Bioen 326 2014 Lecture 27: Cell Adhesion

Motivation for Studying Adhesion

Adhesive Structures

Mechanics of bonds: slip, catch, ideal

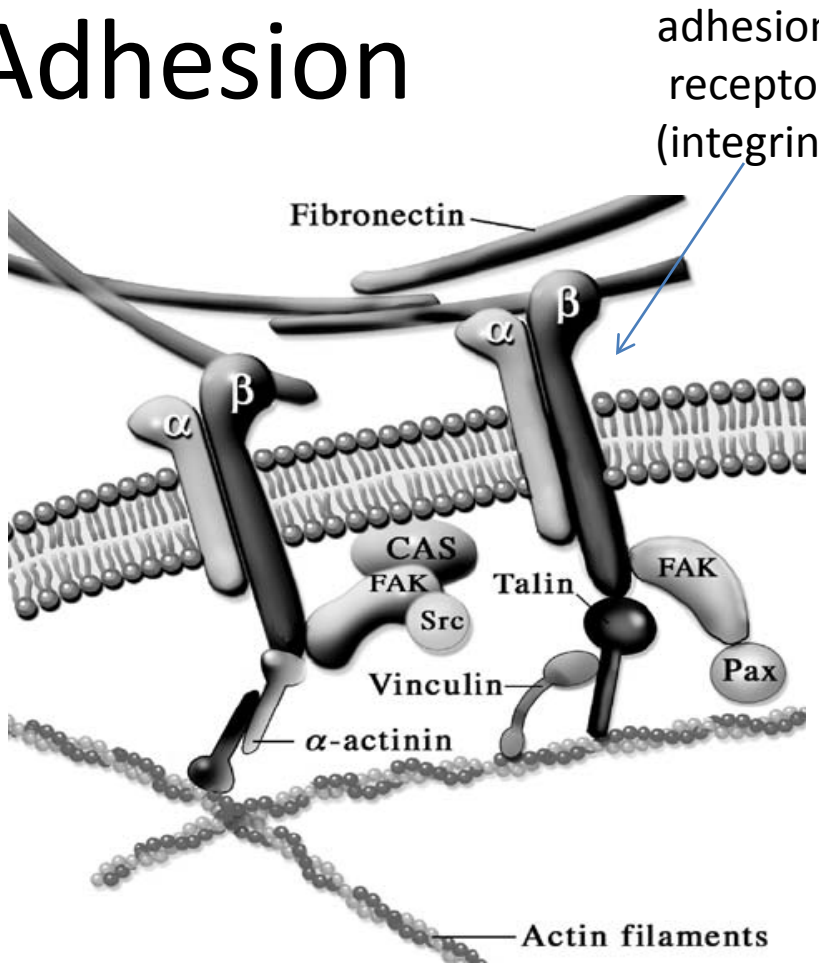
Mechanics of cell adhesion

Cells Bind to Biomacromolecules through **Adhesive Molecules**

- Cells bind to biomacromolecules on cells and tissues
- Cells bind to biomacromolecules from bodily fluid that form a conditioning layer on implanted biomaterials.
- Adhesive molecules on cells are mechanically anchored to stiff structures in the cells in various ways, so provide a mechanical connection between the cell and its environment.

Eukaryotic Adhesion

- **Adhesion receptors** are transmembrane proteins with long extracellular regions that bind an immobilized ligand, and small cytosolic region that anchor to the cytoskeleton and are coupled to signaling pathways.
- Most mammalian cells will initiate **apoptosis** (commit suicide) if their adhesion receptors don't recognize the right ligands and mechanical forces.



from Kamm and Mofrad, Cytoskeletal Mechanics, 2006

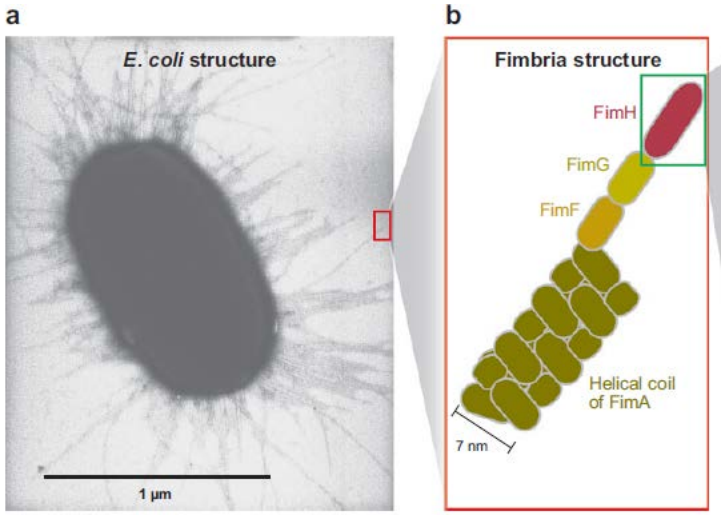
We study mammalian adhesion to…

- control cells in regenerative medicine (e.g. tissue engineering)
- study cancer (metastasized cells don't apoptose when they leave their home tissue).

Bacterial Adhesion

- **Adhesins** are binding proteins, usually on tips of long fibrillar organelles called **fimbraie** or **pili**, that are anchored to the cell wall
- Adhesins are critical to biofilm formation. **Biofilms** are multicellular communities that are 1000-fold more resistant to antibiotics and immune defense than are **planktonic** (swimming or drifting) bacteria.

