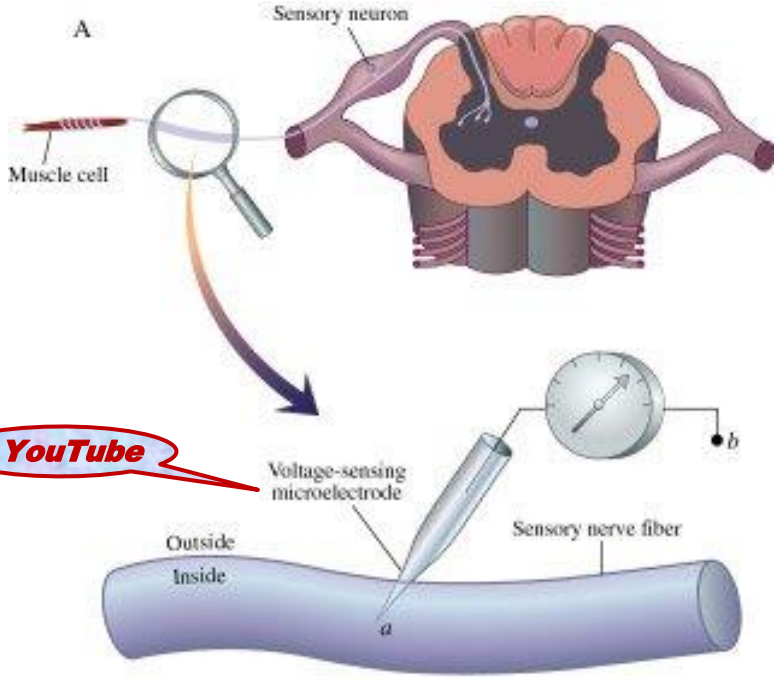


Nerve Conduction

- What is the information signal of a nerve?
- How does that signal move down a nerve fiber?
- What are the molecular mechanisms involved?
- Completely solved in outline
- Mathematics available
  - Many IMPORTANT problems unsolved

Optimization of function

Neuronal Signal



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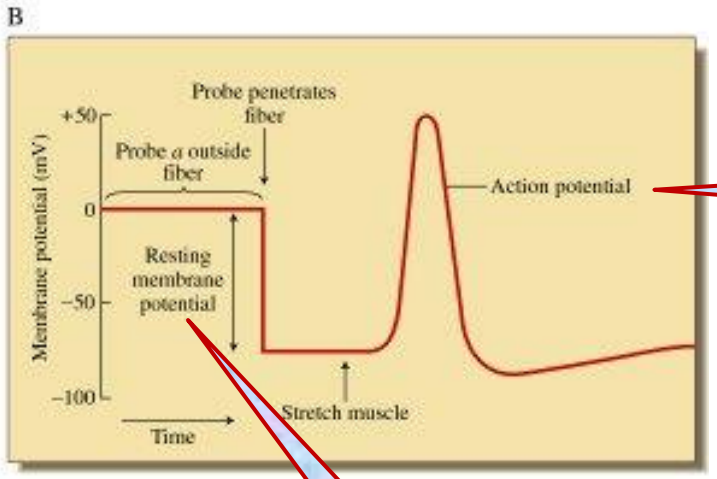

YouTube

YouTube

b NEUROBIOLOGY
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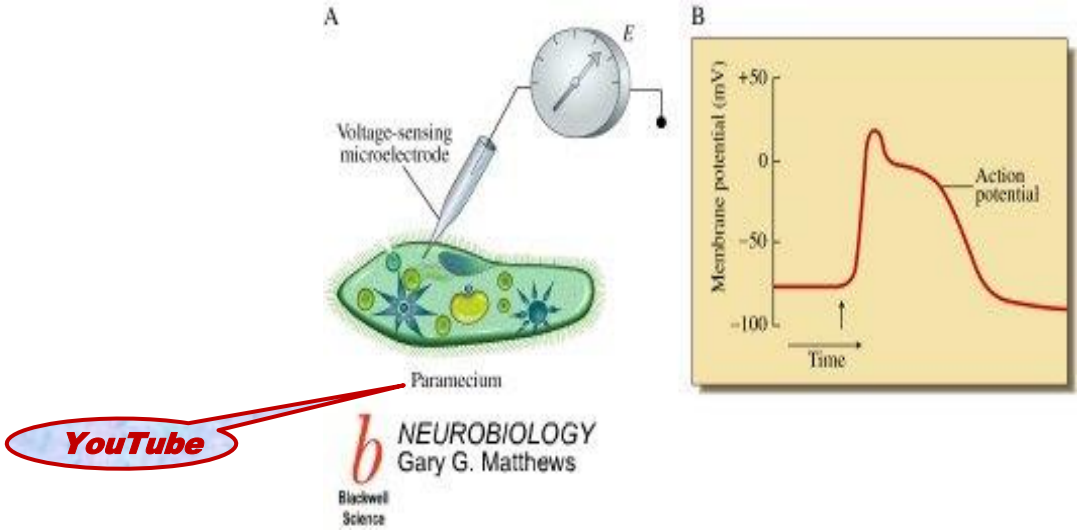
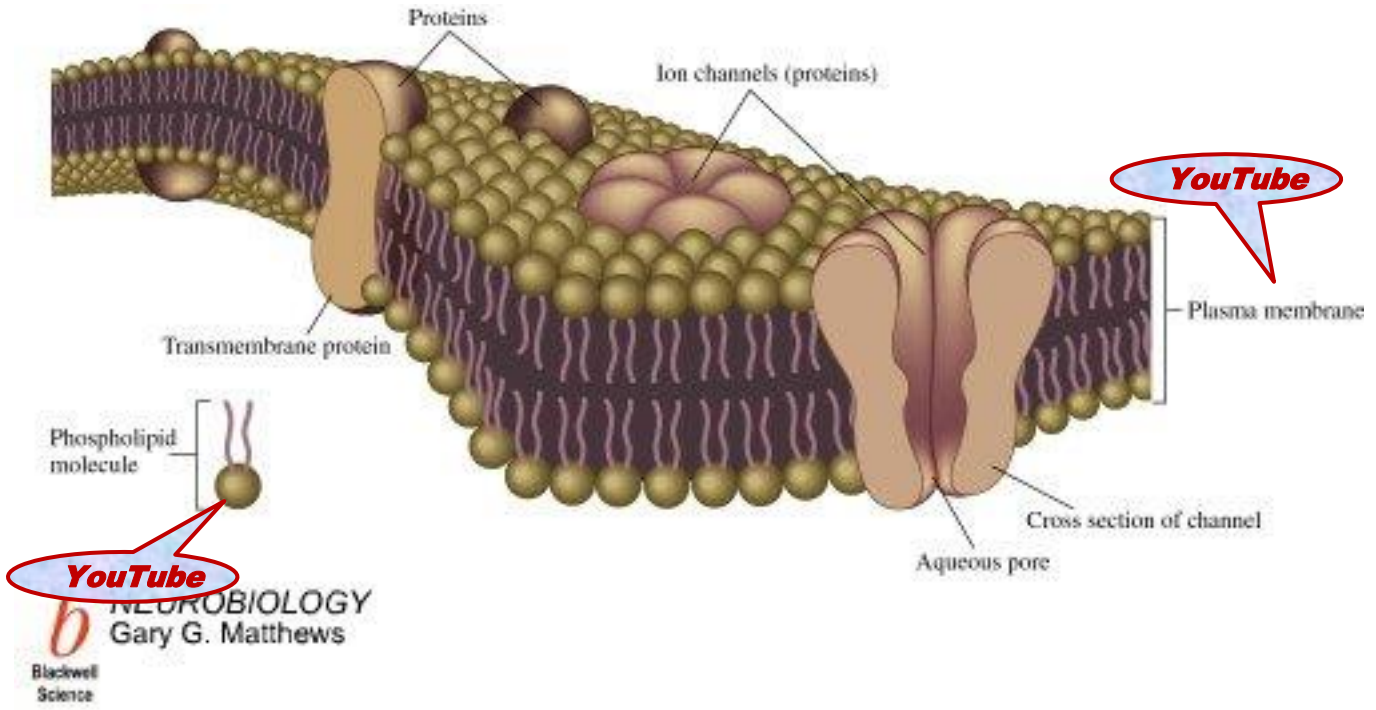
Voltage Signals are Found Throughout Life



Channels Determine Electrical Properties



Resting Potential Set by K Channels

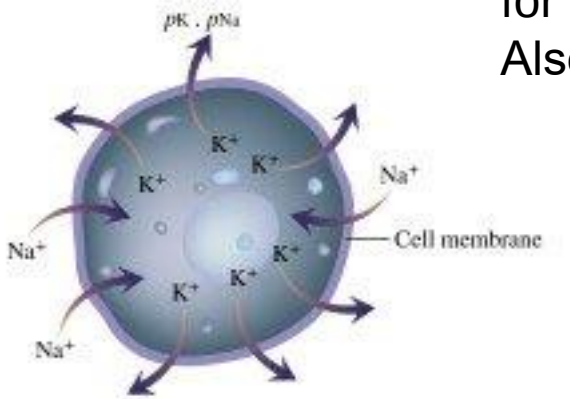
Nernst Potential is the concentration gradient of one ion in electrical units! It is also the "chemical potential" of the ion

YouTube

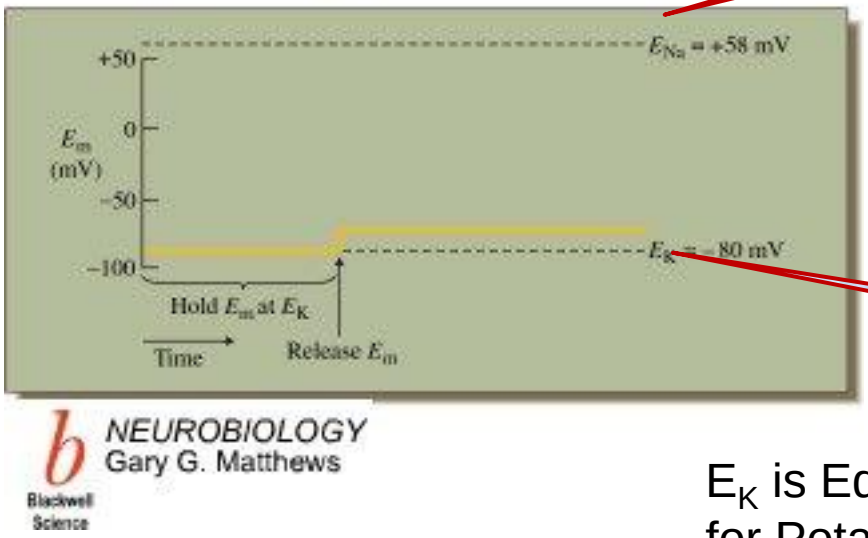
E_{Na} is Equilibrium Potential for Sodium Na
Also called 'Nernst Potential'

$$E_{Na} = \frac{RT}{F} \log_e \frac{[\mathrm{Na}]_{out}}{[\mathrm{Na}]_{in}}$$

[Na] means concentration of Sodium ion



YouTube



YouTube

E_K is Equilibrium Potential for Potassium K
Also called 'Nernst Potential'

YouTube

Nernst Potential

is the concentration gradient of one ion in electrical units!
It is also the "chemical potential" of the ion

YouTube

$$E_{Na} = \frac{RT}{F} \log_e \frac{[\mathrm{Na}]_{out}}{[\mathrm{Na}]_{in}}$$

YouTube

Sodium current = sodium conductance × (Voltage $- \mathbf{E_{Na}}$)

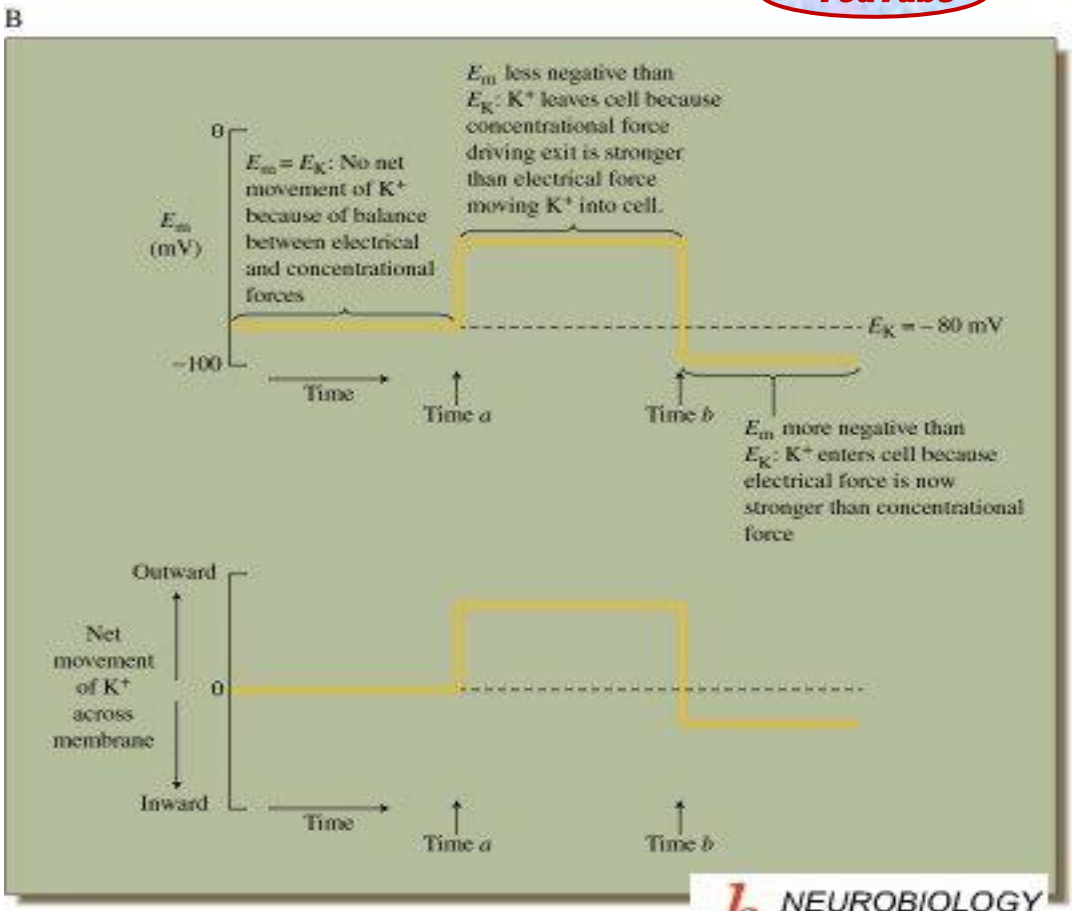
YouTube

Voltage = voltage across membrane = voltage across channel

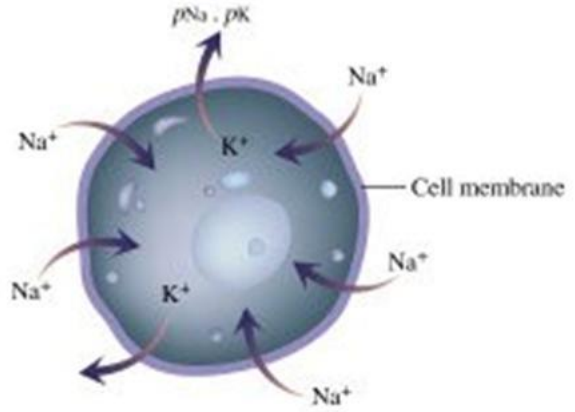
Voltage $- \mathbf{E_{Na}}$ is the DRIVING FORCE for the movement of sodium ions

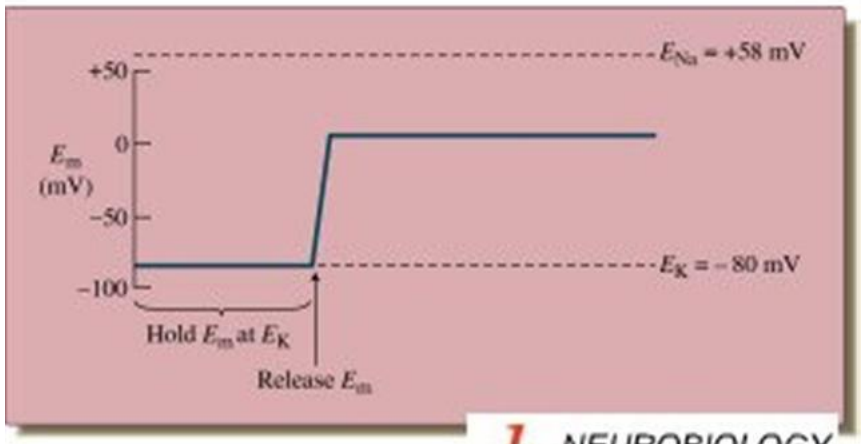
Voltage $- \mathbf{E_{Na}}$ combines electrical and chemical potential into the
ELECTROCHEMICAL POTENTIAL
for the movement of sodium ions

Potential Determined by the Ion with the greatest Conductance



Potential Determined by the Ion with the greatest Conductance





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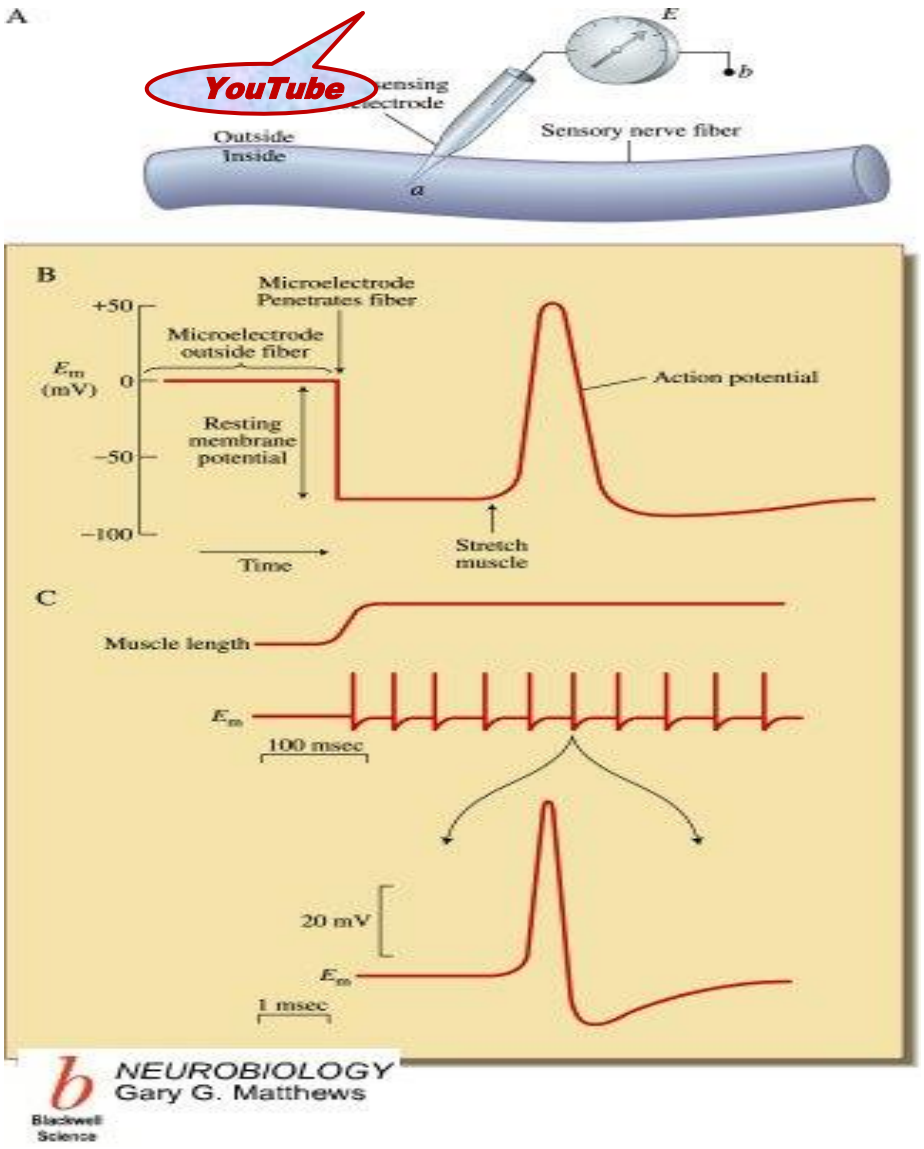
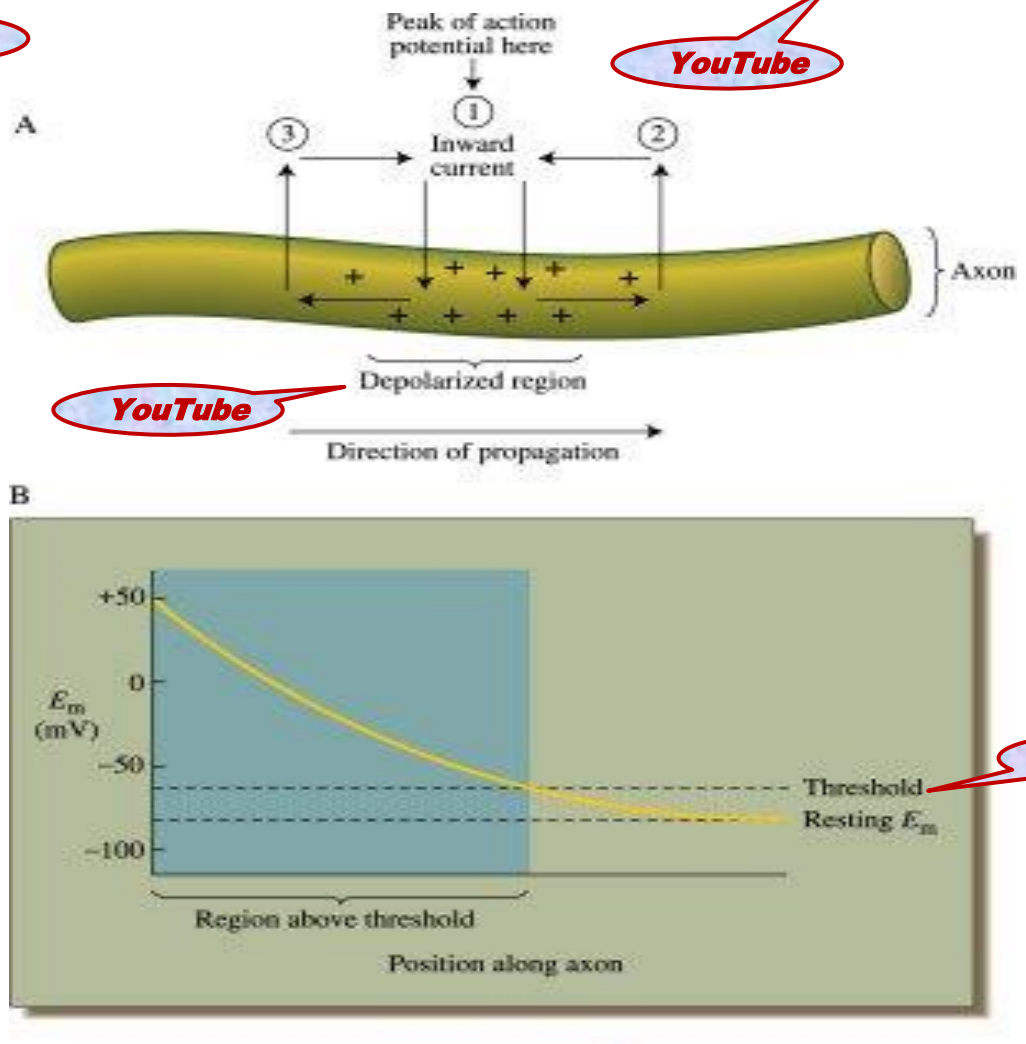
Action Potential



