Measurements of interaction forces in (biological) model systems

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What can force measurements tell us about a system?

Depending on the technique, we might find out about:

- **Attractive and repulsive forces** as a function of distance **within a single molecule, between molecules or between surfaces**:
- **Adhesion**
- **Time-and rate dependence** of interactions
  (can suggest molecular mobility and recovery times of system after probing)
- **Adsorbed layer thickness** (sometimes: molecular size)

Some information is **measured directly** (strength of force, thickness of layer) whereas other things might be **inferred** (mobility, folding, tearing apart).

Expected interactions forces?

Many different ones, superimposed on each other…

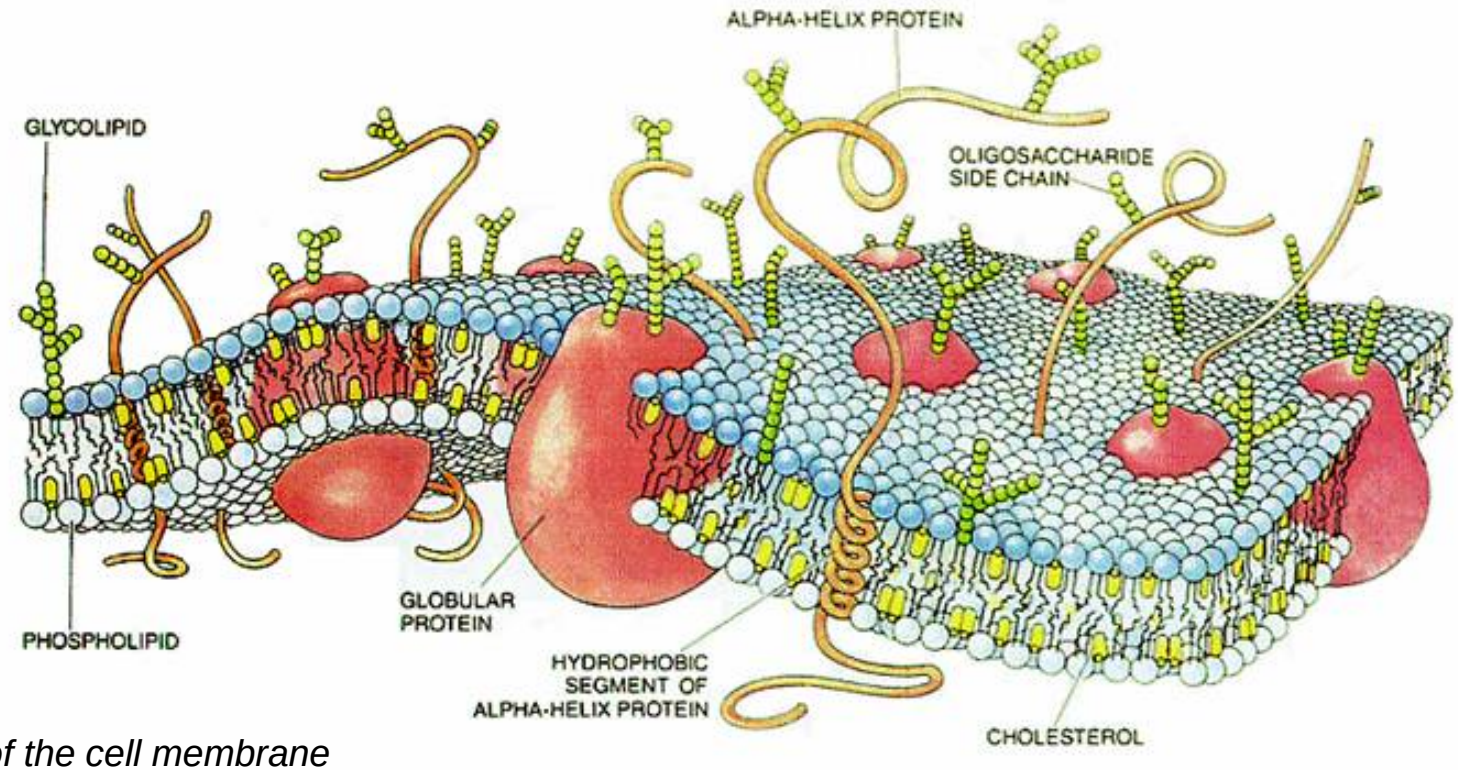
- van der Waals (always there, short range)
- electrostatic (depend on ionic strength)
- steric–entropic (polymer steric forces)
- structural (ordering) (layering between surfaces)
- hydrophobic interactions (what is that? water structure?)
- thermal undulation/fluctuation/ protrusion forces (temporary “bulges”)
- “specific interactions” (for example, receptor–ligand) (depend on orientation, geometry)

Why do we need model systems?

Real systems are typically **complex multicomponent systems:**

😐 **Large** molecules with **non-uniform** composition, “active” and “inactive” parts.

☹ Complex **environment**: Other molecules being within the investigated space or interacting with the same part (or another part) of the molecule of interest.