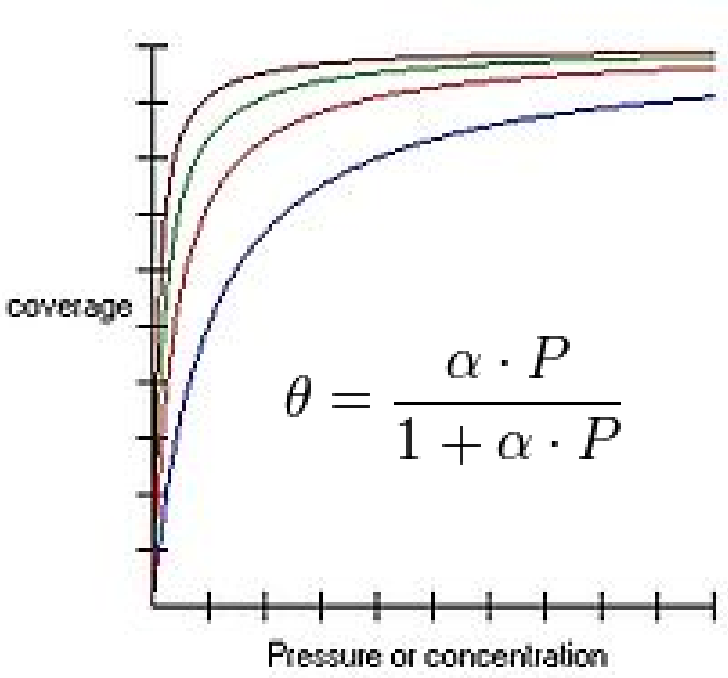
- We study bacterial adhesion to develop **Anti-adhesive therapies** that block adhesion, to leave bacteria susceptible to host defenses; this should provide alternative to antibiotics that does not causes resistance or kill commensal bacteria.



κ_2

Conditioning Layer

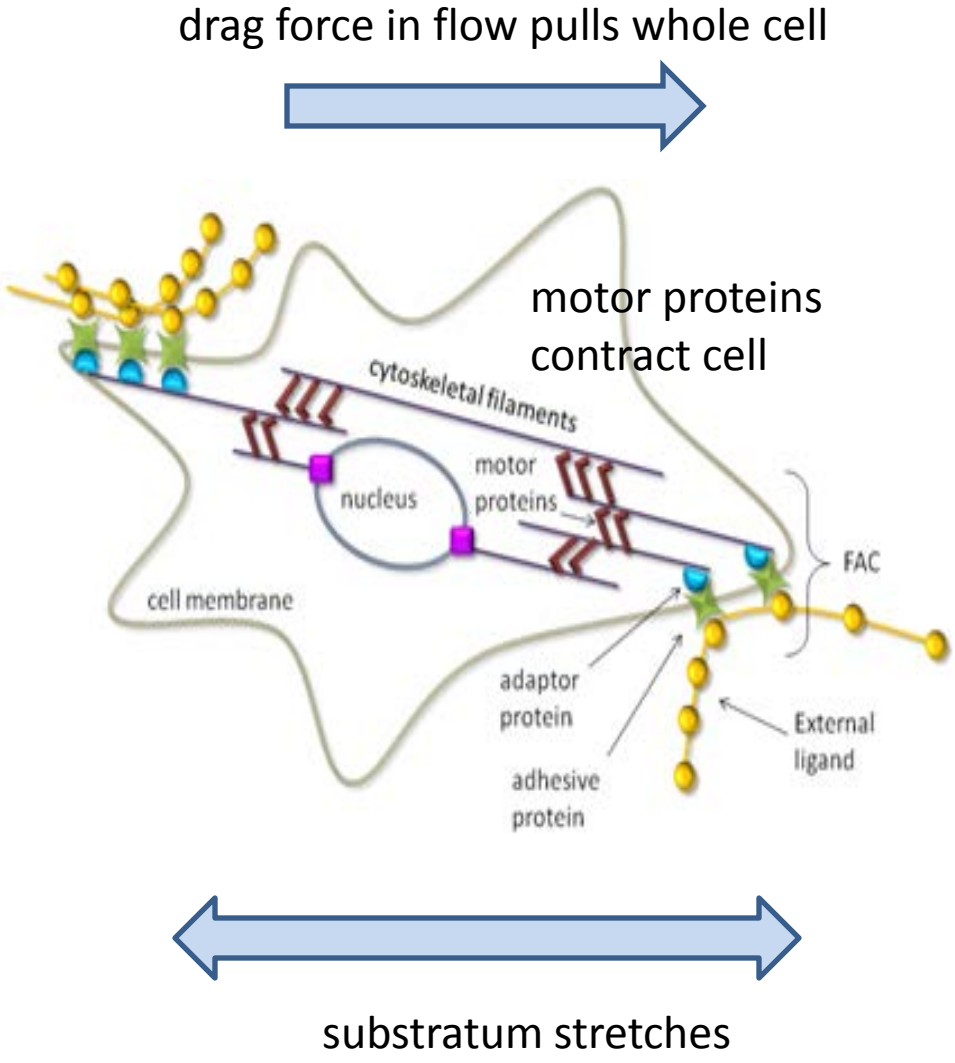
- Abiotic surfaces become coated with macromolecules (e.g. proteins and polysacharides). This is called a conditioning layer.
- Macromolecules generally bind to each other only through specific interactions, so the conditioning layer is a monomer, and binding saturates
- Binding also depends on the interaction energy between the macromolecules and the surface.
- With mixtures of macromoleules, Initial coatings represent P and binding rate, while final coatings reflect highest α*P.
- In blood, fibrinogen and albumen are most abundant.



