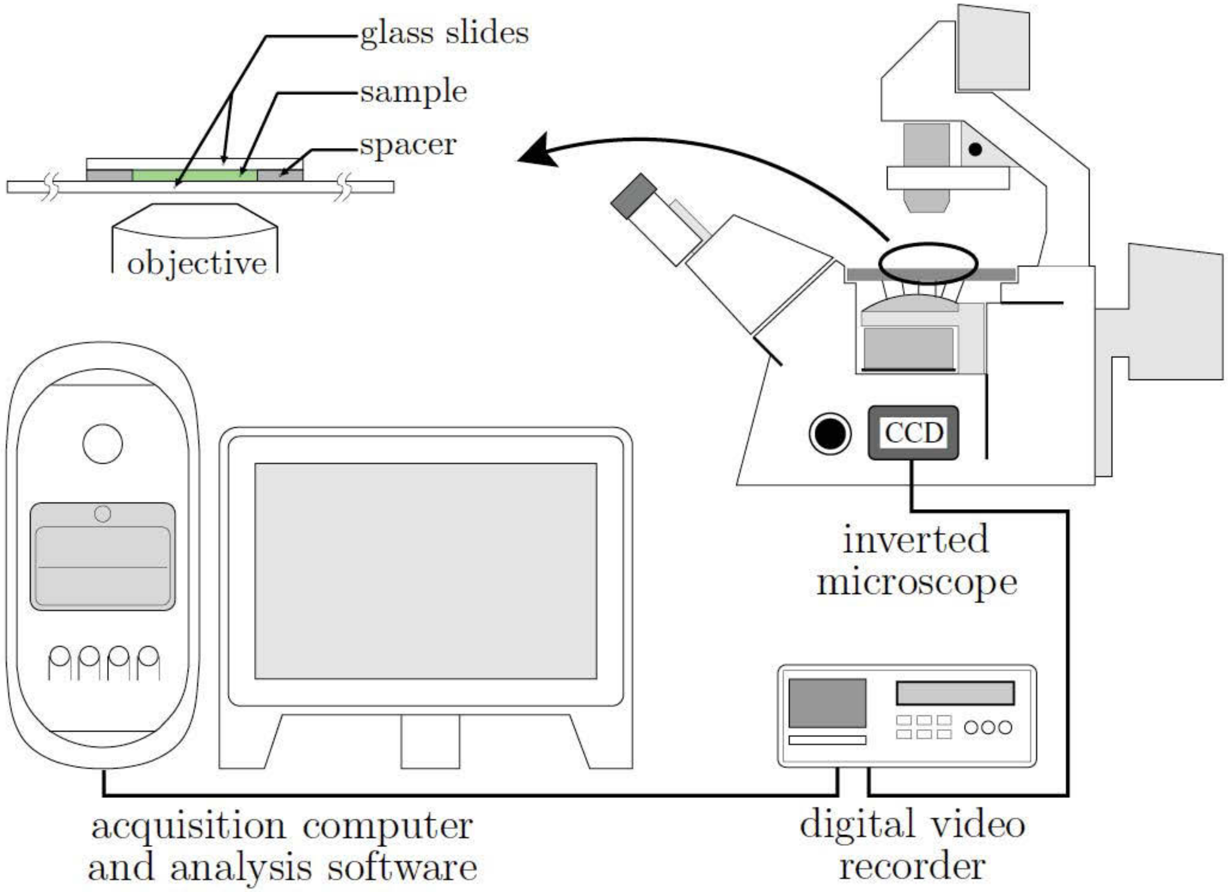
http://en.wikipedia.org/wiki/Fick's_laws_of_diffusion#mediaviewer/File:DiffusionMicroMacro.gif

Particle Tracking Methods (T. Savin)

