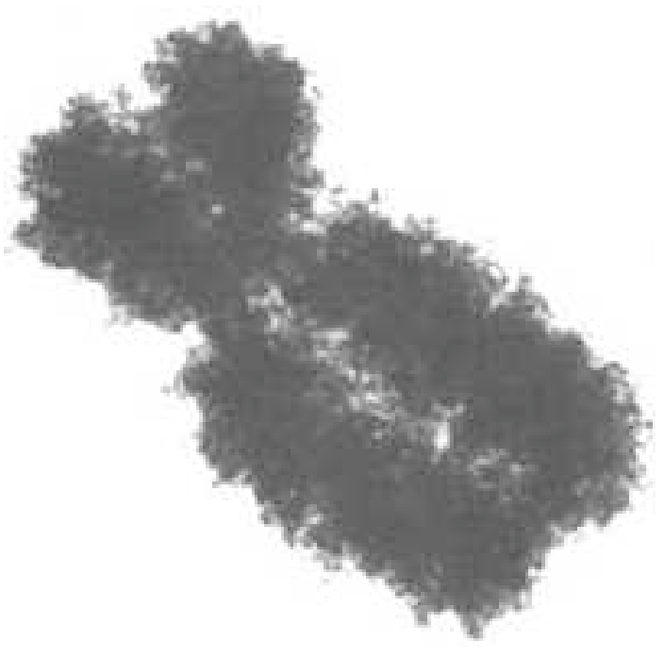
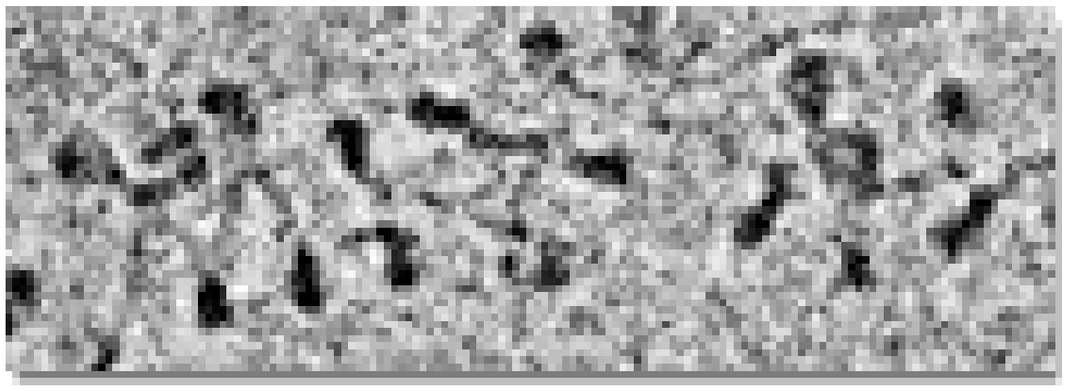
# Chromatin compaction manifested at the mono- and trinucleosomal level



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06.05.06. Santa Barbara

# Motivation

-> Ionic conditions -> $Na^{+}$, $Mg^{2+}$ in vitro switch between open (10nm) and compact (30nm) chromatin fiber conformations

-> Linker histones -> correct compaction of chromatin fibers
multiple roles in transcription and gene regulation
not essential for cell viability
bound to the linker DNA close to the core particle

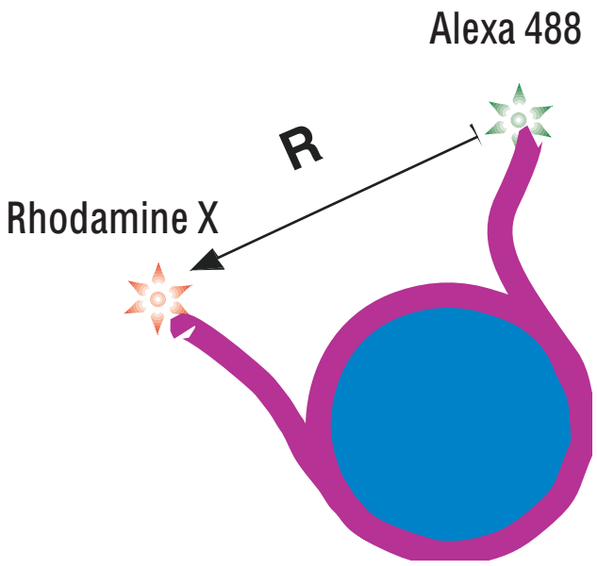
-> Histone acetylation -> de-repression or other types of transcriptional activation;
correlates negatively with the presence of linker histones
depends strongly on the cell cycle.

-> DNA methylation -> correlates with gene silencing
compaction changes in the chromatin

**all these factors influence the electrostatic landscape of the nucleosomes.**

**do they act locally as geometrical changes at the mono- or oligonucleosome level?**

# Preparation



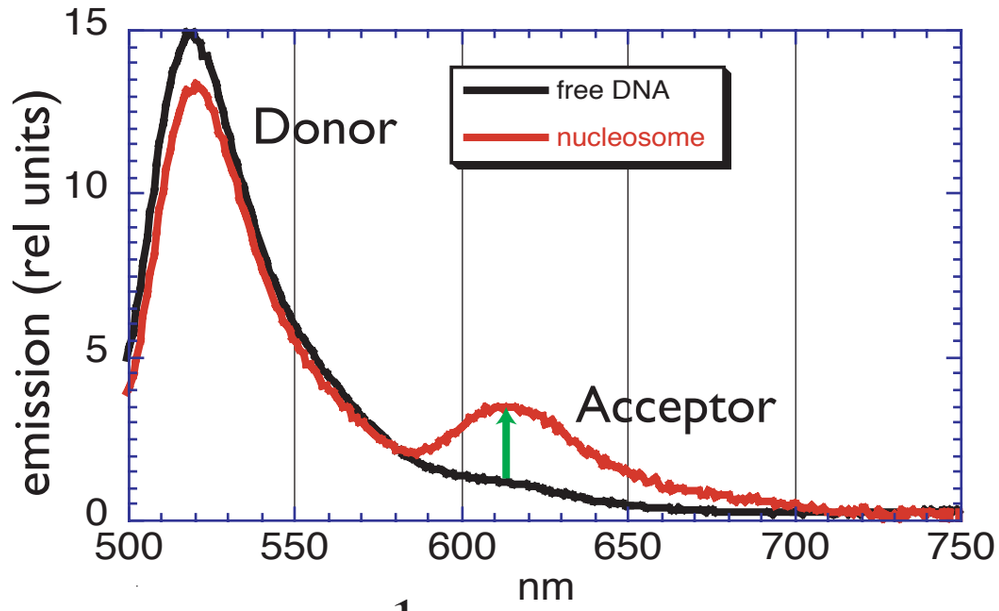

--PCR amplified fragment of positioning (5S rRNA) sequence with fluorescent end-labeled primers (150-223 bp for mono-, 607 bp for trinucleosomes)

--recombinant histones from *X laevis*, (normal or chemically acetylated) or isolated octamers from Hela cells

--reconstitution by stepwise salt dialysis

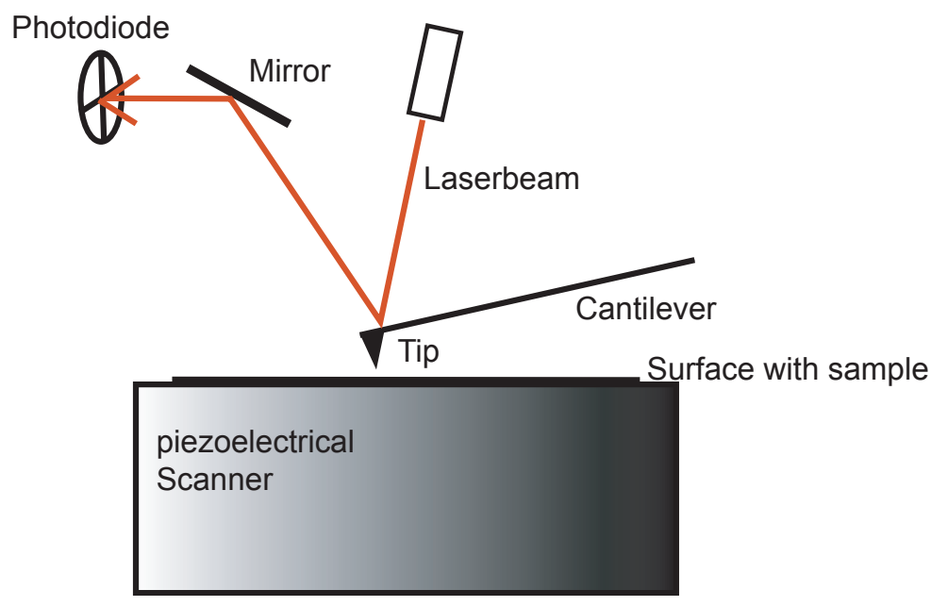
# Measures

## Fluorescence resonance energy transfer




$$E = \frac{1}{1 + (R/R_0)^6}$$

## Scanning force microscopy



# Some structural observations on salt and H1 action

## on chromatin level:

-- increasing salt from 5 to 20 mM NaCl induces closing of the linker DNA arms in oligonucleosomes (van Holde 1985, Buttler 1998)
decreases the internucleosomal distances up to 100mM NaCl (Hamermann2000)

