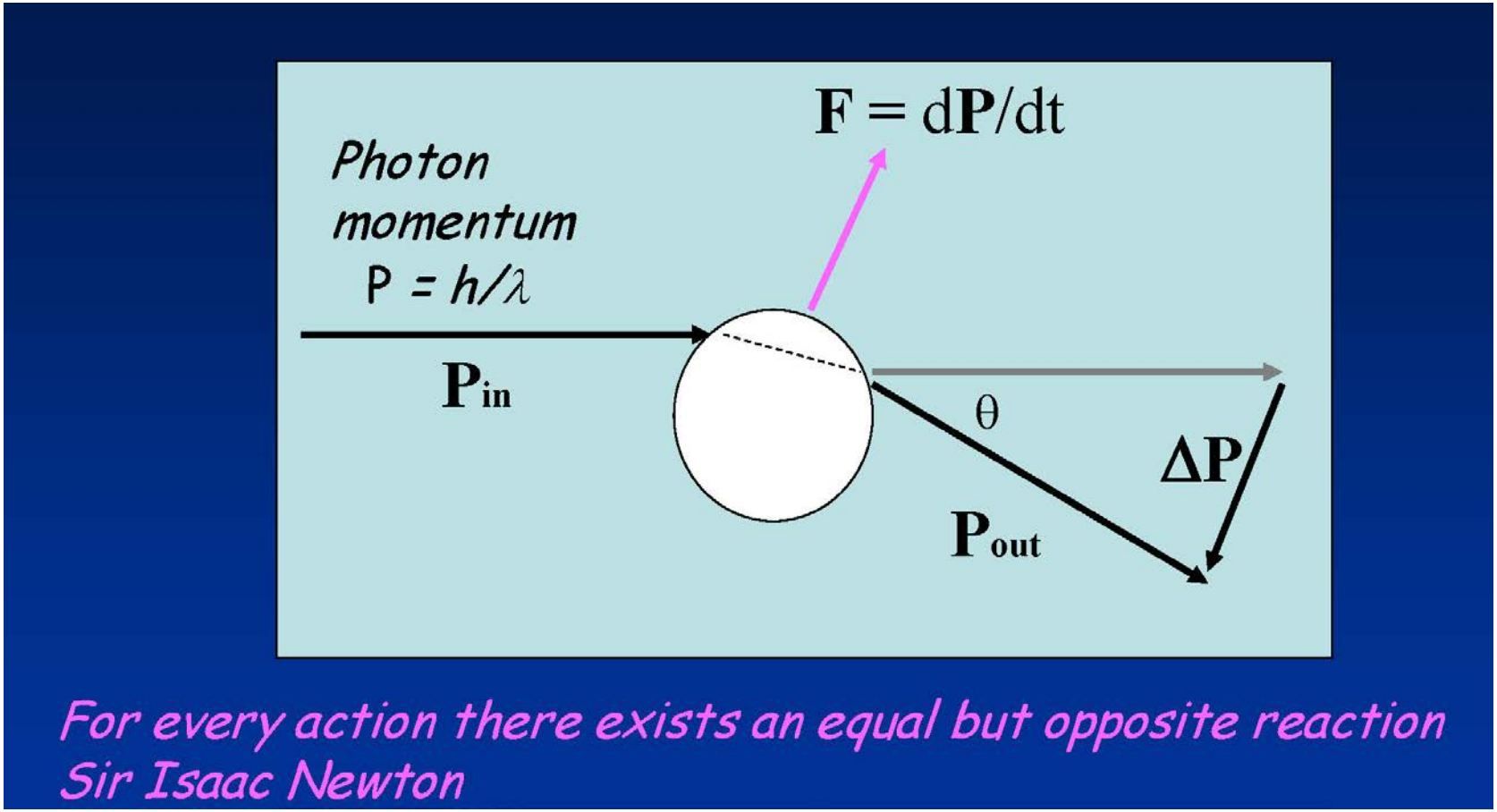
Single Molecule Force Microscopy

Optical Trap

Magnetic Tweezers

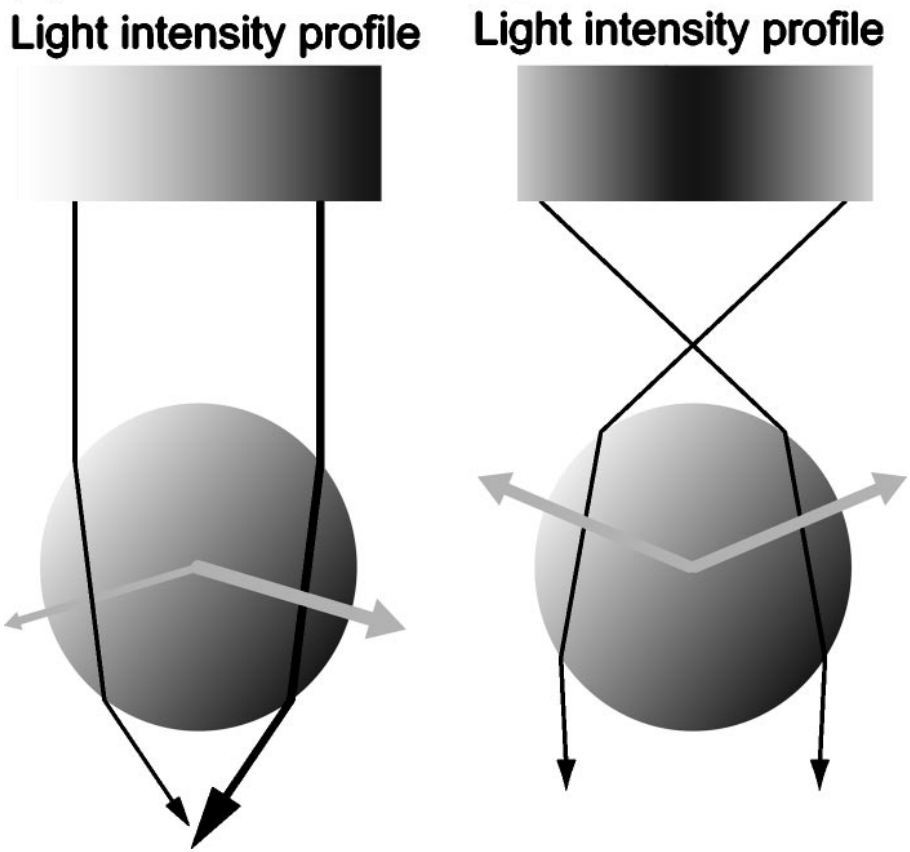
Atomic Force Microscopy

Optical Trapping



A small dielectric sphere (with high index of refraction) of radius r>>λ acts like a lens. The momentum transfers with the deflection of the laser beam.

