*Derivation of isotherm formalisms*

The derivation for the general multimodal isotherm formalism (SMA/HIC Ext. in this work), adapted from Lee et al. [1], Nfor et al. [2], Mollerup et al. [3,4] and Wang et al. [5] and its reduction into the simpler forms is demonstrated in this section. Since the isotherms used in this work have many commonalities, they can all be obtained from the SMA/HIC Ext. expression through making appropriate assumptions in parameter values.

First, the stoichiometry of the multimodal interaction in Eq. **(1)** is shown,

|  |  |
| --- | --- |
|  | (1) |

where protein interacts, reversibly, with charged ligands and hydrophobic ligands . This process displaces salt counterions and releases water molecules and results in the formation of the protein-ligand complex . The equilibrium constant of this reaction is presented in Eq. **(2)**

|  |  |
| --- | --- |
|  | (2) |

and is based on the thermodynamic activities of the components involved in the multimodal interaction. Rewriting in terms of activity coefficients results in Eq. **(3)**,

|  |  |
| --- | --- |
|  | (3) |

with liquid phase concentrations for protein , salt , and water and solid phase concentrations for the protein and salt . Following the assumption presented in Nfor et al. [2], the activity coefficients of all species but the protein are set to unity, resulting in Eq. **(4)**.

|  |  |
| --- | --- |
|  | (4) |

Next, the ligand densities of charged sites and hydrophobic sites are defined in Eqs. **(5) and (6)**, respectively.

|  |  |
| --- | --- |
|  | (5) |
|  | (6) |

Here, and are the steric factors for electrostatic interactions and hydrophobic interactions, respectively. Substituting and into Eq. **(4)** then yields Eq. **(7)**,

|  |  |
| --- | --- |
|  | (7) |

which is further reduced with assumptions shown in Eqs. **(8a)**: activity of water is linearly correlated to adsorbed protein concentration (shown by Wang et al. [5]) with stoichiometric coefficient , **(8b)**: ligand densities and are equal for ligands with 1:1 charged and hydrophobic groups [2], and **(8c)**: the protein solution is treated at infinite dilution, where is the asymmetric activity coefficient and is the activity coefficient of water (set to unity).

|  |  |
| --- | --- |
|  | (8a) |
|  | (8b) |
|  | (8c) |

For simplicity, and ; applying these and the three assumptions results in Eq. **(9)**.

|  |  |
| --- | --- |
|  | (9) |

Since the concentration of water is unchanging, it can be treated as a constant in the equation and be lumped into along with the stoichiometric constant , resulting in Eq. **(10)**.

|  |  |
| --- | --- |
|  | (10) |

This equation represents the equilibrium form of the finalized solution for a single protein component. To represent this in kinetic form, we set where is the adsorption rate constant and is the desorption rate constant. Applying this illustrates that Eq. **(11)** and **(12)** are rate equations in in concentration,

|  |  |
| --- | --- |
|  | (11) |
|  | (12) |

noting that setting to unity removes it from the left-hand side of the equation. This equation is then represented in its multicomponent form and is shown in Eq. **(13)**.

|  |  |
| --- | --- |
|  | (13) |

The asymmetric activity coefficient for protein is next set to the expression provided in Eq. **(14)** [4].

|  |  |
| --- | --- |
|  | (14) |

Further, the expression for is obtained from Wang et al. [5] (exponential dependence on salt concentration).

|  |  |
| --- | --- |
|  | (15) |

Dependencies on pH, using reference pH , can also be applied to in Eq. **(16)**, in Eq. **(17)**, and in Eq. **(18)** if desired [6].

|  |  |
| --- | --- |
|  | (16) |
|  | (17) |
|  | (18) |

Finally, the full set of isotherm formalisms can be obtained by setting the appropriate isotherm parameters to zero (reducing the general SMA/HIC Ext. expression into simpler forms). This is illustrated below in Eqs. **(19-25)** with a visual representation shown in **Figure SI-1**.

Setting terms , , , , and to zero yields the SMA isotherm expression Eq. **(19)**

|  |  |
| --- | --- |
|  | (19) |

Setting terms , , , and to zero yields the SMA isotherm expression Eq. **(20)**

|  |  |
| --- | --- |
|  | (20) |

Setting terms , , and , to zero yields the SMA Ext. isotherm expression Eq. **(21)**

|  |  |
| --- | --- |
|  | (21) |

Setting terms , , and to zero yields the Ottens isotherm expression Eq. **(22)**

|  |  |
| --- | --- |
|  | (22) |

Setting terms to zero yields the Ottens Ext. isotherm expression Eq. **(23)**

|  |  |
| --- | --- |
|  | (23) |

Setting terms and to zero yields the SMA/HIC isotherm expression Eq. **(24)**

|  |  |
| --- | --- |
|  | (24) |

Retaining all terms yields the SMA/HIC Ext. isotherm expression Eq. **(25)**

|  |  |
| --- | --- |
|  | (25) |



**Figure SI-1** Visualization of which isotherm parameters are present in each isotherm formalism. Open boxes denote that the parameter is present in the model and “X” denotes that the parameter is not present in the model.

Alternatively, the spectrum of isotherm formalisms could each be derived from scratch but are expected to have overlap with the provided derivation, which is a convenient way to develop the set of expressions due to their intrinsic commonalities.

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