Principles of AFM force spectroscopy

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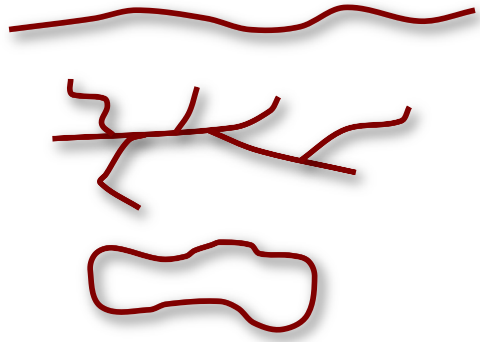
BioAFM Summer School 2026

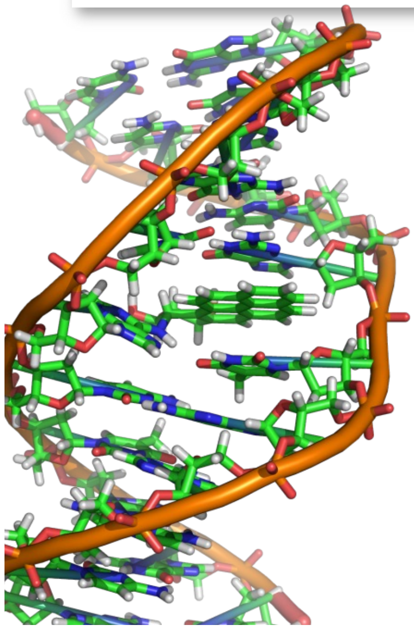
SEMMELWEIS
EGYETEM 1769

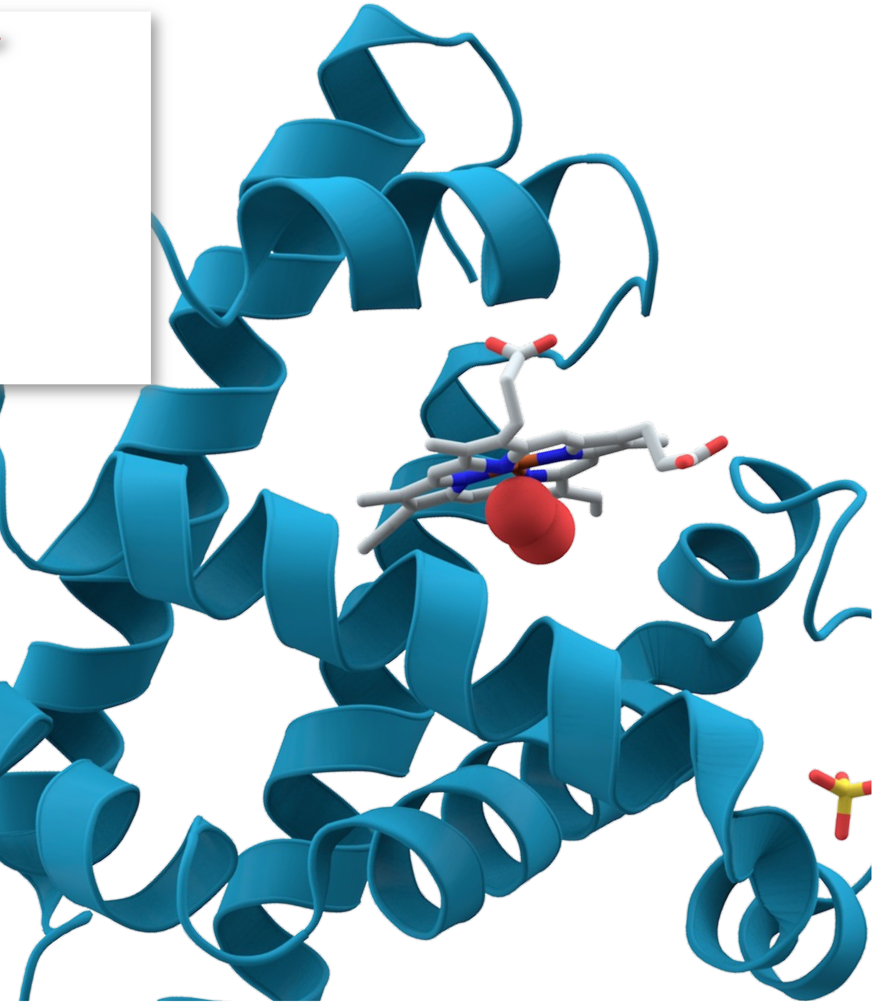
Biomolecules are polymers

Common feature: **Linear primary structure (protein, DNA)**
Strong bonds between monomers (covalent)
Weaker interactions between distant region of polymer chain

