Force as a variable to study biological reactions

Biochemistry under stress!

Radhika Subramanian

QB Lecture-1

Lecture outline

- Lecture-1:
  - Why force??
  - Mechanical processes in biology
    - Motor proteins
    - Mechanosensing
  - Experimental techniques

- Lecture-2:
  - Force → free energy landscape
  - What we can learn from using force to study a mechanical process: RNA unfolding

- Lecture-3:
  - Motor enzymes and mechanochemistry
  - Mechanistic information from force-velocity studies

Why force??

Forces can be an input or an output

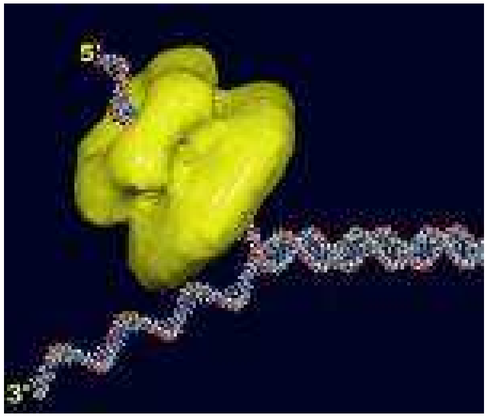
Motor proteins: force generating nanomachines

chemical (or electrochemical) energy → mechanical work

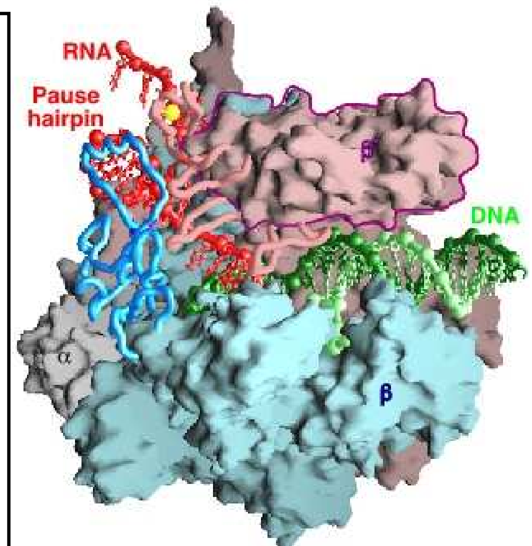
Mechanosensing:

mechanical force → cellular signal

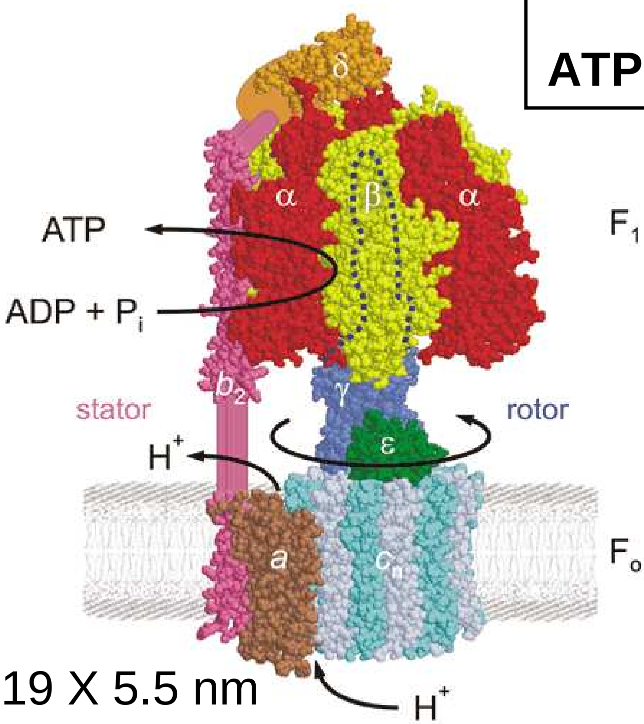
View of cell as a collection of nanomachines



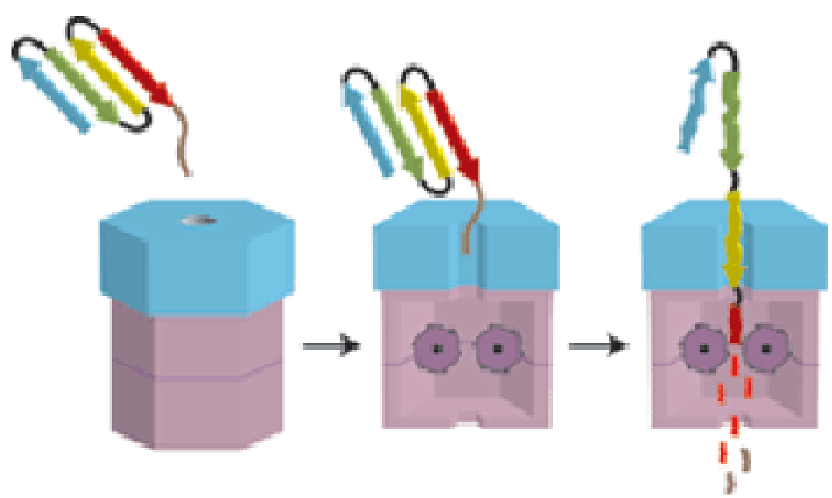
Replication:	**Helicase** **DNA polymerase**
Transcription:	**RNA Polymerase**
Translation:	**Ribosome**
Degradation:	**Proteosome**
ATP synthesis:	**F0F1 ATP synthase**



