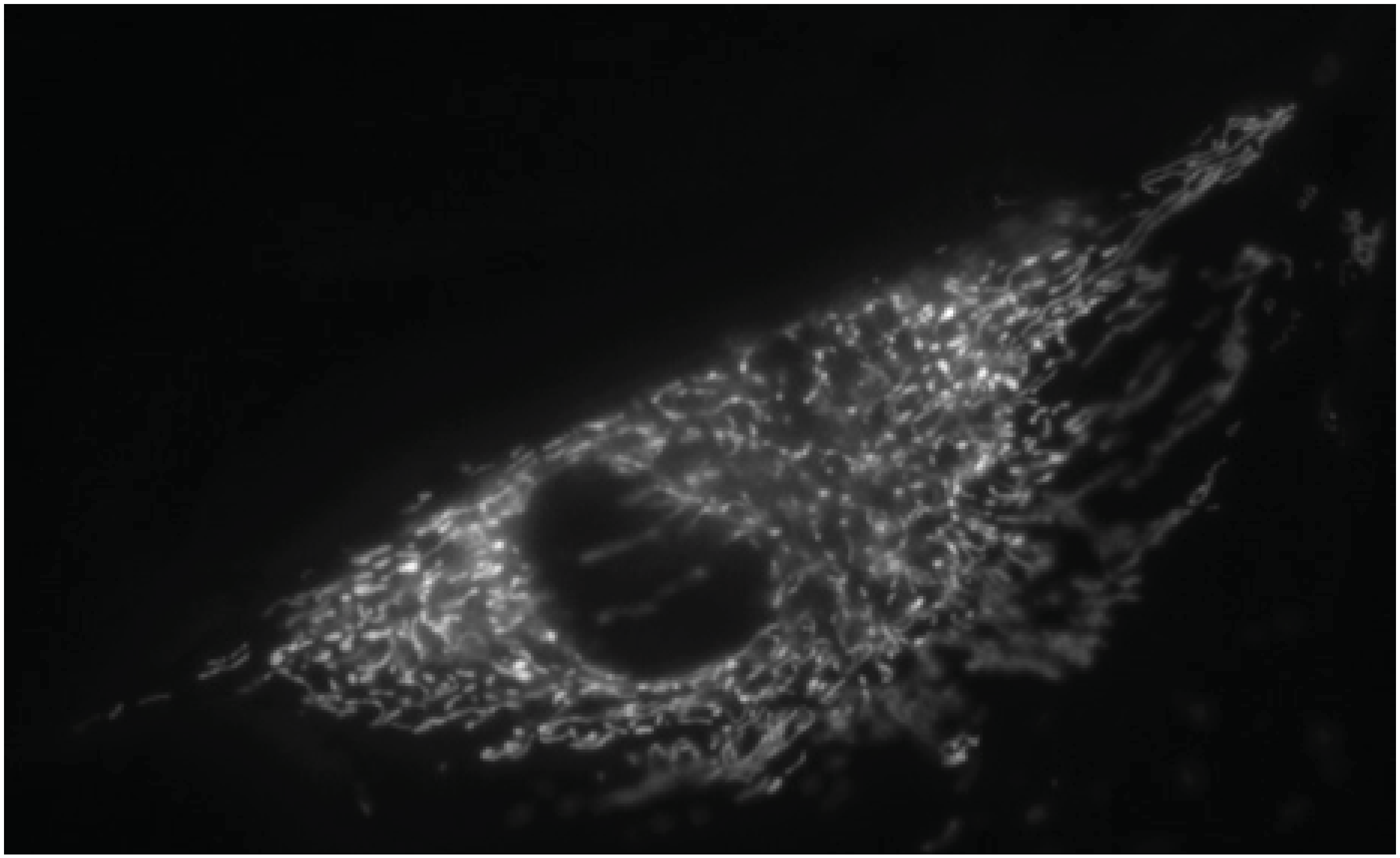
Physical factors that elicit a response

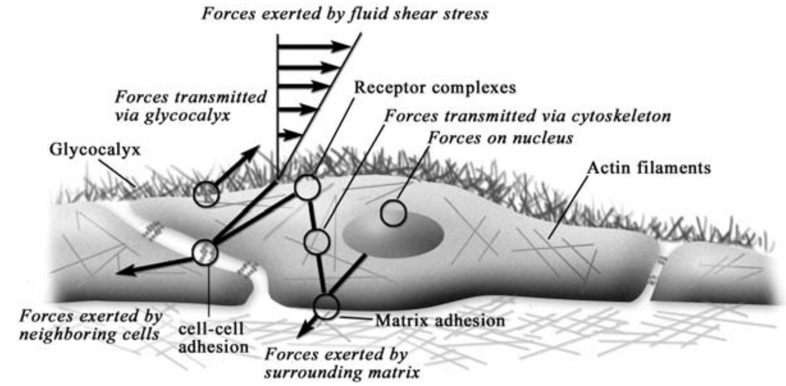
- Fluid dynamic shear stress (> 0.5 Pa)
- Cyclic strain of cell substrate (> 1%)
- Osmotic stress
- Compression in a 3D matrix
- Normal stress (> 500 Pa)
- Mechanical perturbations via tethered microbeads (> 1 nN)

Forces applied at one point in the cell, are transmitted via the cytoskeletal network.



Fibroblast with fluorescent mitochondria forced by a magnetic bead. D. Ingber, P. LeDuc

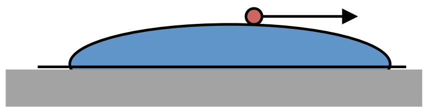
Mechanotransduction: Current theories



Source: Mofrad, Mohammad RK, and Roger D. Kamm, eds. *Cellular Mechanotransduction: Diverse Perspectives from Molecules to Tissues*. Cambridge University Press, 2009.

