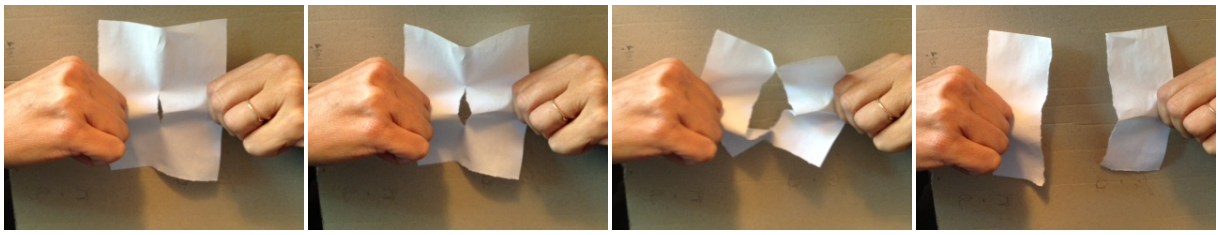
BIOEN 326 2013 LECTURE 24: ELASTIC YIELDING AND PHASE TRANSITIONS

While elastic strain hardening has long been studied for biological and biomaterials, the functional significance and the molecular basis of elastic yielding are only recently understood. Indeed, the community has not settled on a name for the phenomenon, and elastic yielding is simply my choice. Elastic yielding usually occurs when many subunits or elements within a fiber or material switch to an alternative longer conformation. The combination of these many related conformational changes is referred to as a **phase transition**. The phase transition does not occur instantly, so elastic yielding is usually viscous as well. Rapid phase transitions result low hysteresis, low energy loss, and low heating upon repeated changes in stress or strain. This is called **resilience**. In contrast, slow phase transitions result in high hysteresis, which allows **energy dissipation** during rapid stretch and relaxation. Here we address the functional advantages of yielding, resilience and energy absorption, as well as the molecular basis of and elastic yielding and phase transitions.

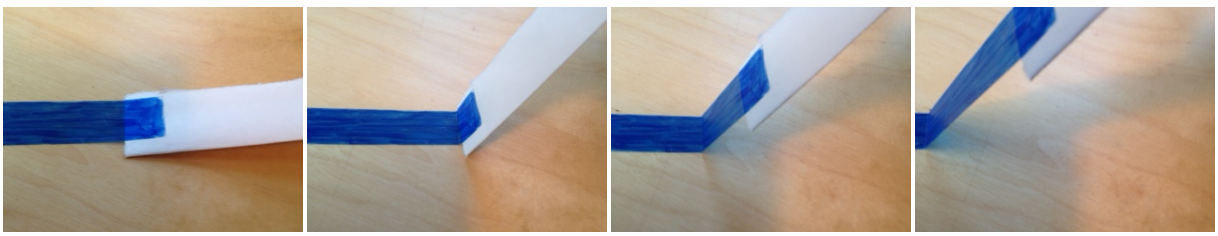
Functional Advantage of Elastic Yielding

As you have seen, the geometry of an object and external load can cause nonuniform stress within the object. When one element is exposed to higher stress than the rest of the object, we refer to this as **stress concentration**. If the stress in this element is high enough to cause material failure, the stress originally supported by this element must transfer to a nearby element, which then fails in turn. This is called **crack propagation**, and leads to total failure of the object or device. Even if a device or component is designed in a way to prevent stress concentration, small discontinuities in the material can cause stress concentration and failure.

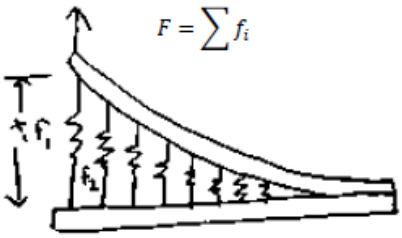
To picture stress concentration, cut a slit in a piece of paper (for example, by folding it and tearing a line.) Then, pull the paper parallel to the slit, and note that it doesn't tear. But, if you pull the material perpendicular to the slit, you can see the stress concentration at the edges of the slit as the crack propagates through the paper:



Essentially the same thing happens when you peel a piece of tape off a surface. If you pull the tape parallel to the surface (as in the left panel below), it is hard to remove, since the stress is distributed over the entire tape, but if you pull the tape at an angle from the surface (right three panels below), the stress is concentrated onto the edge of the adhesive surface.



