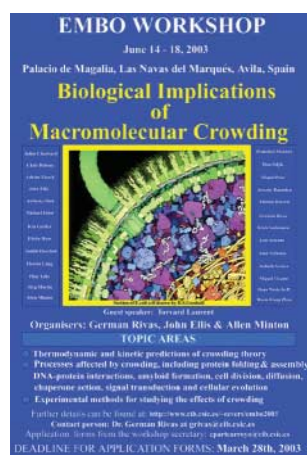


# Life in a crowded world

## Workshop on the Biological Implications of Macromolecular Crowding

Germán Rivas<sup>1</sup>\*, Frank Ferrone<sup>2</sup> & Judith Herzfeld<sup>3</sup>

<sup>1</sup>CSIC, Madrid, Spain, <sup>2</sup>Drexel University, Philadelphia, Pennsylvania, USA, and <sup>3</sup>Brandeis University, Waltham, Massachusetts, USA



This workshop took place at the Palacio de Magalia (Las Navas del Marqués, Avila, Spain), between 14 and 18 June 2003, and was organized by J. Ellis, A. Minton and G. Rivas. Further details on the workshop can be found at <http://www.cib.csic.es/~revers/embo2003/index.htm>

Keywords: crowding; volume exclusion; macromolecular interactions; protein folding and assembly; biochemical rates and equilibria

During recent decades it has gradually become recognized that crowding can considerably alter the reactivity of individual macromolecules, both qualitatively and quantitatively. Crowding can be mimicked experimentally by adding high concentrations of inert synthetic or natural macromolecules, termed crowding agents or crowders, to the system *in vitro* (Ellis, 2001). Experimental and theoretical work has demonstrated substantial (order-of-magnitude) effects of crowding on a broad range of biochemical, biophysical and physiological processes, including—but not limited to—nucleic acid and protein conformation and stability, protein–protein and protein–DNA association equilibria and kinetics (including protein crystallization, protein fibre formation and bundling), catalytic activity of enzymes and cell volume regulation (Zimmerman & Minton, 1993; Minton 1997, 2001; Ellis, 2001).

The fact that biological macromolecules have evolved to function in such crowded environments raises biologically important questions. Why did cells evolve such a highly packed interior? Are there any advantages in being crowded? How do macromolecules fold, associate and travel through the crowded intracellular medium? (Fulton, 1982; Zimmerman & Minton, 1993; Bray, 1998).

The first international workshop on the Biological Implications of Macromolecular Crowding brought together a group of 60 theoreticians and experimentalists to exchange ideas on topics that fell into three categories: first, theoretical predictions of the effects of excluded volume on the rates and/or equilibria of macromolecular reactions; second, experimental and simulation approaches that can be applied to the quantitative characterization of biochemical and biophysical processes in crowded media, both *in vitro* and *in vivo*; and last, experimental findings that indicate that a broad variety of biological phenomena and systems are likely to be substantially influenced by crowding. As many aspects of the theory of crowding are already well developed, this opportunity for interdisciplinary exchange came at a particularly appropriate time.

Some conferences resemble medieval jousting matches during which opponents try to unseat their counterparts in well-known controversies; others are like marketplaces where proponents of new ideas, new theories and new experimental techniques present their 'wares' for acquisition by others who may find that they fill a particular need. Despite the dramatic venue of the conference (namely, a castle in Avila), this meeting had more in common with the market than the tournament. A community of interest that really did not exist before the workshop was assembled, and the

EMBO reports (2004) 5, 23–27. doi:10.1038/sj.embor.7400056

### Introduction

Macromolecules are present as soluble species and/or structural arrays at total concentrations of up to several hundred grams per litre in essentially all physiological compartments. Although local composition varies widely between different systems, it is evident that most macromolecular reactions and processes *in vivo*—as opposed to typical experiments *in vitro* in which the total concentration of macromolecules rarely surpasses 1 g l<sup>-1</sup> (Ralston, 1990; Ellis, 2001)—take place in environments in which macromolecules occupy a considerable fraction (between 10% and 40%) of the total volume (Fulton, 1982; Record *et al.*, 1998). These media are termed 'crowded' or 'volume-occupied' rather than 'concentrated', because no single species of macromolecule is necessarily present at a high concentration. The term 'macromolecular crowding' connotes the non-specific influence of steric repulsions on specific reactions and processes that occur in highly volume-occupied media.

