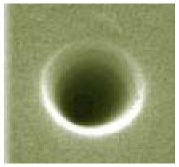
THE · UNIVERSITY · OF · CALIFORNIA · 1868

Assisted Stochastic Sensing of Analytes by a Synthetic Nanopore with Adaptor

Siwy Research Group

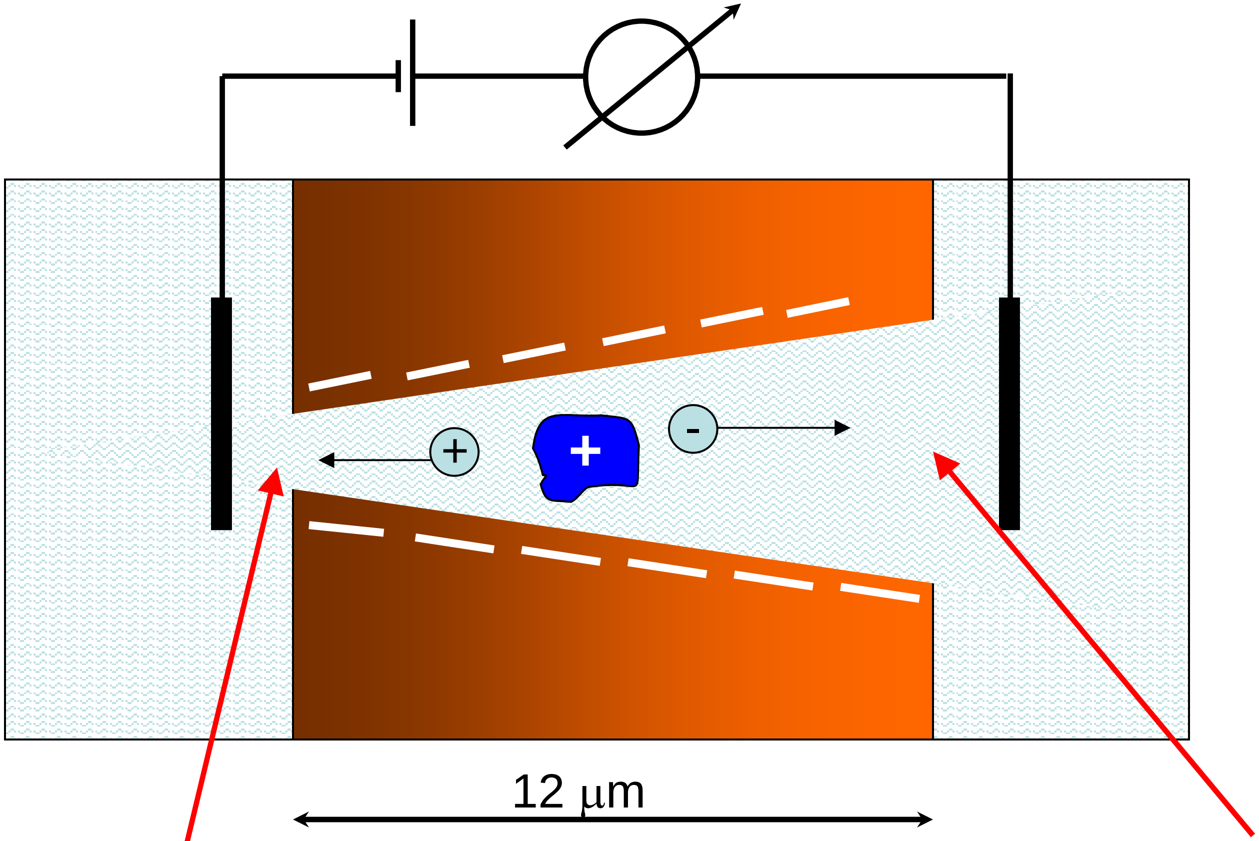
Synthetic Analogues of Biological Channels



http://www.physics.uci.edu/~zsiwy/

Main Object of Our Studies

Our main object of studies is a single nanopore in a polymer film



Several nanometers, typically 2-6 nm

~ 1 μm

We study ionic transport through single conical nanopores

SIWY GROUP

Dr. Ivan
Vlassiouk

Dr. Dragos
Constantin

Graduate students

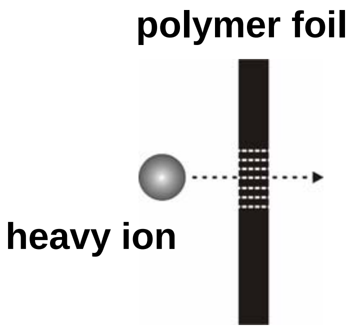
Eric Kalman

Matt Powell

Yan He

Outline

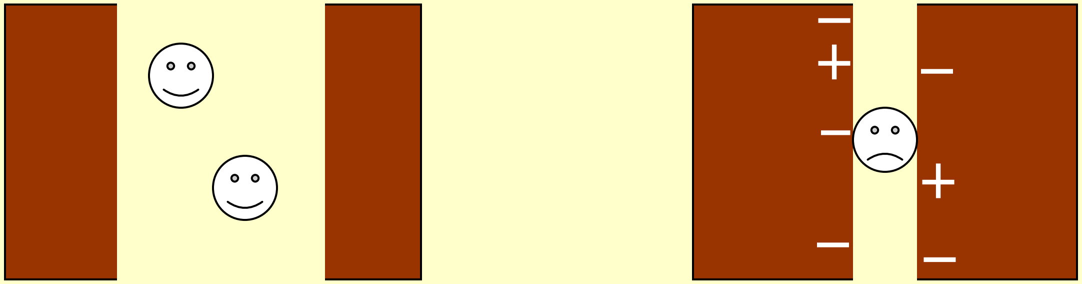
1. Motivation for studies of single nanopores in polymer films.
2. Fabrication of single nanopores by the track-etching technique.



3. Motivation for studying conically shaped nanopores.
4. Characterization of transport properties of synthetic nanopores: ion current rectification (uni- and bipolar diodes), saturation of ion current and cation selectivity.
5. Influence of divalent cations on transport properties of conical nanopores. Preparation of chemical oscillator (singing of divalent cations) and biosensor.
6. Conclusions.

Motivation

Nanometer opening of the pore promotes interactions of translocating ions with pore walls.



These interactions can lead to new transport properties than can be used for building ionic devices and sensors.

Nanopores have very small – sub-femtoliter volumes, therefore very small number of molecules can dramatically change transport properties of nanopores.

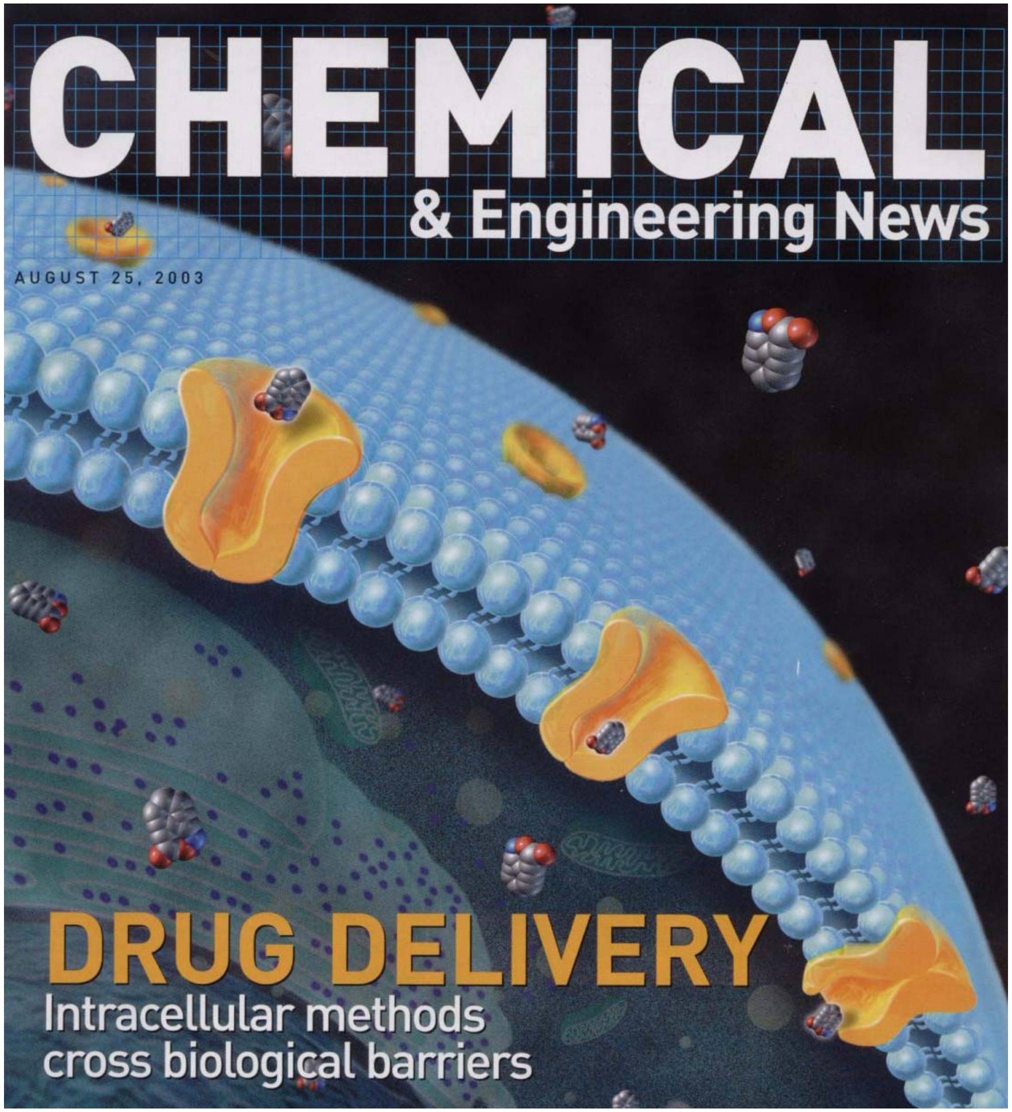
SENSORS

Lessons from Nature
Transport Proteins are Nature's Nanotubes

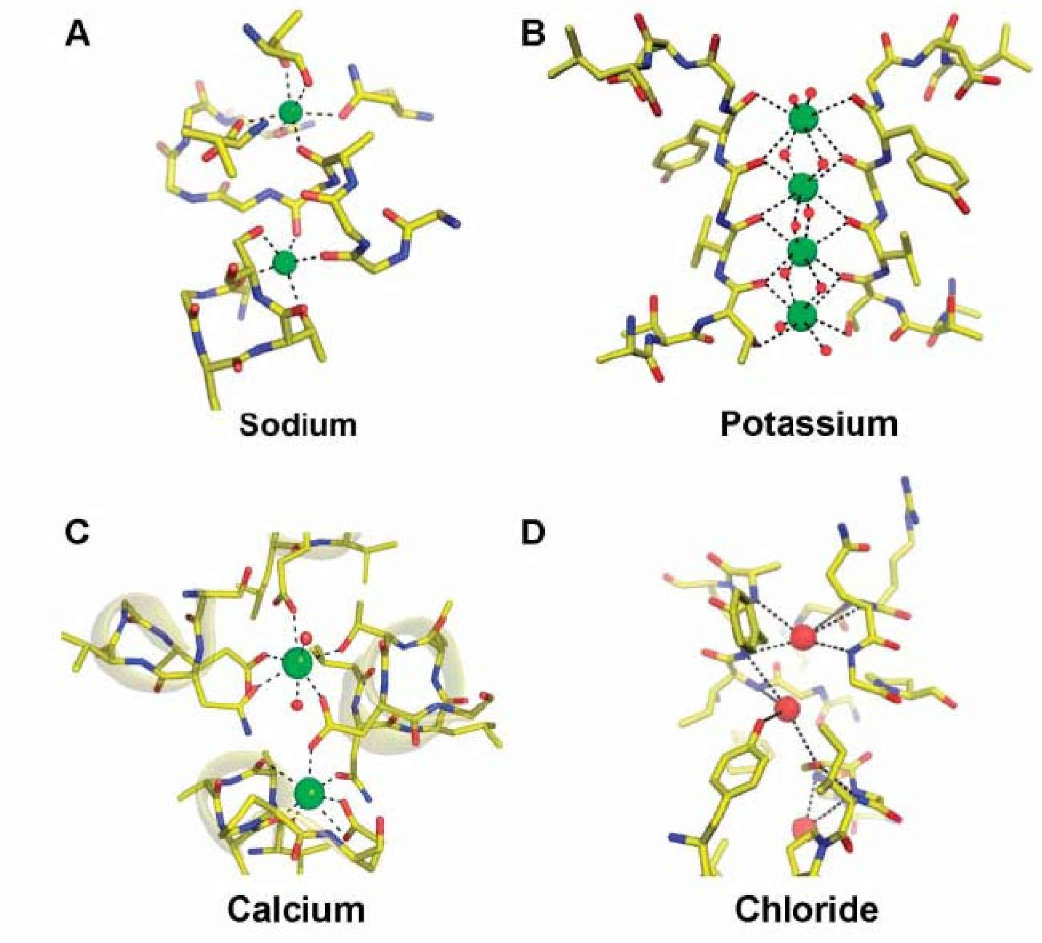
Impermeable lipid bilayer membrane

Membrane-Bound Transport Proteins

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



Interactions Between Ions and Pore Walls Make them Ion Selective

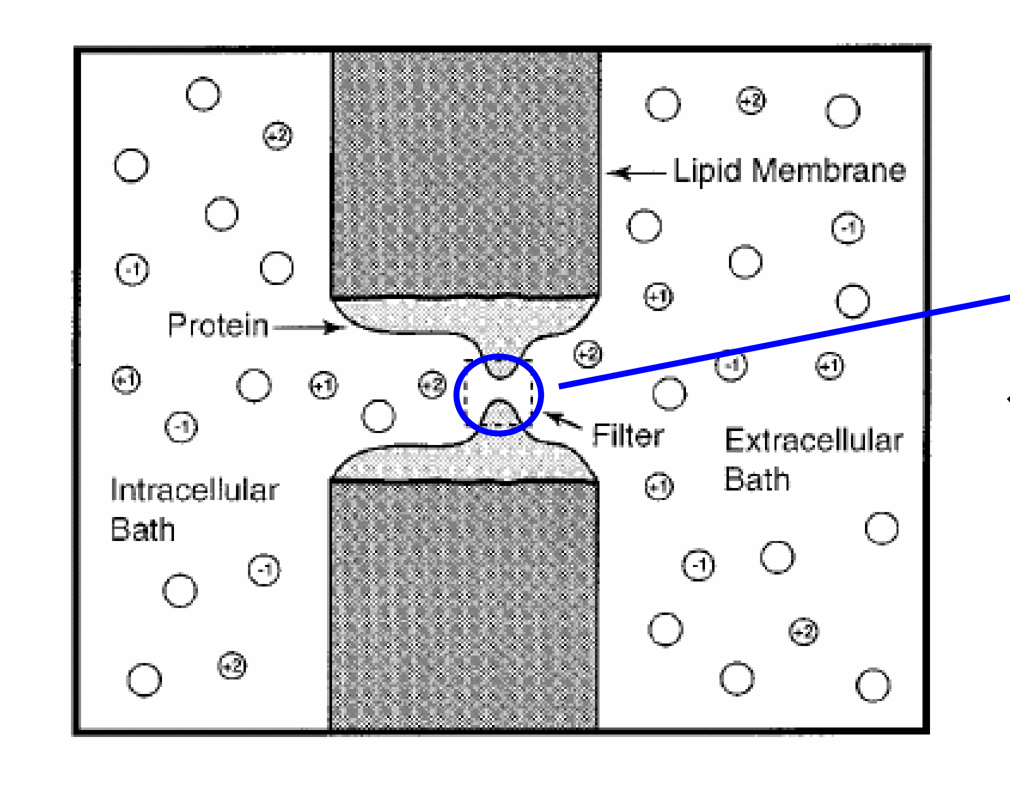


(A) Two binding sites in the LeuT Na^+-dependent pump
(B) Four K^+ binding sites in the KcsA K^+ channel
(C) Ca^{2+} binding sites in the Ca^{2+} ATPase pump
(D) Two central Cl^- binding sites in a mutant ClC Cl^-/H^+ exchanger.

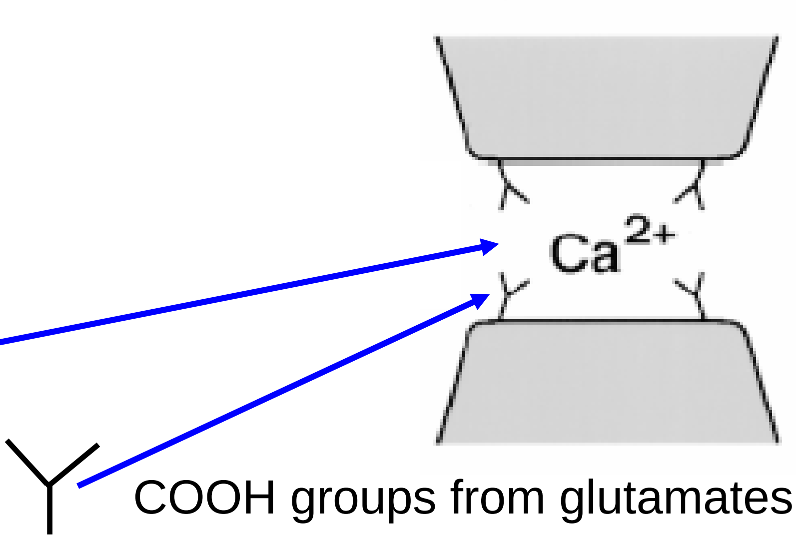
R. MacKinnon, P. Agre 2003

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

Selectivity of L-Type Calcium Channels



W. Nonner, D. Gillespie, D. Henderson, B. Eisenberg,
J. Phys. Chem. **105**, 6427 (2001);



COOH groups from glutamates

E.W. McCleskey, *J. Gen. Physiol.*
113, 765 (1999)

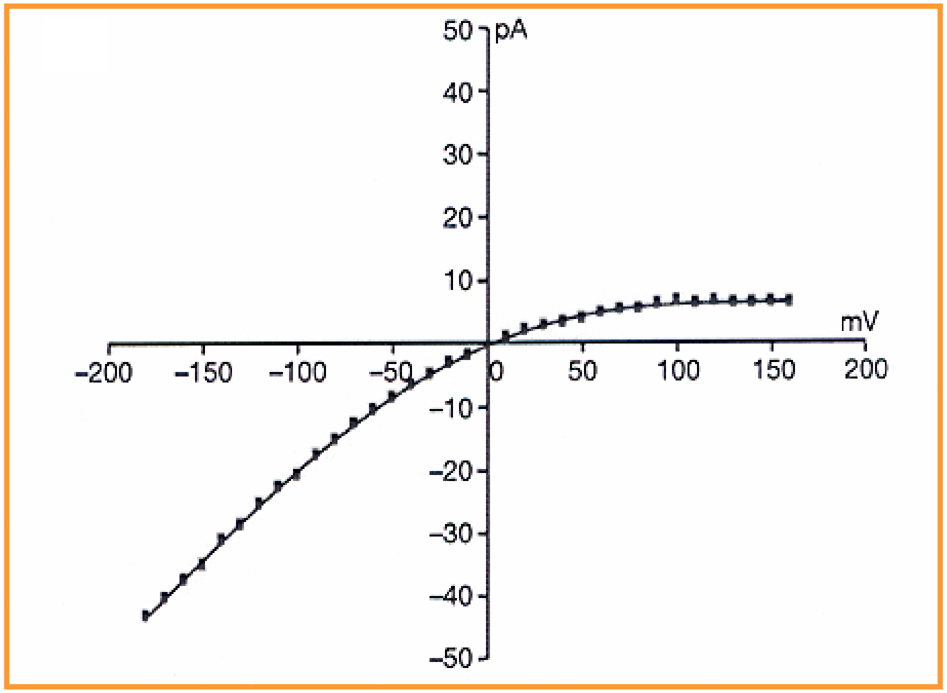
In extra- and intracellular medium:

$$[Ca^{2+}] << [Na^{+}]$$

Ca^{2+} and Na^{+} have basically the same diameter.

