The Abdus Salam
International Centre for Theoretical Physics

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Educational, Scientific and
Cultural Organization

IAEA
International Atomic Energy Agency

2038-24

Conference: From DNA-Inspired Physics to Physics-Inspired Biology

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Computer Simulations of DNA Topology at the Atomic Level

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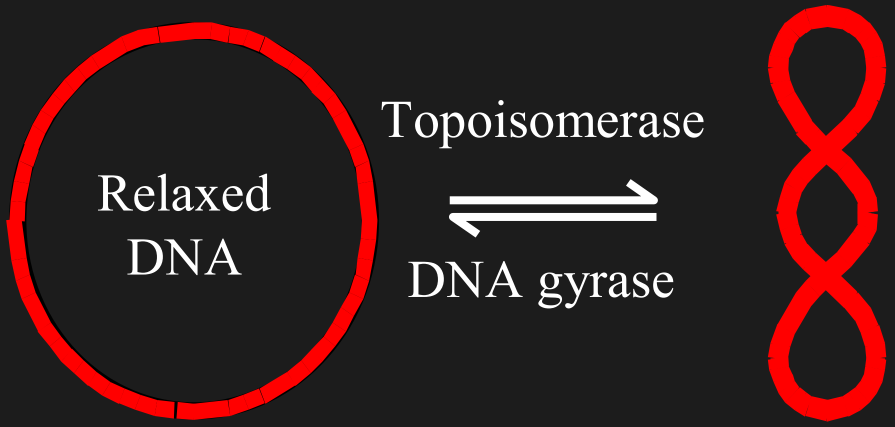
DNA Supercoiling and Packing

In chromosomes, 1-20cm of DNA is densely packaged into only ~1-10µm



Relaxed DNA

Topoisomerase

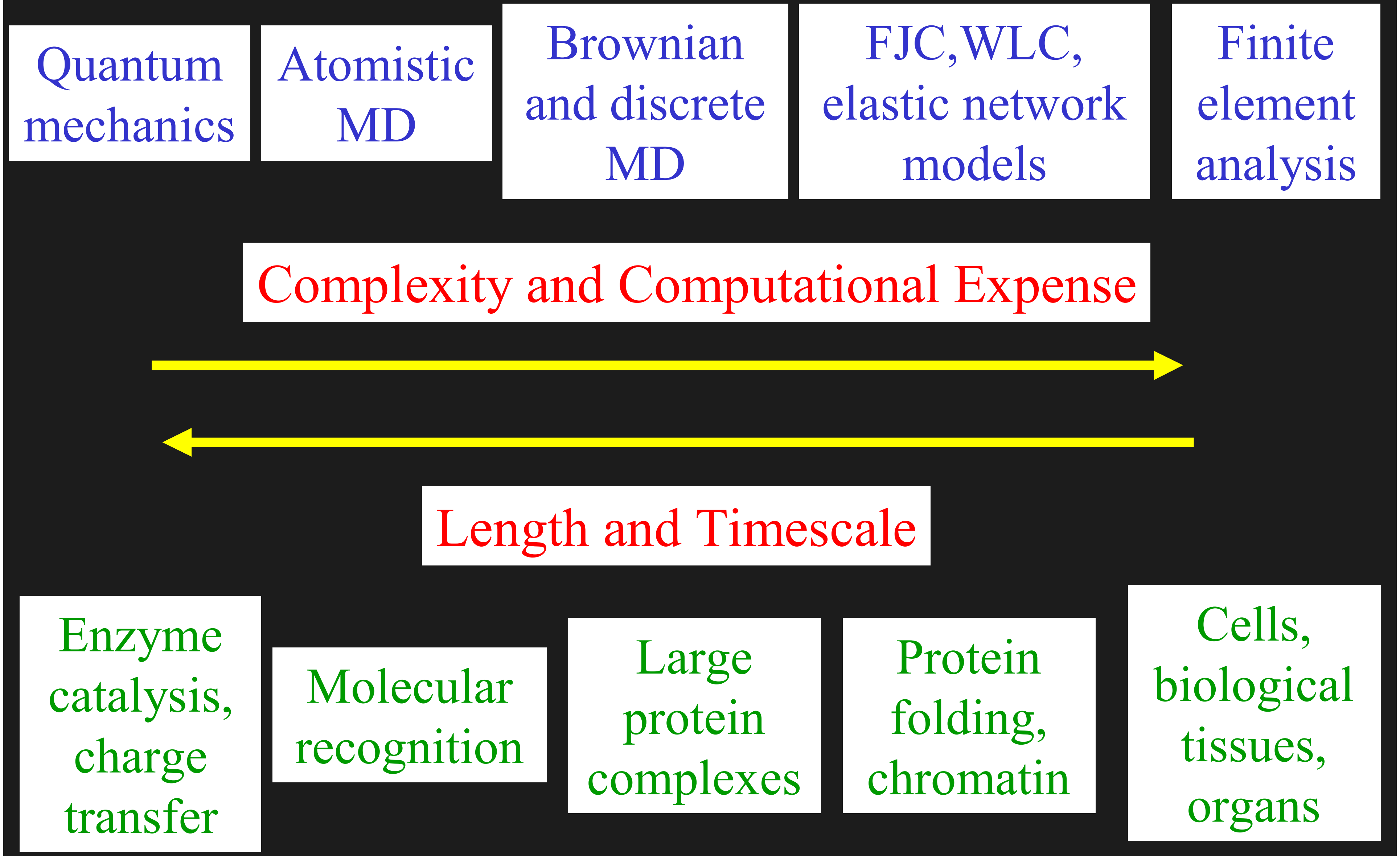
DNA gyrase

Supercoiled DNA

Bacterial DNA is compacted by supercoiling the circular plasmids into higher order helical structures.

Cellular DNA is generally under-wound ~ only thermophiles contain enzymes which can over-wind DNA

The Hierarchy of Biological Models



Quantum mechanics

Atomistic MD

Brownian and discrete MD

FJC,WLC, elastic network models

Finite element analysis

Complexity and Computational Expense

Length and Timescale

Enzyme catalysis, charge transfer

Molecular recognition

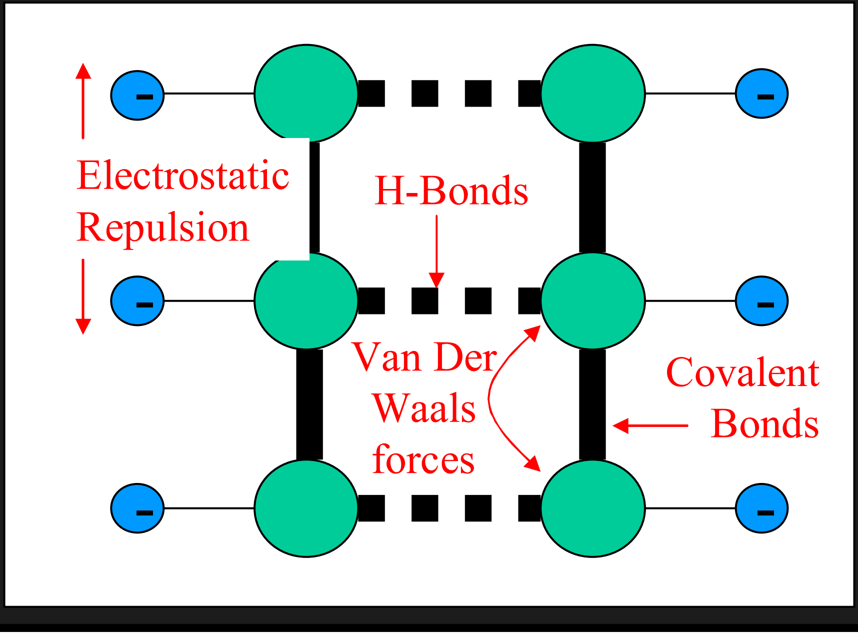
Large protein complexes

Protein folding, chromatin

Cells, biological tissues, organs

Classical (MD) with AMBER

MD represents all of the atoms as classical "balls" possessing atom types which mimic the chemistry of the biomolecule.



$$U_{\text{Total}} = \sum_{\text{Bonds}} K_r \left(r - r_{eq}\right)^2$$

Bonds

$$+ \sum_{\text{Angles}} K_\theta \left(\theta - \theta_{eq}\right)^2 +$$

Angles

$$\sum_{\text{Dihedrals}} \frac{V}{2}\left[1 + \cos(\eta\phi - \gamma)\right] +$$

Dihedrals

$$\sum_{\text{Atoms}} \varepsilon_{ij} \left[\left(\frac{R_{ij}}{r_{ij}} \right)^{12} - \left(\frac{R_{ij}}{r_{ij}} \right)^{6} \right] +$$

