Outline

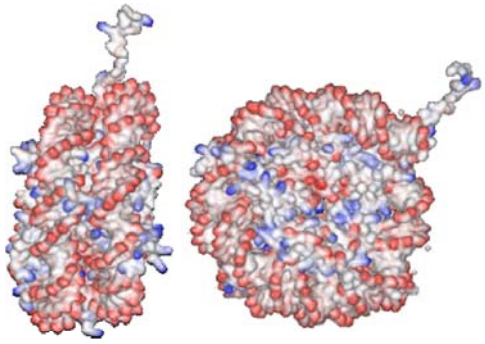
- Modeling the chromatin fiber by a flexible-chain model
- Which chromatin structures can be stable?
- How flexible is chromatin (and does it matter?)
- Unrolling of the DNA from the histone core
- Modelling the nucleosome at intermediate resolution
- How dense is the chromatin fiber in the nucleus?
- Protein-protein interactions in the living cell

dkfz

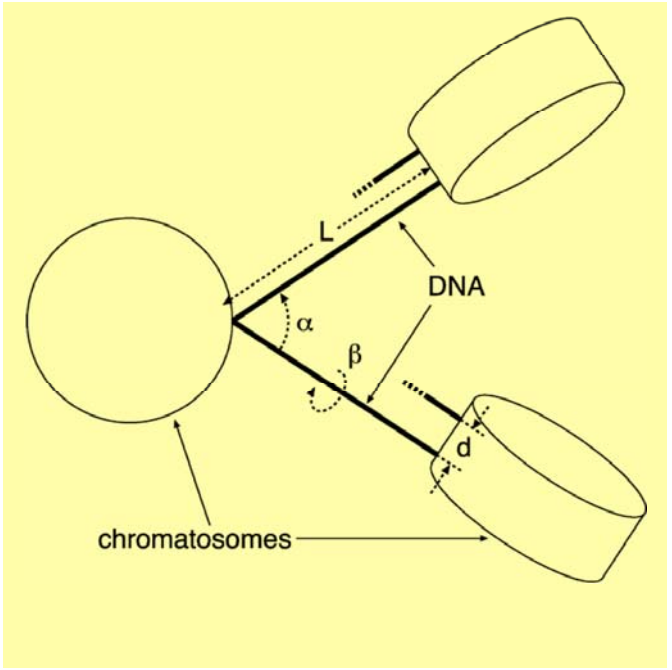
Monte-Carlo model of the chromatin fiber

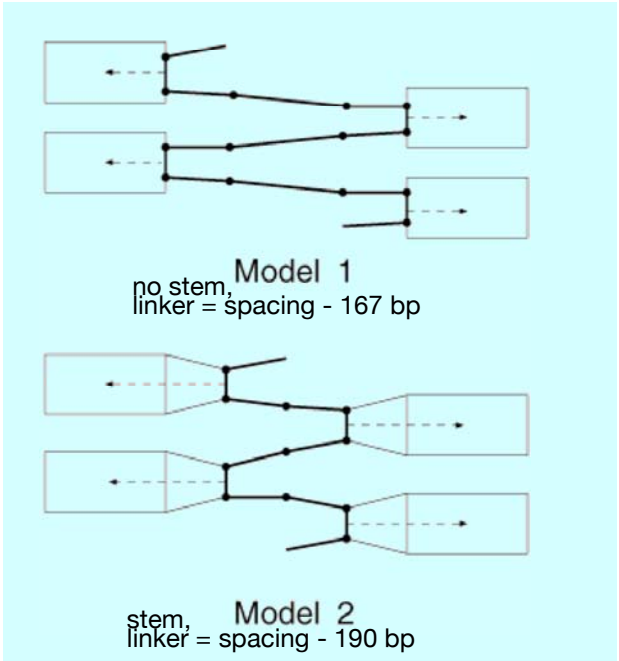
(Wedemann & Langowski, Biophys. J. (2002) 82, 2847-2859

- DNA is approximated as a chain of 10-30 bp segments with known bending, twisting and stretching elasticity
- nucleosomes are modeled as 11.5 * 5.5 nm prolate ellipsoids
- starting conformation either stretched 'bead-on-string' chain or stacked zig-zag



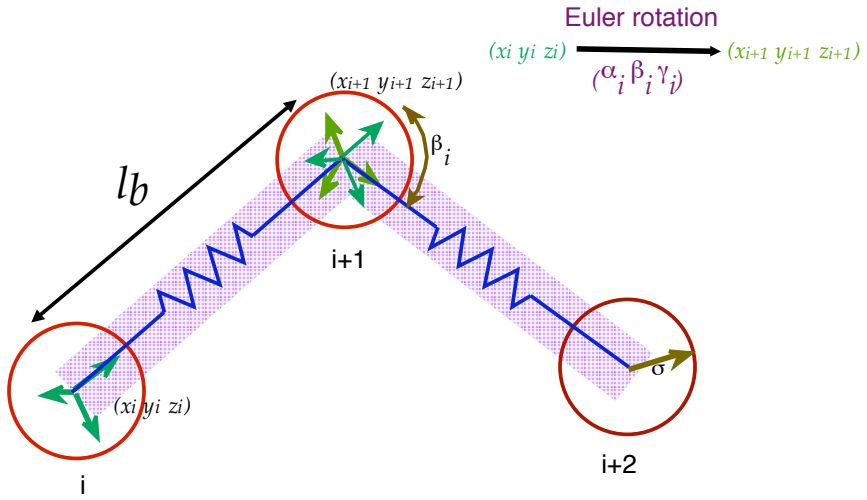
(Luger et al., Nature, 389 (1997) 251)





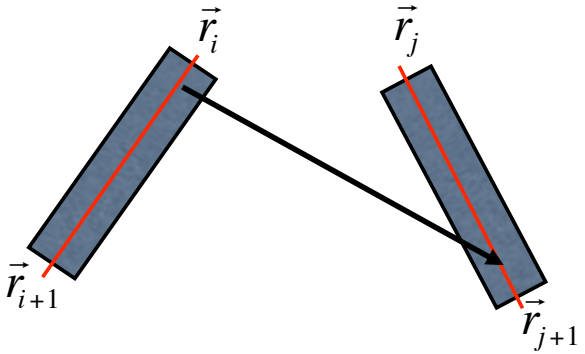
Segmented-chain model of DNA

- DNA segments are:
  - rigid (10-50 bp)
  - connected by harmonic bending, stretching and twisting potentials
  - interact through space by screened Coulomb potential (Debye-Hückel approximation) and hydrodynamic interactions (for the internal motions)



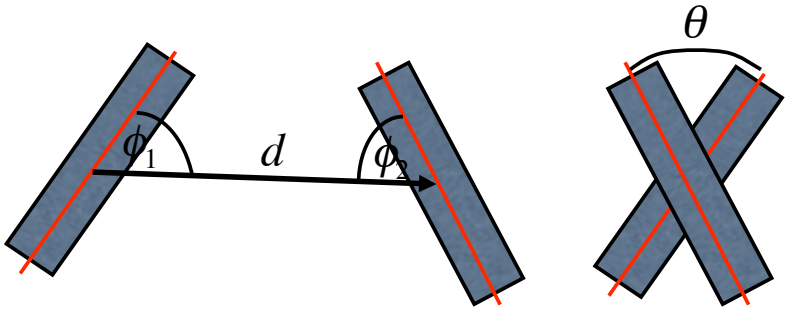
DNA-DNA interaction

- Electrostatic repulsion between backbone phosphates
- Interactions are screened by counterions: Debye-Hückel potential



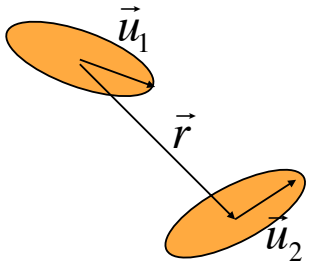
$$E_{ij} = \int_{\vec{r}_i}^{\vec{r}_{i+1}} d\vec{r}_1 \int_{\vec{r}_j}^{\vec{r}_{j+1}} d\vec{r}_2 \frac{e^{-\kappa|\vec{r}_1 - \vec{r}_2|}}{|\vec{r}_1 - \vec{r}_2|}$$

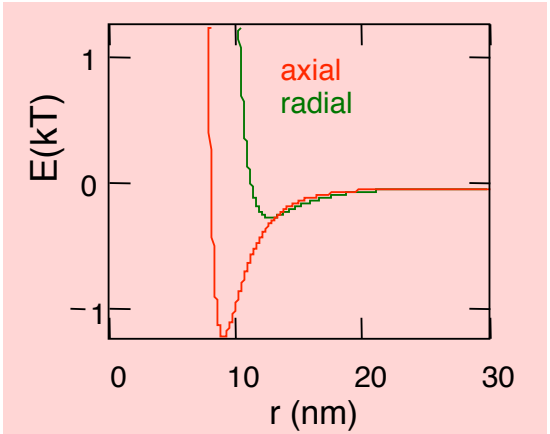
Parameterization in four variables
(through lookup table)

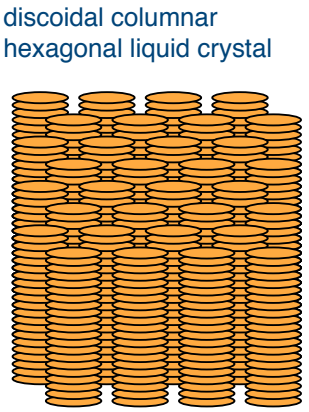


Nucleosome-nucleosome interaction

- Gay-Berne potential: Lennard-Jones type interaction potential between ellipsoids
- Position of potential minimum in radial and axial directions given by equilibrium distances of nucleosomes in the discoidal columnar hexagonal phase (Leforestier & Livolant, 1997)
- Potential depth estimated by comparing of the phase diagram of nucleosome liquid crystals with simulations of a liquid of ellipsoidal particles. Pure discoidal columnar hexagonal phase is only observed for narrow range of potential depths, here E_{min} in radial direction = 0.25 kT, in axial direction = 1.25 kT
- In the simulations, we used E_{min} = 0.1…0.4 kT (no stable chain for smaller values, 'freezing' for larger values)



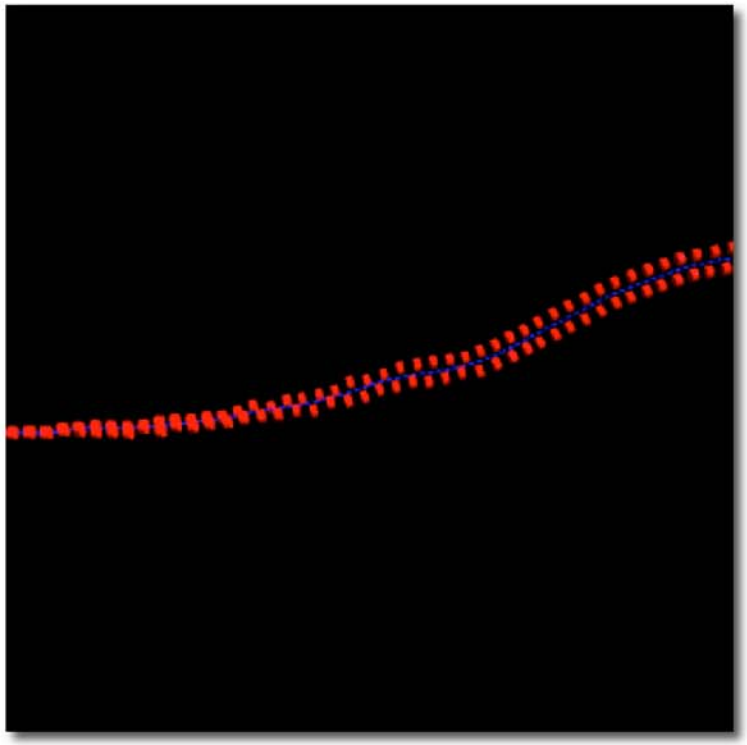




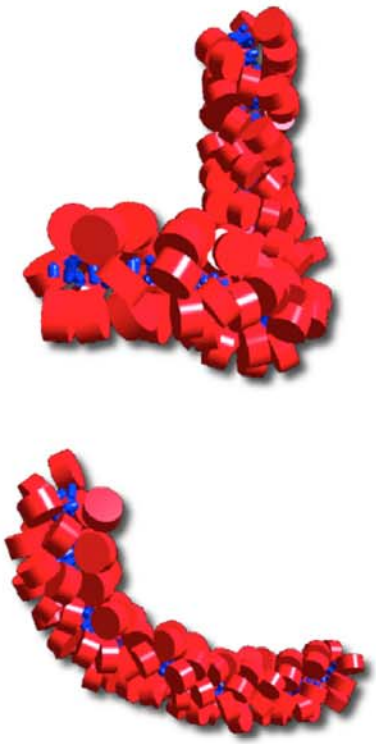
Simulation results

(Wedemann & Langowski, 2002; Aumann et al., 2006)

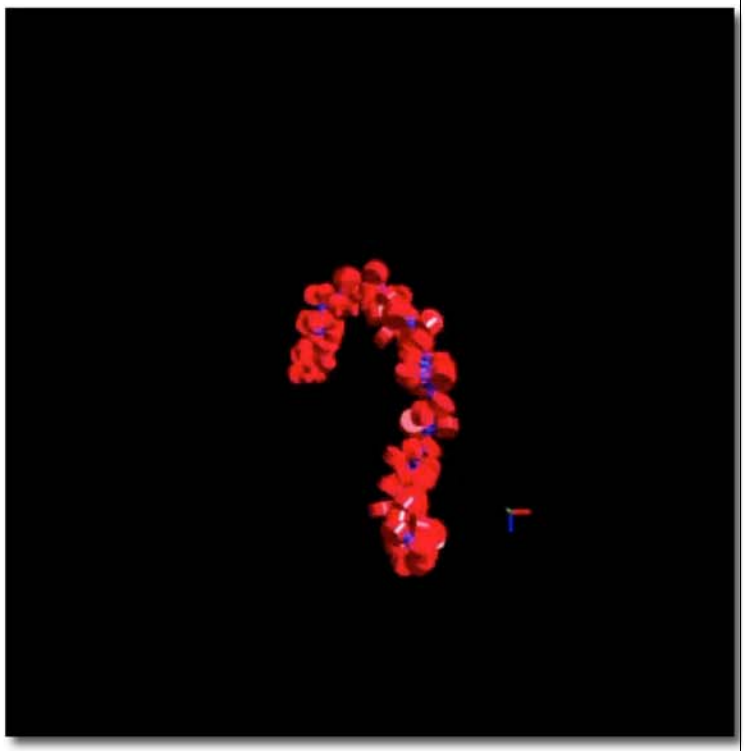
- 100-nucleosome chain, varying linker length, twisting angle, linker opening angle



Condensation from stretched initial state

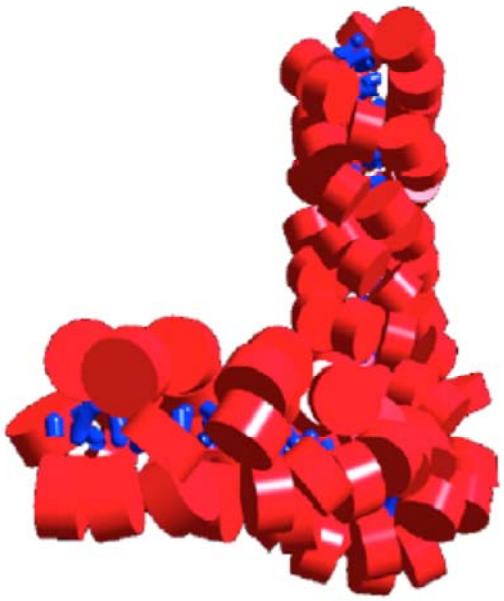


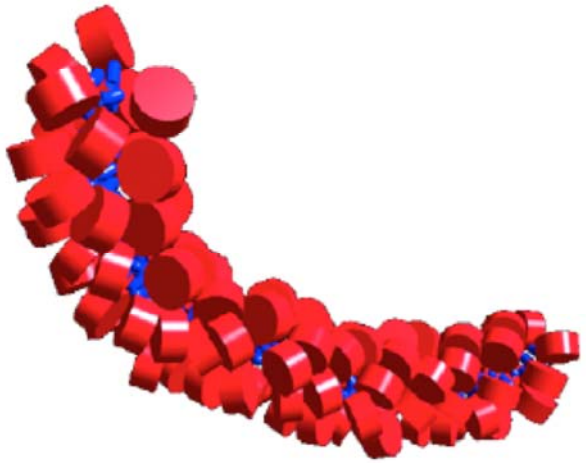
Equilibrium structures



Stretching: 10 pN

Structural parameters of the simulated chromatin fiber





- Linker opening angle α_{eff} = 38°
- Nucleosome twist β_0 = 110°
- Fiber diameter =
  - 32 nm (200 bp, stem)
  - 41 nm (220 bp, stem)
- Linear mass density (ns / 11 nm) =
  - 6.15 (200 bp, stem)
  - 5.5 (175 bp, no stem)
  - 4.5…6.5 (experimental)
  - 4.0 (simulation by Beard & Schlick (2000), 50 bp linker)
- Consecutive nucleosomes are on opposite sides of the fiber (stretched-linker model), the structure forms a two-start helix
- Supported by electron tomography images (Woodcock, Baumeister), and recent tetranucleosome crystallography data (Richmond)

Getting elastic constants from simulated trajectories

A. bending elasticity: persistence length L_p

1. tangent vector $\vec{t}_i = \frac{\vec{c}_i - \vec{c}_{i+1}}{\left|\vec{c}_i - \vec{c}_{i+1}\right|}$ with mass centers **c**, autocorrelation function $\left\langle \vec{t}_i \cdot \vec{t}_j \right\rangle$ decays exponentially with correlation length L_p

2. get L_p from mean squared end-to-end distance: $\left\langle R^2 \right\rangle = 2L_p^2 \left(\frac{L_0}{L_p} - 1 + \exp\left(-\frac{L_0}{L_p} \right) \right)$ (contour length L_0)

B. stretching elasticity: elastic modulus ϵ

1. chain energy E vs. total length L, apply Hooke's law (L_0 is equilibrium length):

$$E_{str} = \frac{1}{2} \frac{YA}{L_0} \left(L - L_0\right)^2 = \frac{1}{2} \frac{\varepsilon}{L_0} \left(L - L_0\right)^2 = \frac{1}{2} D \left(L - L_0\right)^2$$

2. get $\varepsilon = D \cdot L_0$ from length fluctuations: $D = \frac{kT}{\left\langle L^2 \right\rangle - L_0^2}$

Spring constant vs. persistence length

- Assuming that the chromatin chain is homogeneous (like a rubber tube), one can directly estimate its bending flexibility from the stretching elasticity:

Hooke's law: $F = D \cdot \Delta l$

Persistence length from cross-section of elastic material: $L_p = \frac{E \cdot I}{k\,T}$

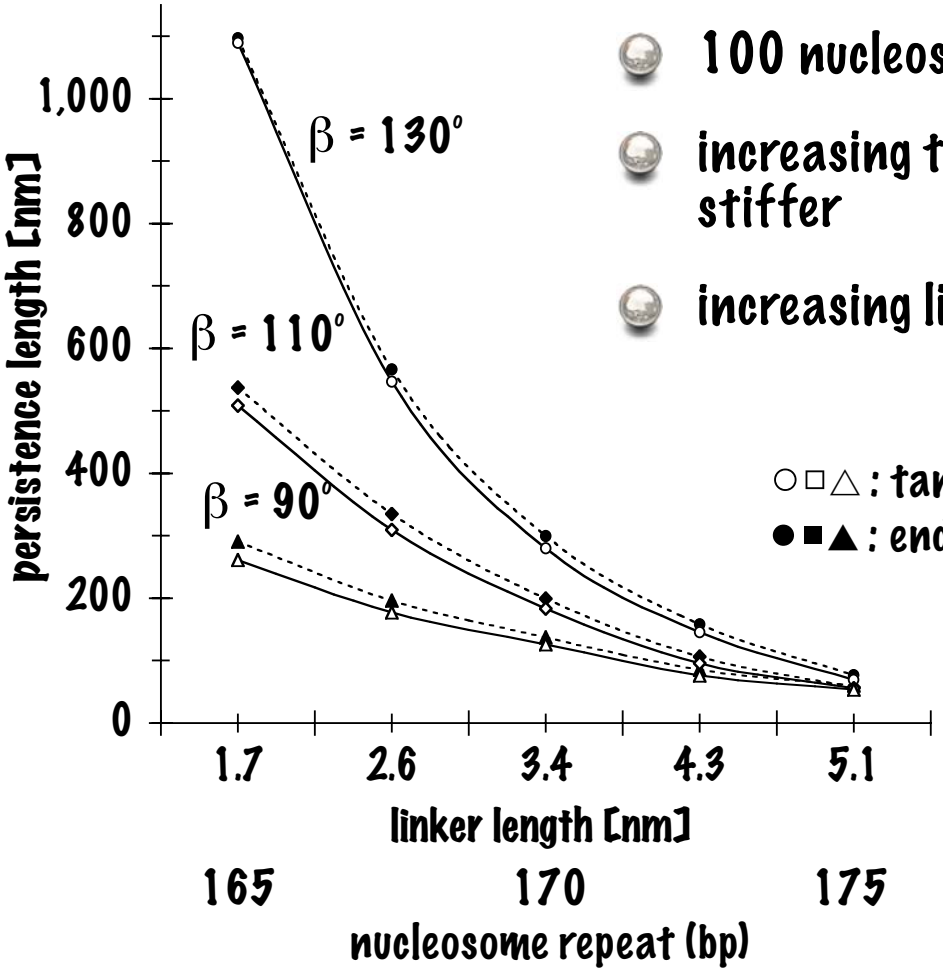
$$F = E \cdot A \cdot \frac{\Delta l}{L_0}$$

i.e., the persistence length of a homogeneous elastic filament with radius R is directly connected to its spring constant D:

$$L_p = \frac{1}{k\,T}\,\frac{F}{A}\,\frac{L_0}{\Delta l}\,I \xrightarrow{I = \frac{1}{4}\pi R^4} L_p = \frac{1}{4}\,\frac{D\,L_0}{k\,T}\,R^2$$

