Outline

- Basic Idea
- Simple Theory
- Design Points
- Calibration of Forces
- Selected Biological Applications

Basic Idea

- First conceived and developed in the mid 1980's by Ashkin, Chu and colleagues at AT&T Bell Laboratories
- Laser tweezers is a method of using radiation pressure to trap atoms, molecules, or larger particles
- With the simplest possible arrangement using a single laser, particles with sizes of several hundred microns down to about 25 nm can be 'trapped' and moved about using the radiation pressure of the EM radiation.
- How does radiation pressure trap such particles?

Radiation Pressure - the Scattering Force

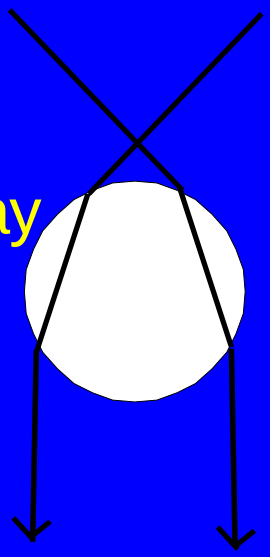
- If a plane EM wave is incident on a particle, the radiation pressure on the particle would propel it along the direction of the beam.
  - since the reflected wave results in a net decrease in forward momentum of the wave and
  - Conservation of momentum for the system composed of the EM wave and the particle then dictates that the particle must sustain a forward momentum
  - A focused 1 W beam striking a particle of radius = 1 wavelength will exert a force of 10 nN, assuming perfect reflection
- This can suspend micron-sized spheres in gravity when the beam intensity is adjusted so as to just balance the sphere's weight. A higher intensity beam would propel the sphere upwards, while a lower intensity beam would allow the sphere to fall but at a reduced acceleration compared to g.

Trapping from Refraction

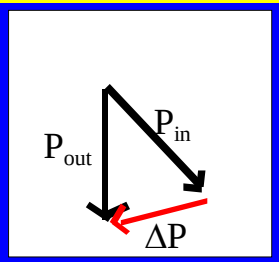
- In addition to the scattering force from reflection there is also another force when the particles refract the incident light
- This additional force tends to trap the particle in the region of highest intensity of light as seen from the following argument

Trapping of a Transparent Sphere

Two equal intensity rays
Note that a ray picture is ok for the Mie regime



Conservation of momentum shown for one of the two beams

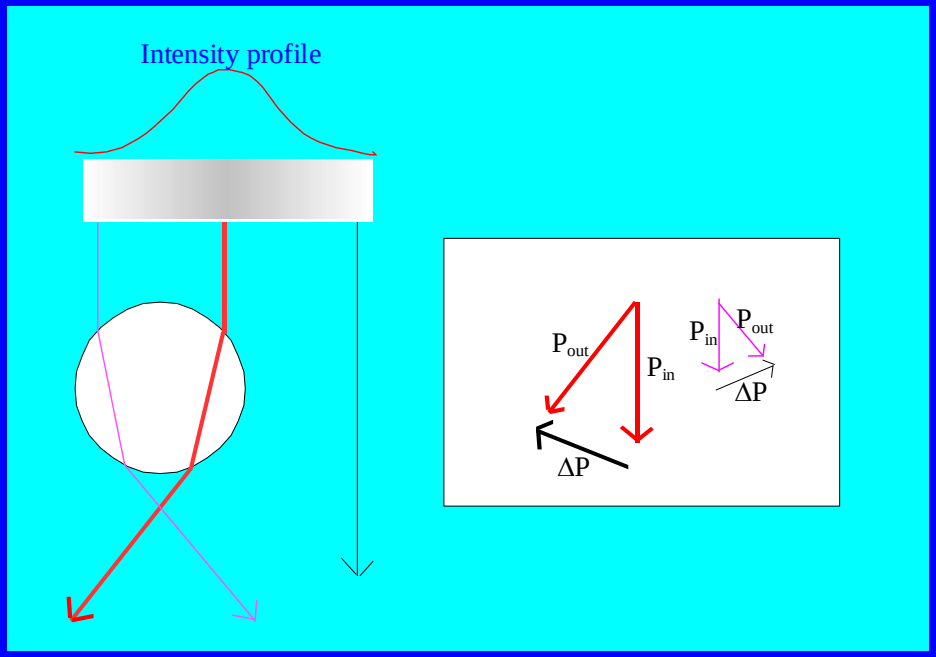


Remember that for a photon
$p = E/c = hf/c = h/\lambda$

- Δp shown is for light beam;
- with the symmetric part, the net Δp for the light is down;
- Δp for particle is opposite

Refraction at the surfaces of a transparent sphere leads to a force directed upwards towards the focal point of the beam - where the intensity is greatest

The Gradient Force



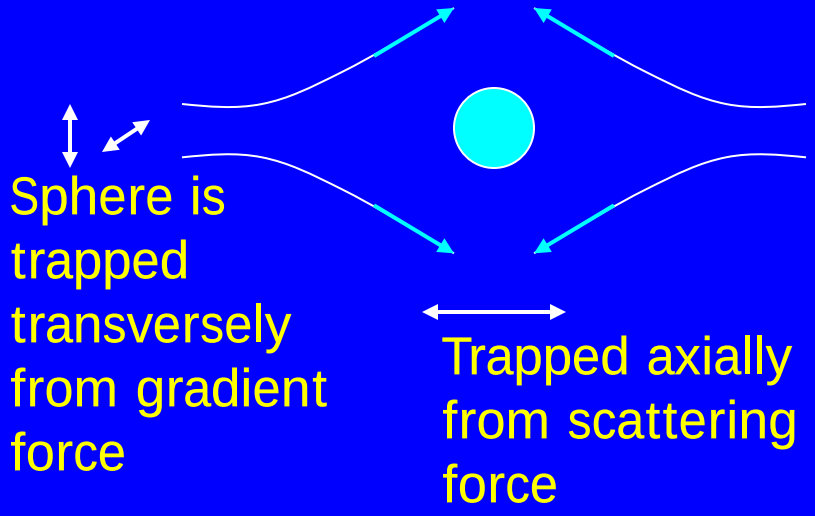
- Dielectric sphere shown off center for a Gaussian profile beam
- Resulting force on particle is larger transverse toward center and net downward toward focus- both acting towards more intense region

Size of Particle

- The ray pictures are fine for Mie scatterers with $d >> \lambda$
  - note that for micron-sized bubbles in glycerol the transverse forces push the bubbles out of the beam, as expected based on reversal of higher and lower indices of refraction
- For Rayleigh scatterers with $d << \lambda$ trapping still occurs but wave optics is needed. Point dipoles and a diffraction-limited focal waist can be used
- For intermediate sized particles $d \approx \lambda$, the region of interest for much biological work, calculations are difficult

First stable single-particle 3-d optical trap

- Two opposing moderately diverging laser beams:



This technique was superseded by using a strongly divergent single laser beam

Basic Ideas of Trapping with Single Laser Beam

- The Gradient Force must be larger than the scattering force to trap a particle
- This can only be achieved with very steep light gradients using high NA lenses
- Typical forces capable of being exerted are in the pN (10^{-12} N) range
- Either the laser beam itself or the sample, sitting on a microscope stage, is moved
- Usually near-infrared laser light with a wavelength of about 1 μm is used with biological samples **- to avoid absorption**
- Experimental station uses a good quality inverted microscope with an optical port for the laser

