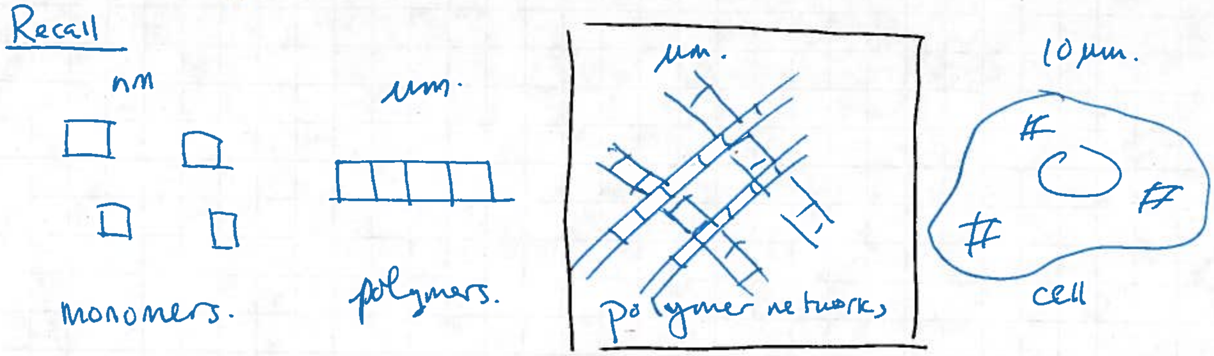
Lecture #3: Polymer Networks & Cytoskeleton [CSK]

Recall



Last time, we derived the deformation of CSK proteins due to tension, torsion, and bending.

We also derived equations for polymerization kinetics. and defined the concept of persistence length.

$$A = \frac{EI}{k_b T}$$

"stiffer filaments are straighter"
" cooler filaments are straighter.

Assuming we know the mechanical properties of individual filaments, what does that actually tell us about the assembly of filaments within a cell?

→ if we knew the structural arrangements of filaments, could we predict the stiffness of overall assembly?

→ How does the filament microstructure affect CSK properties?

→ How can we calculate the macroscopic network properties from the individual microscopic filament properties?

Note: In mechanics, the derivation of macroscopic parameters based on microscopic considerations ⇒ homogenization

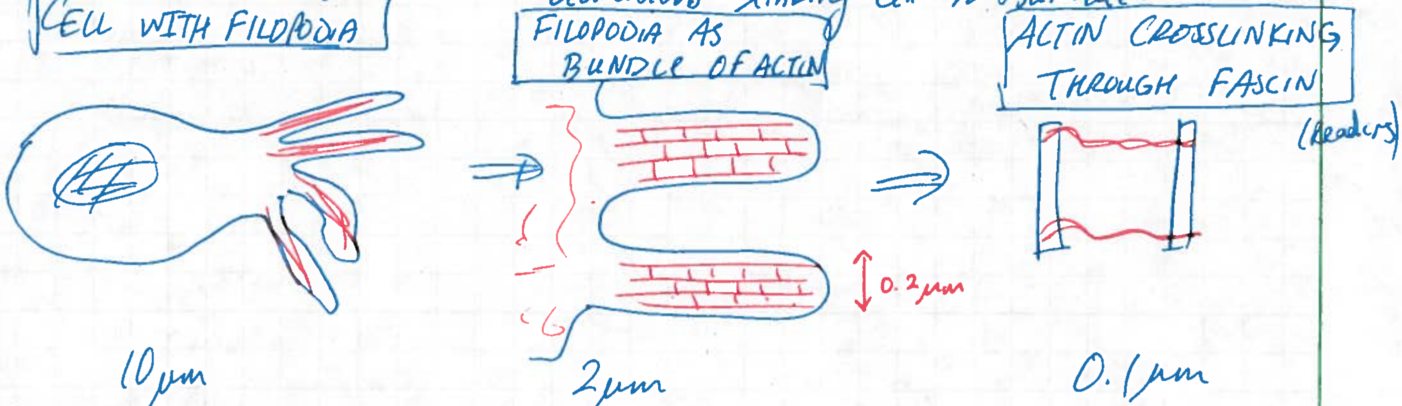
We will cover 3 different examples. to illustrate homogenization.