Cross section moment of inertia of a homogeneous cylinder

Chromatin persistence length depends on internucleosome geometry

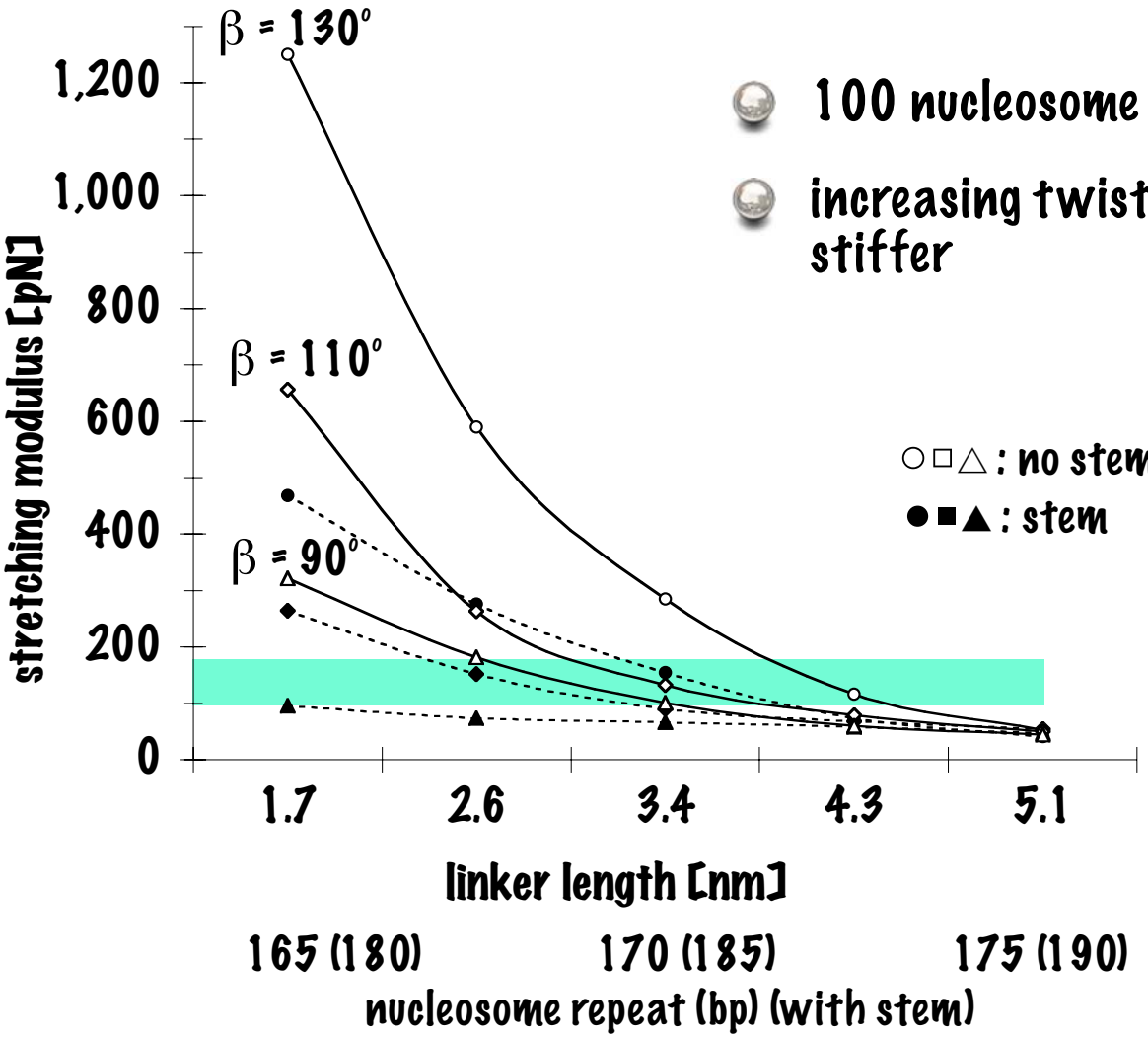


- 100 nucleosome chain, no stem
- increasing twisting angle makes chain stiffer
- increasing linker length makes chain softer

○□△ : tangent angle fluctuations

●■▲ : end to end distances

Chromatin stretching elasticity depends on internucleosome geometry



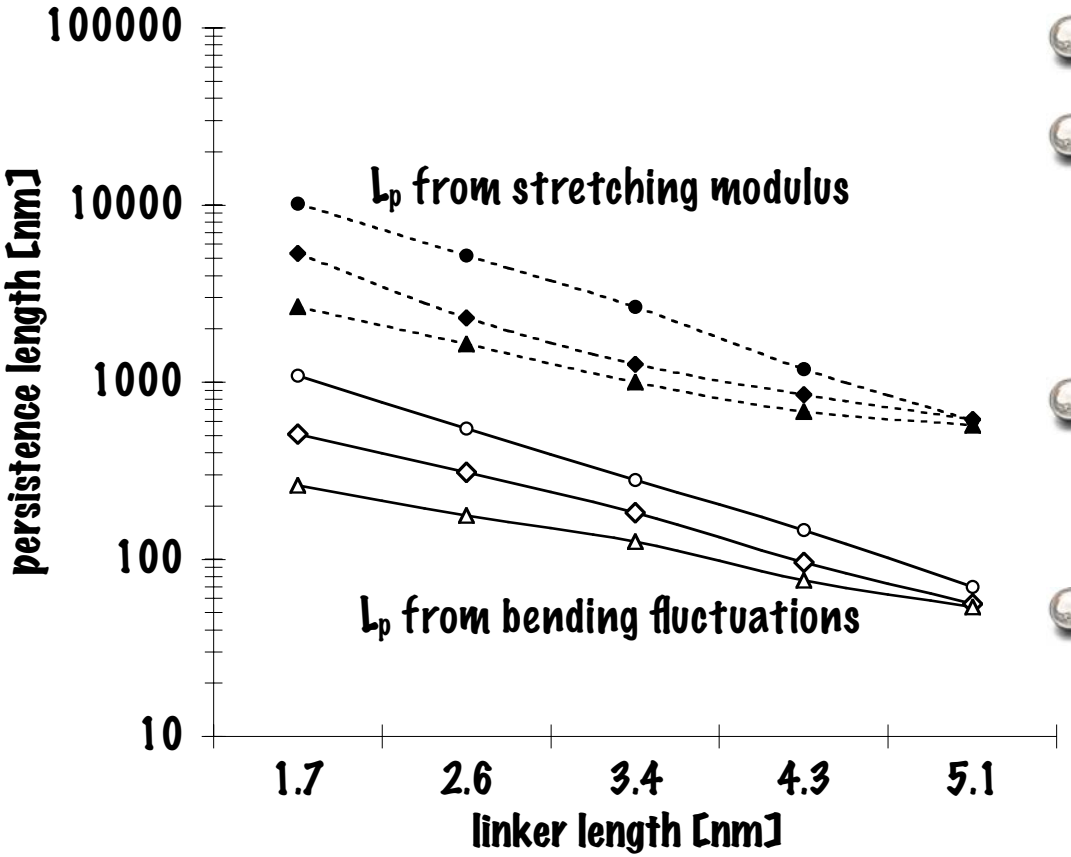
- 100 nucleosome chain, stem vs. no stem
- increasing twisting angle makes chain stiffer

○□△ : no stem

●■▲ : stem

typical experimental values for 180-200 bp repeat corresponding to 2-6 nm linker (with stem)

Chromatin is much more easily bent than stretched



- 100 nucleosome chain, no stem
- persistence length for a hypothetical uniform elastic fiber may be calculated from stretching modulus
- persistence length calculated from bending fluctuations is much smaller ->
- chromatin fiber is anisotropic

Internucleosome potential including histone tails and salt effects

(F. Aumann)

Attractive potential including effects of histone tails and salt dependence (Mangenot et al.)

$$E_{tot} = E_{bend} + E_{stretch} + E_{torsion} + E_{Debye\text{-}Hückel} + E_{Gay-Berne} + E_{pull}$$

| mechanical | DNA-DNA | Nuc-Nuc | stretching

Mangenot et al.:

E_{tails} + E_{electr} + $E_{sphere.}$

attr. | repulsive

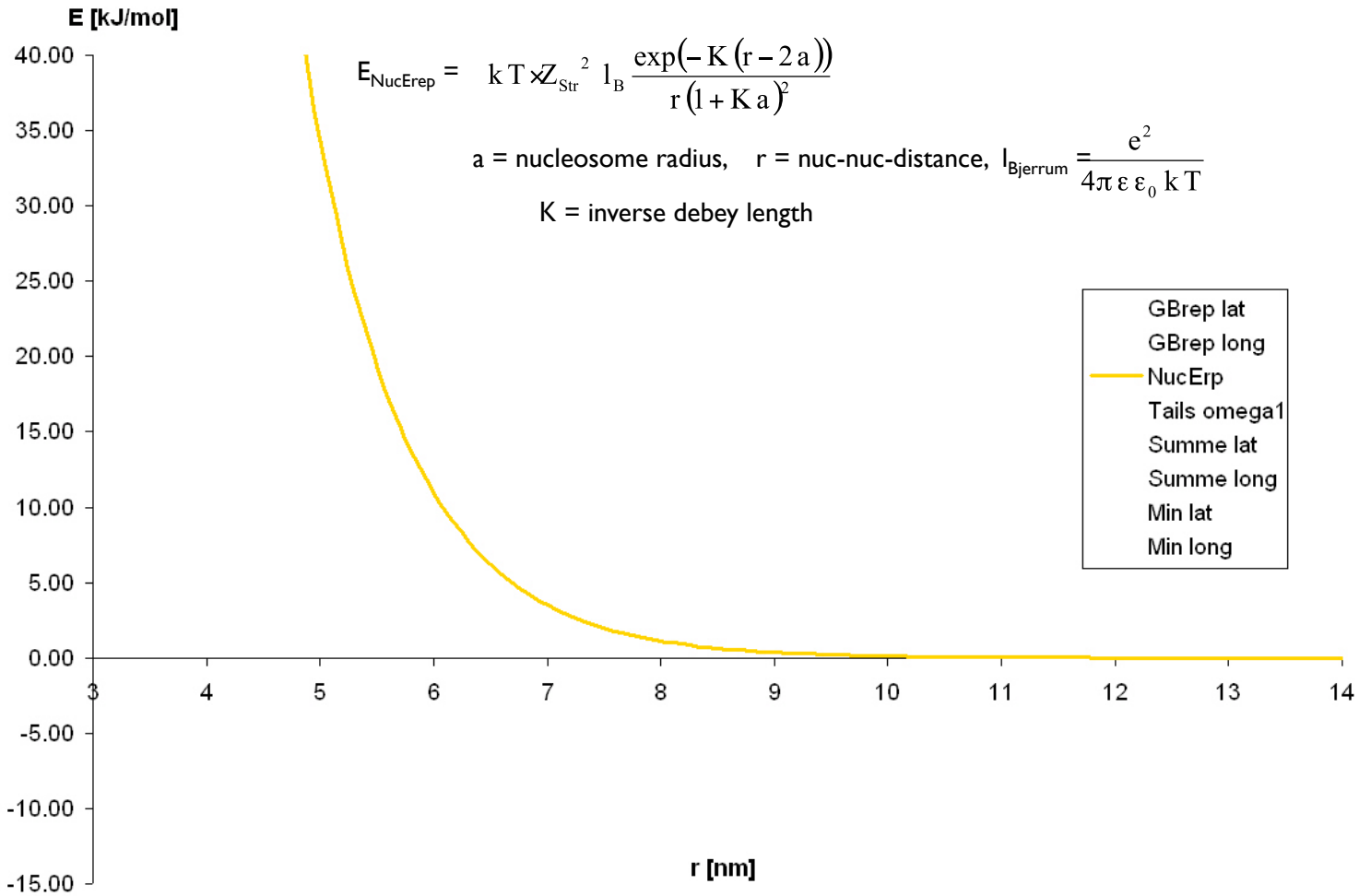
Modification for flat cylinders:

E_{tails} + E_{electr} + $E_{Gay\text{-}Berne\text{-}repulsive}$

attr. | repulsive

Problem: calibration and adjustment of the parameters

Energy vs nucleosome-nucleosome distance

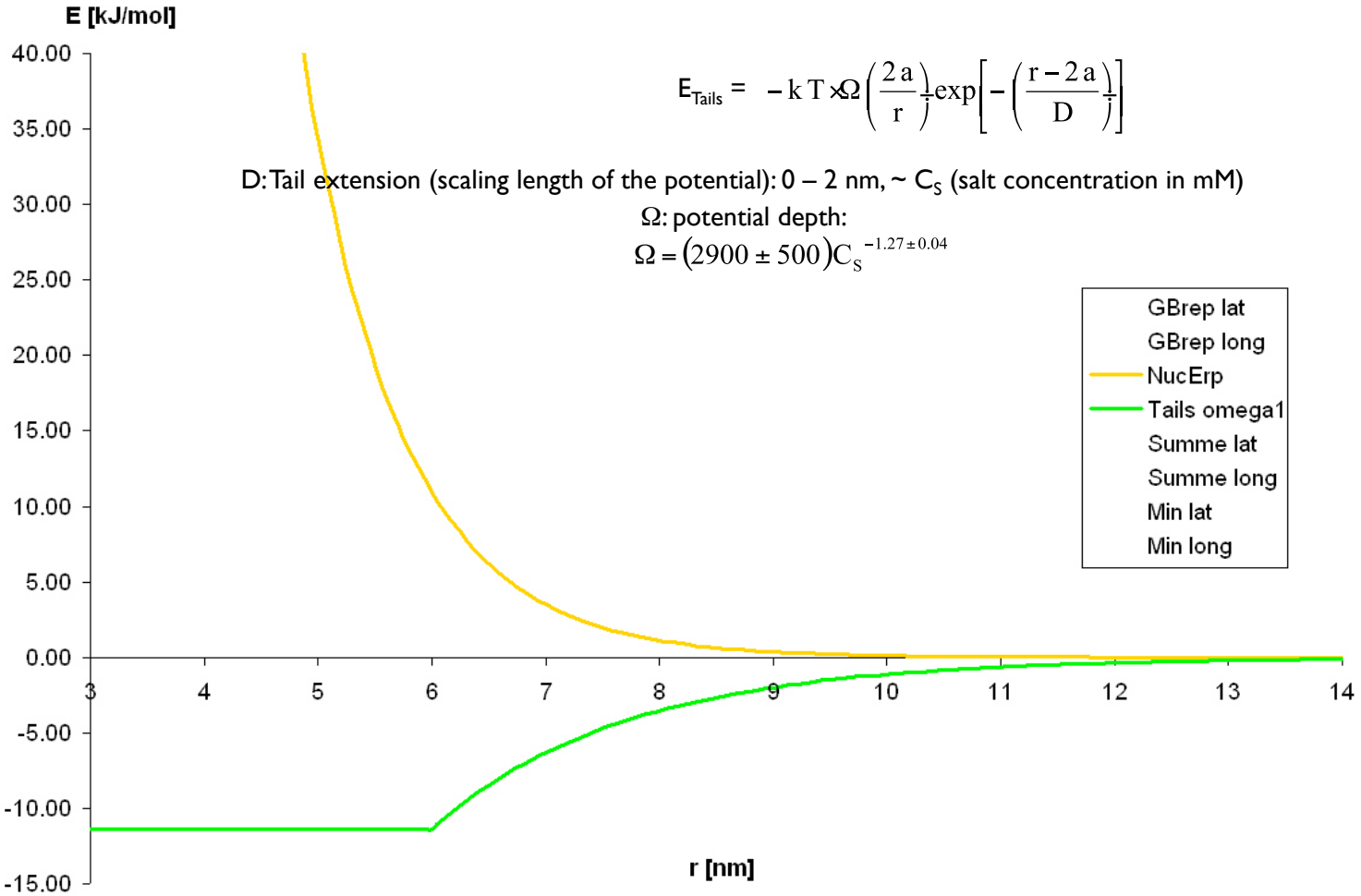


$$E_{NucErep} = k\,T \times Z_{Str}^{\,2}\; l_B \frac{\exp(-K(r-2a))}{r(1+Ka)^2}$$

a = nucleosome radius, r = nuc-nuc-distance, $l_{Bjerrum} = \frac{e^2}{4\pi\,\varepsilon\,\varepsilon_0\,k\,T}$

K = inverse debey length

Energy vs nucleosome-nucleosome distance



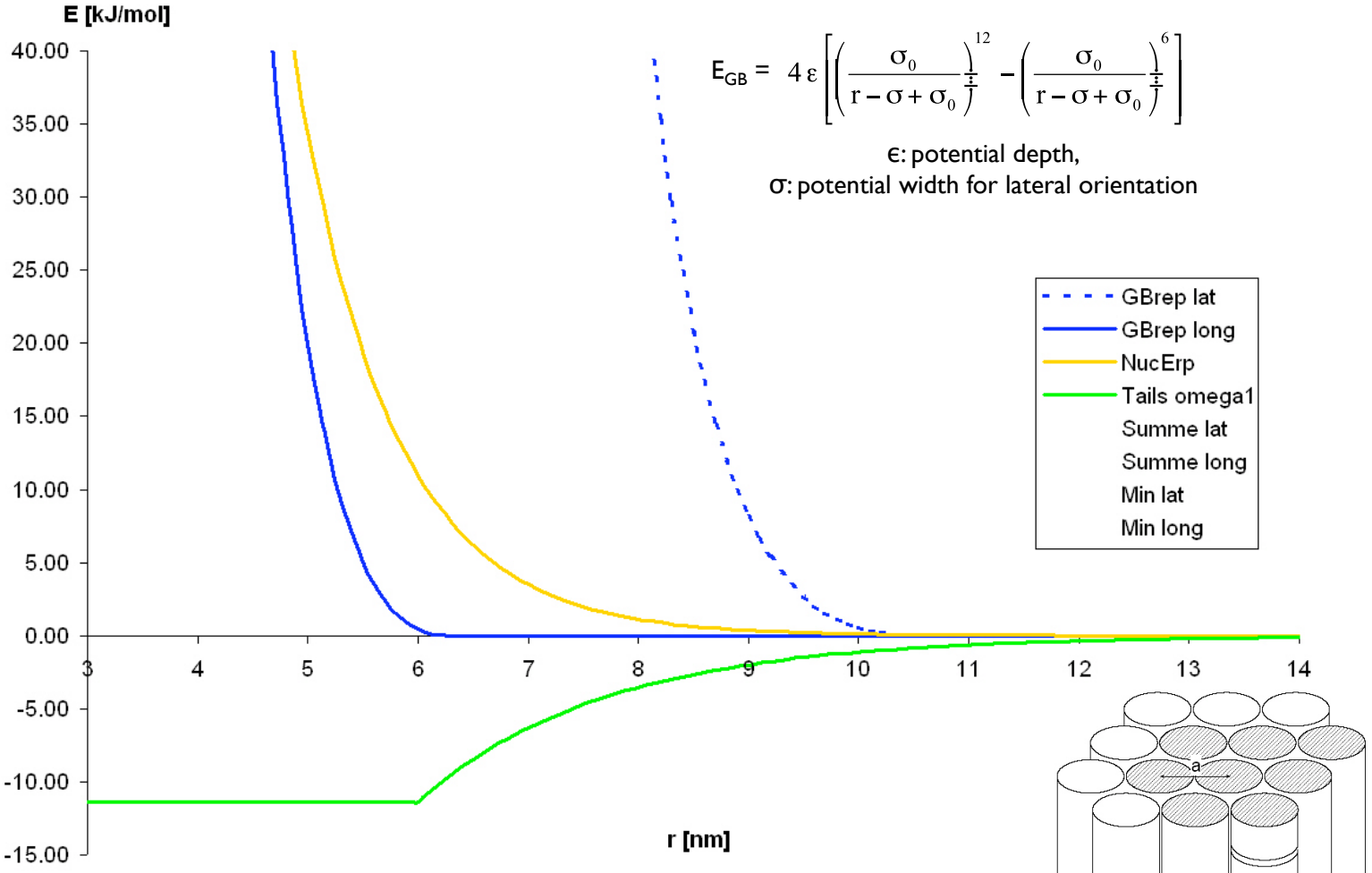
$$E_{Tails} = -kT \times \Omega \left(\frac{2a}{r}\right) \exp\left[-\left(\frac{r-2a}{D}\right)\right]$$

D: Tail extension (scaling length of the potential): 0 – 2 nm, ~ C_S (salt concentration in mM)

Ω: potential depth:

$$\Omega = (2900 \pm 500) C_S^{-1.27 \pm 0.04}$$

Energy vs nucleosome-nucleosome distance

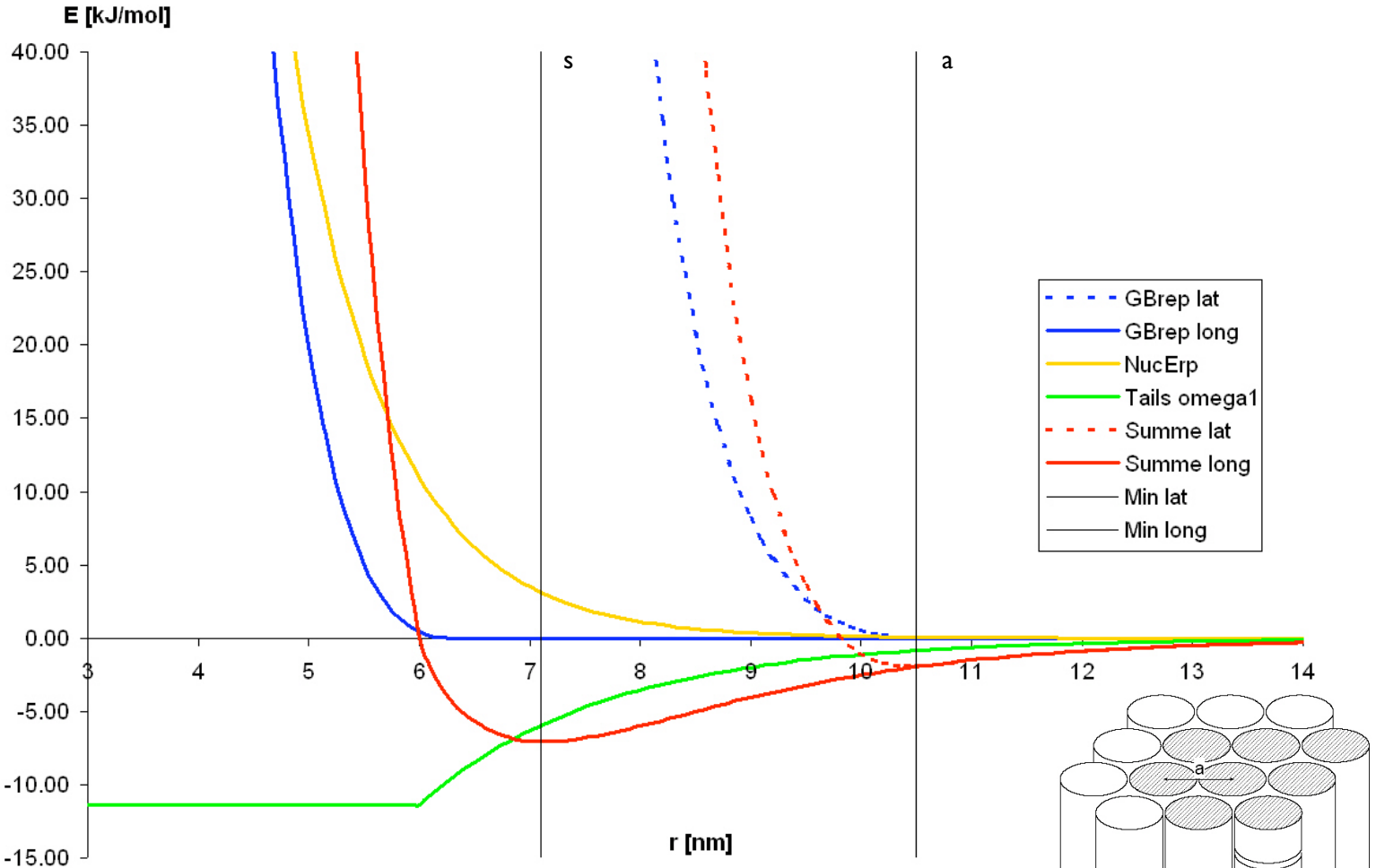


$$E_{GB} = 4\varepsilon\left[\left(\frac{\sigma_0}{r-\sigma+\sigma_0}\right)^{\frac{12}{j}} - \left(\frac{\sigma_0}{r-\sigma+\sigma_0}\right)^{\frac{6}{j}}\right]$$

