Although you were born and educated in the USA, you've worked in the UK for over 11 years. What are your views on the different ways science is done in the two countries? I've probably been away from the States too long to judge! One thing that does stand out, however, is the difference in post-graduate (PhD) education and training. Most students in the UK get funding for a three-year PhD after a three-year undergraduate degree (four years undergraduate in Scotland). This can't provide the same degree of training as occurs in the USA or in those European countries where a Diploma (equivalent of a Master's degree) is a prerequisite to entry into a PhD program. One consequence of this is that too many British PhDs end up not pursuing a productive science career, because they can make a slow start, and everything is rushed at the end.

Part of the problem is money, but even with limited resources, there are opportunities for improvement. For example, the same amount of money could be used to train fewer, more carefully selected students, for longer periods of time — certainly longer than the canonical three years! A step in the right direction is that many of the UK Research Councils, which fund the majority of PhDs, are giving universities more autonomy in how they spend the money dedicated to studentships, so a wholesale restructuring of the PhD experience could in theory begin at a more local level.

Also, in the States it is common practice for recent graduates to work for a year or so in a lab, not only to gain experience but also to think about what they really want to do scientifically, without the competing pressure of finishing coursework. That is less easy to do in the UK, because there aren't that many short-term positions available, and those that are available may be incredibly dull. The UK Research Councils could establish a program in which highly qualified students apply to work in a lab of their choice for a year. This could be done relatively cheaply if there was only a modest stipend and no academic fees to pay (for example, if they didn't actually register for a degree).

Any advice for the students, then? If you don't think that what you're doing is the most interesting thing in the world, you should probably be doing something else, as the other rewards of this job are relatively few. Also, although some people would argue that the history of science is largely irrelevant to the actual practice of science, it's still very useful to see how a field has developed — not only one's own, but related fields as well. It is a good source for general inspiration, as well as for an appreciation of how new technology is so often the primary engine of scientific progress. A few years ago, Cold Spring Harbor Laboratory Press reprinted Horace Freeland Judson's 1979 history of molecular biology, *The Eighth Day of Creation*. Any student using molecular techniques nowadays would do well to read this book.

Is it better to be a post-doc or to run a lab? I like benchwork, so I think it is far, far better to be a post-doc! Paul Nurse gave me a lot of freedom to work on whatever I wanted, which was very lucky. Unfortunately, there are many reasons (some good) why one can't be a post-doc forever. The time to make a move is when you have too many ideas to carry out yourself. Because I have a small group, I still have some time for experiments, which partially compensates for committee meetings, grant writing and reviewing, and lab management. To make a suggestion that significantly helps someone else's project in the lab is a special treat!

What would you do if you weren't a scientist? Dan Mazia used to tell me that if he weren't a scientist he would have gone into advertising. Probably I'd be making things out of wood, or cooking at home.

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Quick guide

Macromolecular crowding

Allen P. Minton

What is macromolecular crowding? The term 'macromolecular crowding' was coined to connote the influence of mutual volume exclusion upon the energetics and transport properties of macromolecules within a crowded, or highly volume-occupied, medium.

Volume exclusion? What's that? Because of steric repulsion, no part of any two macromolecules can be in the same place at the same time. That part of the total volume which cannot be occupied by the center of mass of a particular solute species at a particular instant is called the excluded volume, and the part of total volume that may be occupied is called the available volume (Figure 1). As the fraction of volume occupied by macromolecules of a given size increases, the fraction of volume available to an additional macromolecule of comparable size decreases rapidly, and becomes much less than the fraction of volume available to solvent (water).

In freshman chemistry we are taught that the reactivity of a solute is proportional to its concentration, or number of molecules of solute per unit total volume. In fact, this is only strictly true in the highly dilute limit. In a highly volume-occupied solution, the reactivity of a test solute species is determined by the number of molecules of that solute per unit of *available* volume, which is an effective concentration called the thermodynamic activity. Depending upon the size and shape of the test solute species, and the number density and sizes and shapes of all of the macromolecular solute species in the vicinity of the test

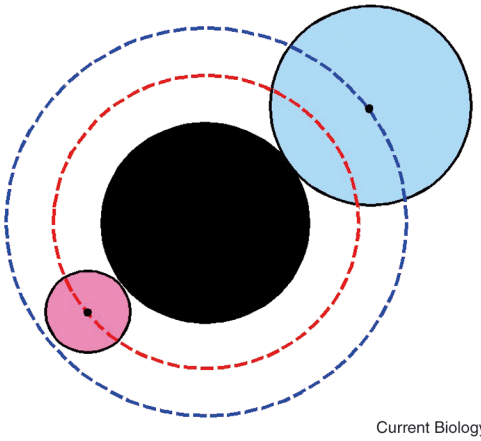

Figure 1. Examples of excluded volume.

For the purpose of estimating excluded volumes, molecules of native protein may be represented by rigid particles of similar size and shape. In this figure, the spheres represent three different species of roughly spherical globular protein. The volume excluded by the black sphere to the red sphere is a spherical volume bounded by the dashed red line. The volume excluded by the black sphere to the blue sphere is a spherical volume bounded by the dashed blue line.

species — termed background species — the effective concentration or activity of the test species may exceed its actual concentration by as much as several orders of magnitude!