<sup>1</sup>Centro de Investigaciones Biológicas, CSIC, Ramiro de Maeztu 9, E-28040 Madrid, Spain

<sup>2</sup>Department of Physics, Drexel University, Philadelphia, Pennsylvania 19104, USA

<sup>3</sup>Department of Chemistry, MS #015, Brandeis University, Waltham, Massachusetts 02454-9110, USA

\*Corresponding author. Tel: +34 91 837 3112; Fax: +34 91 536 0432; E-mail: grivas@cib.csic.es

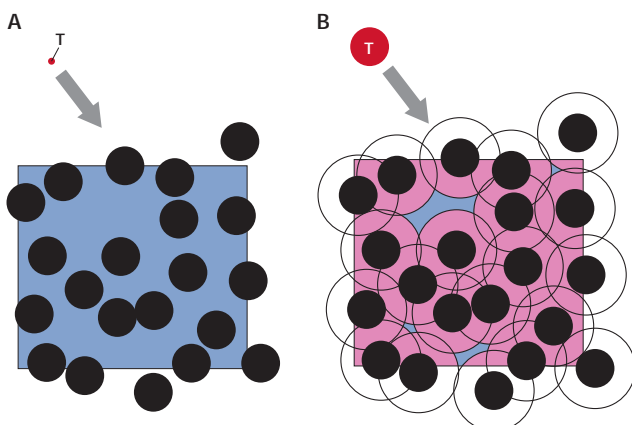
Submitted 1 October 2003; accepted 20 November 2003; published online 19 December 2003

participants tried to decide if and how they could benefit from each other's expertise. Consequently, this report necessarily reads more like a catalogue of goods than a chronicle of champions and challengers.

**Volume exclusion phenomena: entropic consequences**

The mutual impenetrability of solute molecules, due to the Pauli exclusion principle, is arguably the most fundamental intermolecular interaction. In fact, its ubiquity can cause steric repulsions to be taken for granted and their consequences to be overlooked. However, the activity of a solute depends strongly on the volume that is available to the centre of mass of each molecular species. That this steric freedom—or entropy—is a function of the size and shape of the solute is illustrated in Fig 1, which represents a volume element containing spherical macromolecules (in black) that occupy about 30% of the total volume, as is typical of intracellular fluids. The regions that are available to the centre of a test solute molecule (T) are restricted to those that are further from any other solute molecule than the sum of the radii of T and pre-existing solutes. This available volume is indicated by the blue-coloured regions and its complement is referred to as the excluded volume. If T is very small relative to the background macromolecules (Fig 1A), the available volume is almost equal to the total unoccupied volume. But if the size of T is comparable to that of the other solutes (Fig 1B), the available volume is considerably smaller and the contribution of steric repulsion to reduced entropy and increased free energy is correspondingly greater. Clearly, one of the ways in which the system can reduce its free energy is to maximize the available volume (or, alternatively, to minimize the excluded volume). A fundamental chemical consequence of macromolecular crowding is therefore the facilitation of processes that lead to a decrease in excluded volume, namely macromolecular compaction and association (Minton, 2000, 2001; Ellis, 2001). However, it is important to emphasize that although crowding will tend to enhance the association of macromolecules that have an inherent tendency to associate, it will not create this tendency *de novo*.

