Mathematical Descriptions of Ensembles

Note: The following mathematical descriptions of enolate ensembles are based on an ensemble of hexamers composed of two enantiomers, *R* and *S*, which is archived in the supporting information for McNeil, A. J.; Toombes, G. E. S.; Chandramouli, S. V.; Vanasse, B. J.; Ayers, T. A.; O'Brien, M. K.; Lobkovsky, E.; Gruner, S. M.; Marohn, J. A.; Collum, D. B. *J. Am. Chem. Soc.* **2004**, *126*, 5938. Here *R* and *S* will be replaced with the more general definition *A* and *B*. In addition, X_n that defined the mole fraction of the aggregate with *n* subunits of type *A* will be replaced with relative integration, I_n.

a. Ensembles of Aggregates of the Same Aggregation Number

a.1. General Description

A mixture of two lithium enolates, *A* and *B*, of the same aggregation number, *N*, form an ensemble of aggregates generally described as

$$A_n + B_n \rightarrow A_n + A_{n-1}B_1 + A_{n-2}B_2 + A_{n-3}B_3 + \ldots B_n \qquad (1)$$

where *n* is the number of *A* subunits in the aggregate. The following ensembles are most likely:

$$A_2 + B_2 \rightarrow A_2 + A_1B_1 + B_2$$

$$A_4 + B_4 \rightarrow A_4 + A_3B_1 + A_2B_2 + A_1B_3 + B_4$$

$$A_6 + B_6 \rightarrow A_6 + A_5B_1 + A_4B_2 + A_3B_3 + A_2B_4 + A_1B_5 + B_6$$

In the continuous variation experiment, the ratio of *A* and *B* are varied while keeping the total concentration of the two constant. The relative integrations of each aggregate in the ensemble are measured through integration of the corresponding NMR resonances. The independent variable is the mole fraction of subunits *A*, X_A, and the dependent variables are the relative integrations of each aggregated species, I_n. Thus, I_n is predicted as a function of X_A for a given model.

The mathematical description of the equilibria in the above ensembles begins by considering that each aggregate with *n* subunits of *A* and *N*-*n* subunits of *B* has a discrete number of permutations or statistically unequal combinations. For example, a tetrameric aggregate A_2B_2 derives from six statistically discrete combinations (Chart 1). Rather than consider each permutation, ρ, individually, a linear combination of the permutations with the same number of *A* subunits, n_ρ, are treated as a single species, A_nB_{N-n} (Chart 1).

Chart 1. A description of the linear combinations of permutations for ensembles of dimers, tetramers or hexamers.

Dimers	**Tetramers**
$A_2 \Rightarrow AA$	$A_4 \Rightarrow AAAA$
$A_1B_1 \Rightarrow AB + BA$	$A_3B_1 \Rightarrow AAAB + AABA + ABAA + BAAA$
$B_2 \Rightarrow BB$	$A_2B_2 \Rightarrow AABB + ABAB + BAAB + BBAA + BABA + ABBA$
	$A_1B_3 \Rightarrow ABBB + BABB + BBAB + BBBA$
	$B_4 \Rightarrow BBBB$

Hexamers
$A_6 \Rightarrow AAAAAA$
$A_5B_1 \Rightarrow AAAAAB + AAAABA + AAABAA + 3\text{ more}$
$A_4B_2 \Rightarrow AAAABB + AAABAB + AABAAB + 12\text{ more}$
$A_3B_3 \Rightarrow AAABBB + AABABB + ABAABB + 17\text{ more}$
$A_2B_4 \Rightarrow AABBBB + ABABBB + BAABBB + 12\text{ more}$
$A_1B_5 \Rightarrow ABBBBB + BABBBB + BBABBB + 3\text{ more}$
$B_6 \Rightarrow BBBBBB$

The concentrations of each group of permutations, $[A_nB_{N-n}]$, are described by the Boltzmann distribution. The concentrations will depend on

1. Multiplicity (M_n) : The number of permutations, ρ, for which $n_\rho = n$ is the number of ways an aggregate of stoichiometry A_nB_{N-n} can be arranged. The numerical value of M_n is determined with Pascal's triangle or binomial theory to achieve the general result

$$M_n = \frac{N!}{(N-n)! \times n!}$$

2. Free Energy (g_ρ) : Each permutation may have a different energy of assembly. In theory, *AABB* may be a less stable permutation than *ABAB*. In practice, positional isomers occur in only hexamers.

3. Chemical Potential (μ_A and μ_B) : The total concentration of *A*, $[A]_{total}$, and of *B*, $[B]_{total}$, will set the chemical potentials and shift the

likelihood of various species. If $[A]_{total}$ increases relative to $[B]_{total}$, for instance, then $[A_3B_1]$ will increase relative to $[A_1B_3]$.

a.2. Mathematical Description

Consider a given permutation, ρ, with n_ρ subunits of type A and N-n_ρ of type B. The Boltzmann distribution gives its equilibrium concentration as

$$[\rho] = C \times \exp\left(\frac{-g_\rho + n_p\mu_A + (N - n_p)\mu_B}{kT}\right)$$

where C is a constant that relates the concentration of the solution to the activity, g_p is the free energy of assembly of ρ, μ_A is the chemical potential of A, and μ_B is the chemical potential of B. For the proposed experiments, all states for which $n_p = n$ are indistinguishable. The linear combination of concentrations of ρ for which $n_p = n$ is given by

$$[A_nB_{N-n}] = \sum_{\rho;n_\rho=n} [\rho] = C \times \exp\left(\frac{n\mu_A + (N-n)\mu_B}{kT}\right) \times \sum_{\rho;n_\rho=n} \exp\left(\frac{-g_\rho}{kT}\right)$$

$$= C \times \exp\left(\frac{n\mu_A + (N-n)\mu_B}{kT}\right) \times M_n \times \left\langle \exp\left(\frac{-g_\rho}{kT}\right)\right\rangle_{\rho;n_\rho=n}$$

where the average free energy is taken over all states for which $n_\rho = n$. For the remainder of the discussion, the effective variables will be defined as

$$a = \exp\left(\frac{\mu_A}{kT}\right) \qquad b = \exp\left(\frac{\mu_B}{kT}\right) \qquad \phi_n = \left\langle \exp\left(\frac{-g_\rho}{kT}\right)\right\rangle_{\rho;n_\rho=n}$$

where $kT\ln\phi_n$ describes the mean free energy of permutations in $[A_nB_{N-n}]$. ϕ_n can be thought of as a measure of the relative stability among the aggregates. Increasing ϕ_n favors $[A_nB_{N-n}]$ as would be expected if those states have a low free energy.[1] Free energies can only be measured relative to the free energy of a reference state; only the relative energies of the aggregates can be measured, not the absolute values.

Substituting the effective variables into the expression for $[A_nB_{N-n}]$ gives

$$[A_nB_{N-n}] = C \times M_n \times \phi_n \times a^n \times b^{N-n} \qquad (2)$$

---

[1] Though this statement may seem counter intuitive at first glance, one should note that the relationship between ϕ_n and g_ρ is exponential.

Using eq 2 to compute $[A_nB_{N-n}]$, the relative integration, I_n is given by

$$I_n = \frac{\left[A_nB_{N-n}\right]}{\sum_{j=0}^{N}\left[A_jB_{N-j}\right]} = \frac{C \times M_n \times \phi_n \times a^n \times b^{N-n}}{\sum_{j=0}^{N} C \times M_j \times \phi_j \times a^j \times b^{N-j}}$$

$$= \frac{C \times M_n \times \phi_n \times \left(\frac{a}{b}\right)^n \times b^N}{\sum_{j=0}^{N} C \times M_j \times \phi_j \times \left(\frac{a}{b}\right)^j \times b^N} = \frac{M_n \times \phi_n \times \left(\frac{a}{b}\right)^n}{\sum_{j=0}^{N} M_j \times \phi_j \times \left(\frac{a}{b}\right)^j}$$

$$= \frac{M_n \times \phi_n \times \exp\left(\frac{n \times (\mu_A - \mu_B)}{kT}\right)}{\sum_{j=0}^{N} M_j \times \phi_j \times \exp\left(\frac{j \times (\mu_A - \mu_B)}{kT}\right)} \qquad (3)$$

which is independent of the value C.

Summing the number of *A* subunits in the whole ensemble gives the total number of *A* subunits. Thus, the mole fraction of *A*, X_A, is given by

$$X_A = \frac{[A]_{total}}{[A]_{total} + [B]_{total}} = \frac{\sum_{n=0}^{N} n \times [A_nB_{N-n}]}{\sum_{n=0}^{N} N \times [A_nB_{N-n}]} = \frac{\sum_{n=0}^{N} n \times M_n \times \phi_n \times a^n \times b^{N-n}}{\sum_{n=0}^{N} N \times M_n \times \phi_n \times a^n \times b^{N-n}}$$

$$= \frac{\sum_{n=0}^{N} n \times M_n \times \phi_n \times a^n \times \frac{b^N}{b^n}}{\sum_{n=0}^{N} N \times M_n \times \phi_n \times a^n \times \frac{b^N}{b^n}} = \frac{\sum_{n=0}^{N} n \times M_n \times \phi_n \times \left(\frac{a}{b}\right)^n}{\sum_{n=0}^{N} N \times M_n \times \phi_n \times \left(\frac{a}{b}\right)^n} \qquad (4)$$

In general, no simple analytic expression for I_n as a function of X_A and ϕ_n exists. However, the values of X_A and all I_n depend only on the ratio a/b and ϕ_n. Thus, for a given set of ϕ_n and value of X_A, eq 4 uniquely determines a/b because X_A is a strictly monotonic function of a/b and permits I_n and X_A to be evaluated as functions of a/b. This value of a/b can be substituted into eq 3 to calculate the value of any I_n. Functions describing the specific cases of ensembles of dimers, tetramers, or hexamers follow.

The equilibrium mole fractions of different species depends only on the difference in chemical potential between *A* and *B* parameterized by the ratio a/b.