Why is crowding relevant to biology? Biochemical rates and equilibria have traditionally been studied in dilute solution, where the consequences of steric repulsion between solutes are generally negligibly small. In contrast, almost all fluid media in biology contain a high total volume fraction of macromolecules. In special cases, a medium consists primarily of a single species of macromolecule — for example, hemoglobin in hemolysate or albumin in blood serum — but more commonly the medium is highly heterogeneous, as in the case of prokaryotic cytoplasm, containing a mixture of proteins, nucleic acids and polysaccharides in varying proportion. Experiments carried out on solutions containing comparable volume fractions of purified proteins or chemically inert polysaccharides have demonstrated that excluded volume effects in such media can result in the alteration of equilibrium and rate constants by up to several orders of magnitude.

How does crowding affect biochemical equilibria? Crowding is a consequence of steric repulsion, a destabilizing interaction that increases the total free energy or work content of the system. Equilibrium theory predicts that if the composition of a system can change to minimize the total free energy of that system, it will do so. Thus crowding is expected to shift equilibria toward a state of the system in which excluded volume is minimized.

The extent to which a particular macromolecular species excludes volume to its neighbors generally increases with the ratio of surface to volume of that species. Hence crowding exerts a generalized pressure for the reduction of the surface to volume ratio. This is accomplished in two ways. The first is by favoring compact conformations over extended conformations of flexible macromolecules. The second is by favoring both specific macromolecular associations leading to the formation of well-defined oligomeric species, and nonspecific macromolecular associations leading to the formation of large aggregates of native or nonnative species.

How does crowding affect biochemical rates? Crowding can affect reaction rates by two distinct mechanisms. The rate of slow reactions is ordinarily limited by the rate with which reactants pass over a free energy barrier identified as a transition state. In the case of slow reactions, this rate is sufficiently low that the transition state may to a good approximation be treated as if it were in equilibrium with reactant(s) and product(s). Because the attractive interactions that stabilize a complex are ordinarily short-ranged, the transition state of an association reaction tends to resemble the association product more closely than it does the fully separated reactants, and hence exclude a volume to its neighbors similar to that of the fully associated product. For this reason, crowding is expected to increase the association rate constant and have little effect on the dissociation rate constant.

In the case of very fast reactions, the rate of an association is limited by the rate with which reactants encounter each other. This rate is dominated by translational diffusion, which decreases monotonically with increased crowding due to the presence of an increasing number of obstacles. Thus crowding is expected to accelerate slow association reactions and decelerate fast association reactions.

Are all reactions affected equally by crowding? No. One would not expect a reaction to be affected by crowding if it is not accompanied by a significant change in the volume excluded to background solutes. The binding of a small molecule by a macromolecule would thus be essentially unaffected by crowding unless the binding event was linked to a major change in the conformation or the state of association of the macromolecule. On geometric grounds one would not expect crowding by large macromolecules to greatly affect the behavior of small molecules or significantly smaller macromolecules, which can more easily fit into interstices between large molecules. However, both the dynamic and equilibrium behavior of large macromolecules or macromolecular assemblies would be expected to be greatly affected by the presence of high concentrations of smaller macromolecules.

How much of the difference between biochemical reactions

in vitro *and* in vivo *can be attributed to crowding?* The answer to this question depends upon the particular reaction and the microscopic environment in which the reaction is taking place. Early demonstrations of the large effect of crowding on association equilibria and rates were based upon studies of the behavior of mutant and normal hemoglobins in erythrocytes. Hemolysate is a fairly simple fluid containing primarily hemoglobin, and it can be shown that volume exclusion is a dominating factor in this medium.

However, in a more complex heterogeneous environment such as cytoplasm, crowding is probably just one of several nonspecific factors affecting reaction rates and equilibria, such as weak nonspecific associations with background molecules or large structures leading to possible sequestration or adsorption of reactants and/or products.

Nevertheless it is essential to keep in mind that, in a crowded biological fluid, the effects of volume exclusion will always be present and play an important role in influencing macromolecular structure and function, independent of and in addition to the influences of other types of interactions. The ubiquity of this phenomenon in biological fluids has been compared to that of gravity.

Where can I find out more?

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Dark mysteries

Bird plumage traits are the targets of both natural and sexual selection and fascinate evolutionary biologists as many species show quite dramatic variations within their range and between related species. So, genetic changes resulting in plumage variation among closely related groups might represent important evolutionary events. The molecular basis of such differences, however, is unknown in most cases. But sequence variation in the melanocortin-1 receptor (MC1R) gene is associated with melanistic variants in many vertebrates, including several bird species around the world.

The blue-crowned manakin (*Lepidothrix coronata*) is a widely distributed species, exhibiting striking geographic variation in male plumage across its range in southern Central America and western Amazonia. Northern males are black with bright blue crowns whereas southern males are green with lighter blue crowns. In a new study, Z.A. Cheviron and Robb Brumfield at Louisiana State University at Baton Rouge, and Shannon Hackett at the Field Museum of Natural History in Chicago have studied a stretch of coding region in the MC1R gene in 23 individuals spanning the range of male plumage variation (published online in Proceedings B of the Royal Society).

The only variable sites they detected were for synonymous substitutions, none of which were associated with either plumage type. Comparative analysis of the sequences at three amino acid sites thought to be functionally important in pigment variations in other species proved to be similar in these birds. This new study suggests that other mechanisms are at play.

Many loci are known to influence pigmentation in vertebrates so “future studies exploring other candidate genes, especially those with regulatory functions, are likely to provide great insight into our understanding of the evolution of avian plumage colour,” the authors report.

Feather buster: Variations in the MC1R gene do not appear to be linked to pigment differences in the plumage of the blue-crowned manakin. (Photo: Steve Bird, Birdseekers.)