High flexibility of DNA on short length scales probed by atomic force microscopy

Wiggins P. A. et al.
Nature Nanotechnology (2006)

presented by Anja Schwäger
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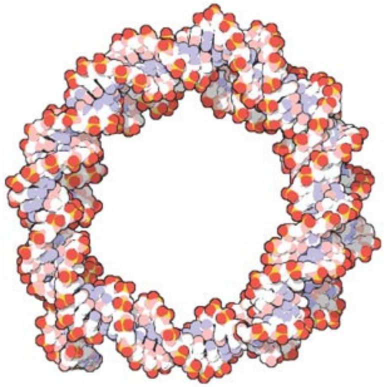
Outline

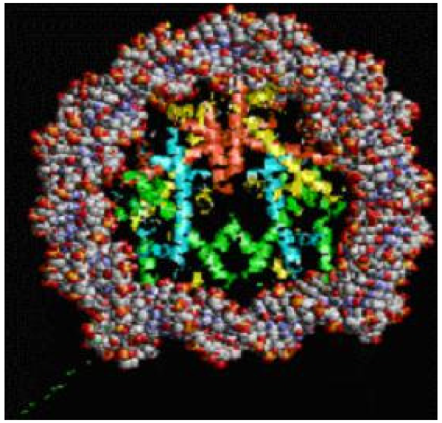
- Theory/Background
  - Elasticity of DNA
  - Aim of study
- Methods
  - AFM
  - Data analysis
- Results
  - New model for DNA elasticity
  - Comparison with other results
- Outlook
  - Improvement of model
  - Summary



DNA mechanics

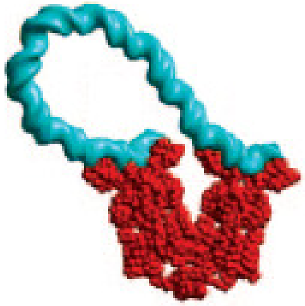
DNA elasticity

- Bending and twisting properties of DNA
  $\Rightarrow$ determine DNA conformation and dynamics
- Elastic properties of DNA
  - double helix relatively stiff for bending
  - unlikely to form sharp bends, high energy needed
- Biological importance of sharp DNA bends on short length scales
  - chromatin
  - packaging of DNA in capsid
  - protein interactions (gene regulation)



http://www.jyi.org/research/re.php?id=419

Nucleosome

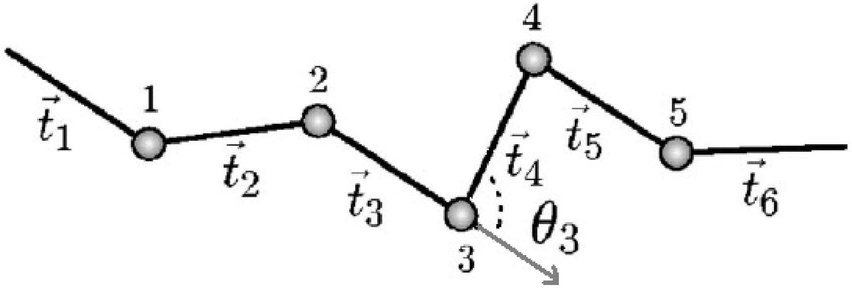


Swigon, PNAS, 2006

Lac Operator

Worm-Like Chain (WLC)

- **Elasticity model** for the description of **semiflexible polymers**
- Molecule as a thin rod with constant and isotrop elastic behaviour
- Approximation (discrete segments):
  - Conformation described as a chain of segments
  - Each segment with classical elastic energy cost for bending (Hooke‘s law)
- WLC is this approximation in the limit of zero segment length



modified according to Wiggins and Nelson, Phys Rev E, 2005

Worm-Like Chain (WLC)

- Important parameter of the WLC:

