The Nanoscale Biophysics of Microscale Cell Adhesion

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Outline

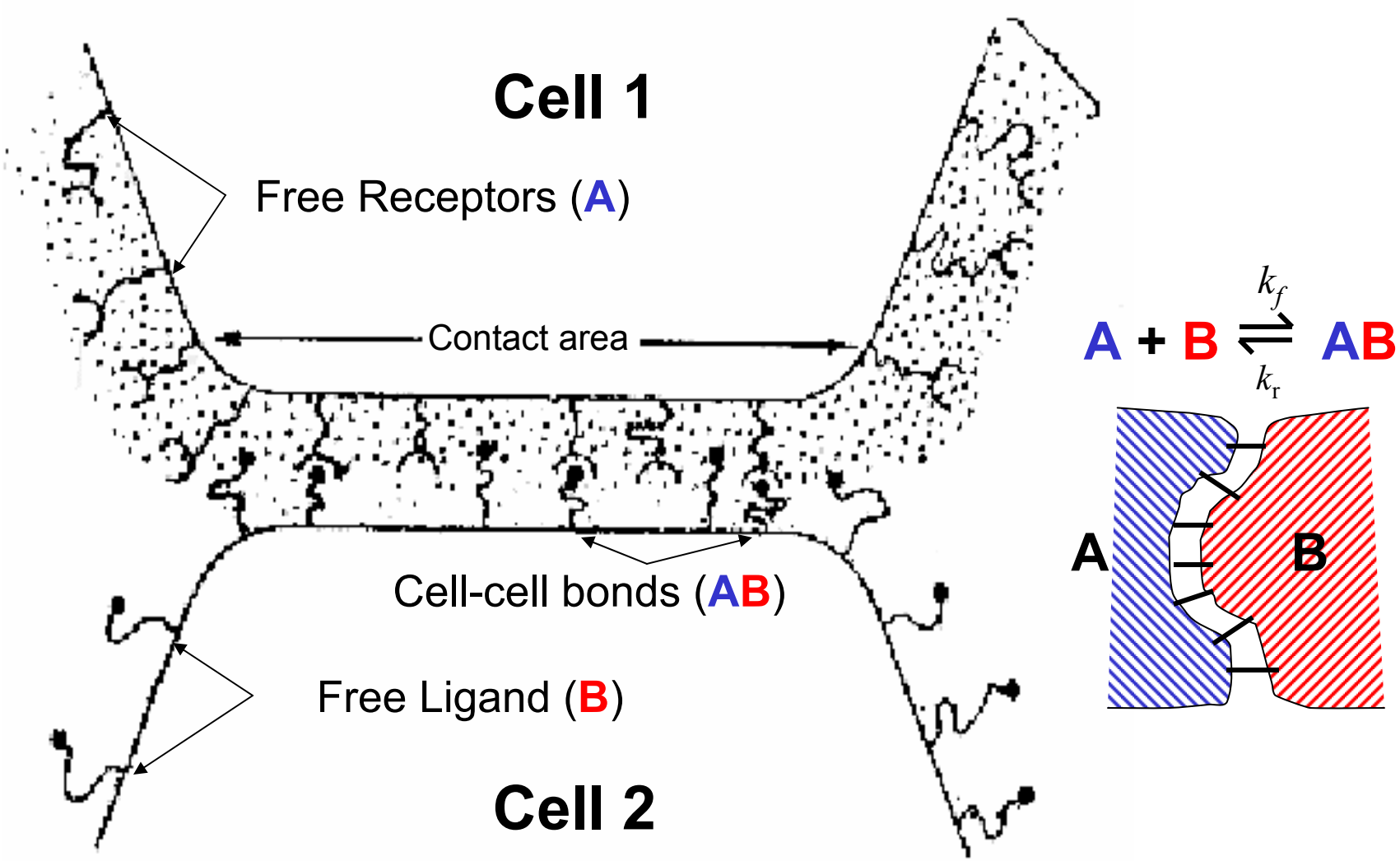
1) Adhesion molecules and review of cell-scale phenomena
2) Force dependence of reaction rates
3) Methods for applying forces to single bonds
4) Force spectroscopy for unbinding
5) Forced unfolding
6) Computational forced unbinding
7) Ancillary topics
8) Summary

Appendix 1—Reaction rates

Appendix 2—Bell model

Appendix 3—Reliability theory

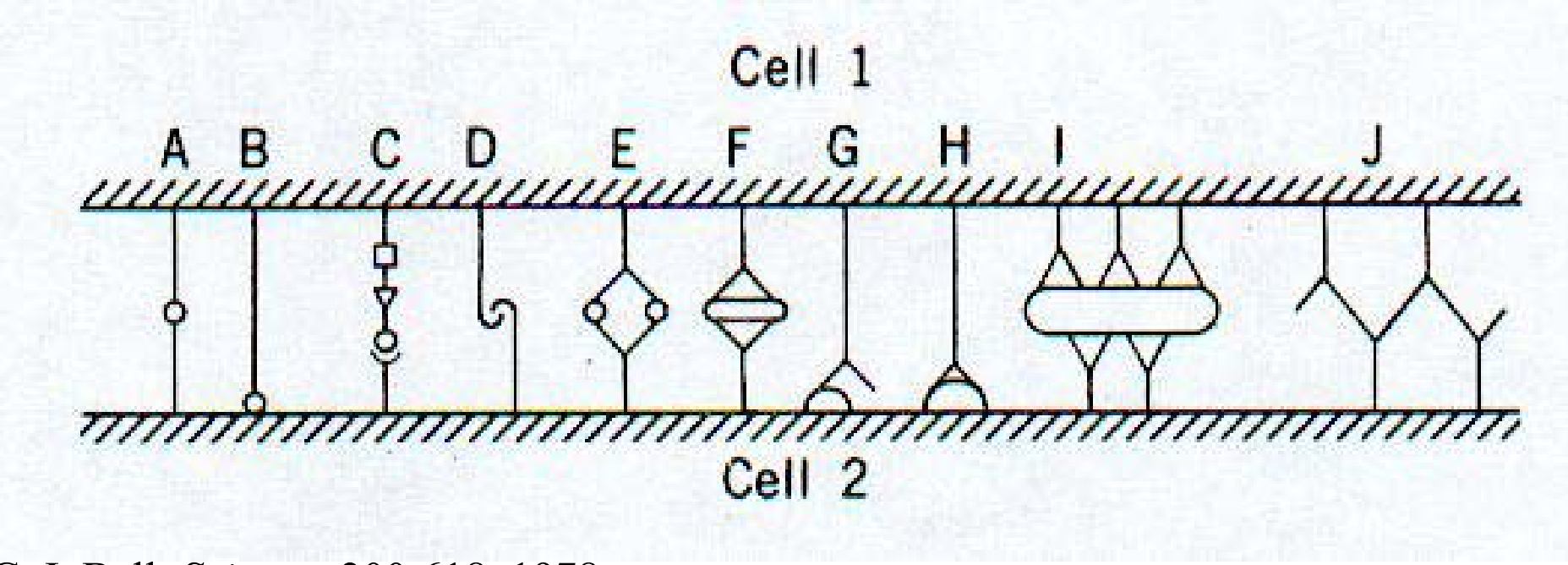
Cell Adhesion: Microscale to Nanoscale



G. I. Bell et al., *Biophys. J.* 45:1051, 1984

Adhesion Molecule Configurations

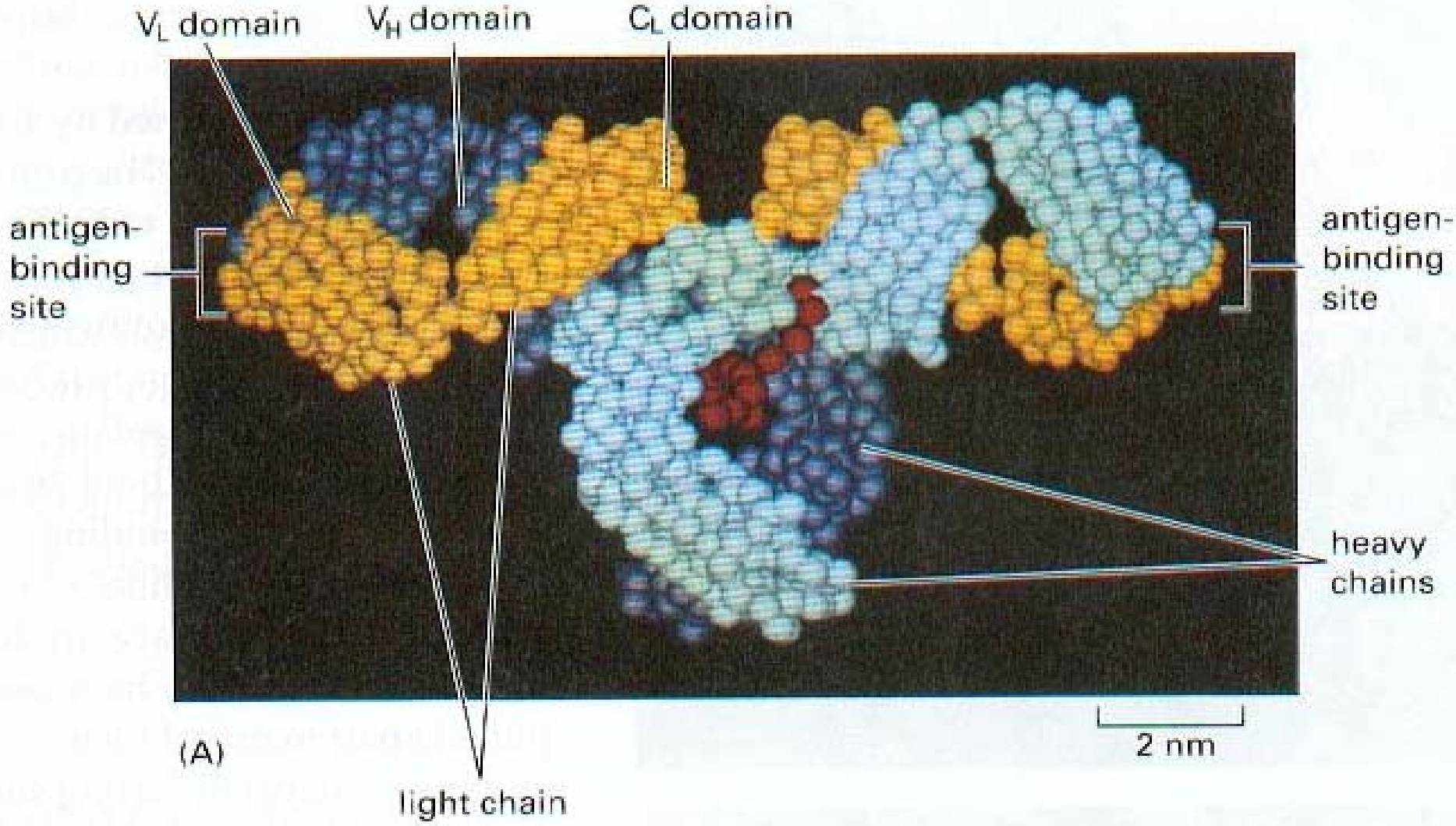
- Molecules can bind by:
- Direct binding (A, B, D)
  - AA: homophylic
  - AB: heterophylic
- Multivalent binding of various sorts (E, F, H, I, J)
- Crosslinking by a third molecule or particle (C,F,I)



G. I. Bell, *Science*, 200:618, 1978

Antibody Detail

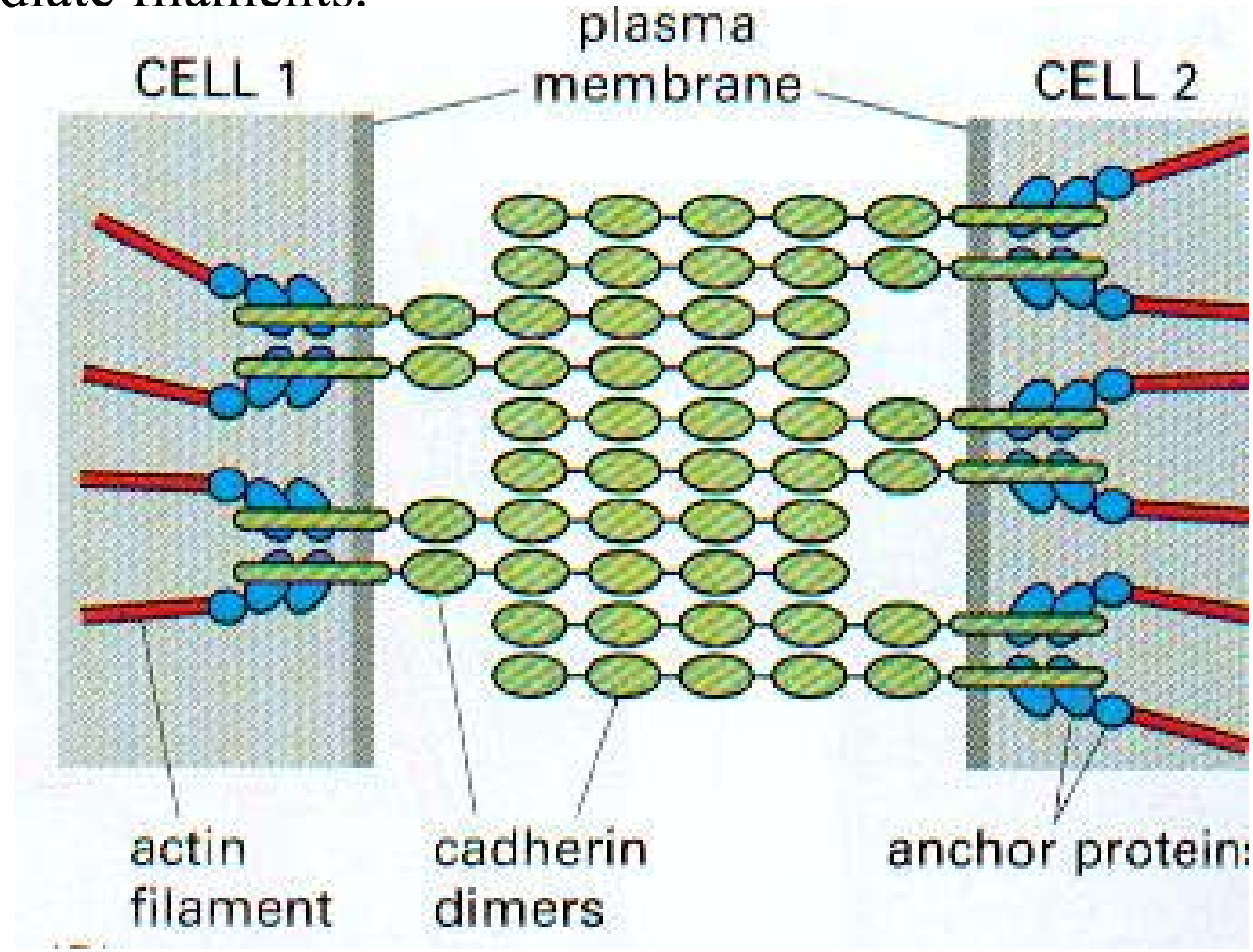
- Until the 1980's antibodies (and lectins) were the only adhesion molecules known



Alberts et al, Molecular Biology of the Cell, 2002

Adhesion Molecules Hold Cells Together

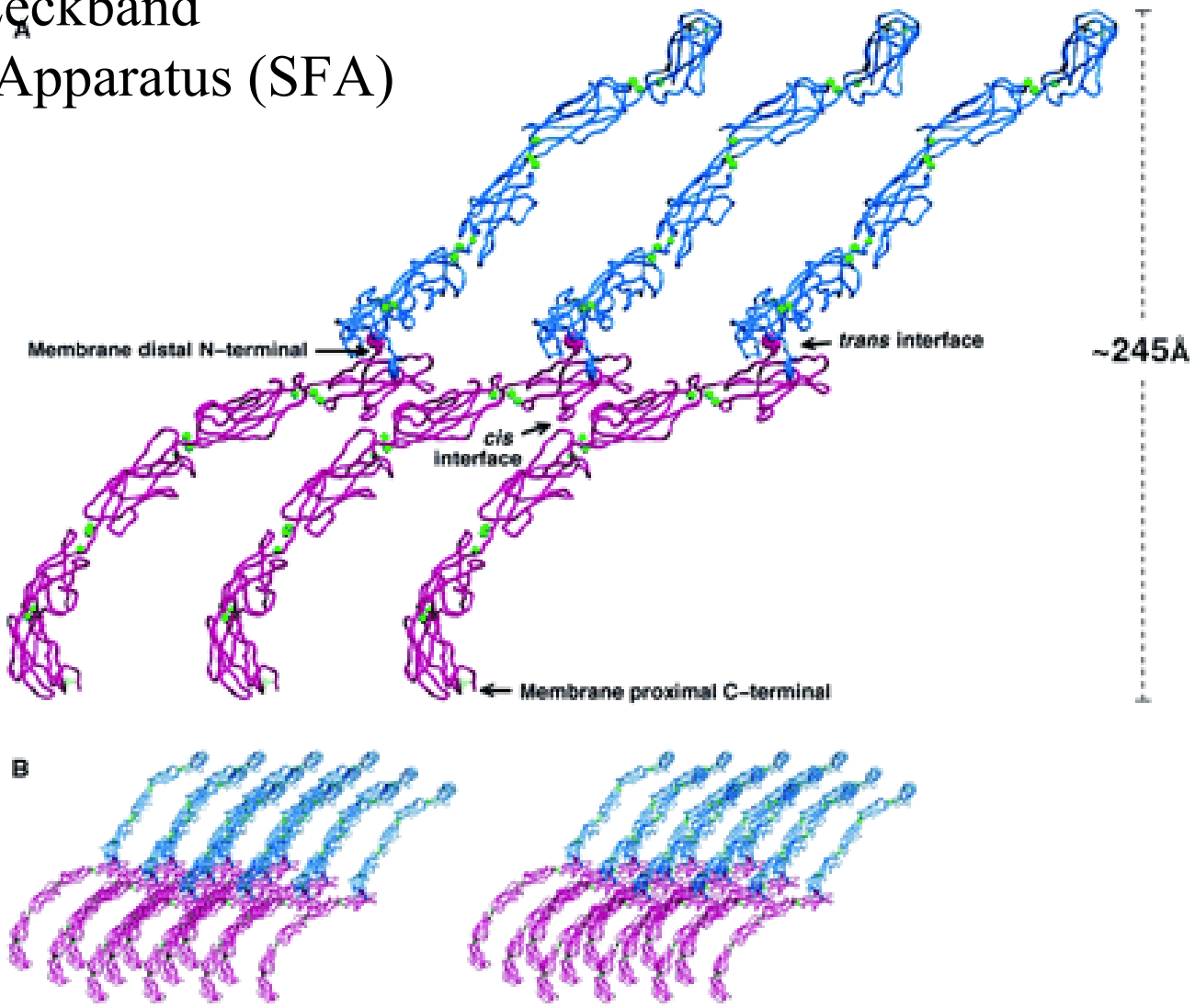
- Tissue mechanical cohesion mediated substantially by Cadherin (Calcium + Adherin) molecules. Cadherins can be bound to actin or intermediate filaments.



Alberts et al, Molecular Biology of the Cell, 2002

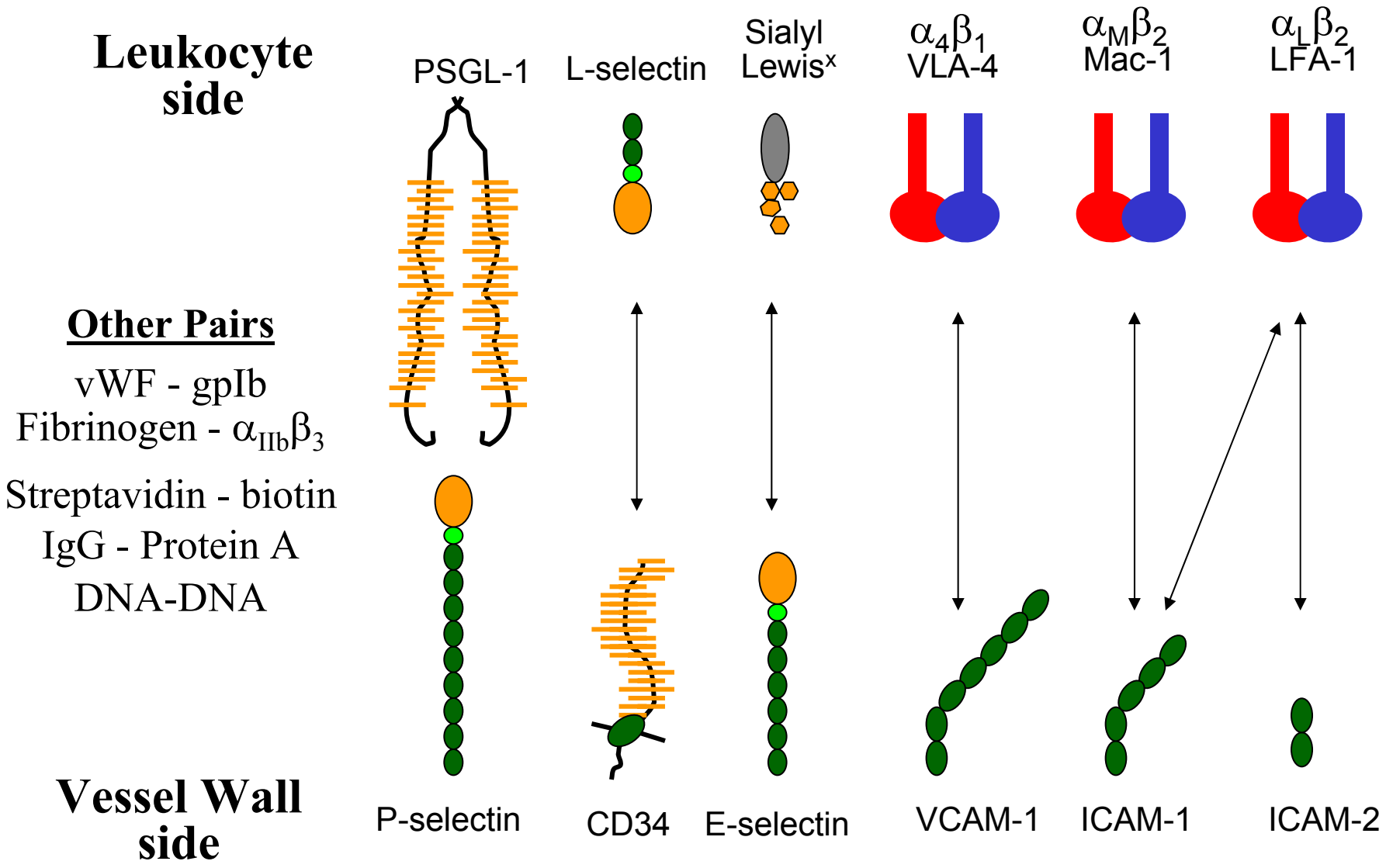
Cadherins

- Cadherins can group together in arrays of bonds.
- Work by Deborah Leckband with Surface Force Apparatus (SFA)



Boggon et al, *Science*, 276:1308, 2002

Receptor-Ligand Pairs for White Cells



Receptor vs Ligand

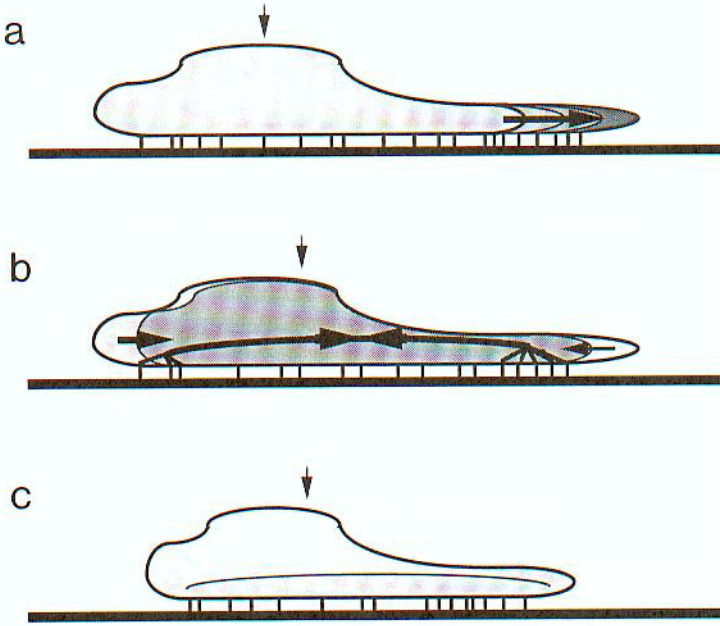
- By convention:
- For molecule-molecule binding:
  - Ligand is molecule dissolved in solution
  - Receptor is molecule bound to a surface (cell or glass/plastic substrate)
- For cell-cell binding:
  - Ligand is molecule mounted on freely suspended cell
  - Receptor is molecule mounted on cell attached to a substrate.

Mechanical Forces in Biology

- Phenomena for which mechanical forces play a major role:
  - Cell Adhesion in flowing blood
  - Cell migration in tissue
  - Cell mechanical deformation and realignment
  - Muscle contraction
- External Forces:
  - Viscous drag
  - Hookean forces
- Intermolecular Forces:
  - Charge-charge interactions
  - charge-dipole, dipole-dipole interactions
  - Hydrophobic interactions
  - Entropic forces
- Energy available ~ kT = 0.027 eV = 4.3 pN·nm at 37°C.

