DNA Biophysics

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Icebreaker!

What is biophysics?

The science of the application of the laws of physics to biological phenomena

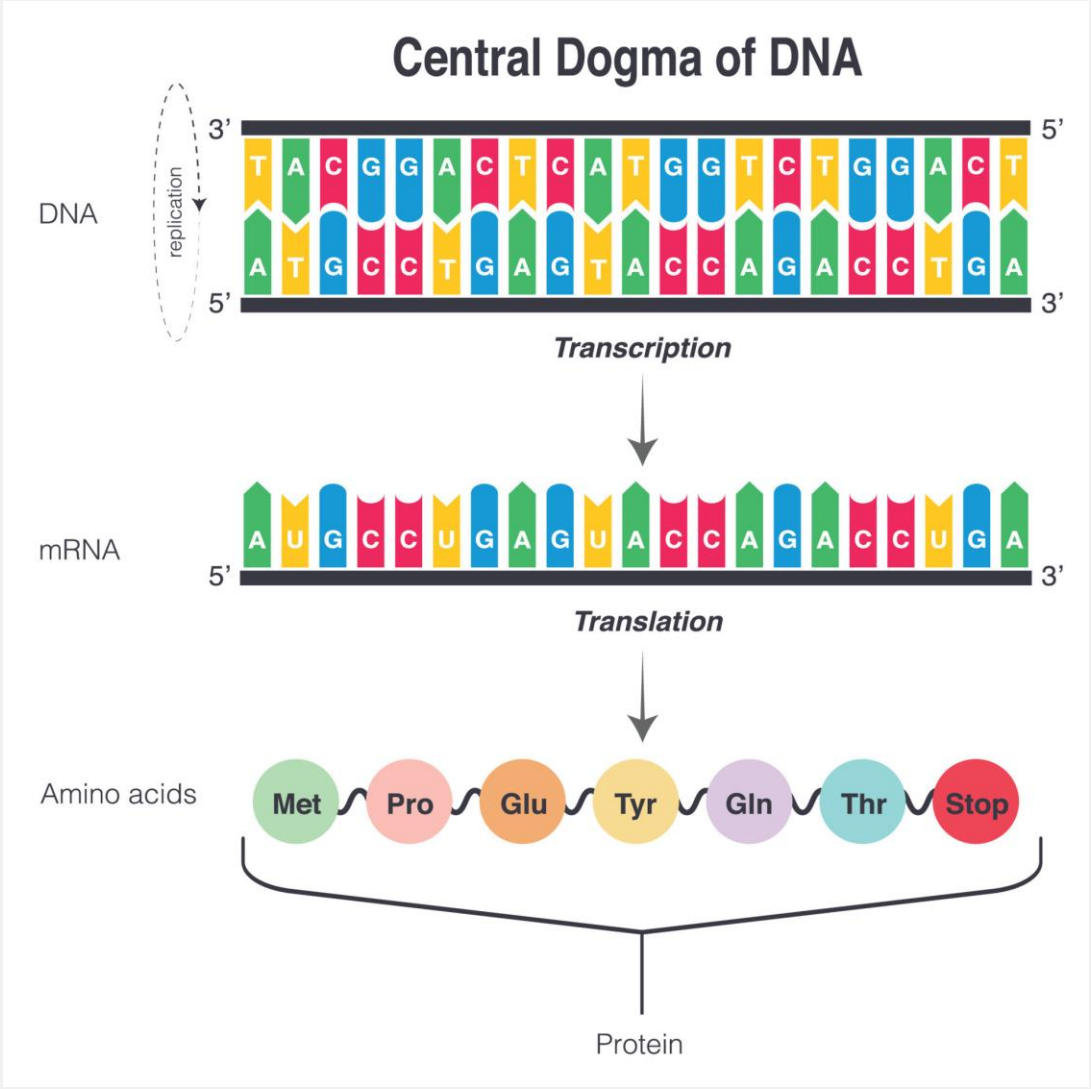
What are some examples of biophysics?

DNA Biophysics

- Today I will tell you about my research in DNA biophysics
- How does twisting up DNA cause it’s structure to change?
- How do we measure this?

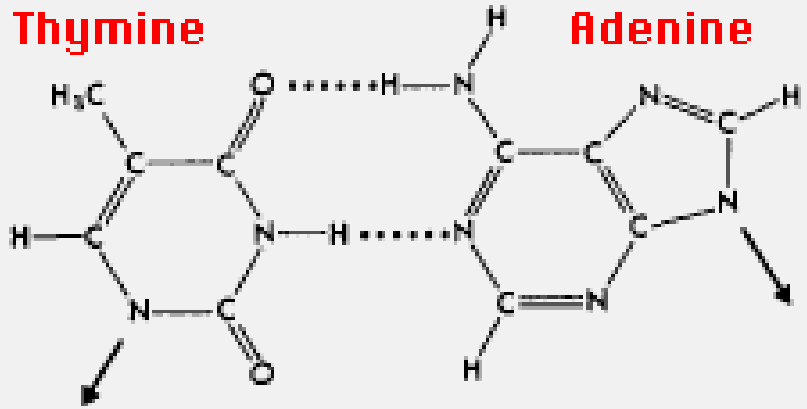
Background Knowledge:
What can you tell me about DNA?

DNA is a set of instructions for your cells to make proteins

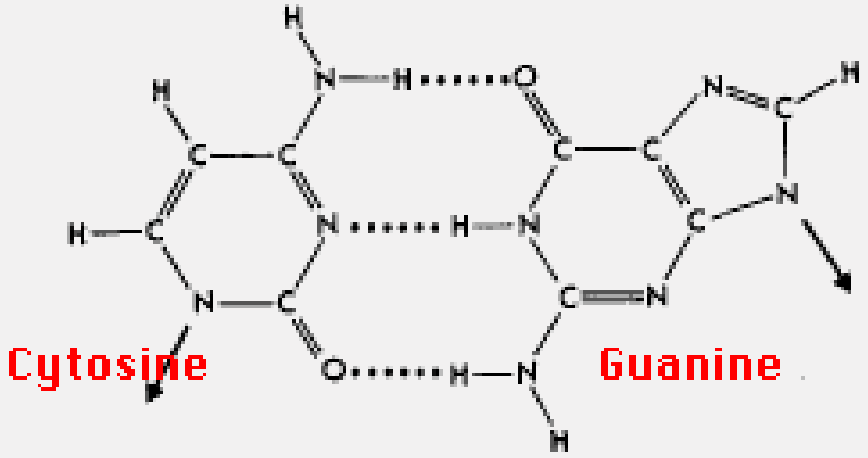


https://www.vecteezy.com/vector-art/7508603-central-dogma-of-dna

DNA Base pairs



- A and T bind with 2 hydrogen bonds
- It takes 0.26 kcal/mol of energy to break these bonds



- G and C bind with 3 hydrogen bonds
- It takes 1.31 kcal/mol of energy to break these bonds

https://bio.libretexts.org/Bookshelves/Introductory_and_General_Biology/Book%3A_Biology_%28Kimball%29/05%3A_DNA/5.04%3A_Base_Pairing_in_DNA_and_RNA

Question 1

You have a circular DNA molecule 3000 bp long, that has a 100bp long stretch that is 90% AT base pairs. If the molecule starts in a relaxed state, how much energy per mole (in kcal/mol) is required to completely denature the AT rich region?

Useful values:

$a = 10.2 \frac{kcal}{mol}$ is the nucleation energy (En required to break the FIRST base pair)

$b_{AT} = 0.26 \frac{kcal}{mol}$ is the energy of breaking one AT pair (next to an already broken bond)

$b_{GC} = 1.31 \frac{kcal}{mol}$ is the energy of breaking one GC pair (next to an already broken bond)

Question 1: solution

You have a circular DNA molecule 3000 bases long, that has a 100bp long stretch that is 90% AT base pairs. If the molecule starts in a relaxed state, how much energy per mole (in kcal/mol) is required to completely denature the AT rich region?

To melt the region, you must nucleate one base pair, then denature 0.9*100 AT pairs and (1-0.9)*100 GC pairs:

$$E = a + 0.9 * 100 * b_{AT} + 0.1 * 100 * b_{GC}$$

$$E \cong 46.7 \frac{kcal}{mol}$$

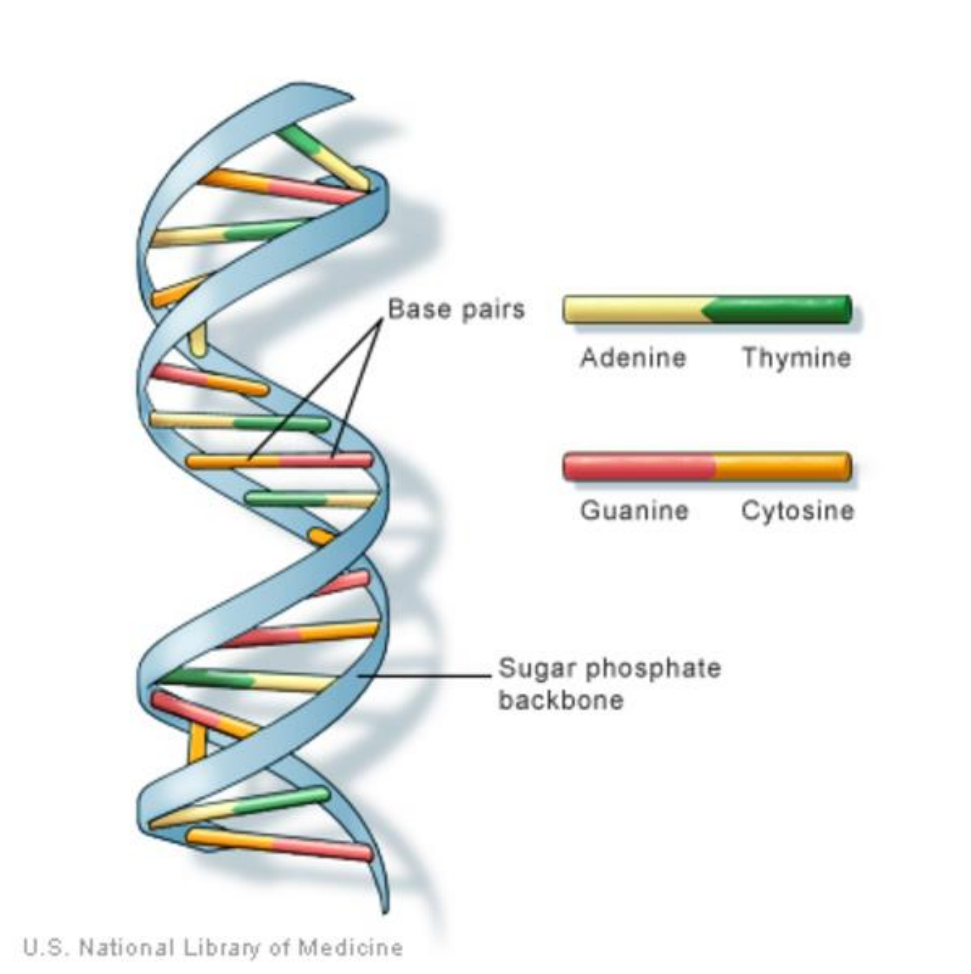
Useful values:

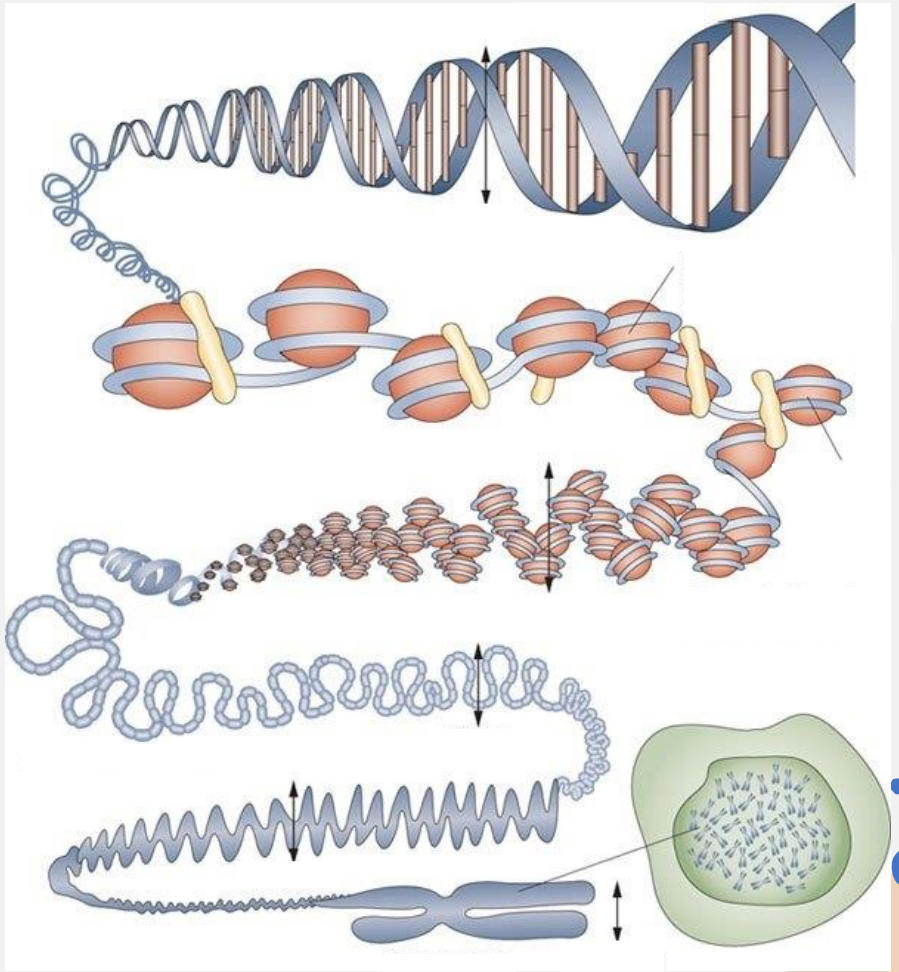
$a = 10.2 \frac{kcal}{mol}$ is the nucleation energy (En required to break the FIRST base pair, in addition to the normal denaturation energy)

$b_{AT} = 0.26 \frac{kcal}{mol}$ is the energy of breaking one AT bond (next to an already broken bond)

$b_{GC} = 1.31 \frac{kcal}{mol}$ is the energy of breaking one GC bond (next to an already broken bond)

DNA structure





Tonna *et al.* 2010, *Nature Reviews Nephrology* (2010)

Question 2: How long is DNA?

Each of your cells contains 46 chromosomes (ie: individual DNA molecules). Combining the DNA from all of your chromosomes, there are 6 billion base pairs of DNA in each of your cells.

a) Assuming that each base pair is 0.34 nm long, if you took all the DNA in one of your cells, stretched it out, and placed them end to end, how long would your DNA be?
b) Assume there are 5 trillion cells in your body. If you take all the DNA in your body and stretch it out end to end, how many times can it go to the sun and back? (The sun is about 150 million km from Earth).

DNA is only about 2nm wide!

Question 2: Solution

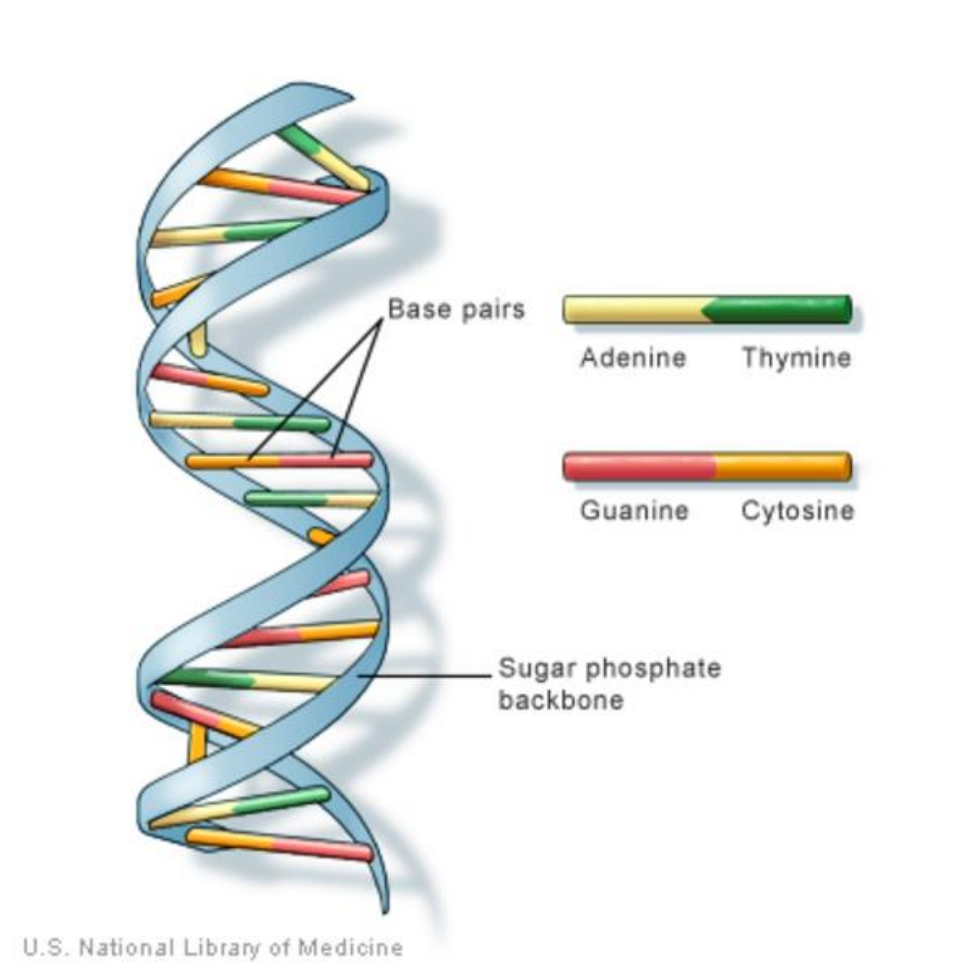
Each of your cells contains 46 chromosomes (ie: individual DNA molecules). Combining the DNA from all of your chromosomes, there are 6 billion base pairs of DNA in each of your cells.

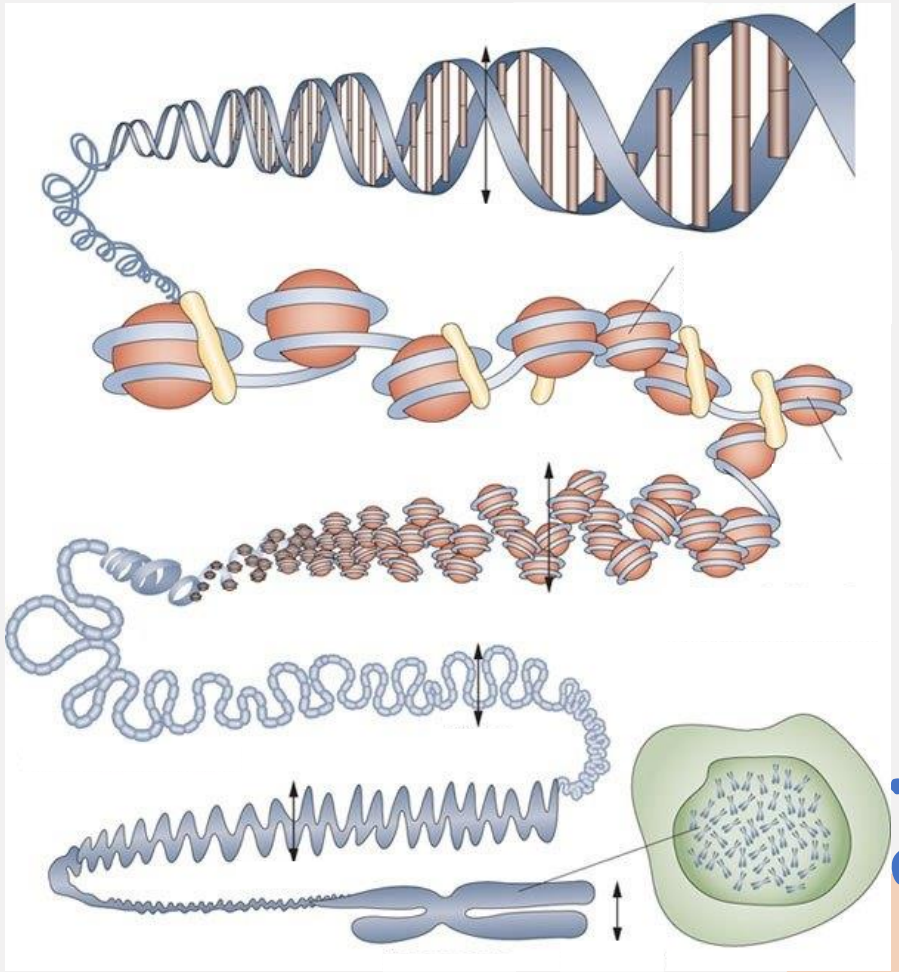
a) Assuming that each base pair is 0.34 nm long, if you took all the DNA in one of your cells, stretched it out, and placed them end to end, how long would your DNA be?
   - 2m

b) Assume there are 10 trillion cells in your body. If you take all the DNA in your body and stretch it out end to end, how many times can it go to the sun and back? (The sun is about 150 million km from Earth).
   - ~70 times (if you got 70000, watch your units!)

DNA is only about 2nm wide!

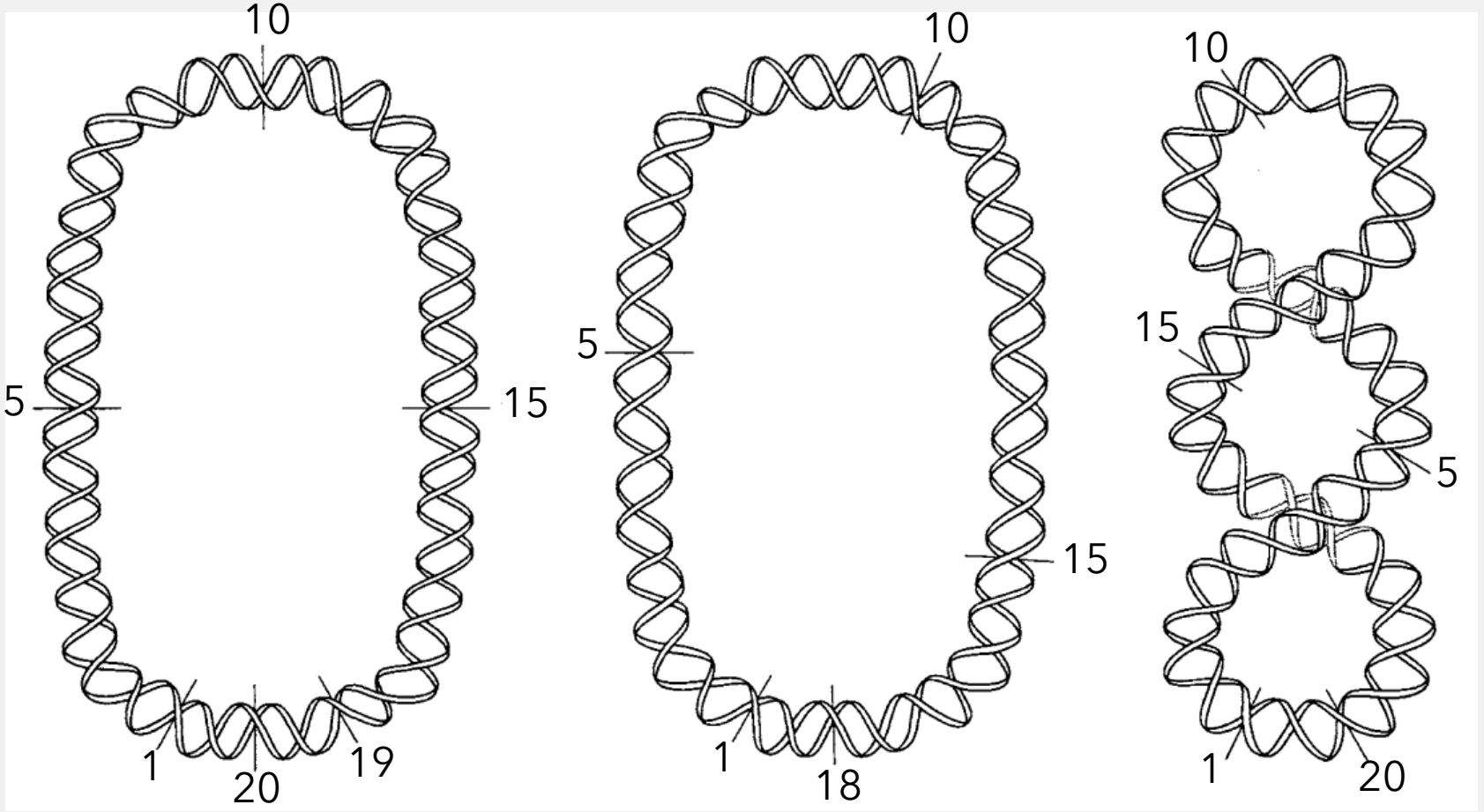
DNA structure





Tonna *et al.* 2010, *Nature Reviews Nephrology* (2010)

How can supercoiling change structure?