Passive Propagation



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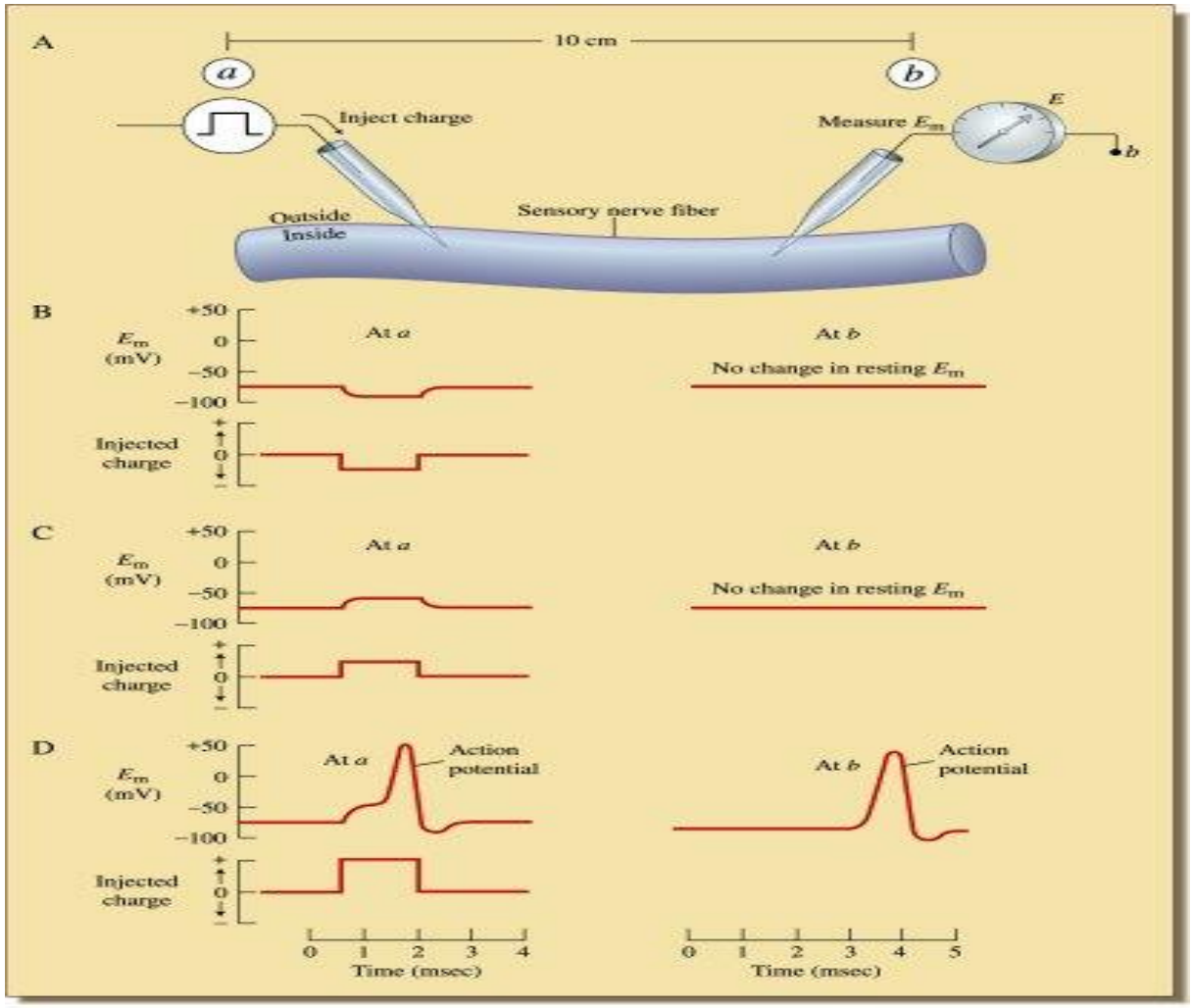
Propagation

Animation of Action Potential

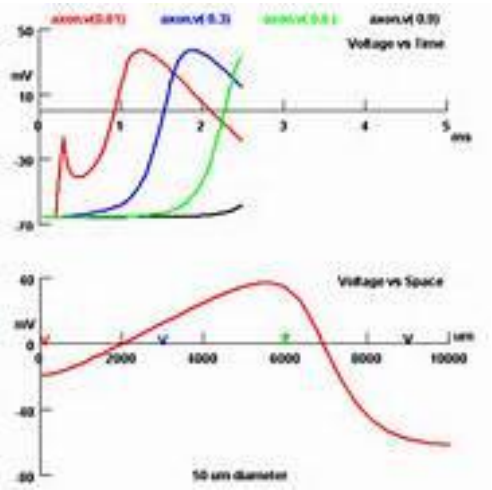
Good References

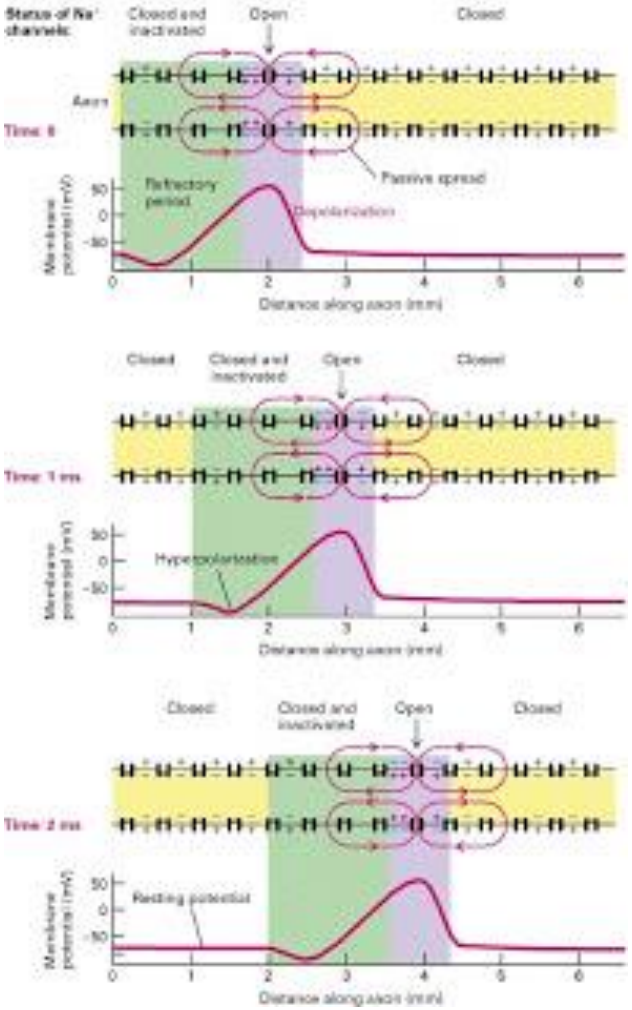
https://www.youtube.com/watch?v=kCdtm_chqKk

https://www.youtube.com/watch?v=oa6rvUJlg7o



Propagation
of
Action Potential





Myelinated Nerve Propagation

YouTube

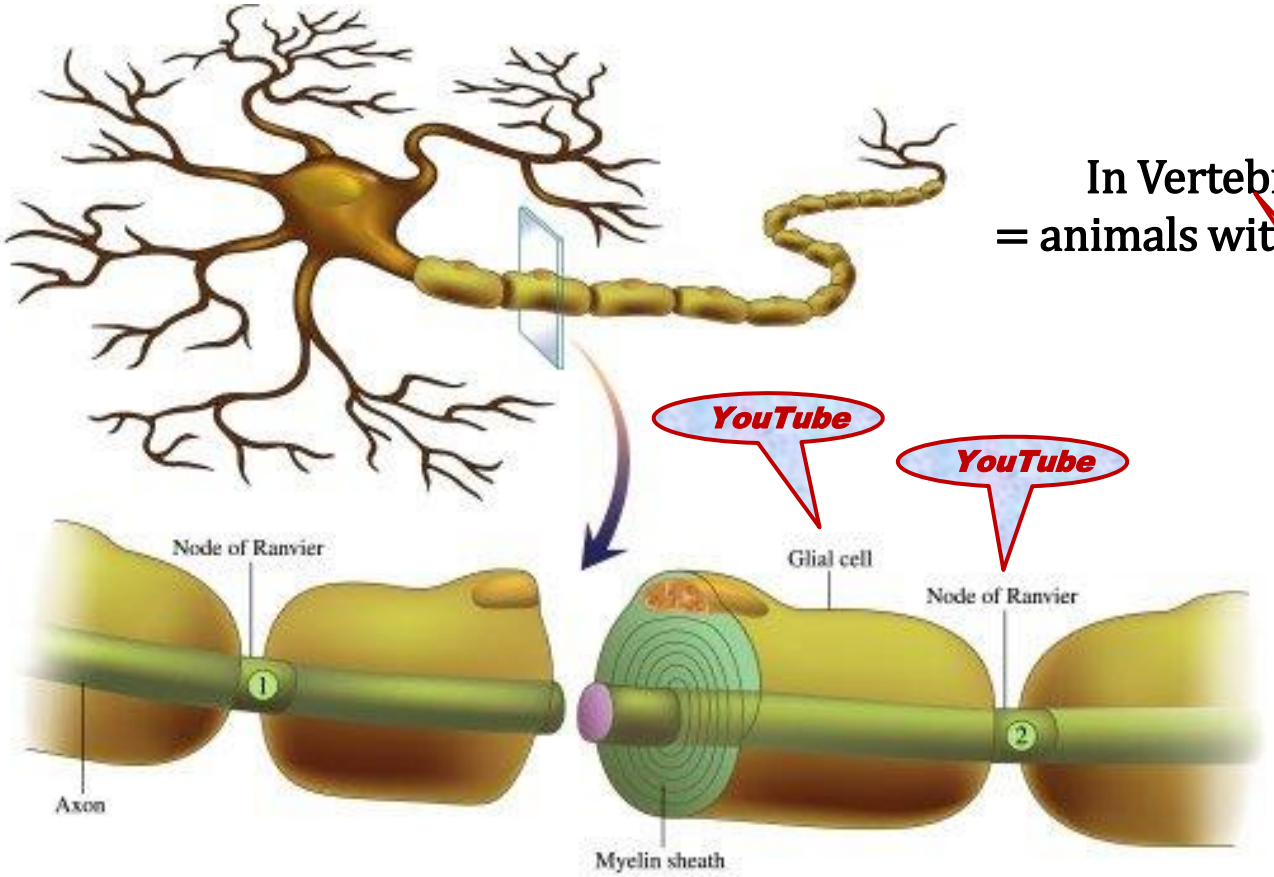
In Vertebrates
= animals with backbones

YouTube

Action Potential Propagation

YouTube

YouTube



Action potential at node 1 depolarizes node 2

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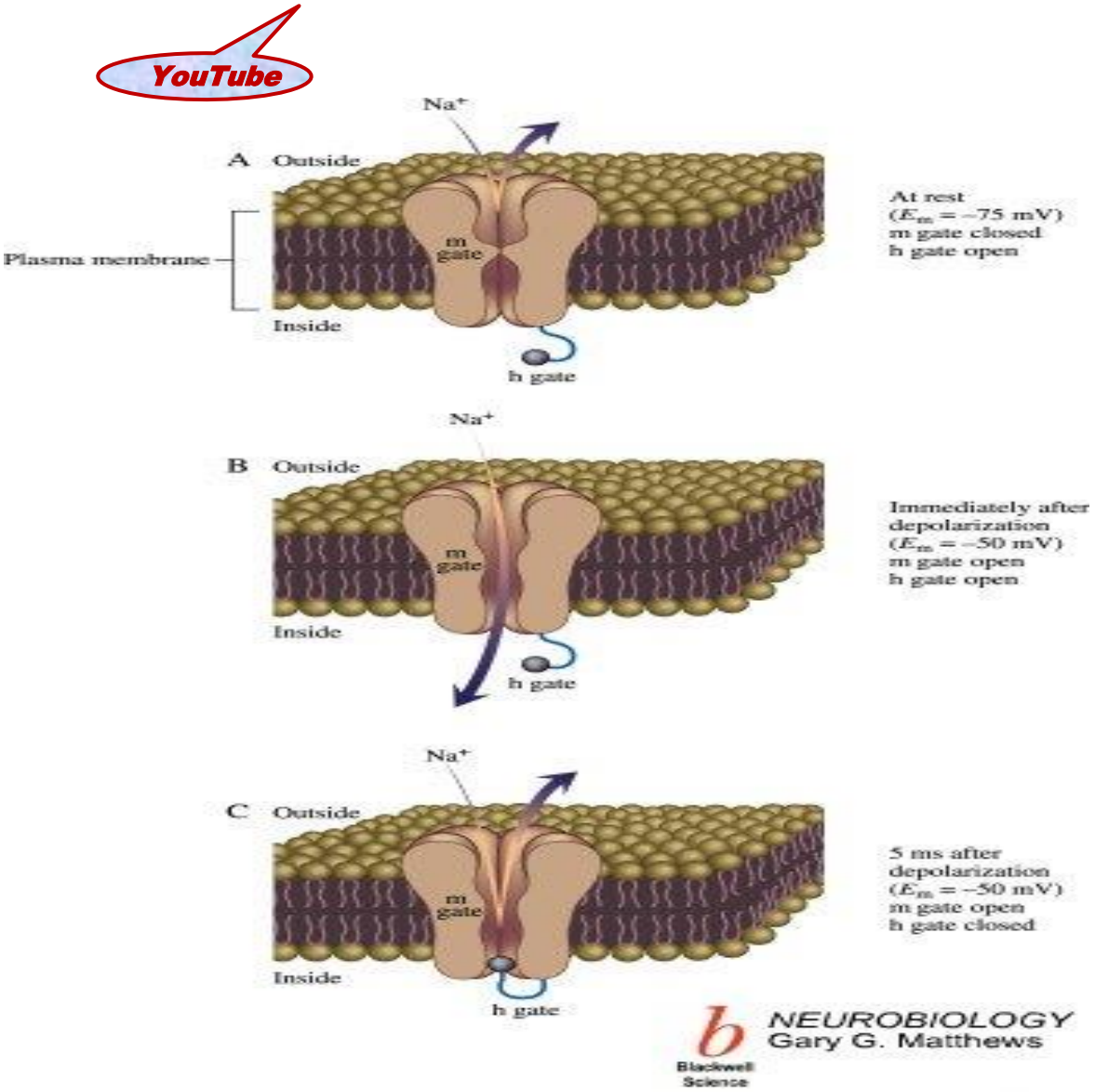
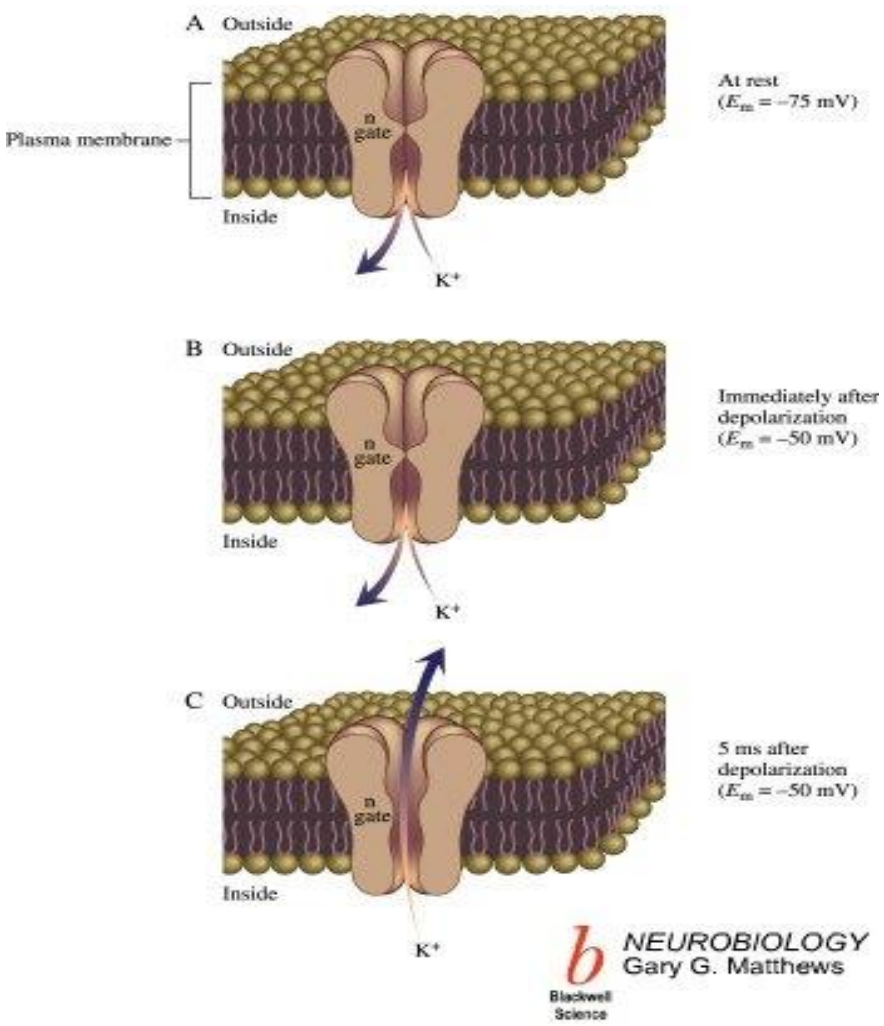
Blackwell Science

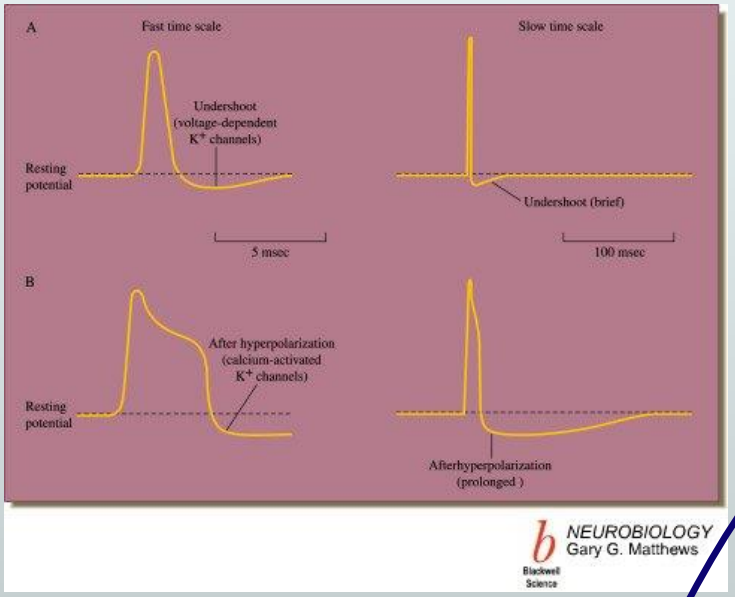
Sodium Na Channel

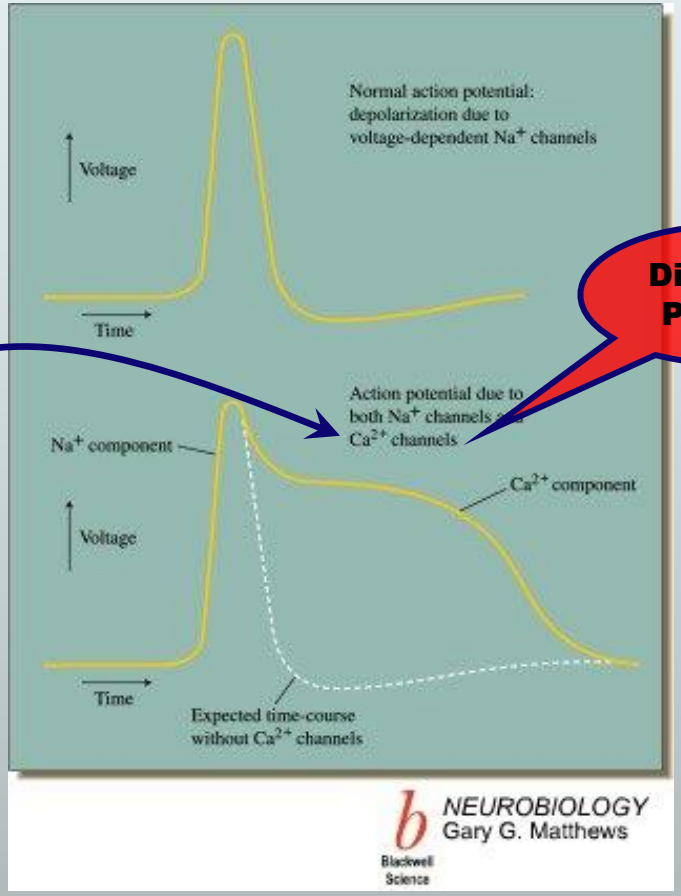


Potassium K channel



Reminder: the Action Potential





Different Protein

One Protein
One Function
(shape of Heart Action Potential)

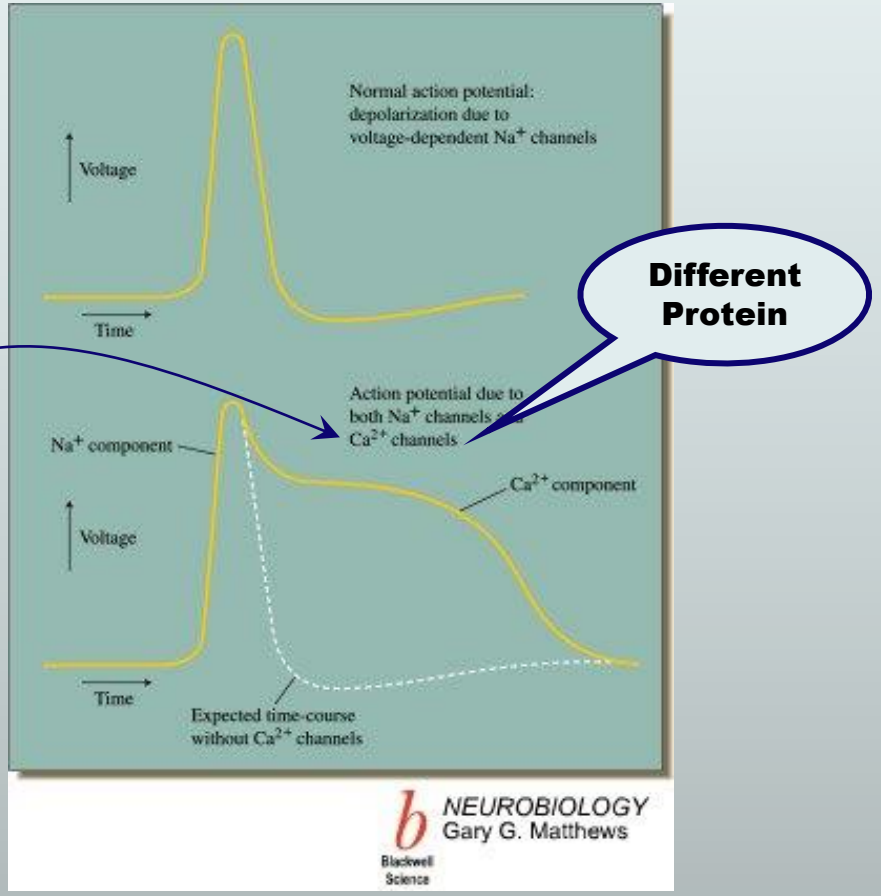
HERG channel
is a KILLER of Students

HERG channel

Sudden Death in STUDENTS
in about 2%
with FIRST USE OF COCAINE

Accumulates with multiple types of drugs, both medical and "recreational"

One Protein
One Function
(shape of Heart Action Potential)



