How Physical Systems Can Help in Studying Function of Biological Systems?

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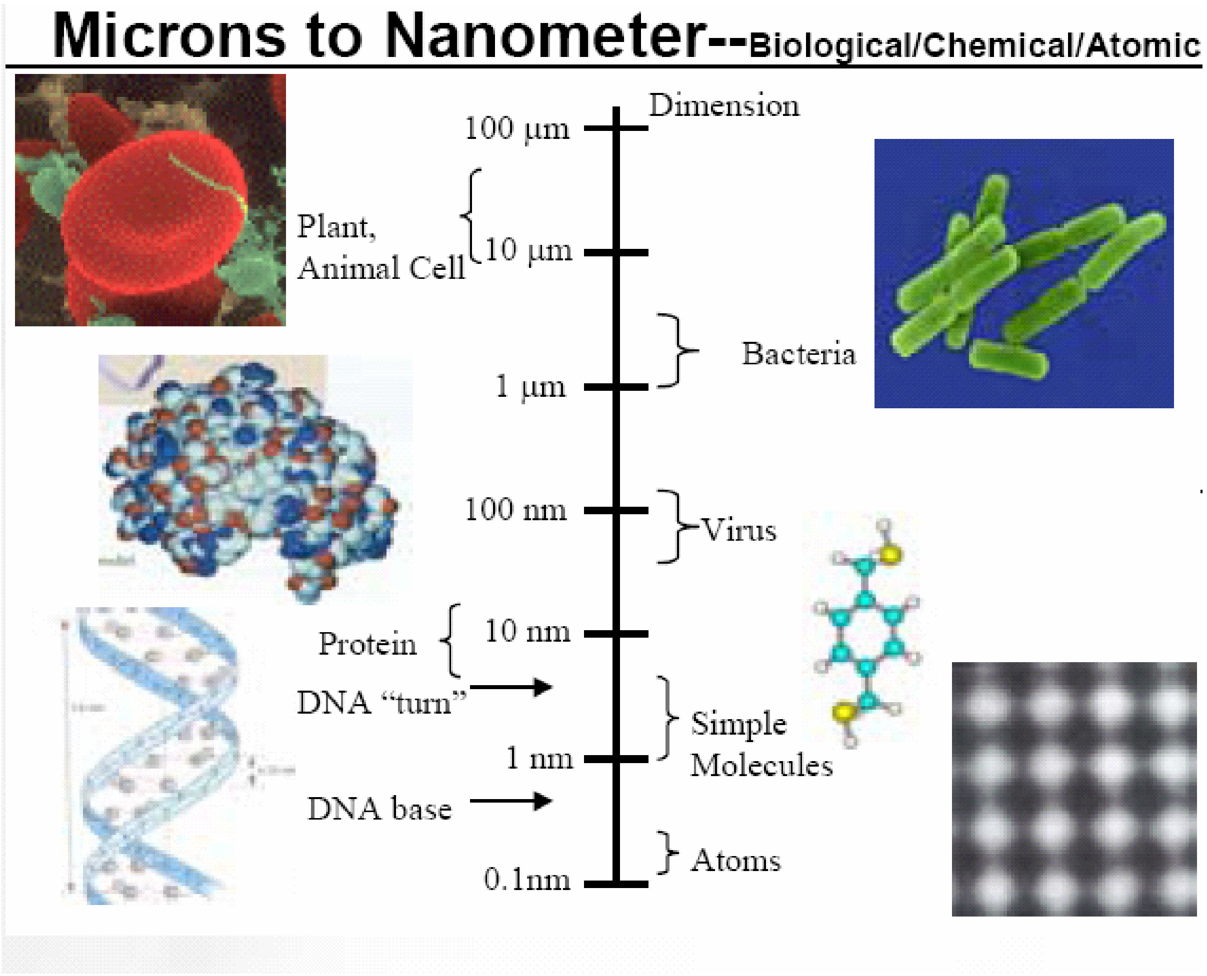
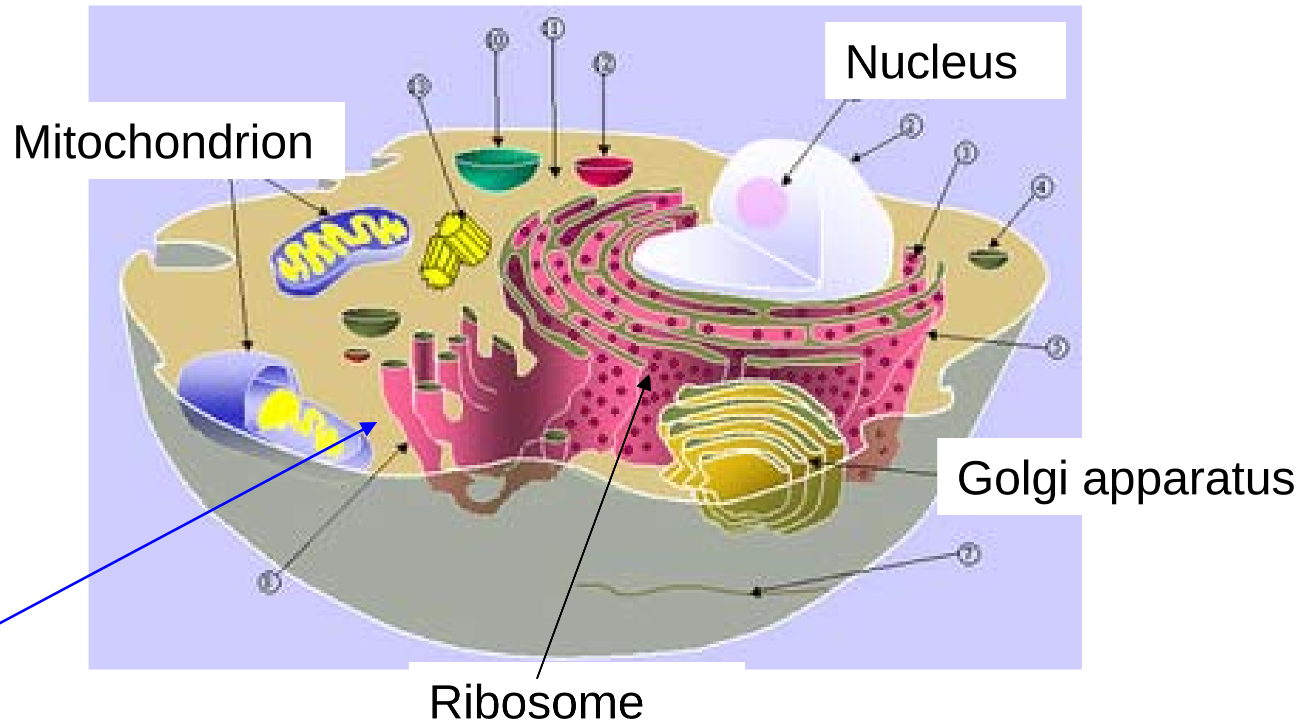
Biological Scales



http://www.ece.purdue.edu/~janes/whats_nano.htm

Scales of Life

Cells – units from which an organisms is built



Nanometer scale, 10^{-9} m

Micrometer scale, 10^{-6} m

http://en.wikipedia.org/wiki/Image:Biological_cell.png

Scales of Life

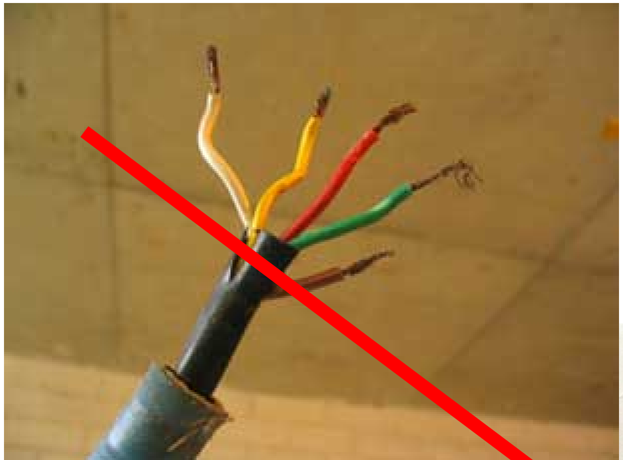
Complexity of Biological Objects

“The closer one looks at [the] performances of matter in living organisms the more impressive the show becomes. The meanest living cell becomes a magic puzzle box full of elaborate and changing molecules, and far outstrips all chemical laboratories of man in the skill of organic synthesis...."

[Max Delbruck]

Although biological objects are complex, and a living organism cannot be described by a collective behavior of individual components, yet the components of a living cell obey the same laws of physics as all other systems”. Studying individual components is crucial in understanding functions of a living organism.

Importance of Wiring - Connections

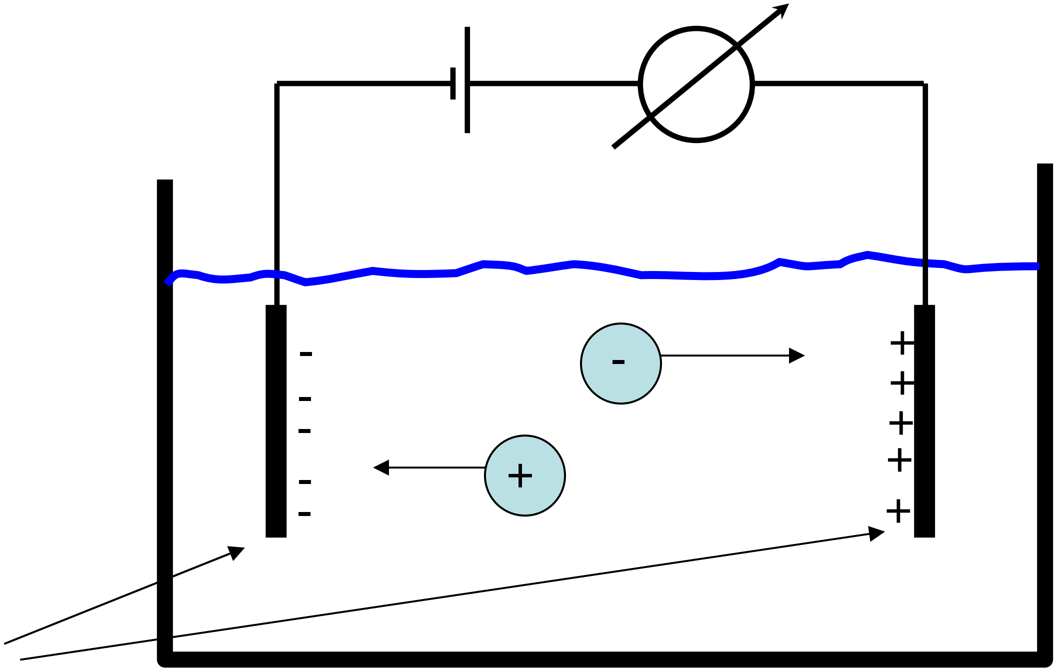


Importance of Wiring - Connections

We are filled 70% with water

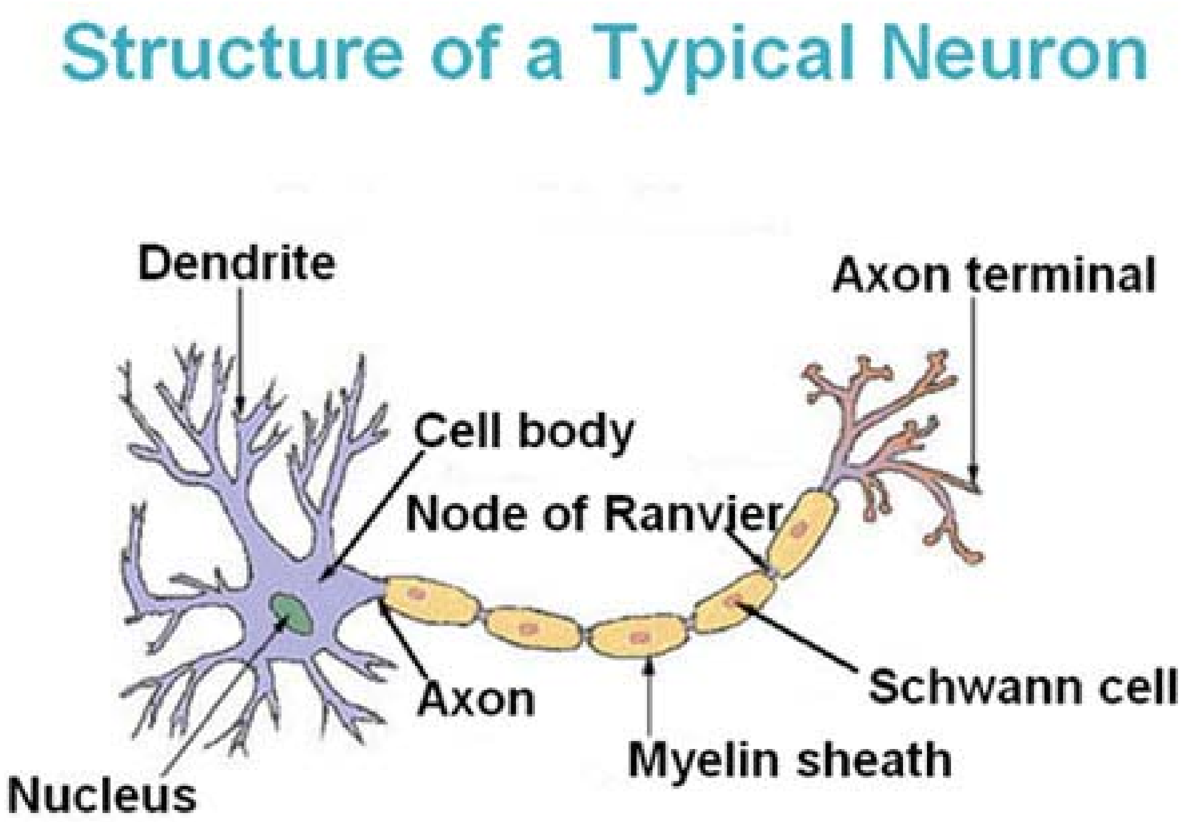
Most important ions: Na^{+}, K^{+}, Cl^{-}, Ca^{2+}

Quantitative description of flow of ions: ion current



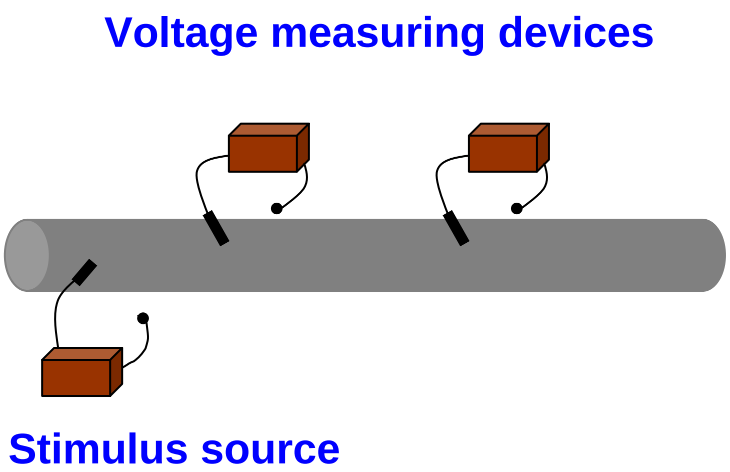
Electrode reactions

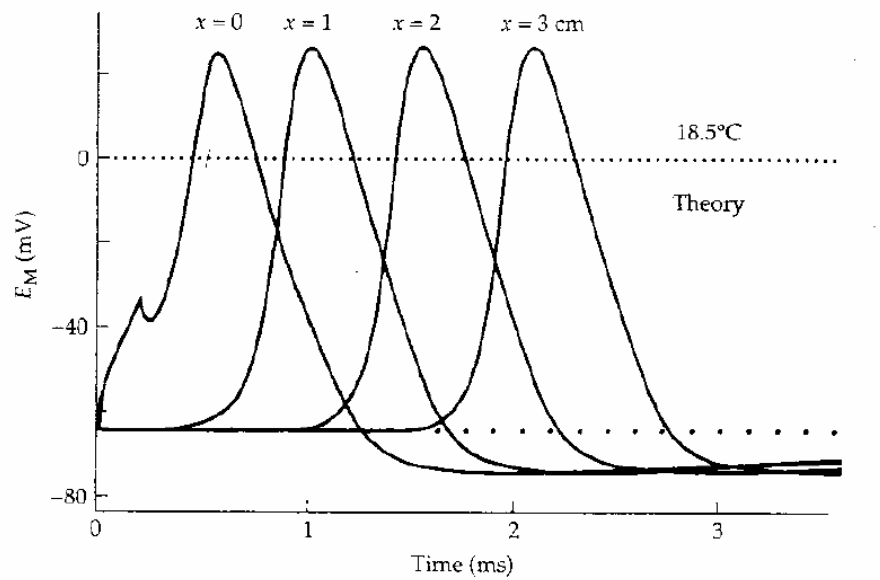
Signal in Neurons



http://en.wikipedia.org/wiki/Image:Neuron.jpg

Sodium and potassium ions play a crucial role in nerve signal transduction





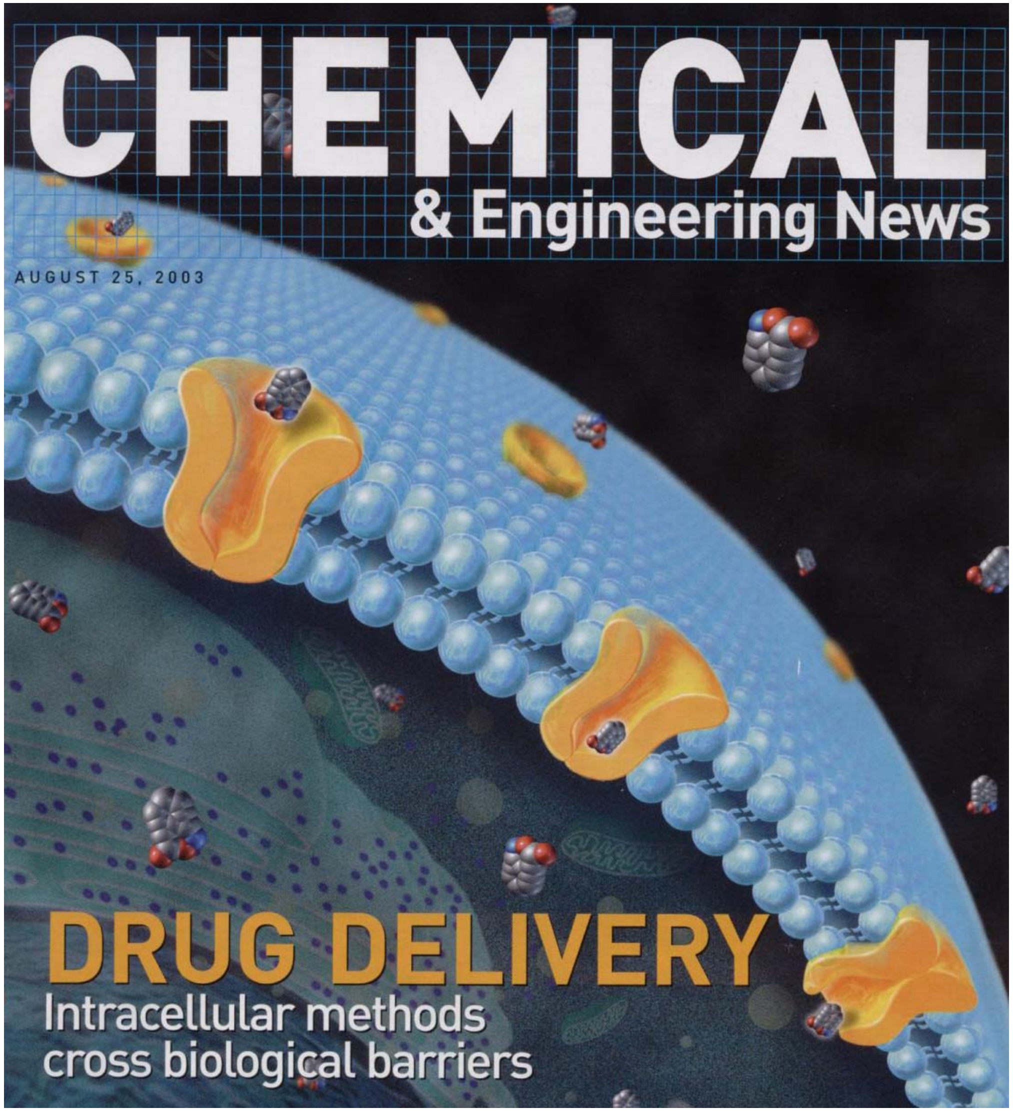
- **Sodium ions pass inside of the cell**
- **Potassium ions flow out of the cell**
- **Action potential moves with speed between 0.1 to 100 m/s.**

