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Biophysical Chemistry 98 (2002) 233–248

Biophysical Chemistry

www.elsevier.com/locate/bpc

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☆

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Received 3 December 2001; received in revised form 21 March 2002; accepted 21 March 2002

Abstract

We have used numerical simulation to demonstrate the potential for macromolecular precipitate solution phase transitions existing within the cell, to play a role in the minimization of changes in location or quaternary state of other macromolecular components, predicted to accompany changes in cell volume. For our modeling we have employed thermodynamic relations that take into account the large effects upon the thermodynamic activity coefficient produced by a solution environment that is highly volume occupied due to the presence of high concentrations of soluble macromolecule. The theoretical approach adopted, along with the simulated results, provide a framework for the interpretation of certain proteins' behavior (e.g. cytoskeletal elements such as tubulin and actin and possibly some prion structures) in response to cell volume change.

Keywords: Cell volume; Entropy buffer; Non-ideality; Cytoskeleton

1. Introduction

As our understanding of the composition and ultra-structure of the cell increases so does our appreciation of the highly complex environment in which the chemistry of the cell takes place [1]. Rather than being the encapsulated dilute aqueous media that we often portray in our in vitro-based assays, the cell is more properly considered as a membrane enclosed mixture of hydrated crystalline phases and solution environments containing a high density of micro-and macro-solutes [2]. The high density of macro-solutes suggests that the solution phases are highly volume occupied or 'crowded' [3]. Such chemical environments often mean that the chemical schema that we use to describe biological interactions occurring in ideal dilute solutions must be modified to account for the non-ideal nature imparted by the reaction conditions. The form of the correction, for equilibrium considerations, requires knowledge of the thermodynamic activity of the molecular species

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0301-4622/02/$ - see front matter
PII: S0301-4622(02)00072-8

of interest, rather than just the concentration per se.

A number of papers [4,5] have explored the possibility that due to the action of volume exclusion by high concentrations of dissolved protein in the cell cytoplasm, relatively small changes in cell volume could produce very large changes in the thermodynamic activity and hence, functional activity of some soluble proteins. These studies identified a likely mechanism for the restoration of basal cell volume by examining the change in solution thermodynamic activity of certain regulatory kinases and phosphatases in a crowded cell environment undergoing volume change. Unlike the condition of a high concentration of soluble non-associating protein encountered in those studies based upon resealed erythrocyte ghosts containing hemoglobin and albumin, the environment inside a cell also contains a large number of different proteins and other macromolecules that polymerize or co-polymerize to form a precipitate-like structure whose chemical state, can in many cases, be defined by relations pertaining to crystal growth [6,7]. In biophysical studies it has been shown that the liquid phase concentration of such molecules in vitro is commonly governed by a first-order phase transition, that is itself, characterized by a definite critical thermodynamic activity of soluble species [8–10]. The maintenance of the critical activity in solution being defined by the interplay between the solution concentration and the thermodynamic activity coefficient. Ready examples of such systems are provided by the many different proteins that constitute the cell cytoskeleton (such as actin microfilaments, intermediate filaments and microtubule networks) which can also account for a major proportion of the total protein within the cell [1,11]. Other prominent examples of protein systems that exist in some form of quasi-equilibrium between fiber and soluble monomer include the formation of the precipitous aggregates that result from (or perhaps cause) the diseases such as Creutzfeldt Jakob disease or Huntingtons Chorea [12].

Under conditions of biological ionic strength the activity coefficient of a macromolecular species can, to a good approximation, be calculated on the basis of the crowded solution as an imperfect gas comprised of hard objects with no intermolecular potential aside from the condition of impenetrability. Under such a scheme each molecule excludes the possibility of any other particle existing in solution within the volume element comprising the co-volume of the two particles in question [3,13]. As the physical manifestation of such excluded volume is dependent upon consideration of only macromolecular species that can be considered to be aqueous per se, any migration of soluble monomer to a precipitate phase would tend to lessen the concentration of total soluble protein and hence aid in the reduction of an excluded volume effect. As the tendency for soluble monomers to form precipitate species would be favored by an increase in the monomer activity coefficient brought about by the effect of excluded volume, the equilibrium between soluble and precipitate forms of the protein potentially presents itself as a means for thereby 'buffering' the effects that would otherwise lead to large changes in the thermodynamic activity of many cell species in response to changes in cell volume and/or alternately cell/cytosol composition.

In short, we investigate the role of the precipitate/solution phase transition of large macromolecular species to act as an entropy buffer[1] and thereby prevent large (and with regards to the cell—possibly harmful) changes in the activity of other solution species. The work utilizes numerical simulation to explore the likelihood and effects of such compensatory change upon certain key processes within the cell. The computer simulations are discussed in light of some available experimental evidence relating to volume regulation and changes in macromolecular composition.

2. Theory section

In this section the aim is to outline the thermodynamic relations describing a first-order phase transition between a soluble macromolecule and its precipitate phase, the means adopted for calculating an activity coefficient of a species in a

[1] The term entropy buffer arises from the fact that the explored effect manifests itself by maximizing available solution volume and hence, at the same time, maximizing the translational entropy of soluble species.

crowded solution, and the procedure for calculating the concentration or quaternary state of a particular protein species that may: (i) migrate across a cell membrane; (ii) undergo a monomer–dimer self association or; (iii) undergo higher order self-association.

Having described the fundamentals of each process a brief overview of the computing strategy used to explore the effect of a change in total solute concentration on both: (i) the precipitate/soluble monomer ratio and; (ii) the tracer proteins concentration or quaternary state will be given.

3. Solution–crystal phase transition

Many reversible helical and linear polymerization processes, at sufficient degree of polymerization, display behavior consistent with crystallization [7]. For the process of crystal growth we can consider the species M as existing in one of two phases: (i) the solution phase; and the (ii) the crystalline or precipitate phase. At constant temperature (T), pressure (P) and number of other species present in solution (n_j) the condition for equilibrium between the two phases is met when there is equality of the chemical potential of species M in each phase [1].

$$(\mu_{M(\mathrm{soln})})_{T,P,nj} = (\mu_{M(\mathrm{ppt})})_{T,P} \tag{1}$$

Expansion of the chemical potential into its usual form along with the reasonable assumption of the constant activity of species M (a_M) in the crystalline array with changing extent of precipitate, yields the characteristic relationship between the activity of monomer in solution and the association equilibrium constant (K_M) governing the phase transition process.

$$(a_M)_{\mathrm{soln}} = 1/K_M \tag{2a}$$

$$K_M = \exp\left(\frac{-\left((\mu^{\circ}_M)_{\mathrm{ppt}} - (\mu^{\circ}_M)_{\mathrm{soln}}\right)}{RT}\right) \tag{2b}$$

where R is the universal gas constant.

