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Two-Dimensional Cytoskeletons Under Stress

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Planar 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 μ and compression modulus K_a of its cytoskeleton in the lipid bilayer plane to which the network is attached (Discher *et al.,* 1994).

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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 $k_BT/K_{sp}S_o^2 = 1/30$, where k_B is Boltzmann's constant, K_{sp} is the network spring constant, and S_o is the equilibrium spring length. At low temperature, the elastic moduli of such a network are $\mu/K_{sp} = \sqrt{3}\,(1 - \sqrt{3}\cdot P/K_{sp})/4$, and $K_a/K_{sp} = \sqrt{3}\,(1 + P/|\sqrt{3}K_{sp}|)/2$, 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_{sp} 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

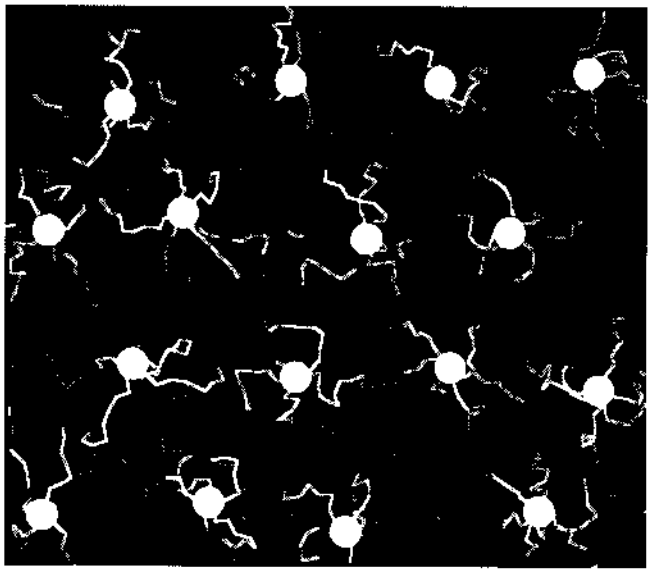

Figure 1. Polymer chain model of the erythrocyte cytoskeleton. The large white disks indicate the locations of the sixfold junction vertices of the chains.

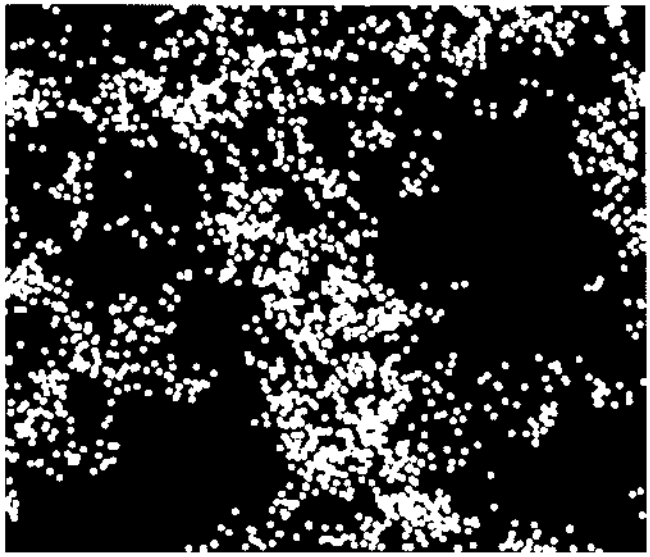

Figure 2. Simulation of randomly diffusing proteins in the bilayer plane, showing locations of the proteins separated by constant time intervals. The proteins are segregated into corrals by their interaction with the cytoskeleton.

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 "corrals" 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).

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Discussion

TAYLOR: Could you clarify what you mean by low temperature in relationship to spectrin?

BOAL: If you look at the motion of the nodes rather than the floppy 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 $K_{sp}S_o^2$, where K_{sp} is the effective spring constant of the network and S_o 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

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 $\beta K_{sp} S_o^2 \sim 30$, what are the units you used? Is the spring constant (K_{sp}) 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_{sp} and kT have different units. K_{sp} is in joules/square meter, so one must use an appropriate length scale to make K_{sp} and kT comparable. The product $K_{sp}S_o^2$, which is an energy, is 30 times kT. The compression modulus K_a, and the shear modulus μ, are both within a factor of two of K_{sp}.

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 around slowly, although the chains themselves are oscillating wildly.

GUNDERSEN: I am very interested in the effect of stretching on the potential corralling of molecules. When vesicles pinch off from membranes—for example, in the flow of proteins from endoplasmic to Golgi reticulum—such a corralling 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 normal 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. However, similar measurements of domain size in erythrocytes are not possible because the size of the beads used in these experiments 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 spectrin 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 anisotropic stresses. The statistical mechanics have not been sufficiently 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 fivefold and sevenfold coordinated sites. This produces modest changes in the moduli, but not the huge differences seen when the nets are depleted.

MACKINTOSH: The examples that I'm thinking of are networks 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 investigators 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 aspiration experiments (involving huge deformations), we have to add some prestress to the stretched cytoskeletons, in order to get better agreement.

SHAFRIR: 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 erythrocytes. This may just mean that percolation theory can't be applied 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.