Relaxed plasmid

Twisted Plasmid

Writhed plasmid

Sinden (1994) DNA Structure and Function, *Academic Press*

Question 3

An enzyme now negatively *supercoils* our 3000 bp long circular DNA by breaking the double-stranded bond, untwisting it 20 complete turns, and sealing it back together. Thus the DNA now has a supercoiling value of $\alpha = -20$. How much energy was added to the DNA to do this?

Energy from supercoiling:

Energy from supercoiling behaves a lot like elastic potential energy, where:

$$E = \frac{K}{2}\alpha^2$$

α is the number of complete twists (+ve α) or untwists (-ve α)

$K = \frac{1368}{N}\frac{kcal}{mol}$ is an experimentally determined constant

N is the length of the molecule in base pairs

Question 3: solution

An enzyme now negatively *supercoils* our 3000 bp long circular DNA by breaking the double-stranded bond, untwisting it 20 complete turns, and sealing it back together. Thus the DNA now has a supercoiling value of $\alpha = -20$. How much energy was added to the DNA to do this?

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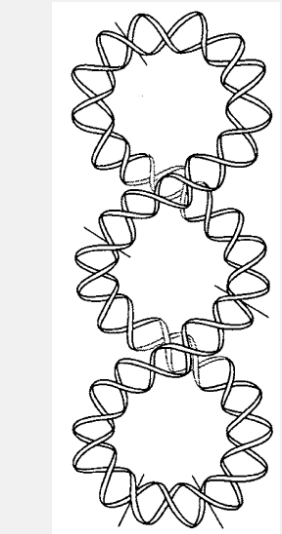
N is the length of the molecule in base pairs

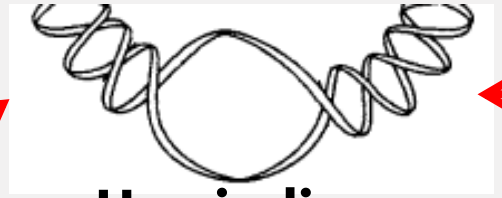
Plugging everything in gives:

91.2 kcal/mol

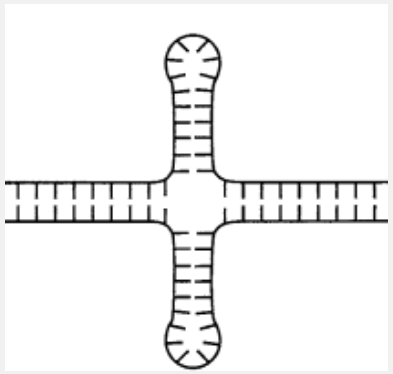
Supercoiling and Structure



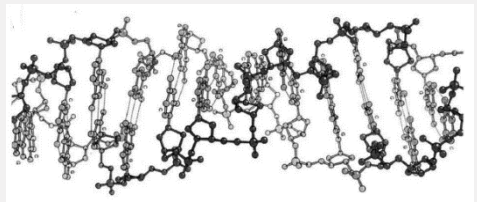
Supercoiled Plasmid



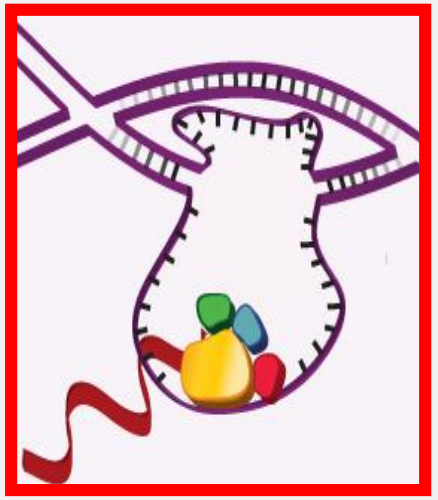
Unwinding



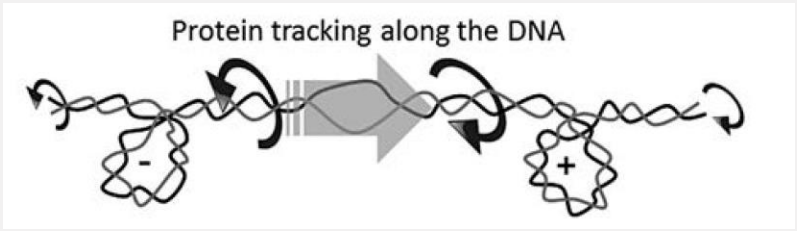
Cruciform



Z-DNA



RNA polymerase binding to an unwound site.

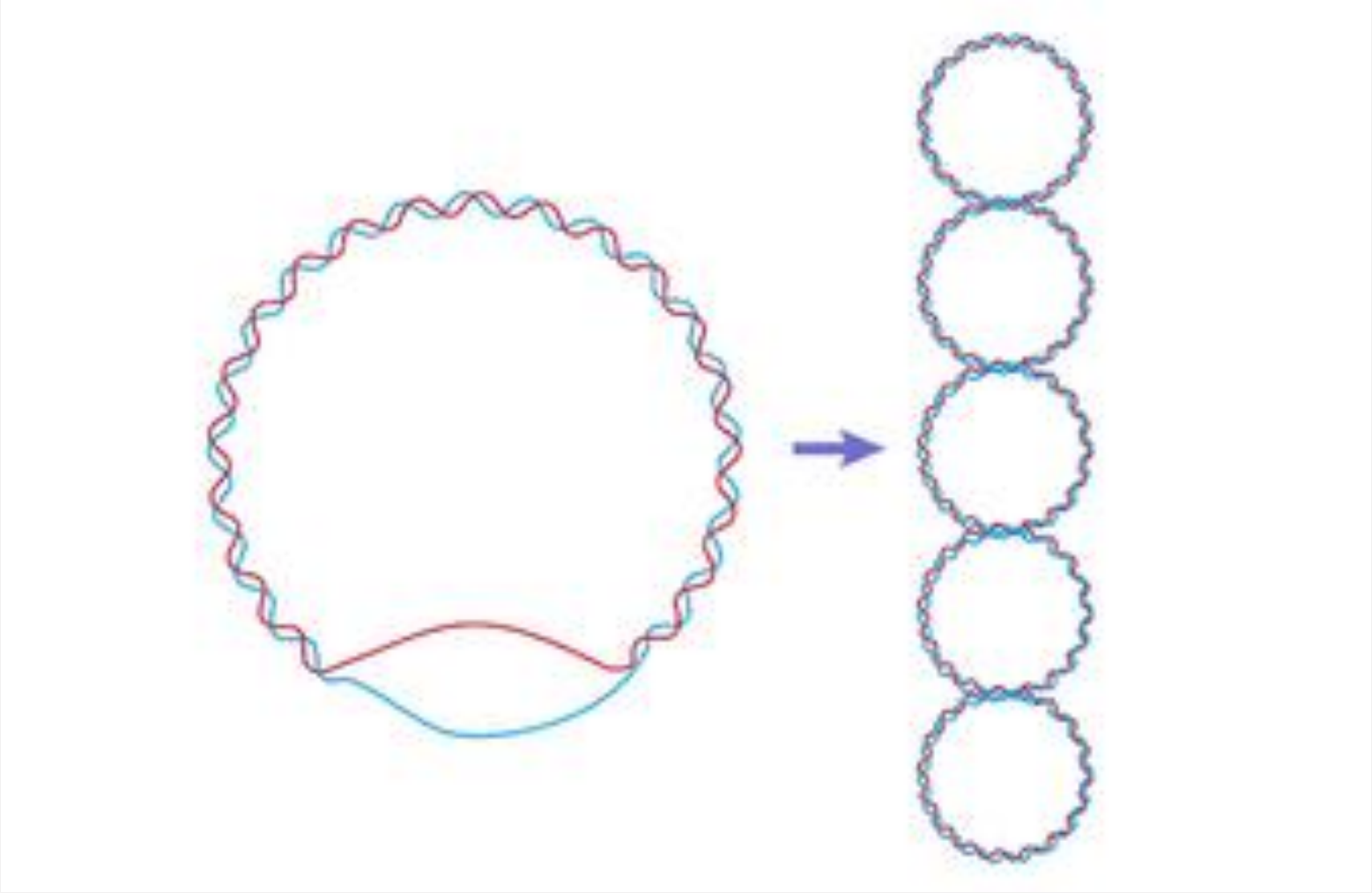


- RNA polymerase and other cell machinery often use open sites to initiate their function.
- RNA polymerase generates positive and negative supercoiling as it transcribes.

Sinden (1994) DNA Structure and Function, *Academic Press*

Kouzine et. al. (2018 Nuclear Arch and Dynamics)

DNA supercoiling



Cell and Molecular Biology – Karp, Figure 10.13

Question 4

When double-stranded DNA is relaxed, there are A=10.4 bp/turn. For the sake of this exercise, assume that when single-stranded DNA is relaxed, it is completely untwisted. Because our DNA is circular, the total number of twists in it is **fixed,** unless we break the double-strand.

a) If the 100 bp long, 90% AT region from question 1 fully denatures in a relaxed molecule, how many full (un)twists (α) must be added to the rest of the molecule for twist to be conserved?
b) If the same region denatures in our supercoiled DNA ($\alpha = -20$), what is the leftover α in the rest of the molecule?

Useful values:

$a = 10.2 \frac{kcal}{mol}$ is the nucleation energy

$b_{AT} = 0.26 \frac{kcal}{mol}$ is the energy of breaking one AT bond

$b_{GC} = 1.31 \frac{kcal}{mol}$ is the energy of breaking one GC bond

N = 3000bp

$\alpha = -20$

$E = \frac{K}{2}\alpha^2$ is the energy from supercoiling

$K = \frac{1368}{N}\frac{kcal}{mol}$ is a constant

A = 10.4 bp/turn

Question 4: solution

When double-stranded DNA is relaxed, there are A=10.4 bp/turn. For the sake of this exercise, assume that when single-stranded DNA is relaxed, it is completely untwisted. Because our DNA is circular, the total number of twists in it is **fixed,** unless we break the double-strand.

a) If the 100 bp long, 90% AT region from question 1 fully denatures in a relaxed molecule, how many full (un)twists (α) must be added to the rest of the molecule for twist to be conserved?

