## Manipulating Single Molecules

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Nature Reviews MCB, November, 2000, 130-136

C. J. Bustamante, J. C. Macosko and G. J. L. Wuite
## Choosing a Method

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<tr>
<th>Methods</th>
<th>$F_{\text{min-max}}$ (N)$\S$</th>
<th>$X_{\text{min}}$ (m)$\S$</th>
<th>Stiffness (N m$^{-1}$)</th>
<th>Applications</th>
<th>Practical advantages</th>
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<tbody>
<tr>
<td>Cantilevers$^*$</td>
<td>$10^{-11}$–$10^{-2}$</td>
<td>$10^{-10}$</td>
<td>0.001–100</td>
<td>Protein/polysaccharides$^{6,64}$</td>
<td>High spatial resolution</td>
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<td>Bond strength$^{65,66}$</td>
<td>Commercially available</td>
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<tr>
<td>Microneedles$^*$</td>
<td>$10^{-12}$–$10^{-10}$</td>
<td>$10^{-9}$</td>
<td>$10^{-6}$–1</td>
<td>Myosin motor force$^{12}$</td>
<td>Good operator control</td>
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<td>DNA/titin strength$^{26,28}$</td>
<td>Soft spring constant</td>
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<td>Flow field$^f$</td>
<td>$10^{-13}$–$10^{-9}$</td>
<td>$10^{-8}$</td>
<td>n.a.</td>
<td>DNA dynamics$^{38}$</td>
<td>Rapid buffer exchange</td>
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<td>RNA polymerase$^{36}$</td>
<td>Simplicity of design</td>
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<td>$10^{-14}$–$10^{-11}$</td>
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<td>DNA entropic elasticity$^{8}$</td>
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<td>Topoisomerase activity$^{41}$</td>
<td>Ability to induce torque</td>
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<td>Protein motors$^{13,14}$</td>
<td>Specific manipulation</td>
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<td>Protein unfolding$^{52}$</td>
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Atomic Force Microscopy (AFM)
AFM Images of Huntingtin
Stretching DNA With AFM

- 1,400 bp (base pair) DNA strand
- Contour Length ~470 nm

Force (pN) vs. Distance (nm) graph
Microneedles

Nature 334, 1988, 74-76  A. Kishino and T. Yanagida
Pulling DNA With Microneedles

PNAS October 13, 1998, 12295-12299
Microneedles and Microscopy

# Manipulation: Force Fields

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Flow Fields

Science 268, 1995, 83-87
Flow Fields and Molecular Motors

Science 287, 2000, 2497-2500
R. J. Davenport, G. J. Wuite, R. Landick and C. J. Bustamante
Movie of RNAP Motor in a Flow
Magnetic Fields
Pulling DNA With Magnets and Flow

Science 258, 1992, 1122-1126  S. B. Smith, L. Finzi and C. Bustamante
Twisting DNA With Magnets

Nature 404, 2000, 901-904  T. R. Strick, V. Croquette and D. Bensimon
Photon Fields
Fishing for DNA With Laser Tweezers
Balancing Signal, Noise and Time

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Bandwidth and Corner Frequency

Equi-partition theorem: $<\Delta x^2> = k_b T / \kappa$

$S/N = F(2\gamma k_B T B)^{-1/2}$

$\gamma$: friction coefficient, $B$: Bandwidth
Stiffness and Drag

\[ \omega_c = \frac{\kappa}{\gamma} \]

- \( \omega_c \): corner frequency
- \( \kappa \): spring constant

- 3.5 \( \mu \)m Bead
- Pipette
- 2.2 \( \mu \)m

- 0.1 \( \frac{pN}{nm} \) = 1 kHz
- 0.1 \( \frac{\mu N \text{sec}^{-1}}{m} \) = 1 kHz
- 180 \( \mu \)m cantilever

- 260 \( \frac{pN}{nm} \) = 40 kHz
- 6.5 \( \frac{\mu N \text{sec}^{-1}}{m} \) = 40 kHz
Using Single Molecule Techniques to Probe Mechanochemistry

Keller & Bustamante Biophysical J. 78:541-56 2000
HIV RT Force Measurements

- **Stall force**: 14 pN
- **Stall time**: 1.9 s
- **Rupture force**: 46 pN
- **Total run time**: 3.6 s

Stretch can be used to estimate length of remaining DNA:
About 28 bases polymerized in this run
Using Force to Find $\Delta x$

\[ v = v_0 e^{-\frac{(\Delta H_x - F\Delta x)}{k_B T}} \]

Log velocity vs. F
7 Å finger motion

Slope at 0 pN
Rate limiting
Force generating

Potential surface

Value at 0 pN?
Single Molecule Imaging

Use fluorescence for:
- Zero force
- High throughput

Keep in mind:
- Non-specific binding
- Bleaching, etc.

Avalanche photodiode and CCD camera for:
- Fast time resolution
- Spatial information
Sample Experimental Design

3' GACCCACCAG 5'

5' CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCGCCGGTGTC 3'

Fluorescently labeled T's (Non-fluorescent G's also in solution)

polymerization

GACCCACCAG 5' PRIMER

ACTGGGTGGTC 3' GATE BASE

T

1 2 3

4 5 6

7 8 9
Fluorescence Persistence Data

![Graph showing fluorescence intensity over time with marked start and end times.](image-url)
Fitting the Data to the Model

10 Bases
\[ k_{\text{poly}} = 95 \text{ bases / sec} \]

20 Bases
\[ k_{\text{poly}} = 106 \text{ bases / sec} \]

30 Bases
\[ k_{\text{poly}} = 105 \text{ bases / sec} \]

40 Bases
\[ k_{\text{poly}} = 106 \text{ bases / sec} \]
Using Temperature to find $E_a$
FRET Study of Transcription
RNA Polymerase Tracking

Stalled Elongation Complex

Mica Surface

Piezo

Piezo Extension vs. Time

Time (ms)

Piezo extension (Angstroms)

18 20 22 24 26 28