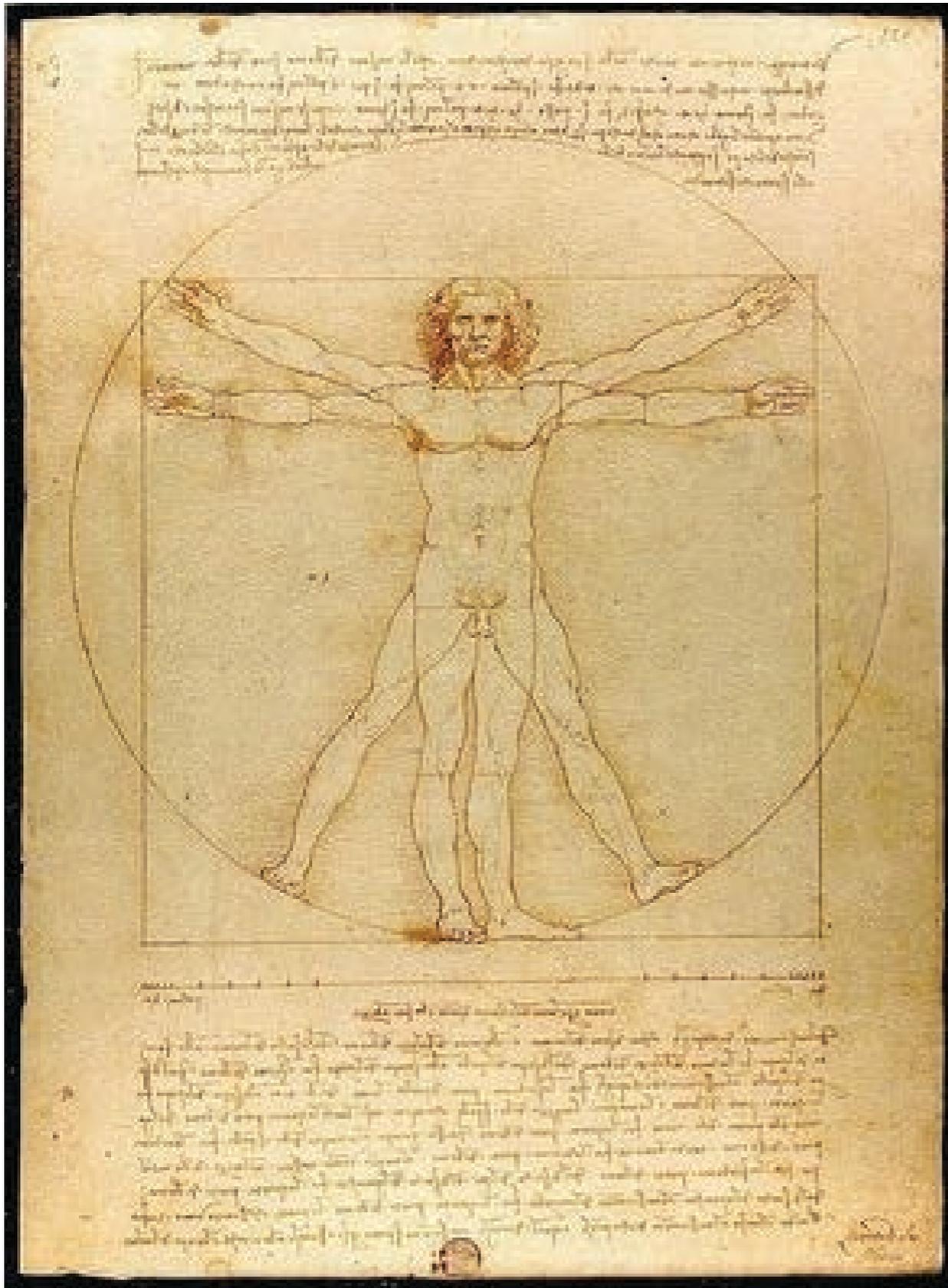


Close encounters with DNA

Aleksei Aksimentiev
Department of Physics
University of Illinois at Urbana-Champaign



WHAT IS LIFE?

The Physical Aspect of the Living Cell

BY

ERWIN SCHRÖDINGER

SENIOR PROFESSOR AT THE DUBLIN INSTITUTE FOR
ADVANCED STUDIES

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



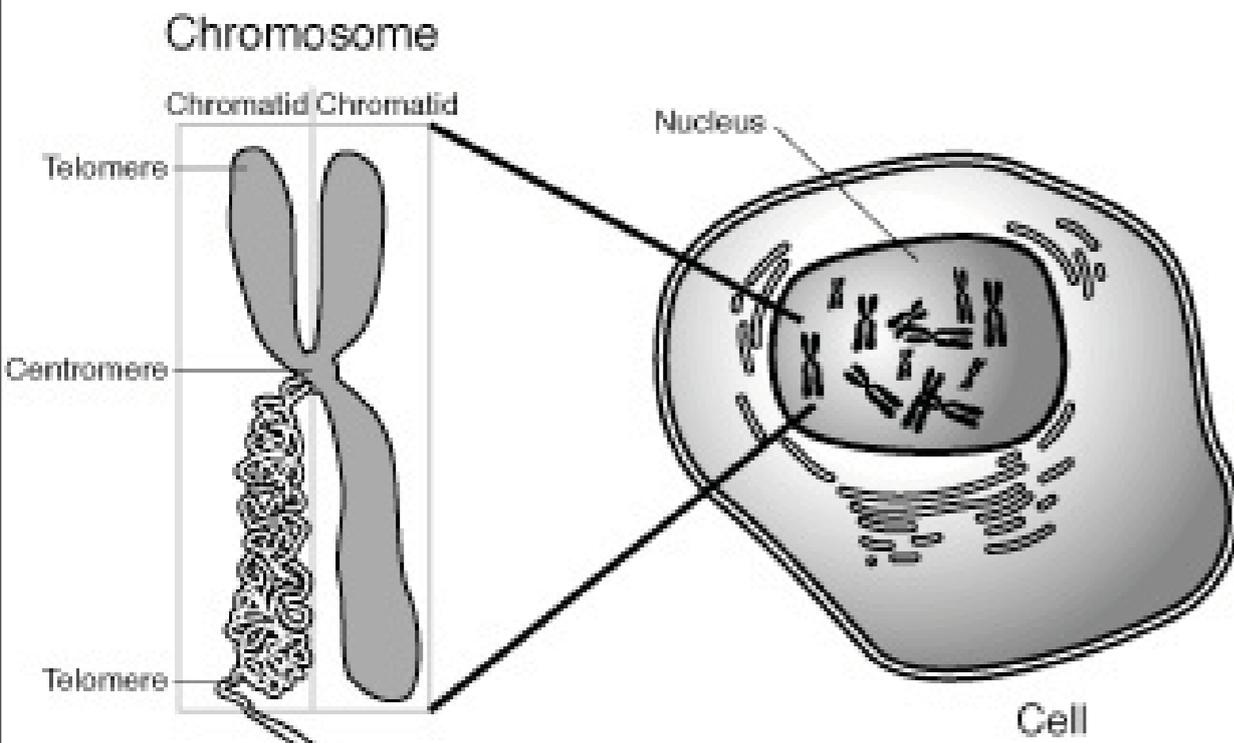
4339

CAMBRIDGE

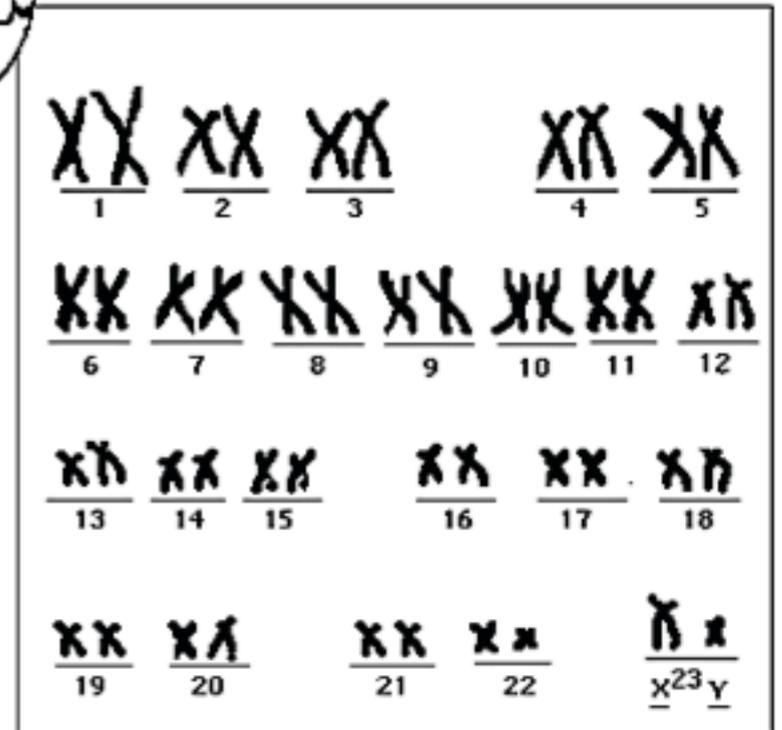
AT THE UNIVERSITY PRESS

1948

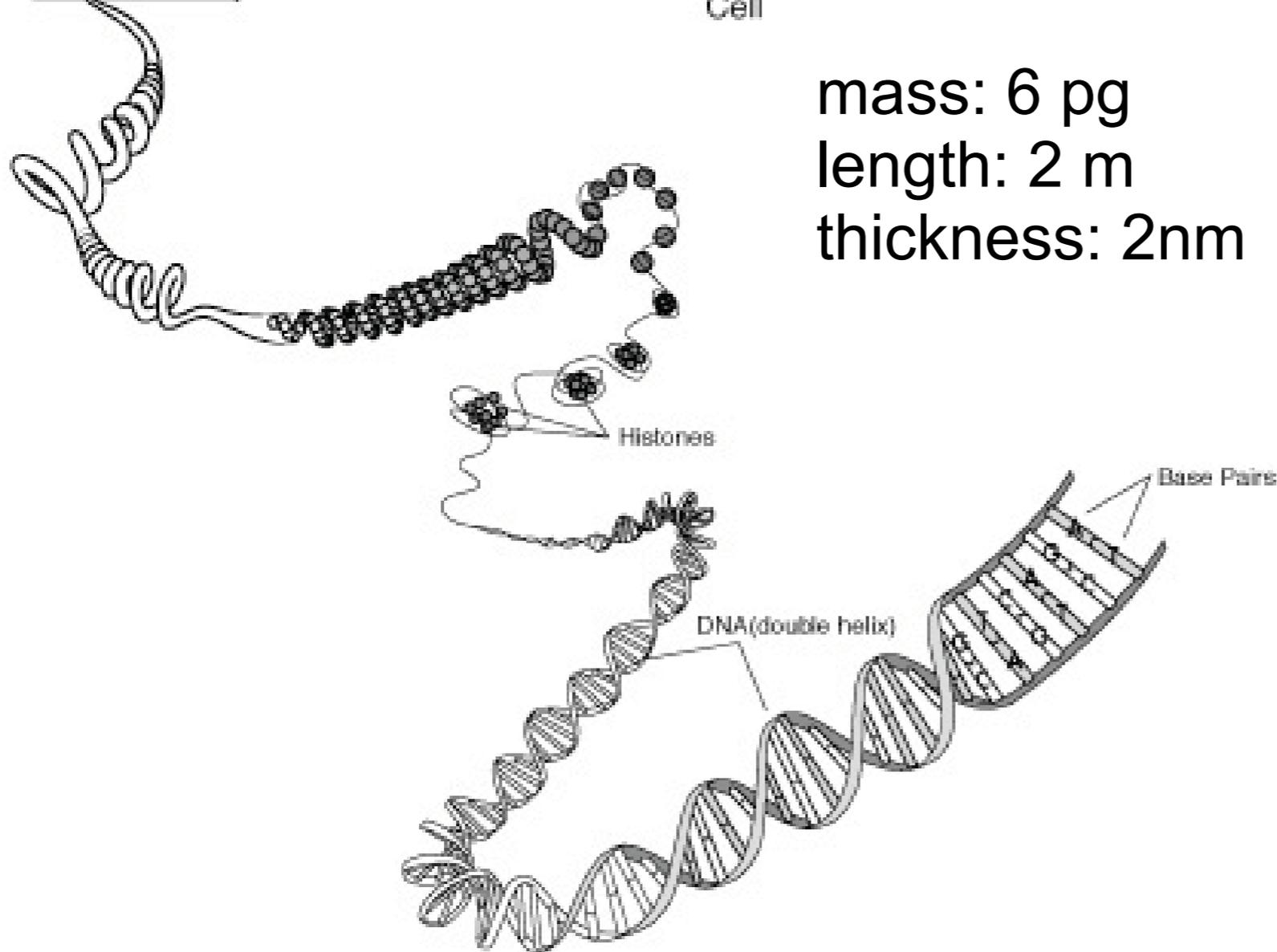
DNA, the blueprint



HUMAN CHROMOSOMES



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



...GTGTGACTCGT
GGTCCGTAATGTC
GTTAATGGTGACC
GTGTGGCCTGATG
GTTAGTGTGTGA...

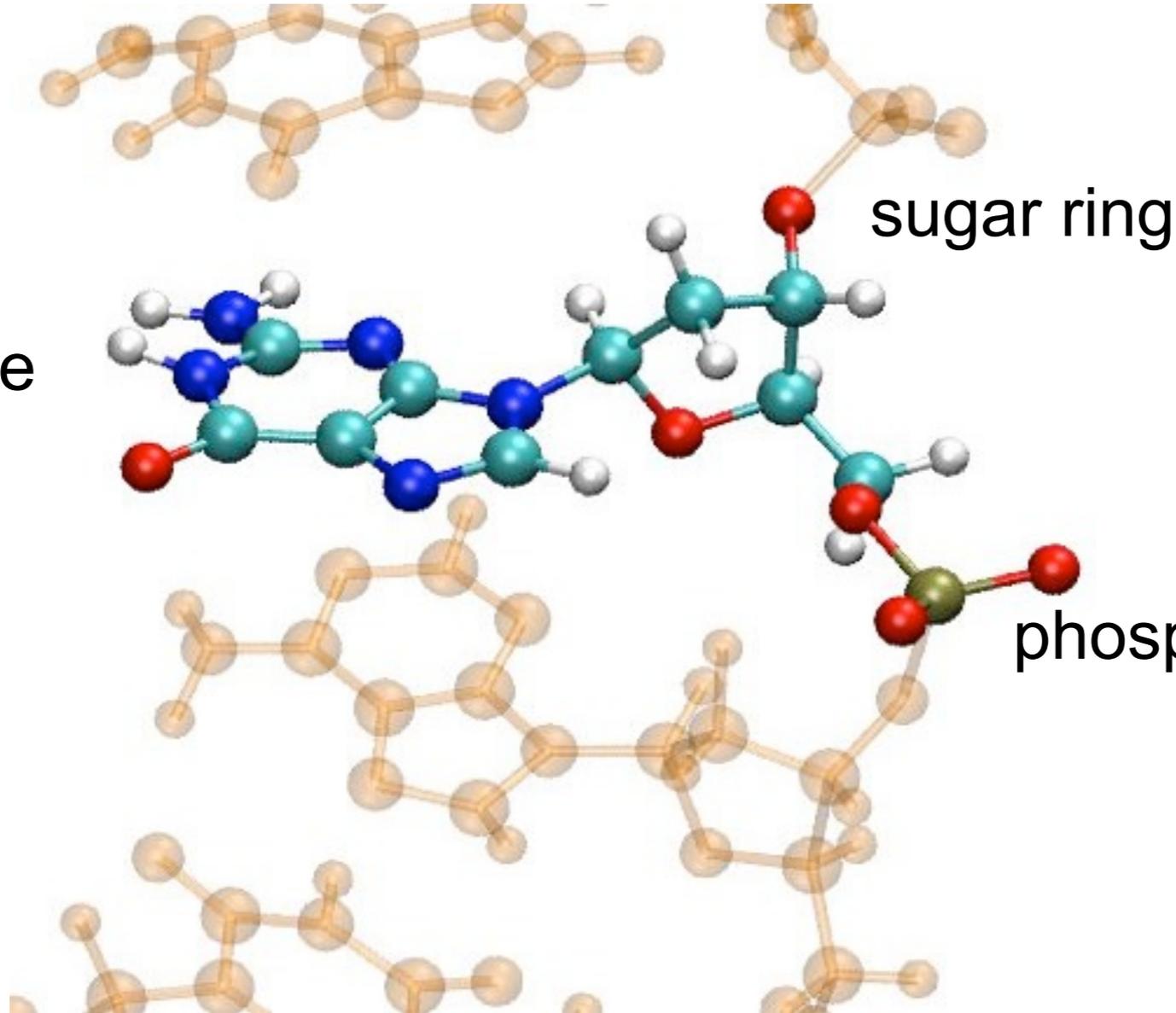
DNA code is written in atoms

Highly charged: 2 electron charges per 0.32nm



Double stranded DNA
(persist. length ~50nm)

base

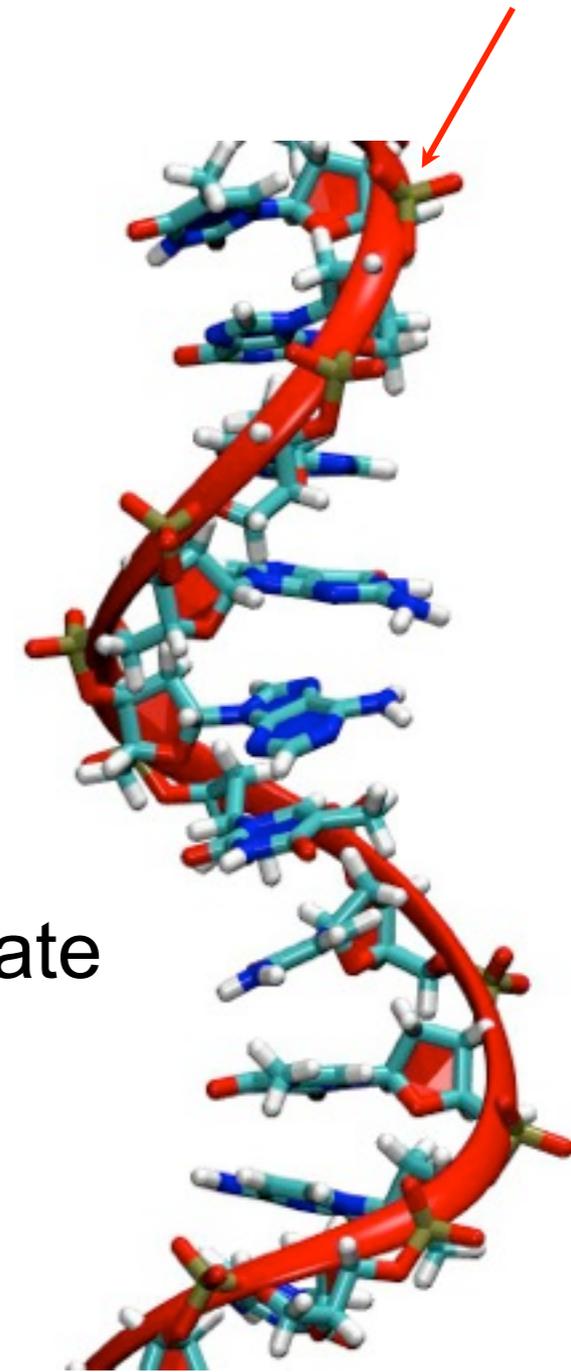


sugar ring

phosphate

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

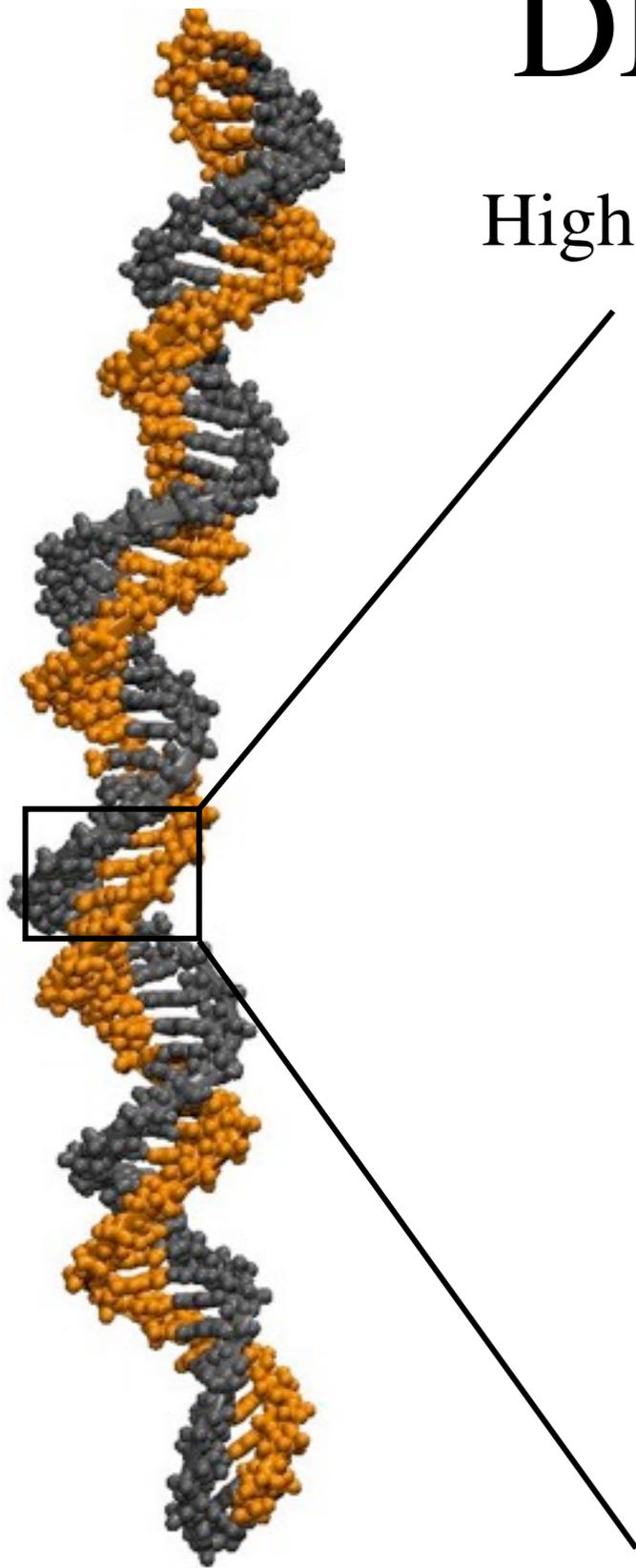
backbone



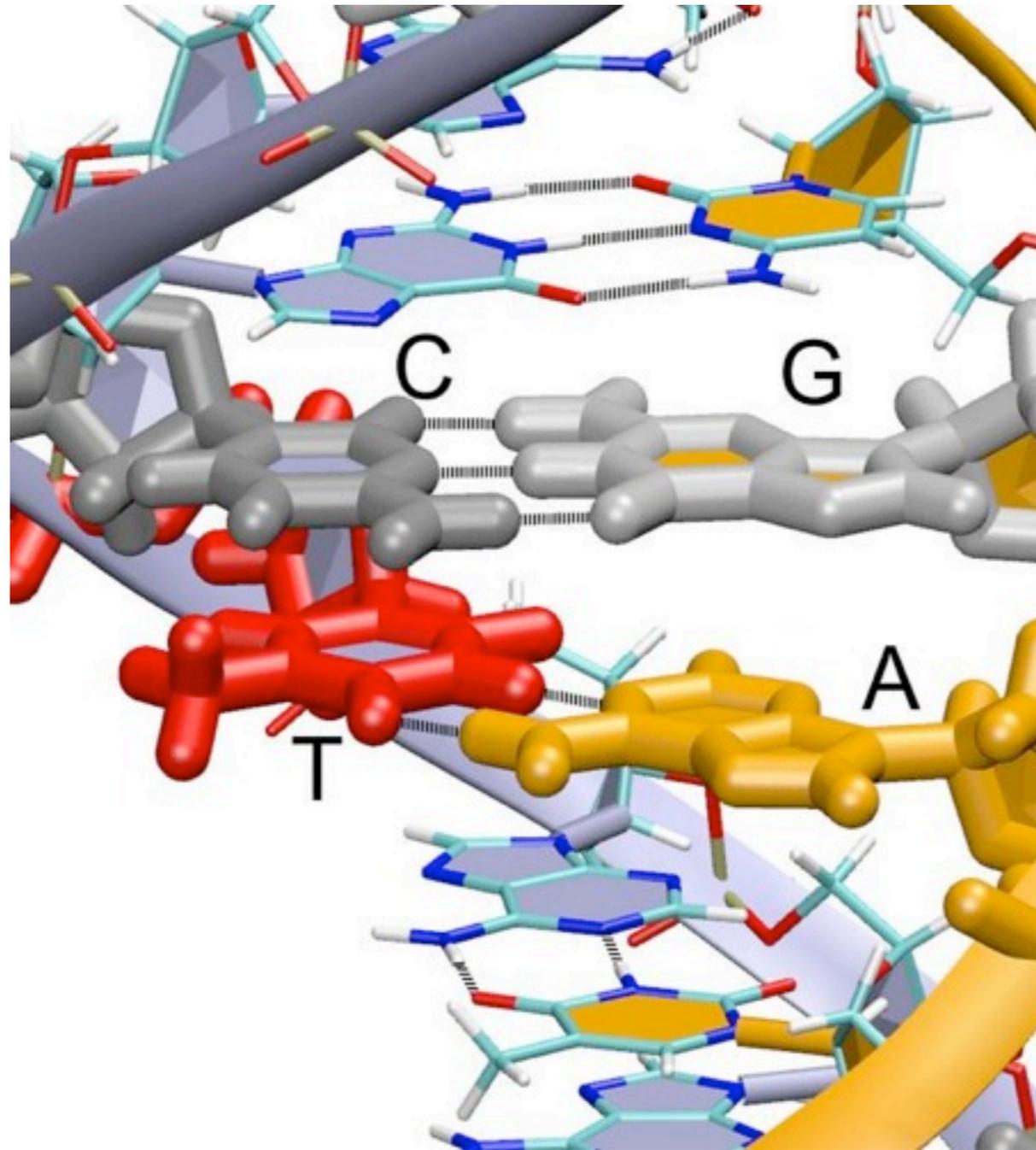
Single stranded DNA
(persist. length ~1.5nm)

DNA code is written in atoms

Highly charged: 2 electron charges per 0.32nm

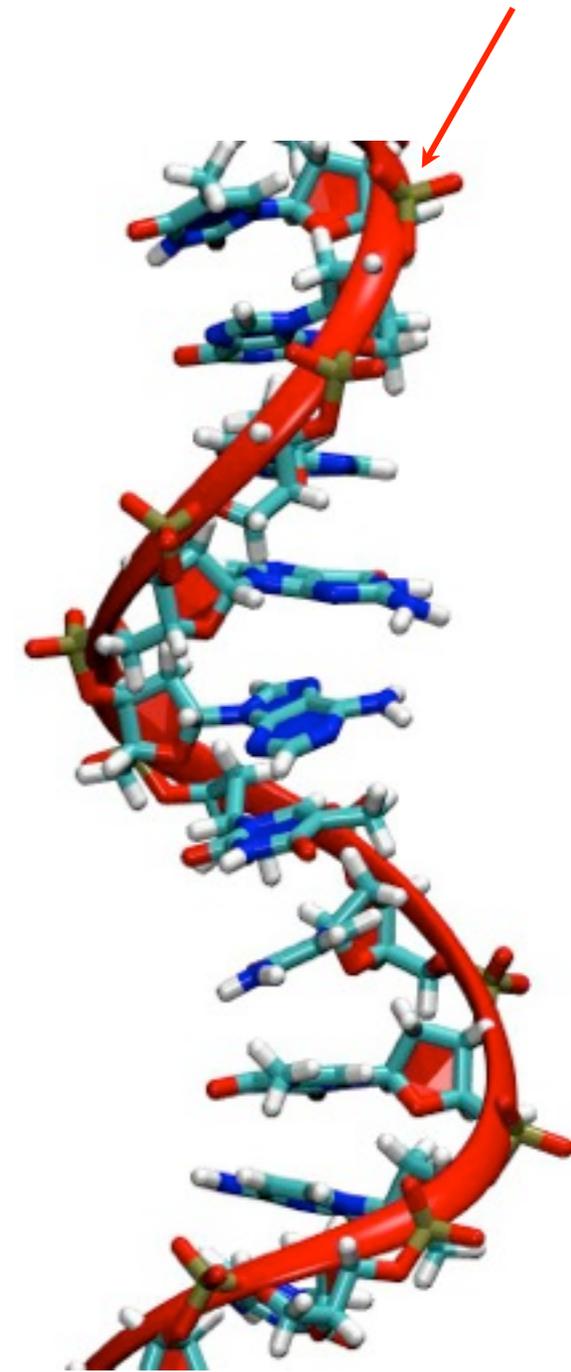


Double stranded DNA
(persist. length ~50nm)



