Close encounters with DNA

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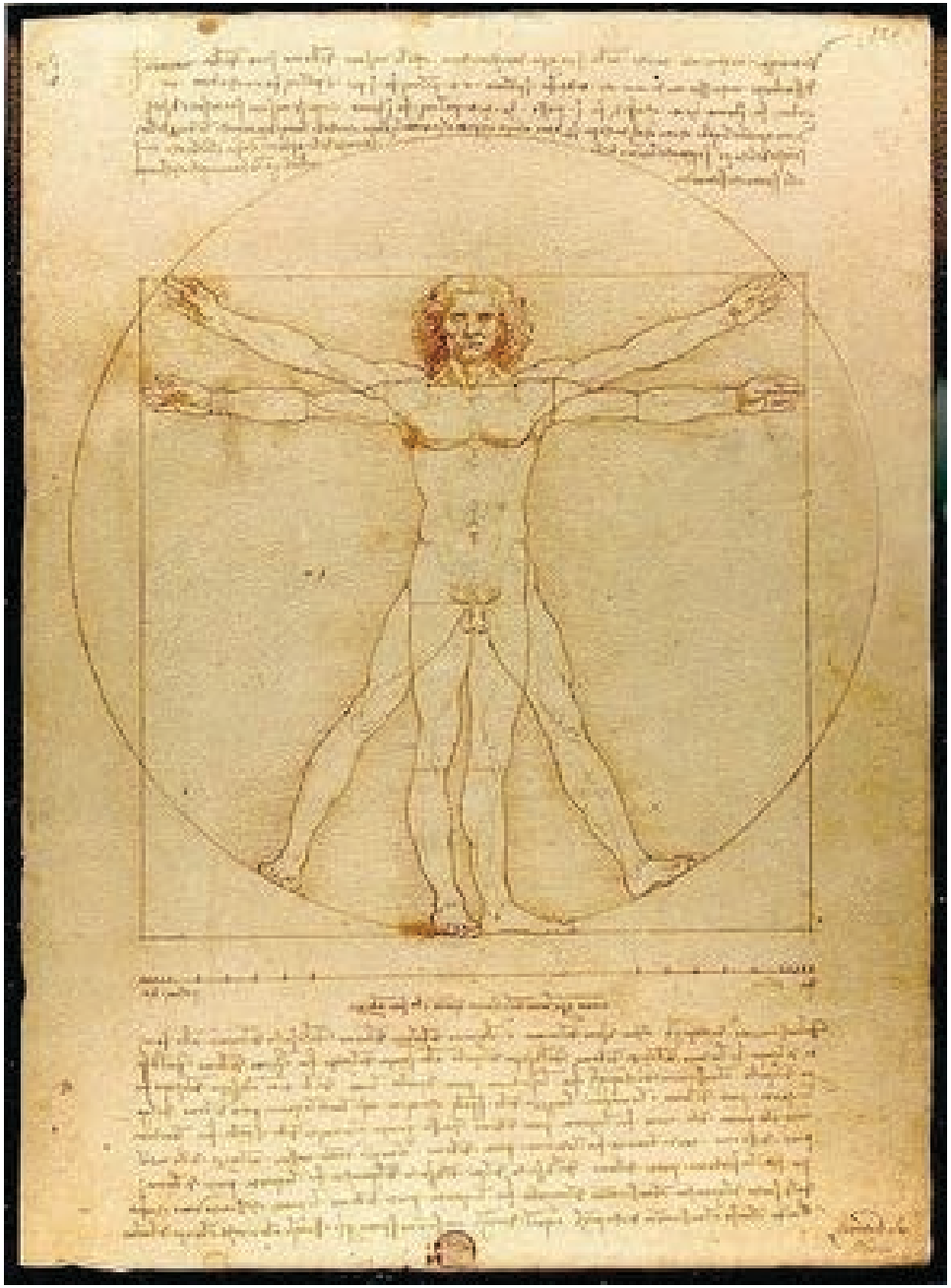


WHAT IS LIFE?

The Physical Aspect of the Living Cell

BY

ERWIN SCHRÖDINGER

SENIOR PROFESSOR AT THE DUBLIN INSTITUTE FOR ADVANCED STUDIES

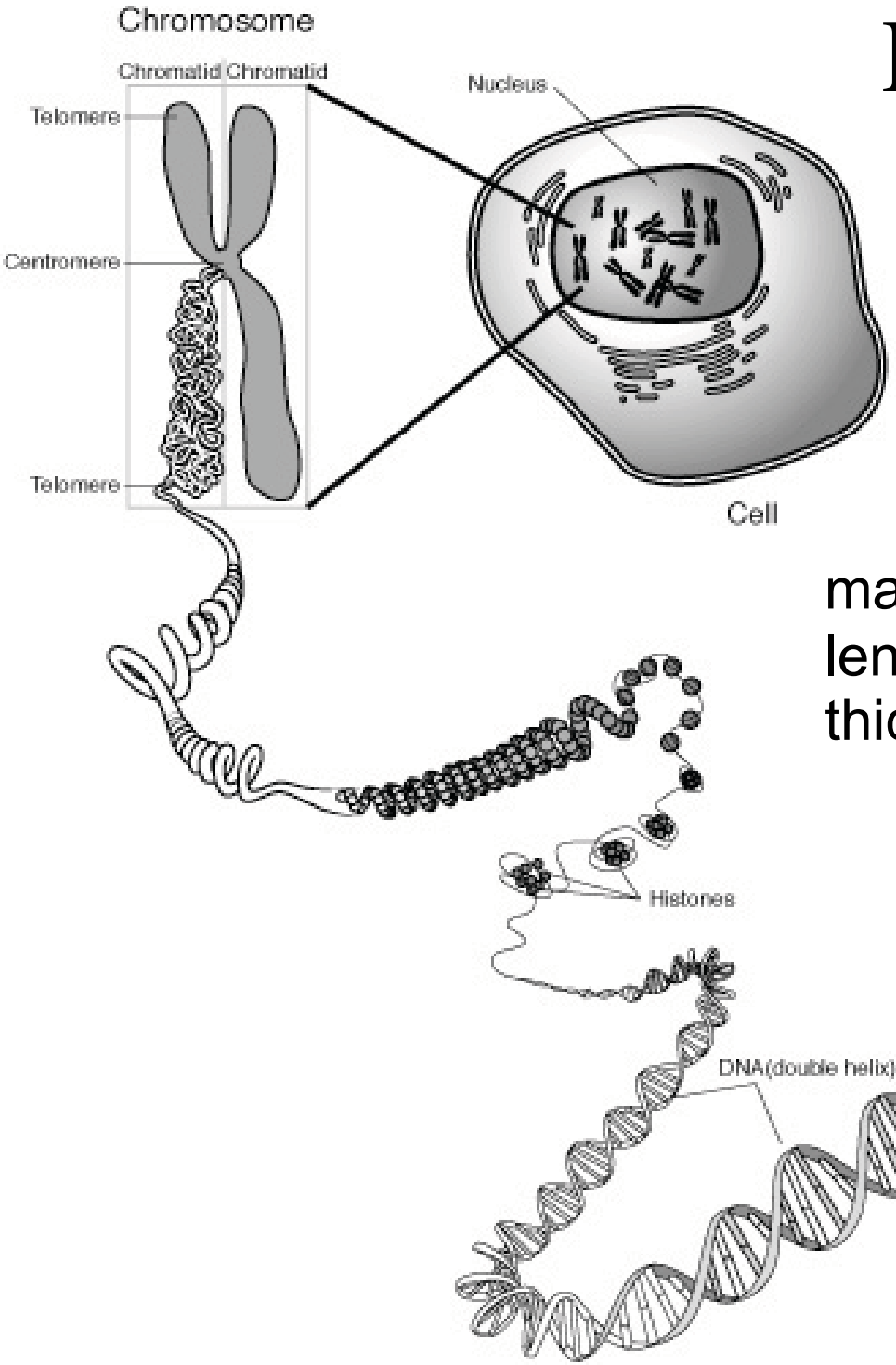
Based on Lectures delivered under the auspices of the Institute at Trinity College, Dublin, in February 1943

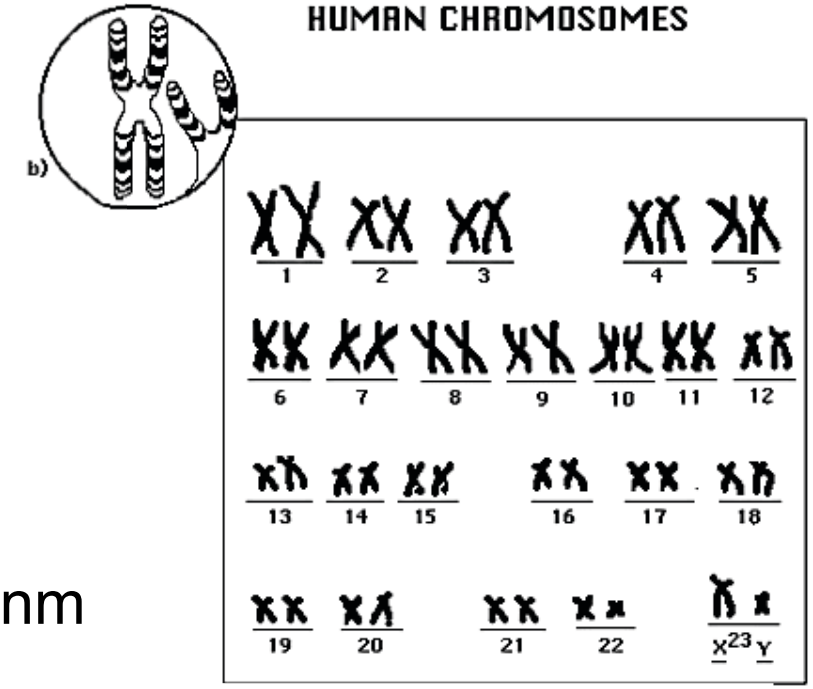
CAMBRIDGE

AT THE UNIVERSITY PRESS

1948

DNA, the blueprint



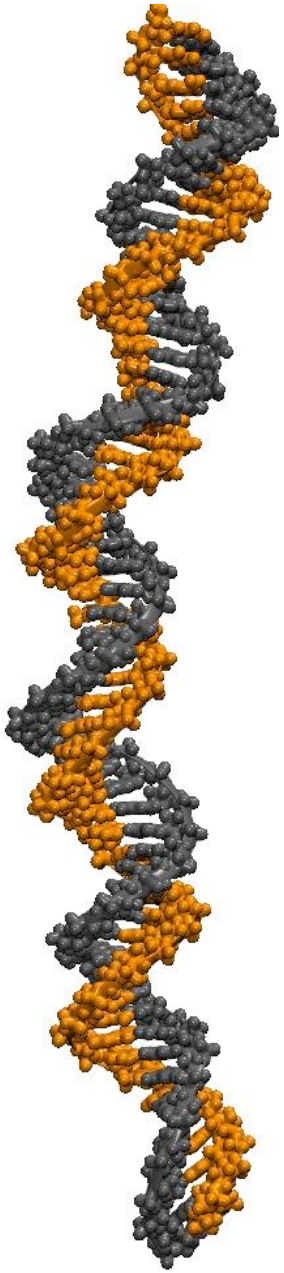


mass: 6 pg
length: 2 m
thickness: 2nm

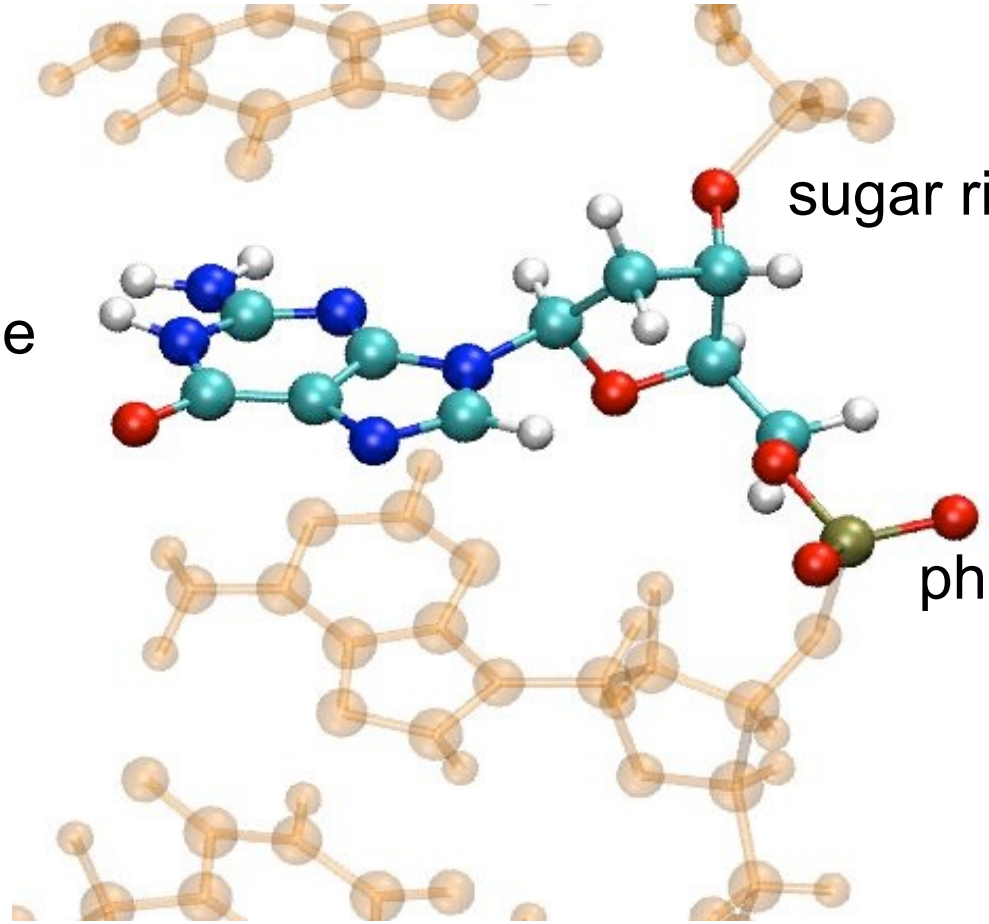
…GTGTGACTCGT
GGTCCGTAATGTC
GTTAATGGTGACC
GTGTGGCCTGATG
GTTAGTGTGTGA…

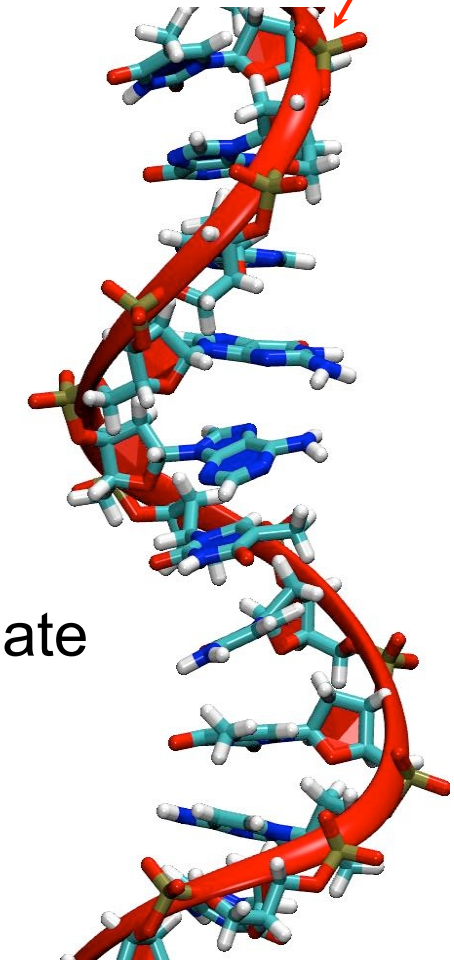
http://www.accessexcellence.org/AB/GG/chromosome.html

DNA code is written in atoms



Highly charged: 2 electron charges per 0.32nm





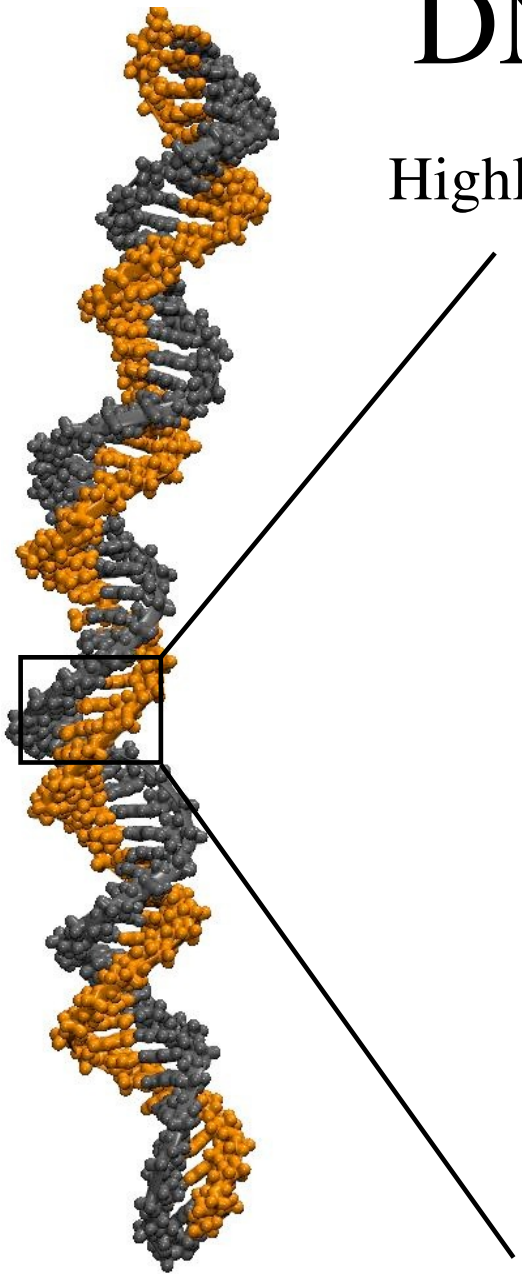
Double stranded DNA
(persist. length ~50nm)