8.5 x 10.5 x 14 nm
25 pN

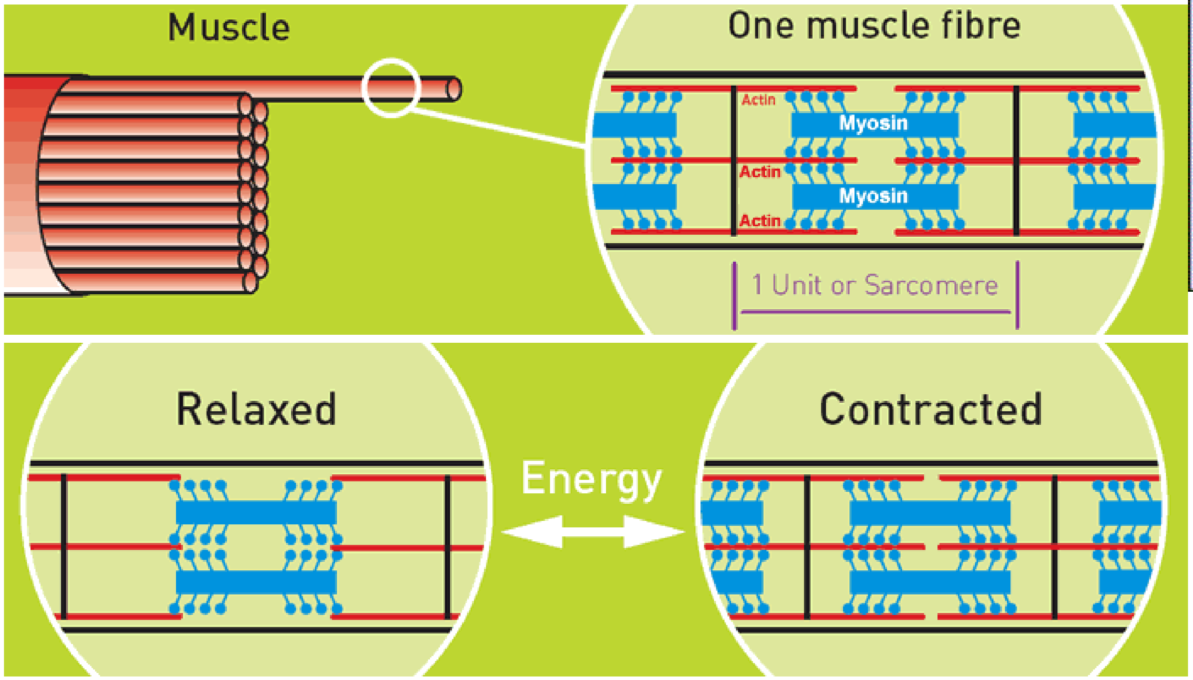


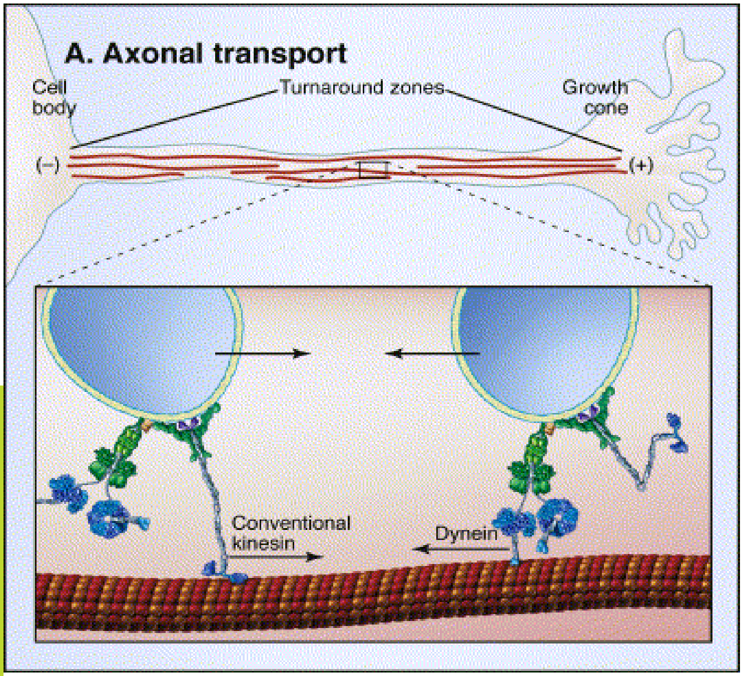
19 X 5.5 nm



Cytoskeletal motors
kinesin, dynein, myosin

- Sub-cellular motility
- Cell division
- Muscle contraction

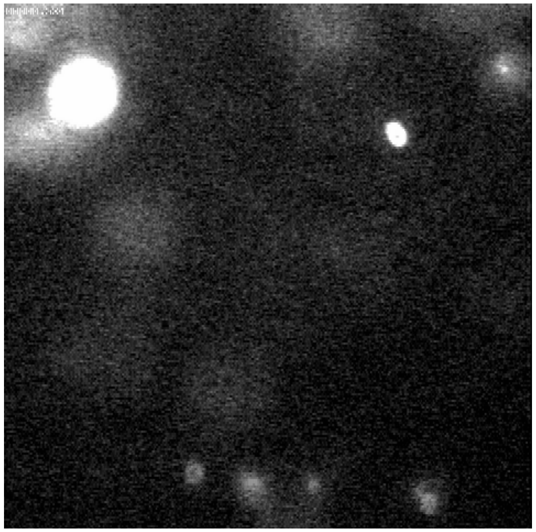


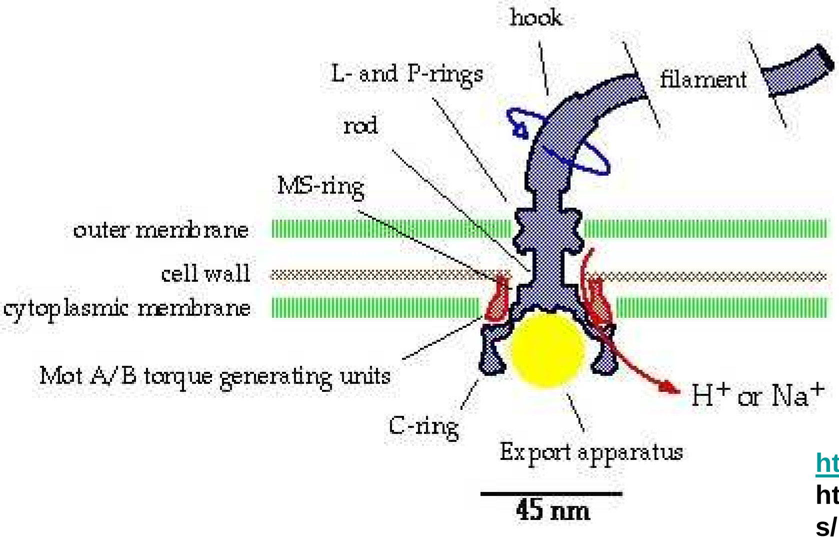


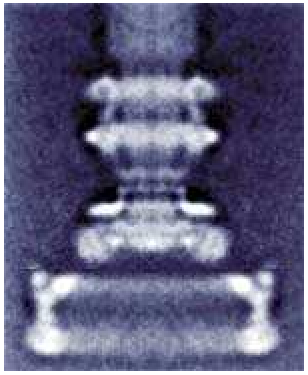
Vale, R.D. The molecular motor toolbox for intracellular transport. *Cell* **112, 467-80 (2003).**

Cellular motility

Flagella





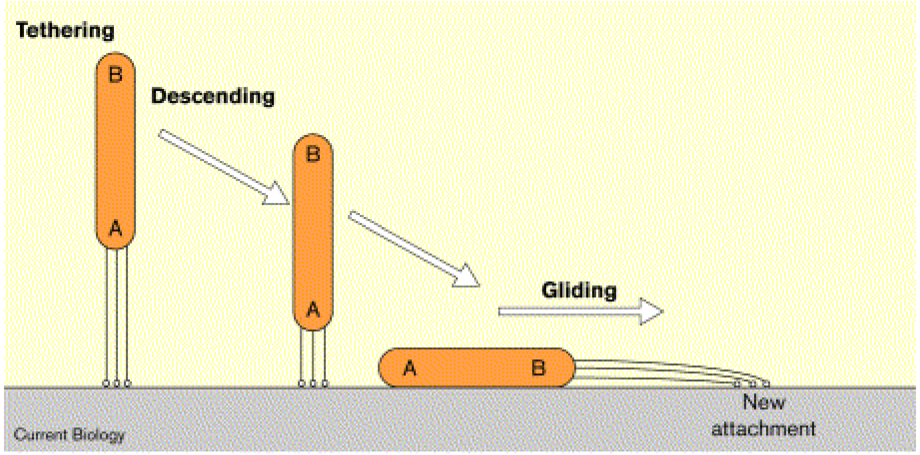


http://www.aip.org/pt/jan00/berg.htm

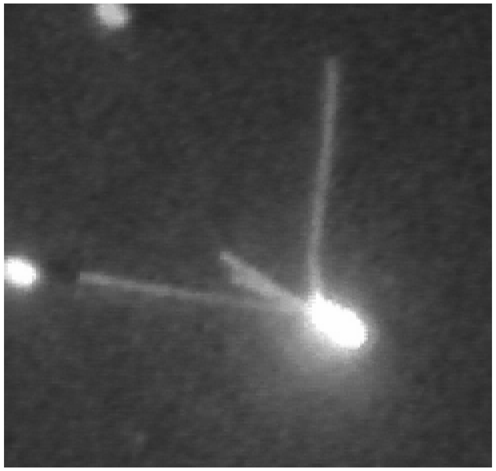
http://www.physics.ox.ac.uk/biophysics/research/flagellar.html

