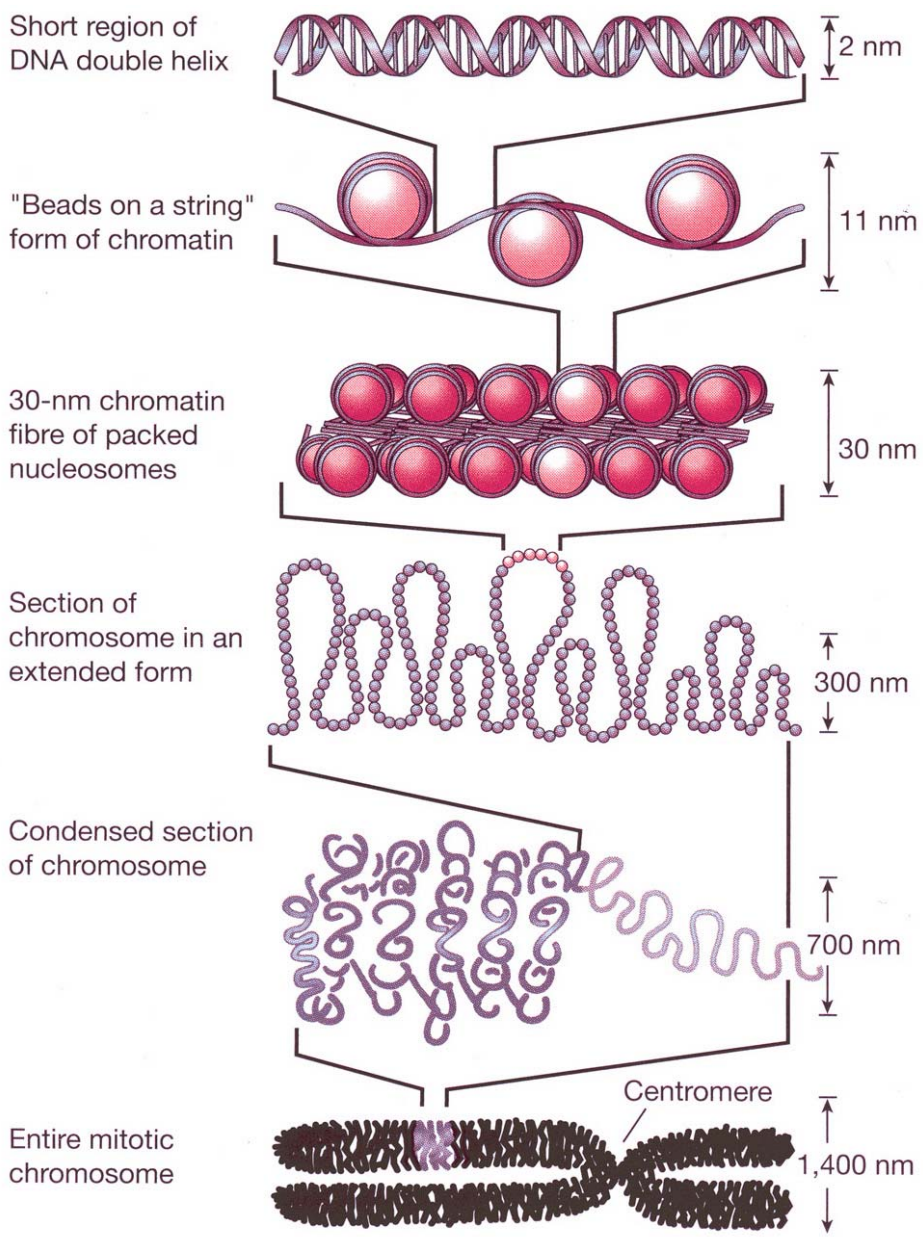
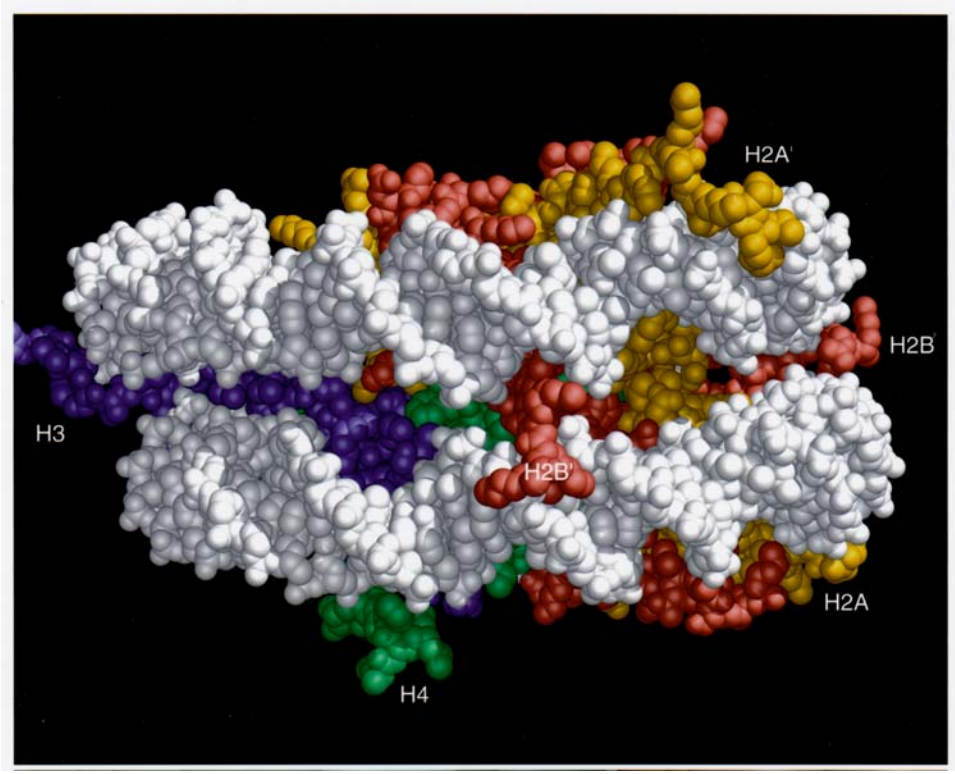
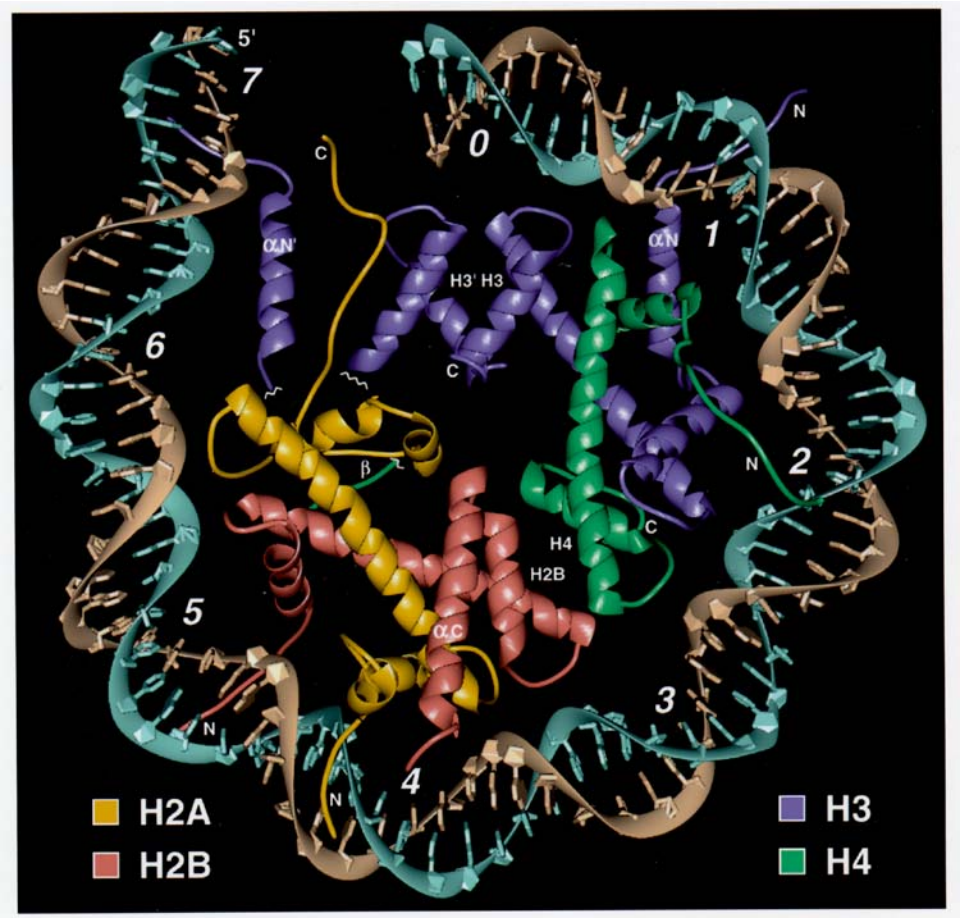


