Two-Dimensional Cytoskeletons Under Stress

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Plumlar triangular networks under stress are predicted to have several interesting properties: a first-order transition to a collapsed state for a range of compressive stresses, and a negative Poisson ratio for a range of tensions (i.e., they expand transversely when stretched longitudinally). When these two-dimensional nets are allowed to fluctuate in three dimensions, they are predicted to be asymptotically rigid at long length scales and to have a universally negative Poisson ratio, even at zero stress (reviewed in Boal, 1996). There are many examples of two-dimensional networks in nature: auditory outer hair cells (Tolomeo et al., 1996) and bacterial cell walls (Ghuysen, 1968) contain few or many layers of networks with square or honeycomb symmetry. Further, not all networks are isotropic: the peptidoglycan network of the bacterial cell wall is anisotropic in the network plane, being stiff in one direction but soft in the other.

One well-studied network is the membrane-associated cytoskeleton of the human red blood cell—a two-dimensional network whose elements are tetramers of the protein spectrin. Although the contour length of a spectrin tetramer is approximately 200 nm, the average separation between the sixfold junctions linking the tetramers is closer to 70 nm (Steck, 1989). Thus, one picture of the erythrocyte cytoskeleton is that of a triangular network of convoluted chains, as shown by the simulation in Figure 1. By mechanically manipulating the erythrocyte, measurements can be made of the shear modulus $\mu$ and compression modulus $K_s$ of its cytoskeleton in the lipid bilayer plane to which the network is attached (Discher et al., 1994).

Although the cytoskeleton chains appear convoluted in the simulation, the chain junctions (the white disks in Fig. 1) fluctuate only slightly around their mean positions. Indeed, the junctions in the simulation behave like those of a spring network with a reduced temperature of $k_B T/K_s S_0^2 = 1/30$, where $k_B$ is Boltzmann's constant, $K_s$ is the network spring constant, and $S_0$ is the equilibrium spring length. At low temperature, the elastic moduli of such a network are $\mu/K_s = \sqrt[3]{3} (1 - \sqrt[3]{3} P/K_s)/4$, and $K_s/K_p = \sqrt[3]{3} (1 + P/(\sqrt[3]{3} K_p))/2$, where $P$ is the in-plane pressure, defined to be negative for networks under tension. These expressions are in rough agreement with experiment if $K_p$ is estimated from the properties of polymer chains. When stretched, the erythrocyte cytoskeleton is predicted to lie close to the bilayer plane and to restrict

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Figure 1. Polymer chain model of the erythrocyte cytoskeleton. The large white disks indicate the locations of the sixfold junction vertices of the chains.
the motion of membrane proteins that extend significantly into the cytoplasm. As shown in Figure 2, membrane proteins that are otherwise freely diffusing may become restricted to localized "corral"s because of their repulsive interactions with the cytoskeleton.

Biological networks contain defects that may alter the mechanical properties from those of networks with perfect triangular, square, or honeycomb symmetry. For example, while a network whose connectivity is sixfold on average may have near-ideal properties, bond-depleted networks may be weak to the point of failure (Mohandas and Evans, 1994). Percolation theory has provided a qualitative description of how the elastic moduli decrease as the average connectivity of the network decreases (reviewed in Saxton, 1990).

**Literature Cited**


**Discussion**

*Taylor*: Could you clarify what you mean by low temperature in relationship to spectrum?

*Boal*: If you look at the motion of the nodes rather than the flexible chains and follow the movement of those nodes, the rms (root mean square) dispersion in the position of these nodes resembles motions at low temperature. The energy scale in this system is provided by $K_p S_0^2$, where $K_p$ is the effective spring constant of the network and $S_0$ is the equilibrium spring length. In these units, the temperature $kT$ is equal to 1/30, which is very low.

*Schwartz*: I want to see if I understand the implication of your model. When the cytoskeletal network is under stress, and the density of sites restricting diffusion increases, you would predict that molecular diffusion would slow. On the other hand, if molecules were confined in a restricted area, would reaction rates increase by stretching the network?

*Boal*: Yes, there is an increase in the local density of proteins, and hence there would be an increase in the reaction rates.

*Schwartz*: In principle, you could effect signaling by molecules that are not actually attached to the cytoskeletal network?

*Boal*: Yes. Let me comment on diffusion. There are two effects in Figure 2: the network is stretched out compared to the equilibrium configuration, so the overall protein density is lower; however, the proteins are concentrated in corrals, so their local density may be higher. One can expect that some effect would arise from the stretching of the network alone. The corralling phenomenon is real.

*Schwartz*: For those of us who think of signaling molecules as being attached to those networks, that is an interesting implication.

*Boal*: If these molecules are attached to the net, they are
PHYSICAL PROPERTIES OF THE CYTOSKELETON

going to spread out more. On the other hand, if they are
corralled, they will bump into each other frequently.