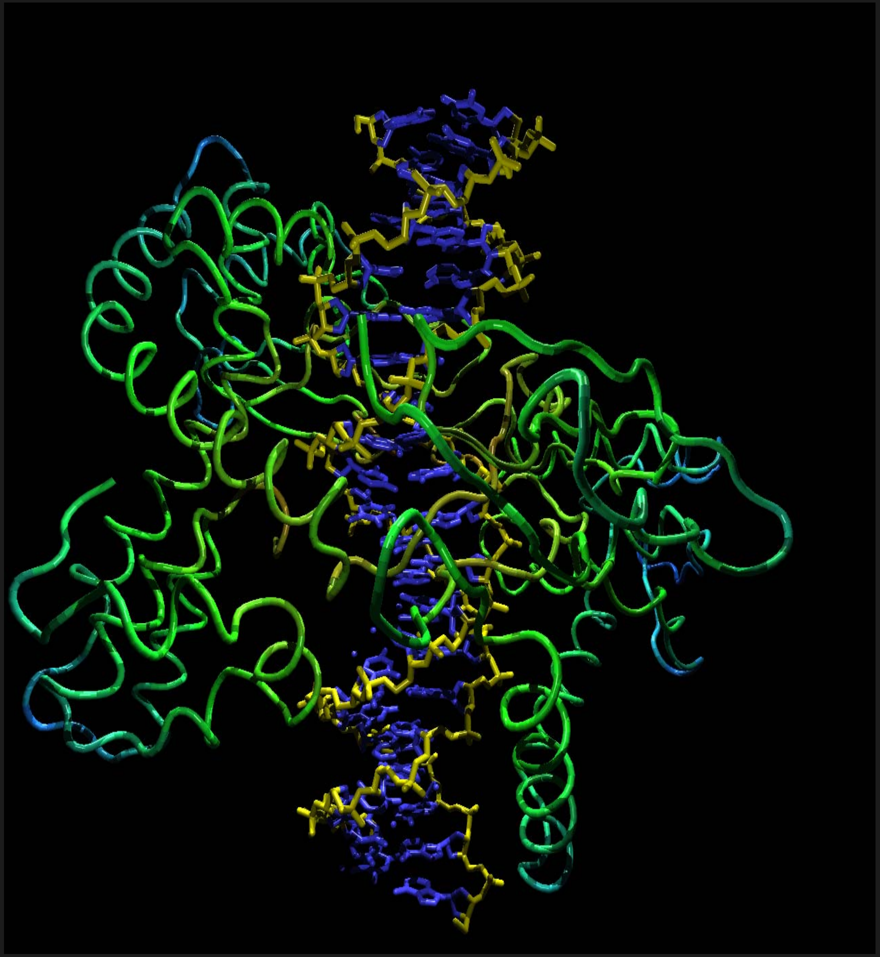
Van der Waals

$$\sum_{\substack{\text{Partial} \\ \text{Charges}}} \frac{q_i q_j}{\varepsilon r_{ij}}$$

Electrostatics

Simulations use the AMBER (BSC0) forcefield with either TIP3P water and PME or the GB/SA implicit solvent model

Topoisomerases



Topoisomerase I (single strand breaks)

Topoisomerases control supercoiling by changing the number of times the two strands wrap around each other

Energy (ATP) is required to increase levels of torsional stress and induce supercoiling

Looped and Circular DNA Structures

The ability of DNA to form either looped or circular DNA structures depends on the bending rigidity of the duplex.

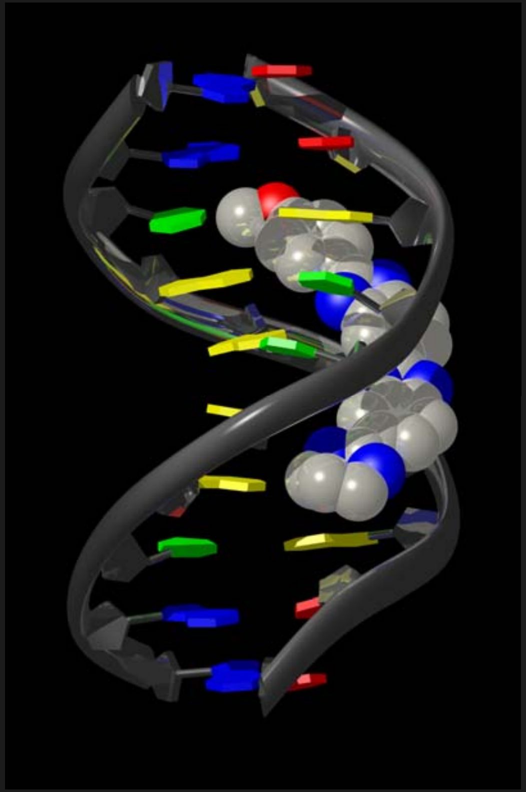
The CAP/DNA complex

DNA circles as small as 60 base pairs have been reported experimentally[1].

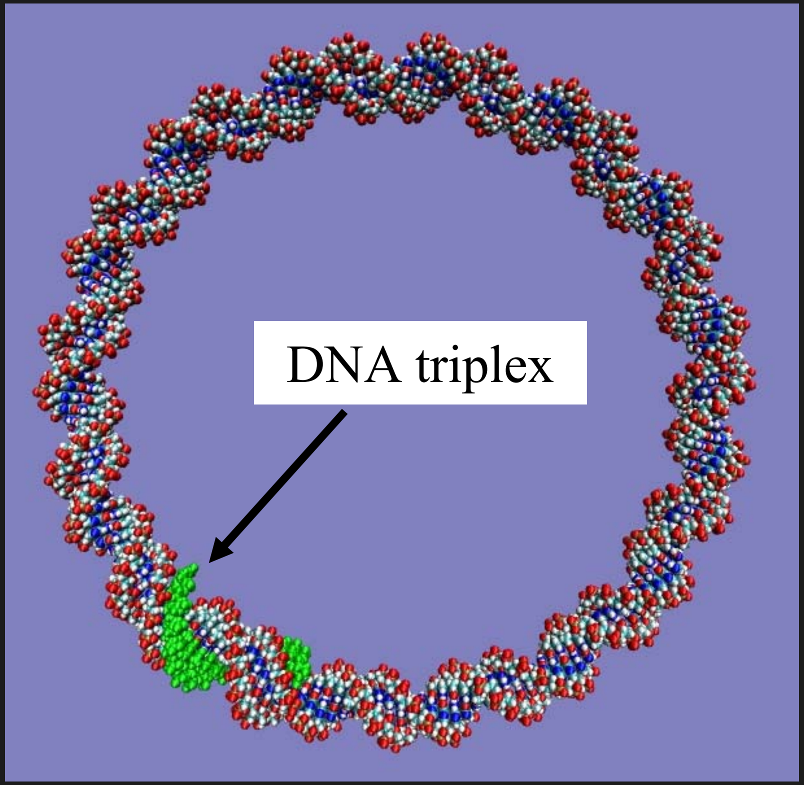
Supercoiling dependent transcription has been studied in circles containing only ~500 base pairs[2].

1. Du *et al* (2008) *Nucleic Acids Res.* 36, 1120.
2. Qureshi, M. *et al* (1997) Biochemistry 36, 12303-16.

Experimental Structural Studies of DNA



BUT



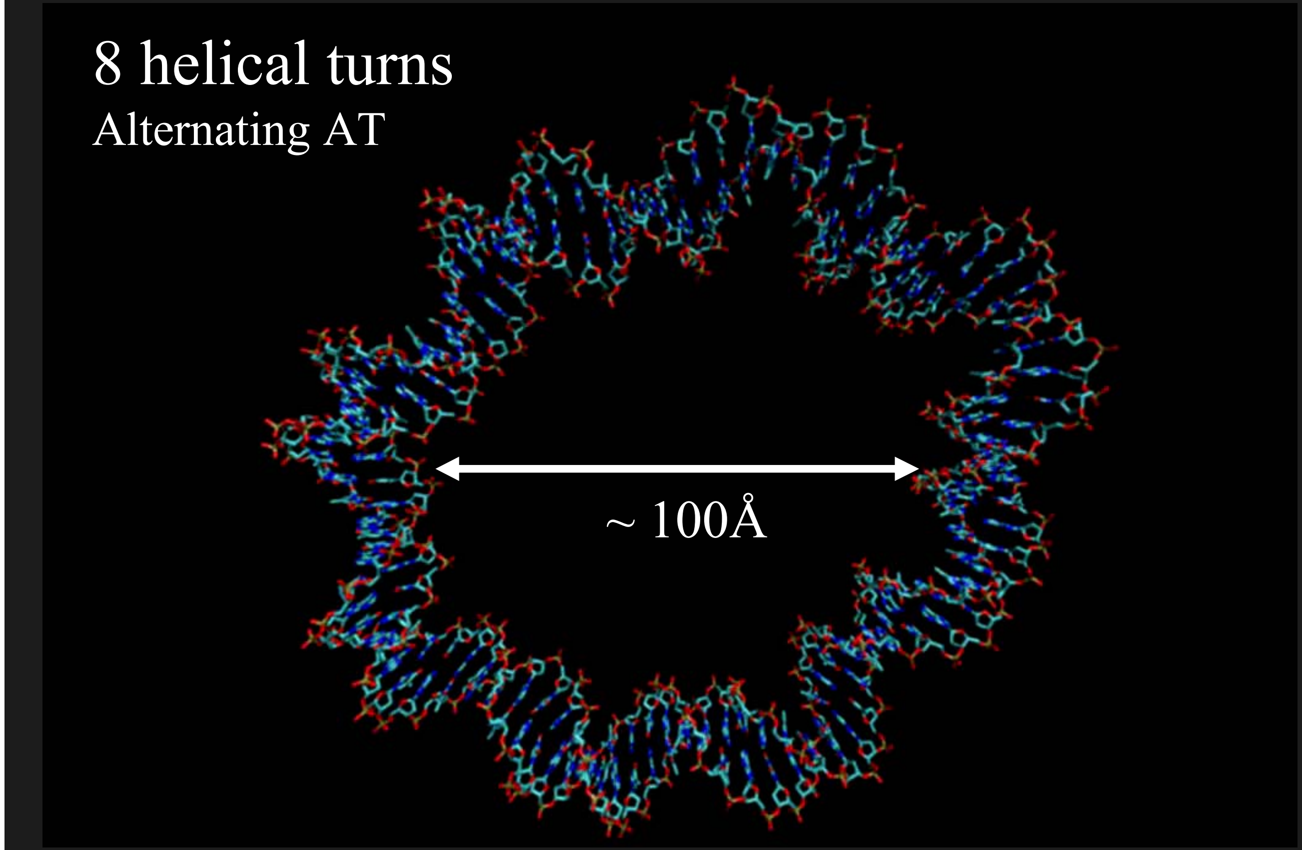
A drug (Hoechst 43254) bound to protein bound to TAAT[1]

Triplex formation is more efficient in negatively supercoiled plasmids[2]

1. Bostock-Smith *et al* (2001) *Nucleic Acids Res.* 29, 693-702
2. Maxwell *et al* (2006) Nucleic Acids Res. 34, e104 .

Atomistic MD Simulations of 90bp DNA Circles

A circle containing ~ 90bp + solvent consists of ~150,000 atoms
1ns takes ~ 48hrs on 32 processors of the UK NGS.