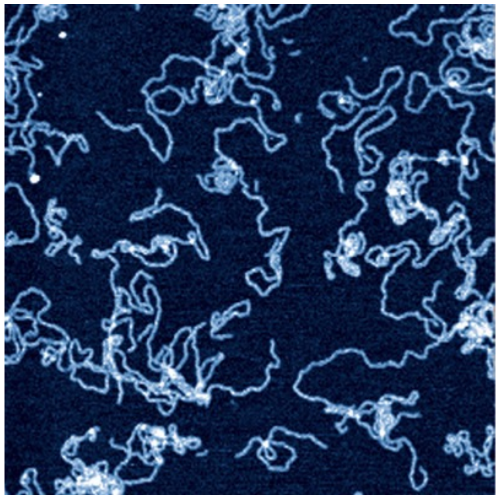
1. Linear
DNA, protein, cellulose
2. Branched
glycogen
3. Circular
mt DNA





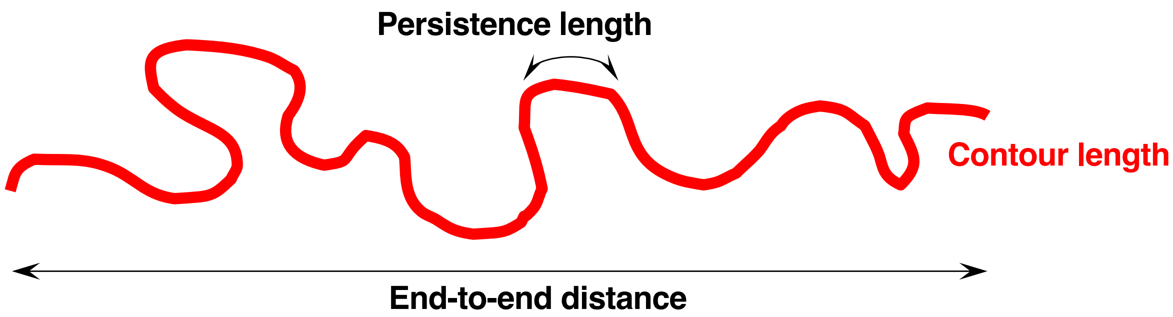


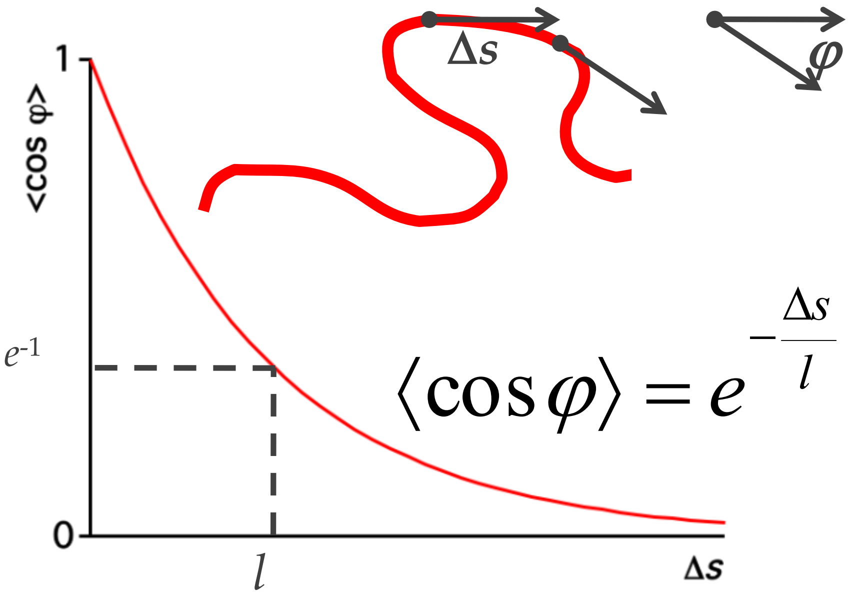
What is the shape of biopolymers?



