

# The physical chemistry of cytoplasm and its influence on cell function: an update

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**ABSTRACT** From the point of view of intermolecular interactions, the cytoplasmic space is more like a crowded party in a house full of furniture than a game of tag in an empty field. Understanding the physical chemical properties of cytoplasm is thus of key importance for understanding cellular function. This article attempts to provide an entrée into the current literature on this subject and offers some general guidelines for thinking about intracellular biochemistry.

**Monitoring Editor**

William Bement  
University of Wisconsin

Received: Apr 2, 2013

Revised: May 8, 2013

Accepted: Jul 2, 2013

## INTRODUCTION

Cellular cytoplasm is the context for all intracellular activities that are not sequestered within membrane-bounded organelles, and thus its physical chemical properties influence key cellular functions, including protein folding, enzyme catalysis, intracellular signaling, intracellular transport, and localization of molecules and organelles, as well as the fate of nanoparticles and therapeutic agents targeted to cells. In 2000, I published a review article in which I attempted to summarize the extensive literature bearing on the nature of the cell interior and in particular the extent to which it departs from the ideal dilute solution often assumed in classical biochemistry (Luby-Phelps, 2000). The principal conclusions of the review were that the aqueous phase of the cytoplasm is not a bag of freely diffusing enzymes but is crowded with macromolecules and that diffusive transport and partitioning of macromolecules and organelles in cytoplasm is highly restricted by steric hindrance, as well as by unexpected binding interactions. The purpose of this perspective is to review developments in the literature since 2000 and place them in context for the readership of *Molecular Biology of the Cell*.

## INTRACELLULAR WATER

The high concentration of macromolecules and the extensive surface area presented by intracellular membranes in eukaryotic cells

has led to proposals that association of intracellular water with surfaces leads to significant effects on its mobility and solvent properties compared with bulk water. If true, this would profoundly affect our understanding of such fundamental cellular processes as diffusion-limited biochemical interactions and protein folding. Experimental support for this view at the time of the previous review lacked direct measurement of water mobility in intact cells under physiological conditions. Since then, such measurements have become available (Jasnin *et al.*, 2008; Persson and Halle, 2008; Stadler *et al.*, 2008), including a study of water relaxation times in cubic micrometer-sized subvolumes within living COS-1 cells (Potma *et al.*, 2001). These studies suggest that at most 10–15% of intracellular water has altered mobility, and that although water molecules in the first layer of hydration may have relaxation times 10- to 15-fold lower than bulk, this does not propagate to water molecules over any significant distance, as the measured overall viscosity of intracellular water is only 70% higher than that of bulk water. Furthermore, water molecules hydrating proteins and other surfaces appear to be readily exchangeable with the bulk. Based on the evidence, there is no reason to suppose that hydration and solvation in the cell cytoplasm are significantly different from what is found in bulk water or that either the rotational or the translational diffusion of solutes in cytoplasm is much affected by the anomalous viscosity of cellular water.

## WHAT'S IN A CROWD?

The recognition that the cell cytoplasm is a highly crowded medium has led to much study and theorizing about the effects of macromolecular crowding on cellular biochemistry (for reviews see Dix and Verkman, 2008; Zhou *et al.*, 2008). Pure crowding effects typically are modeled as hard-sphere repulsive interactions that sterically exclude macromolecules in solution from the volume occupied by their neighbors. According to this excluded-volume model, at high

DOI:10.1091/mbc.E12-08-0617

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Abbreviation used: MSD, mean squared displacement.

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number concentration of macromolecules in solution, open space between molecules is reduced to the point that the free energy cost of making room for an additional molecule is thermodynamically significant. In the absence of other attractive or repulsive interactions between the macromolecules, this free energy cost may promote intermolecular interactions that are energetically unfavorable in dilute solution, much as two people unknown to each other or with little in common may find themselves engaged in conversation at a crowded party. Excluded-volume effects may also stabilize the native conformation of ordered proteins by disfavoring more-extended conformations, much as large arm movements are restricted at a crowded party for fear of hitting other guests. In addition, the crowding molecules present obstacles that may retard long-range translational movement, much as it takes longer to thread one's way around the other guests to cross the room at a crowded party. In the extreme limit, macromolecular crowding might result in confinement of macromolecules within subvolumes of the cytoplasm for significant lengths of time, much as the press of other guests, furniture in the way, and a narrow doorway may temporarily prevent one from moving from one room to another at the crowded house party.

