Lecture: “Cytoskeleton dynamics simulation of the red blood cell” by Ju Li.
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Cytoskeleton dynamics simulation of the red blood cell

Ju Li

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One spectrin tetramer has 39 segments, contour length \( \sim 200 \text{ nm} \).

Room-temperature length \( \sim 80\text{nm} \) due to thermal fluctuations.

Spectrin Elasticity

one segment $\sim 5$ nm

One spectrin tetramer has $\sim 40$ segments, contour length $\sim 200$ nm. Room-temperature length $\sim 80$ nm due to thermal fluctuations.
Worm-like Chain Coarse-Grained Free Energy

\[ V_{\text{WLC}}(L) = \frac{k_B T L_{\text{max}}}{4b} \cdot \frac{3x^2 - 2x^3}{1-x}, \quad x \equiv \frac{L}{L_{\text{max}}} \]
Spectrin-Net Level, Whole Red Blood Cell model (Discher, Boal, Boey, 1998)

\[ V_{\text{total}} = \sum_{i \text{ spectrin link}} V_{\text{WLC}}(L_i) + \sum_{\alpha \text{ triangle}} \frac{C}{A_{\alpha}} + \sum_{\beta, \gamma \text{ triangle}} K_{\text{bend}} (1 - n_{\beta} \cdot n_{\gamma}) \]

+ total volume constraint + total area constraint
Small Cell Simulation
(“volume quench” to get discocyte shape)

\[ \sim 2 \text{ \(\mu\)m} \]

2562 vertices
Stomatocyte - discocyte - echinocyte Sequence

spontaneous curvature parameter

Fig. 2. A sample of observed non-main-sequence shapes, including (top to bottom) nonaxisymmetric discocyte, stomatocyte with triangular mouth, and knizocyte. (Left) Laboratory images reproduced with permission from refs. 27 (Copyright 1981, Biophysical Society), 32 (Copyright 1990, Academic Press), and 2 (Copyright 1973, Springer). (Right) Minimum-energy shapes calculated from our model with values of $\kappa_0$ and $\Delta\kappa_0$ of 0.989 and 0.215%, 0.950 and −0.658%, and 1.000 and 1.144% (from top to bottom) with all other parameters remaining fixed.

required to make them conform. The shape–free–energy functional that incorporates these two effects is

$$F_{\text{ADE}}[S] = \frac{\kappa_0}{2} \int_S dA (2H - C_0)^2 + \frac{\kappa}{2AD} (\Delta A - \Delta A_0)^2,$$

where $D$ is the membrane thickness, $A$ is the membrane area, $\kappa_0$


Stomatocyte–discocyte–echinocyte Sequence

spontaneous curvature parameter

Courtesy of National Academy of Sciences, U. S. A. Used with permission.
Icosahedral network on a sphere
**Geometrically Necessary Disinclinations**

If each carries disinclination charge $60^\circ$, need 12
100% volume
60% volume
To rid of the shape artifacts, melt and quench the network.

GBs freely terminate!
These GBs should be widespread in nature: large viral protein capsids, giant spherical fullerenes, spherical bacterial surface layers, siliceous skeletons of spherical radiolaria (aulosphaera), etc. Sites for chemical reactions, initiation points for bacterial cell division, influence the mechanical response.

Material reference state
for the in-plane shear energy $E_{\text{shear}}$

60% volume: *spherical state* as stress-free reference.
W/ experimental range of parameters and sphere as stress-free reference state, the biconcave shape is only metastable at 60% volume.
With bending energy $E_{\text{bend}}$ only
Canham (1970)
Helfrich (1973)
Optical Tweezers Stretching Simulation
Cross Sectional View
$200 \text{pN} \times 8 \mu\text{m} / 2 = 5000 \text{eV}$!
Why is biconcave the stable equilibrium shape?

\[ E_{\text{bend}} \sim 8\pi \kappa: \quad \kappa \sim 2 \times 10^{-19} \text{ J} \quad \rightarrow \quad E_{\text{bend}} \sim 30 \text{ eV} \]

\[ E_{\text{shear}} \sim \mu \varepsilon^2 A: \quad \mu \sim 8 \mu \text{N/m}, \quad \varepsilon \sim 0.1, \quad A \sim 140 \mu \text{m}^2 \quad \rightarrow \quad E_{\text{shear}} \sim 70 \text{ eV} \]
Material Concept Hypothesis

• In an ideal limit, for any RBC shape, the cytoskeleton will always undergo remodeling in topological connectivity at a slow rate to relax its in-plane *shear* elastic energy to *zero*.

