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Calculation of site affinity constants and cooperativity coefficients for binding of ligands and/or protons to macromolecules

II. Relationships between chemical model and partition function algorithm

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The relationships between the chemical properties of a system and the partition function algorithm as applied to the description of multiple equilibria in solution are explained. The partition functions Z_M, Z_A, and Z_H are obtained from powers of the binary generating functions $J_j = (1 + k_j \gamma_{j,i}[Y])^{i_j}$, where $i_{i_j} = p_{i_j}$, q_{i_j}, or r_{i_j} represent the maximum number of sites in class j, for Y = M, A, or H, respectively. Each term of the generating function can be considered an element $\{i_j\}$ of a vector J_j and each power of the cooperativity factor $\gamma_{j,i}^i$ can be considered an element of a diagonal cooperativity matrix Γ_j. The vectors J_j are combined in tensor product matrices $L_l = \{J_1\}\,[J_2]\ldots[J_j]\ldots$, thus representing different receptor-ligand combinations. The partition functions are obtained by summing elements of the tensor matrices. The relationship of the partition functions with the total chemical amounts T_M, T_A, and T_H has been found. The aim is to describe the total chemical amounts T_M, T_A, and T_H as functions of the site affinity constants k_j and cooperativity coefficients b_j. The total amounts are calculated from the sum of elements of tensor matrices L_l. Each set of indices $\{p_j\ldots, q_j\ldots, r_j\ldots\}$ represents one element of a tensor matrix L_l and defines each term of the summation. Each term corresponds to the concentration of a chemical microspecies. The distinction between microspecies $M_{p_j}A_{q_j}H_{r_j}$ with ligands bound on specific sites and macrospecies $M_PA_QH_R$ corresponding to a chemical stoichiometric composition is shown. The translation of the properties of chemical model schemes into the algorithms for the generation of partition functions is illustrated with reference to a series of examples of gradually increasing complexity. The equilibria examined concern: (1) a unique class of sites; (2) the protonation of a base with two classes of sites; (3) the simultaneous binding of ligand A and proton H to a macromolecule or receptor M with four classes of sites; and (4) the binding to a macromolecule M of ligand A which is in turn a receptor for proton H. With reference to a specific example, it is shown how a computer program for least-squares refinement of variables k_j and b_j can be organized. The chemical model from the free components M, A, and H to the saturated macrospecies $M_PA_QH_R$, with possible complex macrospecies M_PA_Q and AH_R, is defined first. Subsequently, the binary functions compatible with the model, along with the initial values of the site affinity constants k_j, the number of sites in each class, and the cooperativity coefficients b_j, are entered. The chemical model controls the type of tensor product matrices L_l which are generated and the limits of the lower-case letter indices p_j, q_j and r_j which define the terms (microspecies) contributing to the total chemical amounts T_M, T_A, and T_H.

1. Introduction

In preceding papers [1–6], we have shown, by application of the partition function method, how several problems concerning multiple equilibria in solution between receptors and ligands can be resolved. The following fundamental tenets have been achieved.

(1) The relationship [1–5] between the formation function of Bjerrum, $\bar{n}$, and the partition function Z_M for simple combinations MA_Q be-

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tween a receptor M and a ligand A

$$\bar{n} = \partial \ln Z_M / \partial \ln[A] \quad (1)$$

is related [2] to the free energy of association of species MA_Q by

$$\Delta G_F = -RT \ln Z_M \quad (2)$$

In this equation, Z_M is a polynomial [4] whose terms are $\beta_Q[A]^Q$ with $0 \le Q \le Q_l$, each of which gives the probability of finding each species MA_Q in the solution divided by that of finding free M. The partition function as a whole expresses the total probability of finding any species containing M in solutions at variable [A].

(2) The ratio of the partition function for formation Z_M to that for dissociation Z_M^D equals the term $\beta_{Q_l}[A]^{Q_l}$, corresponding to the completely saturated complex MA_{Q_l} [2].

(3) The cumulative constant β_{Q_l} can be factorized [2,4] as the product of stepwise constants K_Q. Ratios between successive stepwise formation constants give the stepwise cooperativity constants K_{γ_Q}, whereas the ratios

$$\beta_Q^{1/Q} / \beta_1 = \gamma_Q \quad (3)$$

between geometric means of formation constants and the first formation constant β_1 yield the average cooperativity factors γ_Q.

(4) Passing to the logarithms of eq. 3 and hence to the plane of free energies and chemical potentials, we have been able to show [3–5] that in several practical cases a cooperativity function $\Gamma_Q(Q)$ exists which, on the logarithmic scale, is empirically found to be a linear function of $(Q-1)$

$$\lg \Gamma_Q = a + b(Q-1) \quad (4)$$

and similar functions for binding of M and H. The values of $\Gamma_Q(Q)$ at each step Q represent the cooperativity factors γ_Q.

(5) The cooperativity factors γ_Q, introduced into the cumulative constants β_Q, modify them in such a way [3–5] that the corrected constants reproduce the behaviour of a model with equal and independent sites; this can be checked on a Scatchard plot, $\bar{n}/[A] = f(\bar{n})$. The observed linearity of the plot proves the exactness of the correction applied. The slope of the line $\bar{n}/[A]$ vs. $\bar{n}$ yields the site affinity constant k.

(6) The cooperativity coefficients b of eq. 4 are amenable to physicochemical interpretation in connection with the charge density of the receptor [5].