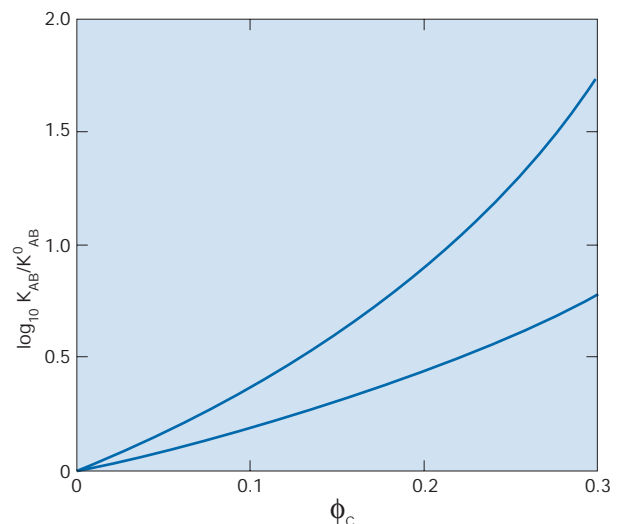
At the workshop, A. Minton (Bethesda, MD, USA) summarized simple statistical-thermodynamic models for predicting the influence



**Fig 1** | Excluded volume (shown in pink and black) and available volume (shown in blue) in a solution of background macromolecules. The two panels show the volume available to the centre of a test molecule (T) either (A) much smaller than or (B) of similar size to the background macromolecules (see the text for details). Reproduced with permission from Minton (2001). (Figure presented at the workshop by A. Minton.)

of excluded volume on macromolecular equilibria and reaction rates in crowded solutions. These are based on the representation of a macromolecular solute as an equivalent hard convex particle of similar size and shape. Such models have proved useful in accounting for a variety of experimental observations, in many cases quantitatively (Hall & Minton 2003). An example of the results that Minton obtained is presented in Fig 2, in which the equilibrium constant for the heteroassociation of two dilute species, A and B, is calculated as a function of the fraction of volume occupied by a third inert species, C. The results indicate that excluded volume alone can lead to a large enhancement of equilibrium association, depending on the relative sizes of A, B and C, and on the degree of volume occupancy by C. For large and elongated macromolecules, orientational ordering can also redistribute available volume, as illustrated by J. Herzfeld (Waltham, MA, USA), who showed that, under certain circumstances, crowding provides a non-specific force towards various forms of spatial ordering of highly anisotropic macrosolutes (Herzfeld, 1996). She reviewed crowding-induced spontaneous alignment and bundling of self-assembled filaments, a phenomenon that has particular significance for cytoskeletal organization.

In addition to the thermodynamic consequences summarized above, crowding can considerably decrease the diffusional mobility of macromolecules in highly volume-occupied environments. Steric interactions therefore also have a strong influence on the rates of diffusion-controlled reactions in crowded solutions and confined spaces, such as cytoskeletal interstices or chaperonin cavities. H.-X. Zhou (Tallahassee, FL, USA) presented theoretical calculations predicting that confinement (Minton, 1992; Eggers & Valentine, 2001) will substantially stabilize the more compact folded state of a protein relative to the unfolded state, whereas crowding is predicted to have a more modest effect on folding stability.



**Fig 2** | Calculated change in the equilibrium constant for heteroassociation of dilute globular protein A (65 kDa) and dilute globular protein B (52 kDa) as a function of the fraction of volume occupied by a 27-kDa inert protein (upper curve) and by a 70-kDa inert protein (lower curve). All proteins are represented by equivalent hard spherical particles with respective radii proportional to the cube root of molar mass; calculations were performed with the scaled particle theory of hard sphere mixtures (Lebowitz *et al*, 1965). For details of the calculation see Minton (1998).

### Studying macromolecular reactions in crowded media

The quantitative characterization of crowding effects on macromolecular reactions presents special challenges to the researcher over and above those that are encountered in the study of these reactions in dilute solution. How does one monitor the behaviour of one or two dilute test species in the presence of far higher concentrations of crowding species? How does one ascertain that any observed effect of crowder species is due to volume exclusion rather than weak associations between the crowder and test species? How can one deal with large deviations from thermodynamic ideality in concentrated solutions? At the workshop, several methods were described that have been used to study macromolecular behaviour in crowded media or have the potential for such applications.