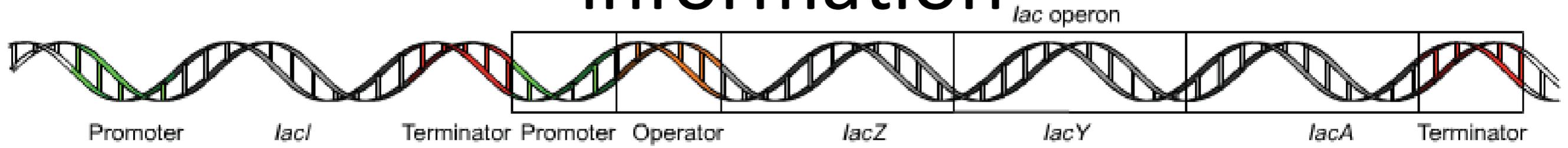
The sequence has direction:
5'-AAGCTGGTTCAG-3'

backbone



Single stranded DNA
(persist. length ~1.5nm)

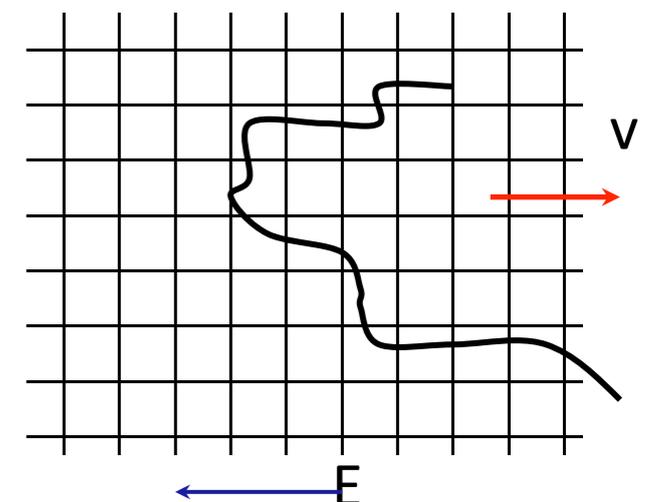
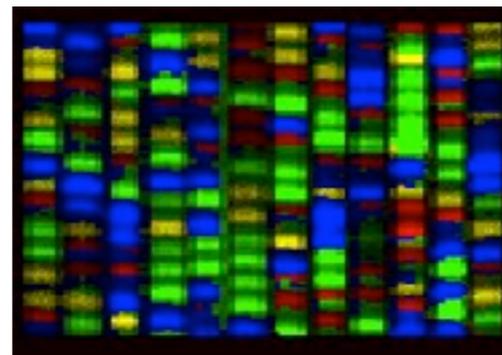
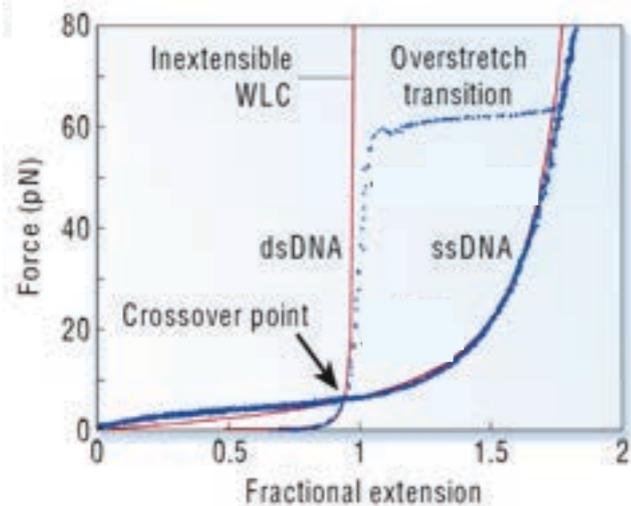
The sequence contains biological information



Central dogma of molecular biology



The physical properties enable functionality

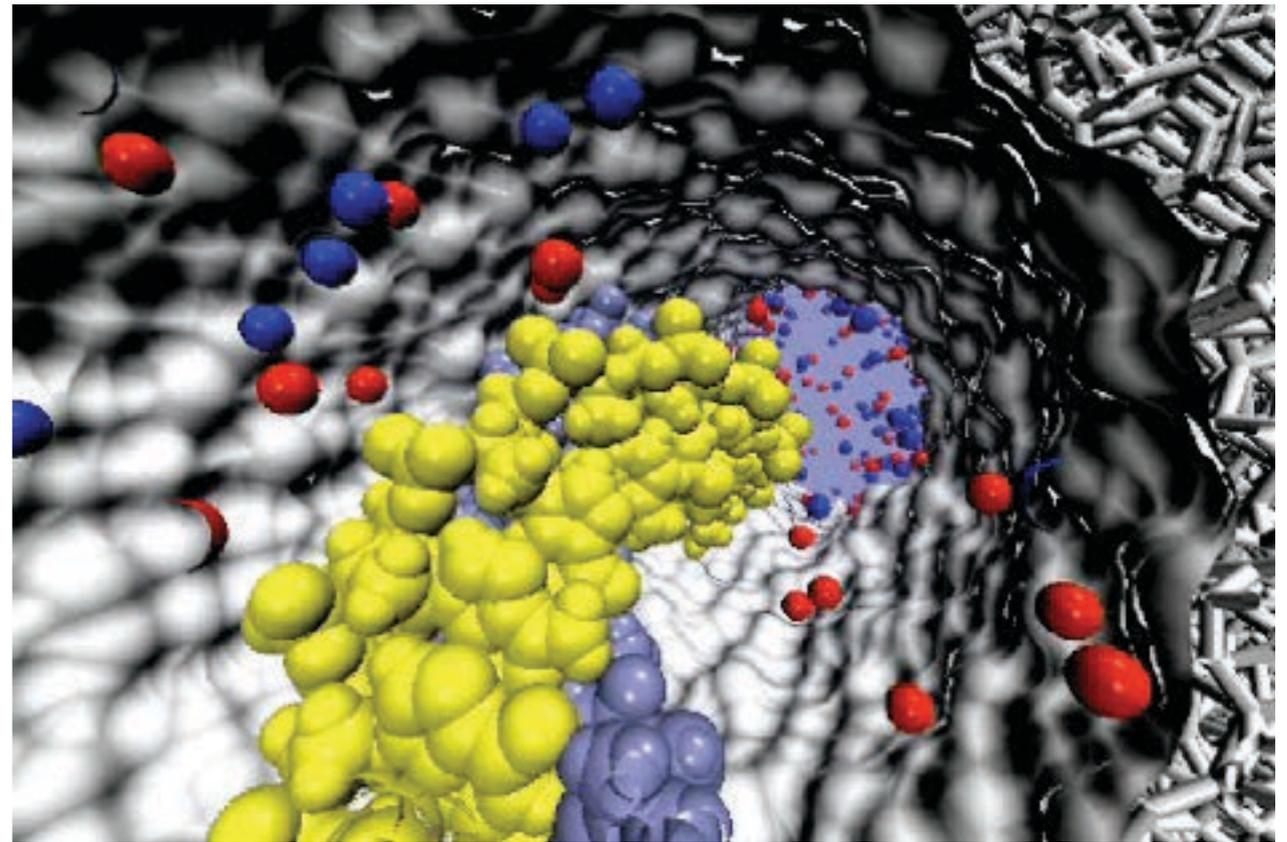


Molecular dynamics simulations, a computational (force) microscope

Massive parallel computer
Blue Waters, ~200,000



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



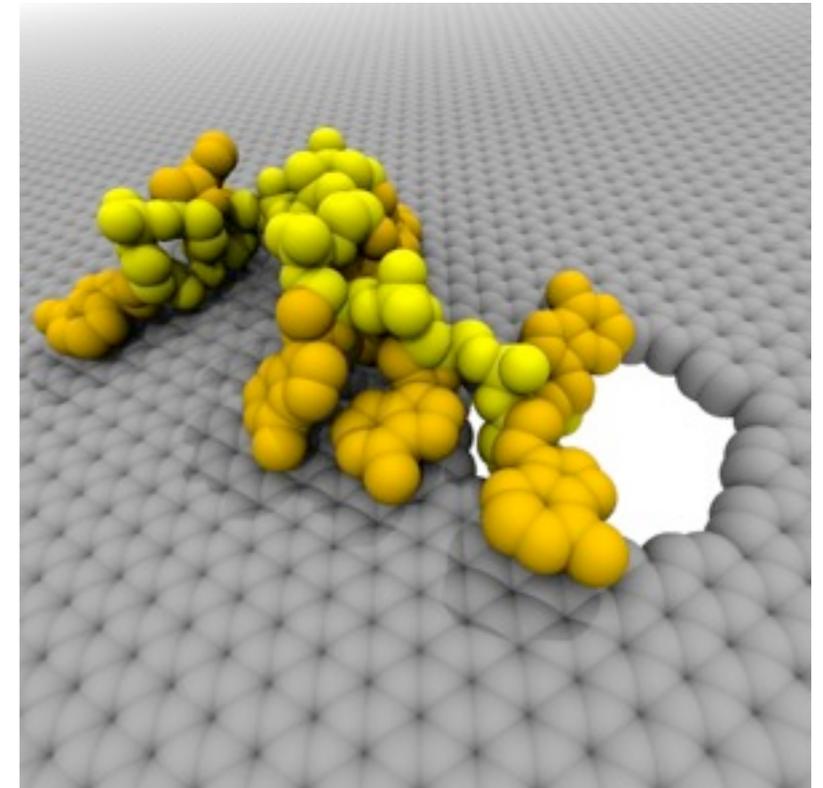
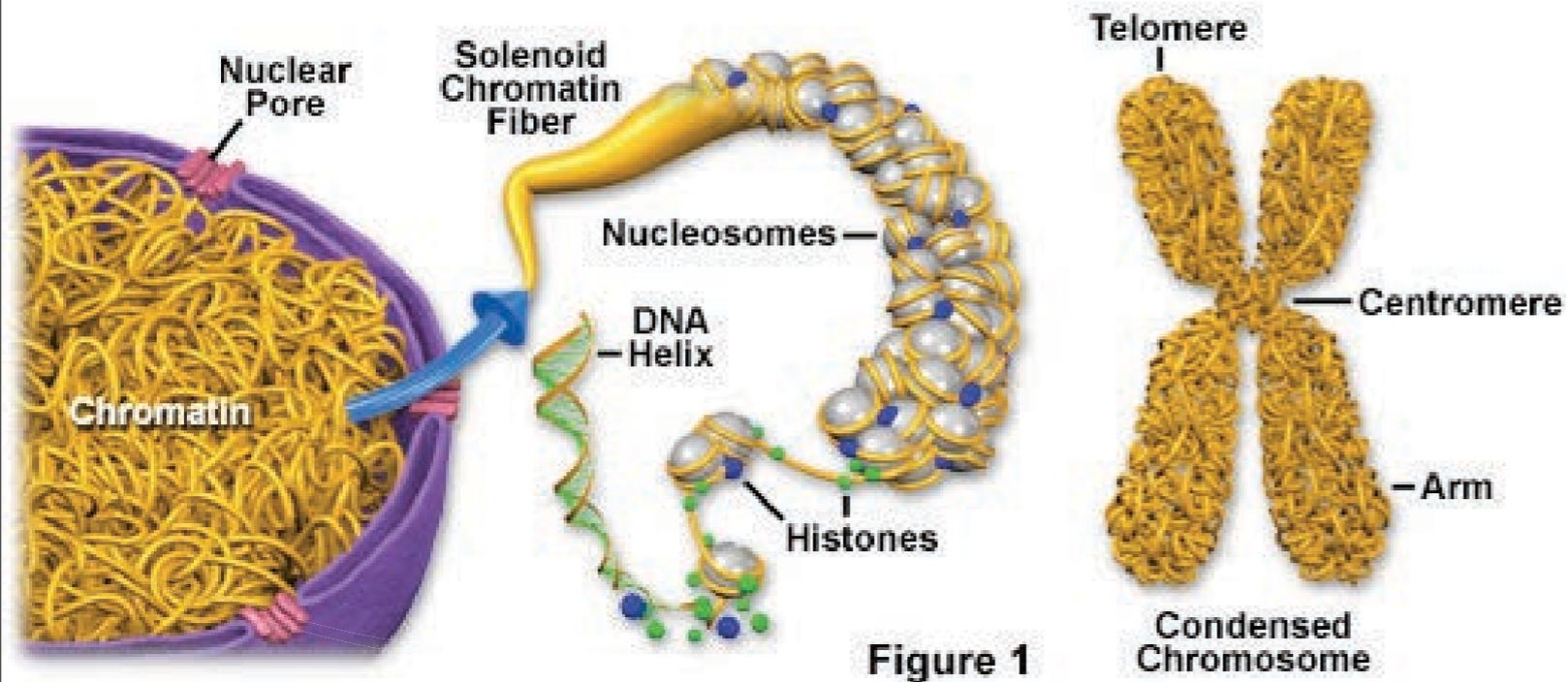
Time scale: $\sim 0.1-100 \mu\text{s}$

Length scale: 10K - 100M
atoms or $(< 50 \text{ nm})^3$

Interaction between atoms is
defined by molecular force field

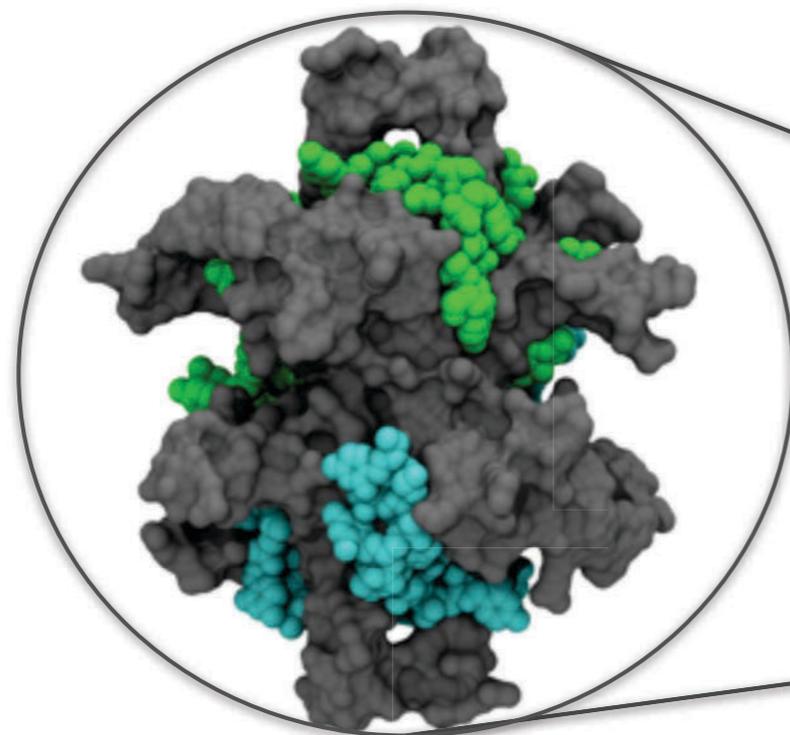
DNA systems

Chromatin and Condensed Chromosome Structure

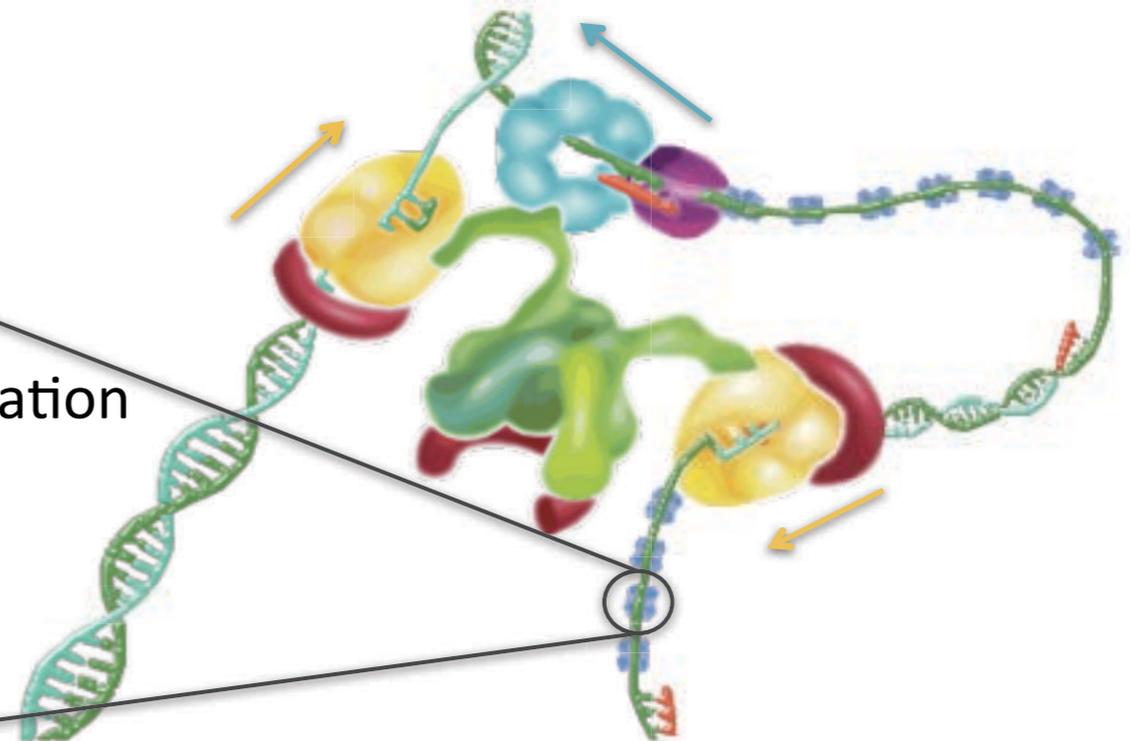


<http://micro.magnet.fsu.edu/cells/nucleus/chromatin.html>

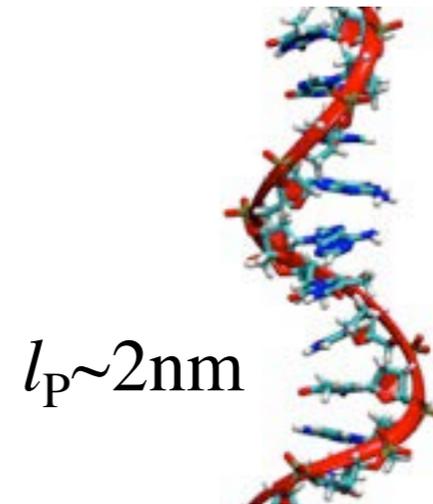
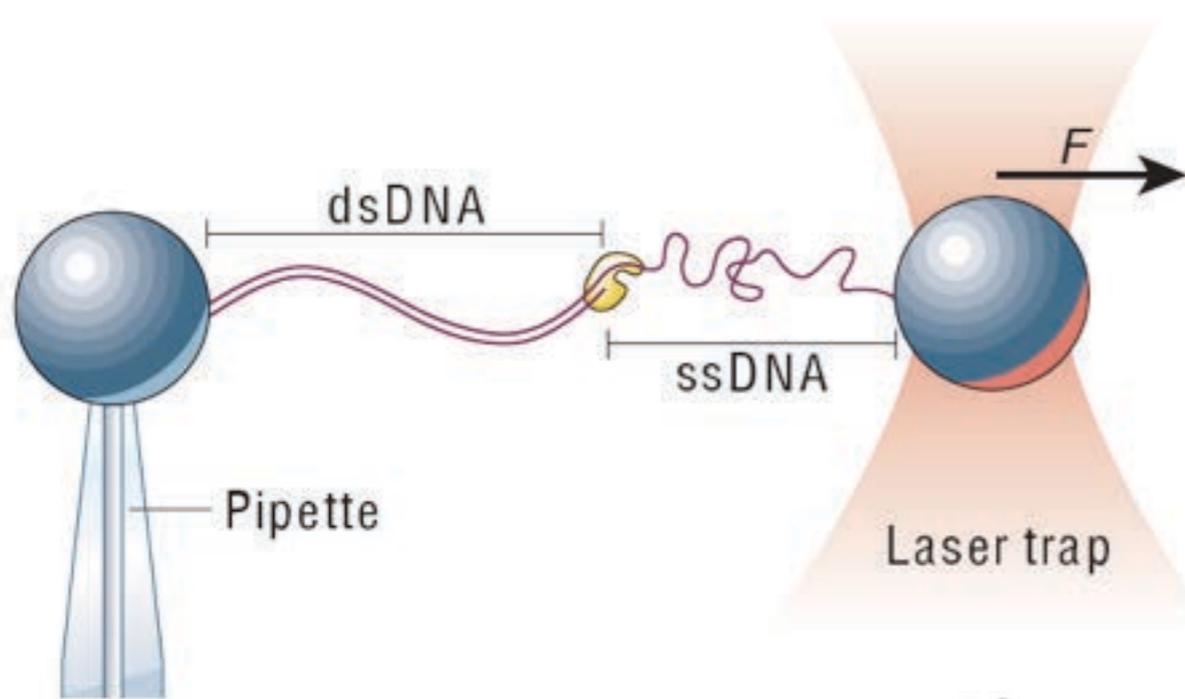
Graphene nanopore sequencing