Lessons from Nature
Transport Proteins are Nature's Nanotubes

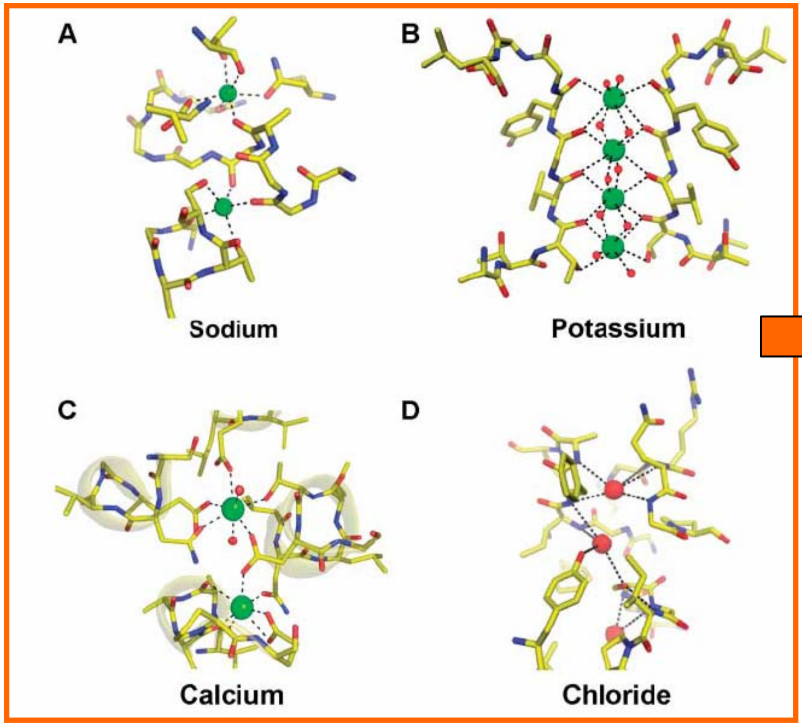
Impermeable lipid bilayer membrane

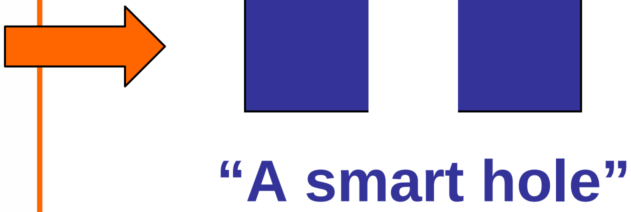
Membrane-Bound Transport Proteins

Allow for highly selective transport of ions, sugars, amino acids, etc. across the lipid bilayer membrane



Ion Channels as NanoDevices and Smart Holes





E. Gouaux, R. MacKinnon, *Science* **310**, 1461 (2005).

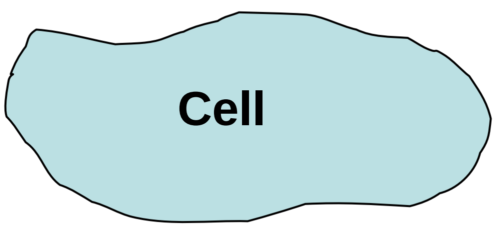
- Voltage-gated channels
- Ligand-gated channels
- Mechano-sensitive channels

Ion channels are selective for one type of ion

Symbiosis of Physical and Biological Approaches to Study Transport Phemomena in Biological Systems

How People Saw/Treated Ion Channels at the beginning of XX Century

Almost Everything We Know about Ion Channels Came from Electrical Measurements



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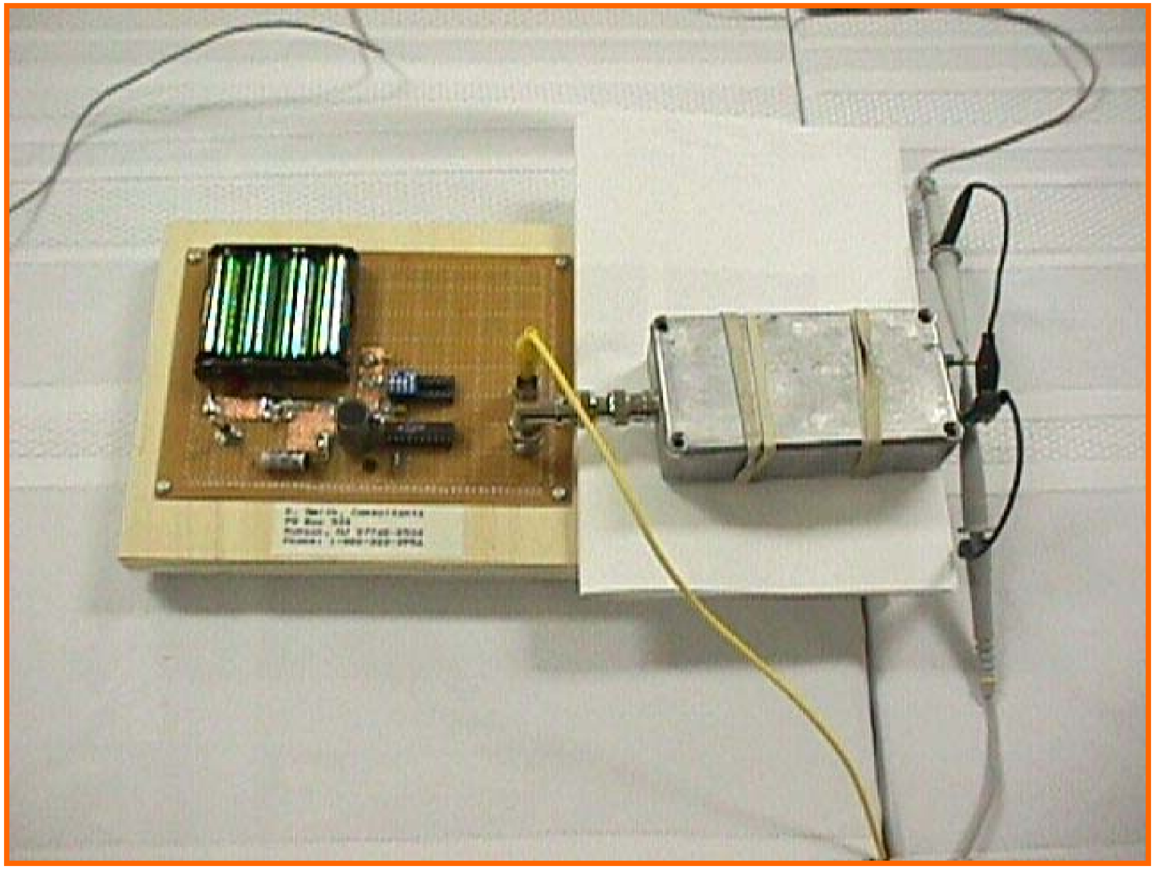
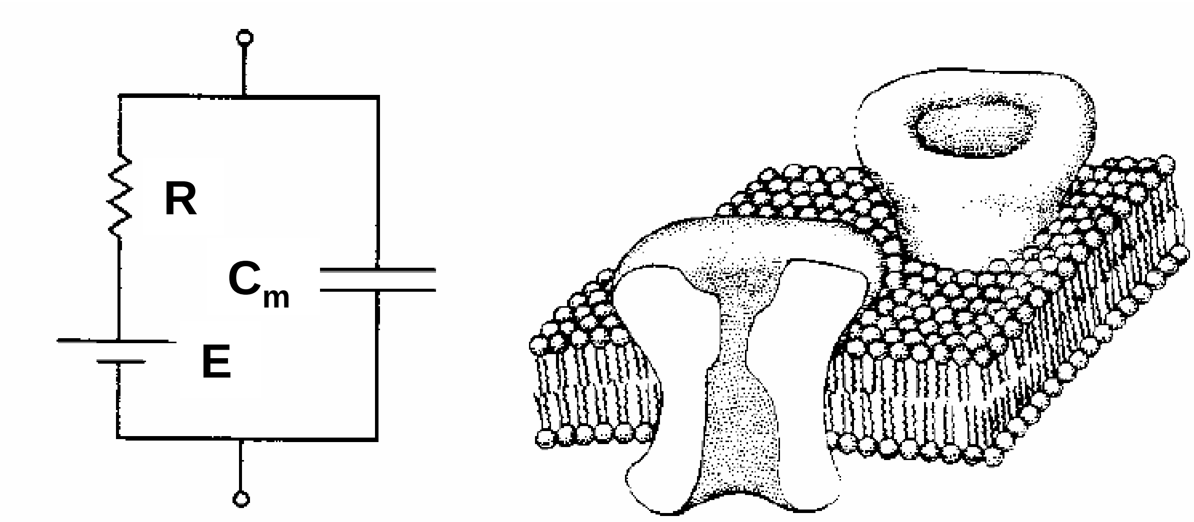


1909 Nobel Prize in Chemistry

www.emcesd.com/seminars/Ellsem21.jpg

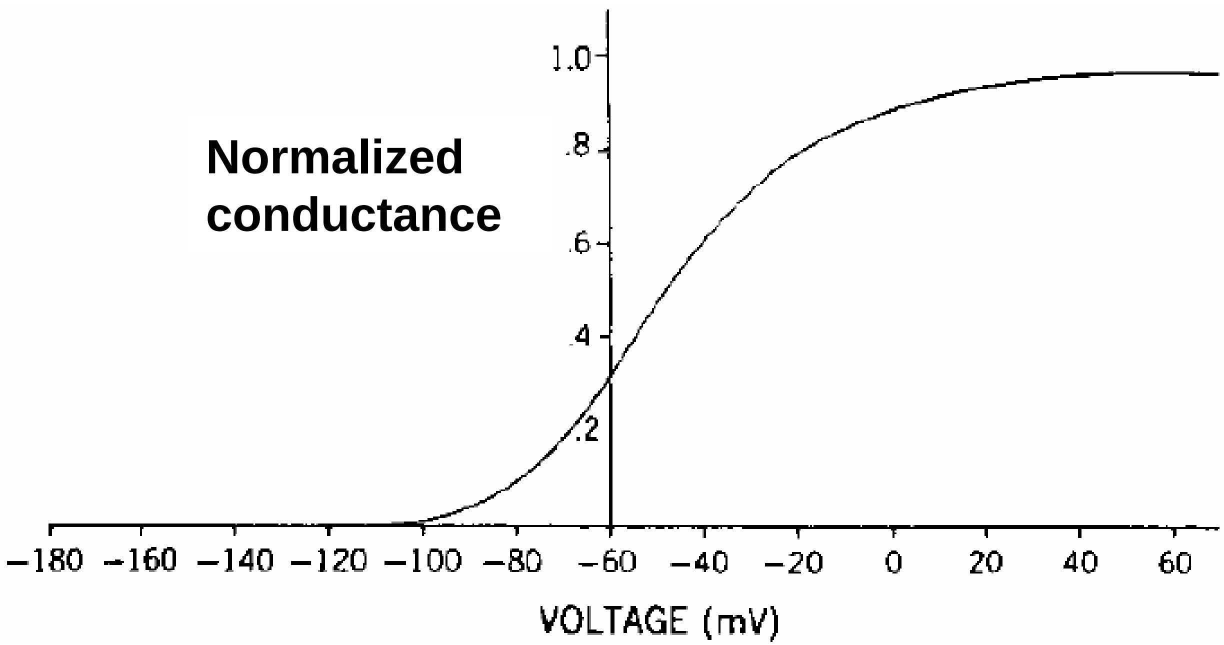
What Did We Know about Channels and Membranes in 40'/50'ties?

Not much..



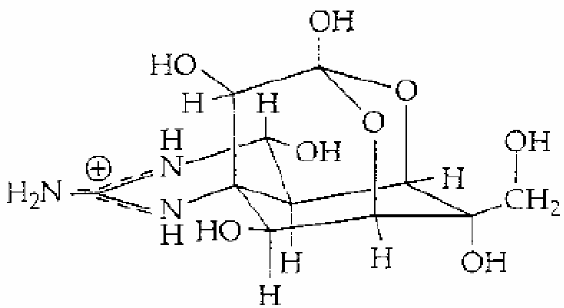
B. Hille, *Ionic Channels of Excitable Membranes*, 2nd ed.; Sinauer: Sunderland, MA, ,1992.

Excitability of the Membrane

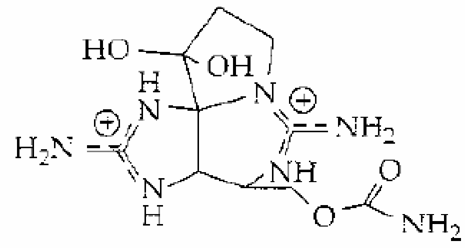


$$I(t) = [g(t)](V - V_0)$$

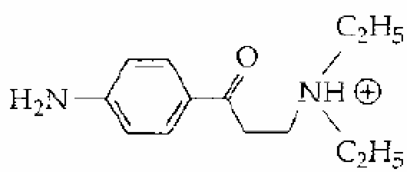
Information Was Obtained from Studies with Blockers of Ion Channels



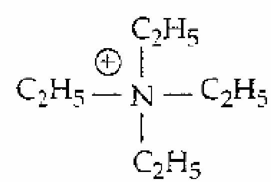
TTX



STX



Procaine



TEA

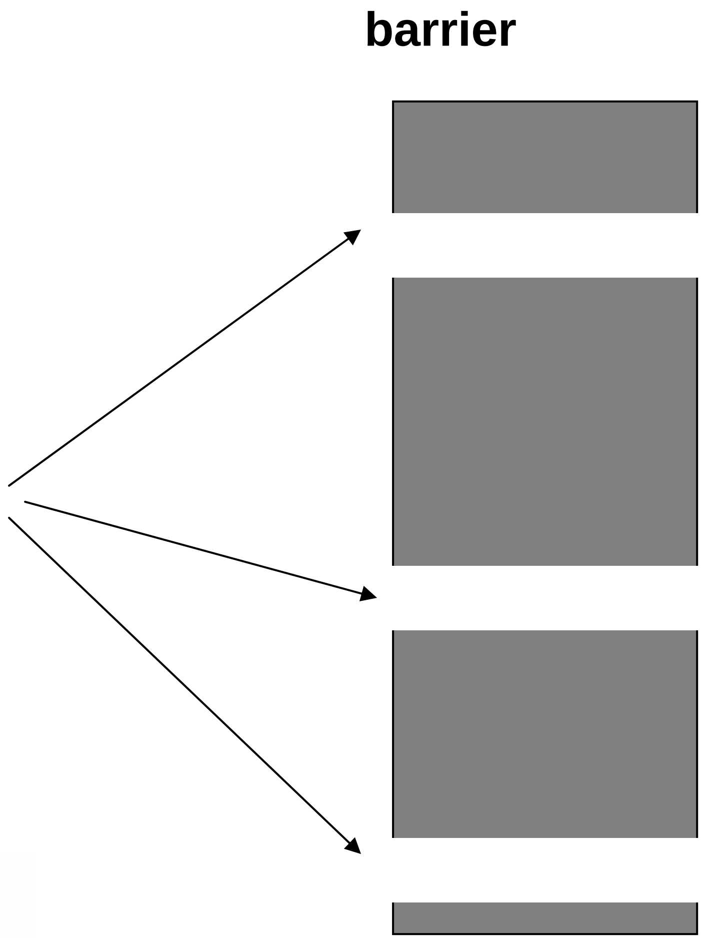
Chemical agents selectively block sodium (potassium) current indicating existence of separate paths for sodium and potassium transport through the pore.

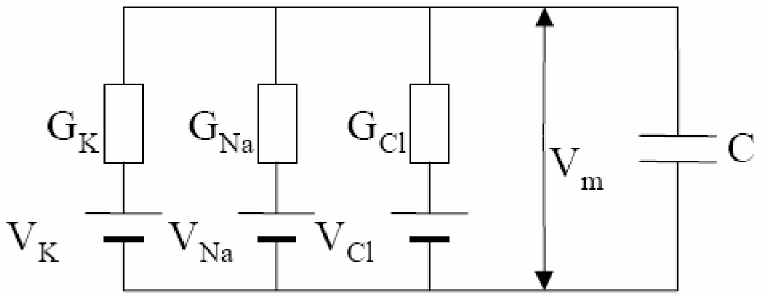
B. Hille, *Ionic Channels of Excitable Membranes*, 2nd ed.; Sinauer: Sunderland, MA, ,1992.