Felsenfeld, G. & Groudine, M. (2003), *Nature* 421: 448-453

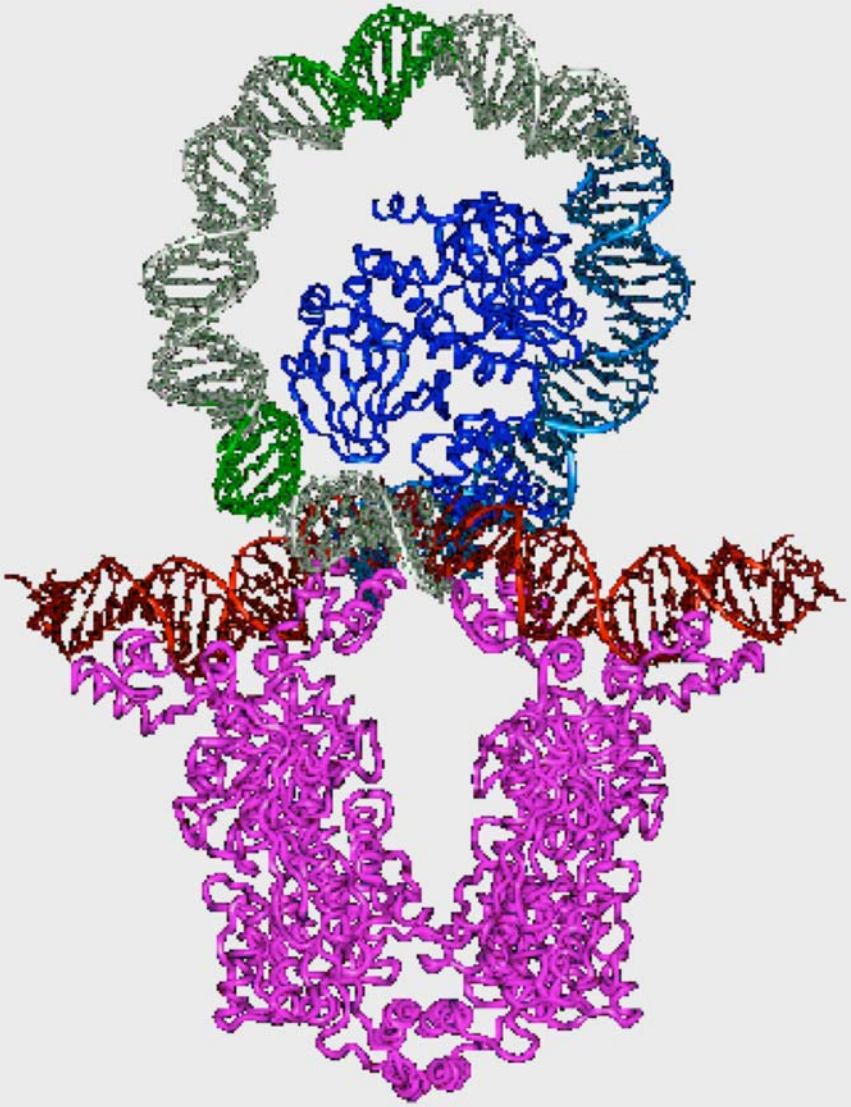
Most eukaryotic DNA is sharply looped





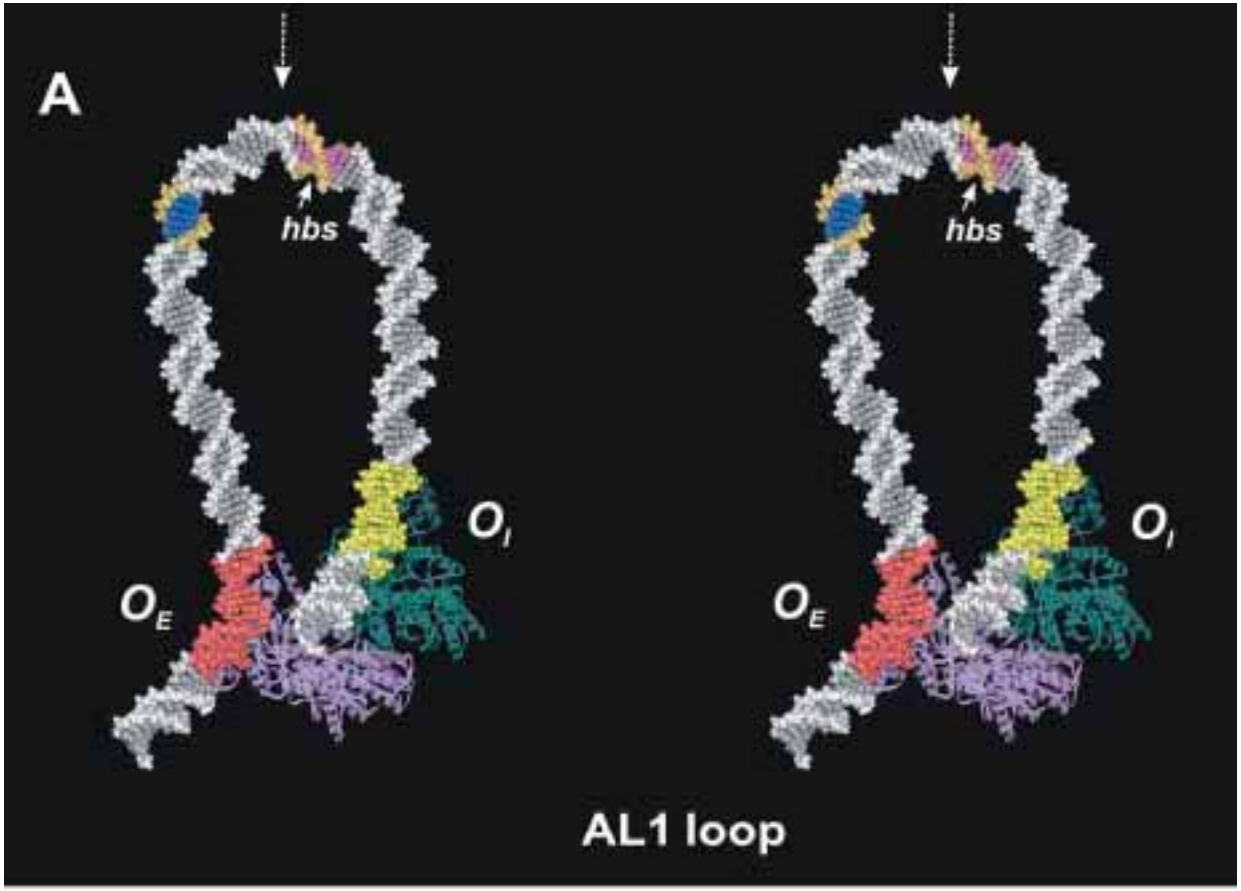
Luger et al., 1997

Sharply looped DNA in the *lac* operon



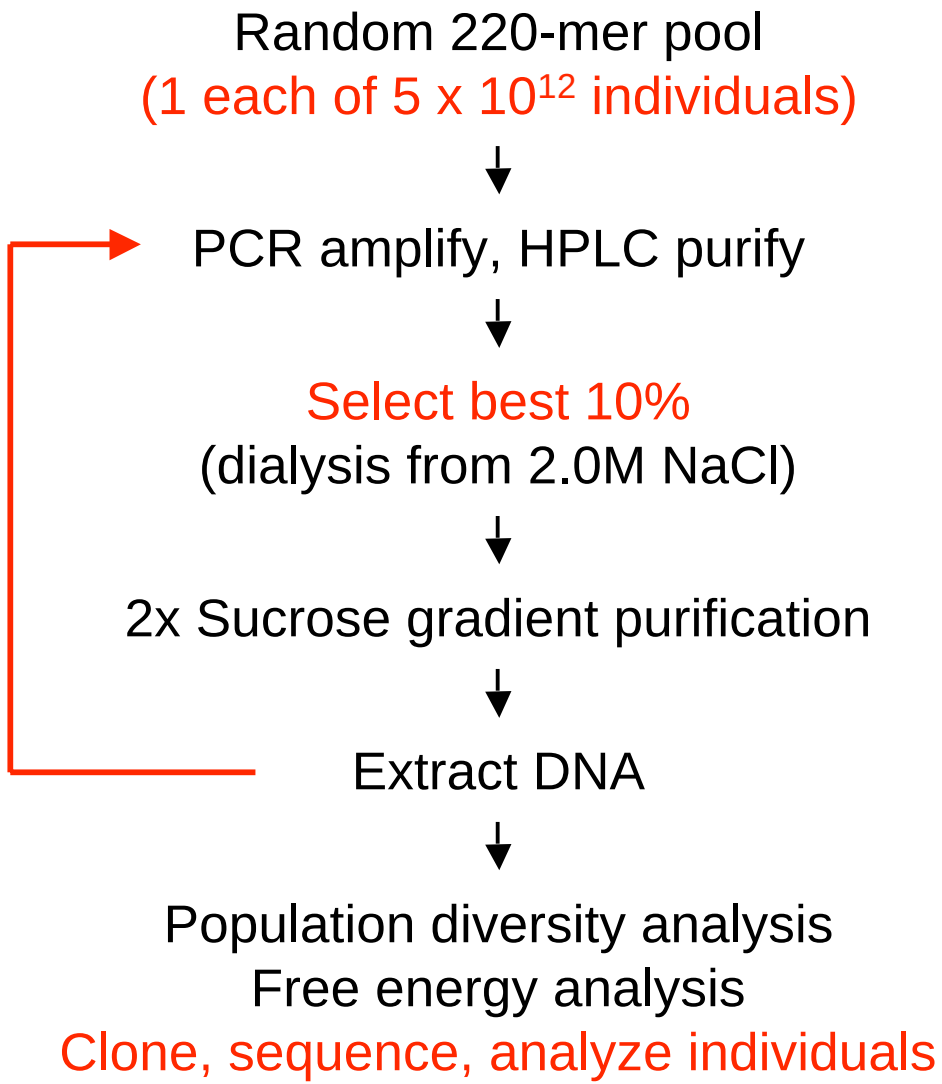
Lewis et al., 1996

Sharply looped DNA in the Gal repressosome



Semsey et al., 2004

Physical selection for stable nucleosome formation on chemically synthetic random DNAs



Lowary & Widom, 1998

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

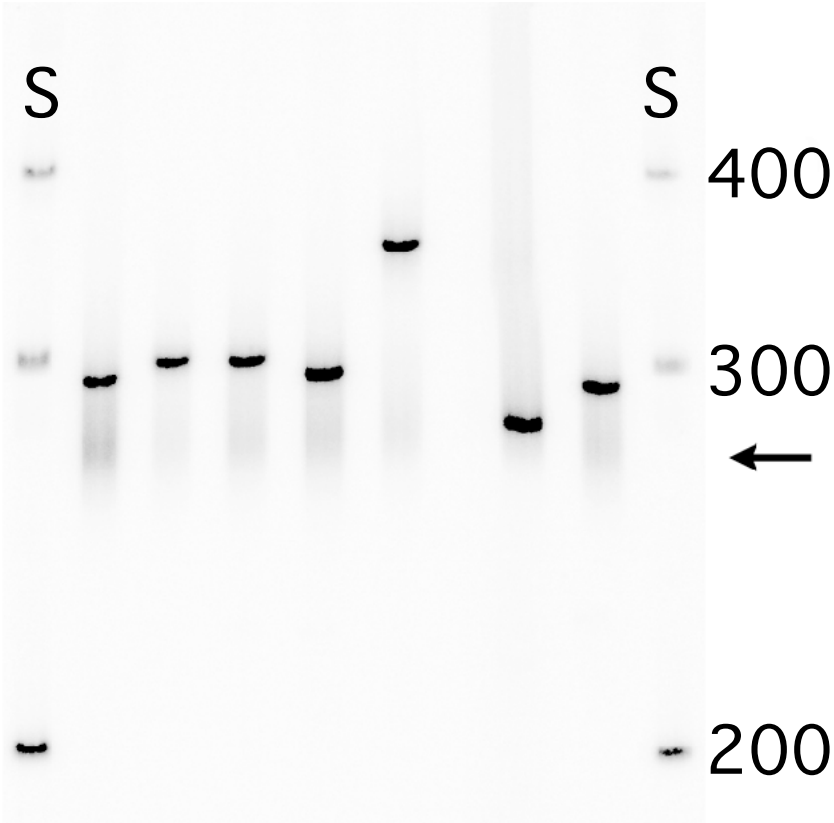
Lowary & Widom, 1998
Thåström et al., 1999
Widom, 2001
Thåström et al., 2004

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

Widom, 2001
Cloutier & Widom, 2004

Nucleosome positioning sequences are especially bent or bendable

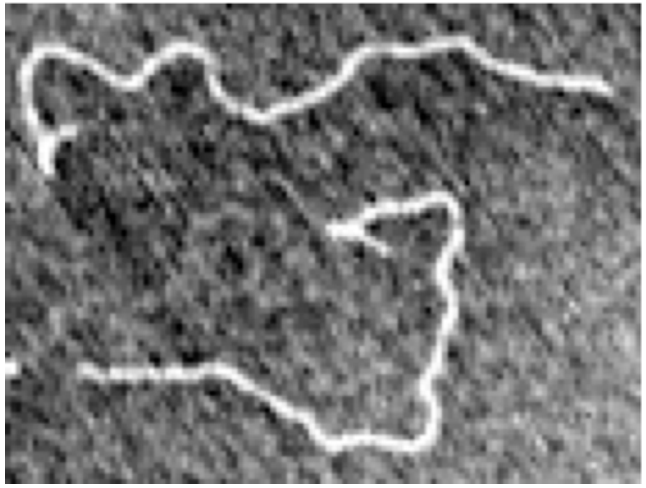


Lowary & Widom, 1998

Sharp bending from static bends?

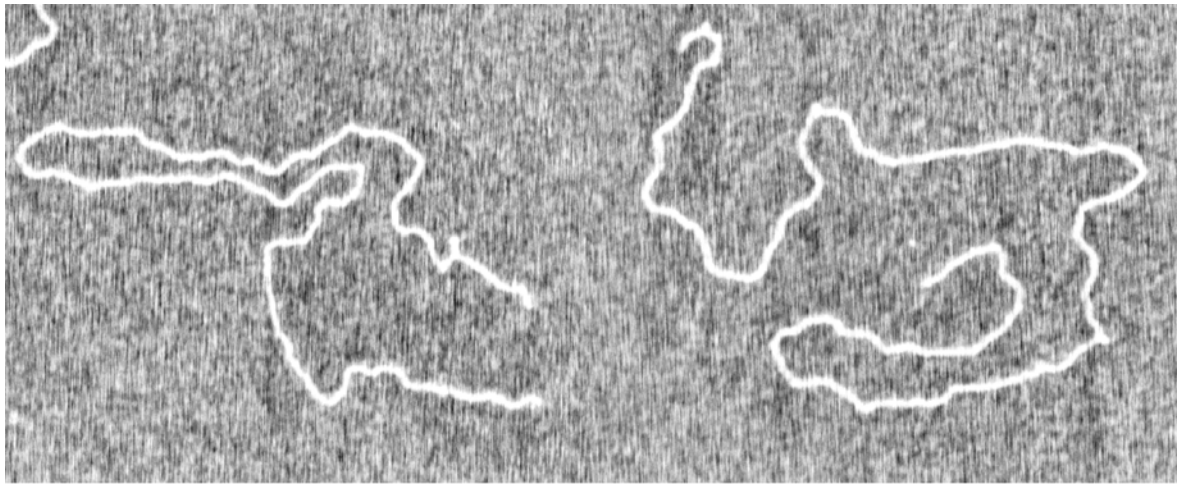
- A-tracts may be the most-bent of all sequences
- The most-bent A-tracts are bent by ~19° per 10-mer (i.e., ~2° / bp)
- DNA in nucleosomes is bent by ~4.5° / bp
- The standard deviation of basepair roll is ~4–6° / bp

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



Greene, Hersam, & Widom

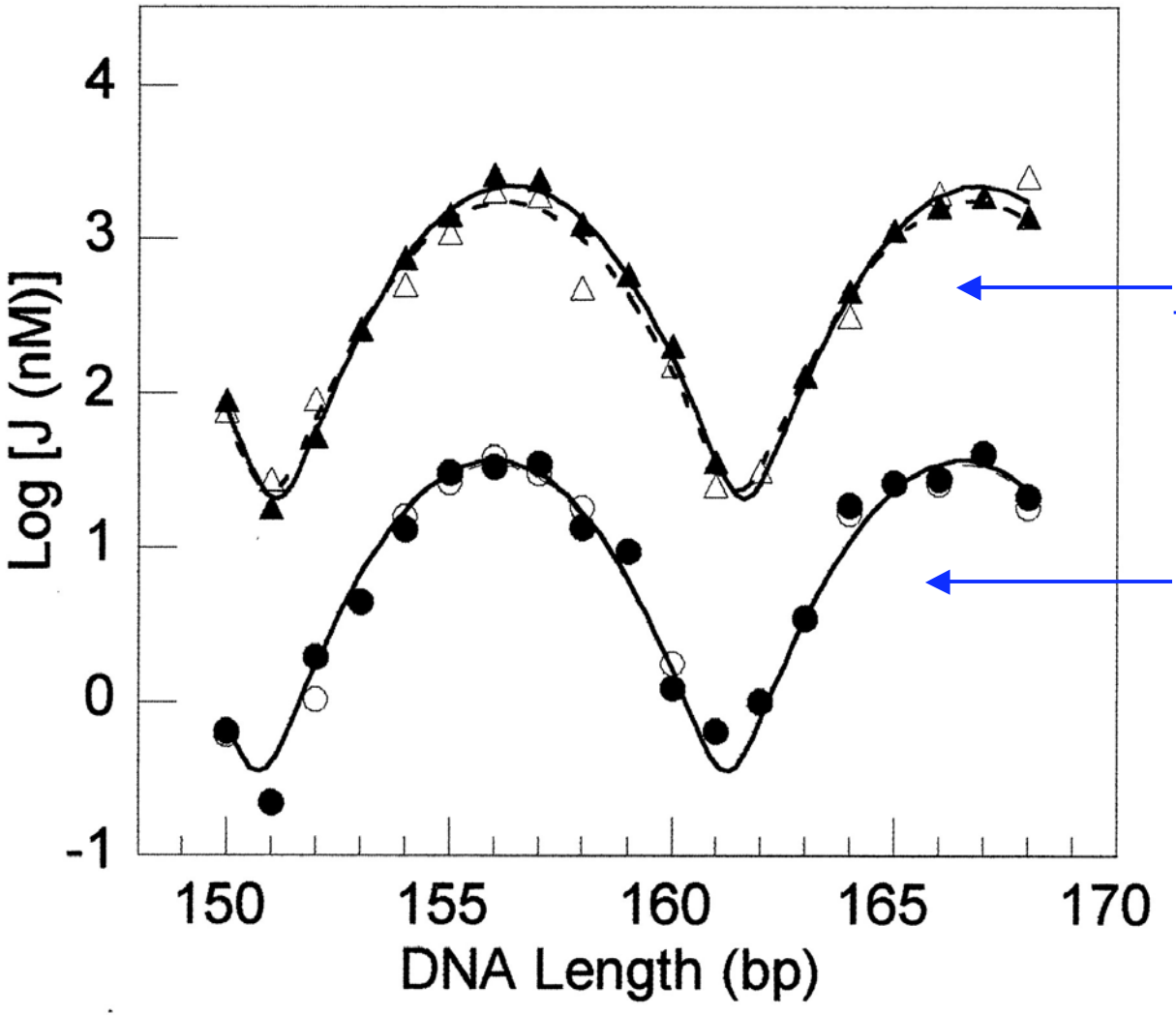
Selected nucleosome-forming DNAs are much less bent than A-tracts



