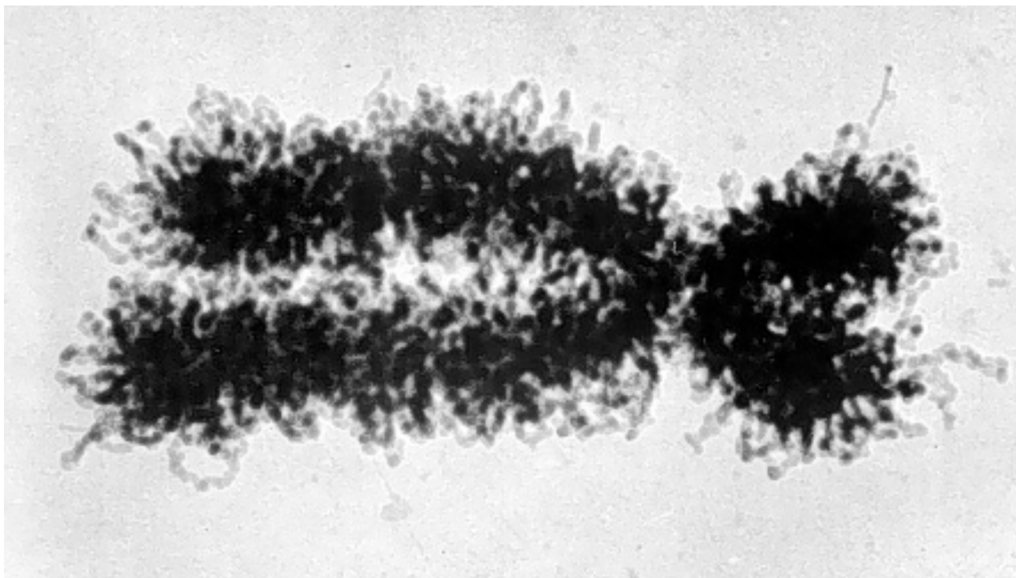
DNA Flexibility

Electron micrograph of a metaphase chromosome

•cm's of DNA

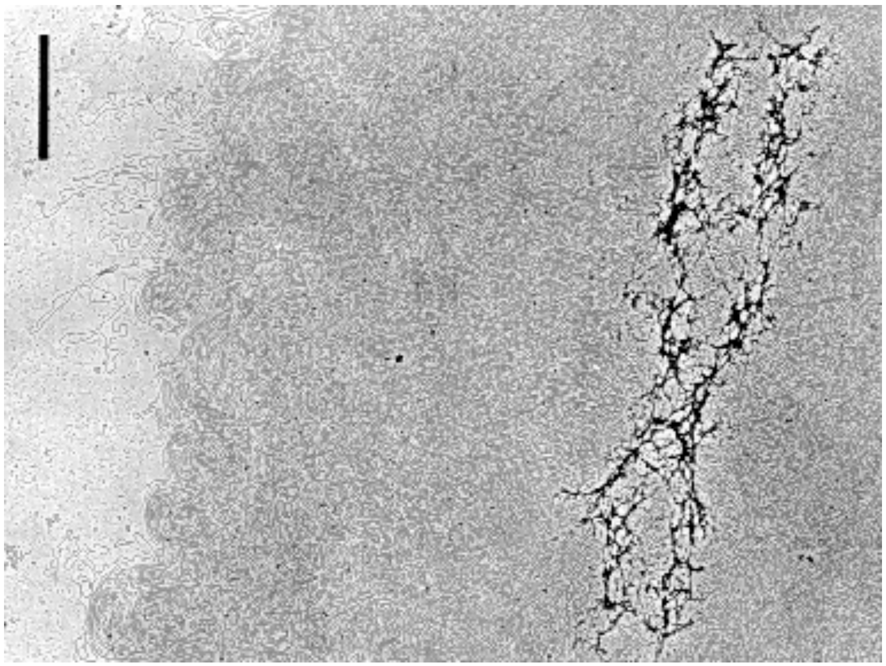


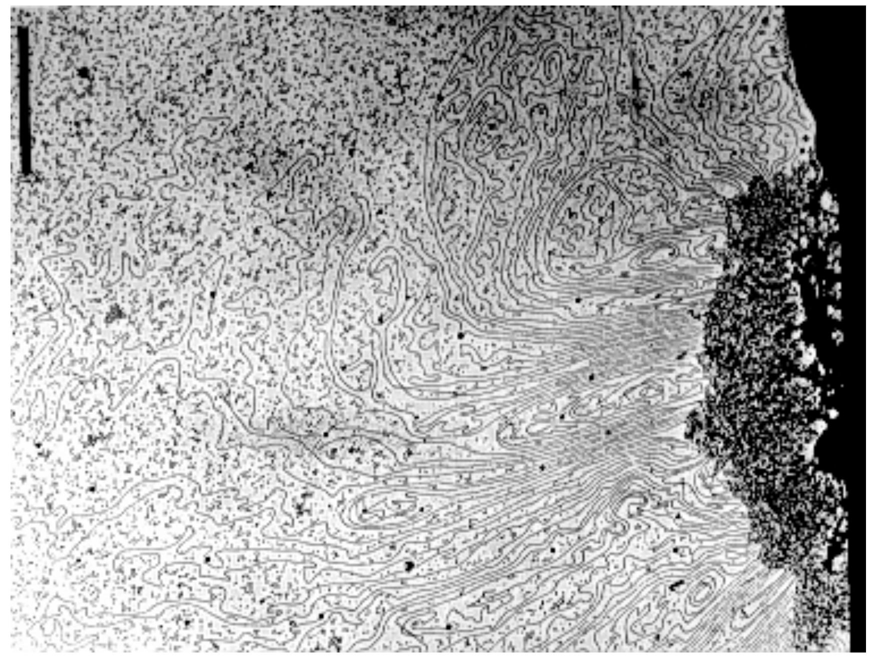
Few hundred nm's

few μ's

Marsden & Laemmli

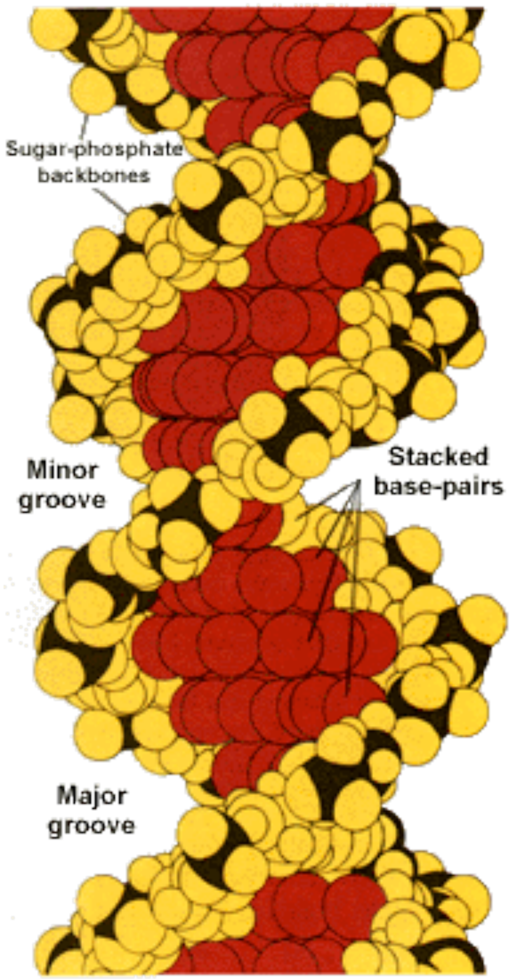
Metaphase chromosome, after removal of histones





Paulson & Laemmli

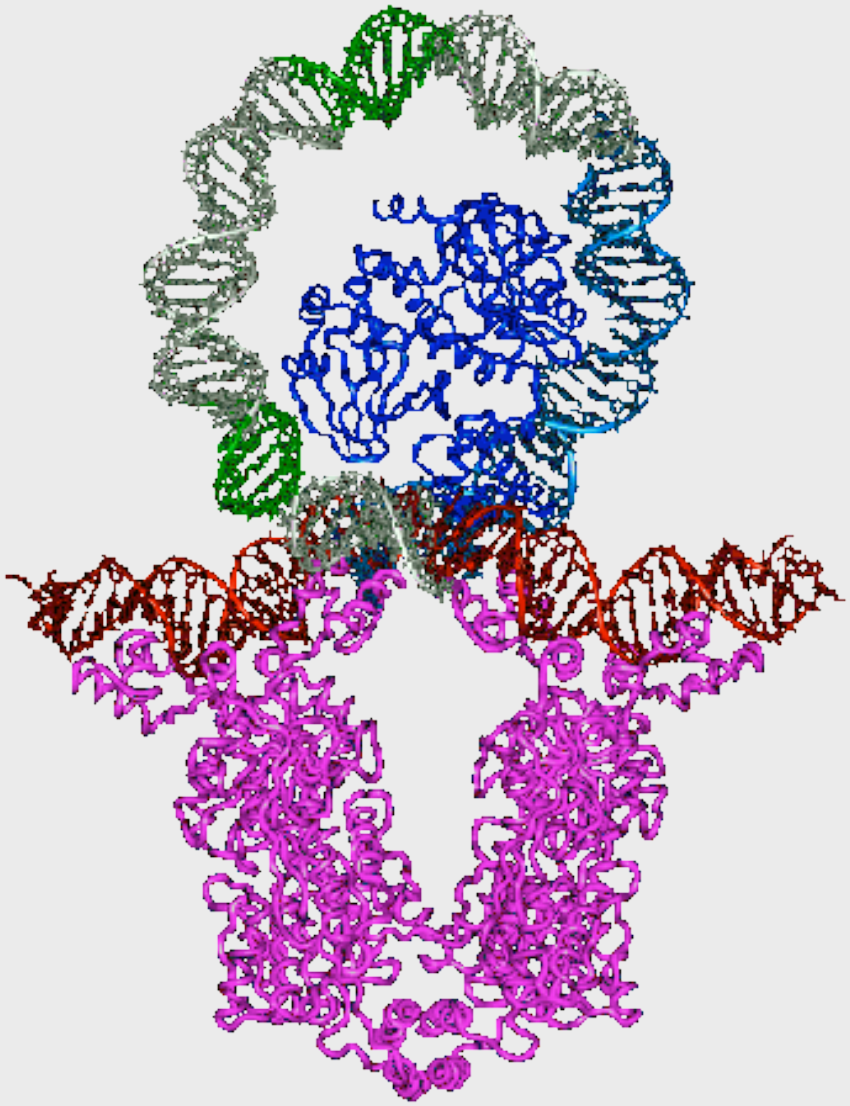
DNA is a stiff polymer



- Phosphate-phosphate repulsion
- Hard sphere repulsions of bases

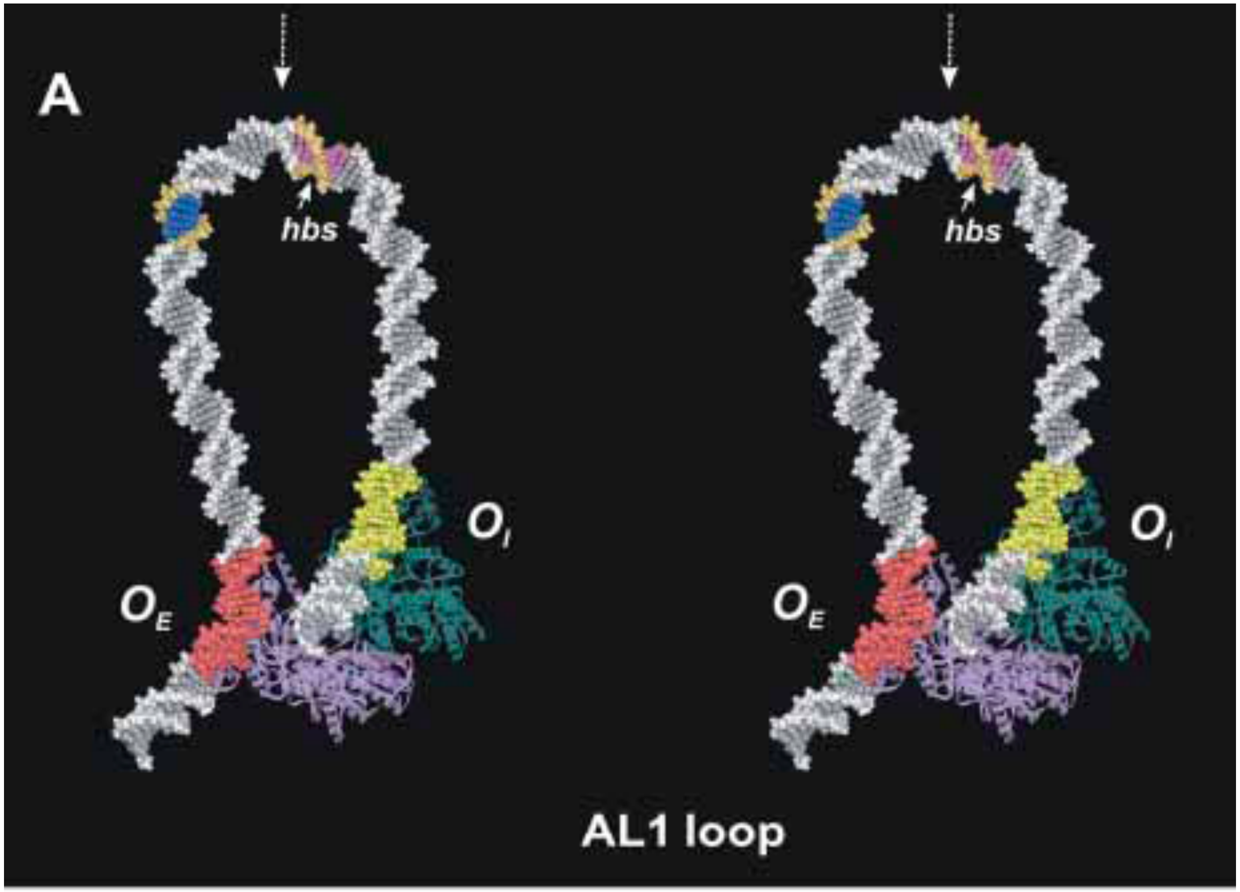
Alberts et al., 4th ed.

