Close encounters with DNA

Aleksei Aksimentiev
Department of Physics
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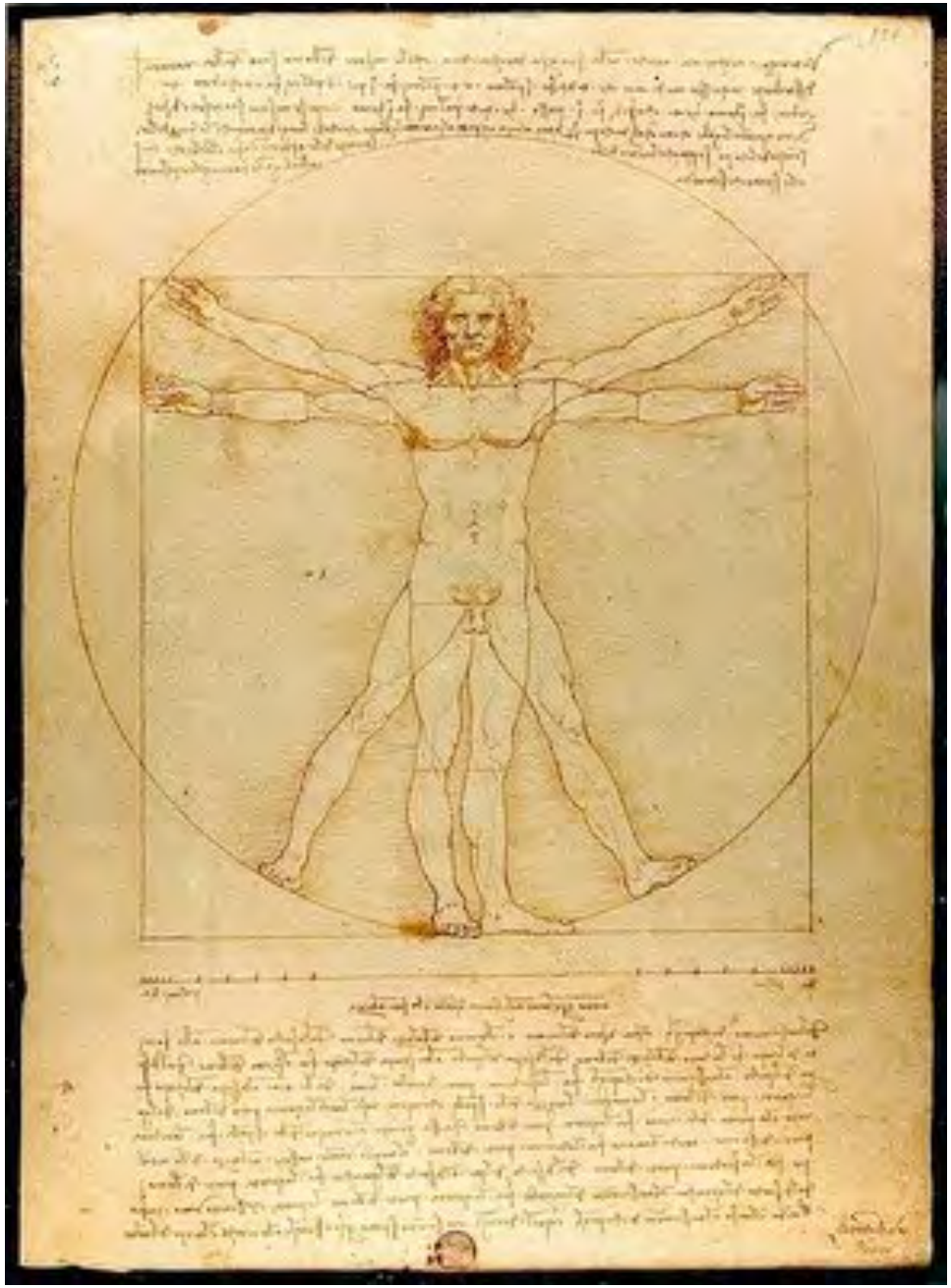


WHAT IS LIFE?

The Physical Aspect of the Living Cell

BY

ERWIN SCHRÖDINGER

SENIOR PROFESSOR AT THE DUBLIN INSTITUTE FOR ADVANCED STUDIES

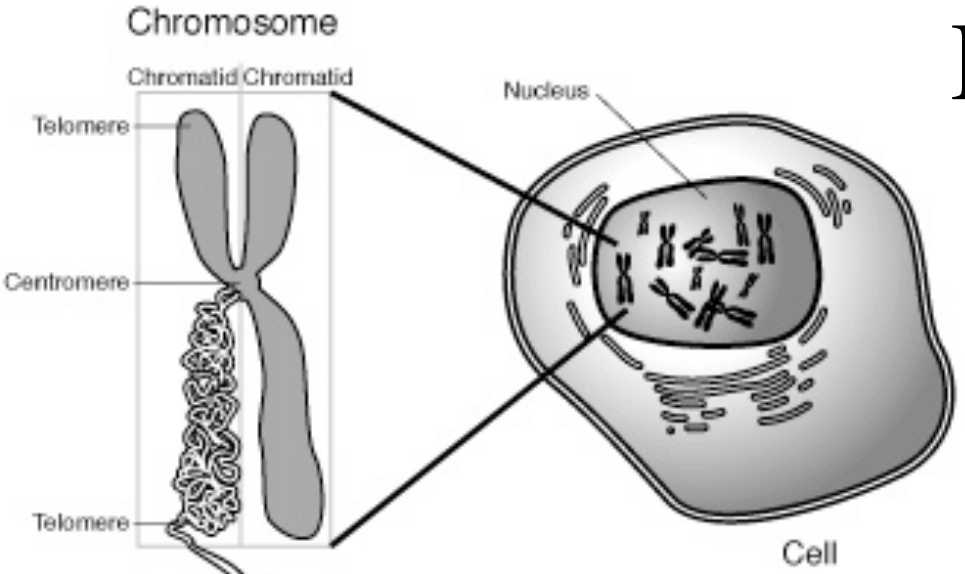
Based on Lectures delivered under the auspices of the Institute at Trinity College, Dublin, in February 1943

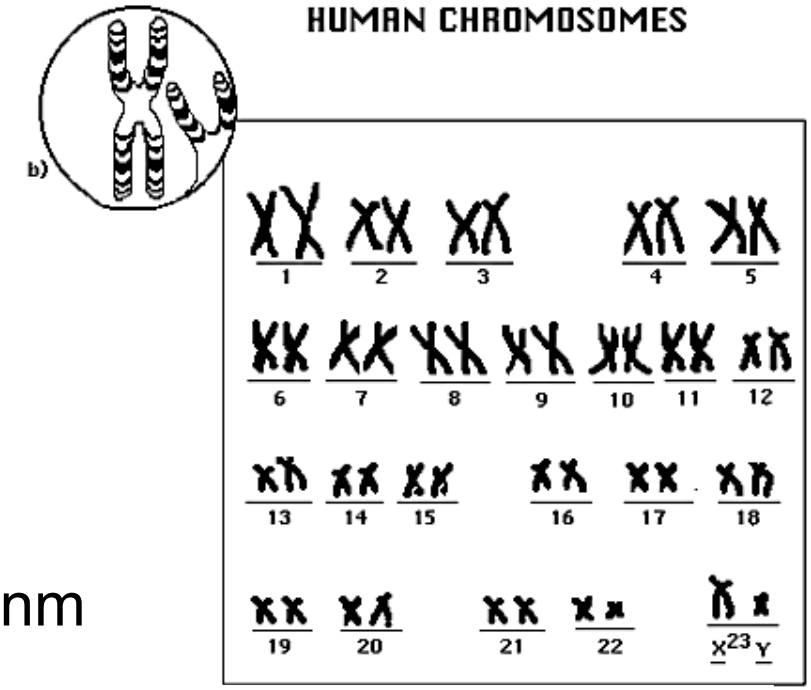
CAMBRIDGE

AT THE UNIVERSITY PRESS

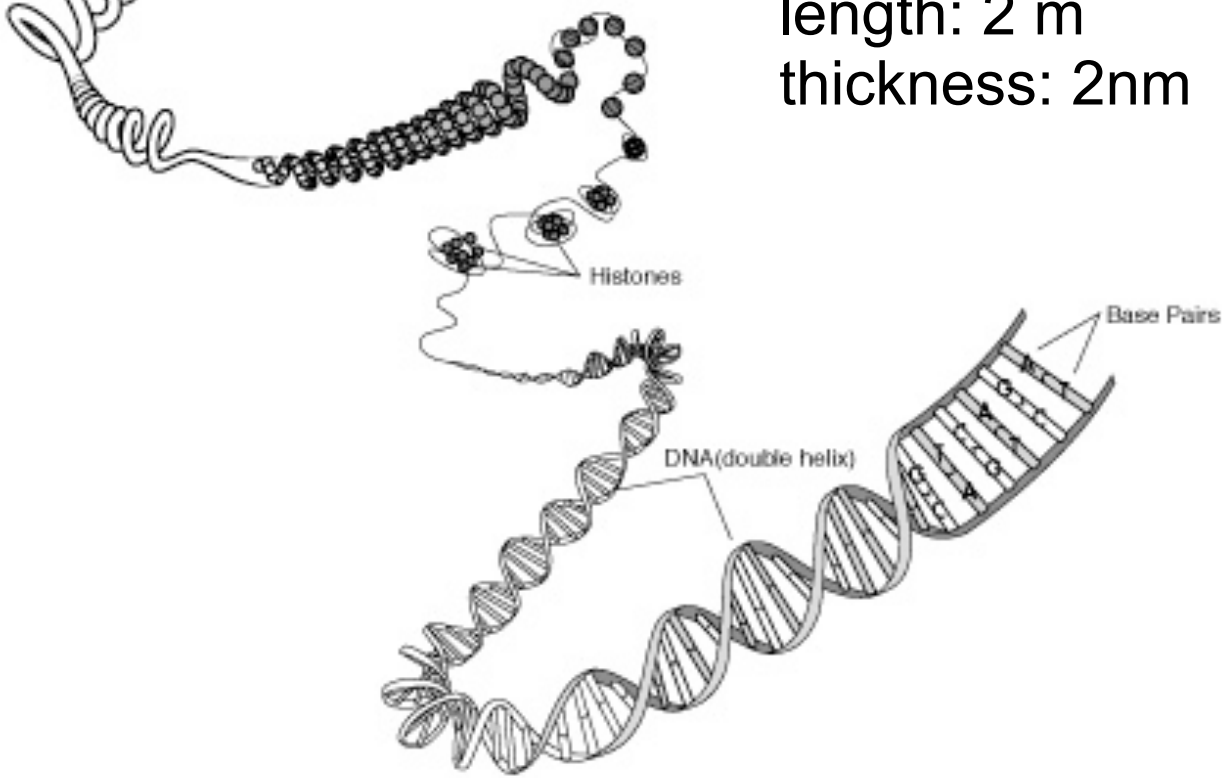
1948

DNA, the blueprint





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

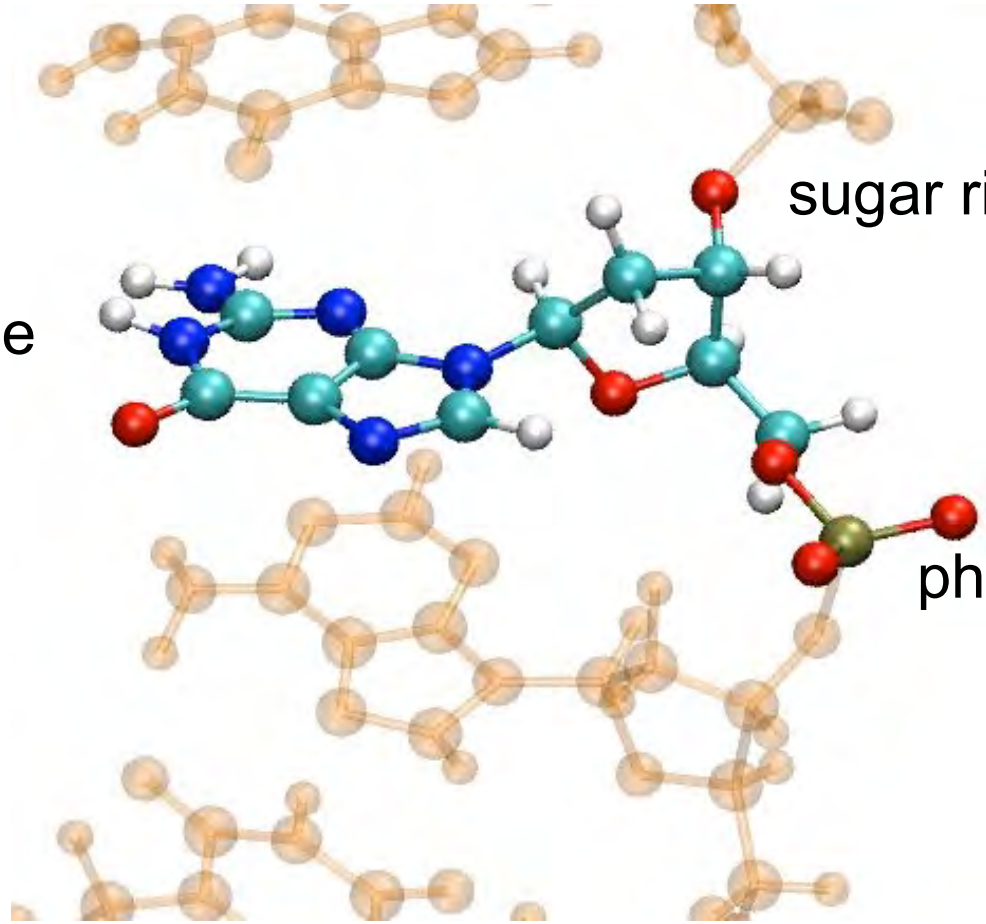


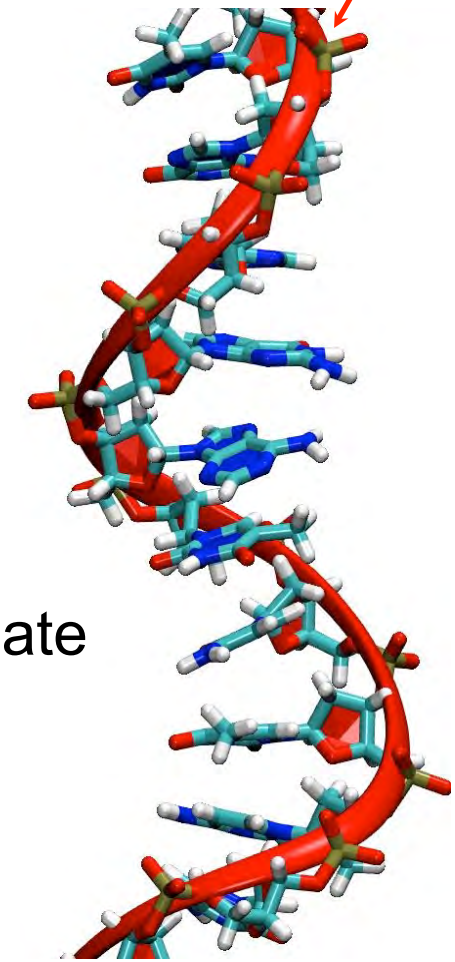
…GTGTGACTCGT
GGTCCGTAATGTC
GTTAATGGTGACC
GTGTGGCCTGATG
GTTAGTGTGTGA…

http://www.accessexcellence.org/AB/GG/chromosome.html

DNA code is written in atoms

Highly charged: 2 electron charges per 0.32nm





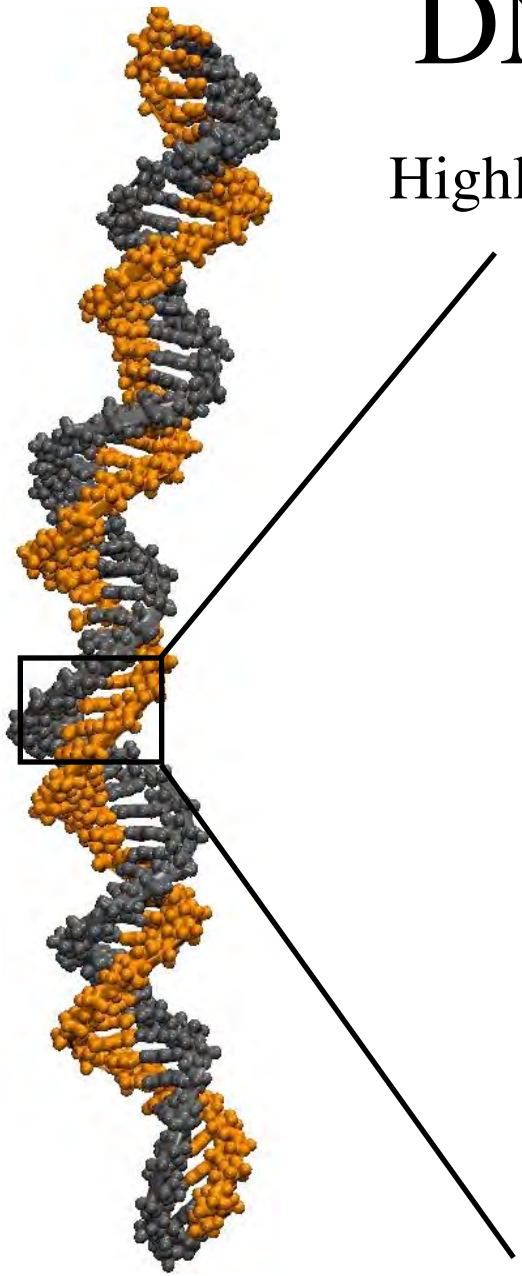
Double stranded DNA
(persist. length ~50nm)

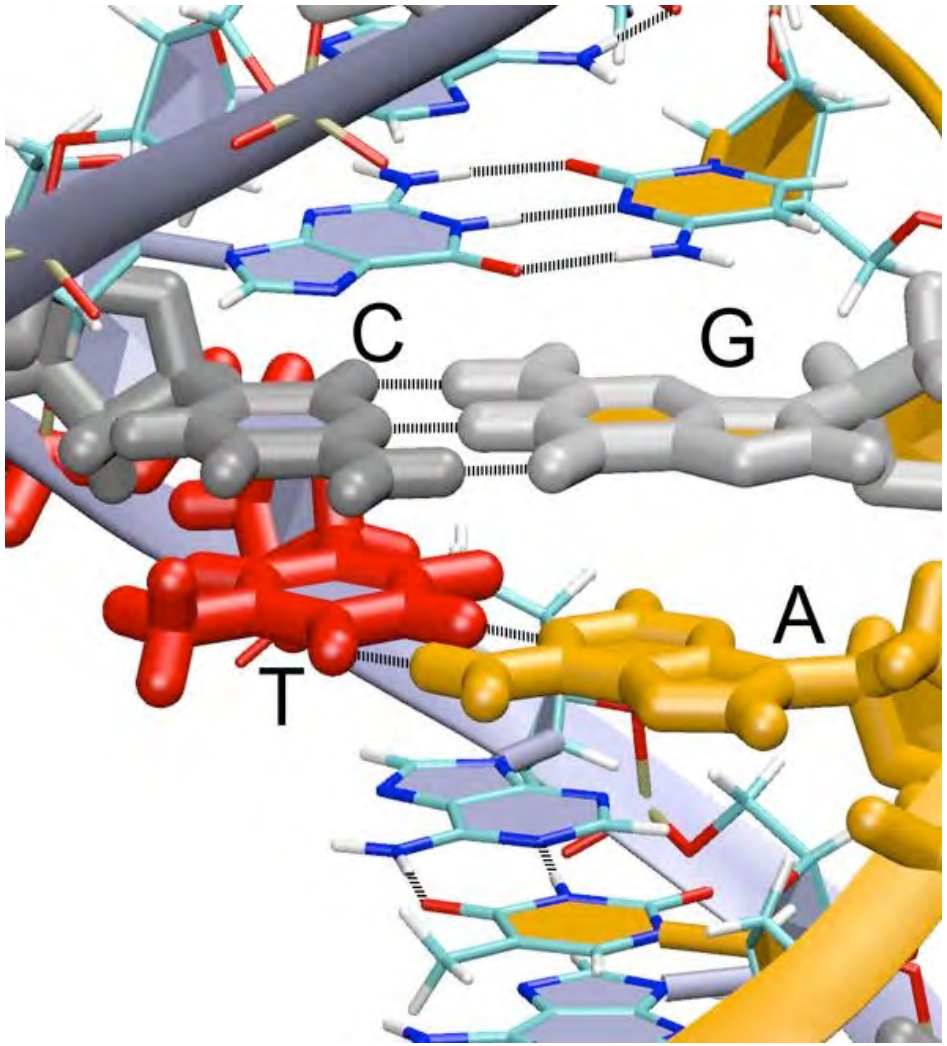
The sequence has direction:
5'-AAGCTGGTTCAG-3'