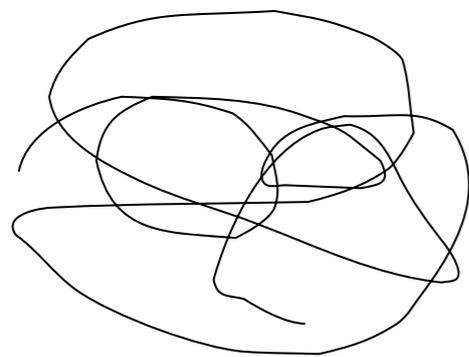
DNA replication and repair



Micromechanics of single DNA molecules

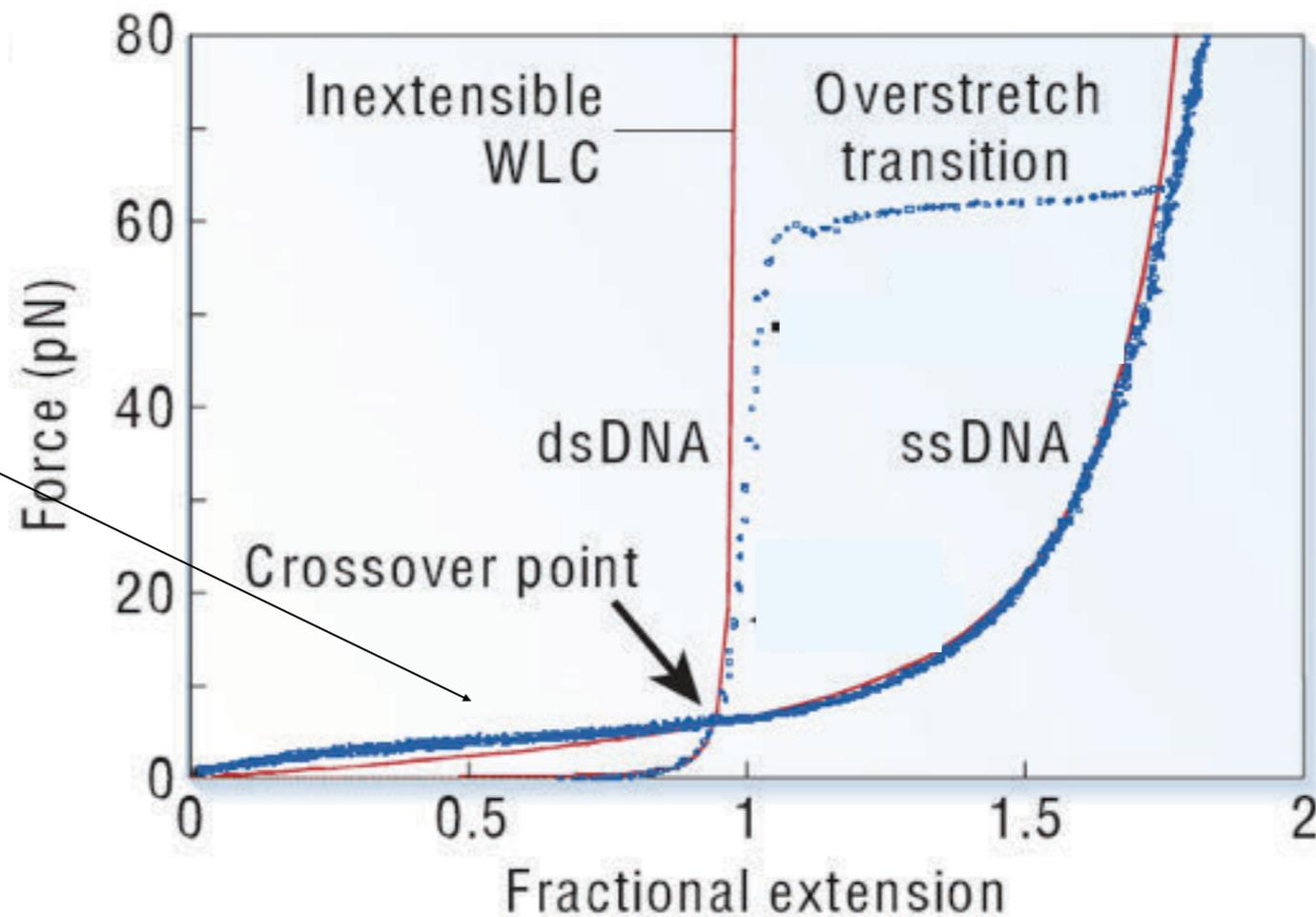


$l_p \sim 50\text{nm}$

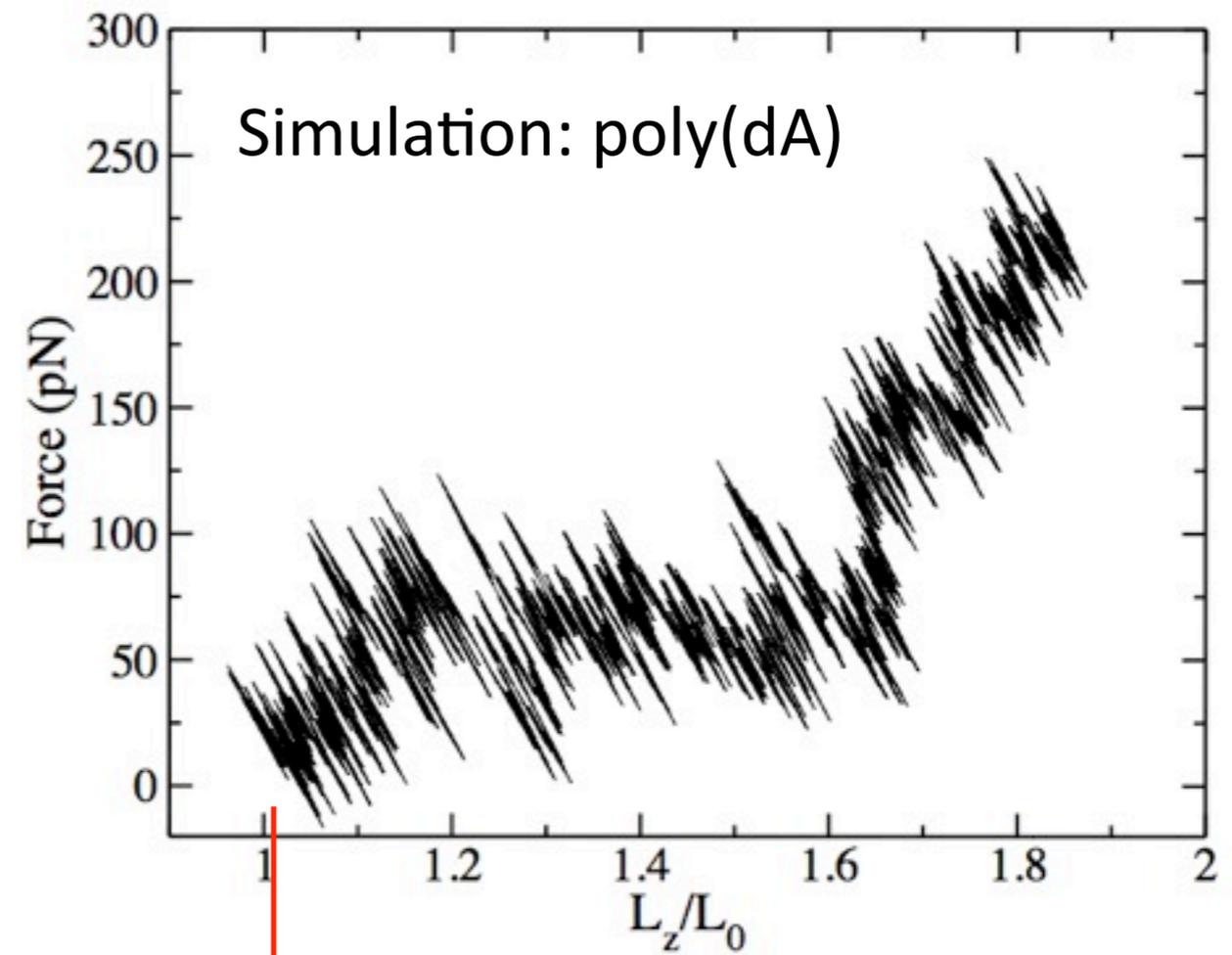
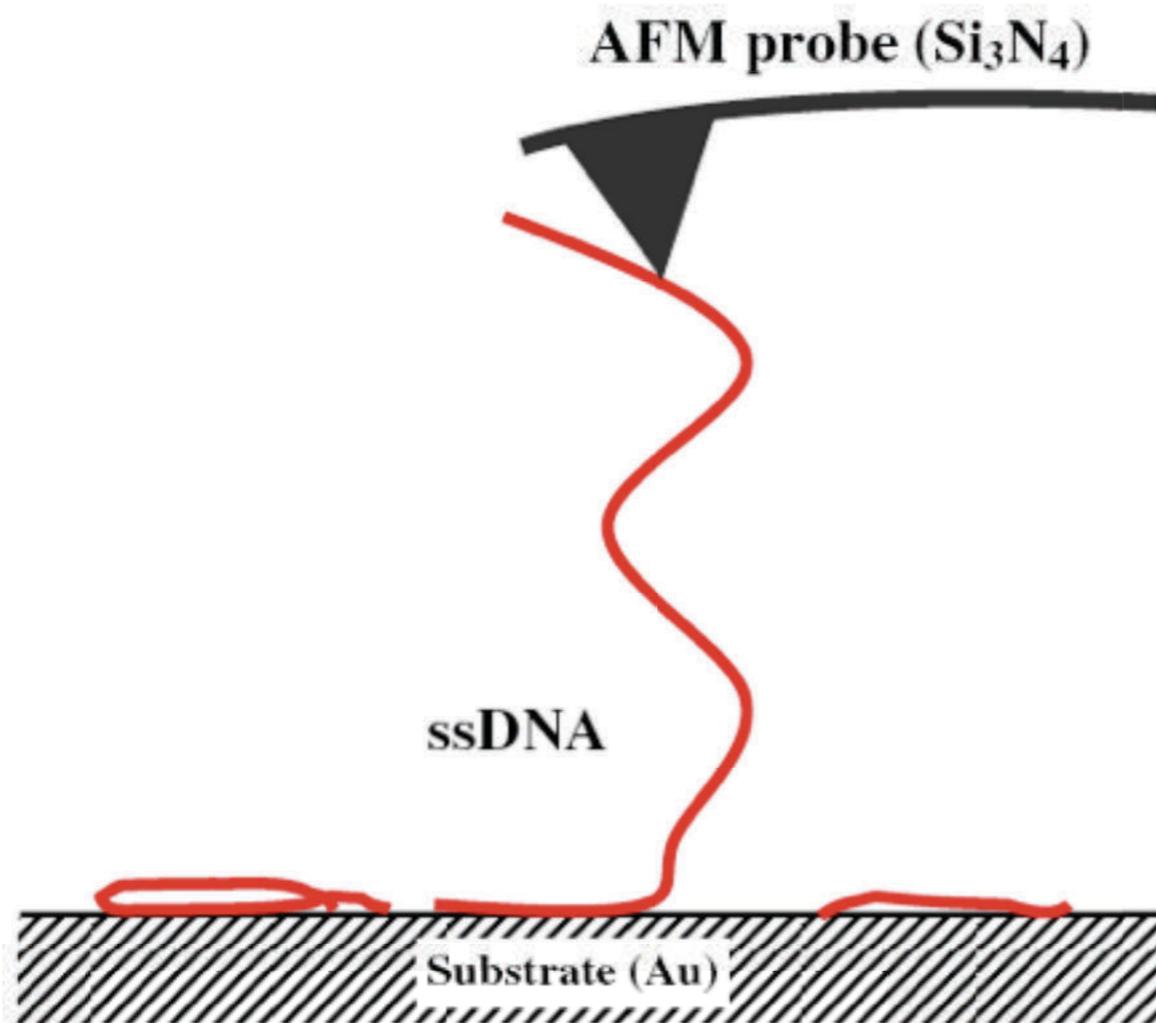


Polymer coil

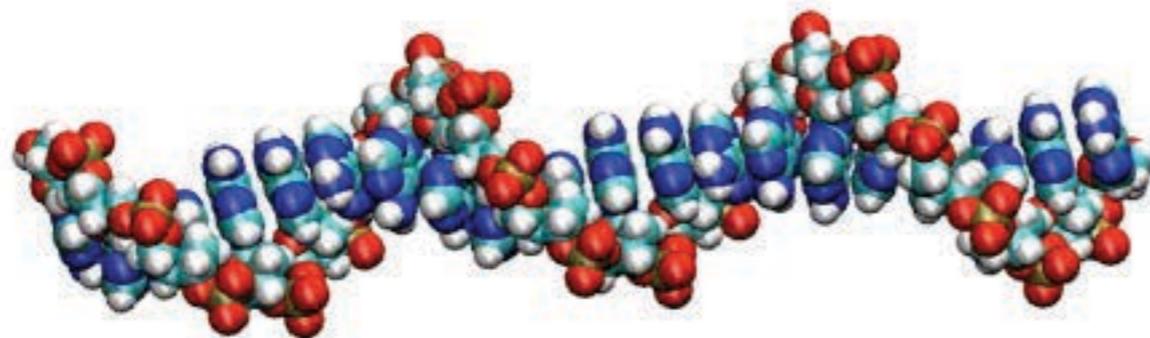
Bustamante et al.,
Nature 421:423 (2003)



Stretching ssDNA with external force



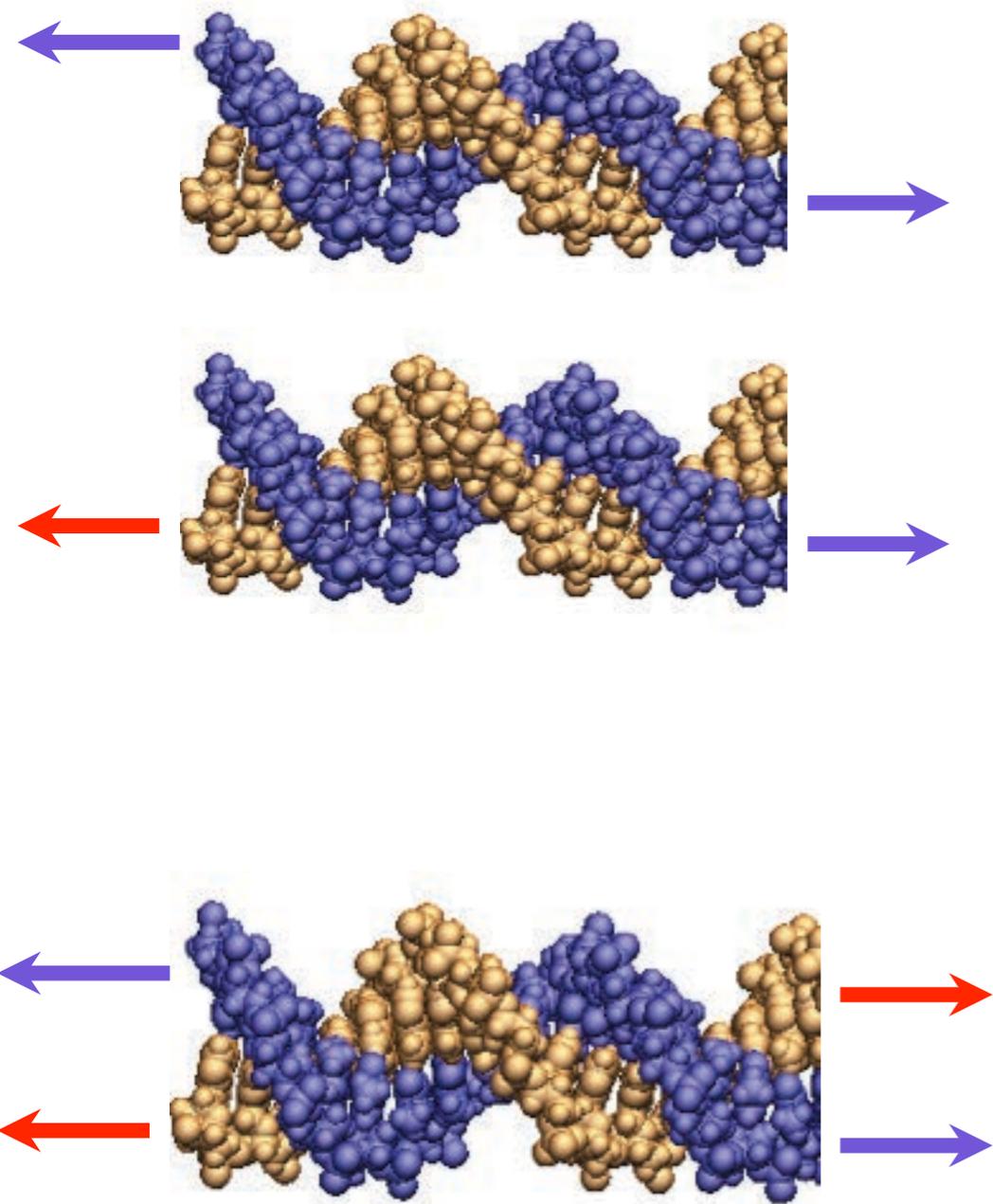
This
end
is
fixed



$V = \text{const}$



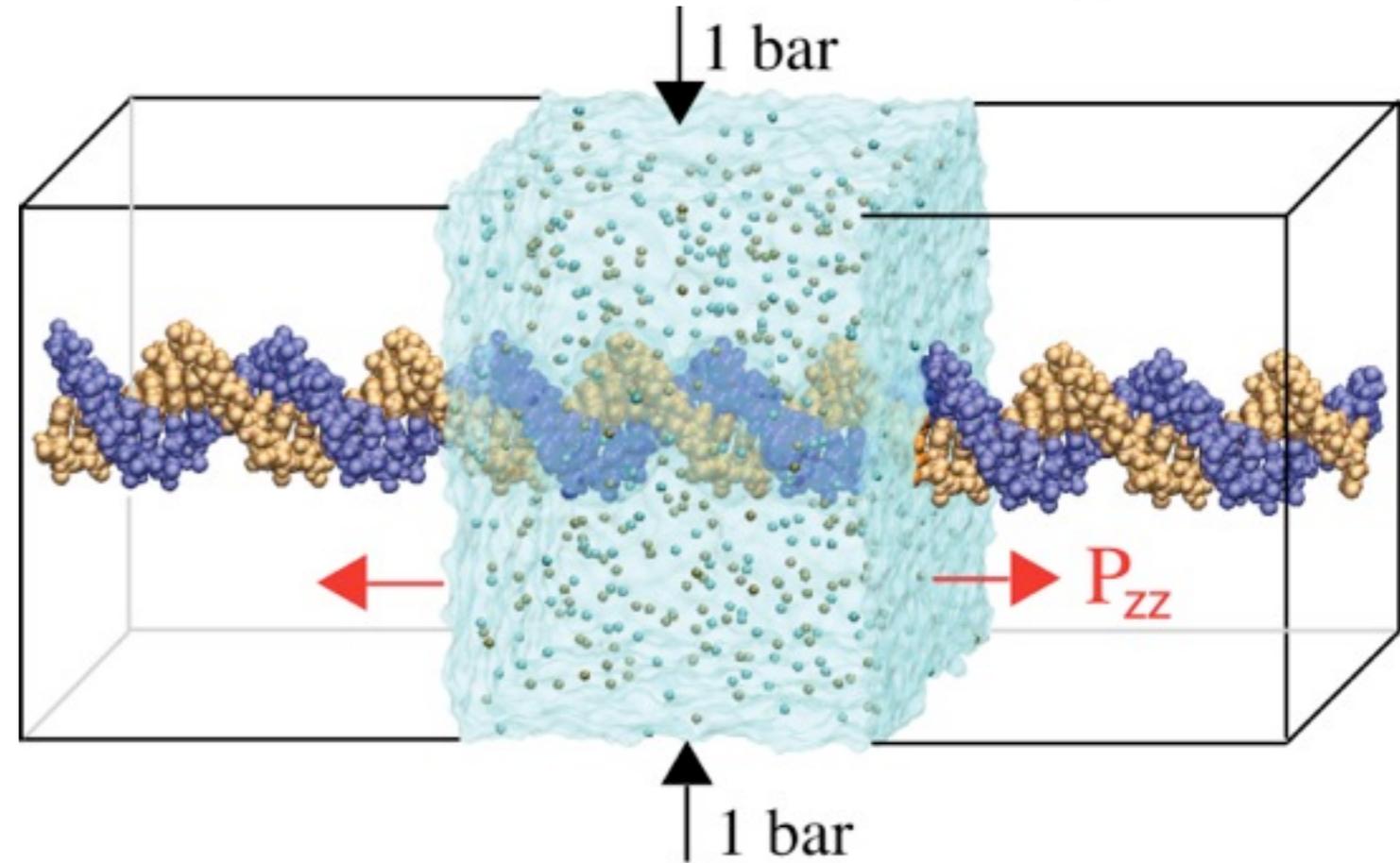
Stretching dsDNA



Applied force simulations

Periodic boundary conditions in 3D

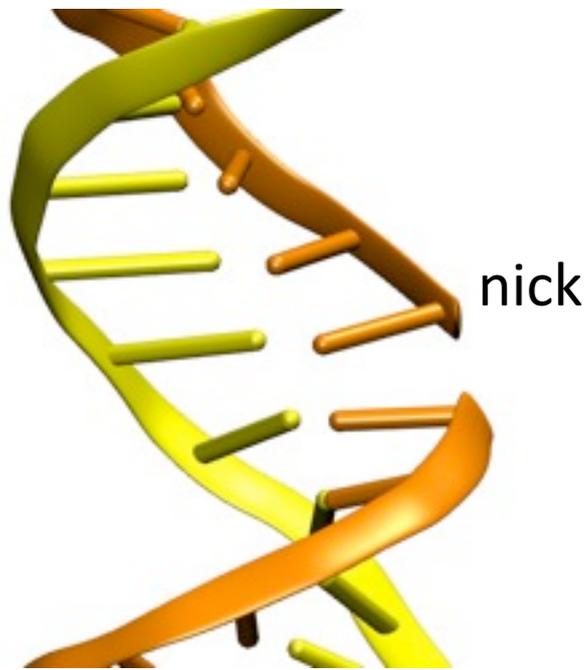
DNA is effectively infinite



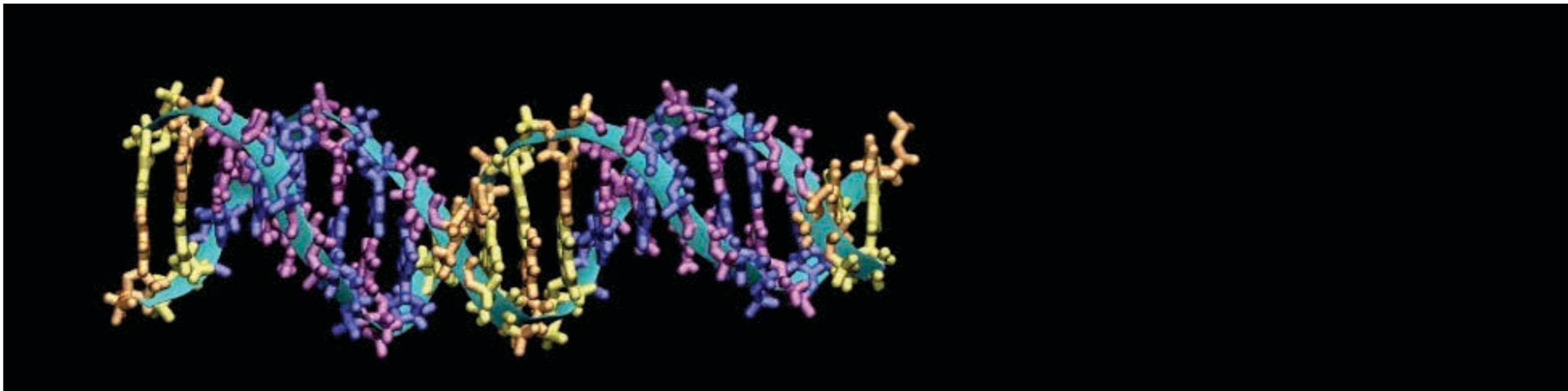
$P_{zz} < 0$ stretches the system in Z direction.

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:

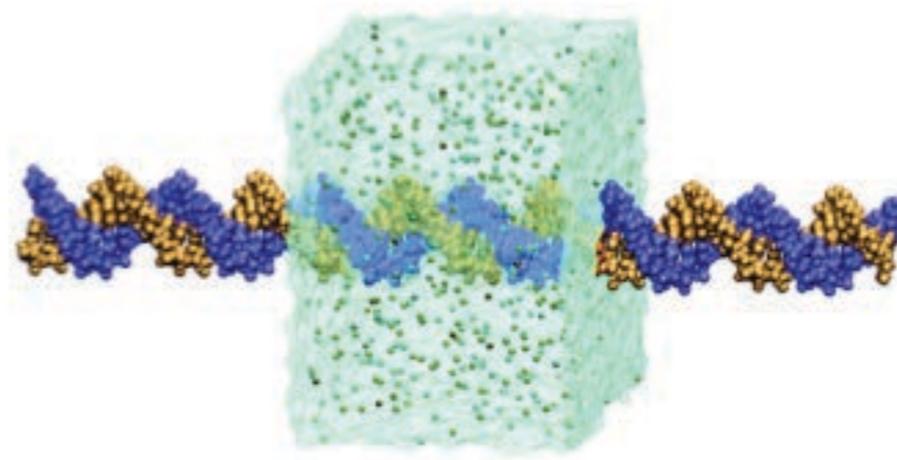
Mechanical Properties of dsDNA Mini Tutorial



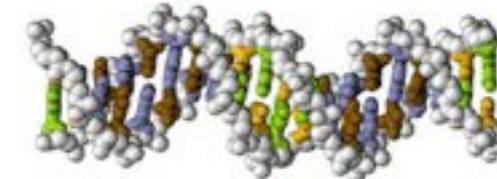
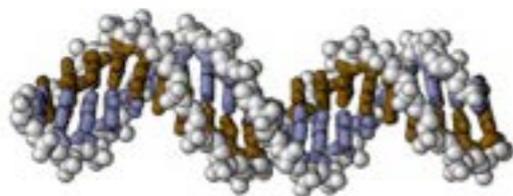
Shu-Han Chao



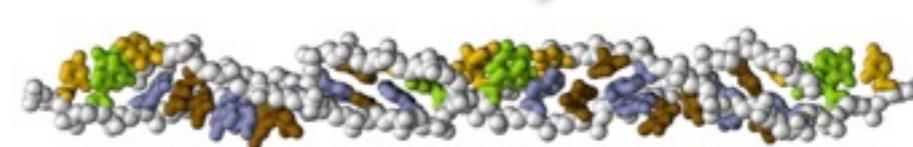
Jejoong Yoo



Part 1. Structure of DNA and Simulation System

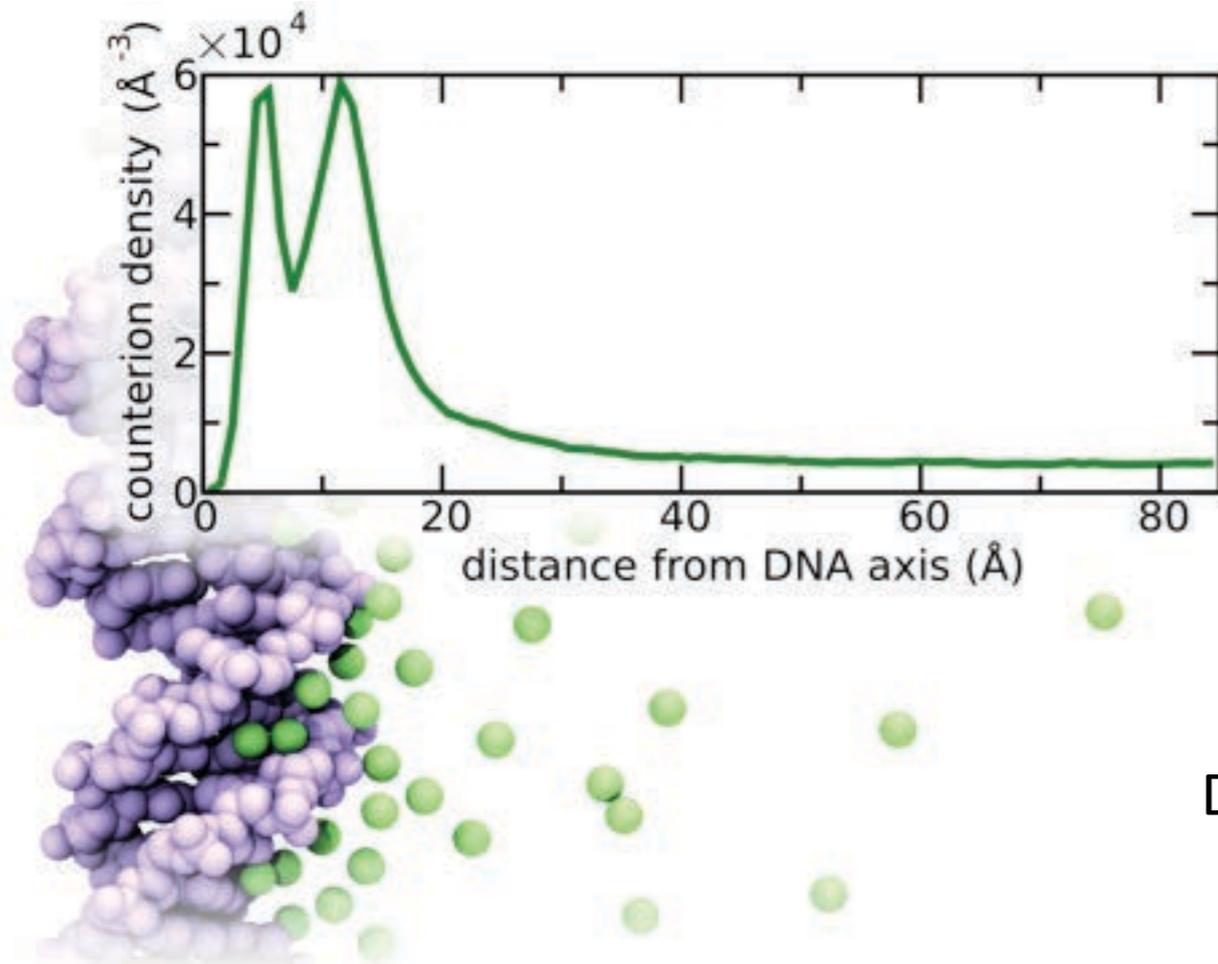
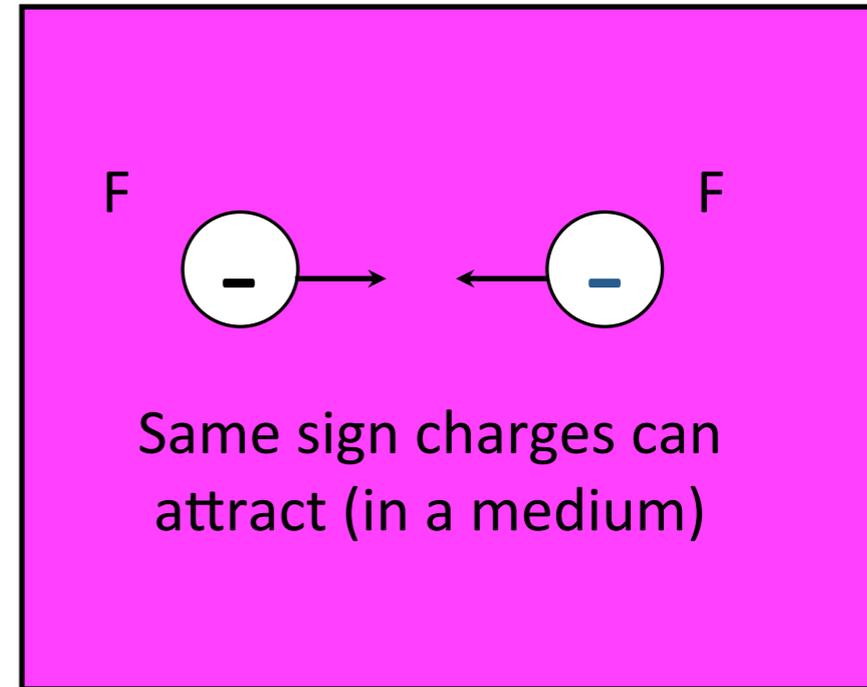
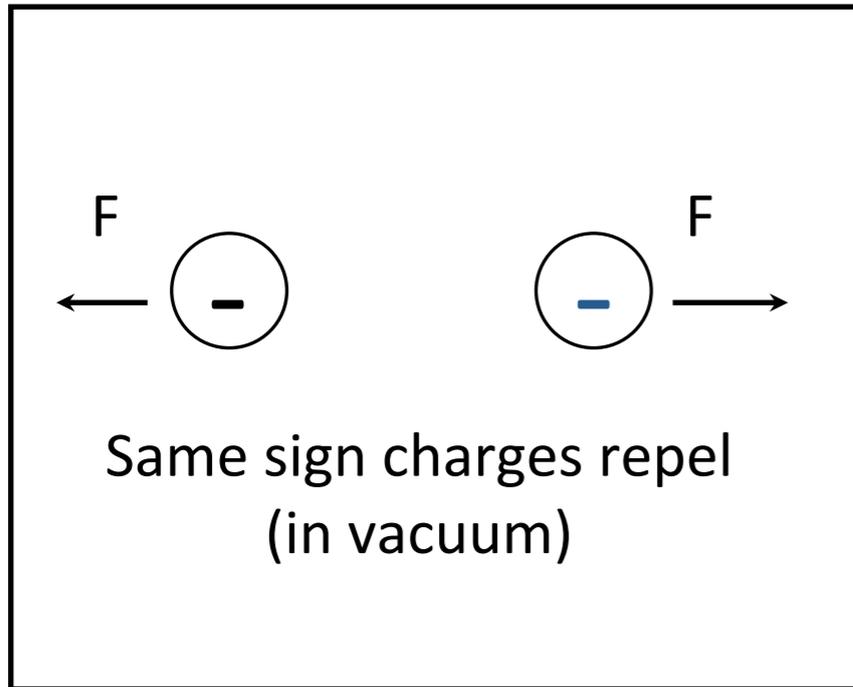


Part 2. Stretching dsDNA (torsionally constrained)



Part 3. Stretching Nicked DNA (torsionally unconstrained)