- Standard video microscopy tracking setup

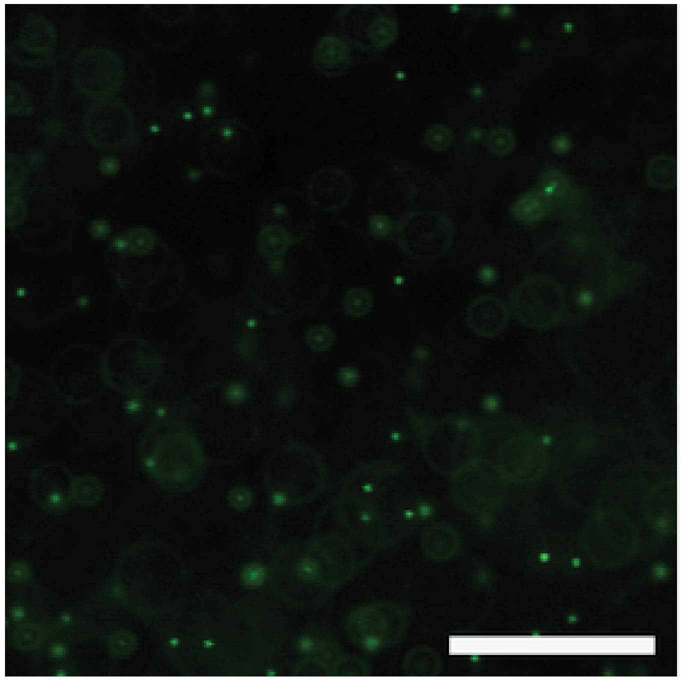
IDL programs



Video Microscopy Particle Tracking

- Tracking algorithms

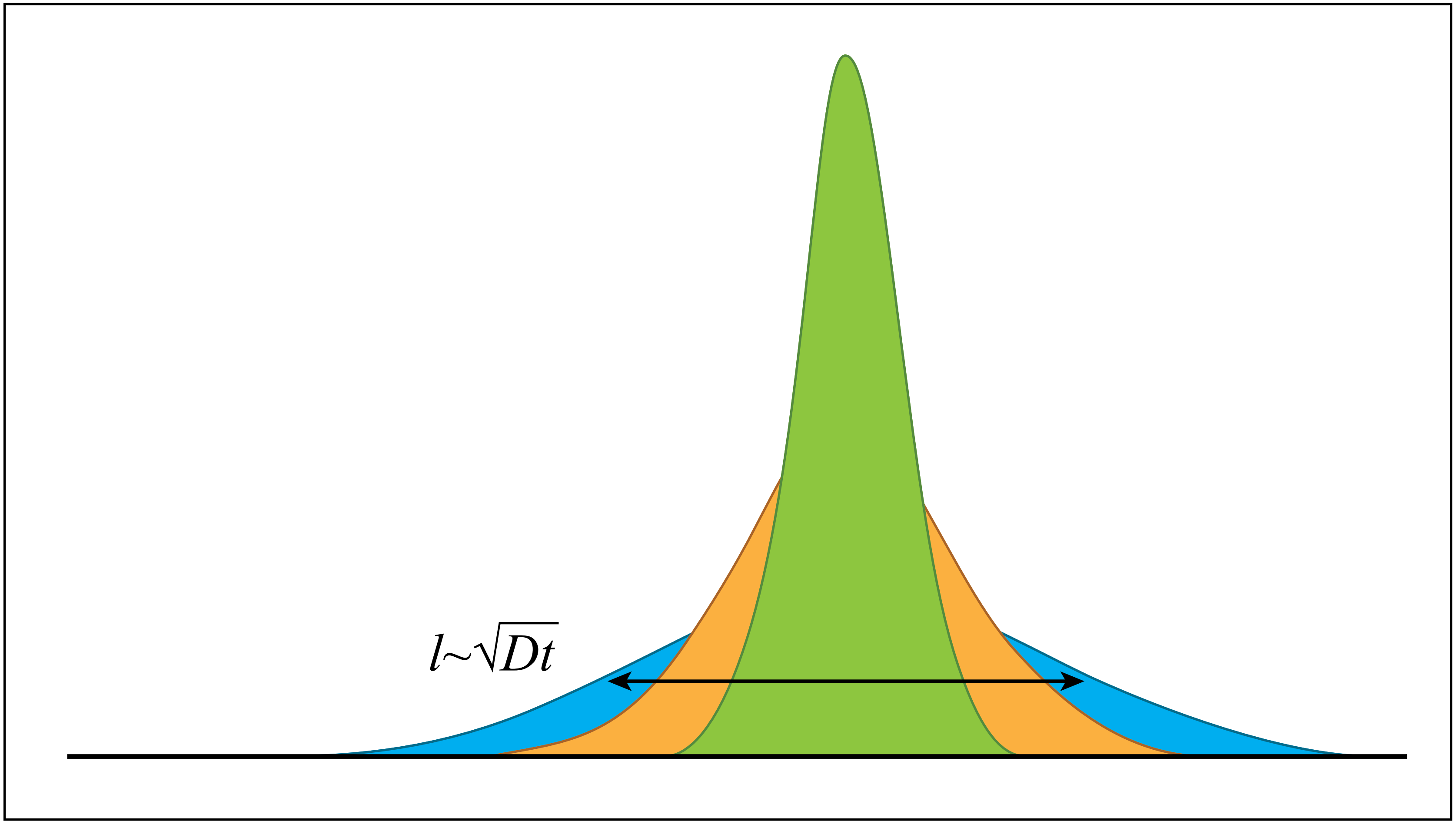
Example:
1 μm diameter
spheres in
water, T=25°C