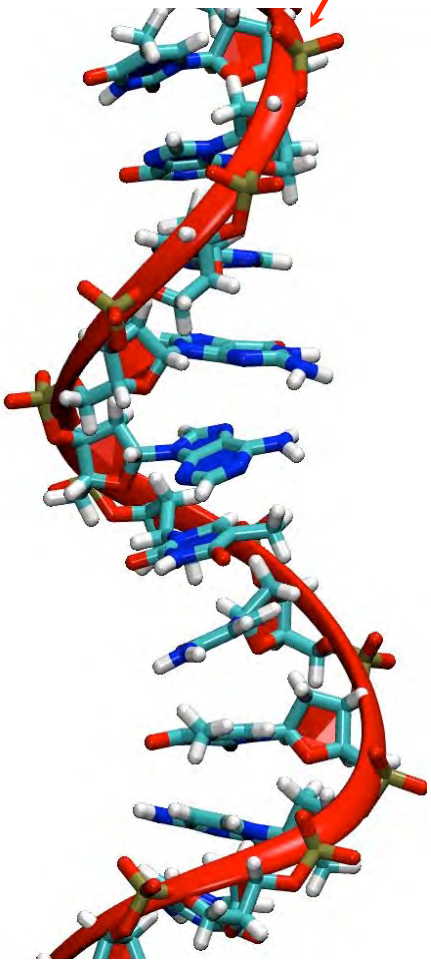
Single stranded DNA
(persist. length ~1.5nm)

DNA code is written in atoms

Highly charged: 2 electron charges per 0.32nm





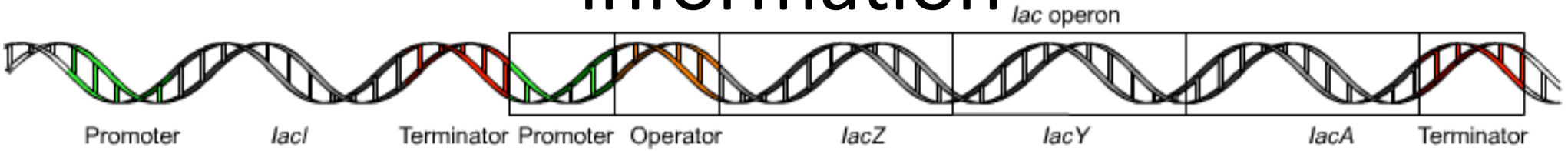


Double stranded DNA
(persist. length ~50nm)

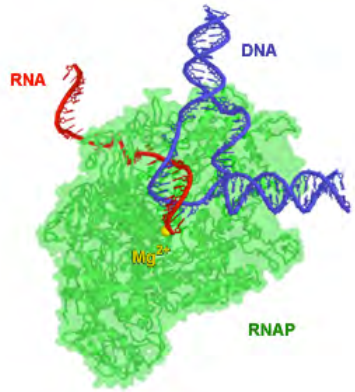
The sequence has direction:
5'-AAGCTGGTTCAG-3'

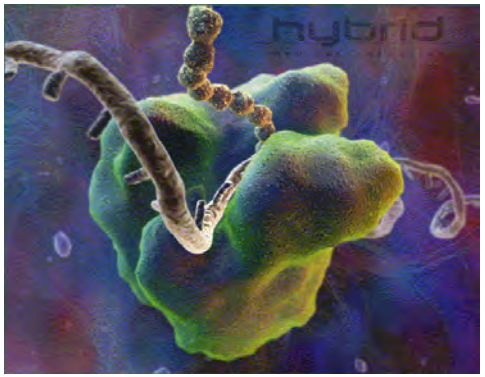
Single stranded DNA
(persist. length ~1.5nm)

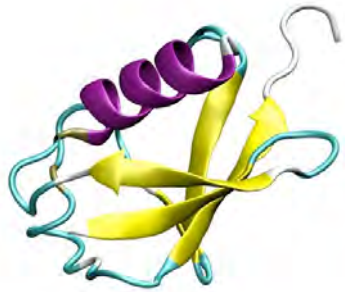
The sequence contains biological information



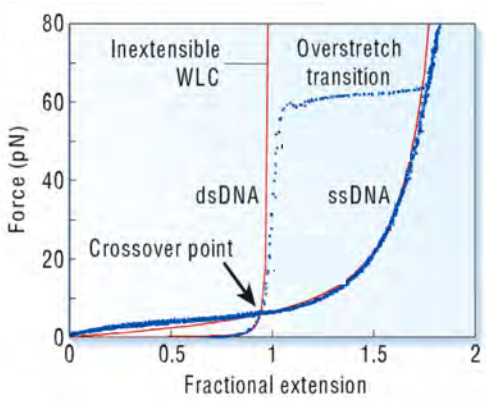
Central dogma of molecular biology

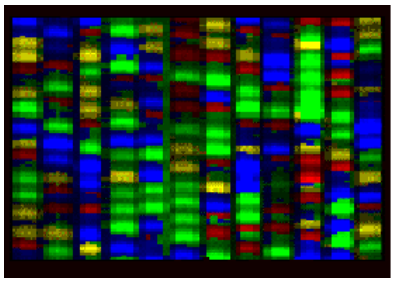


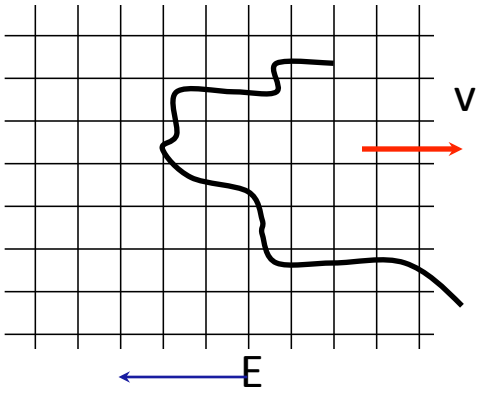




The physical properties enable functionality



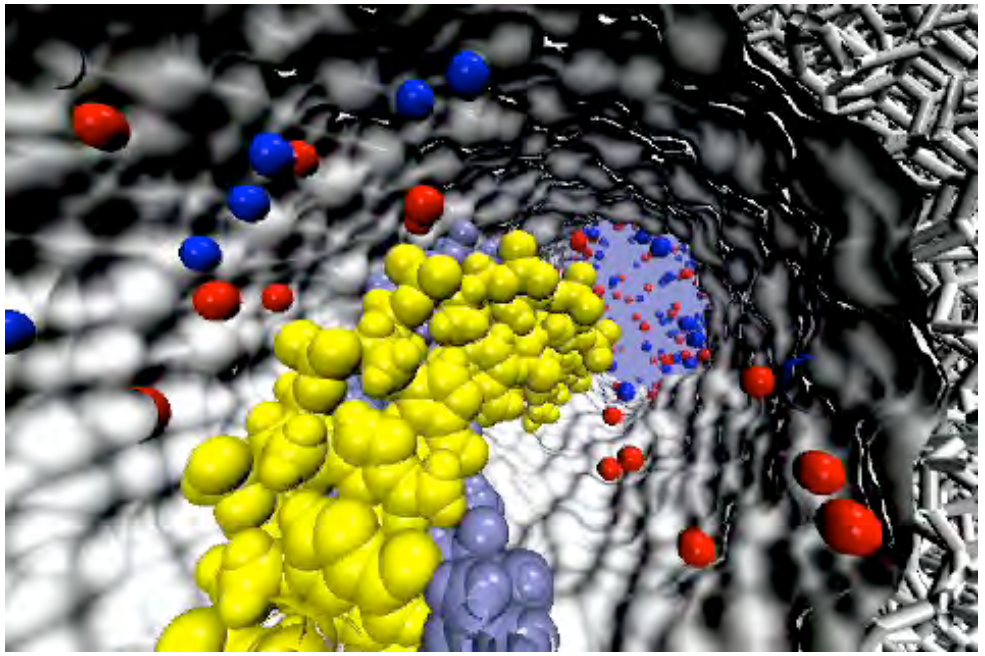




Molecular dynamics simulations, a computational (force) microscope

Massive parallel computer
Blue Waters, ~200,000

Atoms move according to classical mechanics (F= ma)

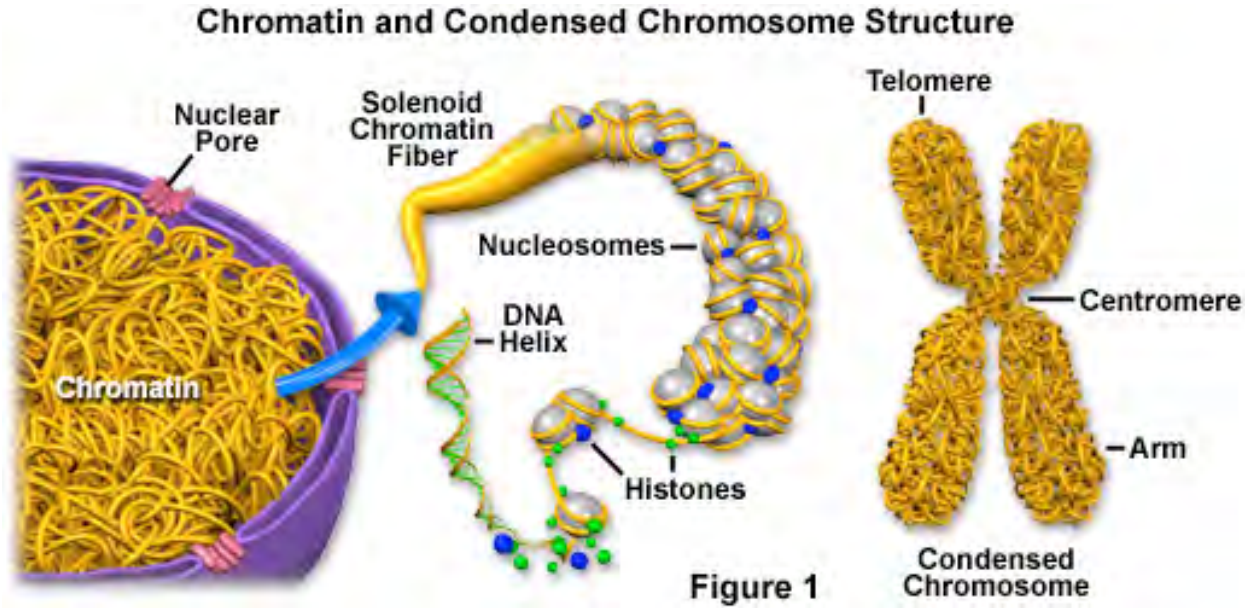


Time scale: ~ 0.1-100 μs

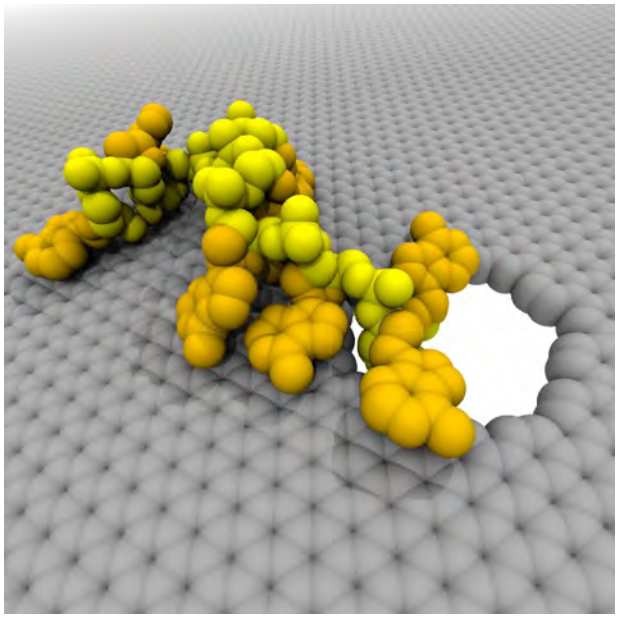
Length scale: 10K - 100M atoms or (< 50 nm)^3

