Homework II - The cytoskeleton

due Thu 05/03/12, 12:50pm, edu-128

You can drop off late homework in a box in front of Durand 217. Please mark clearly with date and time @drop off. We will take off 10 points for each 24 hours late.

Problem 1 - Filament buckling

In class, we have discussed filament buckling of actin polymers. We have seen that the critical buckling force of filopodia

\[ F_{cr} = \frac{\pi^2 EI}{4L_{fil}^2} = \frac{\pi^2 E I}{4L_{fil}^2} \]

can be increased by fascin which can create tightly crosslinked filament bundles.

- In class, we have assumed that there are about \( n = 30 \) actin filaments in one filopodium. Assume we do not know the exact number \( n \). What is the minimum number of actin filaments to obtain a critical filopodia length of \( L_{fil} = 5 \mu m \) assuming that the actin filaments are (i) loosely assembled (4.2.9) and (ii) tightly crosslinked (4.2.12).

- How would the minimum number of required actin filaments \( n \) change if you assume a critical filopodia length of \( L_{fil} = 2.5 \mu m \)?
Problem 2 - Forces on the cell membrane

To gain a better feeling for stresses that the cytoskeleton might induce on the cell membrane, this problem deals with the membrane pressure resulting from microtubules. Consider a generic spherical cell of radius 10μm with a tubulin heterodimer concentration of C = n/V = 5μM.

Figure. Microtubules are made up of rings of thirteen tubulin heterodimers which are 8 nm in diameter. The rings of tubulin heterodimers form a cylinder of thirteen protofilaments.

- Calculate the total length of microtubules that could be made from this amount of protein if each heterodimer is approximately 8 nm in diameter. Take into account that microtubules are made up of rings of thirteen tubulin heterodimers. Remember that 1μM = 1 μmol/liter and that 1 mol contains 6.02 x 10^23 heterodimers.

homework 02 - problem 02

Problem 3 - Cell mechanics research

The recent manuscript “Biomechanics: Cell Research and Applications for the Next Decade” by Discher, Dong, Fredberg, Guilaub, Ingber, Janmey, Kamm, Schmid-Schönbein and Weinbaum discusses the challenges for cell mechanics now and in the future.

- Read the manuscript carefully and summarize it with approximately 150 words.
- The authors have identified ten major accomplishments in cell mechanics. List all the ten accomplishments by title.
- Select your favorite past accomplishment and describe it in less than 100 words.
- The authors have identified seven major challenges for the future. List all the seven future challenges by title.
- Select your favorite future challenge and describe it in less than 100 words.

homework 02 - problem 03

Problem 4 - Final project

Inspired by the recent manuscript on major accomplishments and challenges in cell mechanics,

- Identify a title for your final project.
- Identify five key words for your final project.
- Write a tentative abstract of approximately 150-200 words.

Papers in the past have generally been about 4-6 pages long, two column, with about 3-5 figures and 8-12 references. Here are some examples of individual projects.

- Predicting microtubule structure using molecular dynamics
- The primary cilium: A well-designed fluid flow sensor
- The tensegrity paradigm
- Mechanotransduction in hair cells: Translating sound waves into neural signals
- Modeling cell membrane dynamics
- Theoretical and experimental study of the penetration of the cell membrane
- Integriin and its role in mechanotransduction
- Finite element analysis of micropipette aspiration