Trapping a Glass Bead



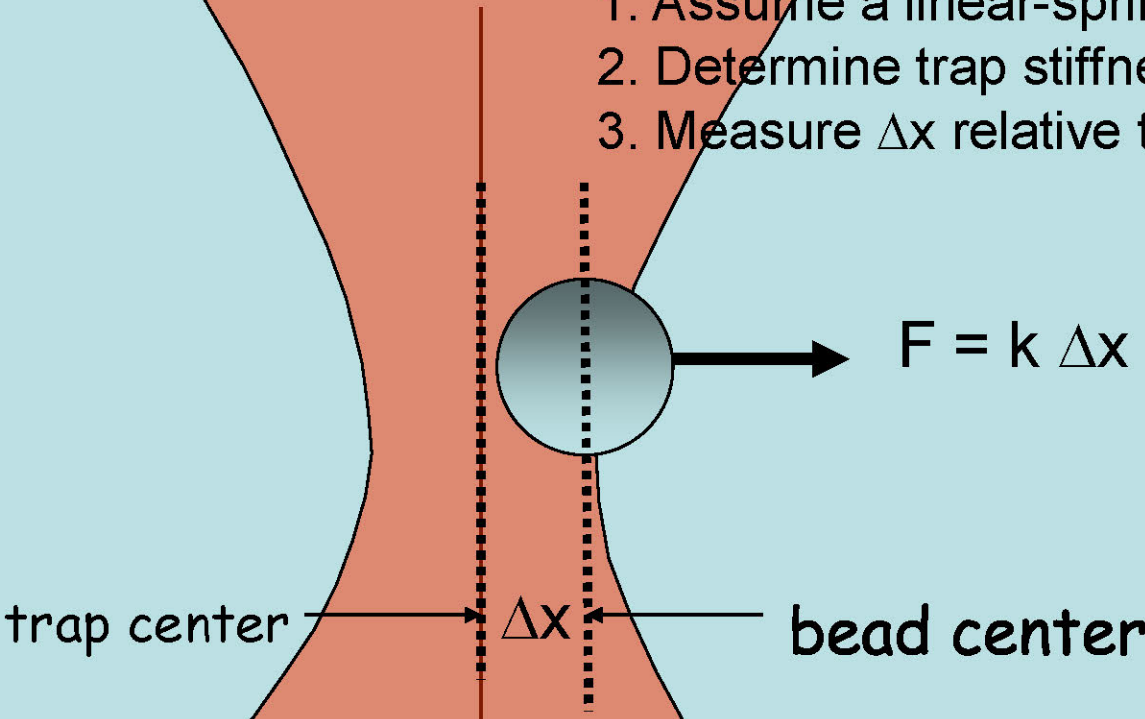
(Right) When the bead is in the center of the focused laser beam, forces that push the bead to right and left cancel out each other.

(Left) If the sphere moves off the trap, the refracted light exerts radial forces to push the sphere back on the axis.

The diameters of the bead usually range from 200 nm to 5 μm, to achieve trapping potential larger than kT.

Estimating Forces

1. Assume a linear-spring restoring force
2. Determine trap stiffness k
3. Measure Δx relative to trap center

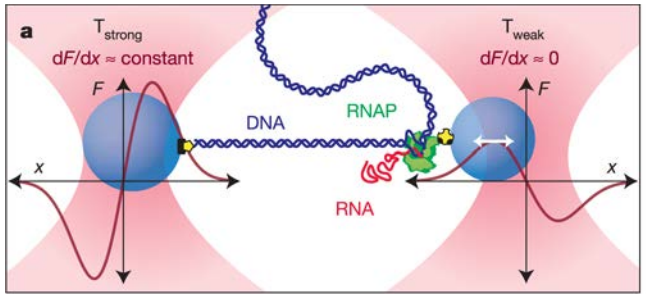


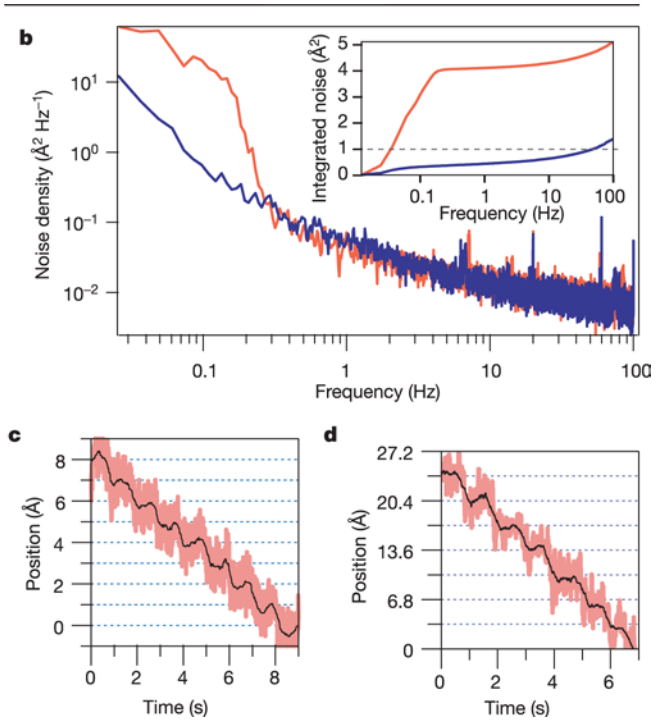
For a Gaussian beam, the intensity gradient is almost linear near the beam center. Therefore, trap acts as a Hookean spring ($F = -k.\Delta x$)

Technical Capabilities

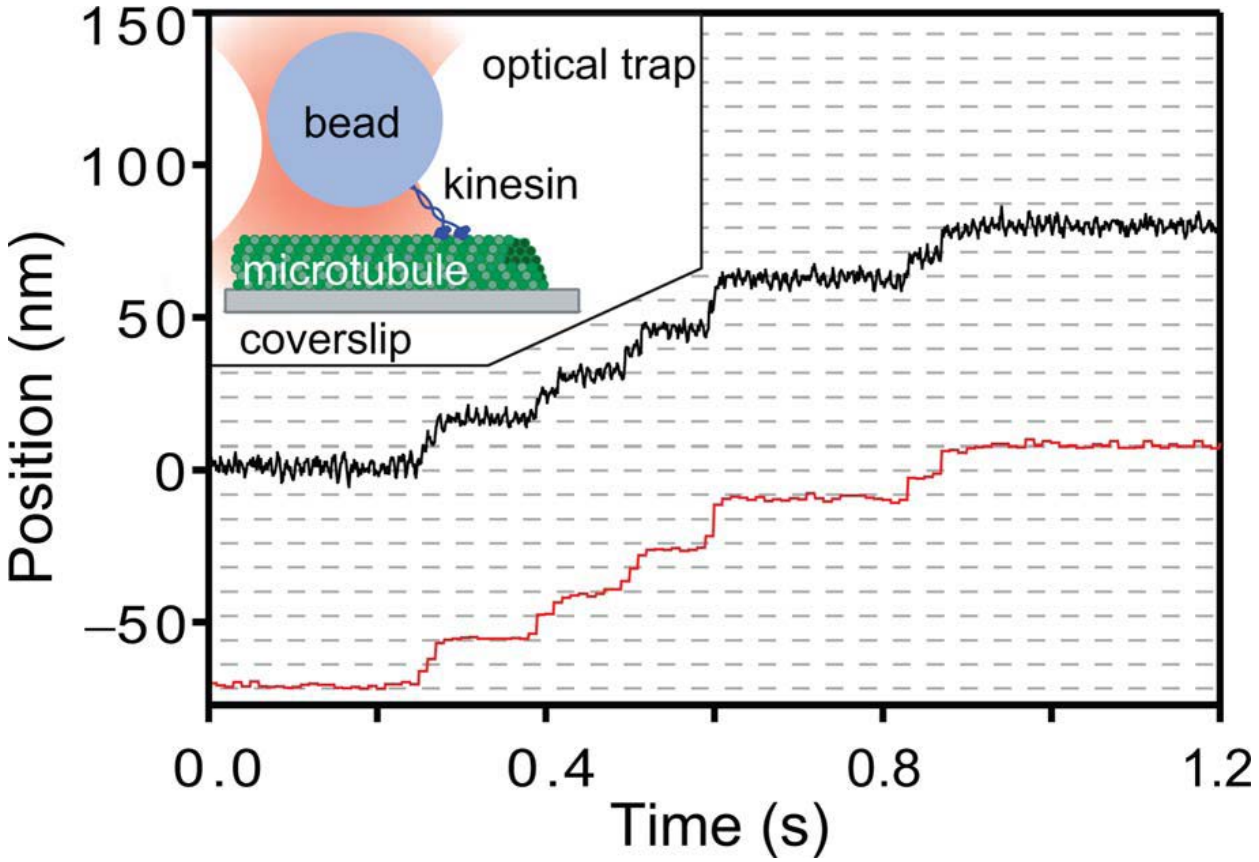
Single biological molecules are too small to be trapped. They need to be attached to a large bead to achieve pN levels of forces.

