DNA Replication
(Copying DNA Prior to Cell Division)
(movies)

http://www.dnai.org/a/index.html
Alberts Figure 4-5
What actually happens: Okazaki fragments

Alberts Figure 5-12

Alberts Figure 5-13
Nanopore Analysis of Macromolecular Structure & Function
Tech talk, Google, 6/1/07

Dave Deamer and Mark Akeson
Biomolecular Engineering & Chemistry Departments
University of California, Santa Cruz
Nanoscale Pores

• Fundamentals of nanopore analysis
• Energy, size, dynamics
• Structural dynamics of dsDNA
• High speed sequencing

Song et al., Science 1996

Li, Golovchenko, Branton et al. 2002
$\alpha$-hemolysin

song at al., science 1996.
Hemolysin pore size: Comparable to enzymes and ribosomes

$E. \text{coli}$
Exonuclease I  $\alpha$-Hemolysin

Ribosome (Noller & coworkers)
SELF-ASSEMBLY OF THE HEMOLYSIN PORE

Membrane insertion

Hagan Bayley, Scientific American
$\alpha$-hemolysin

2.0 nm

100 mV

1 M KCl
70 µl
Hemolysin, dsDNA and ssDNA

- dsDNA: 2.2 nm
- ssDNA/RNA: 1.3 nm
- 10 bp (1 Turn of DNA Helix)
- 3.4 nm
- 13 nm
DNA hairpins in the nanopore

Senior, PNAS, v 85, 1988
A nanopore can detect single base pair differences between DNA hairpins.
One 6bp hairpin molecule
Vercoutere et al., Nature Biotechnology 2001
Dwell time is predicted by duplex stability

The graph shows a linear relationship between the average duration (log ms) and the free energy change ($\Delta G^\circ$) for hairpin formation (kcal/mol). The x-axis represents $\Delta G^\circ$ Hairpin Formation (kcal/mol), while the y-axis represents the average duration (log ms). The graph includes data points for 3bp, 4bp, 5bp, 6bp, and 8bp.
The diagram illustrates the comparison between a 6bp and a 6bpA14 loop structure. The 6bp loop contains the sequence TTGCGAAT with a ΔG of -8.2 kcal/mol, while the 6bpA14 loop has the sequence TTGCGAATA with an A base added and a ΔG of -4.3 kcal/mol. The A base in 6bpA14 stabilizes the structure compared to 6bp.
Detection of a single base pair mismatch
Discrimination among individual Watson-Crick base-pairs
hairpin is captured
Molecular Machines, DNA, and Nanopores

Google
1 June 2007

Biophysics Laboratory
Biomolecular Engineering & Chemistry Departments
University of California, Santa Cruz
Cost of DNA Sequencing in dollars per base pair
Moore’s law extrapolation

Cost per finished bp in US Dollars

$1000 per mammalian genome
High Speed DNA Sequencing

Cost per finished bp in US Dollars

$1000 per mammalian genome
Single Molecule Sequencing
Using a Nanoscale Pore

U.C. Davis, 1991
NHGRI HIGH SPEED SEQUENCING MEETING
Marco Island, Florida April 2006

![Photo of attendees at the NHGRI High Speed Sequencing Meeting 2006]

![Graph showing the increase in Nanopore Articles Year⁻¹ from 1996 to 2005, with notable publications in UCSC and PNAS]

- UCSC
- PNAS

Year:
- '96
- '97
- '98
- '99
- '00
- '01
- '02
- '03
- '04
- 2005

Nanopore Articles Year⁻¹:
- 0
- 50
- 100
- 150
- 200
- 250
- 300

Note: The graph shows a significant increase in Nanopore research publications from 1996 to 2005, with notable contributions from UCSC and PNAS publications.
Nanoscale Pores

1) Technology based on first principles

2) Relevant scales

Guo et al., 1996

Li, Golovchenko et al.
WHAT ACCOUNTS FOR THE GROWING POPULARITY OF NANOPORES FOR DNA & RNA ANALYSIS?

1) BASED ON FIRST PRINCIPLES LEADING TO A ROBUST INSTRUMENT THAT IS CONCEPTUALLY STRAIGHT-FORWARD.