Interactions Between Ions and Pore Walls Make them Better Devices

Another Reason Why Biochannels are “Smart Holes”: Ion Channels are Responsive!!



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

Many biological channels have transport characteristics similar to diodes and transistors!

Fabrication of Nanopores With Controlled Geometry and Pore Chemistry

Creating Ionic Devices Working Similar to Biochannels – Regulating Transport of Ions and Charged Molecules

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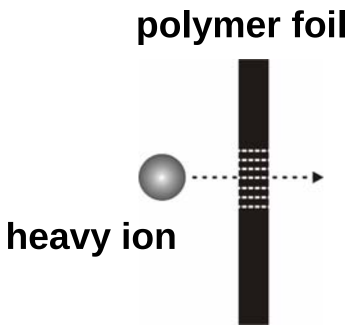

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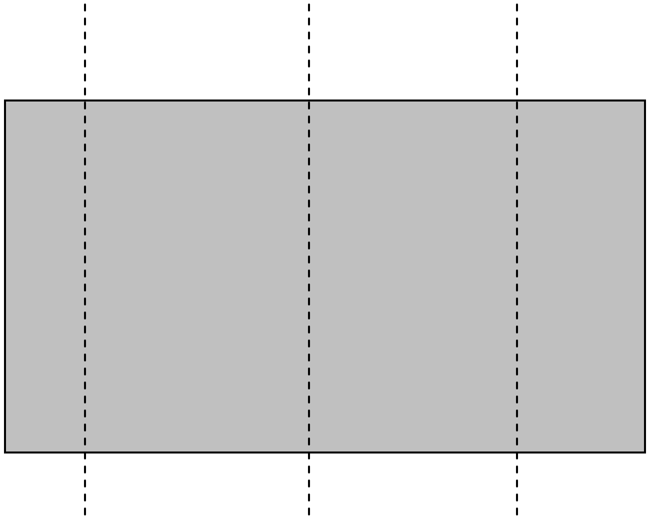
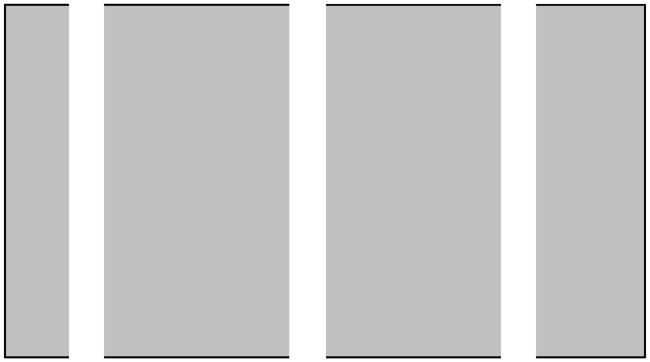
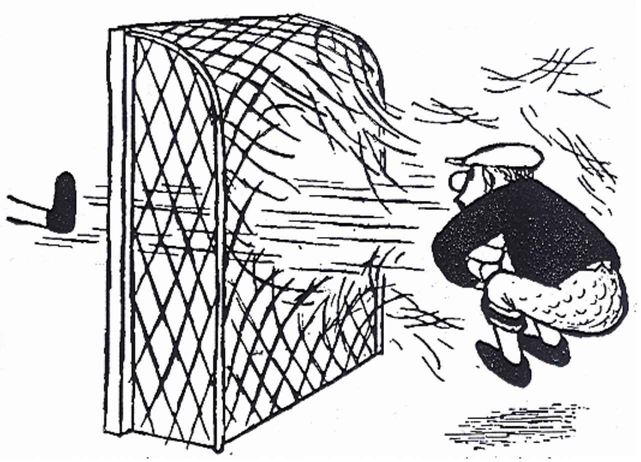
Heavy Ions as a Working Tool

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



Chemical etching



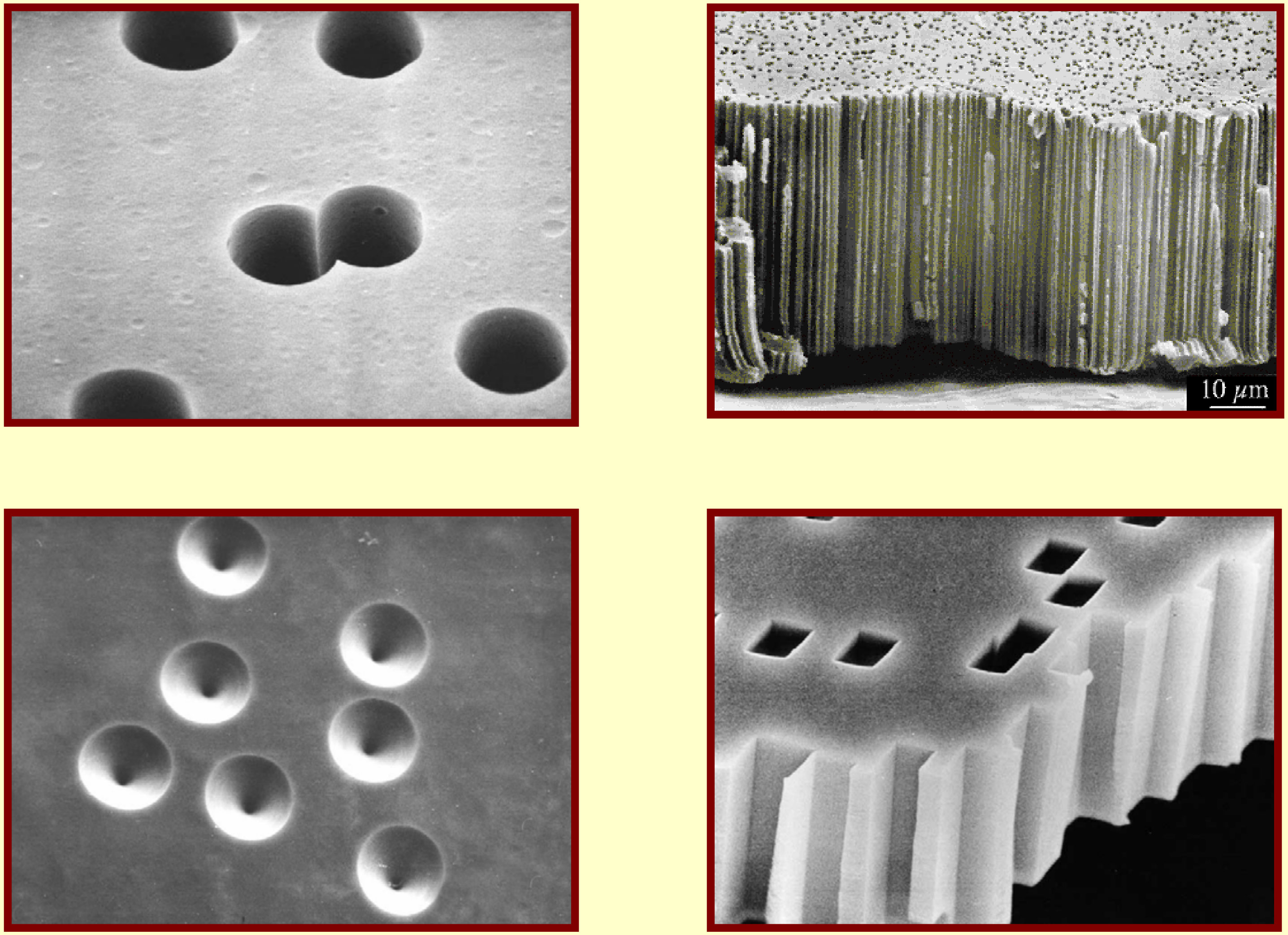


E. Loriot

Linear accelerator
UNILAC, GSI
Darmstadt, Germany

1 ion → 1 latent track → 1 pore !

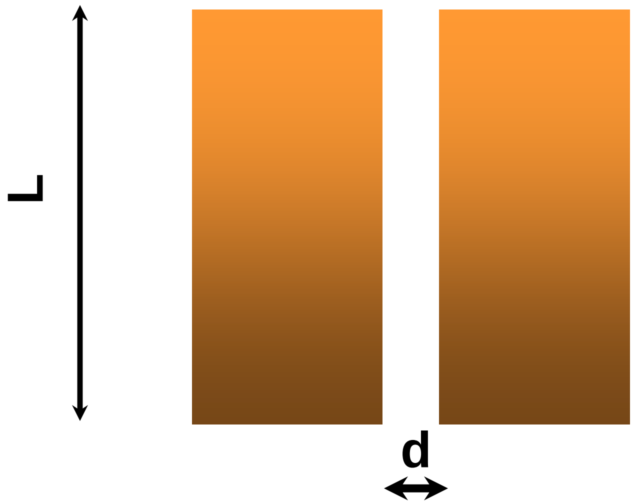
A Short Glimpse at the “Product" of Track Etching Technique



http://www. Iontracktechnology.de

Why Do We Want to Work with Asymmetric Pores?

Cylindrical pore



Tapered cone

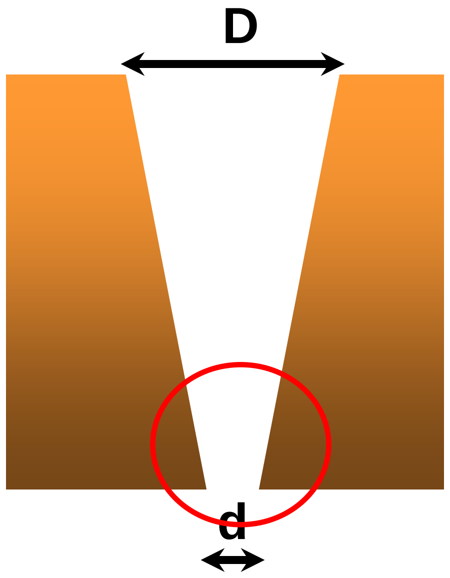
$$R_1 = \frac{4L}{\kappa\pi d^2} \gg R_2 = \frac{4L}{\kappa\pi dD}$$

Example for 1 V, 1 M KCl, L = 10μm

d=1 nm results in current of **3.9 pA.**

d=1 nm, D=2 μm, results in current of **~740 pA**.

Focusing of Resistance in a Conical Nanopore



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

D = 1 μm

d = 3 nm

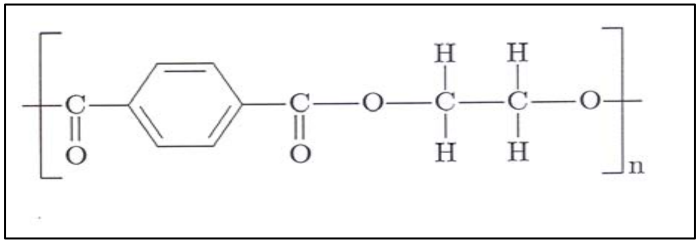
L = 12 μm

50% of total resistance is focused over 36 nm

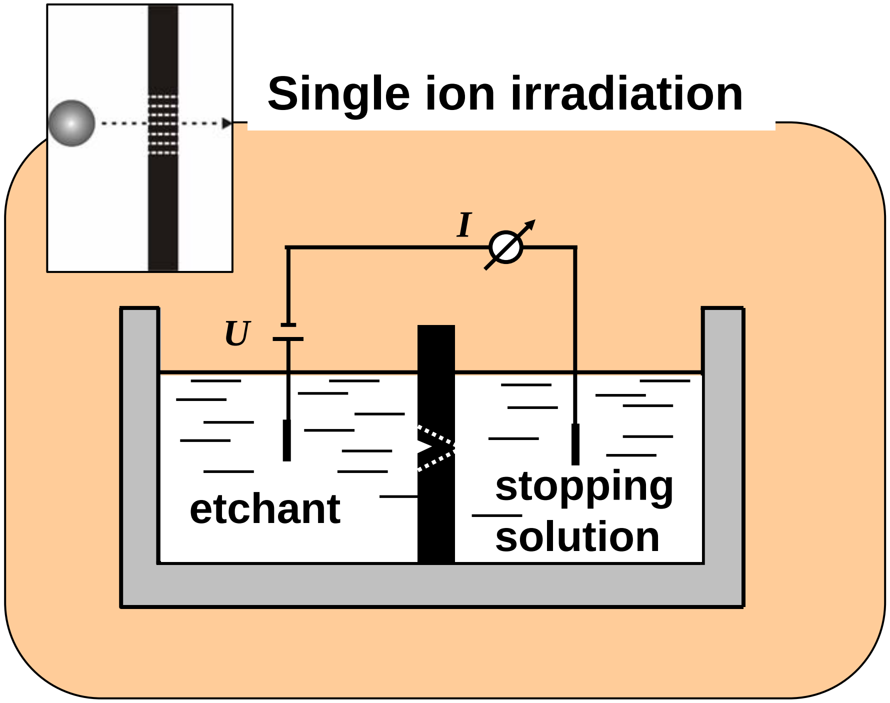
80% of total resistance is focused over 140 nm

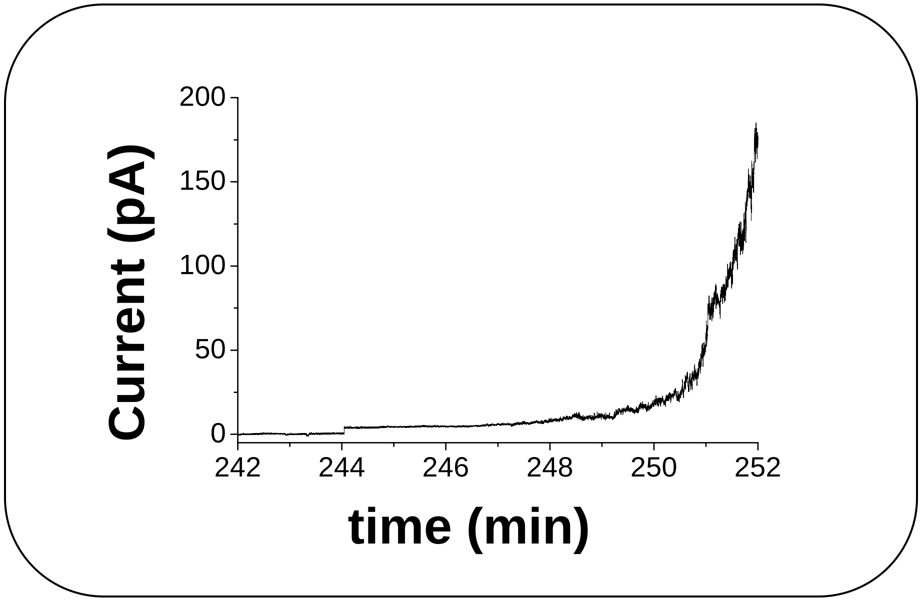
Volume of this part of the pore is sub-femtoliter!!

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



Polyethylene Terephthalate (PET)
(RN 12 Hoechst)

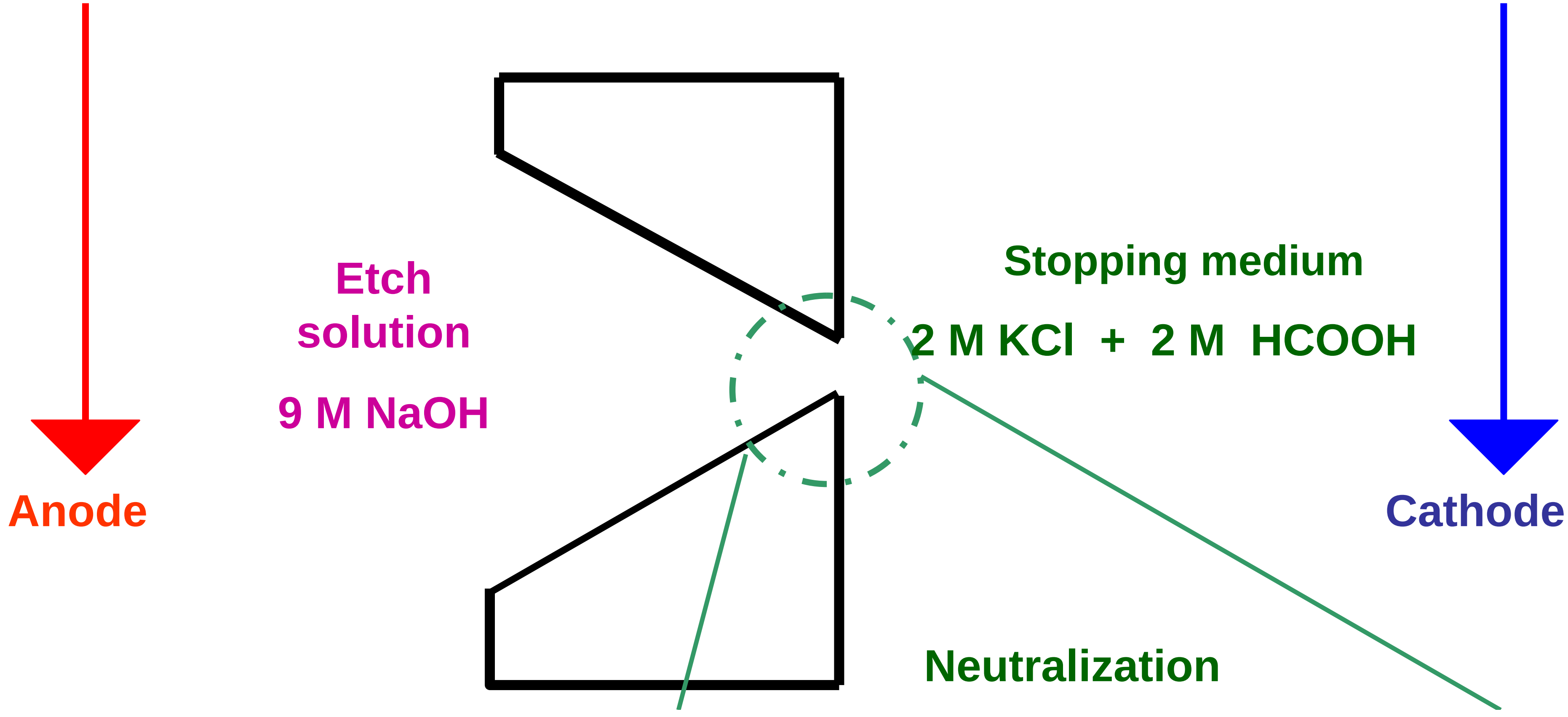




Z. Siwy et al. *Nucl. Instr. Meth. B* **208**, 143-148 (2003); *Applied Physics A* **76**, 781-785;
Surface Science **532-535**, 1061-1066 (2003).