Interaction between atoms is defined by molecular force field

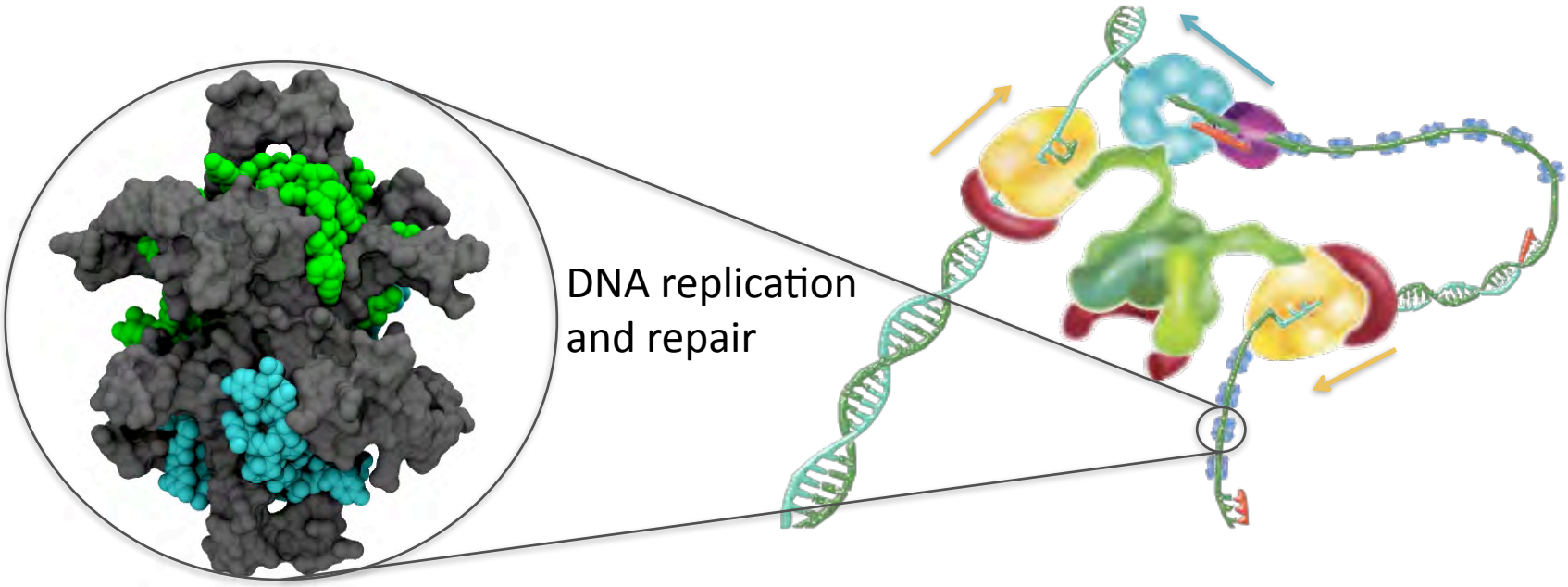
DNA systems



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

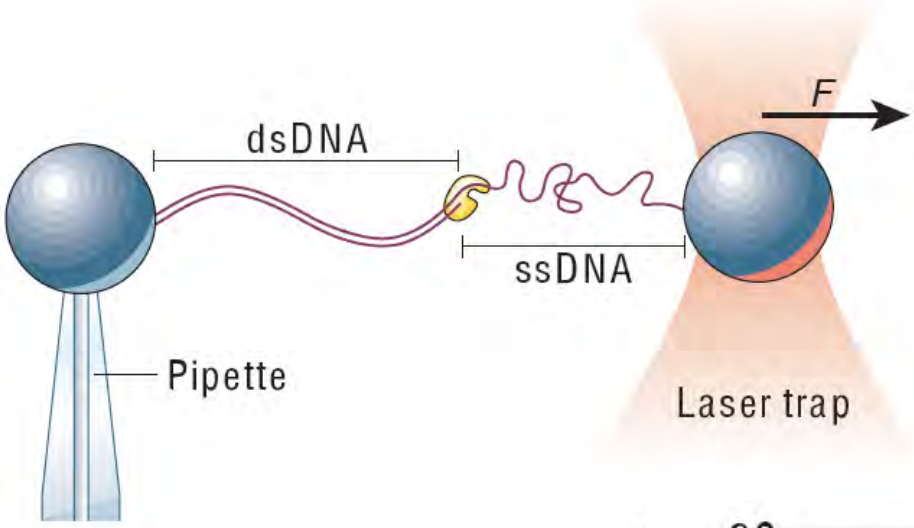


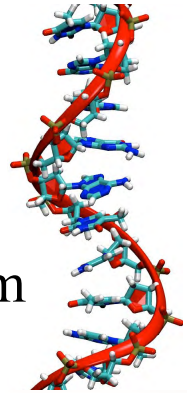
Graphene nanopore sequencing



DNA replication and repair

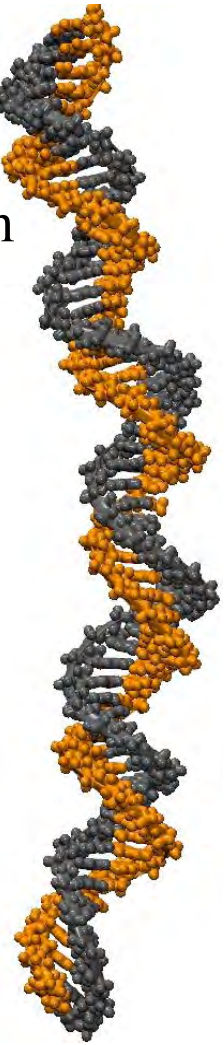
Micromechanics of single DNA molecules

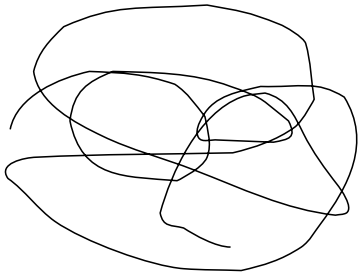




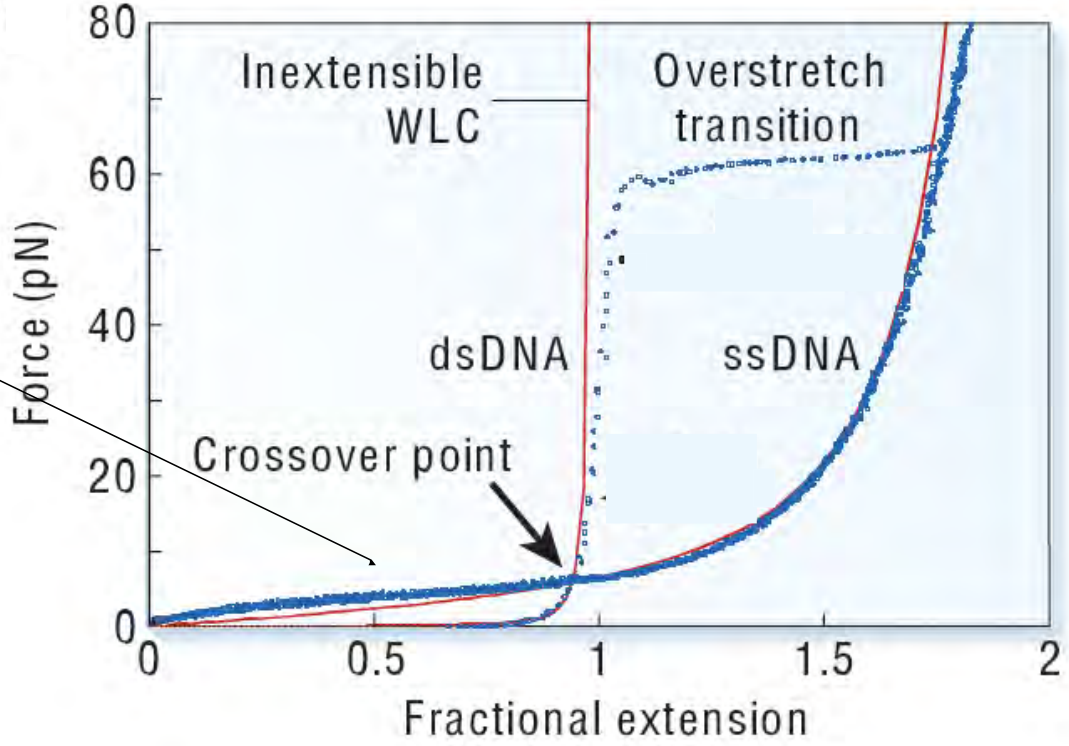
l_P~2nm

l_P~50nm



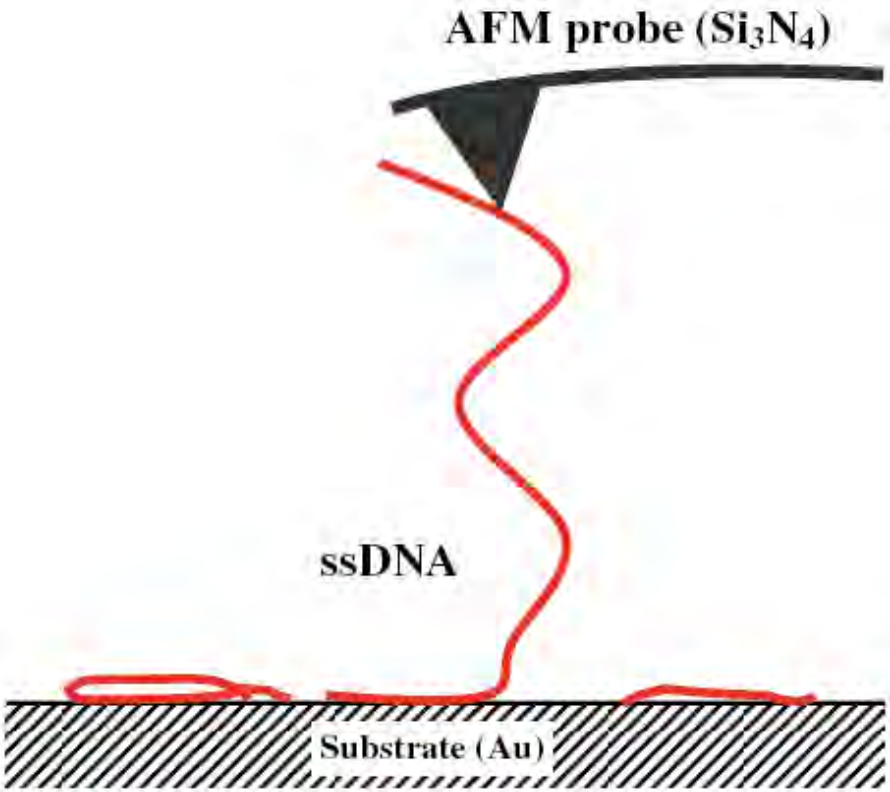


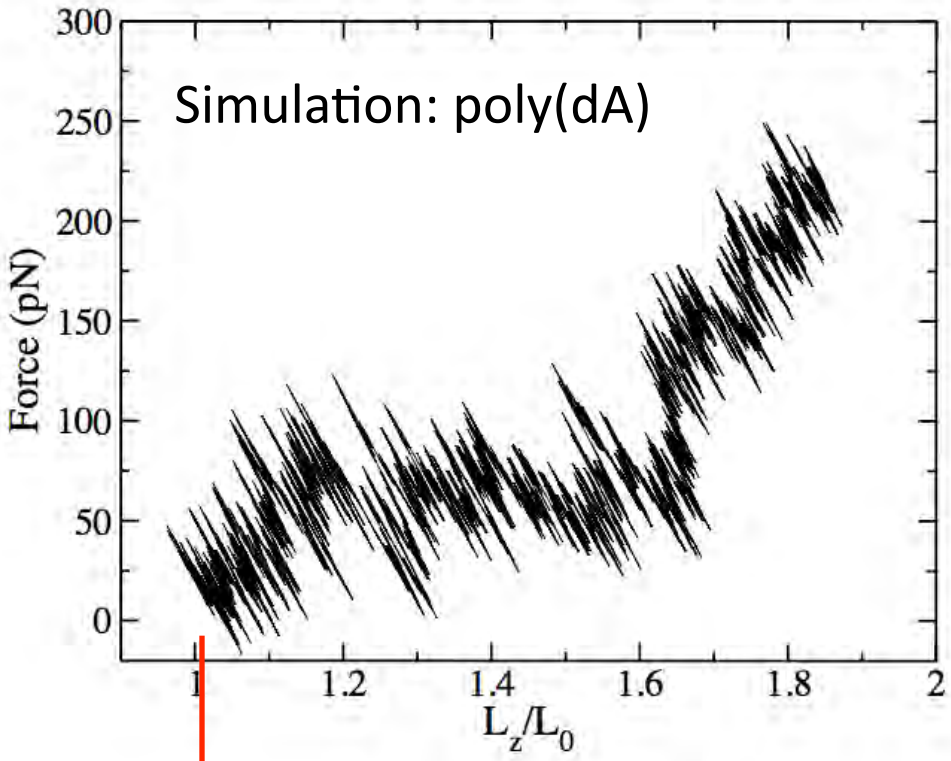
Polymer coil



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

Stretching ssDNA with external force

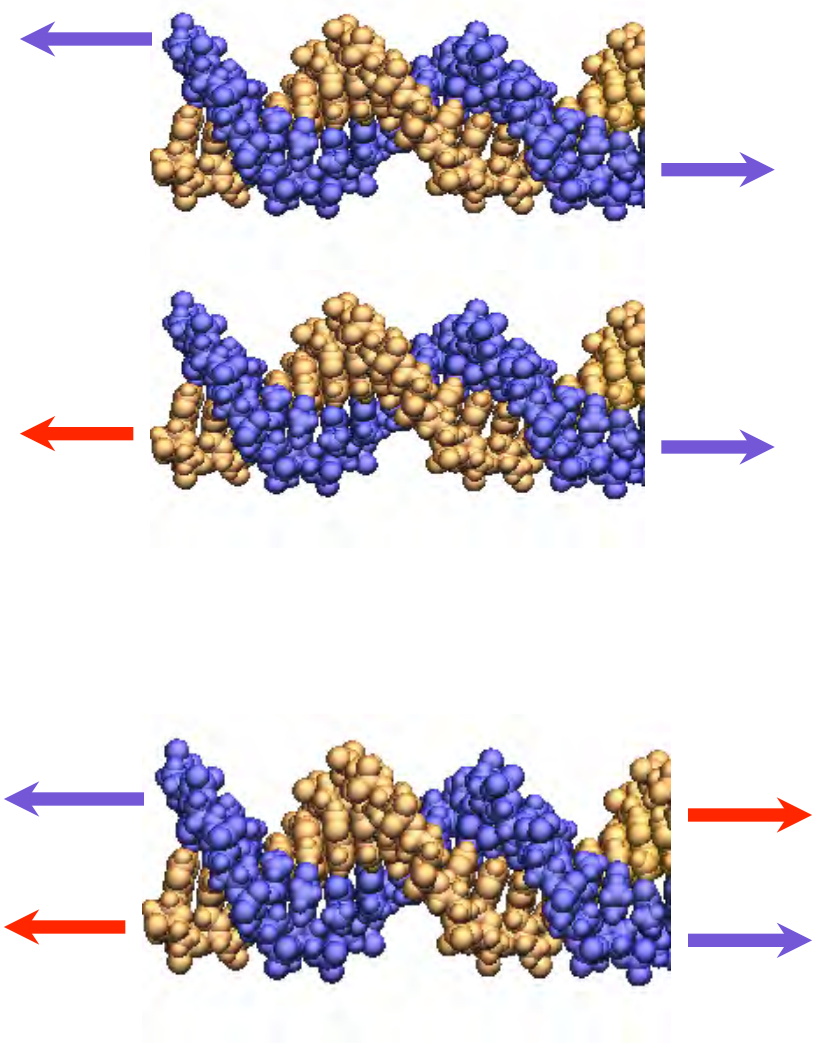




This end is fixed

V=const

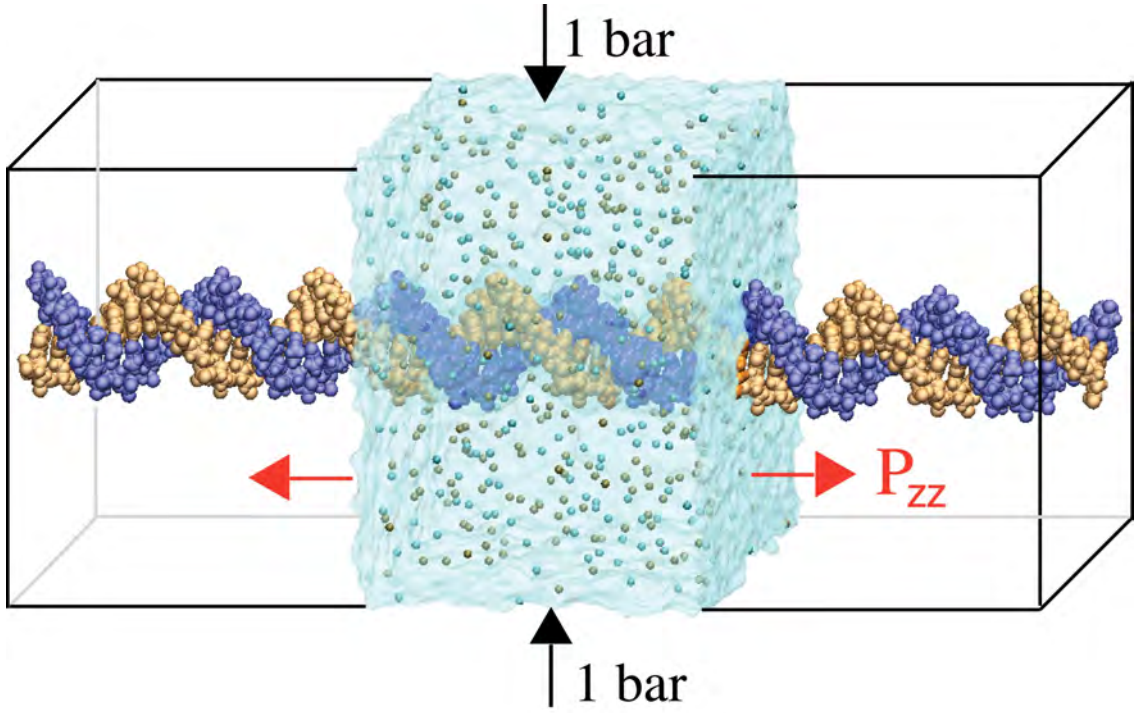
Stretching dsDNA



Applied force simulations

Periodic boundary conditions in 3D

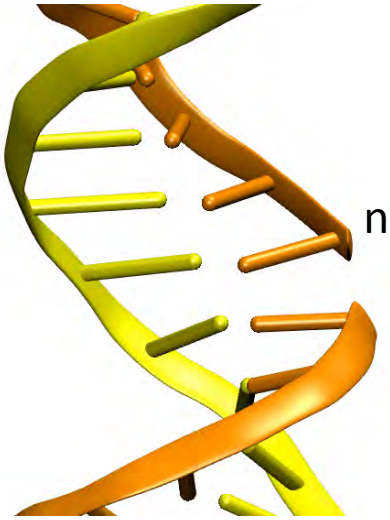
DNA is effectively infinite

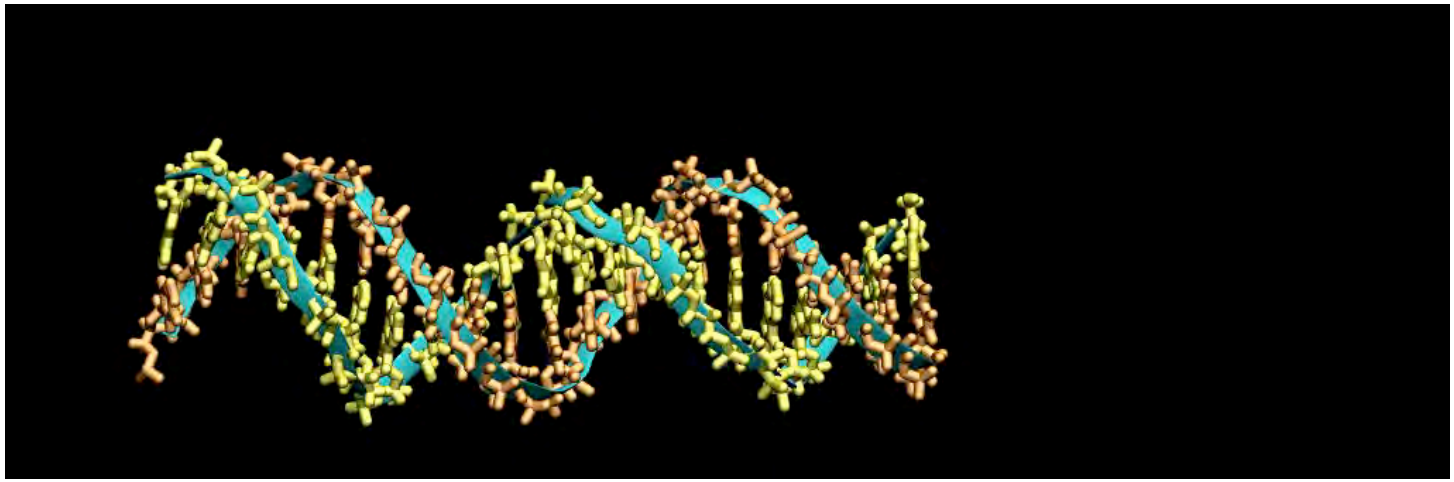


$P_{zz} < 0$ stretches the system in Z direction.

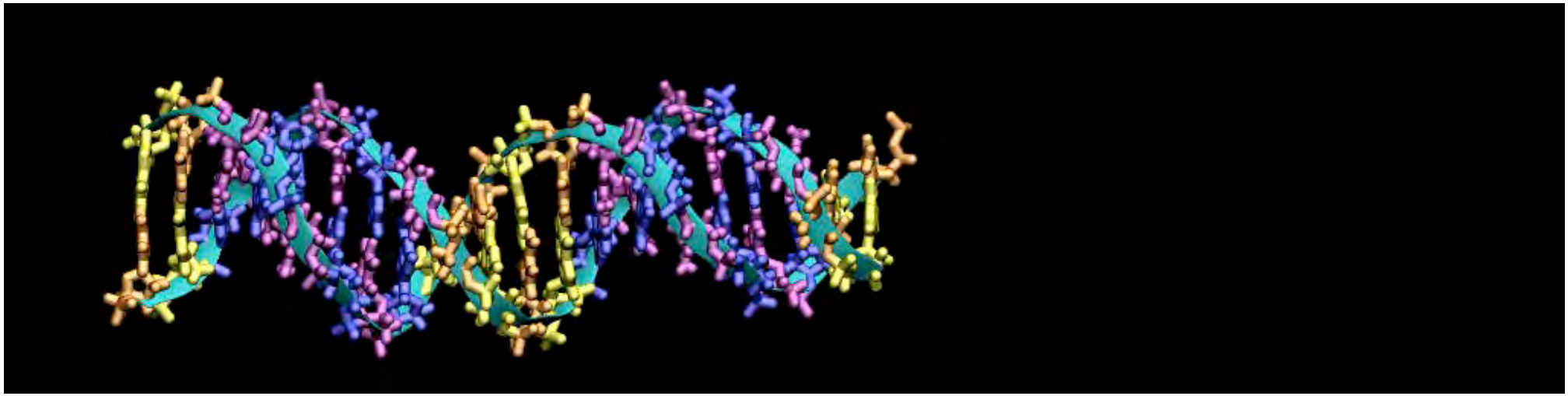
Simulations using anisotropic pressure control

Different modes of dsDNA stretching





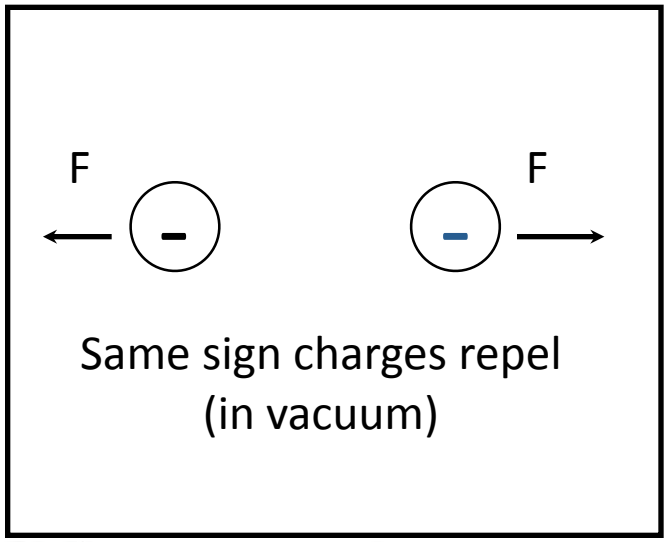
Nicked: force increases gradually as DNA unwinds

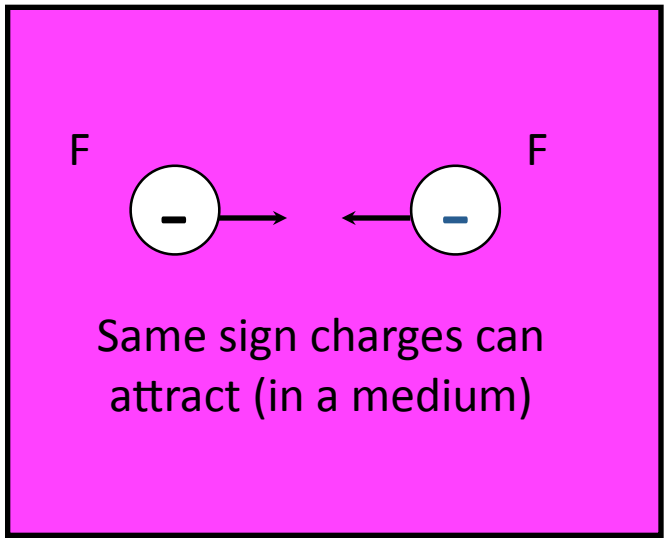


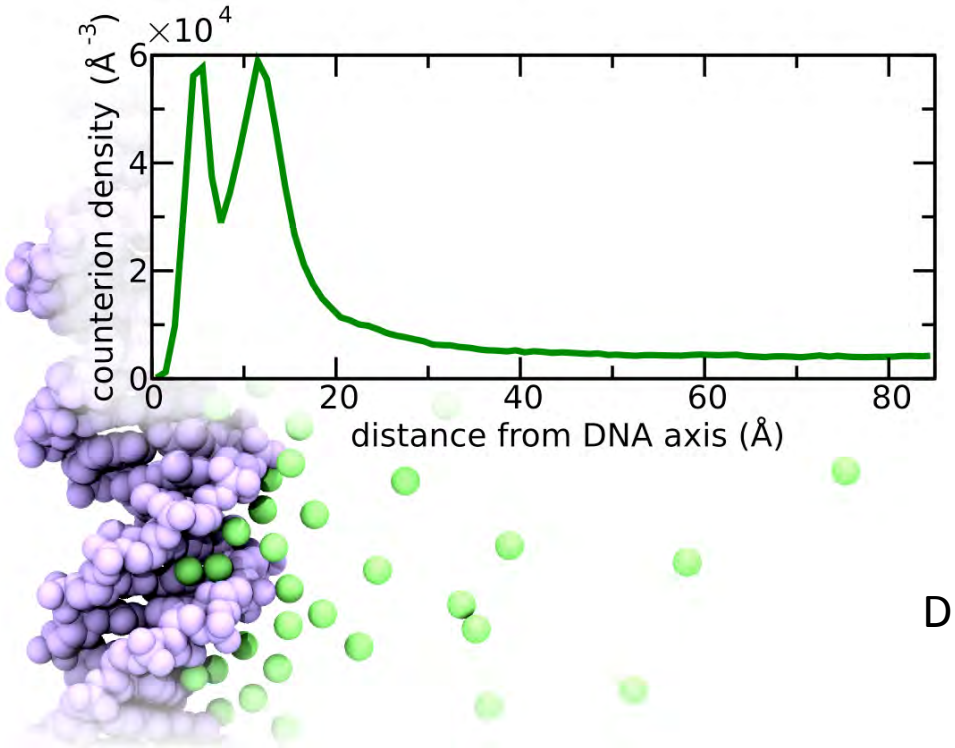
Torsionally constrained: DNA pops, force-extension curve is non-monotonous

Luan and Aksimentiev, PRL **101**:118101 (2008)