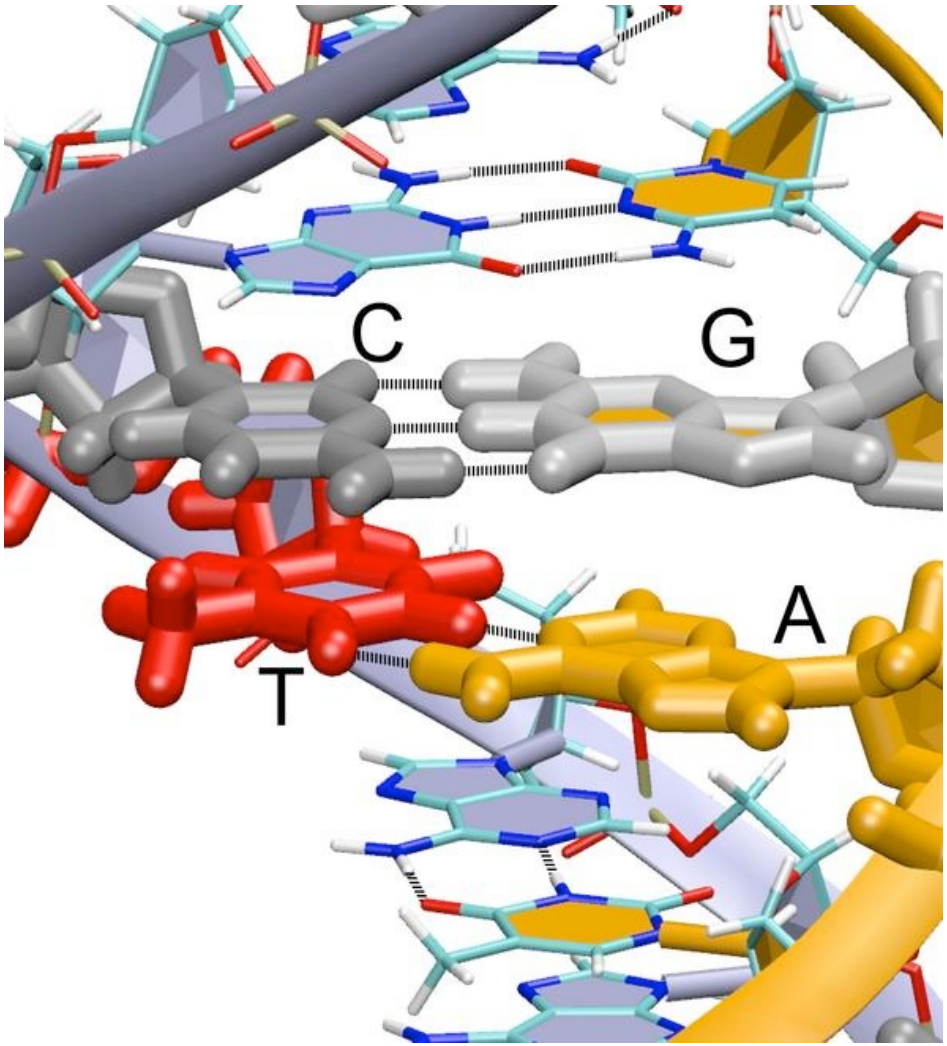
The sequence has direction:
5'-AAGCTGGTTCAG-3'

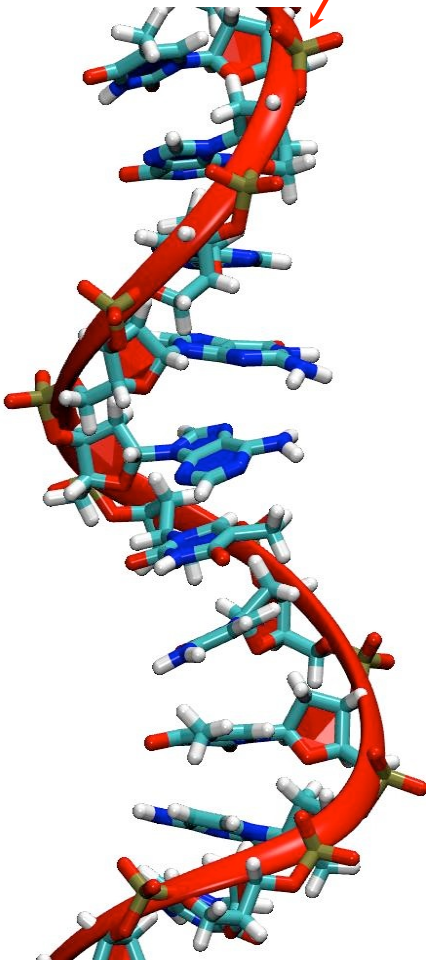
Single stranded DNA
(persist. length ~1.5nm)

DNA code is written in atoms

Highly charged: 2 electron charges per 0.32nm





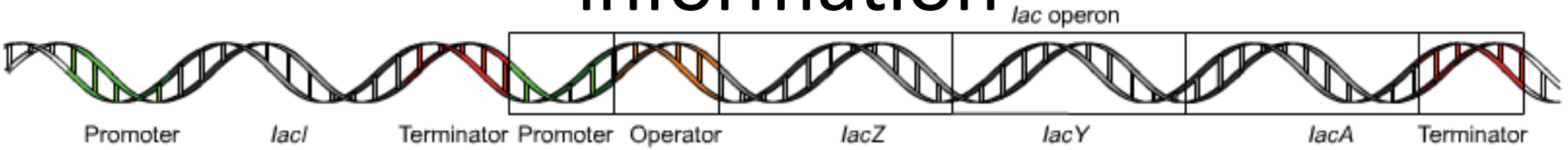


Double stranded DNA
(persist. length ~50nm)

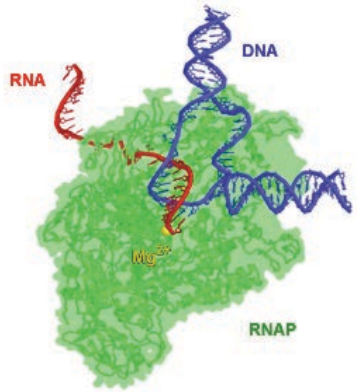
The sequence has direction:
5'-AAGCTGGTTCAG-3'

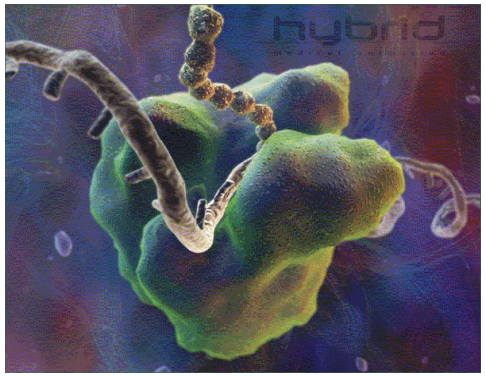
Single stranded DNA
(persist. length ~1.5nm)

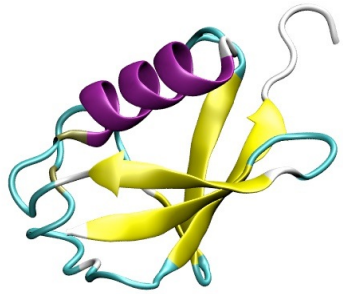
The sequence contains biological information



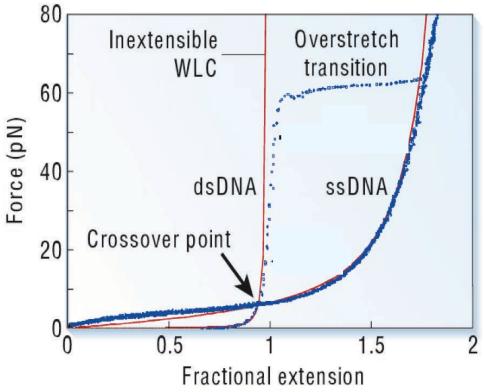
Central dogma of molecular biology

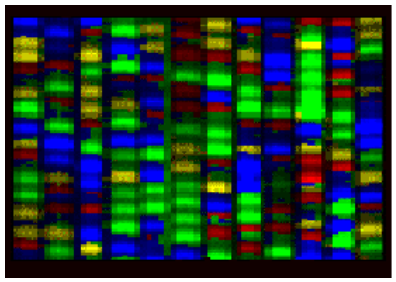


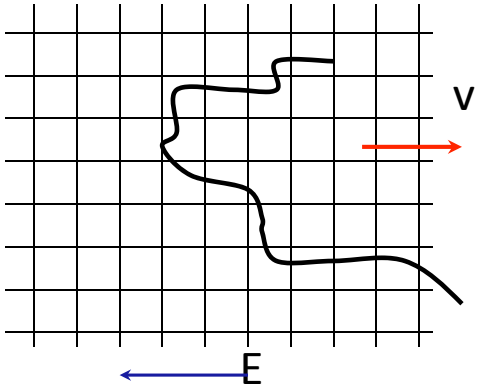




The physical properties enable functionality



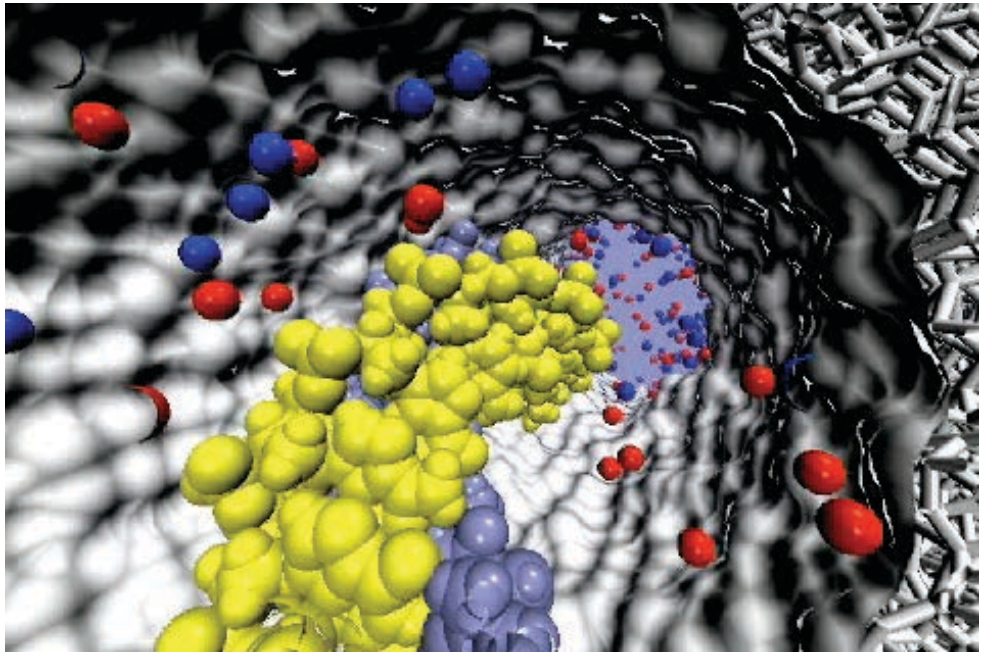




Molecular dynamics simulations, a computational (force) microscope

Massive parallel computer
Blue Waters, ~200,000

Atoms move according to classical mechanics (F= ma)



<u>Time scale</u>: ~ 0.1-100 μs

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

Interaction between atoms is defined by molecular force field

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Condensed Matter

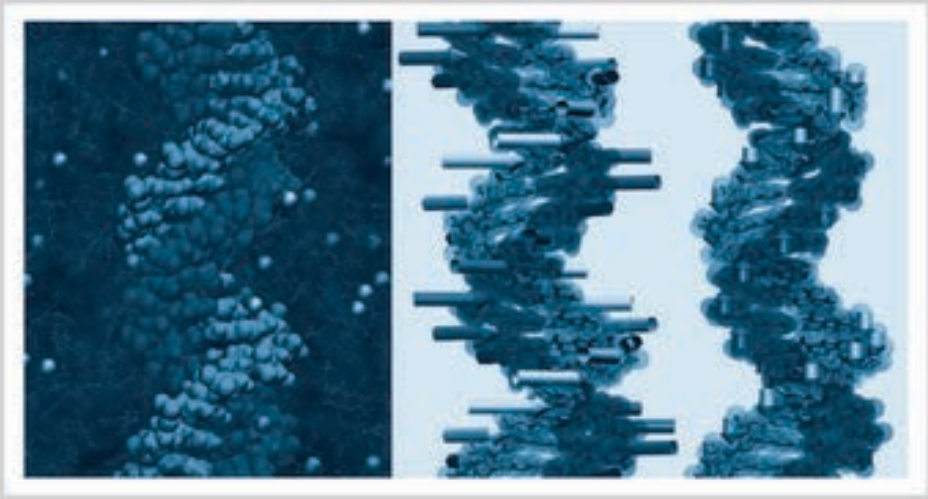
Journal of Physics: Condensed Matter 26: 413101 (2014)

Volume 26 Number 41 15 October 2014

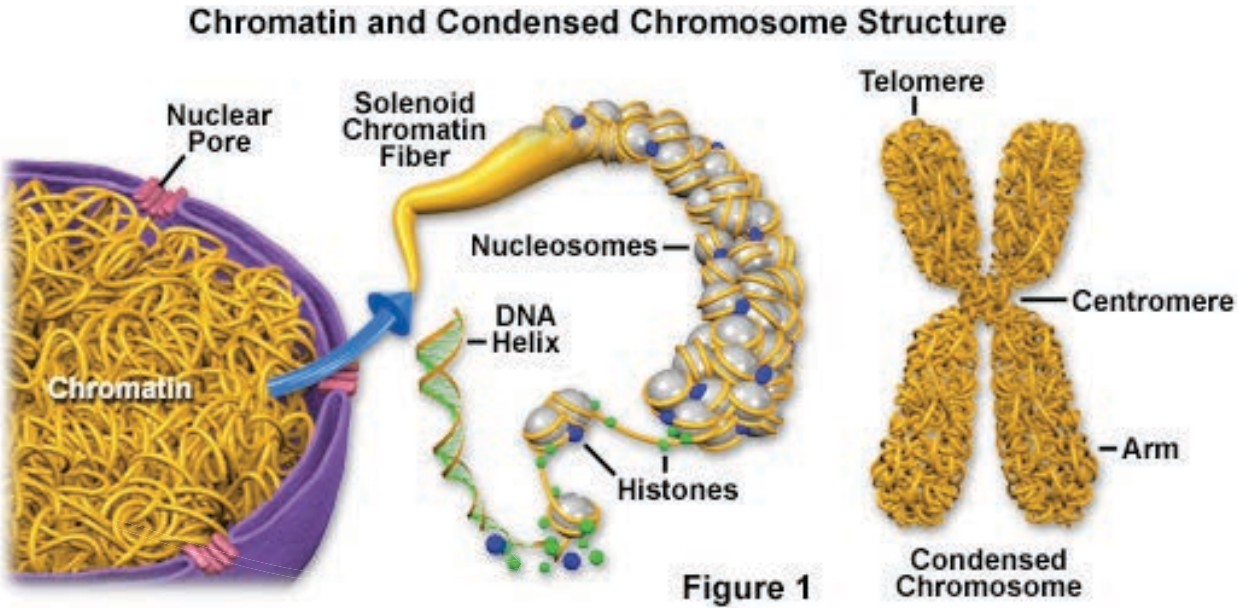
Topical review

Close encounters with DNA

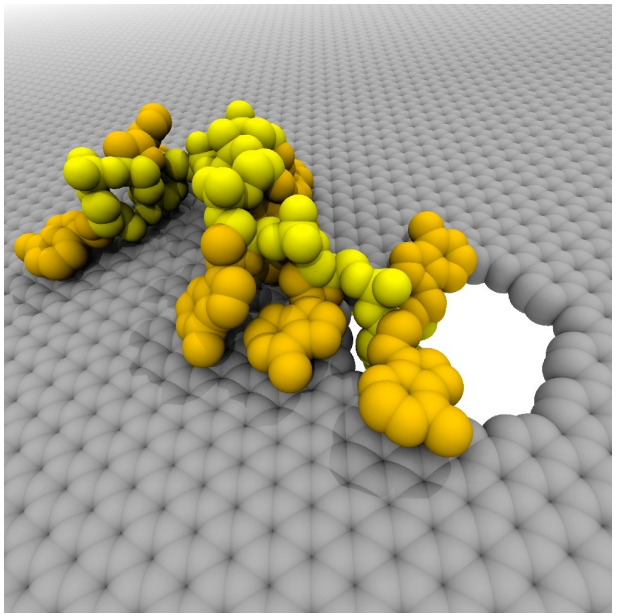
C Maffeo, J Yoo, J Comer, D B Wells, B Luan and A Aksimentiev