  Persistence length of a polymer

  Measure for molecule stiffness / the resistance of the chain to bending

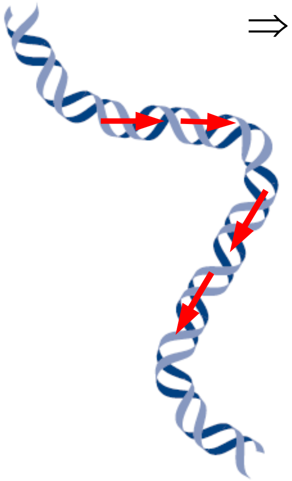
  (length over which correlation of tangent vector directions is lost
  ≈ distance that separates bends)

short persistence length

⇒ more flexible

long persistence length

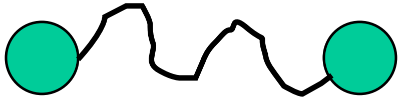
⇒ stiffer



Podgornik, Nature Nanotechnology, 2006

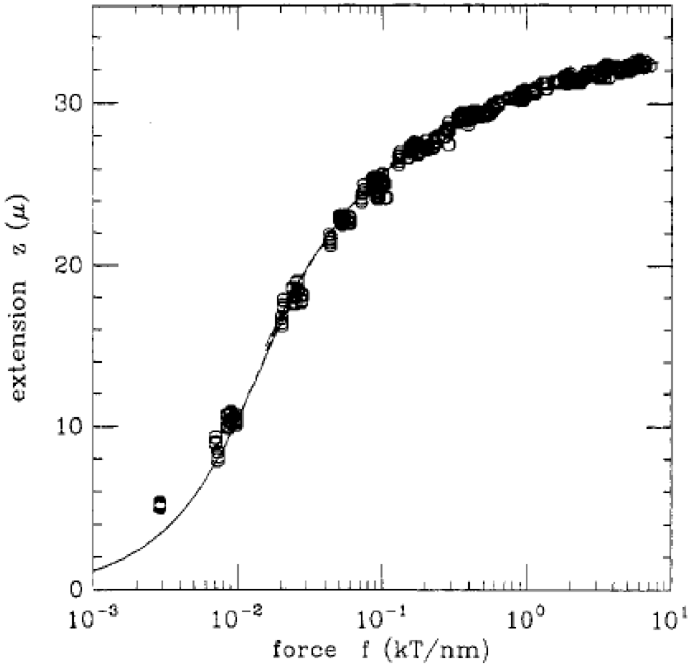
WLC description of DNA

Example: Stretching of DNA



- Simple description of the behaviour of a polymer chain as response to extension force
  - reduction of entropy with straightening

- Length scale longer than persistence length
  - results for L = 32.8 µm
  - persistence length of double helix DNA **$L_P \approx$ 50 nm (150 bp)**



Marko and Siggia, Macromolecules, 1995

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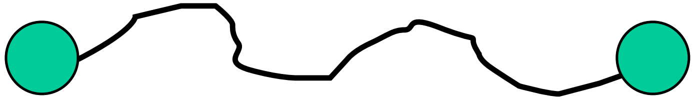

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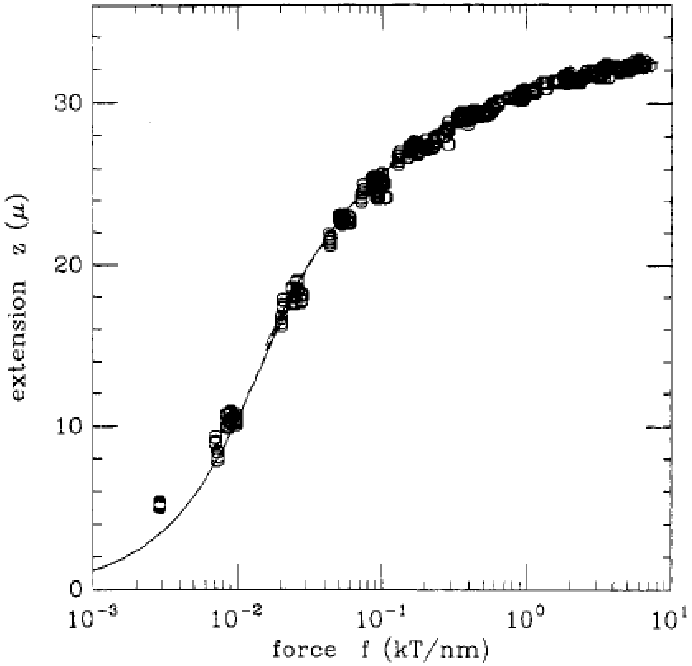