(7) The cumulative formation constants β_Q are not independent of each other because they depend on common site constants k and common cooperativity coefficients b. Therefore, they cannot be used as independent parameters to be refined in a least-squares process for determination of the agreement between observed and calculated values of the total chemical amounts T_M, T_A, and T_H which are related to the concentrations by $[T_M] = T_M/V$, $[T_A] = T_A/V$, etc.

$$[T_M] = [M] + \sum_{Q=1}^{Q_l} \beta_Q [M][A]^Q \quad (5)$$

and

$$[T_A] = [A] + \sum_{Q=1}^{Q_l} Q\beta_Q [M][A]^Q \quad (6)$$

Therefore, the constants β_Q must be expressed as functions of successive powers of the site affinity constants k and cooperativity factors γ_Q (a of eq. 4 is always nearly zero), which are the real independent variables of the system [5].

(8) Interrelations of the same type as those between formation constants β_Q hold for the cumulative formation constants β_{PQR} for complexes $M_P A_Q H_R$. The need arises to express β_{PQR}, and hence the total amounts T_M, T_A, and T_H, as functions of several site affinity constants k_j and cooperativity coefficients b_j. To this end, the relationship (item 1, above) between the formation function $\bar{n}$ and partition function Z_M can be extended [5] to more complex cases where self-association of the receptor takes place

$$\bar{n} = \bar{n}_M^A + \bar{n}_M^M = \partial \ln Z_M / \partial \ln[A] + \partial \ln Z_M / \partial \ln[M] \quad (7)$$

(9) By application [5] of eq. 7, the mass balance equations giving the total chemical amounts T_M, T_A, and T_H of components M, A, and H, are expressed in concentration units as functions of

Z_M, Z_A, and Z_H, respectively:

$$[T_M] = [M]\{Z_M + [M]\,\partial Z_M/\partial[M]\} \quad (8)$$

$$[T_A] = [A]\{Z_A + [A]\,\partial Z_A/\partial[A]\} \quad (9)$$

$$[T_H] = [H]\{Z_H + [H]\,\partial Z_H/\partial[H]\} \quad (10)$$

(10) A method for generating partition functions Z_M, Z_A, and Z_H has been studied [6]. The partition functions are obtained from tensor products of binary generating functions. There is one generating function for each class j

$$J_j = \left(1 + k_j\gamma_{j,i}[Y]\right)^{i_{t_j}} \quad (11)$$

where $i_j = p_j$, q_j, or r_j, for Y = M, A, or H, respectively. Each class j of binding sites has i_t sites. Each polynomial (eq. 11) is associated with a vector J_j, whose elements

$$\{i_i\} = m_{i,j}\left(k_j\gamma_{j,i}[Y]\right)^{i_{t_j}} \quad (12)$$

are the terms of the polynomial; note that the value of the index of each element is equal to the power of the same element. The values of the cooperativity factors can be expressed in a separate diagonal cooperativity matrix Γ_j, whose elements

$$\{\Gamma_{j,i}\} = \gamma_{j,i}^{i_j} = \exp\left[b_j(i_j - 1)i_j\right] \quad (13)$$

are the i_j-th powers of the cooperativity factors. The relationship between eqs. 11 and 13 can be represented in matrix notation

$$\Gamma_j^{-1}J_j = J_j^* \quad (14)$$

where J_j^* is the vector associated with the polynomial, eq. 11, for every cooperativity factor $\gamma_{j,i} = 1$ and Γ_j^{-1} is a diagonal matrix whose elements are reciprocals of eq. 13.

(11) The vectors J_j are combined, according to rules and limits which depend on the chemical model chosen, in tensor matrices

$$L_l = \{J_1\}[J_2]\ldots[J_j]\ldots \quad (15)$$

where $\{J_j\}$ indicates a column vector and $[J_j]$ a row vector. The elements

$$\{l\} = \{i_1, i_2\ldots, i_j\ldots\} \quad (16)$$

of the tensor matrices L_l are defined by a combination of lower-case letter indices $i_j = p_j$, q_j, and r_j, depending on the component vectors J_j. Each index represents a value calculated via eqs. 12 and 13. The whole matrix of the indices forms the index space, which provides a simple bookkeeping method to access all of the information necessary to perform the calculation of concentrations of the species. In this way, one needs only to manipulate the indices during the course of the calculations, and then the actual values corresponding to the indices can be inserted at the end. Each element of the matrix indicates a chemical microspecies $M_{p_j}A_{q_j}H_{r_j}$. The chemical microspecies differ from each other due to the occupation of classes of sites. The summation of the concentrations of all microspecies with the same stoichiometric ratio yields the concentration of macrospecies $M_PA_QH_R$, as shown in fig. 1.

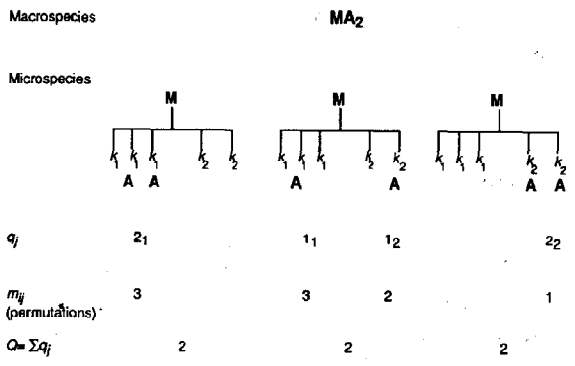

Fig. 1. Possible microspecies compatible with the macrospecies MA_2 in a receptor with two classes j of sites. Number of sites: $q_{t_1} = 3$, $q_{t_2} = 2$.

