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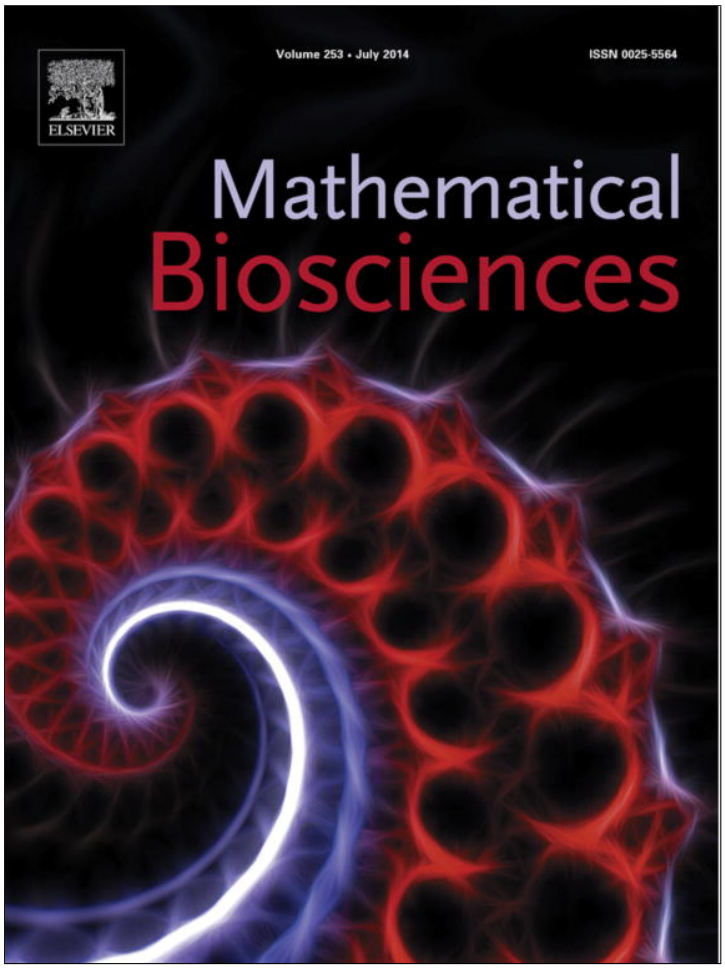

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A generalized free-solvent model for the osmotic pressure of multi-component solutions containing protein–protein interactions

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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.)
ν_{ij} net number of moles of solvent component i interacting with protein j
$\nu_{ja,A:B}$ 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.
$\nu_{jab,A:B:C}$ 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.
$\nu_{ij,Z}$ moles of solvent species i bound to the protein j homo-multimer with Z units
$\nu_{jj,Z}$ moles of protein j forming a homo-multimer with Z units
$\nu_{ija,A:B}$ 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
$\nu_{ijab,A:B:C}$ 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
$\nu_{\text{solvent/homo-multimer}}$ moles of solvent bound to the homo-multimers
$\nu_{\text{solvent/hetero-multimer}}$ moles of solvent bound to the hetero-multimers
$\nu_{\text{solvent/monomer}}$ moles of solvent bound to the free monomers
$\nu N^{\text{II}}_{\text{homo-multimers}}$ moles of diffusible species (water and salt) bound to the homo-multimers
$\nu N^{\text{II}}_{\text{hetero-multimers}}$ moles of diffusible species (water and salt) bound to the hetero-multimers
$\nu N^{\text{II}}_{\text{monomers}}$ 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
$2 \rightarrow (p+1)$ proteins
$(p+2) \rightarrow n$ 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

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

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

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

where $\nu_{ij,Z}$ 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

$$\sum N^{\mathrm{II}}_{\text{hetero-multimers}} = \begin{bmatrix} \sum_{j=2}^{p}\sum_{a=j+1}^{p+1}\sum_{A=\mathrm{i}}\sum_{B=\mathrm{i}}\beta_{ja,A:B}N_j^{\mathrm{II}} + \cdots \\ \cdots + \sum_{j=2}^{p-1}\sum_{a=j+1}^{p}\sum_{\substack{b=j+2\\ b\neq a}}^{p+1}\sum_{A=\mathrm{i}}\sum_{B=\mathrm{i}}\sum_{C=\mathrm{i}}\beta_{jab,A:B:C}N_j^{\mathrm{II}} + \cdots \end{bmatrix} \tag{5}$$

where N_j^{II} is the moles of protein species j in solution initially, $\beta_{ja,A:B}$ is the fractional amount of protein j forming a hetero-multimer with protein a containing A units of protein j and B units of protein a, and $\beta_{jab,A:B:C}$ is the 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.

Here, Eq. (5) only accounts for the protein hetero-multimers in which two and three unique protein species form hetero-multimers. The first term is the moles of the hetero-multimers formed from two unique protein species. The second term is the moles of the hetero-multimers formed from three unique protein species. Each term includes the hetero-multimers in which multiple molecules of protein j bind to multiple molecules of another protein(s) to form hetero-multimers of various homo-multimers (*i.e. jjaaab* is a hetero-multimer formed by a homo-dimer (*jj*), a three protein species homo-trimer (*aaa*), and a monomer (*b*)). Additional terms can be included in Eq. (5) to account for hetero-multimers of any number of unique proteins. For all hetero-multimers, solute–solvent interactions need to be included (Eq. (6)) and the moles of these hetero-multimers need to be removed from the available monomers (Eqs. (7) and (8)).

The moles of solvent bound to the hetero-multimers are

$$\sum \nu N^{\mathrm{II}}_{\text{hetero-multimers}} = \begin{bmatrix} \sum_{\substack{i=1\\ i\neq 2\to p+1}}^{n}\sum_{j=2}^{p}\sum_{a=j+1}^{p+1}\sum_{A=\mathrm{i}}\sum_{B=\mathrm{i}}\nu_{ija,A:B}\beta_{ja,A:B}N_j^{\mathrm{II}} + \cdots \\ \cdots + \sum_{\substack{i=1\\ i\neq 2\to p+1}}^{n}\sum_{j=2}^{p-1}\sum_{a=j+1}^{p}\sum_{\substack{b=j+2\\ b\neq a}}^{p+1}\sum_{A=\mathrm{i}}\sum_{B=\mathrm{i}}\sum_{C=\mathrm{i}}\nu_{ijab,A:B:C}\beta_{jab,A:B:C}N_j^{\mathrm{II}} + \cdots \end{bmatrix} \tag{6}$$

where $\nu_{ija,A:B}$ is the 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, and $\nu_{ijab,A:B:C}$ is the 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.

The moles of monomeric proteins which remain in solution as free monomers (not forming homo- or hetero-multimers) is given as

$$\sum N^{\mathrm{II}}_{\text{monomers}} = \sum_{j=2}^{p+1}\begin{pmatrix} 1-\sum_{Z=\mathrm{ii}}\nu_{jj,Z}\alpha_{j,Z} + \cdots \\ \cdots - \sum_{\substack{a=2\\ a\neq j}}^{p+1}\sum_{A=\mathrm{i}}\sum_{B=\mathrm{i}}\nu_{ja,A:B}\beta_{ja,A:B} + \cdots \\ \cdots - \sum_{\substack{a=2\\ a\neq j}}^{p+1}\sum_{\substack{b=2\\ b\neq j\\ b\neq a}}^{p+1}\sum_{A=\mathrm{i}}\sum_{B=\mathrm{i}}\sum_{C=\mathrm{i}}\nu_{jab,A:B:C}\beta_{jab,A:B:C} + \cdots \end{pmatrix} N_j^{\mathrm{II}} \tag{7}$$

where $\nu_{jj,Z}$ is the moles of protein j forming homo-multimers of Z units, $\nu_{ja,A:B}$ is the moles of protein j interacting with protein a with A units of protein j and B units of protein a, and $\nu_{jab,A:B:C}$ is the moles of protein j interacting with proteins a and b with A units of protein j, B units of protein a, and C units of protein b.

The remaining moles of monomeric protein j is calculated by subtracting the moles of protein j forming homo-multimers (second term) and hetero-multimers (third and fourth terms) from the initial total moles of protein j monomers added, N_j^{II}.

The moles of solvent bound to the free monomers are

$$\sum \nu N^{\mathrm{II}}_{\text{monomers}} = \sum_{\substack{i=1\\ i\neq 2\to p+1}}^{n}\sum_{j=2}^{p+1}\nu_{ij}\begin{pmatrix} 1-\sum_{Z=\mathrm{ii}}\nu_{jj,Z}\alpha_{j,Z} + \cdots \\ \cdots - \sum_{\substack{a=2\\ a\neq j}}^{p+1}\sum_{A=\mathrm{i}}\sum_{B=\mathrm{i}}\nu_{ja,A:B}\beta_{ja,A:B} + \cdots \\ \cdots - \sum_{\substack{a=2\\ a\neq j}}^{p+1}\sum_{\substack{b=2\\ b\neq j\\ b\neq a}}^{p+1}\sum_{A=\mathrm{i}}\sum_{B=\mathrm{i}}\sum_{C=\mathrm{i}}\nu_{jab,A:B:C}\beta_{jab,A:B:C} + \cdots \end{pmatrix} N_j^{\mathrm{II}} \tag{8}$$

where ν_{ij} is the moles of solvent species i bound to protein j.

The final moles of free-solvent in compartment II is

$$\begin{aligned} &N_1^{\mathrm{II}} - \sum \nu_{\text{solvent/homo-multimer}} N^{\mathrm{II}}_{\text{homo-multimers}} + \cdots \\ &\cdots - \sum \nu_{\text{solvent/hetero-multimer}} N^{\mathrm{II}}_{\text{hetero-multimers}} + \cdots \\ &\cdots - \sum \nu_{\text{solvent/monomer}} N^{\mathrm{II}}_{\text{monomers}} \end{aligned} \tag{9}$$

where N_1^{II} is the initial moles of solvent. The second, third, and fourth terms are the moles of water bound to the homo-multimers, hetero-multimers, and free monomers, respectively, and are given by Eqs. (10)–(12)

$$\sum \nu_{\text{solvent/homo-multimer}} N^{\mathrm{II}}_{\text{homo-multimers}} = \sum_{j=2}^{p+1}\sum_{Z=\mathrm{ii}}\nu_{1j,Z}\alpha_{j,Z}N_j^{\mathrm{II}} \tag{10}$$

$$\sum \nu_{\text{solvent/hetero-multimer}} N^{\mathrm{II}}_{\text{hetero-multimers}} = \begin{bmatrix} \sum_{j=2}^{p}\sum_{a=j+1}^{p+1}\sum_{A=\mathrm{i}}\sum_{B=\mathrm{i}}\nu_{1ja,A:B}\beta_{ja,A:B}N_j^{\mathrm{II}} + \cdots \\ \cdots + \sum_{j=2}^{p-1}\sum_{a=j+1}^{p}\sum_{\substack{b=j+2\\ b\neq a}}^{p+1}\sum_{A=\mathrm{i}}\sum_{B=\mathrm{i}}\sum_{C=\mathrm{i}}\nu_{1jab,A:B:C}\beta_{jab,A:B:C}N_j^{\mathrm{II}} + \cdots \end{bmatrix} \tag{11}$$

$$\sum \nu_{\text{solvent/monomer}} N^{\mathrm{II}}_{\text{monomers}} = \sum_{j=2}^{p+1}\nu_{1j}\begin{pmatrix} 1-\sum_{Z=\mathrm{ii}}\nu_{jj,Z}\alpha_{j,Z} + \cdots \\ \cdots - \sum_{\substack{a=2\\ a\neq j}}^{p+1}\sum_{A=\mathrm{i}}\sum_{B=\mathrm{i}}\nu_{ja,A:B}\beta_{ja,A:B} + \cdots \\ \cdots - \sum_{\substack{a=2\\ a\neq j}}^{p+1}\sum_{\substack{b=2\\ b\neq j\\ b\neq a}}^{p+1}\sum_{A=\mathrm{i}}\sum_{B=\mathrm{i}}\sum_{C=\mathrm{i}}\nu_{jab,A:B:C}\beta_{jab,A:B:C} + \cdots \end{pmatrix} N_j^{\mathrm{II}} \tag{12}$$

where ν_{1j} is the moles of water bound to monomeric protein species j.

The mole fraction of free-solvent in compartment II is

$$x_1^{\mathrm{II}} = \frac{\begin{bmatrix} N_1^{\mathrm{II}} - \sum \nu_{\text{solvent/homo-multimer}} N^{\mathrm{II}}_{\text{homo-multimers}} + \cdots \\ \cdots - \sum \nu_{\text{solvent/hetero-multimer}} N^{\mathrm{II}}_{\text{hetero-multimers}} - \sum \nu_{\text{solvent/monomer}} N^{\mathrm{II}}_{\text{monomers}} \end{bmatrix}}{\begin{bmatrix} \sum_{\substack{i=1\\ i\neq 2\to p+1}}^{n} N_i^{\mathrm{II}} + \sum N^{\mathrm{II}}_{\text{homo-multimers}} + \sum N^{\mathrm{II}}_{\text{hetero-multimers}} + \sum N^{\mathrm{II}}_{\text{monomers}} + \cdots \\ \cdots - \sum \nu N^{\mathrm{II}}_{\text{homo-multimers}} - \sum \nu N^{\mathrm{II}}_{\text{hetero-multimers}} - \sum \nu N^{\mathrm{II}}_{\text{monomers}} \end{bmatrix}} \tag{13}$$

In compartment I, the total moles of solvent is $\sum_{\substack{i=1\\ i\neq 2\to p+1}}^{n} N_i^{\mathrm{I}}$, thus the mole fraction of free-solvent in compartment I is

$$x_1^{\mathrm{I}} = \frac{N_1^{\mathrm{I}}}{\sum_{\substack{i=1\\ i\neq 2\to p+1}}^{n} N_i^{\mathrm{I}}} \tag{14}$$

Inserting Eqs. (13) and (14) into Eq. (1) yields the generalized free-solvent model for any number of proteins which have

solute–solvent interactions and may participate in solute–solute interactions in the form of homo-multimers and hetero-multimers. It is important to note that this development of the free-solvent model does not consider protein homo-multimers and protein hetero-multimers as unique and individual protein species (*i.e.* a dimer is not considered as one of the protein species j, but rather $(\alpha_{j,\mathrm{ii}}N_j^{\mathrm{II}})$ is the dimer species); since the homo-multimers and hetero-multimers have unique hydration and ion binding values, they are unique hydrated macromolecules.

2.2. Determining the physical parameters of the free-solvent model

The number of physical parameters (*i.e.* hydration, ion binding, protein–protein interactions, and fractional amounts of the homo- and hetero-multimer forms) which are required in the free-solvent model are a minimum of $2p$ for p proteins if only monomers are considered (*i.e.* no homo-multimers or hetero-multimers are formed).

For protein solutions containing protein–protein interactions in the form of homo- and/or hetero-multimers, the number of physical parameters required to describe the crowded protein osmotic pressure via the free-solvent model are

$$2(p + l_{\text{homo-multimer}} + l_{\text{hetero-multimer}}) + 2(l_{\text{homo-multimer}} + \sum_{h=2} l_{\text{hetero-multimer},h}\eta_h) \tag{15}$$

where p is the number of protein species, $l_{\text{homo-multimer}}$ is the number of homo-multimers formed, $l_{\text{hetero-multimer}}$ is the number of hetero-multimers formed, $l_{\text{hetero-multimer},h}$ is the number of hetero-multimers forming the h multimer (*i.e.* $h = 2$ for two protein species interactions, $h = 3$ for three protein species interactions, etc.), and η_h is the 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.).

The total number of hydration and ion binding parameters are each given by the first term,

$$(p + l_{\text{homo-multimer}} + l_{\text{hetero-multimer}})$$

and the total number of protein–protein interactions and fractional amount parameters are each given by

$$(l_{\text{homo-multimer}} + \sum_{h=2} l_{\text{hetero-multimer},h}\eta_h)$$

Thus in order to utilize the generalized free-solvent model for predicting the osmotic pressure of crowded protein solutions, methods, independent of the crowded protein osmotic pressure, need to be used to determine the hydration and ion binding of the unique species, as well as the protein–protein interactions and fractional amount of proteins forming homo- and hetero-multimers.