Picture of Membrane

Pathways for sodium and potassium, most probably separate

LACK OF DIRECT EVIDENCE





Hodgkin-Huxley Model for Signal Transduction

The Nobel Prize in Physiology and Medicine

1963

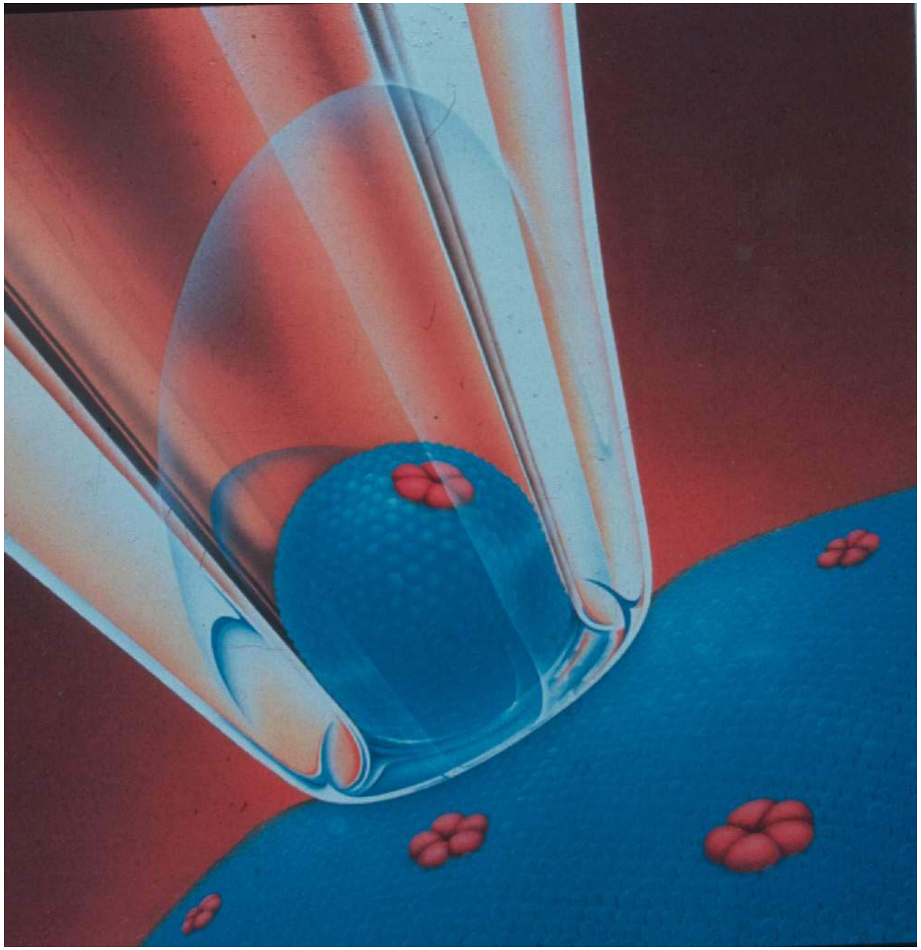
$$\frac{a}{2R_i\theta^2}\frac{d^2V}{dt^2} = C_m\frac{dV}{dt} + G_K n^4\left(V - V_K\right) + G_{Na}m^3h\left(V - V_{Na}\right) + G_L\left(V - V_L\right)$$

Picture of nerve axon as an excitable medium, capable of transmitting nonlinear waves of excitation over long distances without loss of signal strength or character of that wave.

This is all based on Hodgkin-Huxley biophysical measurements that contained no direct evidence for individual ion channels or structure of membrane!

Patch Clamp Setup

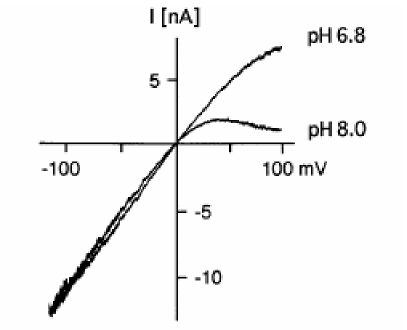
Recordings from One Molecule



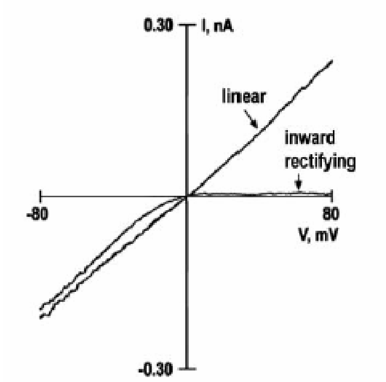
E. Neher & B. Sakmann

1991

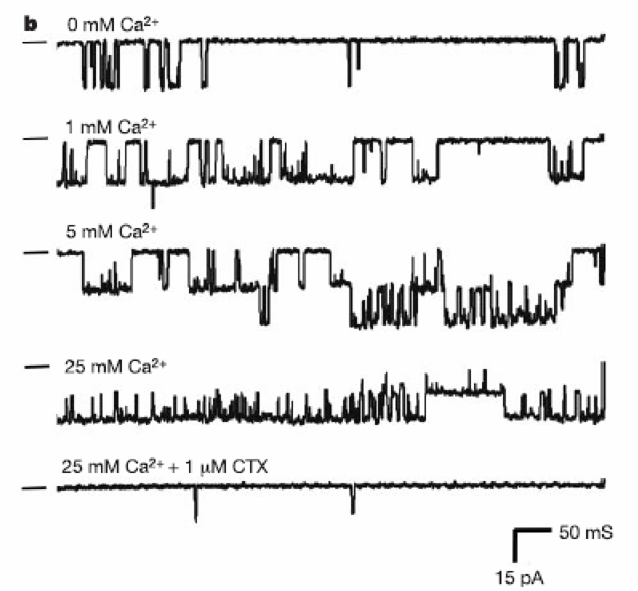
More Complete Picture of Membrane and Channels



T. Baukrowitz et al. *EMBO J.* **18**, 847 (1999)



M. Nishida, R. MacKinnon *Cell* **111**, 957 (2002)



Y. Jiang et al. *Nature* **417**, 515 (2002).

Still no direct picture of channels, channels were recognized by conductance and chemical response.

Now We Can See the Channels

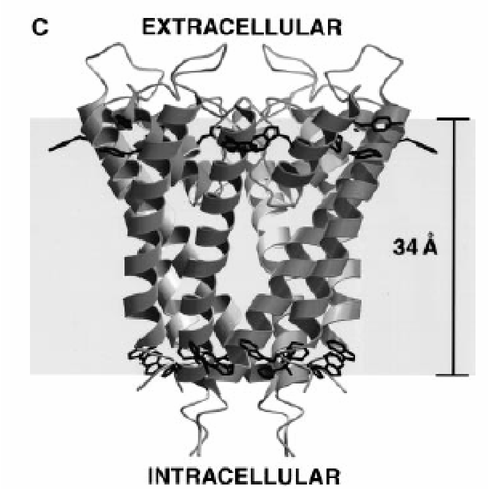
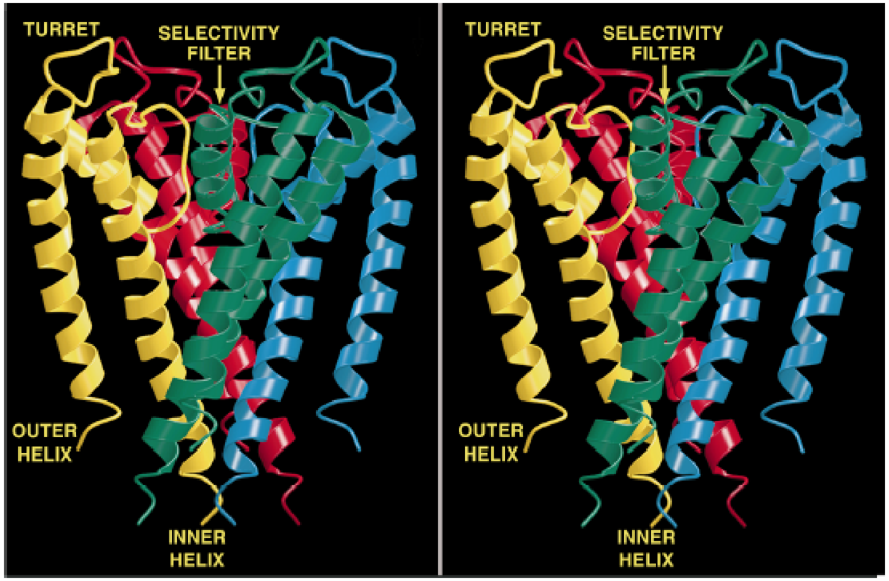


Fig. 3. Views of the tetramer. (**A**) Stereoview of a ribbon representation illustrating the three-dimensional fold of the KcsA tetramer viewed from the extracellular side. The four subunits are distinguished by color. (**B**) Stereoview from another perspective, perpendicular to that in (A). (**C**) Ribbon representation of the tetramer as an integral-membrane protein. Aromatic amino acids on the membrane-facing surface are displayed in black. (**D**) Inverted teepee architecture of the tetramer. These diagrams were prepared with MOLSCRIPT and RASTER-3D (*33*).



R. MacKinnon, P. Agre 2003

D.A. Doyle, J. M. Cabral, R.A. Pfuetzner, A. Kuo, J.M. Gulbis, S.L. Cohen, B.T. Chait, R. MacKinnon, Science **280**, 69 (1998).

Relation Between Structure and Function

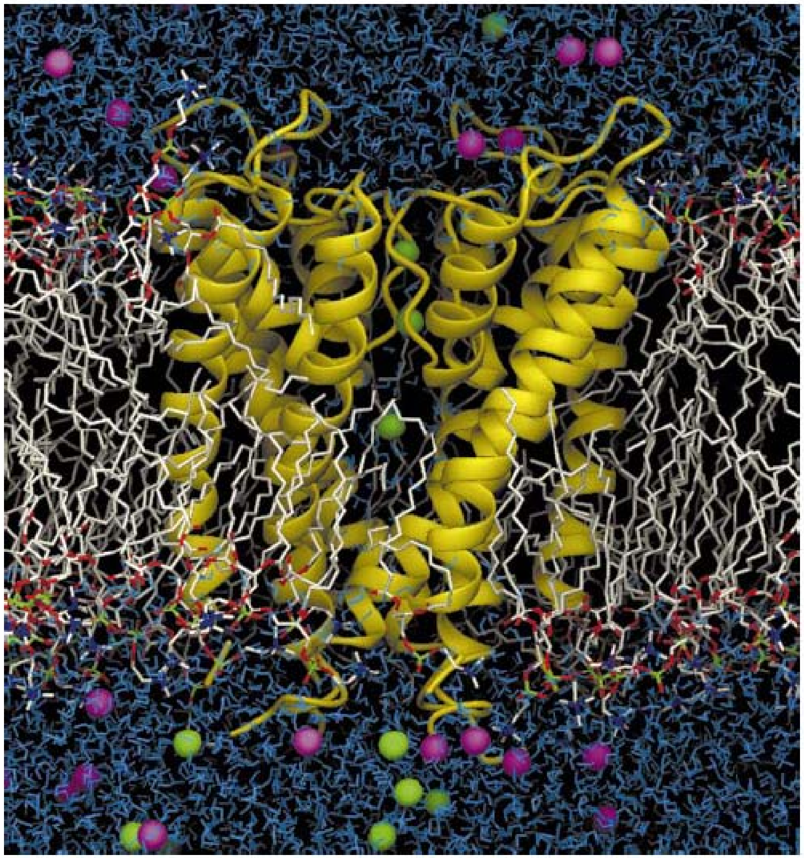
Electrical Measurements

Function

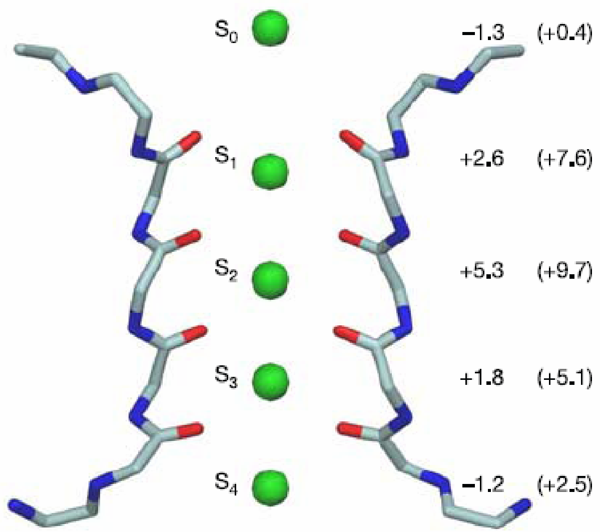
STRUCTURE

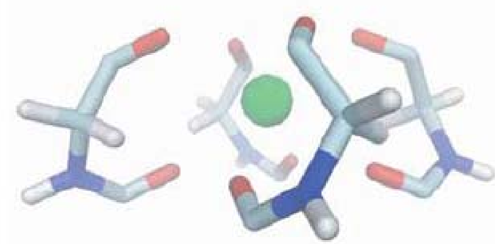
Which parts of the complex structures are responsible for various features like voltage gating, selectivity?

Focusing on "Important" Part of the System



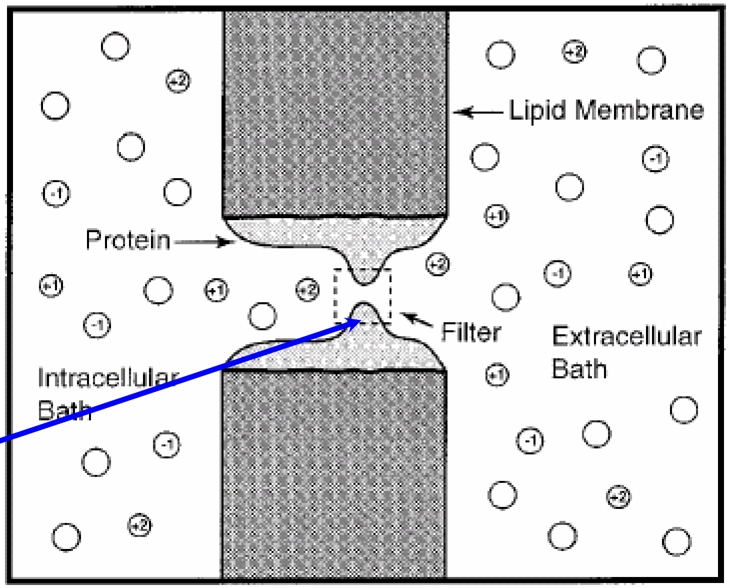
S. Berneche, B. Roux, Nature **414**, 73 (2001)





S.Y. Noskov, S. Berneche, B. Roux, Nature **431**, 830 (2004)

Selectivity of L-Type Calcium Channels



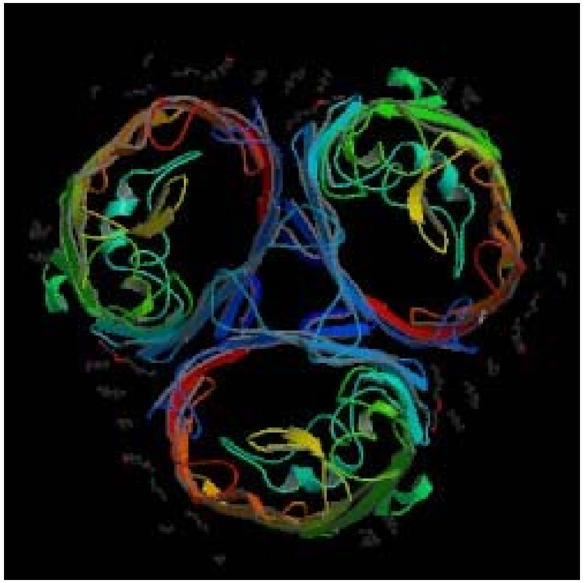
Four COOH groups

There are two binding sites for calcium ions

S.W. Jones, *J. Bioenergetics and Biomembranes*, **30**, 299 (1998); E.W. McCleskey, J. Gen. Physiol. **113**, 765 (1999); W. Nonner, D. Gillespie, D. Henderson, B. Eisenberg, *J. Phys. Chem.* **105**, 6427 (2001);

Calcium channel is selective for calcium ions although concentration of Ca^{2+} << than concentration of Na^{+} and K^{+}

Which Part of the Channel is Responsible for Ca Selectivity ? – Importance of Electrostatics

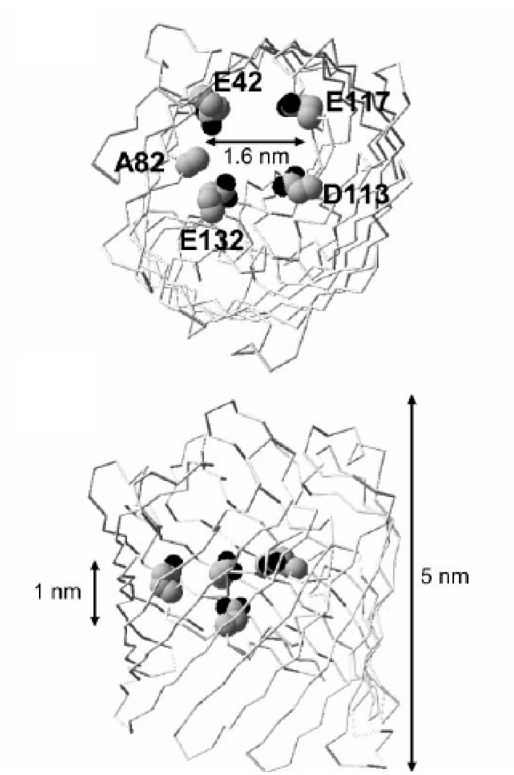


OmpF porin functions to regulate osmotic pressure between the cell and its surroundings. It has one **e**, it is basically not selective.

H. Miedema, A. Meter-Arkema, J. Wierenga, J. Tang, B. Eisenberg, W. Nonner, H. Hektor, D. Gillespie, W. Meijberg, *Biophys. J.* **87**, 3137 (2004).

Protein data bank:
http://www.rcsb.org/pdb/cgi/explore.cgi?job=chains&pdbId=2OMF&page=

Introduction of 2 additional COOH groups turns non-selective OmpF channel into Calcium Selective Channel

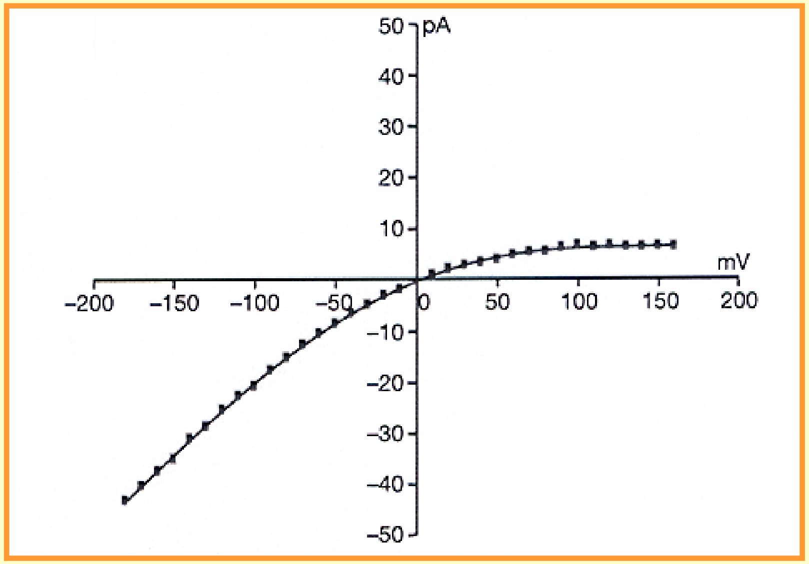


The mutant is calcium selective!

H. Miedema, A. Meter-Arkema, J. Wierenga, J. Tang, B. Eisenberg, W. Nonner, H. Hektor, D. Gillespie, W. Meijberg, *Biophys. J.* **87**, 3137 (2004).