For the purposes of calculation, the average chemical potential of A and B is such that

$$a + b = 1 \leftrightarrow \frac{a}{b} = \frac{a}{1-a}$$

This assumption restricts the values of a and b to between 0 and 1 and has no effect on any calculated quantity because eqs 3 and 4 depend only on the ratio of a/b.

a.3. Equations for Dimers

Consider an ensemble of dimers

$$A_2,\ AB,\ \text{and}\ B_2$$

where $N = 2$. The total mole fraction of A for an ensemble of dimers is

$$X_A = \frac{\sum_{n=0}^{N} nM_n \phi_n a^n b^{N-n}}{\sum_{n=0}^{N} NM_n \phi_n a^n b^{N-n}} = \frac{2\phi_1 a^1 b^1 + 2\phi_2 a^2}{2\phi_0 b^2 + 4\phi_1 a^1 b^1 + 2\phi_2 a^2}$$

$$= \frac{\phi_1 a^1 b^1 + \phi_2 a^2}{\phi_0 b^2 + 2\phi_1 a^1 b^1 + \phi_2 a^2}$$

where $M_0 = 1$, $M_1 = 2$, and $M_2 = 1$. The experimentally measured I_n are

$$I_0 = \frac{C \times M_n \times \phi_n \times a^n \times b^{N-n}}{\sum_{j=0}^{N} C \times M_j \times \phi_j \times a^j \times b^{N-j}} = \frac{\phi_0 b^2}{\phi_0 b^2 + 2\phi_1 a^1 b^1 + \phi_2 a^2}$$

$$I_1 = \frac{2\phi_1 a^1 b^1}{\phi_0 b^2 + 2\phi_1 a^1 b^1 + \phi_2 a^2}$$

$$I_2 = \frac{\phi_2 a^2}{\phi_0 b^2 + 2\phi_1 a^1 b^1 + \phi_2 a^2}$$

a.4. Equations for Tetramers

For an ensemble of tetramers ($N = 4$)

A_4, A_3B_1, A_2B_2, A_1B_3, and B_4

the total mole fraction of A is

$$X_A = \frac{\sum_{n=0}^{N} nM_n\phi_n a^n b^{N-n}}{\sum_{n=0}^{N} NM_n\phi_n a^n b^{N-n}} = \frac{4\phi_1 a^1 b^3 + 12\phi_2 a^2 b^2 + 12\phi_3 a^3 b^1 + 4\phi_4 a^4}{4\phi_0 b^4 + 16\phi_1 a^1 b^3 + 24\phi_2 a^2 b^2 + 16\phi_3 a^3 b^1 + 4\phi_4 a^4}$$

$$= \frac{\phi_1 a^1 b^3 + 3\phi_2 a^2 b^2 + 3\phi_3 a^3 b^1 + \phi_4 a^4}{\phi_0 b^4 + 4\phi_1 a^1 b^3 + 6\phi_2 a^2 b^2 + 4\phi_3 a^3 b^1 + \phi_4 a^4}$$

where $M_0 = 1$, $M_1 = 4$, $M_2 = 6$, $M_3 = 4$, $M_4 = 1$. The experimentally measured relative integrations, I_n, are

$$I_0 = \frac{C \times M_n \times \phi_n \times a^n \times b^{N-n}}{\sum_{j=0}^{N} C \times M_j \times \phi_j \times a^j \times b^{N-j}} = \frac{\phi_0 b^4}{\phi_0 b^4 + 4\phi_1 a^1 b^3 + 6\phi_2 a^2 b^2 + 4\phi_3 a^3 b^1 + \phi_4 a^4}$$

$$I_1 = \frac{4\phi_1 a^1 b^3}{\phi_0 b^4 + 4\phi_1 a^1 b^3 + 6\phi_2 a^2 b^2 + 4\phi_3 a^3 b^1 + \phi_4 a^4}$$

$$I_2 = \frac{6\phi_2 a^2 b^2}{\phi_0 b^4 + 4\phi_1 a^1 b^3 + 6\phi_2 a^2 b^2 + 4\phi_3 a^3 b^1 + \phi_4 a^4}$$

$$I_3 = \frac{4\phi_3 a^3 b^1}{\phi_0 b^4 + 4\phi_1 a^1 b^3 + 6\phi_2 a^2 b^2 + 4\phi_3 a^3 b^1 + \phi_4 a^4}$$

$$I_4 = \frac{\phi_4 a^4}{\phi_0 b^4 + 4\phi_1 a^1 b^3 + 6\phi_2 a^2 b^2 + 4\phi_3 a^3 b^1 + \phi_4 a^4}$$

a.5. Equations for Hexamers

When $N = 6$ as in the case for an ensemble of hexamers

$$A_6,\ A_5B_1,\ A_4B_2,\ A_3B_3,\ A_2B_4,\ A_1B_5, \text{ and } B_6$$

the total mole fraction of A is defined as

$$X_A = \frac{\sum_{n=0}^{N} nM_n\phi_n a^n b^{N-n}}{\sum_{n=0}^{N} NM_n\phi_n a^n b^{N-n}}$$

$$X_A = \frac{6\phi_1 a^1 b^5 + 30\phi_2 a^2 b^4 + 60\phi_3 a^3 b^3 + 60\phi_4 a^4 b^2 + 30\phi_5 a^5 b^1 + 6\phi_6 a^6}{6\phi_0 b^6 + 36\phi_1 a^1 b^5 + 90\phi_2 a^2 b^4 + 120\phi_3 a^3 b^3 + 90\phi_4 a^4 b^2 + 36\phi_5 a^5 b^1 + 6\phi_6 a^6}$$

$$= \frac{\phi_1 a^1 b^5 + 5\phi_2 a^2 b^4 + 10\phi_3 a^3 b^3 + 10\phi_4 a^4 b^2 + 5\phi_5 a^5 b^1 + \phi_6 a^6}{\phi_0 b^6 + 6\phi_1 a^1 b^5 + 15\phi_2 a^2 b^4 + 20\phi_3 a^3 b^3 + 15\phi_4 a^4 b^2 + 6\phi_5 a^5 b^1 + \phi_6 a^6}$$

where $M_0 = 1$, $M_1 = 6$, $M_2 = 15$, $M_3 = 20$, $M_4 = 15$, $M_5 = 6$, and $M_6 = 1$. The experimentally measured relative integrations, I_n, are

$$I_0 = \frac{\phi_0 b^6}{\phi_0 b^6 + 6\phi_1 a^1 b^5 + 15\phi_2 a^2 b^4 + 20\phi_3 a^3 b^3 + 15\phi_4 a^4 b^2 + 6\phi_5 a^5 b^1 + \phi_6 a^6}$$

$$I_1 = \frac{6\phi_1 a^1 b^5}{\phi_0 b^6 + 6\phi_1 a^1 b^5 + 15\phi_2 a^2 b^4 + 20\phi_3 a^3 b^3 + 15\phi_4 a^4 b^2 + 6\phi_5 a^5 b^1 + \phi_6 a^6}$$

$$I_2 = \frac{15\phi_2 a^2 b^4}{\phi_0 b^6 + 6\phi_1 a^1 b^5 + 15\phi_2 a^2 b^4 + 20\phi_3 a^3 b^3 + 15\phi_4 a^4 b^2 + 6\phi_5 a^5 b^1 + \phi_6 a^6}$$

$$I_3 = \frac{20\phi_3 a^3 b^3}{\phi_0 b^6 + 6\phi_1 a^1 b^5 + 15\phi_2 a^2 b^4 + 20\phi_3 a^3 b^3 + 15\phi_4 a^4 b^2 + 6\phi_5 a^5 b^1 + \phi_6 a^6}$$

$$I_4 = \frac{15\phi_4 a^4 b^2}{\phi_0 b^6 + 6\phi_1 a^1 b^5 + 15\phi_2 a^2 b^4 + 20\phi_3 a^3 b^3 + 15\phi_4 a^4 b^2 + 6\phi_5 a^5 b^1 + \phi_6 a^6}$$

$$I_5 = \frac{6\phi_5 a^5 b^1}{\phi_0 b^6 + 6\phi_1 a^1 b^5 + 15\phi_2 a^2 b^4 + 20\phi_3 a^3 b^3 + 15\phi_4 a^4 b^2 + 6\phi_5 a^5 b^1 + \phi_6 a^6}$$

$$I_6 = \frac{\phi_6 a^6}{\phi_0 b^6 + 6\phi_1 a^1 b^5 + 15\phi_2 a^2 b^4 + 20\phi_3 a^3 b^3 + 15\phi_4 a^4 b^2 + 6\phi_5 a^5 b^1 + \phi_6 a^6}$$

a.6. Maximum of I_n as a function of X_A

I_n depends on X_A and has a maximum at a particular value of X_A. When all aggregates in the ensemble have the same aggregation number, the maximum occurs when $X_A = n/N$ as shown by examining eq 3. The maximum value of I_n occurs when the derivative of eq 3 with respect to a/b is zero. The derivative is given by

$$\frac{d(I_n)}{d\left(\frac{a}{b}\right)} = \frac{M_n \times \phi_n \times n \times \left(\frac{a}{b}\right)^{n-1}}{\sum_{j=0}^{N} M_j \times \phi_j \times \left(\frac{a}{b}\right)^{j}} - \frac{\left(M_n \times \phi_n \times \left(\frac{a}{b}\right)^{n}\right) \times \sum_{j=0}^{N} M_j \times \phi_j \times j \times \left(\frac{a}{b}\right)^{j-1}}{\left(\sum_{j=0}^{N} M_j \times \phi_j \times \left(\frac{a}{b}\right)^{j}\right)^2}$$

$$= n \times \left(\frac{a}{b}\right)^{-1} \times \frac{M_n \times \phi_n \times \left(\frac{a}{b}\right)^{n}}{\sum_{j=0}^{N} M_j \times \phi_j \times \left(\frac{a}{b}\right)^{j}} - \frac{M_n \times \phi_n \times \left(\frac{a}{b}\right)^{n}}{\sum_{j=0}^{N} M_j \times \phi_j \times \left(\frac{a}{b}\right)^{j}} \times N \times \left(\frac{a}{b}\right)^{-1} \times \frac{\sum_{j=0}^{N} j \times M_j \times \phi_j \times \left(\frac{a}{b}\right)^{j}}{\sum_{j=0}^{N} N \times M_j \times \phi_j \times \left(\frac{a}{b}\right)^{j}}$$

$$= n \times \left(\frac{a}{b}\right)^{-1} \times I_n - I_n \times N \times \left(\frac{a}{b}\right)^{-1} \times X_A$$

$$= N \times I_n \times \left(\frac{a}{b}\right)^{-1} \times \left(\frac{n}{N} - X_A\right)$$

Setting the derivative equal to zero

$$0 = \frac{d(I_n)}{d\left(\frac{a}{b}\right)} = N \times I_n \times \left(\frac{a}{b}\right)^{-1} \times \left(\frac{n}{N} - X_A\right)$$

the maximum value of I_n occurs when $X_A = n/N$. This derivation requires that all aggregates have the same aggregation number.

b. Ensembles of Aggregates with Different Aggregation Numbers

b.1. General Description

Until this point, ensembles in which all aggregates are of the same aggregation number have been discussed. However, ensembles in which the homoaggregates are of different aggregation numbers could exist. For example, a mixture of subunits A and B could yield an ensemble of monomers and dimers

$$A,\ B,\ A_2,\ AB,\ \text{and}\ B_2$$

or an ensemble of dimers and tetramers

$$A_2,\ AB,\ B_2,\ A_4,\ A_3B_1,\ A_2B_2,\ A_1B_3,\ \text{and}\ B_4$$

Ensembles of these types have yet to be experimentally observed or explored, but they are plausible.