Movies from http://www.rowland.org/labs/bacteria/

Pilus

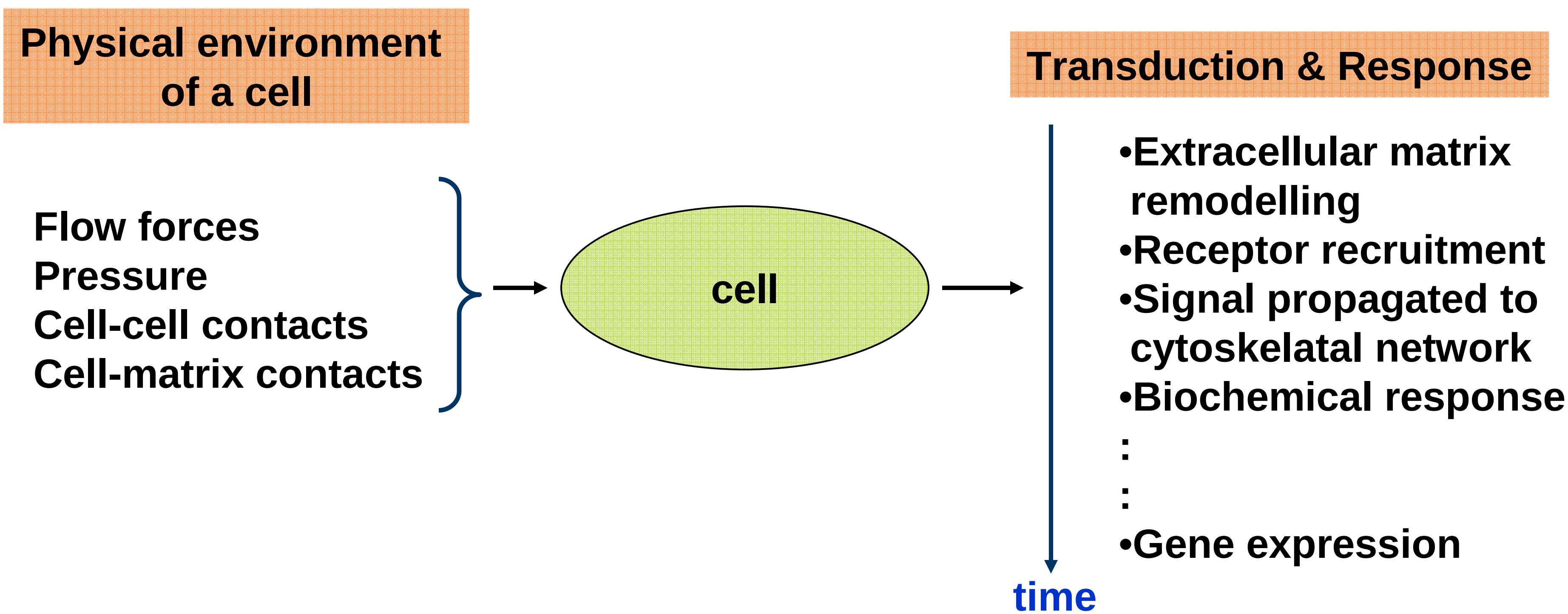


Kaiser, D. Bacterial motility: how do pili pull? *Curr Biol* **10, R777-80 (2000).**

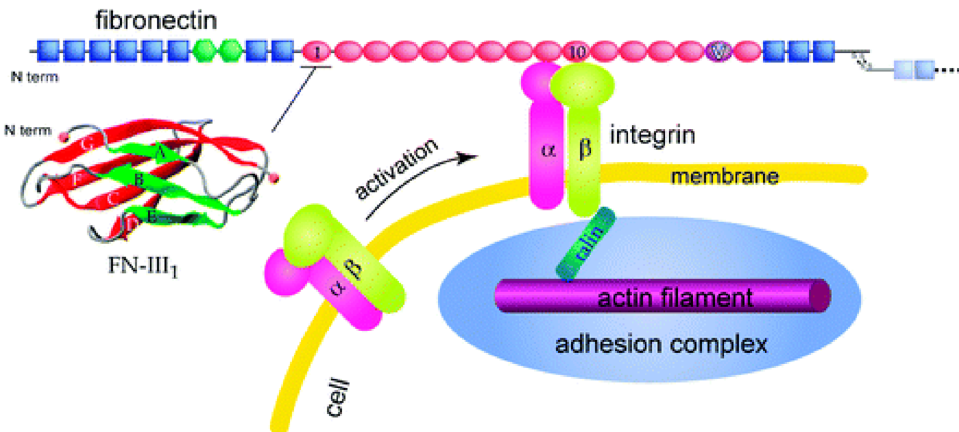


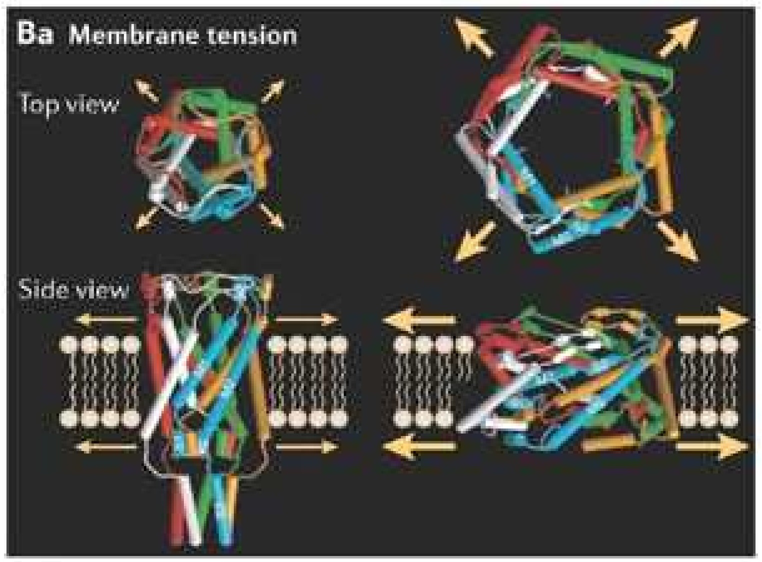
Mechanosensing, transduction and response

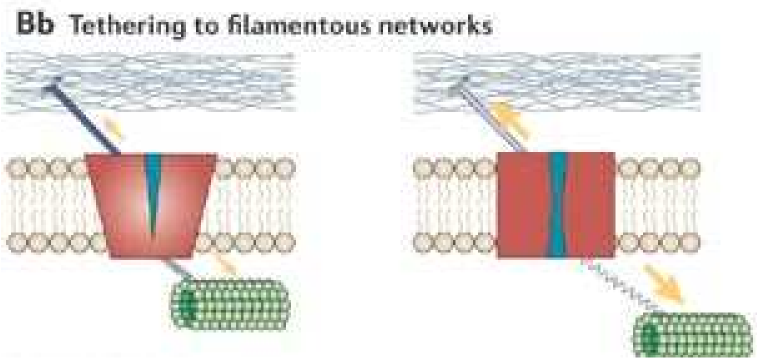
physical signal → primary sensing → transduction → response



Primary sensing by “force receptors”

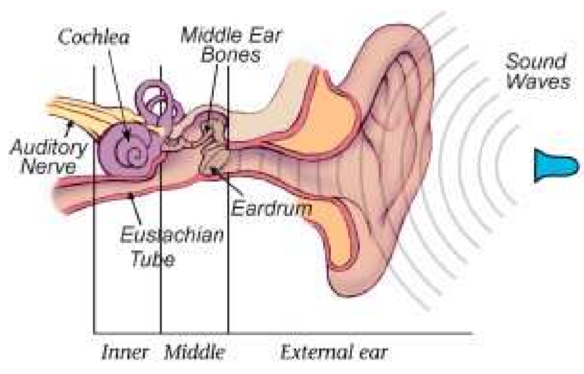


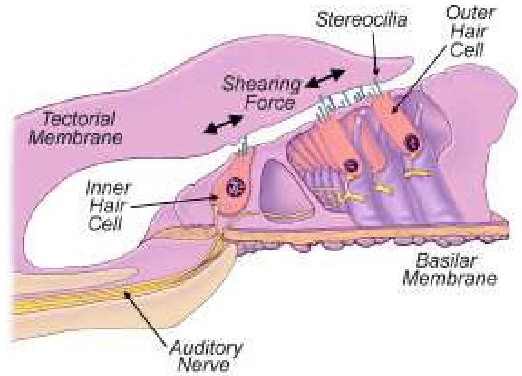


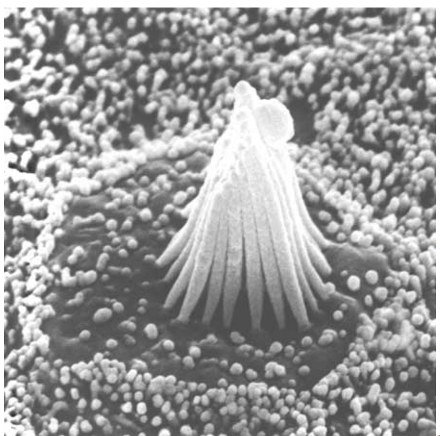


Gao, M., Sotomayor, M., Villa, E., Lee, E.H. & Schulten, K. Molecular mechanisms of cellular mechanics. *Phys Chem Chem Phys* **8, 3692-706 (2006).**
Vogel, V. & Sheetz, M. Local force and geometry sensing regulate cell functions. *Nat Rev Mol Cell Biol* **7, 265-75 (2006).**