Interesting physical properties

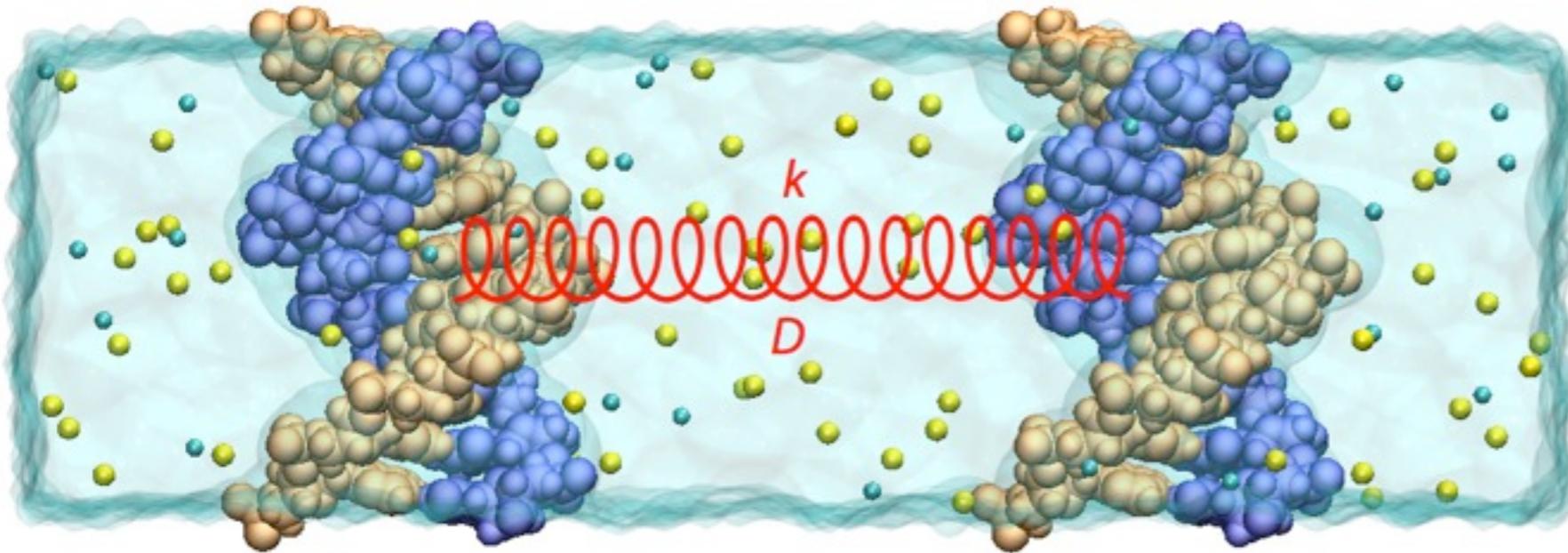


Effective attraction between DNA is observed when counterions have charge $\geq 2e$

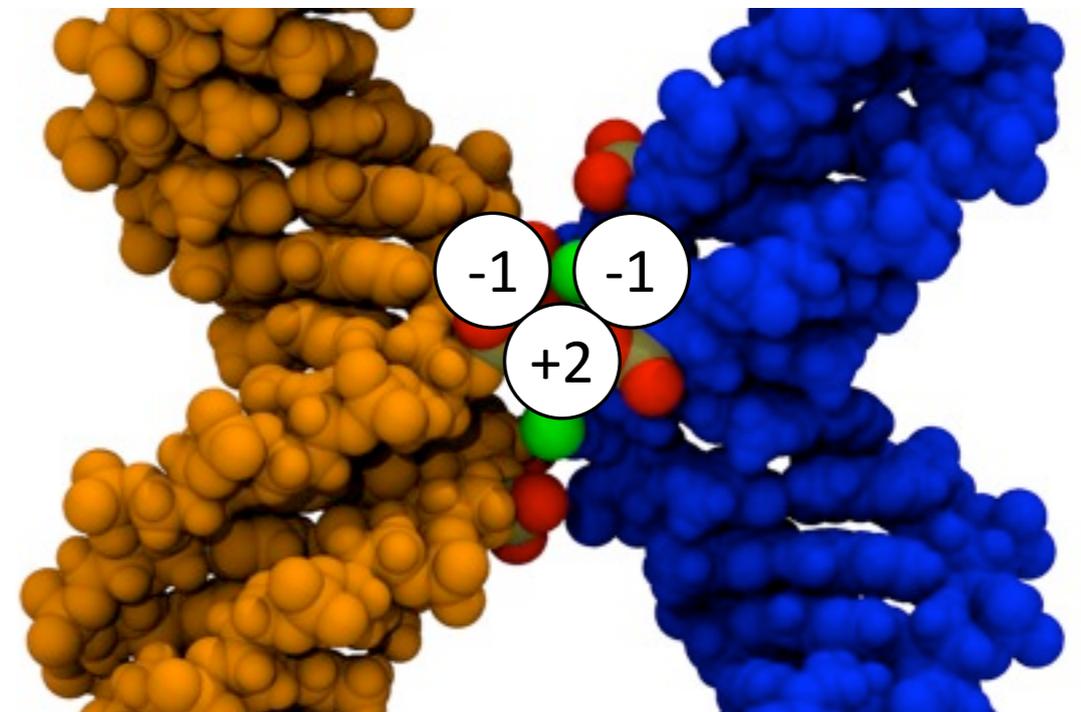
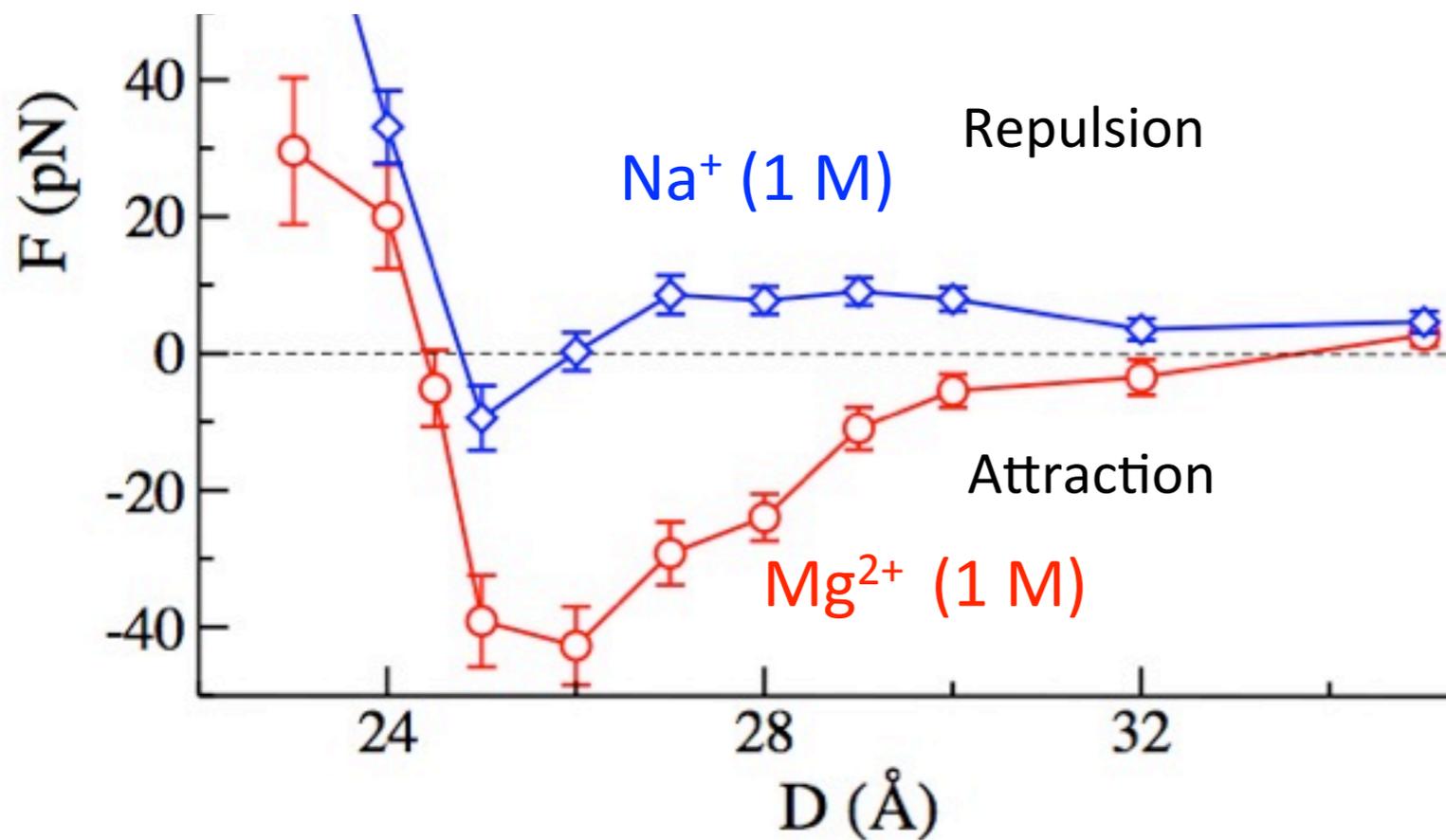
DNA lives in water and is surrounded by counterions

Direct MD simulation of DNA-DNA force

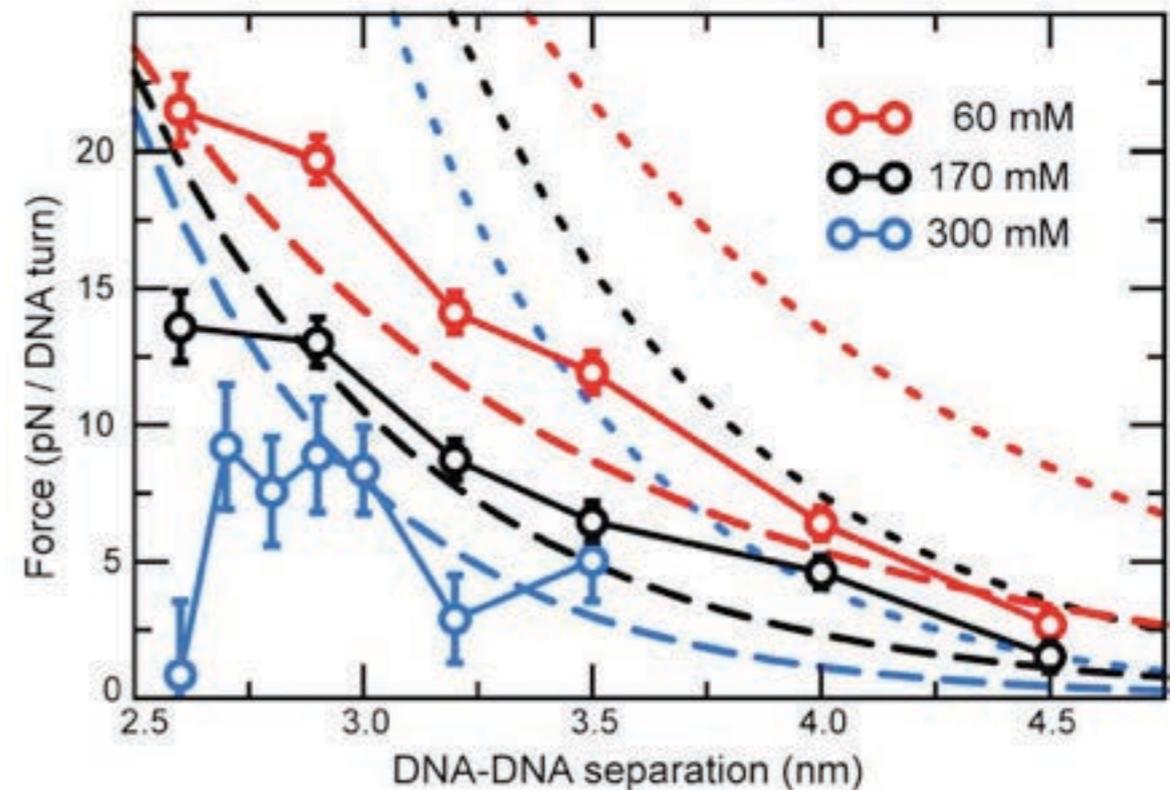
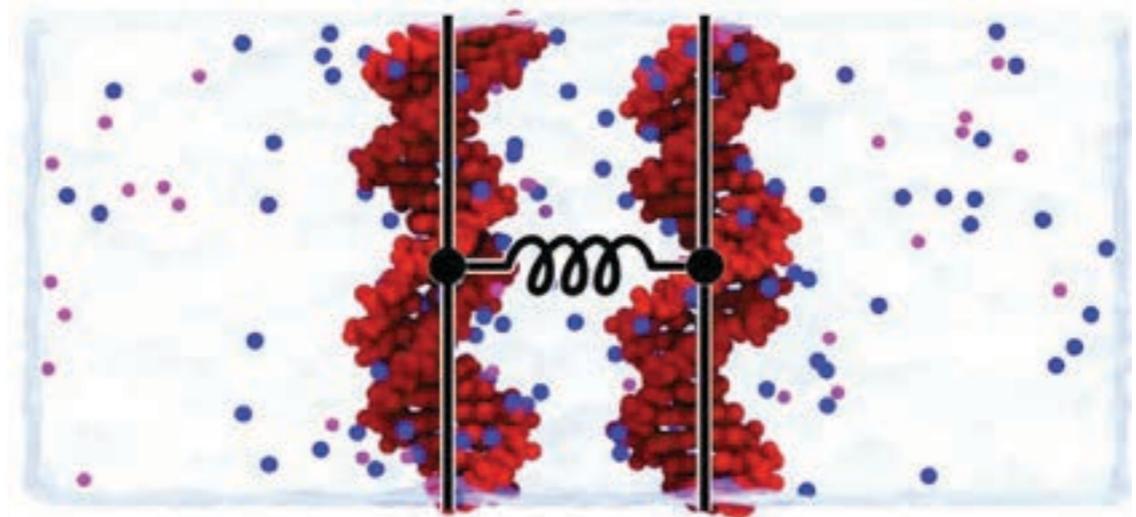
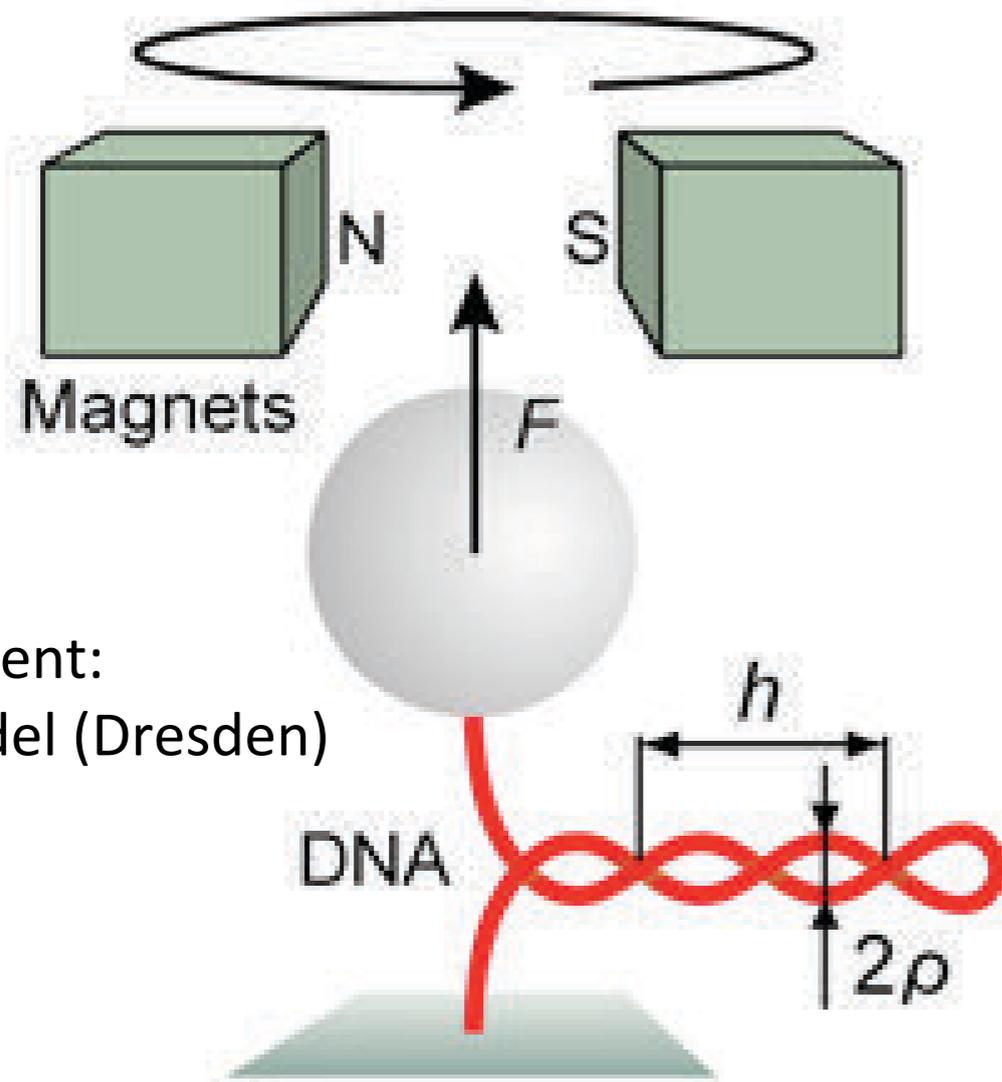
J. Am. Chem. Soc. 130, 15754 (2008)



A virtual spring measures the effective force



Simulations of side-by-side DNA repulsion are in good agreement with experiment



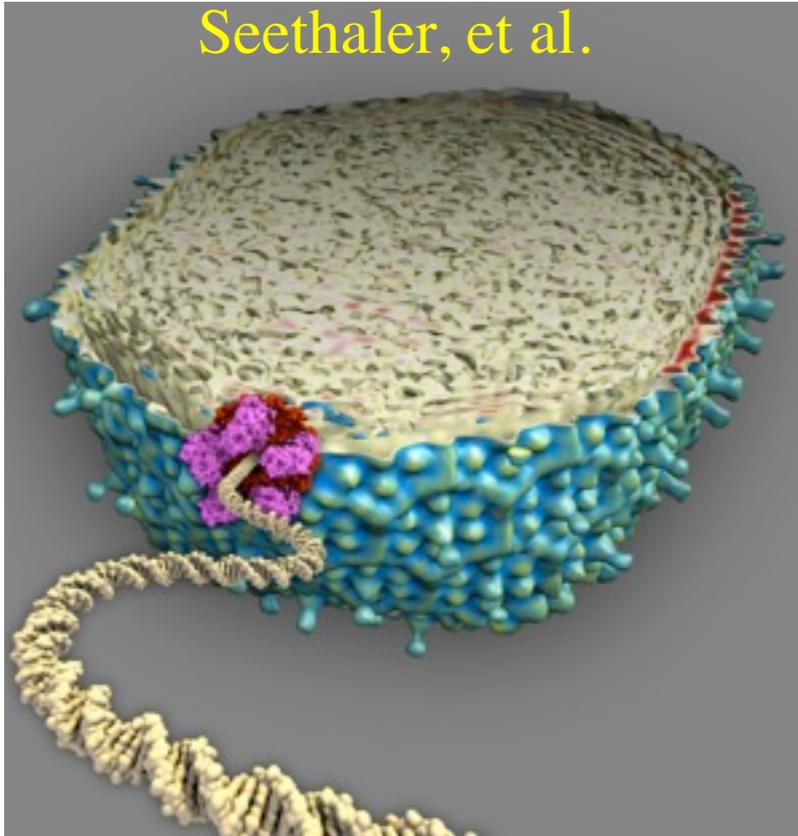
Experiment:
Ralf Seidel (Dresden)

Phys. Rev. Lett. 105, 158101 (2010)

In monovalent electrolytes, the effective charge of dsDNA is about 41% of its nominal charge

MD simulation of dense DNA arrays

Seethaler, et al.



What we control

DNA density (or harmonic constraint radius)

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

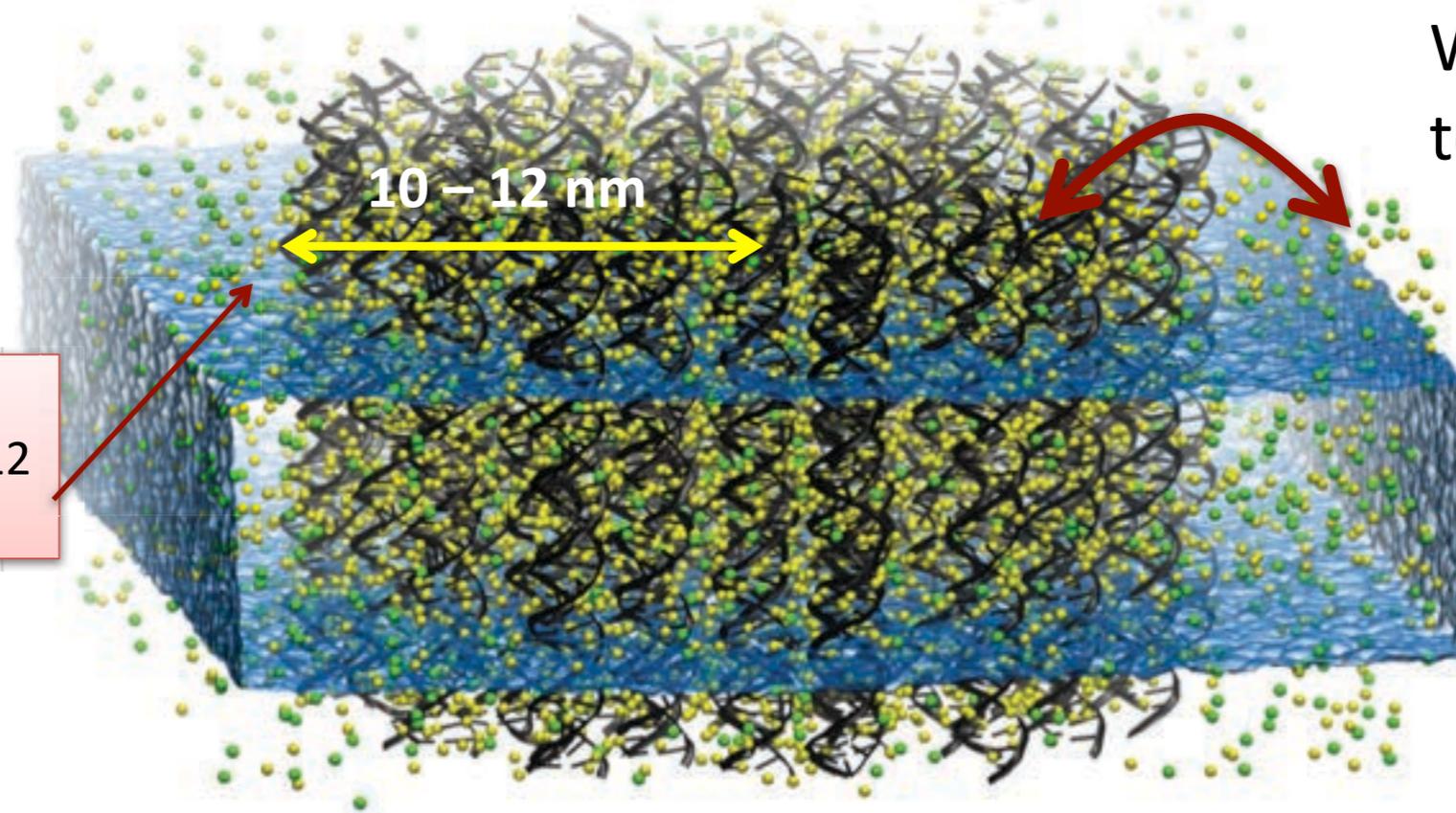
$[\text{Mg}^{2+}]_{\text{buf}} \sim 0 \text{ or } 20 \text{ mM}$

What we measure

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

DNA / ion distribution:

DNA / ion diffusion inside the array

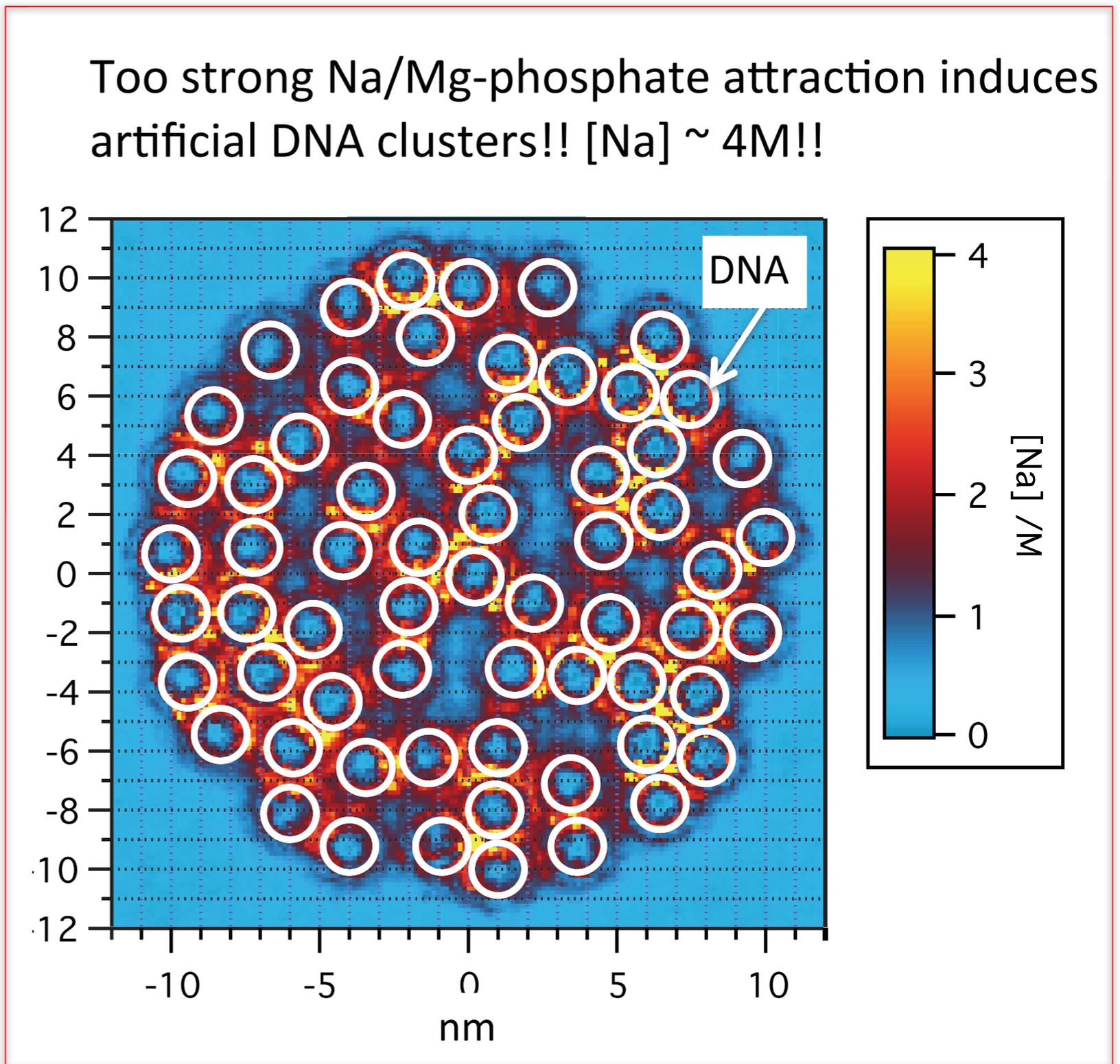
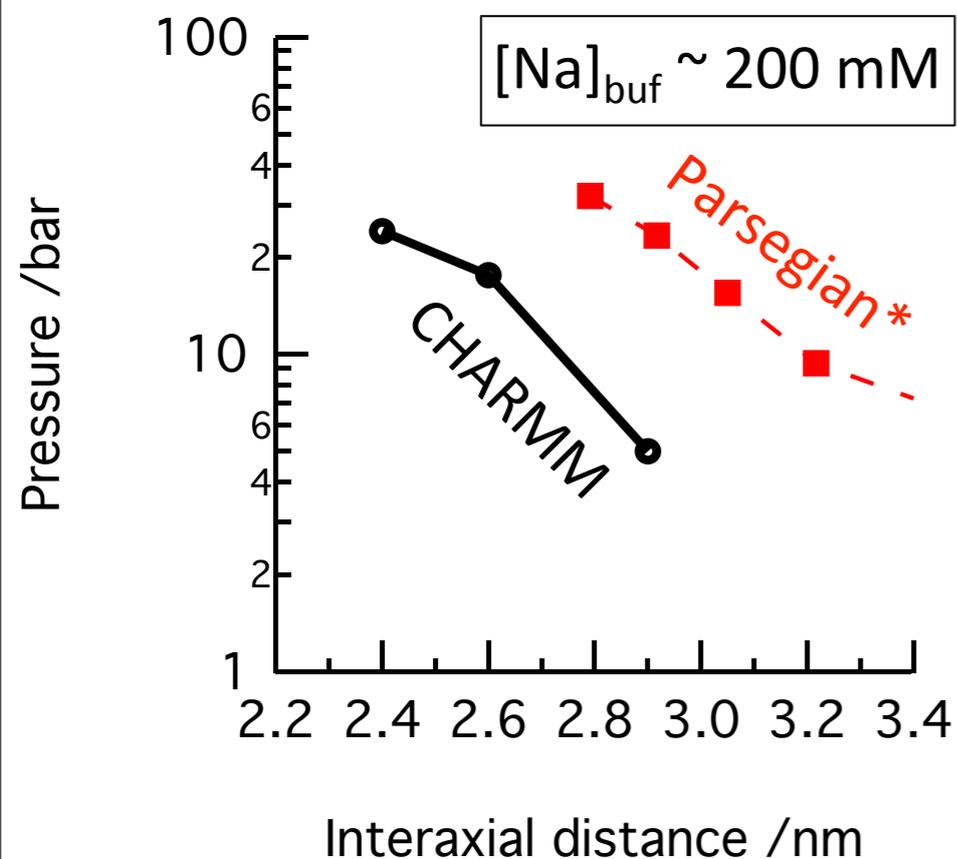
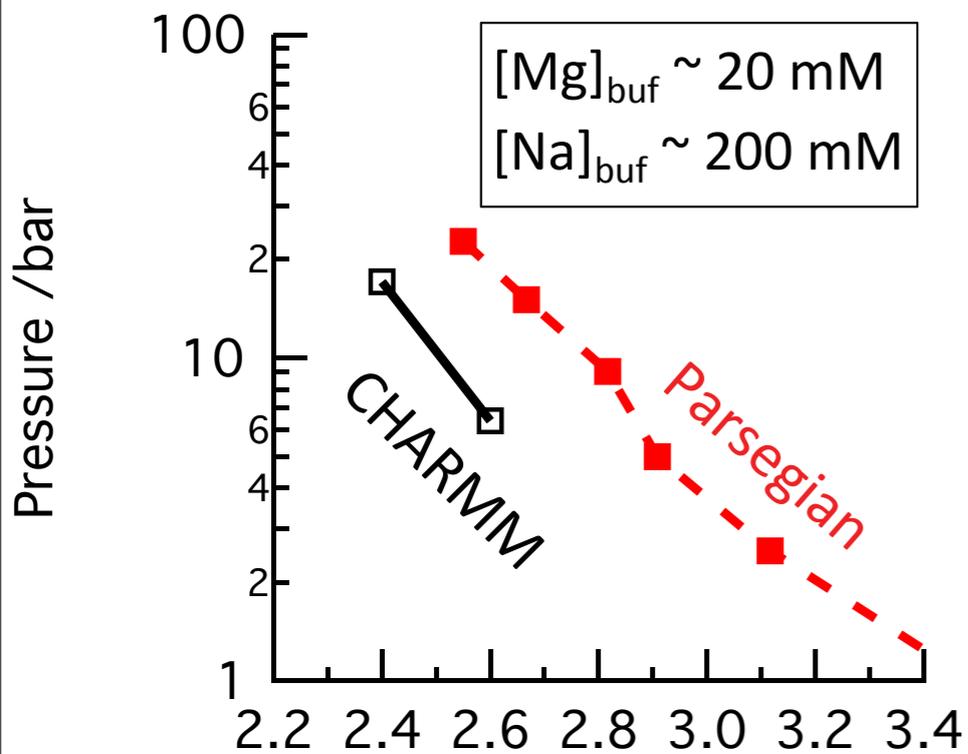