From *The molecules of the cell membrane*
by M.S. Bretscher, Scientific American, 1985, 253(4), 86-90

Model systems

☺ Measure on "**active**" component?
(isolate component/segment and see what interaction it has)

☺ **No other components** in solution than the ones under investigation
(might eliminate some type of interaction force from the system).

☺ Chosen **concentration/composition** (might be needed to detect force).

So, model systems have to be strongly **simplified**? ☹
How can we know if our results are relevant for the real system?

Can often **keep**:
☺ **Size** of molecules (or work on known fragments)
☺ **Natural** (as opposed to synthetic) components (need to be pure)
☺ **Ionic strength** (solution conditions)

Might have to **sacrifice** (initially):
😐 side chains and additional functionalities
😐 neighboring molecules of same/different kind

Good techniques for measuring forces at the nanoscopic and molecular scale:

1) **Optical tweezers** (laser tweezers)
   - single molecules attached to micrometer-sized bead
   - force sensitivity 1-100 **pN**, distance resolution 10 nm.

2) **Atomic force microscopy** (**AFM**, or scanning probe microscopy SPM)
   - sharp tip (radius 10-100 nm) or small bead (0.5-10 μm)
   - probes single molecule or larger area
   - force sensitivity 1 **pN**, distance resolution sometimes 1 nm,
     relative distance measured to 0.1 nm.

3) **Surface Forces Apparatus (SFA)**
   - two smooth, large surfaces (radius 1-2 cm) interacting
   - absolute distance (sample thickness) measurement
   - force sensitivity 10 nN, absolute distance resolution **0.1 nm**.

1) Optical (laser) tweezers:

- Particles trapped at focal point of focused laser beam
  (momentum change in light wave balanced by momentum change in particle)

$\Rightarrow$ Particle is drawn to focal point of light.

- Can a) position particles and b) measure force needed to move them
  (relationship between displacement and force: ***F*** = ***kx***)

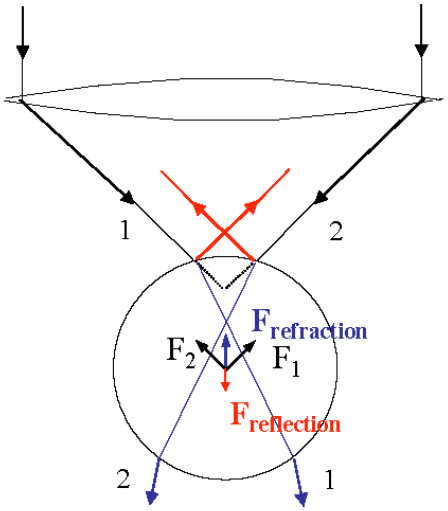


Figure 2. Schematic diagram showing the force on a dielectric sphere due to both reflection and refraction of two rays of light.

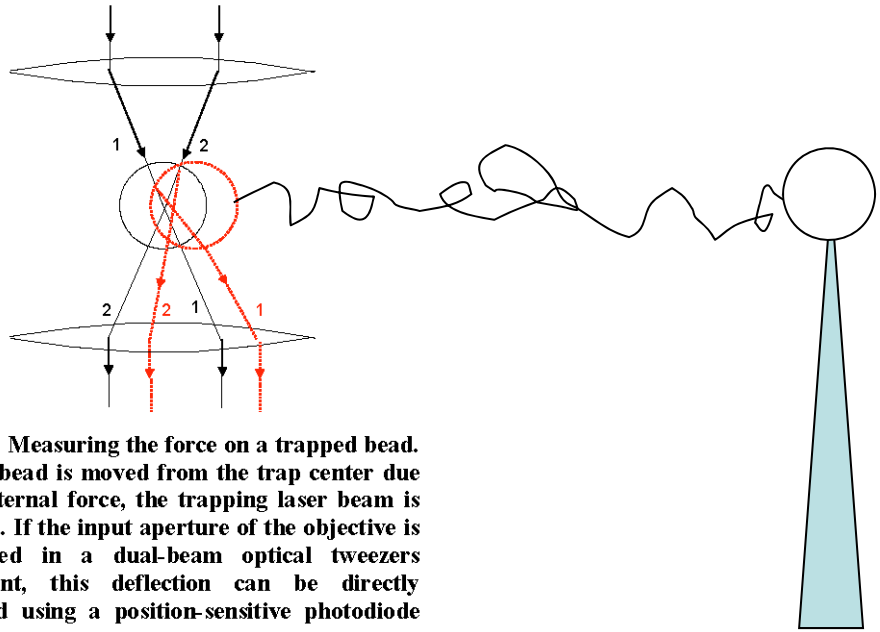
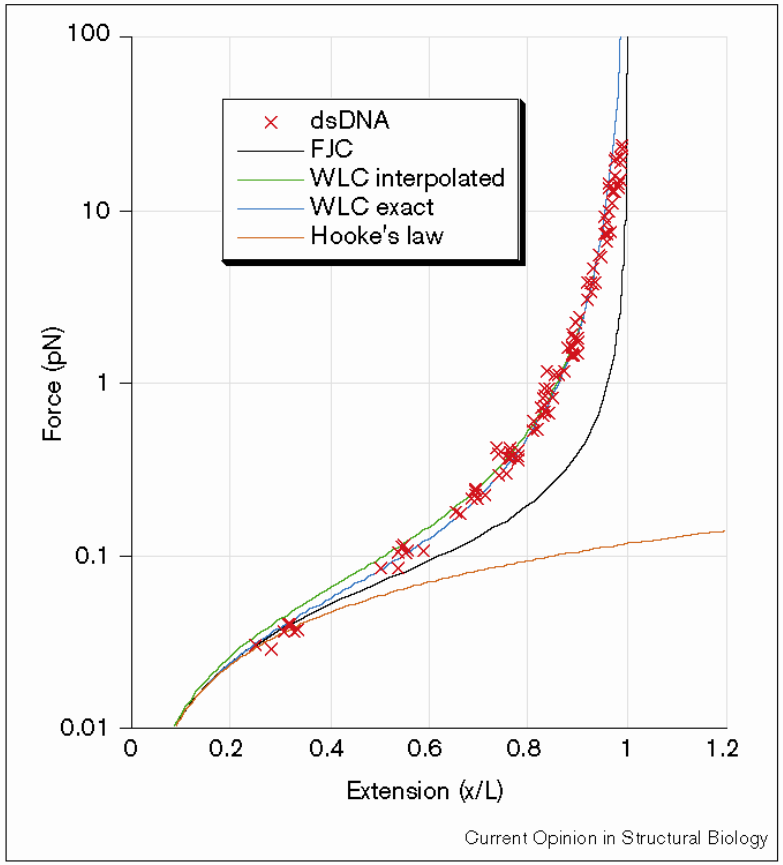