Chemical & Electro-Stopping Technique to Prepare Conical Pores

For polyethylene terephthalate



Etch solution

9 M NaOH

Stopping medium

2 M KCl + 2 M HCOOH

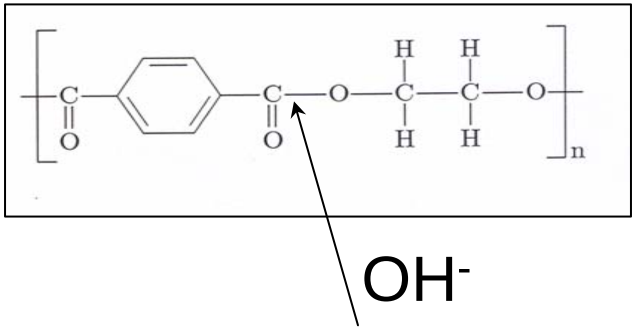
Anode

Cathode

Neutralization

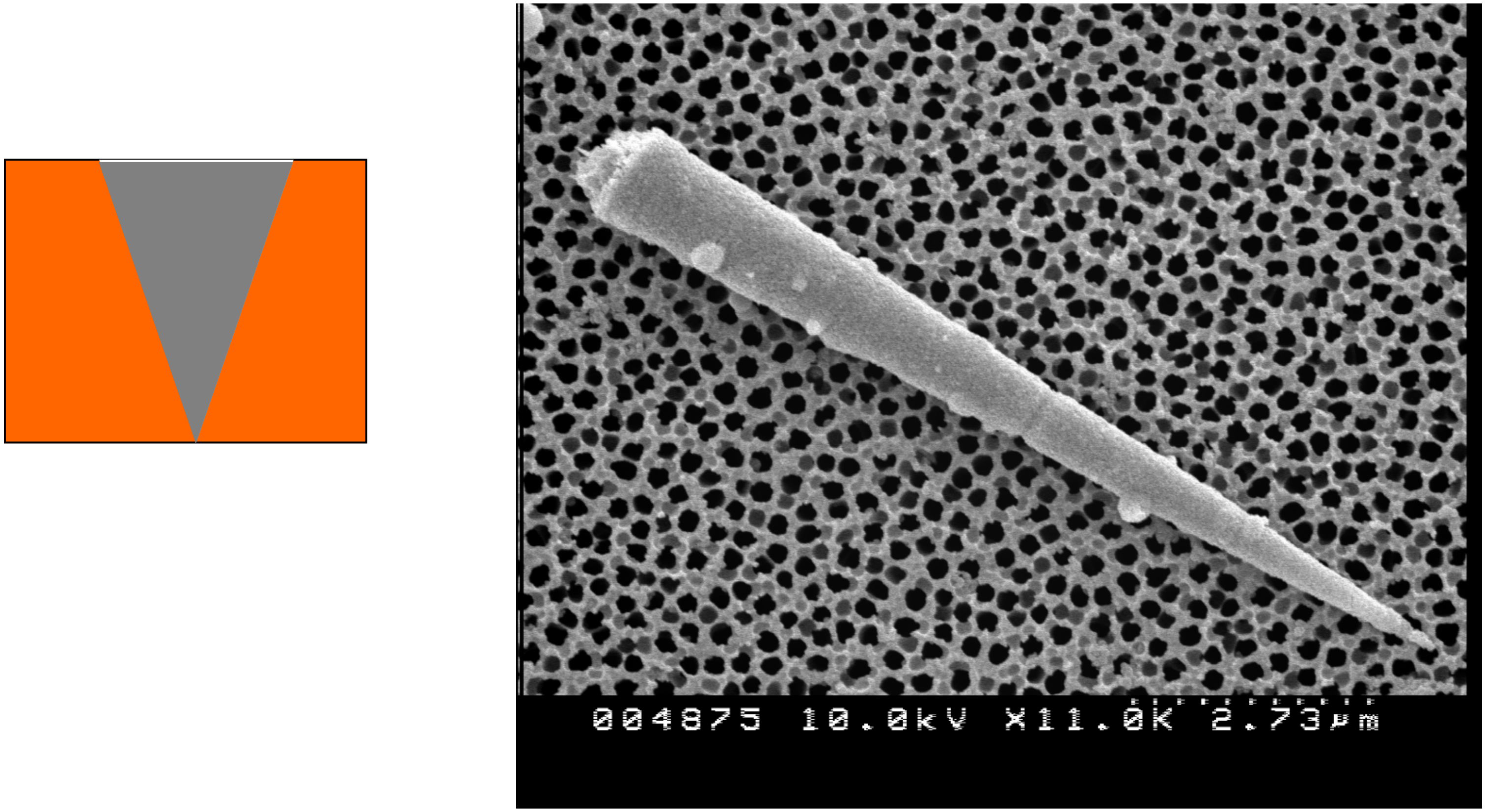
$HCOOH + OH^- \rightarrow HCOO^- + H_2O$

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



The surface density of COOH groups was estimated to be ~ 1.0 per nm^2

Gold Replica of a Single Conical Pore



P. Scopece et al. *Nanotechnology* **17**, 3951 (2006)

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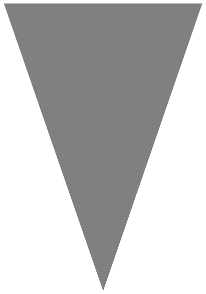

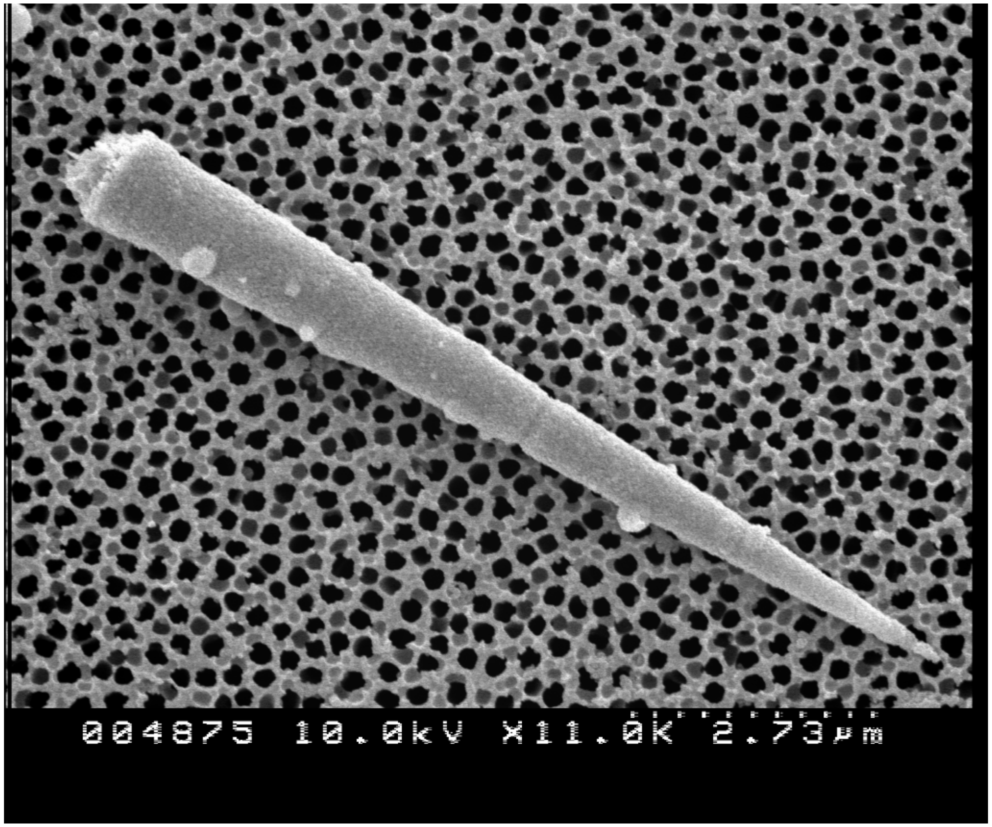

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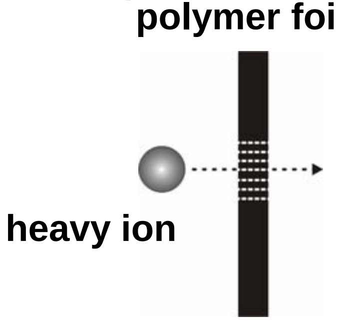

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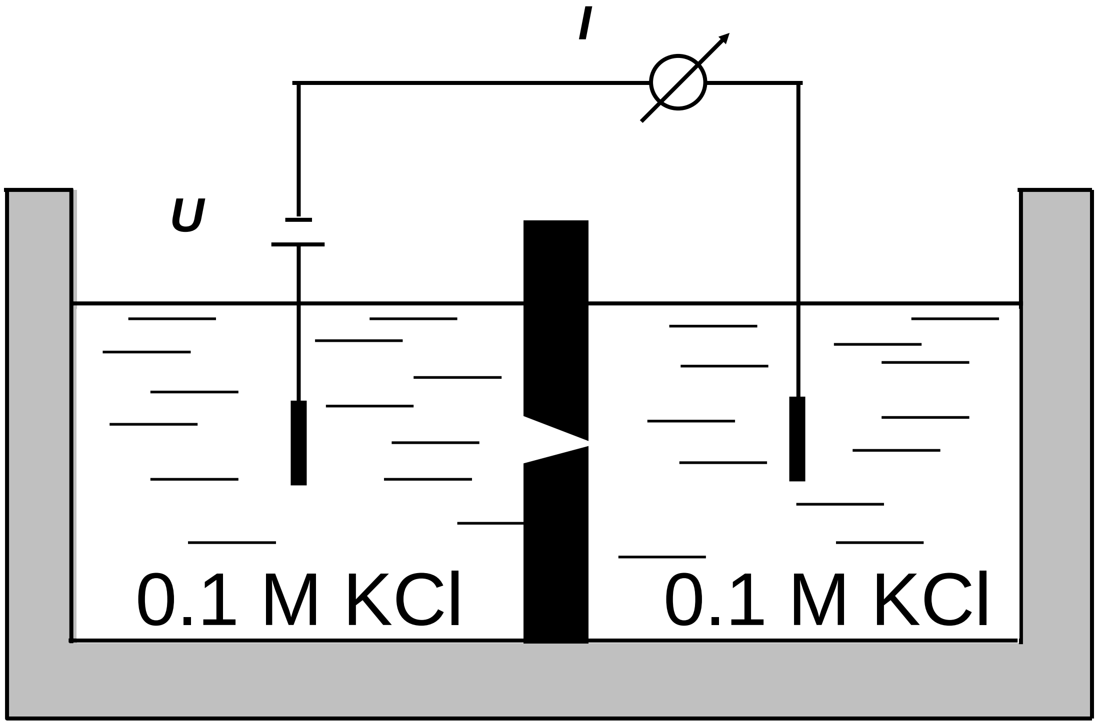
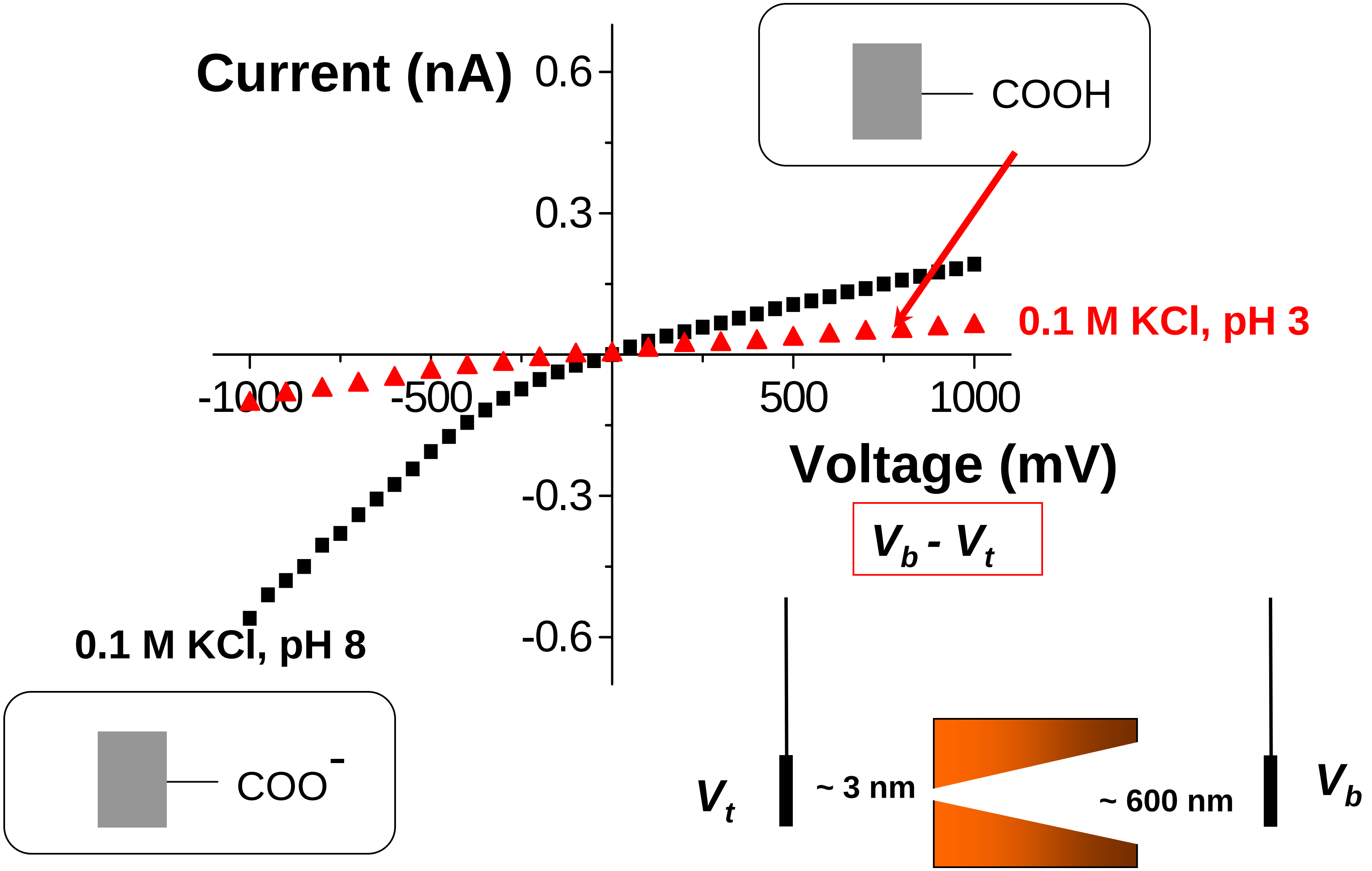
Conductivity Cell Used for Recording Current-Voltage Curves



Our pores exhibit a number of properties that are unique to NANOpores

1.

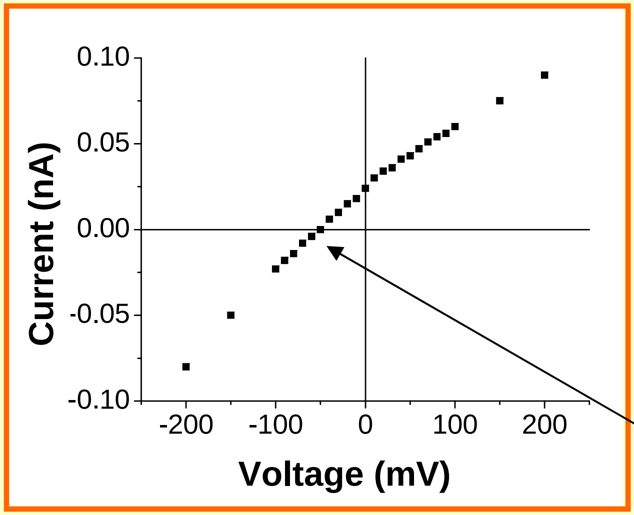
Single Conical Nanopores Rectify Ion Current

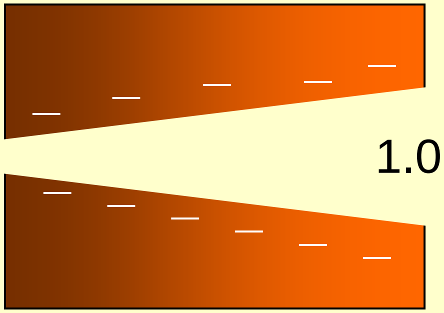


Z. Siwy et al. *Europhys. Lett*. **60**, 349 (2002); Z. Siwy et al. *Surface Science* **532-535**, 1061 (2003)

2.

Synthetic Nanopores Are Ion Selective

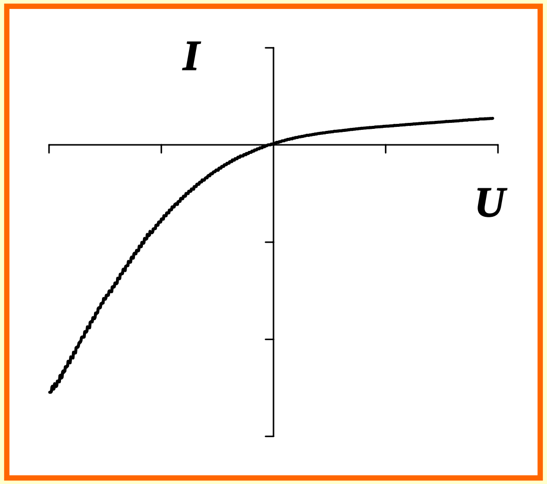


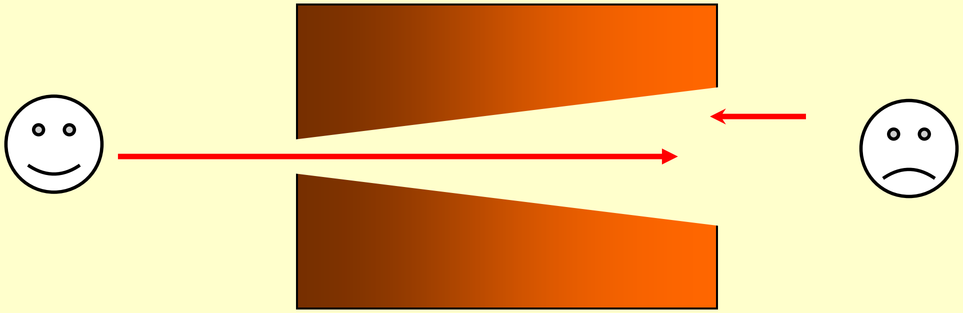


0.1 M KCl

1.0 M KCl

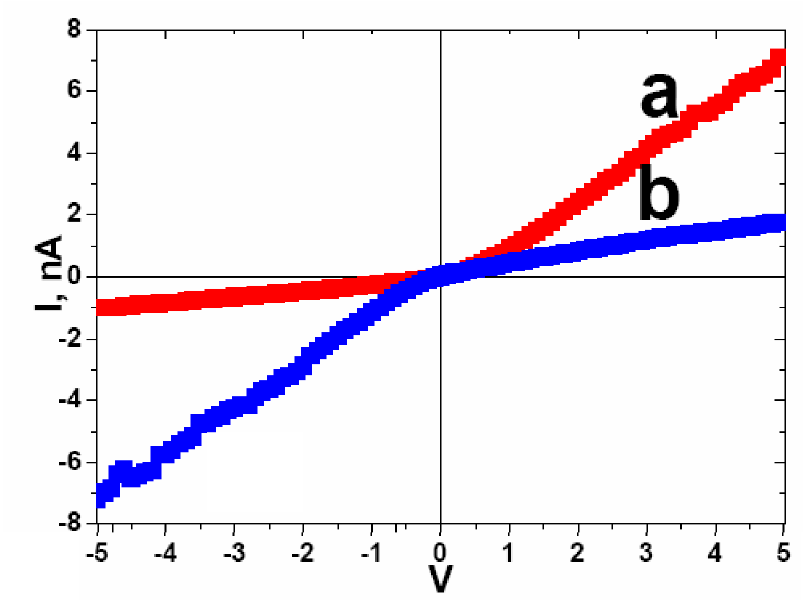
K^+ selectivity

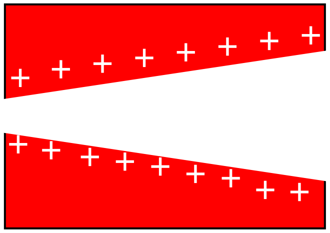




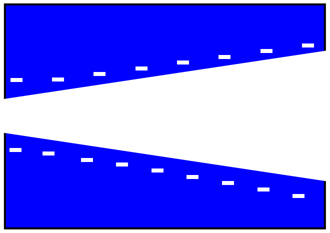
Z. Siwy, A Fulinski, *Phys. Rev. Lett*. **89**, 198103 (2002); *Am. J. Phys*. **72**, 567 (2004)