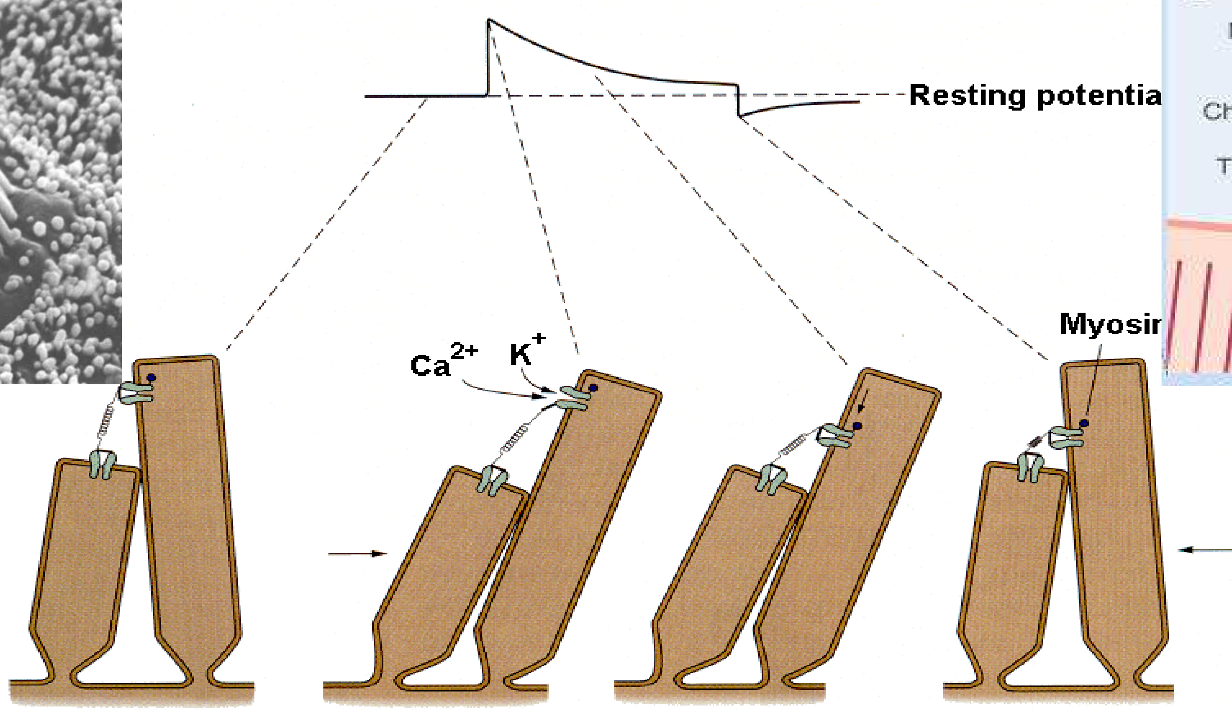
Mechanosensing in hair cells

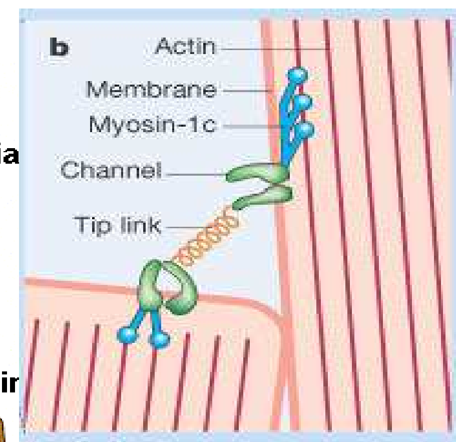






stereocilia



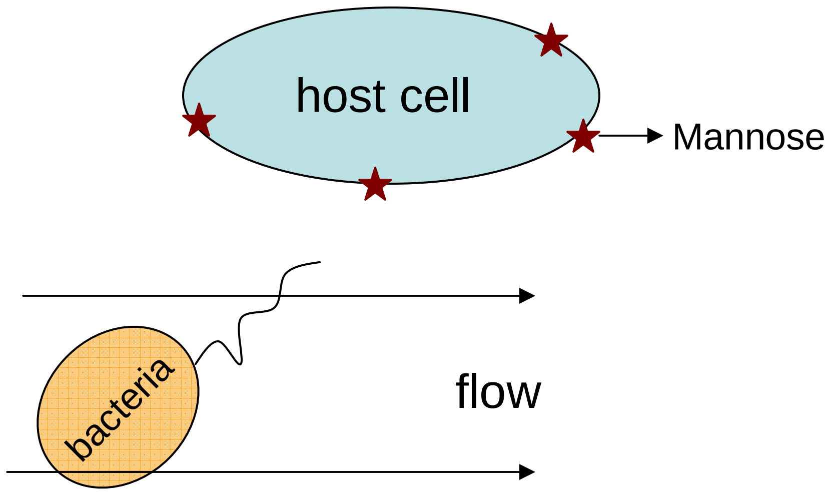


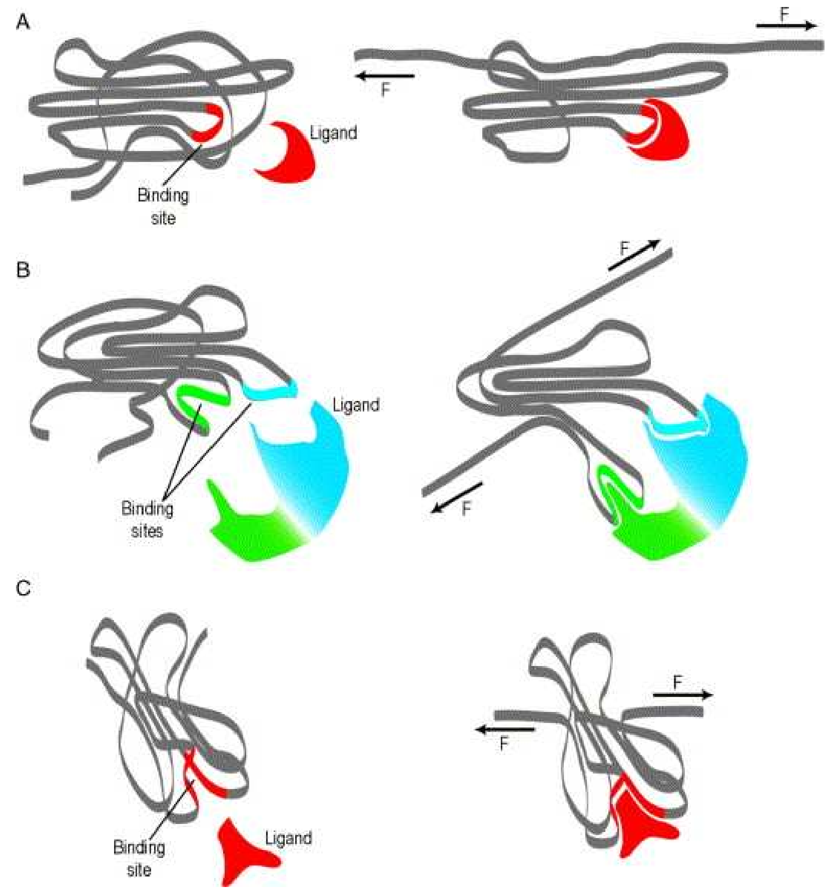
http://www.bcm.edu/oto/research/cochlea/Hearing/index.html

http://www.unmc.edu/Physiology/Mann/mann4b.html

Mechanosensing by "Catch Bonds"