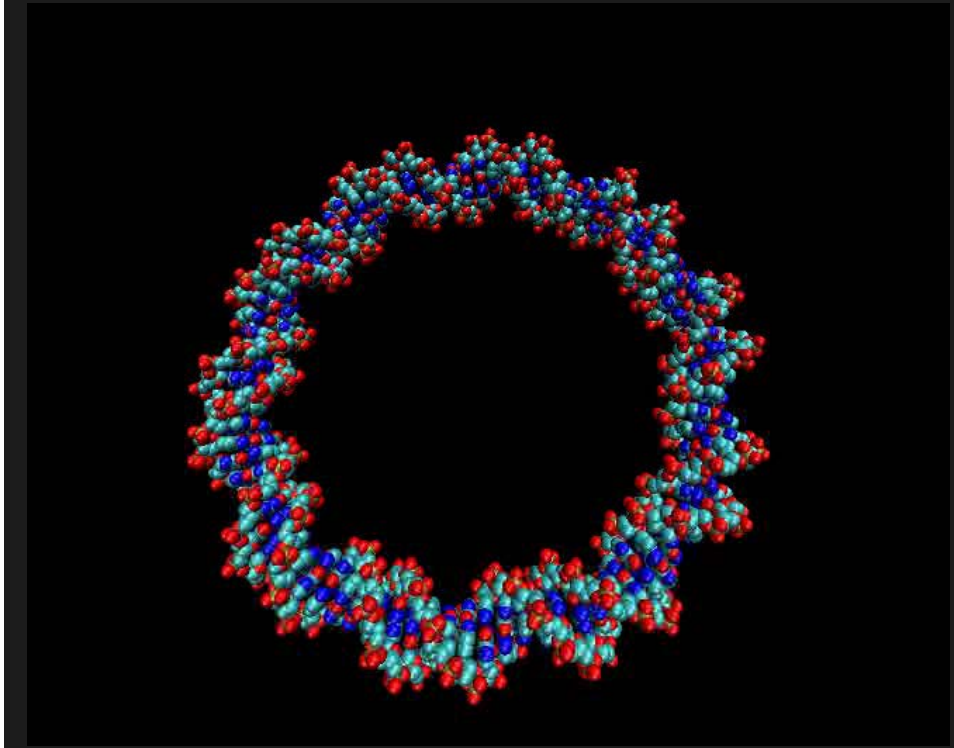


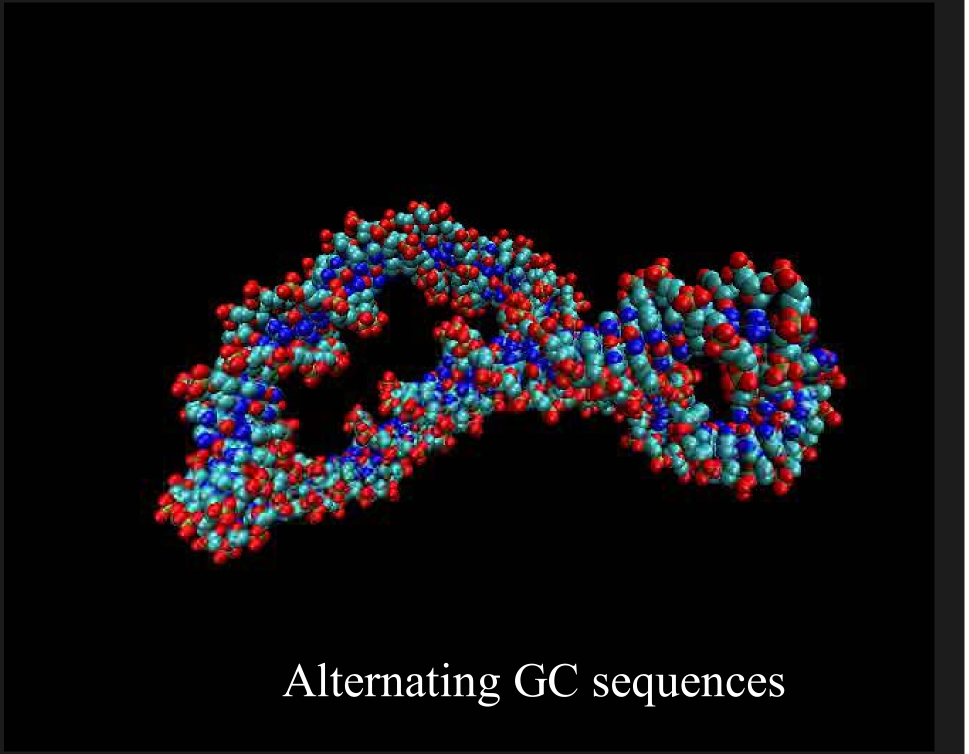
See also Lankas *et al* (2006) *Structure* 14, 1527.

90bp of DNA possesses 8.5 helical turns when torsionally relaxed

Over-twisted DNA Circles

DNA overtwisted by 1.5 turns undergoes a buckle instability (~20ns) to form a writhed structure which relaxes when one strand is nicked.

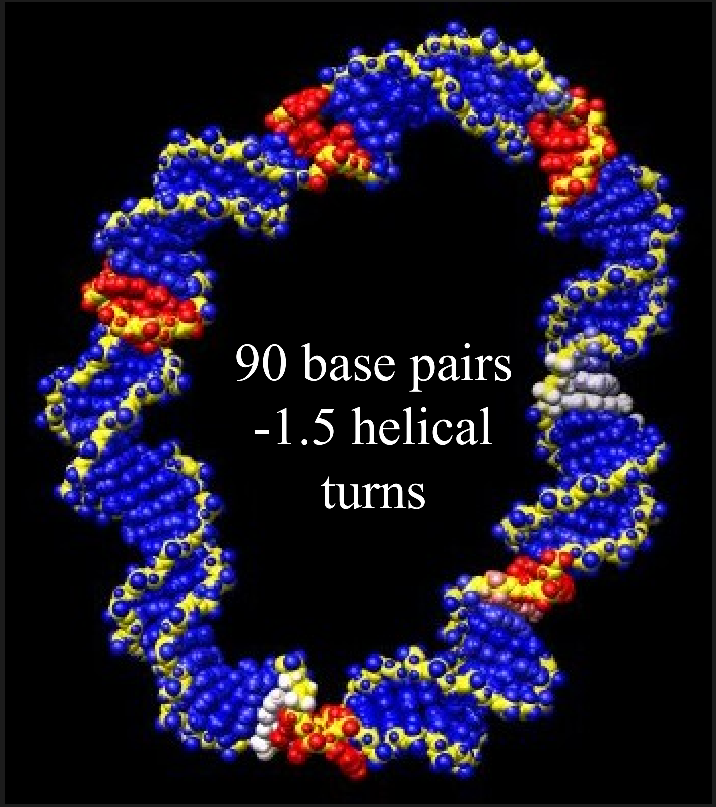




Alternating GC sequences

Under-twisted DNA circles

We observe a distinct asymmetry between over- and under-wound DNA nano-circles.