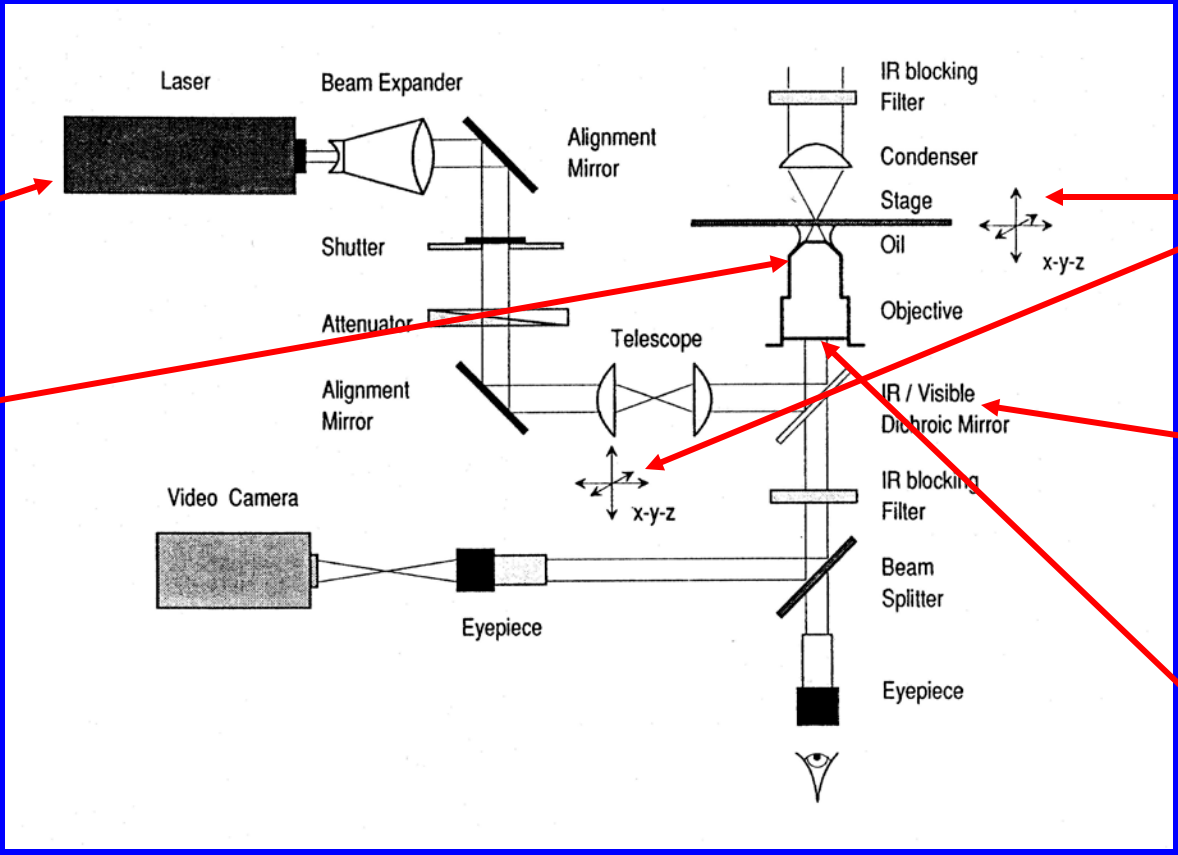
Design Features

- Single-mode laser brought to a tight focus in object plane using high NA objective (note large ~50% transmission loss in near IR)
- Want beam waist diameter to fill back focal plane of objective - usually use a beam expander for this
- Want means of shuttering trap beam and of adjusting beam intensity
- Beam steering usually desired - can be done in one of at least 4 ways

Schematic

CW-TEM$_{oo}$ mode IR

High NA oil-immersion objective



independent motion of sample and trap

reflects IR and transmits vis

Telescope lenses chosen so beam fills objective pupil

Beam Steering

xyz Moveable lens piezo drive at 100Hz

Single-mode optical fiber steered in xyz

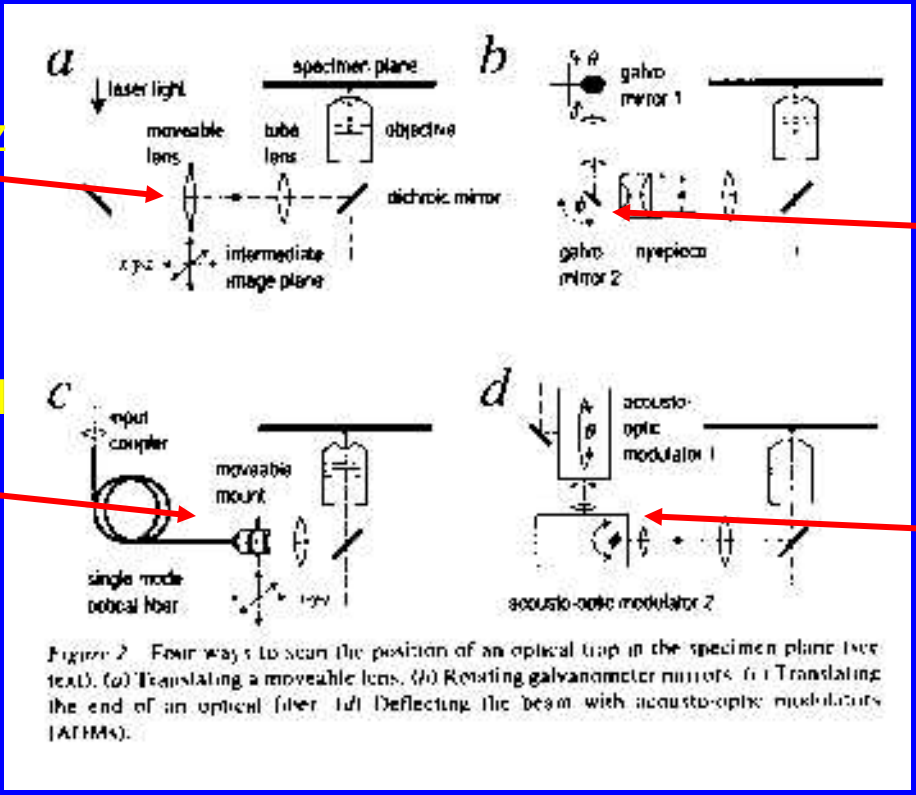


Figure 2 Four ways to scan the position of an optical trap in the specimen plane (see text). (*a*) Translating a moveable lens. (*b*) Rotating galvanometer mirrors. (*c*) Translating the end of an optical fiber. (*d*) Deflecting the beam with acousto-optic modulators (AOMs).