homework 02 - problem 04
the swimming velocity of mammalian sperm

MODELS AND MECHANISMS OF LEUKOCYTE EXTRAVASATION
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ABSTRACT | Leukocyte extravasation is an innate immune system process by which white blood cells leave the circulatory system and enter the inflamed tissue or site of infection. Although leukocyte migration across the walls of microvessels was observed over 200 years ago, the molecular mechanisms of the migration were not clarified until the early part of the twentieth century. Today, leukocyte extravasation is thought to occur in a multistep cascade involving the initial capture of the leukocyte by the endothelium, rolling of the leukocyte, slow rolling, arrest, adhesion, and spreading of the white blood-cell, intravascular crawling, and transmigration of the leukocyte across the endothelium. Over the past two decades, a number of molecular and mechanical models have surfaced that view the leukocyte as a shell membrane, a hard sphere defined by Goldman hydrodynamics and liquid droplets. By exploring each leukocyte model in the context of the extravasation process, a greater insight into the importance of integrin and selectin-based mechanotransduction as well as the shear-flow-induced physics of extravasation can be gained. Such understanding can be particularly valuable in the context of drug and cancer development – two radically different processes that mimic leukocyte extravasation.
4.1 mechanics of the cytoskeleton

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

- Could we then predict the **stiffness of the overall assembly**?
- How does the filament microstructure affect **cytoskeletal properties**?
- How can we calculate the **macroscopic network properties** from the individual microscopic filament properties?

Figure 4.1: The cytoskeleton provides structural stability and is responsible for forces during cell locomotion. Microtubules are thick hollow cylinders reaching out from the nucleus to the membrane, intermediate filaments can be found anywhere in the cytosol, and actin filaments are usually concentrated close to the cell membrane.

**three examples**

- **fiber bundle model** for filopodia
- **network model** for red blood cell membranes
- **tensegrity model** for generic cell structures
4.2 fiber bundle model for filopodia

**microstructural arrangement of actin**

*Figure 4.1.* A crawling cell, drawn to scale, is shown with three areas enlarged to show the arrangement of actin filaments. The actin filaments are shown in red, with arrowheads pointing toward the plus end. Stress fibers are contractile and exert tension. The cell cortex underlies the plasma membrane. Filopodia are spike-like projections of the plasma membrane that allow a cell to explore its environments.

*Alberts, Johnson, Lewis, Raff, Roberts, Walter (2002)*

**assembly of crosslinked actin filaments**

*Figure 4.3.* Bundles of actin filaments tightly crosslinked through fascin are known as filopodia. The mechanical properties of filopodia play an essential role in various different physiological processes including hearing, cell migration, and growth. Filopodia are very thin structures approximately 0.2 μm in diameter, they can easily extend up to 2.0 μm, they typically polymerize and depolymerize at rates of approximately 10 μm/min. The mechanical properties of filopodia play an essential role in various different physiological processes, including hearing, cell migration, and growth. Despite their importance to cell function, the structural architecture responsible for their overall mechanical behavior remains largely unknown.

**pushing the envelope**

**simplified model for cell locomotion**

- protrusion ... polymerization at the leading edge of the cell
- attachment ... formation of focal adhesions to link the cell to the surface
- retraction ... contraction of stress fibers to retract the rear of the cell

*Figure 4.4.* Single-celled amoeba crawling around by using actin polymerization to push out pseudopods to explore new territory. Organisms move in complex patterns within the cell.

*Alberts, Johnson, Lewis, Raff, Roberts, Walter (2002)*

**4.2 fiber bundle model for filopodia**

**pushing the envelope - critical length**

Newton’s third law: actio = reactio

\[ F_{\text{fil}} = F_{\text{mem}} \]

\[ F_{\text{fil}} = \frac{\pi^2 EI}{2L^2} = \frac{\pi^2 EI}{4L} \]

\[ F_{\text{mem}} \approx 5 \sqrt{\pi} r_{\text{act}} \text{ pN/\text{nm}} \]

\[ \frac{\pi^2 EI}{4L^2_{\text{crit}}} = 5 \sqrt{\pi} r_{\text{act}} \text{ pN/\text{nm}} \]

\[ L_{\text{crit}} = \frac{\pi}{2} \sqrt{\frac{EI}{5 \sqrt{\pi} r_{\text{act}} \text{ pN/\text{nm}}}} \]

- \( E = 1.9 \cdot 10^8 \text{ N/m}^2 = 1.9 \text{ GPa} \)
- \( r_{\text{act}} = 3.5 \text{ nm} \)
- loose assembly
- tightly crosslinked