2.2.1. Protein hydration

The methods for determining protein hydration have been reviewed in literature [13–16]. Briefly, many methods are available which attempt to quantify protein hydration, such as ^{17}O NMR, yet it is difficult to determine exact values due to the variety of methods and the associated errors [14–17]. Nevertheless, the consensus is that a globular protein contains about 1 g H_2O/g protein [16]. If this value of hydration is utilized in the free-solvent model, the prediction of the osmotic pressure at near-saturation concentrations will result in a deviation of the predicted and experimental values. Thus, a more exact value for protein hydration is required to ensure that the predicted osmotic pressure is nearly identical to the experimental measurements.

An alternative method of calculating the hydration of a protein which yields a more accurate value is to utilize the solvent accessible surface area (SASA) of the protein. This method requires that the molecular structure has been deposited into the Protein Data Bank (PDB) or can be determined using either NMR or X-ray crystallography. It has been previously shown that the hydration of a protein directly correlates to a monolayer of water, specifically 15.2 ± 0.5 molecules of water per nm^2 of SASA, and thus, if the SASA is known, the protein hydration can be determined [18,19].

2.2.2. Protein-ion binding

Various methods exist for determining the ion binding to proteins, such as the electromotive force (EMF), distribution method, and isopiestic method, which have been reviewed in literature [20–27]. These methods have been used to quantify the number of ions interacting with proteins in various solution properties including salt types, salt concentration, pH, and protein concentration.

2.2.3. Protein–protein binding and fractional amounts of homo- and hetero-multimers

To determine the presence and quantity of the number of proteins interacting to form homo- and hetero-multimers which are in a given multi-component solution, methods such as HPLC and light scattering can be used.

For the fractional amounts of homo-multimers, it is important to note that the fractional amount of each homo-multimer is restricted to $0 \leqslant \alpha_{j,Z} \leqslant 0.5$. The maximum is 0.5 since if a homo-dimer is the only homo-multimer and the entire solution is homo-dimers (*i.e.* no monomers exist in solution), when the fractional amount is 50% of the monomeric protein, every mole of the monomeric protein is consumed. The sum of all fractional amounts of each protein j forming homo-multimers is also restricted to $0 \leqslant \sum_{Z=\mathrm{ii}} \alpha_{j,Z} \leqslant 0.5$.

Similarly, the fractional amount of each hetero-multimer is restricted to $0 \leqslant \beta \leqslant 1$ since if a single hetero-multimer is formed, and the entire solution is hetero-multimers (*i.e.* 100% fractional amount), every mole of the monomeric protein is consumed. The sum of all fractional amounts of each protein j forming homo-multimers is also restricted to $0 \leqslant \sum_{A=\mathrm{i}}\sum_{B=\mathrm{i}} \beta_{ja,A:B} + \sum_{A=\mathrm{i}}\sum_{B=\mathrm{i}}\sum_{C=\mathrm{i}} \beta_{jab,A:B:C} + \cdots \leqslant 1$.

For a given single protein, j, and a known molar ratio of monomer:homo-multimer, $\alpha_{j,Z}$ is calculated for each homo-multimer by

$$\frac{\left(\begin{array}{l} 1 - \sum_{Z=\mathrm{ii}} \nu_{jj,Z}\alpha_{j,Z} + \cdots \\ \cdots - \sum_{\substack{a=2 \\ a\neq j}}^{p+1} \sum_{A=\mathrm{i}}\sum_{B=\mathrm{i}} \nu_{ja,A:B}\beta_{ja,A:B} + \cdots \\ \cdots - \sum_{\substack{a=2 \\ a\neq j}}^{p+1} \sum_{\substack{b=2 \\ b\neq j \\ b\neq a}}^{p+1} \sum_{A=\mathrm{i}}\sum_{B=\mathrm{i}}\sum_{C=\mathrm{i}} \nu_{jab,A:B:C}\beta_{jab,A:B:C} + \cdots \end{array}\right)}{\alpha_{j,Z}|_{j,Z}} = \Phi|_{j,Z} \tag{16}$$

where $\alpha_{j,Z}|_{j,Z}$ is the fractional amount of the homo-multimer at a specific homo-multimer, Z, for protein j and $\Phi|_{j,Z}$ is the molar ratio of the monomer to the specific homo-multimer, Z, for protein j for a specific homo-multimer. The hetero-multimer terms (terms 3 and 4 in the numerator of the left hand side) are evaluated for protein j only.

For the fractional amounts of hetero-multimers, the two protein hetero-multimers are given by

$$\frac{\left(\begin{array}{l} 1 - \sum_{Z=\mathrm{ii}} \nu_{jj,Z}\alpha_{j,Z} + \cdots \\ \cdots - \sum_{\substack{a=2 \\ a\neq j}}^{p+1} \sum_{A=\mathrm{i}}\sum_{B=\mathrm{i}} \nu_{ja,A:B}\beta_{ja,A:B} + \cdots \\ \cdots - \sum_{\substack{a=2 \\ a\neq j}}^{p+1} \sum_{\substack{b=2 \\ b\neq j \\ b\neq a}}^{p+1} \sum_{A=\mathrm{i}}\sum_{B=\mathrm{i}}\sum_{C=\mathrm{i}} \nu_{jab,A:B:C}\beta_{jab,A:B:C} + \cdots \end{array}\right)}{\beta_{ja,A:B}|_{ja,A:B}} = \Gamma|_{ja,A:B} \tag{17}$$

and for three protein hetero-multimers,

$$\frac{\left(\begin{array}{l}1-\sum_{Z=\mathrm{ii}}\nu_{jj,Z}\alpha_{j,Z}+\cdots\\ \cdots-\sum_{\substack{a=2\\a\neq j}}^{p+1}\sum_{A=\mathrm{i}}\sum_{B=\mathrm{i}}\nu_{ja,A:B}\beta_{ja,A:B}+\cdots\\ \cdots-\sum_{\substack{a=2\\a\neq j}}^{p+1}\sum_{\substack{b=2\\b\neq j\\b\neq a}}^{p+1}\sum_{A=\mathrm{i}}\sum_{B=\mathrm{i}}\sum_{C=\mathrm{i}}\nu_{jab,A:B:C}\beta_{jab,A:B:C}+\cdots\end{array}\right)}{\beta_{jab,A:B:C}|_{jab,A:B:C}}=\Gamma|_{jab,A:B:C} \tag{18}$$

where $\beta_{ja,A:B}|_{ja,A:B}$ is the fractional amount of the two protein hetero-multimer at a specific hetero-multimer, *ja*, $\beta_{jab,A:B:C}|_{jab,A:B:C}$ is the fractional amount of the three protein hetero-multimer at a specific hetero-multimer, *jab*, $\Gamma|_{ja,A:B}$ is the molar ratio of the monomer to the specific two protein hetero-multimer, *ja*, and $\Gamma|_{jab,A:B:C}$ is the molar ratio of the monomer to the specific three protein hetero-multimer, *jab*.

Eqs. (16)–(18) need to be solved for each protein, *j*, in solution. If more than 1 multimer forms, the system of equations must be solved to obtain all $\alpha_{j,Z}$, $\beta_{ja,A:B}$, $\beta_{jab,A:B:C}$, etc.

For example, if a single protein solution forms a homo-dimer ($\nu_{22,\mathrm{ii}}=2$ mol monomer/mol dimer) and homo-trimer ($\nu_{22,\mathrm{iii}}=3$ - mol monomer/mol trimer), and if the molar ratio is 3:1:2 for the monomer:dimer:trimer ($\Phi|_{2,\mathrm{ii}}=3/1$ and $\Phi|_{2,\mathrm{ii}}=3/2$), Eq. (16) yields two equations

$$\frac{1-2\alpha_{2,\mathrm{ii}}-3\alpha_{2,\mathrm{iii}}}{\alpha_{2,\mathrm{ii}}}=\frac{3}{1}$$

and

$$\frac{1-2\alpha_{2,\mathrm{ii}}-3\alpha_{2,\mathrm{iii}}}{\alpha_{2,\mathrm{iii}}}=\frac{3}{2}$$

which yields fractional amounts of $\alpha_{2,\mathrm{ii}}=0.091$ and $\alpha_{2,\mathrm{iii}}=0.182$.

3. Case scenarios

Here, representative examples of how the generalized free-solvent model (Eq. (1) with Eqs. (13) and (14) substituted) can be used are shown. In each case, the model is reduced to include only relevant parameters. All cases consider only a single monovalent salt species. Cases 1, 2, and 3 reduce the generalized free-solvent model for single protein solutions, Cases 4, 6, and 7 reduce the generalized free-solvent model for binary protein solutions, and Cases 5 and 8 reduce the generalized free-solvent model for ternary protein solutions.

3.1. Case 1: single protein forming a homo-dimer

Given a solution with a single protein ($p=1$, $n=3$), *A*, that only forms a homo-dimer (*i.e. AA*). The free-solvent mole fractions (Eqs. (13) and (14)) are

$$x_1^{\mathrm{II}}=\frac{N_1^{\mathrm{II}}-\sum\nu_{\mathrm{solvent/homo\text{-}multimer}}N^{\mathrm{II}}_{\mathrm{homo\text{-}multimers}}-\sum\nu_{\mathrm{solvent/monomer}}N^{\mathrm{II}}_{\mathrm{monomers}}}{\sum_{\substack{i=1\\i\neq 2}}^{3}N_i^{\mathrm{II}}+\sum N^{\mathrm{II}}_{\mathrm{homo\text{-}multimers}}+\sum N^{\mathrm{II}}_{\mathrm{monomers}}-\sum\nu N^{\mathrm{II}}_{\mathrm{homo\text{-}multimers}}-\sum\nu N^{\mathrm{II}}_{\mathrm{monomers}}} \tag{19}$$

and

$$x_1^{\mathrm{I}}=\frac{N_1^{\mathrm{I}}}{\sum_{\substack{i=1\\i\neq 2}}^{3}N_i^{\mathrm{I}}}=\frac{N_1^{\mathrm{I}}}{N_1^{\mathrm{I}}+N_3^{\mathrm{I}}} \tag{20}$$

The terms in Eq. (19) are

$$\sum N^{\mathrm{II}}_{\mathrm{homo\text{-}multimers}}=\sum_{j=2}^{3}\sum_{Z=\mathrm{ii}}\alpha_{j,Z}N_j^{\mathrm{II}}=\alpha_{2,ii}N_2^{\mathrm{II}} \tag{21}$$

$$\begin{aligned}\sum\nu N^{\mathrm{II}}_{\mathrm{homo\text{-}multimers}}&=\sum_{\substack{i=1\\i\neq 2}}^{3}\sum_{j=2}^{2}\sum_{Z=\mathrm{ii}}\nu_{ij,Z}\alpha_{j,Z}N_j^{\mathrm{II}}\Rightarrow\sum\nu N^{\mathrm{II}}_{\mathrm{homo\text{-}multimer}}\\&=\nu_{12,\mathrm{ii}}\alpha_{2,\mathrm{ii}}N_2^{\mathrm{II}}+\nu_{32,\mathrm{ii}}\alpha_{2,\mathrm{ii}}N_2^{\mathrm{II}}\end{aligned} \tag{22}$$

$$\sum\nu_{\mathrm{solvent/homo\text{-}multimer}}N^{\mathrm{II}}_{\mathrm{homo\text{-}multimer}}=\sum_{j=2}^{2}\sum_{Z=\mathrm{ii}}\nu_{1j,Z}\alpha_{j,Z}N_j^{\mathrm{II}}=\nu_{12,\mathrm{ii}}\alpha_{2,\mathrm{ii}}N_2^{\mathrm{II}} \tag{23}$$

$$\sum N^{\mathrm{II}}_{\mathrm{monomers}}=\sum_{j=2}^{2}\left(1-\sum_{Z=\mathrm{ii}}\nu_{jj,Z}\alpha_{j,Z}\right)N_j^{\mathrm{II}}=(1-\nu_{22,\mathrm{ii}}\alpha_{2,\mathrm{ii}})N_2^{\mathrm{II}} \tag{24}$$

$$\begin{aligned}\sum\nu N^{\mathrm{II}}_{\mathrm{monomers}}&=\sum_{\substack{i=1\\i\neq 2}}^{3}\sum_{j=2}^{2}\nu_{ij}\left(1-\sum_{Z=\mathrm{ii}}\nu_{jj,Z}\alpha_{j,Z}\right)N_j^{\mathrm{II}}\\&\Rightarrow\sum\nu N^{\mathrm{II}}_{\mathrm{monomers}}\\&=\nu_{12}(1-\nu_{22,\mathrm{ii}}\alpha_{2,\mathrm{ii}})N_2^{\mathrm{II}}+\nu_{32}(1-\nu_{22,\mathrm{ii}}\alpha_{2,\mathrm{ii}})N_2^{\mathrm{II}}\end{aligned} \tag{25}$$

and

$$\begin{aligned}\sum\nu_{\mathrm{solvent/monomer}}N^{\mathrm{II}}_{\mathrm{monomers}}&=\sum_{j=2}^{2}\nu_{1j}\left(1-\sum_{Z=\mathrm{ii}}\nu_{jj,Z}\alpha_{j,Z}\right)N_j^{\mathrm{II}}\\&=\nu_{12}(1-\nu_{22,\mathrm{ii}}\alpha_{2,\mathrm{ii}})N_2^{\mathrm{II}}\end{aligned} \tag{26}$$

Substituting Eqs. (21)–(26) into Eq. (19) and reducing yields

$$x_1^{\mathrm{II}}=\frac{N_1^{\mathrm{II}}-(\nu_{12,\mathrm{ii}}\alpha_{2,\mathrm{ii}}+\nu_{12}(1-\nu_{22,\mathrm{ii}}\alpha_{2,\mathrm{ii}}))N_2^{\mathrm{II}}}{N_1^{\mathrm{II}}+N_3^{\mathrm{II}}+(\alpha_{2,ii}+(1-\nu_{22,\mathrm{ii}}\alpha_{2,\mathrm{ii}})-(\nu_{12,\mathrm{ii}}+\nu_{32,\mathrm{ii}})\alpha_{2,\mathrm{ii}}-(\nu_{12}+\nu_{32})(1-\nu_{22,\mathrm{ii}}\alpha_{2,\mathrm{ii}}))N_2^{\mathrm{II}}} \tag{27}$$

For this case, $p=1$ and $l_{\mathrm{homo\text{-}multimer}}=1$, thus there are a total of 6 parameters (Eq. (15)) in order to predict the osmotic pressure for this solution: 2 hydration, 2 ion binding, 1 protein–protein interaction, and 1 fractional amount of protein.

3.2. Case 2: single protein forming a homo-trimer

Given a solution with a single protein ($p=1$, $n=3$), *A*, that only forms a homo-trimer, the free-solvent model mole fractions (Eqs. (13) and (14)), after substitution and reduction, are

$$x_1^{II} = \frac{N_1^{II} - (v_{12,iii}\alpha_{2,iii} + v_{12}(1 - v_{22,iii}\alpha_{2,iii}))N_2^{II}}{N_1^{II} + N_3^{II} + (\alpha_{2,iii} + (1 - v_{22,iii}\alpha_{2,iii}) - (v_{12,iii} + v_{32,iii})\alpha_{2,iii} - (v_{12} + v_{32})(1 - v_{22,iii}\alpha_{2,iii}))N_2^{II}} \tag{28}$$

and

$$x_1^{I} = \frac{N_1^{I}}{N_1^{I} + N_3^{I}} \tag{29}$$

Identical to the free-solvent model solution for the homo-dimer case, $p = 1$ and $l_{\text{homo-multimer}} = 1$, thus there are a total of 6 parameters with 2 hydration, 2 ion binding, 1 protein–protein interaction, and 1 fractional amount of protein.

3.3. Case 3: single protein forming a homo-dimer and homo-trimer

For a solution containing a single protein ($p = 1$, $n = 3$), A, that forms a homo-dimer and a homo-trimer, the free-solvent model mole fractions (Eqs. (13) and (14)) are

$$x_1^{II} = \frac{N_1^{II} - (v_{12,ii}\alpha_{2,ii} + v_{12,iii}\alpha_{2,iii} + v_{12}(1 - v_{22,ii}\alpha_{2,ii} - v_{22,iii}\alpha_{2,iii}))N_2^{II}}{\left[N_1^{II} + N_3^{II} + N_2^{II}\begin{pmatrix}\alpha_{2,ii} + \alpha_{2,iii} + (1 - v_{22,ii}\alpha_{2,ii} - v_{22,iii}\alpha_{2,iii}) - (v_{12,ii} + v_{32,ii})\alpha_{2,ii} + \cdots \\ \cdots - (v_{12,iii} + v_{32,iii})\alpha_{2,iii} - (v_{12} + v_{32})(1 - v_{22,ii}\alpha_{2,ii} - v_{22,iii}\alpha_{2,iii})\end{pmatrix}\right]} \tag{30}$$

and

$$x_1^{I} = \frac{N_1^{I}}{N_1^{I} + N_3^{I}} \tag{31}$$

Here, $p = 1$ and $l_{\text{homo-multimer}} = 2$, giving a total of 10 parameters where 3 are hydration, 3 are ion binding, 2 are protein–protein interactions, and 2 are fractional amounts of protein.