For ensembles of more than one aggregation number, the absolute concentration of the subunits impacts the equilibrium concentration of the various aggregates as a direct result of the equilibrium between the two aggregation states. Consequently, the maximum of the curve for a particular aggregate in the Job plot does not necessarily occur at the stoichiometry of the aggregate (Figure 1). In these ensembles, the position of the maximum depends on both the a/b ratio and the absolute subunit concentration. Conversely, for ensembles of a single aggregation number, the absolute concentration of the subunits affects all aggregates equally and, therefore, does not need to be considered in the mathematical descriptions of the ensembles.

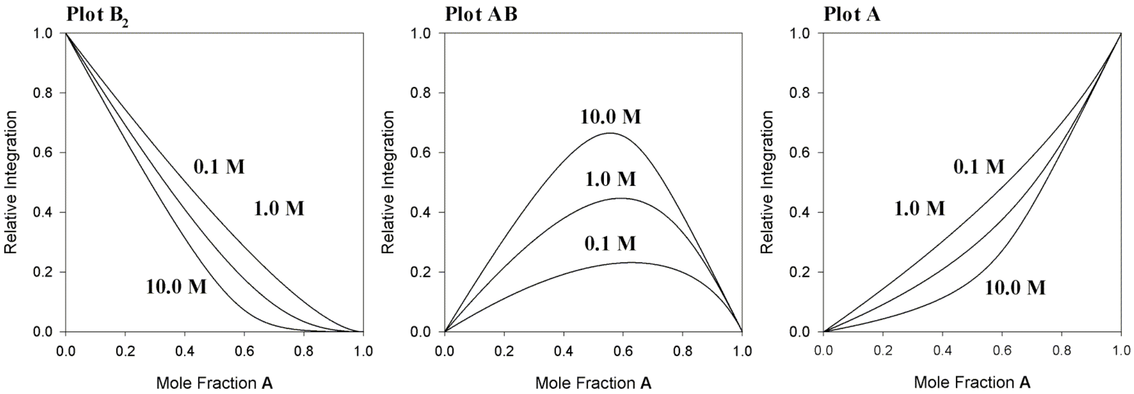

Figure 1. Job plots of a monomer/dimer ensemble of A, AB, and B_2 at different absolute total subunit concentrations.

b.2. Ensemble of Monomers and Dimers

An ensemble of monomers and dimers

$$A, B, A_2, AB, \text{ and } B_2$$

accounts for the simultaneous presence of all aggregates in the ensemble. A discussion of how to apply this general description to a specific ensemble follows.

The effective variables are defined as

$$a = \exp\left(\frac{\mu_A}{kT}\right) \qquad b = \exp\left(\frac{\mu_B}{kT}\right) \qquad \phi_{N_n} = \left\langle \exp\left(\frac{-g_{\rho,N}}{kT}\right)\right\rangle_{\rho;n_\rho = n}$$

where the designation N_n refers to an aggregate of N total subunits with n subunits of type A. The total subunit concentration in the ensemble is

$$[A]_{total} = [A] + 2[A_2] + [AB] \qquad\qquad [B]_{total} = [B] + 2[B_2] + [AB]$$

$$[A]_{total} + [B]_{total} = [A] + [B] + 2[A_2] + 2[AB] + 2[B_2]$$

From eq 2

$$\left[A_n B_{N-n}\right] = C \times M_{N_n} \times \phi_{N_n} \times a^n \times b^{N-n}$$

$$\left[B\right] = C \times \phi_{1_0} \times b$$

$$\left[A\right] = C \times \phi_{1_1} \times a$$

$$\left[B_2\right] = C \times \phi_{2_0} \times b^2$$

$$\left[AB\right] = 2 \times C \times \phi_{2_1} \times a \times b$$

$$\left[A_2\right] = C \times \phi_{2_2} \times a^2$$

By substitution

$$\left[A\right]_{total} + \left[B\right]_{total} = C(\phi_{1_0} b + \phi_{1_1} a) + 2C(\phi_{2_0} b^2 + 2\phi_{2_1} ab + \phi_{2_2} a^2)$$

For ensembles with only one aggregation number, X_A and I_n are uniquely determined by ϕ_n and a/b and are independent of the total subunit concentration. In contrast, the total subunit concentration is important for ensembles with more than one aggregation number, and X_A and I_{Nn} depend on ϕ_{Nn}, a, and b. For convenience, the value of a and b can be expressed as

$$\alpha = \frac{a}{a+b} \qquad \chi = a + b \qquad a = \alpha\chi \qquad b = (1-\alpha)\chi$$

where χ can be thought of as the sum of the activities of *A* and *B*, and α is the proportion of χ resulting from *a*. By substitution

$$\left[A\right]_{total} + \left[B\right]_{total} = \chi C(\phi_{1_0}(1-\alpha) + \phi_{1_1}\alpha) + \chi^2 C(2\phi_{2_0}(1-\alpha)^2 + 4\phi_{2_1}\alpha(1-\alpha) + 2\phi_{2_2}\alpha^2)$$

$$0 = 2\chi^2 C(\phi_{2_0}(1-\alpha)^2 + 2\phi_{2_1}\alpha(1-\alpha) + \phi_{2_2}\alpha^2) + \chi C(\phi_{1_0}(1-\alpha) + \phi_{1_1}\alpha) - \left(\left[A\right]_{total} + \left[B\right]_{total}\right)$$

$$0 = 2\chi^2 CD_{total} + \chi CM_{total} - \left(\left[A\right]_{total} + \left[B\right]_{total}\right)$$

where,

$$M_{total} = (\phi_{1_0}(1-\alpha) + \phi_{1_1}\alpha) \qquad D_{total} = (\phi_{2_0}(1-\alpha)^2 + 2\phi_{2_1}\alpha(1-\alpha) + \phi_{2_2}\alpha^2)$$

Using the quadratic equation to solve for χ,

$$\chi = \frac{-CM_{total} \pm \sqrt{C^2 M_{total}^2 + 8CD_{total}\left(\left[A\right]_{total} + \left[B\right]_{total}\right)}}{4CD_{total}}$$

$$= \sqrt{\frac{C^2 M_{total}^2}{16C^2 D_{total}^2} + \frac{8CD_{total}\left(\left[A\right]_{total} + \left[B\right]_{total}\right)}{16C^2 D_{total}^2}} - \frac{M_{total}}{4D_{total}}$$

$$= \sqrt{\frac{M_{total}^2}{16D_{total}^2} + \frac{\left(\left[A\right]_{total} + \left[B\right]_{total}\right)}{2CD_{total}}} - \frac{M_{total}}{4D_{total}}$$

$$= \frac{M_{total}}{4D_{total}} \times \left(\sqrt{1 + \frac{8D_{total}\left(\left[A\right]_{total} + \left[B\right]_{total}\right)}{CM_{total}^2}} - 1\right)$$

$$= \frac{M_{total}}{4D_{total}} \times \frac{\left(\sqrt{1 + \frac{8D_{total}\left(\left[A\right]_{total} + \left[B\right]_{total}\right)}{CM_{total}^2}} - 1\right) \times \left(\sqrt{1 + \frac{8D_{total}\left(\left[A\right]_{total} + \left[B\right]_{total}\right)}{CM_{total}^2}} + 1\right)}{\sqrt{1 + \frac{8D_{total}\left(\left[A\right]_{total} + \left[B\right]_{total}\right)}{CM_{total}^2}} + 1}$$

$$= \frac{2 \times \left([A]_{total} + [B]_{total} \right) / C}{M_{total} + \sqrt{M_{total}^2 + 8 \times D_{total} \left([A]_{total} + [B]_{total} \right) / C}}$$

where only the positive result is accepted because a negative chemical potential is not physically possible. The above equations can be used to describe X_A such that

$$\mathrm{X}_A = \frac{[A]_{total}}{[A]_{total} + [B]_{total}} = \frac{C\left(2\phi_{2_1}\chi^2\alpha(1-\alpha) + \left(2\phi_{2_2}\chi^2\alpha^2\right) + \chi\phi_{1_1}\alpha\right)}{C\left(2\chi^2 D_{total} + \chi M_{total}\right)}$$

$$= \frac{2\phi_{2_1}\chi\alpha(1-\alpha) + \left(2\phi_{2_2}\chi\alpha^2\right) + \phi_{1_1}\alpha}{2\chi D_{total} + M_{total}}$$

The relative integrations of all possible aggregated species are defined as

$$I_{1_n} = \frac{N[A_n B_{N-n}]}{[A]_{total} + [B]_{total}} = \frac{M_n C\chi\phi_{1_n}\alpha^n (1-\alpha)^{1-n}}{2\chi^2 C D_{total} + \chi C M_{total}} = \frac{\phi_{1_n}\alpha^n (1-\alpha)^{1-n}}{2\chi D_{total} + M_{total}}$$

$$I_{1_0} = \frac{\phi_{1_0}(1-\alpha)}{2\chi D_{total} + M_{total}} \qquad I_{1_1} = \frac{\phi_{1_1}\alpha}{2\chi D_{total} + M_{total}}$$

$$I_{2_n} = \frac{N[A_n B_{N-n}]}{[A]_{total} + [B]_{total}} = \frac{2M_n C\chi^2\phi_{2_n}\alpha^n (1-\alpha)^{2-n}}{2\chi^2 C D_{total} + \chi C M_{total}} = \frac{2M_n\chi\phi_{2_n}\alpha^n (1-\alpha)^{2-n}}{2\chi D_{total} + M_{total}}$$

$$I_{2_0} = \frac{2\chi\phi_{2_0}(1-\alpha)^2}{2\chi D_{total} + M_{total}}$$

$$I_{2_1} = \frac{4\chi\phi_{2_1}\alpha(1-\alpha)}{2\chi D_{total} + M_{total}}$$

$$I_{2_2} = \frac{2\chi\phi_{2_2}\alpha^2}{2\chi D_{total} + M_{total}}$$

Although the theoretical monomer/dimer ensemble is

A, B, A_2, AB, and B_2

where all aggregation states are populated, simpler ensembles that are more probable based on known aggregation behaviors include

$$A, B \text{ and } AB$$

$$A, AB, \text{ and } B_2$$

In these systems, the general derivation above still applies. Missing aggregates are accounted for by assigning the appropriate ϕ_{Nn} as zero, indicating that the corresponding species have no free energies.

b.3. Ensembles of Dimers and Tetramers

The following ensemble of dimers and tetramers

$$A_2, AB, B_2, A_4, A_3B_1, A_2B_2, A_1B_3, \text{ and } B_4$$

includes all possible dimers and tetramers. Such a complex ensemble seems most likely if both subunits form dimer and tetramer homoaggregates when separate. Simpler, perhaps more deceptive to the experimentalist ensembles in which only one homoaggregate is of a different aggregation number are

$$A_4, AB, \text{ and } B_2$$

$$A_4, A_3B_1, A_2B_2, A_1B_3, \text{ and } B_2$$

As in the monomer/dimer examples, missing aggregates are accounted for by assigning the corresponding ϕ_{Nn} to zero.