Knowledge of the state(s) of association of a biological macromolecule, present at low concentration in its naturally crowded physiological environment, cannot be reliably inferred from the knowledge of the association state of that macromolecule in a solution containing only that species of macromolecule at the same concentration. G. Rivas (Madrid, Spain) introduced non-ideal sedimentation equilibrium, a novel adaptation of a classical methodology that allows the molar mass of a dilute trace macromolecule to be measured in the presence of other macromolecular species at concentrations up to several hundred milligrams per millilitre. This technique has allowed the direct observation of the significant enhancement of the self-association of dilute proteins (fibrinogen, tubulin and the bacterial cell-division FtsZ protein) in concentrated solutions of unrelated proteins and polymers (Rivas *et al*, 1999, 2001). In principle, the method can also be used to detect and characterize heteroassociating systems in crowded media.

Fluorescence-based methods are potentially powerful tools for studying the structural organization and dynamics of tracer proteins in crowded solutions (Brown & Royer, 1997). However, so far there are few examples of cases in which fluorescence assays have been applied to study crowding effects. Fluorescence anisotropy measures the decrease in the rotational brownian motion of a fluorescently labelled tracer protein when it associates with another macromolecule. At the workshop, P. Lillo (Madrid, Spain) described the use of steady-state and time-resolved fluorescence anisotropy to detect the self-association of dilute apomyoglobin in crowded solutions. Fluorescence resonance energy transfer (FRET), which measures the proximity of donors and acceptors that might be attached to different molecules, or to different parts of the same molecule, has been used by E. Haas (Ramat Gan, Israel) to detect crowding-induced changes in the conformation of denatured proteins such as adenylate kinase. These data have potential consequences for the study of protein folding pathways. Diffusion of solutes and macromolecules in cellular compartments is an essential requirement for many cellular processes, including signalling events. A. Verkman (San Francisco, CA, USA) has used fluorescence recovery after photobleaching (FRAP), in conjunction with confocal microscopy and time-resolved anisotropy methods, to measure the diffusional mobility of fluorescently labelled small molecules and macromolecules within the cytoplasm of living cells. Verkman found a 3–4-fold decrease in diffusion coefficients of green fluorescent protein (GFP) in the cytosol and in the endoplasmic reticulum lumen compared with water, which is in accord with crowding theory. He also found that the mobility of some enzymes inside mitochondria was much larger than expected, suggesting that these organelles contain relatively uncrowded channels where some proteins move more rapidly than expected for a uniformly congested

medium (Verkman, 2002). Fluorescence correlation spectroscopy (FCS) may also be used to measure diffusional mobility in crowded media, as pointed out by C. Royer (Montpellier, France). Two-colour FCS is being developed by Verkman as a tool to study correlated motions of different macromolecules, and hence the interactions between them, in the cytoplasm of living cells.

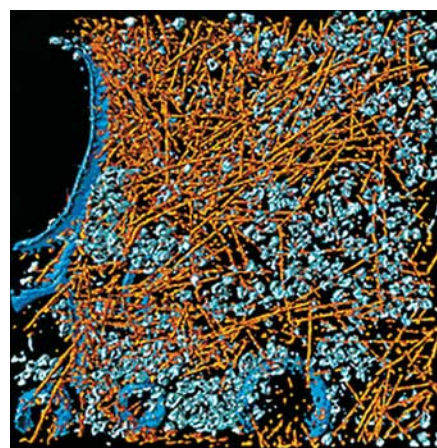
A variety of NMR techniques that are capable of characterizing macromolecular motions and conformations within crowded solutions and within living cells were reviewed by M. Pons (Barcelona, Spain). One of these techniques has recently been used by J. Bryant (Chapel Hill, NC, USA) to detect a conformational change that has been interpreted as the acquisition of structure by a 'natively unfolded' protein under the influence of crowding, both *in vitro* and *in vivo* (Dedmon *et al*, 2002).

The visualization of cells by electron microscopy methods has normally been performed with transmission optics on fixed, dehydrated and stained thin sections. The possible artefacts due to these treatments are avoided in cryoelectron microscopy, which is a powerful and promising tool with which to visualize the crowded nature of cell interiors and to detect and characterize complex macromolecular assemblies in their natural environment (Baumeister, 2002; Grunewald *et al*, 2003), as discussed in the meeting by S. Nickel (Martinsried, Germany). The image shown in Fig 3 is the first cryoelectron microscopy picture of an intact eukaryotic cell that has not been fixed, dehydrated or stained.

