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.

เอกสาร 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)
  \[ \Rightarrow \text{Particle is drawn to focal point of light.} \]

- 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)**

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**Graph 1:**
- **Legend:**
  - dsDNA
  - FJC
  - WLC interpolated
  - WLC exact
  - Hooke’s law

**Force versus extension data (red crosses) for λ phage dsDNA (48,502 bp) pulled by magnetic beads in 10 mM NaCl buffer [4].** The data are fit to a WLC model solved numerically (WLC exact) or using Equation 3 (WLC interpolated), both assuming P = 55 nm. The FJC curve assumes b = 2P = 106 nm. The Hooke’s law force curve is from Equation 2.

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**Graph 2:**
- **Title:** “Unzipping” of “hairpin”

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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).

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)

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 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 $F_{\text{m}}$ 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).
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 (O) and a 0.5% biotin surface (●) in 0.3 mM salt at pH 7.2 and 33 °C (T > Tc). 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 \( D \approx 65 \, \text{Å} \) (\( D_{BA} \approx 20 \, \text{Å} \)), is shown in the inset. (B) Schematic illustration of the biotin and streptavidin molecular configurations during their approach into strong adhesive contact at \( D = 45 \, \text{Å} \) (\( D_{BA} = 0 \, \text{Å} \)).
SFA: Adsorbed layers of a natural glycoprotein from knee joints

“Lubricin” adheres
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.
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.