The effective variables remain defined as

$$a = \exp\left(\frac{\mu_A}{kT}\right) \qquad b = \exp\left(\frac{\mu_B}{kT}\right) \qquad \phi_{N_n} = \left\langle \exp\left(\frac{-g_{\rho,N}}{kT}\right)\right\rangle_{\rho;n_\rho = n}$$

where the designation N_n refers to an aggregate of N total subunits with n subunits of type A. To account for the effect of total subunit concentration on the equilibrium concentration of each aggregate in the ensemble

$$[A]_{total} = 2[A_2] + [AB] + 4[A_4] + 3[A_3B_1] + 2[A_2B_2] + [A_1B_3]$$

$$[B]_{total} = 2[B_2] + [AB] + [A_3B_1] + 2[A_2B_2] + 3[A_1B_3] + 4[B_4]$$

$$[A]_{total} + [B]_{total} = 2\left([B_2] + [AB] + [B_2]\right) + 4\left([A_4] + [A_3B_1] + [A_2B_2] + [A_1B_3] + [B_4]\right)$$

From eq 2, the concentration of dimers is given by

$$\left[A_2\right] = C \times \phi_{2_2} \times a^2$$

$$\left[AB\right] = 2 \times C \times \phi_{2_1} \times a \times b$$

$$\left[B_2\right] = C \times \phi_{2_0} \times b^2$$

Similarly, the concentration of the tetramers is given by

$$\left[A_4\right] = C \times \phi_{4_4} \times a^4$$

$$\left[A_3B_1\right] = 4 \times C \times \phi_{4_3} \times a^3 \times b^1$$

$$\left[A_2B_2\right] = 6 \times C \times \phi_{4_2} \times a^2 \times b^2$$

$$\left[A_1B_3\right] = 4 \times C \times \phi_{4_1} \times a^1 \times b^3$$

$$\left[B_4\right] = C \times \phi_{4_0} \times b^4$$

By substitution,

$$\left[A\right]_{total} + \left[B\right]_{total} = 2C(\phi_{2_2}a^2 + 2\phi_{2_1}ab + \phi_{2_0}b^2) + 4C(\phi_{4_4}a^4 + 4\phi_{4_3}a^3b + 6\phi_{4_2}a^2b^2 + 4\phi_{4_1}a^1b^3 + \phi_{4_0}b^4)$$

As described above

$$\alpha = \frac{a}{a+b} \qquad \chi = a + b \qquad a = \alpha\chi \qquad b = (1-\alpha)\chi$$

By substitution

$$\left[A\right]_{total} + \left[B\right]_{total} = 2\chi^2 C(\phi_{2_2}\alpha^2 + 2\phi_{2_1}\alpha(1-\alpha) + \phi_{2_0}(1-\alpha)^2)$$
$$+4\chi^4 C(\phi_{4_4}\alpha^4 + 4\phi_{4_3}\alpha^3(1-\alpha) + 6\phi_{4_2}\alpha^2(1-\alpha)^2 + 4\phi_{4_1}\alpha^1(1-\alpha)^3 + \phi_{4_0}(1-\alpha)^4)$$

$$\left[A\right]_{total} + \left[B\right]_{total} = 4\chi^4 C T_{total} + 2\chi^2 C D_{total}$$

$$0 = 4\chi^4 C T_{total} + 2\chi^2 C D_{total} - \left(\left[A\right]_{total} + \left[B\right]_{total}\right)$$

where

$$D_{total} = \phi_{2_2}\alpha^2 + 2\phi_{2_1}\alpha(1-\alpha) + \phi_{2_0}(1-\alpha)^2$$

$$T_{total} = \phi_{4_4}\alpha^4 + 4\phi_{4_3}\alpha^3(1-\alpha) + 6\phi_{4_2}\alpha^2(1-\alpha)^2 + 4\phi_{4_1}\alpha^1(1-\alpha)^3 + \phi_{4_0}(1-\alpha)^4$$

As the total concentration of subunits increases, χ^2 increases changing the relative concentrations of dimers and tetramers. The relationship is quadratic. Solving for χ^2 by using the quadratic equation gives

$$\chi^2 = \frac{-2CD_{total} \pm \sqrt{4C^2D_{total}^2 + 16CT_{total}\left(\left[A\right]_{total} + \left[B\right]_{total}\right)}}{8CT_{total}}$$

$$= -\frac{D_{total}}{4T_{total}} \pm \sqrt{\frac{4C^2D_{total}^2 + 16CT_{total}\left(\left[A\right]_{total} + \left[B\right]_{total}\right)}{64C^2T_{total}^2}}$$

$$= -\frac{D_{total}}{4T_{total}} \pm \sqrt{\frac{D_{total}^2}{16T_{total}^2} + \frac{\left(\left[A\right]_{total} + \left[B\right]_{total}\right)}{4CT_{total}}}$$

$$= -\frac{D_{total}}{4T_{total}} \pm \frac{D_{total}}{4T_{total}}\sqrt{1 + \frac{16T_{total}^2\left(\left[A\right]_{total} + \left[B\right]_{total}\right)}{4CT_{total}D_{total}^2}}$$

$$= \frac{D_{total}}{4T_{total}}\left(\sqrt{1 + \frac{4T_{total}\left(\left[A\right]_{total} + \left[B\right]_{total}\right)}{CD_{total}^2}} - 1\right)$$

$$= \frac{D_{total}}{4T_{total}} \times \frac{\left(\sqrt{1 + \frac{4T_{total}\left(\left[A\right]_{total} + \left[B\right]_{total}\right)}{CD_{total}^2}} - 1\right) \times \left(\sqrt{1 + \frac{4T_{total}\left(\left[A\right]_{total} + \left[B\right]_{total}\right)}{CD_{total}^2}} + 1\right)}{\sqrt{1 + \frac{4T_{total}\left(\left[A\right]_{total} + \left[B\right]_{total}\right)}{CD_{total}^2}} + 1}$$

$$= \frac{\left(\left[A\right]_{total} + \left[B\right]_{total}\right)/C}{D_{total} + \sqrt{D_{total}^2 + 4 \times T_{total}\left(\left[A\right]_{total} + \left[B\right]_{total}\right)/C}}$$

The above equations can be used to describe X_A,

$$\mathrm{X}_A = \frac{\left[A\right]_{total}}{\left[A\right]_{total} + \left[B\right]_{total}}$$

$$= \frac{2C\phi_{2_1}\chi^2\alpha\left(1-\alpha\right) + 2C\phi_{2_2}\chi^2\alpha^2 + 4\chi^4C\phi_{4_1}\alpha^1(1-\alpha)^3 + 12\chi^4C\phi_{4_2}\alpha^2(1-\alpha)^2 + 12\chi^4C\phi_{4_3}\alpha^3(1-\alpha)^1 + 4\chi^4C\phi_{4_4}\alpha^4}{4\chi^4CT_{total} + 2\chi^2CD_{total}}$$

$$= \frac{\phi_{2_1}\alpha\left(1-\alpha\right) + \phi_{2_2}\alpha^2 + 2\chi^2\phi_{4_1}\alpha^1(1-\alpha)^3 + 6\chi^2\phi_{4_2}\alpha^2(1-\alpha)^2 + 6\chi^2\phi_{4_3}\alpha^3(1-\alpha)^1 + 2\chi^2\phi_{4_4}\alpha^4}{2\chi^2T_{total} + D_{total}}$$

The relative integrations of the aggregated species are defined as follows

$$I_{2_n} = \frac{N\left[A_n B_{N-n}\right]}{\left[A\right]_{total} + \left[B\right]_{total}} = \frac{2M_n C\chi^2 \phi_{2_n} \alpha^n (1-\alpha)^{2-n}}{4\chi^4 CT_{total} + 2\chi^2 CD_{total}} = \frac{M_n \phi_{2_n} \alpha^n (1-\alpha)^{2-n}}{2\chi^2 T_{total} + D_{total}}$$

$$I_{2_2} = \frac{\phi_{2_2} \alpha^2}{2\chi^2 T_{total} + D_{total}} \qquad I_{2_1} = \frac{2\phi_{2_1} \alpha(1-\alpha)}{2\chi^2 T_{total} + D_{total}} \qquad I_{2_0} = \frac{\phi_{2_0} (1-\alpha)^2}{2\chi^2 T_{total} + D_{total}}$$

$$I_{4_n} = \frac{N\left[A_n B_{N-n}\right]}{\left[A\right]_{total} + \left[B\right]_{total}} = \frac{4M_n C\chi^4 \phi_{4_n} \alpha^n (1-\alpha)^{4-n}}{4\chi^4 CT_{total} + 2\chi^2 CD_{total}} = \frac{2M_n \chi^2 \phi_{4_n} \alpha^n (1-\alpha)^{4-n}}{2\chi^2 T_{total} + D_{total}}$$

$$I_{4_4} = \frac{2\chi^2 \phi_{4_4} \alpha^4}{2\chi^2 T_{total} + D_{total}} \qquad I_{4_3} = \frac{8\chi^2 \phi_{4_3} \alpha^3 (1-\alpha)^1}{2\chi^2 T_{total} + D_{total}} \qquad I_{4_2} = \frac{12\chi^2 \phi_{4_2} \alpha^2 (1-\alpha)^2}{2\chi^2 T_{total} + D_{total}}$$

$$I_{4_1} = \frac{8\chi^2 \phi_{4_1} \alpha^1 (1-\alpha)^3}{2\chi^2 T_{total} + D_{total}} \qquad I_{4_0} = \frac{2\chi^2 \phi_{4_0} (1-\alpha)^4}{2\chi^2 T_{total} + D_{total}}$$

VII. Simulated Ensembles: Influence of Aggregate Stability

Introduction.