30 μm

Crocker and Grier, 1996
http://www.physics.emory.edu/~weeks/idl/

Spread from a point due to molecular diffusion



Entropic forces

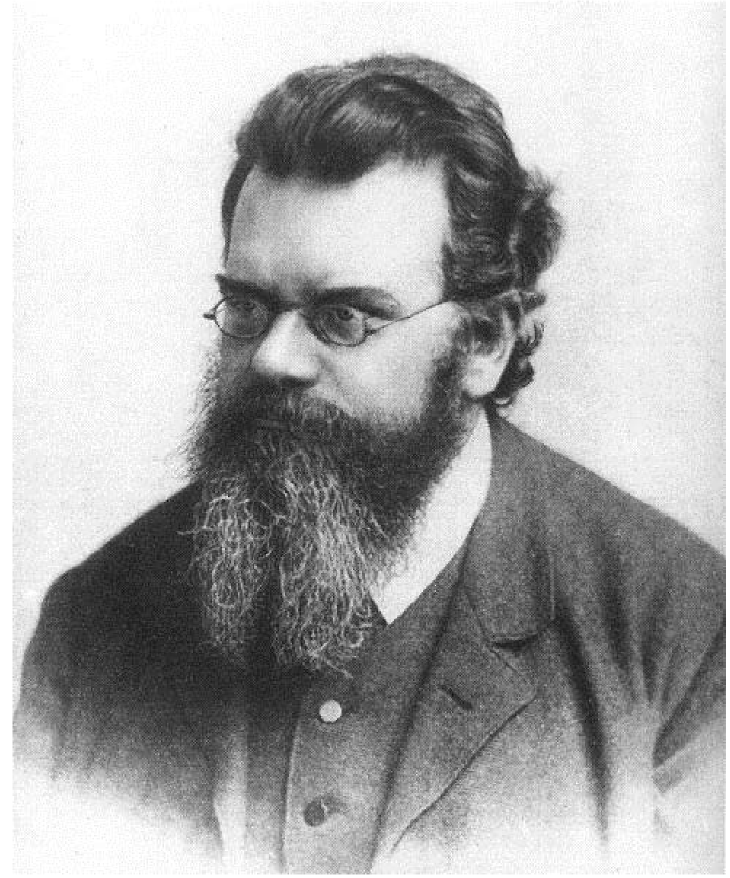


Image in the public domain.

Entropy:

$S = k_B \ln (W)$

k_B = Boltzmann constant

W = multiplicity, i.e. number of microstates

vs.

Review (and a look ahead)

- Random walks and diffusion (see, e.g., Dill & Bromberg)
- Boltzmann statistics

$$P_i = \frac{1}{Q}\exp\left(\frac{-G_i}{k_B T}\right) \qquad Q = \sum_i \exp\left(\frac{-G_i}{k_B T}\right)$$

(note use of free energy, *G*, as opposed to internal energy, *U*, corresponding to an ensemble of states, taking entropy into account; G = H - TS)

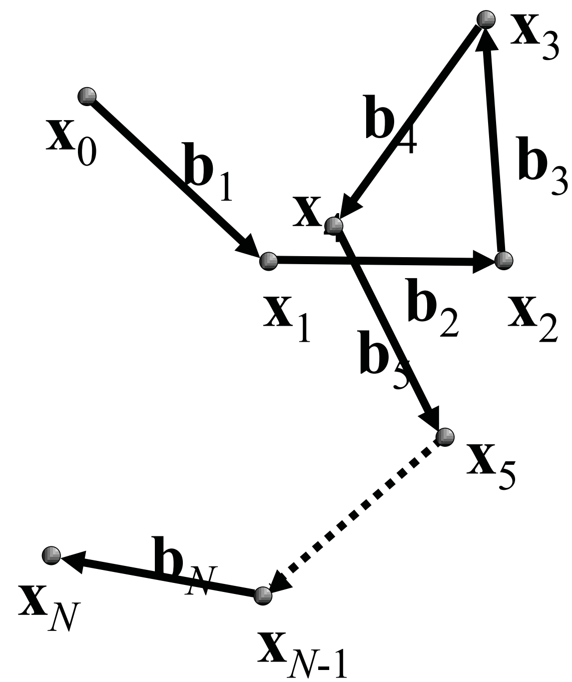
- Thermal energy, $k_B T$ = 4 pN·nm = 0.6 kcal/mole (for T = 300K)
- Persistence length (thermal energy ⟺ bending stiffness)