θϕ Galvanometer driven mirror at few kHz - can make multiple traps - adapted from confocal microscopy

Dual AOM's to steer beam - GHz possible

Design Features - Lasers

- Near IR best for most biological samples - trade off between sample and water absorption regions

Increasing sample absorption in uv-vis

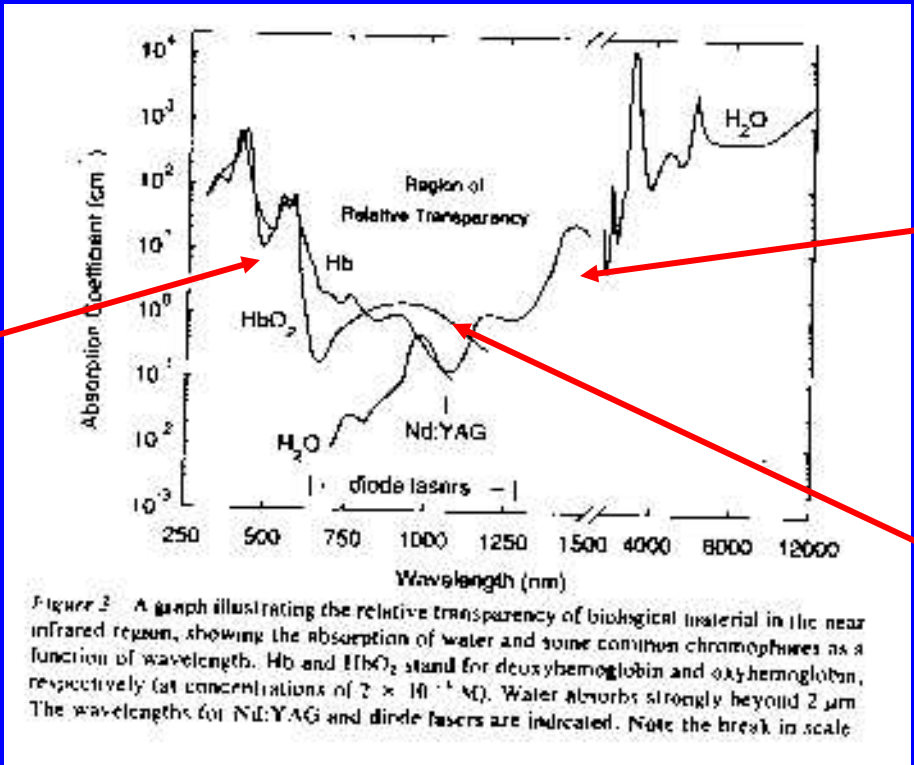


Figure 3 A graph illustrating the relative transparency of biological material in the near infrared region, showing the absorption of water and some common chromophores as a function of wavelength. Hb and HbO_2 stand for deoxyhemoglobin and oxyhemoglobin, respectively (at concentrations of 2×10^{-4} M). Water absorbs strongly beyond 2 μm. The wavelengths for Nd:YAG and diode lasers are indicated. Note the break in scale.

Increasing water absorption in IR

Minimum absorption in near IR

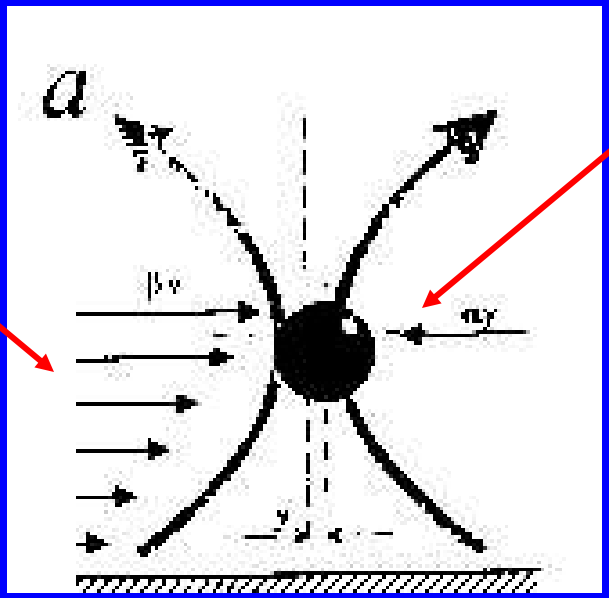
Some Laser Choices

- Nd-YAG at 1.06 mm with 1 W typical power
- Ti:Sapphire tunable in 700 - 1100 nm with 1 W typical power
- Diode laser in 780 - 1330 nm (850 nm typical) with 100 mW of power typical

Calibration of Forces I

- For usual situation in aqueous solvents Reynolds number $Re = va\rho/\eta$ is small so drag force is $F = -\beta v$, where for spheres $\beta = 6\pi\eta a$
- Two basic ways to measure trapping force:

Variable fluid flow from left provides transverse drag force but shear tends to push spheres into cover slide



balanced by trapping force - spring like with stiffness α

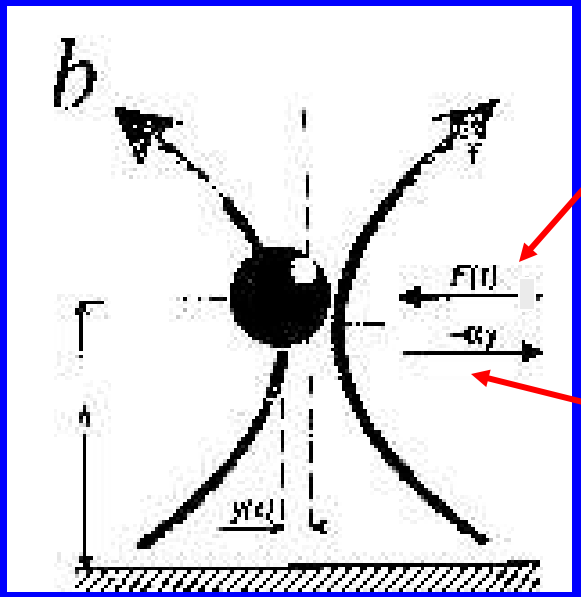
Video recording can determined transverse forces at which sphere leaves trap for v up to ~20μm/s, measured after sphere leaves

Calibration of Forces II

- Second method:
  - Variation has stage stationary and trap moved
  - Trap force is proportional to beam Intensity

velocity of stage at which sphere escapes is measured

Note that near cancellation of trap force, the scattering force leads to increased distance from coverslip



entire fluid chamber is moved at variable speed to left providing drag force - correction to β needed for proximity to coverglass

balanced by trapping force

Measuring Trap Stiffness

- If y is transverse displacement from trap center then $\beta dy/dt + \alpha y = F(t)$, where F(t) is an external force (in simplest case thermal Langevin force)
- This gives Brownian motion in a parabolic potential well with $\langle y^2 \rangle = kT/\alpha$
- Therefore thermal fluctuation analysis can be used to determine α independent of drag force

Handles

- Most biological macromolecules do not refract the laser beam sufficiently to produce trapping.
- Often spheres are attached to provide “handles” to trap
- Non-specific and specific “linkers” to bind spheres are available with spheres in range of 50 nm - 100 μm

Manipulations

- Maximum trapping force is a few 10's of pN. What can this do?
- About 10 pN is needed to move a 1 μm diameter sphere in water at 0.5 mm/s
- **Can** trap bacteria or sperm, move cells, displace organelles within cells, bend/twist biopolymers, ...
- **Can not** pull cytoskeletal assemblies apart nor stop chromosomal motion during mitosis

Selected Applications

- Bacteria Flagella & Rotary Motor
- Kinesin Motor
- Myosin-Actin Motor

  (note estimates of 100 different motors in a cell)
- Polymer Elasticity -- Titan
- DNA
- Cell Fusion
- Future

Bacterial Motility I

- E. coli are driven by several flagella that are turned by a membrane-bound rotary motor (F_1-ATPase) powered by a proton gradient across the membrane

- This same protein is responsible for generating ATP in our bodies from the mitochondrial inner membranes -