1) Fiber bundle model for filopodia.
2) Network model for RBC membranes
* 3) Tensegrity model for generic eukaryotic cells.

Fiber Bundle Model of Filopodia

Definition What is this ?!?

- thin dynamic cytoplasmic projections composed of tight bundles of long actin filaments.
- the "feelers" as a cell moves & explores extracellular matrix & surfaces of other cells.
- involved in cell motility on the leading edge
  - → once they pick a spot they form focal adhesions linking cell to surface



IMPORTANT Parameters

$d = 0.2\,\mu m$

$l_{extension}: 1.5-20\,\mu m$ (1-5 µm in other texts)

Polymerization rate $= \frac{10\,\mu m}{min}$ 20-30 filaments

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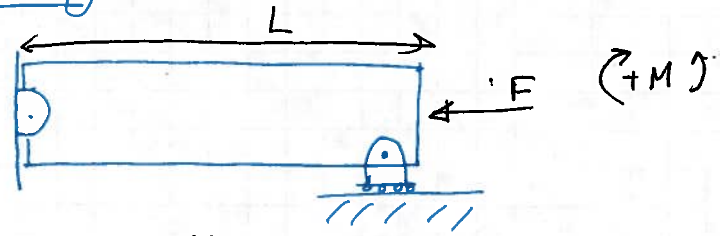
We can model actin filaments within filopodia as elastic beams undergoing buckling.

Assume filopodia is a cylinder w/ radius $r_{filament}$

this cylinder pushes against membrane with F_{fil}

By Newton's 3rd Law we will say $F_{fil} = F_{membrane}$.

Buckling Consider a beam that is loaded axially.



$w(0) = w(L) = 0$

$$\frac{d^2w}{dx^2} = \frac{M(x)}{EI} = -\frac{Fw}{IE}$$

$$\frac{d^2w}{dx^2} + \left(\frac{F}{EI}\right) w = 0 \longrightarrow w(x) = C_1 \sin(kx) + C_2 \cos(kx)$$

to satisfy B.C $y(0) = 0 \Longrightarrow C_2 = 0.$

$$\frac{d^2w}{dx^2} = -C_1 k^2 \sin kx = -\frac{Fw}{EI}$$

$$C_1 \left(k^2 - F/EI\right) \sin(kx) = 0$$

One solution is $k = 0$ or $y(x) = 0.$ ← TRIVIAL.

$$k = \pm\sqrt{F/EI}$$ ← NON TRIVIAL.

$$C_1 \sin\left(L\sqrt{F/EI}\right) = 0 \Longrightarrow L\sqrt{\frac{F}{EI}} = n\pi$$

$$\boxed{F_b = \frac{n^2\pi^2}{L^2} EI}$$

$n =$ different mode.

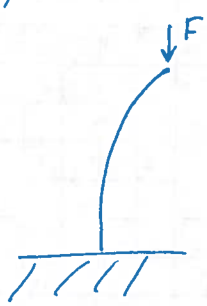
$n = 1 \longrightarrow$ Euler buckling load $\boxed{F_b = \frac{\pi^2 EI}{L^2}}$

Now we have the Euler Buckling Load. What is the critical force a filopodium can bear?

$$F_{fil} = F_{crit} = \frac{\pi^2 E I}{L_{crit}^{\,2}}$$

With Euler Buckling, there are 4 modes and all four have this critical force ~~eq~~ equation.

However, the mode with the highest critical buckling force is below.