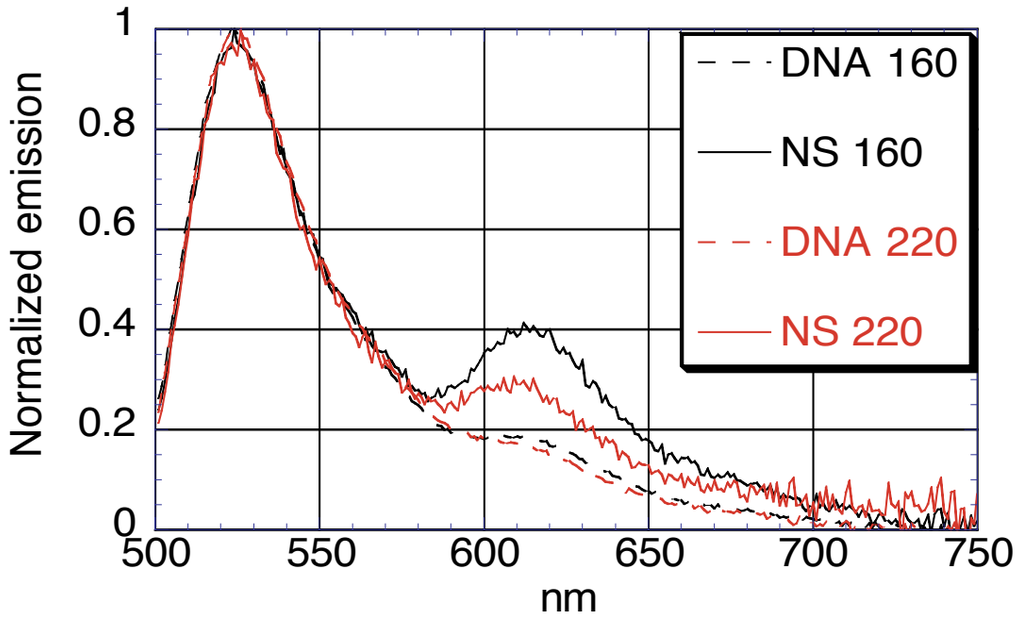
-- linker histones decrease the diameter of the chromatin fiber and the average internucleosomal distance (Carruthers 1998, Zlatanova 1998, Sato 1999)

## on nucleosomal level:

-- evidence that linker DNA arms do not cross in mononucleosomes at low salt conditions (Toth 2001)
neither in tetranucleosomes at high salt conditions (Schalch i2005)

-- linker histones induce stem formation and decrease the entry-exit angle of the linker DNAs (Furrer 1995, Bednar 1998))

# FRET dependence on linker DNA length

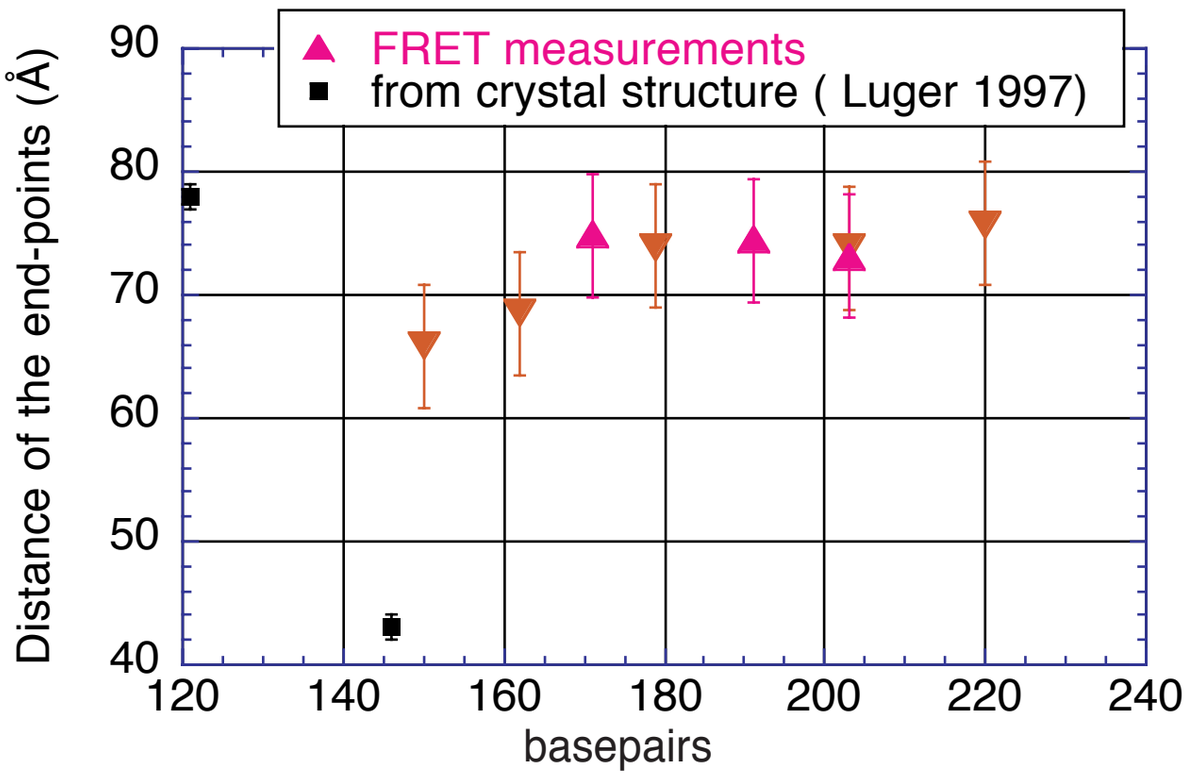


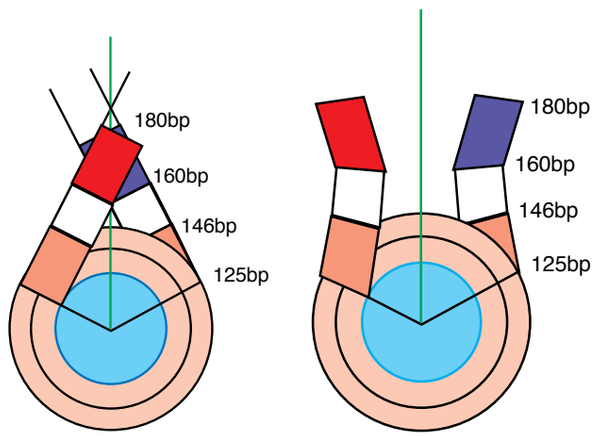

Nucleosomes prepared from Hela histones measured at low salt (5mM NaCl)

FRET signals are averages due to the
--possible asymmetry of the NS positioning
--mobility of the linker DNA
--mobility of the dyes on the $C_6$ linker chain

Averaging gives stronger weight to smaller distances
($E \propto 1/R^6$)