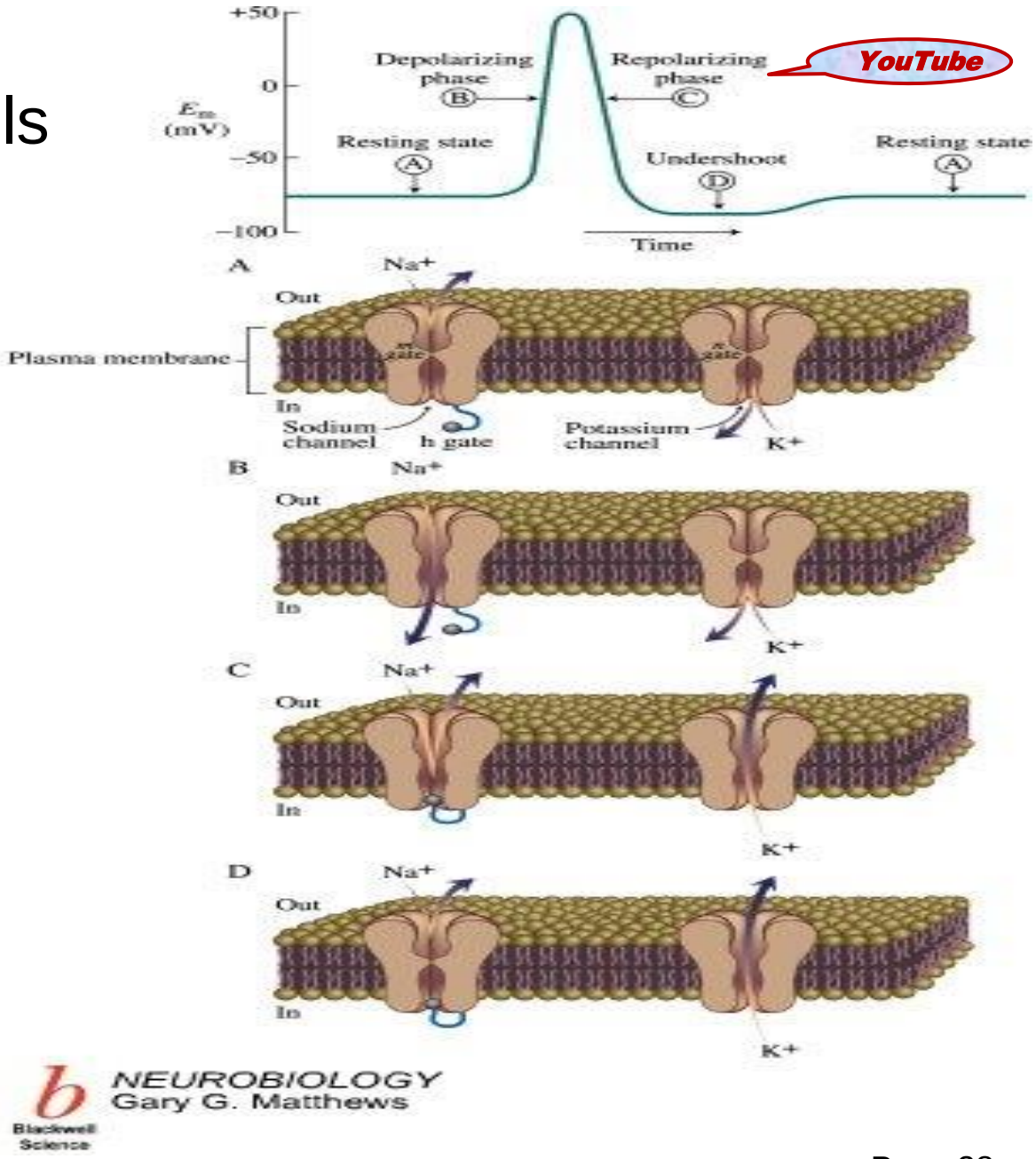
Na & K channels

Animation of Action Potential

Good References

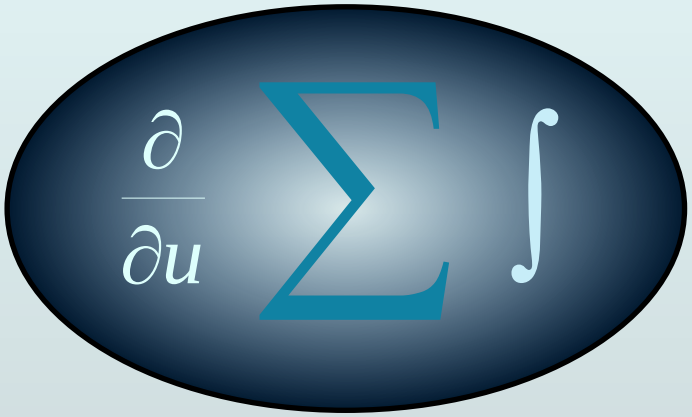
https://www.youtube.com/watch?v=kCdtm_chqKk

https://www.youtube.com/watch?v=oa6rvUJlg7o



Now Discuss Channels

September 2020



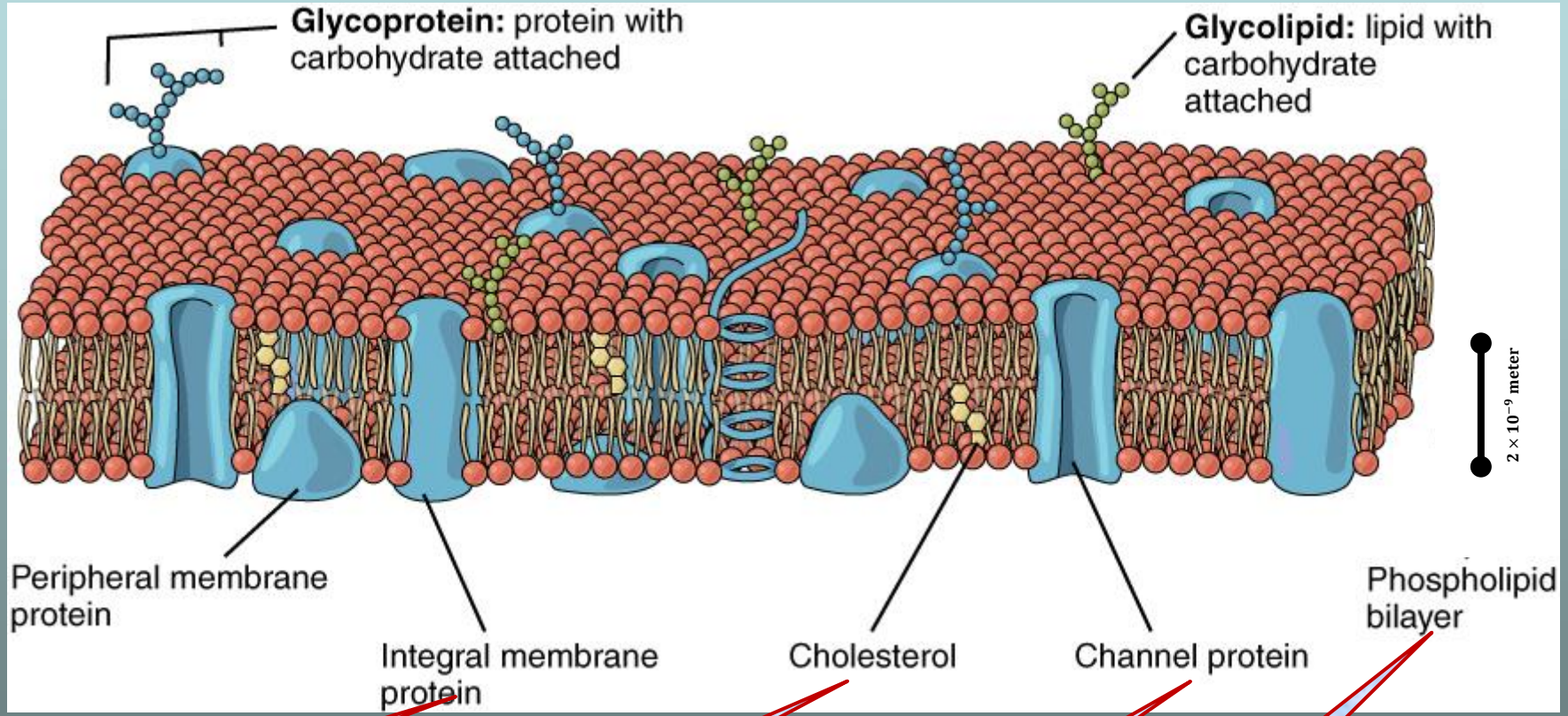
Short Course

From Atoms to Axons

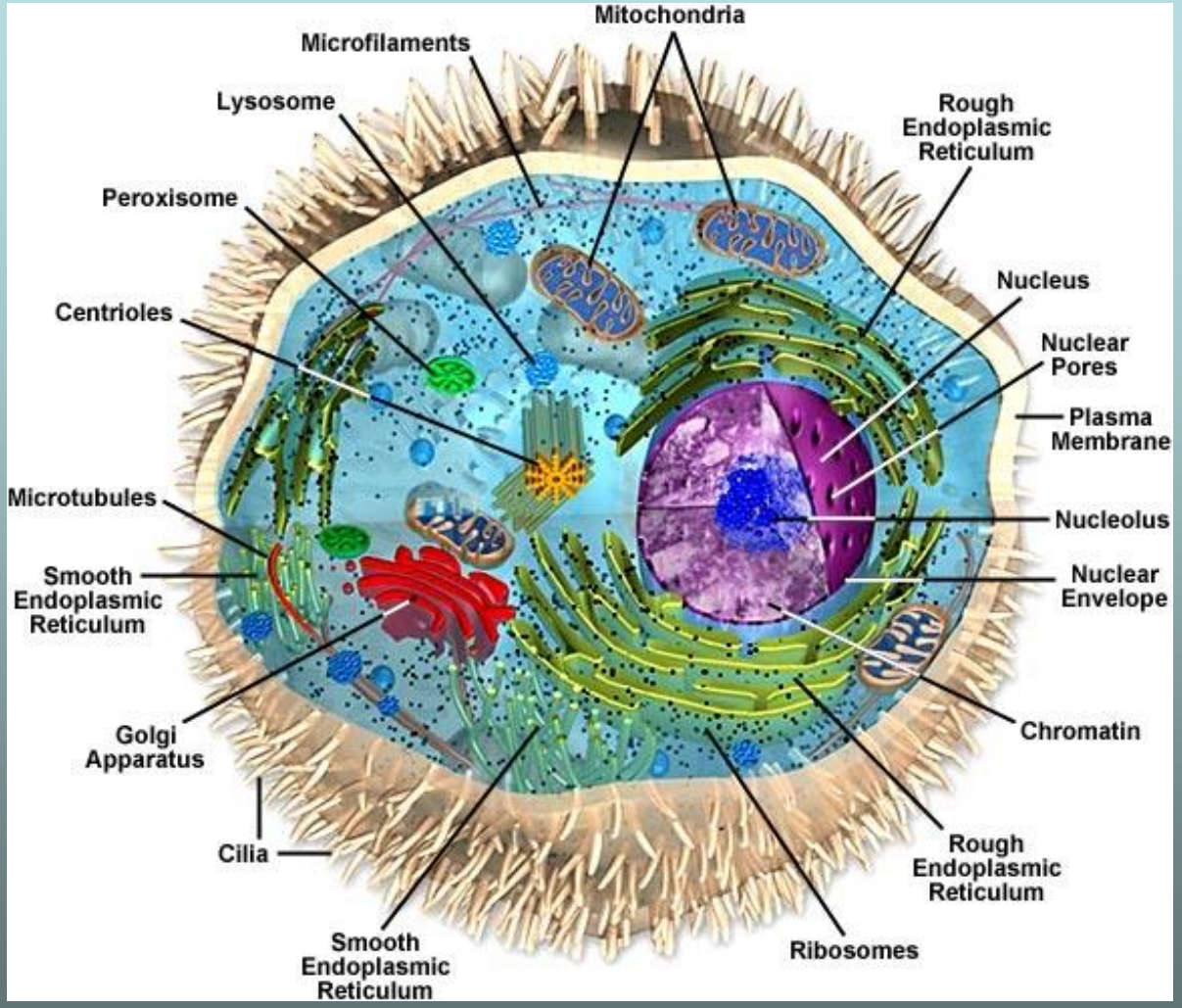
Bob Eisenberg

昆山杜克大学
DUKE KUNSHAN UNIVERSITY

Cells are defined by their Membranes



Most of Life Occurs In Cells with Membranes



10^{-5} meter

Ion Channels are the Valves of Cells

Ion Channels are the Main Controllers of Biological Function

YouTube

YouTube

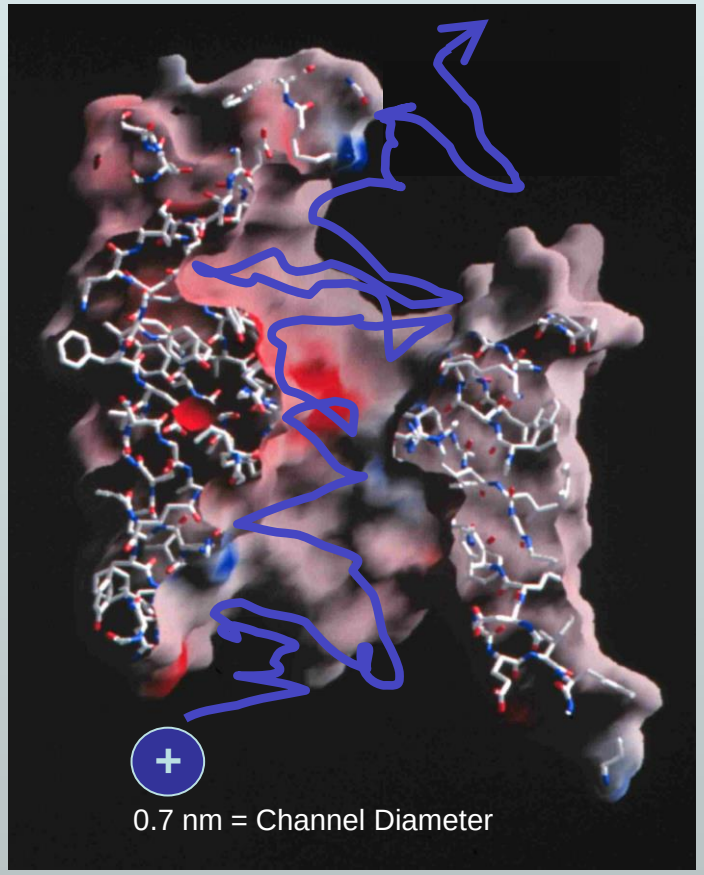
Selectivity

Different Ions carry Different Signals

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

YouTube

YouTube



~30 Å

Figure of ompF porin by Raimund Dutzler

YouTube

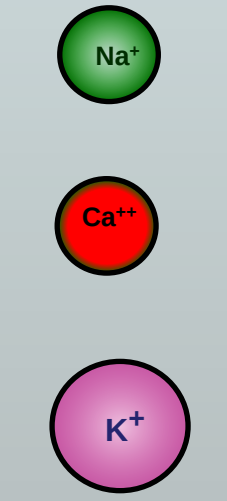
Ions in Water*

are the

Liquid of Life

***Pure H_2O is toxic to cells & proteins**

Hard Spheres



3 Å

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

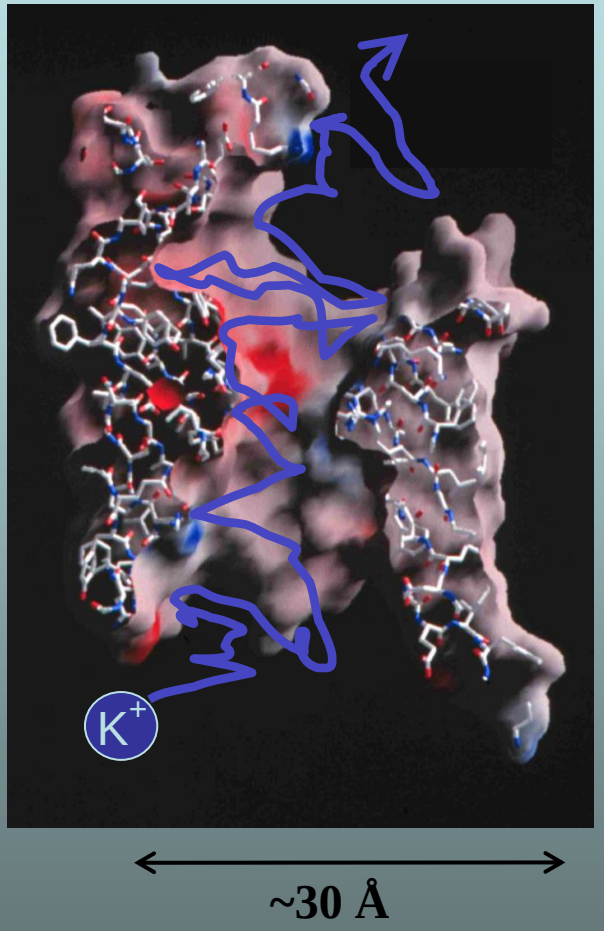
Ion channels produce signals of the nervous system

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

Channels are Devices

Channels are (nano) valves

Valves Control Flow

Classical Theory & Simulations

NOT designed for flow

Thermodynamics, Statistical Mechanics do not allow flow

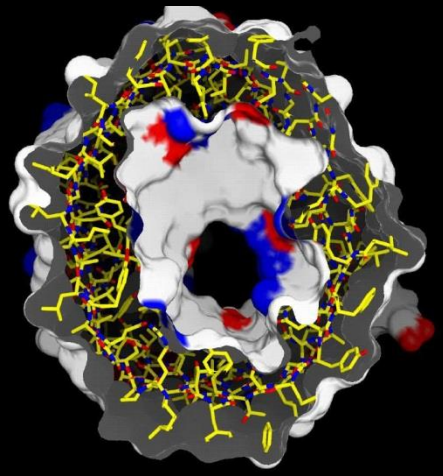
Rate and Markov Models do not Conserve Current

$$A \underset{k_{BA}}{\overset{k_{AB}}{\rightleftharpoons}} B \underset{k_{CB}}{\overset{k_{BC}}{\rightleftharpoons}} C$$

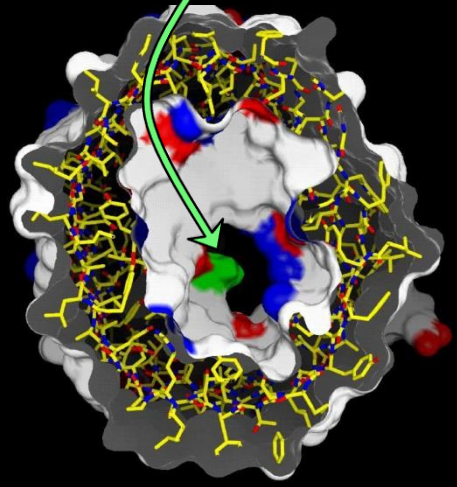
$$I_{AB} - I_{BA} \neq I_{BC} - I_{CB}$$

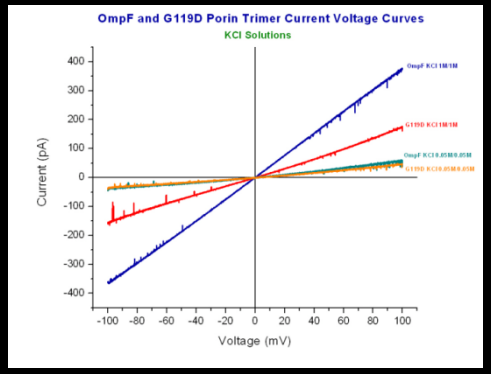
A few atoms make a BIG Difference

Ompf



G119D





Glycine replaced by Aspartate

Structure determined by Raimund Dutzler in Tilman Schirmer's lab

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

Multi-Scale Issues are Always Present

in

Atomic Scale Engineering

Atomic & Macro Scales are
<u>both</u> used by channels
just because
<u>Channels are Nanovalves</u>

By definition: all valves use small structures to control large flows

How do a few atoms control (macroscopic) Biological Function?

Answer, *oversimplified:*

A few atoms control the electric field

Much as they do in transistors

The Electric Field is Strong

If you were standing at arm's length from someone and each of you had

***One percent* more electrons than protons,**

the force
would lift the
Entire Earth!

slight paraphrase of third paragraph, p. 1-1 of
Feynman, Leighton, Sands (1963)
'*The Feynman Lectures on Physics,*
***Mainly Electromagnetism and Matter*'**
also at *http://www.feynmanlectures.caltech.edu/II_toc.html.*

Thermodynamics, Statistical Mechanics, Molecular Dynamics are UNSUITED for DEVICES

Thermodynamics, Statistical Mechanics, Molecular Dynamics have

No inputs, outputs, flows, or power supplies

YouTube

or

DEVICE EQUATIONS

YouTube

Power supply = spatially nonuniform inhomogeneous Dirichlet conditions

Analysis of Devices must be NONEQUILIBRIUM with spatially non-uniform BOUNDARY CONDITIONS

YouTube

Tremendous Opportunity For Applied Mathematics

More important than most Nobel Prizes

THEORETICAL CHEMISTRY MUST BE REBUILT

To deal with FLOW of CURRENT and changes

Where to start?

Why not compute all the atoms?

A different talk!

Multiscale Issues

Journal of Physical Chemistry C (2010)114:20719

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

Journal of Physical Chemistry C (2010)114:20719

Uncalibrated Simulations will not make devices that actually work

Calibration is Hard Work

particularly for Non-Ideal systems

with

Correlations, Finite Size effects, and Flows

Where to start?

Mathematically ?

Physically ?

.

Reduced Models are Needed

Reduced Models are Device Equations

like Input Output Relations of Engineering Systems

The device equation is the mathematical statement of how the system works

Device Equations describe 'Slow Variables'

found in some complicated systems

How find a Reduced Model?

Biology is Easier than Physics

Reduced Models Exist*

for important biological functions

or the

Animal would not survive

to reproduce

***Evolution provides the existence theorems and uniqueness conditions so hard to find in theory of inverse problems.**

(Some biological systems – the human shoulder – are not robust,
probably because they are incompletely evolved,
i.e they are in a local minimum 'in fitness landscape' .
I do not know how to analyze these.
I can only describe them in the classical biological tradition.)

Reduced models exist

because

they are the adaptation

created by evolution

to perform a biological function

like selectivity

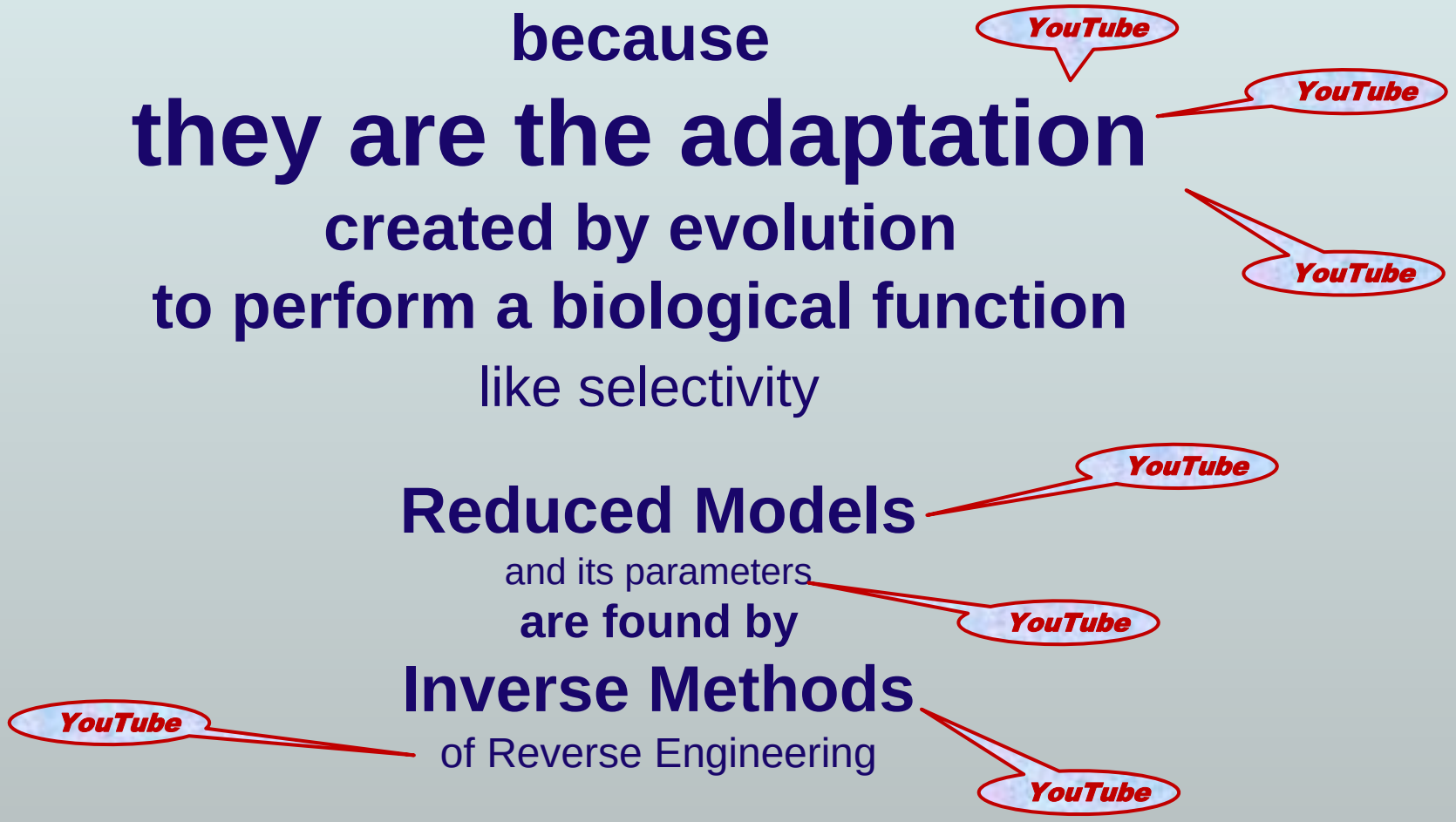
Reduced Models

and its parameters

are found by

Inverse Methods

of Reverse Engineering



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

Working Hypothesis:

Crucial Biological Adaptation is

Crowded Ions *and* Side Chains

Wise to use the Biological Adaptation
to make the reduced model!