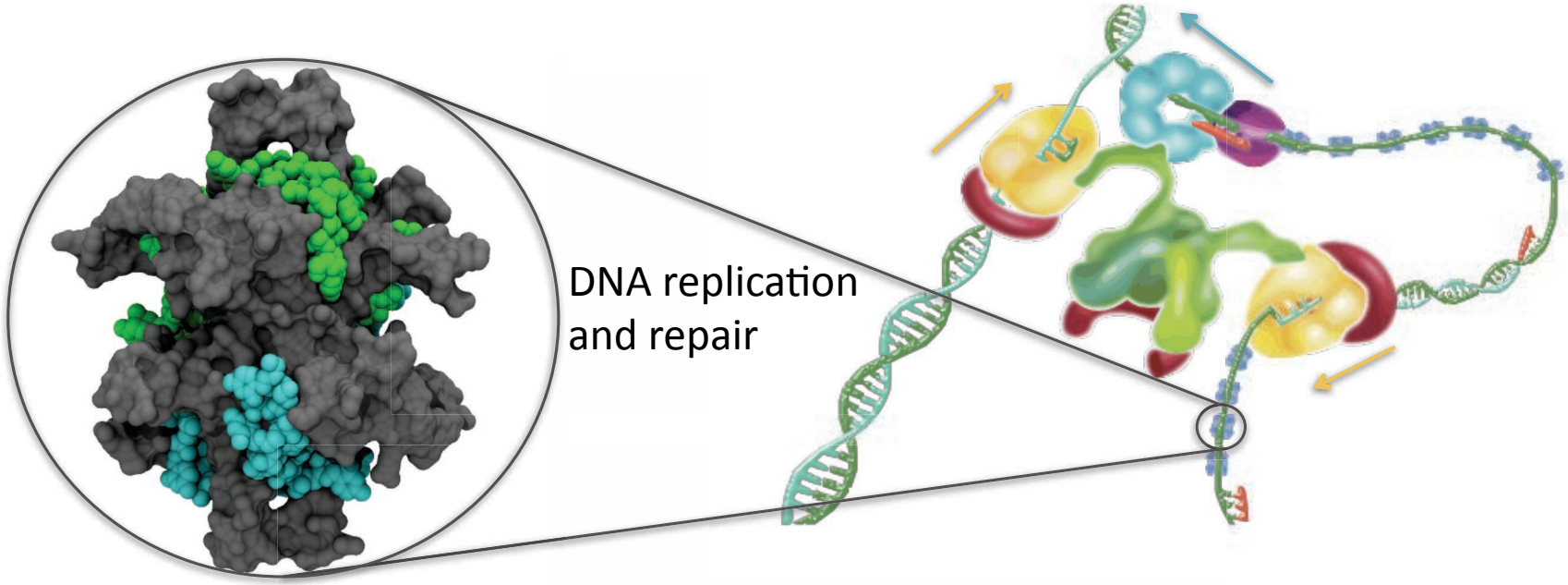
DNA systems



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

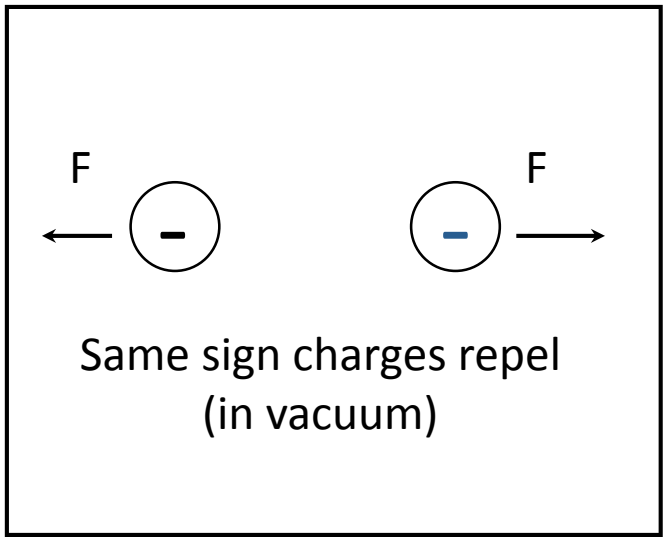


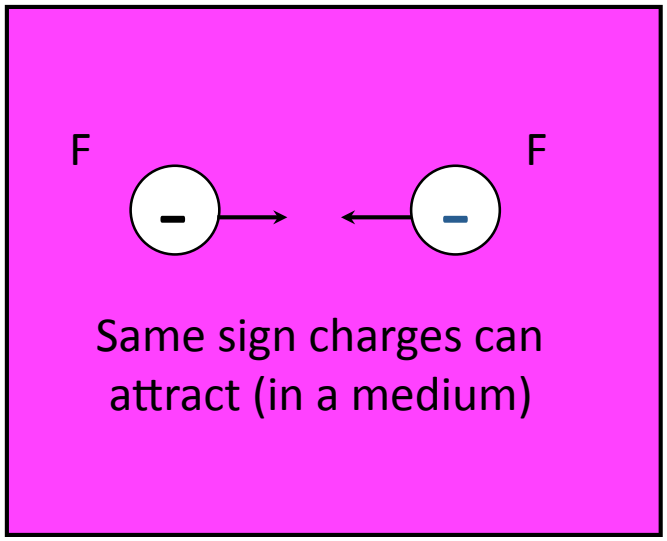
Graphene nanopore sequencing

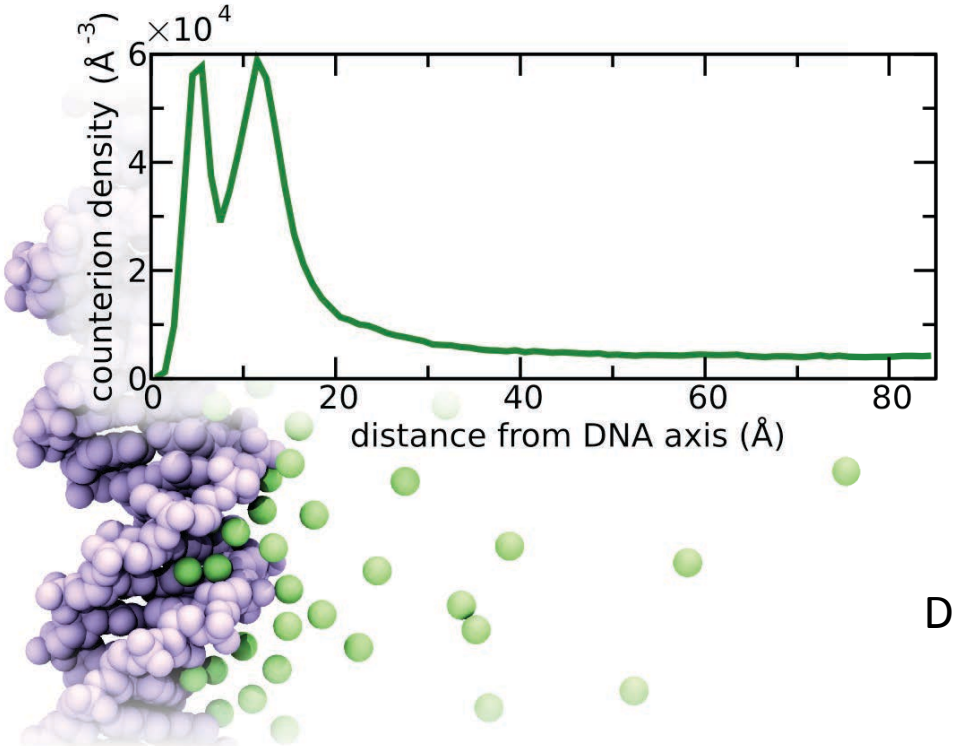


DNA replication and repair

Interesting physical properties





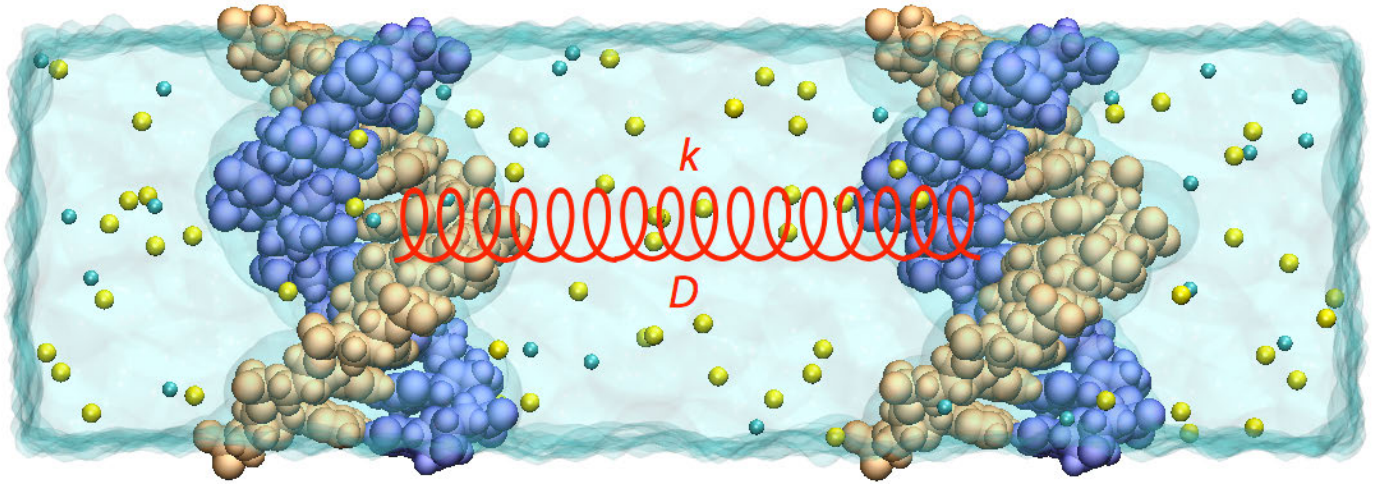


Effective attraction between DNA is observed when counterions have charge ≥ 2e

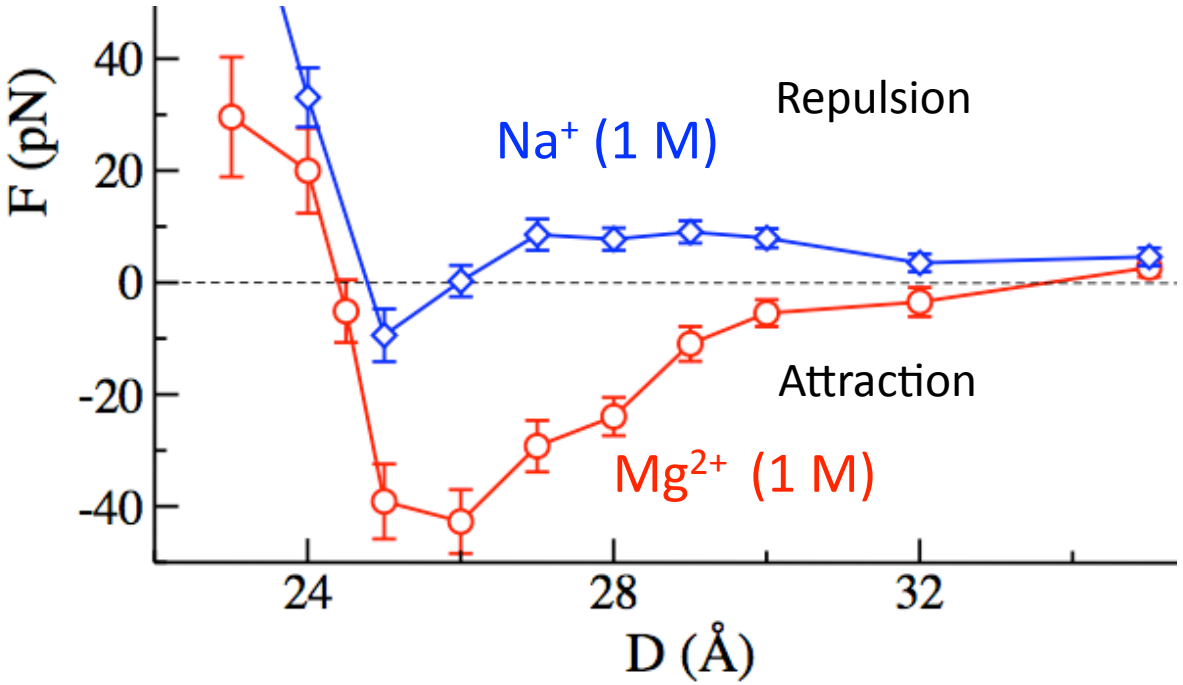
DNA lives in water and is surrounded by counterions

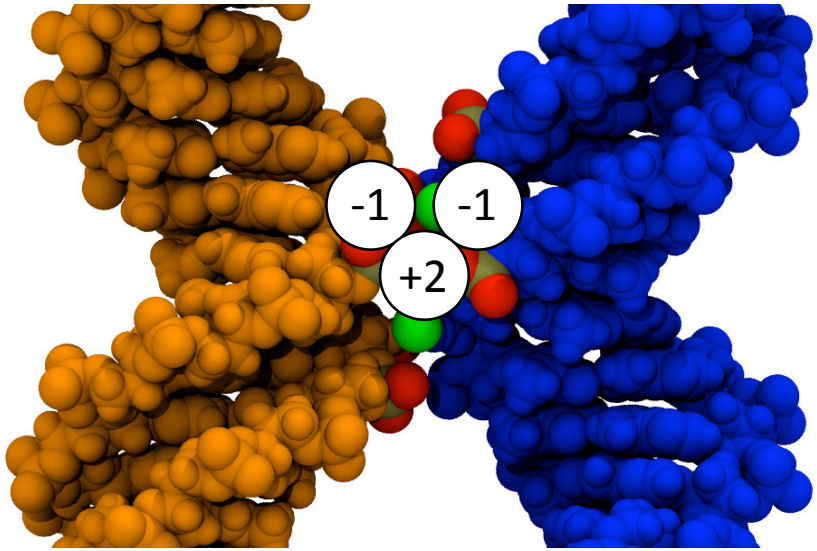
Direct MD simulation of DNA-DNA force

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

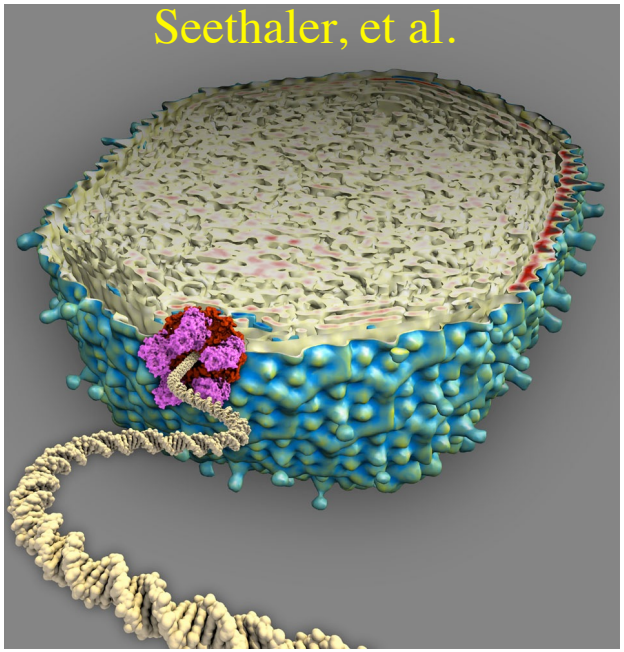


A virtual spring measures the effective force





MD simulation of dense DNA arrays



What we control

DNA density (or harmonic constraint radius)

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

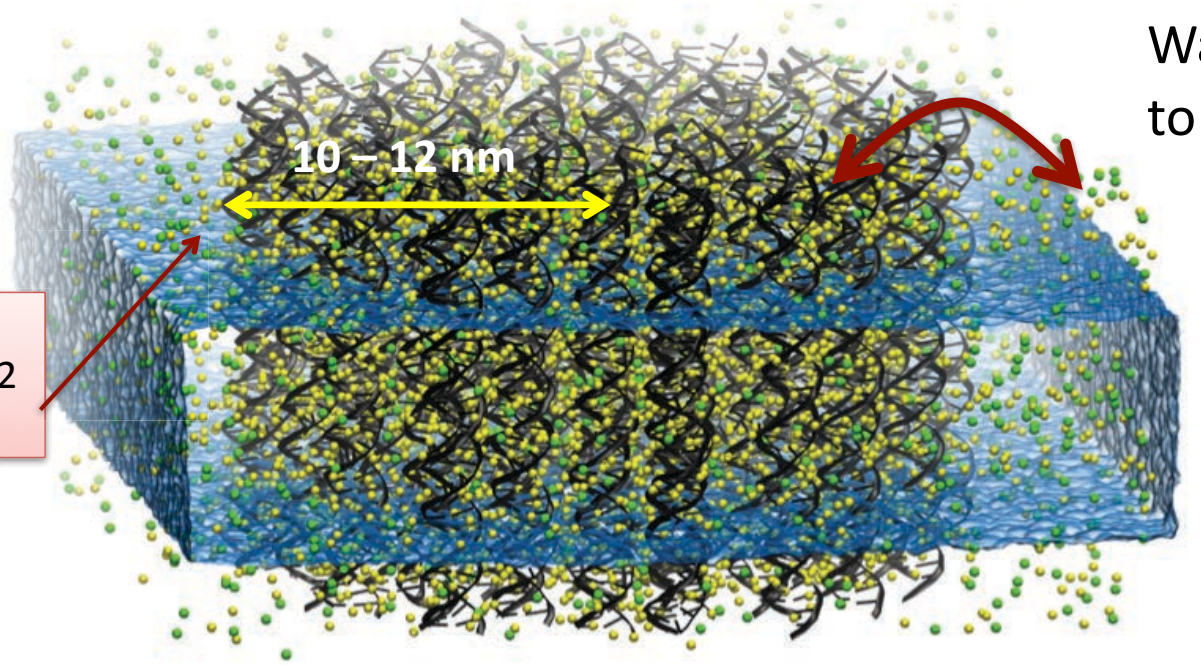
$[Mg^{2+}]_{buf}$ ~ 0 or 20 mM

What we measure

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

DNA / ion distribution:

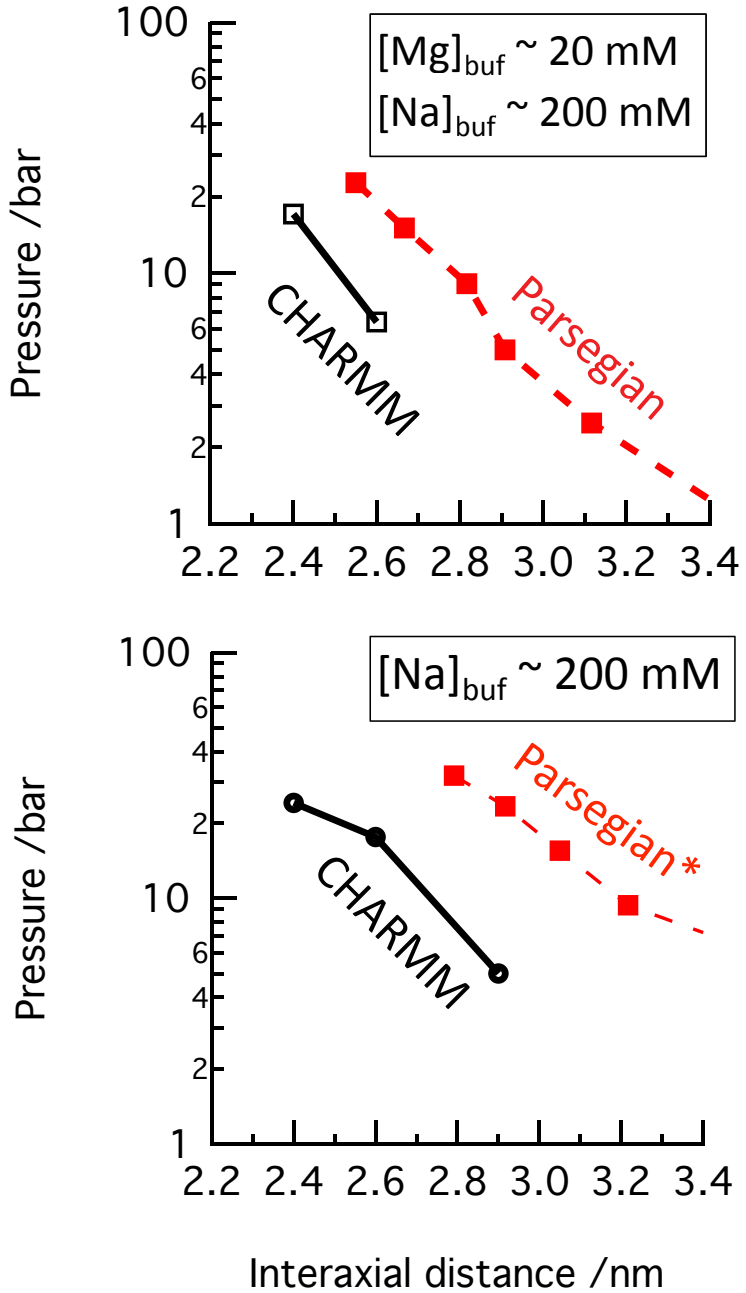
DNA / ion diffusion inside the array



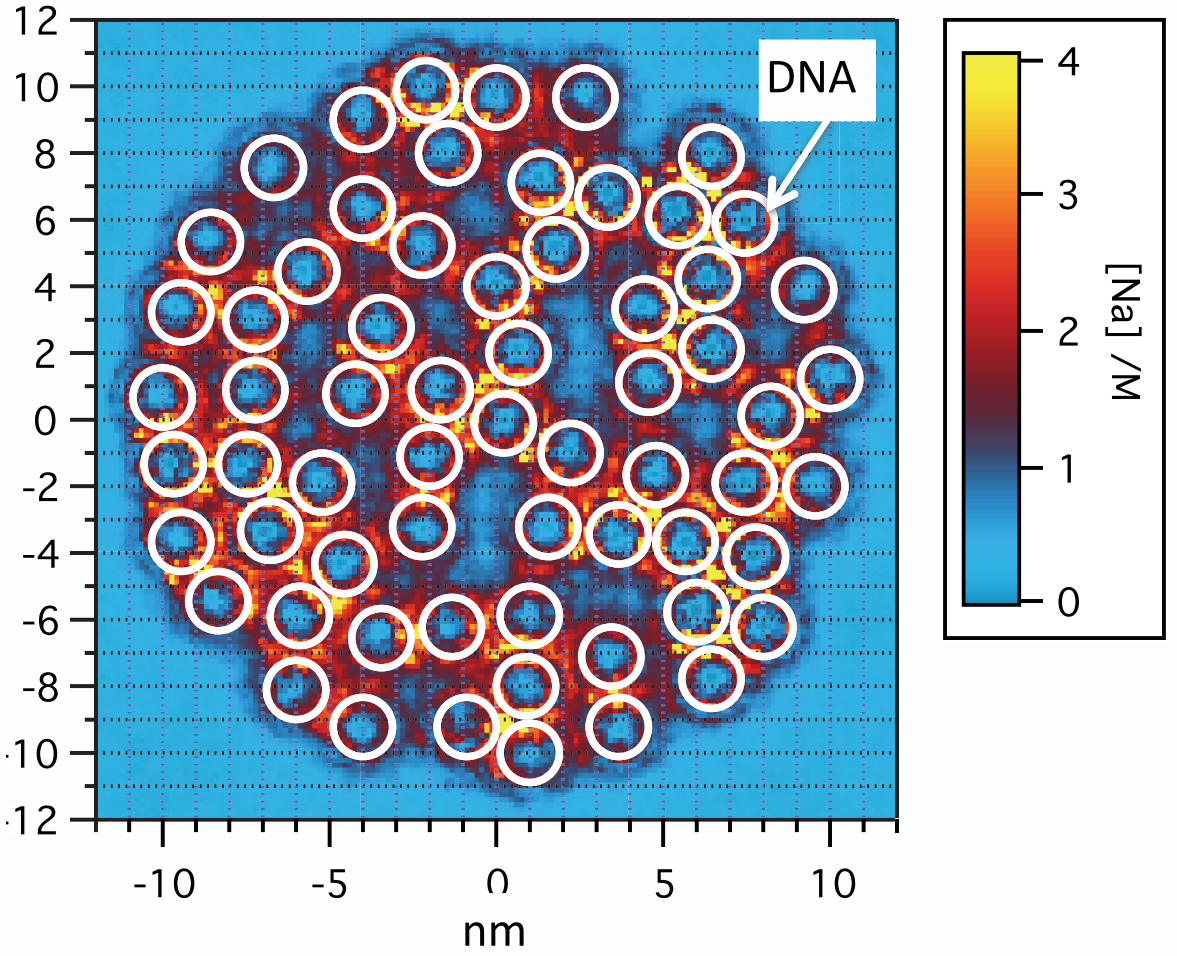
Water / salt free to move

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

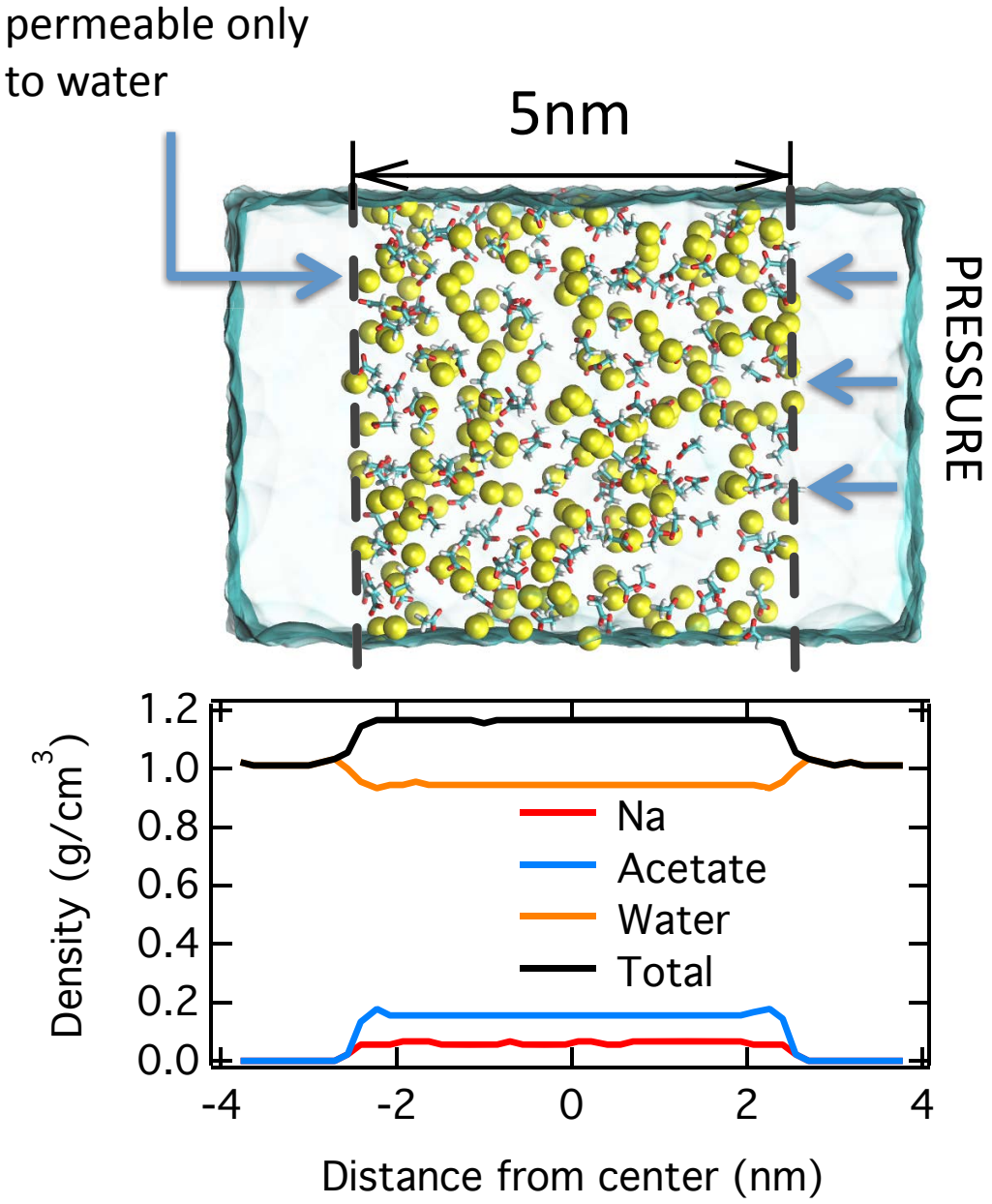


Too strong Na/Mg-phosphate attraction induces artificial DNA clusters!! [Na] ~ 4M!!



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

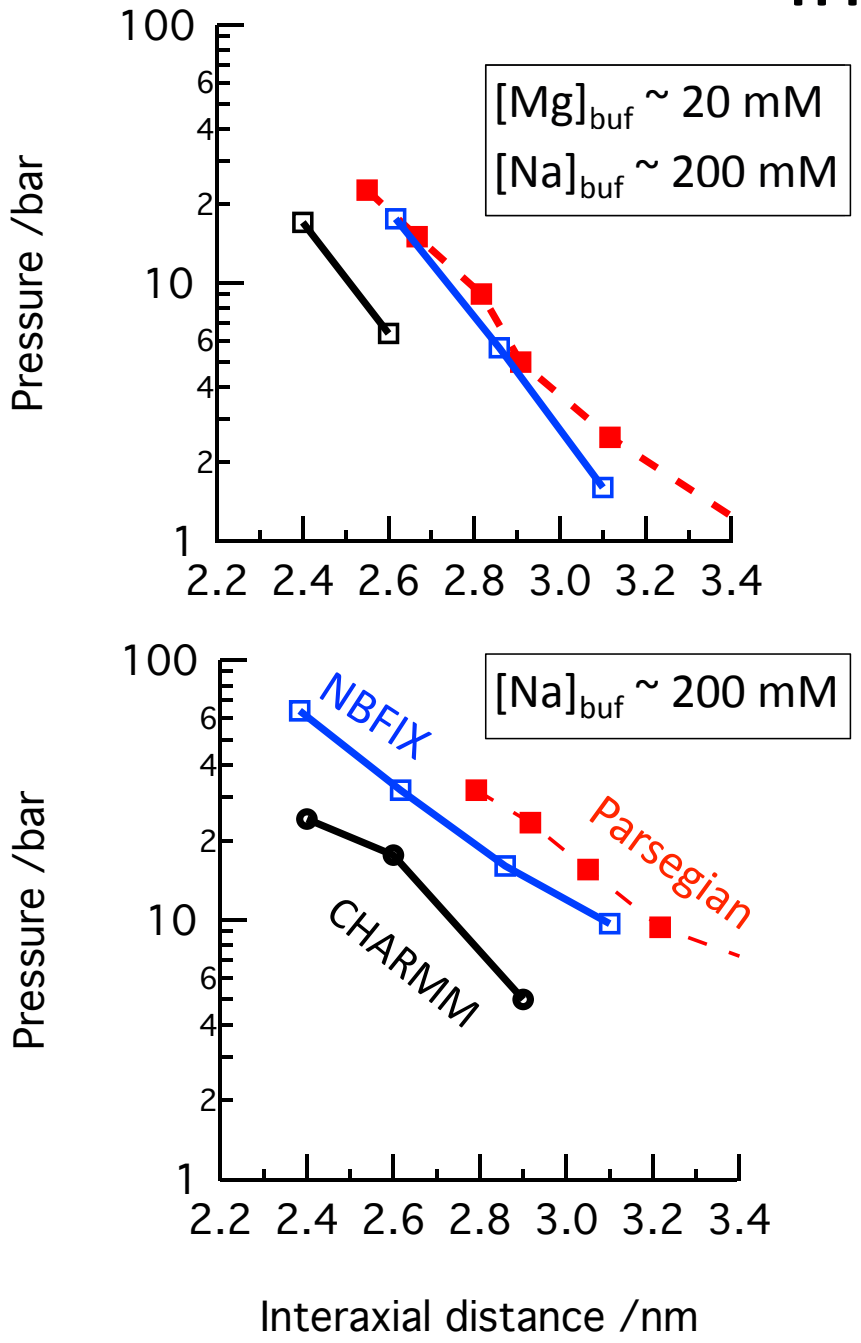
Recalibrate ion-DNA parameters using osmotic pressure data

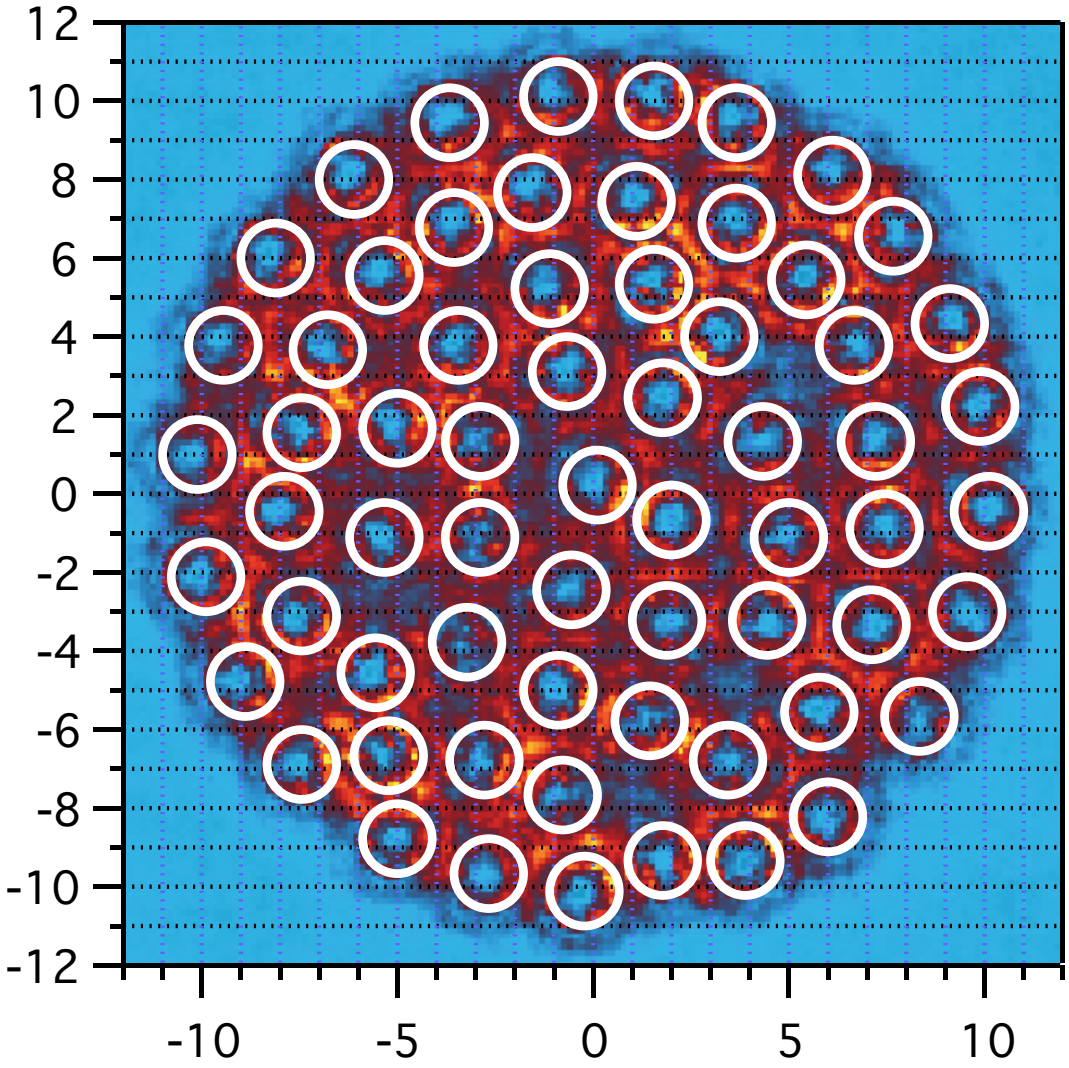


- Osmotic pressure is directly related to ion-pair formation: $\pi = \phi cRT$
- Pros: modify only ion-DNA phosphate interaction, without altering ion-water interaction.
- Cons: nothing.

* Luo & Roux, JPCL (2009)

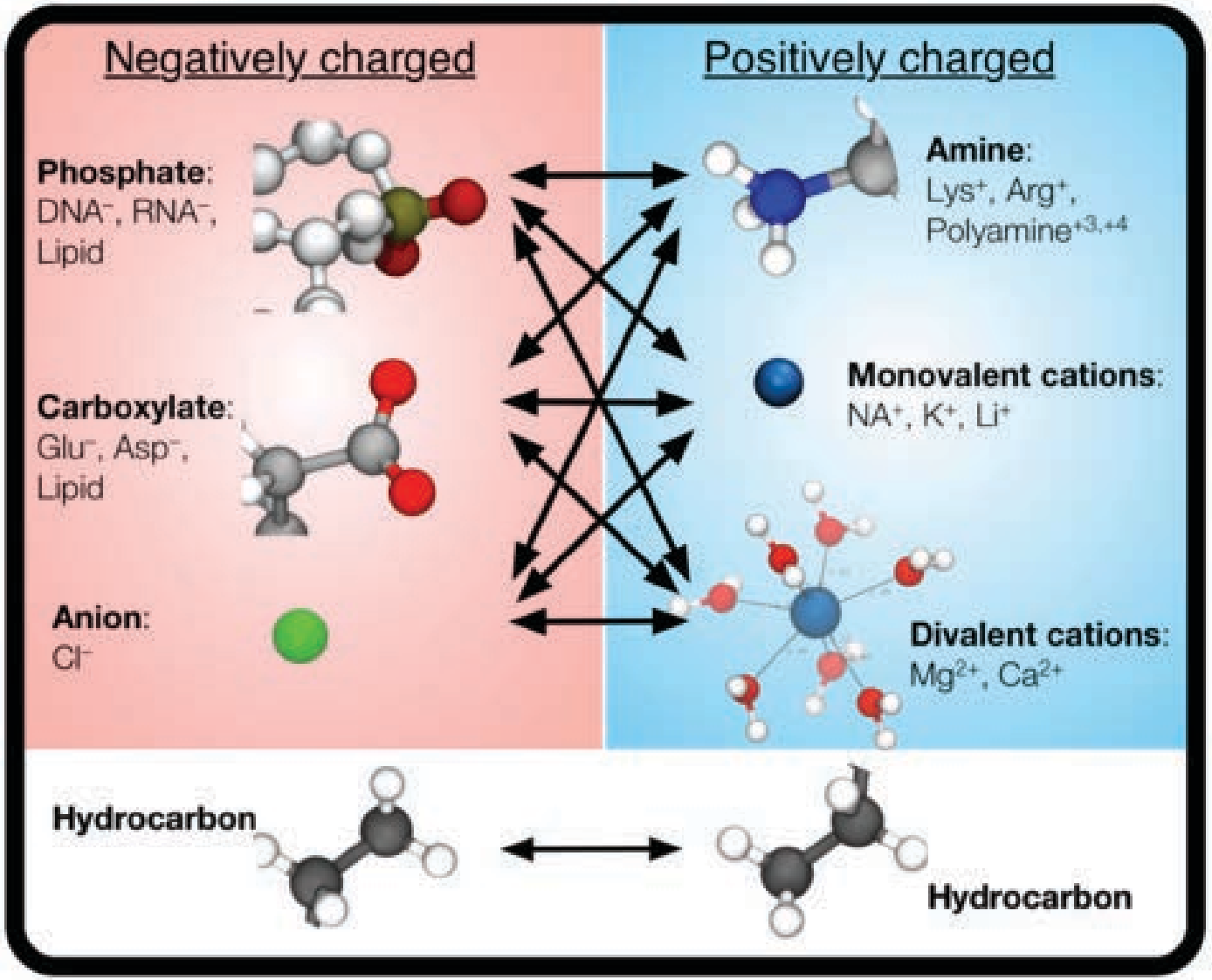
Improved parametrization of ion-DNA interactions





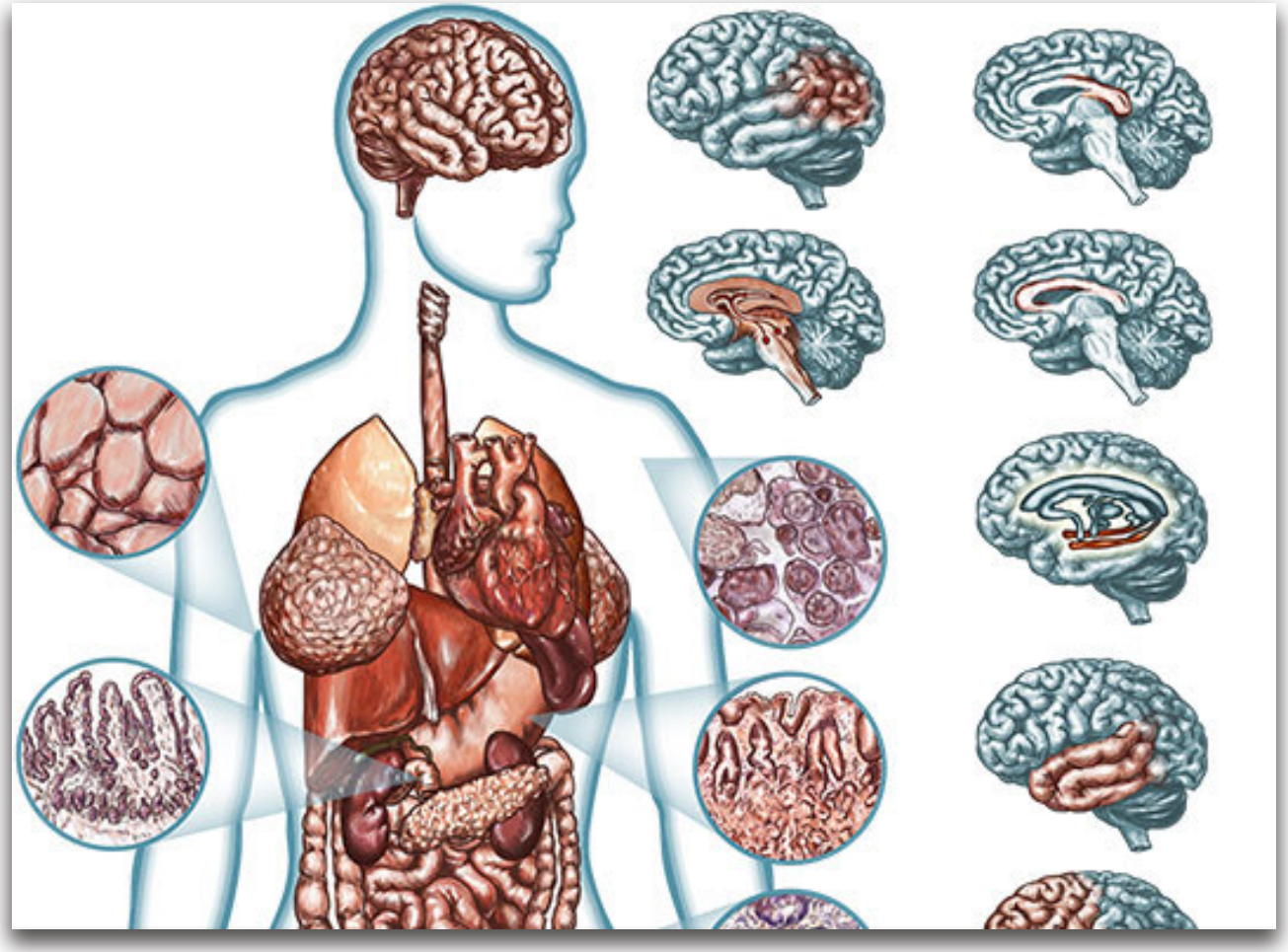
J. Phys. Chem. Lett. 3:45 (2012)