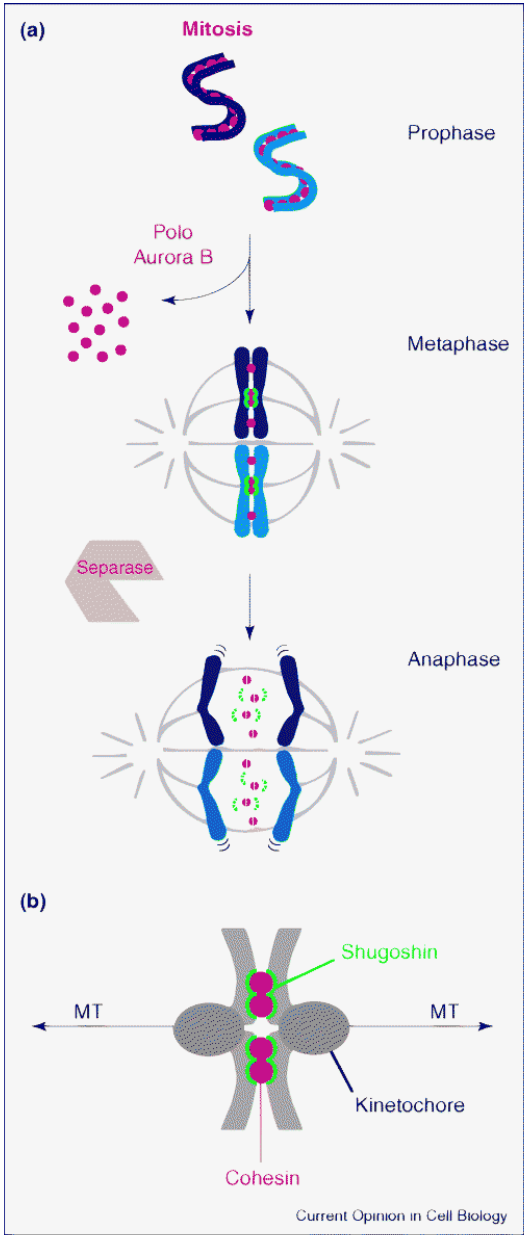
short → long lived attachments at increasing forces

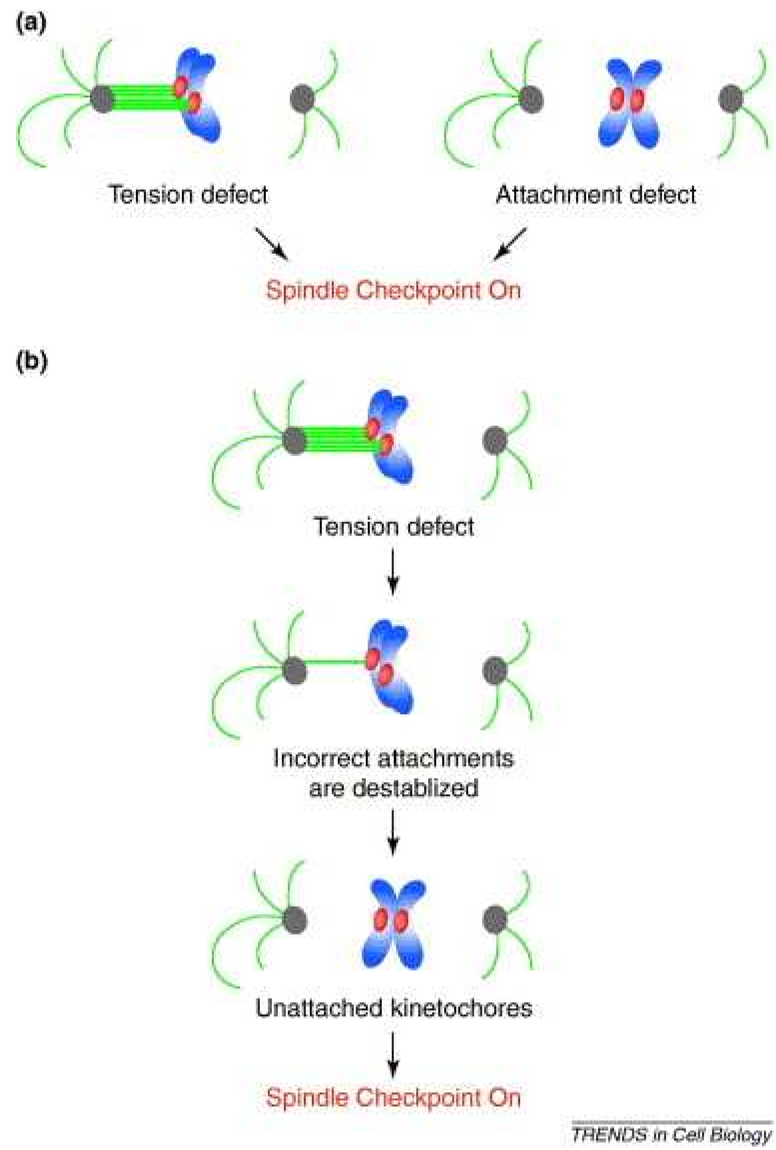




Grandi, F. et al. Hierarchical mechanochemical switches in angiostatin. *Chembiochem* **7, 1774-82 (2006).**