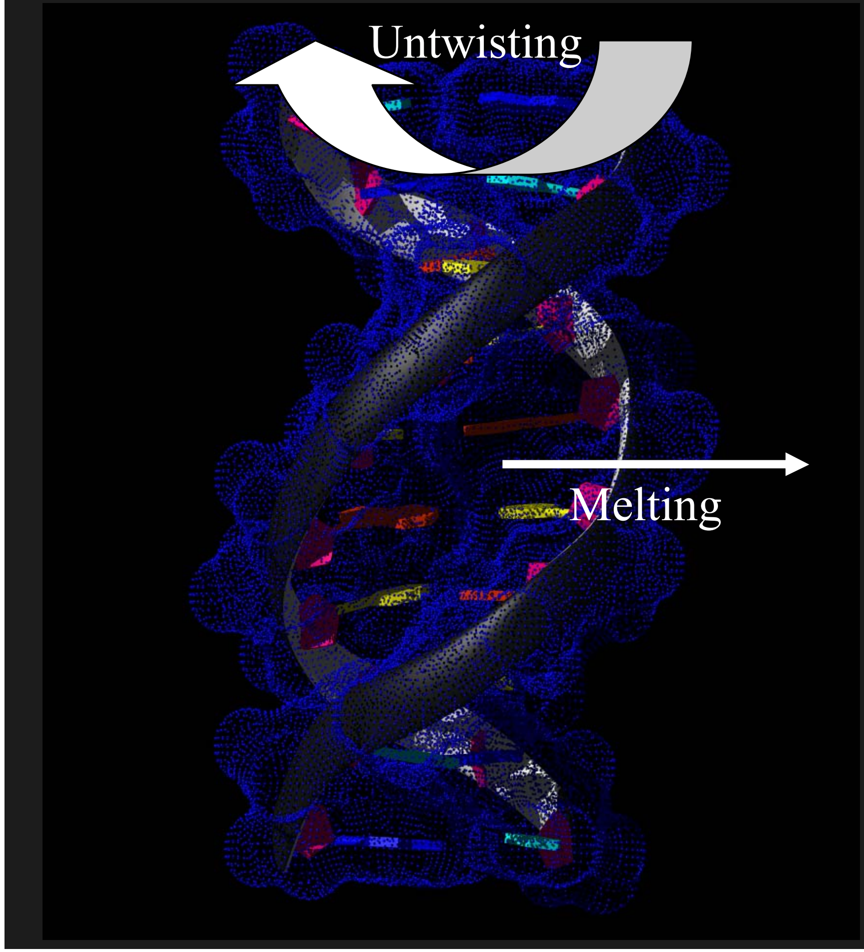
Equivalently under-wound DNA structures denature whenever the minor groove is on the inside of the circle.

Bending the DNA into a circle breaks the helical symmetry of the linear molecule ~ increasing structural diversity.

Could this be used to *in vivo* to fine tune genetic control?

Twisting, Supercoiling and Transcription

Bacteria placed under stress respond using dramatic changes in the levels of supercoiling to alter gene expression.



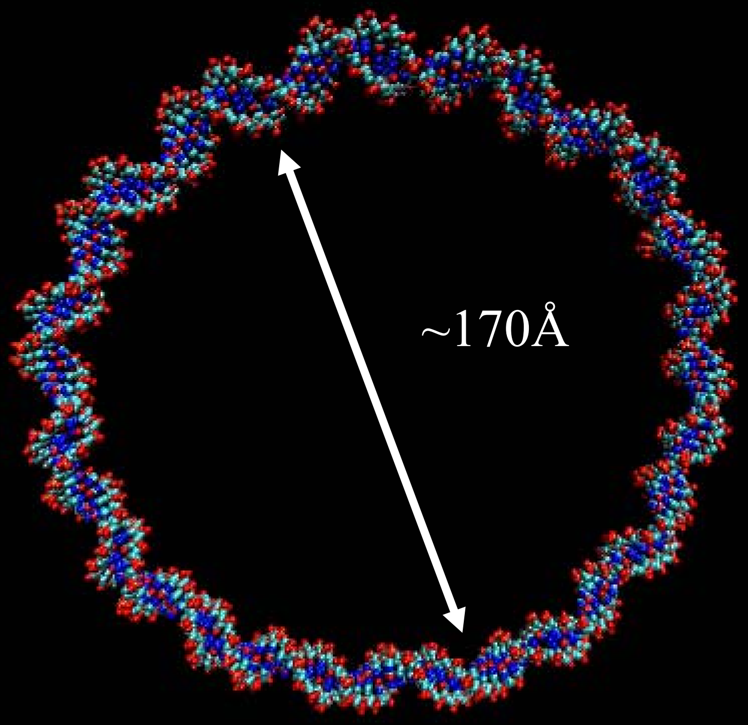
Under-winding is thought to promote cellular processes which require strand separation (eg transcription, translation, replication) by destablising the duplex.

Untwisting weakens the base stacking and leads to strand separation.
Over-winding does not.

The Importance of the Environment 1

Under-winding lead to negative supercoiling only for larger circles (>118 base pairs) in high salt conditions (1M GB/SA)

178 base pairs at -2 turns in 1M salt

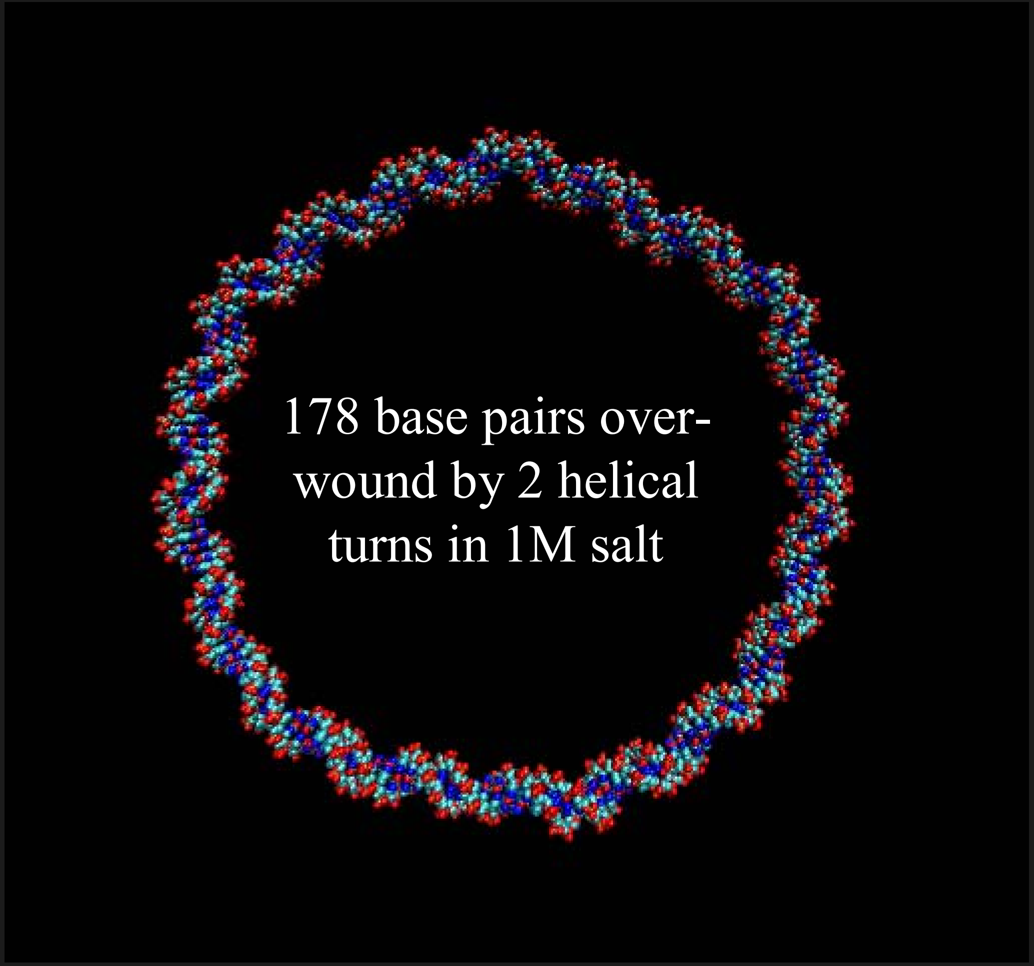


Simulations use an implicit solvent model

Reducing the salt to 0.01M (GB/SA) leads to rapid unwinding due to electrostatic repulsion between crossed DNA strands

Caution: Implicit solvent models give correct structures but poor dynamics due to a lack of solvent friction