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

Greene, Hersam, & Widom

Especially stable nucleosomes have especially flexible DNA



DNA cyclization assays

With 30-mer segment from stable nucleosome

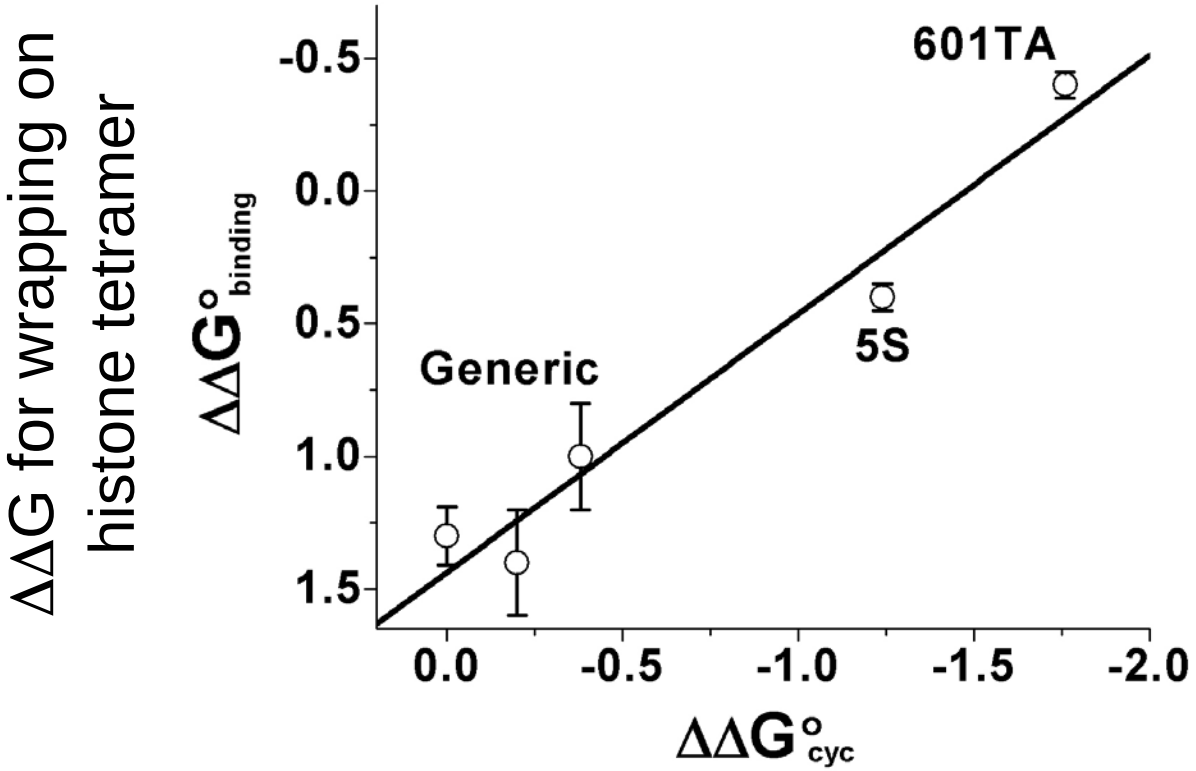
$P_{30\text{-mer}}$ ~ 27 nm

With 30-mer random sequence segment

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

Roychoudhury, et al., 2000

Especially flexible DNAs make especially stable nucleosomes and vice-versa



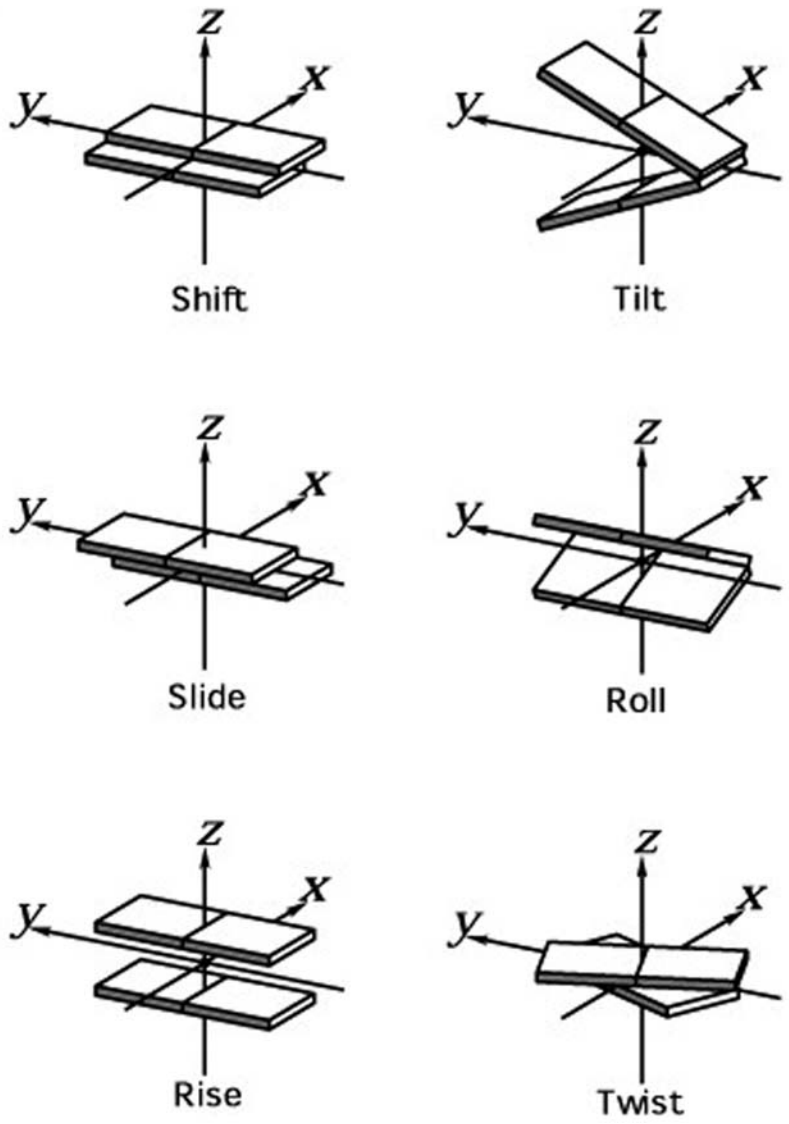
Cloutier & Widom, 2004

Especially flexible DNAs make especially stable nucleosomes and vice-versa

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

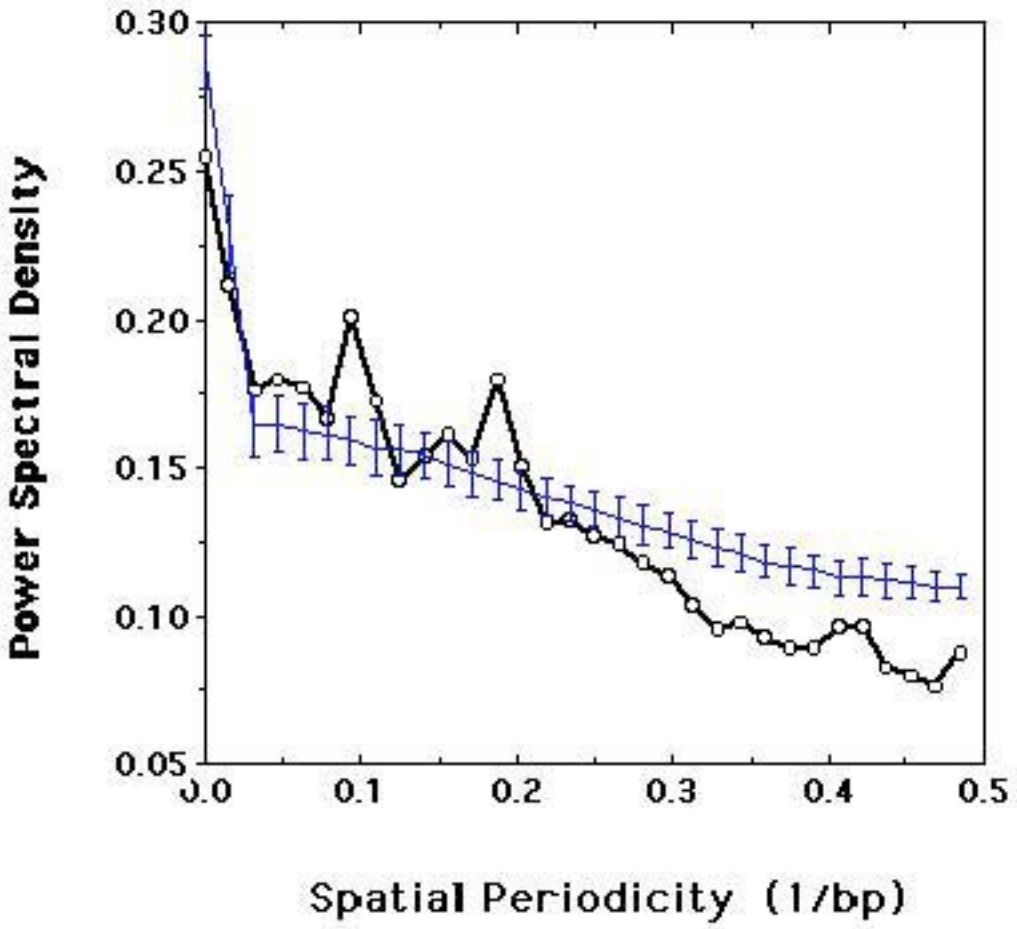
Widom, 2001
Thåström et al., 2004
Segal et al., 2006, in press