However, stress concentration depends on the mechanical properties of the material as well as the geometrical considerations described above. Consider an idealized description of an adhesive tape, held to a hard surface by a series of parallel fibers. The i^{th} fiber supports a force f_i. The force on the tape, F, equals the sum of forces on the individual $F = \sum f_i$. In turn, the force on the fibers depends on their extension x_i and mechanical properties. If the fibers have linear behavior ($f_i = kx_i$), then force is highest on the most extended fiber at the edge of the peel (stress concentration), so this will be the first to break, redistributing force onto the neighboring fibers (crack propagation). If the fibers exhibit strain-hardening, the situation is even worse. However, if the fibers yield, many fibers will be exposed to the same yield force $(f_i = f_{yield})$ in spite of varied extensions. **Thus, yielding elasticity prevents stress concentration and crack propagation**. Because of this, adhesives are often made of a ductile material that yields plastically into long fibers. However, plastic deformation is irreversible so these adhesives are damaged after one use. In contrast elastic yielding allows repeated use.



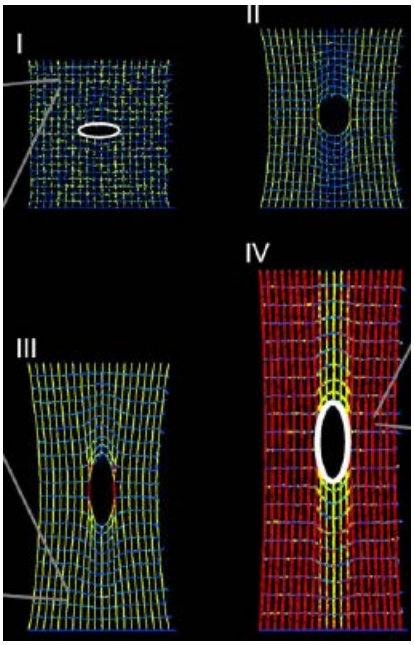


Figure 4. Yielding prevents tearing. Force on fibers is: red > yellow > blue. From (3)

Elastic yielding can also protect materials from tearing. In one study (3), simulations of a network of spectrin showed that when the material is pulled perpendicular to a tear, the individual fibers stretch until the hole re-orients to be parallel to the pull direction, so there is little or no stress concentration at the edges of the tear. Spectrin is responsible for most of the elastic behavior of red blood cells, so this may help prevent tearing when blood cells squeeze through small capillaries. The full functional significance of this elastic yielding remains an open area of research, but the advantages of preventing crack propagation are clear.

Functional Advantages of Resilience and Energy Absorbtion

Elastic yielding describes the behavior of materials when stressed slowly enough to reach equilibrium, so that stress is purely a function of strain, with no history dependence. However, the kinetics, or speed at which equilibrium is reached is also important to material behavior, particularly when strain or stress fluctuates rapidly. In some applications, it is important to transmit stress to strain rapidly within losing energy, so resilience is important. For example, tendons are highly resilient, because we want the stress created by our muscles to be converted to strain, or movement, without wasting energy or heating the tendon. Tendons in moth's wings operate are very resilient even at high frequencies. In other applications, such as earthquake protection, it is important to dissipate vibrational energy without permanent deformation.

Molecular Basis of Elastic Yielding.

Elastic yielding can occur in materials as different in structure and stiffness as metallic crystals, proteins and lipid membranes. These materials all undergo a **phase transition**: the nanostructure elements within the material switches from a native phase that is shorter in length to an alternative phase that is longer in length. This phase transition absorbs a lot of energy, and essentially buffers the force to remain near some critical force at which both phases are equal in energy. This is similar to how a phase transition such as melting vs freezing of ice acts to maintain the temperature of a bath near the phase transition (freezing point) temperature as energy is added or removed from the bath.