Tension sensing during chromosome segregation

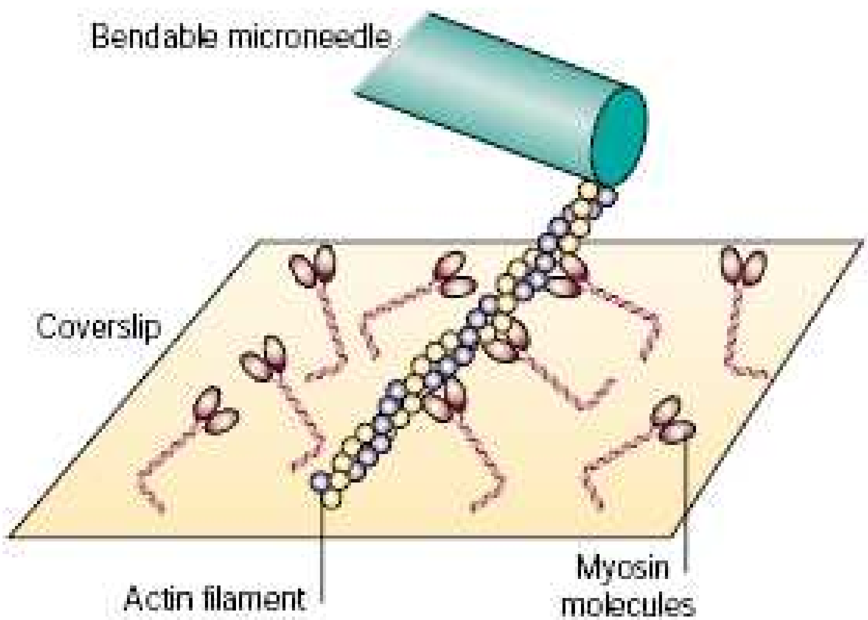


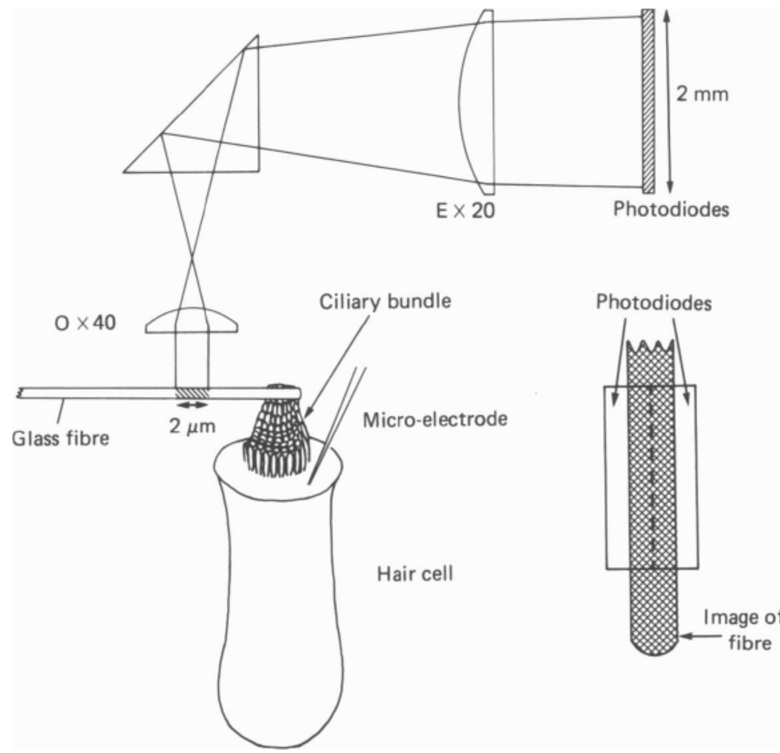


Pinsky, B.A. & Biggins, S. The spindle checkpoint: tension versus attachment. *Trends Cell Biol* **15**, 486-93 (2005).
Watanabe, Y. Shugoshin: guardian spirit at the centromere. *Curr Opin Cell Biol* **17**, 590-5 (2005).

Techniques

Microneedle or glass fibres

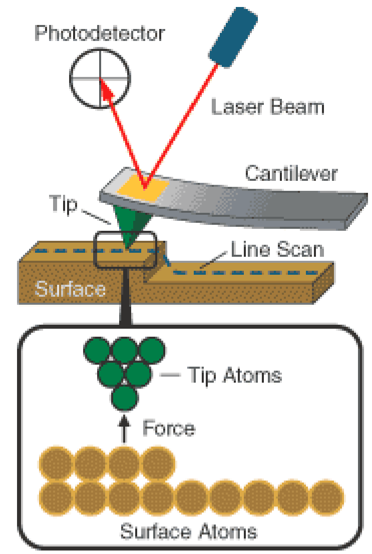


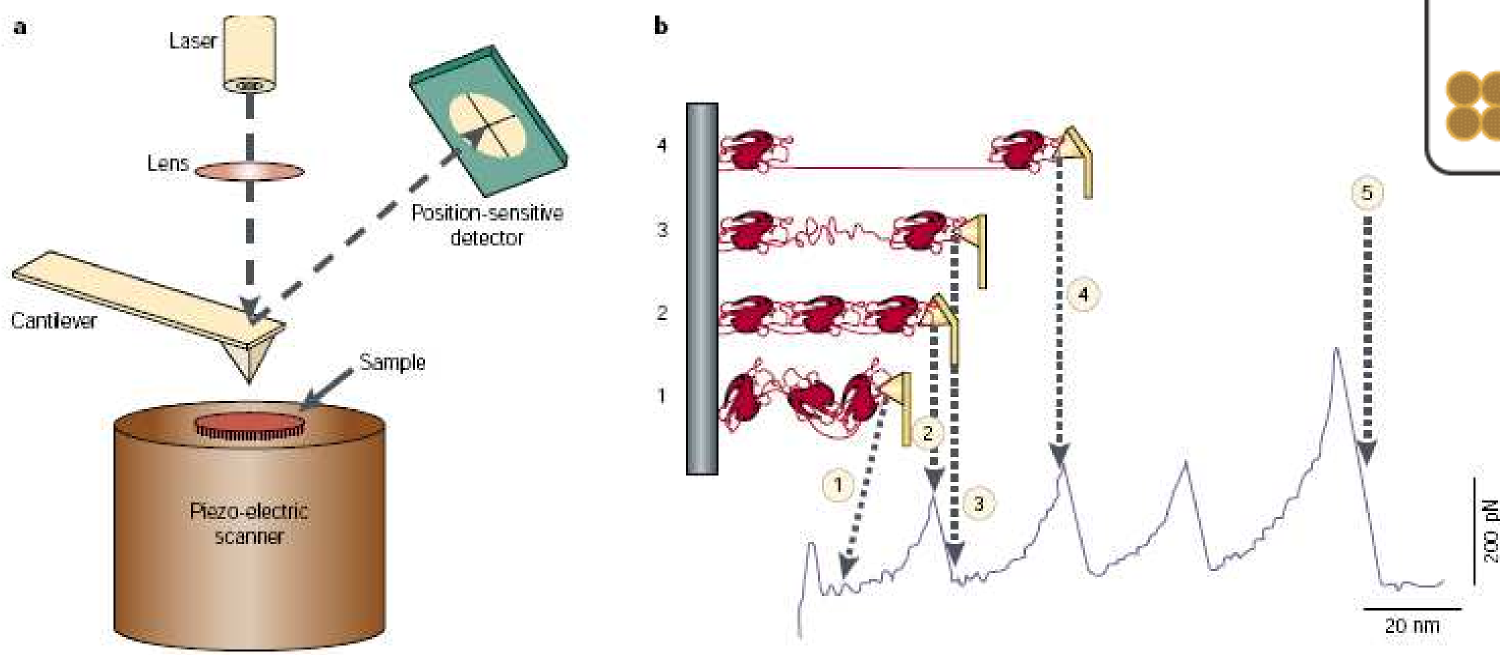


Force range: 1-100 pN
Position resolution: 1 nm

Bustamante, C., Macosko, J.C. & Wuite, G.J. Grabbing the cat by the tail: manipulating molecules one by one. *Nat Rev Mol Cell Biol* **1, 130-6 (2000).**
Crawford, A.C. & Fettiplace, R. The mechanical properties of ciliary bundles of turtle cochlear hair cells. *J Physiol* **364, 359-79 (1985).**

Atomic force microscopy



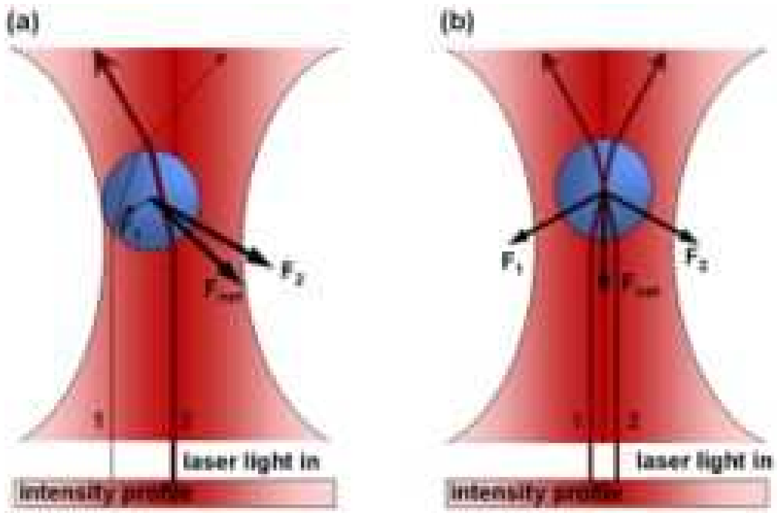


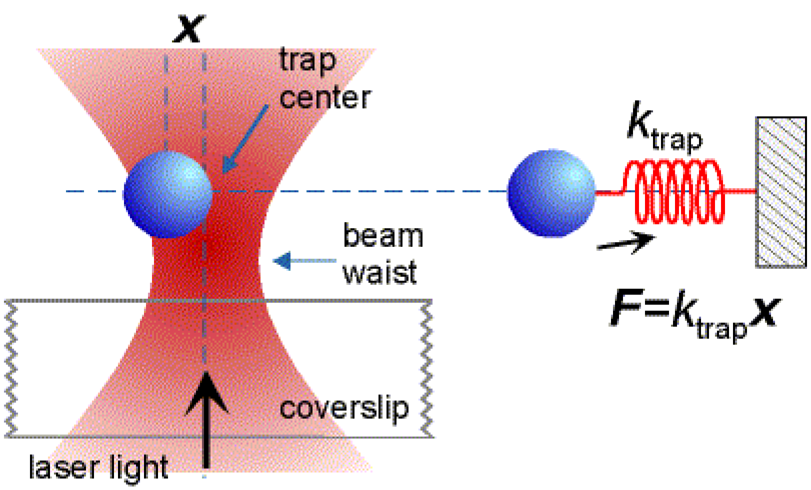
Force range: 10 pN -10 uN
Position resolution: 0.1 nm