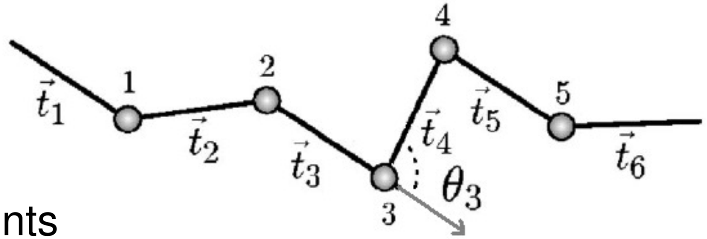
Marko and Siggia, Macromolecules, 1995

WLC description of DNA

Bending energy of a conformation

Chain of segments of length ℓ with **harmonic energy** cost per segment:

$$\boxed{E_{\mathrm{WLC}}(\theta) = \tfrac{1}{2}\, k_{\mathrm{B}}T\, (L_P/\ell)\, (\theta_i)^2}$$



modified according to Wiggins and Nelson, Phys Rev E, 2005

θ: bending angle between segments

L_P: persistence length of DNA

k_BT: thermal energy

Energy rises quadratically with bending angle

⇒ **Boltzmann distribution**: large bending angles will occur rarely

Motivation of experiments

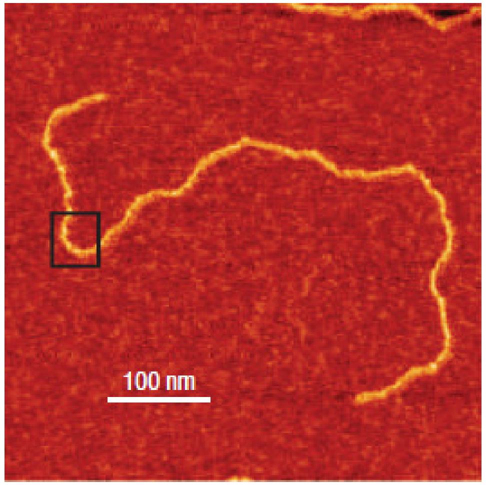
- Previous studies of DNA mechanics focus on longer length scales
- Cyclization studies of **short DNA fragments**: short circles form much more easily than expected from WLC theory
- **Test WLC** model on short length scales (few nanometers)

⇒ Measure model predicted **bending angle frequencies** directly in equilibrium DNA conformations