3.4. Case 4: two proteins forming a hetero-dimer

For a solution containing two proteins ($p = 2$, $n = 4$), A and B, that form a hetero-dimer, AB, the free-solvent mole fractions (Eqs. (13) and (14)) are

$$x_1^{II} = \frac{N_1^{II} - \sum v_{\text{solvent/hetero-multimer}} N_{\text{hetero-multimers}}^{II} - \sum v_{\text{solvent/monomer}} N_{\text{monomers}}^{II}}{\sum_{\substack{i=1 \\ i\neq 2\to p+1}}^{n} N_i^{II} + \sum N_{\text{hetero-multimers}}^{II} + \sum N_{\text{monomers}}^{II} - \sum v N_{\text{hetero-multimers}}^{II} - \sum v N_{\text{monomers}}^{II}} \tag{32}$$

and

$$x_1^{I} = \frac{N_1^{I}}{\sum_{\substack{i=1 \\ i\neq 2\to 3}}^{4} N_i^{I}} = \frac{N_1^{I}}{N_1^{I} + N_4^{I}} \tag{33}$$

The terms in Eq. (32) are

$$\sum N_{\text{hetero-multimers}}^{II} = \sum_{j=2}^{2}\sum_{a=j+1}^{3}\sum_{A=i}\sum_{B=i}\beta_{ja,A:B}N_j^{II} = \beta_{23,i:i}N_2^{II} \tag{34}$$

$$\sum v N_{\text{hetero-multimers}}^{II} = \sum_{\substack{i=1 \\ i\neq 2\to 3}}^{4}\sum_{j=2}^{2}\sum_{a=j+1}^{3}\sum_{A=i}\sum_{B=i} v_{ija,A:B}\beta_{ja,A:B}N_j^{II}$$
$$\Rightarrow \sum v N_{\text{hetero-multimers}}^{II} = v_{123,i:i}\beta_{23,i:i}N_2^{II} + v_{423,i:i}\beta_{23,i:i}N_2^{II} \tag{35}$$

$$\sum v_{\text{solvent/hetero-multimer}} N_{\text{hetero-multimers}}^{II} = \sum_{j=2}^{2}\sum_{a=j+1}^{3}\sum_{A=i}\sum_{B=i} v_{1ja,A:B}\beta_{ja,A:B}N_j^{II}$$
$$\Rightarrow \sum v_{\text{solvent/hetero-multimer}} N_{\text{hetero-multimers}}^{II} = v_{123,i:i}\beta_{23,i:i}N_2^{II} \tag{36}$$

$$\sum N_{\text{monomers}}^{II} = \sum_{j=2}^{3}\left(1 - \sum_{\substack{a=2 \\ a\neq j}}^{3}\sum_{A=i}\sum_{B=i} v_{ja,A:B}\beta_{ja,A:B}\right)N_j^{II}$$
$$\Rightarrow \sum N_{\text{monomers}}^{II} = (1 - v_{23,i:i}\beta_{23,i:i})N_2^{II} + (1 - v_{32,i:i}\beta_{32,i:i})N_3^{II} \tag{37}$$

$$\sum v N_{\text{monomers}}^{II} = \sum_{\substack{i=1 \\ i\neq 2\to 3}}^{4}\sum_{j=2}^{3} v_{ij}\left(1 - \sum_{\substack{a=2 \\ a\neq j}}^{3}\sum_{A=i}\sum_{B=i} v_{ja,A:B}\beta_{ja,A:B}\right)N_j^{II}$$
$$\Rightarrow \sum v N_{\text{monomers}}^{II} = (v_{12} + v_{42})(1 - v_{23,i:i}\beta_{23,i:i})N_2^{II} + (v_{13} + v_{43})(1 - v_{32,i:i}\beta_{32,i:i})N_3^{II} \tag{38}$$

and

$$\sum v_{\text{solvent/monomer}} N_{\text{monomers}}^{II} = \sum_{j=2}^{3} v_{1j}\left(1 - \sum_{\substack{a=2 \\ a\neq j}}^{3}\sum_{A=i}\sum_{B=i} v_{ja,A:B}\beta_{ja,A:B}\right)N_j^{II}$$
$$\Rightarrow \sum v_{\text{solvent/monomer}} N_{\text{monomers}}^{II} = v_{12}(1 - v_{23,i:i}\beta_{23,i:i})N_2^{II} + v_{13}(1 - v_{32,i:i}\beta_{32,i:i})N_3^{II} \tag{39}$$

Substituting Eqs. (34)–(39) into Eq. (32) and reducing yields

$$x_1^{II} = \frac{N_1^{II} - v_{123,i:i}\beta_{23,i:i}N_2^{II} - v_{12}(1 - v_{23,i:i}\beta_{23,i:i})N_2^{II} - v_{13}(1 - v_{32,i:i}\beta_{32,i:i})N_3^{II}}{\left[N_1^{II} + N_4^{II} + (\beta_{23,i:i} + (1 - v_{23,i:i}\beta_{23,i:i}))N_2^{II} + (1 - v_{32,i:i}\beta_{32,i:i})N_3^{II} + \cdots \right.}$$
$$\left. \cdots - ((v_{123,i:i} + v_{423,i:i})\beta_{23,i:i} + (v_{12} + v_{42})(1 - v_{23,i:i}\beta_{23,i:i}))N_2^{II} - (v_{13} + v_{43})(1 - v_{32,i:i}\beta_{32,i:i})N_3^{II}\right] \tag{40}$$

Here, $p = 2$, $l_{\text{hetero-multimer}} = 1$, $l_{\text{hetero-multimer},2} = 1$, and $\eta_2 = 2$, thus there are a total of 10 parameters (Eq. (15)) where 3 are hydration, 3 are ion binding, 2 are protein–protein interactions, and 2 are fractional amounts of protein.

3.5. Case 5: three proteins forming a hetero-trimer

For a solution containing three proteins ($p = 3$, $n = 5$), A, B, and C, that form a hetero-trimer, ABC, the free-solvent mole fractions (Eqs. (13) and (14)) are

$$x_1^{\mathrm{I}} = \frac{N_1^{\mathrm{I}}}{\sum_{\substack{i=1\\ i\neq 2\to 4}}^{5} N_i^{\mathrm{I}}} = \frac{N_1^{\mathrm{I}}}{N_1^{\mathrm{I}} + N_5^{\mathrm{I}}} \tag{41}$$

Eqs. (34)–(39), rather than being for the hetero-dimer, are in the hetero-trimer form, and when combined with Eq. (32) and reduced, becomes

$$x_1^{\mathrm{II}} = \frac{\begin{bmatrix} N_1^{\mathrm{II}} - v_{1234,\mathrm{i:i:i}}\beta_{234,\mathrm{i:i:i}}N_2^{\mathrm{II}} - v_{12}(1 - v_{234,\mathrm{i:i:i}}\beta_{234,\mathrm{i:i:i}})N_2^{\mathrm{II}} + \cdots \\ \cdots - v_{13}(1 - v_{324,\mathrm{i:i:i}}\beta_{324,\mathrm{i:i:i}})N_3^{\mathrm{II}} + -v_{14}(1 - v_{423,\mathrm{i:i:i}}\beta_{423,\mathrm{i:i:i}})N_4^{\mathrm{II}} \end{bmatrix}}{\begin{bmatrix} N_1^{\mathrm{II}} + N_5^{\mathrm{II}} + (\beta_{234,\mathrm{i:i:i}} + (1 - v_{234,\mathrm{i:i:i}}\beta_{234,\mathrm{i:i:i}}))N_2^{\mathrm{II}} + (1 - v_{324,\mathrm{i:i:i}}\beta_{324,\mathrm{i:i:i}})N_3^{\mathrm{II}} + \cdots \\ \cdots + (1 - v_{423,\mathrm{i:i:i}}\beta_{423,\mathrm{i:i:i}})N_4^{\mathrm{II}} + \cdots \\ \cdots - ((v_{1234,\mathrm{i:i:i}} + v_{5234,\mathrm{i:i:i}})\beta_{234,\mathrm{i:i}} + (v_{12} + v_{52})(1 - v_{234,\mathrm{i:i:i}}\beta_{234,\mathrm{i:i:i}}))N_2^{\mathrm{II}} + \cdots \\ \cdots - (v_{13} + v_{53})(1 - v_{324,\mathrm{i:i:i}}\beta_{324,\mathrm{i:i:i}})N_3^{\mathrm{II}} - (v_{14} + v_{54})(1 - v_{423,\mathrm{i:i:i}}\beta_{423,\mathrm{i:i:i}})N_4^{\mathrm{II}} \end{bmatrix}} \tag{42}$$

where $p = 3$, $l_{\text{hetero-multimer}} = 1$, $l_{\text{hetero-multimer},3} = 1$, and $\eta_3 = 3$, which yield a total of 14 parameters: 4 hydration, 4 ion binding, 3 protein–protein interactions, and 3 fractional amounts of protein.

3.6. Case 6: two proteins forming a hetero-dimer of homo-dimers

For a solution containing two proteins ($p = 2$, $n = 4$), A and B, that form a hetero-dimer, $AABB$, of homo-dimers, AA and BB, the free-solvent mole fractions (Eqs. (13) and (14)), after substitution and reduction, are

$$x_1^{\mathrm{II}} = \frac{N_1^{\mathrm{II}} - v_{123,\mathrm{ii:ii}}\beta_{23,\mathrm{ii:ii}}N_2^{\mathrm{II}} - v_{12}(1 - v_{23,\mathrm{ii:ii}}\beta_{23,\mathrm{ii:ii}})N_2^{\mathrm{II}} - v_{13}(1 - v_{32,\mathrm{ii:ii}}\beta_{32,\mathrm{ii:ii}})N_3^{\mathrm{II}}}{\begin{bmatrix} N_1^{\mathrm{II}} + N_4^{\mathrm{II}} + (\beta_{23,\mathrm{ii:ii}} + (1 - v_{23,\mathrm{ii:ii}}\beta_{23,\mathrm{ii:ii}}))N_2^{\mathrm{II}} + (1 - v_{32,\mathrm{ii:ii}}\beta_{32,\mathrm{ii:ii}})N_3^{\mathrm{II}} + \cdots \\ \cdots - ((v_{123,\mathrm{ii:ii}} + v_{423,\mathrm{ii:ii}})\beta_{23,\mathrm{ii:ii}} + (v_{12} + v_{42})(1 - v_{23,\mathrm{ii:ii}}\beta_{23,\mathrm{ii:ii}}))N_2^{\mathrm{II}} - (v_{13} + v_{43})(1 - v_{32,\mathrm{ii:ii}}\beta_{32,\mathrm{ii:ii}})N_3^{\mathrm{II}} \end{bmatrix}} \tag{43}$$

and

$$x_1^{\mathrm{I}} = \frac{N_1^{\mathrm{I}}}{N_1^{\mathrm{I}} + N_4^{\mathrm{I}}} \tag{44}$$

Similar to the hetero-dimer case, ($p = 2$, $l_{\text{hetero-multimer}} = 1$, $l_{\text{hetero-multimer},2} = 1$, and $\eta_2 = 2$), there is a total of 10 parameters: 3 hydration, 3 ion binding, 2 protein–protein interactions, and 2 fractional amounts of protein parameters.

3.7. Case 7: two proteins forming a hetero-dimer of a homo-dimer and monomer

For a solution containing two proteins ($p = 2$, $n = 4$), A and B, that form a hetero-dimer, AAB, of a homo-dimer, AA, and a monomer, B, the free-solvent mole fractions (Eqs. (13) and (14)), after substitution and reduction, are

$$x_1^{\mathrm{II}} = \frac{N_1^{\mathrm{II}} - v_{123,\mathrm{ii:i}}\beta_{23,\mathrm{ii:i}}N_2^{\mathrm{II}} - v_{12}(1 - v_{23,\mathrm{ii:i}}\beta_{23,\mathrm{ii:i}})N_2^{\mathrm{II}} - v_{13}(1 - v_{32,\mathrm{i:ii}}\beta_{32,\mathrm{i:ii}})N_3^{\mathrm{II}}}{\begin{bmatrix} N_1^{\mathrm{II}} + N_4^{\mathrm{II}} + (\beta_{23,\mathrm{ii:i}} + (1 - v_{23,\mathrm{ii:i}}\beta_{23,\mathrm{ii:i}}))N_2^{\mathrm{II}} + (1 - v_{32,\mathrm{i:ii}}\beta_{32,\mathrm{i:ii}})N_3^{\mathrm{II}} + \cdots \\ \cdots - ((v_{123,\mathrm{ii:i}} + v_{423,\mathrm{ii:i}})\beta_{23,\mathrm{ii:i}} + (v_{12} + v_{42})(1 - v_{23,\mathrm{ii:i}}\beta_{23,\mathrm{ii:i}}))N_2^{\mathrm{II}} - (v_{13} + v_{43})(1 - v_{32,\mathrm{i:ii}}\beta_{32,\mathrm{i:ii}})N_3^{\mathrm{II}} \end{bmatrix}} \tag{45}$$

and

$$x_1^{\mathrm{I}} = \frac{N_1^{\mathrm{I}}}{N_1^{\mathrm{I}} + N_4^{\mathrm{I}}} \tag{46}$$

Similar to the hetero-dimer case, ($p = 2$, $l_{\text{hetero-multimer}} = 1$, $l_{\text{hetero-multimer},2} = 1$, and $\eta_2 = 2$), there is a total of 10 parameters: 3 hydration, 3 ion binding, 2 protein–protein interactions, and 2 fractional amounts of protein.