Sharply looped DNA in the *lac* operon



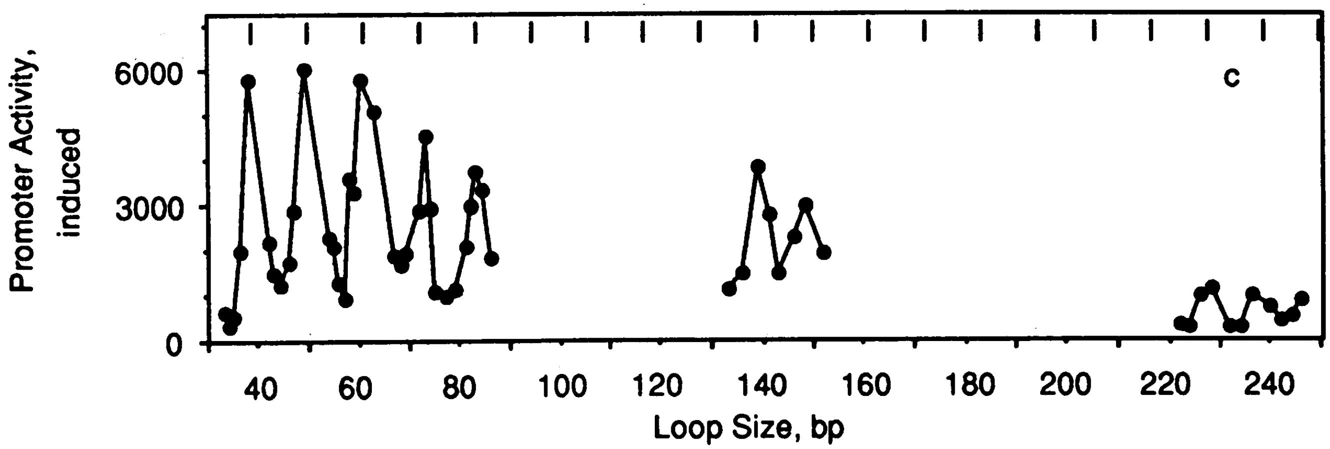
Lewis et al., 1996

Sharply looped DNA in the Gal repressosome

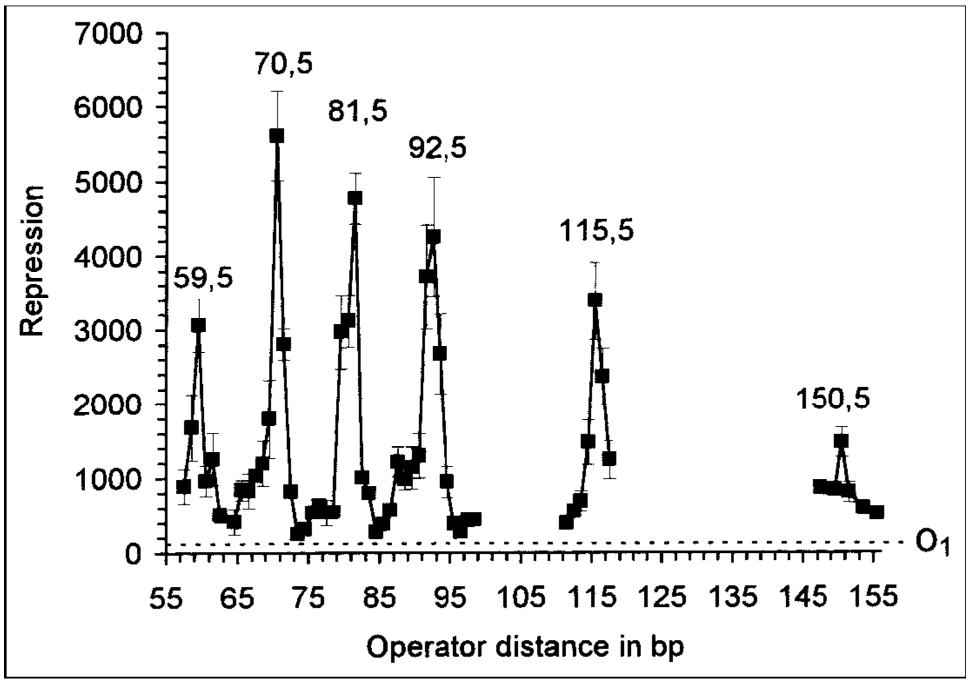


Semsey et al., 2004

Sharply looped and *twisted* DNA *in vivo*



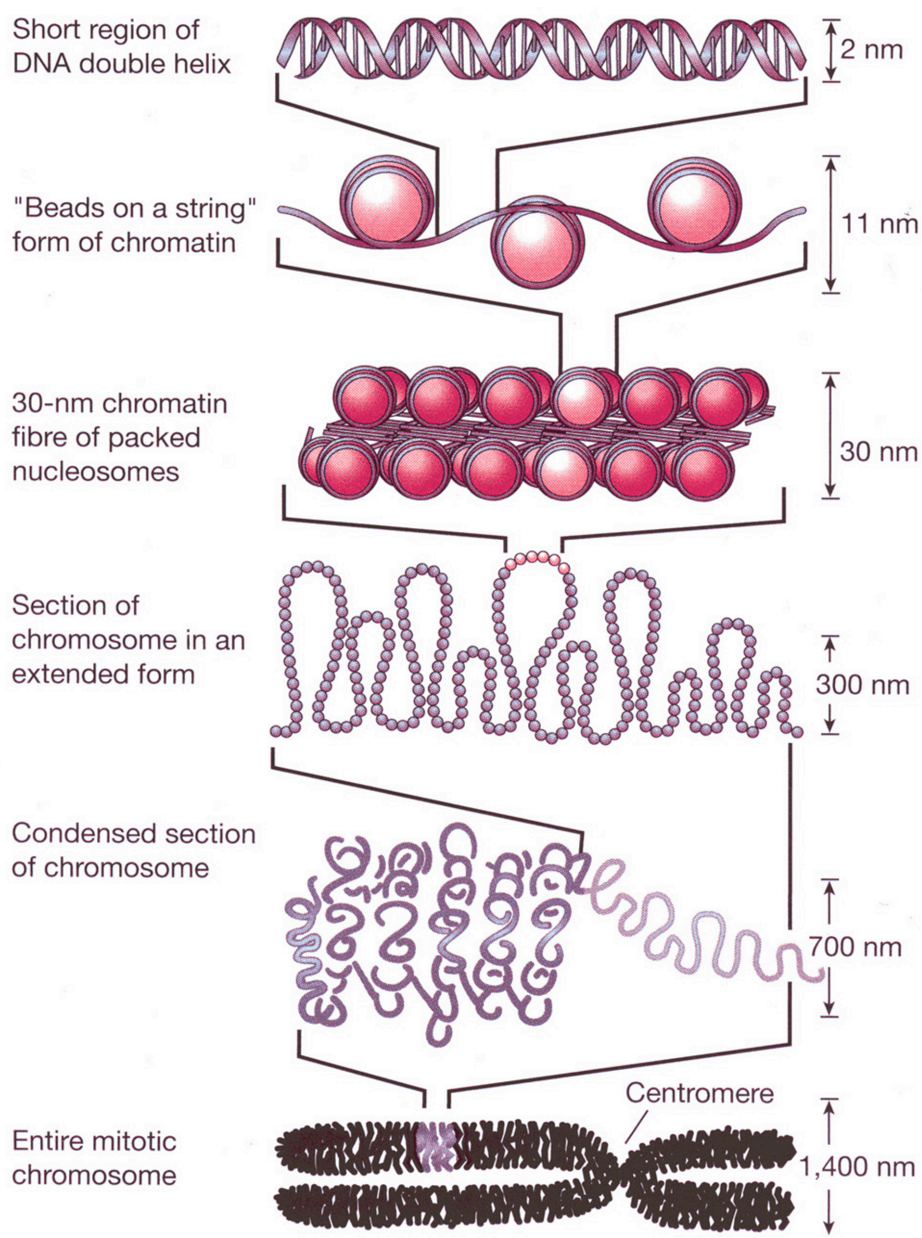
ara*CBAD*



lac

Lee & Schleif, 1989;
Müller et al., 1996

Hierarchical DNA folding in eukaryotic chromosomes

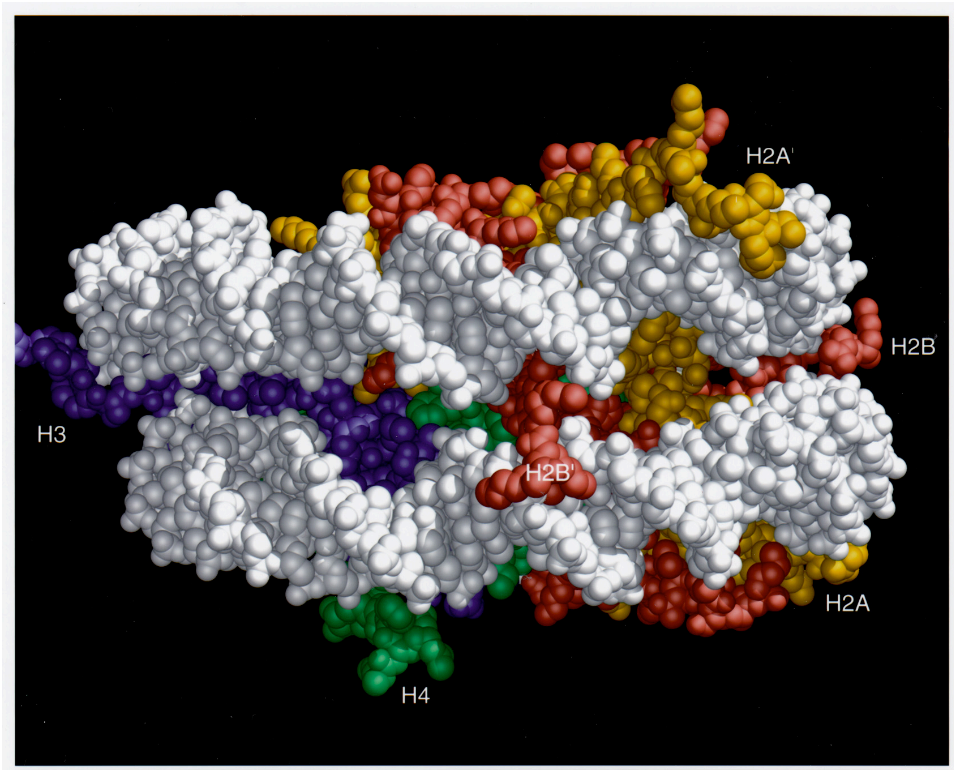


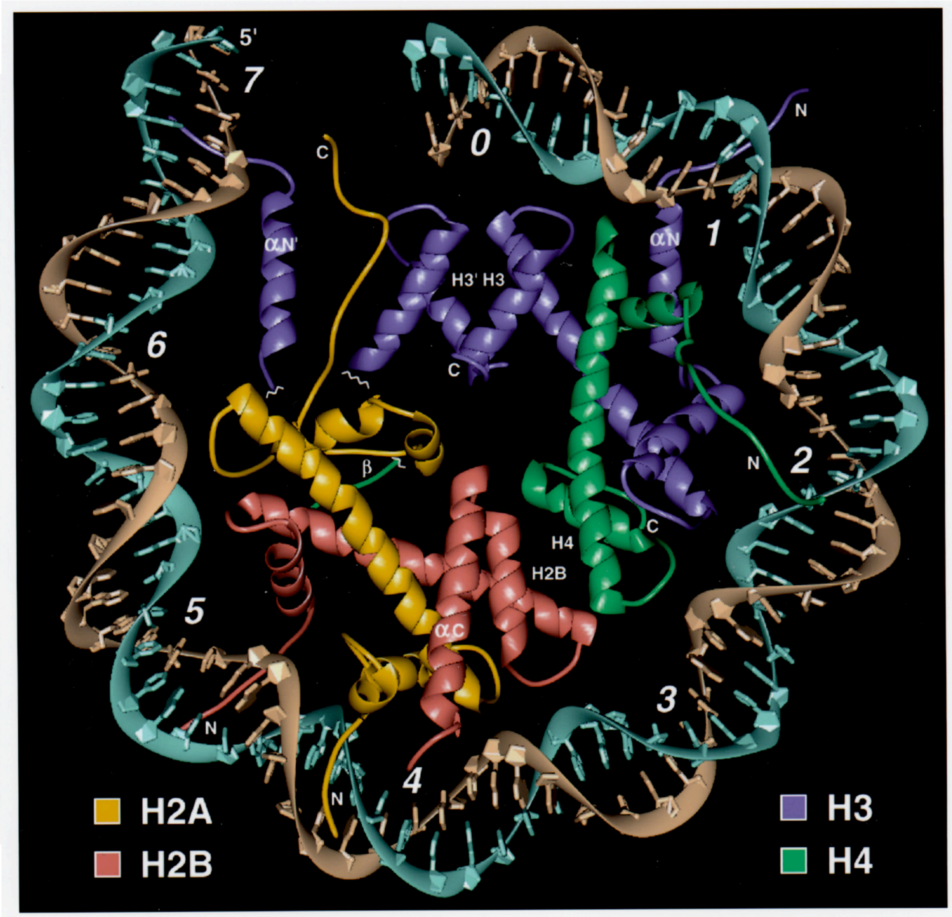
DNA double helix

Nucleosomes

Felsenfeld & Groudine,,
Nature 421: 448-453 (2003)