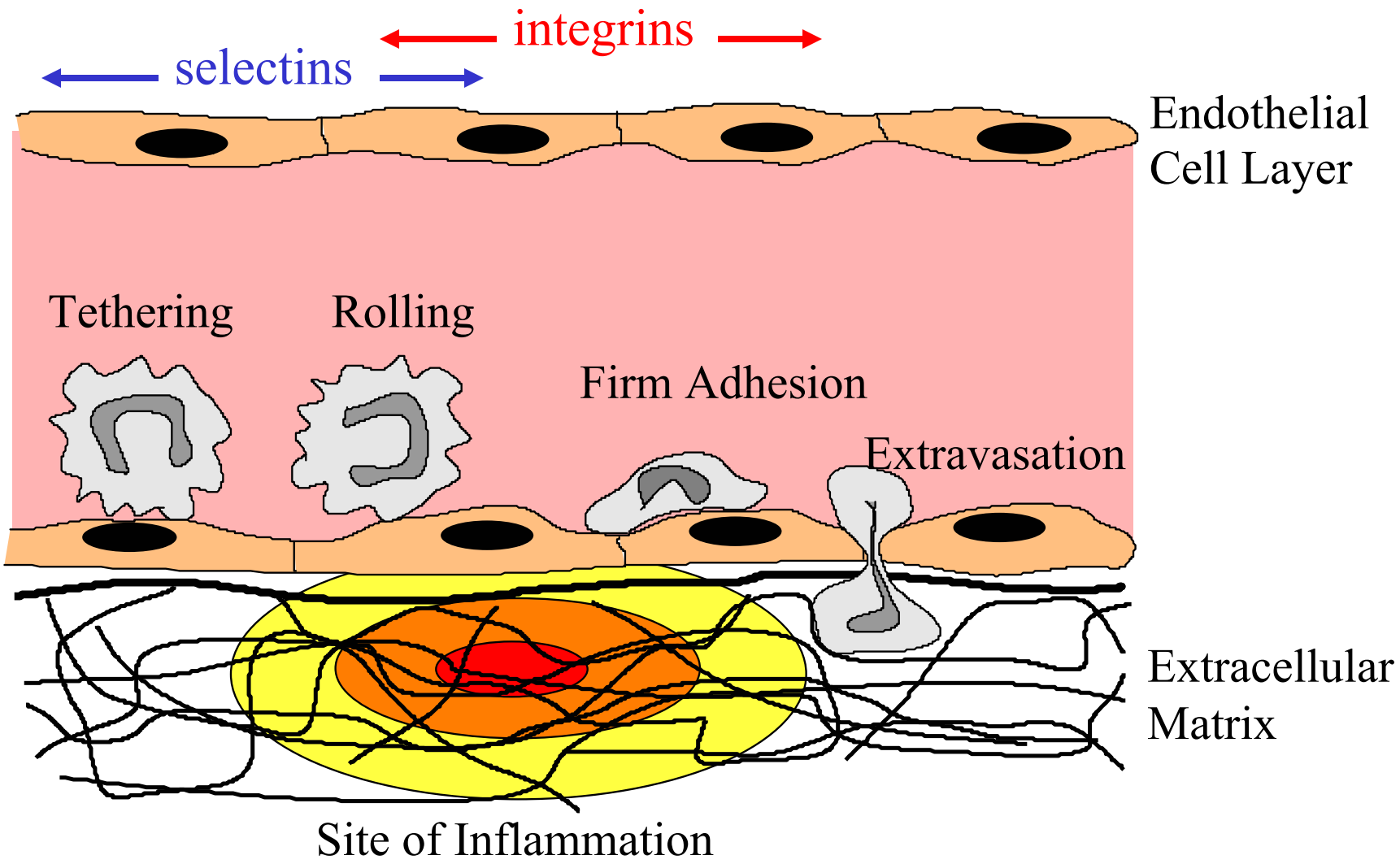
Cell Migration

- Many cells (e.g. fibroblasts, cancer cells, white blood cells) can migrate through extracellular matrix using adhesion molecules for "traction"
- Adhesion needs to be strong enough to support traction, but not so strong that it locks the cell in place ("ice" vs "glue")
- Although this picture (and many experiments) show cells moving in 2-D, true motion is in all 3 dimensions
- Cells also need to secrete proteases to dissolve extracellular matrix ahead of the cell



Lauffenburger & Linderman, *Receptors*, Oxford, 1993

Rolling



Adapted from Springer, *Cell* 76:301, 1994

Order of Magnitude for Bond Strength

- For a long time (and even today) researchers have been trying to quantify "bond strength".
- Bell asked whether there was any meaningful answer to this.
  - Suppose a free energy change E_o is required to disrupt the bond.
  - Suppose further that the distance through which one must pull the bond to get it to dissociate is r_o.
- One can then define a bond strength, f_o as:

$$f_o = \frac{E_o}{r_o}$$

- Consider an antibody. Suppose:
  - $E_o \sim 0.37$ eV or ~13 kT
  - Dimension of the antibody binding cleft is $r_o \sim 0.5$ nm,
- Get

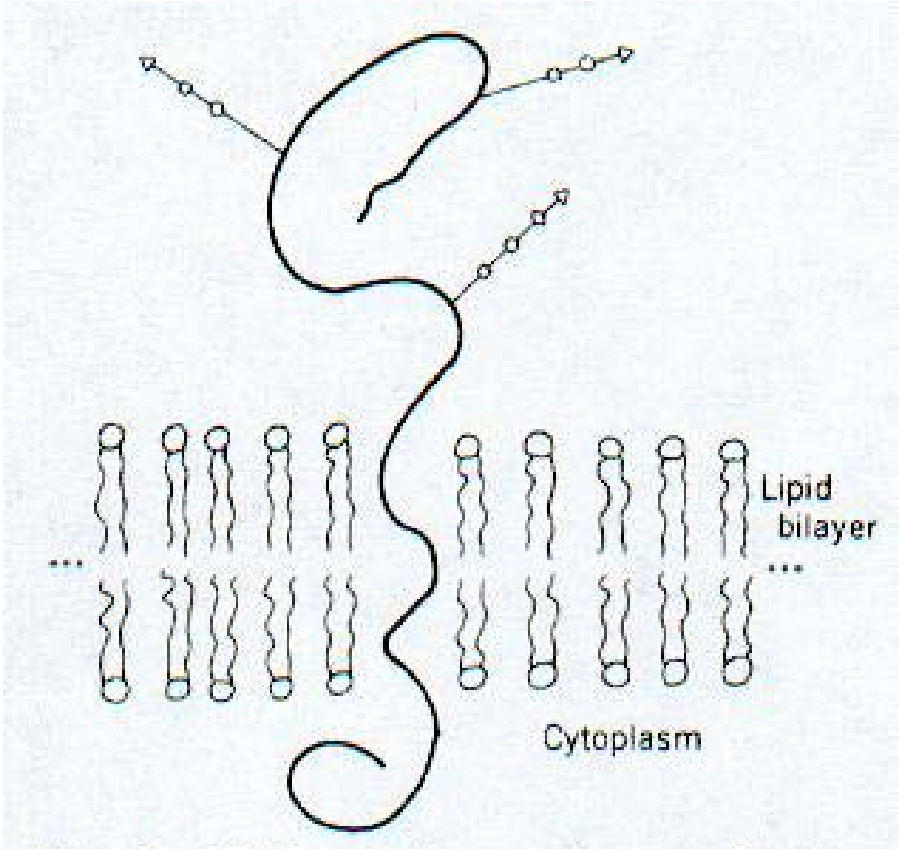
$$f_o = \frac{E_o}{r_o} = \frac{(13)(4.3\ \text{pN}\cdot\text{nm})}{0.4\ \text{nm}} = 133\ \text{pN}$$

G. I. Bell, *Science*, 200:618, 1978

Bond Extraction from Membrane

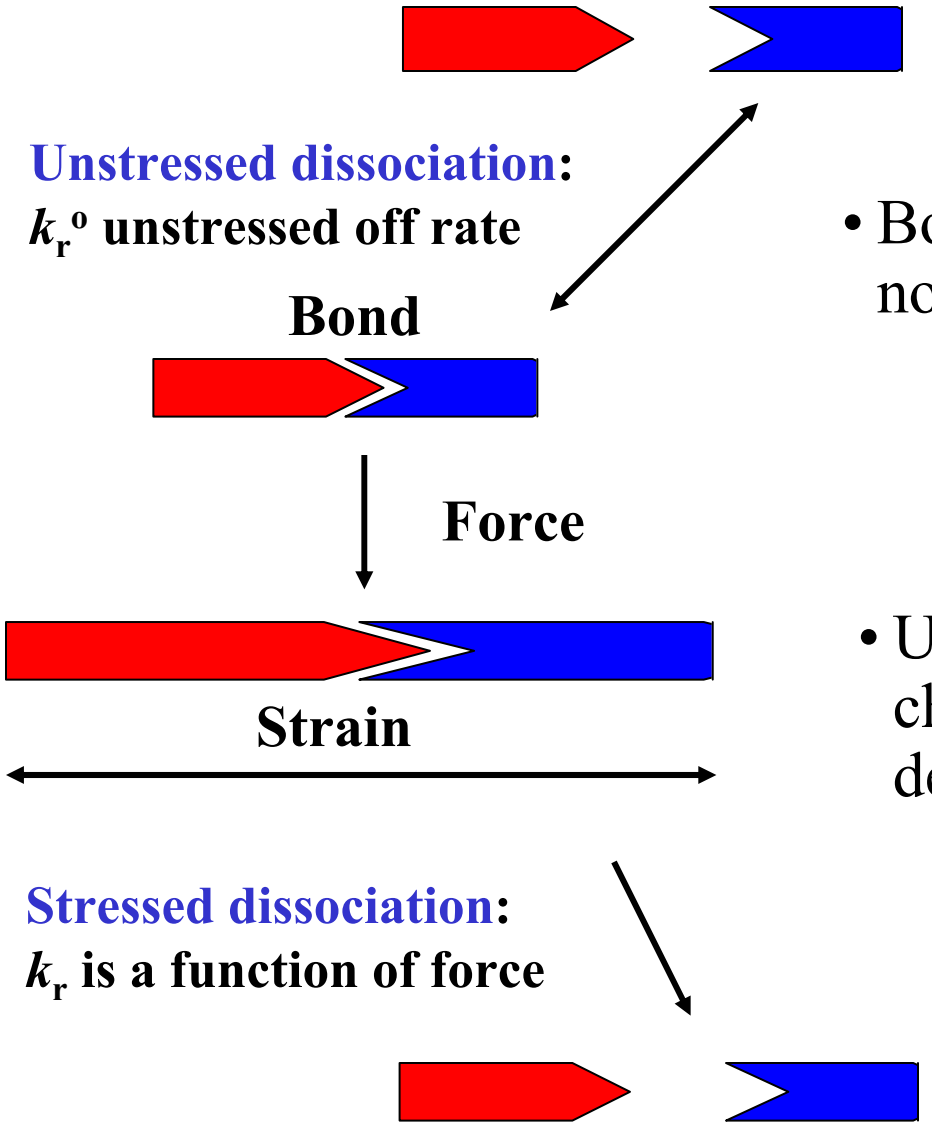
- One can estimate the force to uproot a receptor from the membrane during forced unbinding using $f_o \sim E_o/r_o$:
- Calculate free energy change when hydrophobic amino acid residues moved into water and hydrophilic residues
- Bell gets that $E_o \sim 2.6$ eV/molecule
- For r_o choose thickness of bilayer:

  so $r_o \sim 4$ nm.
- We get $f_o \sim E_o/r_o = 100$ pN
- **This is same order of magnitude as force to rapidly break a bond!**
- One could do better if protein bound to cytoskeleton
- This cytoskeletal linkage could be target for regulation of migration



G. I. Bell, *Science*, 200:618, 1978

Force Dependence of Reaction Rates



- Bonds will dissociate even under no applied force.

- Under load the reaction rate changes. Reverse reaction rate, k_r depends on applied force/bond, f

Bell Model

- Bond Dissociation is a barrier crossing process:

$$k_r(f) = k_r^o(r_o, E_o, f) \exp[\Delta E(r_o, f)/kT]$$

Bell Model applies for a "sharp" transition state:

$\Delta E = r_o f$; k_r^o = constant

$$k_r(f) = k_r^o \exp\left[r_o f / k_B T \right]$$

where:

k_r^o is k_r when $f = 0$

k_B is Boltzmann's constant

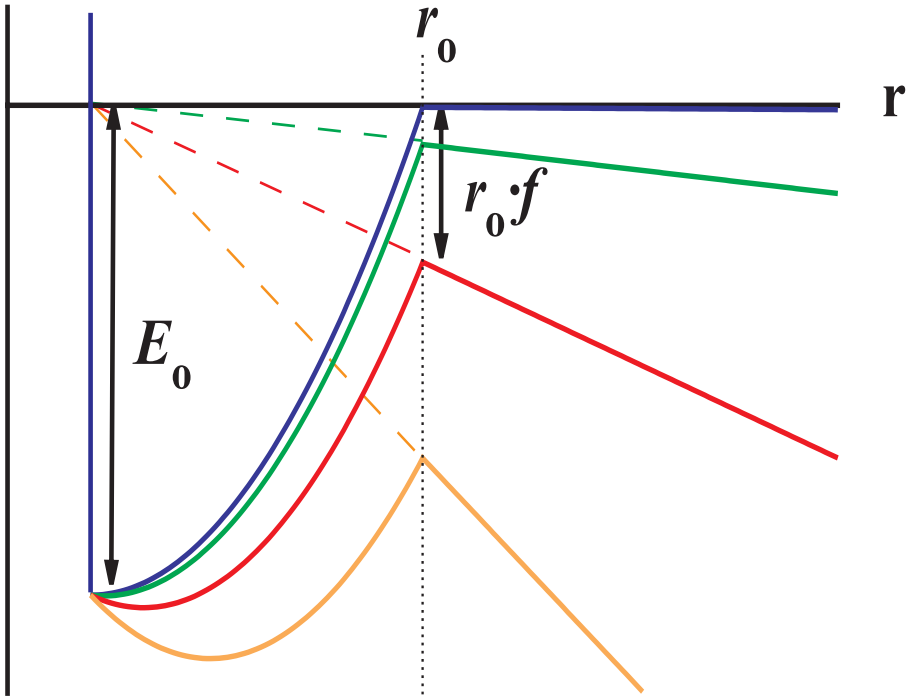
T is absolute temperature

r_o is "reactive compliance"

$r_o > 0$: slip bond

$r_o = 0$: ideal bond

$r_o < 0$: "catch" bond (Marshall et al., *Nature*, 423: 190-193, 2003 and Thomas et al., *Cell*, 109:913-923, 2002)

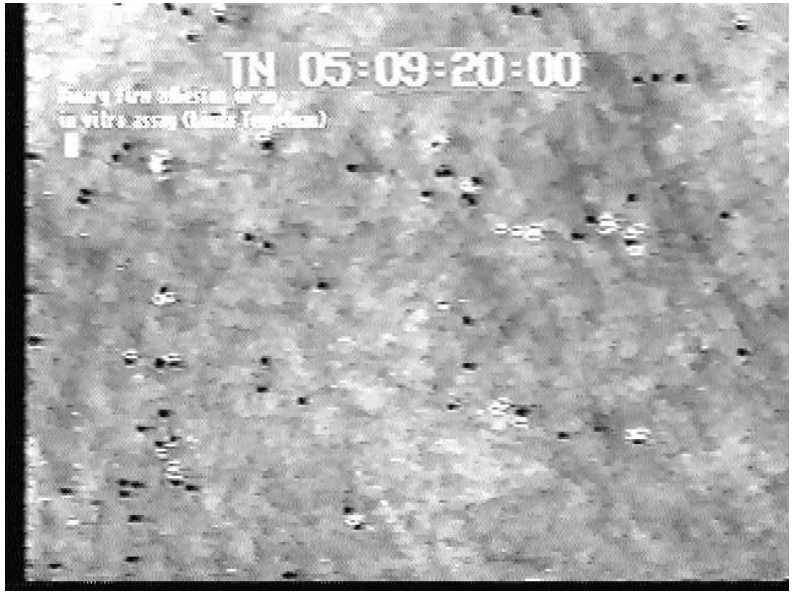


Bell, *Science*, 200:618 1978; Evans, *Faraday Discuss.* 111:1-16, 1998

Adhesive Phenotype

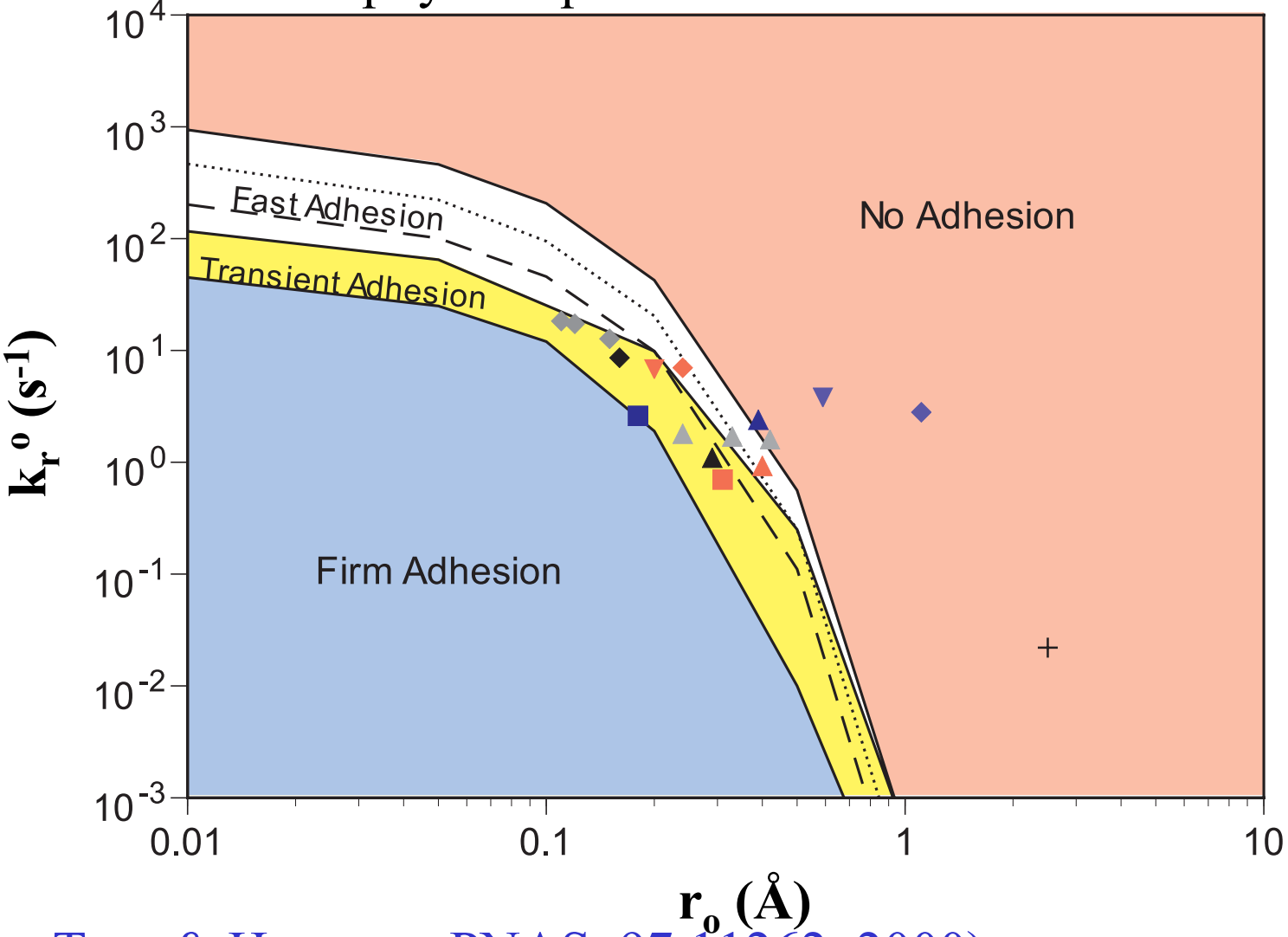
Cell-free rolling

Sudden firm arrest



State Diagram for Adhesion

Computer simulations of microscale adhesion illustrate the importance of nanoscale bond biophysical parameters



(Chang, Tees & Hammer, PNAS, 97:11262, 2000)

Kramers Transition State Theory

- Start from a modified Smoluchowski equation for diffusion current of states, j, in the additional presence of an external applied force f.

$$j = D\left[\left(f - \frac{dU}{dx}\right)\frac{\sigma}{kT} - \frac{d\sigma}{dx}\right]$$

- Integrate diffusion current from bottom of potential to transition state, and find the reaction rate:

$$k_r(f) = \frac{D}{l_{well} l_{ts}(f)} \exp\left\{\left[-U_{well} + \Delta U(f)\right]/kT\right\}$$

- Here:
  - D = diffusion constant
  - l_{well} ~ localization of states at bottom of potential well
  - $l_{\text{ts}}(f)$ ~ width of potential
  - U_{well} = depth of potential well at $f = 0$
  - $\Delta U(f)$ = reduction in barrier height with f

Evans & Ritchie, *Biophys. J.* 72:1541, 1997

Evans-Kramers Theory

- Grouping terms that contain force:

$$k_r(f) = \frac{k_r^o}{l_{ts}(f)} \exp[\Delta E(f)/kT]$$

- where

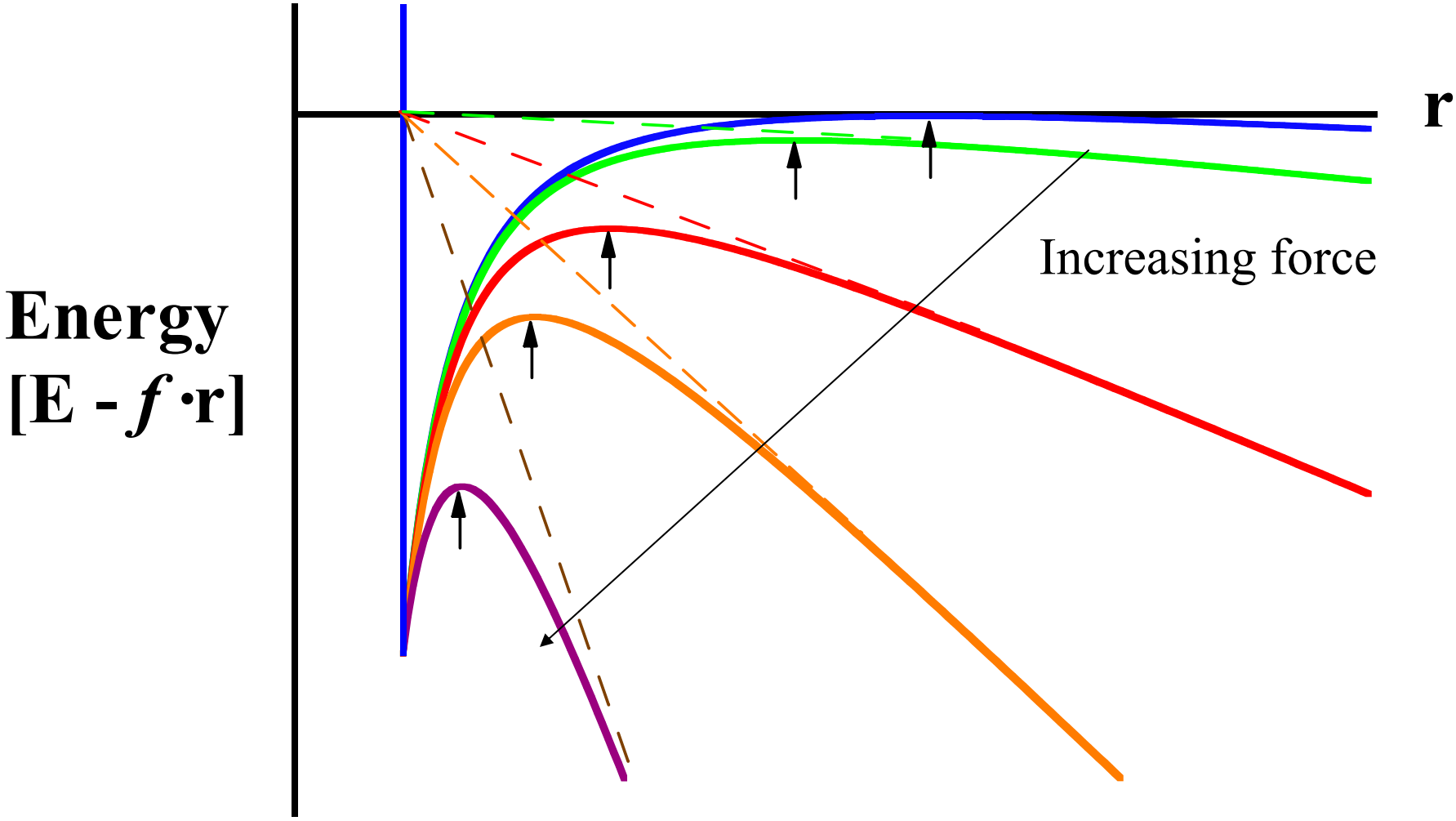
$$k_r^o = \frac{D}{l_{well}} \exp\left[-E_{well}/kT\right]$$

- Thus, given a potential, one can find a k_{off} that depends on only a few parameters.

Evans & Ritchie, *Biophys. J.* 72:1541, 1997

Effect of Force on Power Law Potential

- Suppose we have hard core repulsion at short range and the van der Waals-like form $U = -C/r^6$ beyond some distance



Adapted from Evans & Ritchie, *Biophys. J.* 72:1541, 1997

k_{off} vs Force Relations

Other forms for force dependence of rates:

- Spring: Dembo et al, *Proc. Roy. Soc. Lond. B* 234:55, 1988

  $k_{off} = k_{off}^{o} \exp(\beta f^2/kT)$; $\beta = (\sigma\text{-}\sigma_{ts})/2\sigma^2$

- Power Law: Evans et al, *Biophys. J.* 59:838, 1991

  $k_{off} = k_{off}' (r_o f/kT)^a$

- Modified Power Law: Evans & Ritchie, Biophys. J. 72:1541, 1997

  $k_{off} = k_{off}'' (r_o f/kT + r_o f_o/kT)^a$

- Evans & Ritchie–combined forms:

  $k_{off} = k_{off}''' (r_o f/kT)^a \exp(r_o f/kT)$

Force Application Techniques

- Two classes of methods are available for applying pN scale forces to bonds:

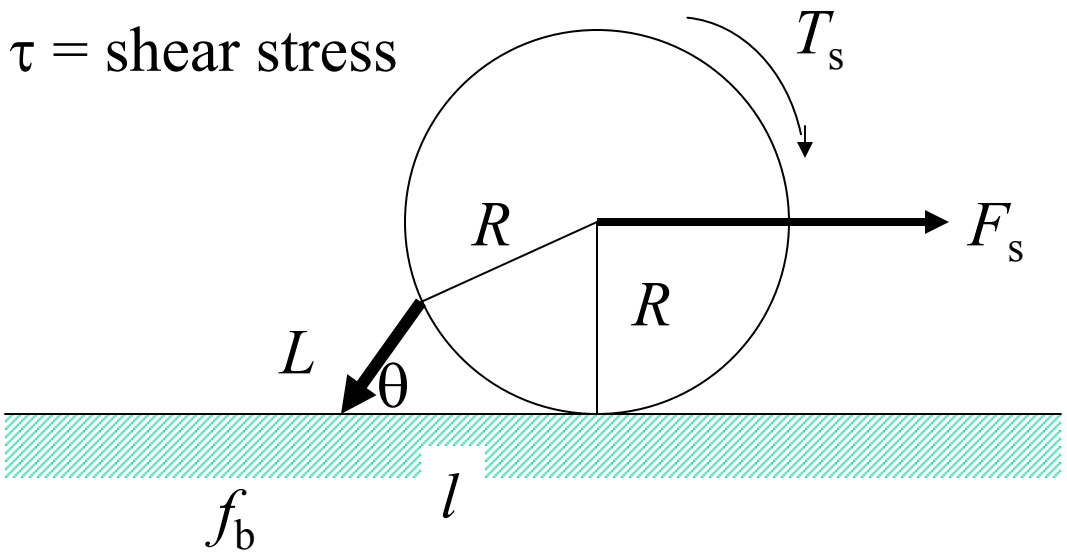
1) Hydrodynamic drag on a micron scale particle

2) Sensitive springs

Hydrodynamic Drag on a Particle

- **Geometry**: $\theta = \arctan(R/l) + \arccos((L^2+l^2)/2L(R^2+l^2)^{1/2}))$
- **Force balance**: $f_b \cos\theta = F_s = 32.05\tau R^2$
- **Torque balance**: $f_b l \sin\theta = T_s + RF_s = 43.91\tau R^3$
- **Microvillus extension**: $f_b = k_1(L-L_o)$
- **Tether formation**: $f_b = F_o + k_2\,(dL/dt)$

Shao et al., PNAS, 95:6803, 1998

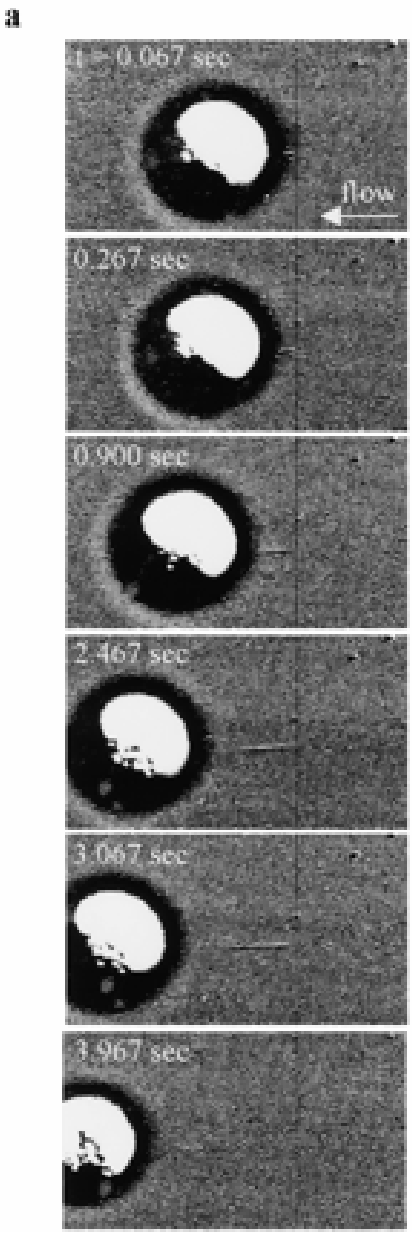


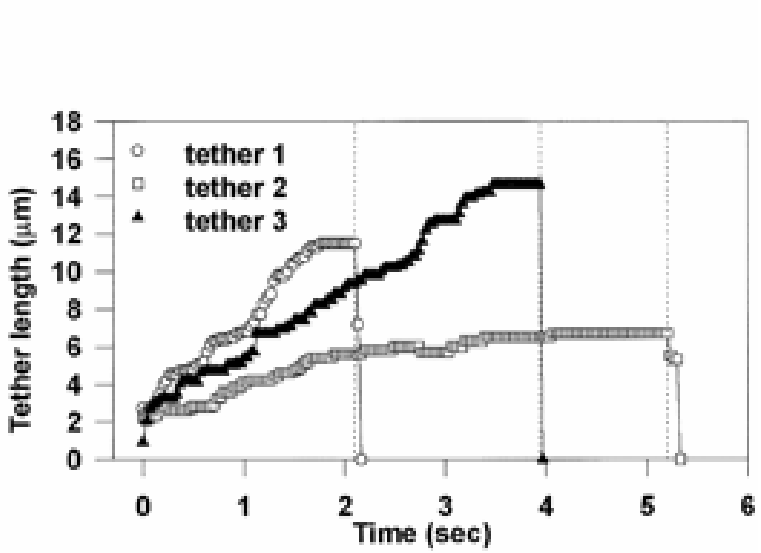
See also:

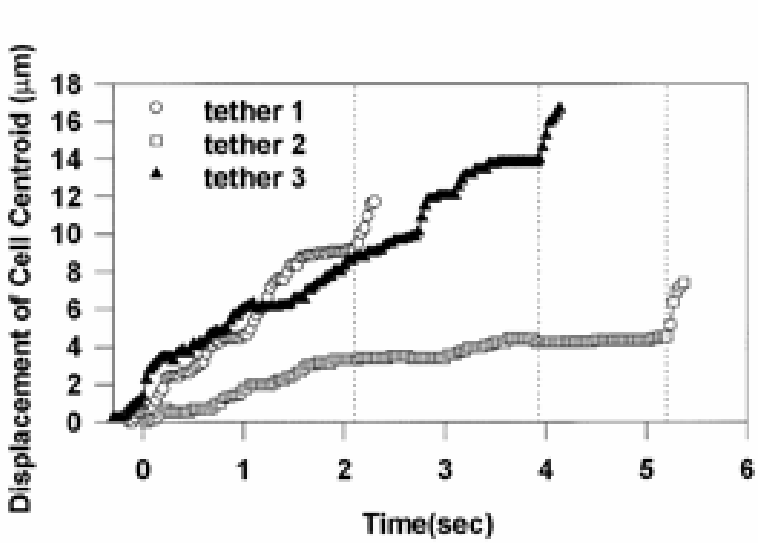
Pierres et al., *J. Biological Chemistry*, 270:26586-26592, 1995 and

