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

I. Generation of partition functions and mass balance equations

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The thermodynamics of binding of a ligand A and/or proton H to a macromolecule M is treated by the partition function method. In complex systems, the representation of the equilibria by means of cumulative constants β_{PQR} used as coefficients in partition functions Z_M, Z_A, and Z_H is ill-suited to least-squares refinement procedures because the cumulative constants are interrelated by common cooperativity functions $\Gamma_j(i)$ and common site affinity constants k_j. There is therefore the need to express Z_M, Z_A, Z_H as functions of site constants k_j and cooperativity coefficients b_j. This is done by developing an algebra of partition functions based on the following concepts: (i) factorability of partition functions; (ii) binary generating function $J_j = (1 + k_j[Y])^{i_j}$ for each class j of sites, represented by column $\{J_j\}$ and row $[J_j]$ vectors; (iii) cooperativity between sites of one class described by functions $\Gamma_j(i)$, represented by diagonal matrices Γ_j; (iv) probability of finding microspecies represented by elements of tensor product matrix $L_l = \{J_1\}[J_2]$; (v) statistical factors m_{ij} obtained from Newton polynomials, J_j; (vi) power operators $O_{i'}$, $O_{(i-1)'}$, and $O_{(i_l-1)'}$, transforming vectors J_j; and (vii) operators O_i or $O_{(i-1)}$ indicating tensor products of i or $(i-1)$ vectors J_j. Vectors J_j combined in tensors L_l give rise to both an affinity/cooperativity space and a parallel index space. The partition functions Z_M, Z_A, and Z_H and the total amounts T_M, T_A, and T_H can be obtained as an appropriate sum of elements of matrices L_l, each of which is represented in an index space by a combination $p_1, p_2, \ldots q_1, q_2, \ldots r_1, r_2, \ldots$ of indices i_j. From these indices the contribution of that element to partition function Z_M, Z_A, or Z_H and to total amount T_M, T_A, or T_H is calculated in the affinity/cooperativity space as product of factors: $[i_l!/i!(i_l - i)!]k_j^i(\exp[b_j(i-1)i])[X]^i$, i being any index p, q, r and X any component M, A, or H. Future applications of this algorithm to practical problems of macromolecule-ligand-proton equilibria are outlined.

1. Introduction

One of the main problems facing biochemists and biophysical chemists is the interpretation of the thermodynamics of binding of haemoglobin to oxygen and to protons, or of the binding of enzymes or macromolecules to substrate or to protons [1–29]. The experimental methods employed to study such systems include potentiometry, dialysis, spectrophotometry, calorimetry, oxygen pressure control, sedimentation, etc. While experimental data are presented in different ways, the most common representation of such data is as the saturation fraction $\bar{\theta}$ of sites occupied as a function of the logarithm of free ligand concentration. Another popular representation is the Scatchard plot $\bar{n}/[A] = f(\bar{n})$ where $\bar{n}/[A]$ is plotted vs. $\bar{n}$. The formation function $\bar{n}$ is the average number

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of ligands bound per molecule of receptor and [A] is the concentration of free ligand [3]. The value of $\bar{n}$ is usually determined from the ratio of the mass balance of bound A to the complete mass balance of M. The difference between $\bar{\theta}$ and $\bar{n}$ is that $\bar{\theta}$ is a fraction of sites occupied, whereas $\bar{n}$ denotes the number of ligands per receptor molecule.

The interpretation of the experimental data can be carried out following several schemes. Every method tries to reproduce the experimental data using a set of equilibrium constants. The equilibrium constants are expressed either as:
(1) cumulative formation constants $\beta_{PQR} = [M_P A_Q H_R][M]^{-P}[A]^{-Q}[H]^{-R}$ (when $P = 1$ and $R = 0$, only index Q is given, so that $\beta_Q = [MA_Q][M]^{-1}[A]^{-Q}$; or (2) stepwise equilibrium constants, $K_Q = [MA_Q][A]^{-1}[MA_{Q-1}]^{-1}$; or (3) specific site affinity constants k.

The constants chosen are introduced into functions such as the binding isotherm or saturation fraction (given here for a case where $P = 1$ and $R = 0$)

$$\bar{\theta} = \bar{n}/N = \left\{\left(\sum_1^{Q_t} Q\beta_Q[A]^Q\right)/\left(\sum_0^{Q_t}\beta_Q[A]^Q\right)\right\}/Q_t \tag{1}$$

where N is the number of possible binding sites per receptor molecule. The first summation denotes the total concentration of bound ligand A. The second summation represents the total concentration of receptor M which, when multiplied by Q_t, gives the total concentration of binding sites, since, in this simple case, $Q_t = N$ is the number of binding sites per receptor molecule. Alternatively, a function employed is the so-called binding polynomial (allowing for self-association of M):

$$B_M = \sum_1^{P_t}\sum_0^{Q_t} P\beta_{PQ}[M]^{(P-1)}[A]^Q \tag{2}$$

with $\beta_{10} = 1$ and P_t and Q_t being maximum values of P and Q, respectively.

Numerical solutions of these equations have been obtained by means of computer programs [15,26]. In order to achieve acceptable agreement between the observed and calculated values of the functions, eqs. 1 and 2, the introduction of imaginary roots for some β_{PQR} values was proposed. On the other hand, in the field of thermodynamics of complexes between metal cations and small ligands or between ligands and protons, the algorithms used are based almost exclusively on the mass balance equations

$$[T_M] = \sum_1^{P_t}\sum_0^{Q_t}\sum_0^{R_t} P\beta_{PQR}[M]^P[A]^Q[H]^R \tag{3}$$

$$[T_A] = \sum_0^{P_t}\sum_1^{Q_t}\sum_0^{R_t} Q\beta_{PQR}[M]^P[A]^Q[H]^R \tag{4}$$

$$[T_H] = \sum_0^{P_t}\sum_0^{Q_t}\sum_1^{R_t} R\beta_{PQR}[M]^P[A]^Q[H]^R \tag{5}$$

The programs described in refs. 29–33 search for the best values of β_{PQR} by means of least-squares refinements, by minimizing the difference between the observed and calculated values of T_M, T_A, and T_H. Irrespective of the parameters to be refined, the optimization of the parameters is performed in nonlinear least-squares procedures by minimizing the sum of squares of the deviations for all data points

$$U = \sum\left\{(T_{M,o} - T_{M,c})^2 + (T_{A,o} - T_{A,c})^2 + (T_{H,o} - T_{H,c})^2\right\} \tag{6}$$

where $T_{M,c}$, $T_{A,c}$, and $T_{H,c}$ are values calculated from the approximate starting values of the parameters, with $T_{M,o}$, $T_{A,o}$, and $T_{H,o}$ being those observed.