(12) The type and number of tensor matrices L_l necessary to calculate the total amounts T_M, T_A, and T_H are governed by the chemical model which defines the possible macrospecies $M_PA_QH_R$, from the free components M, A, and H up to the saturated complex.

The algorithms dealing with vectors J_j and tensor L_l have been presented in detail in the preceding article [6].

2. Examples

The application of the method of generating partition functions for specific chemical systems is illustrated below by referring to a series of examples of gradually increasing complexity, including binding to receptors with both one and different classes of sites, parallel noncompetitive binding of A and H to M, and successive binding of H to A which in turn binds to M.

With respect to the preceding paper [6], some changes have been introduced in the symbols. The vectors J_{γ_j}, which represented binary generating functions containing the cooperativity factors γ_Q, are indicated here (cf. eq. 14) simply as J_j. On the other hand, vectors indicating binary generating functions without cooperativity are now designated by J_j^*. This change has been introduced because binary generating functions with cooperativity are those usually employed in practice and use of the subscript γ would be burdensome. Moreover, the distinction is made between index $i_j = p_j$, q_j, or r_j representing exponents, coefficients, or subscripts and the corresponding letters in brackets $\{i_j\} = \{p_j\}$, $\{q_j\}$, or $\{r_j\}$ which in the index space represent an element of J_j or a factor of an element of L_l. A combination of indices $\{p_j \ldots q_j \ldots r_j \ldots\}$ represents an element of L_l. For all other symbols, referral is made to the glossary (see p. 14 of ref. 6) in the previous article.

The procedure has been devised taking into account potentiometric titrations, where the analytical response is logarithmic to the base 10. The values of cooperativity coefficients b_j and logarithms of the equilibrium constants are expressed on the same scale. The pH-metric measurements are normally performed by using a glass electrode, calibrated against known H^+ concentrations in solutions containing the same amount of inert salt.

We assume that the effect of the ionic strength is kept constant by addition of adequate amounts of inert salt. In this way, the effects of ionic strength are included in the constants k_j and cooperativity coefficients b_j, without explicit use of activities. Corrections for the dependence of the constants on ionic strength are currently under investigation in our laboratories.

Some caution is required concerning the expression of concentrations. If the components are metals or small organic or inorganic ligands, the concentrations are expressed in general in mol dm^{-3}. When dealing with macromolecules or polyelectrolytes, there is the problem of using adequate concentration units. The partition function is conceptually a molar quantity and the concentration cannot be expressed in g l^{-1} or monomer unit concentration. It is therefore necessary to know the approximate molecular weight of the macromolecule or polyelectrolyte and preferentially to use substances with low polydispersity.

The extension of the procedure to other physicochemical techniques where the analytical response is proportional to the concentration, such as calorimetric or spectrophotometric determinations, requires a slightly different approach, which deserves separate treatment.

2.1. Example 1: Base A protonated at a unique class of r_i sites (complexes AH_R)

For reasons that will become apparent when treating the development of the computer program, for each component M, A, and H the self-association binary generating functions (vectors J_j) are defined first. If no direct self-association takes place, then the self-association constants k_j with $j = 1, 2, \ldots$ are assigned a value of zero, as are the coefficients of the cooperativity functions, $b_j = 0$. Therefore, J_1 and J_2, for two components, are reserved for the definition of self-association. Since in this example we assume no self-association, the value of q_1 corresponding to J_1 and that of r_2 corresponding to J_2 are 1. To illustrate the point, we present the equations for the general case AH_R on the left and those for a specific case, AH_4, on the right. The vectors J_j, formed by the terms of the polynomials J_j, and the diagonal matrices Γ_j, formed by powers of the cooperativity

factors $\gamma_{j,i}$, for this example are therefore

vectors J_j	matrices Γ_j	Example $R = 4$ (elements of vector J_j)	
$J_1 = (1 + 0[\mathrm{A}])$,	$\gamma_{1,q_1}^{(q_1-1)} = \exp(2.3 \cdot 0(q_1 - 2)(q_1 - 1))$	$J_1 = \{1\}$	(17)
$J_2 = (1 + 0[\mathrm{H}])$,	$\gamma_{2,r_2}^{(r_2-1)} = \exp(2.3 \cdot 0(r_2 - 2)(r_2 - 1))$	$J_2 = \{1\}$	(18)
$J_3 = (1 + k_3\gamma_{3,r}[\mathrm{H}])^r$	$\gamma_{3,r_3}^{r_3} = \exp(2.3 \cdot b_3(r_3 - 1)r_3)$	$J_3 = \{\{1\}, \{4k_3[\mathrm{H}]\}, \{6k_3^2\gamma_{3,2}^2[\mathrm{H}]^2\}, \{4k_3^3\gamma_{3,3}^3[\mathrm{H}]^3\}, \{k_3^4\gamma_{3,4}^4[\mathrm{H}]^4\}\}$	(19)

where $\gamma_{3,2}^2 = \exp(2.3 \cdot 2b_3)$, $\gamma_{3,3}^3 = \exp(2.3 \cdot 6b_3)$, and $\gamma_{3,4}^4 = \exp(2.3 \cdot 12b_3)$. Each term of the polynomial and hence each term of J_3 is calculated from eqs. 12 and 13 as