$$l_p = \frac{K_b}{k_B T} \qquad K_b = EI$$

More on this later!

Gaussian chain

- Consider a macromolecular chain comprised of “*N*” segments of length “*b*” (the Kuhn length)
- Collection of rigid connected segments is approximated as a random walk with a Gaussian probability distribution
- Valid for $N \gg 1$; $R \ll (Nb)$
- Force-extension curve:
  - not valid in the limit $F \rightarrow$ infinity



$$F = \frac{3k_BT}{Nb^2} R = \frac{3k_BT}{2l_p} \frac{R}{L_c}$$

Freely-jointed chain

- Similar to Gaussian chain, but does not assume a Gaussian probability distribution
- Self avoiding and imposes maximum length

$$F_{FJC} = \frac{k_B T}{b}\left[\frac{3R}{L_c} + \frac{9}{5}\left(\frac{R}{L_c}\right)^3 + \frac{297}{115}\left(\frac{R}{L_c}\right)^5\right]$$

- Note that this agrees with the Gaussian chain for small forces (R/L_c << 1).

Worm-like chain

- Polymer is treated as a flexible rope rather than a collection of freely-jointed rigid rods
- Bending stiffness accounted for directly
- Enthalpic contributions important
- Use Fourier transform methods and equipartition of energy

$$F_{WLC} = \frac{kT}{l_p}\left[\frac{1}{4}\left(1-\frac{R}{L_c}\right)^{-2} - \frac{1}{4} + \frac{R}{L_c}\right]$$

Summary of models

Gaussian chain	$F_{GC} = \frac{3kT}{Nb^2} R = \frac{3kT}{2l_p} \frac{R}{L_c}$
Freely-jointed chain (approx.)	$F_{FJC} = \frac{k_B T}{2l_p} \left[\frac{3R}{L_c} + \frac{9}{5} \left(\frac{R}{L_c} \right)^3 + \frac{297}{115} \left(\frac{R}{L_c} \right)^5 \right]$
Worm-like chain (approx.)	$F_{WLC} = \frac{kT}{l_p} \left[\frac{1}{4} \left(1 - \frac{R}{L_c} \right)^{-2} - \frac{1}{4} + \frac{R}{L_c} \right]$

How much force is required to stretch a typical strand of DNA by 10% of its contour length?

