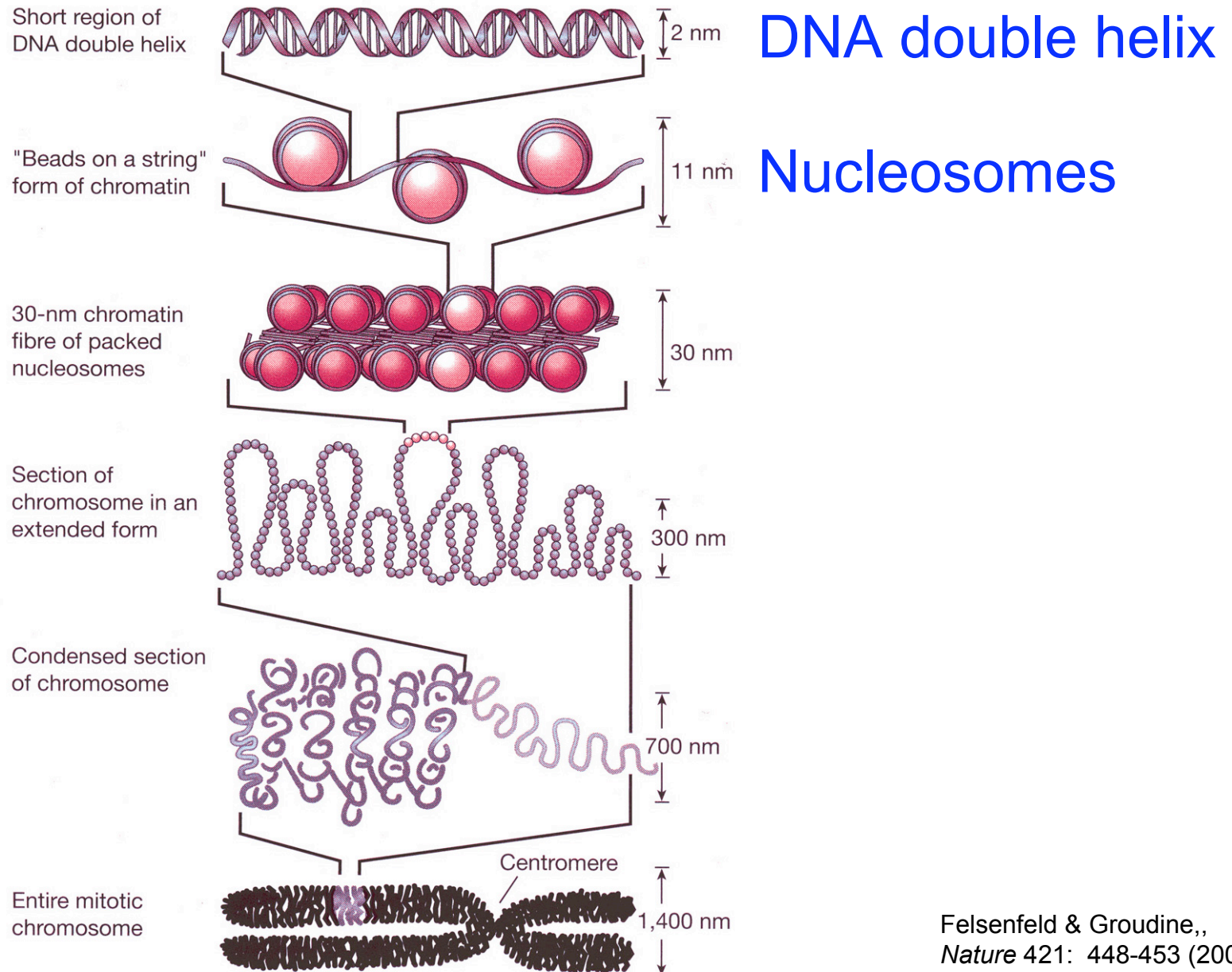


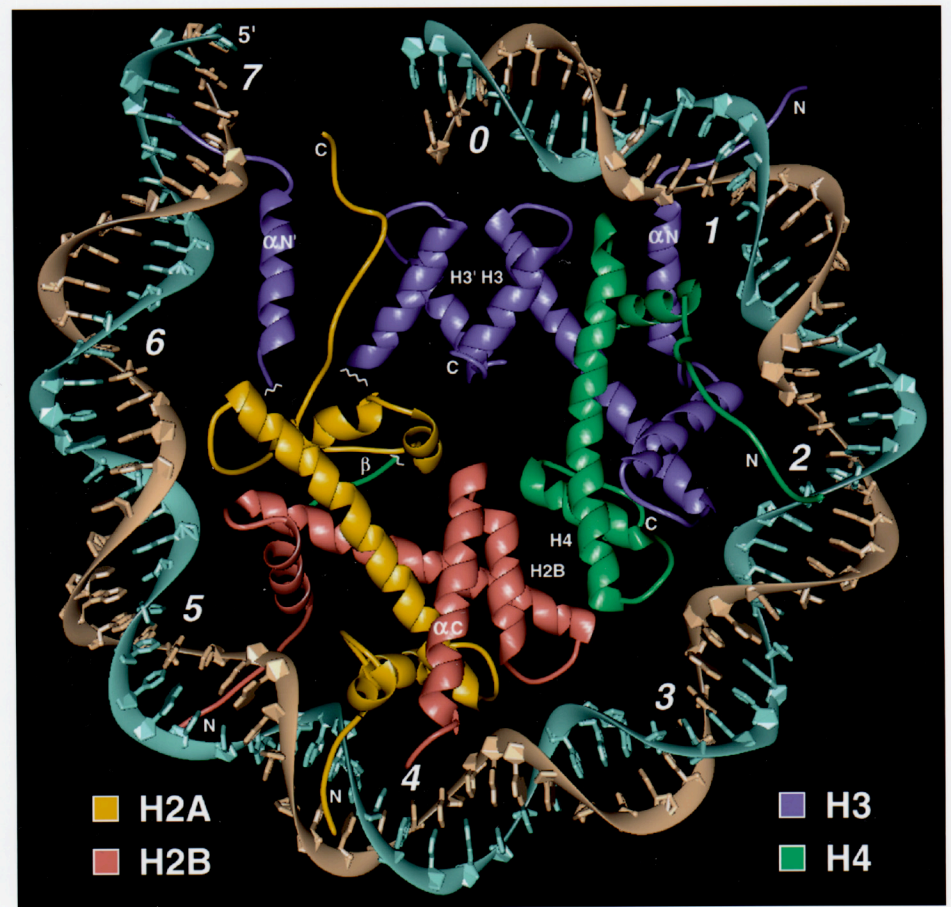
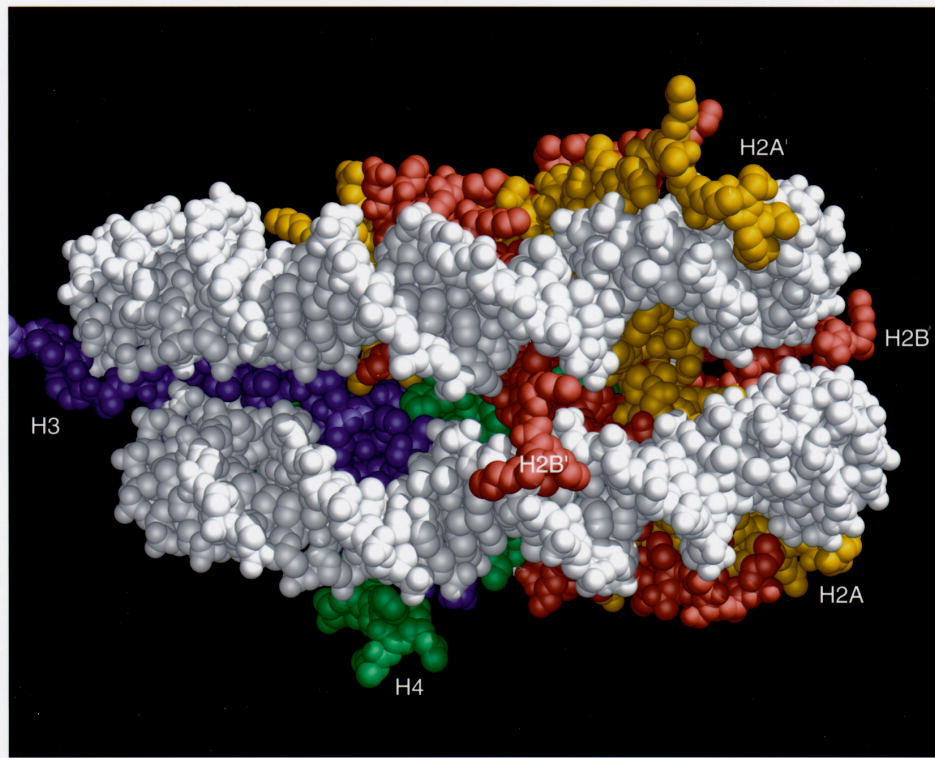
DNA Looping and Gene Regulation: Sequence-dependence of DNA Flexibility

Hierarchical DNA folding in eukaryotic chromosomes



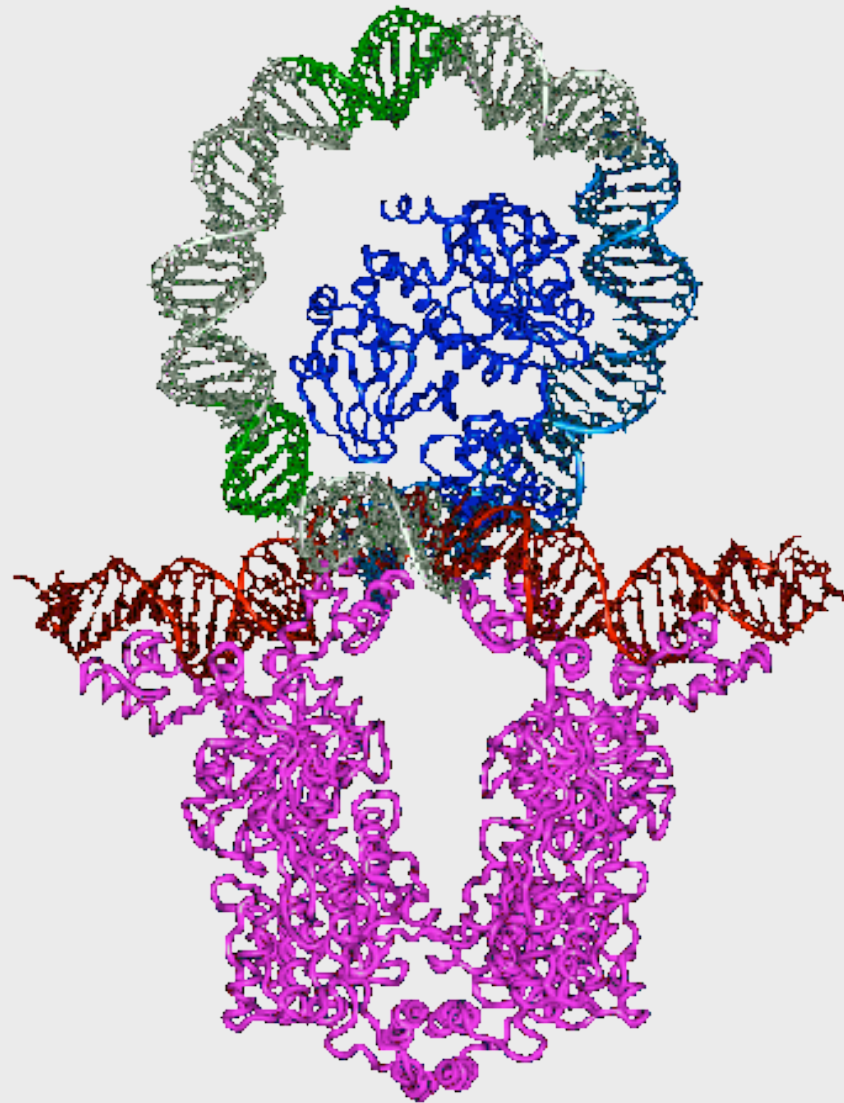
Felsenfeld & Groudine,,
Nature 421: 448-453 (2003)