4. Calculation of an activity coefficient in a crowded solution

The thermodynamic activity of a chemical species is the hypothetical value of the ideal concentration to which the actual concentration would have to be raised or lowered to account for its behavior with regards to basic chemical processes, for instance, the extent of its reversible interaction with other molecules in solution. The calculation of a species activity usually takes the form of a functional modification of the molecules (i) solution concentration (C_i) by an activity coefficient (γ_i) which in its simplest form can be represented by Eq. (3).

$$a_i = \gamma_i C_i \tag{3}$$

The degree of non-ideal behavior, as manifested by a change in the value of the activity coefficient from unity, is a reflection of the difference in free energy between how a real molecule having finite shape, charge and size interacts with its real solution environment *vs*. how a real particle would interact with a hypothetical ideal solution environment comprised of solute molecules that exist in solution as uncharged, shapeless point masses.[2] Although a number of procedures exist for numerical calculation of the activity coefficient a few based on simplifying approximations have shown their utility in their application to in vitro solutions of proteins designed to approximate crowded cellular environments. Particularly encouraging in this regard is the finding that physical characteristics such as sedimentation equilibrium behavior [14–16] and osmotic pressure dependencies [17] of concentrated protein solutions near their iso-ionic point can often be adequately represented by models for the activity coefficient based on treatment of the protein solution as an imperfect gas [18] consisting of hard impenetrable particles having no additional intermolecular potential (aside from the condition of impenetrability) and dimensions commensurate with those determined from the X-ray crystallography or hydrodynamic measurements. Indeed, even for macromolecules in solution that possess significant charge, simple modifications of the hard particle model that either incorporate the charge effects somewhat explicitly [14] or alternately account for them indirectly by

[2] Therefore, it is a measure of the difference in energy between solute–solute interactions between the non-ideal case and the ideal case (in which there are no solute–solute interactions by definition).

allowing the hard particle dimensions to vary (i.e. hypothetical particle size increases for repulsive potential and decreases for attractive potential [19]) have proved to be significantly robust for the description of the observed solution properties. In this vein we adopt the Scaled Particle Theory approach [20–22] based on the hard particle approximation for evaluation of a solution activity coefficient. Although rigorously derived elsewhere [20–22] we describe the approach in the appendix with just sufficient detail to provide the reader with an understanding as to how reduction in the available volume brought about by molecular crowding can act to produce a significant non-ideality effect—the foundation upon which this simulation-based work is built.

5. The three generalized test cases

5.1. Partition of a protein between two phases—one of which is non-ideal

For a species that can partition between two phases the transport mechanism can be denoted by,

$$(B)_\alpha \rightleftarrows (B)_\beta \tag{4}$$

and the condition for partition equilibrium given by Eq. (5).

$$(\mu_B)_\alpha = (\mu_B)_\beta \tag{5}$$

Here α and β refer to two solution environments separated by a semi-permeable membrane. The membrane precludes the migration of inert crowder and cytoskeletal component but allows the free passage of tracer hence, enabling the two solutions to be regarded as two distinguishable phases. If α is the only non-ideal solution environment and K_p is the partition constant describing the ratio of activities of the tracer particle within the two phases then we express the ratio of activities by Eq. (6).

$$K_p = \exp\frac{\left[(\mu_B^\circ)_\alpha - (\mu_B^\circ)_\beta\right]}{RT} = \frac{(a_B)_\alpha}{(a_B)_\beta} \tag{6}$$

The observed partition constant ($(K_p)_{\mathrm{obs}}$) that would be recorded from an experimenters' perspective would be given by (given that the β phase is considered ideal)

$$(K_p)_{\mathrm{obs}} \equiv \frac{(C_B)_\alpha}{(C_B)_\beta} = \frac{K_p(\gamma_B)_\beta}{(\gamma_B)_\alpha} \tag{7}$$

By solving Eq. (7) with the constraint of mass conservation we can calculate the concentration of tracer protein in the β phase according to,

$$(C_B)_\beta = \frac{(K_P)_{\mathrm{obs}}(C_B)_{\mathrm{tot}}}{1+(K_P)_{\mathrm{obs}}} \tag{8}$$

5.2. Dimerization of a protein within a non-ideal crowded solution phase

For a species that can exist as a monomer–dimer equilibrium the overall reaction mechanism can be defined by Eq. (9).

$$2B \rightleftarrows B_2 \tag{9}$$

The association equilibrium constant describing the extent of dimer formation (K_{DIM}) is given by,

$$K_{\mathrm{DIM}} = \frac{(a_{B_2})}{(a_B)^2} \tag{10}$$

The apparent association constant ($(K_{\mathrm{DIM}})_{\mathrm{obs}}$) for any given extent of crowding is therefore,

$$(K_{\mathrm{DIM}})_{\mathrm{obs}} \equiv \frac{(C_{B_2})}{(C_B)^2} = \frac{K_{\mathrm{DIM}}(\gamma_B)^2}{\gamma_{B_2}} \tag{11}$$

By expressing the free monomer concentration in Eq. (11) in terms of the total and dimer concentrations we can calculate the concentration of dimer formed at equilibrium via application of the analytical solution for quadratic equations,

$$C_{B_2} = \frac{\left[(4(K_{\mathrm{DIM}})_{\mathrm{obs}}(C_B)_{\mathrm{tot}}+1) - \sqrt{(4(K_{\mathrm{DIM}})_{\mathrm{obs}}(C_B)_{\mathrm{tot}}+1)^2 - 16(K_{\mathrm{DIM}})_{\mathrm{obs}}^2(C_B)_{\mathrm{tot}}^2}\right]}{8(K_{\mathrm{DIM}})_{\mathrm{obs}}} \tag{12}$$

5.3. Higher order complex formation of a protein in a non-ideal crowded solution phase

For higher order complex formation (in this paper we consider trimer formation as the simplest example) the overall reaction mechanism can be written,

$$nB \rightleftarrows B_n \tag{13}$$

with the multimerization constant, K_M, defined[3] as

$$K_M = \frac{(a_{B_n})}{(a_B)^n} \tag{14}$$

and the experimentally observed association constant ($(K_M)_{obs}$) in the presence of crowder given by Eq. (15).

$$(K_M)_{obs} \equiv \frac{(C_{B_n})}{(C_B)^n} = \frac{K_M(\gamma_B)^n}{\gamma_{B_n}} \tag{15}$$

Expansion of Eq. (15) in terms of total and complexed concentrations for the case of trimer formation ($n=3$) yields the cubic polynomial

$$\begin{aligned} &-27(K_M)_{obs}(C_{B_3})^3 + 27(K_M)_{obs}(C_B)_{tot}(C_{B_3})^2 \\ &\quad -[9(K_M)_{obs}(C_B)^2_{tot} + 1]C_{B_3} \\ &\quad +(K_M)_{obs}(C_B)^3_{tot} = 0 \end{aligned} \tag{16}$$

Solutions to Eq. (16) were found numerically using an iterative bisection procedure. Physically meaningless solutions were filtered out by the imposition of concentration restraints that lead to the rejection of unrealistic roots.

6. The computational strategy

For all cases we start by considering a solution system that has a volume of 5 μl and is subject to a constant external pressure. This solution is composed of four components: (i) water; (ii) inert crowder (X); (iii) polymerizable component (M) and tracer molecule (B). We will regard (at least initially) only the three macromolecules explicitly and treat water as a continuous like ether—the case dictated by the McMillan and Mayer solution theory [18].

The number of molecules of each component in solution is set initially by proclamation. A total concentration of protein representative of cellular environs of 300 mg/ml [23] was used as a starting value for the sum of inert crowder and soluble polymerizable component. The concentration of tracer protein present was typically at 10^{-1} mg/ml or less. All macromolecular species constituting the crowder component were assigned a relative molecular mass of 50 000 and a spherical shape defined by a radius of approximately 3 nm in accordance with values obtained from experimental studies of prokaryotic cells [12].[4] In order to be absolutely identifiable with a spherically compact globular protein the tracer molecule was set to be a sphere of 2.5 nm of relative molecular mass 50 000.

After having established the initial parameter values the next required step involved: (i) the determination of the numerical value of the equilibrium constant that would be compatible with the proclaimed activity of the soluble polymerizable component; (ii) the calculation of the activity coefficients for the various forms of the tracer component (allowing the determination of the tracers initial quaternary state or concentration in the crowded phase).

