

## BIOEN 326 2014 LECTURE 24: STRAIN HARDENING ELASTICITY

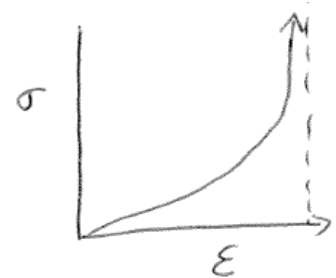
Some materials, including some rubbers, gels, and biological tissues, can withstand large strains (often many fold their length) but then recover their original shape upon relaxation. This means that these materials are stretching elastically, and have a very high elastic limit, so they are referred to as **elastomers**. This is very important for materials that have a large working strain range. These materials must stretch or compress large amounts in their normal operating conditions. For example, arteries must expand and contract with pulsatile flow and to control blood flow in different parts of the body.

However, unlike solids, these materials rarely display linear elasticity throughout this region. Instead, they exhibit elastic strain hardening, elastic yielding, or both. These behaviors are **reversible (elastic)** and thus are sharply distinguished from the **plastic** strain hardening and yielding of metals and polymeric solids, in that the latter are **irreversible**.

Many elastomers exhibit **hysteresis**, but unless we note otherwise, we will ignore that by assuming that we are pulling slowly enough that the load and unload curves are the same.

### Functional Advantage of Elastic Strain Hardening

The **tangent Young's modulus** is defined as the derivative of the stress strain curve:  $E_T(\epsilon) = \frac{d\sigma}{d\epsilon}(\epsilon)$ . If the tangent Young's Modulus increases with strain, we call this **strain hardening**. Materials that exhibit strain hardening within the elastic limit are usually are very pliable, to allow large strains, but above some point, they become much stiffer. Examples of strain-hardening elastomers are collagen networks in the arterial wall, rubbers, elastin networks in tissues like skin, and many cross-linked polymer gels including those made of elastin, polyethylene glycol (PEG) and collagen.



In biomechanics, these materials are often said to exhibit a **J-shaped stress-strain curve**. When the material has a very soft initial region before hardening dramatically, this initial region may be called the **toe region**.

The functional advantage of a strain-hardening material is to allow large deformations within the working range, while preventing excess deformation when stress goes beyond the working range. For example, while arteries need to expand or contract several-fold to modulate blood pressure or blood flow, we don't want them to expand too much when there is a spike of high blood pressure. Strain hardening limits the strain allowed with the stress goes beyond the normal working range.

### Molecular Structure of Elastomers.

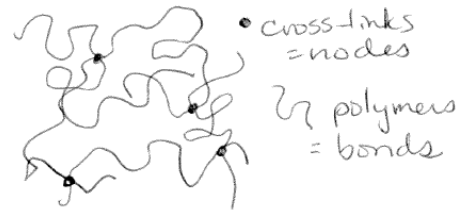
There are several structural causes of elastic strain hardening.

#### Entropic polymers.

First, the material may be made up on individual bonds that exhibit a nonlinear strain hardening behavior. Consider a hydrogel, which is a material that is made up of unstructured

hydrophilic polymers cross-linked together in an aqueous environment. In this case, the cross-links form the nodes and the polymer lengths between cross-links are the bonds. As we learned before, the effect of force on these bonds lengths will determine to a large degree the elastic properties of this material. Thus, we consider the force-distance dependence,  $f(r)$ , of an unstructured polymer in an aqueous environment.

entropic polymer gel:



We assume that the polymer does not interact with itself preferably over the water that surrounds it, since that would cause it to collapse and exclude the water, making it a solid rather than a hydrogel. What determines the shape and length of each polymer in this case? Some polymers, like polypeptide or polysaccharide chains, or single stranded DNA, are composed of many subunits that can rotate quite freely around the polymer bonds that connect the subunits together. In this case, the polymer is equally likely to take on any conformation. That is, it is just as likely to take either of the following two forms:



Indeed, the assumption that there are no self-interactions means that the **enthalpic energy, H**, of all conformations is the same as long as each subunit is in its low-energy conformation.