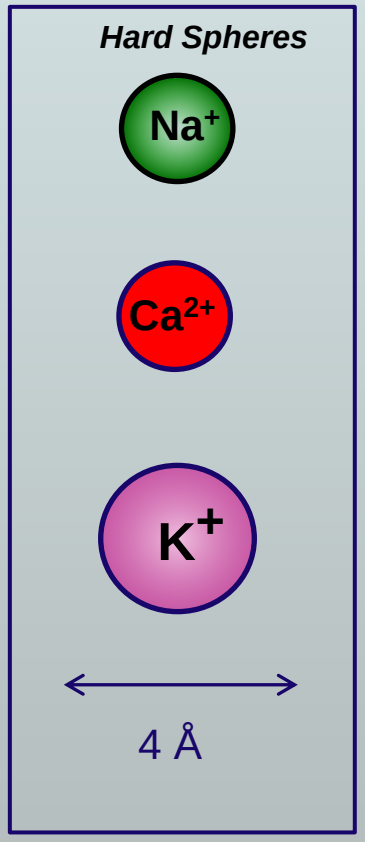
Reduced Models allow much easier Atomic Scale Engineering

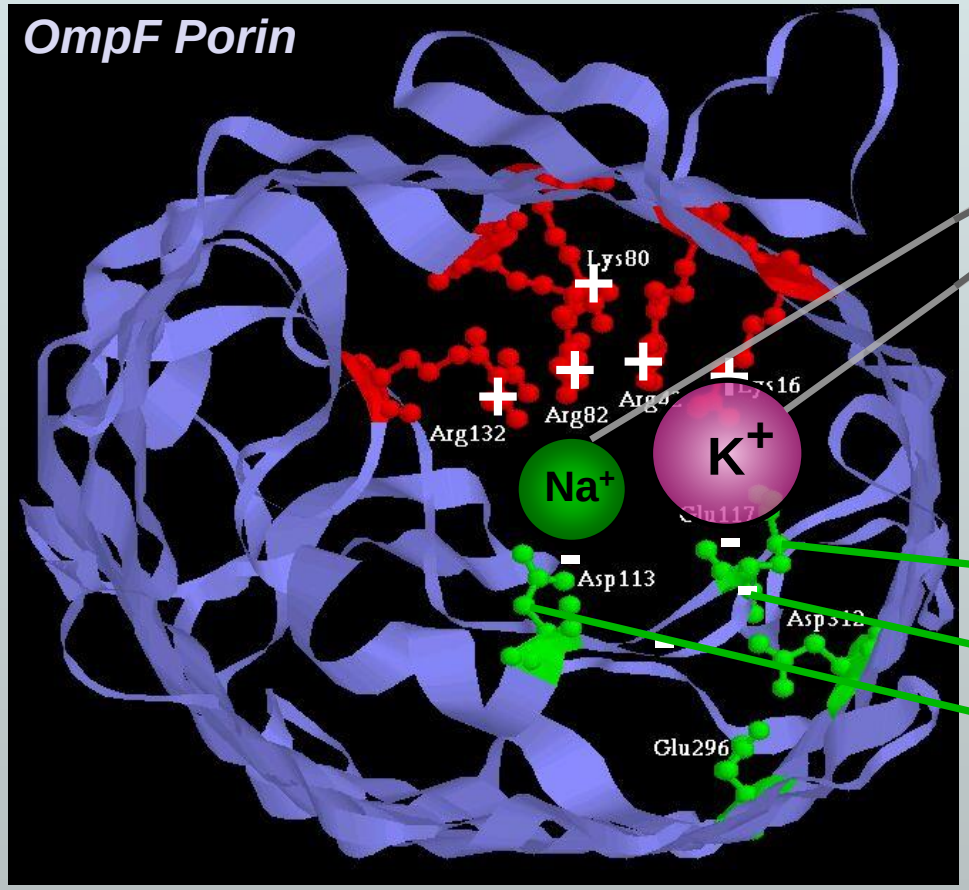
Physical basis of function

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

YouTube

Induced Fit of Side Chains

YouTube

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

Figure adapted from Tilman Schirmer

Crowded Active Sites

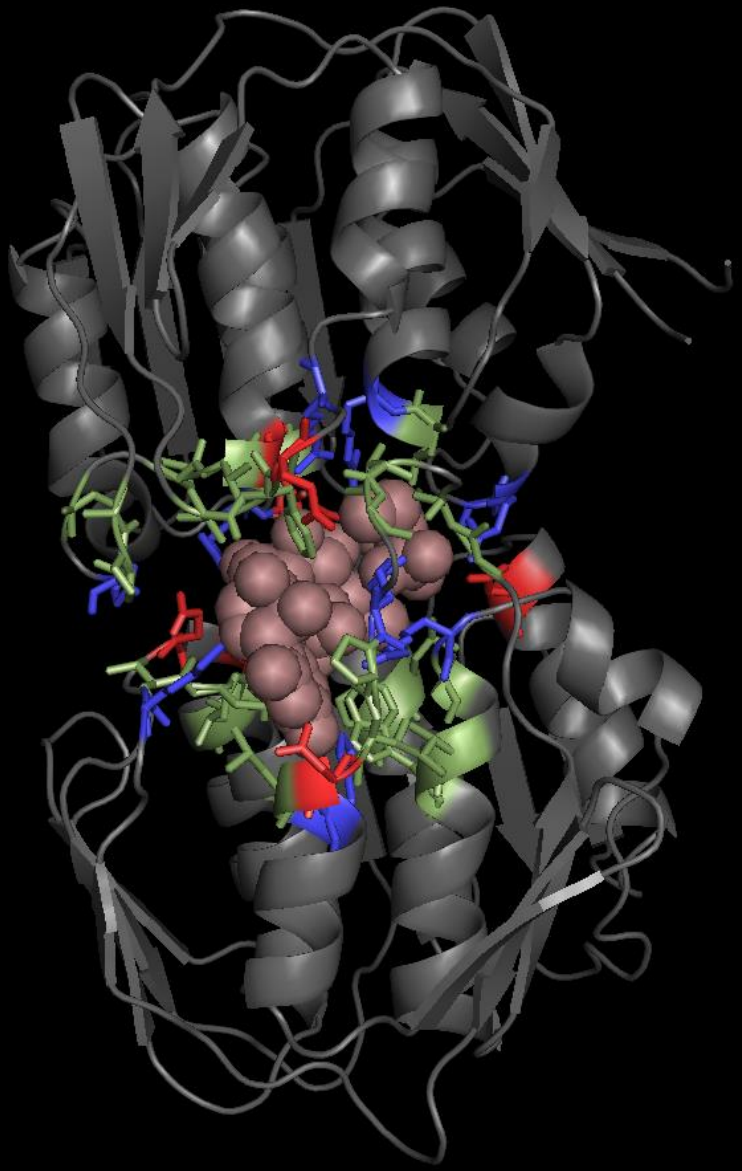
in 573 Enzymes

Enzyme Type		Catalytic Active Site Density (Molar)			Protein
		Acid (positive)	*Basic* (negative)	*Total*	*Elsewhere*
	Total (n = 573)	**10.6**	**8.3**	**18.9**	**2.8**
EC1	Oxidoreductases (n = 98)	7.5	4.6	12.1	2.8
EC2	Transferases (n = 126)	9.5	7.2	16.6	3.1
EC3	Hydrolases (n = 214)	12.1	10.7	22.8	2.7
EC4	Lyases (n = 72)	11.2	7.3	18.5	2.8
EC5	Isomerases (n = 43)	12.6	9.5	22.1	2.9
EC6	Ligases (n = 20)	9.7	8.3	18.0	3.0

*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

Don't worry!

Crowded Charge
is GOOD

It enables
SIMPLIFICATION

by exploiting a biological fact
(an adaptation)

Charges are Crowded where they are important!

Where do we begin?

Crowded Charge
enables
Dimensional Reduction*
to a
Device Equation

Essence of Engineering
is knowing
What Variables to Ignore!
WC Randels
in Warner IEEE Trans CT 48:2457 (2001)

***Dimensional reduction = ignoring some variables**

Three Channel Types

RyR, Ca_V = EEEE, and **Na_V** = DEKA

analyzed <u>successfully</u>*

in a wide range of solutions

by the

‘All Spheres’ Primitive Model

Implicit solvent model of open channel

ions and protein side chains are hard spheres in this model

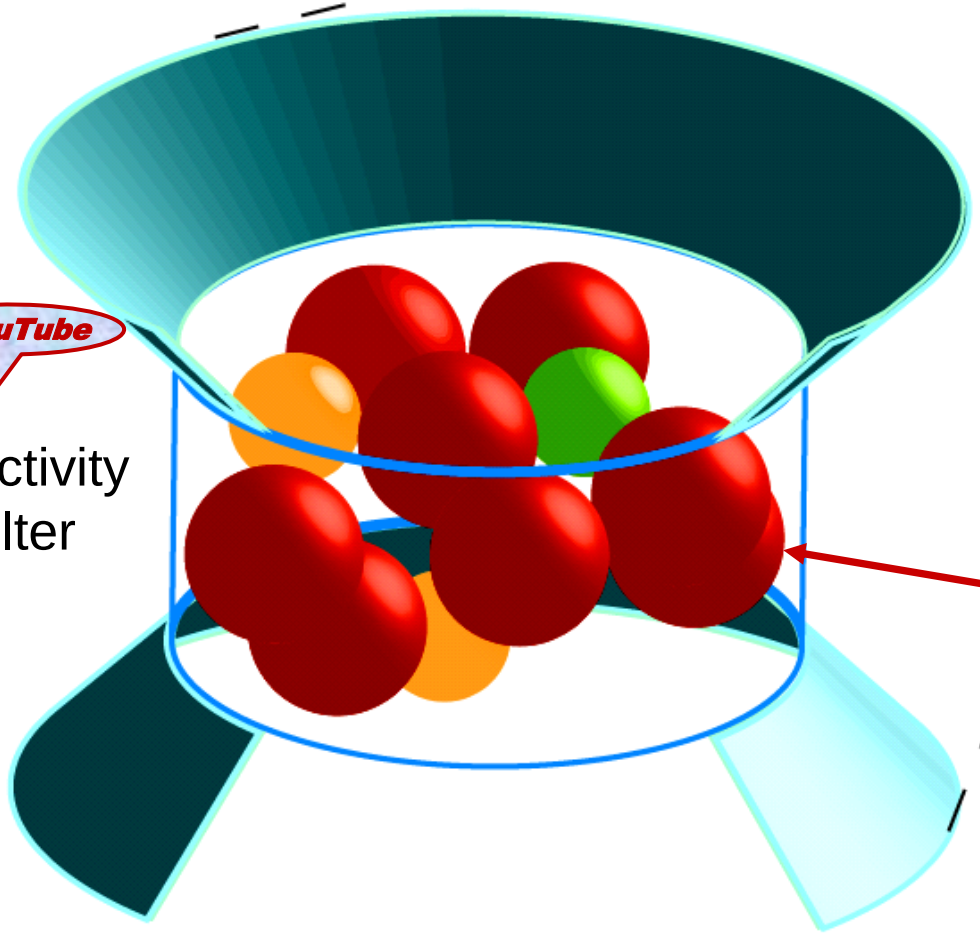
* Many methods have been used in more than 30 papers since Nonner and Eisenberg, 1998

Selectivity Filter

Crowded with Charge

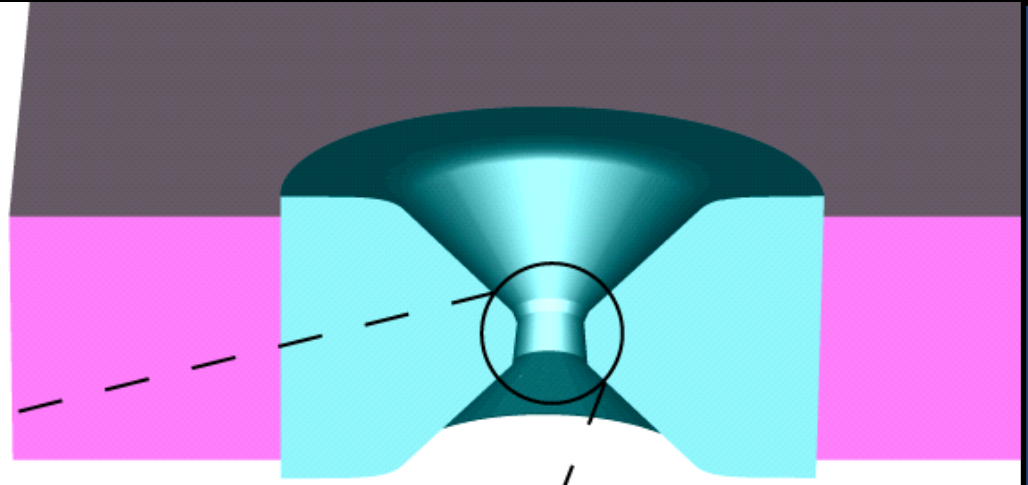
L type Ca Channel

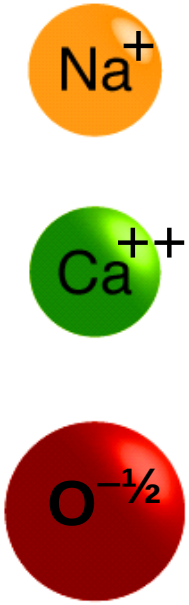
YouTube



YouTube

Selectivity Filter





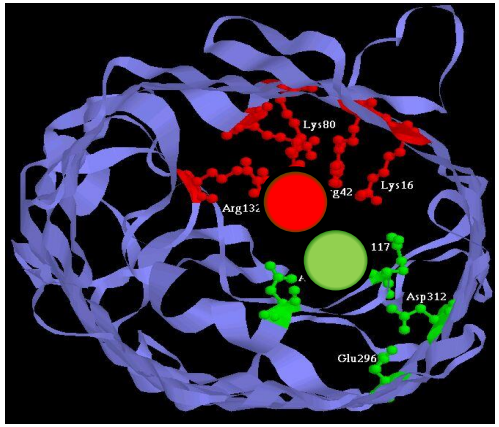
“Side Chains”

Wolfgang Nonner

Experiments have 'engineered' channels (5 papers) including

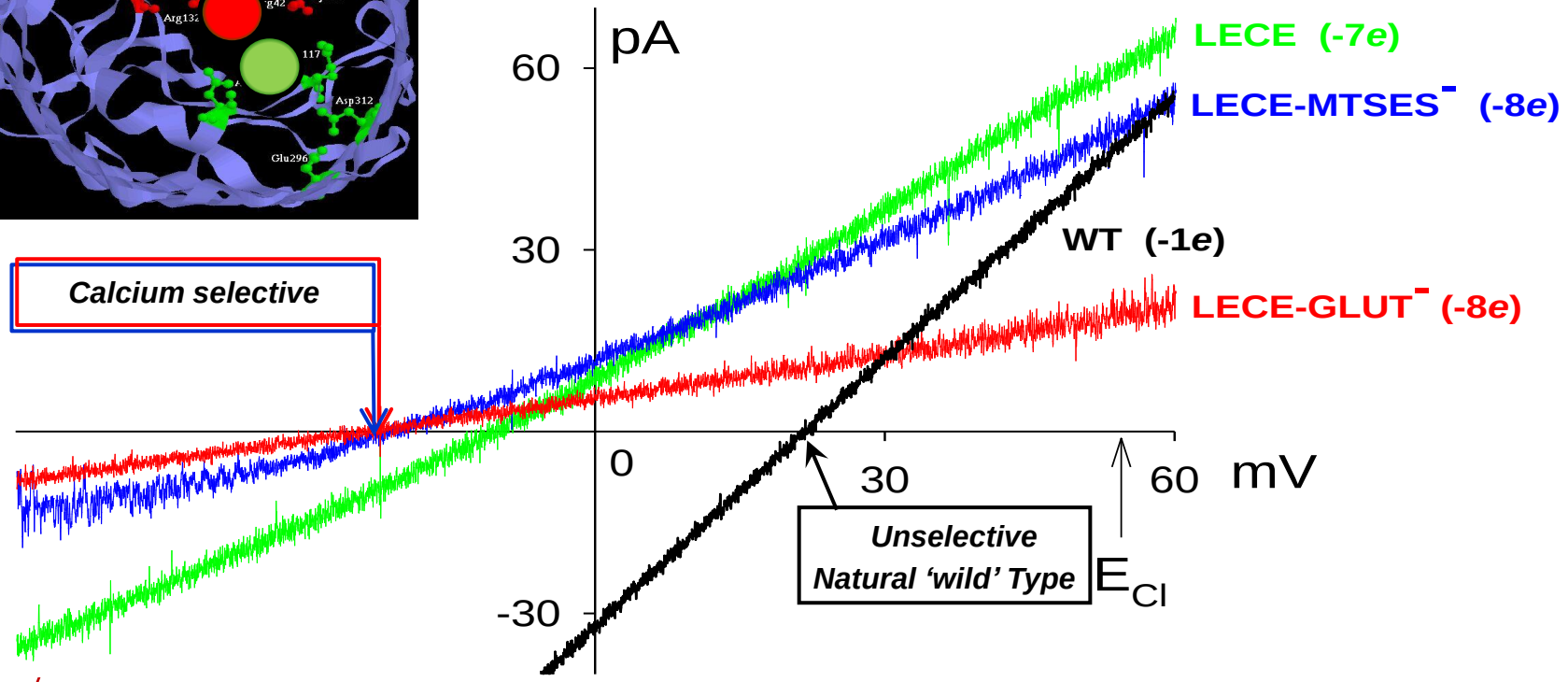
Two Synthetic Calcium Channels

Atomic Scale



Designed by Theory

Glutathione derivatives



As density of permanent charge increases, channel becomes calcium selective

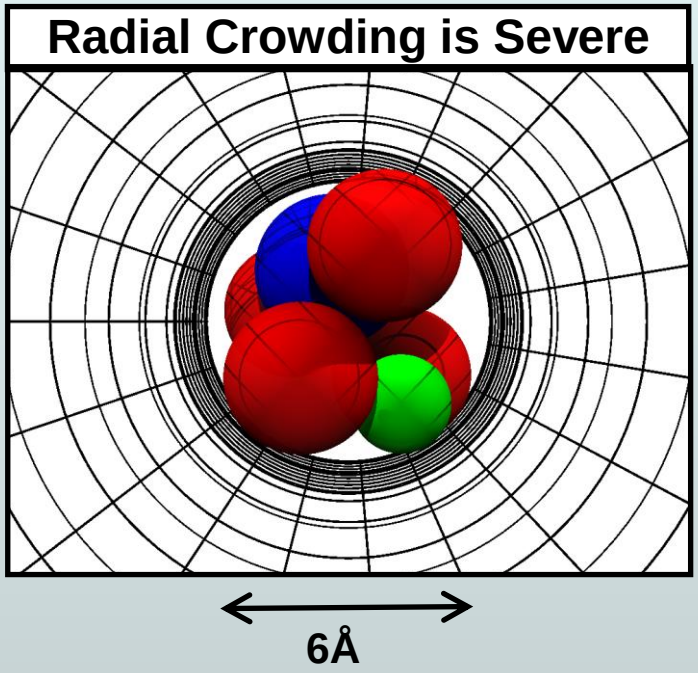
$E_{rev} \rightarrow E_{Ca}$ in 0.1M ||1.0 M $CaCl_2$; pH 8.0

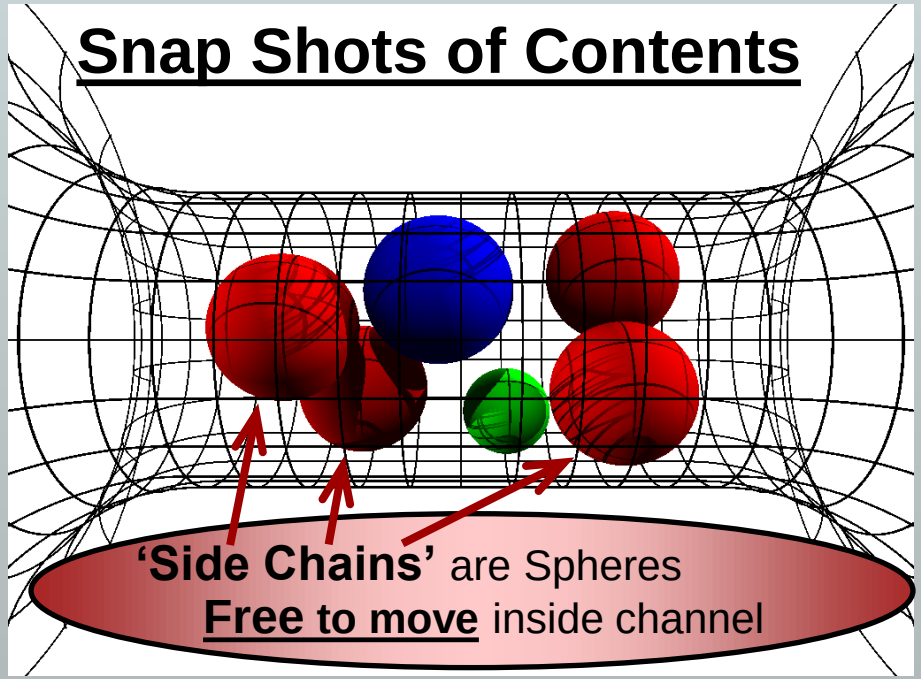
Macro Scale

built by Henk Miedema, Wim Meijberg of BioMade Corp. Groningen, Netherlands 57

***Miedema** et al, Biophys J 87: 3137–3147 (2004); 90:1202-1211 (2006); 91:4392-4400 (2006)*

Crowded Ions





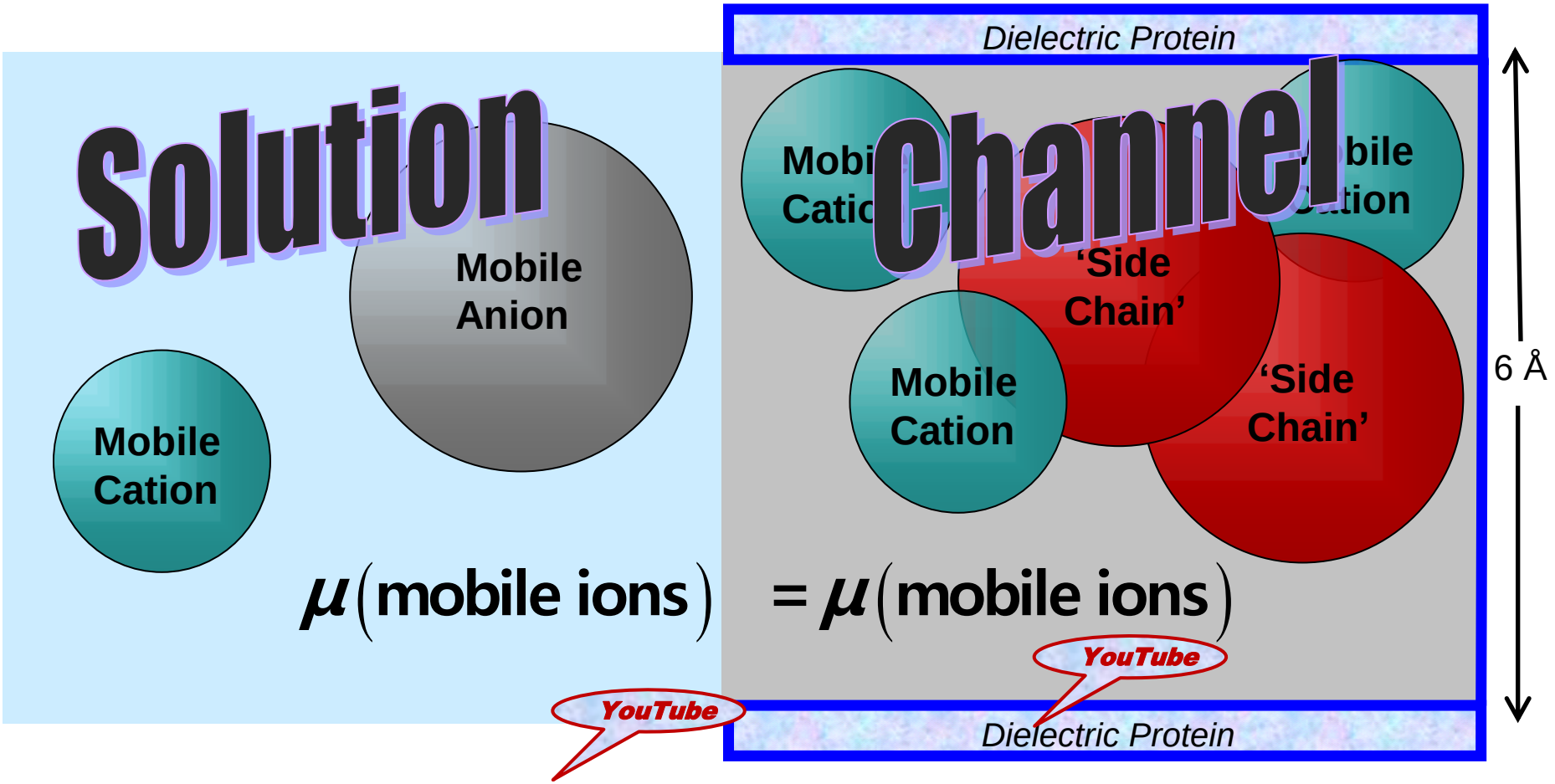
Ion Diameters *'Pauling' Diameters*	
Ca^{++}	1.98 Å
Na^{+}	2.00 Å
K^{+}	2.66 Å
'Side Chain' Diameter	
Lysine K	3.00 Å
D or E	2.80 Å
Channel Diameter 6 Å	

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

Ion 'Binding' in Crowded Channel



Classical Donnan Equilibrium of Ion Exchanger

large mechanical forces

YouTube

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.

Many methods

give nearly identical results:

Equilibrium Multiscale

MSA (mean spherical approximation
SPM (primitive solvent model)
DFT (density functional theory of fluids),
MC-loc (MC with localized side chains)

Non-equilibrium Multiscale

DFT-PNP (Poisson Nernst Planck)
EnVarA (Energy Variational Approach)
DMC *Dynamic Monte Carlo*
NP-LEMC *(Nernst Planck Local Equilibrium Monte Carlo)*
Steric PNP
Poisson Fermi; *etc.*

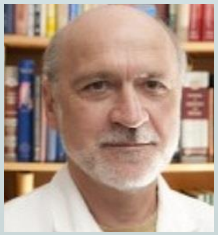
Charge-Space Competition

Monte Carlo Methods

YouTube

Dezső Boda

Doug Henderson



Wolfgang Nonner

Dirk Gillespie

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.