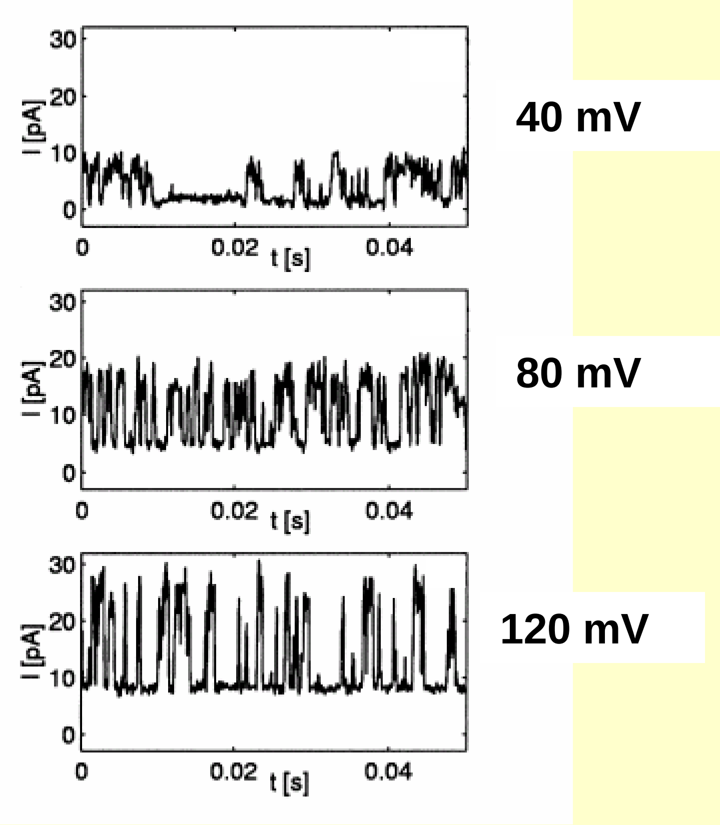
Voltage-Gating of Biochannels

The ion currents are rectified



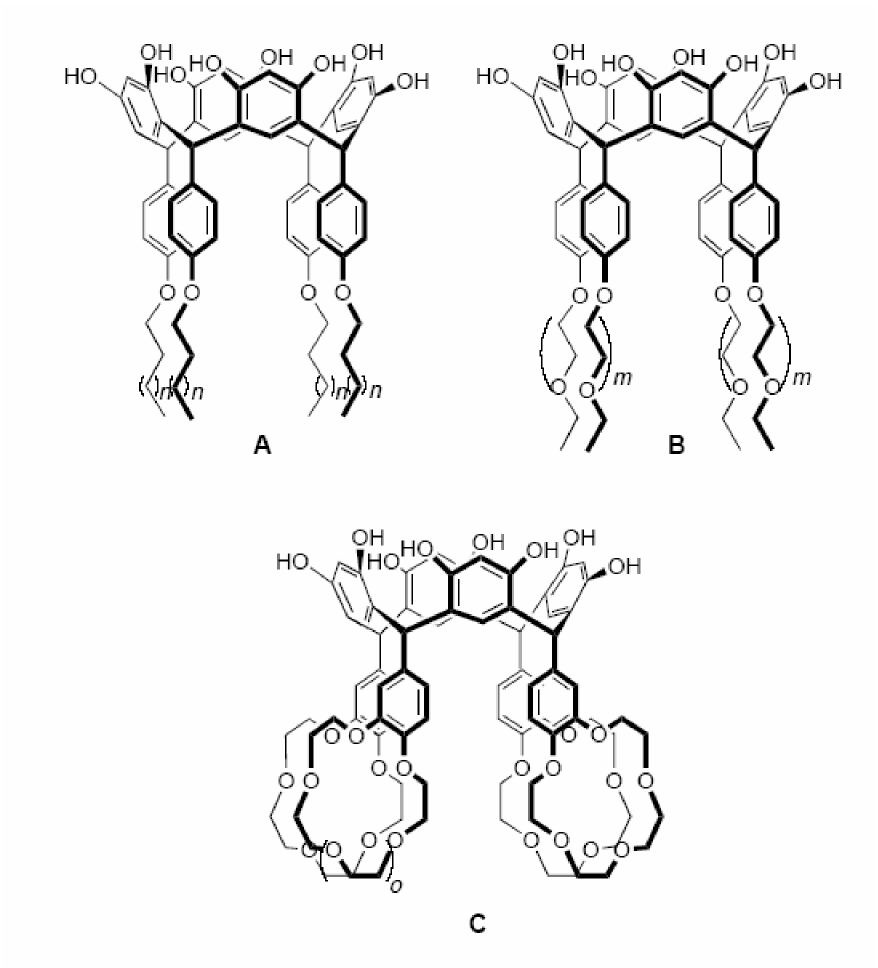
Y. Jiang, A. Lee, J. Chen, M. Cadene, B.T. Chait, R. MacKinnon, *Nature* **417** (2002) 515.

Ion current switches between discrete levels in a voltage-dependent manner

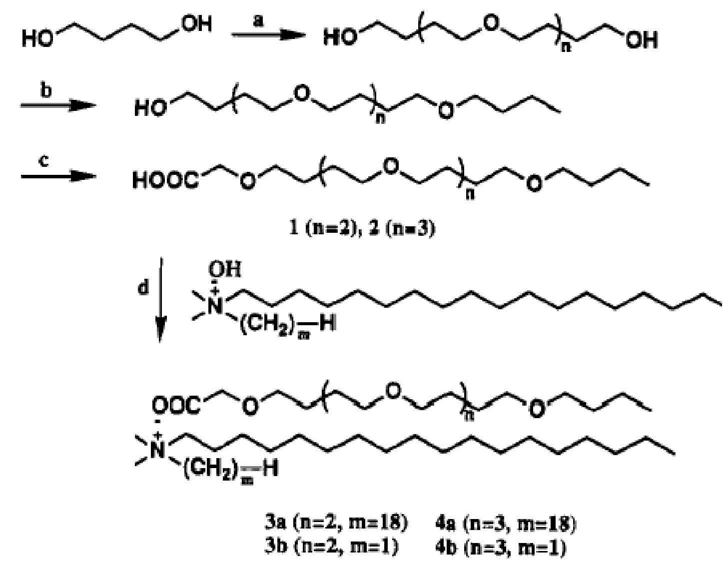


BK channel (P.N.R. Usherwood)

Synthetic NOT-PROTEIN PORES



A.J. Wright, S.E. Matthews, W.B. Fischer, P.D. Beer, *Chem. Eur. J.* **7**, 3474 (2001)



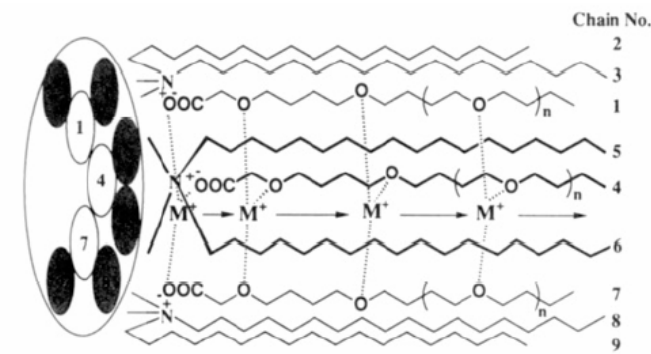
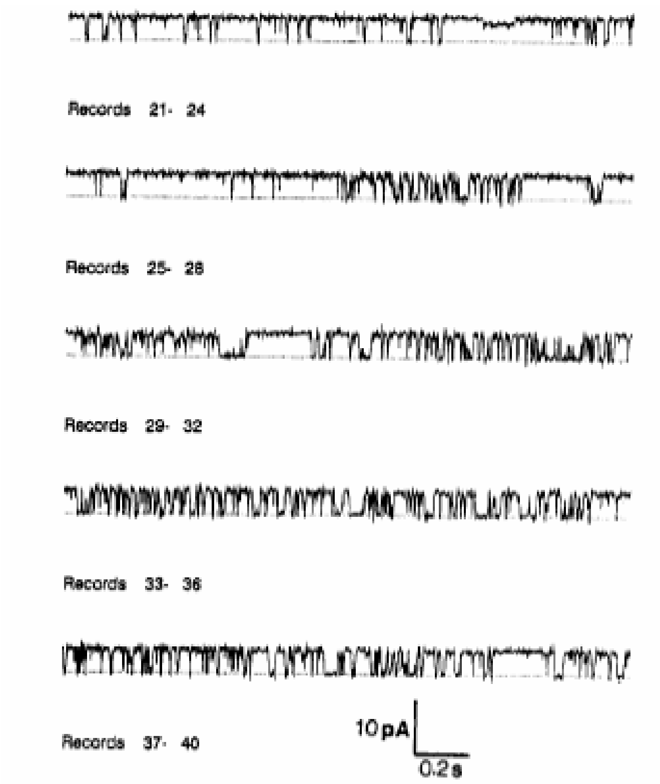
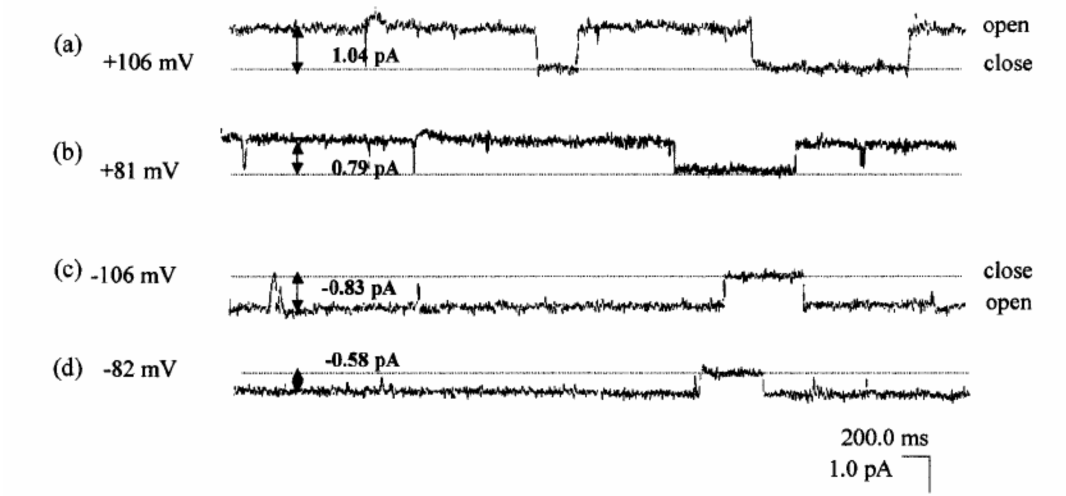


Figure 5. Schematic representation of a hypothetical half ion channel and cation movement in the channel.

Y. Kobuke, K. Ueda, M. Sokabe, J. Am. Chem. Soc. **114**, 7618 (1992).

Gating with Non-Protein Nanopores



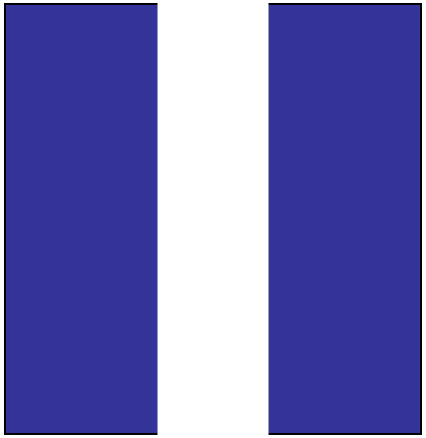


Y. Kobuke, K. Ueda, M. Sokabe, J. Am. Chem. Soc. **114**, 7618 (1992).

C. Goto, M. Yamamura, A. Satake, Y. Kobuke, J. Am. Chem. Soc. **123**, 12152 (2001)

Physicists Can Think About Even a More Crazy Approach

Grafting a feature that we believe plays a crucial role in a given function into an entirely synthetic system.

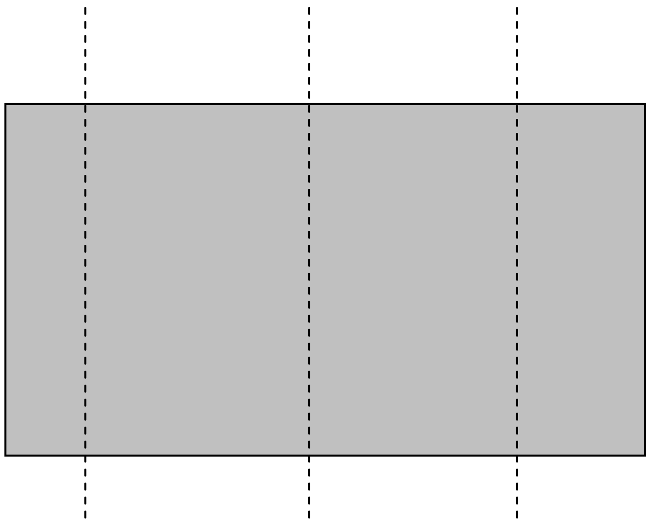


A synthetic “hole”

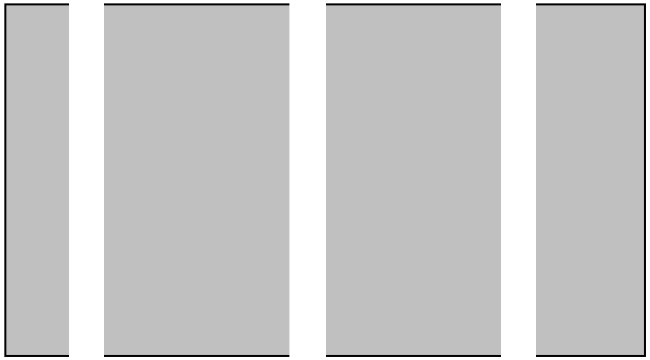
Focusing on basic physical and chemical effects underlying transport properties of biological channel.

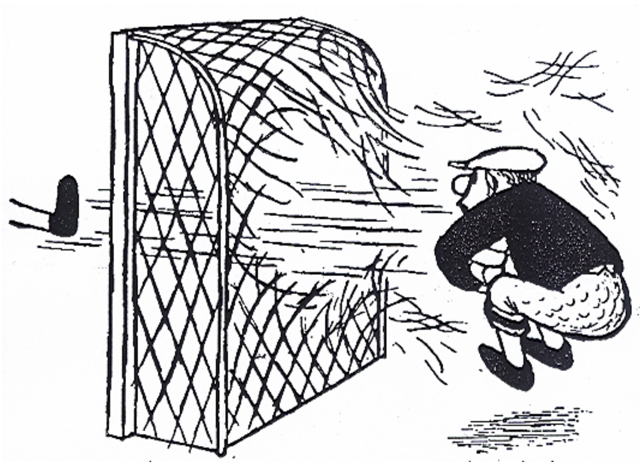
Heavy Ions as a Working Tool

e.g. Xe, Au, U
(~2.2 GeV i.e. ~ 15% c)



Chemical etching



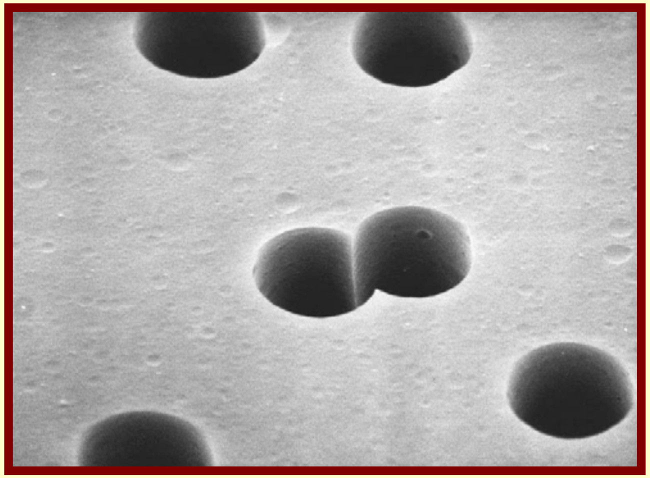


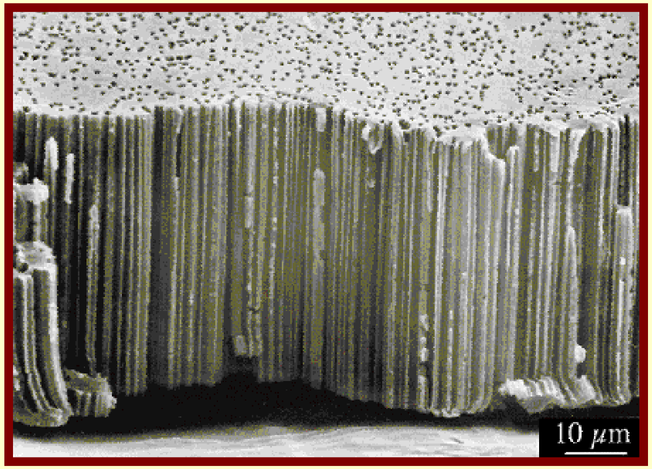
E. Loriot

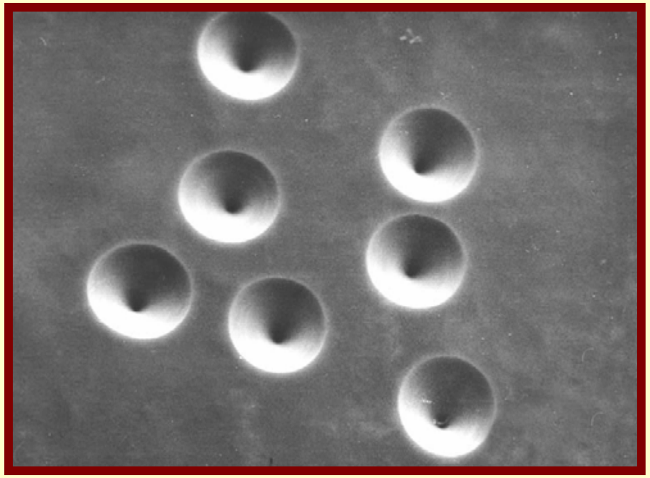
Linear accelerator
UNILAC, GSI
Darmstadt, Germany

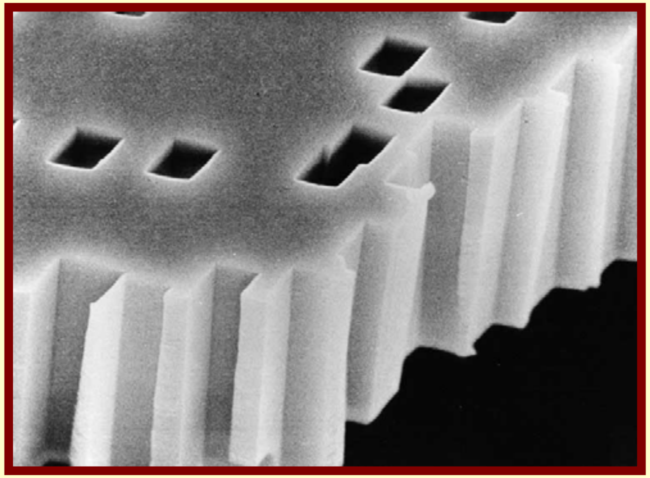
1 ion → 1 latent track → 1 pore !