Time resolution is on the order of 100 µsec. (limited by the roll off frequency of the bead molecule system)
Traps can achieve 1 A precision in bead position.(limited by vibrational, thermal noise and laser beam pointing stability)



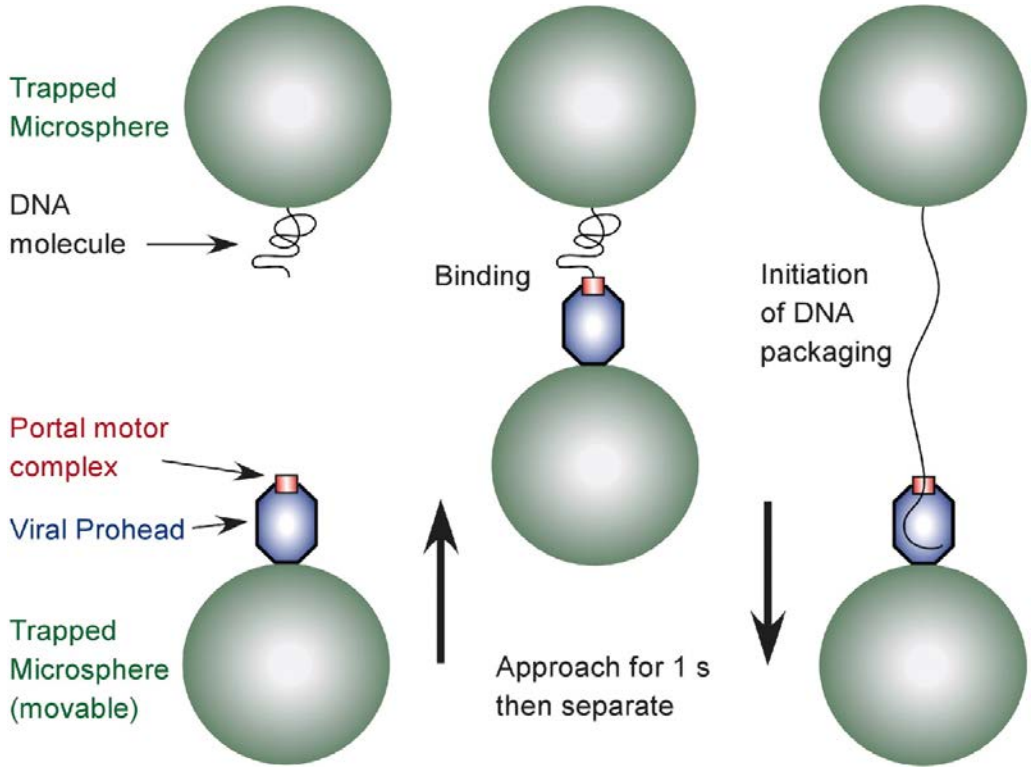


Trapping Geometries-1



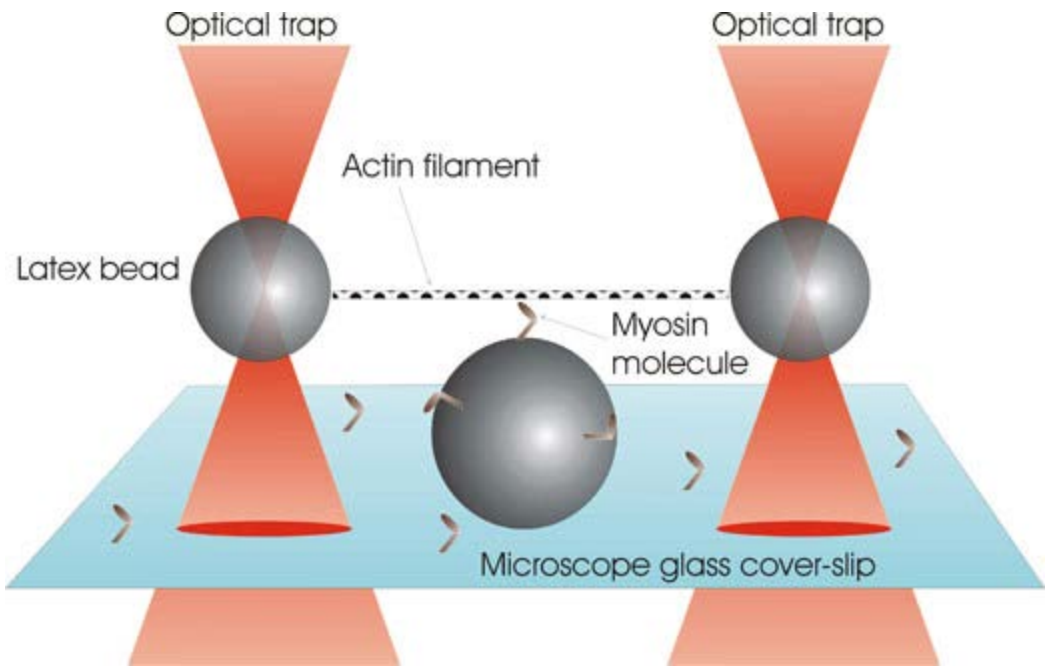
Single motor protein movement along surface immobilized microtubules

Trapping geometries-2



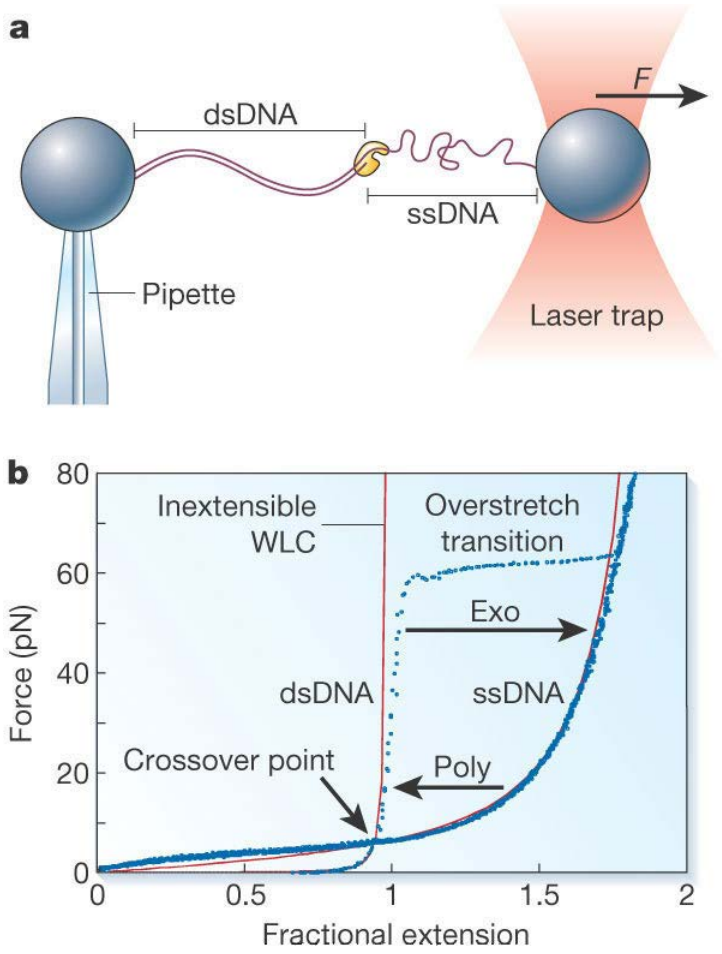
DNA packaging motor bound to a dual beam trap