At this stage we have defined the system in the thermodynamic sense, that is, we have specified the temperature, pressure and composition. The next event to occur that is common to all programmed variants discussed in this paper is the initiation of a sequence of volume reduction. This cycle involves decreasing the cell volume in a quasi-static fashion by 0.25% for each, of a number of incremental steps.[5] The sequence continues until the cell volume is equal to 75% of its initial volume. After each incremental volume change the molar concentration of each component in solution is recalculated and from these new values the value of the activity coefficients of the pertinent species are determined.

For the simplest case where no fraction of the total protein is made up of soluble polymerizable component, the activity coefficient of the tracer protein can be calculated directly (as the tracer protein by definition is sufficiently dilute to not

[3] (That pertains only to the case of an n-body concerted reaction and assumes that no intermediate associative states exist).

[4] These values should be recognized as reflecting a condensed 'average' value for the shape and size of the myriad of differently shaped and sized soluble cellular components.

[5] The quasi-static nature of the volume reduction sequence is emphasized to reinforce the fact that we are considering the system in terms of the equilibrium limit—therefore representing one limiting case of a system which, for the case of a cell undergoing volume change, is never at true thermodynamic equilibrium.

influence the extent of macromolecular crowding in solution). Knowledge of the activity coefficients then allows solutions to be calculated for the partition or association phenomenon operating on the tracer protein in each particular case.

For the situation in which some finite portion of the crowding material is constituted by the soluble precipitable component (and hence able to reversibly polymerize in response to volume change), an extra iterative procedure is implemented before calculation of the tracer components activity coefficient. The first step of this additional cycle involves the calculation of the activity coefficient of the soluble polymerizable component based on the total concentration of crowding species. Subsequent calculation of the free concentration of precipitable component using this activity coefficient and Eq. (2a) provides a new and improved guess of the true liquid phase concentration of the polymerizable component. This more accurate value of the solution phase concentration then allows the more accurate calculation of the polymerizable monomers' activity coefficient—allowing the iterative process to begin again until the specified convergence minimum is reached. Satisfaction of the convergence criteria yields a defined equilibrium concentration of the soluble monomer component capable of polymerization. This value can then be used to calculate the total crowder concentration and hence allow for the determination of the activity coefficients and physical state of the tracer protein.

7. Results

This investigation has employed numerical simulation to determine what possible role, an intracellular macromolecular system that: (i) is capable of reversible polymerization governed by a first-order phase transition; and (ii) comprises a demonstrable fraction of the cells total soluble macromolecular content—can play in the 'buffering' of changes in the location or quaternary state of other cellular macromolecules, expected to eventuate as a result of changes in cell volume (and hence total intracellular protein concentration) altering their thermodynamic activity via excluded volume effects.

To outline the effect that the existence of such a polymerizable system inside a cell might play, we have simulated a situation in which the polymerizable system constitutes exactly 1/12th (25 mg/ml)[6] of the total intracellular soluble macromolecular content (set at 300 mg/ml—see [23]). The other 11/12th (275 mg/ml) of the soluble macromolecular content existing in the cytoplasm of the cell is considered as wholly inert and therefore, not capable of any form of self-association. The simulation exercises consist of examining the effect of volume change upon the behavior of some additional hypothetical tracer species (tracer as it exists at trace amounts) for conditions where the

i. polymerizable system can either condense (according to a first-order phase transition),
ii. the distinct pool of monomer comprising the polymerizable system can alternately behave, like the majority of the soluble macromolecular content, as inert and hence incapable of undergoing the precipitate like phase transition.

The two cases are described pictorially by Fig. 1. The results of the numerical calculations for the effect of volume reduction on the concentration of soluble monomer belonging to the 1/12th pool are shown by Fig. 2. Note that for the case where the monomer is incapable of polymerization, the concentration of the monomer, comprising the distinct pool, increases with decreasing volume. However, for the situation in which the distinct monomer pool is capable of precipitation, we see that the concentration of soluble monomer decreases to virtually zero after a 20% decrease in cell volume. The situation described by Figs. 1 and 2 has been outlined first, as this describes the exact behavior that the distinct pool of polymerizable monomer will follow in each of the three special cases (describing the effect of this event upon a tracer species) to be discussed.

7.1. Partition of a protein between two phases—one of which is non-ideal

In this section we examine the effect of having a portion of the cells total soluble macromolecule

[6] One-twelfth was chosen as it describes a slightly low estimate of the fraction of the total protein existing as either tubulin or microtubules in mouse brain cells [11].

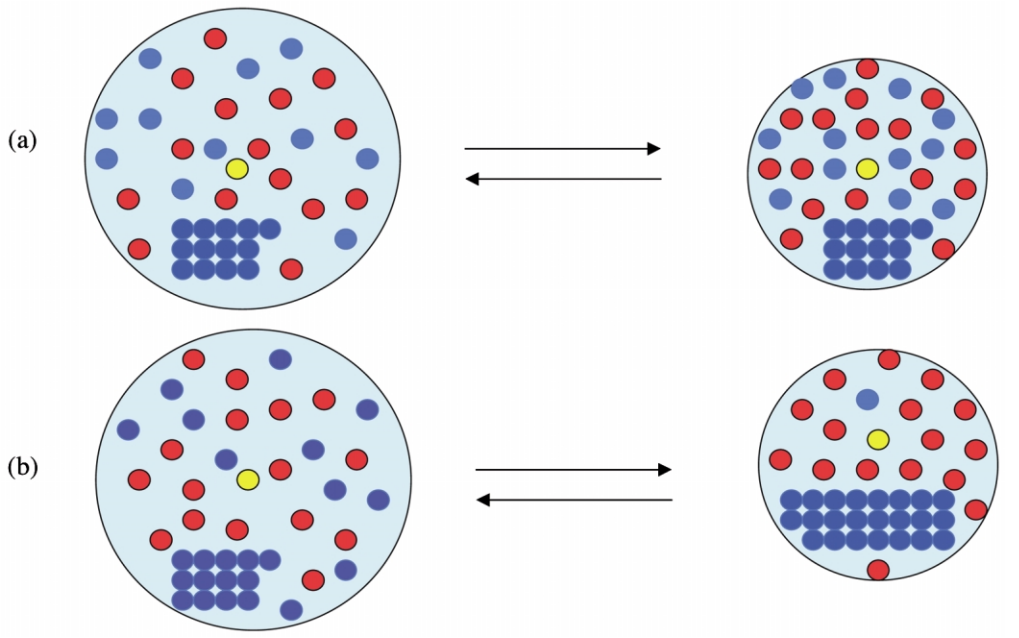

Fig. 1. Basic principle behind this investigation. Represents cases where cell undergoes reversible volume change where (A) no part of the total crowding component (red and blue species) can undergo a precipitate forming phase transition thereby increasing the solution phase macromolecule concentration and hence concomitantly, the thermodynamic activity of a tracer species (yellow). (B) Some minor fraction of the total crowding component (blue species) can undergo a precipitate forming phase transition thereby lessening both the increase in the solution phase macromolecule concentration and the increase in the thermodynamic activity of tracer species that would be expected to occur with the reduction in cell volume.

concentration capable of undergoing a precipitate phase transition, on the partition behavior of a tracer protein in response to changes in cell volume. The partition of the tracer species is considered to occur between a non-ideal cytoplasmic phase and a second ideal phase having an unchanging volume of 0.5 μl. The ideal partition constant, K_p was assigned a value of 1×10^{-4}. Fig. 3 describes the changes in the activity coefficient of the tracer protein in the cytoplasmic phase for the cases in which the 1/12th monomer pool is capable (lower curve) and incapable (upper curve) of undergoing a first-order phase transition to a precipitate state. It can be noted that the presence of a monomer pool which is 'competent',[7] amongst the total concentration of crowder macromolecules, acts to reduce the rise in the predicted activity coefficient brought about by the volume reduction sequence, in comparison to the case where no fraction of the soluble macromolecular cellular content is competent. The difference between the two cases spans over two orders of magnitude after a reduction in cell volume of 25%.

