Evanescent wave scattering: a high resolution probe for conformational changes of biomolecules

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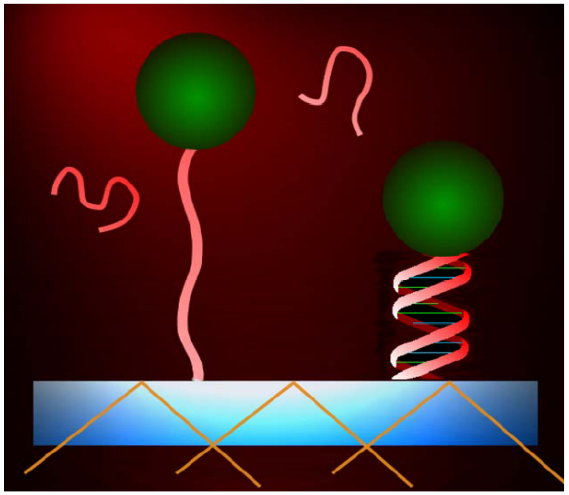
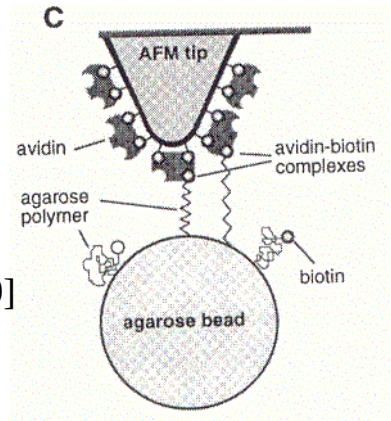
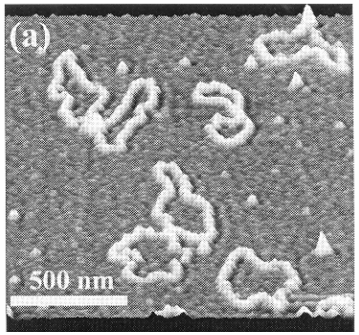


Mechanical probes for single molecules



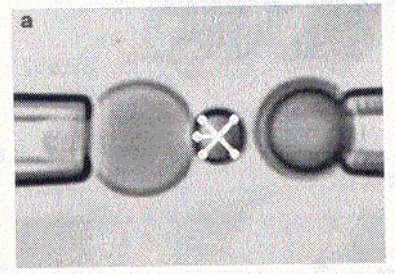
[*Wong et al.* 99]

stiff probes (AFM)
→ spatial resolution



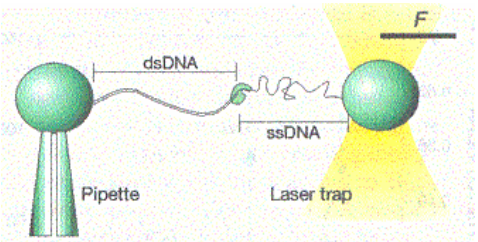
[*Viani et al.* 99]

soft probes (bio-membrane)
→ small forces

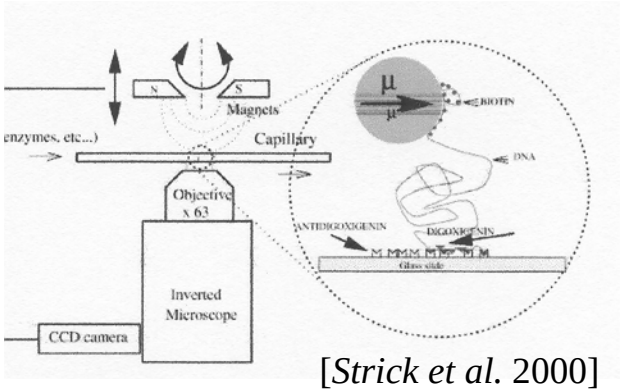


[*Merkel et al.* 99]

Intermediate
(optical tweezers /
magnetic force)

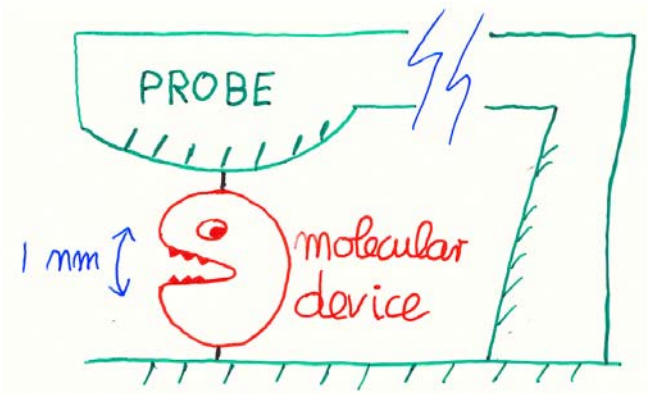


[*Wuite et al.* 2000]



[*Strick et al.* 2000]

nm scale probes & thermal noise



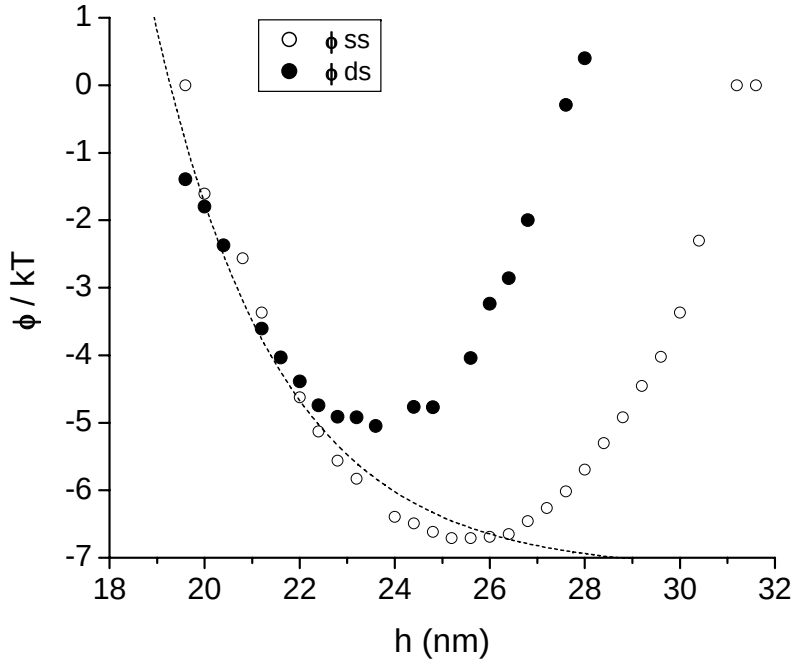
Thermal motion of the probe:

$$\frac{1}{2}\mathrm{K} \; < x^2 > = \frac{1}{2} kT$$

$$\Rightarrow \quad < x^2 > = kT / K$$

$$< F^2 > = \; < (K\, x)^2 > = K\, kT$$

Fundamental limitation: $< F^2 >^{1/2} \; < x^2 >^{1/2} = \; kT$ $\qquad kT \approx 4 \;\; pN \times nm$

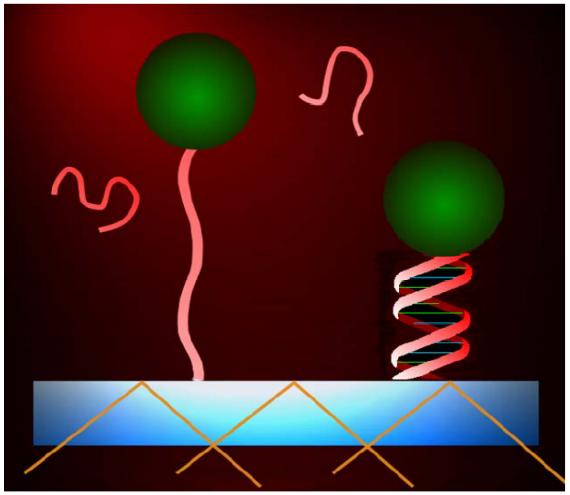


Bead-slide interaction:
ss and ds tether

Single molecule bead-based assay

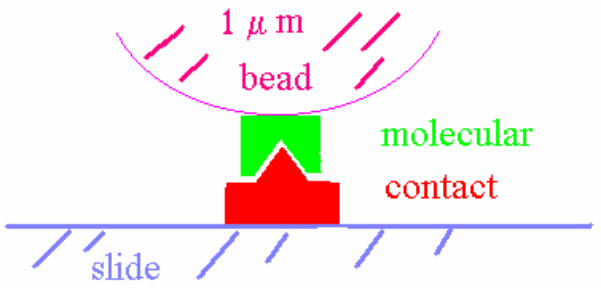
[ChemPhysChem **7**, 555 (2006)]

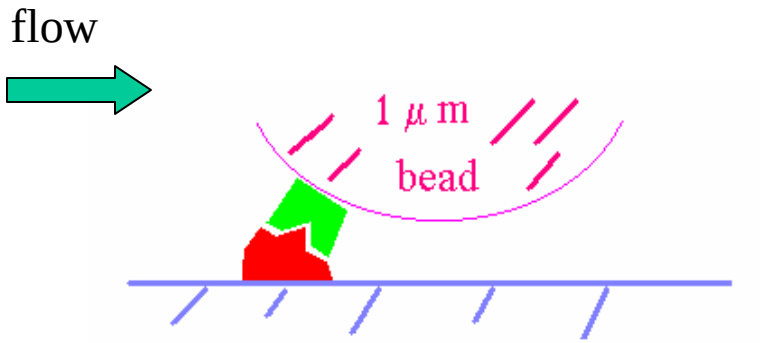
• make a single molecular contact (protein, DNA oligomer)



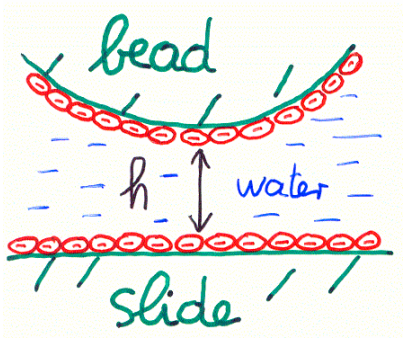
• nm scale conformational changes in the tether are detected by monitoring the displacement of the bead relative to the slide

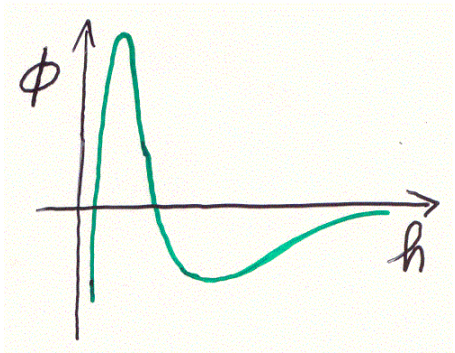
• force can be applied on the contact (flow / magnetic bead)





Bead-slide interaction:





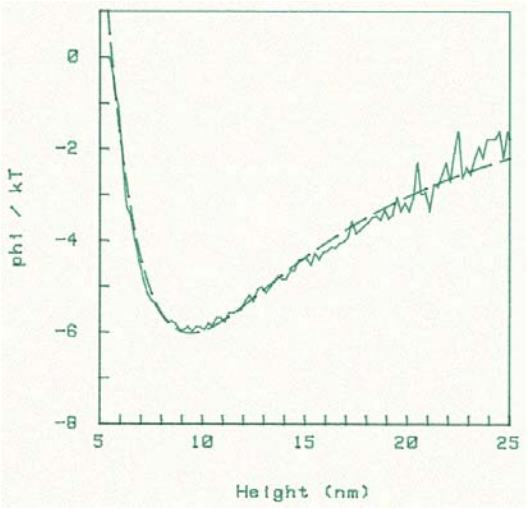
DLVO potential:

$$\phi = A e^{-h/\Delta} - \frac{B}{h}$$

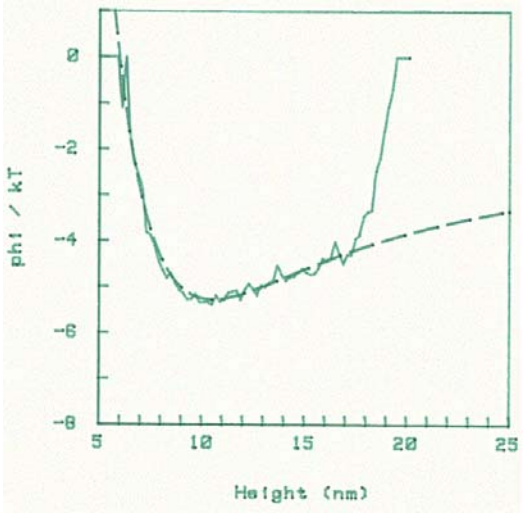
Δ = Debye length
$A \propto R$, surface charge
$B \propto R$, Hamaker const.