Many of the predicted effects of macromolecular crowding have been demonstrated to occur in vitro in well-defined model systems. Several studies have shown that macromolecular crowding can promote protein folding (e.g., Hong and Gierasch, 2010; Stagg *et al.*, 2011) and stabilize the compact conformation of isolated metaphase chromatin (Hancock, 2012). Fewer results are available for the effects of crowding on reaction kinetics, but a temperature-dependent increase in  $K_{cat}$  has been reported for glucose-6-phosphate dehydrogenase in well-defined crowded media (Norris and Malys, 2011). It is now clear, however, that in the more complex intracellular environment, entropic excluded-volume effects are likely to be counteracted by enthalpic contributions from uncharacterized weak attractive or repulsive forces, with results that are not predictable a priori (Inomata *et al.*, 2009; Elcock, 2010; Schlesinger *et al.*, 2011; Wang *et al.*, 2012; Zhang *et al.*, 2012). An additional complication is that in complex mixtures like cytoplasm, crowded with multiple species of macromolecules of differing size, shape, and flexibility, some species may spontaneously demix and condense into stable droplet phases dispersed in the bulk, with unpredictable effects on any particular component (Long *et al.*, 2005). Thus it now seems that bottom-up approaches such as experiments in well-defined model systems in vitro or simulations in silico will provide only very general insight when considering the dynamics of a specific macromolecule in the cytoplasm of a specific cell.

### ANOMALOUS DIFFUSION (SUB OR SUPER?)

A variety of experimental measurements suggest that long-range translational diffusion of macromolecules in the cytoplasm may not match the expectations of normal diffusion in dilute aqueous solution, for which mean-squared displacement (MSD) is a linear function of elapsed time ( $MSD \propto t^x$ , where  $x = 1$ ). Over the past decade, a concept called anomalous diffusion has been adopted from the realm of physics to describe the diffusion of macromolecules in cells. In anomalous diffusion the relationship of MSD with time is nonlinear: cases in which the measured diffusion coefficient appears to decrease with elapsed time are referred to as subdiffusion ( $x < 1$ ), whereas cases in which the apparent diffusion coefficient increases with elapsed time are referred to as superdiffusion ( $x > 1$ ). Although subdiffusion is more often applied to cytoplasm, a recent theoretical treatment proposes that superdiffusion is more likely (Goychuk, 2012). This is a very active area of research, modeling, and simulation that so far has generated more heat than light

regarding whether intracellular diffusion is anomalous, what the value of its exponent is, and what the detailed mechanism might be. Experimental data from the various studies on diffusion in living cells are difficult to reconcile due to the nonoverlapping time and spatial scales of different methods of measurement, and the conclusions drawn from models and simulations often are difficult to test experimentally. A recent article by Saxton (2012) succinctly summarizes the state of play and calls for development of a set of reproducible standard samples as positive controls that could be used to exclude the contributions of differing experimental conditions, methodologies, and artifacts to the experimental data, as well as to test the predictions of various mechanistic models that have been proposed. Although anomalous diffusion clearly has implications for understanding any cellular process that depends on sampling of the cytoplasmic volume by diffusive transport, it is difficult to predict its effects without a clearer understanding of the extent to which anomalous diffusion actually describes intracellular dynamics. Reaction kinetics may be either faster or slower, depending on the type of anomalous diffusion and the time and distance scale under consideration.

### PHASE SEPARATION AND MICROCOMPARTMENTATION

The idea of aqueous phase separation as a self-organizing force in the cell interior dates back to the father of modern cell biology, E. B. Wilson, who proposed that non-membrane-bound compartments such as P-granules and Cajal bodies could be explained by the principles of colloid chemistry (Wilson, 1899). A colloid is a liquid with two phases: a microscopic droplet phase dispersed in a continuous phase. Homogenized whole milk is the classic example. Since Wilson's time, the idea of phase separation as a mechanism for cellular microcompartmentation has gone in and out of vogue (Welch and Clegg, 2010). Currently its popularity is resurging, partly as a result of renewed appreciation for how crowded the cytoplasm is. Crowding-induced phase separation is a well-studied phenomenon in colloid science. Phase separation of immiscible proteins in a crowded solution typically leads to formation of liquid droplets enriched in one or a subset of interacting proteins (Weber and Brangwynne, 2012). Other macromolecules and small solutes may partition into the droplet phase. In crowded solutions with many different protein species, the total protein concentration in droplets is not necessarily higher than in the surrounding medium, and thus there may be no difference in refractive index to make them visible by microscopy. Liquid droplets tend to adopt a minimum-energy, spherical shape unless deformed by external forces. They are dynamic in the sense that proteins readily exchange in and out of the droplet and that droplets encountering each other may coalesce. Examples of well-known intracellular inclusions that exhibit droplet behavior include P-bodies in germline cells of *Caenorhabditis elegans* and Cajal bodies in the nucleus (Hyman and Simons, 2012), as well as intracellular lipid droplets. Recent studies suggest that lipid droplets are not merely a trivial result of immiscibility between hydrophobic lipids and aqueous cytoplasm but instead may be the locus of lipid metabolism (Walther and Farese, 2012) and also may serve as an intermediate compartment in the endoplasmic reticulum-associated protein degradation pathway (Jo *et al.*, 2013).