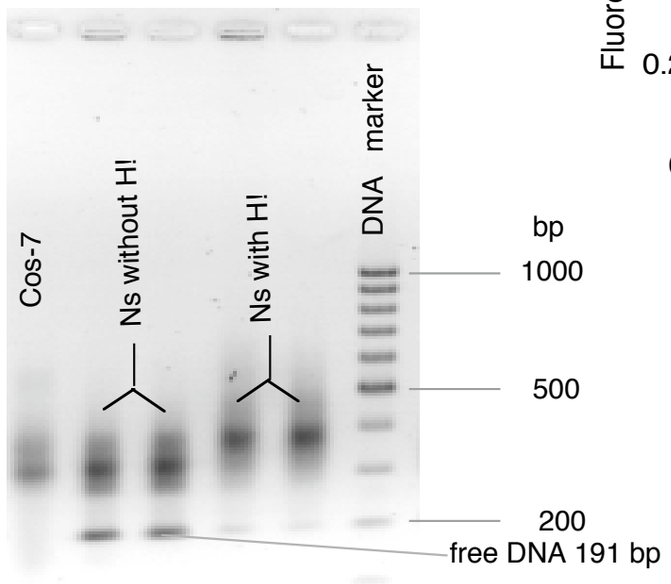
## Calculated end-to end distances

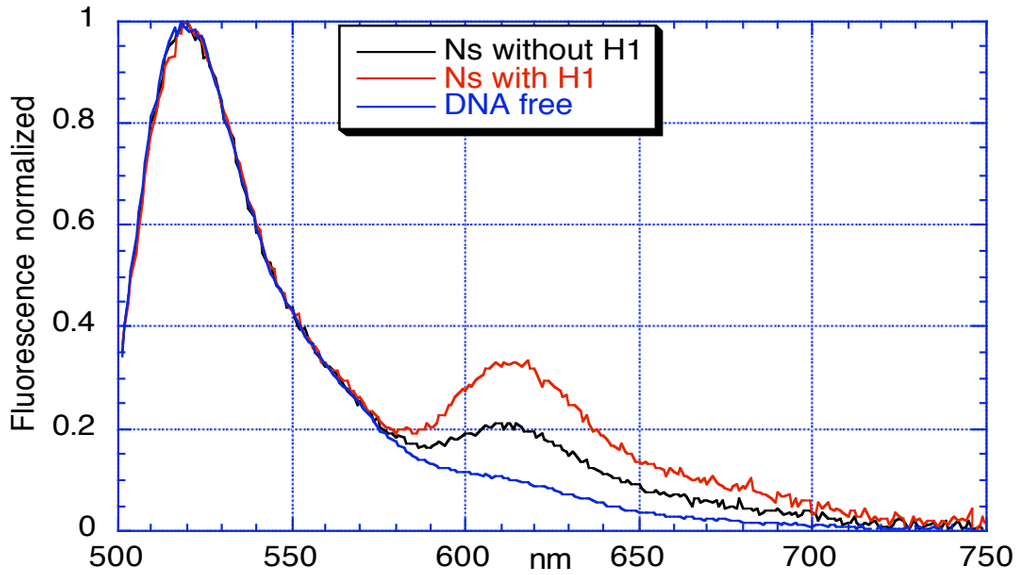


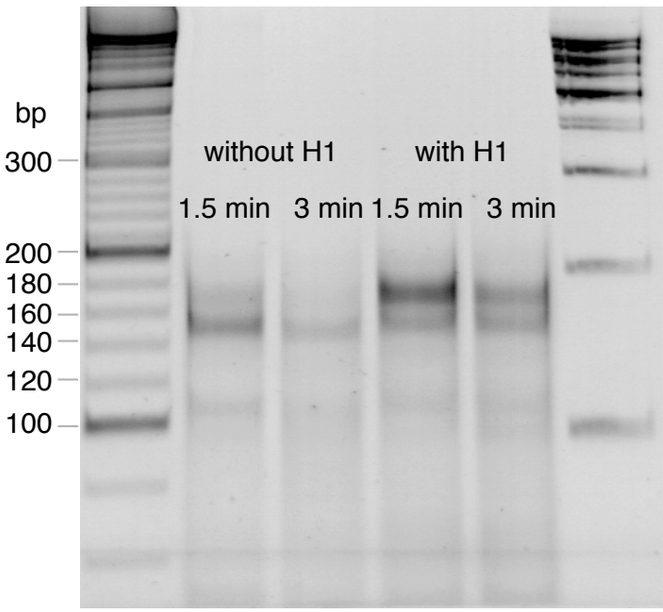

Linker DNA arms of reconstituted mononucleosomes do not cross

# H1 incorporation decreases the distance between the linker arms




Nucleosomes reconstituted from 191 bp DNA labeled with Alexa 488 and rhodamin X measured at 5mM NaCl
0.3 < H1: Nucleosome < 0.5




Calculated end to end distances

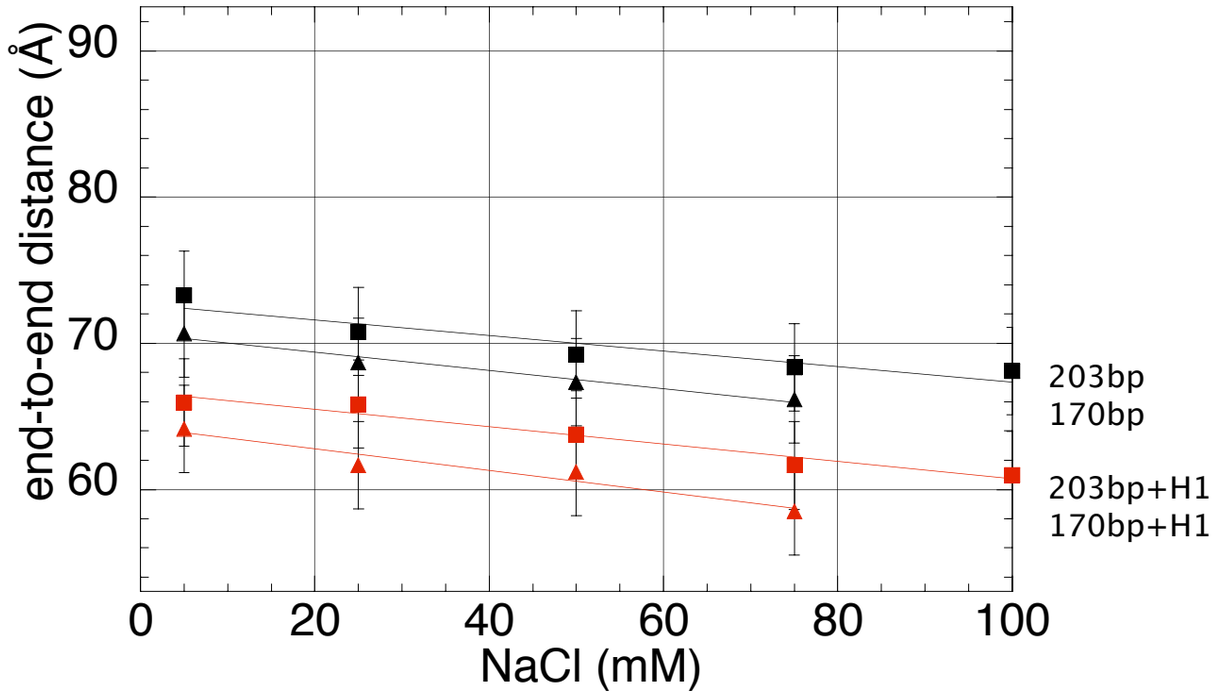
without H1 77±5 Å
with H1 69±5 Å

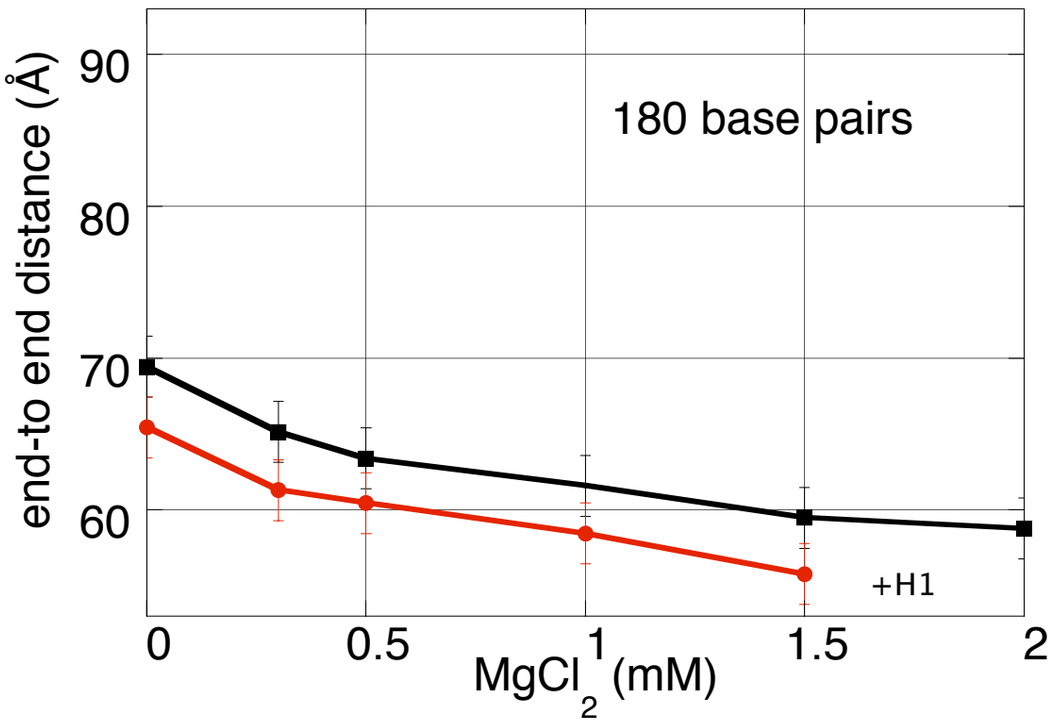
Binding of H1 is demonstrated by decrease in mobility of the nucleosome in 2% agarose gel.

MNase digestion shows stop at approx.170 bp with H1 (similarly to isolated nucleosomes) indicating correct positioning of the linker histones
(0.3 units of MNase/$\mu$g of DNA, 25° C, 1mM $CaCl_2$)

Incorporation of H1 increases the energy transfer for all measured DNA lengths (150-220 bp): the linker arms approach each other

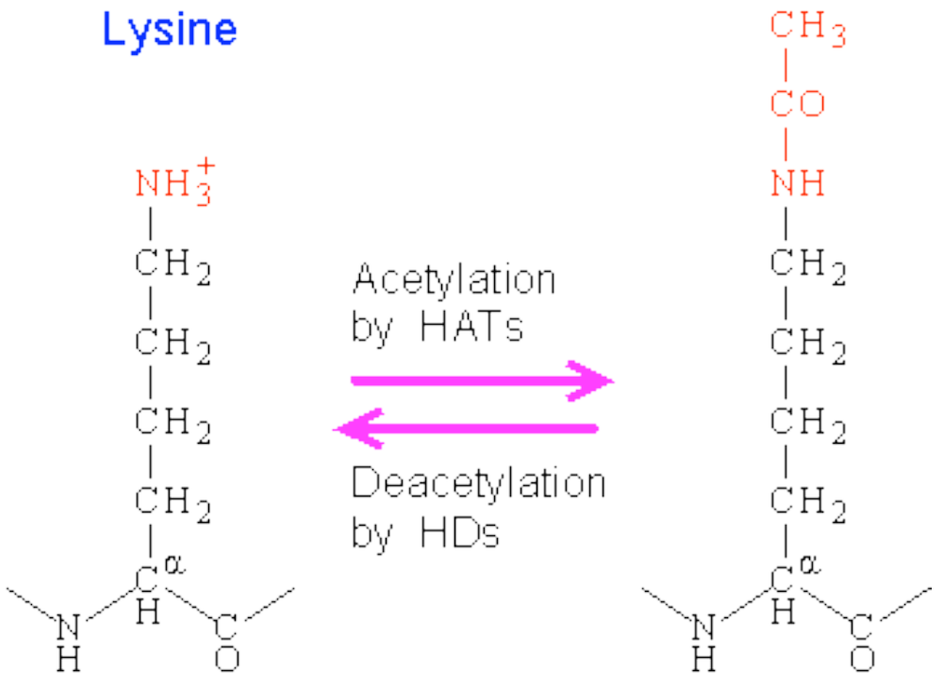
# Salts lead to a moderate approach of the linker DNA ends