Finally, K. Lipkow (Cambridge, UK) and co-workers have used computational biology to understand how chemotactic signals move through a crowded cell (Bray, 2002). Their efforts complement experimental observations and have led to a computer program, Smoldyn, that allows the simulation of the movement and interaction of a large number of particles in a structured environment.

### Effects of crowding on protein folding

The acquisition of the native structure of a protein *in vivo* takes place within the crowded intracellular environment. In principle, crowding could interfere with folding by favouring the aggregation of unfolded proteins, an effect that might be overcome by the action of



**Fig 3** | Cell compartments are crowded. Shown is a three-dimensional reconstruction of part of the cytoplasm of a *Dictyostelium discoideum* cell, produced by the Baumeister group (Medalia *et al*, 2002). Actin filaments are shown in orange, ribosomes and other macromolecular assemblies in grey, and membrane structures in blue. (Figure presented at the workshop by S. Nickel.)

molecular chaperones (van den Berg *et al*, 1999, 2000). The enhanced efficiency of the bacterial chaperones GroEL and GroES in crowded solutions was reviewed by J. Martin (Tübingen, Germany). It was suggested that the enhancement might be due to an increase in the association between GroEL and GroES, which caps the internal cavity within which partly unfolded polypeptides are protected from aggregation while they attain their native conformation (Martin & Hartl, 1997; Martin, 2002). A. Elcock (Iowa City, IA, USA) has simulated the release of rhodanese from the GroEL cavity in crowded solutions by using brownian dynamics; his results suggest that crowding inhibits the escape of partly unfolded proteins from the cavity, thereby providing the partly unfolded protein with a greater opportunity to achieve its fully native conformation (Elcock, 2003).

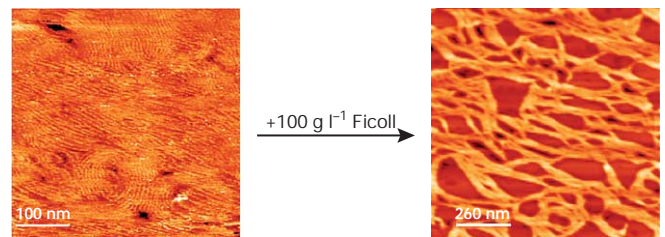
### Effects of crowding on protein assembly

One of the more dramatic effects of crowding seems to be the enhancement of the rate and extent of the formation of fibrous or rod-like large protein assemblies (Minton, 2000), and this theme was addressed in the meeting in both theoretical and experimental terms. Sickle haemoglobin causes the serious disease of sickle-cell anaemia because it forms polymers under physiological conditions of high protein concentrations and low oxygen tensions (Eaton & Hofrichter, 1990). This assembly problem was one of the first effects of crowding to be studied (Minton, 1977; Ross & Minton, 1977; Ferrone *et al*, 1985). Extensive data showing that crowded haemoglobin monomers behave as almost perfect hard spheres have allowed the mechanisms of polymerization to be formulated in thermodynamic terms so that the effects of crowding and additional interactions between concentrated polymers can be included naturally (Han & Herzfeld, 1998). F. Ferrone (Philadelphia, PA, USA) described how the essential features of crowding could be incorporated into models of polymerization kinetics and showed recent tests confirming that this basic approach was correct (Ivanova *et al*, 2000; Jasuja *et al*, 2002).