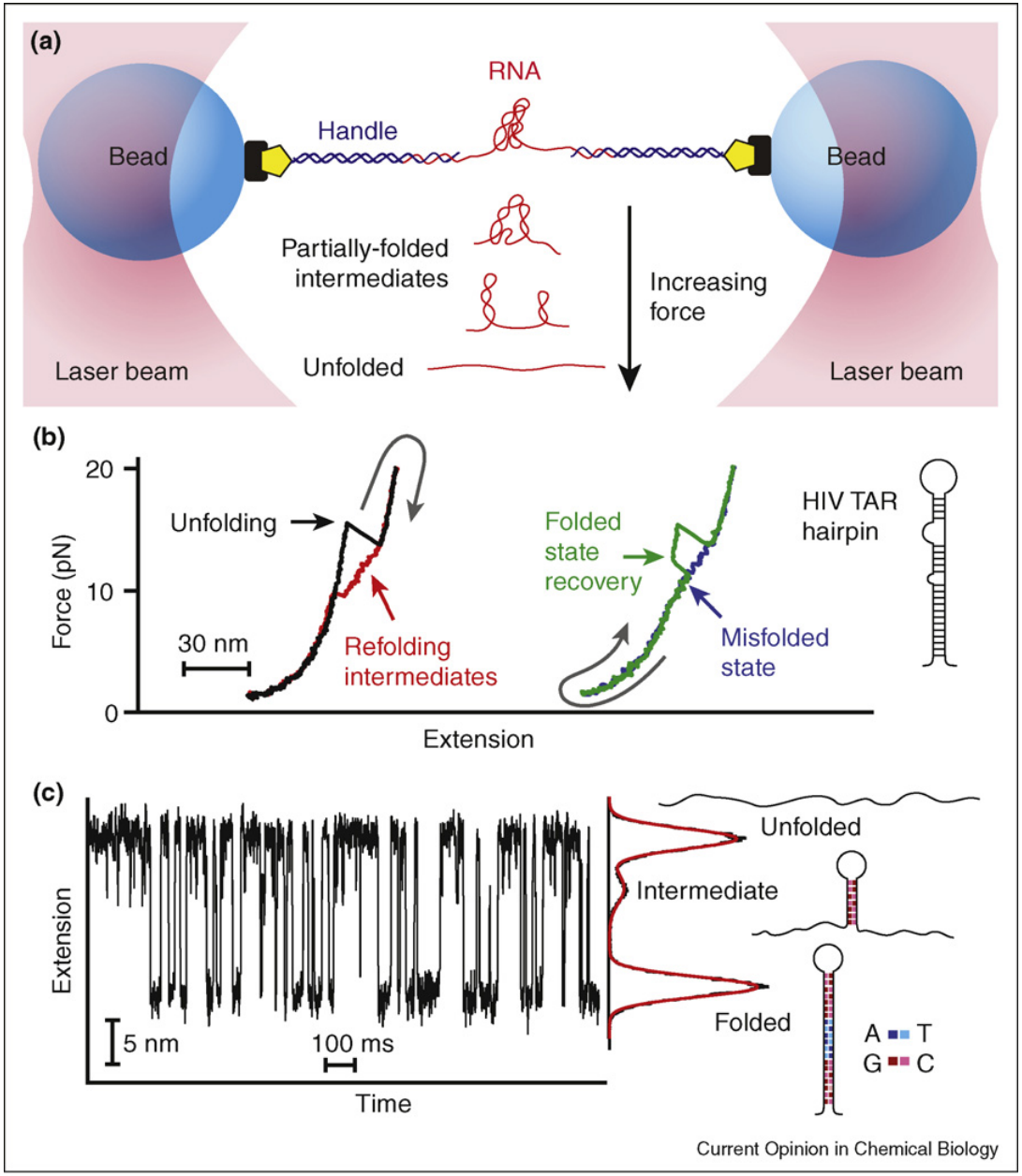
Trapping Geometry-3



Three Bead Optical Trapping for Nonprocessive Motors: Actin Filament is attached to a dual beam and myosin II motors are on an immobilized bead.

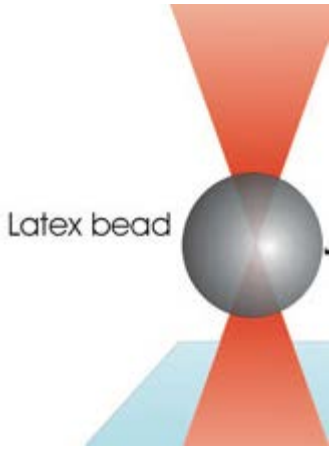
Elastic Properties of DNA

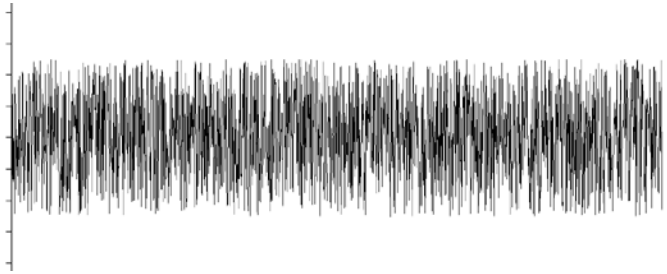



(a)
RNA
Handle
Bead
Bead
Partially-folded
intermediates
Increasing
force
Unfolded
Laser beam
Laser beam
(b)
20
10
0
Force (pN)
Unfolding
30 nm
Refolding
intermediates
Folded
state
recovery
Misfolded
state
HIV TAR
hairpin
Extension
(c)
Extension
5 nm
100 ms
Time
Unfolded
Intermediate
Folded
A T
G C
Current Opinion in Chemical Biology

Calibrating the Force Constant

1. Equipartition



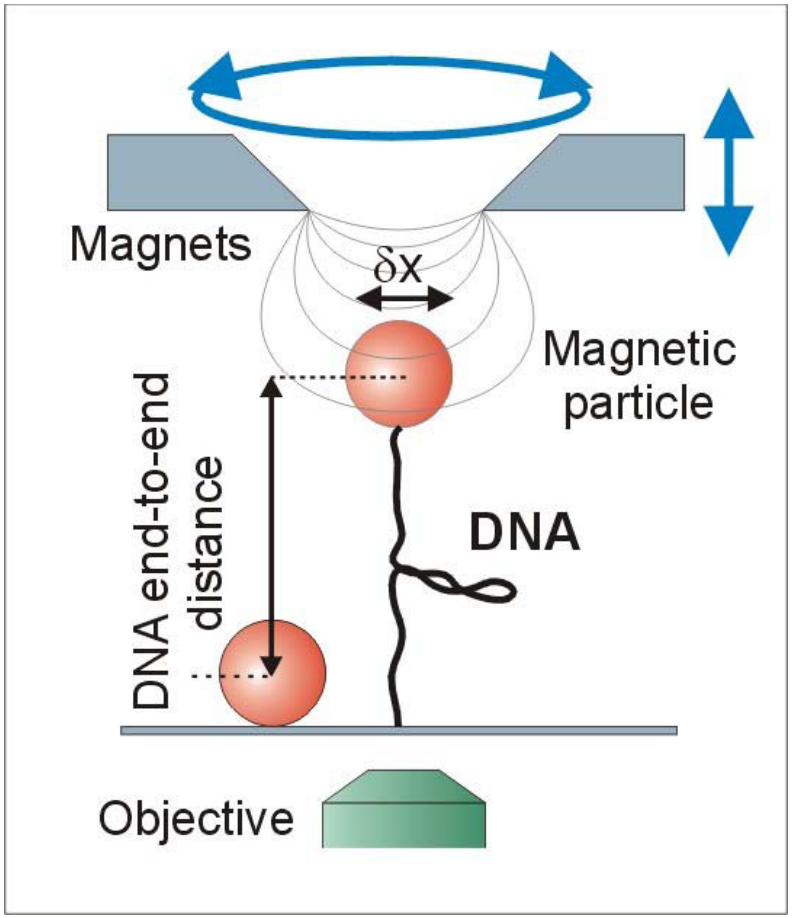


- Position of the trapped bead will fluctuate by thermal motion.
- Energy in each axis is equal to ½ $k_B T$
- Energy stored in trap is ½ $\kappa(\Delta x)^2$

$$\frac{1}{2}\kappa < \Delta x >^2 = \frac{1}{2} k_B T \qquad \kappa = \frac{k_B T}{<\Delta x>^2}$$