Metallic crystals such as gold-cadmium undergo a phase transition from a crysallographically more ordered (Austenite), to less ordered (Martensite) phase. This phase transition typically allows 10% strain, compared to 0.3% tolerated by most metals within the elastic limit. These materials are thus very ductile, but since the phase transition is reversible, they return to their native shape when force is removed, so are elastic. In contrast, most metals are only ductile in the plastic regime. These phase transitioning metallic crystals are called shape memory allows (SMAs).

Proteins often undergo conformational changes. For example, titin is a protein in muscle that is contains nearly 250 domains in a single polypeptide, each of which folds up into a globular folded structure that is connected to the next by a few amino acids, so that the intact molecule looks like beads on a string, as illustrated in the upper panel of Figure 5. When a polypeptide containining several domains of titin is extended (2), it exhibits strain-hardening behavior as the string of beads stretches like an entropic spring. However, as domains unfold one at a time, the force drops dramatically before increasing as a longer entropic spring, creating a sawtooth patten (Figure 5 middle panel). When the entire titin molecule with hundreds of domains is stretched (4), the longer softer entropic spring and lower strain rate damps out the sawtooth pattern (Figure 5, lower panel), but the strain hardening of the entropic spring (between a and c), yielding due to unfolding (between c and d) and hysteresis due to the rapid strain rate relative to the rate of unfolding and refolding (path b-c-d versus d-e-b) are clearly visible.

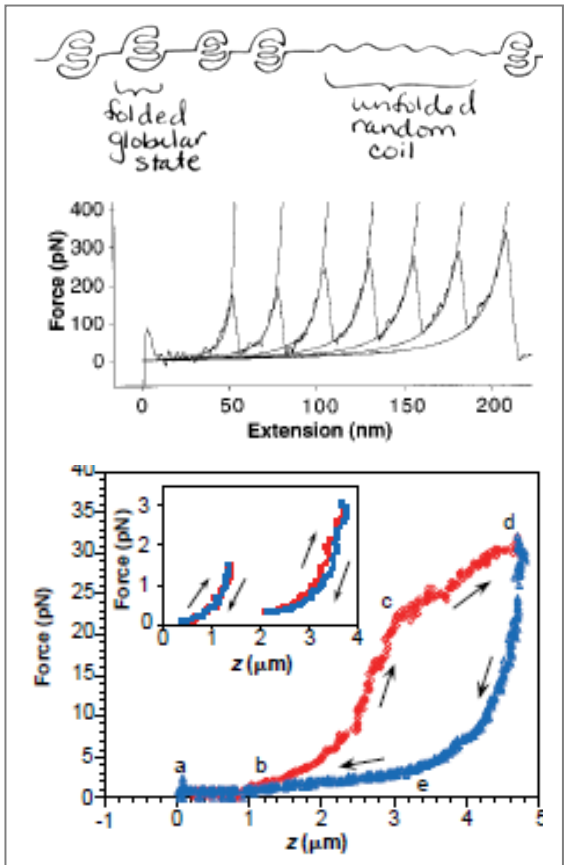


Figure 5. Structure and elastic behavior and of single titin molecules. Upper panel: structure. Middle Panel: force-extension graph for extension of a short segment of titin (2). Upper panel: force-extension graph for extension and relaxation of an entire titin molecule (4).

Quantitative model for Elastic Behaviors due to Phase Transitions

To understand phase transitions, we simply need to apply the molecular biophysics of conformational changes, which we learned previously, to each element or subunit in the material or fiber. We call the state that is lower in energy to be state 1, ($\Delta G^0 = G_2^0 - G_1^0 > 0$). Since $\frac{P_2}{P_1} = \exp\left(-\frac{\Delta G}{k_B T}\right)$, this means that $P_2^0 < P_1^0$, so state 1 is the native state (most probable) without force. When we add a force, f, across the subunit, $\Delta G(f) = \Delta G^0 - f \cdot \Delta x(f)$, where $\Delta x(f) = x_2(f) - x_1(f)$ is the difference in length between the two states at f. If $\Delta x(f) > 0$ for most f, then for some force, the two states will be equally likely: $\Delta G(f) = \Delta G^0 - f \cdot \Delta x(f) = 0$. As before, we call this the **equilibrium force**, f_{eq}, which is defined by $f_{eq}\Delta x(f_{eq}) = \Delta G^0$. That is, if the nonnative state is longer under force, then enough force will induce this state.

