Micro- and nano- particle manipulation by

Optical Tweezers, Acoustic Tweezers, Magnetic Tweezers

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Outline

1. Why Optical, Acoustic, Magnetic Tweezers ?
2. Optical Tweezers (OT) working principle
3. OT particle manipulation in biological applications
4. Acoustic and Magnetic Tweezers working principle
5. AT and MT in biological applications
6. Single molecule biology - force spectroscopy

Optical Tweezers: a Legacy of Arthur Ashkin

Arthur Ashkin
The Nobel Prize in Physics 2018

Born: 2 September 1922, New York, NY, USA 1928 - 2020

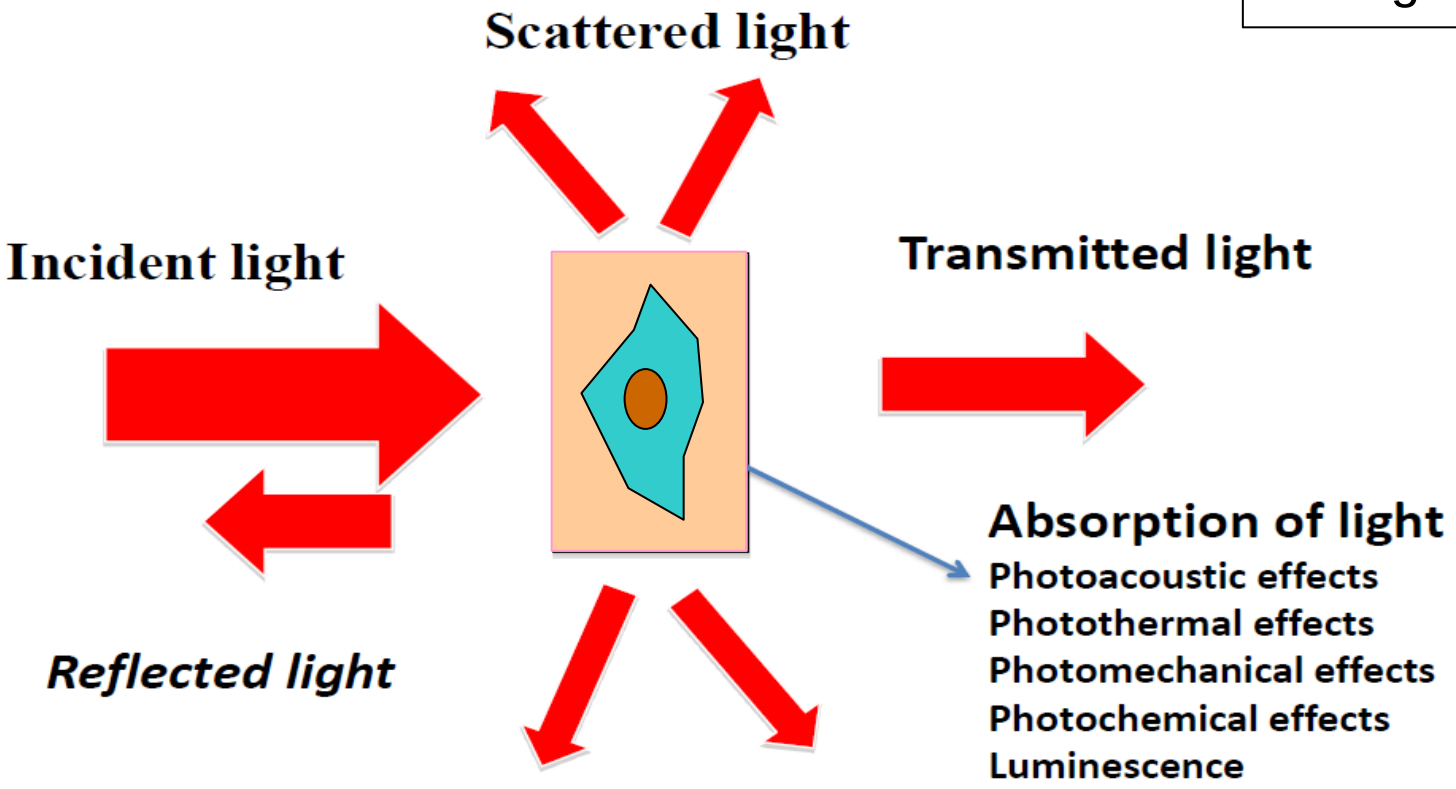
Affiliation at the time of the award: Bell Laboratories, Holmdel, NJ, USA

Prize motivation: "for the optical tweezers and their application to biological systems."

Prize share: 1/2

Light – Sample interaction

Sample:
Living Cell



is governed by physics laws as

Energy and Momentum conservation

and includes **mechanical effects !**

Could Light exert Force on Objects ?

If Yes, How ?

Light is made by photons and a PHOTHON has MOMENTUM

(even if it does not have MASS):

$$p = \frac{h\nu}{c} = \frac{E_p}{c}$$

Momentum ***p*** and energy E_p of a photon

c - light velocity; v – frequency;
λ = c / v – wavelength.

How big is the photon momentum compared with the momentum of an object with mass m, moving at velocity v << c ?

Momentum and Energy of a single photon:

$p \approx 10^{-27}$ N s $E \approx 2$ eV$= 3.2 \times 10^{-19}$ J

Momentum of a single E-coli bacteria swimming in liquid:

Mass: m= 1 pg = 10^{-15} Kg; Velocity: V= 100 nm/s = 10^{-7} m/s

Momentum: $P_{Ecb} = m\,V = 10^{-22}$ N s (N s = kg m / s)

The momentum of a photon is very small !

Nevertheless, even a low power laser beam, has many photons.

Example: laser beam of power $W_{lb} = 1$ mW (energy $E_{lb} = 1$mJ)

The number of photons is: $N = E_{lb}/E \approx 3\ 10^{15}$ →

→ **Momentum** of the laser beam: $P_{lb} = 3\ 10^{-12}$ N s

$$P_{lb} >> P_{Ecb}$$

Can the laser beam influence the motion of the bacteria ?

We need to consider / remember some laws of mechanics

Newton's three laws of motion:

L1. Every object in a state of uniform motion will remain in that state of motion unless an external force acts on it.

L2. Force equals mass times acceleration: **F= m a**

L3. For every action there is an equal and opposite reaction.

+ the laws of conservation of momentum and energy.

If we consider the second law: F= m a ,

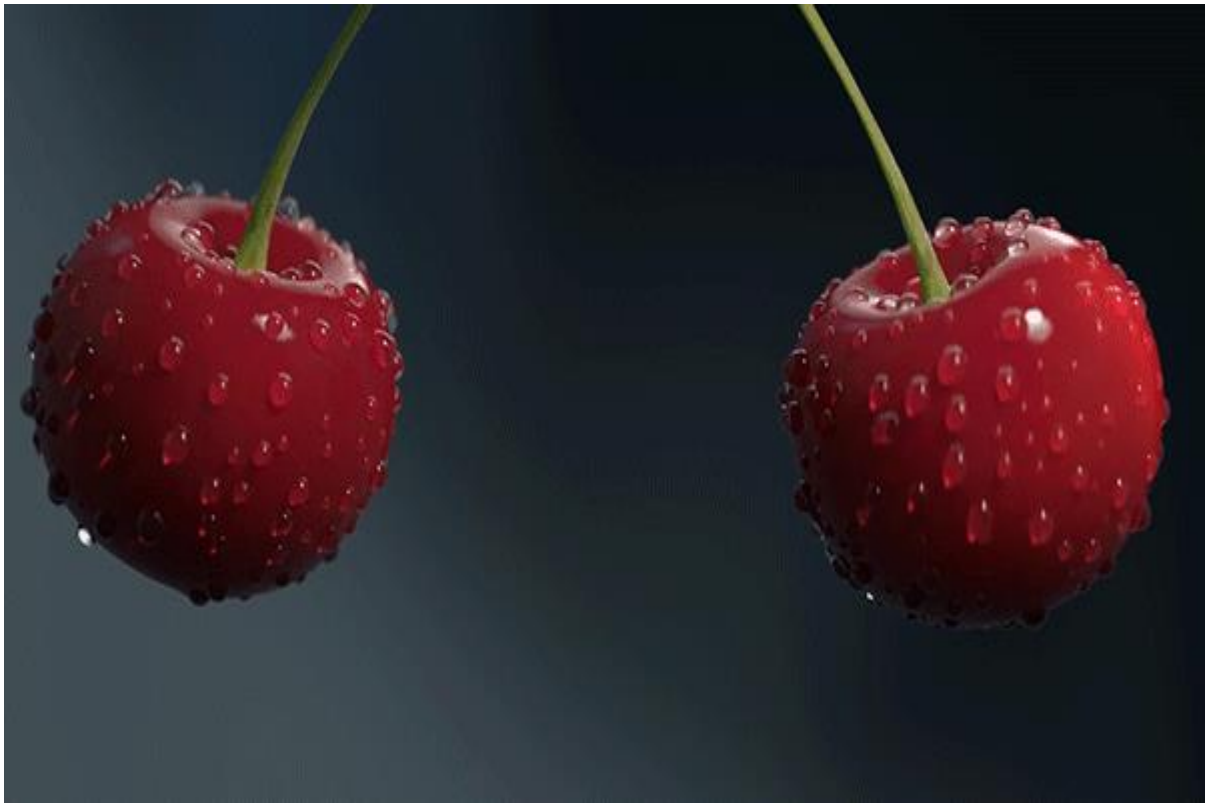
and express acceleration a, as a = ΔV / Δt , we get:

F = m ΔV / Δt = Δ (mV)/ Δt = **Δ P/ Δt,**

which means that:

the change of momentum Δ P in a given time Δt produces force F.

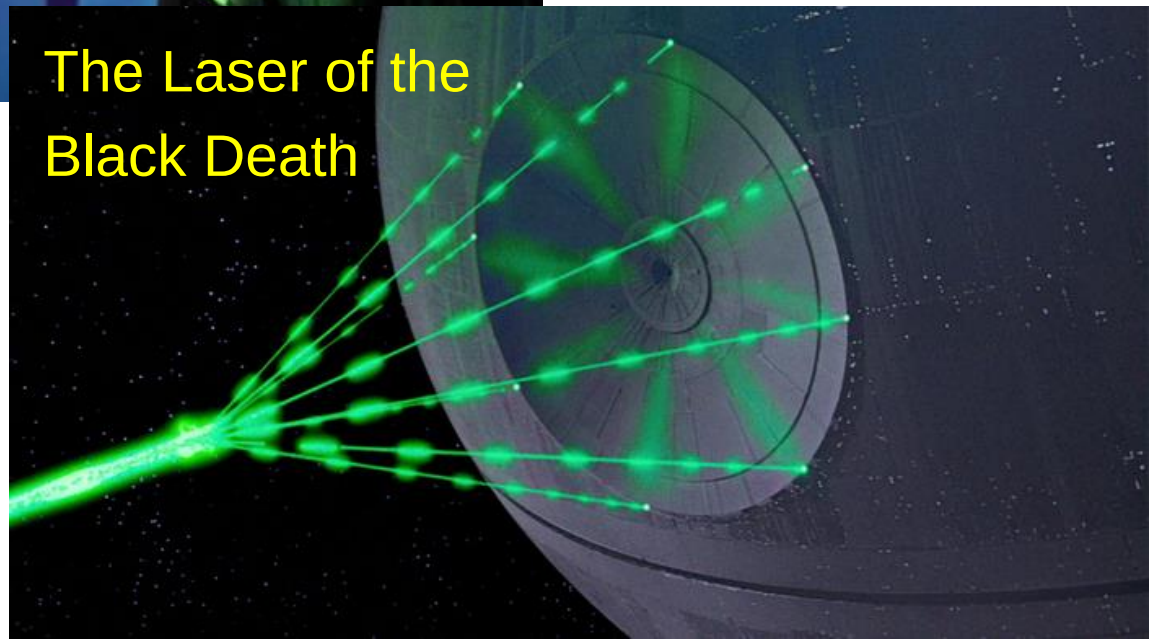
Example of interaction between two objects in motion



Another example (with ellastic and inellastic interaction).

Ellastic: Hammer – Tyre; Inellastic: Hammer-Tom.

Examples of interaction of “object(s)” without mass



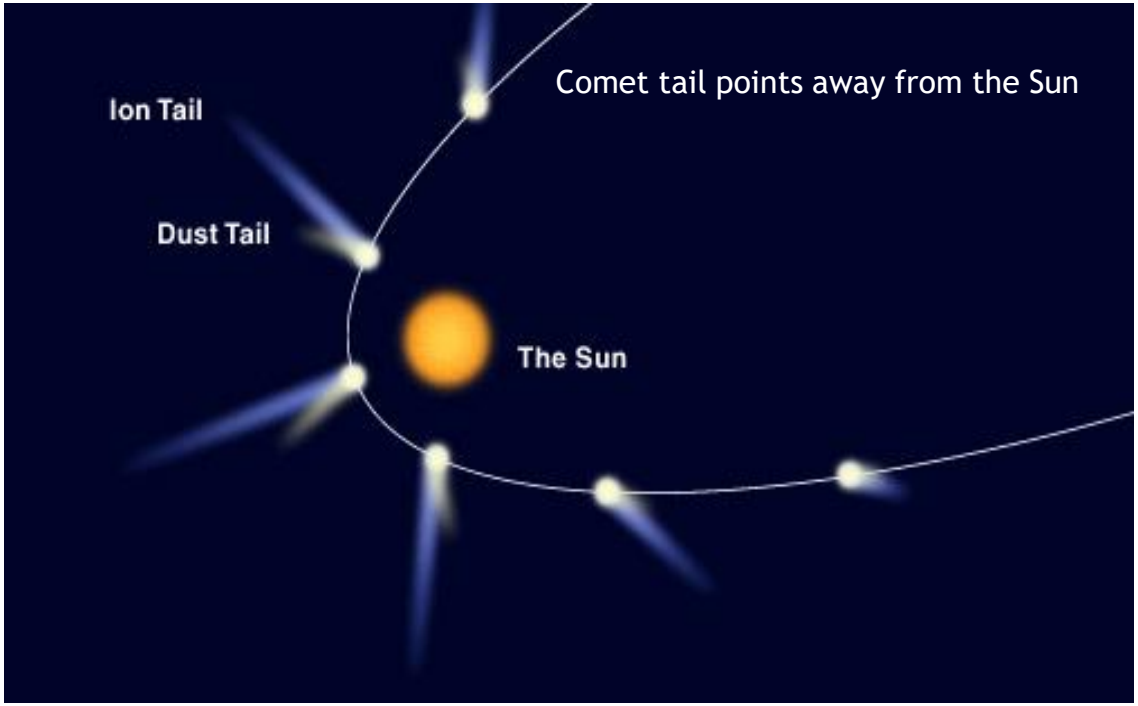
Science Fiction

Star Wars

Light has momentum and can generate force

1619 – Kepler :

Observation of the orientation of the comet tails → suggests that the Sun Light drives the orientation of the comets tail



1873 – Maxwell :

"In a medium in which waves are propagated, there is a pressure in the direction normal to the waves and numerically equal to the energy in unit volume"

1900-1901 Lebedev, Nichols, Hull:

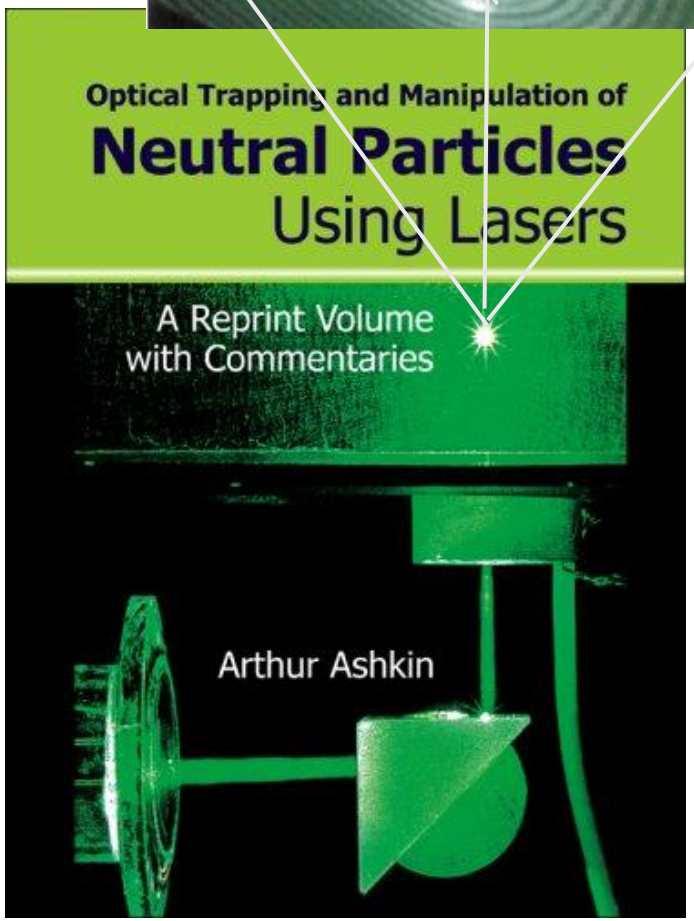
First measurement of the radiation pressure using a torsion balance

Forces generated by light on objects are in general very small and hence the effect is difficult to be detected

→ **use LASER beam and small objects !**

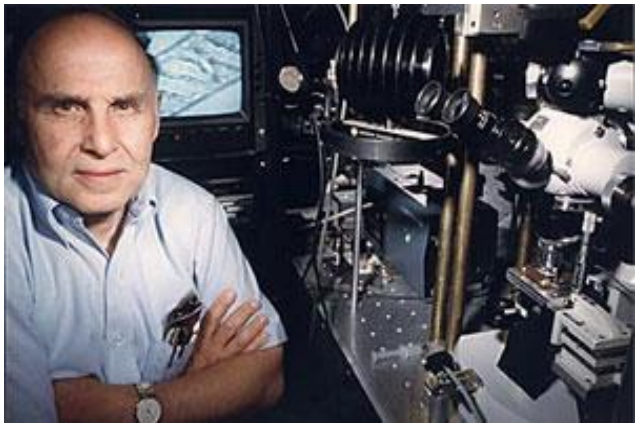
Newton's second law: $F = m\,a$ or $\mathbf{a = F / m}$

Even if the force F is smal, for small objects of small mass m, the effect (measured by acceleration a) can be considerable (detectable and measurable) !

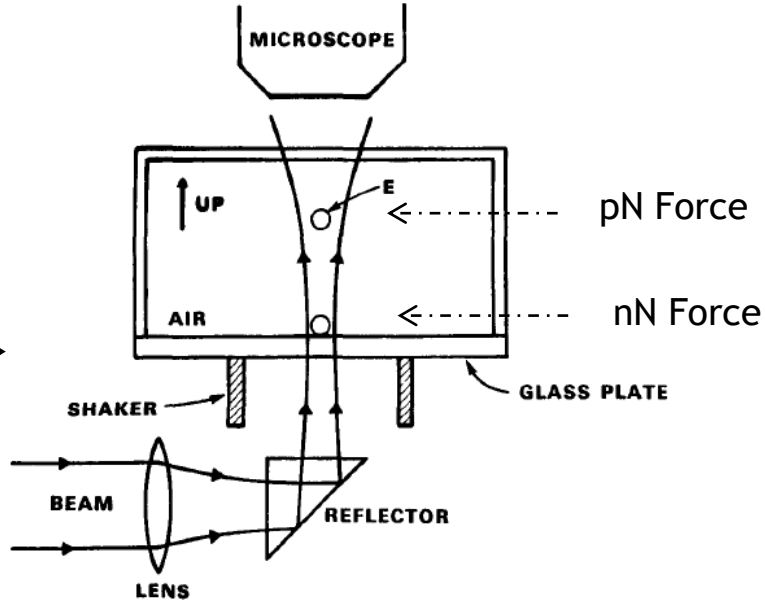


Scientific Publishing 2006

Arthur Ashkin, Bell Labs **(1986)**



Optical levitation of microparticles in air



(hollow silica,beads, diam 50-75 um)

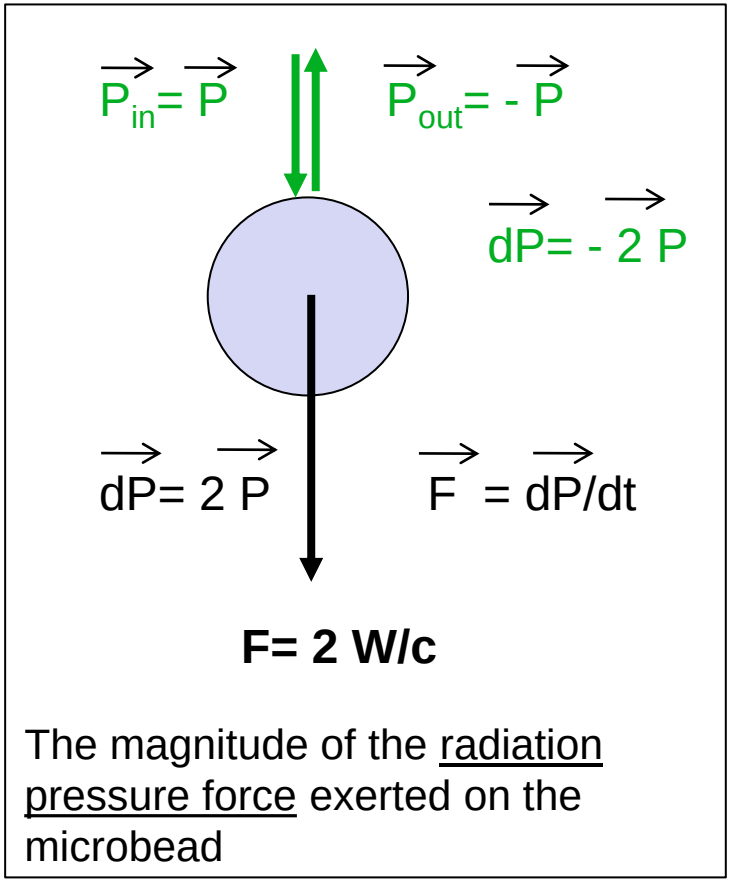
How big is the force exerted by a ray of light reflected perfectly by a microbead ?

Geometrical optics approximation --> light rays

- reflection coefficient R= 1
- (bead diam) d > λ (light wavelength)
- d = 2 μm, λ= 0.5 μm

The magnitude of the momentum associated to the ray of light composed by N photons:

$P = E / c = N h \nu / c$



The magnitude of the radiation pressure force exerted on the microbead

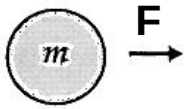
N= 1 photon, -> E≈ 2.5 eV, W≈ 4 x 10^{-19} W -> F≈ 2.7 x 10^{-27} N - very small

N= 10^{15} photons, W ≈ 0.4 mW, F≈ 2.7 x 10^{-12} N = 2.7 pN - **SMALL**

1 pN is the gravitational force of a particle with a mass of 0.1 ng (10^{-10} grams) !

Is the magnitude of this force significant ?

1)



Microbead in free space (vacuum) - no dumping:

F ≈ 2.7 pN - **SMALL** , but also the mass, m, of the microbead is small

m ≈ 8 pg → acceleration **a**≈ F/m = 3.4 x 10^2 [m/s^2] = **34 g** ,

which is very **BIG** !

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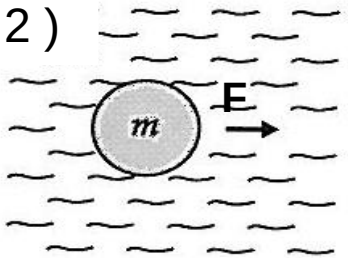
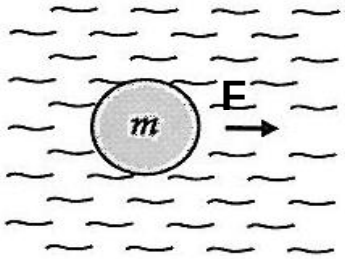
2)



Microbead in liquid - dumping:

F≈ 3.6 pN

refractive index (water) n_m = 1.33; force by light : F= 2 $\mathbf{n_m}$ W/c ;

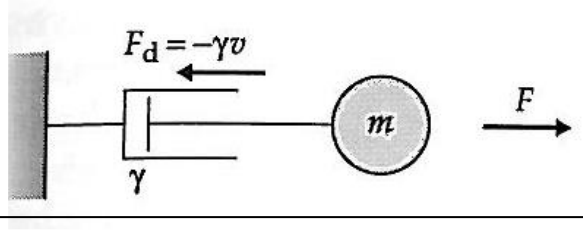


Microbead in liquid - dumping:

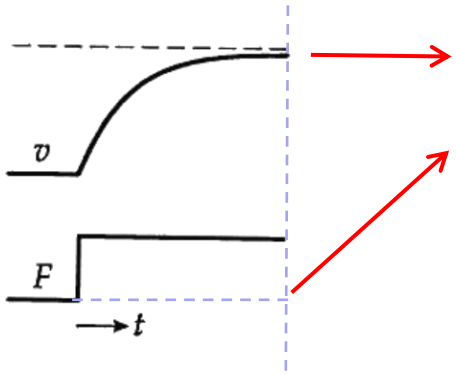
F≈ 3.6 pN

refractive index (water) n_m = 1.33; force by light : F= 2 $\mathbf{n_m}$ W/c ;

mass + dashpot model



$$m\frac{dv}{dt} = F - \gamma v \qquad v(t) = \frac{F}{\gamma}\left[1 - \exp\left(-\frac{t}{\tau}\right)\right]$$



max velocity $v_t = \frac{F}{\gamma}$ **= 360 µm / s**

time constant $\tau = \frac{m}{\gamma}$ **= 0.8 µs**

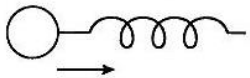
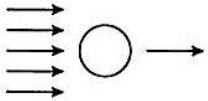
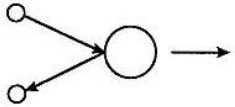
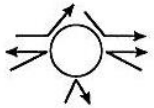
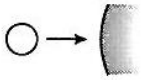
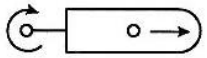
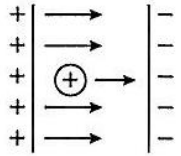
- the max velocity is reached very fast and maintained until the force F is applied.
- When the force is cancelled the particle stops very fast.