- You must have an accurate measure of bead position at high frequencies to monitor the Brownian motion of the bead.
- External noise factors (drift, acoustic noise, mechanical vibration) will overestimate the variance of bead position

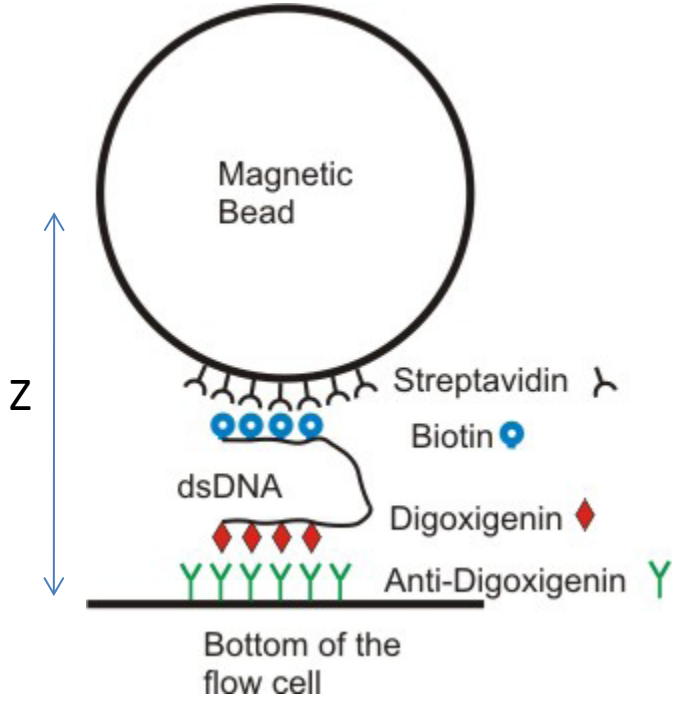
Magnetic Tweezers

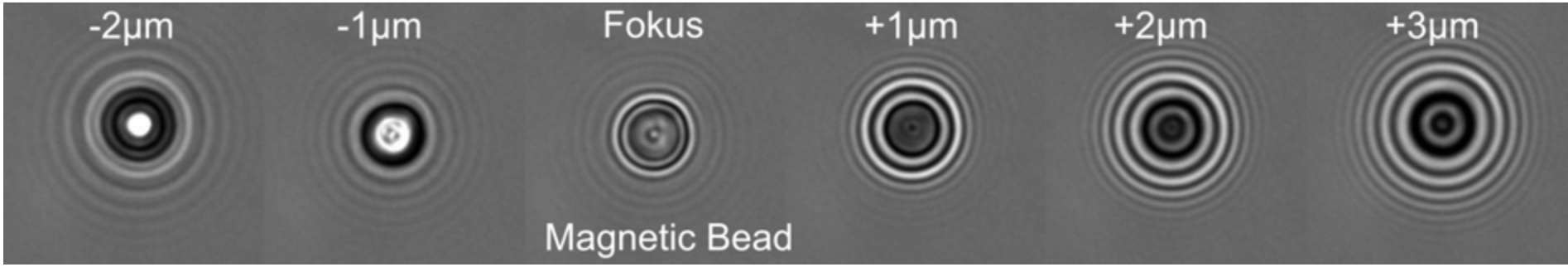


•Magnetic tweezers are a unique tool to manipulate, i.e. stretch and twist, single biomolecules and to read out their response in the form of length changes.

•To apply force and torque, these biomolecules are attached to small superparamagnetic particles, which can be moved in magnetic field gradients.

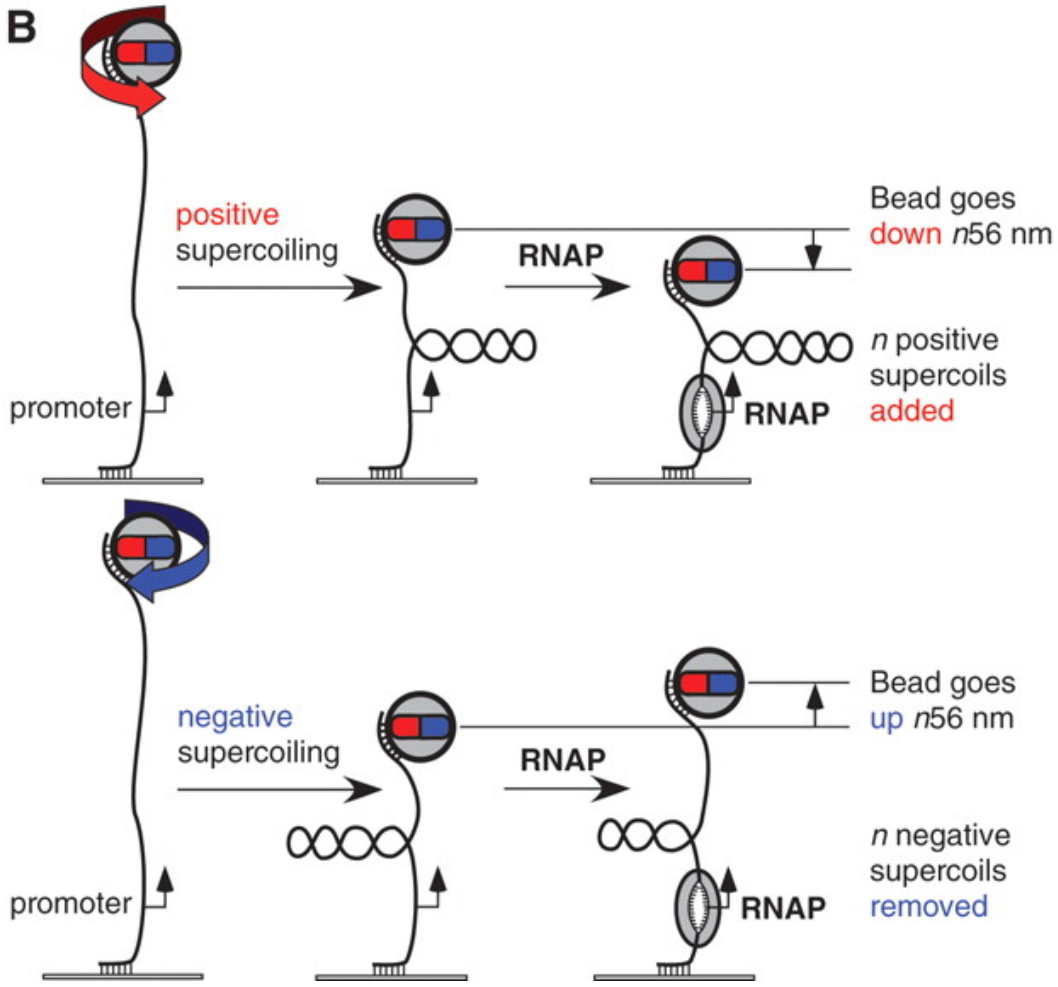
Typical spatial resolution is between 2 and 10 nm and time resolution is around 1 msec





Calibration profile can be obtained to determine z position of the bead with 10 nm precision.