Chang and Hammer, *Langmuir*, 12:2271-2282, 1996

Neutrophils Tethering on P-selectin



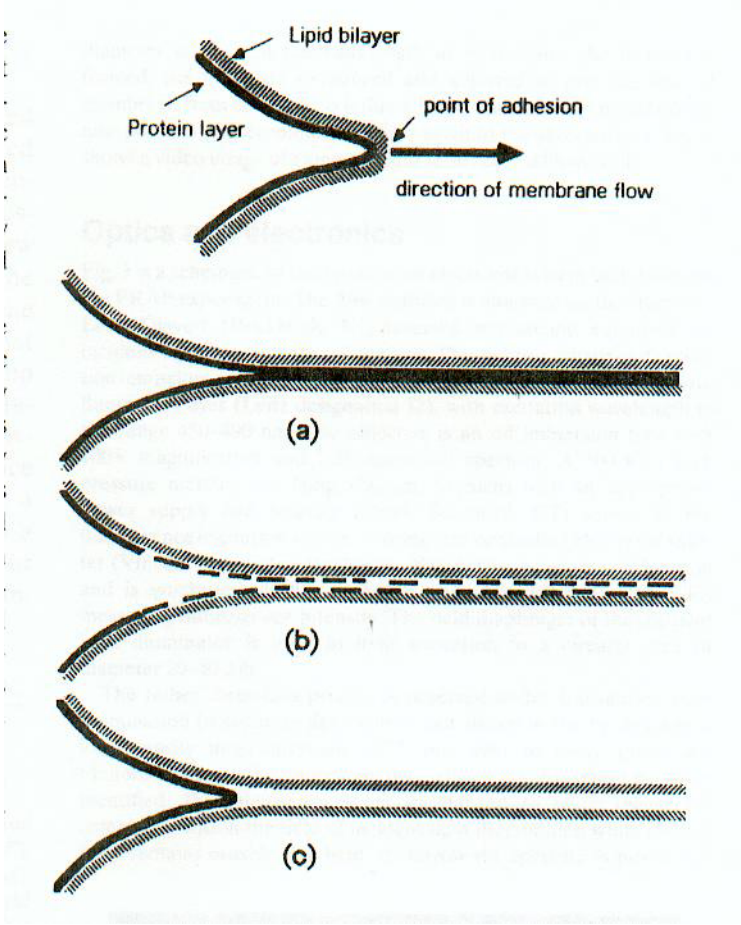




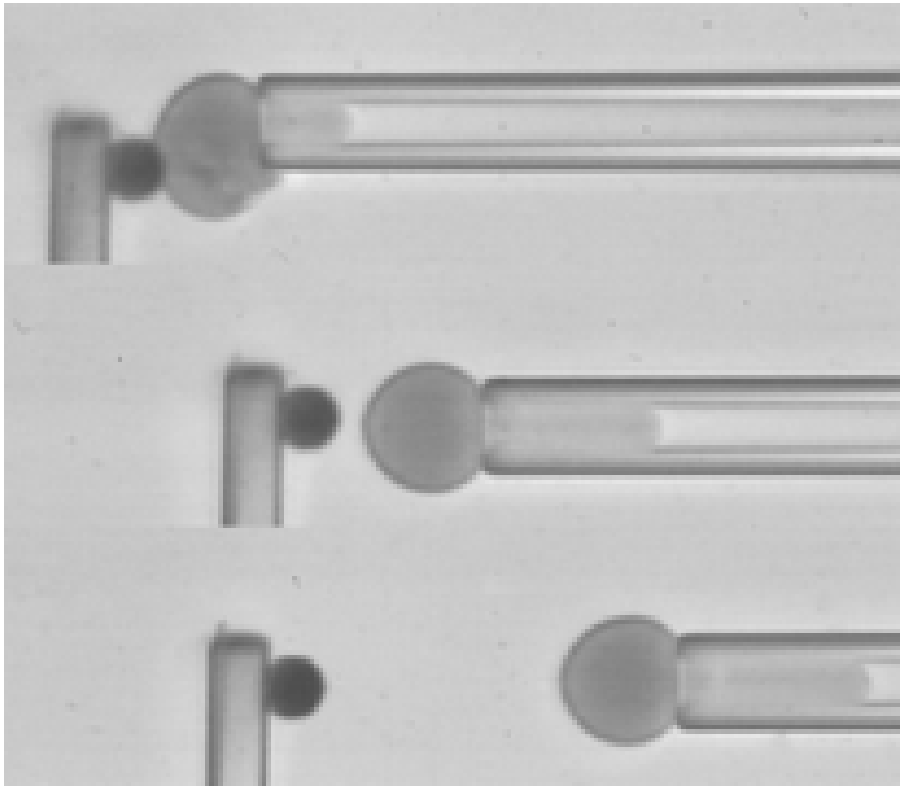
Schmidtke & Diamond, J. Cell Biol., 149:719, 2000

Lipid Bilayer Tethers

- The lipid bilayer can be detached from the cytoskeleton and a cylindrical lipid bilayer tube or tether can be extruded if applied force exceeds ~ 50 pN.



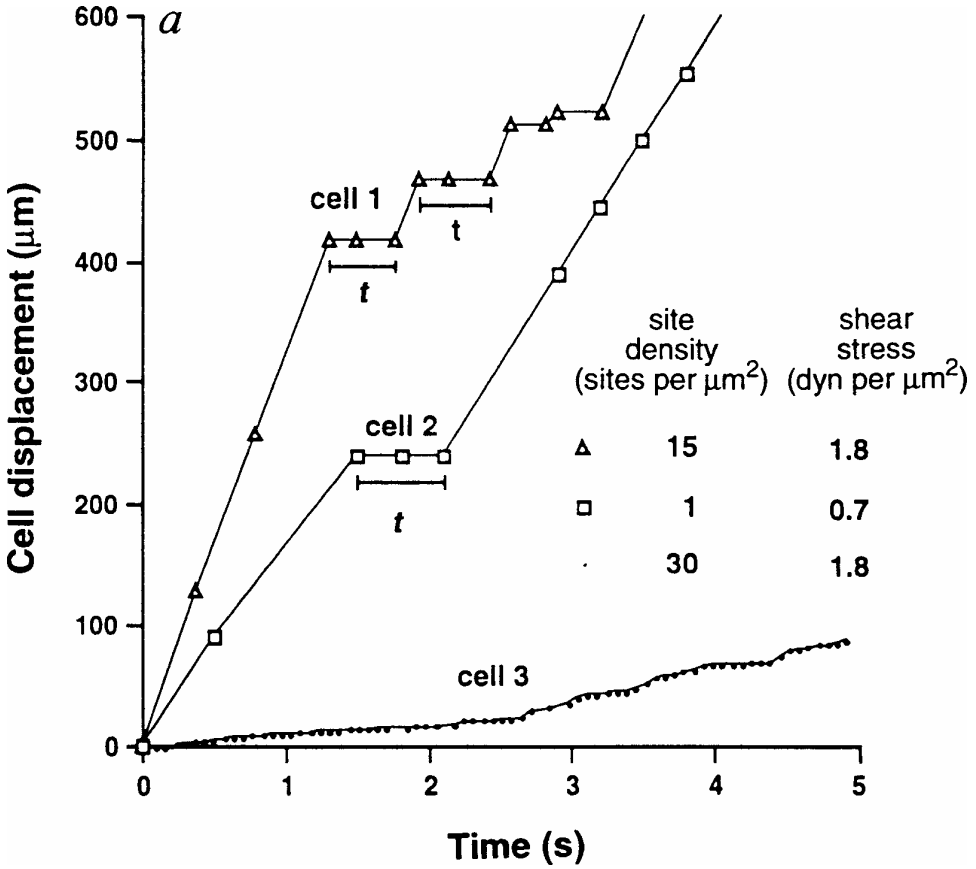
Berk & Hochmuth, Biophys. J. 61:9, 1992

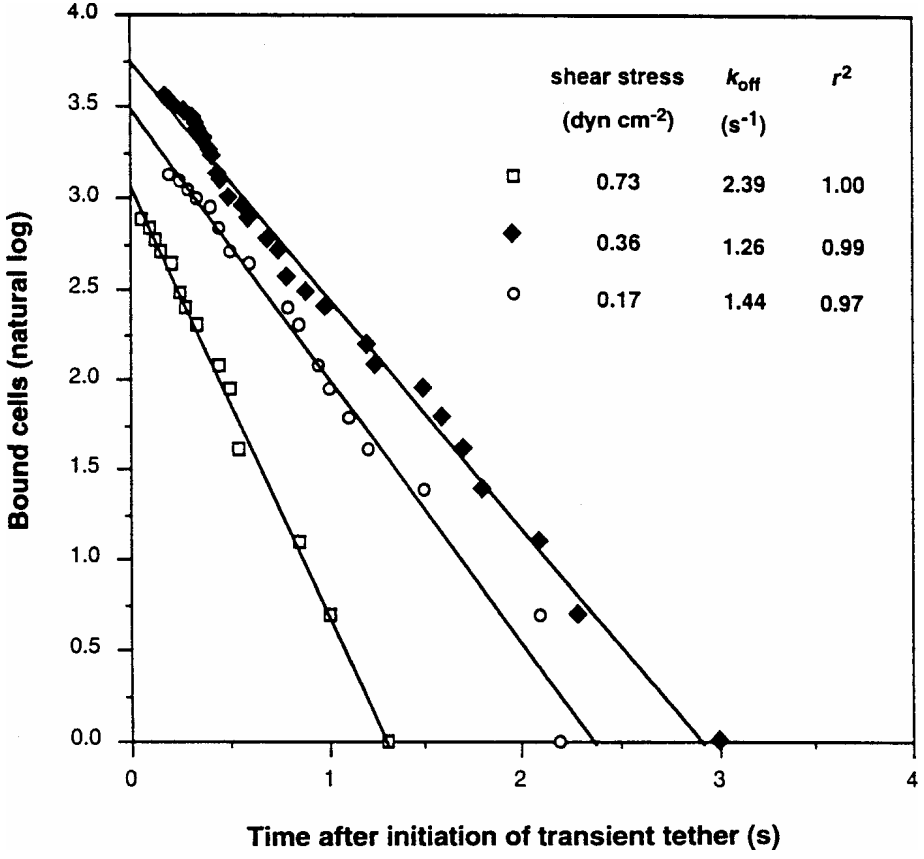


Waugh et al, Blood, 97:1869, 2001

Arrest Duration Distribution

Cell moving over low density of receptor shows pauses



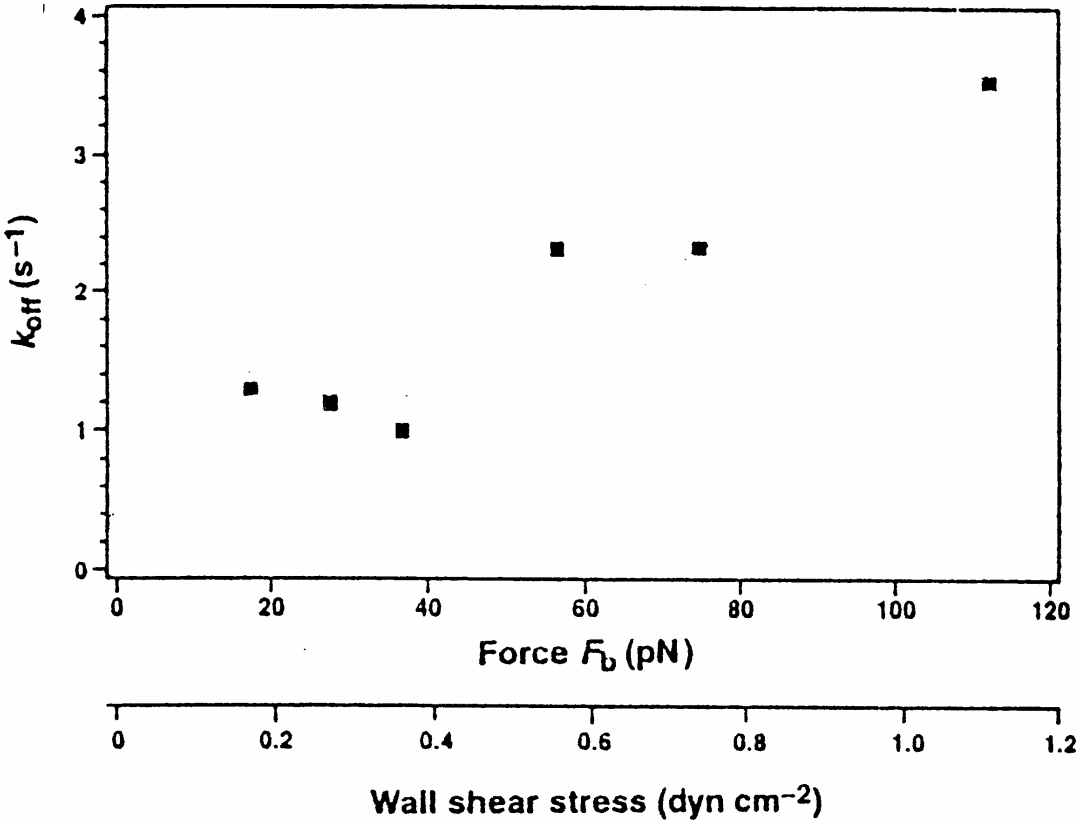


Start with N bound cells. Cells dissociate over time. Number remaining bound follows:

$$N = N_o \exp[-k_{off}\, t] \quad \text{or} \quad \ln N = \ln N_o - k_{off}\, t$$

Alon, Hammer & Springer, Nature 374:539, 1995

P-selectin Dissociation



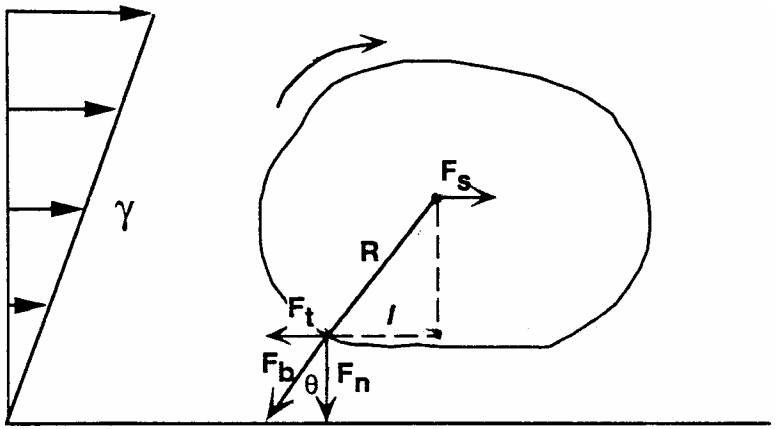
Bell Model

$k_{off} = k_{off}^{o} \exp(r_o f_b / kT)$

Best Fit parameters:

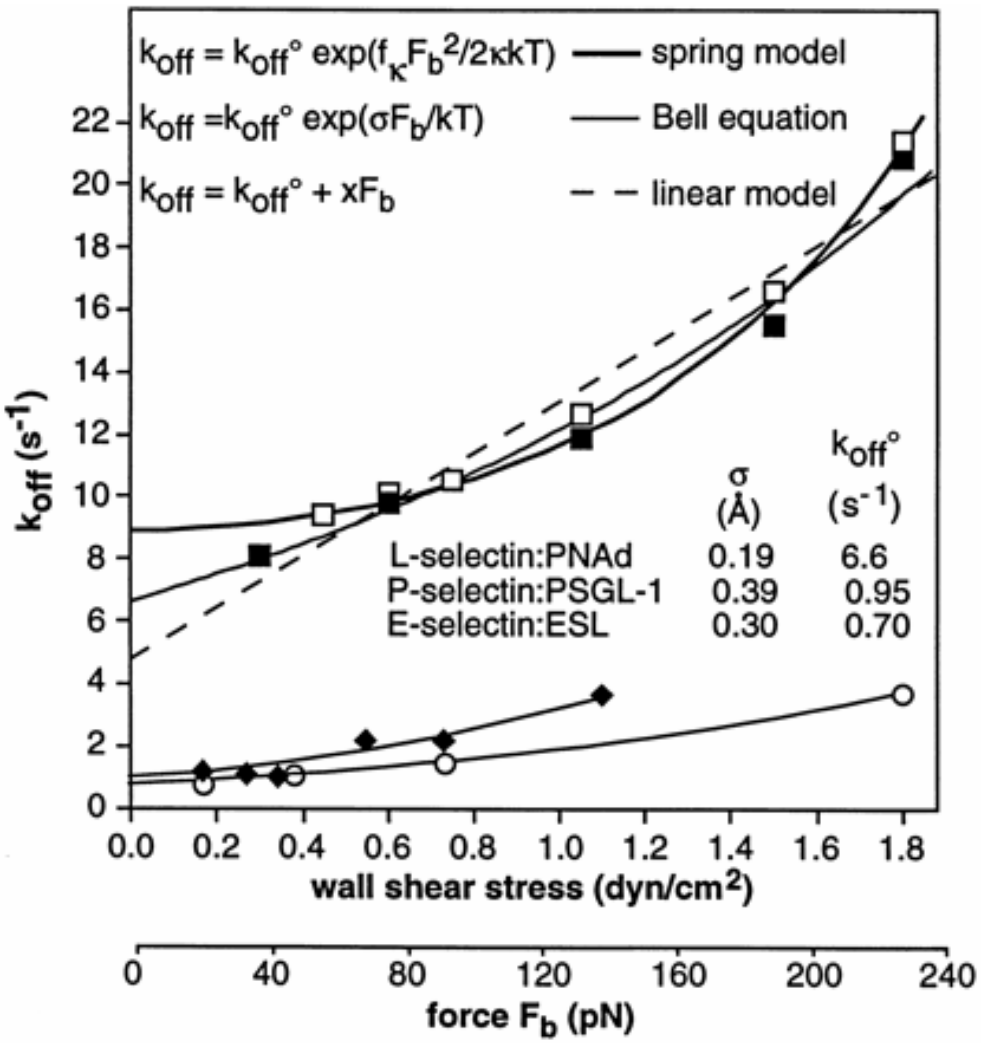
$k_{off}^{o} = 0.95 \pm 0.17\ s^{-1}$

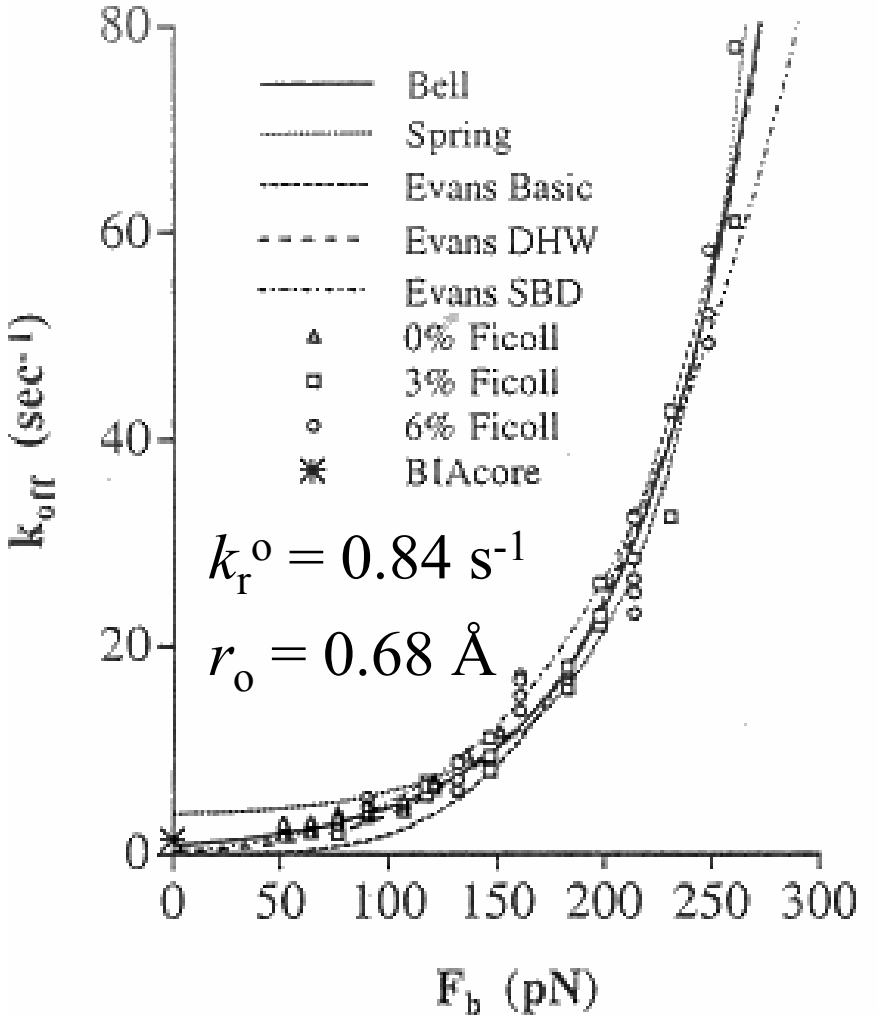
$r_o = 0.49 \pm 0.08$ Å



Alon, Hammer & Springer, *Nature* 374:539, 1995

Selectin Bell Parameters



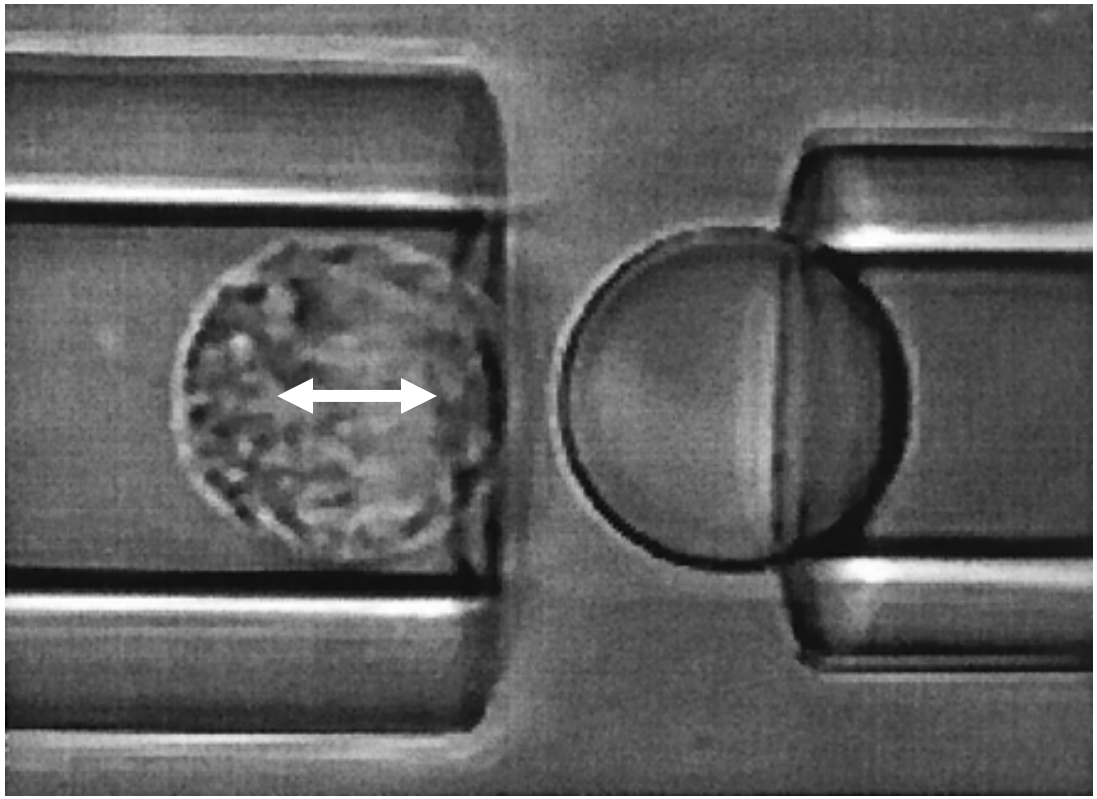


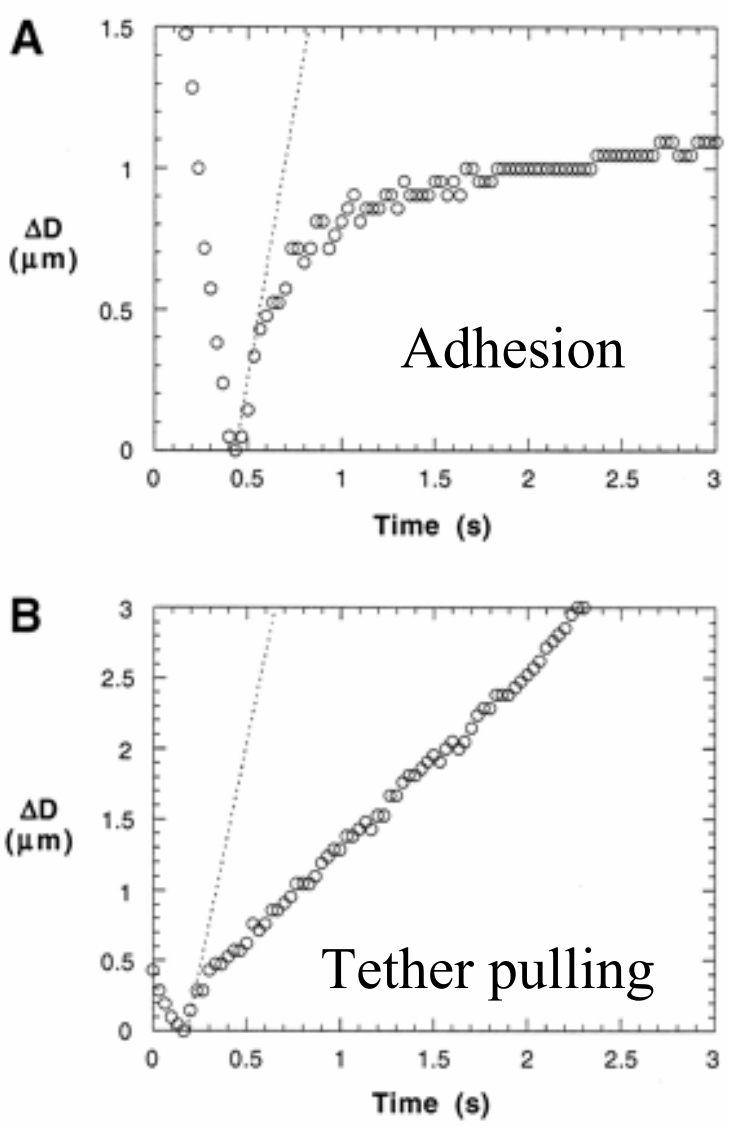
Alon et al., J. Cell Biol, 138:1169, 1997

Chen & Springer, *PNAS* 98:950, 2001

Two Pipette Adhesion Studies

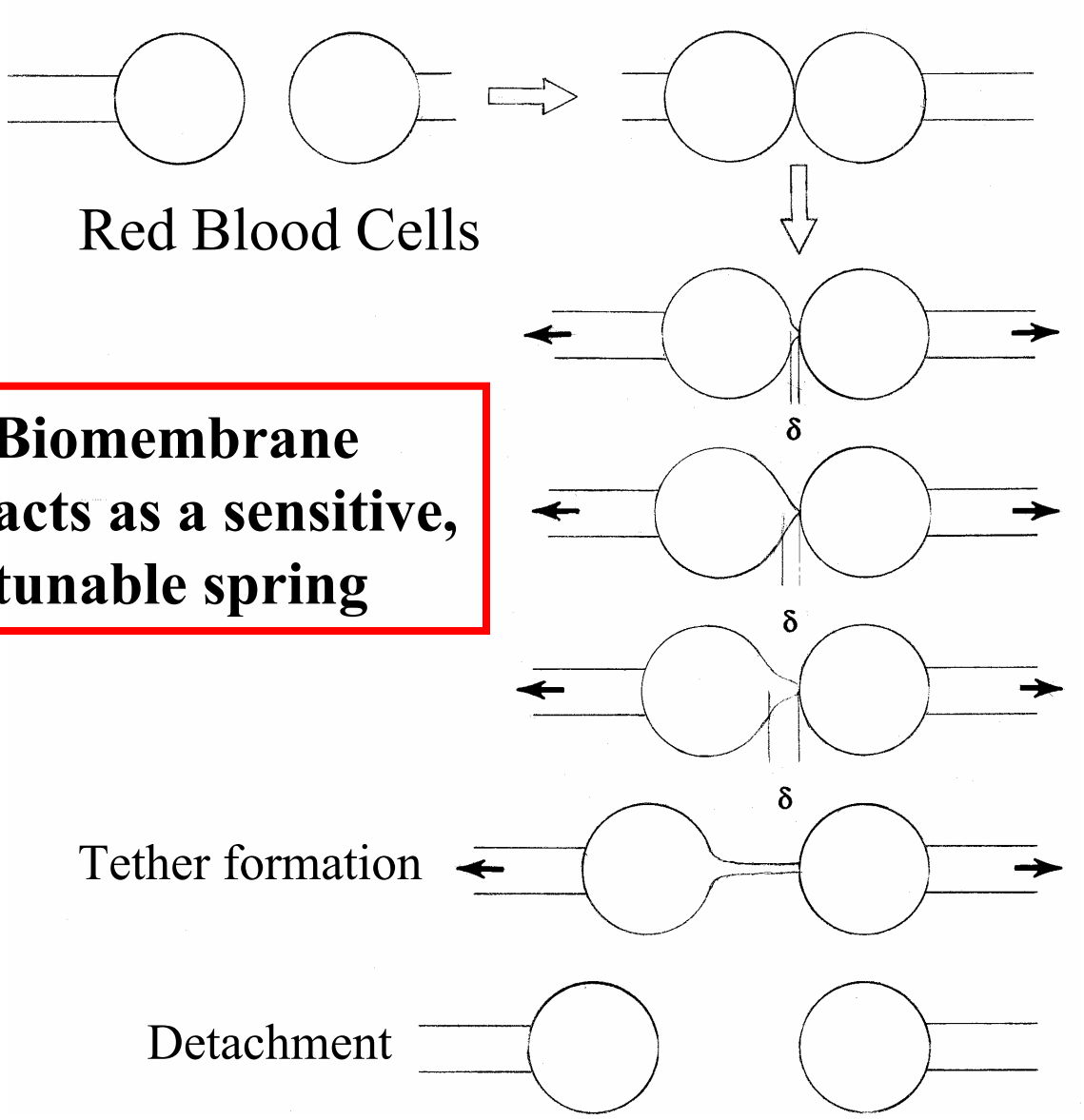
Hydrodynamic force on cell driven back and forth in a micropipette applies pN scale forces to bonds