Figure 6. Measuring the force on a trapped bead. When a bead is moved from the trap center due to an external force, the trapping laser beam is deflected. If the input aperture of the objective is underfilled in a dual-beam optical tweezers instrument, this deflection can be directly measured using a position-sensitive photodiode detector.

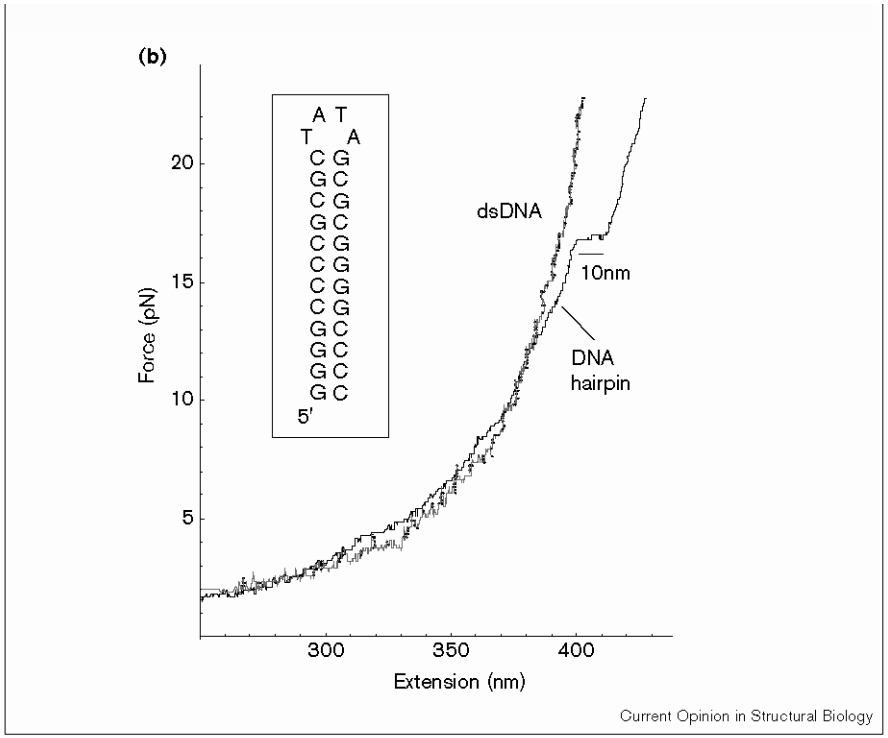
Graphs from Mark Williams, “Biophysics Textbook Online”

Optical tweezers, examples:

Stretching of DNA (before breaking)



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.

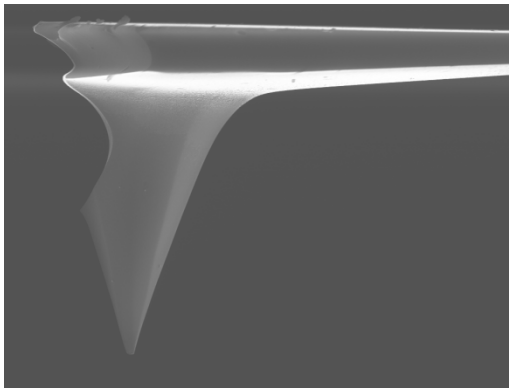


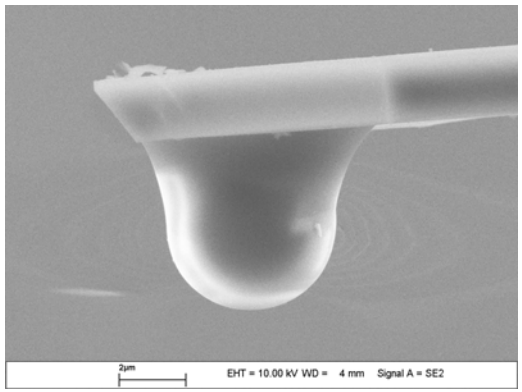
"Unzipping" of "hairpin"