However, if we define  $\vec{r}$  = the end-to-end distance of the polymer, we note that there are many conformations that can result in any small value of  $\vec{r}$ , and look a lot like the conformation on the left above, but are not exactly the same. In contrast, only one can result in  $\vec{r} = L$ , where L is the length of the polymer if all subunits are laid end-to-end, and there are no conformations that result in  $\vec{r} > L$ , unless the subunits themselves are stretching. Thus, if the end-to-end distance  $\vec{r}$  is small, the polymer can still sample many conformations, so has high disorder, or **entropy, S**. Recalling that the Gibb's free energy is:  $G = H - TS$ , where T is temperature, we see that the free energy of the polymer is lower if the ends are held closer together. Because of this, if we stretch an unstructured polymer with an instrument that can measure forces on single molecules (such as an **Atomic Force Microscope**), we observe a strain hardening behavior.

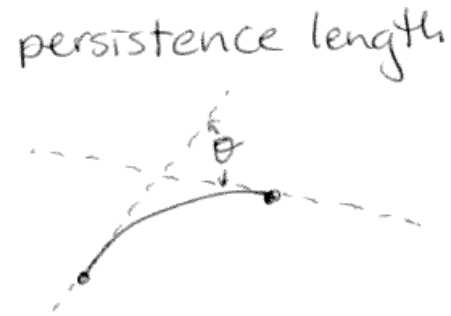
Because of this, we often call these **entropic polymers** or **entropic springs**.

### Worm-Like Chain Model.

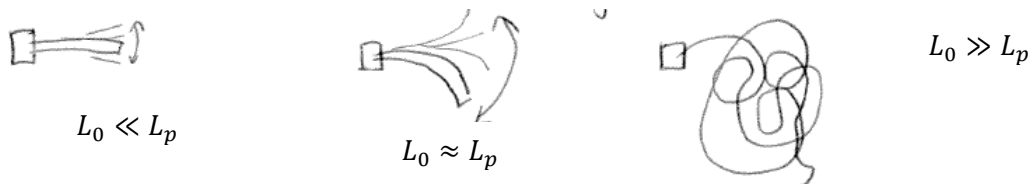
A common model used to describe an entropic polymer is the **worm-like-chain (WLC)** model. This model is best used for fibers that behave like a rigid rod when short, but become flexible at long enough distances, because thermal energy is sufficient to overcome the **flexural rigidity** (EI) of the rod, which controls the ability beams to resist bending in response to lateral forces. . Recall that **thermal energy** represents the energy stored due to random kinetic energy, or motion, of the atoms or molecules in a system. Thermal energy depends on temperature and has the quantity  $RT$  per mole, or  $k_B T$  per molecule, where R is the gas constant and  $k_B$  is the

Boltzman constant. You may recall that the energy per degree of freedom is  $\frac{1}{2}k_B T$ . These random motions jostle all atoms and molecules and other nanoscale particles, pushing them randomly over any energy barriers that are not much higher than thermal energy.

Thus, thermal energy will jostle and bend fibers as long as the bending energy is less than thermal energy. We define the **persistence length,  $L_p$** , of a fiber to be the segment length at which the two ends of the segment face in different directions while the bending energy is around thermal energy. Precisely speaking, we define  $\theta$  = the change in angle between the tangent to the beginning and end of a segment (see figure). Now consider the fluctuations in  $\theta$  that occur over time due to thermal jostling, and calculate the time-average cosine:  $\langle \cos\theta \rangle$ . It can be shown using statistical mechanics that  $\langle \cos\theta \rangle$  decays exponentially from 1 to 0 as segment length,  $s$ , increases, so we define  $L_p$  such that:  $\langle \cos\theta \rangle = e^{-s/L_p}$ . Thus, the persistence length is the segment length at which  $\langle \cos\theta \rangle = 1/e$ . The persistence length is an intrinsic property of the fiber that depends on the structure of the fiber (the flexural rigidity), but not its actual length. It can be shown that  $L_p = \frac{EI}{k_B T}$ .



However, some entropic polymers are more properly considered a **freely jointed chain (FJC)**, in which rigid subunits are linked together at hinges that can rotate freely. The subunit length is often called the **kuhn length,  $b$** . There is a separate model for the FJC, but we will not address this here because it turns out that the FJC and WLC have similar mathematical behaviors if we define  $L_p = b/2$ , and because the scientific community tends to use the WLC model for both types of fibers. Intuitively, it makes sense that the persistence length is less than the kuhn length, because the two segment ends are totally uncorrelated ( $\langle \cos\theta \rangle = 0$ ) at the kuhn length, but not at the persistence length.