$$m_{r_3}\left(k_3\gamma_{3,r_3}[\mathrm{H}]\right)^{r_3} = r_{t_3}!/r_3!(r_{t_3} - r_3)!\left(k_3\gamma_{3,r_3}[\mathrm{H}]\right)^{r_3} \tag{20}$$

with r_3 running from 0 to r_t. The partition function Z_A is obtained as the sum of elements of J_3

$$Z_A = J_3 = 1 + \sum_{r_3=1}^{r_{t_3}} m_{r_3}\left(\gamma_{3,r_3}k_3[\mathrm{H}]\right)^{r_3} \tag{21a}$$

which can yield Z_H by multiplying the summation terms by [A]/[H]

$$Z_H = 1 + \sum_{r_3=1}^{r_{t_3}} m_{r_3}\left(\gamma_{3,r_3}k_3[\mathrm{H}]\right)^{r_3}[\mathrm{H}]^{-1}[\mathrm{A}] \tag{22a}$$

and mass balance equations are obtained from the same elements, multiplied by [A], [H] and by the exponent of [A] or [H], respectively

$$[\mathrm{T_A}] = [\mathrm{A}]J_3 = [\mathrm{A}] + \sum_{r_3=1}^{r_{t_3}} m_{r_3}\left(\gamma_{3,r_3}k_3[\mathrm{H}]\right)^{r_3}[\mathrm{A}] \tag{23a}$$

$$[\mathrm{T_H}] = [\mathrm{H}] + \sum_{r_3=1}^{r_{t_3}} r_3 m_{r_3}\left(\gamma_{3,r_3}k_3[\mathrm{H}]\right)^{r_3}[\mathrm{A}] \tag{24a}$$

Expressed in index space notation, the partition functions (eqs. 21a and 22a), and the total amounts (eqs. 23a and 24a), become eqs. 21b–24b, respectively

$$Z_A = 1 + \sum_{r_3=1}^{r_{t_3}} \{r_3\} \tag{21b}$$

$$Z_H = 1 + \sum_{r_3=1}^{r_{t_3}} \{r_3\}[\mathrm{H}]^{-1} \tag{22b}$$

$$[\mathrm{T_A}] = [\mathrm{A}] + \sum_{r_3=1}^{r_{t_3}} \{r_3\}[\mathrm{A}] \tag{23b}$$

$$[\mathrm{T_H}] = [\mathrm{H}] + \sum_{r_3=1}^{r_{t_3}} r_3\{r_3\}[\mathrm{A}] \tag{24b}$$

In our example, element $\{r_3\} = \{1_3\}$ represents $4k_3[\mathrm{H}]$, $\{r_3\} = \{2_3\}$ represents $6k_3^2\gamma_{3,2}^2[\mathrm{H}]^2$, etc. Note that the value of the index r_3 is always the same as the power to which the variables are raised in the corresponding terms in the affinity space. The subscript r_3 identifies each term of the summation in eq. 23b for this class of sites. In more complex cases each term will be identified by a combination of indices.

The derivatives of the terms $[(\mathrm{T_A})_{r_3}]$, needed for the calculation of the normal equations of least-squares refinement, are

$$(\partial[\mathrm{T_A}]/\partial k_3)_{r_3} = (r_3/k_3)[([\mathrm{T_A}])]_{r_3} \tag{25}$$

$$(\partial[\mathrm{T_A}]/\partial b_3)_{r_3} = (r_3(r_3 - 1))[([\mathrm{T_A}])]_{r_3} \tag{26}$$

$$(\partial[\mathrm{T_A}]/\partial[\mathrm{A}])_{r_3} = m_{r_3}\left(k_3\gamma_{3,r_3}[\mathrm{H}]\right)^{r_3} \tag{27}$$

$$(\partial[\mathrm{T_A}]/\partial[\mathrm{H}])_{r_3} = r_3 m_{r_3}\left(k_3\gamma_{3,r_3}[\mathrm{H}]\right)^{r_3}[\mathrm{H}]^{-1}[\mathrm{A}] \tag{28}$$

Similar equations hold for the terms $[(T_H)_{r_3}]$, where, however, $(\partial T_H/\partial[H])_0 = 1$.

2.2. Example 2: Base A protonated at two classes of sites, with r_{t_3} sites in class $j = 3$ and r_{t_4} in class $j = 4$

In addition to the self-association generating functions, we have the generating functions J_3 and J_4. The generating functions are, apart from J_1 and J_2:

vector J_j matrix Γ_j

$$J_3 = (1 + k_3\gamma_{3,r_3}[H])^{r_{t_3}} \quad \gamma_{3,r_3}^{r_3} = \exp(2.3 \cdot b_3(r_3 - 1)r_3) \quad (27)$$

$$J_4 = (1 + k_4\gamma_{4,r_4}[H])^{r_{t_4}} \quad \gamma_{4,r_4}^{r_4} = \exp(2.3 \cdot b_4(r_4 - 1)r_4) \quad (28)$$

In this case, the partition function Z_A is obtained from the elements of the tensor product

$$L_1 = \{J_3\}[J_4] \quad (29)$$

where $\{J_3\}$ indicates a column vector and $[J_4]$ a row vector. Every column vector can be transposed to a row vector and vice versa.

By representing the terms in the index space, and remembering that $R = r_3 + r_4$, one obtains:

$$Z_A = \sum\{L_1\} = 1 + \sum_{R=1}^{R_t}\{r_3 r_4\} \quad (30)$$

where $\{r_3\}$ and $\{r_4\}$ represent the elements of the vectors J_3 and J_4, respectively, calculated according to eqs. 12 and 13. The $\{r_3 r_4\}$ terms are elements of the matrix L_1 in eq. 29, which are the products of the factors $\{r_3\}$ and $\{r_4\}$. The products are repeated for every combination $r_3 r_4$, within the index limits given in eq. 30.