Most eukaryotic DNA is sharply looped

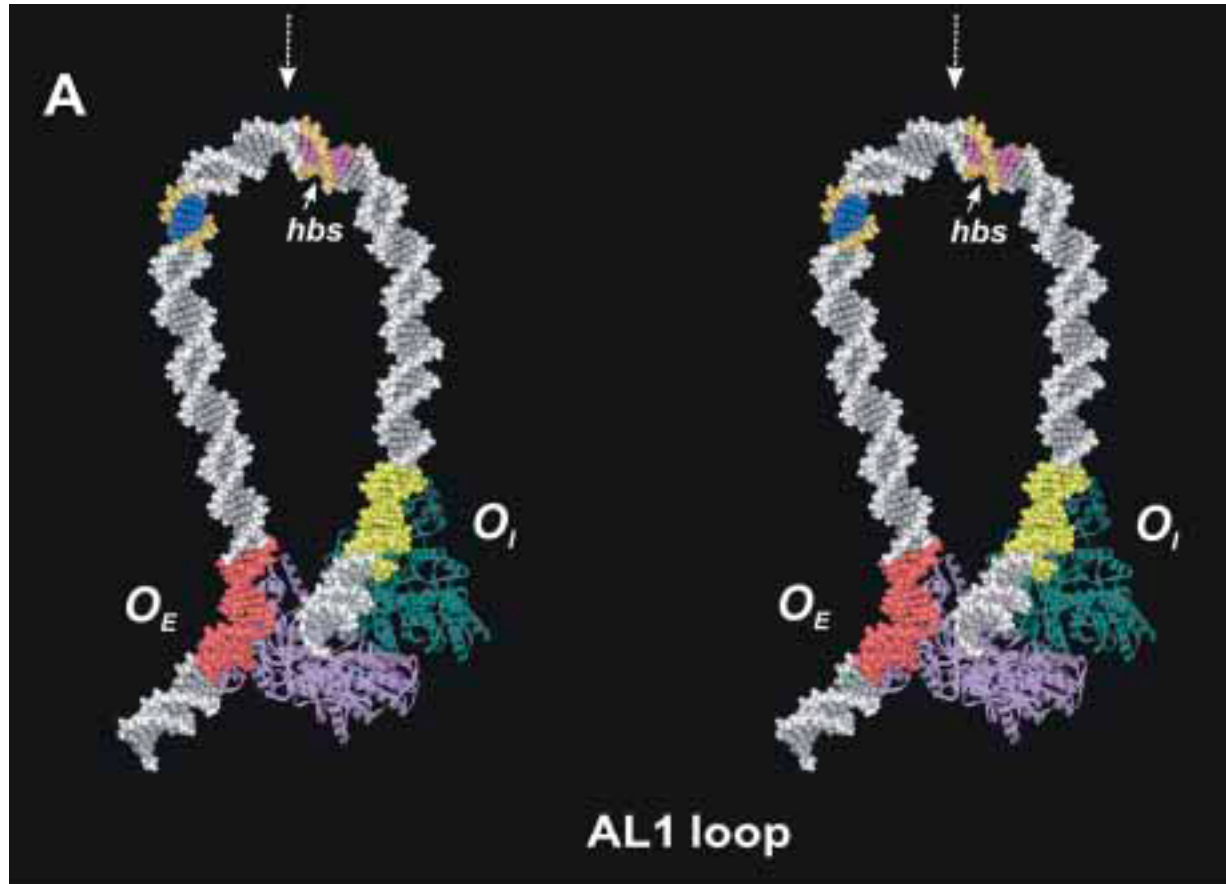


~80 bp per superhelical turn

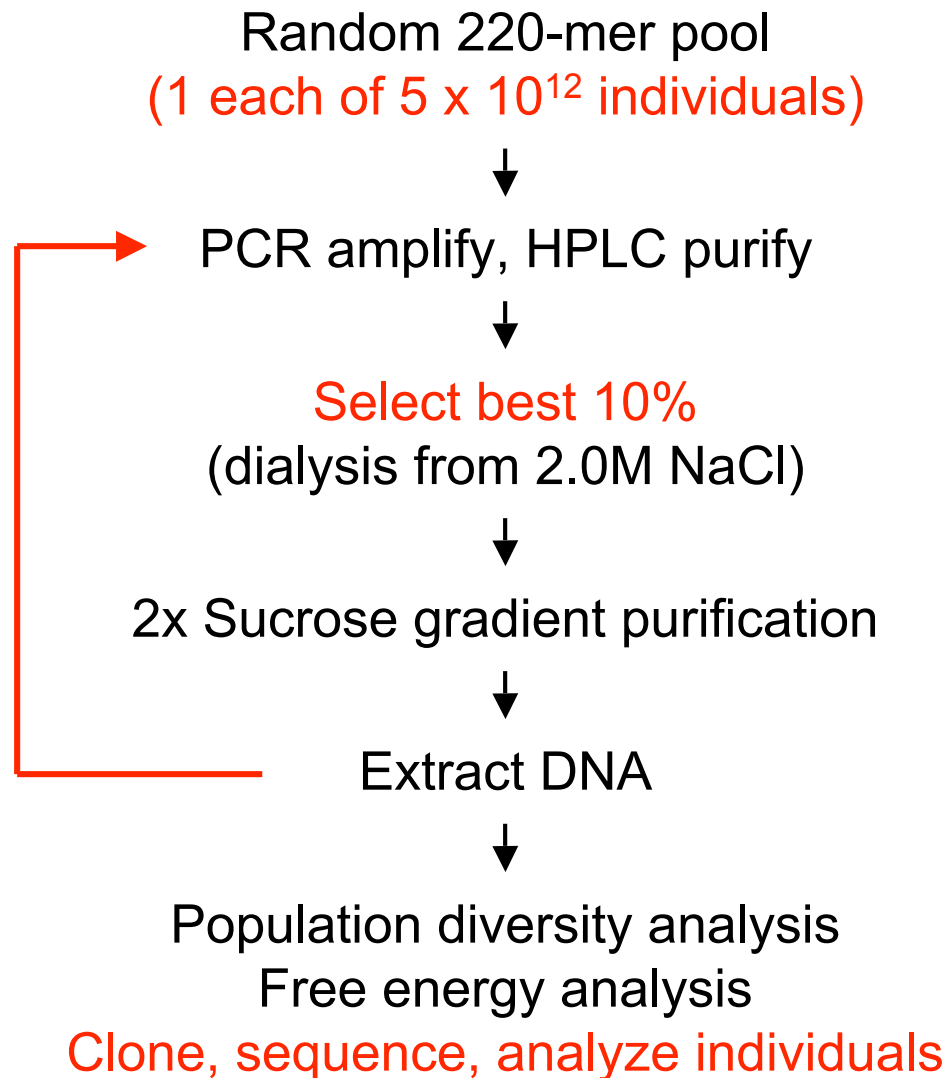
Sharply looped DNA in the *lac* operon



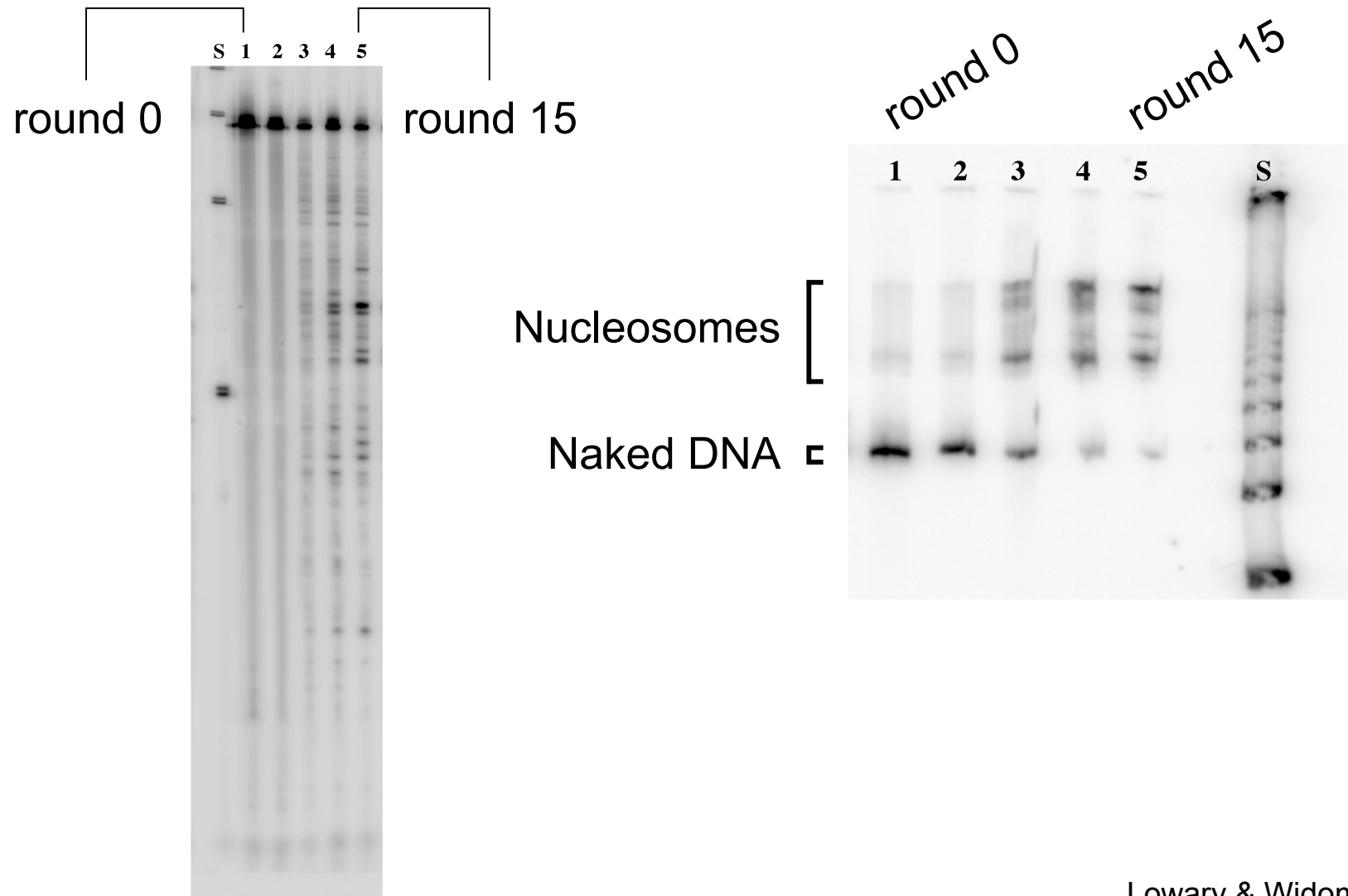
Sharply looped DNA in the Gal repressosome



Physical selection for stable nucleosome formation on chemically synthetic random DNAs



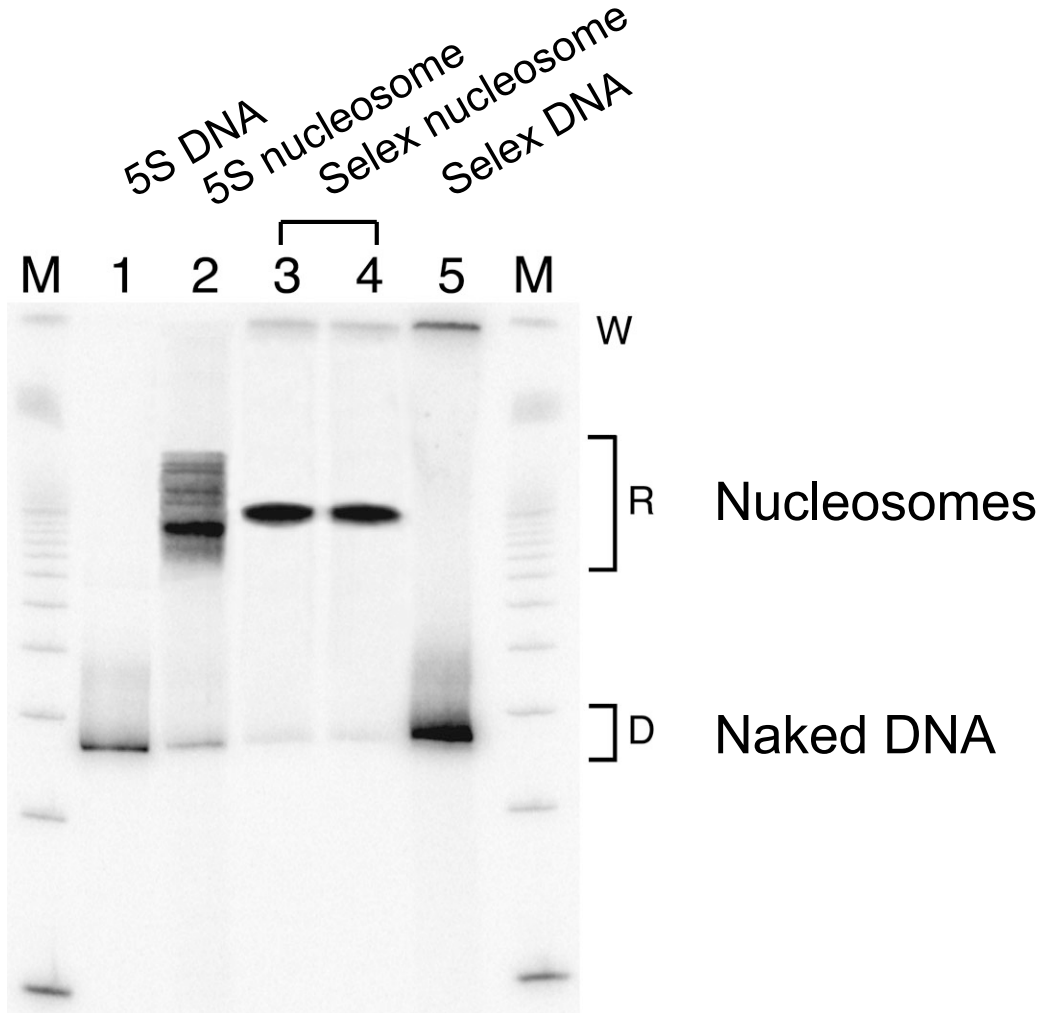
Population diversity decreases, and affinity increases, as selection proceeds



Course of the physical selection

Round #	# of Species	$\Delta\Delta G$ (kcal mol ⁻¹) (rel. to 5S molecule)
0	5×10^{12}	+0.5 ± 0.41 (n=10)
5	$\sim 10^8$	-0.09 ± 0.30 (n=2)
9	$\sim 10^4$	-1.7 ± 0.39 (n=6)
12	~ 100	-2.4 ± 0.37 (n=9)
15	30-50	-2.8 ± 0.32 (n=8)

Selected DNAs are strong nucleosome positioning sequences



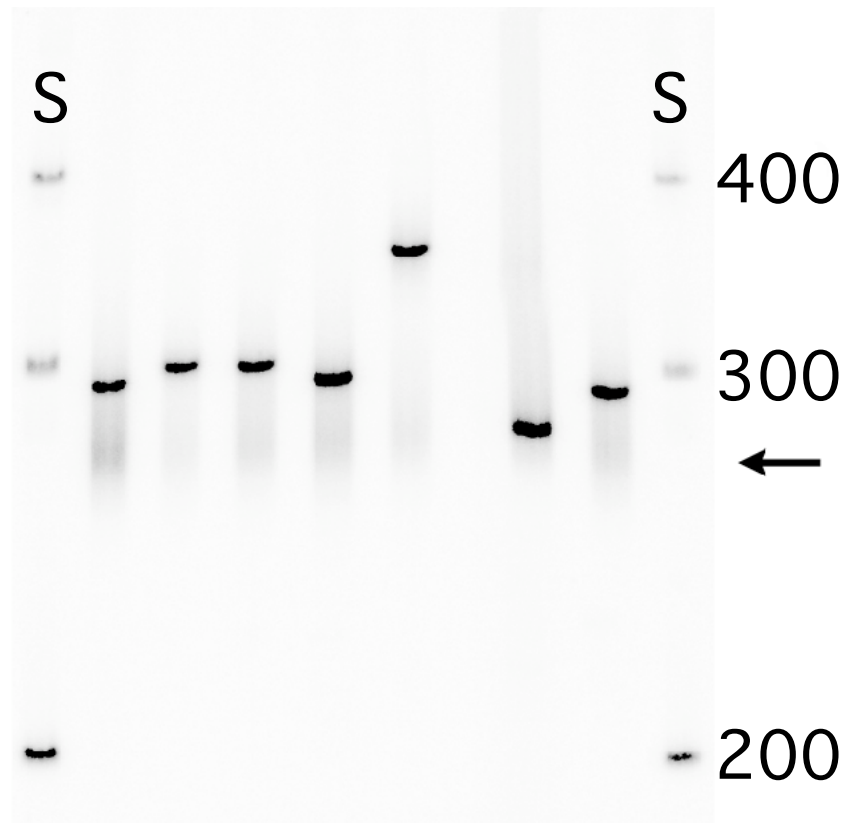
- Differing DNA sequences exhibit a $> 5,000$ -fold range of affinities for nucleosome formation

Lowary & Widom, 1998
Thåström et al., 1999
Widom, 2001
Thåström et al., 2004

Why DNA some sequences have especially high affinity for histone octamer

- More or better bonds
- Appropriately bent
- **More easily bendable**
- Appropriate twist
- More easily twistable

Nucleosome positioning sequences are especially bent or bendable



Bendedness, or *bendability*?