AFM image of dsDNA

Parameters to describe the shape of polymer (The wormlike chain model)





$$\langle \cos \varphi \rangle = e^{-\frac{\Delta s}{l}}$$

Contour length (L): Full length of the chain

End-to-end distance (R): Distance between chain termini.

Persistence length (*l*) : describe the persistence of chain orientation.

Shorter persistence length polymers are more flexible.

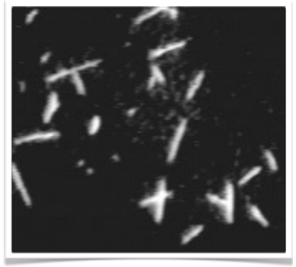
Biopolymer classification based on flexibility

l = persistence length
L = contour length

RIGID

$l >> L$

Microtubules

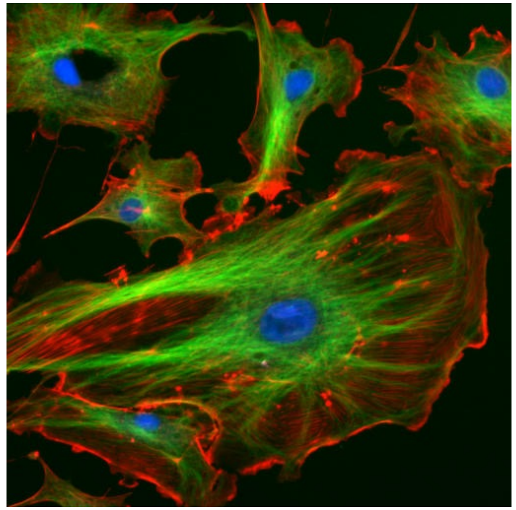


SEMIFLEXIBLE

$l \approx L$

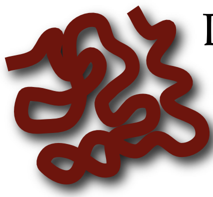
Microfilaments

aktin
tubulin

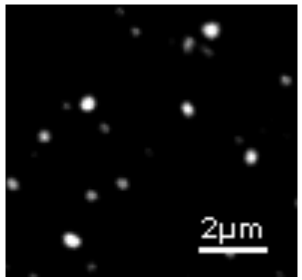


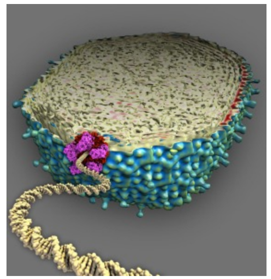
FLEXIBLE

$l << L$



DNA





Are biopolymers elastic?

Yes, but Hooke's law is not valid! Non-linear elasticity.

Entropic elasticity

Thermal energy ($k_B T$) excites bending movements in the chain

↓

The chain's disorder (entropy) increases

↓

The chain shortens

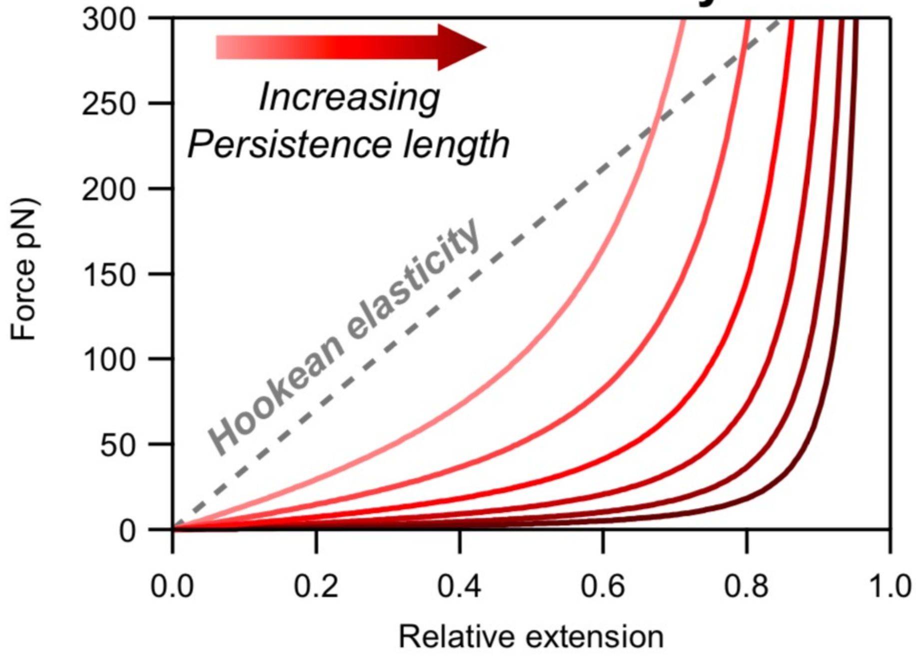
Force is needed to stretch an entropic chain
The wormlike chain (WLC) equation:

$$\boldsymbol{F = \frac{k_B T}{l} \cdot \frac{Z}{L} + \frac{1}{4(1 - Z/L)^2}}$$

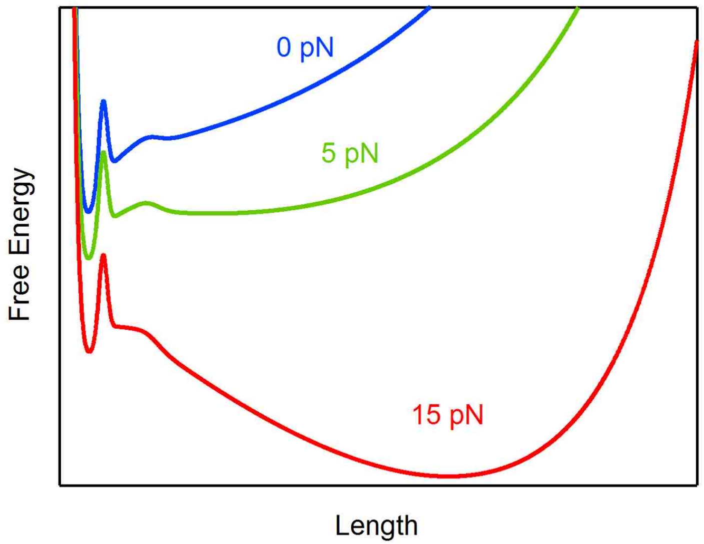
l = **persistence length**
L = contour length
Z = end-to-end distance
Z/L = relative extension

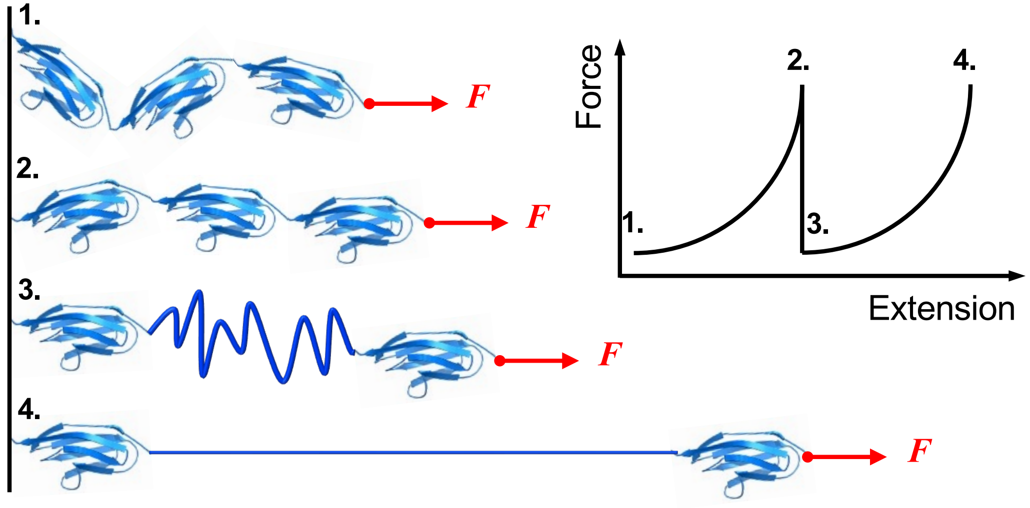
F = force
k_B = Boltzmann constant
T = absolute temperature

