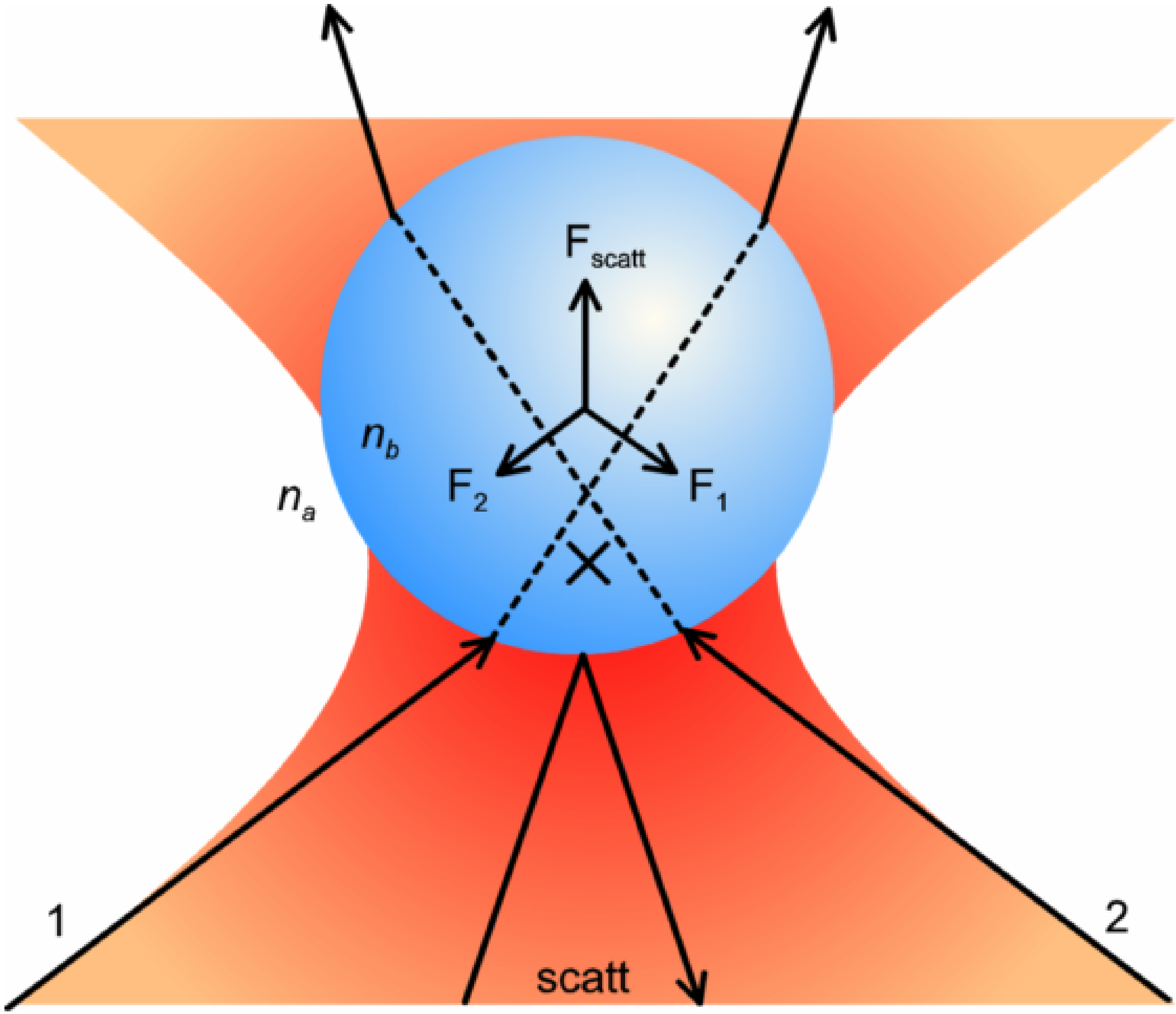
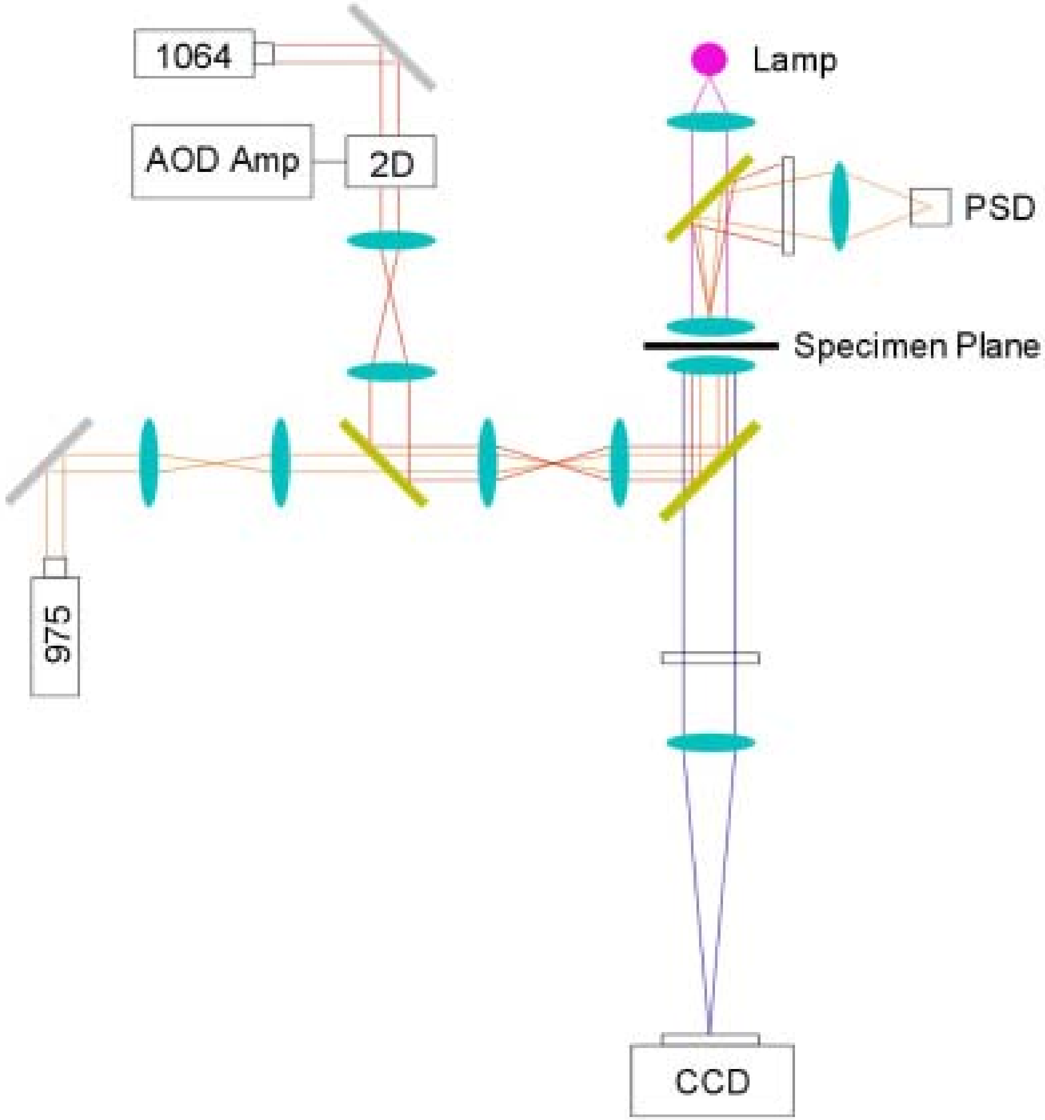


F_scatt
n_b
n_a
F_2
F_1
1
2
scatt

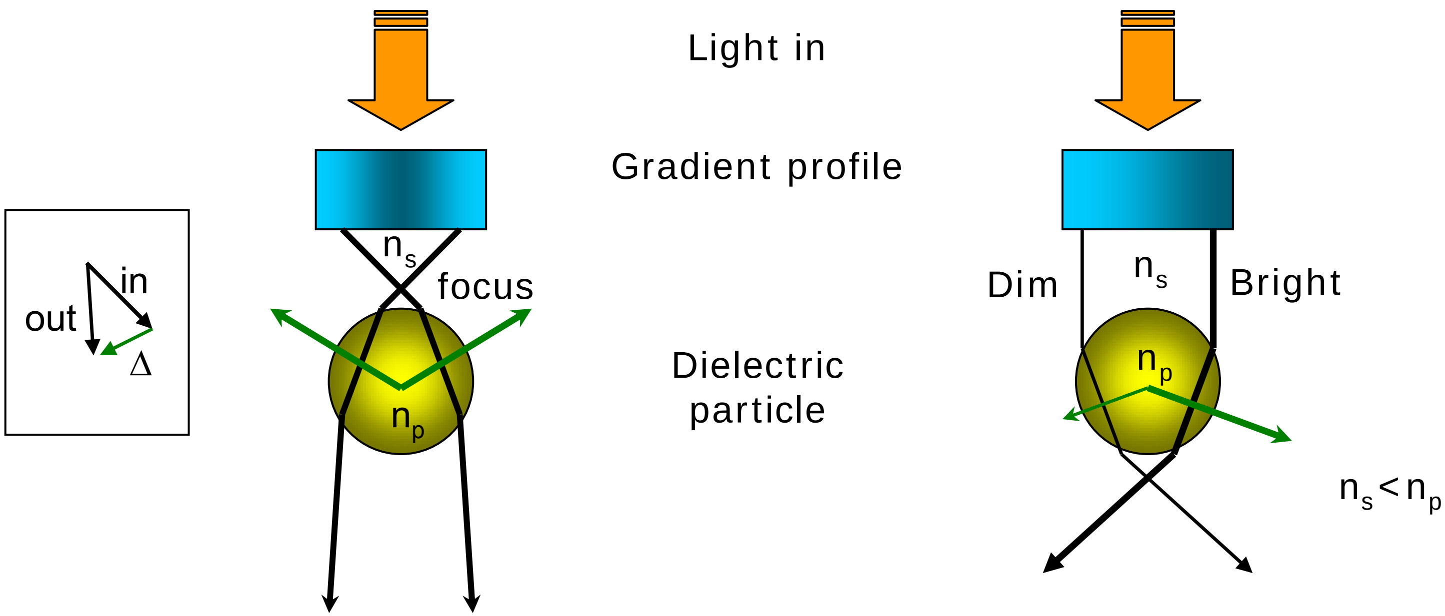
Instrumentation



Optical Trapping at MIT

Comet tails point away from the sun. the dust cloud is pushed away with Radiation pressure.

