Close encounters with DNA

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WHAT IS LIFE?

The Physical Aspect of the Living Cell

BY

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Based on Lectures delivered under the auspices of the Institute at Trinity College, Dublin, in February 1943

CAMBRIDGE

AT THE UNIVERSITY PRESS

1948
DNA, the blueprint

mass: 6 pg
length: 2 m
thickness: 2nm

...GTGTGACTCGT
GGTCCGTAATGTC
GTATATGGTGACC
GTGTGGCCTGATG
GTGTGGCCTGATG...

http://www.accessexcellence.org/AB/GG/chromosome.html
DNA code is written in atoms

Highly charged: 2 electron charges per 0.32nm

Double stranded DNA (persist. length ~50nm)

The sequence has direction: 5’-AAGCTGGTTTCAG-3’

Single stranded DNA (persist. length ~1.5nm)
DNA code is written in atoms

Highly charged: 2 electron charges per 0.32nm

Double stranded DNA (persist. length ~50nm)

The sequence has direction:
5’-AAGCTGGTTCAG-3’

Single stranded DNA (persist. length ~1.5nm)
The sequence contains biological information

Central dogma of molecular biology

The physical properties enable functionality
Molecular dynamics simulations, a computational (force) microscope

Massive parallel computer
Blue Waters, ~200,000 CPUs

Atoms move according to classical mechanics \( (F = ma) \)

Time scale: \(~ 0.1\text{-}100\ \mu\text{s}\)
Length scale: \(10\text{K} - 100\text{M atoms or } (< 50\ nm)^3\)

Interaction between atoms is defined by molecular force field
DNA systems

http://micro.magnet.fsu.edu/cells/nucleus/chromatin.html

Graphene nanopore sequencing

DNA replication and repair
Micromechanics of single DNA molecules

Stretching ssDNA with external force

AFM probe (Si₃N₄)

ssDNA

Substrate (Au)

Simulation: poly(dA)

This end is fixed

V=const
DNA is effectively infinite in 3D. Periodic boundary conditions in 3D imply that the system is considered to be infinite. Additional applied force simulations are shown, with red indicating compression and blue indicating stretching. The figure illustrates that $P_{zz} < 0$ stretches the system in the $Z$ direction.

Simulations using anisotropic pressure control.
Different modes of dsDNA stretching

Nicked: force increases gradually as DNA unwinds

Torsionally constrained: DNA pops, force-extension curve is non-monotonous

Luan and Aksimentiev, PRL 101:118101 (2008)
Visualizing MD Results:
Mechanical Properties of dsDNA Mini Tutorial

Part 1. Structure of DNA and Simulation System

Part 2. Stretching dsDNA (torsionally constrained)

Part 3. Stretching Nicked DNA (torsionally unconstrained)
Interesting physical properties

F
[Diagram: Two negatively charged particles repelling each other.]

Same sign charges repel (in vacuum)

F
[Diagram: Two negatively charged particles attracting each other in a medium.]

Same sign charges can attract (in a medium)

Effective attraction between DNA is observed when counterions have charge ≥ 2e

DNA lives in water and is surrounded by counterions
Direct MD simulation of DNA-DNA force


A virtual spring measures the effective force

![Graph showing force vs. distance with two curves for Na⁺ and Mg²⁺ ions.](image)
Simulations of side-by-side DNA repulsion are in good agreement with experiment

Experiment: Ralf Seidel (Dresden)


In monovalent electrolytes, the effective charge of dsDNA is about 41% of its nominal charge
MD simulation of dense DNA arrays

What we control

DNA density (or harmonic constraint radius)

\[ [\text{Na}^+]_{\text{buf}} \sim 200 \text{ mM} \]

\[ [\text{Mg}^{2+}]_{\text{buf}} \sim 0 \text{ or } 20 \text{ mM} \]

What we measure

Pressure as a function of [ion] & [DNA]

DNA / ion distribution:

DNA / ion diffusion inside the array

Water / salt free to move

Cylindrical harmonic constraint (radius of 10 – 12 nm) only against DNA
The standard MD force field fails to predict internal pressure of a DNA array.

Too strong Na/Mg-phosphate attraction induces artificial DNA clusters!! [Na] ~ 4M!!

Interaxial distance /nm

Recalibrate ion-DNA parameters using osmotic pressure data

- Osmotic pressure is directly related to ion-pair formation: \( \pi = \phi cRT \)
- Pros: modify only ion-DNA phosphate interaction, without altering ion-water interaction.
- Cons: nothing.

* Luo & Roux, JPCL (2009)
Improved parametrization of ion-DNA interactions

\[ [\text{Mg}]_{\text{buf}} \sim 20 \text{ mM} \]
\[ [\text{Na}]_{\text{buf}} \sim 200 \text{ mM} \]

\[ \text{J. Phys. Chem. Lett. 3:45 (2012)} \]
Short dsDNA fragments form end-to-end aggregates

Experimental evidence of end-to-end aggregation of short dsDNA fragments motivated simulations to determine whether aligned DNA fragments would collapse to an end-to-end assembly.

Adapted from Science 318:5854

Chris Maffeo
Proper connection requires a terminal phosphate
Further simulation reveals the strength of the end-to-end DNA interaction

*Nucleic Acids Research* 40:3812 (2012)

The assembly proved stable in the absence of restraints during 600 ns of simulation.

Steered molecular dynamics gave a rupture pathway involving shearing of the DNA ends.