It takes n_{open}/A left-handed twists to fully unwind the denatured region. Thus it takes 9.6 full left-handed (negative) turns. For twist to be conserved, $twist_{absorbed} + \alpha_{leftover} = 0$. Therefore, the rest of the molecule gains +9.6 twists.

a) If the same region denatures in our supercoiled DNA ($\alpha = -20$), what is α in the rest of the molecule?

From a), we know the denaturation adds +9.6 twists, so, since twist is conserved, the total number of twists in the rest of the molecule is

$$twist_{absorbed} + \alpha_{leftover} = -20$$
$$-9.6 + \alpha_{leftover} = -20$$
$$\alpha_{leftover} = -10.4$$

Question 5

Let's consider our 3000 bp long circular DNA molecule, with its 100bp long region that is 90% AT

a) When the molecule is relaxed ($\alpha = 0$), do you expect the AT region to be denatured or double-stranded?
b) When the molecule is supercoiled ($\alpha = -20$) do you expect the AT region to be denatured or double-stranded?
c) Let's say our unwinding region is 90% GC instead of 90% AT. Would that change your answer to a) and b)?

Hint: You want to look at the total energy needed to twist/denature the DNA for the two different states

Useful values:

$a = 10.2 \frac{kcal}{mol}$ is the nucleation energy

$b_{AT} = 0.26 \frac{kcal}{mol}$ is the energy of breaking one AT bond

$b_{GC} = 1.31 \frac{kcal}{mol}$ is the energy of breaking one GC bond

N = 3000bp

$E = \frac{K}{2}\alpha^2$ is the energy from supercoiling

$K = \frac{1368}{N} \frac{kcal}{mol}$ is a constant

A = 10.4 bp/turn

Question 5a): Solution

Let's consider our 3000 bp long circular DNA molecule, with its 100bp long region that is 90% AT

The total energy of a DNA molecule in a given supercoiling/denaturation state can be given by:

$$E_{total} = E_{supercoiling} + E_{denaturation}$$

$$E_{total} = \frac{K}{2}\alpha^2 + an_{nucleations} + b_{AT}n_{openAT} + b_{GC}n_{GCopen}$$

For a relaxed molecule with no denaturation, $\alpha = 0$, $n_{nucleations} = 0$, and $n_{open} = 0$.

Therefore, $E_{TotalClosed} = 0$

For a relaxed molecule with the denatured site open, we get:

$$E_{total} = \frac{K}{2}0^2 + 1\left(10.2\frac{kcal}{mol}\right) + 90\left(0.26\frac{kcal}{mol}\right) + 10\left(1.31\frac{kcal}{mol}\right)$$

$$E_{totalOpen} = 46.7\frac{kcal}{mol}$$

Since $E_{TotalClosed} < E_{TotalOpen}$, the closed state is favoured when there is no supercoiling.

Useful values:

$a = 10.2\frac{kcal}{mol}$ is the nucleation energy

$b_{AT} = 0.26\frac{kcal}{mol}$ is the energy of breaking one AT bond

$b_{GC} = 1.31\frac{kcal}{mol}$ is the energy of breaking one GC bond

N = 3000bp

$E = \frac{K}{2}\alpha^2$ is the energy from supercoiling

$K = \frac{1368}{N}\frac{kcal}{mol}$ is a constant

A = 10.4 bp/turn

Question 5b): Solution

Let's consider our 3000 bp long circular DNA molecule, with its 100bp long region that is 90% AT

The total energy of a DNA molecule in a given supercoiling/denaturation state can be given by:

$$E_{total} = E_{supercoiling} + E_{denaturation}$$

$$E_{total} = \frac{K}{2}\alpha^2 + an_{nucleations} + b_{AT}n_{openAT} + b_{GC}n_{GCopen}$$

For the supercoiled molecule with no denaturation, $\alpha = -20$, $n_{nucleations} = 0$, and $n_{open} = 0$.

Therefore, $E_{TotalClosed} = \frac{K}{2}(-20)^2$ =91.2 kcal/mol

For a relaxed molecule with the denatured site open, we get:

$$E_{total} = \frac{K}{2}(-10.4)^2 + 1\left(10.2\frac{kcal}{mol}\right) + 90\left(0.26\frac{kcal}{mol}\right) + 10\left(1.31\frac{kcal}{mol}\right)$$

$$E_{totalOpen} = 71.36\frac{kcal}{mol}$$

Since $E_{TotalOpen} < E_{TotalClosed}$, the open state is favoured under this much supercoiling.

Useful values:

$a = 10.2\frac{kcal}{mol}$ is the nucleation energy

$b_{AT} = 0.26\frac{kcal}{mol}$ is the energy of breaking one AT bond

$b_{GC} = 1.31\frac{kcal}{mol}$ is the energy of breaking one GC bond

N = 3000bp

$E = \frac{K}{2}\alpha^2$ is the energy from supercoiling

$K = \frac{1368}{N}\frac{kcal}{mol}$ is a constant

A = 10.4 bp/turn

Question 6c): Solution

Let's consider our 3000 bp long circular DNA molecule, with its 100bp long region that is 90% AT

It would not change our answer to a. For b, the energy of the closed state is the same. The energy of the open state is as follows:

$$E_{total} = \frac{K}{2}(-10.4)^2 + 1\left(10.2\frac{kcal}{mol}\right) + 10\left(0.26\frac{kcal}{mol}\right) + 90\left(1.31\frac{kcal}{mol}\right)$$

$$E_{totalOpen} = 155.36\frac{kcal}{mol}$$

Therefore, the closed state is favoured.

Useful values:

$a = 10.2\frac{kcal}{mol}$ is the nucleation energy

$b_{AT} = 0.26\frac{kcal}{mol}$ is the energy of breaking one AT bond

$b_{GC} = 1.31\frac{kcal}{mol}$ is the energy of breaking one GC bond

N = 3000bp

$E = \frac{K}{2}\alpha^2$ is the energy from supercoiling

$K = \frac{1368}{N}\frac{kcal}{mol}$ is a constant

A = 10.4 bp/turn

Question 6

Let's consider our 3000 bp long circular DNA molecule, with its 100bp long region that is 90% AT. Now, it has a second AT rich region that is 150bp long, but only 80% AT.

a) If $\alpha = -20$, which state is preferred? No denaturation, the shorter site denatures, the longer site denatures, both sites denature.
b) If $\alpha = -30$ which state is preferred?

Hint: You want to look at the total energy needed to twist/denature the DNA compared to the relaxed state.

Useful values:

$a = 10.2 \frac{kcal}{mol}$ is the nucleation energy

$b_{AT} = 0.26 \frac{kcal}{mol}$ is the energy of breaking one AT bond

$b_{GC} = 1.31 \frac{kcal}{mol}$ is the energy of breaking one GC bond

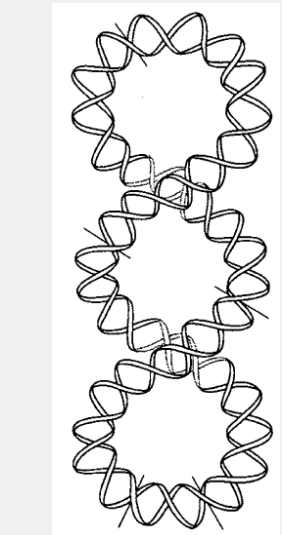
N = 3000bp

$E = \frac{K}{2}\alpha^2$ is the energy from supercoiling

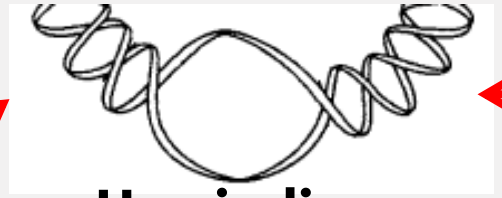
$K = \frac{1368}{N} \frac{kcal}{mol}$ is a constant

A = 10.4 bp/turn

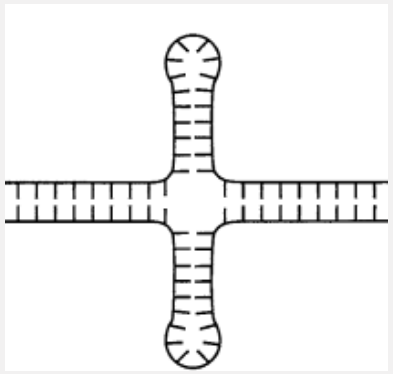
Supercoiling and Structure



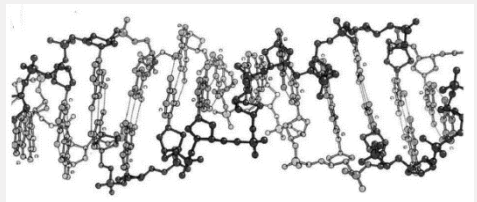
Supercoiled Plasmid



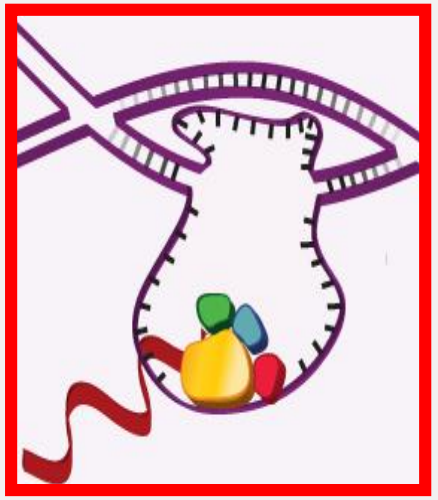
Unwinding



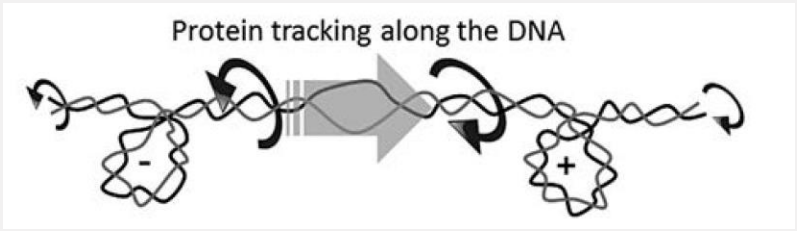
Cruciform



Z-DNA



RNA polymerase binding to an unwound site.