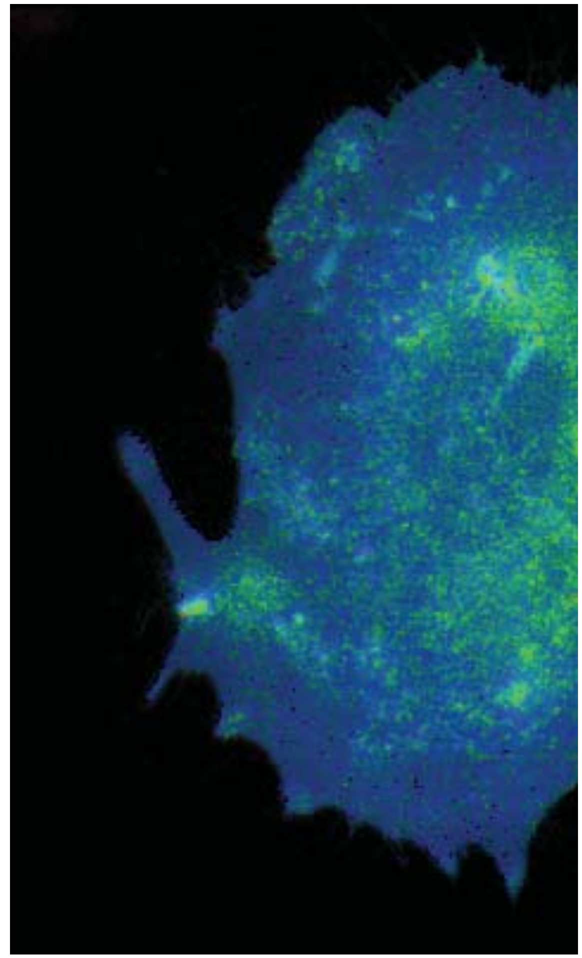
- Changes in membrane fluidity and the diffusivity of transmembrane receptors --> receptor clustering (Butler, 2002, Wang, 2004)
- Direct mechanical effects on the nuclear membrane, DNA, and gene expression (Ingber)
- Stretch-activated ion channels (Gullinsgrud, 2003, 2004)
- Glycocalyx deformation coupling to the cortical cytoskeleton (Weinbaum, 2003)
- Force-induced changes in the conformation of load-bearing proteins (Schwartz, 2001, Jiang, 2003, Bao, 2002)
- Constrained autocrine signaling (Tschumperlin, et al., 2004)

Early events in mechanotransduction: 1) Protein activation progresses in a wave from the site of bead forcing (Wang et al., 2005)