To solve for the equilibrium force, we need an equation for $\Delta x(f_{eq})$. In many cases, we can use the linear approximation, $\Delta x(f) = x_2^0 - x_1^0 + f\left(\frac{1}{\kappa_2} - \frac{1}{\kappa_1}\right)$. This means that our equation for the equilibrium force is $f_{eq} = \frac{\Delta G^0}{\Delta x^0 + f_{eq}\left(\frac{1}{k_2} - \frac{1}{k_1}\right)}$, or $f_{eq}\Delta x^0 + f_{eq}^2\left(\frac{1}{k_2} - \frac{1}{k_1}\right) - \Delta G^0 = 0$, which can be solved with the quadratic formula. If the two states have similar elasticity, or are very stiff, then this reduces to $\Delta x(f) = x_2^0 - x_1^0$, so $f_{eq} = \frac{\Delta G^0}{\Delta x^0}$. However, it is common that state 2 is an entropic spring. We can estimate $\Delta x(f)$ using the linear spring approximation for the spring constant of the entropic spring, $\kappa_2 = \frac{3k_B T}{2L_0 L_p}$ if f_{eq} is sufficiently low, or the full extension approximation, $x_2 = L_0$ if f_{eq} is sufficiently high. We can test our assumption by calculating $x_2(f_{eq})$ after finding f_{eq}, and comparing this to the approximation we used to find it. If neither assumption is appropriate, we need to solve for f_{eq} numerically (eg iteratively or using optimization for $f_{eq}\Delta x(f_{eq}) = \Delta G^0$).

To a first approximation, when stretched slowly enough to avoid hysteresis, a fiber or molecule with N identical subunits will extend from Nx_1^0 to $Nx_1(f_{eq})$ between 0 and f_{eq} according to the elasticity of state 1, but will then yield at f_{eq} to extend from $Nx_1(f_{eq})$ to $Nx_2(f_{eq})$. When force increases further, it will extend according to the elasticity of state 2. This idealized force-extension profile is illustrated in Figure 6. However, there are several things that make the actual force-extension profile of a yielding fiber more complicated.

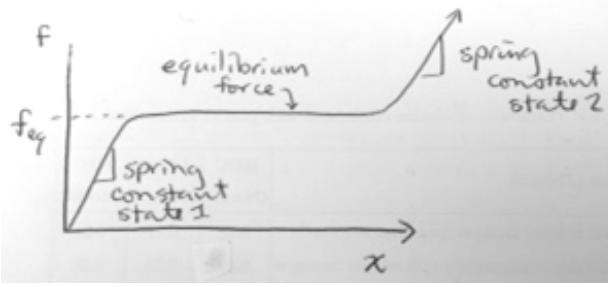


Figure 6. Idealized force extension profile.

First, even if state 1 is globular and acts like a linear spring for an isolated subunit, a string of many subunits will act approximately like an entropic spring with a contour length $Nx_1(f)$ and a persistence length of $x_1(f)$. That is, it will be shorter than $Nx_1(f)$, softer than $\kappa_N = \kappa_1/N$, and will exhibit strain-hardening elasticity. This is illustrated in Figure 7. However, since r approaches L_0 as forces increases for an entropic spring, the length will approximate $Nx_1(f)$.

Indeed, in Figure 5, you can see the near zero force in the initial stages showing that very low forces are sufficient to extend the including a near zero force while waiting to reach theif f_{eq} is sufficiently high. The entropic spring behavior prior to yielding is clear in Figure 5, middle and lower panels. However, we will ignore this for calculations in this class, since even small forces extend enough to ignore the difference.