ϵ: potential depth,
σ: potential width for lateral orientation

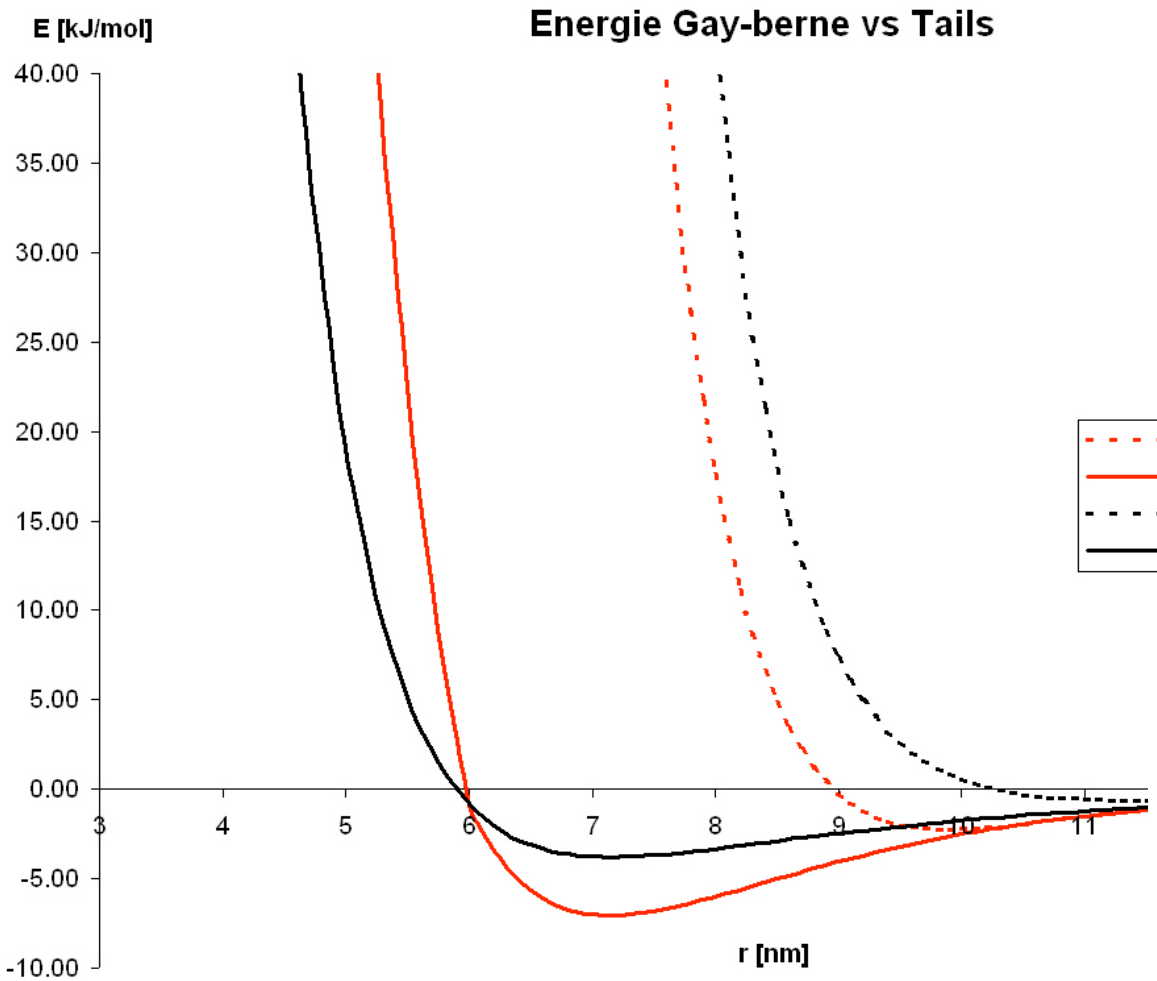
Liquid crystalline phase of nucleosmes (Leforestier et. al)

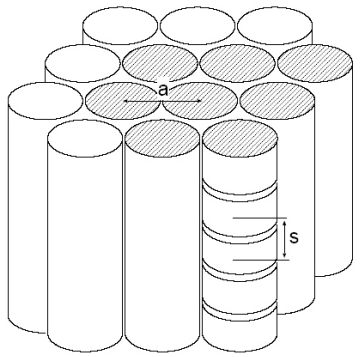
Energy vs nucleosome-nucleosome distance



Liquid crystalline phase of nucleosmes (Leforestier et. al)

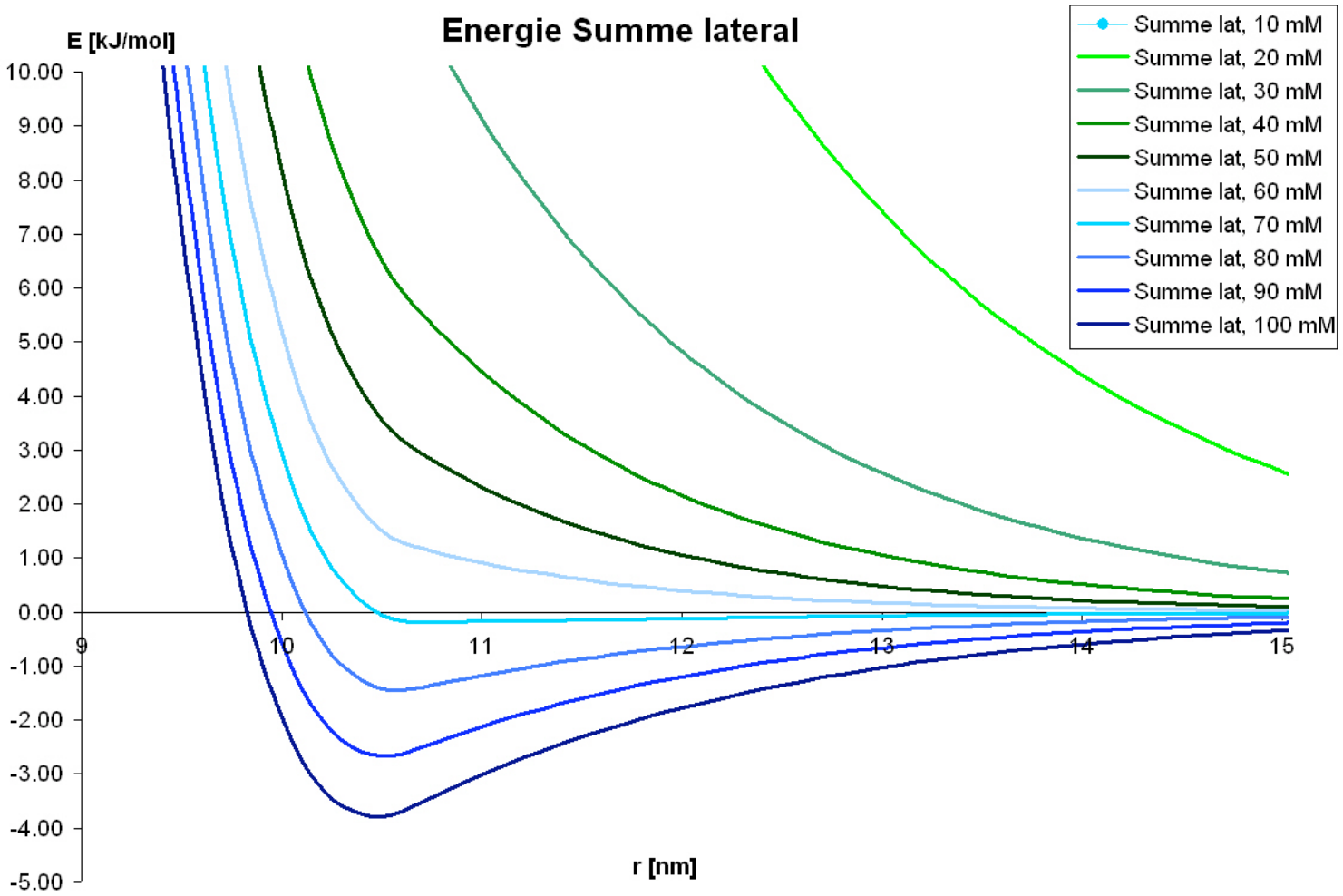
Energy vs nucleosome-nucleosome distance



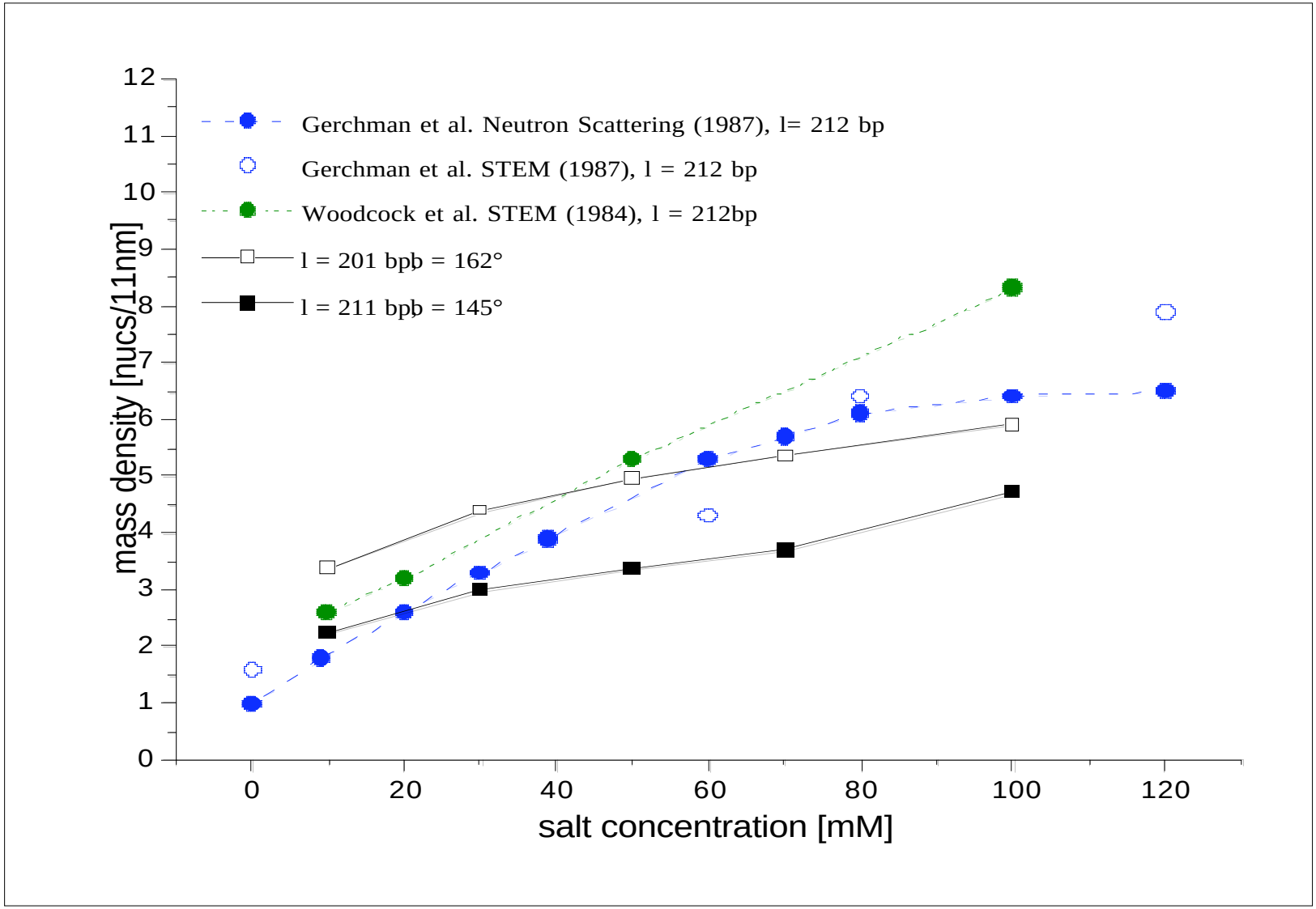


Liquid crystalline phase of nucleosmes (Leforestier et. al)

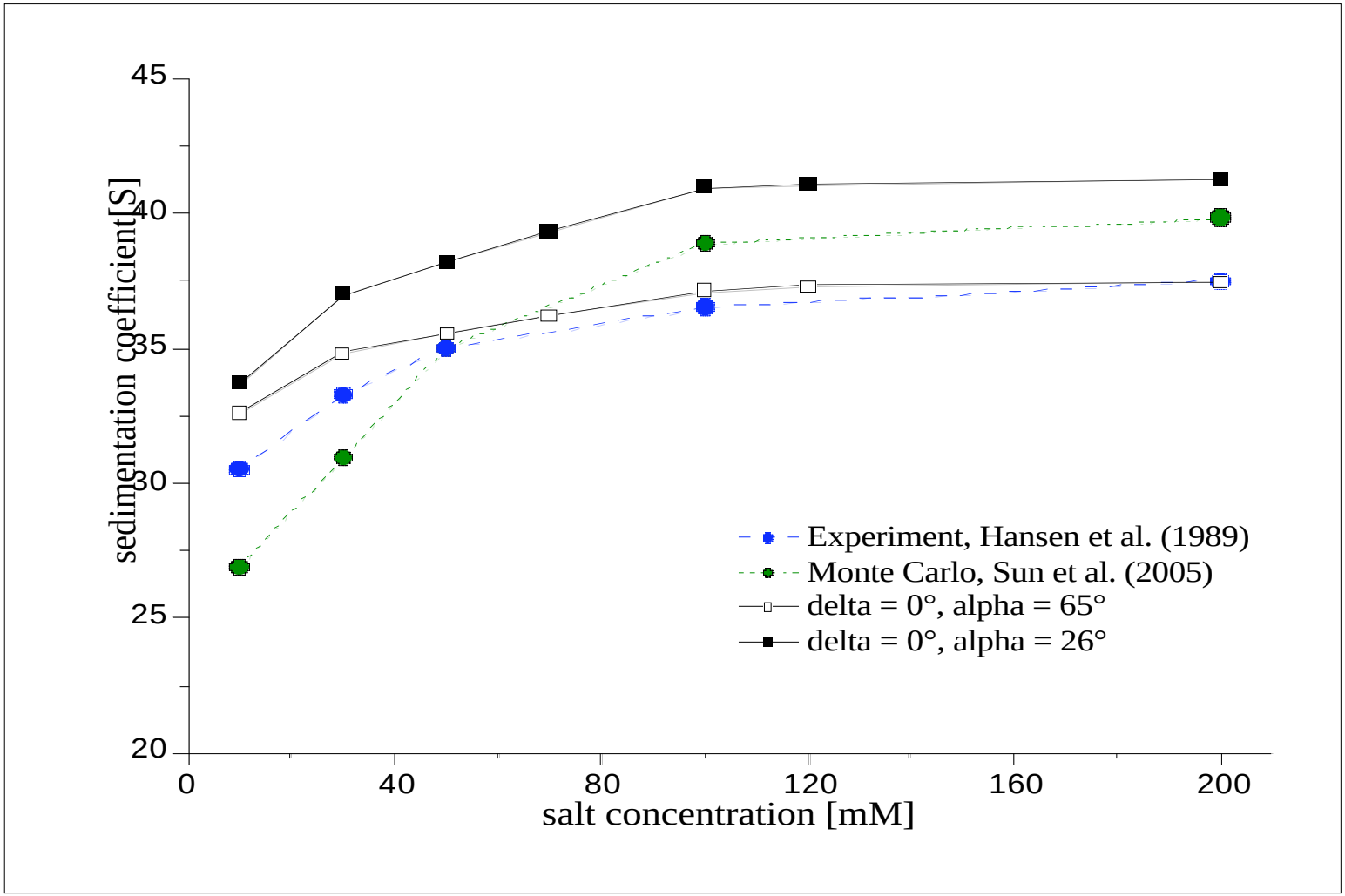
Energy vs nucleosome-nucleosome distance



Chromatin linear mass density increases with salt concentration



Sedimentation coefficient of dodecanucleosome increases with salt concentration

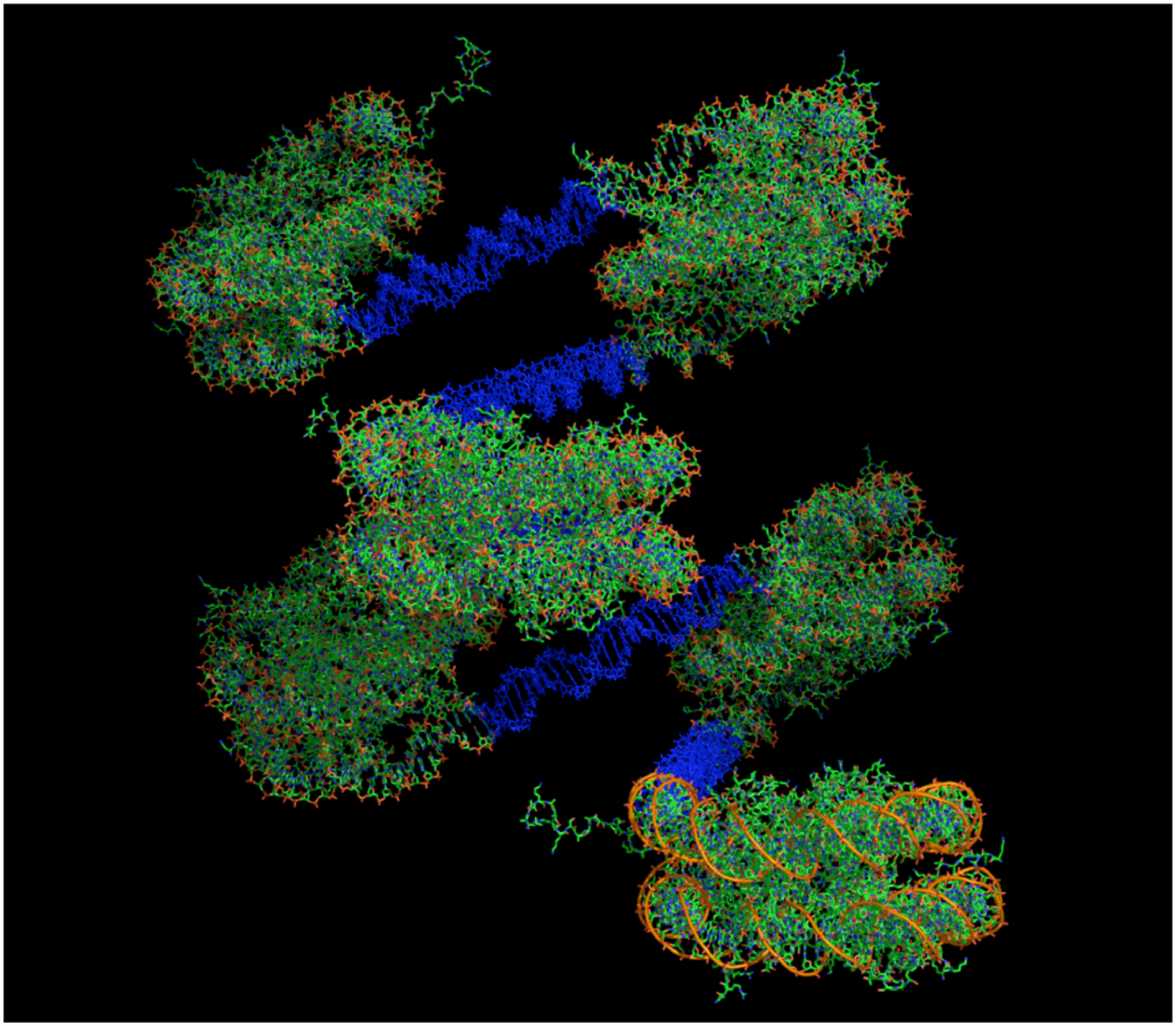


What nucleosome repeats are stable?

1.9Å X-ray structure (Davey et al, 2002)

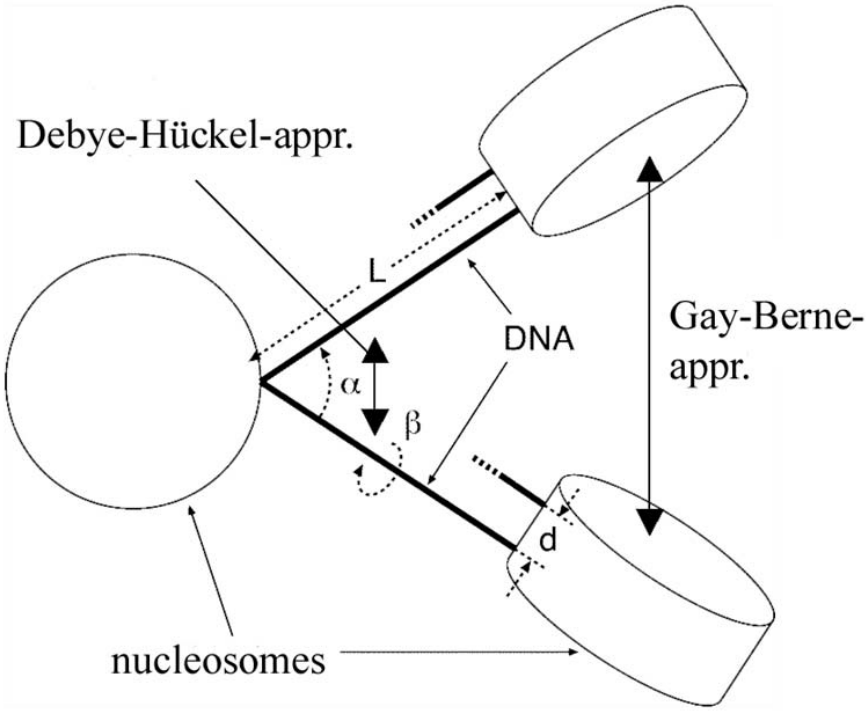
l = 27 bp

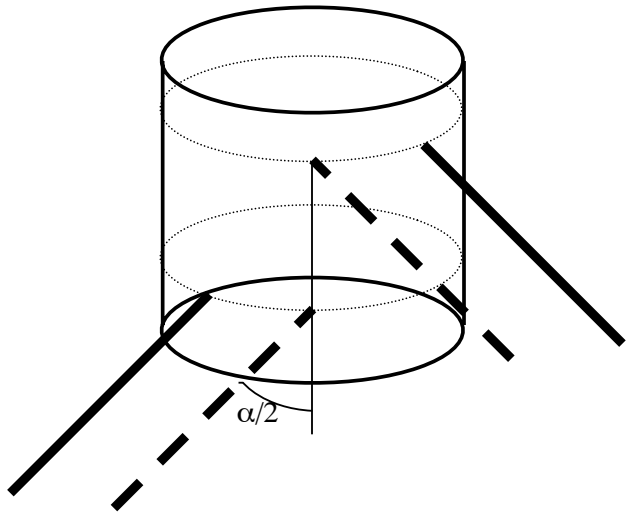
no stem



Nucleosome geometry in the model

Objective: Thermodynamic equilibration of geometrically generated structures and investigating their physical parameters.