CUFIX: Accurate parameterization of non-bonded interactions



http://bionano.physics.illinois.edu/CUFIX

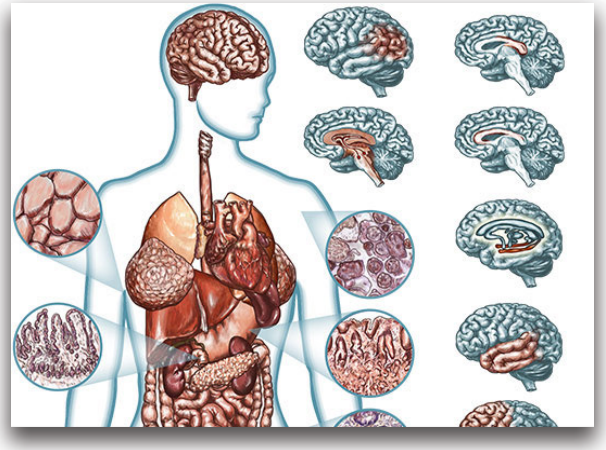
Cells with identical genome can have different functions

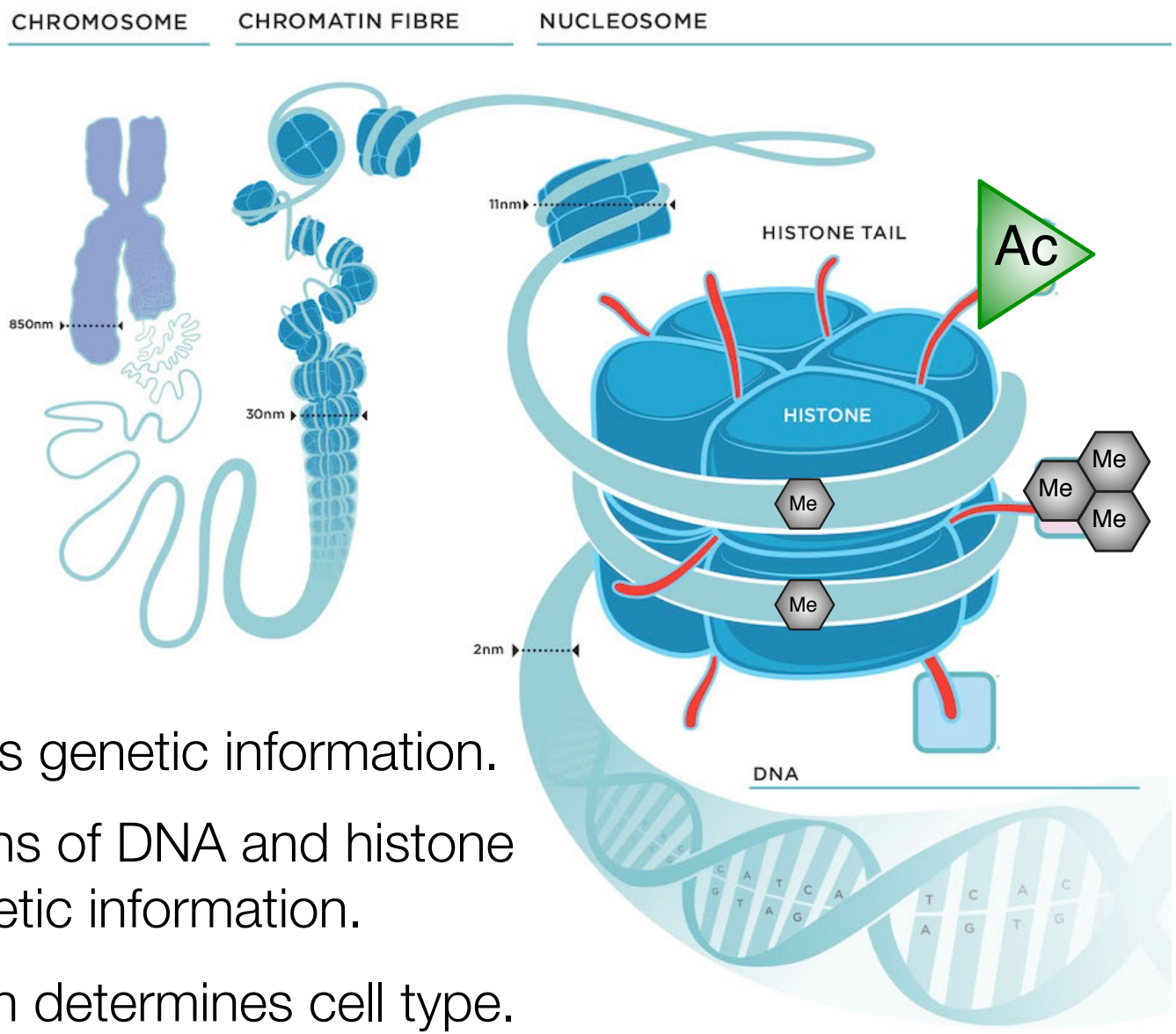


- All our cells contain identical genome sequence that defines ~20,000 genes.
- Depending on the cell type, different sets of genes are turned on.

http://www.roadmapepigenomics.org

Beyond DNA sequence: Epigenetic regulation

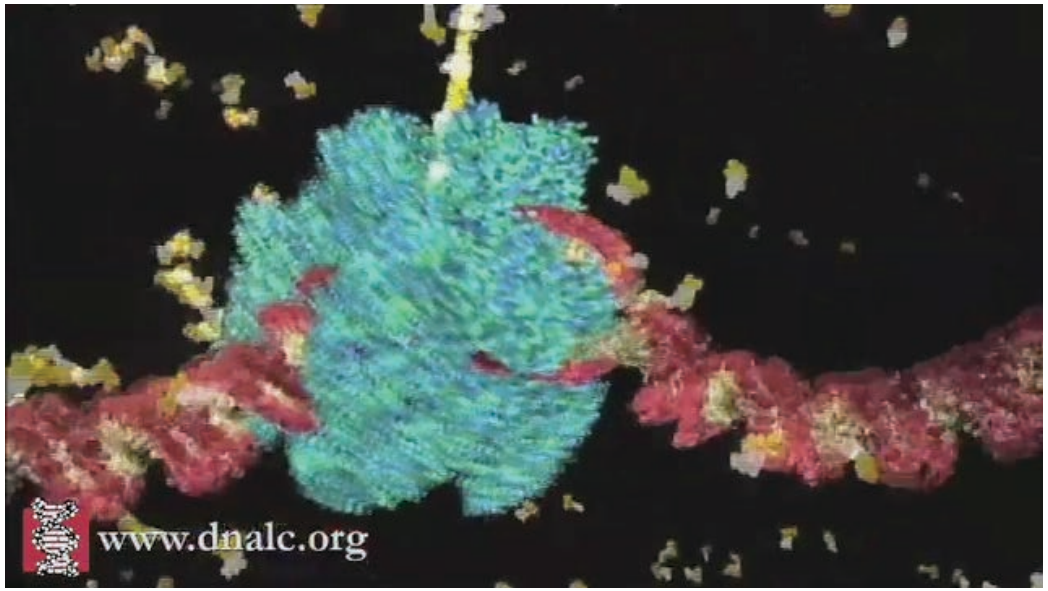


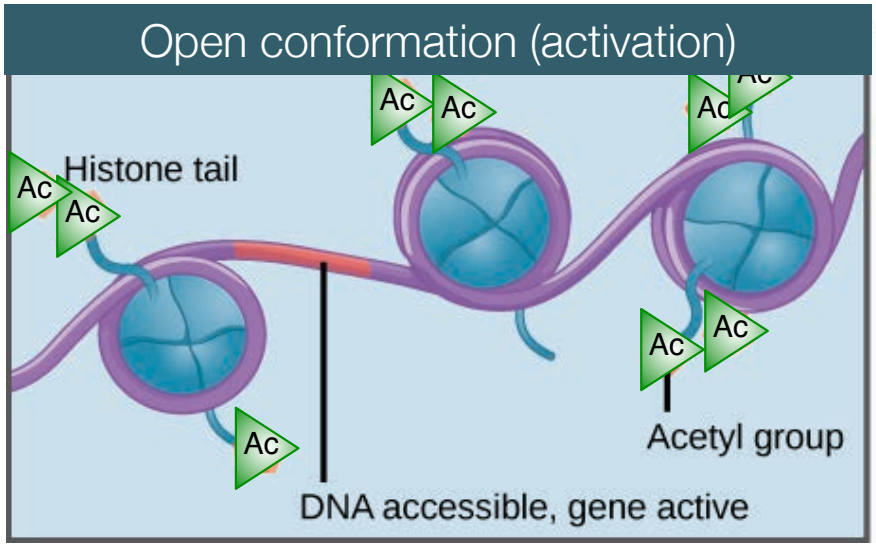


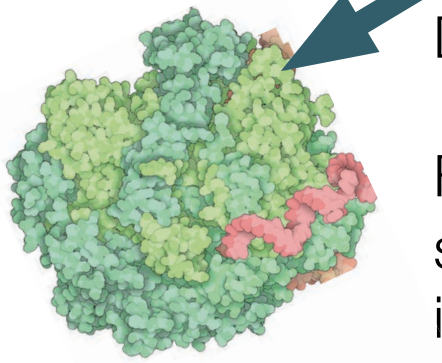
- DNA sequence carries genetic information.
- Chemical modifications of DNA and histone proteins carry epigenetic information.
- Epigenetic information determines cell type.

http://www.resverlogix.com/programs/rvx-208-moa/epigenetics

DNA compaction regulates gene transcription

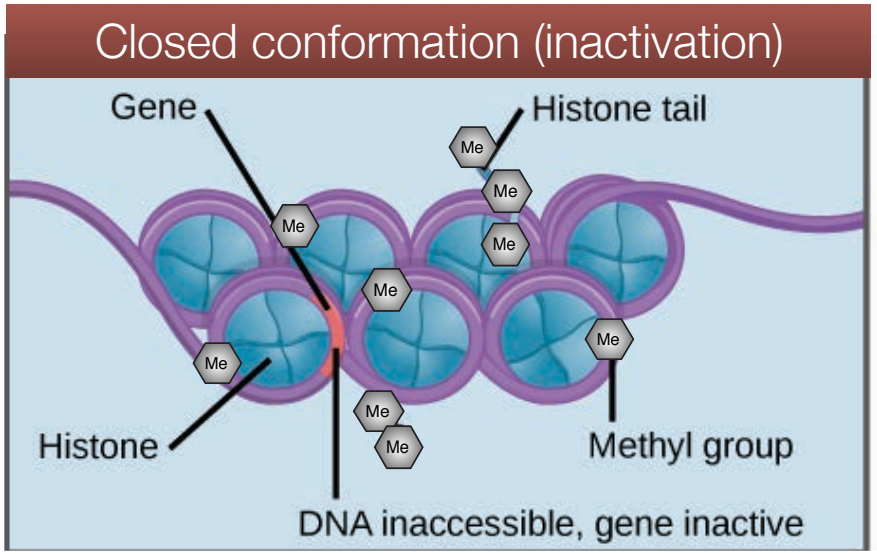






RNA polymerase reads DNA sequence.

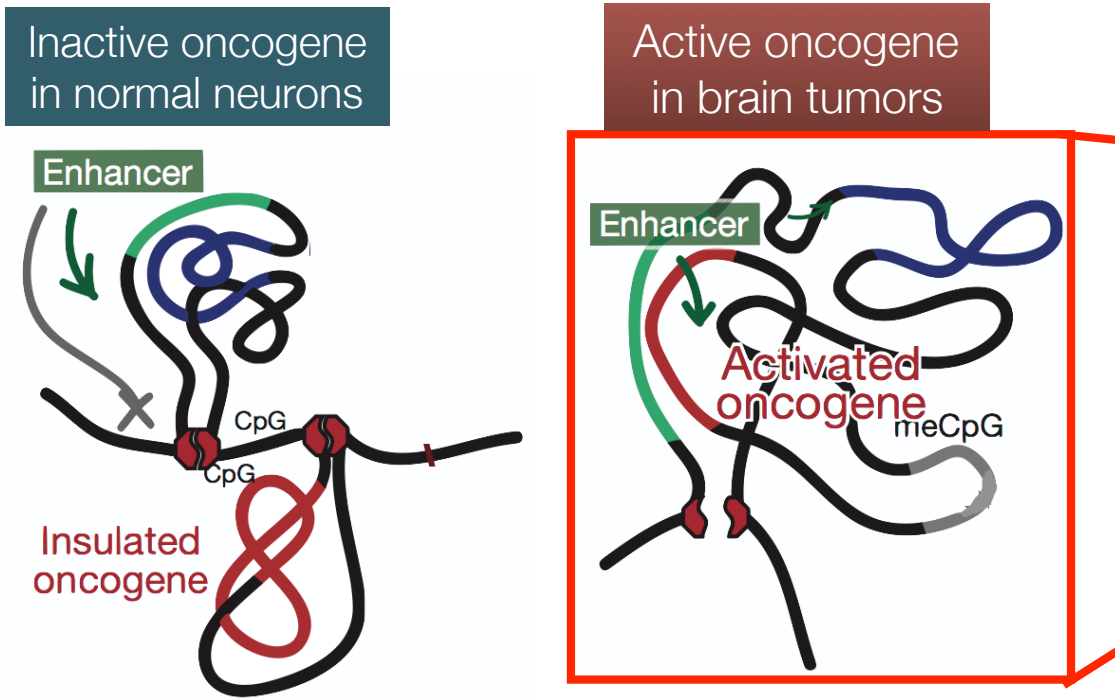
For a given cell, only a subset of ~20,000 genes is accessible to RNA polymerase.



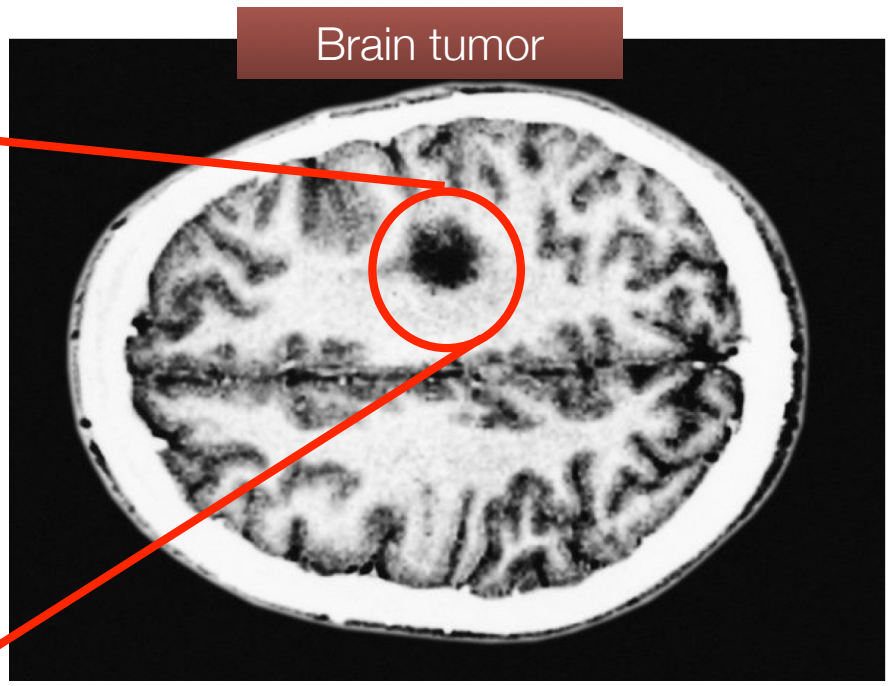
Gibcus & Dekker, Mol Cell 49:5, 773-82

http://bio1510.biology.gatech.edu/module-4-genes-and-genomes/4-7-gene-regulation/

DNA organization in chromosome is a key to understanding development and disease

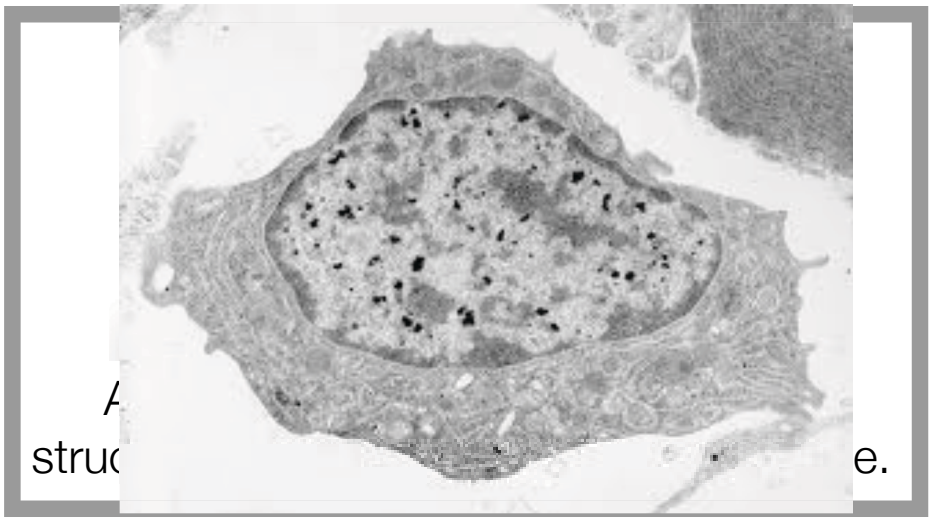


Bernstein and coworkers, *Nature* (2015)



Highlight in New York Times on Dec 23, 2015

- 3D organization of chromatin changes dynamically depending on **developmental** & **aging** stages.
- **Cancer** cells also have their own chromatin organization.



