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

Several nanometers, typically 2-6 nm

12 µm

~ 1 µm

We study ionic transport through single conical nanopores.
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.


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 that 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) Ca²⁺ binding sites in the Ca²⁺ ATPase pump
(D) Two central Cl⁻ binding sites in a mutant ClC Cl⁻/H⁺ exchanger.


COOH groups from glutamates

In extra- and intracellular medium:

$$[\text{Ca}^{2+}] \ll [\text{Na}^+]$$

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

W. Nonner, D. Gillespie, D. Henderson, B. Eisenberg, J. Phys. Chem. 105, 6427 (2001);
Interactions Between Ions and Pore Walls Make them Better Devices

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


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
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.


5. Influence of divalent cations on transport properties of conical nanopores. Preparation of chemical oscillator and biosensor.

6. Conclusions.
Heavy Ions as a Working Tool

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

Chemical etching

Linear accelerator
UNILAC, GSI
Darmstadt, Germany

1 ion → 1 latent track → 1 pore!

E. Loriot
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

\[ R_1 = \frac{4L}{\kappa \pi d^2} \]

\[ \gg \]

Tapered cone

\[ R_2 = \frac{4L}{\kappa \pi dD} \]

Example for 1 V, 1 M KCl, \( L = 10 \mu m \)

\( d=1 \text{ nm} \) results in current of 3.9 pA.

\( d=1 \text{ nm}, D=2 \mu m, \) results in current of \(~740\ pA.\)
Focusing of Resistance in a Conical Nanopore

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

- \( D = 1 \ \mu m \)
- \( d = 3 \ \text{nm} \)
- \( L = 12 \ \mu m \)

- 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)

Chemical & Electro-Stopping Technique to Prepare Conical Pores

For polyethylene terephthalate

- Etch solution: 9 M NaOH
- Stopping medium: 2 M KCl + 2 M HCOOH
- Neutralization: HCOOH + OH⁻ → HCOO⁻ + H₂O
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 nm²
Gold Replica of a Single Conical Pore

Gold Replica of a Single Conical Pore

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.


5. Influence of divalent cations on transport properties of conical nanopores. Preparation of chemical oscillator and biosensor.

6. Conclusions.
Conductivity Cell Used for Recording Current-Voltage Curves

Our pores exhibit a number of properties that are unique to NANO pores.
1. Single Conical Nanopores Rectify Ion Current

2. Synthetic Nanopores Are Ion Selective

Reversing the Direction of Ion Current Rectification

Surface covered with carboxyls COO\(^{-}\) groups

Surface covered with amino NH\(_3\)^{+} groups

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

4. Sensitivity to Charge Patterns in the Nanopore

H. Daiguji, Y. Oka, K. Shirono, *Nano Lett.* 5, 2274 (2005);
Sensitivity to Charge Patterns in the Nanopore

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

0.1 M KCl

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

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:

\[ c(\alpha, t) = c_0, \quad c(L, t) = 0, \quad \text{for } D \gg d \]

\[ c(x) = c_0 \frac{d}{D} \left( \frac{L}{x} - 1 \right) \quad d / 2 \cot \alpha \leq x \leq L \]
Sensitivity to Charge Patterns in OmpF


http://www.gm.uji.es/research_pH_diode.html
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.
5. Influence of divalent cations on transport properties of conical nanopores (singing of divalent cations). Preparation of chemical oscillator and biosensor.
6. Conclusions.
Selectivity of L-Type Calcium Channels

[Image showing a diagram of calcium and sodium ions passing through a membrane.]

In extra- and intracellular medium:

\[ [\text{Ca}^{2+}] \ll [\text{Na}^+] \]

\( \text{Ca}^{2+} \) and \( \text{Na}^+ \) have basically the same diameter.

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

Conductivity Cell Used for Recording Current-Voltage Curves

\[ [\text{Ca}^{2+}] \ll [\text{K}^+] \]
Current-Voltage Curves at Presence of Calcium Ions

Negative incremental resistance


Pore opening 5 nm
Current-Voltage Curves at Presence of Calcium Ions

- 0.1 M KCl
- 0.1 M KCl + 0.01 mM CaCl₂
- 0.1 M KCl + 0.2 mM CaCl₂
- 0.1 M KCl + 0.5 mM CaCl₂
- 0.1 M KCl + 1.0 mM CaCl₂
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.

Gating of Ion Current (I)

![Graph showing voltage versus current for different solutions: KCl and KCl + CaCl₂. The graph indicates a gating effect at -50 mV.]
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)

-900 mV

KCl

KCl + CaCl₂

-800 mV
Power Spectrum of Current Oscillations
Concentration Dependence

For -900 mV

- 0.1 mM CaCl$_2$
- 0.3 mM CaCl$_2$
- 0.7 mM CaCl$_2$
- 1.0 mM CaCl$_2$

Singing of Divalent Cations

- **0.1 M KCl + 0.1 mM Ca^{2+}**
  
- **0.1 M KCl + 0.1 mM Co^{2+}**
Concentration of calcium inside the pore is however a lot higher than in the bulk solution, so we might have nano-precipitation there.

Mechanism of Chemical Oscillations - Nanoprecipitation

Solubility product for CaHPO$_4$ is $\sim 10^{-7}$

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.

Depletion Zone

No opening, because the electric field is not high enough to cause formation of depletion zone and dissolving of precipitate.
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)
DNA characterization using alpha-hemolysin

Hagan Bayley & Paul S. Cremer,
Stochastic sensors inspired by biology.
Application of the System with Calcium/Cobalt to Build Sensors

Detecting Neomycin

![Graph showing the response of sensors to Neomycin](image)

Without Neomycin

15 micromolar Neomycin

0.1 M KCl + 0.2 mM CoCl₂
Detecting Spermine

Without Spermine

15 micromolar Spermine

30 micromolar Spermine

\[ 0.1 \text{ M KCl} + 0.2 \text{ mM CoCl}_2 \]
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