Measured interaction potentials
6 μm diam. glass bead

"free" bead



tethered bead

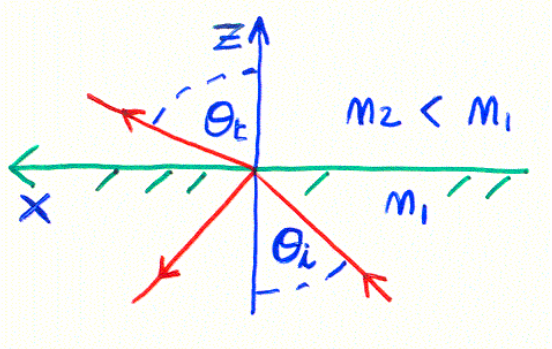


→ bead can be tethered to the surface through a single molecular contact and still move in the secondary minimum of the DLVO potential

Measured Hamaker constant
$A / R = (1.0 \pm 0.3) \times 10^{-14}$ ergs

Evanescent wave scattering

[*Prieve 90, Zocchi 96*]



$$E_t = A\,e^{i(n_2\mathbf{k}_t\cdot\mathbf{x}-\omega t)} \qquad \sin\theta_t/\sin\theta_i = n_1/n_2$$

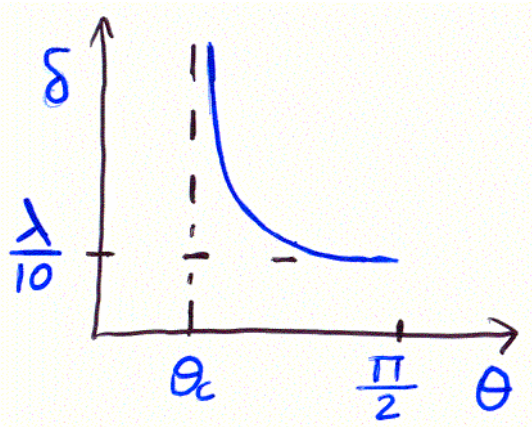
$$E_i = A\,e^{i(n_1\mathbf{k}_i\cdot\mathbf{x}-\omega t)}$$

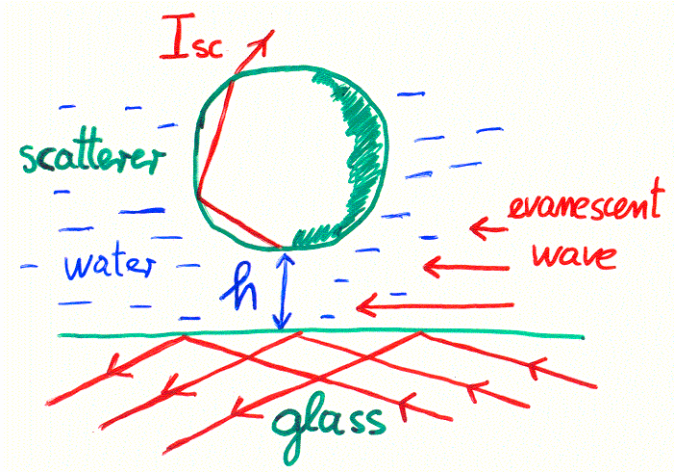
critical angle: $\sin\theta_t = 1 \quad (\sin\theta_c = \frac{n_2}{n_1})$

$$\theta_i > \theta_c \;\Rightarrow\; \sin\theta_t > 1 \Rightarrow\; \cos\theta_t = \sqrt{1-\sin^2\theta_t} = i\sqrt{\sin^2\theta_t - 1}$$

$$E_t = A\,e^{i(n_2 k \sin\theta_t x-\omega t)}e^{-n_2 k\sqrt{\sin^2\theta_t - 1}\,z}$$

$$2\delta = \frac{1}{n_2 k\sqrt{\sin^2\theta_t - 1}} = \frac{\lambda}{2\pi\left[n_1^2\sin^2\theta_i - n_2^2\right]^{1/2}}$$



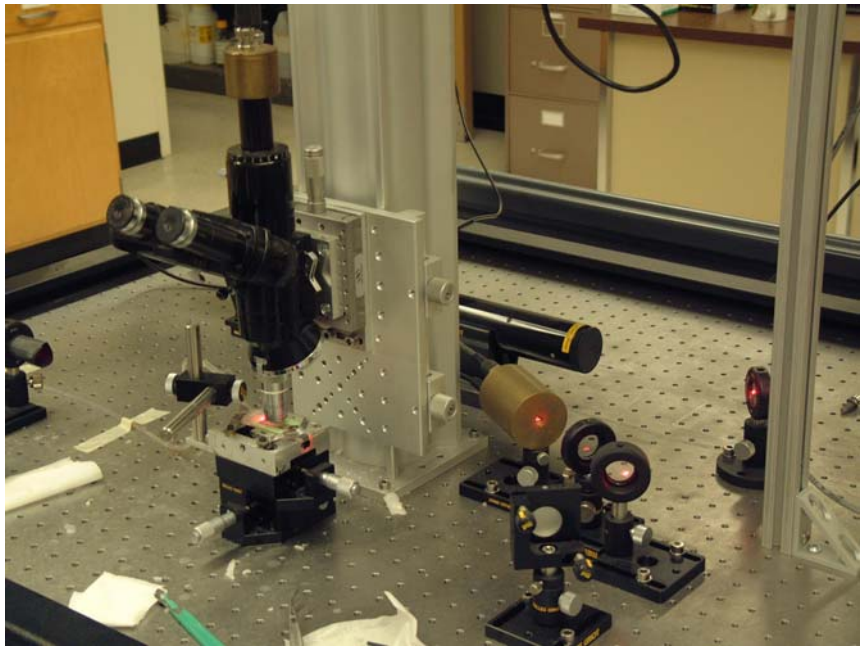


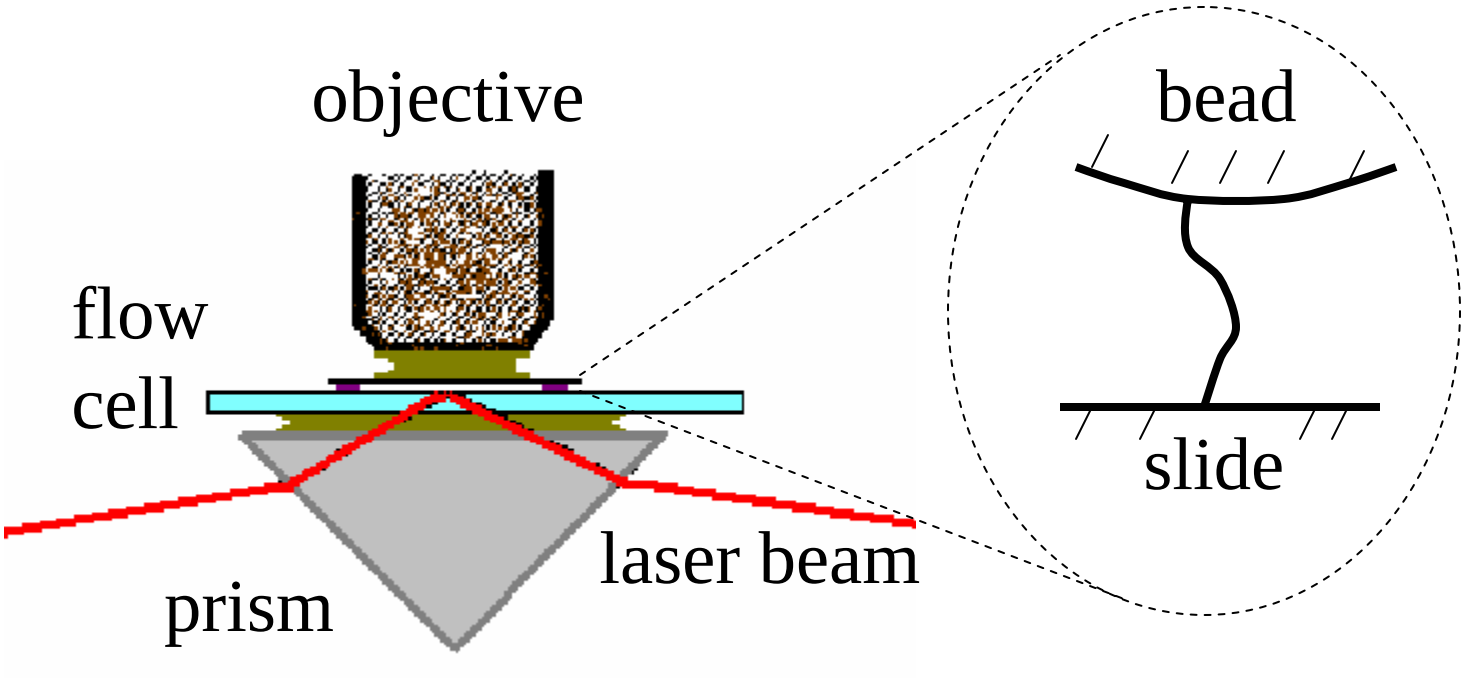
scattered intensity:

$$I_{sc} = I_0\,e^{-h/\delta}$$

$$\delta \approx 86\ nm$$

In practice, a simple setup

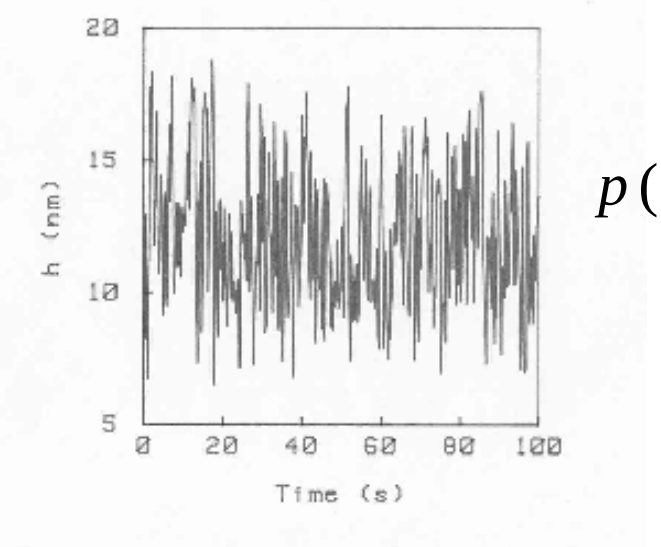




Measurement of single bond rupture force

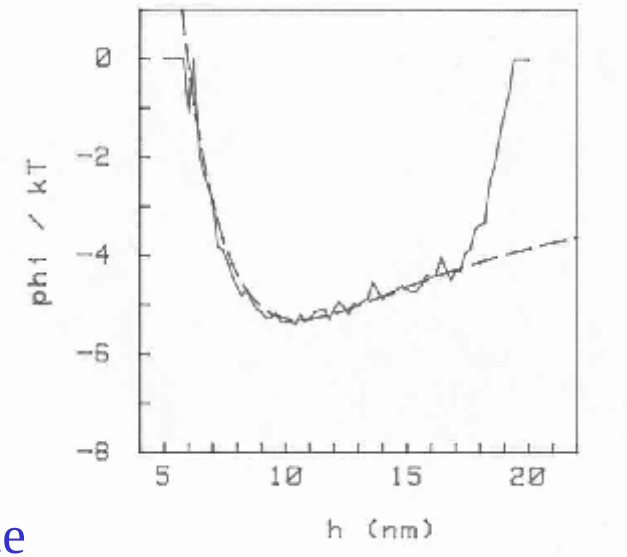
[Biophys. J. **81**, 2946 (2001)]