Sharp bending from static bends?

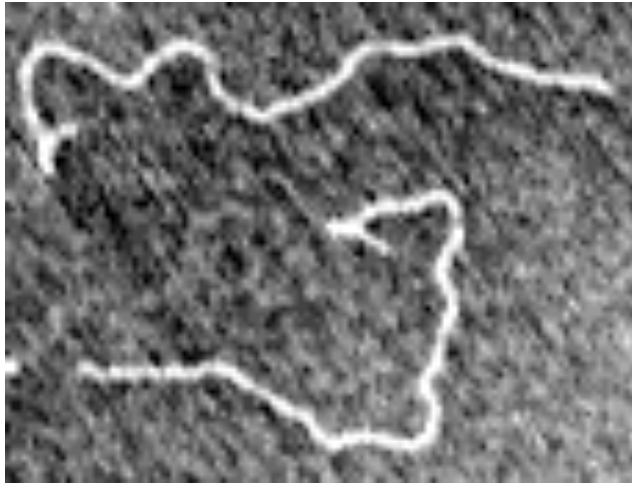
- A-tracts may be the most-bent of all sequences
- The most-bent A-tracts are bent by $\sim 19^\circ$ per 10-mer (i.e., $\sim 2^\circ$ / bp)
- DNA in nucleosomes is bent by $\sim 4.5^\circ$ / bp
- The standard deviation of basepair roll is $\sim 4\text{--}6^\circ$ / bp

Sequence dependence of DNA flexibility

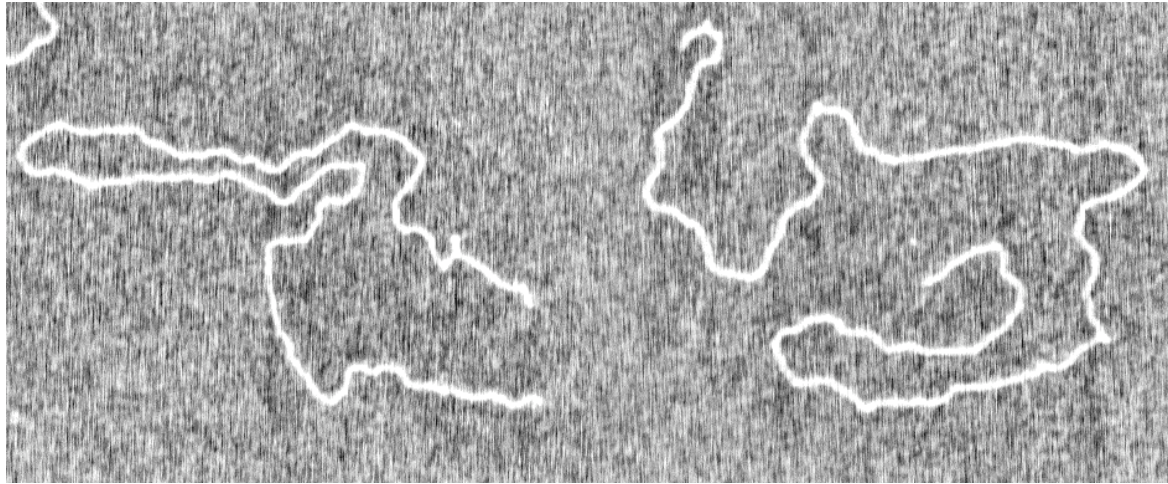
DNA sequences that make stable nucleosomes are especially bent or especially bendable

A-tracts are not very bent (relative, e.g., to sharp DNA loops or to the standard deviation of basepair roll)

J-shapes in AFM images of plasmid molecules with 9 A-tracts near one end



Selected nucleosome-forming DNAs are much less bent than A-tracts



- 3 kb plasmid linked to 30 copies (~6 kb)
of selected sequence 601

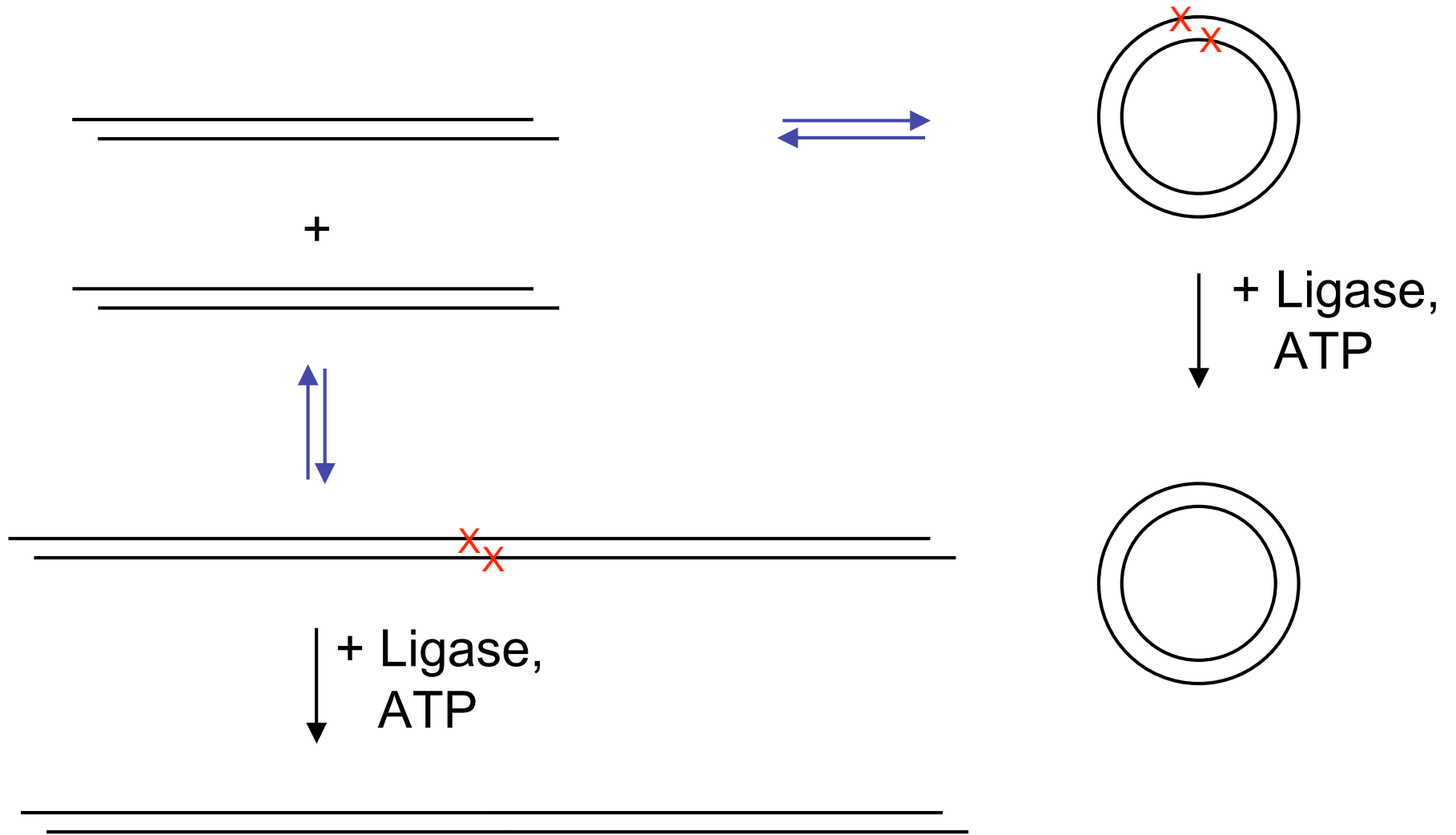
Properties of nucleosome positioning sequences

DNA sequences that make stable nucleosomes are especially bent or especially bendable

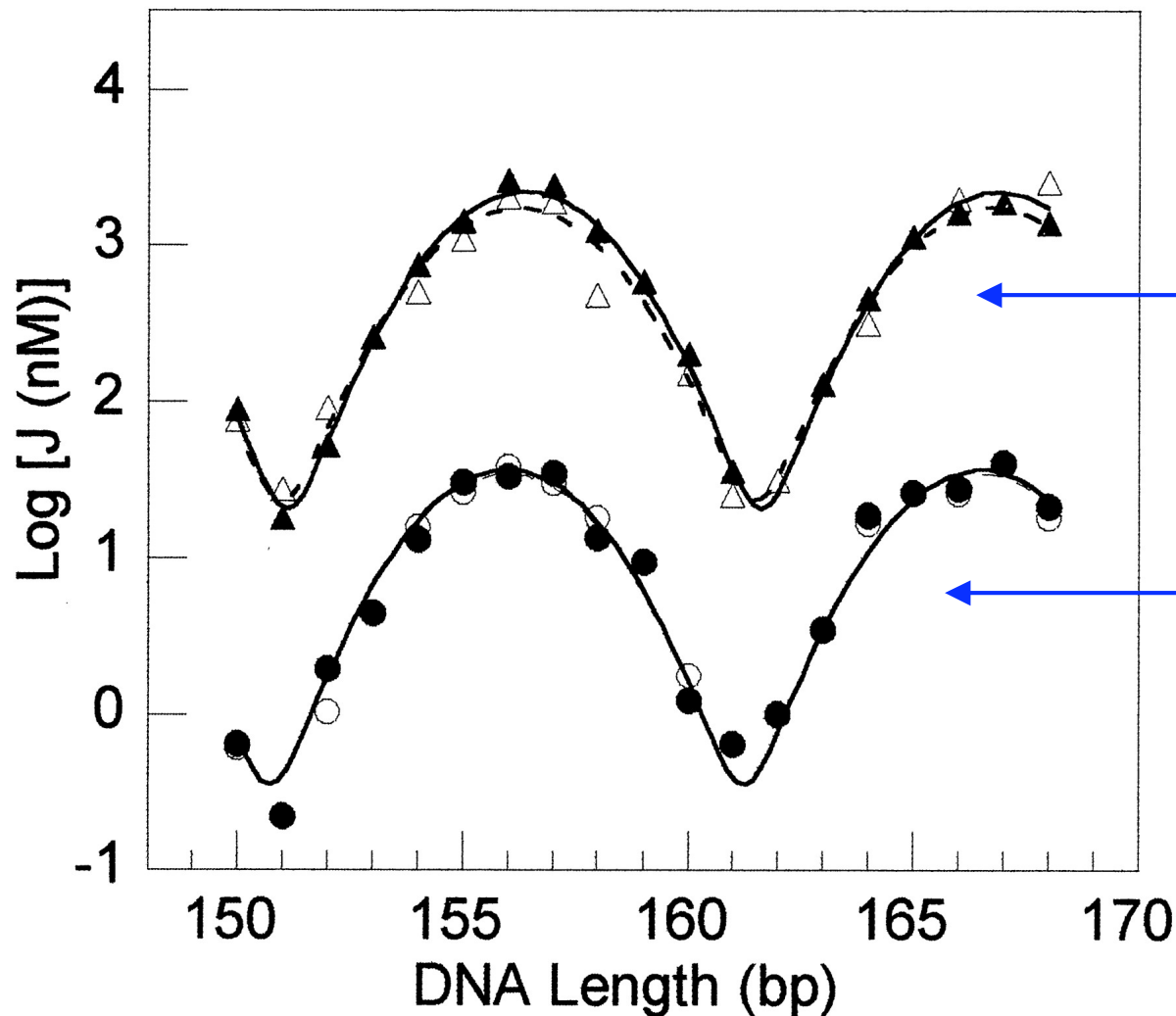
A-tracts are not very bent (relative to sharp DNA loops or to the standard deviation of basepair roll)