θIs the fractional surface coverage
α is related to the binding energy
P is the concentration

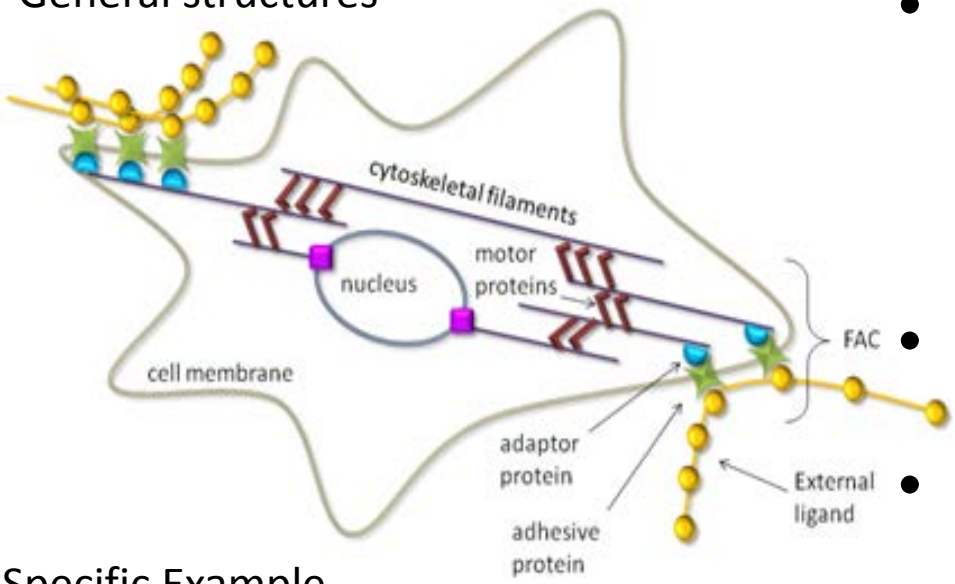
Mechanics of Adhesion

- Adhesive molecules must resist mechanical forces to maintain adhesion in spite of fluid flow, and movement.
- Cells generate mechanical forces across adhesive molecules through cytoskeletal contraction. Essentially, they grab their surroundings and pull.
- Cells have generated methods to resist detaching under these forces.