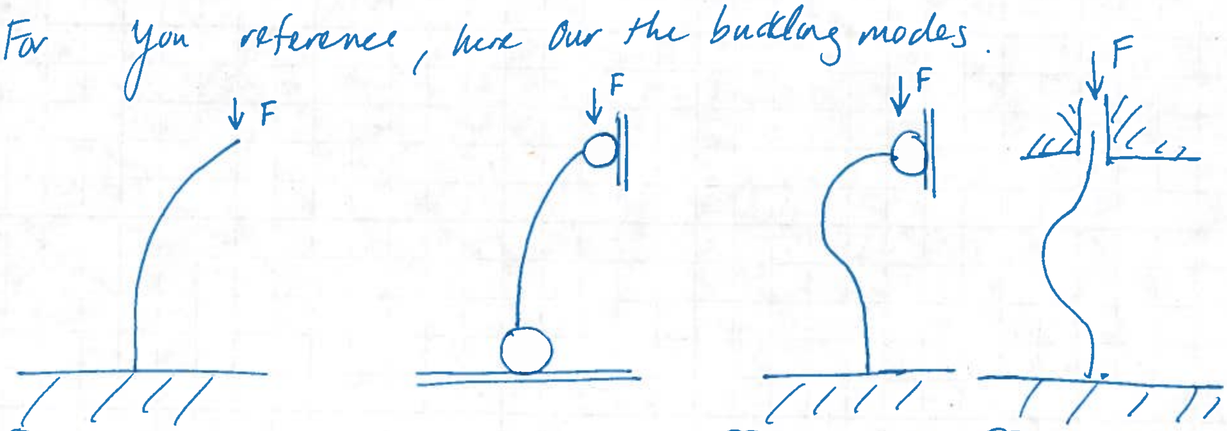
$L_{crit} = 2L$

It is the most flexible with the longest Buckling length.

Thus, As $L_{crit} \downarrow$, $\underline{F_{crit} \uparrow}$

[resistence to buckling]

For you reference, here our the buckling modes.



1. $L_{crit} = 2L_{fil}$
2. $L_{crit} = L_{fil}$
3. $L_{crit} = \frac{L_{fil}}{\sqrt{2}}$
4. $L_{crit} = L_{fil}/2$

Thus, our selection is $L_{crit} = 2L$. Let's plug in!

$$F_{fil} = F_{crit} = \frac{\pi^2 EI}{L_{crit}^2} = \frac{\pi^2 EI}{(2L_{fil})^2} = \frac{\pi^2 EI}{4L_{fil}^2}$$

From lit.

Now, I will just tell you my assumption for membrane force

$$F_{mem} \approx 5 r_{fil} \ \frac{pN}{nm}$$

Derive in class activity.

What is radius of filopodium?

$$A_{fil} = \pi r_{fil}^2$$

$$A_{actin} = n \pi r_{actin}^2$$

filaments

$$\left.\begin{matrix} A_{fil} = \pi r_{fil}^2 \\ A_{actin} = n\pi r_{actin}^2 \end{matrix}\right\} A_{fil} = A_{act} \longrightarrow \boxed{r_{fil} = \sqrt{n}\, r_{act}}$$

So $\boxed{F_{mem} \approx 5\sqrt{n}\, r_{act}}$ $\frac{pN}{nm}$

Note

Experimentally,

$F_{mem} \approx 50\,pN$

What if force exerted by cell membrane is on filopodium cylinder?

$$F_{fil} = F_{mem}$$

$$\frac{\pi^2 EI}{4L_{crit}^2} = 5\sqrt{n}\, r_{act}$$

$$\boxed{L_{crit} = \frac{\pi}{2}\sqrt{\frac{EI}{5\sqrt{n}\, r_{act}}}}$$

Recall

for actin

$E = 1.9\,GPa$

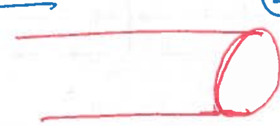
$r_{act} = 3.5\,nm$

$I = \frac{\pi R_{bundle}^4}{4}$

Two cases can be considered about your actin filaments.

Either they are considered in a ① $\boxed{\text{Loose Assembly}}$ so you treat each actin filament separately and $I = n\, I_{actin}$.

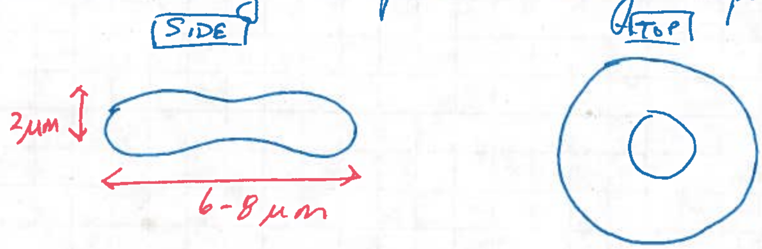
OR ② $\boxed{\text{Tight Assembly}}$ so you treat as one filament so I based on r_{fil}.



Network Model for Red Blood Cells

We just examined the buckling of filopodia — now, let's focus on the cytoskeletal structure. Specifically, the highly structured red blood cell [erythrocyte].