More than 35 papers are available at

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

*M*etropolis *M*onte *C*arlo Simulates Location of Ions

both the mean and the variance

MMC *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, $E_B \neq 0$ and configuration may be accepted

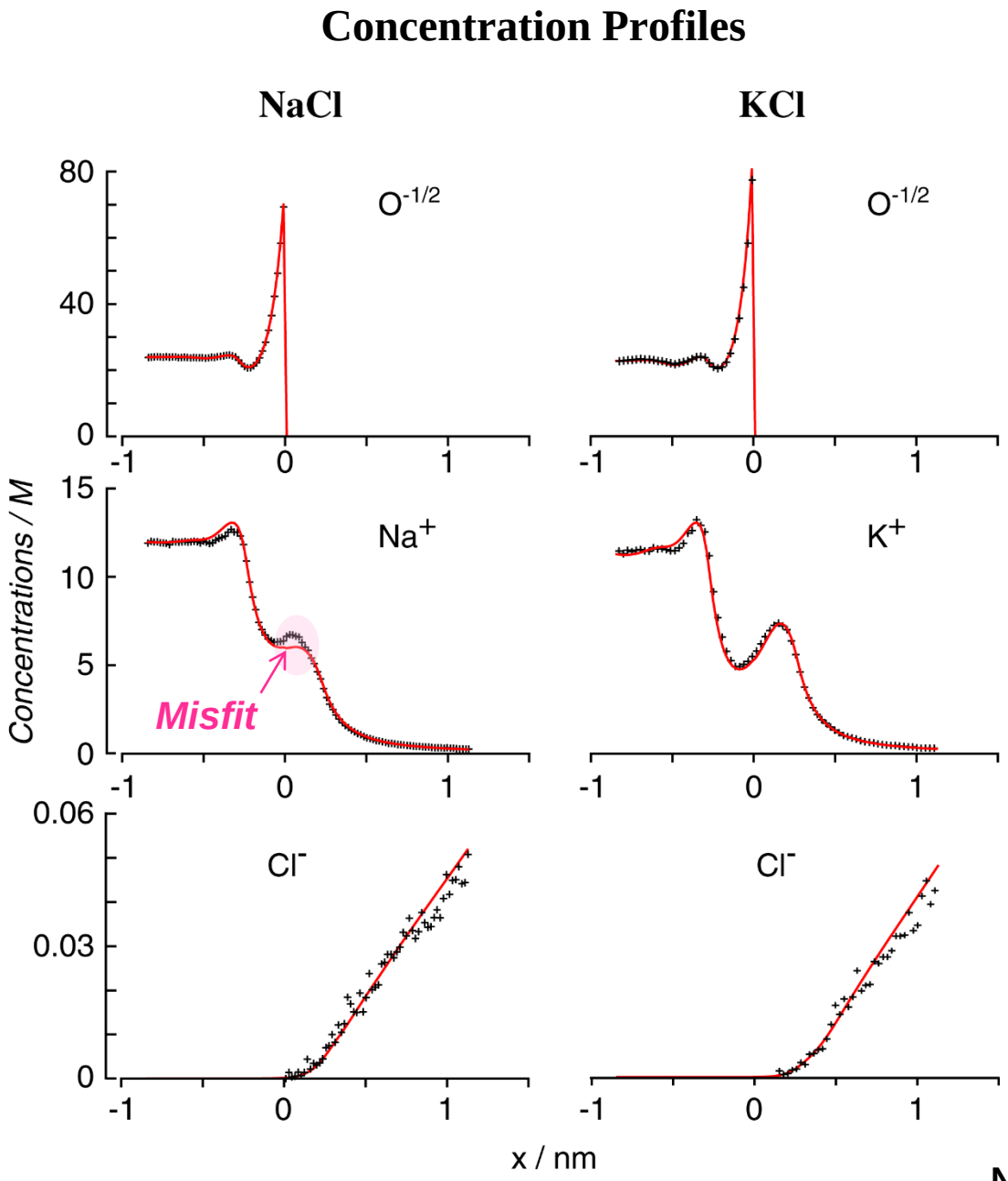
(4.1) If $E_B < E_A$: accept new configuration.

(4.2) If $E_B > E_A$: accept new configuration with probability $\exp\left[-(E_A - E_B)/k_B T\right]$

Key idea

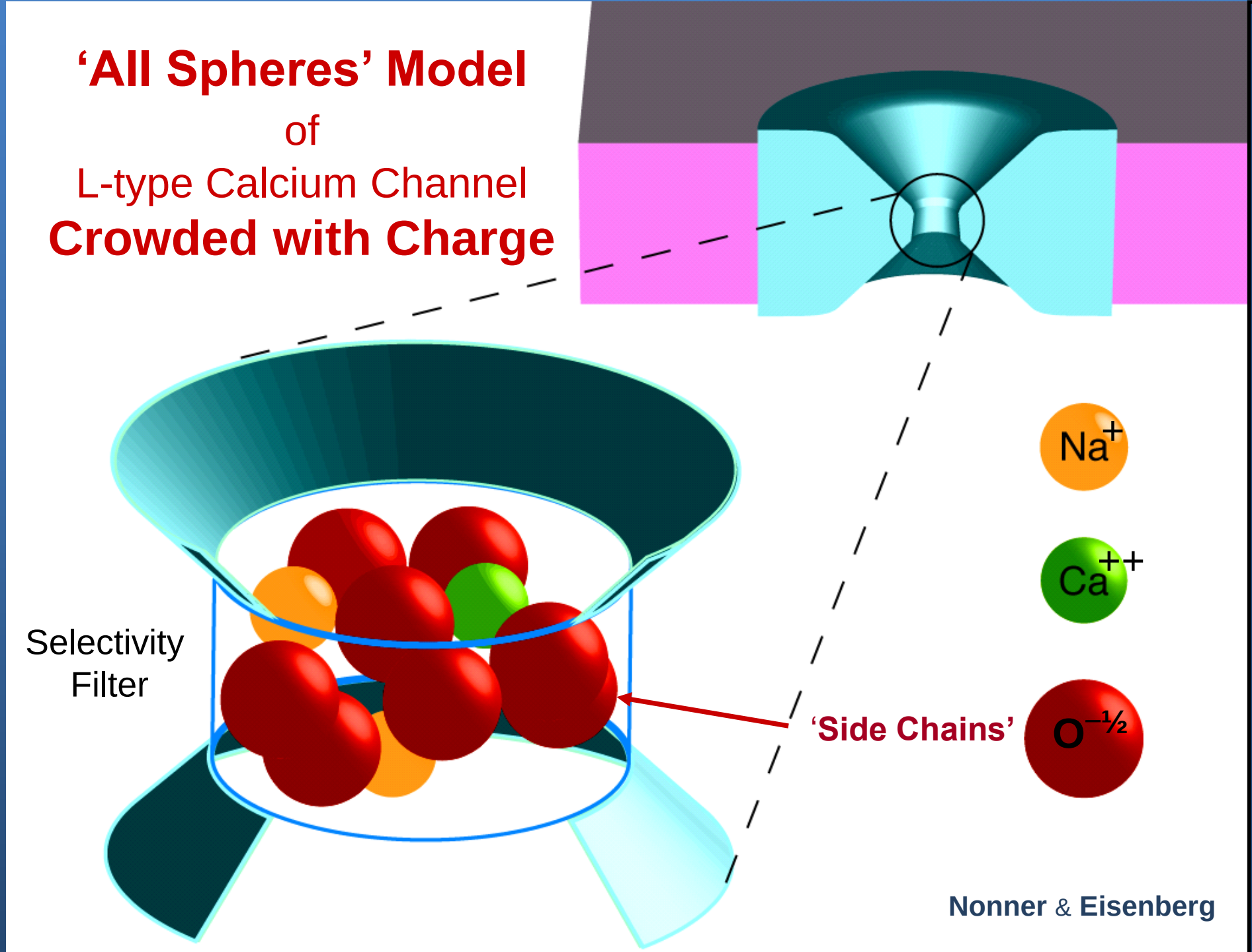
Instead of choosing configurations from uniform distribution, then weighting them with $\exp(-E/k_B T)$, ***MMC*** chooses them with a Boltzmann probability and weights them evenly.

DFT/PNP *vs* Monte Carlo Simulations



Nonner, Gillespie, Eisenberg

‘All Spheres’ Model
of
L-type Calcium Channel
Crowded with Charge



Nonner & Eisenberg

Ca Channel

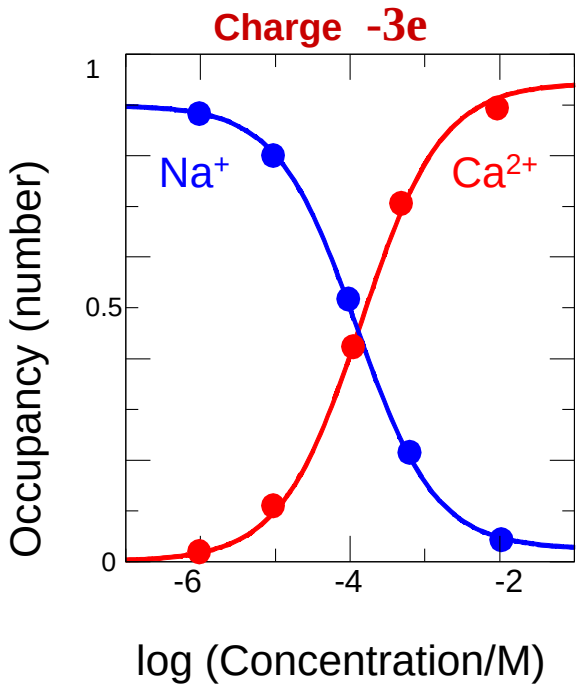
Mutation

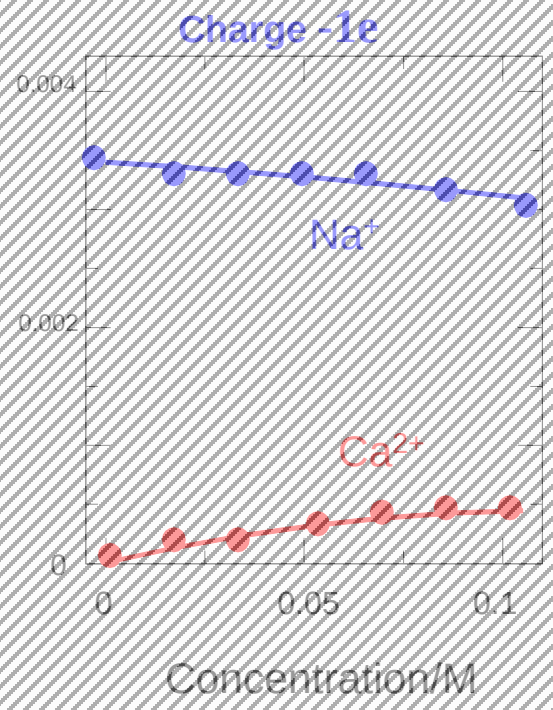
Na Channel

Same Parameters

E
E
E
A

D
E
K
A



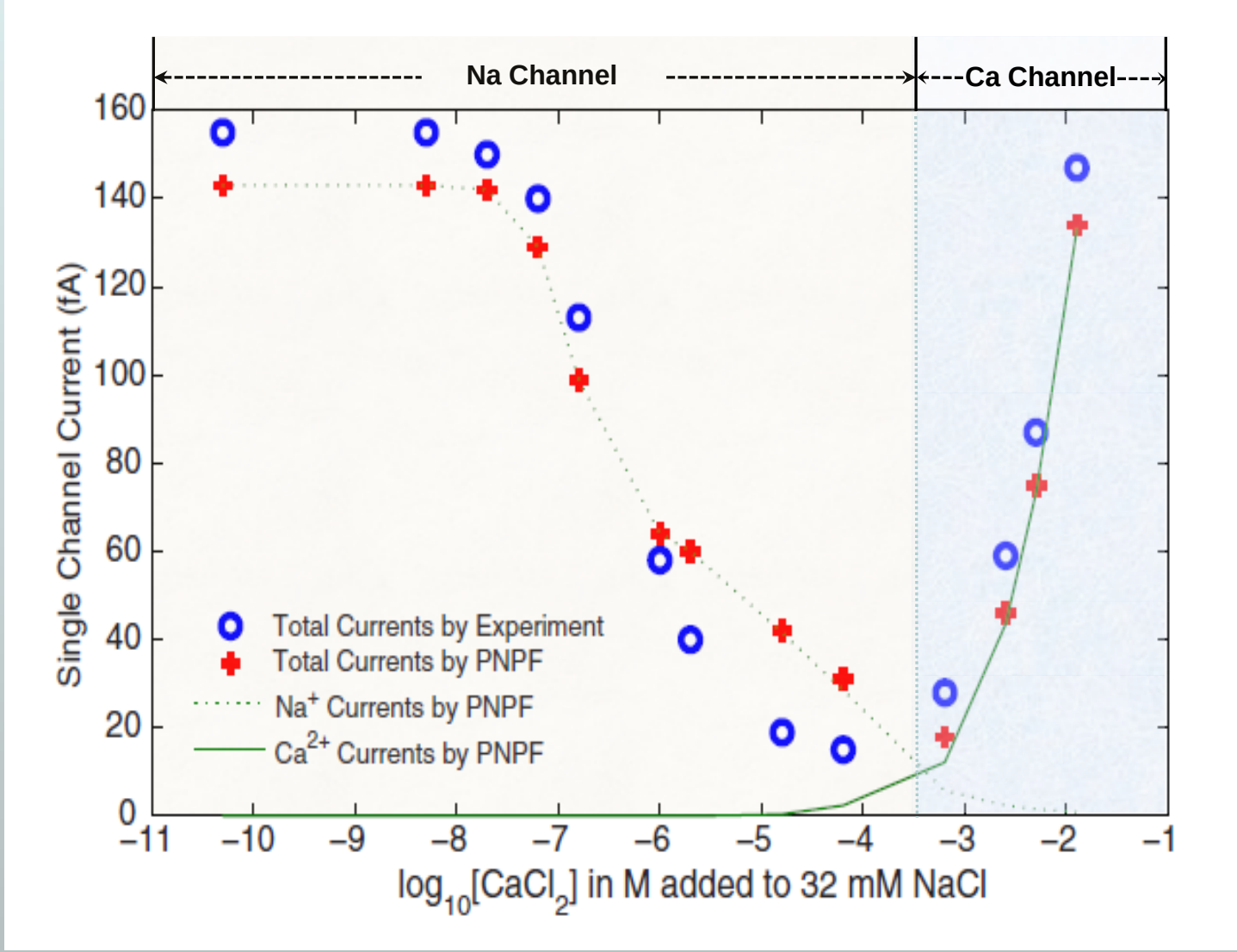


EEEE has full biological selectivity in similar simulations

Boda, et al

Signature of Cardiac Calcium Channel $Ca_V1.n$

Anomalous Mole Fraction (non-equilibrium)*



******Anomalous* **because CALCIUM CHANNEL IS A SODIUM CHANNEL at $[CaCl_2] < 10^{-3.4}$**
Ca^{2+} is conducted for $[Ca^{2+}] > 10^{-3.4}$, but Na^+ is conducted for $[Ca^{2+}] < 10^{-3.}$

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

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

Sodium Channel

Voltage controlled channel responsible for signaling in nerve and coordination of muscle contraction

Challenge

from channologists

Walter Stühmer and **Stefan Heinemann**

Göttingen *Leipzig*

Max Planck Institutes

Can THEORY explain the MUTATION
Calcium Channel into Sodium Channel?

DEEA ⟺ ***DEKA***

Calcium Channel ***Sodium Channel***

Ca Channel

Mutation

Same Parameters

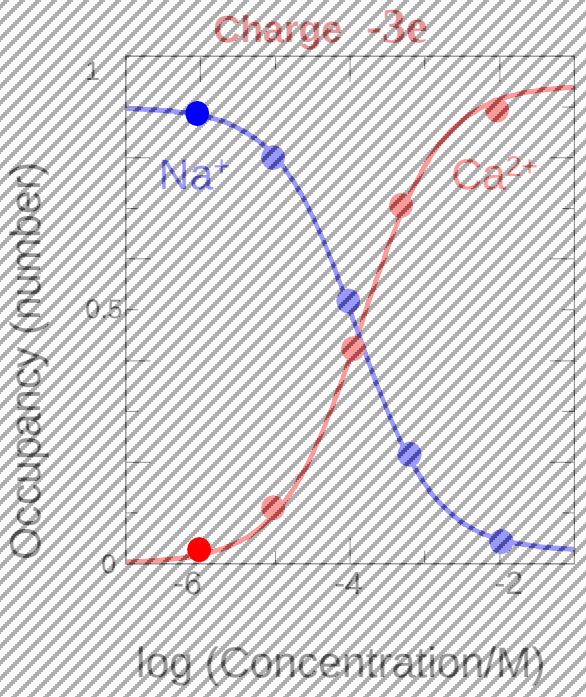
Na Channel

E → D

E → E

E → K

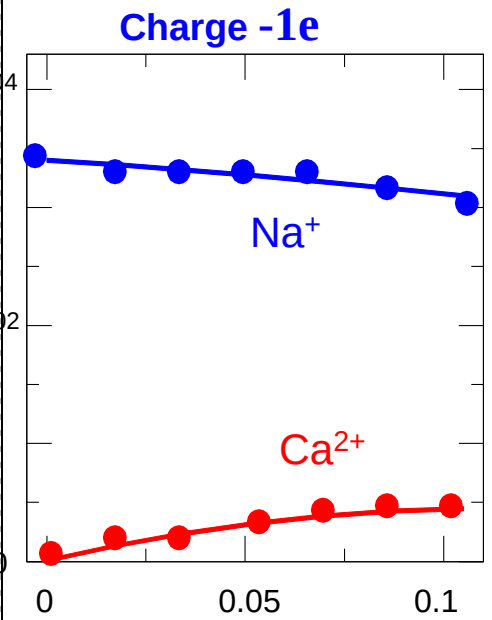
A → A



Mutation

Same Parameters

pH 8



EEEE has full biological selectivity in similar simulations

Monte Carlo simulations of Boda, et al

Nothing was Changed

from the

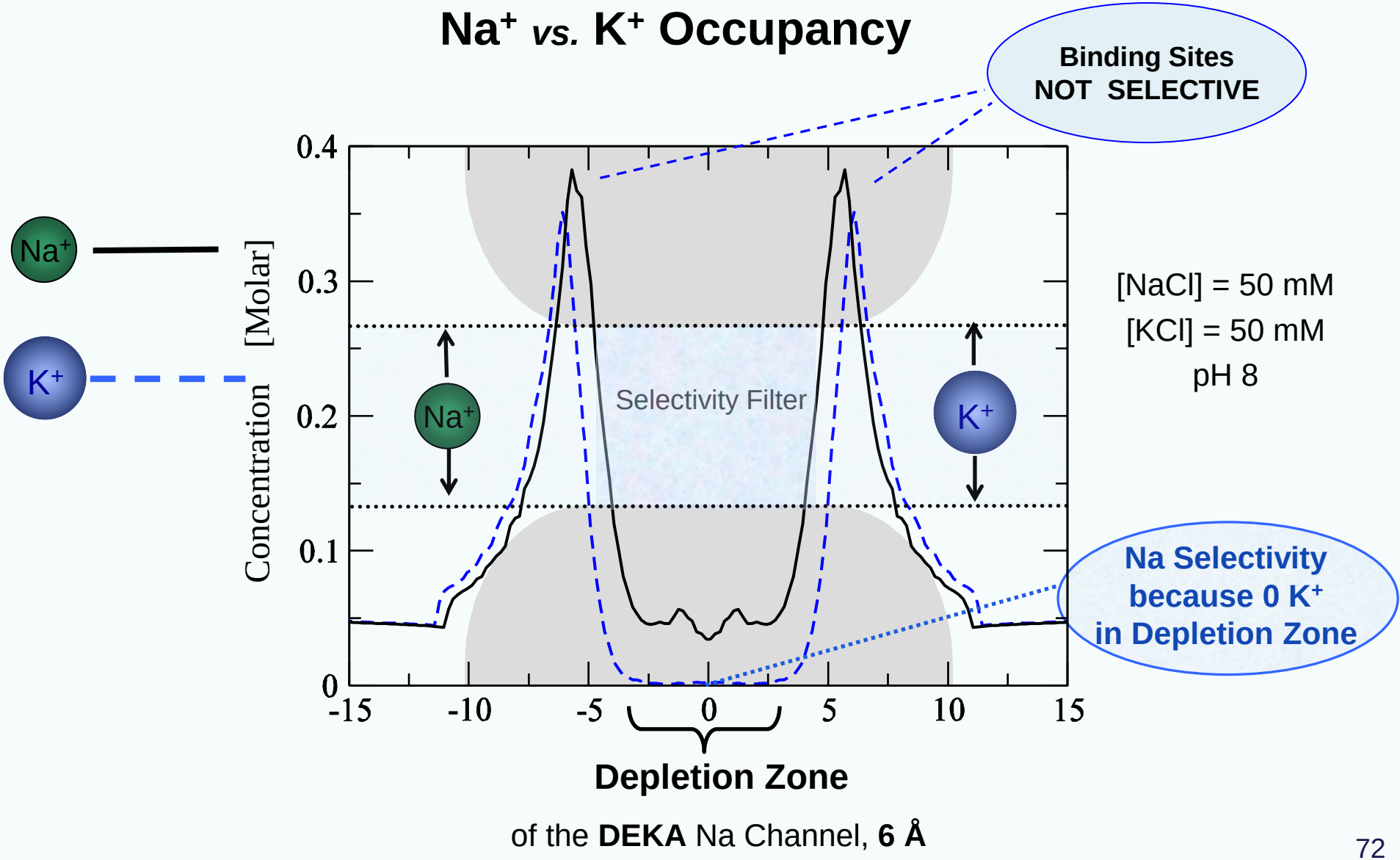
EEEA Ca channel

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

Size Selectivity is in the Depletion Zone



of the **DEKA** Na Channel, **6 Å**

Boda, et al

How?