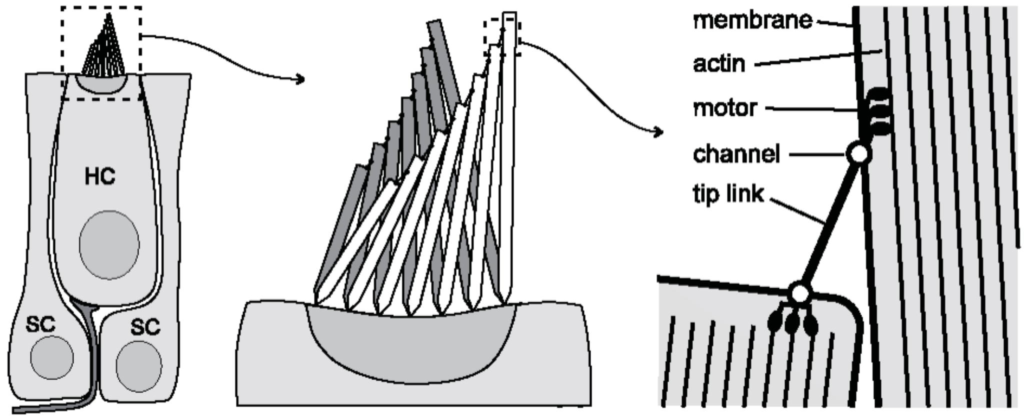
- Response of a membrane-targeted Src reporter.
- Phosphorylation of a domain taken from a c-Src substrate, P130cas, leads to a conformational change that reduces FRET.
- A wave of activation propagates away from the site of forcing at a speed of ~18 nm/s

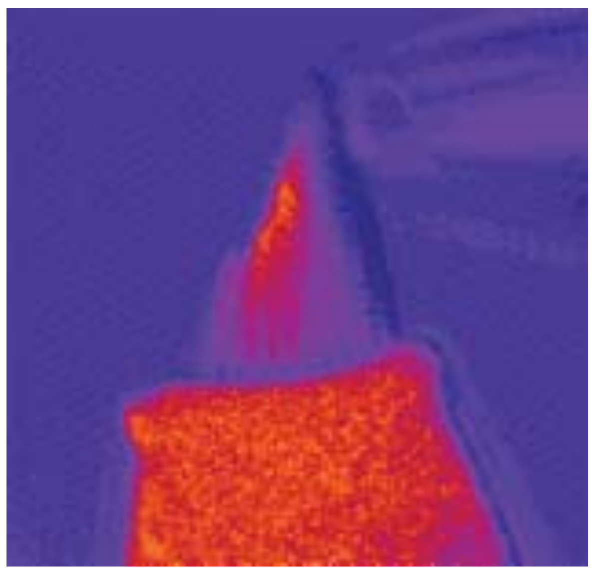
Neither mechanism -- of force transduction or propagation of activation wave -- are understood



Stretch-activated ion channels constitute one method of mechanotransduction

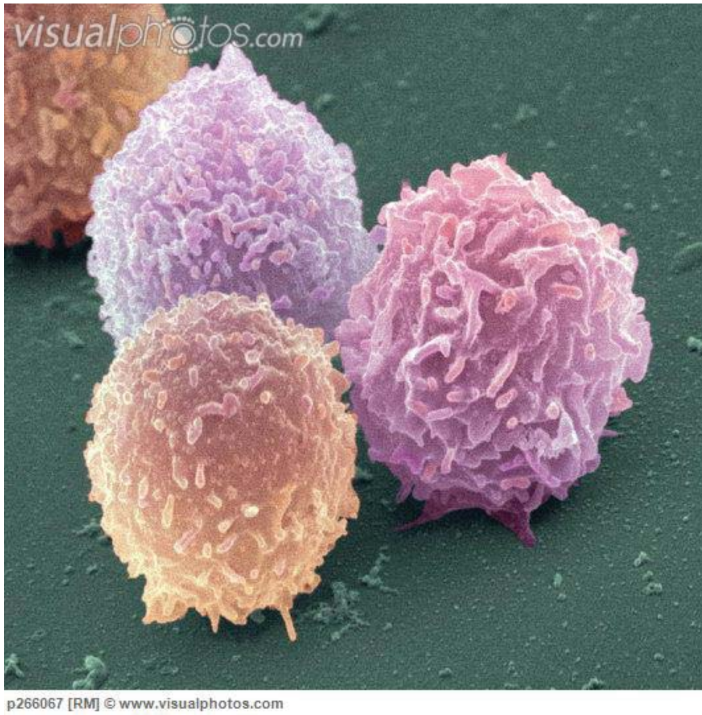
SEM of the stereocilia on the surface of a single hair cell (Hudspeth)