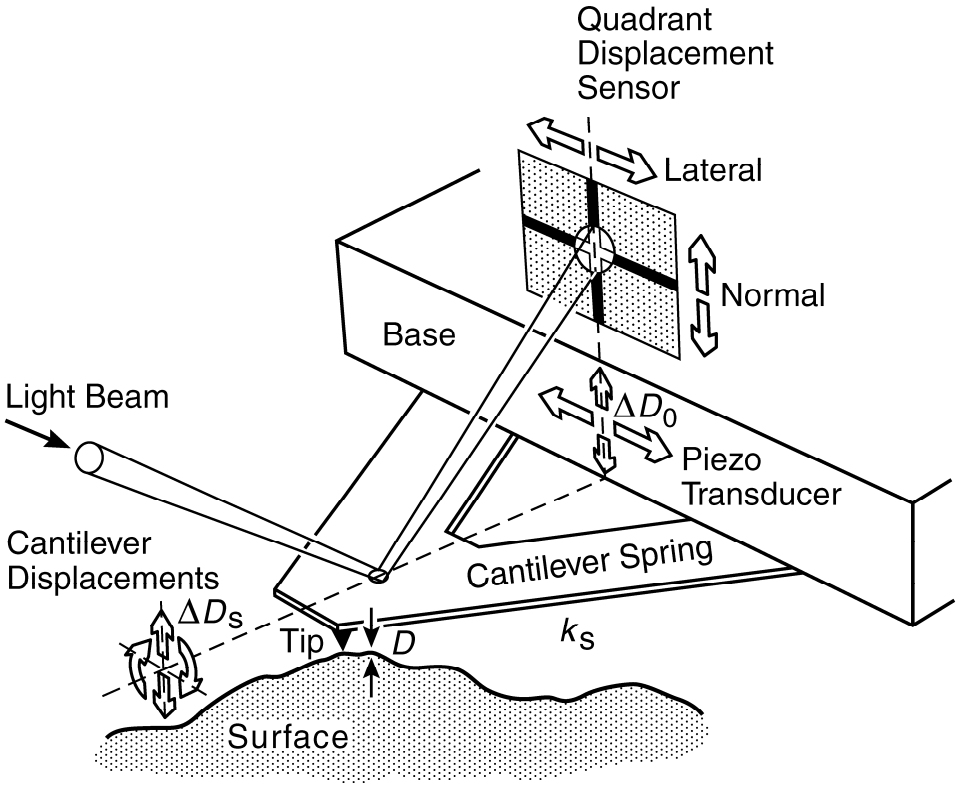
C. Bustamante et al. Single-molecule studies of DNA Mechanics. Current Opinion in Structual Biology (2000) 10:279.

2) Atomic force microscopy (AFM)

- Measure **deflection** of thin cantilever beam when probe interacts with surface
- Scan to make **images** that tell us about molecular size and shape (maybe).
- Can approach and separate quite fast. Difficult to go slowly (piezoelectric elements).