3.8. Case 8: three proteins forming a hetero-trimer and two hetero-dimers

For a solution containing three proteins ($p = 3$, $n = 5$), A, B, and C, that form a hetero-trimer, ABC, and two hetero-dimers, AB and AC, the free-solvent mole fractions (Eqs. (13) and (14)) are

$$x_1^{\mathrm{II}} = \frac{\begin{bmatrix} N_1^{\mathrm{II}} - (v_{123,\mathrm{i:i}}\beta_{23,\mathrm{i:i}} + v_{124,\mathrm{i:i}}\beta_{24,\mathrm{i:i}} + v_{1234,\mathrm{i:i:i}}\beta_{234,\mathrm{i:i:i}})N_2^{\mathrm{II}} + \cdots \\ \cdots - v_{12}(1 - v_{23,\mathrm{i:i}}\beta_{23,\mathrm{i:i}} - v_{24,\mathrm{i:i}}\beta_{24,\mathrm{i:i}} - v_{234,\mathrm{i:i:i}}\beta_{234,\mathrm{i:i:i}})N_2^{\mathrm{II}} + \cdots \\ \cdots - v_{13}(1 - v_{32,\mathrm{i:i}}\beta_{32,\mathrm{i:i}} - v_{324,\mathrm{i:i:i}}\beta_{324,\mathrm{i:i:i}})N_3^{\mathrm{II}} - v_{14}(1 - v_{42,\mathrm{i:i}}\beta_{42,\mathrm{i:i}} - v_{423,\mathrm{i:i:i}}\beta_{423,\mathrm{i:i:i}})N_4^{\mathrm{II}} \end{bmatrix}}{\begin{bmatrix} N_1^{\mathrm{II}} + N_5^{\mathrm{II}} + (\beta_{23,\mathrm{i:i}} + \beta_{24,\mathrm{i:i}} + \beta_{234,\mathrm{i:i:i}} + (1 - v_{23,\mathrm{i:i}}\beta_{23,\mathrm{i:i}} - v_{24,\mathrm{i:i}}\beta_{24,\mathrm{i:i}} - v_{234,\mathrm{i:i:i}}\beta_{234,\mathrm{i:i:i}}))N_2^{\mathrm{II}} + \cdots \\ \cdots + (1 - v_{32,\mathrm{i:i}}\beta_{32,\mathrm{i:i}} - v_{324,\mathrm{i:i:i}}\beta_{324,\mathrm{i:i:i}})N_3^{\mathrm{II}} + (1 - v_{42,\mathrm{i:i}}\beta_{42,\mathrm{i:i}} - v_{423,\mathrm{i:i:i}}\beta_{423,\mathrm{i:i:i}})N_4^{\mathrm{II}} + \cdots \\ \cdots - ((v_{123,\mathrm{i:i}} + v_{423,\mathrm{i:i}})\beta_{23,\mathrm{i:i}} + (v_{12} + v_{52})(1 - v_{23,\mathrm{i:i}}\beta_{23,\mathrm{i:i}} - v_{24,\mathrm{i:i}}\beta_{24,\mathrm{i:i}} - v_{234,\mathrm{i:i:i}}\beta_{234,\mathrm{i:i:i}}))N_2^{\mathrm{II}} + \cdots \\ \cdots - (v_{13} + v_{53})(1 - v_{32,\mathrm{i:i}}\beta_{32,\mathrm{i:i}} - v_{324,\mathrm{i:i:i}}\beta_{324,\mathrm{i:i:i}})N_3^{\mathrm{II}} - (v_{13} + v_{53})(1 - v_{42,\mathrm{i:i}}\beta_{42,\mathrm{i:i}} - v_{423,\mathrm{i:i:i}}\beta_{423,\mathrm{i:i:i}})N_4^{\mathrm{II}} \end{bmatrix}} \tag{47}$$

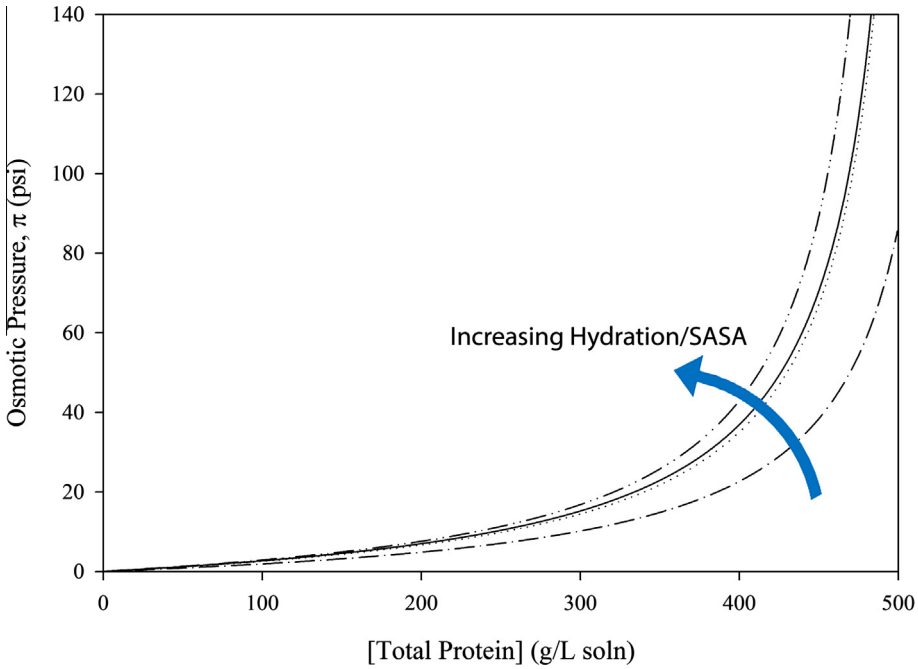

Fig. 1. Osmotic pressure vs. concentration for a theoretical single protein solution, in 0.15 M NaCl, with the formation of a homo-dimer: the effect of changing the hydration and SASA. The generalized free-solvent model is plotted when only the monomer is present with solute–solvent interaction parameters of ν_{12} = 1.177 g H_2O/g monomer and ν_{32} = 8.81 mol NaCl/mol monomer (solid curve). When a homo-dimer occurs and forms at a ratio of 3:1 monomer:dimer ($\alpha_{2,ii}$ = 0.2 and $\nu_{22,ii}$ = 2 mol monomer/mol dimer), with the monomer maintaining its hydration and ion binding, the free-solvent model is plotted for changes to the homo-dimer SASA which affects the homo-dimer hydration at a fixed ion binding (where $\nu_{dimer\text{-}ion} = 2(\nu_{monomer\text{-}ion})$ or $\nu_{32,ii}$ = 17.62 mol NaCl/mol dimer): (1) the homo-dimer has no change to the hydration, $\nu_{12,ii}$ = 1.177 g H_2O/g Dimer (dotted curve); (2) the homo-dimer has a SASA that is 80% of 2 times the monomer SASA (thus $\nu_{12,ii}$ = 0.942 g H_2O/g dimer) (dash-dot curve); and (3) the homo-dimer has a SASA that is 10% more than 2 times the monomer SASA (thus $\nu_{1,ii}$ = 1.295 g H_2O/g dimer) (dash-dot-dot curve).

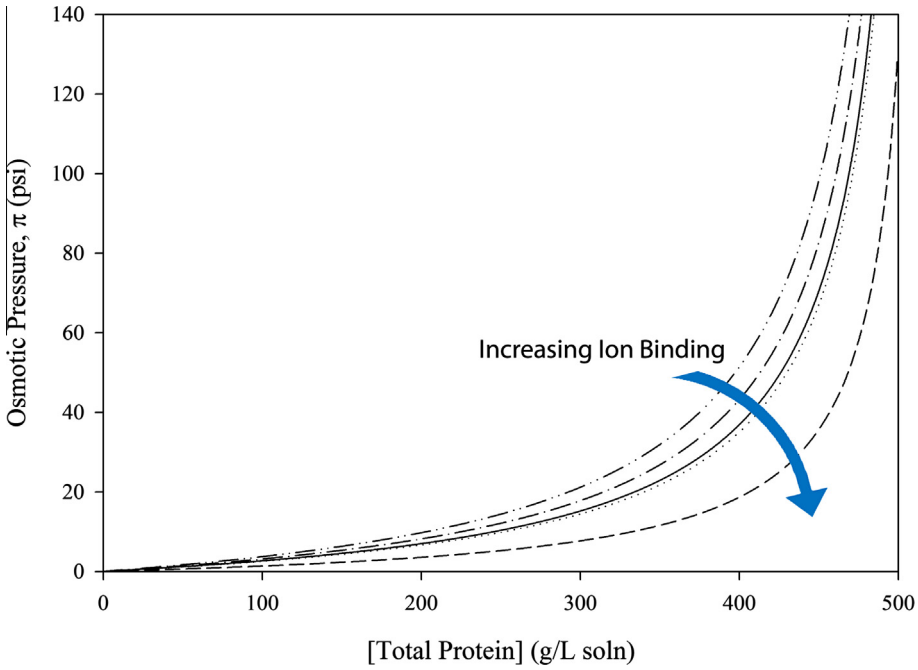

Fig. 2. Osmotic pressure vs. concentration for a theoretical single protein solution, in 0.15 M NaCl, with the formation of a homo-dimer: the effect of changing the ion binding. The generalized free-solvent model is plotted when only the monomer is present with solute–solvent interaction parameters of ν_{12} = 1.177 g H_2O/g monomer and ν_{32} = 8.81 mol NaCl/mol monomer (solid curve). When a homo-dimer occurs and forms at a ratio of 3:1 monomer:dimer ($\alpha_{2,ii}$ = 0.2 and $\nu_{22,ii}$ = 2 mol monomer/mol dimer), with the monomer maintaining its hydration and ion binding, and the homo-dimer having no change to the SASA ($SASA_{dimer} = 2(SASA_{monomer})$ therefore $\nu_{12,ii}$ = 1.177 g H_2O/g dimer), the generalized free-solvent model is plotted when: (1) the ion binding of the homo-dimer is 2 times that of the monomer, $\nu_{32,ii}$ = 17.62 mol NaCl/mol dimer (dotted curve); (2) the homo-dimer ion binding value is 1.5 times that of the monomer, $\nu_{32,ii}$ = 13.22 mol NaCl/mol dimer (dash-dot curve); (3) the homo-dimer ion binding value is the same as the monomer, $\nu_{32,ii}$ = 8.81 mol NaCl/mol dimer (dash-dot-dot curve); and (4) the ion binding of the homo-dimer is 3 times that of the monomer, $\nu_{32,ii}$ = 26.43 mol NaCl/mol dimer (dashed curve).

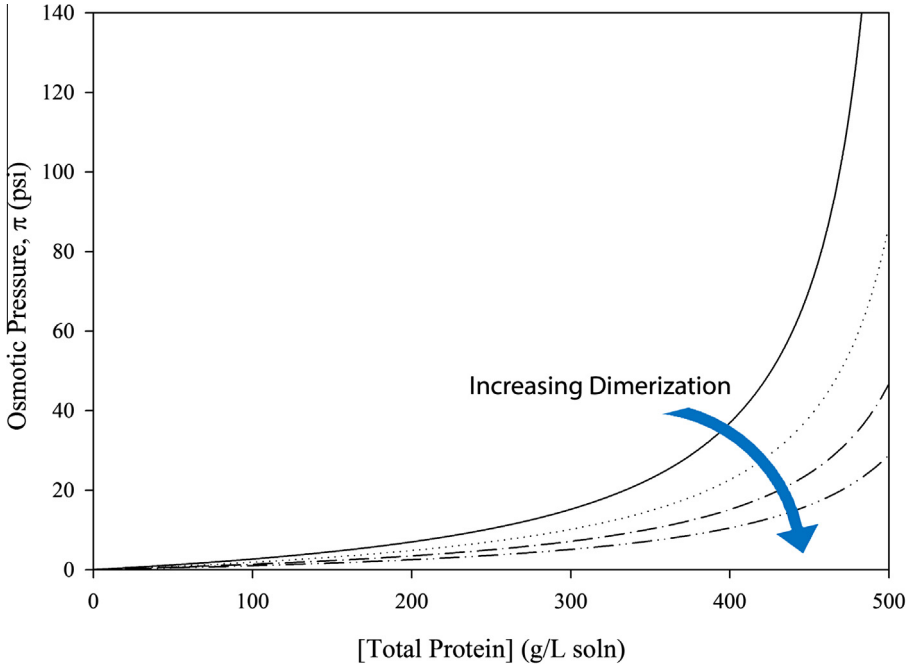

Fig. 3. Osmotic pressure vs. concentration for a theoretical single protein solution, in 0.15 M NaCl, with the formation of a homo-dimer: the effect of increased homo-dimerization. The generalized free-solvent model is plotted when only the monomer is present with solute–solvent interaction parameters of ν_{12} = 1.177 g H_2O/g monomer and ν_{32} = 8.81 mol NaCl/mol monomer (solid curve). The homo-dimers ($\nu_{22,ii}$ = 2 mol monomer/mol dimer) have solute–solvent interaction parameters of $\nu_{12,ii}$ = 0.942 g H_2O/g dimer and $\nu_{32,ii}$ = 17.62 mol NaCl/mol dimer in all cases. The generalized free-solvent model is plotted for various fractional amounts of homo-dimerization: (1) 3:1 monomer:homo-dimer, $\alpha_{2,ii}$ = 0.2 (dotted curve); (2) 1:1 monomer:homo-dimer, $\alpha_{2,ii}$ = 0.33 (dash-dot curve); and (3) 1:3 monomer:homo-dimer, $\alpha_{2,ii}$ = 0.43 (dash-dot-dot curve).

Table 1
Physical parameters used in the generalized free-solvent model for a single protein solution forming a homo-dimer.

Protein	Molecular weight, M_2 (kDa)	Hydration (g H_2O/g Protein)	Solvent accessible surface area (Å^2)	Ion binding (mol Salt/mol Protein)	Fractional amount of homo-dimer, $\alpha_{2,ii}$	Protein–protein binding, $\nu_{22,ii}$ (mol Monomer/mol Mulimer)
Monomer (A)	66.43	1.177	28,553	8.81	N/A	N/A
Homo-dimer (AA)	132.86	Varied: 1.177 0.942 1.295	Varied: 57,106 45,705 62,832	Varied: 17.62 13.22 8.81 26.43	Varied: 0.20 0.33 0.43	2

Table 2
Physical parameters used in the generalized free-solvent model for a binary protein solution forming a hetero-dimer.

Protein	Molecular weight, M_2 (kDa)	Hydration (g H_2O/g Protein)	Solvent accessible surface area (Å^2)	Ion binding (mol Salt/mol Protein)	Fractional amount of hetero-dimer, $\beta_{23,i:i}$, $\beta_{32,i:i}$	Protein–protein binding, $\nu_{23,i:i}$, $\nu_{32,i:i}$ (mol Monomer/mol Mulimer)
Monomer (A)	66.43	1.177	28,553	8.81	N/A	N/A
Monomer (B)	155	1.110	62,830	24.30	N/A	N/A
Homo-dimer (AB)	221.43	0.904	731,001	28.20	Varied: 0.09 0.23 0.33 0.50	1

and

$$x_1^I = \frac{N_1^I}{N_1^I + N_5^I} \tag{48}$$

where p = 3, $l_{\text{hetero-multimer}}$ = 3 ($l_{\text{hetero-multimer},2}$ = 2 and $l_{\text{hetero-multimer},3}$ = 1), η_2 = 2, and η_3 = 3, which yield a total of 26 parameters: 6 hydration, 6 ion binding, 7 protein–protein interactions, and 7 fractional amounts of protein.

4. Theoretical effects of model physical parameters on osmotic pressure

4.1. Single protein solution with homo-dimers

The free-solvent model for a single protein solution, and a monovalent salt, in which a homo-dimer forms (Eqs. (20) and (27) substituted into Eq. (1) is plotted for various homo-dimer

SASA and the corresponding homo-dimer hydration value (Fig. 1), various dimer ion binding values (Fig. 2), and various dimerization ratios (Fig. 3). For all three cases, $v_{22,ii} = 2$ mol monomer/mol dimer, and the first two cases have a constant a ratio of 3:1 monomer:homo-dimer ($\alpha_{2,ii} = 0.2$) (see Tables 1 and 2).

Fig. 1 shows the effect changing the SASA, upon homo-dimerization of a protein, has on osmotic pressure. When there is no change to the SASA upon dimerization (*i.e.* the SASA of the dimer, $SASA_{dimer}$, is two times the value of the monomer SASA, $SASA_{monomer}$), and if the ion binding is twice that of the monomer, such as occurs if dimerization has no effect on the net charge, the osmotic pressure is nearly identical to the osmotic pressure of a purely monomer solution. The lack of a SASA change for the dimer (*i.e.* $SASA_{dimer} = 2(SASA_{monomer})$) is typically observed the protein–protein interactions are weak.

If there is a reduction in the SASA of the dimer (*i.e.* $SASA_{dimer} < 2(SASA_{monomer})$), the osmotic pressure of the solution decreases, while if the SASA of the dimer increases (*i.e.* $SASA_{dimer} > 2(SASA_{monomer})$), the osmotic pressure of the solution increases (Fig. 1). The former case occurs in the majority of strong protein–protein interactions as solvent is displaced and replaced by electrostatic or van der Waals interactions. The latter case can occur if the protein–protein interaction causes a conformational change in one or more of the proteins.

Fig. 2 shows the effect changing the ion binding, upon homo-dimerization of a protein, has on osmotic pressure. When the hydration values are keep constant for the dimer, the loss of ions (reduction in ion binding, $v_{dimer\text{-}ion}$, compared to twice that of the monomer, $v_{monomer\text{-}ion}$) upon dimerization results in an increase in the osmotic pressure, while an increase in the ion binding of the dimer (*i.e.* $v_{dimer\text{-}ion} > 2(v_{monomer\text{-}ion})$) yields a decrease in the osmotic pressure. When a protein–protein interaction occurs, there is typically an effect on the overall net charge of the dimer which causes a shift in the amount of ions bound. However, at the isoelectric point of the protein, the ion binding value of the homo-dimer may be unaffected (*i.e.* $v_{dimer\text{-}ion} = 2(v_{monomer\text{-}ion})$).

