Nanopores and Nanofluidics for Single DNA Studies

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Overview

• Motivation: biological, physical, and technological

• The program:
  • develop and characterize new tools
  • apply these to the study of biological systems

• Nanofluidic devices

• Solid-state nanopores
The importance of biomolecules

• **The machinery of life**
  • the structure and function of biomolecules like DNA, RNA, and proteins tell us how living systems work

• **Biomolecules contain information**
  • The distribution of biomolecules within the cell indicates identity, activity, disease, etc.
  • Sensitivity to different biomolecules is the basis of medical diagnostics

• **Where a physicist fits in…**
  • Developing new tools, and exploring the fundamental science
Studying biomolecules at the nanoscale

Perhaps best way to study a molecule is the most direct: grab it and look at it!
Motivation for “nano-biophysics”

Biomolecules operate...

• Under water
• At micro- and nanometre length scales

Nanofabrication allows...

• The realization of ultra-small devices
• The handling of tiny amounts of fluid

Micro- and nanofluidics...

• Seem naturally suited to studying biological systems!
Silicon-based nano-biophysics
(The "lab-on-a-chip" concept)

Borrows the IC’s “smaller, cheaper, faster” paradigm, and its fabrication technology...
A vision for nanofluidic technology...

- The vision is to manipulate and analyze every component of a cell in molecular detail!

- We need to explore what is possible

Current leading research is focusing on...

**Applications:**
- Protein crystallization
- Protein detection and recognition
- Molecular separations
- Haplotyping
- DNA sequencing

**Science:**
- Single-molecule dynamics (polymers, enzymes, molecular motors)
- Materials science
- Fluid dynamics and electrokinetic phenomena

Any analysis!
Nanofluidic devices

• Confine and transport tiny quantities of fluid and molecules

• Nanochannels are the “wires” of a lab-on-a-chip

SEM image of a 28 nm high nanochannel cross-section

• What are their transport properties?
DNA in a pressure-driven flow

Pressure-driven $\lambda$-DNA motion in a 500 nm high channel

Pressure gradient applied using water columns

Nanochannel

$h=170\text{nm} - 3.8\mu\text{m}$
Length dependent DNA transport

We define the pressure-driven mobility, $\nu$, using

$$\bar{V} = \nu p$$
Length dependent DNA transport

There are two distinct regimes for pressure-driven DNA in channels:

- **Length-dependent regime**
- **Length-independent regime**

![Graph showing mean velocity vs. pressure gradient and channel height](image)
DNA as a random flight polymer

Equilibrium DNA conformations can be modeled using random flight statistics.

The Edwards diffusion equation

\[
\frac{b^2}{6} \nabla^2 P(z, s) = \frac{\partial P(z, s)}{\partial s}
\]

Hard wall boundary conditions

\[
P\left(z = \pm \frac{h}{2}, s\right) = 0
\]

The average density of DNA segments across the channel is given by:

\[
\rho(z) = \frac{1}{L} \int_0^L P(z, s) P(z, L - s)
\]

In our experiments:

\[R_g \approx 0.29\mu m, 0.46\mu m, \text{ and } 0.73\mu m\]
Modeling DNA transport

1. What is the pressure-driven mobility of DNA?
2. Equilibrium DNA conformations and Poiseuille fluid flow should apply in the low-shear limit.

\[
\bar{V} = \int_{-h/2}^{h/2} U_x(z)\rho(z)dz \bigg/ \int_{-h/2}^{h/2} \rho(z)dz
\]

- fluid flow profile
- average DNA segment density
Pressure-driven DNA mobility

Length-independent mobility in thin channels

\[ \rho(z) \propto \sin^2 \left( \frac{\pi z}{h} \right) \]

Length-dependent mobility in large channels

\[ \rho(z) \propto \tanh^2 \left( \sqrt{\pi} \left( \frac{|z| - h/2}{R_g} \right) \right) \]

Modeling the free energy landscape for DNA

Top view

Confinement free energy
Self-exclusion energy
Entropic elasticity
Viscous energy (work)
Fixed contour length

Side view

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Modeling DNA transport: single-pit occupancy

Increasing pressure

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Modeling DNA transport: double-pit occupancy

Increasing pressure

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The predicted transition from stochastic to deterministic transport was observed.