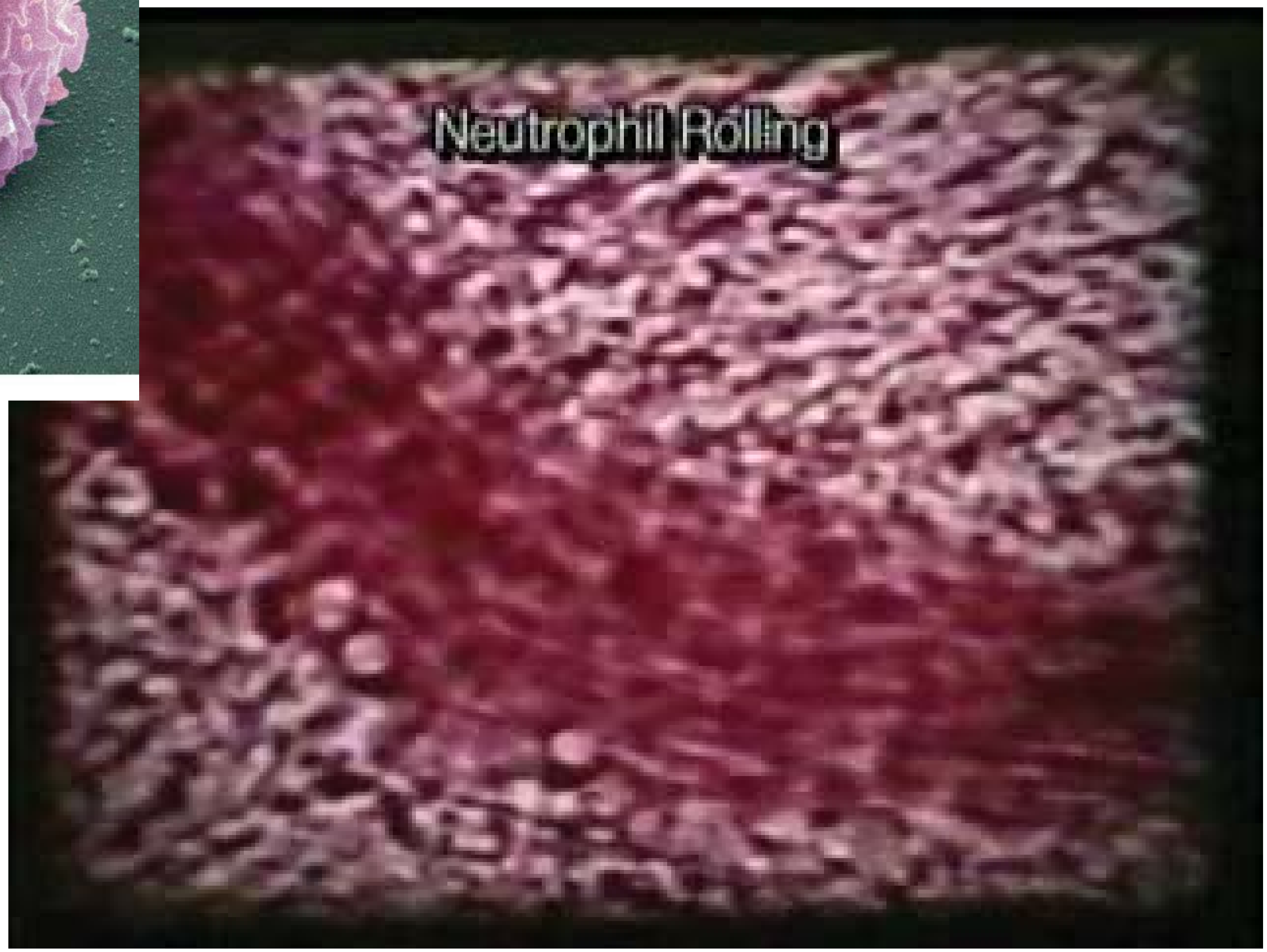


Tension in the tip link activates a stretch-activated ion channel, leading to intracellular calcium ion fluctuations.

