Cell biology

Join the crowd

R. John Ellis and Allen P. Minton

Cells are packed with large molecules. The ramifications of this 'crowding' for a wide range of intracellular processes are only now becoming more generally understood.

All cells contain lots of big molecules, especially proteins, nucleic acids and complex sugars. The very high total concentration of these molecules, or 'macromolecular crowding', has energetic consequences that could affect many aspects of cellular function. Yet most biochemical studies of macromolecular properties are carried out in dilute solutions in which crowding effects do not occur, despite the availability of polymeric compounds whose addition to such solutions mimics the phenomenon. The first international meeting* devoted to the biological implications of macromolecular crowding brought together 60 theoreticians and experimentalists; their interaction revealed both the diversity and the magnitude of crowding effects on cellular processes.

The high total concentration of macromolecules inside cells (up to 400 grams per litre) means that between 5% and 40% of the total volume is physically occupied by these molecules. An even larger fraction of the total volume is unavailable to other molecules of comparable size. For example, in a solution containing 30% by volume of identical globular molecules, less than 1% of the remaining volume is available to an additional molecule of equal size — that is, less than 1% can accommodate such a molecule without displacing one of the molecules already present. The work required to place the additional molecule in this solution is correspondingly much higher than that required to place it in a dilute solution.

However, the effect of volume occupancy on available volume is sensitive to the relative sizes and shapes of the occupying molecules. Any reactions that increase the available volume are theoretically stimulated by crowded conditions. These processes include the binding of macromolecules to one another, the folding of protein and nucleic-acid chains into more compact shapes, and the formation of aggregates, such as the amyloid deposits seen in some neurodegenerative diseases. Another effect of crowding should be to reduce the rate of diffusion by factors up to 10 — depending on the size of the diffusing particle and the degree of occupancy of the medium — compared with the rate in uncrowded buffers. Thus a biochemical reaction might be influenced by crowding if its rate is limited by diffusion or by the stability of macromolecular complexes. The magnitude of these various effects can be estimated by using equations developed to describe model fluids containing hard particles or randomly coiled polymers (A. Minton; J. Herzfeld, Brandeis Univ., Waltham, Massachusetts; R. de Vries, Wageningen Univ.).

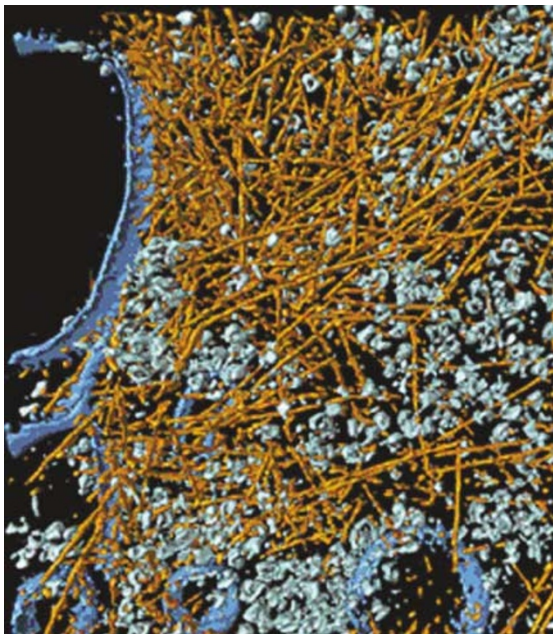

Figure 1 **Crowded interior. This three-dimensional reconstruction shows part of the cytoplasm of an intact motile *Dictyostelium discoideum* cell. The orange linear complexes are actin filaments; ribosomes and other macromolecular complexes are in grey; membranes are in blue. Reprinted with permission from ref. 3.**

How does reality conform to these theoretical expectations? Direct evidence for the crowded state of cell interiors is provided by the technique of cryoelectron tomography, in which thin intact cells are frozen rapidly in liquid ethane. The frozen cells are studied in an electron microscope that takes many pictures over a range of tilt angles; a computer program then reconstructs three-dimensional images with a resolution of 5–6 nanometres. The high density of actin filaments (part of the cellular 'skeleton') and ribosomes (protein-making machines) seen in reconstructed images of the slime mould *Dictyostelium* supports the view that the cytoplasm is filled with large ensembles of macromolecules, which form functional complexes, rather than with freely diffusing and colliding macromolecules (S. Nickell, Max Planck Institute, Martinsried; Fig.1). In addition, direct observation of fluorescent proteins in animal cells — both within the cytoplasm and inside two cellular compartments, the mitochondrion and endoplasmic reticulum — shows that their diffusion rate is reduced by factors in the range 3–8, broadly consistent with predictions (A. Verkman, Univ. California, San Francisco).