Fig. 3 shows the effect increasing the amount of dimers in solution (*i.e.* $\alpha_{2,ii}$ increases) has on osmotic pressure. The osmotic pressure, as the fractional amounts of dimers increase, reduces the osmotic pressure, assuming that the hydration is the same ($SASA_{dimer} > 2(SASA_{monomer})$) and the ion binding value is twice that of the monomer ($v_{dimer\text{-}ion} = 2(v_{monomer\text{-}ion})$). This effect is due to the mole fraction of water for homo-multimers being closer to unity compared to the mole fraction of water for the pure monomer solution. As the total protein concentration, in grams per liter solution, increases, the moles of monomer increase more rapidly than the moles of homo-multimers.

4.2. Binary protein solution

4.2.1. Only monomers are present

The free-solvent model for a binary protein solution, and a monovalent salt, in which only monomers are present (Fig. 4) is modeled. The free-solvent model for a binary protein solution of pure monomers was developed previously by Yousef et al. [12]. As the molar ratio of protein A to protein B is changed, the osmotic pressure is affected such that the greater the molar ratio in favor of protein A (*i.e.* moles A is greater than moles of B, $(A/B) > 1$), the osmotic pressure closes in on the osmotic pressure of the single protein A monomer solution as $B \to 0$. Conversely, when the moles of protein B are greater than the moles of protein A ($(A/B) < 1$), the osmotic pressure closes in on the single protein B monomer solution osmotic pressure as $A \to 0$.

4.2.2. With the formation of hetero-dimers

The free-solvent model for a binary protein solution in which hetero-dimers occur, given by substituting Eqs. (33) and (40) into Eq. (1), is modeled (Fig. 5). As the fractional amount of

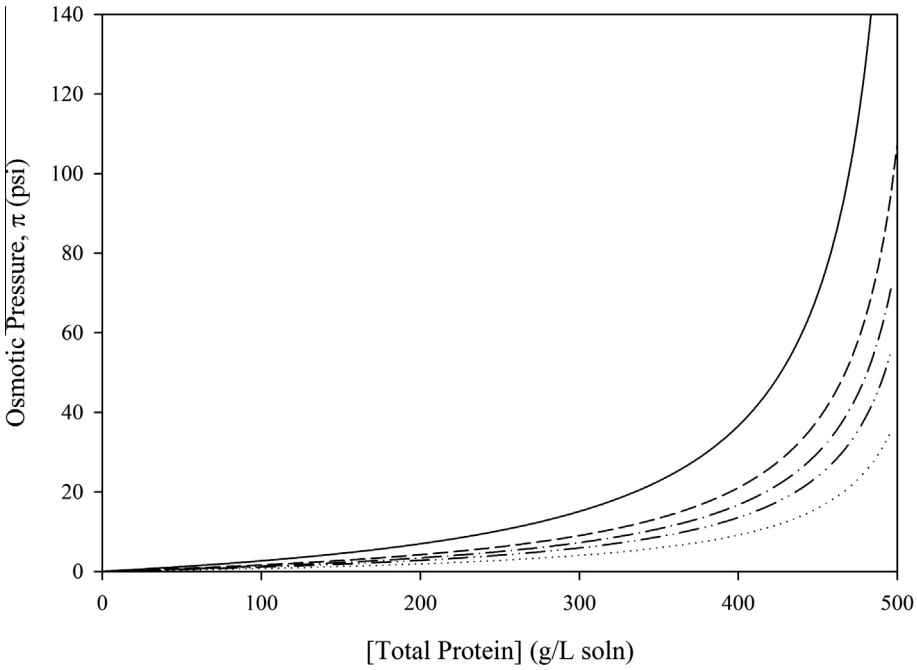

Fig. 4. Osmotic pressure vs. concentration for a theoretical binary protein solution in 0.15 M NaCl: the effect of changing ratio between the two proteins. The generalized free-solvent model for each single protein solution is plotted: protein A with $v_{12} = 1.177$ g H_2O/g Protein A and $v_{32} = 8.81$ mol NaCl/mol Protein A (solid curve) and protein B with $v_{12} = 1.110$ g H_2O/g Protein B and $v_{32} = 24.30$ mol NaCl/mol Protein B (dotted curve). The generalized free-solvent model for various molar ratios between protein A and protein B is plotted: (1) 1:1 A:B (dash-dot curve); (2) 3:1 A:B (dash-dot-dot curve); and (3) 1:3 A:B (dashed curve).

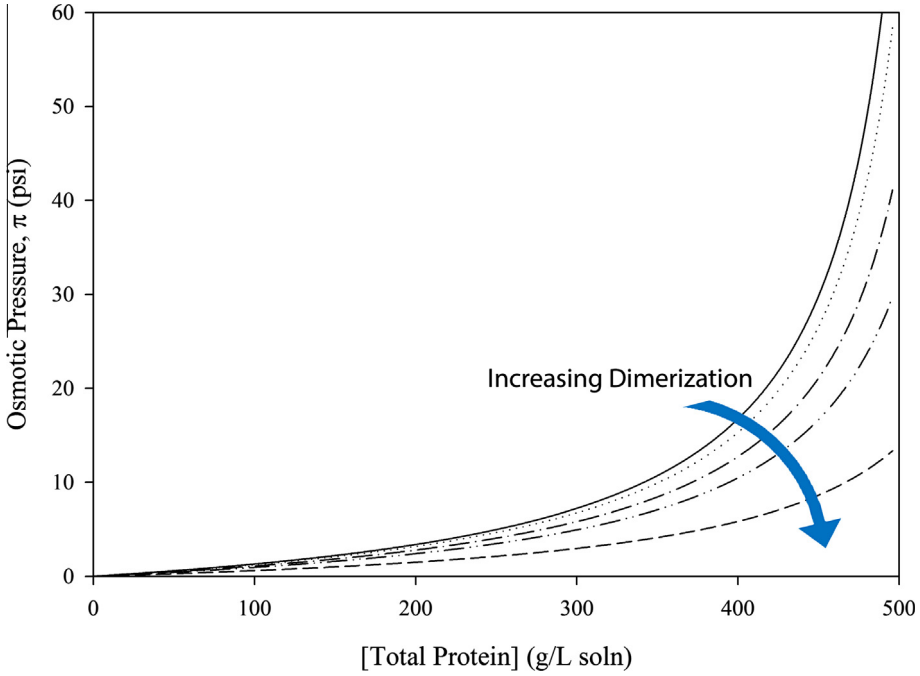

Fig. 5. Osmotic pressure vs. concentration for a theoretical binary protein solution in 0.15 M NaCl with the formation of a hetero-dimer: the effect of increased dimerization. The generalized free-solvent model for a 1:1 A:B binary protein solution is plotted when the solute–solvent parameters are ν_{12} = 1.177 g H_2O/g Protein A, ν_{32} = 8.81 mol NaCl/mol Protein A, ν_{12} = 1.110 g H_2O/g Protein B, and ν_{32} = 24.30 mol NaCl/mol Protein B (solid curve). When a hetero-dimer ($\nu_{23,i:i}$ = 1 mol monomer A/mol dimer AB and $\nu_{32,i:i}$ = 1 mol monomer B/mol dimer AB) occurs with solute–solvent parameters of $\nu_{123,i:i}$ = 0.904 g H_2O/g dimer and $\nu_{423,i:i}$ = 28.02 mol NaCl/mol dimer, the free-solvent model is plotted for various fractional amounts of hetero-dimerization: (1) 10% hetero-dimerization (A:AB = 9:1), $\beta_{23,i:i} = \beta_{32,i:i}$ = 0.1 (dotted curve); (2) 30% hetero-dimerization (A:AB = 7:3), $\beta_{23,i:i} = \beta_{32,i:i}$ = 0.3 (dash-dot curve); (3) 50% hetero-dimerization (A:AB = 1:1), $\beta_{23,i:i} = \beta_{32,i:i}$ = 0.5 (dash-dot-dot curve); and (4) 100% hetero-dimerization (A:AB = 0:1), $\beta_{23,i:i} = \beta_{32,i:i}$ = 1.0 (dashed curve).

dimerization, $\beta_{23,i:i}$, increases, the osmotic pressure decreases when all other parameters are held constant.

5. Discussion

5.1. Weak protein–protein interactions

Weak protein–protein interactions are considered to be those for which no (or minimal) solvent is displaced from the proteins' surfaces (negligible loss in solvent accessible surface area) by the interaction. An example of this is albumin [28,29].

For the scenario when weak protein–protein interactions occur, the free-solvent model can be developed with the assumption that only monomers exist in solution (given that $\nu_{\text{dimer-ion}} = 2(\nu_{\text{monomer-ion}})$). While this assumption is not physiologically true, the displacement of no, or minimal, solvent allows for the hydration value of monomers and homo-multimers to be nearly-identical (if considering the units grams of water per gram of protein). This effect on osmotic pressure is shown in Fig. 1 by comparing the solid curve (which is the pure monomer solution) and the dotted curve (homo-dimer solution with $\text{SASA}_{\text{dimer}} = 2(\text{SASA}_{\text{monomer}})$ and $\nu_{\text{dimer-ion}} = 2(\nu_{\text{monomer-ion}})$).

5.2. Strong protein–protein interactions

Strong protein–protein interactions are considered as all interactions which displace solvent from the surface of each protein, resulting in a change in the SASA for the multimer (*i.e.* the multimer has a binding interface that does not contain solvent). The binding interface between the proteins forming a multimer can be driven by electrostatic or van der Waals interactions.

For any solution in which strong protein–protein interactions occur, the free-solvent model, developed herein, can be used after making the appropriate reduction in terms (similar to the case scenarios presented). Each of these interactions will have unique hydrations, ion binding values, and fractional amounts of protein which need to be considered.

5.3. Robustness and limitations of the generalized free-solvent model

5.3.1. Osmotic pressure prediction

Given that the values of hydration and ion binding are available for all proteins in solution, and that the amount of proteins participating in protein–protein interactions is known, the free-solvent model can provide excellent predictions of the crowded protein osmotic pressure [6,7,30].

While a solution with n species and p proteins can be modeled, there is a limit to the predictive power of the free-solvent model; there will be a point at which the inclusion of additional parameters will have a negligible effect on the predicted osmotic pressure due to the sensitivity of each parameter being reduced.

5.3.2. Osmotic pressure regression

The free-solvent model has also been shown to provide information about the protein, such as hydration, ion binding, and SASA, when concentrated osmotic pressure data is regressed on [6,7,18,19,30–33]. However, with the development of the generalized free-solvent model which considers protein–protein interactions comes some limitations to the ability of the free-solvent model for osmotic pressure data regression. The free-solvent model may have limited success when regressing for multi-component protein solutions due to the large number of parameters which may need to regressed on. This may cause the regressed parameters to have a high covariance and thus remove physiological significance from the values of the parameters. However, if the values of most of the physical parameters are known for the

macromolecules in solution, the regression power of the free-solvent model on the remaining parameters will increase and may yield physiologically meaningful values of the regressed parameters.

5.3.3. Truncating the free-solvent model

In order to recover some of the robustness of the free-solvent model for predicting osmotic pressure or for regressing on osmotic pressure data to determine the values of the physical parameters, the free-solvent model may need to be truncated to remove some of the higher order multimers. This should be done with caution; experimentally determining the values of the hydration or the fractional amounts of the homo- and hetero-multimers can be useful when determining the appropriate terms to remove in order to maintain the robustness of the free-solvent model. Alternatively, a sensitively analysis of the terms in the free-solvent model (mole number, hydration, ion binding, and fractional amount) can be performed to determine the appropriate terms to neglect for truncating the generalized free-solvent model.

6. Conclusions

The free-solvent model is an excellent predictor of the osmotic pressure for concentrated single protein solutions. Here, a generalized free-solvent model for multi-component solutions in which protein–protein interactions occur has been developed. This generalized form of the free-solvent model considers both intra- (homo-multimer) and inter-protein (hetero-multimer) interactions.

Given that the physical parameters are available, experimental data for the osmotic pressure of crowded multi-component protein solutions no longer needs to be obtained because the free-solvent model allows for excellent predictability. This methodology can be extended to even predict the osmotic pressure within a biological system, such as a cell, given that the hydrations, ion binding values, and the fractional amounts of protein are known for each macromolecule.

Acknowledgement

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Appendix A. An example of the generalized free-solvent model considering seven monomeric proteins in solution forming homo- and hetero-multimers

Consider a multi-component protein mixture, containing seven monomeric proteins, in aqueous solution with a single monovalent salt in which homo-multimers and hetero-multimers are observed. For illustration purposes, let the monomeric proteins be labeled protein *A*–*G*. The following homo-multimers are formed in solution: *AA*, *BBB*, *EE*, and *FFFFF*. The hetero-multimers formed are: *BCC*, *CCDD*, *EF*, *ABE*, *BEFG*, and *ABCDEFG*.

A.1. Free-solvent in compartment II

In this solution, the total number of monomeric proteins is seven ($p = 7$) and the total number of species is nine ($n = 9$). The solvent and salt are species 1 and 9, respectively. The proteins are species 2–8.

For the solution in compartment II, the solvent bound to the proteins and their interactions are

$$\sum \nu_{\text{solvent/homo-multimer}} N^{\text{II}}_{\text{homo-multimers}} = \sum_{j=2}^{8} \sum_{Z=\text{ii}} \nu_{1j,Z} \alpha_{j,Z} N^{\text{II}}_{j}$$

$$\sum \nu_{\text{solvent/hetero-multimer}} N^{\text{II}}_{\text{hetero-multimers}} = \left[\begin{array}{l} \displaystyle\sum_{j=2}^{7} \sum_{a=3}^{8} \sum_{A=\text{i}} \sum_{B=\text{i}} \nu_{1ja,A:B} \beta_{ja,A:B} N^{\text{II}}_{j} + \sum_{j=2}^{6} \sum_{a=j+1}^{7} \sum_{\substack{b=j+2 \\ b\neq a}}^{8} \sum_{A=\text{i}} \sum_{B=\text{i}} \sum_{C=\text{i}} \nu_{1jab,A:B:C} \beta_{jab,A:B:C} N^{\text{II}}_{j} + \cdots \\ \cdots + \displaystyle\sum_{j=2}^{5} \sum_{a=j+1}^{6} \sum_{\substack{b=j+2 \\ b\neq a}}^{7} \sum_{\substack{c=j+3 \\ c\neq a \\ c\neq b}}^{8} \sum_{A=\text{i}} \sum_{B=\text{i}} \sum_{C=\text{i}} \sum_{D=\text{i}} \nu_{1jabc,A:B:C:D} \beta_{jabc,A:B:C:D} N^{\text{II}}_{j} + \cdots \\ \cdots + \displaystyle\sum_{j=2}^{2} \sum_{a=j+1}^{3} \sum_{\substack{b=j+2 \\ b\neq a}}^{4} \sum_{\substack{c=j+3 \\ c\neq a \\ c\neq b}}^{5} \sum_{\substack{d=j+4 \\ d\neq a \\ d\neq b \\ d\neq c}}^{6} \sum_{\substack{e=j+5 \\ e\neq a \\ e\neq b \\ e\neq c \\ e\neq d}}^{7} \sum_{\substack{f=j+6 \\ f\neq a \\ f\neq b \\ f\neq c \\ f\neq d \\ f\neq e}}^{8} \sum_{A=\text{i}} \sum_{B=\text{i}} \sum_{C=\text{i}} \sum_{D=\text{i}} \sum_{E=\text{i}} \sum_{F=\text{i}} \sum_{G=\text{i}} \nu_{1jabcdef,A:B:C:D:E:F:G} \beta_{jabcdef,A:B:C:D:E:F:G} N^{\text{II}}_{j} \end{array} \right]$$

$$\sum \nu_{\text{solvent/monomer}} N^{\text{II}}_{\text{monomers}} = \sum_{j=2}^{8} \nu_{1j} \left(\begin{array}{l} 1 - \displaystyle\sum_{Z=\text{ii}} \nu_{jj,Z} \alpha_{j,Z} + \cdots \\ \cdots - \displaystyle\sum_{a=2}^{8} \sum_{A=\text{i}} \sum_{B=\text{i}} \nu_{ja,A:B} \beta_{ja,A:B} + \sum_{a=2}^{8} \sum_{\substack{b=2 \\ b\neq a}}^{8} \sum_{A=\text{i}} \sum_{B=\text{i}} \sum_{C=\text{i}} \nu_{jab,A:B:C} \beta_{jab,A:B:C} + \cdots \\ + \cdots \displaystyle\sum_{a=2}^{8} \sum_{\substack{b=2 \\ b\neq a}}^{8} \sum_{\substack{c=2 \\ c\neq a \\ c\neq b}}^{8} \sum_{A=\text{i}} \sum_{B=\text{i}} \sum_{C=\text{i}} \sum_{D=\text{i}} \nu_{jabc,A:B:C:D} \beta_{jabc,A:B:C:D} + \cdots \\ \cdots + \displaystyle\sum_{a=2}^{8} \sum_{\substack{b=2 \\ b\neq a}}^{8} \sum_{\substack{c=2 \\ c\neq a \\ c\neq b}}^{8} \sum_{\substack{d=2 \\ d\neq a \\ d\neq b \\ d\neq c}}^{8} \sum_{\substack{e=2 \\ e\neq a \\ e\neq b \\ e\neq c \\ e\neq d}}^{8} \sum_{\substack{f=2 \\ f\neq a \\ f\neq b \\ f\neq c \\ f\neq d \\ f\neq e}}^{8} \sum_{A=\text{i}} \sum_{B=\text{i}} \sum_{C=\text{i}} \sum_{D=\text{i}} \sum_{E=\text{i}} \sum_{F=\text{i}} \sum_{G=\text{i}} \nu_{jabcdef,A:B:C:D:E:F:G} \beta_{jabcdef,A:B:C:D:E:F:G} \end{array} \right) N^{\text{II}}_{j}$$