Optical Tweezers Basics



Net force will tend to pull the sphere towards the focus point

Net force will tend to push the sphere down and to the right

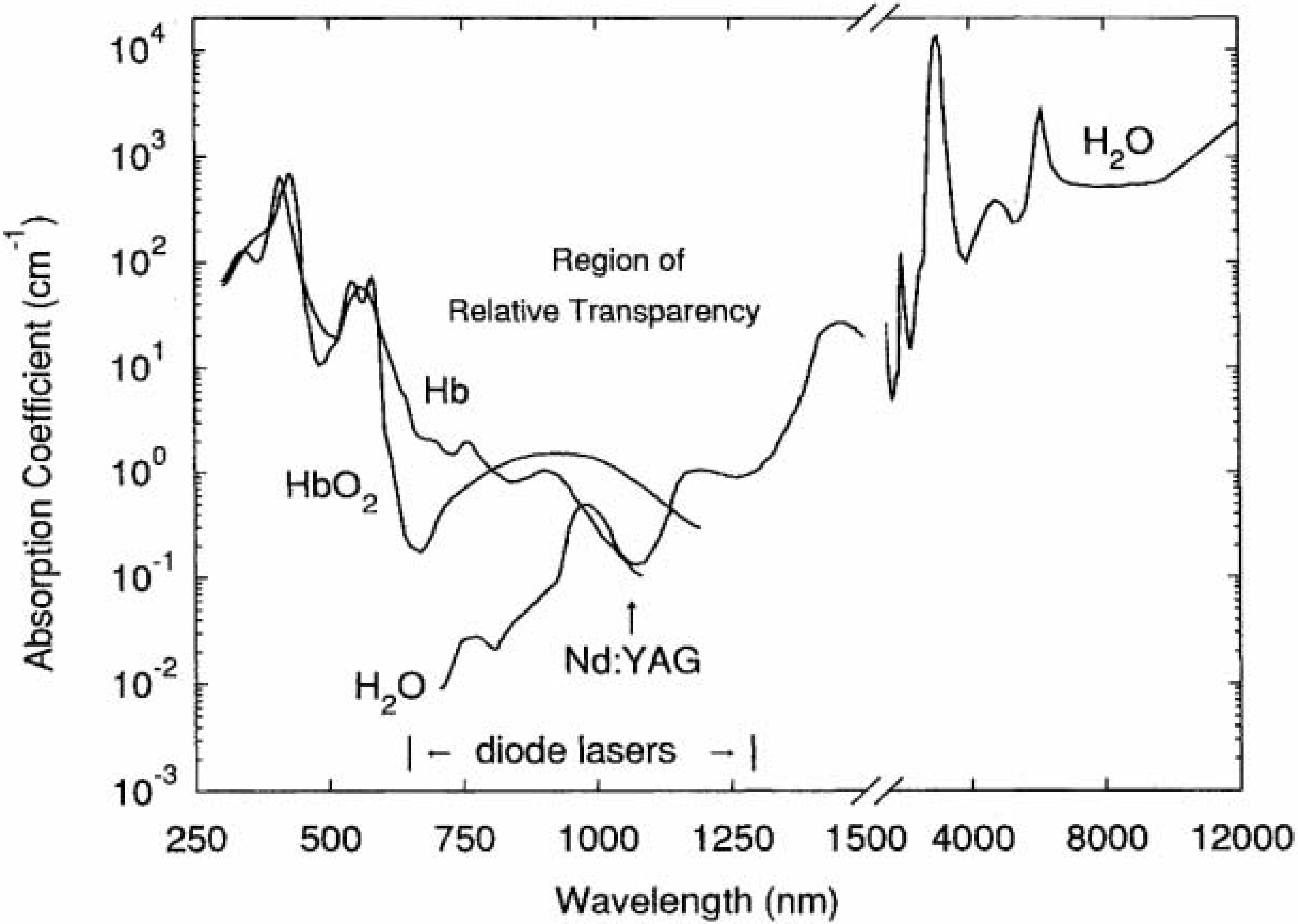
Optical tweezers

Kinesin coated silica bead
High kinesin density

Membrane viscoelasticity

Distorting
Red blood cells

Window of optical transparency

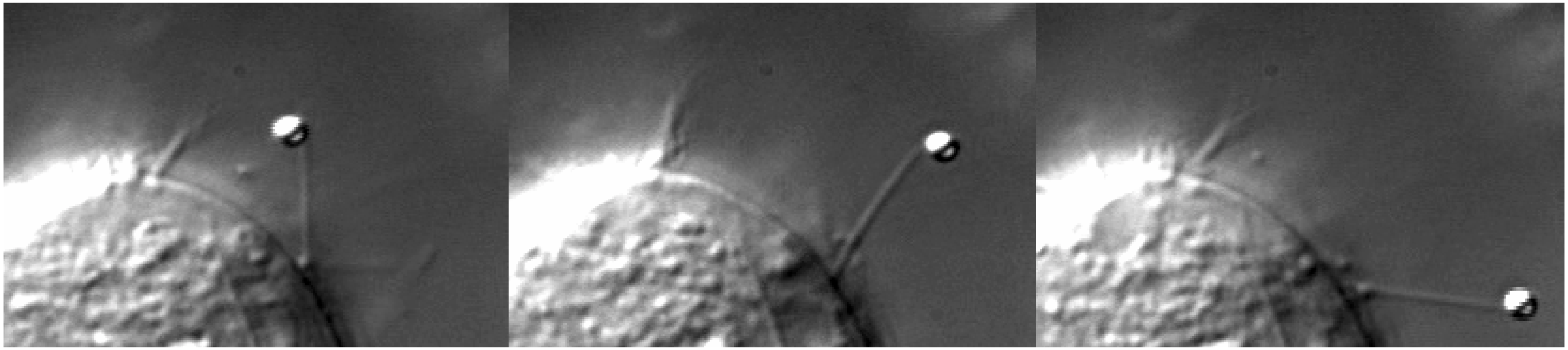


Single molecule mechanical measurements with optical tweezers

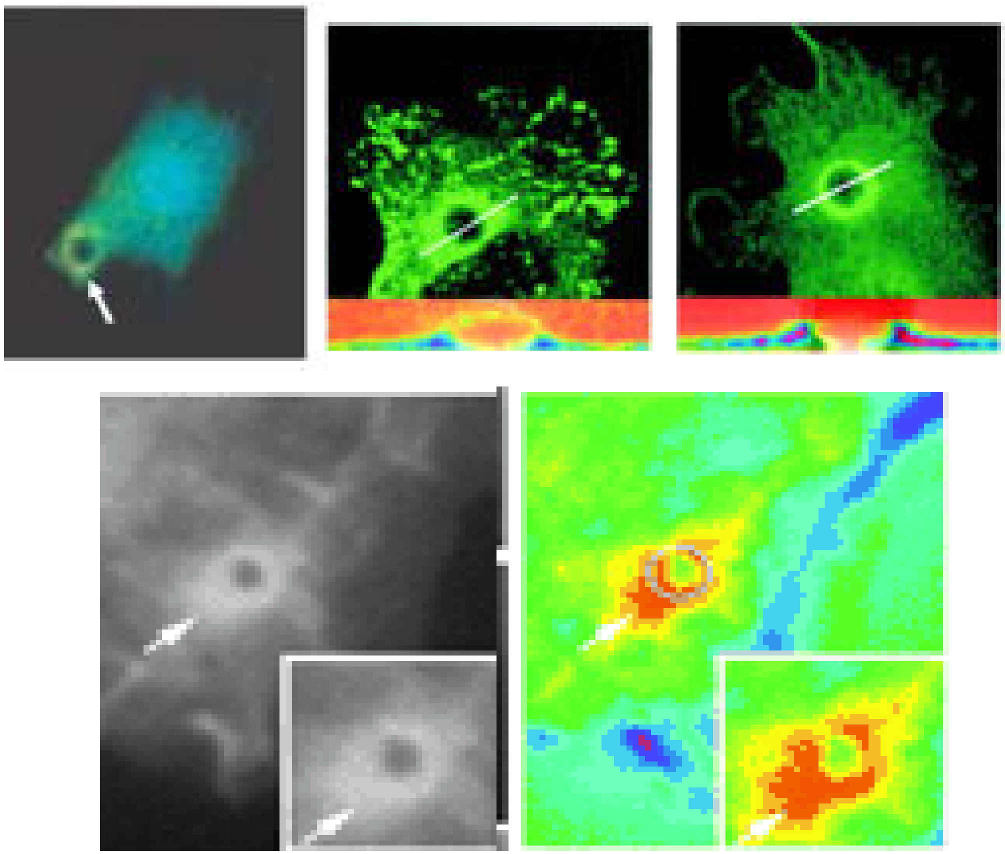
- Force resolution sub pN
- Force range to ~300pN
- Position resolution ~0.3nm
- Self-orienting
- Manipulate with light
- Non-invasive infrared light
- Can synthesize multiple traps