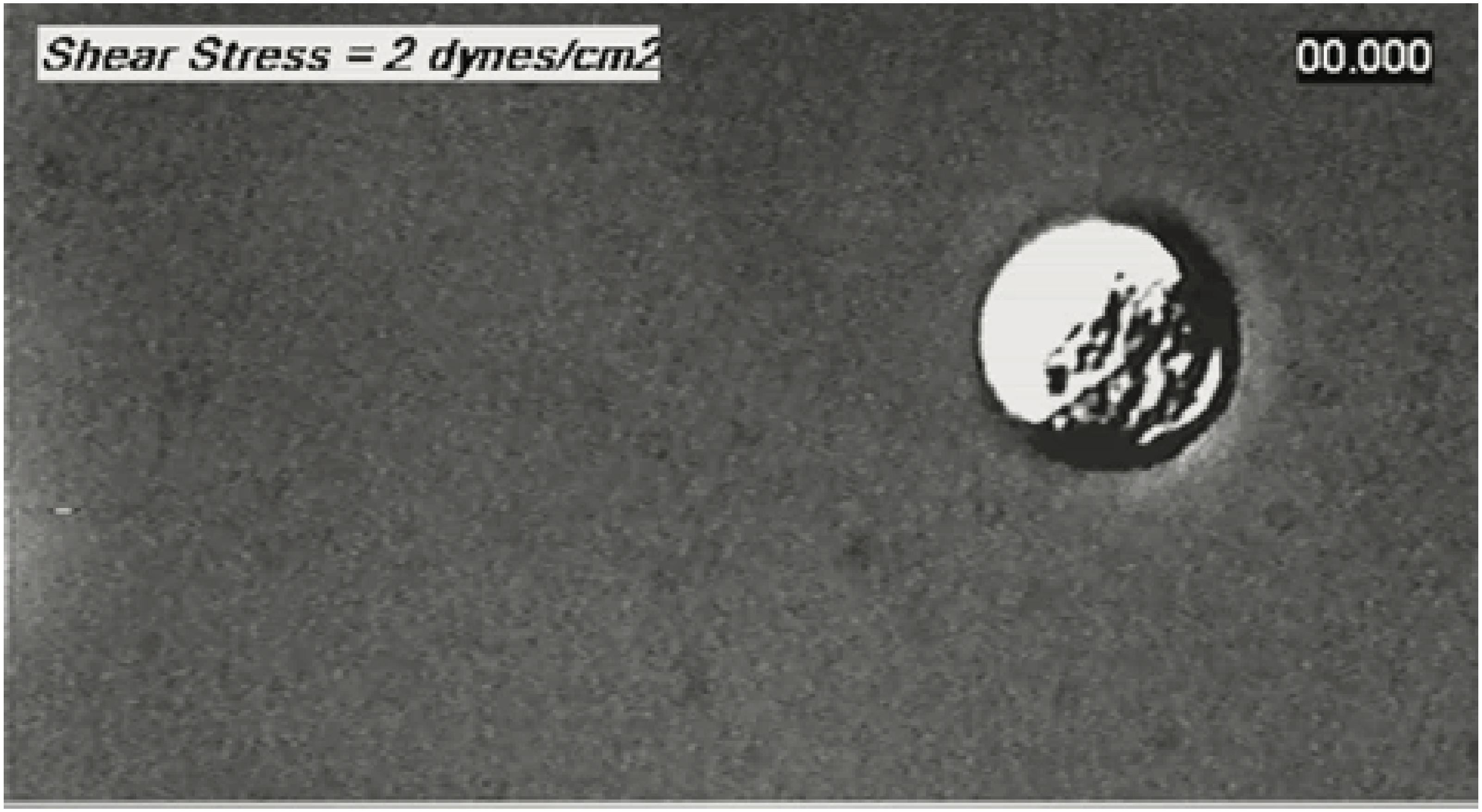
Leukocyte rolling and transient adhesion



http://labs.idi.harvard.edu/springer/pages/multimedia



Courtesy of Timothy A. Springer. Used with permission.



http://cbr.med.harvard.edu/investigators/springer/lab/lab_goodies/
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Leucocyte adhesion/arrest and transmigration