Thermal motion of bound bead

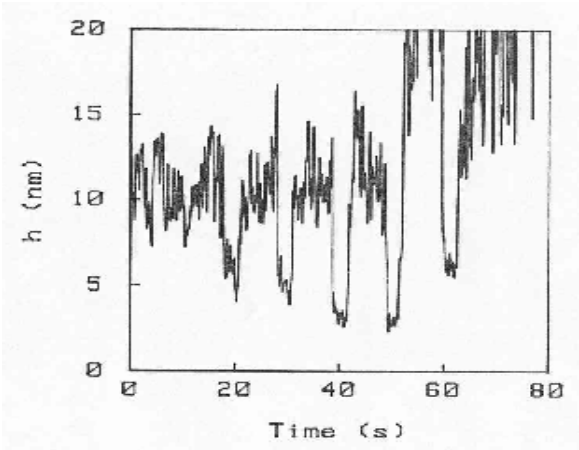


$$p(h) \propto e^{-\phi(h)/kT}$$

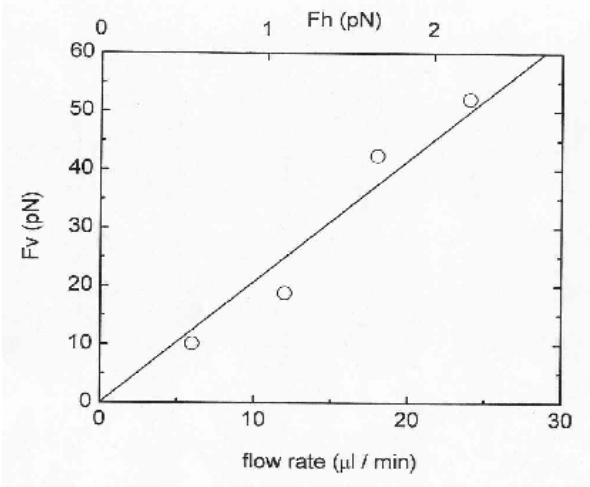
Interaction potential



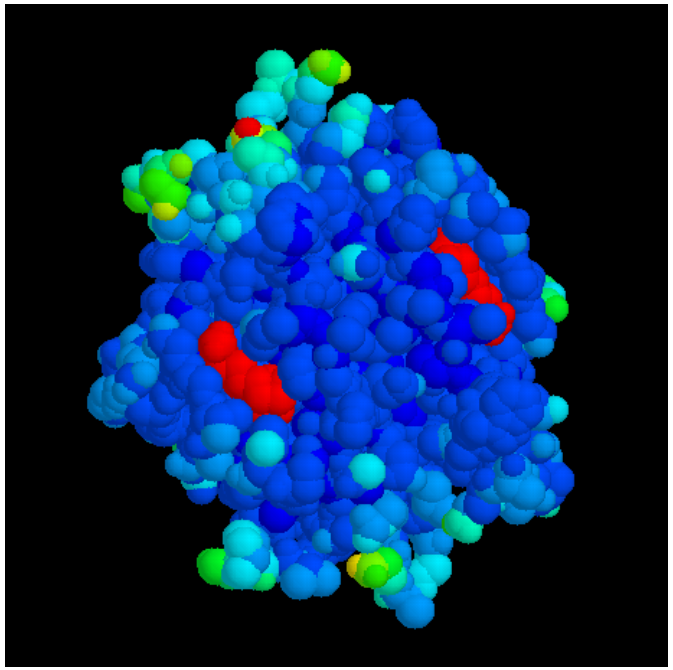
Apply force with a flow



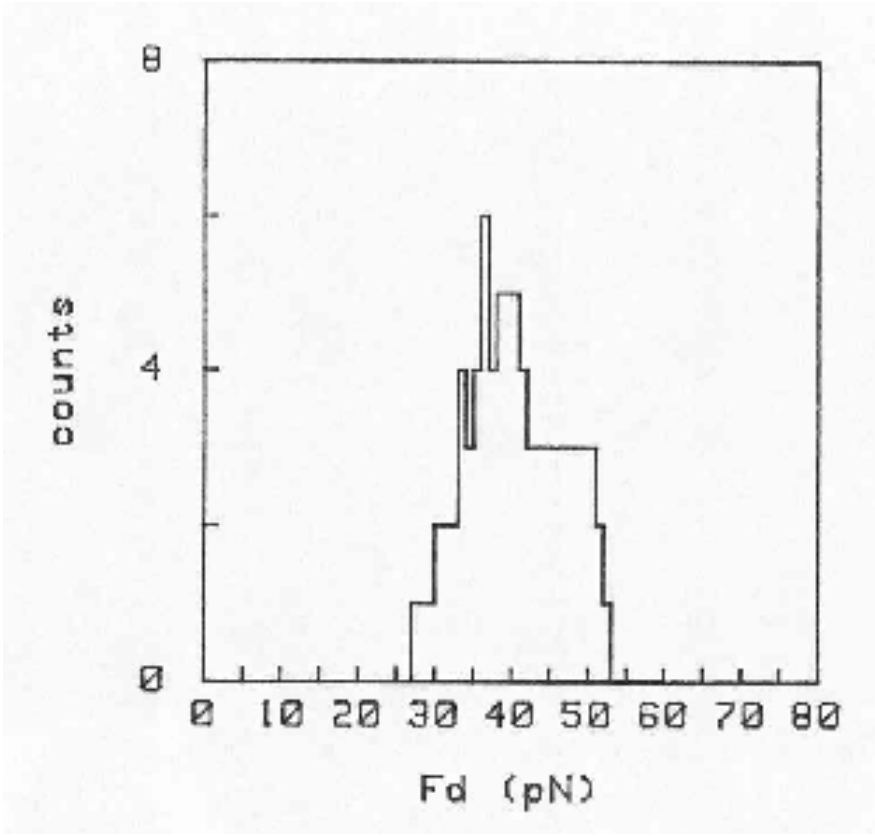
Flow – force calibration



Streptavidin with biotin

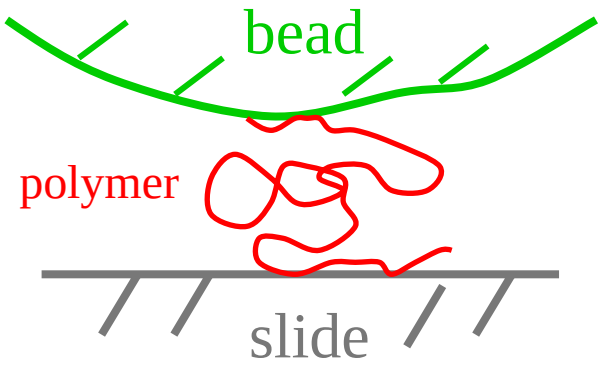


Histogram of bond rupture forces



Entropic spring constant of a single polymer chain

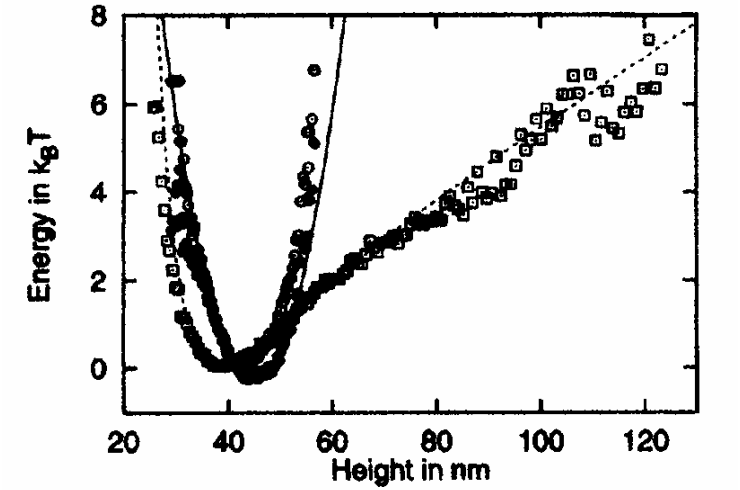
[Phys. Rev. Lett. **79**, 5030 (1997)]



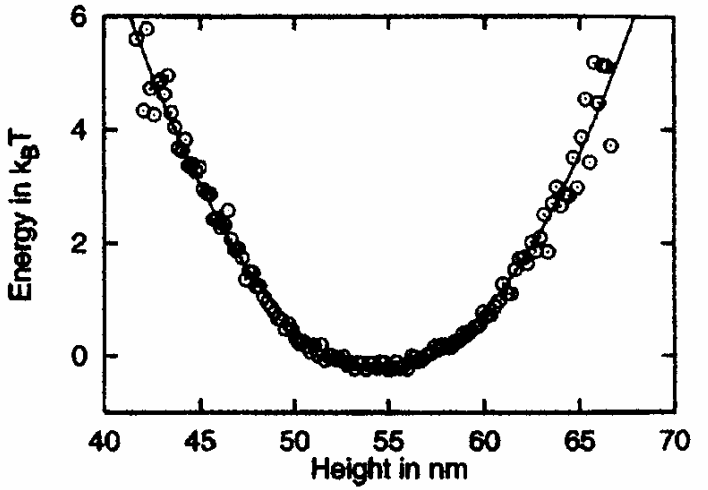
Polystyrene coil (R ~ 50 nm) bridging the bead-slide gap behaves like a spring:

$K \approx 2.9 \times 10^{-4}$ N / m

Interaction potentials with and without tether

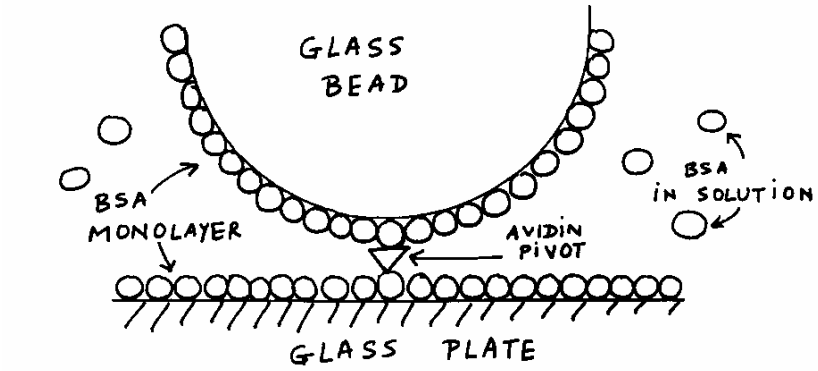


Free energy of the polymer tether



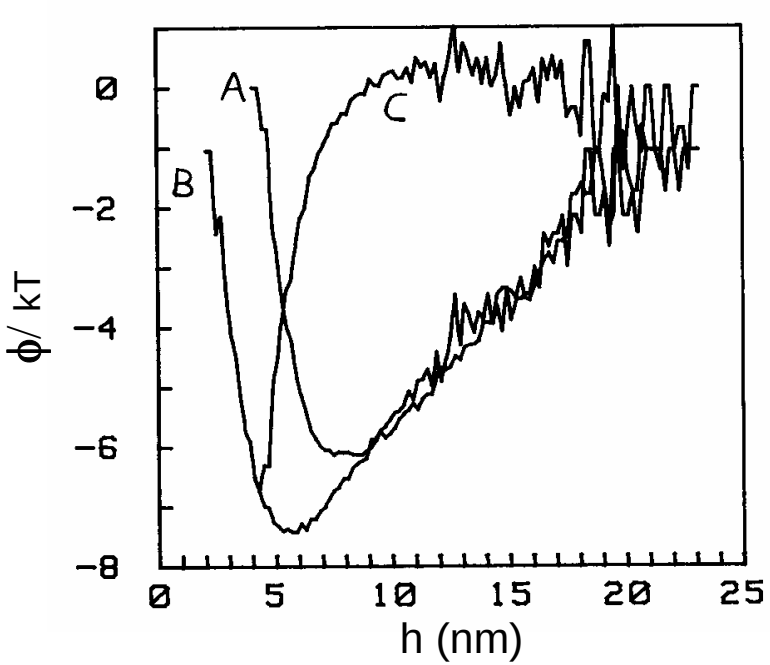
Depletion forces with albumin

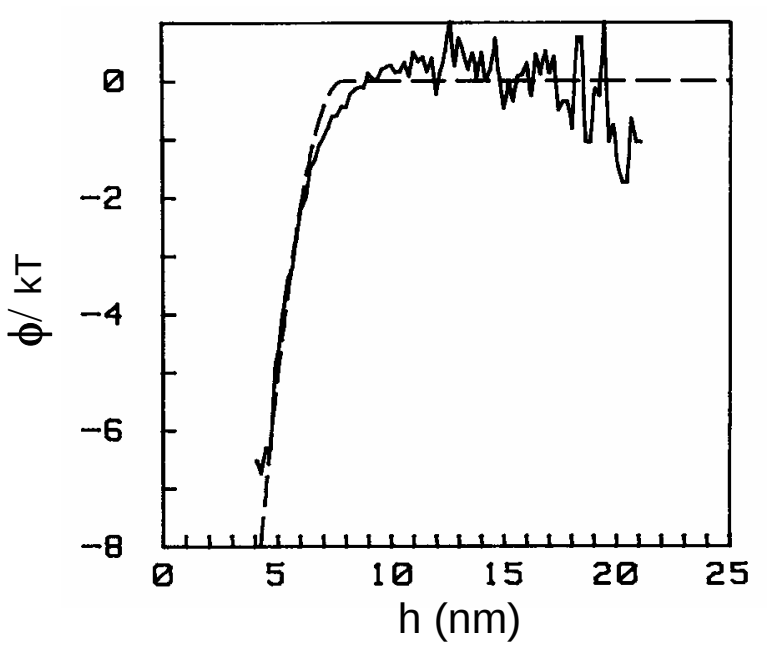
[PNAS **96**, 6711 (1999)]