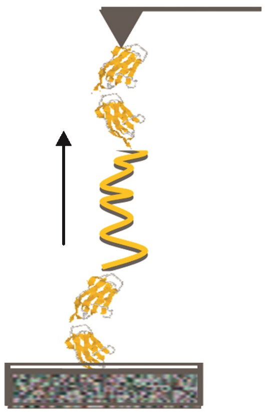
Nonlinear elasticity

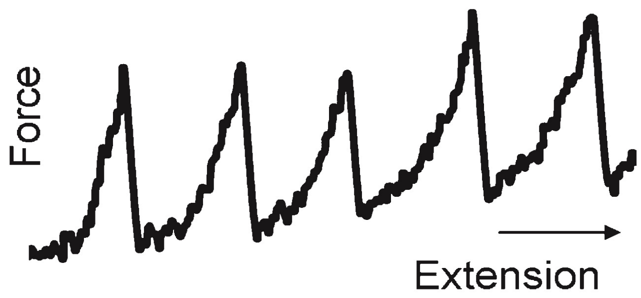


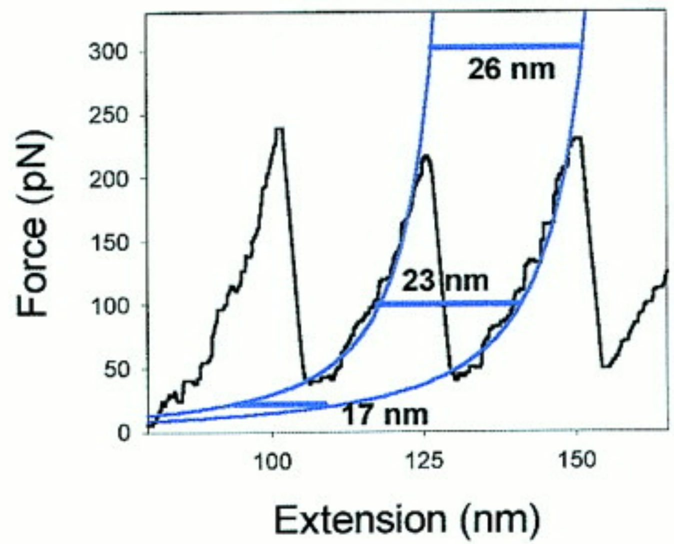
Sawtooth pattern in force spectroscopy





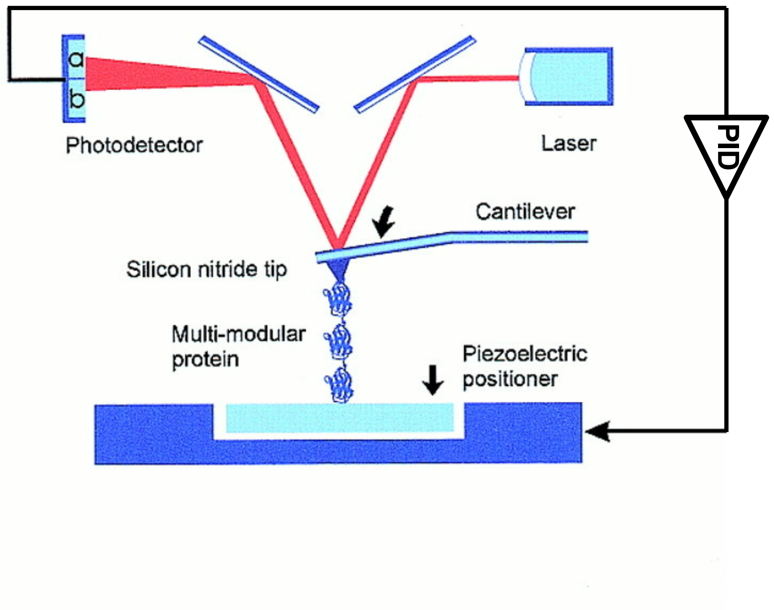


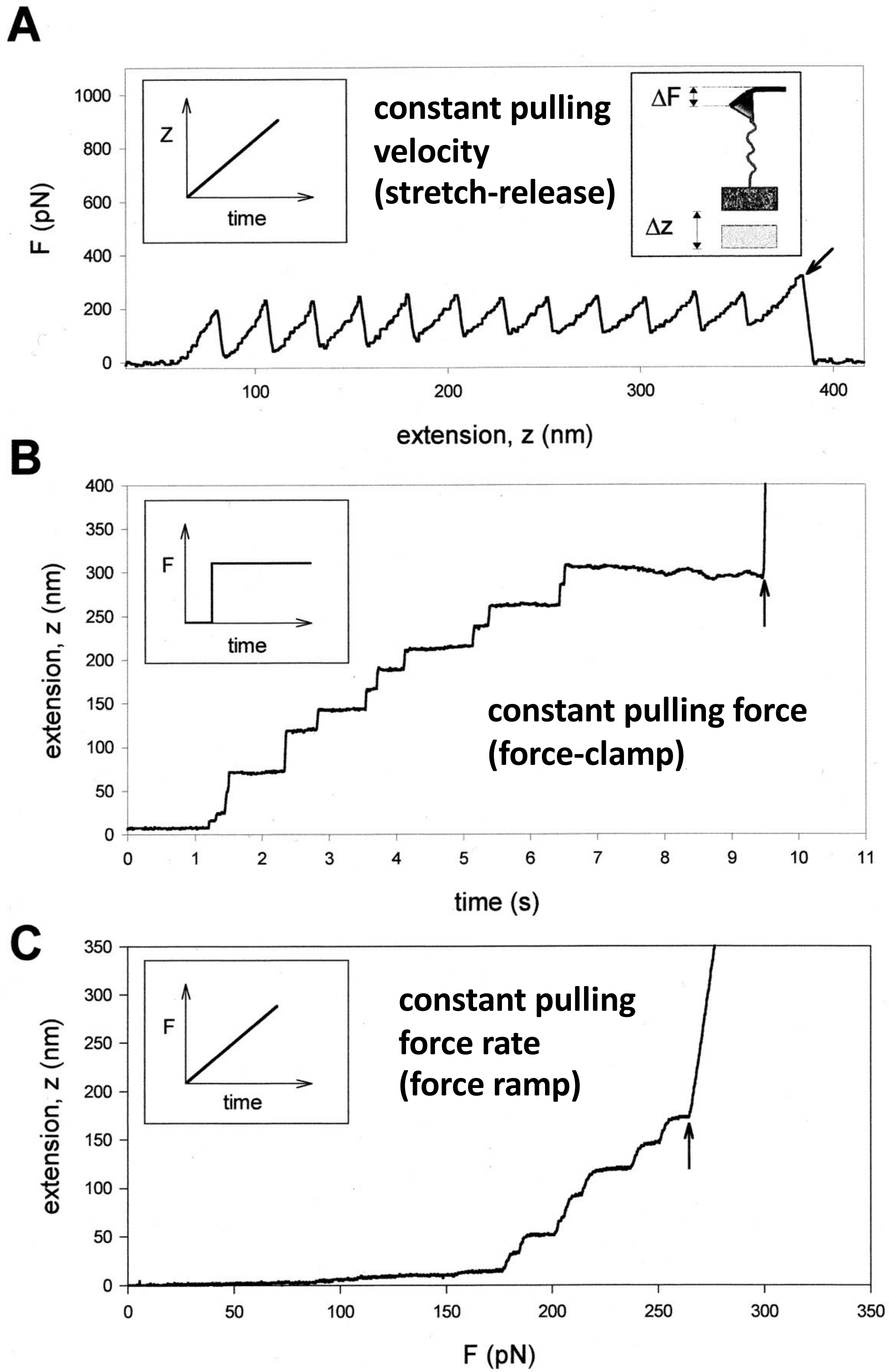




Chem. Soc. Rev.. 2012;41(9):3523-3534. doi:10.1039/c2cs15329g

Force feedback mode





https://doi.org/10.1073/pnas.98.2.468

Dynamic force spectroscopy

1. **In force clamp mode we collect unfolding extension-time curves at different clamp forces**