The above equations reduce to

$$\sum \nu_{\text{solvent/homo-multimer}} N^{\text{II}}_{\text{homo-multimers}} = \nu_{12,\text{ii}}\alpha_{2,\text{ii}}N^{\text{II}}_2 + \nu_{13,\text{iii}}\alpha_{3,\text{iii}}N^{\text{II}}_3 + \nu_{16,\text{ii}}\alpha_{6,\text{ii}}N^{\text{II}}_6 + \nu_{17,\text{v}}\alpha_{7,\text{v}}N^{\text{II}}_7$$

$$\sum \nu_{\text{solvent/hetero-mulitmer}} N^{\text{II}}_{\text{hetero-mulitmers}} = \begin{bmatrix} \nu_{134,\text{i:ii}}\beta_{34,\text{i:ii}}N^{\text{II}}_3 + \nu_{145,\text{ii:ii}}\beta_{45,\text{ii:ii}}N^{\text{II}}_4 + \nu_{167,\text{i:i}}\beta_{67,\text{i:i}}N^{\text{II}}_6 + \nu_{1236,\text{i:i:i}}\beta_{236,\text{i:i:i}}N^{\text{II}}_2 + \cdots \\ \cdots + \nu_{13678,\text{i:i:i:i}}\beta_{3678,\text{i:i:i:i}}N^{\text{II}}_3 + \nu_{12345678,\text{i:i:i:i:i:i:i:i}}\beta_{2345678,\text{i:i:i:i:i:i:i}}N^{\text{II}}_2 \end{bmatrix}$$

$$\sum \nu_{\text{solvent/monomer}} N^{\text{II}}_{\text{monomers}} = \begin{bmatrix} \nu_{12}(1 - \nu_{22,\text{ii}}\alpha_{2,\text{ii}} - \nu_{236,\text{i:i:i}}\beta_{236,\text{i:i:i}} - \nu_{2345678,\text{i:i:i:i:i:i:i}}\beta_{2345678,\text{i:i:i:i:i:i:i}})N^{\text{II}}_2 + \cdots \\ \cdots + \nu_{13}(1 - \nu_{33,\text{iii}}\alpha_{3,\text{iii}} - \nu_{34,\text{i:ii}}\beta_{34,\text{i:ii}} - \nu_{326,\text{i:i:i}}\beta_{326,\text{i:i:i}} - \nu_{3678,\text{i:i:i:i}}\beta_{3678,\text{i:i:i:i}} - \nu_{3245678,\text{i:i:i:i:i:i:i}}\beta_{3245678,\text{i:i:i:i:i:i:i}})N^{\text{II}}_3 + \cdots \\ \cdots + \nu_{14}(1 - \nu_{43,\text{ii:i}}\beta_{43,\text{ii:i}} - \nu_{45,\text{ii:ii}}\beta_{45,\text{ii:ii}} - \nu_{4235678,\text{i:i:i:i:i:i:i}}\beta_{4235678,\text{i:i:i:i:i:i:i}})N^{\text{II}}_4 + \cdots \\ \cdots + \nu_{15}(1 - \nu_{54,\text{ii:ii}}\beta_{54,\text{ii:ii}} - \nu_{5234678,\text{i:i:i:i:i:i:i}}\beta_{5234678,\text{i:i:i:i:i:i:i}})N^{\text{II}}_5 + \cdots \\ \cdots + \nu_{16}(1 - \nu_{66,\text{ii}}\alpha_{6,\text{ii}} - \nu_{67,\text{i:i}}\beta_{67,\text{i:i}} - \nu_{623,\text{i:i:i}}\beta_{623,\text{i:i:i}} - \nu_{6378,\text{i:i:i:i}}\beta_{6378,\text{i:i:i:i}} - \nu_{6234578,\text{i:i:i:i:i:i:i}}\beta_{6234578,\text{i:i:i:i:i:i:i}})N^{\text{II}}_6 + \cdots \\ \cdots + \nu_{17}(1 - \nu_{77,\text{v}}\alpha_{7,\text{v}} - \nu_{76,\text{i:i}}\beta_{76,\text{i:i}} - \nu_{7368,\text{i:i:i:i}}\beta_{7368,\text{i:i:i:i}} - \nu_{7234568,\text{i:i:i:i:i:i:i}}\beta_{7234568,\text{i:i:i:i:i:i:i}})N^{\text{II}}_7 + \cdots \\ \cdots + \nu_{18}(1 - \nu_{7368,\text{i:i:i:i}}\beta_{7368,\text{i:i:i:i}} - \nu_{7234568,\text{i:i:i:i:i:i:i}}\beta_{7234568,\text{i:i:i:i:i:i:i}})N^{\text{II}}_8 \end{bmatrix}$$

Then moles of free-solvent then becomes

$$\begin{aligned} & N^{\text{II}}_1 - (\nu_{12,\text{ii}}\alpha_{2,\text{ii}}N^{\text{II}}_2 + \nu_{13,\text{iii}}\alpha_{3,\text{iii}}N^{\text{II}}_3 + \nu_{16,\text{ii}}\alpha_{6,\text{ii}}N^{\text{II}}_6 + \nu_{17,\text{v}}\alpha_{7,\text{v}}N^{\text{II}}_7) + \cdots \\ & \cdots - (\nu_{134,\text{i:ii}}\beta_{34,\text{i:ii}}N^{\text{II}}_3 + \nu_{145,\text{ii:ii}}\beta_{45,\text{ii:ii}}N^{\text{II}}_4 + \nu_{167,\text{i:i}}\beta_{67,\text{i:i}}N^{\text{II}}_6 + \nu_{1236,\text{i:i:i}}\beta_{236,\text{i:i:i}}N^{\text{II}}_2) + \cdots \\ & \cdots - (\nu_{13678,\text{i:i:i:i}}\beta_{3678,\text{i:i:i:i}}N^{\text{II}}_3 + \nu_{12345678,\text{i:i:i:i:i:i:i:i}}\beta_{2345678,\text{i:i:i:i:i:i:i}}N^{\text{II}}_2) + \cdots \\ & \cdots - (1 - \nu_{22,\text{ii}}\alpha_{2,\text{ii}} - \nu_{236,\text{i:i:i}}\beta_{236,\text{i:i:i}} - \nu_{2345678,\text{i:i:i:i:i:i:i}}\beta_{2345678,\text{i:i:i:i:i:i:i}})N^{\text{II}}_2 + \cdots \\ & \cdots - (1 - \nu_{33,\text{iii}}\alpha_{3,\text{iii}} - \nu_{34,\text{i:ii}}\beta_{34,\text{i:ii}} - \nu_{326,\text{i:i:i}}\beta_{326,\text{i:i:i}} - \nu_{3678,\text{i:i:i:i}}\beta_{3678,\text{i:i:i:i}} - \nu_{3245678,\text{i:i:i:i:i:i:i}}\beta_{3245678,\text{i:i:i:i:i:i:i}})N^{\text{II}}_3 + \cdots \\ & \cdots - (1 - \nu_{43,\text{ii:i}}\beta_{43,\text{ii:i}} - \nu_{45,\text{ii:ii}}\beta_{45,\text{ii:ii}} - \nu_{4235678,\text{i:i:i:i:i:i:i}}\beta_{4235678,\text{i:i:i:i:i:i:i}})N^{\text{II}}_4 + \cdots \\ & \cdots - (1 - \nu_{54,\text{ii:ii}}\beta_{54,\text{ii:ii}} - \nu_{5234678,\text{i:i:i:i:i:i:i}}\beta_{5234678,\text{i:i:i:i:i:i:i}})N^{\text{II}}_5 + \cdots \\ & \cdots - (1 - \nu_{66,\text{ii}}\alpha_{6,\text{ii}} - \nu_{67,\text{i:i}}\beta_{67,\text{i:i}} - \nu_{623,\text{i:i:i}}\beta_{623,\text{i:i:i}} - \nu_{6378,\text{i:i:i:i}}\beta_{6378,\text{i:i:i:i}} - \nu_{6234578,\text{i:i:i:i:i:i:i}}\beta_{6234578,\text{i:i:i:i:i:i:i}})N^{\text{II}}_6 + \cdots \\ & \cdots - (1 - \nu_{77,\text{v}}\alpha_{7,\text{v}} - \nu_{76,\text{i:i}}\beta_{76,\text{i:i}} - \nu_{7368,\text{i:i:i:i}}\beta_{7368,\text{i:i:i:i}} - \nu_{7234568,\text{i:i:i:i:i:i:i}}\beta_{7234568,\text{i:i:i:i:i:i:i}})N^{\text{II}}_7 + \cdots \\ & \cdots - (1 - \nu_{7368,\text{i:i:i:i}}\beta_{7368,\text{i:i:i:i}} - \nu_{7234568,\text{i:i:i:i:i:i:i}}\beta_{7234568,\text{i:i:i:i:i:i:i}})N^{\text{II}}_8 \end{aligned} \tag{A.1}$$

A.2. Total moles in compartment II

The total moles of proteins and the bound solvent in compartment II are

$$\sum N^{\text{II}}_{\text{homo-multimers}} = \sum_{j=2}^{8}\sum_{Z=\text{ii}}\alpha_{j,Z}N^{\text{II}}_j$$

$$\sum \nu N^{\text{II}}_{\text{homo-multimers}} = \sum_{\substack{i=1 \\ i\neq 2-8}}^{9}\sum_{j=2}^{8}\sum_{Z=\text{ii}}\nu_{ij,Z}\alpha_{j,Z}N^{\text{II}}_j$$

$$\sum N^{\text{II}}_{\text{hetero-multimers}} = \begin{bmatrix} \displaystyle\sum_{j=2}^{7}\sum_{a=j+1}^{8}\sum_{A=\text{i}}\sum_{B=\text{i}}\beta_{ja,A:B}N^{\text{II}}_j + \cdots \\ \cdots + \displaystyle\sum_{j=2}^{6}\sum_{a=j+1}^{7}\sum_{\substack{b=j+2 \\ b\neq a}}^{8}\sum_{A=\text{i}}\sum_{B=\text{i}}\sum_{C=\text{i}}\beta_{jab,A:B:C}N^{\text{II}}_j + \cdots \\ \cdots + \displaystyle\sum_{j=2}^{5}\sum_{\substack{a=j+1 \\ a\neq j}}^{6}\sum_{\substack{b=j+2 \\ b\neq j \\ b\neq a}}^{7}\sum_{\substack{c=j+3 \\ c\neq j \\ c\neq a}}^{8}\sum_{A=\text{i}}\sum_{B=\text{i}}\sum_{C=\text{i}}\sum_{D=\text{i}}\beta_{jabc,A:B:C:D}N^{\text{II}}_j + \cdots \\ \cdots + \displaystyle\sum_{j=2}^{2}\sum_{\substack{a=j+1 \\ a\neq j}}^{3}\sum_{\substack{b=j+2 \\ b\neq j \\ b\neq a}}^{4}\sum_{\substack{c=j+3 \\ c\neq j \\ c\neq a \\ c\neq b}}^{5}\sum_{\substack{d=j+4 \\ d\neq j \\ d\neq a \\ d\neq b \\ d\neq c}}^{6}\sum_{\substack{e=j+5 \\ e\neq j \\ e\neq a \\ e\neq b \\ e\neq c \\ e\neq d}}^{7}\sum_{\substack{f=j+6 \\ f\neq j \\ f\neq a \\ f\neq b \\ f\neq c \\ f\neq d \\ f\neq e}}^{8}\sum_{A=\text{i}}\sum_{B=\text{i}}\sum_{C=\text{i}}\sum_{D=\text{i}}\sum_{E=\text{i}}\sum_{F=\text{i}}\sum_{G=\text{i}}\beta_{jabcdef,A:B:C:D:E:F:G}N^{\text{II}}_j \end{bmatrix}$$

$$\sum vN^{\mathrm{II}}_{\text{hetero-multimers}} = \left[\begin{array}{l} \sum_{\substack{i=1\\ i\neq 2\to 8}}^{9}\sum_{j=2}^{7}\sum_{a=j+1}^{8}\sum_{A=\mathrm{i}}\sum_{B=\mathrm{i}} v_{ija,A:B}\beta_{ja,A:B}N_j^{\mathrm{II}} + \cdots \\ \cdots + \sum_{\substack{i=1\\ i\neq 2\to 8}}^{9}\sum_{j=2}^{6}\sum_{a=j+1}^{7}\sum_{\substack{b=j+2\\ b\neq a}}^{8}\sum_{A=\mathrm{i}}\sum_{B=\mathrm{i}}\sum_{C=\mathrm{i}} v_{ijab,A:B:C}\beta_{jab,A:B:C}N_j^{\mathrm{II}} + \cdots \\ \cdots + \sum_{\substack{i=1\\ i\neq 2\to 8}}^{9}\sum_{j=2}^{5}\sum_{\substack{a=2\\ a\neq j}}^{6}\sum_{\substack{b=2\\ b\neq j\\ b\neq a}}^{7}\sum_{\substack{c=2\\ c\neq j\\ c\neq a}}^{8}\sum_{A=\mathrm{i}}\sum_{B=\mathrm{i}}\sum_{C=\mathrm{i}}\sum_{D=\mathrm{i}} v_{ijabc,A:B:C:D}\beta_{jabc,A:B:C:D}N_j^{\mathrm{II}} + \cdots \\ \cdots + \sum_{\substack{i=1\\ i\neq 2\to 8}}^{9}\sum_{j=2}^{2}\sum_{\substack{a=2\\ a\neq j}}^{3}\sum_{\substack{b=2\\ b\neq j\\ b\neq a}}^{4}\sum_{\substack{c=2\\ c\neq j\\ c\neq a\\ c\neq b}}^{5}\sum_{\substack{d=2\\ d\neq j\\ d\neq a\\ d\neq b\\ d\neq c}}^{6}\sum_{\substack{e=2\\ e\neq j\\ e\neq a\\ e\neq b\\ e\neq c\\ e\neq d}}^{7}\sum_{\substack{f=2\\ f\neq j\\ f\neq a\\ f\neq b\\ f\neq c\\ f\neq d\\ f\neq e}}^{8}\sum_{A=\mathrm{i}}\sum_{B=\mathrm{i}}\sum_{C=\mathrm{i}}\sum_{D=\mathrm{i}}\sum_{E=\mathrm{i}}\sum_{F=\mathrm{i}}\sum_{G=\mathrm{i}} v_{ijabcdef,A:B:C:D:E:F:G}\beta_{jabcdef,A:B:C:D:E:F:G}N_j^{\mathrm{II}} \end{array}\right]$$