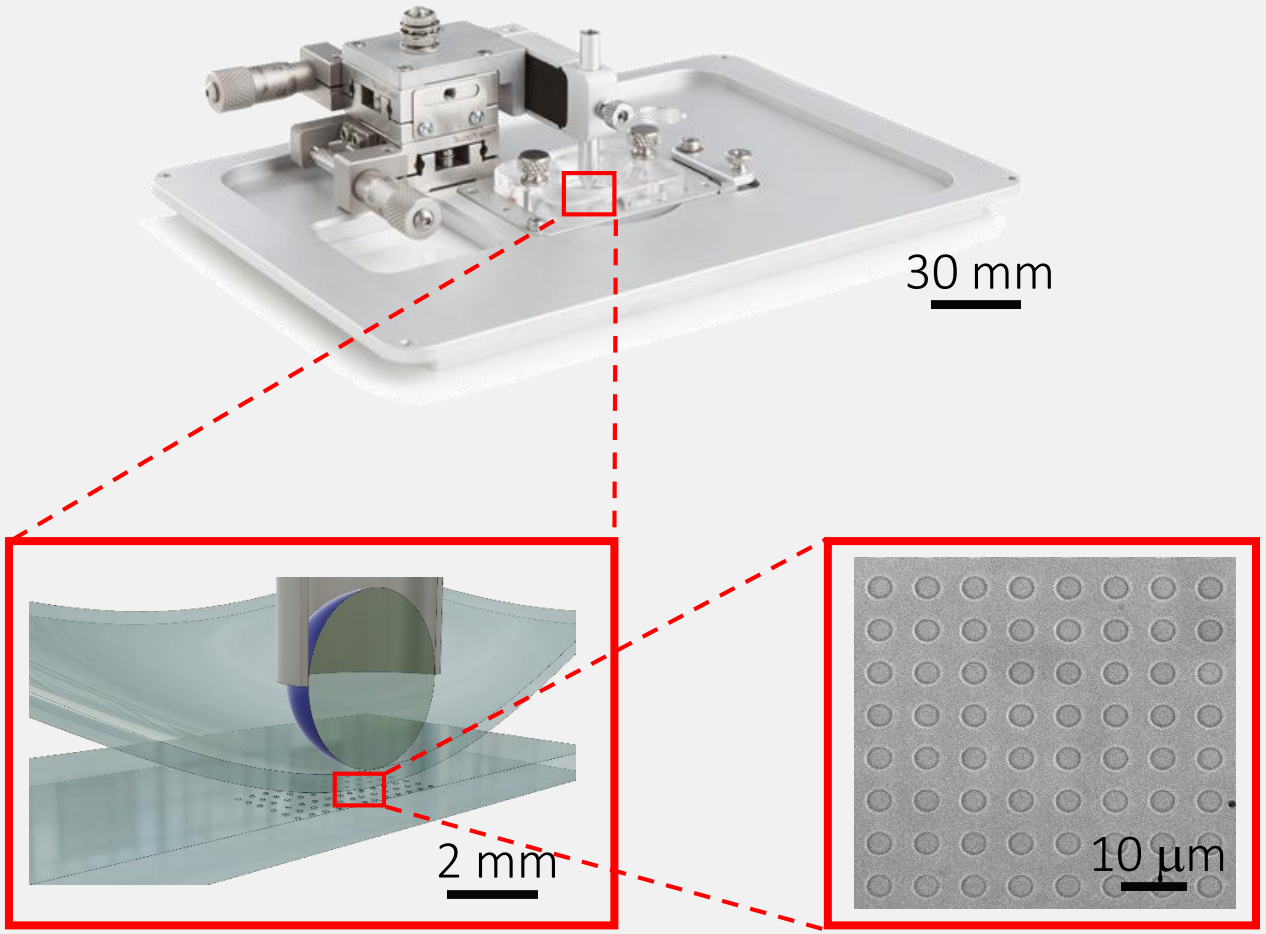
- RNA polymerase and other cell machinery often use open sites to initiate their function.
- RNA polymerase generates positive and negative supercoiling as it transcribes.

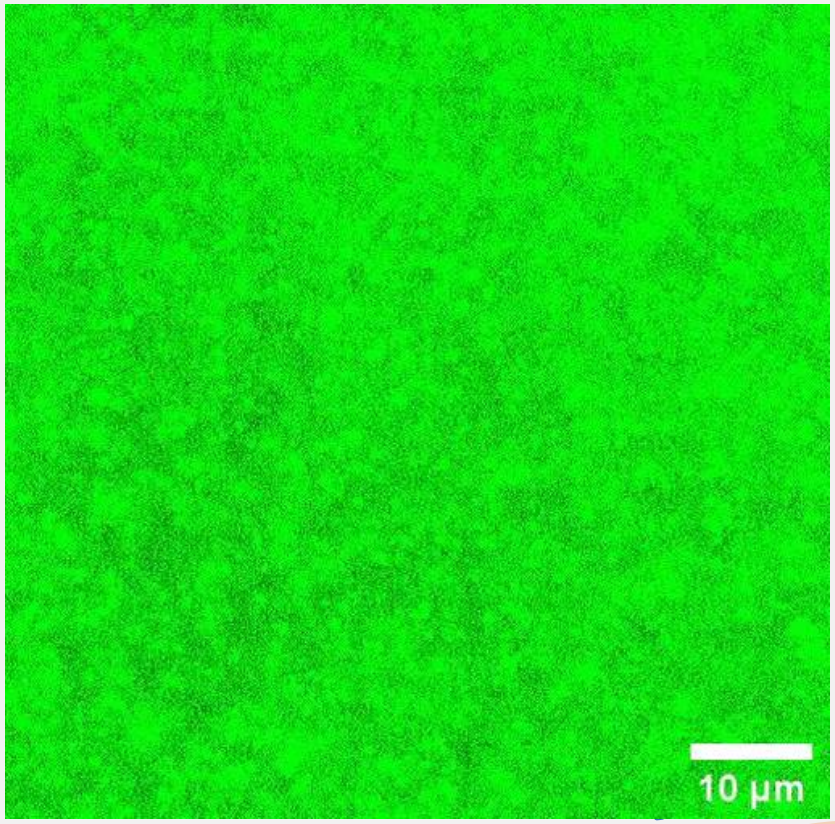
Sinden (1994) DNA Structure and Function, *Academic Press*

Kouzine et. al. (2018 Nuclear Arch and Dynamics)

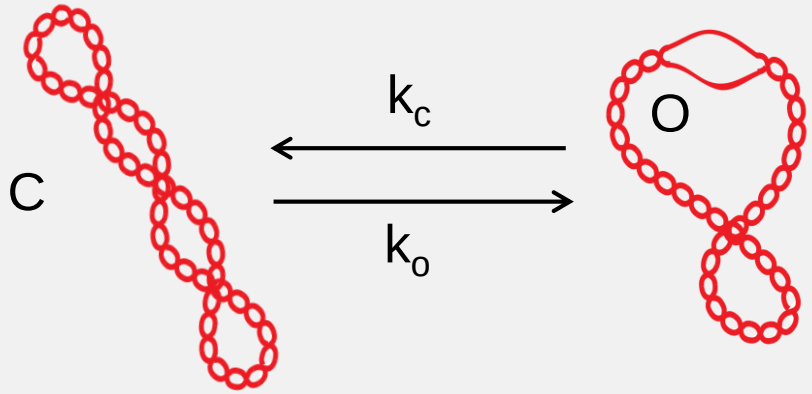
How can we study supercoil-induced unwinding?

Convex Lens-induced Confinement (CLiC)

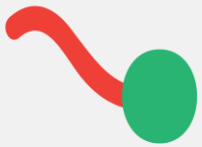


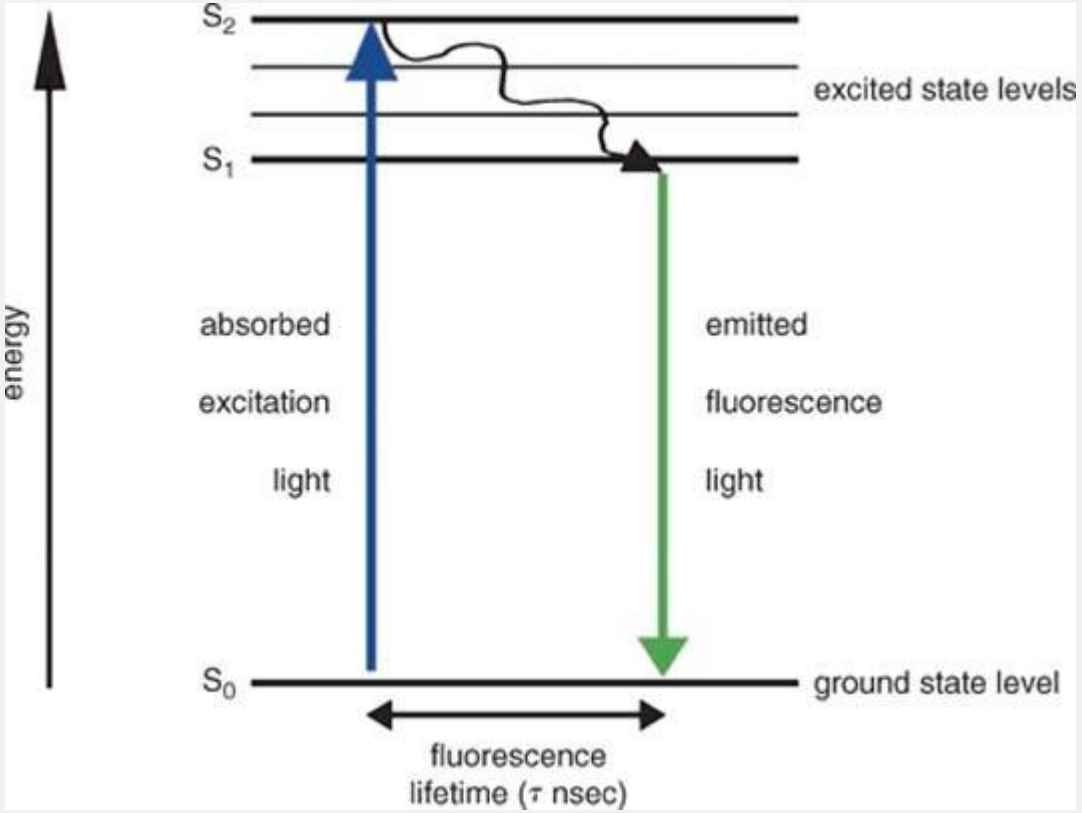


The system: a DNA plasmid with 1 unwinding site



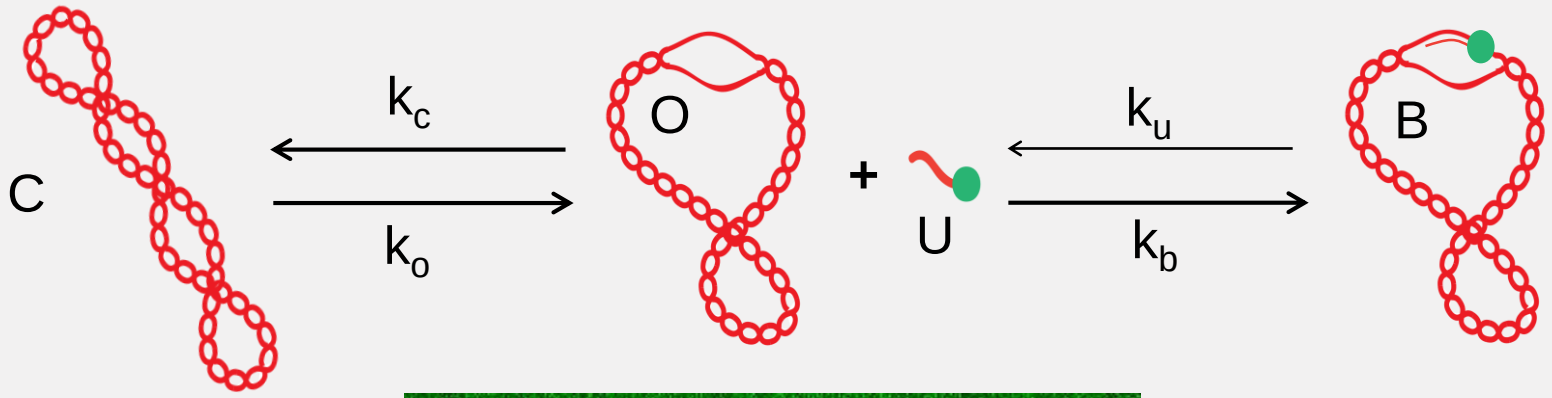
Fluorophores and oligos

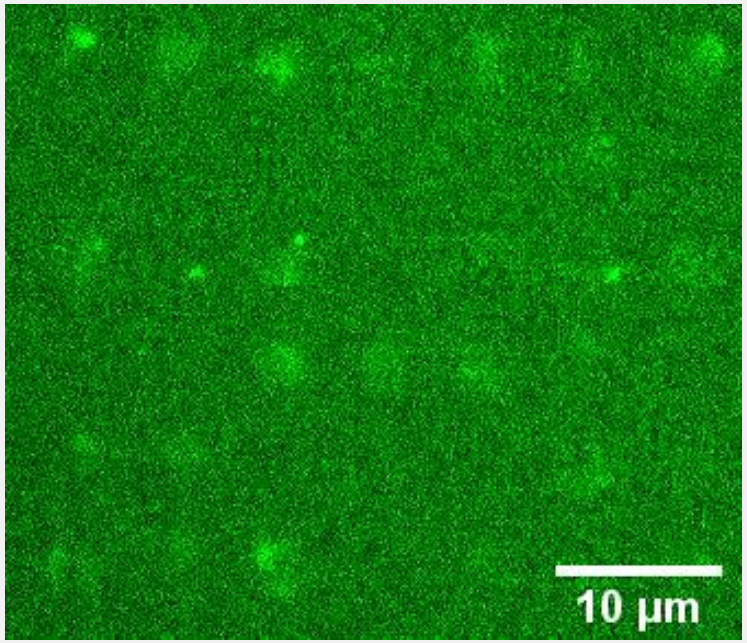




https://www.researchgate.net/publication/23236997_Detecting_Protein-Protein_Interactions_In_Vivo_with_FRET_using_Multiphoton_Fluorescence_Lifetime_Imaging_Microscopy_FLIM/figures?lo=1&utm_source=google&utm_medium=organic

Plasmid-Oligo Binding Model





Question 7:

Why are the molecules moving in the pits?