Reversing the Direction of Ion Current Rectification





Surface covered with amino NH_3^+ groups

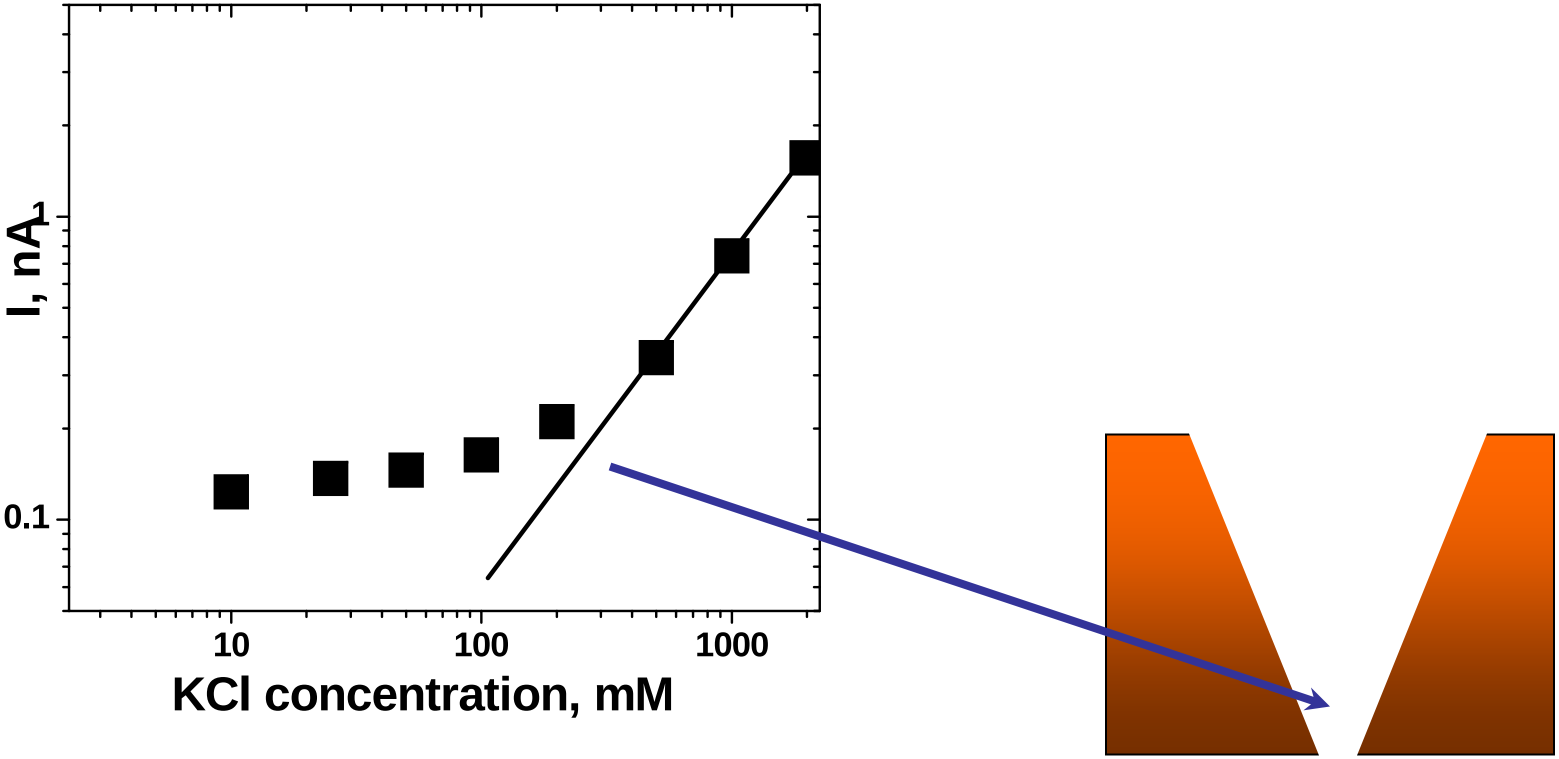


Surface covered with carboxyls COO^- groups

I. Vlassiouk, Z. Siwy, *Nano Lett.* **7**, 552-556 (2007)**;** Z. Siwy, E. Heins, C.C. Harrell, P. Kohli, C.R. Martin. *J. Am. Chem. Soc.* **126**, 10850-10851 (2004); S. Umehara, N. Pourmand, C.D. Webb, R.W. Davis, K. Yasuda, M. Karhanek. *Nano Lett.* **6**, 2486-2492 (2006).

3. Saturation of Ion Currents at Low Concentrations

Currents at 1000 mV recorded for a nanopore with tip 3 nm

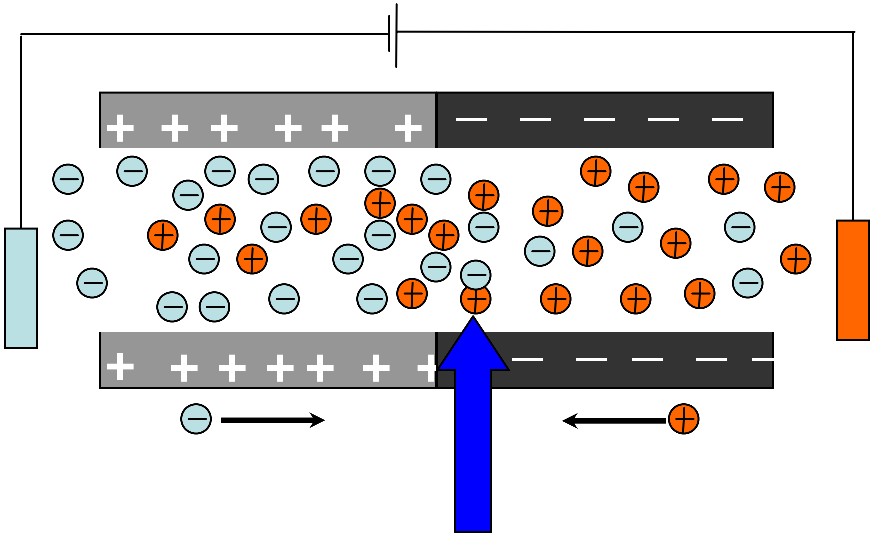


The opening of the pore is comparable to Debye length

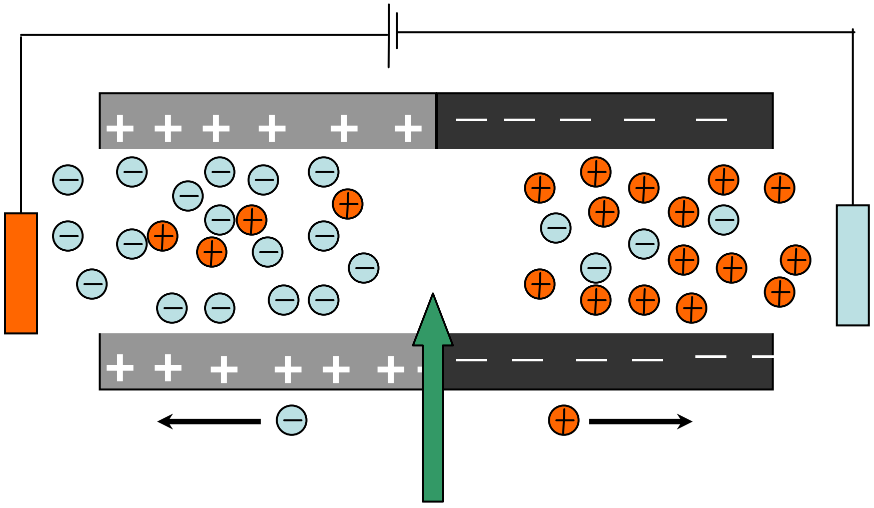
D. Stein, M. Kruithof, C. Dekker, *Phys. Rev. Lett.*, **93**, 035901 (2004)

4.

Sensitivity to Charge Patterns in the Nanopore



Higher concentration than
in the bulk

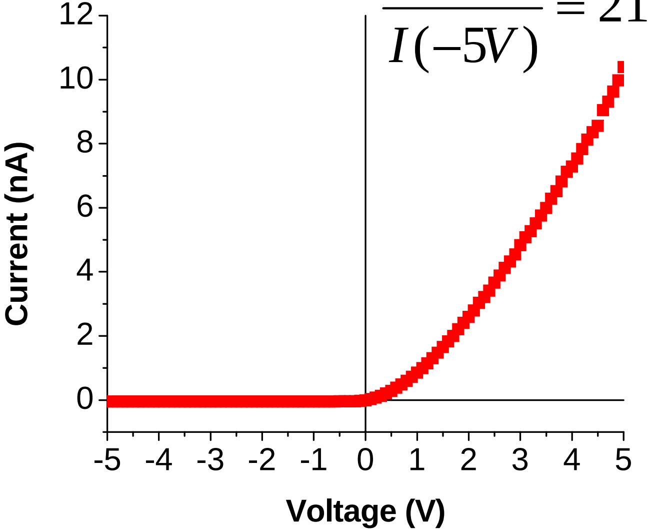


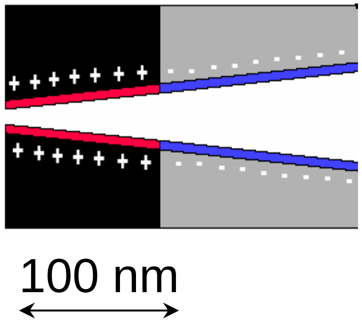
Depletion
zone

H. Daiguji, Y. Oka, K. Shirono, *Nano Lett.* **5**, 2274 (2005);
I. Vlassiouk, Z. Siwy, *Nano Lett.* **7**, 552-556 (2007)

4. Sensitivity to Charge Patterns in the Nanopore

$$\frac{I(+5V)}{I(-5V)} = 217$$





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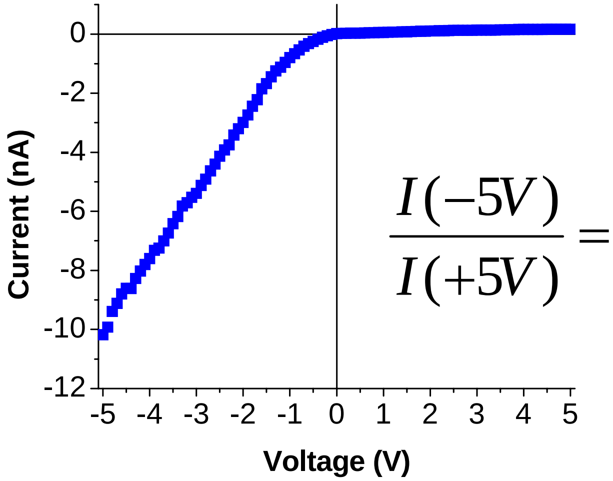
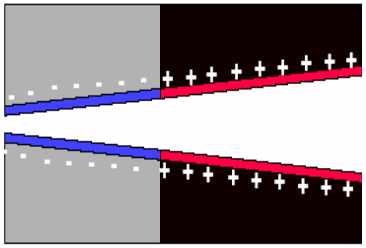
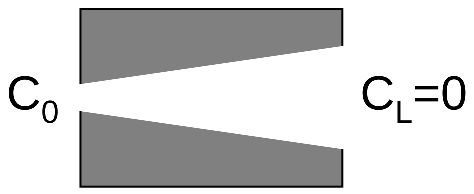


$$\frac{I(-5V)}{I(+5V)} = 61$$

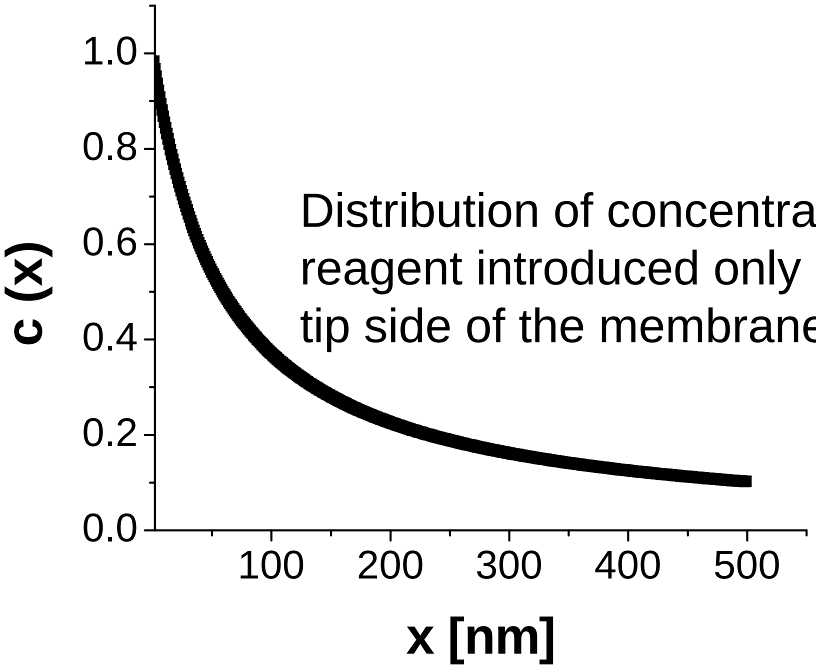


I. Vlassiouk, Z. Siwy, *Nano Lett.* **7**, 552-556 (2007)

Steady-State Solution of Diffusion Problem



Targeted modification of the tip



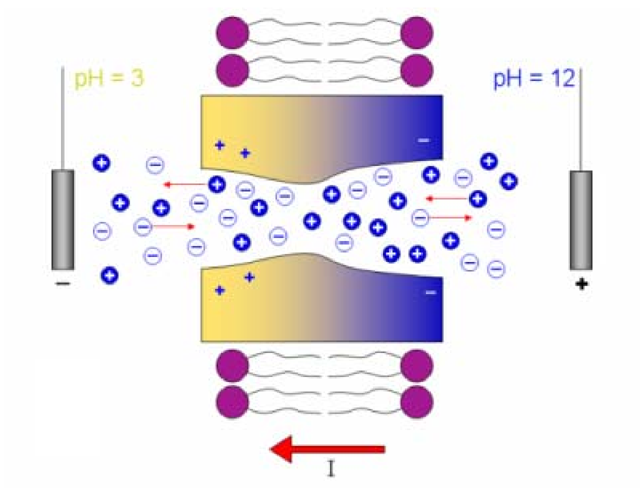
Distribution of concentration of a reagent introduced only on the tip side of the membrane

Solution of diffusion equation with reagent on tip side:

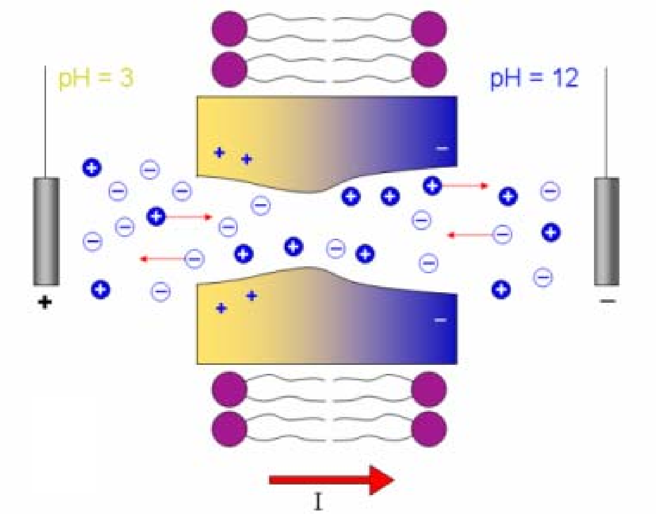
$c(actg\alpha,t)=c_0$, $c(L,t)=0$, for $D >> d$

$$\boxed{c(x)=c_0\frac{d}{D}\left(\frac{L}{x}-1\right)_{d/2ctg\alpha\le x\le L}}$$

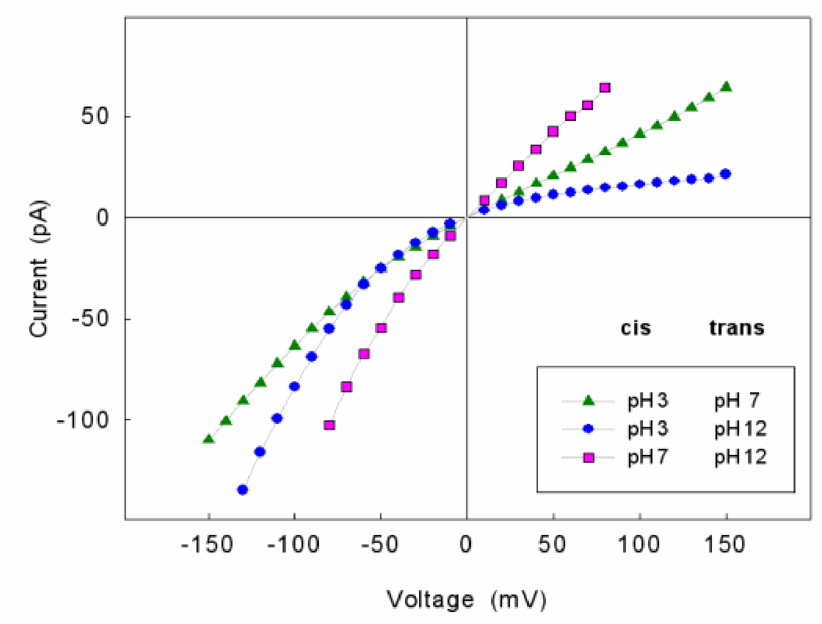
Sensitivity to Charge Patterns in OmpF



Enrichment of ions



Depletion of ions



A. Alcaraz, P. Ramirez, E. Garcia-Gimenez, M.L. Lopez, A. Andrio, V.M. Aguilella. *J. Phys. Chem. B* **110**, 21205-21209 (2006).

http://www.gm.uji.es/research_pH_diode.html

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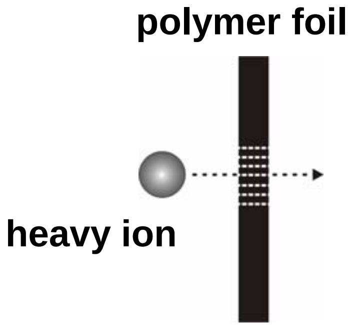