α: angle between incoming and leaving DNA L: length of the linker-DNA

β: angle of twisting between two succeeding nucleosomes

Simulation setup

δ = 180°

α = 0°

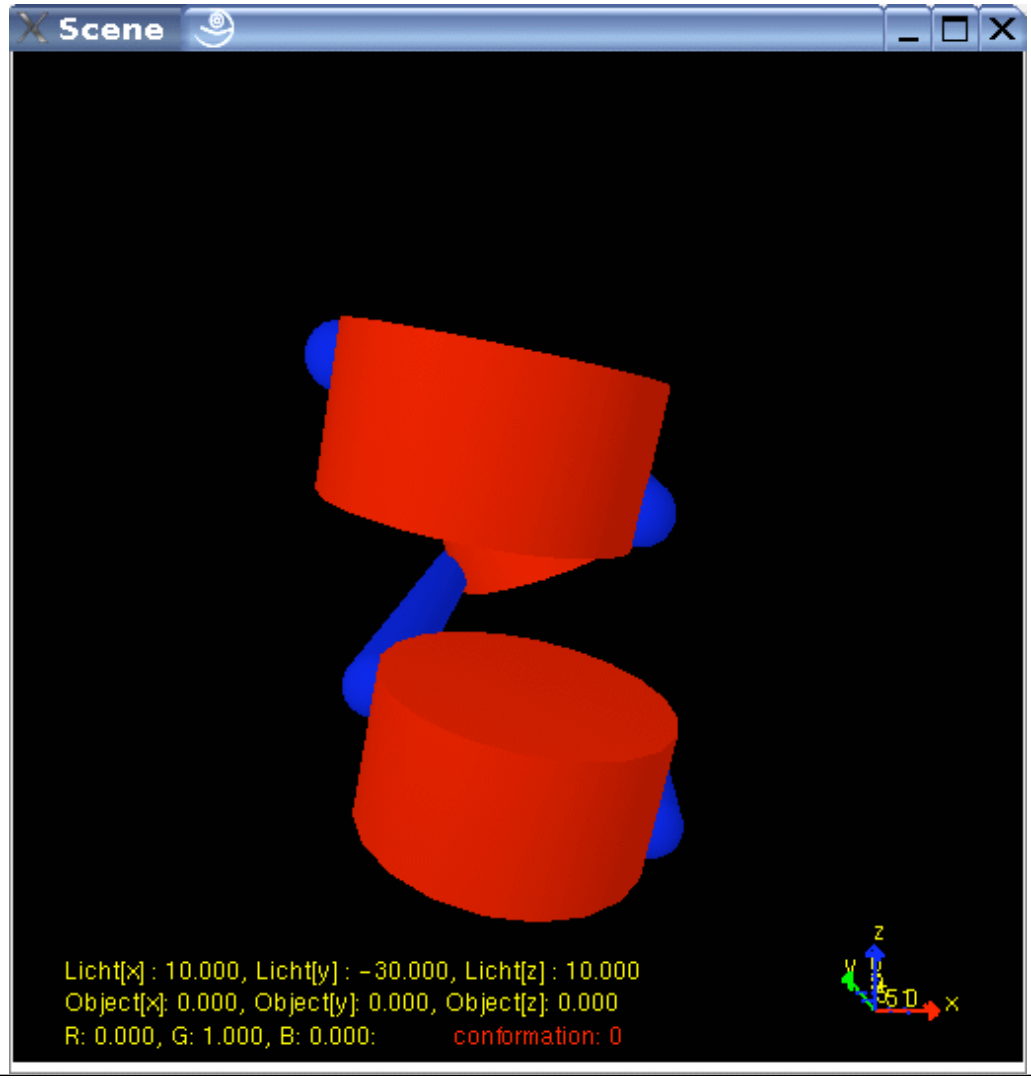
no stem

100 mM salt concentration

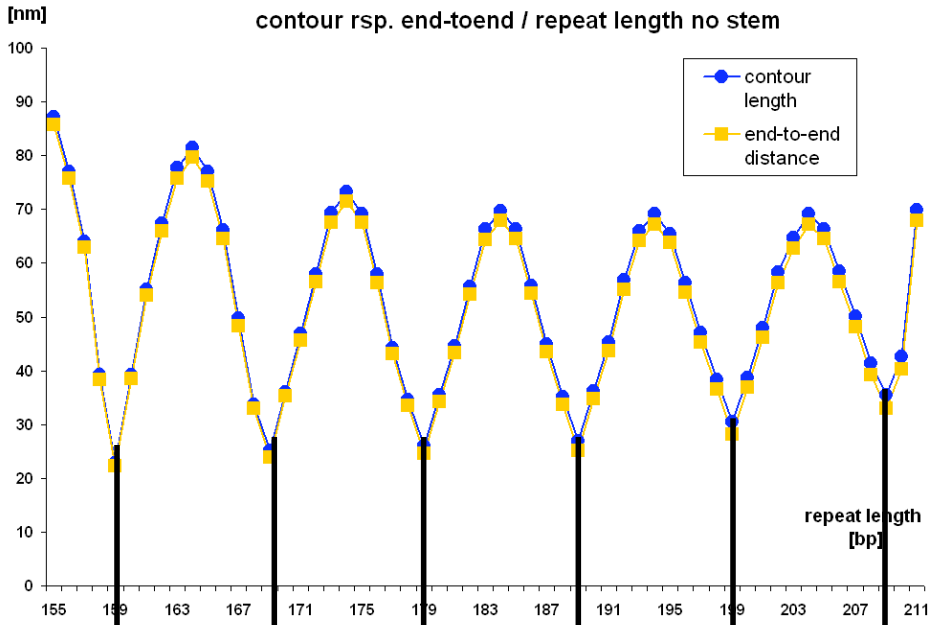
Number of nucleosomes:

26, 100 (Gay-Berne potential)

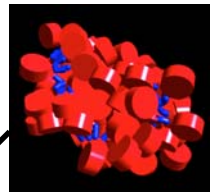
26 (Tails-Potential)

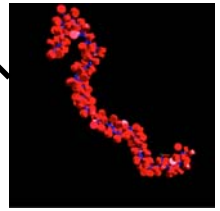


Simulation: results

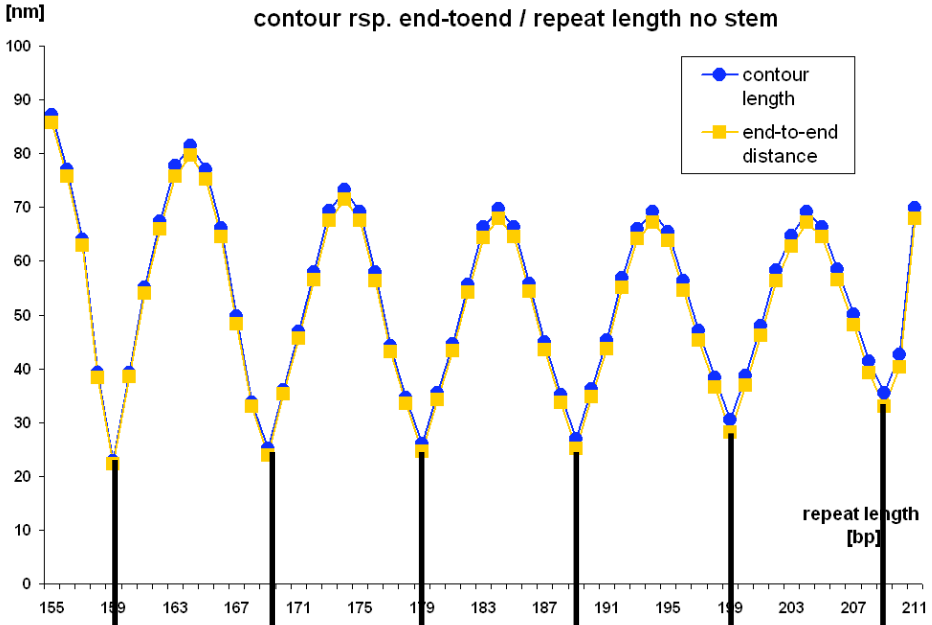


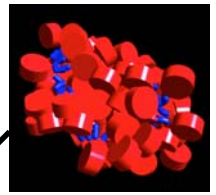
- Conformations for n*10+9 bp are unstable.
- Conformations for n*10+0 bp are stable.

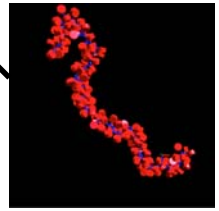




Simulation: results



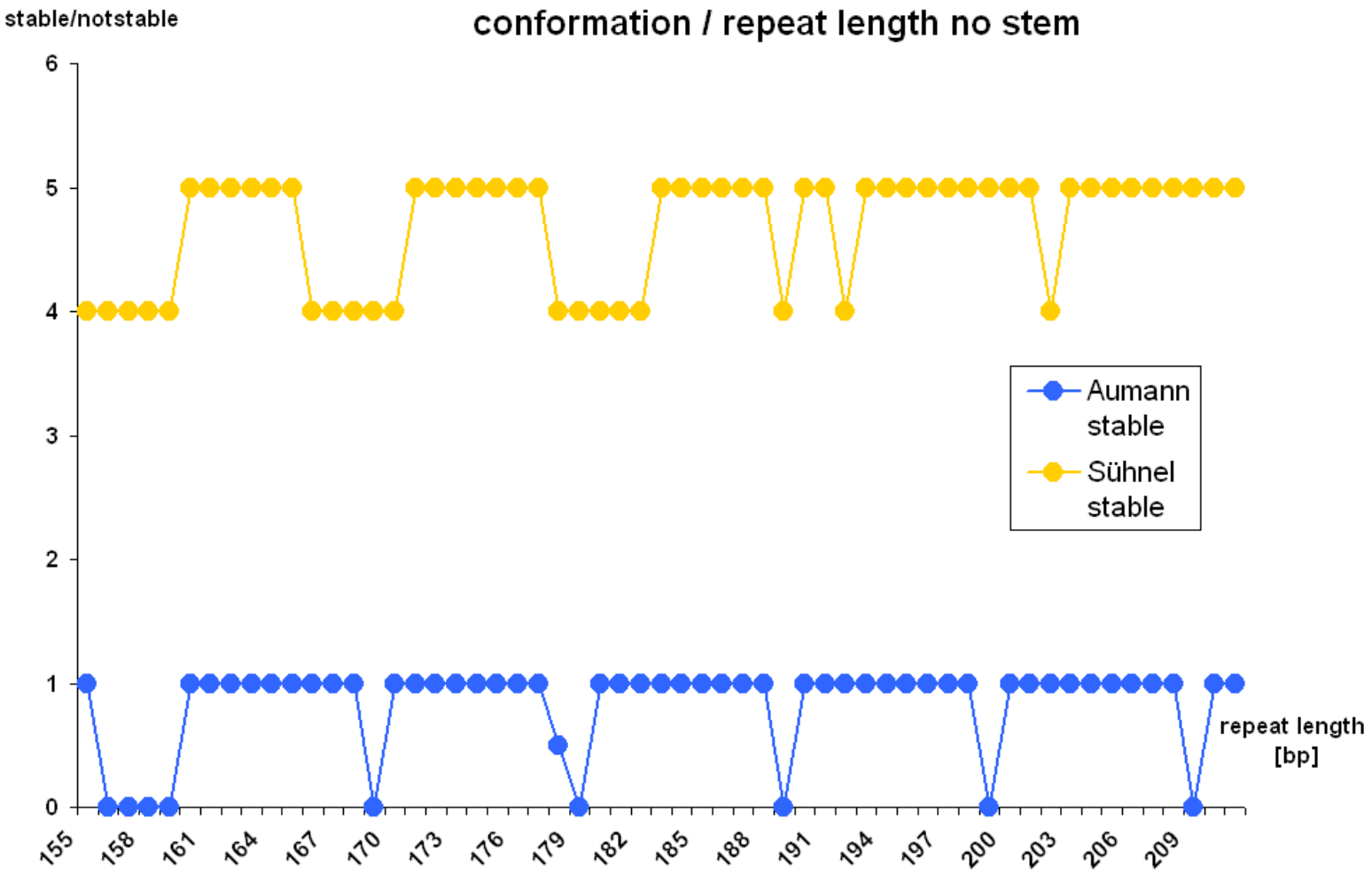




- Conformations for n*10+9 bp are unstable.
- Conformations for n*10+0 bp are stable.

Stability of chromatin fibers of varying repeat

$\alpha = 0°, \delta = 180°$

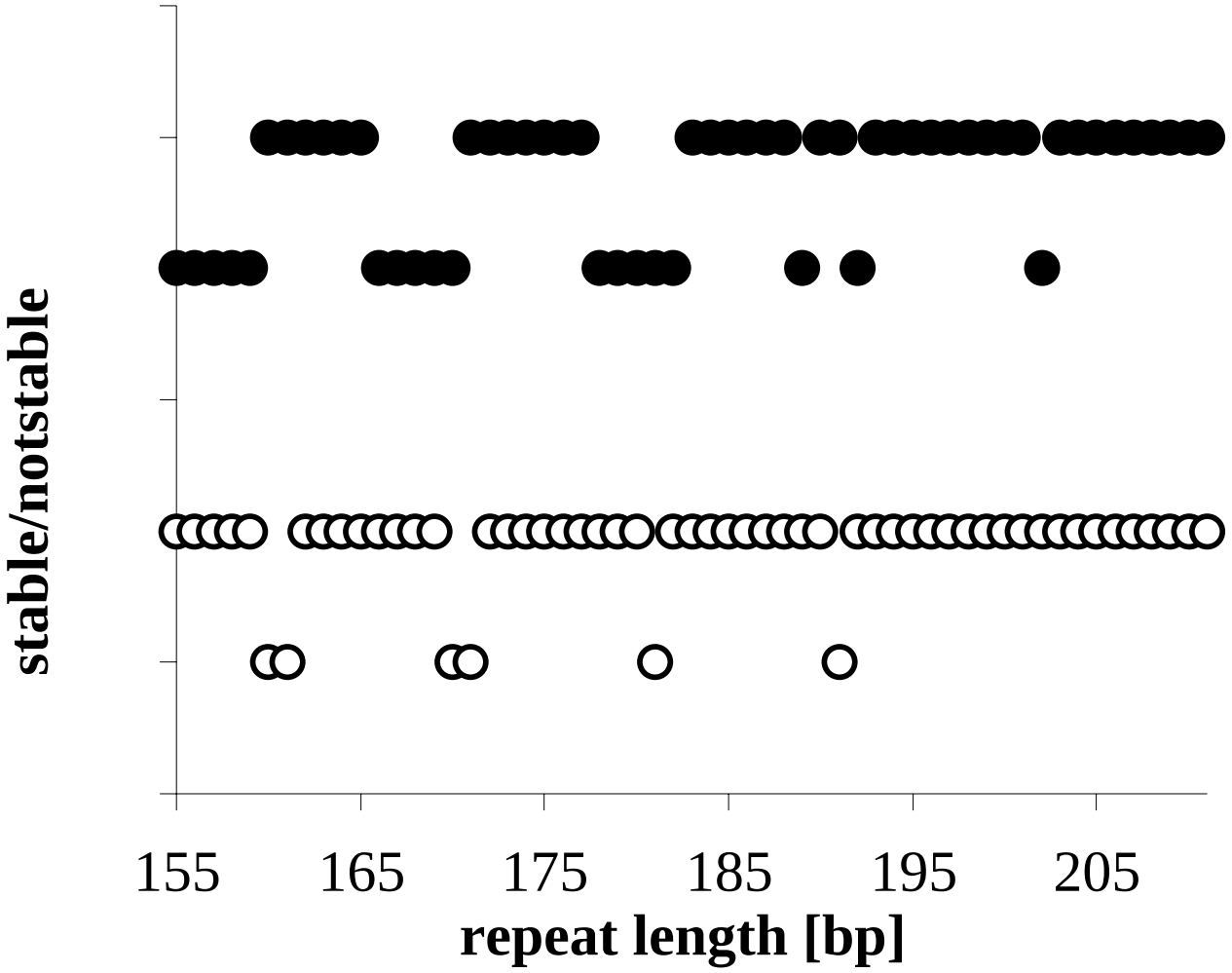


The unstable conformations we found at n*10 + 9 bp agree with those from simple steric analysis

Most of the conformations found unstable from steric analysis are still possible due to thermal fluctuations.

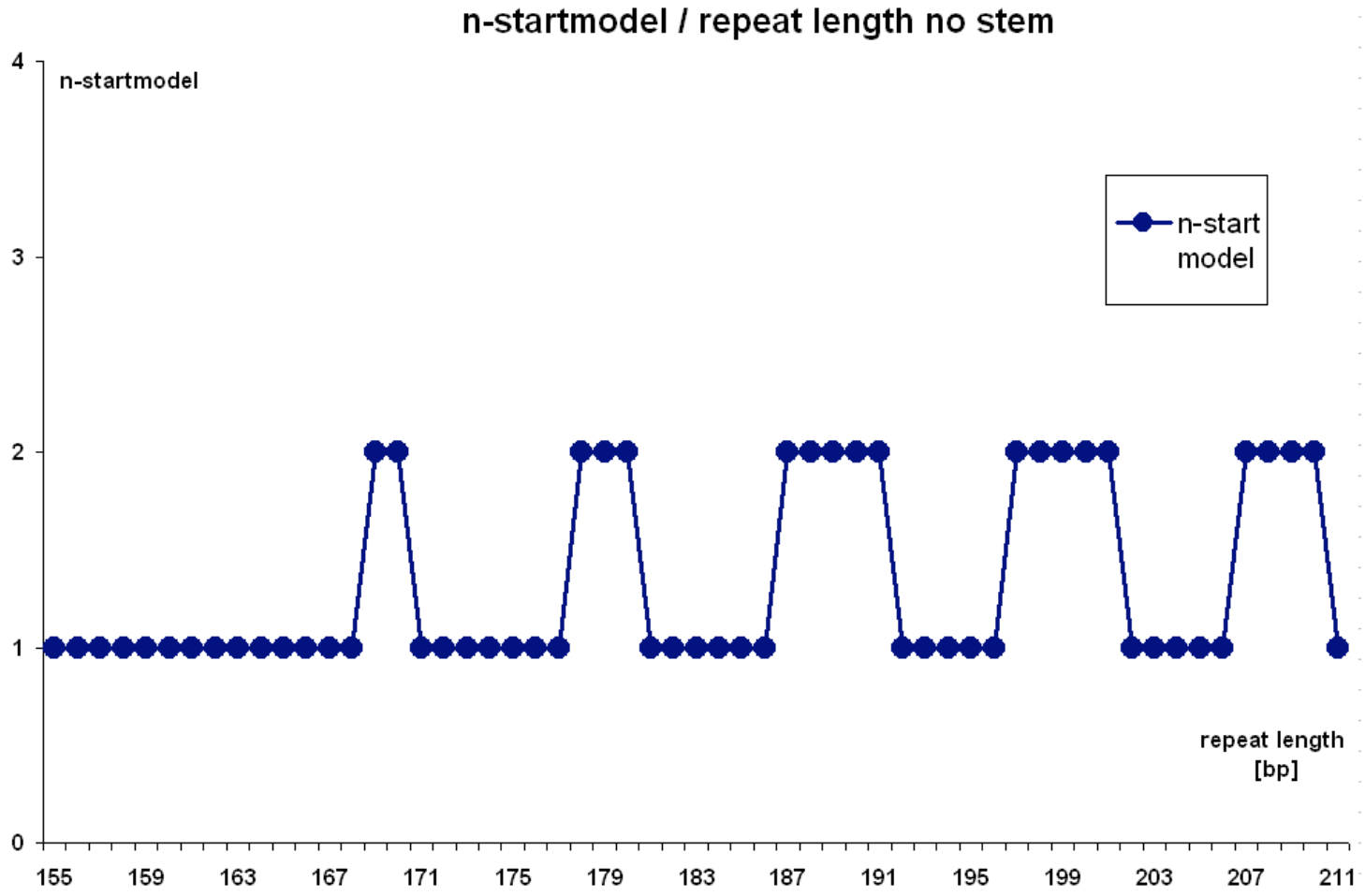
Stability of chromatin fibers of varying repeat

$\alpha = 60°, \delta = 120°$



Analysis of helix repeats

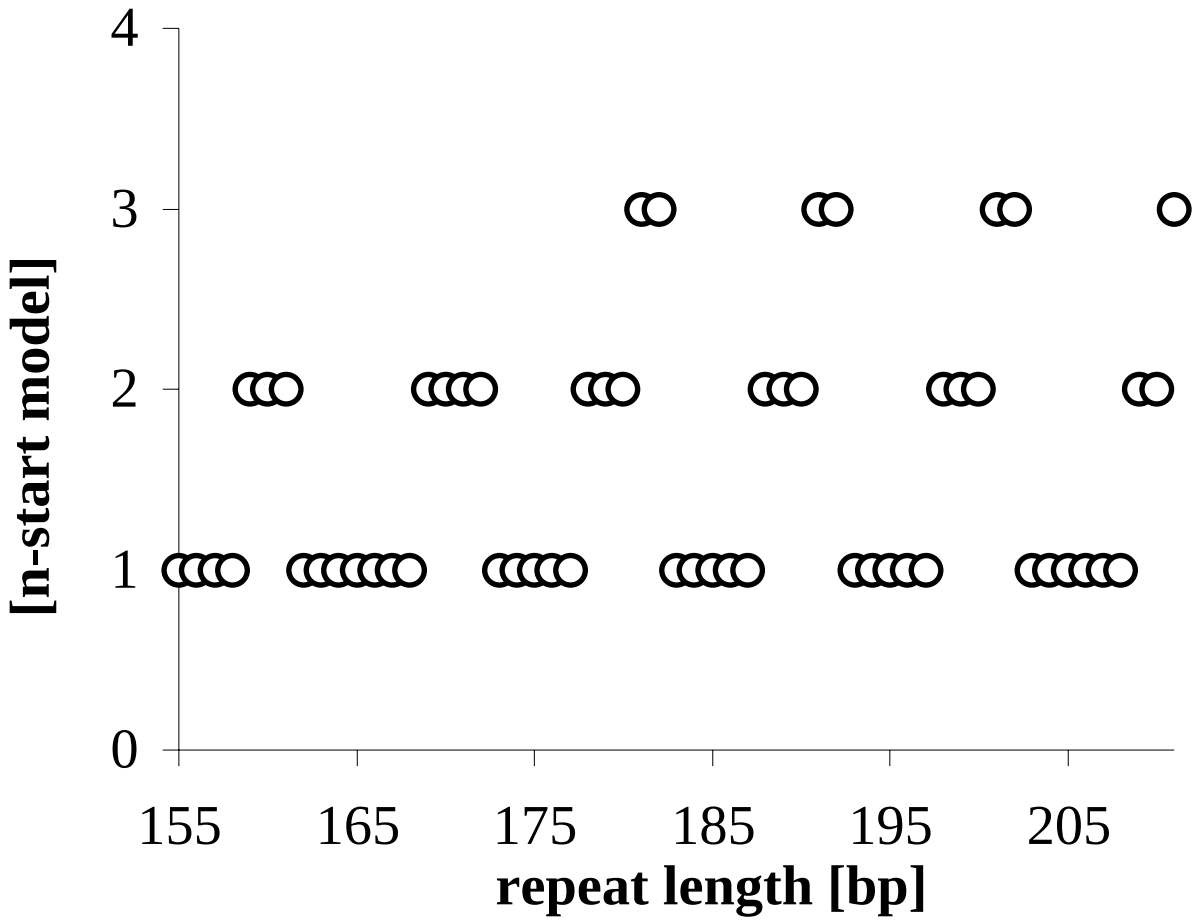
$\alpha = 0°, \delta = 180°$



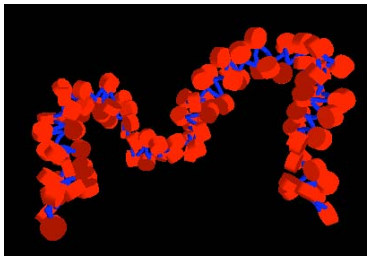
mainly 1-start helices for short linker lengths and 2-start helices for n *10 + 0 bp, no 3 start helices