⇒ Frequencies of bending angles correspond to **bending energy function**

Experimental approach

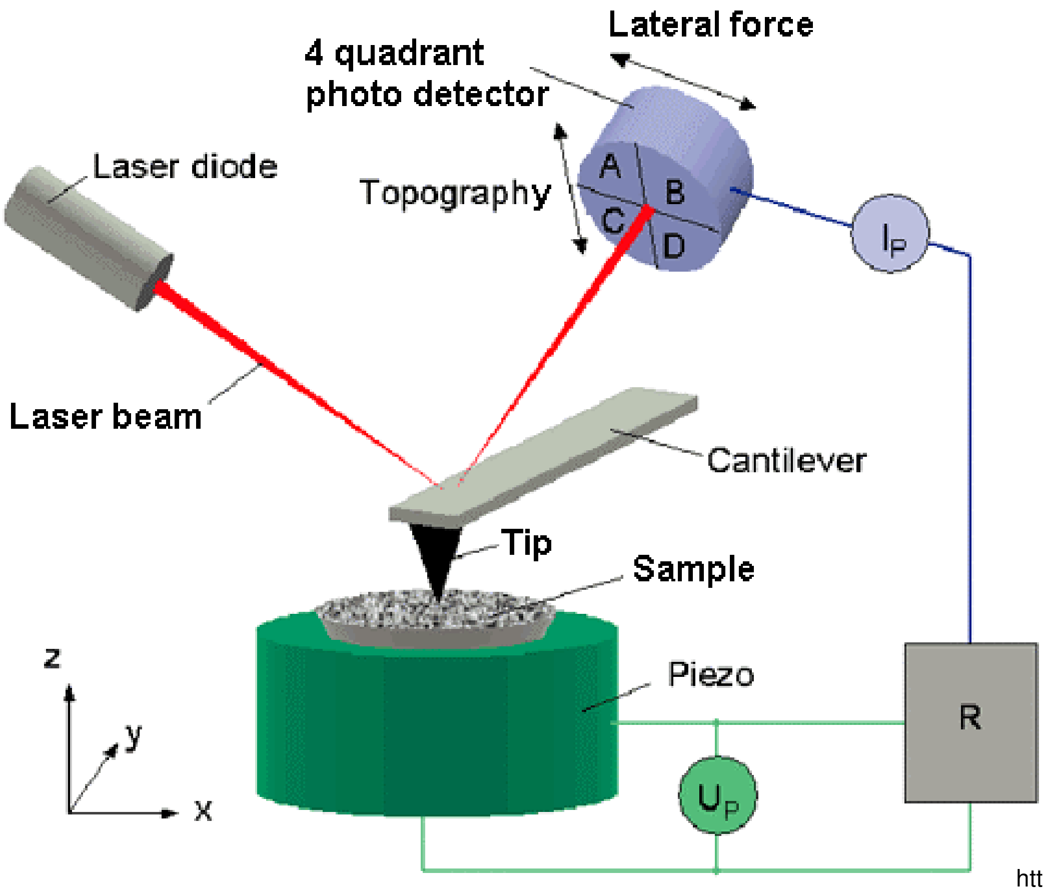
- High-resolution **atomic force microscopy**
- 2743 bp **linear double-stranded DNA** with random sequence, **adsorbed to surface**
- **Two-dimensional** movement possible
- Assume **equilibrium conformation** of adsorbed molecules



Wiggins, Nature Nanotechnology, 2006

Atomic Force Microscopy (AFM)

Principle



Characteristics

- topographical images
- near angstrom resolution (0.1 nm)

