

# Supporting Information for: Synergy in Protein-Osmolyte Mixtures

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## 1. Fundamental Kirkwood-Buff equations<sup>1</sup>

In Kirkwood-Buff Theory, the information on the structure of the solution is summarized in the matrix  $B$  with elements  $B_{ij}$

$$B_{ij} = c_i \delta_{ij} + c_i c_j \mathcal{G}_{ij} = \text{Cov}(N_i, N_j)/V, \quad (\text{S1})$$

where  $\delta_{ij}$  is the Kronecker delta. Chemical potential derivatives can be calculated using the determinant  $|B|$  with cofactors  $|B|_{ij}$

$$\left( \frac{\partial \mu_i}{\partial N_j} \right)_{T,V,N_{k \neq j}} = RT \frac{|B|_{ij}}{V|B|}. \quad (\text{S2})$$

The isochoric ensemble is readily transformed to an isobaric one

$$\left( \frac{\partial \mu_i}{\partial N_j} \right)_{T,p,N_{k \neq j}} = \left( \frac{\partial \mu_i}{\partial N_j} \right)_{T,V,N_{k \neq j}} - \frac{\bar{v}_i \bar{v}_j}{\kappa V}. \quad (\text{S3})$$

Also obtained from  $B$  are the partial molar volume

$$\bar{v}_i = \sum_k c_k |B|_{ik} / \sum_{j,k} c_j c_k |B|_{jk} \quad (\text{S4})$$

and compression coefficient

$$\kappa RT = |B| / \sum_{j,k} c_j c_k |B|_{jk}. \quad (\text{S5})$$

Another very handy volumetric equation is<sup>2,3</sup>

$$\bar{v}_k = RT\kappa - \sum_{i=1}^n \bar{v}_i c_i \mathcal{G}_{ik}, \quad (\text{S6})$$

which can be proven in a straightforward manner. Eq. S6 becomes a true statement after inserting Eqs. S4 and S5, using Eq. S1, and taking into account the cofactor expansion ( $\sum_i B_{ik} |B|_{ij} = |B|$  for  $j = k$  and 0 otherwise).

## 2. Derivation of Eqs. 6 and 8

To calculate  $m$ -values we need to know how much the chemical activity of a protein state changes upon addition of an osmolyte (Eq. 4). The dependence of the chemical activity  $a_k$  of component  $k$  on component  $j$  is

$$-\gamma_{kj} = \sum_i \mathcal{G}_{ik} c_i \gamma_{ij} - \frac{\delta_{jk}}{c_j} + \frac{\delta_{1k} \bar{v}_j}{c_1 \bar{v}_1} \quad (\text{S7})$$

This can be proven using Eqs. S1 and S21 along with the cofactor expansion. The  $m$ -value is given according to Eq. 4 by the difference between the native and denatured states' version of Eq. S7. However, Eq. S7 has  $\gamma_{kj}$  on both sides of the equation. We solve for  $\gamma_{kj}$  in two alternative ways. The variant containing preferential interactions is obtained by first subtracting  $\mathcal{G}_{1k}$  times the Gibbs-Duhem relation (Eq. S24) on the right hand side. Solving for  $\gamma_{kj}$  yields

$$-\gamma_{kj} = \sum_{i \neq 1,k} \frac{\Gamma_{ik}}{1 + \Gamma_{kk}} \gamma_{ij}. \quad \text{for } k \neq \{1, j\} \quad (\text{S8})$$

Eq. S7 may also be solved directly to obtain  $\gamma_{ki}$  as a function of  $\mathcal{G}_{iP}$ . Forming the difference between

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N and D state gives

$$-\frac{m_j}{RT} = \sum_{i \neq 1, P} \gamma_{ij} \Delta \frac{\Gamma_{iP}}{1 + \Gamma_{PP}} = \sum_{i \neq P} \gamma_{ij} \Delta \frac{c_i \mathcal{G}_{iP}}{1 + c_P \mathcal{G}_{PP}}, \quad (\text{S9})$$

remembering the definitions for  $m_j$  and  $\gamma_{ij}$  in Eqs. 4 and 7. When the protein concentration is low,  $\Gamma_{PP}$  and  $c_P \mathcal{G}_{PP}$  vanish, so that Eq. 6 is obtained.