Water / salt free to move

10 – 12 nm

Cylindrical harmonic constraint (radius of 10 – 12 nm) only against DNA

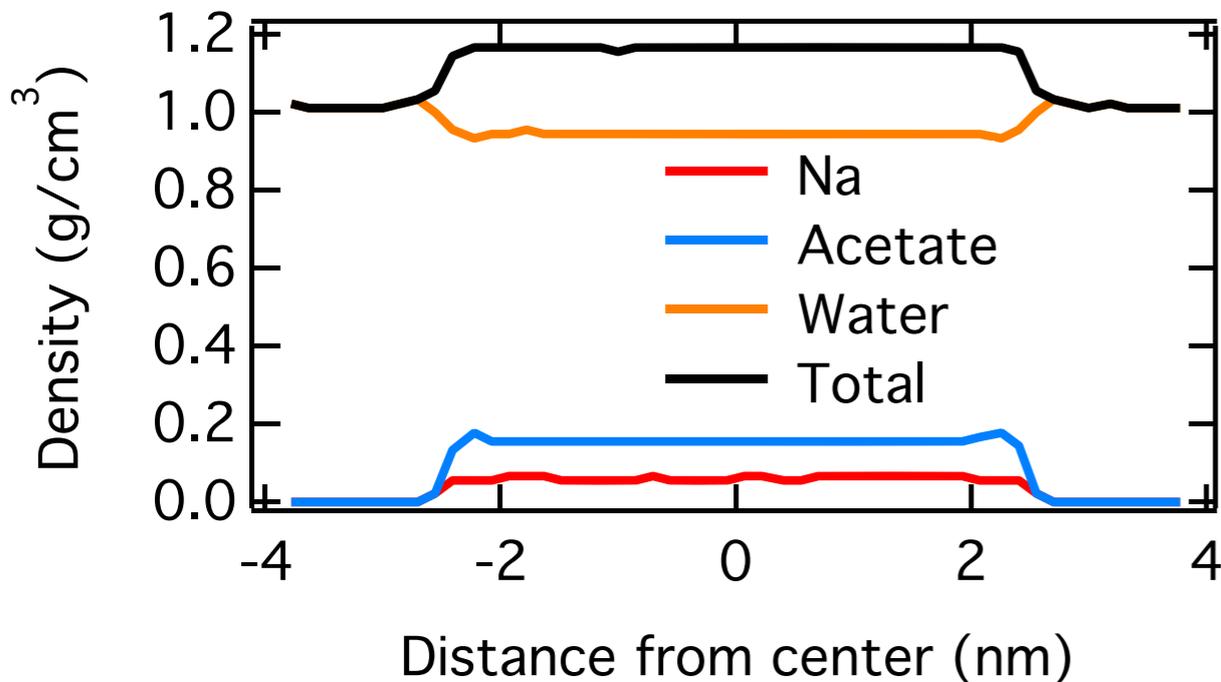
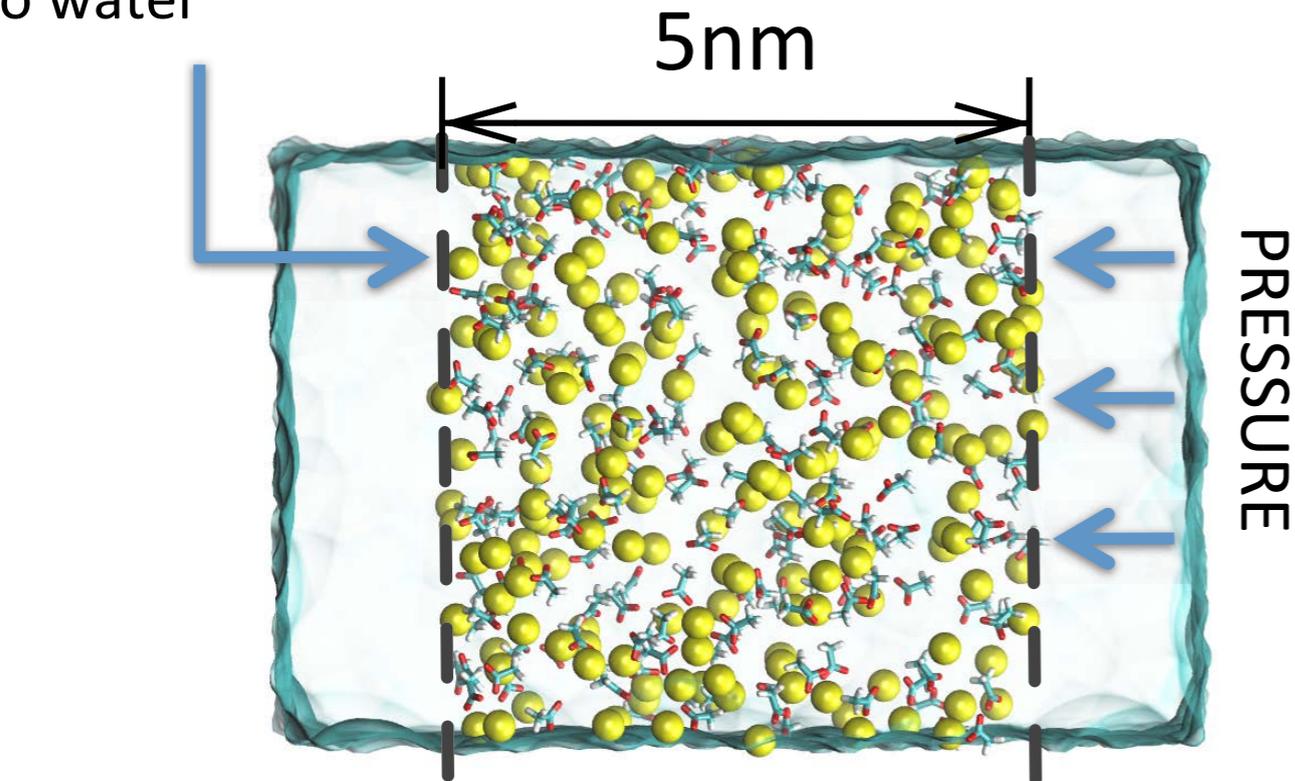
The standard MD force field fails to predict internal pressure of a DNA array



* Rau, D. C.; Lee, B.; Parsegian, PNAS (1984)

Recalibrate ion-DNA parameters using osmotic pressure data

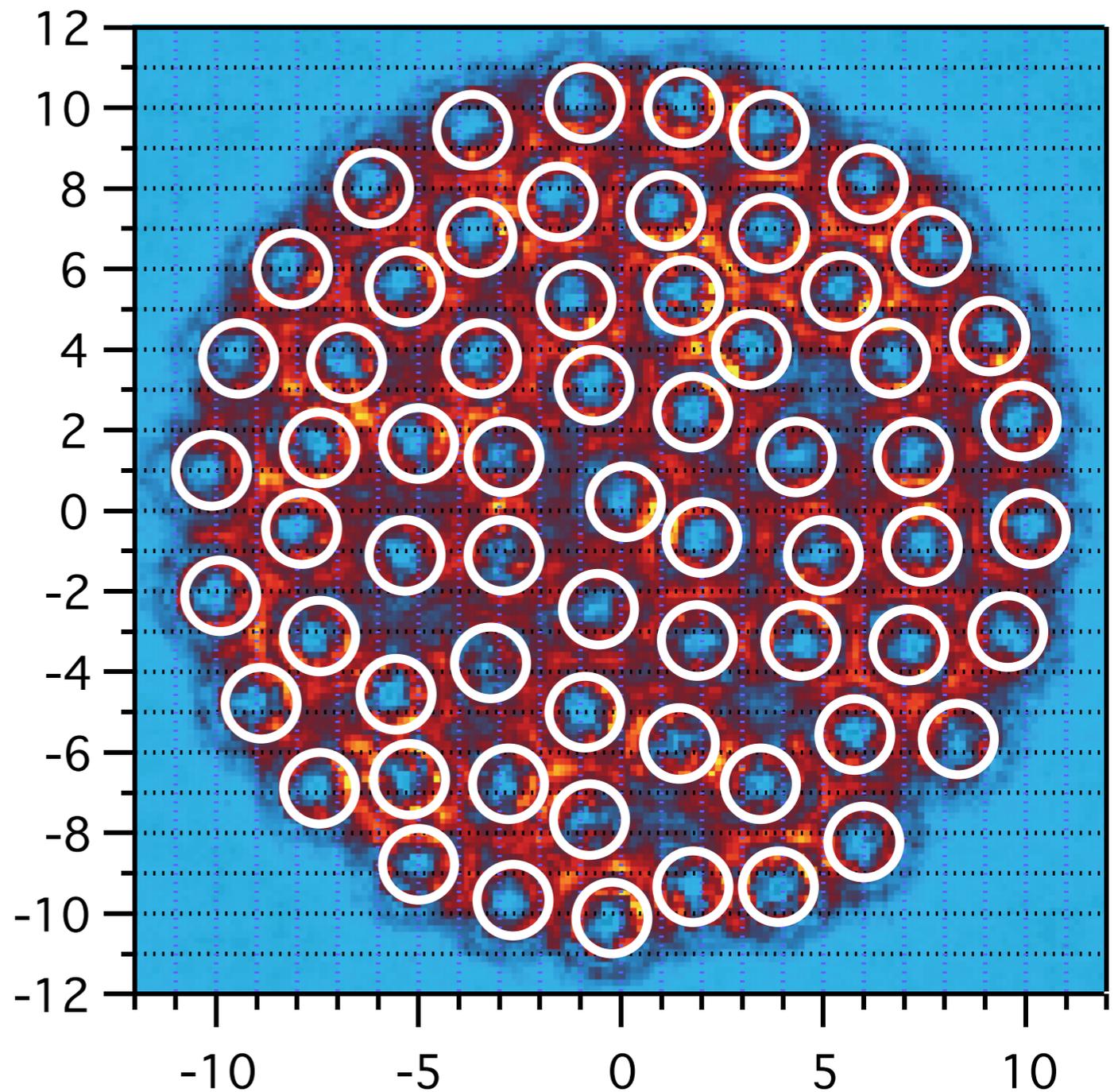
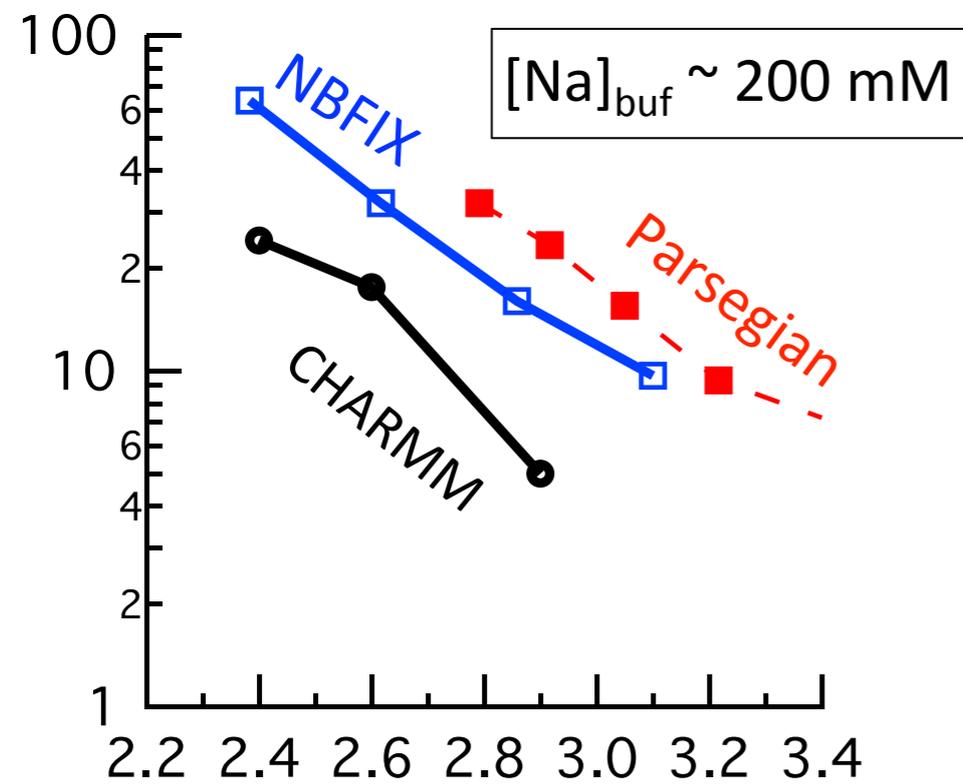
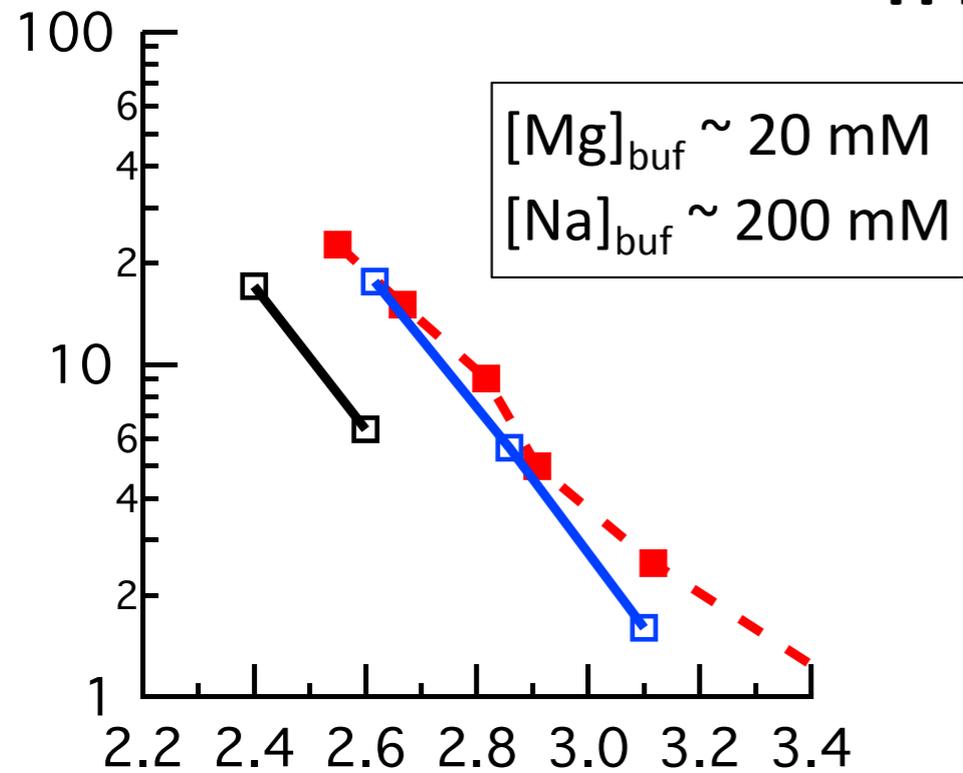
permeable only
to water



- 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)

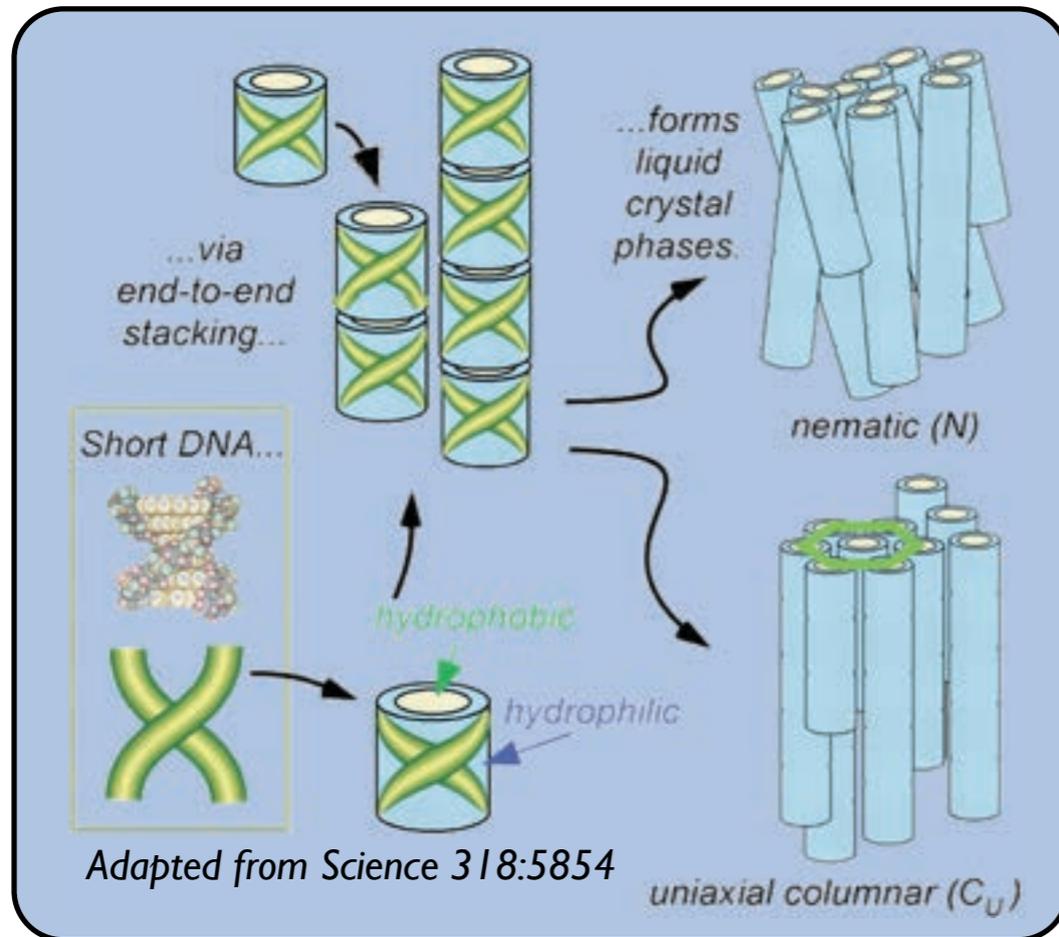
Improved parametrization of ion-DNA interactions



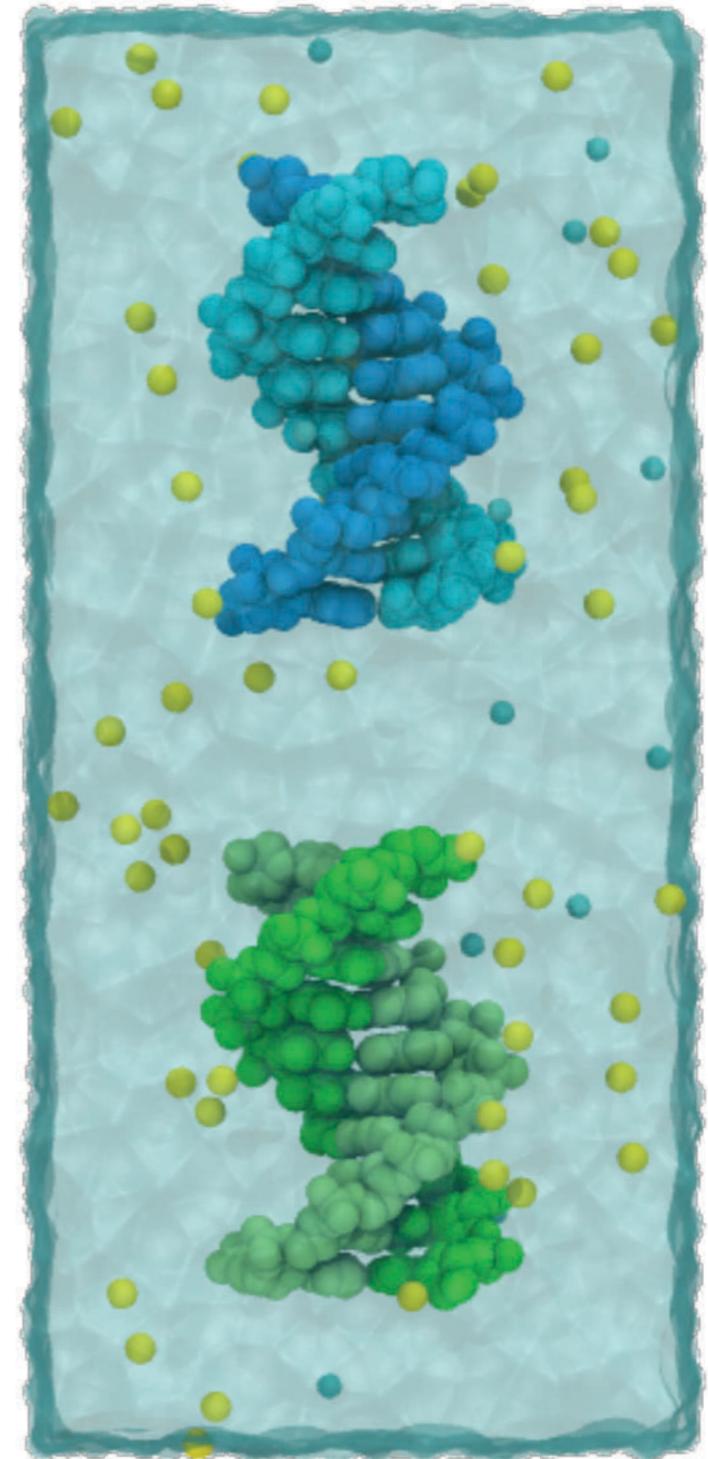
J. Phys. Chem. Lett. 3:45 (2012)

Short dsDNA fragments form end-to-end aggregates

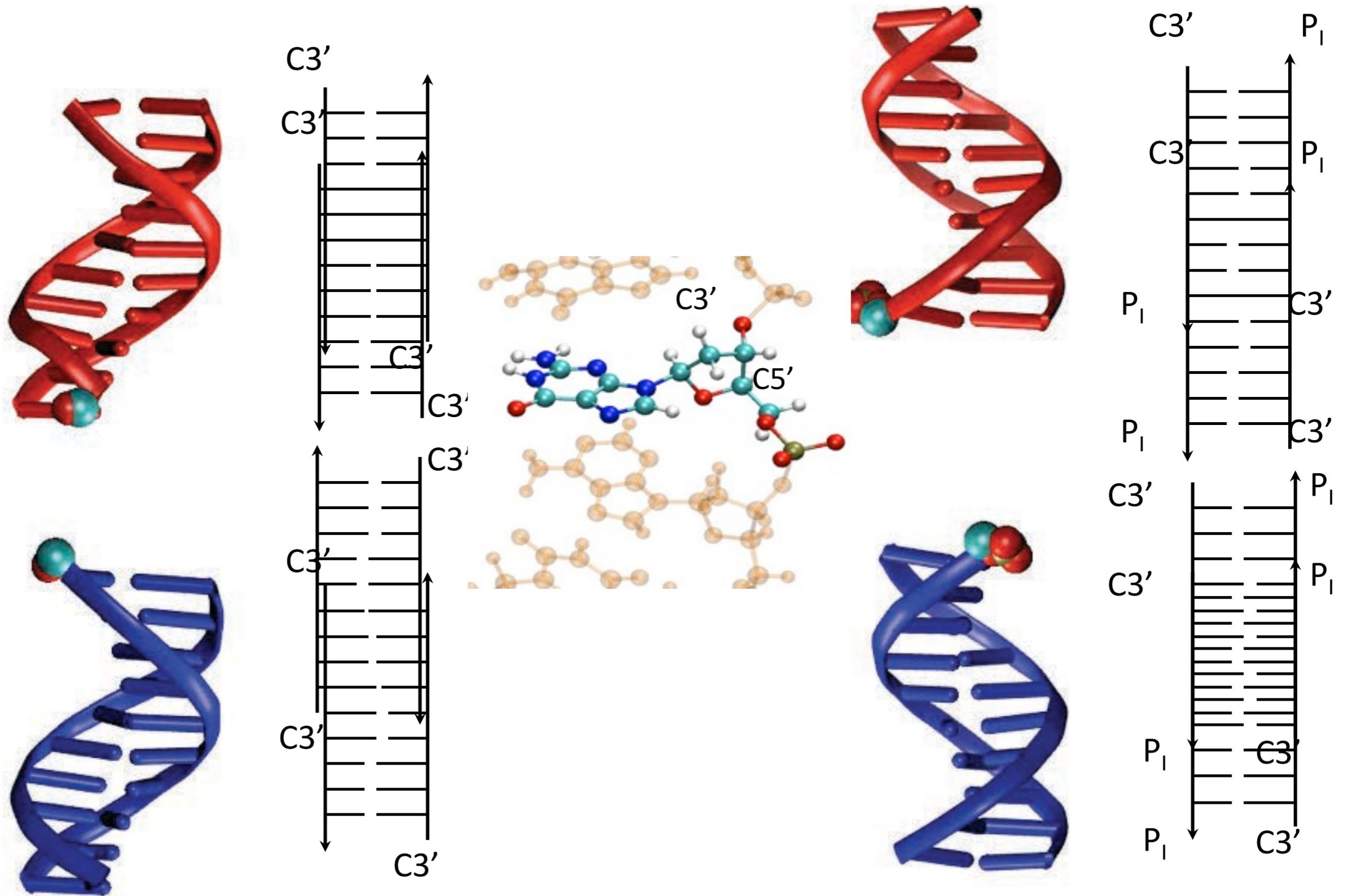
Experimental evidence of end-to-end aggregation of short dsDNA fragments motivated simulations to determine whether aligned DNA fragments would collapse to an end-to-end assembly.