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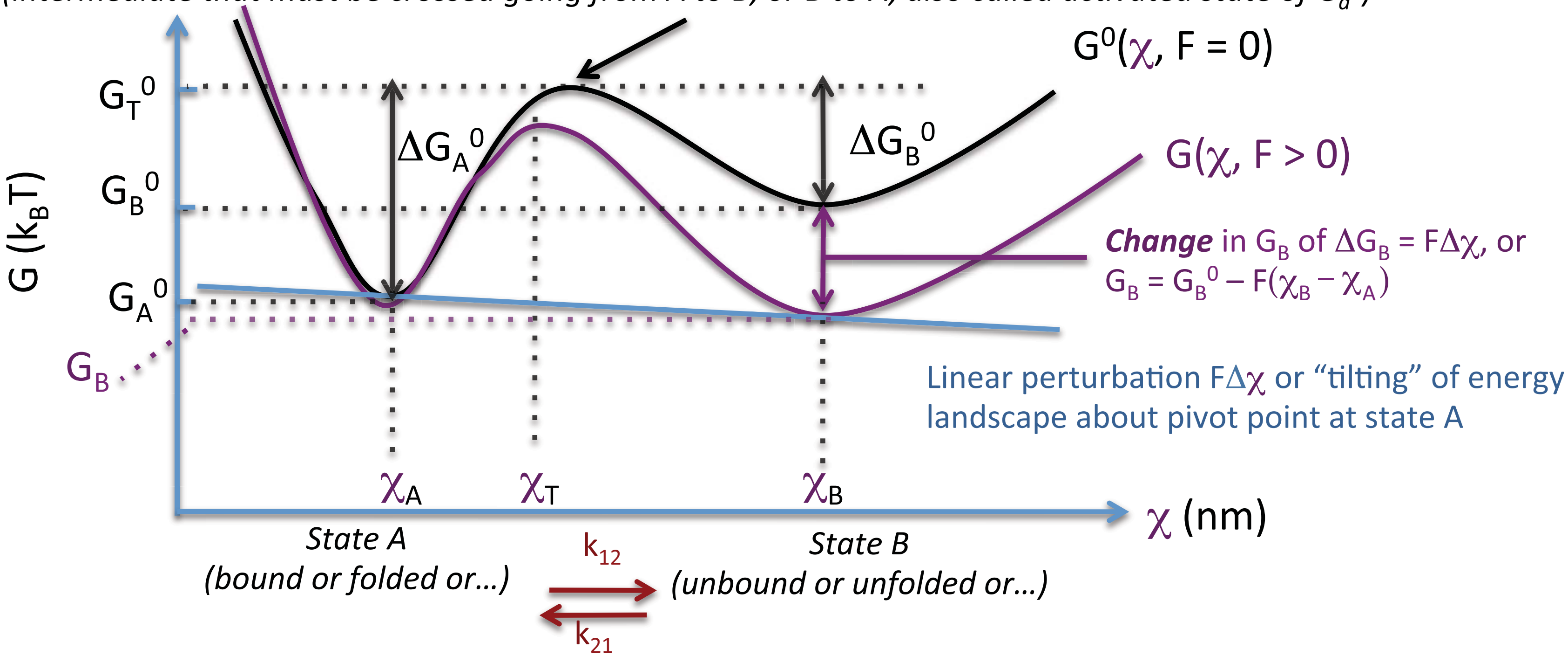
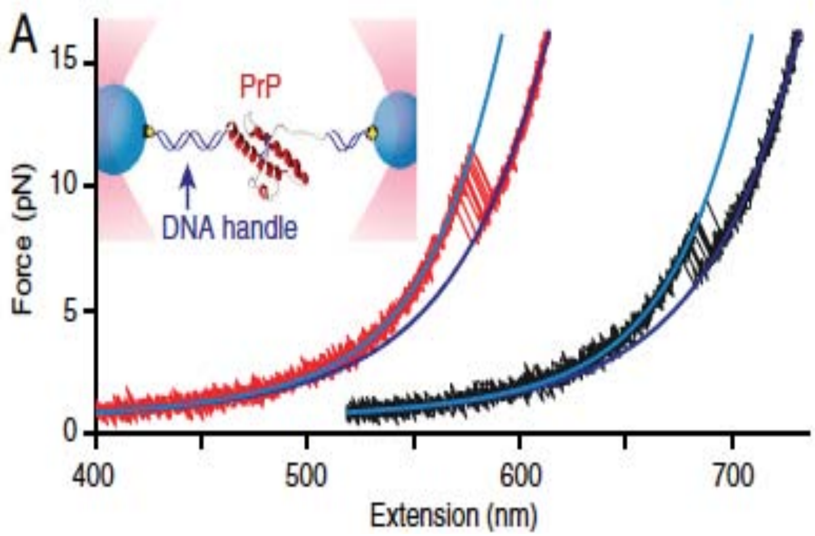
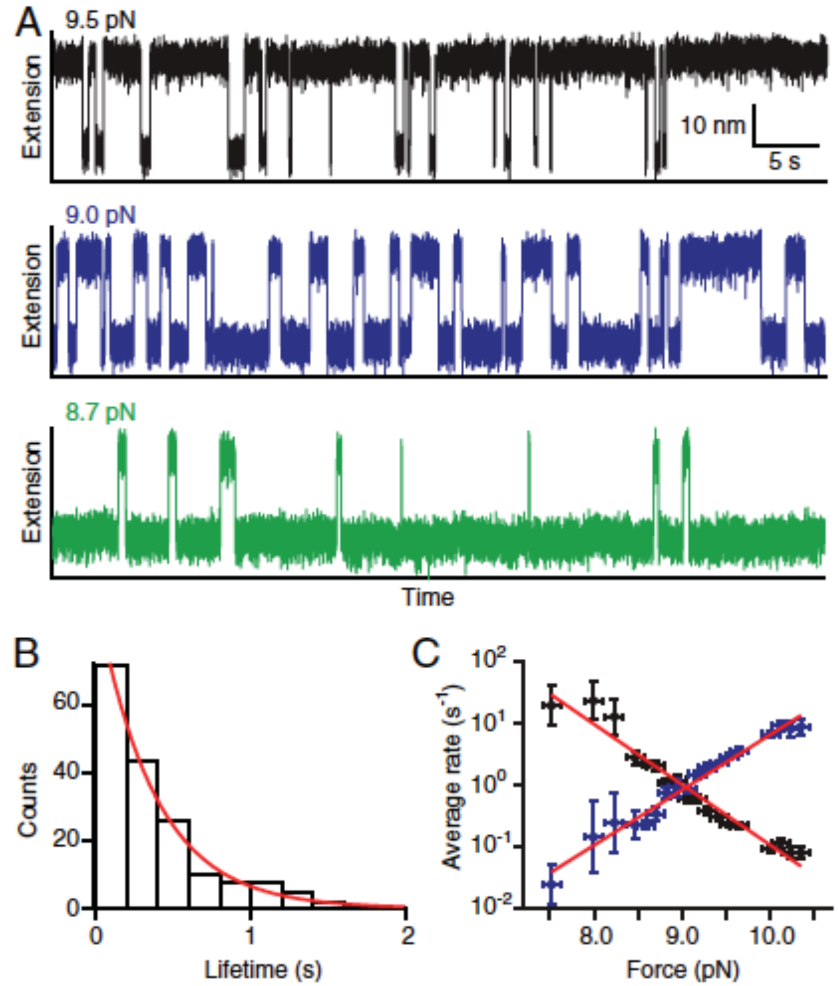
1D Energy Landscape with Two States: Perturbed by extrinsic force