Umbrella sampling revealed that the free energy for the interaction is ~6.5 kcal/mol.
Standard binding free-energy of end-to-end assembly

Result: \( G_{\text{bind}} = -6.3 \pm 1 \text{ kcal/mol} \) for a DNA concentration of 1 M in 0.12 NaCl

\[ G_{\text{bind}} = G_{U_{\theta} \text{ on, } r=r^*} + G_{r^* \rightarrow r^{\dagger}} + G_{r^{\dagger} \rightarrow b} + G_{U_{\theta} \text{ off, } b} \]
Simulation of DNA aggregation

A simulation of 458 DNA fragments (~56 mM) allowed observation of unbiased end-to-end aggregation.

Assuming aggregation kinetics are independent of aggregate length, we extract kinetic rates to obtain an independent estimate of $G_{\text{bind}}$.

\[
G_{\text{bind}} = -k_B T \log \frac{k_{\text{on}}}{k_{\text{off}}} = -5.5 \text{ kcal/mol}
\]

Poisson statistics suggests 1 rupture in 250 ns corresponds to $k_{\text{off}} \sim 67 \mu s^{-1}$. 

Wednesday, November 27, 13
Significance of end-to-end interaction depends on concentration of DNA ends

*Nucleic Acids Research* 40:3812 (2012)

- **Concentration of DNA ends is** too small for significant blunt-ended cyclization
- **End-to-end adhesion may** aid repair of double-stranded DNA breaks, since the DNA ends are held in proximity
- Free energy of end-to-end assembly is DNA concentration dependent: 1.4 kcal/mol for every 10-fold drop in concentration
- High concentration of short DNA fragments results in aggregation and subsequent formation of liquid crystals
- X-ray scattering indicates overall attraction DNA fragments in divalent electrolyte
User-Defined Forces in NAMD

TclBC

GridForces

TclForces

Medium

Medium

Advanced

Estimated completion time: \(\sim 2\) hours/section

*TclBC* and *TclForces*: basic knowledge of *Tcl*
Mitosis and DNA replication

- Mitosis requires replication of a genome
- DNA replication occurs at a replication fork (replisome)
- Can be highly processive: 2900 bases/min (eukaryotes)
- 1000 bases/s in *E. coli*

**Enzymes common to all replisomes:**
- Helicase
- Primase
- Polymerase
- Ligase (not depicted)
- Sliding clamp and clamp loader
- ssb

*Adapted from Mol. Cell 23:155*

*Alberts, Molecular Biology of the Cell, fifth edition*
SSB protects single-stranded DNA

Prevents formation of secondary structure, enzymatic digestion, chemical modification

Single-stranded DNA binding protein (SSB) can bind 35 or 65 nucleotides of ssDNA ($SSB_{35}$ and $SSB_{65}$) with high affinity

Problem: how is SSB removed when it is no longer needed?
Diffusion of ssb along DNA

What is the microscopic mechanisms of SSB diffusion?

How does dissociation of DNA from SSB occur?

What makes an ssb an ssb?

† Ha group, Nature 461:1092
A model is build from an x-ray crystal structure

Unresolved DNA was modeled by the crystallographers (Lohman and Waksman groups, Washington U. School of Medicine) and provided to us via Ruobo Zhou of the Ha group.
Individual nucleotides are loosely bound to SSB

SSB\textsubscript{35} \hspace{1cm} SSB\textsubscript{65}

![Graphs showing RMSD over time for SSB\textsubscript{35} and SSB\textsubscript{65}]
Mechanics of ssb-DNA (dis)assembly

Chemla group, unpublished
All-atom simulations cannot quite reach experiment.

Forces are 1-2 orders of magnitude larger than in experiments.

Little hope of observing diffusion-related events.
Coarse-grained modeling connects with experiment

Coarse-grained models of and single-stranded DNA binding protein (SSB) were developed from all-atom simulation. Excellent agreement was obtained between experiment and simulation suggesting that SSB binds DNA dynamically.

**Future Goal:** Extend model to include base sense and protein–protein interactions to enable diverse studies of the mechanisms of DNA replication and repair, including the following:

- **SSB** saturates DNA during replication; the effect of protein–protein interactions on the structural and dynamical properties remain unexplored.

- **RecA** efficiently displaces tightly-bound SSB molecules from ssDNA—likely an important capability for efficient repair—but the mechanism is elusive.

- **RecA** mediates strand exchange during DNA repair, but must efficiently displace tightly-bound SSB molecules from ssDNA, but the mechanism is elusive.

Excellence agreement was obtained between experiment and simulation suggesting that SSB binds DNA dynamically.
SSB parameterization

Obtain density of all-atom nucleotides

Apply iterative Boltzmann inversion using CG ssDNA to obtain the interaction potential that makes the AA and CG densities match
SSB represented through moving grids

The CG ssDNA can interact with atomically-detailed SSB using Gridforces in NAMD.

Problem: global rearrangements of CG DNA are still slow, and dynamics of SSB–DNA interaction is unrealistic.

Solution: modify NAMD to make grids move in response to forces and torques. Langevin forces and torques are also applied.
DNA (1-site/nt) + SSB simulation trajectory

This simulation is ~400 ns, but smoothed potentials make the kinetics equivalent to ~20 µs. We obtain ~1 µs/day with 1-site model on two processors with 200 nucleotides and one SSB. 2-site SSB parametrization is underway.
Atomistic mechanics of single-stranded DNA Binding-Protein

Chris Maffeo

Scripting system assembly for protein-DNA systems with latest force-field
Difficult!

Pulling SSB
Moderately difficult
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VMD and NAMD