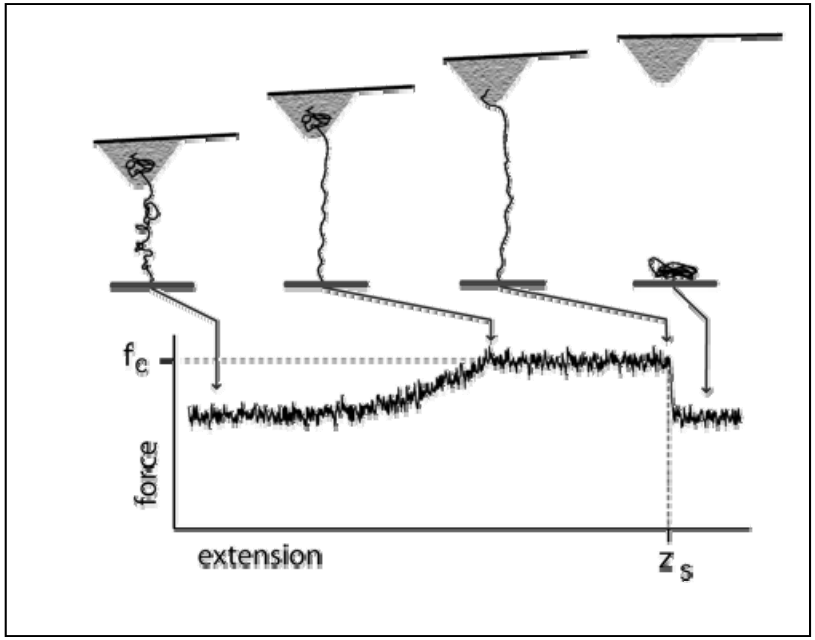


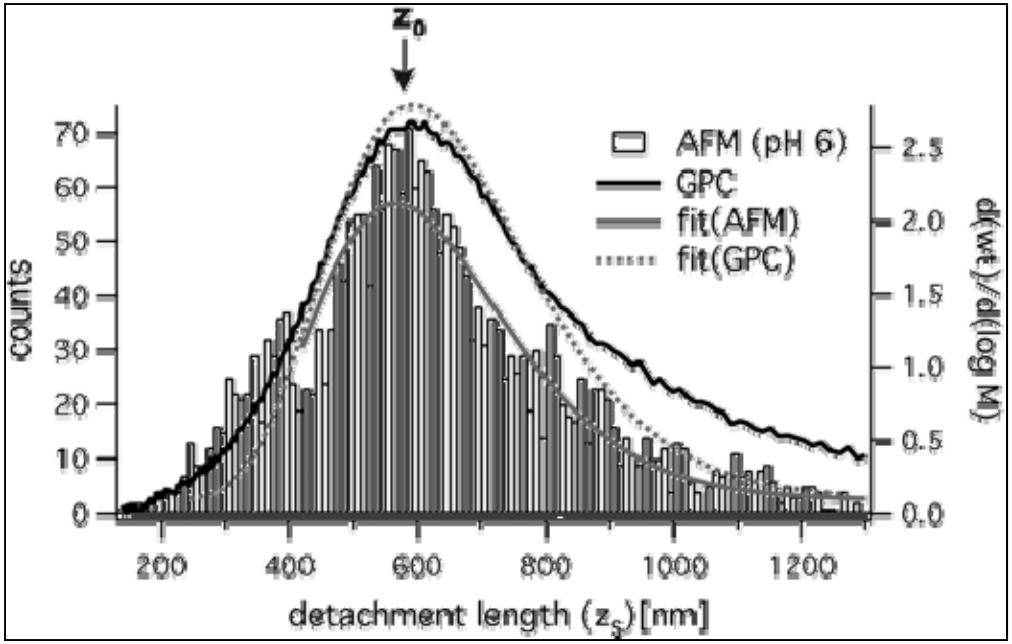
Ruths & Israelachvili, in Handbook of Nanotechnology, 3rd ed., Bhushan, Ed., Springer, Berlin 2010.

Atomic force microscopy, AFM, examples:

Single-molecule force spectroscopy

Pulling linear polymer chain (picking one randomly) and measuring its length:
(**molecules only attached to flat substrate**)





L. Sonnenberg et al. AFM-Based Single Molecule Force Spectroscopy of End-Grafted Poly(acrylic acid) Monolayers. *Macromolecules* **2006,** *39,* 281-288.

AFM: Single-molecule force spectroscopy

- Need to make many measurements to get **statistics** (but AFM is fast)
- Can find out about **relative distances** (e.g., protein unfolding over certain length)
- Cannot know absolute layer thickness (distance from substrate).

Measuring interaction events between **molecules bound on tip and substrate**:

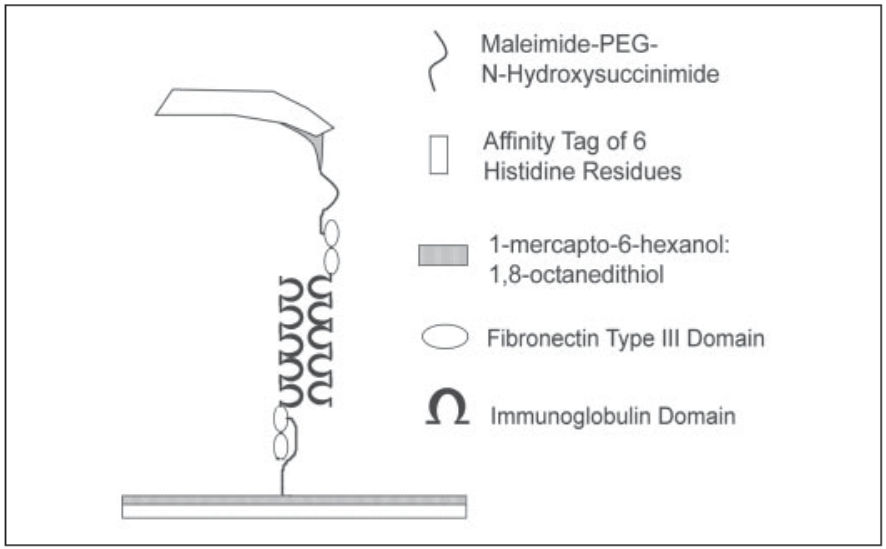


FIGURE 2. **Schematic of the covalent attachment of the protein to both the AFM tip and surface.**