  Every day we synthesize about our own weight in ATP -

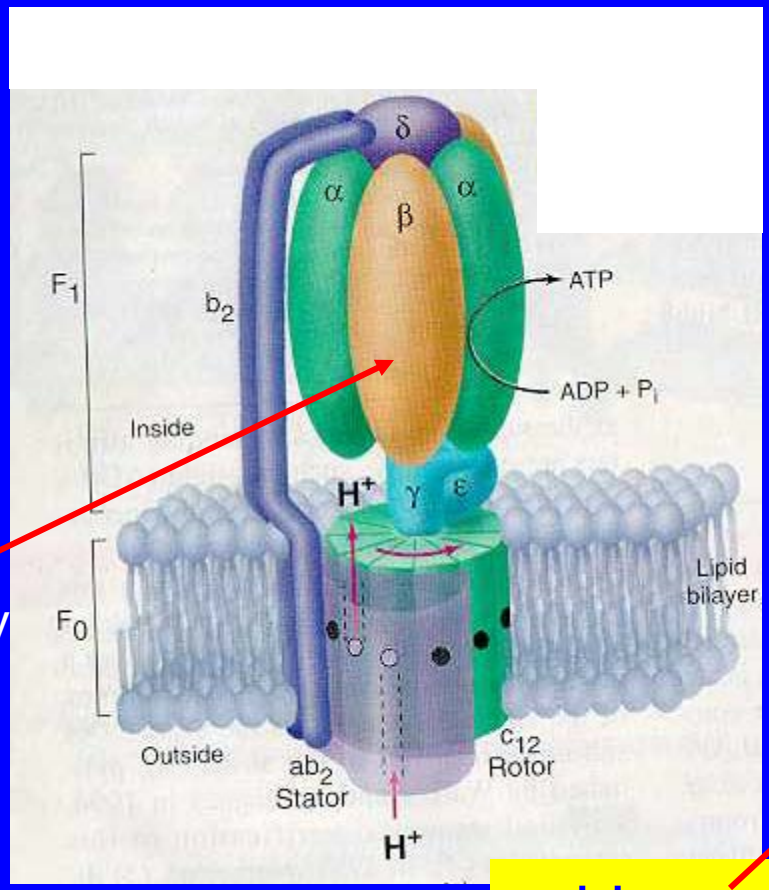
- Bacteria can be trapped optically and measurements made of the torque imparted by rotating flagella

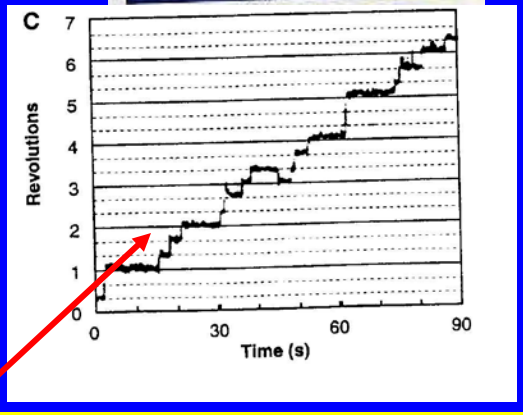
Bacterial Motility II

- More recently, the single F1-ATPase molecule, which has 3-fold rotational symmetry, has been studied by attaching an actin filament of different lengths to the shaft of the motor and either measuring the torque produced as ATP is split and the filament made to rotate, or by rotating the filament backwards and running the motor in reverse to generate ATP
- Discrete rotational steps of 120^{o} were seen in the motor - always rotating counterclockwise for many minutes
- Comparing the work needed to rotate the actin filament with the free energy liberated by an ATP (both about 80 pN-nm) showed that the efficiency of the motor is ~ 100% and it is fully reversible

F1-ATPase Rotary Motor

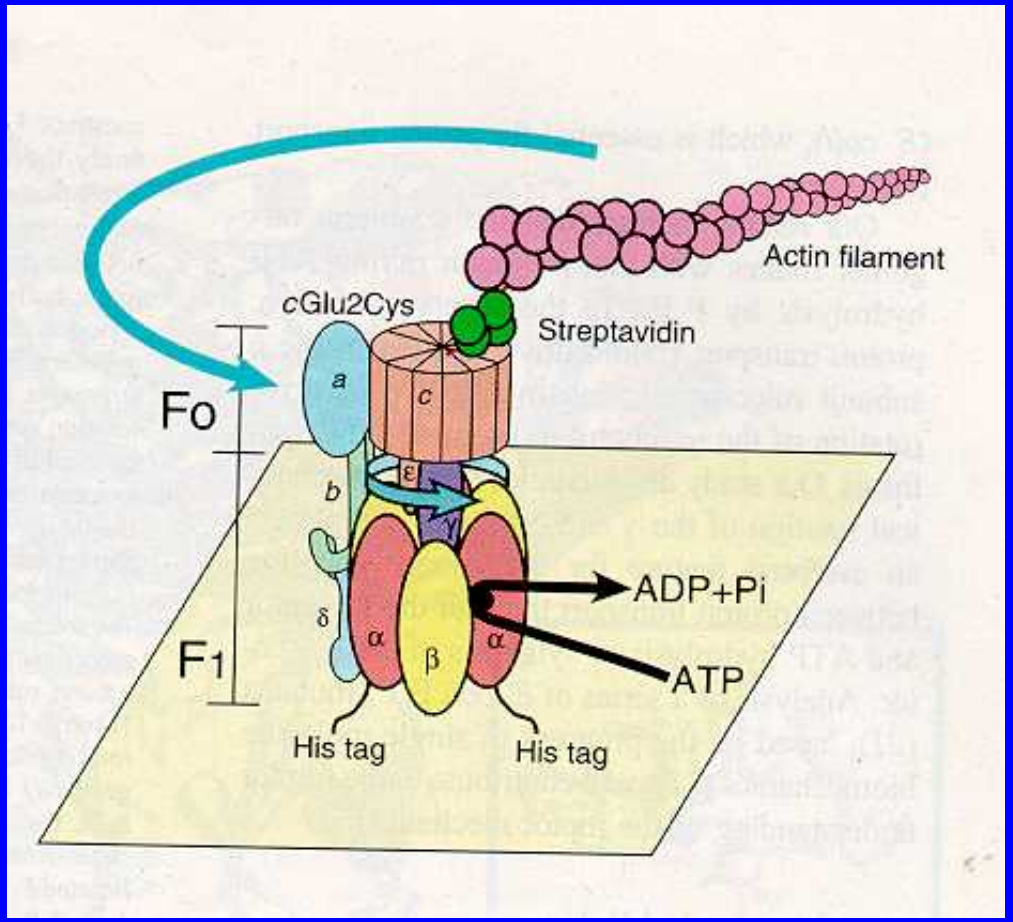
3 pairs of identical subunits - synchronously catalyze ATP to produce rotations