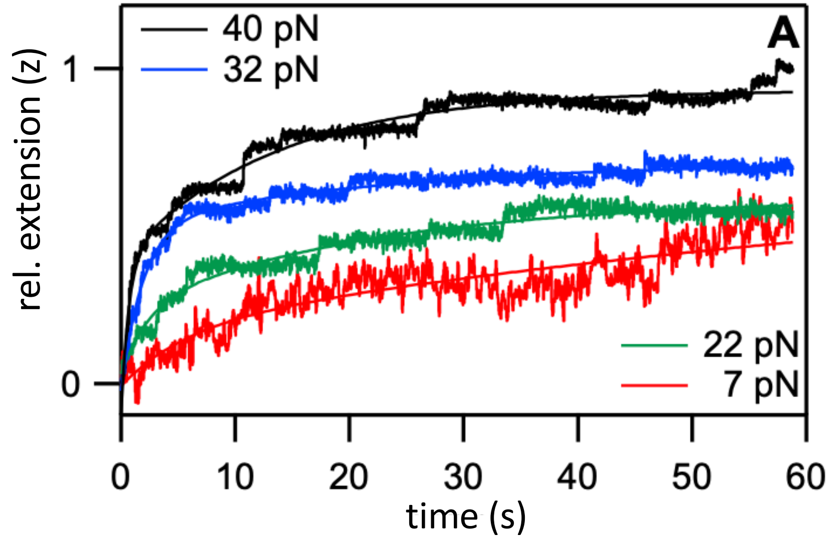
2. **Fit the extension-time curves with exponential to obtain rate constant:** $\boldsymbol{z = z_0 \cdot (1 - e^{-k\cdot t})}$

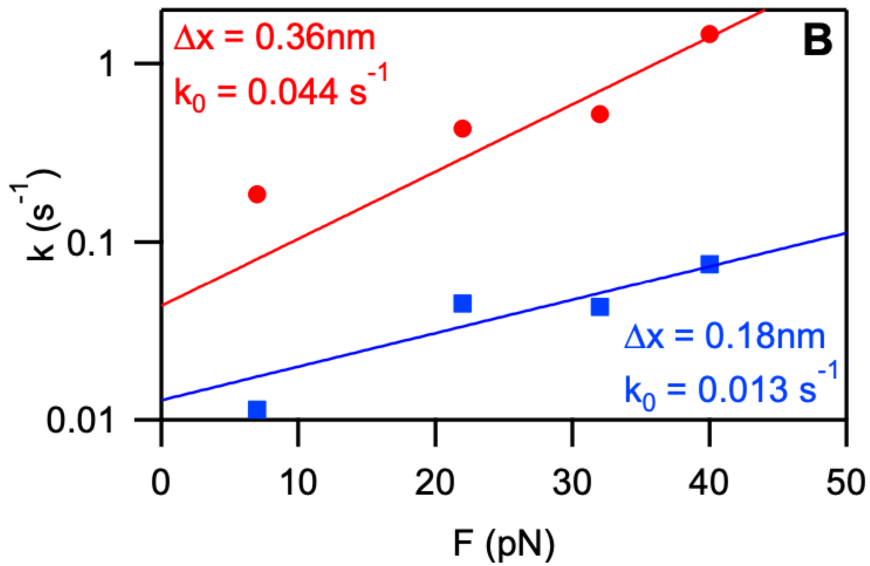
3. **Fit the rate constants versus force graphs by the Bell-Evans model**

$$k = k_0 \cdot e^{\frac{F\cdot\Delta x}{k_B\cdot T}}$$

***k*: unfolding rate at given force (F)**
k_0: spontaneous unfolding rate
***F*: force**
Δx: distance to transition on the reaction coordinate
k_B: Boltzmann const, *T*: abs. temp.