RNA Polymerase



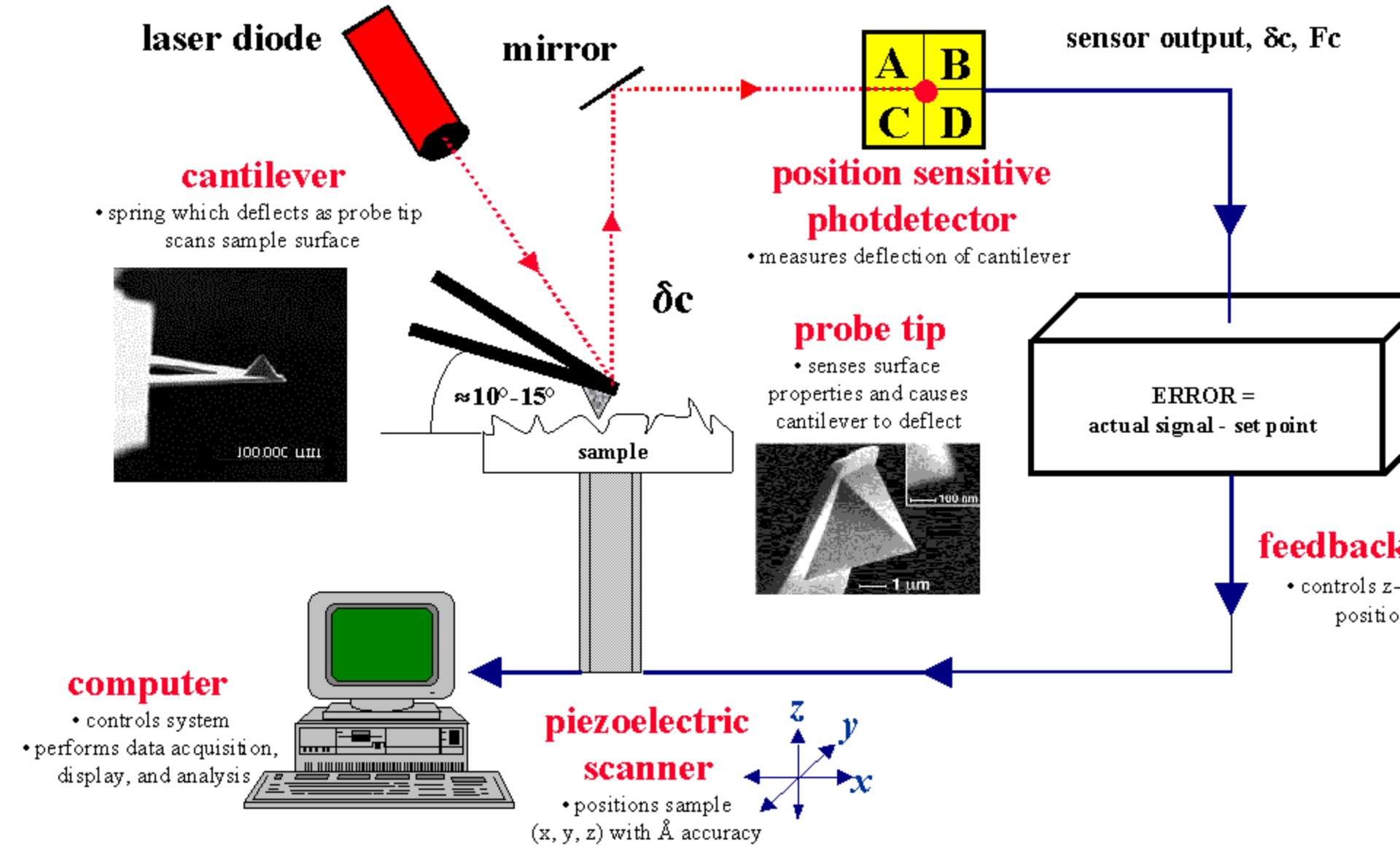
RNAP unwinding adds positive supercoils to the DNA.

If the DNA already contains positive supercoils RNAP activity reduces the distance between the bead and the surface.

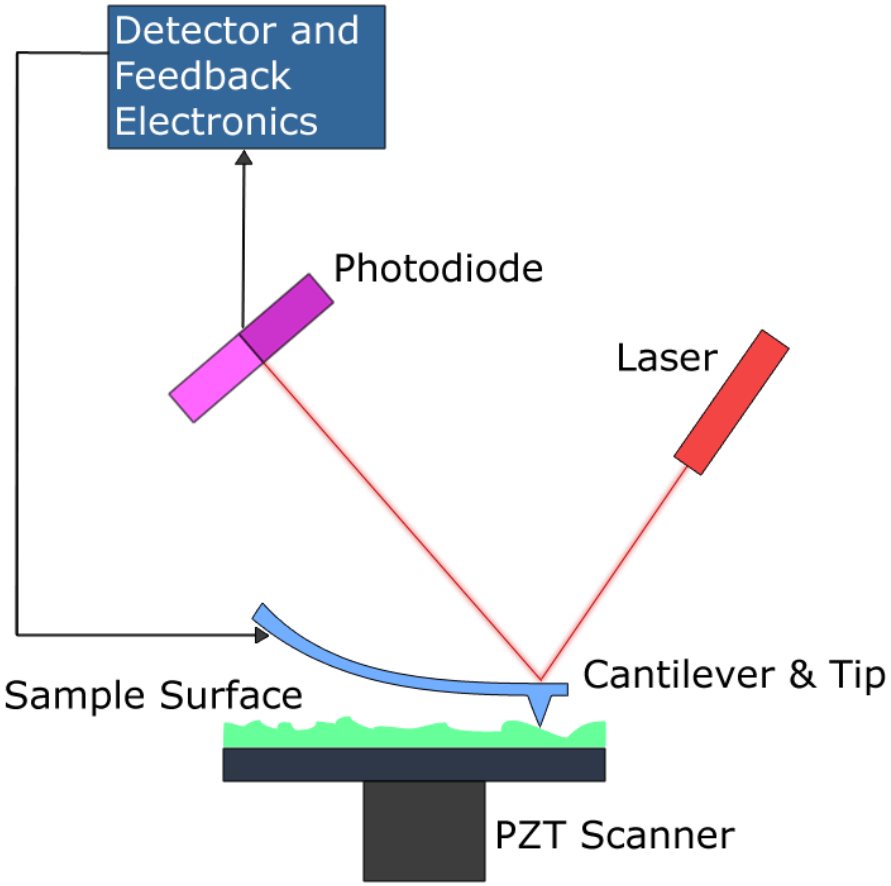
If the DNA contains negative supercoils, bead goes up.

Atomic Force Microscopy (AFM) :

General Components and Their Functions



Position Detection



Measures short range interactions between a scanning probe and object to contour conducting and nonconducting surfaces.