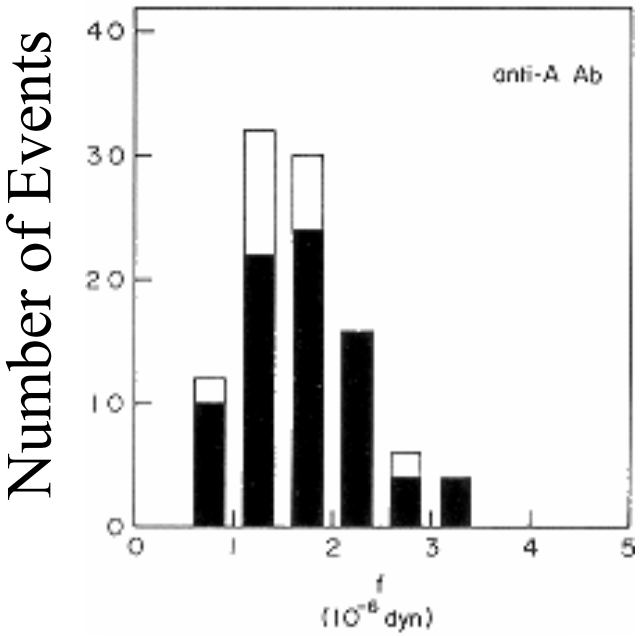


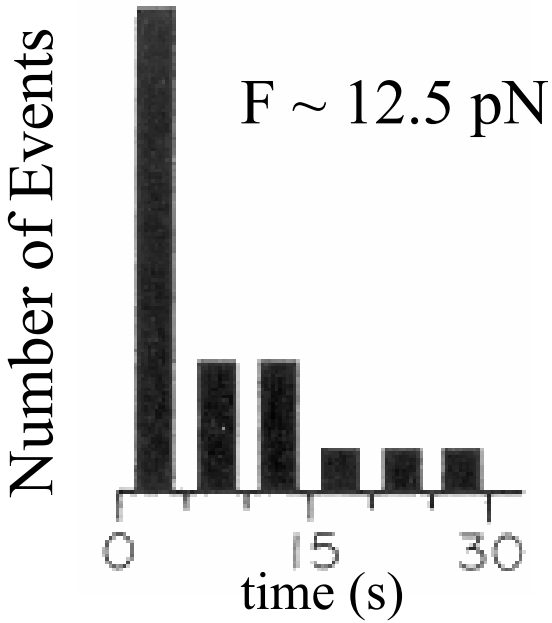
Shao et. al. *PNAS*, 95: 6797-6802, 1998

Biomembrane Force Probe



Biomembrane acts as a sensitive, tunable spring

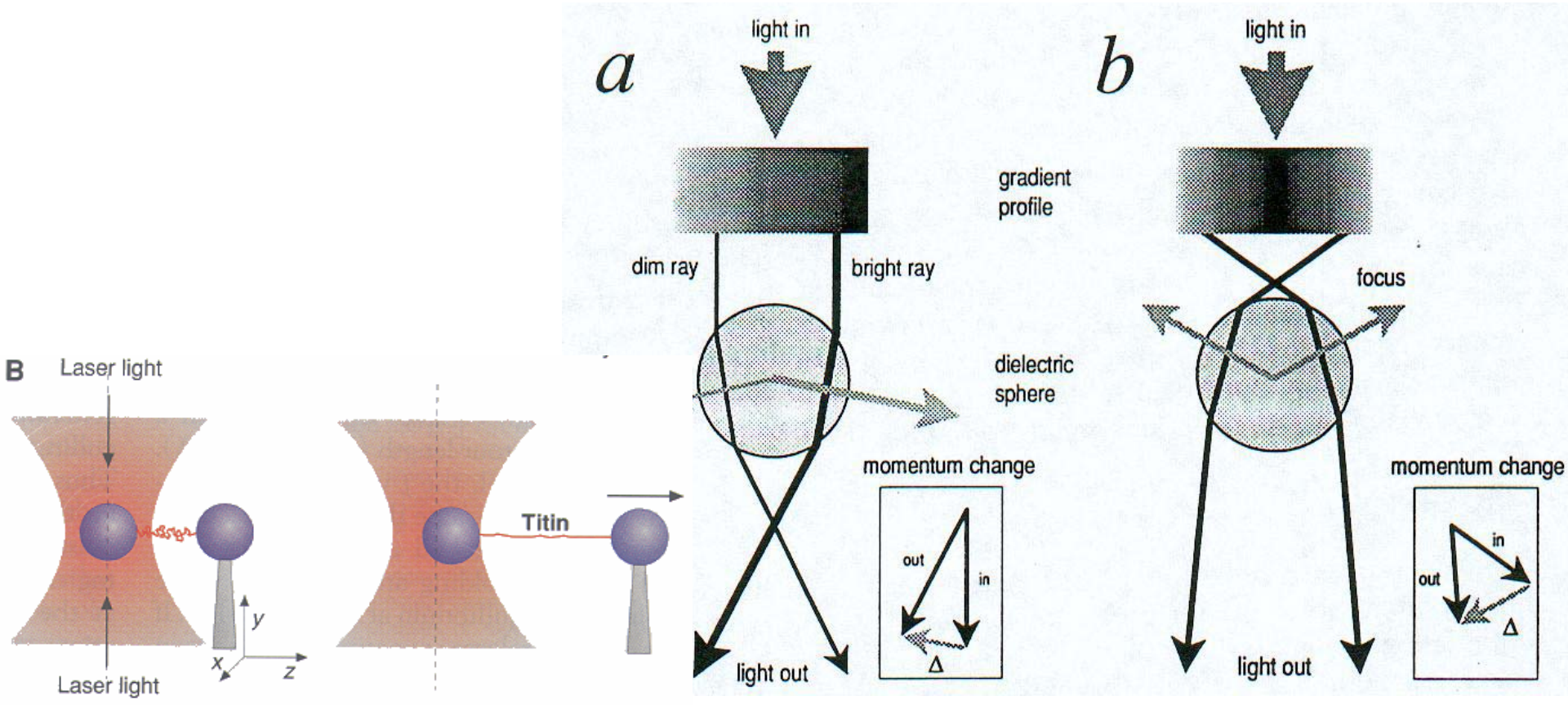




Evans et al, *Biophys. J.*, 59:838, 1991

Optical Tweezers

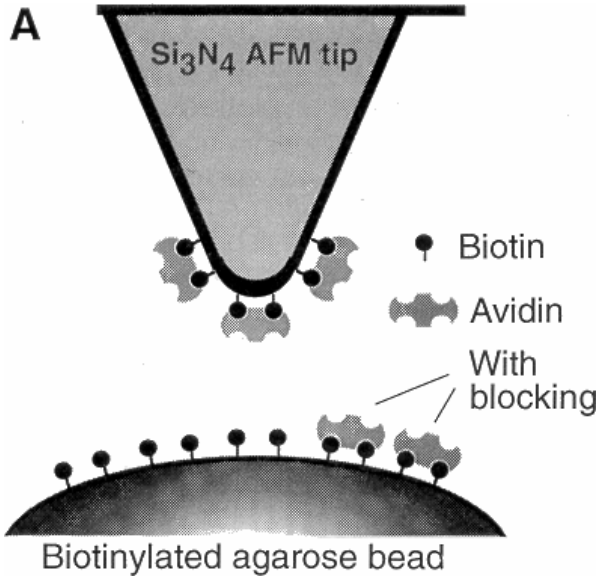
- Light momentum before and after refraction leads to a sideways restoring force toward the center of Gaussian beam and an in-line restoring force towards the focus

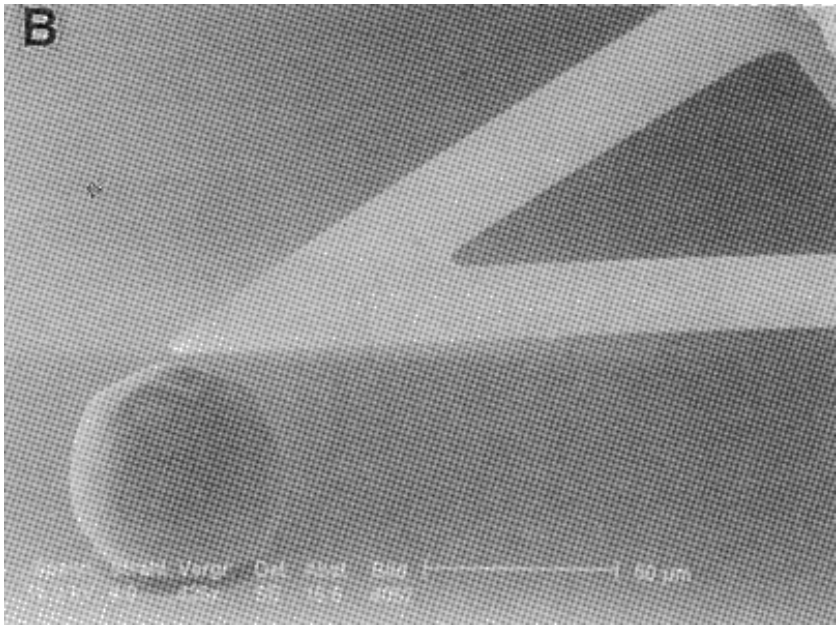


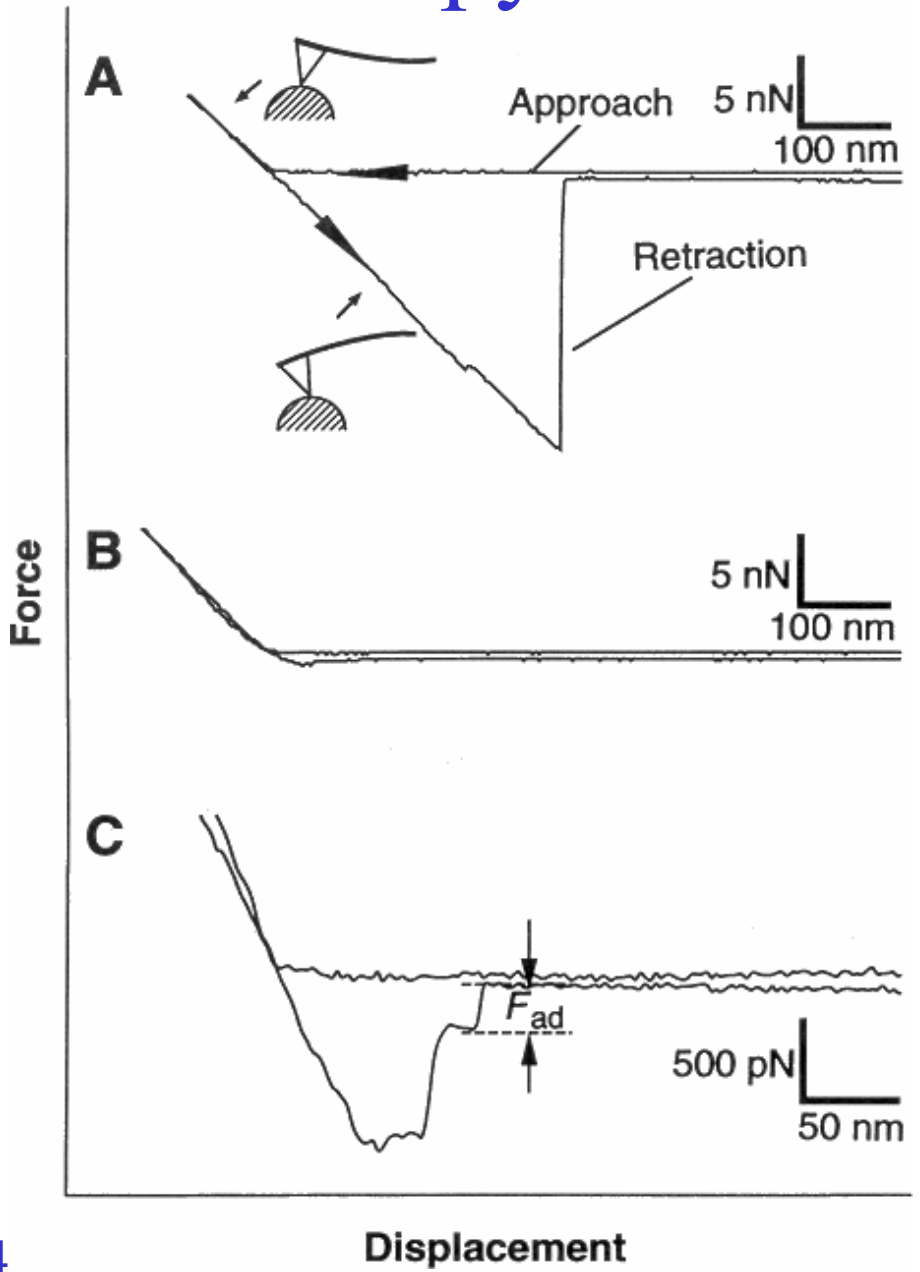
K. Svoboda & S. M. Block, Ann. Rev. Biophys. Biomol. Struct., 23:247-285, 1994

Tskhovrebova et al. *Nature*, 387:308, 1997

Atomic Force Microscopy



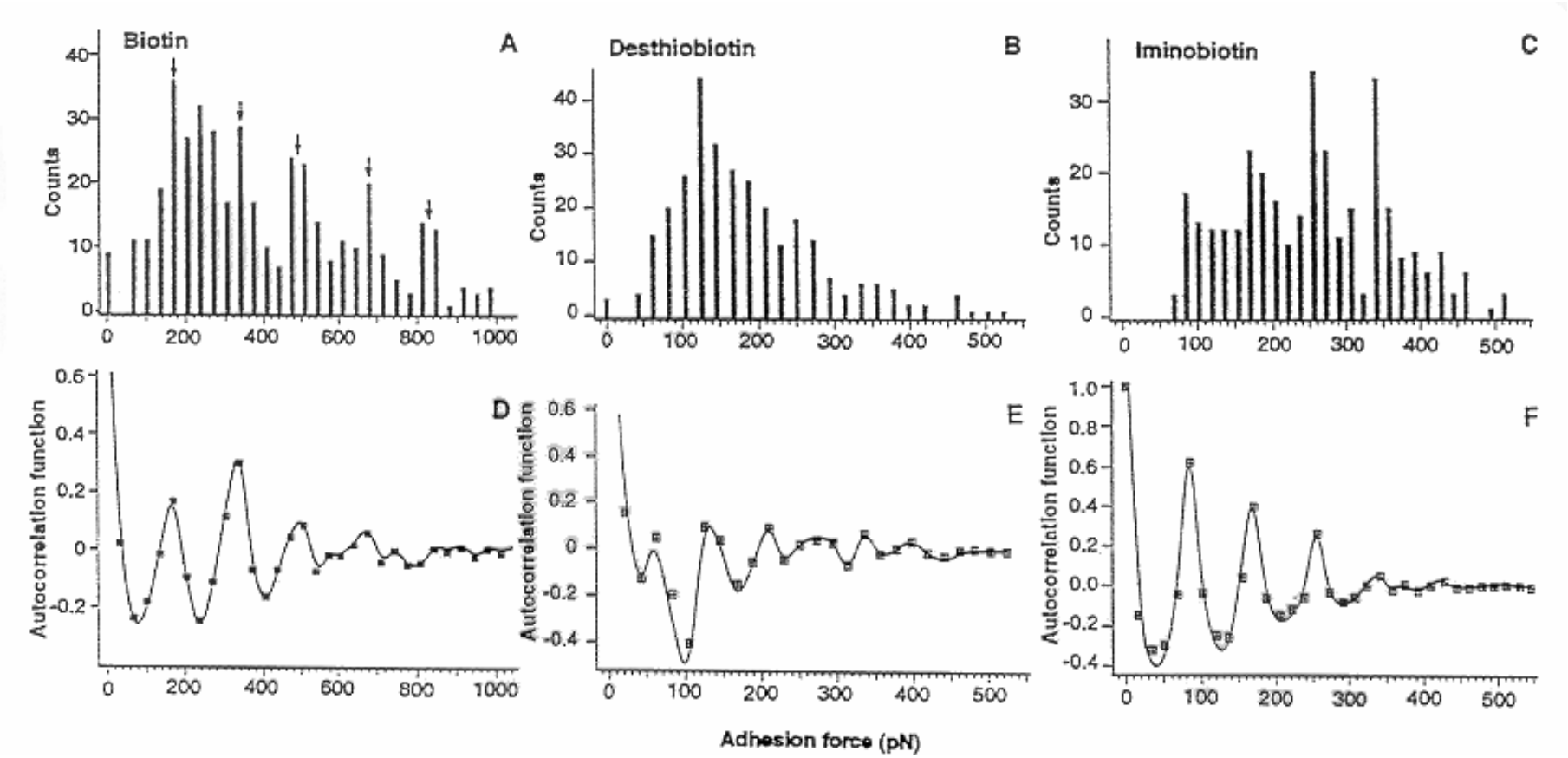




Florin, Moy & Gaub, *Science*, 264:415, 1994

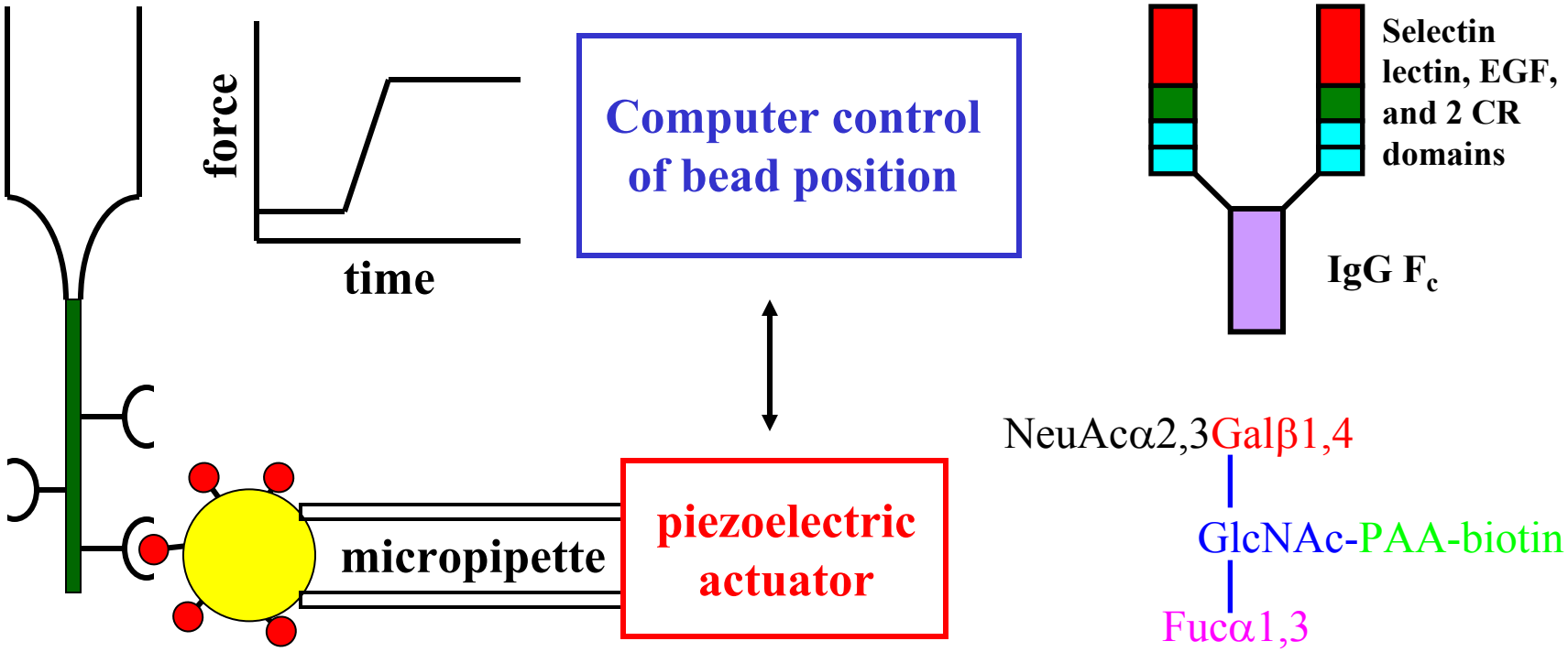
Force Distribution at Break-up from AFM

Evenly spaced peaks can be seen in distribution of force at break-up:



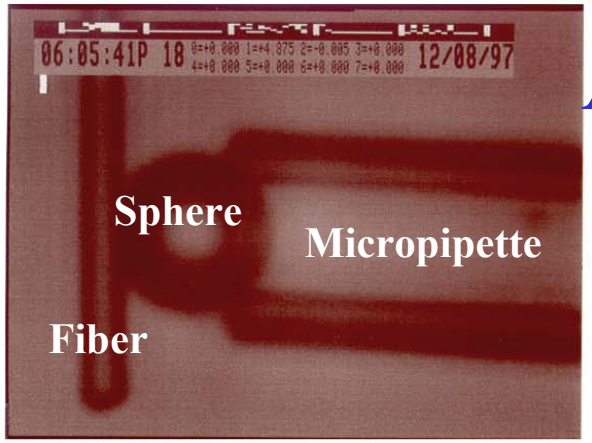
Florin, Moy & Gaub, *Science*, 264:415, 1994

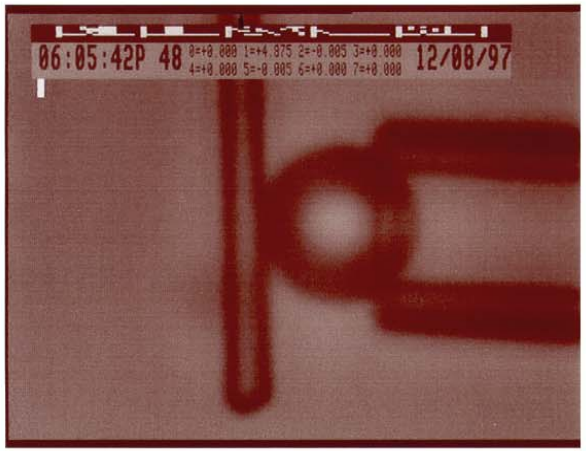
Force Application Device

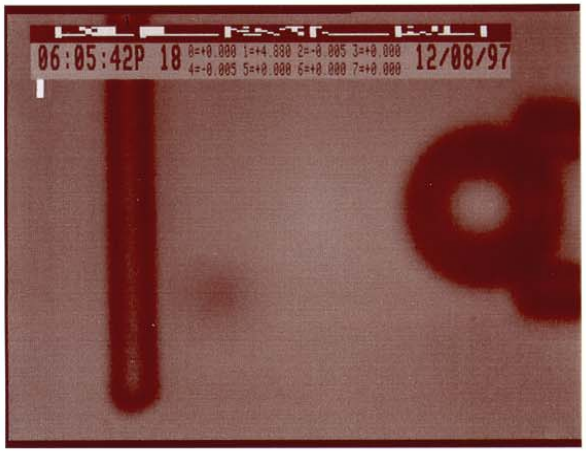


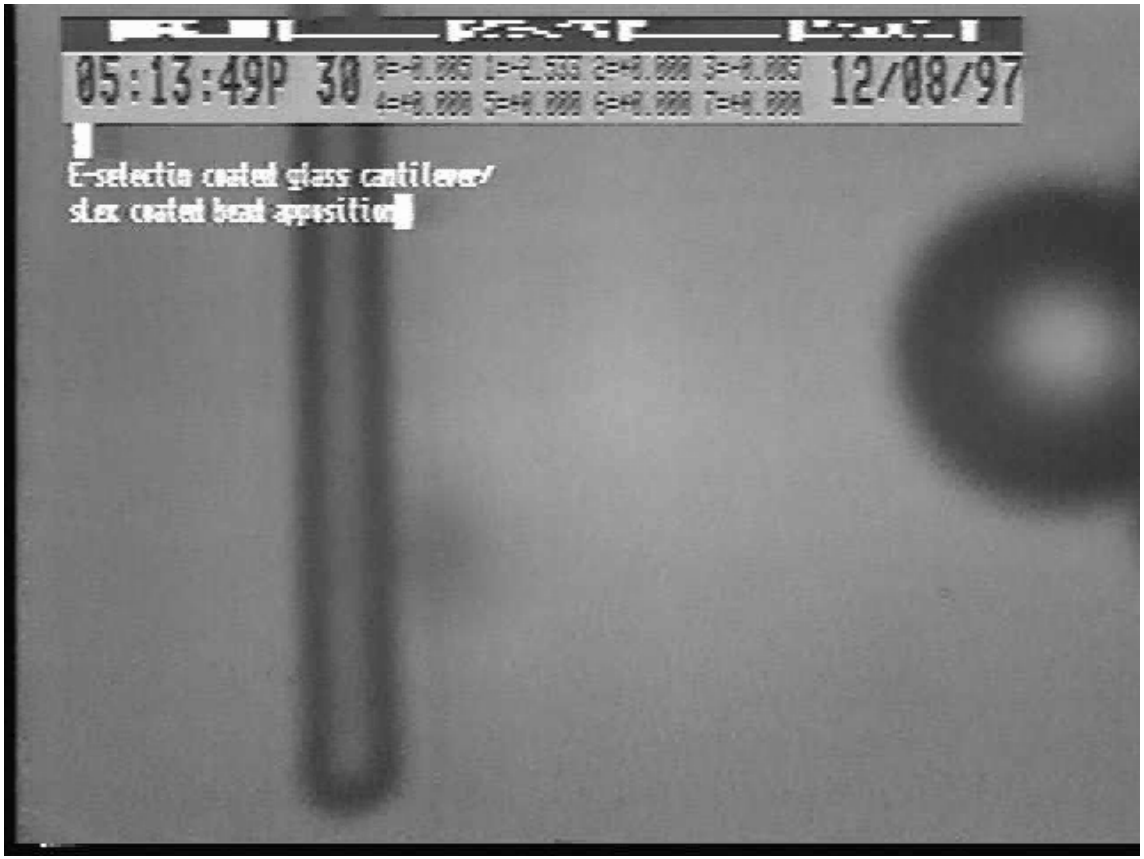
- Force = Spring Constant × Displacement
- Spring Constant of fiber = $(3\pi/64)\ ED^4 / L^3$

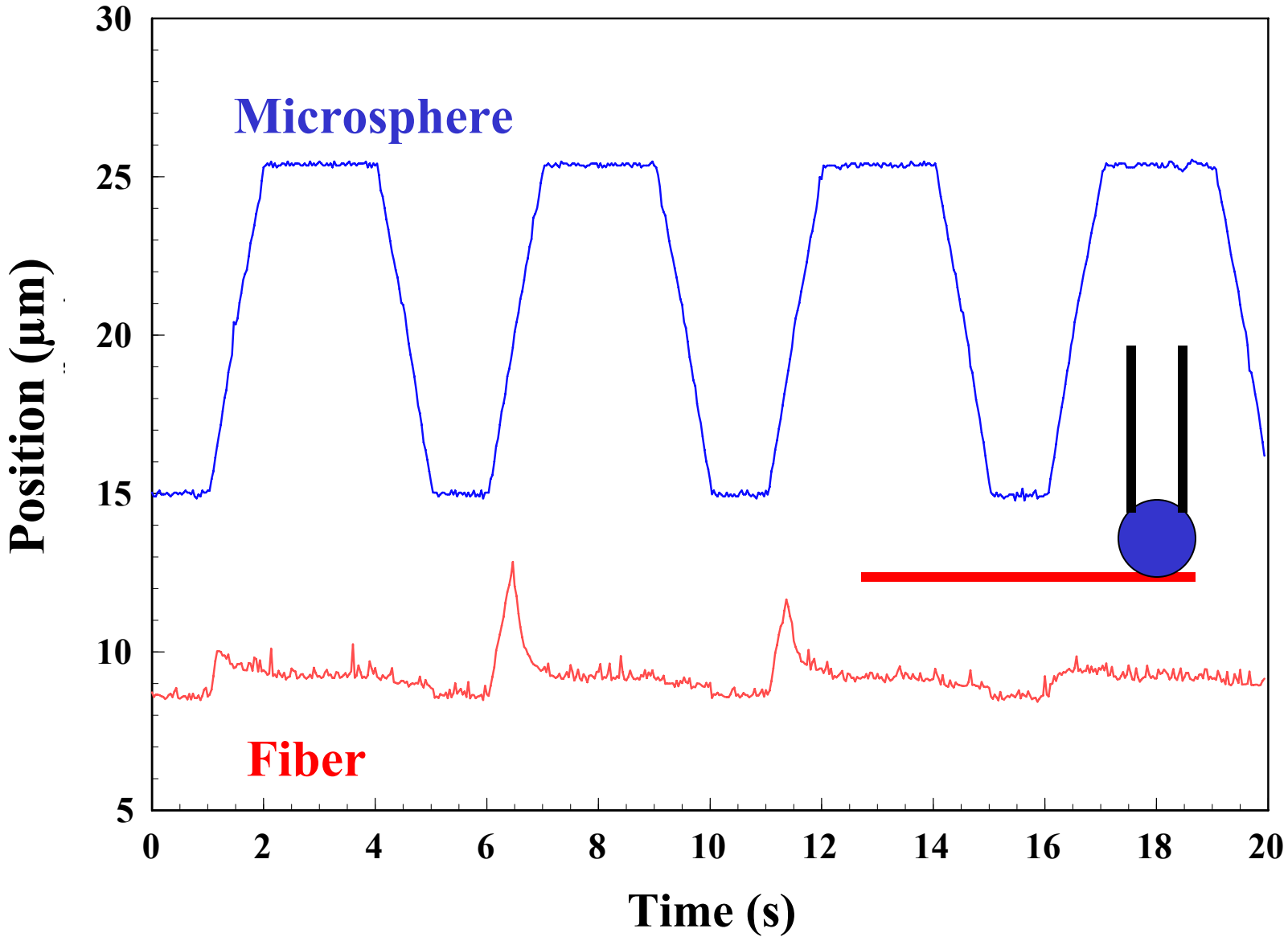
Adhesive Events



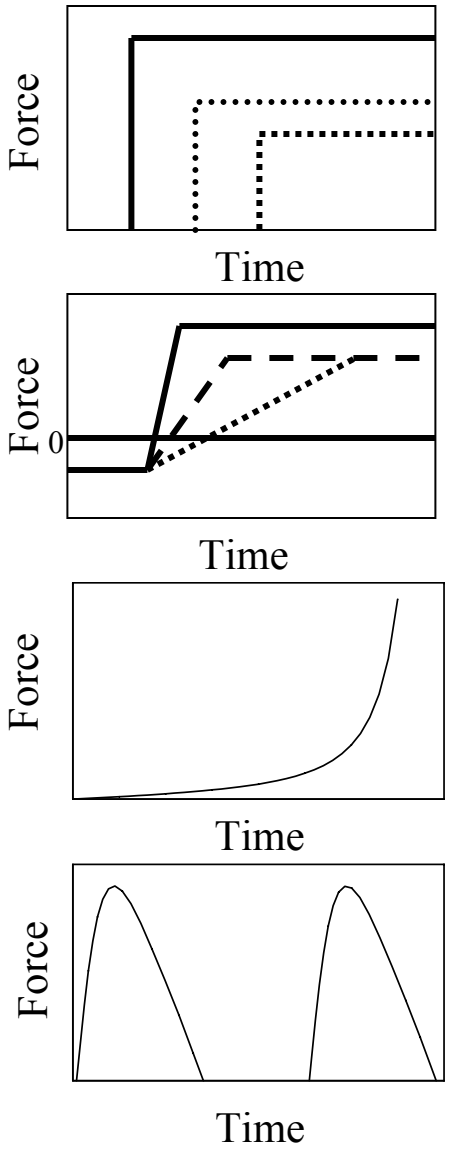







Microsphere
Fiber
Position (μm)
Time (s)
30
25
20
15
10
5
0
2
4
6
8
10
12
14
16
18
20

Loading Rate



In an ideal experiment, one would apply force instantaneously and measure time for bond to break.