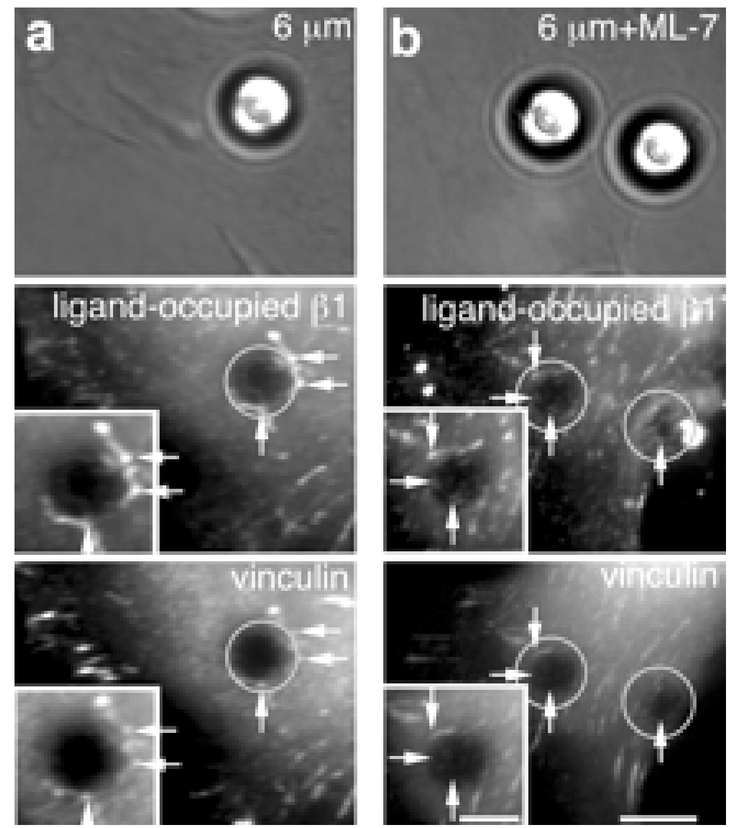
tetris

Christoph Schmidt

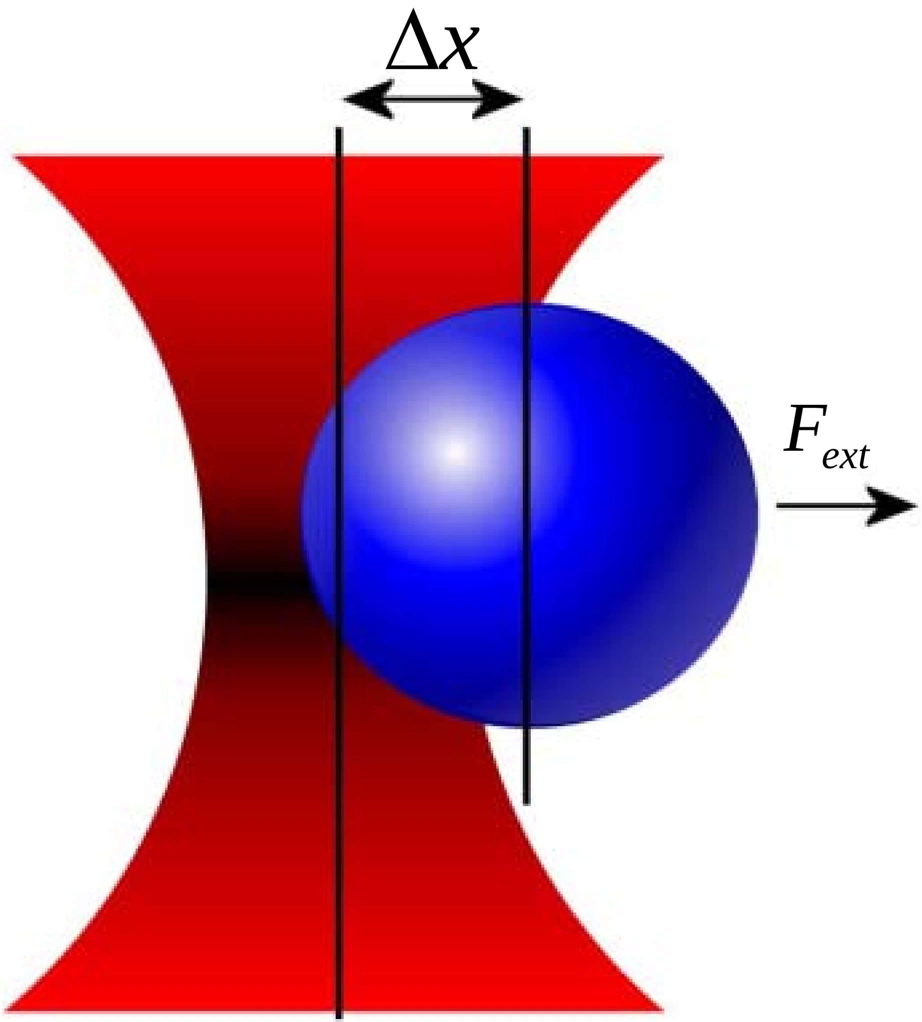


Trapping with Cells, and Fluorescence measurements





Optical Tweezers



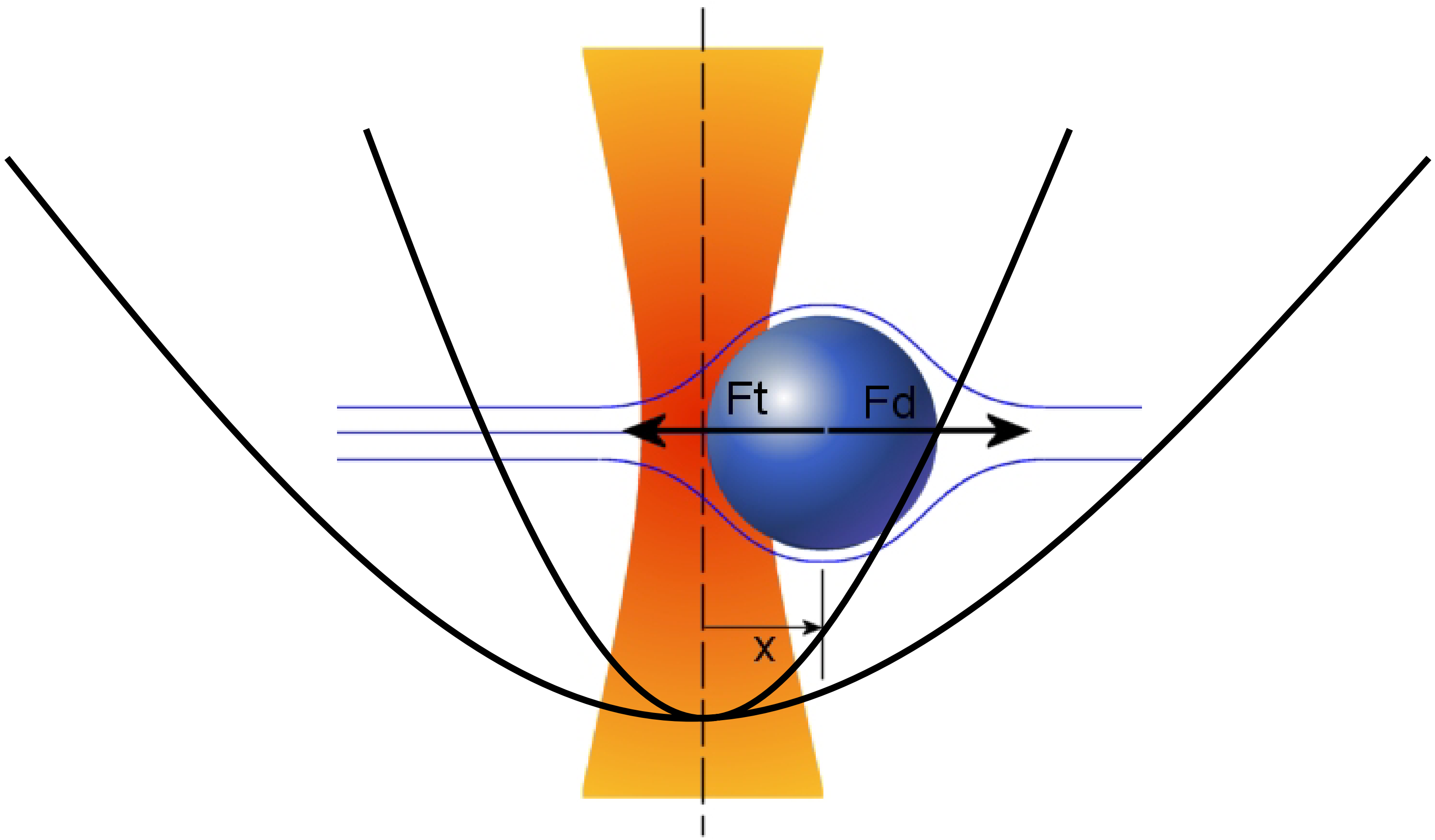
$$F_{ext} = k\Delta x$$

Calibration methods

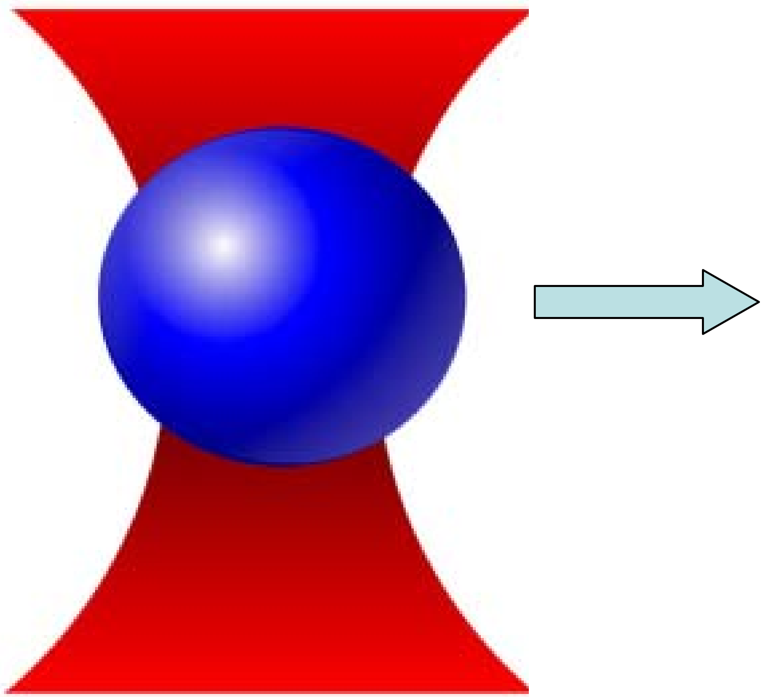
- Calibration of your detectors…
- NIST traceable piezo stage
- Video-track a particle by moving the stage
- Calibrate the video (pixel/nm)
- Move your trap, calibrate your deflector (MHz/pixel X nm/pixel)
- Trap a bead, move over the quad diode (V/MHz… V/nm)

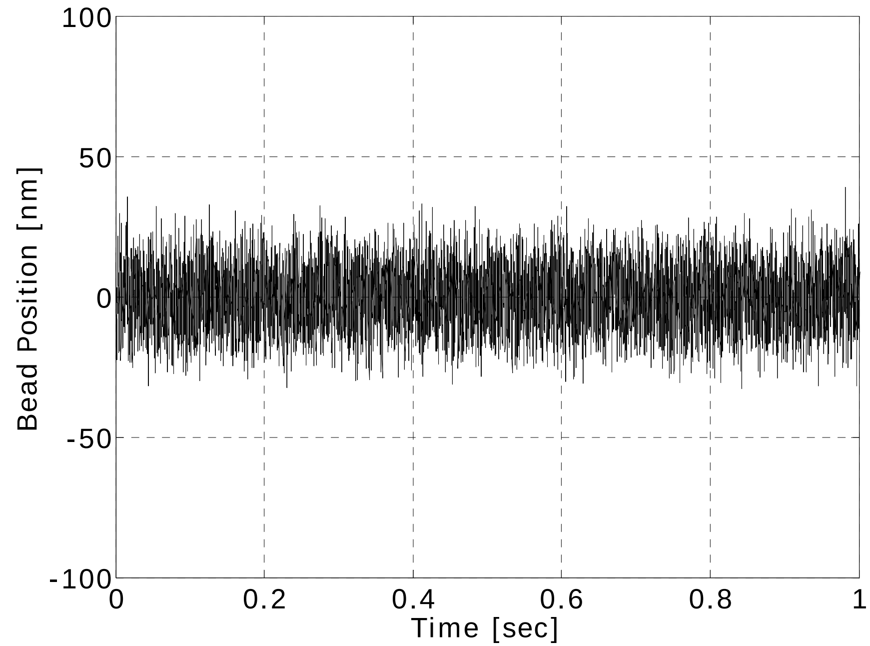
- Calibration of the stiffness of the trap…
- Stokes drag (fluid velocity flow) probes the outer edge of the trap
- (variance in position, know T) probes the center of the trap
- Frequency of changing direction (roll-off method) probes noise sources