Most eukaryotic DNA is sharply looped

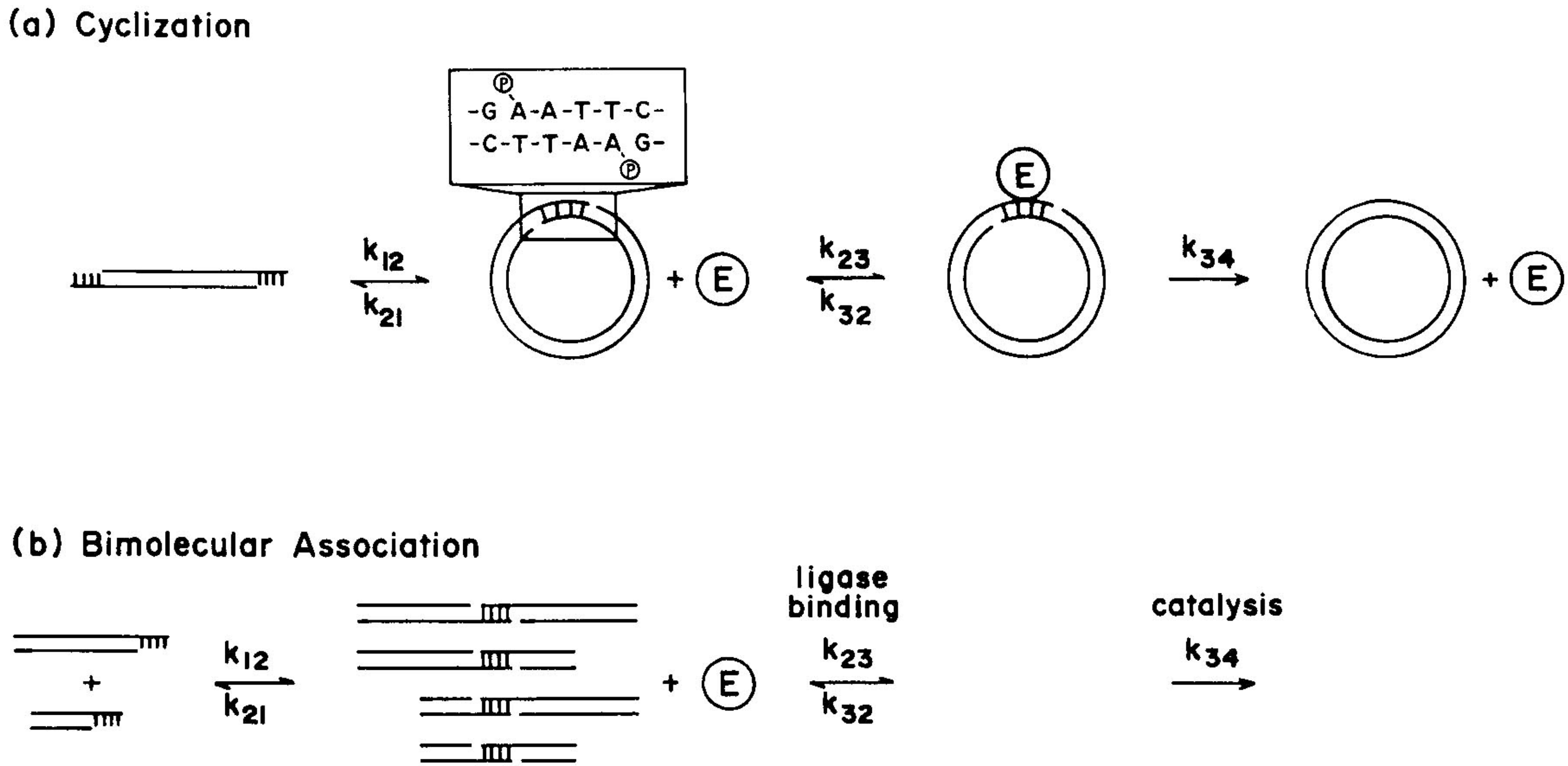




~80 bp per superhelical turn

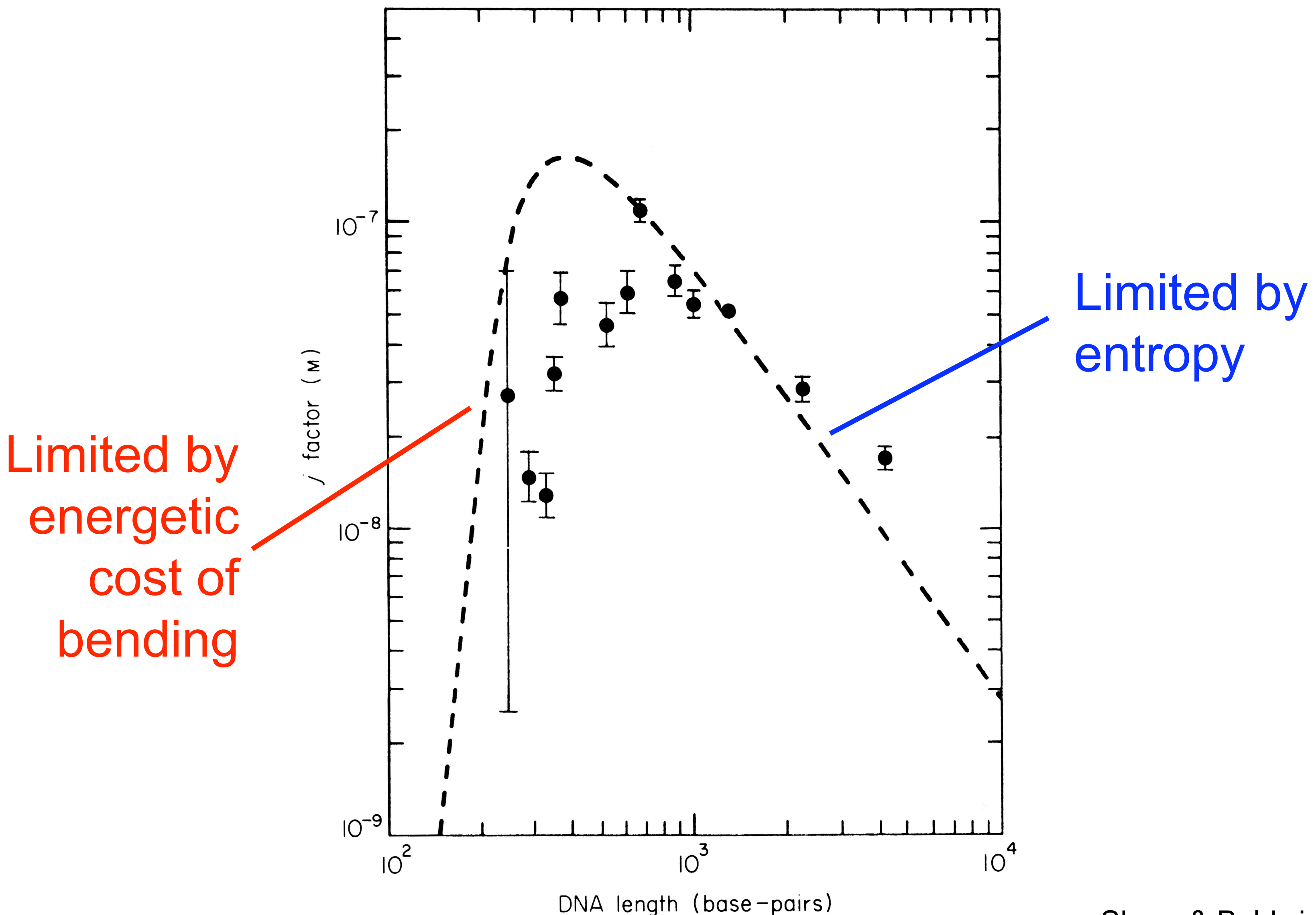
Luger et al., 1997

Cyclization assay for DNA flexibility



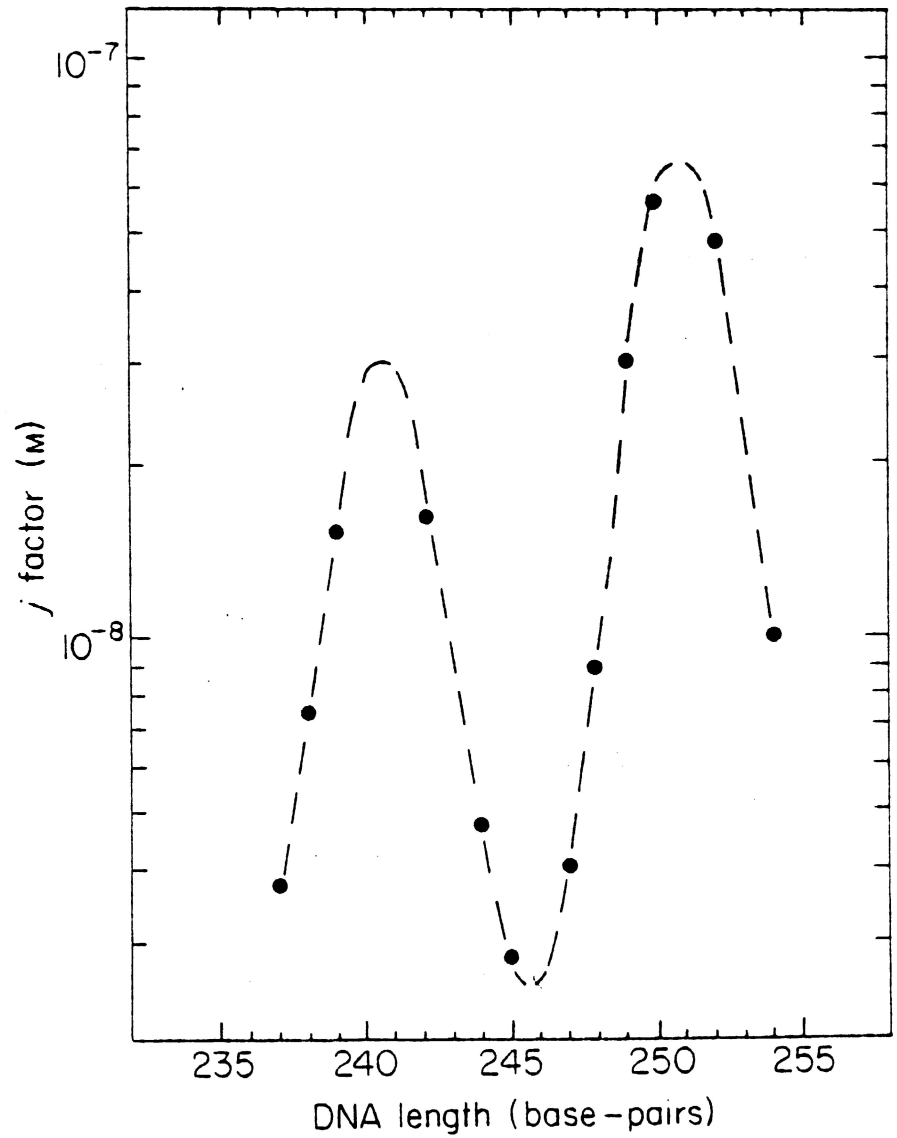
Shore, Langowski, & Baldwin, 1981

J depends on DNA length



Shore & Baldwin, 1983

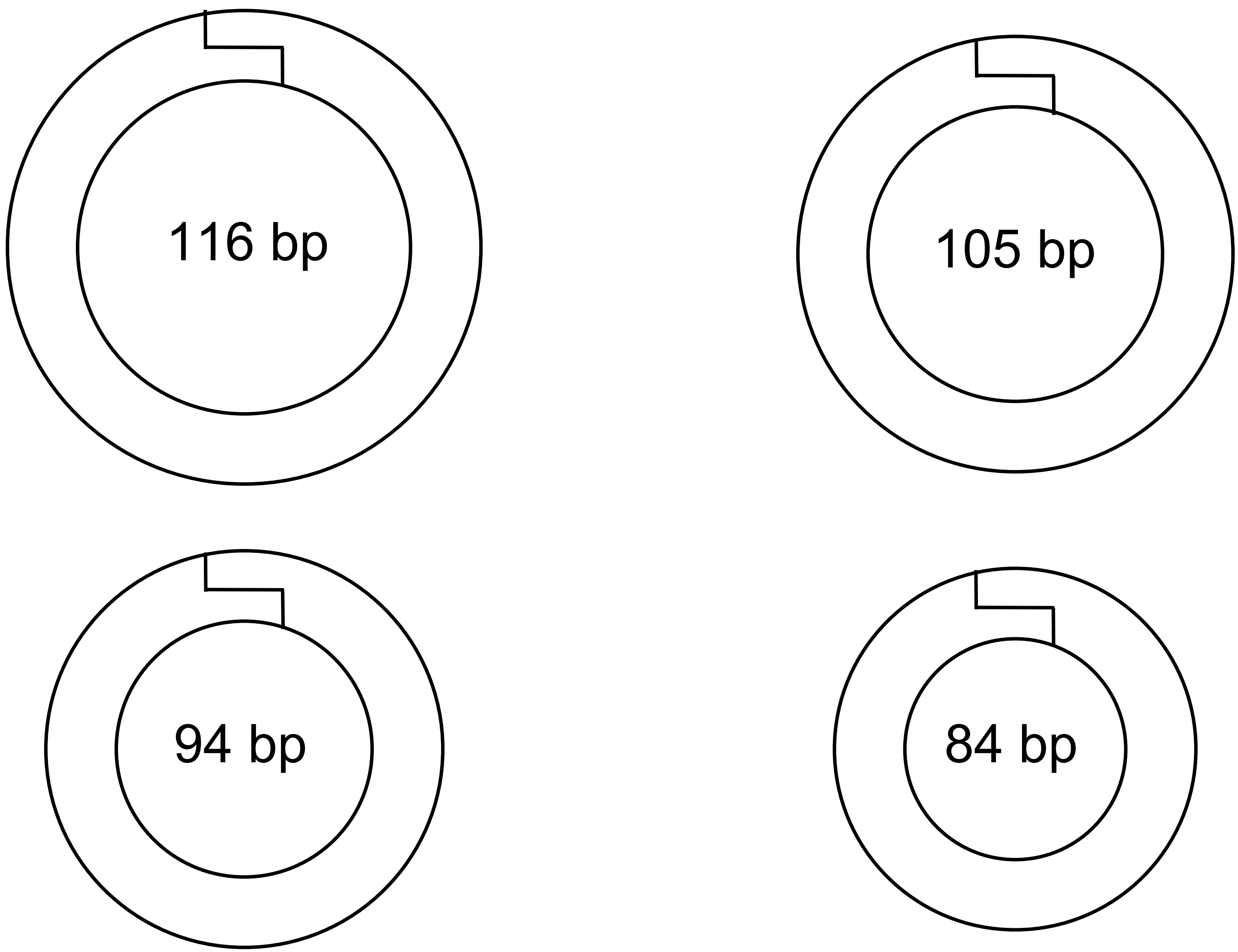
J depends on total DNA *twist*



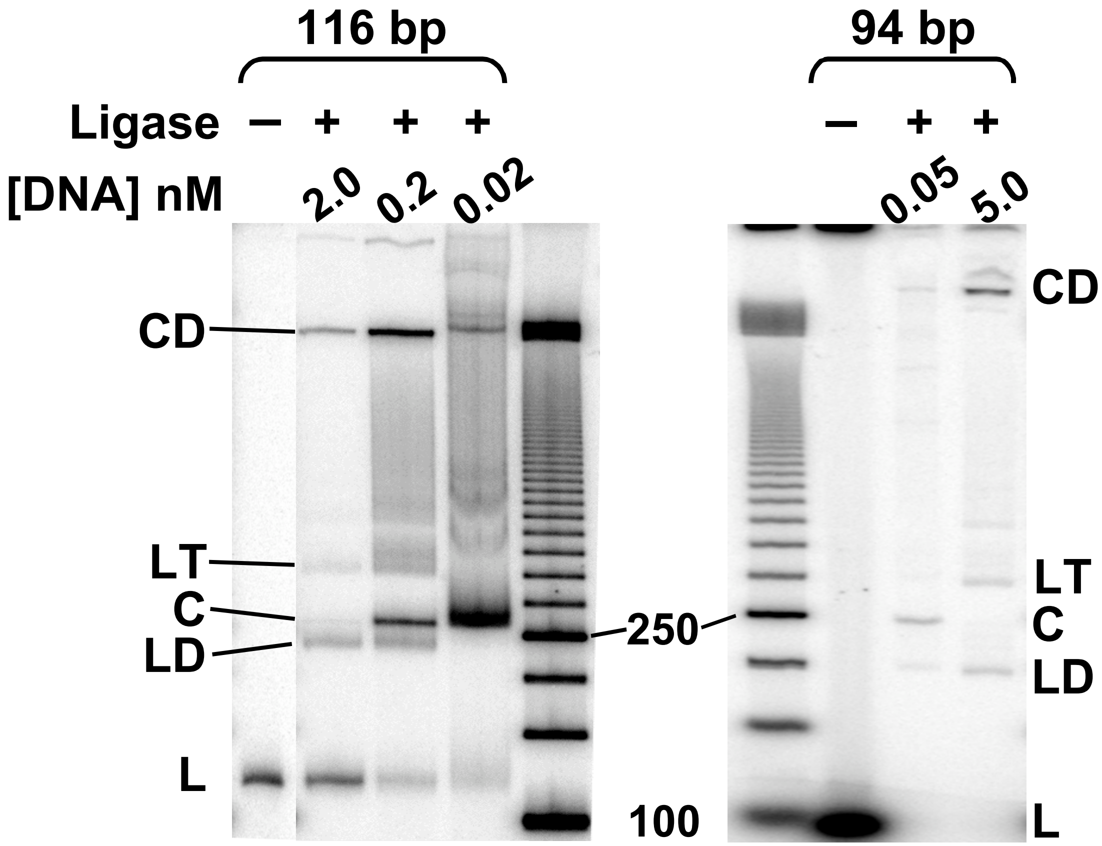
- Period equals DNA helical repeat
- Amplitude reflects the DNA torsional stiffness

Shore & Baldwin, 1983

Very small DNA circles!



116 bp and 94 bp circles are easy to make



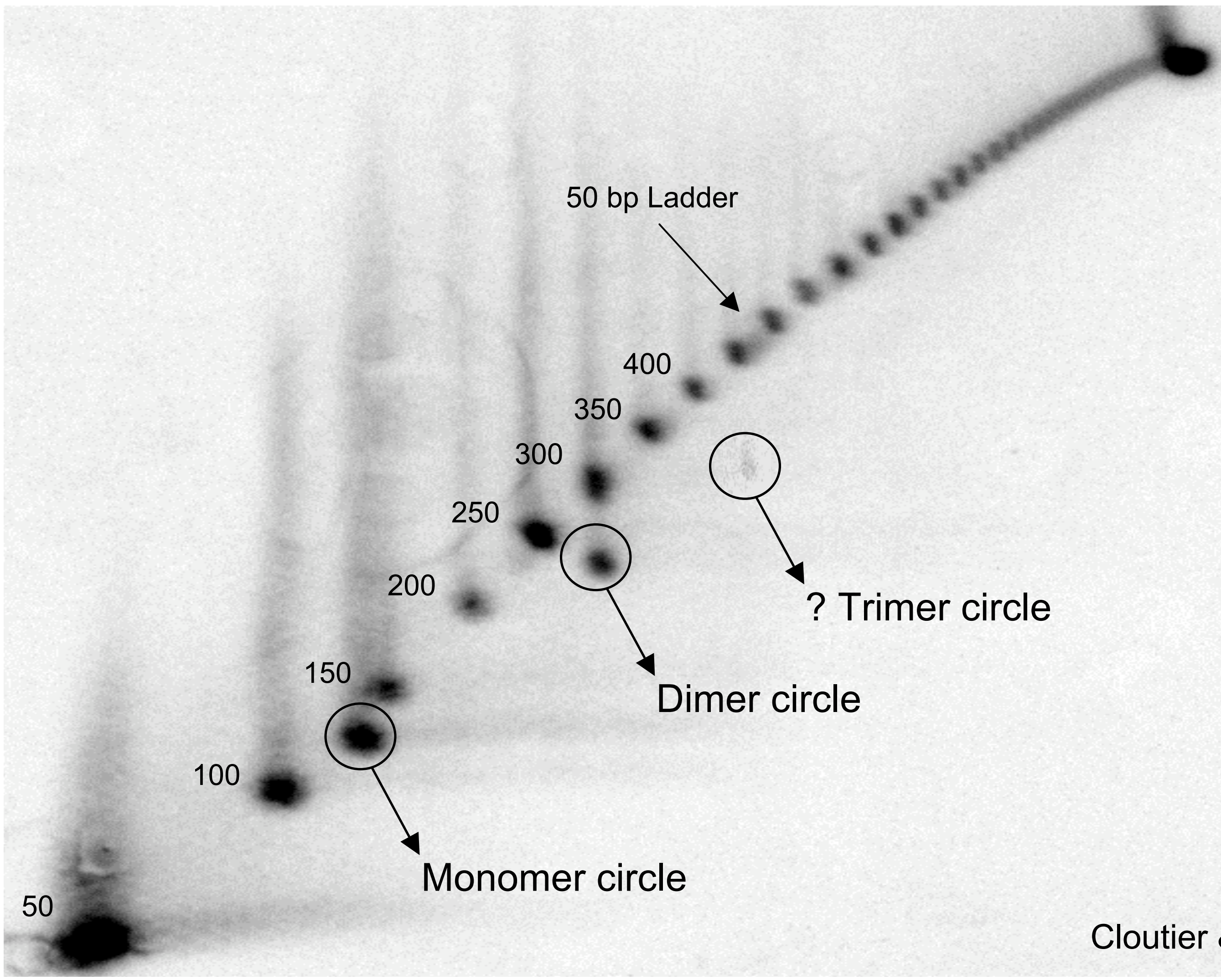
Cloutier & Widom, 2004

How do we know they are circles?

- (Monomeric) circles are favored at low concentration
- Circles run off the ladder of linear oligomers
- Circles run off diagonal in a topology-sensitive 2-D gel assay
- Circles resist digestion by exonuclease