Chris Maffeo



Proper connection requires a terminal phosphate



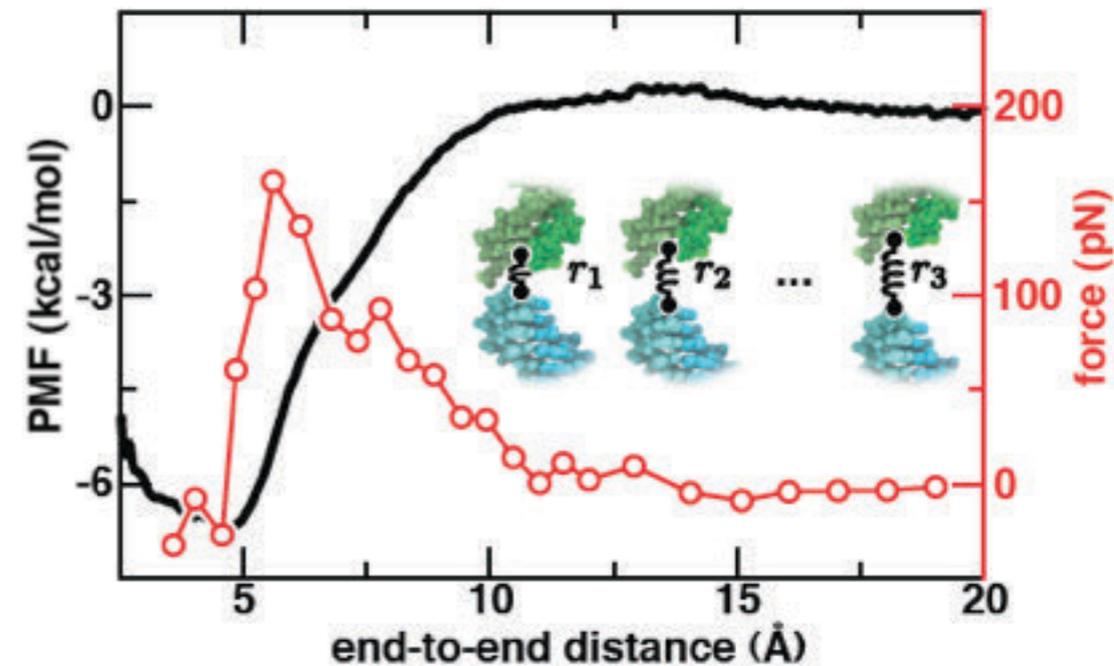
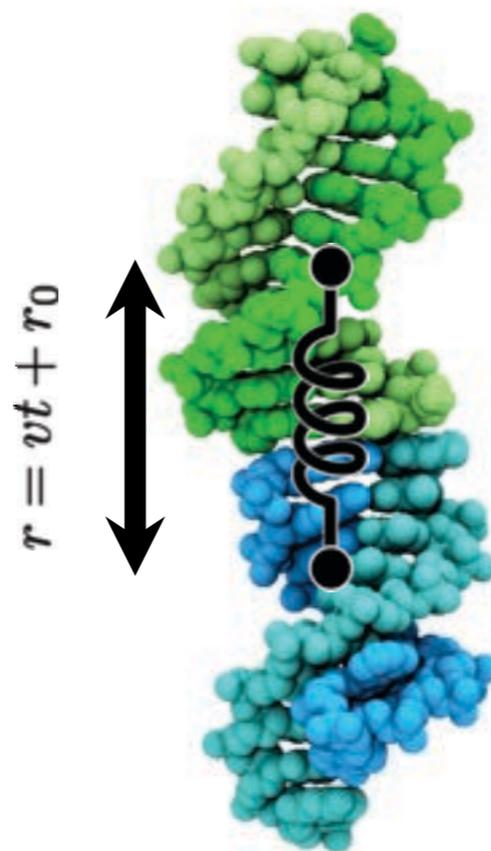
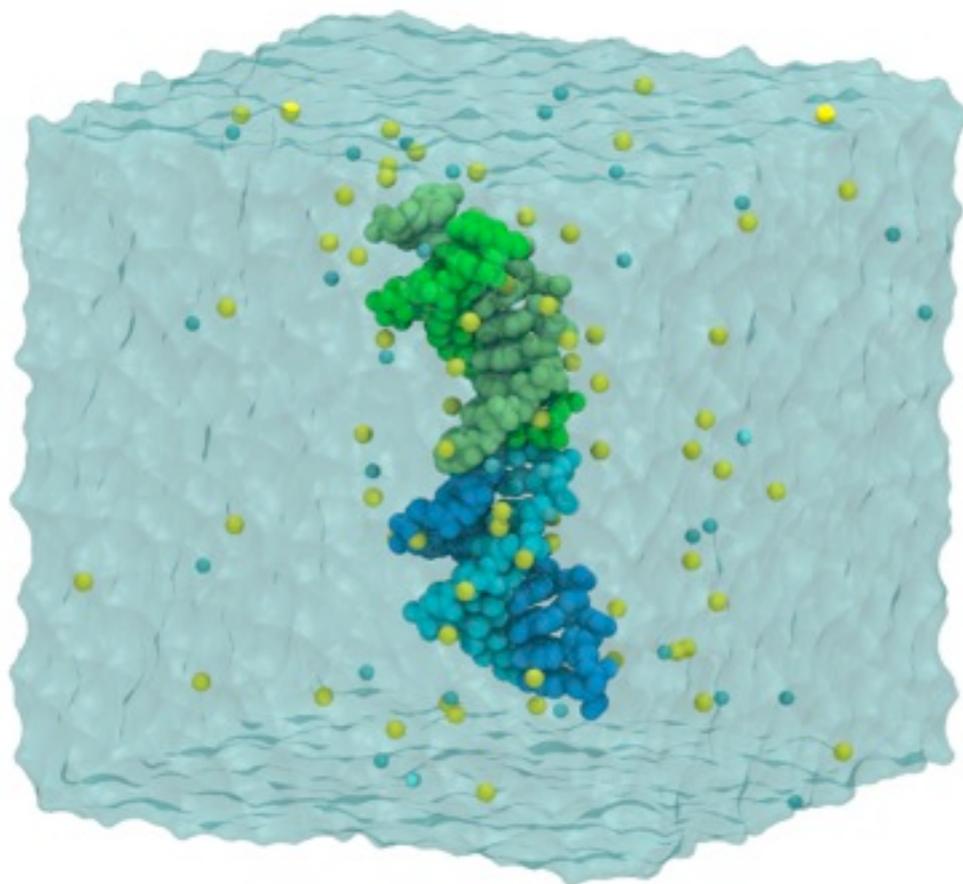
Further simulation reveals the strength of the end-to-end DNA interaction

Nucleic Acids Research 40:3812 (2012)

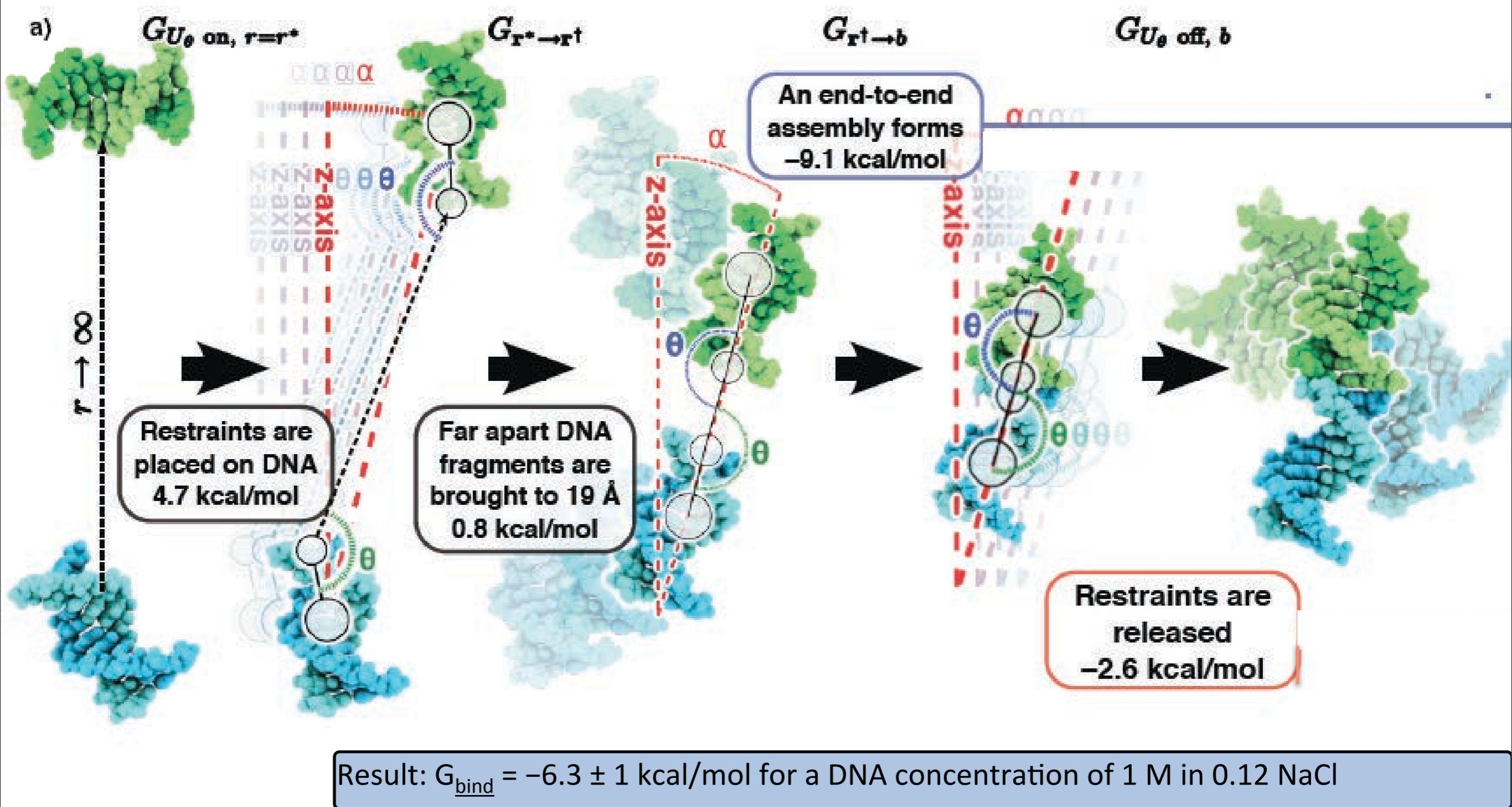
The assembly proved stable in the absence of restraints during 600 ns of simulation.

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

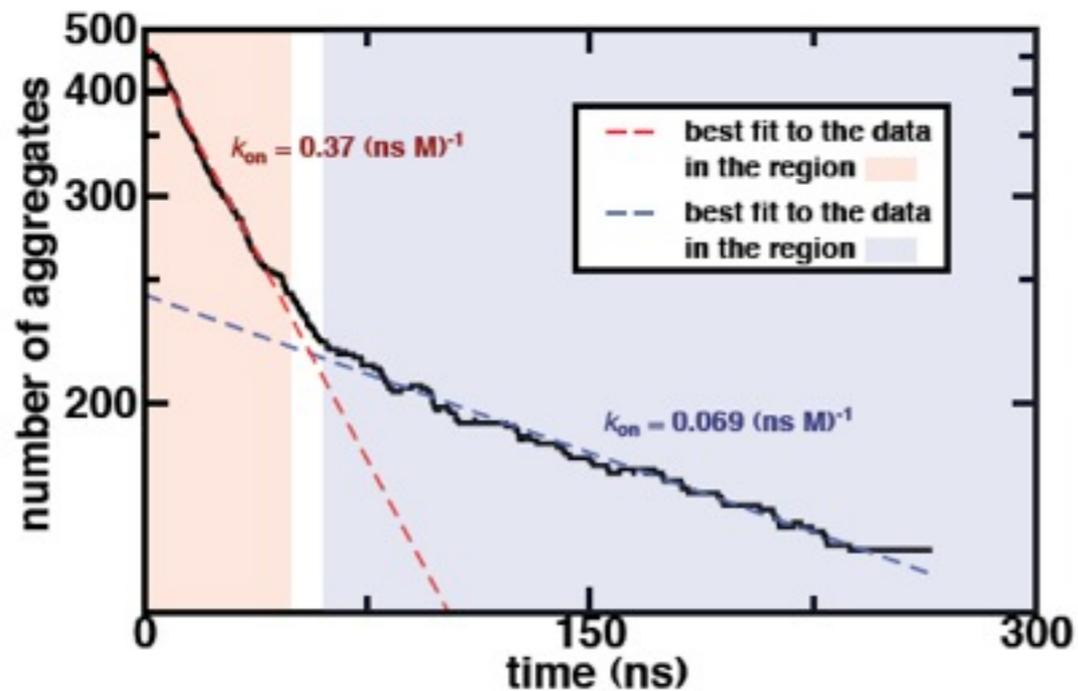


$$G_{\text{bind}} = G_{U_\theta \text{ on}, r=r^*} + G_{r^* \rightarrow r^\ddagger} + G_{r^\ddagger \rightarrow b} + G_{U_\theta \text{ off}, b}$$

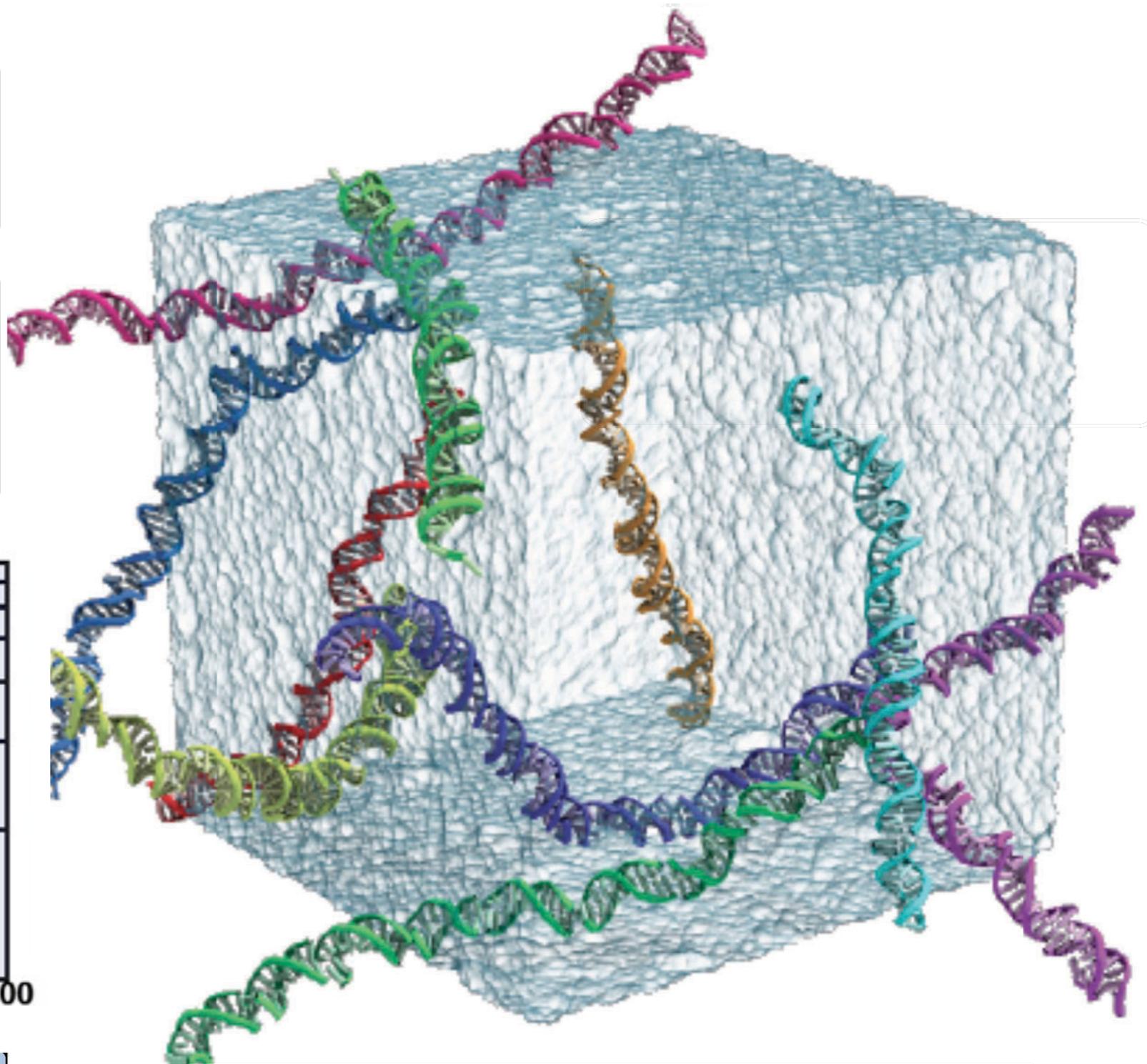
Simulation of DNA aggregation

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_{bind}



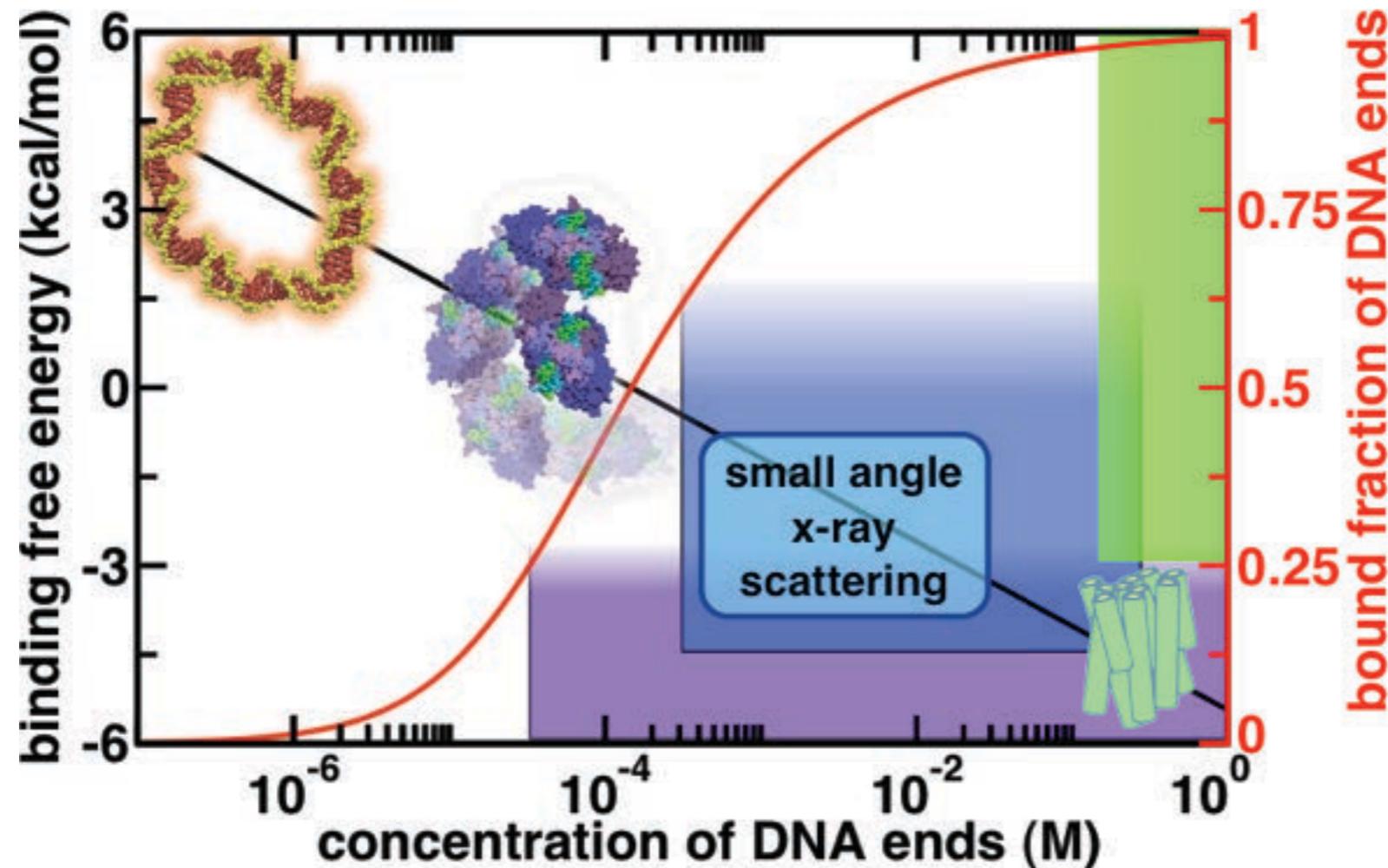
$$G_{\text{bind}} = -k_B T \log k_{\text{on}}/k_{\text{off}} = -5.5 \text{ kcal/mol}$$



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

Significance of end-to-end interaction depends on concentration of DNA ends

Nucleic Acids Research 40:3812 (2012)



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

Concentration of DNA ends is too small for significant blunt-ended cyclization

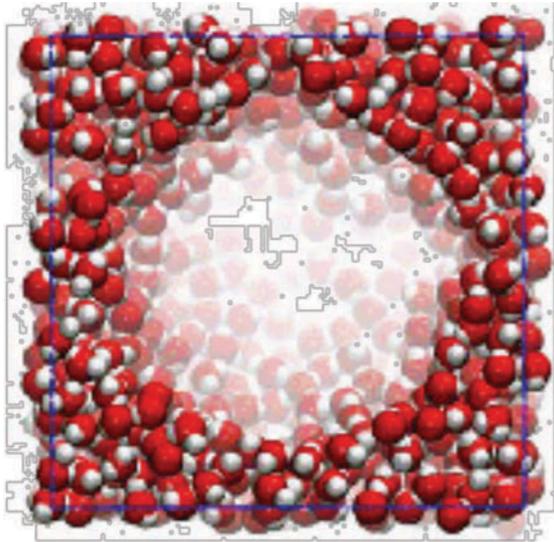
End-to-end adhesion may aid repair of double-stranded DNA breaks, since the DNA ends are held in proximity

User-Defined Forces in NAMD

Maxim Belkin

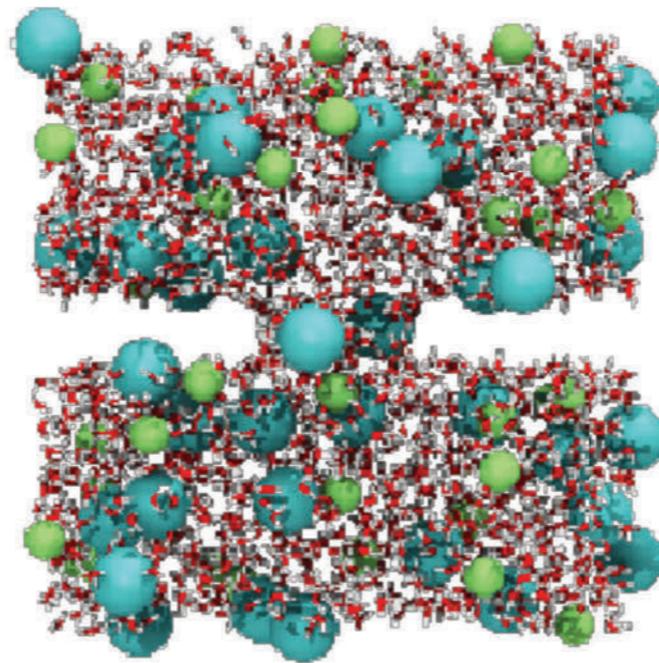


TclBC



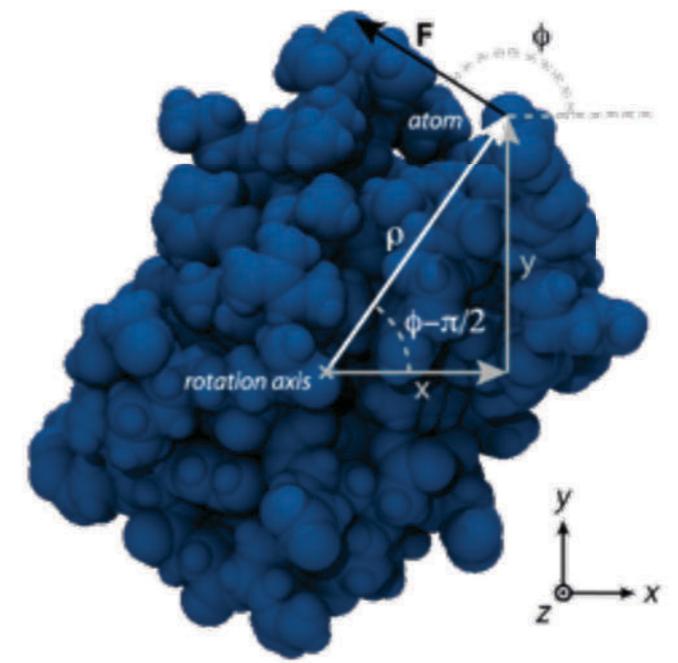
Medium

GridForces



Medium

TclForces



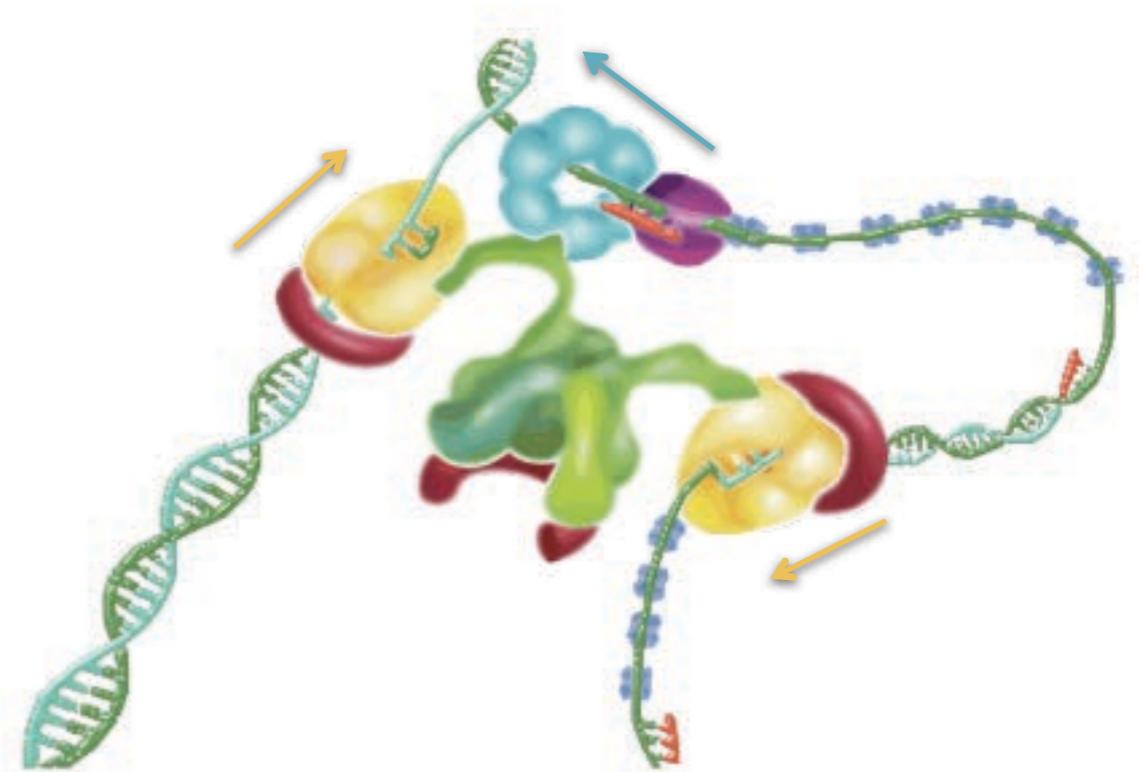
Advanced

Estimated completion time: ~ 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*



Adapted from Mol. Cell 23:155

Enzymes common to all replisomes:

Helicase

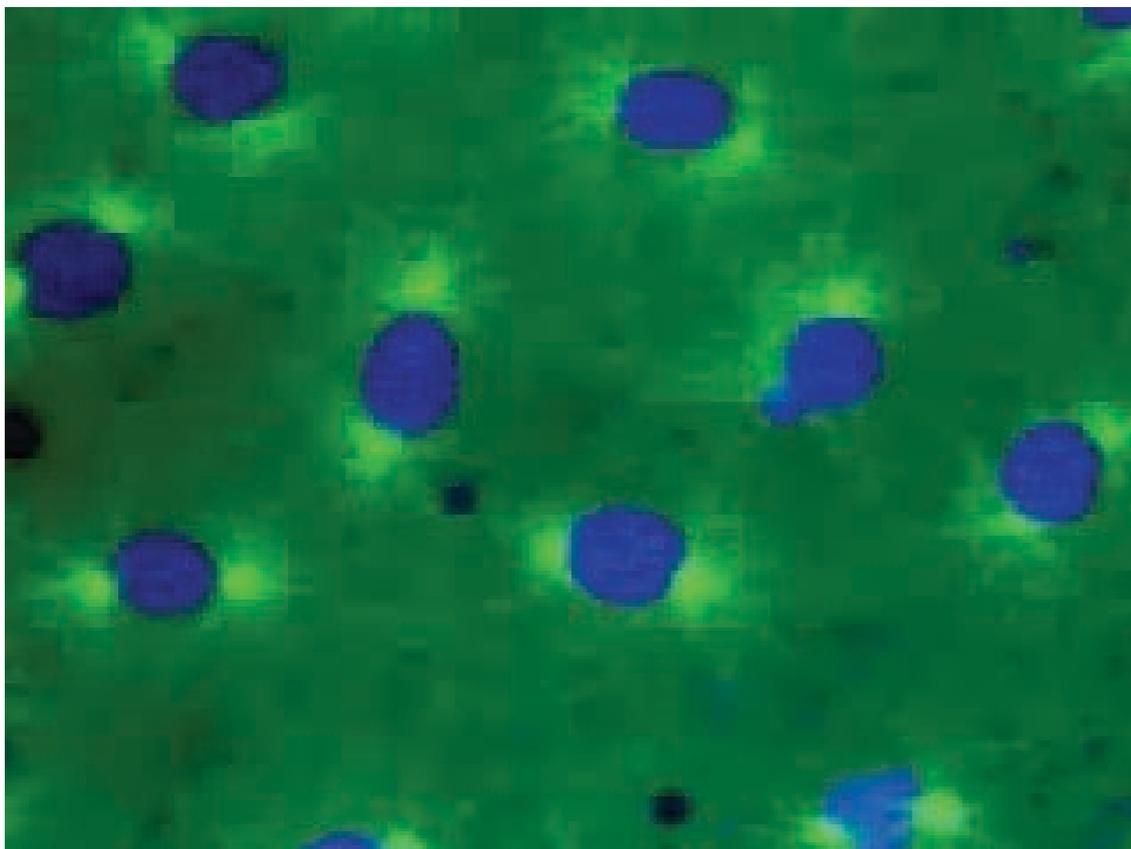
Primase

Polymerase

Ligase (not depicted)

Sliding clamp and clamp loader

ssb

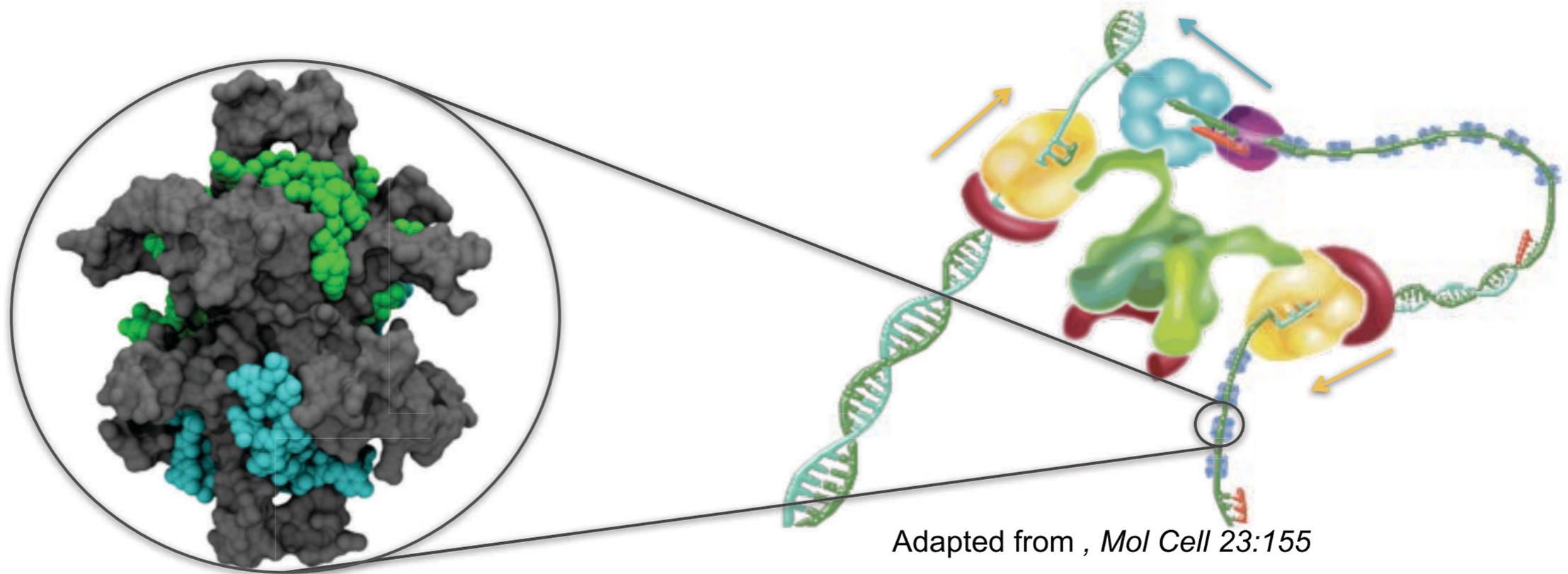


Alberts, Molecular Biology of the Cell, fifth edition

SSB protects single-stranded DNA

Prevents formation of secondary structure, enzymatic digestion, chemical modification

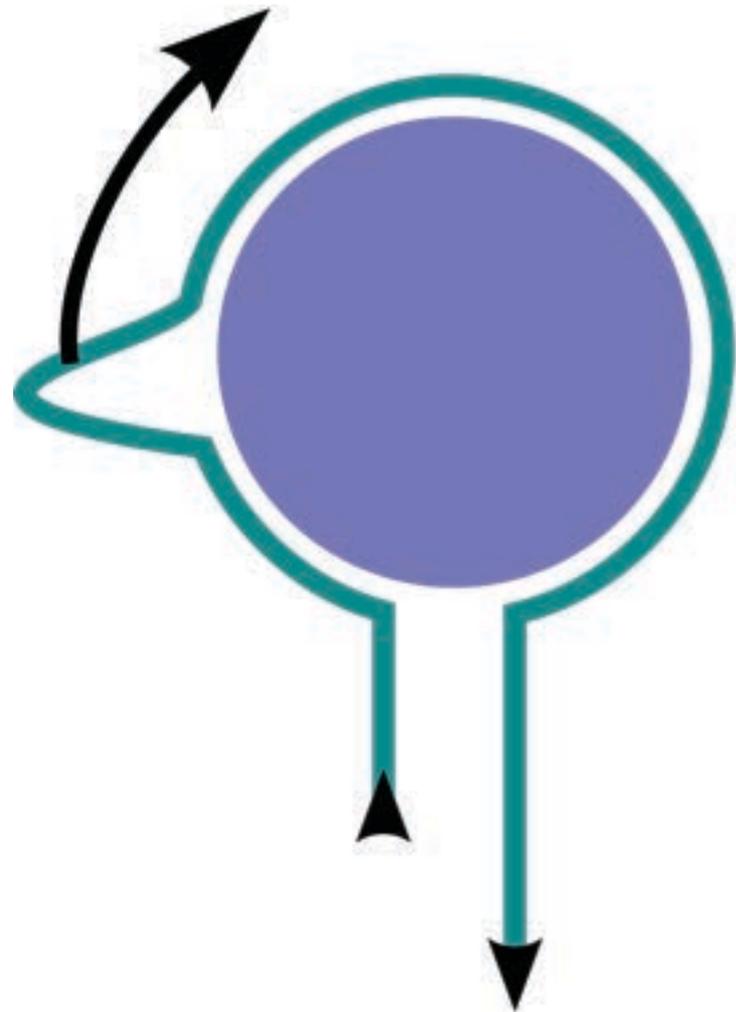
Single-stranded DNA binding protein (SSB) can bind 35 or 65 nucleotides of ssDNA (SSB₃₅ and SSB₆₅) with high affinity



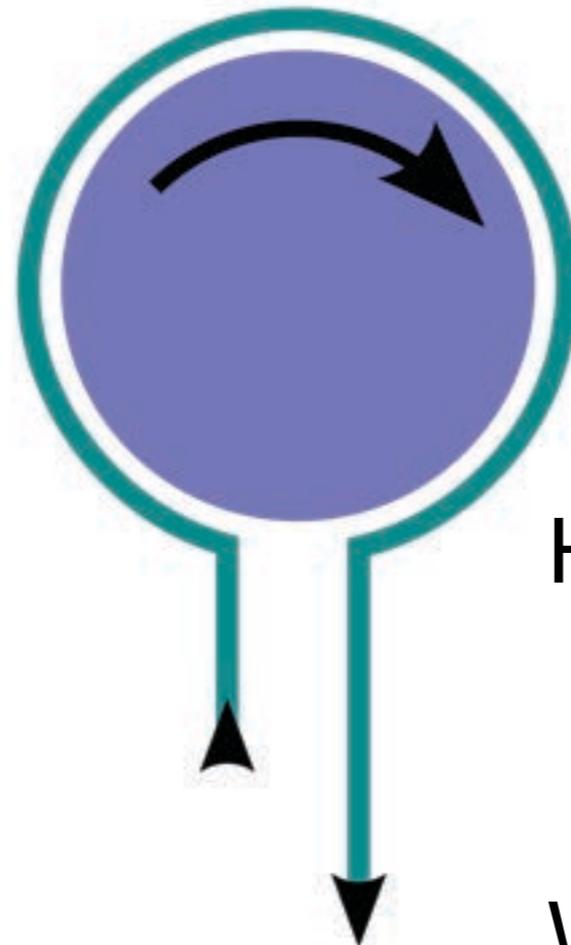
Problem: how is SSB removed when it is no longer needed?

Diffusion of ssb along DNA

reptation



rolling



What is the microscopic mechanisms of SSB diffusion?

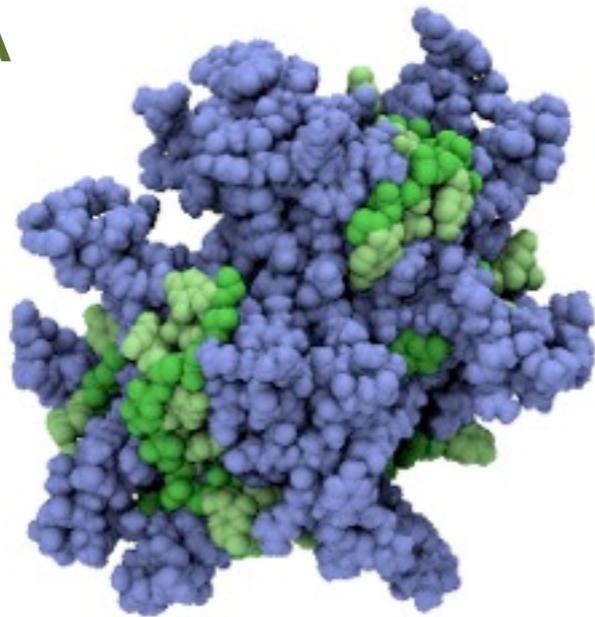
How does dissociation of DNA from SSB occur?

What makes an ssb an ssb?

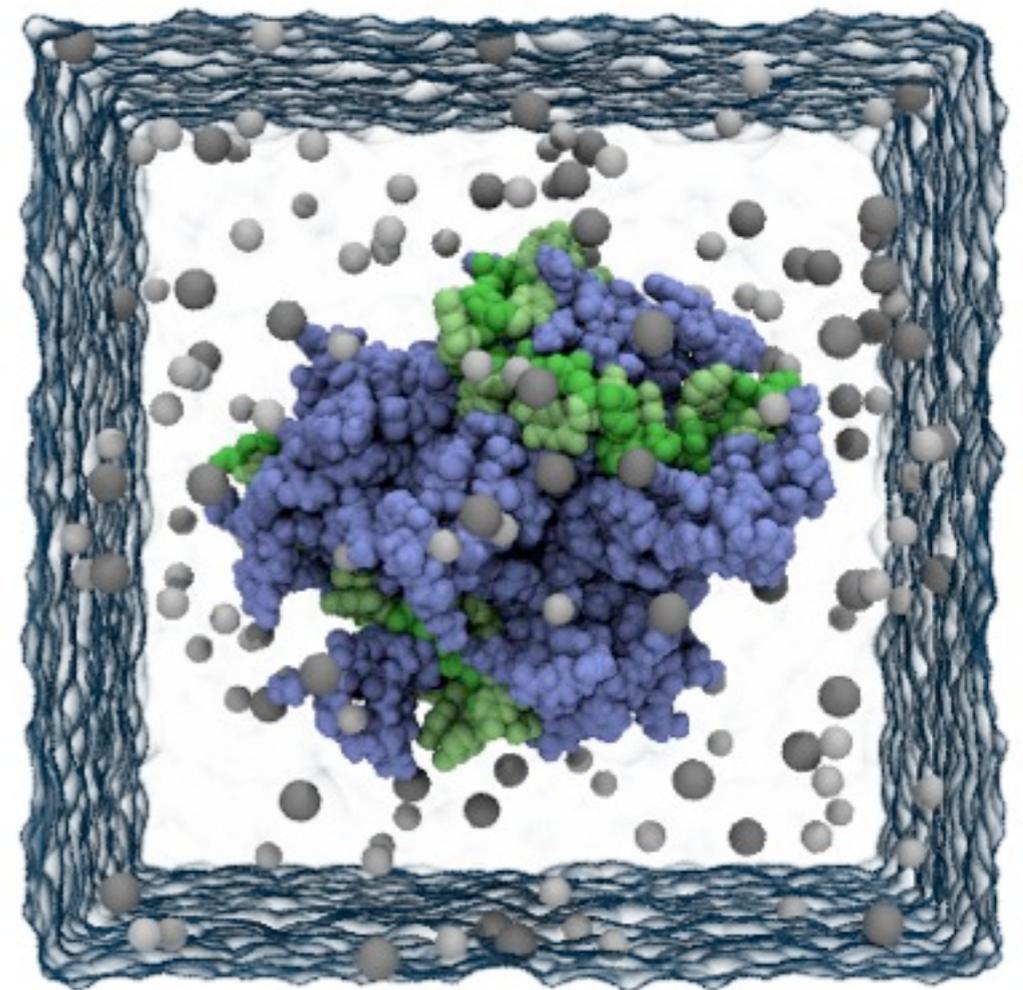
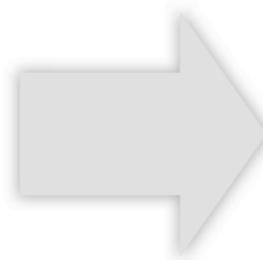
† Ha group, Nature 461:1092

A model is build from an x-ray crystal structure

SSB
DNA



KCl
water

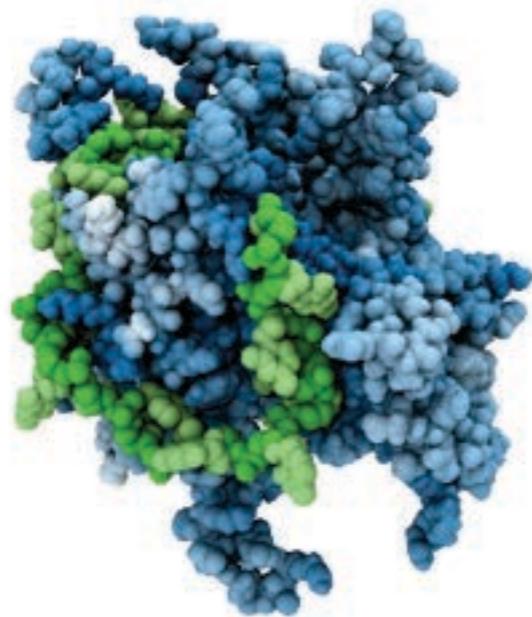


PDB:1EYG

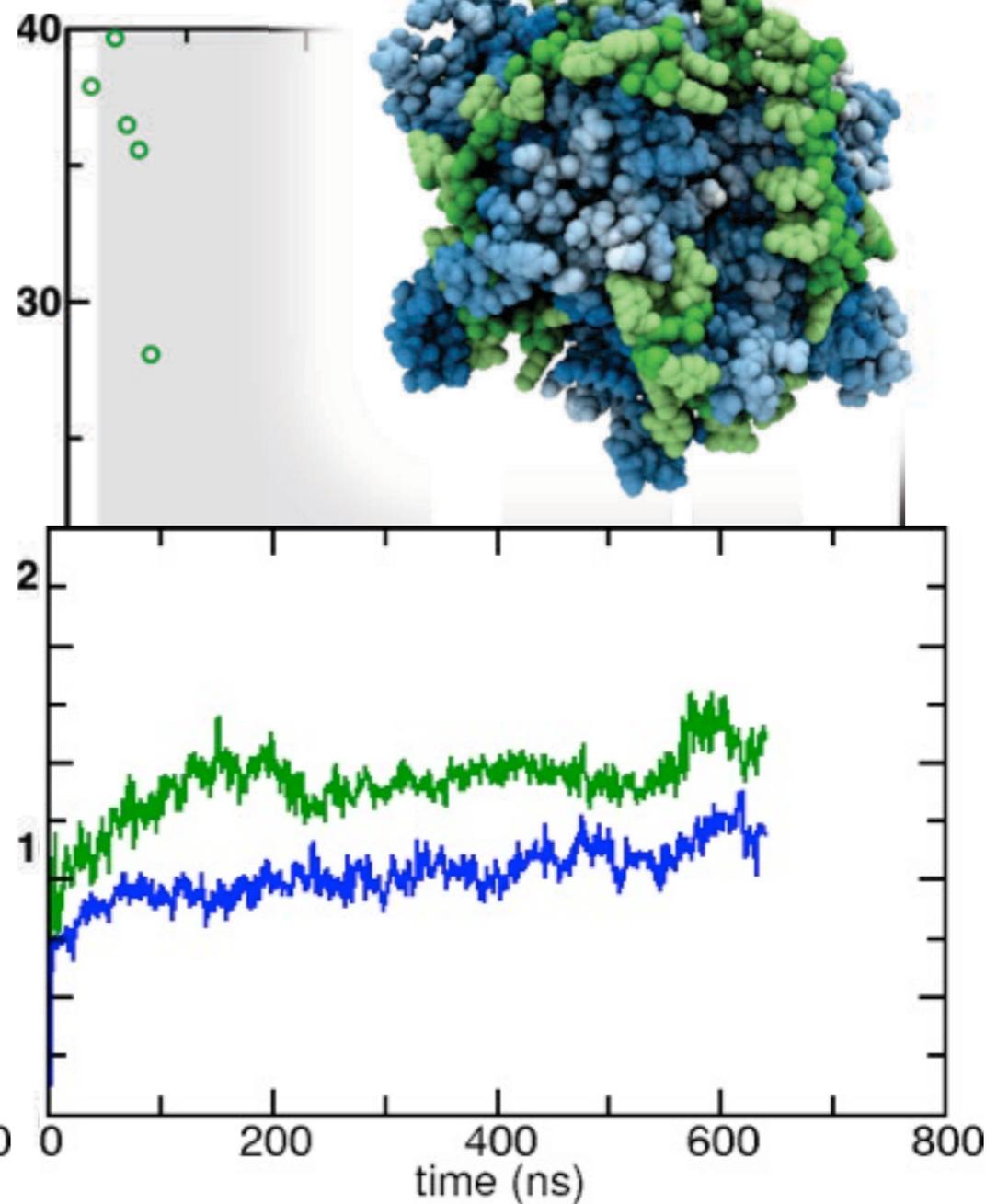
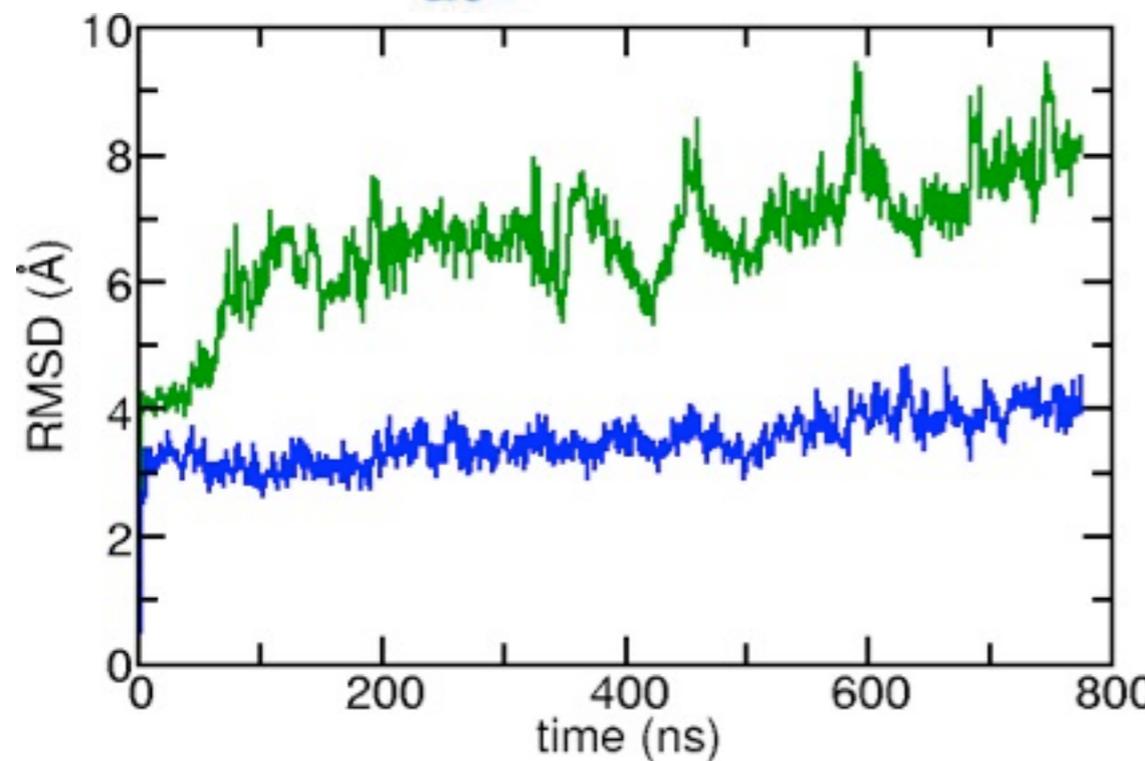
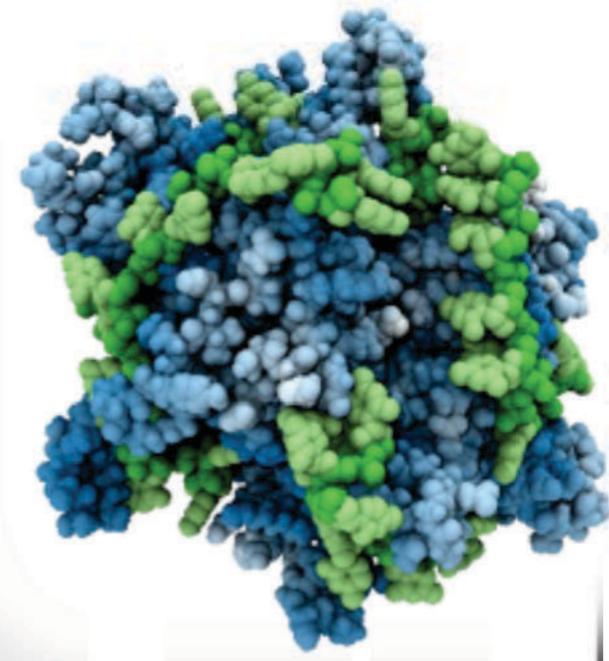
Unresolved DNA was modeled by the crystallographers (Lohman and Waksman groups, Washington U. School of Medicine) and provided to us via Ruobo Zhou of the Ha group.

Individual nucleotides are loosely bound to SSB

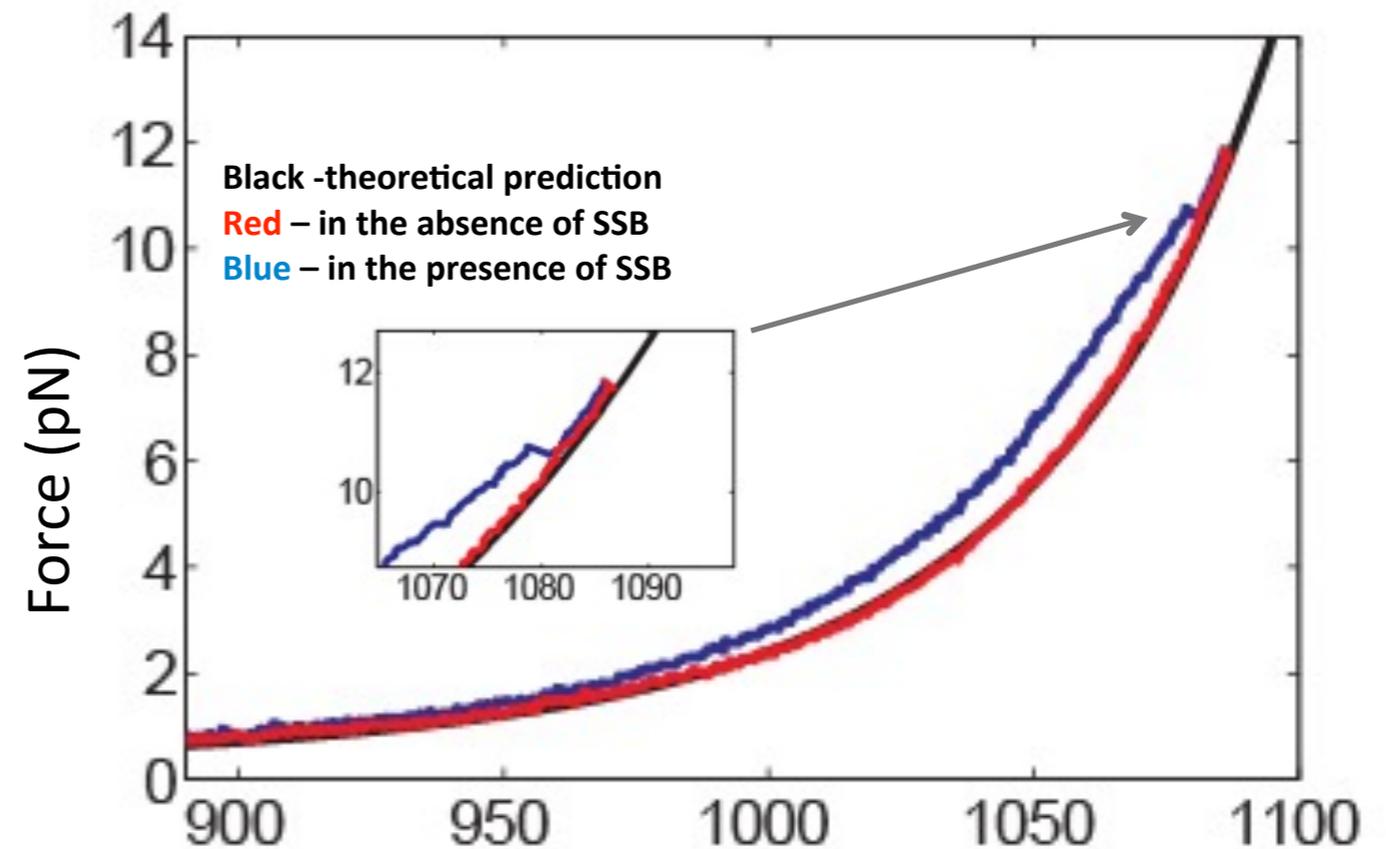
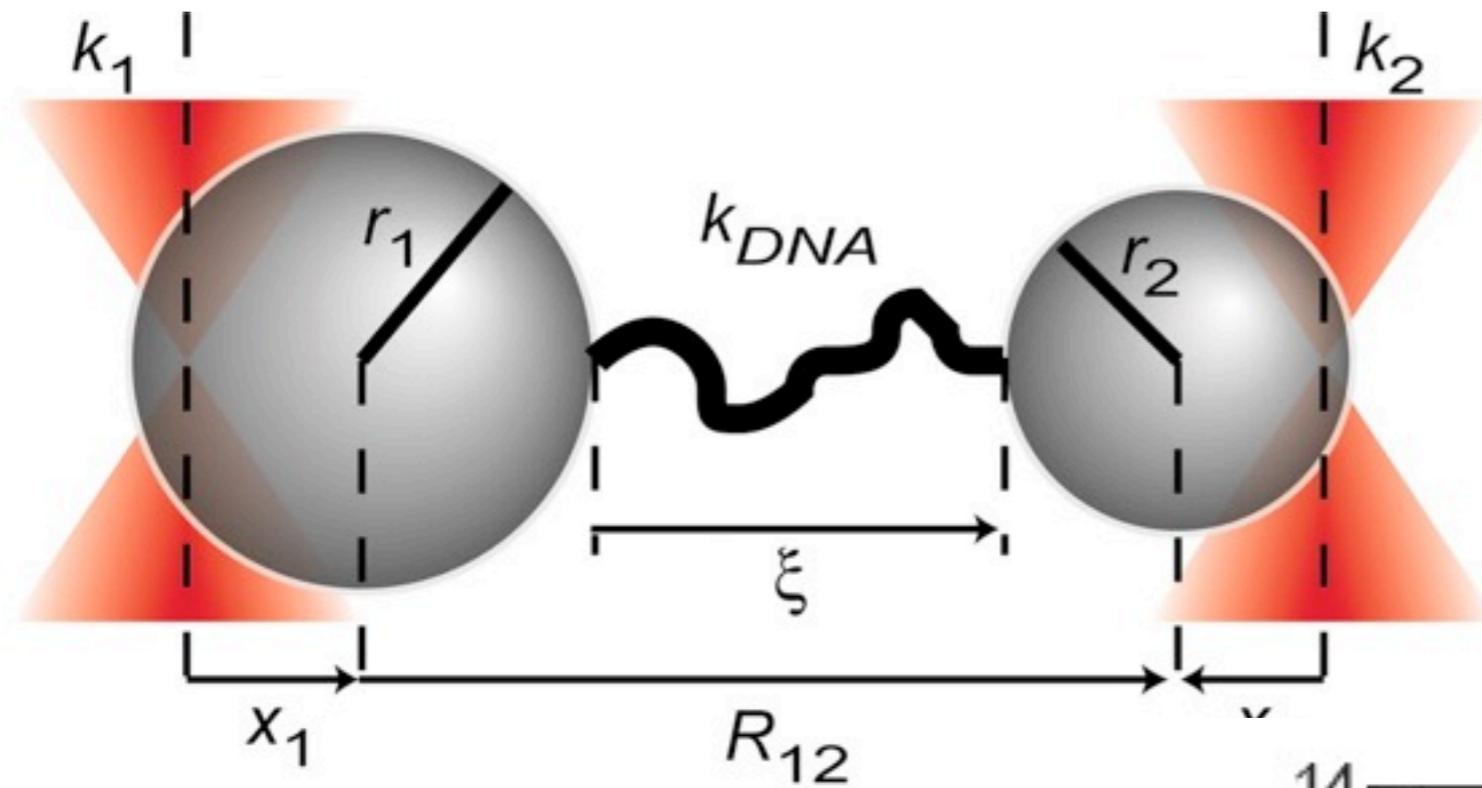
SSB₃₅