Roll-off and Variance



Calibrating Optical Tweezers



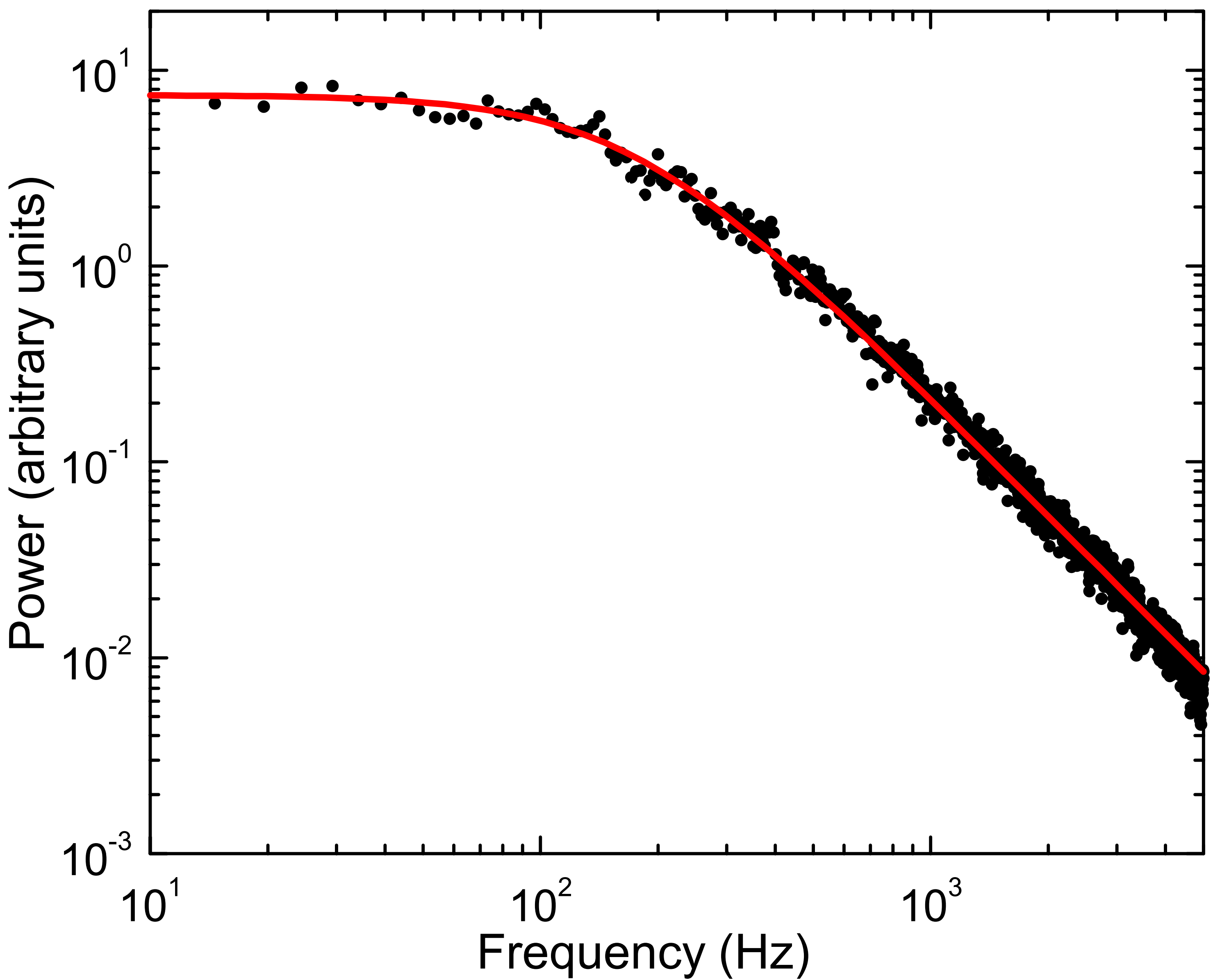


Equipartition

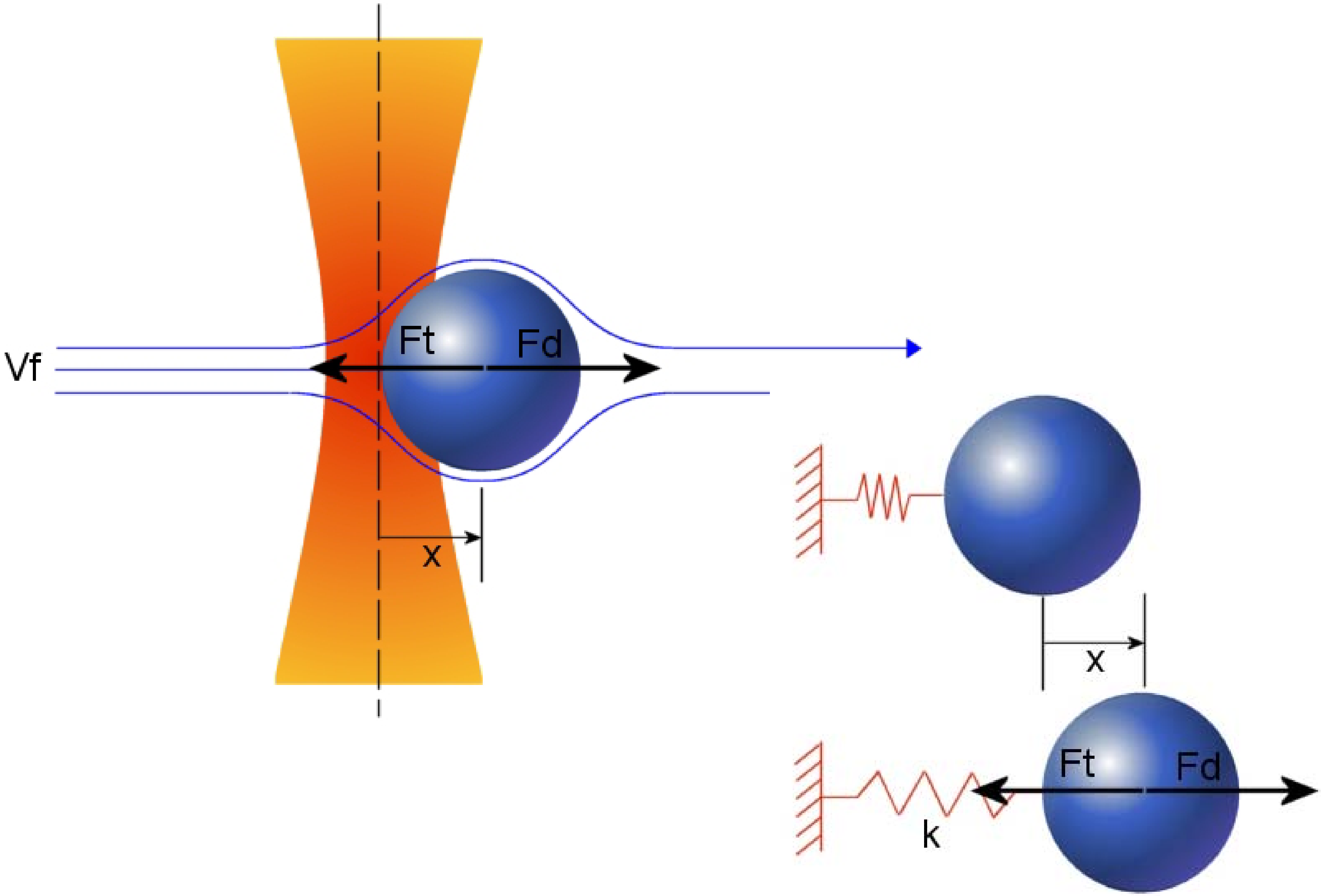
$$k = \frac{k_b T}{<(\bar{x} - x)^2>}$$

Power Spectrum

$$S = \frac{k_b T}{6\pi^3 r \eta \left(f^2 + f_c^2\right)}; k = 12\pi^2 r \eta f_c$$

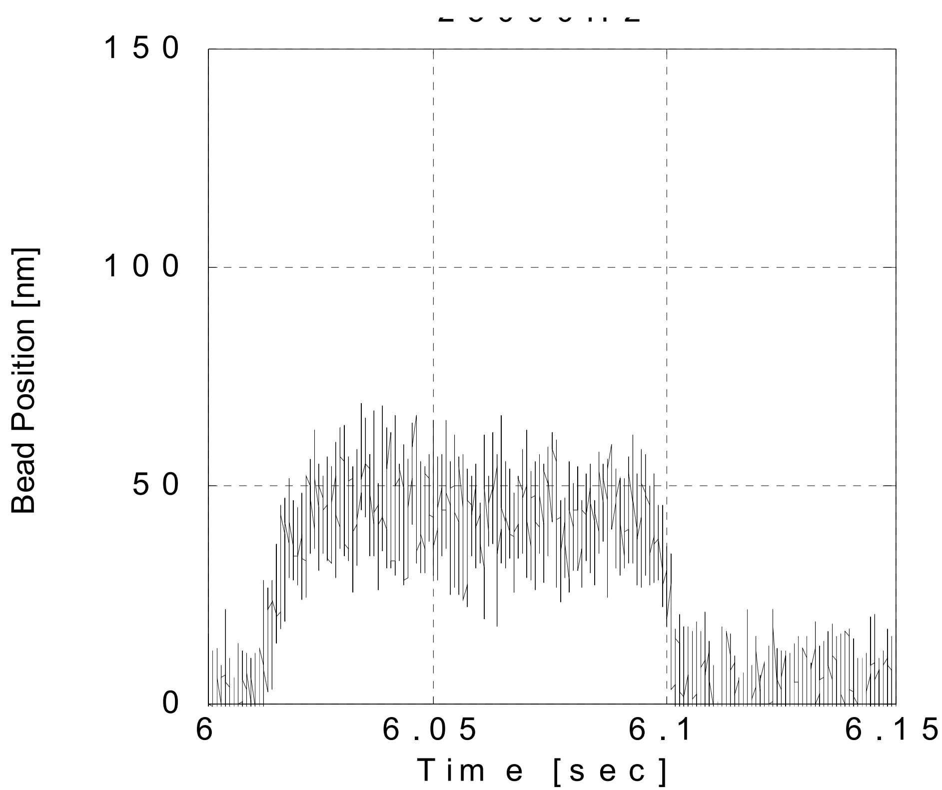


Power (arbitrary units)
10^{1}
10^{0}
10^{-1}
10^{-2}
10^{-3}
10^{1}
10^{2}
10^{3}
Frequency (Hz)



$$F_d = 6\pi\mu a v_f$$

$$F_t = kx$$


150
100
50
0
Bead Position [nm]
6
6.05
6.1
6.15
Time [sec]

Force distorts the Energy Barriers

Force: a time machine

2264 G. Bao / J. Mech. Phys. Solids 50 (2002) 2237–2274

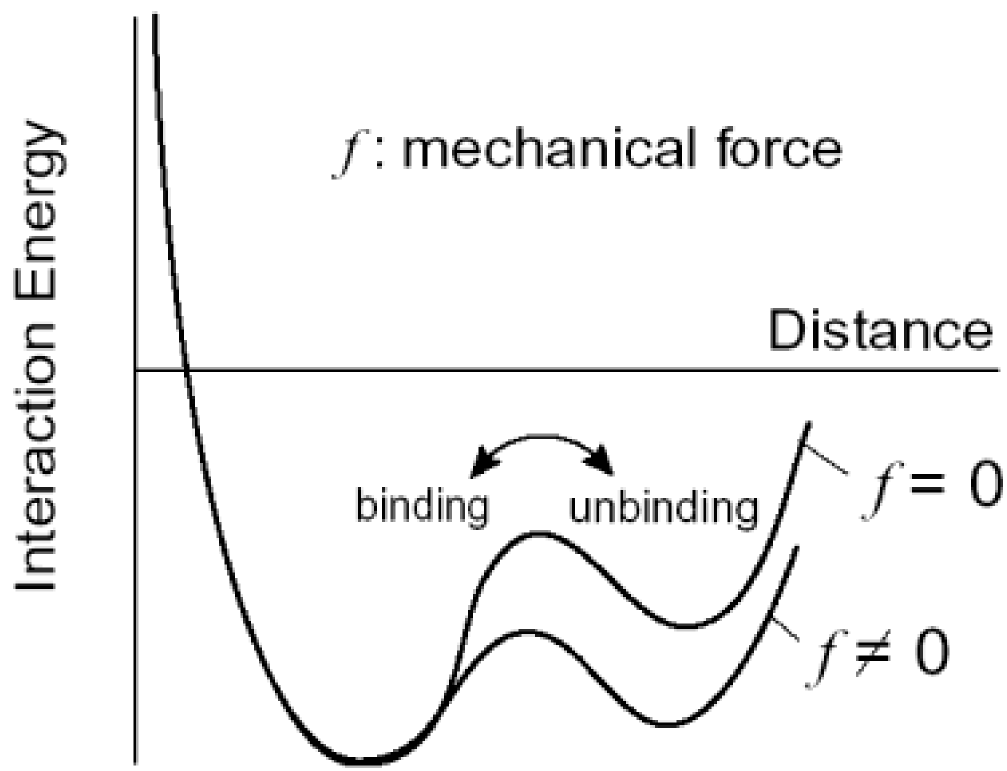
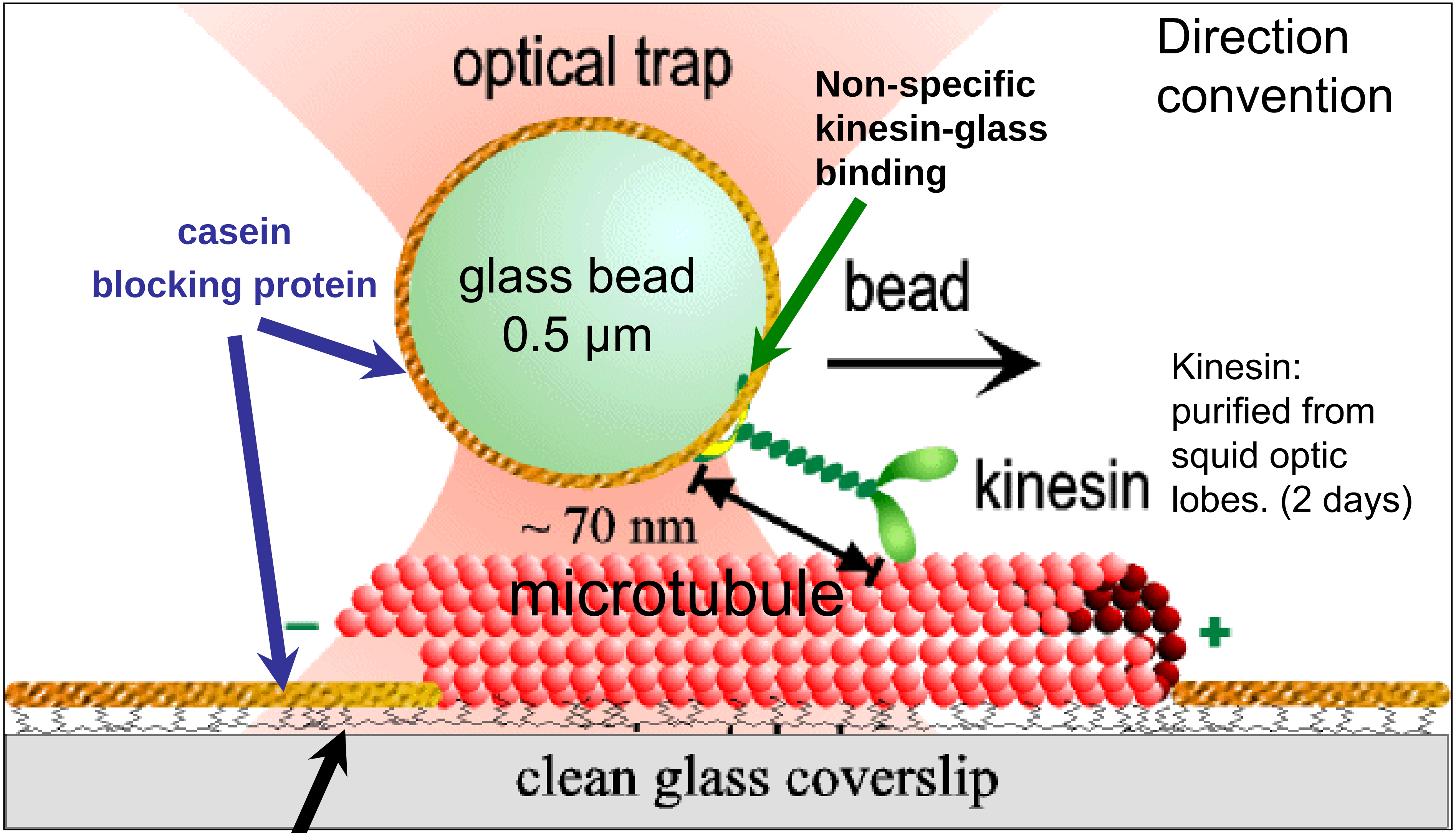


Fig. 14. The applied mechanical force can lower the energy barrier of molecular unbinding, thus influencing receptor–ligand reaction kinetics.