If albumin does not fit in the gap,
there is an interaction energy
= excluded volume × osmotic pressure

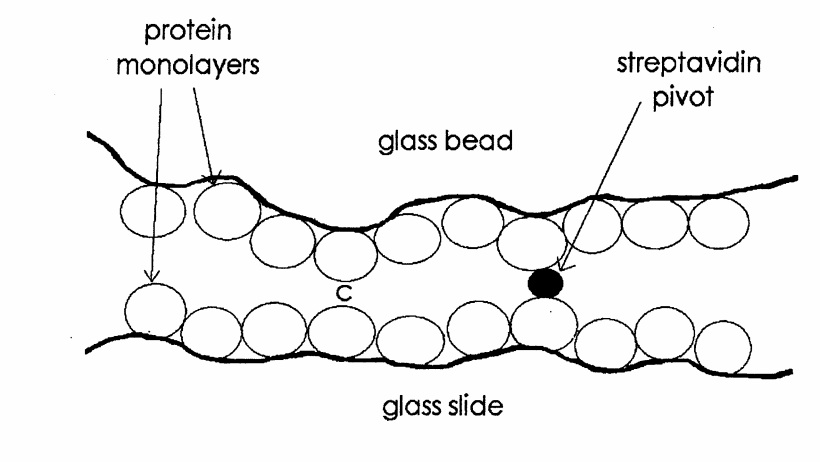
Range of the interaction
= size of the albumin molecule

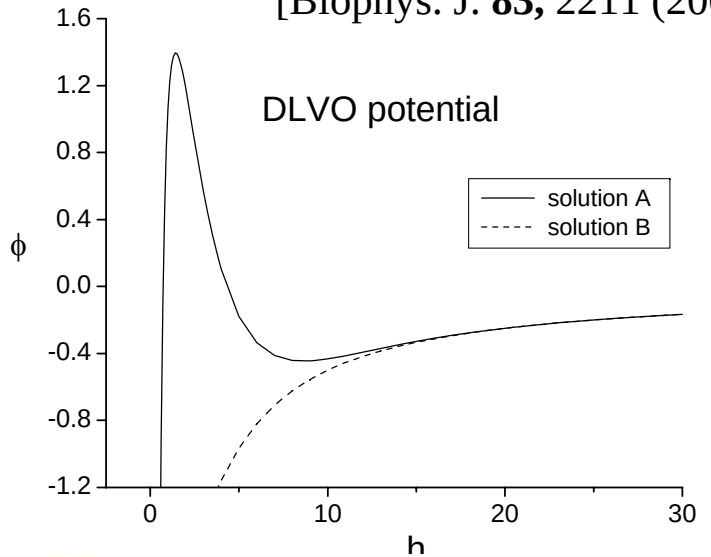


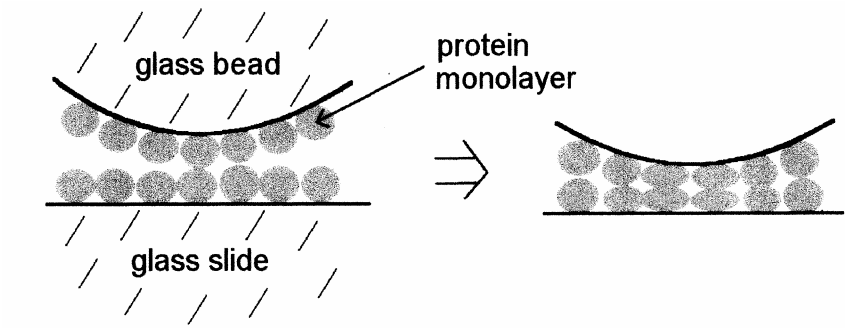


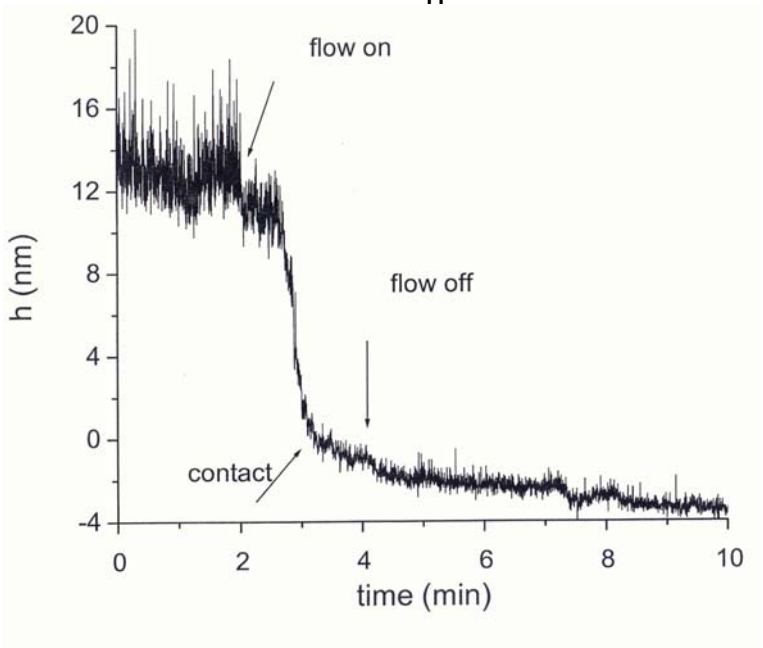
Mechanical deformation of proteins: squeezing a small region (~ 10 molecules) of a protein monolayer

[Biophys. J. **83,** 2211 (2002)]

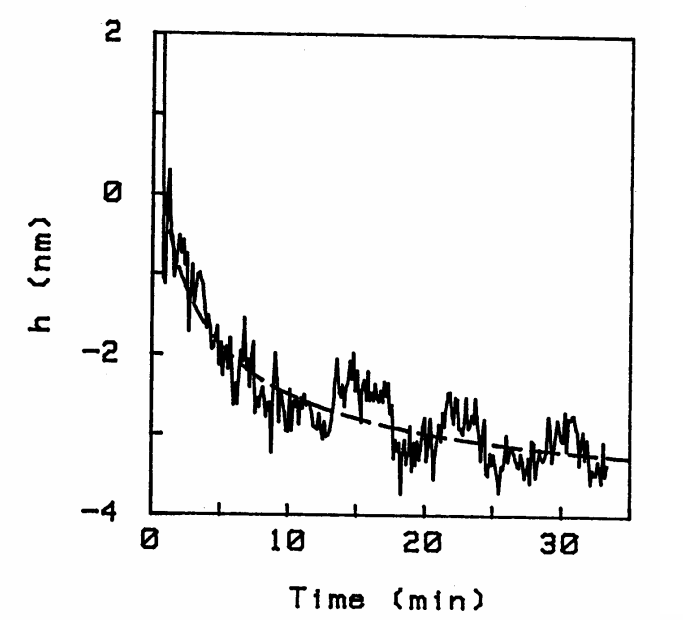


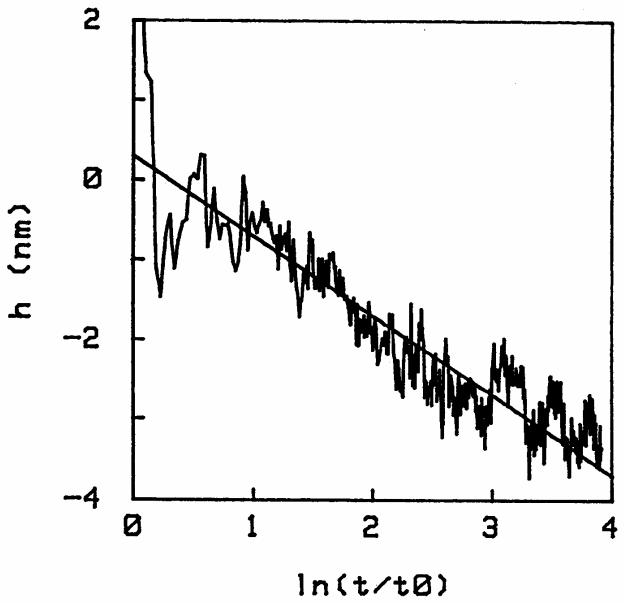




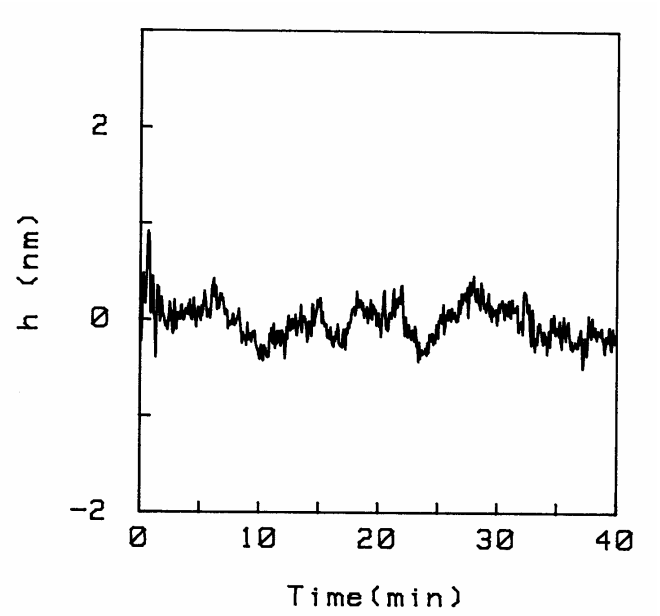


Slow dynamics in the deformation of albumin





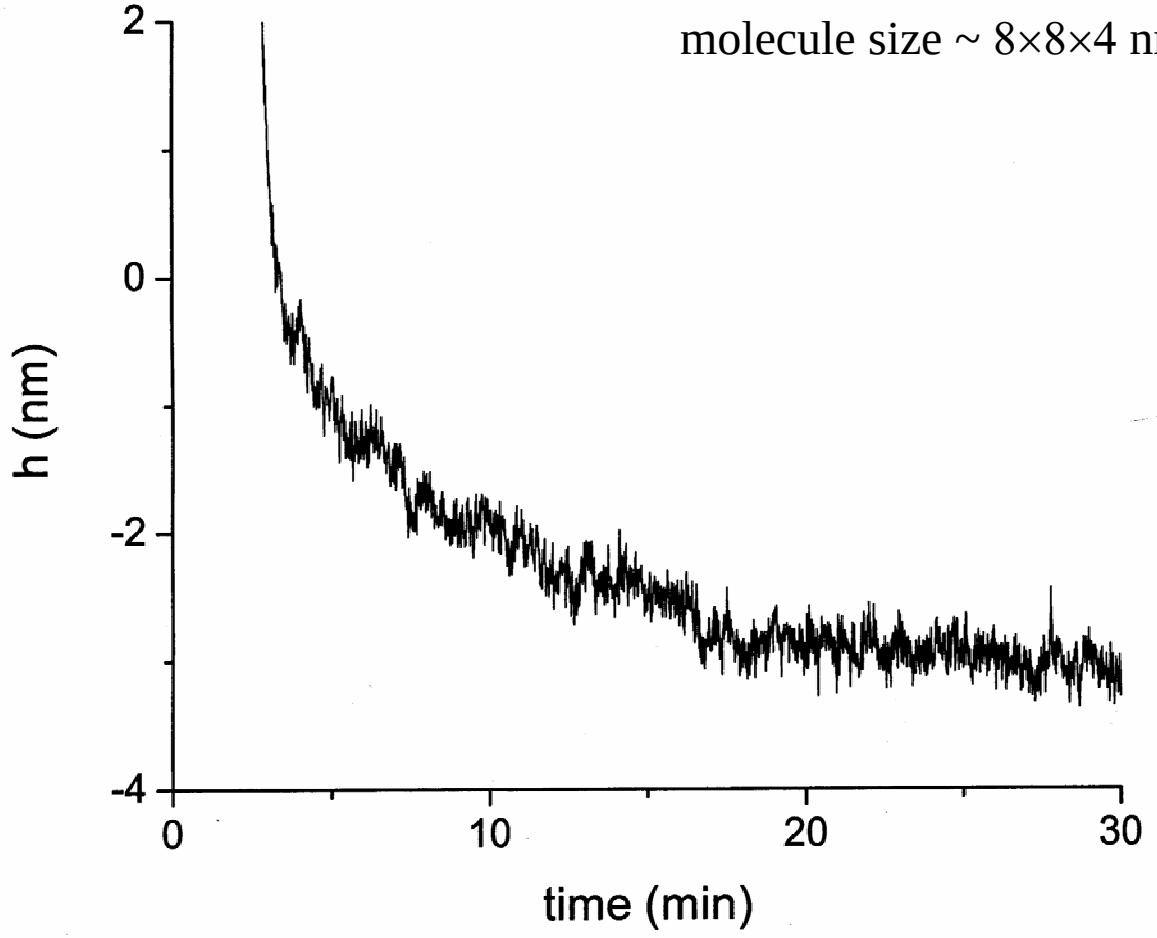
Control (no protein on the surfaces)



- Large deformations (strain ~ 0.37)
- Finite shear modulus
- Slow (logarithmic) dynamics

→ creep !