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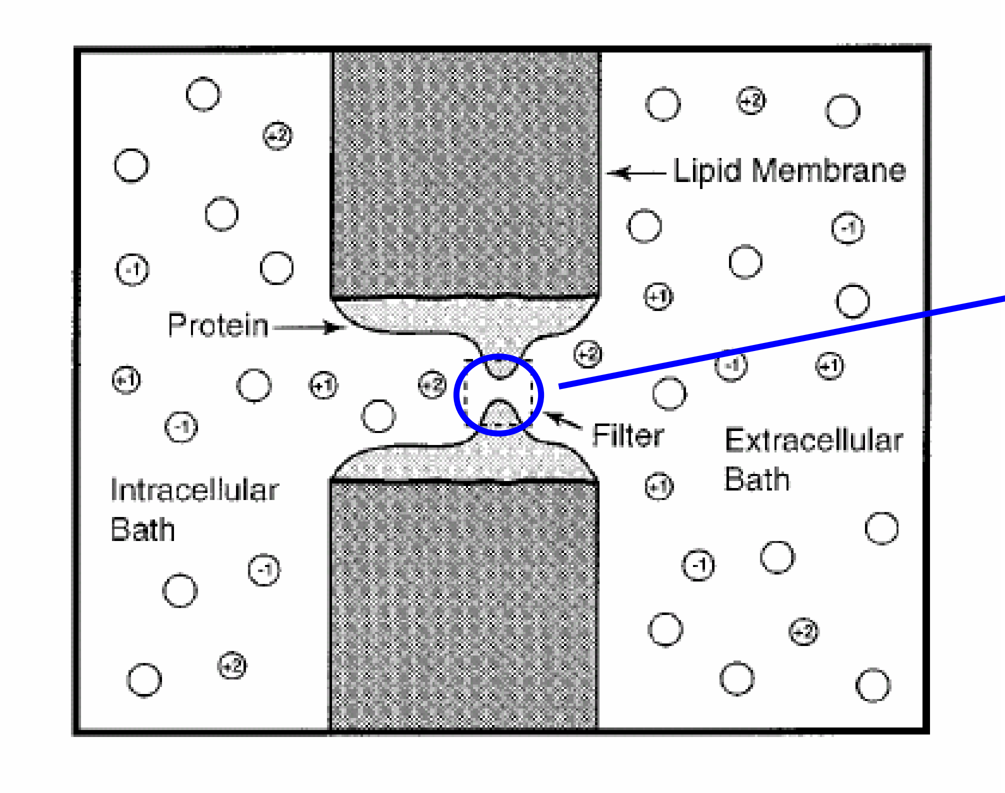
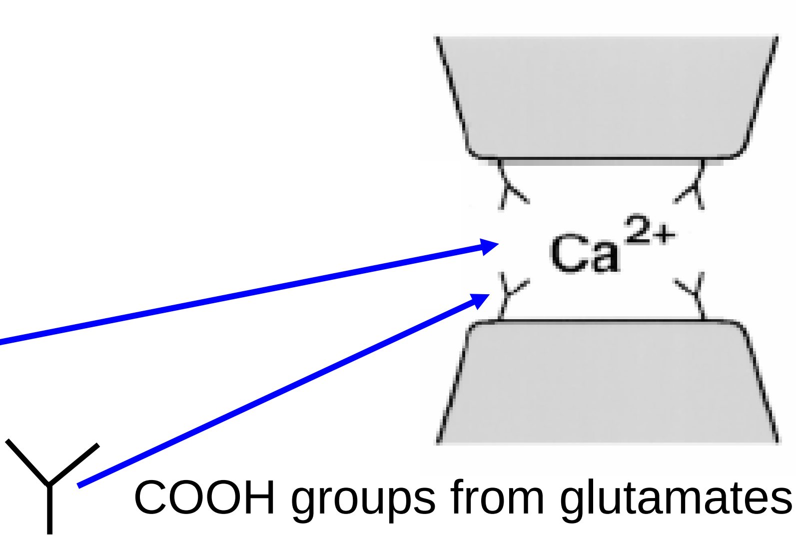
Selectivity of L-Type Calcium Channels



W. Nonner, D. Gillespie, D. Henderson, B. Eisenberg, *J. Phys. Chem.* **105**, 6427 (2001);



COOH groups from glutamates

E.W. McCleskey, *J. Gen. Physiol.* **113**, 765 (1999)

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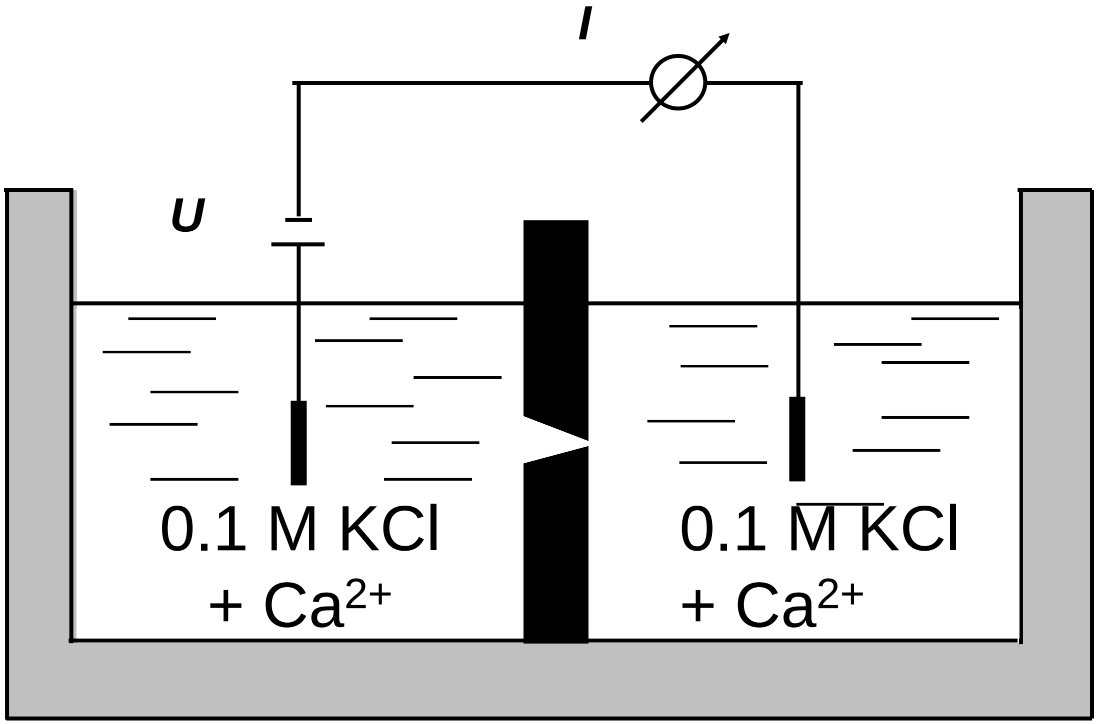
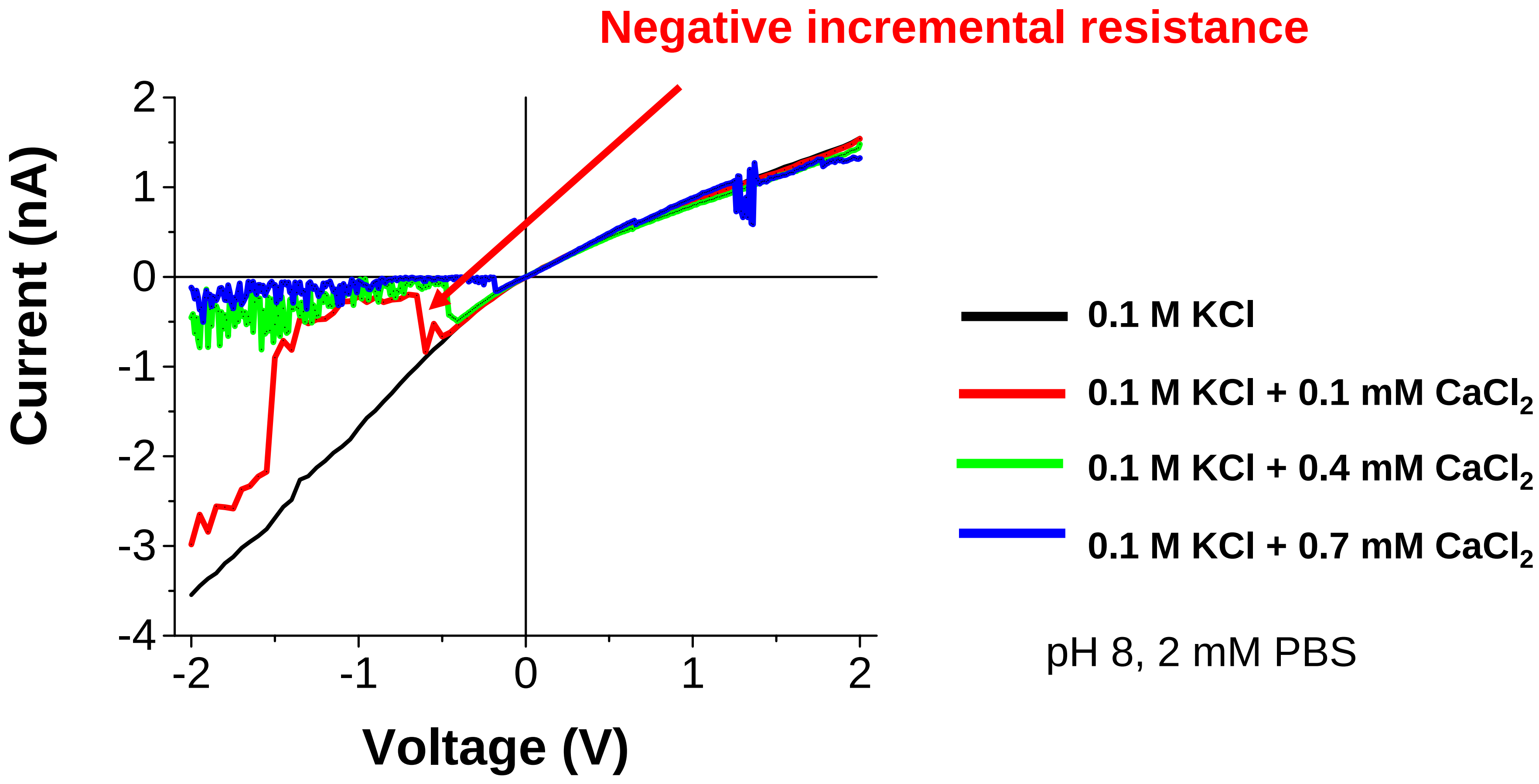


$[Ca^{2+}] << [K^{+}]$

Current-Voltage Curves at Presence of Calcium Ions



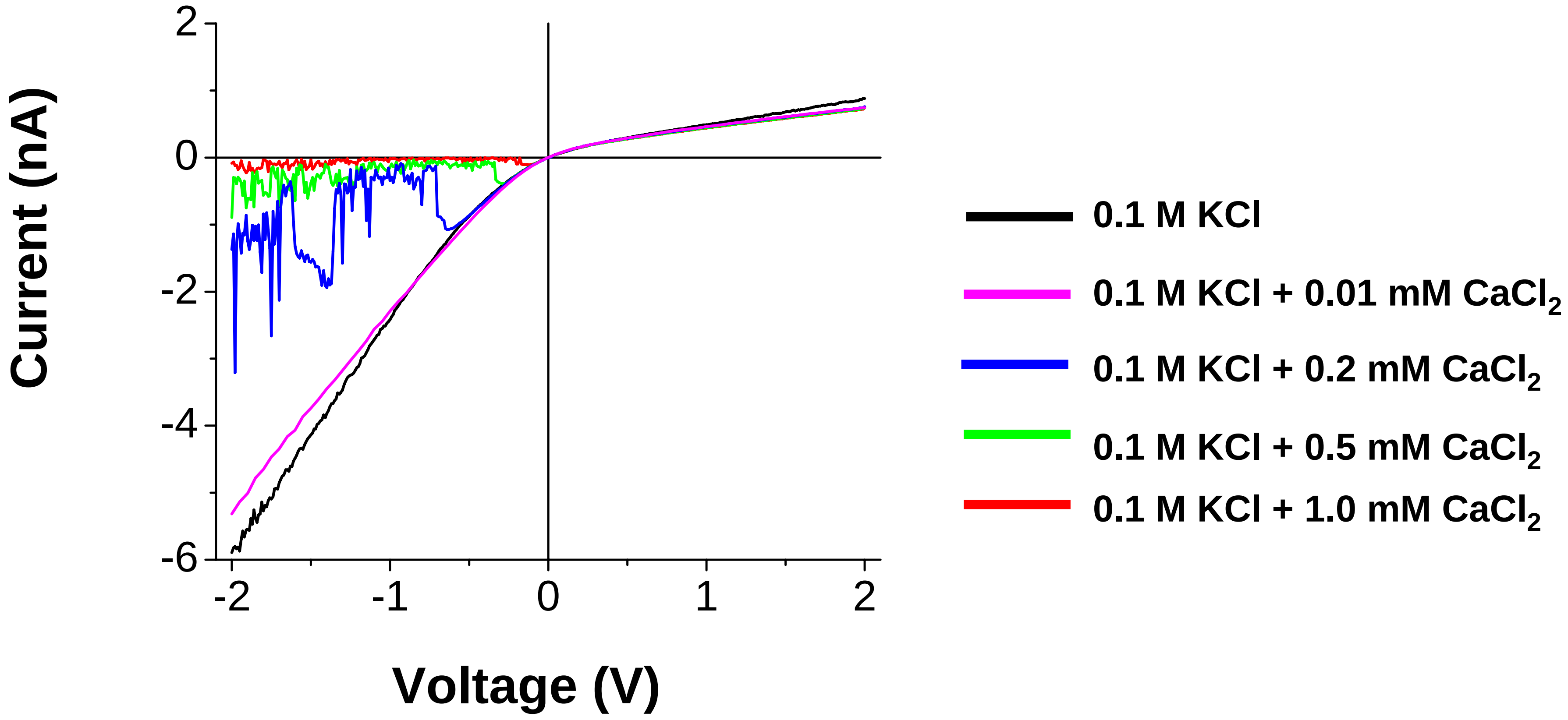
Negative incremental resistance

pH 8, 2 mM PBS

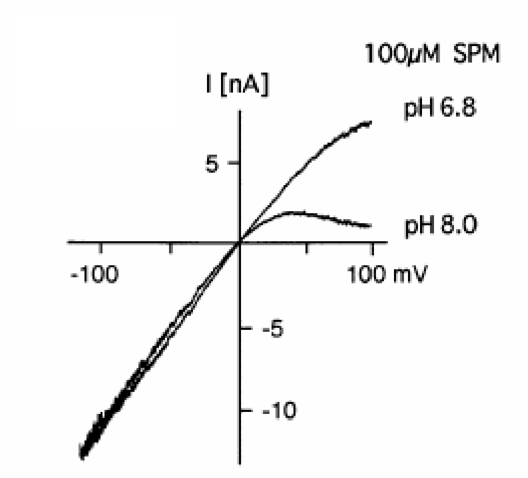
Z. Siwy et al. *Nano Lett.* **6** (2006) 473-477.

Pore opening 5 nm

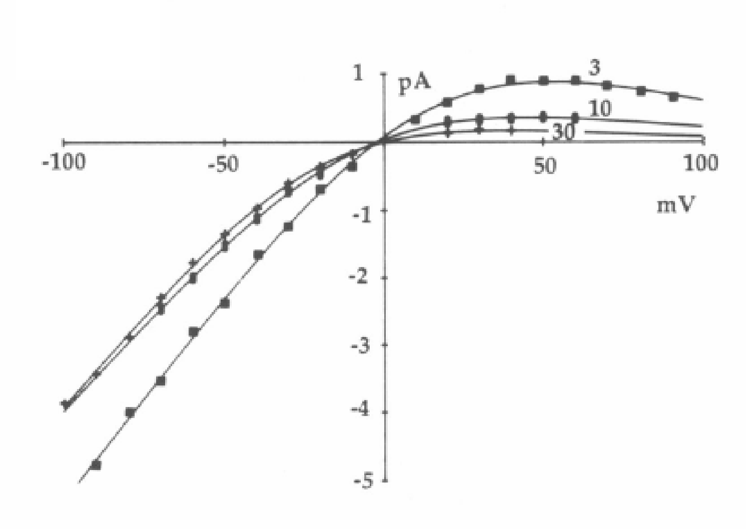
Current-Voltage Curves at Presence of Calcium Ions



Similar Effect of Negative Incremental Resistance was Observed for Biochannels



Effect of spermine on inward-rectifier potassium channel with sulfonylurea receptor

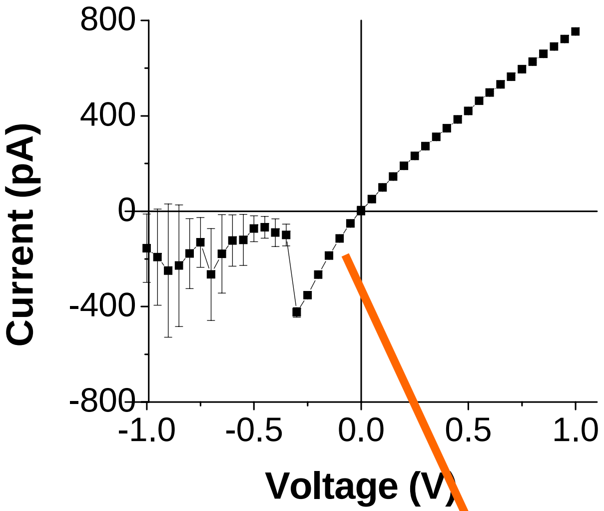


Effect of intracellular magnesium ions on blockage of N-methyl-D-aspartate (NMDA) activated channel.

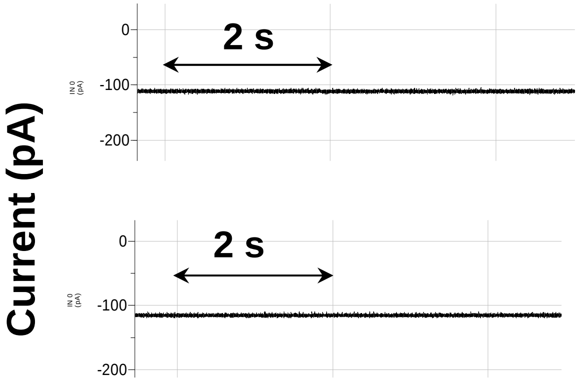
T. Baukrowitz, S.J. Tucker, U. Schulte, K. Benndorf, J.P. Ruppersberg, B. Fakler. 1999 *EMBO J.* **18**: 847-853.

J.W. Johnson, P. Ascher. 1990. *Biophys. J.* **57**: 1085-1090.