The single molecule assay

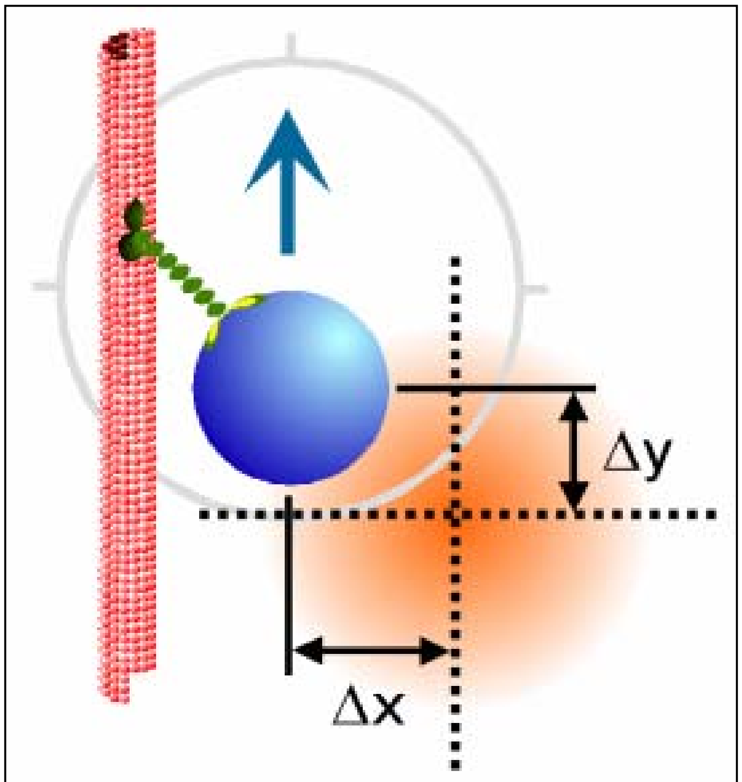


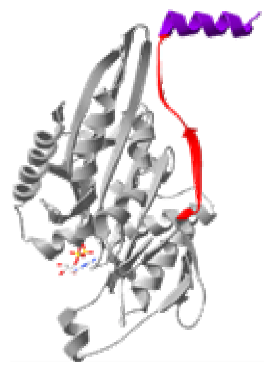
Poly-lysine to "glue" microtubules

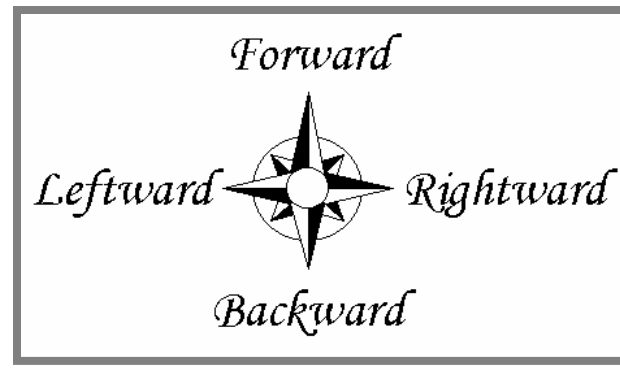
Direction convention

Kinesin: purified from squid optic lobes. (2 days)

Goal: probe kinesin's mechanochemical coupling in 2D

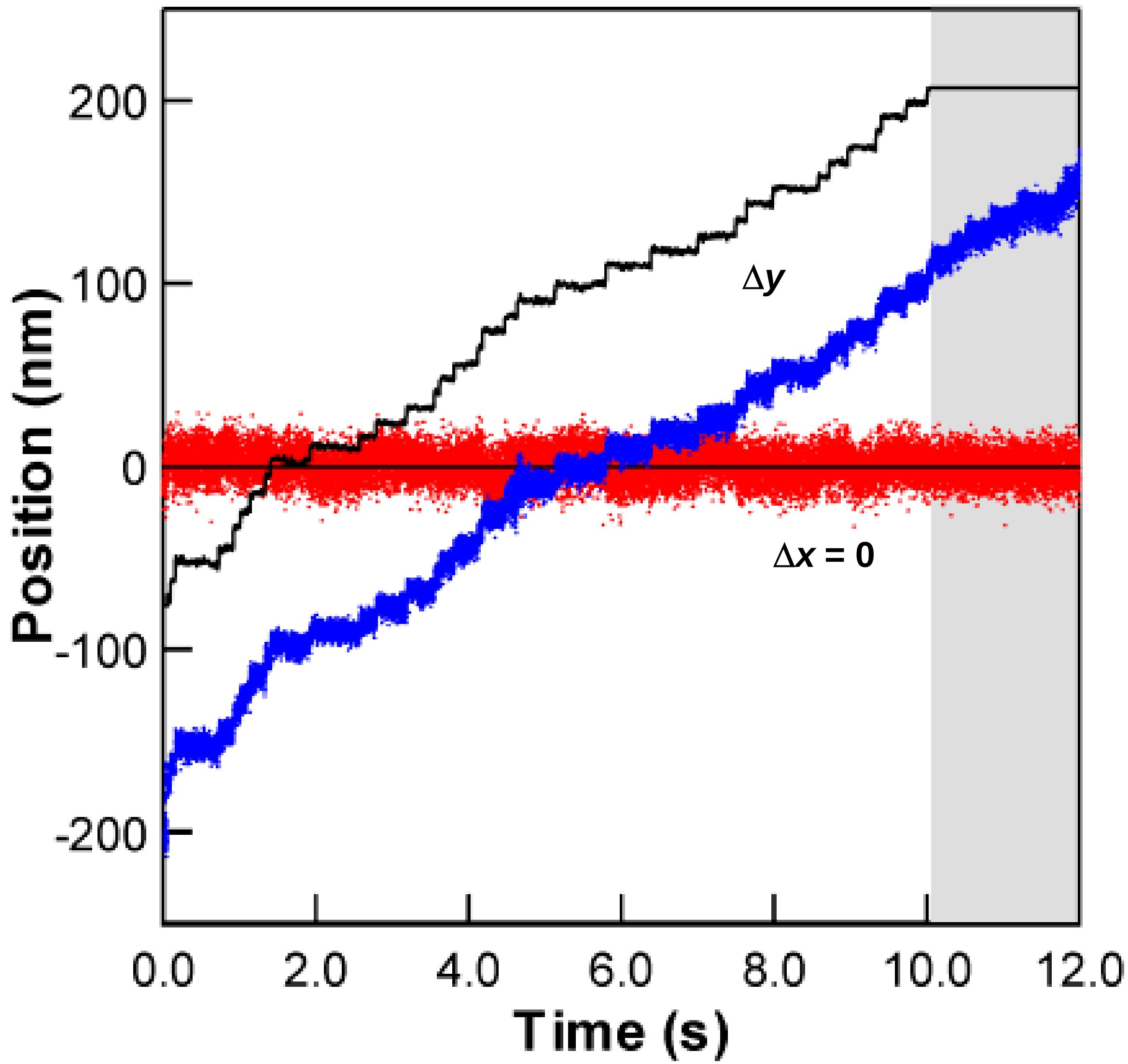


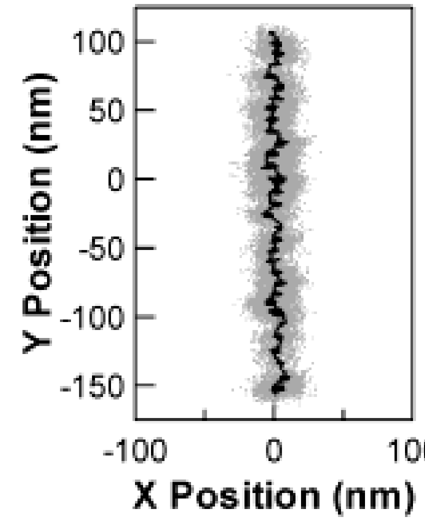


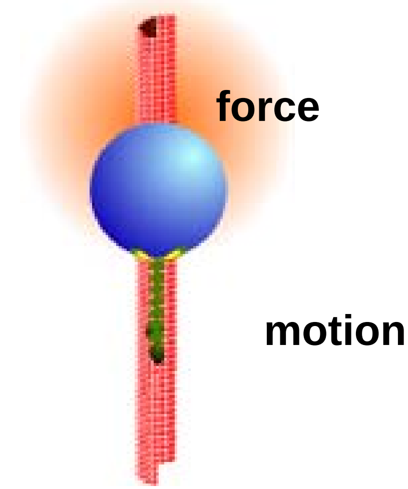


Our computer-controlled trap automatically follows the bead to deliver a constant force as the bead moves.