Nucleosome positioning sequences are much less bent than are A-tracts

Quantitative measurement of J factor



Especially stable nucleosomes have especially flexible DNA



DNA cyclization assays

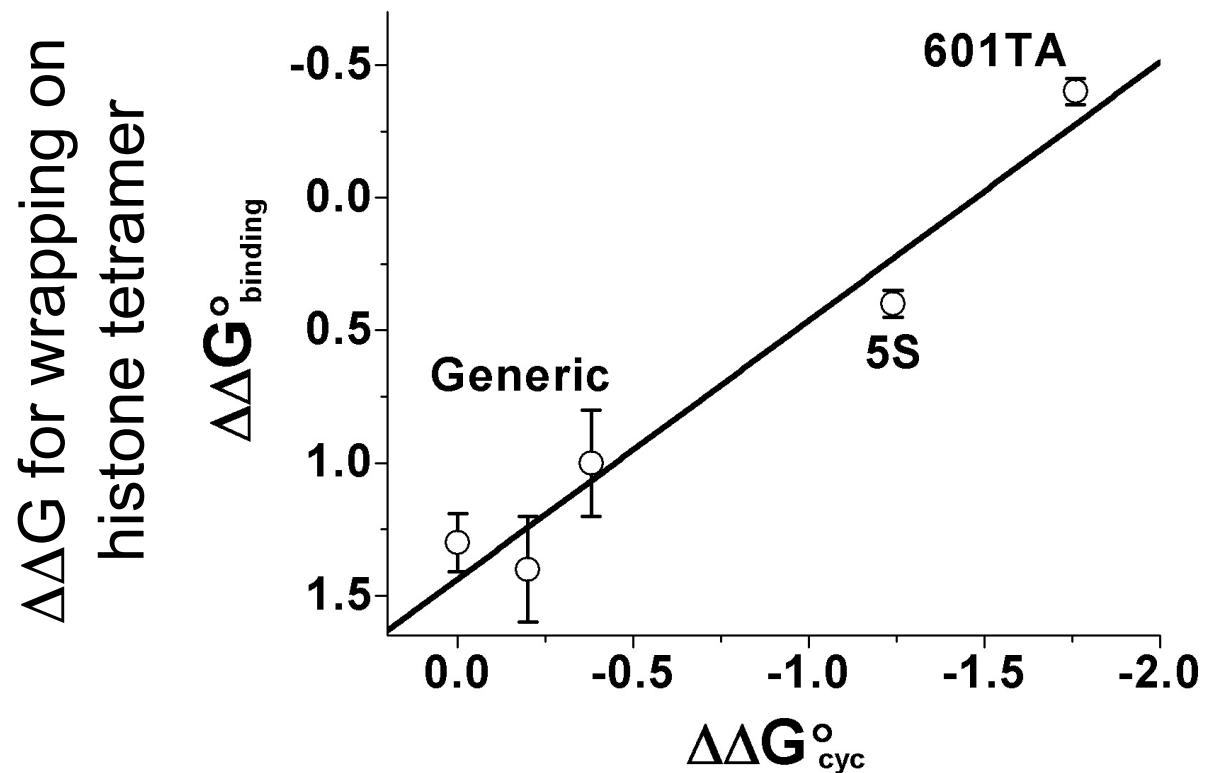
With 30-mer segment from stable nucleosome

$P_{30\text{-mer}} \sim 27 \text{ nm}$

With 30-mer random sequence segment

$P_{30\text{-mer}} \sim 50 \text{ nm}$

Especially flexible DNAs make especially stable nucleosomes and vice-versa



$\Delta\Delta G$ for cyclization of 94 bp DNAs
(Kcal mol⁻¹)

Properties of nucleosome positioning sequences

DNA sequences that make stable nucleosomes are especially bent or especially bendable

A-tracts are not very bent (relative to sharp DNA loops or to the standard deviation of basepair roll)

Nucleosome positioning sequences are much less bent than are A-tracts

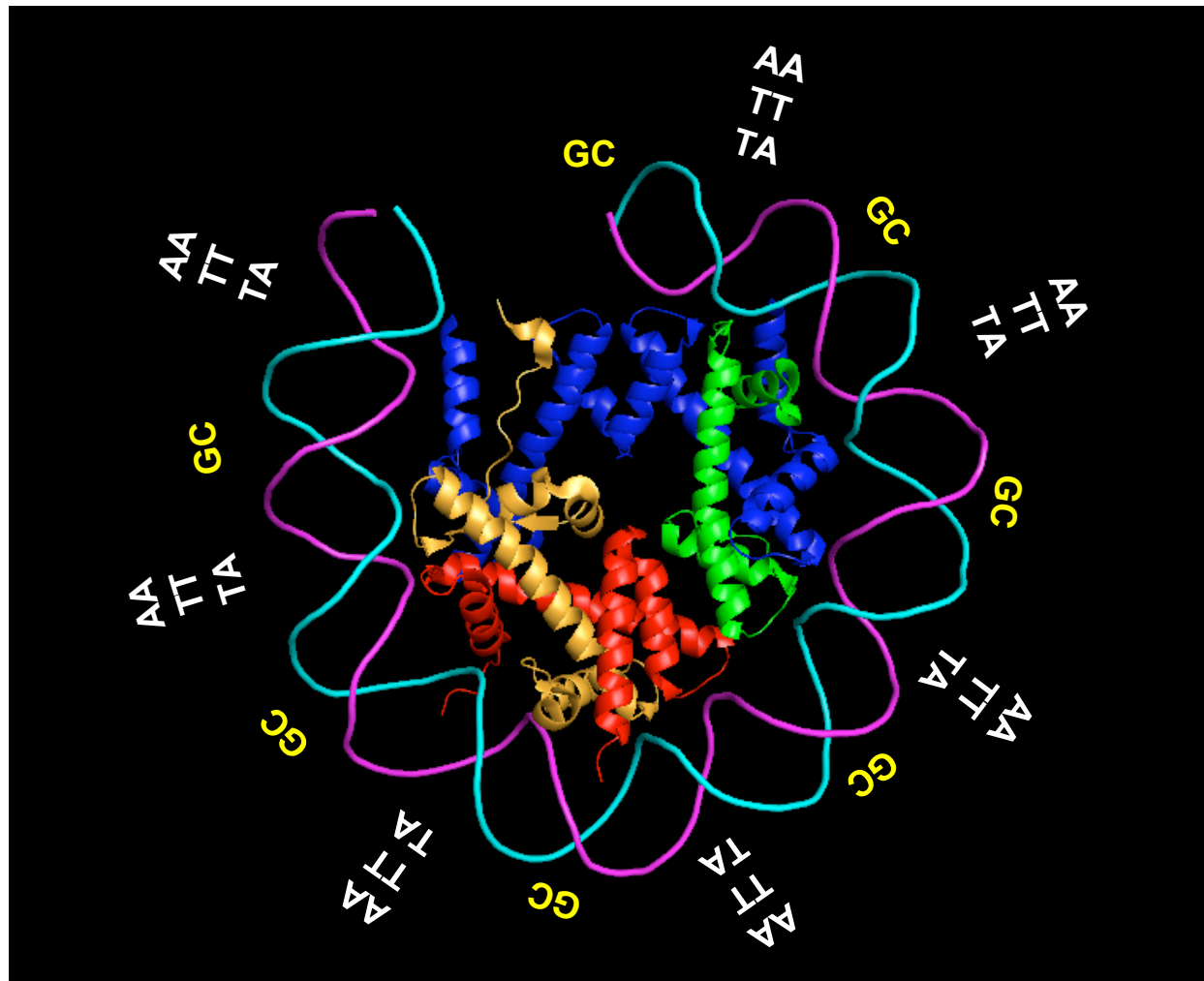
Nucleosome positioning DNA sequences are sequences that are especially *bendable* (especially soft for bending)

Especially flexible DNAs make especially
stable nucleosomes and vice-versa

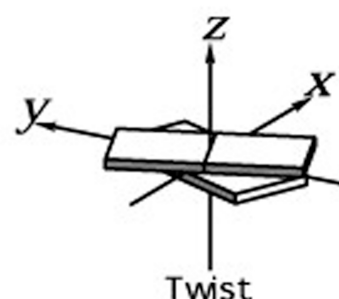
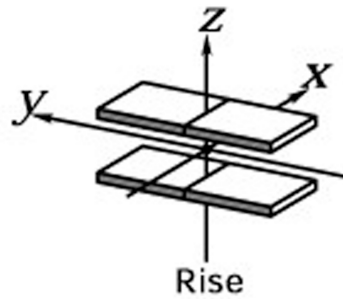
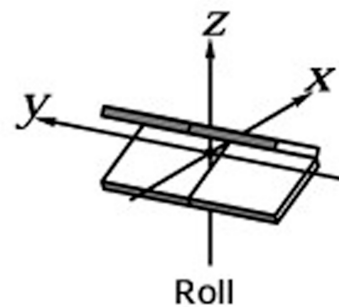
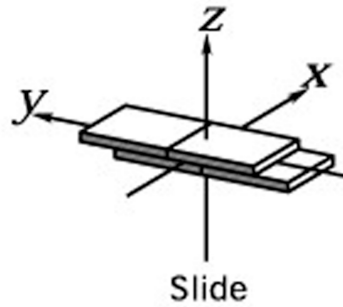
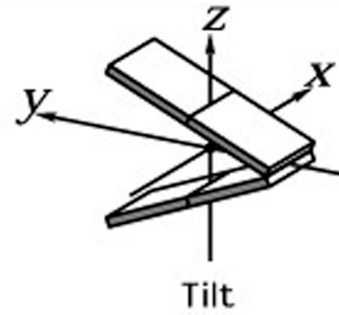
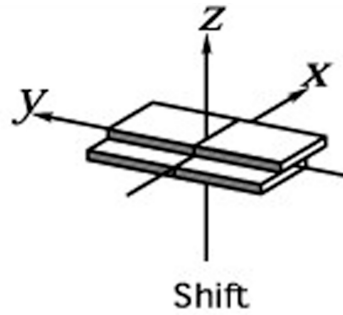
What DNA sequence motifs facilitate DNA
bendability? And, how do they work?

Widom, 2001
Thåström et al., 2004
Segal et al., 2006, in press

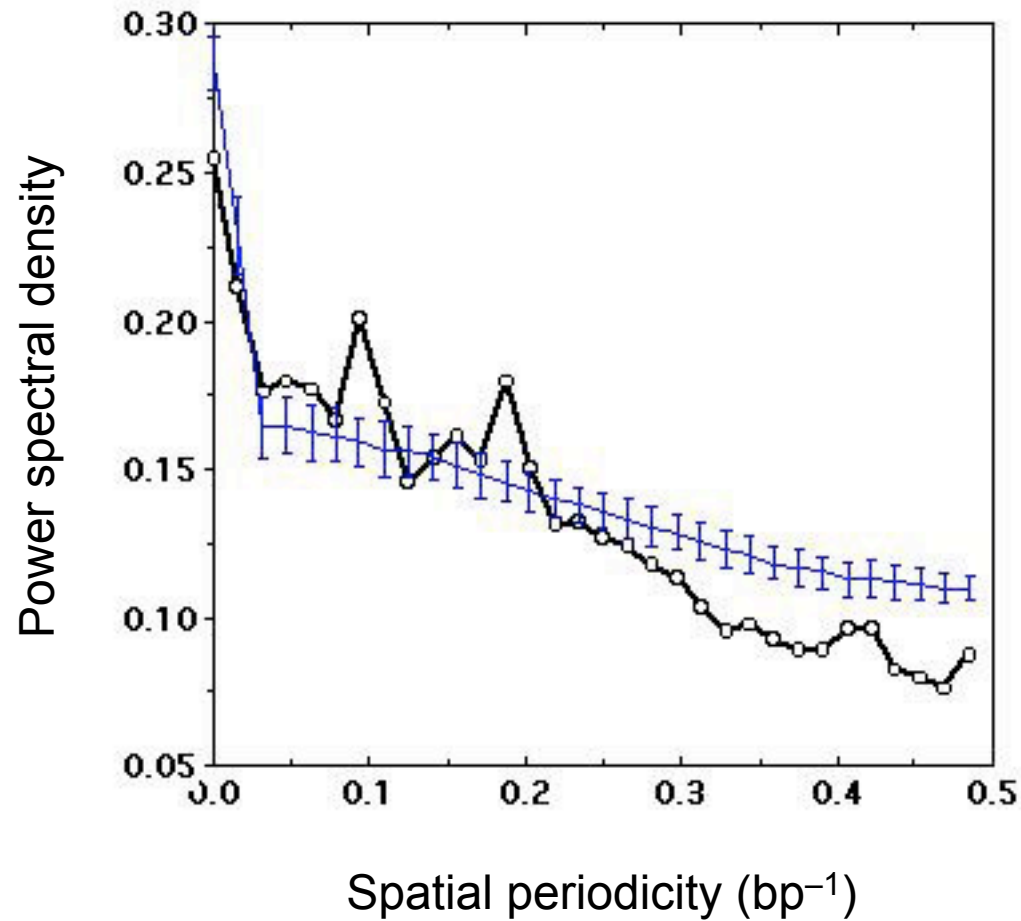
DNA sequence motifs that stabilize nucleosomes and facilitate spontaneous sharp looping



