Silicon Based Nanopore Sensors for Detection of DNA Molecules

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Introduction

- Nano for Bio:
  - Electrical Sequence Sensor
  - Biophysics of Interactions
- Bio for Nano:
  - Single molecule conductivity
  - Replacement of end-of-roadmap devices

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**Decrease in the Cost of Finished DNA Sequencing**

![Graph showing the decrease in the cost of finished DNA sequencing](http://campus.queens.edu/faculty/jannr/Genetics/images/FG13_01dogma.jpg)

**MOORE'S LAW**

![Graph showing Moore's Law](http://www.intel.com/technology/mooreslaw/index.htm)

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**Service, 2006**
Biological Nanopores

- 2.6 nm α-hemolysin channel
- Patterns to discriminate targets
- DNA–nanopore

[Image of DNA through an α-HL channel: Kasianowicz et al., 1996]

[Graph: Meller et al., 2000]

[Image: Howorka et al., 2001]
Solid-state Nanopores

- Why solid-state nanopores?
- DNA Characterization

- Filter Membrane with DNA Selectivity

[Chang et al., 2004]

[Chang et al., 2004]

[Chang et al., 2004]

[Kohli et al., Science, 2004]
Selective Nanopore Channels (NPCs) Motivation

- Nanopores
- Selectivity towards specific Targets

- Protein Ion Channels

[Kasianowicz et al., 2006]
Selective NPC Arrays
Motivation

• Miniature DNA analyzer
  – Single molecule events: Nanoscale
  – Interface: Macroscale

• Nanopores for interactions of:
  – DNA-DNA
  – DNA-protein

• Applications:
  – Personalized Medicine
    • Therapeutics
    • Diagnostics
  – Forensics, Legal (e.g. Paternity)
  – Gene Identification
  – Gene Disorder
The Big Picture

End Goals:

- Design Single Molecule Selectivity
- Detection of Specific Genes
NPC Fabrication

Key steps

- EBL to write dots
- TMAH etch
- Pore in SOI
- Oxidation
- Shrinking in TEM

Pore shrinking temporal profile of a nanopore channel

All Images at 1,000,000X. (NPC-2)
Selective NPCs
Overview

<table>
<thead>
<tr>
<th>Pore</th>
<th>Approximate Diameter (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPC-1</td>
<td>20</td>
</tr>
<tr>
<td>NPC-2</td>
<td>17</td>
</tr>
<tr>
<td>NPC-3</td>
<td>16</td>
</tr>
<tr>
<td>NPC-4</td>
<td>16</td>
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[Iqbal et al., Nature Nanotechnology, April 2007]
Selective NPCs Measurement Setup

- Custom LabView Software
- PC Data Acquisition Card
- Current PreAmplifier
- Ag/AgCl Electrodes
Selective NPC-1 Measurements and Analysis

- NPC-1: 20 nm dia
- **MM-DNA** target vs. Subsequent PC-DNA
  - Faster Translocation
  - Smaller Mean Passage Time
- 1MM-DNA target
  - before and
  - after PC-DNA

<table>
<thead>
<tr>
<th></th>
<th>NPC-1</th>
<th>1MM-DNA (in 120 min)</th>
<th>PC-DNA (in 120 min)</th>
<th>1MM-DNA after PC-DNA (in 120 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Signature of pulses</td>
<td>τ (ms)</td>
<td>I₀ (pA)</td>
<td>τ (ms)</td>
<td>I₀ (pA)</td>
</tr>
<tr>
<td>Mean</td>
<td>178.8</td>
<td>28.9</td>
<td>10.2</td>
<td>31.2</td>
</tr>
<tr>
<td>Sigma</td>
<td>260.3</td>
<td>31.7</td>
<td>30.4</td>
<td>27.8</td>
</tr>
<tr>
<td>Number of pulses</td>
<td>3,353</td>
<td>96,876</td>
<td>2,896</td>
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<tr>
<td>Signature of pulses</td>
<td>$\tau$ (ms)</td>
<td>$I_b$ (pA)</td>
<td>$\tau$ (ms)</td>
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<td>Mean</td>
<td>178.8</td>
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Selective NPC-2
Measurements and Analysis

- Smaller Pore
- More Selective
- More $I_b \rightarrow$ Higher % of the NPC blocked

One-base Mismatch

Perfect Complementary
Selective NPC-2 Measurements and Analysis

<table>
<thead>
<tr>
<th>NPC-2</th>
<th>3MM-DNA in 120 minutes</th>
<th>2MM-DNA in 120 minutes</th>
<th>1MM-DNA in 120 minutes</th>
<th>PC-DNA in 120 minutes</th>
<th>3MM-DNA after PC-DNA in 120 minutes</th>
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<tr>
<td>Signature of pulses</td>
<td>( \tau ) (ms)</td>
<td>( I_b ) (pA)</td>
<td>( \tau ) (ms)</td>
<td>( I_b ) (pA)</td>
<td>( \tau ) (ms)</td>
</tr>
<tr>
<td>Mean</td>
<td>32.8</td>
<td>22.4</td>
<td>7.1</td>
<td>23.2</td>
<td>42.9</td>
</tr>
<tr>
<td>Sigma</td>
<td>69.2</td>
<td>8.6</td>
<td>7.9</td>
<td>10.1</td>
<td>84.4</td>
</tr>
<tr>
<td>Number of pulses</td>
<td>7,866</td>
<td>15,693</td>
<td>300</td>
<td></td>
<td>238,560</td>
</tr>
</tbody>
</table>