Interesting physical properties





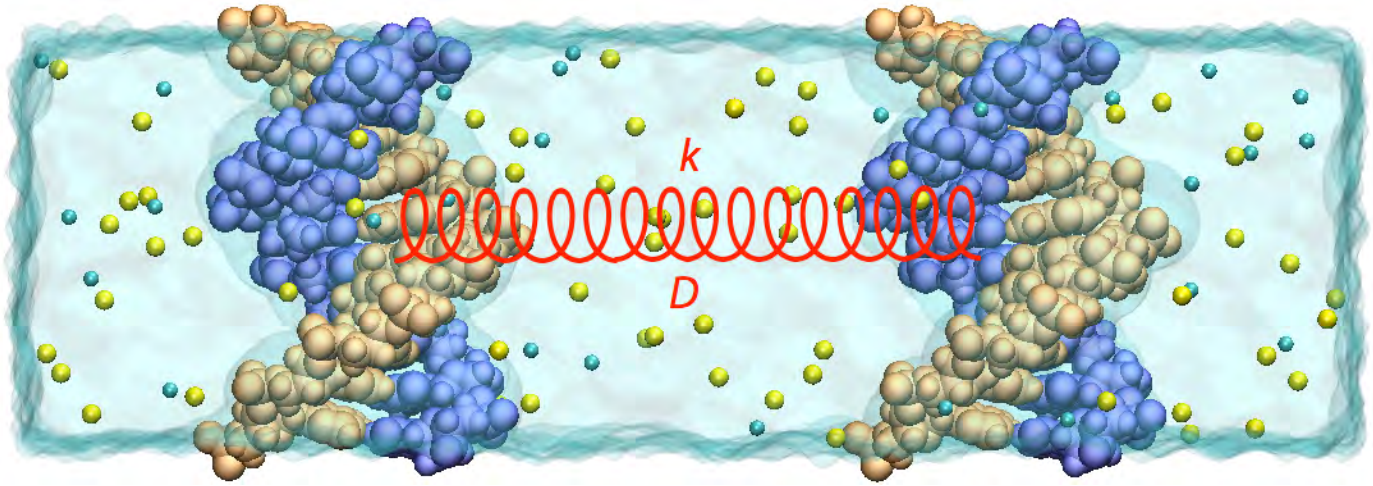


Effective attraction between DNA is observed when counterions have charge ≥ 2e

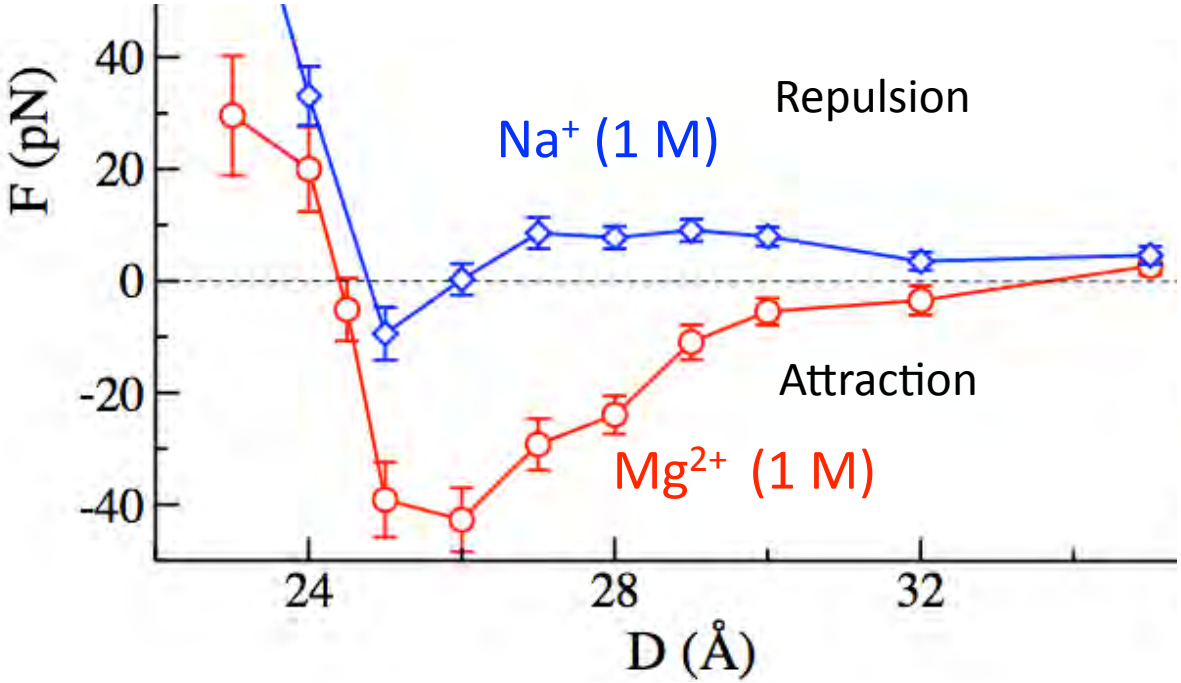
DNA lives in water and is surrounded by counterions

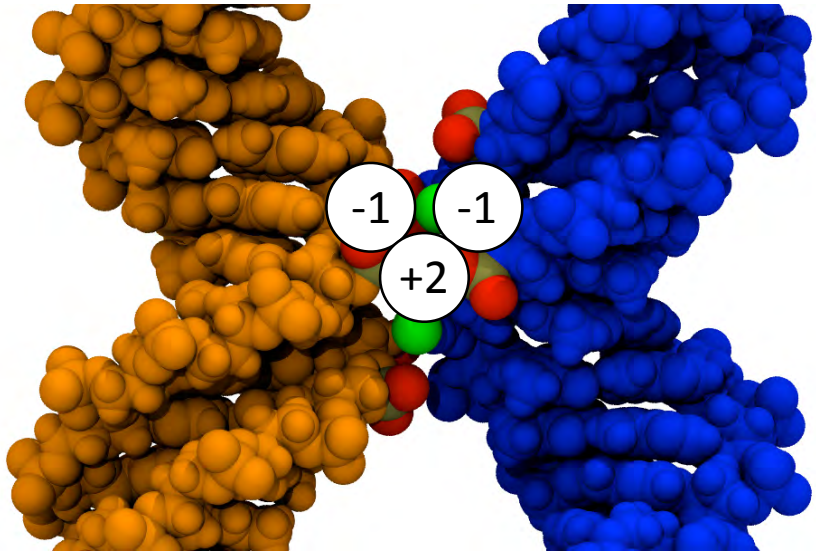
Direct MD simulation of DNA-DNA force

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

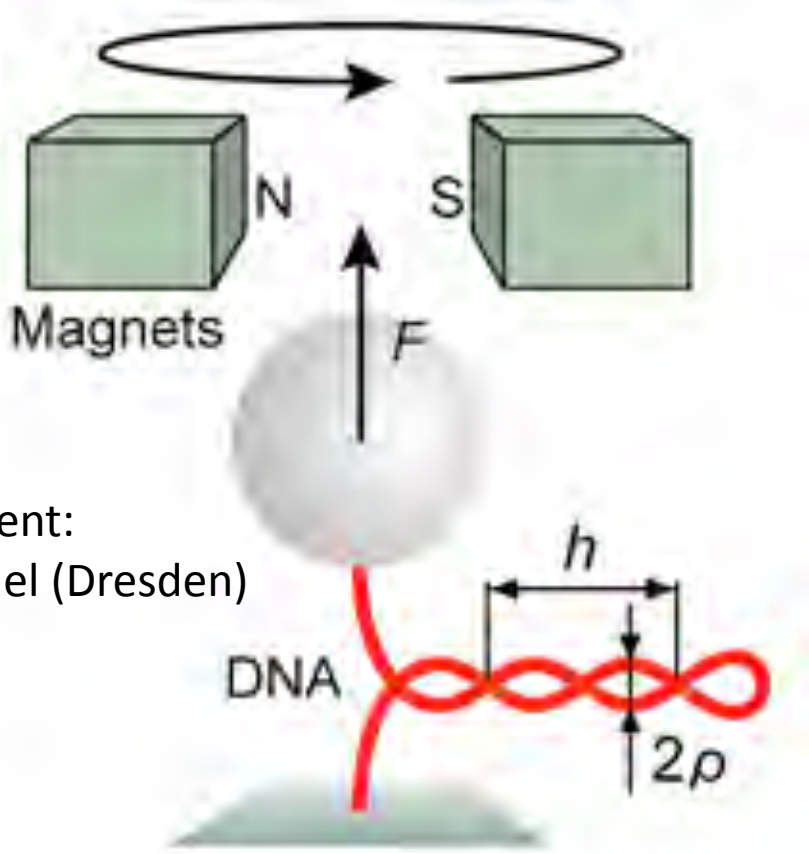


A virtual spring measures the effective force

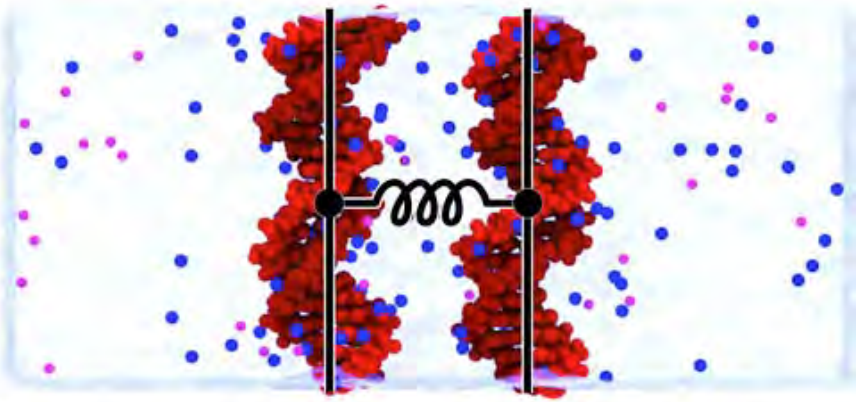


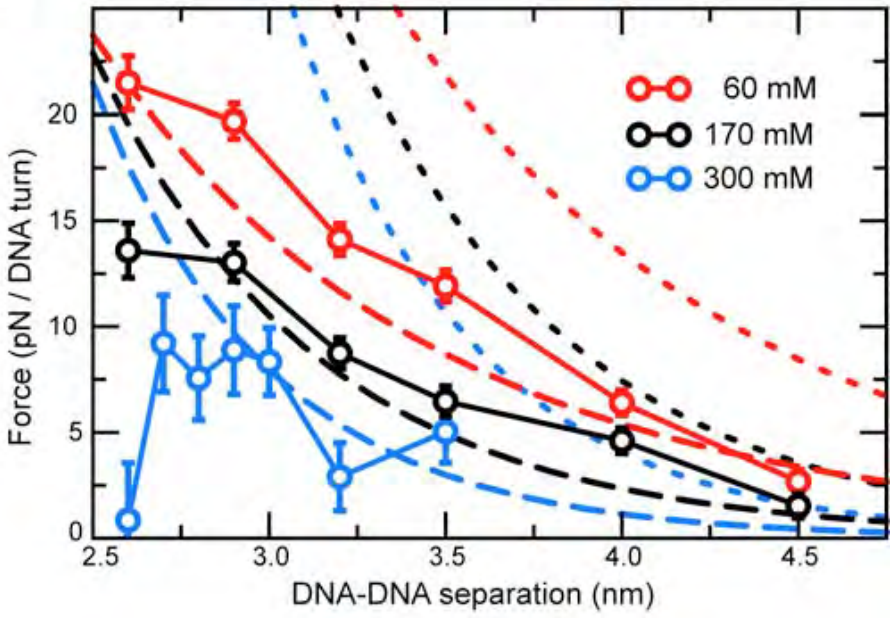


Simulations of side-by-side DNA repulsion are in good agreement with experiment



Experiment:
Ralf Seidel (Dresden)



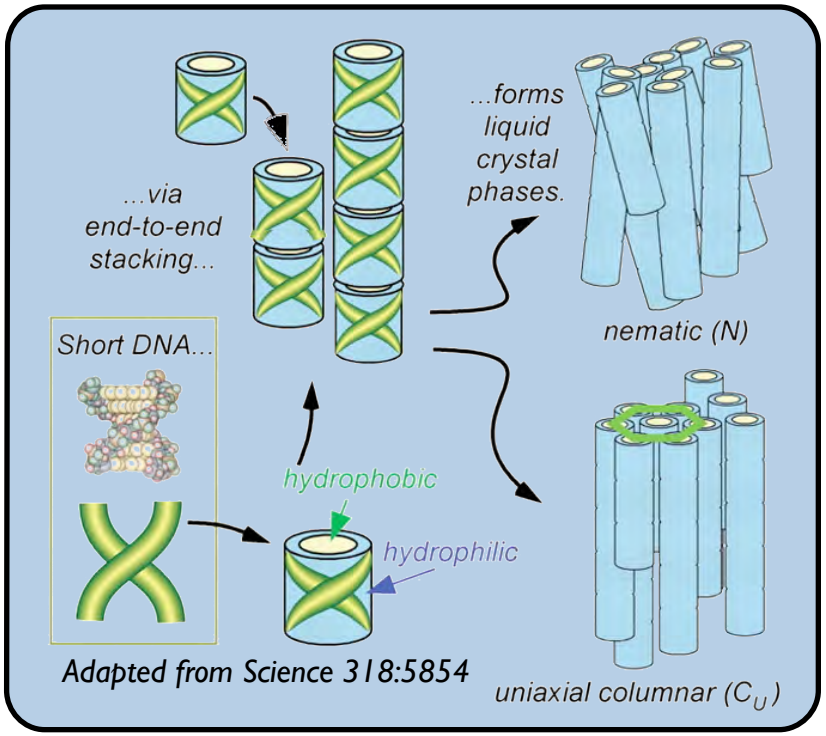


Phys. Rev. Lett. 105, 158101 (2010)

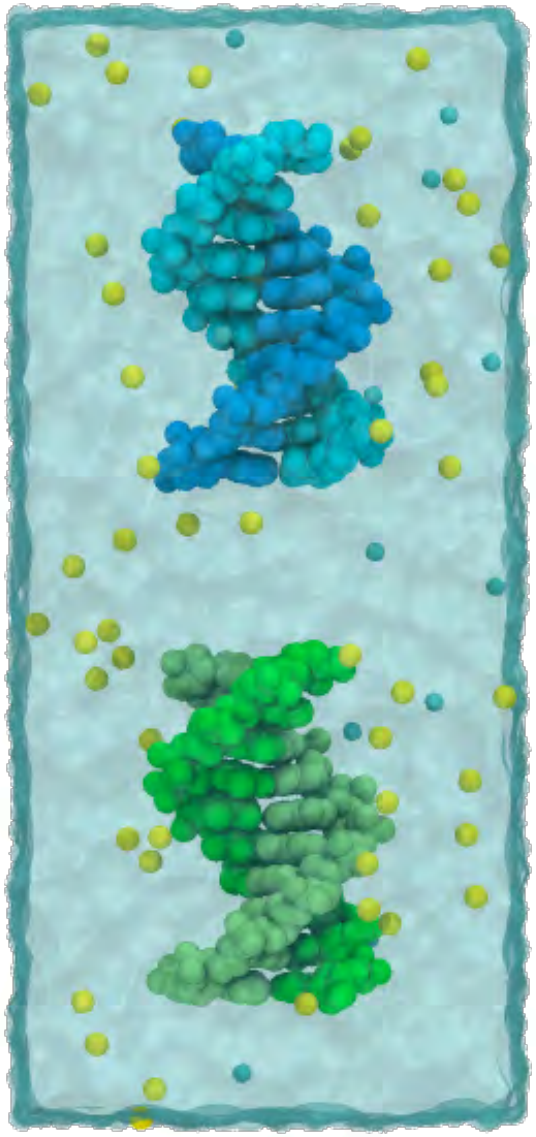
In monovalent electrolytes, the effective charge of dsDNA is about 41% of its nominal charge

Short dsDNA fragments form end-to-end aggregates

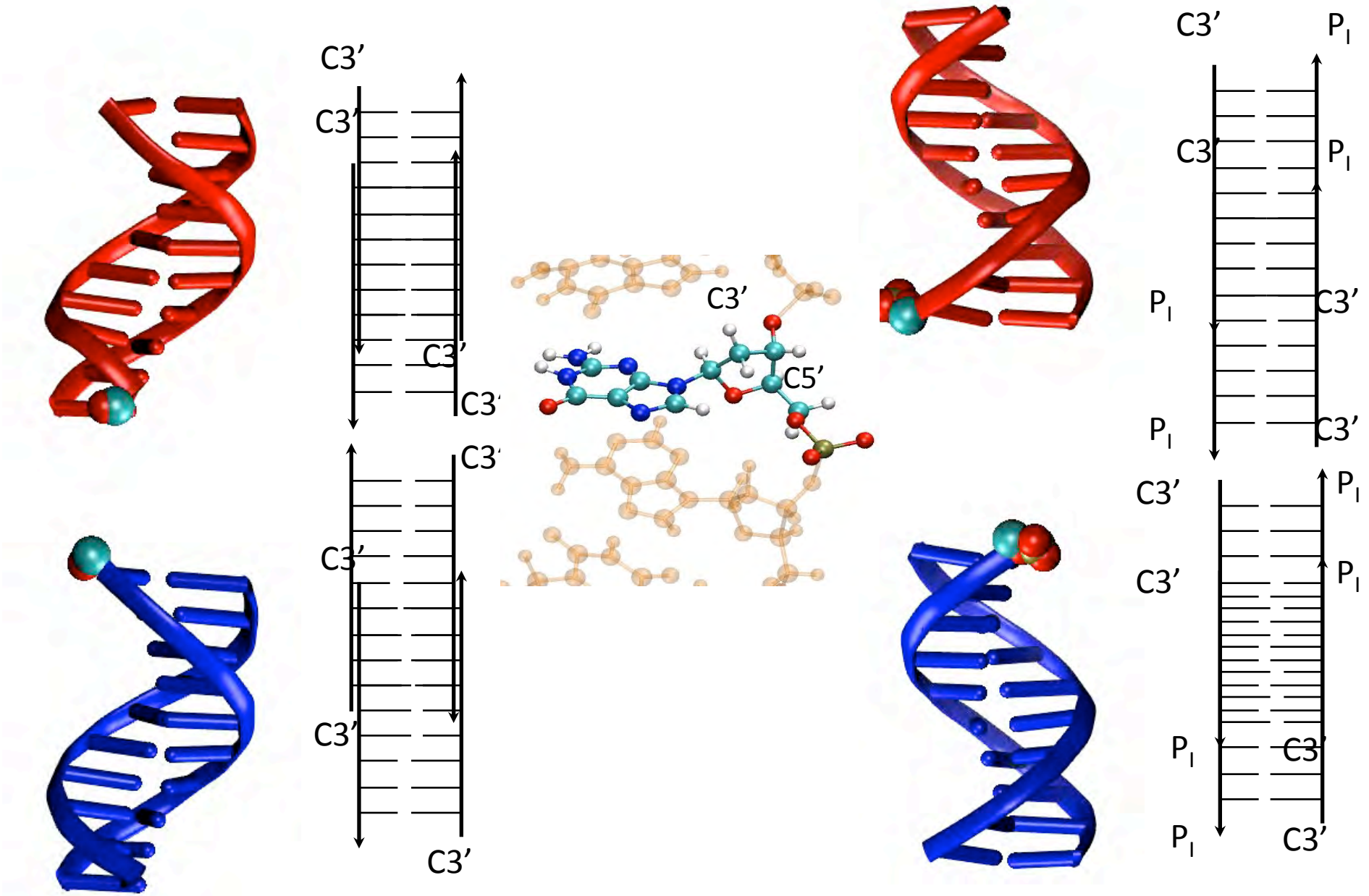
Experimental evidence of end-to-end aggregation of short dsDNA fragments motivated simulations to determine whether aligned DNA fragments would collapse to an end-to-end assembly.