Usually Complex Unsatisfying Answers*

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

* Gillespie, D., Energetics of divalent selectivity in the ryanodine receptor. *Biophys J (2008). 94: p. 1169-1184*

* Boda, *et al,* Analyzing free-energy by Widom's particle insertion method. *J Chem Phys (2011) 134: p. 055102-14*

Calculations and experiments done at pH 8

Simple
Independent§ Control Variables*

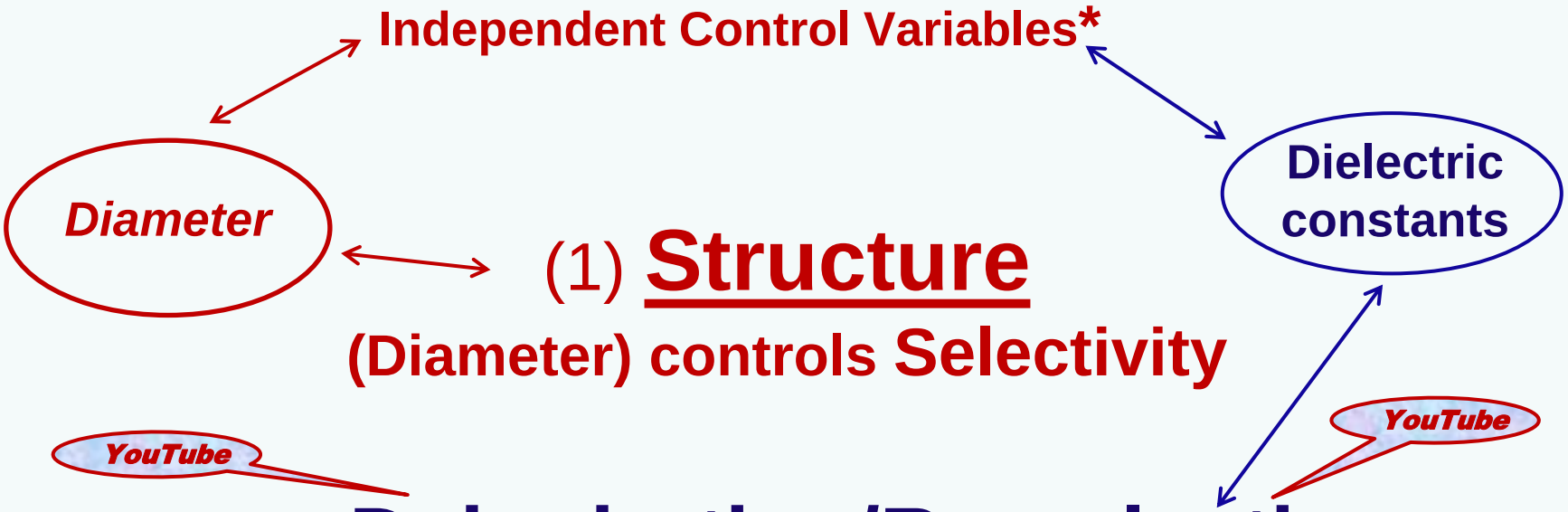
for DEKA Na^+ channel

but only in a special case
and not for other channels

Amazingly simple, not complex
for the most important selectivity property
of DEKA Na^+ channels

Control variab**le = position of **gas pedal** or dimmer on **light switch
§ Gas pedal and **brake pedal are** (hopefully**) independent control variables**

OUTPUT of Calculations NOT Assumed or Manipulated



Both emerge from calculations

**Control variables emerge as outputs*

*Control variables are **not** inputs*

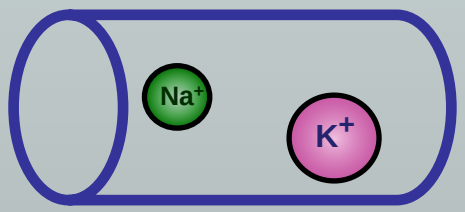
Structure (diameter) controls Selectivity

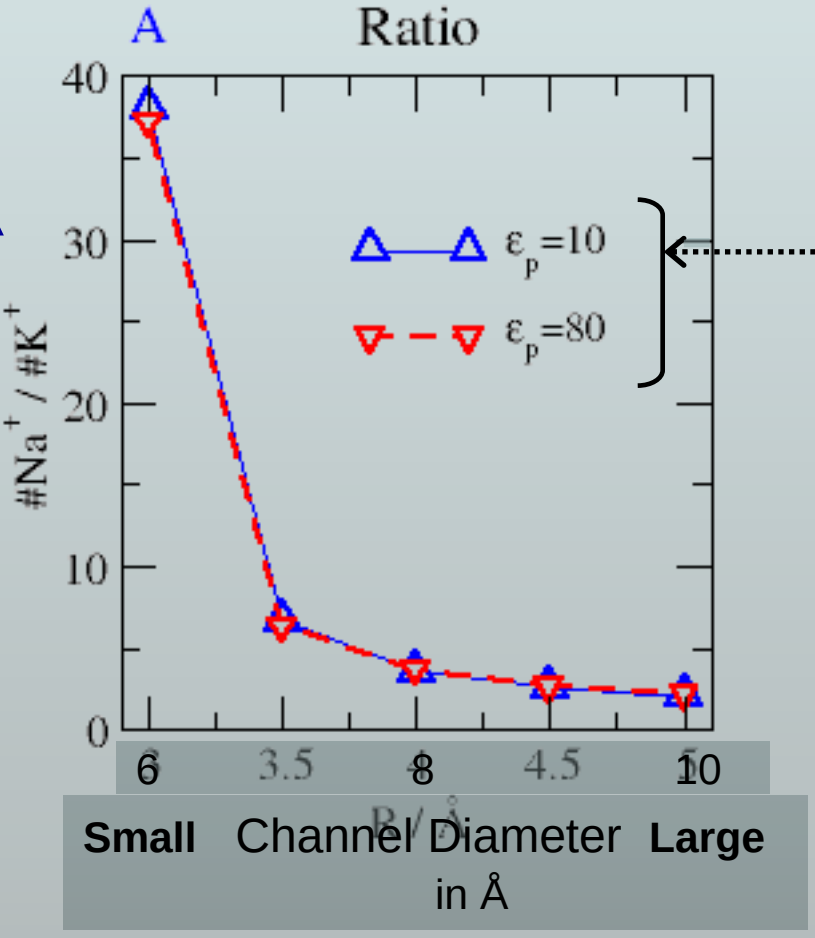
Control Variable emerged as output
Control Variables are not inputs

Monte Carlo calculations of the DEKA Na channel

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

Selectivity
for small ion



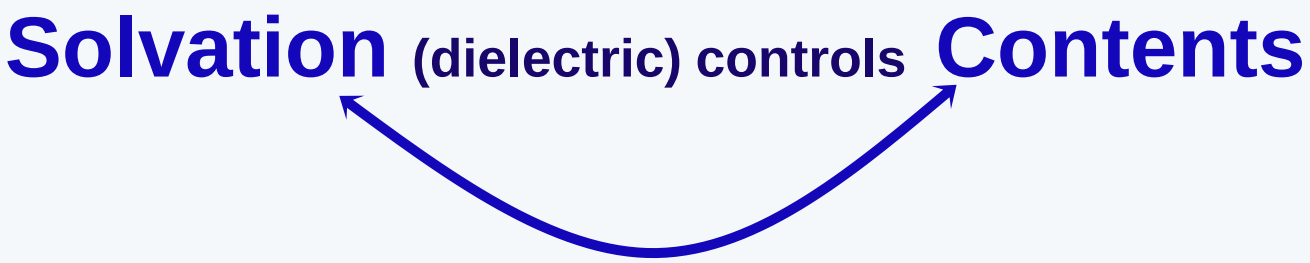


Na^+ 2.00 Å

K^+ 2.66 Å

*in DEKA Na channel

Boda, et al

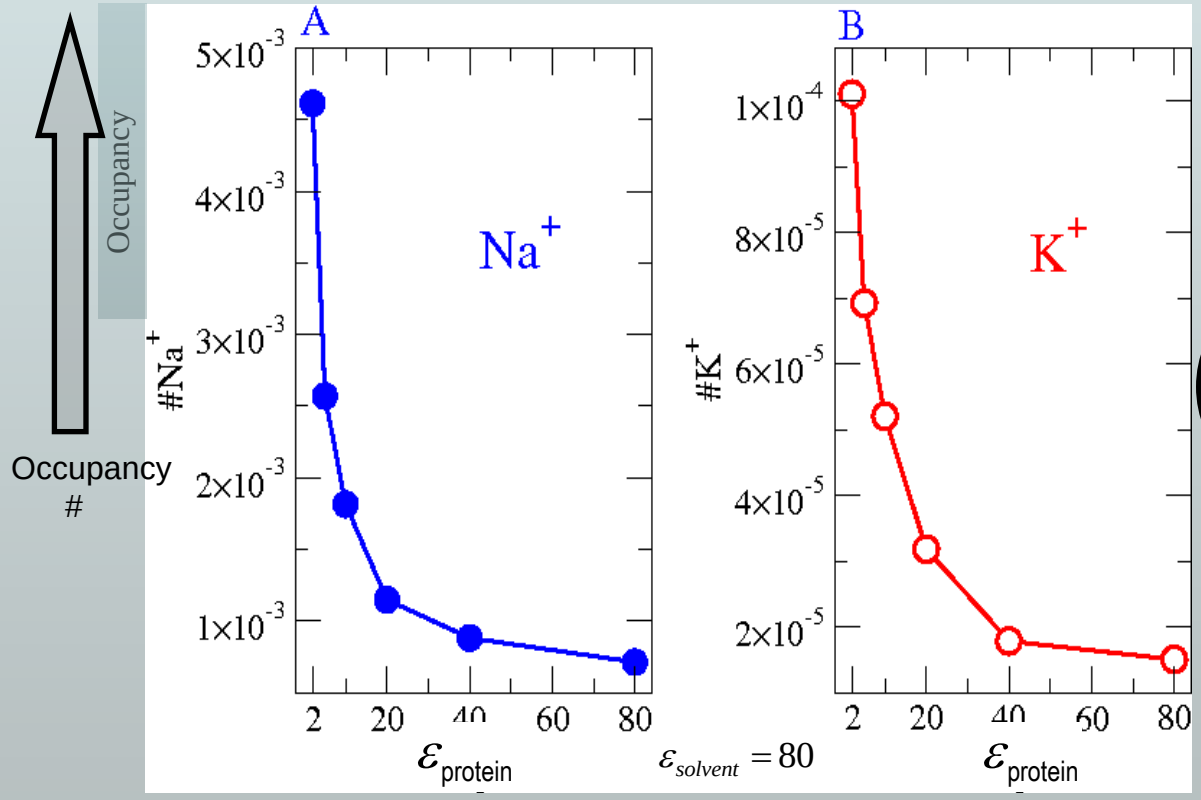


Control Variable emerged as <u>output</u>
Control Variables are <u>not</u> inputs

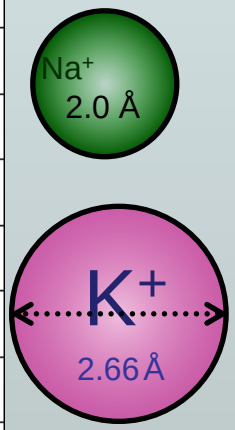
Monte Carlo calculations of the DEKA Na channel

Occupancy # Depends on Protein Dielectric

Protein Dielectric 'Amplifies' Charge & Electrostatic effects



DEKA Na Channel, 6 Å



Size Selectivity ratio does not depend on protein dielectric

Boda, et al

What does the protein do?

Channel and Contents
form a
Self-Organized Structure YouTube

with Side Chains at position of
Minimum Free Energy

Protein Fits the Substrate

‘Induced Fit Model of Selectivity’

YouTube

for

Biologists:
a Word Picture

How does Calcium Selectivity Work?
qualitatively

How does it work qualitatively?

(for biologists)

2 Ca^{++}

are

LESS CROWDED

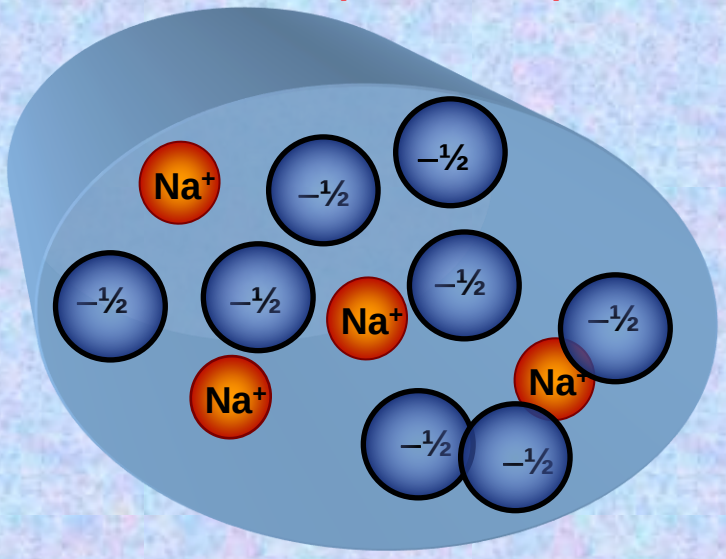
than 4 Na^{+}

Selectivity from Crowded Charges

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

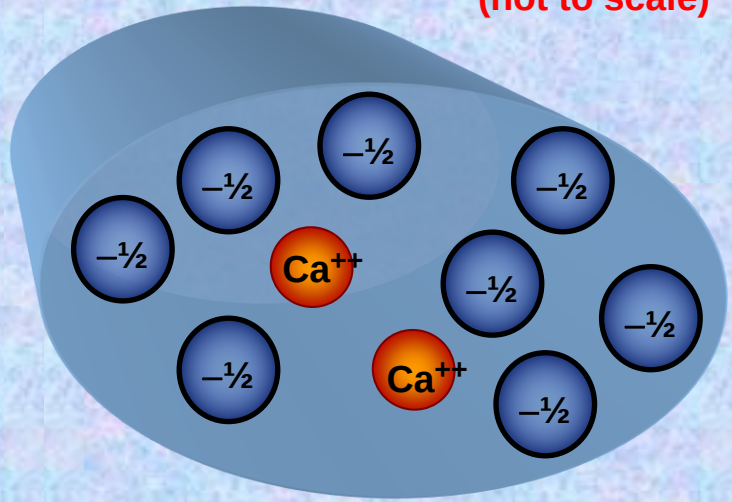
Ca Channel Filled with Na^{+}

(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

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

This is the
Self-organized
Induced fit model
of Koshland
and the founders of enzymology,

Made specific by
Mathematics and Computation

Selectivity Depends on Structure

Structure

is the

Computed Consequence

of Forces

in these models

We can actually compute the structures that determine Selectivity

Best Evidence is from the

RyR Receptor

YouTube

Dirk Gillespie

Dirk_Gillespie@rush.edu

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

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

1. Gillespie, D., Energetics of divalent selectivity in a calcium channel: the ryanodine receptor case study. *Biophys J, 2008. 94(4): p. 1169-1184.*
2. Gillespie, D. and D. Boda, Anomalous Mole Fraction Effect in Calcium Channels: A Measure of Preferential Selectivity. *Biophys. J., 2008. 95(6): p. 2658-2672.*
3. Gillespie, D. and M. Fill, Intracellular Calcium Release Channels Mediate Their Own Countercurrent: Ryanodine Receptor. *Biophys. J., 2008. 95(8): p. 3706-3714.*
4. Gillespie, D., W. Nonner, and R.S. Eisenberg, Coupling Poisson-Nernst-Planck and Density Functional Theory to Calculate Ion Flux. *Journal of Physics (Condensed Matter), 2002. 14: p. 12129-12145.*
5. Gillespie, D., W. Nonner, and R.S. Eisenberg, Density functional theory of charged, hard-sphere fluids. Physical Review E, 2003. 68: p. 0313503.
6. Gillespie, D., Valisko, and Boda, Density functional theory of electrical double layer: the RFD functional. *Journal of Physics: Condensed Matter, 2005. 17: p. 6609-6626.*
7. Gillespie, D., J. Giri, and M. Fill, Reinterpreting the Anomalous Mole Fraction Effect. The ryanodine receptor case study. Biophysical Journal, 2009. 97: p. pp. 2212 - 2221
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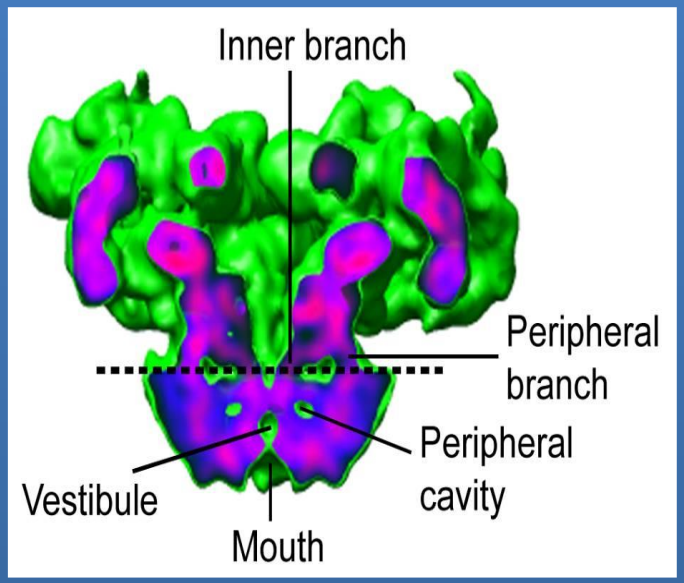
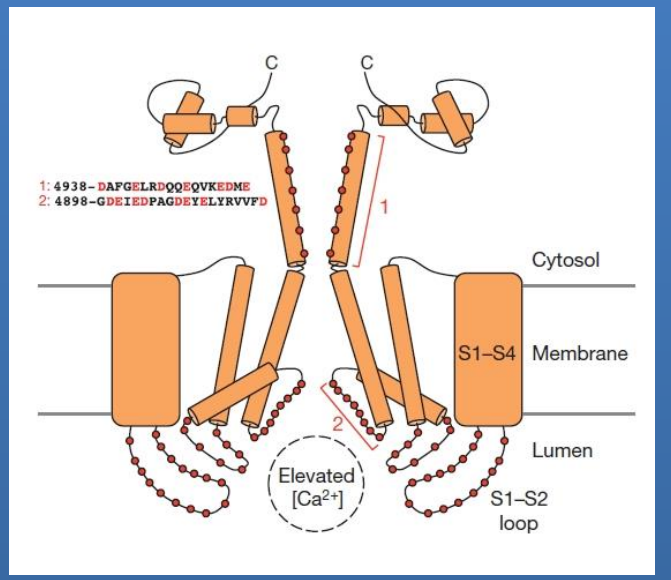
13. Wang, Y., L. Xu, D. Pasek, D. Gillespie, and G. Meissner, Probing the Role of Negatively Charged Amino Acid Residues in Ion Permeation of Skeletal Muscle Ryanodine Receptor. *Biophysical Journal, 2005. 89: p. 256-265.*
14. Xu, L., Y. Wang, D. Gillespie, and G. Meissner, Two Rings of Negative Charges in the Cytosolic Vestibule of T Ryanodine Receptor Modulate Ion Fluxes. *Biophysical Journal, 2006. 90: p. 443-453.*

RyR

Ryanodine Receptor redrawn in part from Dirk Gillespie, with thanks!

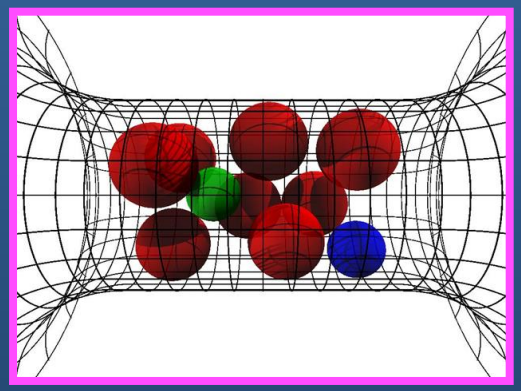


Samsó et al, 2005, Nature Struct Biol 12: 539



Zalk, et al 2015 Nature 517: 44-49.

All Spheres Representation



- **4 negative charges D4899**
- **Cylinder 10 Å long, 8 Å diameter**
- **13 M of charge!**
- **18% of available volume**
- **Very Crowded!**
- **Four lumenal E4900 positive amino acids overlapping D4899.**
- **Cytosolic background charge**

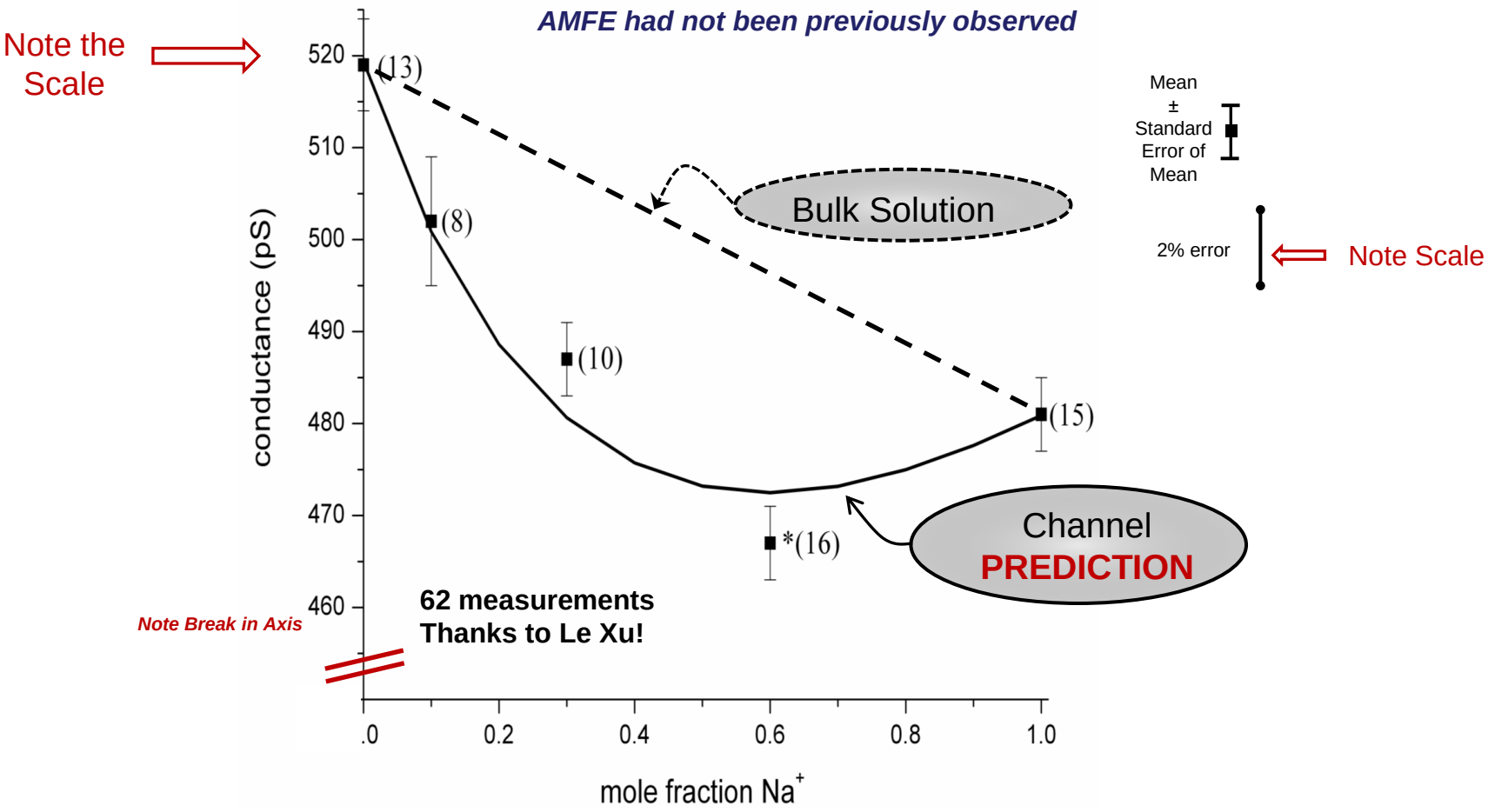
Solved by DFT-PNP (Poisson Nernst Planck)

DFT-PNP
gives location
of Ions and 'Side Chains'
as OUTPUT

Other methods
give nearly identical results

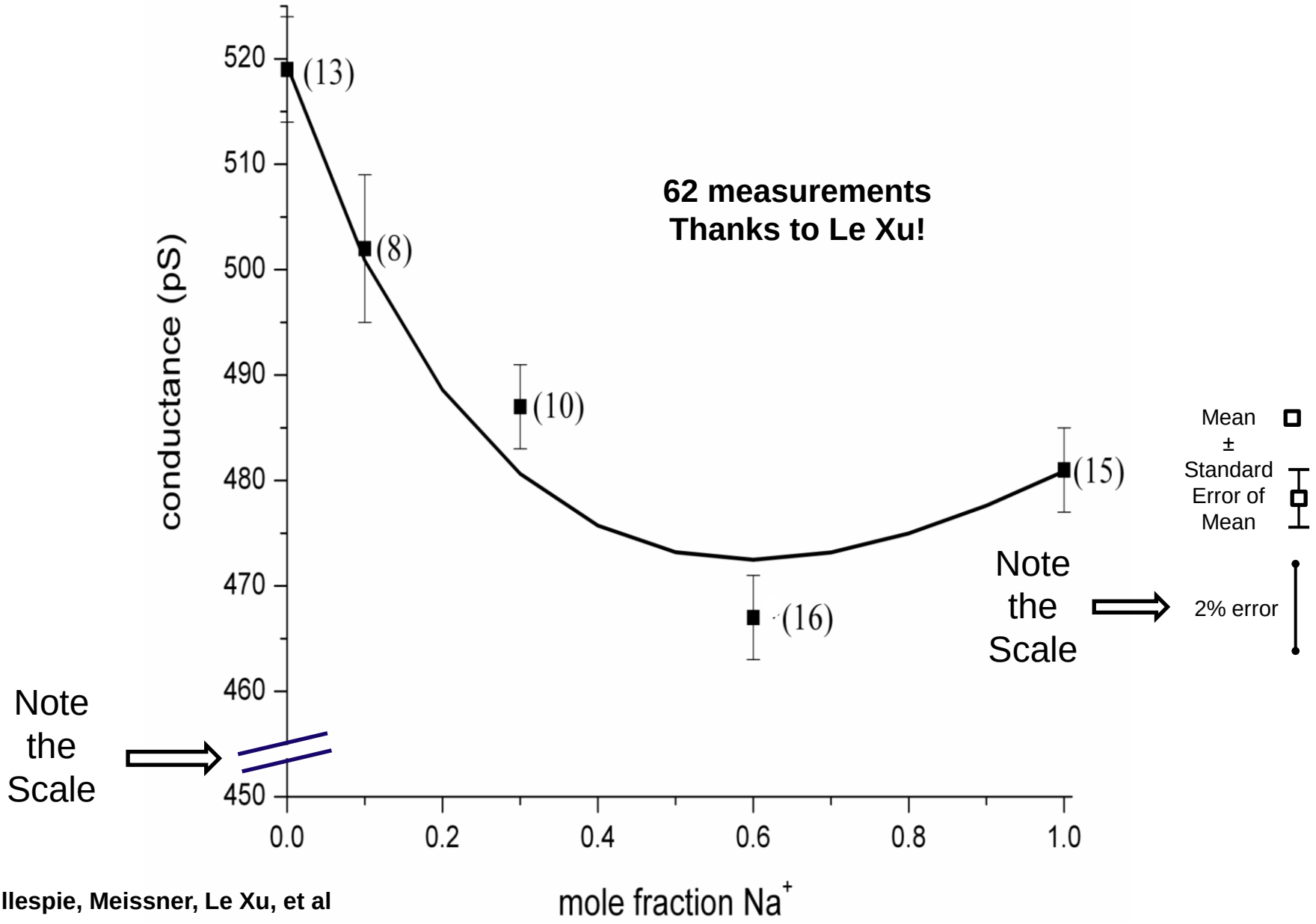
DFT (Density Functional Theory of fluids, *not electrons*)
MMC (Metropolis Monte Carlo))
SPM (Primitive Solvent Model)
EnVarA (Energy Variational Approach)
Non-equil MMC (Boda, Gillespie) several forms
Steric PNP (simplified EnVarA)
Poisson Fermi (replacing Boltzmann distribution)