We show now that

$$\mathcal{G}_{kP} = RT\kappa - \frac{\bar{v}_P + \gamma_{Pk}}{c_P \gamma_{PP}} - \sum_{i \neq 1, P} \frac{\gamma_{Pi} c_i (\mathcal{G}_{ki} - RT\kappa)}{c_P \gamma_{PP}}, \quad (\text{S10})$$

holds, which also (using Eq. 3) leads to

$$\Gamma_{kP} = -\frac{\gamma_{Pk} c_k}{\gamma_{PP} c_P} - \sum_{i \neq 1, P} \frac{\gamma_{Pi} c_i}{\gamma_{PP} c_P} \Gamma_{ki}. \quad (\text{S11})$$

For a proof, we first consider two sums. The first one is

$$\sum_i \gamma_{Pi} c_i = \frac{v_P}{\kappa RT} - \frac{|B|_{P1}}{v_1 |B|} \quad (\text{S12})$$

where we first used Eq. S21, and then Eqs. S4 and S5. The other sum is treated similarly with the initial use of the definition in Eq. S1, and then of Eq. S6:

$$\sum_i \gamma_{Pi} c_i \mathcal{G}_{ki} = -\gamma_{Pk} - \frac{|B|_{P1}}{v_1 |B|}. \quad (\text{S13})$$

The difference between Eq. S13 and  $\kappa RT$  times Eq. S12 is then solved for  $\mathcal{G}_{Pk}$  to result in Eq. S10. The sum can be written to exclude water ( $i=1$ ), because  $\gamma_{P1}$  is zero (see Eq. S23).

Note that for low concentrations of protein ( $P$ ) the term  $c_P \gamma_{PP}$  becomes unity – a simplification of Eqs. S10 and S11 that we use in the main text.

### 3. Base transform to the molar scale

We would like to calculate  $(\partial \mu_m / \partial c_k)_{c_{l \neq 1, k}}$  and need to convert Eq. S3 to be a function of molarities  $c_i$ , rather than particle numbers  $N_i$ . One of the  $N_i$  has to be excluded from this transformation to retain a proper extensive property that defines the size of the system. It is convenient to choose the principal solvent water for this purpose. The base

transform from  $(T, p, N_1, N_{i \neq 1})$  to  $(T, p, N_1, c_{i \neq 1})$  is<sup>4</sup>

$$\left( \frac{\partial}{\partial c_k} \right)_{c_{l \neq 1, k}} = \sum_{i \neq 1} \left( \frac{\partial N_i}{\partial c_k} \right)_{c_{l \neq 1, k}} \left( \frac{\partial}{\partial N_i} \right)_{N_{l \neq i}}. \quad (\text{S14})$$

As indices,  $T$ ,  $p$ , and  $N_1$  are omitted here and in the following, because all partial derivatives have  $(T, p, N_1)$  as base elements, except for one case where specifically  $V$  is used in place of  $p$ . The primary unknown in Eq. S14 is

$$\left( \frac{\partial N_i}{\partial c_k} \right)_{c_{l \neq 1, k}} = V \left( \delta_{ik} + \frac{c_i \bar{v}_k}{c_1 \bar{v}_1} \right), \quad (\text{S15})$$

where the partial molar volumes are defined as  $\bar{v}_i = (\partial V / \partial N_i)_{N_{l \neq i}}$ .

First, we prove Eq. S15 for  $i = k$ . For this purpose we need the relation

$$\left( \frac{\partial N_i}{\partial N_k} \right)_{c_{l \neq 1, k}, i \neq 1, k} = c_i \left( \frac{\partial V}{\partial N_k} \right)_{c_{l \neq 1, k}, i \neq 1, k} \quad (\text{S16})$$

(where we used  $N_i = c_i V$ ), and the base transform

$$\left( \frac{\partial}{\partial N_k} \right)_{c_{l \neq 1, k}} = \left( \frac{\partial}{\partial N_k} \right)_{N_{l \neq k}} + \sum_{i \neq 1, k} \left( \frac{\partial N_i}{\partial N_k} \right)_{c_{l \neq 1, k}} \left( \frac{\partial}{\partial N_i} \right)_{N_{l \neq i}}. \quad (\text{S17})$$