The Importance of the Environment 2

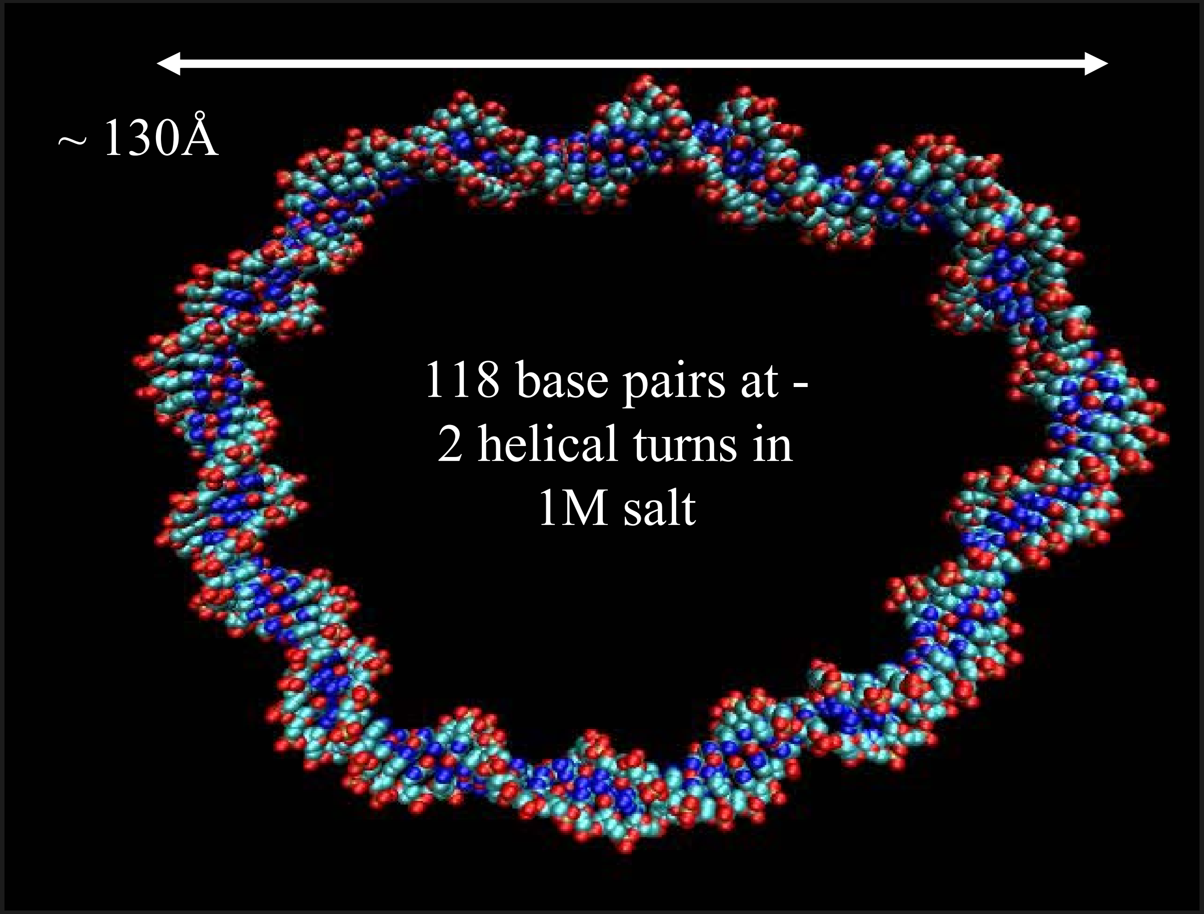


178 base pairs over-wound by 2 helical turns in 1M salt

The over-wound 178 base pair circle at 1M salt forms a supercoil with two crossing points.

When the salt is reduced to 0.01M the circle refolds to form a structure containing only one crossing point.

“Frustrated” DNA Circles



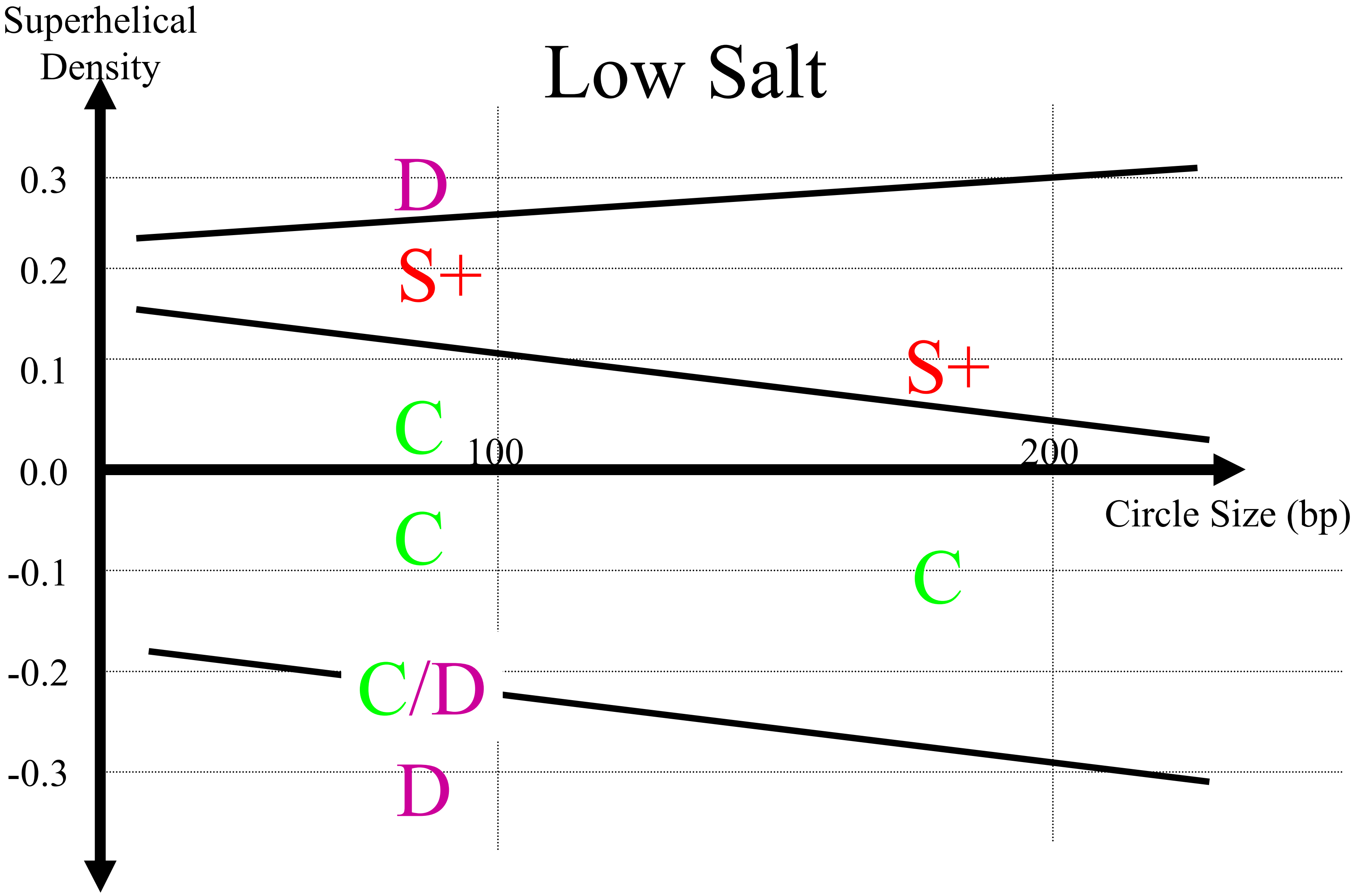
Supercoiling relieves torsional stress but requires sharp bends at the apices

This bending stress is larger for smaller circles

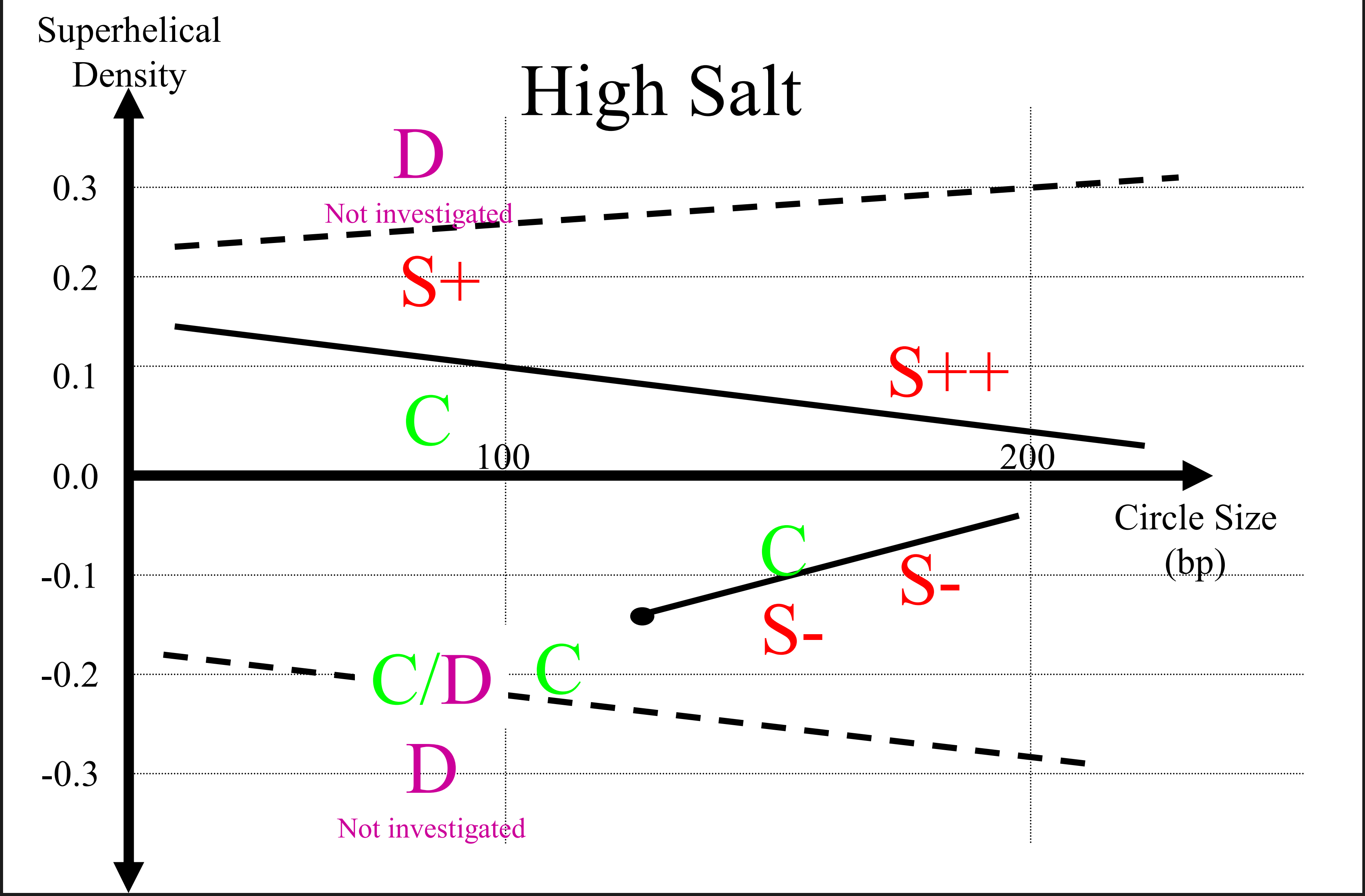
These terms almost balance for circles of 118 base pairs.

