Conference: From DNA-Inspired Physics to Physics-Inspired Biology

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Shape and Dynamics of a Single Molecule of DNA

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“Bodies of microscopically visible size suspended in liquids must, as a result of thermal molecular motions, perform motions of such magnitude that these motions can easily be detected by a microscope.”

— A. Einstein

*Annalen der Physik* 17, 549 (1905)
The ABEL trap

Anti-Brownian ELectrokinetic trap

• Track the motion of a single particle using fluorescence microscopy
• Use real-time feedback to impose an electrokinetic drift that exactly cancels the Brownian motion of a single particle.

Factor of $k$ decrease in particle radius $\rightarrow$
factor of $k^3$ increase in power in optical trap; but only
factor of $k$ increase in speed in ABEL trap.
Gen. 2: Digital Feedback, Remote Electrodes

- Purely microfluidic system (no microfab. electrodes)
  - Trapping region connected by 4 fluidic channels to macroscopic electrodes
- Electrogenated products kept away from trapping region
- Feedback force may be either electrophoretic or electroosmotic

Control without feedback

200 nm particles

Molecules of λ-DNA
ABEL trap traps smaller particles than any comparable technology

Polystyrene nanospheres
100 nm
20 nm

CdSe nanocrystal in 50% glycerol

Lipid vesicle

Tobacco Mosaic Virus

B-Phycoerythrin in 40% sucrose

GroEL in 50% glycerol

structure

structure

Polymer Dynamics

Theory

Rouse
- Beads only have nearest-neighbor forces

Zimm
- Beads have hydrodynamic coupling to other beads → Nonlinear equation of motion
- Mean-field approximation linearizes equations of motion

De Gennes
- Calculated Scaling laws and relaxation times based on Rouse and Zimm models.

Experiment

Dynamic Light Scattering

Force-extension curves

http://www.stanford.edu/group/blocklab/RNAP.html

Stretching and flow

Phys. Rev. Lett. 95, 018301

Trapping molecules in equilibrium
DNA single molecules at equilibrium

SAMPLE
- λ-DNA labeled with YOYO-1
- Contour length ~ 20 μm
- Radius of gyration ~ 700 nm

DATA
- Images 32 x 32 pixels
- One image every 4.5 ms
- 21 separate molecules studied
- ~ 60,000 video frames

Many ways to slice the data
Mean density distribution is not Gaussian

Mean intensity relative to C.M.

Experimental distribution has fat tails

\[ P(x) = \frac{3}{2\pi R_G^2} \int_0^1 \frac{3 \exp\left(-\frac{3x^2}{2R_G^2(3\varepsilon^2 - 3\varepsilon + 1)}\right)}{(3\varepsilon^2 - 3\varepsilon + 1)} \, d\varepsilon \]

Still only 1 free parameter: \( R_G \)

\( R_G: 689 \text{ nm} \)
Center of mass diffusion reflects conformational dynamics

Pseudo-free trajectories

“What the trajectory would have been if the trap were off”

1) Extract the mobility from the measured displacements and voltages

2) Residuals are due purely to Brownian motion:

\[ C^{(4)}(\tau) = \text{corr} \left( \Delta x(t + \tau)^2, \Delta x(t)^2 \right) \]

Probe fluctuations in \( D \) with 4\(^{th} \) order correlations

Pseudo-free trajec

Measured trajec

4\(^{th} \) order correlation shows fluctuations in \( D \)
DNA internal correlations probe mechanical response function

Most general 2-point covariance:

$$C(r_1, r_2, \tau) = \langle \delta I(r_1, t + \tau) \delta I(r_2, t) \rangle$$

where: \( \delta I \equiv I - \langle I \rangle_t \)

Covariance related to mechanical response via Fluctuation-Dissipation Theorem:

$$\chi(r_1, r_2, \tau) = -\frac{1}{k_B T} \frac{d}{d\tau} C(r_1, r_2, \tau)$$

Fluorescence Correlation Spectroscopy and Dynamic Light Scattering probe low-dimensional projections of \( C(r_1, r_2, \tau) \):

Spatial decay of autocorrelation

Eigenfunctions of $C(r_1, r_2)$

90% of variance in first 34 eigenfunctions

Equal-time covariance matrix:

$C(r_1, r_2) = \langle \delta I(r_1, t) \delta I(r_2, t) \rangle_t$

DNA Normal modes

Stiffness (AU)