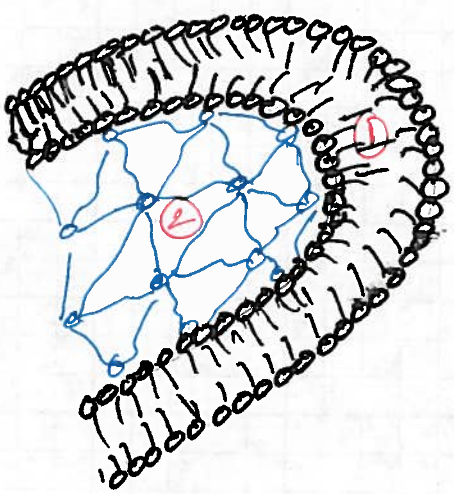
The red blood cell is responsible for delivering O_2 to the body. It has the ability to squeeze through capillaries without bursting.



$V = 90\ fL$ $\longrightarrow$ can swell to $150\ fL$

$SA = 136\ \mu m^2$

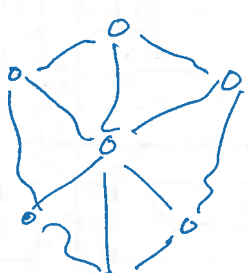
MEMBRANE OF THE RED BLOOD CELL PLAYS A KEY ROLE IN REGULATING SURFACE Deformability and flexibility.



FOR RBC

1. outer membrane = phospholipid bilayer
2. ~~outer~~ Inside surface membrane = network of spectrin tetramers crosslinked through actin
   AKA cytoskeleton

The architecture of the actin-spectrin network has a six fold connectivity.



Is the structure of the red blood cell's cytoskeleton functionally advantageous?

In theoretical and computational mechanics:

Hill condition $\quad W^{macro} = W^{micro}$ $\quad$ (Homogenization)

UPON Deformation, the energy W^{macro} stored in macroscopic continuum should be equivalent to energy W^{micro} stored in microscopic networks.

Our goal is to express the overall macroscopic properties of this network in terms of known microscopic parameters.

For a 2-D continuous sheet, note:

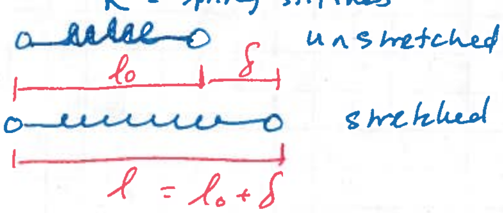
$$W^{macro} = \tfrac{1}{2}\,\kappa\left[\varepsilon_{xx} + \varepsilon_{yy}\right]^2 + \tfrac{1}{2}\mu\left[\varepsilon_{xx} - \varepsilon_{yy}\right]^2 + 2\mu\varepsilon_{xy}^{\,2}$$

bulk modulus. normal strain in x, y direction. shear modulus. shear strain

General idea: express κ and μ in terms of k = spectrin stiffness

So, consider modeling spectrin as a Gaussian chain

k = spring stiffness



Spectrin polymers

r = end to end length = 75nm

L = 200nm (contour)

l = 7.5nm (aprox segment length)

b/c $l \ll L$, $r \ll L$ } Gaussian chain

Gaussian chains are linear entropic springs with spring stiffness

$$k = 3 k_B T \frac{N}{L}$$

Assume
30nm
300K
$L = 200$nm

For a spring, free energy $\quad W^{spr} = \frac{1}{2} k \delta^2 = \frac{1}{2} k\left[l - l_0\right]^2$

Before we start

A short aside on ...

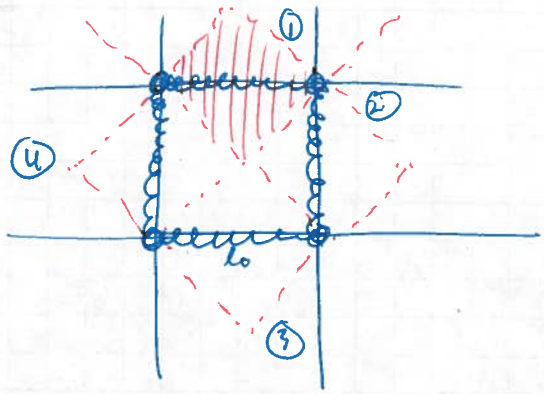
Representative area [or volume] element.