A Short Glimpse at the “Product" of Track Etching Technique





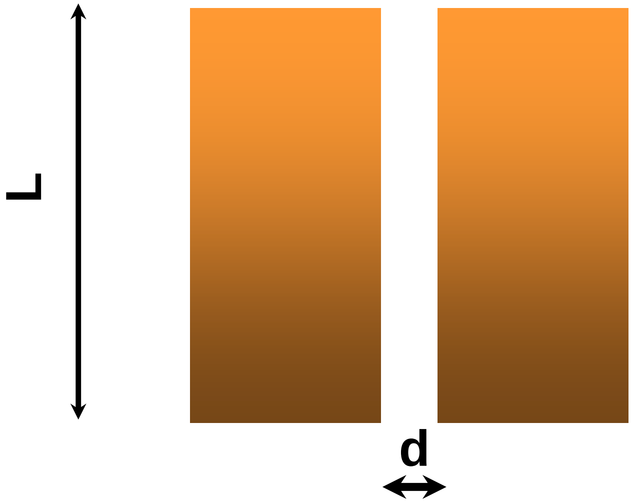




http://www. Iontracktechnology.de

Why Do We Want to Work with Asymmetric Pores?

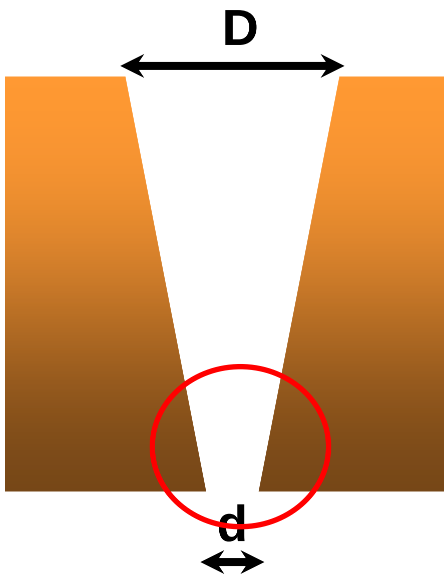
Cylindrical pore



Tapered cone

$$R_1 = \frac{4L}{\kappa\pi d^2} \quad >> \quad R_2 = \frac{4L}{\kappa\pi dD}$$

Focusing of Resistance in a Conical Nanopore



$$R = \frac{4L}{\kappa \pi d D}$$

D = 1 μm

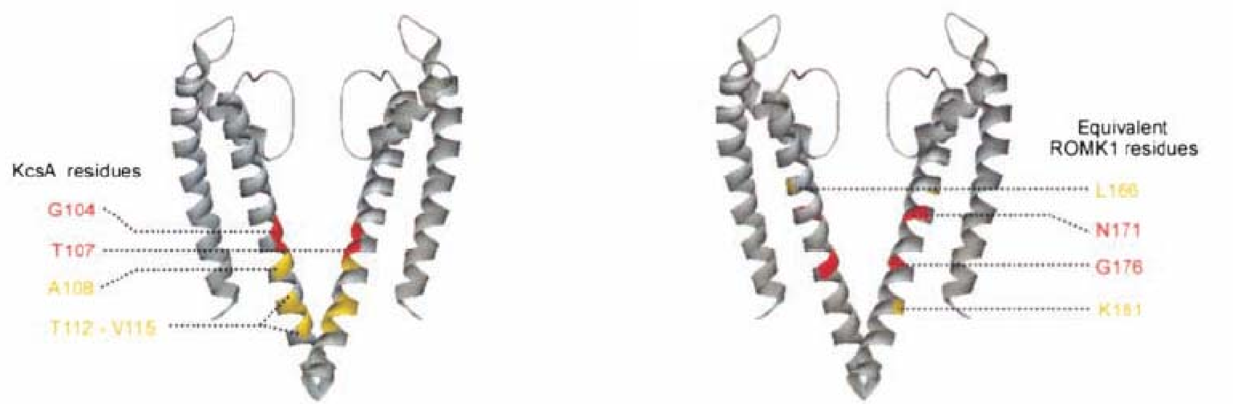
d = 3 nm

L = 12 μm

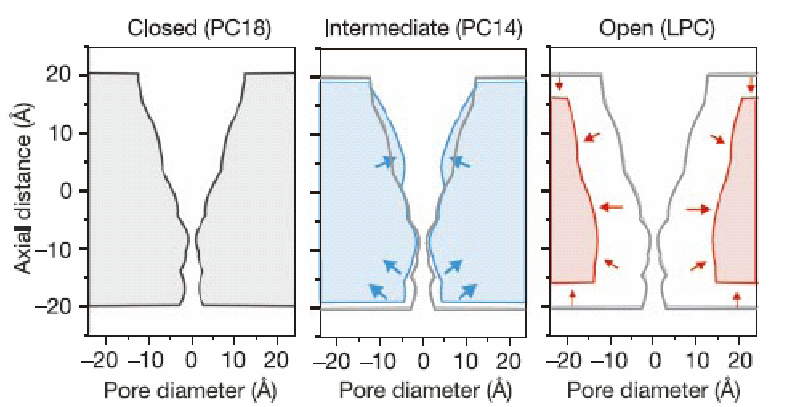
50% of total resistance is focused over 36 nm

80% of total resistance is focused over 140 nm

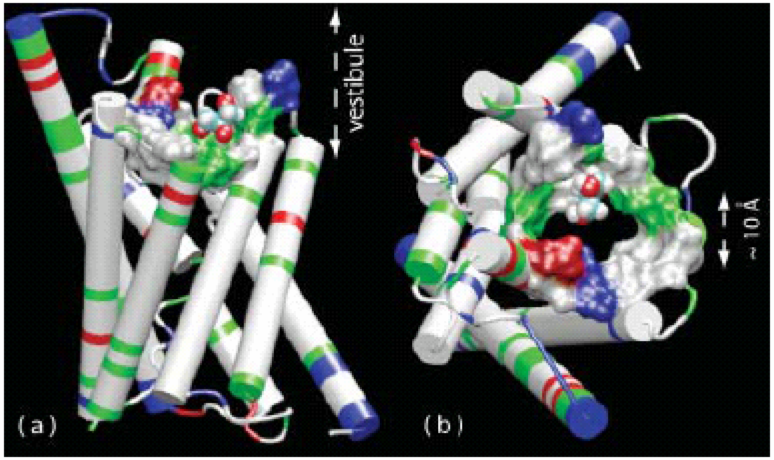
Nature Likes Asymmetry Very Much



Y. Zhou, J.H. Morais-Cabral, R. MacKinnon, Nature 414 (2001) 43

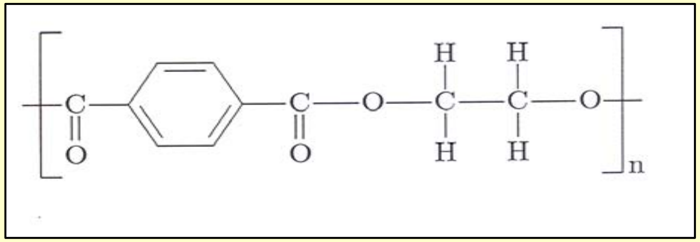


E. Perozo et al. Nature 418 (2002) 942

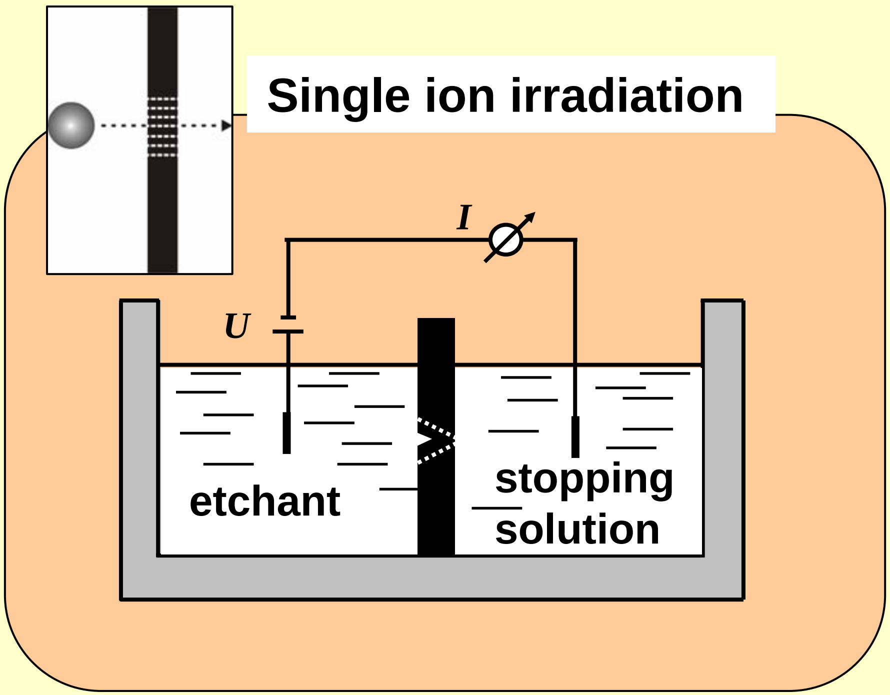


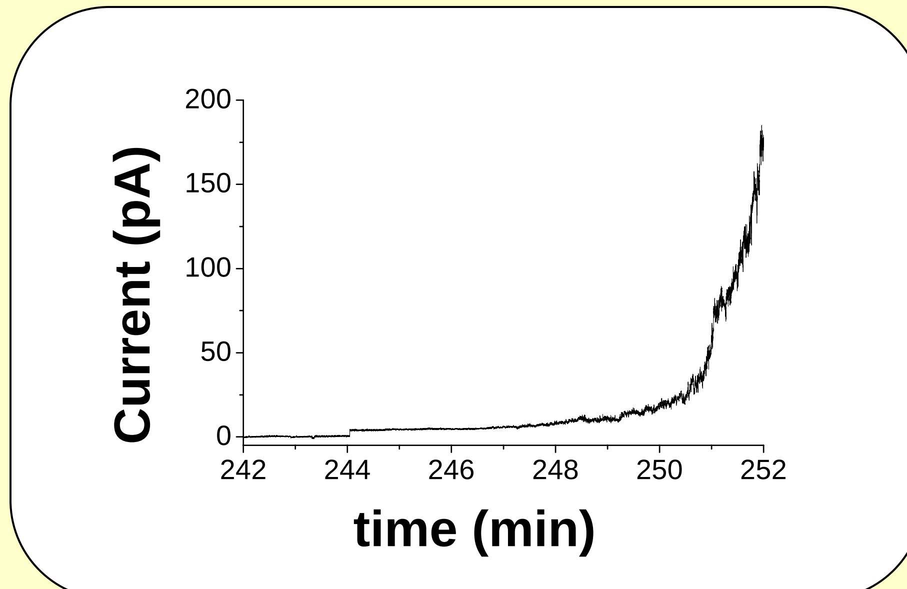
D. Lu, P. Grayson, K. Schulten, Biophys. J. 85 (2003) 2977

Conical Pores are Obtained by Putting Etch Solution on One Side of Membrane and Stop Solution of the Other



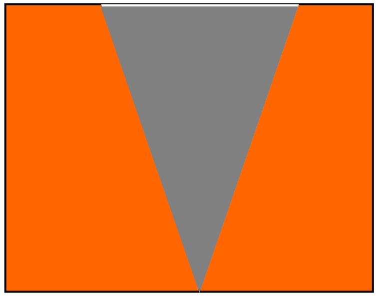
Polyethylene Terephthalate (PET)
(RN 12 Hoechst)

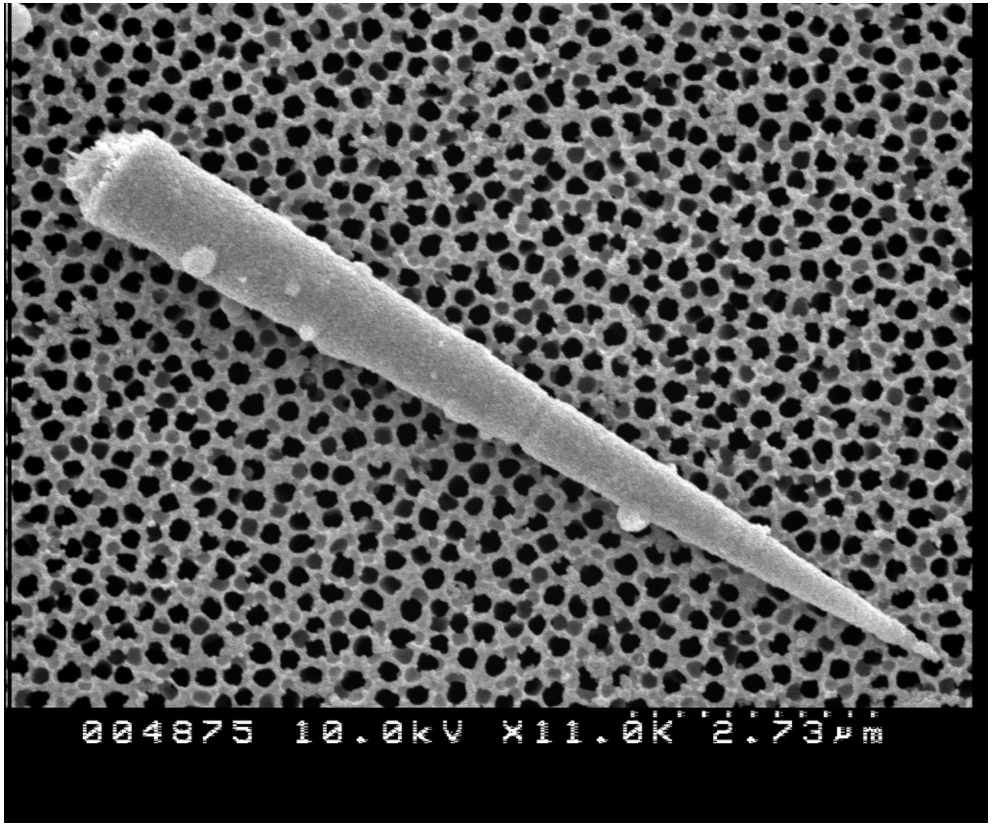




Z. Siwy et al. *Nucl. Instr. Meth. B* **208**, 143-148 (2003); *Applied Physics A* **76**, 781-785; *Surface Science* **532-535**, 1061-1066 (2003).

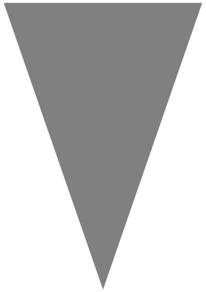
Gold Replica of a Single Conical Pore

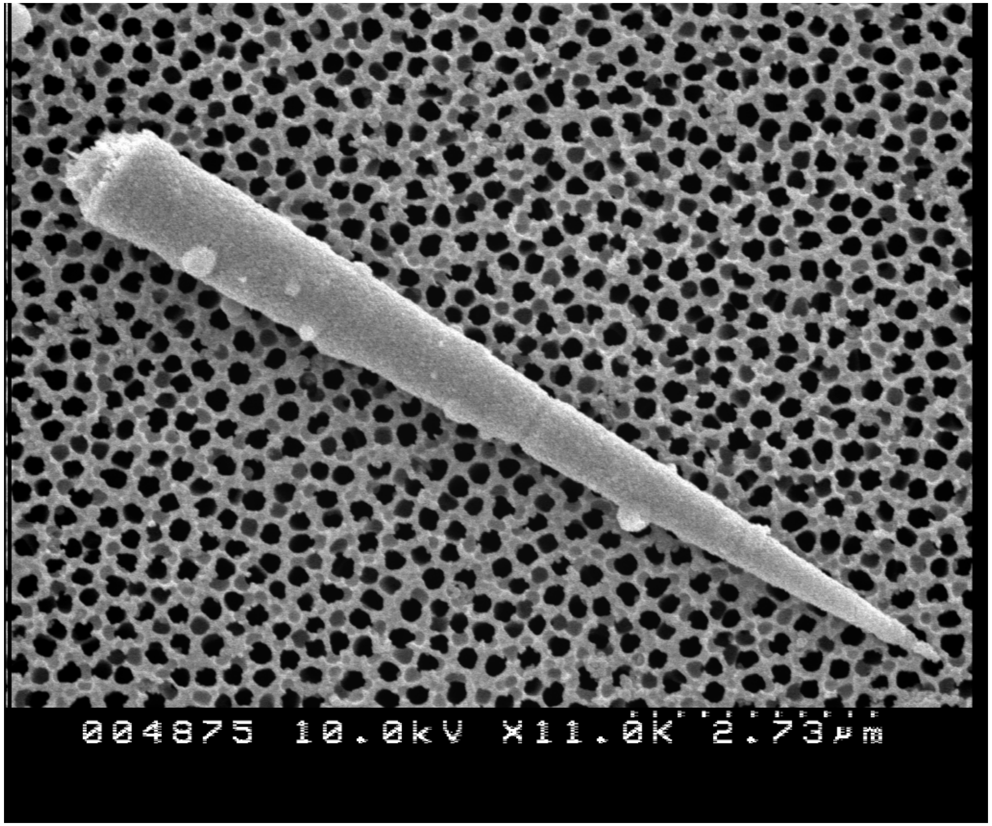




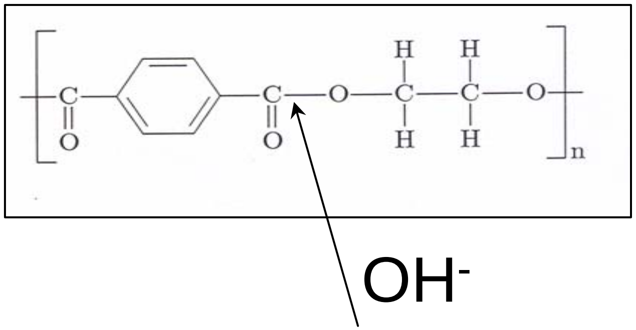
Dept. of Chemistry, Univ. of Florida, Gainesville

