New and Notable

Modeling Nonlinear Red Cell Elasticity

Udo Seifert
Max-Planck-Institut für Kolloid und Grenzflächenforschung, Teltow, Germany

The remarkable elasticity of red cells shows up under a variety of conditions both *in vivo* and in controlled experiments on single cells. Squeezed through narrow capillaries with diameters significantly smaller than the cells themselves, they perfectly recover their biconcave resting shape countless times during their life cycle of about 100 days in the circulatory system. Likewise, this normal biconcave shape undergoes drastic transformations if the physiochemical conditions are changed by, for example, cholesterol depletion, which causes a transition from discocytes to cup-shaped stomatocytes. All these deformations imply both nonlinearity and anisotropy, because the intermediate or final shapes differ substantially from the initial biconcave rest shape. Micropipet aspiration has become the paradigmatic method for the controlled study of strong deformation.

How can the elastic behavior of the red blood cell in these conditions be understood quantitatively on the basis of the molecular architecture of its compound membrane? This question has intrigued biophysicists for nearly 30 years. Based on a body of earlier work, Discher, Boal and Boey have now made big progress in modeling the erythrocyte membrane, as reported in two companion papers in this issue (Boey et al., 1998; Discher et al., 1998). Their success consists in exploiting and finally resolving both the structural duality and the dichotomy of scales of the red cell.

The structural duality resides in the membrane's two-component structure consisting of the lipid bilayer to which a protein network is attached. Clearly the influence of the spectrin net on elasticity is profound, in particular because it endows the cell with some shear resistance. Investigators pursuing a reductionist approach have systematically studied the bare fluid membrane elasticity during the last ten years both theoretically and experimentally by using giant vesicles. These studies have shown that a large variety of shapes and shape transformations can be explained simply on the basis of curvature elasticity and constraints on area and volume (Seifert, 1997). On the other hand, the same studies have also revealed that this simple one-component model membrane lacking the network does not form anything like the spicules of echinocytes.

Network elasticity is much harder to model for strong deformations such as those encountered in aspiration. Continuum models have two related weaknesses (Elgsaeter and Mikkelsen, 1991). First, nonlinear elasticity is difficult to implement without introducing some arbitrariness. Second, the contribution of thermal fluctuations to the configurations of the network can be incorporated easily only on the linear level. On the other hand, computer simulations of discrete networks have been devoted to almost planar configurations with a few exceptions concerning polymerized vesicles (Gompper and Kroll, 1997).

Exploiting the dichotomy of scales between the mesh size of about 70 nm and the scale of the cell about 100 times larger, Discher and co-workers have successfully modeled a whole cell undergoing strong deformation using a two-step simulation approach. First, they simulated an almost planar two-dimensional network modeling each spectrin strand between the junctions as a polymer with about 10 to 20 monomers. Such simulations yield area-pressure characteristics and elastic parameters even for the nonlinear regime where steric interactions between the strands (for strong compression) and finite extendability (for strong stretching) become important. Although it would be desirable to use this model for the whole cell, the computational cost of such a full-scale simulation is still prohibitive. The clever idea of Discher and co-workers is to use these small-scale simulations to calibrate parameter values of a coarse-grained model for the network, keeping only the junctions which interact through two and three body potentials. The functional form of these potentials is chosen to reproduce the elasticity of the more detailed model. In the second step, the network of junctions is put on a closed fluid bilayer membrane with rigidity, thus modeling the compound membrane. Simulation of the whole cell, e.g., in an aspiration experiment, is made possible through this significant reduction in the number of degrees of freedom.

Discher et al. have thus been able to bridge the gap between the mesh size scale and that of the whole cell. As a first illustration, they demonstrate the power of this quantitative approach by comparing the calculated network density profile in the aspirated tongue with that obtained from fluorescence imaging experiments. Such a comparison reveals that the cytoskeleton seems to be prestressed, i.e., the junctions are closer together than they would be in an isolated cytoskeleton. A possible origin of this effect could be the loss of bilayer material in the early stages of cell maturation.

In the future, Discher and colleagues' model (and ramifications still to be developed) can be applied to several interesting problems highlighted in the following incomplete list. First, even though breaking of axisymmetry has not been crucial in the aspiration experiment, this model can

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Address reprint requests to Udo Seifert, Max-Planck-Institut für Kolloid und Grenzflächenforschung, Kantstrasse 55, D-14513 Teltow, Germany. Tel.: 49-3328-46594; Fax: 49-3328-46212; E-mail: useifert@menkar.mpikg-teltow.mpg.de.