For a small particle dumping is dominant over inertia because: *m* -> d^3 , *γ*-> *d*

Example from biology: movement of a bacterium in water. The bacterial motor must be able to generate a force > 0.5 pN to swim through water and stops immediately when motor stops.

Physical forces and their magnitudes at the single molecule level

Table 2.1 Examples of forces acting on molecules

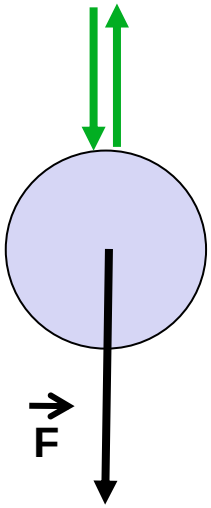
Type of force	Diagram	Approximate magnitude
Elastic		1–100 pN
Covalent		10,000 pN
Viscous		1–1000 pN
Collisional		10^{-12} to 10^{-9} pN for 1 collision/s
Thermal		100–1000 pN
Gravity		10^{-9} pN
Centrifugal		$< 10^{-3}$ pN
Electrostatic and van der Waals		1–1000 pN
Magnetic		$<< 10^{-6}$ pN

pN

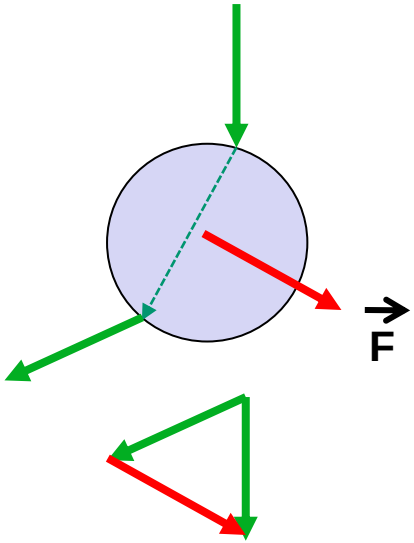
J. Howard, Mechanics of motor protein and the cytoskeleton, Sinauer Associates Inc. , 2001

Force induced by a ray of light by refraction on a bead in water

reflection only
R=1



refraction only,
R=0; $n_b > n_m$



The magnitude of the force:

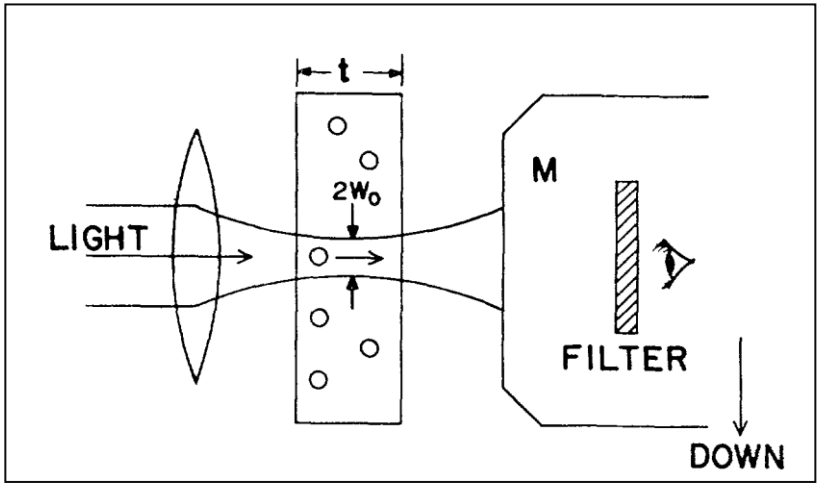
$$F = Q n_m W_{in}/c$$

the incident momentum /s of a ray of power W_{in}

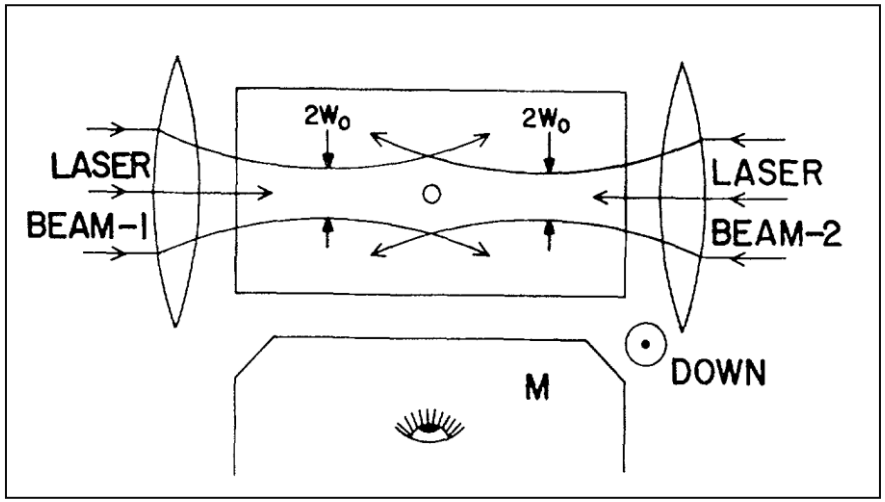
Q - dimensionless factor, $Q \leq 2$
Q - function of shape, material

- If the beam of light is not focused or midly focused, the force always pushes the object forward.
- However, if the beam is tightly focused, there is a force component attracting the object toward the focus → 3D trapping

2D and 3D optical trapping



ONE focused laser beam
2D trapping



TWO focused laser beams,
(counter propagating)
3D trapping

NOTE: focusing through relatively low NA lenses

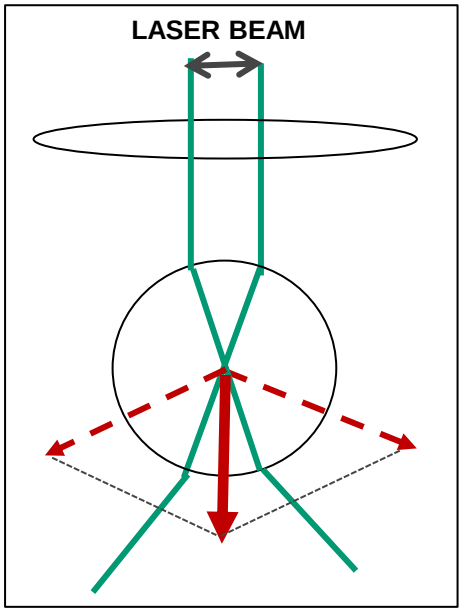
Acceleration and trapping of particles by radiation pressure

A. Ashkin, *Phys. Rev. Lett.* 24, 156 (1970) >5000 citations

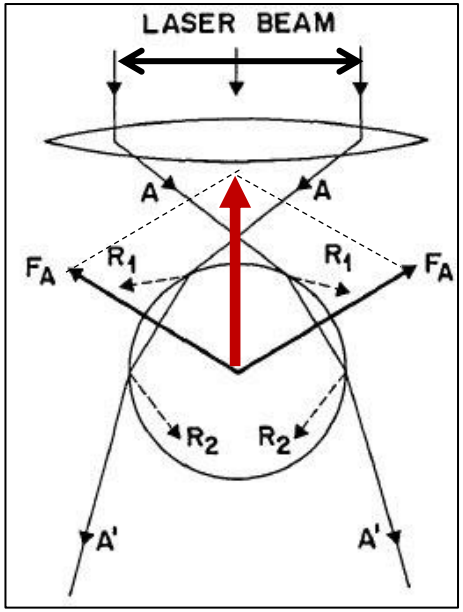
Observation of a single-beam gradient force optical trap for dielectric particles

A. Ashkin, J. M. Dziedzic, J. E. Bjorkholm, and S. Chu, *Opt.Lett.* 11, 288 (1986)

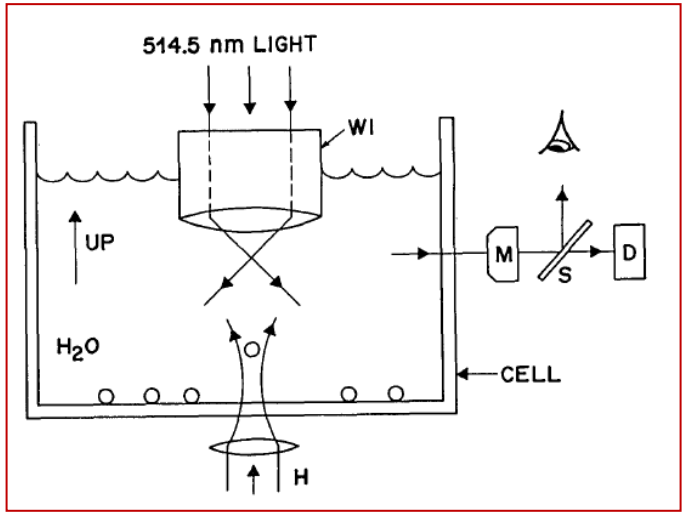
> 6000 citations



Force generated by a **midly** focused laser beam on a transparent microparticle in water.



Force generated by a **tightly** focused laser beam.



Sketch of the basic apparatus.

Size of particles :

10 um (Mie) to 25 nm (Rayleigh)

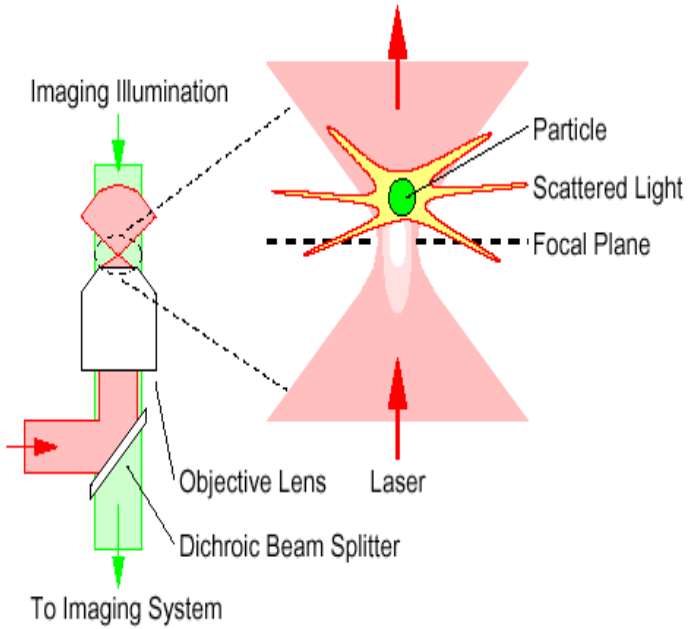
Acceleration and trapping of particles by radiation pressure

A. Ashkin, *Phys. Rev. Lett.* 24, 156 (1970) >5000 citations

What is an Optical / Laser Tweezers ?

A laser beam **tightly** focused through a high Numerical Aperture (NA) objective

A. Ashkin *et al* ***Opt. Lett.*** 1986

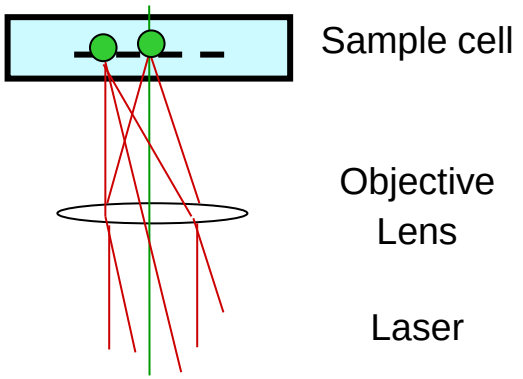


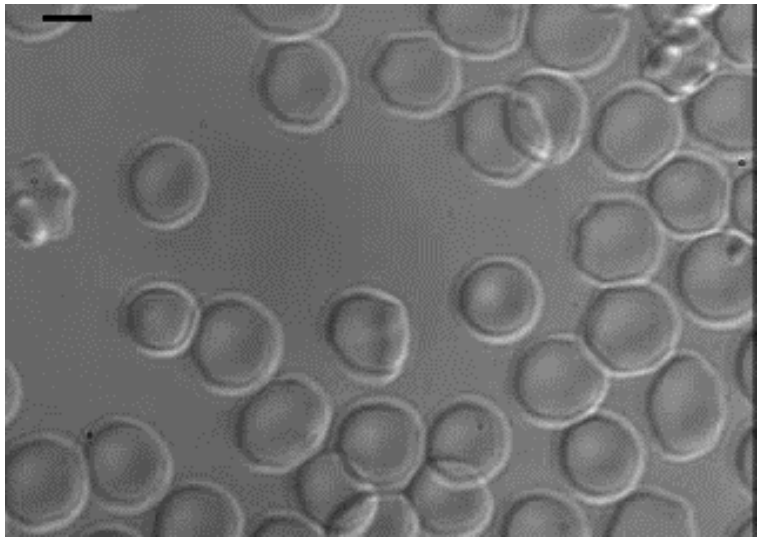
$$F = Q\frac{n_m W}{c}$$

Ex: **F= 1.33 pN** for
W= 1 mW, n_m=1.33, Q=0.3

F – trapping force

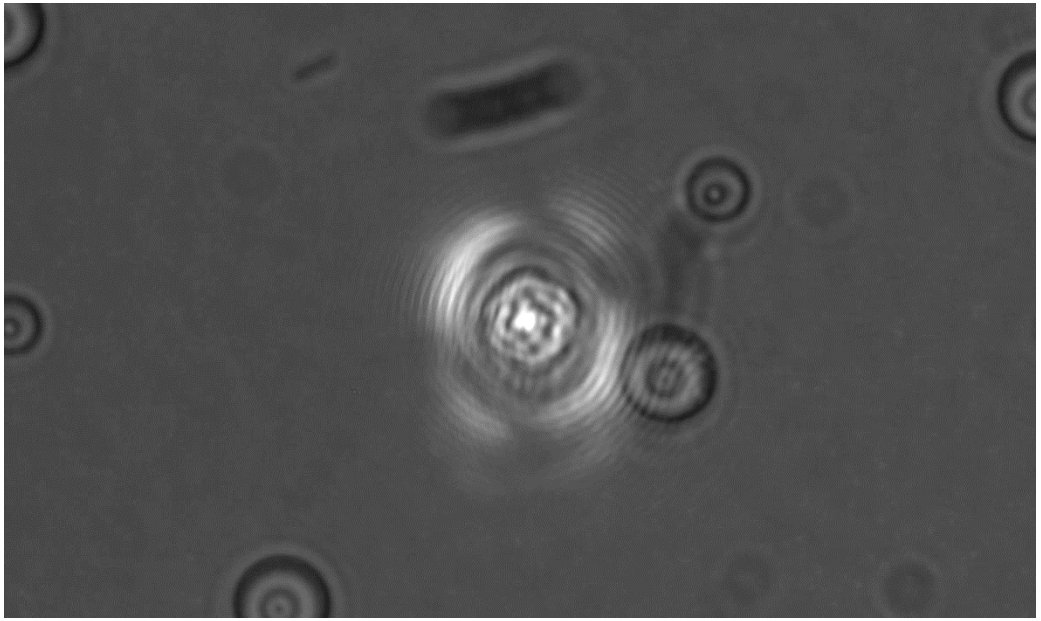
Q – dimensionless efficiency coefficient
W – power of the laser beam
n_m – refractive index of the medium
c – light speed in vacuum





Example of human erythrocyte trapping
2004 - OM Lab

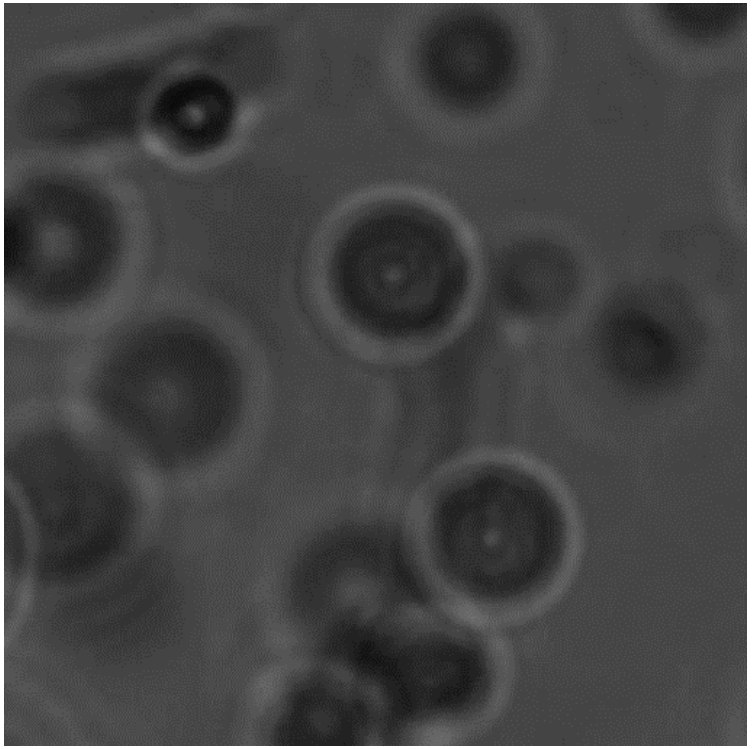
Some examples of trapping from OM Lab



silica microbeads, laser 970 nm,

power at the sample about

P= 5 mW



Optical trap behaves as an

attractor of particles

P= 120 mW

Are there sensitive issues when using optical tweezers to trap biological particles ?

1. The intensity at the trapping position (focal plane) is very high !
   Absorption of light by different components of a biological sample is wavelength dependent !

Is the laser beam damaging the sample ?

If yes, which is the level of damage ?

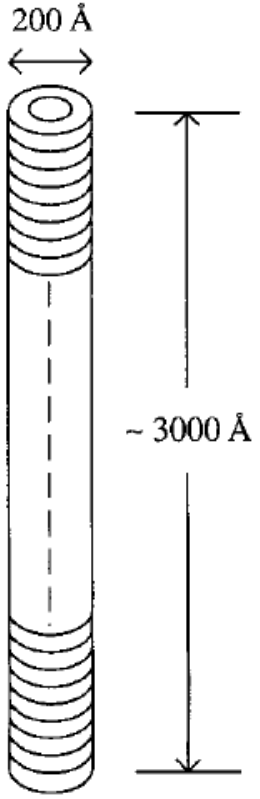
2. Biological samples (e.g. viruses, bacteria, cells) have arbitrary shapes while the laser beam is symmetric.

Does this mismatch prevent trapping ?

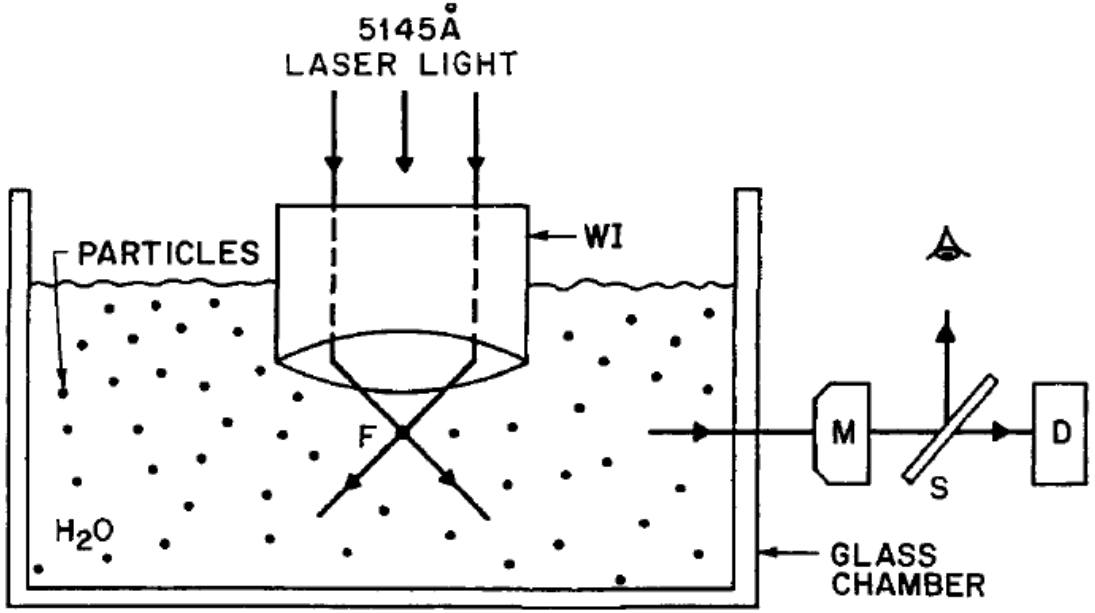
First optical trapping of a biological sample

Tobacco Mosaic Virus (TMV)

TMV
shape & size



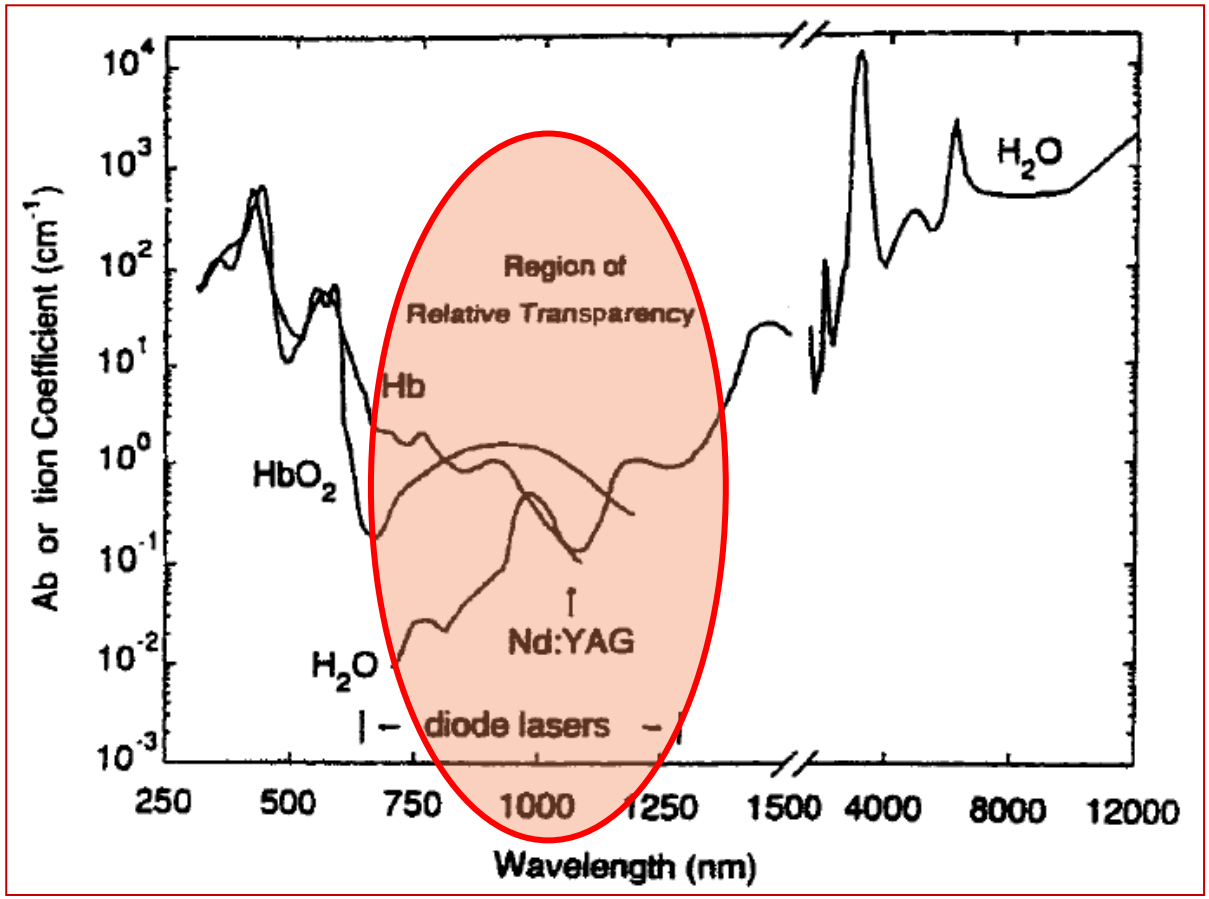
Apparatus used for optical trapping of TMV particles and mobile bacteria



Bacteria (which are slightly larger than Rayleigh particles) trapping was accidentally observed and then rigorously characterized for *E. Coli* in a closed sample cell.

A. Ashkin and J.M. Dziedzic, "Optical trapping and manipulation of viruses and bacteria", *Science* 235, 1517 (1987)

Damage – free trapping of living cells with infrared light



Plot of the optical absorption coefficients of hemoglobin (Hb), oxyhemoglobin (HbCh) and water versus the wavelength.