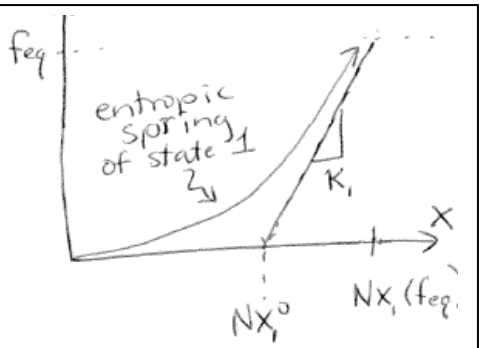


Figure 7. Elasticity of polymer with N subunits in state 1, where each subunit has length $x_1(f) = x_1^0 + f\kappa_1$

Second, the plateau is not flat in a protein like titin where the phase transition occurs independently. An **independent** phase transition means that each subunit undergoes the transition dependent on the force, but independent of the state of the neighboring subunits. Protein unfolding is usually independent since the subunits are already not touching each other when the string of beads is stretched. In this case, the average length of each subunit depends on the probability of existing in each state. $x(f) = P_1(f)x_1(f) + P_2(f)x_2(f)$. The length of a polymer with N subunits is thus determined by a wormlike chain with a contour length $L(x) = Nx(f)$. This means that the length increases gradually from $Nx_1(f)$ to $Nx_2(f)$ over a narrow range of force. This may explain the gradual increases from point c to point d in Figure 5. If we assume Δx is independent of force, we define the **critical force** as $f_c = \frac{k_B T}{\Delta x}$. The critical force increases the equilibrium ratio by e-fold: $K_{eq}(f + f_c) = \exp\left(-\frac{\Delta G(f) - f_c \Delta x}{k_B T}\right) = K_{eq}(f)\exp(1)$. Since $P_2 = \frac{K_{eq}}{1+K_{eq}}$ the polymer changes from 27% to 73% state 2 between $f_{eq} - f_c$ and $f_{eq} + f_c$. In contrast, the yielding at f_{eq} is perfectly flat if the phase transition is **cooperative**. A cooperative phase transition is one where the state of one subunit affects the state of the neighbor, so that the phase transition occurs preferentially at the ends of the polymer. In this case, there is a boundary between the section of polymer in state 1 and that in state 2 (see Figure 8). The boundary moves forward or backwards according to $K_{eq}(f)$ but in due time, it moves all the way to the preferred state.

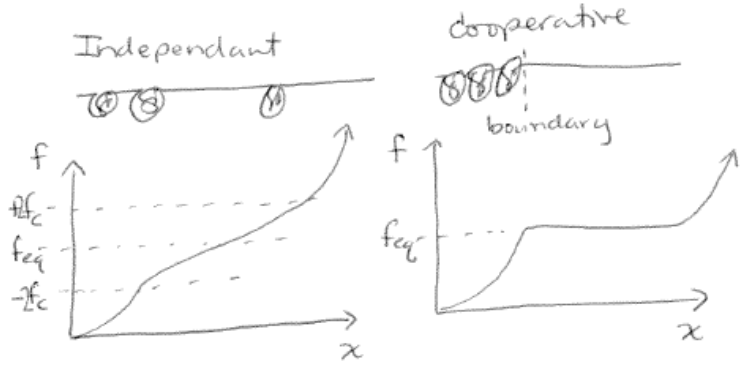


Figure 8. Independent and Cooperative Phase Transitions.

However, even for the independent phase transitions, the slope of the plateau is shallow if $f_c \ll f_{eq}$. In this case, the fiber behavior is primarily affected by the equilibrium force, regardless of whether the transition is cooperative or independent.

Quantitative model of viscoelastic behaviors due to phase transitions.

The discussion above assumed that the material was tested in a way that we only considered the thermodynamic equilibrium, so we did not consider kinetics. However, phase transitions

may also cause creep, stress relaxation, and strain hardening. Here we will consider how to calculate these behaviors on single polymers undergoing phase transitions.

Recall that the equilibrium constant can also be expressed as the ratio of kinetic constants, $K_{eq} = \frac{k_{12}}{k_{21}}$, and that the rate constants are exponentially affected by force as controlled by the distance between each low energy state and the transition state: $k_{12}(f) = k_{12}^0 \exp\left(\frac{f\Delta x_{1t}(f)}{k_B T}\right)$. Calculating $\Delta x_{1t}(f)$ involves the same issues as calculating $\Delta x(f)$, in that you need to determine whether to use the constant estimate Δx_{1t}^0, the linear estimate $\Delta x_{1t}^0 + f\left(\frac{1}{\kappa_t} - \frac{1}{\kappa_1}\right)$, or the full calculation based on nonlinear elasticity of the low energy and transition states.