*4.2 fiber bundle model for filopodia*
4.2 fiber bundle model for filopodia

\[ L_{\text{crit}} = \frac{\pi}{2} \sqrt{\frac{EI}{5 \sqrt{n} r_{\text{act}}}} \text{ pN/nm} \]

moment of inertia \( I = \pi \frac{r_{\text{act}}^4}{4} \)

\( I = n I_{\text{act}} \) with \( I_{\text{act}} = \frac{\pi r_{\text{act}}^4}{4} \)

\( E = 1.9 \cdot 10^9 \text{ N/m}^2 = 1.9 \text{ GPa} \)

\( r_{\text{act}} = 3.5 \text{ nm} \)

Much too low - disagrees with observations of 2\( \mu \text{m} \)

**case I** - loosely assembled actin filaments

\( L_{\text{crit}} = \frac{\pi}{2} \sqrt{\frac{1.9 \cdot 10^9 \text{ N/m}^2 \cdot n \pi/4 [3.5 \cdot 10^{-9}]^4 \text{m}^4}{5 \sqrt{n} 3.5 \cdot 10^{-12} \text{ N}}} \approx 0.17769 \mu \text{m} \cdot n^{1/4} \)

\( n = 30 \text{ filaments} \)

\( L_{\text{crit}} = 0.416 \mu \text{m} \)

**case II** - tightly crosslinked actin filaments

\( L_{\text{crit}} = \frac{\pi}{2} \sqrt{\frac{1.9 \cdot 10^9 \text{ N/m}^2 \cdot n^2 \pi/4 [3.5 \cdot 10^{-9}]^4 \text{m}^4}{5 \sqrt{n} 3.5 \cdot 10^{-12} \text{ N}}} \approx 0.17769 \mu \text{m} \cdot n^{3/4} \)

\( n = 30 \text{ filaments} \)

\( L_{\text{crit}} = 2.278 \mu \text{m} \)

4.3 network model for red blood cells

erithrocytes, red blood cells are essential to deliver oxygen to the body via the blood flow through the circulatory system. They take up oxygen in the lungs and release it while squeezing through the body's capillaries. Adult humans have about 2-3 \( 10^{13} \), 20-30 trillion, red blood cells comprising about a quarter of the total amount of cells in the human body.

4.3 network model for red blood cells

human mature red blood cells are flexible biconcave disks that lack a cell nucleus and most organelles. Typical human erythrocytes have a disk diameter of 6-8\( \mu \text{m} \), a thickness of 2\( \mu \text{m} \), a volume of 90fL, and a surface of 136\( \mu \text{m}^2 \). They can swell to spherical shape of 150fL, without membrane distension. The membrane of the red blood cell plays a key role in regulating surface deformability, flexibility, adhesion to other cells. These functions are highly dependent on its composition. The red blood cell membrane is composed of 3 layers: the glycocalyx on the exterior, which is rich in carbohydrates; the lipid bilayer consisting of lipidic main constituents and transmembrane proteins; and the membrane skeleton, a structural network of proteins located on the inner surface of the lipid bilayer.
4.3 network model for red blood cells

The human red blood cell membrane skeleton is a network of roughly 33,000 protein hexagons that looks like a microscopic geodesic dome.
homogenization - hill-mandel condition

aim. to determine the overall material properties $\kappa$ and $\mu$ of the network of spectrin chains in terms of the spectrin chain stiffness $k$

Energy Approach

$$W^{\text{mac}} = W^{\text{mic}}$$

It has been shown how the central problem is reducible to the calculation of average stress or strain in one or other phase. A more versatile approach stems directly from classical theorems in elasticity and focuses on strain energies.