# of azimuthal nodes

$10^2$

$10^1$
Different Approaches to PCA

Lagrangian Approach

Follow each particle $r_i(t)$, and calculate the covariance $\langle r_i(t)r_j(t + \tau) \rangle$

Leads to Rouse modes for a random walk

Eulerian Approach

Measure quantities in the lab frame, (e.g. $\rho(r, t)$, $j(r, t)$) and calculate the covariance $\langle \rho(r_1, t)\rho(r_2, t+\tau) \rangle$

Eulerian approach depends on interchange symmetry of all particles
Model of the Principal Components

Density distribution of chain
\[ \rho(x_1) = \sum_{\alpha=0}^{N} \delta(x_1 - x_\alpha) \]

Sum over chain elements
Coordinate of chain element

Joint density distribution
\[ \rho(x_1) \rho(x_2) = \sum_{\alpha, \beta=0}^{N} \delta(x_1 - x_\alpha) \delta(x_2 - x_\beta) \]

Expansion in Rouse Modes
\[ x_\alpha = \sum_{\nu=1}^{\infty} c_\nu \cos \left( \frac{\pi \nu \alpha}{N} \right) \]
Gaussian distributed coefficients
\[ \langle c_\nu c_\mu \rangle = 1/(\pi \nu)^2 \delta_{\nu\mu} \]

Formula for joint probability density (with fixed C.M.)
\[ \langle \rho(x_1) \rho(x_2) \rangle = \sum_{\alpha, \beta=0}^{N} \frac{6}{\pi \sqrt{16F(\alpha)F(\beta) - G(\alpha, \beta)^2}} \exp \left[ \frac{-24F(\beta)x_1^2 - 24F(\alpha)x_2^2 + 12G(\alpha, \beta)x_1x_2}{16F(\alpha)F(\beta) - G(\alpha, \beta)^2} \right] \]

\[ F(\alpha) \equiv 3 \frac{\alpha^2}{N^2} - 3 \frac{\alpha}{N} + 1 \]
\[ G(\alpha, \beta) \equiv 3 \left( \frac{\alpha}{N} + \frac{\beta}{N} - 1 \right)^2 + 3 \left( \left| \frac{\alpha}{N} - \frac{\beta}{N} \right| - 1 \right)^2 - 2 \]
Normal modes fit random walk model

<table>
<thead>
<tr>
<th>Data</th>
<th>Analytical</th>
<th>Numerical</th>
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| ![Data Image](image) | Microscopic model based on 2-d random walk  
Analytical expression for covariance matrix; diagonalized numerically | 2-D Rouse polymer |

Eigenfunctions

![Eigenfunctions Images](image)
Simulated Eigenfunctions in 3-D

Computed from 3-D simulations of a random walk polymer

1s

2d

3p

2s

2f
Transitions between eigenstates obey selection rules

\[ \text{Covariance: } \langle a_i(t) a_j(t + 18 \text{ ms}) \rangle \]

Also saw signs of nonlinear hydrodynamics...
Dynamics of Eigenstates

Dynamics of diagonal and off-diagonal mode covariance

Power-law scaling of mode relaxation times

\[ \tau_k \sim k^{-1/2} \]
Nonlinear dynamics in DNA

Fit dynamics to linear model:

\[ a(n + 1) = M a(n) + \xi(n) \]

Look for structure in residuals:

\[ \tilde{\rho}^{(3)}_{mn}(\tau) = \frac{\langle \xi_m^2(t + \tau) a_n(t) \rangle}{\text{var}(\xi_m) \text{var}(a_n)^{1/2}} \]
What’s Next

• More statistics, better statistics
• Biophysical experiments (GroEL)
• New instrumentation:

Maxwell’s Demon
Use feedback to “violate” the second law

Fabrication
Trapping in a photopolymerizable medium

Orientation
Use quadrupolar and AC fields to orient molecules
Summary

ABEL trap allows one to:

• Trap, manipulate, and study individual fluorescent molecules in solution

We have, for the first time:

• Trapped single molecules of DNA in equilibrium

Questions for the Audience:

• What can be learned about transport coefficient ($\mu(t)$ and $D(t)$) from an observation of a trajectory?

• How to calculate $\mu(t)$ and $D(t)$ for objects with complex shapes and complex patterns of surface charge?

• Are there analytical approximations for the PCA eigenfunctions and eigenvalues of a random walk? Are there other scenarios where this analysis is useful?
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