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be used to investigate distinctly nonaxisymmetric shapes such as echinocytes that would be difficult to study with continuum models. In such a study one should allow for the expected partial separation between network and bilayer. Second, in this model both the bilayer and the network are still homogeneous, whereas the bilayer membrane of real cells consists of various lipids and integrated proteins and the real network has a significant number of defects. How does this inhomogeneity affect and how is it affected by strong deformations? Such a question can be addressed by decorating this model with additional particles modeling the proteins and by introducing network defects. Depending on the interactions of proteins with both the membrane and the network, enrichment or depletion will occur in a manner sensitive to local curvature and network distortion.

Finally, an outstanding challenge will be to extend this approach to study of the dynamics of shape changes induced by external hydrodynamic fields such as shear or capillary flow. This will require incorporating long-range hydrodynamic interactions into the model, a task recently achieved in the simpler model system of fluid bilayer vesicles (Kraus et al., 1996). Discher and colleagues' model and its future refinements can take us closer to a quantitative understanding of the remarkable mechanochemical properties of the red blood cell.

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Run, Don't Hop, through the Nearest Calcium Channel

Richard Horn

Department of Physiology, Institute of Hyperexcitability, Jefferson Medical College, Philadelphia, Pennsylvania 19107, USA

At first glance ion permeation in calcium (Ca^{2+}) channels presents a collection of wildly contradictory facts (Tsien et al., 1987). In the absence of divalent cations this channel behaves like a perfectly respectable cation-selective channel that discriminates poorly among alkali metal cations, much like an acetylcholine receptor channel. Monovalent current, carried for example, by Na^+ ions, is blocked by submicromolar concentrations of Ca^{2+}, indicating a high affinity of the channel for Ca^{2+}. However, in physiological solutions, in which Ca^{2+} concentration is in the millimolar range, these channels select strongly ($\sim$1000:1) for Ca^{2+} over Na^+, and are capable of admitting a substantial Ca^{2+} influx despite the 100-fold higher concentration of extracellular Na^+. The high Ca^{2+} conductance indicates low free energy barriers for a Ca^{2+} ion to traverse the pore. But how is the channel capable of conducting $\sim 10^6$ Ca^{2+} ions/s if it binds Ca^{2+} so tightly? Moreover, how does raising the Ca^{2+} concentration switch off the high permeability to Na^+?

These paradoxes have intrigued biophysicists for the past two decades. The commonly accepted explanation for the above phenomena is that the Ca^{2+} channel is a single-file pore capable of binding at least two Ca^{2+} ions at discrete sites (Tsien et al., 1987). The first Ca^{2+} ion binds with high affinity, and thus is capable of blocking the current carried by monovalent cations, which have a much lower affinity for the open channel. The binding of a second Ca^{2+} ion reduces the affinity of both divalent cations for the pore, perhaps by electrostatic repulsion; and now the pore is less sticky for Ca^{2+} ions, which can therefore permeate readily. The selectivity of Ca^{2+} over Na^+ is a consequence of selective binding of the divalent cation. This heuristic account can be modeled quantitatively by a rate theory model of ions hopping from one binding site to the next over a small number of free energy barriers.

Enter PNP2 (Nonner and Eisenberg, 1998), and all of the sacred tenets of the rate theory description are called into question. With this Poisson-Nernst-Planck model, the successor of PNP0 and PNP1, there are no Ca^{2+} binding sites, there is no single filing, and the biophysical fingerprint of the Ca^{2+} channel is predicted under conditions in which the pore is occupied on average by less than one Ca^{2+} ion. Furthermore, the authors argue on physical chemical grounds that some assumptions underlying Eyring rate theory models of Ca^{2+} channels must be invalid.

Certainly the successes of this paper are stunning. Nonner and Eisenberg (1998) begin with a rather featureless permeation pathway composed of a central 6 Å $\times$ 10 Å "pore proper" with conical aqueous vestibules extending into the bulk solution. Ions flow through the channel, obeying laws of bulk electrodiffusion. Using five single-valued measurements (e.g., the maximum conductances of Ca^{2+} and Na^+ at high concentrations) from the Ca^{2+} channel literature, they are able to predict most of the published biophysical properties of Ca^{2+} channels under a wide variety of conditions (i.e., solution compositions and voltage). For example, the model predicts de novo 1) the "anomalous mole fraction effect" in which Ca^{2+} blocks at low concentration and permeates well at high concentration, 2) typical current-voltage relationships over a variety of ionic conditions, 3) the saturation of Ca^{2+} currents in the range of tens of mM, and 4) the voltage-dependent block of currents by protons. The ro-

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