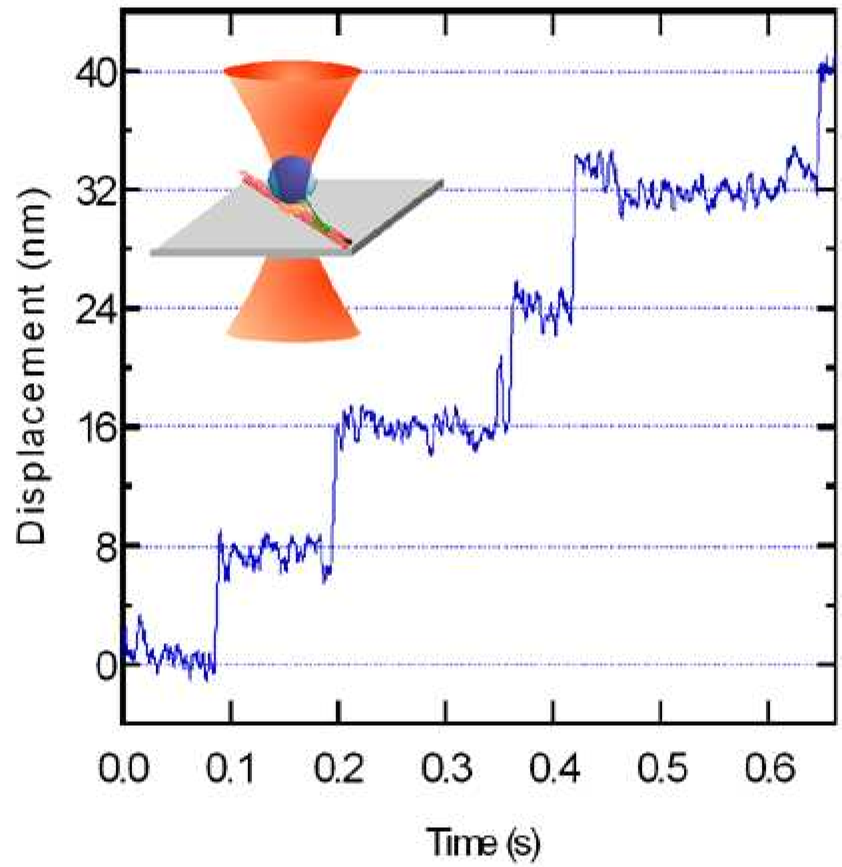
Bustamante, C., Macosko, J.C. & Wuite, G.J. Grabbing the cat by the tail: manipulating molecules one by one. *Nat Rev Mol Cell Biol* **1, 130-6 (2000).**
http://www.molec.com/what_is_afm.html