These rate constants can be used to write a differential equation for the probability of being in state 2: $\frac{dP_2}{dt} = k_{12}(f)P_1 - k_{21}(f)P_2$. Combining this with $P_1 = (1 - P_2)$ gives

$$\frac{dP_2}{dt} = k_{12}(f) - \big(k_{12}(f) + k_{21}(f)\big)P_2$$

We can use this to solve for the probability of being in state 2 if we know the initial condition for P_2, and have an equation for $f(t)$. If there is a polymer with a large number of subunits, N, then we can ignore the stochastic switching between states for each subunit, and use the probability to calculate the fraction in state 2 over time: $N_2(t) = NP_2(t)$. From the fraction of subunits in each state and the lengths $x_1\big(f(t)\big)$ and $x_2\big(f(t)\big)$ we can calculate the approximate length of the polymer, as $X(t) = NP_2(t)\, x_2\big(f(t)\big) + N(1 - P_2(t)x_1\big(f(t)\big)$. Calculating the exact length is complicated if one of the conformations is a linear spring as a single subunit but an entropic spring as a polymer of subunits, but even in that case, the effect is usually small.

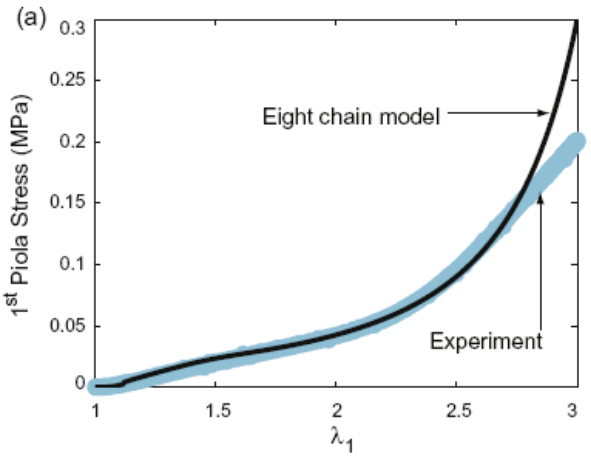
We can use this differential equation approach to predict or understand responses of yielding materials to various viscoelastic tests. However, for tests in which we control the length, the situation requires iterative solutions since we don't know how much of this controlled length is used by each phase. If we control the force, we can use the differential equation to solve it, but this requires that we repeatedly determine $r(f)$ for worm-like chains, for which we don't have an analytic equation. In all these cases, force is changing over time, so the coefficients in the differential equation are not constant, so we can't use Laplace transforms to solve them. Instead, we must use numerical methods to solve the nonlinear differential equations. Between these many issues, this is beyond the scope of this class.

However, if we perform a creep test, or any other experiment where the force is changed with a step function after being held at one force long enough to stop changing length, the force is a constant, so the transition rates are constant with respect to time, so we can use Laplace Transforms to solve the differential equations. In the homework, you will use this approach to demonstrate that a material will exponentially approach the new length at the sum of the two rates. For example, if we stretch a material to induce the phase transition to state 2, and then remove force, it will recover to the length that corresponds to P_2^0 at a rate $k_{12}^0 + k_{21}^0$. An exponential decay or approach has reached 99% of its final value by 5 time constants, so the transition is essentially complete by $T = \frac{5}{k_{12}(f) + k_{21}(f)}$.

We can use this fact to estimate how slow a polymer must be stretched to avoid hysteresis; For any force, we can estimate the strain at that force from the equation above for $Nx(f)$, and the time needed to reach equilibrium from the equation above for T, and divide to get a strain rate.

Materials properties.