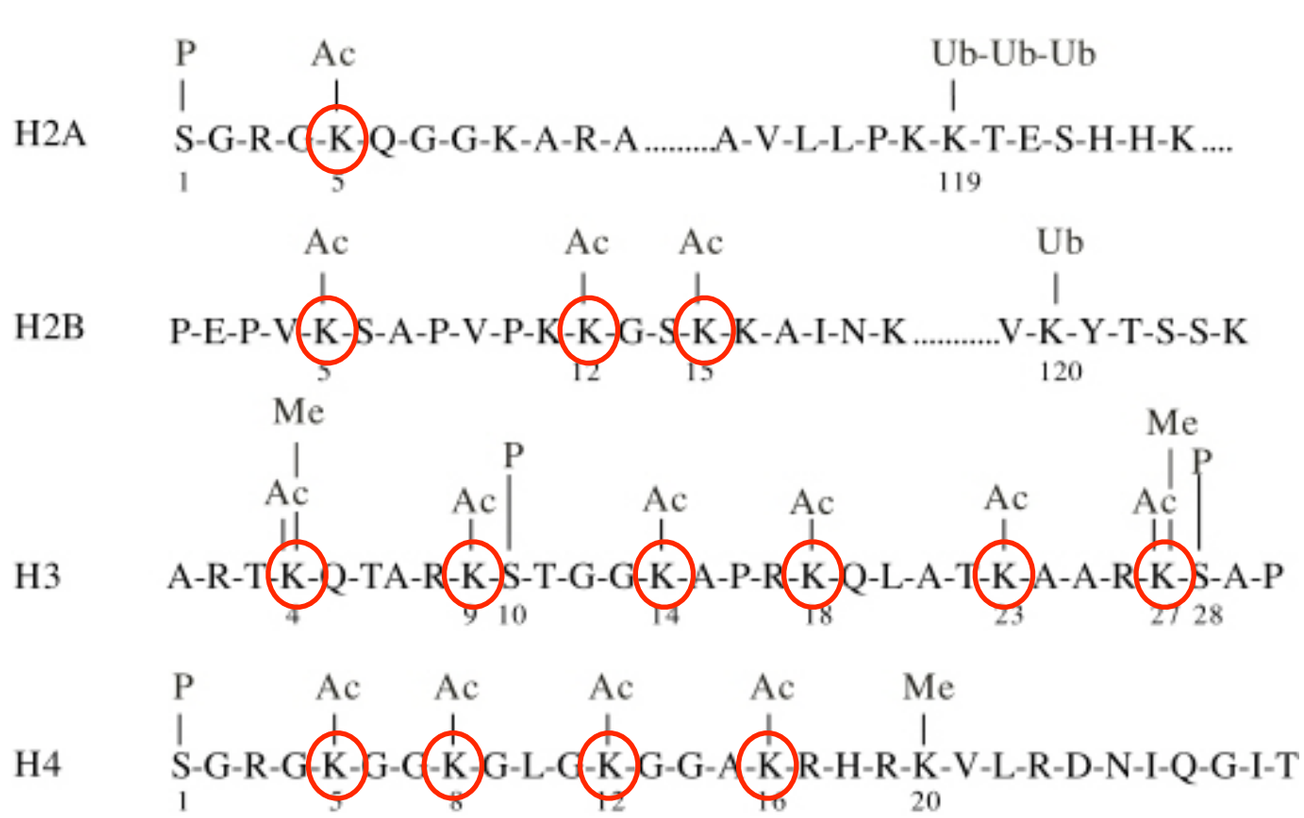



# Compaction by salts and linker histones is additive

# Histone acetylation




## chemically :

acetyl phosphate or acetyl adenylate

## biochemically :

Histone AcetylTransferase + acetyl coenzym A in equilibrium with deacetylases

# Structural effects observed on histone acetylation

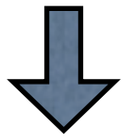
on chromatin level:
- assembly efficiency is not influenced (Cotten 1985)
- remodeling is facilitated (Krajewski2000)
- thermal and mechanical stability is slightly lowered (Yau 1982, Brower-Toland 2005)
- compactness is decreased (EM, AUC) (Garcia-Ramirez 1995)
- DNAse sensitivity and flexibility is increased (Krajewski1998)

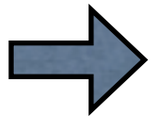
on nucleosomal level:
- no significant change in hydrodynamic parameters (Ausio 1986)
- increased helicity (CD) of the H4 tail (Wang 2000)
- histone tail – linker DNA interaction is not weakened (laser cross linking) (Mutskov 1998))

# Acetylation

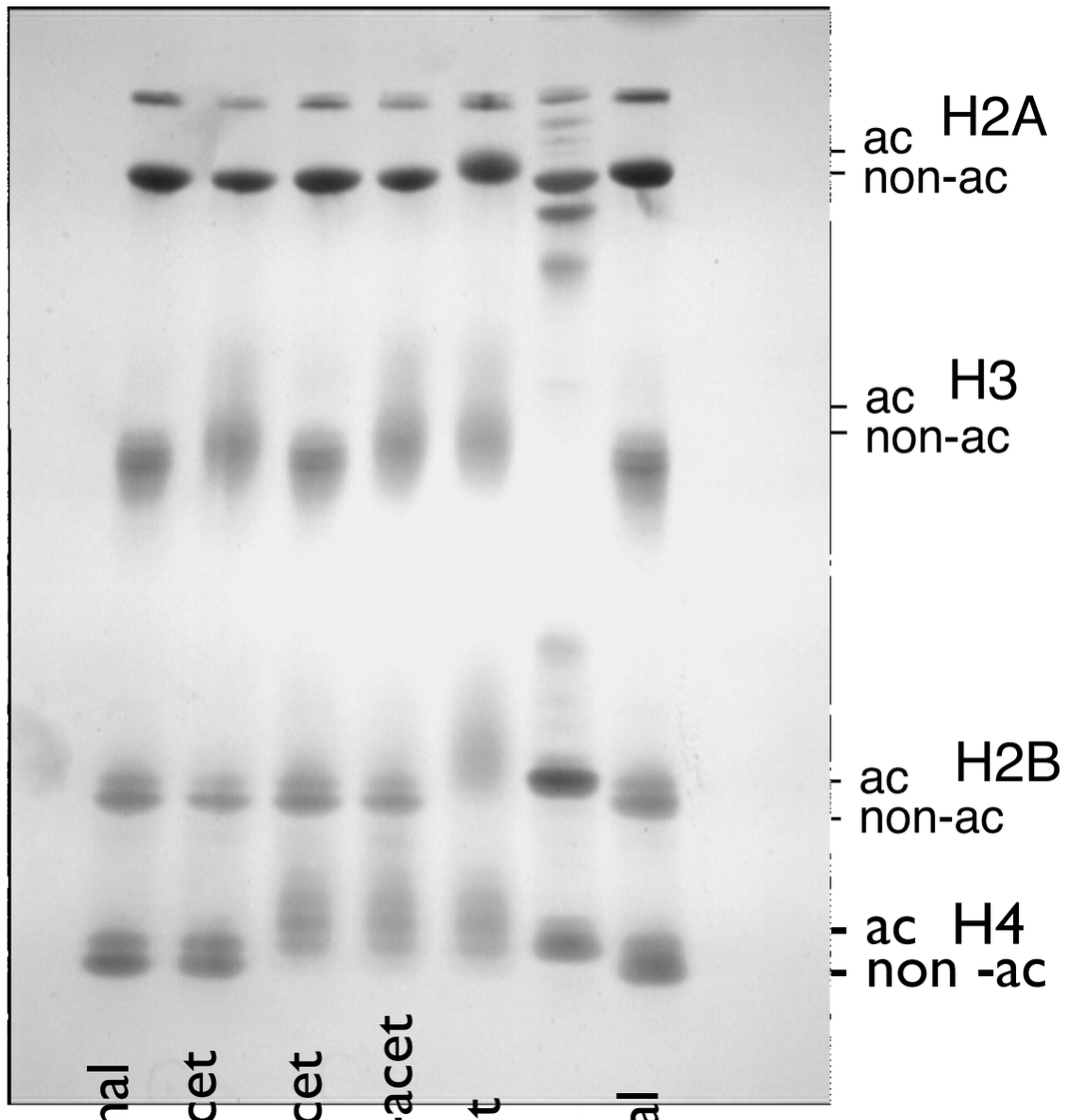
Selective chemical acetylation of recombinant histones

octamer reconstruction



FPLC purification 

TAU (Triton-X/urea/acetic acid) gel of recombinant histone octamers



# Mass spectrometry

## reflectron InSource Decay

T3 sequencing

Acetylated / total lysins

| | N terminal (45 AA) | C terminal ( 25-50 AA) |
|---|---|---|
| H2A | 0-3 / 5 | 0-1(2) / 6 |
| H2B | 0-3 / 12 | 0-1 / 5 |
| H3 | 0-3(4) / 8 | 0-(1) / 2 |
| H4 | 0-3 / 7 | 1 / 3 |

## N-termini acetylation of different H4 histones

K: acetylated K:dimethylated

Xenopus, recombinant

43 Da - **SGRGKGGKGLGKGGAKRHRKPAI…**

(purification artifact)

Xenopus, chemically acetylated

**SGRGKGGKGLGKGGAKRHRKPAI…**

(statistical)