Fig. 4 describes the translated effect of the reduction in the thermodynamic activity coefficient (brought about by having a competent pool of monomer comprising 1/12th of the total soluble macromolecule concentration) on the actual concentration of tracer protein in the second (non-cytosolic) phase. As can be noted the presence of the competent pool diminishes significantly, the tendency for the tracer protein to leave the cytosolic phase and enter the second phase.

[7] Competent in the sense that it is capable of undergoing the phase transition, incompetent referring to the situation in which it is incapable of undergoing the phase transition.

7.2. Dimerization of a protein within a non-ideal crowded solution phase

Here we examine the effect, of a fraction of the cells total soluble crowder concentration being governed by a first-order phase transition, on the dimerization of a tracer protein in response to changes in cell volume. The dimer species was considered as a sphere of radius 1.26 times the radius of the tracer monomer. The tracer protein total concentration was set at 2.5×10^{-6} mg/ml and the dimerization association equilibrium constant was set at 1×10^{8} M^{-1}.

Fig. 5 describes the effect of the presence of an incompetent or competent fraction of the total macromolecular concentration pool, on the activity coefficient of monomer and dimer tracer species in response to volume change. We note that (as expected for identical geometries) the effect upon the tracer monomer activity coefficient is identical to the preceding case, however, there exists a large difference between the activity coefficient predicted for the tracer dimer species for the case where some part of the total crowding pool is able to undergo the precipitate phase transition *vs*. the case where it cannot.

From Fig. 6 we see that the predicted effect, for the case where a competent precipitable monomer pool exists as a fraction of the total crowding pool, on the extent of dimerization in response to volume change, is to lessen the extent of dimer formation when compared to the alternate case where no part of the total macromolecule component is able to self-associate.

7.3. Higher order complex formation of a protein in a non-ideal crowded solution phase

The final case that we consider is the effect, of a fraction of the total macromolecule concentration being either competent or incompetent, on the formation of a trimer of tracer from a tracer monomer in response to volume change. The trimer tracer was modeled as a sphere of 1.442 times the radius of a tracer monomer. The ideal trimerization association equilibrium constant was set at 5×10^{8} M^{-2} and the total concentration of the tracer was made equal to 0.01 mg/ml.

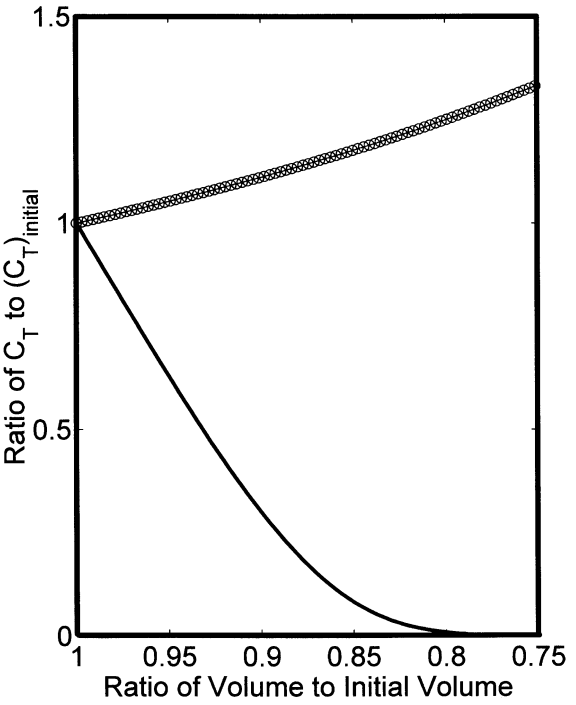

Fig. 2. Numerical calculation of the ratio of the change in the soluble concentration of the fractional component of total macromolecule that is considered as having the potential for undergoing a precipitate forming first-order phase transition, vs. the fractional change in cell volume. The upper line (circles) represents the case where the fractional component is incapable of forming a precipitate. The lower line describes the case where the fractional component is capable of undergoing the phase transition in response to volume change. (The polymerizable component initially constituted a 1/12 of the total soluble macromolecule concentration—25 mg/ml out of a total of 300 mg/ml.)

Fig. 7 describes the effect on the predicted change in the activity coefficient for trimer and monomer species in response to cell volume change for the two cases reflecting either the ability, or inability, of some part of the total crowding medium to reversibly undergo a precipitate forming phase transition. We note from Fig. 7 that the effect upon the activity coefficient of the trimer is over five orders of magnitude over the entire range of reduction in cell volume.

Fig. 8 describes how the effect of volume reduction on the tracer species activity coefficient

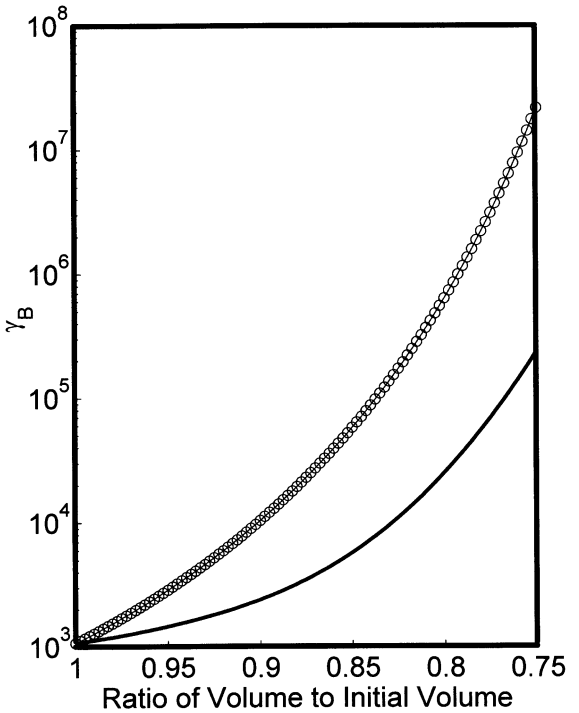

Fig. 3. Special Case 1: Tracer capable of partition equilibrium—calculation of the thermodynamic activity coefficient of the tracer species in the non-ideal cytosolic phase, on the basis of Eq. (10) in response to changes in cell volume (tracer having spherical geometry, radius equaling 3 nm). The upper line (circles) describes the case where the fractional component is incapable of forming a precipitate, the lower line describes the case where the fractional component is capable of undergoing the phase transition in response to a change in cell volume.

is manifested in terms of the actual concentration of the higher order complex. In the case where some of the total crowding component has the ability to polymerize, the effect is quite dramatic when compared to the alternate case. At the 20% reduction in cell volume stage less than 25% of the tracer species is present as trimer whereas, for the case where no part of the total crowder component can polymerize, nearly 50% of the tracer is present as trimer. It can be recalled from Fig. 2 that the effective 'buffering' range of the fractional pool of crowder is exhausted at the 20% stage of the cell volume decrease.

8. Discussion

8.1. Parallels in biology

Throughout the text, we have been careful to describe the components involved in the simulation exercises as macromolecules, crowding material and tracer species, without placing undue emphasis as to their possible biological nature within the cell. In this section we speculate as to some possible parallels of the process investigated here, with the biology of the cell.