Gold Replica of a Single Conical Pore



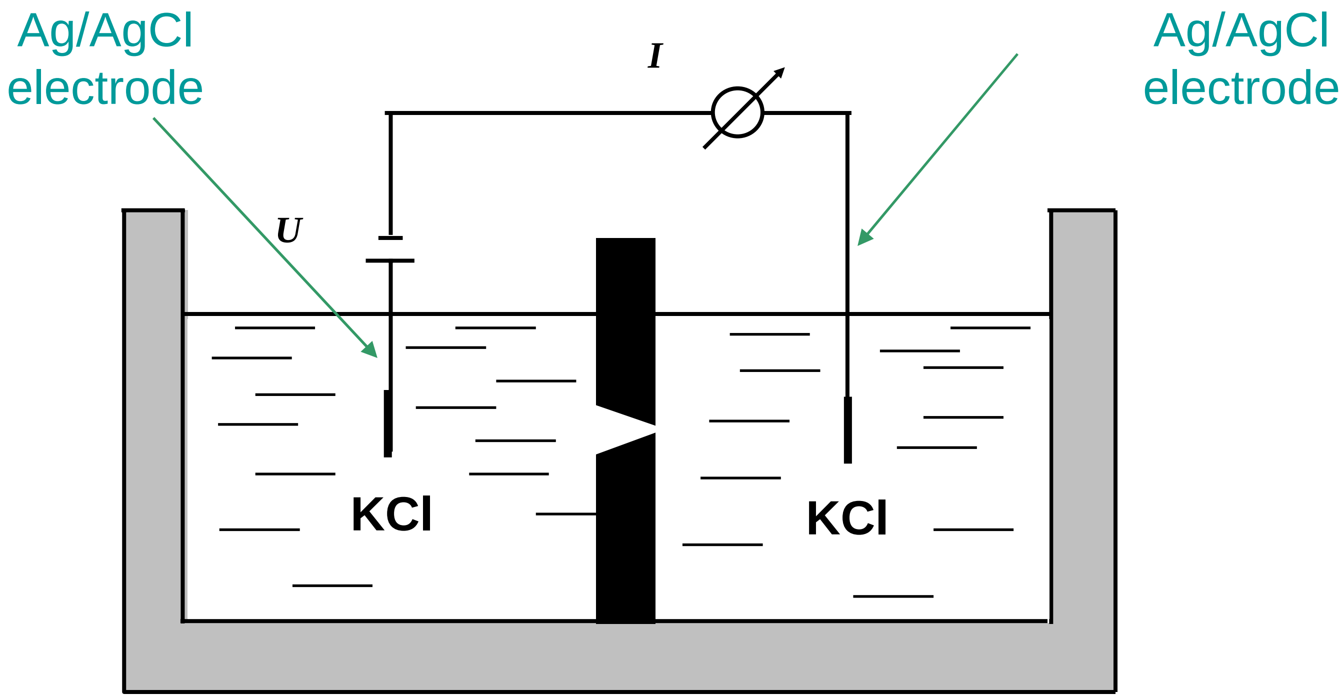


Hydrolysis of Ester Bonds with NaOH in PET Causes Formation of COOH Groups

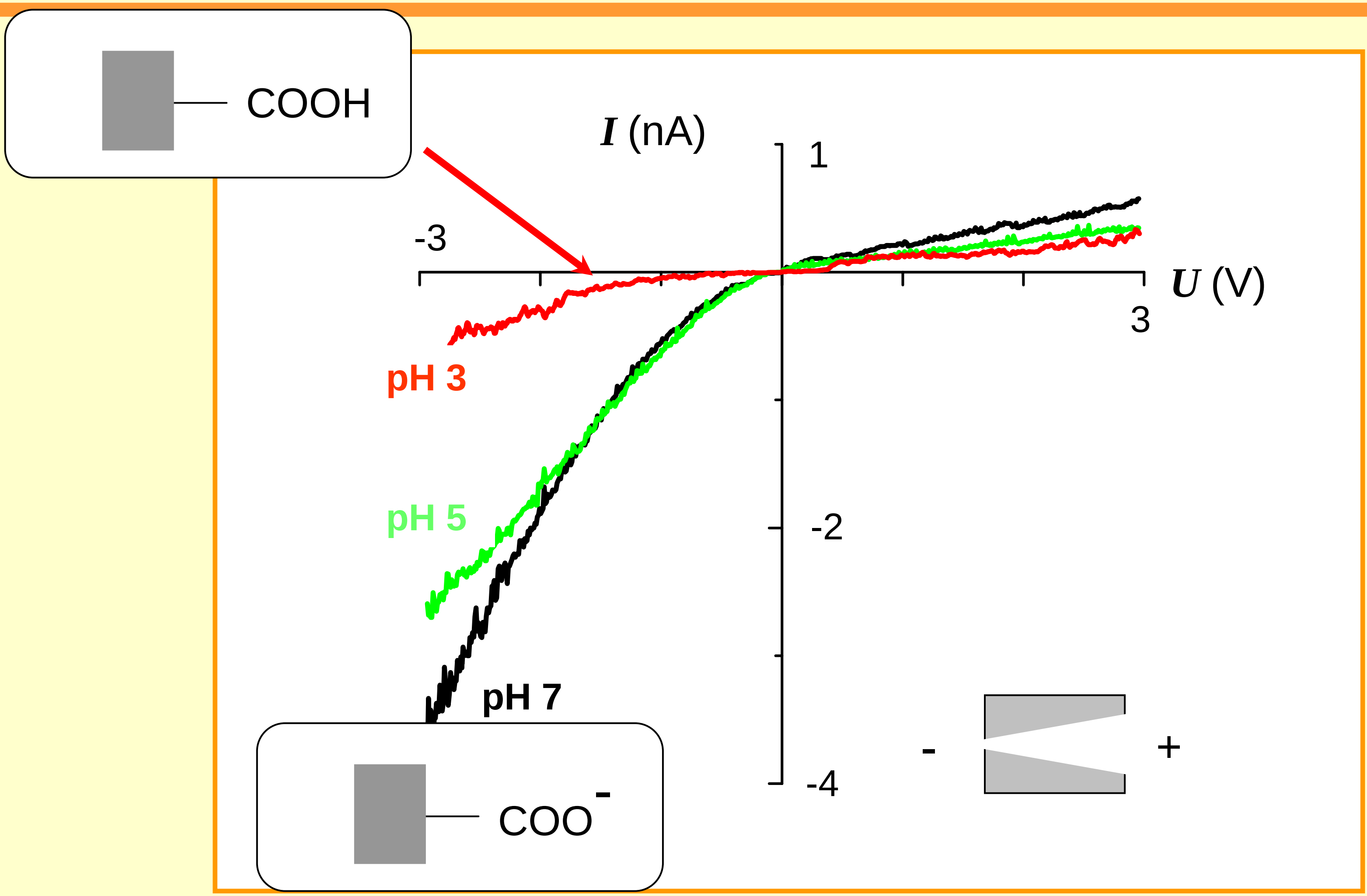


The surface density of COOH groups was estimated to be ~ 1.5 per nm^2

Experimental Set-up



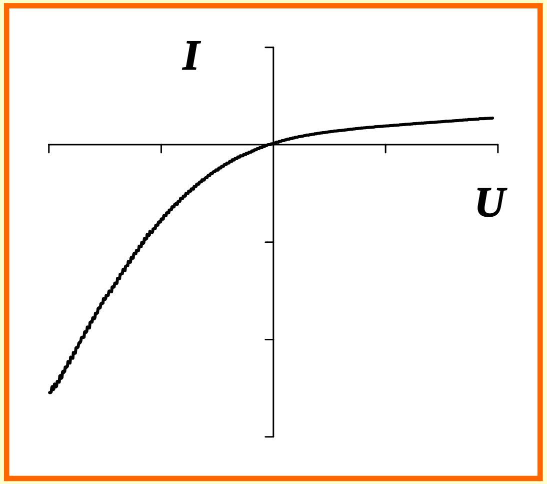
Current-Voltage Characteristics of Single Conical Pores

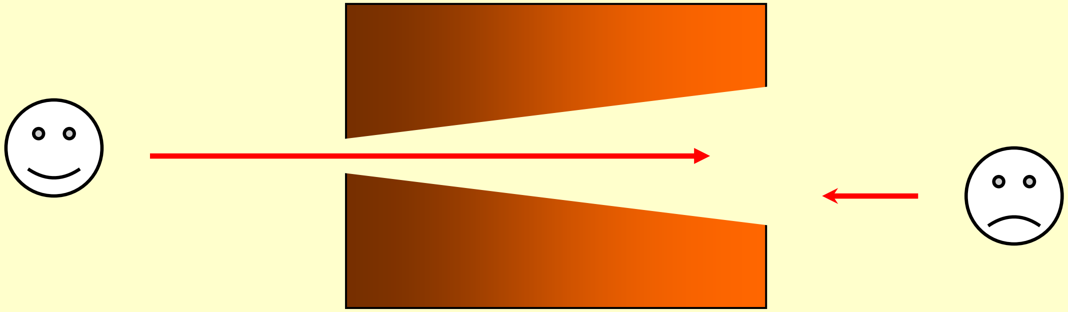


Z. Siwy, Gu Y., Spohr H., Baur, D., Wolf-Reber A., Spohr, R., Apel, P., Korchev Y.E. *Europhys. Lett*. **60**, 349 (2002); Z. Siwy, Apel P. Baur D., Dobrev, D.D., Korchev Y.E., Neumann R., Spohr R., Trautmann, R., *Surface Science* **532-535**, 1061 (2003)

Are the Nanopores Ion Selective?

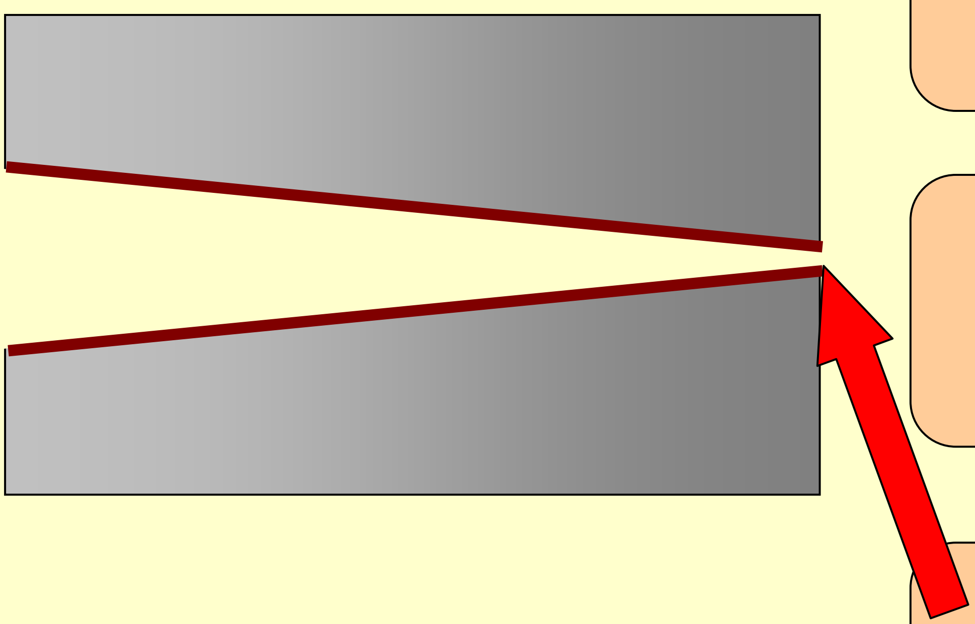
PET and Kapton pores are cation selective $t^+ \sim 0.90$





Z. Siwy, A Fulinski, Phys. Rev. Lett. **89**, 198103 (2002); Am. J. Phys. **72**, 567 (2004).

Which features are crucial for rectification?



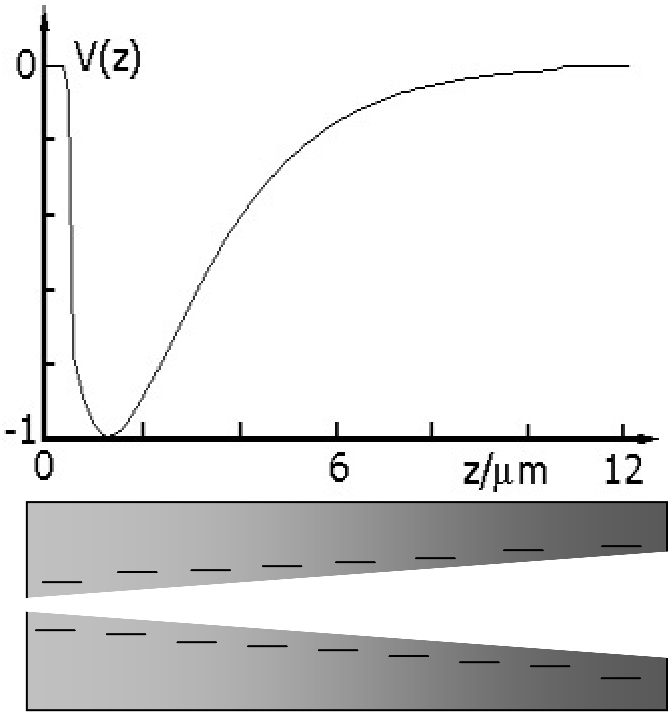
Asymmetric shape of the pore

The pore has to be charged

The diameter of the pore has to be very small !

Why do Asymmetric Nanopores Rectify?

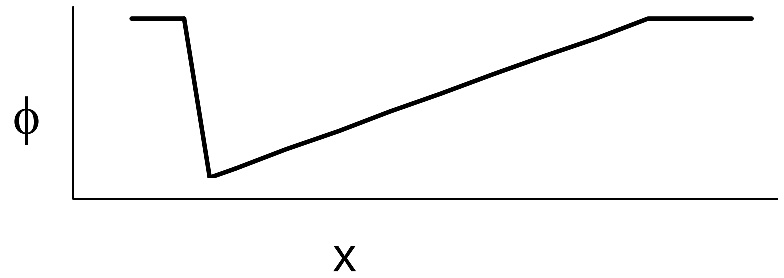
The profile of electrostatic potential *V*(*z*) inside an asymmetric pore

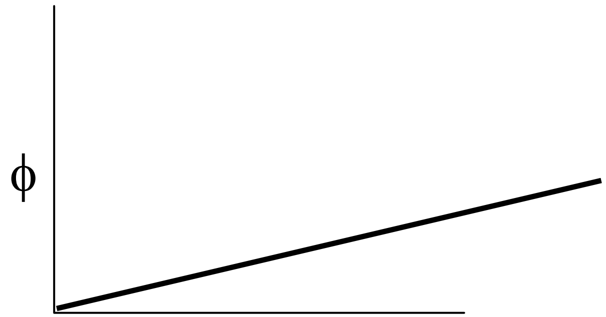


Siwy Z., Fulinski A. *Phys. Rev. Lett.* **89**, 198103 (2002); Siwy Z., Fulinski A. *The American Journal of Physics* 74 (2004) 567; Siwy Z., Heins E., Harrell C., Kohli P., Martin C.R. J. Am. Chem. Soc. **126** 10850 (2004).

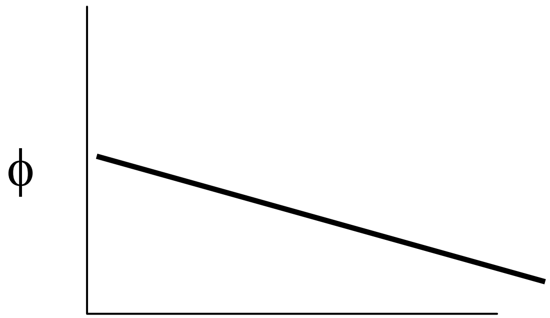
Existence of Electrostatic Trap

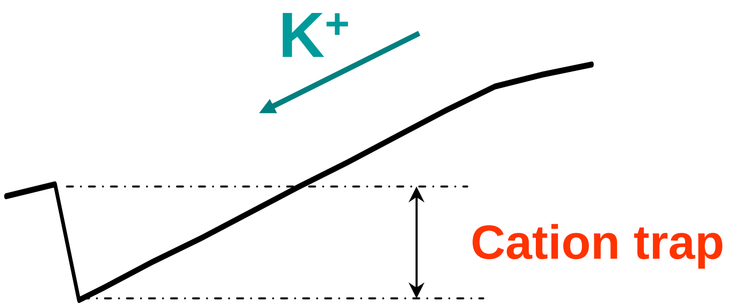
Shape of electrostatic potential inside a conical pore with negative surface charge

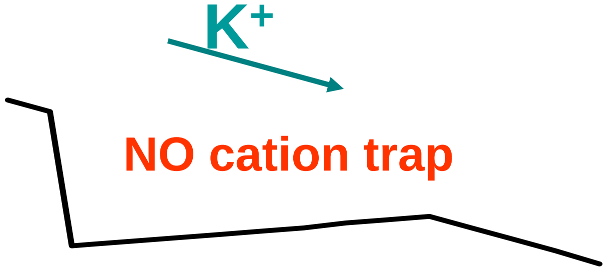




External voltage





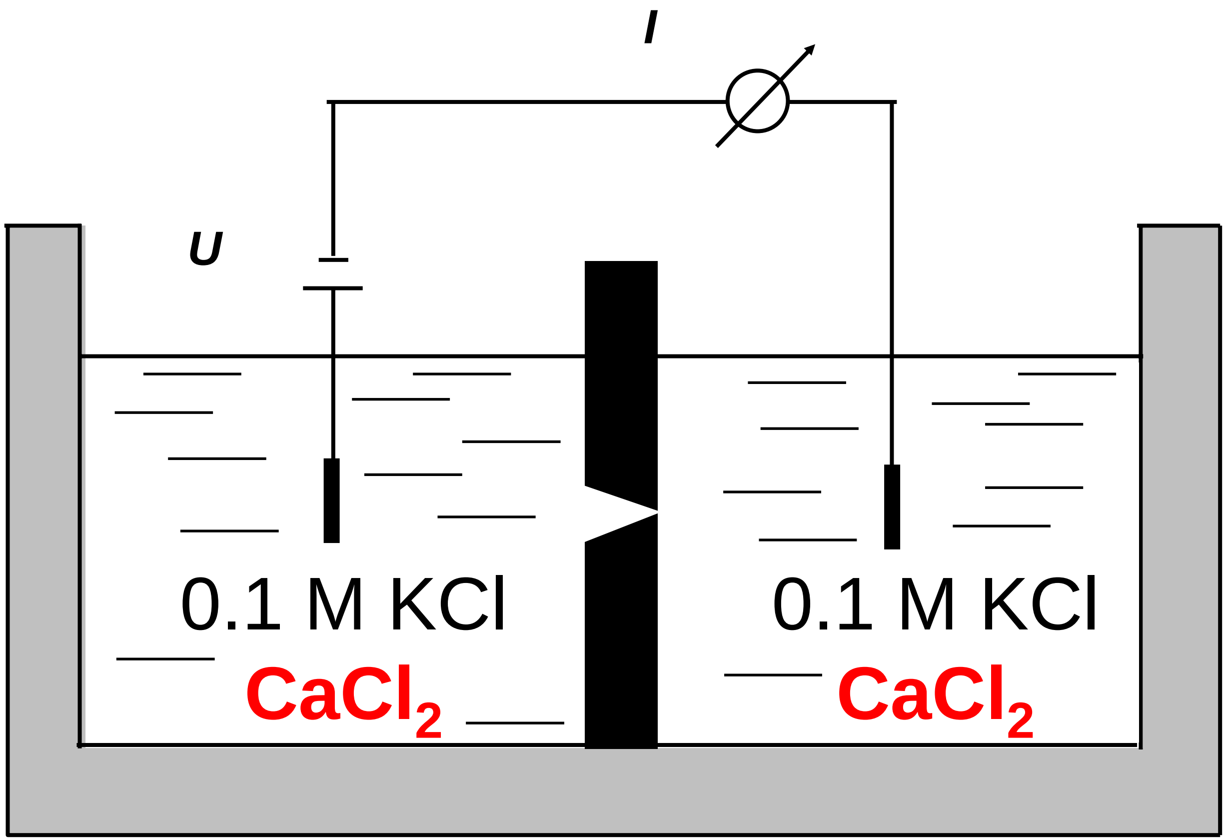


Z. Siwy, E. Heins, C.C. Harrell, P. Kohli, C.R. Martin,
J. Am. Chem. Soc. **126** 10850 (2004).

Model of ion current rectification in KCl assumes therefore a simple superposition of internal potential with externally applied voltage