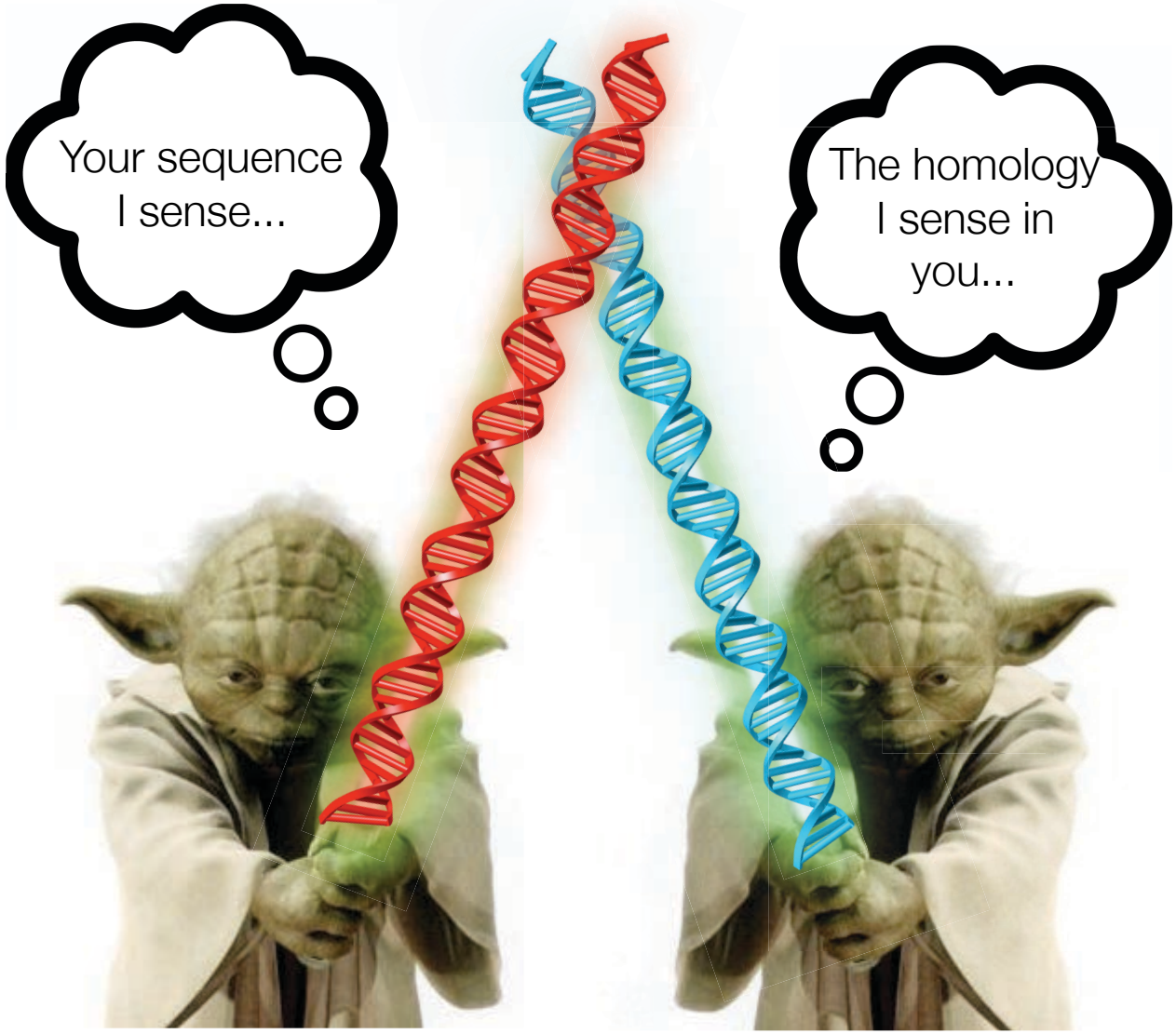
A
struc e.

0.001


DNA senses the
sequence of neighbors

DNA telepathy (?!)

Jejoong Yoo



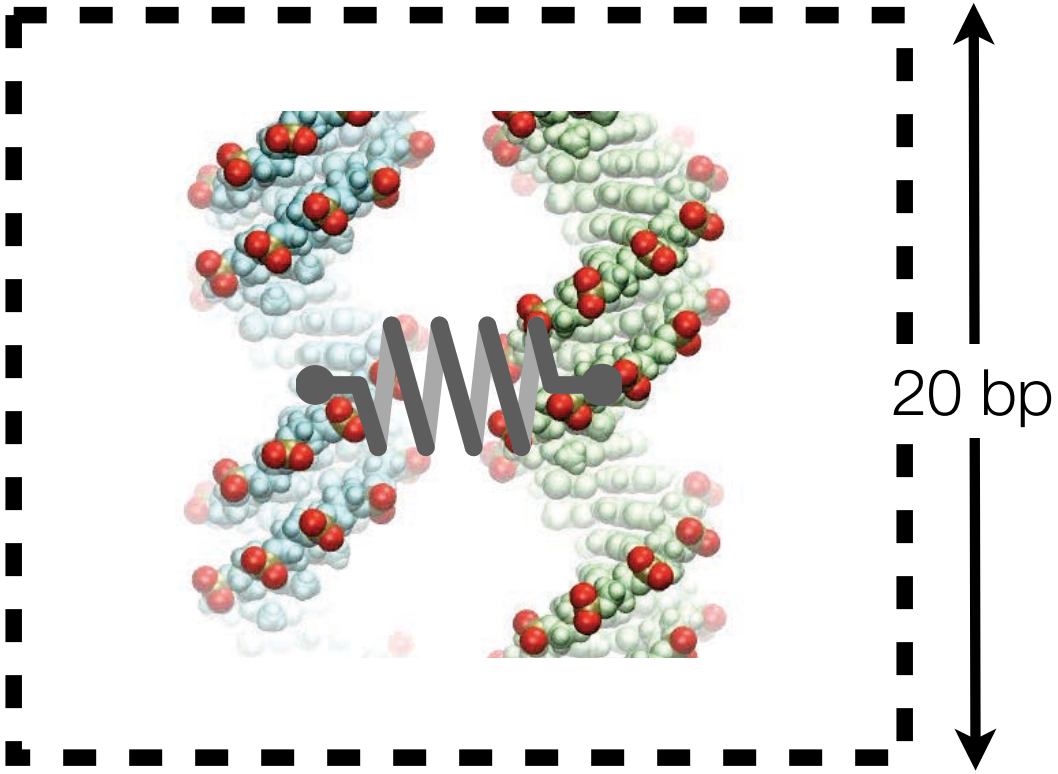
Leikin and Kornyshev

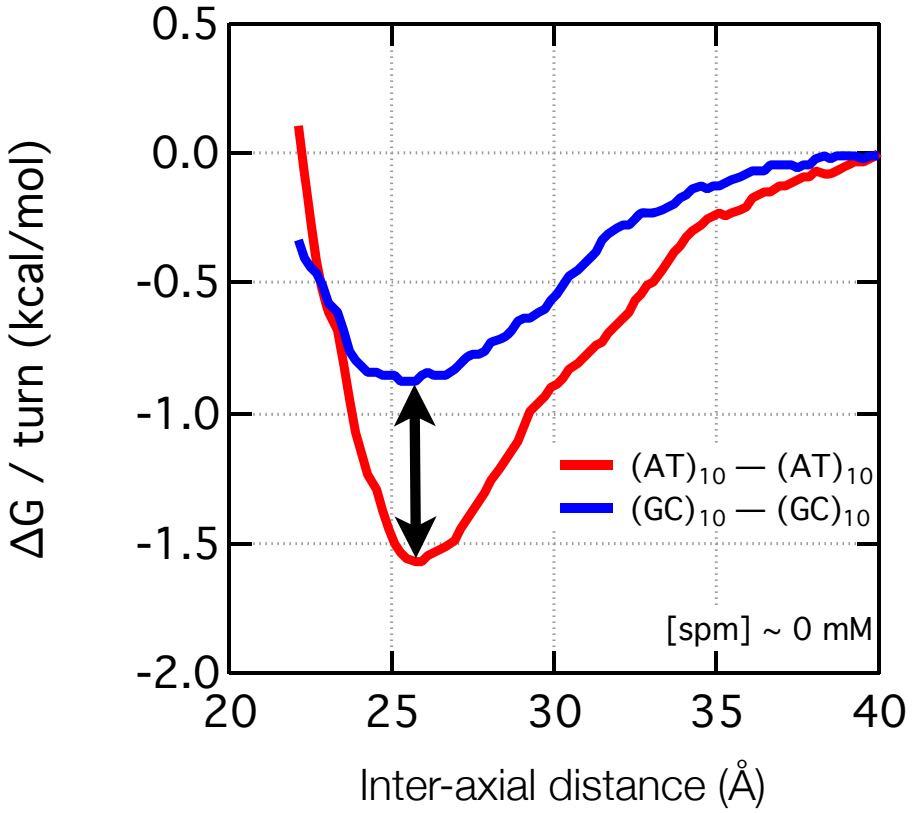
Journal of Physical Chemistry B (2008)

DNA can recognize complementary sequence without any mediator protein

- **DNA telepathy** means sensing and colocalization of homology sequences
- But, often debated due to lack of evidence.
- We validate this hypothesis.

Free energy calculations predict **stronger attraction** for DNA molecules having **higher AT contents**

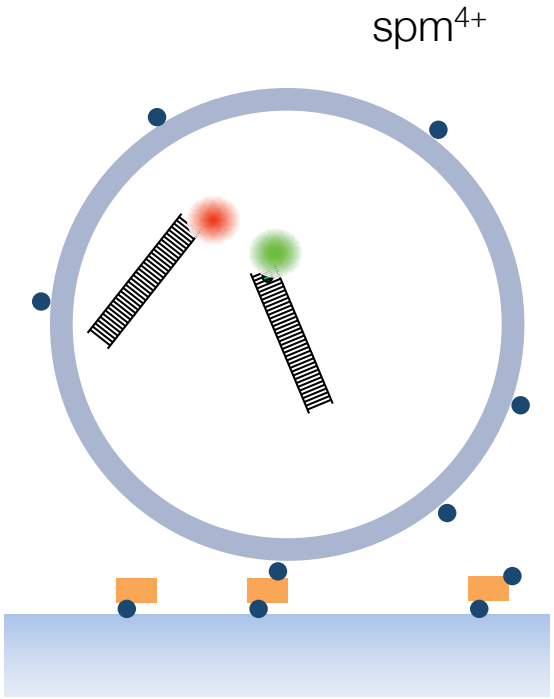


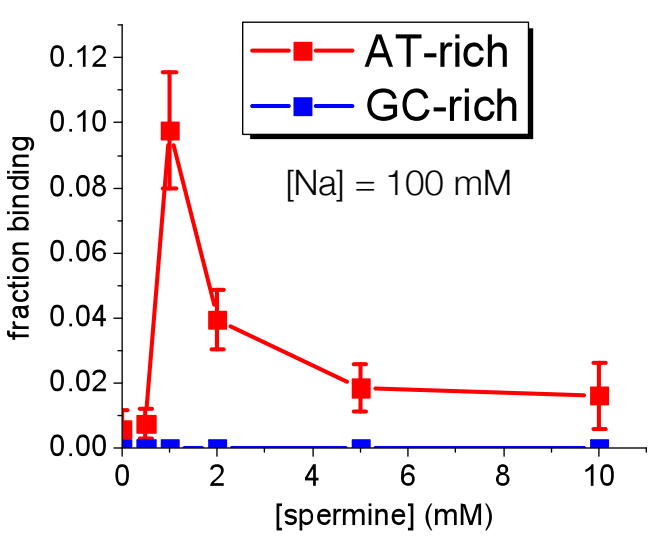


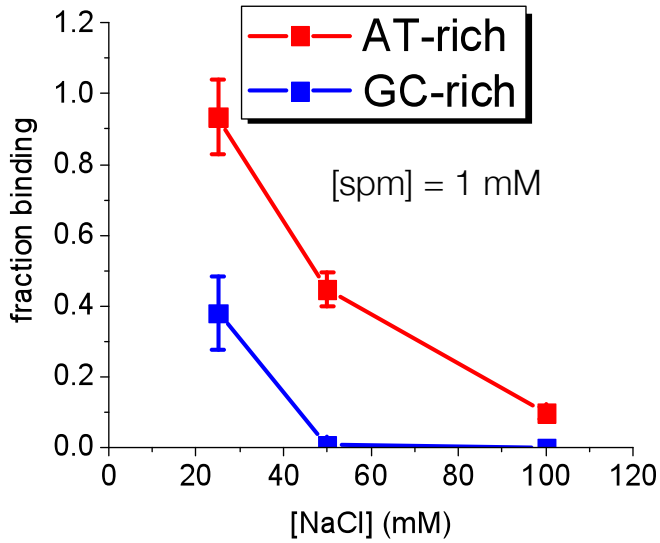
- AMBER99bsc0 force field with custom refinement of amine-phosphate interactions.
- Sub-mM level of spermine; just enough to neutralize DNA.
- Fully atomistic including explicit water.
- Umbrella sampling.

FRET experiments confirm MD predictions

Hajin Kim (Ha group)



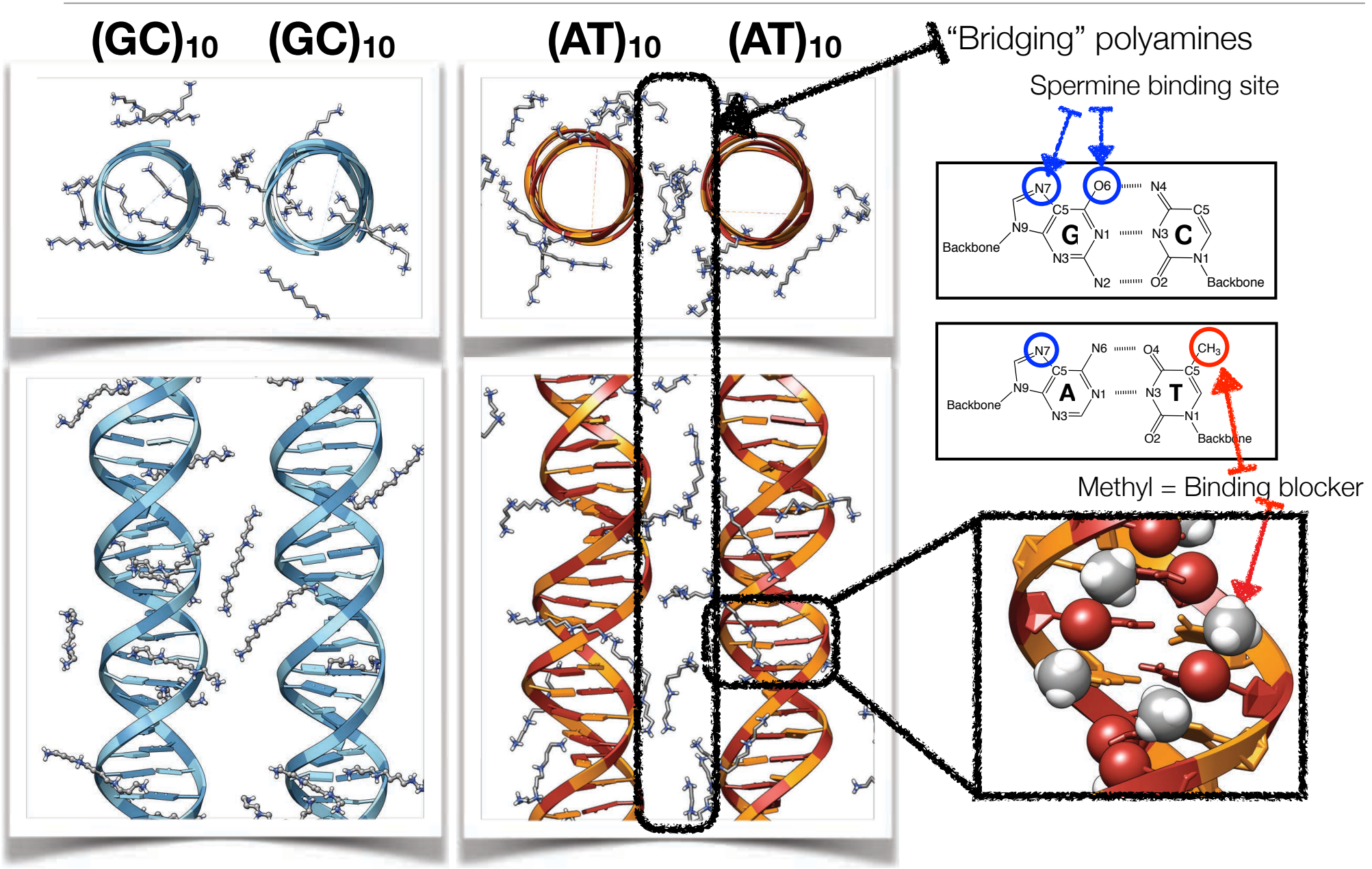




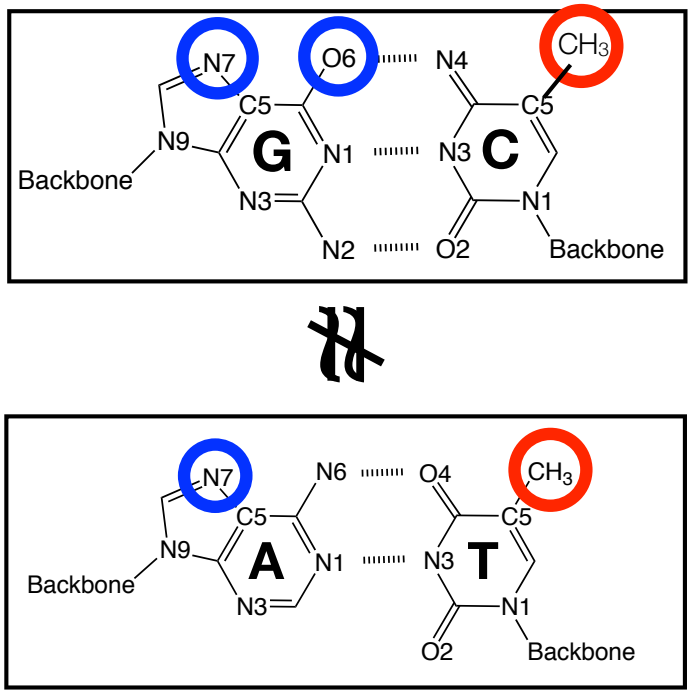
- FRET signal indicates binding of two double stranded DNA

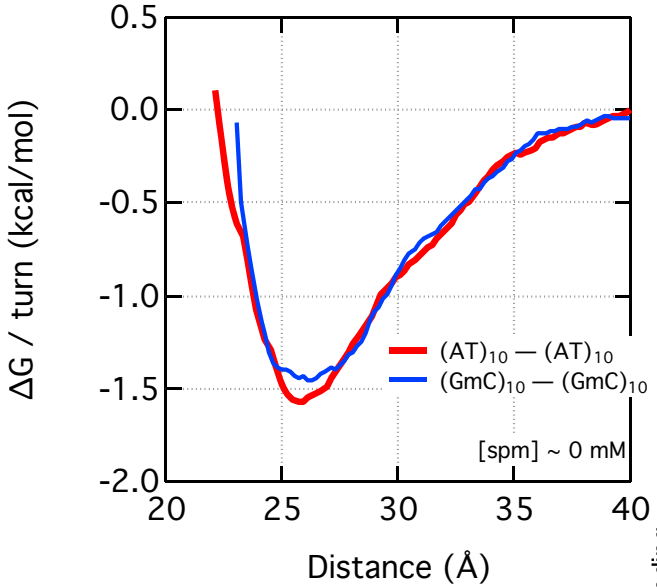
Center for the Physics of Living Cells

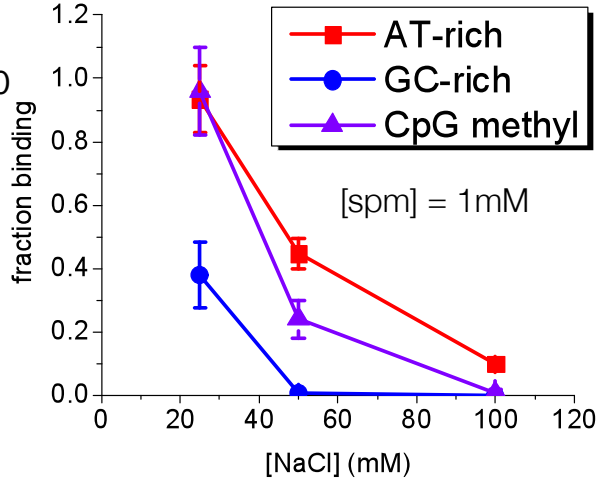
AT-rich segments form **clusters** better because they **share** polyamines with neighbors