The other partition function in index space notation is

$$Z_H = 1 + \sum_{R=1}^{R_t}\{r_3 r_4\}[H]^{-1}[A] \quad (31)$$

The corresponding mass balance equations are obtained again from L_1

$$[T_A] = [A] + \sum_{R=1}^{R_t}\{r_3 r_4\}[A] \quad (32)$$

$$[T_H] = [H] + \sum_{R=1}^{R_t}(r_3 + r_4)\{r_3 r_4\}[A] \quad (33)$$

The derivatives of the individual terms in eqs. 32 and 33 are similar in form to eqs. 25–28.

2.3. Example 3: Macromolecule M, combined with ligand A and proton H

In this case, the complexes formed include MA_Q, MH_R, and MA_QH_R with different (non-competing) sites on M for A and H. There is no interaction between A and H. The sites on M for A and H are of two classes each: for A, q_{t_4}, q_{t_5}; for H, r_{t_6}, r_{t_7}. There is no self-association; thus the generating function vectors J_1–J_3 are not given. The generating function vectors and the cooperativity matrices are

vector J_j matrix Γ_j

$$J_4 = (1 + k_4\gamma_{4,q_4}[A])^{q_{t_4}} \quad \gamma_{4,q_4}^{q_4} = \exp(2.3 \cdot b_4(q_4 - 1)q_4) \quad (34)$$

$$J_5 = (1 + k_5\gamma_{5,q_5}[A])^{q_{t_5}} \quad \gamma_{5,q_5}^{q_5} = \exp(2.3 \cdot b_5(q_5 - 1)q_5) \quad (35)$$

$$J_6 = (1 + k_6\gamma_{6,r_6}[H])^{r_{t_6}} \quad \gamma_{6,r_6}^{r_6} = \exp(2.3 \cdot b_6(r_6 - 1)r_6) \quad (36)$$

$$J_7 = (1 + k_7\gamma_{7,r_7}[H])^{r_{t_7}} \quad \gamma_{7,r_7}^{r_7} = \exp(2.3 \cdot b_7(r_7 - 1)r_7) \quad (37)$$

The tensor product is

$$L_1 = J_4 J_5 J_6 J_7 \quad (38)$$

and the partition functions are obtained from the elements of L_1, expressed in index space, as follows:

$$Z_M = 1 + \sum_{Q+R=1}^{Q_t+R_t}\{q_4 q_5 r_6 r_7\}[M][A] \quad (39)$$

$$Z_A = 1 + \sum_{Q=1}^{Q_t}\sum_{R=0}^{R_t}\{q_4 q_5 r_6 r_7\}[M][A] \quad (40)$$

$$Z_H = 1 + \sum_{Q=0}^{Q_t}\sum_{R=1}^{R_t}\{q_4 q_5 r_6 r_7\}[M][H] \quad (41)$$

Each term of the summations is labelled with the indices of the elements of L_1. The factors of the elements are calculated from eqs. 12 and 13, e.g., $\{q_4\} = m_{q_4}(k_4\gamma_{4,q_4}[A])^{q_4}$. Each element $\{q_4 q_5 r_6 r_7\}$ is the product of four factors each of which is calculated by repeated application of eqs. 12 and 13. All of the terms are calculated for every possible combination of the four indices, within the limits given. The mass balance equations are ob-

tained from the same elements of L_1

$$[T_M] = [M] + \sum_{Q+R=1}^{Q_t+R_t} \{q_4 q_5 r_6 r_7\}[M] \quad (42)$$

$$[T_A] = [A] + \sum_{Q=1}^{Q_t} \sum_{R=1}^{R_t} q_j \{q_4 q_5 r_6 r_7\}[M] \quad (43)$$

$$[T_H] = [H] + \sum_{Q=1}^{Q_t} \sum_{R=1}^{R_t} r_j \{q_4 q_5 r_6 r_7\}[M] \quad (44)$$

2.4. Example 4: Macromolecule M with two classes of sites, binding ligand A, which is a receptor with two classes of sites for proton H

Two types of complexes $M(AH_R)_Q$ (or $MA_QH_{R'}$ with $R' = Q \times R$) and AH_R are formed in this case. In this example, H binds only to A, which may in turn bind to M. The numbers of sites on M are q_{t_4} and q_{t_5}, those on A for binding H being r_{t_6} and r_{t_7}. There is no self-association.

The generating function vectors and cooperativity matrices are:

receptor	vector J_j	matrix Γ_j	
M	$J_4 = (1 + k_4\gamma_{4,q_4}[A])^{q_{t_4}}$	$\gamma_{4,q_4}^{q_4} = \exp(2.3 \cdot b_4(q_4 - 1)q_4)$	(45)
M	$J_5 = (1 + k_5\gamma_{5,q_5}[A])^{q_{t_5}}$	$\gamma_{5,q_5}^{q_5} = \exp(2.3 \cdot b_5(q_5 - 1)q_5)$	(46)
A	$J_6 = (1 + k_6\gamma_{6,r_6}[H])^{r_{t_6}}$	$\gamma_{6,r_6}^{r_6} = \exp(2.3 \cdot b_6(r_6 - 1)r_6)$	(47)
A	$J_7 = (1 + k_7\gamma_{7,r_7}[H])^{r_{t_7}}$	$\gamma_{7,r_7}^{r_7} = \exp(2.3 \cdot b_7(r_7 - 1)r_7)$	(48)

By examining the chemical model, the tensor products needed for calculation of the mass balance equations can be inferred. They are

$$L_1 = J_4[(O_qJ_6)(O_qJ_7)]\,J_5[(O_qJ_6)(O_qJ_7)] \quad (49)$$

and

$$L_2 = J_6J_7 \quad (50)$$

Note that these expressions include the cooperativity factors although the cooperativity matrices are not explicitly presented. It should be borne in mind that they are contained in J_j, as shown in eq. 14.