G_A^0 =	free energy of state A, in absence of constant applied force F
G_A =	free energy of state A, in presence of constant applied force F, and = $G_A^0 - F(\chi_A - \chi_A) = G_A^0$
G_B =	free energy of state B, in presence of constant applied force F, and = $G_B^0 - F(\chi_B - \chi_A)$
ΔG_A^0 =	activation energy that must be overcome to get from state A to transition state T under no F, and = $G_T^0 - G_A^0$
p_A^0 =	probability of being in state A in absence of constant F, given by Boltzmann distbn = $1/Z \exp(-G_A^0/k_BT)$ where Z is partition funct.
K_{eq}^0 =	equilibrium constant in absence of F; dimensionless ratio of probabilities of being in state B to state A, defined as $p_B^0/p_A^0 = \exp(G_A^0 - G_B^0)/k_BT = \exp -[(G_B^0 - G_A^0)/k_BT = -\Delta G_{AB}^0/k_BT$
K_{eq} =	equilibrium constant in presence of F; dimensionless ratio of probabilities of being in state B to state A, defined as $p_B/p_A = \exp -[(G_B - G_A)/k_BT = \exp\{-[\Delta G_{AB}^0 - F(\chi_B - \chi_A)]/k_BT\} = K_{eq}^0 \exp\{[+F(\chi_B - \chi_A)]/k_BT\}$
k_{12}^0 =	transition rate from A to B governed by the activation energy barrier moving from A to B, in absence of constant F, in [1/sec] and = $C \exp(-\Delta G_A^0/k_BT)$, where C is a pre-exponential frequency factor (Lecture #4)
k_{12} =	transition rate from A to B in the presence of constant F, in [1/sec] and = $C \exp(-(\Delta G_A^0 - F(\chi_T - \chi_A))) = k_{12}^0 \exp(+F(\chi_T - \chi_A))$. Note $k_{21} = C \exp(-(\Delta G_B^0 - F(\chi_T - \chi_B))) = k_{21}^0 \exp(+F(\chi_T - \chi_B))$