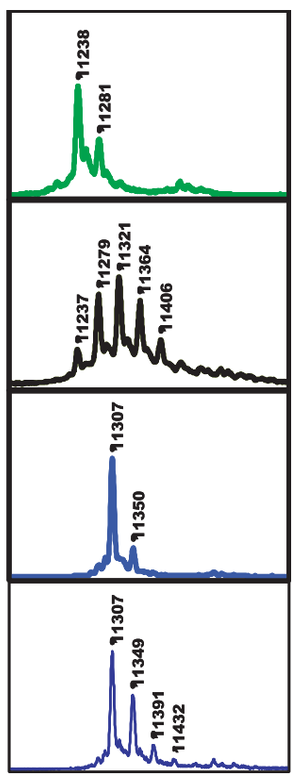
HeLa, normal

**SGRGKGGKGLGKGGAKRHRKPAI…**

HeLa, hyper-acetylated

**SGRGKGGKGLGKGGAKRHRKPAI…**

(Resemann 2005



# Acetylation

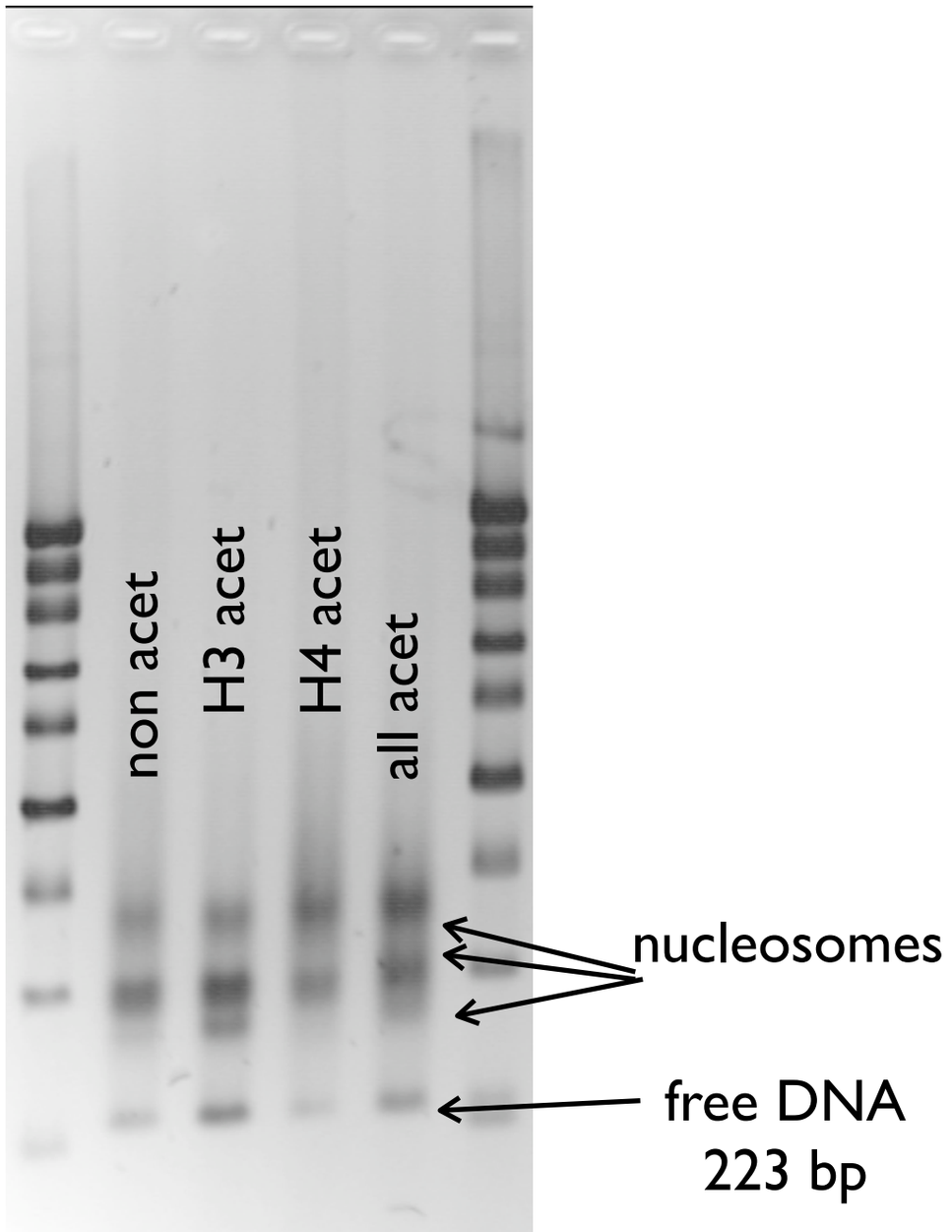
Selective chemical acetylation of recombinant histones

octamer folding

FPLC purification

nucleosome reconstitution

Agarose gel of reconstituted nucleosomes



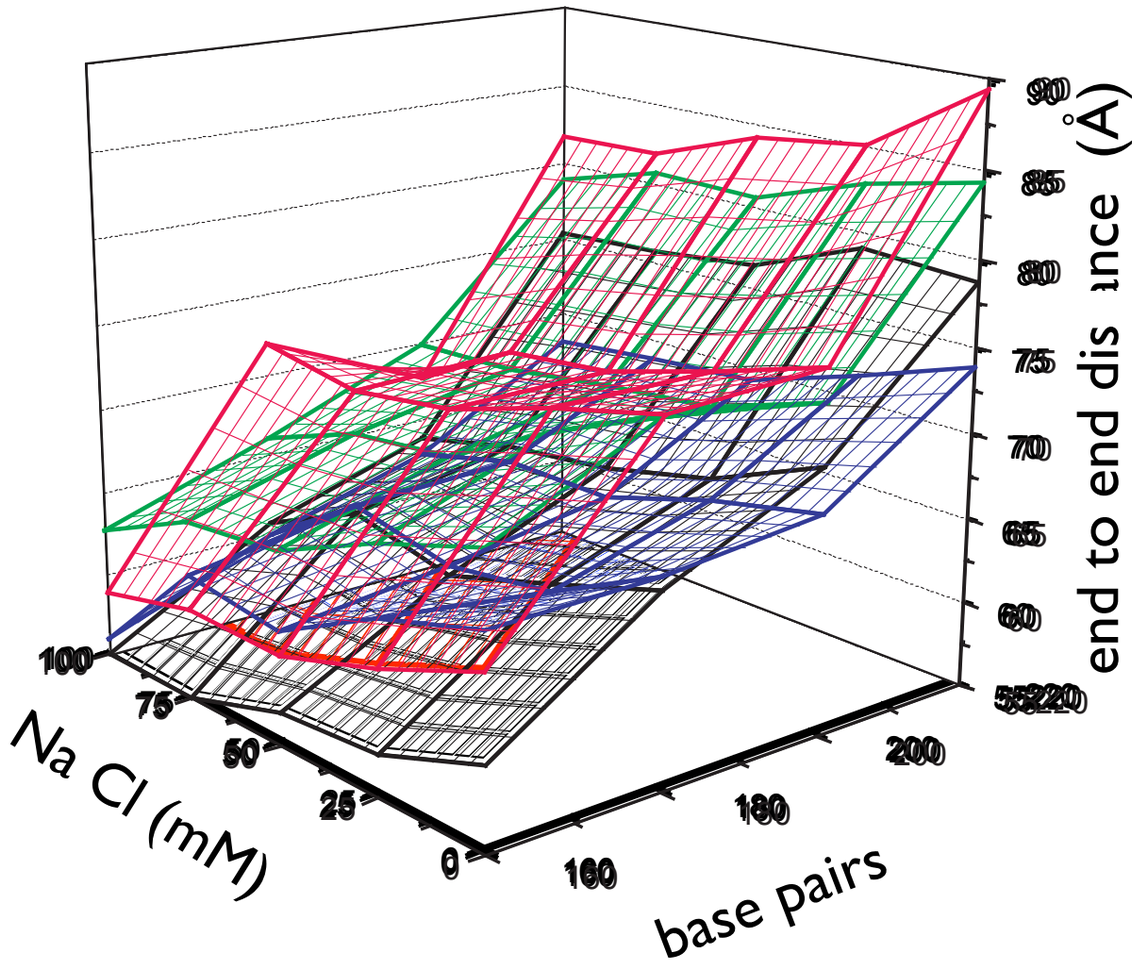
# The linker DNA opening changes differentially with histone acetylation

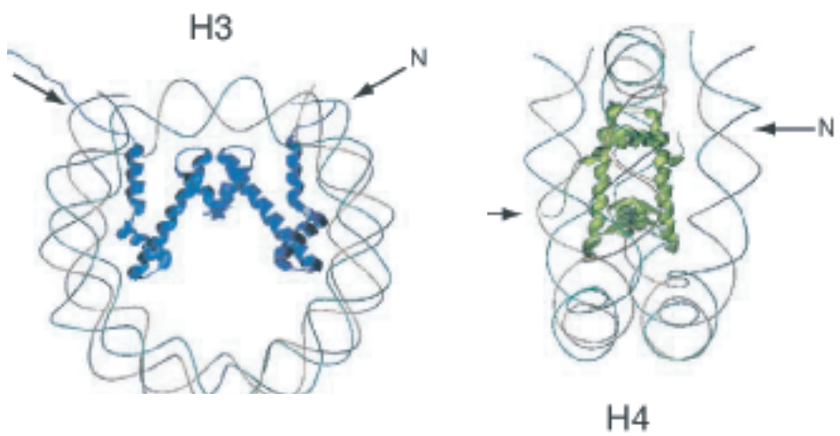
non acetylated octamers

**all acetylated**

**H4 acetylated**

**H3 acetylated**



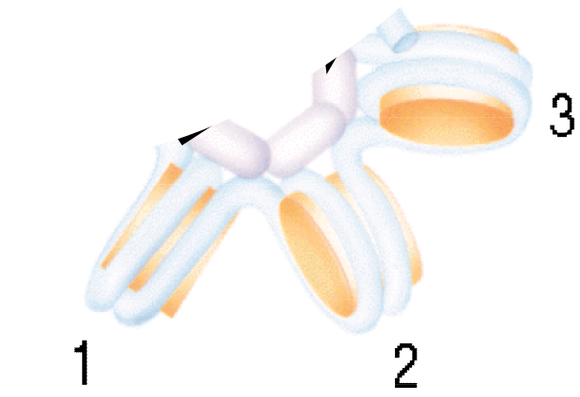

Acetylation of all histones or H3 only opens the linker DNA arms.