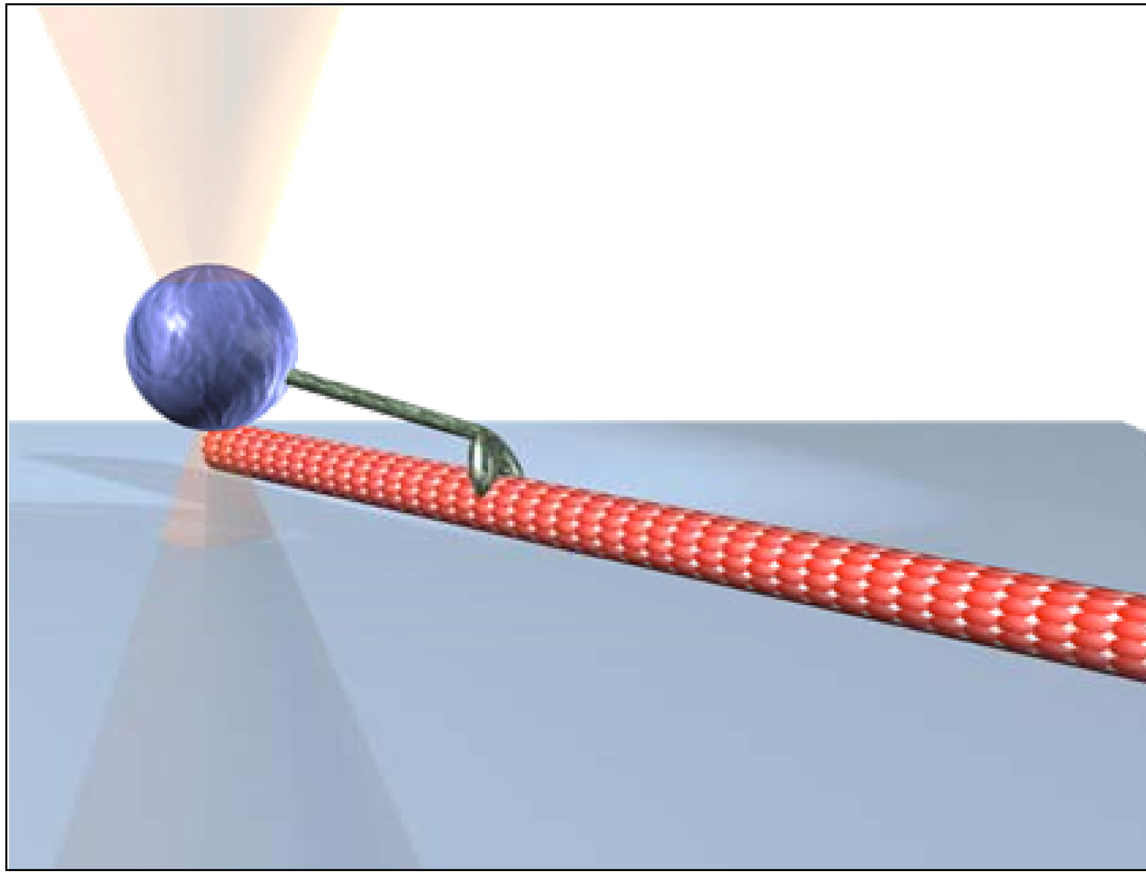
KINESIN MOVEMENT WITH FORWARD LOAD





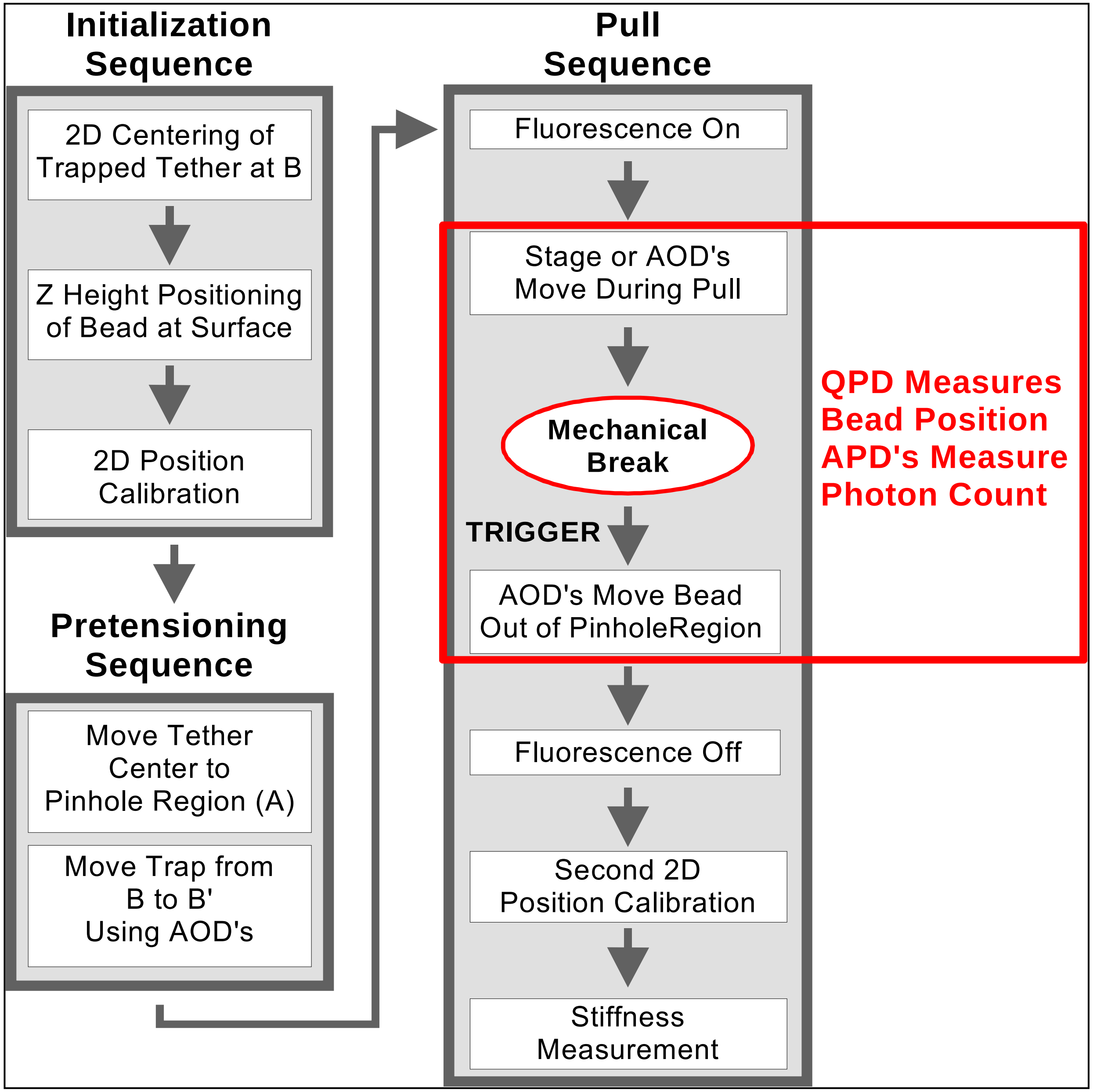


Kinesin movie



Lang with Charles L. Asbury, Joshua W. Shaevitz and Steven M. Block

Automated: procedure ~3min/event

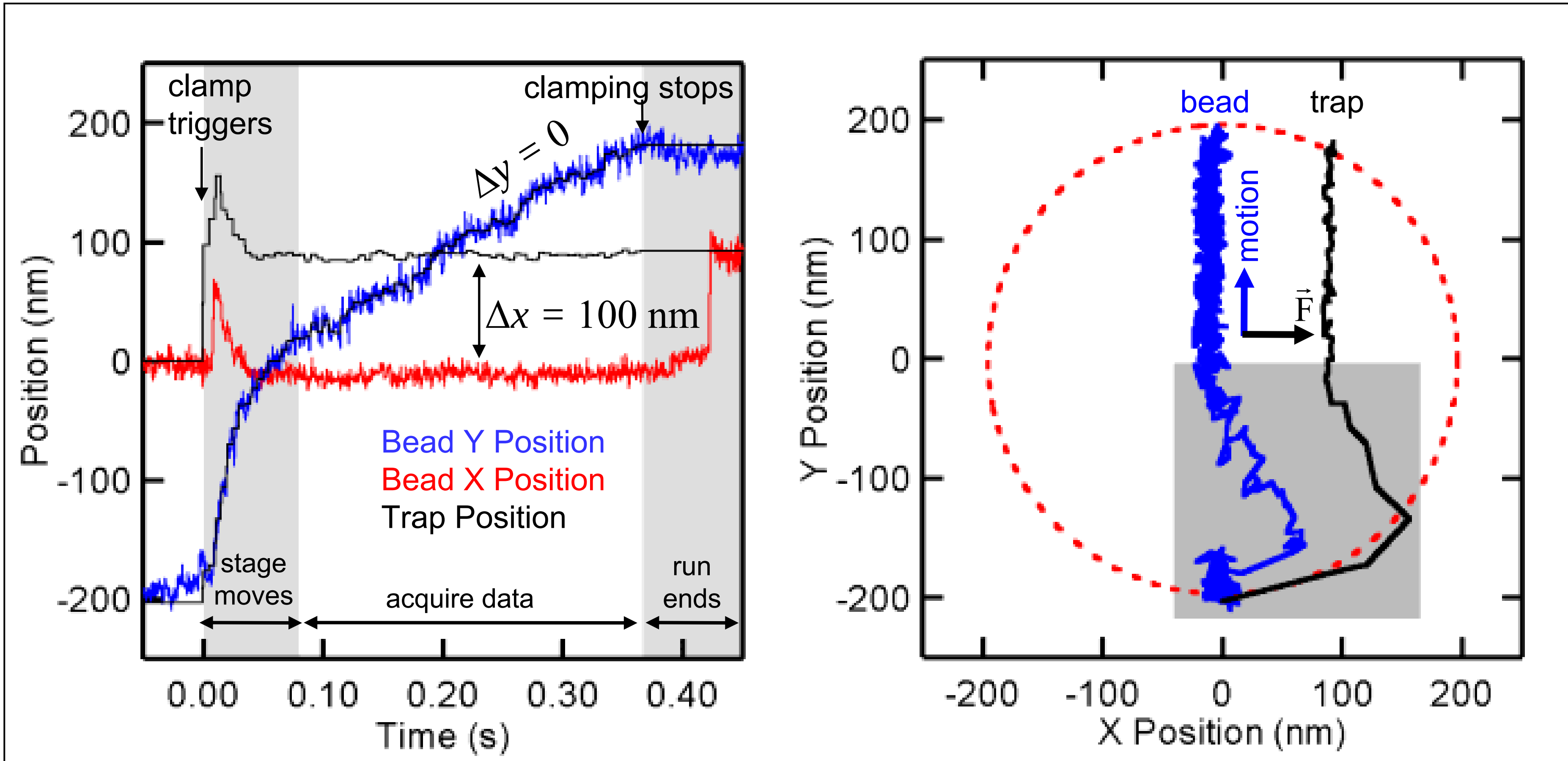


Each event includes a Position calibration Precise centering and stiffness measurement

Many events/slide

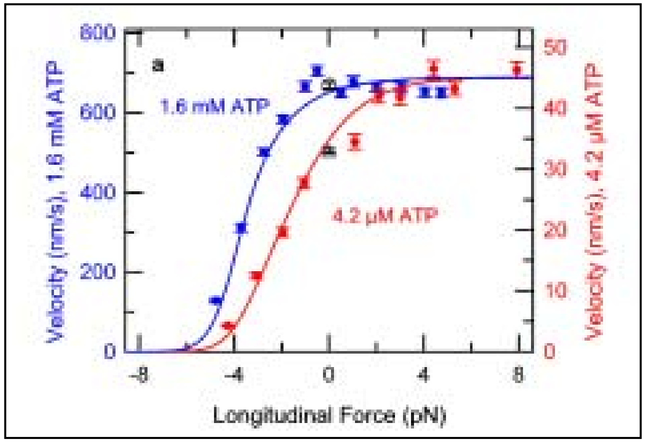
Quickly build Distributions.