AMFE for Na^+/Cs^+ mixtures
Predicted before measurements
AMFE had not been previously observed



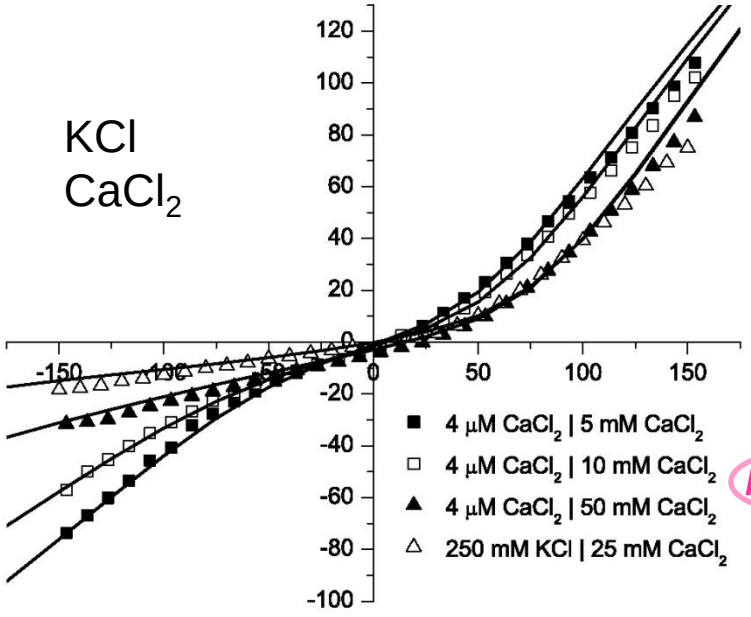
Gillespie, Meissner, Le Xu, et al

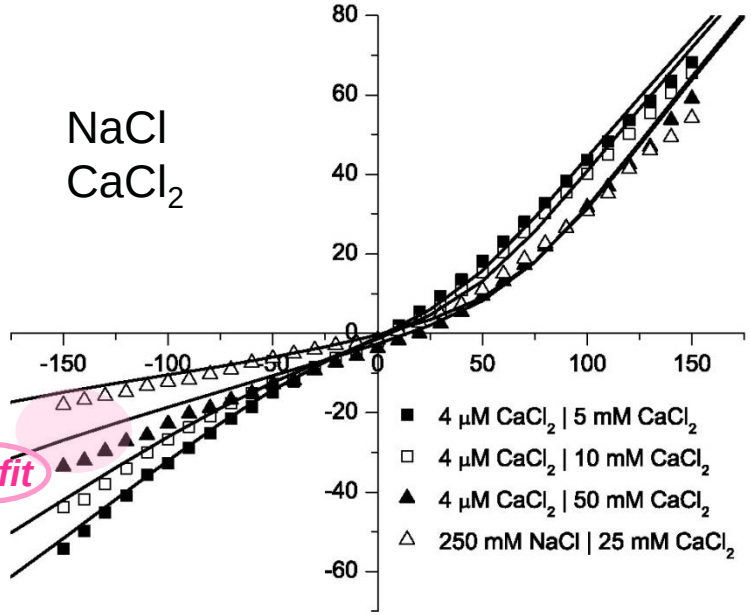
The model predicted an AMFE for Na^+/Cs^+ mixtures before it had been measured

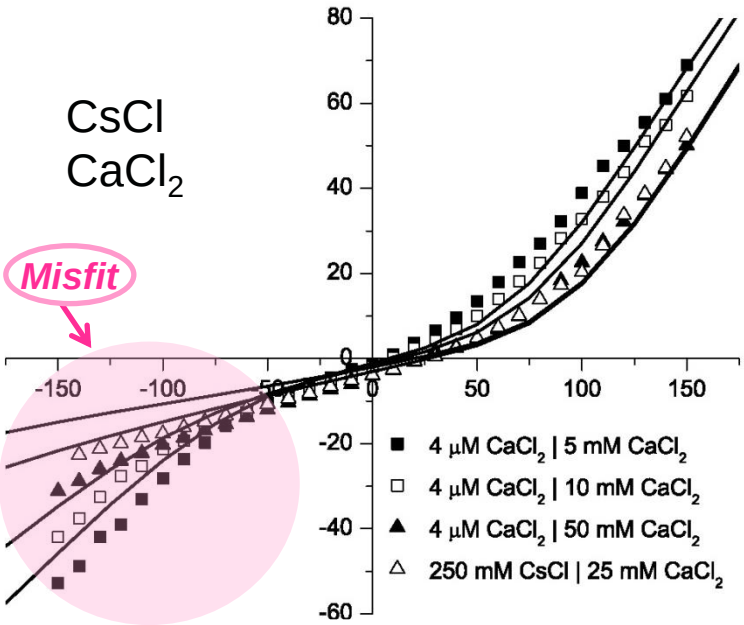


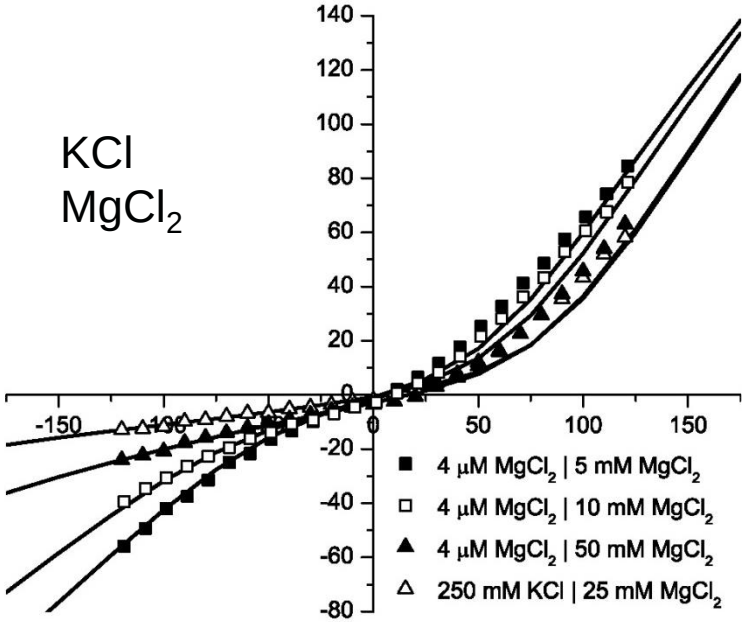
Gillespie, Meissner, Le Xu, et al

Divalents


KCl
CaCl2
4 μM CaCl2 | 5 mM CaCl2
4 μM CaCl2 | 10 mM CaCl2
4 μM CaCl2 | 50 mM CaCl2
250 mM KCl | 25 mM CaCl2

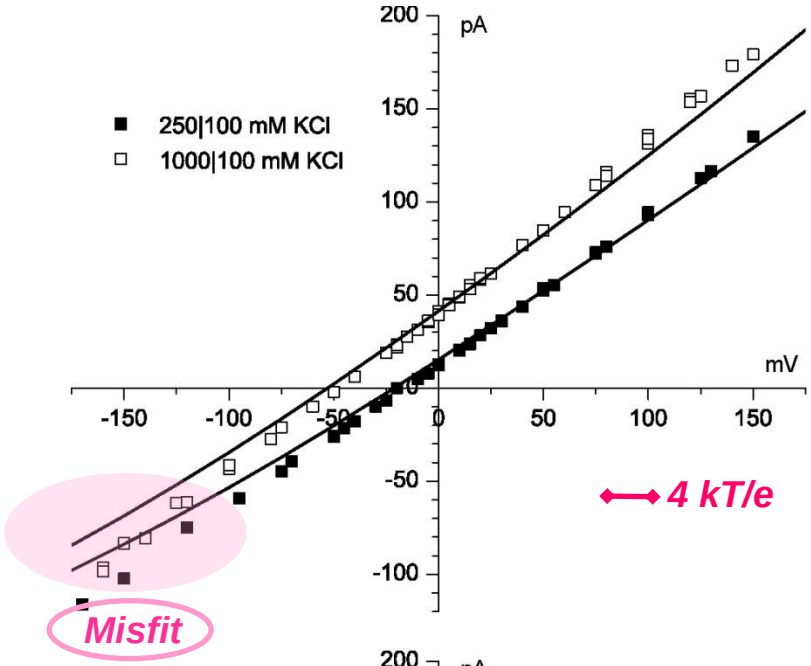

NaCl
CaCl2
Misfit
4 μM CaCl2 | 5 mM CaCl2
4 μM CaCl2 | 10 mM CaCl2
4 μM CaCl2 | 50 mM CaCl2
250 mM NaCl | 25 mM CaCl2

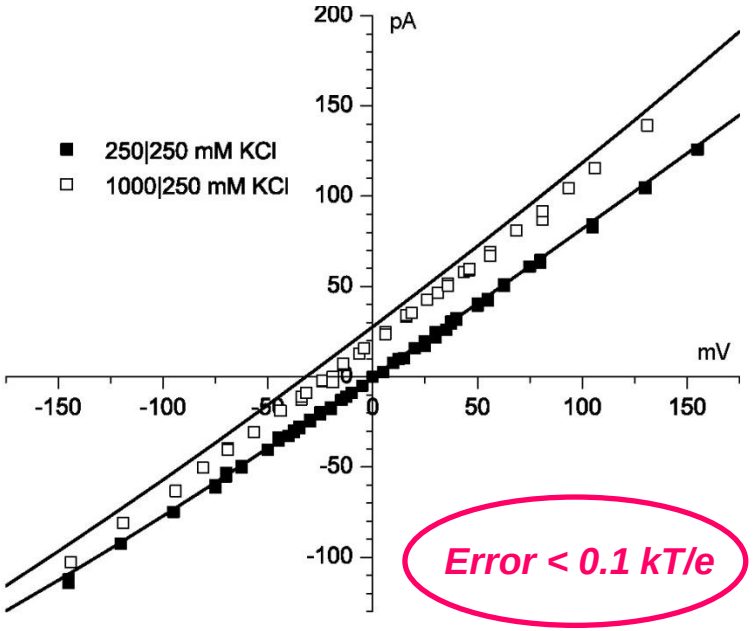

CsCl
CaCl2
Misfit
4 μM CaCl2 | 5 mM CaCl2
4 μM CaCl2 | 10 mM CaCl2
4 μM CaCl2 | 50 mM CaCl2
250 mM CsCl | 25 mM CaCl2

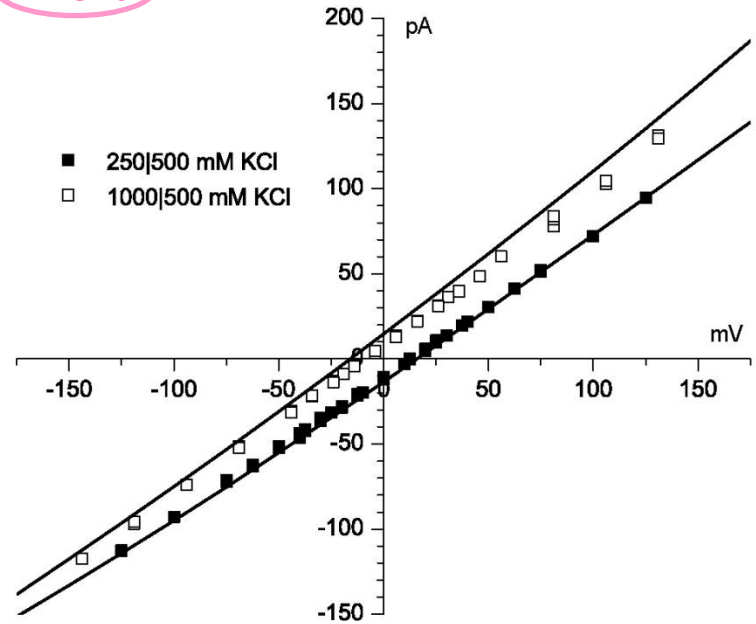

KCl
MgCl2
4 μM MgCl2 | 5 mM MgCl2
4 μM MgCl2 | 10 mM MgCl2
4 μM MgCl2 | 50 mM MgCl2
250 mM KCl | 25 mM MgCl2

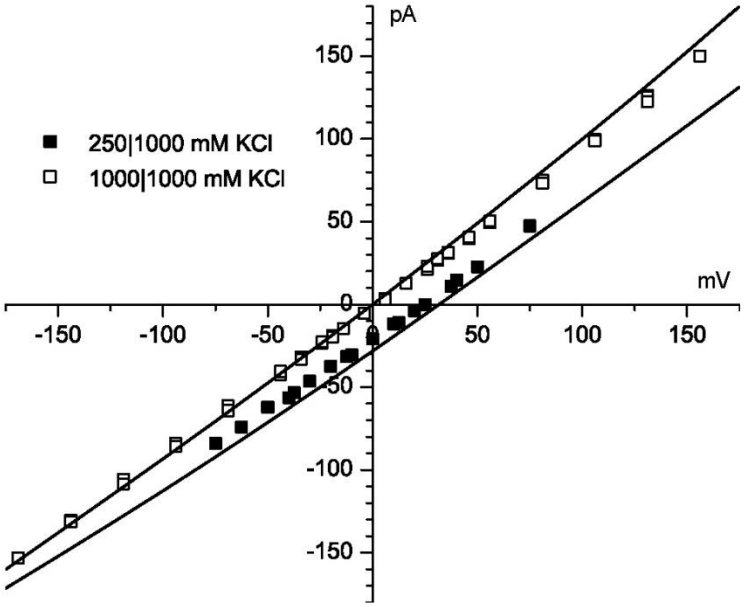
KCl

Gillespie, Meissner, Le Xu, et al

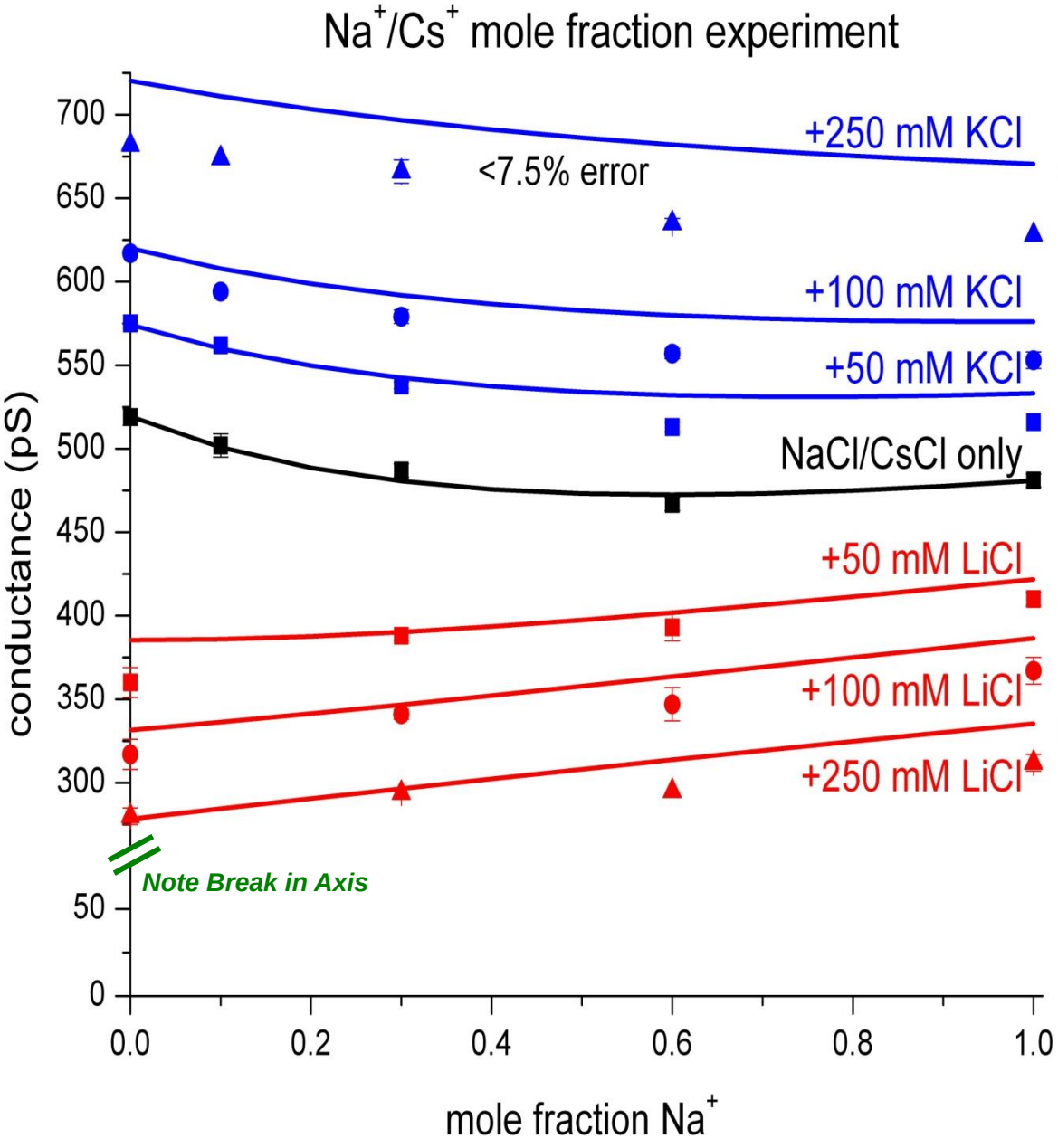








The model predicted that AMFE disappears



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.