There exists a large number of candidates in the cell for the 'entropy buffer'—the macromolecule composing a sizeable fraction of the cells total soluble macromolecule composition capable of undergoing a reversible first-order phase transition to a precipitate phase. Chief amongst these are the

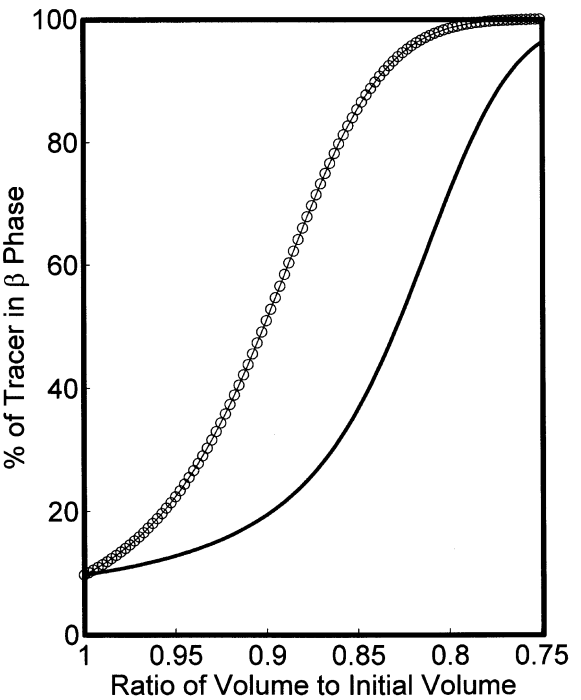

Fig. 4. Special Case 1: Tracer capable of partition equilibrium—calculation of the change in the percentage of tracer existing in the non-ideal phase in response to a change in cell volume for the case where a fraction of the total crowding component is (lower line) and is not (upper line) capable of forming a precipitate (the partition constant equal to 1×10^{-4}).

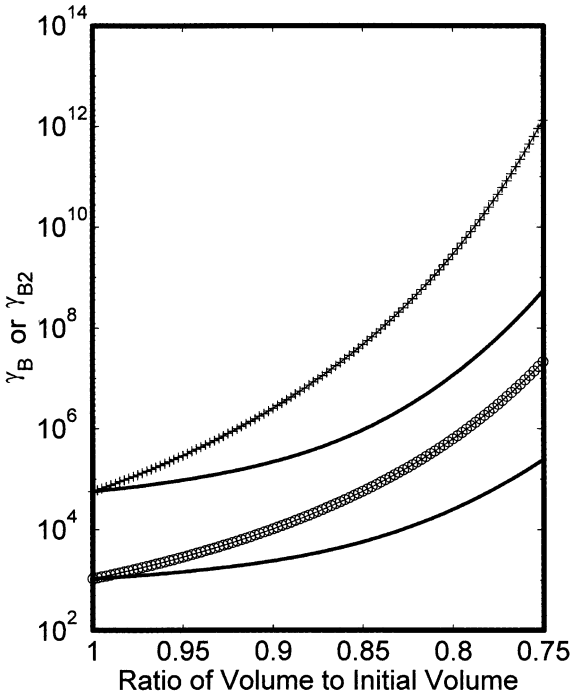

Fig. 5. Special Case 2: Tracer capable of dimerization—calculation of the thermodynamic activity coefficient of the tracer species existing in the monomeric (lower two curves) and dimeric form (upper curves) (tracer dimer having a spherical geometry of 1.26 times the radius of the monomer). The lines having only lines describe the case where a 1/12th fraction of the total crowding material is capable of forming a precipitate, the lines with symbols (+ and o) describe the case where no fractional component is capable of undergoing the phase transition in response to a change in cell volume.

cell cytoskeletal components, actin and tubulin which undergo polymerization behavior akin to a first-order phase transition to form the crystal-like, micro-filaments and micro-tubules, respectively [9,10]. Interesting in this regard are the great number of studies that have associated changes in the cell cytoskeleton with changes in cell volume which are in somewhat qualitative agreement with the behavior outlined above [24–28]. However, it must be cautioned that other experimental investigations into cytoskeletal behavior in response to volume change have produced results that are apparently inconsistent to the above predicted behavior—seemingly especially so for the polymerization of tubulin[8] [29]. As such we must resist against the tendency for simple generalizations in terms of the outlined model. It must be appreciated that a wide variety of changes are taking place in a cell in response to cell volume change, for example ionic strength and ionic composition and osmolyte concentration in addition to intracellular macromolecular crowding [31,32]. The effect of these other changes, once considered [33,34], will undoubtedly modify the way that we might interpret changes in the cell cytoskeleton in response

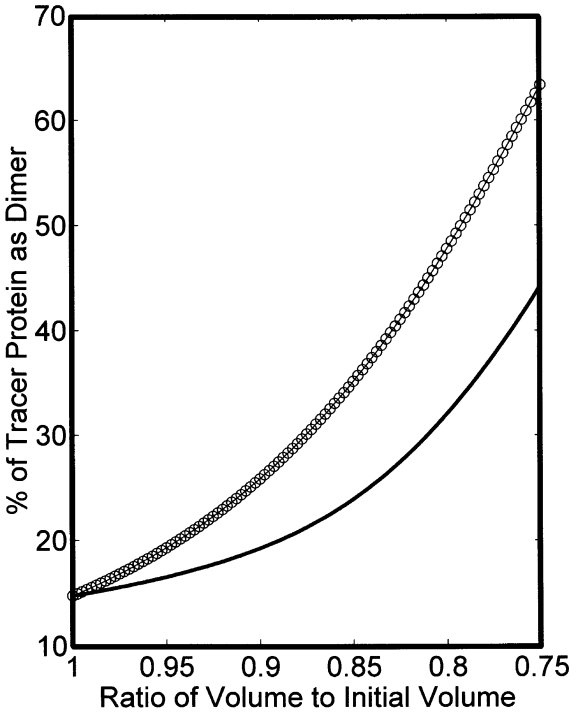

Fig. 6. Special case 2: Tracer capable of dimerization—calculated change in the percentage of monomer existing as dimer in response to a change in cell volume for the two distinct cases. The upper line (+) describes the case where no fractional component of the total crowding material is capable of forming a precipitate, the lower line describes the case where some fractional component is capable of undergoing the phase transition.

[8] An interesting experimental observation supportive of the proposed behavior comes from the in vitro study of osmotic pressure associated with a 5.5-mg/ml tubulin solution—the osmotic pressure decreasing upon polymerization of the protein (see Charmasson [30]).

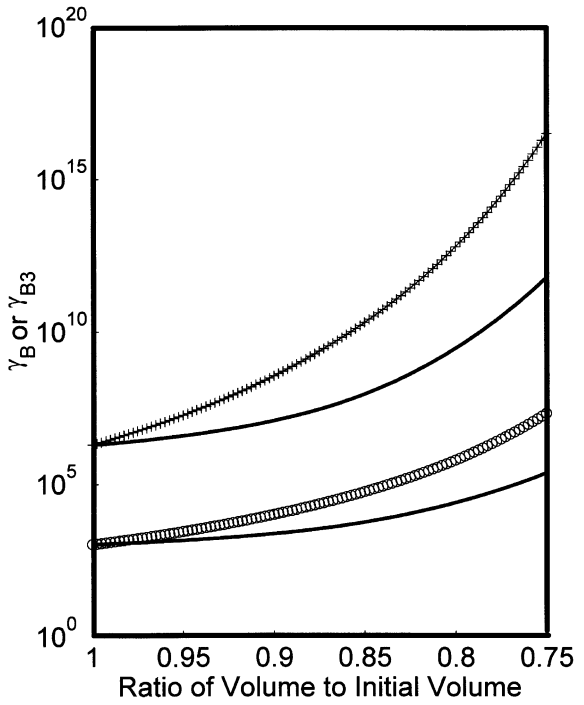

Fig. 7. Special case 3: Tracer capable of higher degree complex formation—calculation of the thermodynamic activity coefficient of the tracer species for monomer (lower two curves) and trimer form (upper curves) in response to changing cell volume (tracer trimer having a spherical geometry of 1.442 times the radius of the monomer). The lines having symbols (+ and o) describe the case where a 1/12th fraction of the total crowding material is capable of forming a precipitate, the lines not having symbols describe the alternate case.

to volume change, on the basis of the simplified arguments described above. However, it is here that we see the great power of the construction of our argument, developed as it is, in terms of free energy functions. We realize that any other additional effect will not dismiss the arguments made so far, but rather will have to be considered in addition to the effects described above.