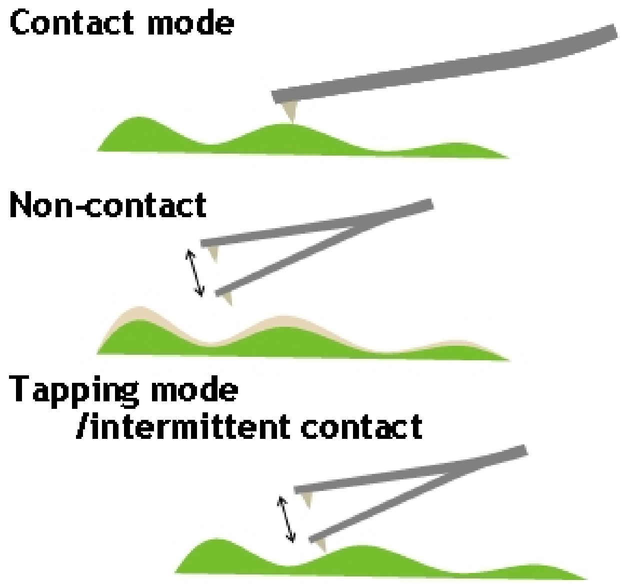
Biological application

- imaging of living cells
- measurement in physiological buffers

http://www.nanomat.de/datenblaetter/42000.htm

AFM operation modes

Typical modes



http://en.wikibooks.org/wiki/Nanotechnology/AFM

- Tapping mode usually used for soft biological materials

Experimental and simulated AFM data

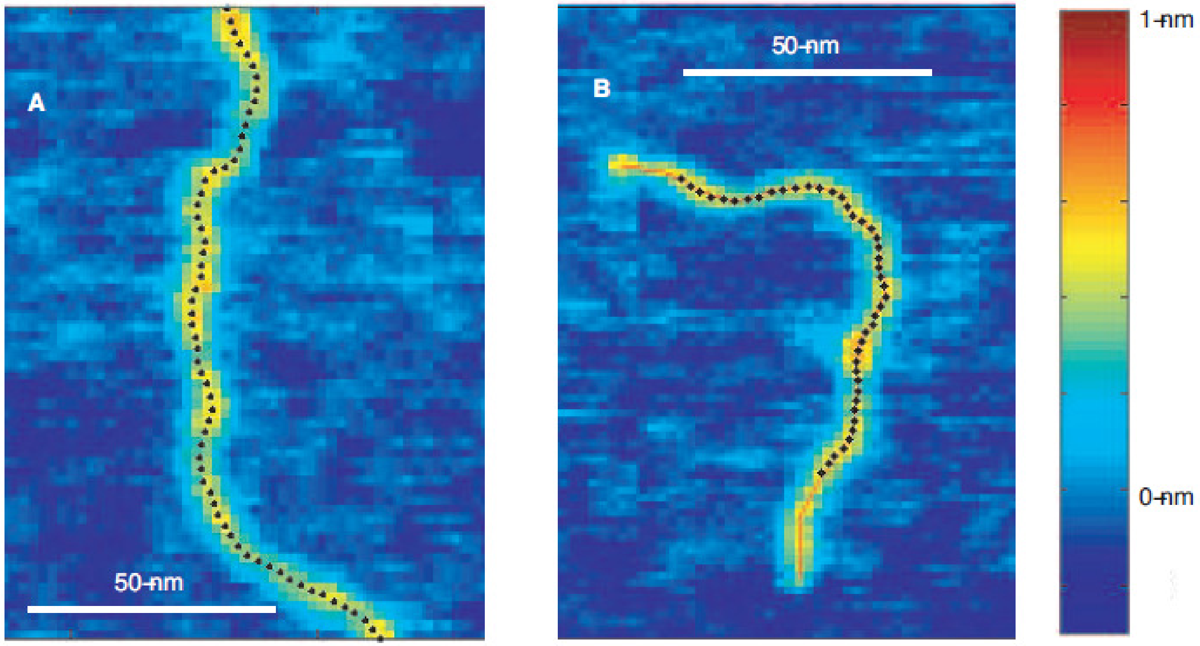
- Linear DNA molecules adsorbed to mica (silicate surface) with different concentrations of Mg^{2+} ions
- AFM measurement in tapping mode **in air**
  - height profile of equilibrium conformations
- Simulation of AFM data on the basis of WLC
  - generation of WLC conformations, 2D chains with bends
  - bending angle distribution according to WLC prediction
  - convertion to simulated AFM images
  - same analysis as experimental conformations

⇒ Exclude that conformations are artifacts of AFM measurement

Image analysis

Automatic tracing of DNA contours

- Set points with a distance of 2.5 nm on the contour
- Length ℓ of segments 2.5 nm

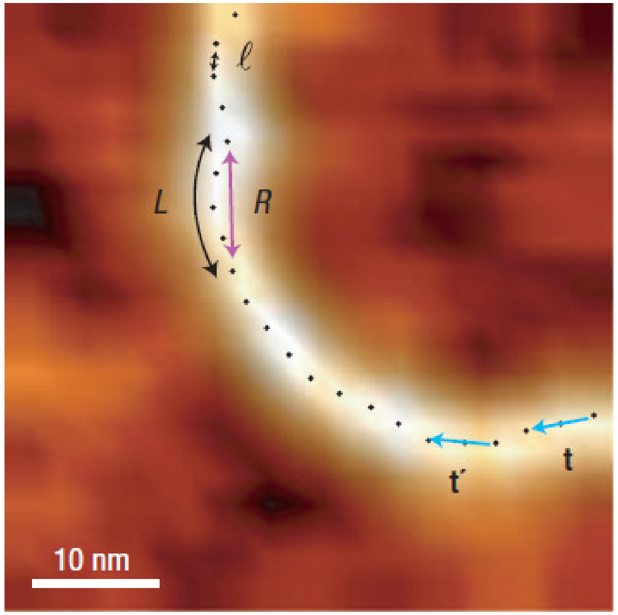


experimental image **simulated image**

Wiggins, Nature Nanotechnology, 2006

Measurement of angles

- Two points of chain separated by the **contour length *L***
- **Tangents** of these points t and t‘
- Angle between two tangents
- Measured for all points on complete chain and different *L*