In the Real World, force cannot be applied simultaneously

Examples of loading rates:

Method	Time required to apply 100 pN
AFM:	1 ms - 1 s
Hydrodynamic:	10 ms - 10 s
Micropipette:	10 ms - 100 s
MD:	$\sim 10^{-10}$ s [!]

Measuring Bell model Parameters

From Reliability Theory of failure, the probability density for *single* bond dissociation in the interval $(t, t + dt)$ is:

$$p(t, f) = k_r^o(f) \exp\left\{ - \int_o^t k_r[f(t')]dt' \right\}.$$

Find the mode, or peak force, f_{crit} for this distribution ($\partial p/\partial f = 0$). Assume linear loading $f = r_f t$, where r_f is the force loading rate.

$$k_r(f_{crit}) = r_f \frac{\partial}{\partial f} \ln k_r(f) \Big|_{f = f_{crit}}$$

Substitute Bell model: $k_r = k_r^o \exp[r_o f/kT]$ and get:

$$f_{crit} = \frac{kT}{r_o} \ln\left(\frac{r_o}{k_r^o kT} \right) + \frac{kT}{r_o} \ln r_f$$

Evans & Ritchie, *Biophys J.*, 72:1541, 1997

Measuring Bell model Parameters

- Plot most likely force at break-up, f_{crit} vs loading rate, r_f.

$$f_{crit} = \frac{kT}{r_o}\ln\left(\frac{r_o}{k_r^o kT}\right) + \frac{kT}{r_o}\ln r_f$$

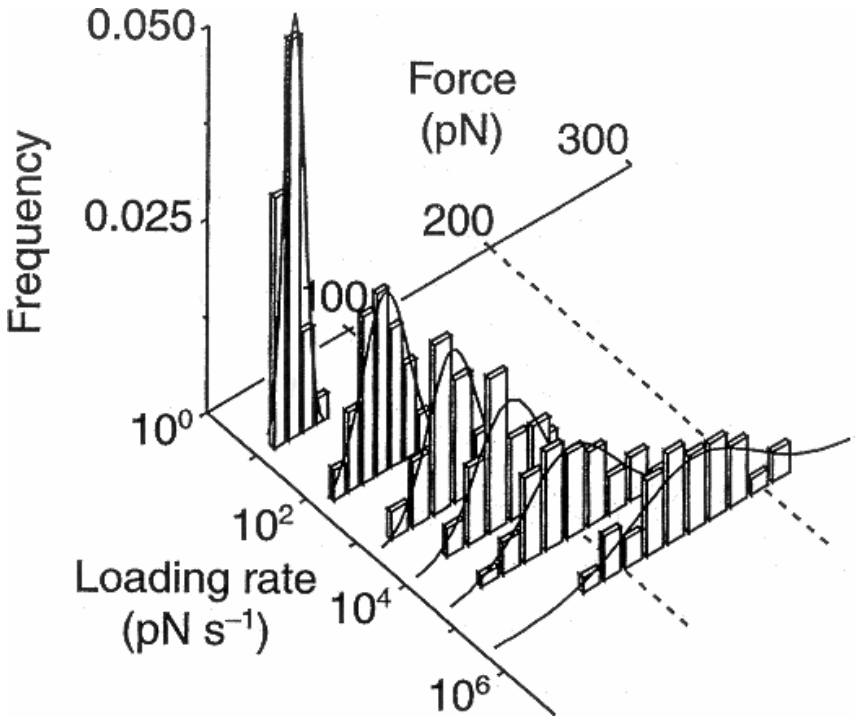
- So:

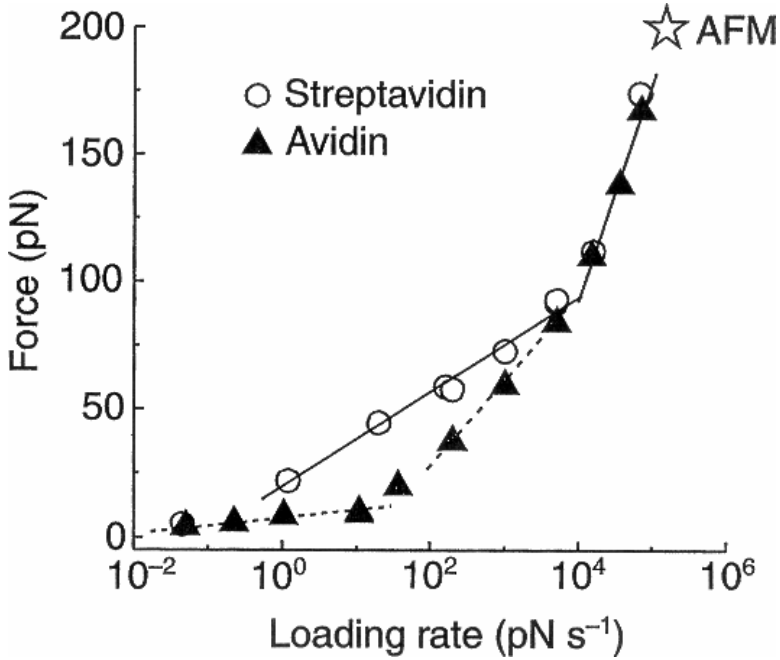
$$\text{slope} = \frac{kT}{r_o} \Rightarrow r_o = \frac{kT}{\text{slope}};$$

$$\text{intercept} = \frac{kT}{r_o}\ln\left[\frac{r_o}{k_r^o kT}\right] \Rightarrow k_r^o = \frac{e^{\text{-intercept/slope}}}{\text{slope}}.$$

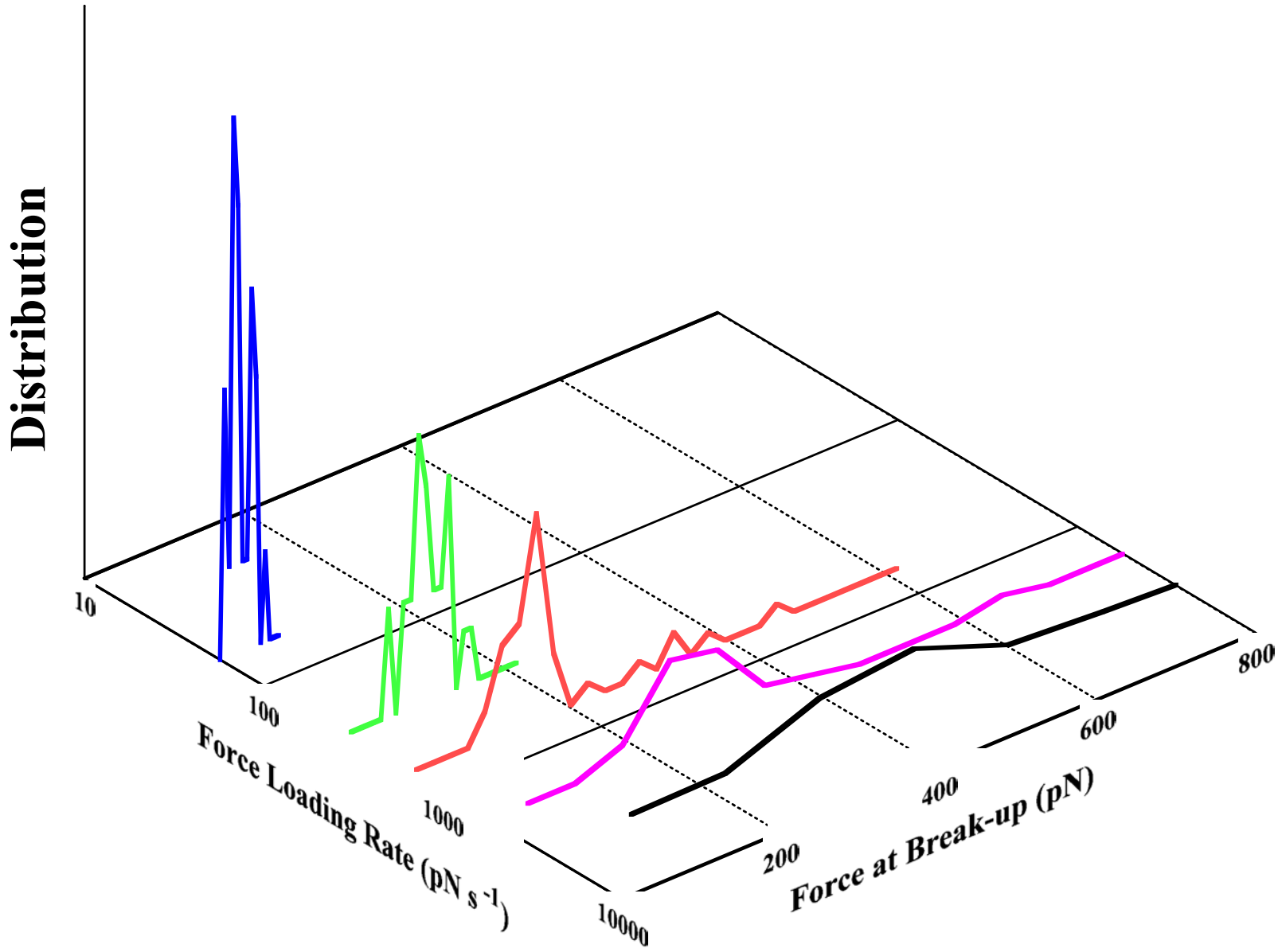
Note that at room temperature, $kT = 4.1$ pN·nm

Force Spectroscopy

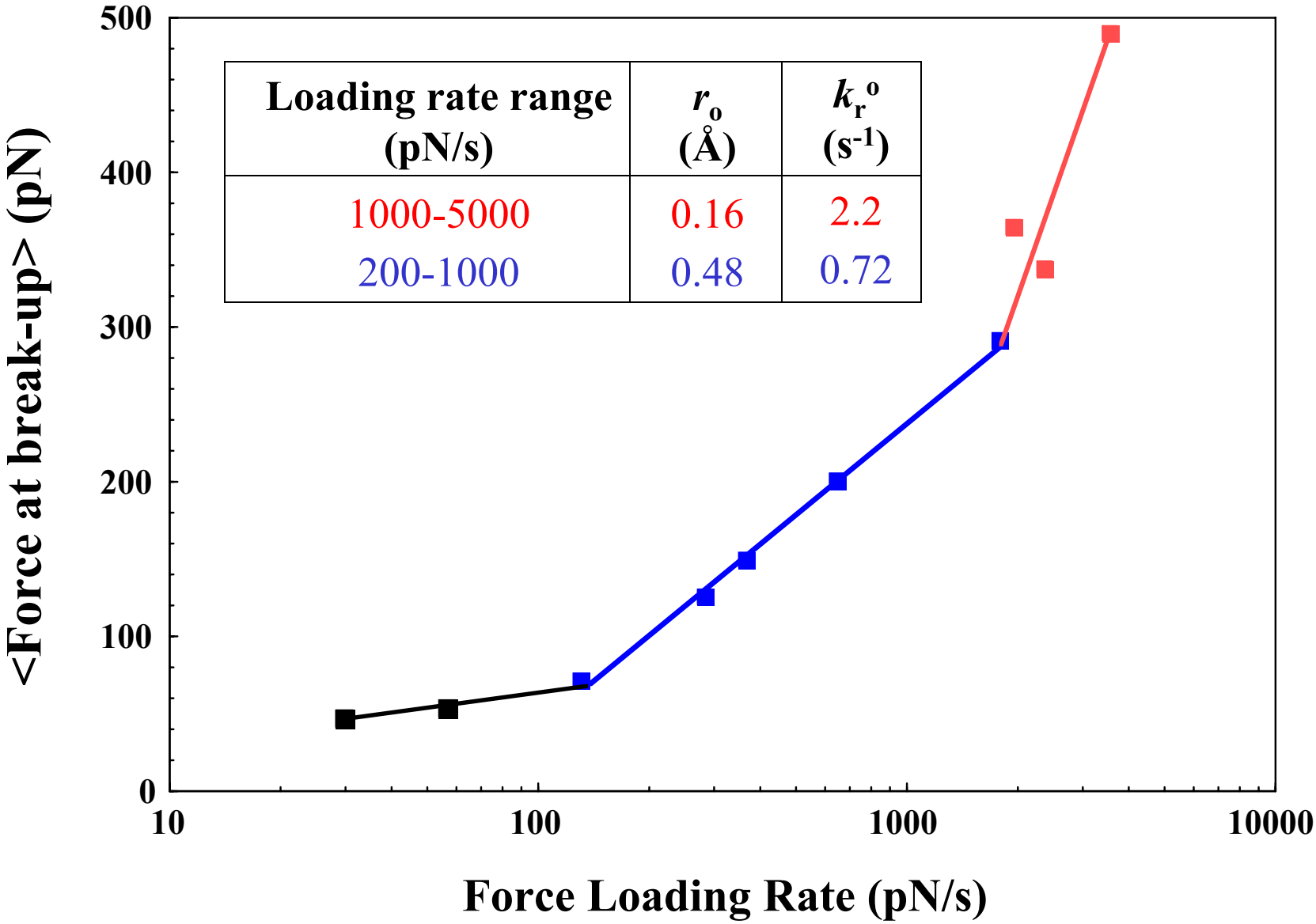




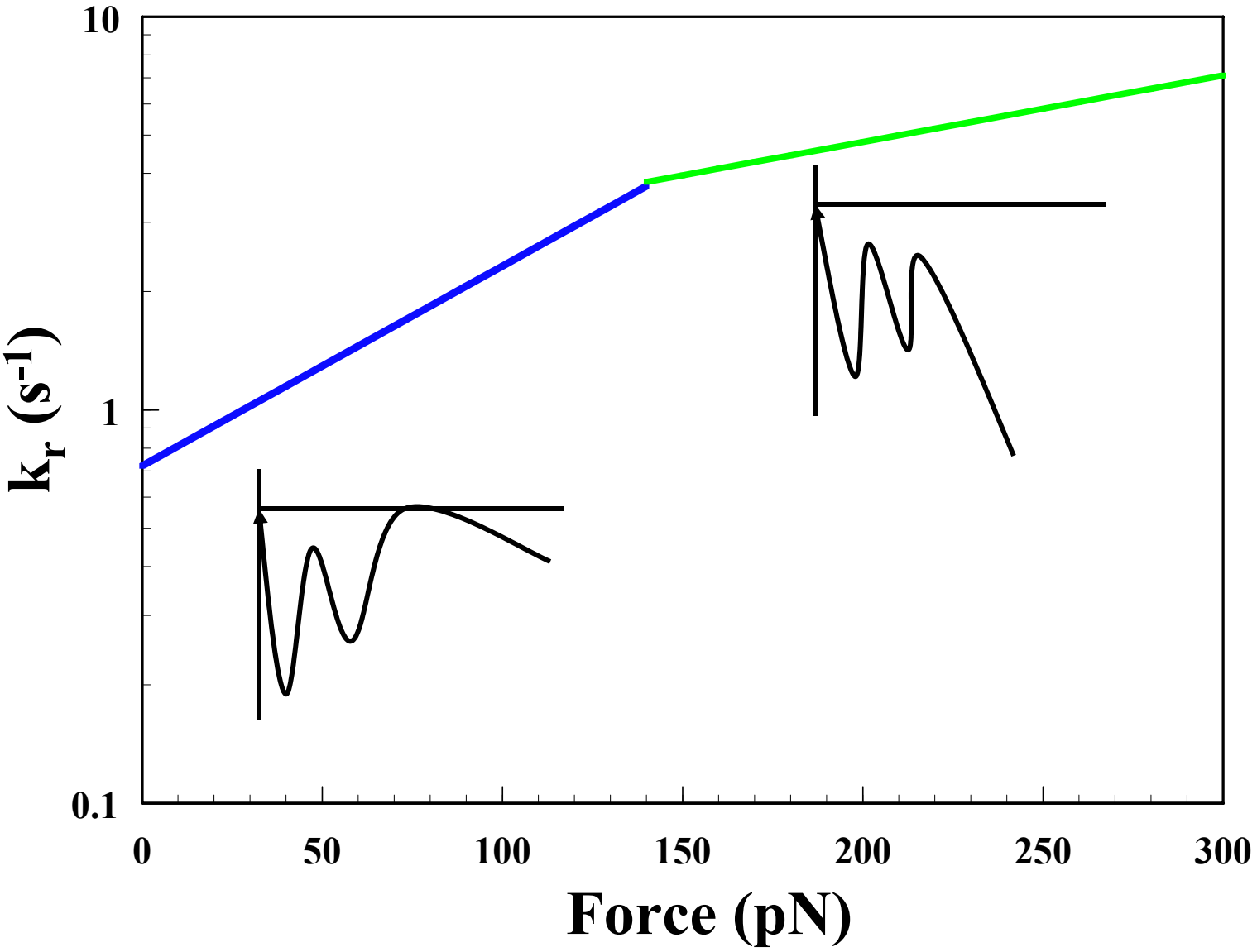
Merkel et al, *Nature*, 397:50, 1999


Distribution
10
100
1000
10000
Force Loading Rate (pN s -1)
200
400
600
800
Force at Break-up (pN)

Observed Force vs Loading Rate



Bell Model Regime



Tees & Goetz. *News in Physiological Sciences*, 18:186-190, 2003

Mean vs Mode

Note that the derivation given previously applies when f_{crit} is the "peak" force, or **mode** of the time distribution. The **mean** of the distribution follows a different relation:

$$\langle f_{break} \rangle = r_f \langle t \rangle = r_f \int tp(t, f)dt$$

or

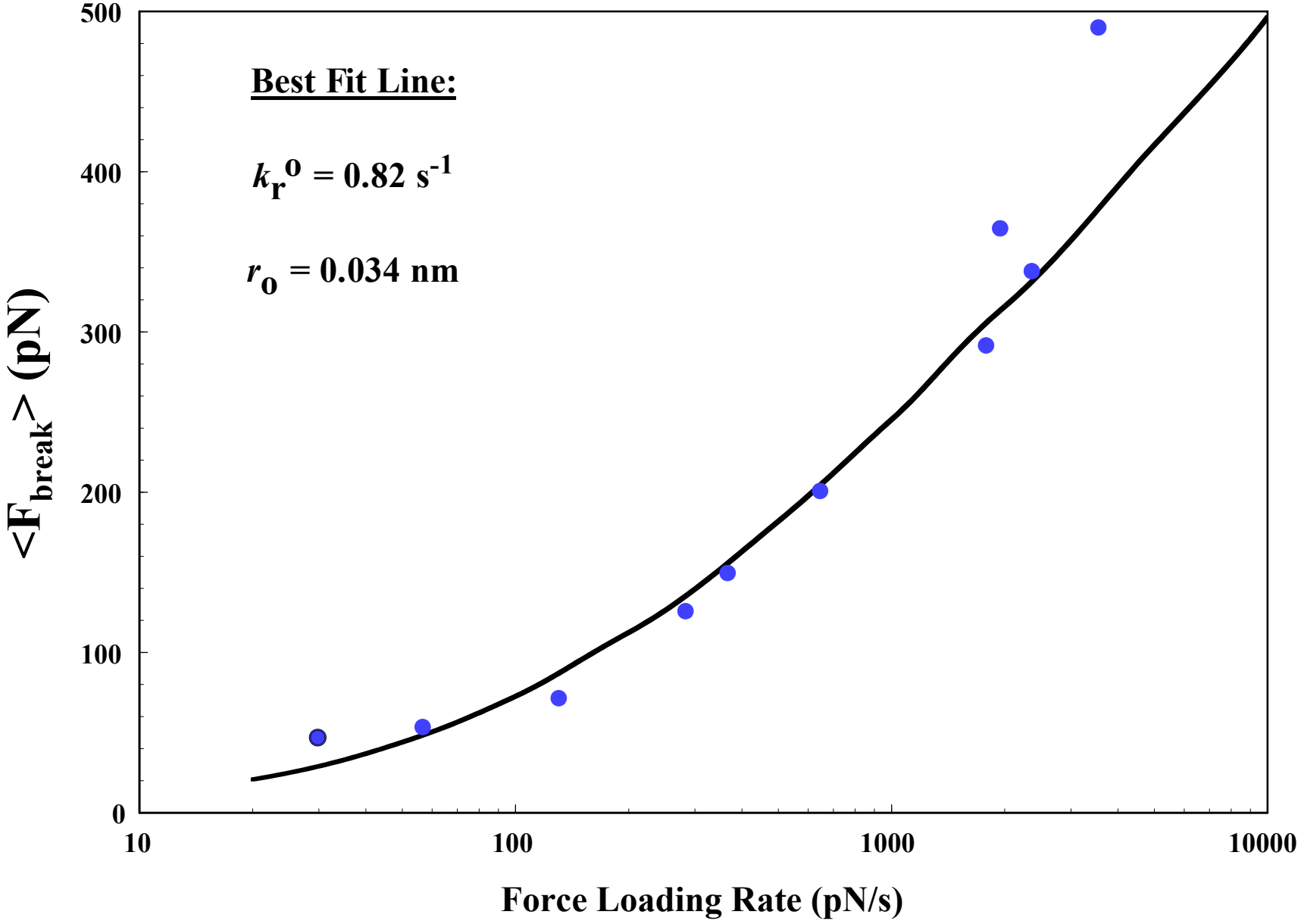
$$\langle f_{break} \rangle = \frac{kT}{r_o} \exp\left[\frac{k_r^o kT}{r_f r_o}\right] E_1\left(\frac{k_r^o kT}{r_f r_o}\right)$$

where $E_1(a)$ is the exponential integral

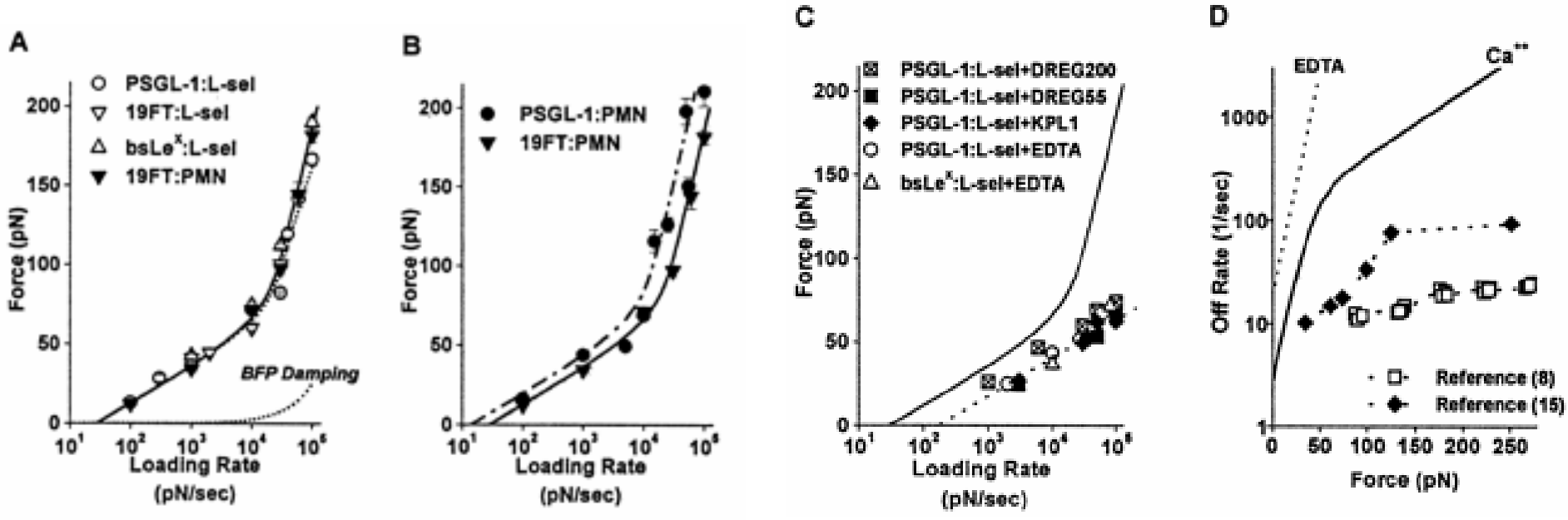
$$E_1(a) = \int_1^\infty \exp(at)/t\, dt$$

Loading rate in rolling experiments:

~100 pN/(0.1—0.01 s) = 1000—10,000 pN/s.


Best Fit Line:
$k_r^o = 0.82\ s^{-1}$
$r_o = 0.034$ nm
500
400
300
200
100
0
10
100
1000
10000
$\langle F_{break} \rangle$ (pN)
Force Loading Rate (pN/s)

L-selectin data using BFP



Evans et al, PNAS, 98:3784-3789, 2001

Protein Unfolding

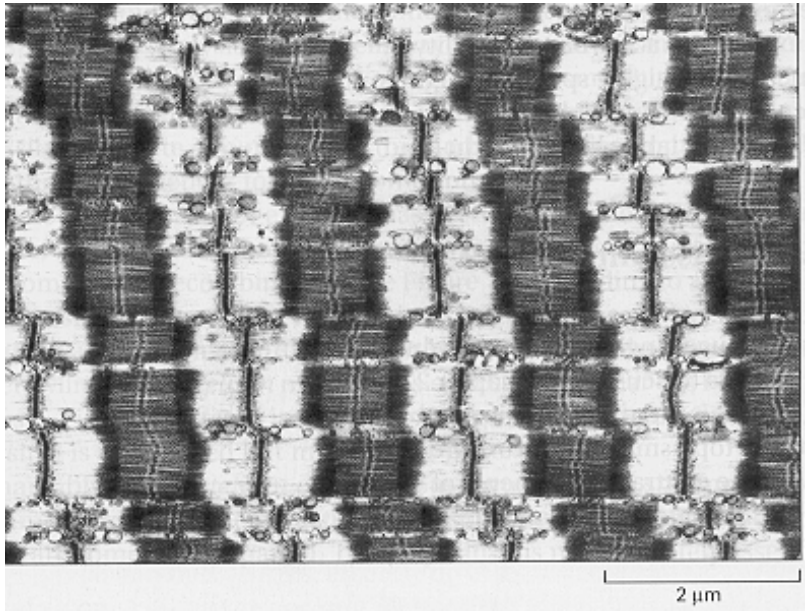
- Giant muscle protein Titin has been unfolded by applied forces using AFM and Optical Tweezers. This process has also been modeled:
  - Kellermayer et al., *Science*, 276:1112, 1997 (Bustamante lab)
  - Rief et al., Science, 276:1109-1112, 1997 (Gaub lab)
  - Tskhovrebova et al. *Nature*, 387:308, 1997
  - Carrion-Vazquez et al., PNAS, 96:3694, 1999
  - Lu & Schulten, Biophys. J. 79:51-65, 2000

- Many other proteins, carbohydrates and DNA have since been exposed to applied forces to study the barriers to deformation. See the following:
  - Wang et al., Stretching DNA with optical tweezers, Biophys. J. 72:1335-1346, 1997.
  - Rief et al., Sequence dependent mechanics of single DNA molecules, *Nature Structural Biology*, 6:346-349, 1999.
  - Marszalek et al., Polysaccharide elasticity governed by chair-boat transitions of the glycopyranose ring., Nature, 396:661-664, 1998.