Damage – free trapping of living cells

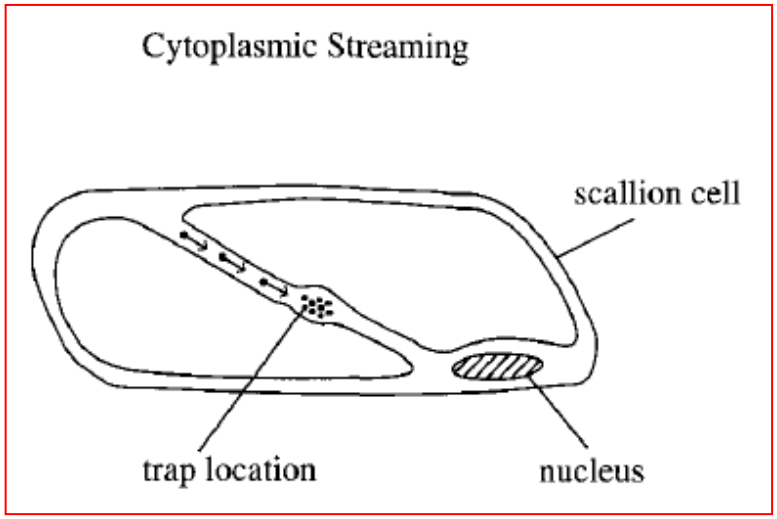
A. Ashkin, J.M. Dziedzic, T. Yamane, "Optical trapping and manipulation of single cells using infrared laser beams", *Nature* 330, 769 (1987)

Ashkin: "We tried red blood cells, plant cells, and the huge number of different types of protozoa, diatoms, and single cells of algae one can find in pond water." **One can trap almost any type of cells with IR beam without, or with limited damage.**

Not only were the cell types quite varied, but also their sizes and shapes. Shape and optical properties of particles are crucial to the trapping process.

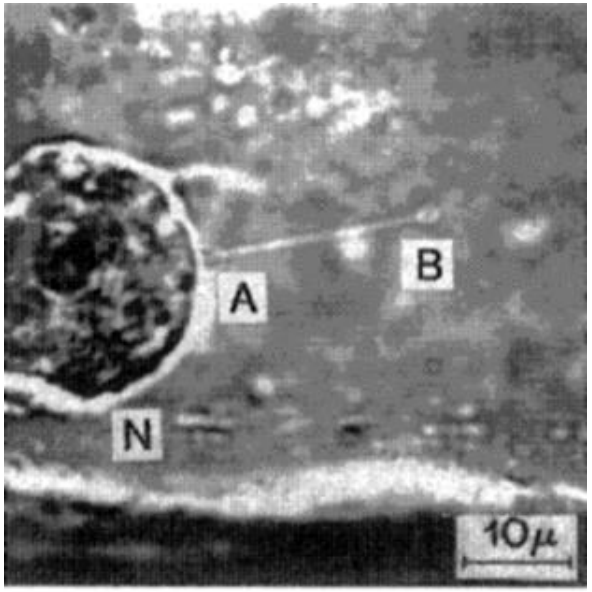
Optical tweezer-type traps are very tolerant of shape particle variation .

Intra-cellular trapping



Internal cell manipulation. Collection of particles and a blob of cytoplasm trapped within a streaming channel of cytoplasm inside a living scallion cell. When released, they simply move on.

A. Ashkin and J. M. Dziedzic, Internal cell manipulation using infrared laser traps, *Proc. Natl. Acad. Sci. USA* ***86,*** 7914 (1989).



Nobel Prize in Physics 2018

Arthur Ashkin invented optical tweezers that grab particles, atoms, viruses and other living cells with their laser beam fingers.

This new tool allowed Ashkin to realise an old dream of science fiction – using the radiation pressure of light to move physical objects.

He succeeded in getting laser light to push small particles towards the centre of the beam and to hold them there. Optical tweezers had been invented.

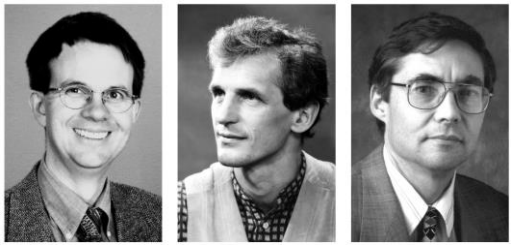
A major breakthrough came in 1987, when Ashkin used the tweezers to capture living bacteria without harming them. He immediately began studying biological systems and optical tweezers are now widely used to investigate the machinery of life.

Prize motivation for Arthur Ashkin:

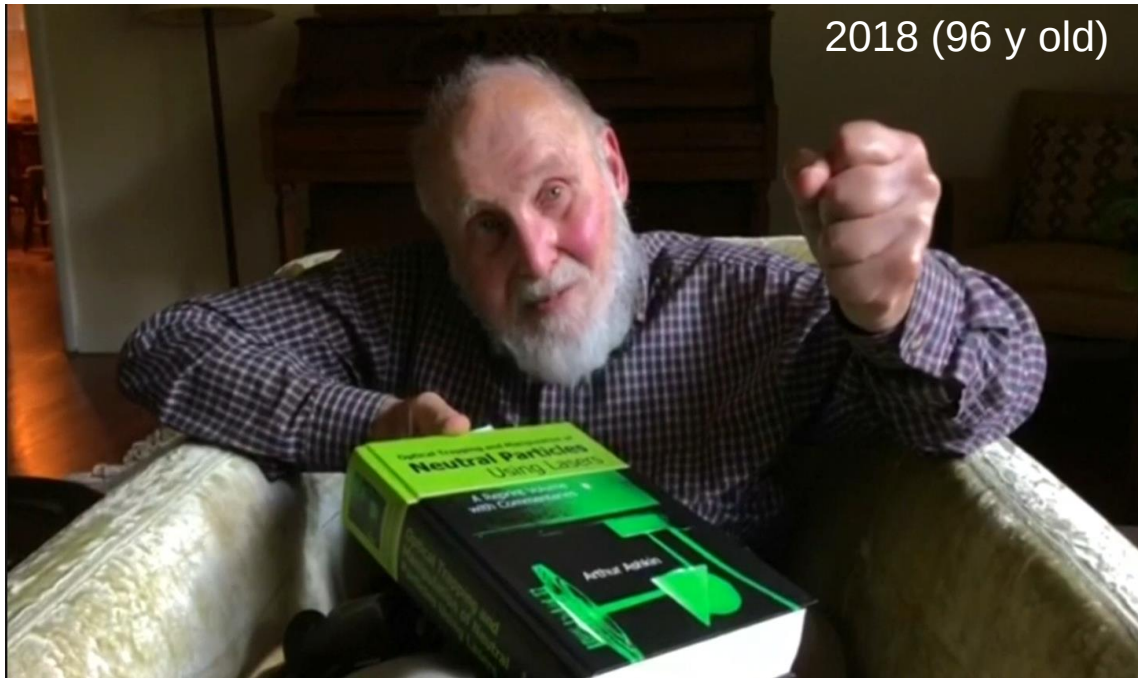
"for the optical tweezers and their application to biological systems."

Other Two Nobel Prizes in Physics – related to optical trapping

The Nobel Prize in Physics 1997 was awarded jointly to Steven **Chu**, Claude **Cohen-Tannoudji** and William D. **Phillips** "for development of methods to cool and trap atoms with laser light."



The Nobel Prize in Physics 2001 was awarded jointly to Eric A. **Cornell**, Wolfgang **Ketterle** and Carl E. **Wieman** "for the achievement of Bose-Einstein condensation in dilute gases of alkali atoms, and for early fundamental studies of the properties of the condensates."



Arthur Ashkin

Noble Prize in Physics

2018

Optical Tweezers – some properties

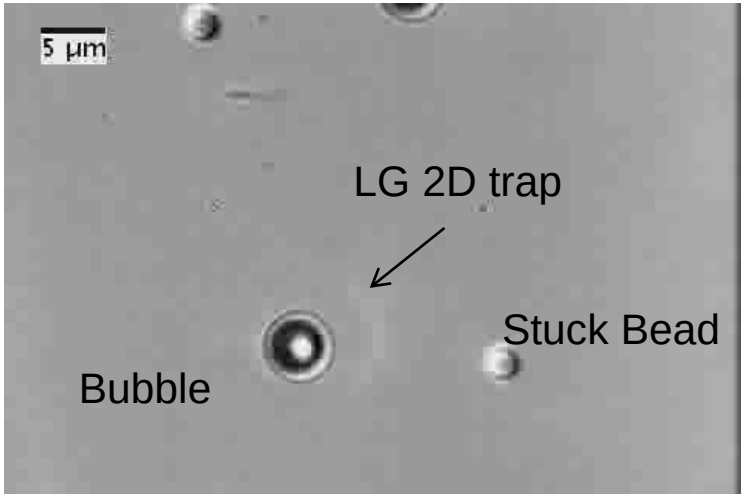
What type of particles can be trapped ?

- **Material:**
  - Dielectric (polystyrene, silica);
  - Metallic (gold, silver, copper);
  - **Biological** (cells, macro-molecules, intracellular structures, DNA filaments);
  - Low index (ultrasound agent contrast); crystal or amorphous material.
- **Size:** 20 nm – 20 μm
- **Shape:** spherical, cylindrical, arbitrary.

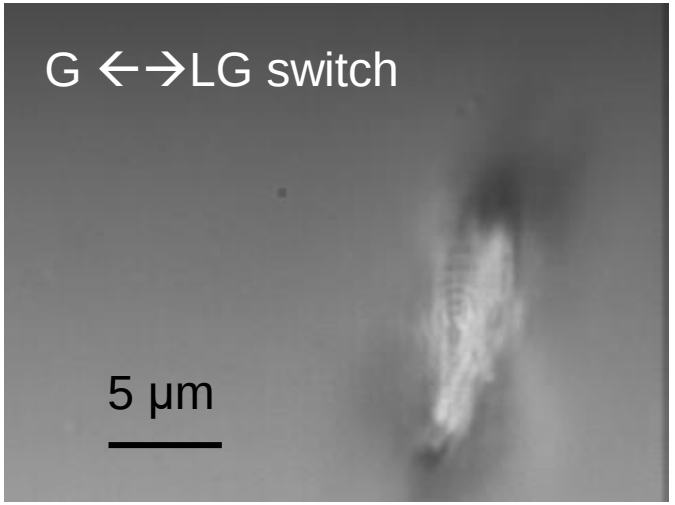
Range of forces that can be applied and measured : **0.1 – 100 pN**

Some examples of optical manipulation from OM Lab

Ultrasound Contrast Bubble – LG 2D trap



Very simple rotor - piece of glass

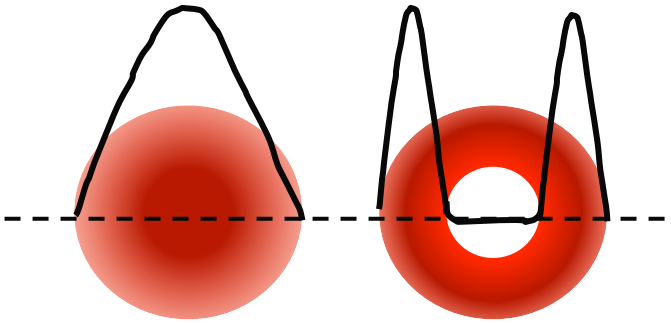


Gaussian

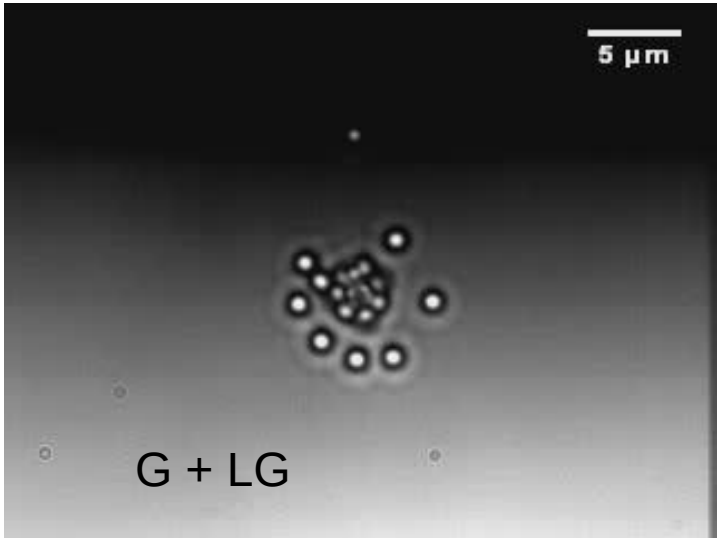
TEM 00

Laguerre Gaussian

TEM 01

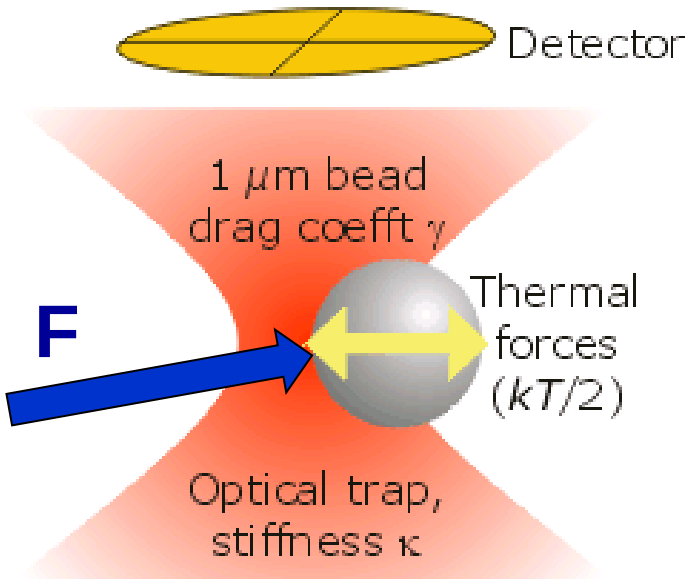


LG OAM transfer to silica bead



OAM = Optical Angular Momentum

Using the trapped bead to probe external forces



Measuring the displacement Δ of the particle and knowing the stiffness of the trap K we get F:

$$F = K \Delta$$

F = (Fx, Fy, Fz) Force

K = (Kx,Ky,Kz) stiffness of the trap

Δ = (Δx, Δy, Δz) Displacement

OT allows measuring forces in 3D !

Typical values for **OT : K_{OT} = 0.001 – 10 pN/nm**

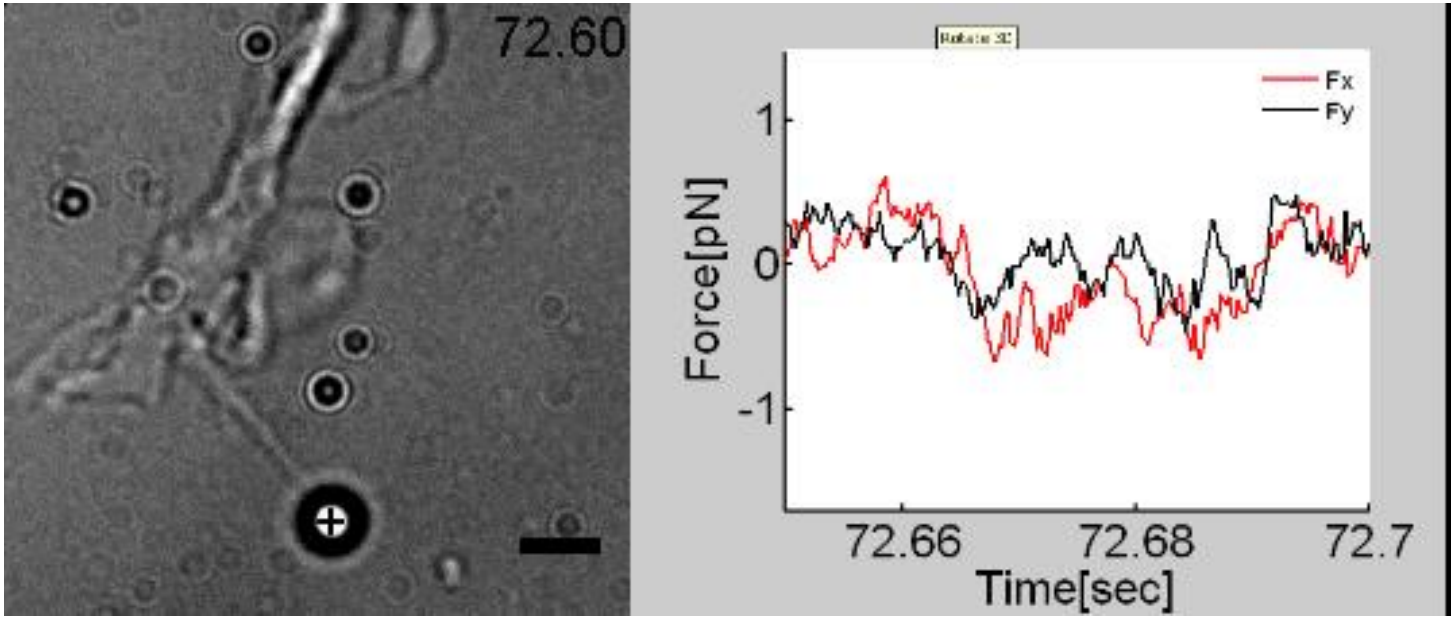
Typical values for **AFM: K_{AFM} = 10 – 1000 pN/nm**

OT and AFM are Complementary Techniques

Measuring the forces exerted by neuronal cells during development

Force exerted by Filopodia - Protrusion

Acquisition rate: 20Hz; Scale Bar = 2μm; Time in seconds



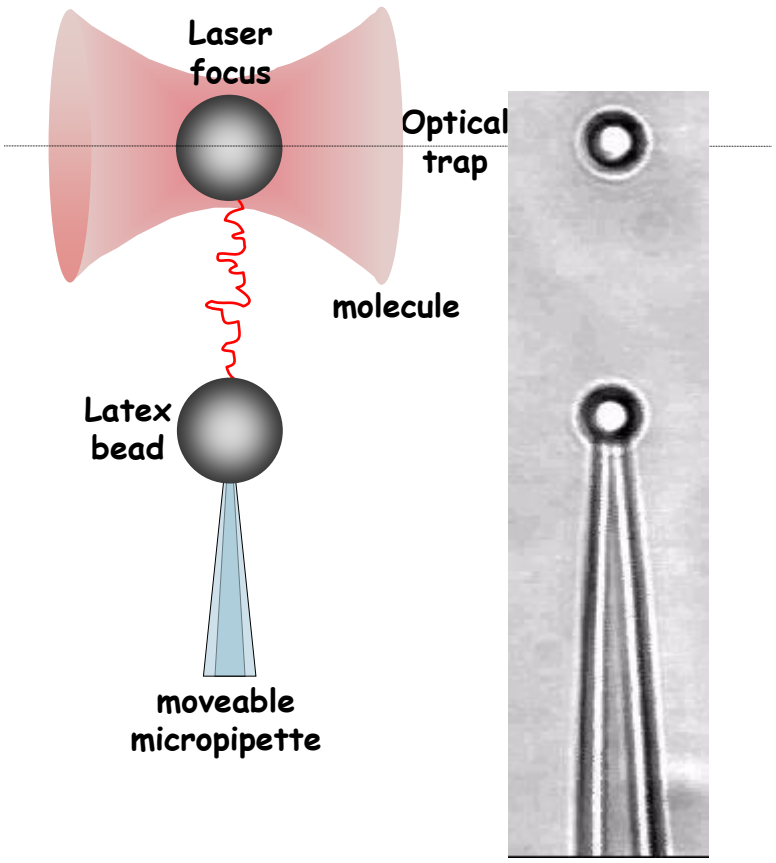
2 Days In Vitro hippocampal neuron from mouse

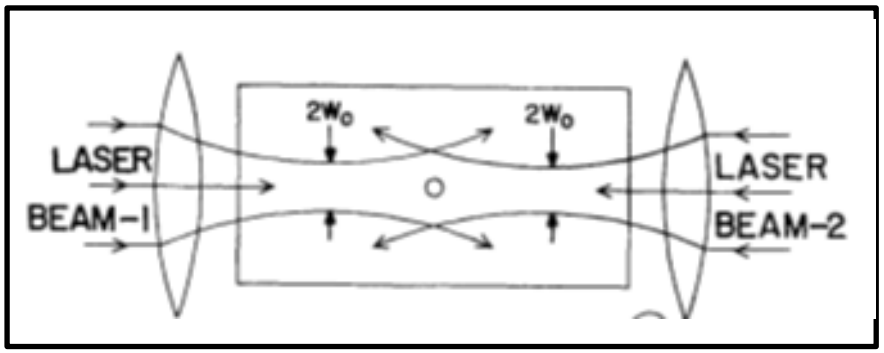
The force and protrusion due to actin polymerization of the bundle of actin filaments in the filopodia is observed.

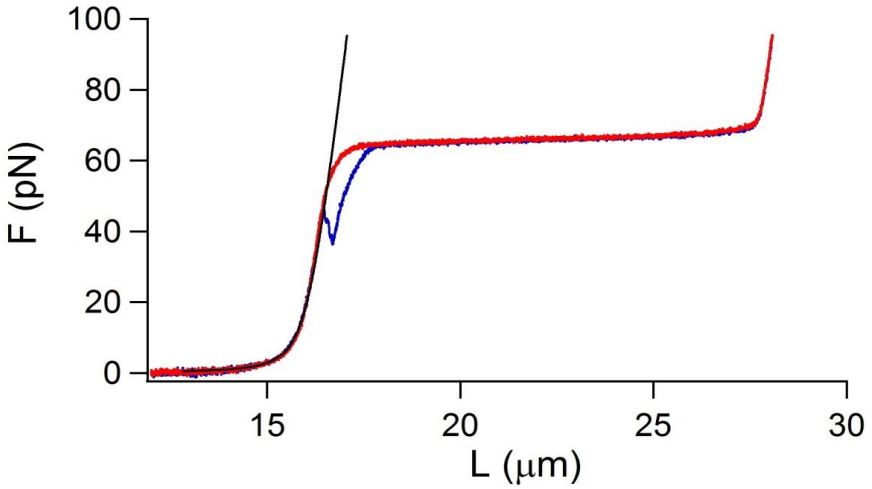
Cojoc, D, … & Torre, V, PLoS One 2 (10), e1072 (2007)

Difato, F, Pinato, G & Cojoc, D, *Int. J. Mol. Sci.* **14**, 8963 (2013) - REVIEW

Measuring force and length range when stretching λ-phage DNA



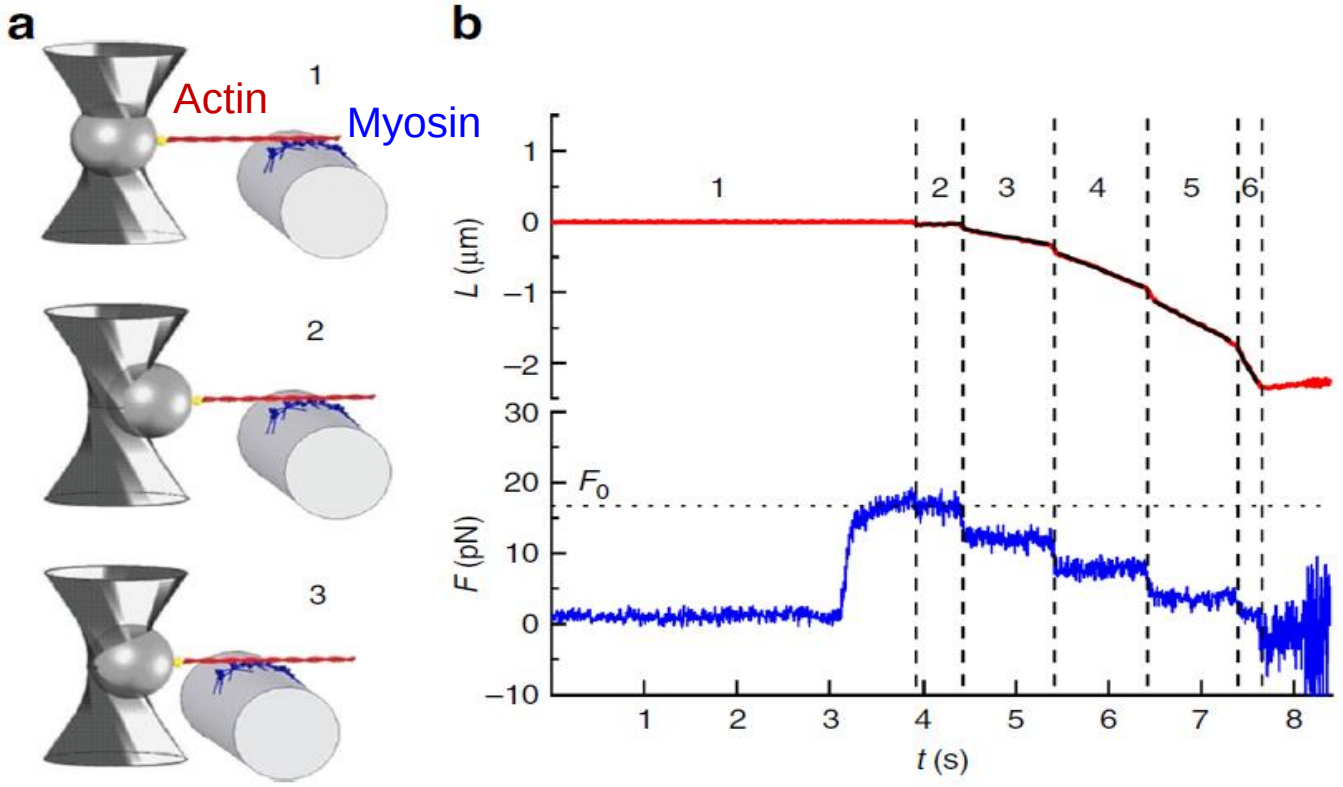




The molecule undergoes a highly cooperative structural change at ~65 pN that implies 70% elongation and is likely involved in the modulation of the access to genetic information .

collab V. Lombardi, P. Bianco, Florence Univ.