We carried out detailed simulations of Job plots hoping to understand: (1) how changes in the relative aggregate stabilities deviate from statistical and influence the appearance of the Job plots; (2) how alternative (more complex) aggregate distributions might masquerade as simpler ensembles; (3) how erroneous models would be detected in an experimental setting. The headings in the following sections are based on the model used to simulate the data. Within these sections we will consider the influence of relative aggregate energies (ϕ), changes in absolute concentrations, and consequences of fitting the data to incorrect models.

a. Ensemble: $\mathbf{A_2}$-**AB**-$\mathbf{B_2}$.

Figure 1 presents curves **I-III** which represent simulated $\mathbf{A_2}$-**AB**-$\mathbf{B_2}$ data at low (**I**), intermediate (**II**), and high (**III**) relative stability of **AB** (ϕ_1). By definition, any combination of ϕ values results in a plot that exhibits symmetry about the x-axis at $X_\mathbf{A} = 0.5$ and the maximum of the **AB** curve at $X_\mathbf{A} = 0.5$. The appearance of the plot will be independent of the absolute concentrations of the subunits.

One might ask how to rule out alternative models in which one of the homoaggregates (**A** for illustration) is a monomer or tetramer (**A** or $\mathbf{A_4}$, respectively.) Can a least-squares fit to models based on **A**-**AB**-$\mathbf{B_2}$ or $\mathbf{A_4}$-**AB**-$\mathbf{B_2}$ ensembles rule out such a possibility? To explore the ability of least-squares fits to distinguish between the models, theoretical data from $\mathbf{A_2}$-**AB**-$\mathbf{B_2}$ at low, intermediate and high values of ϕ_1 were fit to alternative models **A**-**AB**-$\mathbf{B_2}$ and $\mathbf{A_4}$-**AB**-$\mathbf{B_2}$. The choice of absolute concentration is arbitrary and, at best, emblematic. In each case, the goodness of fit was evaluated by the sum of the absolute values of the residuals, the absolute difference between the data and the fit summed over all curves. Of course, fitting the $\mathbf{A_2}$-**AB**-$\mathbf{B_2}$ data to the correct $\mathbf{A_2}$-**AB**-$\mathbf{B_2}$ model would give no residuals.

Figure 2 illustrates $\mathbf{A_2}$-**AB**-$\mathbf{B_2}$ data (symbols) at low (III), intermediate (I) and high (II) ϕ_1 fit to the **A**-**AB**-$\mathbf{B_2}$ (line) model (derived on pp Sxx-Sxx). In each fit, the maximum of the **AB** curve lies toward the **A** side of the plot. Plot IV shows the sums of the residuals as a function of $\boldsymbol{X}_\mathbf{A}$ for plots I-III. The fit is least able to distinguish between the two models when the **AB** aggregate is at an extreme. The fit to the statistical $\mathbf{A_2}$-**AB**-$\mathbf{B_2}$ (Plot I) ensemble is the worst and clearly not the correct model. Ensembles of **A**-**AB**-$\mathbf{B_2}$ are most likely to be misinterpreted as $\mathbf{A_2}$-**AB**-$\mathbf{B_2}$ when the mixed aggregate is in either high or low integrations relative to the homoaggregates.

Figure 3 demonstrates the analogous experiment when $\mathbf{A_2}$-**AB**-$\mathbf{B_2}$ data is fit as $\mathbf{A_4}$-**AB**-$\mathbf{B_2}$. The resulting fits are near mirror images of the fits to **A**-**AB**-$\mathbf{B_2}$. The curve for the **AB** aggregate lies toward the $\mathbf{B_2}$ side of the plot. The statistical $\mathbf{A_2}$-**AB**-$\mathbf{B_2}$ most obviously displays the mismatch of the models.

b. Ensemble: A, AB, B_2.

The curves in Figure 4 were generated from a model in which a mixture of a monomer (**A**) and dimer (**B_2**) afford a mixed dimer (**AB**). The curves correspond to varying values of ϕ for dimer **AB**. Unlike the ensemble of pure dimers, the curves lack symmetry about the x-axis at X_A=0.5; the maximum of the **AB** aggregate does *not* appear at X_A=0.5. The curves appear most symmetric when the mixed **AB** aggregate is highly favored and most asymmetric when the relative stability of **AB** is low. Consequently, at high populations of **AB**, a **A-AB-B_2** ensemble could easily masquerade a simpler **A_2-AB-B_2** ensemble. Fits of **A-AB- B_2** theoretical data to **A_2-AB-B_2** produce the inverse of the plots in Figure 2; the lines would be the data, the symbols the fit, and the residuals would remain the same. Pairs of ensembles possess inherent differences that will reveal themselves regardless of which ensemble is the data and which is the model.

Theoretical **A-AB-B_2** fit to the **A_2-AB-B_4** model, however, present an important advisory (Figure 5). The subtle differences between the data and the incorrect model at all values of ϕ suggest difficulty distinguishing these two ensembles by Job plot experiments. The residuals' gradual and continuous decrease as the mole fraction of **A** increases offers the most visual clue to the incongruent fit. As in the previous examples, the mismatched model is most evident when the mixed aggregate has a maximum intensity around 0.5.

c. Ensemble: A_4-A_3B_1-A_2B_2-A_1B_3-B_4.

Figure 6 presents curves **I-III** which represent simulated **A_4-A_3B_1-A_2B_2-A_1B_3-B_4** data at low (**I**), intermediate (**II**), and high (**III**) relative stability of **A_4** (ϕ_4). The intermediate case, which is statistically distributed, is the only example which is symmetric about the x-axis at $X_A = 0.5$. Deviations from statistical distributions result in asymmetric plots; however, the maxima of the curves remain at the stoichiometry of the aggregate.

When characterizing an ensemble of tetrameric aggregates, one might consider whether the ensemble is actually **A_2-A_3B_1-A_2B_2-A_1B_3-B_4**. Application of the **A_2-A_3B_1-A_2B_2-A_1B_3-B_4** model to the **A_4-A_3B_1-A_2B_2-A_1B_3-B_4** data (Figure 7) affords poor fits. A plot (Plot IV) of the residuals reveals that as the dimeric aggregate increases in stability, the overall goodness of fit decreases.

Conclusion. The subtle complexities associated with the myriad of possible enolate ensembles draws attention to the importance of considering multiple models when assigning aggregation numbers to homoaggregated enolates. Fits of ensembles cannot stand alone as unambiguous aggregation assignments although they are essential. Though they may be technically difficult, control experiments such as varying the absolute lithium concentration and solvent swaps play vital roles by providing corroborating evidence for or against a particular model. Unequivocal enolate aggregation assignments require a conglomeration of supporting evidence.

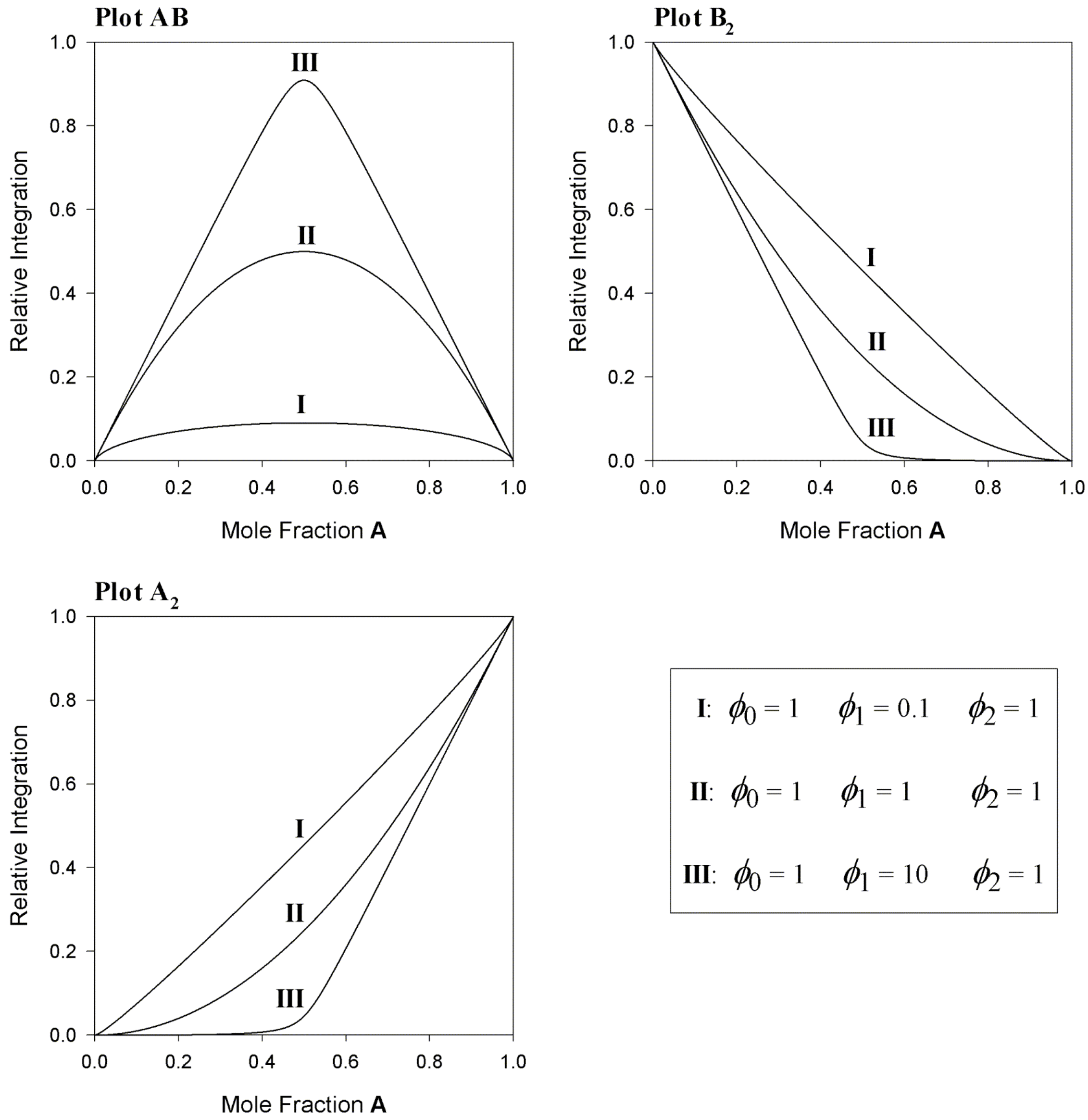

Figure 1. Plots of a dimer ensemble, $\mathbf{A}_2$-$\mathbf{AB}$-$\mathbf{B}_2$ at low (I), intermediate (II), and high (III) ϕ_1. The curves for each aggregated species are grouped as indicated.