Average of 5 independent experiments with BSA monolayers

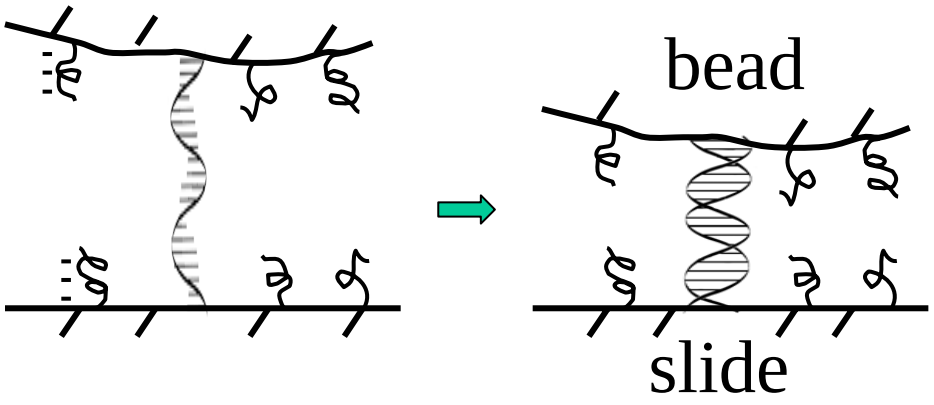


Protein behaves like a solid (finite shear modulus)
Logarithmic dynamics (creep).

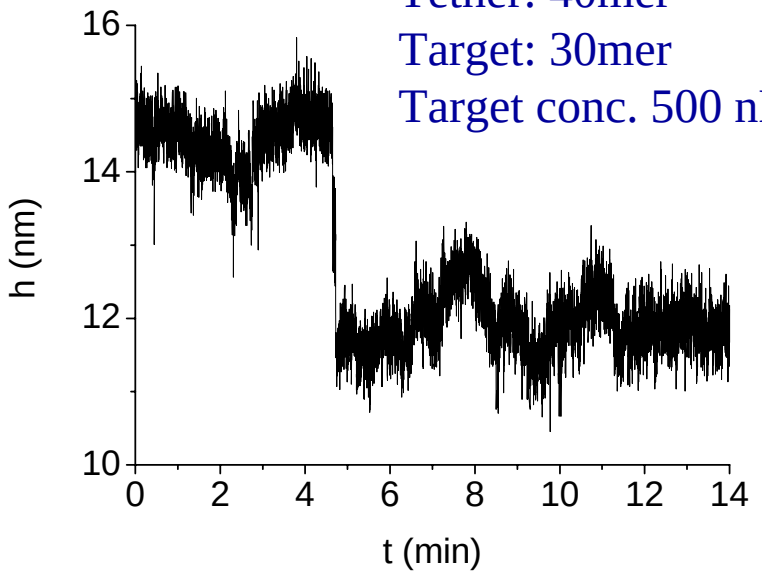
Detecting nm scale conformational changes

I. DNA hybridization assay (single tether)

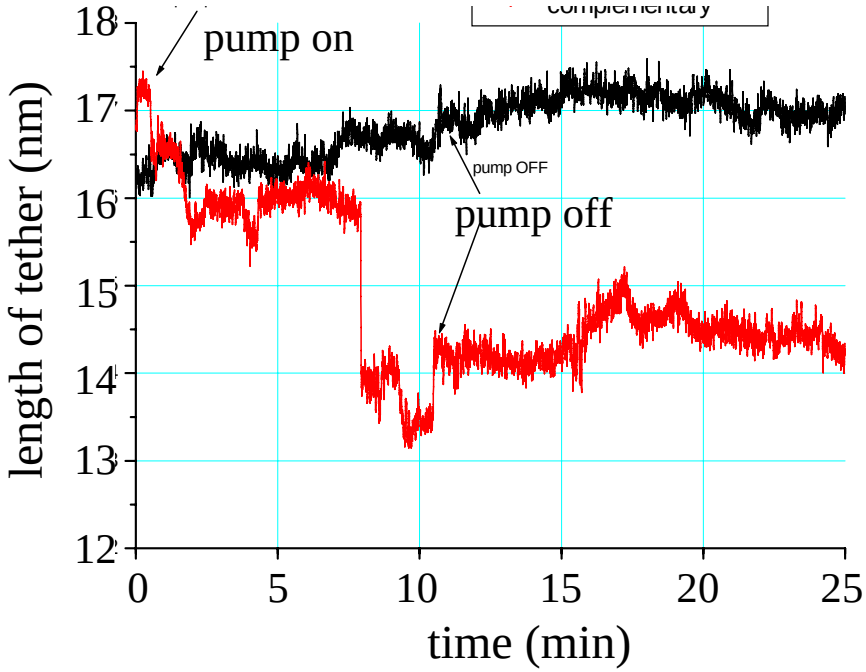
[PNAS **100**, 7605 (2003)]



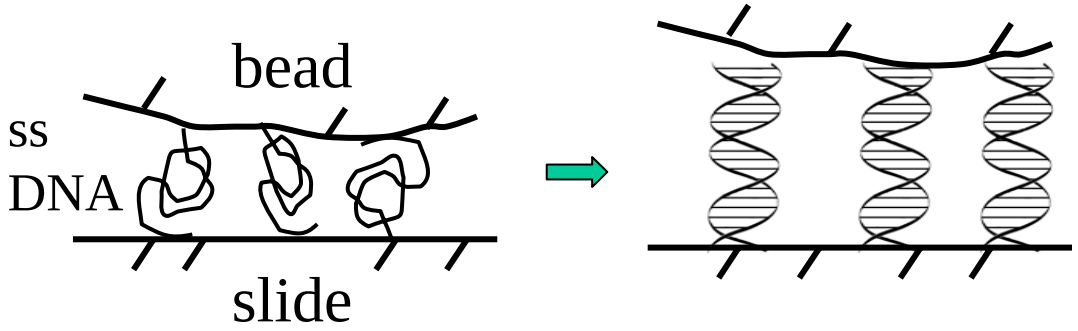
Tether: 40mer
Target: 30mer
Target conc. 500 nM

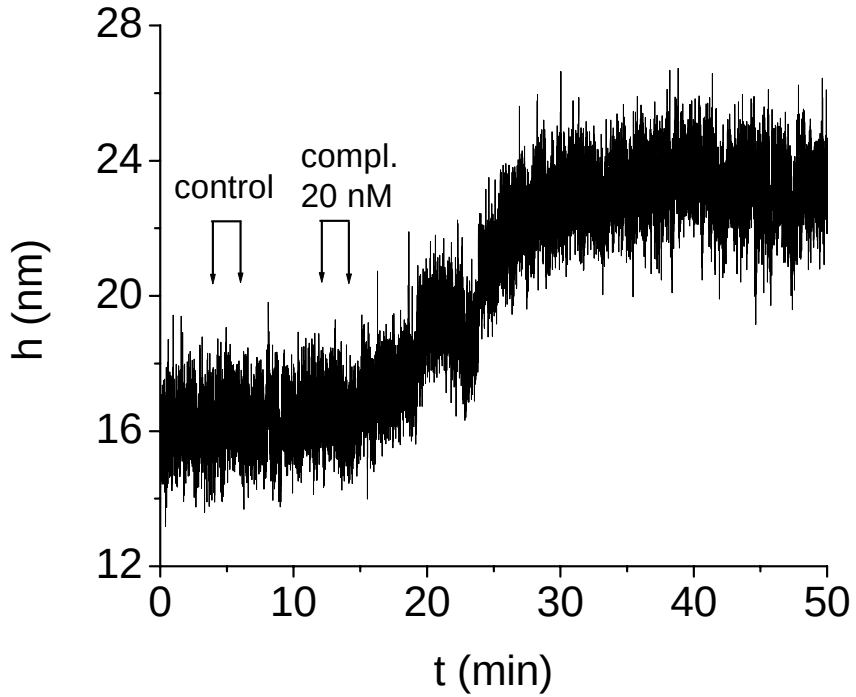


Tether: 30mer
Target: 30mer



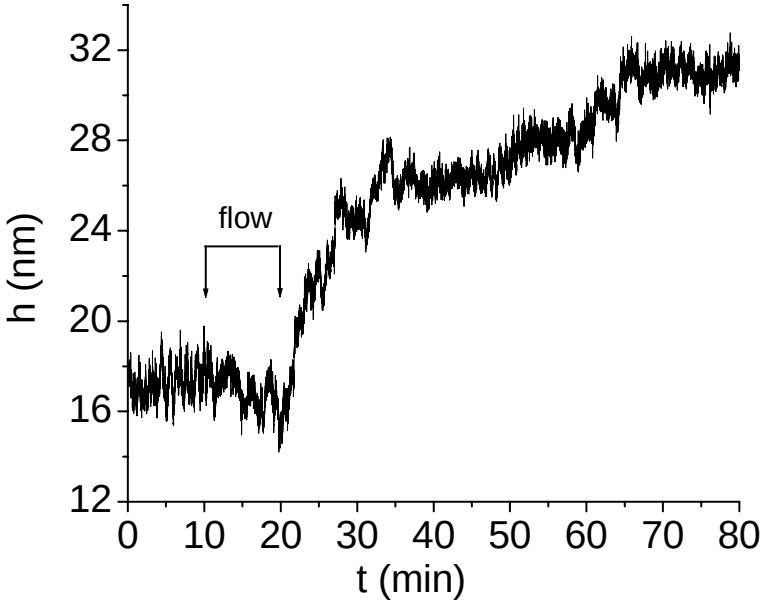
Multiple tethers
(exploiting the coil-to-helix transition)



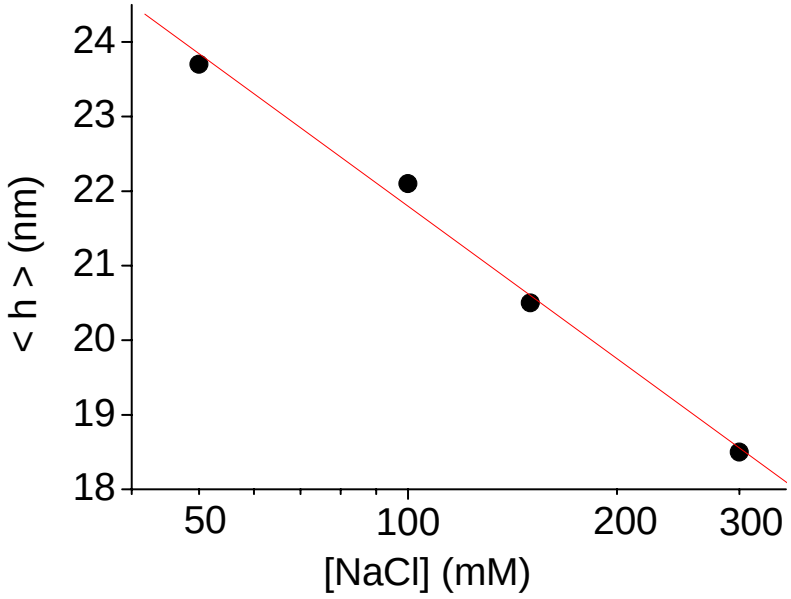


Electrostatic effects

Surface charge build up repels the bead from the surface:

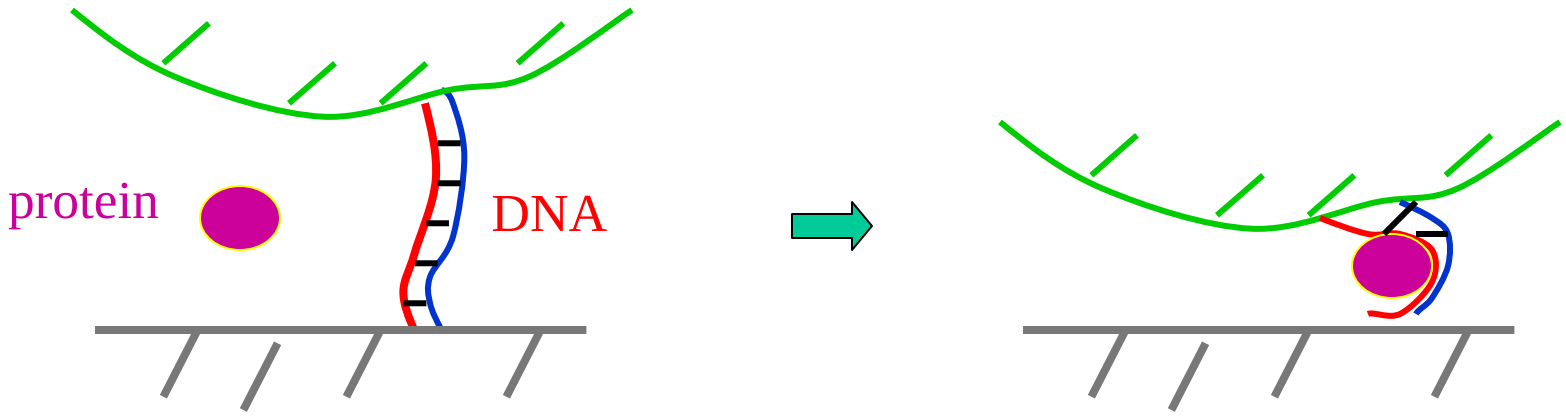


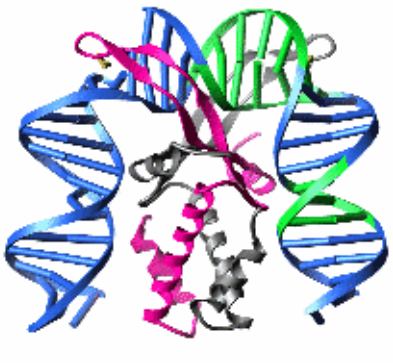
Screening the electrostatic repulsion displaces the bead towards the surface:



II. Protein-DNA binding dynamics

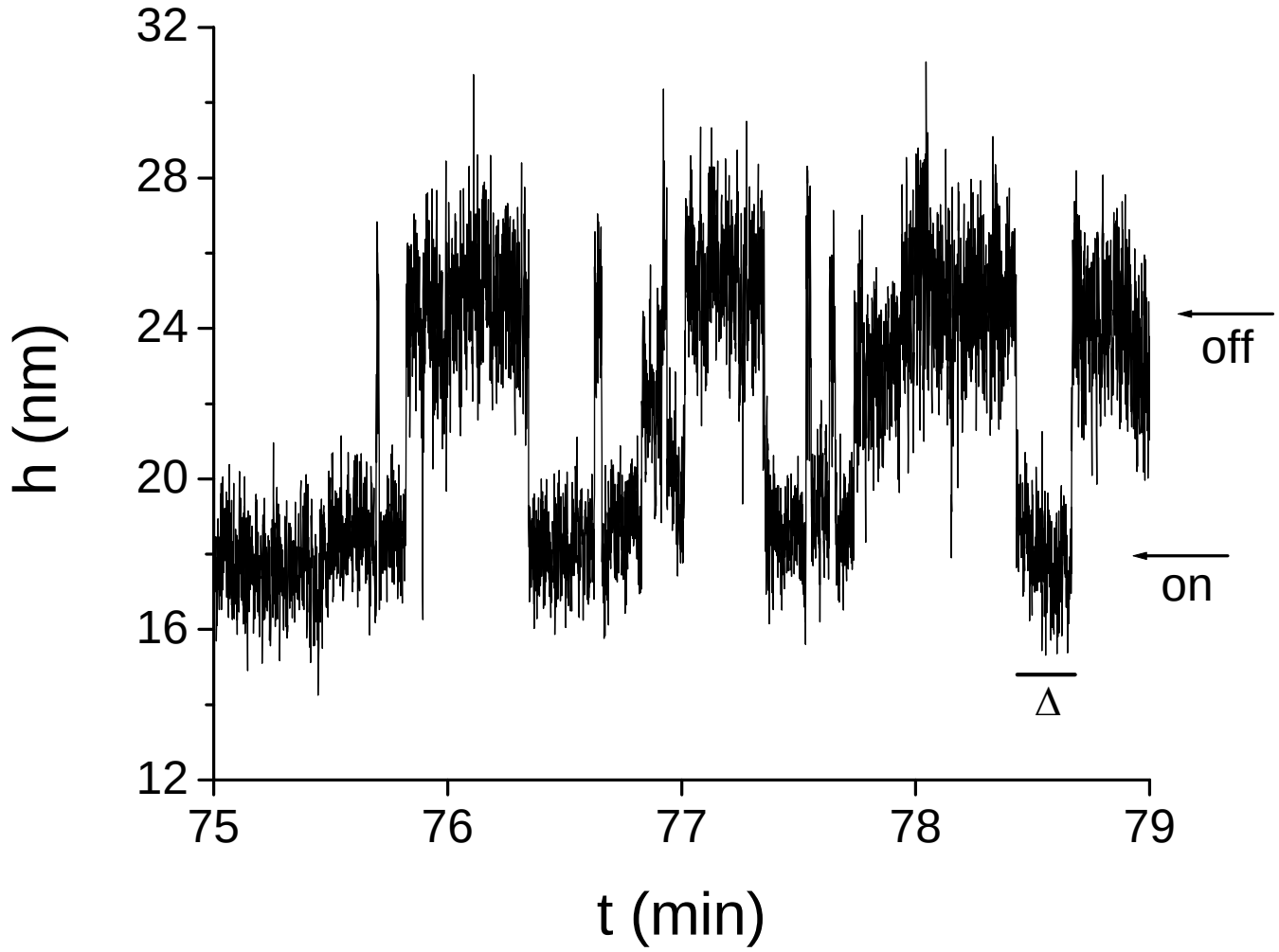
[Phys. Rev. Lett. **94**, 118101 (2005)]



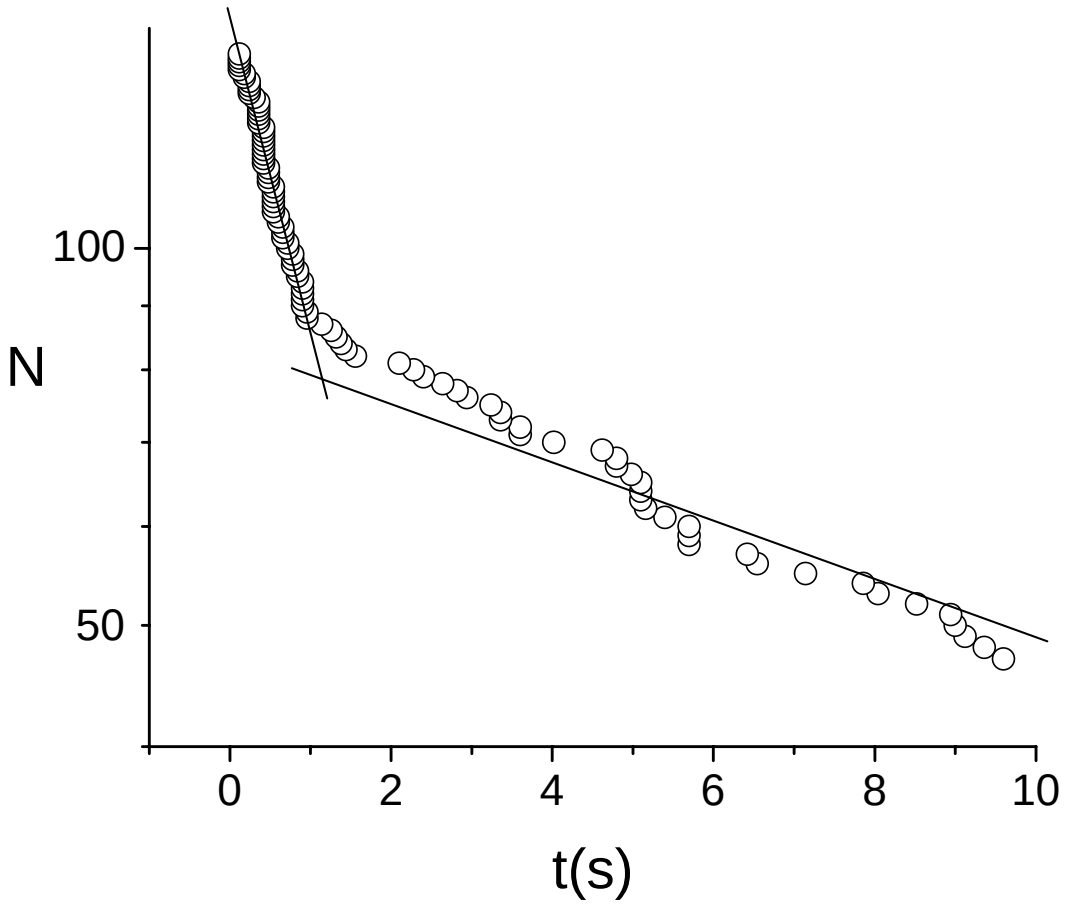


IHF protein of E.coli
involved in the integration of the viral DNA into the Bacterial DNA

Single IHF molecules binding and falling off the 76 bp tether (single binding site)



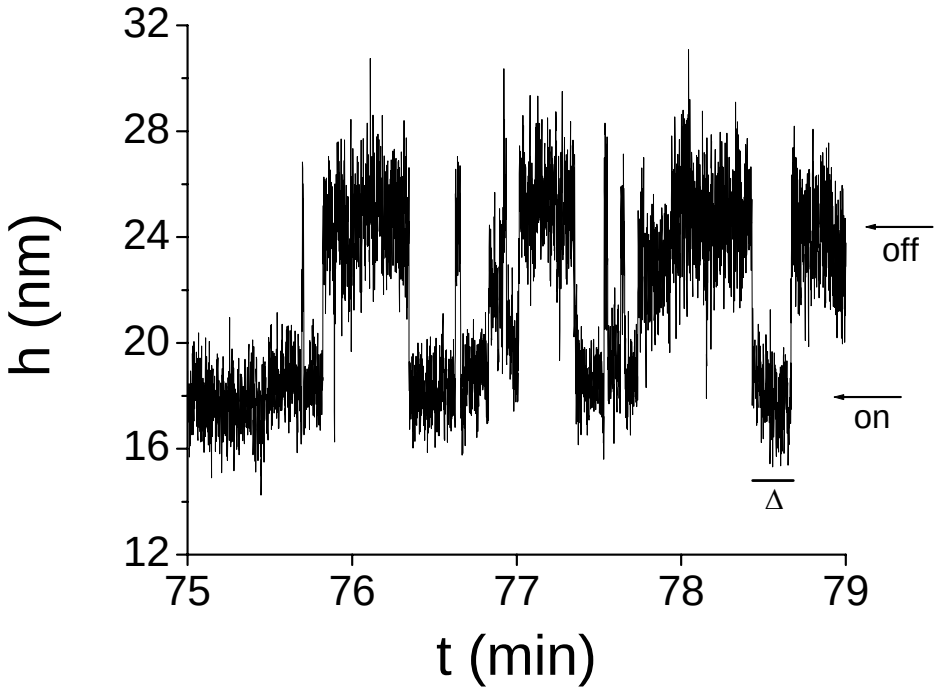
Two off - rates
→ two modes of binding

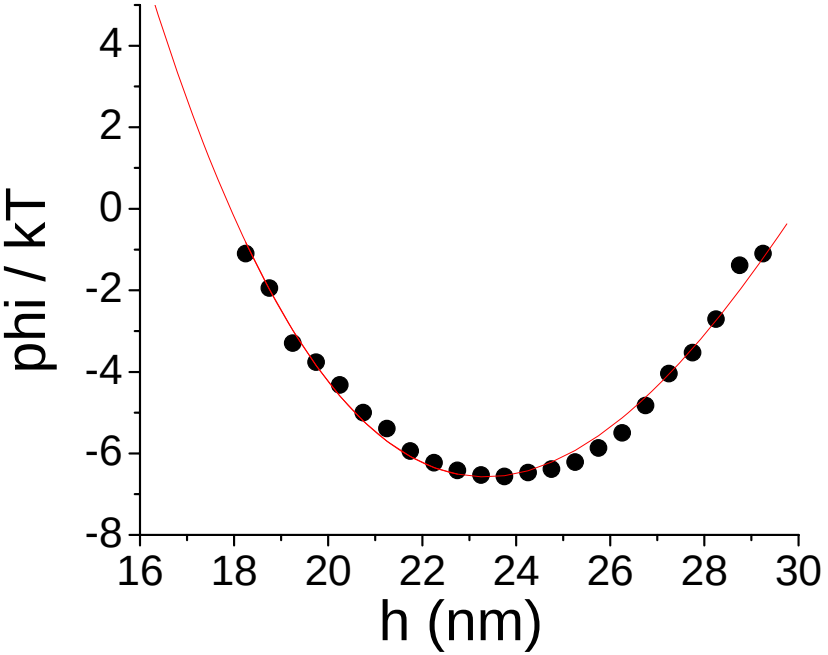


What is the mechanical tension on the DNA ?