Gating of Ion Current (I)

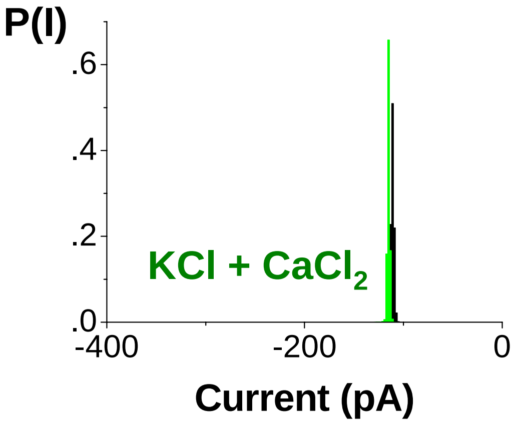


-50 mV



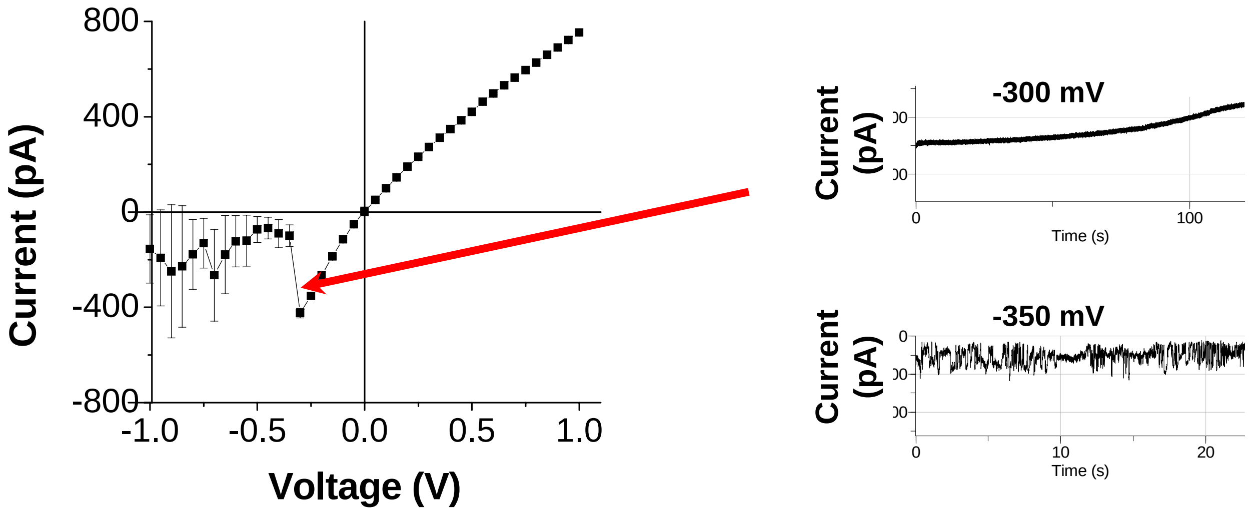
KCl

KCl + $CaCl_2$



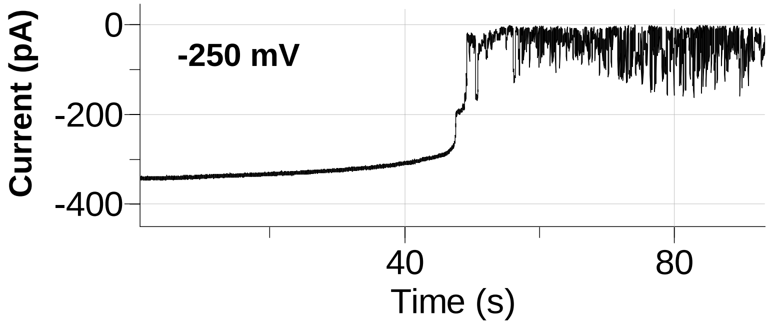
Time Series of Ion Current – Looking Closely at Negative Incremental Resistance

0.1 M KCl
0.3 mM $CaCl_2$

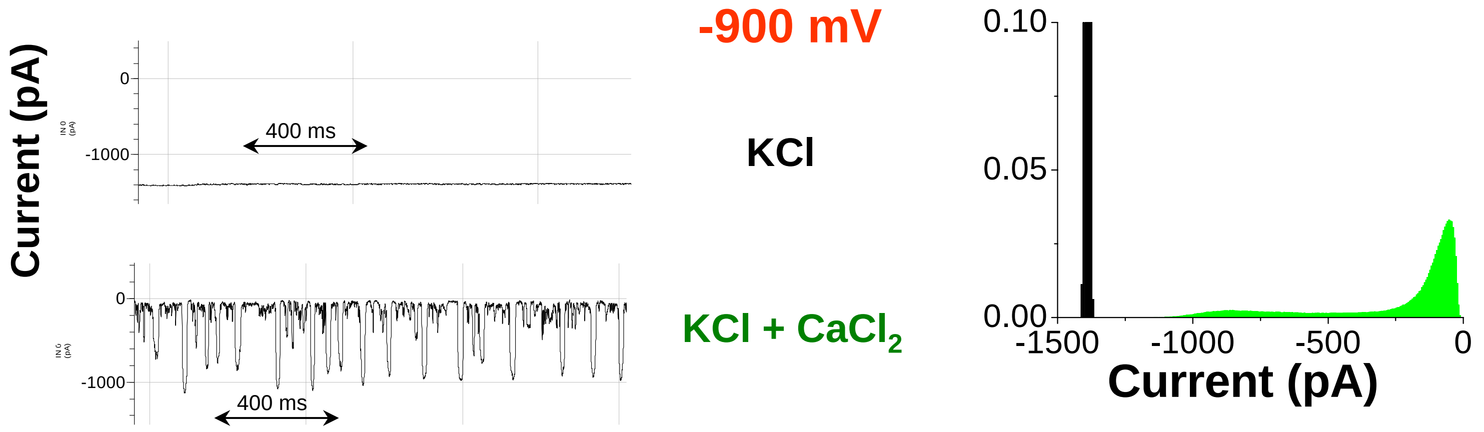


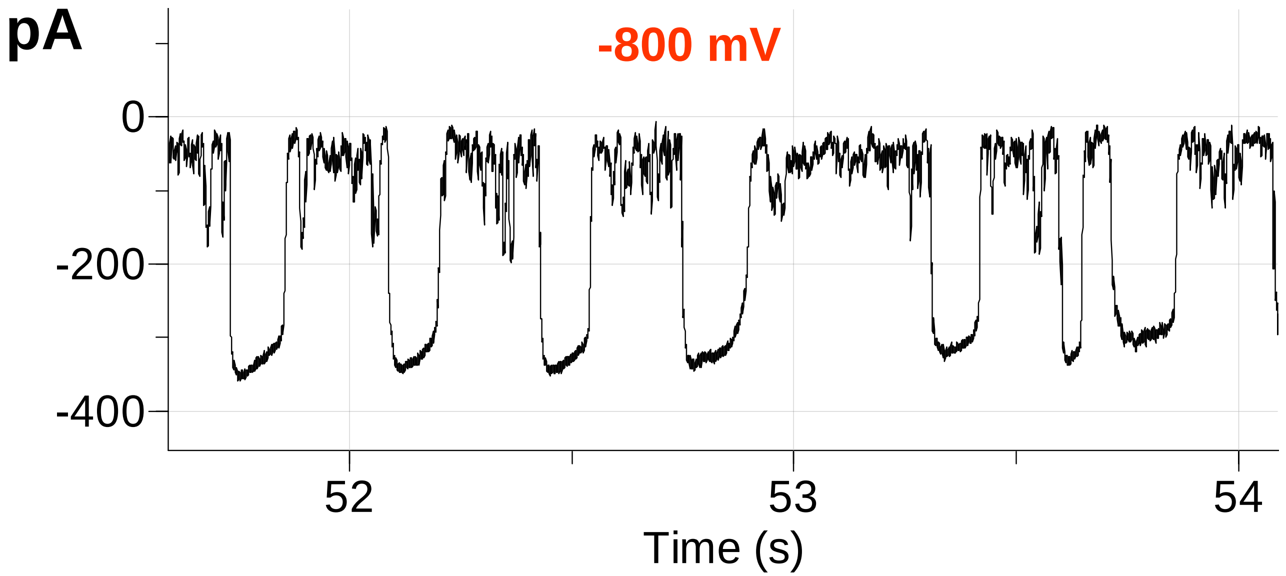
For higher Ca^{2+} concentrations, the closing occurs more abrupt

0.1 M KCl
0.7 mM $CaCl_2$

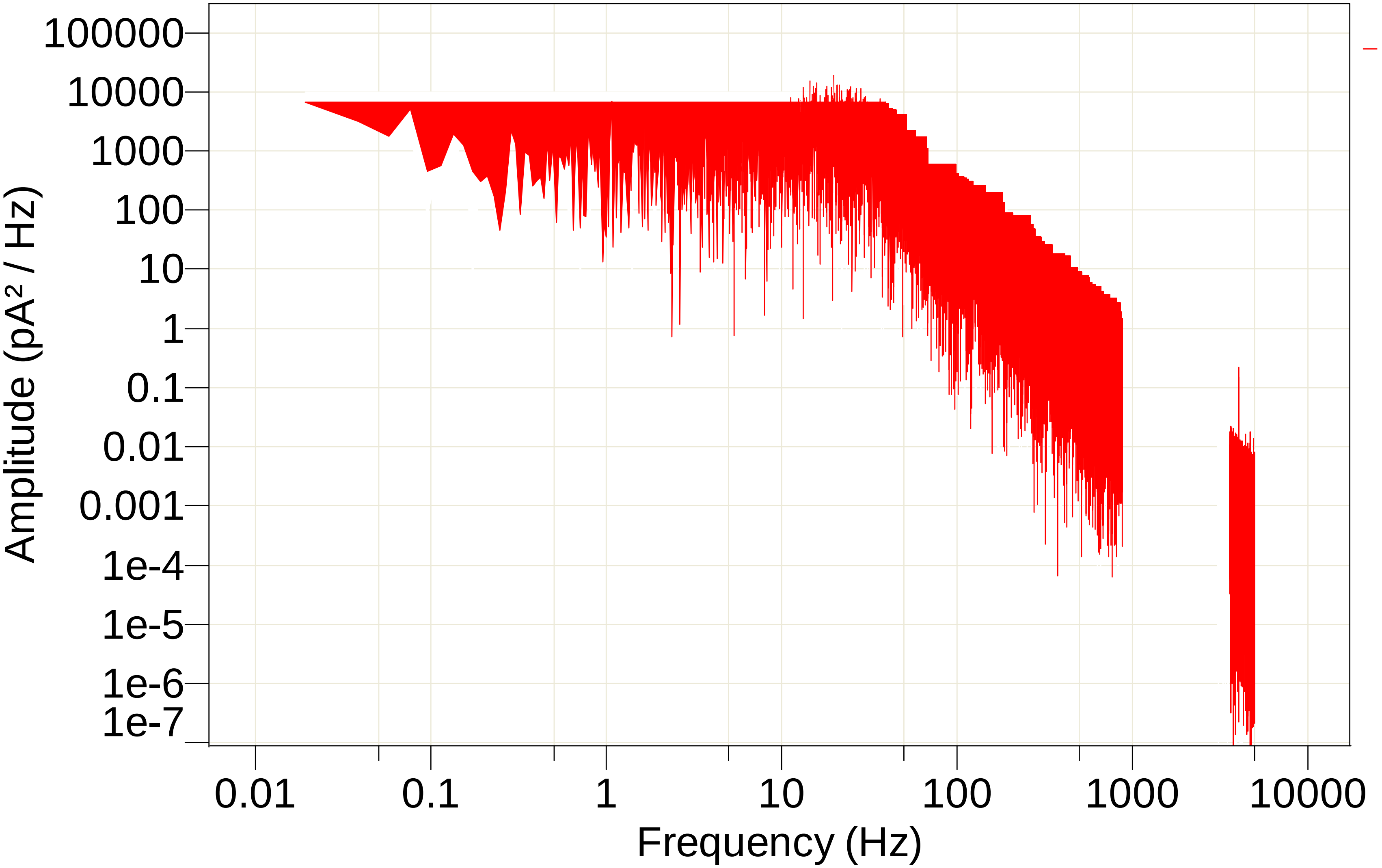


Gating of Ion Current (II)





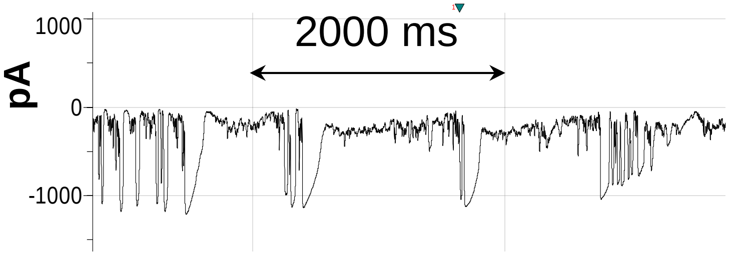
Power Spectrum of Current Oscillations



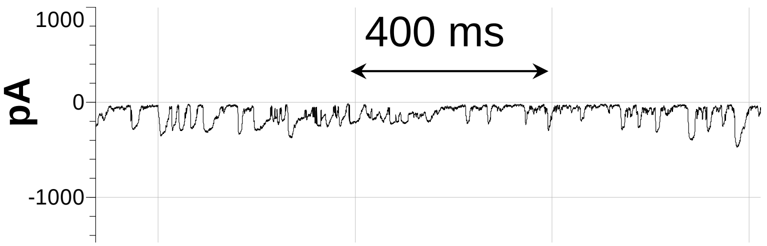
Concentration Dependence

For -900 mV

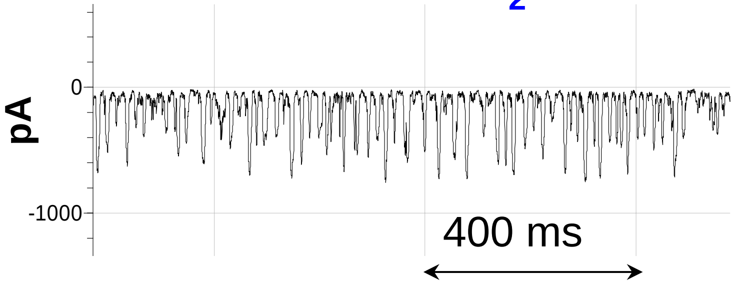
0.1 mM $CaCl_2$



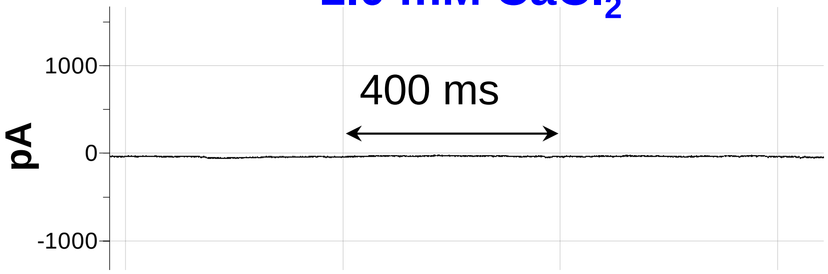
0.7 mM $CaCl_2$



0.3 mM $CaCl_2$



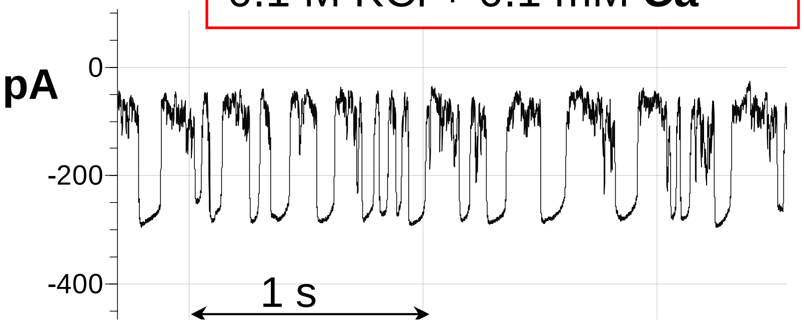
1.0 mM $CaCl_2$



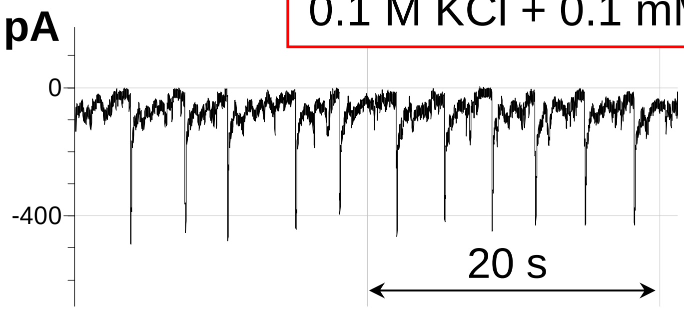
Z. Siwy et al. *Nano Lett.* **6** (2006) 1729-1734.

Singing of Divalent Cations

0.1 M KCl + 0.1 mM ***Ca***$^{2+}$



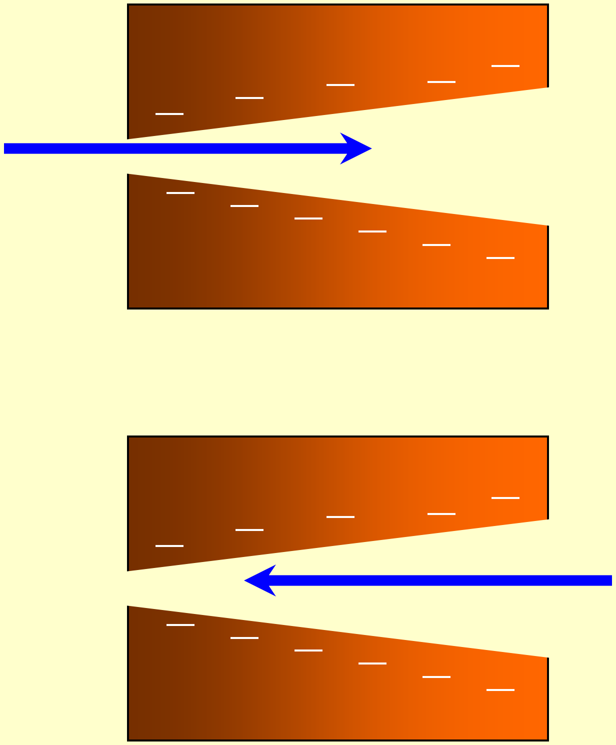
0.1 M KCl + 0.1 mM ***Co***$^{2+}$

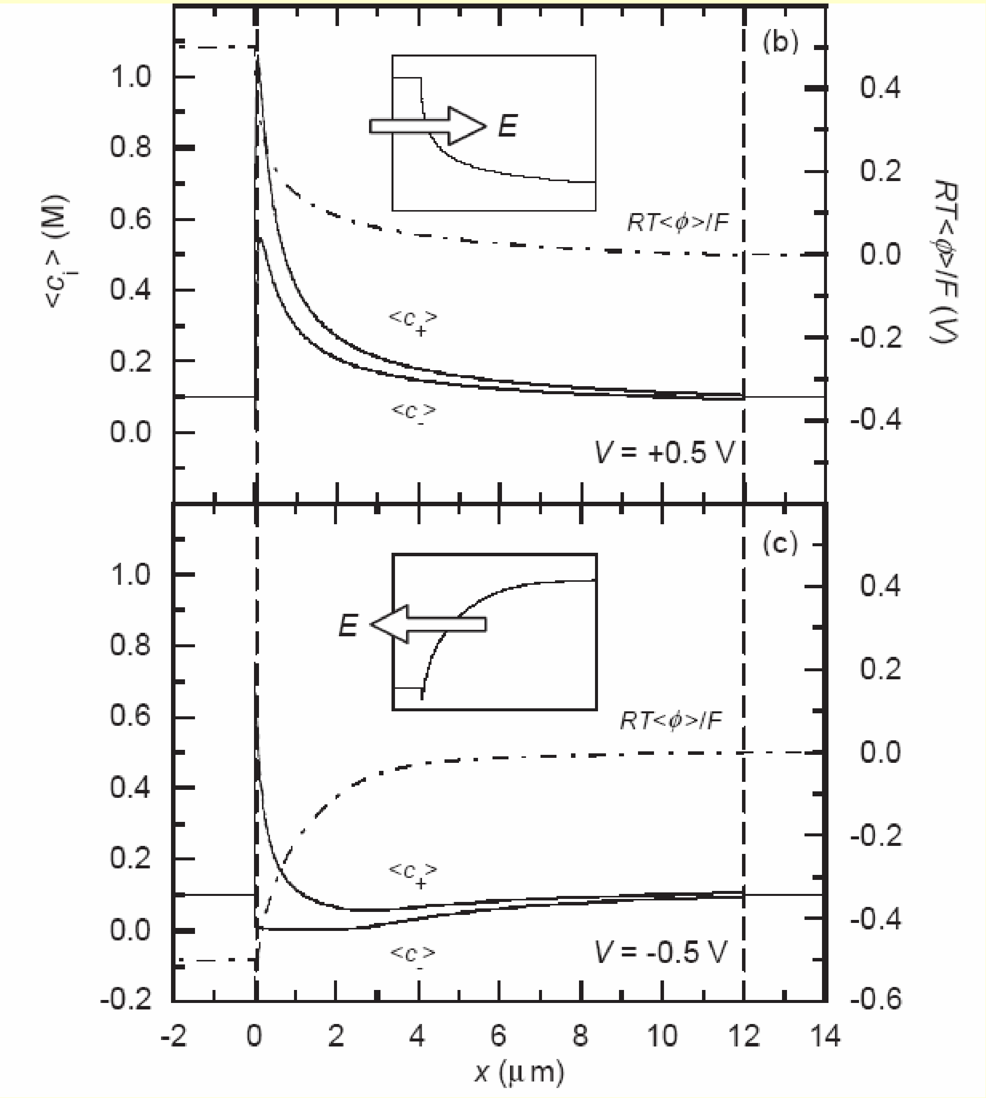


Ca^{2+} 🔊

Co^{2+} 🔊

Mechanism of Chemical Oscillations - Nanoprecipitation





Concentration of calcium inside the pore is however a lot higher than in the bulk solution, so we might have nano-precipitation there.