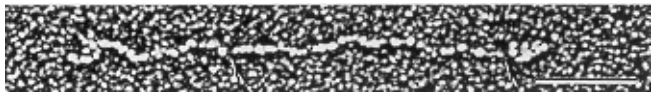
Titin Unfolding

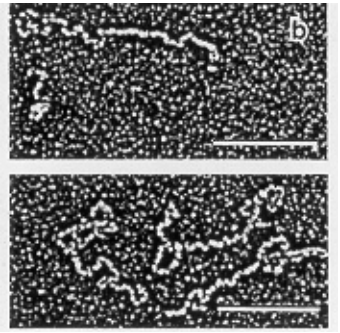
(Carrion-Vazquez et al., *PNAS*, 96:3694, 1999)

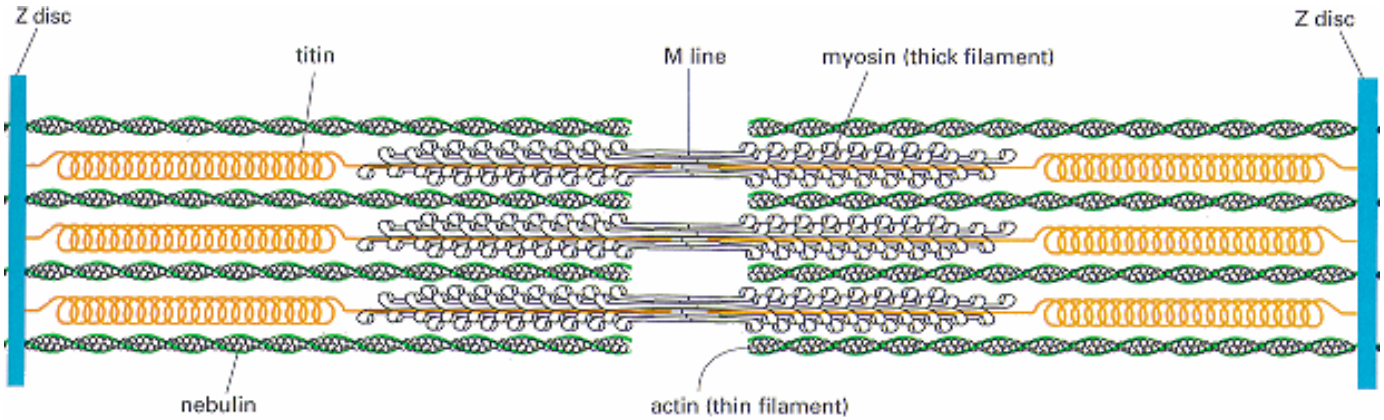
Giant muscle protein Titin can be unfolded by applied force



Micrographs:
Wang et al., *PNAS*, 81:3685, 1984





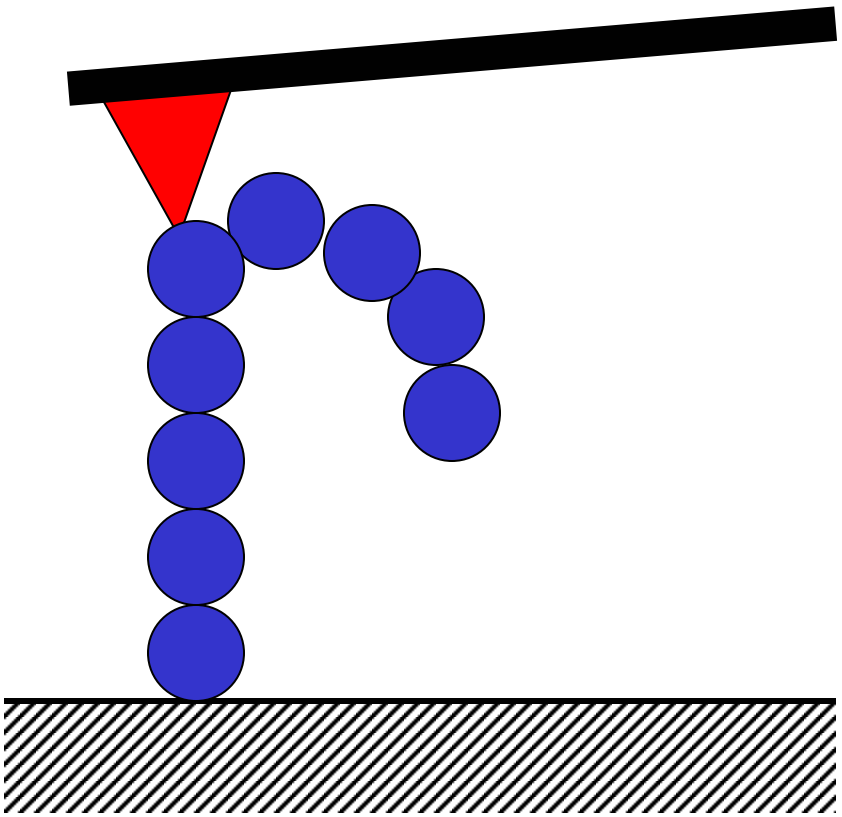


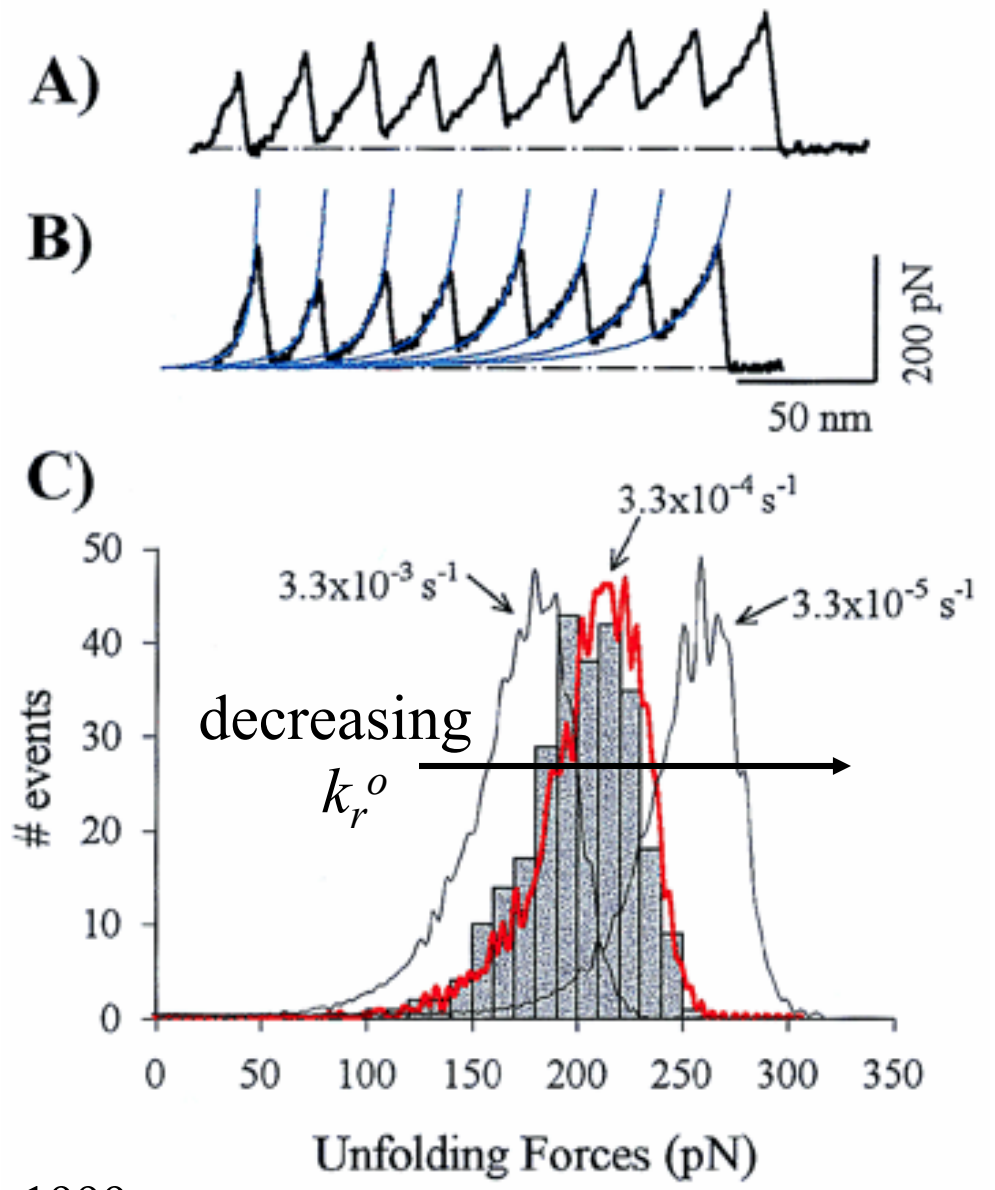
Alberts et al., Molecular Biology of the Cell, 1994

PEVK

Forced Unbinding of Titin Ig Domains

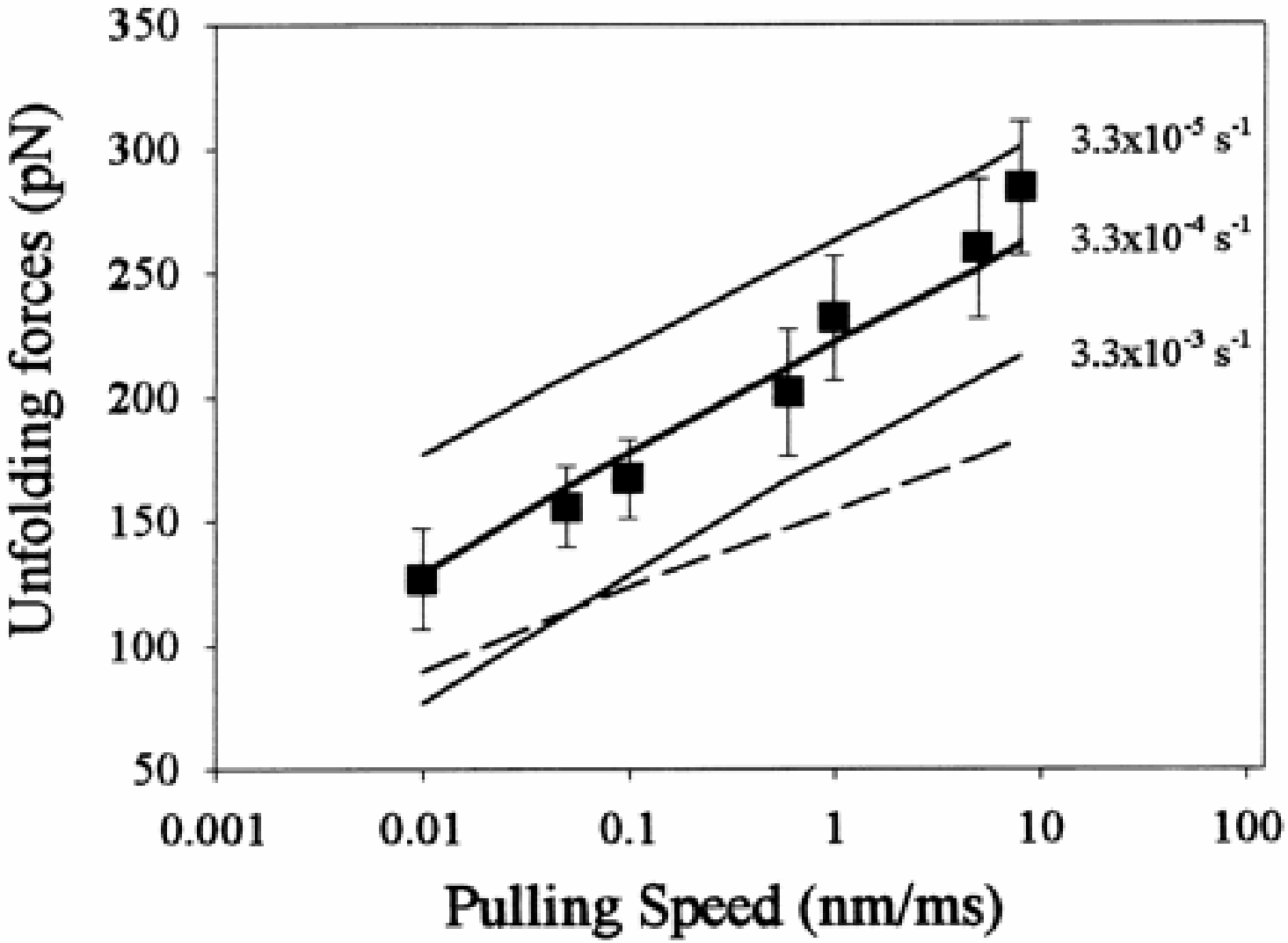
8-mer of I27 domain of Titin





Carrion-Vazquez et al., *PNAS*, 96:3694, 1999

Unfolding Force vs Loading Rate

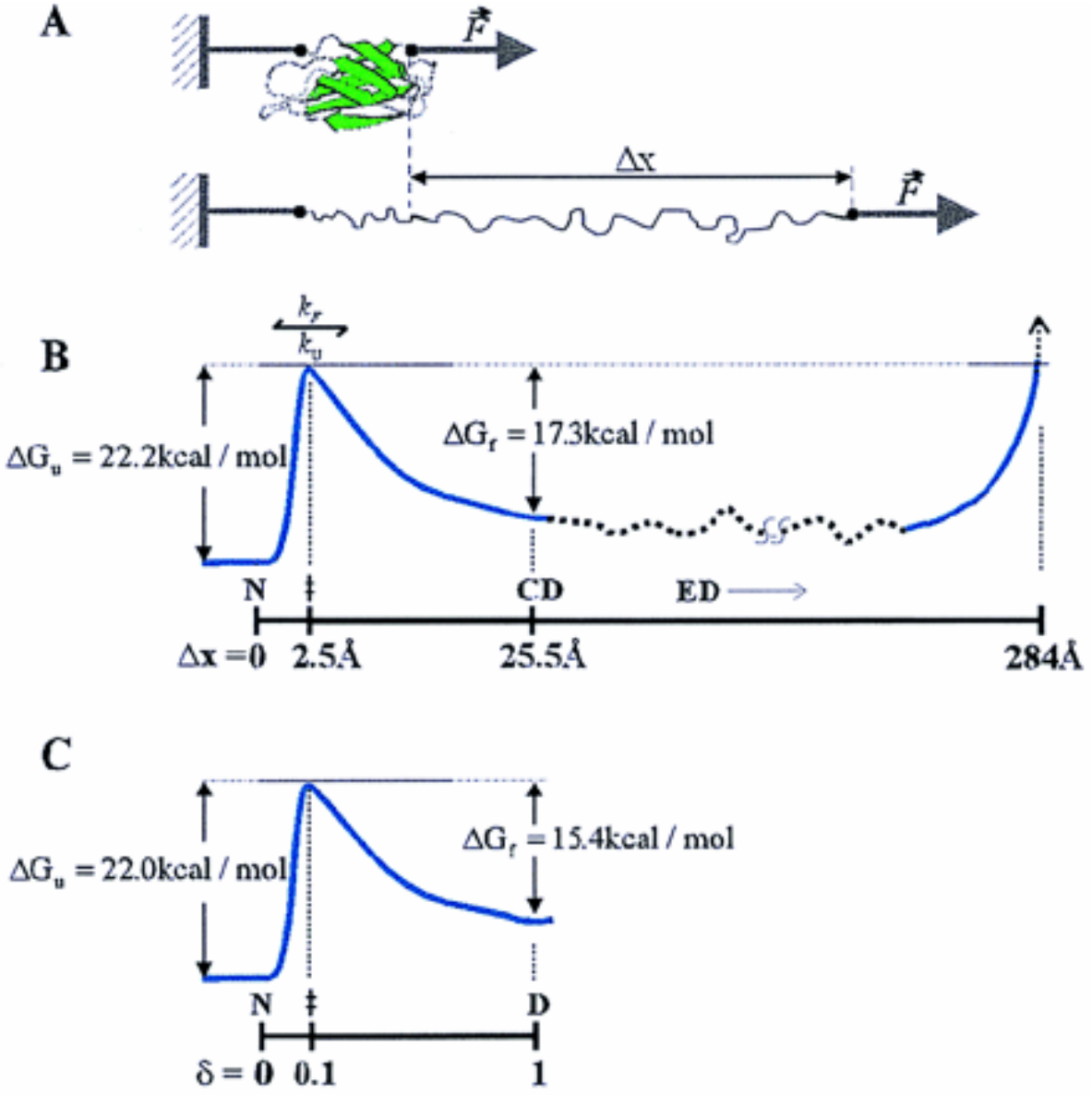


Carrion-Vazquez et al., *PNAS*, 96:3694, 1999

Unfolding Mechanism

Energetics of forced unfolding similar to that for chemical unfolding.

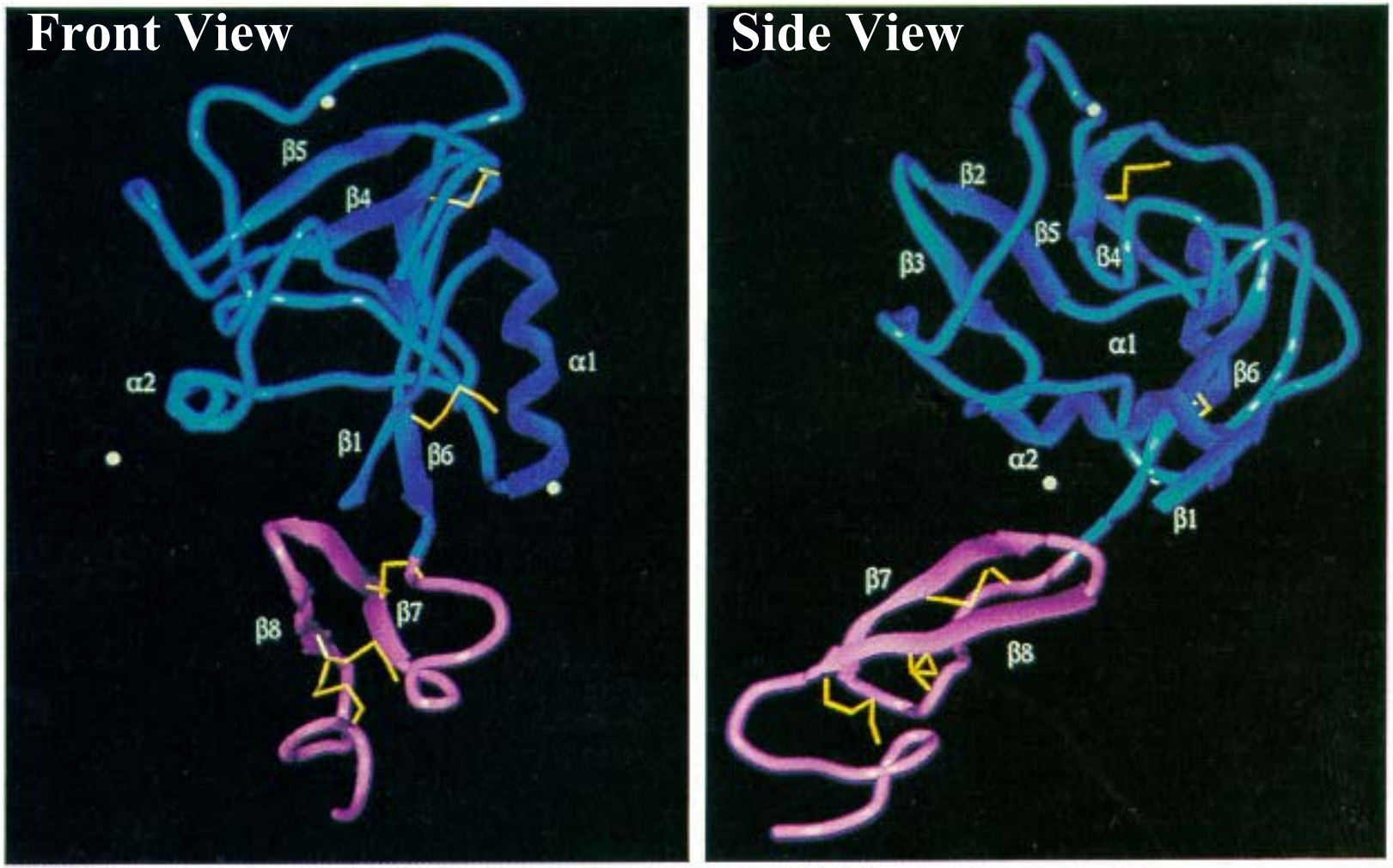
Forced unfolding may thus be used to study chemical pathways.



Carrion-Vazquez et al., *PNAS*, 96:3694, 1999

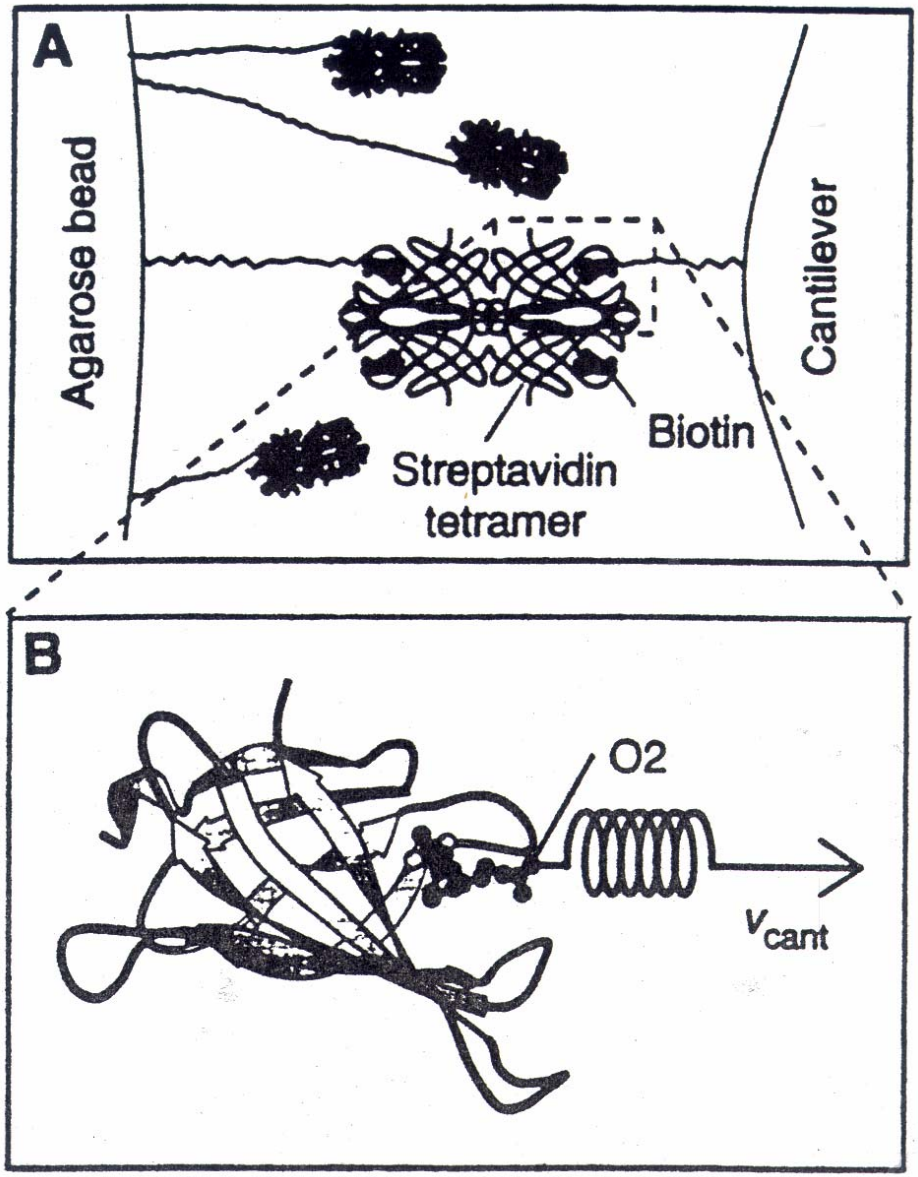
E-selectin Structure

The structure of E-selectin coupled to its carbohydrate ligand has been solved.



Graves et al., *Nature*, 367:532, 1994; Somers et al., *Cell*, 103:467, 2000

Molecular Dynamics Simulations



- Forced unbinding of receptor-ligand systems has been simulated with Molecular Dynamics for Streptavidin-biotin
- Can only simulate nanosecond scale time series - orders of magnitude faster loading than in experiments

Grubmüller et al., *Science*, 271:997, 1996

Bonds in Series and Parallel

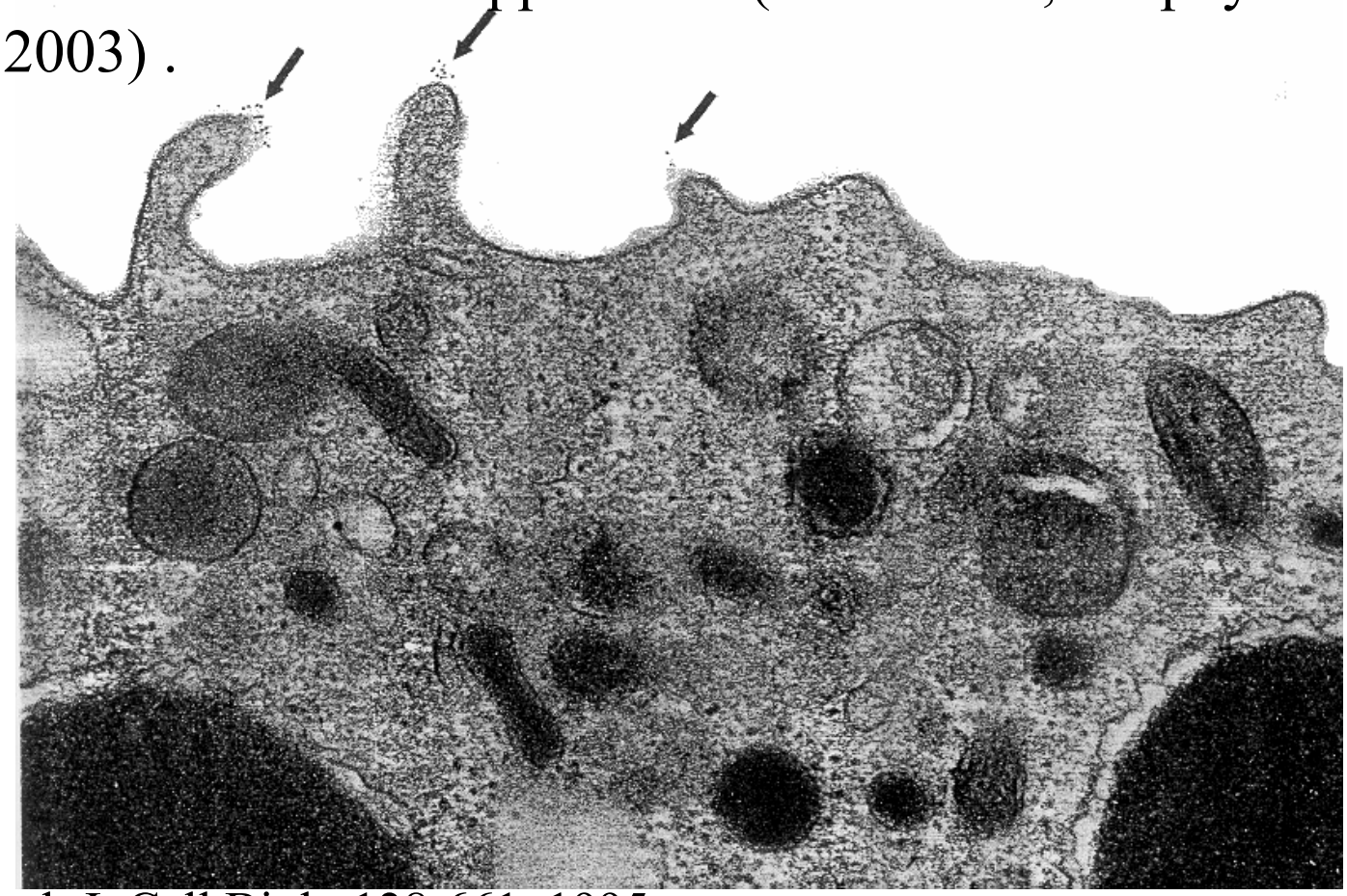
- Bonds in series with an elastic element:
  - Evans, E & K. Ritchie. Strength of a weak bond connecting flexible polymer chains. *Biophys. J.* 76:2439-2447, 1999.