At the end of the refinement, one obtains

$$\sigma = (U/3(n-m))^{1/2} \tag{7}$$

where n is the number of data points and m denotes the number of β values calculated, giving the average standard deviation in the amount of each component. Alternatively, other experimental data, e.g., pH values, can be fitted.

In the equilibria with small molecules, the cumulative constants can have only real values and if the model is correct there is no need for imaginary roots to achieve agreement between observed and calculated data. These computer pro-

grams often fail when applied to equilibria with receptors possessing several binding sites. These difficulties are commonly due to interrelations between equilibrium constants (the cooperativity effect) and/or self-association. It is the purpose of the present paper to develop a procedure for the treatment of data which should overcome the difficulties encountered by biochemists in handling systems with macromolecules and by chemists in handling systems involving small molecules with multiple binding sites.

2. Partition functions, cooperativity functions and site constants

As described in our previous publications, it is useful for the development of this treatment to define partition functions [35–38]:

$$Z_M = \sum_{P=1}^{P_t} \sum_{Q=0}^{Q_t} \sum_{R=0}^{R_t} \beta_{PQR}[M]^{(P-1)}[A]^Q[H]^R \quad (8)$$

$$Z_A = \sum_{P=0}^{P_t} \sum_{Q=1}^{Q_t} \sum_{R=0}^{R_t} \beta_{PQR}[M]^P[A]^{(Q-1)}[H]^R \quad (9)$$

$$Z_H = \sum_{P=0}^{R_t} \sum_{Q=0}^{Q_t} \sum_{R=1}^{R_t} \beta_{PQR}[M]^P[A]^Q[H]^{(R-1)} \quad (10)$$

each of which gives the total concentration of species divided by the free M, or A, or H, respectively. These values are proportional to the probability of finding in the unit volume any species containing M, A, or H, respectively. Each term of Z_M equals the ratio of concentrations $[M_P A_Q H_R]/[M]$, which in turn is equal to $\beta_{PQR}[M]^P[A]^Q[H]^R$. Similarly, for Z_A the ratio of concentrations is $[M_P A_Q H_R]/[A]$ which equals $\beta_{PQR}[M]^P[A]^{(Q-1)}[H]^R$ and for Z_H the ratio of concentrations is $[M_P A_Q H_R]/[H]$ which equals $\beta_{PQR}[M]^P[A]^Q[H]^{(R-1)}$. The terms $\beta_{100} = [M]/[M] = \beta_{010} = [A]/[A] = \beta_{001} = [H]/[H] = 1$. It is important to note that in aqueous solution, R can be negative, because of hydrolysis.

The relationships between partition functions and total concentrations have been shown [38] to be:

$$[T_M] = [M]\{Z_M + [M]\partial Z_M/\partial[M]\} \quad (11)$$

$$[T_A] = [A]\{Z_A + [A]\partial Z_A/\partial[A]\} \quad (12)$$

$$[T_H] = [H]\{Z_H + [H]\partial Z_H/\partial[H]\} \quad (13)$$

The product $[M]Z_M$ gives the total concentration of species containing M. The product $[M]^2\partial Z_M/\partial[M]$ adjusts the total for the stoichiometric factor to yield $[T_M]$. Eqs. 11–13 are generally valid even in the case of the self-association of macromolecule M or ligand A.

For simple cases where $P = 1$ and $R = 0$ (i.e., the complexation of one or more ligands A to one receptor M), the partition function,

$$Z_M = 1 + \sum_{Q=1}^{Q} \beta_Q[A]^Q \quad (14)$$

is related to the formation function $\bar{n}$ of Bjerrum [39]

$$\begin{aligned}\bar{n} &= \left([MA] + 2[MA_2] + \ldots Q_t\left[MA_{Q_t}\right]\right)/ \\ &\quad \left([M] + [MA] + \ldots\left[MA_{Q_t}\right]\right) \\ &= ([T_A] - [A])/[T_M] \\ &= \partial \ln Z_M/\partial \ln[A] \end{aligned} \quad (15)$$

The Bjerrum function $\bar{n}$ is useful when plotted against ln[A] in that the resulting plot, taken from raw experimental data $[T_A]$, $[T_M]$ and [A], provides insight into the kinds of complexes which are formed. Specifically, when plots are constructed at different values of $[T_M]$, it is possible to distinguish between mononuclear and polynuclear (self-association) reactions. The area on the Bjerrum plot under the curve of $\bar{n}$ vs. ln[A] is proportional to the free energy of formation of the complexes described by Z_M. The cumulative constant β_{Q_t} of the completely saturated macromolecule can be determined and factorized as the product of stepwise formation constants:

$$\beta_{Q_t} = \prod_{1}^{Q_t} K_Q \quad (16)$$

Hence, the standard free energy of formation of

this saturated complex can be expressed as the sum of stepwise partial molar quantities, viz., the chemical potentials

$$\Delta\mu_Q^{\varnothing} = -RT\ln K_Q \tag{17}$$

Any cooperativity effect, either positive or negative, can be determined from the variation of stepwise constants. One can calculate the ratios between successive constants

$$K_Q/K_{Q-1} = K_{\gamma_Q}s \tag{18}$$

where s is a statistical factor and K_{γ_Q} denotes the cooperativity factor for that case. Alternatively, one can calculate the ratios between the geometric means of constants $\beta_Q^{1/Q}$ and affinity constants β_1 for binding of the first unperturbed ligand

$$\beta_Q^{1/Q}/\beta_1 = \gamma_Q s \tag{19}$$

In this approach, γ_Q represents a geometric average cooperativity factor. We have found the latter cooperativity factor γ_Q to be more useful than K_{γ_Q} in interpreting the behavior of systems with small molecules [38,40].

Ratios between constants $\beta_Q^{1/Q}$ correspond to differences between areas on the Bjerrum plot. These differences are expressed as

$$\Delta\mu_{\gamma_Q} = (1/Q)\left(\sum_{Q=1}^{Q}\Delta\mu_Q^{\varnothing}\right) - \Delta\mu_1^{\varnothing} + \Delta\mu_s \tag{20}$$

and provide a measure of the cooperativity effect. Upon examination of the experimental data, it is found for many compounds that such differences turn out to be linear functions of $(Q-1)$. Thus, we refer to the existence of cooperativity functions, $\Gamma(Q)$ of the form

$$\lg\Gamma(Q) = a + b(Q-1) \tag{21}$$

where a and b are derived empirically. For protonation of A or M the cooperativity functions are given by

$$\lg\Gamma(R) = a + b(R-1) \tag{22}$$

The value of the cooperativity function $\Gamma(Q)$ at point Q is henceforth designated as the cooperativity factor, γ_Q.