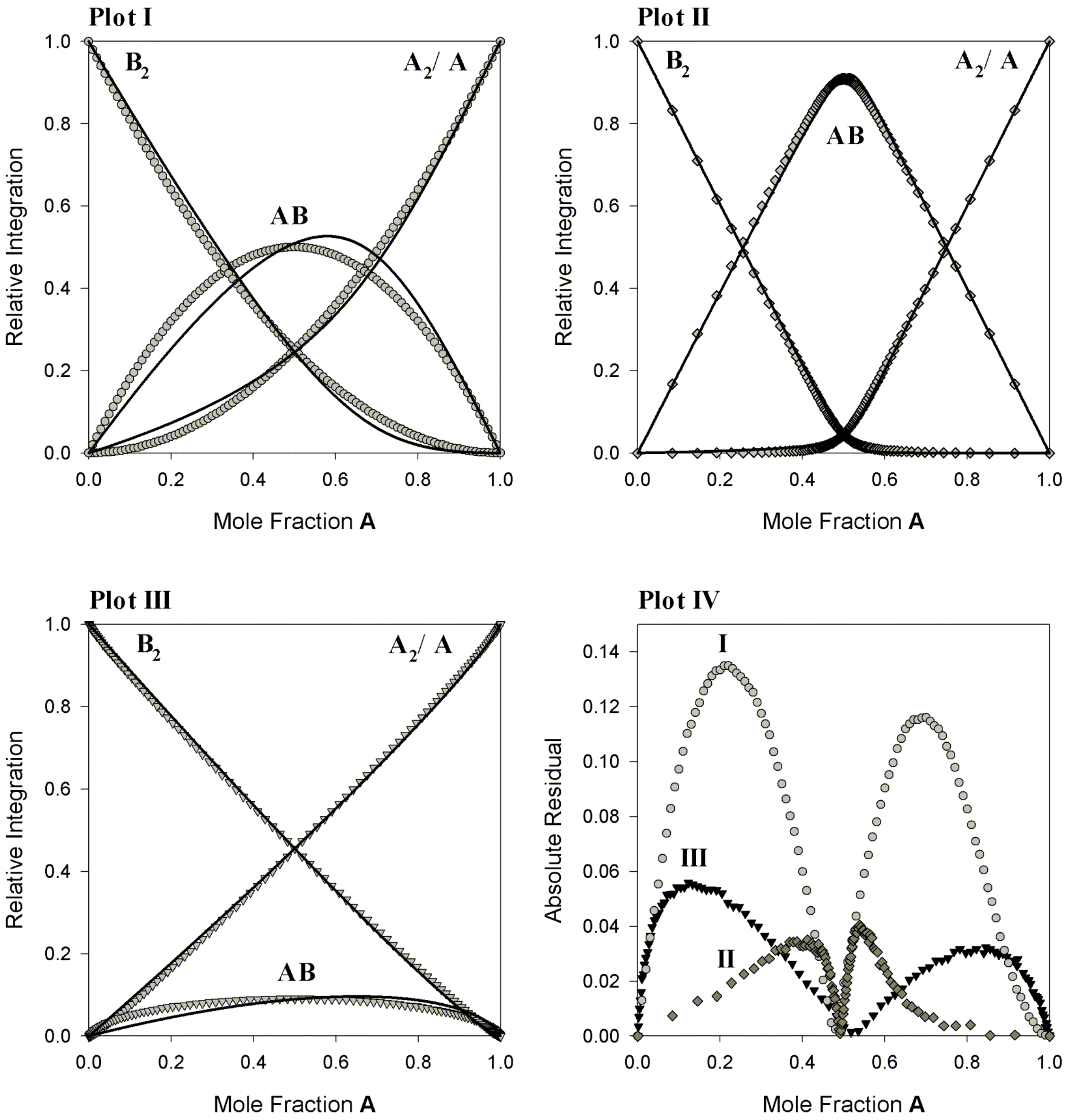

Figure 2. $\mathbf{A_2}$-**AB**-$\mathbf{B_2}$ data (symbols) at low (III), intermediate (I) and high (II) ϕ_1 fit to the **A**-**AB**-$\mathbf{B_2}$ (line) model. The absolute residual is plotted as a function of mole fraction of **A** in plot IV. Plot I) $\mathbf{A_2}$-**AB**-$\mathbf{B_2}$: $\phi_0 = \phi_2 = 1$, $\phi_1 = 1$; **A**-**AB**-$\mathbf{B_2}$ (0.10 M): $\phi_{10} = 0$, $\phi_{11} = 0.542$, $\phi_{20} = 0.549$, $\phi_{21} = 1.886$, $\phi_{22} = 0$, rms = 0.030. Plot II) $\mathbf{A_2}$-**AB**-$\mathbf{B_2}$: $\phi_0 = \phi_2 = 1$, $\phi_1 = 10$; **A**-**AB**-$\mathbf{B_2}$ (0.10 M): $\phi_{10} = 0$, $\phi_{11} = 0.167$, $\phi_{20} = 0.177$, $\phi_{21} = 8.088$, $\phi_{22} = 0$, rms = 0.009. Plot III) $\mathbf{A_2}$-**AB**-$\mathbf{B_2}$: $\phi_0 = \phi_2 = 1$, $\phi_1 = 0.1$; **A**-**AB**-$\mathbf{B_2}$ (0.10 M): $\phi_{10} = 0$, $\phi_{11} = 1.272$, $\phi_{20} = 1.673$, $\phi_{21} = 0.532$, $\phi_{22} = 0$, rms = 0.011.

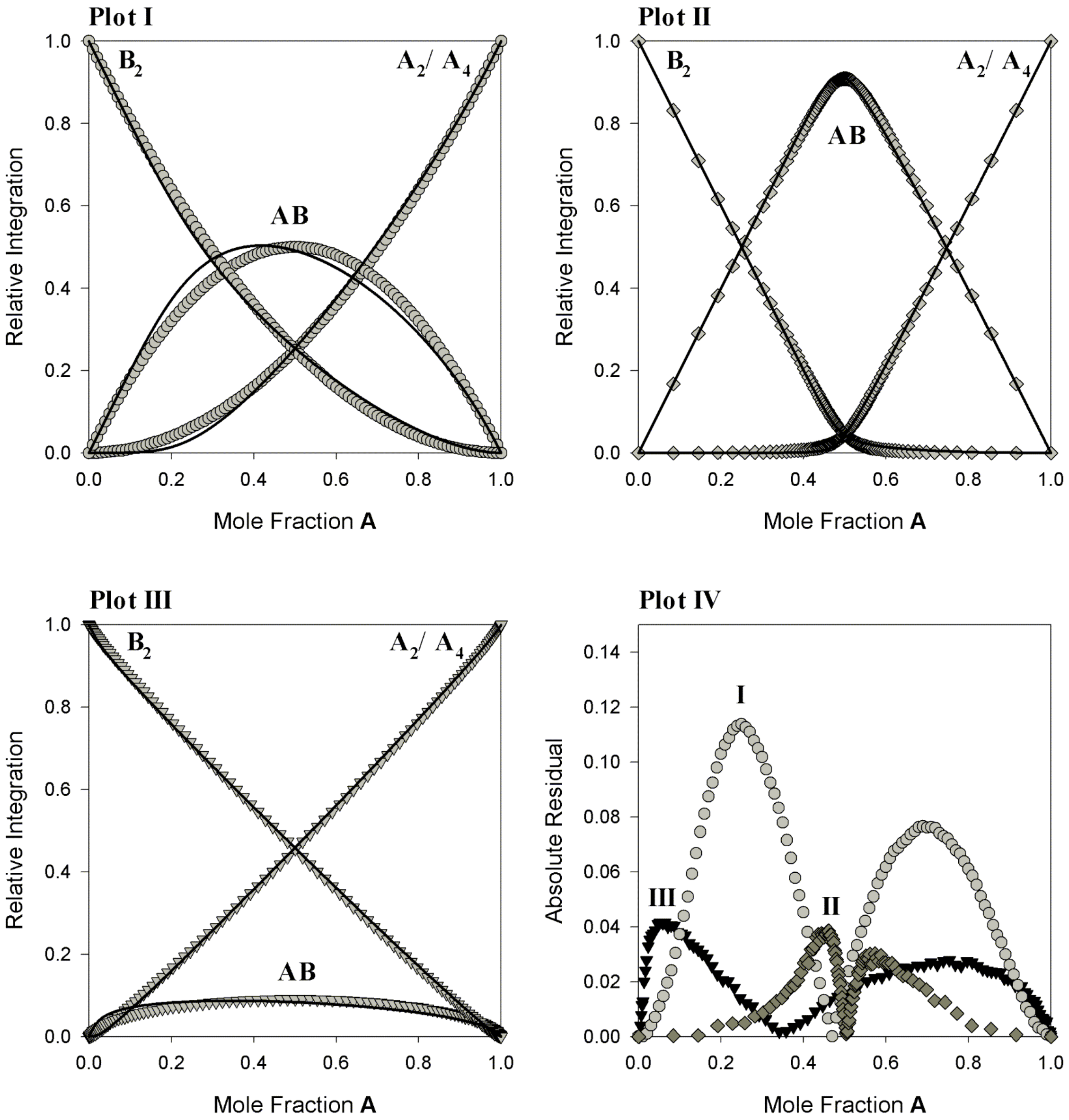

Figure 3. $\mathbf{A_2}$-$\mathbf{AB}$-$\mathbf{B_2}$ data (symbols) at low (III), intermediate (I) and high (II) ϕ_1 fit to the $\mathbf{A_4}$-$\mathbf{AB}$-$\mathbf{B_2}$ (line) model. The absolute residual is plotted as a function of mole fraction of **A** in plot IV. Plot I) $\mathbf{A_2}$-$\mathbf{AB}$-$\mathbf{B_2}$: $\phi_0 = \phi_1 = \phi_2 = 1$; $\mathbf{A_4}$-$\mathbf{AB}$-$\mathbf{B_2}$ (0.10 M): $\phi_{20} = 1.646$, $\phi_{21} = 0.526$, $\phi_{22} = 0$ $\phi_{40} = \phi_{41} = \phi_{42} = \phi_{43} = 0$, $\phi_{44} = 1.284$, rms = 0.022. Plot II) $\mathbf{A_2}$-$\mathbf{AB}$-$\mathbf{B_2}$: $\phi_0 = \phi_2 = 1$, $\phi_1 = 10$; $\mathbf{A_4}$-$\mathbf{AB}$-$\mathbf{B_2}$ (0.10 M): $\phi_{20} = 0.551$, $\phi_{21} = 1.968$, $\phi_{22} = 0$ $\phi_{40} = \phi_{41} = \phi_{42} = \phi_{43} = 0$, $\phi_{44} = 0.978$, rms = 0.008. Plot III) $\mathbf{A_2}$-$\mathbf{AB}$-$\mathbf{B_2}$: $\phi_0 = \phi_2 = 1$, $\phi_1 = 0.1$; $\mathbf{A_4}$-$\mathbf{AB}$-$\mathbf{B_2}$ (0.10 M): $\phi_{20} = 7.939$, $\phi_{21} = 0.188$, $\phi_{22} = 0$ $\phi_{40} = \phi_{41} = \phi_{42} = \phi_{43} = 0$, $\phi_{44} = 6.386$, rms = 0.008.