Cervera et al. *Europhys. Lett.* **71**, 35 (2005)

Mechanism of Chemical Oscillations - Nanoprecipitation

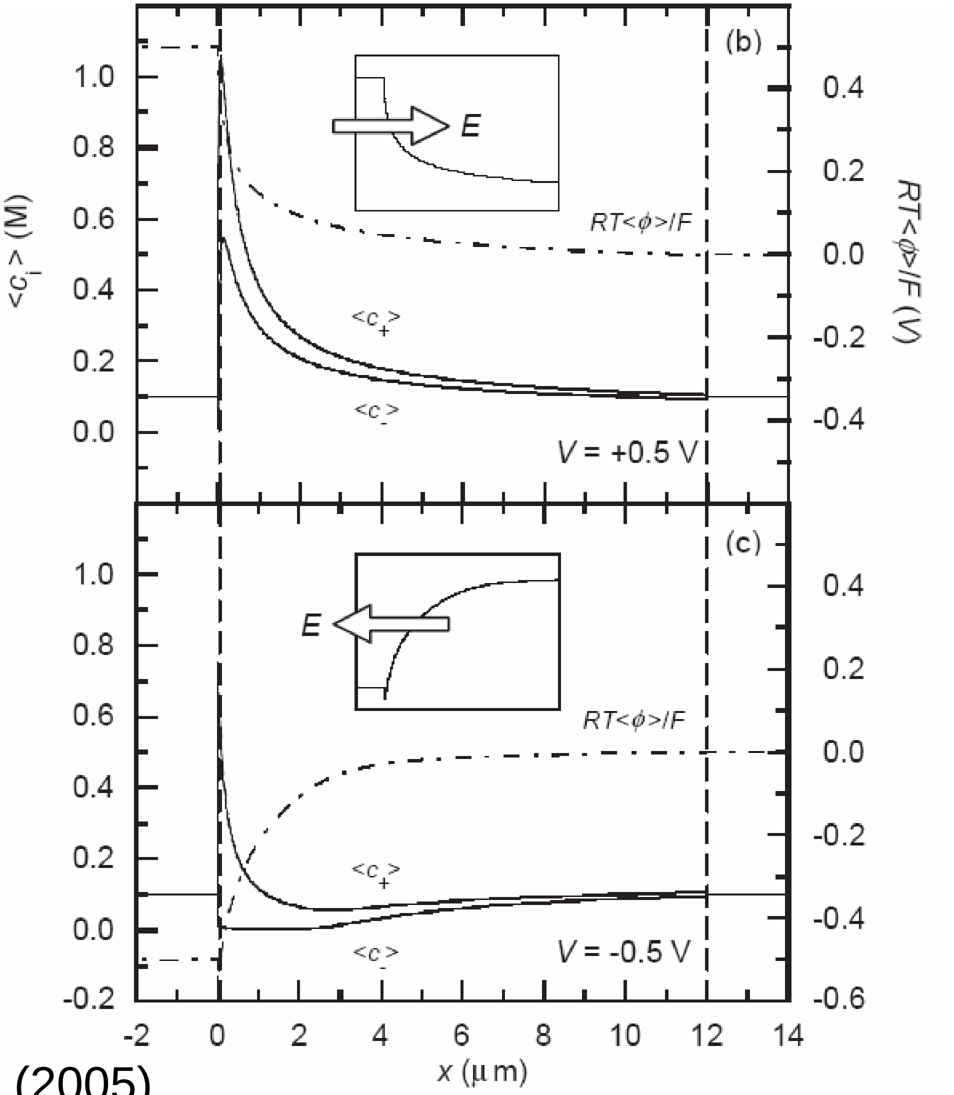
Solubility product for $CaHPO_4$ is ~ 10^{-7}

Concentration of HPO_4 is 10^{-3} M

Concentration of Ca^2 is 10^{-5} - 10^{-4} M

In bulk solution the salt is soluble

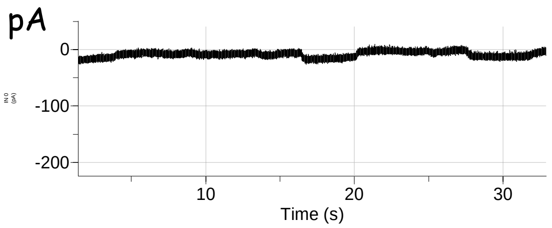
Buffers – are they “innocent”?

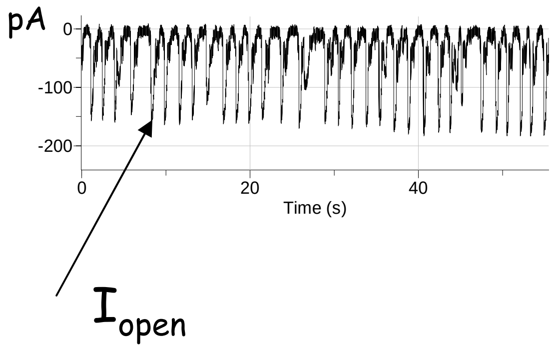


Concentration of calcium inside the pore is however a lot higher than in the bulk solution, so we might have nano-precipitation there.

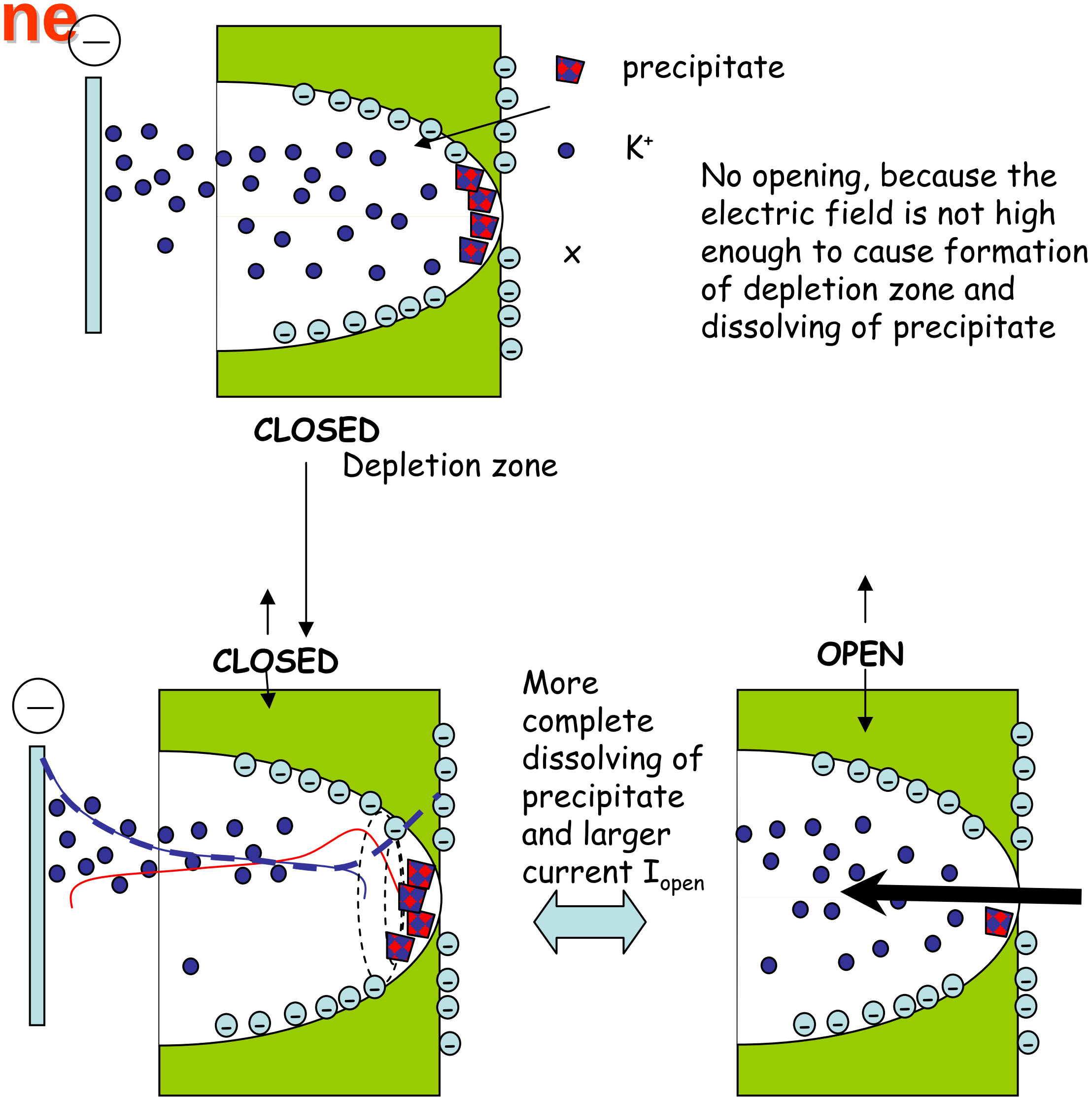
Cervera et al. *Europhys. Lett.* **71**, 35 (2005)

Depletion Zone





I_{open}

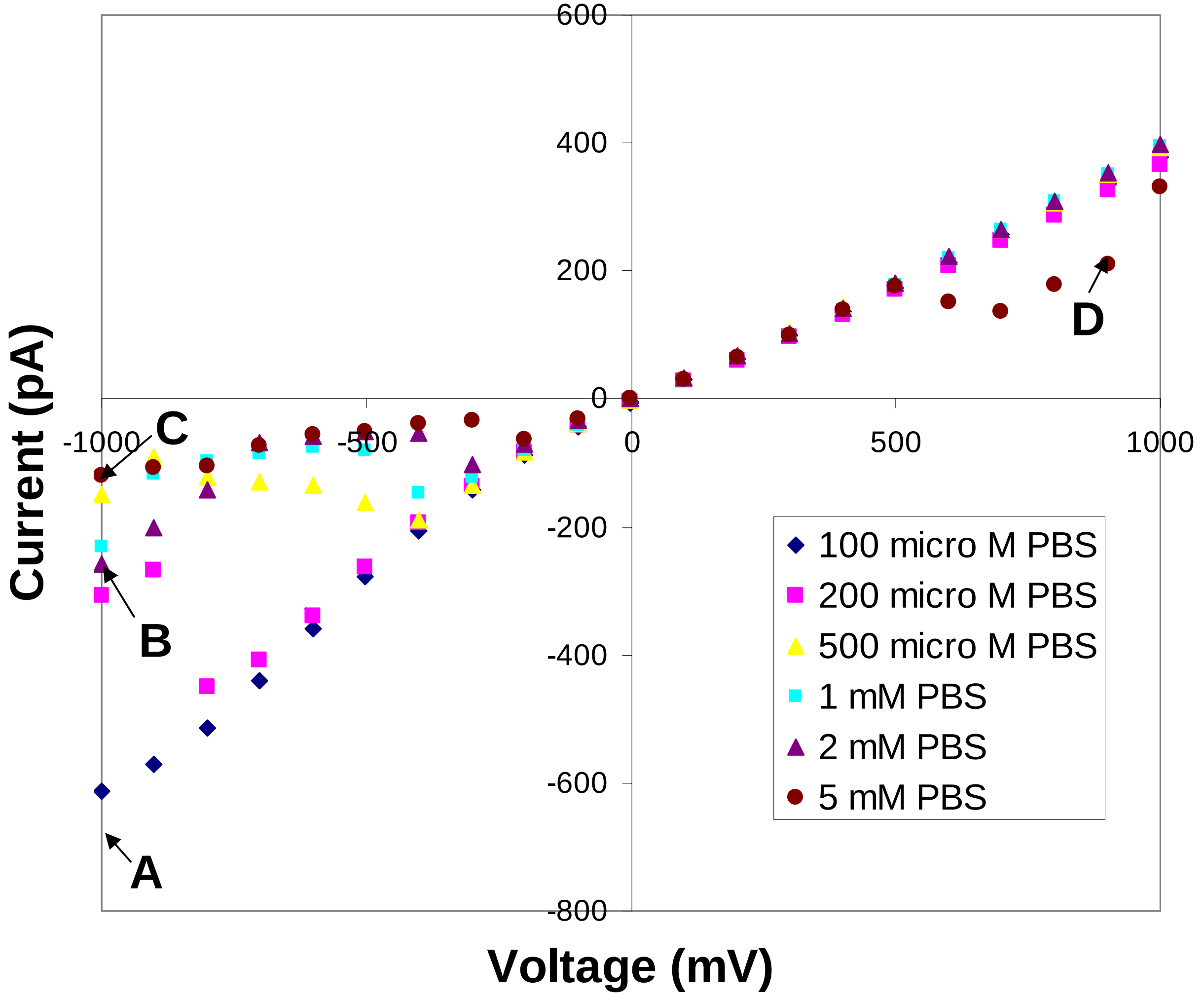


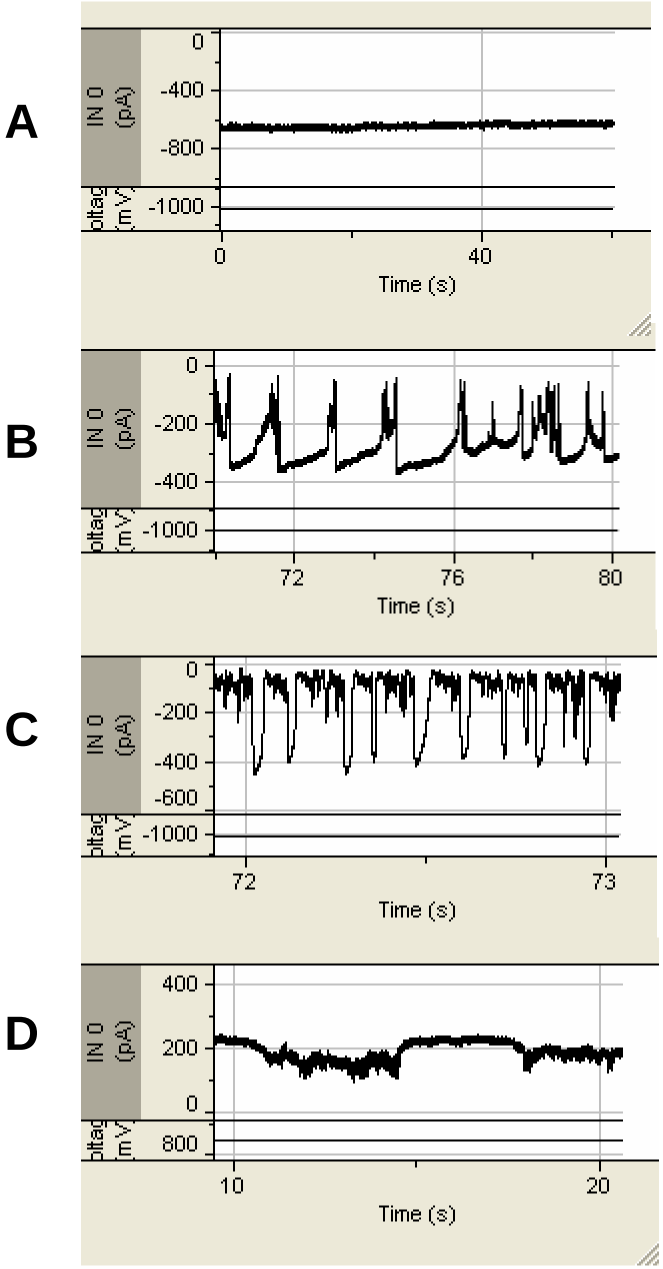
No opening, because the electric field is not high enough to cause formation of depletion zone and dissolving of precipitate

More complete dissolving of precipitate and larger current I_{open}

Dependence on Phosphate Buffer Concentrations

0.1 M KCl + 0.2 mM $CaCl_2$ + buffer
Small opening = 2 nm

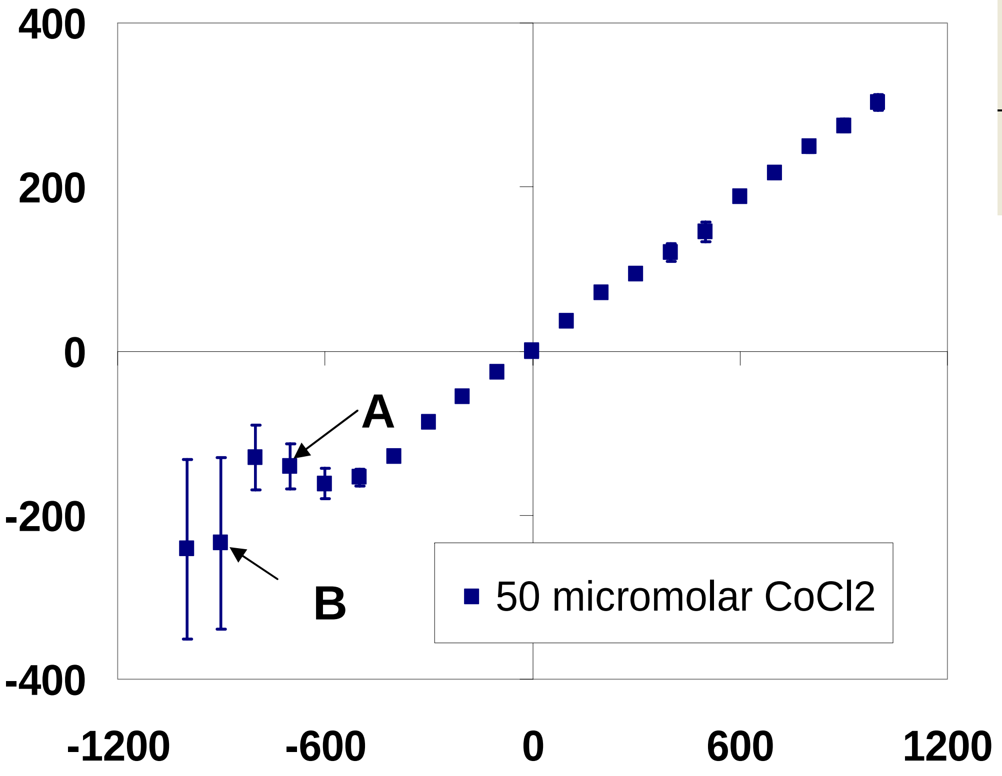


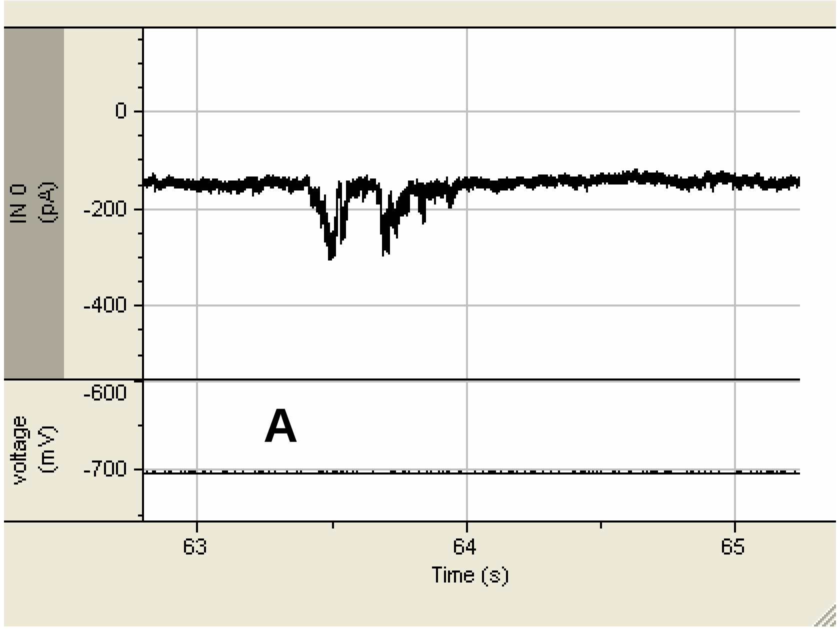


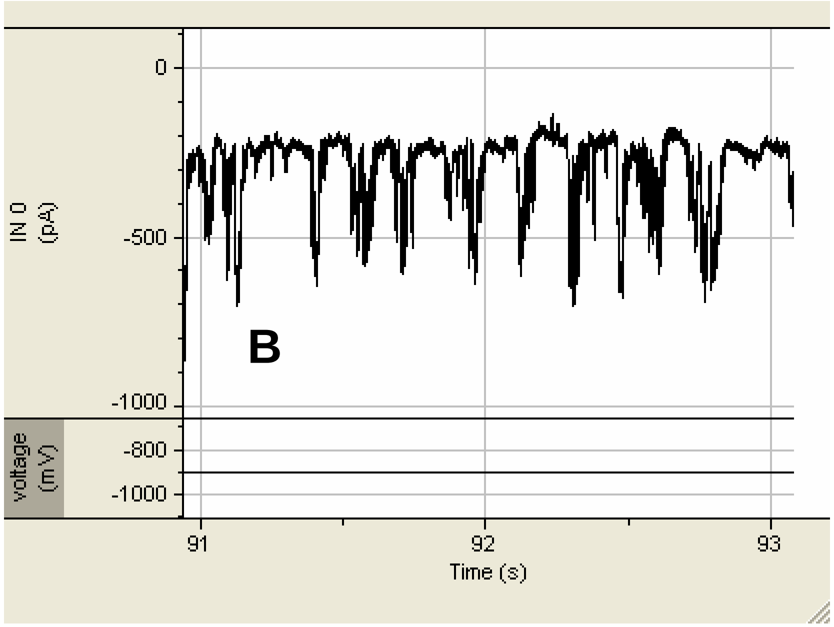
Precipitation of $Co(OH)_2$

Formation of cobalt hydroxide!

