Biosensing with Ionic Diodes and Non-Equilibrium Fluctuations of Ion Currents in Single Nanopores

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Three Projects Will be Discussed

I. Building a sensor

Bacillus anthracis

II. Electrostatic interactions in ionic liquids

Ionic liquids – media which consist only of ions

III. 1/f noise in nanopores

Behavior of ion currents in time; voltage gated noise properties

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$S(f), \text{pA}^2/\text{Hz}$
GOAL
Label-free sensor for antigens that are bioterrorism agents

Prototype: Monitoring infection with *Bacillus anthracis*

Infection with *Bacillus anthracis* results in γDFGA in the blood at the levels that are higher than 20 ng/ml (~10 pM γDFGA).
Prototype
Detection of polyglutamic acid from *B. anthracis*

Monoclonal antibody for polyglutamic acid
Prof. T. Kozel, University of Nevada

We perform this reaction inside a sub-femtoliter volume of a single nanopore

**Binding of polyglutamic acid has two consequences that we will use in sensing:**

1) Very few molecules “fit” in the tip of the pore – *the pore will be thus partially blocked.*

2) Placing heavily negatively charged molecules *will change local electric fields*, which can ENHANCE the detection signal.
What Do We Need to Make This Sensor Happen?

IONIC DIODE is a component that allows an ionic flow only in one direction, blocking it in the other.

Ionic diode is very sensitive to charges on the pore walls.

Rectification degrees of several hundreds: 
\[
\frac{I(+V)}{I(-V)}
\]

1. Preparation of Nanopores of Known Geometry

2. Studies of How Transport Properties of Nanopores Depend on the Pore Surface Charge
1. Irradiation with e.g. Xe, Au, U
(\sim 2.2\, \text{GeV i.e. \sim 15\% c})

2. Chemical etching

1 ion \rightarrow 1 \text{ latent track} \rightarrow 1 \text{ pore!}

Chemical & Electro-Stopping Technique to Prepare Conical Pores

For polyethylene terephthalate (PET)

Etch solution
9 M NaOH

Acidic stopping medium
2 M KCl + 2 M HCOOH

Neutralization
HCOOH + OH⁻ → HCOO⁻ + H₂O

HCOOH + OH⁻ → HCOO⁻ + H₂O
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 ~ 1.0 per nm²
Relation Between the Surface Charge Pattern and Transport Properties

Sensitivity of the Current-Voltage Curves on Surface Charge
Single Conical Nanopores Rectify Ion Current

Ionic current rectifier: \( I(-V) > I(+V) \)

Sign of the Surface Charge Influences Current-Voltage Characteristics

Flipping of the I-V Curve Was Also Observed with Polyimide Pores

**Surface Patterns** and Their Influence on I-V

*Ionic diodes*

Depletion zone


HIGH Conductance State of Nanopore

BIPOLAR DEVICE – current carried by both + and –
How Did We Do That?

GOAL!

The negative groups (COO⁻) at the narrow opening have to be changed into groups with positive charges, e.g. NH₃⁺

Collaboration with Prof. Ken Shea
Steady-State Solution of Diffusion Problem

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

\[ c(x) = c_0 \frac{a}{A} \left( \frac{L}{x} - 1 \right) \]

Targeted modification of the tip

Only the region of the pore close to the tip with high enough EDC and amines concentration will be modified!
Modification Chemistry

Ethylene diamine + EDC, 0.1 M KCl, pH 5.5

0.1 M KCl, pH 5.5
An Ionic Diode Made From a Nanopore with a Positive Tip

An Ionic Diode Made From a Nanopore with a Negative Tip

0.1 M KCl, pH 5.5

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

Unipolar Diodes from UC Berkeley

Sensitivity to Charge Patterns in OmpF Pore


http://www.gm.uji.es/research_pH_diode.html
Diode Pattern Realized in OmpF Pore

Preparation of Ionic Bipolar Junction

P. Apel, Dubna
Step-by-Step Modifications

Salt concentration determines the potential in the pore and thus the leakage current level in BJT.
3. We are almost ready to make a sensor
Summary: How to Tune Current-Voltage Curves Of Nanopores by the Surface Charge

Surface charge patterns AND Corresponding current-voltage curves

Changes of the surface pattern are induced upon binding of an analyte
What were the steps for developing a method to detect polyglutamic acid from *Bacillus anthracis*?

Making a prototype for something that we know
Prototype of the Sensor for Avidin and Streptavidin

KCl as the background electrolyte
Prototype of the Sensor for Avidin

Nanopore with the tip modified with biotin; 10 mM KCl, pH 7.0

Current (nA)
Voltage (V)

With avidin 0.5 μM, 2 h
With biotin
Tip modified with biotin
Avidin on top
Is Non-Specific Adsorption a Problem?