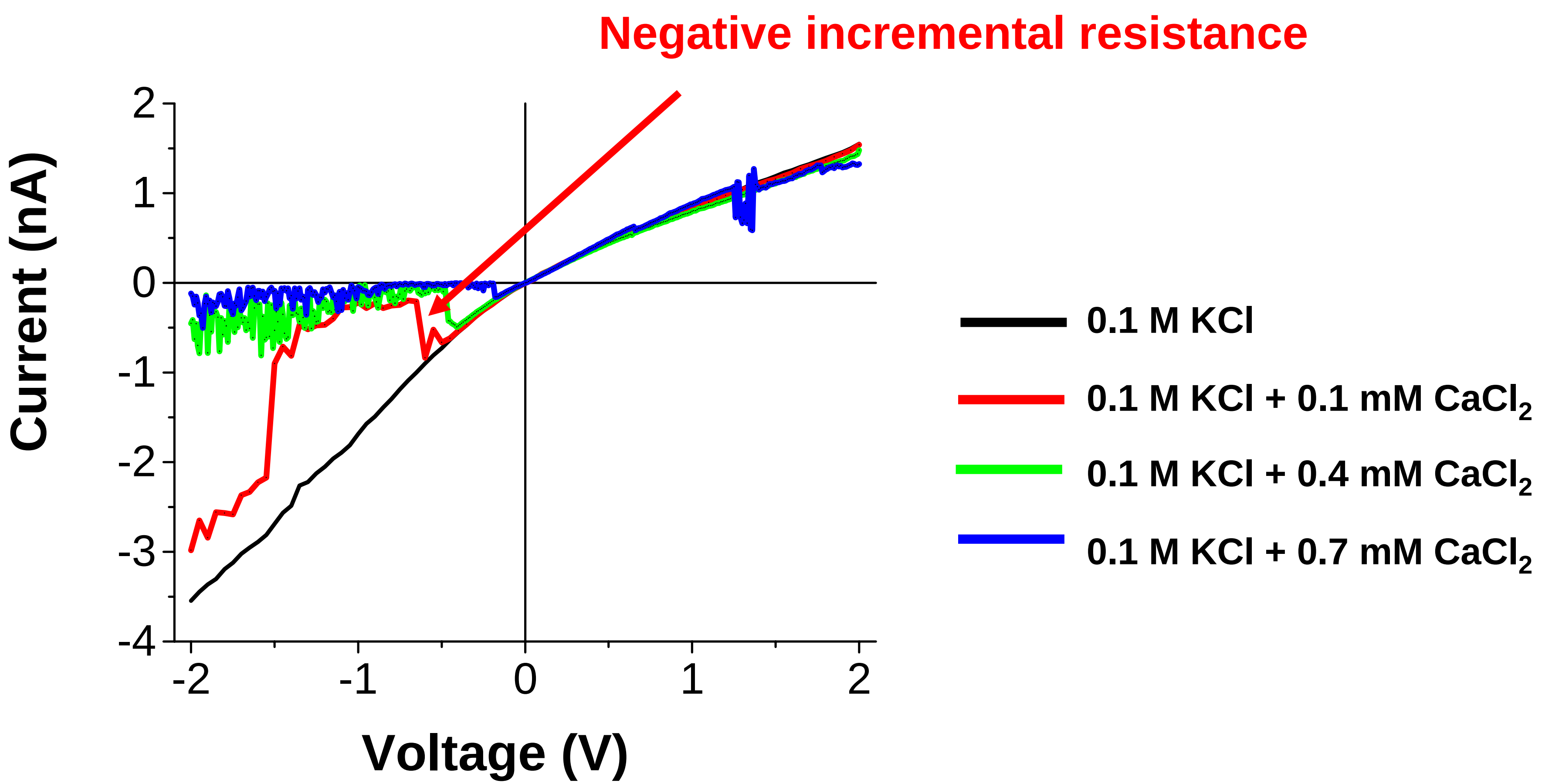
Importance of COOH in Ca Sensitivity

$K_d = 10^{-8}$ M



Concentration of Ca^2 << Concentration of K^+

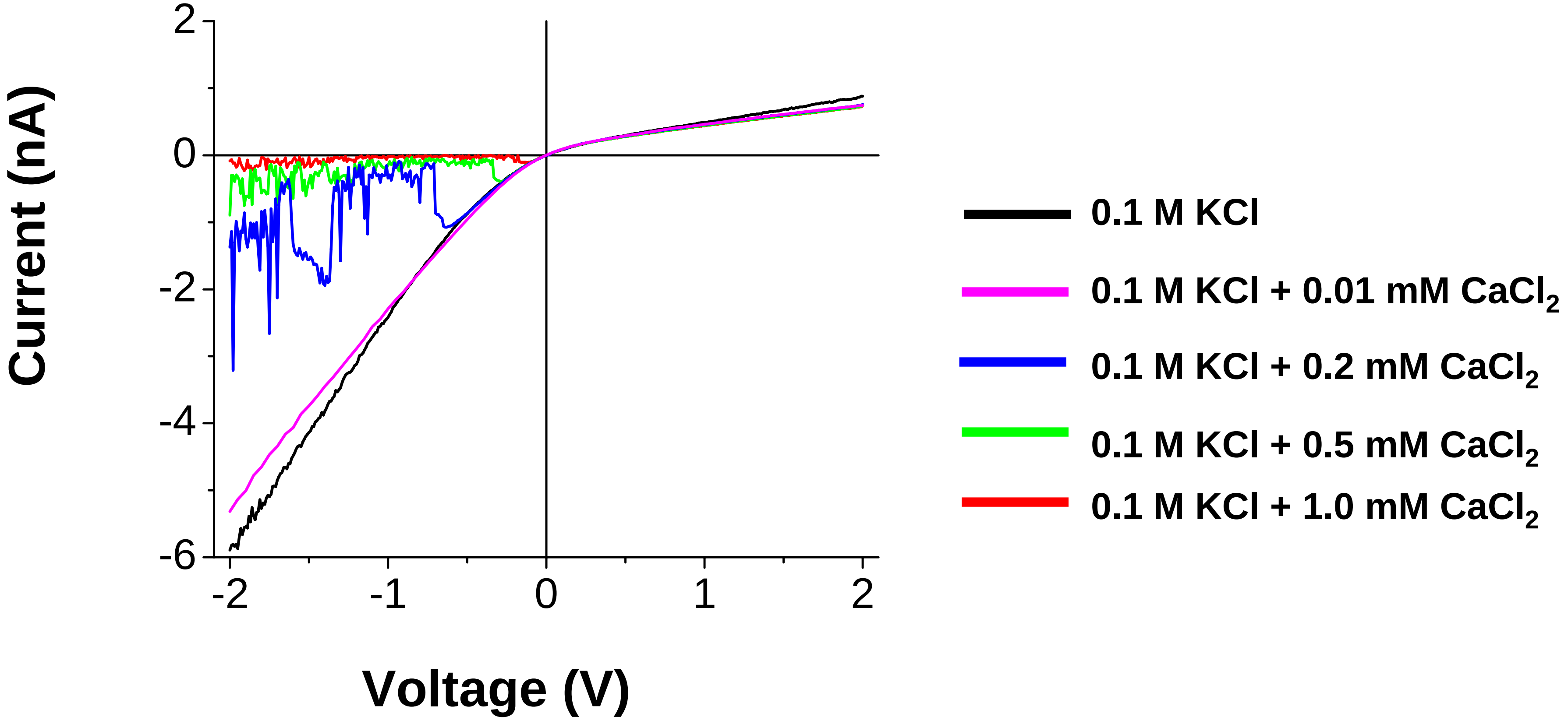
Current-Voltage Curves at Presence of Calcium Ions



Small opening: 5 nm

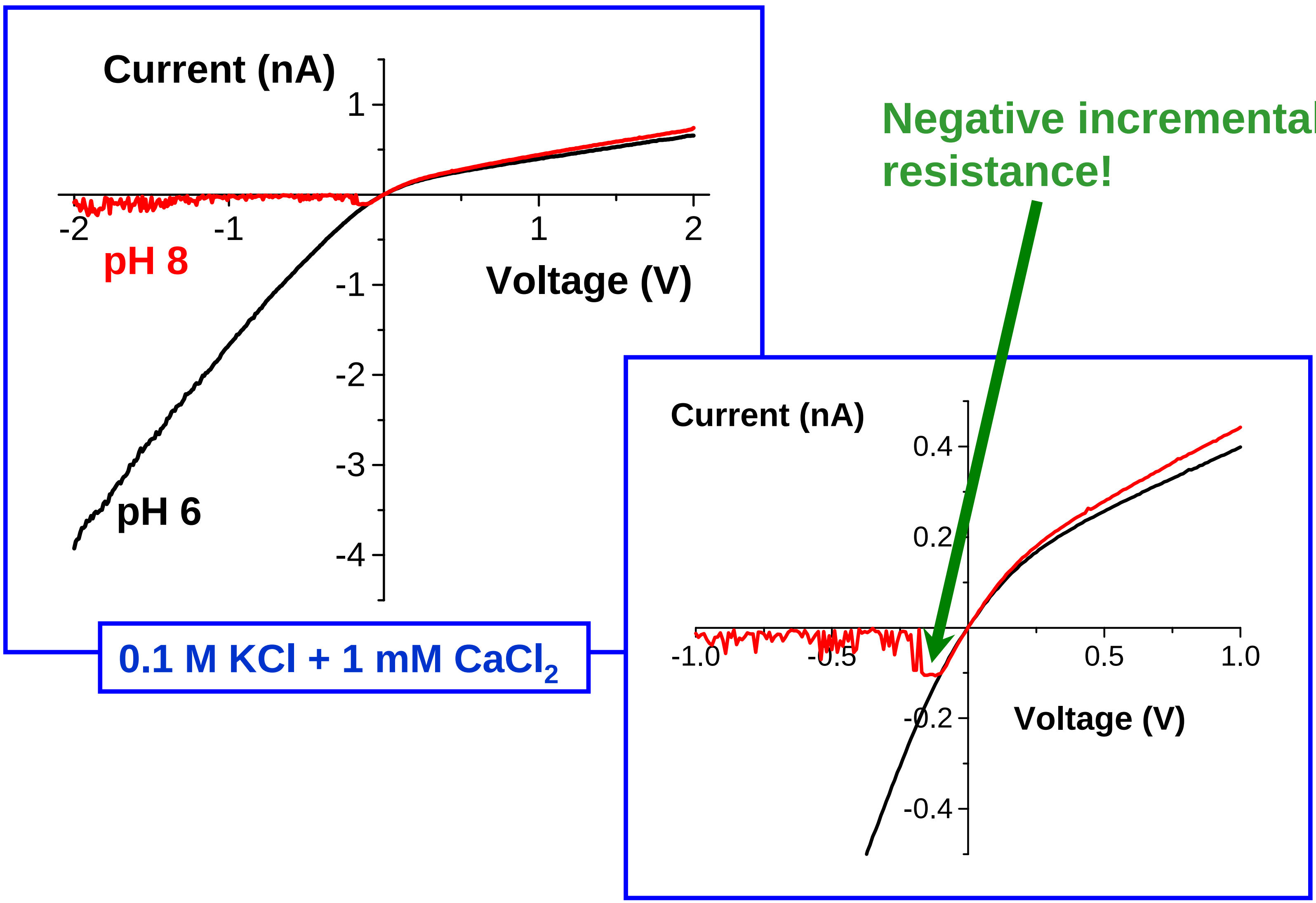
Z. Siwy, M.R. Powell, E. Kalman, R.D. Astumian, R.S. Eisenberg, *Nano Lett.* ASAP, Feb. 2, 2006.

Current-Voltage Curves at Presence of Calcium Ions

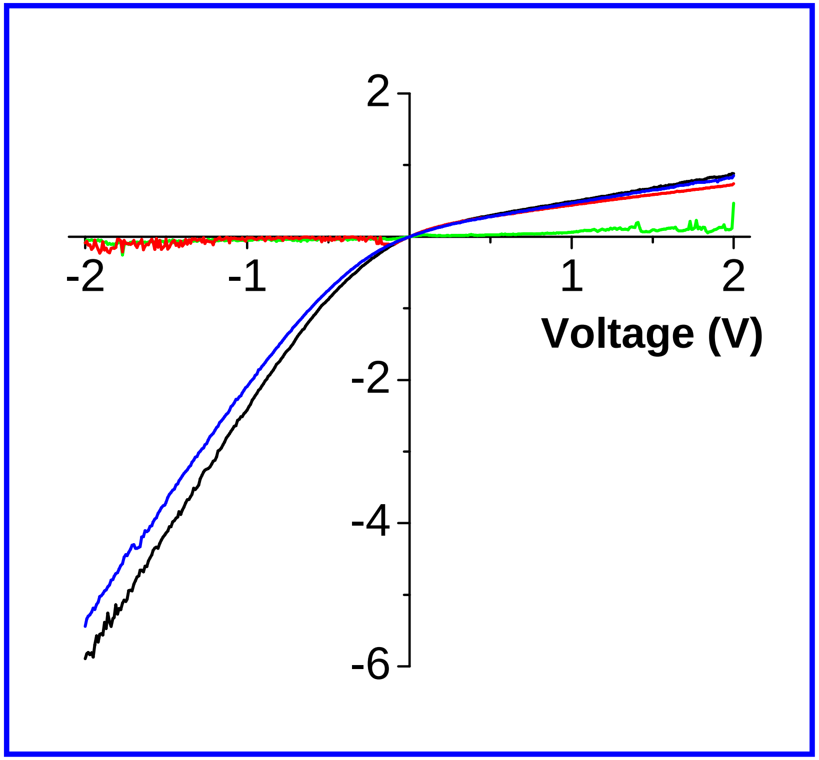


Small opening: 9 nm

Importance of Surface Charge

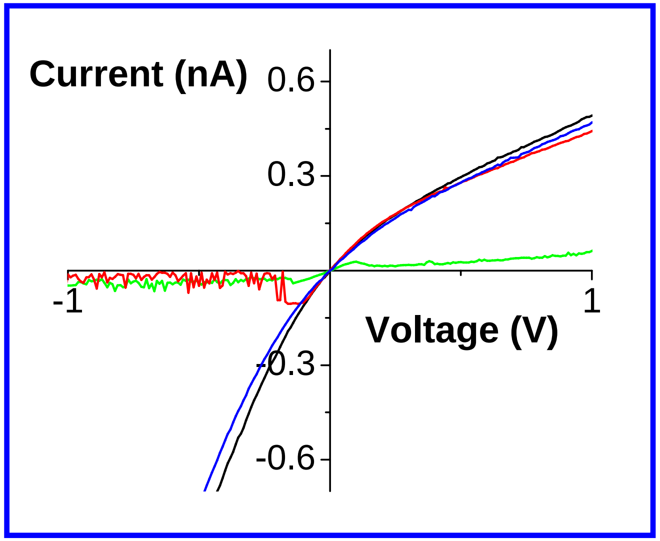


What About Other Divalent Cations?



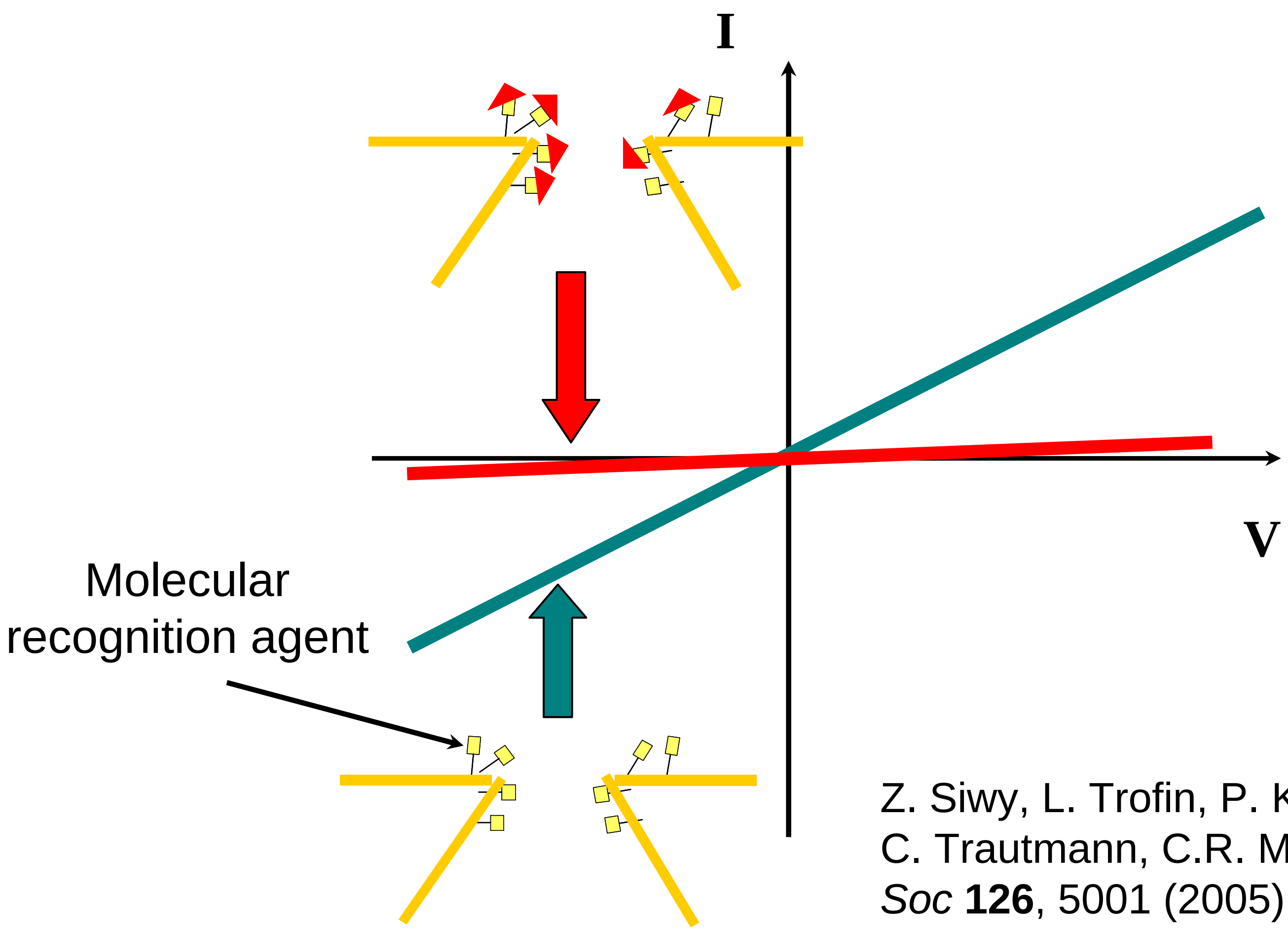
pH 8

- **0.1 M KCl**
- **0.1 M KCl + 1 mM $MgCl_2$**
- **0.1 M KCl + 1 mM $CaCl_2$**
- **0.1 M KCl + 0.1 mM $ZnSO_4$**