The validity of the cooperativity function approach has been tested on Scatchard plots in which $\bar{n}/[A]$ is plotted vs. $\bar{n}$. When experimentally determined cumulative constants are used in systems which are affected by cooperativity, nonlinear Scatchard plots (concave upward or downward) are obtained. In the case for the binding of A to three sites on M ($P_t = 1$, $Q_t = 3$, $R_t = 0$), the following holds true:

$$\bar{n}/[A] = \left(\beta_1 + 2\beta_2[A] + 3\beta_3[A]^2\right)/ \left(1 + \beta_1[A] + \beta_2[A]^2 + \beta_3[A]^3\right) \tag{23}$$

If the β_Q values are corrected for values of the cooperativity coefficients γ_Q calculated from eq. 21

$$\bar{n}_{corr}/[A] = \frac{\beta_1 + 2\beta_2/\gamma_2^2[A] + 3\beta_3/\gamma_3^3[A]^2}{1 + \beta_1[A] + \beta_2/\gamma_2^2[A]^2 + \beta_3/\gamma_3^3[A]^3} \tag{24}$$

linear Scatchard plots are obtained. The site affinity constant k is given by the slope of the line. The slopes b in eqs. 21 and 22 are amenable to physicochemical interpretation. In benzene polycarboxylic acids, the slope is proportional to the charge density of the base [40]. If a proton is added to a base bearing a positive charge the cooperativity effect clearly indicates repulsion [40]. The procedure is applicable even when the receptor possesses different classes j of sites for the same ligand.

The existence of cooperativity functions $\Gamma(Q)$ and $\Gamma(R)$ explains why the application of least-squares methods to the determination of constants fails when there are several sites. The parameters β_{PQR} to be refined in the mass balance equations are not independent from one another. Specifically, the constants β depend on the site constants k and on the values of the coefficients a and b in eqs. 21 and 22. Usually, the value of a is close to zero. On the other hand, partition functions depend on cumulative constants. Therefore, partition functions Z_M, Z_A, and Z_H can simply be expressed as functions of site binding constants k_j and coefficients b_j of the cooperativity functions of several classes j of sites. These partition func-

tions are written in the form (note the use of lower-case indices in this case):

$$Z_M = 1 + \sum_q \sum_r m_q k_{MA}^q \exp\{b(q-1)q\}[A]^q \times m_r k_{MH}^r \exp\{b(r-1)r\}[H]^r \quad (25)$$

when only one class of MA interactions and one of MH interactions are present. The same can be written for Z_A and Z_H. The coefficients m_q and m_r represent statistical correction factors. Indices p, q and r (or in general i) represent the number of ligands bound within a class. These define single terms contributing to the cumulative constants β to be introduced, as shown below, into the mass balance equations (eqs. 11–13). In this particular case, values of k_{MA}, k_{AH}, b_A, and b_H, can be assigned as parameters to be optimised in a least-squares process. By using this procedure the introduction of imaginary roots for β_{PQR} is avoided.

Simple model molecules can be chosen for which the values of site constants and cooperativity functions depend on the same kinds of electrostatic, inductive, steric and solvent effects acting on the macromolecules. Therefore, they can be used to obtain initial values in the least-squares refinement process for large molecules.

3. Partition functions, vectors and tensors

The generation of partition functions for simple systems is relatively straightforward. However, for binding to complex molecules, the mathematics is rather involved. The remainder of this paper is devoted to developing a method for the generation of partition functions for the general case.

With the aim of preparing a program to compute mass balance equations as functions of site constants and cooperativity functions represented by k_j and Γ_j, we present an algebra of partition functions going through a series of examples of increasing complexity.

The construction of the total partition functions is based on the following concepts:

(i) The probability of occupation for a group consisting of more than one class of sites is proportional to the product of the probabilities of occupation of each class (factorability of partition functions).

(ii) Each binary generating function J_j expresses the probability with each class and can be represented by column $\{J_j\}$ and row $[J_j]$ vectors.

(iii) The cooperativity effect is expressed by functions $\Gamma_j(i)$, which are introduced to modify the generating functions by means of diagonal matrices Γ_j.

(iv) The product of two binary generating functions J_1 and J_2 is represented by tensor products, $L_I = \{J_1\}[J_2]$.

(v) Statistical factors m_{p_j}, m_{q_j}, or m_{r_j} (or in general m_{i_j}) from Fermi-Dirac statistics are used within the binary generating function and therefore appear in their products.

(vi) Power operators O transform vectors J_j according to certain rules which depend on the chemical model which is chosen.

We need a notation for indices to define the terms of the partition functions, and designate them using lower-case letters p_{1_j}, $p_{2_j}, \ldots, q_{1_j}$, $q_{2_j}, \ldots, r_{1_j}$, $r_{2_j}, \ldots$, where p, q, and r represent individual sites and subscript j is used to differentiate between classes of sites. The value of j indicates the position of that class of sites in the list of binary generating functions. The relationship of the indices to P, Q, and R of the cumulative constants β_{PQR} is in general $P = \sum p_j$, $Q = \sum q_j$, and $R = \sum r_j$. The cumulative constants, however, are the sum of terms with the same $\sum p_j$, $\sum q_j$, and $\sum r_j$, respectively, but obtained with every combination of single values, e.g., $R = 4$ for two classes $j = 1$ and $j = 2$ with three sites of class 1 and four sites of class 2 is formed by terms (r_1, r_2) labelled (0,4), (1,3), (2,2), and (3,1). In chemical terms, indices referred to by capitals define species with a given stoichiometry, whereas lower-case letters differentiate the microspecies.

The binary generating functions can be used to obtain the complete partition functions Z_M, Z_A, and Z_H by combining them using tensor algebra. Each generating function J_j corresponds to one class j of p_{i_j}, q_{i_j}, or r_{i_j} (or in general i_{i_j}) binding sites between a receptor X (X = M, A, H) and a ligand Y (Y = M, A, H).

Each generating function J_j is given by a power i_{t_j} of the binomial

$$J_j = (1 + k_j[\mathrm{Y}])^{i_{t_j}} \tag{26}$$

which corresponds to Z_X for the case where no cooperativity effect exists and there is only one class of sites. Here, i_{t_j} is the total number of sites within each class j. The resulting polynomial consists of $(i_{t_j} + 1)$ terms and is represented as a whole by a one-column vector $\{J_j\}$ whose terms are obtained from eq. 26 and labelled by indices i_j or a row vector $[J_j]$ obtained and labelled in the same way. For example, the row vector representing the binary generating function for three sites is expressed as:

$$[J_j] = [1, 3k[\mathrm{Y}], 3k^2[\mathrm{Y}]^2, k^3[\mathrm{Y}]^3] \tag{27}$$

with indices 0_j, 1_j, 2_j, and 3_j, respectively. These vectors can also be considered as first-order tensors. Ideally, the total partition function could be obtained as $(i_t - 1)$ tensor products of the simplest binomial $(1 + k[\mathrm{Y}])$, but this is not always appropriate (see the appendix). Rather, in some cases a cooperativity function $\Gamma(i_j)$ must be introduced, which makes the expressions for the binary generating function and tensor product not equivalent. Indeed, these expressions correspond to different physicochemical hypotheses, as shown below. A detailed explanation concerning tensor powers and successive tensor products is provided in the appendix.