- 3MM-DNA target before and after PC-DNA
  - Reduced \( \tau \)
  - Enhanced flux

Recognition/Binding sites activation after PC-DNA
Selectivity towards Single-Base Mismatch

- Channel-Molecular Interaction
  - MM-DNA vs. PC-DNA
  - Expectation
- PC-DNA
  - Interactions with Binding Sites
  - Faster and More than MM-DNA
- MM-DNA
  - Electrostatic Friction
  - Mechanical Resistance
  - Inability to open HPL

\[ J = \frac{n}{\tau} (c_1 - c_2) \]
Selective NPC-3 Bias Effects

- Electrophoretic Bias $\rightarrow$ 100 mV vs. 200 mV
- Consistent trend
  - DNA moves *slow* at decreased Bias
  - *Consistent* pulse trend between 1MM- & PC-DNA ($\tau$, $I_b$ and frequency)
Selective NPC-4
Temporal Viability

- **1:1 Mixture of One Mismatch and PC-DNA**
- **Behavior Shift from MM to PC-DNA**

(a) for the first 10 minutes, (b) for minute 11 to 20, (c) for minute 21 to 30, (d) for minute 31 to 40, (e) for minute 41 to 50, (f) for minute 51 to 60.
Selective NPC-4 Transitions

- Two regimes of Flux with respect to Time
- Flux Regimes $\rightarrow$ Effect of Binding Sites Activation
Diffusive Transport with Channel Interaction

- Protein channels provide **selective** pathways
- **Binding sites** affect transport
- PC-DNA transport with interactions
  - Shorter Mean First Passage Time ($\tau$)
  - Higher Flux ($J$)
- The flux of particles **interacting with channel**

\[
\begin{align*}
n &= \frac{L}{2} \left< e^{-\phi} \right> \\
\tau &= \frac{L^2}{2D} \frac{1}{\left< e^{\phi} \right> \left< e^{-\phi} \right>}
\end{align*}
\]

\[
J = \frac{D}{L} \frac{1}{\left< e^{\phi} \right> \left< e^{-\phi} \right>} (c_1 - c_2)
\]

[Bauer and Nadler, 2005 and 2006]
Channel Interaction
Attractive vs. Repulsive Potential

Key Assumptions
- **PC/HPL:** Attractive Potential
- **MM/HPL:** Repulsive Potential
- Magnitudes of Potentials
- $\phi$ span part of the channel

(a) Attractive potential (b) Repulsive potential, spanning part of the channel
Diffusive Transport with Channel Interaction

\[ w = 1 \]
\[ J_{PC} = \frac{D}{L} \frac{1}{e^{-\phi_{PC}}} c_1 \]
\[ J_{MM} = \frac{D}{L} \frac{1}{e^{\phi_{MM}}} c_1 \]

\[ \frac{J_{PC}}{J_{MM}} = e^{\phi_{MM}} e^{\phi_{PC}} \]

\[ w < 1 \]
\[ \tau = \left( \frac{L^2}{2D} \right) w(1 - w) e^{\phi} \]
\[ J_{PC} = J_o \frac{1}{1 - w} \]
\[ J_{MM} = J_o w e^{-\phi_{MM}} \]

\[ \frac{\tau_{MM}}{\tau_{PC}} = e^{\phi_{MM} - \phi_{PC}} \]

\[ J_{PC} > J_o > J_{MM} \]
\[ \tau_{MM} > \tau_{PC} > \tau_o \]
Free Energy and Melting Temperature

HPL-DNA Probe:

\[
\text{Amine-C6-5'} \quad \text{CCAACGGTTG} \quad \text{3'} \quad \text{GTTGGTGT}\]

Stem at 1 is 5 bp long, Loop = 10. \( \Delta G = -3.0, T_M = 74.8 \)

PC-/HPL-DNA:

\[
\text{Amine-C6-5'} \quad \text{CCAACGGTTGGTTGTGGTTG} \quad \text{3'} \quad \text{Sense}
\]

3' CCAACCAACACCAACC 5' Antisense

Stack at 6 is 15 bp long. \( \Delta G = -21.0, T_M = 34.4 \)

IMM-/HPL-DNA:

\[
\text{Amine-C6-5'} \quad \text{CCAACGGTTGGTTGTGGTTG} \quad \text{3'} \quad \text{Sense}
\]

3' CCAACCAACACTAACC 5' Antisense

Stack at 6 is 10 bp long. \( \Delta G = -11.0, T_M = -1.9 \)

Free energy \( \Delta G \): Stability of the duplex

\( T_M \): Temperature above which the duplex denatures into two single strands
NN Effects - $\Delta G$ Contributions

$\Delta G_{\text{MM}(0.1 \text{ M KCl})} = 0.93 \text{ kCal/mol}$

Vs.

$\Delta G_{\text{PC}(0.1 \text{ M KCl})} = -0.6 \text{ kCal/mol}$

Thus

$|\Delta G_{\text{MM}}| > |\Delta G_{\text{PC}}|$

in terms of chemical potentials

$|\phi_{\text{MM}}| > |\phi_{\text{PC}}|$
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