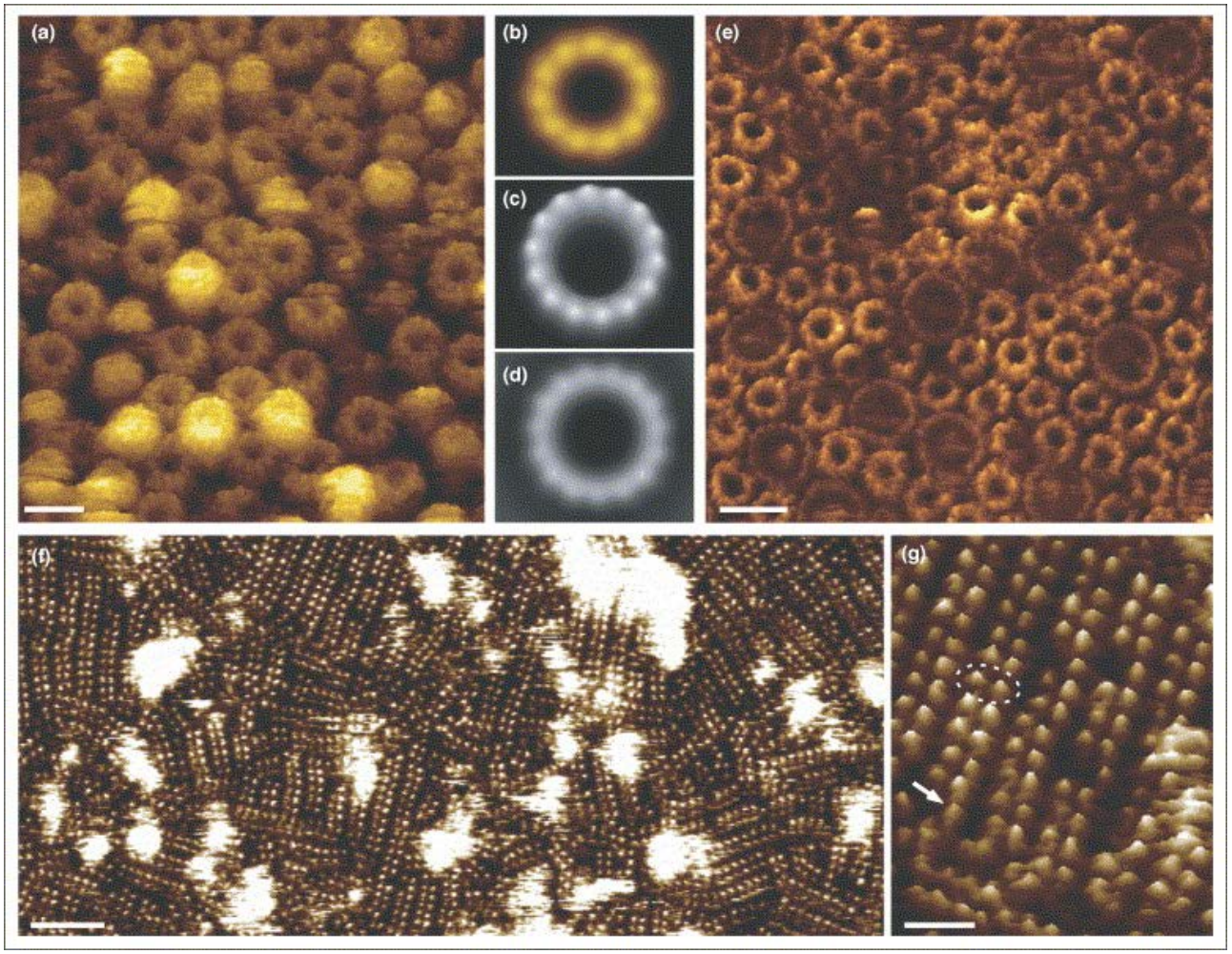
Recording of the force at every pixel is used to reconstruct the surface topography.

Constant Force Mode: Force on AFM is kept constant by feedback loop. The image detects the height change for each pixel and reflects the sample topography.

Oscillation Mode: AFM head is oscillated sinusoidally and feeedback loop ensures the probe touches the surface at the minima of the downward movement. This interaction changes the amplitude, which is reconstructured to a topograph.

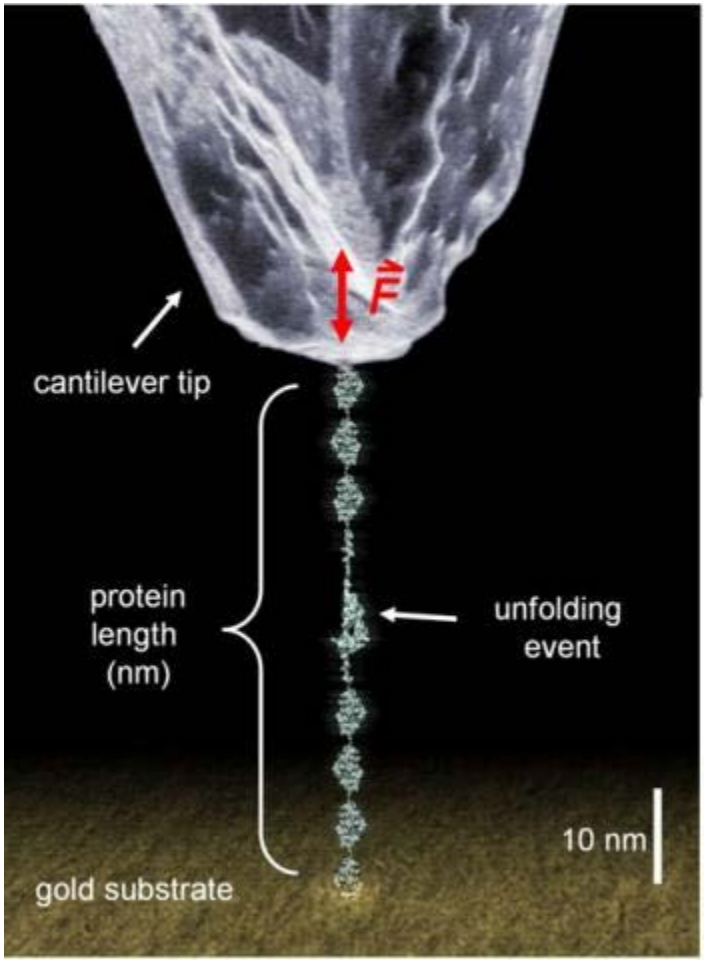
AFM can achieve ~0.5 nm precision in xy imaging.
Vertical resolution is in Angstrom range.

AFM Images



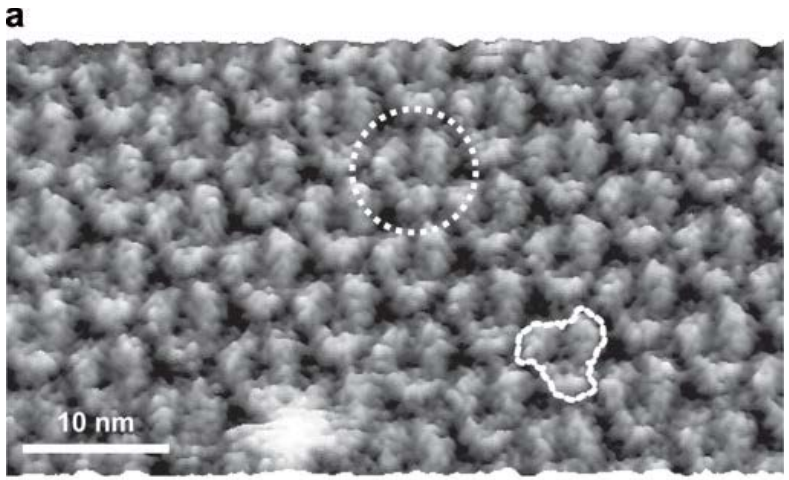
a-d) Fo-F1 ATPase, e) the mixture of light harvesting complex, small rings with ~50A diameter, and a reaction center, f-g) Rho dimers on a cell membrane

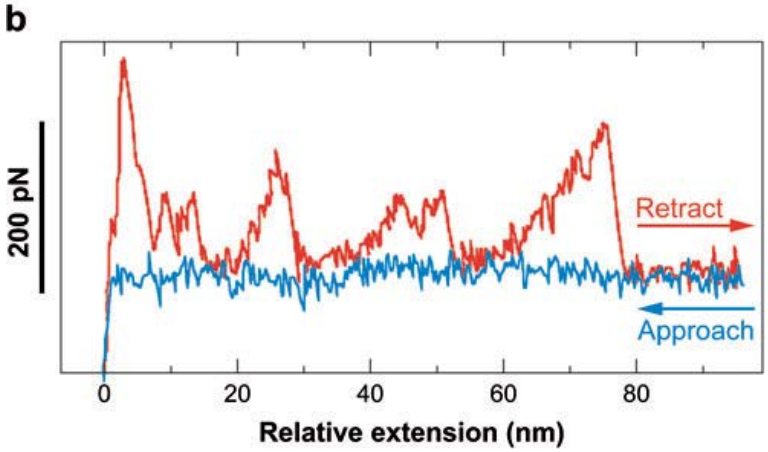
Folding Studies



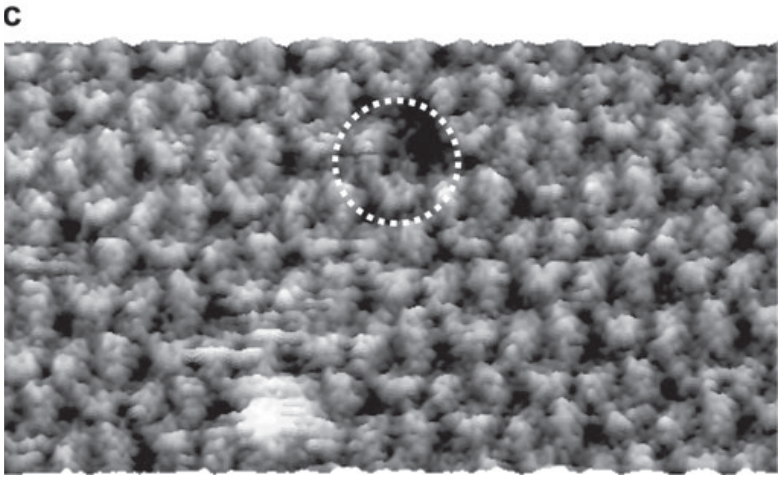
AFM tip can also be used as a single molecule spectroscope