Multiplication of two generating functions is carried out in the process of developing the total partition function and can be represented by a tensor product

$$L_l = \{J_1\}[J_2] \tag{28}$$

where l is a number in a list of matrices. The labelling $L_{1,2}$, according to the component vectors, is sometimes used instead of l. In this way, we obtain a second-order tensor, represented by a matrix with elements $\{i_1, i_2\}$ of dimensions $i_1 = 2$ and $i_2 = 3$, as shown by this relatively simple example, in which the cooperativity effect is ignored (i.e., it is assumed that the sites are independent and that the site constant is valid for all binding sites):

$$L_l = \{J_1\}[J_2] = \begin{bmatrix} 1 \\ 2k_1[\mathrm{Y}] \\ k_1^2[\mathrm{Y}]^2 \end{bmatrix} [1, 3k_2[\mathrm{Y}], 3k_2^2[\mathrm{Y}]^2, k_2^3[\mathrm{Y}]^3] \tag{29}$$

The products yields the following matrix

i_2 / i_1	0	1	2	3
0	1	$3k_2[\mathrm{Y}]$	$3k_2^2[\mathrm{Y}]^2$	$k_2^3[\mathrm{Y}]^3$
1	$2k_1[\mathrm{Y}]$	$2k_1 3k_2[\mathrm{Y}]^2$	$2k_1 3k_2^2[\mathrm{Y}]^3$	$2k_1 k_2^3[\mathrm{Y}]^4$
2	$k_1^2[\mathrm{Y}]^2$	$k_1^2 3k_2[\mathrm{Y}]^3$	$k_1^2 3k_2^2[\mathrm{Y}]^4$	$k_1^2 k_2^3[\mathrm{Y}]^5$

(30)

The above representation might be used in a case where J_1 and J_2 are the only generating functions describing a chemical system, with receptor X = M and ligands $\mathrm{Y}_1 = \mathrm{Y}_2 = [\mathrm{A}]$, which is binding in two different classes or ways. In this case, each element of the matrix is a term of the total partition function Z_M, which is obtained as the following sum of all the elements

$$Z_M = \sum_{Q=0}^{Q_t} m_{q_1} k_1^{q_1} m_{q_2} k_2^{q_2} [\mathrm{A}]^{q_1} [\mathrm{A}]^{q_2} \tag{31}$$

where $Q = q_1 + q_2$ and thereby combines all the permutations of the q values. For example, the term in the sum, eq. 31, corresponding to $Q = 2$ is in turn the sum of the diagonal elements in the matrix, eq. 30, for which the power of [Y] is 2. The other partition function Z_A can be obtained from the matrix (eq. 30) by multiplying each term by [M] and dividing by [A]. The term (0,0) remains unchanged in value at unity.

If more than two generating functions are needed to construct the partition functions, the successive tensor products are applied as described in the appendix.

4. Affinity-cooperativity space and index space

In order to introduce the cooperativity effect, the elements of the matrix binary generating functions J_1 and J_2, when multiplied by the respective

cooperativity factors, are equivalent to the terms of the polynomials

$$J_{\gamma_1} = (1 + k_1\gamma_{1,i}[\mathrm{A}])^{i_{i_1}} \tag{32}$$

$$J_{\gamma_2} = (1 + k_2\gamma_{2,i}[\mathrm{A}])^{i_{i_2}} \tag{33}$$

The binary generating functions J_{γ_1} and J_{γ_2} refer to J_1 and J_2 corrected for the cooperativity effect, respectively. Note that the values of the cooperativity function $\Gamma_j(i_j)$ are different for each value of i_j and are given by $\gamma_{j,i}$. The terms of the polynomials J_{γ_j} are obtained from vectors $\{J_j\}$ by vector multiplication with a diagonal matrix $\Gamma_j(i_j)$, whose elements are given by $\gamma^i_{j,i} = \exp(b_j i_j(i_j - 1))$. It should be borne in mind that the number of elements or dimension of the matrix Γ_j is the same as the number of elements in vector J_j, namely $(i_{t_j} + 1)$. In doing so, we obtain

$$J_j\Gamma_j(i_j) = J_{\gamma_j} = \left\{ m_{t_j} k_j^i [\mathrm{Y}]^i \exp\left(i_j(i_j - 1)b_j\right)\right\} \tag{34}$$

where

$$m_{i_j} = i_{t_j}!/i_j!(i_{t_j} - i_j)! \tag{35}$$

The elements of the matrix (tensor) L_I, obtained by tensor multiplication of vectors J_{γ_1} and J_{γ_2}, can be considered as being the points of a space (affinity and cooperativity space) (fig. 1), where the coordinates along the spatial axes are the elements of the vectors J_{γ_1} and J_{γ_2}. The single points within the affinity and cooperativity space (a.c.s.) are elements of the matrix given by the vector products of the respective coordinate elements. Each cell of a.c.s. which contains an element can be labelled by the two indices of the component terms of their generating coordinates. Therefore, we can imagine an index space parallel to the a.c.s. and providing index information about the positions in the matrices of the various terms. This principle is illustrated in fig. 1, where only the coordinates of the a.c.s. are shown (e.g., $2k_1\gamma_{1,1}$) along with the index space superimposed to show the i_j indices corresponding to the rows and colums of a.c.s. elements. For example, the indices of coordinates $2k_1\gamma_{1,1}$ are 1,0. Note that the individual elements of the space are missing from fig. 1. Fig. 2 shows the elements of the space added.

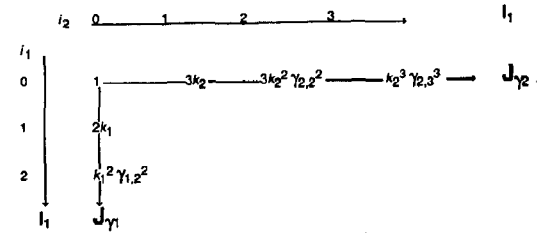

Fig. 1. Affinity and cooperativity space (a.c.s.) with axes J_{γ_1} and J_{γ_2} and index space (i.s.) with axes I_1 and I_2. The cooperativity factors are $\gamma^i_{j,i} = \exp[i(i-1)b_j]$.