STEWART: I want to follow up on Ed Taylor’s question. In
your equation $\beta K_\mu S_\mu^2 \sim 30$, what are the units you used? Is
the spring constant ($K_\mu$) in that expression on the order of $kT$?
Or, depending upon the units, is it much less than $kT$, perhaps
two or more orders of magnitude less?

BOAL: $K_\mu$ and $kT$ have different units. $K_\mu$ is in joules/square
meter, so one must use an appropriate length scale to make $K_\mu$
and $kT$ comparable. The product $K_\mu S_\mu^2$, which is an energy, is
30 times $kT$. The compression modulus $K_\mu$ and the shear modu-
lus $\mu$ are both within a factor of two of $K_\mu$.

STEWART: If we applied the sort of energy involved in $kT$
to the system, would this produce a large or small change in
terms of the difference between nodes?

BOAL: A small change. Basically, the nodes are vibrating
slowly, although the chains themselves are oscillating
wildly.

GUNDERSEN: I am very interested in the effect of stretching
on the potential coralling of molecules. When vesicles pinch
off from membranes—for example, in the flow of proteins
from endoplasmic reticulum to Golgi dictendum—such a coralling
of molecules may occur. I'm wondering if you have any comments
on this?

BOAL: I cannot comment on that in my own research, but I
am familiar with experiments on rat kidney cells. These
cells show a strong tendency to form corals or domains. The
domains are typically 500–700 nm, reflecting the fact that the
cytoskeletons in these kidney cells are presumably much looser,
or of a much larger scale system, than in the erythrocyte. How-
ever, similar measurements of domain size in erythrocytes are not
possible because the size of the beads used in these experi-
ments is comparable to the domain size in the erythrocyte.

GUNDERSEN: With respect to the pinching off of vesicles,
proteins on the vesicles may actually be affecting the clustering
phenomenon.

MACKINTOSH: Although your talk focused primarily on spec-
trin networks, you also mentioned anisotropic stresses. Can you
look at anisotropic stresses in the lamellopodium?

BOAL: Not yet. We have done some general work on aniso-
tropic stresses. The statistical mechanics have not been suffi-
ciently investigated and, before studying biological systems,
that is where my laboratory has been focusing. In principle,
there is no reason why we cannot study these stiffer, longer
systems, such as the lamellopodium.

MACKINTOSH: Several people have suggested that you can
create defects in polymer networks, removing cross-links and
enhancing the modulus, without weakening the material. These
are rather special cases, yet they are supported by simulations.
This is a fundamental property of entropic elasticity.

BOAL: There has been a lot of work on generic changes to
the triangulation of triangulated nets; for example, having five-
fold and sevenfold coordinated sites. This produces modest
changes in the modulus, but not the huge differences seen when
the nets are depleted.

MACKINTOSH: The examples that I'm thinking of are net-
works that have zero shear modulus at zero temperature, like a
square lattice.

INGBER: Studies on lipid domains and stretch-activated ion
channels are looking for the type of information that you have.
It might be interesting to see how your kinetic phenomena
match up with some of those channel systems. In the type of
experiments you have described, it seems that most investiga-
tors pull on the outer curvature of the red blood cell. Does the
dimple in the middle of the cell have the same mechanical properties
as the outer rim?

BOAL: I do not think they differ at all. Even in our studies,
there is a slightly different average connectivity at the edges
compared to the center. But when you inflate the cell first and
then pull, there are no differences. A question would be, has
the cytoskeleton relaxed during the inflation process such that
an initially inhomogeneous connectivity has relaxed away?

INGBER: There must be some prestress or internal stress, to
maintain that kind of curvature.

BOAL: If we compare our stretched cytoskeletons with aspira-
tion experiments (involving huge deformations), we have to
add some prestress to the stretched cytoskeletons, in order to
get better agreement.

SHAPIRO: You cited discrete percolation theory, but from
what I saw in your picture of this network (Fig. 1), it does not
appear to be discrete. Did you try to simulate that (Boal: That
is not my work.) with a continuous percolation model?

BOAL: Mike Saxton, at University of California—Davis, has
looked at a variety of percolation models. As I recall, in no
cases did the predicted value of the shear modulus agree with
the experimentally observed value for spectrin-depleted eryth-
rocytes. This may just mean that percolation theory can’t be ap-
plied to this system because of the structure of the spectrin
network. For example, connectivity in spectrin-depleted red
blood cells may be different from that in the normal blood
cell. However, there may be some experimental bias in these
measurements. When researchers collect the samples on which
to conduct the aspiration, they select blood cells where they
can attach the micropipette onto the surface. Even though the
sample has a global average spectrin content, the specific cells
chosen for investigation may not have the same spectrin content
as the global average.