Basepair steps as fundamental units of DNA mechanics

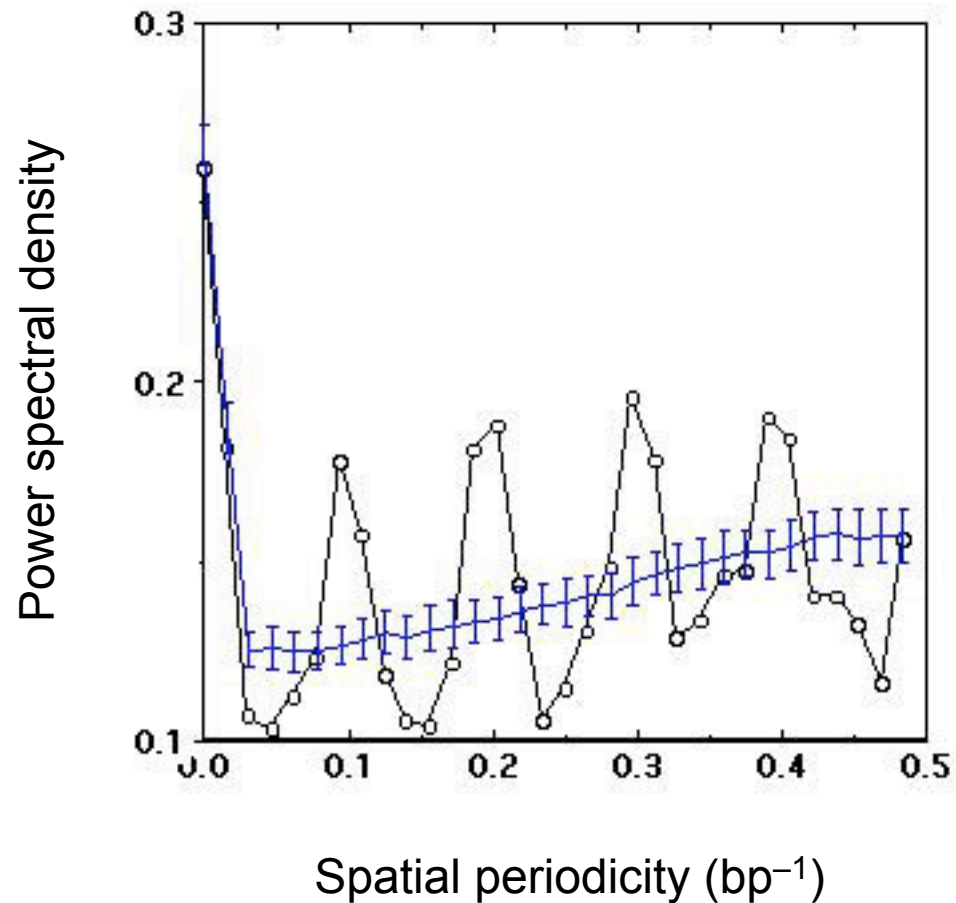


Liquid-like ~ 10 bp spacings of AA dinucleotides in selected nucleosome forming DNAs



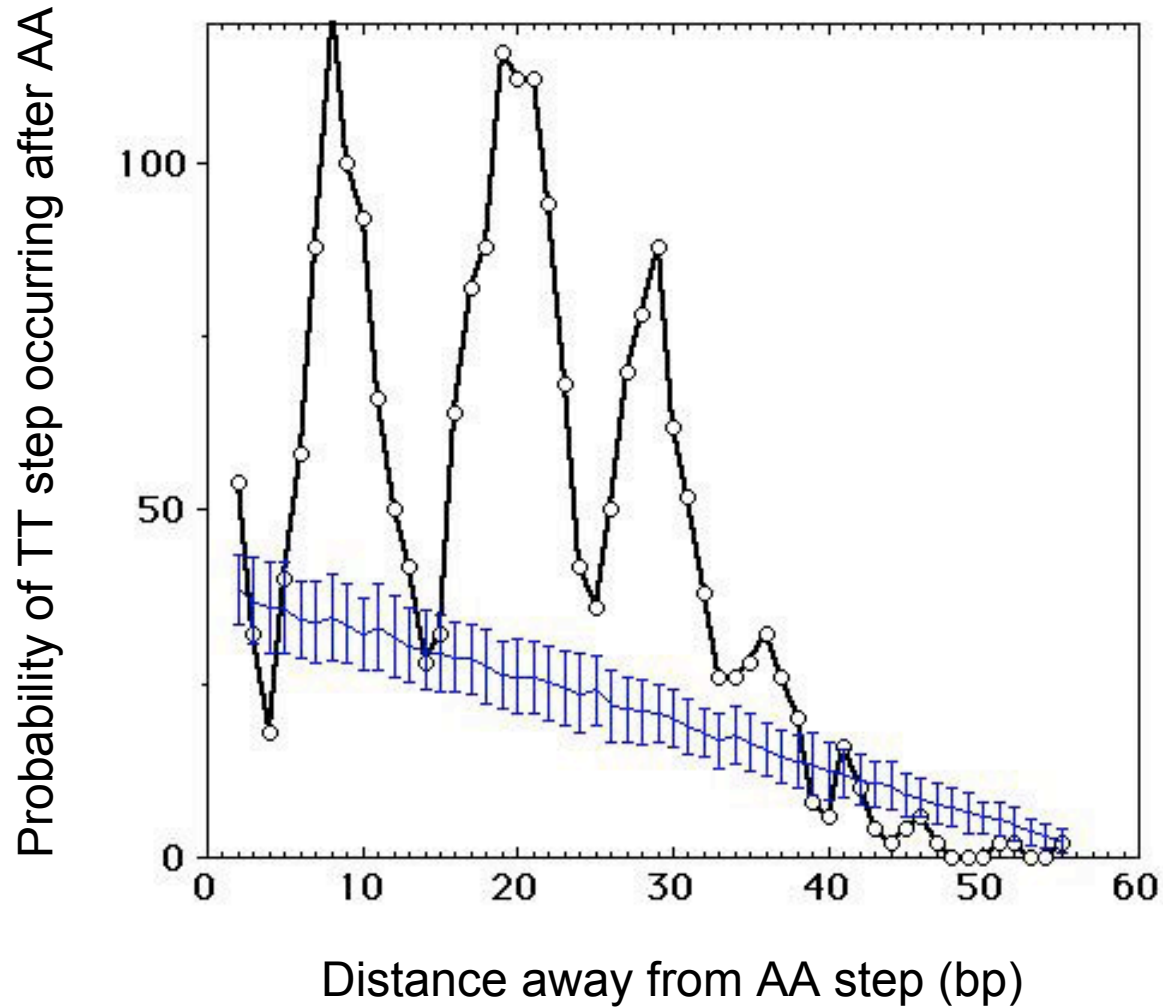
Lowary & Widom, 1998
Bailey et al., 2000

Crystal-like ~ 10 bp spacings of TA dinucleotides in selected nucleosome forming DNAs



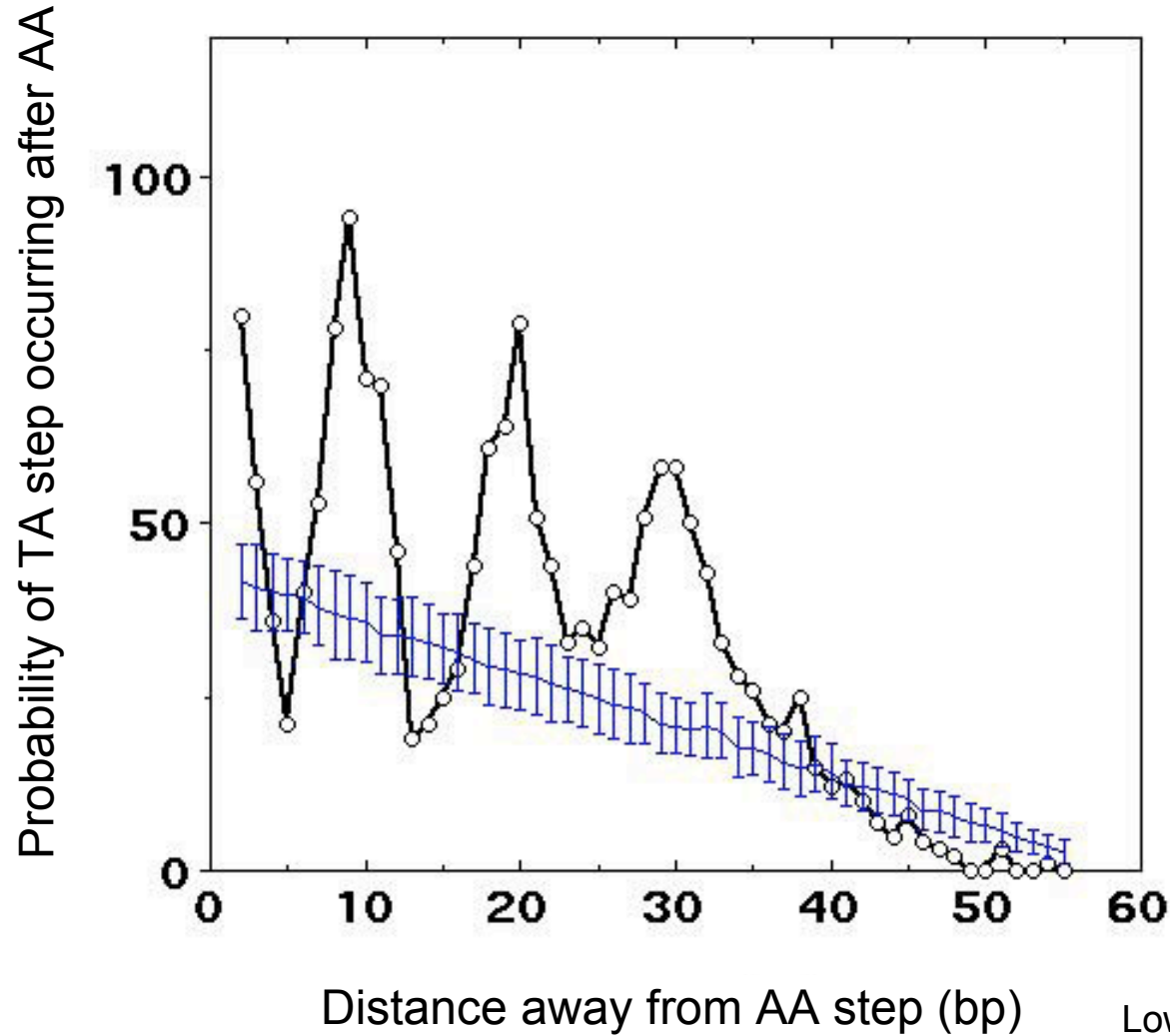
Lowary & Widom, 1998
Bailey et al., 2000

Probability of TT at distance λ bp from an AA, in selected nucleosome forming DNAs



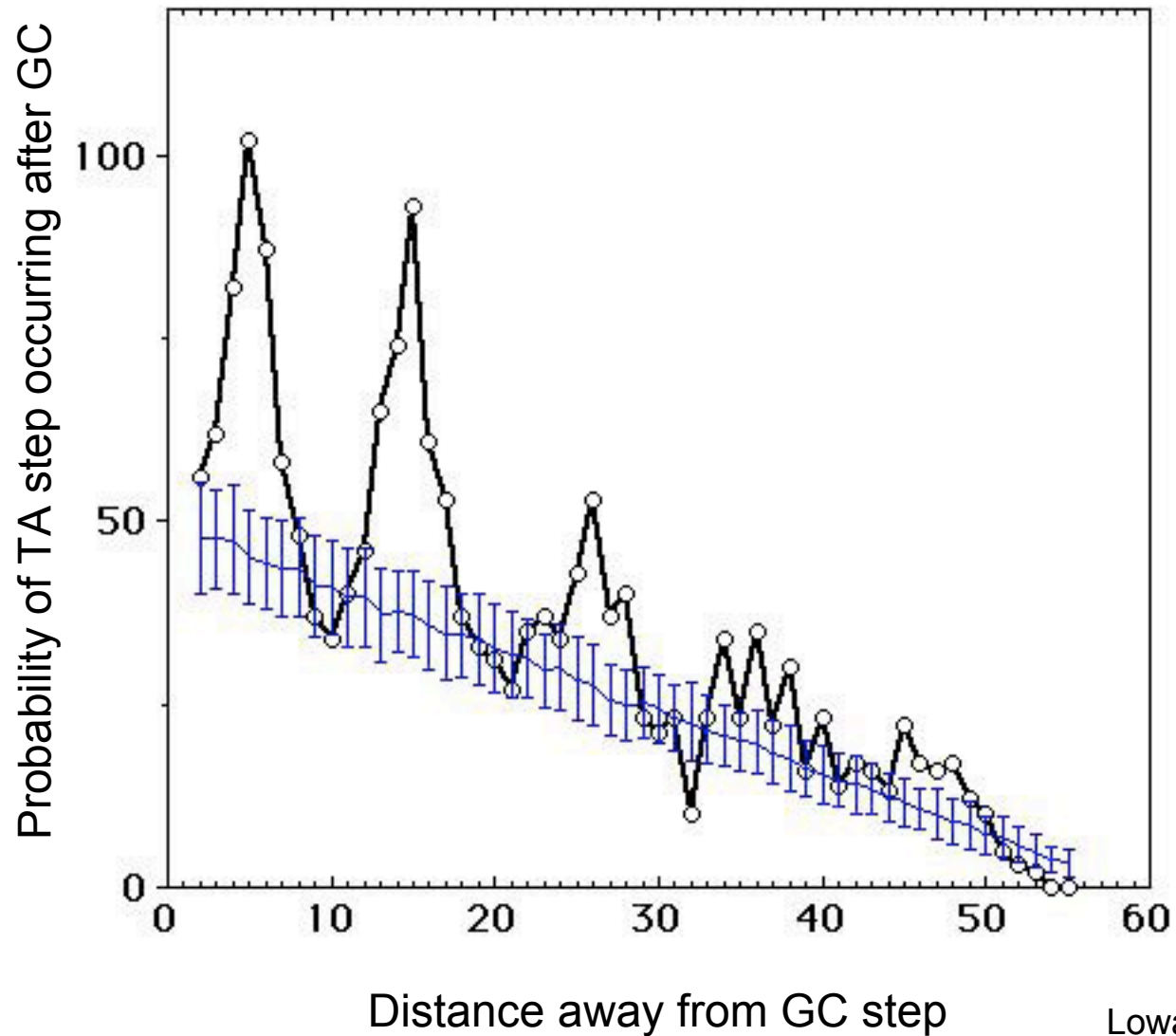
Lowary & Widom, 1998
Bailey et al., 2000

Probability of TA at distance λ bp from an AA, in selected nucleosome forming DNAs