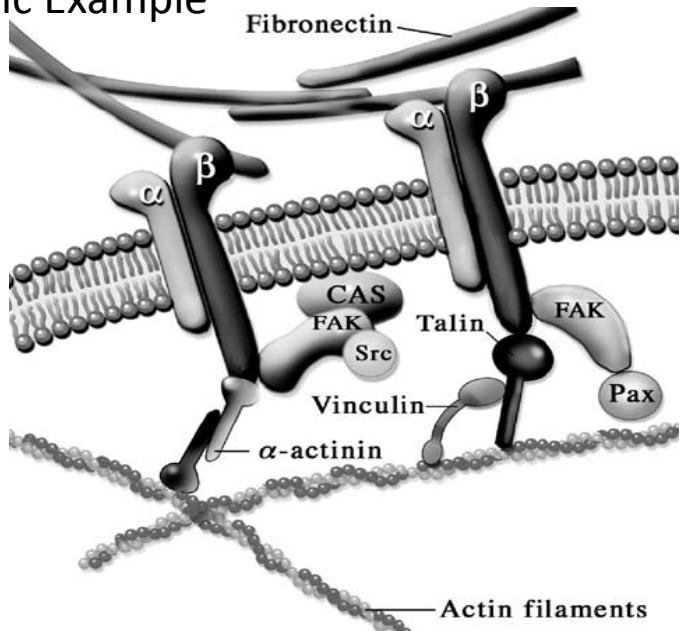


Structures in Eukaryotic Adhesion

General structures



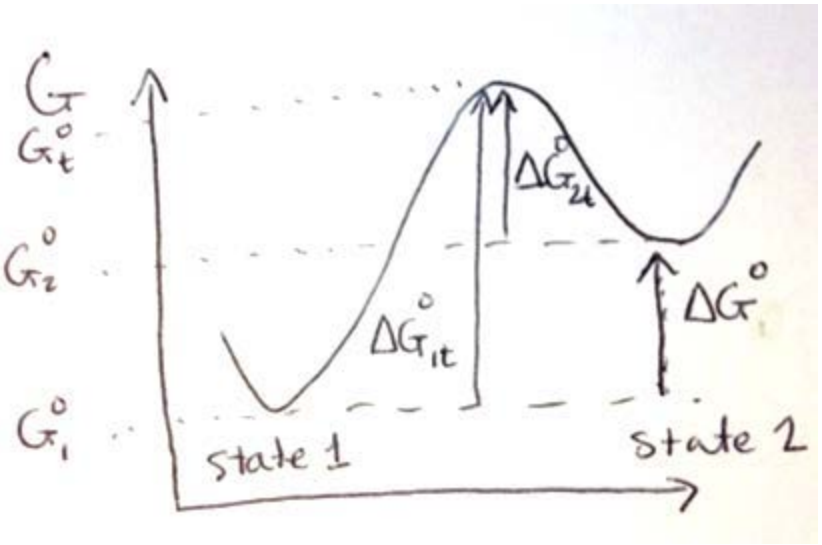
Specific Example

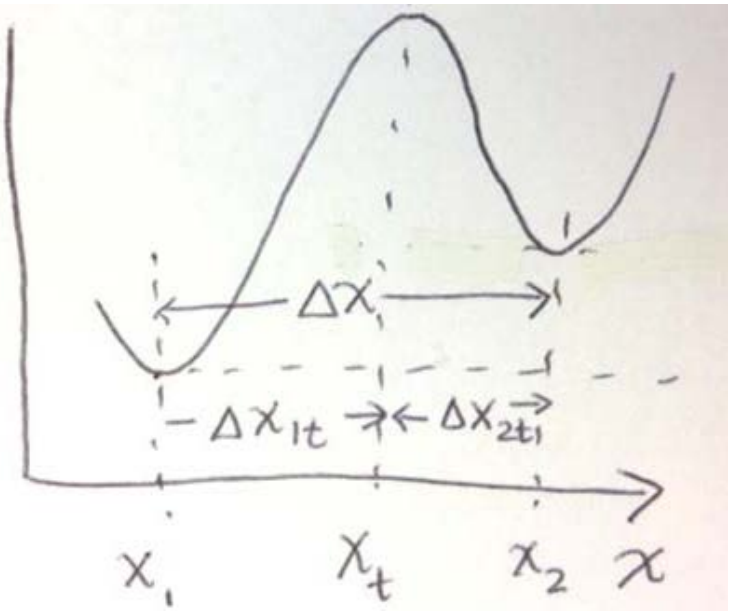


- **Immobilized Ligand:** extracellular matrix protein, receptor on another cell, conditioning layer on biomaterial.
- **Adhesion receptors** (e.g. integrin, cadherin, selectin)
- **Adaptor protein** connects to cytoskeleton (eg talin, vinculin, alpha-actinin)
- **Signaling Molecules**: eg FAK, Src
- **Focal Adhesion Complex**: a cluster of all of the above.
- **Cytoskeleton**: actin filaments, microtubules, intermediate.
- **Motor proteins**: (e.g. myosin II)

Bond Mechanics

- We consider binding as a state change, so we again use the molecular biomechanics knowledge we learned earlier.
- We call the bound state to be state 1 and the unbound is state 2.
- Thus, the unbinding rate, koff, is the transition rate from state 1 to state 2, which we called previously k12.





How Long Do Bonds Last?

- the unbinding rate without is determined by the height of the energy barrier: Just as $k_{12}^0 = A\exp\left(-\frac{\Delta G_{1t}^0}{k_B T}\right)$, we now have:

$$k_{off}^0 = A\exp\left(-\frac{\Delta G_{1t}^0}{k_B T}\right)$$

In some of these notes, drop the subscript 1t and use Δx for short.

- Probability of bond remaining bound (or number remaining bound) follows ODE:

$$\frac{dP}{dt} = -k_{off}P$$

solution is:

$$P(t) = \mathrm{P}(0)\exp\left(-k_{off}t\right)$$

- Mean of exponential distribution is inverse of rate constant, so average lifetime is:

$$\langle\tau\rangle = \frac{1}{k_{off}}$$

Effect of force on bonds

- Recall that the effect of force on rate constants depends on the difference in length of the initial vs transition state. Thus, for unbinding:

$$k_{off}(f) = k_{off}^{0} \exp\left(\frac{f\Delta x_{1t}(f)}{k_B T}\right)$$

- Since lifetime is the inverse of the rate constant, the lifetime under force is

$$\tau(f) = \tau_0 \exp\left(\frac{-f\Delta x_{1t}(f)}{k_B T}\right)$$

Slip Bonds are Inhibited by Force

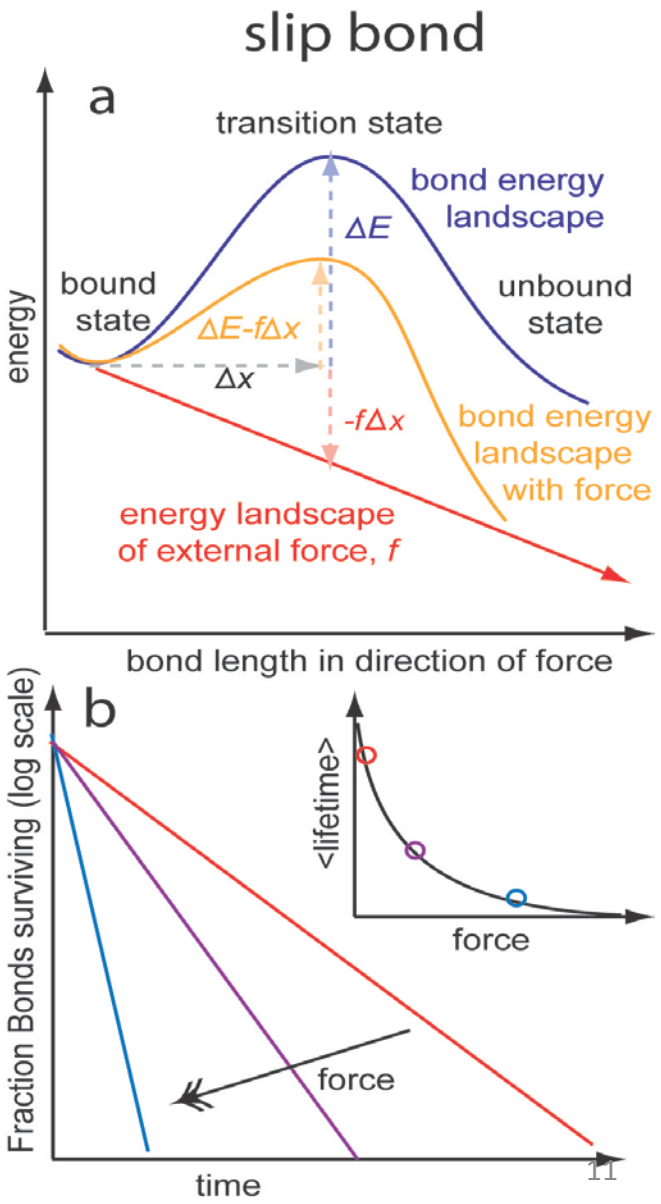
- Since bond lifetime under force is:

$$\tau(f) = \tau_0 \exp\left(\frac{-f\Delta x_{1t}(f)}{k_B T}\right)$$

- Then bond lifetime is exponentially decreased by force as long as

$$\Delta x_{1t} > 0$$

- This is shown in the energy landscape here, since the transition state is to the right of the bound state.
- We often use constant approximation for Δx_{1t} assuming bound and transition states have same spring constants