If only H4 is acetylated, the outer parts of the linker DNAs approach

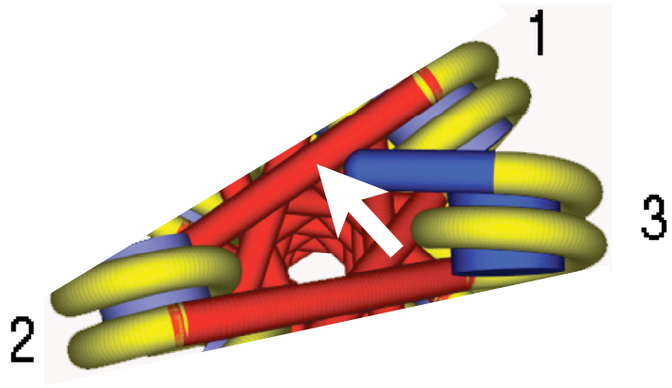
(Toth et al 2006)

# Trinucleosomes

607 bp DNA

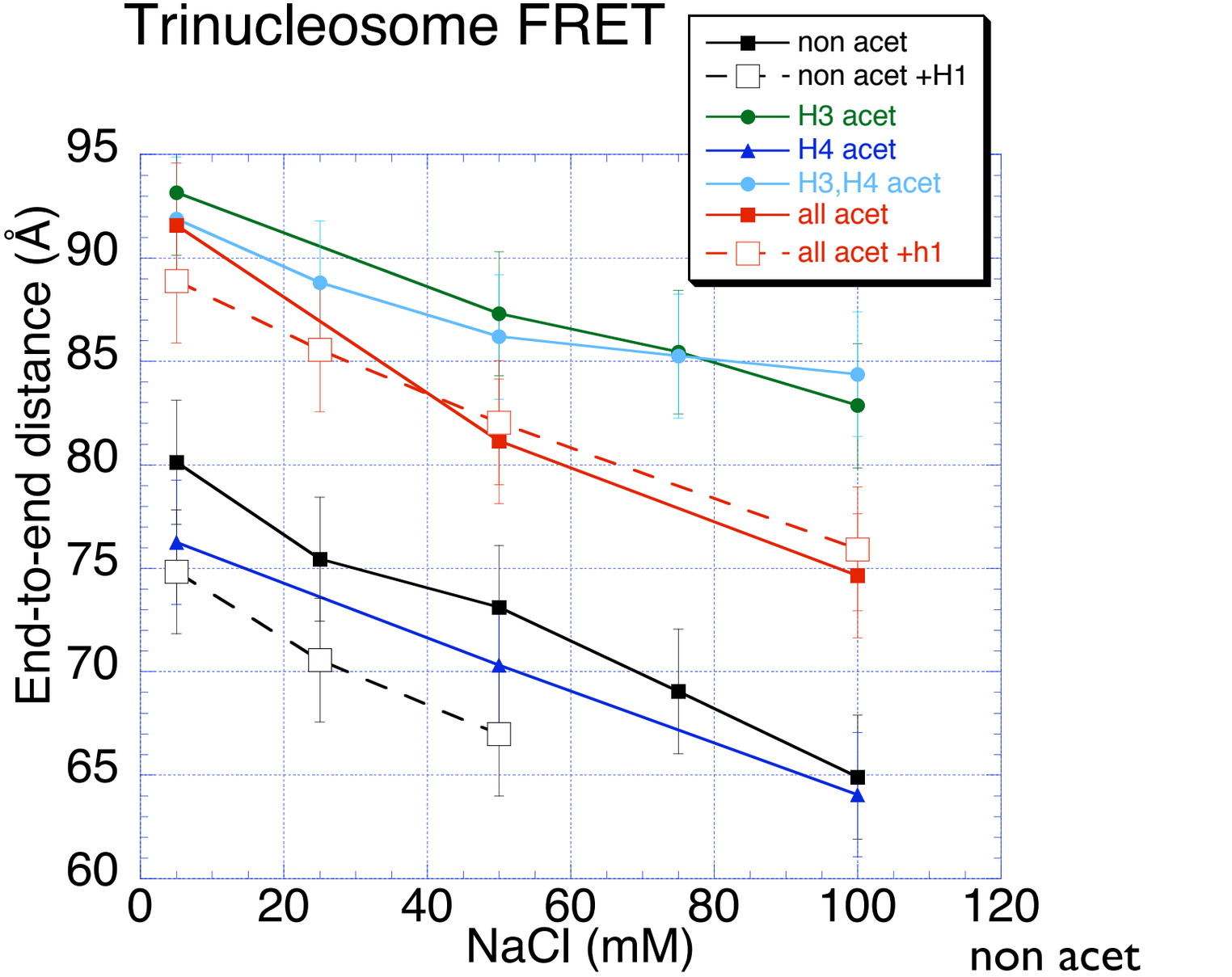


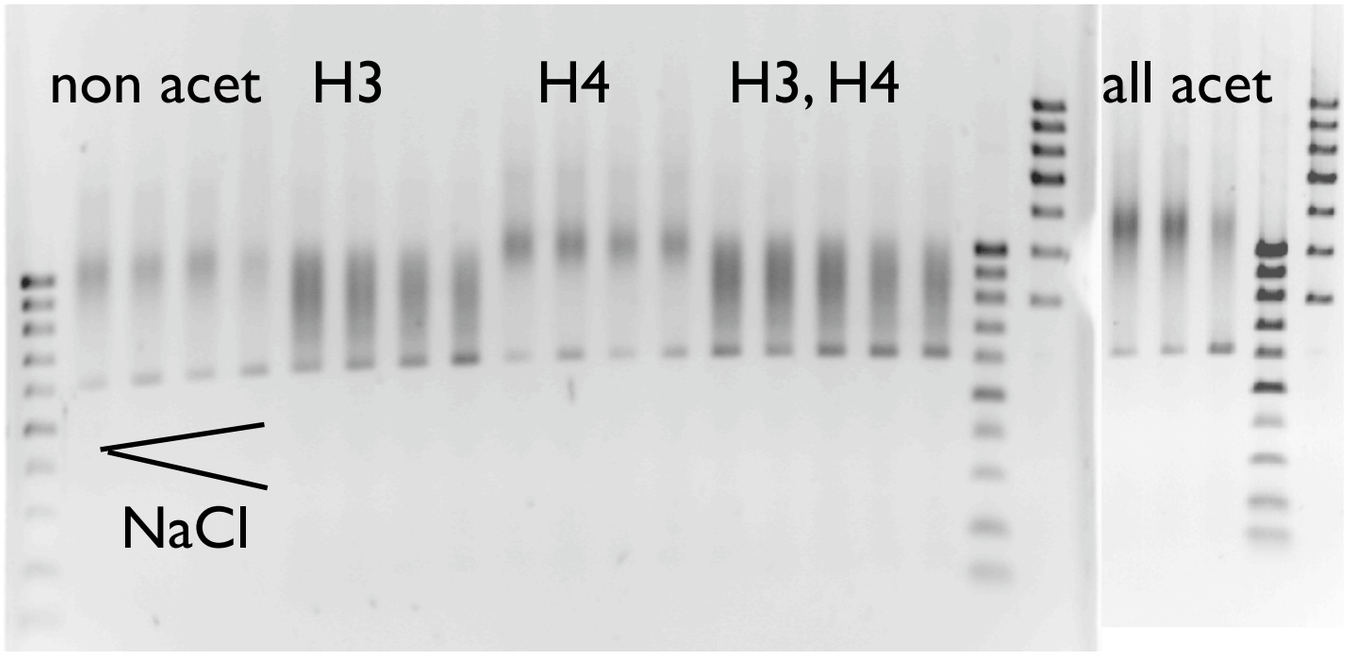

30nm



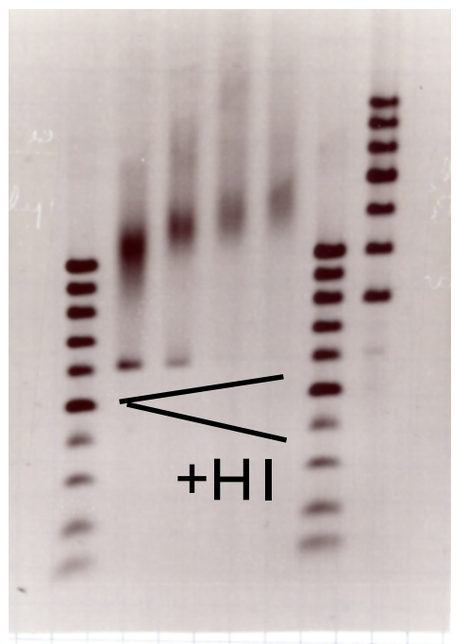

30nm

607 bp long DNA +
differently acetylated
histone octamers




non acet



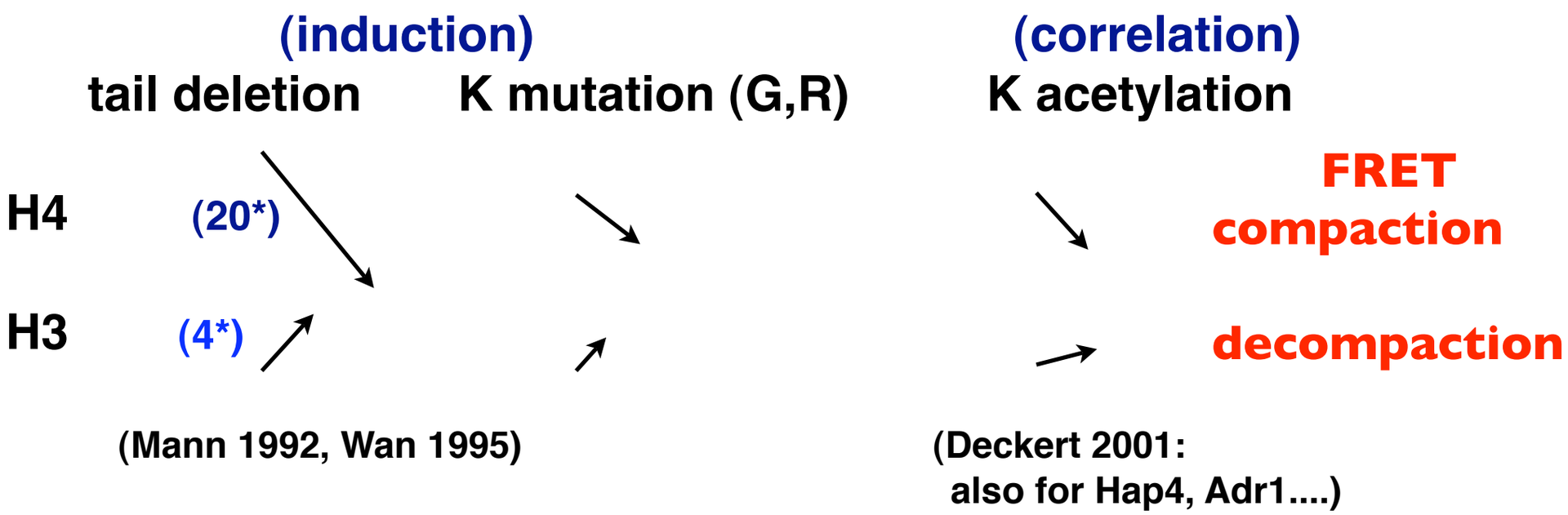
## compared to mononucleosomes:

stronger salt dependent compaction