A myosin II nanomachine mimicking the striated muscle,

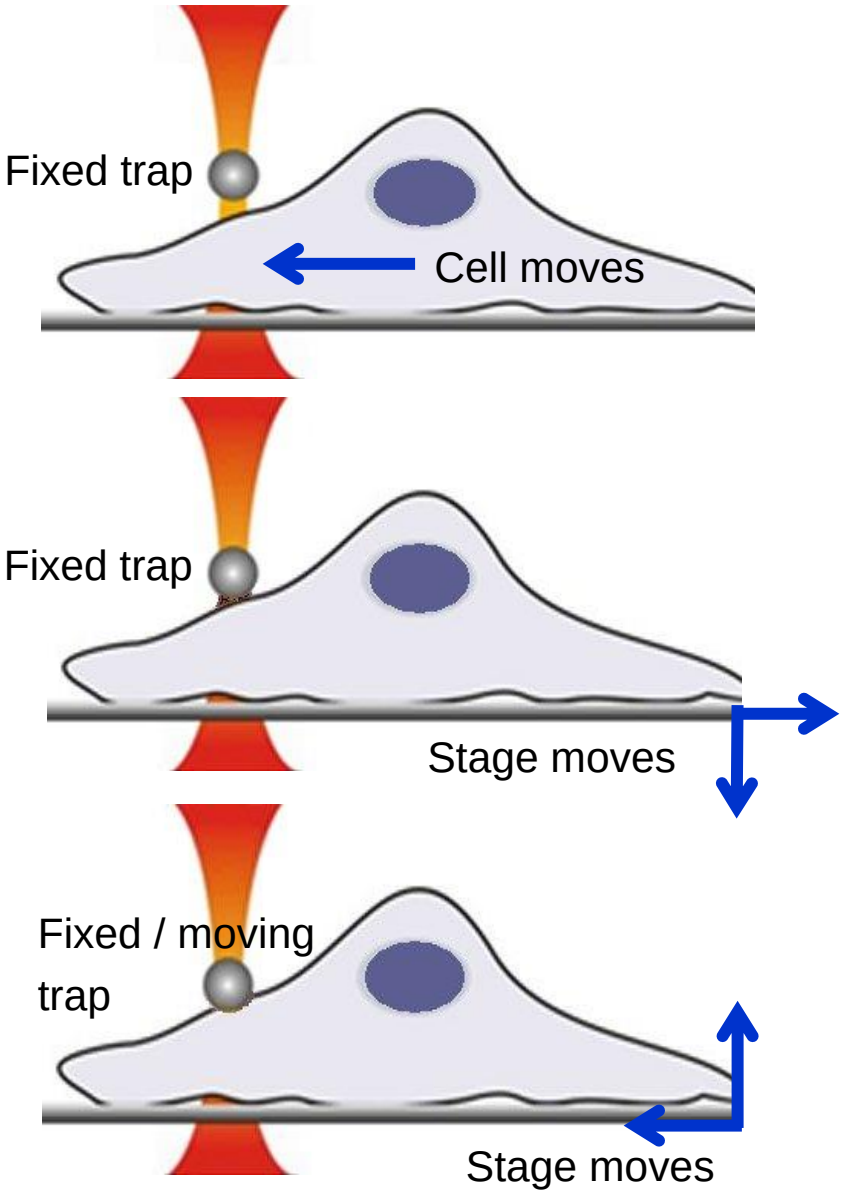


a. Schematic representation of three snapshots during the phases of the interaction between the actin filament and the motors.
b. Recording of the relative sliding (red) and force (blue) during interaction. Phase 1, following the formation of the first bonds between the actin filament and myosin motors, the force rises in position feedback to the maximum isometric value ~17 pN.

Pertici et al., *Nature Communications*, (2018) 9:3532.

OT local probing living cells

(touch - pull - push approaches)



Touch / intercept

Measure forces when full cell or part of the cell moves

Pull (Coated beads)

Local adhesion / binding

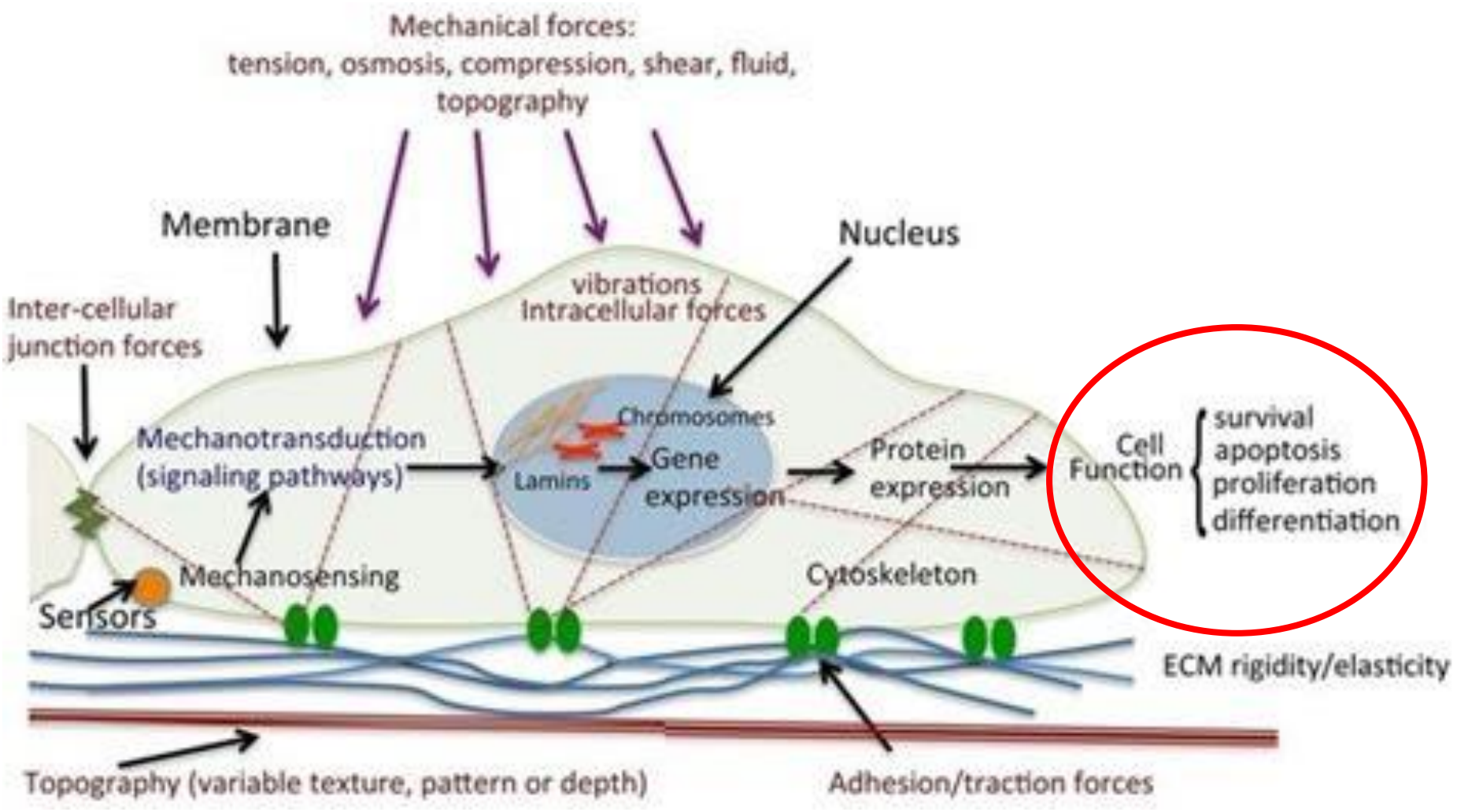
Local viscoleasticity (tether membrane)

Push

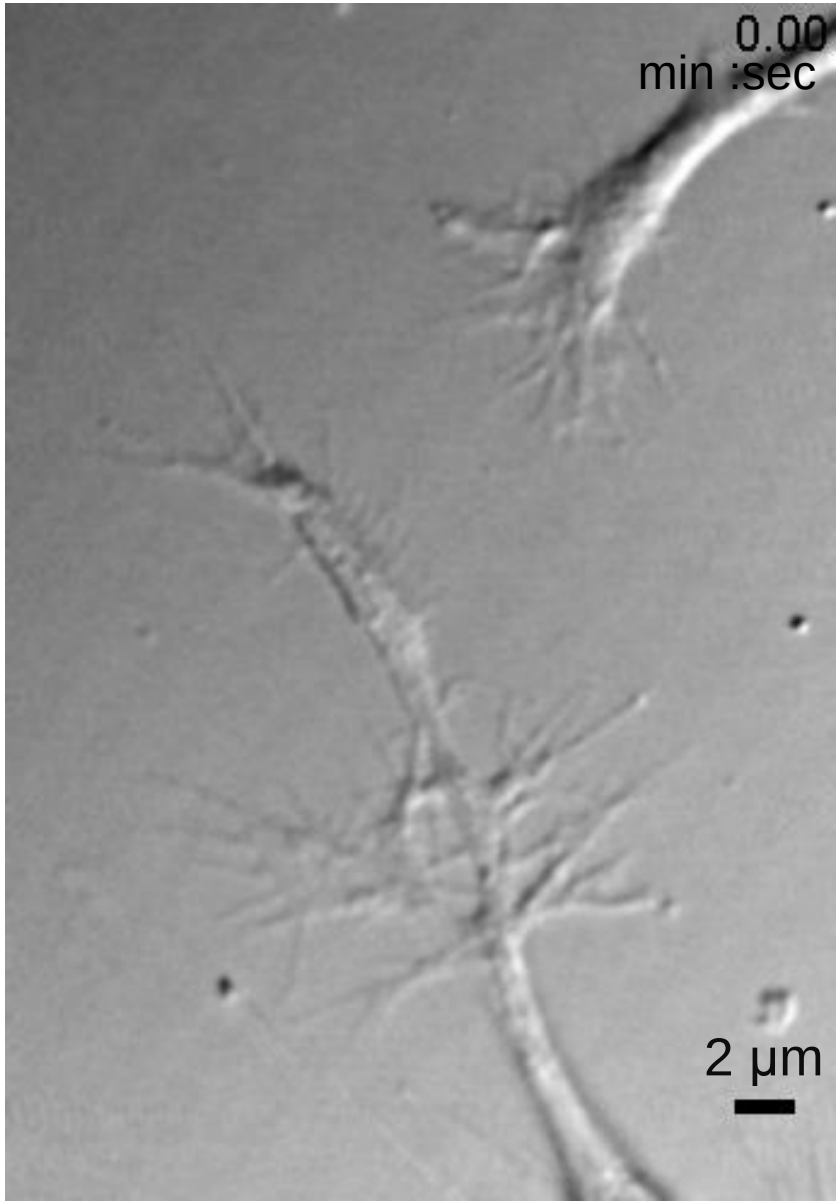
Local viscoelastic properties

Local cell stressing

Cell mechanotransduction – cell function



Neuronal development (pre and post natal)



Neurons release biochemical cues which are intercepted and interpreted by their nearby neurons but

they interact also mechanically

➢ The **Growth Cone (GC)** searches and detects molecular signposts that are displayed by the nearby developing neuron and the environment.

➢ **GC** responds to these signs by advancing, pausing and turning until it reaches its proper destination

F. Difato *et al* (2006) OM-Lab & SISSA

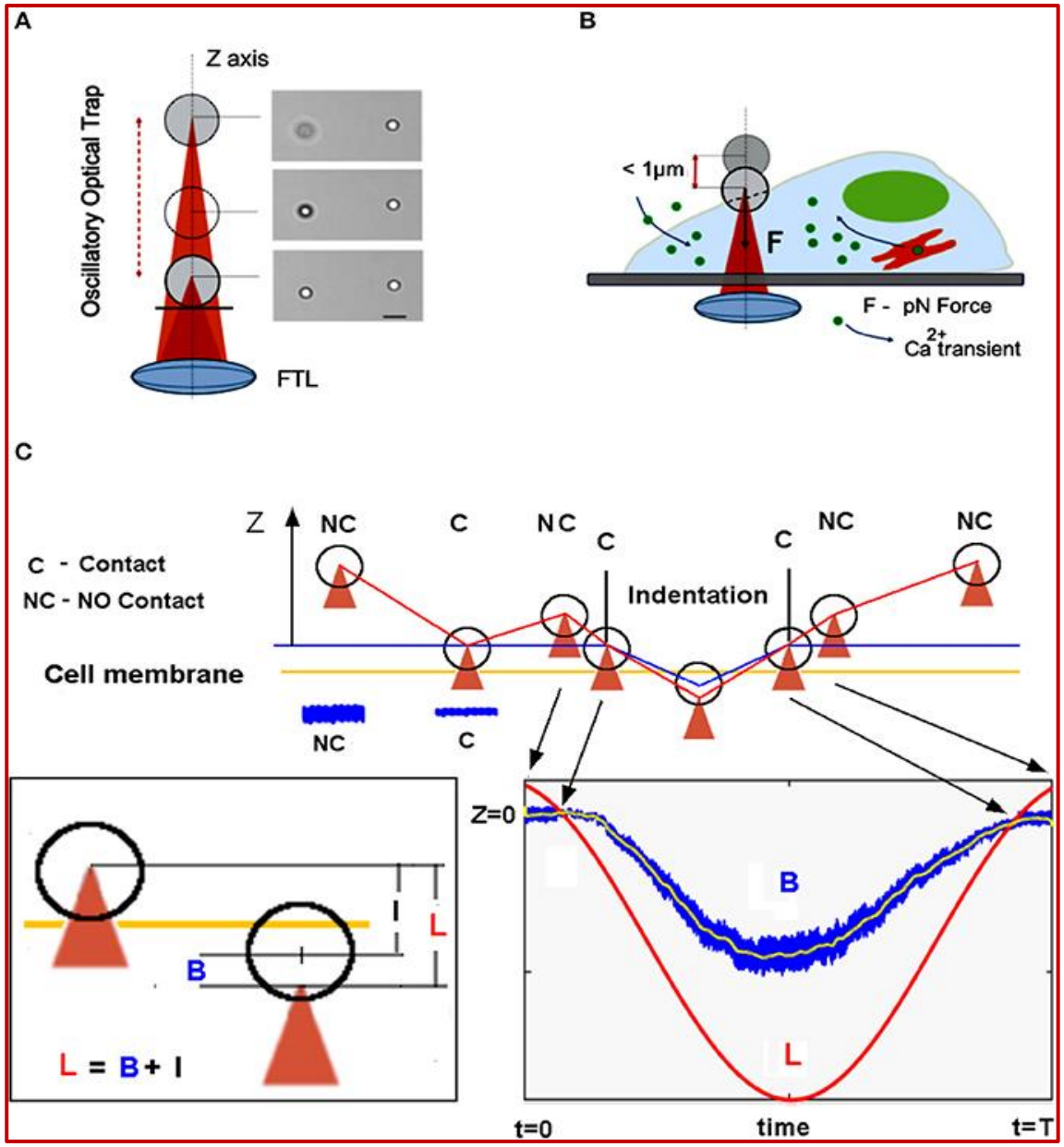
Mechanotransduction studies how cells sense physical forces and the cellular signal transduction in response to mechanical stimuli.

Piconewton forces, characteristic for OT, are in the range of forces expressed by neurons during development, cell-cell and cell ECM interaction.

Transduction of the mecanical stimulus applied by OT to the cell can be investigated on the same optical microscopy platform (e.g. Calcium signaling).

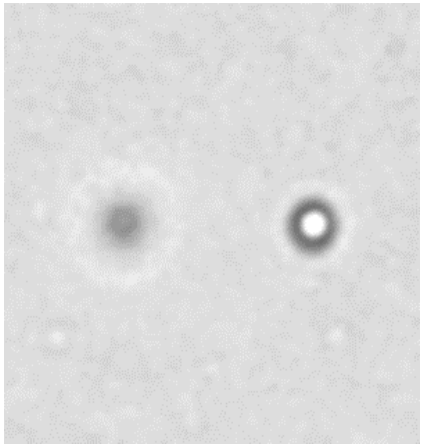
We demonstrate cell mechanotransduction in neurons, using very small (piconewton forces), applied with unprecedent high spatial and temporal resolution.

Cell membrane indentation and cellular calcium transients



Overview of the mechanical stimulation

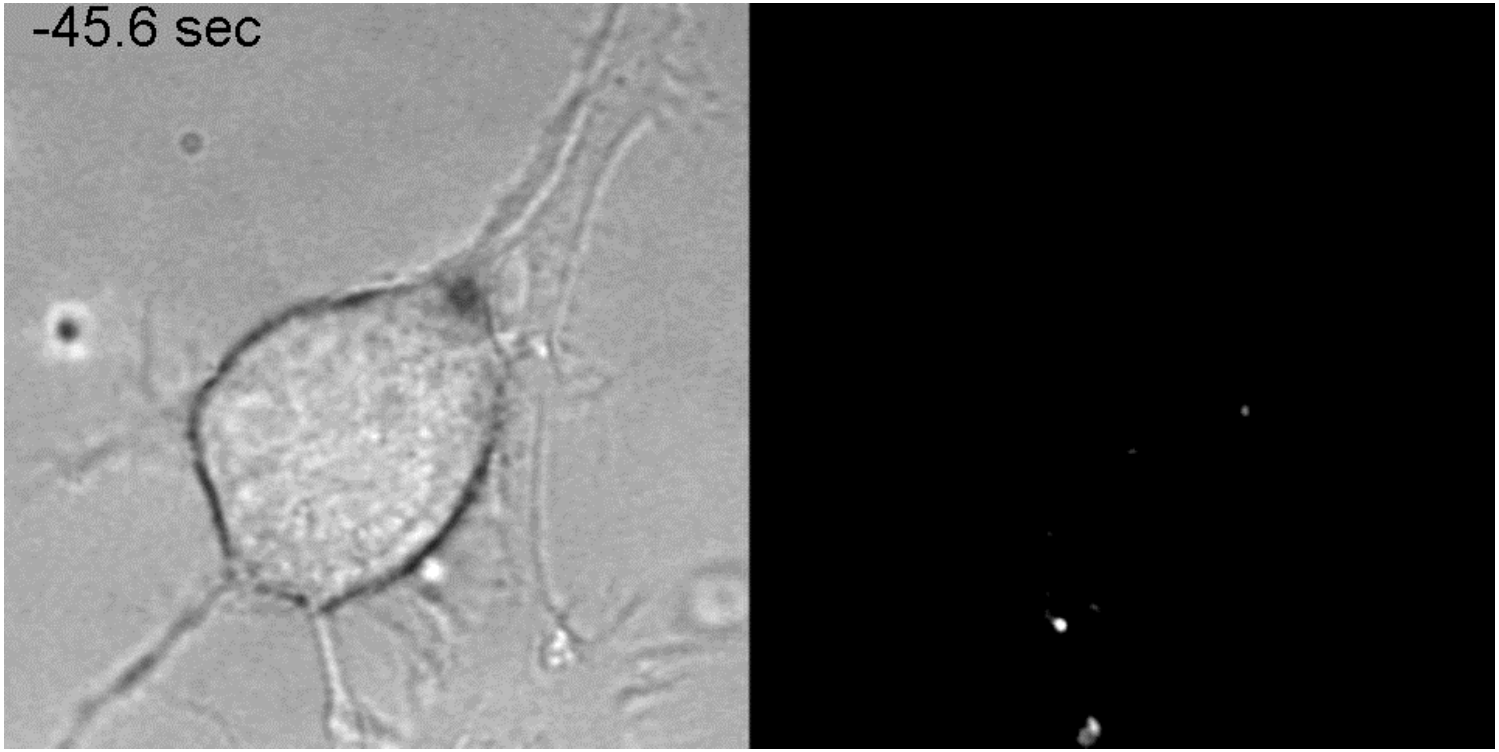
Dynamic Optical Trap



F. Falleroni *et al*, Frontiers Cell Neurosci, 2018

Ca^{2+} transients evoked by calibrated mechanical stimulations

Brightfield | Calcium Imaging

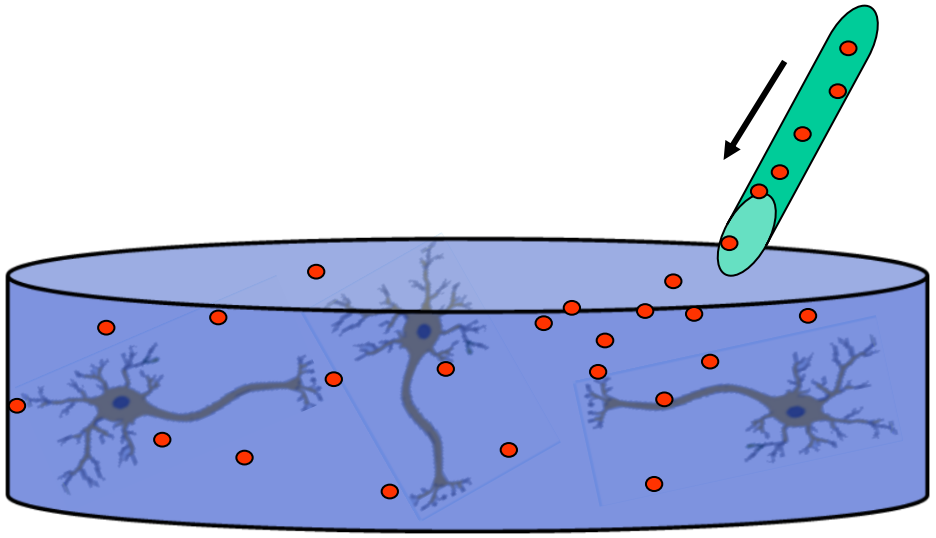


mouse neuroblastoma NG108-15

F. Falleroni *et al*, Frontiers Cell Neurosci, 2018

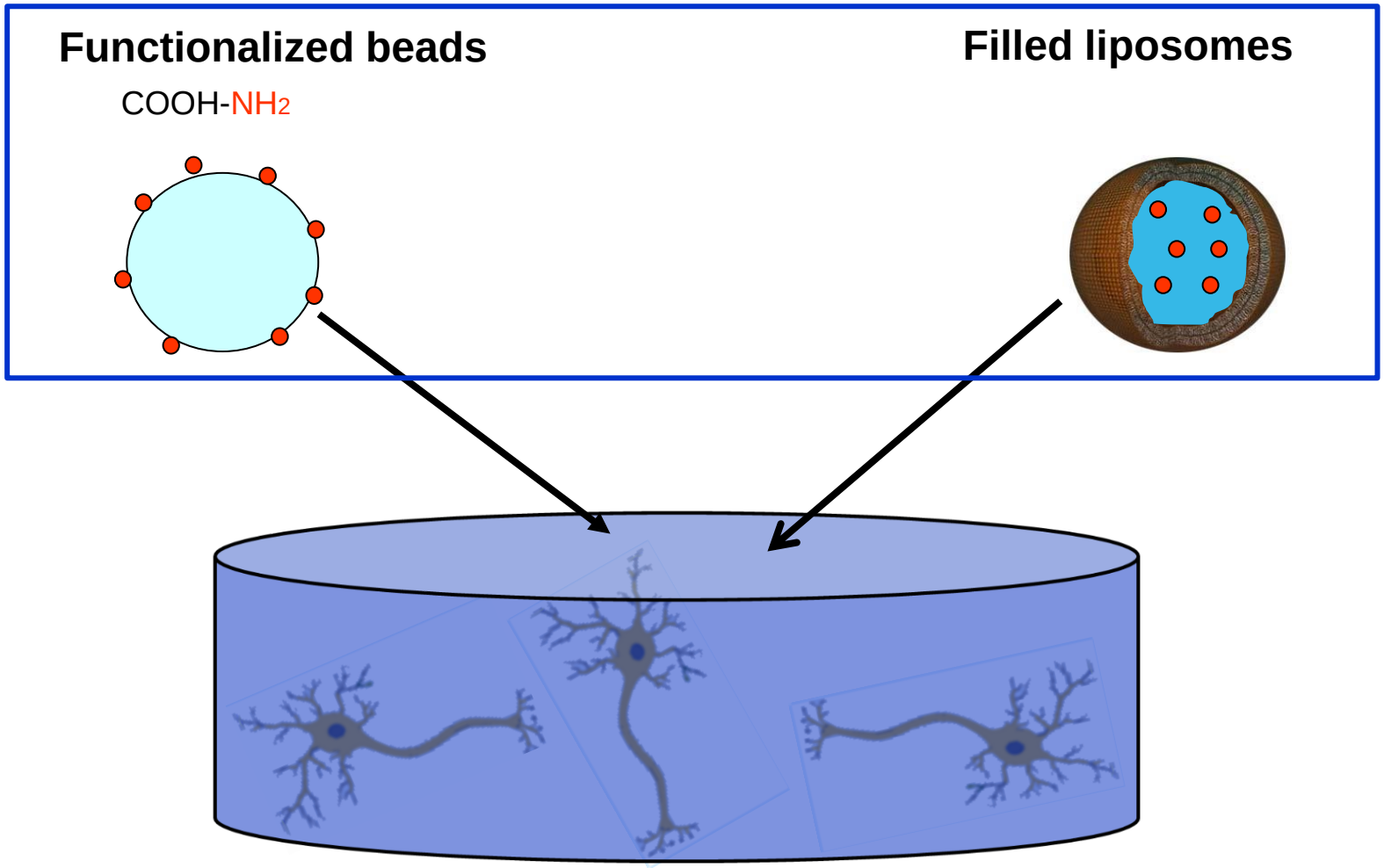
Create physiological inspired experimental conditions !

