Case studies in the context of disease states

1. P. Falciparum malaria

- Red blood cell (RBC) - merozoite
- 7-8 μm
- Optical tweezers: changes in RBC deformability
- Large deformation stretching of healthy and infected cells
- Healthy cells undergo 80% stretch
- Infected cells cannot deform

Biochemistry: PFEMP, KAHRP, RESA

- Phospholipid bilayer anchored to spectrin network by some proteins.
- RESA - spectrin, KAHRP bound to ankyrin & actin: known interactions.
- How are biochemistry & mechanics related?

- Is the increase in stiffness due to change in membrane or presence of granular particles from infection?

\[ m_1 > m_2 > m_3 \]

Knock-out \((m_3)\) experiments:

- From mechanics to biochemistry and gene inactivation

RESA (green) parasites (blue/purple) band 3 = red blood cells (red)
if \( \Delta \text{RESA} \): purple, but no more green.
the presence of RESA protein significantly stiffens the infected cell
\[ \mu_2 > \mu_1, \quad \mu_3 \approx \mu_1 \quad \text{and} \quad \mu_4 > \mu_1 \]
in-plane shear modulus: from \( \sim 8 \) to \( \sim 15-20 \mu \text{N/m} \)
(J.P. Mills, M. Diez 2006)
- now known what specific site on spectrum RESA binds
- RESA (ring-stage erythrocyte surface antigen) affects stiffness in ring stage
RESA's effects most dominant during febrile episodes (41°C) (not trophozoite)

2. Effect of SPC-induced reorganization of keratin network in human epithelial Panc-1 cells
- Pancreatic cancer has high mortality, hard to detect.
- Sphingosylphosphorylcholine (SPC) induced keratin rearrangement in cancer cells (fluorescence)
collapse in perinuclear region within 1 hour
- how are single cell mechanical properties affected?
  increased propensity to metastatic invasions of cancer cells?
  microplate experiments:

- Lyosphosphatiideic acid (LPA) promotes actin stress-fiber formation: no effect
  on cancer cell deformability (stiffness and lack of hysteresivity conserved)
- circumstantial evidence for role of single cell mechanical properties in cancer progressi"
Membrane & cytoskeleton mechanics

What are the primary structural elements in the cell?
How do cells interact with their environment?
How do cells generate force?
How do cells migrate?

- cell simplified by membrane + cytoskeleton + nucleus
  but more complex, heterogeneous, crowded in reality!
  neutrophils present microvilli, and a dense cortex of actin, with fluid inside.
- the cytoskeleton is made of actin microfilaments: 7-9 \text{um} \phi, 15 \text{um} l_p
  microtubules: 25 \text{um} \phi, 6000 \text{um} l_p
  intermediate filaments: 10 \text{um} \phi, (less dynamic family)
  persistence length, bending stiffness and Young's modulus

\[ l_p = \frac{k_B}{k_B^T} \]
\[ K_B = E I = E \frac{\pi}{4} a^4 \]
\[ E \sim \text{GPa} ! \]

- the membrane is a lipid bilayer (2 leaflets of hydrophobic tails / hydrophilic heads)
  lipids can organize into micelles, bilayers or liposomes.
  glycolipids, phospholipids, and proteins are anchored in membrane
  fluid mosaic model: molecule freely diffuse within membrane, and membrane sheets easily

- forces are transmitted through membrane by adhesion receptors (anchored in both ECM and cells are not passive, they can generate force (muscles), cytoskeleton)
  cells are dynamic: can change their modulus in seconds and become activated (migrate)
  example of neutrophils deforming in capillaries / microchannels

- membrane deformation and moduli

\[ N = \frac{E h}{2(1-\nu^2)} \frac{\Delta A}{A_0} = K_e \frac{\Delta A}{A_0} \]
\[ M_x = -\frac{E t}{12(1-\nu^2)} \frac{\partial^2 u_3}{\partial x_2^2} \]
\[ N_{12} = \sigma_{12} h = 2G e_{12} \]
\[ N_{12k} = K_B \frac{\partial^2 u_3}{\partial x_2^2} \]

\{ N: surface tension
  \tau or h: thickness of membrane}
Derive governing equations for linear deformations and the reduced forms for bending or tension dominance.

\[
\frac{K_B}{N\lambda^2} \gg 1 \quad K_B \left( \frac{\partial^4 u_1}{\partial x_1^4} + 2 \frac{\partial^4 u_3}{\partial x_1^2 \partial x_2^2} \right) \quad \text{etc} \quad \gamma 
\]

- cell peeling experiments to measure \( K_B \approx 10^{-18} \text{ N m} \)
- micropipette aspiration experiments to characterize viscoelastic responses.

But membranes are more complicated than a mere sheet: liquid vehicles exhibit fluctuations - dominated (entropic) regime and an elastic (enthalpic) regime when inflated.

\[
\frac{\Delta A}{A_0} \approx \frac{k_B T}{8 \pi K_B} \ln \left( \frac{N^2 K_B}{\pi^2 K_B} \right) + \frac{N}{K_e}
\]

- cell adhesion and membrane receptors:
  - integrins to form tight junctions
  - cadherins
  - N-CAM
  - selectins
  - gap junctions (signaling)
  - hemi-deshmosomes

Measure (adhesion): patterned deformable substrates
- force generation: on pillars/substrates
- on bead polymer gel (fluorescent markers = displacement)

Observe size of focal adhesions, \( \sigma_{FA} \approx 5 \text{ kPa} \) \( \gg \) shear flow regardless of size of FA.