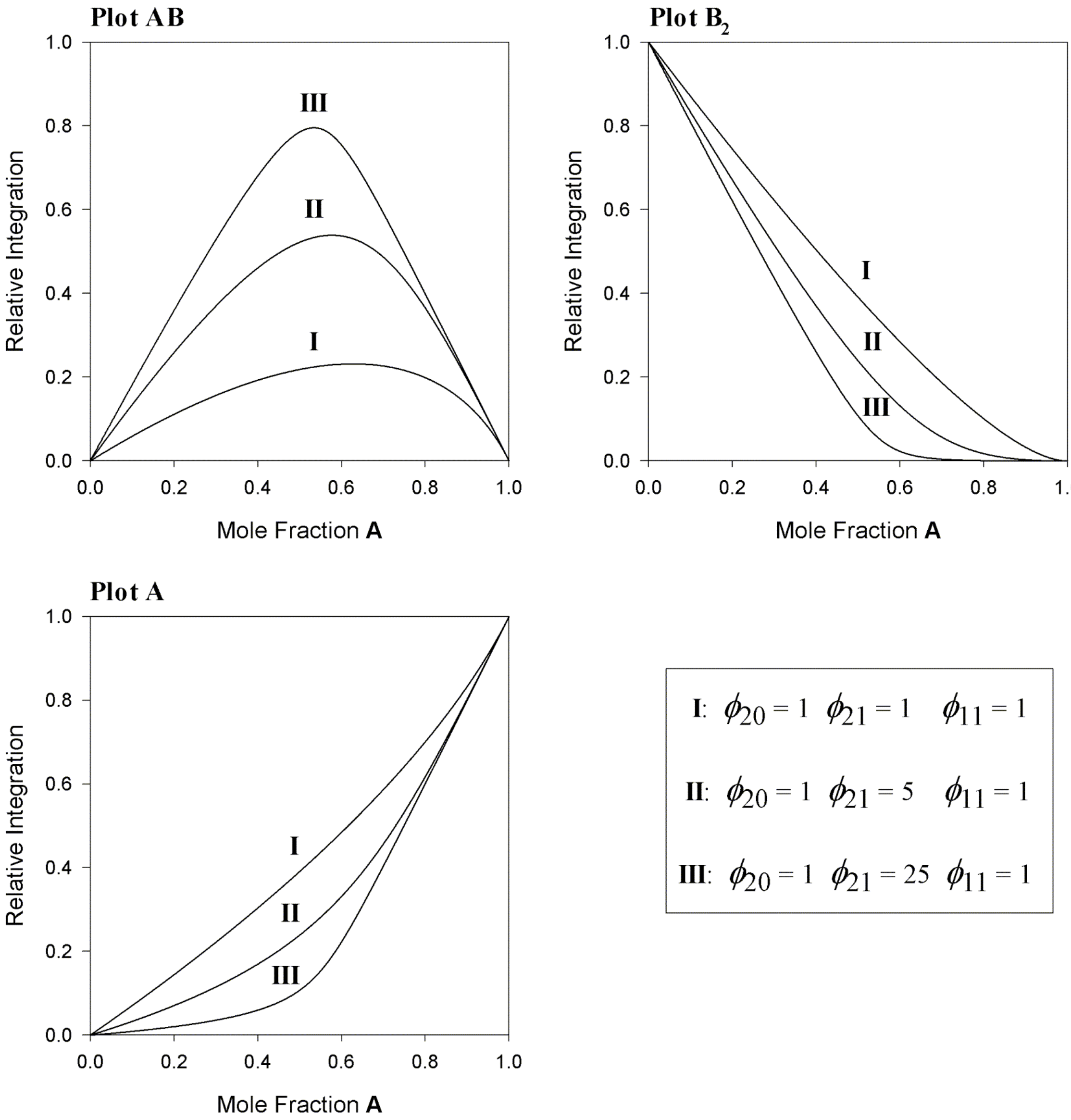

Figure 4. Plots of a monomer-dimer ensemble, **A**-**AB**-**B**$_2$ at low (I), intermediate (II), and high (III) ϕ_{21}. The curves for each aggregated species are grouped as indicated.

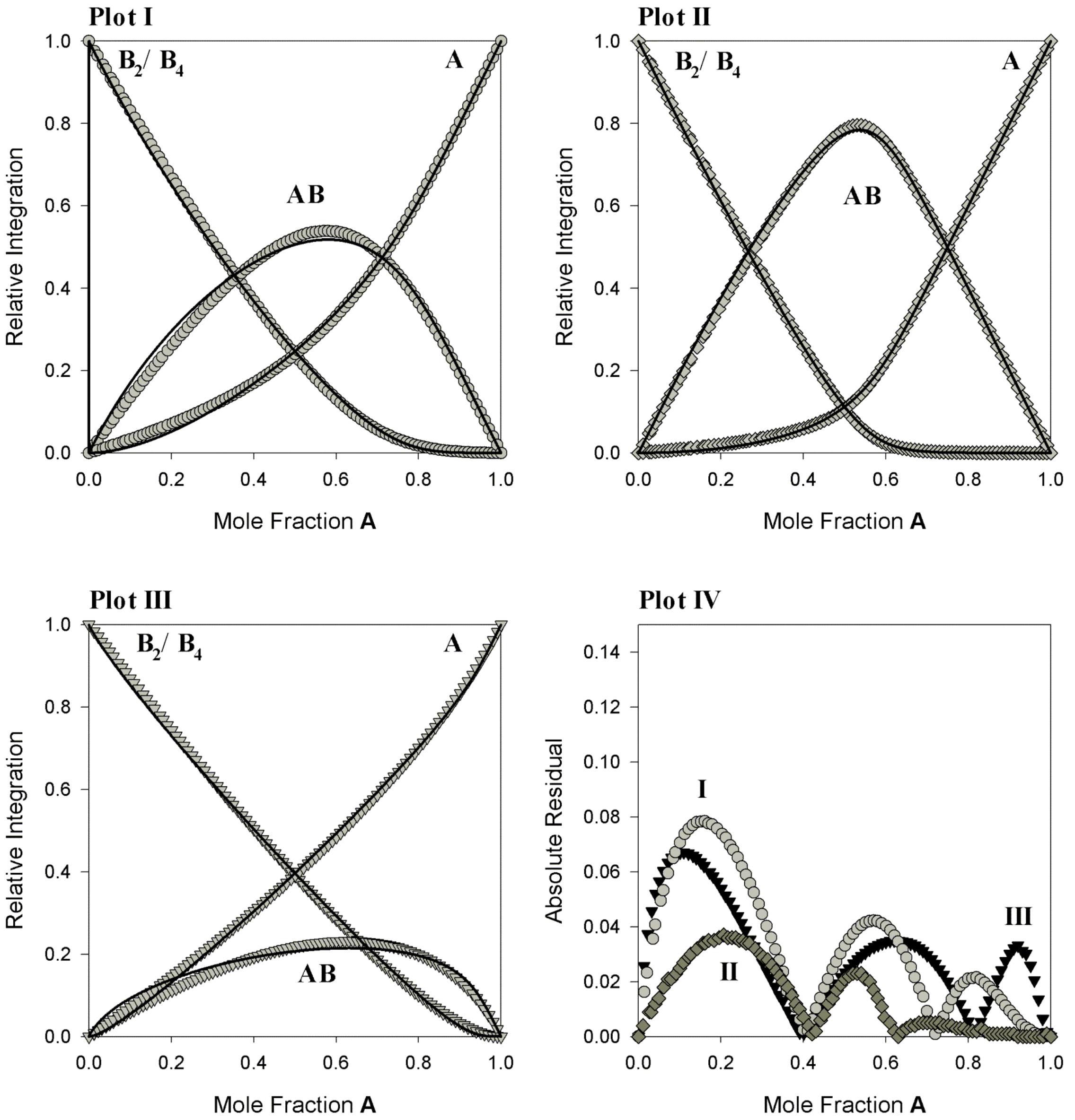

Figure 5. **A**-**AB**-**B**$_2$ data (symbols) at low (III), intermediate (I) and high (II) ϕ_{21} fit to the **A**$_2$-**AB**-**B**$_4$ (line) model. The absolute residual is plotted as a function of mole fraction of **A** in plot IV. Plot I) **A**$_2$-**AB**-**B**$_2$: $\phi_{20} = \phi_{11} = 1$, $\phi_{21} = 5$; **A**$_2$-**AB**-**B**$_4$ (0.10 M): $\phi_{20} = 0$, $\phi_{21} = 0.52$, $\phi_{22} = 1.44$ $\phi_{40} = 1.34$; $\phi_{41} = \phi_{42} = \phi_{43} = \phi_{44} = 0$, rms = 0.014. Plot II) **A**$_2$-**AB**-**B**$_2$: $\phi_{20} = \phi_{11} = 1$, $\phi_{21} = 25$; **A**$_2$-**AB**-**B**$_4$ (0.10 M): $\phi_{20} = 0$, $\phi_{21} = 1.11$, $\phi_{22} = 0.97$ $\phi_{40} = 1.01$; $\phi_{41} = \phi_{42} = \phi_{43} = \phi_{44} = 0$, rms = 0.010. Plot III) **A**$_2$-**AB**-**B**$_2$: $\phi_{20} = \phi_{11} = 1$, $\phi_{21} = 1$; **A**$_2$-**AB**-**B**$_4$ (0.10 M): $\phi_{20} = 0$, $\phi_{21} = 0.188$, $\phi_{22} = 1.92$ $\phi_{40} = 1.84$; $\phi_{41} = \phi_{42} = \phi_{43} = \phi_{44} = 0$, rms = 0.012.