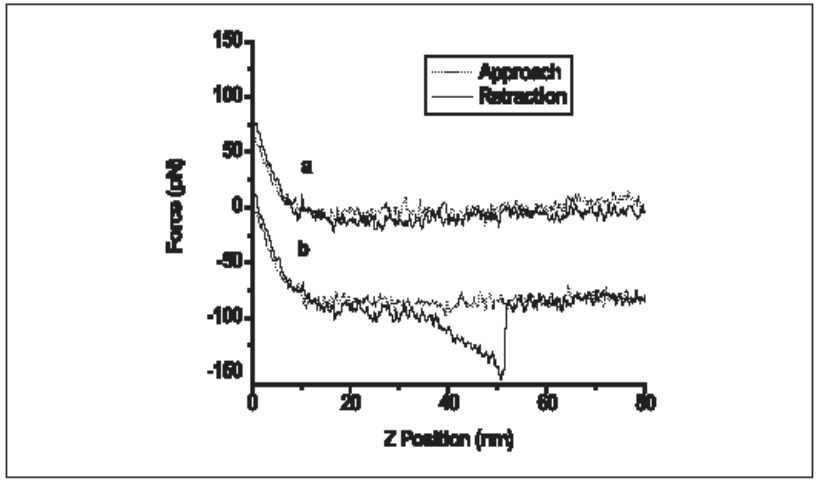


FIGURE 5. **Force extension profiles obtained from measurements between full-length NCAM extracellular domains showing both the absence (*a*) and occurrence (*b*) of a binding event.**

Wieland, Julie A.; Gewirth, Andrew A.; Leckband, Deborah E. Single Molecule Adhesion Measurements Reveal Two Homophilic Neural Cell Adhesion Molecule Bonds with Mechanically Distinct Properties. Journal of Biological Chemistry (2005), 280, 41037.

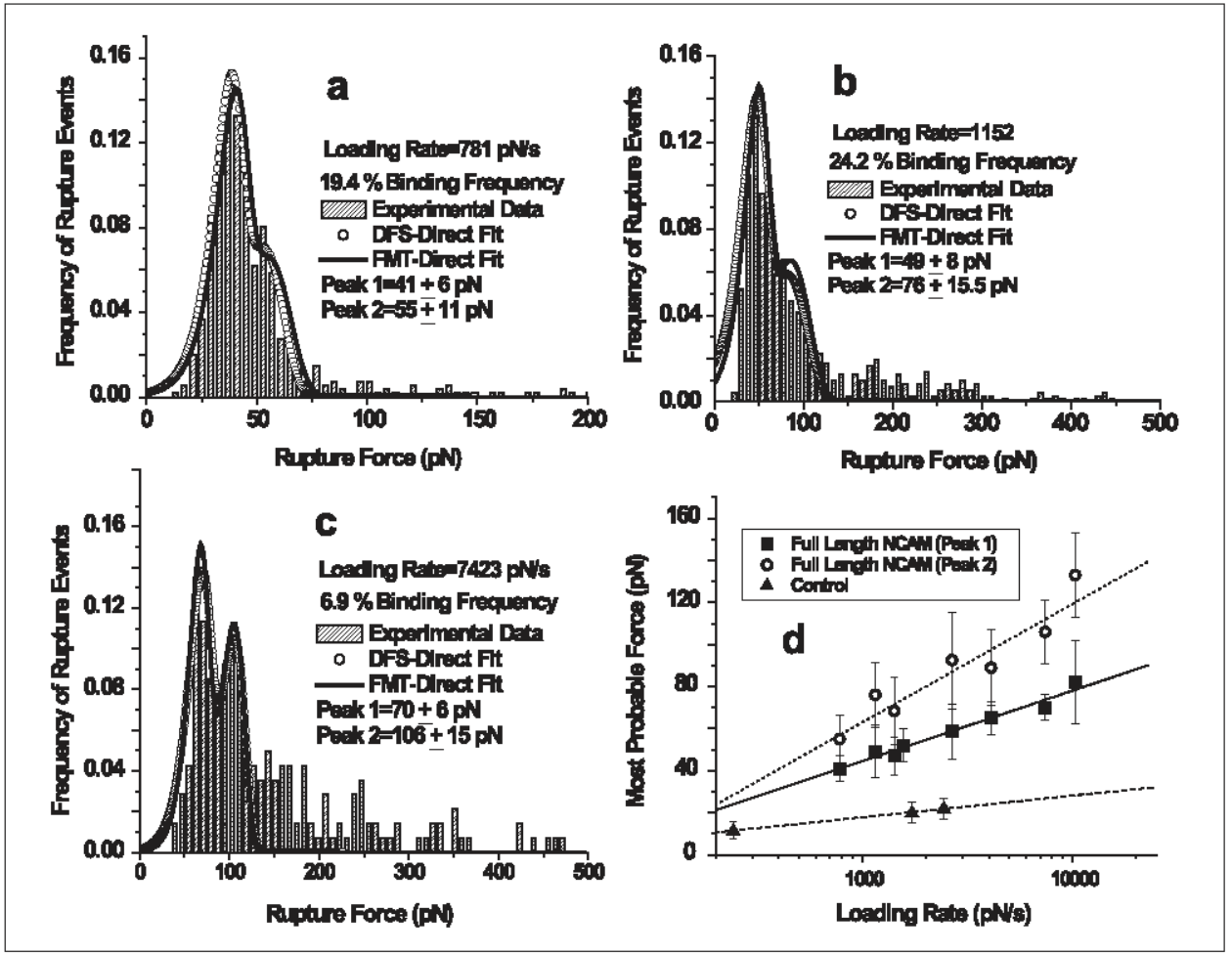
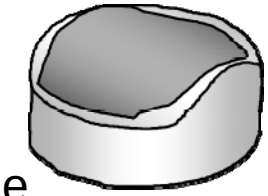


FIGURE 7. **Histograms of the rupture force measured between full-length NCAM extracellular domains at different loading rates (*a*–*c*).** Plot of the most probable rupture force F_m *versus* the logarithm of the loading rate (dynamic force spectrum) for both peaks 1 and 2 (*d*). *d* also shows the results of control measurements (*triangles*).