This system undergoes large thermal fluctuations between circular and supercoiled structures

A Phase Diagram for Nanocircle Topology 1



A Phase Diagram for Nanocircle Topology 2



Thermodynamics and Supercoiling

Supercoiling is easier when:

i) The circles are larger ~ bending energy at the apices is reduced (end loops can be larger)

ii) The salt concentration is high ~ better electrostatic screening

iii) Circles are over-wound rather than under-wound

Harris, Laughton, Liverpool *Nucleic Acids Res.* **36,** 21-29, 2008

Liverpool, Harris, Laughton *Phys. Rev. Lett.* **100,** 238103, 2008

Can we use our theoretical methods to quantify the changes in enthalpy and entropy when supercoils form?

Thermodynamics from Experiment

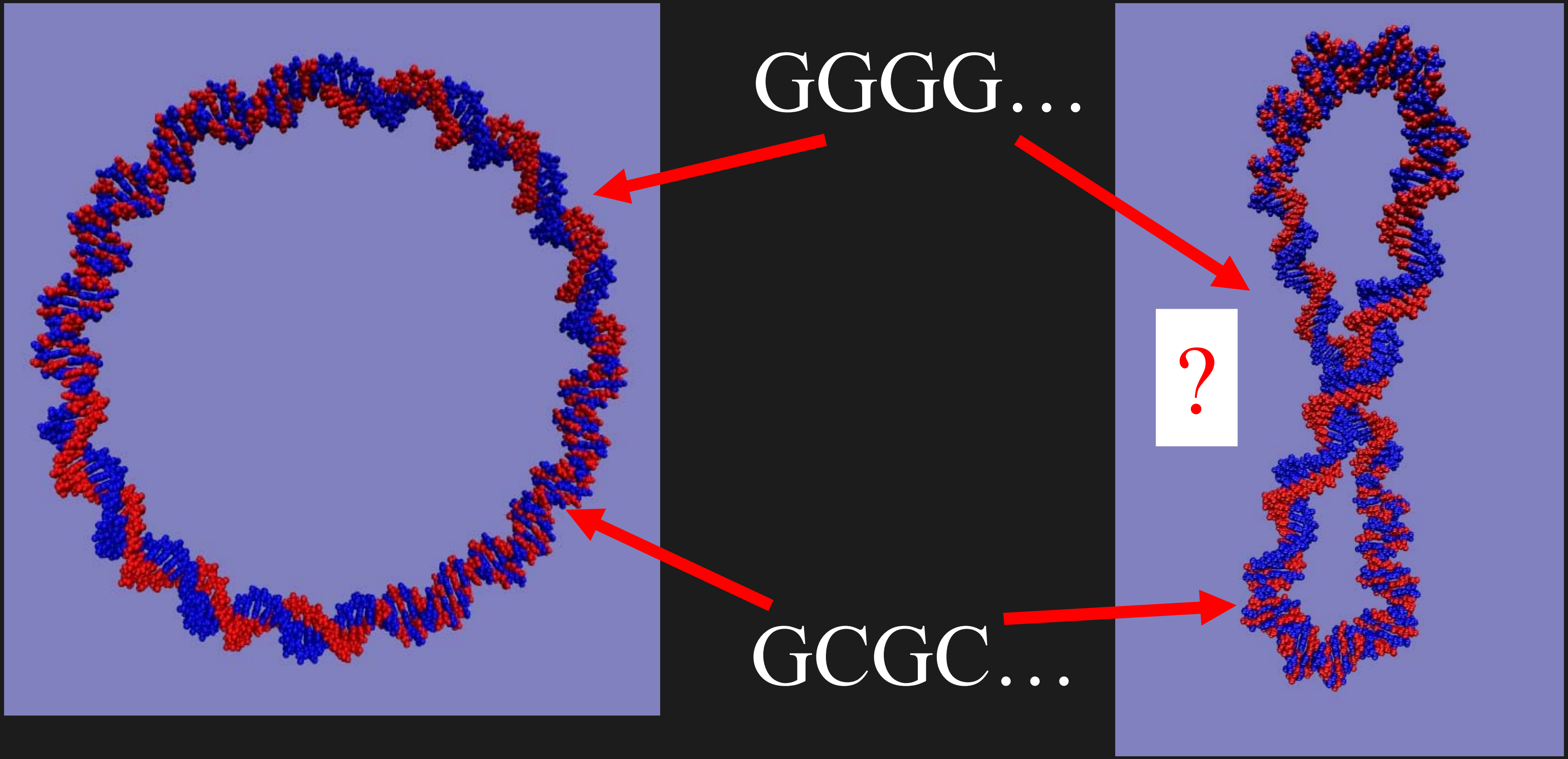
Experimental measurements show that 178 base pairs negatively supercoils in high salt, but that topoisomerase can only supercoil sequences larger than 148 base pairs[1]

Larger DNA sequences supercoil more easily experimentally, as we also observe in the simulations

Future calculations aim to make a quantitative comparison of the thermodynamics

1. A. D. Bates & A. Maxwell (1989) EMBO, 8, 1861.
2. Bednar *et al* (1994) J. Mol. Biol. 235, 825.
2. Fogg, J.M. *et al* (2006) J. Phys. Cond. Mat., 18, S145-S159

“Programmable” Sequences



GGGG…

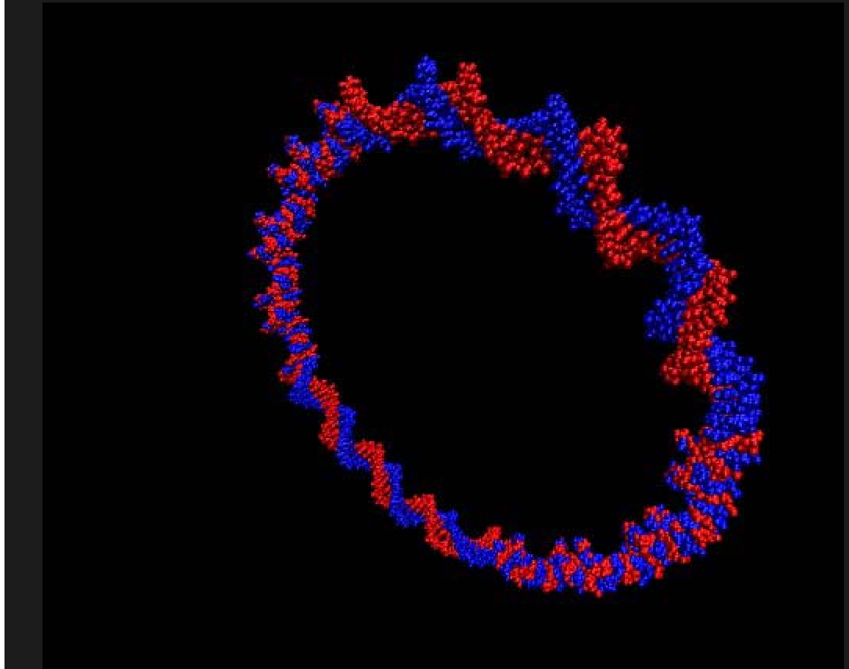
?

GCGC…

The alternating GCGC… sequences are less rigid than GGGG due to differences in base stacking.

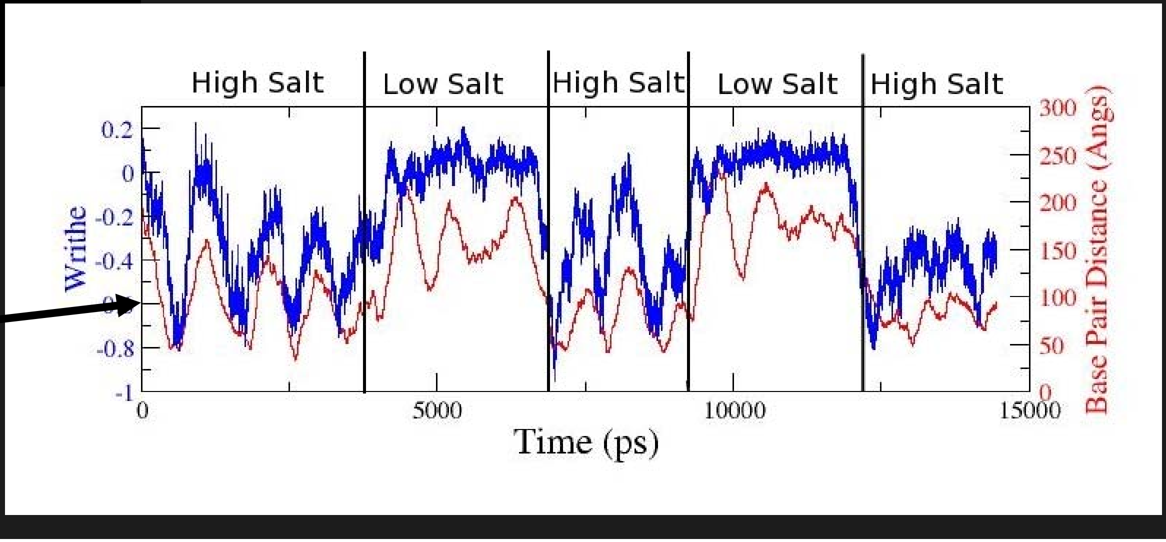
Lankas *et al* (2003) *Biophys J.* **85**, 2872