Catch Bonds are Activated by Force

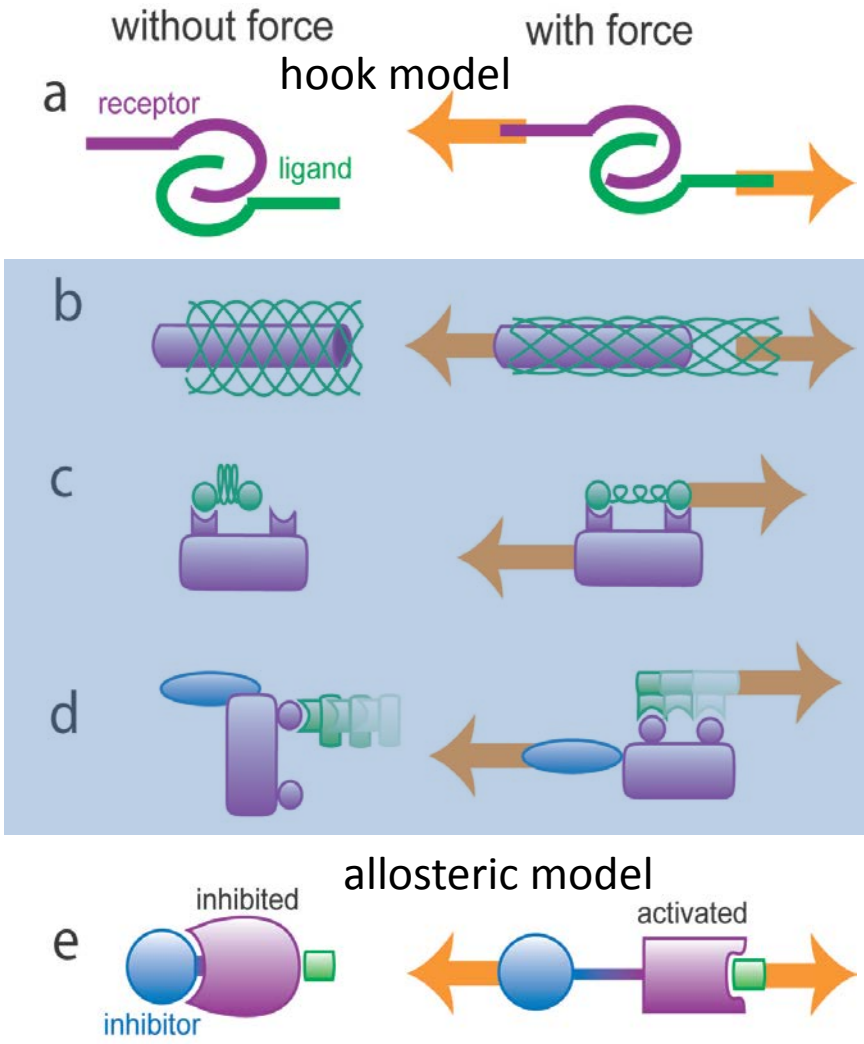
- All models require that an unbinding pathway has a transition state that is shorter than the bound state.

$$\Delta x_{1t}(f) < 0$$

Hook model: transition state brings the hook together.

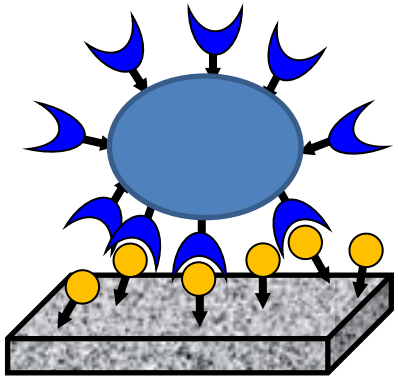
Allosteric model: allosteric change between long high-affinity to short low-affinity.

Don't worry about the remaining models



Catch Bonds are Common

Cell adhesive molecules



selectins

Marshall (2003) Nature

GPIb/VWF

Yago (2008) J Clin Invest

Integrins

Kong (2009) J Cell Bio

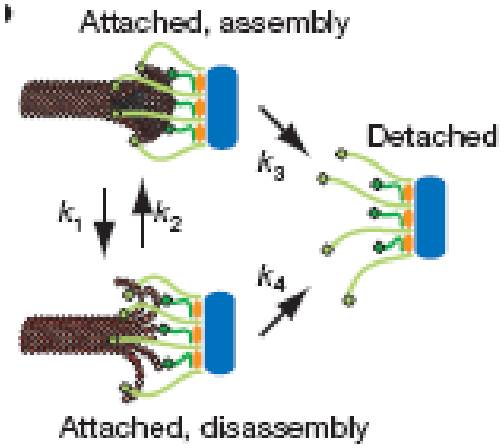
Cadherins

Rakshit (2012)PNAS

Intracellular bonds exposed to force

Actin-myosin

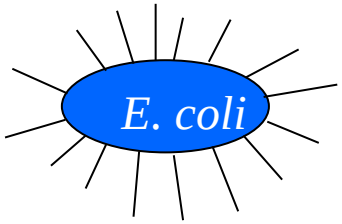
Guo (2006) PNAS



Kinetochore-MT

Akiyoshi (2010) Nature

Bacterial adhesive molecules



FimH/mannose

Yakovenko (2008) JBC

shear-enhanced <u>bacterial adhesion:</u>

- *E. Coli* P-pili(Nilsson 2006)
- *E. coli* CFA/I (Tchesnokova 2010)
- *Pseudomonas* (Lecuyer 2011)
- *Staph epi*(Weaver 2011)
- Strept gordonii (Ding 2010)

If Bonds are Exposed to Force, Catch Bonds are the Rule

Biophysics Model for Catch Bonds

- Two unbinding pathways.