weaker dependence on H1
similar selective compaction on acetylation

**H4 and H3 acetylation have opposite effects on the linker DNA distance and trinucleosome compaction**

# Biological relevance?

**Relation to GAL1 activation (yeast):**




(Mann 1992, Wan 1995)

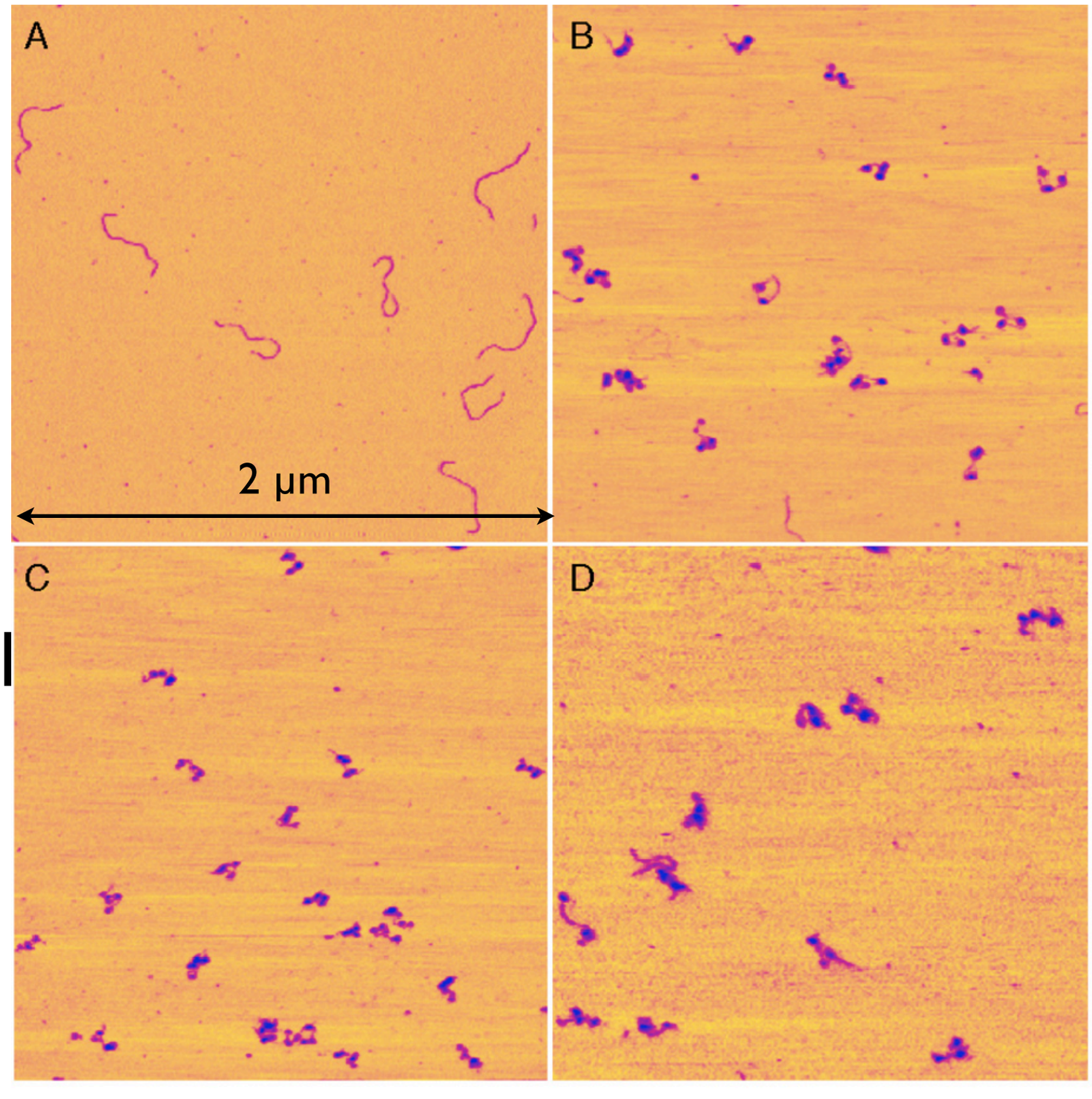
(Deckert 2001:
also for Hap4, Adr1....)

**acetylated ≠ decompacted = active**

# AFM(M.Bussiek)

samples trapped on polylysine covered mica
imaged in solution (10mM NaCl)