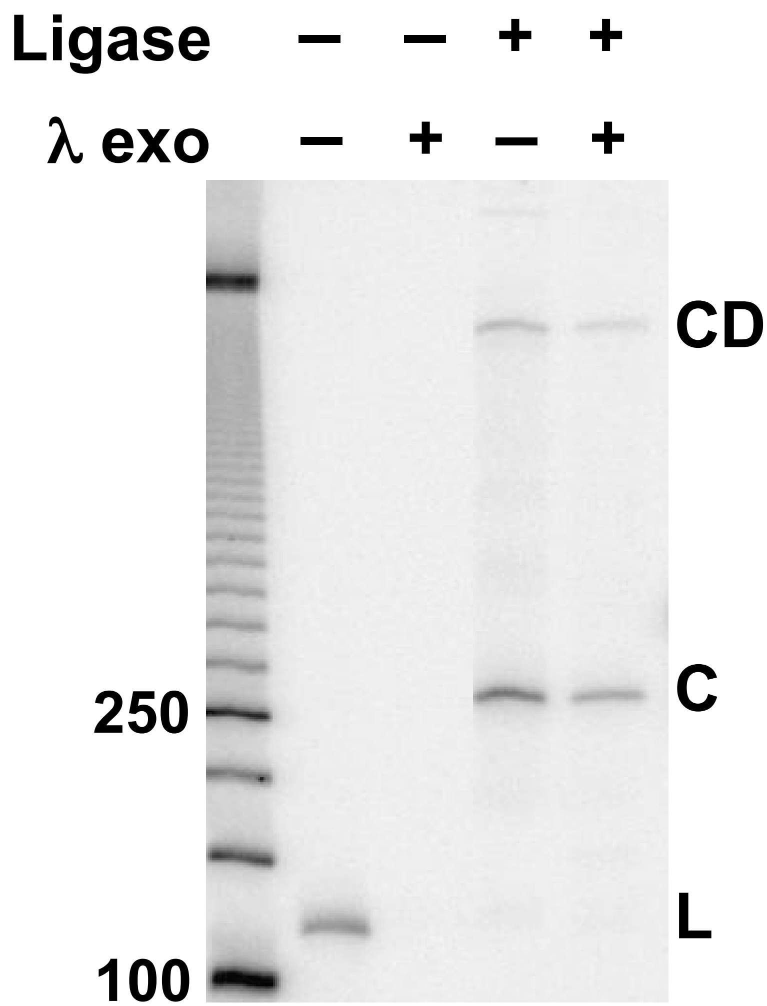
Circles run off the diagonal in 2-D gel

Ligation of 116 bp DNA at 100 pM



Cloutier & Widom, 2004

Circles resist digestion by exonuclease

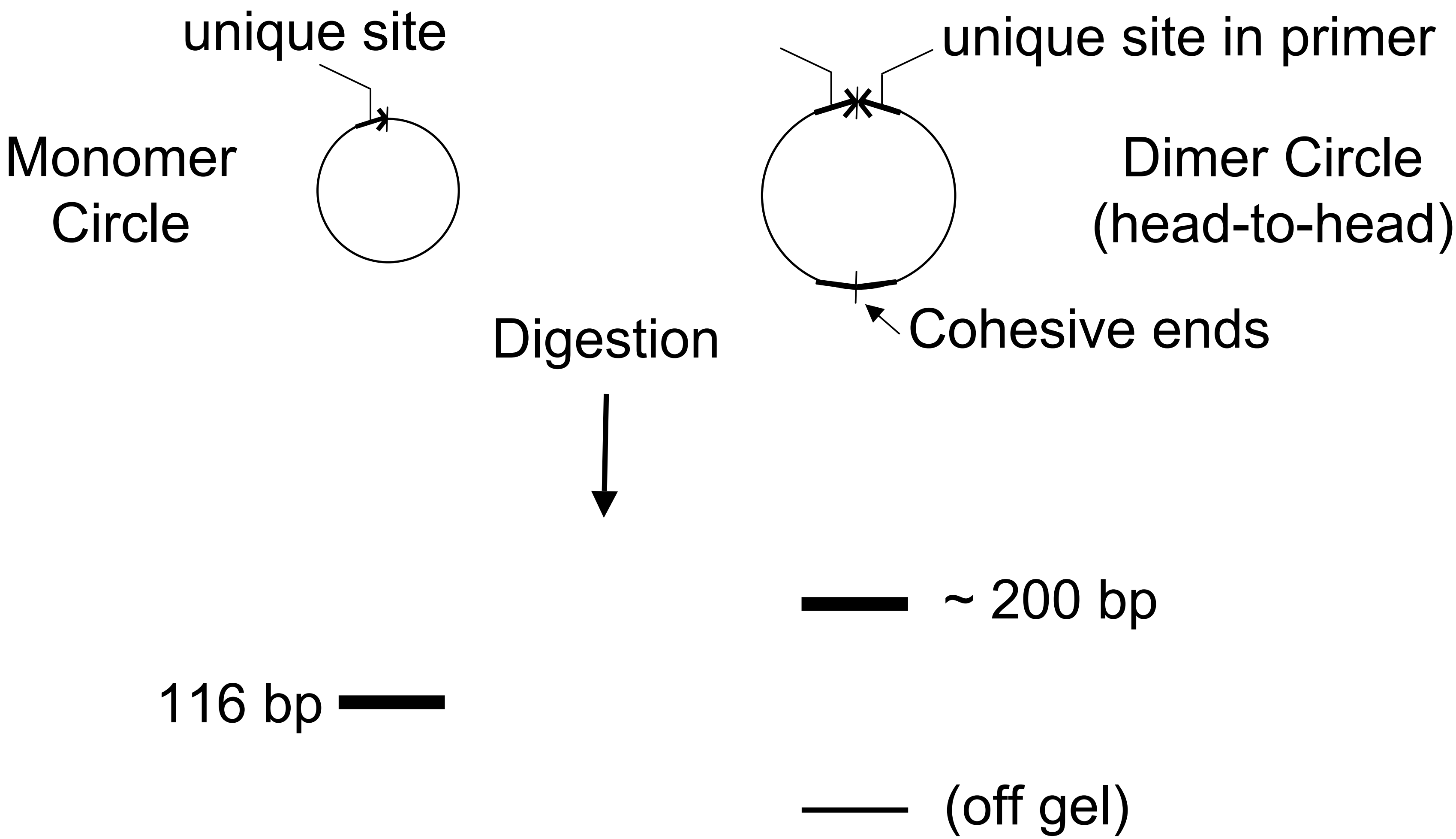


Cloutier & Widom, 2004

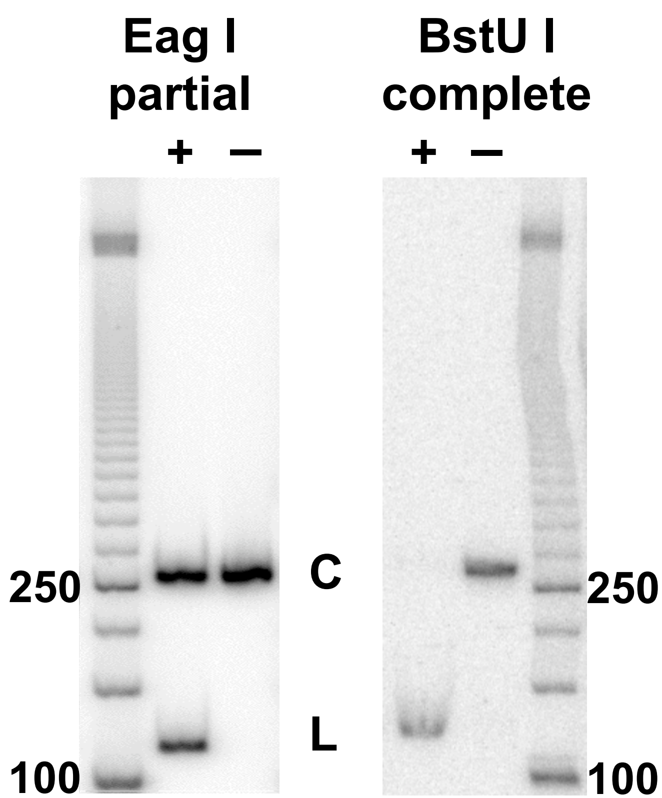
How do we know circles are monomeric?

- Monomeric circles are favored at low concentration
- Monomer circles run near monomer linears in agarose gels
- Partial restriction digestion yields only linear monomer
- Complete restriction digestion nearby cohesive site yields only linear monomer

Restriction enzyme digestion distinguishes monomers from oligomers



Restriction enzyme digestion distinguishes monomers from oligomers

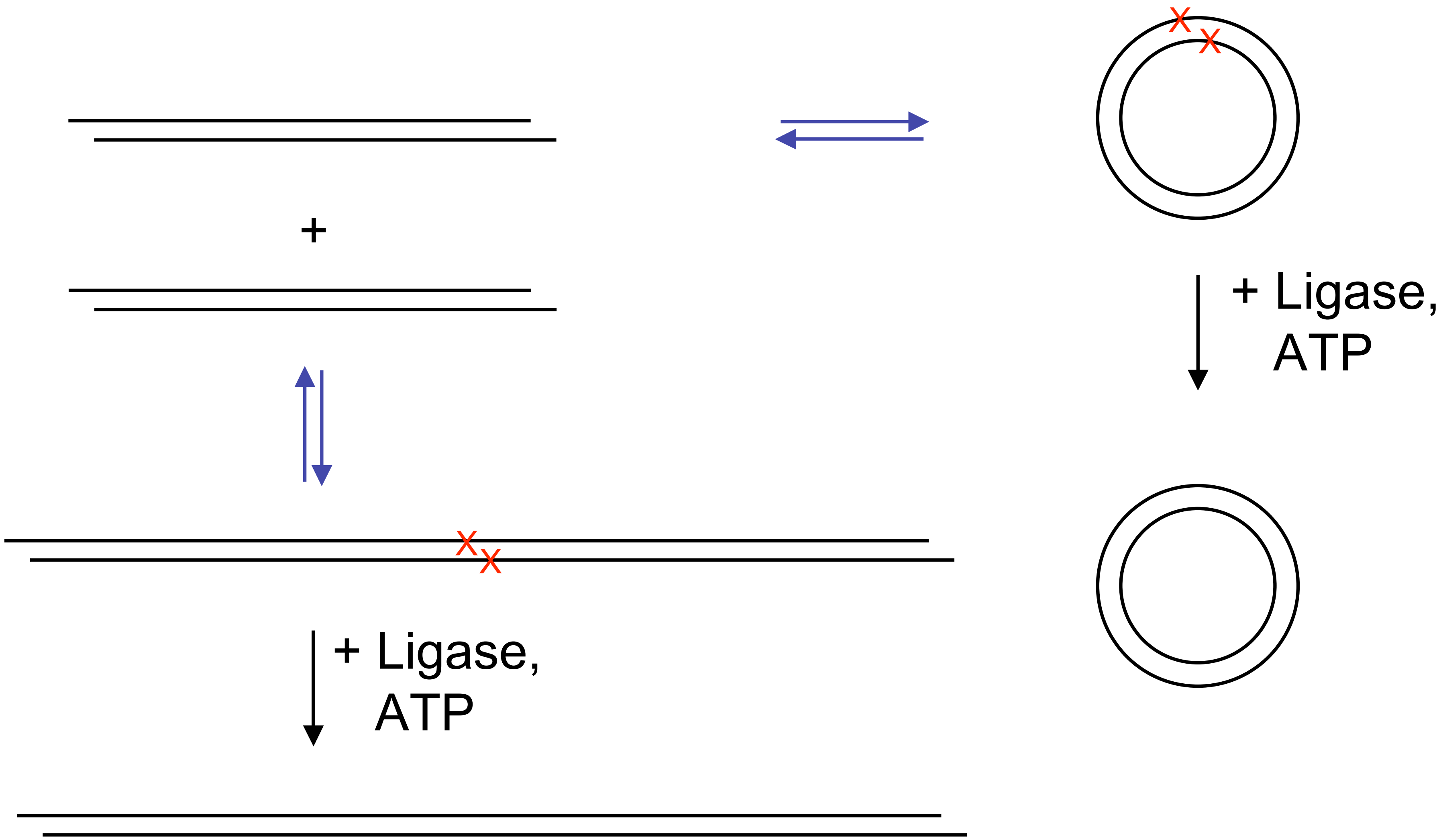


- Partial digestion by Eag I yields only linear monomer

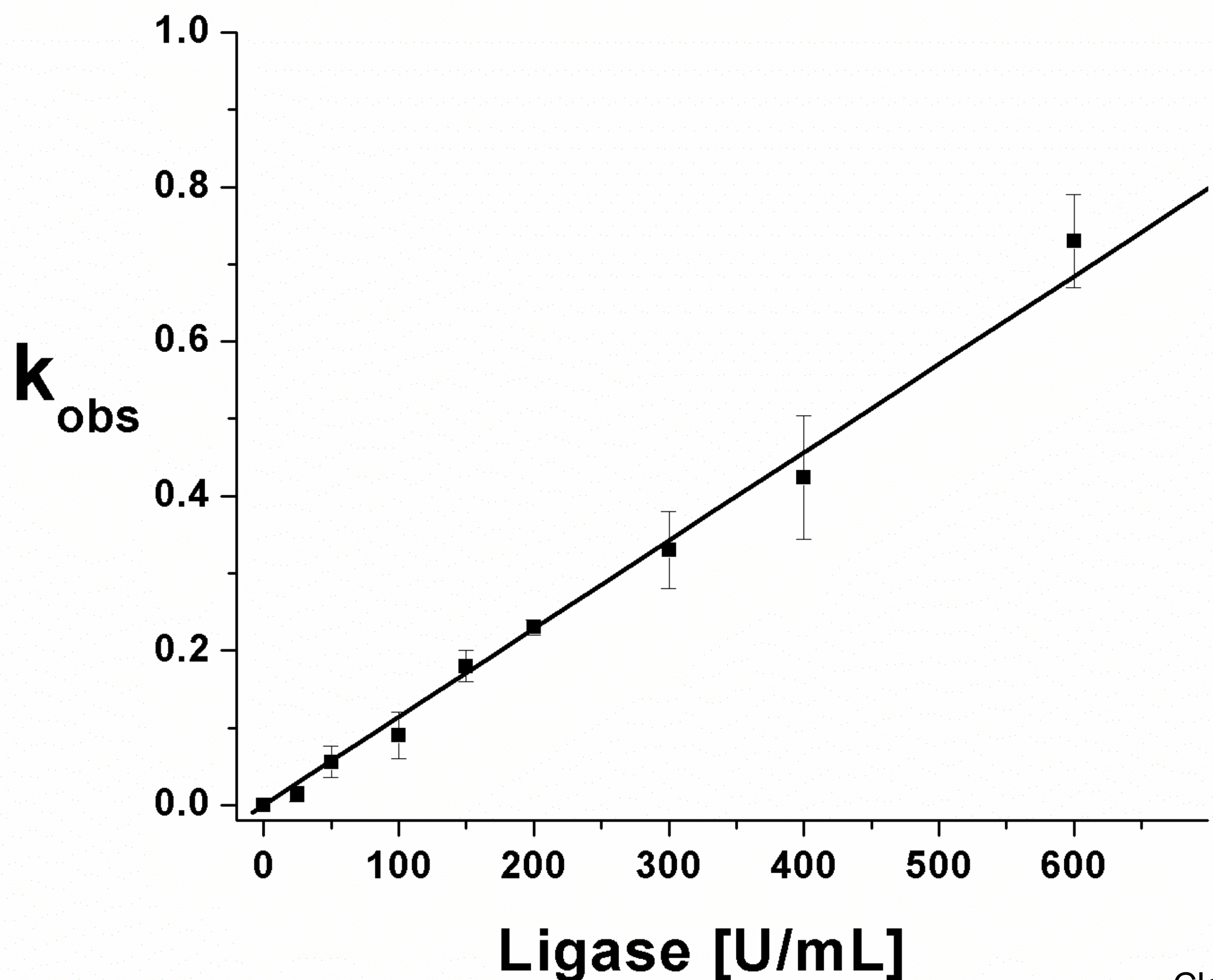
- Complete restriction digestion by BstU I yields only linear monomer

