A model of intracellular organization

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Almost everything we know about biological chemistry comes from experiments on dilute samples of macromolecules (proteins, DNA, RNA, polysaccharides, etc.). By "dilute," I mean macromolecular concentrations of 10 g per liter or less. Such conditions are astonishingly different from those inside living cells (1, 2). A cell's cytoplasm is crowded with macromolecules (Fig. 1), their total concentrations exceeding hundreds of grams per liter (3). In terms of total protein concentration, the inside of a cell is like egg white. However, the cytoplasm is not only crowded but also organized. An article in this issue of PNAS by Long *et al.* (4) describes a simple model system that mimics this organization.

Arthur Kornberg was among the first to grasp the practical importance of what we now call macromolecular crowding. He and his laboratory had been struggling for 10 years to make DNA replication work *in vitro* when they discovered in 1981 that a high concentration of a synthetic polymer, poly(ethylene glycol), sets the system in motion. The polymer mimicked the crowding found in intact cells and stabilized the binding of essential proteins to the origin of replication. Kornberg thought crowding so important that he made it one of the "Ten Commandments"—Thou Shalt Correct for Extract Dilution with Molecular Crowding (5).

In the 1980s, Steven Zimmerman showed the generality of macromolecular crowding effects, and Allen Minton (6) developed a theoretical framework to describe its effects. One of Zimmerman's discoveries—that adding poly(ethylene glycol) increases the efficiency of DNA ligation—is used every day by molecular biologists.

Descriptions of macromolecular crowding are based on two effects, excluded volume and binding. Let us use protein stability as a test case to illustrate these effects. Many globular proteins exist in only two thermodynamic states at equilibrium. The native state is biologically active and compact; much of its potential surface area is buried in its tightly packed interior. The denatured state is biologically inactive and exposes much more surface to the solvent than does the native state. Excluded volume is another way of saying "two entities cannot be in the same place at the same time" or "there is less free space in a crowded solution." The excluded volume effect stabilizes proteins because the lack of space in a crowded solution favors the more compact native state over the more open denatured state. The other effect, binding, can be stabilizing or destabilizing. If the crowding molecules interact more strongly with the native state, the protein is stabilized, and vice versa.

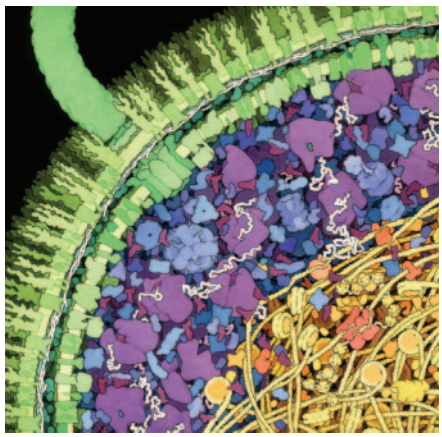

Fig. 1. A cross section of an *Escherichia coli* cell. Cell wall, concentric membranes, transmembrane proteins, and a flagellum with its motor are shown in green. Cytoplasm is shown in blue and purple. Nucleic acids are shown in yellow. (Reprinted with permission of David S. Goodsell, The Scripps Research Institute, La Jolla, CA.)

Given the ubiquity, utility, and detailed molecular descriptions of macromolecular crowding, it is remarkable that most biological chemists are only now beginning to realize the importance of studying biochemistry under crowded conditions (7, 8). Clearly, there is much to do, and most enlightened investigators are taking the straightforward approach by making biophysical measurements on proteins of interest in the presence and absence of high concentrations of some soluble protein or synthetic polymer (9–11) and even in living cells (12, 13). The article by Long *et al.* (4) raises the bar by confronting the organization of the cellular cytoplasm.

Biology exploits differential distribution to control chemistry inside cells.

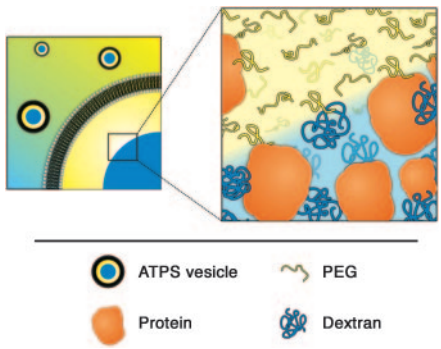

Fig. 2. An illustration of the model cell used by Long *et al.* (4). ATPS, aqueous two-phase system; PEG, poly(ethylene glycol).