DNA
607bp

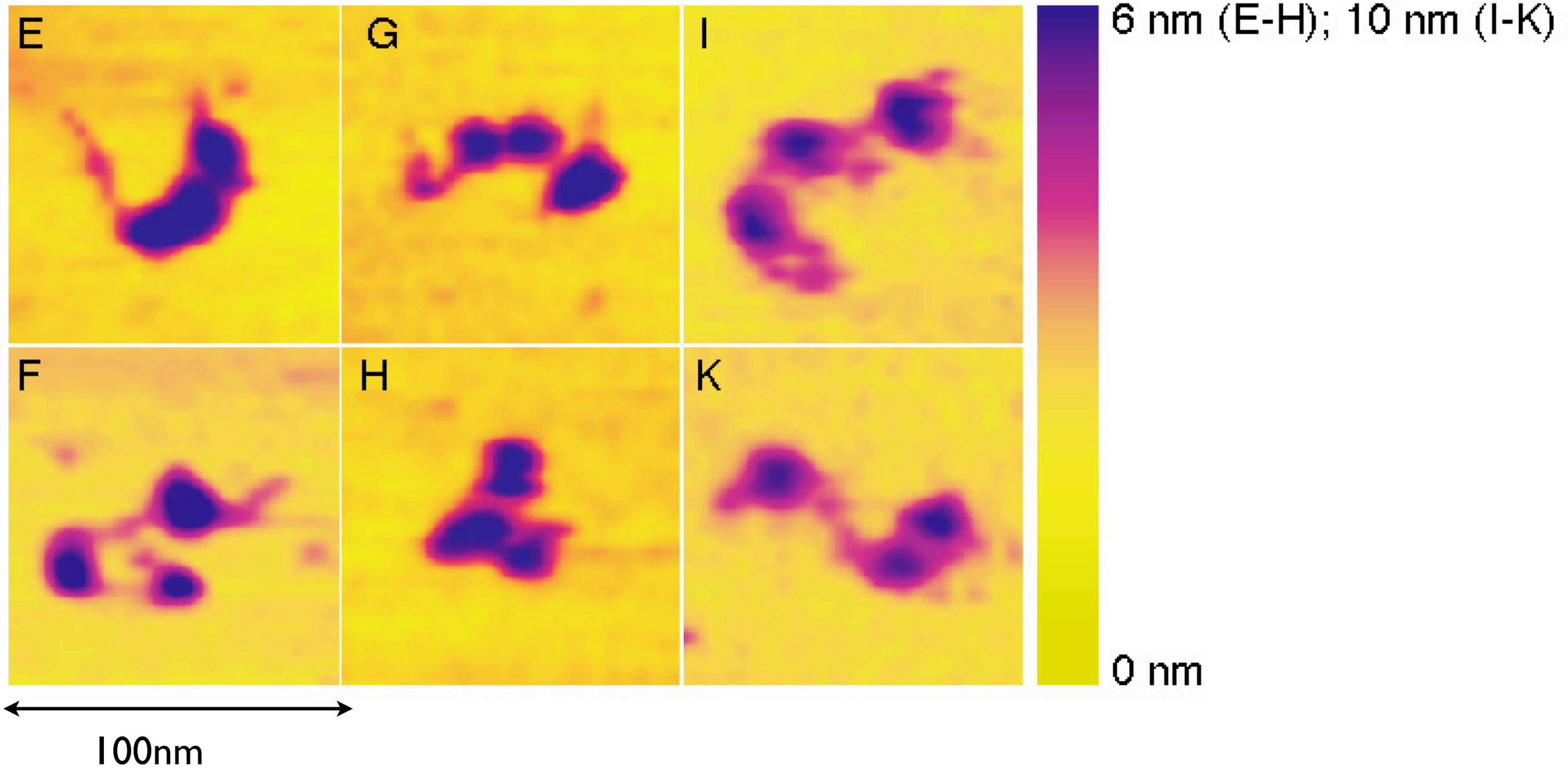



Tri norm
+H1

Tri normal

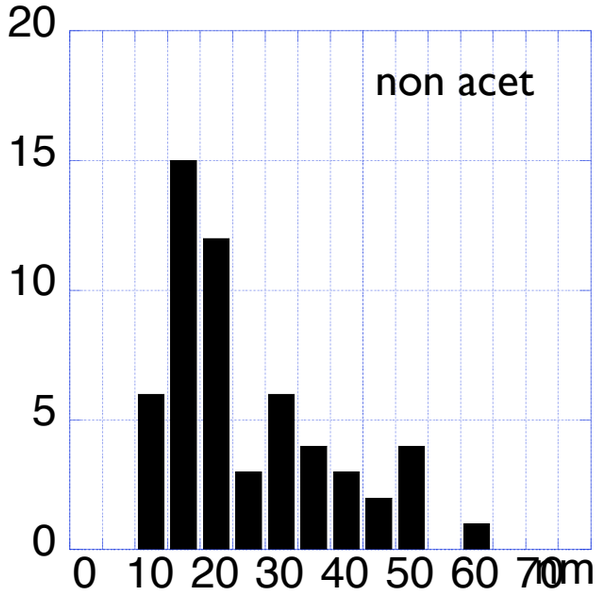
Tri all acet.

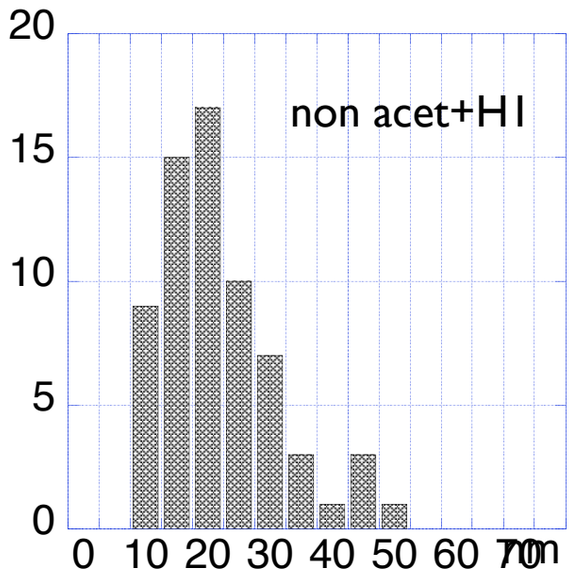
non acet. non acet+HI all acet.

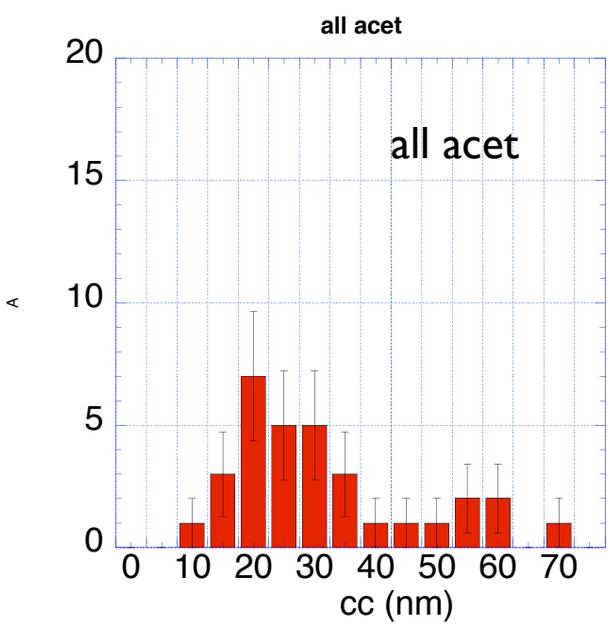



- DNA ends may be buried inside the particle
- center-to-center distances and internucleosomal angle are less sensitive to the adhesion process

## center to center distance

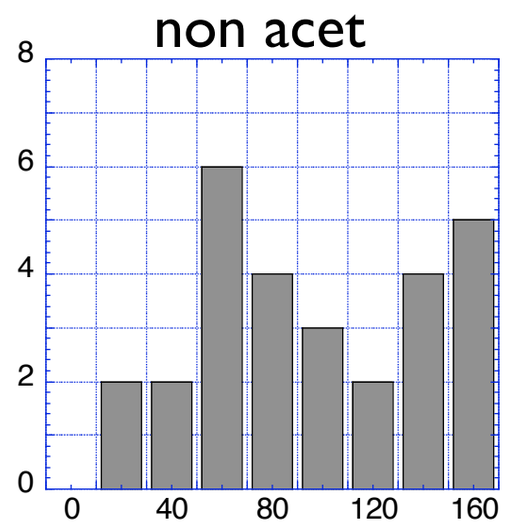


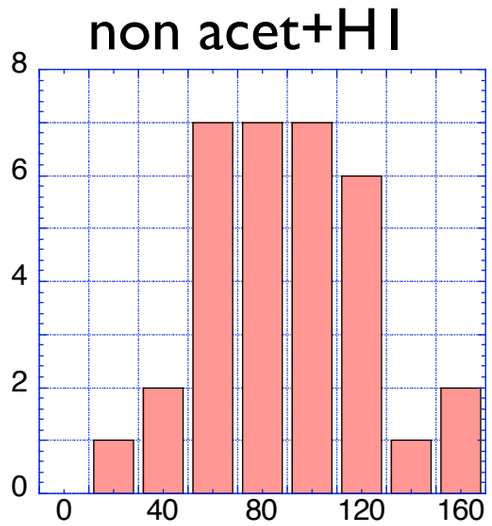




| | center-to-center | | end-to-end |
|---|---|---|---|
| | peak(nm) | mean (nm) | FRET (nm) |
| non acet | 19.2±7 | 27.6±13.2 | 7.7±0.3 |
| non acet+H1 | 21.8±10.4 | 24.3±9 | 7.3±0.3 |
| all acet | 26.8±11.9 | 31.5±10 | 8.9±0.3 |

## trinucleosomal angle



# Conclusions:

salts: increasing salt compacts mono- and (even more) trinucleosomal structure
linker histone: closes the linker DNA arms, decreases the internucleosomal distance and the trinucleosomal angle
histone acetylation: acetylation of all core histones (dominated by the effect of H3) decompacts the mono and trinucleosomes. Acetylation of H4 and H3 has opposite effect on the linker DNA distances as well as on the trinucleosome compaction. This may explain findings about anticorrelation between H4 acetylation and gene activation.

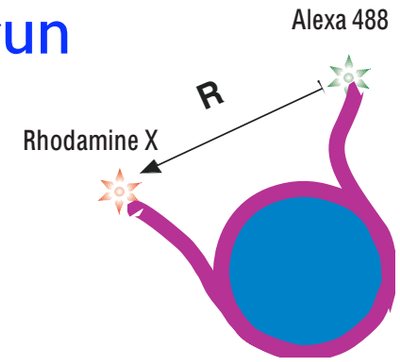
**The same factors determine trinucleosome compaction and linker DNA distance in a similar way.**
**This suggests that the linker DNA geometry determines the chromatin fiber conformation.**

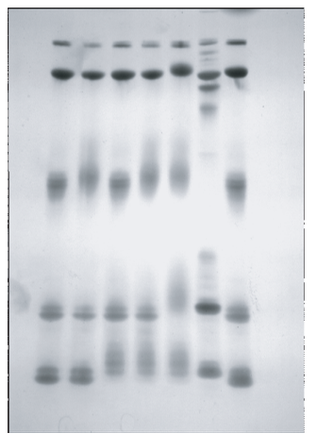
Next step: single pair FRET to access the distribution and kinetics of the linker DNA distances and nucleosome mobility

# In collaboration with

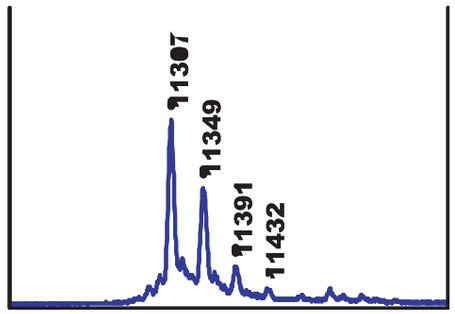
Jörg Langowski
Biophysics of Macromolecules

Nathalie Brun



Anja Resemann
(Bruker Daltonics)




Martina Schnölzer,
Wilma Dormeyer (DKFZ)

Malte Bussiek