SSB₆₅

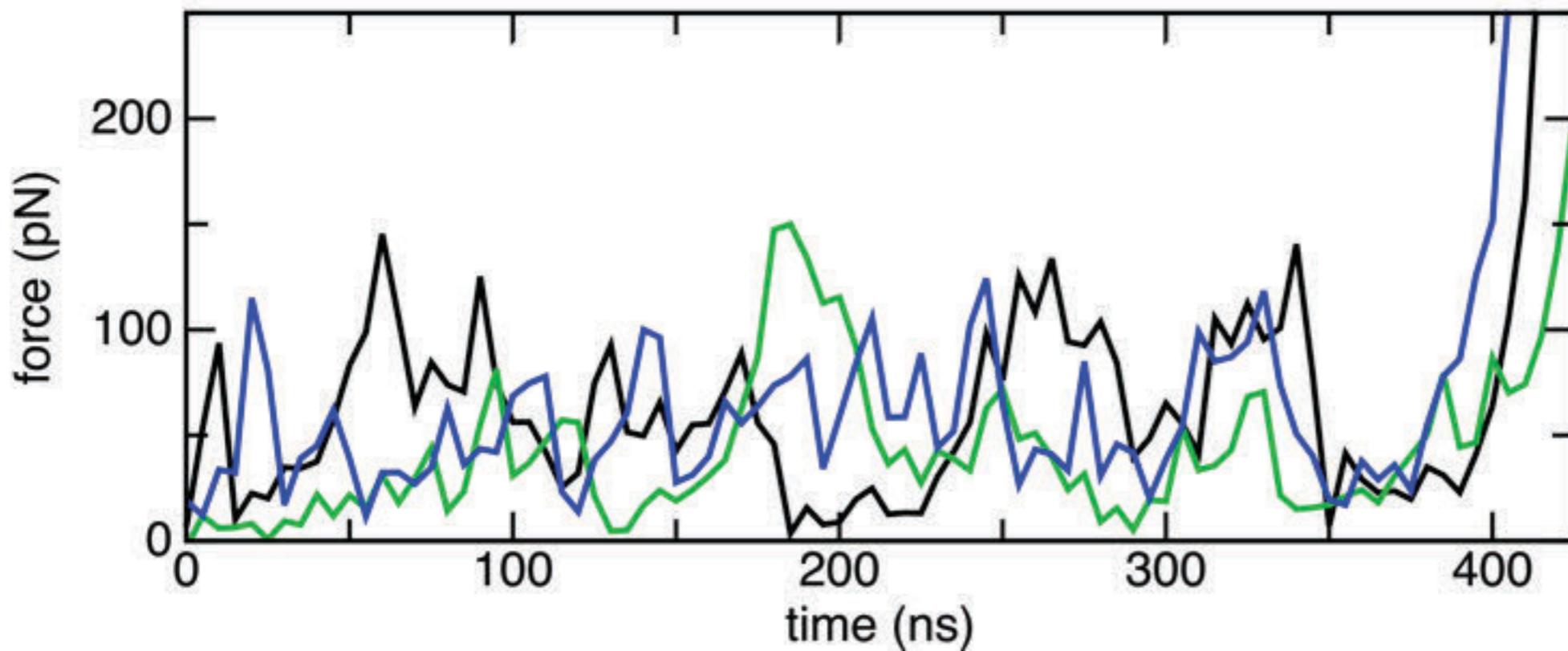
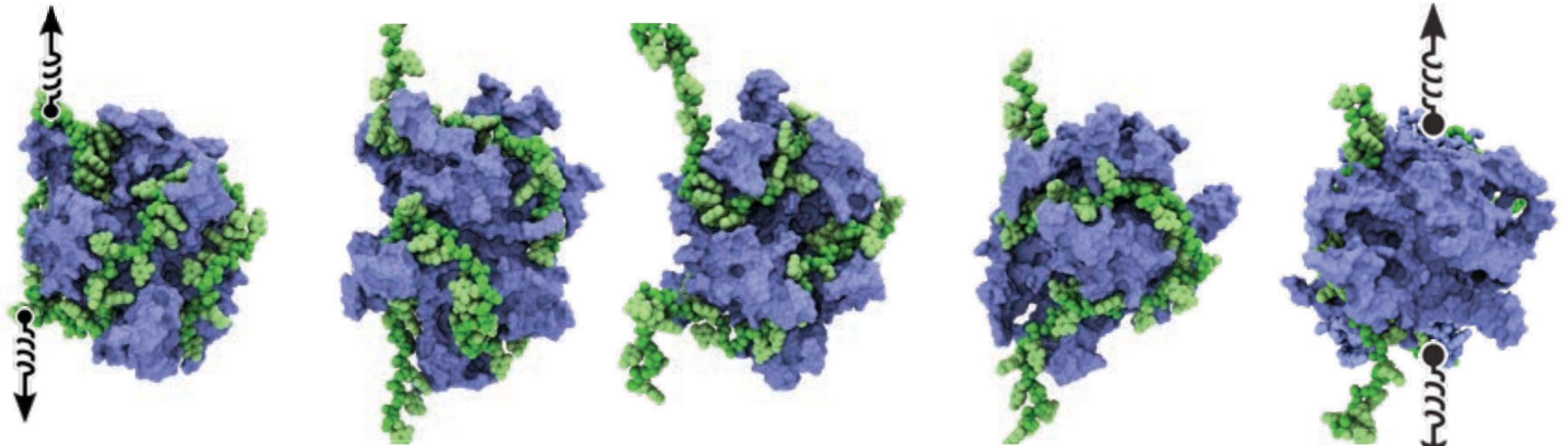


Mechanics of ssb-DNA (dis)assembly



Chemla group, unpublished

All-atom simulations cannot quite reach experiment

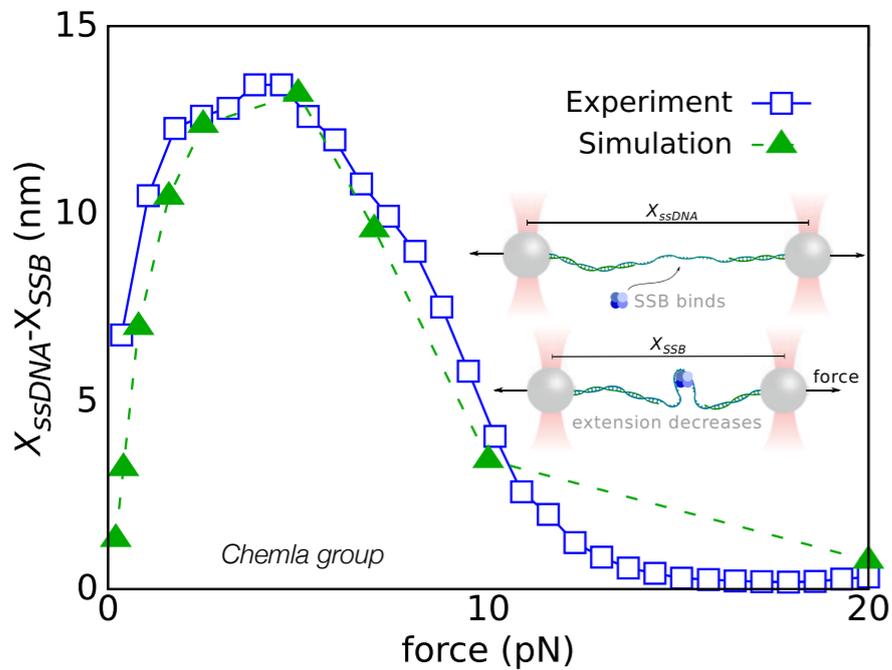
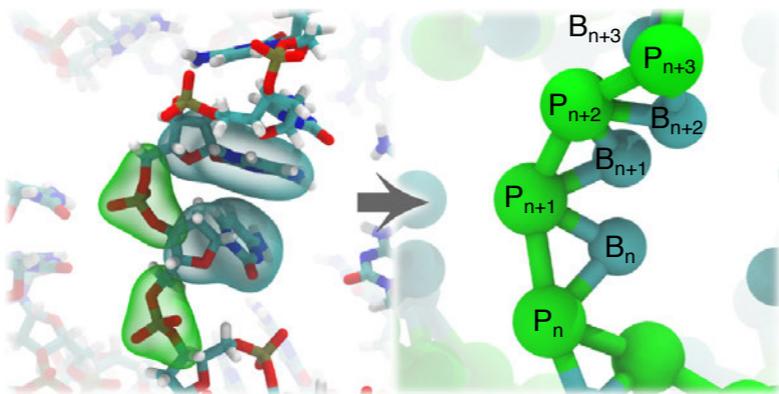


Forces are 1-2 orders of magnitude larger than in experiments

Little hope of observing diffusion-related events

Coarse-grained modeling connects with experiment

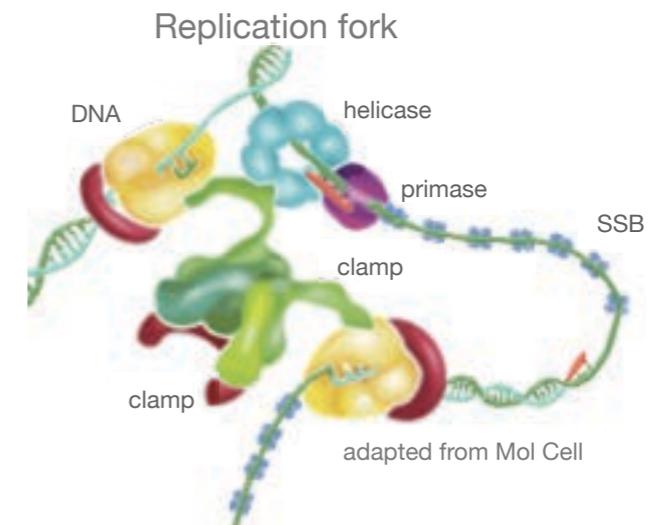
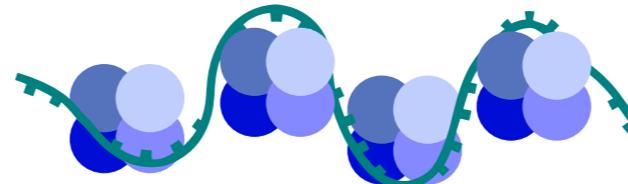
Coarse-grained models of and single-stranded DNA binding protein (SSB) were developed from all-atom simulation



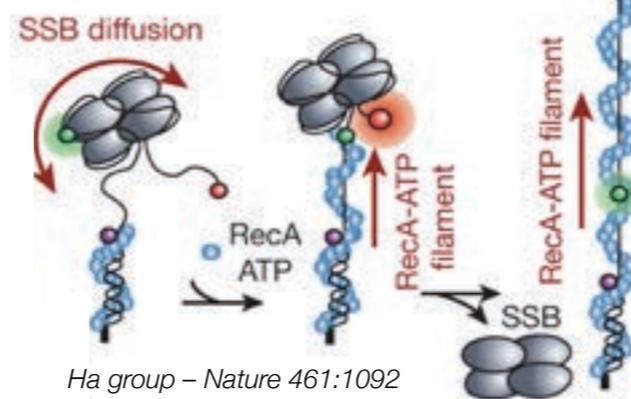
Excellent agreement was obtained between experiment and simulation suggesting that SSB binds DNA dynamically

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

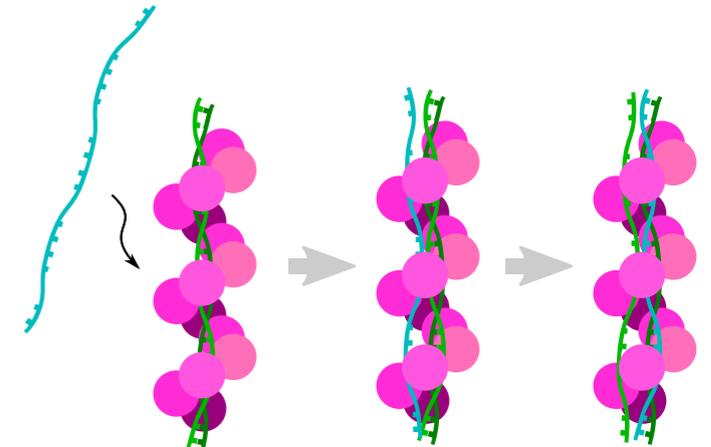
SSB saturates DNA during replication; the effect of protein-protein interactions on the structural and dynamical properties remain unexplored



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

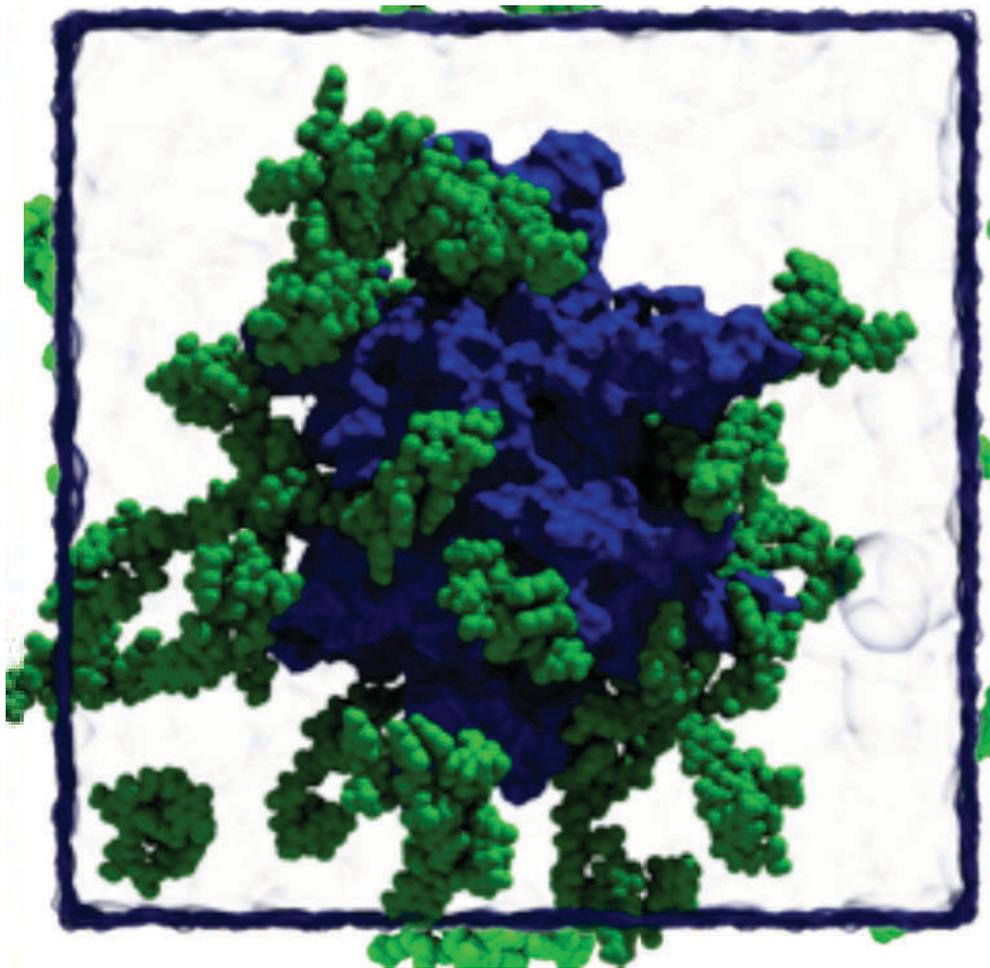


RecA mediates strand exchange during DNA repair, but must efficiently displace tightly-bound SSB molecules from ssDNA, but the mechanism is elusive

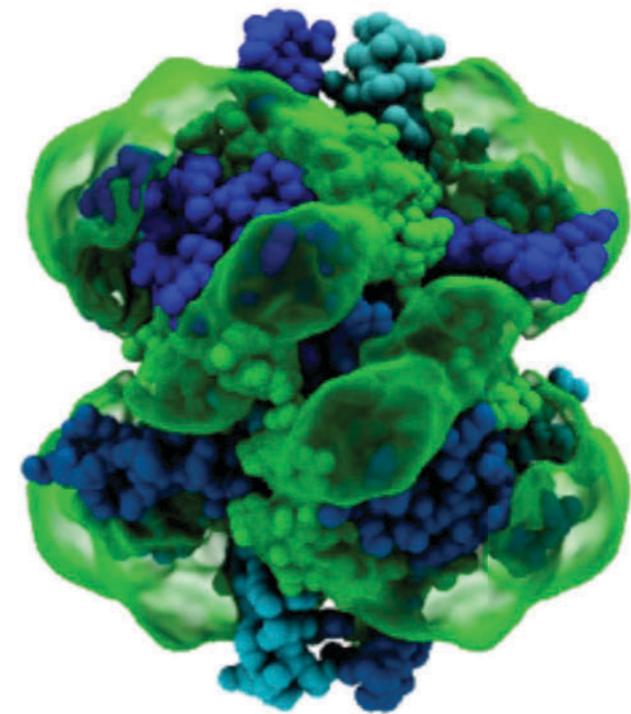


SSB parameterization

Obtain density of all-atom nucleotides



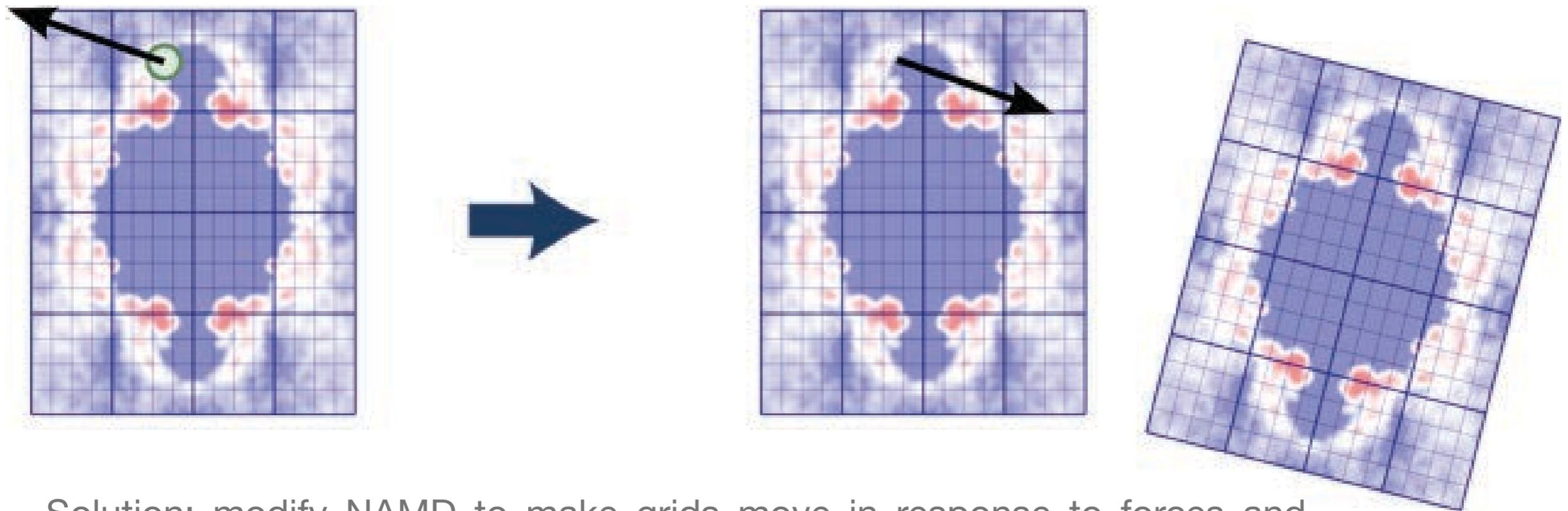
Apply iterative Boltzmann inversion using CG ssDNA to obtain the interaction potential that makes the AA and CG densities match



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



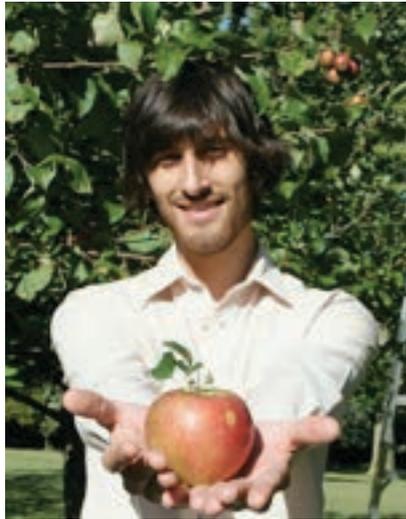
Solution: modify NAMD to make grids move in response to forces and torques. Langevin forces and torques are also applied.

DNA (1-site/nt) + SSB simulation trajectory

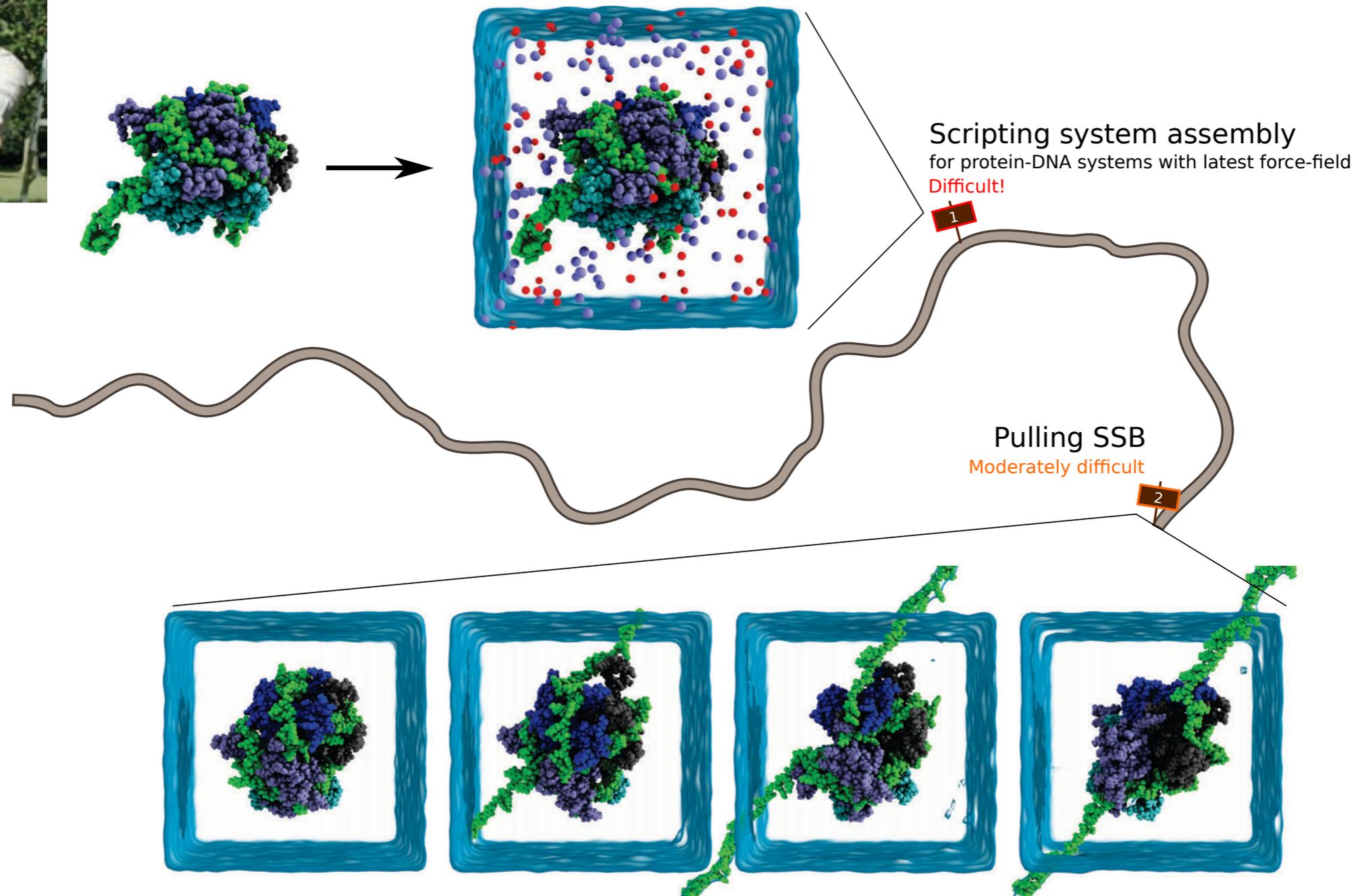


This simulation is ~400 ns, but smoothed potentials make the kinetics equivalent to ~20 μ s. We obtain ~1 μ s/day with 1-site model on two processors with 200 nucleotides and one SSB. 2-site SSB parametrization is underway.

Atomistic mechanics of single-stranded DNA Binding-Protein



Chris Maffeo



Acknowledgements

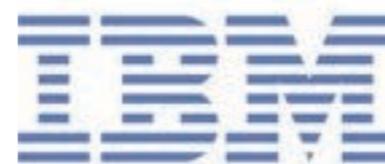
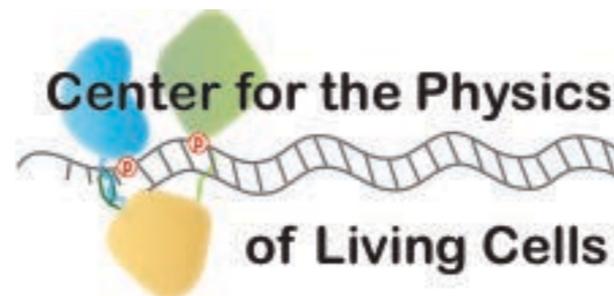
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(UIUC)

J Gundlach
Ian Derrington
(UW Seattle)

Cees Dekker
Magnus Jonsson
(TU Delft)



VMD and NAMD



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