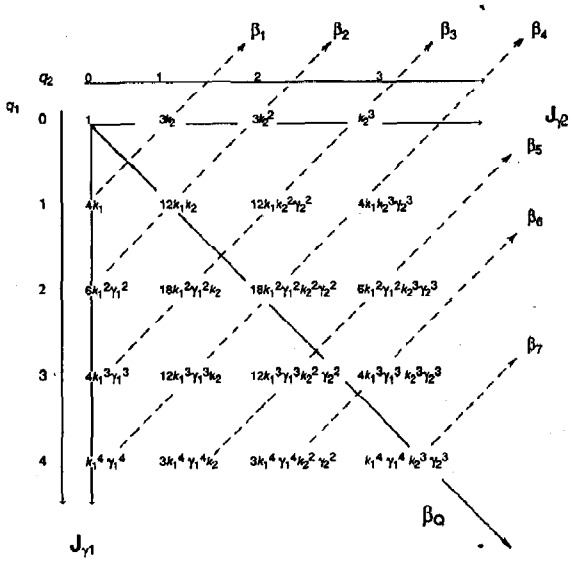

Fig. 2. Transformation from site constants k_j to cumulative constants β_Q in affinity and cooperativity space. J_{γ_1} and J_{γ_2} are the original axes, β_Q the transformed axis. $\beta_Q = \sum$ (elements of Q-th diagonal).

The tensor algebra described above is useful in demonstrating how the cumulative constants β_{PQR} are unsuitable for describing the binding equilibria because they represent a reference frame of nonorthogonal axes. The relationship between the site constants k_j and β_{PQR} can be visualized from fig. 2 as a transformation of axes J_{γ_1} and J_{γ_2} to axis β_Q. By transforming the axes in fig. 2 from J_{γ_1} and J_{γ_2} to the unique diagonal axis β_Q, we obtain a new reference frame where each coordinate along the β_Q axis equals the sum of the terms on the

diagonal of the original reference frame. For example,

$$\beta_2 = 6k_1^2 + 12k_1k_2 + 3k_2^2\gamma_{2,2}^2 \quad (36)$$

The principles of the pattern recognition or factor analysis [41] state that the proper solution of a problem depends on the appropriate rotation and/or translation of axes, so as to obtain the projection where the dominant factors are clearly separated (or orthogonality is achieved). In fact, each term or cell along the diagonal perpendicular to the unique β_Q axis is a function of different powers of k_1 and k_2, whereas the J_{γ_1} and J_{γ_2} axes represent the separate effects k_1 and k_2. Note that, when one multiplies each term by the value of the appropriate cooperativity factor $\gamma_{j,i}$, the bias of coordinates and their unsuitability as independent parameters to be refined in a least-squares process become even more apparent. Therefore we can recognize that:

(i) the representation of the system with reference to the cumulative constants β_{PQR} is not suitable for the identification of the physicochemical parameters controlling the equilibria; and

(ii) the reference frame of site affinity binary generating functions J_{γ_1}, J_{γ_2}, if correctly chosen, satisfies the necessary orthogonality conditions.

5. Power operators

Complicated situations arise when compounds containing three components are formed, for example, when combination takes place between a receptor M and a ligand A, which is in turn the receptor for a ligand H. If M has two sites and A three, compounds with stoichiometric composition $M(AH_3)_2$ are possible, which increases the number of binding sites for H from three to six. The statistics and cooperativity must be expressed and calculated accordingly. Analogous situations arise when self-association of macromolecule M occurs. In this case, each additional M binding to the first one can be considered as a different ligand, thus producing ternary compounds with stoichiometry $MA_q(MA_q)_{(p-1)}$.

In order to resolve such problems, we introduce special mathematical operators. In the process of obtaining the tensor products $\{J_1\}[J_2]$, we use a vector power operator $O_{i'}$ (or $O_{(i-1)'}$) with a primed index to expand the second vector J_2 prior to the multiplication which is executed element by element. In this way, the statistics associated with all the possible combinations of sites is accounted for, yielding according to index i_1 of the first vector, $i_2' = i_1 \cdot i_{t_2}$ sites. In the case of self-associating components, the primed index of the expanded vector $J_{2'}$ becomes $i_2' = [(i_1 - 1) + 1] \cdot i_{t_2}$ because of the operator $O_{(i-1)'}$. Note that in this case the expression for $i_{2'}$ contains $(i_1 - 1)$ indicating the term of the generating function $J_1 = (1 + k_1[Y])^{(i_1-1)}$.

The transformation of J_2 indicated by $O_{i'}$ yields the transformed row vector $J_{2'}$, according to the rule defining the operator. This rule is a function of the index i_1 of the element of the column vector J_1 (or J_{γ_1}). Then, the transformed vector $J_{2'}$, transposed to a column vector, can be multiplied by the cooperativity matrix $\Gamma_2(i')$, where the index i' is that of the transformed vector. The sequence can be written

$$\{O_{i'}[J_2]\}\Gamma_2(i') = \{J_{\gamma_{2'}}\} \quad (37)$$

Finally, the tensor multiplication produces the matrix

$$L_{1,2} = \{J_{\gamma_1}\}\{J_{\gamma_{2'}}\}^{\mathrm{T}} = \{J_{\gamma_1}\}[J_{\gamma_{2'}}] \quad (38)$$

or, for the entire transformation written in compact form

$$L_l = \{J_{\gamma_1}\}[\{O_{i'}[J_2]\}\Gamma_2(i')] \quad (39)$$

L_l (or $L_{1,2}$) is a triangular matrix (lower left triangular).

For example, we suppose that the abovementioned combination between receptor M and ligand A is described by vector J_1 with sites $i_{t_1} = q_l = 2$ without cooperativity and the combination between receptor A and ligand H by vector J_2, with sites $i_{t_2} = r_l = 3$. The latter vector is modified, before multiplication with each element of index i_1 of the former vector, by the power operator $O_{i'}$, which has the meaning: 'raise the polynomial J_2 to the i_1-th power before applying the cooperativity function $\Gamma_2(r')$, where r' is the index of the expanded vector'. The following example illustrates this point:

$$\boldsymbol{L}_{1,2} = \boldsymbol{J}_1 \boldsymbol{O}_{i'} \boldsymbol{J}_2 = \begin{bmatrix} 1 \\ 2k_1[\mathrm{A}] \\ k_1^2[\mathrm{A}]^2 \end{bmatrix} \left[\left\{ \boldsymbol{O}_{i'} \left[1 \; 2k_2[\mathrm{H}] \; k_1^2[\mathrm{H}]^2 \right] \right\} \boldsymbol{\Gamma}_2(r') \right] \tag{40}$$

