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A generalized free-solvent model for the osmotic pressure of multi-component solutions containing protein–protein interactions

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ABSTRACT

The free-solvent model has been shown to have excellent predictability of the osmotic pressure for single and binary non-interactive proteins in aqueous solutions. Here the free-solvent model is extended to be more generalized by including the contributions of intra- and inter-protein interactions to the osmotic pressure of a solution in the form of homo- and hetero-multimers. The solute–solvent interactions are considered to be unique for each homo- and hetero-multimer in solution. The effect of the various generalized free-solvent model parameters on the osmotic pressure are examined for a single protein solution with a homo-dimer, a binary protein solution with no protein–protein interactions, and a binary protein solution with a hetero-dimer. Finally, the limitations associated with the generalized free-solvent model are discussed.

1. Introduction

The non-ideal osmotic pressure of proteins at high concentrations has been a source of significant interest for many decades. While clearly concentrated solutions may exist in many separations processes, even in the cell, concentrations of crowded proteins (multiple species) can be as high as 400 g/L [1,2]. While many models have focused on protein–protein interactions to fundamentally explain this phenomena but with little success. The majority of these models use a virial expansion model which is based on McMillan–Mayer Theory [3]. Once more, these models lack physically realistic parameters and are unable to confidently predict the osmotic pressure of concentrations near-saturation.

More recently, we have reexamined a free-solvent model to elucidate the physics of these systems. Briefly, the original free-solvent model was developed by van Laar [4] and further by Lewis and Randall [5]. van Laar [4] proposed that solvent–solute interactions are coupled to the observed non-ideal behavior of the osmotic pressure and he argued that the mole fraction is the appropriate concentration variable to describe osmotic pressure. Recently, Yousef et al. [6,7] revised the free-solvent model to include protein-ion binding. Further, the revised free-solvent model [6,7] describes the solute as a hydrated macromolecule, which contains a monolayer of water and bound ions, and, upon correcting the mole fraction for these interactions, provides excellent predictions for the observed osmotic pressure of single and binary non-interacting protein solutions. Once more, the free-solvent model is based on two independently measurable physical parameters, protein hydration and protein-ion binding, [8–12].

While previous developments of the free-solvent model have fully described the solutions modeled (*i.e.* pH and salt(s)), a generalized free-solvent model in which intra- and inter-protein interactions occur does not exist. Therefore, a more generalized free-solvent model should provide a more physically realistic model of the osmotic pressure of interacting proteins in aqueous solutions. Here, the generalized free-solvent model is developed for multi-component solutions which protein–protein interactions can occur in the form of homo-multimers (intra-species interactions) and hetero-multimers (inter-species interactions).

2. The generalized free-solvent model

2.1. Development of the free-solvent model to include protein–protein interaction

The free-solvent model has been described in detail elsewhere for non-interactive protein solutions [6,7]. The following is the development of the generalized free-solvent model which accounts for protein hydration and ion binding as well as protein–protein interactions.

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Nomenclature

$l_{\text{homo-multimer}}$ number of homo-multimers formed
$l_{\text{hetero-multimer}}$ number of hetero-multimers formed
$l_{\text{hetero-multimer},h}$ number of hetero-multimers forming the h multimer
N_i^k initial number of moles of species i in compartment k
N^k initial total number of moles in compartment k
$N^{\text{II}}_{\text{homo-multimers}}$ moles of protein forming homo-multimers (intra-protein interactions) in chamber II
$N^{\text{II}}_{\text{hetero-multimers}}$ moles of protein forming hetero-multimers (inter-protein interactions) in chamber II
$N^{\text{II}}_{\text{monomers}}$ moles of protein remaining as free (unbound) monomers in chamber II
R ideal gas constant
T temperature
$\bar{V}_i$ specific volume of species i