“liquefaction”, “slow-flowing glass”

• At the timescale of optical tweezers stretching, the above relaxation is not significant, so large shear energy can be injected temporarily.
Stillinger-Weber liquid on curved surface:

no shear energy can survive long!
RBC cytoskeleton at reduced spectrin density

very large holes start to percolate ...
## Extreme Statistics of Cytoskeletal Defects in RBC

<table>
<thead>
<tr>
<th></th>
<th>actin#</th>
<th>spectrin#</th>
<th>largest polygon hole</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal</td>
<td>28673</td>
<td>81718</td>
<td>6</td>
</tr>
<tr>
<td>degree-4.5</td>
<td>26880</td>
<td>57523</td>
<td>8</td>
</tr>
<tr>
<td>degree-4</td>
<td>24372</td>
<td>48012</td>
<td>11</td>
</tr>
<tr>
<td>degree-3.5</td>
<td>21504</td>
<td>37416</td>
<td>22</td>
</tr>
<tr>
<td>degree-3</td>
<td>18637</td>
<td>26837</td>
<td>35</td>
</tr>
</tbody>
</table>

But this is basically from a “geometrical” simulation
no biophysical basis, yet.
Intermediate Summary

• Spectrin-level and continuum FEM analyses indicate our optical tweezers experiments give approximately the same in-plane shear modulus as micropipette aspiration experiments: \( \mu = 5 \text{ to } 10 \times 10^{-6} \text{ N/m} \).

• Stabilization of biconcave equilibrium shape strongly suggests the cytoskeleton undergoes slow but constant remodeling topologically to always relax the in-plane shear elastic energy to zero.

• Connection to single-molecule stretching experiments ("intermolecular potential development").
CGMD model with *breakable* actin-spectrin junction

\[ \kappa_{\text{bare}} = 2 \times 10^{-20} \text{ J} \]

\[ F = \alpha \frac{4\pi\kappa_{\text{bare}}}{3r_0}, \quad \alpha \text{ chosen to be 0.36} \]

approximate A–A nearest neighbor repulsion due to membrane
We also put soft \((0.1k_{BB})\) confinement potential on A and B in \(z\) to mimic interaction with the membrane without actually simulating the membrane.
temperature

system size

pressure
Pure shear deformation at 300K and strain rate $3 \times 10^5$/s
Stress-strain curve at 300K and no ATP

- ~8μN/m
- ~30μN/m
Defect statistics at 300K with no ATP
A broken link 5-fold defect
Corrugation due to buckling: elevated / depressed in height
Now add ATP (0.5eV random kinetic energy to green ball):
hit rate = 100/\mu s per spectrin end
Defect statistics at 300K, ATP hit rate 100/µs
Now turn off ATP hits, "anneal" at 300K...

Miraculously, the system recovers, within CGMD simulation timescale.
A more reasonable ATP hit rate: 10/µs. Simultaneously, also shear deform.

ATP hit rate = 10/µs/spectrin, strain rate = 3× 10^5/s

shear stress [µN/m]

engineering shear strain

~3.4µN/m

completely fluidized
ATP hit rate = 10/µs
ATP hit rate = 1/µs:

ATP hit rate = 1/µs, strain rate = 3×10^5/s

- plastic displacement burst
- initial slope ~ 8µN/m
ATP hit rate = 1/µs
ATP hit rate = 2/µs:
two plastic displacements… also longer

ATP hit rate = 2/µs, strain rate = 3 \times 10^5 /s

initial slope
still 8\mu N/m!
ATP hit rate = 5/µs:
large-strain resistance collapses, manifest global yield
Schematic model of the red cell membrane, with the vertical and horizontal interaction of its components indicated. Estimated frequencies of mutations in different membrane proteins in HS and HE/HPP are as follows. Vertical interaction: hereditary spherocytosis: band 3, ~20%; protein 4.2, ~5%; ankyrin, ~45%; spectrin, ~30%. Horizontal interaction: hereditary elliptocytosis/hereditary pyropoikilocytosis: β spectrin, ~5%; α spectrin, ~80%; protein 4.1, ~15%. The relative position of the various proteins is correct, but the proteins and lipids are not drawn to scale.

Figure by MIT OCW. After Tse and Lux, 1999.

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Coarse Grain Molecular Dynamic Modeling
Shear Deformation

![Graph showing shear stress vs. engineering shear strain with a linear relationship and a marker for 10 μN/m and ~25 μN/m.]

- 10 μN/m
- ~25 μN/m
Shear Deformation and Promoted
Dimer – Dimer Dissociation

ATP hit rate 10/µs/spectrin, strain rate = 3 x 10⁷/s

Plastic displacement burst

~10 µN/m

ATP hit rate 50/µs/spectrin, strain rate = 3 x 10⁷/s

Fluidized

~10 µN/m
Summary

• A minimal CGMD model with *breakable* actin-spectrin junction has been developed, with physically reasonable parameters and behavior.

• ATP hydrolysis is modeled as stochastic kinetic energy transfer. As ATP hit rate rises, we see initiation of plastic displacement excursions, followed by macroscopic yield, and eventually, complete fluidization.

• Practical timescale of CGMD able to simulate recovery.