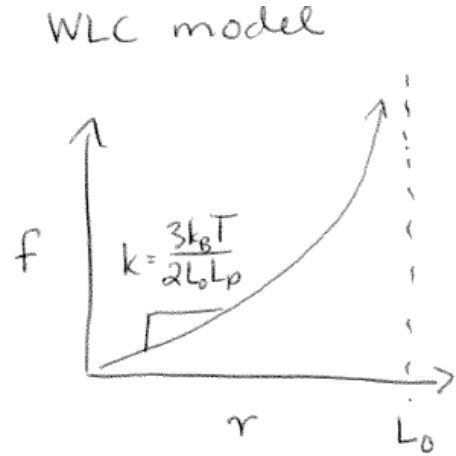
We define the **contour length,  $L_0$** , of the fiber to be the distance along all the curves of the fiber, which is the same as the end to end distance if the fiber were completely straight. The contour length is an extrinsic property of the fiber. If the contour length is shorter than the persistence length ( $L_0 << L_p$ ), the fiber is rigid, but a polymer with a contour length in excess of the persistence length ( $L_0 >> L_p$ ) is wiggly and soft.

Now we define  $\vec{r}$  to be the end-to-end distance of the fiber. This depends on the specific conformation of the fiber, which is changing all the time due to thermal fluctuations, so it is often more useful to consider  $\vec{r}$  to be a time-averaged value. Note that  $\vec{r}$  is a vector, which indicates the length as well as direction from the start to the end of the fiber. The average value of this distance is trivial, since the fiber is as likely to go in any direction:  $\langle \vec{r} \rangle = 0$ . Therefore, it is

more useful to characterize the end-to-end distance by  $r_{rms} = \sqrt{\langle \vec{r}^2 \rangle}$ . Although we skip the complicated derivation here, it can be shown that  $r_{rms} = \sqrt{2L_0L_p \left[ 1 - \frac{L_p}{L_0} \left( 1 - e^{-\frac{L_0}{L_p}} \right) \right]}$ . If we assume that the polymer is soft and wiggly, that is,  $L_0 \gg L_p$ , then this can be approximated as:  $r_{rms} = \sqrt{2L_0L_p}$ . Thus, this is the value that we would use for  $r_0$  in the estimation of the Young's modulus of a material made of these fibers. Further calculations of the Young's modulus is left for the reader, in this week's homework.

Now we consider what happens if we apply external force to the fiber. In this case, we define  $r = \langle \vec{r} \rangle$ , is the end-to-end distance (in the direction in which force is applied). The WLC model for an entropic polymer provides the following

equation:  $f(r) = \frac{k_B T}{L_p} \left( \frac{1}{4 \left( 1 - \frac{r}{L_0} \right)^2} - \frac{1}{4} + \frac{r}{L_0} \right)$ . (Note that this is response to force, so is  $f$ , not  $f_b$ ). Using First order approximation from the Taylor Expansion, you can show that, for sufficiently small  $r$ ,  $f \approx \frac{3k_B T}{2L_p} \frac{r}{L_0}$ . Thus, the spring constant for the WLC within its linear range, is  $k = \frac{3k_B T}{2L_p L_0}$ . It



turns out that this approximation is fairly good within a fairly large range,  $r < L_0/3$ .

One example of an entropic polymer is Tropoelastin, a protein in skin, arteries, lungs and other highly elastic tissues. Tropoelastin is 830 amino acid long polypeptide that is rich in proline and glycine, which tend to form unstructured random coils rather than beta sheets and alpha helices, and does not take on a discrete low-energy structure as do most proteins. An enzyme called lysyl hydroxylase catalyzes the covalent cross-linking of lysine residues in tropoelastin to create a network called elastin.

Entropic polymers also describe proteins that normally fold up into a globular structure of alpha-helices and/or beta sheets, but have been unfolded by mechanical force. Thus, the unfolded state of a protein like titin is actually a worm-like chain; if the force or strain are sufficiently low, the protein acts like a soft spring with  $k = \frac{3k_B T}{2L_p L_0}$ , but at higher forces, the nonlinearity comes into play. At very high forces, the distance approaches  $L_0$ .