What factors affect how fast a molecule moves?

Solutions:

Why are the molecules moving in the pits?

- Water molecules are constantly bumping into them, causing them to move
- All molecules are moving a bit because they have a non-zero (Kelvin) temperature. When tens of thousands of small water molecules bump into a larger molecule, they can impart enough kinetic energy that the larger molecule moves a visible distance

What factors affect how fast a molecule moves?

- Molecule size
- Molecule shape
- Viscosity of the liquid
- External forces

Stokes-Einstein equation

$$D = \frac{k_B T}{6\pi\eta r}$$

- D is the diffusion coefficient with dimensions Length^2/Time
- k_B is the Boltzmann constant
  - $k_B = (1.38 * 10^{-23}\ m^2\ kg\ s^{-2}\ K^{-1})$
- T is temperature in Kelvin
  - $T_{inKelvin} = T_{inCelcius} + 273.15$
- r is the radius of the particle
  - We are assuming the particle is a sphere
  - This is a good approximation for long pieces of DNA
- η is viscosity
  - $\eta_{water} \cong 0.001 \frac{kg}{m\ s}$

Question 8

The oligo probe has an approximate average radius of 4nm.

The target plasmid has an approximate average radius of 130nm.

What is the diffusion coefficient for each molecule moving through water at room temperature?

$$D = \frac{k_B T}{6\pi\eta r}$$

- D is the diffusion coefficient with dimensions Length^2/Time
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  - This is a good approximation for long pieces of DNA
- η is viscosity
  - $\eta_{water} \cong 0.001 \frac{kg}{m\ s}$

Solution

The oligo probe has an approximate average radius of 4nm.

The target plasmid has an approximate average radius of 130nm.

What is the diffusion coefficient for each molecule moving through water at room temperature?

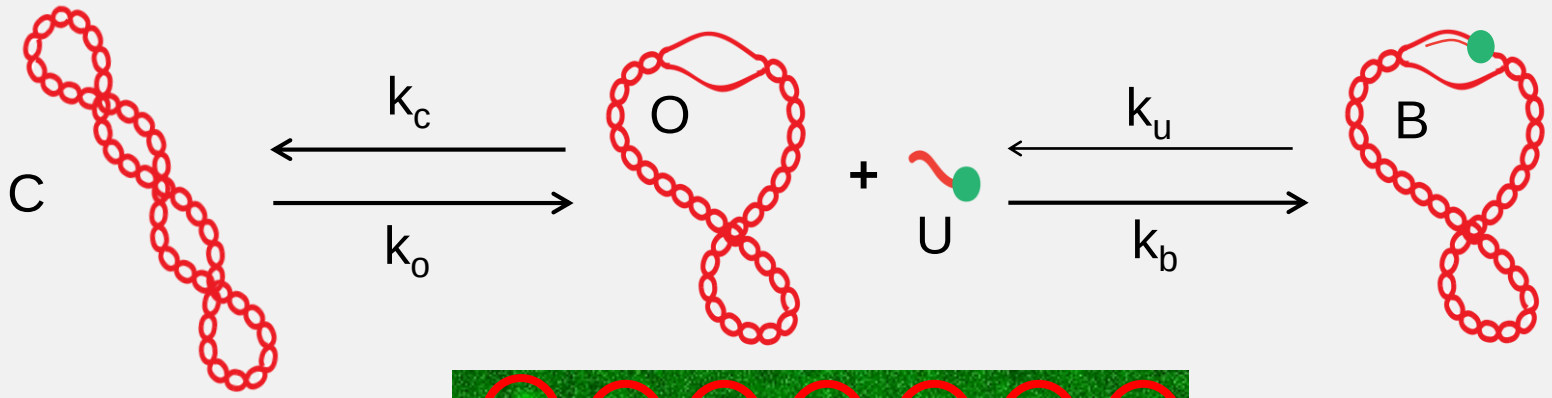
Small probe, $\mathrm{D} \cong 5.4 * 10^{-11} \frac{m^2}{s} = 54 \frac{\mu m^2}{s}$

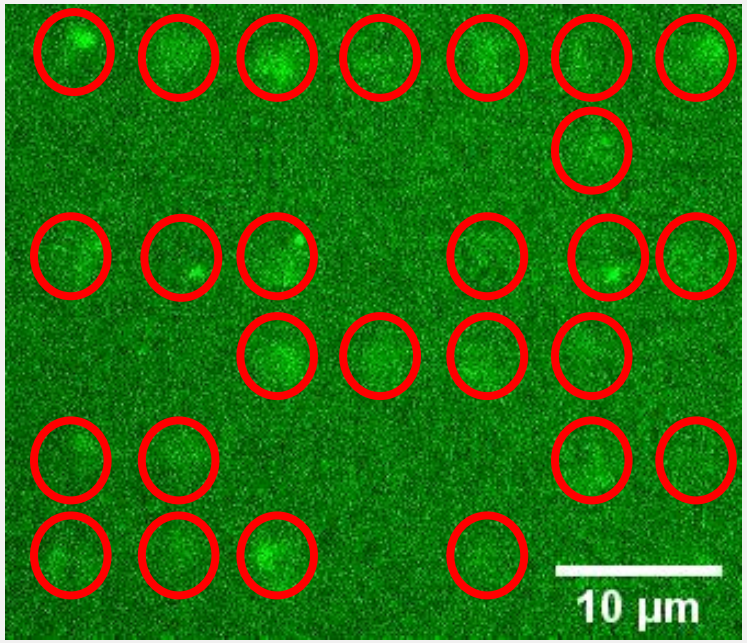
Large probe, $D \cong 1.66 * 10^{-12} \frac{m^2}{s} = 1.66 \frac{\mu m^2}{s}$

$$D = \frac{k_B T}{6\pi\eta r}$$

- D is the diffusion coefficient with dimensions Length^2/Time
- k_B is the Boltzmann constant
  - $\mathrm{k_B} = (1.38 * 10^{-23}\ m^2\ kg\ s^{-2}\ K^{-1})$
- T is temperature in Kelvin
  - $T_{inKelvin} = T_{inCelcius} + 273.15$
- r is the radius of the particle
  - We are assuming the particle is a sphere
  - This is a good approximation for long pieces of DNA
- η is viscosity
  - $\eta_{water} \cong 0.001 \frac{kg}{m\ s}$

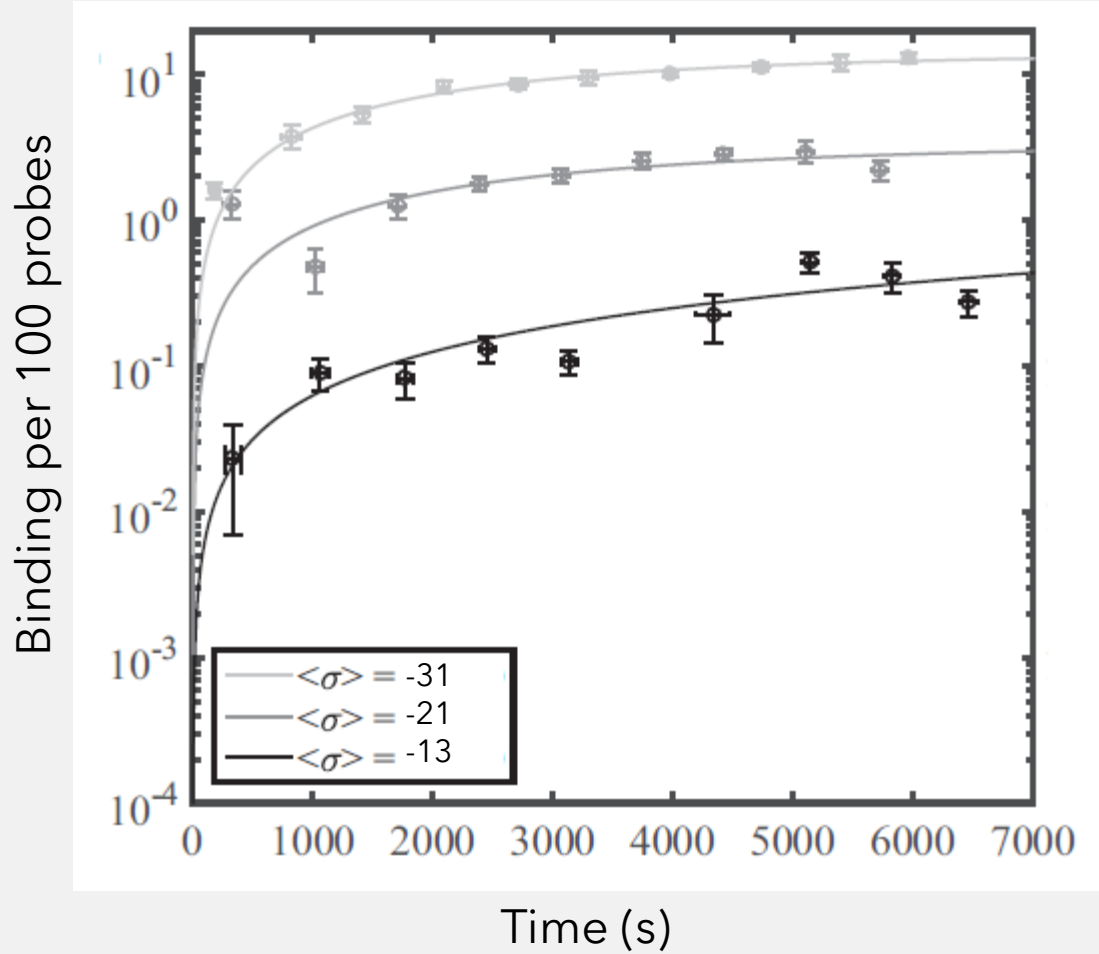
Plasmid-Oligo Binding Model





Does unwinding increase with supercoiling?