State probabilities, rate constants and transition times





Courtesy of Michael Woodside. Used with permission.
Source: Yu, Hao et al. "Energy Landscape Analysis of Native Folding of the Prion Protein yields the Diffusion Constant, Transition Path Time, and Rates." *Proceedings of the National Academy of Sciences* 109, no. 36 (2012): 14452-7.

$$k_+ = C \exp\left[-\frac{\left(G_a - G_1\right) - F\left(x_a - x_1\right)}{k_B T}\right]$$

Essential equations

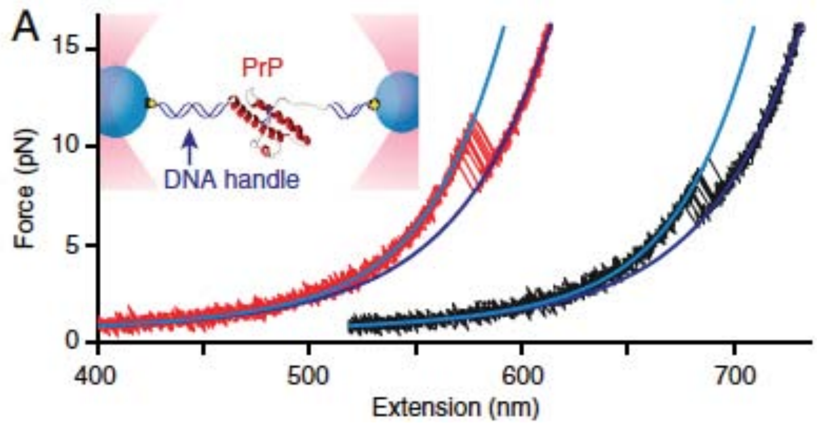
$$\frac{p_2}{p_1} = K_{eq} = \frac{k_+}{k_-} = \exp\left[-\frac{\left(G_2 - G_1\right) - F\left(x_2 - x_1\right)}{k_B T}\right]$$

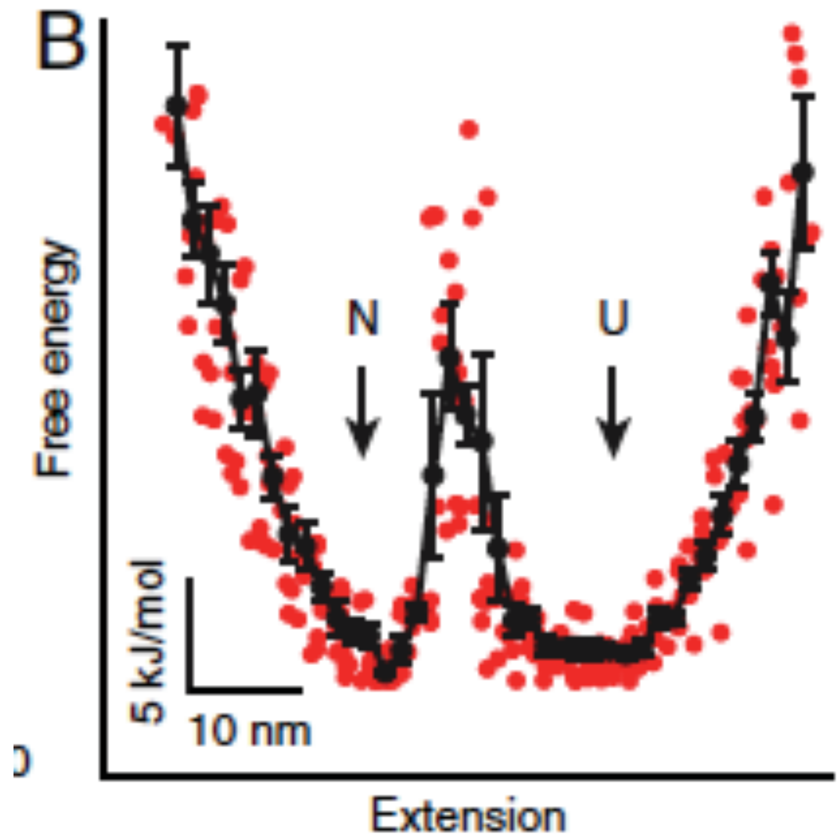
$$k_+ = C\exp\left[-\frac{\left(G_a - G_1\right) - F\left(x_a - x_1\right)}{k_B T}\right]$$

$$k_- = C\exp\left[-\frac{\left(G_a - G_2\right) - F\left(x_a - x_2\right)}{k_B T}\right]$$

56

Measured energy landscape for PrP at F = 9.1 pN





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