The discovery that some fibers exhibit elastic yielding is still very new. In most cases, much of the research performed on this topic is at the nanoscale level of single fibers, and the role in macroscopic (or even microscopic) behaviors still remains to be determined. Estimating the tangent Young's modulus from the force-extension of the fibers is not accurate, because at larger strains, the yielding of single fibers is off-set by the many mechanisms for strain-hardening including orientation of fibers, and the strain-hardening that occurs before or after yielding. For these reasons, it is hard to predict whether the yielding or strain or hardening behaviors will dominate the elastic properties of the materials. However, even if the material does not exhibit yielding, yielding of the fibers may protect these materials from crack propagation.



Fibrin is one of the few proteins for which people have performed force microscopy of individual molecules and thin fibers, and also the elastic properties of a network, or material. Fibrinogen is the most abundant protein in the blood and is composed of both a long section of alpha helices and a few globular domains. When initiated by a clotting cascade, fibrinogen polymerizes into thin fibrin fibers through covalent end-to-end interactions, then to thicker fibers through lateral interactions, and finally a cross-linked network referred to as the fibrin clot. Force experiments on individual fibers or monomers show that they yield when the alpha helices extend and the globular domains unfold. The material (a clot) exhibits the nonlinear behavior shown by the blue curve in the figure on the right (5). Note that the initial behavior is nearly linear, followed by strain hardening, with the yielding behavior barely or not at all visible. A multi-scale model of a network of fibers that each behave like a single fibrin fiber nearly reproduced this behavior, as shown by the black line (5).

Examples of yielding fibers

<u>Unfolding domains</u>

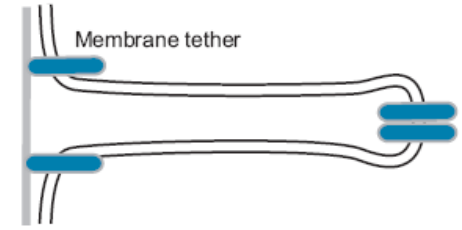
Titin is not the only protein that exhibits unfolding under force. The extracellular protein fibronectin also has many "type 3" domains that unfold under force, allowing fibronectin fibers to elongate up to four-fold their native length when pulled by contracting cells. Fibronectin fibers are laid down by fibroblasts during wound repair and form a matrix for cells to invade as part of the healing process, much as tissue scaffolds are used in tissue engineering. Indeed, most extracellular proteins and adhesive molecules have domains like this that may unfold under force, although most have not been studied for their mechanical properties.

Alpha helical proteins.

Some structural proteins are primarily composed of alpha helices that do not fold up into a globular structure but instead form long fibers. Often, three alpha helices wrap around each other to form a coiled coil with two alpha helices, or even three. This is distinct from the collagen triple helix, which is not an alpha helix because each polypeptide is nearly extended before coiling around each other. If an alpha helix is stretched, it undergoes a cooperative phase transition at an equilibrium force from helical coil to extended as the amino acid at either end of the helix breaks the hydrogen bonds characteristic of an alpha helix.

Alpha helical proteins include nearly all intermediate filaments, which make up the third class of cytoskeletal proteins. Until recently, the role of these filaments was not understood, since they seemed so much softer than actin filaments and microtubules, which are the best understood cytoskeletal proteins. However, it has recently been recognized that they may play a large role in preventing tearing. Another protein with a similar role is spectrin, which forms a network just inside the membrane of red blood cells. Many of the proteins that connect the adhesive anchors like integrins and cadherins to the cytoskeleton are also alpha helical.

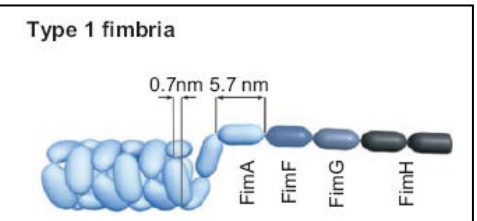
Membrane tethers.



Membrane tether. (1)

Most adhesive proteins are integral membrane proteins that are attached to the cytoskeleton on their cytosolic side, but they can often detach from the cytoskeleton during cell adhesion under force. When this happens, the protein remains anchored in the lipid bilayer, and pulls a column of lipid called a membrane tether when the adhesive protein is pulled, as during blood cell adhesion in strong arterial flow. (1). The phase transition in this case is the switch of each phospholipid molecule from its preferred flat curvature in the cell membrane to the unfavorable highly curved state inside the membrane tether, which has a circular cross-section with a small radius. The energy difference between the two states depends on the phospholipid composition of the membrane. Membrane tethers appear to be critical for cell adhesion in flow, by buffering the force on the adhesive molecules.