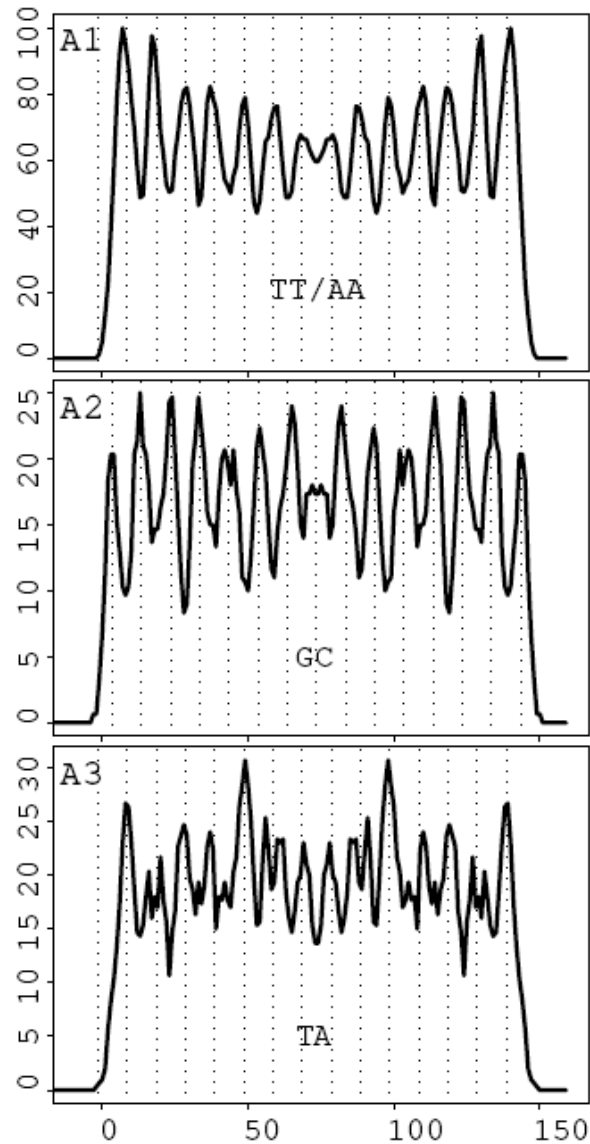
Lowary & Widom, 1998
Bailey et al., 2000

GC steps occur out of phase with AA, TT, & TA steps in high affinity nucleosome-forming DNAs

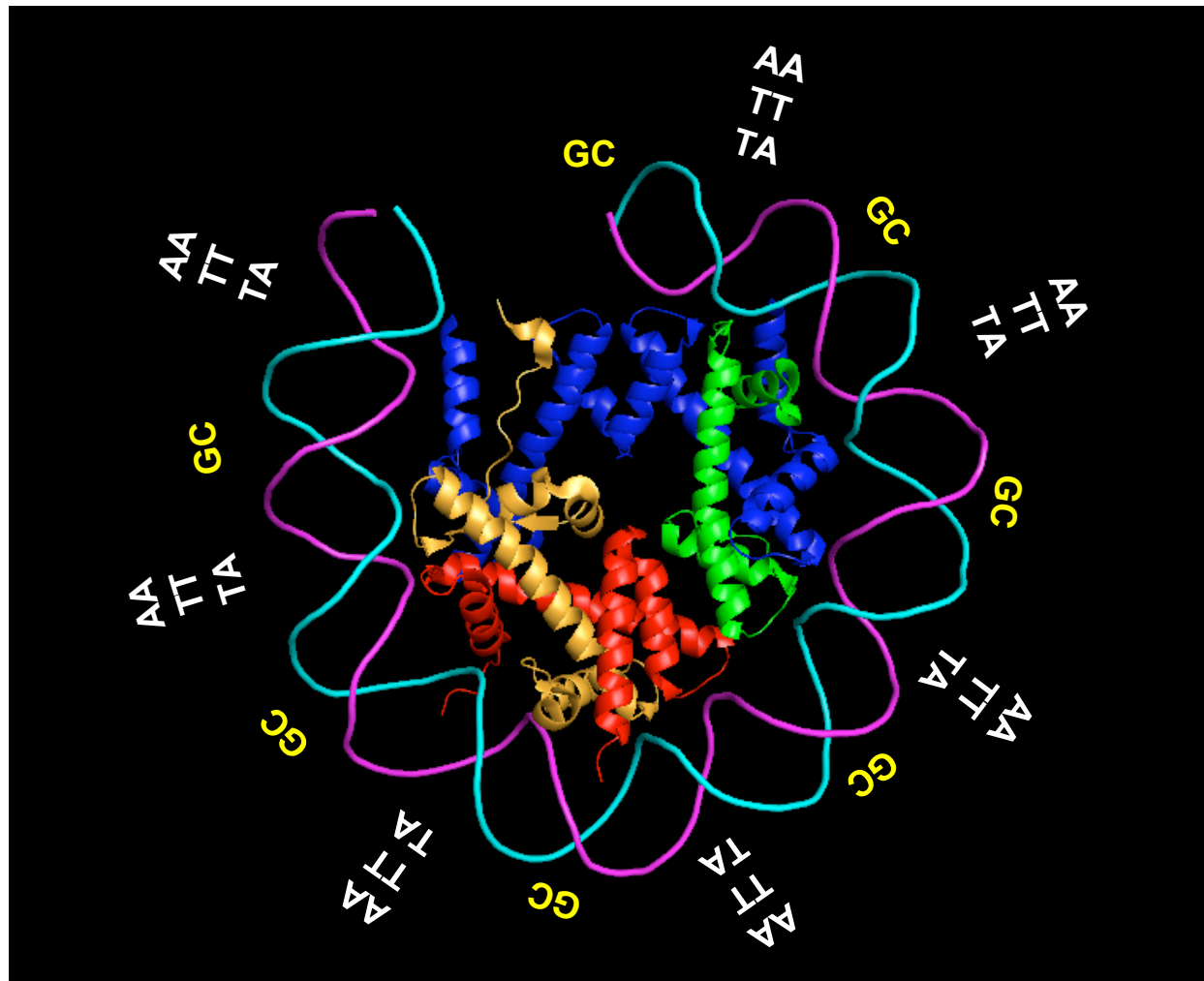


Lowary & Widom, 1998
Bailey et al., 2000

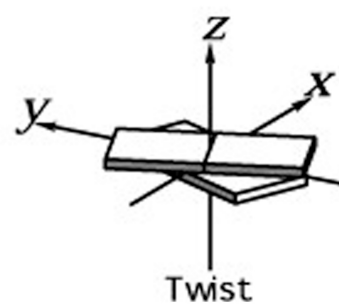
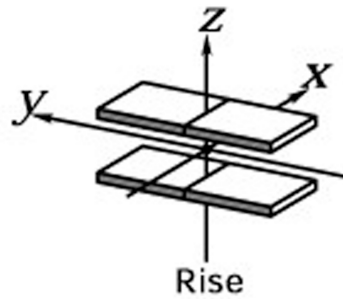
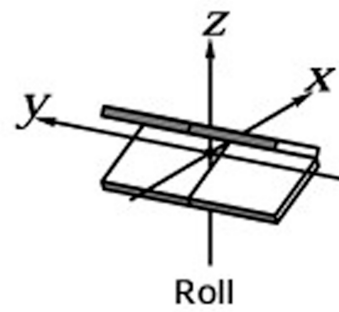
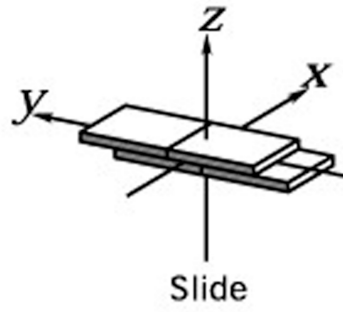
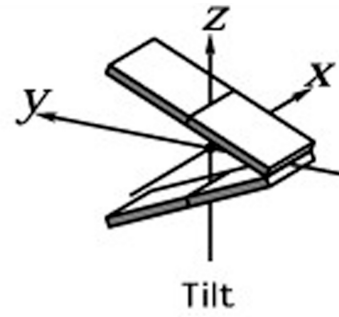
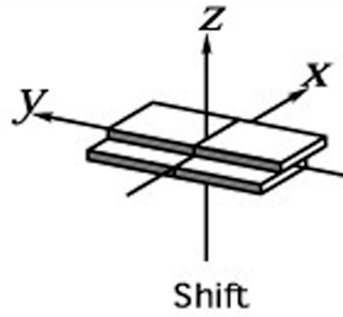
Locations of dinucleotide steps in alignments of natural nucleosome DNA sequences



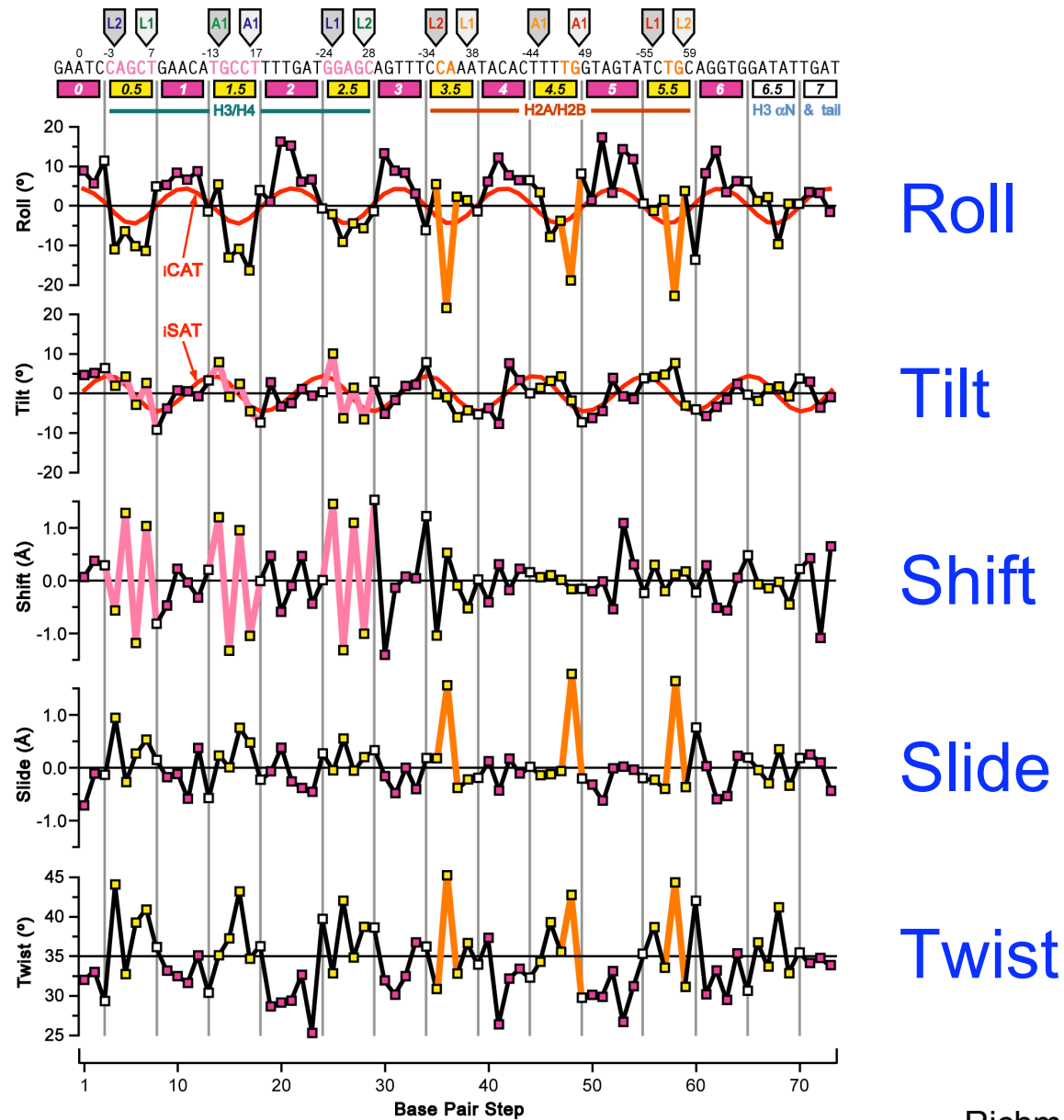
DNA sequence motifs that stabilize nucleosomes and facilitate spontaneous sharp looping



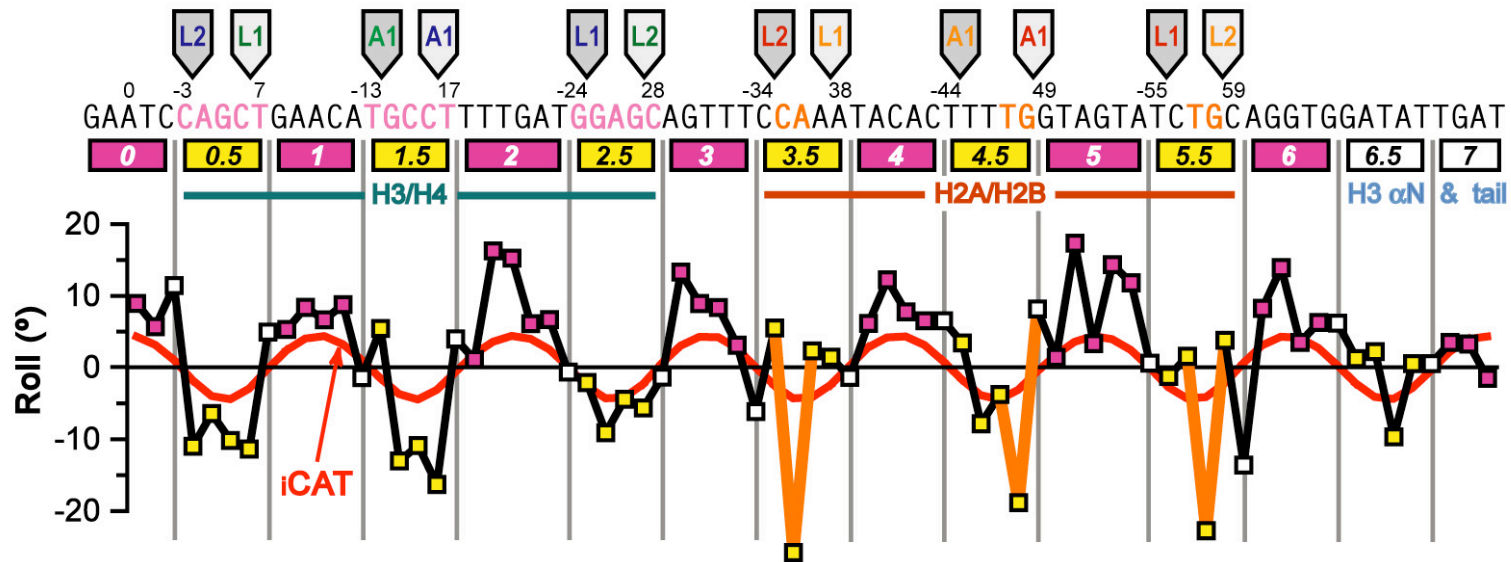
Basepair steps as fundamental units of DNA mechanics