evidence of 120° rotations - each step due to 1 ATP splitting

Controlling F_1ATPase

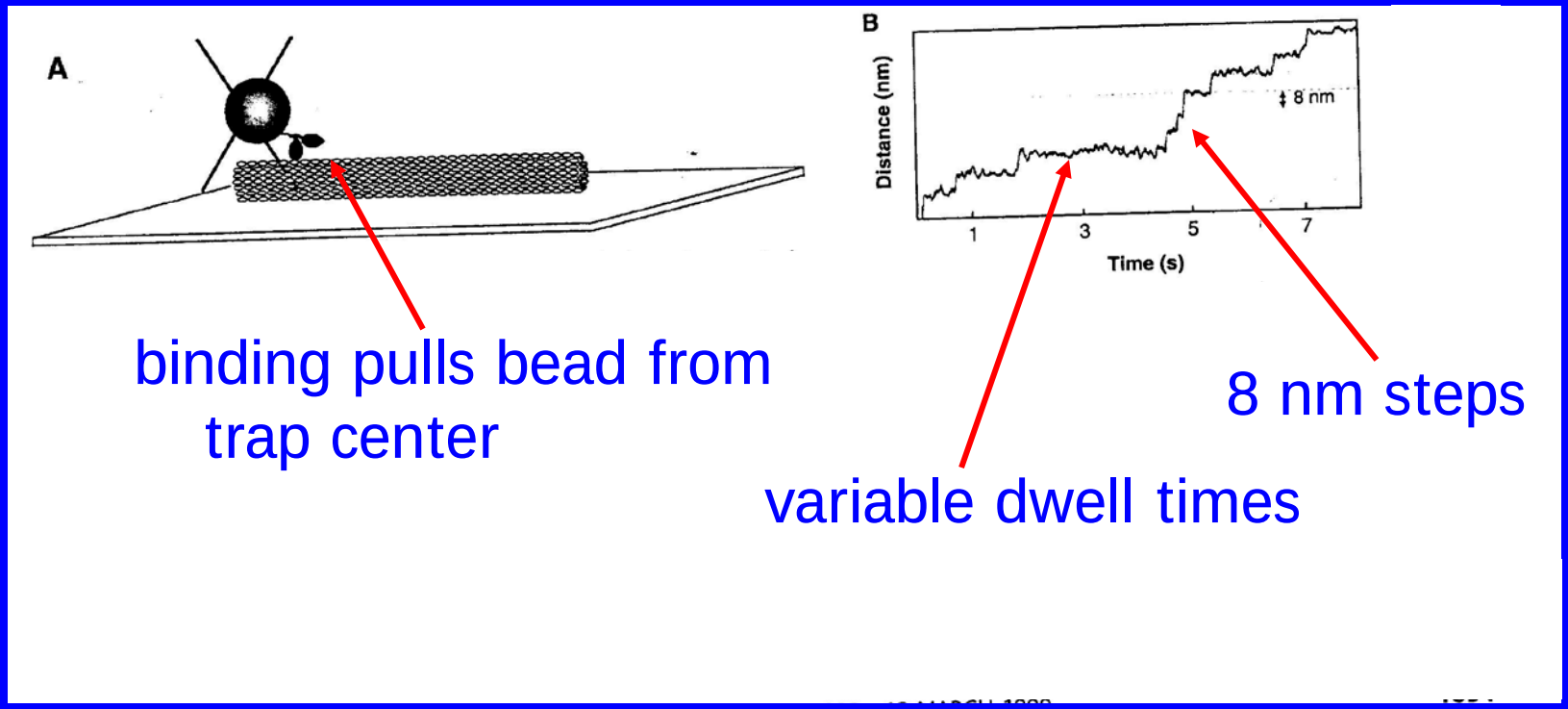


From Science, November 99

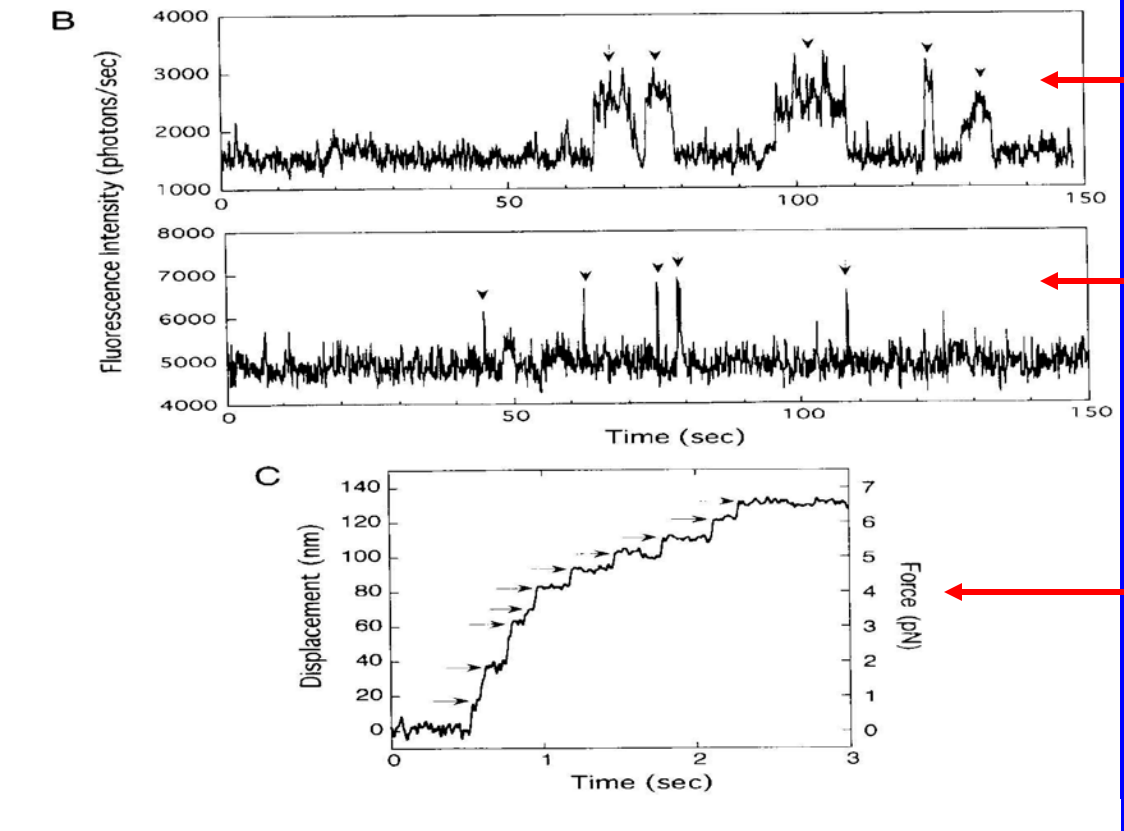
The Linear Motor Protein Kinesin

- Kinesin is a two-headed dimer that transports vesicles along microtubules (hollow tubes made of tubulin dimers)
- When kinesin, by diffusion, finds a microtubule, it remains attached for many catalytic (ATP) cycles and travel for several mm before detaching
- When attached to a silica bead, kinesin can be trapped and brought near a fixed microtubule - measurements show kinesin executes 8 nm steps with variable dwell times between steps and generates about 6 pN of force; each step requires 1 ATP splitting