In contrast to haemoglobin assembly, in which crowding is an intrinsic factor (because of the high solubility of the protein *in vivo*), D. Hall (Cambridge, UK) presented theoretical work on tubulin assembly that illustrated the way in which added macromolecular crowders can affect the distributions of polymer lengths (Hall, 2002). Herzfeld also presented some theoretical work that described how crowders can cause the demixing and bundling of filamentous proteins (Herzfeld, 1996). The influence of macromolecular crowders on protein assembly was considered experimentally by J. González (Madrid, Spain) and A. Fink (Santa Cruz, CA, USA). Under conditions that resemble the crowded intracellular environment of bacteria, the essential cell-division protein FtsZ forms filaments that tend to align and form dynamic ribbons of dimensions that would fit into the Z-ring *in vivo* (Fig 4). This crowding effect on FtsZ was observed in the presence of GTP and K<sup>+</sup> ions (ligands that promote FtsZ assembly in dilute solutions) but not in the presence of GDP (González *et al*, 2003). Fink showed that crowding has significant effects on the rate of formation of amyloid fibres of  $\alpha$ -synuclein, a process that is implicated in the pathogenesis of Parkinson's disease (Uversky *et al*, 2001).

### Other biological processes influenced by crowding

Crowding also has implications for genome structure and function because it influences both the structural organization of DNA and the interactions between DNA and proteins (Zimmerman, 1993; Zimmerman & Murphy, 1996). At the workshop, R. de Vries



**Fig 4** | Crowding-induced formation of ribbons of the bacterial cell division FtsZ protein as monitored by atomic force microscopy. The left-hand image shows the thin FtsZ filaments observed *in vitro* in the absence of crowding. The right-hand image shows the FtsZ ribbons formed after the addition of 100 g l<sup>-1</sup> Ficoll 70, a 70-kDa inert crowder. (Figure presented at the workshop by J. González; see González *et al*, 2003.)

(Wageningen, the Netherlands) reviewed the current theoretical understanding of the interaction of DNA with flexible polymers, which is relevant to the compaction of DNA and nucleoids under crowded conditions. Related data presented by J.-L. Sikorav (Gif-sur-Yvette, France) described the acceleration of DNA processing by the crowding-enhanced condensation of DNA.

Crowding is an important factor in the compensation mechanisms that buffer essential macromolecular interactions in *Escherichia coli* against large variations in the tonicity of its environment, as reported by S. Cayley (Madison, WI, USA). *E. coli* adapts to large changes in growth osmolarity by making large changes in the amounts of cytoplasmic water and osmolytes such as cytoplasmic K<sup>+</sup> ions. Crowding effects resulting from changes in the amount of water seem to compensate for the effects of changes in cytoplasmic K<sup>+</sup> ions and contribute to maintaining protein–nucleic-acid equilibria and kinetics in the range required for function *in vivo* (Record *et al*, 1998). In related work, H. Westerhoff (Amsterdam, the Netherlands) applied an *in silico* strategy (see [www.siliconcell.net](http://www.siliconcell.net)) to yeast glycolysis and to the *E. coli* phosphotransferase system (PTS). The simulated yeast glycolysis behaves similarly to its real counterpart, suggesting that crowding in yeast cells does not affect the glycolytic pathway. However, crowding has to be invoked to simulate *in silico* the rapid rate of metabolite transfer between successive enzymes in the PTS system *in vivo*: the results *in silico* correspond to the experimental data when macromolecular crowding is assumed both to increase the association rate constants and to decrease the dissociation rate constants of the PTS complexes (Rohwer *et al*, 1998).

Finally, perhaps one of the most dramatic consequences of a crowded milieu is the reversal of biochemical reactions. R. Roy (New Delhi, India) showed that crowding could cause enzymes to synthesize polypeptides rather than catalyse peptide hydrolysis as they would in a dilute solution (Somalinga & Roy, 2002).

### What next?

Despite the recent advances presented at this workshop, it is clear that many questions still need to be answered for a better understanding of the biological implications of macromolecular crowding. Do high concentrations of 'inert' macromolecules provide a good (valid) simulation of the interior of cells? What would be better? Is there a perfect crowder? How should the crowded cell be simulated numerically? How should the non-equilibrium effects of crowding be addressed? How can one reconstitute a biological process *in vitro*? What about surface phenomena (such as life and surface chemistry)?