Analysis of helix repeats

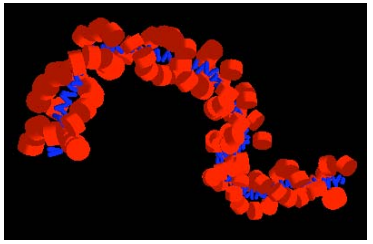
α = 60°, δ = 120°: 1- 2- and 3-start helices



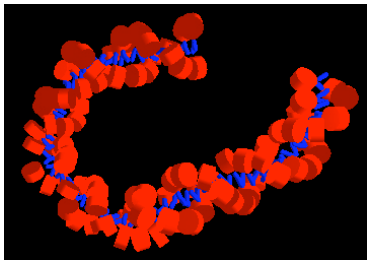
Fiber conformations at different persistence lengths



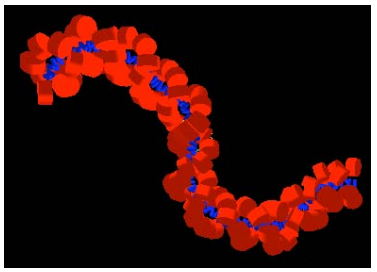
L_p = 30 nm
rpt = 202, no stem
β = 137°



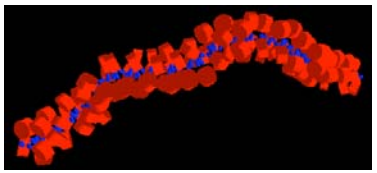
L_p = 48 nm
rpt = 204
β = 120°



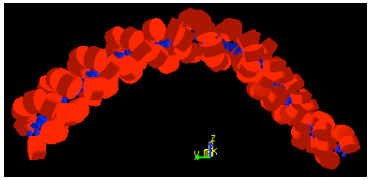
L_p = 52 nm
rpt = 205
β = 110°



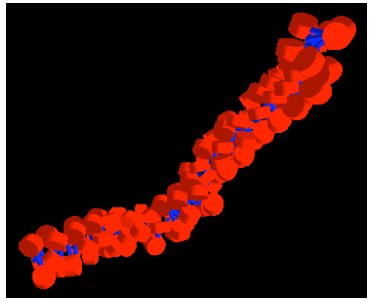
L_p = 97 nm
rpt = 200
β = 110°



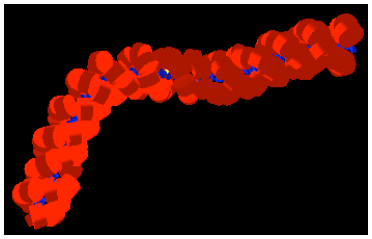
L_p = 117 nm
rpt = 199
β = 309°



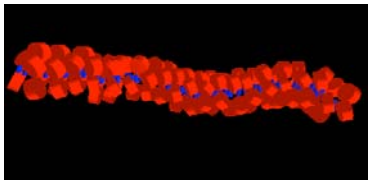
L_p = 146 nm
rpt = 198
β = 110°



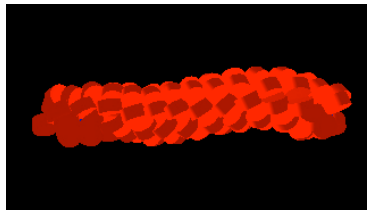
L_p = 150 nm
rpt = 195, no stem
β = 257°



L_p = 220 nm
rpt = 195
β = 110°



L_p = 235 nm
rpt = 198
β = 274°

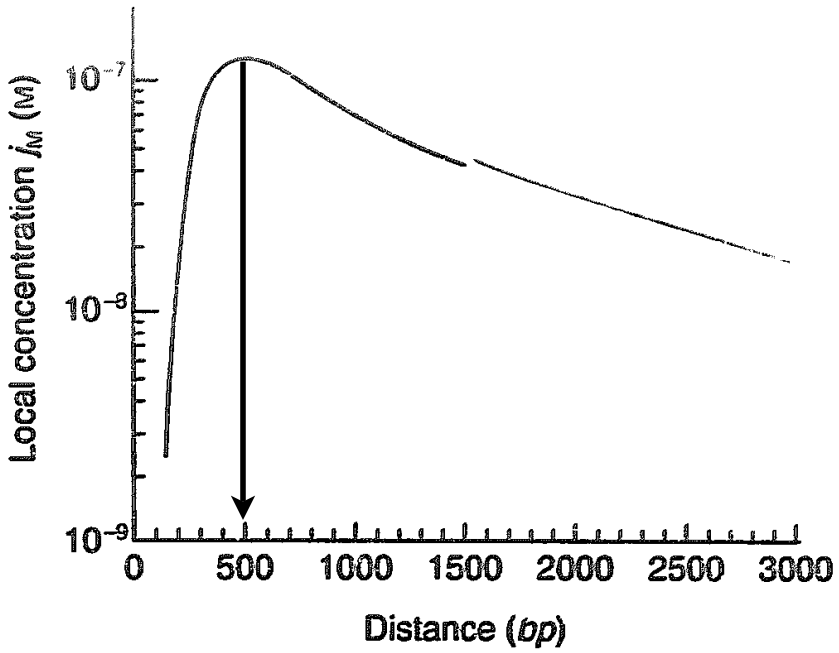


L_p = 378 nm
rpt = 195
β = 171°

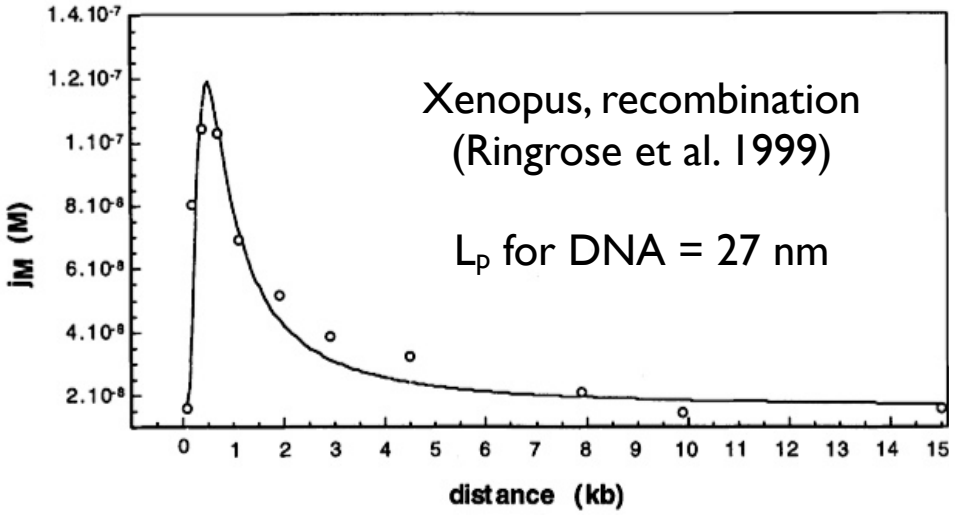
Chromatin flexibility from looping probability

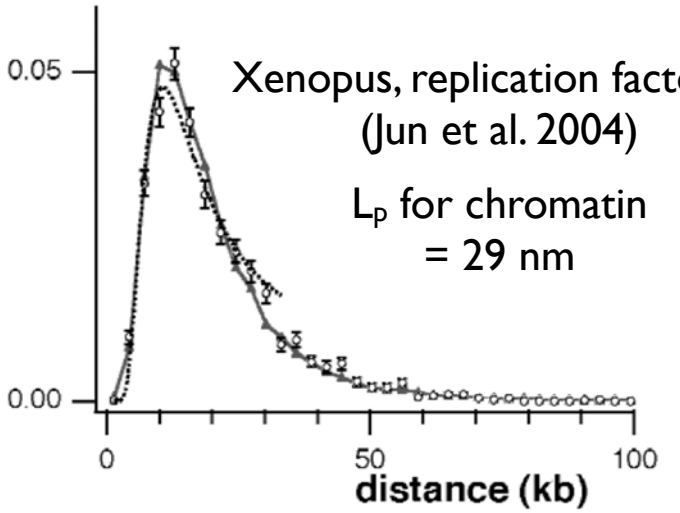
Cyclization probability for DNA: maximum at 3.3 L_p

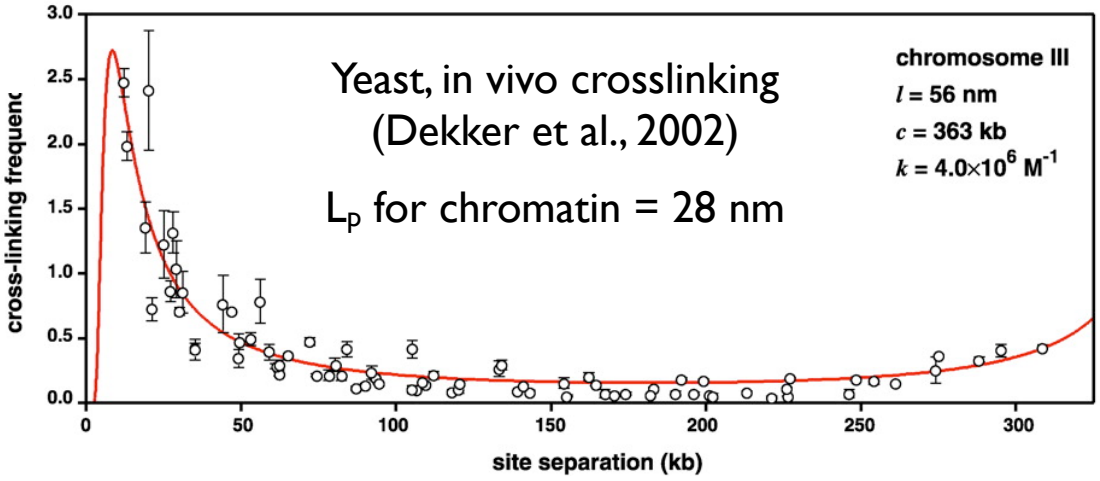
(Shimada-Yamakawa theory; Fig. from Rippe, von Hippel, JL, TIBS 1995)



Chromatin flexibility from looping probability







max looping probability at 1-15 kb

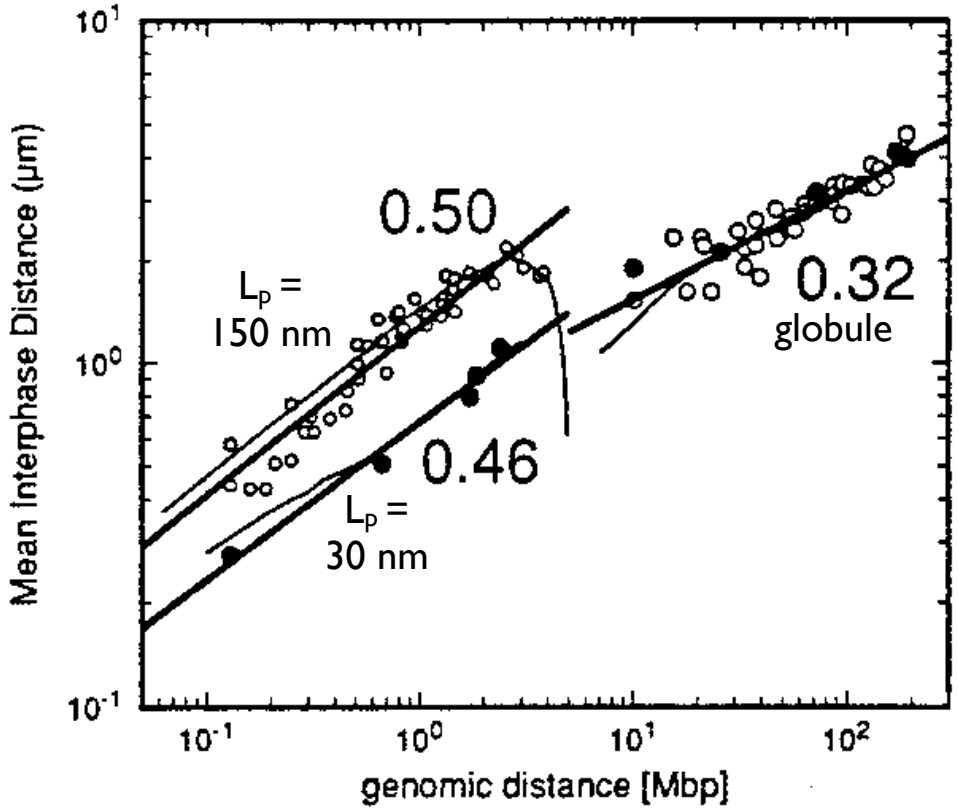
=> L_p values quite low

But: distances measured in kb, not nm =>

L_p depends on compaction

Chromatin flexibility from marker distances

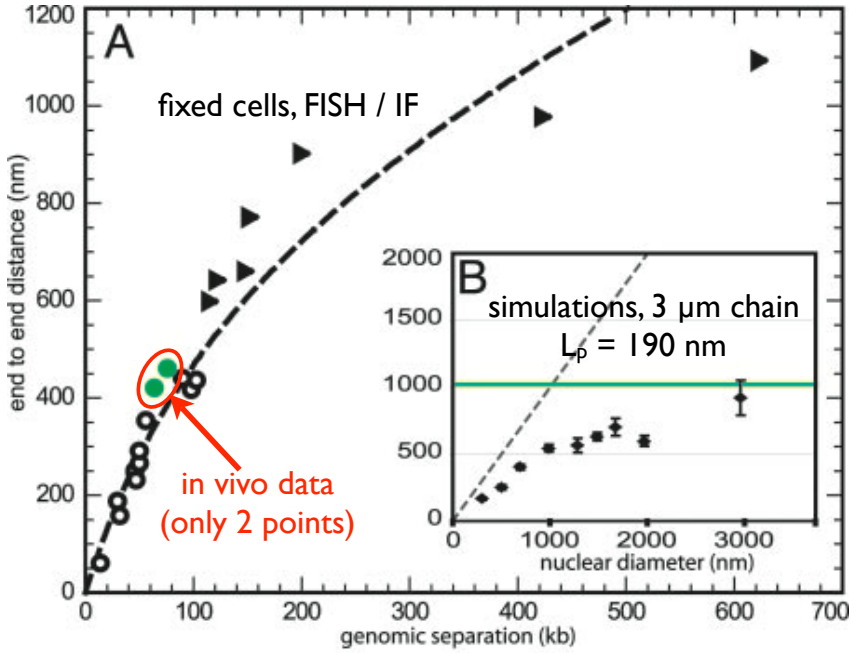
- L_p 30-150 nm depending on conditions (Münkel et al., JMB 1999)

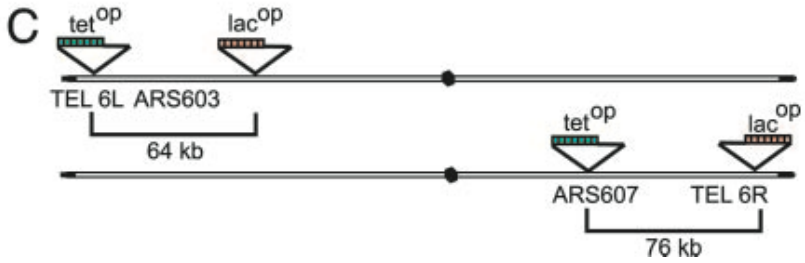


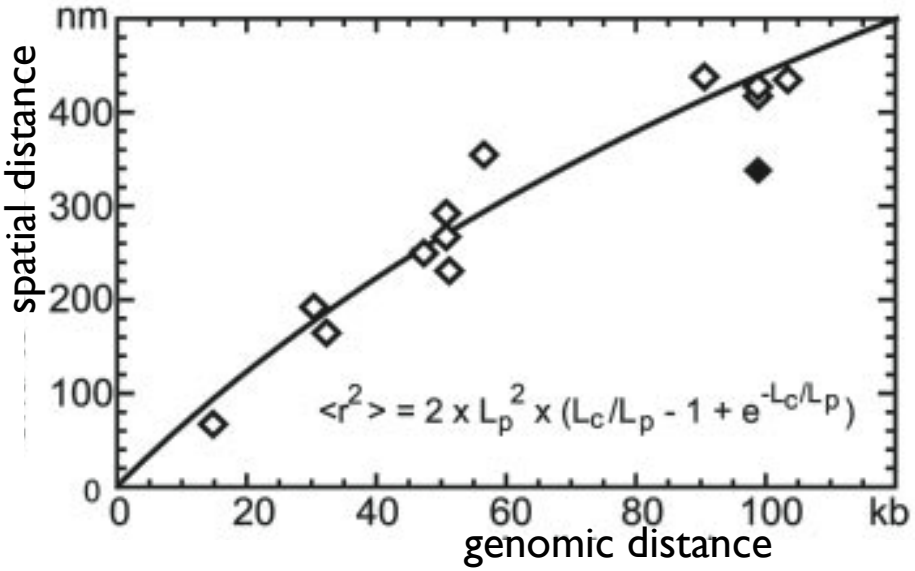
data taken from van den Engh et al. (1992) and Yokota et al. (1995)

Measuring chromatin flexibility in yeast cells

(Bystricky et al. PNAS 2005)



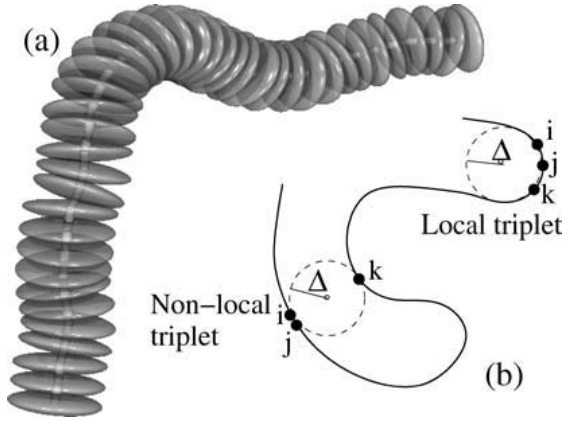




- spatial vs. genomic distance shows flexible wormlike chain behavior
- results:
  - L_p = 167±95 nm
  - mass density 110±32 bp/nm
    ('classical' solenoid 100 bp/nm)
- deviations for long distances
  (influence of restricted nuclear volume)

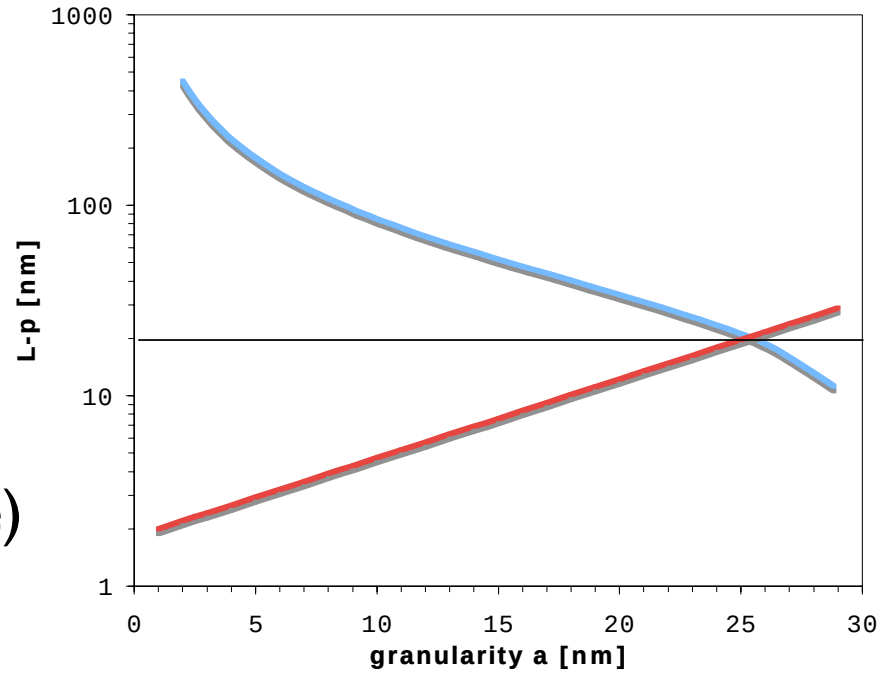
What is the *mimimum* persistence length of the 30 nm fiber (if it exists)?