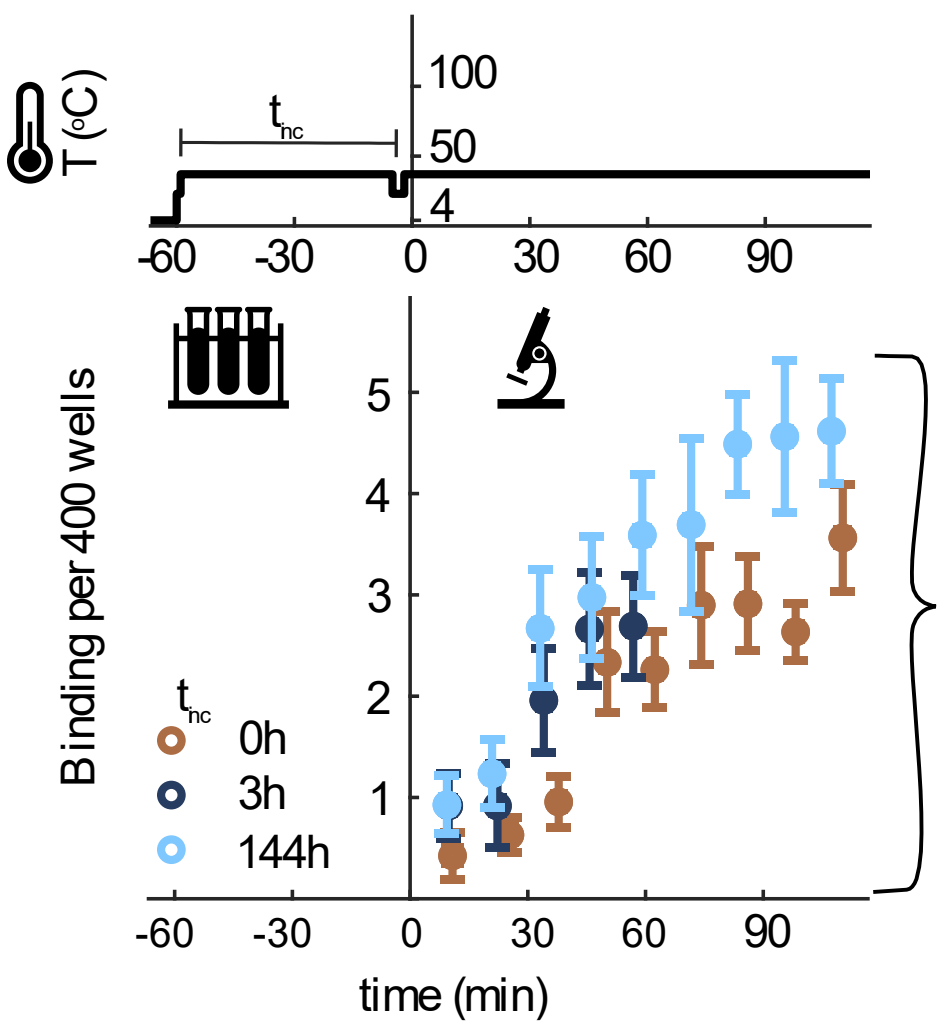
Measuring Binding vs Time vs Supercoiling

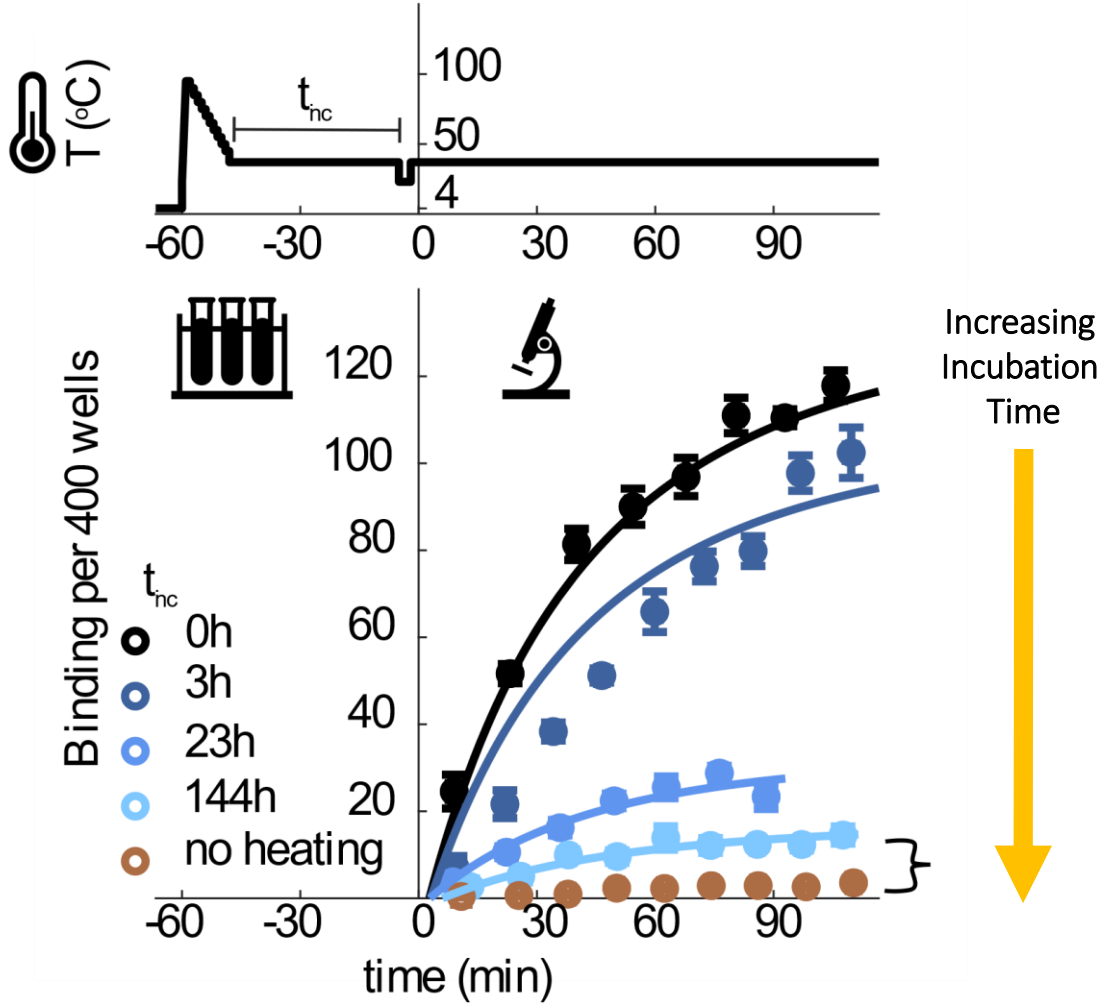


Scott et al, 2018

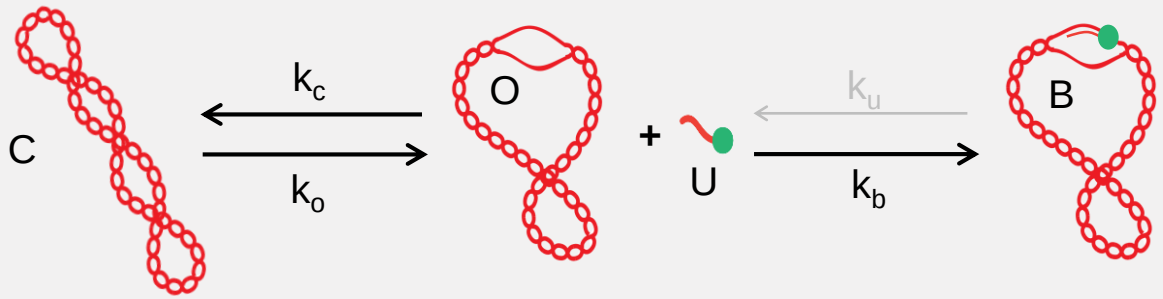
What are the out-of-equilibrium dynamics of the supercoil-induced unwinding region?