Basepair steps as fundamental units of DNA mechanics



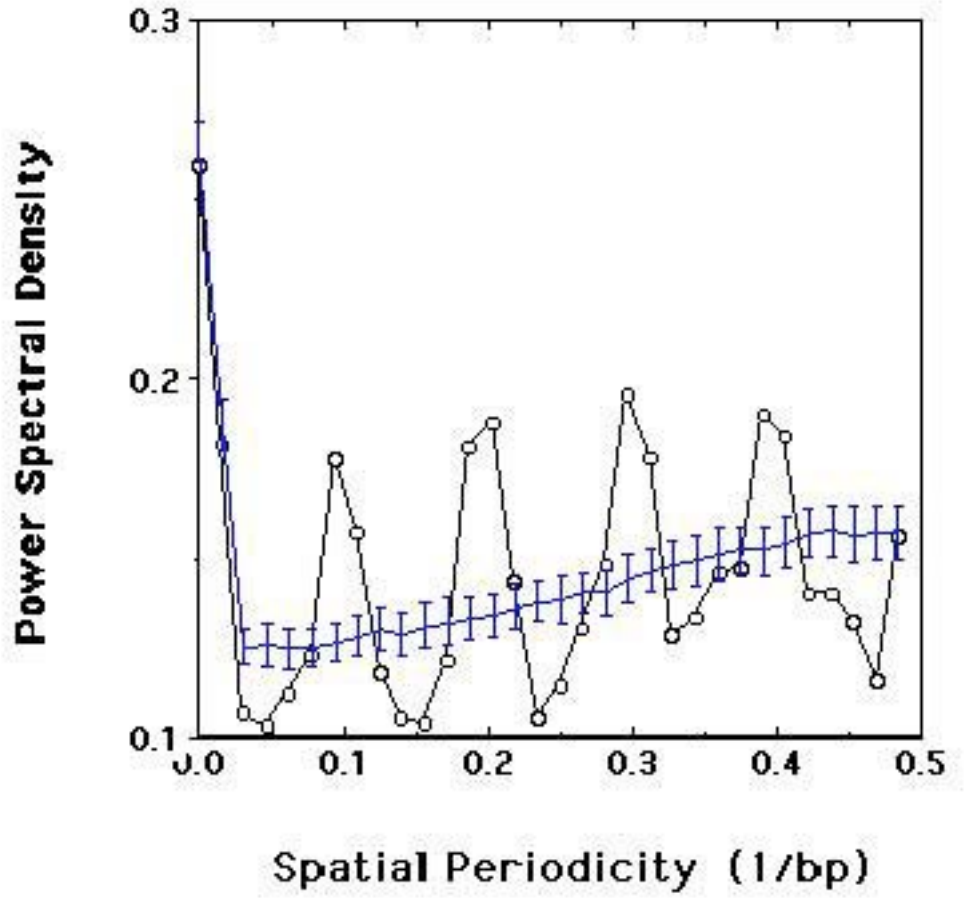
Olson
Calladine & Drew

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



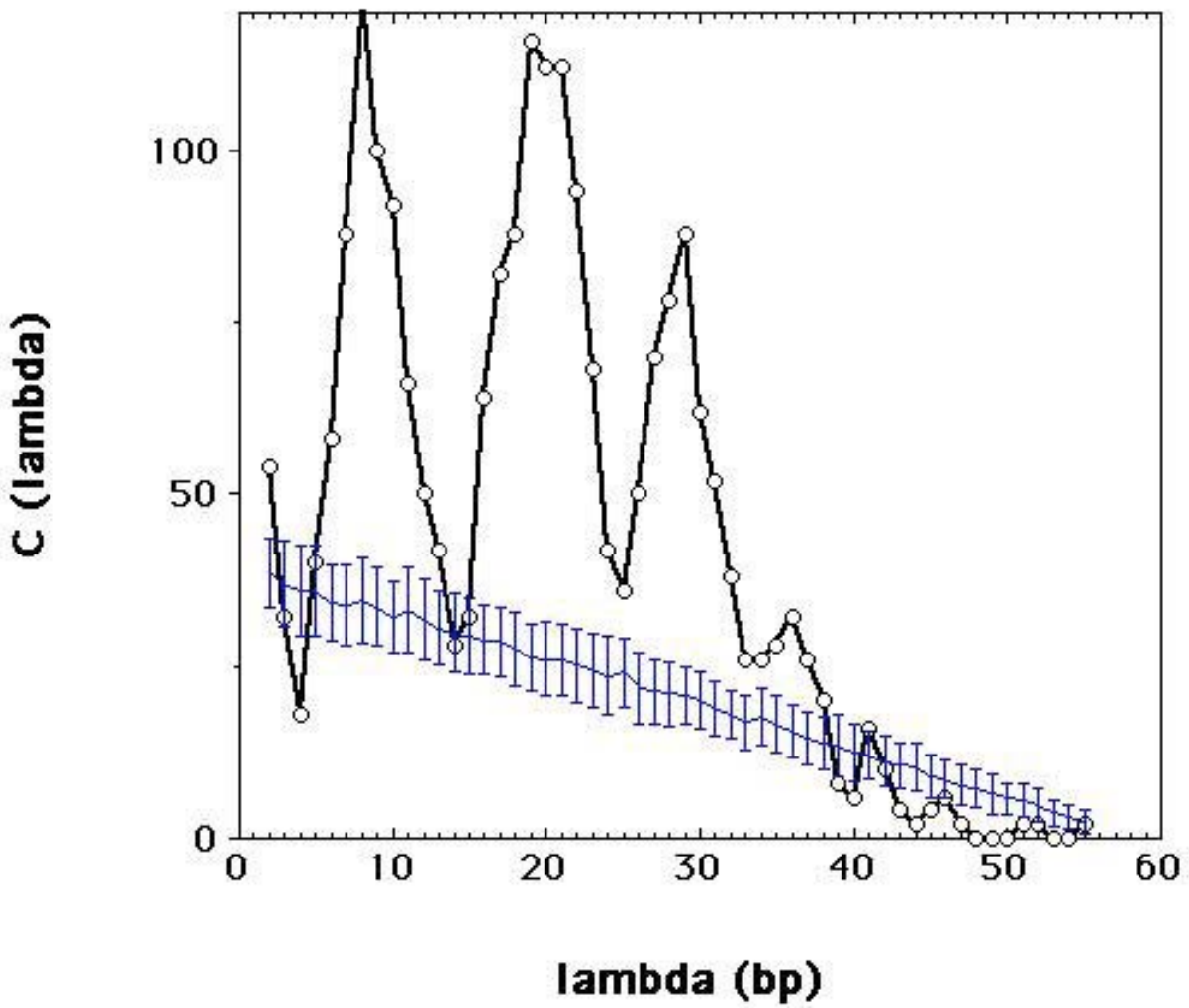
Lowary & Widom, 1998

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



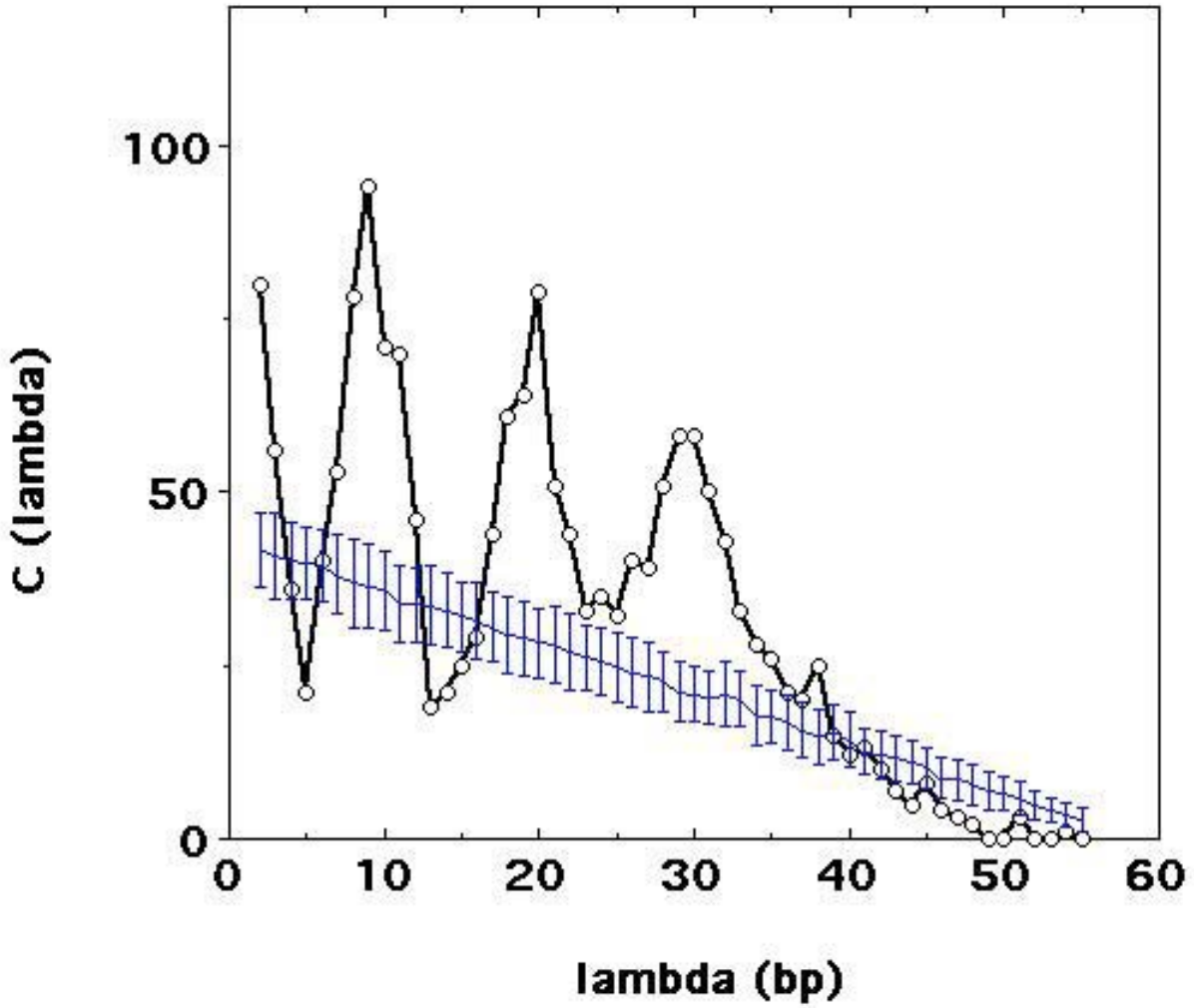
Lowary & Widom, 1998

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



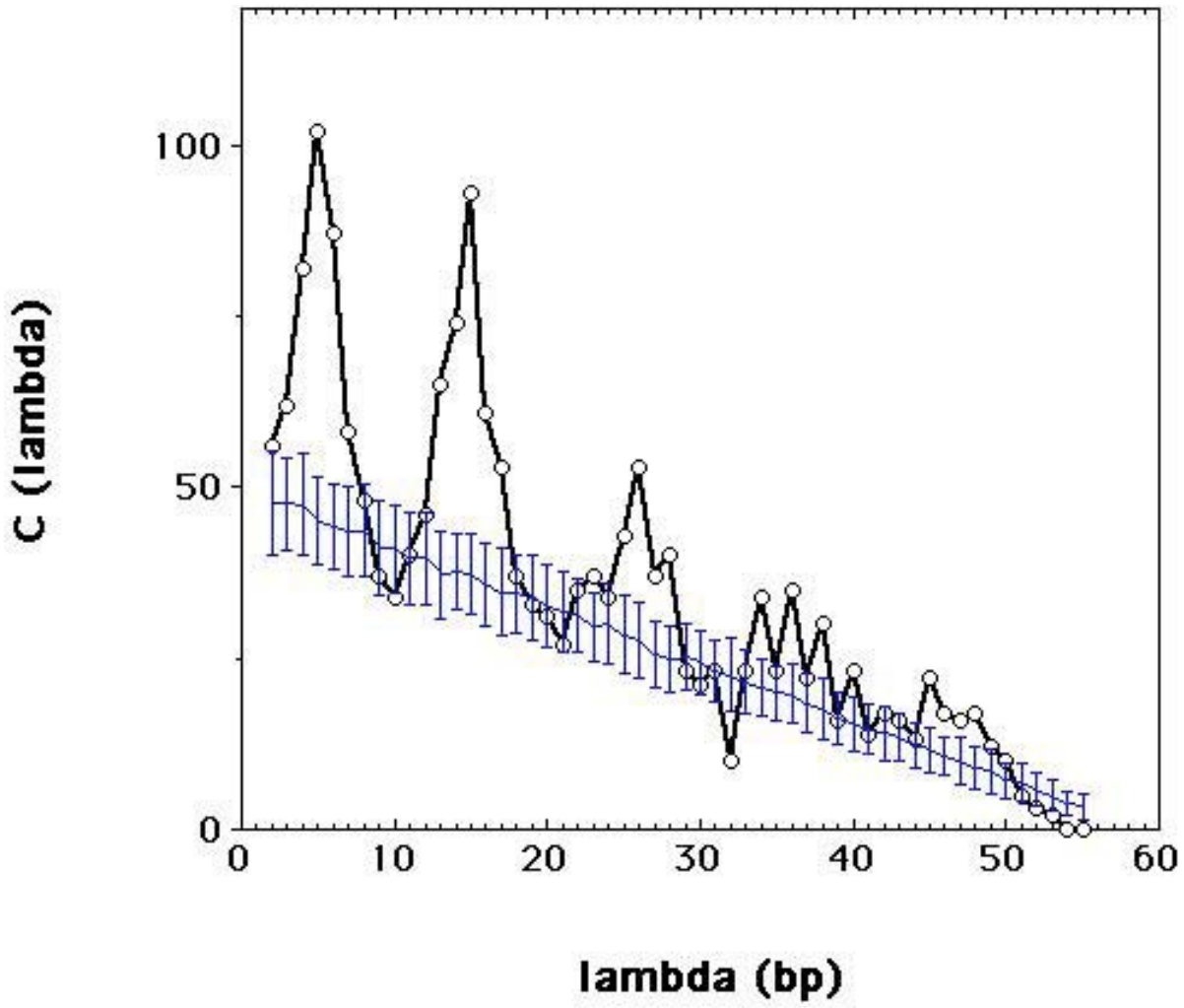
Bailey et al., 2000

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



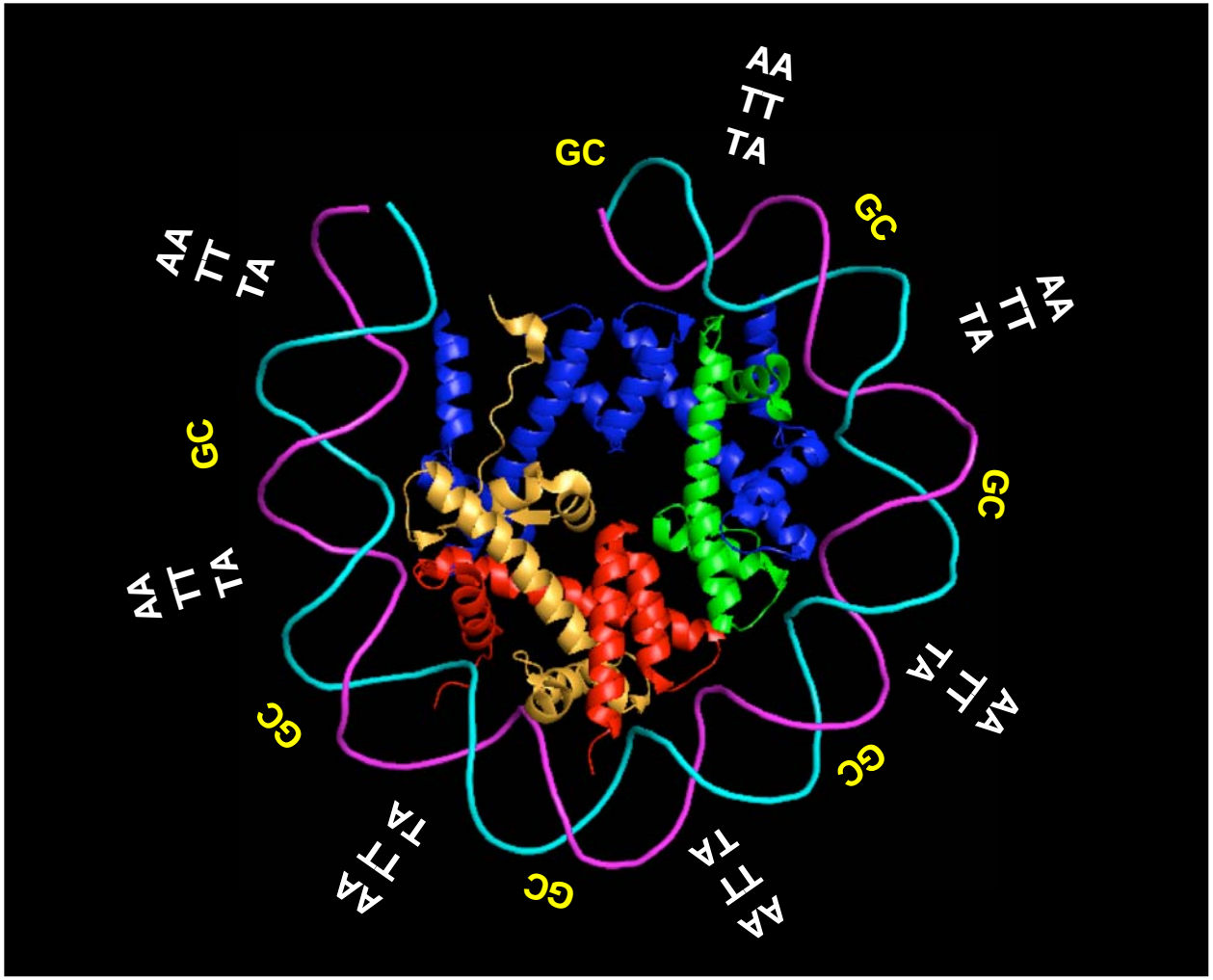
Bailey et al., 2000

Probability of GC at distance λ bp from a TA, in selected nucleosome forming DNAs



Bailey et al., 2000

DNA sequence motifs that stabilize nucleosomes and facilitate spontaneous sharp looping



Segal, Wang, & Widom

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

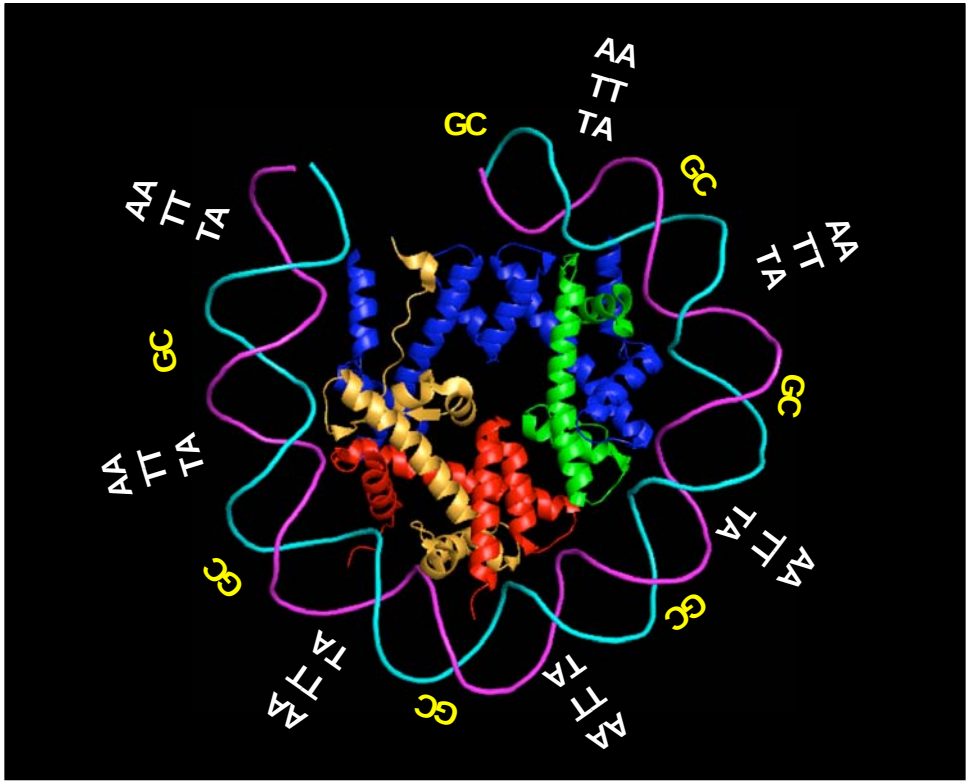
2: Johnson, Zhu, & Wartell (1998), ΔG^0 (±0.09) (kcal mol^{-1}) for basepair stacking

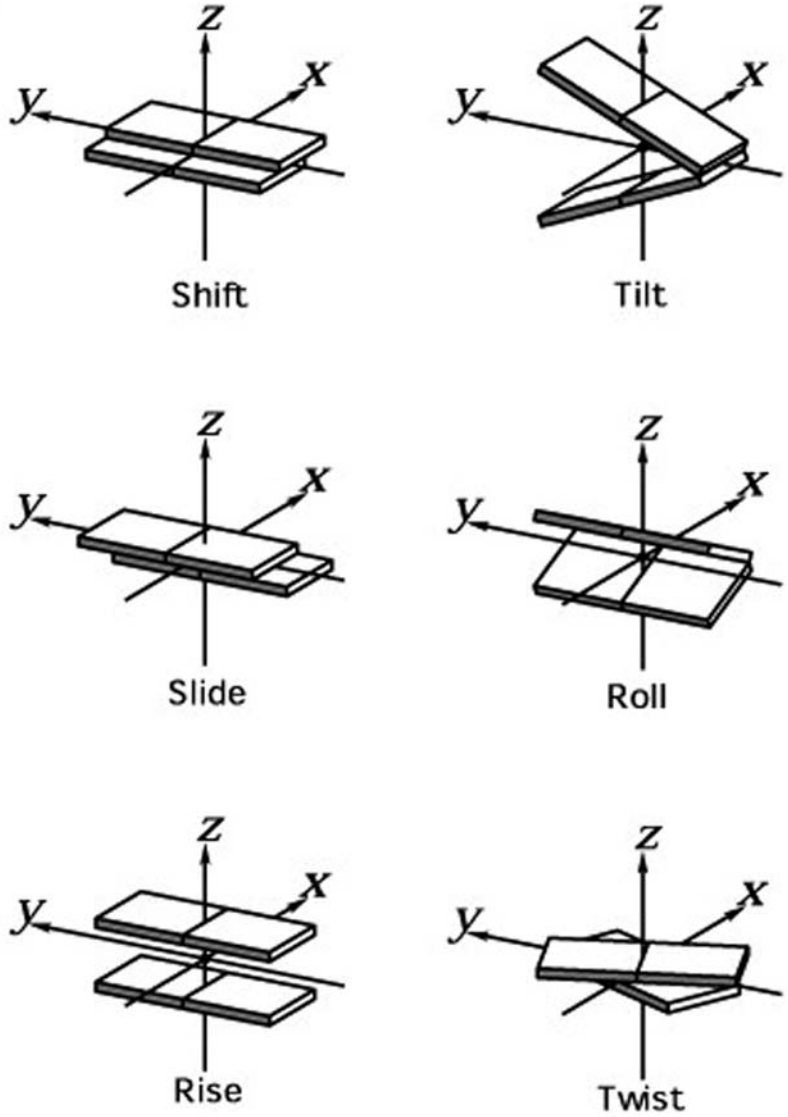
Most-nonrandom dinucleotides in selected nucleosome DNAs

2-mer	# Occurrences	(actual-expected) / 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

Lowary & Widom, 1998

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





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

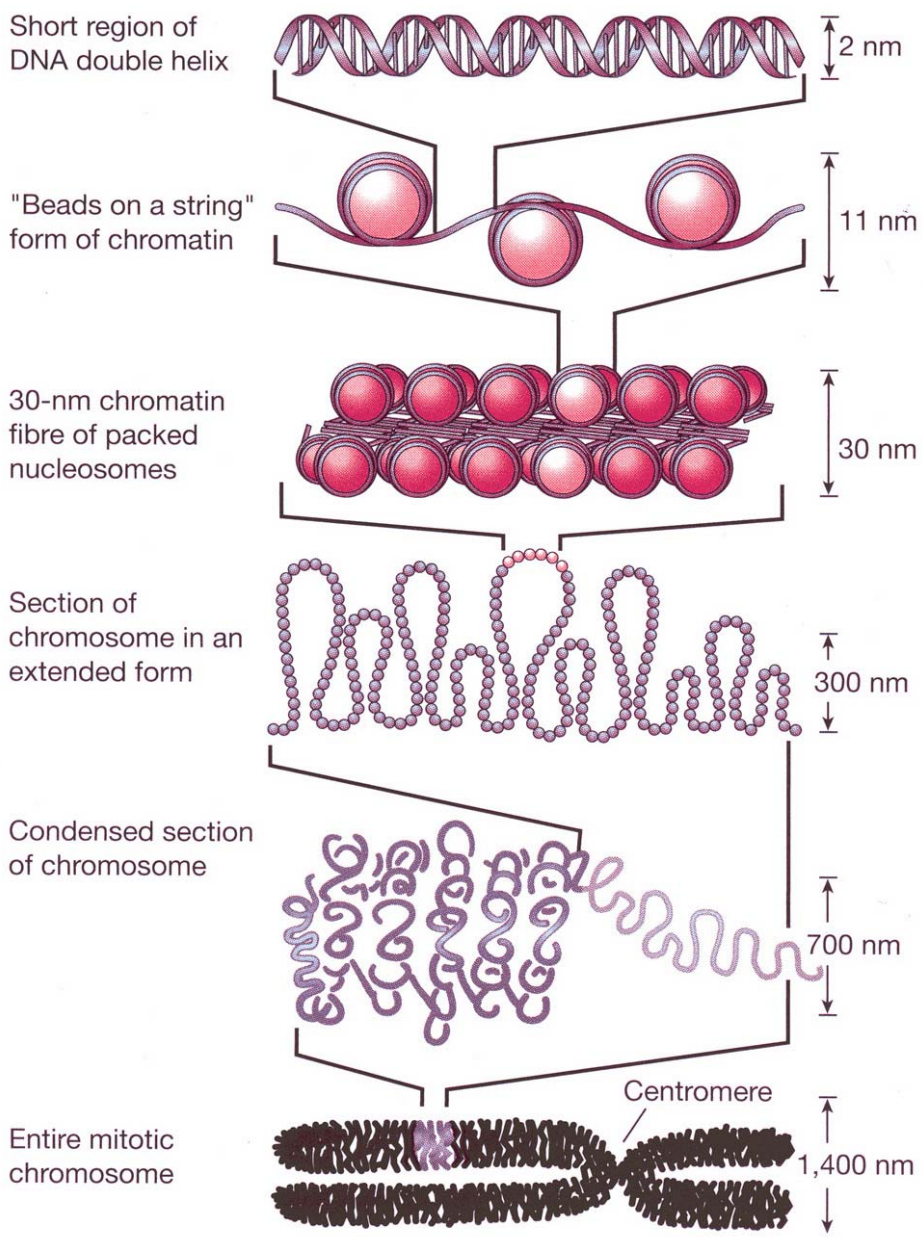
Olson, W.K. et al., (1998); updated Feb 2001