First consider four fold connectivity. We need to represent each Spring over an area.

We do this by considering Symmetry.

Look at the red outline.

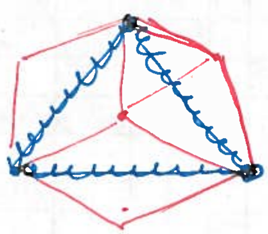
Now look at the shaded region. This is the representative area is for 1 spring.



Notice by symmetry, the total representative area.

$$\sum_{i=1}^{4} A_i^{spr} = 4A^{spring} = 2\underline{A_{square}} = 2\, l_0^2$$

For 6 fold, it works the same way except for 3 springs:



$$\sum_{i=1}^{3} A_i^{spring} = 3A^{spring} = 2\, A_{triangle}.$$

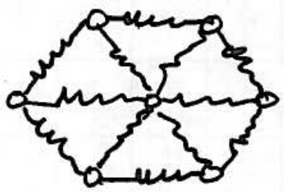
Thus

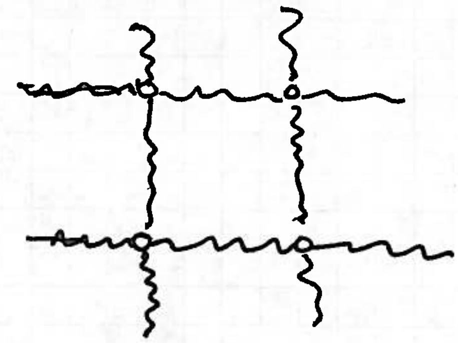
$$\sum_{i=1}^{3} A_i^{spring} = 2\left[\frac{1}{2} l_0 h_0\right]$$

$$= \frac{\sqrt{3}}{2} l_0^2$$

Back to the proof!

SIX FOLD (Lecture) — FOUR FOLD (IN CLASS)

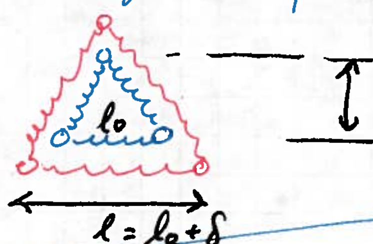




CASE 1: EXTENSION

Consider the smallest unit cell or representative volume element

Extend the triangle isotropically.



SIDE NOTE

representative area for spring

For 1 spring, area is

We want a way to represent the spring as area

Required Network Energy Density

$$W^{micro} = \frac{\sum_{i=1}^{3} W_i^{spring}}{\sum_{i=1}^{3} A_i^{spring}}$$

We know $W^{spring} = \frac{1}{2} k \delta^2$ for one spring. Thus,

$$\sum_{i=1}^{3} W_i^{spr} = 3\, W^{spr} = 3\left[\tfrac{1}{2} k \delta^2\right]$$

The area of the spring system is just

$$\sum_{1}^{3} A_i^{spr} = 2\, A^{triangle} = 2\left[\tfrac{1}{2} l_0 h_0\right]$$

$$= \tfrac{1}{2}\sqrt{3}\, l_0^2$$

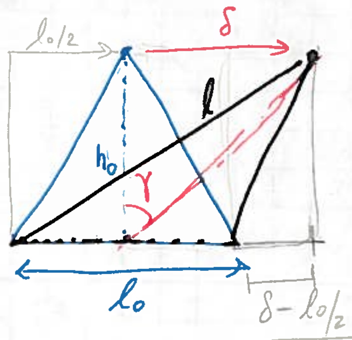
So $W^{micro} = \dfrac{3(\frac{1}{2} k \delta^2)}{\frac{1}{2}\sqrt{3}\, l_0^2} \rightarrow \boxed{W^{micro} = \sqrt{3}\, k \left[\dfrac{\delta}{l_0}\right]^2}$

For six fold $\quad \varepsilon_{xx} = \dfrac{\delta}{l_0} \quad \varepsilon_{yy} = \dfrac{\delta}{l_0} \quad \varepsilon_{xy} = 0$