Methylated CpG is as attractive as AT

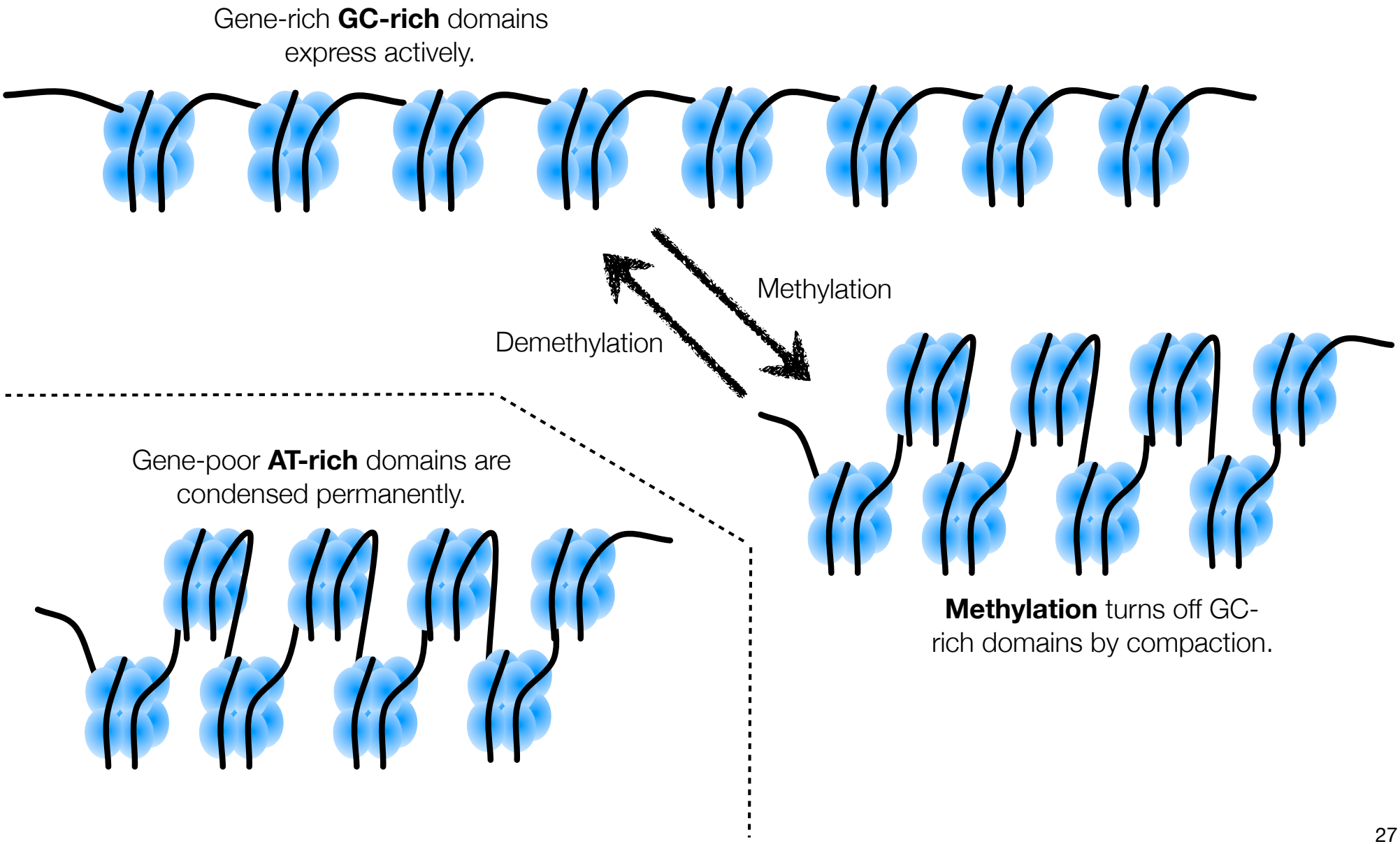




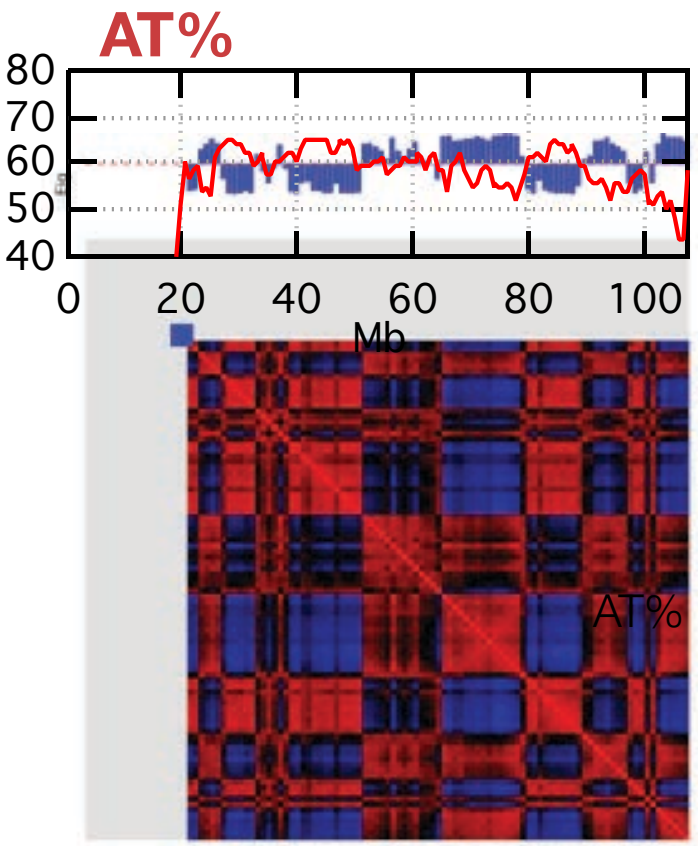


- Possible epigenetic gene inactivation mechanism ?

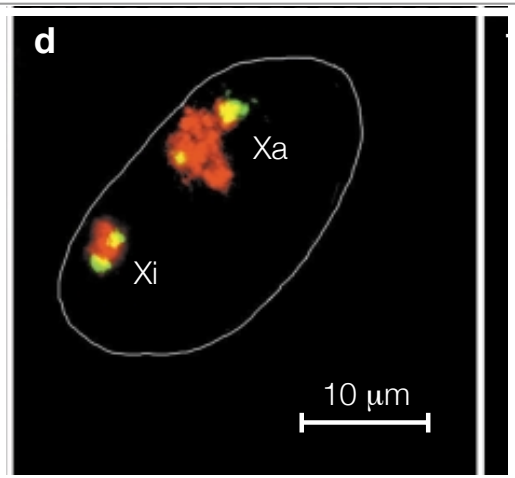
Implications to chromatin folding & gene regulations



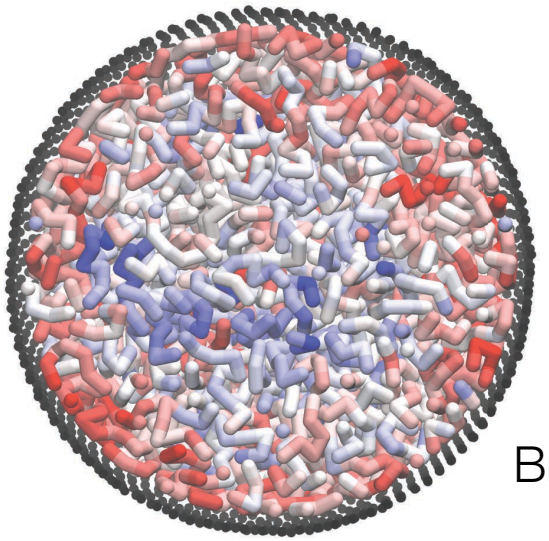
Implications to chromatin folding & gene regulations

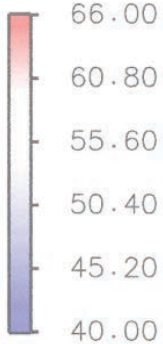


chromosome 14



Highly methylated inactive X (Xi) chromosome is more compact than active X (Xa).





AT%

- 1 bead = 1 Mb.
- Inter-bead interactions depend on AT-content

Brownian dynamics simulations of chromatin folding in nucleus

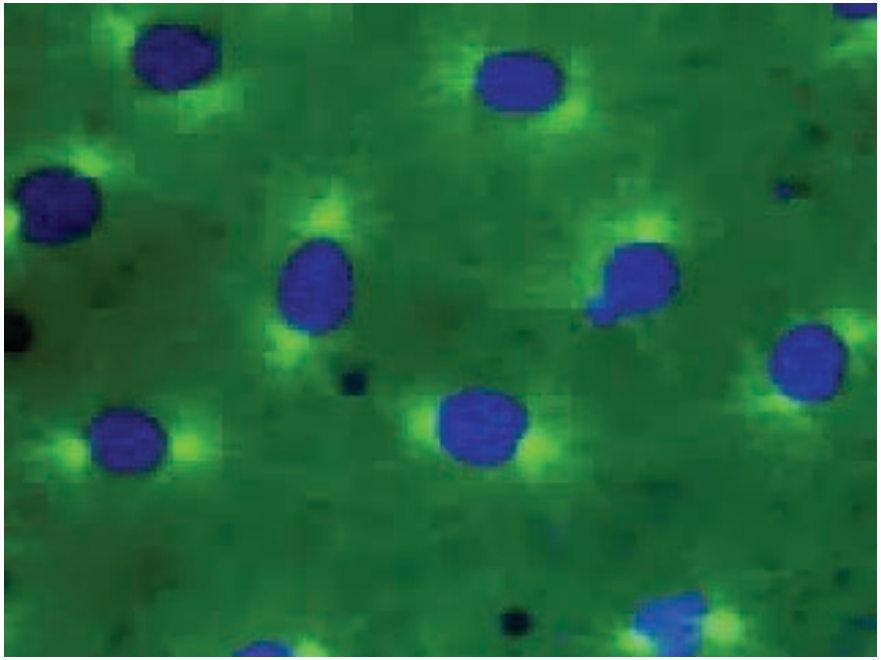
Baù, D. et al., *NSMB*, 18(1), pp. 107-14.

Cremer, T. & Cremer, C., 2001,, *Nat Rev Genet*, 2(4), pp. 292-301.

Bickmore, W.A. & van Steensel, B., 2013, *Cell*, 152(6), pp. 1270-84.

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

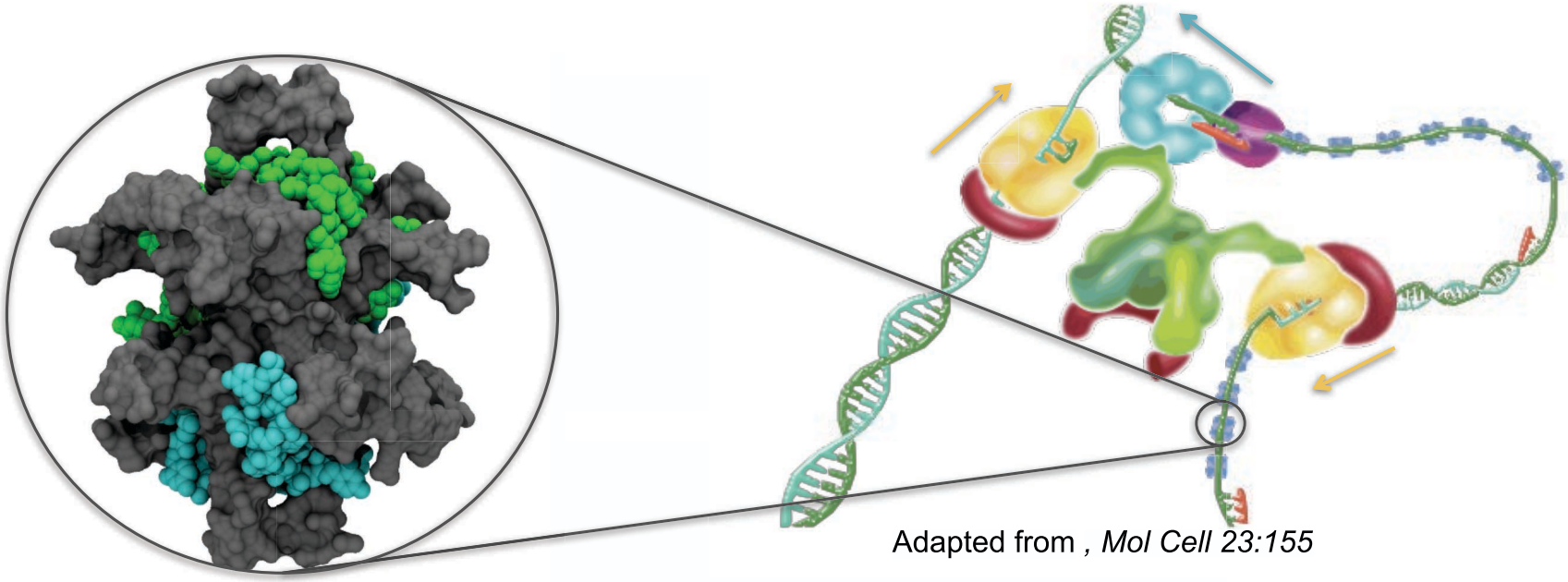
Adapted from Mol. Cell 23:155

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



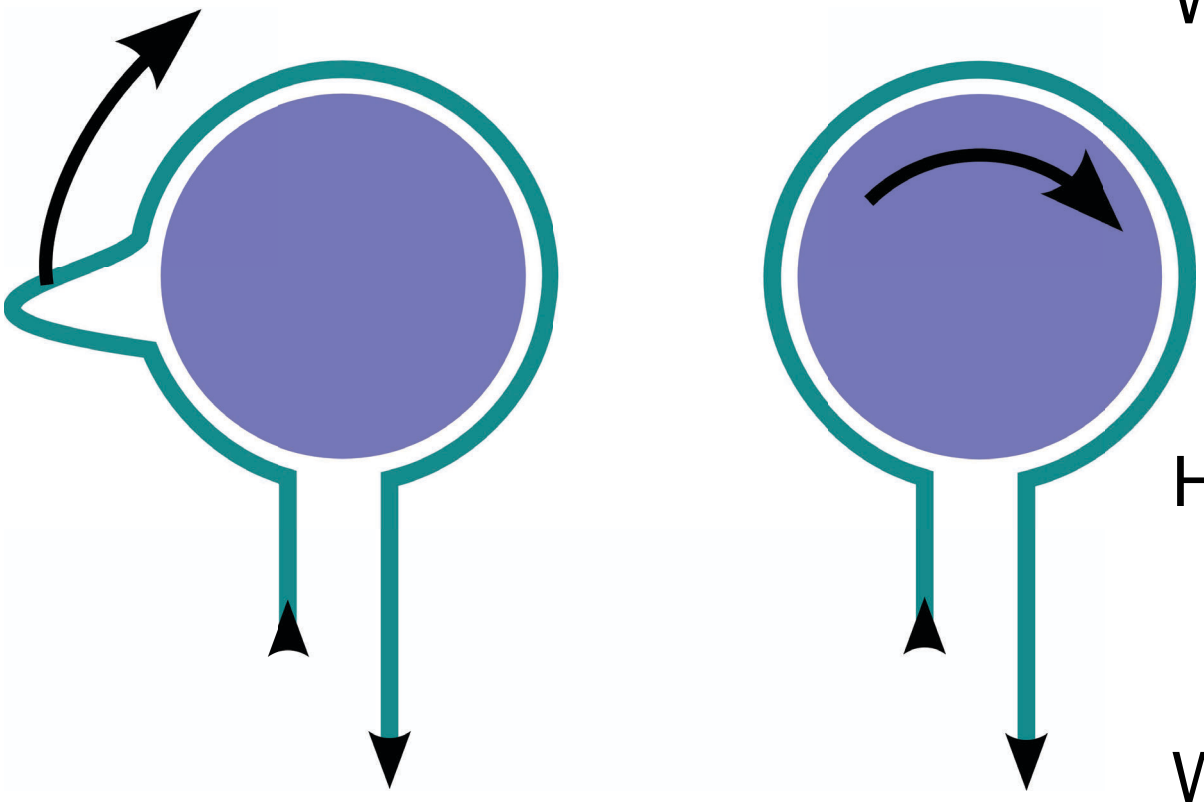
Adapted from , *Mol Cell 23:155*

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?

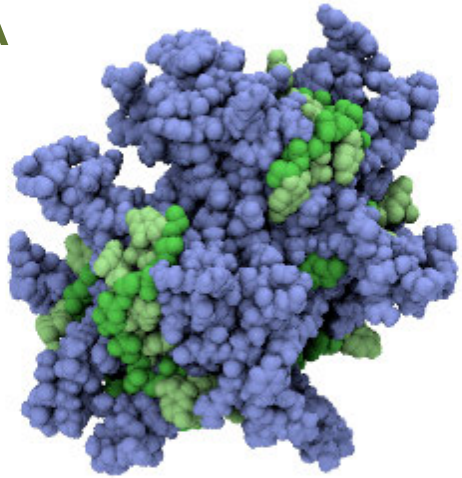
How does dissociation of DNA from SSB occur?

What makes an ssb an ssb?