$$= \begin{bmatrix} 1 & [1] & & & \\ (2k_1[\mathrm{A}]) & [1 \; 2k_2[\mathrm{H}] & k_2^2\gamma_{2,2}^2[\mathrm{H}]^2] & & \\ (k_1^2[\mathrm{A}]^2) & [1 \; 4k_2[\mathrm{H}] & 6k_2^2\gamma_{2,2}^2[\mathrm{H}]^2 & 4k_2^3\gamma_{2,3}^3[\mathrm{H}]^3 & k_2^4\gamma_{2,4}^4[\mathrm{H}]^4] \end{bmatrix} \tag{41}$$

giving the matrix

$r \rightarrow$ / $q \downarrow$	0	1	2	3	4
0	1				
1	$2k_1[\mathrm{A}]$	$2k_1[\mathrm{A}]2k_2[\mathrm{H}]$	$2k_1[\mathrm{A}]k_2\gamma_{2,2}^2[\mathrm{H}]^2$		
2	$k_1^2[\mathrm{A}]^2$	$k_1^2[\mathrm{A}]^2 4k_2[\mathrm{H}]$	$k_1^2[\mathrm{A}]^2 6k_2^2\gamma_{2,2}^2[\mathrm{H}]^2$	$k_1^2[\mathrm{A}]^2 4k_2^3\gamma_{2,3}^3[\mathrm{H}]^3$	$k_1^2[\mathrm{A}]^2 k_2^4\gamma_{2,4}^4[\mathrm{H}]^4$

(42)

The matrix, eq. 42, defines every microspecies with principal receptor M.

Actually, in the combinations between M, A, and H, there are two ways of combining the generating functions, corresponding to two different physical situations. The first is to assume that the cooperativity effect among binding Hs is independent of which A serves as the receptor, and this is the case considered in eqs. 37–42. The second case describes the situation where the binding of H to A is affected by cooperativity effects acting only among H binding to the same A. In this case we use a tensor power operator $\boldsymbol{O}_i$ in the tensor product $\boldsymbol{J}_{\gamma_1} \boldsymbol{O}_i \boldsymbol{J}_{\gamma_2}$, where $\boldsymbol{O}_i$ with unprimed index signifies: 'at each element of index i_1 of $\boldsymbol{J}_{\gamma_1}$, calculate the i_1-th tensor power of $\boldsymbol{J}_{\gamma_2}$ after the cooperativity function $\boldsymbol{\Gamma}_2(r)$ has been applied to $\boldsymbol{J}_2$'. Note that the unprimed index r is that of the unmodified vector. In compact notation, the whole set of operations can be written in comparison with eq. 39

$$\boldsymbol{L}_{1,2} = \{ \boldsymbol{J}_{\gamma_1} \} \left[\boldsymbol{O}_i \{ \{ \boldsymbol{J}_2 \} \boldsymbol{\Gamma}_2(i) \} \right] \tag{43}$$

In the example given of the mentioned combination between M, A, and H, in which cooperativity is restricted within each unit AH_R, the resulting matrix, $\boldsymbol{L}_{1,2}$, in comparison to eq. 42, has row $q = 2$ transformed into submatrix

$$k_1^2[\mathrm{A}]^2 \times \begin{bmatrix} 1 & 2k_2[\mathrm{H}] & k_2^2\gamma_{2,2}^2[\mathrm{H}]^2 \\ 2k_2[\mathrm{H}] & 4k_2^2\gamma_{2,2}^2[\mathrm{H}]^2 & 2k_2^3\gamma_{2,2}^2[\mathrm{H}]^3 \\ k_2^2\gamma_{2,2}^2[\mathrm{H}]^2 & 2k_2^3\gamma_{2,2}^2[\mathrm{H}]^3 & k_2^4\gamma_{2,2}^2[\mathrm{H}]^4 \end{bmatrix} \tag{44}$$

The difference between $\boldsymbol{O}_{i'}$ and $\boldsymbol{O}_i$ centers on the point at which we introduce the cooperativity function, using the cooperativity matrix $\boldsymbol{\Gamma}_2(i)$ which alters each element of vector $\boldsymbol{J}_2$ according to information given by the index of the element of $\boldsymbol{J}_1$. In physical terms, the primed index operator $\boldsymbol{O}_{i'}$ is used when the cooperativity effect and statistics act over all the ligands bound to q units of A, irrespective of which particular A is involved; therefore, the matrix $\boldsymbol{\Gamma}_2(i')$ modifies each term of the modified polynomial $J_{2'} = (1 + k[\mathrm{H}])^{q \cdot r_1}$, after expansion by $\boldsymbol{O}_{i'}$.

On the other hand, the use of the unprimed index operator $\boldsymbol{O}_i$ indicates that cooperativity occurs among Hs bound to the same A; therefore, the cooperativity matrix $\boldsymbol{\Gamma}_2(i)$ modifies the terms of $\boldsymbol{J}_2$ prior to modification of $\boldsymbol{J}_2$ by $\boldsymbol{O}_i$. Note in particular that, in the example of the final terms of eqs. 42 and 43, $\gamma_{2,4}^4 \neq \gamma_{2,2}^2 \gamma_{2,2}^2$, because the value of the cooperativity factor $\gamma_{j,i}$ changes with index i.

The same choice concerning cooperativity can be made in self-associating complexes. In one case, the cooperativity effects are considered to extend over all the ligands in any particular self-associating unit, and in this treatment, the vector operator $\boldsymbol{O}_{(i-1)'}$ with primed index is chosen. Alternatively, a model can be assumed that considers the cooperativity effect to be restricted within each monomeric unit of the self-associating assembly, and in this case the operator $\boldsymbol{O}_{(i-1)}$ with unprimed index is applied.

Another case for the use of a vector power operator is $\boldsymbol{O}_{(i_t-i)'}$, which is used for the solution of the possibility in which A and H compete for the same sites on the receptor M. The result of $\boldsymbol{O}_{(i_t-i)'}$ is the contraction of the second generating function J_2. This is shown in the following example.

If the generating functions are J_1 with index $i_1 \equiv q$ for the binding of M and A and J_2 with index $i_2 = r$ for the binding of M and H, then the tensor product is obtained as in the following example where M has three sites:

$$\boldsymbol{L}_{1,2} = \{\boldsymbol{J}_1\}\left[\left\{\boldsymbol{O}_{(i_t-i)'}[\boldsymbol{J}_2]\right\}\boldsymbol{\Gamma}_{j_2}(i')\right] \tag{45a}$$

$$= \begin{bmatrix} 1 \\ 3k_1[\mathrm{A}] \\ 3k_1^2[\mathrm{A}]^2 \\ k_1^3[\mathrm{A}]^3 \end{bmatrix} \boldsymbol{O}_{3-q}\left[(1+k_2[\mathrm{H}])^{3-q}\right]\boldsymbol{\Gamma}_{j_2}(q')] \tag{45b}$$

where the primed index i' is that of the contracted vector $\boldsymbol{J}_{2'}$, giving the following upper triangular matrix:

r / q	0	1	2	3
0	1	$3k_2[\mathrm{H}]$	$3k_2^2\gamma_{2,2}^2[\mathrm{H}]^2$	$k_2^3\gamma_{2,3}^3[\mathrm{H}]^3$
1	$3k_1[\mathrm{A}]$	$3k_1[\mathrm{A}]2k_2[\mathrm{H}]$	$3k_1[\mathrm{A}]k_2^2\gamma_{2,2}^2[\mathrm{H}]^2$	
2	$3k_1^2[\mathrm{A}]^2$	$3k_1^2[\mathrm{A}]^2k_2[\mathrm{H}]$		
3	$k_1^3[\mathrm{A}]^3$			

(46)

Tensors of higher order (see the appendix) are obtained with multicomponent complexes, and are represented by multidimensional matrices of matrices.