Bacterial fimbriae.



Bacterial fimbriae. (1)

Most gram negative bacteria express adhesive molecules on the tips of helical fimbriae. While alpha helices are secondary structural motif, these fimbriae form a helical coil in their quaternary structure, which refers to the interactions between domains or subunits. (The figure below is from Thomas 2008, Annual Review of Bioengineering 10 p. 39). Many different adhesive organelles have been tested and shown to exhibit elastic yielding with strains of up to 10. The universal nature of this motif suggests that the structure has a functional role, which the Thomas Lab suggests is to hold force near f_{eq}.

DNA helix.

DNA undergoes a phase transition to uncoil the helix, which allows a fractional lengthening.

Summary.

- The functional role of elastic yielding is prevention of **stress concentration** and **crack propagation**. This helps prevent adhesive failure and material tearing.
- Elastic yielding is caused by **phase transitions**.
- The **equilibrium force f_{eq}** is the force at which this phase transition occurs when the material is pulled slowly enough to avoid hysteresis. f_{eq} is related to the free energy difference between the two states in standard (no force) conditions, and the difference in length between the two states as a function of force: $f_{eq} = \Delta G^0/\Delta x(f_{eq})$. Depending on the form of the equation for $\Delta x(f)$, this may have a simple analytical solution or may require an iterative numerical solution.
- A **cooperative** phase transition occurs when subunits stabilize the structure of neighbors, and is typical for helices and alloys. This results in a flat plateau at f_{eq}, so that the overall length is $L(f) = Nx_1(f)$ if $f < f_{eq}$, and $L(f) = Nx_2(f)$ if $f > f_{eq}$.
- An **independent** phase transition occurs when subunits do not interact with neighbors, and is typical for folding proteins or polymers. This results in a gradual plateau centered around f_{eq} and extending for several times $f_c = \frac{k_B T}{\Delta x(f_{eq})}$ on either side, so that the overall length at f is $L(t) = NP_2\, x_2(f) + NP_1 x_1(f)$.
- The viscous properties of phase transitions are determined by the kinetic rate constants, according to the differential equation $\frac{dP_2}{dt} = k_{12}(f) - \big(k_{12}(f) + k_{21}(f)\big)P_2$ and the equation for length, $L(t) = NP_2(t)\, x_2\big(f(t)\big) + N(1 - P_2(t)x_1\big(f(t)\big)$. At a constant force, the material creeps at time constant $\big(k_{12}(f) + k_{21}(f)\big)^{-1}$.
- Materials made up of yielding fibers may not clearly show the yielding behavior at the macroscale but can still benefit by avoiding stress concentration and crack propagation, which occur at the nanoscale.
- Examples of yielding materials include alloys that change crystalline structures, uncoiling helices of all types (alpha helical proteins, bacterial fimbriae, DNA), unfolding proteins, and changing curvative of lipid membranes.

References

1. Thomas W (2008) Catch bonds in adhesion. *Annu Rev Biomed Eng* 10:39-57.
2. Rief M, Gautel M, Oesterhelt F, Fernandez JM, & Gaub HE (1997) Reversible unfolding of individual titin immunoglobulin domains by AFM [see comments]. *Science* 276(5315):1109-1112.
3. Ackbarow T, Sen D, Thaulow C, & Buehler MJ (2009) Alpha-helical protein networks are self-protective and flaw-tolerant. (Translated from eng) *PLoS One* 4(6):e6015 (in eng).
4. Kellermayer MS, Smith SB, Granzier HL, & Bustamante C (1997) Folding-unfolding transitions in single titin molecules characterized with laser tweezers. (Translated from eng) *Science* 276(5315):1112-1116 (in eng).
5. Purohit PK, Litvinov RI, Brown AE, Discher DE, & Weisel JW (2011) Protein unfolding accounts for the unusual mechanical behavior of fibrin networks. (Translated from eng) *Acta Biomater* 7(6):2374-2383 (in eng).