Higher levels of resolution for intact cells can be achieved with the use of nuclear magnetic resonance techniques to study the conformation of proteins expressed at high levels. For instance, a bacterial protein that lacks ordered structure in uncrowded buffers seems to acquire a degree of persistent structure when crowding agents are added to dilute solutions of the pure protein — and, more significantly, when expressed in the bacterium *Escherichia coli* (J. Bryant, Univ. North Carolina, Chapel Hill).

A type of crowding called confinement refers to situations in which macromolecules find themselves inside small compartments. Such compartments include those created by cytoskeletal structures or by the central cage of the chaperonin proteins — inside which newly synthesized proteins can fold, protected from crowding-enhanced, non-productive aggregation with other folding chains. Theory predicts that such confinement will stabilize compact shapes more than extended shapes and will enhance the rates of reactions leading to compaction (H.-X. Zhou, Florida State Univ., Tallahassee). These predictions agree qualitatively with observations that encapsulating proteins inside small pores in hydrated silica glasses enhances their stability when heated (D. Eggers, San José State Univ.; J.-M. Yuan, Drexel Univ., Philadelphia).

Another observation is that the efficiency of the bacterial chaperonins GroEL and GroES — which work together to help certain proteins fold — is enhanced by crowding agents. This might be due to an enhancement of the association between GroEL and GroES; the latter caps the internal cavity of GroEL and prevents the escape of an encapsulated polypeptide (J. Martin, Max Planck Institute, Tübingen). And it might be because crowding reduces the probability that an encapsulated polypeptide can diffuse away from an uncapped GroEL before folding completely (A. Elcock, Univ. Iowa, Iowa City).

* *EMBO Workshop: Biological Implications of Macromolecular Crowding.* Palacio de Magalia, Spain, 14–18 June 2003 (www.cib.csic.es/~revers/embo2003).

Crowding also seems to affect the way in which bacteria adapt to large changes in the concentration of osmotically active molecules in their environment: bacteria grown under widely disparate osmotic environments show significant differences in their intracellular concentrations of macromolecules, as well as of smaller molecules (S. Cayley, Univ. Wisconsin–Madison). Other processes influenced by crowding include: the regulation of metabolic pathways associated with signal transduction (H. Westerhoff, Free Univ. Amsterdam); the extraordinary stability of the crystallin proteins in the lens of the eye (J. Clauwaert, Univ. Antwerp); and the synthesis of compact proteins from peptide fragments by enzymes that catalyse the opposite reaction, polypeptide breakdown, in the absence of crowding (R. Roy, Natl Inst. Immunol., New Delhi). Moreover, when DNA is in the crowding-induced compact conformation — rather than the worm-like coiled shape seen in dilute solution — several enzyme-catalysed DNA-processing reactions are accelerated by many orders of magnitude (J. L. Sikorav, CEA-Saclay, Gif-sur-Yvette).

One of the more dramatic effects of crowding is the stimulation of the rate and extent of formation of rod-like protein aggregates. Examples are the amyloid fibres of synuclein that are implicated in Parkinson's disease (A. Fink, Univ. California, Santa Cruz), microtubules (D. Hall, Univ. Cambridge), and large arrays of the protein FtsZ, essential to bacterial division (J. Gonzalez, CIB-CSIC, Madrid). Also, the rate of formation of fibres of deoxygenated haemoglobin S (the mutant form found in patients with sickle-cell anaemia) can vary over several orders of magnitude depending on the degree of crowding. This dependence can be accounted for quantitatively over the entire range of experimental observation by relatively simple hard-particle models of excluded volume (F. Ferrone, Drexel Univ.).