This workshop drew attention to the fact that excluded volume effects arising from steric repulsion in crowded media will always be present *in vivo*, independently of the presence or absence of other types of interaction. Although a cell compartment can contain microregions that might be more or less crowded than suggested by cell-average concentrations, there will be conditions under which the effects of crowding must be taken into account when analysing the equilibria and dynamics of macromolecular reactions in physiological environments. Moreover, crowding must also be considered in total genome and proteome analyses to develop quantitative global models of intracellular processes. As the field is still emerging, workshops such as this one will serve to support efforts in an area that we feel will ultimately be recognized as an important interdisciplinary aspect of integrative biology.

## ACKNOWLEDGEMENTS

We thank J. Ellis and A. Minton for their comments on the manuscript, and A. Minton and O. Medalia for kindly providing Figs 2 and 3, respectively.

## REFERENCES

- Baumeister W (2002) Electron tomography: towards visualizing the molecular organization of the cytoplasm. *Curr Opin Struct Biol* **12**: 679–684
- Bray D (1998) Signalling complexes: biophysical constraints on intercellular communication. *Annu Rev Biophys Biomol Struct* **27**: 59–75
- Bray D (2002) Bacterial chemotaxis and the question of gain. *Proc Natl Acad Sci USA* **99**: 7–9
- Brown MP, Royer C (1997) Fluorescence spectroscopy as a tool to investigate protein interactions. *Curr Opin Biotech* **8**: 45–49
- Dedmon MM, Patel CN, Young GB, Pielak GJ (2002) FlgM gains structure in living cells. *Proc Natl Acad Sci USA* **99**: 12681–12684
- Eaton WA, Hofrichter J (1990) Sick cell hemoglobin polymerization. *Adv Protein Chem* **40**: 63–279
- Eggers DK, Valentine JS (2001) Molecular confinement influences protein structure and enhances thermal protein stability. *Protein Sci* **10**: 250–261
- Elcock AH (2003) Atomic-level observation of macromolecular crowding effects: escape of a protein from the GroEL cage. *Proc Natl Acad Sci USA* **100**: 2340–2344
- Ellis RJ (2001) Macromolecular crowding: obvious but underappreciated. *Trends Biochem Sci* **26**: 597–604
- Ferrone FA, Hofrichter J, Eaton WA (1985) Kinetics of sickle hemoglobin polymerization. II. A double nucleation mechanism. *J Mol Biol* **183**: 611–631
- Fulton AB (1982) How crowded is the cytoplasm? *Cell* **30**: 345–347
- González JM, Jiménez M, Vélez M, Mingorance J, Andreu JM, Vicente M, Rivas G (2003) Essential cell division protein FtsZ assembles into one monomer-thick ribbons under conditions resembling the crowded intracellular environment. *J Biol Chem* **278**: 37664–37671
- Grunewald K, Medalia O, Gross A, Steven AC, Baumeister W (2003) Prospects of electron cryotomography to visualize macromolecular complexes inside cellular compartments: implications of crowding. *Biophys Chem* **100**: 577–591
- Hall D (2002) On the role of the macromolecular phase transitions in biology in response to change in solution volume or macromolecular composition: action as an entropy buffer. *Biophys Chem* **98**: 233–248
- Hall D, Minton AP (2003) Macromolecular crowding: qualitative and semi-quantitative successes, quantitative challenges. *Biochim Biophys Acta* **1649**: 127–139
- Han J, Herzfeld J (1998) Interpretation of the osmotic behavior of sickle cell hemoglobin solutions: different interactions among monomers and polymers. *Biopolymers* **45**: 299–306
- Herzfeld J (1996) Entropically driven order in crowded solutions: from liquid crystals to cell biology. *Acc Chem Res* **29**: 31–37
- Ivanova M, Jasuja R, Kwong S, Briehl RW, Ferrone FA (2000) Nonideality and homogeneous nucleation of sickle hemoglobin. *Biophys J* **79**: 1016–1022