6. Calculation of partition functions and mass balance equations

The number and type of vectors and tensors necessary for the calculation of partition functions and mass balance equations depend on the chemical model assumed. For a case where complexes of the type MA_Q, MH_R, and $\mathrm{MA}_Q\mathrm{H}_R$ all form, we need three vectors, $\boldsymbol{J}_1$–$\boldsymbol{J}_3$, to determine whether self-association occurs. If self-association does not take place as in the present model, these vectors are given null constants, k_1–k_3. If the complexes MA_Q and MH_R each have two classes of sites, four other vectors are needed, namely, $\boldsymbol{J}_4$, $\boldsymbol{J}_5$, $\boldsymbol{J}_6$ and $\boldsymbol{J}_7$, corresponding to the indices q_4, q_5, r_6 and r_7, respectively. If no complexes of the type AH_R are formed, according to the hypothetical chemical reaction scheme, then we need only one matrix, $\boldsymbol{L}_1$, to calculate tensor products, since they are all of the form having a single M reacting with A, H, or both. From $\boldsymbol{L}_1$, the partition functions, expressed in index space, are

$$Z_{\mathrm{M}} = 1 + \sum_{Q+R=1}^{Q_t+R_t} \{q_4q_5r_6r_7\}[\mathrm{M}][\mathrm{M}]^{-1} \tag{47}$$

$$Z_{\mathrm{A}} = 1 + \sum_{Q=1}^{Q_t}\sum_{R=0}^{R_t} Q\{q_4q_5r_6r_7\}[\mathrm{M}][\mathrm{A}]^{-1} \tag{48}$$

$$Z_{\mathrm{H}} = 1 + \sum_{Q=0}^{Q_t}\sum_{R=1}^{R_t} R\{q_4q_5r_6r_7\}[\mathrm{M}][\mathrm{H}]^{-1} \tag{49}$$

In a similar fashion, from $\boldsymbol{L}_1$ the mass balance equations expressed in index space are obtained as:

$$[\mathrm{T}_{\mathrm{M}}] = [\mathrm{M}] + \sum_{Q+R=1}^{Q_t+R_t} \{q_4q_5r_6r_7\}[\mathrm{M}] \tag{50}$$

$$[T_A] = [A] + \sum_{Q=1}^{Q_t} \sum_{R=0}^{R_t} Q\{q_4 q_5 r_6 r_7\}[M] \quad (51)$$

$$[T_H] = [H] + \sum_{Q=0}^{Q_t} \sum_{R=1}^{R_t} R\{q_4 q_5 r_6 r_7\}[M] \quad (52)$$

The combination of indices in the brackets in eqs. 47–52 indicates the product of four factors, each of which is of the form

$$\left[i_t!/i_j!(i_t - i_j)!\right] k_j^{i_j} \left(\exp\left[b_j(i_j - 1)i_j\right]\right)[X]^{i_j} \quad (53)$$

Note that for each index $i_j = 0$, the corresponding factor equals 1.

It is significant that each term contributing to the mass balance equations is analytically derivable with respect to the variables to be refined, namely, k_j, b_j, [M], and [A], if [H], $[T_M]$, $[T_A]$, and $[T_H]$ have been experimentally determined.

More details of the procedures to be followed will be presented in the following article [42].

7. Conclusions

The partition function represents a very efficient mathematical algorithm for dealing with many kinds of equilibria in solution. The existence of cooperativity effects within classes of sites and the inadequacy of cumulative or Adair constants to describe the equilibria have previously inhibited the study of many important systems. The introduction of imaginary roots [15,26] does not seem a convincing answer to this problem. In contrast, the classes of sites with specific cooperativity effects are strictly connected with the concept of cooperons introduced by Brunori et al. [28] and should be verifiable by computer. The development of the partition function method through cooperativity coefficients and site affinity constants may provide an answer to many of the questions currently under debate in solution thermodynamics.

Examples of the kinds of problems to which this method may be applied include reexamination of the protonation of ribonuclease [43], protonation of polymethacrylic acids [44], and investigation of equilibria between metal and organic ligands in aqueous solutions where many species coexist in a narrow pH range [45]. This procedure may also be applicable to the reappraisal of many of the systems that have been previously interpreted by means of cumulative constants.

Other kinds of problems concern the influence of ionic strength on both site affinity constants and cooperativity coefficients. The variation in lg k_j with ionic strength is connected with the so-called Debye length l_D in Debye-Hückel theory. The influence of ionic strength on b_j is correlated with the so-called Bjerrum length, l_B, which depends on the overlap between adjacent charges. The binding of metals and of protons to polyelectrolytes (Buffle [46]) is modelled by introducing operative conditional equilibrium constants which change with pH and other conditions. New insights into these systems may also be gained by treating them with the algorithm described herein.

All these systems are measured experimentally by pH-metric or in general potentiometric titrations. A typical titration experiment involves a solution of known receptor concentration being titrated pH-metrically by a base or acid in the presence of a known amount of ligand.

Particular mention should be made for the case of hemoglobin. The equilibria of hemoglobin with oxygen are generally studied by monitoring the oxygen pressure. The amount of oxygen ligand T_A varies during the course of the experiment. It has been shown, however, that this problem can also be solved [38] by means of the partition function method. Another problem with hemoglobin is that the equilibria are studied at constant pH in order to avoid difficulties due to the Bohr effect. This restriction should no longer be necessary because equilibria with oxygen, or other ligands, and protons can be calculated by the partition function method in the same system simultaneously.

Insight can also be gained by considering other experimental approaches. For instance, in calorimetric measurements, the calculated distribution of the heat evolved is made on the basis of the concentration of species. However, species with the same stoichiometry with indexes P, Q, R may correspond to mixtures of microspecies with different $p_1, p_2 \ldots q_1, q_2, \ldots$, $r_1, r_2, \ldots$ sites and with different cooperativity effects. The approach described herein should be useful in the calculation

of the enthalpy from calorimetric measurements. Similarly, spectroscopic determinations could be improved using the present procedure.