Chris Maffeo



Proper connection requires a terminal phosphate



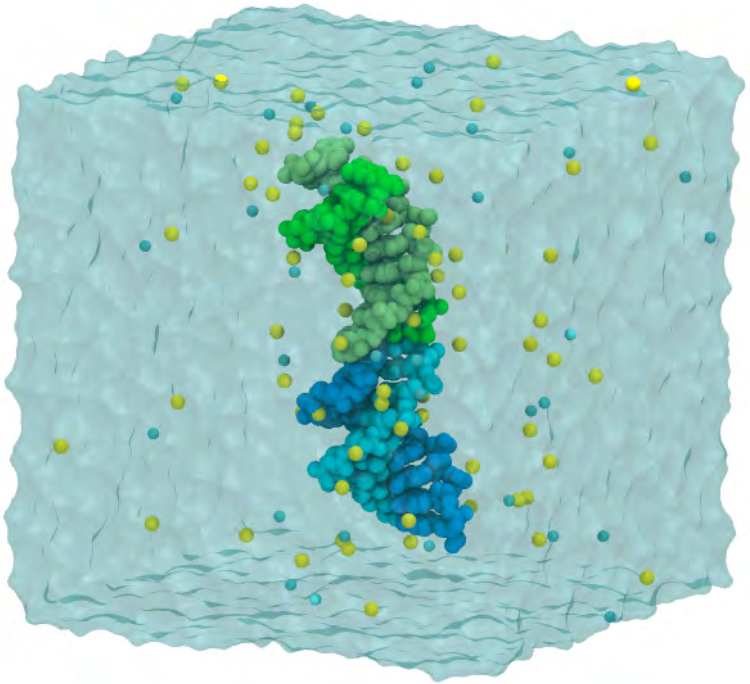
Further simulation reveals the strength of the end-to-end DNA interaction

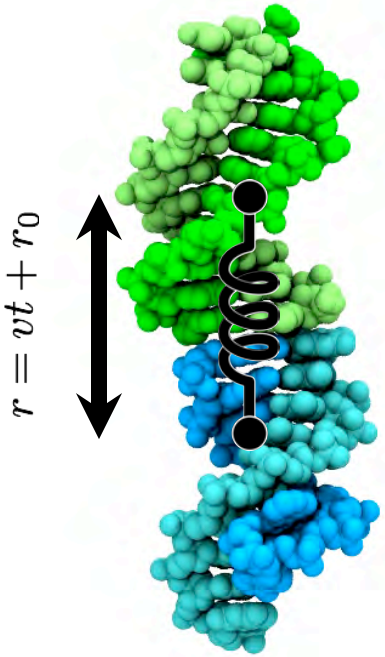
Nucleic Acids Research 40:3812 (2012)

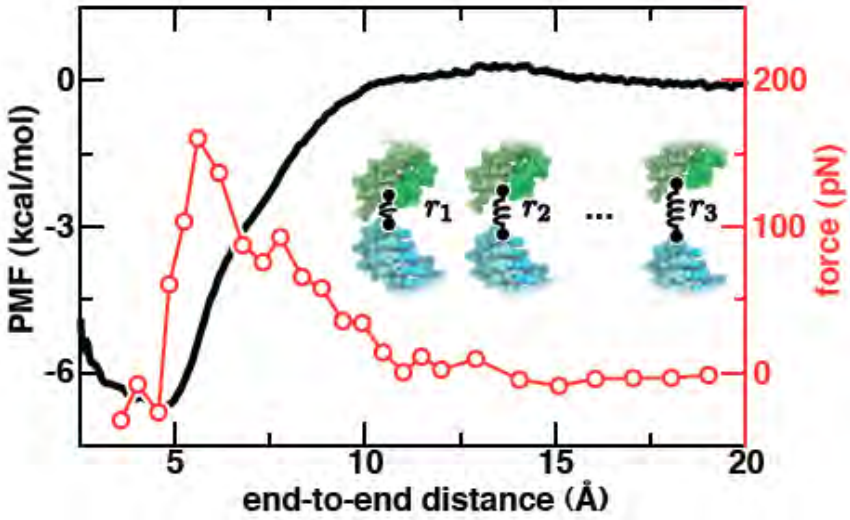
The assembly proved stable in the absence of restraints during 600 ns of simulation.

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

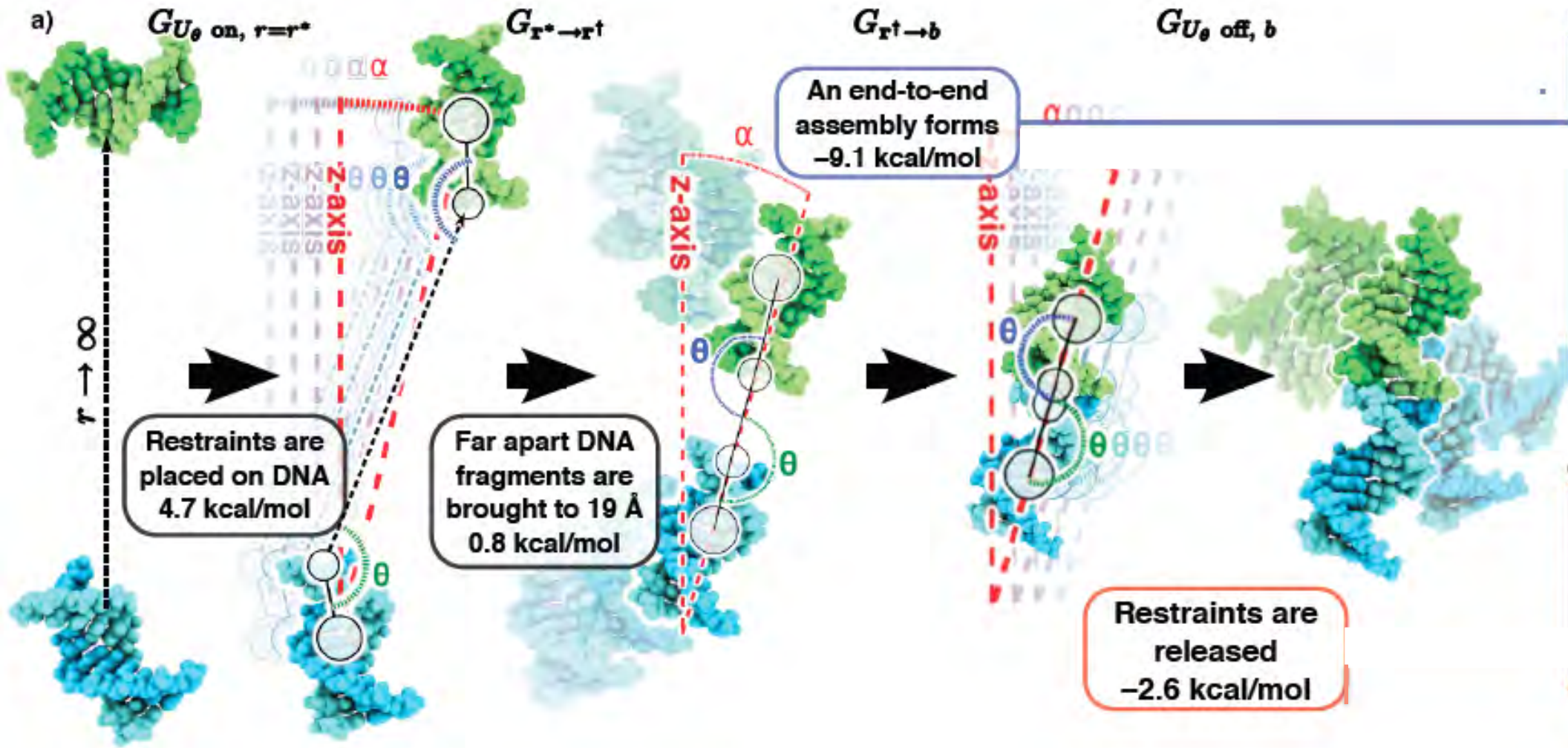
Umbrella sampling revealed that the free energy for the interaction is ~6.5 kcal/mol







Standard binding free-energy of end-to-end assembly



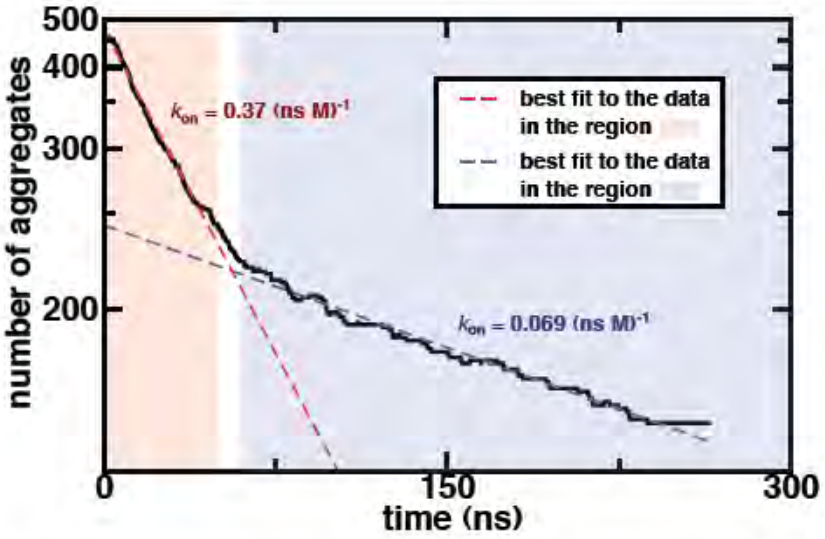
Result: $G_{\underline{bind}}$ = −6.3 ± 1 kcal/mol for a DNA concentration of 1 M in 0.12 NaCl

$$G_{\text{bind}} = G_{U_\theta \text{ on, } r=r^*} + G_{\mathbf{r}^* \to \mathbf{r}^\dagger} + G_{\mathbf{r}^\dagger \to b} + G_{U_\theta \text{ off, } b}$$

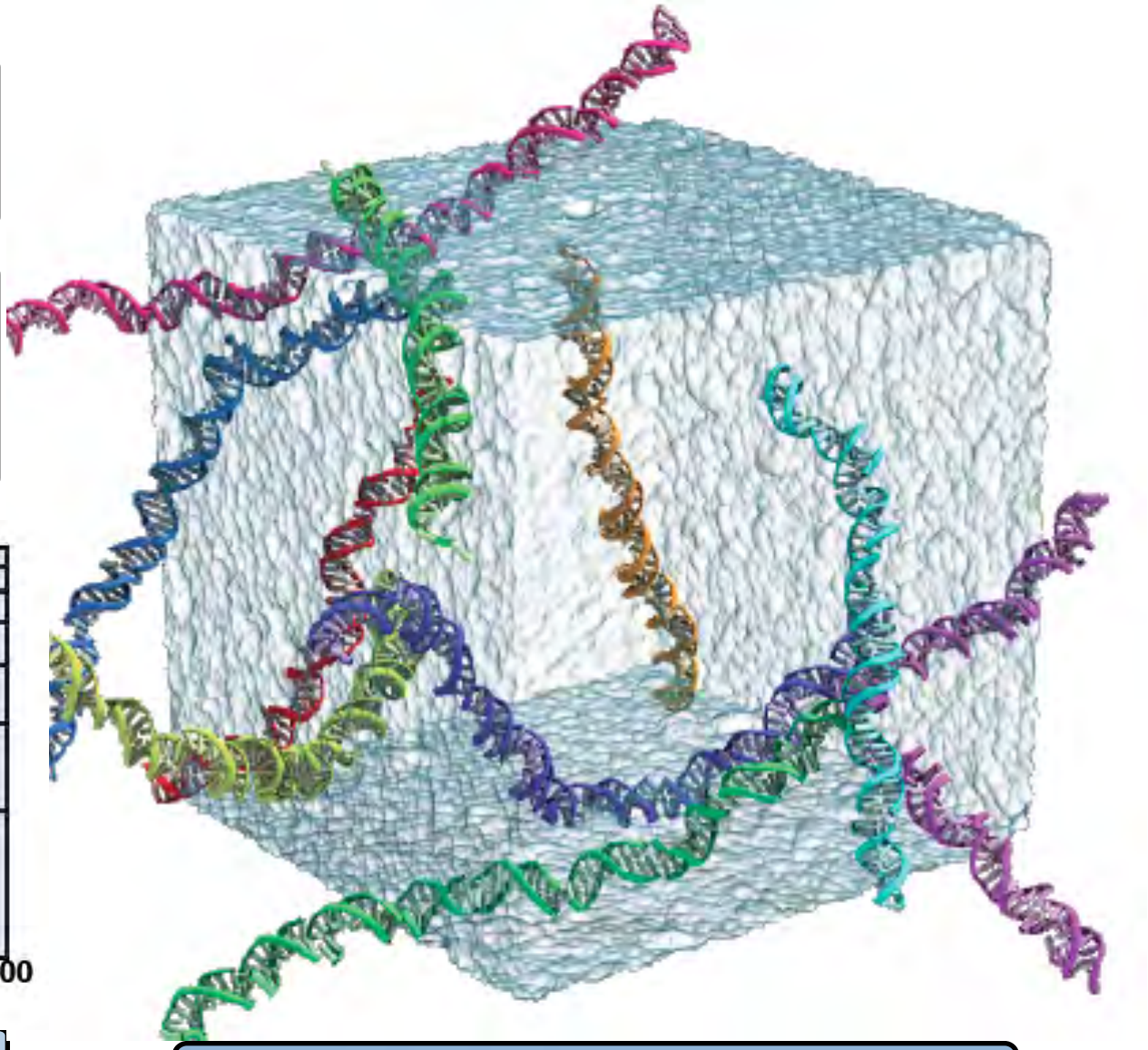
Simulation of DNA aggregation

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}



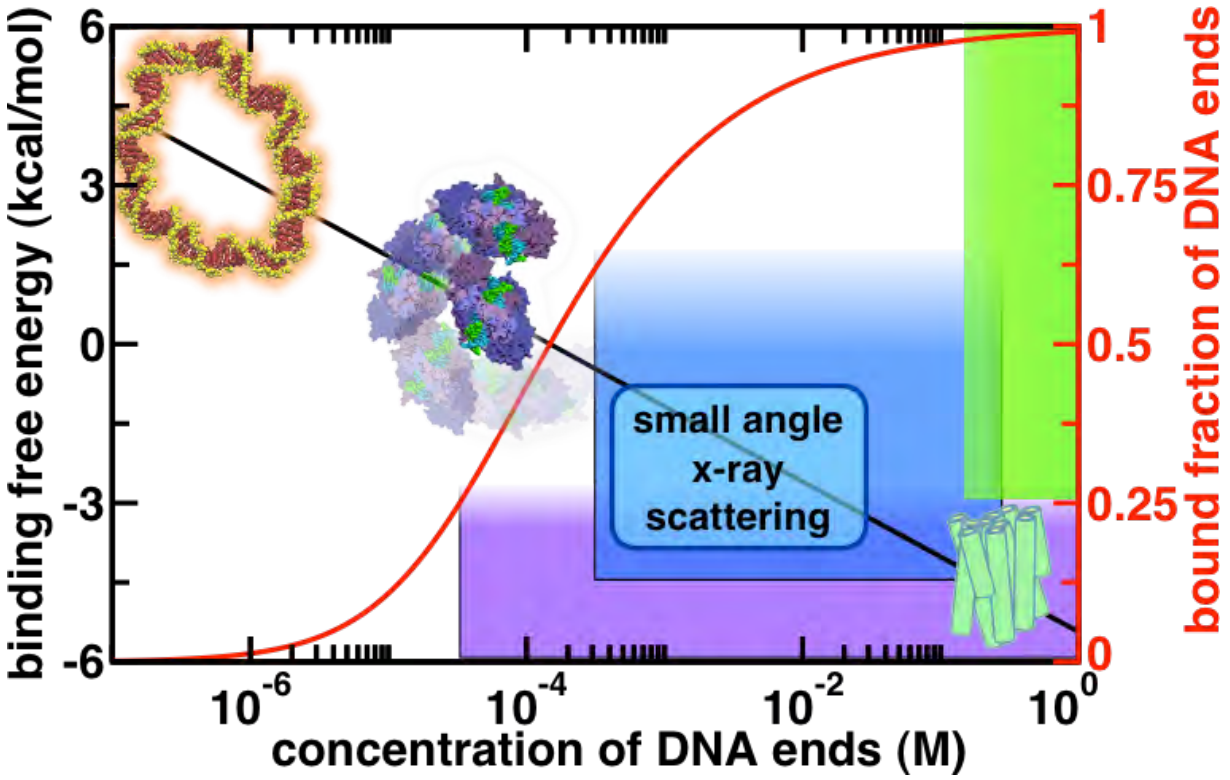
$G_{bind} = -k_B T \log k_{on}/k_{off} = -5.5$ kcal/mol



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

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

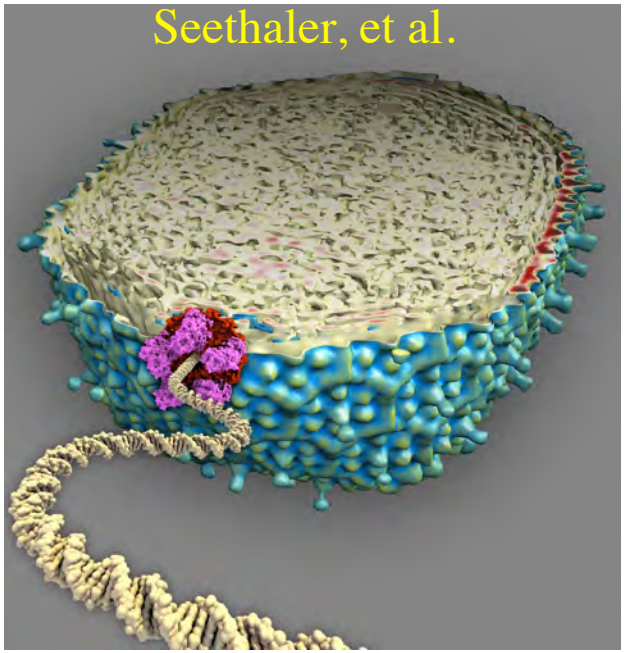
High concentration of short DNA fragments results in aggregation and subsequent formation of liquid crystals

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

MD simulation of dense DNA arrays



What we control

DNA density (or harmonic constraint radius)

$[Na^+]_{buf}$ ~ 200 mM

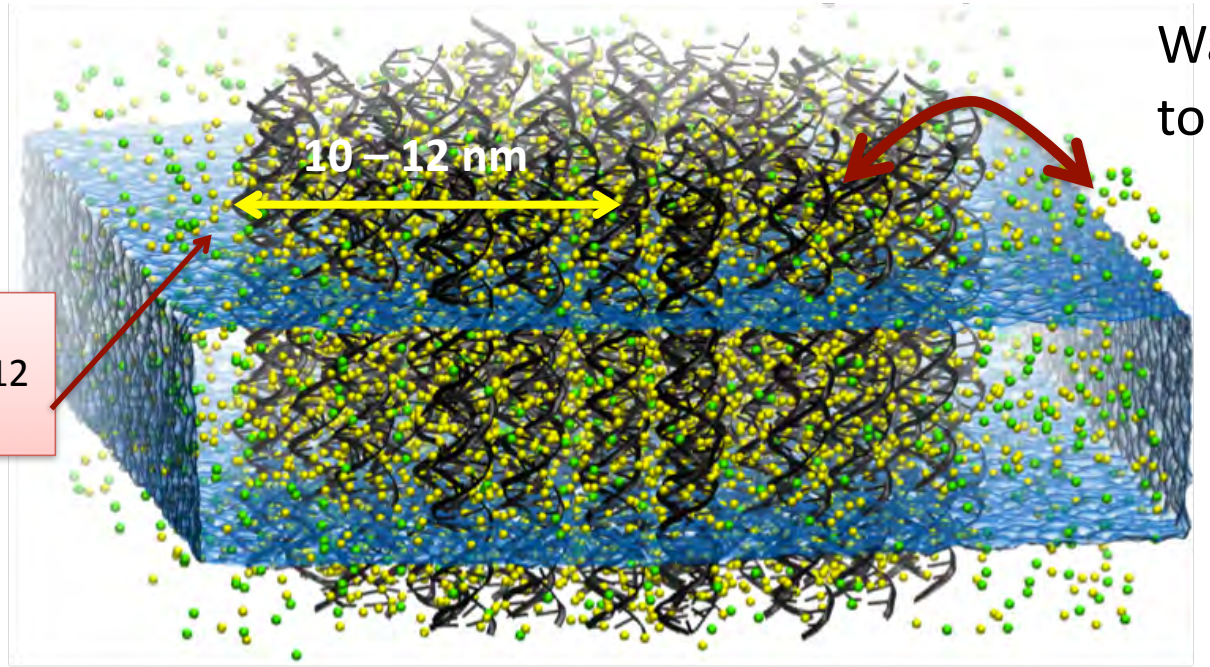
$[Mg^{2+}]_{buf}$ ~ 0 or 20 mM

What we measure

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

DNA / ion distribution:

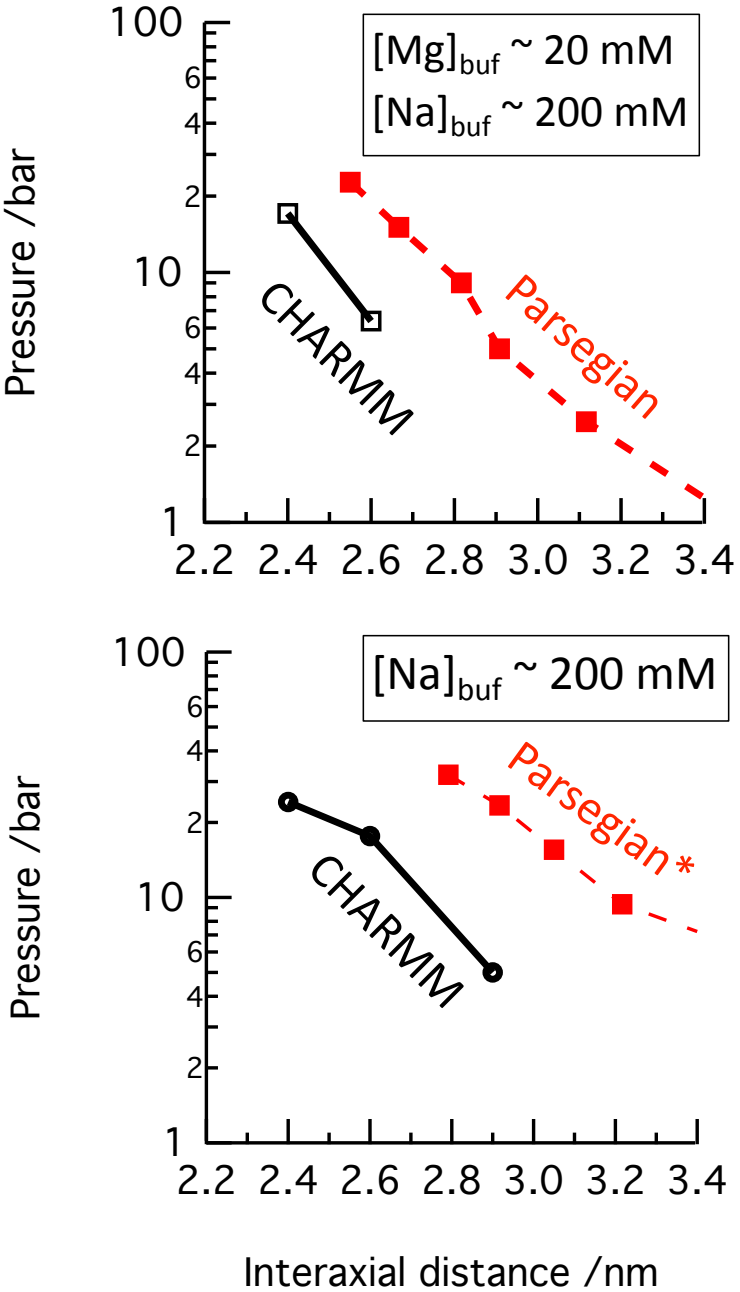
DNA / ion diffusion inside the array



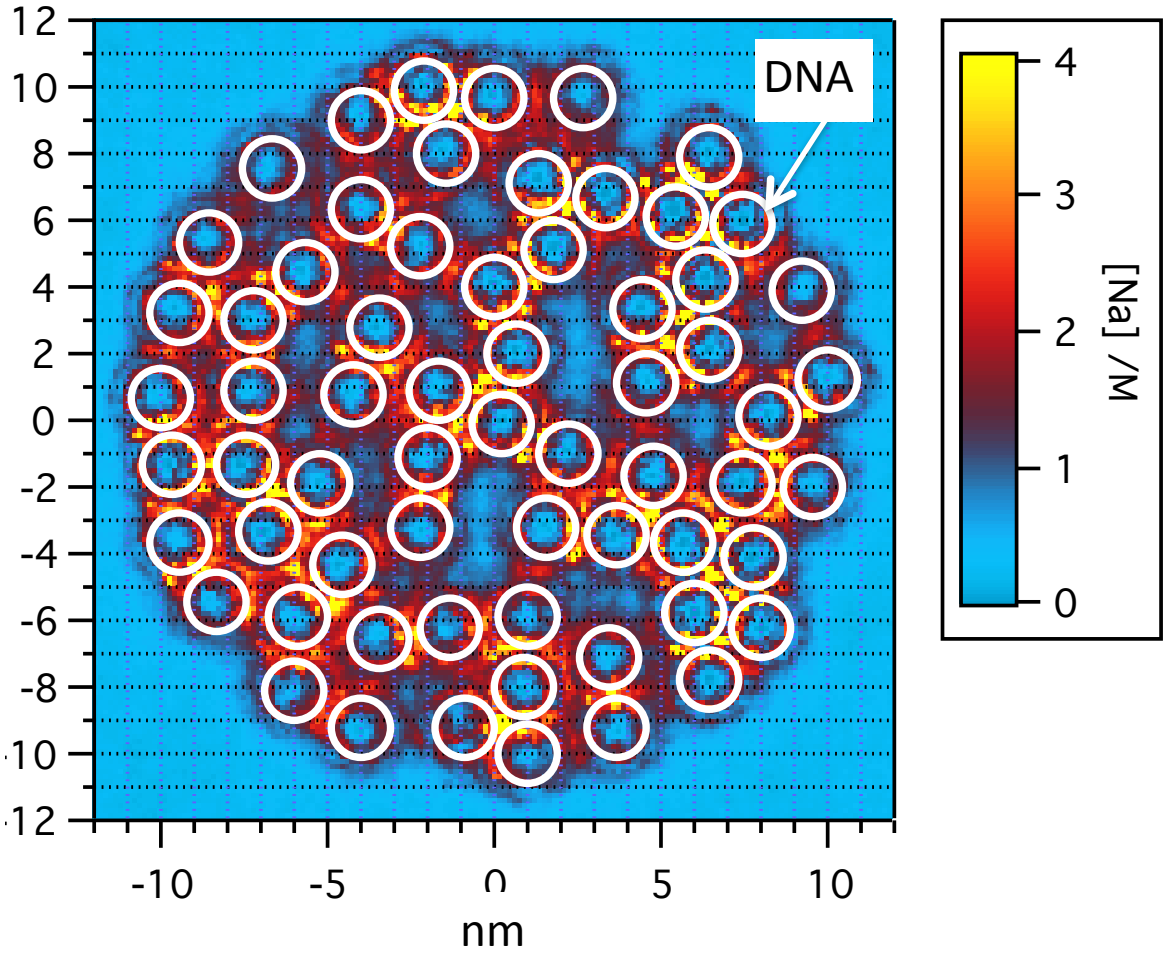
Water / salt free to move

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

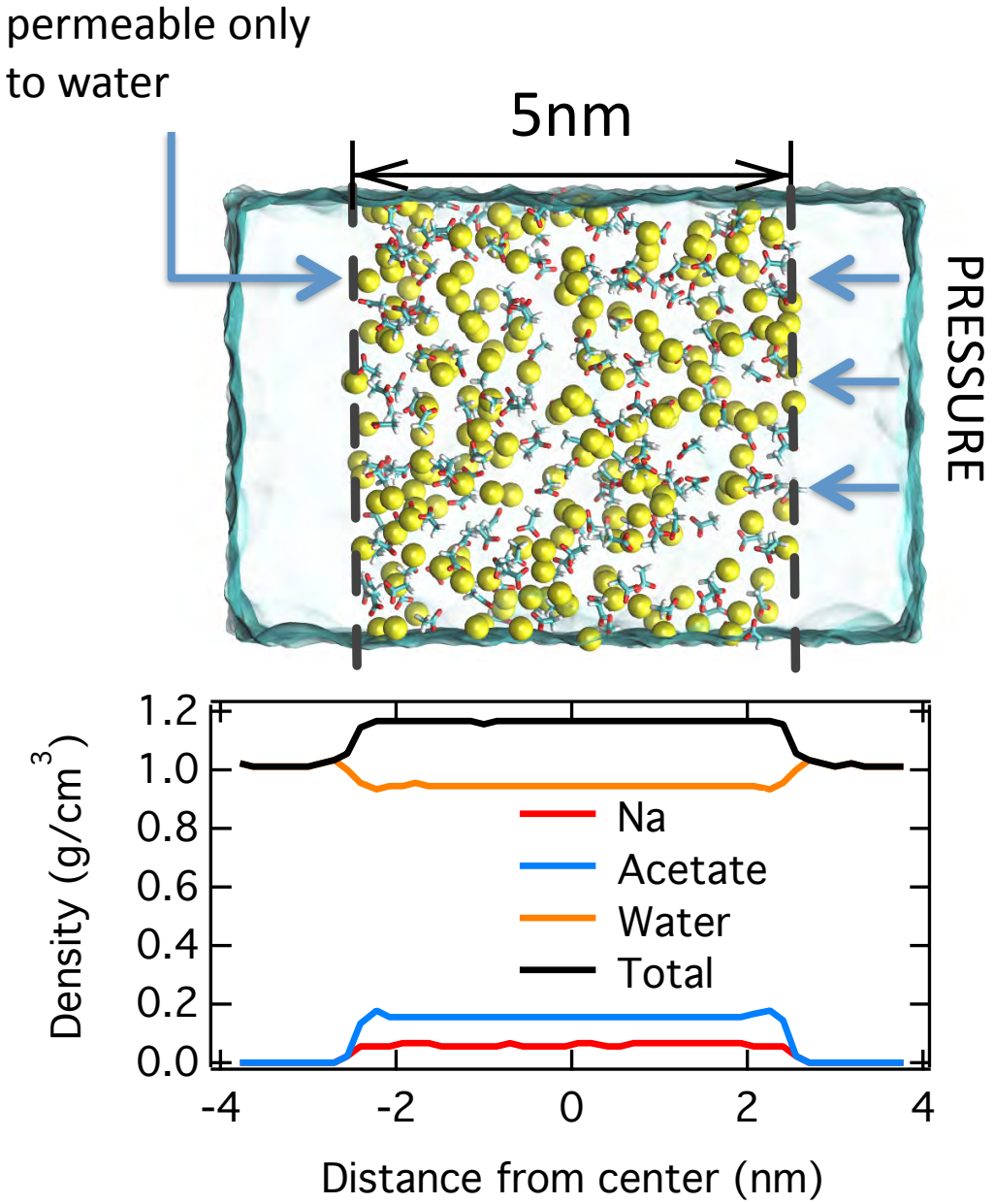


Too strong Na/Mg-phosphate attraction induces artificial DNA clusters!! [Na] ~ 4M!!



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

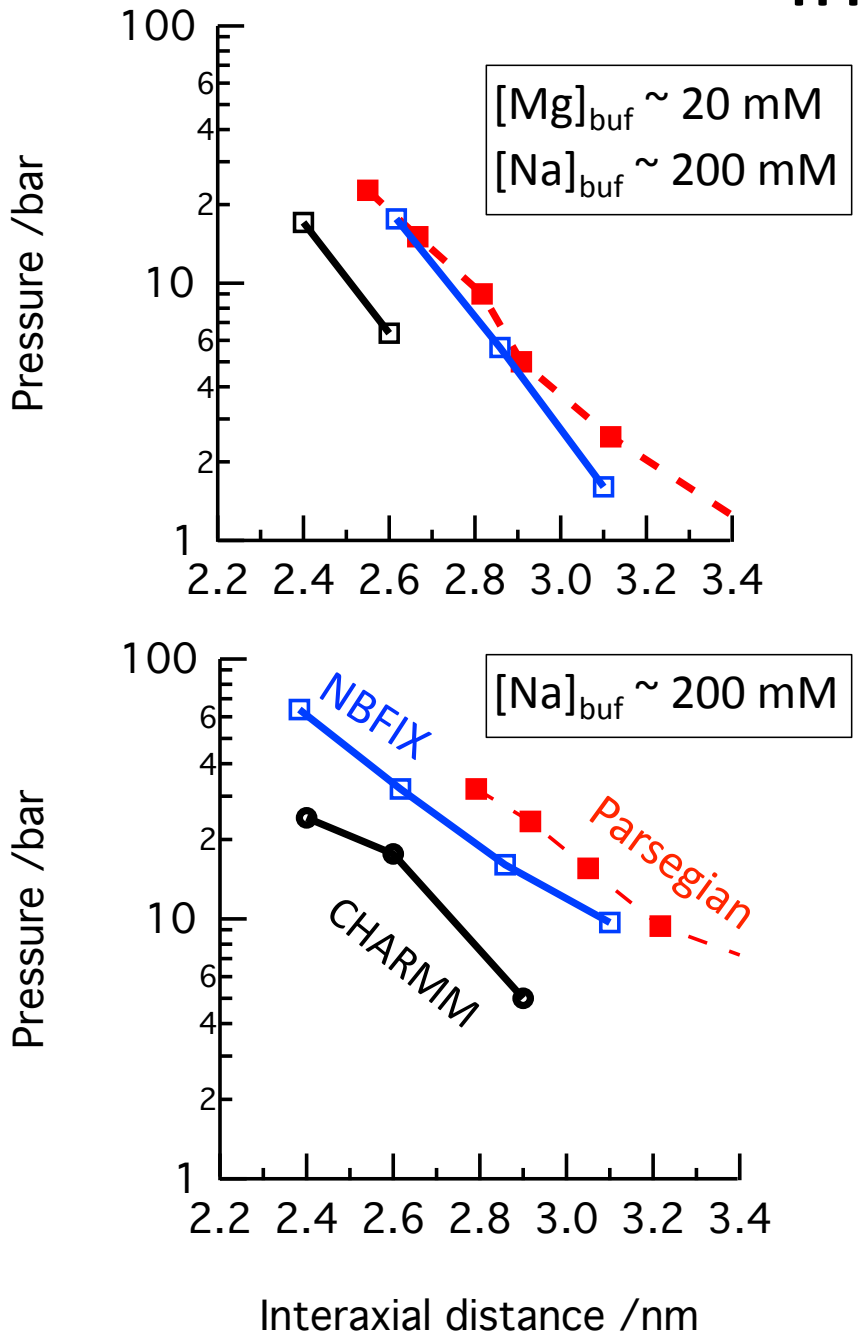
Recalibrate ion-DNA parameters using osmotic pressure data

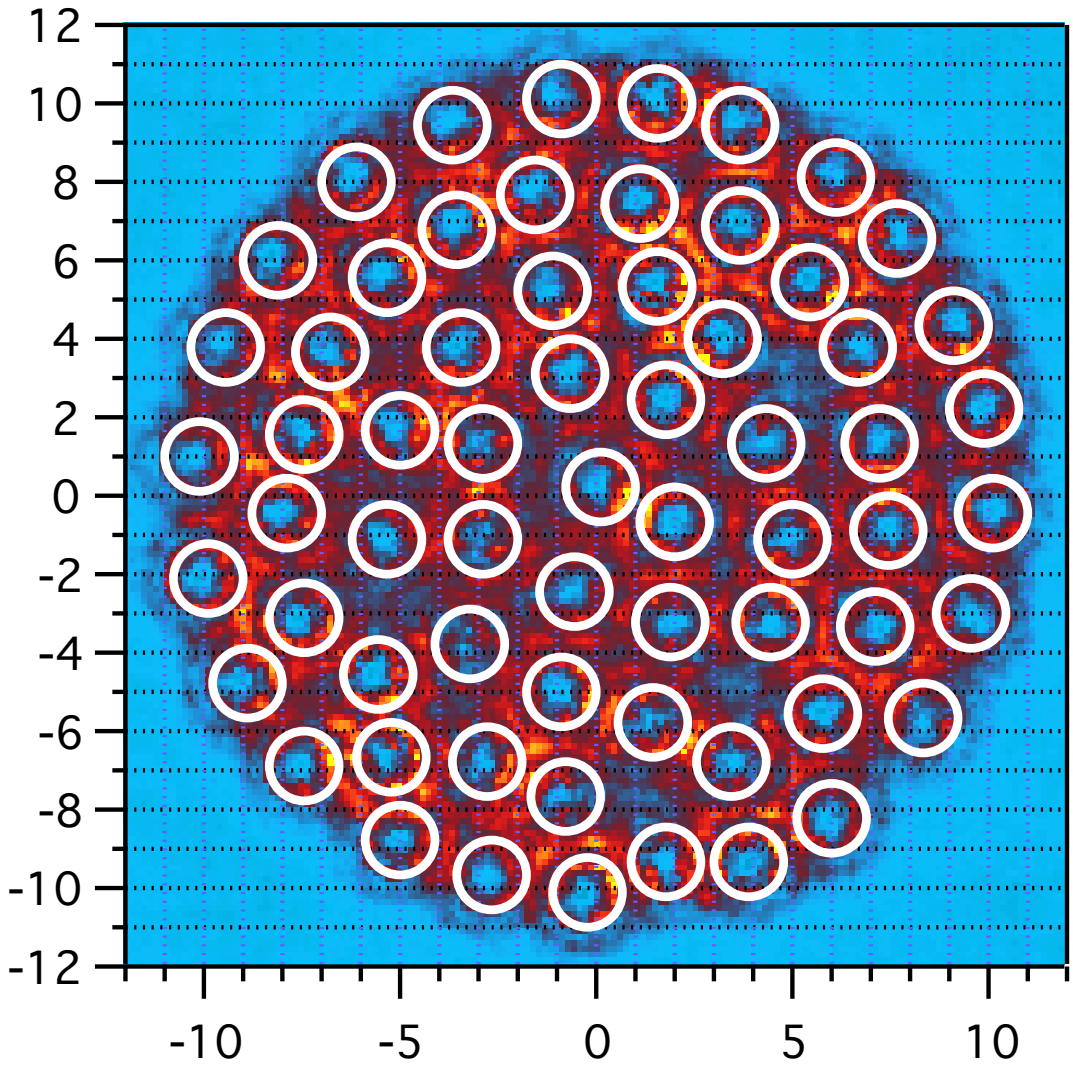


- Osmotic pressure is directly related to ion-pair formation: $\pi = \phi cRT$
- Pros: modify only ion-DNA phosphate interaction, without altering ion-water interaction.
- Cons: nothing.

* Luo & Roux, JPCL (2009)

Improved parametrization of ion-DNA interactions

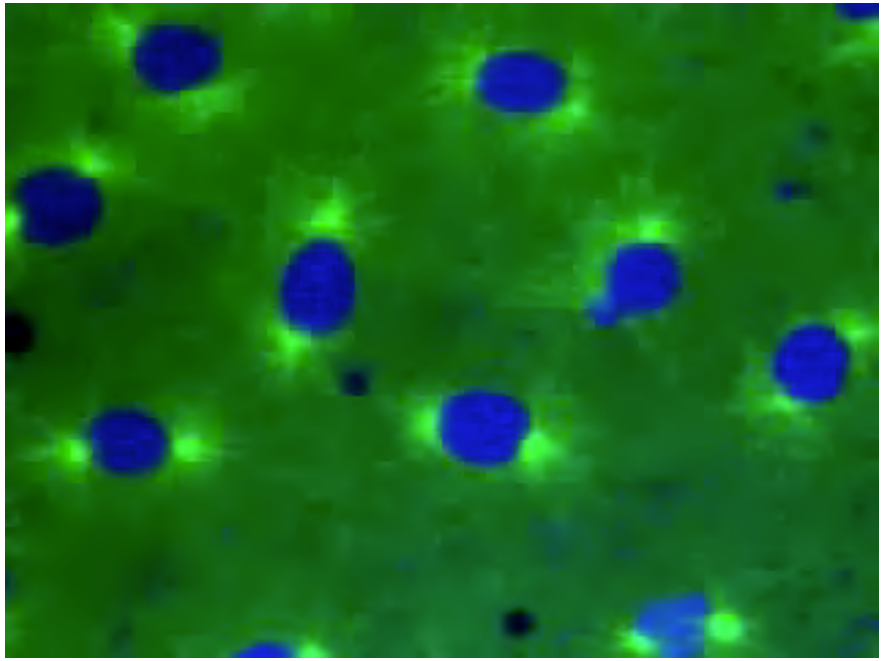




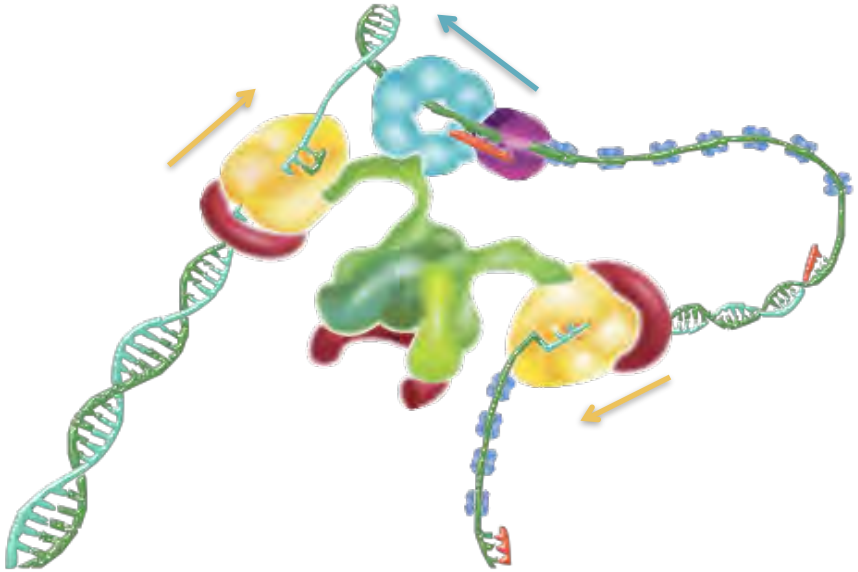
J. Phys. Chem. Lett. 3:45 (2012)