Cloutier & Widom, 2004

Quantitative measurement of J factor

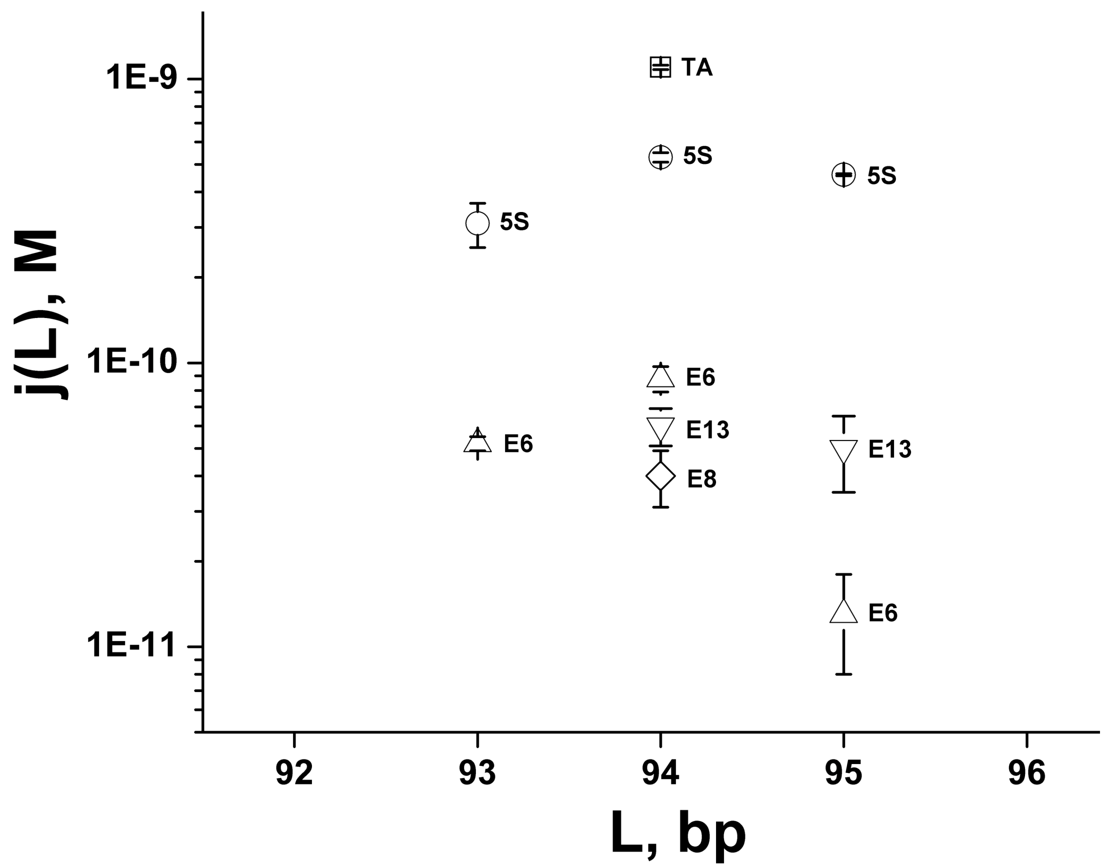


Cyclization reactions with 94bp DNAs are first order in ligase concentration



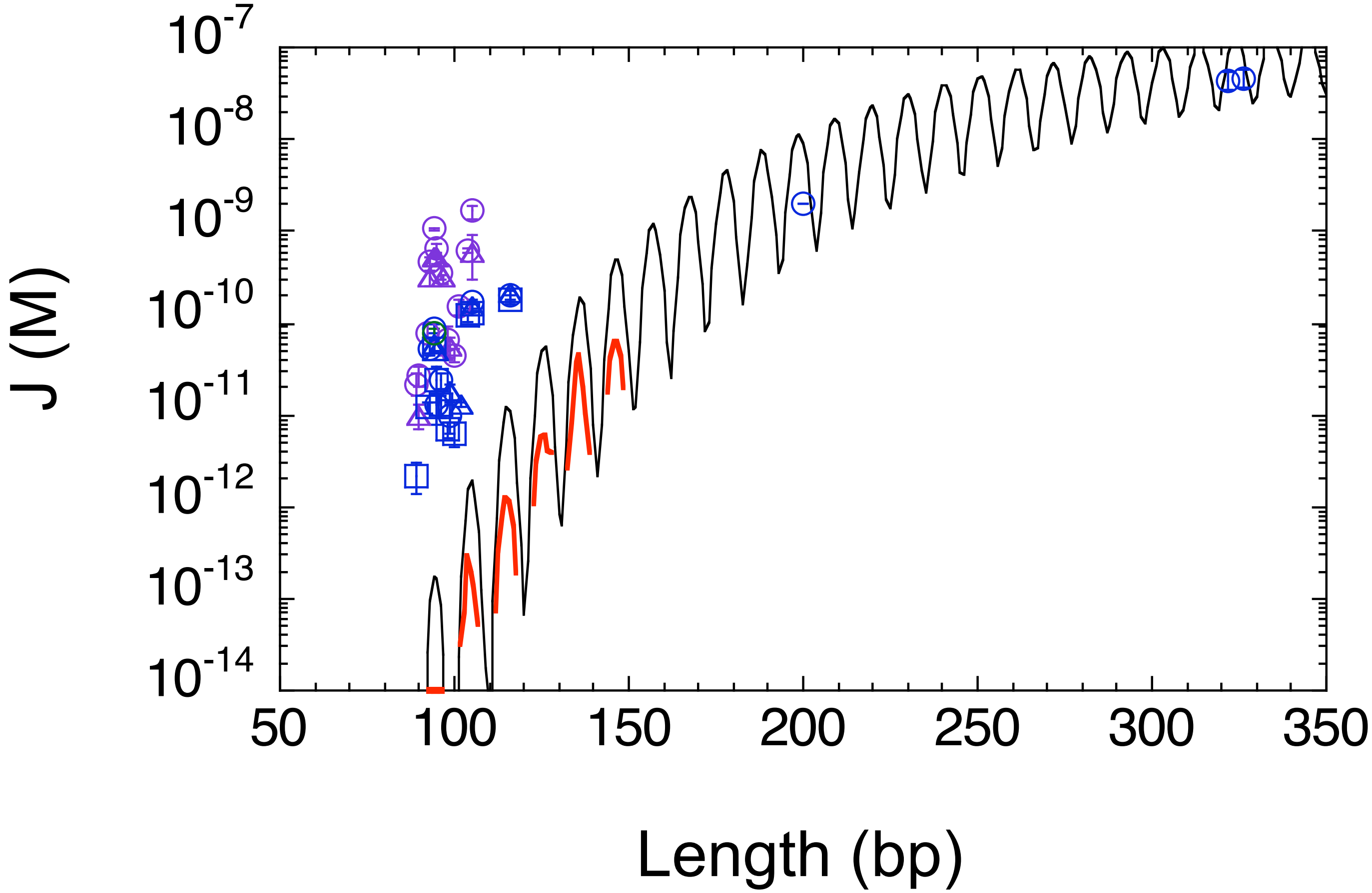
Cloutier & Widom, 2004

Differing DNA sequences differ in inherent cyclizability



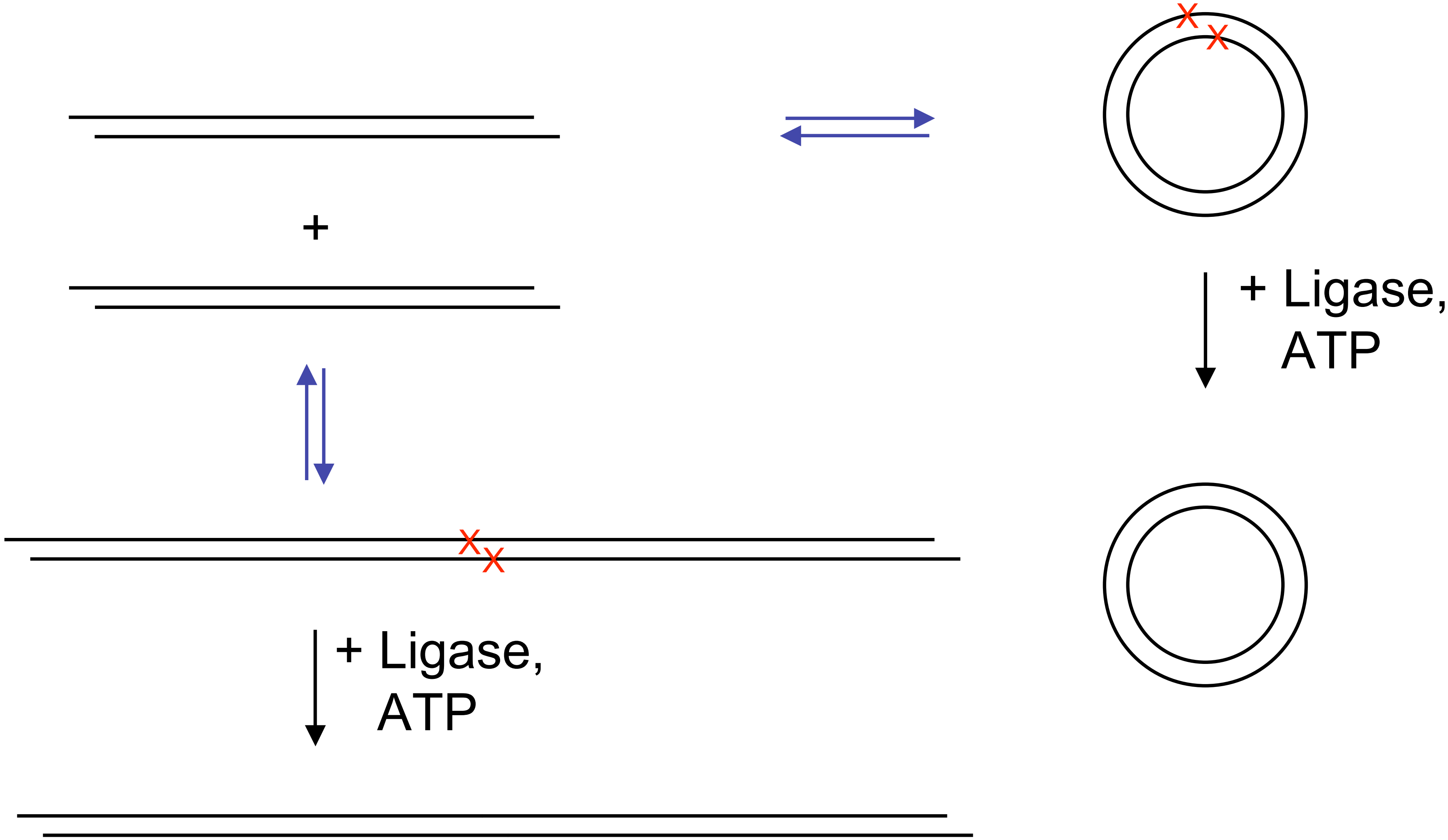
Cloutier & Widom, 2004

Sharply bent DNA appeared to be much softer for sharp looping than predicted

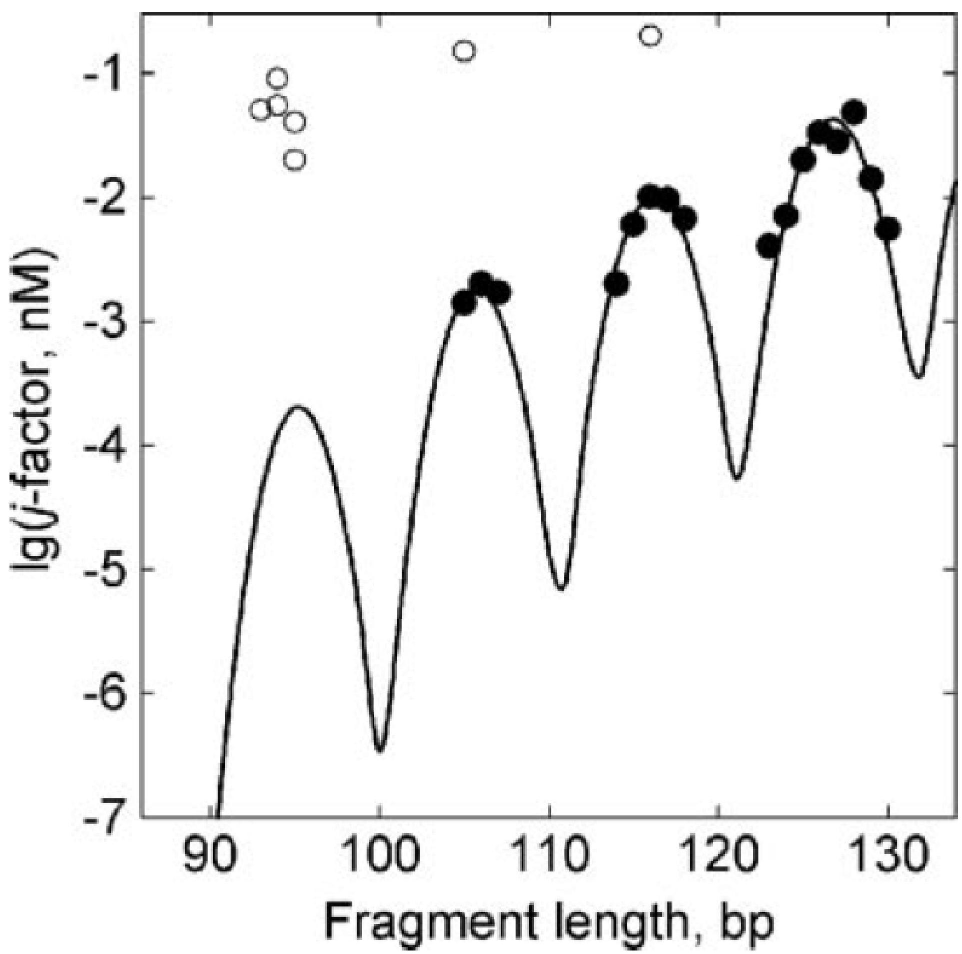


Cloutier & Widom, 2005

Quantitative measurement of J factor



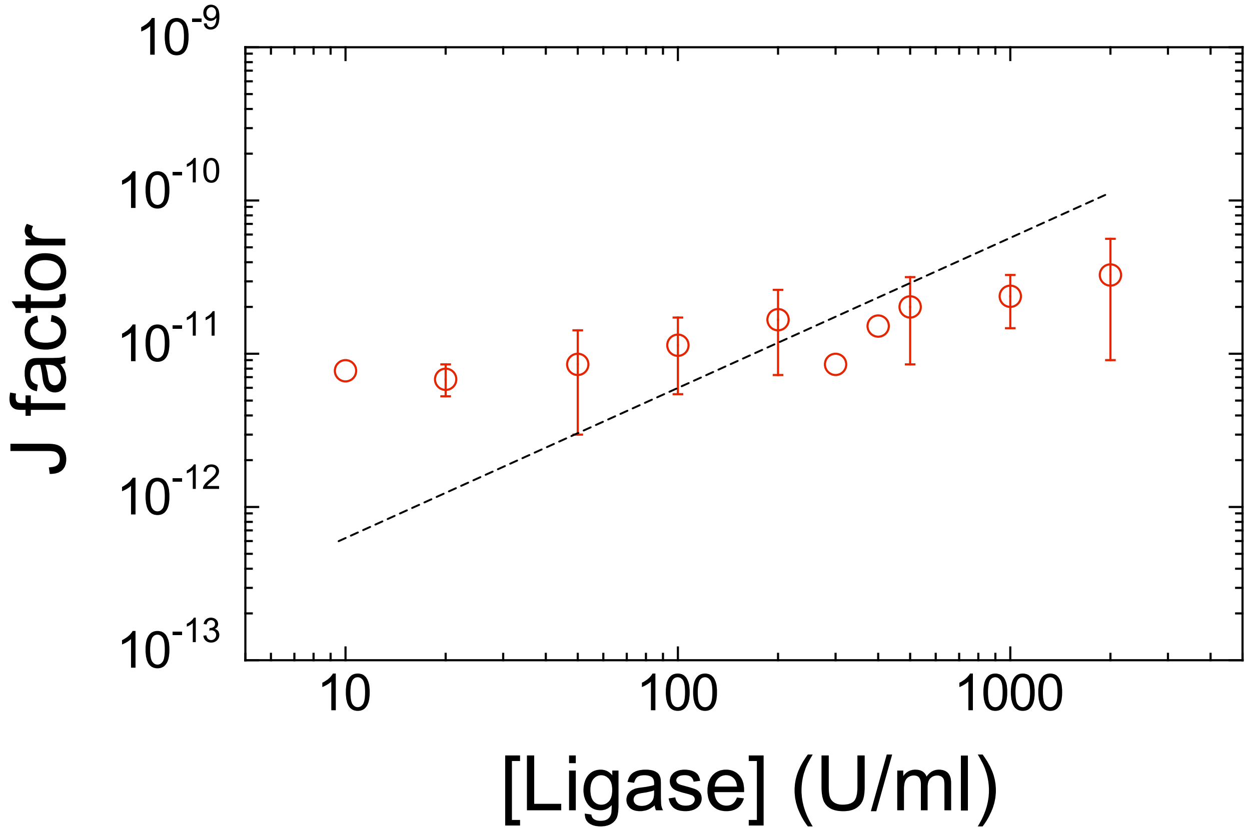
DNA may *not* be softer for sharp looping than predicted



Du et al., 2005

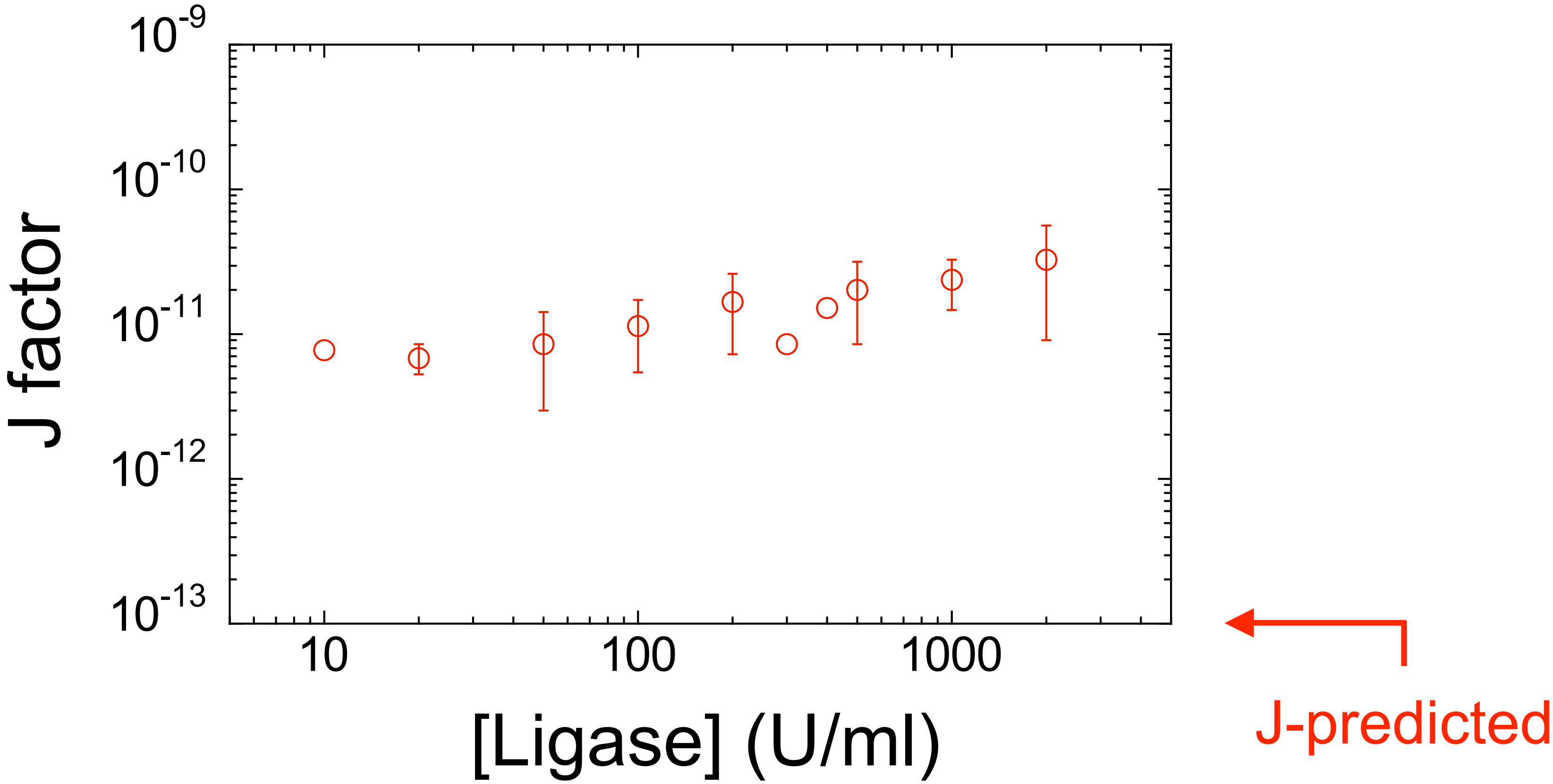
Measured J factors are independent of [ligase]

•bimolecular joining reactions, too, are first order in [ligase]