Highly enriched tetranucleotides

Tetranucleotide	Actual # Occur.	Expected #	(actual-expected) / 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

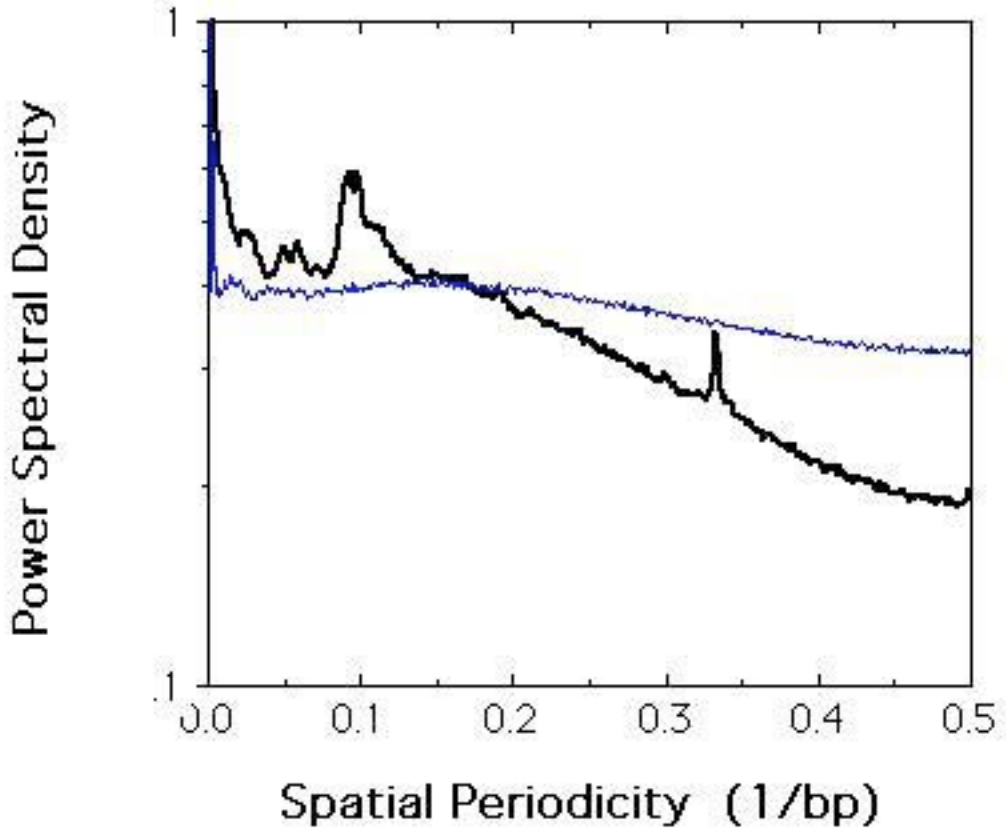
$p<10^{-8}$

Lowary & Widom, 1998



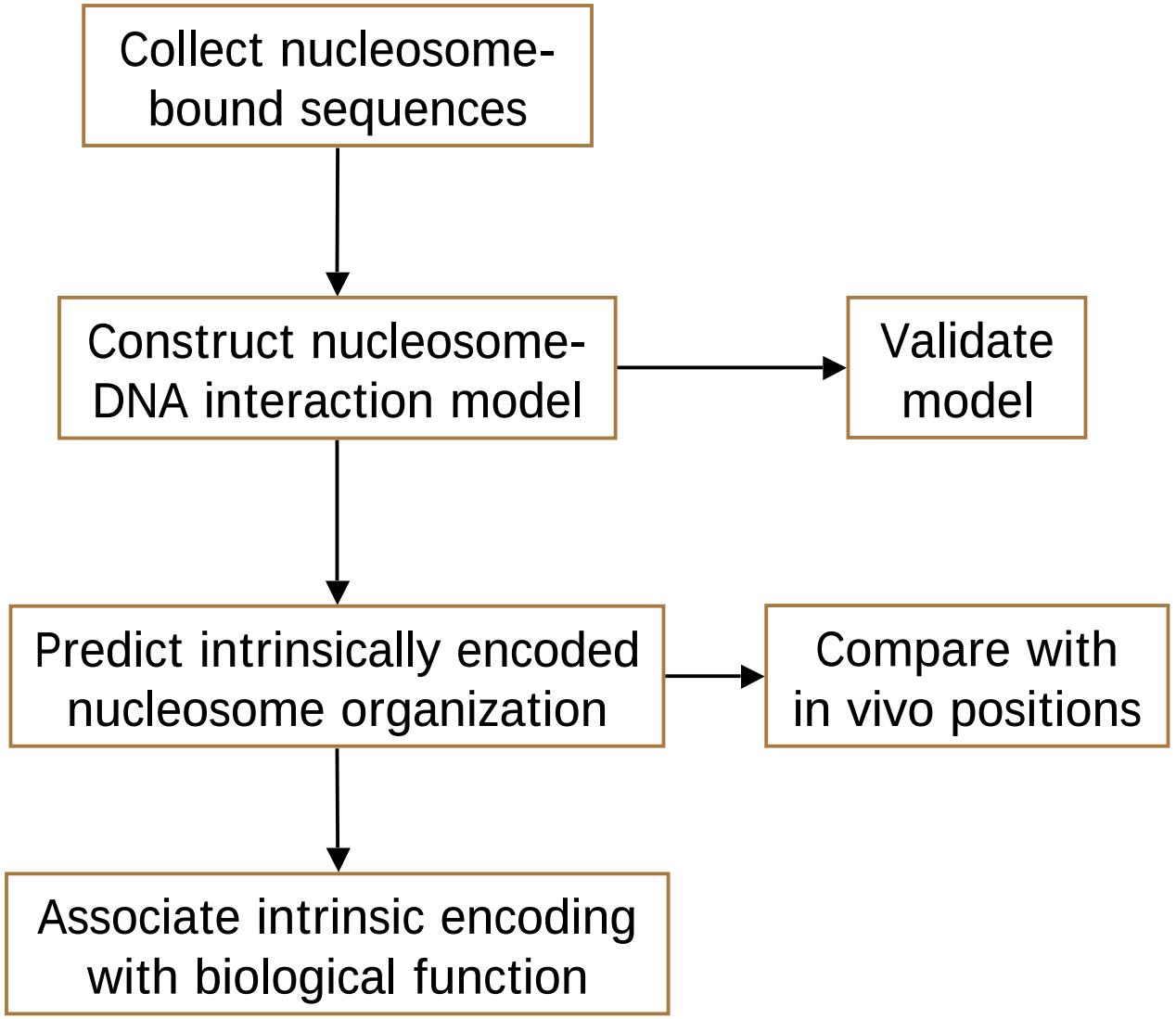
Felsenfeld, G. & Groudine, M. (2003), *Nature* 421: 448-453

~10.2 bp periodicity of AA/TT steps in the *C. elegans* genome

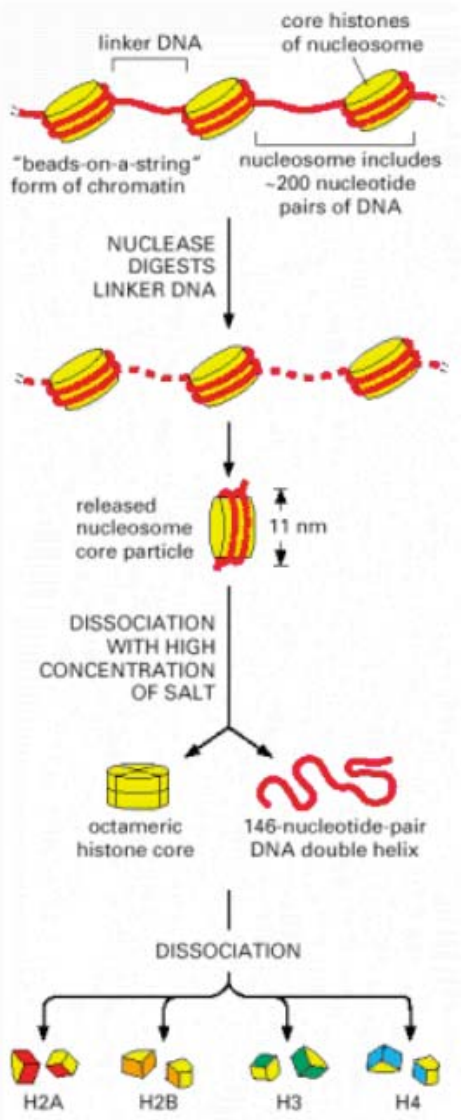


Widom, 1996

Understanding and predicting the genome's nucleosome-forming potential



Isolation of natural nucleosome core DNA



Alberts et al., 4th ed., Fig. 4–24 (2002)

Center alignment of yeast nucleosome DNAs

ACGTAGCTGTAGTGTACTGACGTACGTCGTC

ACTAGCTGATACGGAGACCCGCGCGATTTTGCGGTC

ACTGTTCGTCGTGTGTGTGTGCTGCTGTAGACTTGTGTG

TACTGTTTTTATTTTGCGGGCATGCTTGT

Center align

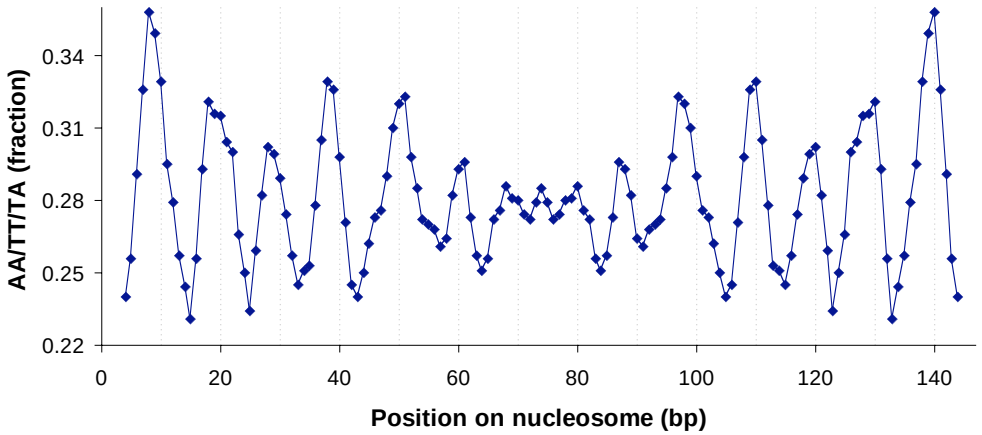
ACGTAGCTGTAGTGTACTGACGTACGTCGTC

ACTAGCTGATACGGAGACCCGCGCGATTTTGCGGTC

ACTGTTCGTCGTGTGTGTGTGCTGCTGTAGACTTGTGTG

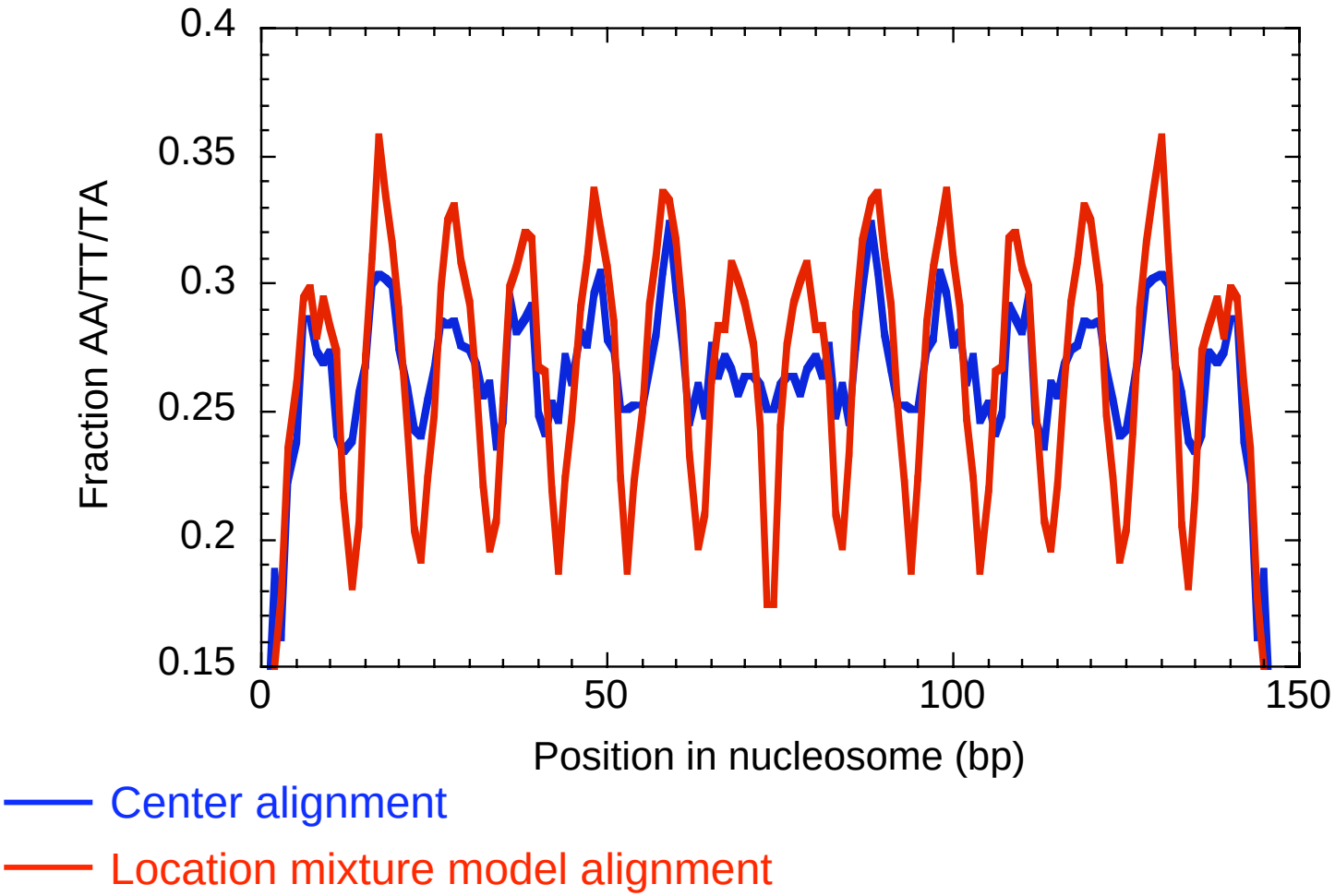
TACTGTTTTTATTTTGCGGGCATGCTTGT

- ~10bp periodicity of AA/TT/TA
- Same period for GC, out of phase with AA/TT/TA
- Signals important for DNA bending
- NO signal from alignment of randomly chosen genomic regions