k_BT = 4 pN·nm

l_p = 50 nm

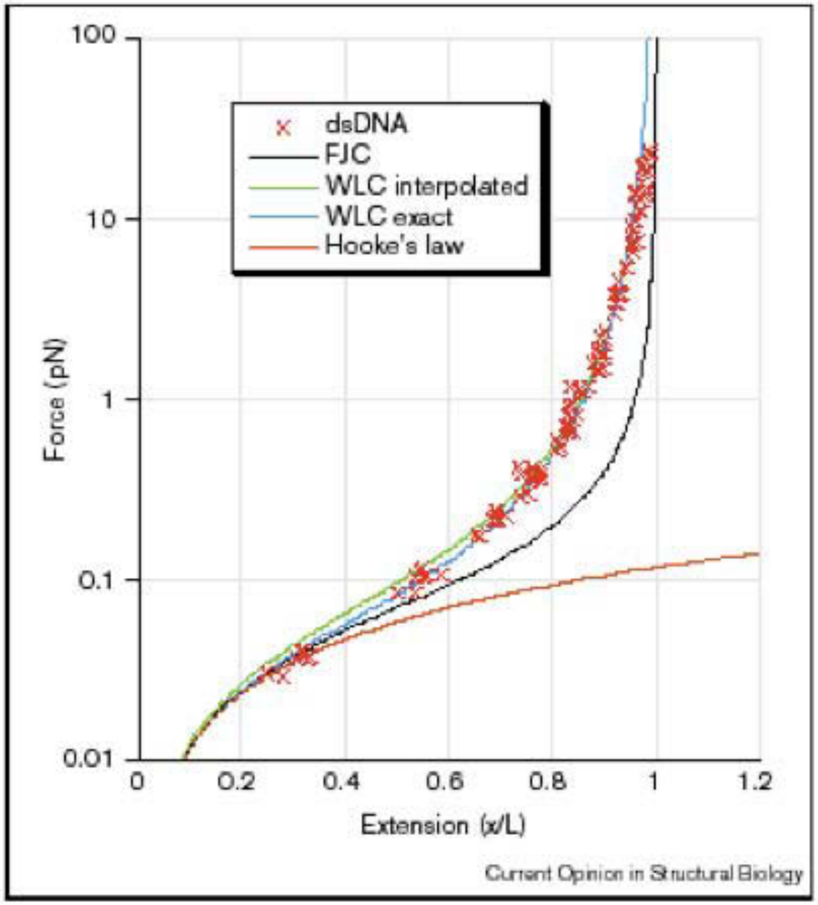
R/L_c = 0.01

L_c = 15 μm

F = ??

$$F = \frac{3k_BT}{Nb^2}R = \frac{3k_BT}{2l_p}\frac{R}{L_c}$$

DNA extension -- comparison of Gaussian chain (Hooke's law) FJC and WLC.

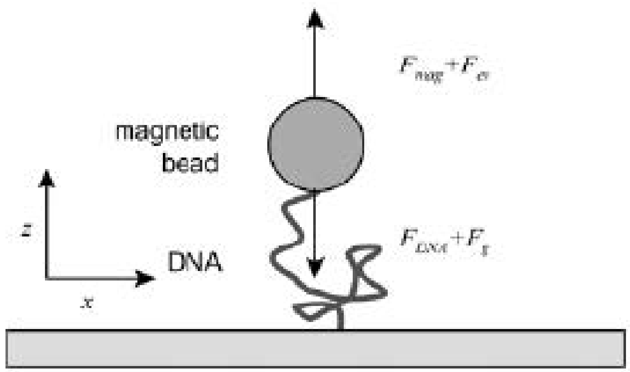


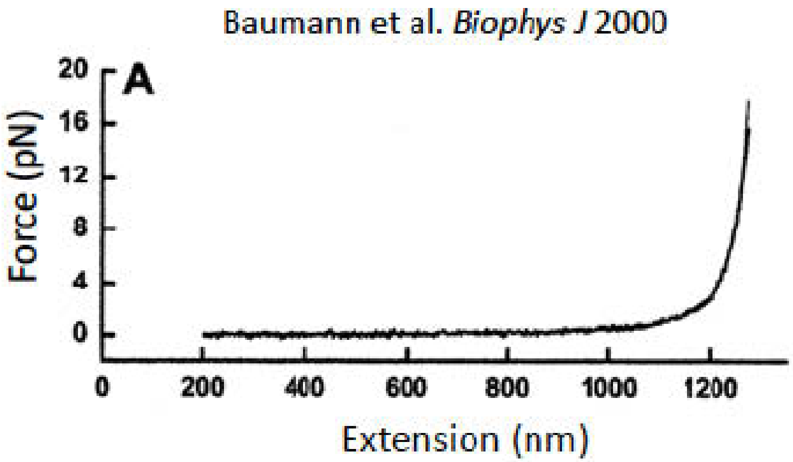
Force versus extension data (red crosses) for λ phage dsDNA (48,502 bp) pulled by magnetic beads in 10 mM Na^+ buffer [4]. The data are fit to a WLC model solved numerically (WLC exact) or using Equation 3 (WLC interpolated), both assuming P = 53 nm. The FJC curve assumes b = 2P = 106 nm. The Hooke's law force curve is from Equation 2.