The unprimed index operator O_q (see the preceding paper) in eqs. 49 and 50 means for example: 'calculate the q_4-th tensor power of the following vector J_6 and J_7' and indicates that the cooperativity effect exists only within protons H bound to the same ligand A. The operator acts on J_j in a different fashion before each multiplication by individual terms of the previous J_j vector. For example, before multiplying 2_4 by the elements of J_6, we take the second tensor product of J_6 by itself; and before multiplying 3_4 by the elements of J_6, we take the third tensor product of J_6 by itself, etc. The choice of O_q instead of $O_{q'}$ is made on physicochemical grounds and could be changed if the agreement between observed and calculated data were not satisfactory. The use of these operators is illustrated below. Note that, in eq. 49, the operator O_q is repeated 2×2 times, two for each class of sites on M for binding of A, multiplied by two, the classes of A for binding of H, according to the chemical model we are testing. The tensor power operator O_q introduces the cooperativity effect separately into J_6 and J_7.

If it is required to test the hypothesis that the cooperativity effects extend over all the protons, irrespective of which particular receptor A is bound, the operator $O_{q'}$ is applied to J_6^* (cf. eq. 14). The operator $O_{q'}$ signifies: 'raise the polynomial J_6 to the q_4-th power before applying the cooperativity function $\Gamma_6(r')$, where r' is the index of the expanded vector'. The product

$$(O_qJ_6^*)\Gamma_{6,r'} = O_q(\Gamma_{6,r}^{-1}J_6)\Gamma_{6,r'} \quad (51)$$

is substituted for (O_qJ_6) in eq. 49. The physicochemical meaning of the operators O_i and

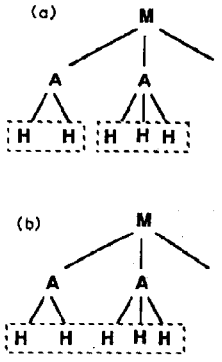

Fig. 2. Physicochemical grounds of (a) tensor power and (b) vector power. (a) Operator $O_{q'}$; the cooperativity effect is limited within the group of H binding to the same A. (b) Operator O_q; the cooperativity extends to all protons H, irrespective of to which particular A they are bound. Dashed boxes indicate limits of cooperativity effects.

$O_{i'}$ is illustrated in fig. 2. It is assumed that in this example we accept the model with restricted cooperativity, and use the operator O_q in eq. 49.

Again from the chemical model, the limits of the indices in the summations for the total chemical amounts can be derived

$$[T_M] = [M] + \sum_{Q+R=1}^{Q_t+R_t} \{q_4\{o_{q_4}r_6\}\{o_{q_4}r_7\}q_5\{o_{q_5}r_6\} \times \{o_{q_5}r_7\}\}[M] \quad (52)$$

$$[T_A] = [A] + \sum_{Q=1}^{Q_t}\sum_{R=0}^{R_t} q_j\{q_4\{o_{q_4}r_6\}\{o_{q_4}r_7\}q_5 \times \{o_{q_5}r_6\}\{o_{q_5}r_7\}\}[M] + \sum_{R=1}^{R_t}\{r_7r_8\}[H] \quad (53)$$

$$[T_H] = [H] + \sum_{Q=0}^{Q_t}\sum_{R=1}^{R_t} r_j\{q_4\{o_{q_4}r_6\}\{o_{q_4}r_7\}q_5 \times \{o_{q_5}r_6\}\{o_{q_5}r_7\}\}[M] + \sum_{R=1}^{R_t} r_j\{r_6r_7\}[A] \quad (54)$$

This example is explained in more detail in section 3.

3. Development of computer program

The development of the computer program can be better explained with reference to a working case analogous to example 4.

A macromolecule M is the receptor for a ligand A, which is in turn the receptor for a ligand H. The model constitutes the first computer input (table 1) with indication of: (i) components M, A, and H; (ii) types of complexes $M(AH_R)_Q$, AH_R; (iii) receptors and ligands; (iv) classes of sites, with number of sites in each class; and (v) saturated complexes.

The next input (table 2) consists of the data for construction of vectors J_j and cooperativity matrices Γ_j, site constants k_j, cooperativity coefficients b_j, maximum number of sites i_{t_j}. Subsequently, we provide the computer with the rules (table 3) for calculating those products of vectors J_j which are necessary to obtain the tensor matrices L_l.

The next input (table 4) contains the instructions to calculate the partition functions Z_M, Z_A, and Z_H, bearing in mind however, that calculation of the numerical values of the partition functions is not necessary if one's goal is the evaluation of the total chemical amounts. Finally, the instructions for the calculation of total amounts T_M, T_A, and T_H, are given in table 5.