Polypeptide chains have been measured to have a persistence length of anywhere from 0.3 to 1.0 nm. The contour length per amino acid is 0.3 nm. The variation may be partially due to the amino acid composition of the polypeptide, but may also be largely due to differences in measurement methods and curve fitting procedures, since the persistence length must be calculated indirectly by fitting the WLC equation to experimental data.

## Other molecular structures that cause strain hardening

### Fiber re-orientation

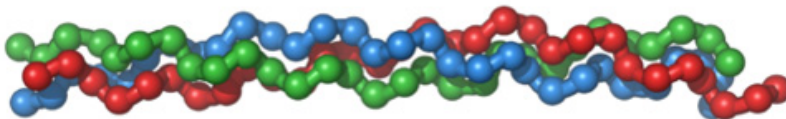
Another cause of strain hardening elastic behavior is that a material made up of cross-linked fibers can be quite soft while the fibers orient with the direction in which stress is applied, but then becomes quite stiff once the fibers are finished reorienting and further strain can only occur as a result of stretching of the individual fibers. Consider, for example, stretching a woven cloth. In this case, the Young's modulus at low forces does not reflect bond *length* spring constants, but rather bond *angle* spring constants. For many crosslinked polymers, the bond angles are very soft.



Thus, even if the hydrogel is composed of fibers that are not entropic polymers and do not exhibit strain hardening, the material will often exhibit strain hardening due to this re-orientation. An example of this is that a woven cloth exhibits strain hardening. (It also exhibits anisotropy, since it is stiff when pulled parallel to either fiber direction, and soft but strain hardening on the diagonal).

### Mixed materials.

Another cause of strain hardening behavior is the presence of mixed fiber types and of kinks. For example, the ligaments and arteries are made up primarily of collagen fibers. Tropocollagen has three polypeptides that form a triple helix. Unlike an alpha helix, the triple helix is not a tight coil. Instead, each peptide is nearly elongated, and the three twist slowly around each other.



(from <http://en.wikipedia.org/wiki/Collagen>)

This structure cannot extend much when stretched, which makes ligaments and arteries fairly stiff. However, most tissues made of collagen exhibit a “toe” region at low strains where the material is very soft. It turns out that collagen is kinked in the native tissue. Often, elastin in the tissue maintains the native shape and pulls the collagen into this kinked conformation, so at low strains, the soft elastin stretches and the kinks in the collagen straighten, and the tangent young's modulus is low. However, at higher strains, the collagen itself must stretch, and because it is stiffer, the material is also stiffer.

## Macroscale models of strain hardening.

To predict the behavior of a nonlinear material in various situations, it is useful to have a quantitative model for the stress as a function of strain. This can be used in numerical simulations of nonlinear materials, and in some cases, in analytic calculations as well. The WLC model only supplies the force-distance relationship of entropic polymers, not the materials properties. While we can estimate the Young's modulus from the WLC model, our assumptions in that derivation assume small deformations, and an unrealistic square uniform crystalline structure. We thus do not have a model for strain hardening at the materials level.

Different models have been proposed based on their simplicity and ability to empirically fit stress-strain curves of strain-hardening materials. An **empiric model** (also called a **black box model**) is a model that fits the data but is not based on any theory, and thus does not reflect or assume an underlying mechanism. The parameters in an empiric model do not represent any physical characteristic which could be measured independently. In contrast, a **parametric model** (also called a **gray box** or **mechanistic model**) is a model based on the underlying mechanism, in which the parameters in theory could be measured directly in a different type of experiment such they represent a physical property. For example, our model for the Young's modulus of a material,  $E = k/r_0$ , is a parametric model, in which the parameters  $k$  and  $r_0$  represent bond characteristics that can be measured chemically.

A commonly used empirical model for strain hardening elastic materials was developed by YC Fung. Here we consider just the one-dimensional version, neglecting for now lateral stresses and strains:  $\sigma(\epsilon) = \frac{E}{\beta}(e^{\beta\epsilon} - 1)$ . This model has two parameters,  $E$  (in Pa), and  $\beta$ (unitless). In this model, it can be shown that  $E$  is the Young's modulus at small deformations and  $\beta$  introduces the nonlinearity, and determines how much strain is needed before the nonlinear behavior is obvious.