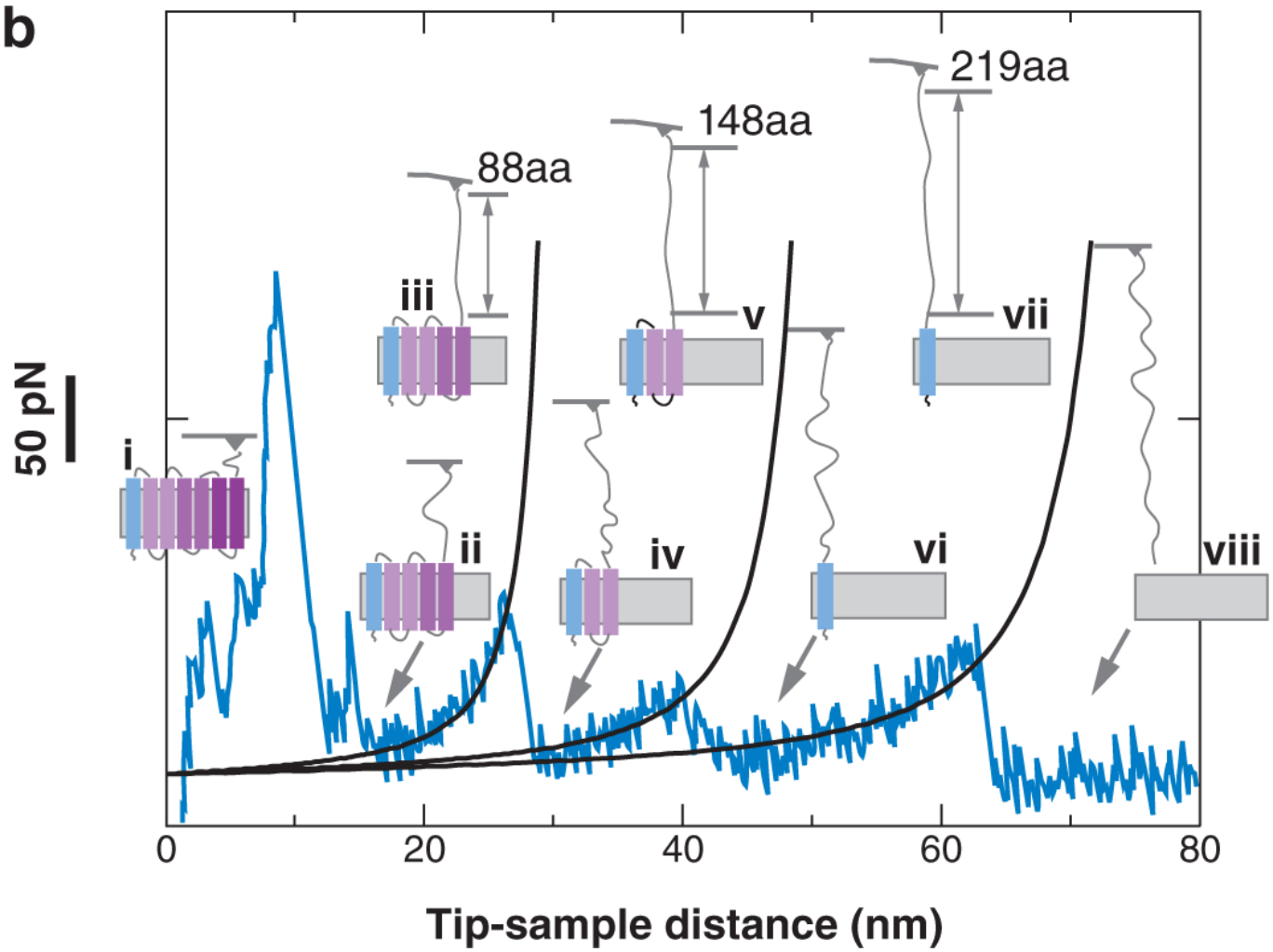
Membrane Protein Unfolding





Controlled unfolding of one bacteriorhodopsin (trimers) from native purple membrane.



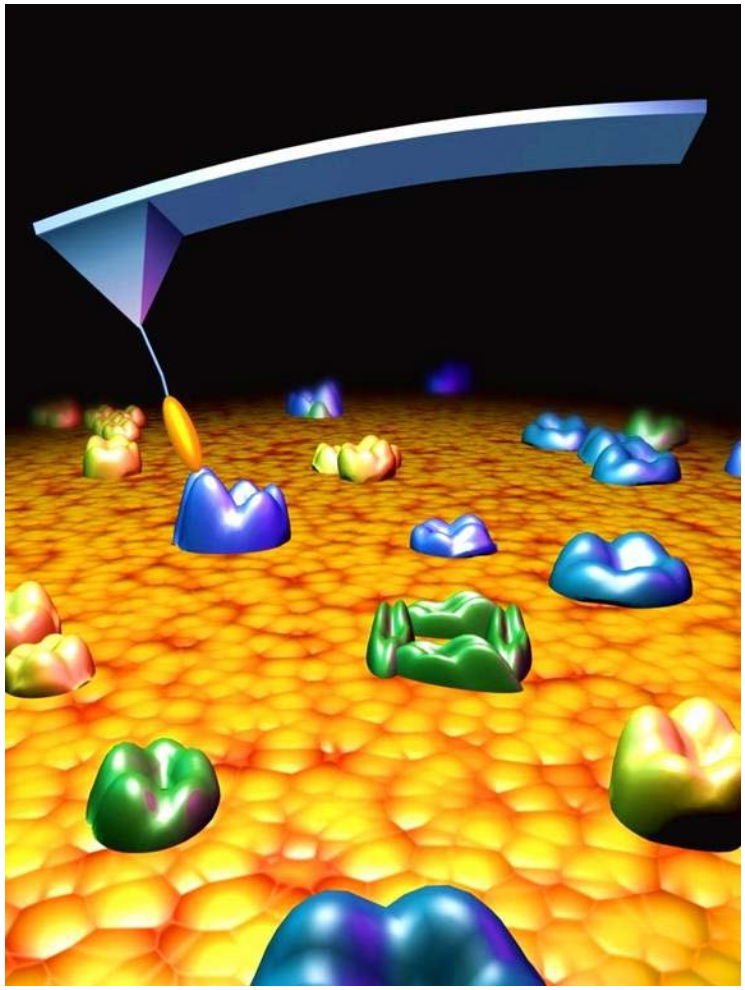


Unlike water soluble proteins that unfold at one step, membrane proteins unfold in a stepwise manner, each step representing an unfolding intermediate.

Advantages

- Biological sample can be investigated in buffer at RT.
- Bypasses the labeling procedures.
- Studies proteins in their native environment
- Offers outstanding SNR
- can be used as single molecule force spectroscope
- is well suited to study surfaces.

Future Directions



AFM is particularly well suited to study membrane proteins.

AFM head is 'fishing' for molecular sites recognized by an antibody tethered to the probe by a fine polymer thread.

AFM can be performed at a high speed to study protein dynamics.