doi:10.1242/jcs.138461

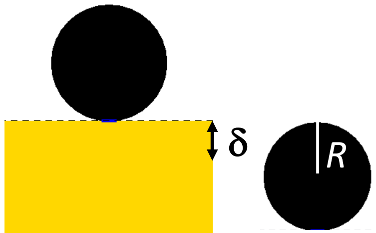




Cellular mechanics

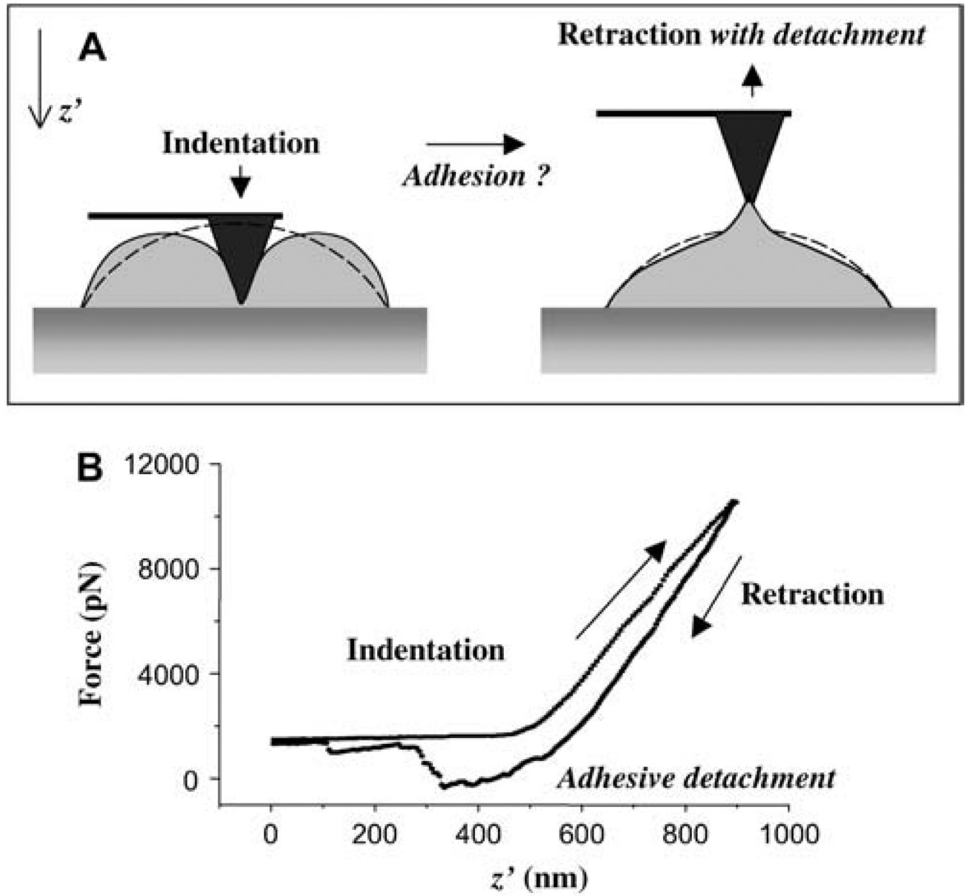
Hertz model

(Pressing a sphere into an elastic object)

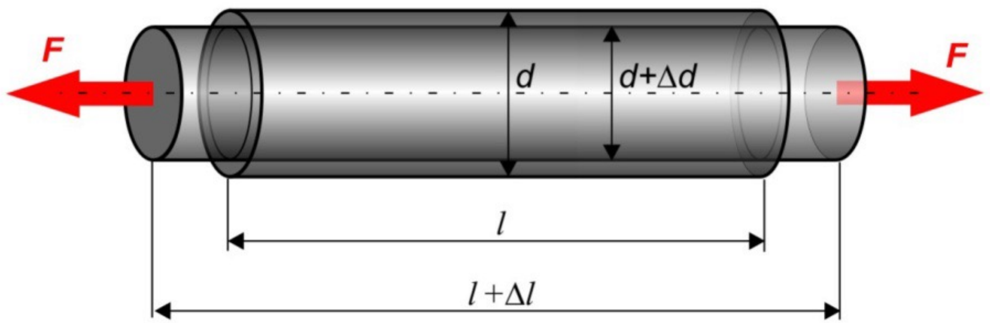


$$F = \frac{4}{3}\frac{E}{1-\nu^2}\sqrt{R}\,\delta^{3/2}$$

F: **Force (N)**
E: **Young's modulus (stiffness) (Pa)**
ν: **Poisson's ratio (uasually 0.5 for cells)**
R: **radius of spherical probe**
δ: **indentation distance**



Poisson's ratio



$$\frac{\Delta d}{d} = -\nu \frac{\Delta l}{l}$$

ν: Poisson's ratio (no dimension)

$\nu_{max} = 0.5$ (*usually between 0.3-0.5*)

Elastic deformation

Stress

$$\sigma = \frac{F}{A_0}$$

Dimenson: $\left[\frac{N}{m^2} = Pa\right]$

Strain (deformation)

$$\varepsilon = \frac{\Delta l}{l_0}$$

Dimensionless: $\left[\frac{m}{m}\right]$

Hooke's law:

$$\sigma = E\varepsilon$$

Stiffness (Young's modulus):

$$E = \frac{\sigma}{\varepsilon}$$

Dimenson: $[Pa]$