The researchers start by noting that the inside of a cell is probably partitioned into different thermodynamic phases, analogous to the white and yolk of an egg. Such organization could have many functions (14); one recent in-cell study shows how organization might facilitate metabolism (15). Until now, however, it was unclear how to study cellular partitioning outside living cells. Long *et al.* (4) take a bottom-up approach by making a partitioned, artificial cell and showing that partitioning can affect the distribution of biological macromolecules.

Their model cell (Fig. 2) is like an egg, with a lipid-bilayer shell, a poly(ethylene glycol)-rich white, and a dextranrich yolk. They chose these aqueous polymer mixtures because the solution separates into two phases by changing the temperature or by changing the osmotic pressure outside the "shell" (for instance, by adding sugar). They prove the structure of these artificial cells by fluorescently labeling the components and visualizing the cells with microscopy. Unlike eggs, where scrambling the yolk and white is irreversible, the researchers prove the reversibility of their phase separation by using heat or osmotic stress to change conditions and

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using microscopy to show the appearance and disappearance of phase separation.

An important observation comes from spiking the inside of the artificial cell with proteins or DNA. The researchers find that these biopolymers are differentially distributed across the phases. That is, DNA or protein has a higher concentration on one side of the boundary than on the other. The reason for the difference is that the investigators designed the system so that one of the biopolymers interacts more favorably with one of the phases. For instance, they exploited the fact that dextran is made from sugars by adding a sugar-binding protein. This protein preferentially found its way to the dextran-rich phase. In another experiment, they modified some of the poly(ethylene glycol) with biotin and added a biotin-binding protein. This protein preferred the poly(ethylene glycol)-rich phase. Despite the system's simplicity, the concentration differential across the phases is a healthy 4- to 7-fold. If such differential distribution occurs in such a simple model, it seems likely that biology exploits this phenomenon to control the chemistry inside cells.

The differential distribution of solutes is important, but this effect occurs between phases even when they are not encased in a shell. The shell imparts two properties that break new ground and show off the properties of this cell mimic. First, the shell allows the differential distribution of solutes to be manipulated by changing the environment outside the shell. In one experiment, the distribution of single-stranded DNA was altered by using sugar in the external solution to suck some of the water out of the cells. Real cells probably use this property to detect and react to external changes in environment. The second property is the speed at which the distribution can be established. The model cell's small volume combined the large contact area between the phases allows an equilibration that requires hours to days without the shell to occur in minutes in the cell mimic. This ability to speed reaction is one of the reasons cells are the basic unit of life.

This work is not the last word in models, however. Comparing Figs. 1 and 2 shows that real cells are more complicated than the model. Also, the synthetic polymers used in the study are not found in real cells, and the investigators cannot exactly control the concentrations of materials inside their model. Nevertheless, this work by Long *et al.* (4) should be seen as a challenge to make more realistic models. For instance, it is easy to imagine models in which the level of activity of an enzyme is controlled in both space and time by altering the enzyme's distribution in a model cell.

Biochemistry has much to learn from polymer chemistry. This statement is especially true when it comes to understanding macromolecular crowding. The model cell architecture itself is reminiscent of core-shell polymer particles fabricated with different cargos on their interior (16). Macromolecular crowding also has analogies in polymer physics. For example, when the concentration of polymer segments in solution becomes comparable to the concentration of segments on the interior of an isolated "random coil" polymer chain there are dramatic macroscopic consequences. Above this concentration, entanglements between chains become prevalent, and viscoelastic behavior dramatically changes (17). Biological macromolecules generally are not random coils, but even when specific macromolecular conformations persist in crowded solutions of anisometic-shaped macromolecules, mesophases (liquid crystalline phases) can spontaneously form and give rise to long-range, orientationally correlated molecules. This correlation has consequences ranging from anisotropic diffusivity to DNA compaction (18). Liquid crystallinity, in turn, offers the possibility of coupled, and therefore abrupt, structural transformations. For example, a mesophase order-to-disorder transition could alter gene expression by destabilizing regulatory proteins. I hope this is only the beginning of many bottom-up polymer-chemistry-driven models of cellular organization.

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