In our simple model we have restricted the entropy buffer to a single class of molecule that can undergo a precipitate—solution phase transition. This in itself is a generalization necessitated by the author's desire for a simplified system for the demonstration of a principle. However, it could be expected that there exists a number of candidates capable of undergoing some form of phase transition to a precipitate state (to be removed from consideration in solution phase calculations). If true, this engenders the exciting possibility of the existence of a range of systems that may 'buffer' the thermodynamic activity of the cells components over different ranges of cell volume change. Indeed, evaluation of biological problems within the perspective of the arguments outlined in this paper, may provide us with an extra string to our bow in the discussion of precipitous like aggregates occurring at different stages of the cells age or life cycle.

Another interesting possibility not fully explored in this simulation exercise, was the converse role played in the growth of precipitate phases by changes in the total cell crowder concentration

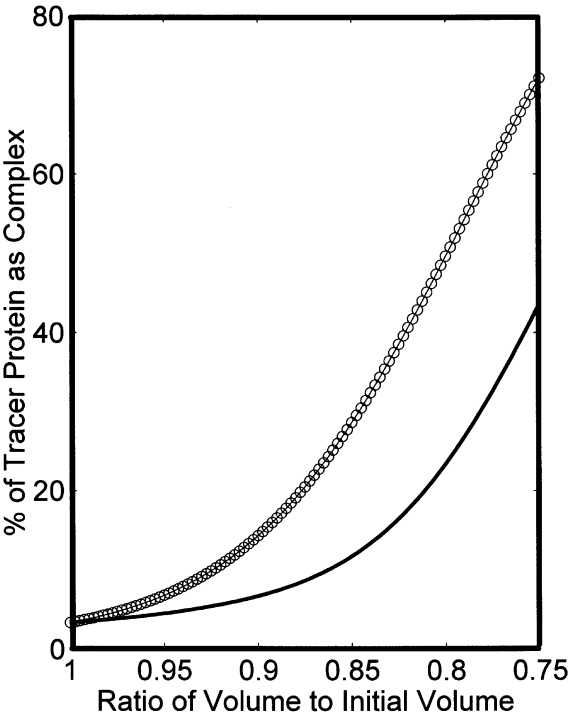

Fig. 8. Special case 3: Tracer capable of higher degree complex formation—predicted change in the percentage of monomer existing as trimer in response to a change in cell volume for the two distinct cases. The upper line (o) describes the case where no fractional component of the total crowding material is capable of forming a precipitate, the lower line describes the case where some fractional component is capable of undergoing the phase transition in response to changes in cell volume.

brought about by changes in cell volume or cell composition. Indeed the passage of macromolecule into the precipitate phase in response to changes in composition or volume may provide the physical 'switch' triggering the growth of cytoskeletal or other like structures.

The simulations describing the behavior of 'tracer species' location or quaternary state in response to changes in cell volume were conducted with the aim of shedding light on how individual macromolecular systems existing in the cell would respond to an osmotic challenge. Although necessarily simplified to a gross extent, there exists numerous parallels between the systems described and events occurring in the living cell. The partition case was meant to describe the possible passage of a protein between two separate phases existing within the cell, for example, the passive diffusion of mitogen activated protein kinase (MAPK) between the cytosol and the nucleus through the nuclear pores [35]. The simulations describing the effect of an entropy buffer on the behavior of a tracer capable of either dimerization or higher complex formation revealed the ability of the entropy buffer to minimize changes in the quaternary of the tracer. Changes in quaternary state of enzymes have been associated with changes to their functional enzymic activity. Ready examples of such quaternary state regulated systems include the deactivation of the p66 form of HIV-1 reverse transcriptase upon transition from the monomer to dimeric form [36] and the reduction in the enzymic activity of glyceraldehyde-3-phosphate upon formation of the tetramer form the monomer form [37].

We see in all of the simulation cases, that the effect of having an entropy buffer present was to minimize changes to the status quo existing before the imposed change.[9] If we acknowledge that changes in location or quaternary state would affect the enzymic function, we begin to realize the possible importance of the existence of such an entropy buffer to the maintenance of the cells normal functions.

[9] As such this whole exercise might be disingenuously described as a protracted demonstration of Le Chatelier's principle.

8.2. Limitations of the model

The model described is admittedly extremely simplified. This was done for two reasons. The first reason was to make the problem both mathematically tractable and understandable for, both the biochemist reading the work, and the biochemist writing the work. The second reason was that a major goal of this exercise was the conveyance of a conceptual framework, that could be put to use in the discussion of events affecting the cell, for instance changes in cell volume.

One obvious criticism of the simulations is the doubtfulness of using a sphere to describe the shape of a particle formed as a result of the coupling of a number of smaller hard spheres—such as we have done in the dimerization and multimerization cases. To maintain the reader's confidence it should be recognized that the use of the spherical shape instead of any other perhaps more realistic shape, say for instance, a dumb-bell, leads to a lessening of the estimate of the energy required for the particles existence. In this sense our simplifying assumption of constant spherical geometry is acting to quiet any tendency for exaggeration of the proposed buffering effect by ignoring the possibilities (and intricacies) of factoring in shape dependencies of the tracer component into the model. With regards to our selection of an average dimension and size for the intra-molecular crowding component it must be realized that our 'effective sphere' of 50 000 g/mole and radius equal to 3 nm represents an averaged value derived from experimental studies of cell cytosol containing many particles of different size and shape.[10]

[10] For the author's confidence similar calculations to those explored within this paper were carried out using the same total weight concentration of soluble intracellular component but with a geometry for the crowding component defined by a molecular weight of 50 000 g/mole and a spherical radius of 2.5 nm—the case representing a cell composed of wholly spherical globular proteins as the only intracellular soluble species. These calculations showed lower estimates for the increase in the activity coefficients and subsequent behavioral trends of the tracer protein but exhibited the same general tendencies outlined by cases explored within this paper. The results of these additional calculations are available upon request from the author.

We have not incorporated into the model the fact that proteins such as actin and tubulin often require the hydrolysis of a nucleotide triphosphate, such as ATP or GTP, for successful polymerization. Nor have we accounted for the fact that many times these proteins, in their cytoskeletal form, are associated with various accessory proteins such as microtubule-associated proteins (MAPs), or actin binding proteins (ABPs). However, the experimentally recorded phase transition behavior of these proteins, when either in pure form, or as a mixture containing accessory proteins, can be empirically described by a first-order process—thus justifying the approach adopted in the simulation strategy.