Classical bath administration of molecules rarely reflects the physiological conditions in which molecules are locally released at low concentrations, creating spatial and temporal gradients.

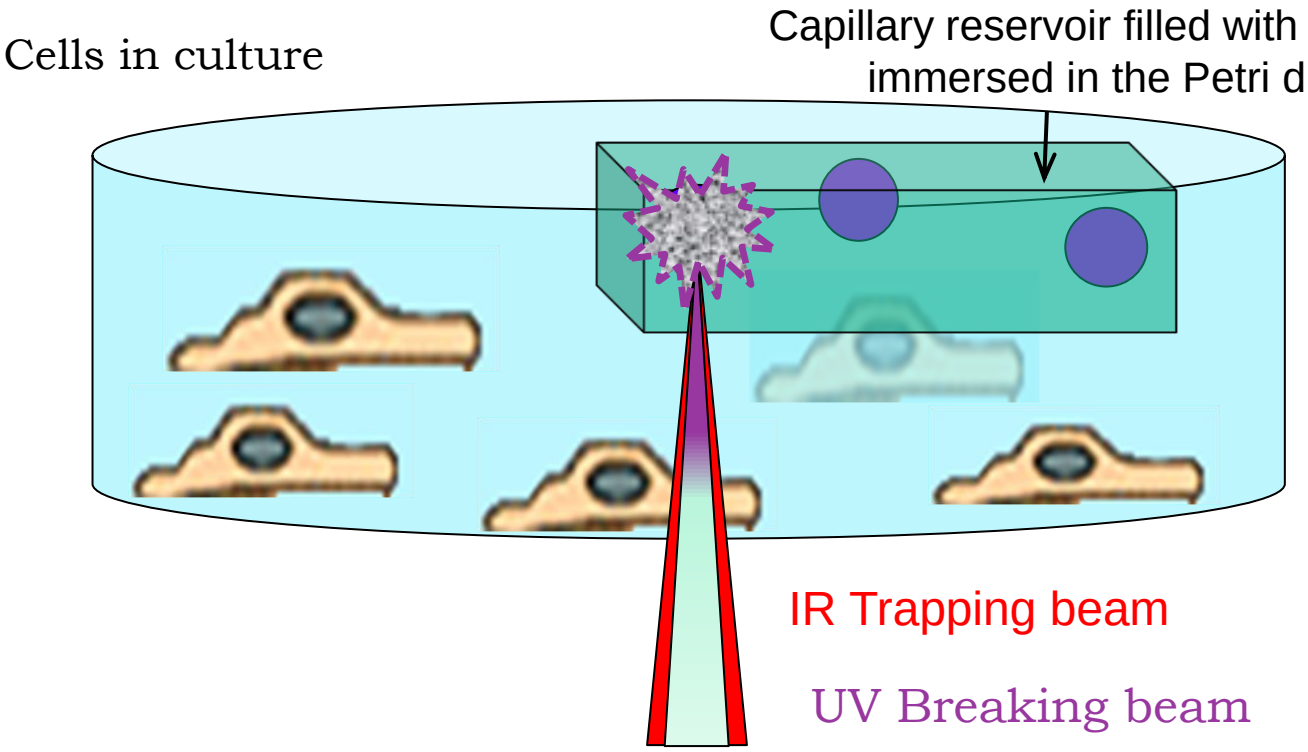


Local stimulation using micro/nano vectors

Active molecules (e.g. guidance cues) are cross-linked to the surface of **microbeads** or encapsulated in **liposomes** (lipid vesicles)



Vector - Cell Positioning by Optical Manipulation

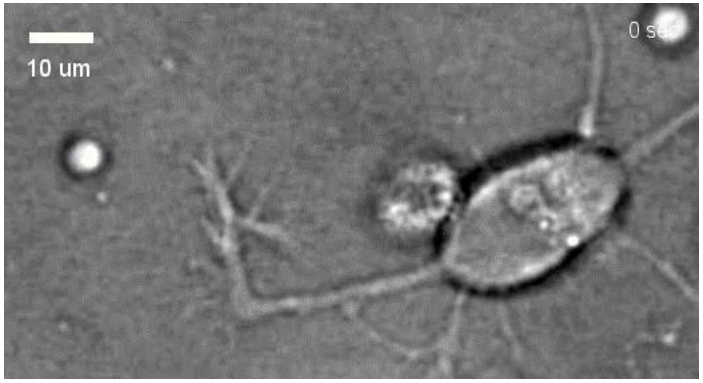


and delivered by:

- contact (beads or microsources) – D'Este *et al* Integrative Biology (2011)

- photolysis of liposomes Sun B, Chiu DT , J ACS (2003)

Focal stimulation of hippocampal neurons by guidance cues encapsulated in liposomes



Netrin-1

Growth Cone turning

Proof of concept

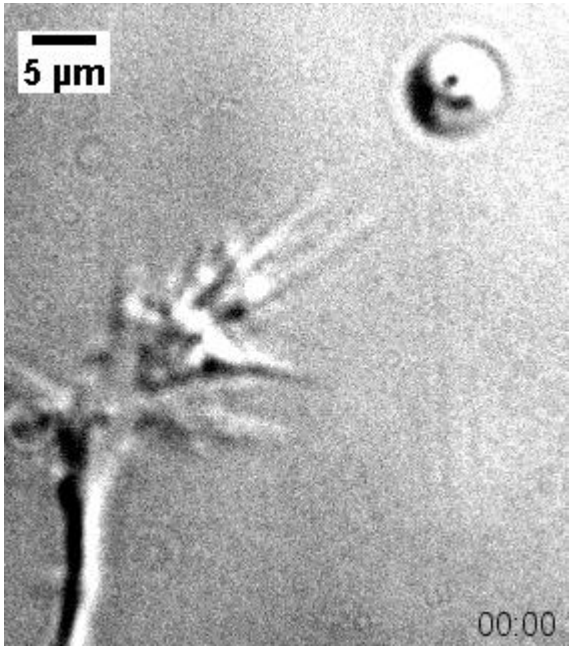
Pinato G, *et al* J. Eur. Opt. Soc. – Rap. Comm. 6, 11042, (2011)

SemA3

A more quantitative study:

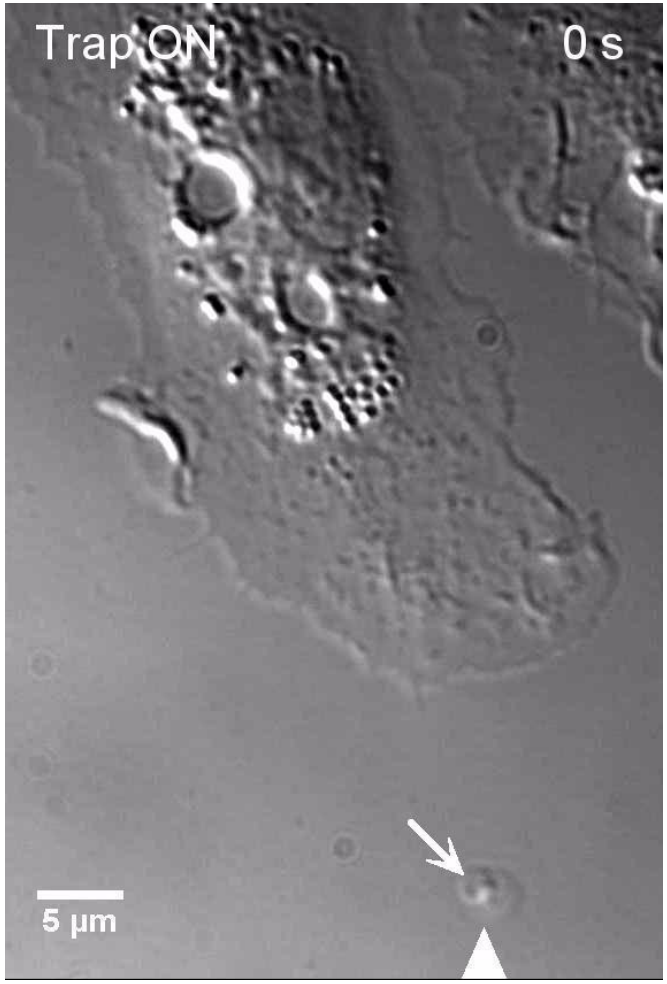
Less than 5 Netrin-1 molecules initiate attraction but 200 Sema3A molecules are necessary for repulsion

Pinato G *et al* Sci. Rep. 2, 675 (2012)



Collaboration with the group of prof. **Vincent Torre,** Neurobiology Sector**, SISSA, Trieste**

Extracellular Vesicles (EV)



EV from microglial cells on a microglia cell.

- EV are circular membrane structures released by most cells which represent highly conserved mediators of intercellular communication.
- EV carry proteins, lipids and genetic materials and transfer these cellular components between cells by different mechanisms, such as endocytosis, macropinocytosis or fusion.
- Temporal and spatial dynamics of vesicle-cell interaction still remain largely unexplored

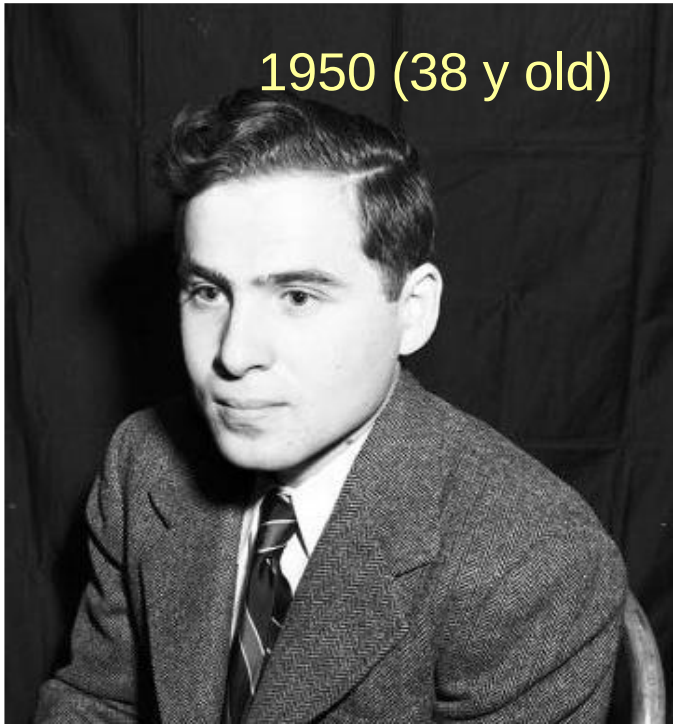
Collaboration:
Claudia Verderio - CNR-Institute of Neuroscience Milan
Roberto Furlan – San Raffaelle, Milan
Giuseppe Legname – SISSA, Trieste

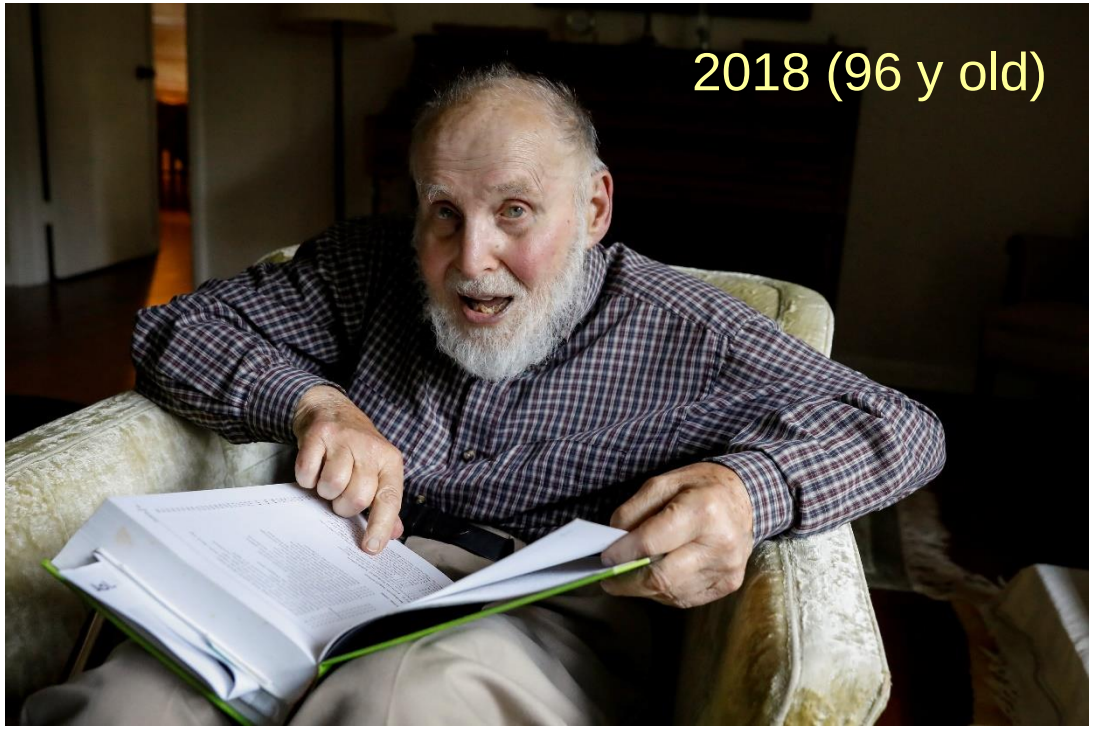
Prada I *et al* BioTehniques, (January 2016)

Optical Tweezers Microscopy

- Light has momentum, change of momentum generates force
- Optical Tweezers (OT): Laser beam tightly focused on micro/nano objects in liquid
- Forces applied and measured by OT: 1 - 200 pN
- OT with IR laser can be applied to living cells and biomolecules without damaging them
- OT is implemented on a microscope platform → trap and manipulate what you see and see what you manipulate (ex. Mechanotransduction)

Thank you Arthur Ashkin !





Magnetic Tweezers (MT)

The first MT was assembled in 1996 by Strick et al who used them to explore elasticity of supercoiled DNA

Magnetic Tweezers are scientific instruments for the manipulation and characterization of biomolecules or polymers. These apparatus exert forces and torques to individual molecules or groups of molecules. It can be used to measure the tensile strength or the force generated by molecules.

Strick TR, Allemand J-F, Bensimon D, Croquette V. The elasticity of a single supercoiled DNA molecule. *Science* (1996) **271**:1835.

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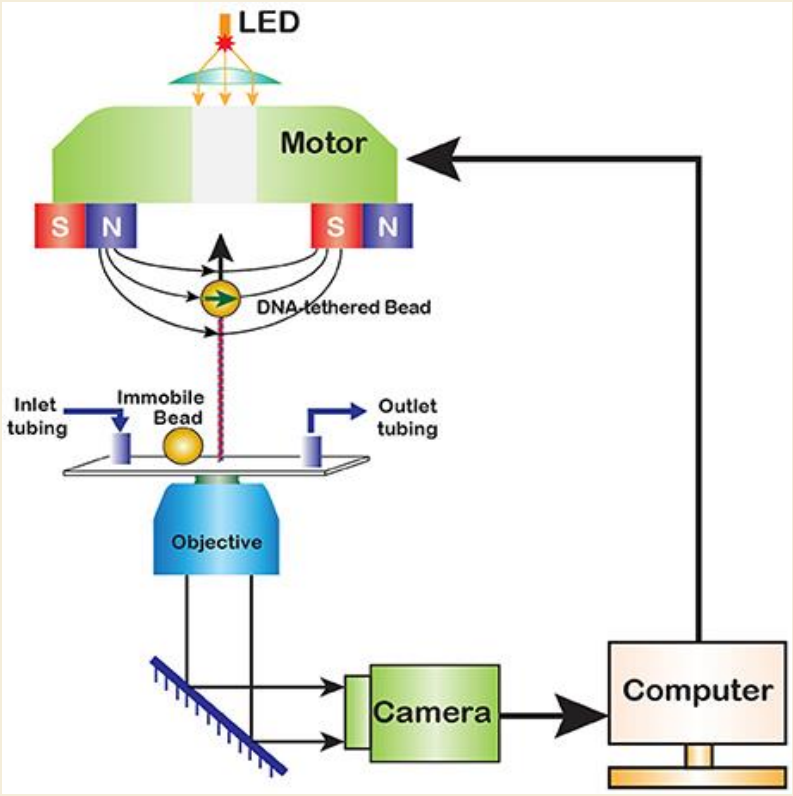


Schematic of magnetic tweezers setup

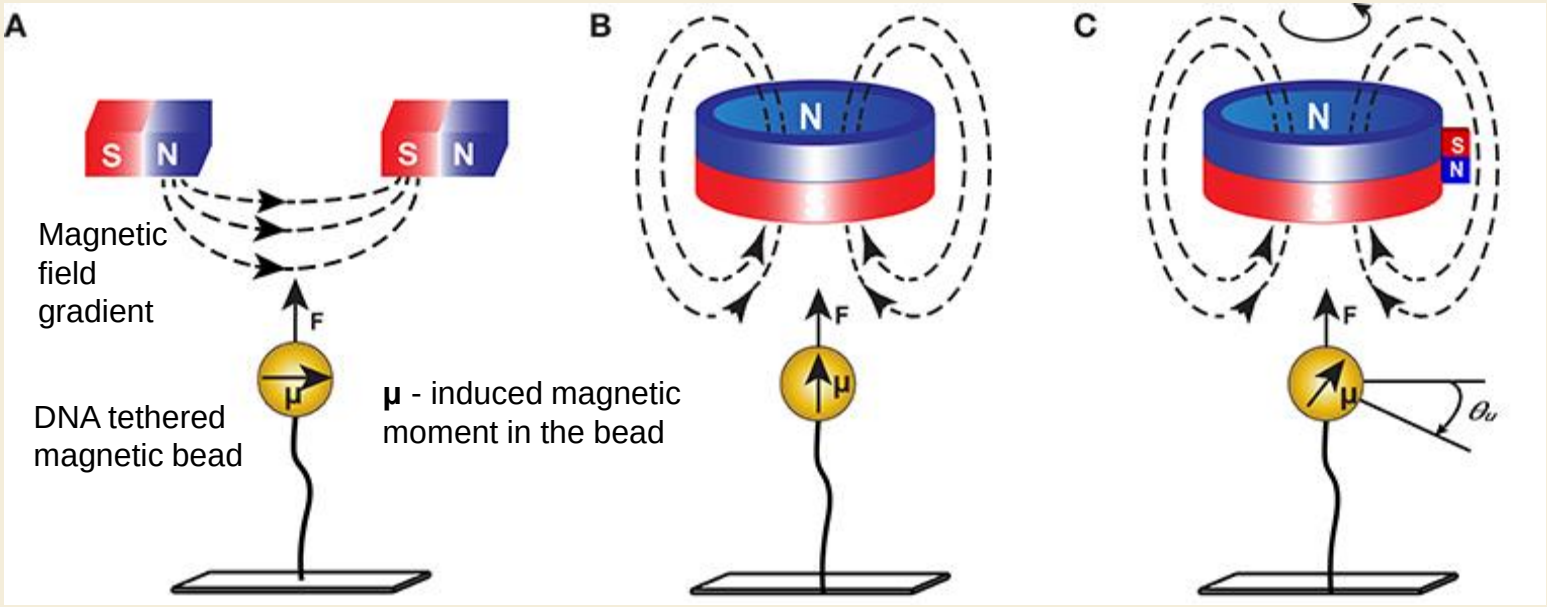
A paramagnetic bead (e.g. FeO) is tethered to the surface of a flow cell via a functionalized DNA molecule.

Permanent magnets produce magnetic field that pulls the bead in the direction of the field gradient.

The magnets can be translated or rotated to alter the stretching force or twist the DNA.

Strick TR, Allemand J-F, Bensimon D, Croquette V. The elasticity of a single supercoiled DNA molecule. *Science* (1996) **271**:1835.

Orientation of magnetic field and magnetic forces.



(A) A pair of horizontally placed magnets induces a horizontal magnetic moment (μ) in the bead. Vertical translation of the magnets induces translation of the bead. Rotation of the magnets around the tether axes induces rotation of the bead.

(B) Cylindrical magnets exert a vertical magnetic field to the tether axis.

(C) Magnetic torque tweezers: a small horizontal field gradient in addition to a strong vertical one.

R. Sarkar, V. Rybenkov, A Guide to Magnetic Tweezers and Their Applications, Frontiers in Physics 4, 2016.

The force F experienced by the bead in MT:

$$\vec{F} = \frac{V_b\,\chi}{\mu_0}\nabla\left|\vec{B}\right|^2$$

$$\vec{B} = \mu_0\,(1+\chi)\,\vec{H}$$

$\vec{B}$ – the magnetic flux density (magnetic induction) [T] = [N/Am]

V_b - the bead volume [m^3]

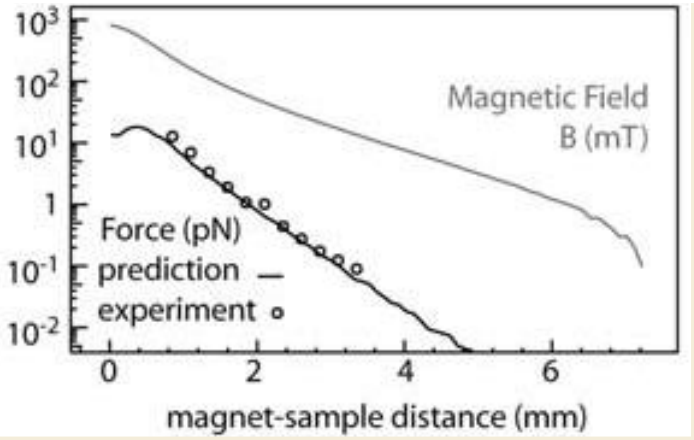
μ_0 - permeability of free space 1.26 10^{-6} [H/m]=[T m/A]

χ - magnetic susceptibility of the bead (material) [-]

The force F is a function of

- the gradient of the magnetic field
- the volume of the particle (bead)
- the material (paramagnetic χ>0, superparamagnetic and ferromagnetic χ>>0)

Force vs magnet – sample distance



Ex. Using NdFeB (Neodymium Iron Boron) magnets and micrometric beads (2.5 um), the MT can produce forces F of 10-20 pN at a distance of about 1 mm, which is sufficient for most of single molecule applications.

The force experienced by a magnetic bead in MT has an expression similar to the gradient force experienced by a dielectric bead in OT :

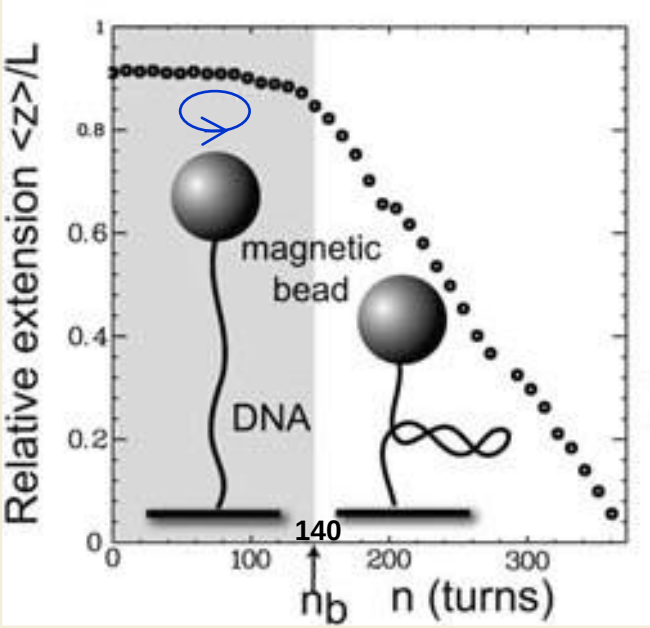
Magnetic Tweezers MT		**Optical Tweezers OT**
$\vec{m} = V\chi\vec{H} = \frac{V\chi}{\mu_0}\vec{B}$	Induced **dipole moment**	$\vec{p} = \alpha\vec{E} = 4\pi\varepsilon_0 V\vec{E}$
$U = -\vec{m}\cdot\vec{B}$	The interaction dipole moment – field (H or E) creates a **potential:**	$U = -\vec{p}\cdot\vec{E}$
$\vec{F} = -\nabla U = \frac{V\chi}{\mu_0}\nabla\left\|\vec{B}\right\|^2$	For a varying field the **force** is**:**	$\vec{F} = -\nabla U = 4\pi\varepsilon_0 V\nabla\left\|\vec{E}\right\|^2$

MT characteristics

- The force due to a magnetic field is analogous to the optical gradient force, with a potential energy due to the response of the particle to an external field.
- MT allows creating an uniform force over a large area, thus enabling many systems to be probed at once → **increase the throughput**.
- The orientation of the field can also be manipulated independent of the gradient, permitting **controlled rotation**, attractive for studying phenomena such as DNA supercoiling.

Twisting DNA with MT

The ease with which DNA can be twisted in a MT setup by rotating the magnets makes this system an optimum choice for the study of coiled (and braided) DNA and its interactions with proteins (e.g., topoisomerases).