Optical tweezers

Force range: 0.1-100 pN
Position resolution: 1 nm

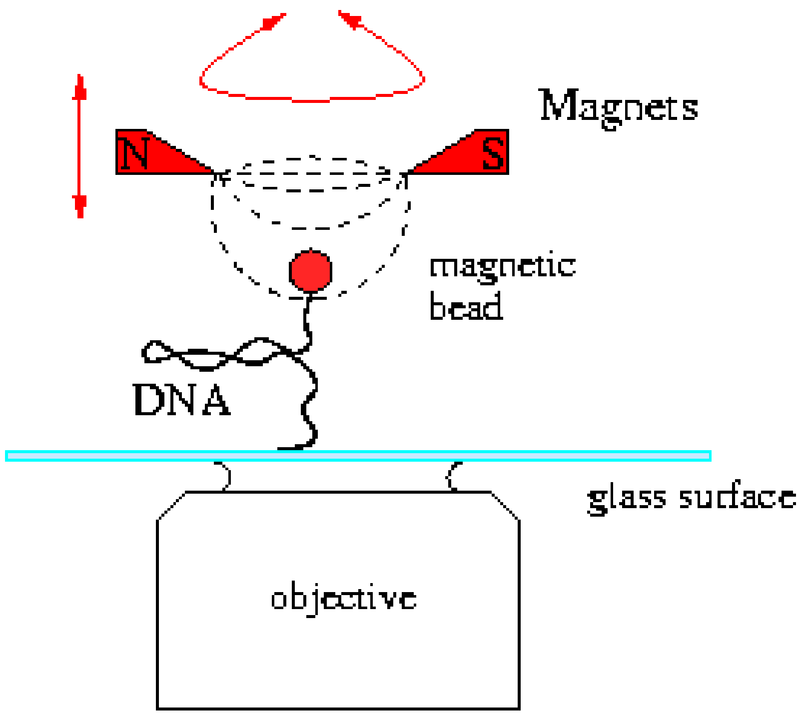






http://www.stanford.edu/group/blocklab/Optical%20Tweezers%20Introduction.htm

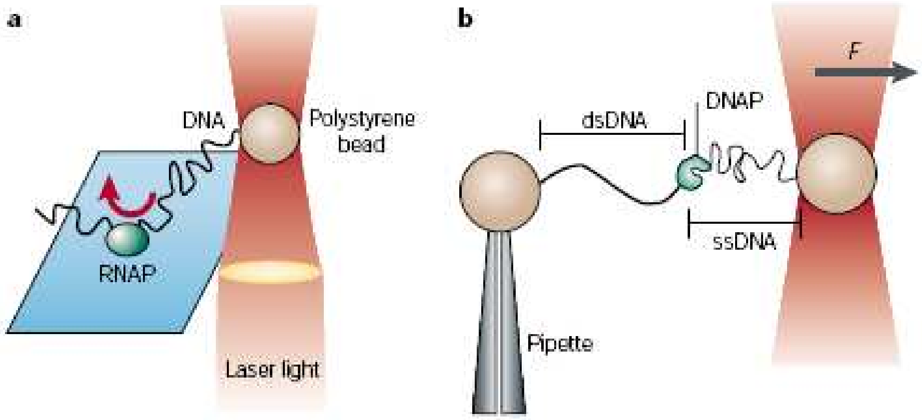
Magnetic tweezers

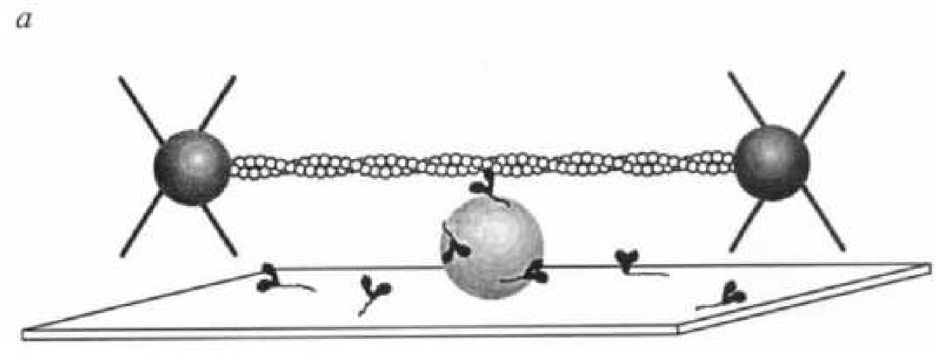


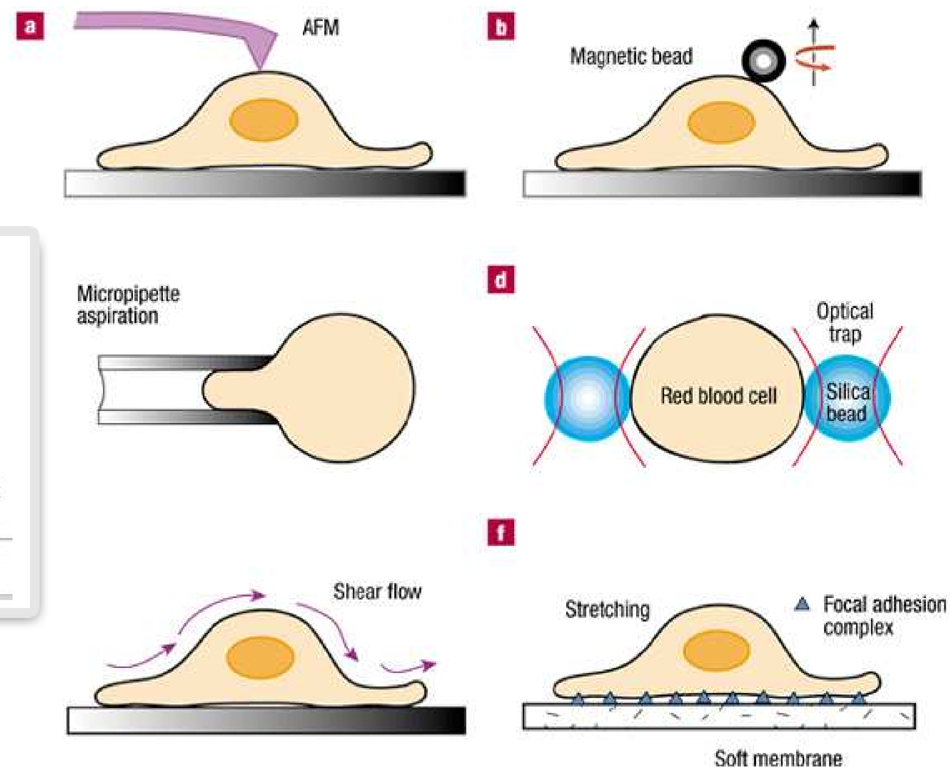
Force range: 0.01-10 pN
Position resolution: 10 nm

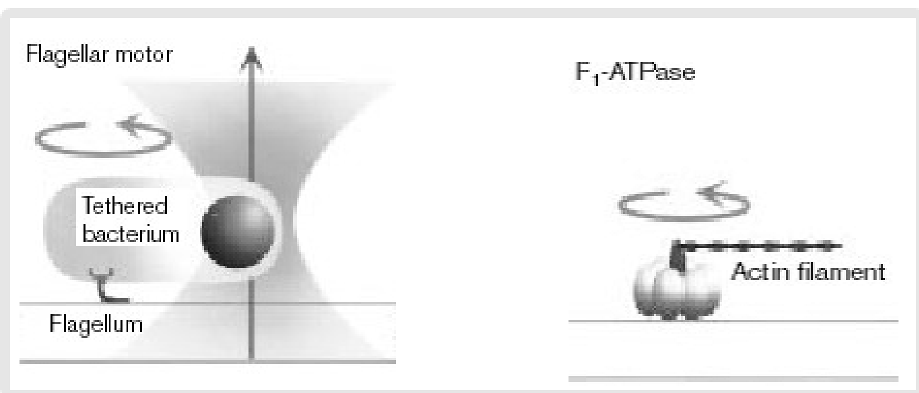
Figure from http://www.picotwist.com/index.php?content=smb&option=mag

Various experimental designs

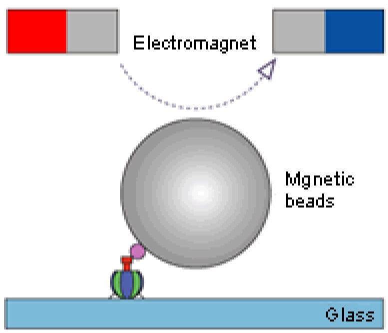


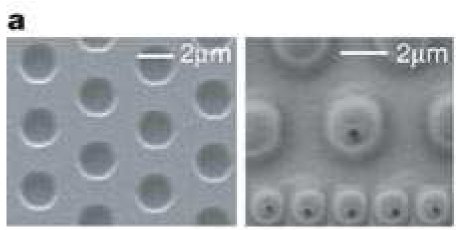


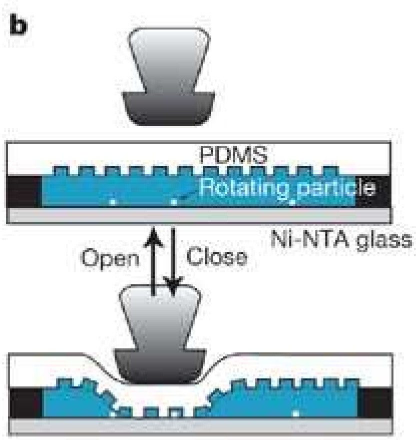


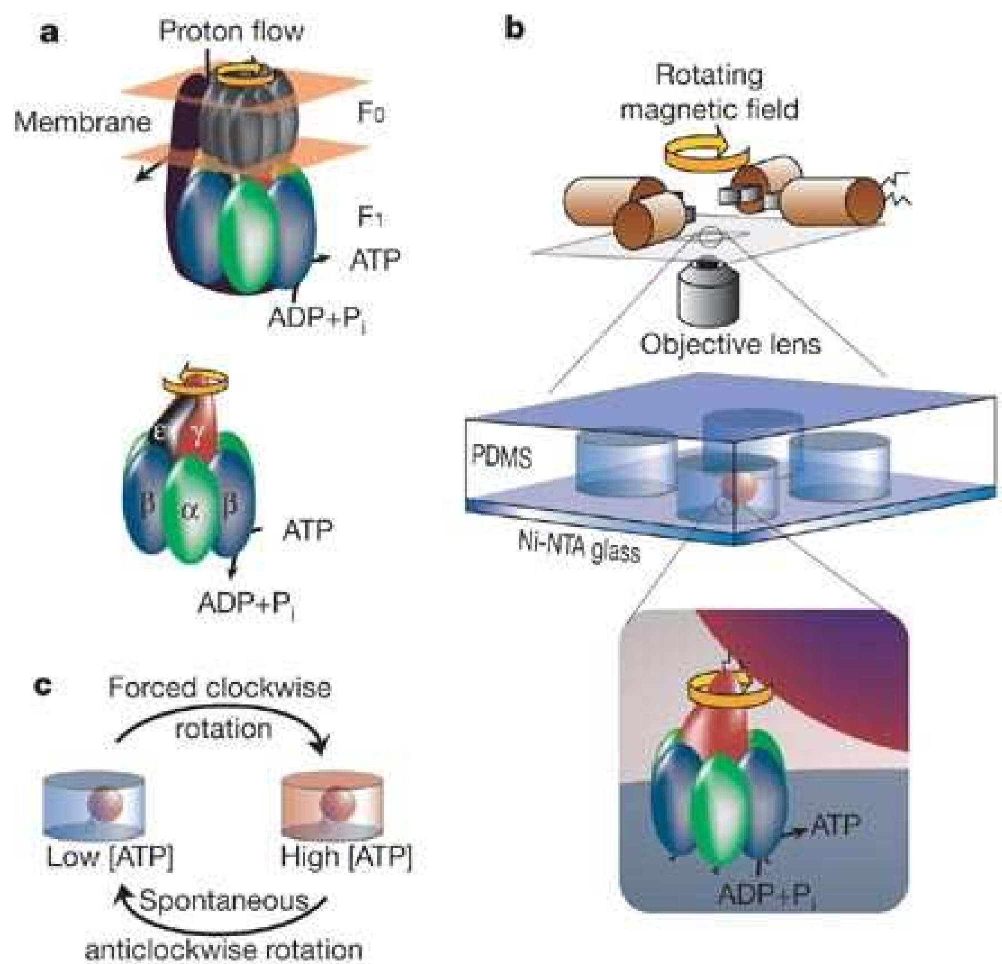


A “cool” experiment !









Rondelez, Y. et al. Highly coupled ATP synthesis by F1-ATPase single molecules. *Nature* **433**, 773-7 (2005).