We have used the term 'entropy buffer' because the buffering principle outlined stems from considering non-ideality from the simple standpoint of excluded volume—the effects outlined are thus derived from modulating the available volume of the soluble components, and therefore necessarily, their translational entropy. We have not explored the role of molecular charge in the construction of the hypothetical non-ideal solution environment, although as described in the introduction this is often a legitimate approximation in the consideration of globular proteins at physiological ionic strength. The decision to set the extent of the amount of crowding entropy buffer to 1/12th of the total soluble concentration of macromolecule was done with vague notions that the proteins making up the cytoskeleton constitute this percentage or greater in many cell types [1,11]. Indeed even the assessment of the total intracellular soluble macromolecule concentration is an approximation derived by Zimmerman and Trach from physico-chemical studies of the bacterial cell of *E. coli* [23]. It is important to appreciate, however, that the principles outlined are independent of the parameter values chosen—the effect is a general one that will happen to some extent in all cases, the exact magnitude to be determined from future experimental observation.

9. Conclusions

In short, we have provided a fairly solid thermodynamic argument for subsequent interpretation of at least one aspect of the effect of cell volume or cell composition change within the cell. By use of numerical simulation we have demonstrated the possibility that macromolecular precipitate solution phase transitions existing within the cell could play a role in the minimization of changes in location or quaternary state of other macromolecular components, predicted to accompany changes in cell volume (or conversely macromolecular composition). We have coined the phrase 'entropy buffering' to describe the physical principle underlying the process behind the reduction of the increase in the thermodynamic activity coefficient of the other cellular species.

Acknowledgments

Initially, I would like to acknowledge Dr Leonard Nourse for a very interesting discussion that prompted this investigation—'Damien what would happen if the tubulin was the crowding material'. I would like to acknowledge the following people for reading the manuscript and offering helpful advice on improving both the form and content—Dr Jan Wolff, Dr Herman Edskes, Dr Kenji Sasahara, Dr Harry Saroff, Dr Peter McPhie, Dr Steven Zimmerman and Dr Allen Minton. I would additionally like to acknowledge my supervisor Dr Allen Minton for support and encouragement shown whilst working in his lab. Finally I express my gratitude to the US government for financial assistance in the form of a Fogarty Visiting Fellowship.

Appendix A: The development of the Scaled Particle Theory

As a prelude to the development of the Scaled Particle Theory approach for the determination of an activity coefficient we first identify what the activity coefficient constitutes in terms of the change in free energy of the system. In this vein a more instructive manner of expressing the chemical potential of a species is as follows,

$$\mu_i = (\mu_i)_{\text{ideal}} + (\mu_i)_{\text{non-ideal}} = \mu_i^\circ + RT\ln C_i + RT\ln\gamma_i \quad \text{(A1)}$$

where μ_i° refers to the standard state chemical potential.[11]

This formulation allows us to identify the activity coefficient of a species with the excess free energy required to bring about the existence of the real species in solution as compared to a hypothetical ideal species, requiring that

$$\gamma_i = e^{(\mu_i)\text{non-ideal}/RT} \tag{A2}$$

The Scaled Particle Theory [20] is a technique that has found success in predicting the activity coefficient of a macromolecular species (size $\gg$ intermolecular distance of the solvent) in solution. The method is based on the premise that the interaction between any two solute molecules, designated here as *A* and *B*, that have respective radii of r_A and r_B and are separated by a distance r_{AB} will be governed by a hard sphere interaction potential, $U(r_{AB})$. The hard sphere interaction potential implies an infinite intermolecular potential if the center of the incoming particle lies within the co-volume of any neighbor and a zero interaction potential if the center of the incoming particle lies outside the co-volume of its neighbor—all of which may be summarized in a mathematical sense by Eq. (A3).

$$\begin{aligned} r_{AB} < r_A + r_B &\Rightarrow U(r) = \infty \\ r_{AB} \geq r_A + r_B &\Rightarrow U(r) = 0 \end{aligned} \tag{A3}$$

With this concept on board a simple version of the Scaled Particle Theory applicable to spherical solutes can be developed in the following manner

(i.) Realize that the **probability of insertion of a real particle** (P_A) into a volume occupied solution such that its center will be lie outside the co-volume that it shares with all of the other particles in solution **will always be greater than the probability of insertion of a point particle except at the zero limit** of real particle size where the two probabilities will be equal. We can formally write out this probability (and therefore the free energy) of insertion for each particle (real and point) in terms of the characteristic dimensions of the incoming particle and solution components and relate the two probabilities via an inequality ($\geq$).[12]

Which may be expressed in compact form as,

$$\begin{aligned} P_A(r_A = 0) &= 1 - \rho_B \frac{4}{3}\pi(r_A + r_B)^3 \\ P_A(r_A \geq 0) &\geq P_A(r_A = 0) \\ W_A &= RT\ln(P_A) \end{aligned} \tag{A4}$$

where ρ_B is the number density of other solute molecules of type *B* already present in solution.

(ii.) The derivative of the work expression (at dimensions $r_A = 0$) with respect to the dimensions of the inserted particle is taken and evaluated. Knowledge of the value of this derivative for dimensions equal to zero along with the assumption of continuity for the derivative expression over the interval in question, allows for the expression of the work of insertion of a real particle by use of a Taylor series expansion about the zero point into the positive direction of the dimensions of the inserted particle.

$$\begin{aligned} W_A(r_A \geq 0) = W_A(r_A = 0) &+ \left(\frac{dW_A(r_A = 0)}{dr_A}\right) r_A \\ &+ 0.5\left(\frac{d^2W_A(r_A = 0)}{dr_A^2}\right) r_A^2 + ... \end{aligned} \tag{A5}$$

(iii.) The Taylor polynomial is truncated at the cubic term—with the final term in the work polynomial being replaced by the integral of the pressure, *P*, volume isotherm for the insertion of a

[11] More correctly we should write $\mu_i = \mu_i^{\circ} + RT \ln (C_i/C_i^{\circ}) + RT\ln(\gamma_i/\gamma_i^{\circ})$, however, we define our standard state such that C_i° and γ_i° are both equal to one.

[12] As a conceptual aid the actual probability of the successful insertion of a real particle into a volume occupied space can be thought of as the product of two probabilities, the second conditional upon the first. The first probability expresses the likelihood that the center of the particle to be inserted lies outside the physical volume occupied by the particles already present in solution (water being treated as a continuum). The second probability reflects the likelihood that the potential site for insertion lies outside the co-volume that would be shared by the incoming particle and the other particles in solution. Where this calculation becomes difficult for a real particle (i.e. dimensions >0) lies in the complex manner in which particles in solution share co-volume. The Scaled Particle Theory affords one avenue for solving the problem by expanding the total probability around the zero dimension point of the incoming particle—a starting point where the problem is completely solvable.

particle of dimensions equal to the final dimensions of the particle into a non-ideal gas. (This term necessarily depending upon the equation of state adopted to describe the non-ideality of the gas).

$$W_A(r_A \geq 0) = W_A(r_A = 0) + \left(\frac{dW_A(r_A = 0)}{dr_A} \right) r_A + 0.5 \left(\frac{d^2 W_A(r_A = 0)}{dr_A^2} \right) r_A^2 + P \frac{4}{3} \pi (r_A + r_B)^3 \quad \text{(A6)}$$

In this simulation exercise we utilize the Scaled Particle Theory developed by Gibbons [21] which although developed along the same basic argument, is a slightly more sophisticated version than the simple scheme outlined above. The exact form of the Gibbons Scaled Particle Theory is given by

$$\ln \gamma_A = -\ln Q + aBR_A/Q + bAR_A^2/Q + a^2 B^2 R_A^2/(2Q^2) + c\{\nu/Q + B^2 C/(3Q^3) + AB/Q^2\} R_A^3 \quad \text{(A7)}$$

where,

$$\nu = \sum_i \nu_i$$

$$A = a \sum_i \nu_i R_i$$

$$B = b \sum_i \upsilon_i R_i^2$$

$$C = a^2 \sum_i \upsilon_i R_i^2$$

$$Q = 1 - c \sum_i \upsilon_i R_i^3$$

and,

$\nu_i = w_i N_A/(1000 M_i)$ (number density per ml)

$R_i = \{M_i \upsilon_i / N_A c\}^{1/3}$ (characteristic radius)

where M_i is the relative molecular mass, υ_i is the partial specific volume and w_i the weight concentration of the ith species. N_A is Avagadro's number.