- thick flexible chain with thickness Δ, granularity a (only local thickness constraints):



(Toan et al. Biophys J 2005)

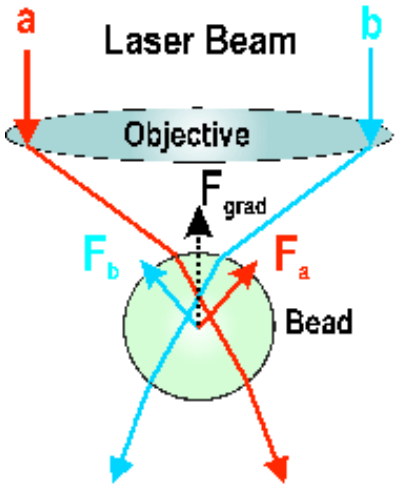
$$L_p = -\frac{a}{\ln\left(1 - a^2/4\Delta^2\right)}$$



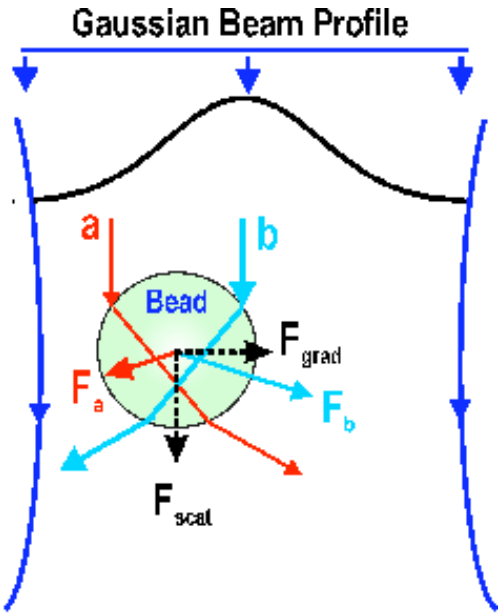
- chromatin fiber, Δ = 15 nm: minimum L_p = 20 nm, a = 25 nm
- for a = 10 nm (internuc. distance) $L_p \approx$ 100 nm

Stretching chromatin with optical tweezers

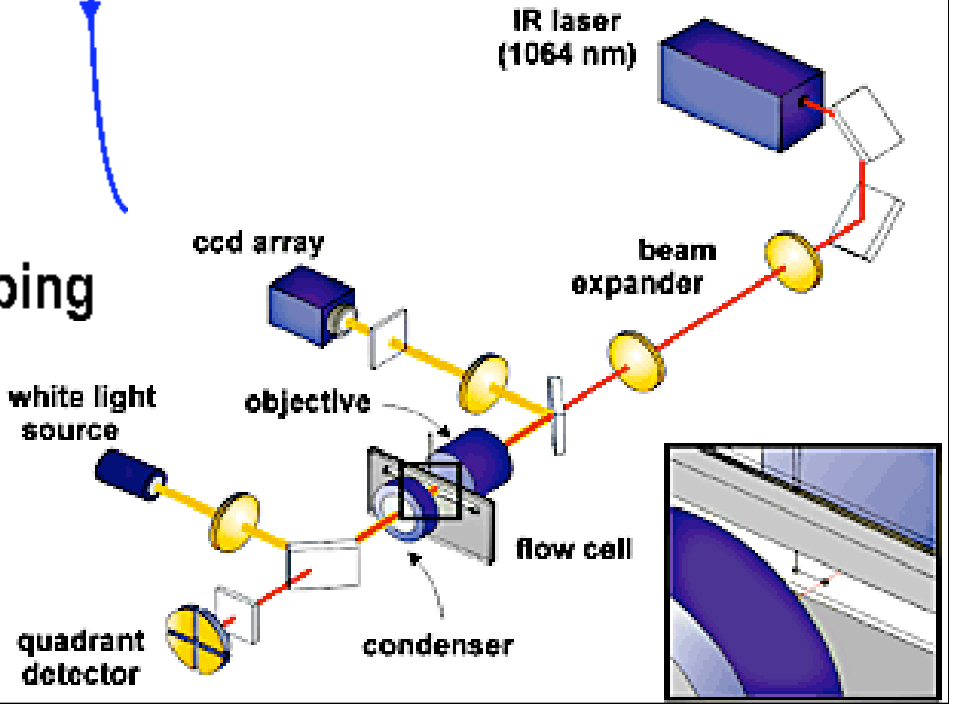
Bennink et al. 2001



Axial Trapping

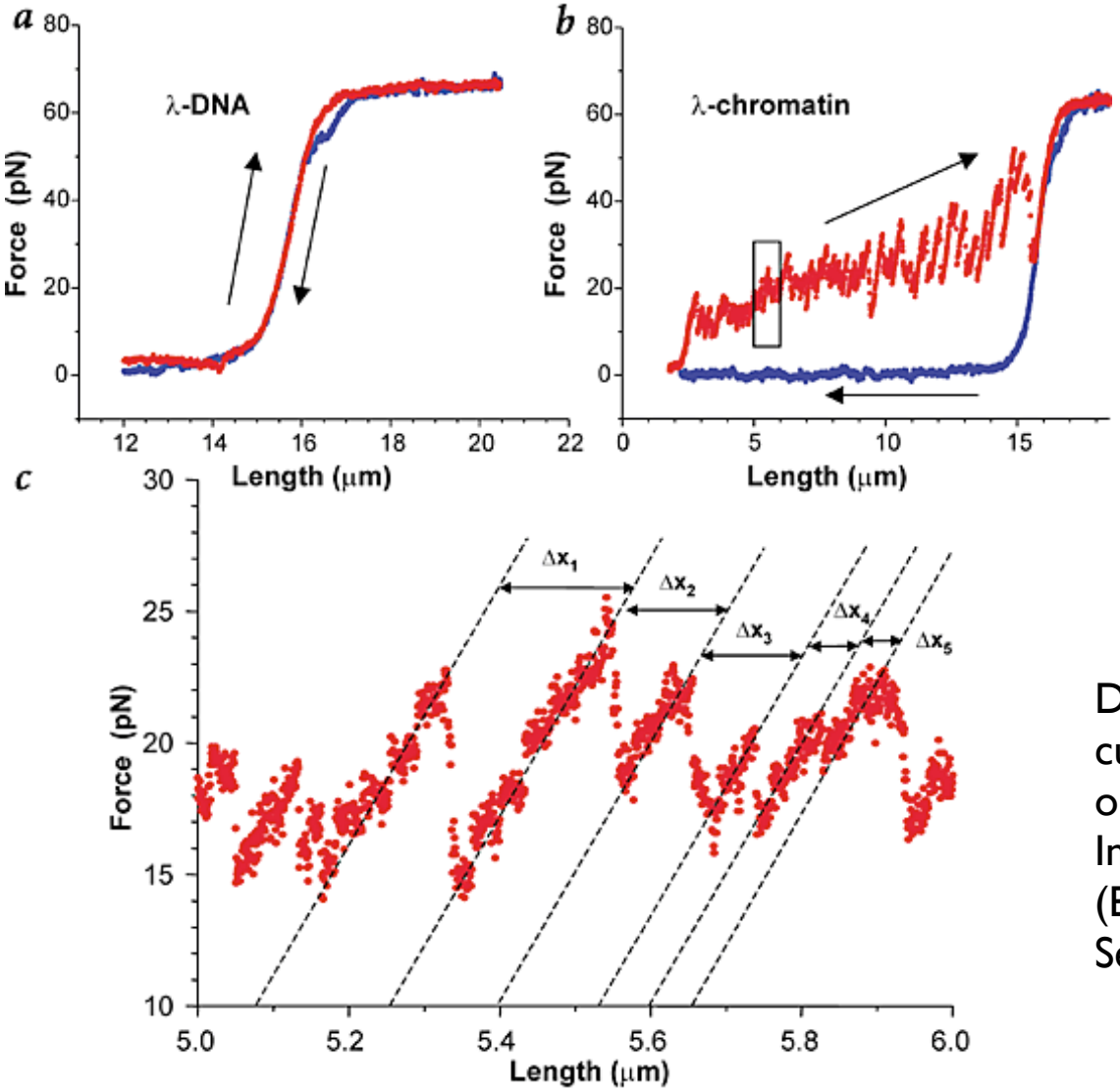


Lateral Trapping



Stretching chromatin with optical tweezers

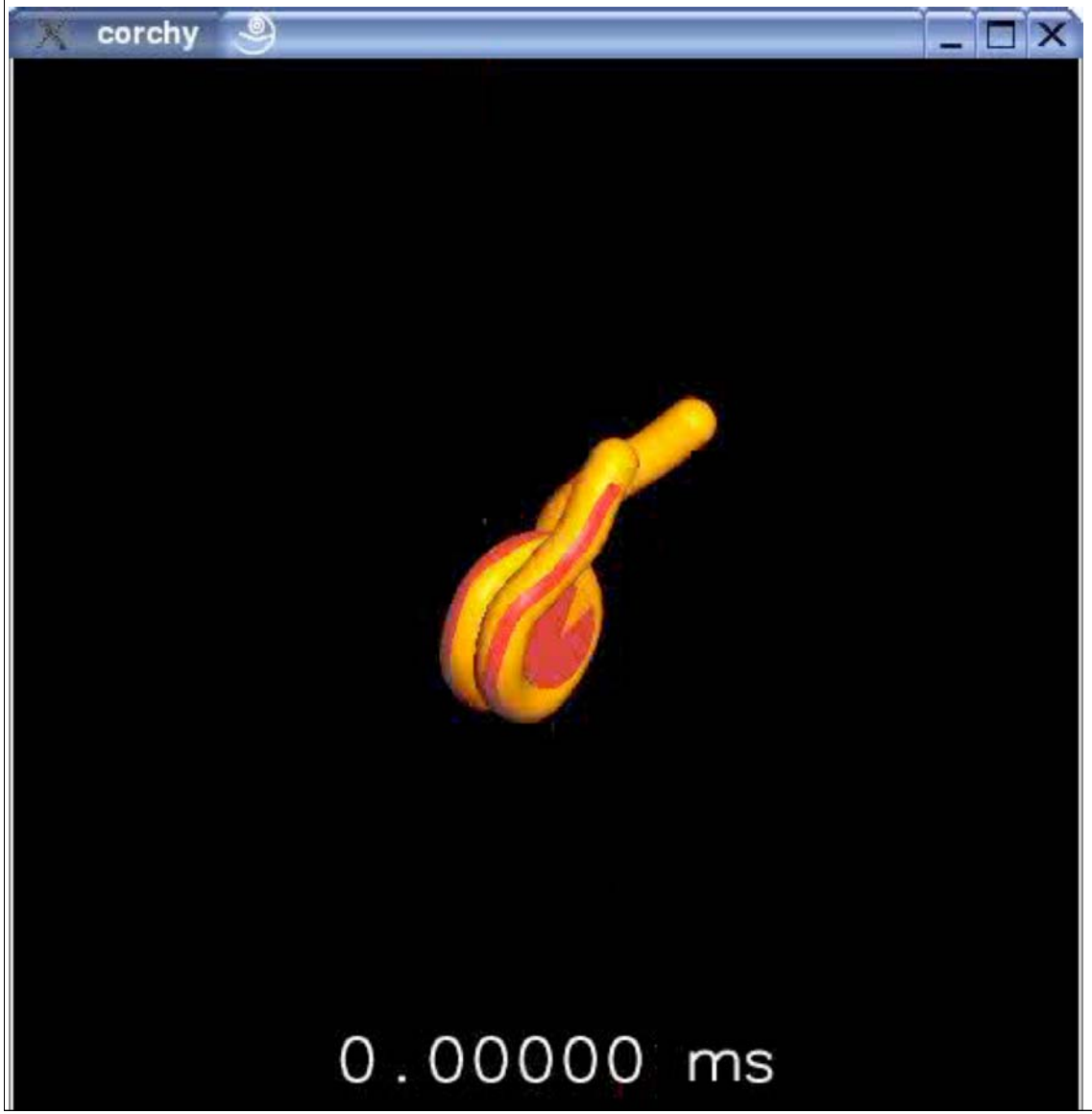
Bennink et al. 2001



Discrete steps in the stretching curve correspond to unwrapping of one or more nucleosomes. Intermediate stops are found (Brower-Toland et al. 2002)
See also Pope et al. 2005

Nucleosome formation and unrolling - BD simulations

(Wocjan, Klenin and Langowski, unpublished)



220 bp DNA

binding potential:
2 kT / base pair (146 bp bound on nucleosome)

compare to elastic energy:
120 kT for bending one persistence length by 11π

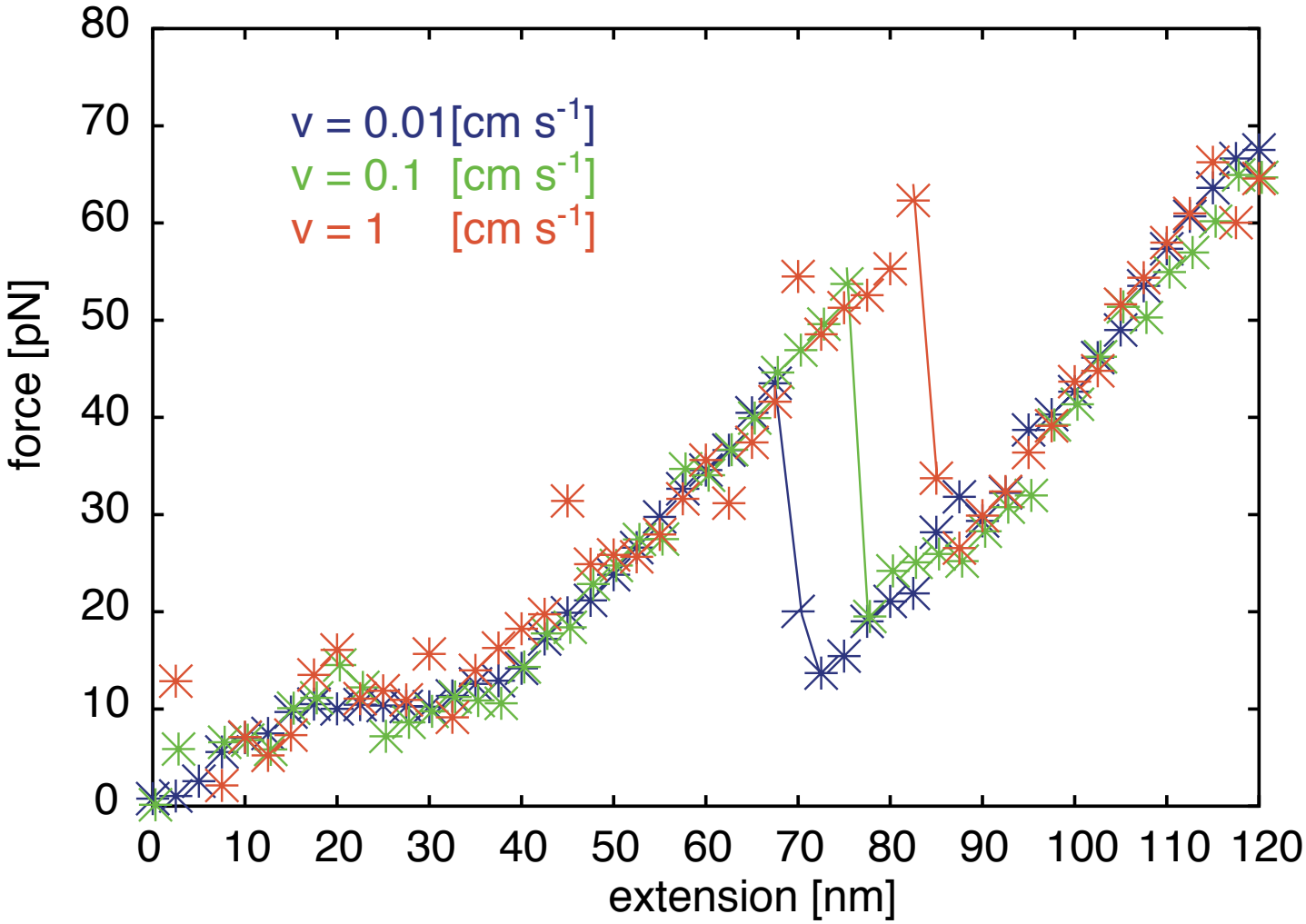
stretching force: 16 pN

DNA-histone core interaction parameters

U_0	depth of potential well [k T]	15
r_0	radius of potential well [nm]	0.5
Θ_0	critical angle [grad]	90
k_0	energy coefficient outside $\Theta \in [-\Theta_0, \Theta_0]$ [0.1]	0.1
$\vec{r}$	vector to middle point of linear segement	-
H_{nuc}	height of cylinder [nm]	4.180
R_{nuc}	radius of cylinder [nm]	4.180
-	number of turns	1.750

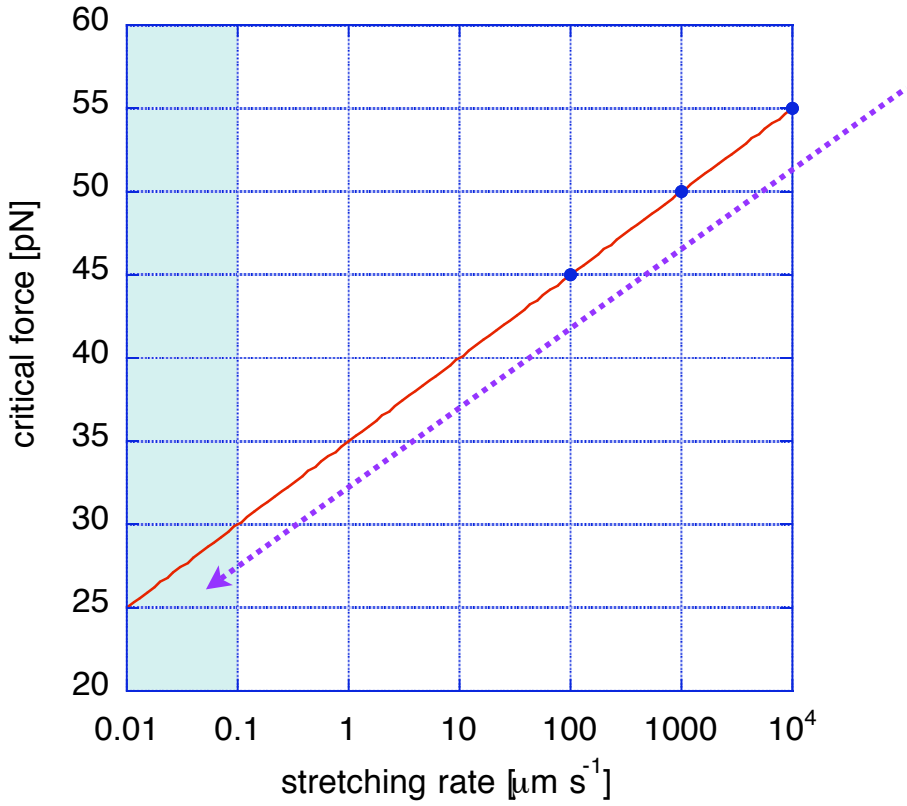
BD simulations of nucleosome unrolling

Effect of loading rate



BD simulations of nucleosome unrolling

Effect of loading rate



- Typical stretching rates used in experiments: 10-100 nm s^{-1} per nucleosome
- theory of dynamic force spectroscopy predicts logarithmic dependence of unbinding force on loading rate:

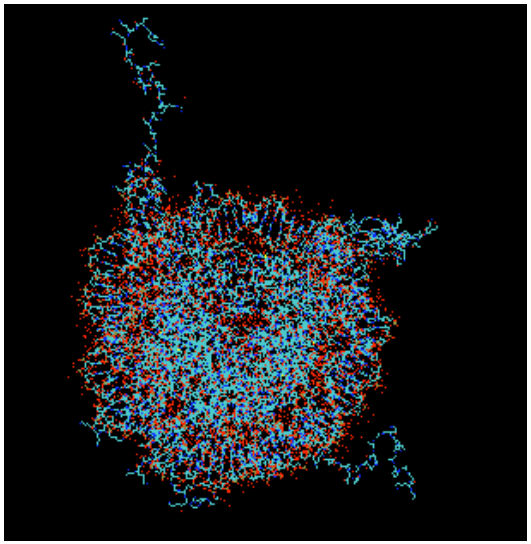
$$F^* = \frac{k_B T}{d}\left[\ln\left(\frac{1}{N}\frac{dF}{dt}\right) - \ln\left(\frac{k_B T k_{off}}{d}\right)\right],$$

- data still needs to be converted from dx/dt to dF/dt; simulations at even lower loading rates

Coarse-grained molecular dynamics of the nucleosome

(Karine Voltz, Joanna Trylska, Valentina Tozzini, Vandana Kurkal, Jeremy Smith, J.L.)

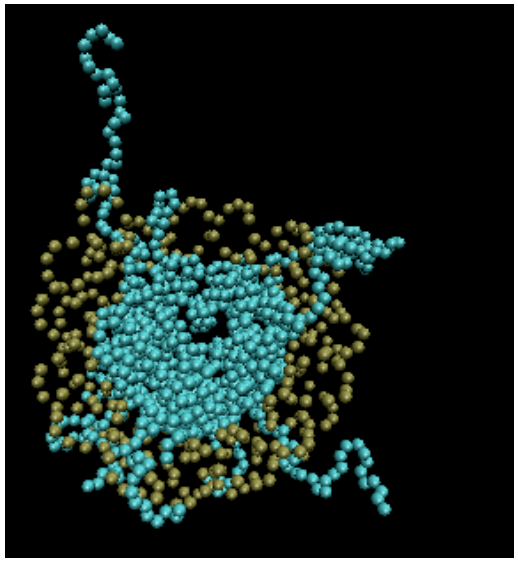
All-atom model:



16 000 atoms (nucleosome), 160 000 (solvent)

did ≈ 2 ns explicit-solvent MD
(CHARMM 27 force field)

Coarse-grained model:



Amino acids represented by beads centered on C_α

Nucleotides represented by beads centered on P

1266 beads, μs timescale

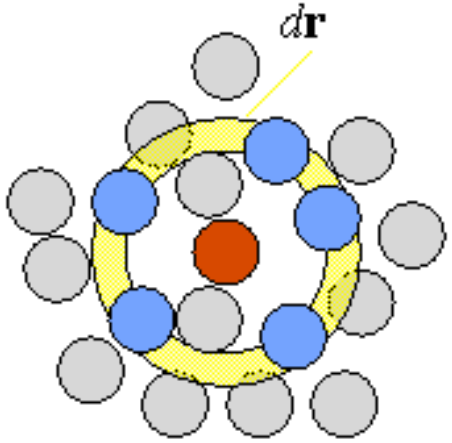
force fields?