Example: *L* = 3 x 2.5 nm
contour length between t and t‘

Wiggins, Nature Nanotechnology, 2006

Statistical evaluation

- **Angle Distribution $G(\theta; L)$**:
  Probability distribution of angles between tangents
  - angles between tangents t_i and t_{i+1} separated by L measured for many molecules
  - histogram of angles
- Curve measures the **bending energy $E(\theta)/k_B T$** ($E(\theta)$ in units of $k_B T$) of the segments of length L

  Bending angle distribution:

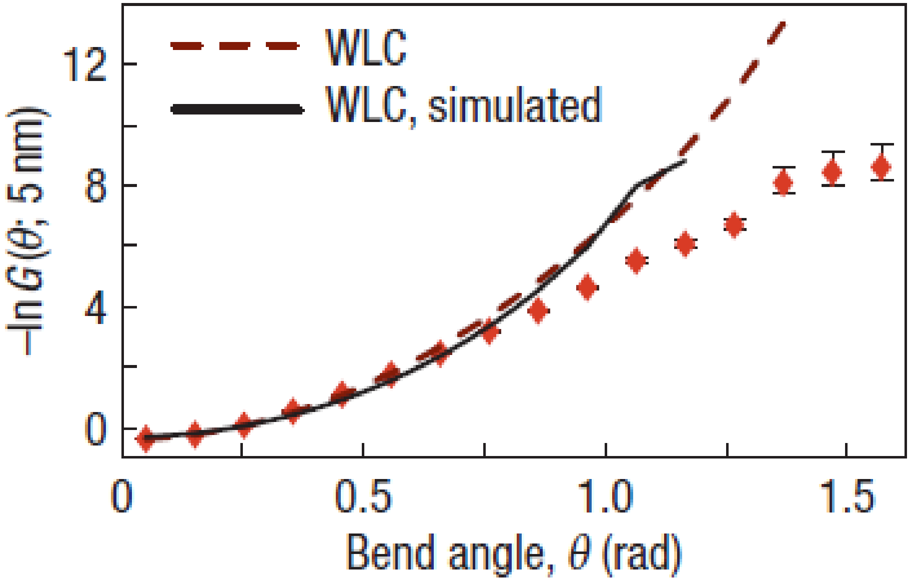
  $$g(t_{i+1}|t_i) = q^{-1} \exp[-E(\theta_i)/k_B T]$$

  Negative logrithm:

  $$-\ln(g) = \ln(q) + E(\theta_i)/k_B T$$

Results

Dots: measured angle distribution for ***L* = 5 nm** (negative logarithm)



Wiggins, Nature Nanotechnology, 2006

- Distribution calculated from theoretical model (WLC) does not match the measured data for large angles
- Values of measured data are much lower (= higher frequency of large angles)
- Simulated WLC data match theoretical curve

Results

- Segments with large bends much more frequent than predicted by WLC
  - large bends (≥ 1.1 rad) 30 times more frequent
- Elastic energy for high bending angles must be lower than predicted
- Energy function is almost linear

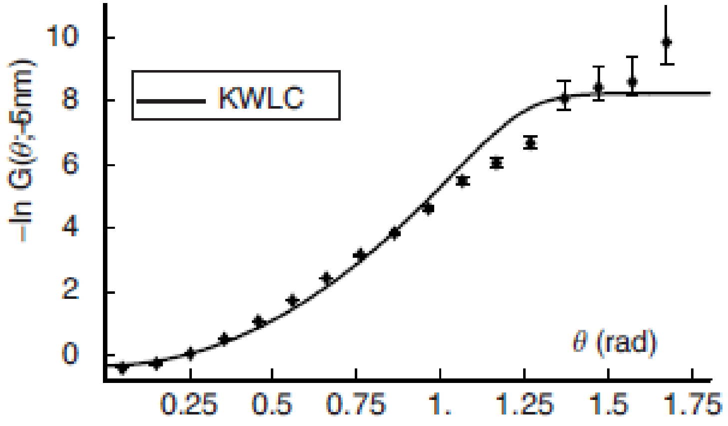
⇒ Hooke‘s law does not apply for the elastic energy on small length scale

Other experiments

- Cyclization studies by Cloutier and Widom
- Sharply looped DNA highly bendable and also **highly twistable**, DNA more flexible than predicted by models
- Theories of DNA flexibility:

Melted bubbles, single-stranded regions with high flexibility, faciliate loop formation

Kinkable worm-like chain
Extension of the WLC that includes spontaneous kink formation when curvature is high



Wiggins, Nature Nanotechnology, 2006

⇒ Local alternative conformations with high flexibility

Develoment of new model

- Equation that fits measured data
- **Linear ‘sub-elastic chain‘ (LSEC)** model

$$\boxed{E_{\mathrm{LSEC}}(\theta) = \alpha\, |\theta|\, k_{\mathrm{B}}T}$$

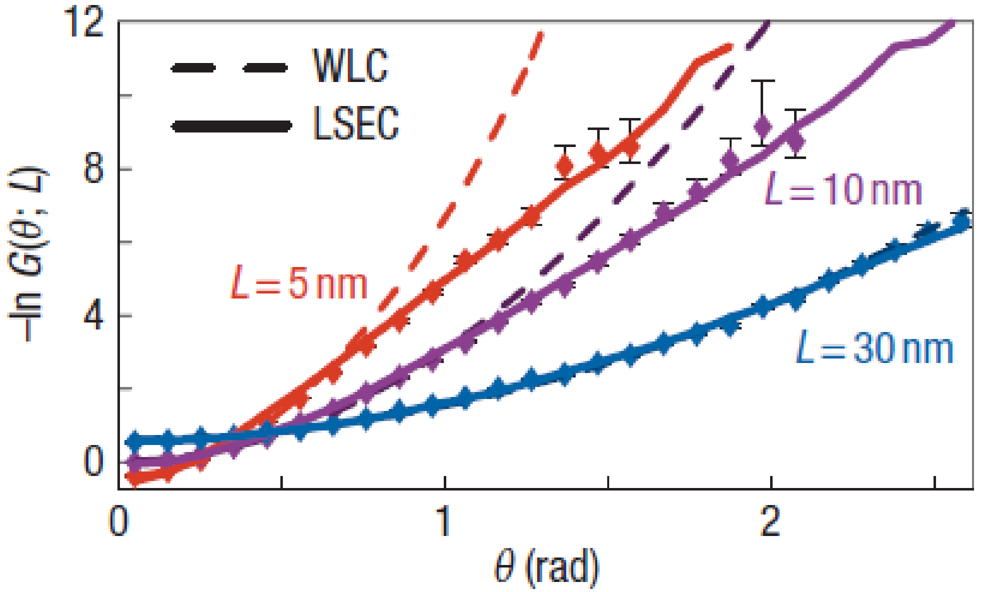
θ: bending angle between segments

α: dimensionless constant, dependent on ℓ

$k_B T$: thermal energy

Non-harmonic (linear) energy function, “softer“ for sharp bending

LSEC on long scale

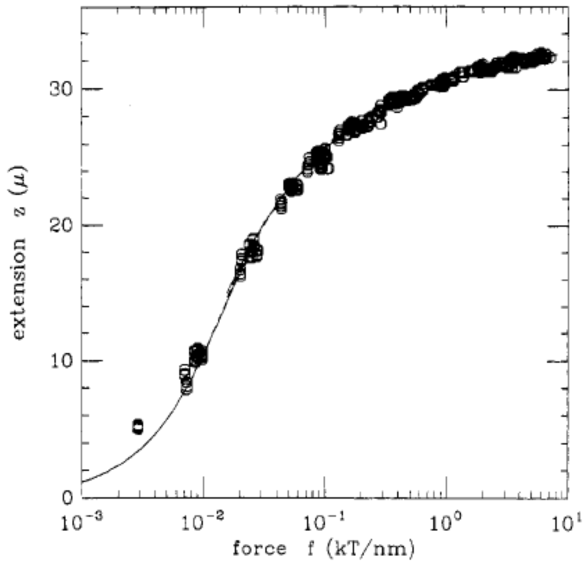


Wiggins, Nature Nanotechnology, 2006

- WLC describes behaviour for **long** contour length correctly
- LSEC model fits data on **all** length scales

LSEC on long scale

- Application of LSEC on other experimental data
- Force-extension experiment correctly described by LSEC