We apply Eq. S17 to  $V$  and use Eq. S16, which results in

$$\left( \frac{\partial V}{\partial N_k} \right)_{c_{l \neq 1, k}} = \frac{\bar{v}_k}{\phi_1 + \phi_k}, \quad (\text{S18})$$

where we used the fact that the volume fractions  $\phi_i = c_i \bar{v}_i$  add up to unity. Inserting Eq. S18 into Eq. S16 and the result into Eq. S17 gives

$$\begin{aligned} \left( \frac{\partial c_k}{\partial N_k} \right)_{c_{l \neq 1, k}} &= \left( \frac{\partial c_k}{\partial N_k} \right)_{N_{l \neq k}} + \sum_{i \neq 1, k} \frac{c_i \bar{v}_k}{\phi_1 + \phi_k} \left( \frac{\partial c_k}{\partial N_i} \right)_{N_{l \neq i}} \\ &= \frac{1}{V} \frac{\phi_1}{\phi_1 + \phi_k}. \end{aligned} \quad (\text{S19})$$

Upon inversion (exchanging  $c_k$  and  $N_k$  as axes) Eq. S19 yields Eq. S15 (for  $k = i$ ).

Now, we prove Eq. S15 for  $i \neq k$ . We get

$$\begin{aligned} \left( \frac{\partial c_k}{\partial N_i} \right)_{c_l \neq 1, k} &= \left( \frac{\partial c_k}{\partial N_i} \right)_{N_l \neq i} + \left( \frac{\partial N_k}{\partial N_i} \right)_{c_l \neq 1, k} \left( \frac{\partial c_k}{\partial N_k} \right)_{N_l \neq k} \\ &+ \sum_{n \neq 1, i, k} \left( \frac{\partial N_n}{\partial N_i} \right)_{c_l \neq 1, k} \left( \frac{\partial c_k}{\partial N_n} \right)_{N_l \neq n} = \frac{1}{V} \frac{c_1 \bar{v}_1}{c_i \bar{v}_k}, \end{aligned} \quad (\text{S20})$$

using Eqs. S16 and S18, and recognizing that  $(\partial N_n / \partial N_i)_{c_n, c_i} = c_n / c_i$ . Again, inversion leads to Eq. S15 ( $i \neq k$ ).

To finish up the transform we insert Eq. S15 into Eq. S14, apply the derivative to the chemical potential  $\mu_m$ , and express it in terms of Kirkwood Buff integrals by use of Eqs. S2 and S3. This expression is simplified using Eqs. S4 and S5. Noting also  $\sum_{i>1} c_i \bar{v}_i = (1 - \bar{v}_1 c_1)$ , yields

$$\left( \frac{\partial \mu_m / RT}{\partial c_k} \right)_{c_l \neq 1, k} = \frac{|B|_{mk}}{|B|} - \frac{\bar{v}_k}{\bar{v}_1} \frac{|B|_{m1}}{|B|}. \quad (\text{S21})$$

Using Eq. S2, this results in

$$\left( \frac{\partial \mu_m}{\partial c_k} \right)_{c_l \neq 1, k} = \left( \frac{\partial \mu_m}{\partial c_k} \right)_{V, N_l \neq k} - \frac{\bar{v}_k}{\bar{v}_1} \left( \frac{\partial \mu_m}{\partial c_1} \right)_{V, N_l}. \quad (\text{S22})$$

Note that

$$\left( \frac{\partial \mu_m}{\partial N_1} \right)_{c_l \neq 1} = 0 \quad (\text{S23})$$

holds, since the number of water molecules serves as the one and only scaling factor for the size of the system; and in the macroscopic limit no chemical potential should depend on the size of the system.

Incidentally, Eq. S22 also implies that the Gibbs-Duhem relation is valid in terms of molarities

$$\sum_{i \geq 1} c_i \left( \frac{\partial \mu_i / RT}{\partial c_j} \right)_{T, p, N_1, c_l \neq 1, j} = 0. \quad (\text{S24})$$

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