Correlated deformations for sharp DNA wrapping



Structural basis of sharply looped protein–DNA complexes



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

Base step thermodynamic stability does not explain sequence preferences for sharp DNA bending

Base step	ΔG^0 (1)	Base step	ΔG^0 (2)
TA/TA	-0.58	TA/TA	0.76
AT/AT	-0.88	AT/AT	0.51
AA/TT	-1.00	AA/TT	0.26
AG/CT	-1.28	AG/CT	0.15
GA/TC	-1.30	AC/GT	-0.05
AC/GT	-1.44	GA/TC	-0.16
CA/TG	-1.45	CA/TG	-0.26
CC/GG	-1.84	CC/GG	-0.68
CG/CG	-2.17	GC/GC	-1.02
GC/GC	-2.24	CG/CG	-1.07

1: Allawi & SantaLucia (1997), ΔG^0 (± 0.06) (kcal mol⁻¹)
for base pair formation

2: Johnson, Zhu, & Wartell (1998), ΔG^0 (± 0.09) (kcal
mol⁻¹) for basepair stacking

Most-nonrandom dinucleotides in selected nucleosome DNAs

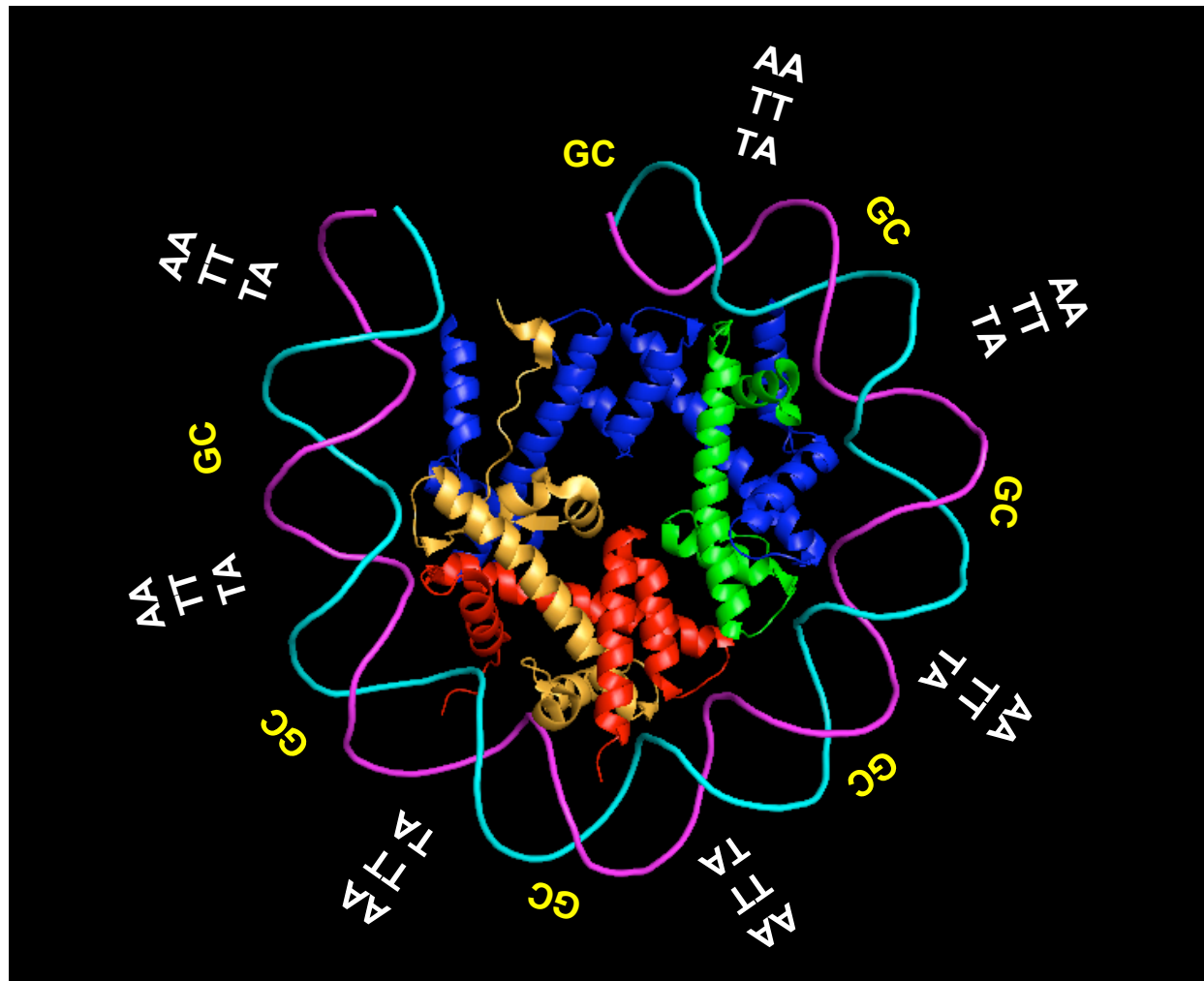
2-mer	# Occurrences	<u>(actual-expected)</u> std. dev.
ta	1094	6.4
at	850	-4.9
ct	1196	4.5
tg	1012	-4.0
gc	1350	3.9
ca	1012	-3.7

Basepair stacking energies capture some but not all of the sequence preferences

$\Delta G_{\text{stacking}}$ (kcal mol⁻¹)

	A	T	G	C
5'-A	-1.11	-1.34	-1.06	-1.81
5'-T	-0.19	-1.11	-0.55	-1.43
5'-G	-1.43	-1.81	-1.44	-2.17
5'-C	-0.55	-1.06	-0.91	-1.44

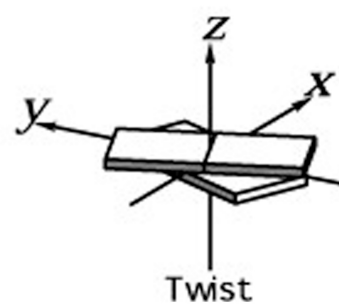
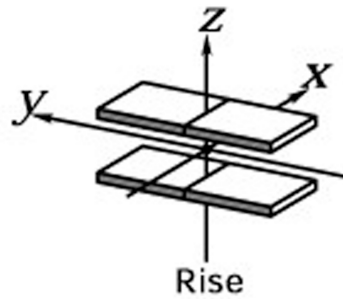
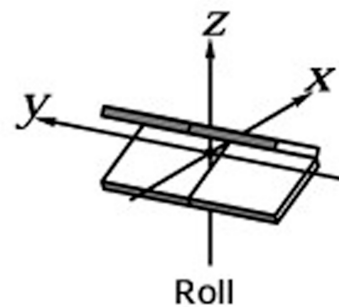
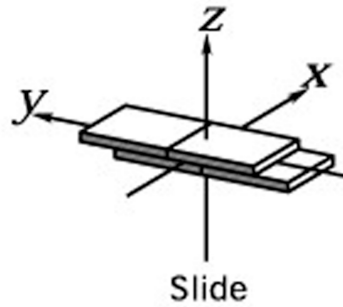
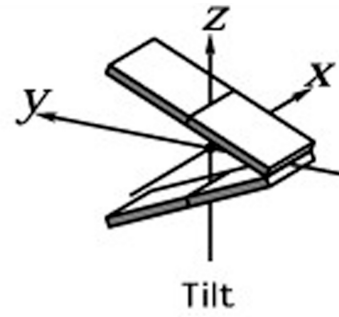
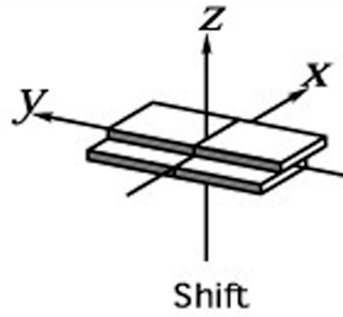
DNA sequence motifs that stabilize nucleosomes and facilitate spontaneous sharp looping



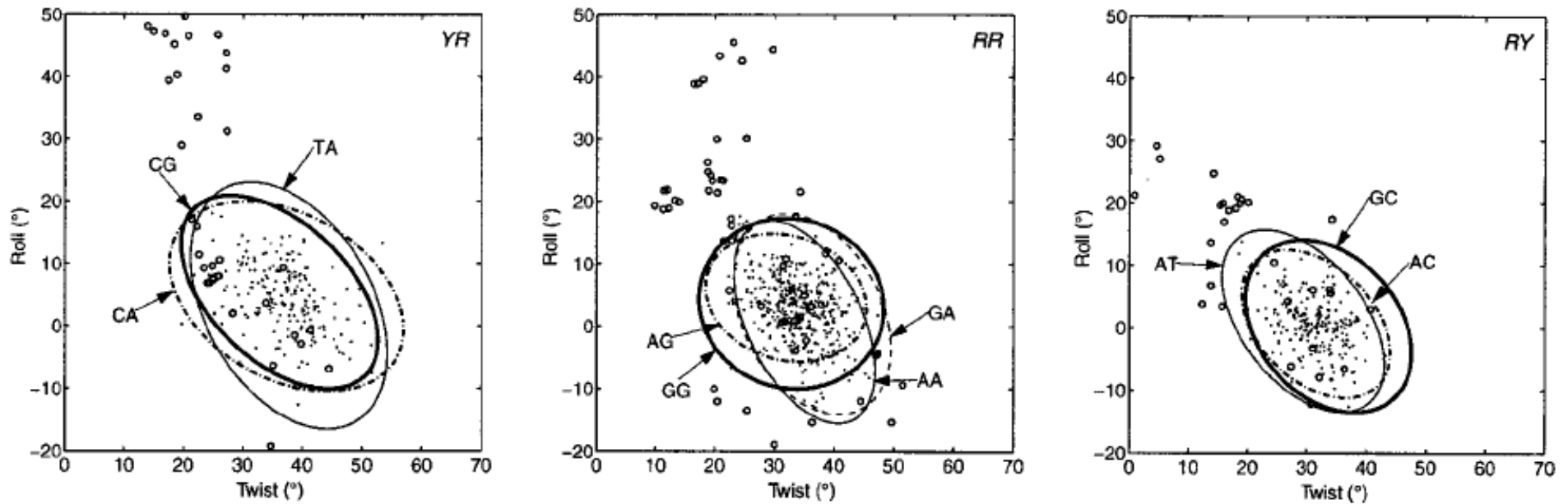
Flexibility of DNA for bending via basepair roll

Base step	Avg roll (degrees)	Dispersion
CA/TG	5.2	4.6
CG/CG	5.1	5.3
CC/GG	4.7	4.3
AG/CT	3.5	3.8
TA/TA	2.7	5.7
GA/TC	2.1	5.2
AC/GT	1.6	3.6
AT/AT	1.1	3.7
AA/TT	.8	4.5
GC/GC	.8	4.5

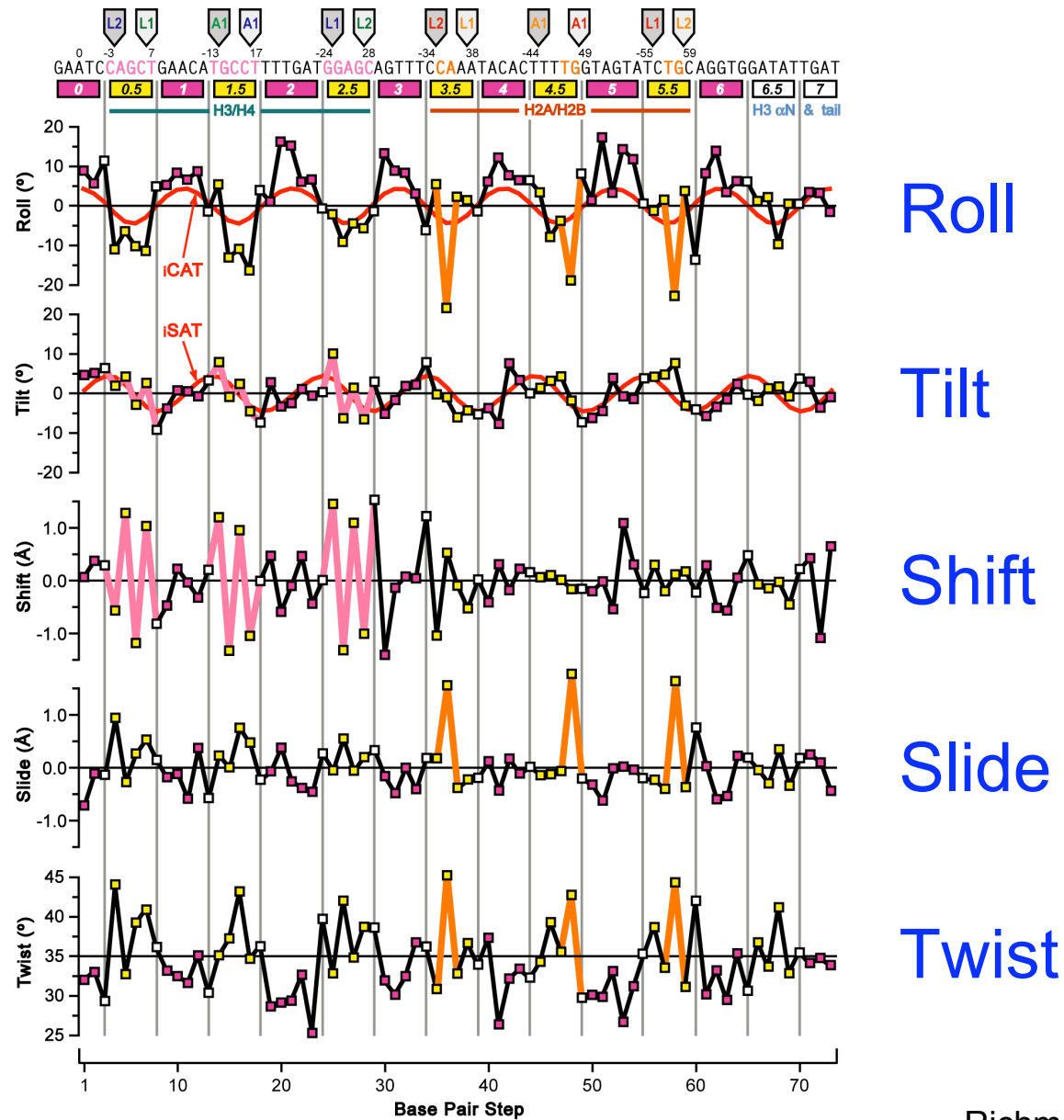
Basepair steps as fundamental units of DNA mechanics