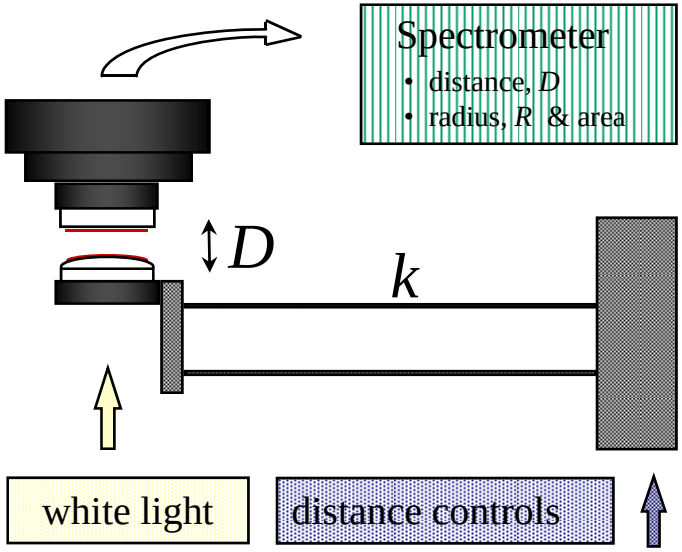
Wieland, Julie A.; Gewirth, Andrew A.; Leckband, Deborah E. Single Molecule Adhesion Measurements Reveal Two Homophilic Neural Cell Adhesion Molecule Bonds with Mechanically Distinct Properties. Journal of Biological Chemistry (2005), 280, 41037.

3) Surface Forces Apparatus (SFA)

- Measure deflection of macroscopic spring, force is an average over large area
- Need dust-free sample, homogeneous over 100x100 microns



- Can measure absolute distances and deformation/shape of sample at micrometer scale laterally and 0.1 nm in height
- Can go slowly (hours), cannot go really fast because of hydrodynamic effects.



Surface forces apparatus, SFA, examples:

Biotin–streptavidin interaction, **supported membranes**:

Lower limit for receptor–ligand interaction strength deduced from measured force, for **known density**.

Binding so strong that lipid is pulled out from the membrane (cannot separate receptor–ligand bond again).