OHM’S LAW

ELECTROPHORETIC TRANSPORT
Size of the nanopore sensor relative to a cell

~ 1:5000 the diameter of a mammalian cell

Human Embryo (8 Cell Stage)
The $\alpha$–Hemolysin Pore is the Scale of Interesting Structures Within DNA and RNA

- dsDNA: 22 Å
- ssDNA/RNA: 13 Å
- 10 bp (1 Turn of DNA Helix): 34 Å
- GTP
- GDP
- 50S
- 30S
- mRNA
Scale of the electric field across a model pore

<table>
<thead>
<tr>
<th>Field Strength (V/cm)</th>
<th>200 mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well</td>
<td>100 Å</td>
</tr>
<tr>
<td>Sensor</td>
<td>50 cm</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Field Strength (V/cm)</th>
<th>5000 V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well</td>
<td>100 Å</td>
</tr>
<tr>
<td>Sensor</td>
<td>50 cm</td>
</tr>
</tbody>
</table>
A 6 \( K_b T \) DIFFERENCE BETWEEN INDIVIDUAL MOLECULES IS EASILY DISCERNED

\[
GTP \rightarrow GDP + Pi \quad \sim 12 \ K_b T \text{ at Physiological Temp}
\]
Small Angle X-Ray Scattering (SAXS) of HK97 Phage Prohead in Solution

X-ray scattering measurements were performed on Beam Line 4-2 at the Stanford Synchrotron Radiation Laboratory, Menlo Park, CA. Courtesy of Kelly Lee (Scripps).
Particle flux through the nanopore detector is high.

**SAXS (Stanford Synchrotron)**

- Flux
  - Particles/mm²-s
  - $10^{13}$ photons

**alpha-HL Nanopore**

- $10^{15}$ ions
## Devices Used for Analyzing Dynamics of RNA/DNA and Associated Enzymes

<table>
<thead>
<tr>
<th>Device</th>
<th>High Throughput?</th>
<th>Linkers Required?</th>
<th>Frequency Attained</th>
<th>1 nt Measurements</th>
<th>Cost of State-of-the-Art Instrument for Biological Polymers</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFM</td>
<td>No</td>
<td>Yes</td>
<td>NA</td>
<td>NA</td>
<td>$150,000</td>
</tr>
<tr>
<td>Laser Tweezers</td>
<td>No</td>
<td>Yes</td>
<td>1 Hz</td>
<td>10,000 Hz</td>
<td>$$$!</td>
</tr>
<tr>
<td>Nanopore</td>
<td>1000’s min⁻¹</td>
<td>No</td>
<td></td>
<td></td>
<td>$500</td>
</tr>
</tbody>
</table>
Making solid state nanopores: *Ion Beam Sculpting at Harvard*

**Sputter Etching**
- **Ar⁺ Beam**
- **Si₃N₄**
- **Cavity**
- **Nanopore**
- **Low Temperature**

**Lateral transport**
- **Ar⁺ Beam**
- **Si₃N₄**
- **Pore**
- **Nanopore**
- **Room Temperature**
Ion Beam Sculpting System

- Ion Gun
  - Duty Cycle
  - Flux
- Si$_3$N$_4$ membrane
- Ion focusing optics
- Ion energy analyzer

- Ion Gun Control
- Single ion detector
- Stop signal
- Counter
**Closing a Hole**

**EM Images**

- Top image: 70 nm
- Bottom image: 1.8 nm

**Graph:**
- **Ar^+ (Counts/sec)**
- **Time Sputtered (sec)**
- Ar^+ (Energy) = 3 KeV
  - Si$_3$N$_4$ = 500 nm, 28°C
  - Flux = 28 Ar^+/sec/nm$^2$
  - Sputter on 200 ms/sec
Tunneling into Nanotube-DNA Complex

- Potential Energy
- Tunnelling electrons
- Nanotube Emitter
- Water
- DNA-Nanotube Collector
- Applied voltage
Implementing Device Fabrication

(a) Nanotube across FIB pore
(b) Nanotube across a nanopore after ALD
(c) Nanotube articulated nanopore
SELF-ASSEMBLY UNDER PHYSIOLOGICAL CONDITIONS