- cell adhesion and the rolling leukocyte: Bell equation = rate for unbinding if

\[
k_r = k_r^0 \exp \left( \frac{-f}{k_B T} \right)
\]

- different measurement techniques cover different orders of magnitude in force/displacement
- magneto-chemometry
- optical tweezers
- magnetic trap
- atomic force microscopy
- substrate deformation
- embedded particle tracking
- micropipette aspiration
- ...
Mechanical properties of the cytoskeleton

Books:  
- Muscle reflexes and locomotion  
- Biological physics  
- Molecular driving forces  
- Mechanisms of motor proteins and cytoskeleton  

T. McMahan  
P. Nelson  
Bill & Bromberg  
J. Howard

- What are the physical laws governing elasticity, contraction, remodeling?  
  that play roles in adhesion, spreading, crawling, invaginating

22,000 human genes, 100,000 proteins, intricate connectivity, maps

How do we understand such complexity?  
- Splitters: normative reductionist biology  
- Lumpers: seek integrative unifying networks

- systems biology: complete parts list, detailed interactive maps insufficient to predict integrative function?

\[
\begin{align*}
\text{CSK} & = ? \\
\text{mechanism} & = ? \\
\text{protein-protein interactions} & = ?
\end{align*}
\]

* can we establish laws?

Paush & Kroger, Nature 2006: A bottom-up approach to cell mechanics

- Portrait of CSK elasticity, contraction and remodeling:

  4 hallmarks: soft & shear-thinning  
  scale-free dynamics  
  aging & rejuvenation  
  hopping, intermittency

# 1 - in the stiffness universe, \( E \approx 16 \text{ kPa} \) for actin, \( E \approx 10 \text{ Pa} \) for actin gels!

explanations: volume fraction (dilution), bending (not only stretching), non-affine relations, prestress (exist in cells, not in actin gels)

Phillips & Quake, Physics Today 2006: in biology, a confluence of energy scales

covalent, ATP hydrolysis, hydrogen bonds, thermal energies \( \sim 10^{-18} \text{ to } 10^{-20} \text{ J} \)

all terms come together in active biology

biology is very crowded! densely packed (not dilute) space
what is the stiffness of biology? Young’s modulus \( E = \frac{F}{A} = \frac{\text{ATP hydrolysis energy}}{\text{volume}} \)

stress in biology? traction microscopy
measure deformation of substrate to infer forces exerted
the cell is in a state of tension everywhere
stiffness controlled by prestress (tension) in cells

Butler AJT ’02, Stamenovic JAP ’04, Gandol PRL’06
speculation of tensegrity
Kumar BJ ’06 laser nanoscissors cut stress fibers

#2 Magnetic twisting cytometry: creep compliance

\[ E(t) \]

\[ \tau \sim 1s \]

to explain experimental data, fit parameters

but if you cover 5 orders of magnitude in frequency,
slow dynamics
  \{ creep \( t^{x-1} \sim t^{0.2} \)
  approach to equilibrium slower than exp.
  no instantaneous elasticity
  no distinct relaxation times

\[ G'(\omega) \sim G_0 \left( \frac{j \omega}{\omega_s} \right)^{x-1} + j \omega \mu \]

structural damping law

Alcaraz BJ ’03, Smith BJ ’05: AFM,
Desprat BJ ’05: microplates, Fabry: MTC

also conformation of nucleus
2-point microchemistry Hoffmann: MAS2
Cell biomechanics - 7.

\[
\begin{align*}
G' & \sim x^{-1} \\
G & \sim x & (G^* \sim j \omega x^{-1}) \\
\end{align*}
\]

- slope \( x \) plays a key role in dynamics
- \( x = 1 \) Hookean solid
- \( x = 2 \) Newtonian fluid

... non universal exponent, has "temperature-like" properties
... glass transition theory.

- semi-flexible polymers:
- what accounts for the behavior of gels? active gels \( K_B \) stretching stiffness
- \( l_p = \frac{K_B}{k_B T} \) persistence length
- \( l_p \sim 17 \text{um} \) not very flexible! \( (l_p \sim 50 \text{nm}) \)

- elasticity: "mechanical" spring constant at zero temperature \( K_M \sim E a^2 l_p^{-1} \)
- "thermal" spring constant at finite temperature \( K_T \sim a^2 l_p^{-1} \)
- which dominates? softer spring will dominate even if \( l < l_p \)

- thermal effects can dominate even if \( l < l_p \)
- dynamics: \( \rho \) density of filaments, \( a \) radius, \( \zeta \) drag coefficient
- \( G^*(f) \sim (\rho K_B l_p + 15) \cdot (4 \pi \zeta \Pi f + K_B) \)
- observed in cells as well.
- at high \( f \) frequency.

slope in gels 3/4

\#3 
- aging and rejuvenation
  - molecule in an energy landscape (that describes all possible molecular configurations)
  - to remodel, the system must overcome energy barriers (Boltzmann, Eyring processes)
    - \( E \gg k_B T \), rearrangements cannot be driven by \( k_B T \) only
    - kinetics would progressively slow down \( \rightarrow \) aging
    - no steady state, trapping in deeper wells
  - but ATP hydrolysis in biology \( \sim 25 k_B T \) \( \Rightarrow \) rejuvenation
  - shearing stress can give energy