$$\sum N^{\mathrm{II}}_{\text{monomers}} = \sum_{j=2}^{8}\left(\begin{array}{l} 1 - \sum_{Z=\mathrm{ii}} v_{jj,Z}\alpha_{j,Z} + \ldots \\ \ldots - \sum_{\substack{a=2\\ a\neq j}}^{8}\sum_{A=\mathrm{i}}\sum_{B=\mathrm{i}} v_{ja,A:B}\beta_{ja,A:B} - \sum_{\substack{a=2\\ a\neq j}}^{8}\sum_{\substack{b=2\\ b\neq j\\ b\neq a}}^{8}\sum_{A=\mathrm{i}}\sum_{B=\mathrm{i}}\sum_{C=\mathrm{i}} v_{jab,A:B:C}\beta_{jab,A:B:C} + \ldots \\ \ldots - \sum_{\substack{a=2\\ a\neq j}}^{8}\sum_{\substack{b=2\\ b\neq j\\ b\neq a}}^{8}\sum_{\substack{c=2\\ c\neq j\\ c\neq a}}^{8}\sum_{A=\mathrm{i}}\sum_{B=\mathrm{i}}\sum_{C=\mathrm{i}}\sum_{D=\mathrm{i}} v_{jabc,A:B:C:D}\beta_{jabc,A:B:C:D} + \ldots \\ \ldots - \sum_{\substack{a=2\\ a\neq j}}^{8}\sum_{\substack{b=2\\ b\neq j\\ b\neq a}}^{8}\sum_{\substack{c=2\\ c\neq j\\ c\neq a\\ c\neq b}}^{8}\sum_{\substack{d=2\\ d\neq j\\ d\neq a\\ d\neq b\\ d\neq c}}^{8}\sum_{\substack{e=2\\ e\neq j\\ e\neq a\\ e\neq b\\ e\neq c\\ e\neq d}}^{8}\sum_{\substack{f=2\\ f\neq j\\ f\neq a\\ f\neq b\\ f\neq c\\ f\neq d\\ f\neq e}}^{8}\sum_{A=\mathrm{i}}\sum_{B=\mathrm{i}}\sum_{C=\mathrm{i}}\sum_{D=\mathrm{i}}\sum_{E=\mathrm{i}}\sum_{F=\mathrm{i}}\sum_{G=\mathrm{i}} v_{jabcdef,A:B:C:D:E:F:G}\beta_{jabcdef,A:B:C:D:E:F:G} \end{array}\right) N_j^{\mathrm{II}}$$

$$\sum vN^{\mathrm{II}}_{\text{monomers}} = \sum_{\substack{i=1\\ i\neq 2\to 8}}^{9}\sum_{j=2}^{8} v_{ij}\left(\begin{array}{l} 1 - \sum_{Z=\mathrm{ii}} v_{jj,Z}\alpha_{j,Z} + \ldots \\ \ldots - \sum_{\substack{a=2\\ a\neq j}}^{8}\sum_{A=\mathrm{i}}\sum_{B=\mathrm{i}} v_{ja,A:B}\beta_{ja,A:B} - \sum_{\substack{a=2\\ a\neq j}}^{8}\sum_{\substack{b=2\\ b\neq j\\ b\neq a}}^{8}\sum_{A=\mathrm{i}}\sum_{B=\mathrm{i}}\sum_{C=\mathrm{i}} v_{jab,A:B:C}\beta_{jab,A:B:C} + \ldots \\ \ldots - \sum_{\substack{a=2\\ a\neq j}}^{8}\sum_{\substack{b=2\\ b\neq j\\ b\neq a}}^{8}\sum_{\substack{c=2\\ c\neq j\\ c\neq a}}^{8}\sum_{A=\mathrm{i}}\sum_{B=\mathrm{i}}\sum_{C=\mathrm{i}}\sum_{D=\mathrm{i}} v_{jabc,A:B:C:D}\beta_{jabc,A:B:C:D} + \ldots \\ \ldots - \sum_{\substack{a=2\\ a\neq j}}^{8}\sum_{\substack{b=2\\ b\neq j\\ b\neq a}}^{8}\sum_{\substack{c=2\\ c\neq j\\ c\neq a\\ c\neq b}}^{8}\sum_{\substack{d=2\\ d\neq j\\ d\neq a\\ d\neq b\\ d\neq c}}^{8}\sum_{\substack{e=2\\ e\neq j\\ e\neq a\\ e\neq b\\ e\neq c\\ e\neq d}}^{8}\sum_{\substack{f=2\\ f\neq j\\ f\neq a\\ f\neq b\\ f\neq c\\ f\neq d\\ f\neq e}}^{8}\sum_{A=\mathrm{i}}\sum_{B=\mathrm{i}}\sum_{C=\mathrm{i}}\sum_{D=\mathrm{i}}\sum_{E=\mathrm{i}}\sum_{F=\mathrm{i}}\sum_{G=\mathrm{i}} v_{jabcdef,A:B:C:D:E:F:G}\beta_{jabcdef,A:B:C:D:E:F:G} \end{array}\right) N_j^{\mathrm{II}}$$

and reduce to

$$\sum N^{\mathrm{II}}_{\text{homo-multimers}} = \alpha_{2,\mathrm{ii}}N_2^{\mathrm{II}} + \alpha_{3,\mathrm{iii}}N_3^{\mathrm{II}} + \alpha_{6,\mathrm{ii}}N_6^{\mathrm{II}} + \alpha_{7,\mathrm{v}}N_7^{\mathrm{II}}$$

$$\sum vN^{\mathrm{II}}_{\text{homo-multimers}} = \left[\begin{array}{l} v_{12,\mathrm{ii}}\alpha_{2,\mathrm{ii}}N_2^{\mathrm{II}} + v_{92,\mathrm{ii}}\alpha_{2,\mathrm{ii}}N_2^{\mathrm{II}} + v_{13,\mathrm{iii}}\alpha_{3,\mathrm{iii}}N_3^{\mathrm{II}} + v_{93,\mathrm{iii}}\alpha_{3,\mathrm{iii}}N_3^{\mathrm{II}} + \cdots \\ \cdots + v_{16,\mathrm{ii}}\alpha_{6,\mathrm{ii}}N_6^{\mathrm{II}} + v_{96,\mathrm{ii}}\alpha_{6,\mathrm{ii}}N_6^{\mathrm{II}} + v_{17,\mathrm{v}}\alpha_{7,\mathrm{v}}N_7^{\mathrm{II}} + v_{97,\mathrm{v}}\alpha_{7,\mathrm{v}}N_7^{\mathrm{II}} \end{array}\right]$$

$$\sum N^{\mathrm{II}}_{\text{hetero-multimers}} = \left[\begin{array}{l} \beta_{34,\mathrm{i:ii}}N_3^{\mathrm{II}} + \beta_{45,\mathrm{ii:ii}}N_4^{\mathrm{II}} + \beta_{67,\mathrm{i:i}}N_6^{\mathrm{II}} + \beta_{236,\mathrm{i:i:i}}N_2^{\mathrm{II}} + \cdots \\ \cdots + \beta_{3678,\mathrm{i:i:i:i}}N_3^{\mathrm{II}} + \beta_{2345678,\mathrm{i:i:i:i:i:i:i}}N_2^{\mathrm{II}} \end{array}\right]$$

$$\sum vN^{\mathrm{II}}_{\text{hetero-multimers}} = \left[\begin{array}{l} v_{134,\mathrm{i:ii}}\beta_{34,\mathrm{i:ii}}N_3^{\mathrm{II}} + v_{934,\mathrm{i:ii}}\beta_{34,\mathrm{i:ii}}N_3^{\mathrm{II}} + v_{145,\mathrm{ii:ii}}\beta_{45,\mathrm{ii:ii}}N_4^{\mathrm{II}} + v_{945,\mathrm{ii:ii}}\beta_{45,\mathrm{ii:ii}}N_4^{\mathrm{II}} + \cdots \\ \cdots + v_{167,\mathrm{i:i}}\beta_{67,\mathrm{i:i}}N_6^{\mathrm{II}} + v_{967,\mathrm{i:i}}\beta_{67,\mathrm{i:i}}N_6^{\mathrm{II}} + v_{1236,\mathrm{i:i:i}}\beta_{236,\mathrm{i:i:i}}N_2^{\mathrm{II}} + v_{9236,\mathrm{i:i:i}}\beta_{236,\mathrm{i:i:i}}N_2^{\mathrm{II}} + \cdots \\ \cdots + v_{13678,\mathrm{i:i:i:i}}\beta_{3678,\mathrm{i:i:i:i}}N_3^{\mathrm{II}} + v_{93678,\mathrm{i:i:i:i}}\beta_{3678,\mathrm{i:i:i:i}}N_3^{\mathrm{II}} + \cdots \\ \cdots + v_{12345678,\mathrm{i:i:i:i:i:i:i:i}}\beta_{2345678,\mathrm{i:i:i:i:i:i:i:i}}N_2^{\mathrm{II}} + v_{92345678,\mathrm{i:i:i:i:i:i:i:i}}\beta_{2345678,\mathrm{i:i:i:i:i:i:i:i}}N_2^{\mathrm{II}} \end{array}\right]$$

$$\sum N^{\mathrm{II}}_{\mathrm{monomers}} = \begin{bmatrix} (1-\nu_{22,\mathrm{ii}}\alpha_{2,\mathrm{ii}}-\nu_{236,\mathrm{i:i:i}}\beta_{236,\mathrm{i:i:i}}-\nu_{2345678,\mathrm{i:i:i:i:i:i:i}}\beta_{2345678,\mathrm{i:i:i:i:i:i:i}})N_2^{\mathrm{II}}+\cdots \\ \cdots+(1-\nu_{33,\mathrm{iii}}\alpha_{3,\mathrm{iii}}-\nu_{34,\mathrm{i:ii}}\beta_{34,\mathrm{i:ii}}-\nu_{326,\mathrm{i:i:i}}\beta_{326,\mathrm{i:i:i}}-\nu_{3678,\mathrm{i:i:i:i}}\beta_{3678,\mathrm{i:i:i:i}}-\nu_{3245678,\mathrm{i:i:i:i:i:i:i}}\beta_{3245678,\mathrm{i:i:i:i:i:i:i}})N_3^{\mathrm{II}}+\cdots \\ \cdots+(1-\nu_{43,\mathrm{ii:i}}\beta_{43,\mathrm{ii:i}}-\nu_{45,\mathrm{ii:ii}}\beta_{45,\mathrm{ii:ii}}-\nu_{4235678,\mathrm{i:i:i:i:i:i:i}}\beta_{4235678,\mathrm{i:i:i:i:i:i:i}})N_4^{\mathrm{II}}+\cdots \\ \cdots+(1-\nu_{54,\mathrm{ii:ii}}\beta_{54,\mathrm{ii:ii}}-\nu_{5234678,\mathrm{i:i:i:i:i:i:i}}\beta_{5234678,\mathrm{i:i:i:i:i:i:i}})N_5^{\mathrm{II}}+\cdots \\ \cdots+(1-\nu_{66,\mathrm{ii}}\alpha_{6,\mathrm{ii}}-\nu_{67,\mathrm{i:i}}\beta_{67,\mathrm{i:i}}-\nu_{623,\mathrm{i:i:i}}\beta_{623,\mathrm{i:i:i}}-\nu_{6378,\mathrm{i:i:i:i}}\beta_{6378,\mathrm{i:i:i:i}}-\nu_{6234578,\mathrm{i:i:i:i:i:i:i}}\beta_{6234578,\mathrm{i:i:i:i:i:i:i}})N_6^{\mathrm{II}}+\cdots \\ \cdots+(1-\nu_{77,\mathrm{v}}\alpha_{7,\mathrm{v}}-\nu_{76,\mathrm{i:i}}\beta_{76,\mathrm{i:i}}-\nu_{7368,\mathrm{i:i:i:i}}\beta_{7368,\mathrm{i:i:i:i}}-\nu_{7234568,\mathrm{i:i:i:i:i:i:i}}\beta_{7234568,\mathrm{i:i:i:i:i:i:i}})N_7^{\mathrm{II}}+\cdots \\ \cdots+(1-\nu_{8367,\mathrm{i:i:i:i}}\beta_{8367,\mathrm{i:i:i:i}}-\nu_{8234567,\mathrm{i:i:i:i:i:i:i}}\beta_{8234567,\mathrm{i:i:i:i:i:i:i}})N_8^{\mathrm{II}} \end{bmatrix}$$

$$\sum \nu N^{\mathrm{II}}_{\mathrm{monomers}} = \begin{bmatrix} \nu_{12}(1-\nu_{22,\mathrm{ii}}\alpha_{2,\mathrm{ii}}-\nu_{236,\mathrm{i:i:i}}\beta_{236,\mathrm{i:i:i}}-\nu_{2345678,\mathrm{i:i:i:i:i:i:i}}\beta_{2345678,\mathrm{i:i:i:i:i:i:i}})N_2^{\mathrm{II}}+\cdots \\ \cdots+\nu_{92}(1-\nu_{22,\mathrm{ii}}\alpha_{2,\mathrm{ii}}-\nu_{236,\mathrm{i:i:i}}\beta_{236,\mathrm{i:i:i}}-\nu_{2345678,\mathrm{i:i:i:i:i:i:i}}\beta_{2345678,\mathrm{i:i:i:i:i:i:i}})N_2^{\mathrm{II}}+\cdots \\ \cdots+\nu_{13}(1-\nu_{33,\mathrm{iii}}\alpha_{3,\mathrm{iii}}-\nu_{34,\mathrm{i:ii}}\beta_{34,\mathrm{i:ii}}-\nu_{326,\mathrm{i:i:i}}\beta_{326,\mathrm{i:i:i}}-\nu_{3678,\mathrm{i:i:i:i}}\beta_{3678,\mathrm{i:i:i:i}}-\nu_{3245678,\mathrm{i:i:i:i:i:i:i}}\beta_{3245678,\mathrm{i:i:i:i:i:i:i}})N_3^{\mathrm{II}}+\cdots \\ \cdots+\nu_{93}(1-\nu_{33,\mathrm{iii}}\alpha_{3,\mathrm{iii}}-\nu_{34,\mathrm{i:ii}}\beta_{34,\mathrm{i:ii}}-\nu_{326,\mathrm{i:i:i}}\beta_{326,\mathrm{i:i:i}}-\nu_{3678,\mathrm{i:i:i:i}}\beta_{3678,\mathrm{i:i:i:i}}-\nu_{3245678,\mathrm{i:i:i:i:i:i:i}}\beta_{3245678,\mathrm{i:i:i:i:i:i:i}})N_3^{\mathrm{II}}+\cdots \\ \cdots+\nu_{14}(1-\nu_{43,\mathrm{ii:i}}\beta_{43,\mathrm{ii:i}}-\nu_{45,\mathrm{ii:ii}}\beta_{45,\mathrm{ii:ii}}-\nu_{4235678,\mathrm{i:i:i:i:i:i:i}}\beta_{4235678,\mathrm{i:i:i:i:i:i:i}})N_4^{\mathrm{II}}+\cdots \\ \cdots+\nu_{94}(1-\nu_{43,\mathrm{ii:i}}\beta_{43,\mathrm{ii:i}}-\nu_{45,\mathrm{ii:ii}}\beta_{45,\mathrm{ii:ii}}-\nu_{4235678,\mathrm{i:i:i:i:i:i:i}}\beta_{4235678,\mathrm{i:i:i:i:i:i:i}})N_4^{\mathrm{II}}+\cdots \\ \cdots+\nu_{15}(1-\nu_{54,\mathrm{ii:ii}}\beta_{54,\mathrm{ii:ii}}-\nu_{5234678,\mathrm{i:i:i:i:i:i:i}}\beta_{5234678,\mathrm{i:i:i:i:i:i:i}})N_5^{\mathrm{II}}+\cdots \\ \cdots+\nu_{95}(1-\nu_{54,\mathrm{ii:ii}}\beta_{54,\mathrm{ii:ii}}-\nu_{5234678,\mathrm{i:i:i:i:i:i:i}}\beta_{5234678,\mathrm{i:i:i:i:i:i:i}})N_5^{\mathrm{II}}+\cdots \\ \cdots+\nu_{16}(1-\nu_{66,\mathrm{ii}}\alpha_{6,\mathrm{ii}}-\nu_{67,\mathrm{i:i}}\beta_{67,\mathrm{i:i}}-\nu_{623,\mathrm{i:i:i}}\beta_{623,\mathrm{i:i:i}}-\nu_{6378,\mathrm{i:i:i:i}}\beta_{6378,\mathrm{i:i:i:i}}-\nu_{6234578,\mathrm{i:i:i:i:i:i:i}}\beta_{6234578,\mathrm{i:i:i:i:i:i:i}})N_6^{\mathrm{II}}+\cdots \\ \cdots+\nu_{96}(1-\nu_{66,\mathrm{ii}}\alpha_{6,\mathrm{ii}}-\nu_{67,\mathrm{i:i}}\beta_{67,\mathrm{i:i}}-\nu_{623,\mathrm{i:i:i}}\beta_{623,\mathrm{i:i:i}}-\nu_{6378,\mathrm{i:i:i:i}}\beta_{6378,\mathrm{i:i:i:i}}-\nu_{6234578,\mathrm{i:i:i:i:i:i:i}}\beta_{6234578,\mathrm{i:i:i:i:i:i:i}})N_6^{\mathrm{II}}+\cdots \\ \cdots+\nu_{17}(1-\nu_{77,\mathrm{v}}\alpha_{7,\mathrm{v}}-\nu_{76,\mathrm{i:i}}\beta_{76,\mathrm{i:i}}-\nu_{7368,\mathrm{i:i:i:i}}\beta_{7368,\mathrm{i:i:i:i}}-\nu_{7234568,\mathrm{i:i:i:i:i:i:i}}\beta_{7234568,\mathrm{i:i:i:i:i:i:i}})N_7^{\mathrm{II}}+\cdots \\ \cdots+\nu_{97}(1-\nu_{77,\mathrm{v}}\alpha_{7,\mathrm{v}}-\nu_{76,\mathrm{i:i}}\beta_{76,\mathrm{i:i}}-\nu_{7368,\mathrm{i:i:i:i}}\beta_{7368,\mathrm{i:i:i:i}}-\nu_{7234568,\mathrm{i:i:i:i:i:i:i}}\beta_{7234568,\mathrm{i:i:i:i:i:i:i}})N_7^{\mathrm{II}}+\cdots \\ \cdots+\nu_{18}(1-\nu_{8367,\mathrm{i:i:i:i}}\beta_{8367,\mathrm{i:i:i:i}}-\nu_{8234567,\mathrm{i:i:i:i:i:i:i}}\beta_{8234567,\mathrm{i:i:i:i:i:i:i}})N_8^{\mathrm{II}}+\cdots \\ \cdots+\nu_{98}(1-\nu_{8367,\mathrm{i:i:i:i}}\beta_{7368,\mathrm{i:i:i:i}}-\nu_{8234567,\mathrm{i:i:i:i:i:i:i}}\beta_{8234567,\mathrm{i:i:i:i:i:i:i}})N_8^{\mathrm{II}} \end{bmatrix}$$