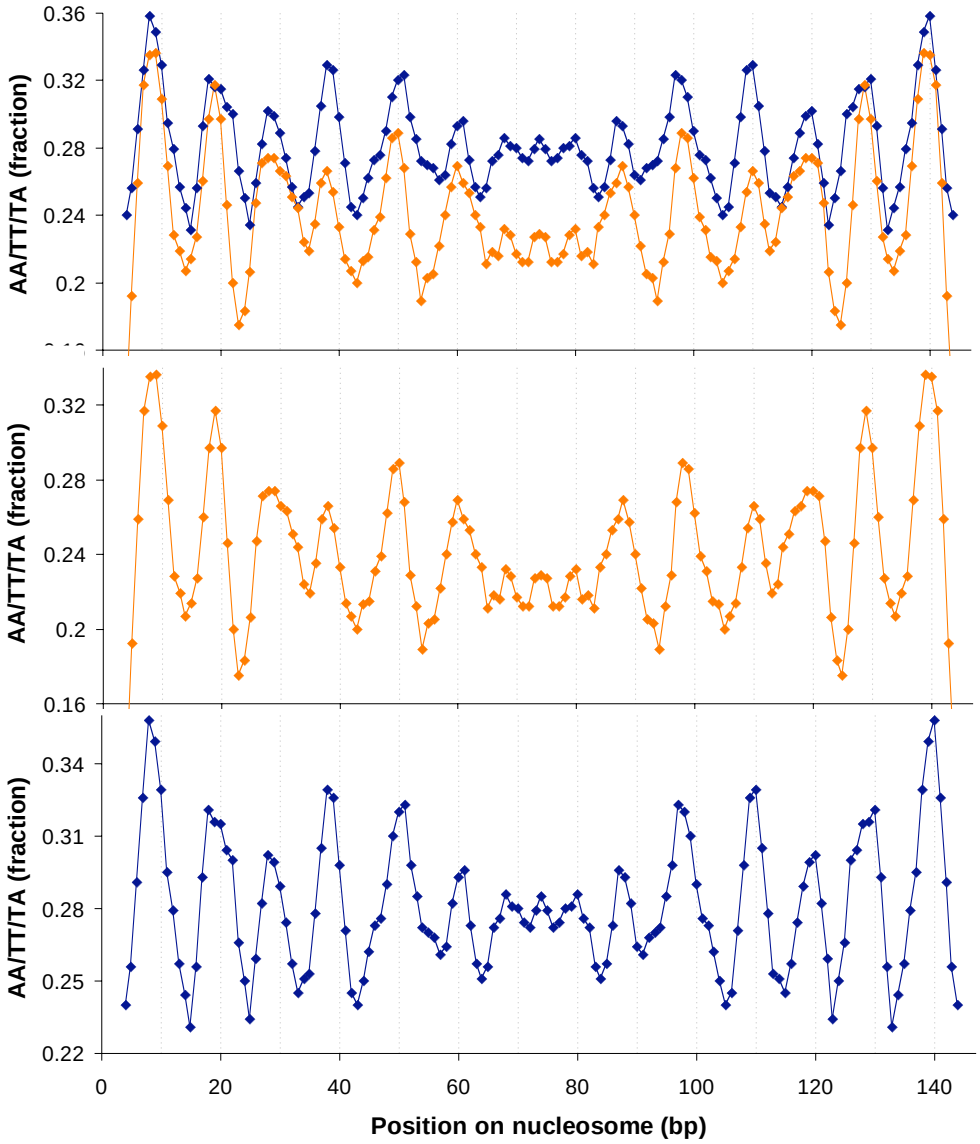
Yeast (in vivo nucleosomes)

Location mixture model alignment *vs* center alignment



Wang & Widom, 2005

Center alignment of chicken nucleosome DNAs

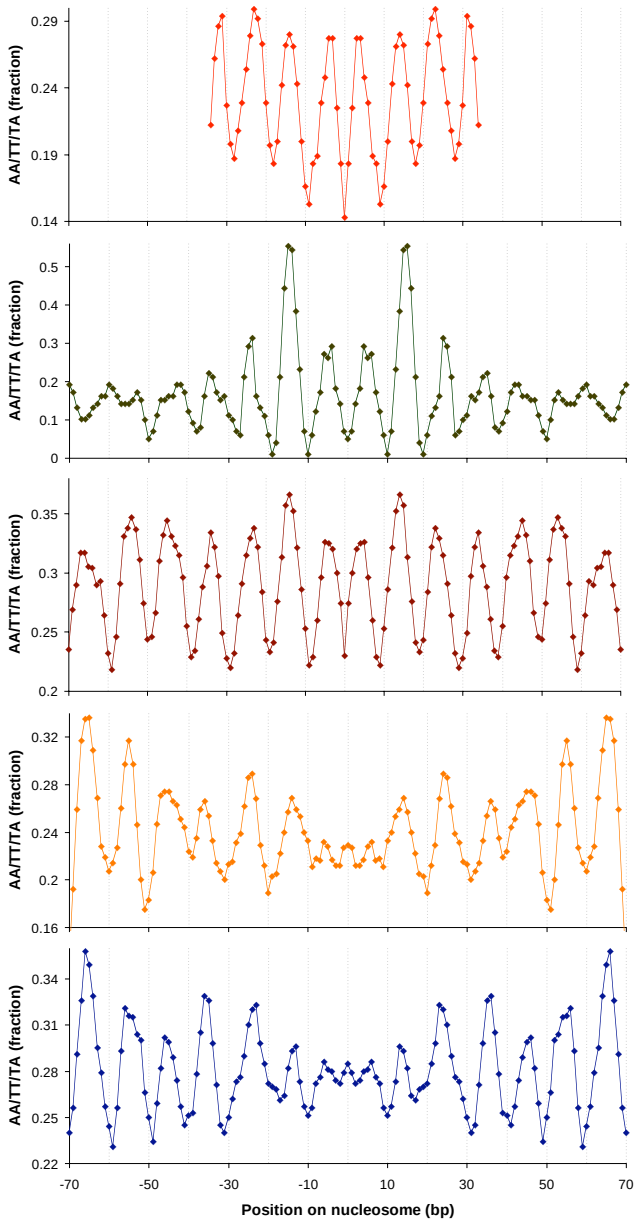


Chicken + Yeast merge

Chicken (in vivo) (Satchwell et al., 1986)

Yeast (in vivo)

Alignments of nucleosomes selected in vitro



Mouse (in vitro) (Widlund et al., 1997)

Random DNA (in vitro) (Lowary & Widom, 1998)

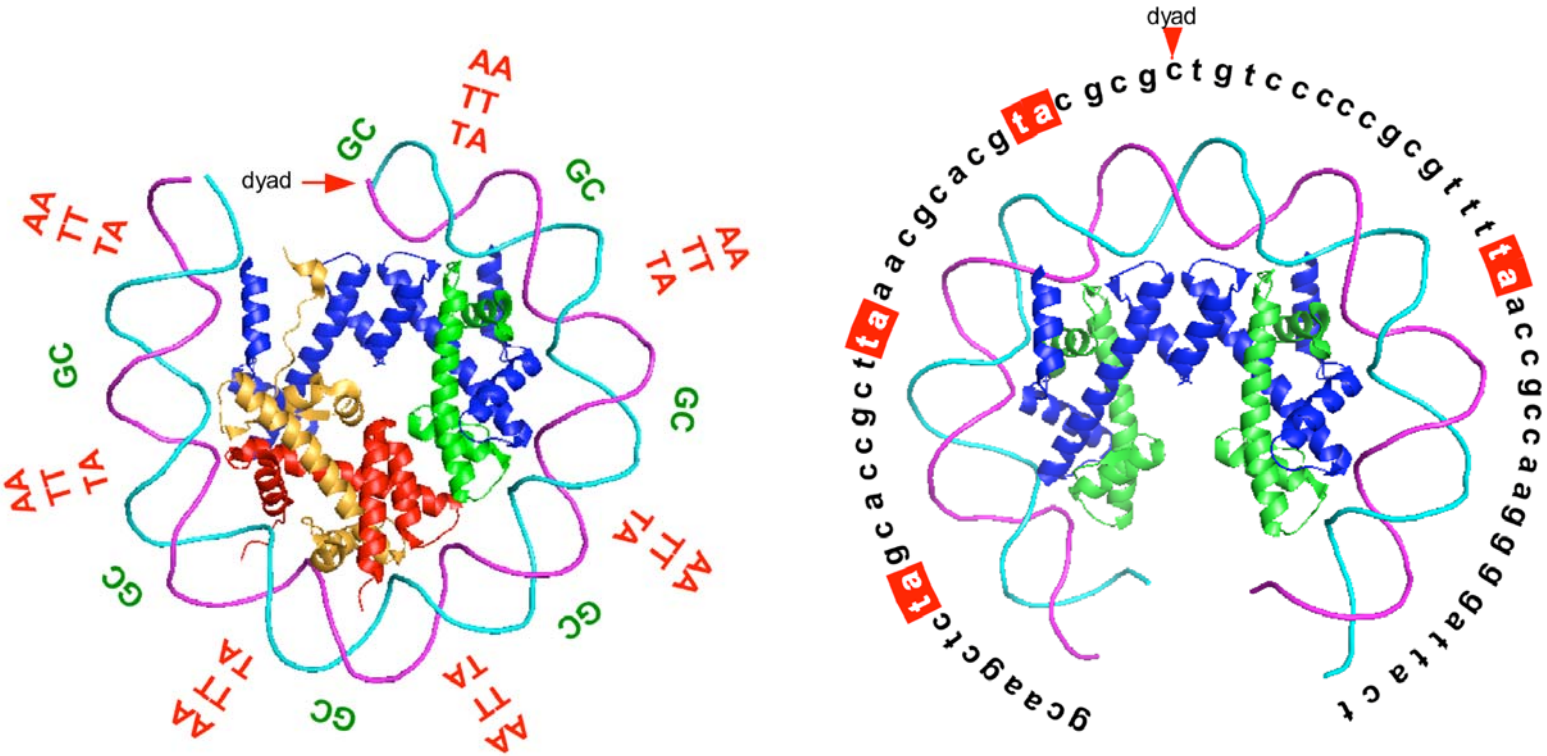
Yeast (in vitro)

Chicken (in vivo) (Satchwell et al., 1986)

Yeast (in vivo)

In vitro experimental validation of histone-DNA interaction model

- Adding key motifs increases nucleosome affinity
- Deleting motifs or disrupting their spacing decreases affinity



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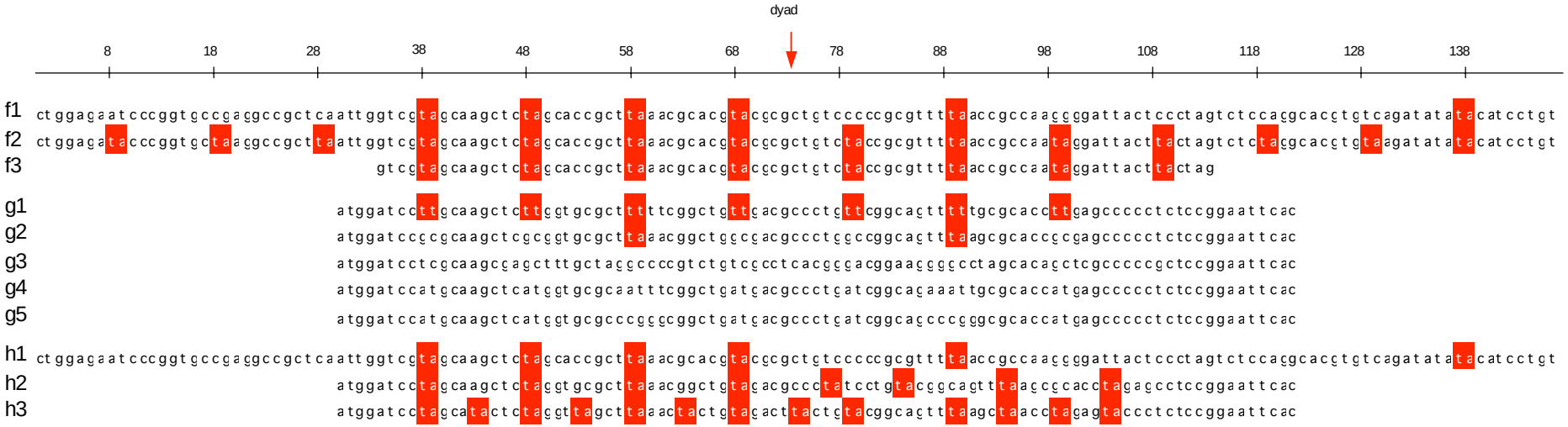
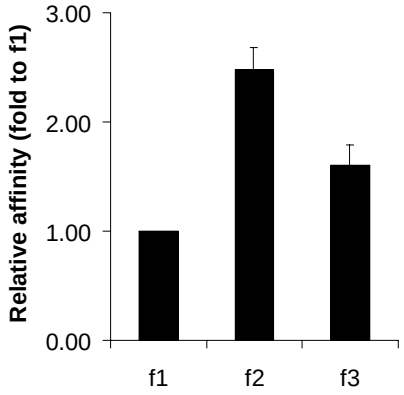
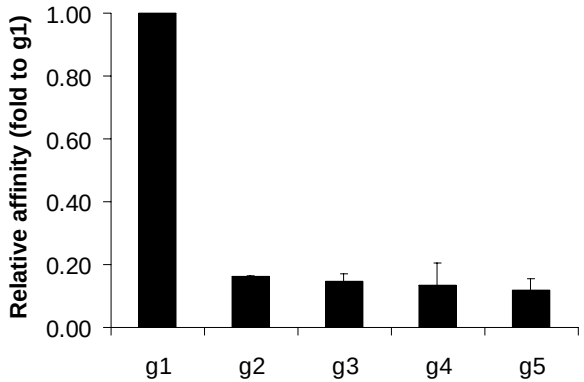
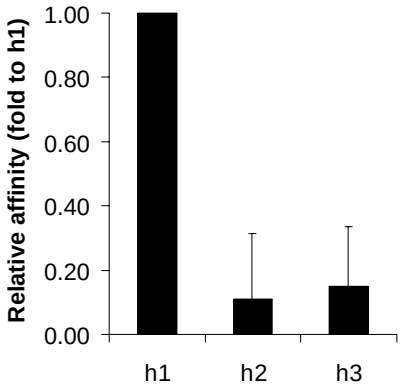








Summary

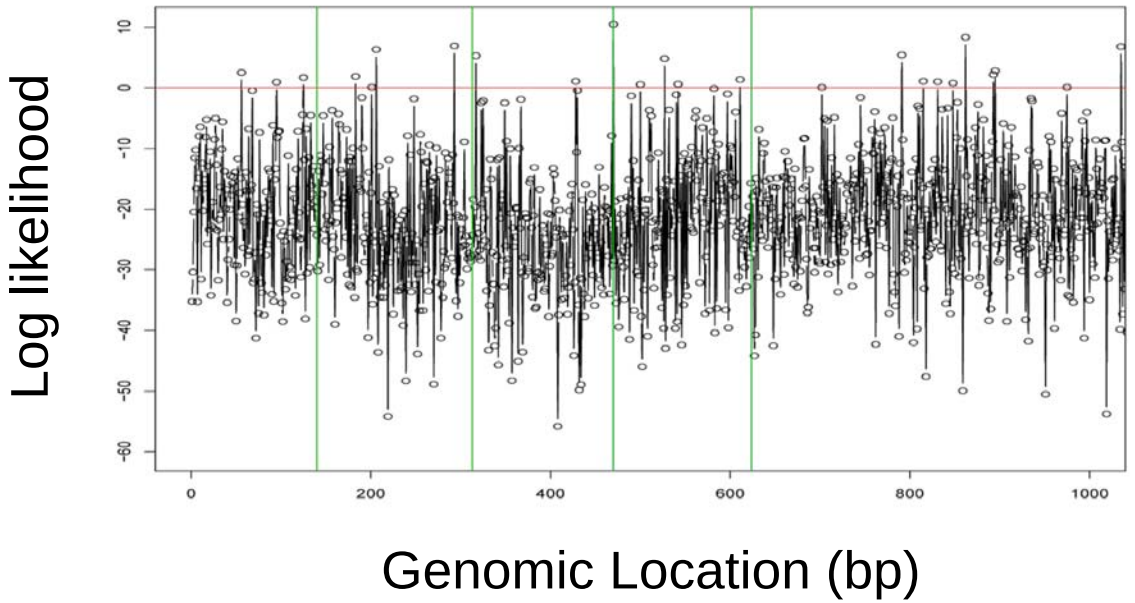
Differing DNA sequences exhibit a > 5,000-fold range of affinities for nucleosome formation

We have a predictive understanding of the DNA sequence motifs that are responsible

Sequences matching these motifs are abundant in eukaryotic genomes, and are occupied by nucleosomes in vivo

Placing nucleosomes on the genome

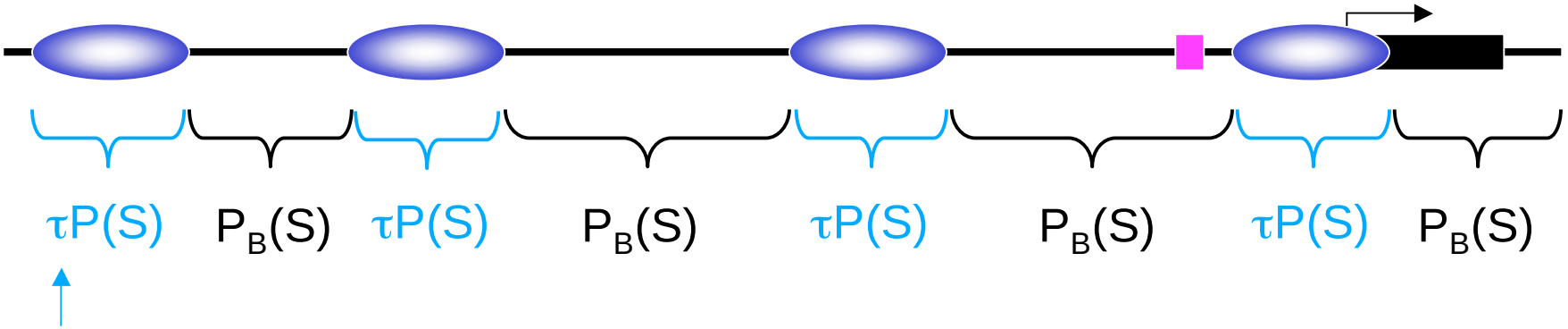
A free energy landscape, not just scores and a threshold !!