- Jasuja R, Ivanova M, Ferrone FA (2002) Heterogeneous nucleation and crowding in sickle hemoglobin: an analytical approach. *Biophys J* **82**: 399–406
- Lebowitz JL, Helfand E, Praestgaard E (1965) Scaled particle theory of fluid mixtures. *J Chem Phys* **42**: 774–779
- Martin J (2002) Requirement for GroEL/GroES-dependent protein folding under nonpermissive conditions of macromolecular crowding. *Biochemistry* **41**: 5050–5055
- Martin J, Hartl FU (1997) The effect of macromolecular crowding on chaperonin-mediated protein folding. *Proc Natl Acad Sci USA* **94**: 1107–1112
- Medalia O, Weber I, Frangakis AS, Nicastro D, Gerisch G, Baumeister W (2002) Macromolecular architecture in eukaryotic cells visualized by cryoelectron microscopy. *Science* **298**: 1209–1213
- Minton AP (1977) Non-ideality and the thermodynamics of sickle-cell hemoglobin gelation. *J Mol Biol* **110**: 89–103
- Minton AP (1992) Confinement as a determinant of macromolecular structure and reactivity. *Biophys J* **63**: 1090–1100
- Minton AP (1998) Molecular crowding: analysis of effects of high concentrations of inert cosolutes on biochemical equilibria and rates in terms of volume exclusion. *Methods Enzymol* **295**: 127–149
- Minton AP (2000) Implications of macromolecular crowding for protein assembly. *Curr Opin Struct Biol* **10**: 34–39
- Minton AP (2001) The influence of macromolecular crowding and macromolecular confinement on biochemical reactions in physiological media. *J Biol Chem* **276**: 10577–10580
- Ralston GB (1990) The effect of 'crowding' in protein solutions. *J Chem Educ* **67**: 857–860
- Record TM Jr, Courtenay ES, Cayley S, Guttman HJ (1998) Biophysical compensation mechanisms buffering *E. coli* protein–nucleic acid interactions against changing environments. *Trends Biochem Sci* **23**: 190–194
- Rivas G, Fernandez JA, Minton AP (1999) Direct observation of the self-association of dilute proteins in the presence of inert macromolecules at high concentration via tracer sedimentation equilibrium: theory, experiment, and biological significance. *Biochemistry* **38**: 9379–9388
- Rivas G, Fernández JA, Minton AP (2001) Direct observation of the enhancement of non-cooperative protein assembly by macromolecular crowding: indefinite self-association of the bacterial cell division protein FtsZ. *Proc Natl Acad Sci USA* **98**: 3150–3155
- Rohwer JM, Postma PW, Kholodenko BN, Westerhoff HV (1998) Implications of macromolecular crowding for signal transduction and metabolite channeling. *Proc Natl Acad Sci USA* **95**: 10547–10552
- Ross PD, Minton AP (1977) Analysis of non-ideal behavior in concentrated hemoglobin solutions. *J Mol Biol* **112**: 437–452
- Somalinga B, Roy R (2002) Volume exclusion effect as a driving force for reverse proteolysis. *J Biol Chem* **277**: 43253–43261
- Uversky VN, Cooper EM, Bower KS, Li J, Fink AL (2001) Accelerated  $\alpha$ -synuclein fibrillation in crowded milieu. *FEBS Lett* **515**: 99–103
- van den Berg B, Ellis RJ, Dobson CM (1999) Effects of macromolecular crowding on protein folding and aggregation. *EMBO J* **18**: 6927–6933
- van den Berg B, Dobson CM, Ellis RJ (2000) Macromolecular crowding perturbs protein refolding kinetics: implications for folding inside the cell. *EMBO J* **19**: 3870–3875
- Verkman AS (2002) Solute and macromolecular diffusion in cellular aqueous compartments. *Trends Biochem Sci* **27**: 27–32
- Zimmerman SB (1993) Macromolecular crowding effects on macromolecular interactions: some implications for genome structure and function. *Biochim Biophys Acta* **1216**: 175–185
- Zimmerman SB, Minton AP (1993) Macromolecular crowding: biophysical, biochemical, and physiological consequences. *Annu Rev Biophys Biomol Struct* **22**: 27–65
- Zimmerman SB, Murphy LD (1996) Macromolecular crowding and the mandatory condensation of DNA in bacteria. *FEBS Lett* **390**: 245–248



Germán Rivas



Frank Ferrone



Judith Herzfeld