- Parallel Bonds:
  - Tees, D. F. J., J. T. Woodward, and D. A. Hammer. 2001. Reliability theory for receptor-ligand bond dissociation. *Journal of Chemical Physics,* 114:7483-7496.
  - Seifert, U. 2000. Rupture of multiple parallel molecular bonds under dynamic loading. *Physical Review Letters*, 84:2750-2753.
  - Seifert, U. 2002, Dynamic strength of adhesion molecules: role of rebinding and self-consistent rates. *Europhysics Letters*, 58:792-798, 2002.

Microvilli and Membranes

Cell membranes are ruffled (see figure below). Ruffles (microvilli) can stretch (Shao et al, PNAS, 95:6797-6802, 1998).

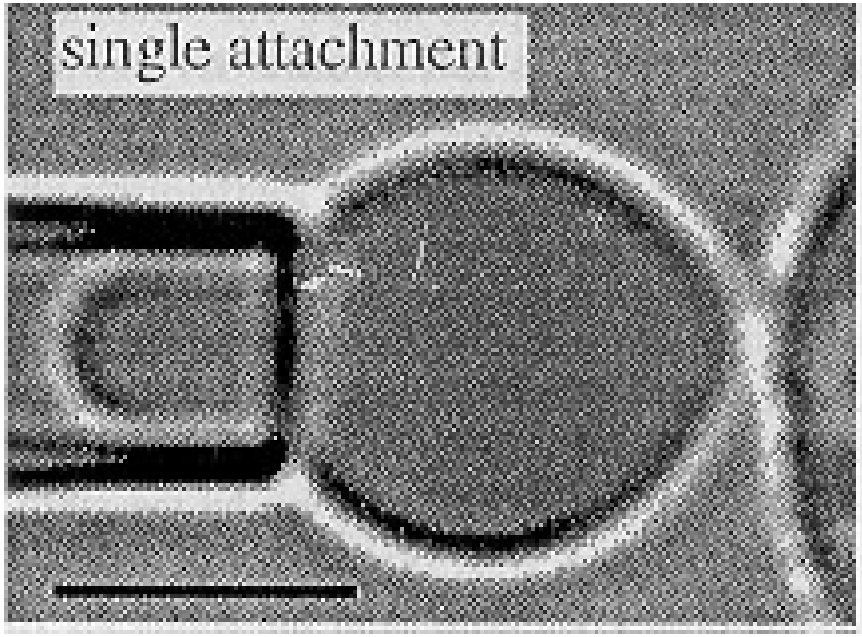
Cell membranes can be lysed by tension. Critical lysis tension depends on rate of force application (Evans et al, Biophys. J. 85:2342-2350, 2003) .

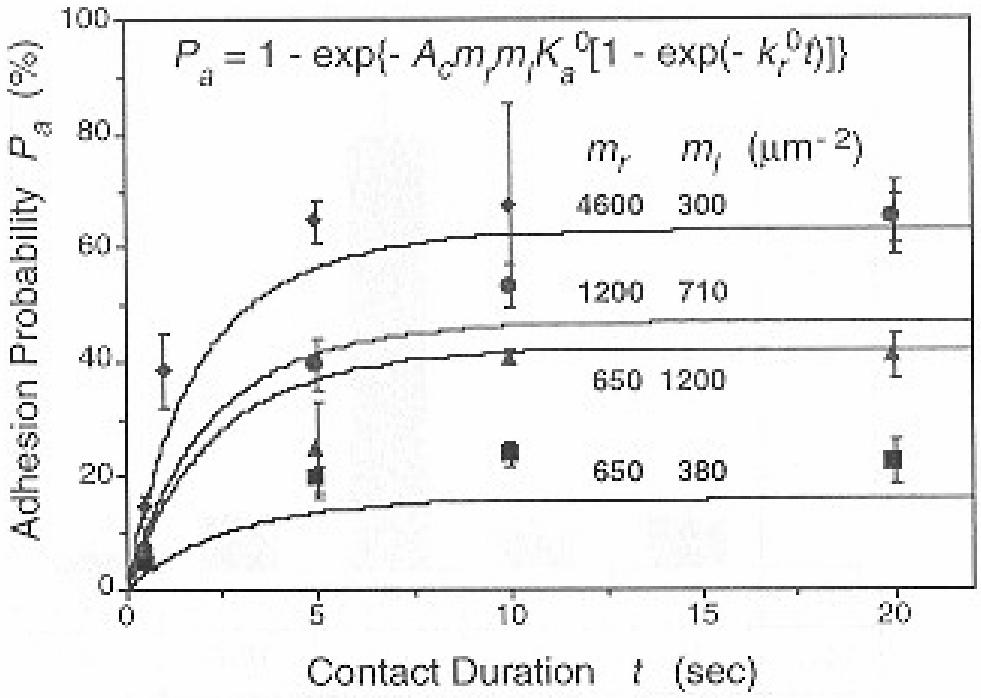


Moore et al, J. Cell Biol., 128:661, 1995

Bond Formation Rate

Adhesion frequency can be used to determine forward rates for bonds using spring as a sensor for single adhesion events.





Chesla et al., *Biophys. J.*, 75:1553-1572, 1998

Summary

- Obvious Applications:
  - The white blood cell rolling paradigm provides a system in which applied forces are physiologically relevant and hence Bell parameters are required for building models. Parameters are also needed so that other systems can be modeled (cell migration, cell realignment in flow, molecular motors, diffusion of receptors in membranes).

- Likely Applications:
  - Force Spectroscopy measurements of intermolecular interaction potential of mean force for receptor-ligand unbinding and protein unfolding should be related to molecular structure

- Open Questions:
- The effect of bonds in series and bonds in parallel has not been sufficiently studied.
  - What constitutes a “parallel” bond.
  - How close do bonds have to be before they can be lumped together or treated separately.

Useful Reference Books

- Biology:
  - Alberts et al., *Molecular Biology of the Cell*: ($102) This introductory cell and molecular biology textbook is a standard reference, good for both the undergraduate and graduate level.

- Biophysics:
  - P. Nelson, *Biological Physics: Energy, Information and Life,* W.H. Freeman and Co., New York, 2003: ($106) is a new, detailed and very readable introduction to a wide range of topics in biological physics.
  - D. L. Lauffenburger and J. L. Linderman *Receptors: models for binding, trafficking, and signaling* ($50) is a good reference for receptor-ligand binding, signaling, cell adhesion and migration.

- Ancillary topics:
  - Jacob Israelachvili, *Intermolecular and Surface Forces, 2nd ed*, ($78) is still the standard reference for intermolecular forces.
  - Paul C. Hiemenz and Raj Rajagopalan *Principles of Colloid and Surface Chemistry, 3rd ed* ($70) is an excellent reference for colloidal phenomena, diffusion and Brownian motion.

Useful References from the Literature

- **Biophysics of Cell Adhesion:**
  - Bell, G. I. 1978. Models for the specific adhesion of cells to cells. *Science (Washington D.C.),* 200:618-627.
  - Dembo, M., D. C. Torney, K. Saxman, and D. Hammer. 1988. The reaction limited kinetics of membrane-to-surface adhesion and detachment. *Proceedings Royal Society of London. B. Biological Sciences,* 234:55-83.
  - Evans, E., D. Berk, and A. Leung. 1991. Detachment of agglutinin-bonded red blood cells I. Forces to rupture molecular-point attachments. *Biophysical Journal,* 59:838-848.
  - Tees, D. F. J., J. T. Woodward, and D. A. Hammer. 2001. Reliability theory for receptor-ligand bond dissociation. *Journal of Chemical Physics,* 114:7483-7496.
- **Force Spectroscopy:**
  - Evans, E. 1999. Energy landscapes of biomolecular adhesion and receptor anchoring at interfaces explored with dynamic force spectroscopy. *Faraday Discussions,* 111:1-16.
  - Evans, E. 2001. Probing the relation between force-lifetime-and chemistry in single molecular bonds. *Annual Review of Biophysics and Biomolecular Structure,* 30:105-128.
  - Evans, E. and K. Ritchie. 1997. Dynamic strength of molecular adhesion bonds. *Biophysical Journal,* 72:1541-1555.
  - Merkel, R. 2001. Force spectroscopy on single passive biomolecules and single biomolecular bonds. *Physics Reports*, 346:343-385.
  - Merkel, R., P. Nassoy, A. Leung, K. Ritchie, and E. Evans. 1999. Energy landscapes of receptor-ligand bonds explored with dynamic force spectroscopy. *Nature,* 397:50-53. (PDF)
  - Seifert, U. 2000. Rupture of multiple parallel molecular bonds under dynamic loading. *Physical Review Letters*, 84:2750-2753.
  - Seifert, U. 2002, Dynamic strength of adhesion molecules: role of rebinding and self-consistent rates. *Europhysics Letters*, 58:792-798, 2002.
  - Tees D.F.J. et al. 2001. A microcantilever device to assess the effect of force on the lifetime of selectin-carbohydrate bonds. *Biophysical Journal,* 82:668-682.
  - Zhu, C. et al, 2002. Measuring receptor/ligand interactions at the single-bond level: Experimental and interpretative issues. *Annals of Biomedical Engineering*, 30:305-314.

Appendix 1– Receptor/Ligand Kinetics

- The Master Equation and reaction kinetics is an area that is not covered in most physicists' education.
- The following appendix covers the basics and introduces Surface Plasmon Resonance (SPR) one of the many techniques for calculating kinetic rate parameters.

- For further information and an excellent introduction to cell signaling networks, see Lauffenburger & Linderman, *Receptors*, Oxford, 1993

Receptor vs Ligand

- For cell-cell adhesion there is no obvious distinction between receptor and ligand.
- For many other situations, however, a soluble molecule binds to a surface bound molecule (e.g. soluble insulin binds to an insulin receptor, or various neurotransmitters secreted by one cell bind to receptors on another cell)
- By convention, we shall define for cases like this:
  - Ligand is a molecule that is dissolved in solution
  - Receptor is a molecule that is bound to a surface (cell or inert substrate like glass or plastic)
- If one cell type that is freely suspended in solution binds to a cell type that is fixed to a surface (e.g. a blood cell attaching to an endothelial cell) then:
  - Ligand is a molecule mounted on the freely suspended cell
  - Receptor is a molecule mounted on the cell attached to a substrate.

Homophilic & Homotypic

- The following types of binding have special names:

- The following refer to cell types
  - Homotypic: same ***cell*** types bind to one another
  - Heterotypic: different ***cell*** types bind to one another

- The following refer to the adhesion molecules that bind the cells together.
  - Homophilic: same ***molecule*** types bind to one another
  - Heterophilic: different ***molecule*** types bind to one another

- Adhesion can be homotypic and heterophilic
- Adhesion can be homotypic and homophilic
- Adhesion can be homophilic and heterotypic
- Adhesion can be homophilic and homotypic

Monovalent Binding

- For the receptor-ligand reaction:

$$R + L \underset{k_r}{\overset{k_f}{\rightleftharpoons}} C$$

- We can write a simple Master Equation that states that the rate of accumulation of bound complex C is equal to the rate at which molecules associate to form C less the rate at which C dissociates into its components:

$$\frac{dC}{dt} = k_f RL - k_r C$$

- Here
  - C is the concentration of product,
  - R is the concentration of receptor
  - L the concentration of ligand.
- The units for all of these is mol/L or M. k_f is the forward reaction rate ($M^{-1}s^{-1}$) and k_r is the reverse reaction rate [s^{-1}]

Monovalent Binding Master Equation

- One can go further by applying "conservation laws":

$$R_T = R + C \quad \text{and} \quad L_o = L + C$$

- where R_T = total number of receptors and L_o = initial ligand concentration. We thus obtain:

$$\frac{dC}{dt} = k_f \left(R_T - C\right)\left(L_o - C\right) - k_r C$$

- To simplify this, suppose that L_o is very much larger than C and thus ligand isn't depleted much by the reaction from its initial value, L_o. We then get:

$$\frac{dC}{dt} = k_f \left(R_T - C\right) L_o - k_r C$$

- As one may check that with the initial condition $C(t = 0) = C_o$, the solution to this equation is:

$$C(t) = C_o \exp\left[-\left(k_f L_o + k_r\right)t\right] + \left(\frac{k_f L_o R_T}{k_f L_o + k_r}\right)\left\{1 - \exp\left[-\left(k_f L_o + k_r\right)t\right]\right\}$$

- As $t \rightarrow \infty$, (i.e. at equilibrium):

$$C_{eq} = \left(\frac{k_f L_o R_T}{k_f L_o + k_r}\right)$$

Lauffenburger & Linderman, *Receptors*, Oxford, 1993

K_D and K_A

- One can simplify the equilibrium concentration a bit, by using the ratio $K_D = k_r/k_f$:

$$C = \frac{k_f L_o R_T}{k_f L_o + k_r} = \frac{L_o R_T}{L_o + k_r/k_f} = \frac{L_o R_T}{L_o + K_D}$$

- K_D is called the dissociation constant. A related constant is $1/K_D = k_f/k_r = K_A$, the association constant. We then have:

$$C = \frac{k_f L_o R_T}{k_f L_o + k_r} = \frac{\left(k_f/k_r\right) L_o R_T}{\left(k_f/k_r\right) L_o + 1} = \frac{K_A L_o R_T}{1 + K_A L_o}$$

- When $K_A = K_D = 1$, we get

$$C = \frac{L_o R_T}{1 + L_o}$$

Lauffenburger & Linderman, *Receptors*, Oxford, 1993

Product Equilibrium Concentration

- One can then see how the concentration of product, C_{eq}, changes as the ligand concentration is varied. We had:

$$C_{eq} = \frac{L_o R_T}{L_o + K_D}$$

- When $L_o >> K_D$, we get:

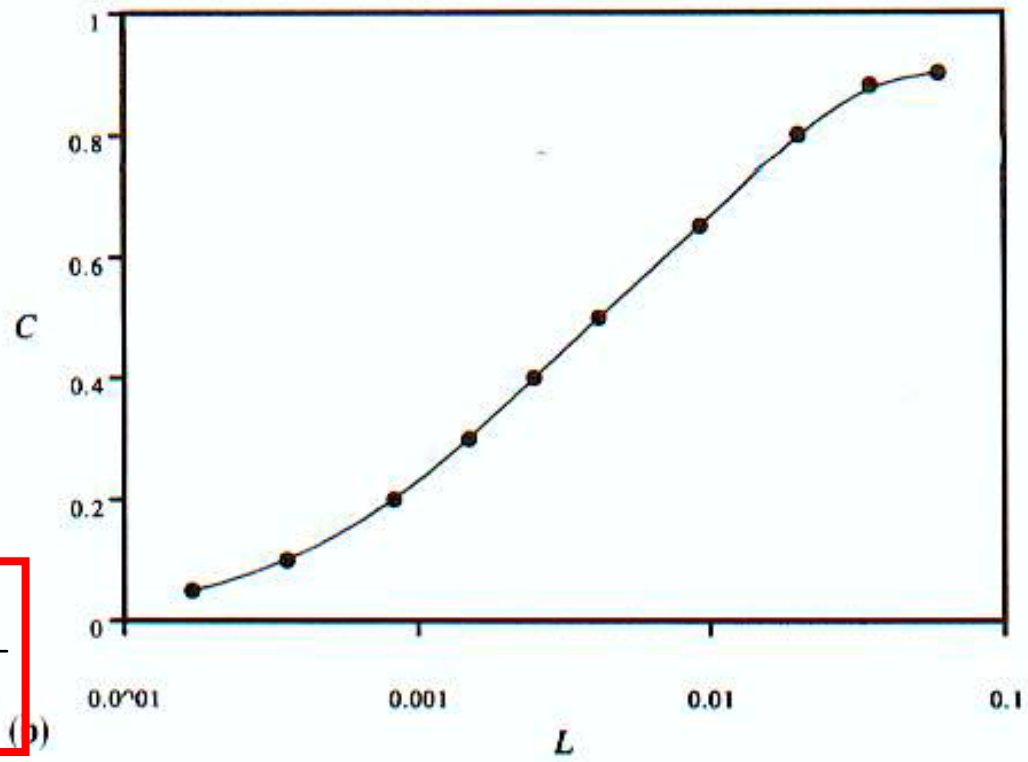
$$C_{eq} = \frac{L_o R_T}{L_o} = R_T$$

- When $L_o << K_D$, we get:

$$C_{eq} = \frac{R_T}{K_D} L_o$$

- When $L_o = K_D$, we get:

$$C_{eq} = \frac{K_D R_T}{K_D + K_D} = \frac{K_D R_T}{2K_D} = \frac{R_T}{2}$$



Lauffenburger & Linderman, *Receptors*, Oxford, 1993

Meaning of K_D and K_A: Affinity

- The K_A and K_D give estimates of Affinity.
- A system where the reaction goes almost to completion (i.e $C_{eq} \sim R_T$ and hence there will be very little free R at equilibrium) is considered to be "high affinity". It will have a large K_A (e.g. 10^9 or 10^{12} M^{-1}) or a tiny K_D (e.g. nM or pM).
- A system where the reaction goes only partly to completion at equilibrium is "low affinity" and it will have a small K_A (e.g. 10^3 M^{-1}) or a large K_D (e.g. mM)
- K_D's from pM to M are observed with biological receptor ligand bonds. Evolution seems to have tailored the affinity to the function to be performed.

Scatchard Plots I

- How do we measure K_D and the other rates? For K_D, we can do some clever rewriting of the equilibrium product concentration:

$$C_{eq} = \frac{L_o R_T}{L_o + K_D}$$

- Move the denominator to the LHS and rearrange

$$C_{eq} L_o + C_{eq} K_D = L_o R_T$$

$$C_{eq} K_D = -C_{eq} L_o + R_T L_o$$

- We finally obtain:

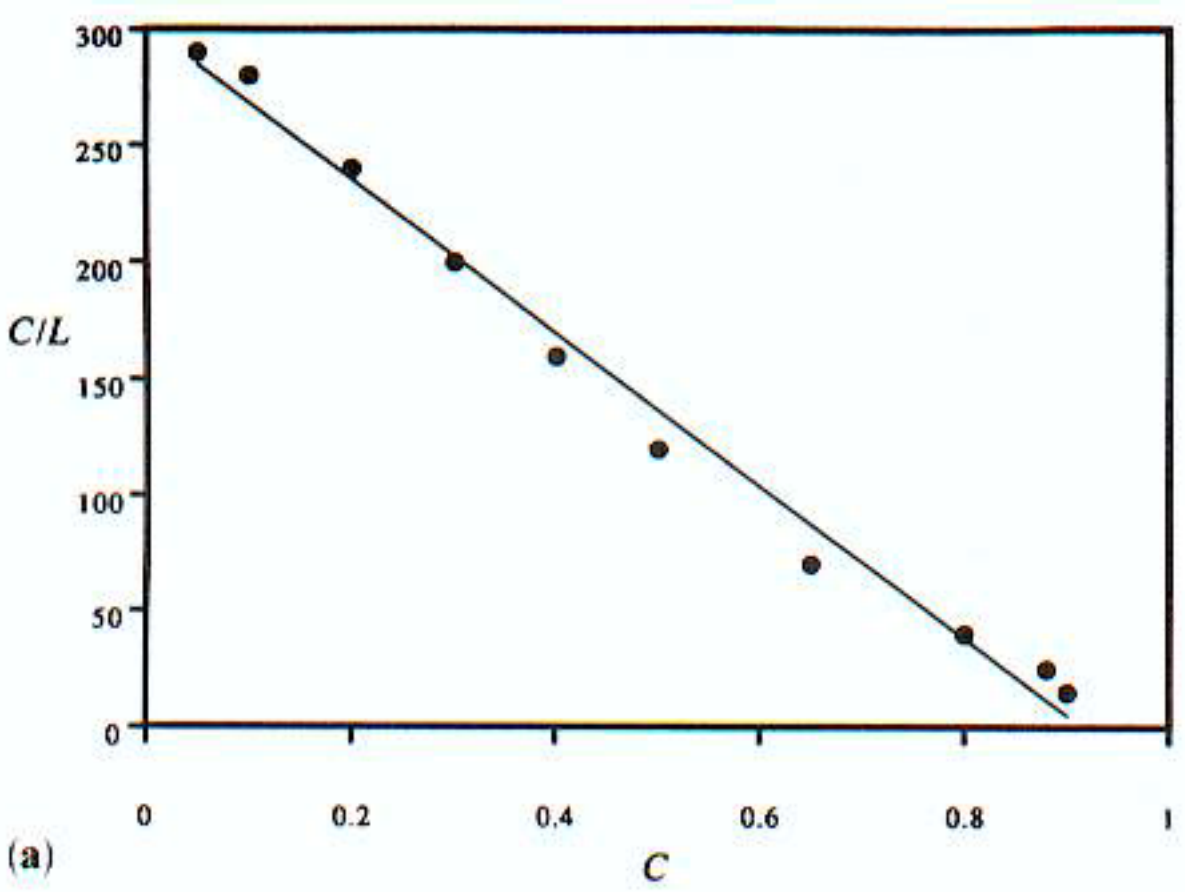
$$\frac{C_{eq}}{L_o} = -\frac{C_{eq}}{K_D} + \frac{R_T}{K_D}$$

- If we plot C/L (i.e. bound/free ligand) vs C, we should have a straight line with slope = $-1/K_D$ and C/L intercept = R_T/K_D. A plot that shows this is called a Scatchard Plot.

Lauffenburger & Linderman, *Receptors*, Oxford, 1993

Scatchard Plots II

- The following is a sample Scatchard Plot (for benzodiazepine binding to rat brain cells.



- NB: The *C*'s have to be equilibrium values for a given ligand concentration

Lauffenburger & Linderman, *Receptors*, Oxford, 1993

Reaction Kinetics I: Formation

- The affinity is a ratio of the forward and reverse reaction rates.
- Can get high affinity if k_f and k_r are both fast
- Can also get high affinity if k_f and k_r are both slow, however.
- Exact values of k_f and k_r are important. It is thus necessary to examine kinetics of the reaction. We had:

$$C(t) = C_o \exp\left[-\left(k_f L_o + k_r\right)t\right] + \left(\frac{k_f L_o R_T}{k_f L_o + k_r}\right)\left\{1 - \exp\left[-\left(k_f L_o + k_r\right)t\right]\right\}$$

- For the case of bond formation, suppose that there is initially no bound complex, C (i.e. $C_o = 0$):

$$C(t) = \left(\frac{k_f L_o R_T}{k_f L_o + k_r}\right)\left\{1 - \exp\left[-\left(k_f L_o + k_r\right)t\right]\right\}$$

- This can be written:

$$C(t) = A\left[1 - \exp\left(-k_{obs} t\right)\right]$$

- Where $A = L_o R_T/(L_o + K_D)$ and $k_{obs} = k_f L_o + k_r$ are effective concentrations and rates respectively.

Bond Kinetics II: Dissociation

- Suppose the concentration of bound complex, C has built up to some equilibrium value, C_{eq}. Suppose further that one could then wash out all of the free ligand, so $L_o = 0$. The original equation:

$$C(t) = C_o \exp\left[-\left(k_f L_o + k_r\right)t\right] + \left(\frac{k_f L_o R_T}{k_f L_o + k_r}\right)\left\{1 - \exp\left[-\left(k_f L_o + k_r\right)t\right]\right\}$$

- then becomes:

$$C(t) = C_{eq} \exp\left(-k_r t\right)$$

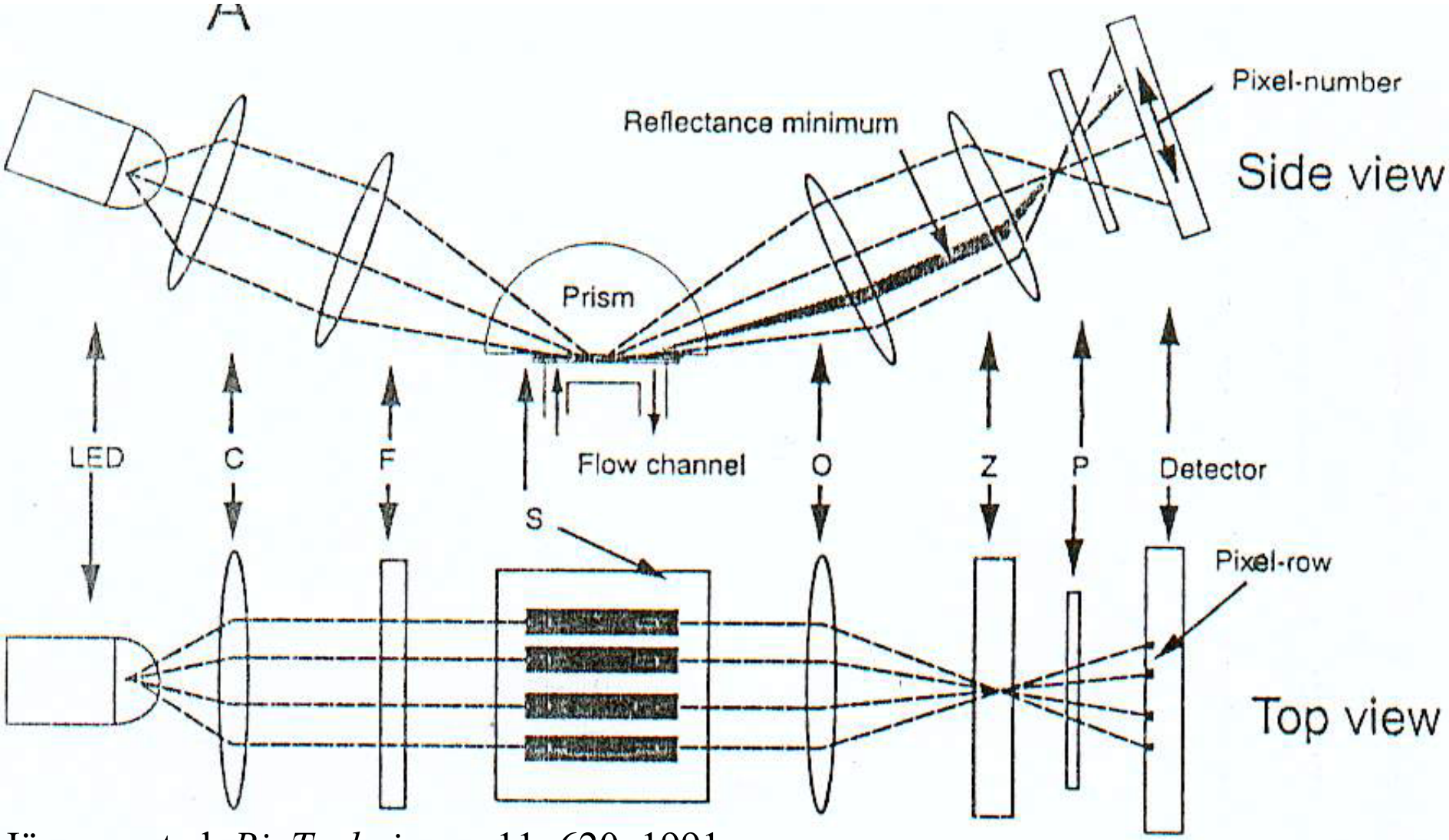
- This is a simple exponential decay.

Surface Plasmon Resonance

- A surface plasmon is a quantum of vibration in the free electron gas in a metal.
- Plasmons can be induced by photons. When the incident light direction is just right, energy is absorbed in creation of surface plasmons.
- Angle for maximum absorption is very sensitive to index of refraction on other side of interface.
- Use absorption as an indicator of index of refraction.
- Index of refraction next to surface depends on concentration of molecules (and proteins) next to the surface
- Pharmacia Diagnostics patented a surface plasmon resonance technique for measuring the kinetics of receptor-ligand binding
- Device uses a special microfluidics chip to flow in or wash out ligand.
- Device for Biospecific Interaction Analysis (BIA) is marketed under trade name BIAcore.

SPR: Geometry

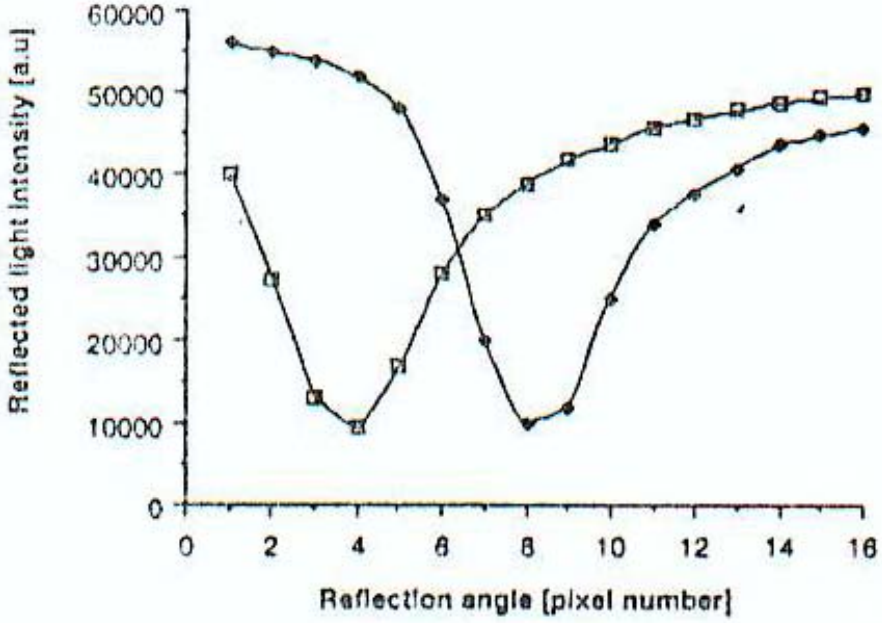
- Beam of laser light impinges on a metal film that lies underneath a flow channel.