2-methoxyethylamine

Incubation with 0.5 μM avidin

After incubation with avidin

10 mM KCl, pH 6

control
Finding Isoelectric Point (Pl) pl of the mAb for γDPGA

Monoclonal antibody for polyglutamic acid

Current (nA) vs Voltage (V) graph

- pH 4.8
- pH 6.0
- pH 8.0
Sensing Polyglutamic Acid


Incubation with 1 μM DPGA for 0.5 h and measuring I-V curves in 10 mM KCl
Sensing Signal

Current (nA) vs. Voltage (V)

- pH 4.8
- pH 6.0
- pH 8.0

Rectification degree $I(+5V)/I(-5)$

Before adding $\gamma$DPGA

After adding $\gamma$DPGA

Polyglutamic acid

Voltage (V)
Rectification can become a sensing signal when a molecule specifically binds to the pore walls.
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$S(f), \text{pA}^2/\text{Hz}$

$I/f 0.62$

$I/f 1.25$
Ionic Liquids – Frustrated Solids

• Ionic liquids, or molten salts, are liquids composed completely of ions (i.e. there is no solvent) at or below 100° C.
Unique Properties of Ionic Liquids

- Negligible vapor pressure
- Stability over wide range of temperatures
- Non-corrosive and non-toxic
- Hygroscopic
- More viscous than water
- Wide electrochemical windows

The choice of cation and anion affords a great deal of customization in terms of physical and chemical properties
Why Are Ionic Liquids “liquid” at RT?

- Weakly-coordinating anions and charge delocalization
- Large ions increase lattice energy
- Asymmetry
  - The more asymmetric the ions, the more difficult it is to pack them efficiently into a crystal

Ionic Liquids – a Fascinating System for Condensed Matter Physics

Ions of ionic liquids cannot be treated as point charges.
So What is the Structure of the Double-Layer and Range of Interactions?

The classical theory, which treats ions as points can hardly be used here:

Classical Debye layer length:

\[ k^{-1} = \left[ \frac{\varepsilon_0 \varepsilon k_B T}{2e^2 C_{bulk}} \right]^{1/2} \]

For ionic liquids \( k^{-1} \sim 5\text{Å} \)

Very different information from the literature:

How to Check the Influence of the Restricted Geometry on Transport

1. Measuring ionic conductivity $\kappa$ of ionic liquids through nanopores of different diameters.

2. Existence of ion rectification will point to electric interactions of range similar to the pore radius.

Conductivity in Cylindrical and Conical Pores

Cylindrical pore

Tapered cone

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

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

From Current-Voltage Curves
Ionic Liquids Experiments

1-butyl-3-methylimidazolium methyl sulfate

$T_m = 269 \text{ K}$

1-butyl-3-methylimidazolium 2-(2-methoxyethoxy) ethyl sulfate

$T_m = 201 \text{ K}$
It has been shown that the viscosity of ionic liquids varies significantly with modest changes in temperature. For [BMIM][CH3O4S]:

\[ \eta_1 = 0.28899 \text{ Pa} \cdot \text{s at } T_1 = 293 \text{ K} \]
\[ \eta_2 = 0.21319 \text{ Pa} \cdot \text{s at } T_2 = 298 \text{ K} \]

\[ \frac{\kappa_1}{\kappa_2} = 0.68 \]
\[ \frac{\eta_2}{2 \eta_1} = 0.73 \]


It is not surprising, but it is worth noting that the larger anion results in an overall lower value for $\kappa$. 
Rectification in Current-Voltage Curves?

Conical pore with 12 nm diameter

Conical pore with 8 nm diameter
Surface Patterns Can Enhance Rectification

The first example of an ionic diode for ionic liquids

Studying transport of ionic liquids in nanopore provides insight into steric, electric and maybe even van der Walls interactions with surfaces.
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- $1/f^{0.62}$
- $1/f^{1.25}$

$U$
1/f Noise is Pretty Much Everywhere Including Nanopores

S. Bezrukov and M. Winterhalter: 1/f noise results from switching a pore between various conducting states.


Studies of 1/f Noise in Fluctuating 2 nm Polymer Pores at Low Voltages

Smeets et al. 1/f noise could be related to the presence of air bubbles, this would also relate to conductance changes.

Equilibrium ion current fluctuations:

\[
\frac{S(f)}{\langle I \rangle^2} = \frac{H}{fN_c}
\]

\[\alpha = 1.1 \times 10^{-4}\]
1/f Noise in Conical **Rectifying** Nanopores

\[ I \propto V^{0.79} \quad \text{pH 8} \]

\[ I \propto V^{1.23} \quad \text{pH 3} \]

\[ S(f) \propto 1/f^{1.25} \quad \text{pH 8} \]

\[ S(f) \propto 1/f^{0.62} \quad \text{pH 3} \]

\[ V \propto I \]

\[ S(1 \text{ Hz})/I^2 \]

\[ \text{Exponent } \alpha \]

M.R. Powell, C. Martens, Z.S. Siwy - submitted
1/f Noise in Cylindrical NON-Rectifying Nanopores

Current (nA)

Voltage (mV)

S(f), pA²/Hz

f, Hz

S(1Hz)/<I>2

Voltage, mV

9.0x10⁻⁹

6.0x10⁻⁹

3.0x10⁻⁹
We found non-equilibrium 1/f noise in conical rectifying nanopores
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