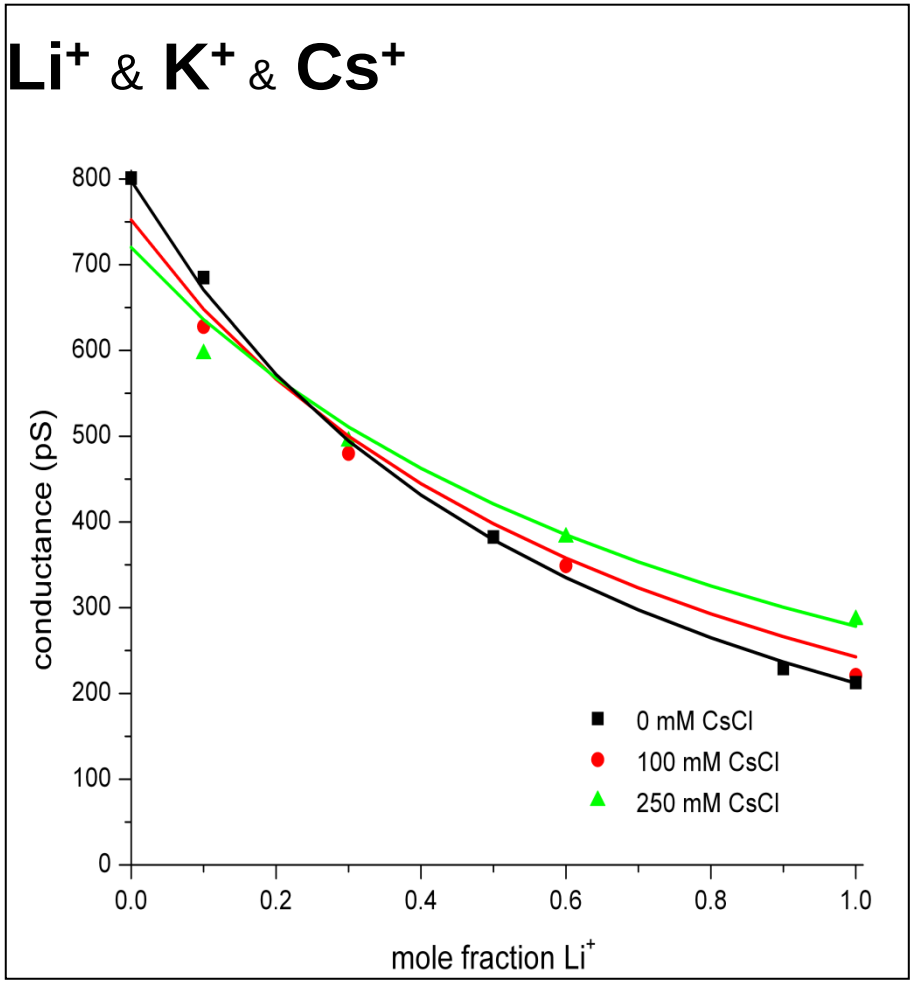
Error < 0.1 kT/e

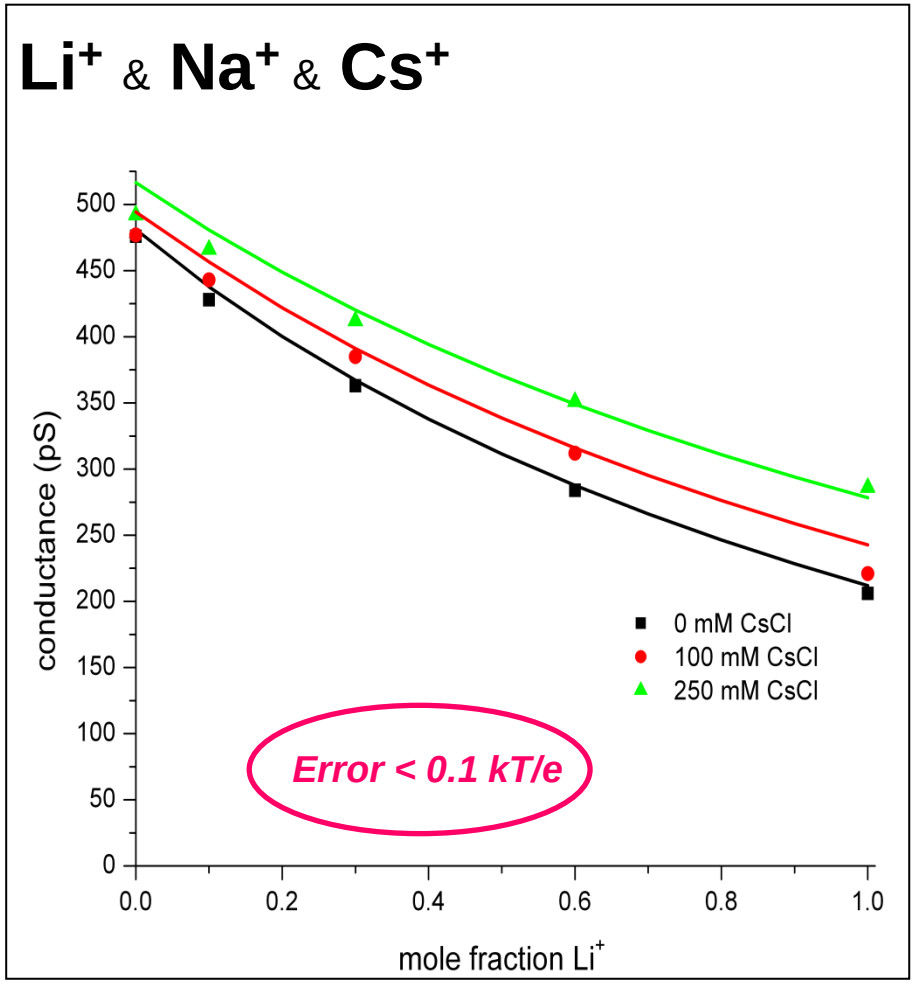
Gillespie, Meissner, Le Xu, et al

Mixtures of THREE Ions

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

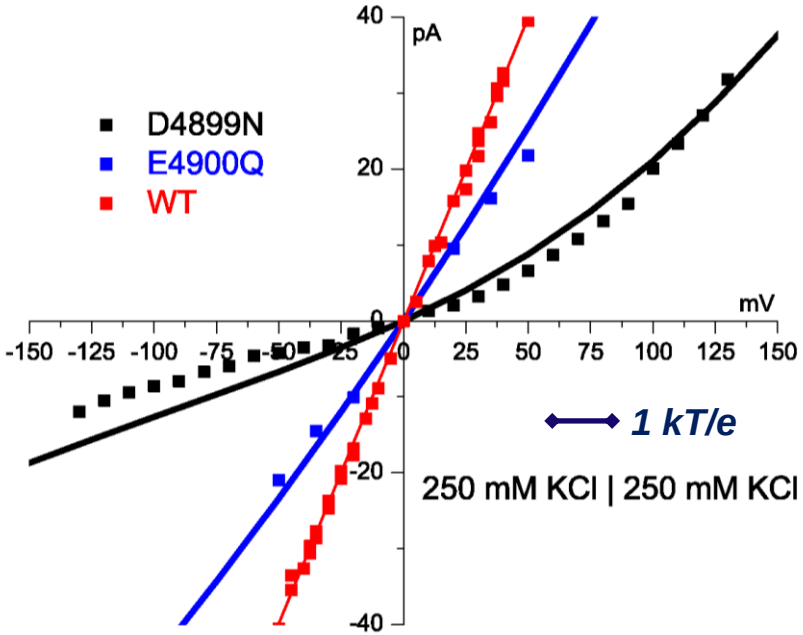
Gillespie, Meissner, Le Xu, et al



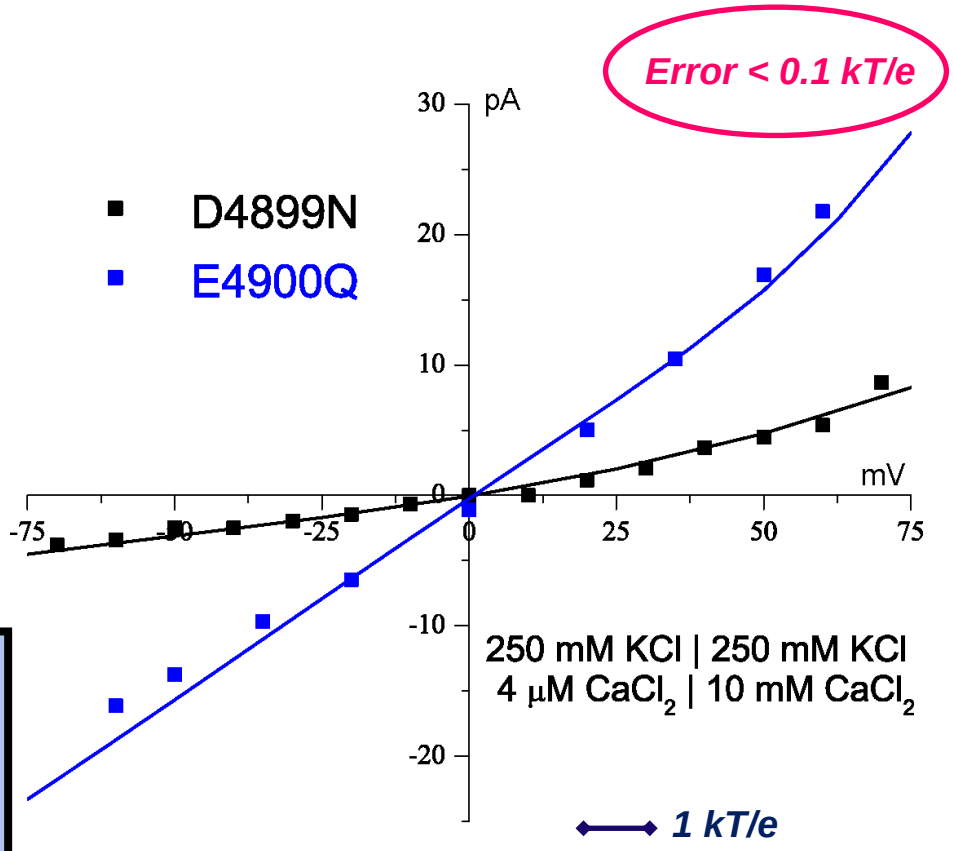


Theory fits Mutation with Zero Charge

Theory Fits Mutant in K



Theory Fits Mutant in K + Ca



Protein charge density

wild type* 13 M ⇨ 0 M *in* D4899

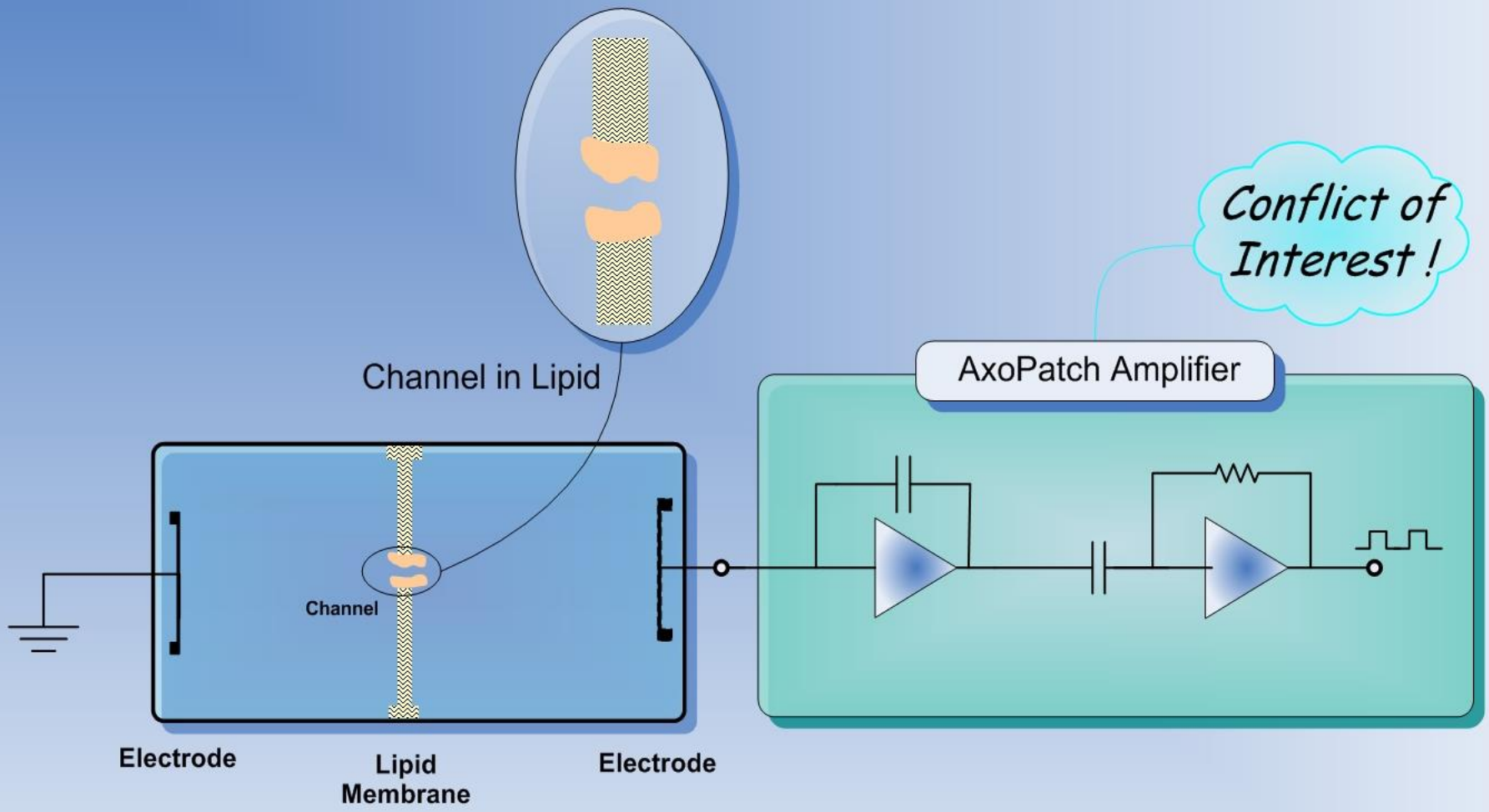
Solid Na^+Cl^- is 37 M

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

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

Experimental Detail

Single Channel Recording



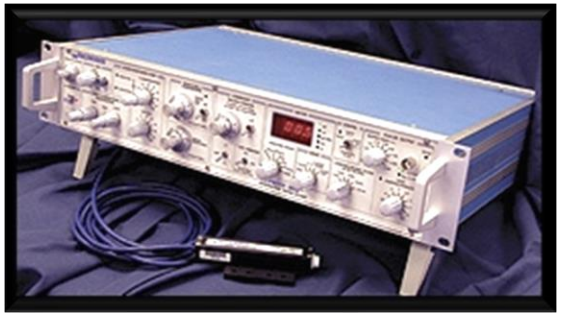
Channel in Lipid Bilayer Set-up

Recording Current from One Protein Molecule

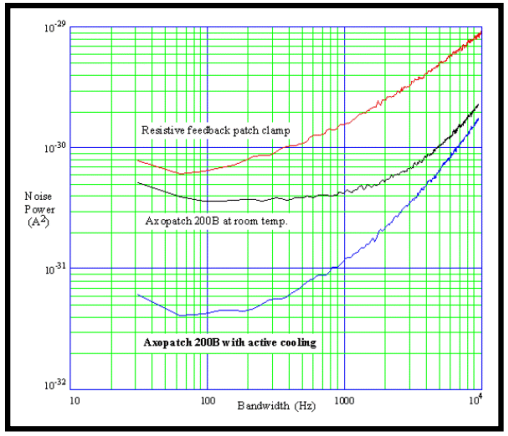
Thousands of
Molecular Biologists
Study Channels
every day,

One protein molecule at a time

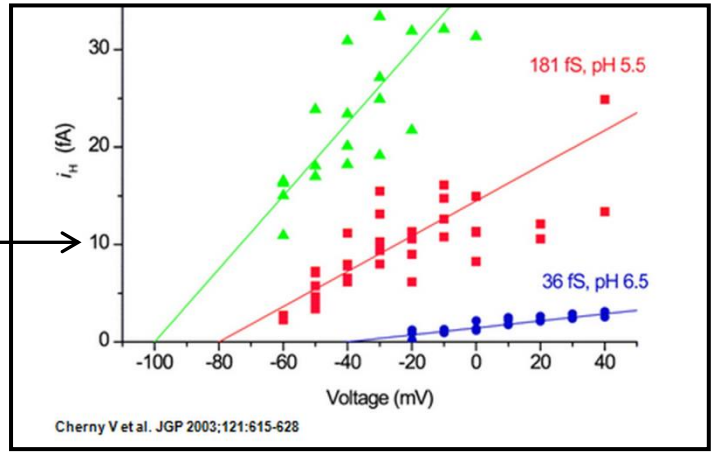
This number is not an exaggeration.
We have sold >10,000 AxoPatch amplifiers



AxoPatch 200B



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



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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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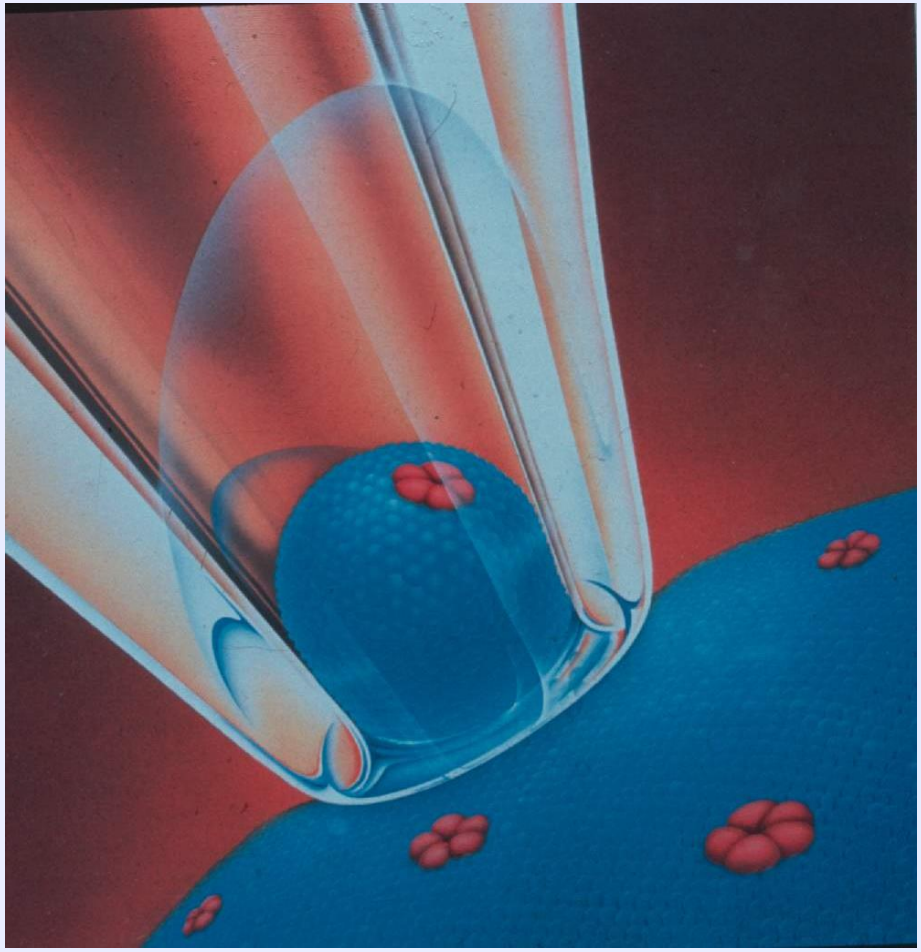


YouTube

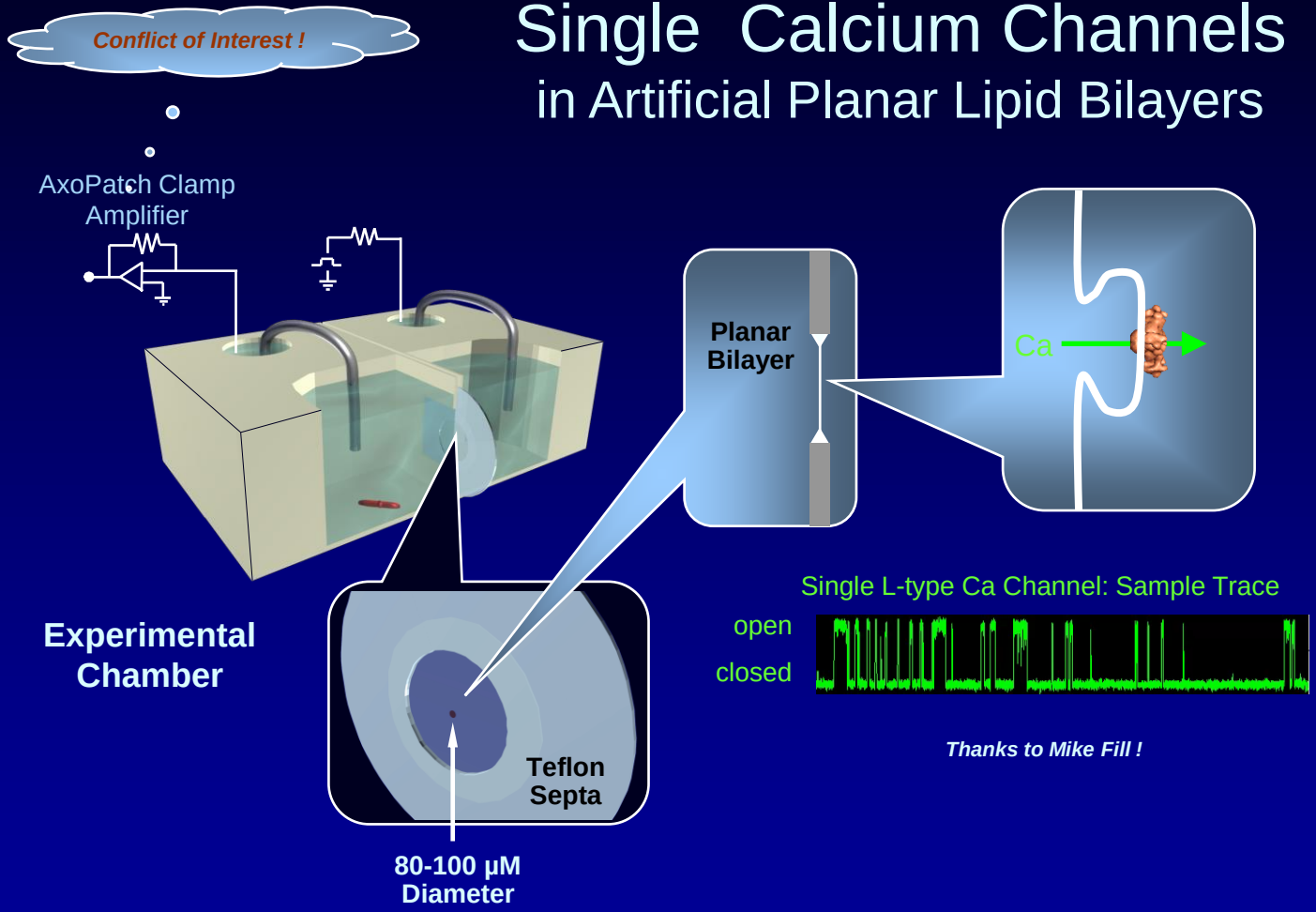
Patch Clamp Setup

Recordings from One Molecule

YouTube

Patch clamp and
Bilayer apparatus clamp
ion concentrations
in the baths and the
voltage across membranes.

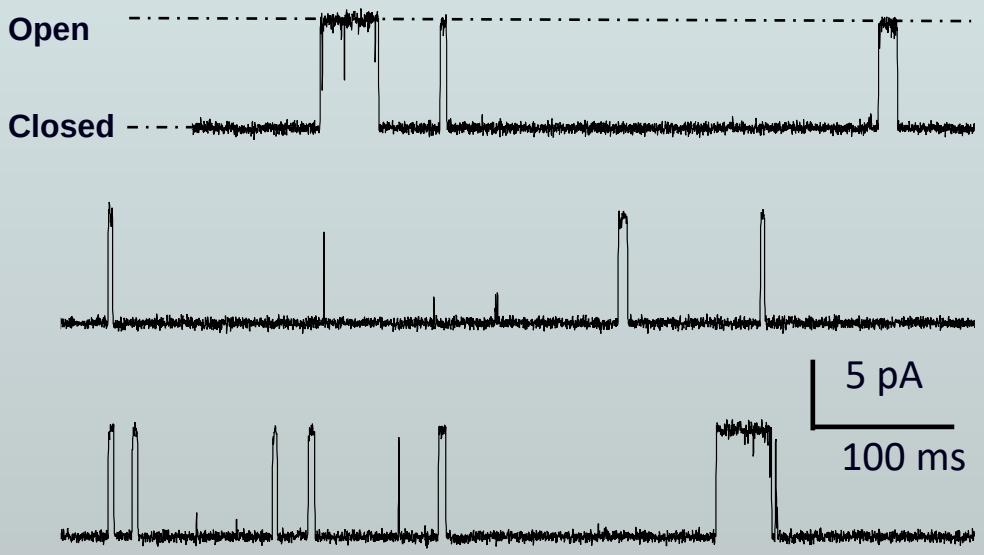
Single Calcium Channels
in Artificial Planar Lipid Bilayers



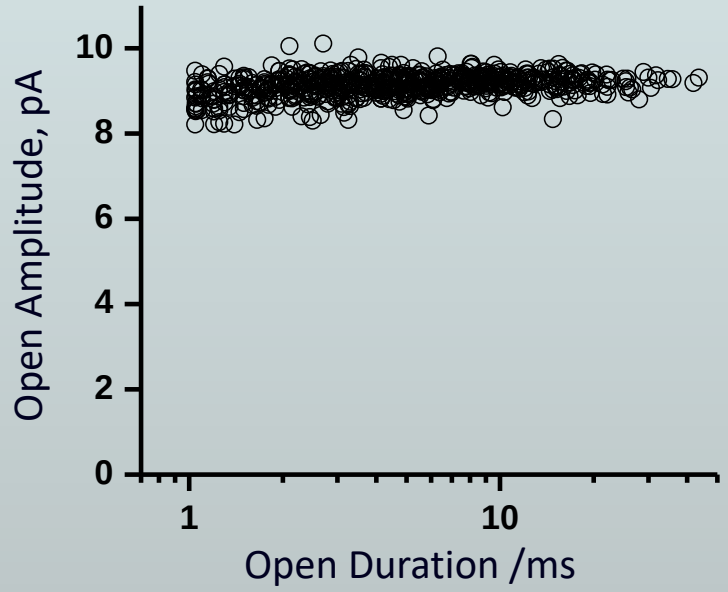
Channel Structure Does Not Change

once the channel is open

Current vs. time



Amplitude vs. Duration

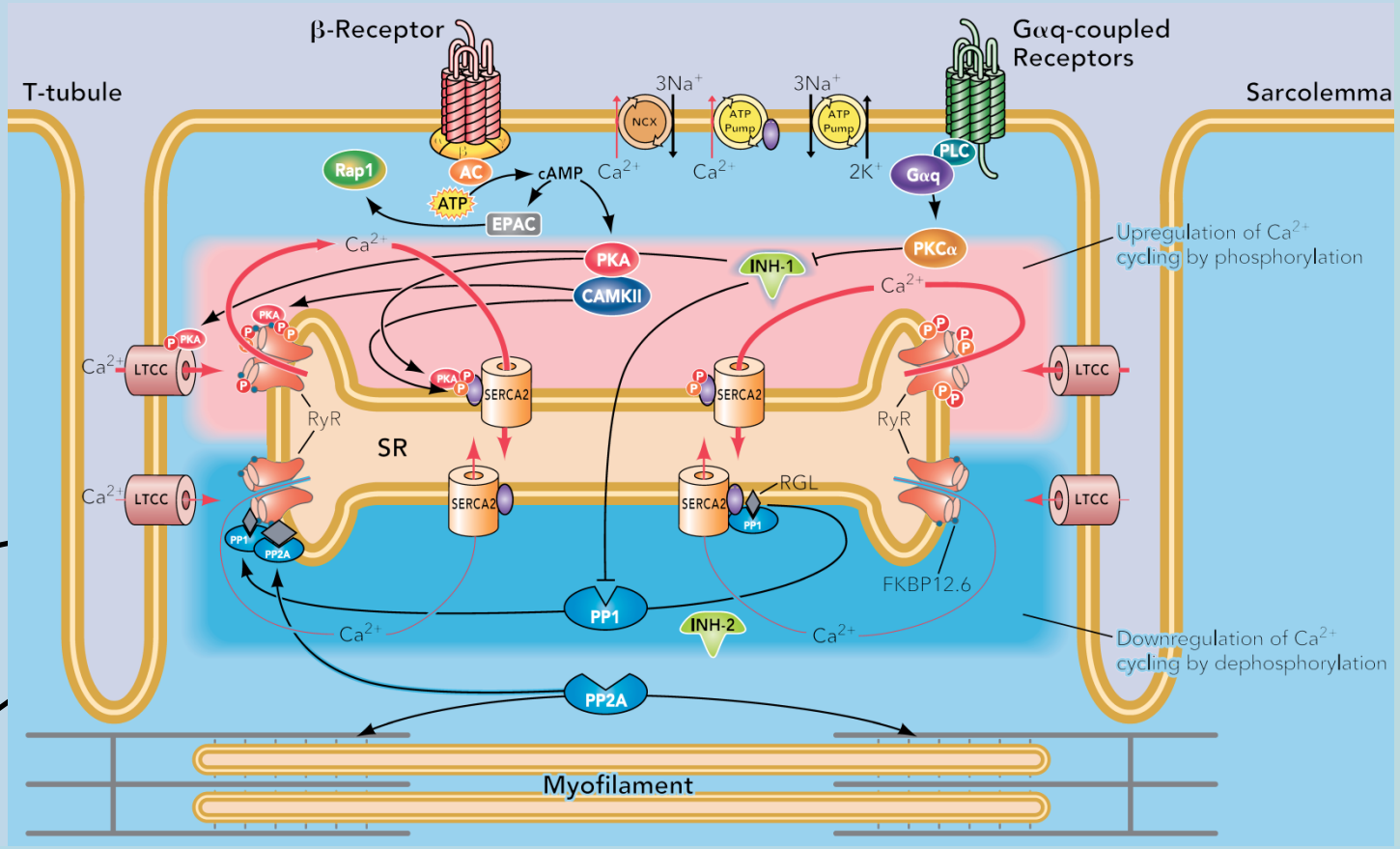


Lowpass Filter = 1 kHz Sample Rate = 20 kHz

<u>Typical</u> Raw Single Channel Records

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

Cardiac Muscle Cell



Ikeda et al, Physiology 23: 6 (2008)

Vocabulary

Life extends over many Scales
with a
Hierarchy of Structures
one on top of another

So **Life Needs Many Words**
to define the Structures
And how they work

Extensive Vocabulary is Needed
Extensive Vocabulary is a Barrier for Students
I mark most of these in my lectures with
a call out

and I will provide a
Vocabulary List

Vocabulary

Extensive Vocabulary is Needed
Extensive Vocabulary is a Barrier for Students

Best Source for Vocabulary are Video Clips, often from YouTube

or from educational websites like MIT

or from Wikipedia

Or from general Internet Search

and I will provide a Vocabulary List

Lecture 2 & 3 Vocabulary List

1) Kinase
2) Nerve Conduction
3) Resting potential
4) Action Potential
5) Phospholipid molecule
6) Plasma membrane
7) Ion Channel
8) K^+ = potassium ion
9) Myelin
10) Glial Cell
11) Node of Ranvier
12) Myelinated Nerve
13) Unmyelinated Nerve
14) Selectivity
15) Acid
16) Base
17) Ions
18) Valves
19) Power Supply
20) Device Equation
21) Boundary Conditions
22) Calibration
23) Correlation
24) Adaptation
25) Evolution
26) Inverse Problem
27) Reverse Engineering
28) Reduced Model
29) Side Chain
30) Amino acid residue
31) Induced Fit Enzymes
32) Enzyme
33) Active Site
34) Catalytic Active Site
35) Dimensional Reduction
36) Primitive Model of ionic solutions
37) Electrolyte solution
38) Implicit solvent
39) RyR channel
40) CaV channel
41) Na_V channel
42) L type Calcium channel
43) Lysine
44) Donnan Equilibrium
45) Ion exchanger
46) Dielectric
47) Monte Carlo
48) Metropolis Monte Carlo
49) AMFE
50) Anomalous Mole Fraction Effect
51) Solvation
52) Dehydration
53) Dielectric Constant
54) Cardiac Muscle
55) Patch Clamp
56) Bilayer
57) Lipid