Roll–twist covariance for YR , RR , and RY steps



Correlated deformations for sharp DNA wrapping



Elastic energy of dinucleotide step

$$E = E_0 + \frac{1}{2} \sum_{i=1}^6 \sum_{j=1}^6 f_{ij} \Delta\theta_i \Delta\theta_j$$

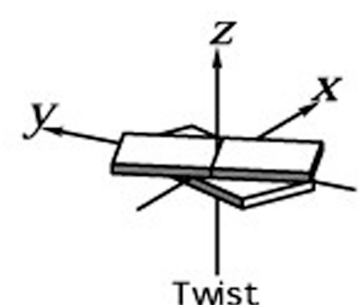
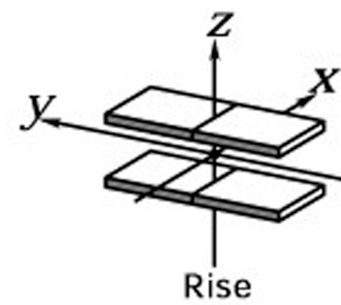
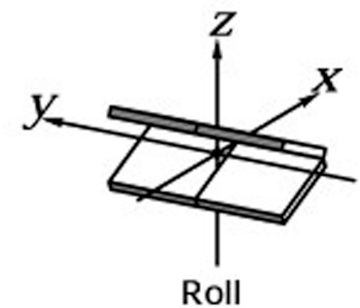
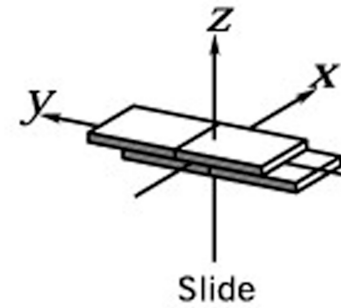
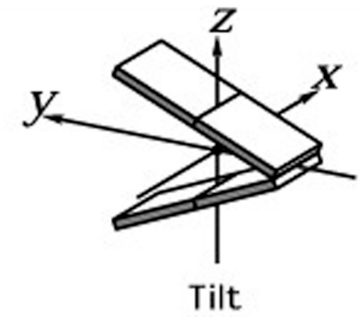
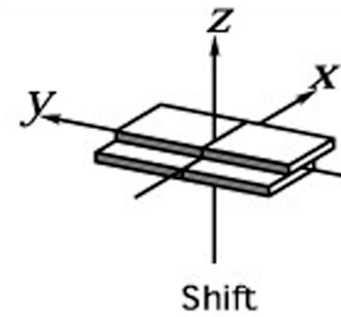
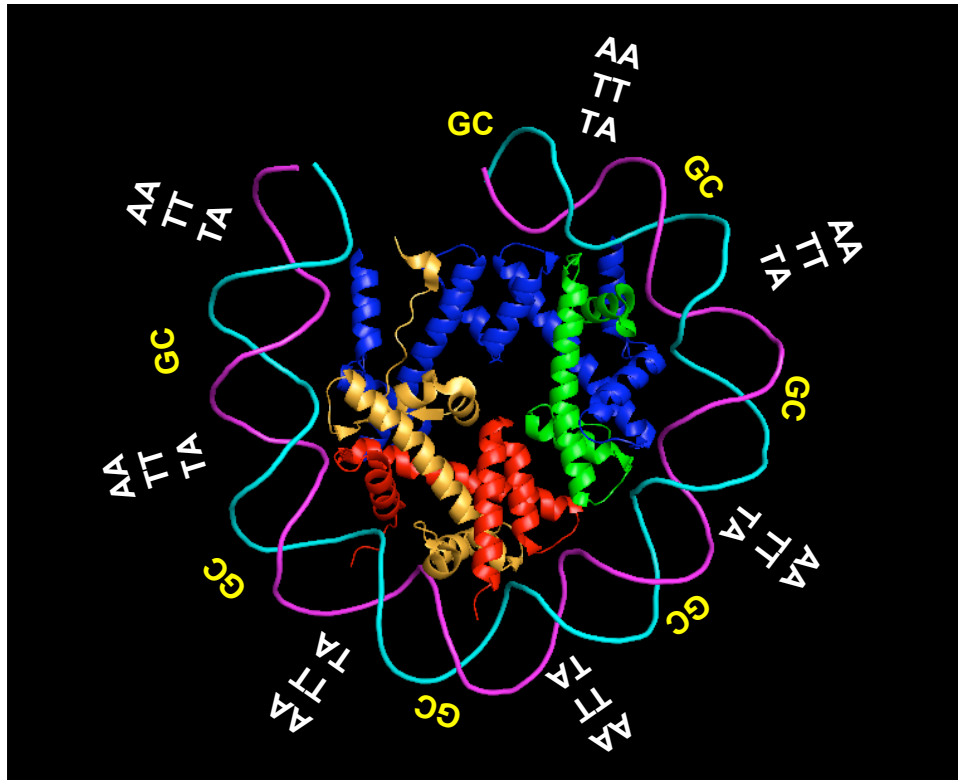
- Knowledge-based harmonic potential

E_0 = minimum energy for step

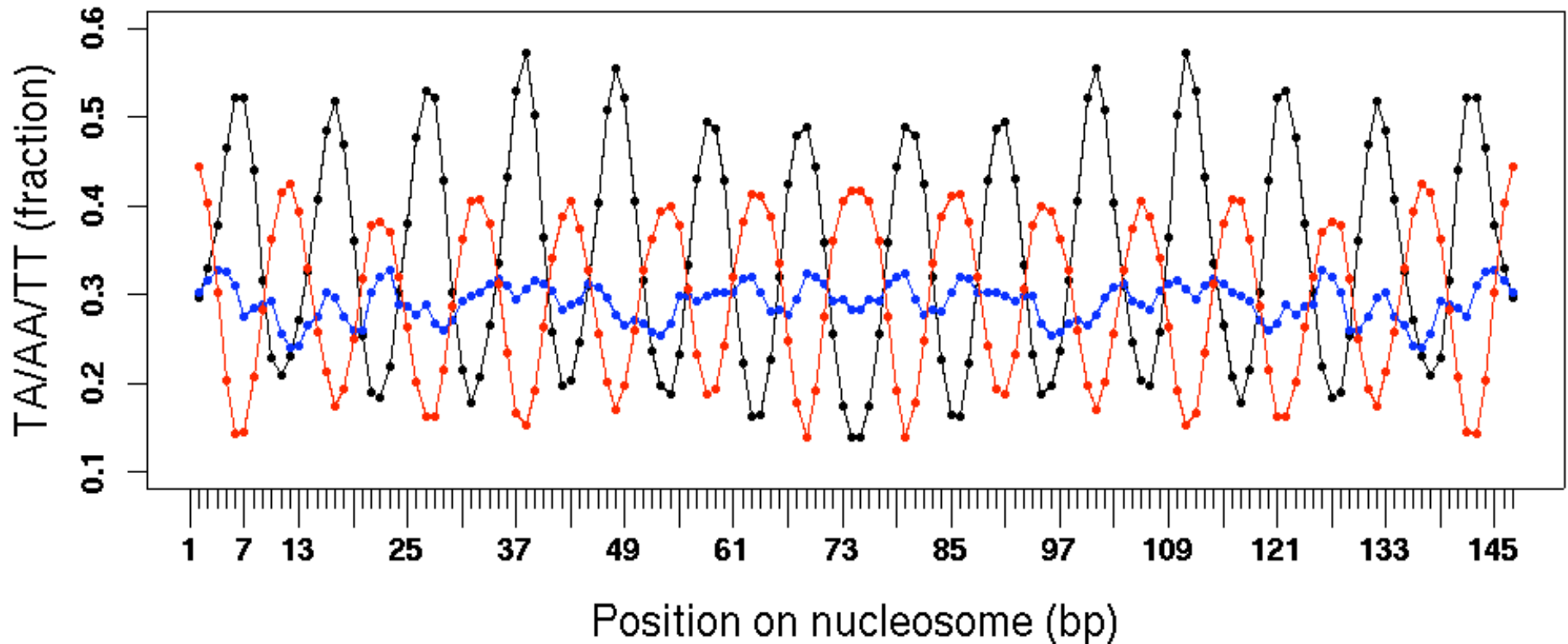
f_{ij} = elastic constants impeding deformation; calculated from dispersion of parameters in X-ray crystal structures, assuming harmonic potential

$\Delta\theta_i = \theta_i - \theta_i^0$,
= fluctuation of step parameter from equilibrium

Toward a proper free energy model for the sequence-dependent cost of DNA wrapping

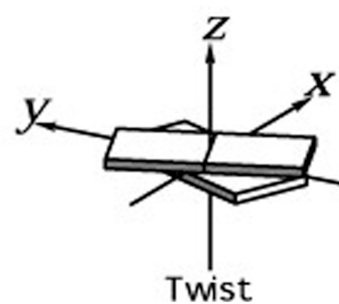
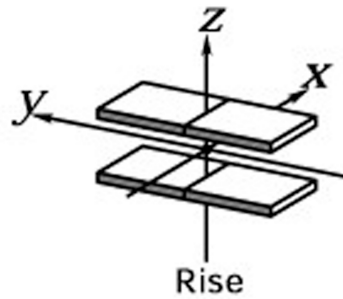
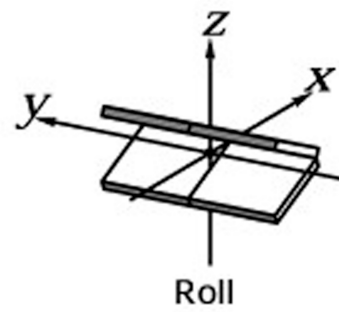
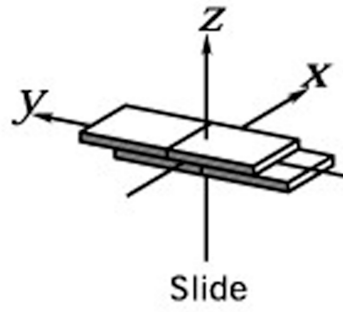
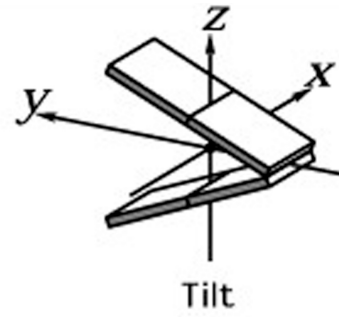
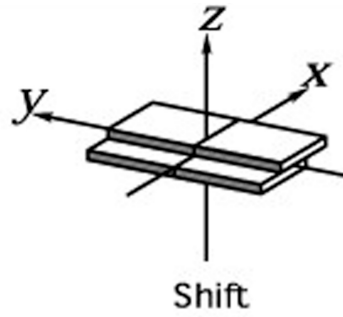


Dinucleotide step elasticity model correctly classifies high and low affinity nucleosome forming sequences



- Predicted good sequences
- Predicted bad sequences
- Arbitrary sequences

Basepair steps as fundamental units of DNA mechanics



Most-strongly correlated trinucleotides

Tri-nt Pair	Separ. (bp)	Actual # Occur.	<u>(actual-expected)</u> std. dev.
cta : tag	11	48	27.1
cta : cta	10	44	25.5
taa : tag	10	41	24.2
cta : tta	10	41	24.0
tag : tag	10	44	22.1
tta : tag	11	34	21.2

Highly enriched tetranucleotides

Tetranucleotide	Actual # Occur.	Expected #	<u>(actual-expected)</u> std. dev.
ctag	152	65 ± 9	10.0
taga	124	57 ± 9	7.8
tcta	124	58 ± 9	7.8
agag	104	67 ± 8	4.6

$p < 10^{-8}$

Acknowledgements

Sequence-dependence of DNA flexibility

Peggy Lowary

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John Reeve (Ohio State U.)

Timothy Cloutier

Julie Dohm

Mark Greene

Mark Hersam (Northwestern U. Mat. Sci. Eng.)

Karissa Fortney

Eric Siggia (Rockefeller U.)

Alexandre Morozov (Rockefeller U.)

Dan Grilley