Parameterisation of the force-field:

(J. Trylska & V. Tozzini, Biophys. J., 2005)

$$E = \frac{1}{2}k_{1-2}(r - r_0)^2 + \frac{1}{2}k_{1-3}(r - r_0)^2 + \frac{1}{2}k_{1-4}(r - r_0)^2 + A_{P,C\alpha}(r_0)\left[1 - \exp(-\alpha_{P,C\alpha}(r - r_0))\right]^2$$

Radial Distribution Function (RDF) (g(r)): distribution of the distances separating each pair of beads of the system



- RDF calculated for Cα-Cα pairs and P-P pairs.
- RDF calculated on 6 X-ray structures from the PDB: 1KX5, 1KX4, 1KX3, 1AOI, 1EQZ, 1F66

Parameterisation of the force-field:

(J. Trylska & V. Tozzini, Biophys. J., 2005)

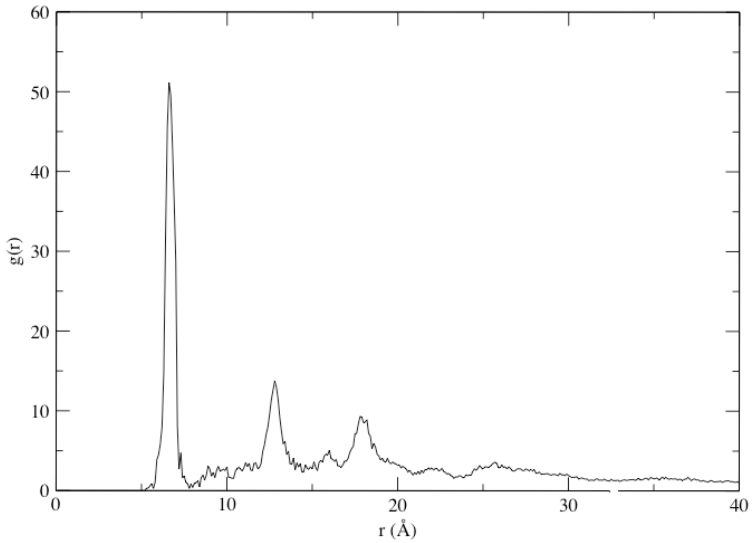
$$E = \frac{1}{2}k_{1-2}(r - r_0)^2 + \frac{1}{2}k_{1-3}(r - r_0)^2 + \frac{1}{2}k_{1-4}(r - r_0)^2 + A_{P,C\alpha}(r_0)\left[1 - \exp(-\alpha_{P,C\alpha}(r - r_0))\right]^2$$

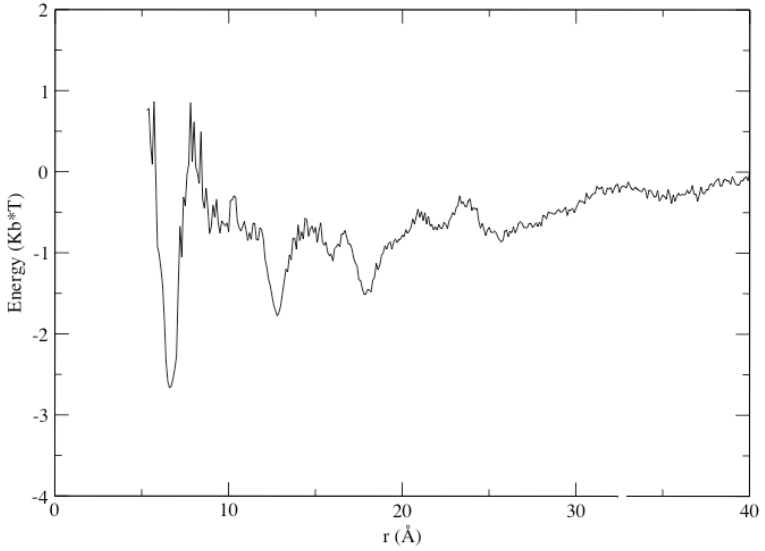
Radial Distribution Function (RDF) (g(r)): distribution of the distances separating each pair of beads of the system

Free energy from Boltzmann equation:

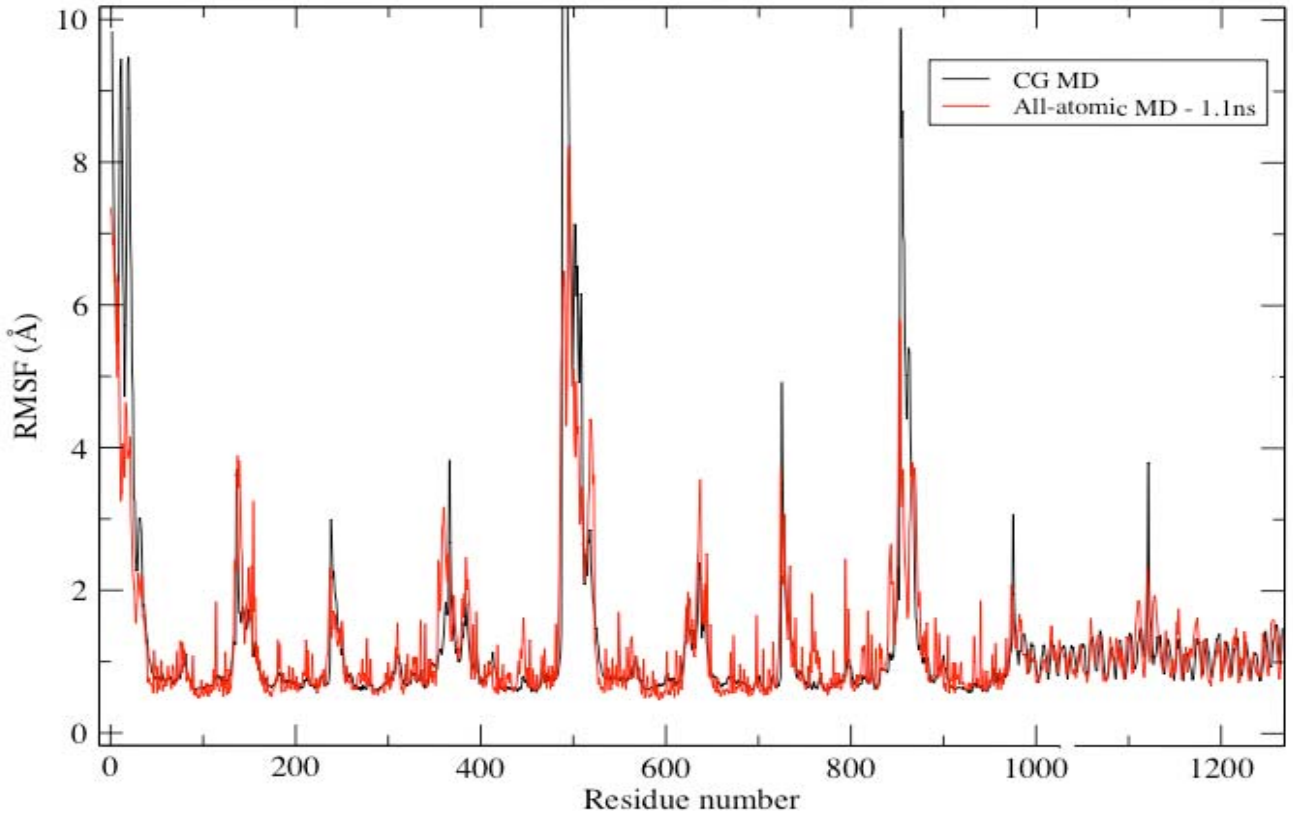
$$W(r) = -k_B T \ln(g(r))$$

g(r)





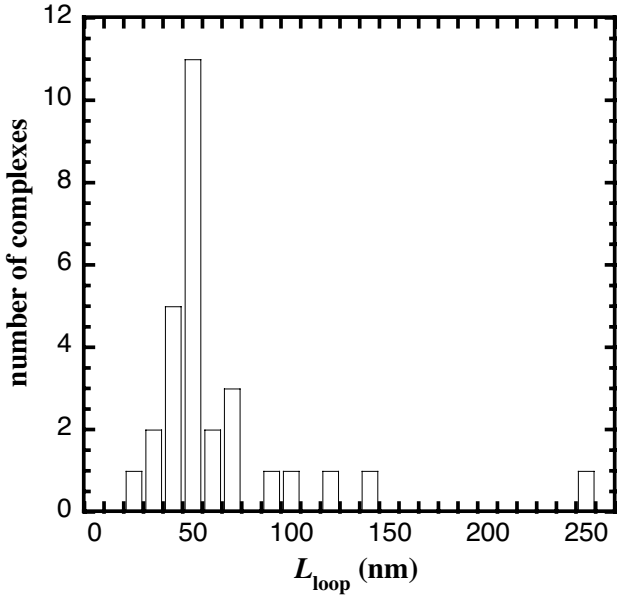
First test simulations with the coarse-grained model: Comparison of RMS fluctuations with all-atom MD

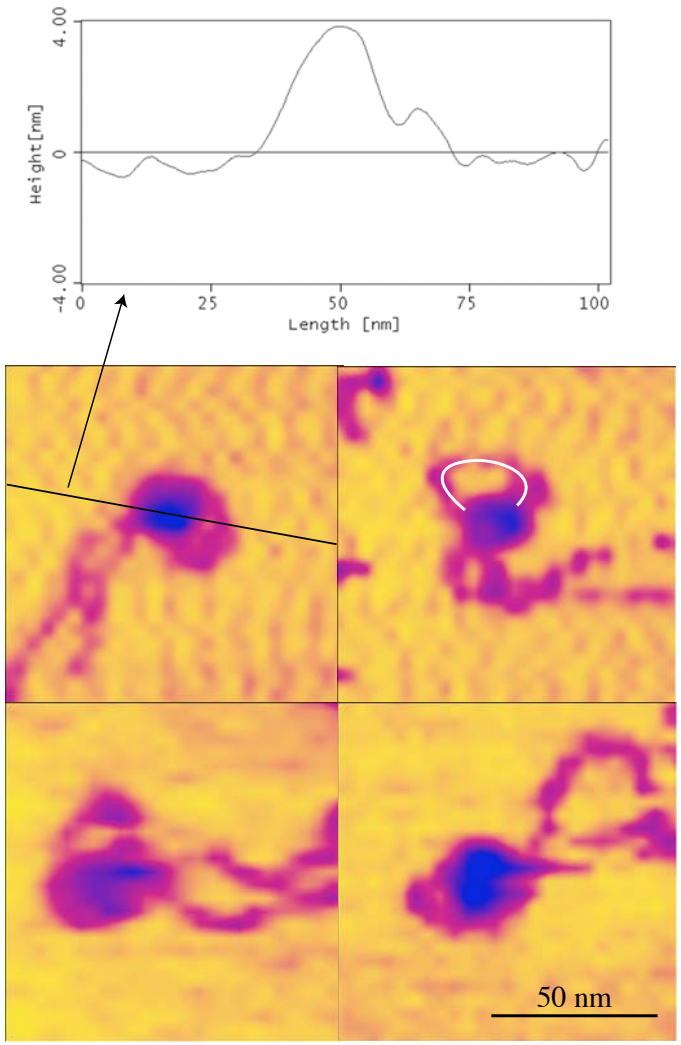


DNA loop formation on nucleosomes in superhelical DNA

(Malte Bussiek, Katalin Tóth, Nathalie Brun, JL, JMB 2005)

- Nucleosomes on superhelical DNA are very often found at DNA 'crossings'
- These structures consist of a DNA loop on on side of the nucleosome and DNA entering and exiting on the other
- Most probable loop size around 50 nm, order of magnitude compatible with the 100 nm predicted by Kulic and Schiessel

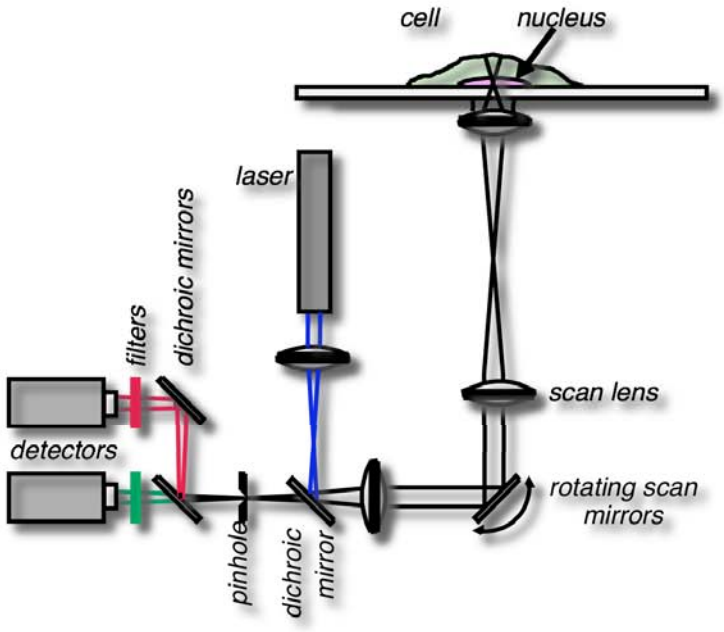


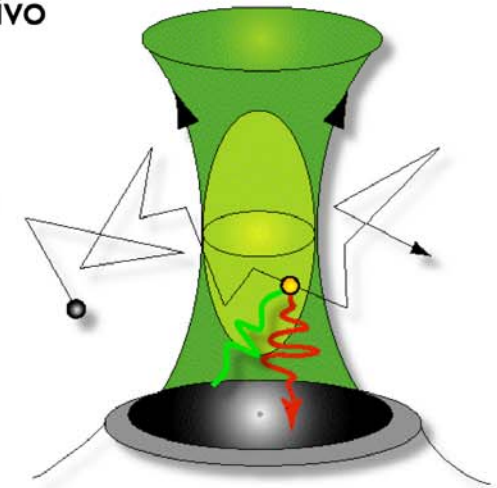


Fluorescence correlation spectroscopy (FCS) – the Fluorescence Fluctuation Microscope

(Wachsmuth, Tewes, Langowski, European Patent No. 0941470 (2001))

- FCS - Fluorescence fluctuations from single molecules moving through a confocal observation volume
- Determine:
  - Diffusion coefficient and concentration of fluorescent probes
  - Association constants of biomolecules
  - Mobility of fluorescent probes in vivo





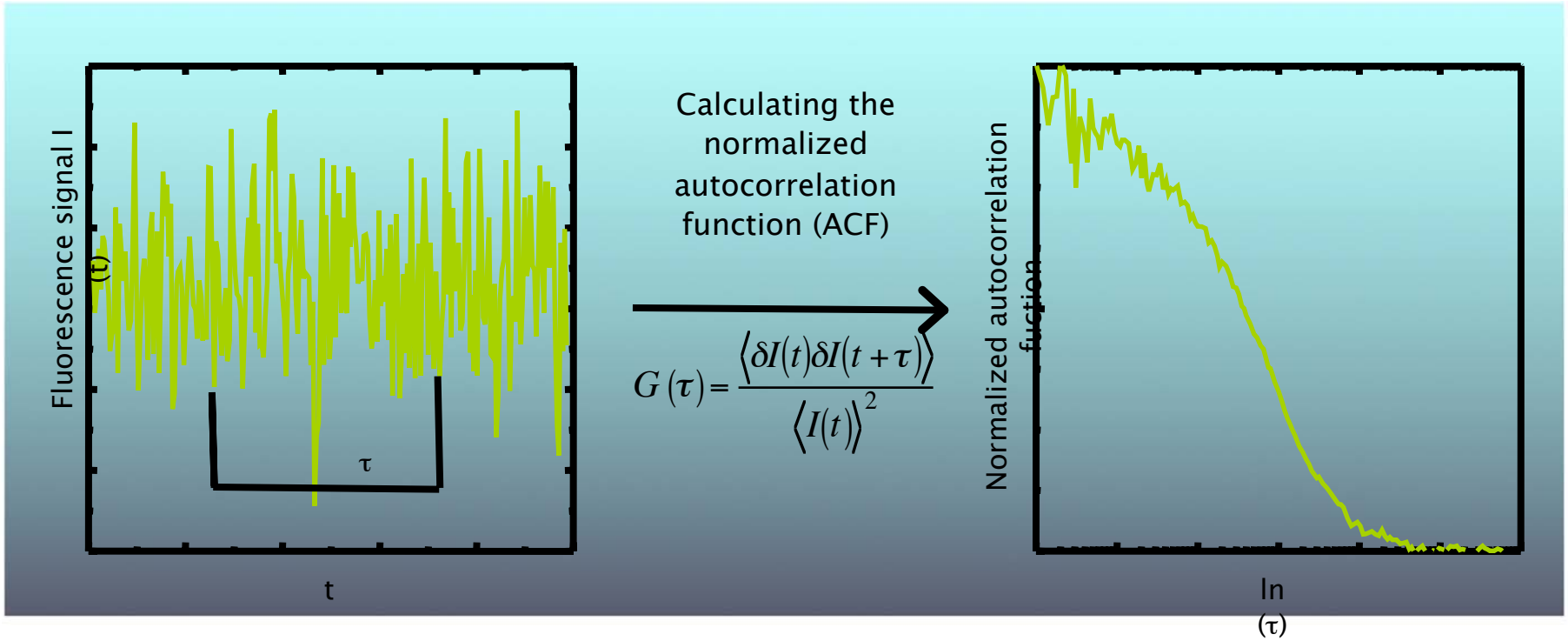
Our instrument (in-house construction):

Two color channel confocal FCS module

Operating modes: FCS, cross correlation, photobleaching, photon count histogram, single-molecule FRET

Positioning of FCS focus spot and imaging through integrated galvanometer scanner

FCS autocorrelation function



Fitting the autocorrelation function to appropriate model functions yields

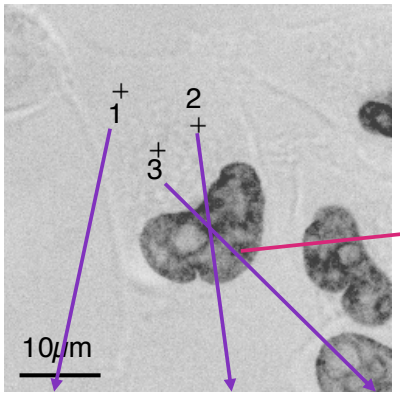
- the diffusion coefficient
- the concentration

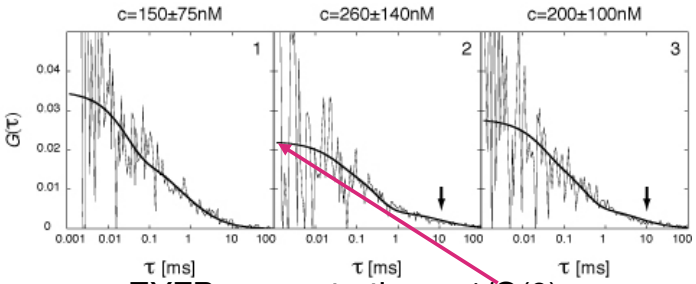
of several species with different hydrodynamic properties

Measuring absolute chromatin density by FCS

(Weidemann et al., JMB 2003)

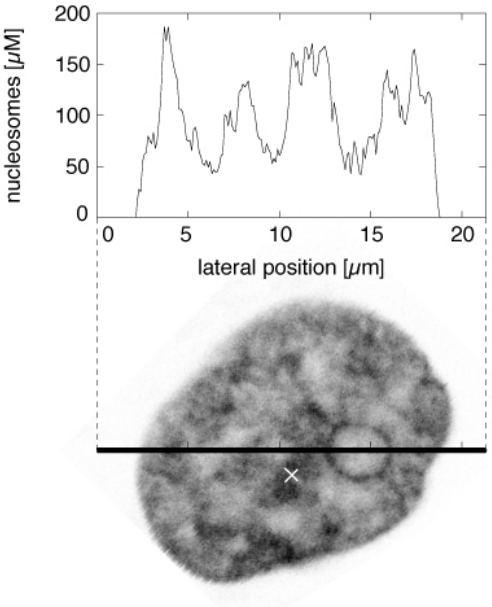
- Strategy:
  - measure concentration and brightness of free EYFP-H2B in the cytoplasm
  - calculate concentration of EYFP-H2B in the nucleus from its measured brightness
  - measure incorporation ratio of EYFP-H2B into chromatin by FCS on chromatin fragments
  - all this data taken together yields nucleosome concentration





EYFP concentration ∝ 1/G(0)

5% fluorescent histone incorporated into chromatin (determined by FCS)



Cross-section through HeLa cell with nucleosome concentration

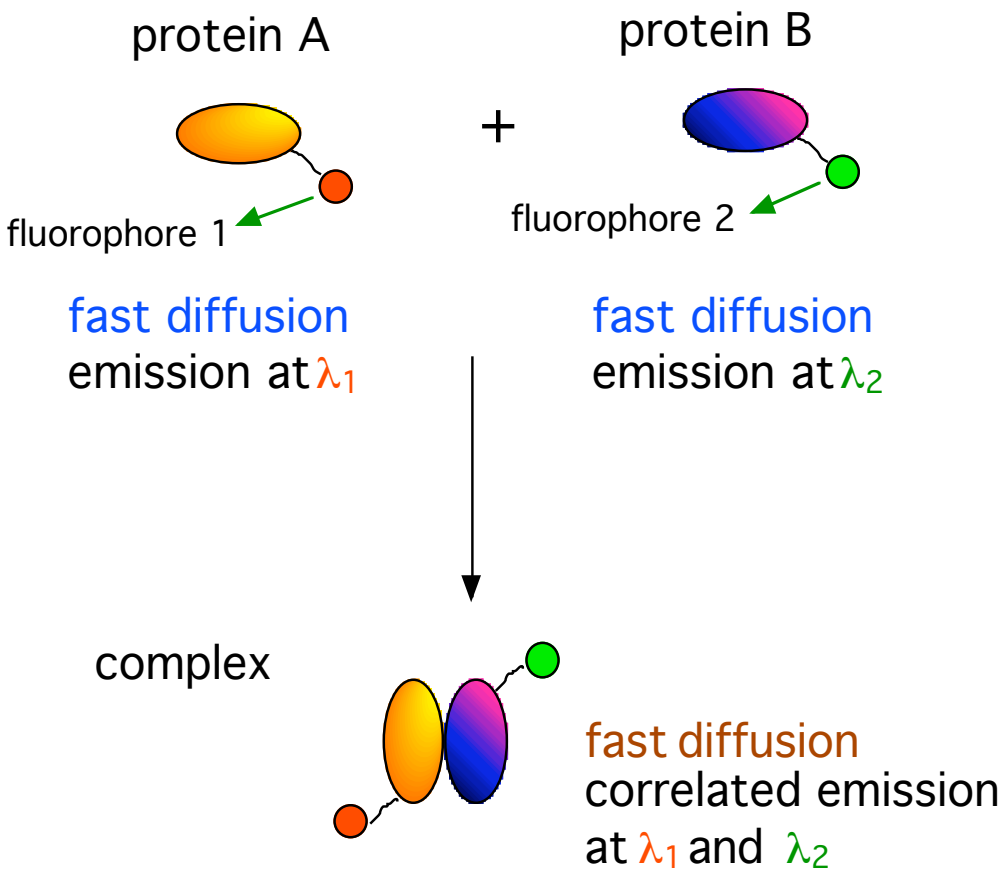
Volume density of chromatin fiber in interphase HeLa cell is about 5 to 10%
Chromatin network is highly penetrable for proteins < 200000

Measuring interactions between biomolecules by FCCS: association of two macromolecules of similar size

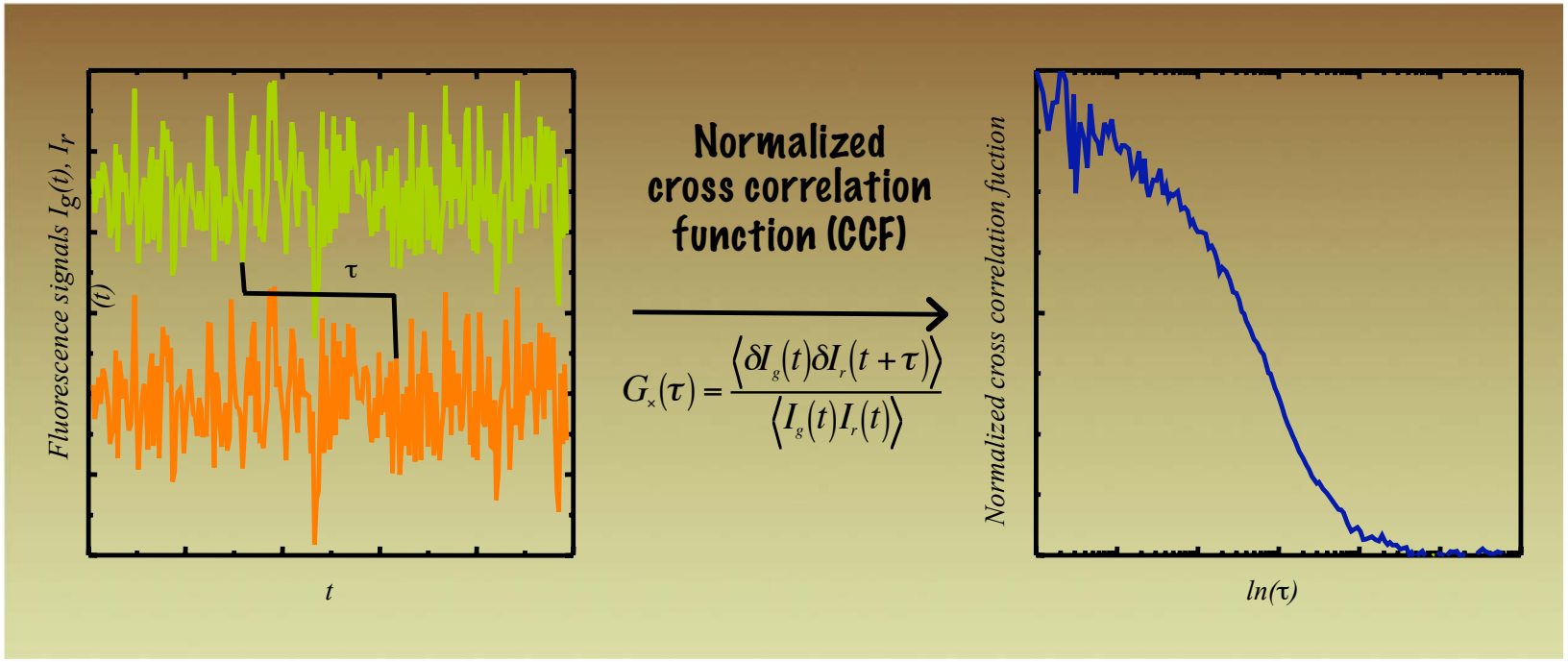
Example:
protein-protein interaction

Here the complex cannot be distinguished from the components by its diffusion coefficient.
By simultaneous detection at two wavelengths only those particles will be seen that carry both fluorophores -> only the complex is detected.