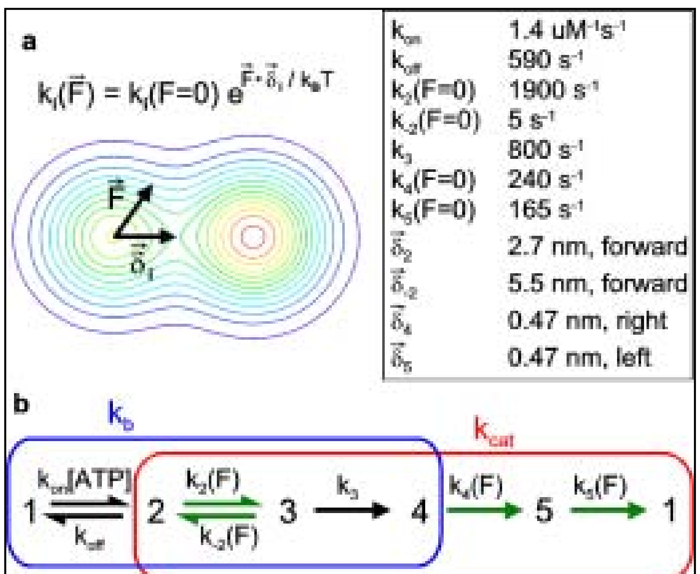
Motion under sideways load

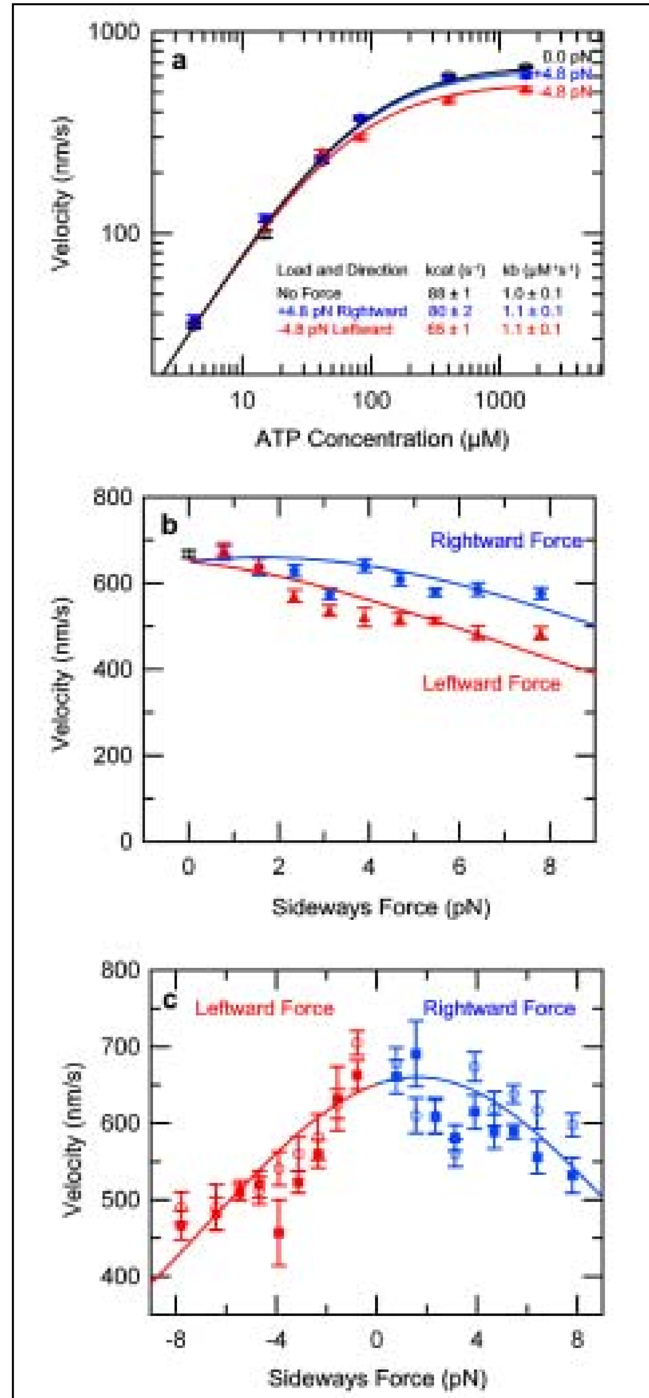


Long constant force records
De-coupled from motion that prepares the load

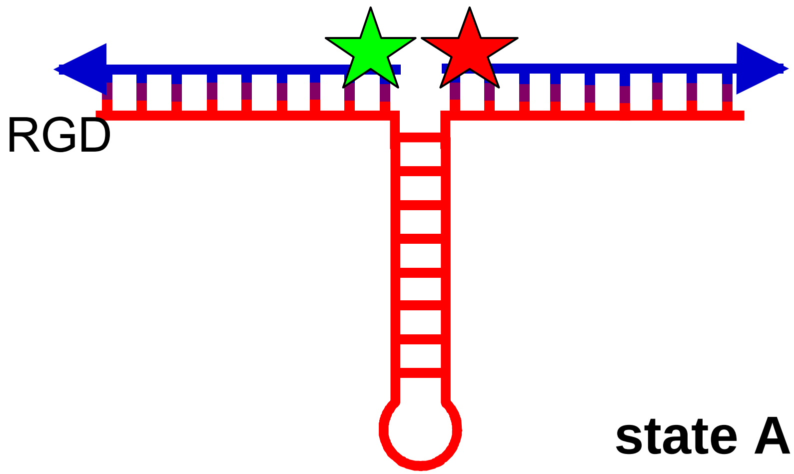
Global fit to the 2D kinesin motility data:

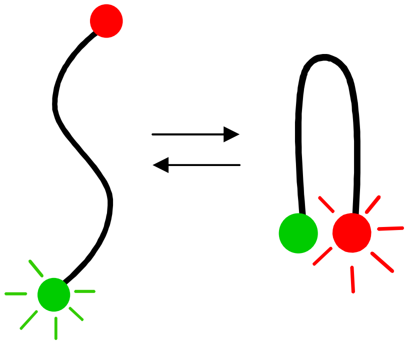


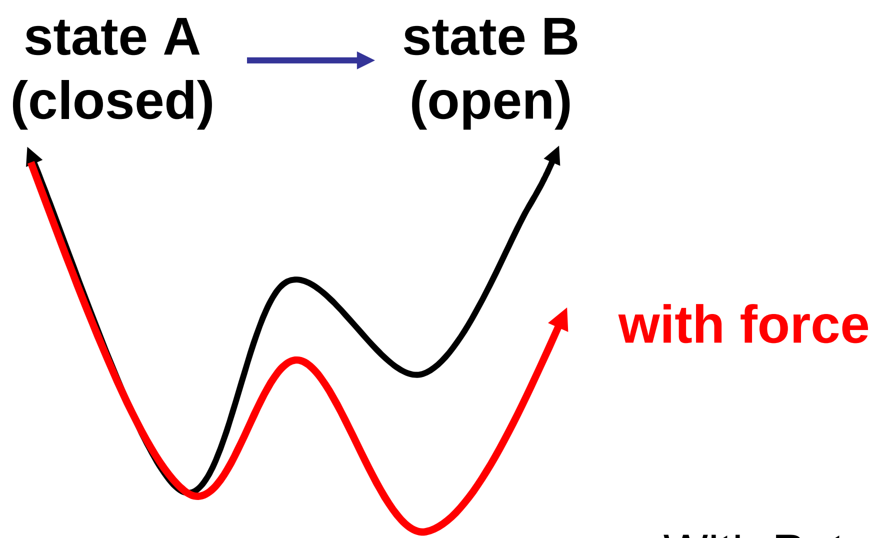




PNAS, 100, 2351-2356 (2003)





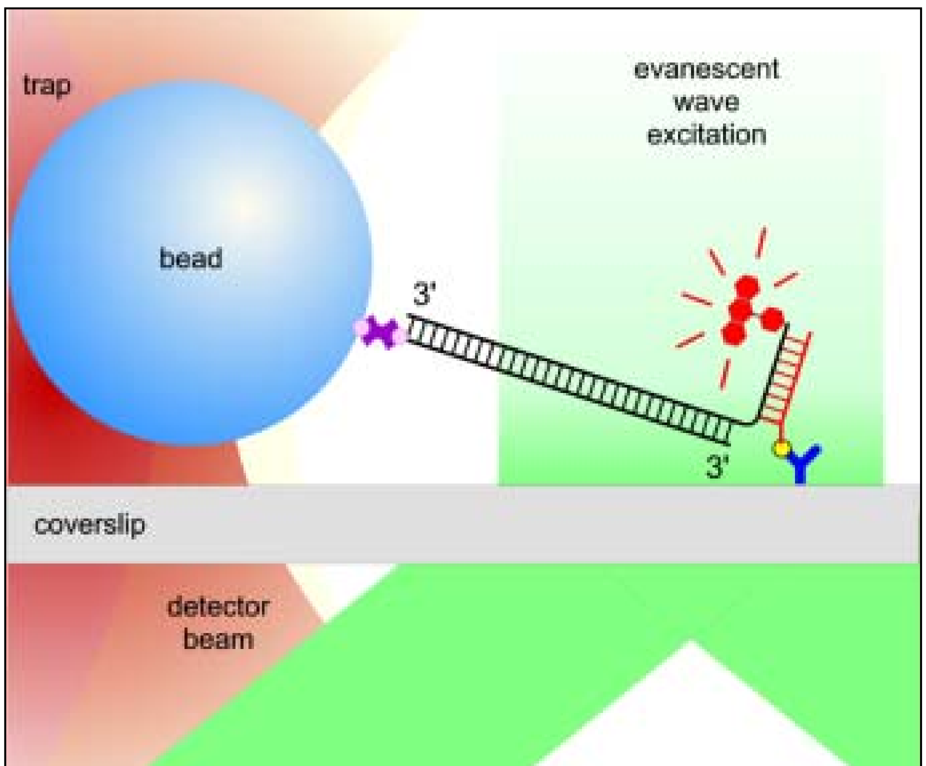


With Peter So

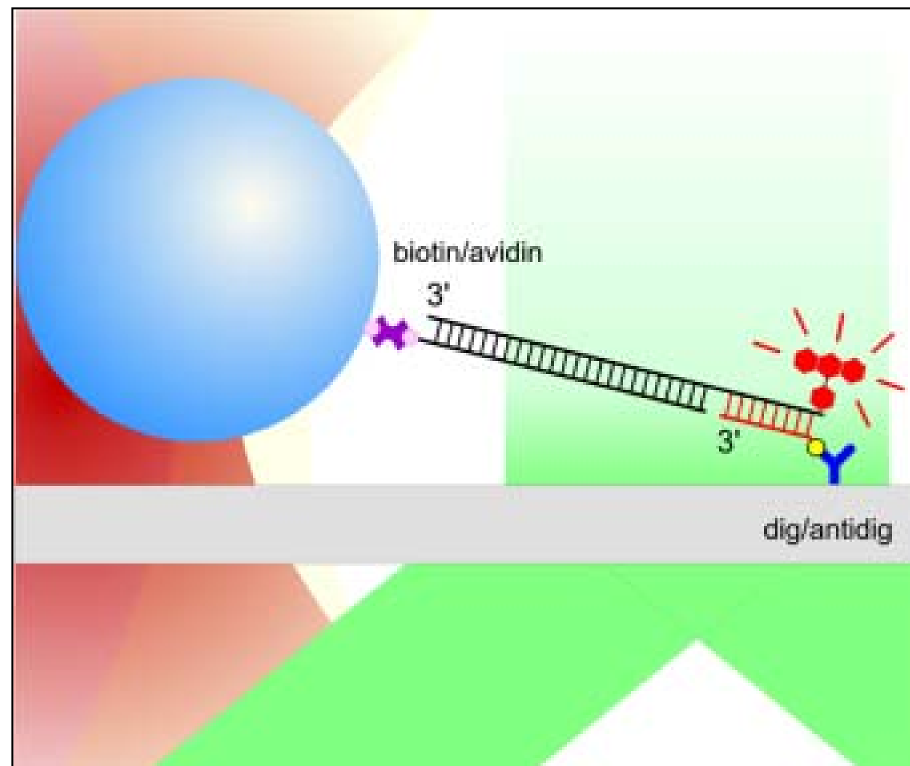
Journal of Biology
BioMed Central
Research article
Combined optical trapping and single-molecule fluorescence
Matthew J Lang*†‡, Polly M Fordyce§ and Steven M Block*¶