Temperature Perturbation





Reaction Model



Our system

$C \overset{k_o}{\rightarrow} O$

$O \overset{k_c}{\rightarrow} C$

$U + O \overset{k_b}{\rightarrow} B$

As differential equations

$$\frac{dO}{dt} = k_o C - k_c O - k_b OU$$

$$\frac{dC}{dt} = k_c O - k_o C$$

$$\frac{dU}{dt} = -k_b OU$$

$$\frac{dB}{dt} = k_b OU$$

Initial conditions

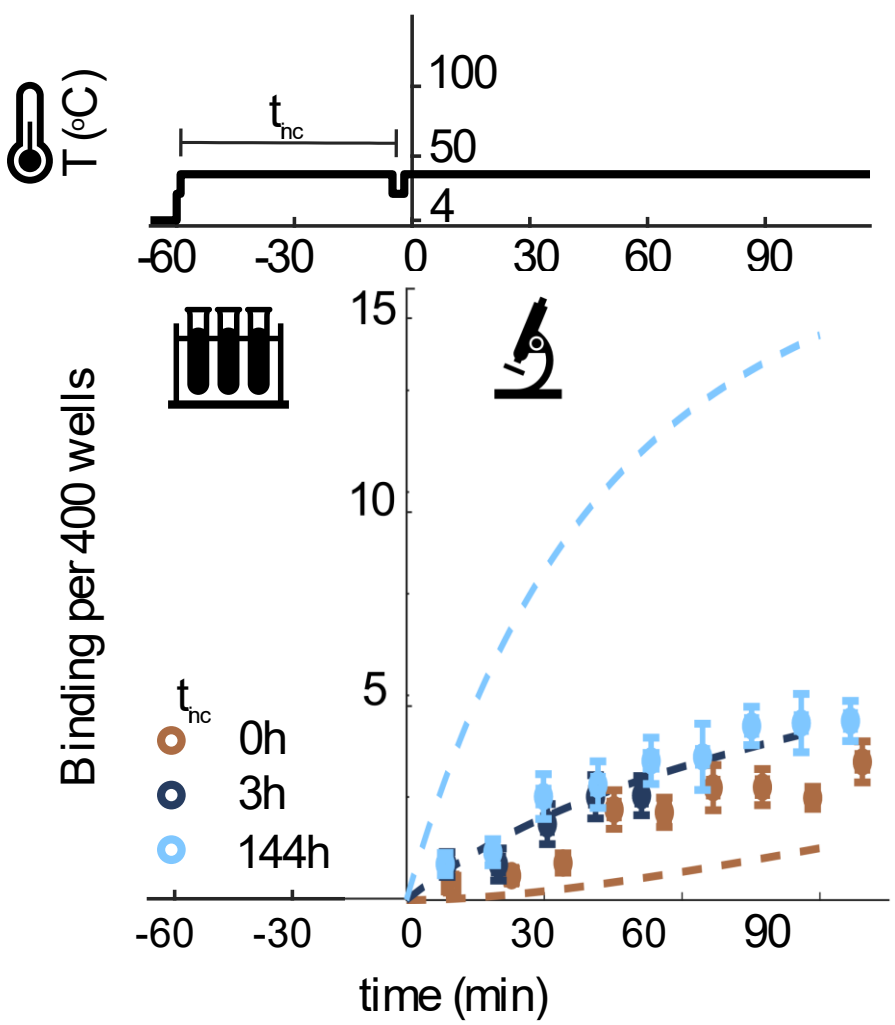
$O_0 = O_0$

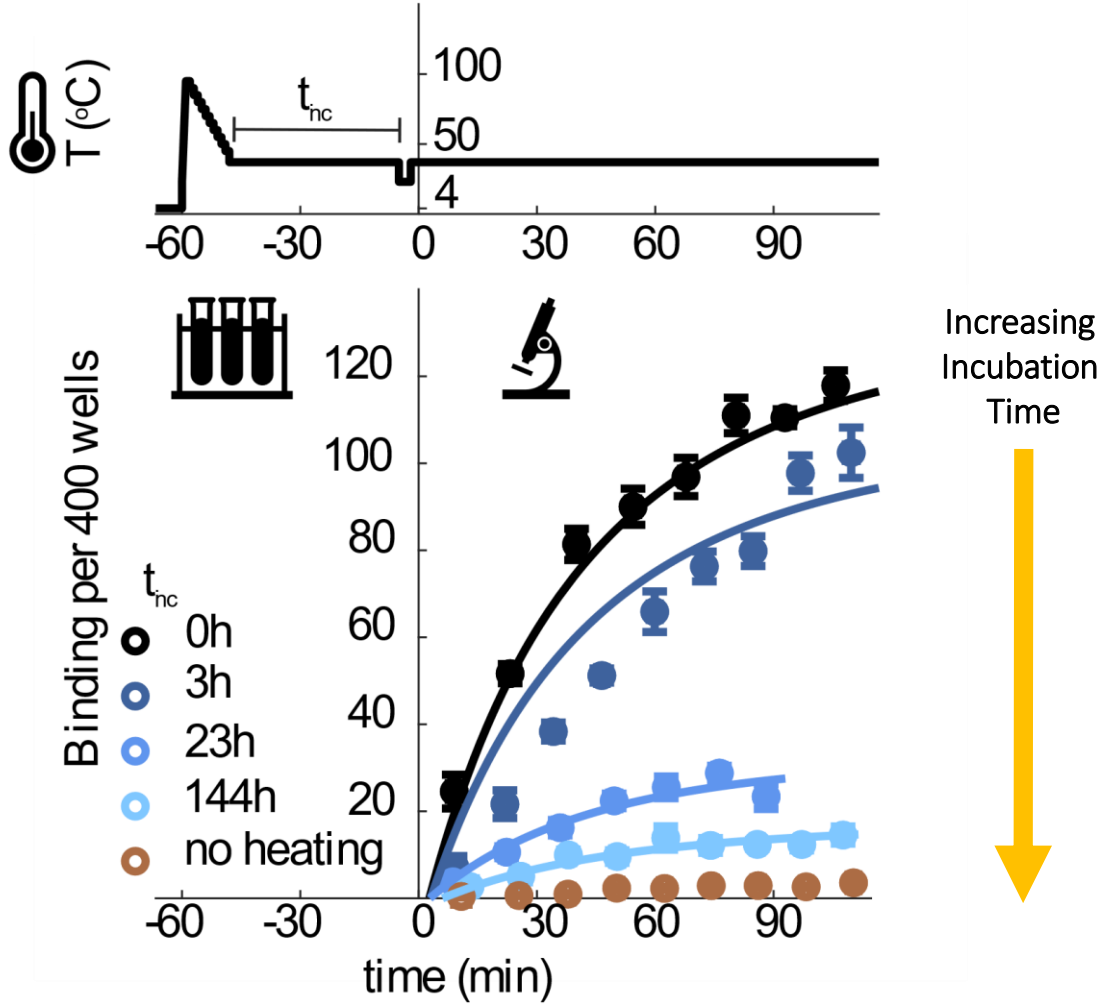
$C_0 = 21.1nM - C_0$

$U_0 = 0.752nM$

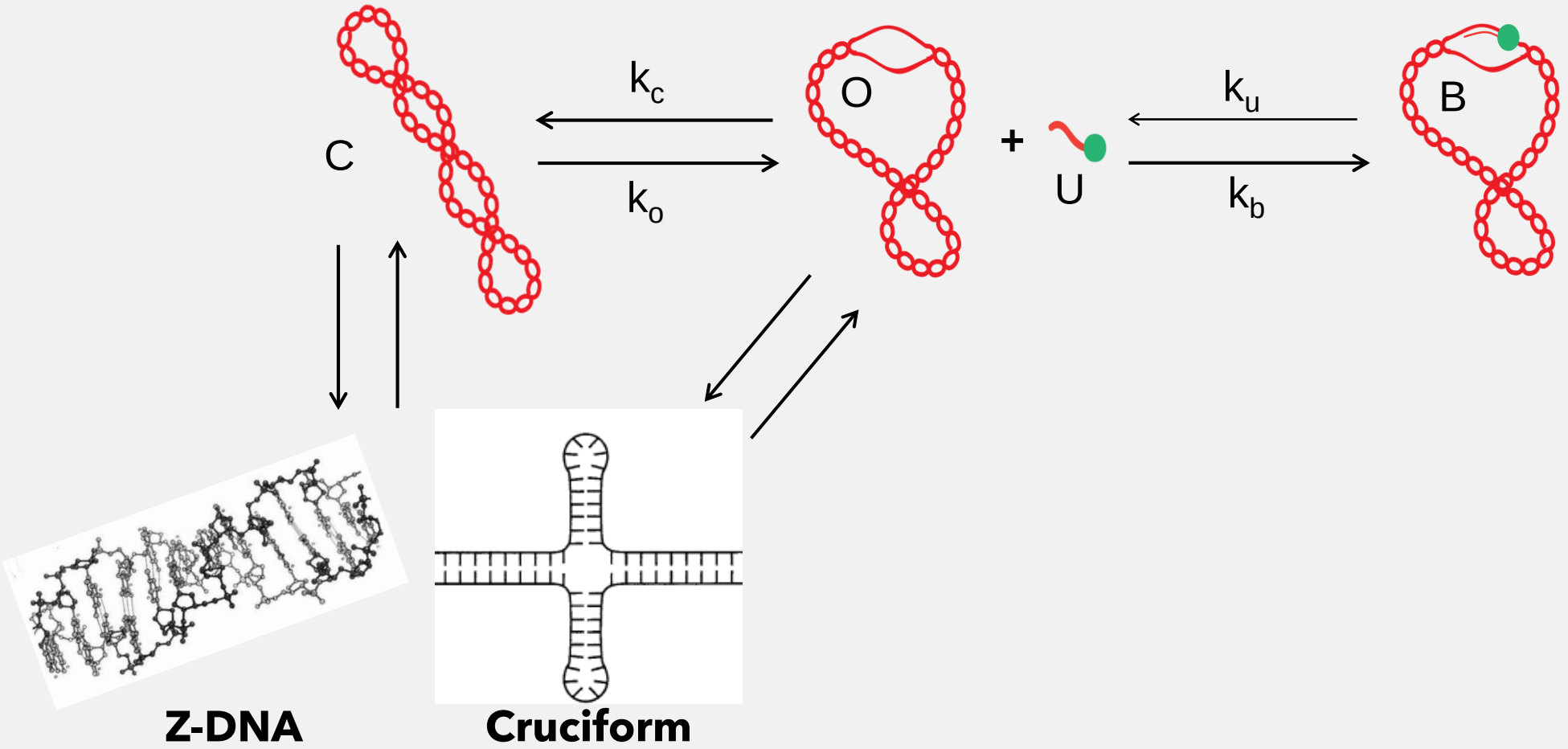
$B_0 = 0$

Temperature Perturbation

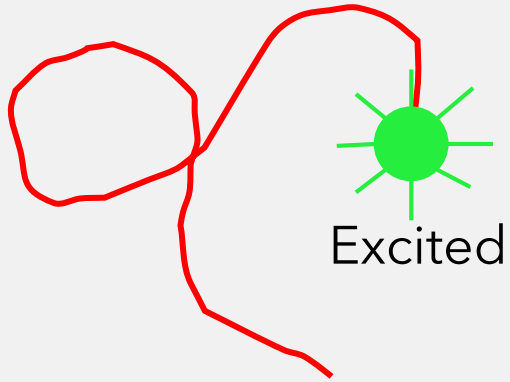




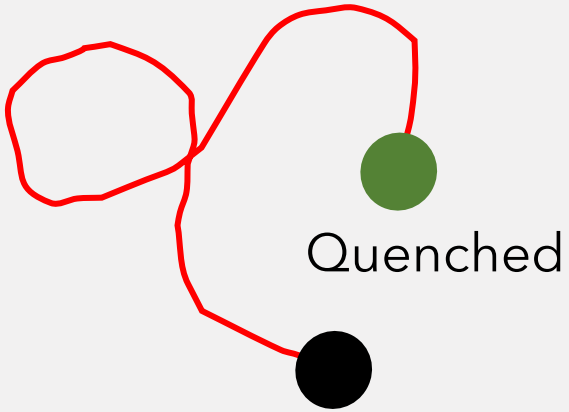
Plasmid-Oligo Binding Model



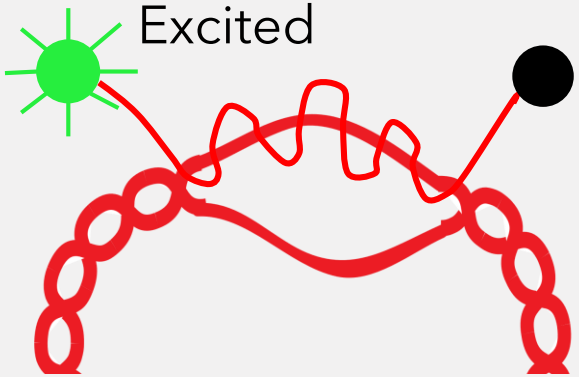
Future Investigations: Developing a new probe



Current Probe

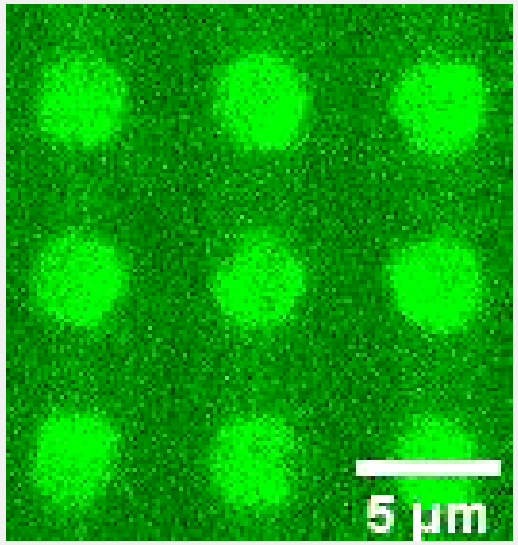


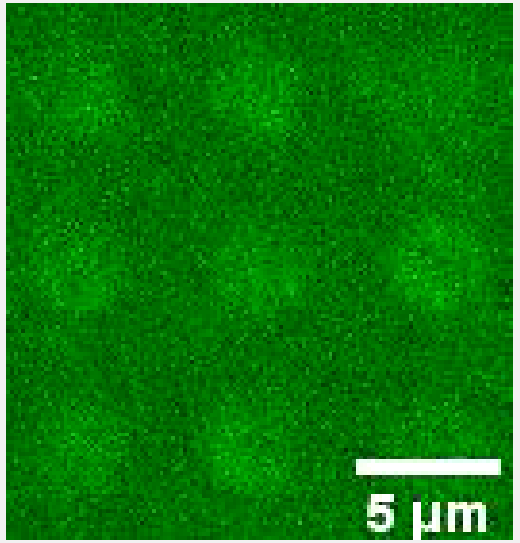
Molecular Beacon

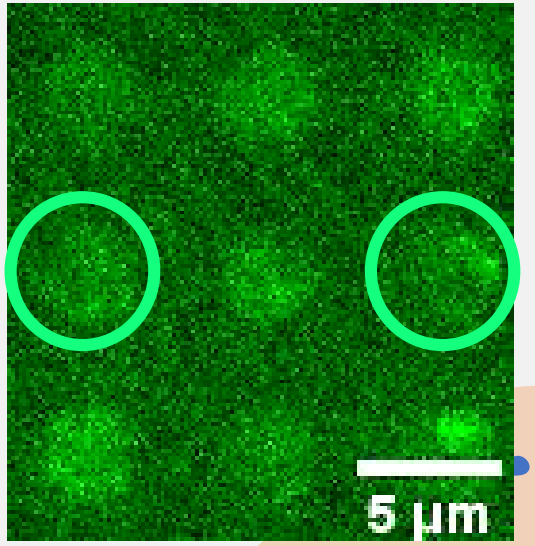


Beacon-plasmid Binding

50 nM of probes in pits







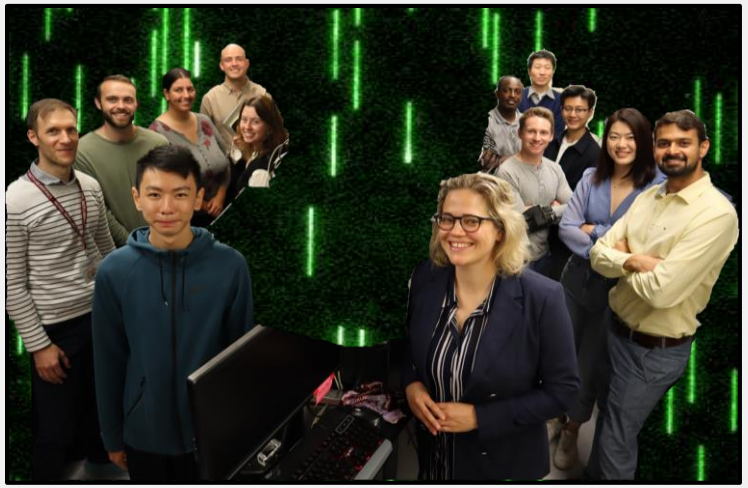
Conclusion

Supercoiled DNA drives structural transitions

These transitions compete among each other

CLiC can help us understand the dynamics of these competitions

Acknowledgements



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