4.3 network model for red blood cells

single spring energy

free energy $W^{\text{spr}}$ of a single spring

$$W^{\text{spr}} = \frac{1}{2} k \delta^2 = \frac{1}{2} k (l - l_0)^2$$

where $\delta = l - l_0$

$$k \quad \text{unstretched spring}$$

$$l_0 \quad \text{stretched spring}$$

$k \ldots \text{spring stiffness}$

Figure 4.7: Spectrin can be modeled as Gaussian chain which we can conceptually replace by an equivalent linear entropic spring with a spring stiffness of $k = 3kT N / L$. The strain energy of this spring can then be expressed as $W^{\text{spr}} = \frac{1}{2} k \delta^2$.

different network kinematics

six-fold connected network

four-fold connected network

Figure 4.6: Microstructural architecture of a six-fold and four-fold connected network. The theory of homogenization helps to explain why nature prefers a six-fold connected network geometry.

4.3 network model for red blood cells

discrete microscopic network energy

extension

$$W^{\text{mic}} = \frac{1}{2} \sum_{i=1}^{3} A_i^{\text{spr}}$$

$$\sum_{i=1}^{3} W_i^{\text{spr}} = 3 W^{\text{spr}} = 3 \left( \frac{1}{2} k \delta^2 \right)$$

$$\sum_{i=1}^{3} A_i^{\text{spr}} = 3 A^{\text{spr}} = \frac{1}{2} \sqrt{3} l_0^2$$

$$W^{\text{mic}} = \frac{3}{2} \sqrt{3} l_0^2 = \sqrt{3} k \left[ \frac{\delta}{l_0} \right]^2$$
4.3 network model for red blood cells

\[ W^{\text{mac}} = \frac{1}{2} \kappa \left[ \varepsilon_{xx} + \varepsilon_{yy} \right]^2 + \frac{1}{2} \mu \left[ \varepsilon_{xx} - \varepsilon_{yy} \right]^2 + 2 \mu \varepsilon_{xy}^2 \]

micro-to-macro kinematics
\[ \varepsilon_{xx} = \varepsilon_{yy} = \delta / l_0 \quad \varepsilon_{xy} = 0 \]

\[ W^{\text{mac}} = W^{\text{mic}} \]
\[ \frac{1}{2} \kappa \left[ \frac{\delta}{l_0} + \frac{\delta}{l_0} \right]^2 = \sqrt{3} k \left[ \frac{\delta}{l_0} \right]^2 \]
\[ \kappa = \frac{1}{2} \sqrt{3} k \]

4.3 network model for red blood cells

\[ W^{\text{mic}} = \frac{\sum_{i=1}^{3} W_i^{\text{spr}}}{\sum_{i=1}^{3} A_i^{\text{spr}}} \]
\[ \sum_{i=1}^{3} W_i^{\text{spr}} = \frac{1}{2} k \left[ 0 + \frac{1}{2} \delta \right]^2 + \frac{1}{4} k \left[ -\frac{1}{2} \delta \right]^2 + \frac{1}{4} k \delta^2 \]
\[ \sum_{i=1}^{3} A_i^{\text{spr}} = 3 A^{\text{spr}} = \frac{1}{2} \sqrt{3} l_0^2 \]
\[ W^{\text{mic}} = \frac{1}{4} k \frac{\delta^2}{\sqrt{3} l_0^2} = \frac{\sqrt{3}}{6} k \left[ \frac{\delta}{l_0} \right]^2 \]

4.3 network model for red blood cells

\[ W^{\text{mac}} = \frac{1}{2} \kappa \left[ \varepsilon_{xx} + \varepsilon_{yy} \right]^2 + \frac{1}{2} \mu \left[ \varepsilon_{xx} - \varepsilon_{yy} \right]^2 + 2 \mu \varepsilon_{xy}^2 \]

micro-to-macro kinematics
\[ \varepsilon_{xx} = 0 \quad \varepsilon_{yy} = 0 \]
\[ \varepsilon_{xy} = 0 \]

\[ W^{\text{mac}} = W^{\text{mic}} \]
\[ 2 \mu \left[ \frac{1}{\sqrt{3} l_0} \right]^2 = \frac{\sqrt{3}}{6} k \left[ \frac{\delta}{l_0} \right]^2 \]
\[ \mu = \frac{1}{4} \sqrt{3} k \]