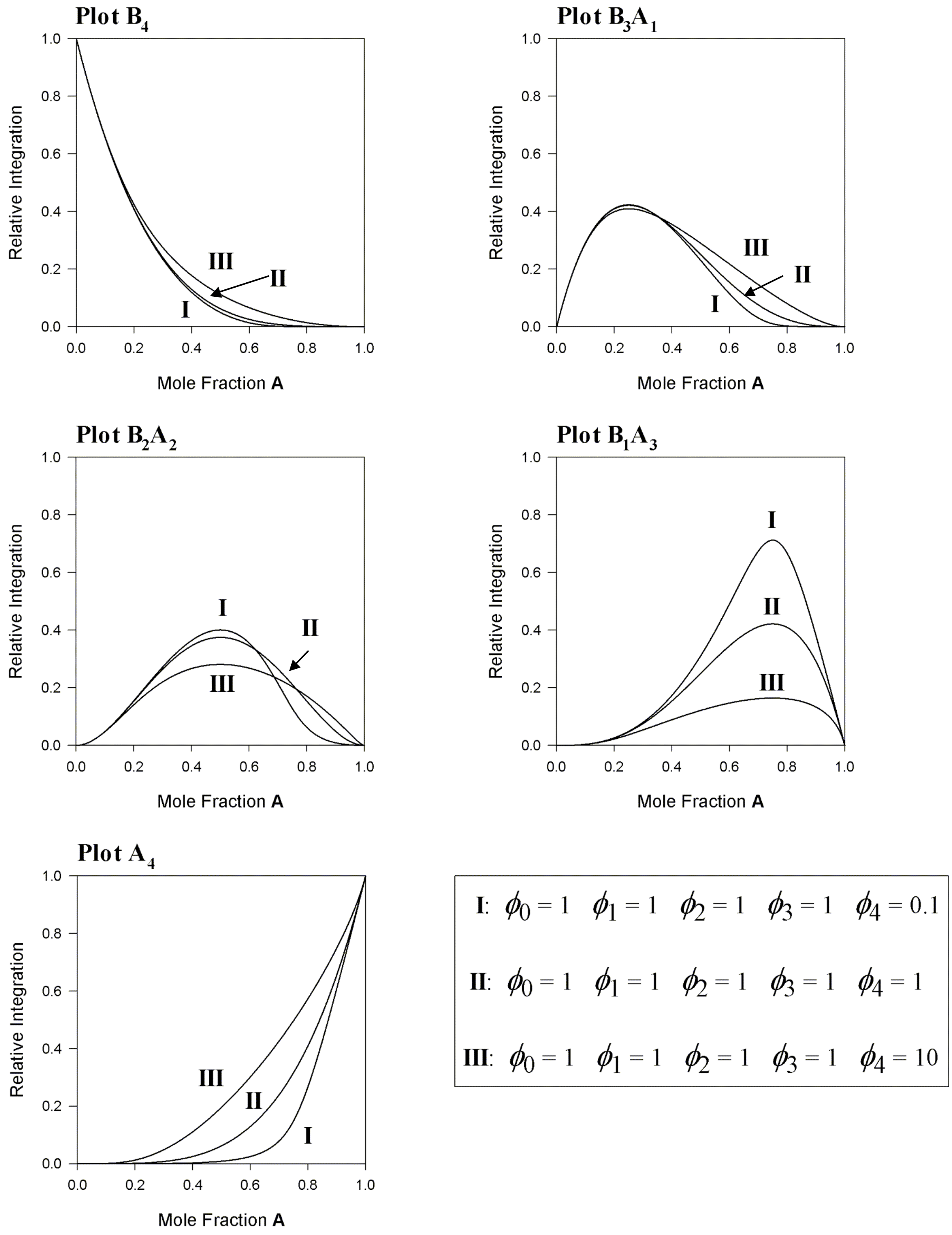

Figure 6. Plots of a tetramer ensemble, $\mathbf{A_4}$-$\mathbf{A_3B_1}$-$\mathbf{A_2B_2}$-$\mathbf{A_1B_3}$-$\mathbf{B_4}$ at low (I), intermediate (II), and high (III) ϕ_4. The curves for each aggregated species are grouped as indicated.

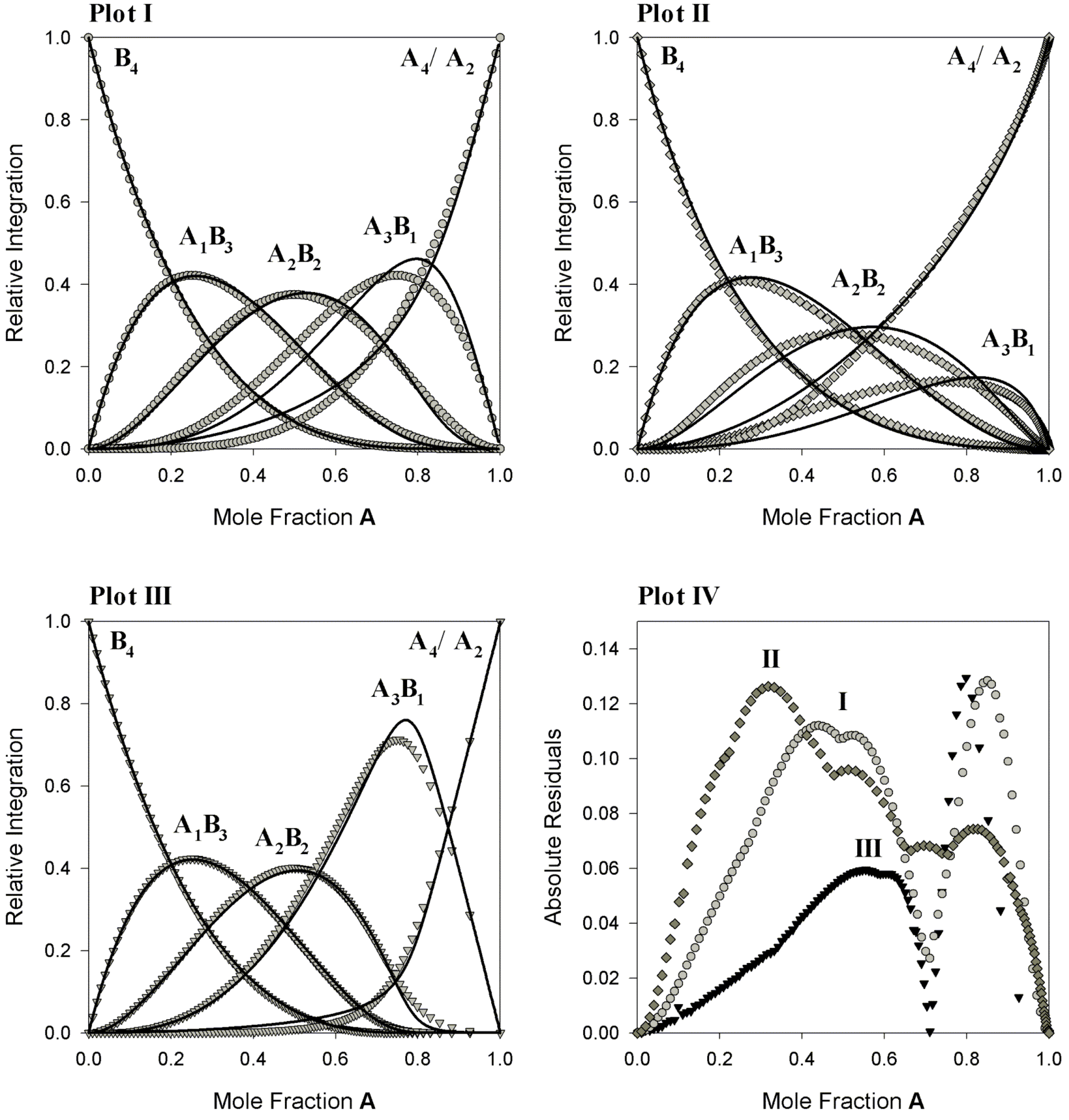

Figure 7. **A_4-A_3B_1-A_2B_2-A_1B_3-B_4** data (symbols) at low (III), intermediate (I) and high (II) ϕ_4 fit to the **A_2-A_3B_1-A_2B_2-A_1B_3-B_4** (line) model. The absolute residual is plotted as a function of mole fraction of **A** in plot IV. Plot I) **A_4-A_3B_1-A_2B_2-A_1B_3-B_4**: $\phi_0 = \phi_1 = \phi_2 = \phi_3 = \phi_4 = 1$; **$A_2$-$A_3B_1$-$A_2B_2$-$A_1B_3$-$B_4$** (0.20 M): $\phi_{20} = \phi_{21} = 0$, $\phi_{22} = 0.207$, $\phi_{40} = 0.932$, $\phi_{41} = 1.026$, $\phi_{42} = 1.098$, $\phi_{43} = 0.959$, $\phi_{44} = 0$, rms = 0.020. Plot II) **A_4-A_3B_1-A_2B_2-A_1B_3-B_4**: $\phi_0 = \phi_1 = \phi_2 = \phi_3 = 1$, $\phi_4 = 10$; **A_2-A_3B_1-A_2B_2-A_1B_3-B_4** (0.20 M): $\phi_{20} = \phi_{21} = 0$, $\phi_{22} = 0.427$, $\phi_{40} = 1.719$, $\phi_{41} = 1.332$, $\phi_{42} = 0.858$, $\phi_{43} = 0.379$, $\phi_{44} = 0$, rms = 0.016. Plot III) **A_4-A_3B_1-A_2B_2-A_1B_3-B_4**: $\phi_0 = \phi_1 = \phi_2 = \phi_3 = 1$, $\phi_4 = 0.1$; **A_2-A_3B_1-A_2B_2-A_1B_3-B_4** (0.20 M): $\phi_{20} = \phi_{21} = 0$, $\phi_{22} = 0.080$, $\phi_{40} = 0.320$, $\phi_{41} = 0.583$, $\phi_{42} = 1.066$, $\phi_{43} = 1.933$, $\phi_{44} = 0$, rms = 0.013.