† Ha group, Nature 461:1092

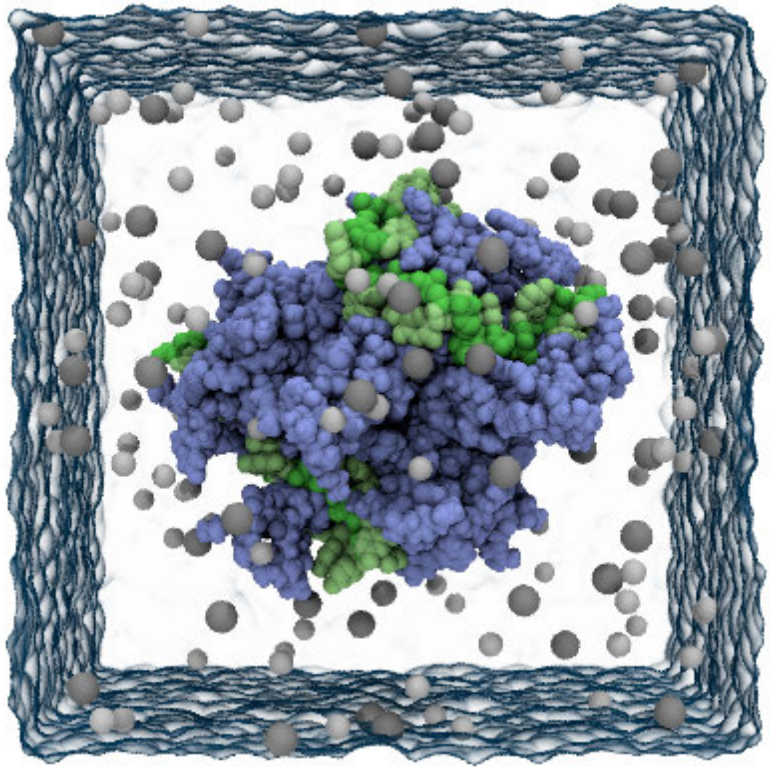
A model is build from an x-ray crystal structure

SSB
DNA



PDB:1EYG

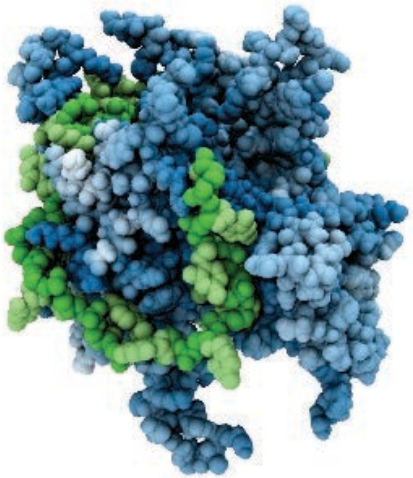
KCl
water

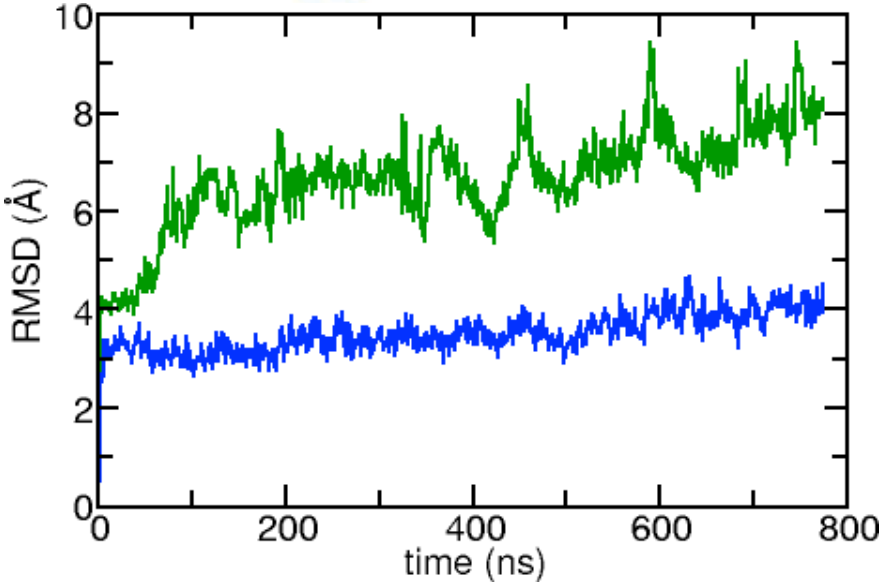


Unresolved DNA was modeled by the crystallographers (Lohman and Waksman groups, Washington U. School of Medicine) and provided to us via Ruobo Zhou of the Ha group.

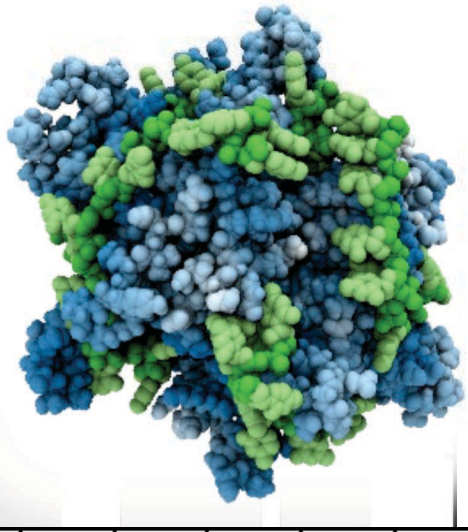
Individual nucleotides are loosely bound to SSB

SSB_{35}

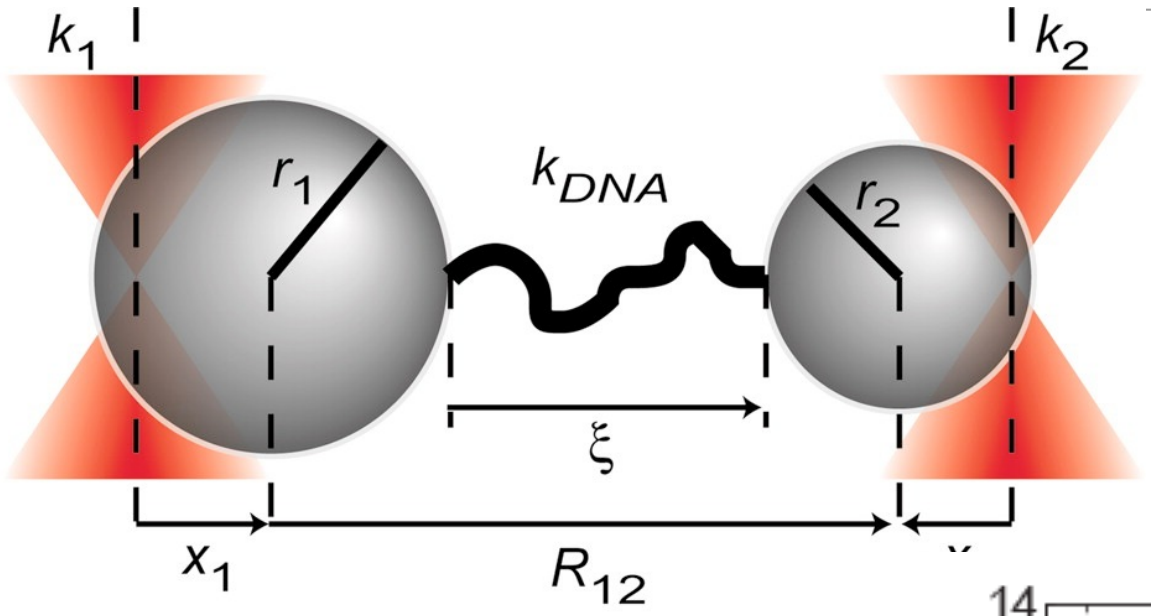


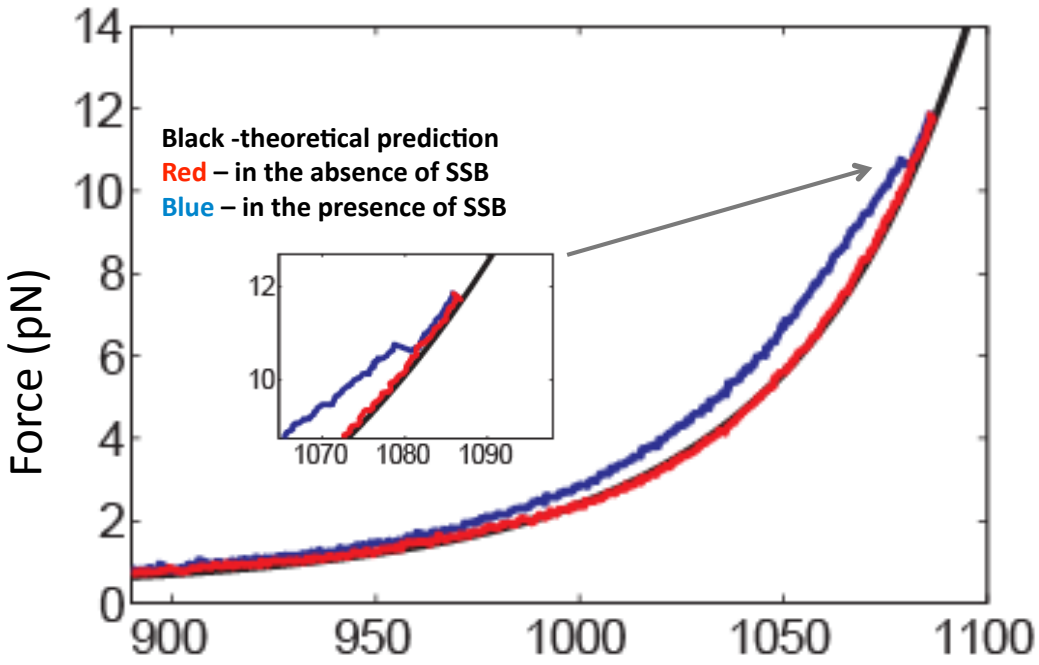


SSB_{65}



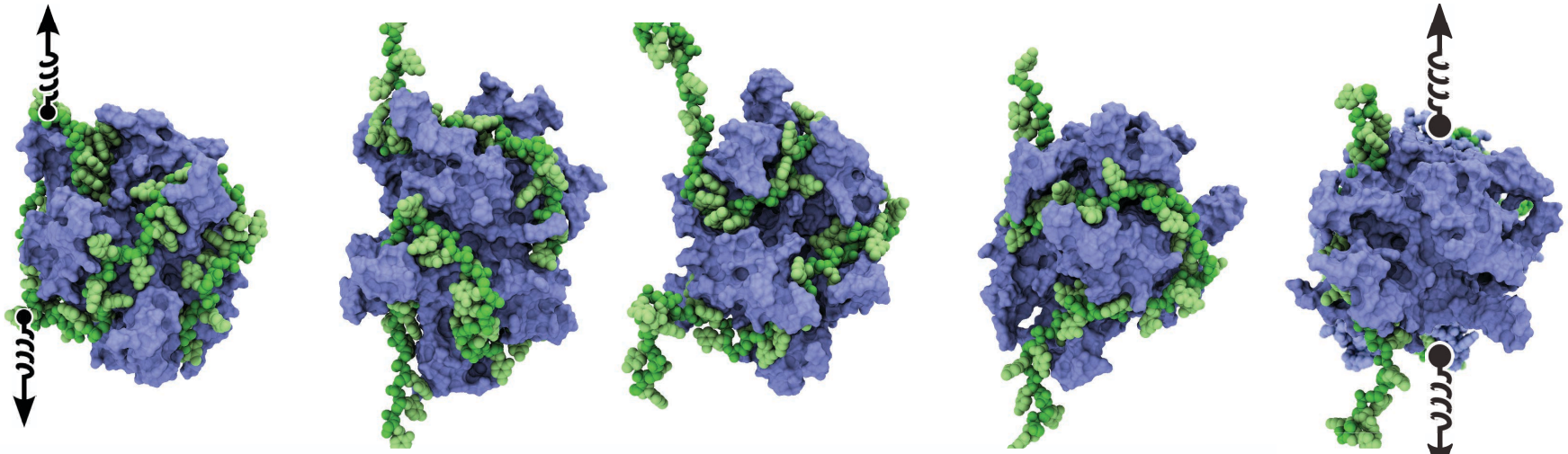
Mechanics of ssb-DNA (dis)assembly

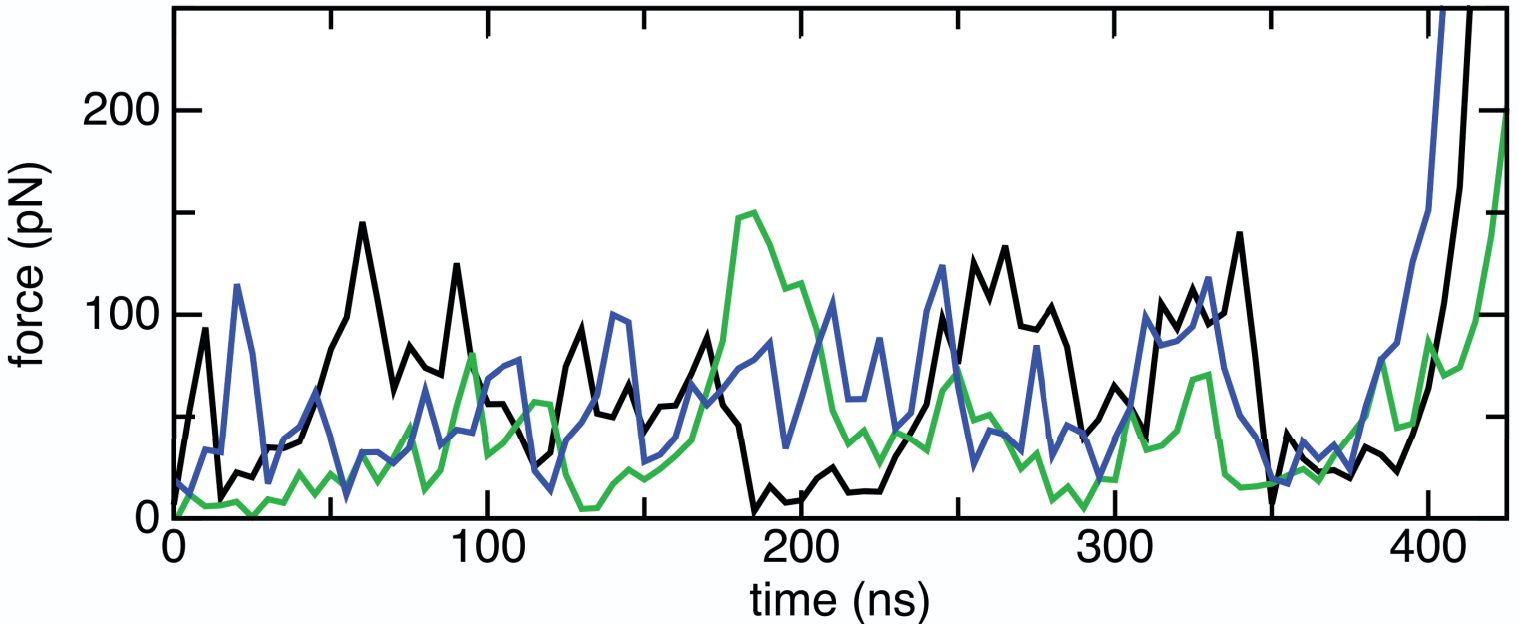




Chemla group, unpublished

All-atom simulations cannot quite reach experiment

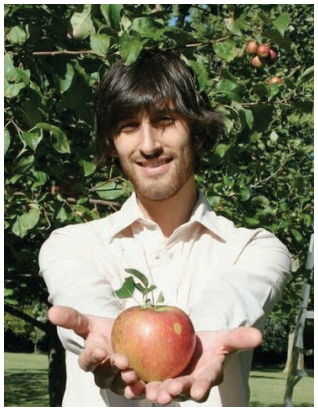




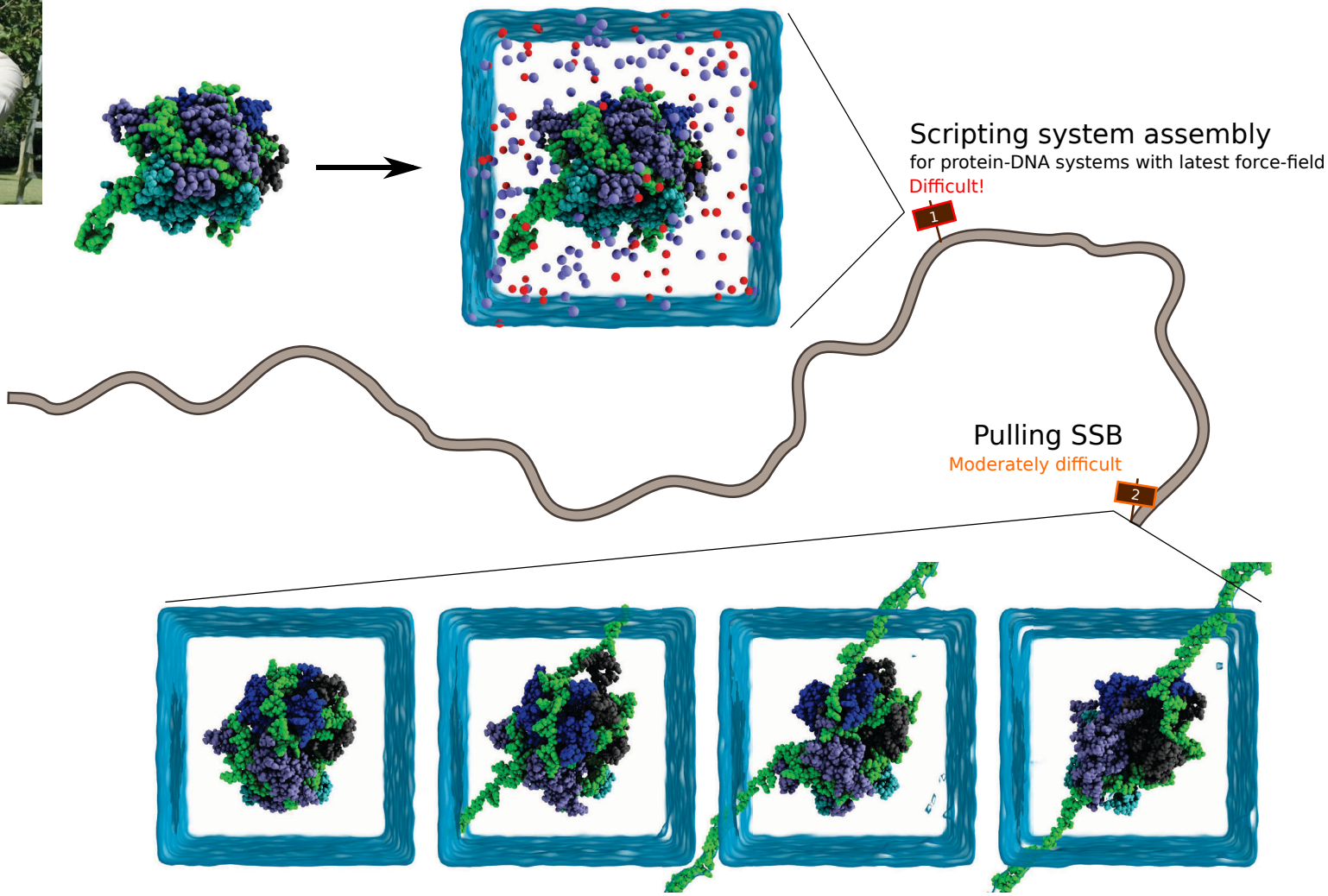
Forces are 1-2 orders of magnitude larger than in experiments

Little hope of observing diffusion-related events

Atomistic mechanics of single-stranded DNA Binding-Protein



Chris Maffeo



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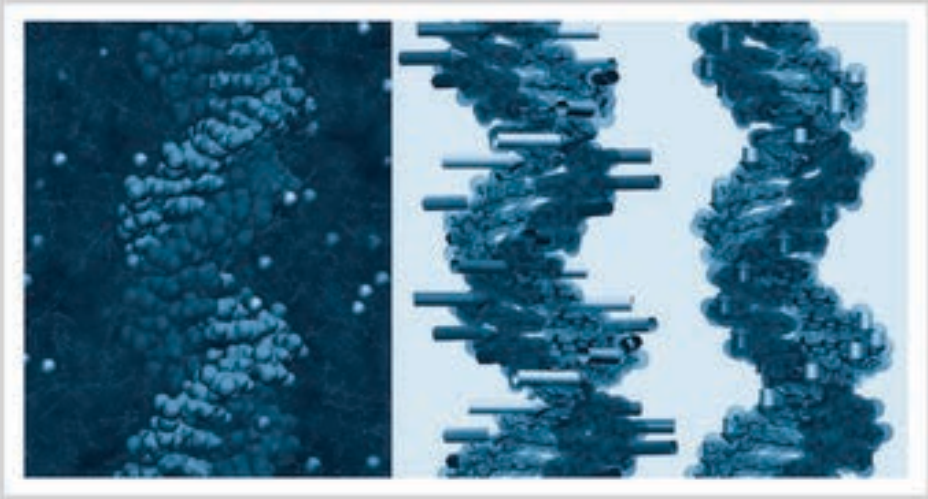
Topical review

Close encounters with DNA

C Maffeo, J Yoo, J Comer, D B Wells, B Luan and A Aksimentiev



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Ian Derrington
(UW Seattle)

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(TU Delft)

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VMD and NAMD