Single Kinesin Molecule-Microtubule Interactions



Single Kinesin on a Microtubule



ATP* lifetime (~10s) when detached from microtubule

ATP* lifetime (~0.1s) when attached to microtubule

8 nm steps with 6 pN forces per ATP split

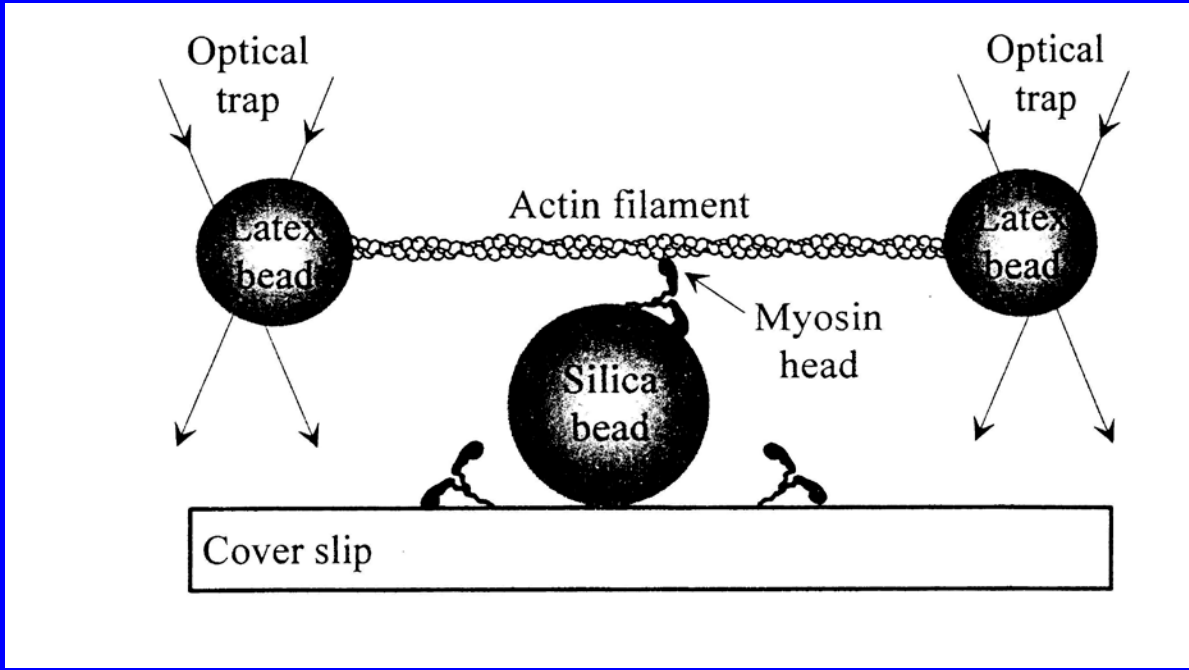
Latest on Kinesin

- Recent work has shown that Kinesin moves along one head- or foot- at a time
- Each step has one ATP binding to the front foot, causing a 15 amino acid “neck linker” region to associate with a nearby region and stiffen; this stiffening pulls the rear foot off the microtubule, causing it to swing ahead to be the new front foot
- This type of motion is very different from the myosin motor, discussed next

Myosin-Actin Forces I

- Myosin II (skeletal) is also two-headed and interacts with a helical polymer (actin) using ATP; myosin moves along the actin filament at a much faster rate than kinesin moves along microtubules, although generating about the same 6 pN force
- However myosin only has 1 power stroke per attachment to actin, making it difficult to use the same geometry as for the kinesin experiments - so the actin filament is held at both ends in traps while the myosin is attached to static silica beads
- Because the power stroke step size is less than 15 nm (the exact value is still in dispute) and the Brownian diffusion of the actin filament, when in a very weak trap (so the load myosin sees is minimal), is about 50 nm it is difficult to measure the step size of the power stroke
- Myosin stays bound to actin after the power stroke until ATP binds, so measurements at low ATP extend their duration making them more distinct compared to thermal noise

Single Myosin-Actin Filament Interactions I



Myosin-Actin Forces II

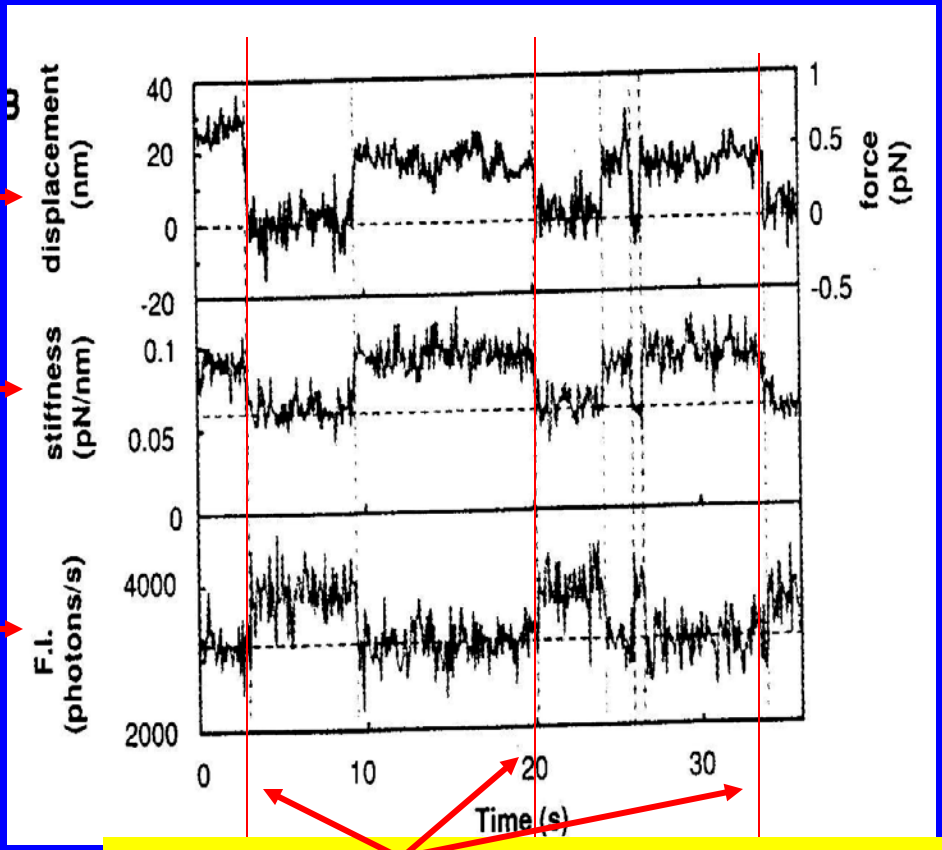
- One way to measure step size is to measure the increase in stiffness constraining actin-attached bead diffusion as a signature of myosin binding
- In experiments where the myosin and actin were optimally aligned, the mean bead displacement was about 10 nm while when orthogonal the mean displacement was 0
- Experiments on non-muscle myosins show differences in power stroke step size as well as a longer dwell time before release from actin
- Simultaneous measurement of force/displacement generated, stiffness and fluorescence signal from ATP show a 1 to 1 coupling between ATP turnover and the mechanical cycle of binding and releasing actin