Recent reports show that purified components of the N-WASP signaling pathway (Li *et al.*, 2012) and RNA-binding proteins in a cell lysate (Kato *et al.*, 2012) spontaneously phase separate under certain conditions in vitro. Overexpression of the protein interaction domains of two binding partners in the N-WASP signaling platform resulted in formation of similar liquid droplets in tissue culture cells (Li *et al.*, 2012). In these studies, phase separation was found to

depend on multivalent weak interactions between low-complexity repeat domains and/or disordered hydrophobic domains. Further experimentation on living cells is required to decide whether and how these observations are relevant physiologically.

An intriguing area of emerging research is the structure and function of bacterial microcompartments that encapsulate several enzymes of a metabolic pathway and sequester their substrates and intermediates (Yeates *et al.*, 2011). These microcompartments have a highly organized icosahedral protein shell similar to virus capsids. Small pores in the walls of the shell are postulated to permit gated exchange of small molecules between the shell interior and the cytoplasm. No analogous structures have been reported for higher organisms, but several metabolic pathways have been reported to form supramolecular assemblies microscopically visible as foci or fibers (O'Connell *et al.*, 2012).

## SIZE MATTERS AND THINGS CHANGE WITH TIME

Regardless of the details of the physical chemistry of cytoplasm, certain general concepts are clear. Anything targeted to the cell surface by receptor specific ligands or on nanoparticles will enter the cell primarily by endocytosis, and their transport will reflect the behavior and fate of the endocytic vesicle containing them unless there is some mechanism of escape from the endocytic compartment. Overexpressed proteins and agents delivered directly into the cytoplasm by methods that bypass the endocytic pathway will be subject to the same constraints on diffusion as endogenous intracellular solutes. It is inaccurate and misleading to think of cytoplasm as a homogeneous medium like a dilute solution, with a single viscosity that characterizes the rotational mobility of small molecules, the long-range translational diffusion of solutes, and the consistency of the bulk. The observed mobility of solutes in crowded, complex mixtures such as the cell interior will depend on the size of the solute and the time/space interval over which it is observed. In the absence of binding, the rotational and translational mobility of small molecules, such as ions and small organic solutes, will be unaffected by crowding or by obstruction due to fixed obstacles and should reflect the viscosity of intracellular water, which current evidence suggests is essentially like bulk water. Even macromolecules the size of a typical globular protein (~3 nm in radius) may diffuse normally over extremely short distances or on very short time scales because the probability of encountering barriers to diffusion in this space-time regime is relatively low. Thus reaction rates that depend on diffusion of the reactants over short distances will be relatively unaffected by excluded-volume effects on diffusion and will approximate those measured in dilute solution. For macromolecule-sized solutes on longer time and distance scales, it is necessary to consider the possible effects of crowding, obstruction by fixed obstacles, and transient confinement on solute mobility. Predicting these from first principles is very difficult, if not impossible, and for real biological molecules in the cytoplasm of living cells additionally depend on the specific size, shape, and deformability of the molecule under study, as well as on the effects of weak attractive or repulsive forces. To the extent that they experience transient binding interactions or partition into droplet phases, the mobility of molecules of any size may be slowed further. In this regard, two recent studies indicate that binding interactions are the dominant factors responsible for the extremely low mobility of globular proteins observed in *Escherichia coli* (Nenninger *et al.*, 2010; Wang *et al.*, 2011).

## LOCATION, LOCATION, LOCATION

The cytoplasmic compartment is inhomogeneous at nearly every length scale. In addition to randomly distributed local inhomogene-

ity driven stochastically by crowding and phase separation, nonrandom localization of intracellular vesicles, organelles, and supramolecular assemblies is a hallmark of eukaryotic cells. It is becoming clear that in prokaryotes, as well as in eukaryotes, individual protein and RNA molecules may also be nonrandomly localized within the cytoplasmic compartment (Nevo-Dinur *et al.*, 2012). An extensive literature suggests that the concentrations of even small signaling molecules such as cAMP and Ca<sup>2+</sup> may be locally regulated. It is important to remember that reported values for the physical properties of cytoplasm are spatially and temporally averaged and thus may not well describe the conditions in any particular subvolume of the cell.

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