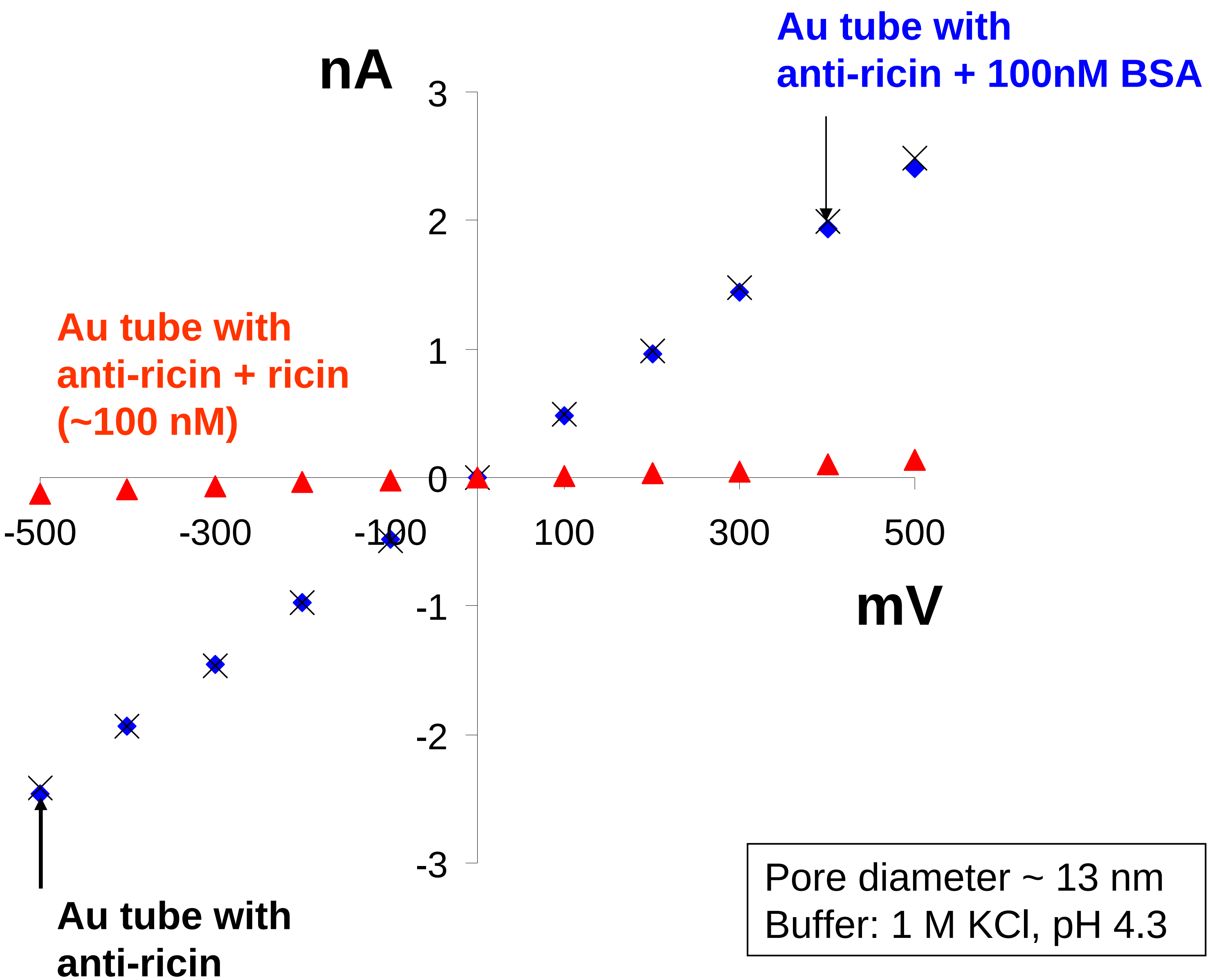
Interactions of Ions Passing Through a Nanopore with Pore Walls as a Basis for Biosensors

Detection signal: current-voltage characteristic



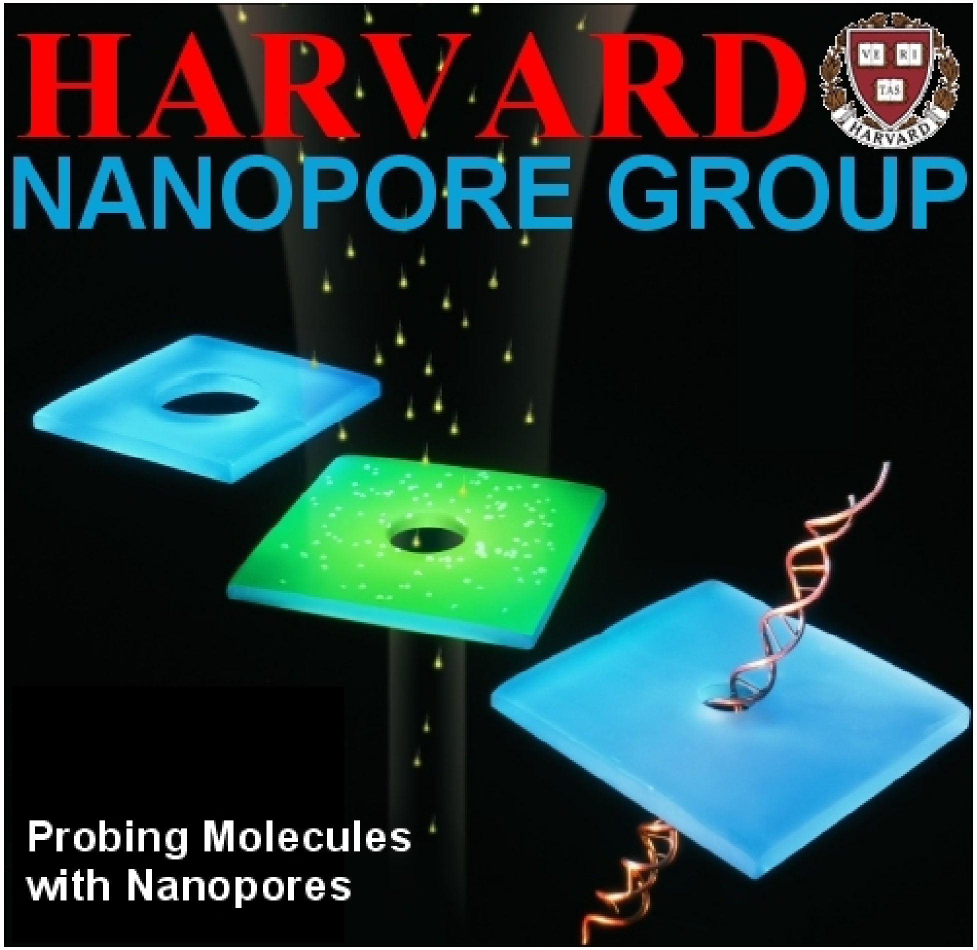
Z. Siwy, L. Trofin, P. Kohli, L.A. Baker, C. Trautmann, C.R. Martin *J. Am. Chem. Soc* **126**, 5001 (2005).

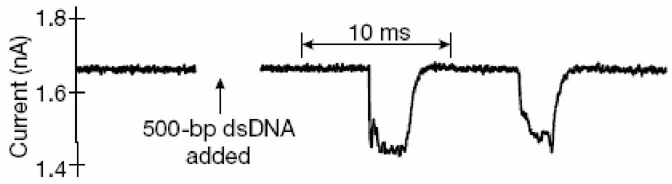
Sensing Biowarfare Ricin



Pore diameter ~ 13 nm
Buffer: 1 M KCl, pH 4.3

Detecting Single Molecules with Nanopores

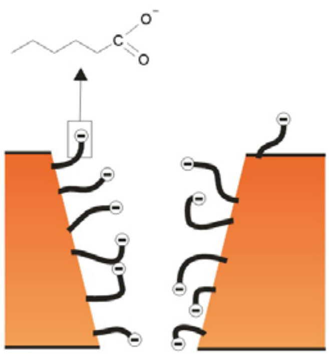


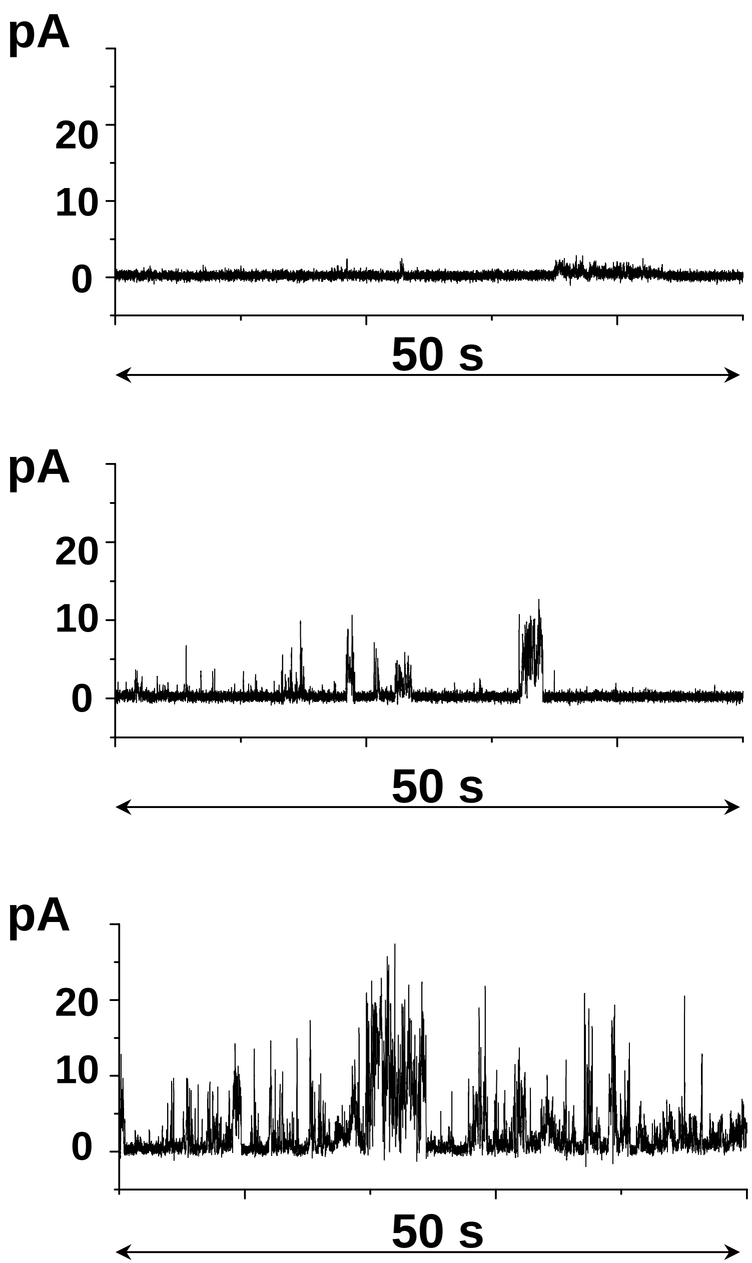


http://www.mcb.harvard.edu/branton/

J. Li, D. Stein, C. McMullan, D. Branton, M.J. Aziz, J.A. Golovchenko, Nature 412 (2001) 166.

Voltage-Dependent Fluctuations in Time





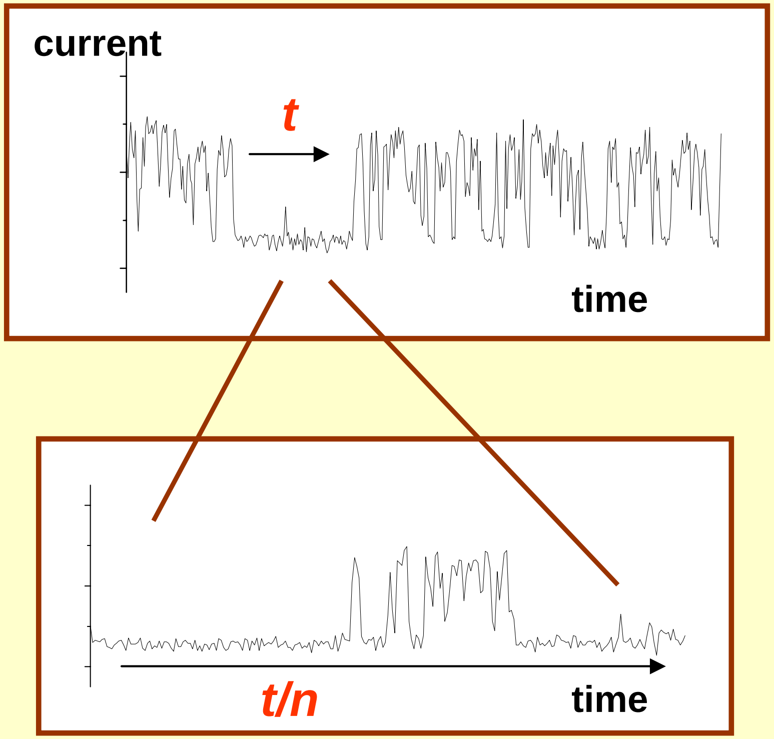
60 mV

125 mV

175 mV

Fluctuations of ion current are self-similar in time

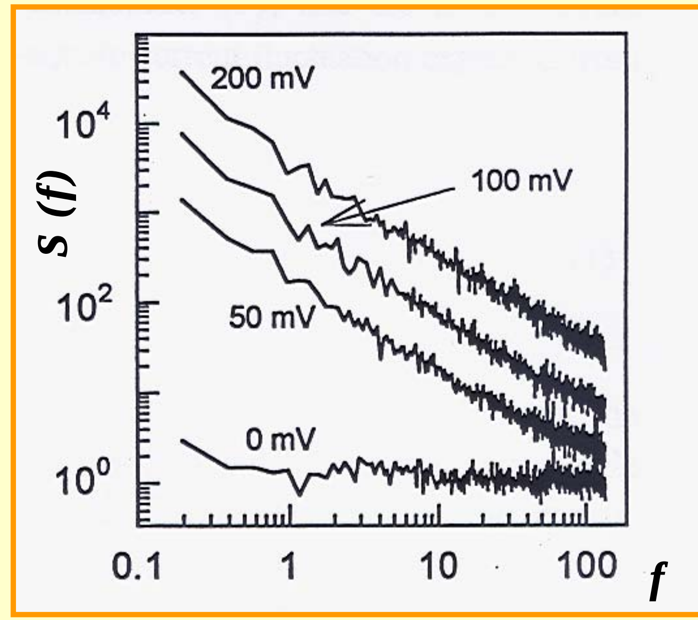
The closer we look the more we see !



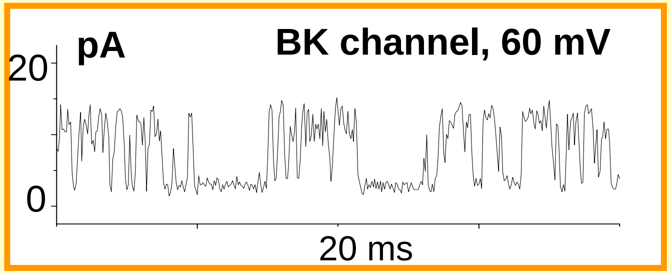
L.S. Liebovitch, *Fractals and Chaos Simplified for the Life Sciences*, Oxford University Press, New York, 1998

POWER SPECTRA

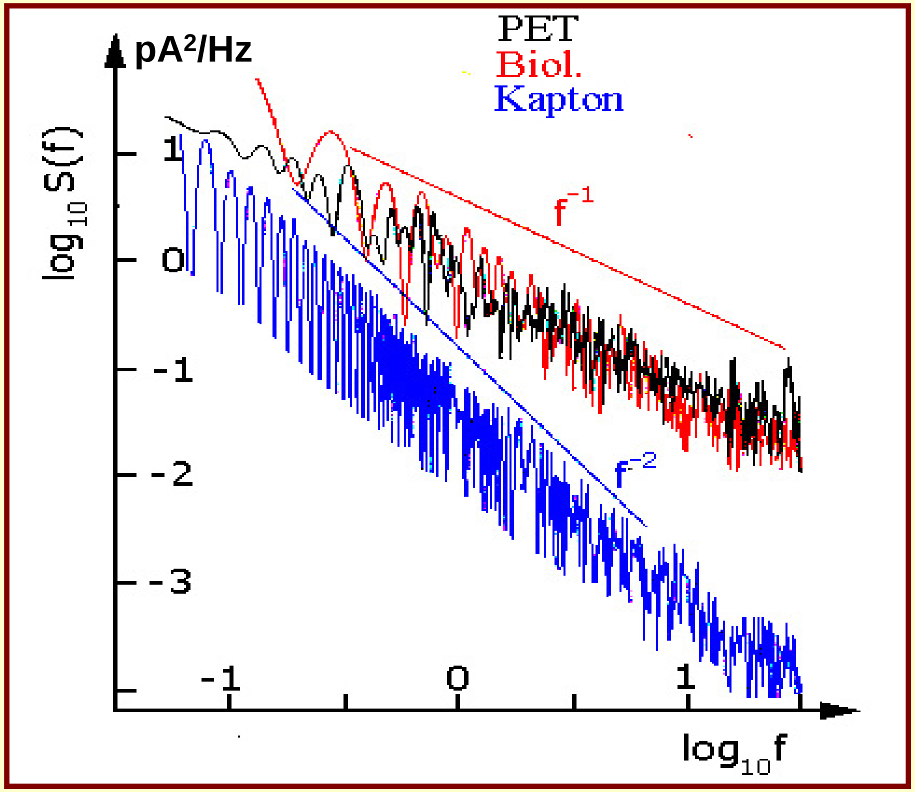
Studies of the origin of $1/f^{\alpha}$ noise in membrane channels currents



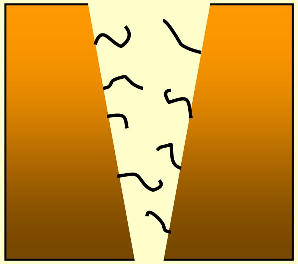
The spectral density through a single ion channel; S.M. Bezrukov, in *Proc. First Int. Conf. on Unsolved Problems of Noise, Szeged 1996*, edited by C. R. Doering, L. B. Kiss, and M. F. Schlesinger.



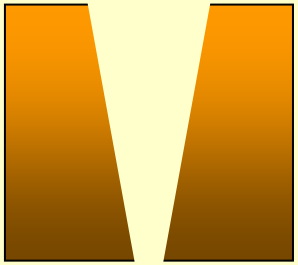
Power spectra



The 1/f noise "reflects the complex hierarchy of equilibrium protein dynamics that modulate channel conductance" (S.M. Bezrukov & M. Winterhalter, *Phys. Rev. Lett.* **85**, 202 (2000)



1/f noise !!



No 1/f noise !!

Siwy Z., Fulinski A. *Phys. Rev. Lett*. **89**, 158101 (2002): AIP Conference Proceedings Vol 665(1) pp. 273-282, May 28, (2003).

CONCLUSIONS

1. Combining Physical, Biological and Chemical Approaches enables one to answer intriguing scientific questions.
2. Experiments and modeling working together bring new insights into studied phenomena.
3. Synthetic systems give new opportunities to focus on basic physical and biological phenomena underlying a given biological function.

Acknowledgments

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- **Birgitta Schiedt, GSI, Germany**
- **Prof. Clare Yu, University of California, Irvine**
- **Prof. Bob Eisenberg, Rush Medical Center, Chicago**
- **Prof. Andrzej Fulinski, Jagellonian University, Poland**

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