  Here we drop the subscript 1t on the Δx, but it still refers to transition states

- Catch pathway is inhibited by force

$$\Delta x_c < 0$$

- Slip pathway is activated by force

$$\Delta x_s > 0$$

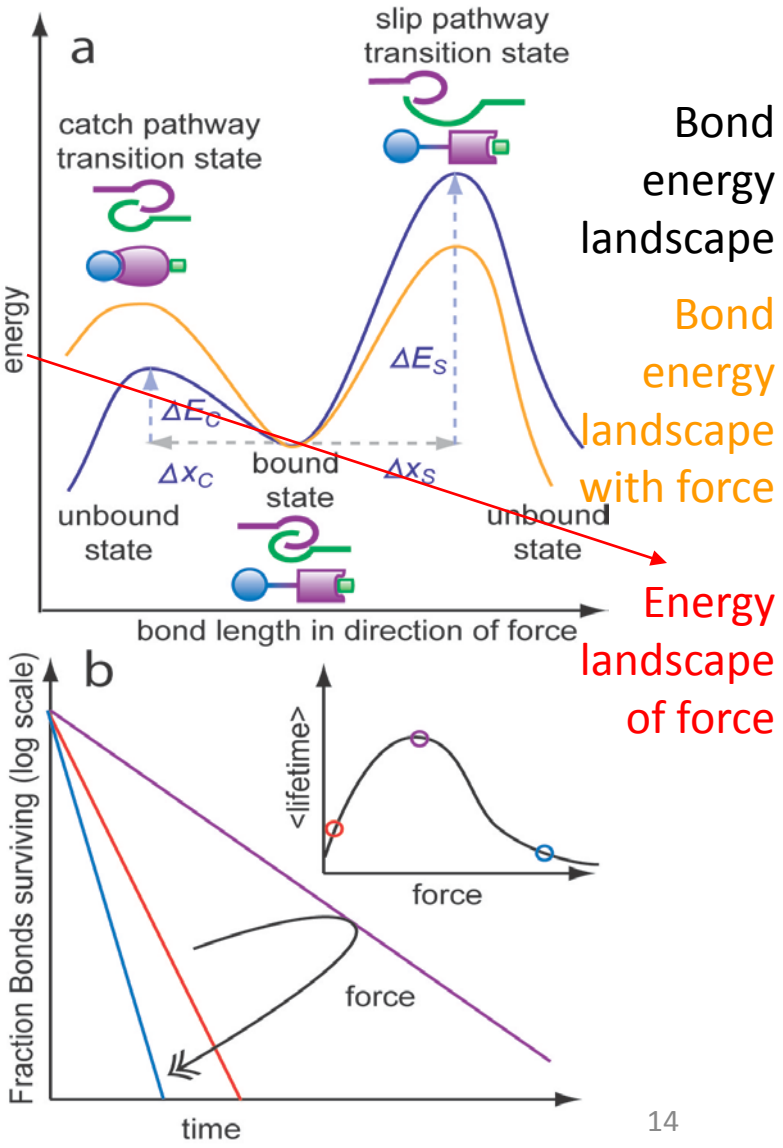
- Catch pathway is faster than slip pathway in absence of force

$$k_c^0 \gg k_s^0$$

- Total unbinding rate is sum of two pathways.

$$k_{off}(f) = k_s^0 \exp\left(\frac{f\Delta x_s}{k_B T}\right) + k_c^0 \exp\left(\frac{f\Delta x_c}{k_B T}\right)$$

- Biphasic response to force, with longest lifetime at optimum force.

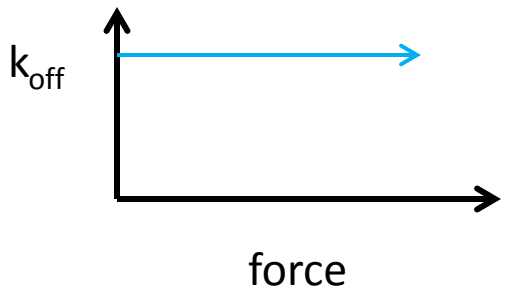


Explanation of previous slide

- When force is added, it changes the bond energy landscape because $\Delta G(f) = \Delta G^0 - f\Delta x$.
- Thus, pathway with $\Delta x<0$ has $\Delta G(f)$ increase with force, so rate of transition through this pathway decreases. This is the catch pathway.
- Conversely, slip pathway gets faster with force.
- The requirement that $k_c^0 \gg k_s^0$ means the energy barrier on the catch pathway should be smaller without force, and is necessary or the slip pathway is always faster, and catch pathway is irrelevant.

Ideal Bonds are Unaffected by Force

- Unbinding pathway has no length change
- We recently discovered that this occurs when rate limiting step is when the door to the binding pocket flips open; detachment then follows rapidly.



How much force Needed to Break Bonds?

- None – single bonds will break in due time without any force.
- If you increase force until bond breaks, you will measure the rupture force, but it depends on loading rate.
- You can calculate the parameters (k_{off}^0 and Δx) by measuring the rupture force (f*) at multiple loading rates (lr, in pN/sec).

$$\langle f^* \rangle = \frac{k_B T}{\Delta x} \cdot \ln\left(\frac{lr}{k_{off}^0} \cdot \frac{\Delta x}{k_B T} \right)$$

Here we drop the subscript 1t on the Δx, but it still refers to transition state

- Takes more force at higher loading rates, since that means less time allowed for bonds to break on their own.