- Nucleosomes occupy 147 bp and exclude 157 bp

Equilibrium configurations of nucleosomes on the genome

- One of *very many* possible configurations

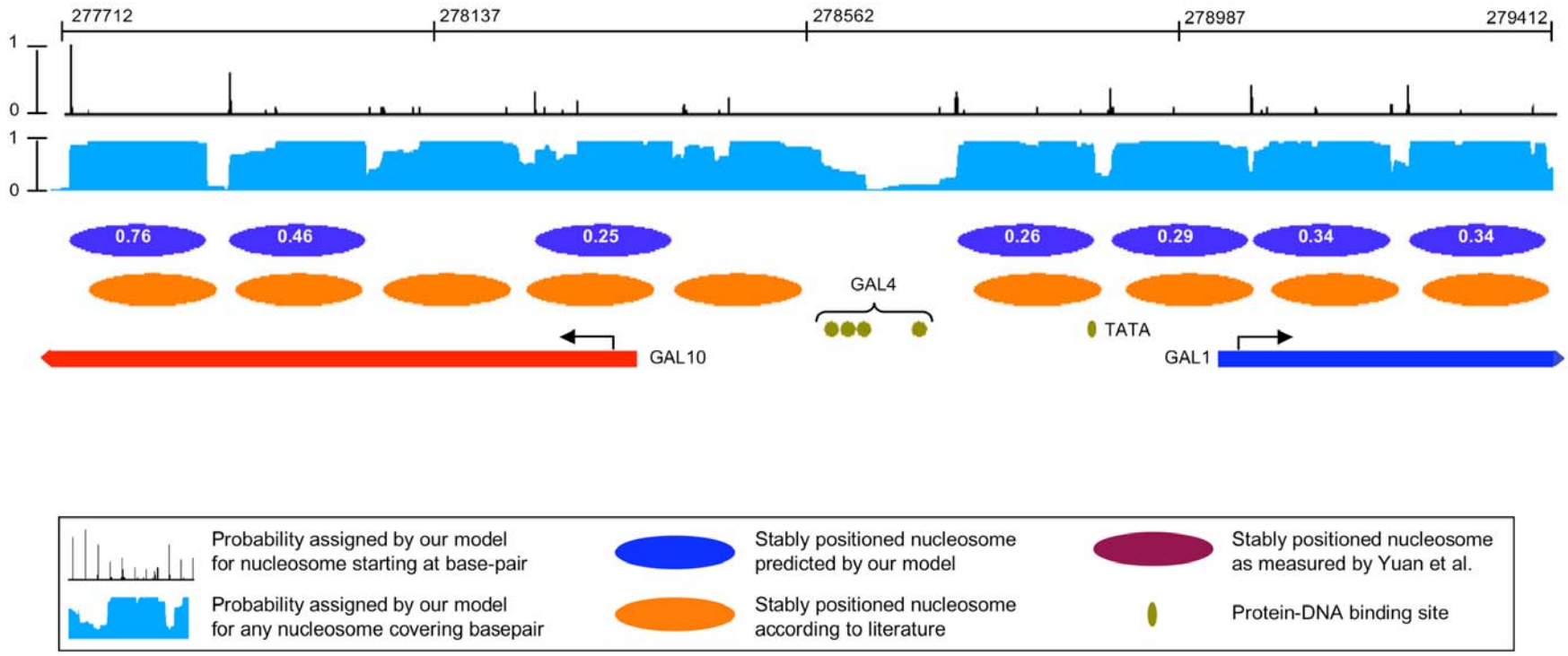


Probability of placing a nucleosome starting at each allowed basepair *i* of S

Probability of *any* nucleosome covering position *i* (≡ average occupancy)

Locations *i* with high probability for starting a nucleosome (≡ stable nucleosomes)

Nucleosome coding potential at the *GAL1–10* locus: predicted distribution compared to experimental



Summary

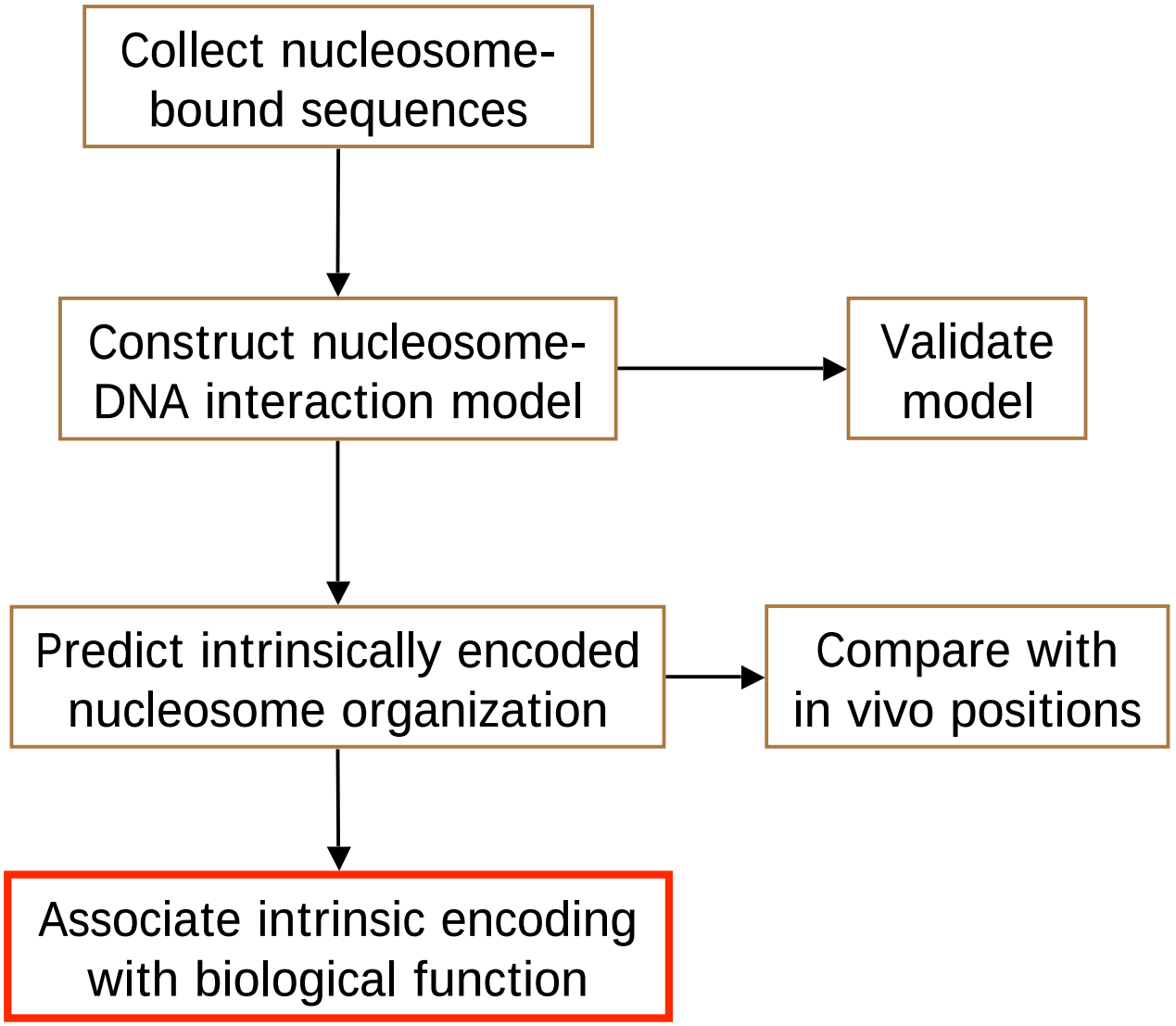
Differing DNA sequences exhibit a > 5,000-fold range of affinities for nucleosome formation

We have a predictive understanding of the DNA sequence motifs that are responsible

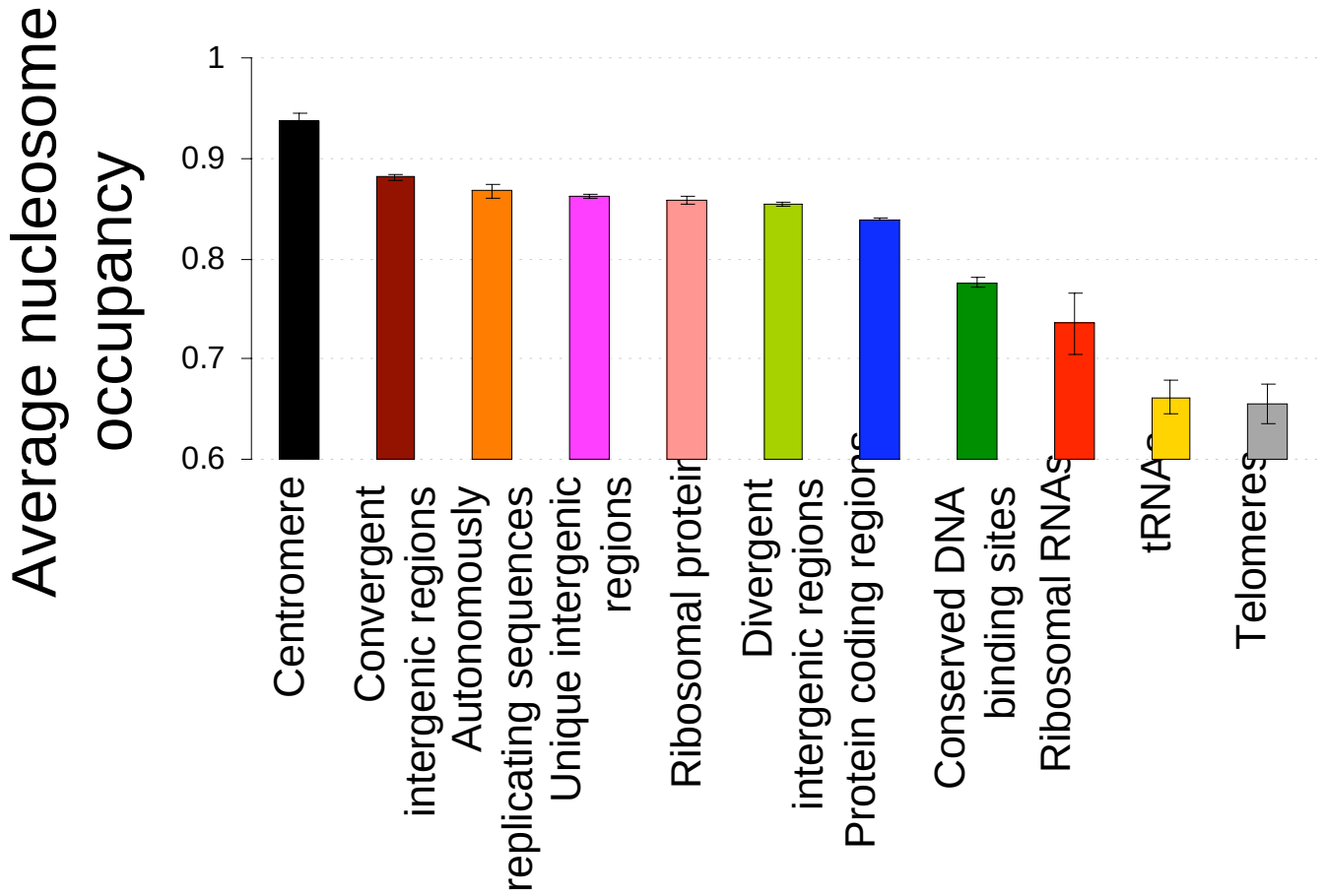
Sequences matching these motifs are abundant in eukaryotic genomes, and are occupied by nucleosomes in vivo

A model based only on these DNA sequence motifs and nucleosome-nucleosome exclusion explains ~50% of in vivo nucleosome positions

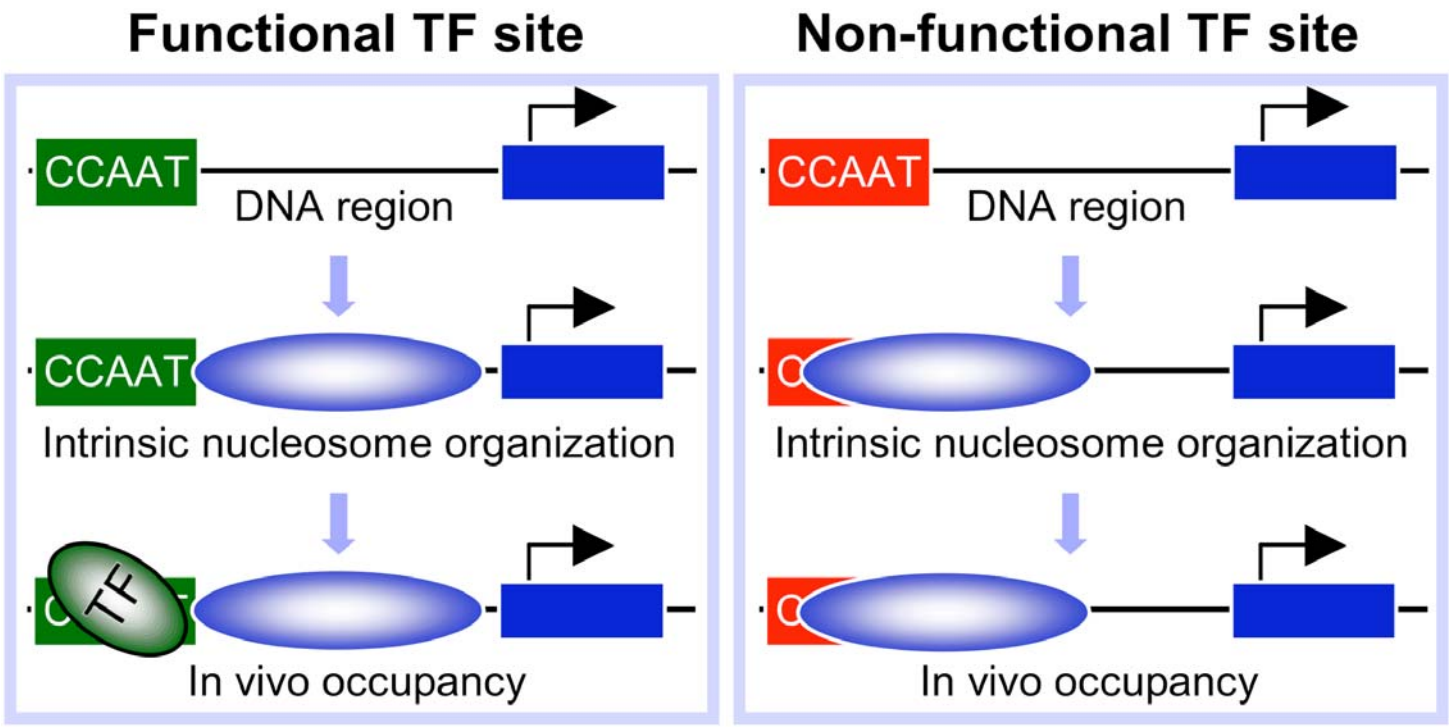
Understanding and predicting the genome's nucleosome-forming potential