Membrane insertion

Hagan Bayley, Scientific American
$\alpha$-hemolysin

100 mV

1 M KCl

70 µl

2.0 nm
Reading along one strand:
Baldarelli CIP
Reading duplex ends in the pore vestibule
One 6bp hairpin molecule

pA vs Duration (log ms)
Vercoutere et al., Nature Biotechnology 2001
B form DNA

3.4 Å

22 Å
hairpin is captured
Sequence-Dependent Dynamic Changes in Duplex Structure
rise ($D_z$)  
slide ($D_y$)  
propeller twist ($\omega$)
A) 9bpGACG

B) Number of Observations

UL 'B'

UL 'A'

C2’ endo Sugar Pucker

C3’ endo Sugar Pucker

'B' Form DNA

'A' Form DNA & RNA
4) Coupling processive DNA/RNA enzymes to pores

- **E. coli** Exonuclease I
- **E. coli** Klenow Fragment
- **E. coli** Ribosomes

*Biophysics Laboratory, Department of Biomolecular Engineering, U.C. Santa Cruz*
E. coli Exonuclease I

13 nucleotides

Catalytic Domain
Proof-reading 3′ - 5′ Exonuclease of *E. coli* DNA polymerase III

Hamdan et al. *Structure* 2002
Biochemistry is confirmed on the pore using 3′-P ssDNA vs 3′-OH ssDNA.
Single-molecule analysis of DNA-protein Complexes using nanopores


Biophysics Laboratory,
Center for Biomolecular Science & Engineering, U.C. Santa Cruz
Lone 64mer Oligonucleotide Event Profile
1uM:1uM Exo I to 64mer Oligonucleotide
VARYING FORCE THROUGH RAMPED VOLTAGE

![Graph showing varying force through ramped voltage](image)
Polymerases and Nanopore

Biophysics Laboratory,
Biomolecular Engineering, U.C. Santa Cruz
Structure & Function of Klenow Fragment

Bell and Baker, *Cell* 1998
DNA sequencing with chain-terminating inhibitors
(DNA polymerase/nucleotide sequences/bacteriophage φX174)

Fig. 7. Mechanism for enzymatic DNA replication.
Binding States Revealed in Single Events
Mean dwell time = 4 ms
Current = 21 +/- 2 pA
Field-programmable Gate Array (FPGA) Hardware Enables Fast Control Logic Execution

FPGA Combines Speed and Flexibility for Fast Signal Monitoring and Voltage Control

- **FPGA** – multiple tasks in parallel with no overhead
- **PC with DAQ card** – tasks are performed in series with overhead of OS

Images taken from: apple.com, fluke.com, ni.com, and xilinx.com
Transition from Ternary to Binary Complex is Detectable in Real Time

a) Ternary complex indicated by ~100 ms event at 24 pA.
b) Transition to Binary complex indicated by drop to 20 pA.
c) Duplex shears after 5-10 ms.

Nearly ALL events longer than 20 ms display this pattern.
Correct dNTP absent: 15/589 (2.5%) events > 20 ms.
Correct dNTP present: 505/839 (60%) events > 20 ms.
Hypothesis: If event is longer than 20 ms, ternary complex is present.
<table>
<thead>
<tr>
<th></th>
<th>Total No. of events</th>
<th>No. of long (&gt;20ms) events</th>
<th>No. of times 2 sequential long events occurred</th>
<th>No. of times 3 sequential long events occurred</th>
<th>No. of times 4 sequential long events occurred</th>
<th>No. of times 5 sequential long events occurred</th>
</tr>
</thead>
<tbody>
<tr>
<td>14 bp</td>
<td>686</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>14 bp + KF</td>
<td>589</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>14bp + KF + dGTP</td>
<td>839</td>
<td>505</td>
<td>306</td>
<td>178</td>
<td>108</td>
<td>67</td>
</tr>
</tbody>
</table>
Initial FSM-Controlled Nanopore Experiments Demonstrate Real-Time Detection and Reaction to Complexes

3 ms to detect + 20 ms time threshold.

For this data, after 20 ms, voltage set to zero, and molecule exits pore.

455 events median = 23 ms (1.4)