Single Myosin-Actin Filament Interactions II

bead displacement parallel to actin axis

stiffness of trap even without much displacement

used total internal reflection microscopy to see ATP changes



Note that when force/displacement end, ATP spike occurs

Single Molecule Elasticity - Titan

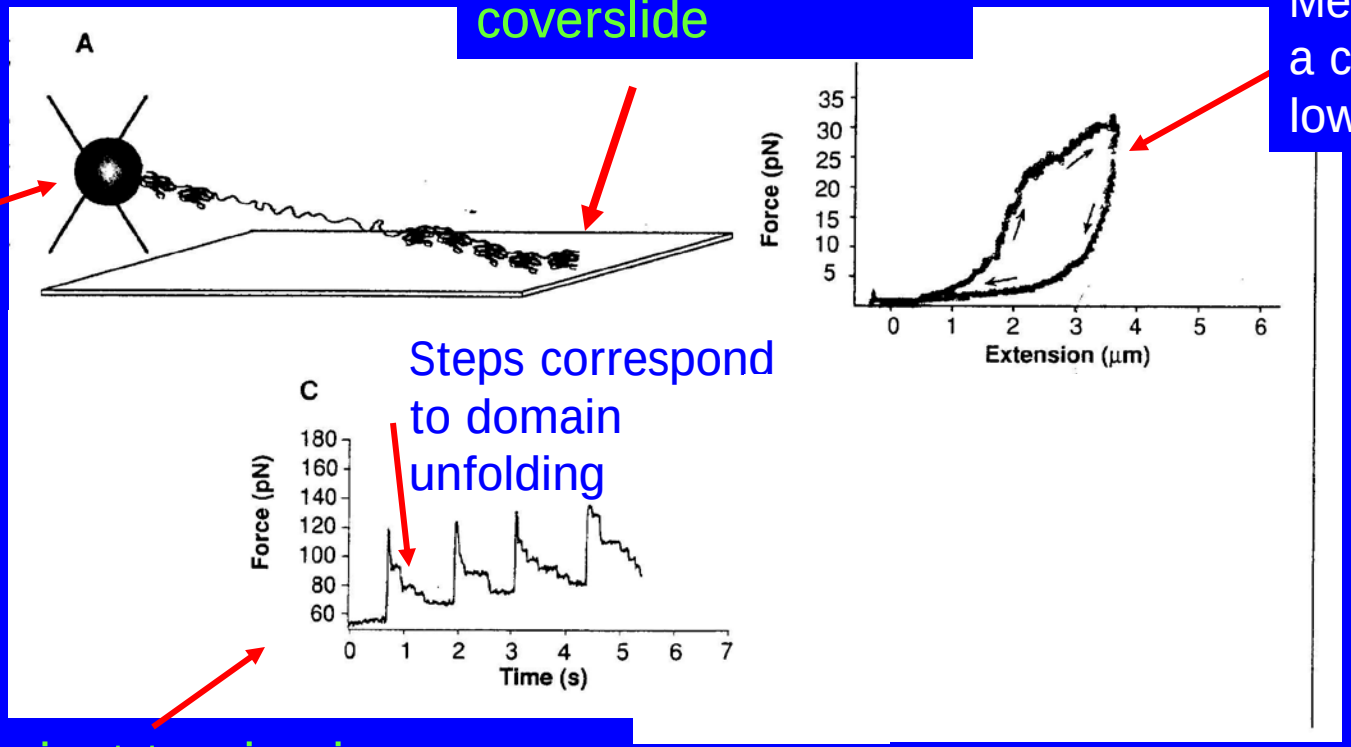
- Movable traps can be used to stretch biopolymers beyond their normal range, even unfolding their tertiary conformation
- Titan is protein responsible for the structural integrity and elasticity of relaxed muscle
- Titan is about 1 mm long when extended but has several folded domains
- As increasing stretching force is applied and the force-extension diagram mapped, different unfolding regimes can be identified corresponding to the unfolding of different domains
- This curve shows hysteresis because the re-folding only occurs at very low applied forces

Single Titin Molecule Elasticity

One end fixed to coverslide

Measured with a compliant or low force trap

Bead attached via antibody



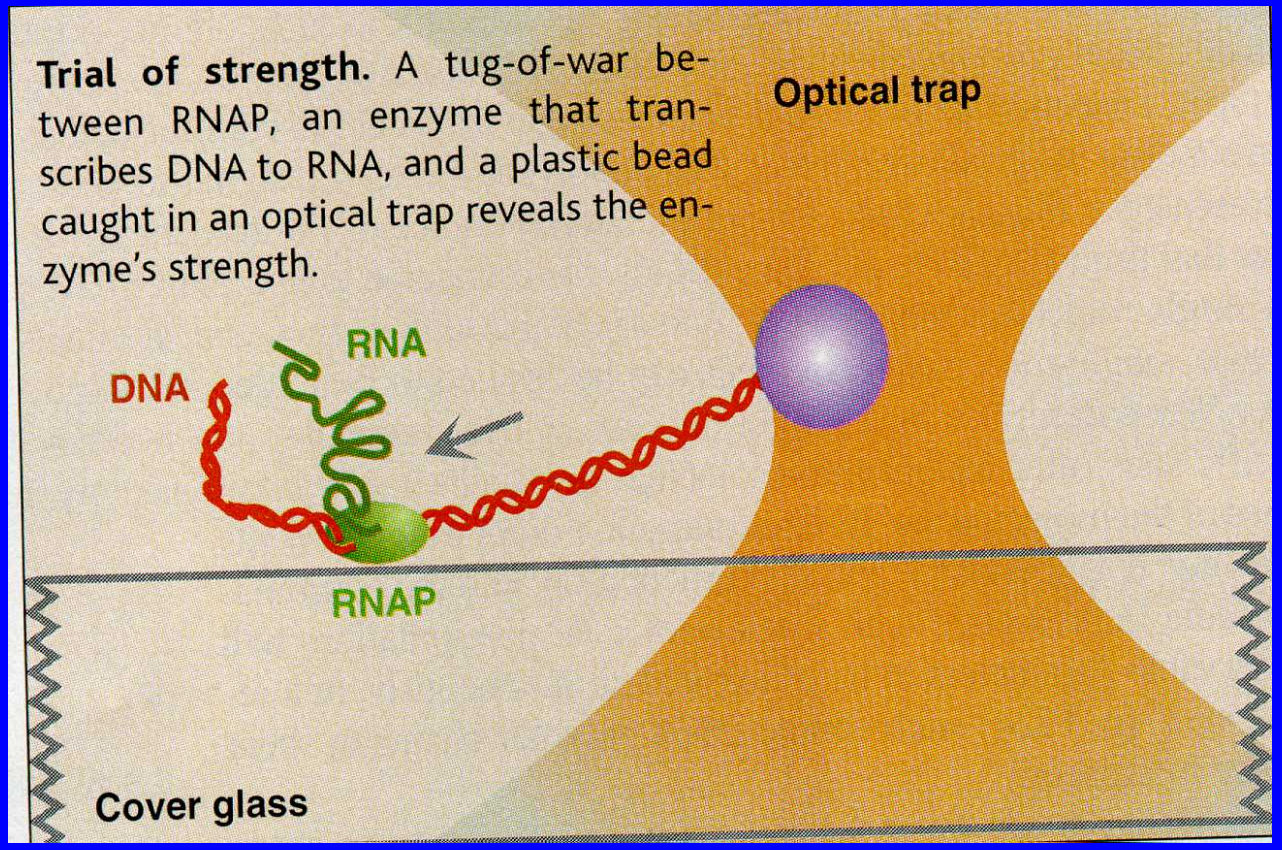
Steps correspond to domain unfolding

Transient tension jumps applied and relaxation probed

DNA

- Very long, robust molecule - good to study individual particle properties via “nanometry” = various optical single-particle methods
- In a recent series of reports, the action of RNA polymerase (RNAP), which transcribes DNA into RNA, on DNA has been studied
- A single molecule of RNAP was fixed to a glass slide; one end of a DNA strand had a bead attached which is optically trapped. The DNA was brought near the RNAP and the force it exerted on the DNA was measured to be 25 pN - about 4 times that of myosin!! - the most powerful single molecule force yet studied- probably needed to unzip DNA so it can be copied