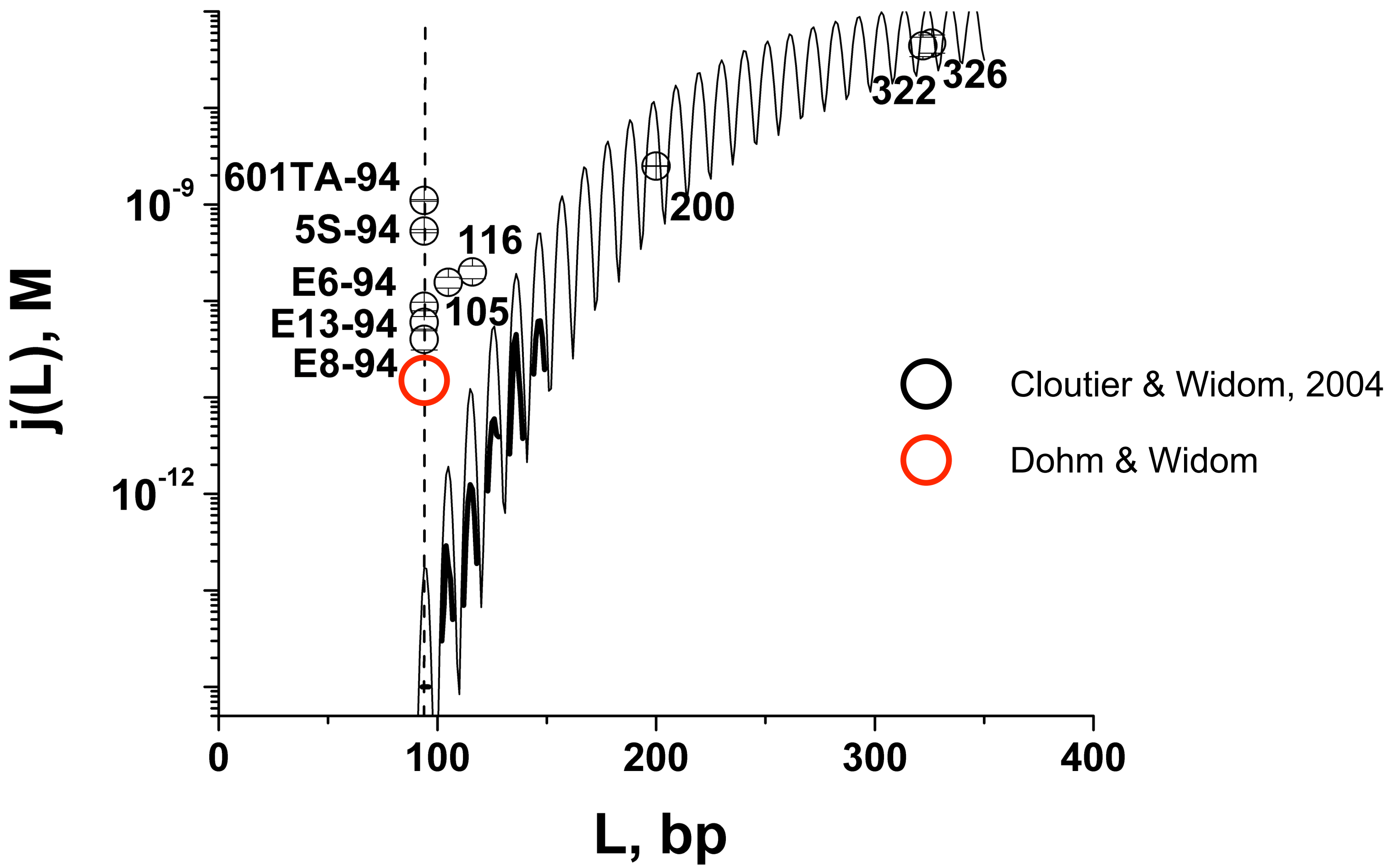
J. Dohm

Measured J factors greatly exceed prediction

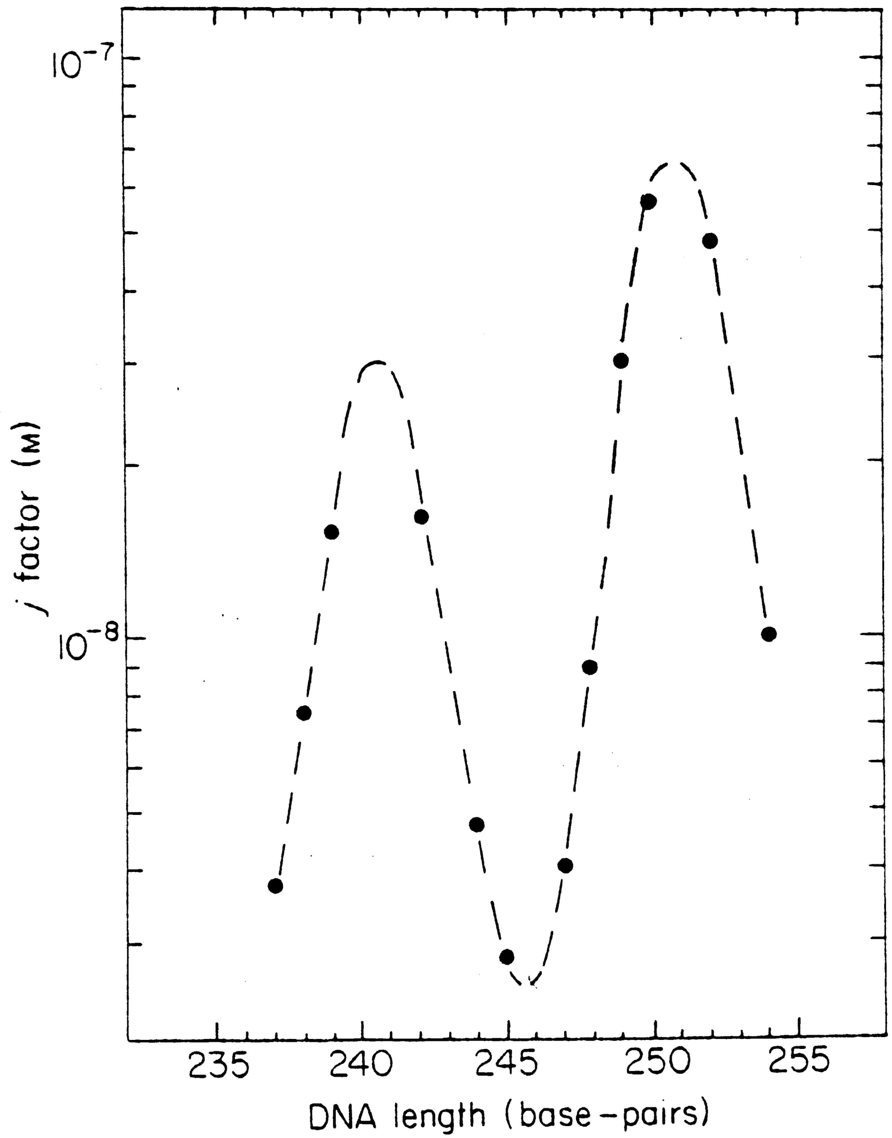


J. Dohm

Measured J factors greatly exceed prediction



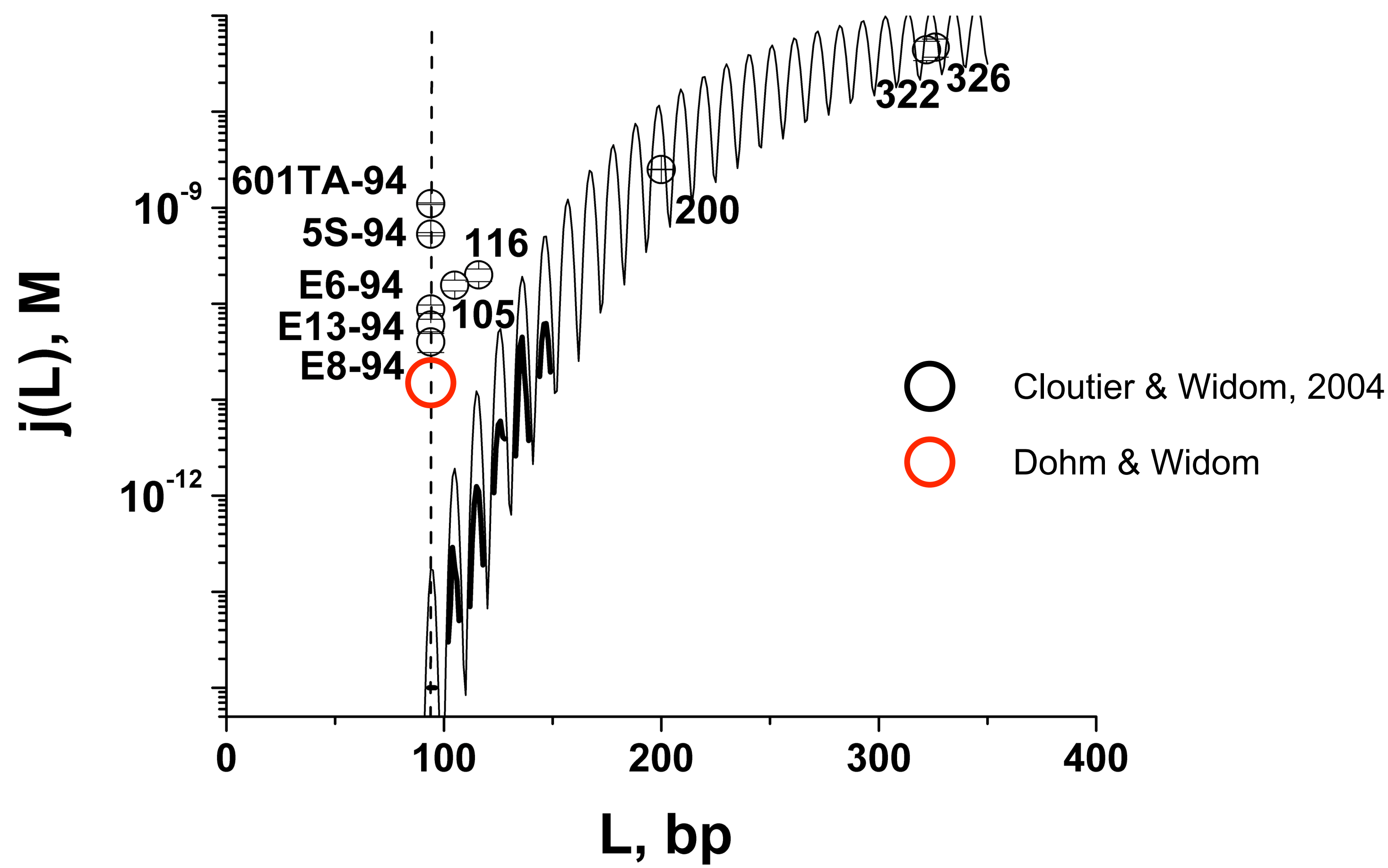
J depends on total DNA *twist*



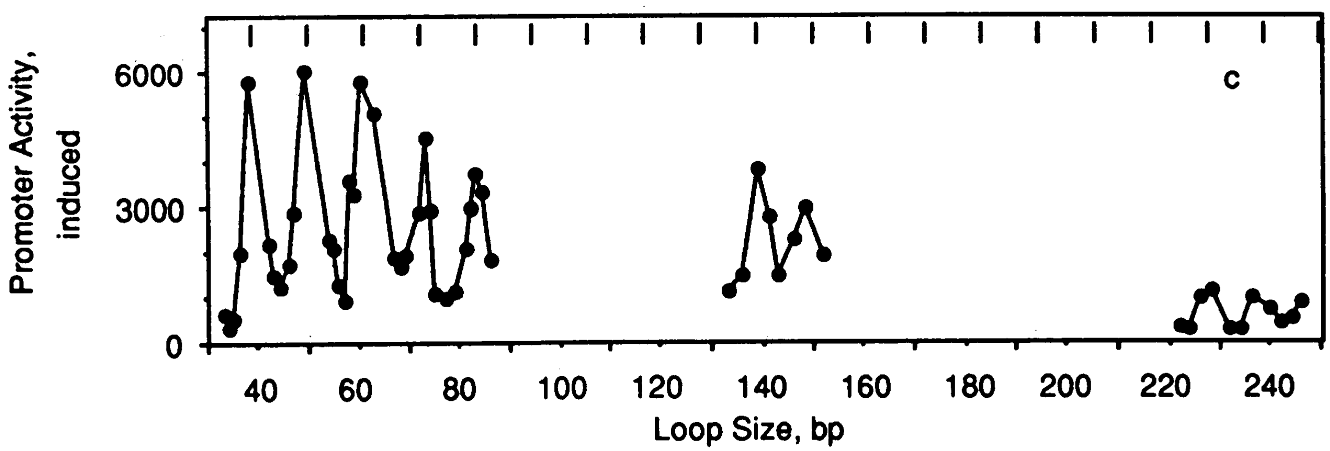
- Period equals DNA helical repeat
- Amplitude reflects the DNA torsional stiffness

Shore & Baldwin, 1983

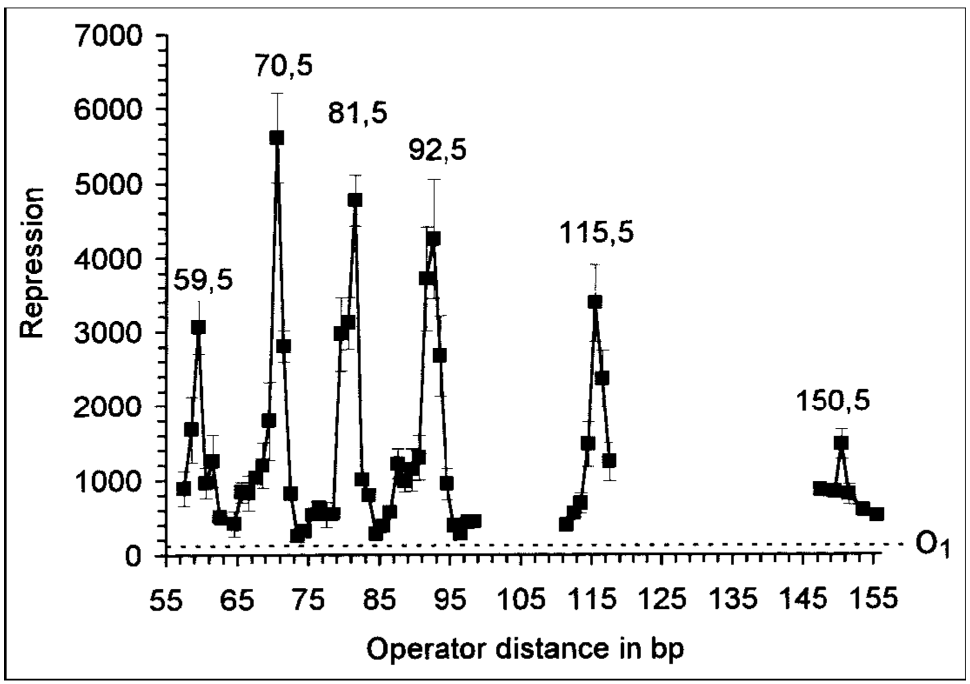
Measured J factors greatly exceed prediction



Sharply looped and *twisted* DNA *in vivo*



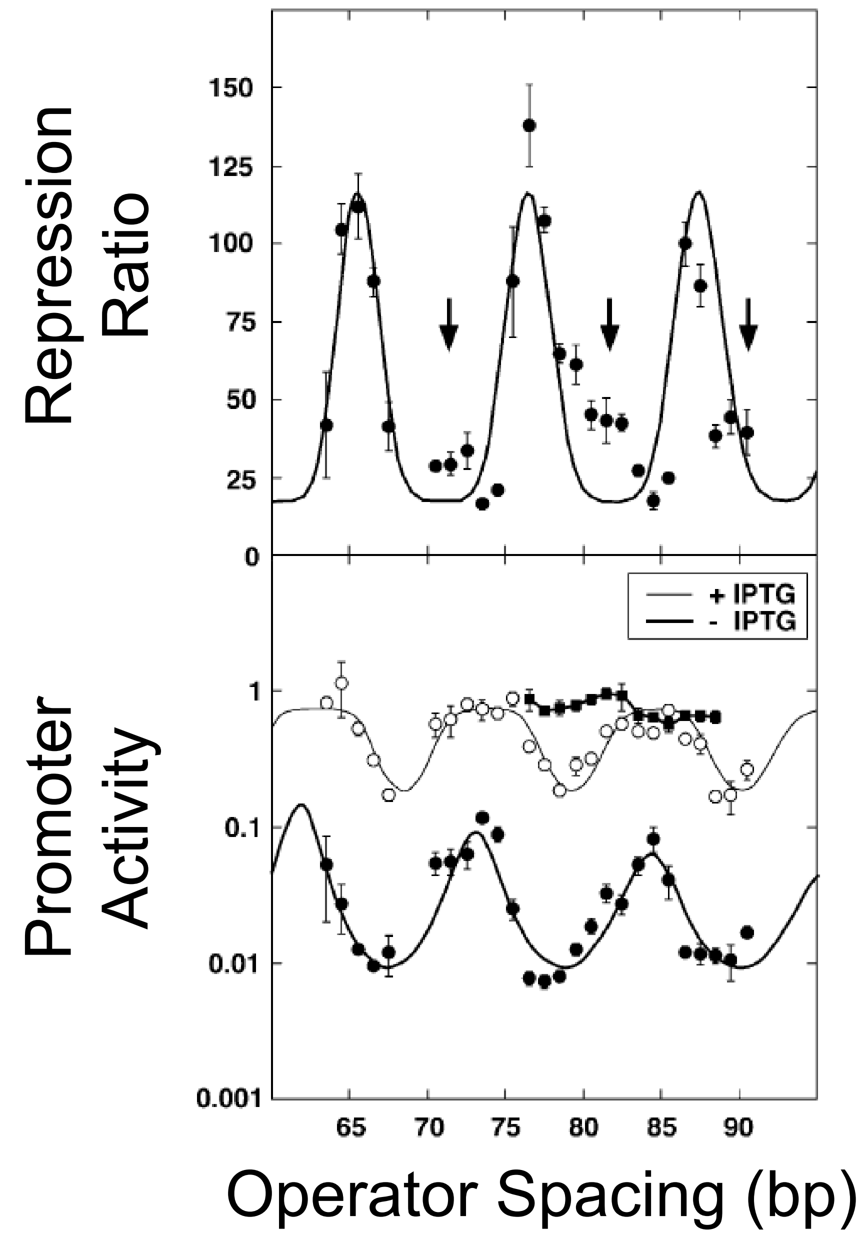
araCBAD



lac

Lee & Schleif, 1989;
Müller et al., 1996

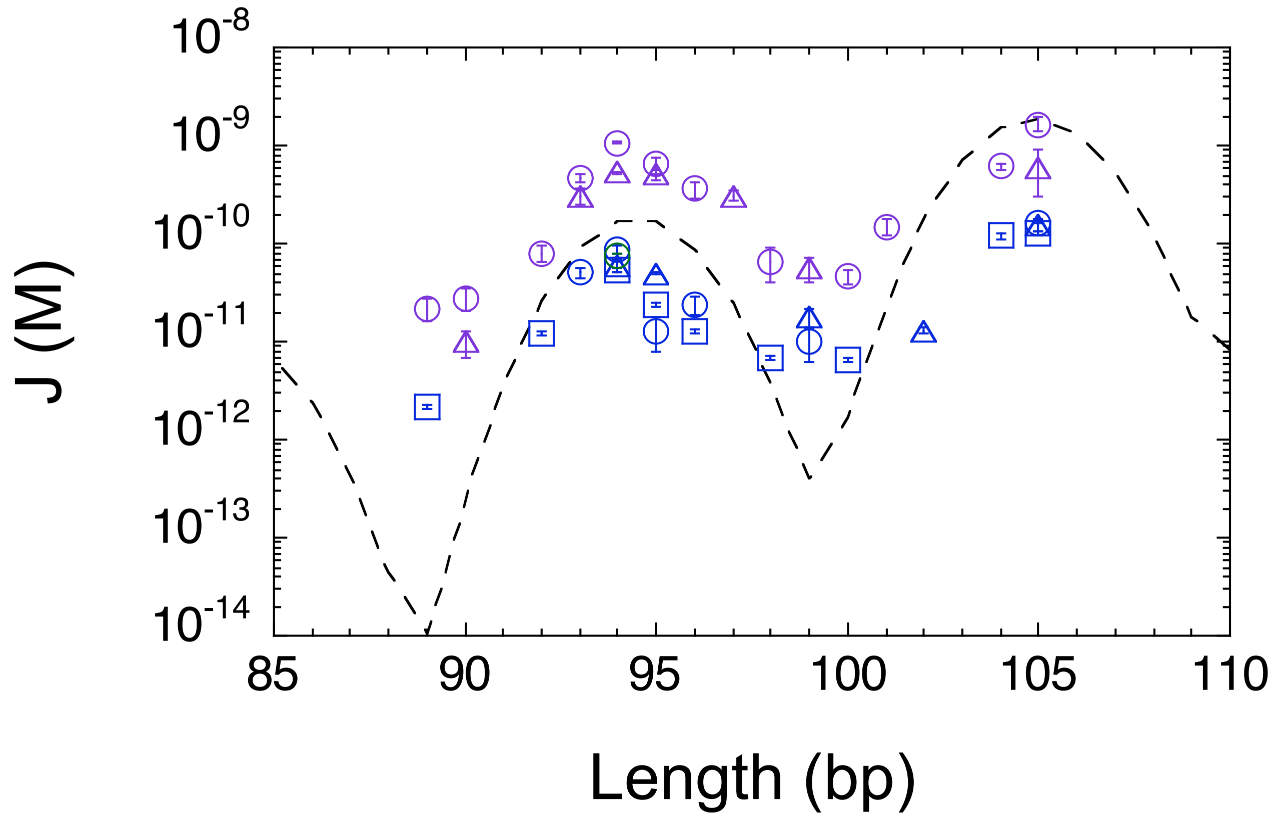
Sharply looped and *twisted* DNA, *in vivo*