BULK MODULUS

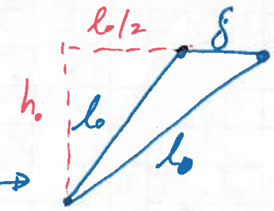
$$W^{macro} = \frac{1}{2} K \left[\frac{\delta}{l_0} + \frac{\delta}{l_0}\right]^2 = \sqrt{3}\, k \left[\frac{\delta}{l_0}\right]^2 = W^{micro} \rightarrow \boxed{K = \tfrac{1}{2}\sqrt{3}\, k}$$

Case 2: Shear



$h_0 = \frac{\sqrt{3}}{2} l_o$

if we redraw $\longrightarrow$



Deformed length of left spring

$$l = \sqrt{h_o^2 + \left(\frac{l_o}{2} + \delta\right)^2} = \sqrt{\frac{3}{4} l_o^2 + \frac{l_o^2}{4} + l_o\delta + \delta^2}$$

$$l = \sqrt{l_o^2 + l_o\delta + \delta^2} = l_o\sqrt{1 + \frac{\delta}{l_o} + \frac{\delta^2}{l_o^2}}$$

Since δ is small

$$l \approx l_o\sqrt{1 + \delta/l_o}$$

Now for some magic.

Note $\left(1 + \delta/2l_o\right)^2 = 1 + \frac{\delta}{l_o} + \frac{\delta^2}{4l_o^2} \approx 1 + \delta/l_o$

So $$l \approx l_o\sqrt{\underbrace{\left(1 + \frac{\delta}{2l_o}\right)^2}_{\text{Complete the square}}} \approx \boxed{l_o + \frac{\delta}{2} = l}$$

This implies left spring $\uparrow \delta/2$.

In a similar manner, it can be shown right spring $\downarrow \delta/2$.

Now, $$\sum_{i=1}^{3} W_i^{spr} = \sum_{i=1}^{3} \frac{1}{2} k\left[l_i - l_o\right]^2$$

$$= \underbrace{\frac{1}{2}k\left[+\delta/2\right]^2}_{\text{left spring}} + \underbrace{\frac{1}{2}k\left[-\delta/2\right]^2}_{\text{right spring}} + \underbrace{0}_{\text{bottom spring does not deform}}$$

$$\sum W^{spr} = \frac{1}{4}k\delta^2$$

Again, $$\sum_{i=1}^{3} A_i^{spr} = \frac{\sqrt{3}}{2} l_o^2$$

Thus, $$W^{micro} = \frac{\frac{1}{4}k\delta^2}{\frac{\sqrt{3}}{2} l_o^2} = \frac{\sqrt{3}}{6} k\left[\frac{\delta}{l_o}\right]^2$$

Here, $\varepsilon_{xx} = \varepsilon_{yy} = 0$

$$\varepsilon_{xy} = \frac{1}{2}\left[\frac{\delta x}{y} + \cancel{\frac{\delta y}{x}}^{\,0}\right]$$

$$\varepsilon_{xy} = \frac{1}{2}\left[\frac{\delta}{h_o}\right] = \frac{1}{\sqrt{3}}\frac{\delta}{l_o}$$

$\rightarrow W^{micro} = W^{micro}$

$$2\mu\left[\frac{1}{\sqrt{3}}\frac{\delta}{l_o}\right]^2 = \frac{\sqrt{3}}{6}k\left[\frac{\delta}{l_o}\right]^2$$

$$\boxed{\mu = \frac{\sqrt{3}}{4} k_{spr}}$$

TENSEGRITY

→ "tensional integrity"

↳ BUILDING TECHNIQUE WHERE MECHANICAL INTEGRITY OF STRUCTURE MAINTAINED BY INTERNAL MEMBERS → under tension or compression

EXAMPLE IN ARCHITECTURE: GEODESIC DOMES

FROM DON INGBER LAB ...

THIS THEORY WAS ADAPTED TO CELLS WHERE

- actin microfilaments generate tension
- microtubules under compression
- there are interconnections b/w actin & MT's

FROM Stamenovic & Coughlin ...