### Summary

- Strain hardening materials are an advantage when the material must be soft to undergo large strains during normal working use, but must also resist excess strain that would cause damage when stress is abnormally high.
- Strain hardening can occur at the macroscale because 1) the nanoscale fibers in the material are entropic polymers, 2) the fibers in the material orient with the force, and 3) the material has more than one type of fiber that dominate stiffness at different strains.
- The WLC model,  $f(r) = \frac{k_B T}{L_p} \left( \frac{1}{4 \left(1 - \frac{r}{L_0}\right)^2} - \frac{1}{4} + \frac{r}{L_0} \right)$ , describes the force-extension curve of an entropic polymer, which is approximately  $f(r) \approx \frac{3k_B T}{2L_p L_0} r$  in the linear regime ( $r < L_0/3$ ). The persistence length can be calculated from the flexural rigidity of a beam:  $L_p = \frac{EI}{k_B T}$
- The Fung model describes the stress-strain diagram of a strain hardening material:  $\sigma(\epsilon) = \frac{E}{\beta}(e^{\beta\epsilon} - 1)$ .

**Example. Actin filament as WLC.**

- Estimate the persistence length of an actin filament.
- If a cell is 100um long, could cells use an actin filament to push two ends of the cell apart?
- If an actin filament were to grow to a contour length of 500 um, what would be the tensile spring constant in the linear regime?
- how much force would it take to extend such an actin filament to 1/3 of its contour length?
- How about 99% of its contour length?

Answer:

- We know  $L_p = \frac{EI}{k_B T}$ . We know that an actin filament is made of globular subunits, so we can assume a Young's modulus of  $E = 2 \text{ GPa}$ . We will estimate the flexural rigidity by approximating the cross-section as a circle with diameter 5.5 nm. It is actually asymmetric, with a cross-section that is  $7 \times 4 \text{ nm}$ , and is a helix so we cannot simply take the orientation with the smaller value of  $I$ . So we will estimate it as a circle with a diameter that is the average distance across, since we did not learn the more complex formula for this class. Thus,  $I = \frac{\pi}{4} (2.5e - 9m)^4 = 3E - 35 m^4$ , and  $EI = 6.1E - 26Nm^2$ . We divide this by  $k_B T = 4.1E - 21Nm$ , to learn that  $L_p = 1.5E - 5m$ , or  $15 \mu m$ . Experimental measurements have reported persistence lengths of *12 and 18  $\mu m$* .
- The persistence length of actin is much less than the length of a cell so an actin filament the length of a cell would not be a rigid rod and could not be used to push. This is why cells can pull using actin stress fibers, but can only push with actin by cross-linking actin into a stiff network in which the bonds between the nodes are tens to hundreds of nanometers.
- The spring constant of a filament with  $L_0 = 500 \mu m$  in the linear regime is:  $k = \frac{3k_B T}{2L_p L_0} = 8.2E - 13N/m$ .
- To extend to 1/3, the linear approximation should be OK, so  $f = k \frac{L_0}{3} = 3.6E - 17N$ . We can check this with the exact calculation:  $f(L_0/3) = \frac{k_B T}{L_p} \left( \frac{1}{4(1-1/3)^2} - \frac{1}{4} + \frac{1}{3} \right) = \frac{k_B T}{L_p} (0.65) = 2.6E - 16 * 0.65 = 1.8E-16 N/m$ , so my estimation produced an error of  $(0.65-0.5)/0.65 = 23\%$  underestimation.
- We clearly need the exact formula for  $r = 0.99L_0$ .

$$f(0.99L_0) = \frac{k_B T}{L_p} \left( \frac{1}{4(1-0.99)^2} - \frac{1}{4} + 0.99 \right) = 2500 \frac{k_B T}{L_p} = 6.85E - 13N$$

It thus requires only sub-pN forces to extend an actin filament to 99% of contour length. It should be remembered, however, that the worm-like-chain model only applies when  $L_p \ll L_0$ , so this force is the same independent of contour length, as long as this condition holds. Thus, while the actin filament is wiggly, it does not take much force to extend one to be nearly straight.