In order to understand the working process, the use of indices in the calculations involving matrix L_4 is reported in table 6, for the minimum com-

Table 1

Chemical model

Complexes: MA_QH_R, $AH_{R'}$ ($R = QR'$) (receptor M, ligand A and proton H bound to A).

$P = 1$			
$0 \le Q \le 8$	MA_Q	class 4	3 sites
		class 5	5 sites
$0 \le R' \le 5$	$AH_{R'}$	class 6	3 sites
		class 7	2 sites
$0 \le R \le 40$	MA_QH_R	class 4 (6, 7)	15 sites
		class 5 (6, 7)	25 sites
Saturated complexes		MA_8H_{40}	
		AH_5	

Table 2

Generating functions and cooperativity functions

Notes: (1) The numerical values of k_j, i_t, and b_j are hypothetical; (2) in columns 2 and 3, we specify both the identity and concentration of free receptor and ligand. At the very beginning, they are assumed to be approximately equal to the total concentrations.

j	[Receptor] [X]	[Ligand] [Y]	Vector J_j				Matrix Γ_j	
			k_j	i_t			b_j	Indices
				p_t	q_t	r_t		
1	[M]	[M]	0	1			0	$(q_1-1)(q_1-2)$
2	[A]	[A]	0		1		0	$(q_2-1)(q_2-2)$
3	[H]	[H]	0			1	0	$(r_3-1)(r_3-2)$
4	[M]	[A]	10^7		3		−0.2	$q_4(q_4-1)$
5	[M]	[A]	10^2		5		−0.2	$q_5(q_5-1)$
6	[A]	[H]	10^7			3	−0.6	$r_6(r_6-1)$
7	[A]	[H]	10^4			2	−0.6	$r_7(r_7-1)$

plex (free receptor M), completely saturated complex and an arbitrary intermediate combination. The expanded display of the elements of the tensor matrix L_4 is reported in table 7. In table 8 the expanded version of the elements of L_5 is also reported for the combinations between A and H.

The information contained in the elements of the tensor matrices must be calculated in order to pass effectively from index space to affinity/cooperativity space. In fact, the combination of indices of any element represents the product of values which can be calculated by repetitive application of eqs. 12 and 13 (cf. eq. 18); each value is calculated for its own index and with the appropriate k_j, b_j, [X], and [Y] taken from table 2. The resulting products give the terms $[(T_M)_{p,\dots q,\dots r,\dots}]$, $[(T_A)_{p,\dots q,\dots r,\dots}]$, and $[(T_H)_{p,\dots q,\dots r,\dots}]$ contributing to the total amounts

Table 3

Tensor matrices L_l

Notes: (1) Each J_j in part a is assumed to be multiplied by its appropriate cooperativity matrix Γ_j; (2) the unprimed index operator O_q is chosen according to the physicochemical model of cooperativity; (3) the index o_q corresponds to the element of O_q.

l
(a) Products
1 J_1
2 J_2
3 J_3
4 $\{J_1\}\{J_4[(O_qJ_6)(O_qJ_7)]\}\{J_5[(O_qJ_6)(O_qJ_7)]\}$
5 $\{J_2\}\{J_6J_7\}$
(b) Elements
1 $\{p-1=0\}$
2 $\{q-1=0\}$
3 $\{r-1=0\}$
4 $\{(p-1)_1q_4\{\{o_qr_6\},\{o_qr_7\}\},q_5\{\{o_qr_6\},\{o_qr_7\}\}\}$
5 $\{(q-1)_2r_6,r_7\}$

Table 4

Partition functions

Notes: (1) $\{l_4\}$ and $\{l_5\}$ are elements of the tensor matrices L_4 and L_5, respectively (see table 3); (2) the limits of the summations are defined by inspection of table 1 (chemical model); (3) for the sake of consistency we have included $[M][M]^{-1}$ and $[A][A]^{-1}$ for $[X][Y]^{-1}$ in the approriate equations.

$$Z_M = \sum_{Q=1}^{Q=8}\sum_{R=0}^{R=40}\{l_4\}[M][M]^{-1}$$

$$Z_A = \sum_{Q=1}^{Q=8}\sum_{R=0}^{R=40}\{l_4\}[M][A]^{-1} + \sum_{Q=1}^{Q=1}\sum_{R=0}^{R=5}\{l_5\}[A][A]^{-1}$$

$$Z_H = \sum_{Q=0}^{Q=8}\sum_{R=1}^{R=40}\{l_4\}[M][H]^{-1} + \sum_{Q=0}^{Q=1}\sum_{R=1}^{R=5}\{l_5\}[A][H]^{-1}$$

Table 5

Total chemical concentrations

Notes: (1) $\{l_4\}$ and $\{l_5\}$ are elements of the tensor matrices L_4 and L_5, respectively (see table 3); (2) the limits of the summations are defined by inspection of table 1 (chemical model).

$$[T_M] = [M] + \sum_{Q=1}^{Q=8} \sum_{R=0}^{R=40} \{l_4\}[M]$$

$$[T_A] = [A] + \sum_{Q=1}^{Q=8} \sum_{R=0}^{R=40} Q\{l_4\}[M] + \sum_{Q=1}^{Q=8} \sum_{R=0}^{R=40} Q\{l_5\}[A]$$

$$[T_H] = [H] + \sum_{Q=1}^{Q=8} \sum_{R=0}^{R=40} R\{l_4\}[M] + \sum_{Q=1}^{Q=8} \sum_{R=0}^{R=40} R\{l_5\}[A]$$