It seems likely from the observations reported at this meeting, and reviewed recently[1,2], that macromolecular crowding *in vivo* is involved in many aspects of cellular function. Given that crowding was not discovered yesterday, one may wonder why little mention of this phenomenon is found in current textbooks of biochemistry and molecular and cell biology. ■

R. John Ellis is in the Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, UK.
e-mail: jellis@bio.warwick.ac.uk
Allen P. Minton is at the National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, US Department of Health and Human Services, Bethesda, Maryland 20892-0830, USA.
e-mail: minton@helix.nih.gov

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Quantum physics

Entanglement hits the big time

Vlatko Vedral

Entanglement is a quantum phenomenon usually associated with the microscopic world. Now it is clear that its effects are also relevant on macroscopic scales, such as in the magnetic properties of some solids.

Entanglement describes a correlation between quantum mechanical systems, such as photons or atoms, that does not occur in classical, newtonian physics. Under scrutiny since the birth of quantum theory, such correlations have been used to highlight a number of apparent paradoxes at the heart of quantum physics, but their existence has nevertheless been confirmed in a number of different experiments since the beginning of the 1980s. For instance, the entanglement of two photons means that once the state of one photon is known, we immediately know the state of the other; entanglement is also the basis of the quantum computer. But who, apart from possibly a few philosophers of physics and some computer scientists, actually cares about statistical correlations between two or more systems? In other words, do quantum correlations affect anything of any importance in the real, macroscopic world? Amazingly, they do, and the paper by Ghosh *et al.*[1] on page 48 of this issue demonstrates just that.

Ghosh *et al.* report experiments involving the magnetic salt compound $LiHo_{0.045}Y_{0.955}F_4$ (see refs 4–6 in ref. 1). The atoms in this salt all behave like small magnets, interacting with each other as well as adjusting themselves to any external magnetic field (modelled by an Ising chain of interacting spins). The authors[1] investigated the magnetic susceptibility of the system for a range of low temperatures. Susceptibility tells us how much the magnets align with each other when an external magnetic field is applied, so the greater the susceptibility the more the magnets align when the external magnetic field is increased. Our intuition would tell us that the more correlated the magnets, the higher the degree of susceptibility we should expect. Also, as the temperature increases, the magnets heat up and their behaviour becomes more random, less correlated[2,3]. We would therefore expect the susceptibility to decrease with temperature. Experiment shows that it does (Fig. 1a), but how do theoretical predictions compare?

One way of deriving the macroscopic properties of a physical system — known as the 'royal route' in the technical jargon — is by classical statistical mechanics. Here the first and most important task is to construct the partition function of the system under investigation. This is a sum over the various energy states that the system can occupy and tells us how the system distributes itself over the available states in terms of probabilities. Once we have the partition function, we can then compute all the other relevant (and macroscopically observable) properties, such as the internal energy of the system, its pressure, entropy and susceptibility and so on. This is quite remarkable: knowing the different energy states that the system can occupy is sufficient to construct all the other macroscopic quantities needed to describe the physical system completely. But, ultimately, this conclusion is erroneous, as can clearly be seen in the disagreement shown by Ghosh *et al.*[1] between the classical predictions and experimental results (Fig. 1a).

Quantum mechanically, if we wish to fully specify the state of a system, we need to know both the energy levels and the particular states corresponding to these energy levels. Loosely speaking, this means that for a salt such as $LiHo_{0.045}Y_{0.955}F_4$ we need to know the orientations of the magnetic atoms and not just their overall energy. More significantly, these states could, through quantum mechanics, display an excess of correlations between the individual magnets over and above anything allowed in classical physics — they could be entangled[2,3]. And, amazing though it may seem, the presence of this entanglement can make a difference in observed macroscopic quantities. As the quantum correlations are stronger than classical ones, we can predict that the susceptibility will be higher according to quantum mechanics. This is indeed demonstrated by Ghosh *et al.*[1], and they also show that other quantities, such as the heat capacity, depend on the presence of entanglement (see Fig. 2 on page 49).

This work is important for at least two reasons: one is that it is no longer enough for physicists to investigate only the energy spectrum of a system, but some other features — such as entanglement in this case — are of paramount importance in the overall behaviour of the system. The other reason is that even a very small amount of entanglement can produce significant effects in the macroscopic world (Fig. 1b).

Finally, I should like to indulge in a little speculation. It is widely accepted that quantum mechanics is our most accurate