- ASSUME CELLS HAVE $\boxed{6}$ compression elements (struts)

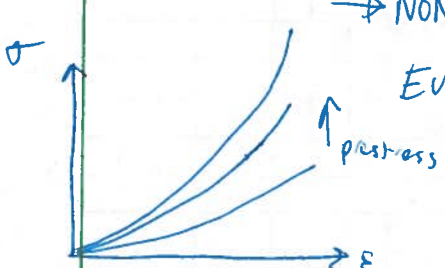
  → WHY SIX? BECAUSE THIS IS THE SMALLEST NUMBER OF STRUTS THAT CAN PROVIDE NON TRIVIAL TENSEGRITY SYSTEM → spatially isotropic

- SIX STRUTS JOINED BY 24 Tension elements (ropes)

  → Gaussian ~~springs~~ chain or entropic linear spring

THE MAIN FEATURE TO COME OUT OF THIS MODEL:

→ NON ZERO FORCE IN CYTOSKELETON TENSION ELEMENTS EVEN IN RESTING STATE



→ CELL HAS A "PRESTRESS", P

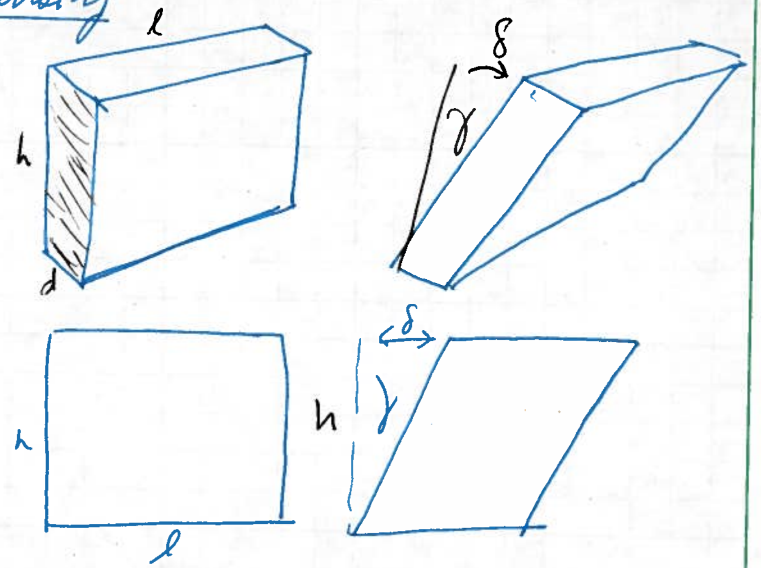
$F_0 = k(l_0 - l)$

THE DERIVATION YIELDS

$$P \propto E_0 = 5.85 \frac{F_0}{l_0^2} \frac{1+4\varepsilon_0}{1+12\varepsilon_0} \qquad \varepsilon_0 = \frac{l_0}{l_r} - 1$$

A short aside ... Another way to understand shear derivation.

Shear modulus & strain energy density



$\tau = G\gamma$

shear stress (τ), shear modulus (G), shear strain (γ)

$$\gamma = \frac{\partial u}{\partial y} + \frac{\partial v}{\partial x} \Rightarrow \frac{\delta}{h} = \tan\gamma$$

small angle approx

$\tan\gamma \approx \gamma$

Strain energy per unit undeformed volume or strain energy density

$$\Delta w_v = \frac{\Delta W}{V} \qquad V = lwh$$

$$\Delta w_v = \frac{1}{2} G \gamma^2$$

Upon inspection $$\frac{\partial^2 \Delta w_v}{\partial \gamma^2} = \frac{\partial^2 [\frac{1}{2} G \gamma^2]}{\partial \gamma^2} = \frac{\partial [G\gamma]}{\partial \gamma} = G$$

Therefore if we know ΔW under some $\gamma \rightarrow G$ is known.

Now consider 2D $\rightarrow$ block is thin so small depth d.

$$N_s = \tau d \quad \Rightarrow N_s = K_s \gamma \qquad K_s = \text{shear modulus.}$$

$$\underline{K_s = Gd} \doteq \frac{\partial^2 \Delta w_a}{\partial \gamma^2}$$

With these relations, we can calculate K_s for RBC.

Our strategy

Find ΔW given γ
Divide by undeformed area

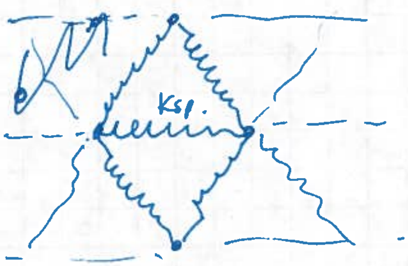
$\Big\} \; \Delta w_a \longrightarrow \underline{K_s}.$

Six fold Connectivity

First, we need to model the polymer network.