Lifetime of Bond Clusters (no force)

- Cells remain adherent for long times because bonds rebind. Binding energy related to equilibrium constant, K_D, which depend on on-rate.

$$\frac{K_D}{[L]} = \exp\left(\frac{\Delta G(L)}{k_B T}\right)$$

$$\frac{k_{off}}{k_{on}[L]} = \frac{K_D}{[L]}$$

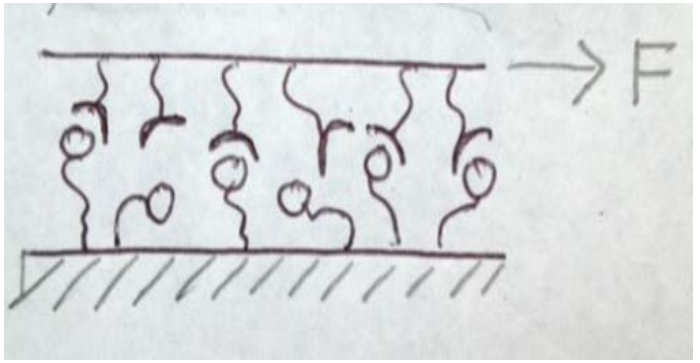
- If bonds can't rebind, lifetime of a cluster of N bonds is approximately log(N) times the lifetime of one bond

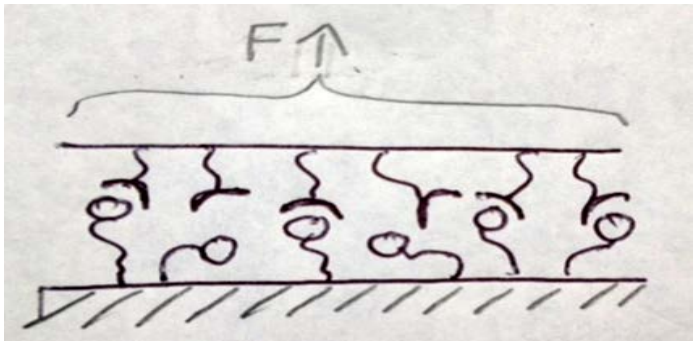
$$\tau_N = \ln(N) \cdot \tau$$

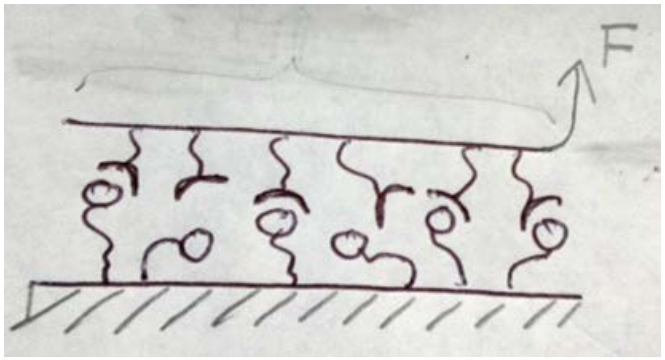
- Rebinding is hard to estimate; depends on geometry; how close is receptor to ligand and how much do they diffuse?
  - high cooperativity: each bond is kept in ideal position to rebind if others remain bound.
  - Then, energy of cluster = N times energy of one.

Cluster of N bonds Under Force

- **Shear force** is applied to pull two surfaces parallel to each other.
  - force per bond is f=F/N, so no stress concentration
  - rebinding is favored since surfaces stay close.
  - cluster is strong
- **Normal force** is applied uniformly across the surfaces to pull the two surfaces directly apart.
  - force per bond is f=F/N, so no stress concentration
  - rebinding is inhibited due to stretch
  - cluster has moderate strength.
- **Peeling force** is applied to the two surfaces at the edge of their contact zone.
  - force per bond depends on location; . f~F on edge bond, so stress concentration.
  - rebinding is inhibited due to stretch
  - cluster is weak.