A Designed DNA Topology



The DNA consistently “folds” so that the flexible sequence lies at the apices

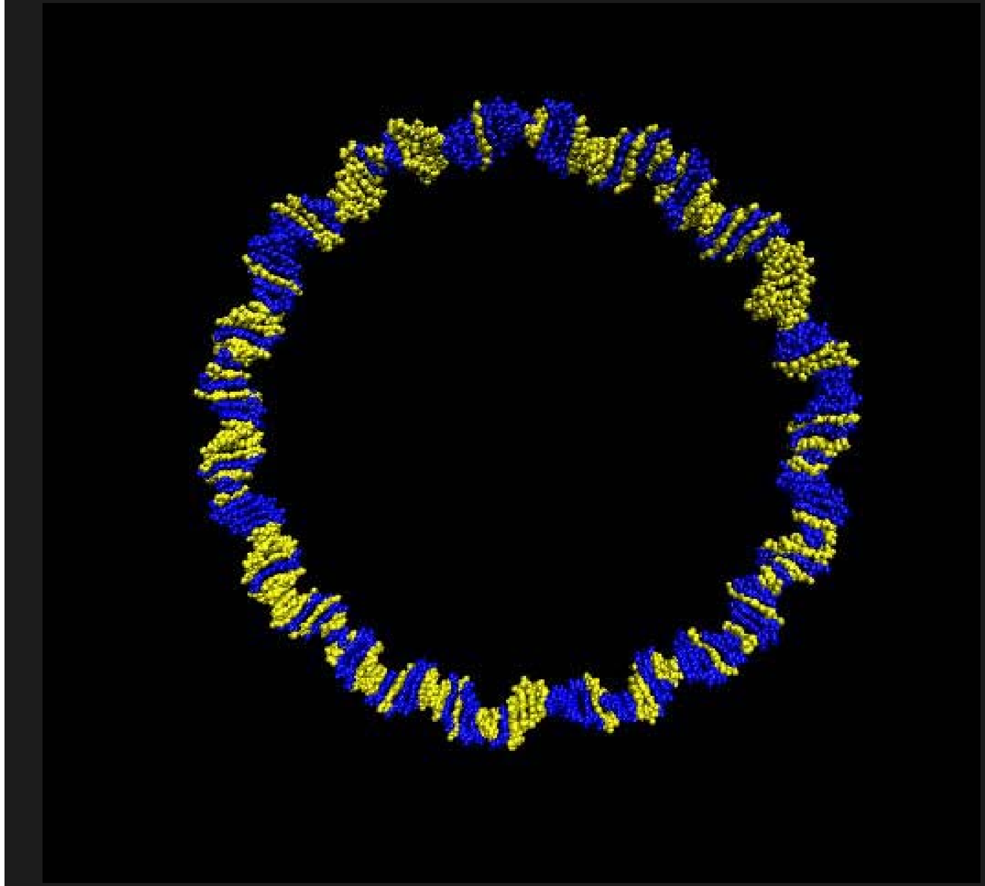
Guanine: Red
Cytosine: Blue

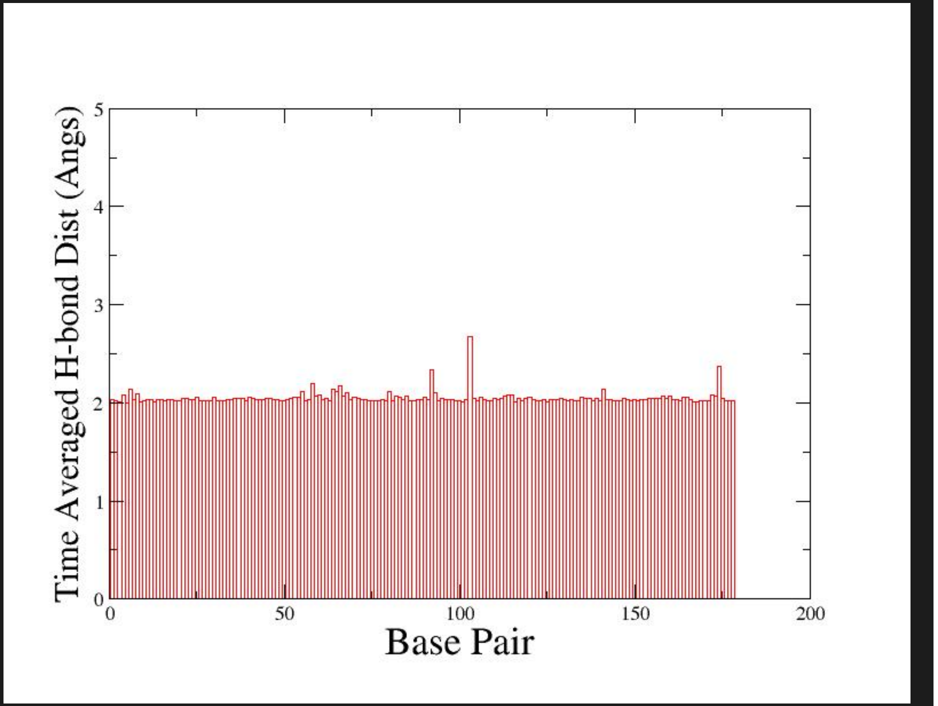


Distance between bases at the crossing point (red line)

Mixed Sequences

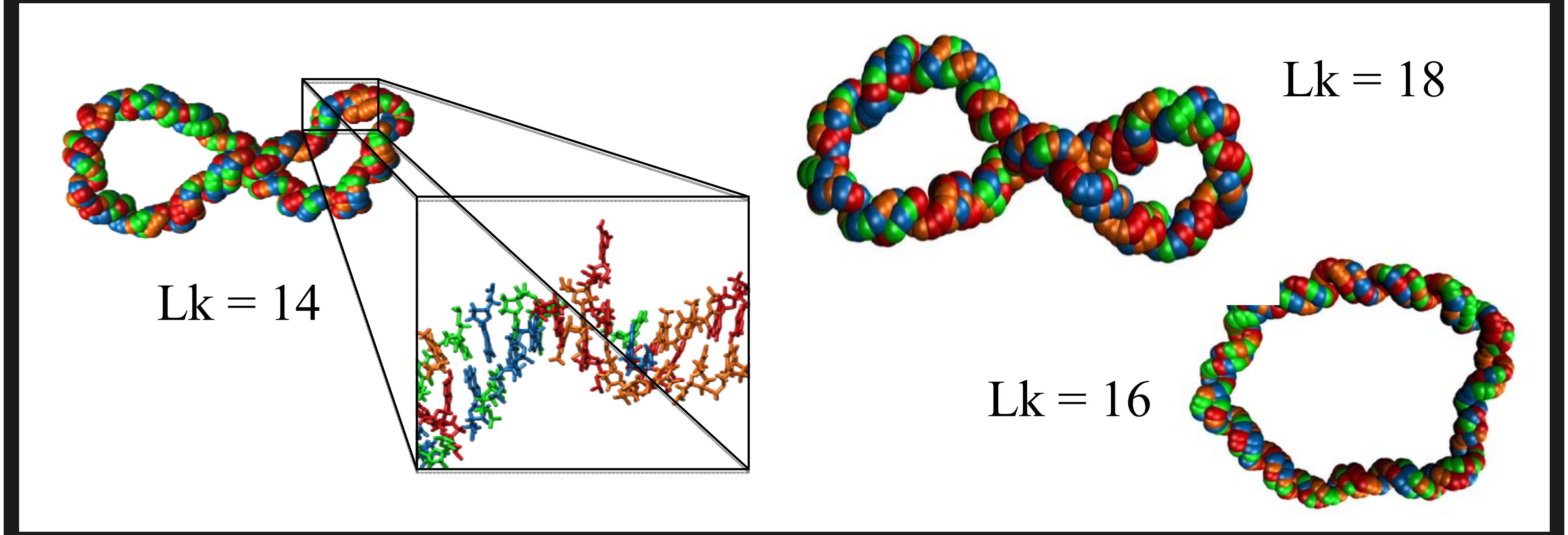
AT rich regions have a tendency to denature during writhing and require careful equilibration