Time trace

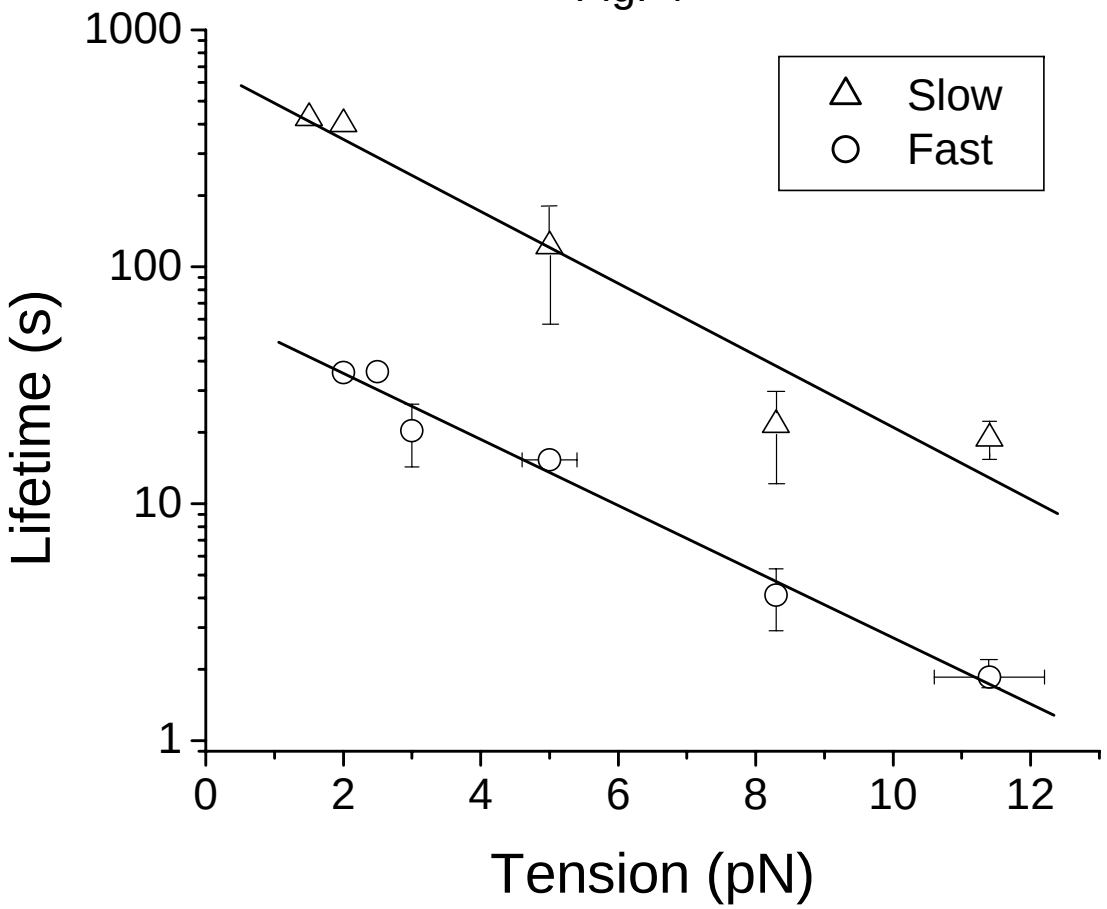
Bead – slide interaction potential
unbound state



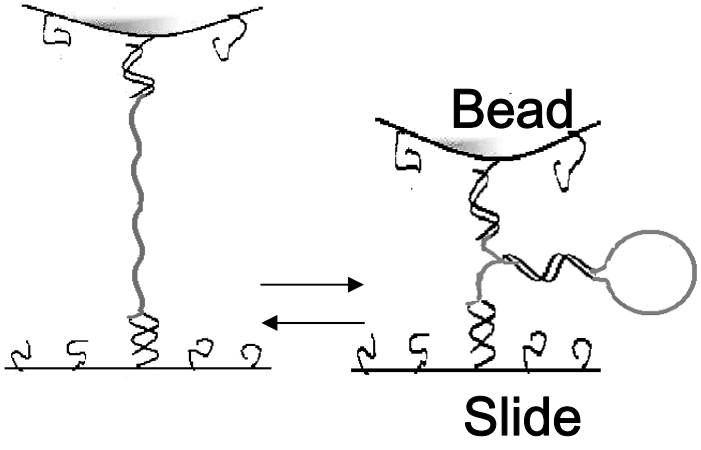


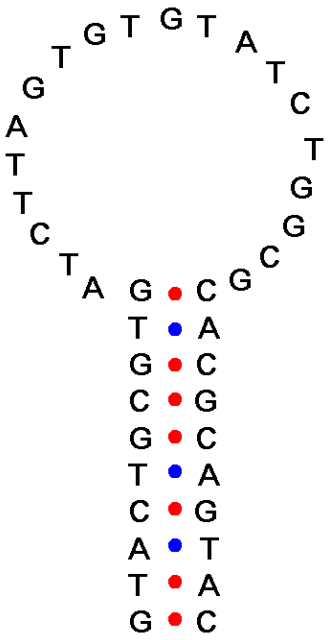
Lifetime of bound state depends on DNA tension