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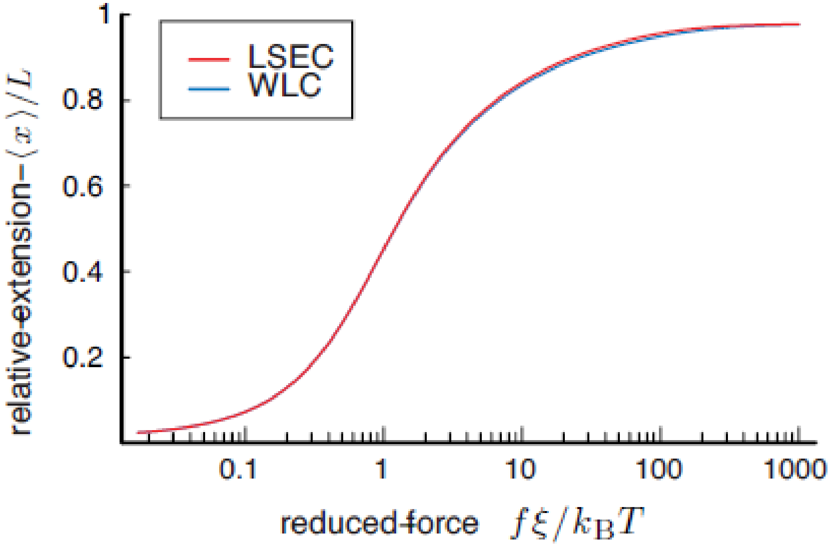

Wiggins, Nature Nanotechnology, 2006

Outlook

Improvement of new model

- Include twisting properties in LSEC model
- Test sequence dependence of DNA elasticity

Application

- Relevance of model to other semiflexible biopolymers
  - Is DNA the only polymer with higher elasticity on short scales?

Criticism

- Further/other experiments that support findings
  (Exclude that results are an artifact of the measurement)

Summary

- DNA flexibility on short length scales is important in many biological processes (e.g. nucleosome formation).
- Using high-resolution AFM the bending behaviour of DNA in such scales can be measured directly.
- Sharp bends in DNA on short length scales are much more frequent than predicted by the WLC model.
- A simple model can describe both the bending behaviour of DNA on short scales and the long-length scale observations (force-extension curve of DNA)

Literature

- Wiggins P.A., Van der Heijden T., Moreno-Herrero F., Spakowitz A., Phillips R., Widom J., Dekker C. and Nelson P.C. (2006) High flexibility of DNA on short length scales probed by atomic force microscopy. *Nature Nanotechnology* **1**, 137-141.
- Cloutier T.E. and Widom J. (2005) DNA twisting flexibility and the formation of sharply looped protein-DNA complexes. *Proc. Natl. Acad Sci.* **102**,3645-3650.
- Podgornik R. (2006) Polymer Physics: DNA off the hook. *Nature Nanotechnology* **1**, 100-101.
- Wiggins P.A. and Nelson P.C. (2006) Generalized theory of semiflexible polymers. *Phys. Rev. E* **73**, 031906.
- Marko J.F. and Siggia E.D. (1995) Streching DNA. *Macromolecules* **28**, 8759-8770.
- Swigon D., Coleman B.D., and Olson W.K. (2006) Modeling the Lac repressor-operator assembly: The influence of DNA looping on Lac repressor conformation. *Proc. Natl. Acad. Sci.* **103**, 9879–9884.
- Wiggins P.A., Philips R. and Nelson P.C.(2005) The exact theory of kinkable elastic polymers. *Phys. Rev. E* **71**, 021909.
- Yan J., Marko J.F. (2004) Localized single-stranded bubble mechanism for cyclization of short double helix DNA. *Phys. Rev. Lett.* **93**, 108108.
- Jena B.P. and Hörber J.K.H. (Editors) *Force microscopy: applications in biology and medicine* (J. Wiley & Sons, Hoboken, New Jersey, 2006)

Additional figues:

- http://www.jyi.org/research/re.php?id=419
- http://www.nanomat.de/datenblaetter/42000.htm
- http://en.wikibooks.org/wiki/Nanotechnology/AFM

...Thank you for your attention!