•*lac* promoter

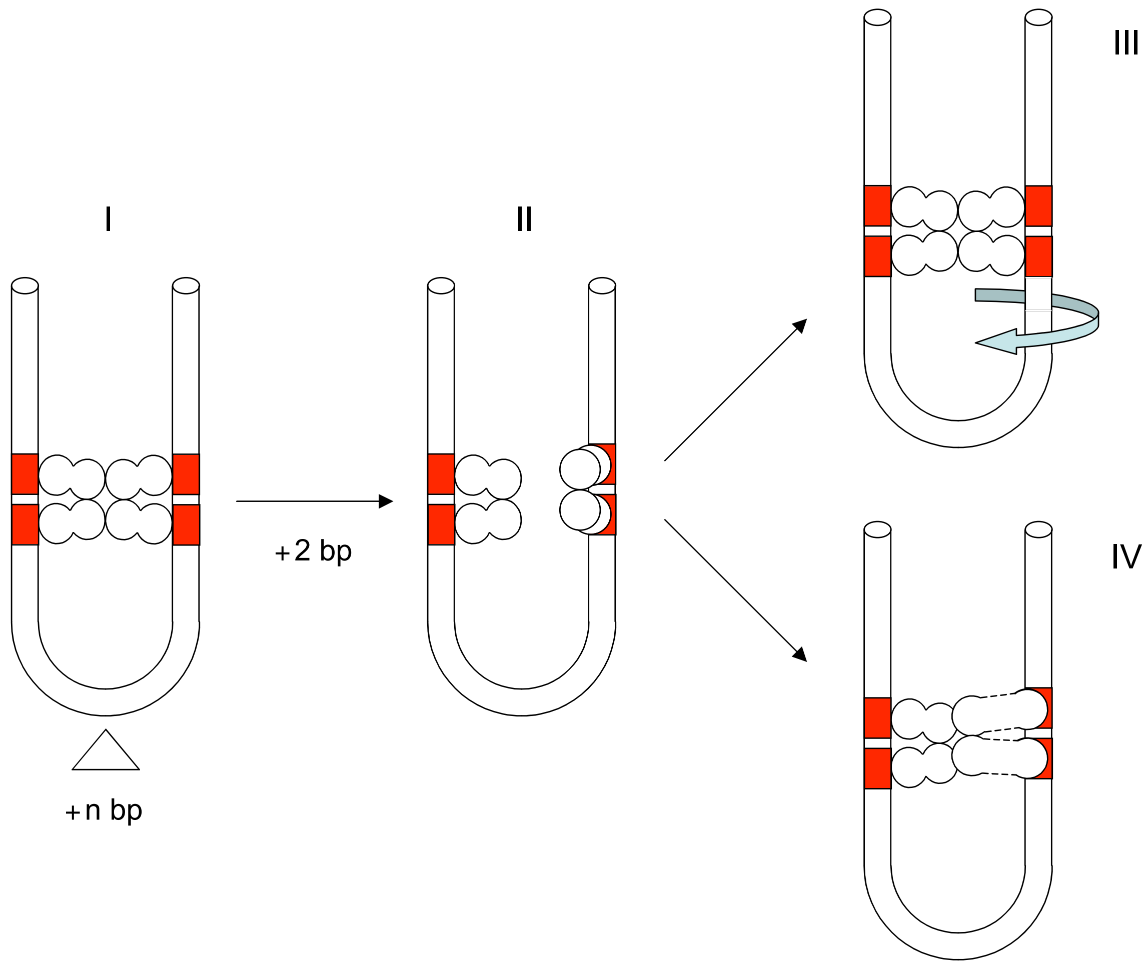
Becker, et al., 2005

Sharply bent DNA also appears to be much softer for twisting than predicted



Cloutier & Widom, 2005

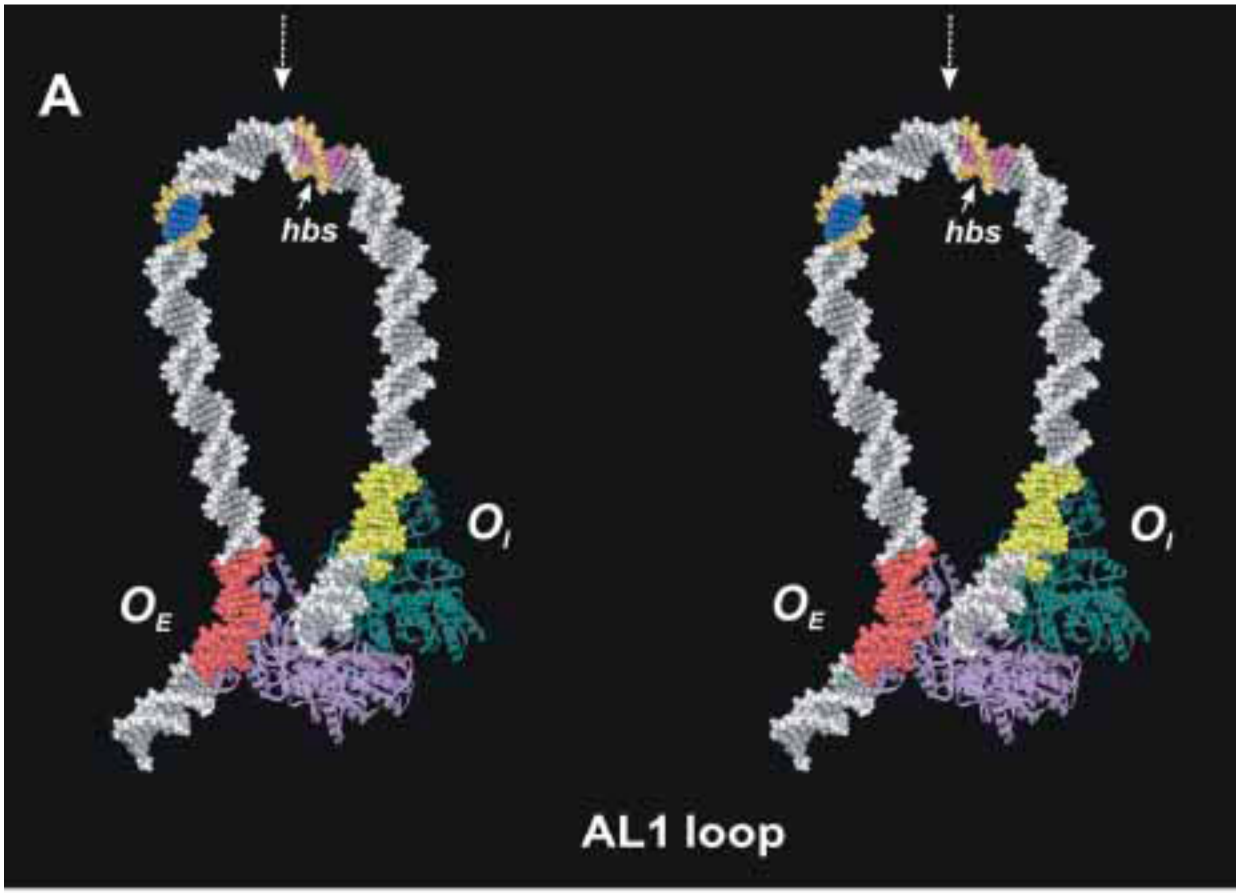
Formation and stability of sharply looped protein–DNA complexes



Cloutier & Widom, 2005

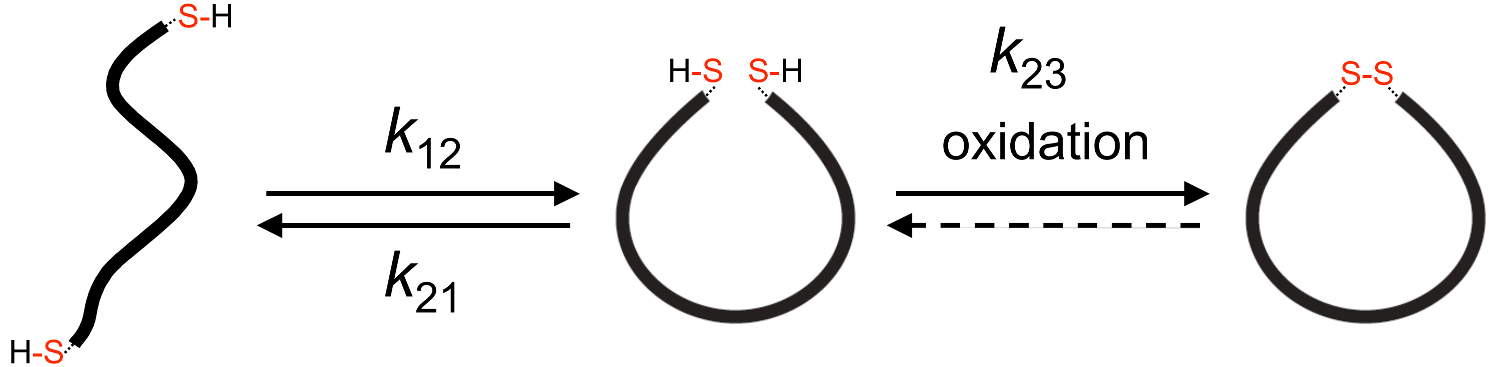
Looping *vs* cyclization

•Sharply looped DNA in the Gal repressosome



Semsey et al., 2004

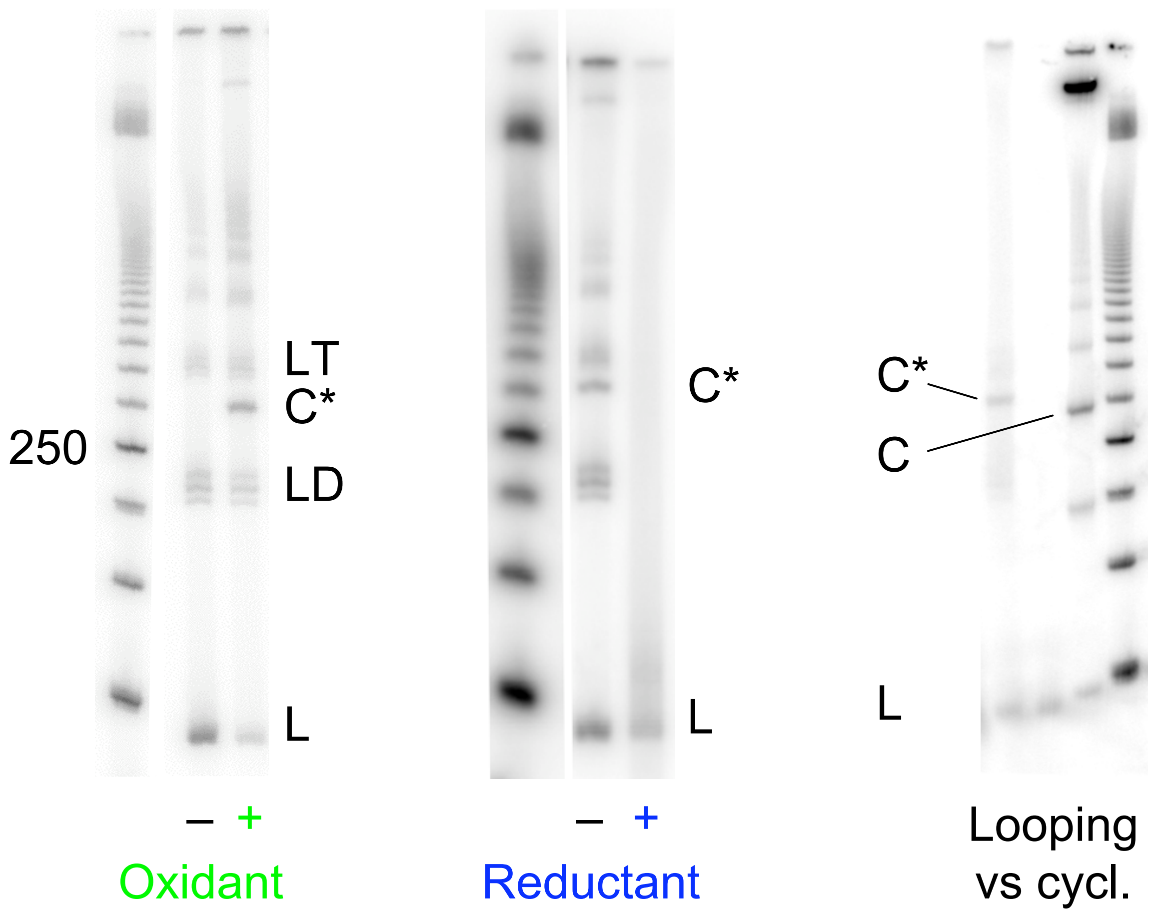
Chemical trapping assay for looping equilibria and kinetics



J. Dohm

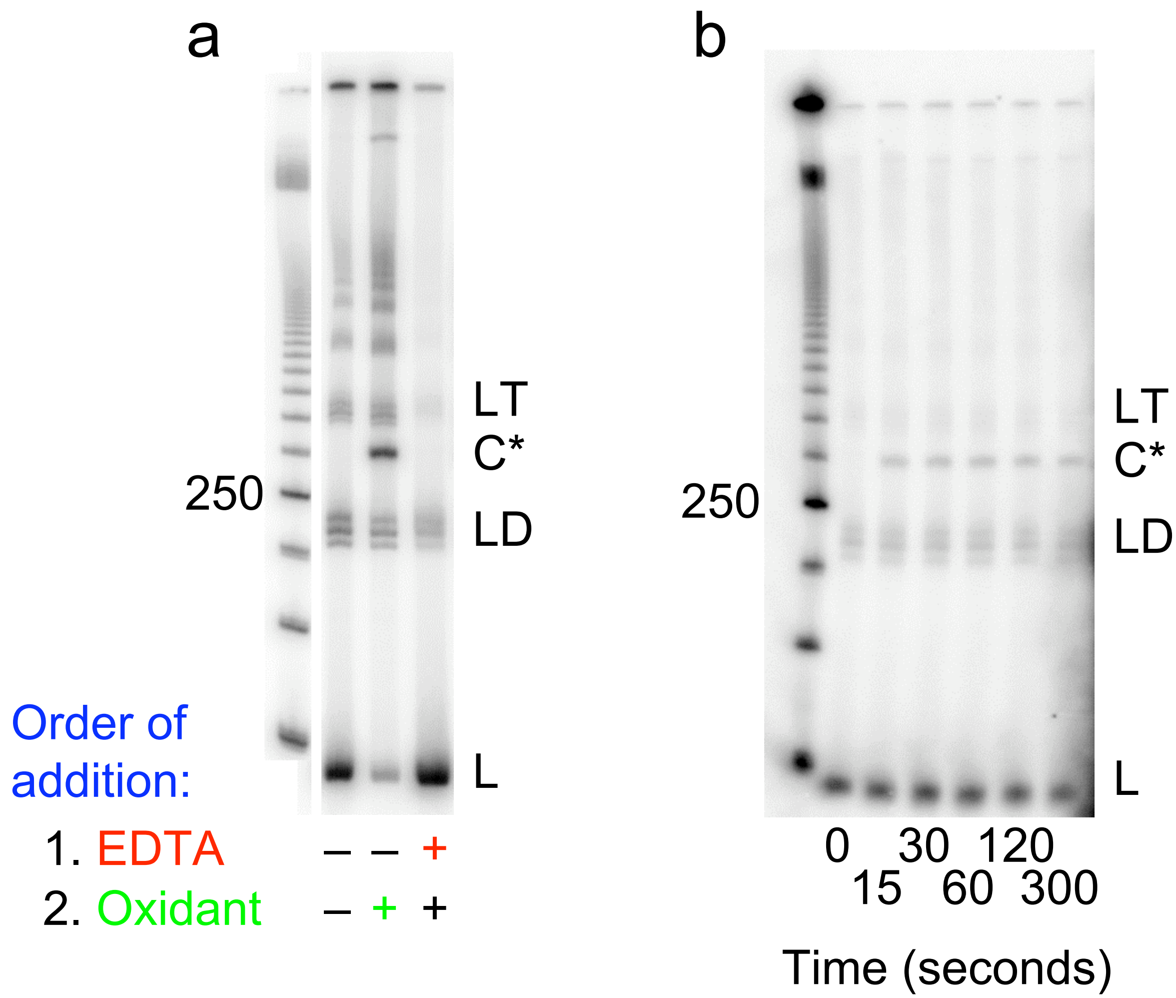
Chemical trapping assay for looping equilibria and kinetics