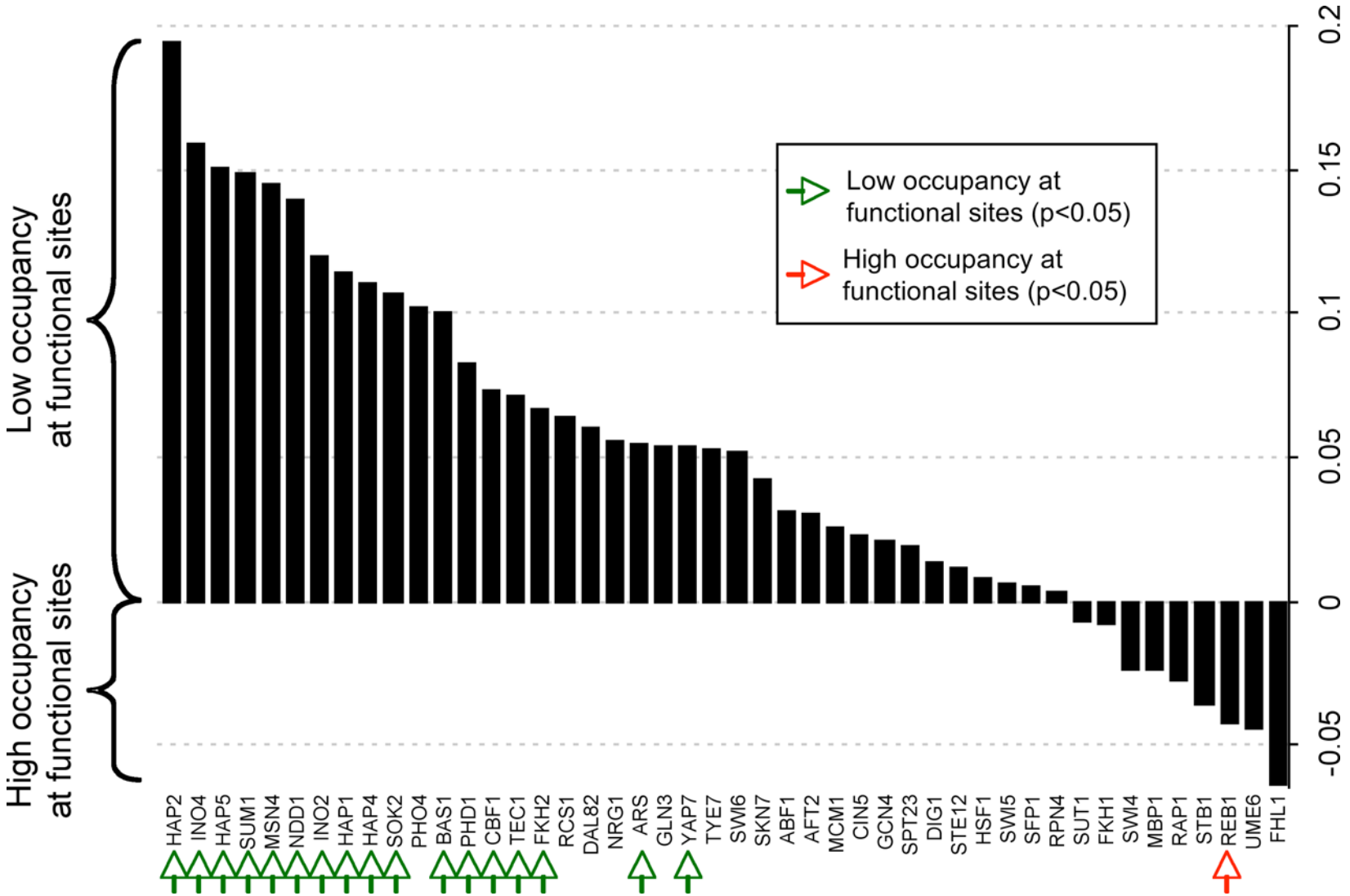
Nucleosome occupancy varies with chromosome region type



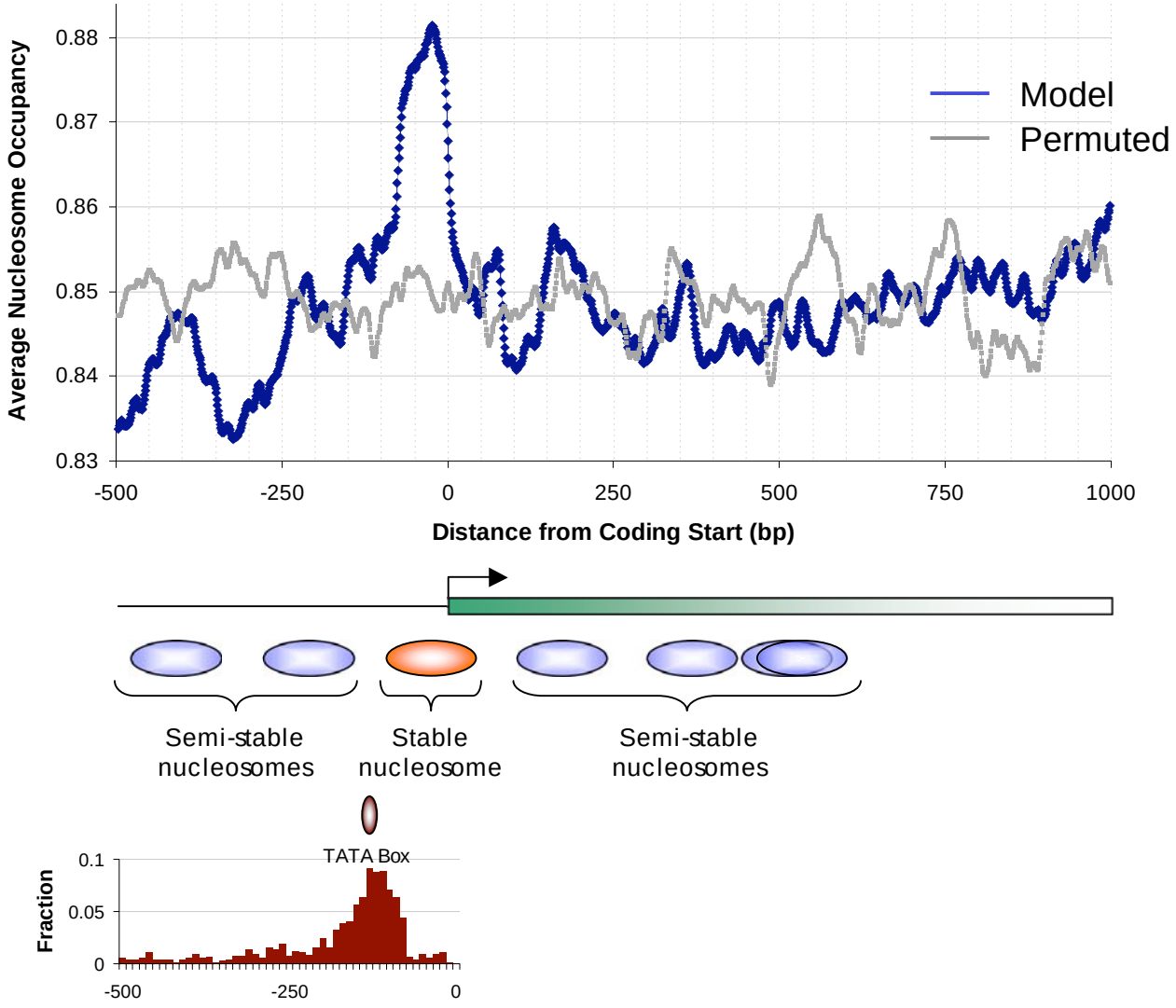
Does the genome's intrinsic nucleosome organization facilitate occupancy of functional binding sites?



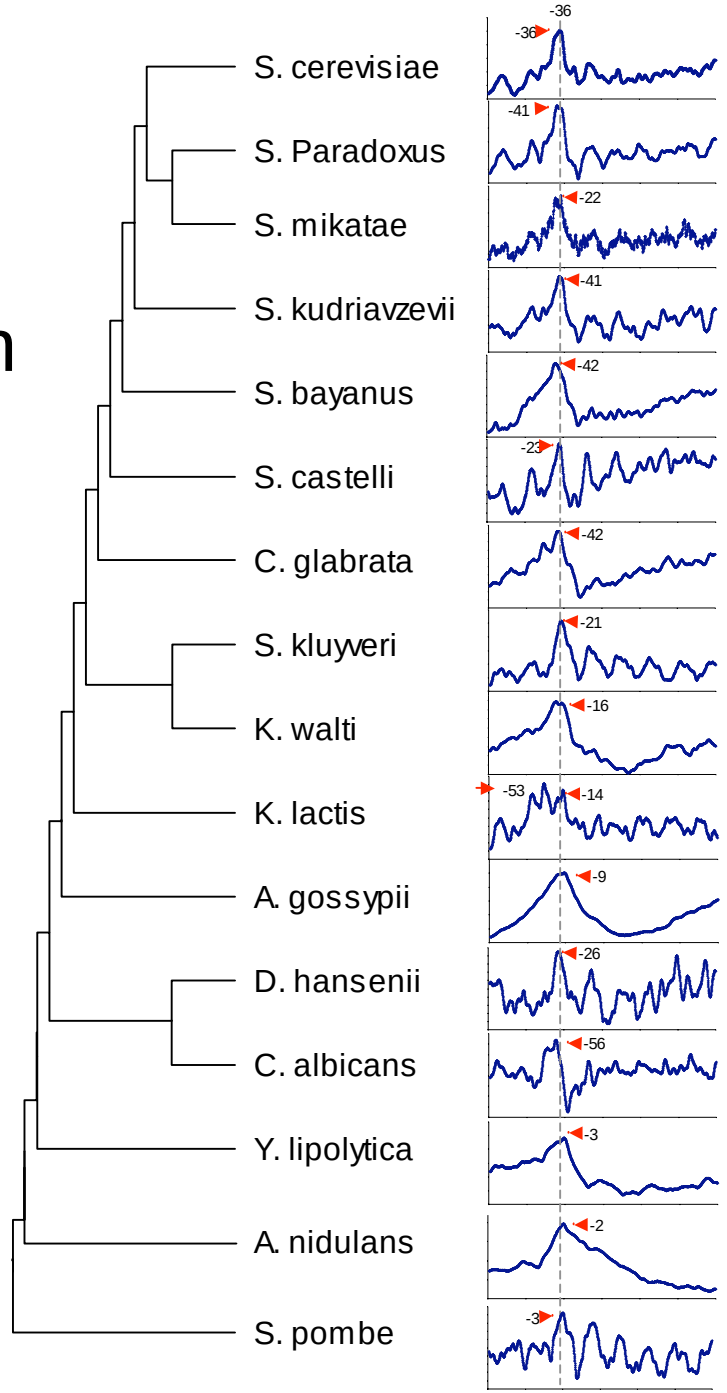
The yeast genome encodes low nucleosome occupancy over functional binding sites



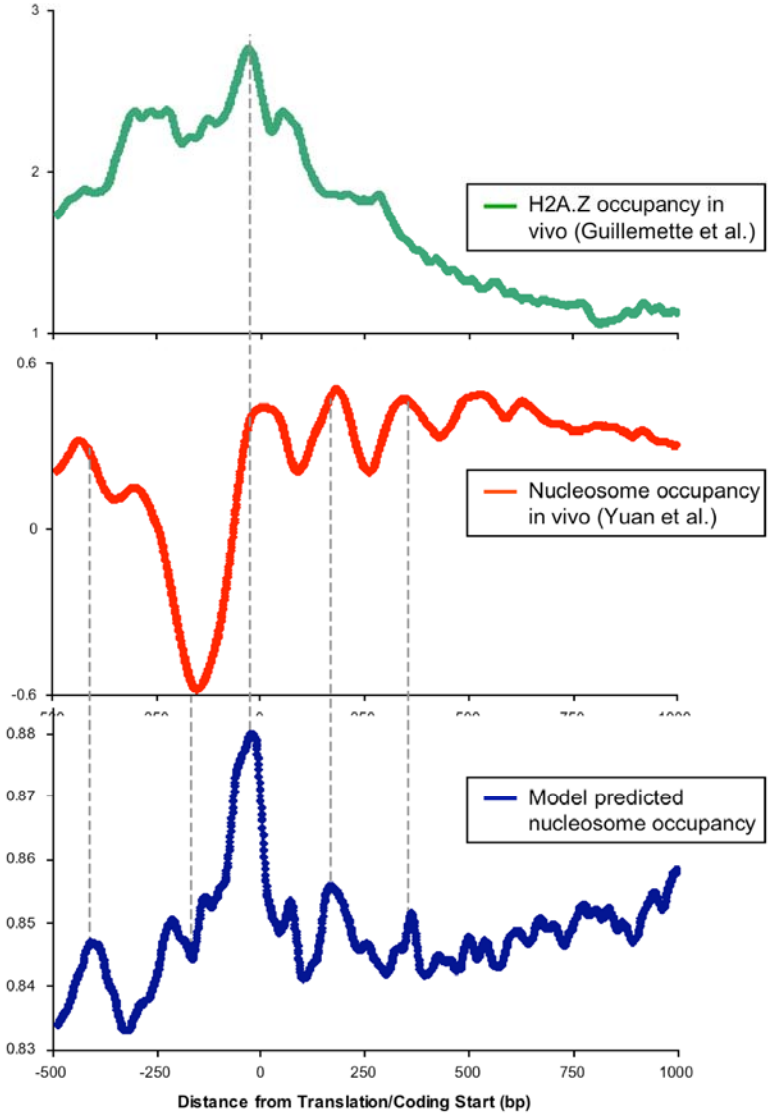
Distinctive nucleosome occupancy adjacent to TATA elements at yeast promoters

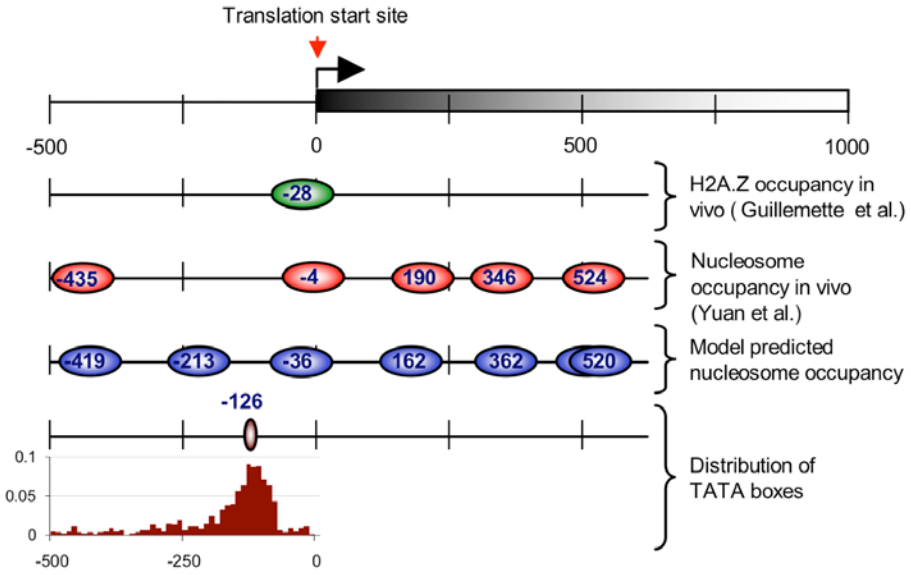


Nucleosome organization near 5’ ends of genes is conserved through evolution



Predicted nucleosome organization near 5’ ends of genes – comparison to experiment





Summary

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

We have a predictive understanding of the DNA sequence motifs that are responsible

Sequences matching these motifs are abundant in eukaryotic genomes, and are occupied by nucleosomes in vivo

A model based only on these DNA sequence motifs and nucleosome-nucleosome exclusion explains ~50% of in vivo nucleosome positions

These intrinsically encoded nucleosome positions are correlated with, and may facilitate, essential aspects of chromosome structure and function

Goals for future (current) work

Include competition with set of sequence-specific DNA binding proteins

Improve the model for nucleosome DNA sequence preferences, and elucidate the underlying mechanics

Elucidate the linker DNA length probability distribution function and sequence preferences

Worry about nucleosome-nucleosome attractive interactions

Test the equilibrium hypothesis

Acknowledgements

Nucleosome positioning in vivo

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