Bustamante et al. 2001

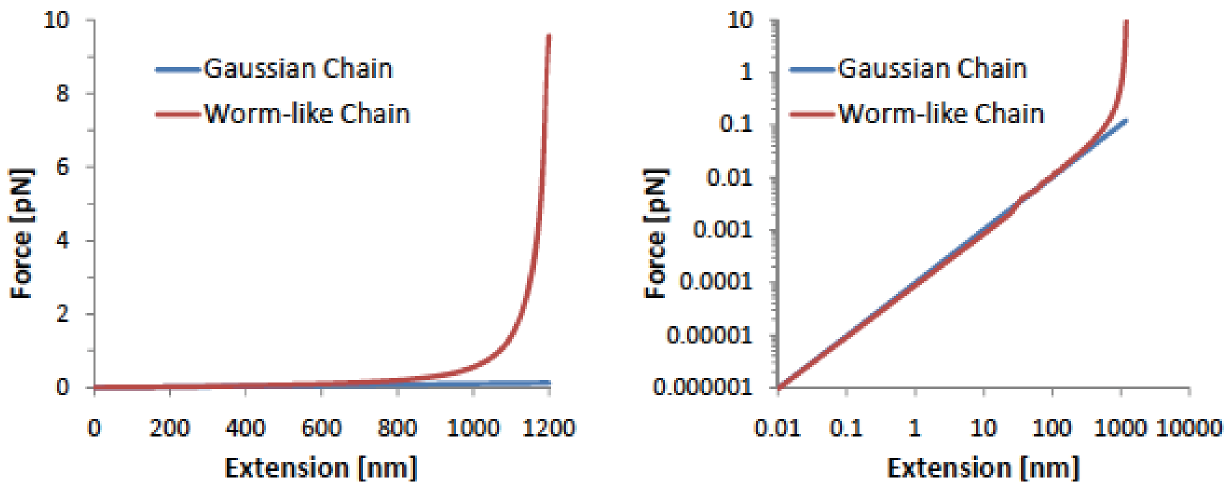
Source: Bustamante, Carlos, Steven B. Smith, et al. "Single-molecule Studies of DNA Mechanics." *Current Opinion in Structural Biology* 10, no. 3 (2000): 279-85.

DNA extension -- comparison of Gaussian chain (Hooke's law) FJC and WLC.





Courtesy of The Biophysical Society. Used with permission.
Source: Baumann, Christoph G., et al. "Stretching of Single Collapsed DNA Molecules." *Biophysical Journal* 78, no. 4 (2000): 1965-78.



But this neglects the effects of internal bonds (H-bonds, ionic or hydrophobic interactions)

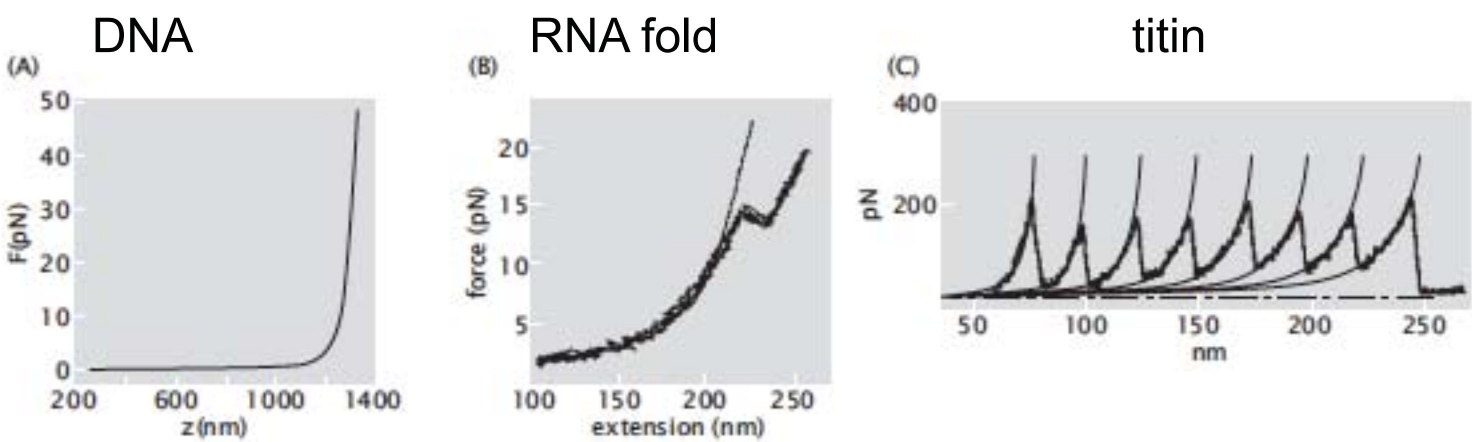


Figure 8.23: Force-displacement curve for a variety of different molecules illustrating the sense in which single molecule experiments serve as the basis of force spectroscopy.

Source: Phillips, Rob, Jane Kondev, et al. Physical Biology of the Cell. Garland Science, 2012.

Each “jump” represents a transition to a different energetic state. How do we account for this?