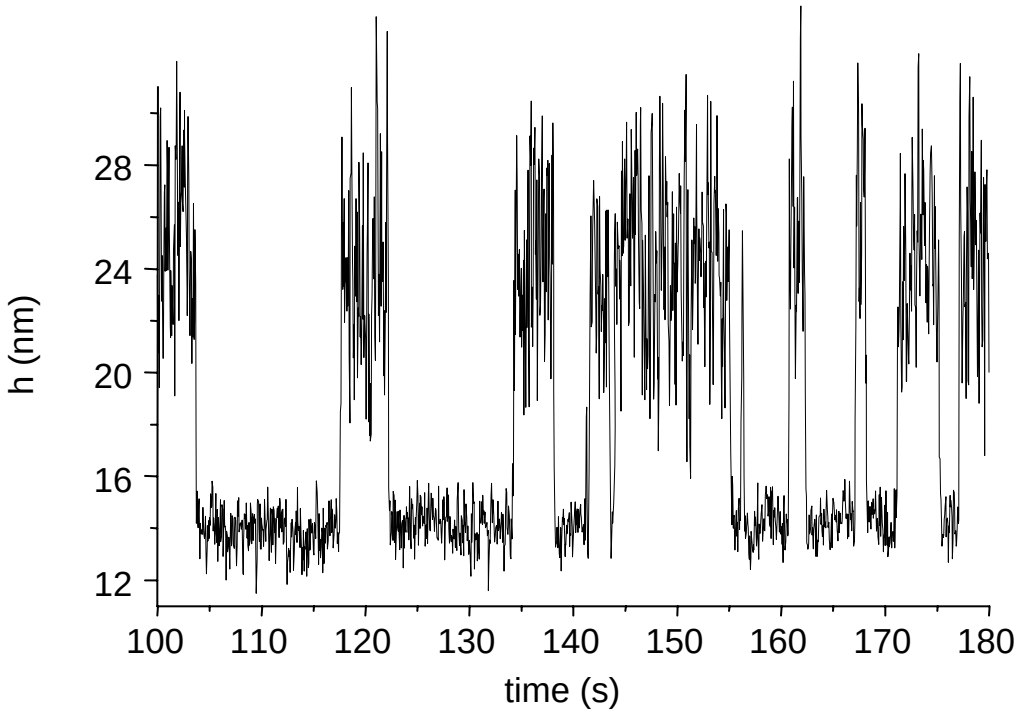
Fig. 4



III. DNA hairpin opening dynamics

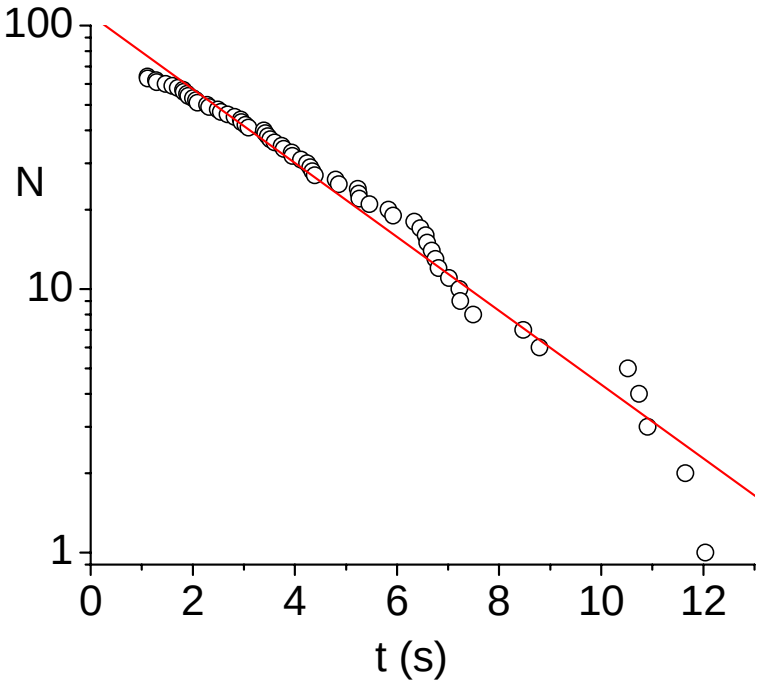




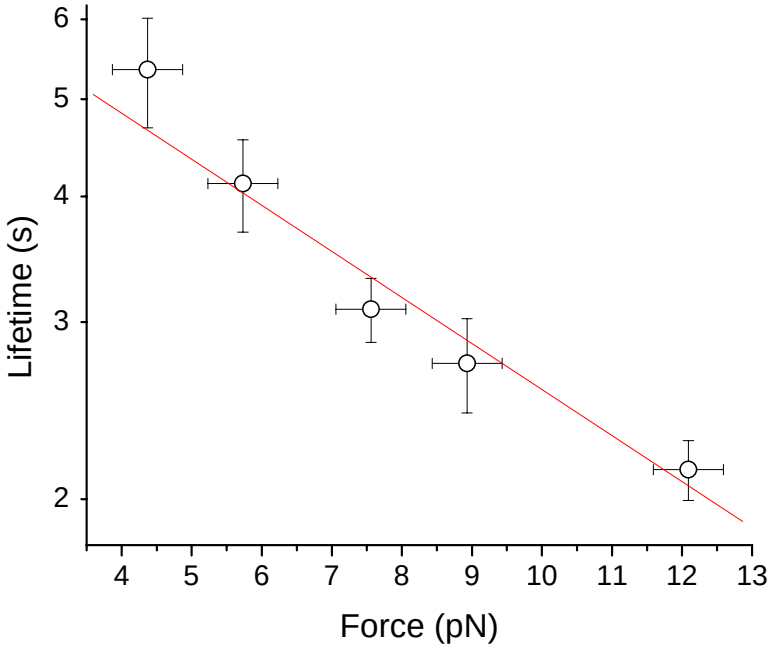


Single hairpin opening and closing

Statistics of residence time in the bound state



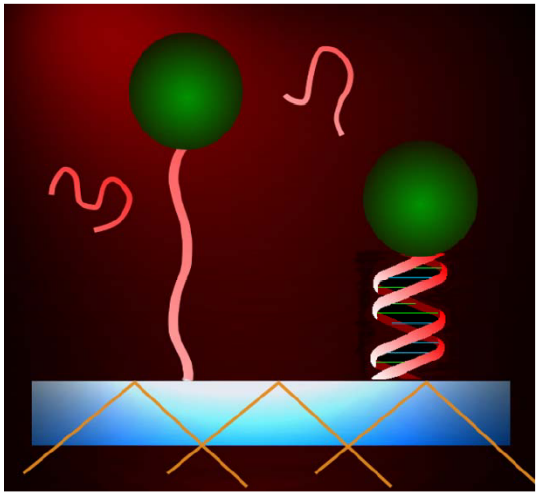
Lifetime vs. force



Conclusions

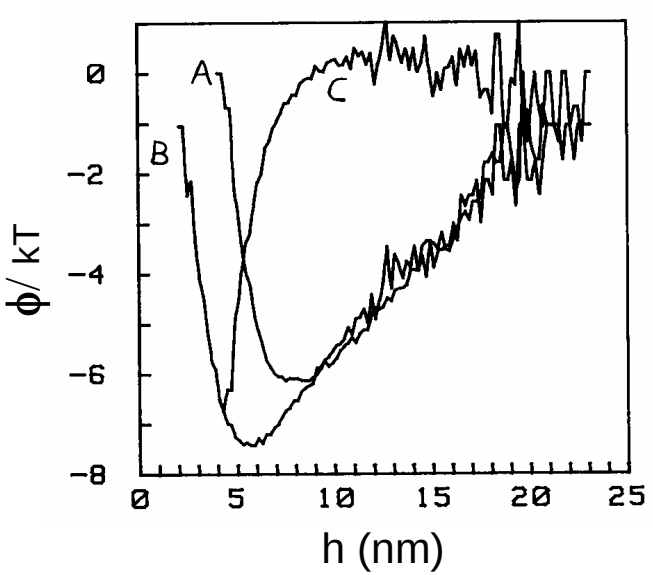
High resolution allows direct observation of nm scale conformational changes in single DNA oligomers:

- hybridization assay
- protein-DNA interaction dynamics
- DNA hairpin dynamics



Measurements of interaction potentials:

- depletion forces
- polymer springs
- bond rupture forces



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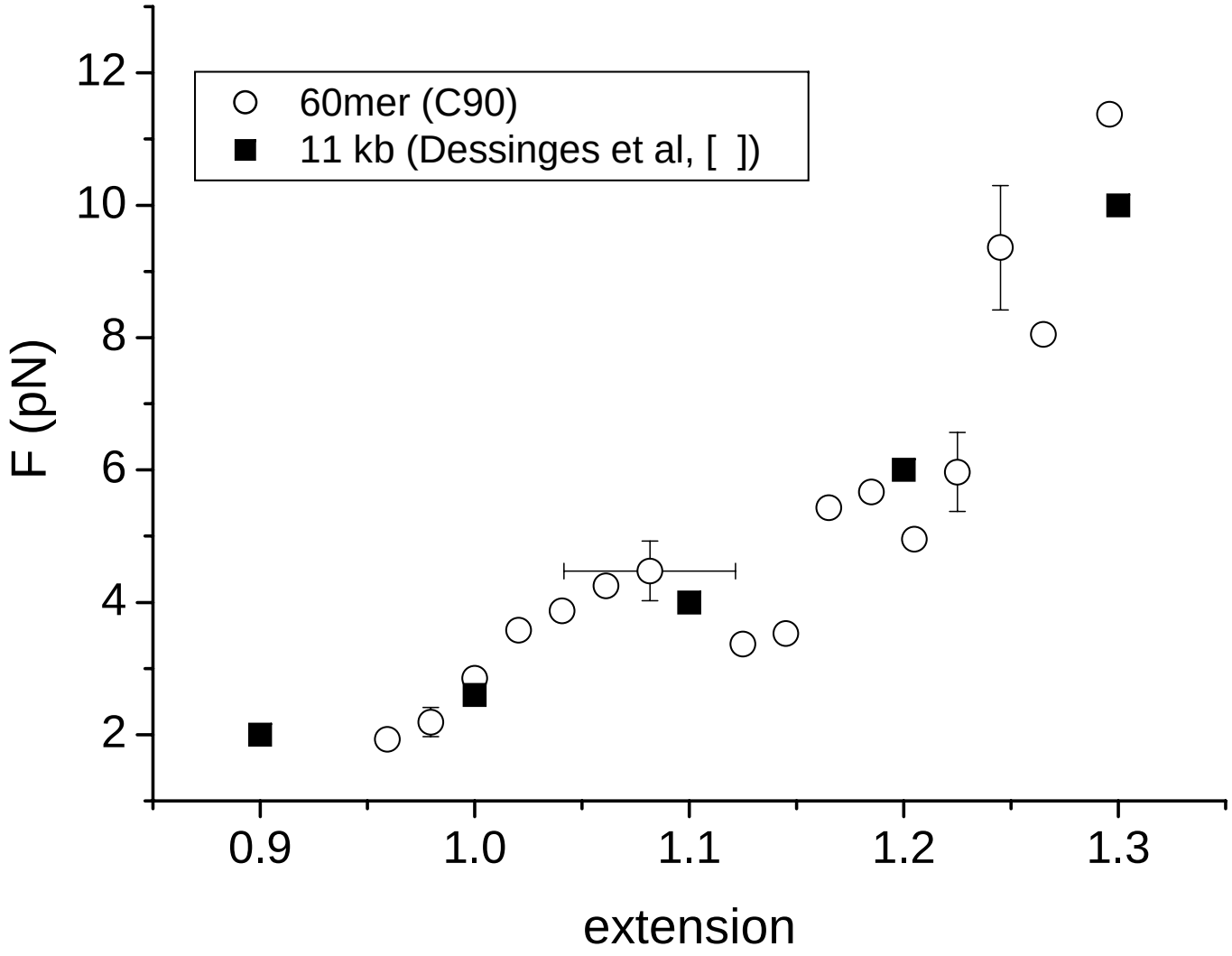
Force-extension curve for the ss tether



Adhesion force:

Work W necessary to separate two surfaces adhering through a contact area Sc:

$W / S_c = 2\gamma = A / (12\ \pi\ D_0^2)$

A: Hamaker constant

$D_0 \approx$ 0.2 nm

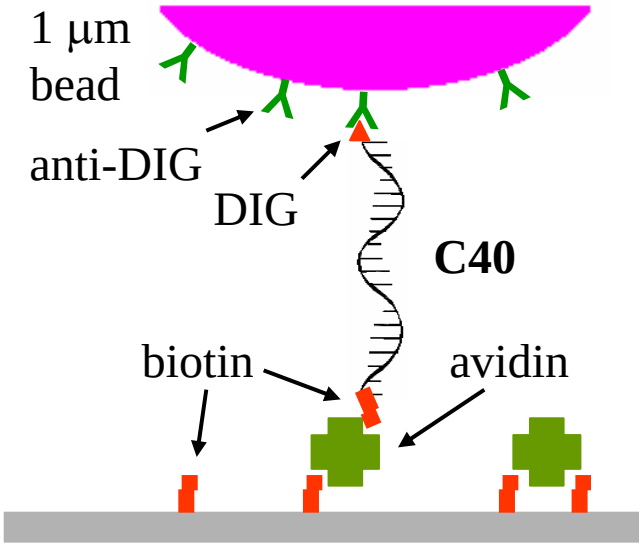
Adhesion force:

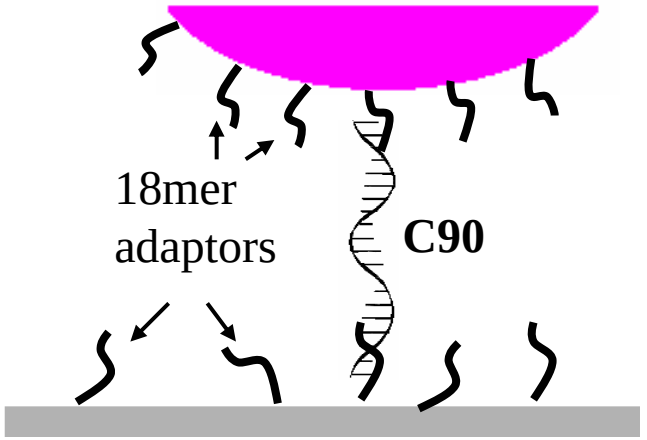
$F_{ad} / S_c = 4\ \gamma / D_0$

I.e. force per unit contact area = stress is independent of contact area

In the experiment: $F_{ad} / S_c \approx 6$ pN / $(nm)^2$

Chemistry of grafting

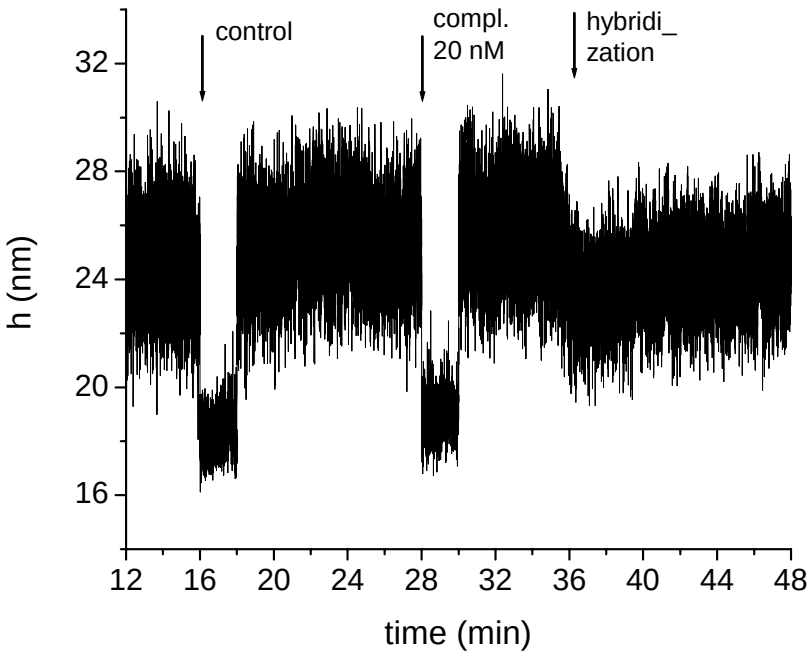




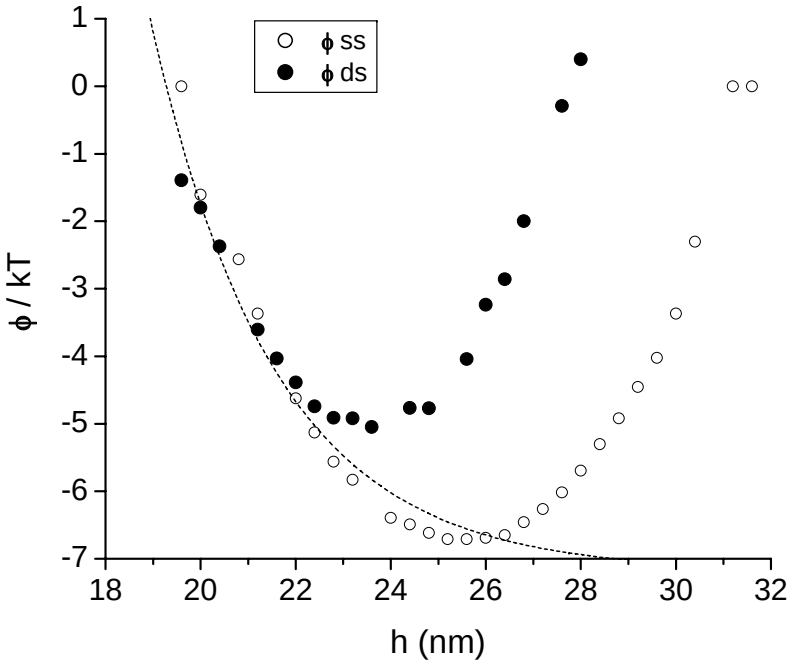
Analysis of tether elasticity confirms ss → ds transition

Tether: 90mer
Target: 60mer

Control 100 nM
Target 20 nM



Bead-slide interaction:
ss and ds tether



Relative extension of the ss:

$$E = 1 + \frac{h(ss) - h_{\max}(ds)}{20}$$