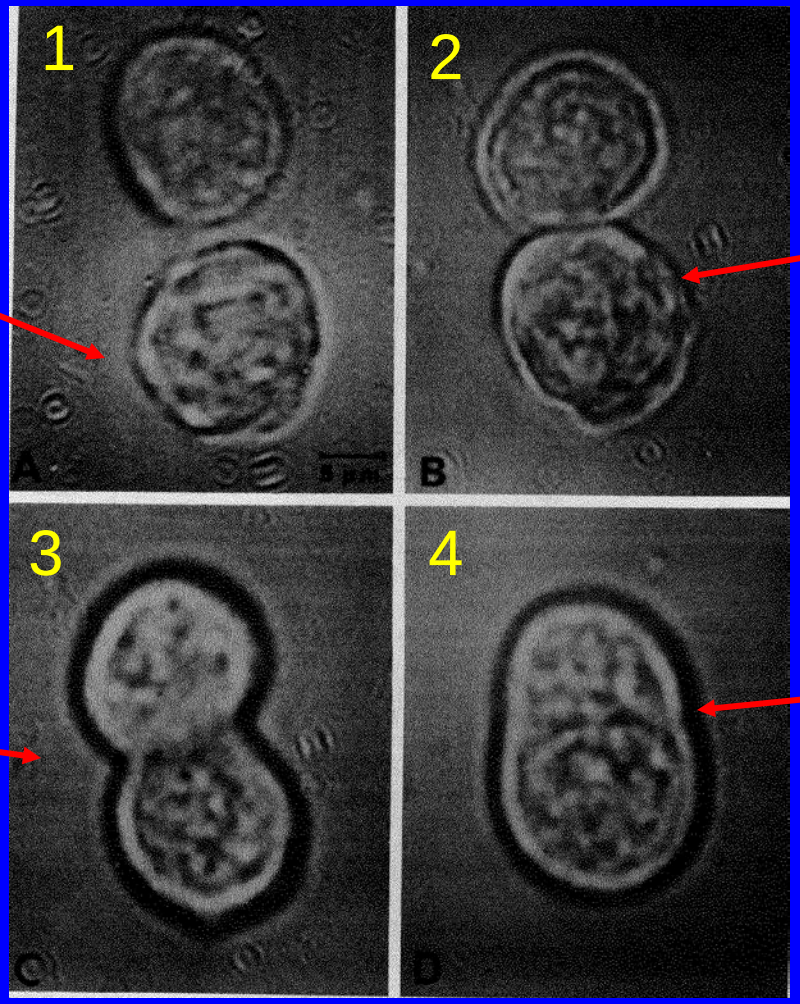
DNA at Work



Science, March 12, 1999

Promoting Cell Fusion

Lower cell is trapped and brought near upper cell



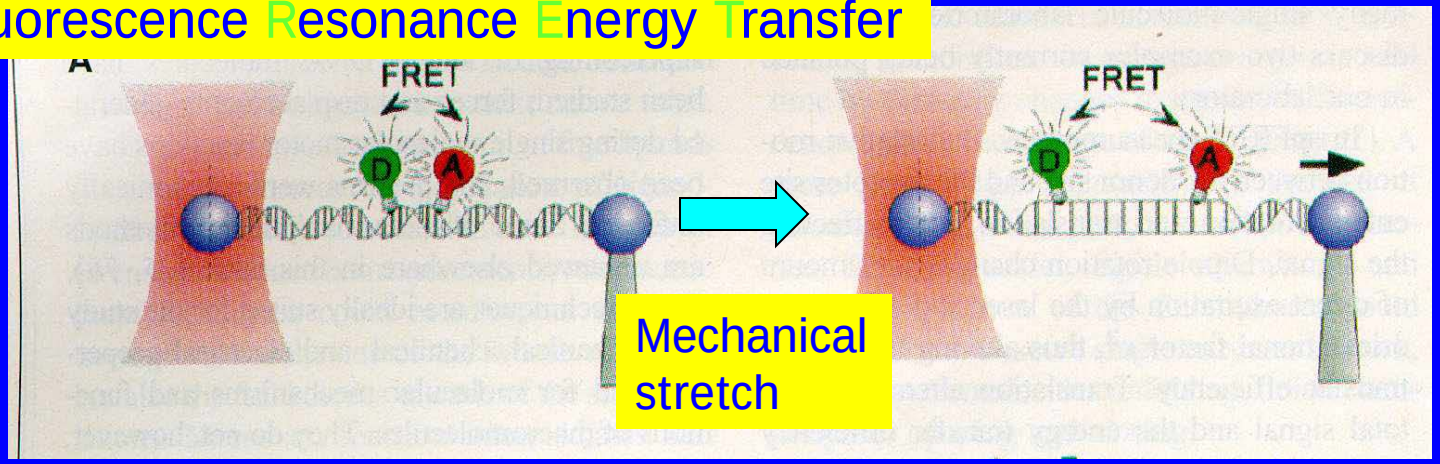
Cells are now in contact

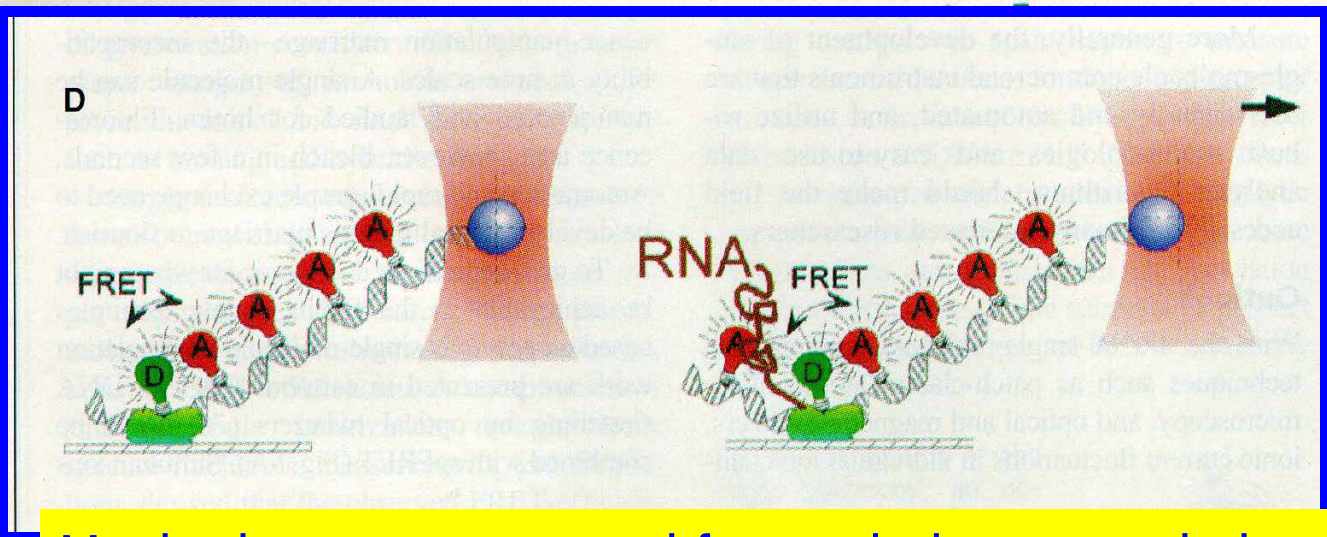
10 uv pulses of laser light at the membrane junction = "laser scissors"

Lead to fusion of the cells (rounding up after 5 minutes)

Future Combined Experiments

Fluorescence Resonance Energy Transfer





Monitoring movement and forces during transcription

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