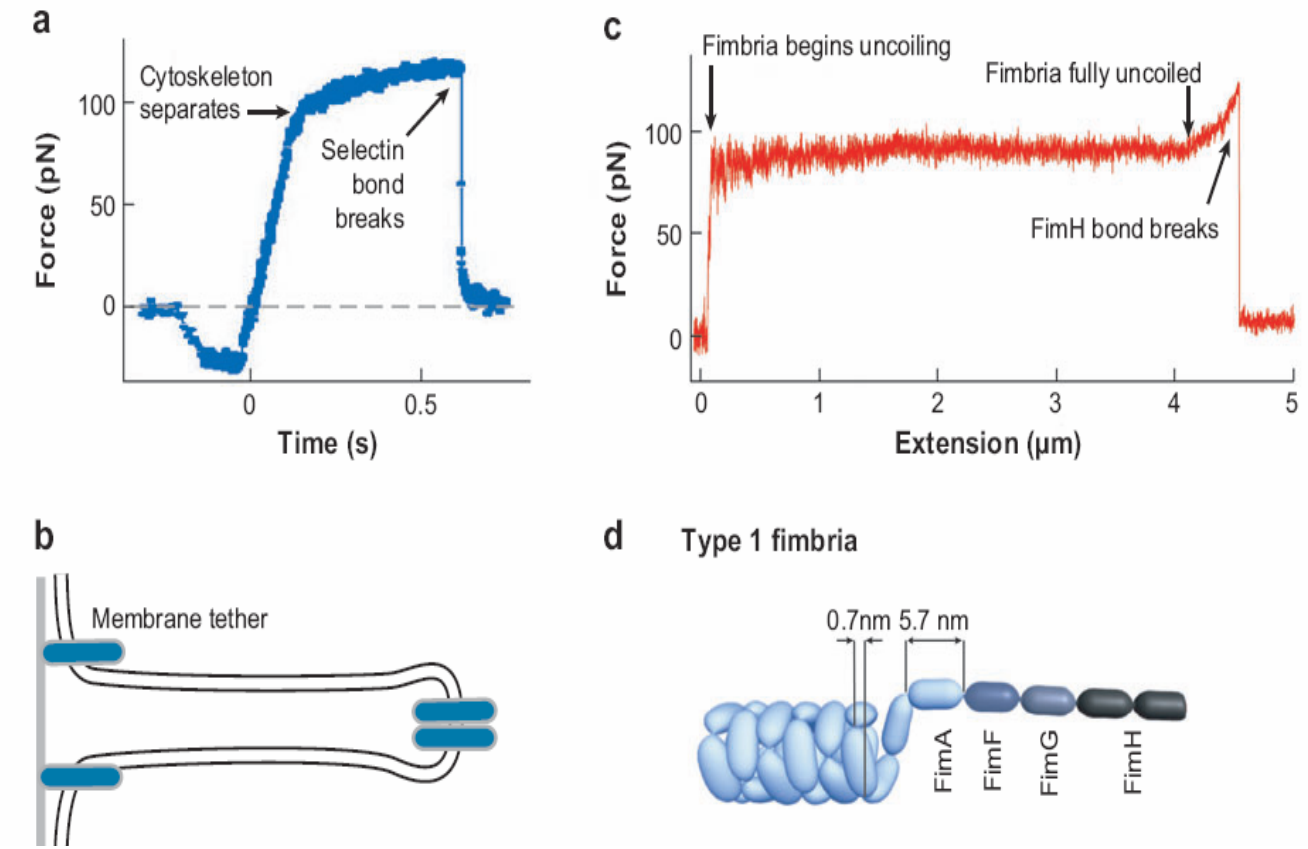






Importance of Yielding Elasticity

- Yielding tethers on bonds can prevent stress concentration even with peeling geometry.

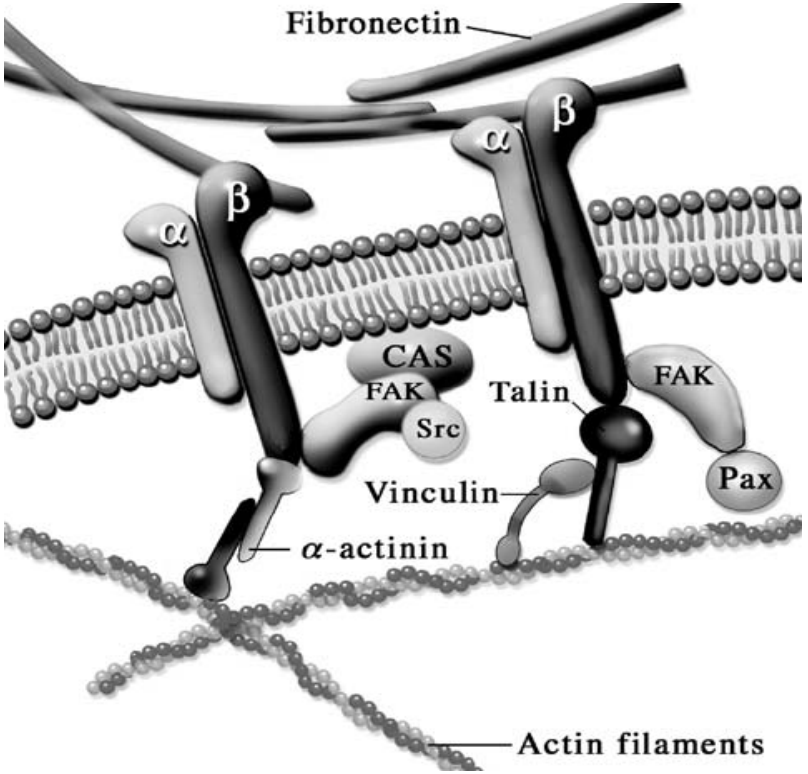


Rebinding Rates in Clusters

- N bonds in Cluster with evenly distributed normal force, F force on cluster, one bond breaks.
  - now N-1 bonds in cluster
  - Consider linear springs, with spring constant k. f=F/(N+1)
  - length of all bonds is x = f/k.
  - for broken bond, position is determined by Boltzman distribution; energy of x = f/k is $\frac{1}{2}kx^2$, which is $\frac{1}{2} f^2/k$. Thus, stiff spring has lower energy than soft springs when stretched enough to bind, so will rebind faster
- Clusters with stiff springs rebind faster under normal force.
- However, soft springs distribute force better over the cluster if bonds are not the same length.

Cluster Strength Depends on …

- Strength of individual bonds (Δx and k_{off})
- Rebinding rates of individual bonds (k_{on})
- Geometry of how force is applied (shearing > normal > peeling)
- Geometry of cluster (are all bonds stretched to similar lengths?
- Mechanical properties of each tether



Focal Adhesion complexes are evolved to have appropriate nanostructure for high strength. They are also evolved to localize signal transduction so cells can sense spatial information.

Summary

- Adhesion occurs through receptor-ligand bonds called **adhesins** or **adhesive receptors**
- Adhesion must resist forces due to external stretch or drag, and cytoskeletal contraction (cells pull).
- Molecular Biophysics controls off-rates:
  - **Slip bonds** are exponentially inhibited by force $k_{off}(f) = k_{off}^{0} \exp\left(\frac{f \Delta x_{1t}(f)}{k_B T}\right)$
  - **catch bonds** are exponentially activated by force (until a peak force, then slip)
  - **Ideal bonds** are unaffected by force.
- **Clusters** of bonds are strong under shear force, moderately strong under normal force, and weak under peeling force, because of differences in rebinding and load distribution.
- **Yielding tethers** stabilize bond clusters by distributing the load well between many bonds. The yield force needs to be in a range where bonds are long-lived.
- **Focal adhesion complexes** are evolved to be mechanically strong.
- Binding strength depends on both cell and ligands on the substrate (in conditioning layer or on tissue or cells to which cell binds)