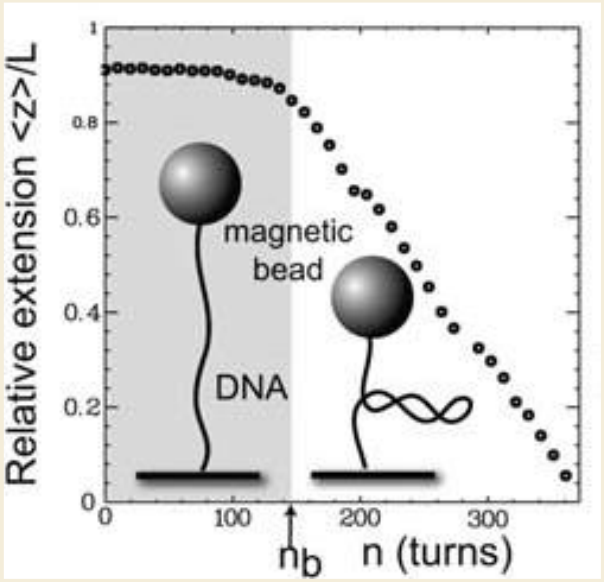


Experimental curve (normalized extension) of one single dsDNA molecule (50 Kb) at F = 1.2 pN. At low number of turns n, the change in DNA extension is small, → the molecule stores torsional energy.

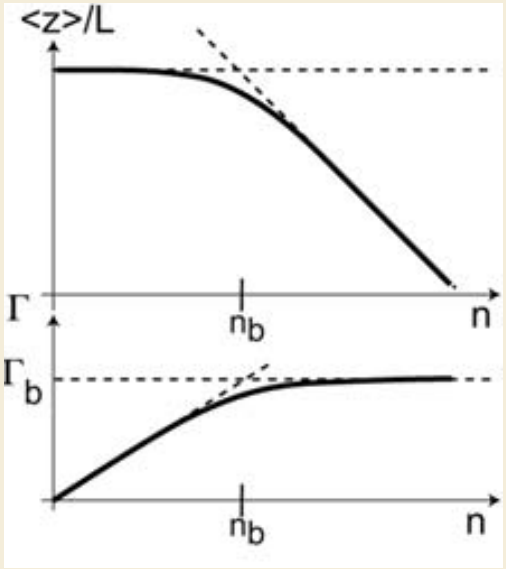
After n = nb ≈ 140, the molecule buckles and starts forming plectonemes. The extension decreases almost linearly.

T. Lionnet, ..., V. Croquette, Cold Spring Harbor Protoc; 2012

Extension vs. supercoiling for one dsDNA



A)



B)

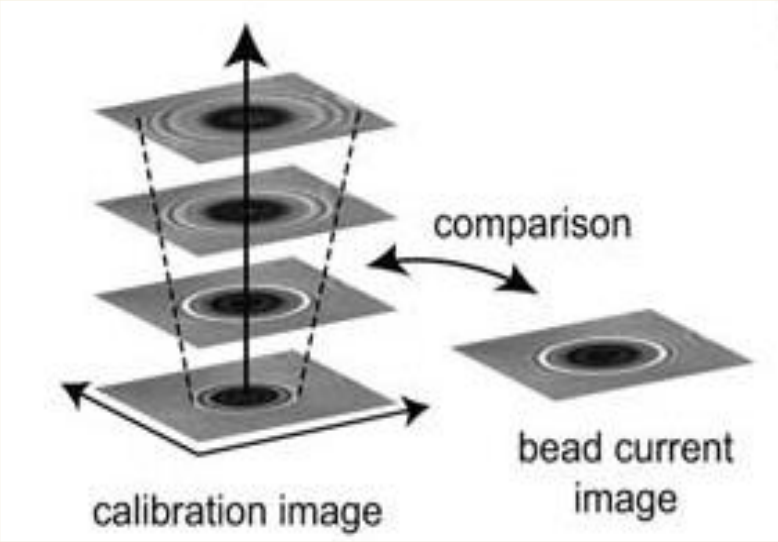
Initial twisting does not change the system's extension, but the torque stored in the tube increases linearly with the number of turns n applied. At n=nb, forming a loop (plectonemes) costs less energy than increasing the torsional energy.

Each additional turn leads to the formation of another loop, so that the extension decreases linearly with n, but the torque Γ = Γb remains constant.

T. Lionnet, ..., V. Croquette, Cold Spring Harbor Protoc; 2012

Magnetic Tweezers **Bead tracking**

Measurement principle of the bead position in z



Due to diffraction the bead image is formed by a series of diffraction rings whose sizes depend on the relative distance between the bead and the focal plane. When the bead is in focus, the rings disappear, but they increase in diameter as the bead moves out of focus.

By precisely stepping the focal plane through a series of positions (e.g., by moving the objective with a piezo-electric device), one forms a stack of calibration images that records the shape of the diffraction rings versus distance from the focal plane.

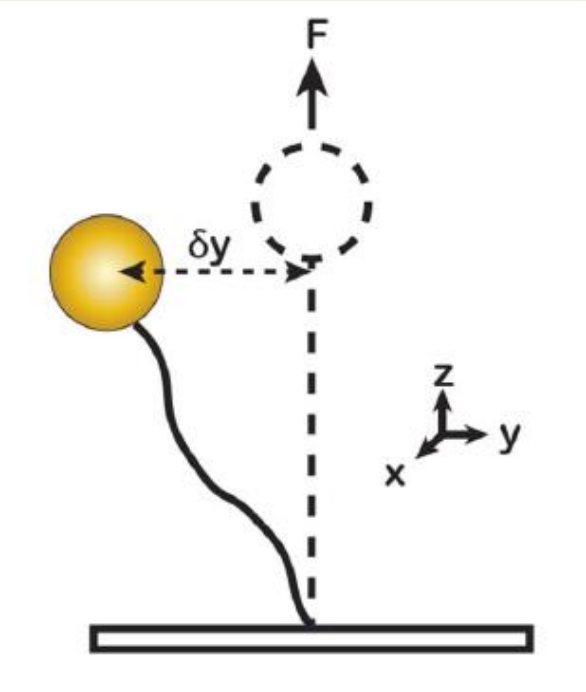
The out-of-focus distance for a new bead image is determined by comparing its diffraction pattern to the calibration stack.

T. Lionnet, ..., V. Croquette, Cold Spring Harbor Protoc; 2012

Magnetic Tweezers **Force calibration**

Bead magnetization varies from one bead to another → calibration using Brownian motion is necessary for each bead

Brownian motion of a DNA tethered magnetic bead in solution.



The tethered bead behaves as a harmonic pendulum with the lateral stiffness:

$$k_y = F/ l.$$

The stiffness can be calculated from the horizontal fluctuation $<\delta y^2>$ of the bead:

$$k_y = kT/ <\delta y^2>$$

or in the frequency domain using the PSD (Power Spectrum Density), in a similar way to Optical Tweezers.

T. Lionnet, ..., V. Croquette, Cold Spring Harbor Protoc; 2012

Acoustic tweezers

Acoustic tweezers spatially and temporally manipulate matter by using the interaction of sound waves with fluid and solid particle.

There are three types of acoustic tweezers:

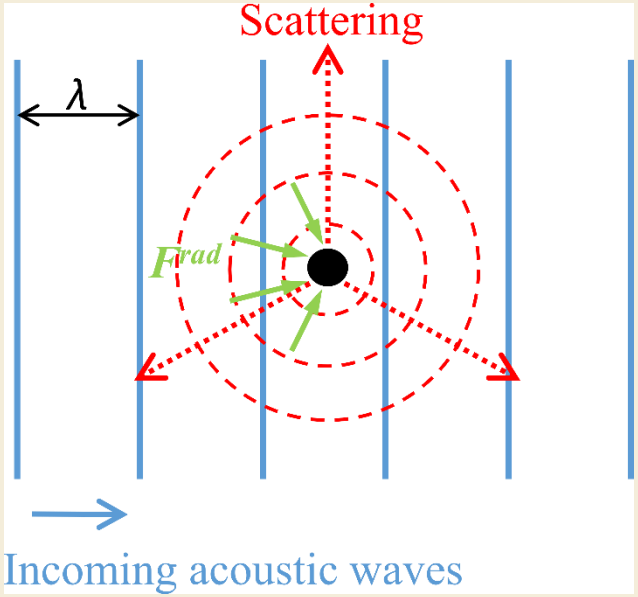
traveling-wave, standing-wave, and **acoustic-streaming tweezers**.

Both standing-wave and traveling-wave tweezers manipulate particles directly via an applied **acoustic radiation force**, whereas acoustic-streaming tweezers indirectly manipulate particles via **acoustically induced fluid flows**.

A. Ozcelik et al, Acoustic tweezers for life sciences, *Nature Methods* (2018) **15**: 1021.

G. Sitters et al, Acoustic Force Spectroscopy, *Nature Methods* (2014) **12**: 47.

Acoustic tweezers



Sketch of the far-field region $r \gg \lambda$ of an incoming acoustic wave ϕ_{in} (vertical lines) of wavelength λ scattering off a small particle (black dot) with radius $a \ll \lambda$, leading to the outgoing scattered wave ϕ_{sc} (circles and arrows).

The radiation force on the particle placed in a standing wave is a gradient force of the form:

$$\boldsymbol{F^{rad} = -\nabla U}$$

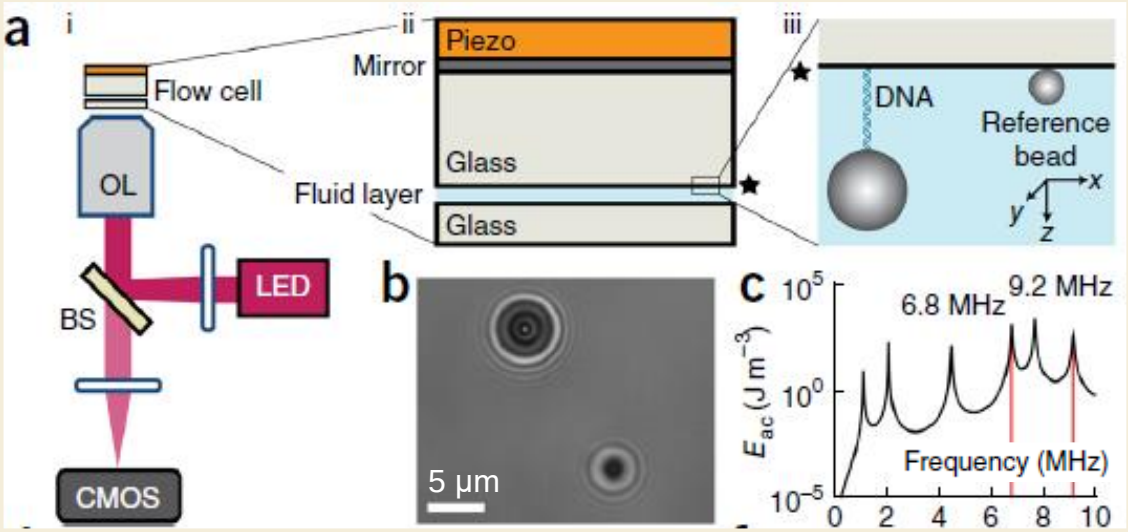
U -acoustic potential energy
V – microbead volume

$$F = -V\nabla\left[\frac{1-\kappa^*}{4}\kappa_m p^2 - \frac{\left(\rho^* - 1\right)}{2\rho^* + 1}\rho_m v^2\right]$$

in which p is the acoustic pressure, v the acoustic velocity, and $\rho^*(=\rho_p/\rho_m)$ and $\kappa^*(=\kappa_p/\kappa_m)$ are the density ratio and compressibility ratio between the particle and the medium, respectively[11]. In the case of polystyrene or silica microspheres in water, the force is dominated by the gradient of the squared acoustic pressure, driving the microspheres toward an acoustic pressure node.

Mikkel Settnes and Henrik Bruus Phys. Rev. E **85**, 016327 –2012

Acoustic Force Spectroscopy Setup



(a, i) Acoustic force device integrated in a flow cell; objective lens (OL); digital camera CMOS, LED light source.

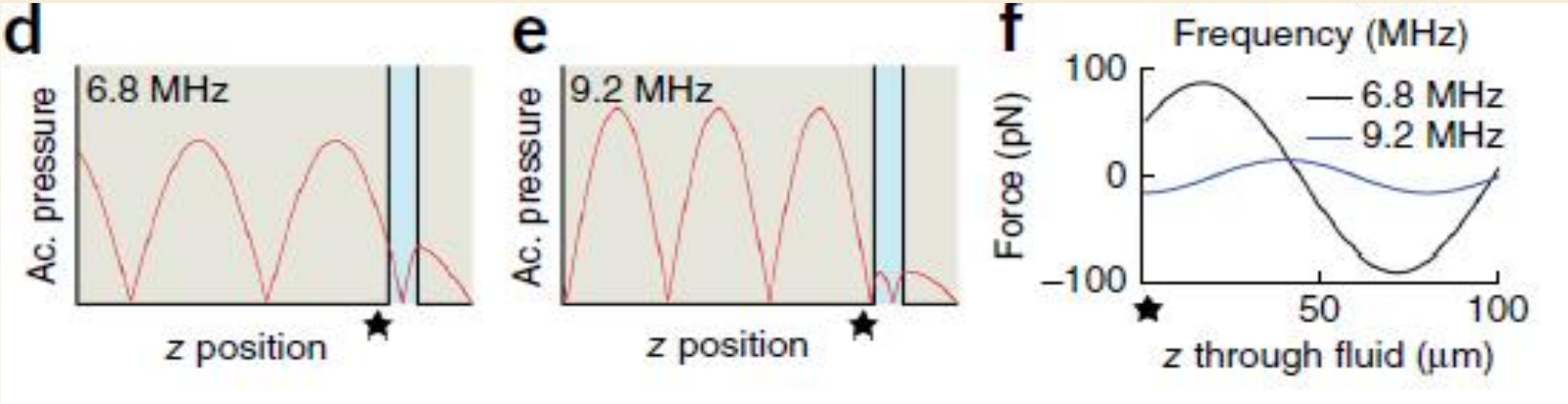
(a, ii) Flow cell: two glass plates with a fluid chamber in between. An acoustic wave-generating piezo plate is attached to the upper glass slide, which has a sputtered mirroring aluminum layer for illumination.

(a, iii) A single DNA molecule, attached at one end to the upper glass plate (black stars) and at the other to a microsphere, is stretched by acoustic forces acting on the microsphere.

(b) Digital camera image of a DNA-tethered polystyrene microsphere (4.5-µm diameter; DNA length, 8.4 kbp) and a silica reference microsphere (1.5-µm diameter).

(c) Theoretical acoustic energy (Eac) of the AFS device driven with a peak-to-peak voltage (Vpp) of 5 V.

G. Sitters et al, Acoustic Force Spectroscopy, *Nature Methods* (2014) **12**: 47

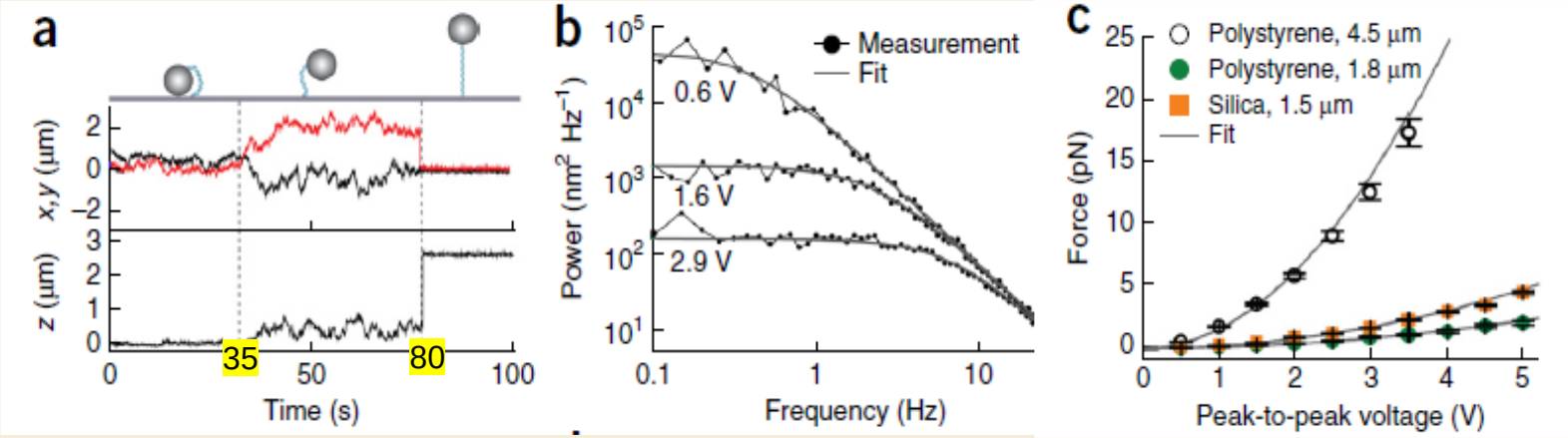


(d,e) Predicted acoustic (Ac.) pressure distribution at 6.8-MHz (d) and 9.2-MHz (e) resonance frequencies across the glass and fluid layers.

(f) Predicted forces for a 4.5-µm-diameter polystyrene microsphere directed along the z direction (0.5- W input power). Microspheres near the upper surface (z = 0) experience a force directed away from the surface at 6.8-MHz resonance and toward the surface at 9.2 MHz.

G. Sitters et al, Acoustic Force Spectroscopy, *Nature Methods* (2014) **12**: 47

AFS applied to tethered DNA.



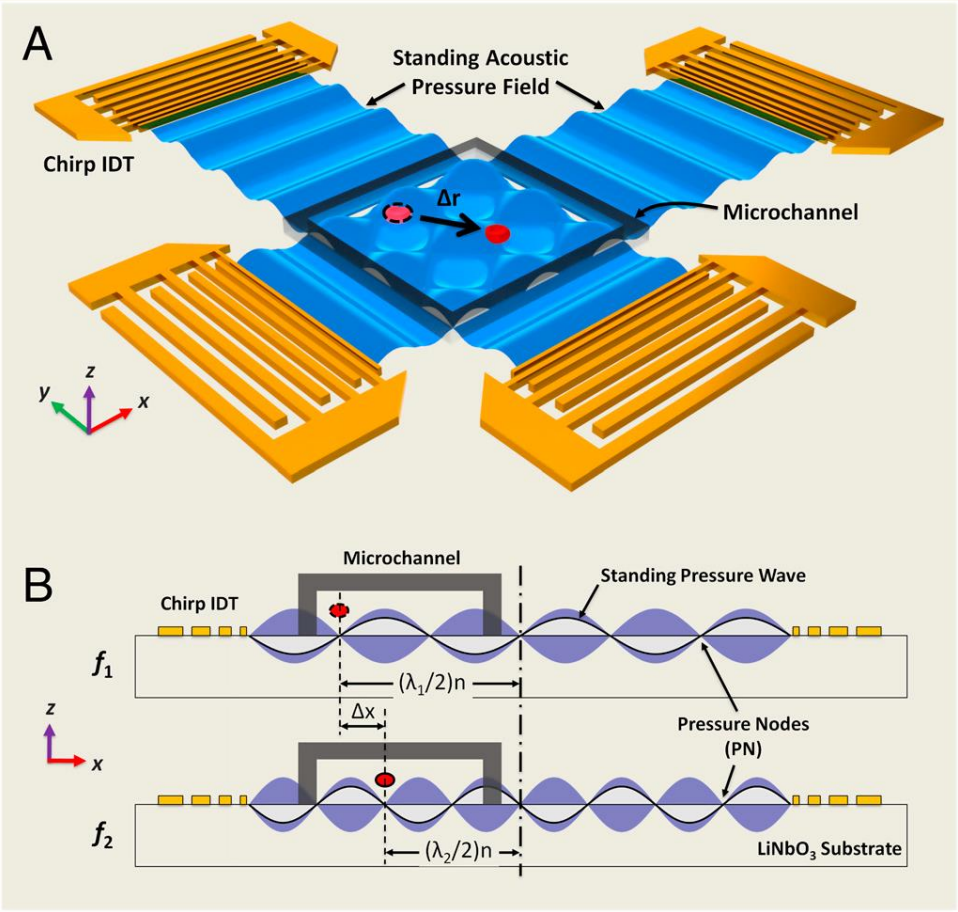
(a) Time traces, *x* (black) and *y* (red) position, of a DNA-tethered microsphere (polystyrene, diameter, 4.5 μm; DNA length, 8.4 kbp). 0- 35 s: piezo driven at 9.0 MHz (peak-to-peak voltage (*V*pp) = 0.5 V), pushing the microsphere toward the surface. 35–80 s: no acoustic force was applied; > 80 s the piezo was driven at 6.7 MHz (*V*pp = 2.4 V), pulling the microsphere away from the surface.

(b) Mean power-spectra values of the microsphere's *x* position using a Lorentzian function. Forces obtained were 0.61, 3.9 and 11.8 pN at *V*pp = 0.6, 1.6 and 2.9 V piezo-driving voltages, respectively.

(c) Forces acting on polystyrene and silica microspheres tethered to the glass surface with DNA (length, 8.4 kbp) as determined from power-spectrum analysis.

G. Sitters et al, Acoustic Force Spectroscopy, *Nature Methods* (2014) **12**: 47

On-chip manipulation of single microparticles using surface acoustic waves (SAW)



Device structure and working mechanism of the SAW

(A) Schematic illustrating a microfluidic device with orthogonal pairs of chirped IDTs for generating standing SAW.
(B) A standing SAW field generated by driving chirped IDTs at frequency f_1 and f_2. When particles are trapped at the n_{th} pressure node, they can be translated a distance Δx:

$$\Delta x = n\,(\Delta\lambda / 2) = n(c/f_1 - c/f_2)/2$$

by switching from f_1 to f_2.

This relationship indicates that the particle displacement can be tuned by varying the pressure node where the particle is trapped.

IDT – Inter Digital Transducer

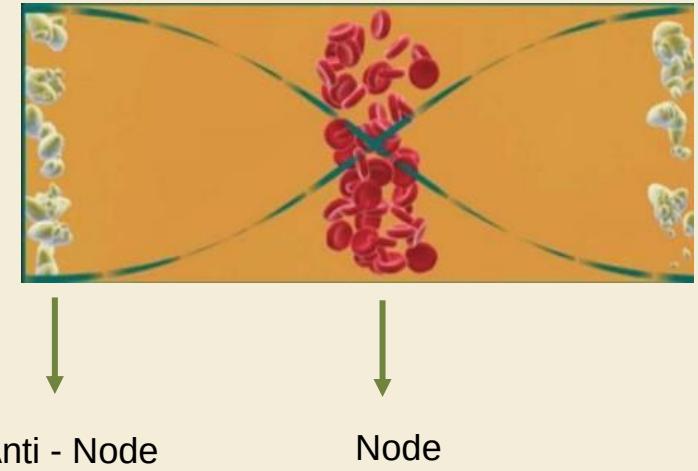
X. Ding et al,, PNAS 109, 11105 (2012).

Single particle manipulation in 2D, by SAW.

10 um polystyrene bead.

X. Ding et al,, PNAS 109, 11105 (2012).

Separation of lipid particles
(formed by triglycerides leaking from cells)
from erythrocytes



J. Shi, Lab Chip 9, 3354 (2009)

ARTICLE

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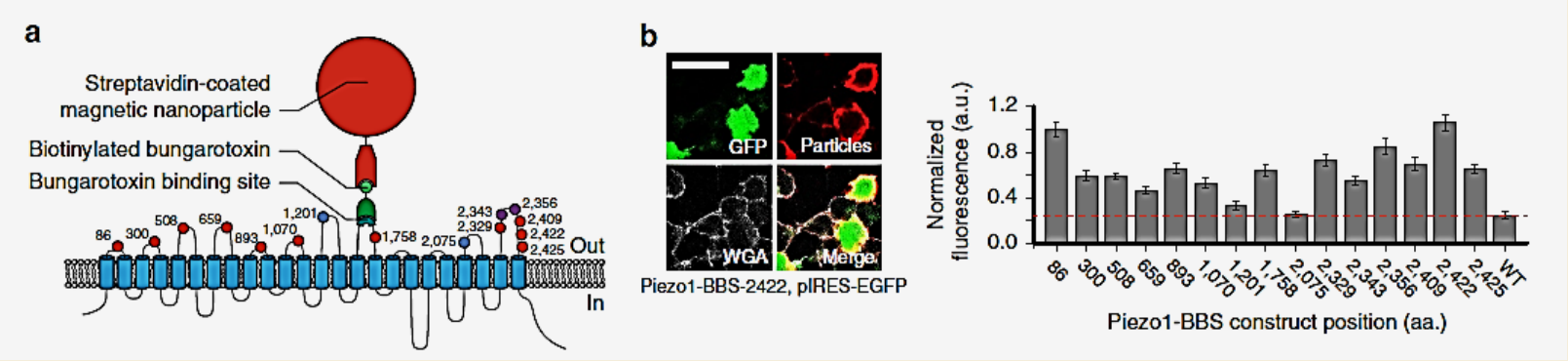
DOI: 10.1038/ncomms12939 OPEN

Localized force application reveals mechanically sensitive domains of Piezo1

Jason Wu[1], Raman Goyal[1] & Jörg Grandl[1]

.... we use magnetic nanoparticles as localized transducers of mechanical force in combination with pressure-clamp electrophysiology to identify mechanically sensitive domains important for activation and inactivation.



Localized force application by nanoparticle labelling and magnetic field generation.

IT allows to bind and apply forces at different domains of the protein !

(a) Piezo1 transmembrane topology with aa. locations of BBS insertions (red, labelled and functional; blue, non-labelled; magenta, non-functional) and schematic of bead labelling strategy.