•94 bp loops



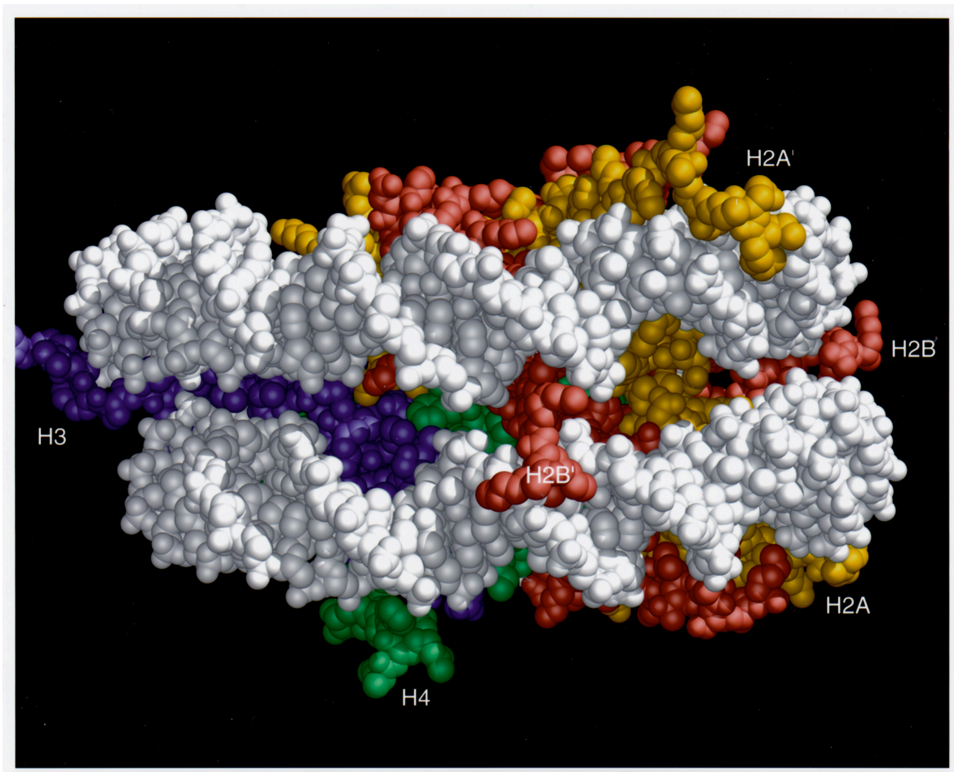
J. Dohm

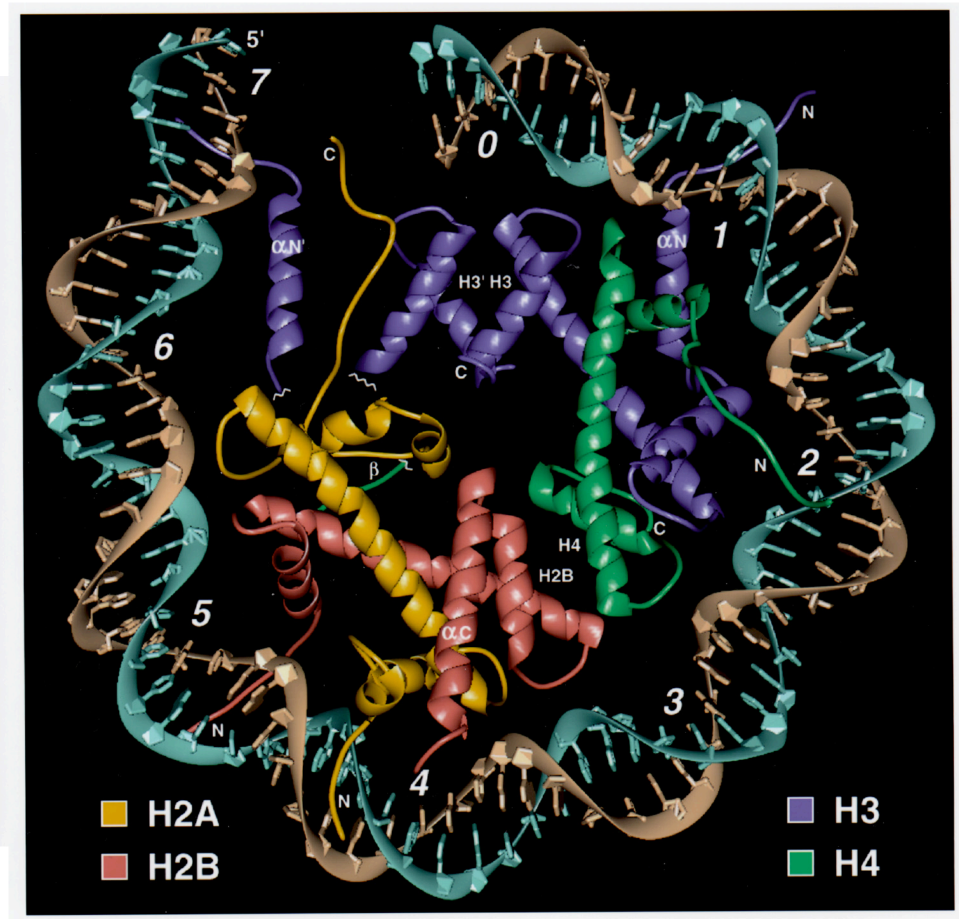
Rapid spontaneous looping of 94 bp DNAs



J. Dohm

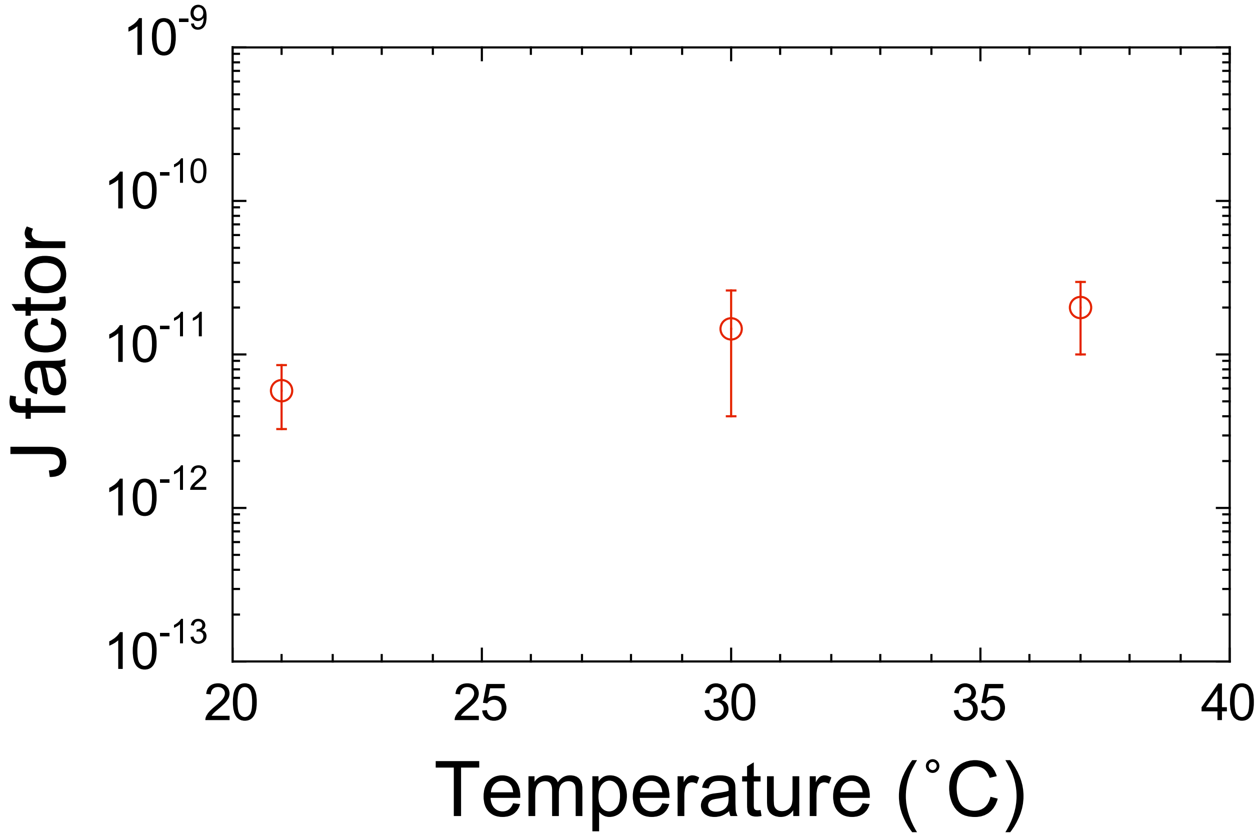
Structural basis of sharply looped protein–DNA complexes





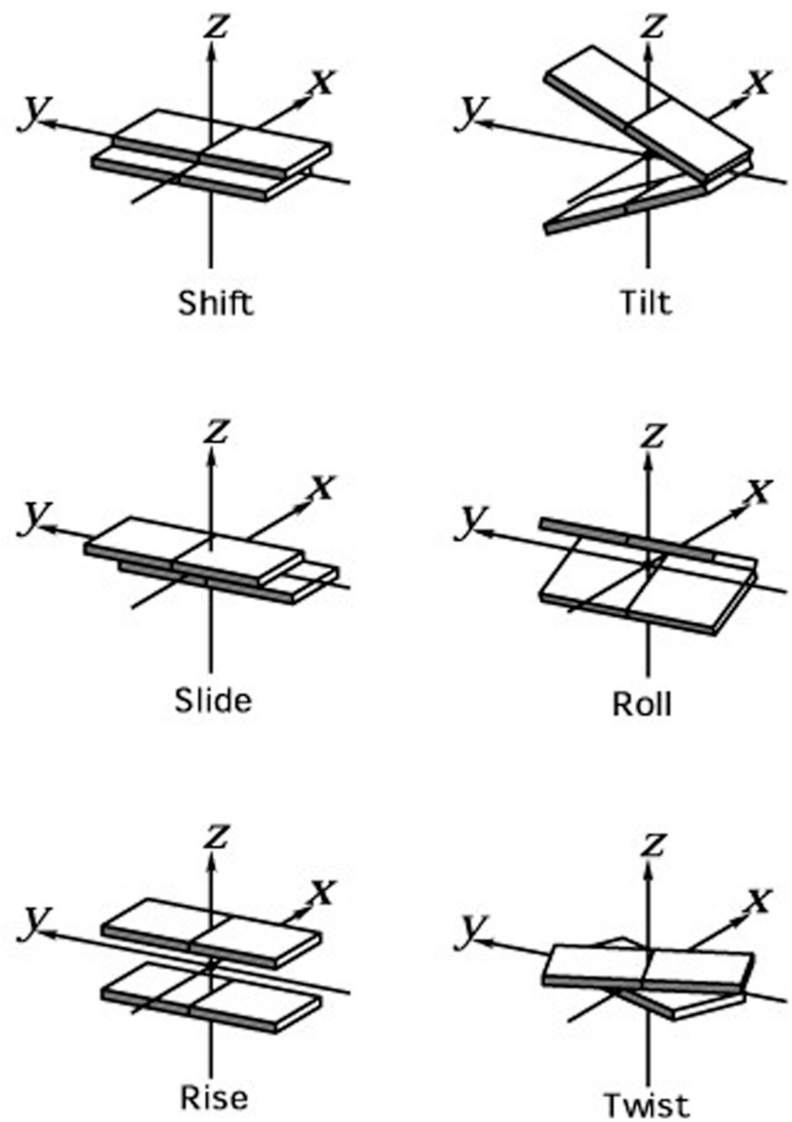
Luger et al., 1997
Richmond & Davey, 2003

J factors are weakly dependent on temperature



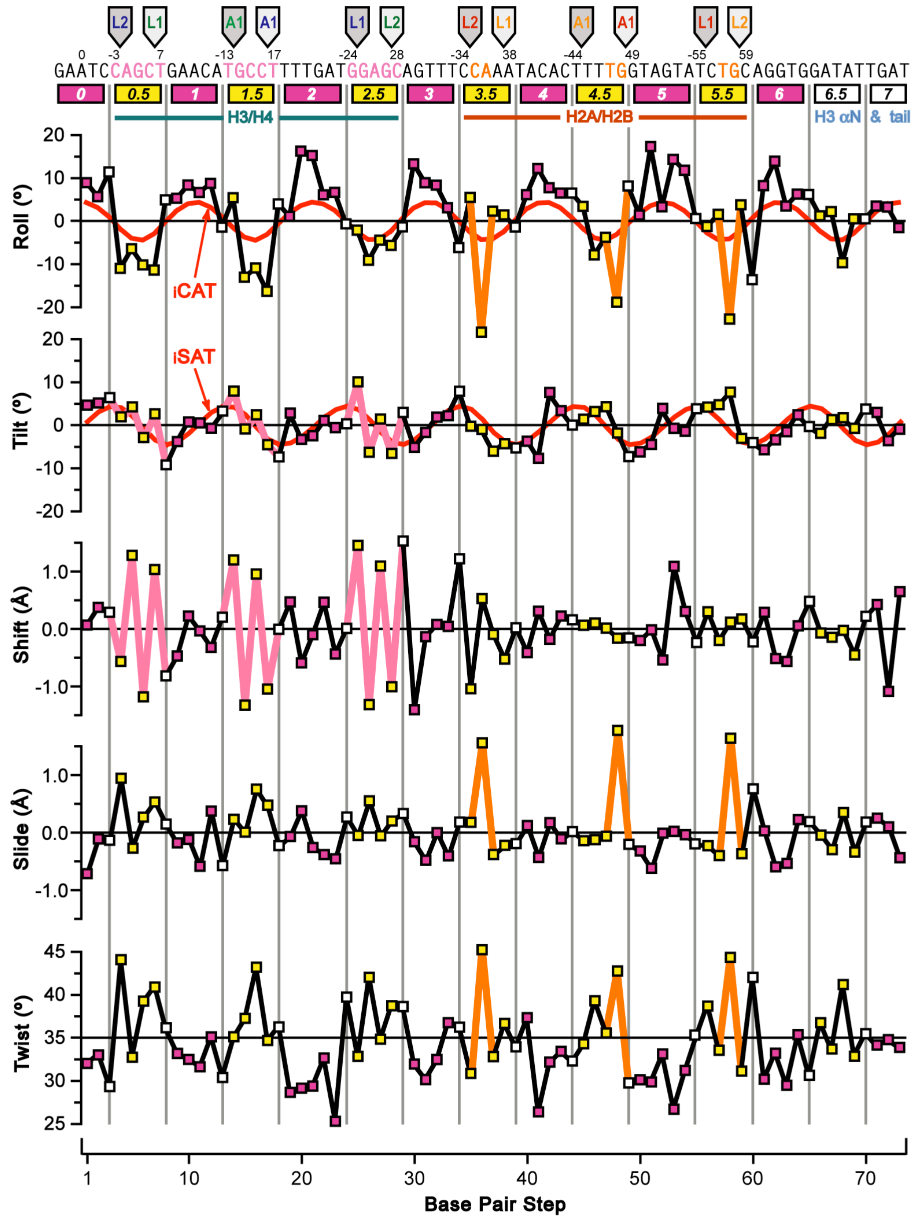
J. Dohm

Basepair steps as fundamental units of DNA mechanics



Zhurkin
Olson

Correlated deformations for sharp DNA wrapping



Roll

Tilt

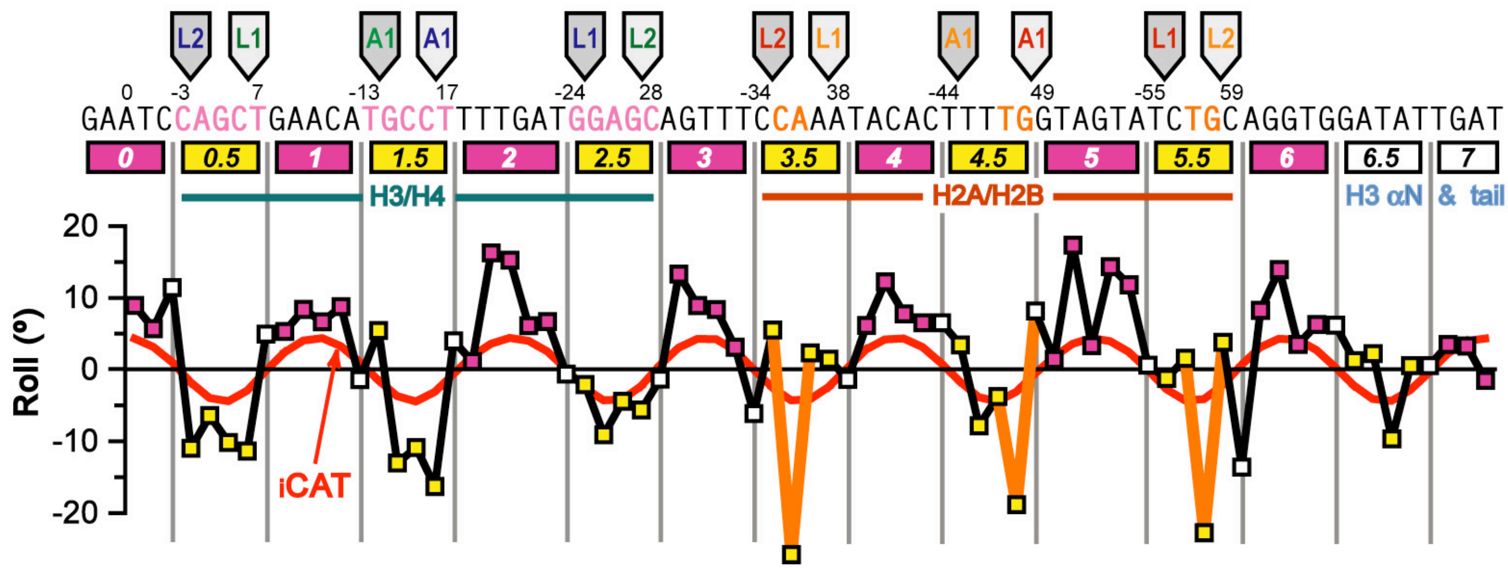
Shift

Slide

Twist

Richmond & Davey, 2003

Structural basis of sharply looped protein–DNA complexes



• Small distortions, and localized larger distortions along the full wrapped DNA length

Richmond & Davey, 2003

Acknowledgements

Tim Cloutier
Julie Dohm
Karissa Fortney
Dan Grilley