In vivo, Spectrin : $L = 200nm$, $l_p = 15nm$ } $l_p \ll L$
spectrin not fully stretched
$R < L$
thus assume entropic springs

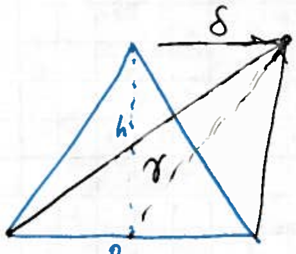
$K_{sp} = \frac{3K_BT}{Lb}$ for six fold network.



$\Rightarrow$

$K_{sp}/2$

Consider an equilateral triangle as a unit cell.

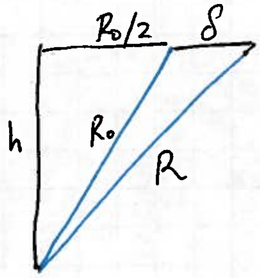


$$h = \sqrt{R_0^2 - (R_0/2)^2} = \frac{\sqrt{3}}{2}R_0$$

If we displace top vetex by small δ

$$\tan\gamma = \frac{\delta}{h} = \frac{2\delta}{\sqrt{3}R_0} \approx \gamma$$

$$\text{so } \delta = \frac{\sqrt{3}R_0}{2}\gamma$$



Defomed length of left spring. R.

$$R = \sqrt{h^2 + \left(\frac{R_0}{2} + \delta\right)^2} = R_0\sqrt{\frac{\delta}{R_0} + \frac{\delta^2}{R_0^2}}$$

Since δ is small

$$R \approx R_0\sqrt{1 + \delta/R_0}$$

Note: $\left(1 + \frac{\delta}{2R_0}\right)^2 = 1 + \frac{\delta}{R_0} + \frac{\delta^2}{4R_0^2} \approx 1 + \frac{\delta}{R_0}$

so $R \approx R_0\sqrt{\left(1 + \delta/2R_0\right)^2} \approx R_0 + \delta/2$

This implies the left spring $\uparrow \delta/2$.

It can be shown in a similar manner the right spring $\downarrow \delta/2$.

$$\Delta W_{sp} = \tfrac{1}{2} K_{sp} (R - R_0)^2$$

The total strain energy $= \sum_{i=1}^{3} \Delta W_i$

$$\Delta W = \Delta W_{sp}^{left} + \Delta W_{sp}^{right} + \Delta W_{sp}^{bottom}$$

$$= \frac{1}{2}\left(\frac{K_{sp}}{2}\right)\left[(R_0 + \delta/2) - R_0\right]^2 + \frac{1}{2}\left(\frac{K_{sp}}{2}\right)\left[(R_0 - \frac{\delta}{2}) - R_0\right]^2 + 0$$

$$\Delta W = \frac{K_{sp}\,\delta^2}{8}$$

$$\Delta w_a = \frac{\Delta W}{A} = \frac{\frac{K_{sp}\delta^2}{8}}{\frac{1}{2} R_0 h} = \frac{\frac{K_{sp}\delta^2}{8}}{\frac{1}{2} R_0 \frac{\sqrt{3}}{2} R_0} = \frac{K_{sp}\,\delta^2}{2\sqrt{3}\,R_0^2}$$

Recall $\gamma = \dfrac{2\delta}{\sqrt{3}\,R_0}$ so $\Delta w_a = \dfrac{\sqrt{3}}{8} K_{sp}\,\gamma^2$

$$K_s = \frac{\partial^2 \Delta w_a}{\partial \gamma^2} = \frac{\partial^2}{\partial \gamma^2}\left[\frac{\sqrt{3}}{8} K_{sp}\,\gamma^2\right]$$

$$\boxed{K_s = \frac{\sqrt{3}\,K_{sp}}{4}}$$

$$\left.\begin{aligned} b &= 30\,nm \\ T &= 300K \\ L &= 200\,nm \end{aligned}\right\} K_s = 0.9\,\mu N/m$$

Experimentally: $K_s = 2.4\,\mu N/m$

Sources of error? Approx as Gaussian chain. Remember $R \to L$, stiffness $\uparrow$ but in Guassian model, linear. Must have $L \gg R$.