(b) Representative images of HEK293T cells expressing Piezo1-BBS-2422-pIRES-EGFP construct, live-labelled with streptavidin-coated nanoparticles, immunostained against streptavidin, and labelled with WGA to confirm membrane localization (green, GFP; red, anti-streptavidin; grey, WGA). Mean fluorescence intensity normalized to BBS-86 (a.u.) of nanoparticle labelling along the cell membrane for all constructs compared with wild-type Piezo1 (WT, red line) (n¼10 cells per transfection, 2–5 transfections; Po0.0001 for all constructs except BBS-1201 and BBS-2075 (P40.01), one-way ANOVA and NP multiple comparison).

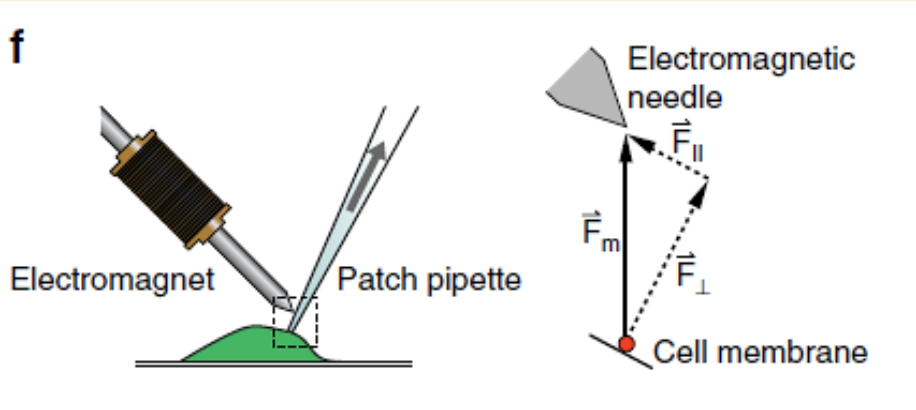
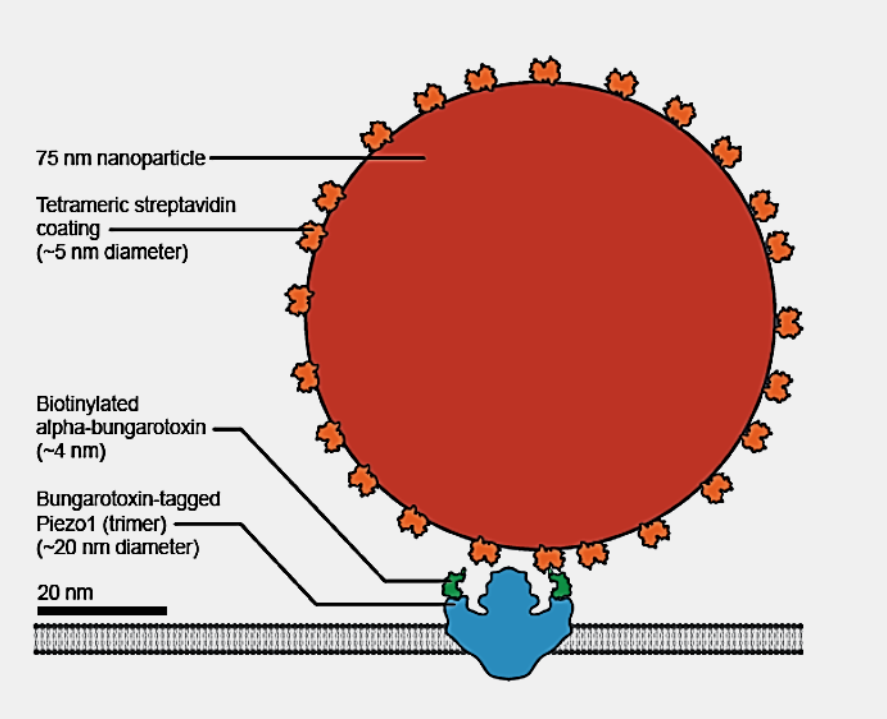


Diagram of patch-clamp pipette and electromagnetic needle

and corresponding force diagram on nanoparticle (Fm, magnetic force vector; F>, force vector normal to patch membrane; F||, force vector parallel to patch membrane).



Scale diagram of BBS-Piezo1 binding complex.

Diagram depicting to-scale relationships of BBS-Piezo1, biotinylated bungarotoxin, and streptavidin-coated nanoparticle in complex.

Try to understand and explain:

The experimental approach to apply forces with EM needle, fluorescence imaging and patch clamp to measure ionic currents.

Force spectroscopy - single molecule

Single molecule methods can provide detailed information about molecular mechanisms and interactions, complementing bulk assays with additional information that is often difficult or impossible to observe using traditional methods Single Molecule Biology https://doi.org/10.1016/j.molcel.2006.10.017

By analyzing the behavior of individual particles, single molecule methods provide access to details of a population's characteristics that are obscured by ensemble averaging.
For instance, these methods allow direct observation of:

- molecular sub-populations
- short-lived or transient states
- rare molecular events
- non-uniform kinetics
- mechanical and thermodynamic information about mechanochemical events in individual molecules

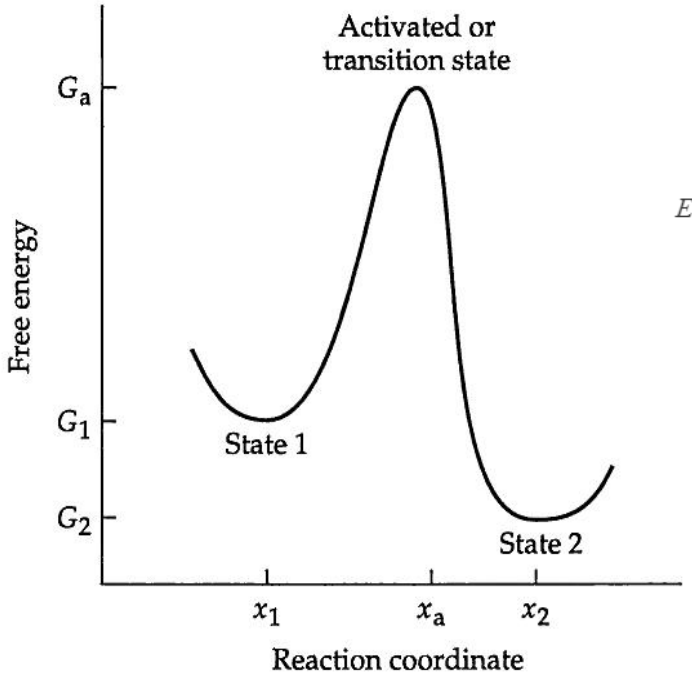
Zlatanova, Mol. Cell. 2006 Single Molecule Biology https://doi.org/10.1016/j.molcel.2006.10.017

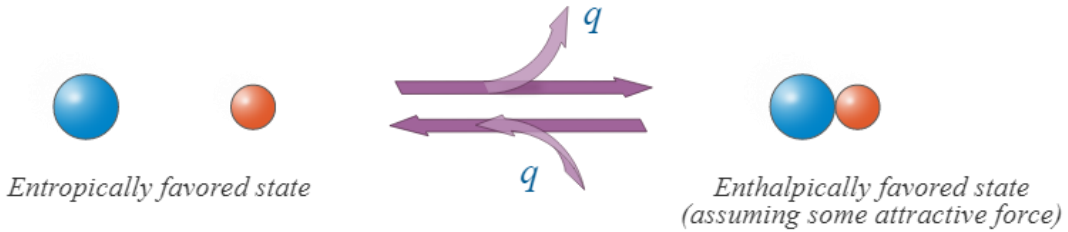
L'energia libera di Gibbs è una funzione di stato usata in termodinamica e termochimica per rappresentare l'energia libera nelle trasformazioni isotermo bariche (cioè a pressione e temperatura costante, come per la maggior parte delle reazioni chimiche), che determina la spontaneità di una reazione.

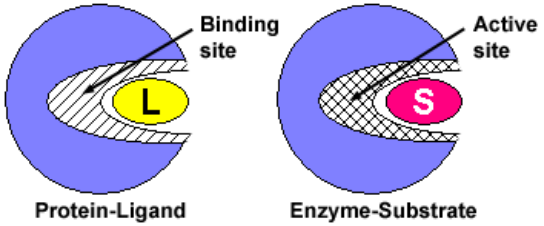
$$\Delta G = \Delta H - T\,\Delta S$$

L'entalpia H posseduta da un sistema termodinamico è una funzione di stato definita come la somma dell'energia interna U e del prodotto della pressione per il volume pV: H= U+pV
L'entropia S è una funzione di stato che viene interpretata come una misura del disordine presente in un sistema fisico qualsiasi.

Example: molecular bond – dissociation rate







Energy landscape for protein molecular transition between two structural states

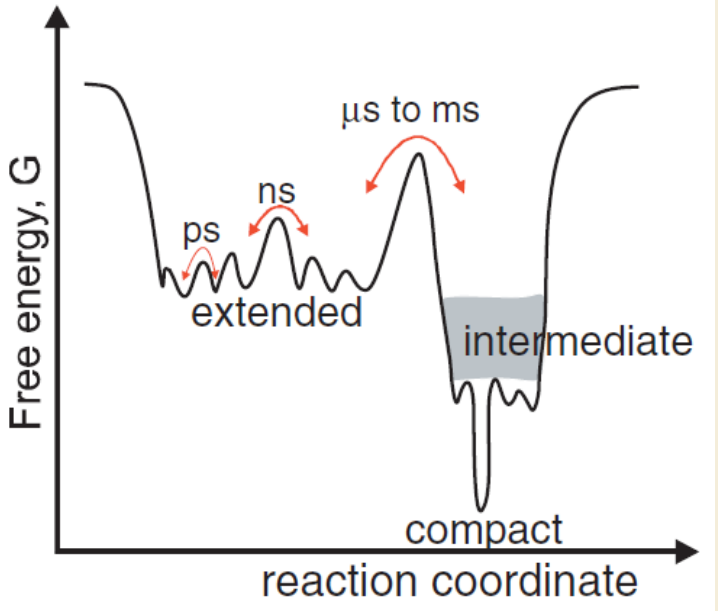
The transition between two strucutral states in a first order reaction is very fast,

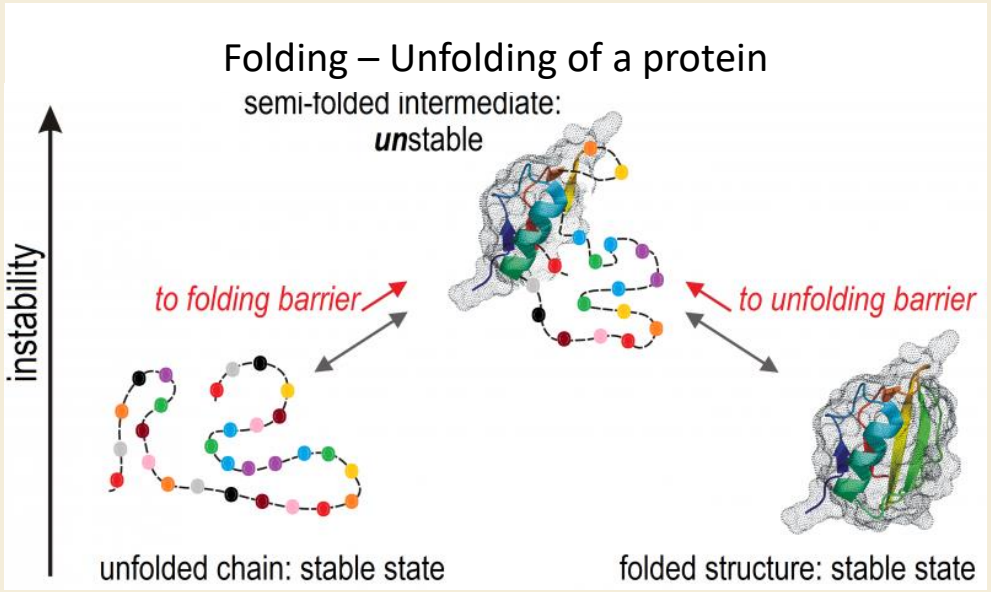
i.e. the duration of the transition is very much shorter than the average lifetimes of the states ($1/k_1$ for E1, and $1/k_{-1}$ for E2).

How fast might the transition be ?

- Covalent chemical changes : about 0.1 picosecond;
- Global conformational changes: about 10 nanoseconds;
- Lifetime of the structural states : > 1 miliseconds .

Energy landscape of protein structures and protein motion time scales





a protein's atoms intramolecular positional fluctuations result in rapid loop and side chain motions at fast time scales nanosecond (ns) and picosecond (ps)

Forward and reverse rate constants are functions of Gibbs free energy

The simplest chemical reaction is the conversion between two species that satisfies:

$$E_1 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E_2 \qquad \frac{d[E_1]}{dt} = -k_1[E_1] + k_{-1}[E_2]$$

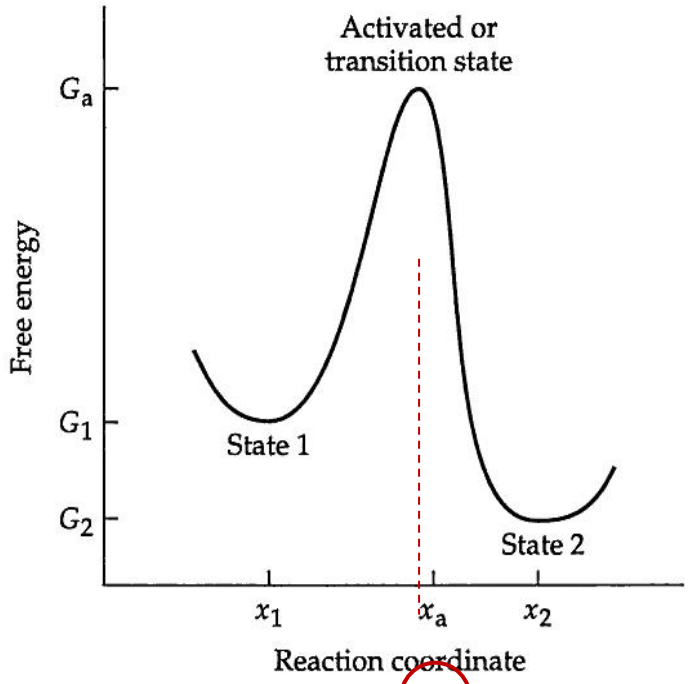
This reaction obeys first-order kinetics because the rate of change depends linearly on the concentrations of species. k_1 and k_{-1} = forward and reverse rate constants [s^{-1}]. (Association and dissociation constants)

When the reaction reaches equilibrium, ($d[E_1]/dt=0$), and:

$$\frac{k_1}{k_{-1}} = \frac{[E_2]}{[E_1]} = K_{eq} = exp\left[-\frac{\Delta G}{KT}\right]$$

The equilibrium constant $\boldsymbol{K_{eq}}$, defined with the concentrations, is equal to the ratio of forward and reverse rate constants.

If the free energy difference between product and reactant ΔG depends on the force, then either the forward or the reverse rate (or both) must depend on force.



Some properties of the first-order reactions can be understood using the idea that the reaction proceeds via a high-energy **activated state**, or **transition state**.

The activated state corresponds to a position **(x_a)** in the reaction coordinate, intermediate between the initial (x_1) and final (x_2) positions.

Assumptions:

- the reactant is in equilibrium with the activated state
- The activated state is equally likely to break to reactant or product with some rate A.

$$k_1 = A\ exp\left[-\frac{\Delta G_{a1}}{KT}\right] \qquad \Delta Ga_1 = G_a - G_1$$

Arrhenius equation; *A*- frequency factor

The force can change the rate of the reaction

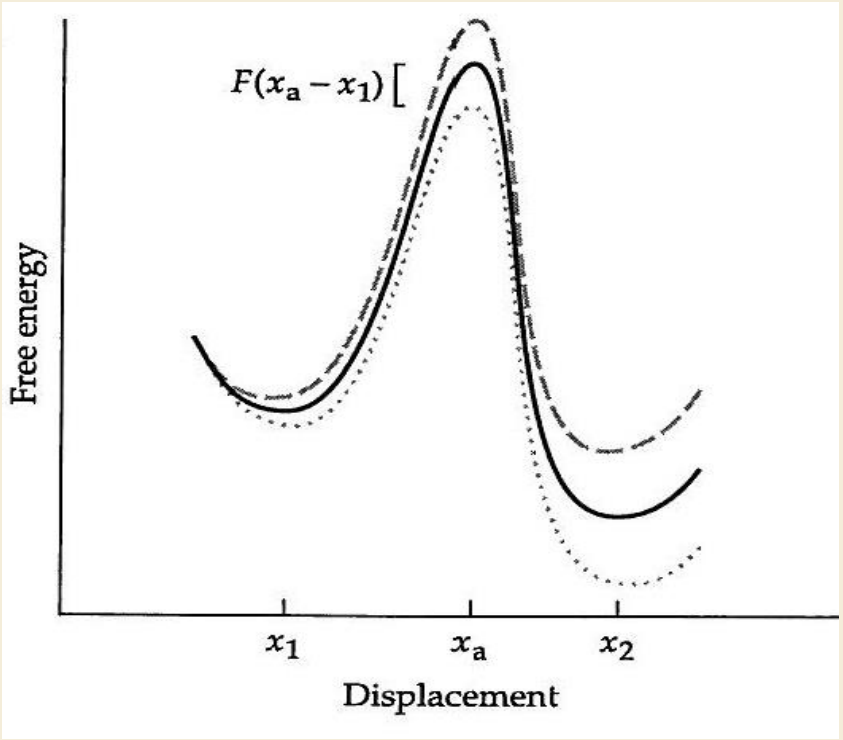
If the state transitions $E_1 \rightarrow E_a \rightarrow E_2$ are associated with displacements x1, xa, and x2 in the direction of the force, F, then the energies of the states will be decreased by Fx1, Fxa, and Fx2, respectively.

This implies:

$$k_1 = A\exp\left[-\frac{\Delta G_{a1} - F\Delta x_{a1}}{kT}\right] = k_1^0 \exp\left[\frac{F\Delta x_{a1}}{kT}\right]$$

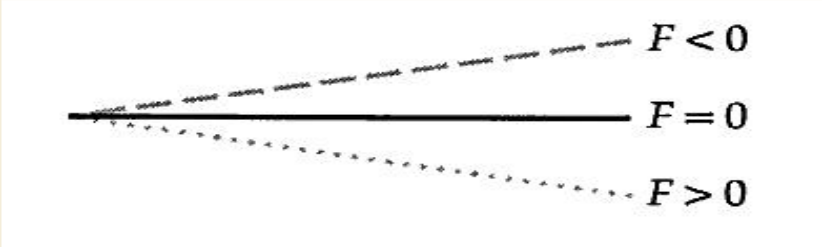
where $\Delta G_{a1} = G_{a1} - G1$ and $\Delta x_{a1} = X_{a1} - x_1$.

An analogous expression holds for k_{-1}.



The effect of force on the reaction rates:

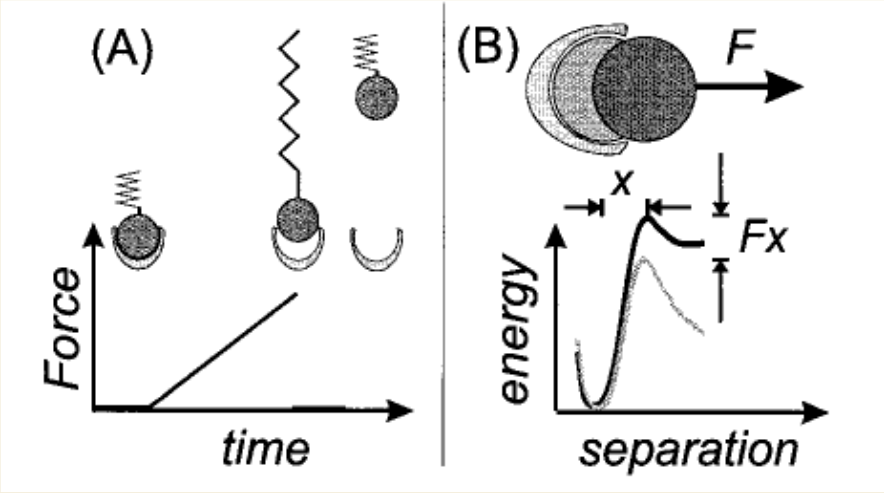
tilting the free energy diagram.



Rate theory of chemical reactions and the effect of force on the chemical rate constant

How to determine the dissociation rate of a ligand – receptor bond using force

Model and design experiment



(A) Direct observation of the dissociation under a mechanical force. The force on a single complex increases until it dissociates. The dissociation is monitored by an abrupt relaxation of the macroscopic spring of a force probe.

(B) The dissociation over a sharp energy barrier is characterized by a decrease of the barrier, giving rise to a characteristic length scale *x*.

Strunz et al, Model Energy Landscapes and the Force-Induced Dissociation of Ligand-Receptor Bonds, Biophys.J 79 (2000) - model

The goal is to determine the dissociation rate k_{off}

Eyring theory → Reaction rate without force

$$k_1^0 = \frac{KT}{h}\, exp\left[-\frac{\Delta Ga_1}{KT}\right]$$

$k_1^0 = k_{off}$ – dissociation rate without force

(1) $k_1 = k_{off}\, exp\left[\frac{F \cdot x}{kT}\right]$ with $x = x_a - x_1$ – dissociation with force

Evans-Ritchie model, assumption:

(2) $F = r \cdot t$ with r – loading rate in $\left[\frac{N}{s}\right]$, t – time [s]

The stochastic nature of the dissociation events is captured by solving the master equation for the probability $N(t)$ to be in the bound state, under increasing load $F = r \cdot t$

(3) $$\frac{dN(t)}{dt} = -k_1 \cdot N(t)$$

This results in a distribution of unbinding forces:

(4) $$P(F) = \frac{k_1(F)}{r}\, N\left(\frac{F}{r}\right)$$

In the limit of large statistics, the distributions of rupture times and forces follow a first-order (Markov) process where time and force are tied together through the loading dynamics.

Evans, Annu. Rev. Biophys Niomol Struct. 2001. 30:105-27; Strunz *et al*, Biophys.J 79 (2000)

(5) $$P(F) = \frac{k_{off}}{r} \cdot \exp\left\{\frac{Fx}{kT} + k_{off}\frac{kT}{R}/\left(1-\exp\frac{Fx}{kT}\right)\right\}$$

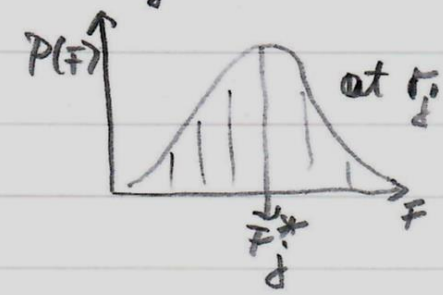
(6) The most probable unbinding/dissociation force

$$F^* = \frac{kT}{x}\ln\left(\frac{x}{kT}\frac{r}{k_{off}}\right) \qquad \frac{kT}{x} = 2$$

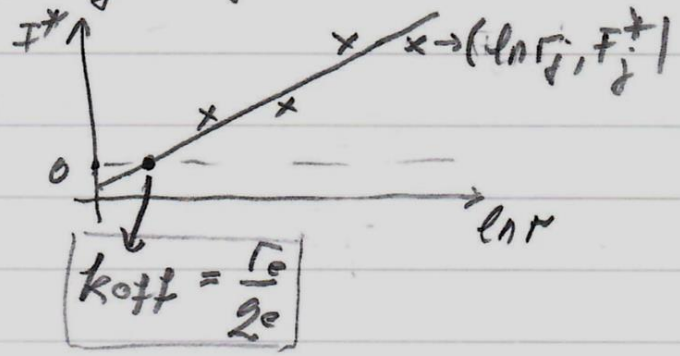
(7) $$F^* = 2\ln\left(\frac{r}{2\cdot k_{off}}\right) = 2\ln r - 2\ln(2\cdot k_{off})$$

F^* linear with $\ln r$

F_j^* measured for loading rate r_j, $j = 1 - N_{experiments}$



$j = 1 - N_{exp}$

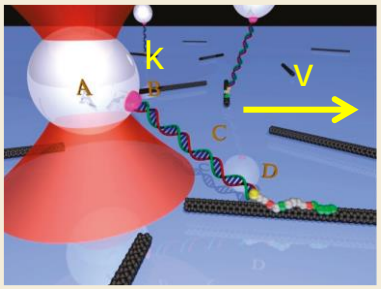


$$k_{off} = \frac{r_e}{2e}$$

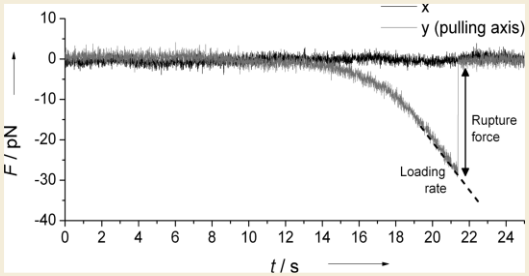
Strunz et al, Model Energy Landscapes and the Force-Induced Dissociation of Ligand-Receptor Bonds, Biophys.J 79 (2000)

Use the force to measure unbinding forces and dissociation rate k_{off} of Ligand to Receptor

How it works in practice ?



1. We need a tool to exert force ($F = k_{probe}\, x$): AFM, OT, MT, AT; the choice depends on the strength of the bond. The probe exert force on the ligand –receptor bond.
2. We need linkers to connect the ligand with the probe (e.g. OT bead, AFM tip) because the probe is much bigger than the ligand molecule.