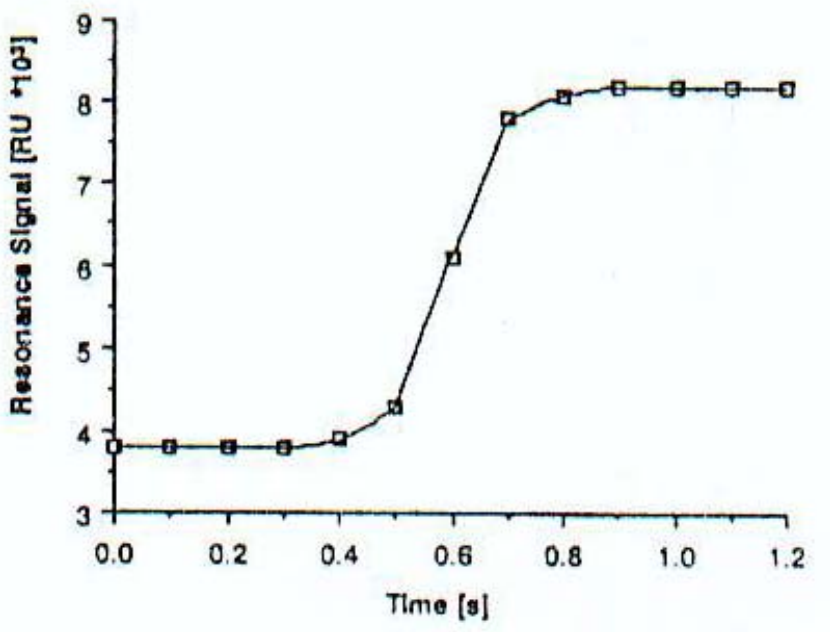


Jönsson et al, *BioTechniques*, 11: 620, 1991

SPR Procedure

- Vary the angle of incidence and observe absorption of light beam as dip in reflected light intensity.
- Monitor optimal angle for surface plasmon resonance as a function of time. This is directly related to index of refraction on the non-illuminated side. Index of refraction is related to concentration of proteins.
- Plot resonance angle vs time in Resonance Units (RU)

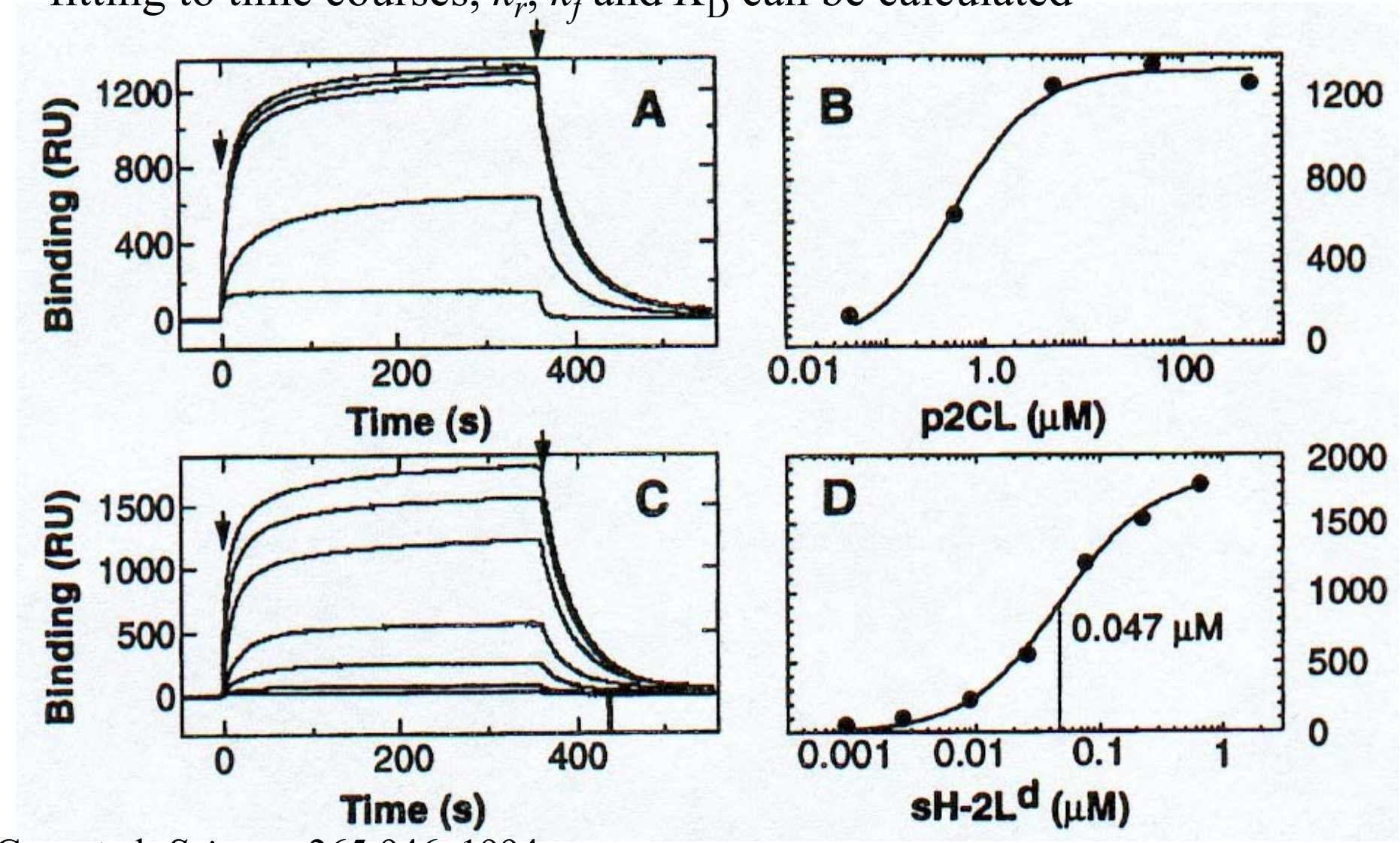




Jönsson et al, *BioTechniques*, 11: 620, 1991

MHC/TCR binding and unbinding

- Resonance units are proportional to concentration of product, *C*. From fitting to time courses, k_r, k_f and K_D can be calculated



Corr et al, *Science*, 265:946, 1994

Appendix 2–The Bell Model

- The Bell model is the most commonly used expression for the force dependence of bond reaction rates.

- The following slides go through the original derivation and give its sources.

- NB: Every author seems to use their own symbol for the parameter that Bell originally called γ. Bell also used r_o for a similar transition state distance. Evans used x_β for this in some of his papers. Springer used the symbol σ. Seifert has used x_b and μ. Other authors feel at liberty to make up yet more symbols. I would propose that you should stick with Bell's original usage and use r_o or γ for the transition state distance.

Bell Model

- Bell proposed what is now called the Bell Model for lifetime, τ, of receptor-ligand bond failure:

$$\tau = \tau_o \exp\left[\left(E_o - \gamma f\right)/kT\right]$$

- Here τ_o is the reciprocal of the natural frequency of oscillations in solids (he says ~10^{-13} s due to damping in liquids it is probably more like 10^{-11} s). This gives the time for return to an energy barrier
- The exponential is a Boltzmann-like probability factor giving probability of escape from the energy barrier
- The energy barrier (change in free energy) for the unbinding is:

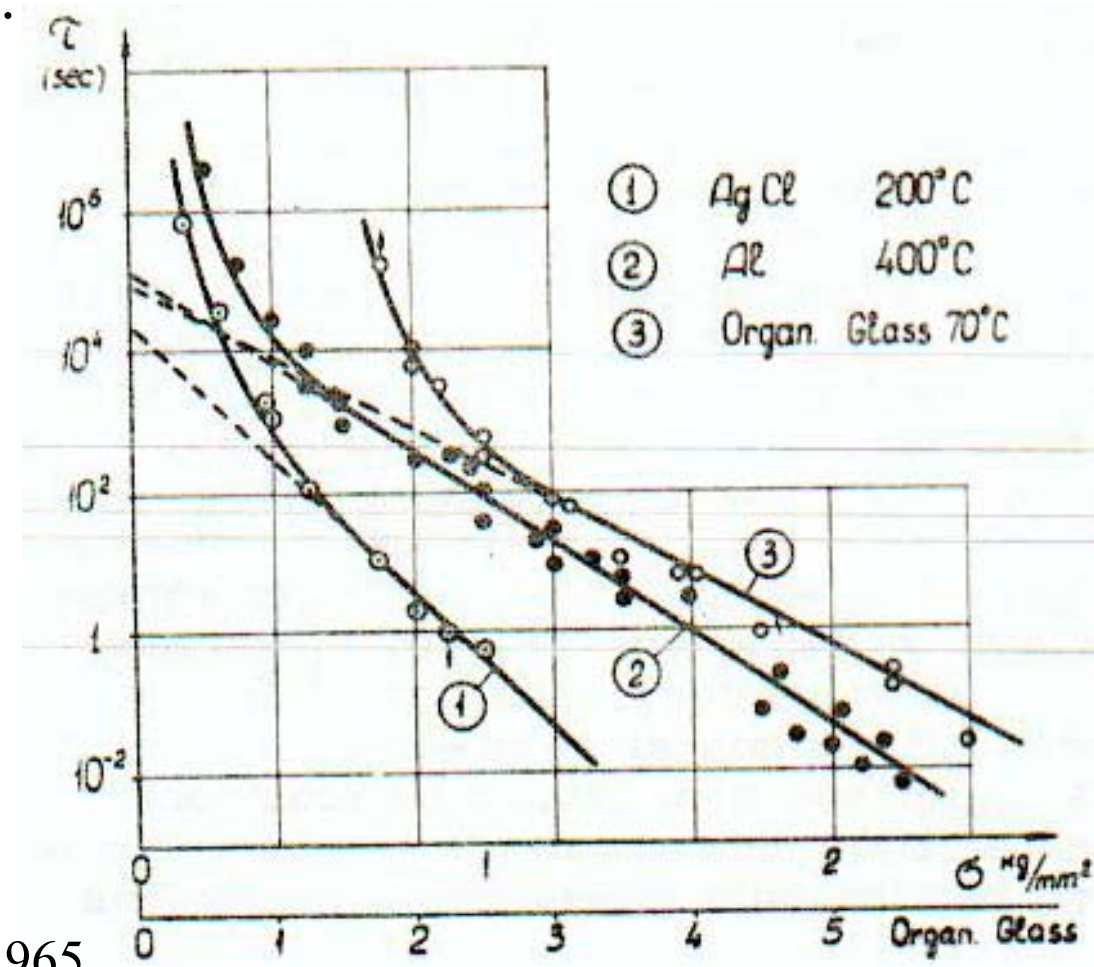
$$E(f) = E_o - \gamma f$$

- E_o is the height of the barrier, γ is the range of the barrier and f is the applied force per bond

G. I. Bell, *Science*, 200:618, 1978

Zhurkov Macroscopic Failure

- S. N. Zhurkov, *Int. J. Fract. Mech.* 1:311, 1965 is cited source for Bell model.
- Zhurkov measured lifetime to rupture for macroscopic wires subjected to tensile stress σ.
- Zhurkov gives the equation that Bell uses without a reference.
- In this figure, τ is lifetime σ is related to tensile stress.



Zhurkov, *Int. J. Fract. Mech*. 1:311, 1965

Bell Model

- One can separate out the E_o part of the exponential and combine it with τ_o to get τ in terms of a zero force lifetime, t_o:

$$\tau = \tau_o \exp\left[E_o/kT\right]\exp\left[-\gamma f/kT\right] = t_o \exp\left[-\gamma f/kT\right]$$

- One can note that $k_r = 1/\tau$, so:

$$k_r = k_r^o \exp\left[\gamma f/kT\right]$$

- Where k_r^o is the zero-force reverse reaction rate.
- This final value is what is usually referred to as the Bell model
- Basic original source for Bell equation is H. A. Kramer's 1940 paper on Transition State Theory (*Physica*, 7:284-304, 1940) but this lacked the force part.
- For a more detailed theory, see Evans & Ritchie, Biophys. J. 72:1541-1555, 1997

Appendix 3: Reliability Theory Analysis of Bonding

- Reliability theory forms the basis for understanding forced unbinding and multiple bonding.

- A wonderful reference for reliability theory that has many of the results derived here, is Petr Beckmann's book *Probability in Communication Engineering*, Harcourt, Brace & World, New York, 1967
- Many of the results here are published in my paper: D.F.J. Tees et al, *J. Chem. Phys.*, 114:7483-7496, 2001

Reliability Theory

- Bond break-up can be modeled using Reliability Theory.

Conditional probability

P(failure in t, $t+dt$| bond survives to t) = $P(t<T<t+dt|T>t) = k_r(t)\,\Delta t$.

Define Reliability: $r(t) = P(T>t)$ i.e. probability that bond breaks after time t.

$$r(t+\Delta t) = [1 - k_r(t)\,\Delta t]\, r(t)$$

Rearranging, taking the limit as $\Delta t \rightarrow 0$ and integrating:

$$\ln r(t) = \exp\left[-\int_0^t k_r(t)dt\right]$$

To get probability density, $p(t)$, note:

$$r(t) = P(T > t) = \int_t^\infty p(t)dt = \rho(\infty) - \rho(t)$$

where $r'(t) = p(t)$. Since $r(\infty) = 0$, $r(t) = -r(t)$ and hence:

$$p(t) = -r'(t) = k_r(t)\exp\left[-\int_0^t k_r(t')dt'\right]$$

P. Beckmann, *Probability in Communication Engineering*, 1967

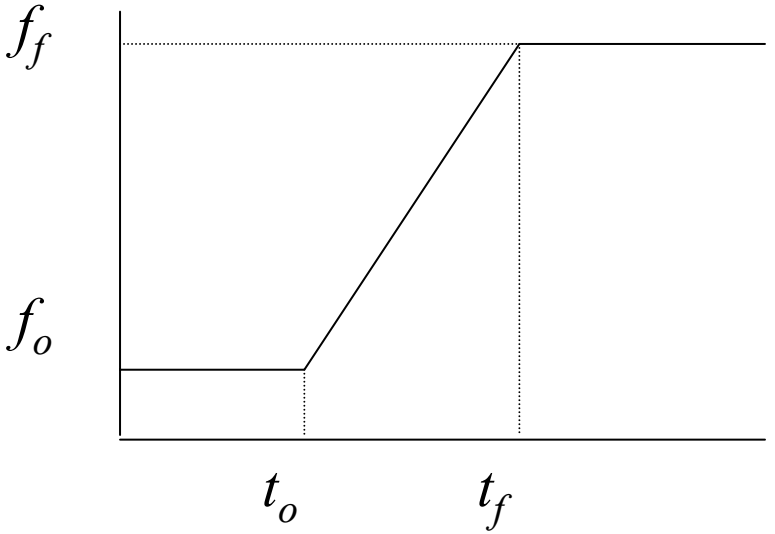
Probability Density and Loading Rate

Reliability Theory leads to different probability densities for break-up depending on the force loading protocol

$$p(t)dt = k_r\left[f(t)\right]\exp\left\{-\int_o^t k_r\left[f(t')\right]dt'\right\}dt.$$

One form for time dependence of force, $f(t)$ is:

$$f(t) = \begin{cases} f_o; t < t_o \\ f_o + r_f(t - t_o); t_o \le t < t_f \\ f_f; t \le t_f \end{cases}$$



r_f = force loading rate (pN/s),
i.e. rate of increase in force.

Tees et al, *J. Chem. Phys.*, 114:7483, 2001

Special Cases

For compactness, set $\beta = k_B T$

Force applied instantaneously ($r_f t_f << 1/k_r(f_f)$), $t_o = 0; f_o = 0; t_f = 0$

$$p(t) = k_r^o \exp\left(\frac{f_f}{\beta}\right) \exp\left[-k_r^o t \exp\left(\frac{f_f}{\beta}\right)\right]$$

Constant linear force ramp from an initial value, f_o , $t_o = 0$, $t_f = \infty$

$$p(t) = k_r^o \exp\left(\frac{f_o + r_f t}{\beta}\right) \exp\left[-k_r^o \exp\left(\frac{f_o}{\beta}\right) \frac{\beta}{r_f} \left\{\exp\left(\frac{r_f t}{\beta}\right) - 1\right\}\right]$$

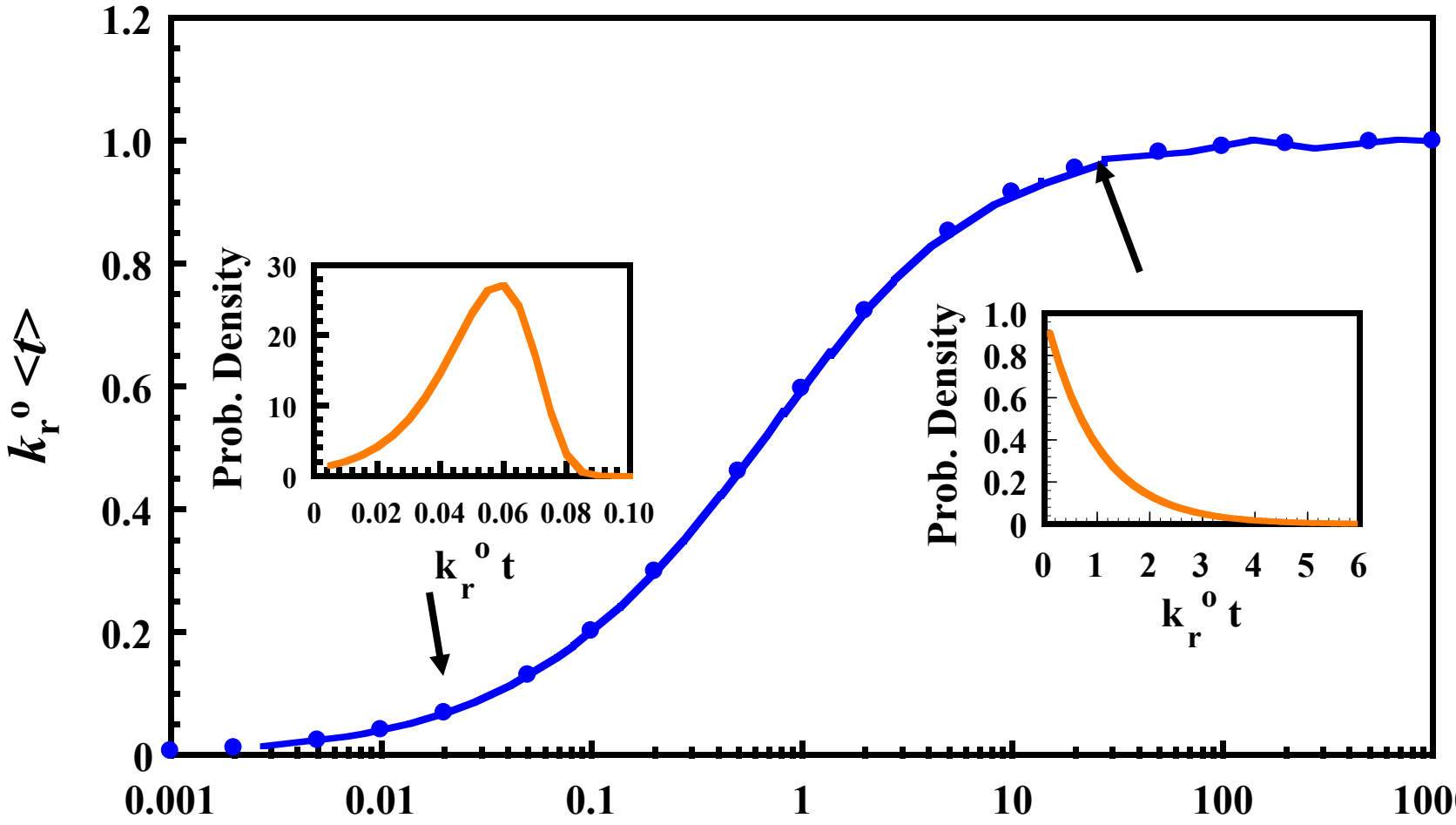
Linear ramp from $f = 0$: $t_o = 0; f_o = 0; t_f = \infty$:

$$p(t) = k_r^o \exp\left(\frac{r_f t}{\beta}\right) \exp\left[-k_r^o \frac{\beta}{r_f} \left\{\exp\left(\frac{r_f t}{\beta}\right) - 1\right\}\right]$$

Tees et al, *J. Chem. Phys.*, 114:7483, 2001

Average Break-up Time for Force Ramp

So: $k_r^0 \langle t \rangle = a \exp(a) E_1(a)$ where $a = k_r^0 k_B T / r_o r_f$



Fast loading ← $k_r^o kT / r_o r_f$ → **Slow loading**

Tees et al, *J. Chem. Phys.*, 114:7483, 2001

<Force> vs Loading Rate for Ramp

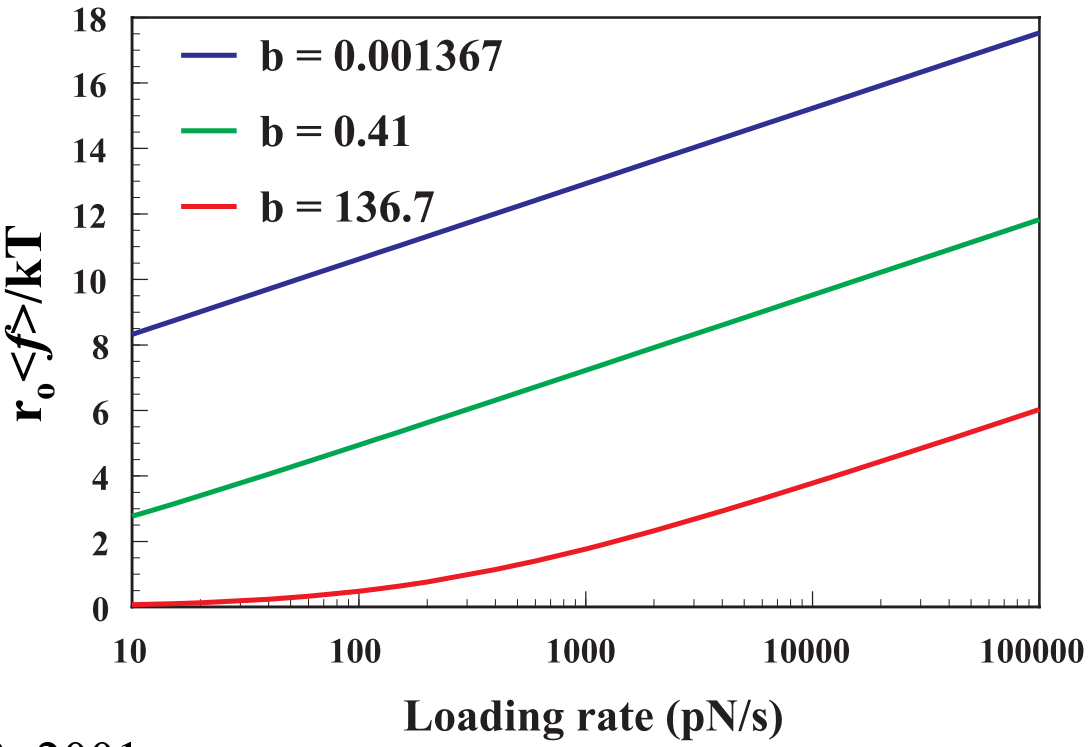
For a constant force ramp, $\langle f \rangle = r_f \langle t \rangle$. Write $\langle f \rangle$ in terms of loading rate using $a = b/r_f$ where $b = k_r^o\, kT/r_o$:

$$\langle f \rangle = \frac{kT}{r_o} \exp\left(\frac{b}{r_f}\right) E_1\left(\frac{b}{r_f}\right) \qquad \text{For } b/r_f \ll 1, \quad \langle f \rangle \sim \frac{kT}{r_o}\left(\ln r_f - \gamma - \ln b\right)$$

Streptavidin-like bonds:
($k_r^o \sim 10^{-4}$ s^{-1}; $r_o \sim 0.3$ nm):
$b = 0.001367$ pN s^{-1}

Antibody-like bonds:
($k_r^o \sim 10^{-2}$ s^{-1}; $r_o \sim 0.1$ nm):
$b = 0.41$ pN s^{-1}

Selectin-like bonds:
($k_r^o \sim 1$ s^{-1}; $r_o = 0.03$ nm):
$b = 136.7$ pN s^{-1}



Tees et al, *J. Chem. Phys.*, 114:7483, 2001

Multiple Bonds

Suppose there are n, **independent** parallel bonds with no reformation allowed. For **each bond**, $P(T>t) = r(t)$. Thus $R(t)$, probability of failure of all bonds after time t is:

$$1\text{-}R(t) = [1\text{-}\, r(t)]^n \text{ or } R(t) = 1\text{-}[1\text{-}\, r(t)]^n$$

From Reliability theory, Probability density,

$$p(t) = \text{-}R'(t) = nr'(t)[1\text{-}r(t)]^{n\text{-}1}$$

or:

$$p(t) = nk_r(t)\exp\left[-\int_0^t k_r(t')dt'\right]\left[1-\exp\left[-\int_0^t k_r(t')dt'\right]\right]^{n-1}$$

Since the bonds must be independent this treatment only applies for $k_r(t) = k_r^{\,o}$, i.e. constant.

$$p(t) = nk_r^o \exp\left(-k_r^o t\right)\left[1-\exp\left(-k_r^o t\right)\right]^{n-1}$$

P. Beckmann, *Probability in Communication Engineering*, 1967

Average Break up Time for Multiple bonds
(No Applied Force)

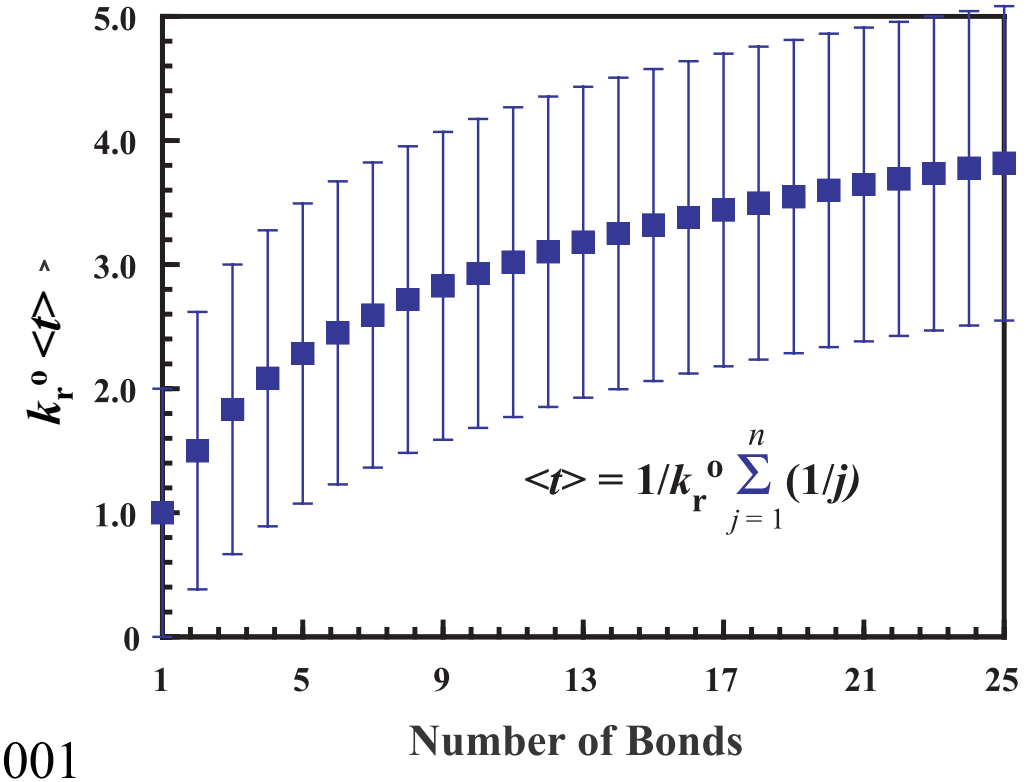
Average break-up time from Reliability Theory:

$$\langle t \rangle = \int_0^\infty t p(t) dt = \int_0^\infty n k_r^o \exp\left(-k_r^o t\right)\left[1-\exp\left(-k_r^o t\right)\right]^{n-1} t dt$$

Use the binomial expansion, exchange the sum and integral, and evaluate. After rearrangement we get:

$$\langle t \rangle = \frac{1}{k_r^o} \sum_{j=1}^{n} \frac{1}{j}$$

Find that $k_r^o \langle t \rangle = H_n$, the *n*th harmonic number



Tees et al, *J. Chem. Phys.*, 114:7483, 2001

Monte Carlo Simulation

- For each of 10,000 successive adhesive events
  - Choose number of bonds, n, (fixed or from Poisson distribution)
  - In each time step, Δt
  - Find instantaneous force/bond (using force history)
  - Calculate probability of break-up using:

$$P_b = (1\text{-}\exp[\text{-}k_r(f)\,\Delta t])$$

  - where $k_r(f) = k_r^o \exp(f/\beta)$ – Bell Model
    $k_r(f) = k_r^o \exp(f/\alpha)^2$ – Dembo model
  - Test each bond to see whether break-up has taken place
  - Update bond number
  - Update force
- Repeat until all bonds are broken
- Compute distribution and moments for break-up times or forces

Effect of Multiple Bonding