Greek
$\alpha_{j,Z}$ fractional amount of protein j forming the homo-multimer of Z units
$\beta_{ja,A:B}$ fractional amount of protein j forming a hetero-multimer with protein a containing A units of protein j and B units of protein a
$\beta_{jab,A:B:C}$ fractional amount of protein j forming a hetero-multimer with proteins a and b containing A units of protein j, B units of protein a, and C units of protein b
η_h size of the h multimer (i.e. $\eta_2 = 2$ for a two protein species interactions, $\eta_3 = 3$ for a three protein species interactions, etc.)
v_{ij} net number of moles of solvent component i interacting with protein j
$v_{ja,A:B}$ moles of solute j bound to the hetero-multimer between proteins j and a with A units of protein j and B units of protein a.
$v_{jab,A:B:C}$ moles of solute j bound to the hetero-multimer between proteins j, a, and b with A units of protein j, B units of protein a, and C units of protein b.
$v_{ij,Z}$ moles of solvent species i bound to the protein j homo-multimer with Z units
$v_{jj,Z}$ moles of protein j forming a homo-multimer with Z units
$v_{ija,A:B}$ moles of solvent species i bound to the hetero-multimer between proteins j and a with A units of protein j, and B units of protein a
$v_{ijab,A:B:C}$ moles of solvent species i bound to the hetero-multimer between proteins j, a, and b with A units of protein j, B units of protein a, and C units of protein b
$v_{\text{solvent/homo-multimer}}$ moles of solvent bound to the homo-multimers
$v_{\text{solvent/hetero-multimer}}$ moles of solvent bound to the hetero-multimers
$v_{\text{solvent/monomer}}$ moles of solvent bound to the free monomers
$vN^{\text{II}}_{\text{homo-multimers}}$ moles of diffusible species (water and salt) bound to the homo-multimers
$vN^{\text{II}}_{\text{hetero-multimers}}$ moles of diffusible species (water and salt) bound to the hetero-multimers
$vN^{\text{II}}_{\text{monomers}}$ moles of diffusible species (water and salt) bound to the free monomers
π osmotic pressure

Superscripts
I compartment I (solvent)
II compartment II (solution)

Subscripts
1 solvent
$2 \to (p+1)$ proteins
$(p+2) \to n$ salts
h type of multimer (*i.e.* $h = 2$ for a two protein species interactions, $h = 3$ for a three protein species interactions, etc.)
i individual species
j individual monomeric protein species
k compartment of the osmometer
n number of individual species
p number of individual monomeric proteins

For a two-chamber osmometer, with the chamber containing the proteins in aqueous solution denoted as compartment II and the chamber containing only the solvent and diffusible ions (proteins are absent) denoted as compartment I, the free-solvent model, with the mole fraction chosen as the appropriate composition variable, describes the osmotic pressure, π, as [6]

$$\pi - \frac{RT}{\bar{V}_1}\ln\frac{x_1^{\text{II}}}{x_1^{\text{I}}} \tag{1}$$

where the free-solvent mole fraction, x_1, is the remaining moles of solvent that are not bound to the protein.

For a solution containing n distinct species with p proteins, where species 1 is the solvent, species 2 through $(p+1)$ are the proteins, and species $(p+2)$ through n are the remaining diffusible solvent components, the initial total moles of the solution in compartment II is $\sum_{i=1}^{n} N_i^{\text{II}}$, where i denotes each individual species.

The final total moles of solution in compartment II, after solute–solvent and solute–solute interactions occur, is

$$\sum_{\substack{i=1\\ i\neq 2\to p+1}}^{n} N_i^{\text{II}} + \sum N^{\text{II}}_{\text{homo-multimers}} + \sum N^{\text{II}}_{\text{hetero-multimers}} + \cdots + \sum N^{\text{II}}_{\text{monomers}} - \sum vN^{\text{II}}_{\text{homo-multimers}} - \sum vN^{\text{II}}_{\text{hetero-multimers}} - \sum vN^{\text{II}}_{\text{monomers}} \tag{2}$$

where the first term is the moles of solvent and salt species, the second term is the moles of protein forming homo-multimers (intra-protein interactions), the third term is the moles of protein forming hetero-multimers (inter-protein interactions), the fourth term is the moles of protein remaining as free (unbound, non-interacting) monomers, and the fifth, sixth, and seventh terms are the moles of diffusible species (water and salt) bound to the homo-multimers, hetero-multimers, and free monomers, respectively.

The moles of proteins forming homo-multimers is given as

$$\sum N^{\text{II}}_{\text{homo-multimers}} = \sum_{j=2}^{p+1}\sum_{Z=\text{ii}}\alpha_{j,Z}N_j^{\text{II}} \tag{3}$$

where N_j^{II} is the moles of protein species j in solution initially and $\alpha_{j,Z}$ is the fractional amount of protein j forming the homo-multimer of Z units.

The moles of solvent bound to the homo-multimers are

$$\sum vN^{\text{II}}_{\text{homo-multimers}} = \sum_{\substack{i=1\\ i\neq 2\to p+1}}^{n}\sum_{j=2}^{p+1}\sum_{Z=\text{ii}} v_{ij,Z}\alpha_{j,Z}N_j^{\text{II}} \tag{4}$$

where $v_{ij,Z}$ is the moles of solvent species i bound to the protein j homo-multimer with Z units.

The moles of monomeric proteins forming hetero-multimers is given as