* Measurement procedure:

1. Pull the "construct" (ligand + linker) with a force F:

$F = r \cdot t$, r - load rate $\left[\frac{N}{s}\right]$, t - time [s]

e.g. for OT: stiffness of the trap $k_{OT} = 0.001 - 0.5 \left[\frac{pN}{nm}\right]$ $F = k \cdot x_B$

Force is applied by moving the ligand (fixed substrate) with a constant velocity $\vec{v}$; $\vec{v} = 20 - 2000 \left[\frac{nm}{s}\right]$

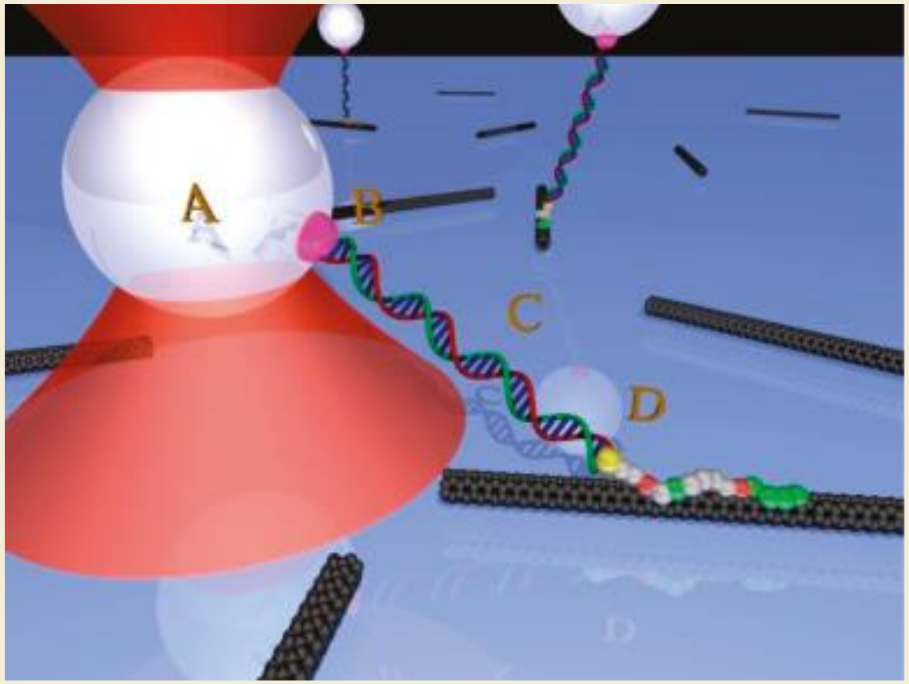
The load rate, r, will be then $r = \vec{v} \cdot k$

r= 0.02 – 1000 [pN/s]

Aubin-Tam et al, Adhesion through single peptide aptamers dx.doi.org/10.1021/jp1031493 J. Phys. Chem. A 2011

How the experiment is developed

Schematics of optical tweezers pulling on a single peptide aptamer molecule linked to a carbon nanotube.



The optical trap captures a bead (A) that is linked to an aptamer (D) via a DNA molecule (C) and a biotin/streptavidin linkage (B).

Adhesion through Single Peptide Aptamers

Aptamers are biomolecules with specific binding affinity, enabling applications in sensing, diagnostic, drug delivery, imaging, and therapy.

Peptide aptamers typically contain 8-20 amino-acids and bind materials or biomolecules.

They can be engineered via selection from large libraries of random sequences ($\sim 10^{10}$) by directed evolution techniques such as phage display.

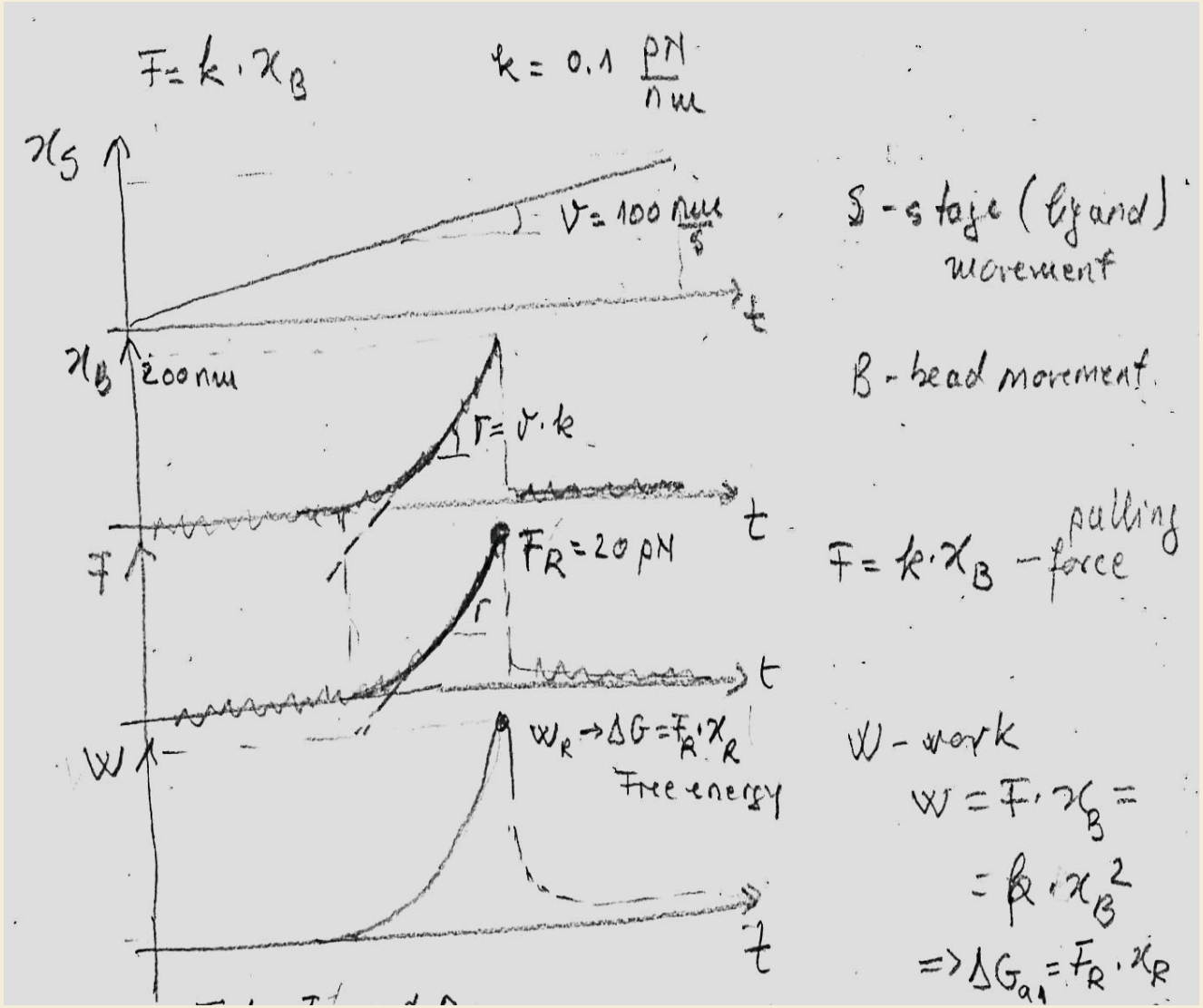
Aubin-Tam et al, Adhesion through single peptide aptamers

dx.doi.org/10.1021/jp1031493 |J. Phys. Chem. A 2011

2)

Force ramp approach

Single experiment



The force F increases with time t: F(t)= r t

Force is calculated measuring the displacement x_B: F(xB) = k x_B

The load rate r = v k and is the slope of the tangent to the measured displacement of the bead.

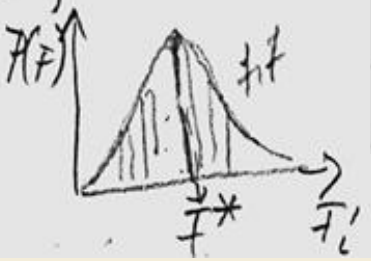
3) Take F_R and r:

→ One measurement is not enough (stochastic behavior)

→ $N \gg 50$ measurements necessary → repeat:

$$\Rightarrow \{F_R^i\}_{i=1-N}, r_1$$

plot the probability distribution $P(F)$ and determine F^*



$$P(F) = \frac{k_{off}}{r} \cdot \exp\left\{\frac{Fx}{kT} + k_{off}\frac{kT}{R}\Big/\left(1-\exp\frac{Fx}{kT}\right)\right\}$$

k_{off} *and x as free parameters*

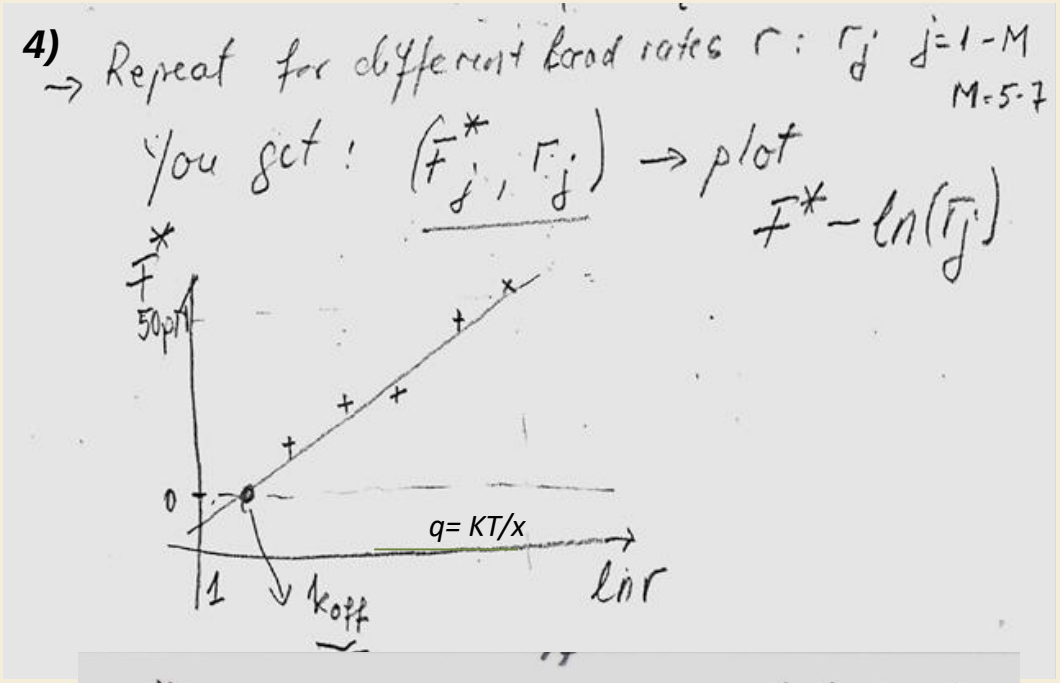
F* is found after multiple experiments

The most probable unbinding/dissociation force

$$F^* = \frac{kT}{x}\ln\left(\frac{x}{kT}\frac{r}{k_{off}}\right) \qquad \frac{kT}{x} = 2$$

Having more than one dataset at different load rates ***r*** is crucial to extrapolate the value of k_{off} in absence of load F=0, (i.e. natural thermal off-rate), that is the most relevant parameter the assay can return.

$$k_{off}(F^*) = k_{off} \cdot \exp(F^* x / KT)$$



$$F^* = 2\ln\left(\frac{r}{2\cdot k_{off}}\right) = 2\ln r - 2\ln(2\cdot k_{off})$$

F^* linear with ln r

dataset	r [nN/s]	F^* (pN)	[nm] x_{a1}	$k_{off}(F)$ [1/s]
1	10	20	0.3	0.13
⋮	⋮	⋮	⋮	⋮
j	40	50	0.2	0.21
⋮	⋮	⋮	⋮	⋮
M	80	70	0.15	0.34

fit F^* parameters k_{off}, x_{a1}

Experiments are repeated for different loads

The slope is q= KT/x

F=0* → $k_{off} = r_0 / q$

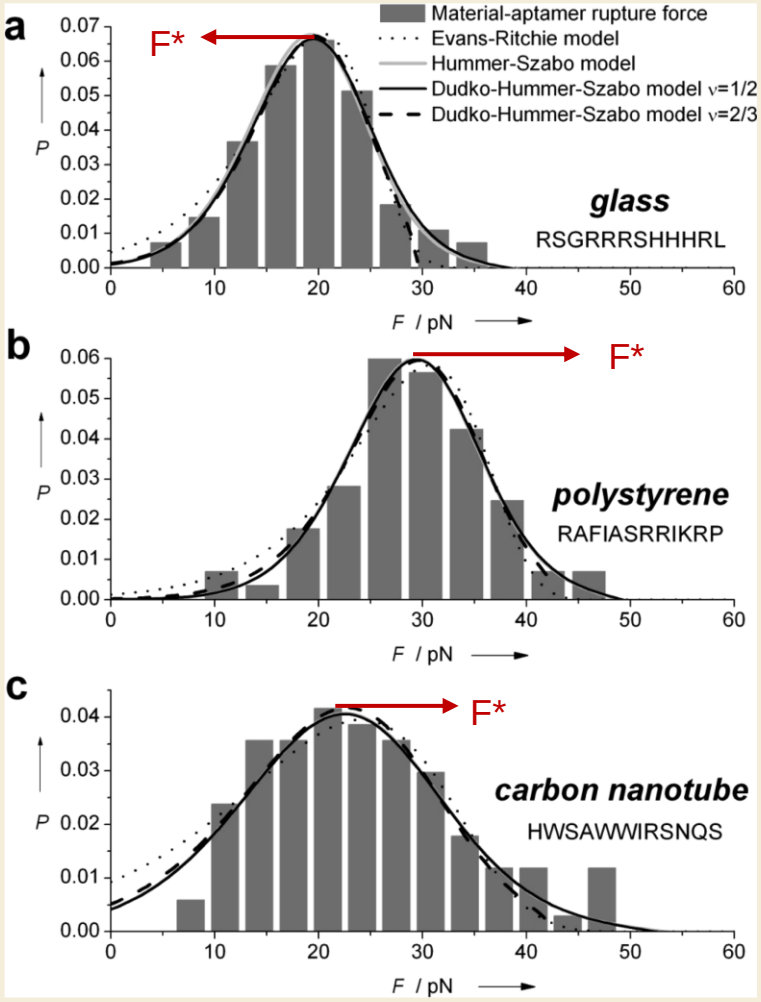
r_0 – *extrapolated load rate r for F*=0*

$k_{off}(F^*) = k_{off} \cdot \exp(F^* x / KT)$

model prediction

Rupture-force probability *P(F)* distributions

for peptide aptamer binding to :



(a) glass,

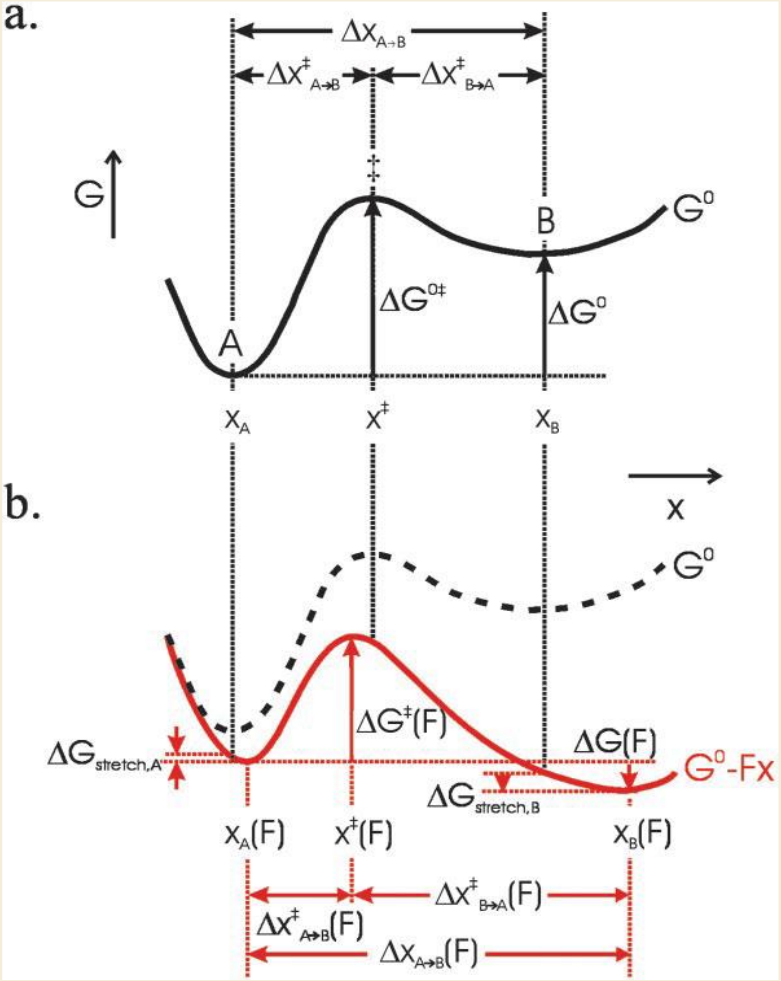
(b) polystyrene, and

(c) carbon nanotubes

$\tau_0^a = {}^1/_{k_{off}}$	Evans–Ritchie model	
interaction	$\tau_0{}^a$	$x^{\ddagger\, b}$
glass/aptamer	96.9	0.747
polystyrene/aptamer	109.5	0.652
CNTs/aptamer	20.7	0.404

[a] Units are s. [b] Units are nm. [c] $\Delta G^{\ddagger}$ is in $k_B T$ units.

The effect of force on the free energy of a two-state system, where x represents the mechanical reaction coordinate.



(a) No applied force.

(b) Red curve: positive applied force.

The application of force lowers the energy of both the transition state ‡ and state B relative to state A, which increases the rate of the forward reaction and the population of state B, respectively.

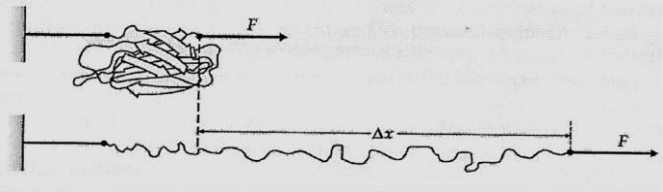
The positions of the free energy minima (x_A and x_B) and maximum ($x‡$) shift to longer and shorter x, respectively, with a positive applied force. Their relative shifts in position depend on the local curvature of the free energy surface.

The free energy change of states A and B upon stretching is $\Delta G_{stretch\ A,\ B}$

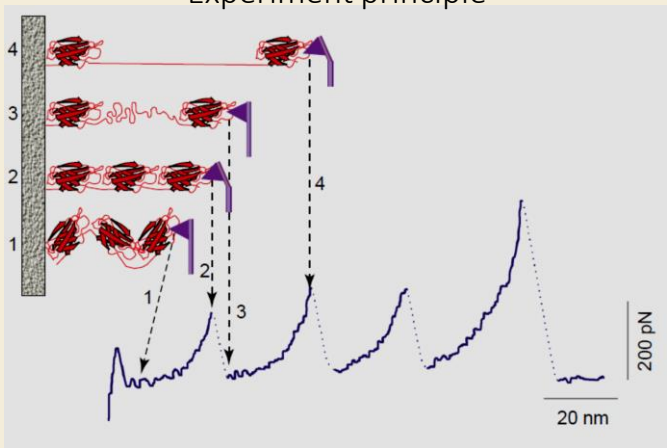
2020 Annual Review of Biochemistry Single-Molecule Studies of Protein Folding with Optical Tweezers Carlos Bustamante et all Review

Unfolding rate of a single protein domain

AFM was used to reversibly unfold immunoglobulin modules (IG27) domain. And measure the unfolding rate, which was compared with that obtained from chemical unfolding using a denaturant reaction.



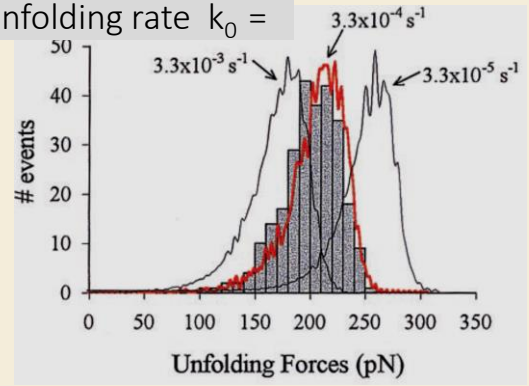
Experiment principle



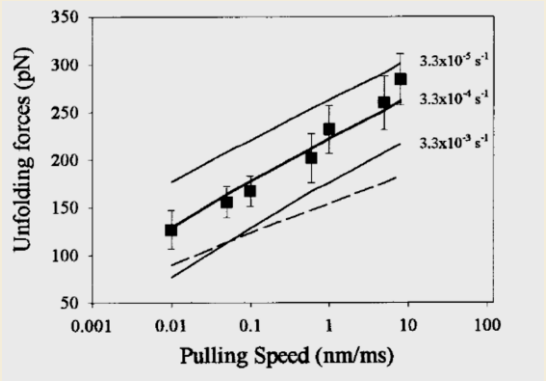
Force-extension relationship for recombinant poly(I27)

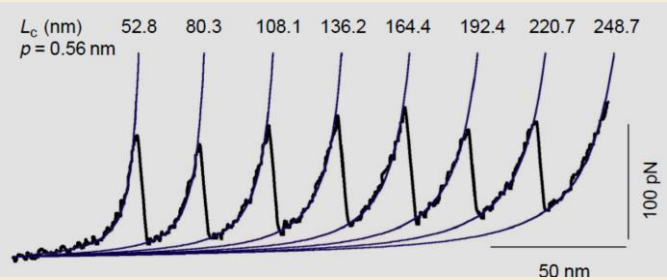
Unfolding force frequency histogram

Unfolding rate k_0 =



Unfolding force vs pulling speed





WLC model (Worm Like Chain)

$$F(x) = \frac{kT}{p}\left[\frac{1}{4}\left(1-\frac{x}{L_c}\right)^2 - \frac{1}{4} + \frac{x}{L_c}\right]$$

unfolding distance
Δx=0.25 nm,
pulling rate v = 0.6 nm/ms.

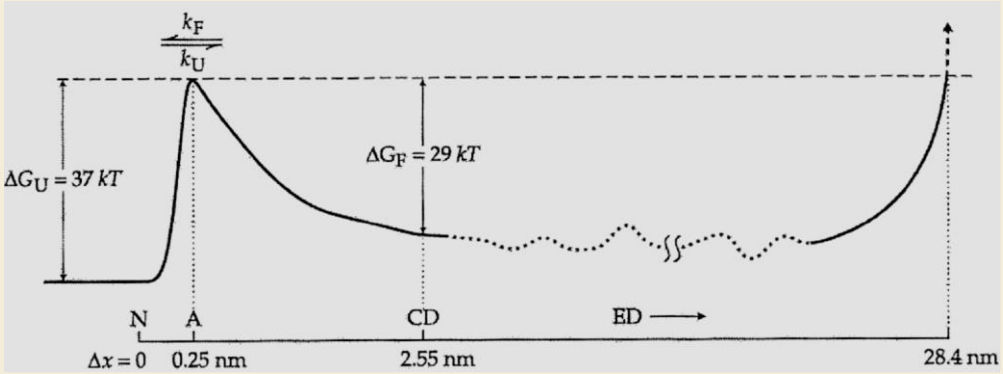
Carrion-Vasquez et al, PNAS 96:3494, 1999

Mechanical and chemical unfolding of a single protein a comparison

Chemical unfolding rate $k_0 = 4.9 \times 10^{-4}\ s^{-1}$

Unfolding rate of a single protein domain

Diagram of the unfolding pathway for an Ig domain as determined by using AFM



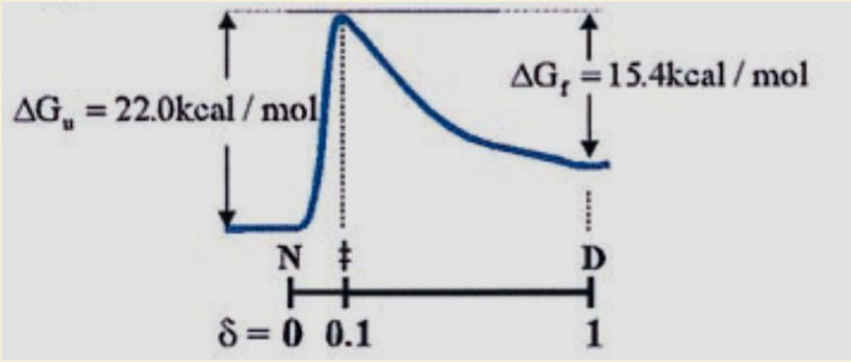
N = native state, A = activated state, CD = compact disordered state, ED = extended state

Free energies are calculated from the rates constant using Eyring rate theory.

A small strain of 0.25 nm, about 5% of the length of the folded protein (5.1 nm), is enough to completely destabilize the structure and lead to unfolding.

The folding of the protein requires the formation of a nearly fully folded transition state.

Diagram of the unfolding pathway for an Ig domain as determined by using chemical denaturants



Carrion-Vasquez et al, PNAS 96:3494, 1999

Mechanical and chemical unfolding of a single protein a comparison

Summary (very short ☺)

1. Optical, Acoustic, Magnetic Tweezers as manipulation tools without mechanical contact
2. OT, AT, MT exert forces in the range 1 – 200 pN
3. Biological applications: manipulation of cells, molecules, probes
4. Single molecule biology - force spectroscopy