Time [minutes] 0 5 10

2 µm

Low pressure

High pressure

Position

Time [seconds] 0 10 20

2 µm

Direction of fluid flow
Electro-fluidics
manipulating charged molecules with electrostatic fields

[Diagram of electro-fluidics with labels for electrostatic fields, DNA, gate electrodes, effective potential energy, and position.]

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The electric double layer

Debye electrostatic screening length $\lambda_D$

charged surface

compact layer (Stern layer)

diffuse layer

Debye screening length: $\lambda_D \sim 1$ nm (0.1M)
10 nm (1mM)
100 nm (10 µm)
(for monovalent salt)

positive ion
negative ion

neutral fluid

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Measuring repulsive electrostatic forces on a single molecule in solution
A charged rod near a charged wall

Our simple model of DNA interacting with a charged nanochannel wall follows the method described by Onsager for colloidal particles:

The energy of a charged rod at a given distance and angle is:

The excluded width due to electrostatics incorporates the Boltzmann factor follows:

This can be integrated, and is well approximated by:

We expect an excluded region near each wall that is a few times the Debye screening length.
Confinement induces reproducible changes in the size of a polymer

D.J. Bonthuis, C. Meyer, D. Stein, and C. Dekker

Solid-state nanopores
Basic principles of nanopore sensing

A biological nanopore as an electronic molecule sensor

“Low resolution RNA sequencing”

Fabricating nanopores in a transmission electron microscope
Single-DNA detection using a solid-state nanopore

DNA length discrimination by single-molecule electrophoresis

The translocation time is a measure of molecular length
The nanopore senses molecular folds!
Extracting sequence information using sequence-specific binding

MIZF is a zinc finger protein that binds to dsDNA in a sequence-specific manner.
Controlling the translocation of DNA using optical tweezers

Our goal is to generate a "DNA barcode" that contains sequence information in the electrical signal.

Optical tweezers & nanopores first demonstrated by:
Electrostatically gated nanopores
(mimicking biology by opening and closing a pore)
Probing DNA by transverse electronic tunneling

Perhaps metallic carbon nanotubes would make the ideal tunneling electrodes?
Nanopores milled through CNT’s embedded in a silicon nitride membrane

TEM of buried CNT

...with nanopore (9 nm gap)

CNT

TEM of buried CNT

...with nanopore (12 nm gap)

CNT

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Idea: Sequence DNA by combining nanopores with mass spectrometry

**Appeal:**
1) Contrast; 2) Single-ion sensitivity; 3) Bandwidth; 4) Robustness
Conclusions

• Individual molecules can be manipulated and studied in new ways thanks to “Lab-on-a-chip”-style nanostructures.

• Certain physical phenomena become particularly important to the behavior of devices at the nanoscale:
  • Statistical properties
  • Electrostatic effects
  • Molecular size exclusion

• Exciting opportunities for science and technology still await at the nanoscale
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Ion beam sculpting a nanopore

Idea: gain nanometre control by incorporating feedback

The feedback-controlled ion beam sculpting apparatus
Discovery of a new matter transport phenomenon

- Temperature = 28°C
- Incident energy = 3kV
- Incident flux = 47 Ar
- Pulsed beam duty cycle: 200ms on/1s total

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A surface diffusion model of ion beam sculpting

The surface diffusion model predicts the flux dependence of nanopore formation

Information from a nanopore signal

The duration and the amplitude of an event provide information about the translocating molecule.
DNA translocation distributions

a) 3 nm SiN pore

b) 10 nm SiN pore

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A study of individual translocation events reveals two distinct populations.