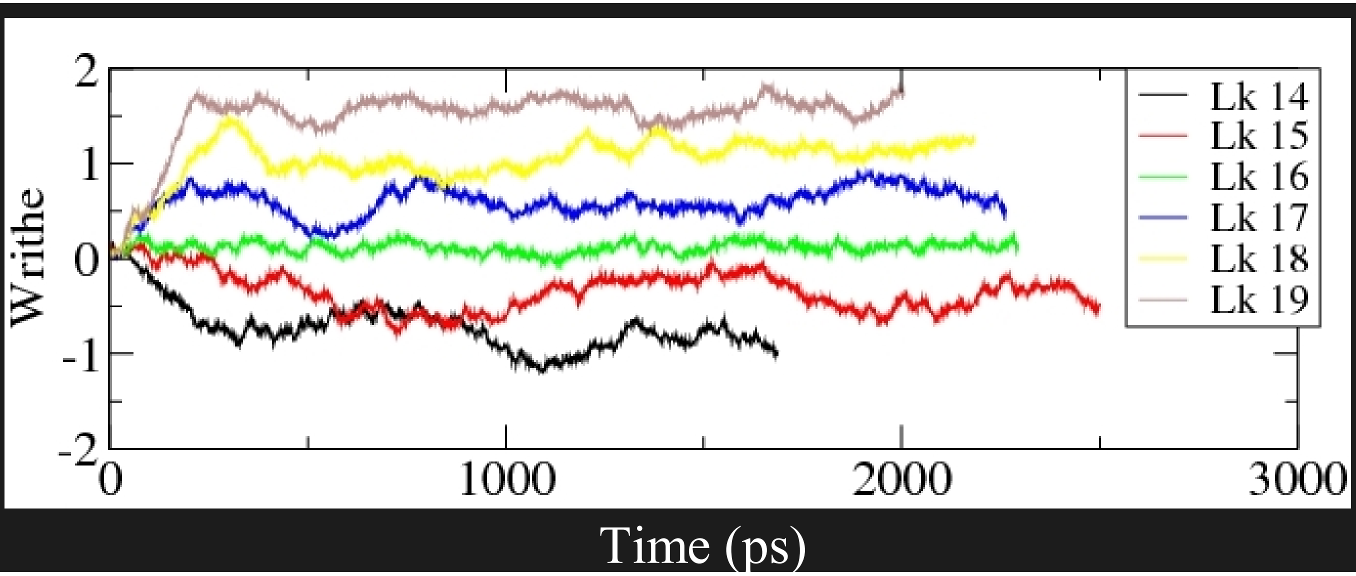




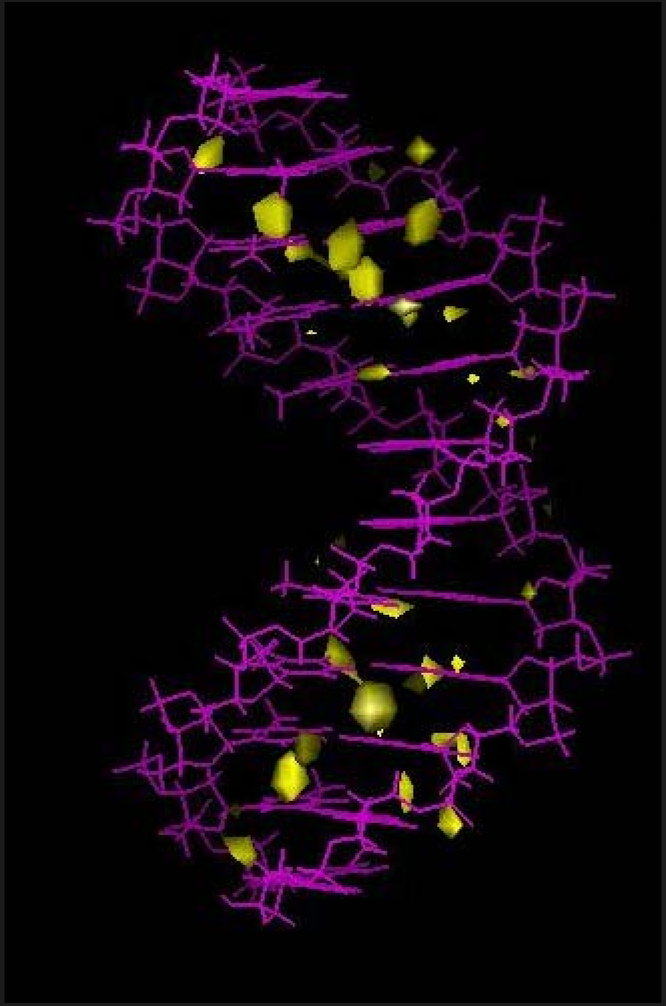
Adenine/Thymine: Yellow
Guanine/Cytosine: Blue

Topoisomers of a Mixed Sequence





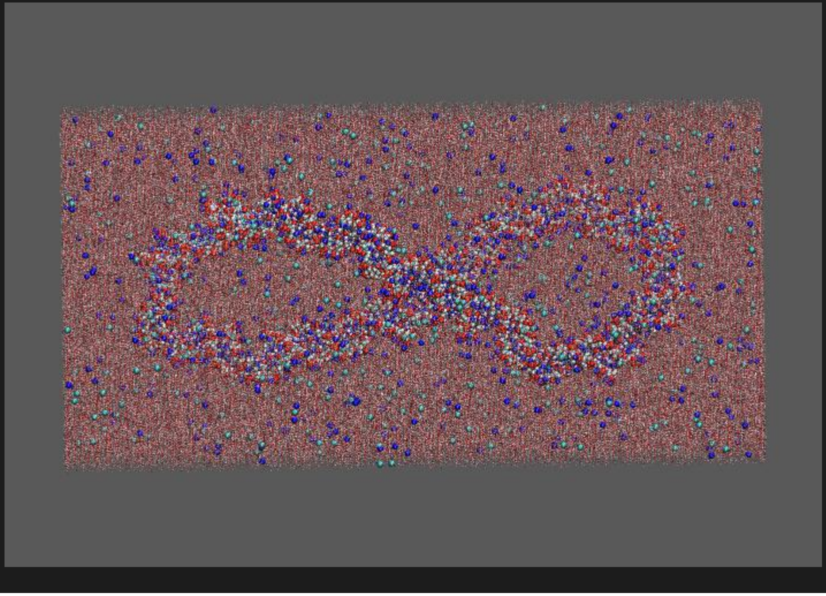
The Spine of Hydration



The spine of hydration in AT-rich DNA

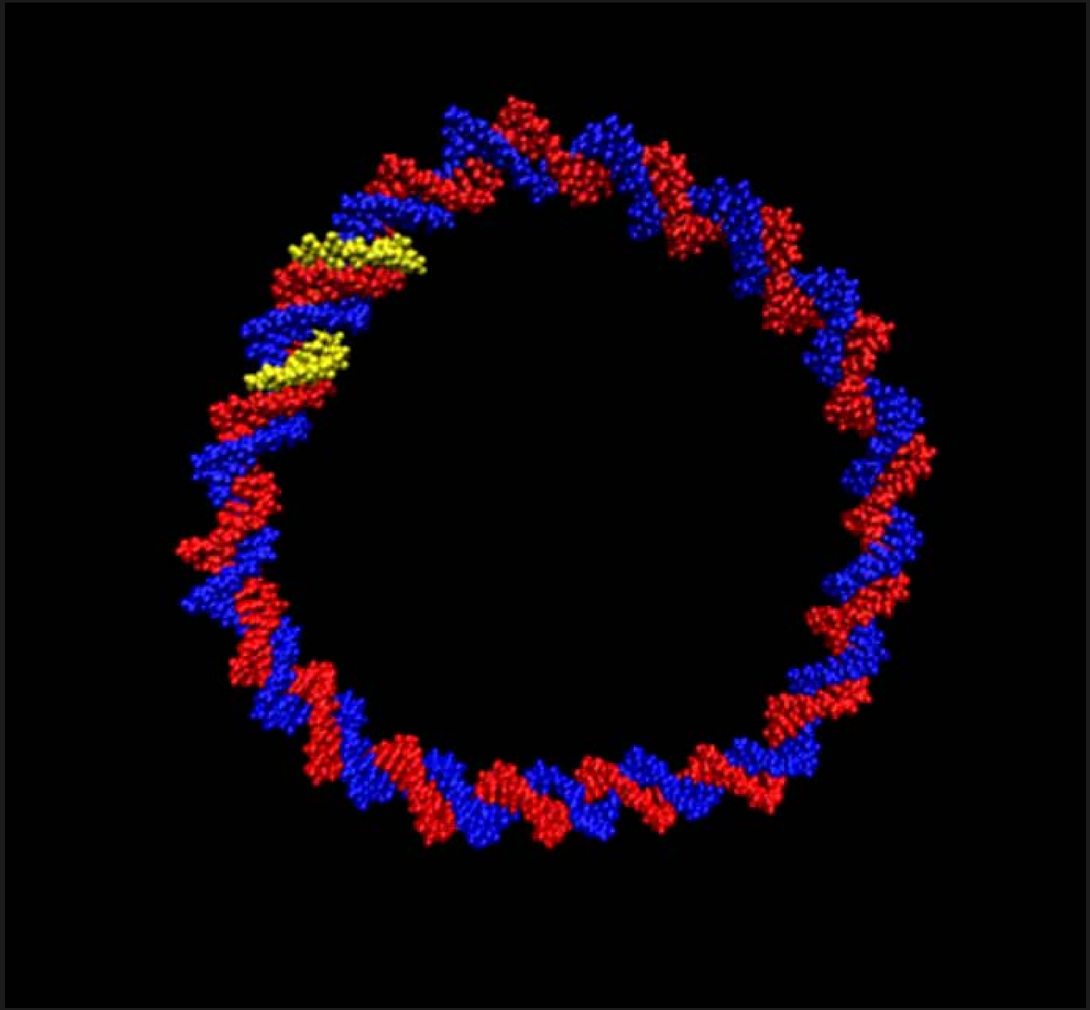
A run of AT bases in linear DNA will have a narrow minor groove ~ this is responsible for the "Spine of Hydration"

But what happens when the DNA is supercoiled?



Trial run in explicit solvent ~ 1 million atoms
~128 processors on Hector UK supercomputer

DNA recognition and Supercoiling



Concluding remarks

Writhing is favoured by:

1) Larger circles
2) High salt
3) More flexible sequences
4) Over-winding: Because under-winding leads more readily to denaturation

Thermal fluctuations can produce interesting effects at the nanoscale such as frustration and entropic allostery.

The lowest free energy state is often determined by a delicate balance of large but compensating terms.

This enables biomolecules to be highly responsive to changes in the environment ~ as is necessary for living systems