Force-induced strand separation of ds DNA

Geometry for "Unzipping" Force

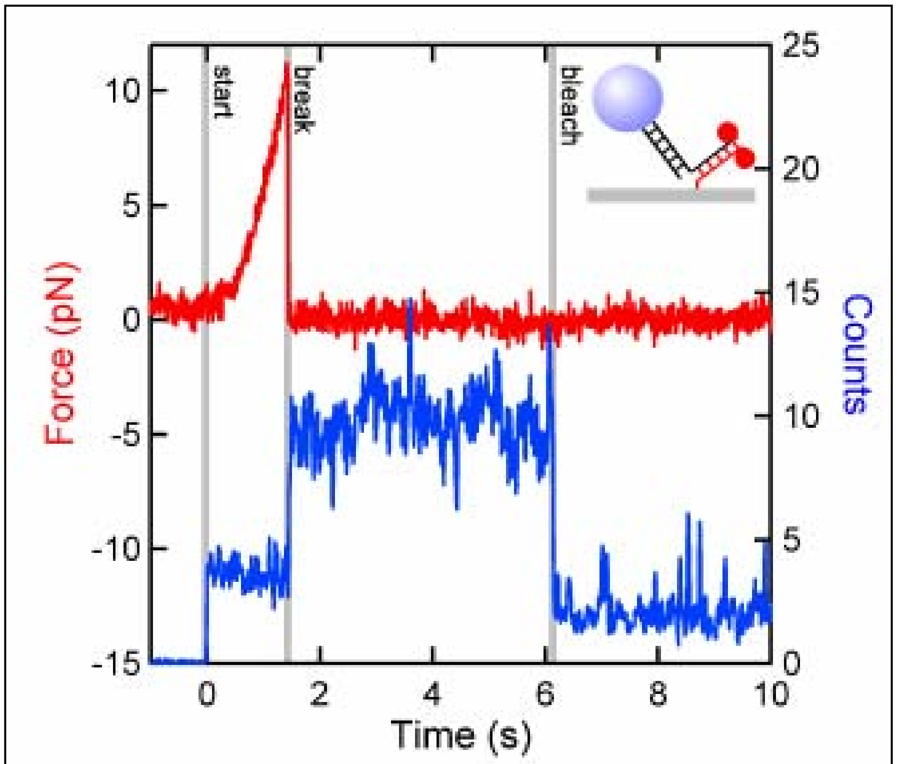


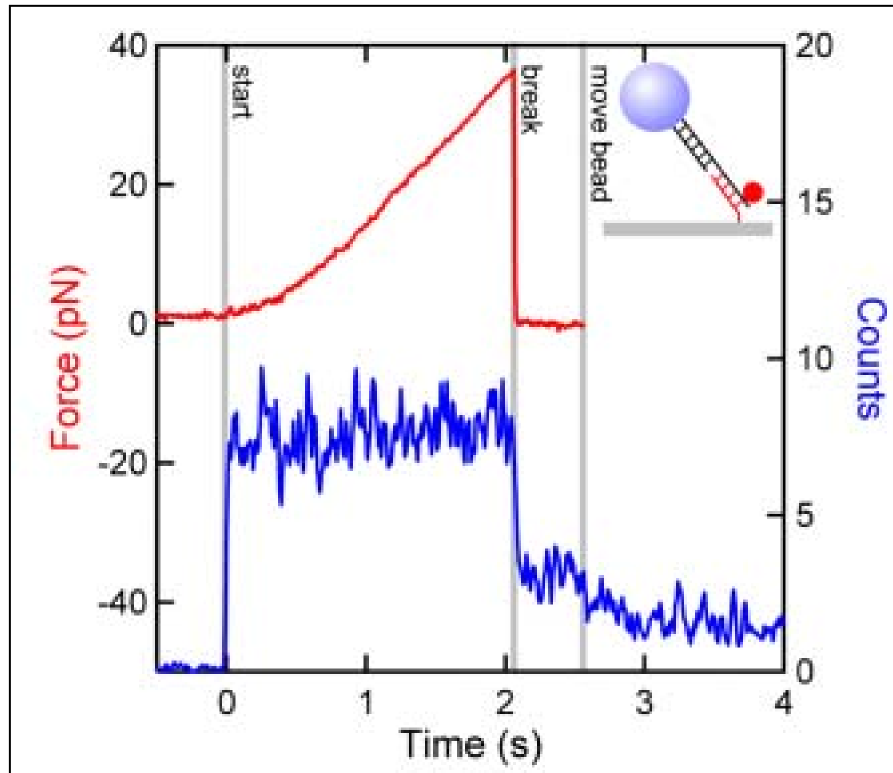
Geometry for "Shearing" Force



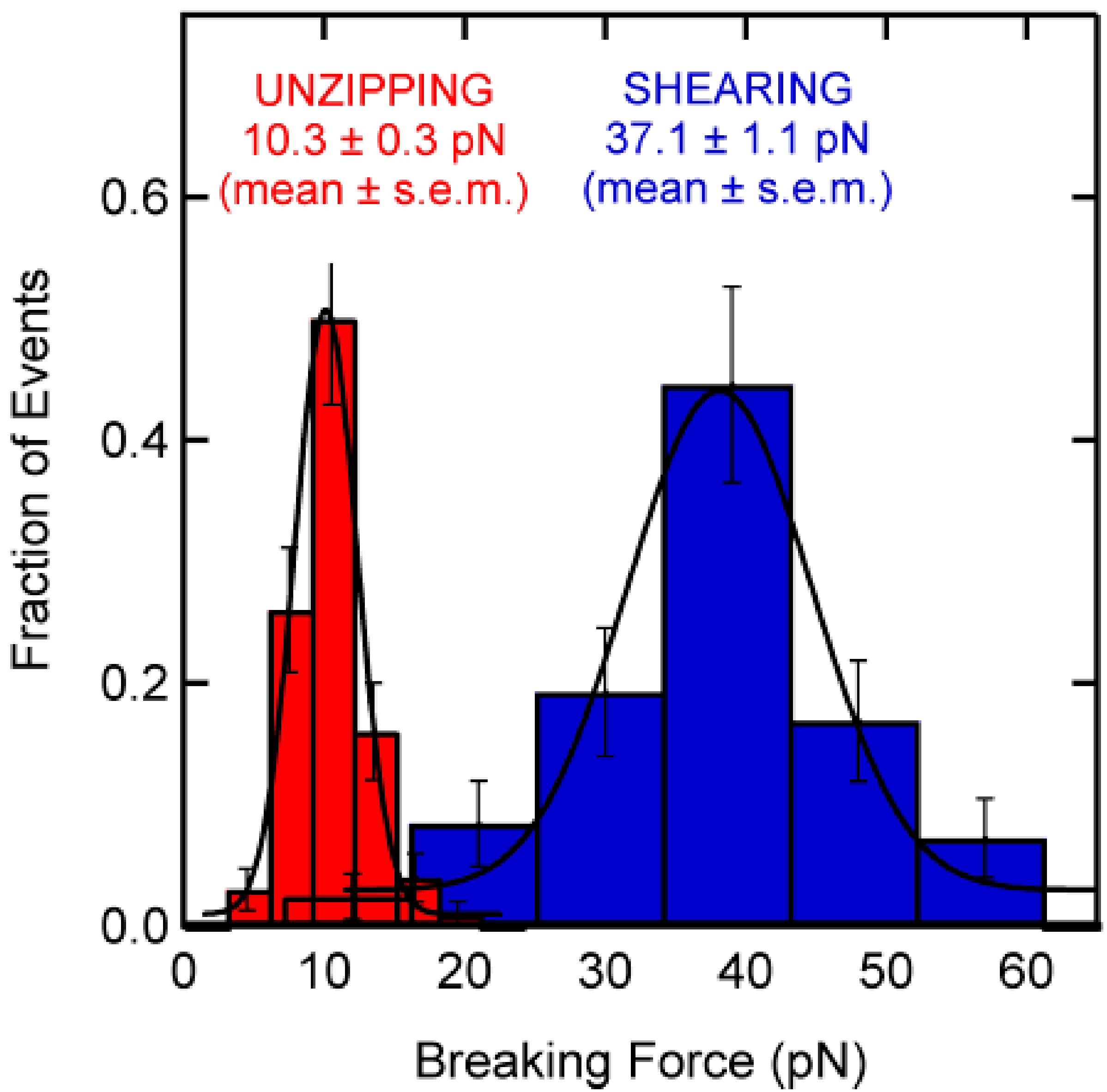
Chromophores on adjacent base pairs unquench at the mechanical break.

HIGHER RUPTURE FORCES FOR SHEARING





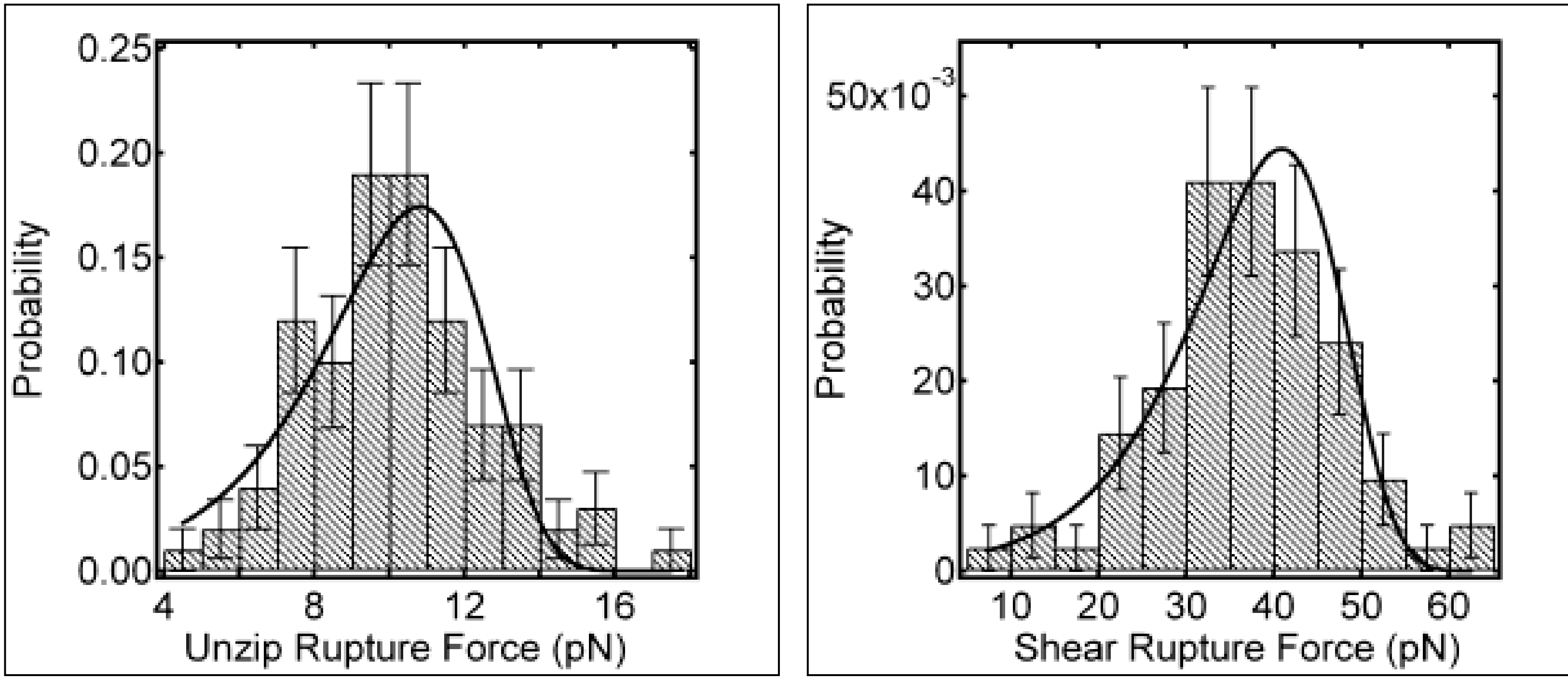
COMPARING SHEARING AND UNZIPPING



Probability distributions of unbinding forces.

$$p(F) = \frac{\nu_0}{\partial F / \partial t} e^{\frac{Fx}{k_B T}} Exp\left[\frac{\nu_0 k_B T}{\partial F / \partial t \, x} \left(1 - e^{\frac{Fx}{k_B T}} \right) \right]$$

Evans, E. & Ritchie, K. (1997) *Biophys. J.* **72,** 1541-1555



ν0 = 0.03±0.01
x = 1.9±0.2 nm
~ 11 pN/s
10.3±0.5 pN
(FWHM 5 pN, N=100).

ν0 = 0.021±0.009 s-1
x = 0.49±0.05 nm
~ 24 pN/s
37.1±1.1 pN
(FWHM 15 pN, N=83).

Probability distributions of unbinding forces, along with fits to the probability distribution function discussed in the text. **A** Distribution of unzipping rupture forces. The most probable unzipping rupture force is 10.3±0.5 pN (FWHM 5 pN, N=100). **B** Distribution of shearing rupture forces, with probability distribution function fit. The most probable shearing rupture force is 37.1±1.1 pN (FWHM 15 pN, N=83).

Home built optical traps

e-coli spinners

BE.309 Bioinstrumentation laboratory

David Appleyard