The total moles in compartment II is

$$\begin{aligned}
&N_1^{\mathrm{II}}+N_9^{\mathrm{II}}+\alpha_{2,\mathrm{ii}}N_2^{\mathrm{II}}+\alpha_{3,\mathrm{iii}}N_3^{\mathrm{II}}+\alpha_{6,\mathrm{ii}}N_6^{\mathrm{II}}+\alpha_{7,\mathrm{v}}N_7^{\mathrm{II}}+\beta_{34,\mathrm{i:ii}}N_3^{\mathrm{II}}+\beta_{45,\mathrm{ii:ii}}N_4^{\mathrm{II}}+\beta_{67,\mathrm{i:i}}N_6^{\mathrm{II}}+\cdots \\
&\cdots+\beta_{236,\mathrm{i:i:i}}N_2^{\mathrm{II}}+\beta_{3678,\mathrm{i:i:i:i}}N_3^{\mathrm{II}}+\beta_{2345678,\mathrm{i:i:i:i:i:i:i}}N_2^{\mathrm{II}}+\cdots \\
&\cdots+(1-\nu_{22,\mathrm{ii}}\alpha_{2,\mathrm{ii}}-\nu_{236,\mathrm{i:i:i}}\beta_{236,\mathrm{i:i:i}}-\nu_{2345678,\mathrm{i:i:i:i:i:i:i}}\beta_{2345678,\mathrm{i:i:i:i:i:i:i}})N_2^{\mathrm{II}}+\cdots \\
&\cdots+(1-\nu_{33,\mathrm{iii}}\alpha_{3,\mathrm{iii}}-\nu_{34,\mathrm{i:ii}}\beta_{34,\mathrm{i:ii}}-\nu_{326,\mathrm{i:i:i}}\beta_{326,\mathrm{i:i:i}}-\nu_{3678,\mathrm{i:i:i:i}}\beta_{3678,\mathrm{i:i:i:i}}-\nu_{3245678,\mathrm{i:i:i:i:i:i:i}}\beta_{3245678,\mathrm{i:i:i:i:i:i:i}})N_3^{\mathrm{II}}+\cdots \\
&\cdots+(1-\nu_{43,\mathrm{ii:i}}\beta_{43,\mathrm{ii:i}}-\nu_{45,\mathrm{ii:ii}}\beta_{45,\mathrm{ii:ii}}-\nu_{4235678,\mathrm{i:i:i:i:i:i:i}}\beta_{4235678,\mathrm{i:i:i:i:i:i:i}})N_4^{\mathrm{II}}+\cdots \\
&\cdots+(1-\nu_{54,\mathrm{ii:ii}}\beta_{54,\mathrm{ii:ii}}-\nu_{5234678,\mathrm{i:i:i:i:i:i:i}}\beta_{5234678,\mathrm{i:i:i:i:i:i:i}})N_5^{\mathrm{II}}+\cdots \\
&\cdots+(1-\nu_{66,\mathrm{ii}}\alpha_{6,\mathrm{ii}}-\nu_{67,\mathrm{i:i}}\beta_{67,\mathrm{i:i}}-\nu_{623,\mathrm{i:i:i}}\beta_{623,\mathrm{i:i:i}}-\nu_{6378,\mathrm{i:i:i:i}}\beta_{6378,\mathrm{i:i:i:i}}-\nu_{6234578,\mathrm{i:i:i:i:i:i:i}}\beta_{6234578,\mathrm{i:i:i:i:i:i:i}})N_6^{\mathrm{II}}+\cdots \\
&\cdots+(1-\nu_{77,\mathrm{v}}\alpha_{7,\mathrm{v}}-\nu_{76,\mathrm{i:i}}\beta_{76,\mathrm{i:i}}-\nu_{7368,\mathrm{i:i:i:i}}\beta_{7368,\mathrm{i:i:i:i}}-\nu_{7234568,\mathrm{i:i:i:i:i:i:i}}\beta_{7234568,\mathrm{i:i:i:i:i:i:i}})N_7^{\mathrm{II}}+\cdots \\
&\cdots+(1-\nu_{7368,\mathrm{i:i:i:i}}\beta_{7368,\mathrm{i:i:i:i}}-\nu_{7234568,\mathrm{i:i:i:i:i:i:i}}\beta_{7234568,\mathrm{i:i:i:i:i:i:i}})N_8^{\mathrm{II}}+\cdots \\
&\cdots-(\nu_{12,\mathrm{ii}}\alpha_{2,\mathrm{ii}}N_2^{\mathrm{II}}+\nu_{92,\mathrm{ii}}\alpha_{2,\mathrm{ii}}N_2^{\mathrm{II}}+\nu_{13,\mathrm{iii}}\alpha_{3,\mathrm{iii}}N_3^{\mathrm{II}}+\nu_{93,\mathrm{iii}}\alpha_{3,\mathrm{iii}}N_3^{\mathrm{II}})+\cdots \\
&\cdots-(\nu_{16,\mathrm{ii}}\alpha_{6,\mathrm{ii}}N_6^{\mathrm{II}}+\nu_{96,\mathrm{ii}}\alpha_{6,\mathrm{ii}}N_6^{\mathrm{II}}+\nu_{17,\mathrm{v}}\alpha_{7,\mathrm{v}}N_7^{\mathrm{II}}+\nu_{97,\mathrm{v}}\alpha_{7,\mathrm{v}}N_7^{\mathrm{II}})+\cdots \\
&\cdots-(\nu_{134,\mathrm{i:ii}}\beta_{34,\mathrm{i:ii}}N_3^{\mathrm{II}}+\nu_{934,\mathrm{i:ii}}\beta_{34,\mathrm{i:ii}}N_3^{\mathrm{II}}+\nu_{145,\mathrm{ii:ii}}\beta_{45,\mathrm{ii:ii}}N_4^{\mathrm{II}}+\nu_{945,\mathrm{ii:ii}}\beta_{45,\mathrm{ii:ii}}N_4^{\mathrm{II}}+\nu_{167,\mathrm{i:i}}\beta_{67,\mathrm{i:i}}N_6^{\mathrm{II}})+\cdots \\
&\cdots-(\nu_{967,\mathrm{i:i}}\beta_{67,\mathrm{i:i}}N_6^{\mathrm{II}}+\nu_{1236,\mathrm{i:i:i}}\beta_{236,\mathrm{i:i:i}}N_2^{\mathrm{II}}+\nu_{9236,\mathrm{i:i:i}}\beta_{236,\mathrm{i:i:i}}N_2^{\mathrm{II}}+\nu_{13678,\mathrm{i:i:i:i}}\beta_{3678,\mathrm{i:i:i:i}}N_3^{\mathrm{II}})+\cdots \\
&\cdots-(\nu_{93678,\mathrm{i:i:i:i}}\beta_{3678,\mathrm{i:i:i:i}}N_3^{\mathrm{II}}+\nu_{12345678,\mathrm{i:i:i:i:i:i:i}}\beta_{2345678,\mathrm{i:i:i:i:i:i:i}}N_2^{\mathrm{II}}+\nu_{92345678,\mathrm{i:i:i:i:i:i:i}}\beta_{2345678,\mathrm{i:i:i:i:i:i:i}}N_2^{\mathrm{II}})+\cdots \\
&\cdots-\nu_{12}(1-\nu_{22,\mathrm{ii}}\alpha_{2,\mathrm{ii}}-\nu_{236,\mathrm{i:i:i}}\beta_{236,\mathrm{i:i:i}}-\nu_{2345678,\mathrm{i:i:i:i:i:i:i}}\beta_{2345678,\mathrm{i:i:i:i:i:i:i}})N_2^{\mathrm{II}}+\cdots \\
&\cdots-\nu_{92}(1-\nu_{22,\mathrm{ii}}\alpha_{2,\mathrm{ii}}-\nu_{236,\mathrm{i:i:i}}\beta_{236,\mathrm{i:i:i}}-\nu_{2345678,\mathrm{i:i:i:i:i:i:i}}\beta_{2345678,\mathrm{i:i:i:i:i:i:i}})N_2^{\mathrm{II}}+\cdots \\
&\cdots-\nu_{13}(1-\nu_{33,\mathrm{iii}}\alpha_{3,\mathrm{iii}}-\nu_{34,\mathrm{i:ii}}\beta_{34,\mathrm{i:ii}}-\nu_{326,\mathrm{i:i:i}}\beta_{326,\mathrm{i:i:i}}-\nu_{3678,\mathrm{i:i:i:i}}\beta_{3678,\mathrm{i:i:i:i}}-\nu_{3245678,\mathrm{i:i:i:i:i:i:i}}\beta_{3245678,\mathrm{i:i:i:i:i:i:i}})N_3^{\mathrm{II}}+\cdots \\
&\cdots-\nu_{93}(1-\nu_{33,\mathrm{iii}}\alpha_{3,\mathrm{iii}}-\nu_{34,\mathrm{i:ii}}\beta_{34,\mathrm{i:ii}}-\nu_{326,\mathrm{i:i:i}}\beta_{326,\mathrm{i:i:i}}-\nu_{3678,\mathrm{i:i:i:i}}\beta_{3678,\mathrm{i:i:i:i}}-\nu_{3245678,\mathrm{i:i:i:i:i:i:i}}\beta_{3245678,\mathrm{i:i:i:i:i:i:i}})N_3^{\mathrm{II}}+\cdots \\
&\cdots-\nu_{14}(1-\nu_{43,\mathrm{ii:i}}\beta_{43,\mathrm{ii:i}}-\nu_{45,\mathrm{ii:ii}}\beta_{45,\mathrm{ii:ii}}-\nu_{4235678,\mathrm{i:i:i:i:i:i:i}}\beta_{4235678,\mathrm{i:i:i:i:i:i:i}})N_4^{\mathrm{II}}+\cdots \\
&\cdots-\nu_{94}(1-\nu_{43,\mathrm{ii:i}}\beta_{43,\mathrm{ii:i}}-\nu_{45,\mathrm{ii:ii}}\beta_{45,\mathrm{ii:ii}}-\nu_{4235678,\mathrm{i:i:i:i:i:i:i}}\beta_{4235678,\mathrm{i:i:i:i:i:i:i}})N_4^{\mathrm{II}}+\cdots \\
&\cdots-\nu_{15}(1-\nu_{54,\mathrm{ii:ii}}\beta_{54,\mathrm{ii:ii}}-\nu_{5234678,\mathrm{i:i:i:i:i:i:i}}\beta_{5234678,\mathrm{i:i:i:i:i:i:i}})N_5^{\mathrm{II}}+\cdots \\
&\cdots-\nu_{95}(1-\nu_{54,\mathrm{ii:ii}}\beta_{54,\mathrm{ii:ii}}-\nu_{5234678,\mathrm{i:i:i:i:i:i:i}}\beta_{5234678,\mathrm{i:i:i:i:i:i:i}})N_5^{\mathrm{II}}+\cdots \\
&\cdots-\nu_{16}(1-\nu_{66,\mathrm{ii}}\alpha_{6,\mathrm{ii}}-\nu_{67,\mathrm{i:i}}\beta_{67,\mathrm{i:i}}-\nu_{623,\mathrm{i:i:i}}\beta_{623,\mathrm{i:i:i}}-\nu_{6378,\mathrm{i:i:i:i}}\beta_{6378,\mathrm{i:i:i:i}}-\nu_{6234578,\mathrm{i:i:i:i:i:i:i}}\beta_{6234578,\mathrm{i:i:i:i:i:i:i}})N_6^{\mathrm{II}}+\cdots \\
&\cdots-\nu_{96}(1-\nu_{66,\mathrm{ii}}\alpha_{6,\mathrm{ii}}-\nu_{67,\mathrm{i:i}}\beta_{67,\mathrm{i:i}}-\nu_{623,\mathrm{i:i:i}}\beta_{623,\mathrm{i:i:i}}-\nu_{6378,\mathrm{i:i:i:i}}\beta_{6378,\mathrm{i:i:i:i}}-\nu_{6234578,\mathrm{i:i:i:i:i:i:i}}\beta_{6234578,\mathrm{i:i:i:i:i:i:i}})N_6^{\mathrm{II}}+\cdots \\
&\cdots-\nu_{17}(1-\nu_{77,\mathrm{v}}\alpha_{7,\mathrm{v}}-\nu_{76,\mathrm{i:i}}\beta_{76,\mathrm{i:i}}-\nu_{7368,\mathrm{i:i:i:i}}\beta_{7368,\mathrm{i:i:i:i}}-\nu_{7234568,\mathrm{i:i:i:i:i:i:i}}\beta_{7234568,\mathrm{i:i:i:i:i:i:i}})N_7^{\mathrm{II}}+\cdots \\
&\cdots-\nu_{97}(1-\nu_{77,\mathrm{v}}\alpha_{7,\mathrm{v}}-\nu_{76,\mathrm{i:i}}\beta_{76,\mathrm{i:i}}-\nu_{7368,\mathrm{i:i:i:i}}\beta_{7368,\mathrm{i:i:i:i}}-\nu_{7234568,\mathrm{i:i:i:i:i:i:i}}\beta_{7234568,\mathrm{i:i:i:i:i:i:i}})N_7^{\mathrm{II}}+\cdots \\
&\cdots-\nu_{18}(1-\nu_{7368,\mathrm{i:i:i:i}}\beta_{7368,\mathrm{i:i:i:i}}-\nu_{7234568,\mathrm{i:i:i:i:i:i:i}}\beta_{7234568,\mathrm{i:i:i:i:i:i:i}})N_8^{\mathrm{II}}+\cdots \\
&\cdots-\nu_{98}(1-\nu_{7368,\mathrm{i:i:i:i}}\beta_{7368,\mathrm{i:i:i:i}}-\nu_{7234568,\mathrm{i:i:i:i:i:i:i}}\beta_{7234568,\mathrm{i:i:i:i:i:i:i}})N_8^{\mathrm{II}}
\end{aligned} \tag{A.2}$$

The free-solvent model for such a solution is Eq. (A.1) divided by Eq. (A.2).

Assuming that the number of moles of each monomeric protein species is known, there are 82 unknowns, all of which are physically realistic and independently measurable. Of these unknowns, 17 are hydration values, 17 are ion binding, 24 are protein–protein interactions, and 24 are fractional amounts of the monomeric proteins.

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