0.1 M KCl + 50 micromolar $CoCl_2$ +
~500 micromolar NaOH (pH 10.4)



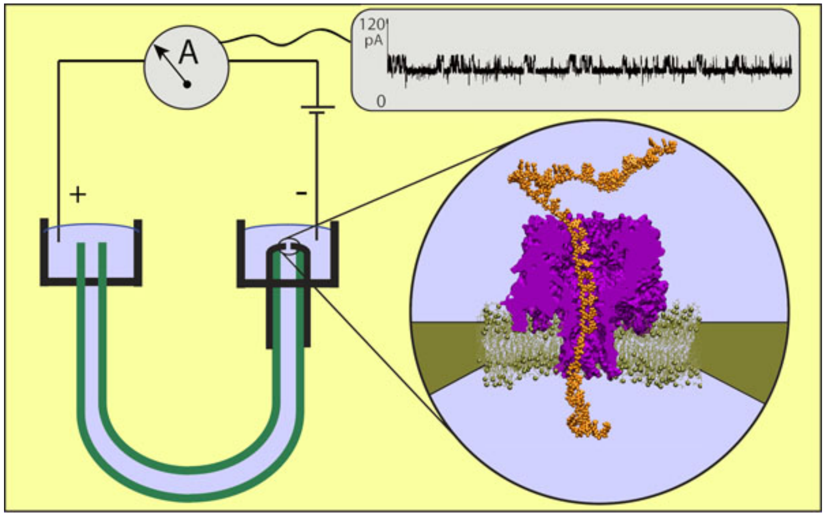




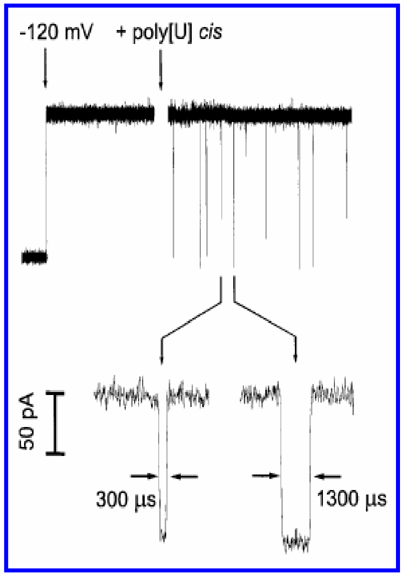
Nanopore DNA Translocations

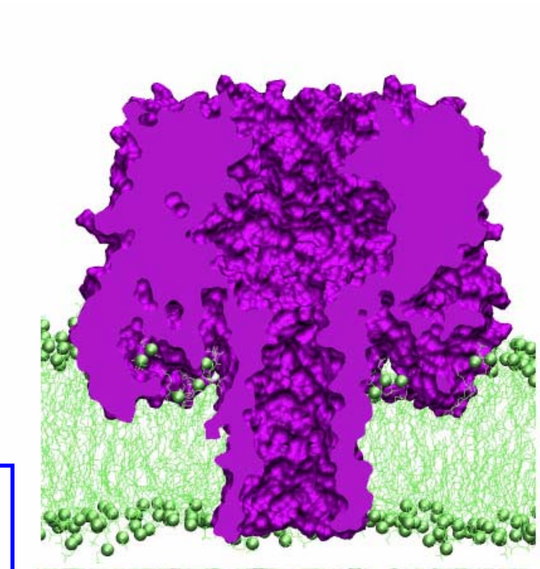
DNA characterization using alpha-hemolysin

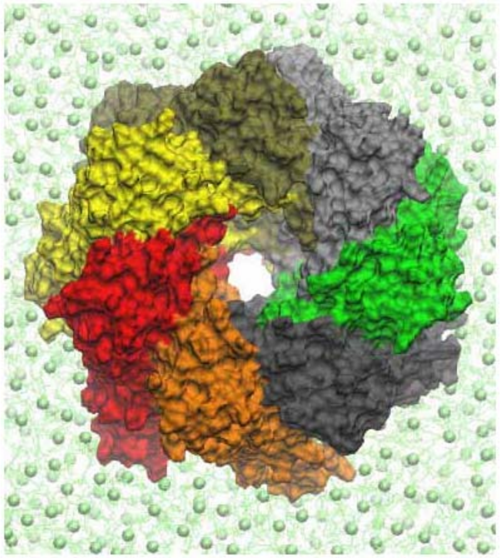
Kasianowicz,J., Brandin, E., Branton, D., & Dreamer, D. W. Characterization of individual polynucleotide molecules using a membrane channel. *Proc. Natl. Acad. Sci. USA* **93**, 13770-13773 (1996).



http://www.ks.uiuc.edu/Research/hemolysin/



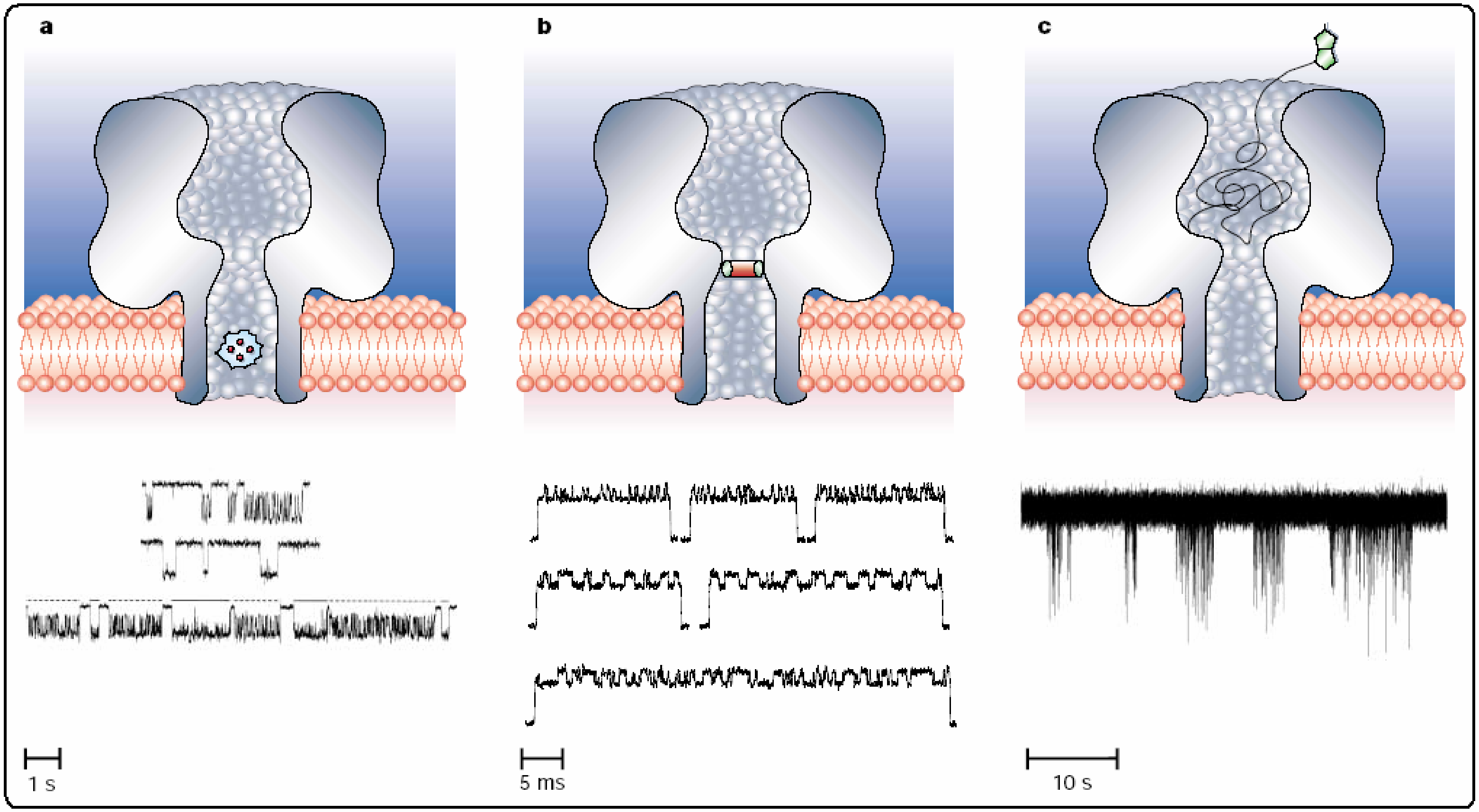




http://www.ks.uiuc.edu/Research/hemolysin/

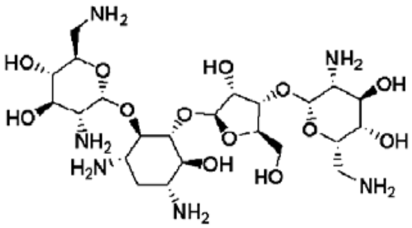
Stochastic Sensing with α - Hemolysin

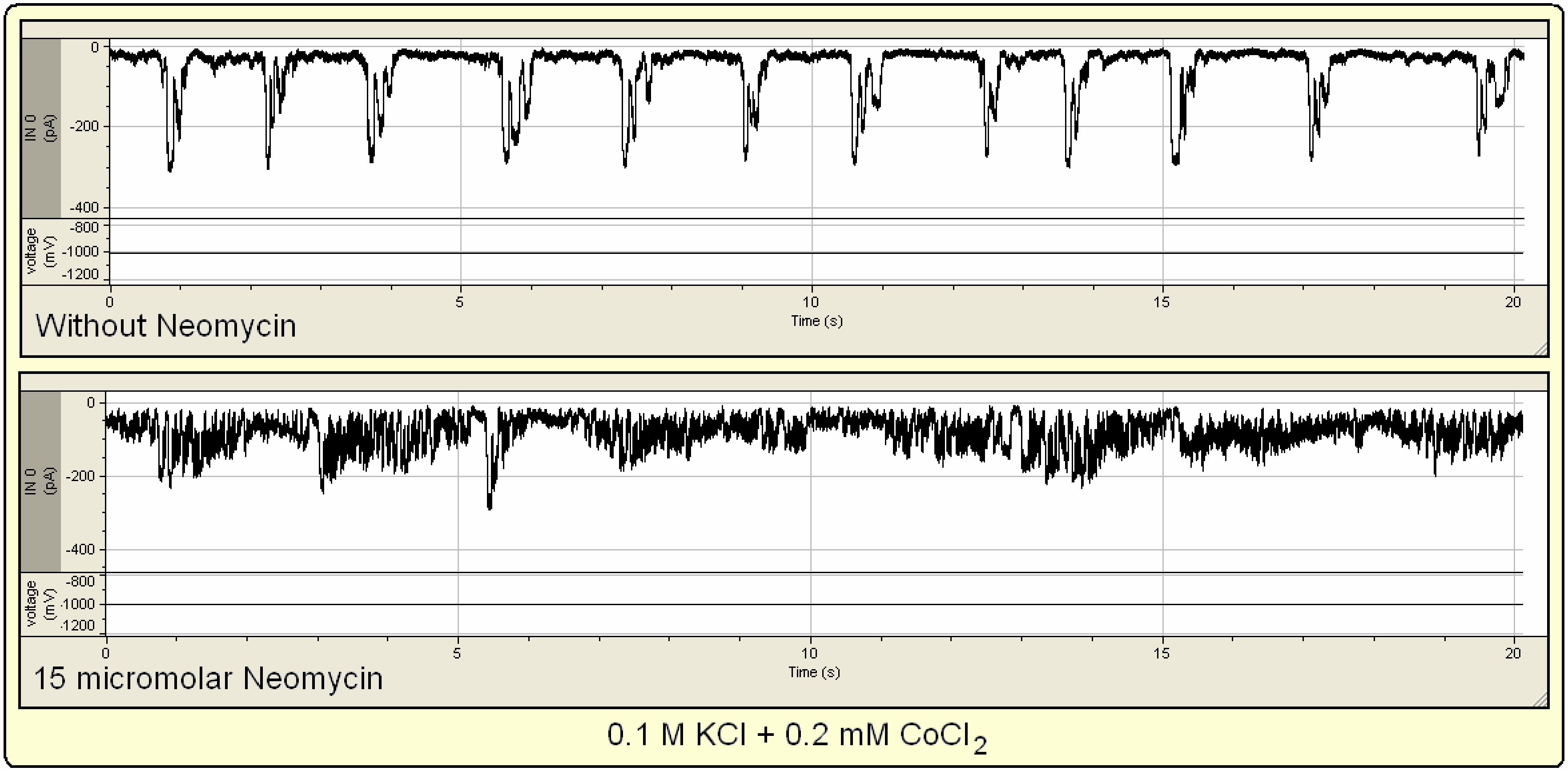
Hagan Bayley & Paul S. Cremer,
Stochastic sensors inspired by biology.
Nature **413**, 226-230 (2001).



Application of the System with Calcium/Cobalt to Build Sensors

Detecting Neomycin



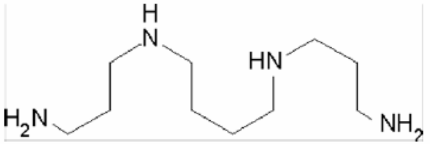


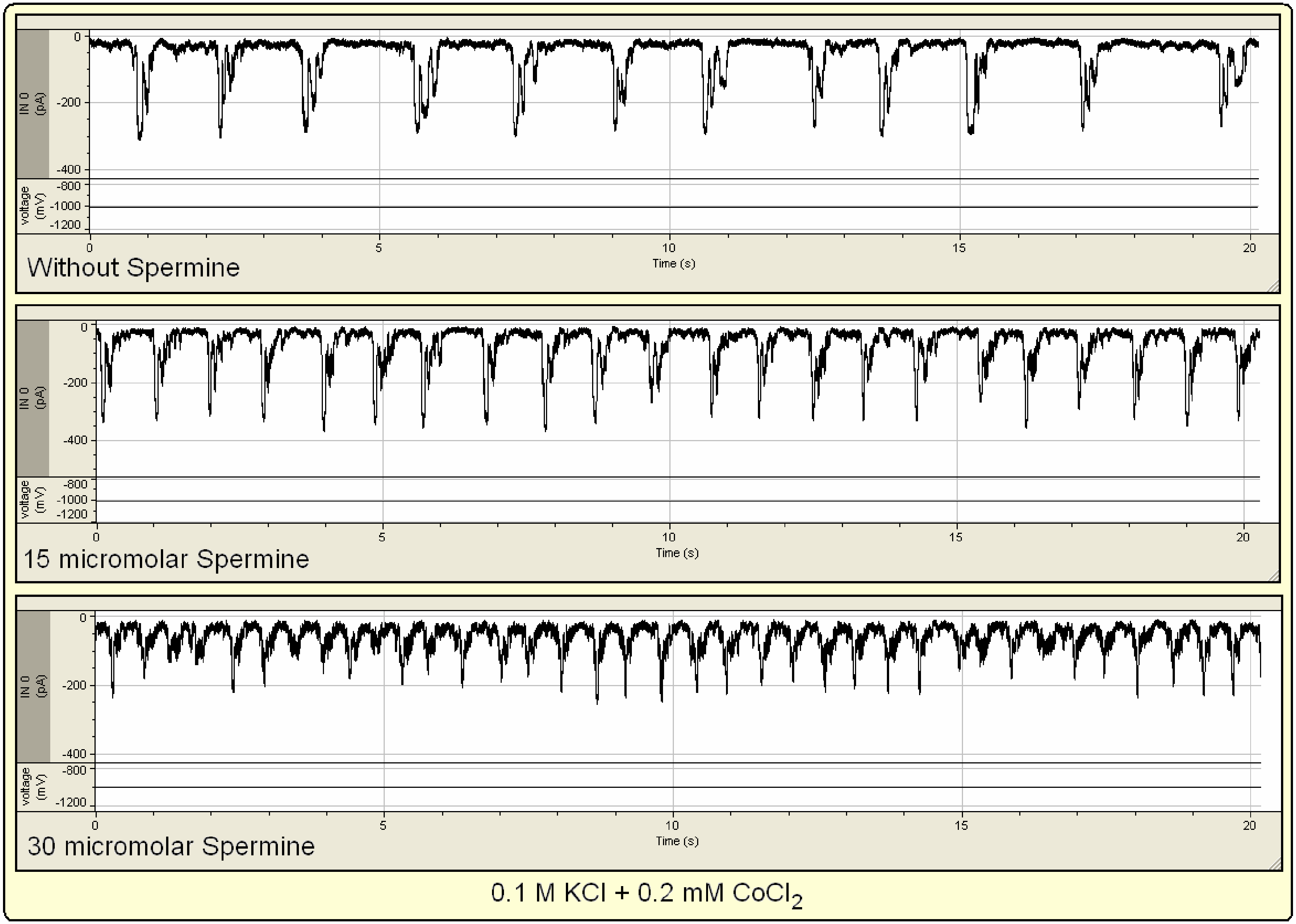
Without Neomycin

15 micromolar Neomycin

0.1 M KCl + 0.2 mM $CoCl_2$

Detecting Spermine





Without Spermine

15 micromolar Spermine

30 micromolar Spermine

0.1 M KCl + 0.2 mM $CoCl_2$

Conclusions

1. Unipolar and Bipolar ionic diodes were prepared on the basis of conical nanopores with tailored surface chemistry.
2. The principle of operation of the bipolar diode is analogous to that of a bipolar semiconductor diode.
3. We have prepared a system of a single conical nanopore that operates far from equilibrium. Ion current through such a pore oscillates/fluctuates in time and possibly can be used as a biosensor.

Acknowledgments

- **Bob Eisenberg, Rush Medical Center, Chicago**
- **Gesellschaft fuer Schwerionenforschung (GSI), Darmstadt, Germany**
- **Dr. Christina Trautmann, GSI, Germany**
- **Prof. Ken Shea, UCI**
- **Mike Sullivan, George Mason University**
- **Ken Healy, University of College Cork, Ireland**

Alfred P. Sloan Foundation

Institute for Complex Adaptive Matter

Institute for Surface and Interface Science