Correlated emission is quantitated by measuring the cross-correlation function of the two color channels: fluorescence cross correlation spectroscopy



Two-color cross correlation



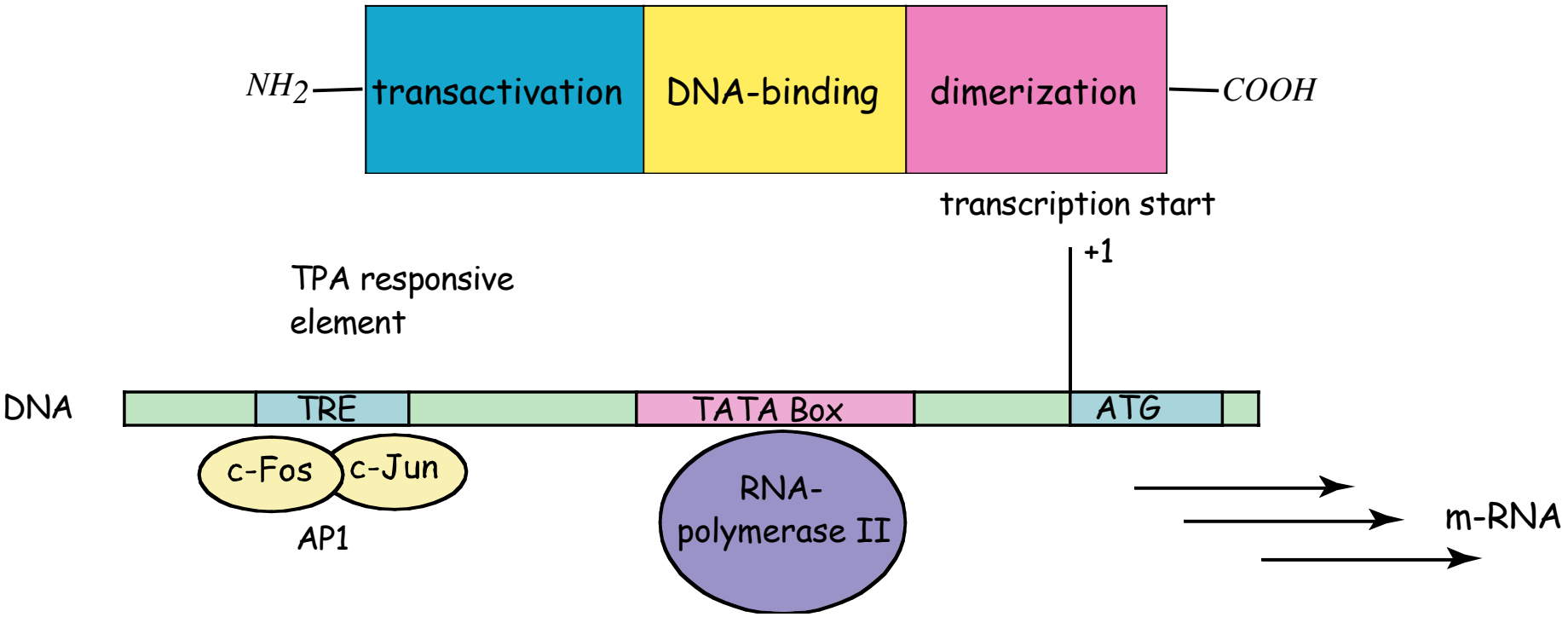
- Fitting the cross correlation function to appropriate model functions yields
  - the diffusion coefficient
  - the fraction of double labelled species

(Weidemann et al., Single Mol. 3 (2002) 49-61)

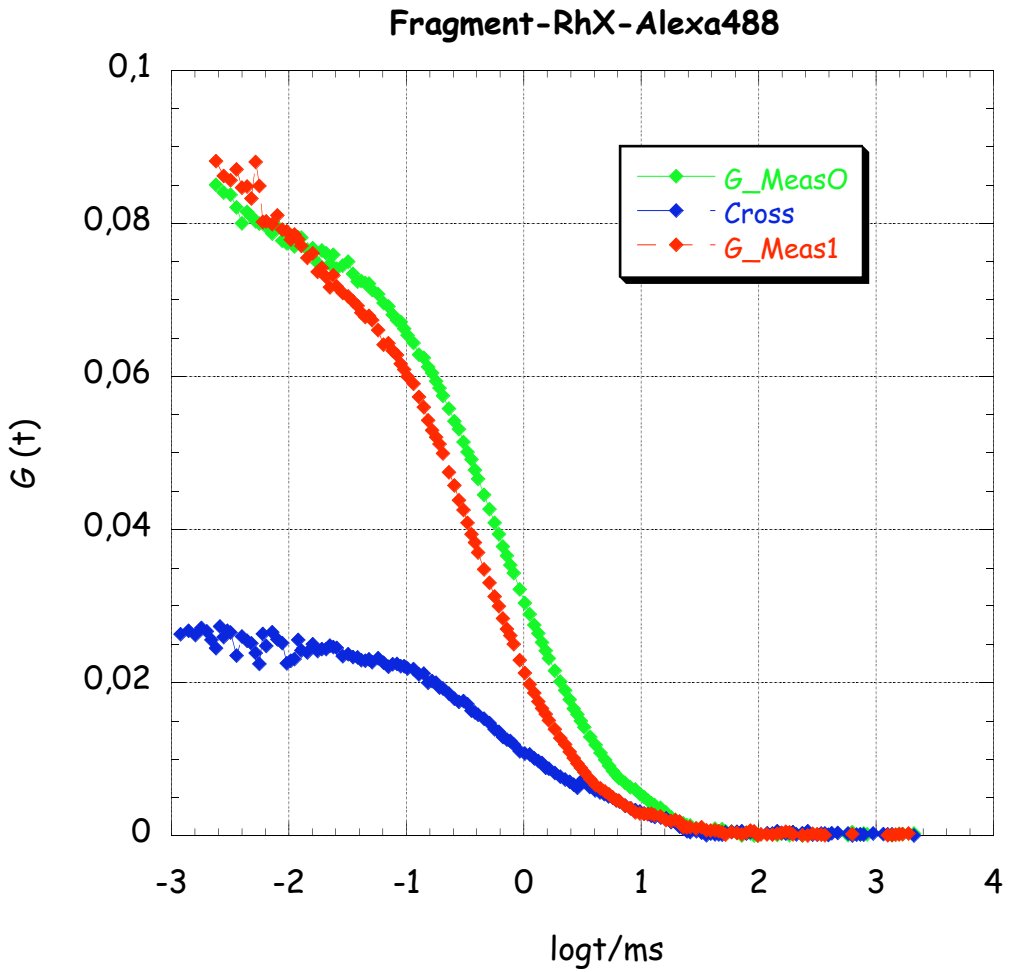
In-vivo study of protein-protein interactions with FCCS

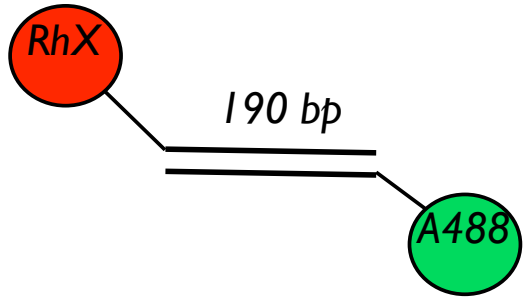
(Nina Baudendistel, JL, 2005 ChemPhysChem)

- The AP-1 system is a group of inducible transcription activator proteins
  - variable subunits: c-Fos, c-Jun, JunB, JunD, FraI
  - major components are c-Fos and c-Jun monomers
  - all proteins have a leucin-zipper
  - dimerization is required for DNA-binding
  - does dimerization occur before or upon DNA binding?



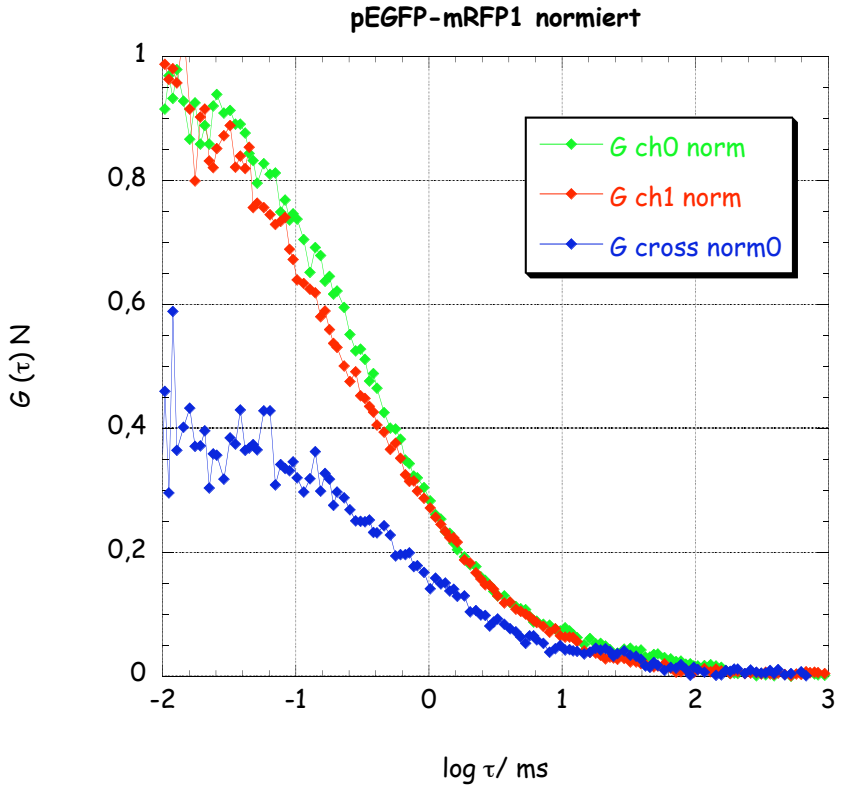
FCCS control: double-labeled DNA



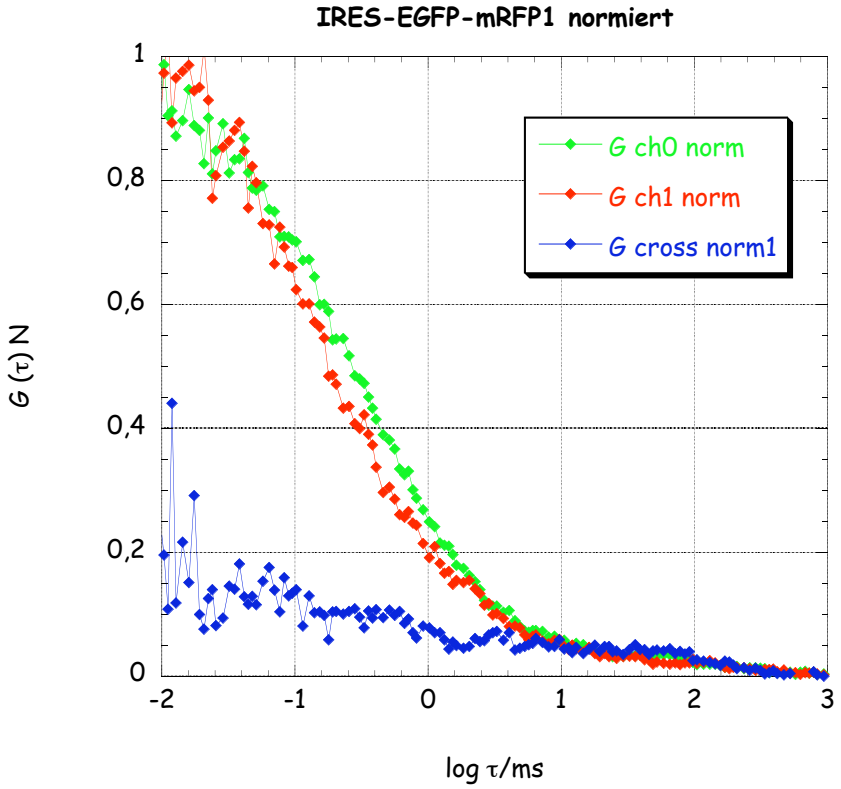


—>42% cross correlation
for 100% double-labeled fragment

FCCS: controls

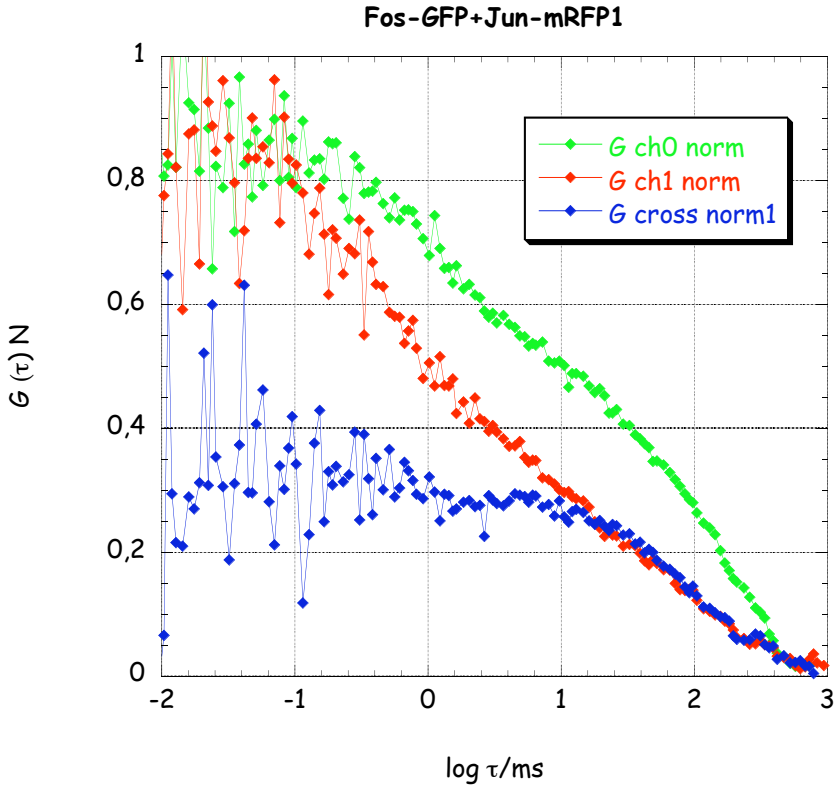


EGFP-mRFP fusion

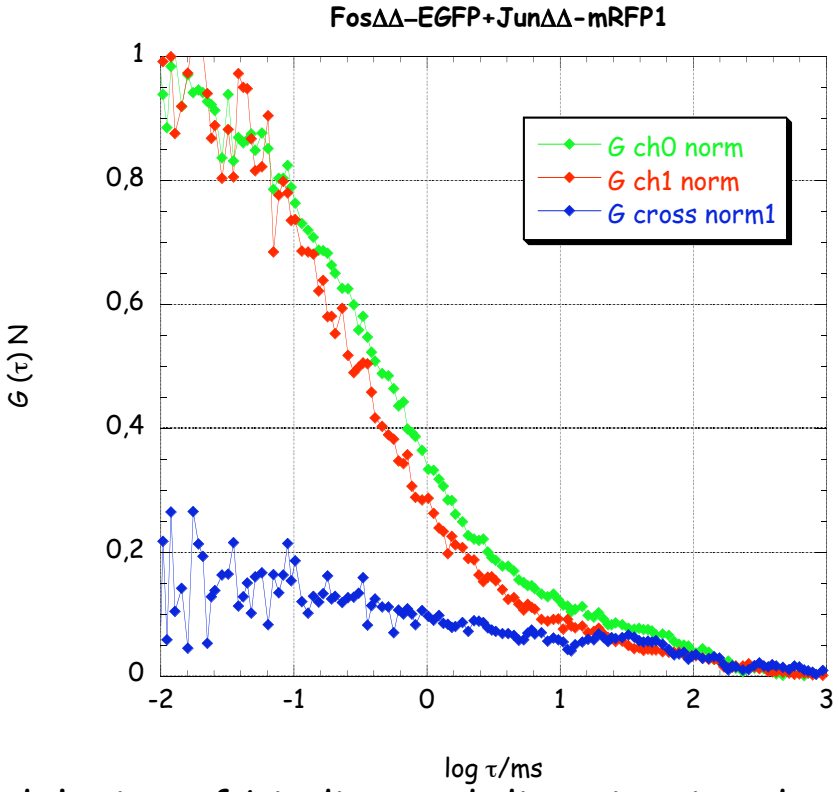


EGFP-mRFP separate expression

FCCS: Fos-EGFP + Jun-mRFP

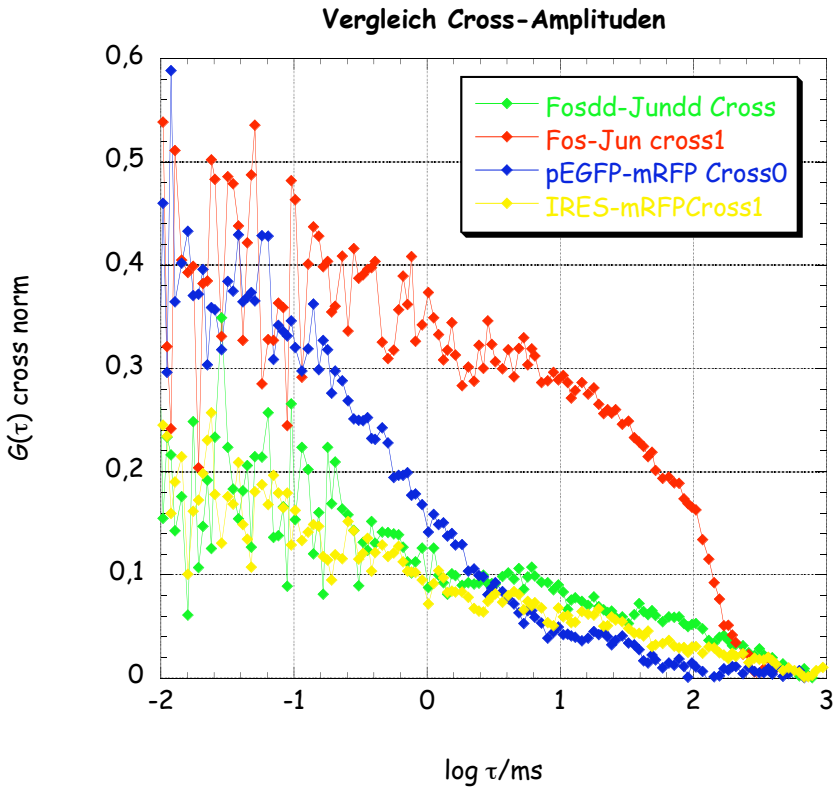


full proteins show interaction
(positive cross correlation)



deletion of binding and dimerization domain -> no interaction
(background cross correlation)

Interacting Fos and Jun show slow diffusion



FCCS shows that the correlated signal corresponds to a DNA-bound component

-> no free Fos-Jun dimer detected

-> majority of Fos-Jun dimer bound to DNA

People + Money

- DKFZ, Division Biophysics of Macromolecules:
  - Frank Aumann (chromatin fiber model)
  - Karine Voltz (coarse-grained nucleosome model), co-adviser Jeremy Smith, Heidelberg
  - Tomasz Wocjan (nucleosome unrolling)
- Alumni
  - Nina Baudendistel (FCCS of protein-protein interaction), now BASF
  - Malte Bussiek (AFM of nucleosomes), now Bennink group, U of Twente
  - Lutz Gehlen (yeast chromatin flexibility), now Gasser group, FMI Basel
  - Konstantin Klenin (MC and BD models, nucleosome unrolling), now Wenzel group, Karlsruhe Research Center
  - Michael Tewes (FCS module), now CAESAR, Bonn
  - Malte Wachsmuth (FFM, intranuclear mobility), now Institut Pasteur, Seoul
  - Waldemar Waldeck, Gabriele Müller (cell biology)
  - Gero Wedemann (chromatin fiber model) now FH Stralsund
  - Thomas Weidemann (nucleosome counting), now Novartis, Vienna
- Support
  - Volkswagen foundation program 'Physics, Chemistry and Biology with single molecules'
  - DFG priority program 'Supramolecular Structures'
  - Spatz group, Biophysical Chemistry, Heidelberg (collaboration on intracellular mobility)