Table 6

Tensor matrix L_4

Note: (1) The intermediate set is a possible combination of indices arbitrarily chosen.

l
1 $\{p-1=0\}$
2 $\{q-1=0\}$
3 $\{r-1=0\}$
4 $\{(p-1)_1, q_4\{(O_q r_6), (O_q r_7)\}, q_5\{(O_q r_6), (O_q r_7)\}\}$
5 $\{(q-1)_2, r_6, r_7\}$

Minimum, maximum, and intermediate elements (indices)

(a) Minimum

4 $\{0_1\}$

(b) Maximum

4 $\{0_1 3_4 3_6 3_6 3_6 2_7 2_7 2_7 5_5 3_6 3_6 3_6 3_6 3_6 2_7 2_7 2_7 2_7 2_7 2_7\}$

(c) Intermediate

4 $\{0_1 2_4 3_6 2_6 0_7 1_7 3_5 1_6 2_6 1_6 2_7 1_7 2_7\}$

Table 7

Tensor matrix L_4, with stoichiometric indices

Notes: (1) The expanded elements group together on the same line p_j and $P = \Sigma p_j, q_j$ and $Q = \Sigma q_j, r_j$ and $R = \Sigma r_j$; (2) the intermediate set is the same combination of indices shown in table 6.

Expanded elements			PQR
(a) Minimum			
$(p-1)$ 0_1			1
(b) Maximum			
$(p-1)$ 0_1			1
q	3_4	5_5	8
r	$3_6 3_6 3_6 2_7 2_7 2_7$	$3_6 3_6 3_6 3_6 3_6 2_7 2_7 2_7 2_7 2_7$	40
(c) Intermediate			
$(p-1)$ 0_1			1
q	2_4	3_5	5
r	$3_6 2_6 0_7 1_7$	$1_6 2_6 1_6 2_7 1_7 2_7$	15

Table 8

Tensor matrix L_5, with stoichiometric indices

	Expanded elements	P	Q	R
Minimum				
$(q-1)$	0_2		1	
Maximum				
$(q-1)$	0_2		1	
r	$3_6 2_7$			5
Intermediate				
$(q-1)$	0_2		1	
r	$1_6 2_7$			3

Table 9

Storage of data for least-squares normal equations

Notes: (1) The headings ∂k_j, ∂b_j, ∂[X] represent $\partial[(T_X)_{p,\ldots q,\ldots r,\ldots}]/\partial k_j$, etc. Note: (2) The table indicates in parentheses the equation whereby the appropriate derivatives are obtained. Each new value corresponding to a different set of indices is added to the total already present in that memory position.

Term	$[T_X]$	∂k_4	∂k_5	∂k_6	∂k_7	∂b_4	∂b_5	βb_6	∂b_7	∂[M]	∂[A]	∂[H]
$[(T_M)_{p,\ldots q,\ldots r,\ldots}]$	$[T_M]$	(25)	(25)	(25)	(25)	(26)	(26)	(26)	(26)	(27)	(28)	(28)
$[(T_A)_{p,\ldots q,\ldots r,\ldots}]$	$[T_A]$	(25)	(25)	(25)	(25)	(26)	(26)	(26)	(26)	(28)	(27)	(28)
$[(T_H)_{p,\ldots q,\ldots r,\ldots}]$	$[T_H]$	(25)	(25)	(25)	(25)	(26)	(26)	(26)	(26)	(28)	(28)	(27)

T_M, T_A, and T_H, respectively. The value of the contributing terms for each total amount can then be used to obtain, by means of eqs. 23–26, the derivatives with respect to the variables under refinement. In the case at hand, we need on the whole 12 addresses for storing the totals for the normal equations of the least-squares process. These totals are collected in table 9, where each cell indicates the numbering of the equation to be used to calculate each coefficient.

4. Conclusions

The application of the partition function algorithm to model cases has confirmed that it is a very potent tool for dealing with the multiple equilibria in solution. The use of index space, parallel to affinity/cooperativity space, renders the expressions relatively simple and suitable for translation into computer language.

An important feature of this algorithm is that it is strictly connected to and controlled by the chemical model. The possible generating function vectors J_j with respective maximum number of sites are selected on the basis of the combinations between components M, A, and H assumed in the complexes $M_P A_Q H_R$. Each vector J_j corresponds to one class of sites and the number of sites can be inferred from chemical and physicochemical information. Self-associating complexes are defined by the model and are promptly transformed into input information by the appropriate J_j vector. The type of cooperativity in self-associating or successive binding complexes, whether restricted within a single unit or extended to every unit, is distinguished by applying the unprimed index operator O_i or the primed index operator $O_{i'}$. If the model supposes competitivity for sites by different ligands, then the appropriate primed operator $O_{(i_r-i)'}$, can be introduced (cf. paper I).

The scheme has been developed for three components M, A, and H, but can be extended to more ligands B, C, etc.

The number of tensor matrices L_l used for the calculation of the total amounts T_M, T_A, and T_H is derived from the number of independent classes of receptors of the chemical model. The limits of the indices in the summations for the calculation of the partition functions and total chemical amounts are chosen by inspection of the number of sites within one class and the combinations of the chemical model. The chemical model also provides the basis for definition of the statistical coefficients. All this means that the method presented here is very useful for assessing any chemical model, either differing in stoichiometry of the complexes, in self-association of the units, or in kind and extension of the cooperativity effect. Therefore, it appears very promising for applications to complicated systems such as those involving binding to biological macromolecules.

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