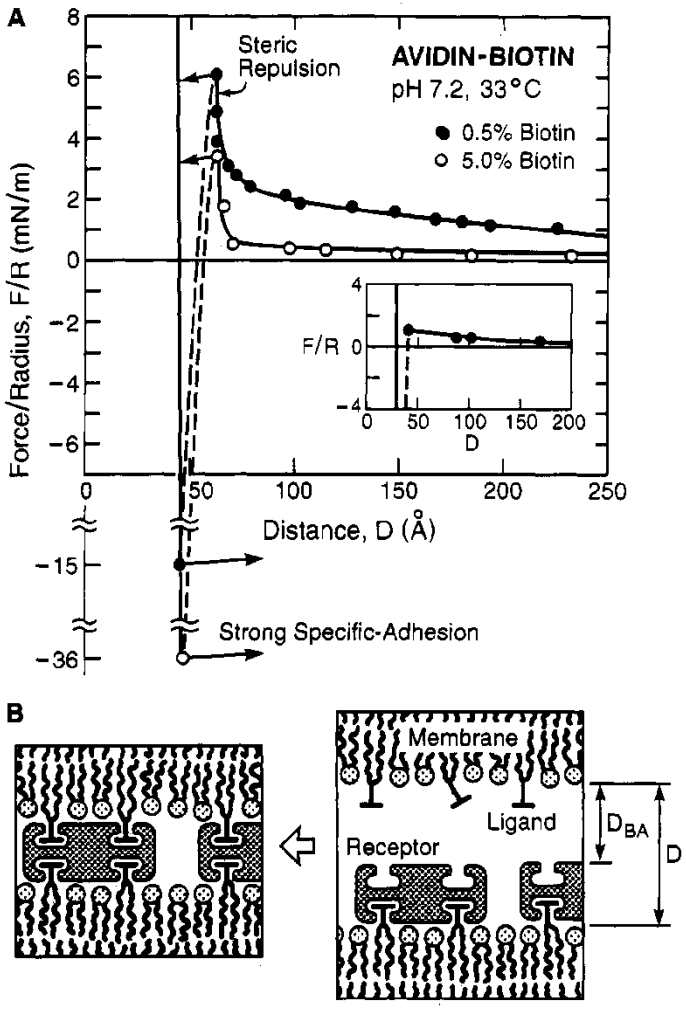


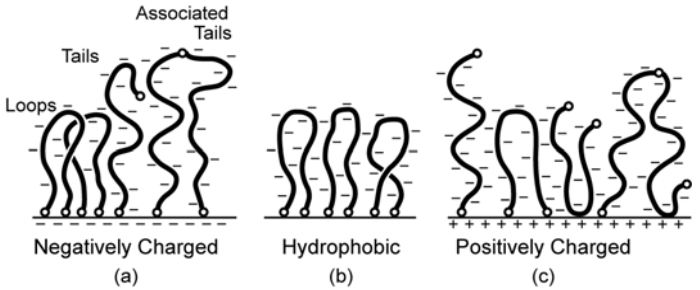
FIGURE 6: (A) Measured force–distance profiles for a streptavidin surface interacting with a 5% biotin surface (○) and a 0.5% biotin surface (●) in 0.3 mM salt at pH 7.2 and 33 °C ($T > T_c$). At this temperature, the outer monolayers are in the fluid state. The equilibrium force–distance profile, demonstrating the absence of the time-dependent steric force barrier at $D \approx 65$ Å ($D_{BA} \approx 20$ Å), is shown in the inset. (B) Schematic illustration of the biotin and streptavidin molecular configurations during their approach into strong adhesive contact at $D = 45$ Å ($D_{BA} = 0$ Å).

D. Leckband et al., Direct force measurements of specific and non-specific protein interactions. Biochemistry, 1994, 33: 4611.

SFA:

Adsorbed layers of a natural glycoprotein

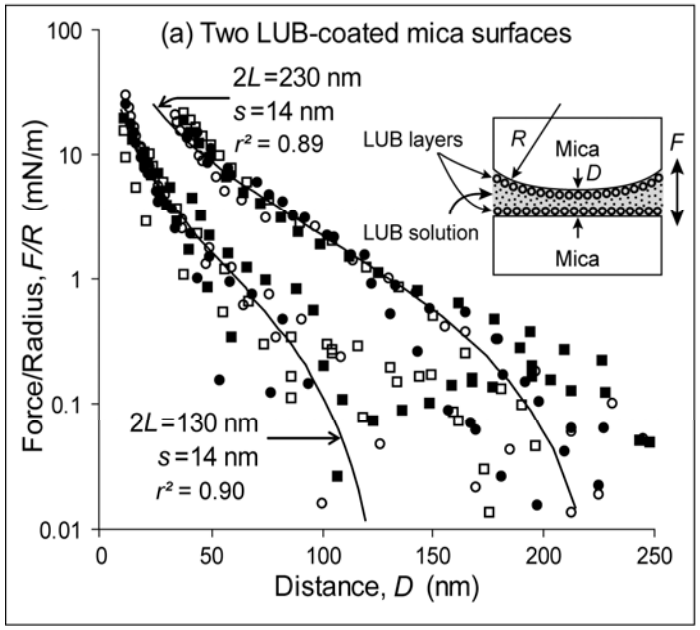
from knee joints

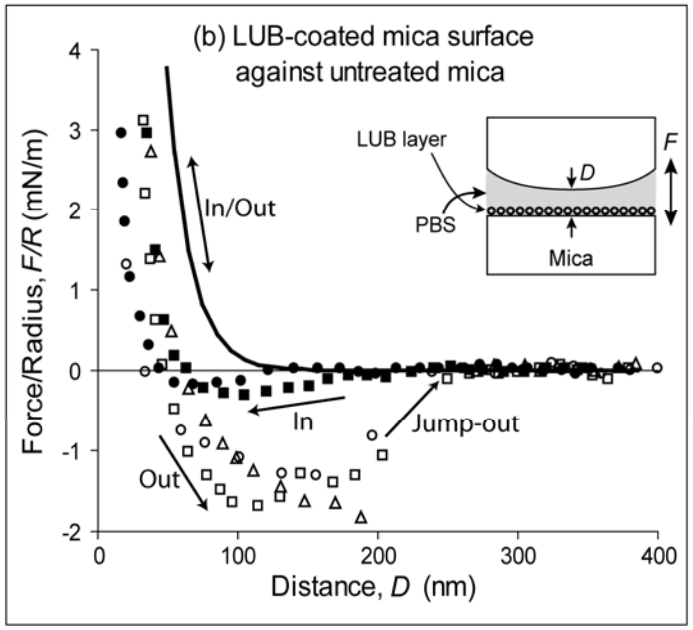


"Lubricin" adhers

1) to negatively and positively charged hydrophilic surfaces, and
2) to hydrophobic ones, with **similar adsorbed amount and similar structure** (**polymer brush** configuration).

Low friction at pressures similar to in knee joint, protects surfaces from damage.





B. Zappone et al.
Biophysical Journal 2007

Limitations/concerns when using these techniques:

All of them require a lot of **skill**. The results are difficult to **interpret** (need to separate the various components of the measured overall force).

How do we know that what we are measuring is **representative** of what happens in the real (biological) system? Have we constructed the right model system?

Some final thoughts:

A lot of information can be obtained from force measurements, but a careful check of **conditions** is necessary.

Also, we have to keep in mind that an investigation of a **single molecule** might not tell us exactly what happens in a real system.

Probing with an external, large probe might **perturb** the system.