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*



Alberts, Molecular Biology of the Cell, fifth edition



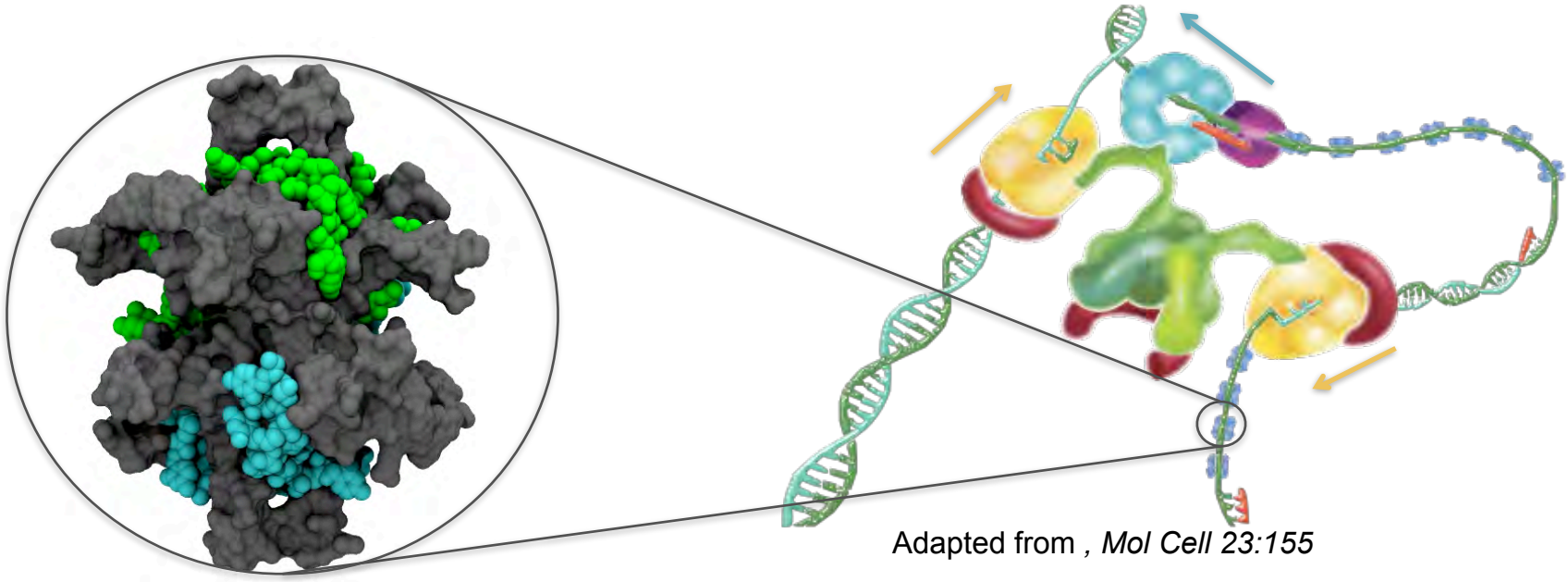
Adapted from Mol. Cell 23:155

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

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

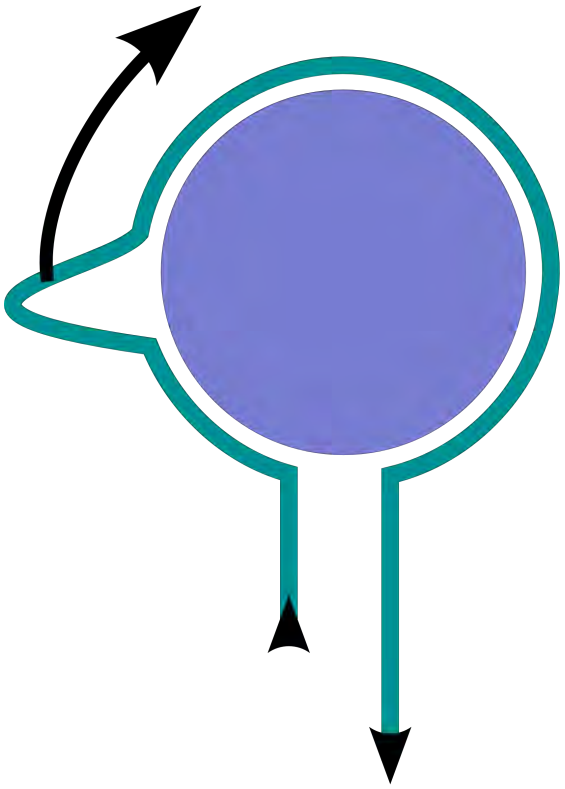


Adapted from , *Mol Cell 23:155*

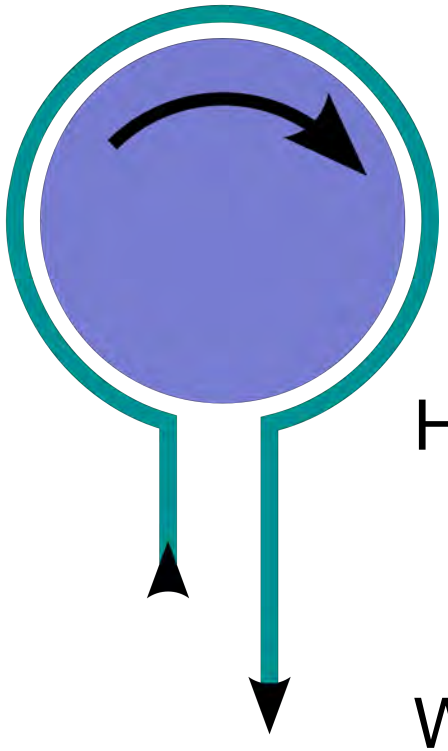
Problem: how is SSB removed when it is no longer needed?

Diffusion of ssb along DNA

reptation



rolling



What is the microscopic mechanisms of SSB diffusion?

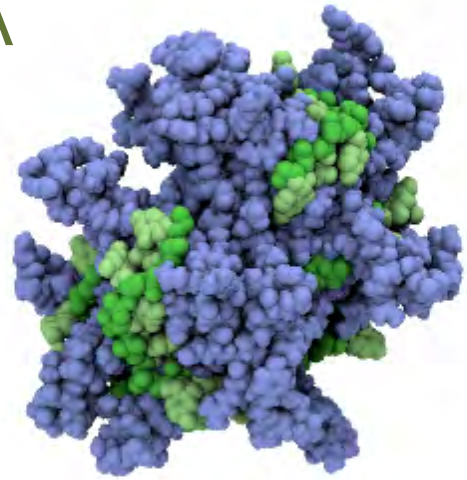
How does dissociation of DNA from SSB occur?

What makes an ssb an ssb?

† Ha group, Nature 461:1092

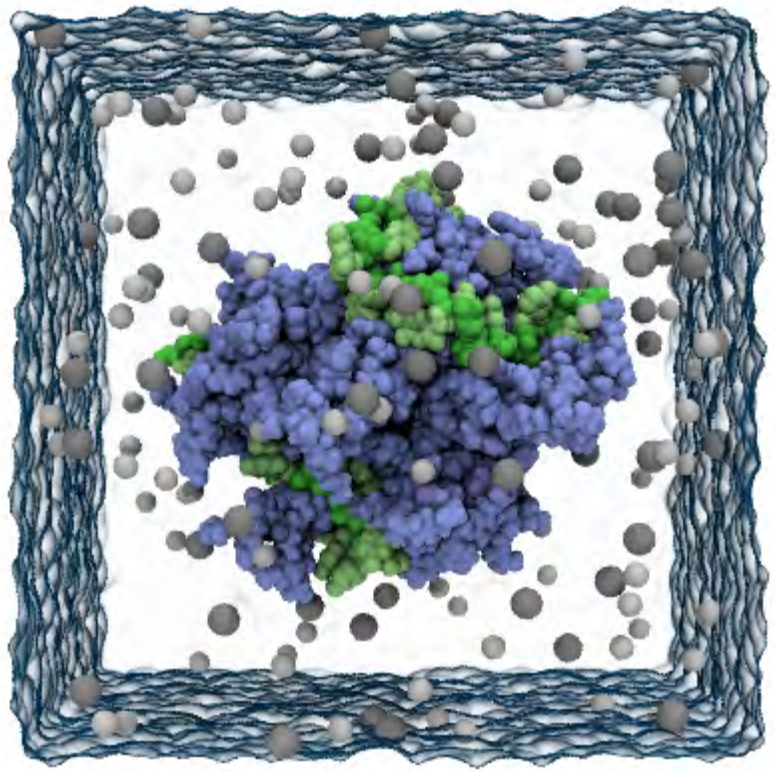
A model is build from an x-ray crystal structure

SSB
DNA



PDB:1EYG

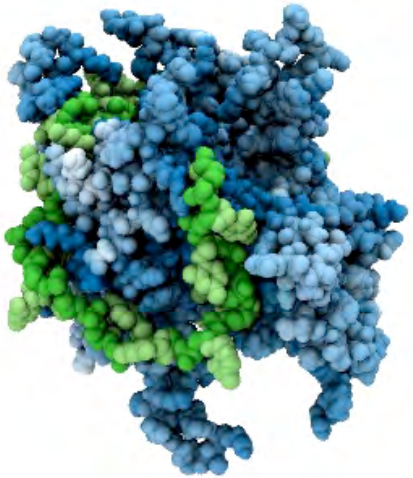
KCl
water

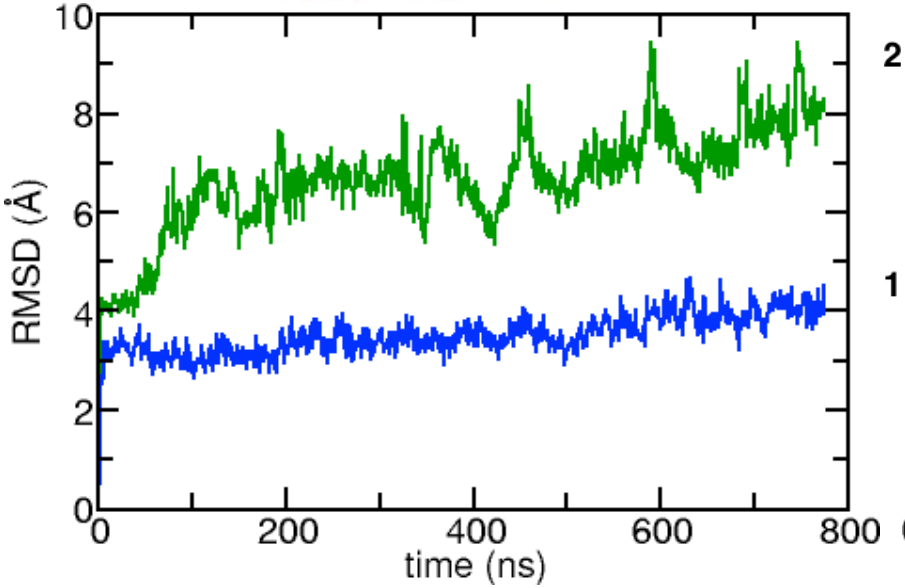


Unresolved DNA was modeled by the crystallographers (Lohman and Waksman groups, Washington U. School of Medicine) and provided to us via Ruobo Zhou of the Ha group.

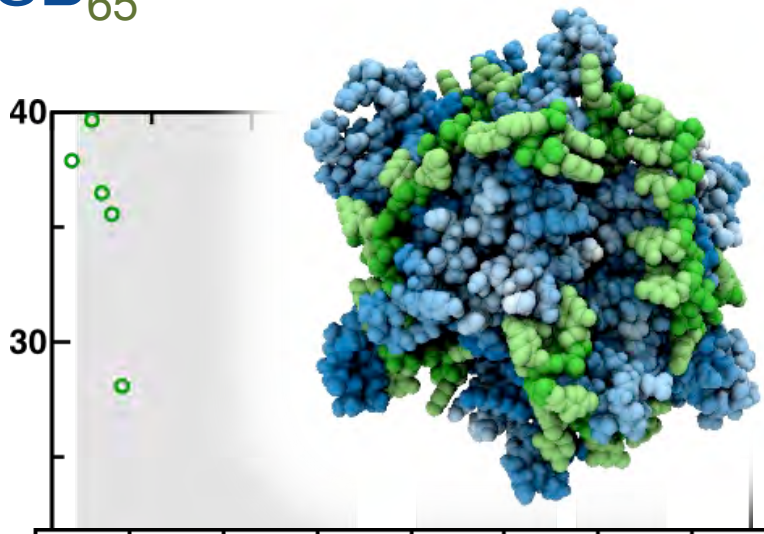
Individual nucleotides are loosely bound to SSB