For spherical hard particles the scaled shape parameters are given by,

$$a = 1$$

$$b = 4\pi$$

$$c = 4\pi/3 \quad \text{(A8)}$$

References

[1] A.B. Fulton, How crowded is the cytoplasm?, Cell 30 (1982) 345–347.

[2] M. Hoppert, F. Mayer, Principles of macromolecular organization and cell function in bacteria and archaea, Cell Biochem. Biophys. 31 (1999) 247–284.

[3] A.P. Minton, The effect of volume occupancy upon the thermodynamic activity of proteins: some biochemical consequences, Mol. Cell. Biochem. 55 (1983) 119–140.

[4] A.P. Minton, G. Graig Colclasure, J.C. Parker, Model for the role of macromolecular crowding in regulation of cellular volume, Proc. Nat. Acad. Sci. USA 89 (1992) 10504–10506.

[5] G. Craig Colclasure, J.C. Parker, Cytosolic protein concentration is the primary volume signal for swelling-induced [K-Cl] cotransport in dog red cells, J. Gen. Physiol. 98 (1992) 881–892.

[6] F. Oosawa, S. Asakura, Thermodynamics of the Polymerization of Protein. Chapter 3, Academic Press, 1975.

[7] F. Oosawa, M. Kasai, Theory of linear and helical aggregations of macromolecules, J. Mol. Biol. 4 (1962) 10–21.

[8] R.A. Lindner, G. Ralston, Macromolecular crowding: effects on actin polymerisation, Biophys. Chem. 66 (1997) 57–66.

[9] F. Gaskin, C.A. Cantor, M.L. Shelanski, Turbidimetric studies of the in vitro assembly and disassembly of porcine neurotubules, J. Mol. Biol. 89 (1974) 737–758.

[10] C. Frieden, Actin and tubulin polymerization: the use of kinetic methods to determine mechanism, Ann. Rev. Biophys. Biophys. Chem. 14 (1985) 189–210.

[11] P.J. Anderson, The structure and amount of tubulin in cells and tissues, J. Biol. Chem. 254 (1979) 2168–2171.

[12] S.B. Prusiner, in: S.B. Prusiner (Ed.), Prion Biology and Disease (Chapter 1), Cold Spring Harbor Laboratory Press, New York, 1999.

[13] P.R. Wills, W.D. Comper, D.J. Winzor, Thermodynamic nonideality in macromolecular solutions: interpretation of virial coefficients, Arch. Biochem. Biophys. 300 (1993) 206–212.

[14] P.R. Wills, D.R. Hall, D.J. Winzor, Interpretation of thermodynamic non-ideality in sedimentation equilibrium experiments on proteins, Biophys. Chem. 84 (2000) 217–225.

[15] J. Behlke, O. Ristau, Analysis of the thermodynamic non-ideality of proteins by sedimentation equilibrium experiments, Biophys. Chem. 76 (1999) 13–23.

[16] A.P. Minton, M.S. Lewis, Self-association in highly concentrated solutions of myoglobin: a novel analysis

of sedimentation equilibrium of highly nonideal solutions, Biophys. Chem. 14 (1981) 317–324.
[17] A.P. Minton, A molecular model for the dependence of the osmotic pressure of bovine serum albumin upon concentration and pH, Biophys. Chem. 57 (1995) 65–70.
[18] W.G. McMillan, J.E. Mayer, The statistical thermodynamics of multi-component systems, J. Chem. Phys. 13 (1941) 276–305.
[19] A.P. Minton, H.P. Edelhoch, Light scattering of bovine serum albumin solutions: extension of the hard particle model to allow for electrostatic repulsion, Biopolymers 21 (1979) 1308–1314.
[20] H. Reiss, H.L. Frisch, J.L. Lebowitz, Statistical mechanics of rigid spheres, J. Chem. Phys. 31 (1959) 369–380.
[21] R.M. Gibbons, The scaled particle theory for particles of arbitrary shape, Mol. Phys. 17 (1969) 81–86.
[22] R.C. Chatelier, A.P. Minton, Sedimentation equilibrium in macromolecular solutions of arbitrary concentration. I. Self-associating proteins, Biopolymers 26 (1987) 507–524.
[23] S.B. Zimmerman, S.O. Trach, Estimation of macromolecule concentrations and excluded volume effects for the cytoplasm of *Escherichia coli* (1991), J. Mol. Biol. 222 (1991) 599–620.
[24] T.H. Larsen, H. Dalen, R. Boyle, M.M. Souza, M. Lieberman, Cytoskeletal involvement during hypo-osmotic swelling and volume regulation in cultured chick cardiac myocytes, Histochem. Cell. Biol. 113 (2000) 479–488.
[25] J.W. Mills, The cell cytoskeleton: possible role in volume controls, Curr. Topics Memb. Transp. 30 (1987) 75–101.
[26] J. Zhang, T.H. Larsen, M. Lieberman, F-actin modulates swelling-activated chloride current in cultured chick cardiac myocytes, Am. J. Physiol. 273 (1997) C1215–1224.
[27] E.M. Schwiebert, J.W. Mills, B.A. Stanton, Actin-based cytoskeleton regulates a chloride channel and cell volume in a renal cortical collecting duct cell line, J. Biol. Chem. 269 (1994) 7081–7089.
[28] J.H. Henson, Relationships between the actin cytoskeleton and cell volume regulation, Microsc. Res. Tech. 47 (1997) 155–162.
[29] R.N. Melmed, P.J. Karanian, R.D. Berlin, Control of cell volume in the J774 macrophage by microtubule disassembly and cyclic AMP, J. Cell Biol. 90 (1981) 761–768.
[30] R. Charmasson, Osmotic effects of tubulin (brain contractile protein) polymerization. A possible role in cell salt and water regulation, Physiol. Chem. Phys. 13 (1981) 11–14.
[31] F. Lang, G. Busch, H. Völkl, The diversity of volume regulatory mechanisms, Cell Physiol. Biochem. 8 (1998) 1–45.
[32] M.M. Garner, M.B. Burg, Macromolecular crowding and confinement in cells exposed to hypertonicity, Am. J. Physiol. 266 (1994) C877–C892.
[33] D.R. Hall, M.P. Jacobsen, D.J. Winzor, Stabilizing effect of sucrose against irreversible denaturation of rabbit muscle lactate dehydrogenase, Biophys. Chem. 57 (1995) 47–54.
[34] F. Lang, G. Busch, M. Ritter, et al., Functional significance of cell volume regulatory mechanisms, Physiol. Rev. 78 (1998) 247–306.
[35] M. Adachi, M. Fukuda, E. Nishida, Two co-existing mechanisms for nuclear import of MAP kinase: passive diffusion of a monomer and active transport of a dimer, EMBO J. 18 (1999) 5347–5358.
[36] J.F. Cabodevilla, L. Odriozola, E. Santiago, J.J. Martinez-Irujo, Factors affecting the dimerization of the p66 form of HIV-1 reverse transcriptase, Eur. J. Biochem. 268 (2001) 1163–1172.
[37] A.P. Minton, J. Wilf, Effect of macromolecular crowding upon the structure and function of an enzyme: glyceraldehyde-3-phosphate dehydrogenase, Biochemistry 20 (1981) 4821–4826.