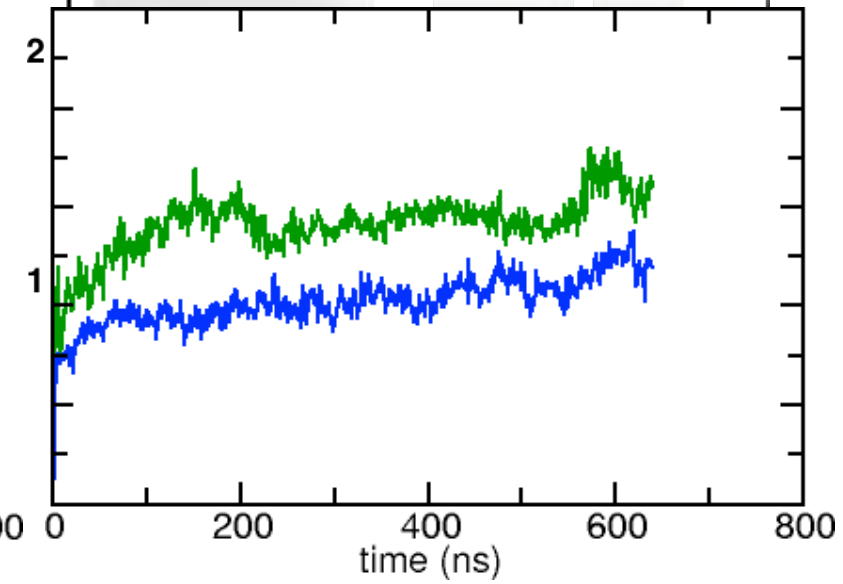
SSB_{35}



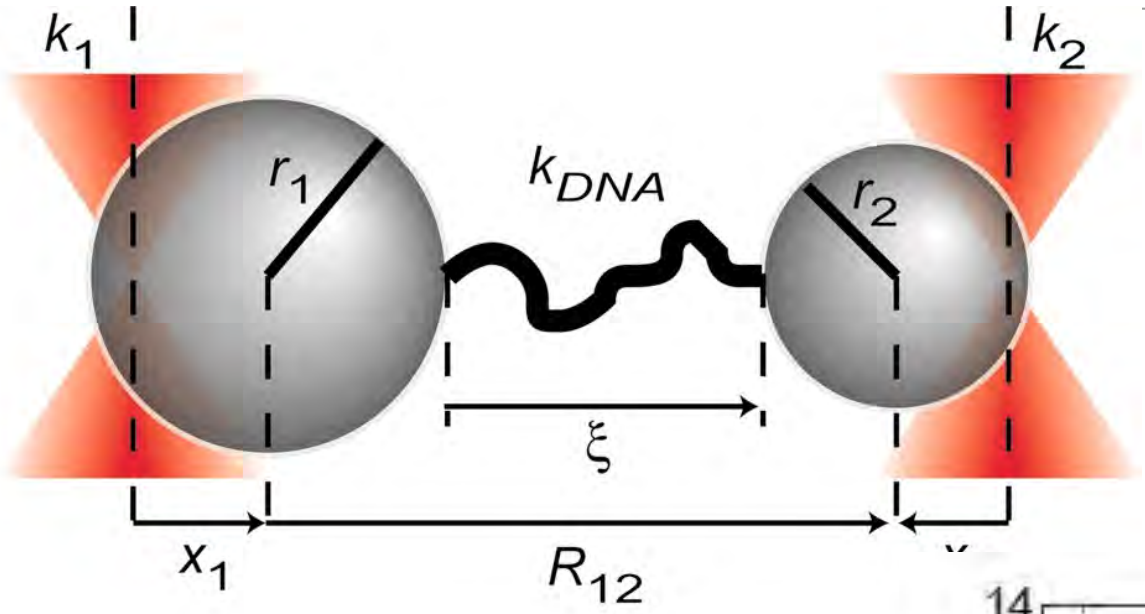


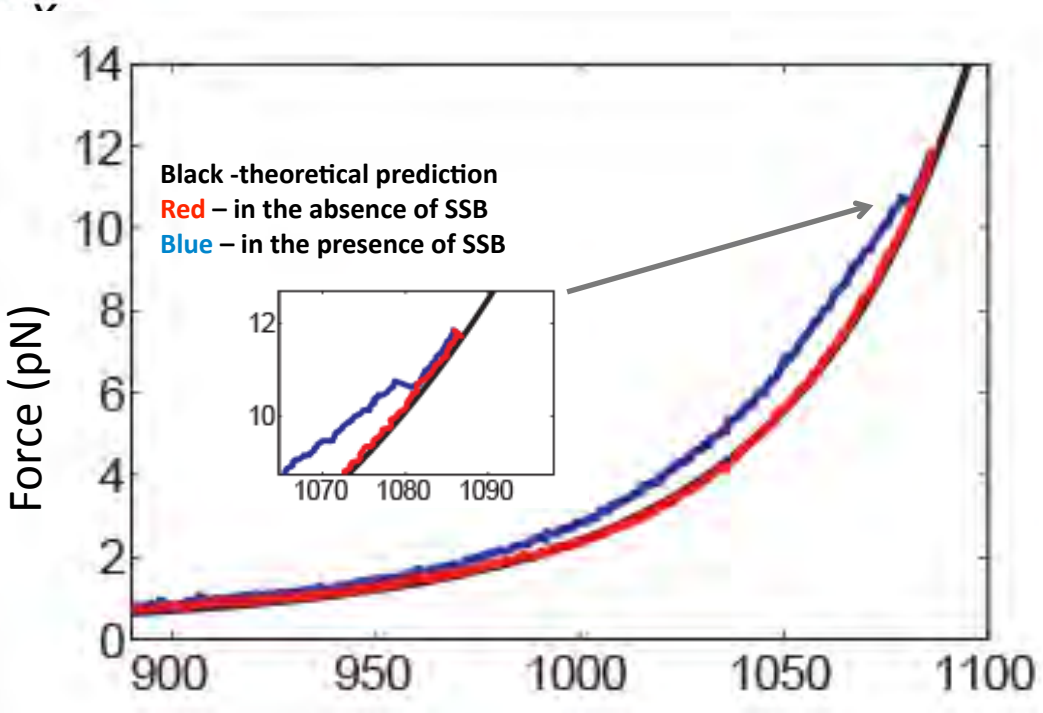
SSB_{65}





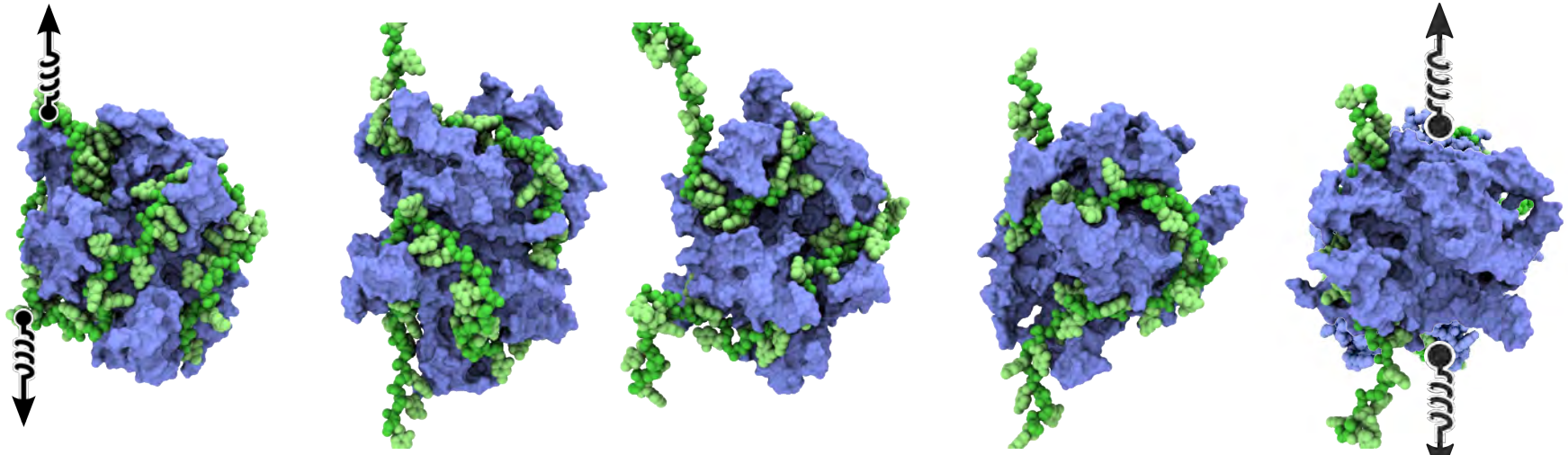
Mechanics of ssb-DNA (dis)assembly

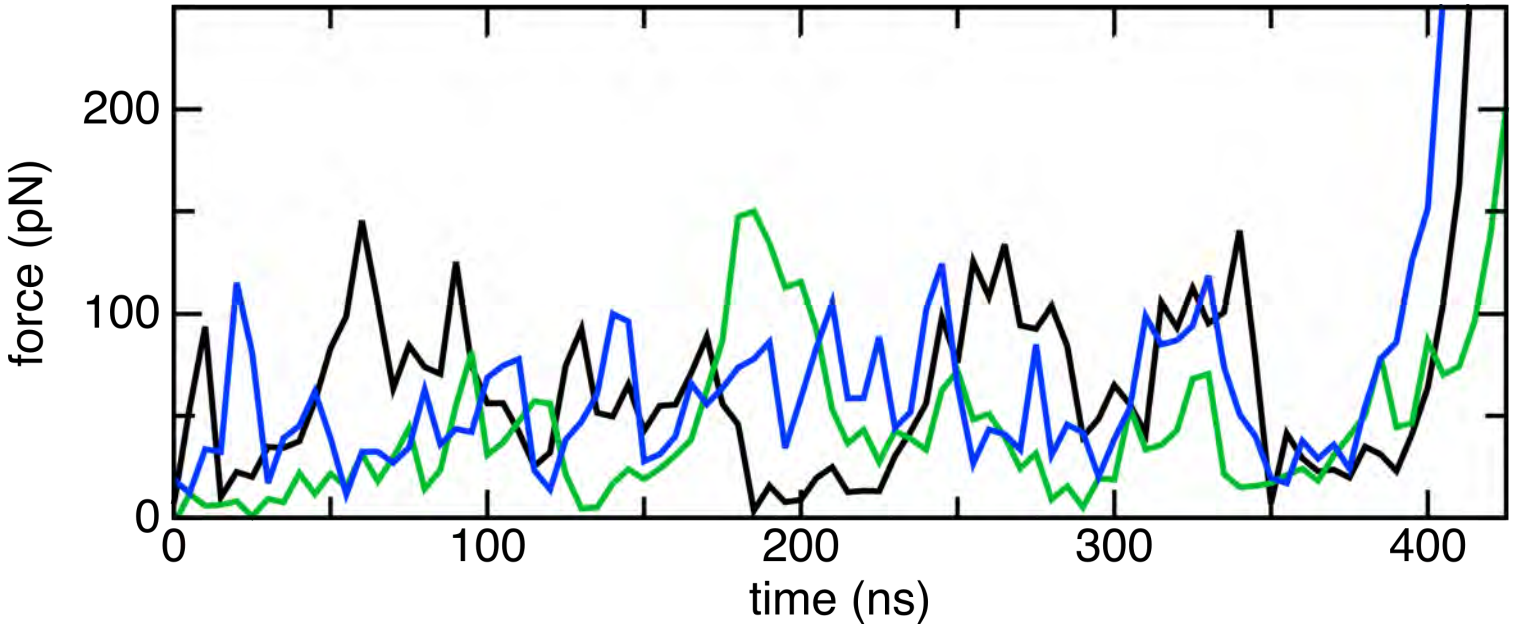




Chemla group, unpublished

All-atom simulations cannot quite reach experiment



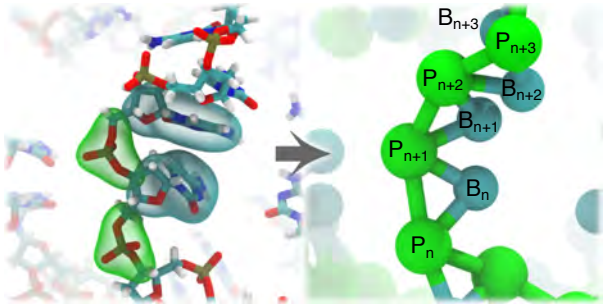


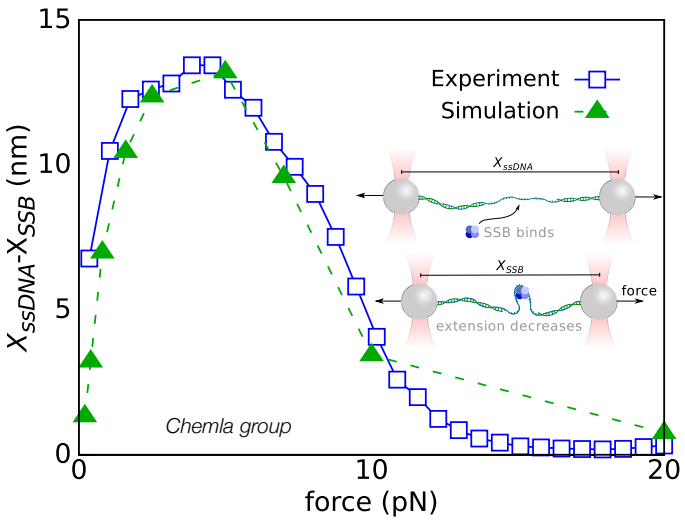
Forces are 1-2 orders of magnitude larger than in experiments

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

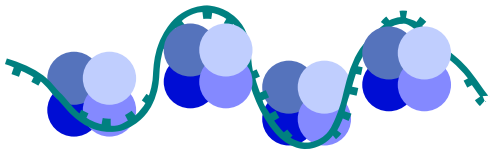




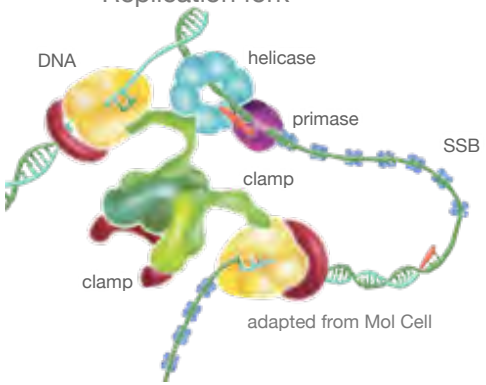
Excellent agreement was obtained between experiment and simulation suggesting that SSB binds DNA dynamically

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

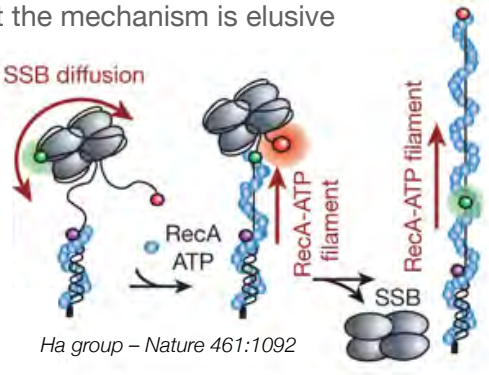


Replication fork



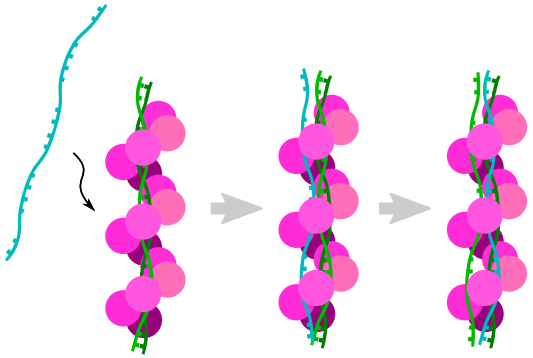
adapted from Mol Cell

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



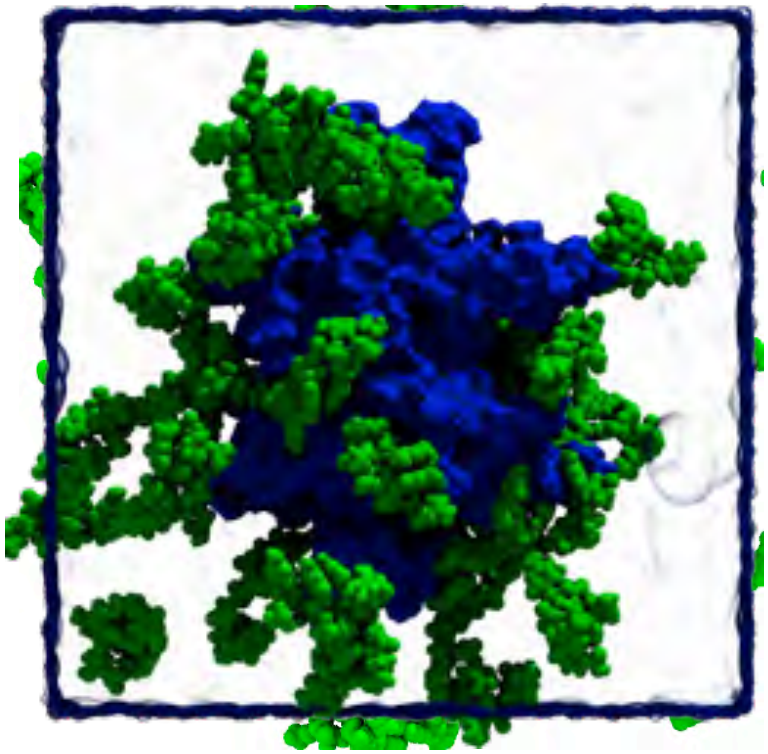
Ha group – Nature 461:1092

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

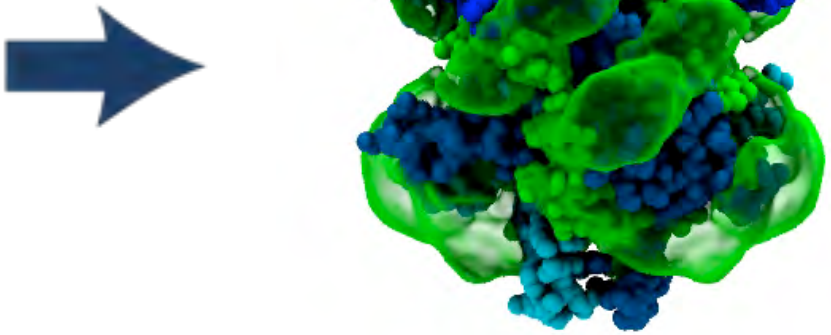


SSB parameterization

Obtain density of all-atom nucleotides



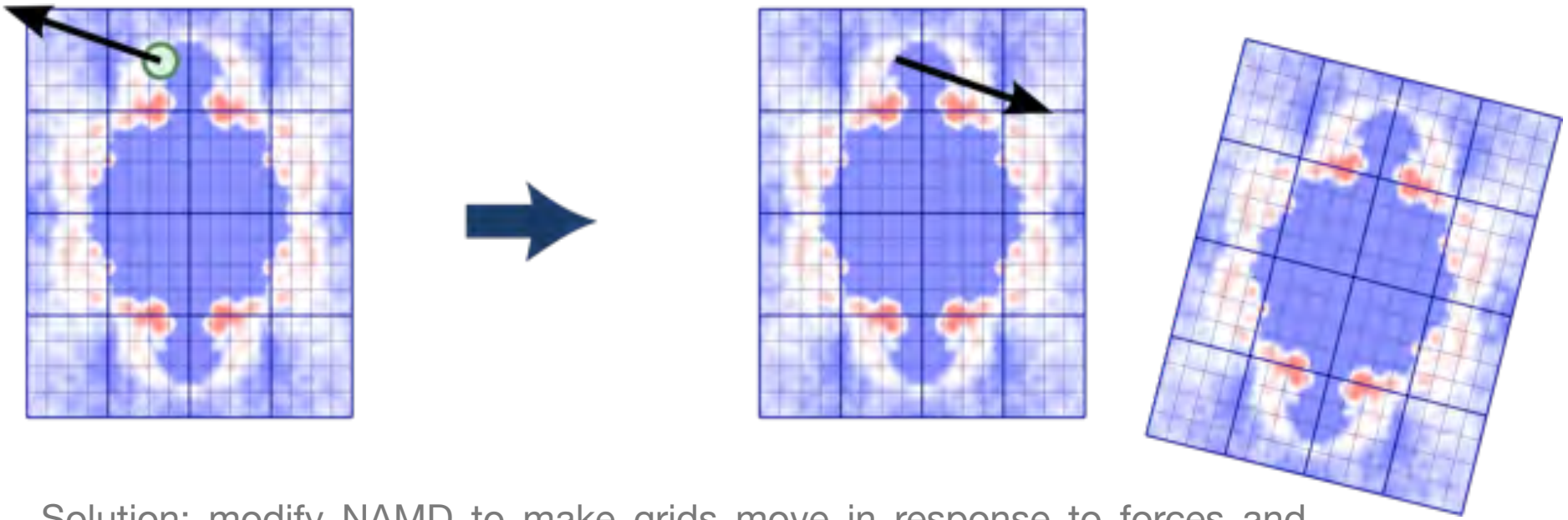
Apply iterative Boltzmann inversion using CG ssDNA to obtain the interaction potential that makes the AA and CG densities match



SSB represented through moving grids

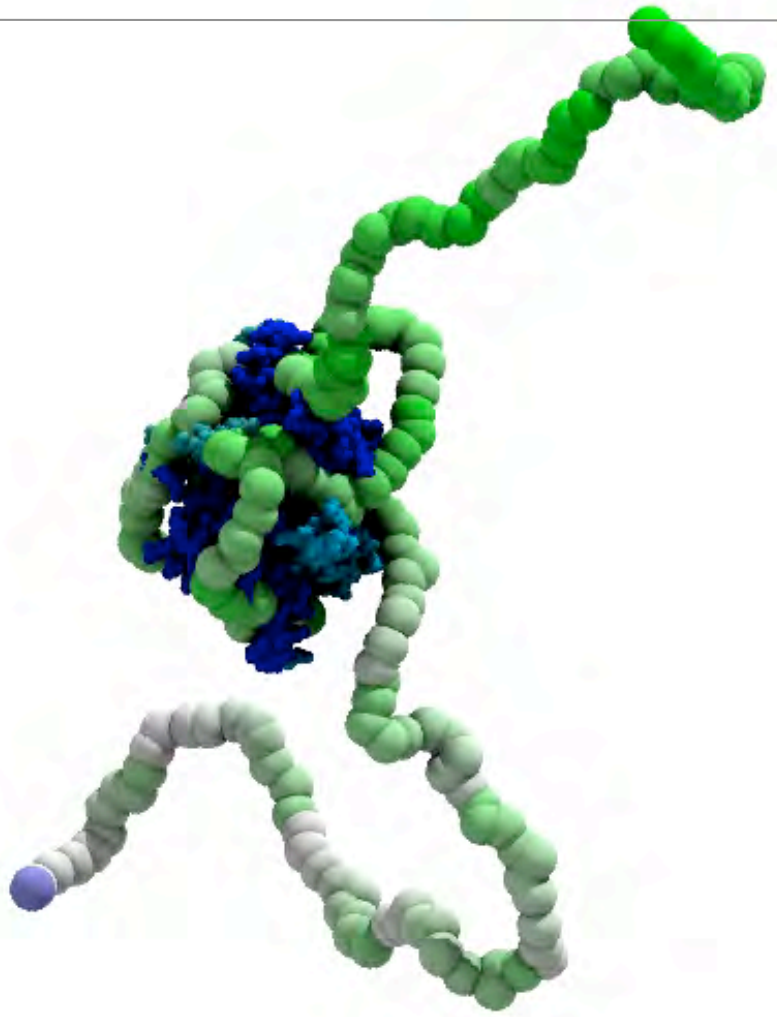
The CG ssDNA can interact with atomically-detailed SSB using Gridforces in NAMD.

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.

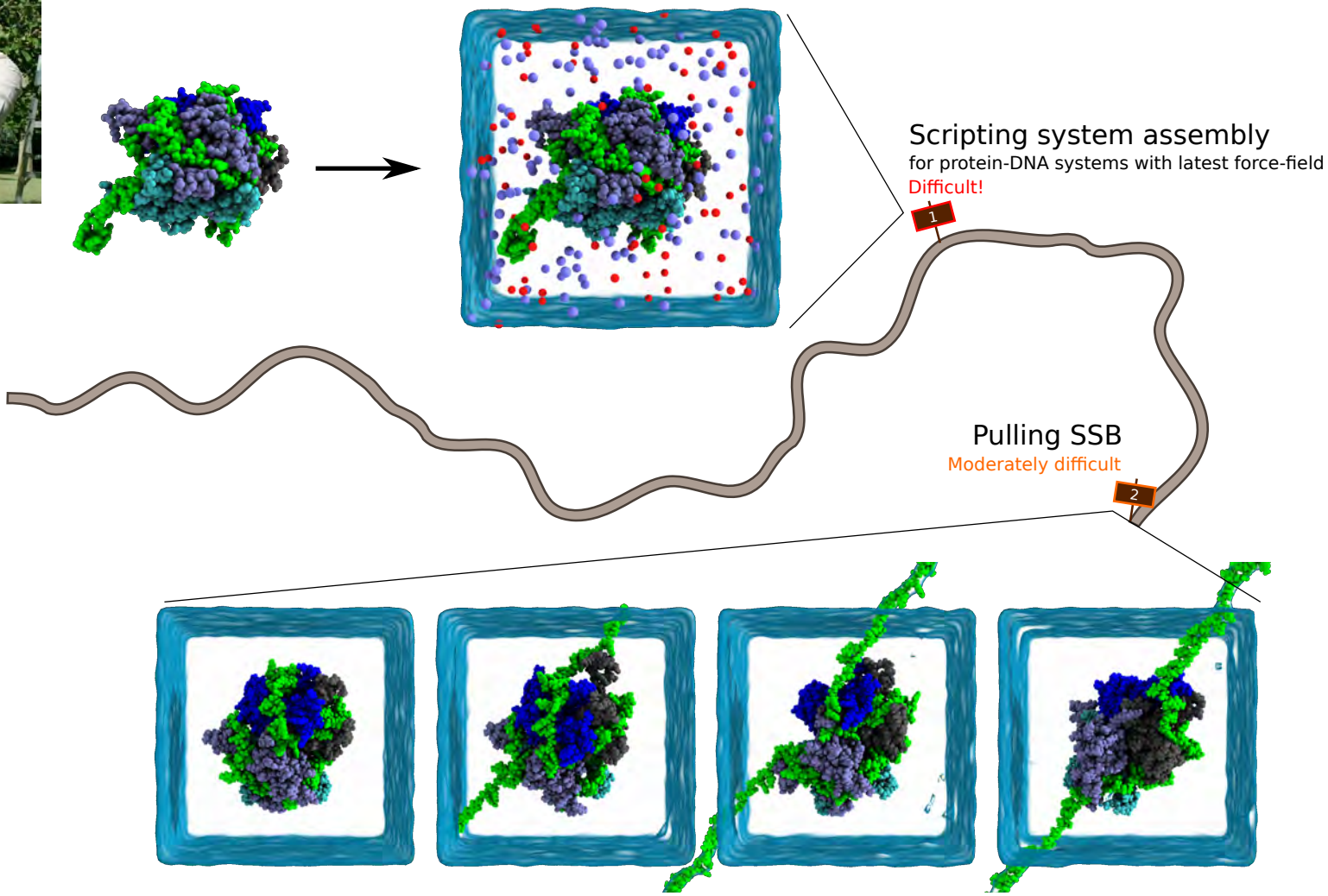
DNA (1-site/nt) + SSB simulation trajectory



This simulation is ~400 ns, but smoothed potentials make the kinetics equivalent to ~20 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



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Chen-Yu Lu
Shu-Han Chao
Karl Dekker

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Hajin Kim
(UIUC)

J Gundlach
Ian Derrington
(UW Seattle)

Cees Dekker
Magnus Jonsson
(TU Delft)

VMD and NAMD