Appendix

The tensor product is a multiplication operation performed by a column vector on a row vector, whereby each element of the column vector multiplies all the elements of the row. In our notation

$$\{J_1\}[J_2] = L_{1,2} \tag{A1}$$

or L_l where l is an index in a housekeeping list. A simple example is

$$\{J_1\}[J_2] = \begin{bmatrix} 1 \\ k[\mathrm{A}] \end{bmatrix} [1 \; k[\mathrm{A}]] = \begin{bmatrix} 1 & k[\mathrm{A}] \\ k[\mathrm{A}] & k^2[\mathrm{A}]^2 \end{bmatrix} \tag{A2}$$

Other examples are provided by eqs. 28–30. The vectors commute. In fact,

$$[J_j]^T = \{J_j\} \tag{A3}$$

where the superscript T indicates the transpose of vector or matrix and the same holds true for the inverse transformation from row to column vector. Thus, we can calculate

$$\{J_2\}[J_1] = L_{2,1} = L_{1,2}^T \tag{A4}$$

Eq. A2 represents the second power of vector J_1. In order to calculate a tensor power of higher order following the unitary vector method [12,20,35], we can transform the matrix $L_{1,2}$ into a column vector via post-multiplication by a column vector composed of as many unities as the columns of $L_{1,2}$

$$\begin{bmatrix} 1 & k[\mathrm{A}] \\ k[\mathrm{A}] & k^2[\mathrm{A}]^2 \end{bmatrix} \begin{bmatrix} 1 \\ 1 \end{bmatrix} = \begin{bmatrix} 1 + k[\mathrm{A}] \\ k[\mathrm{A}] + k^2[\mathrm{A}]^2 \end{bmatrix} \tag{A5}$$

The resulting column vector can multiply a row vector again in a tensor product. In general, if we indicate by e_j the unitary vector, we can calculate the n-th tensor power

$$J_j^n = \{J_1\}[J_2]\{e_2\} \ldots [J_j]\{e_j\} \cdots [J_{n-1}]\{e_{n-1}\} \times [J_n] = L_{1,2 \ldots j \ldots n}\{e_n\} \tag{A6}$$

The resulting vector J_j^n can be decomposed into a sum of more vectors, each of which retains its own index. Each term is finally identified by a series of indices.

For the sake of simplicity, however, we do not apply the unitary vectors e_j. The tensor products or powers are performed by multiplying each element of a matrix by all the elements of a row vector

$$L_{1,2}[J_j] = L_{1,2,j} \tag{A7}$$

or each element of a column vector by all the elements of a matrix

$$[J_j]^T L_{1,2} = L_{j,1,2} \tag{A8}$$

The labelling of the elements of the resulting tensor matrix is straightforward, viz., by addition of the index of the element of the vector.

The same rules as applied for powers can be employed in successive tensor products of vectors presenting different dimensions.

With reference to the unitary vector method, we note that one can obtain the partition function expression or a binary generating function by post-multiplying the matrix L_l by a unitary vector e_j with the same number of elements as the columns of L_l, and then by pre-multiplying by a unitary vector e_j with the same number of elements as the rows of L_l, e.g.

$$\begin{bmatrix} 1 & 1 \end{bmatrix} \begin{bmatrix} 1 & k[\mathrm{A}] \\ k[\mathrm{A}] & k^2[\mathrm{A}]^2 \end{bmatrix} \begin{bmatrix} 1 \\ 1 \end{bmatrix} = \left[1 + k[\mathrm{A}] + k[\mathrm{A}] + k^2[\mathrm{A}]^2\right] \tag{A9}$$

This result is the polynomial $(1 + k[\mathrm{A}])^2$ and it could be either a partition function or a binary generating function for two sites. As a generating function, we represent eq. A9 by a vector J_j

$$J_j = \left[1 \; 2k[\mathrm{A}] \; k^2[\mathrm{A}]^2\right] \tag{A10}$$

The nonequivalence of eqs. A9 and A10 in the present context derives from the fact that we can choose to introduce the cooperativity function into

eq. A10, and not into J_1 and hence neither into eq. A9, by post-multiplication by the diagonal matrix Γ_j

$$J_j\Gamma_j = J_{\gamma_j} = \left[1\ 2k[\mathrm{A}]\ k^2\gamma_{j,2}^2[\mathrm{A}]^2\right] \qquad \text{(A11)}$$

We can take advantage of this property, whenever we wish to restrict the cooperativity effect within the realm of a group of sites by selecting the stage at which the cooperativity function is introduced. Beyond this stage, one calculates tensor powers instead of polynomial vectors of higher dimensions. Examples are given in eqs. 43 and 45, and in general the question pertains to the application of primed index operators $O_{i'}$ and unprimed index operators O_i.

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Glossary: List of symbols

Symbol	Meaning
M	macromolecule or metal
A	ligand
H	proton
[M],[A],[H]	concentration of free or unbound component (charges omitted)
$[M_P A_Q H_R]$	concentration of species $M_P A_Q H_R$
β_{PQR}	cumulative equilibrium constant for species $M_P A_Q H_R$
K_Q	stepwise constant for $MA_{Q-1} + A \rightleftarrows MA_Q$
K_{γ_Q}	stepwise cooperativity constant for step Q
$\bar{\theta}$	saturation fraction
$\bar{n}$	average number of ligand A bound to M
Z_M, Z_A, Z_H	partition function for M, A, and H, respectively
B_M, B_A, B_H	polynomial for binding to M, A, and H, respectively
P_t, Q_t, R_t	maximum value of index P, Q, R respectively
p_t, q_t, r_t	maximum value (sites) of index p, q, r, respectively
T_M, T_A, T_H ($[T_M]$,$[T_A]$,$[T_H]$)	total chemical amount (concentration) of M, A, and H, respectively
U	sum of squares of the residuals
σ	estimated standard deviation
s	statistical correction factor
ΔG	Gibbs free energy change
$\Delta\mu_Q^{\circ}$	standard chemical potential change for step Q
$\Delta\mu_{\gamma_Q}$	chemical potential change for cooperativity at step Q
$\Delta\mu_{\gamma_q}$	chemical potential change for cooperativity at step q
$\Gamma(p)$, $\Gamma(q)$, $\Gamma(r)$	cooperativity function
γ_p, γ_q, γ_r, γ_i	cooperativity factor at step p, q, r, i, respectively
$\gamma_{j,p}$, $\gamma_{j,q}$, $\gamma_{j,r}$, $\gamma_{j,i}$	cooperativity factor for steps of class j
a, b	coefficient of the cooperativity function
a_j, b_j	coefficient of the cooperativity function of class j
$\partial Z_M / \partial[A]$	partial derivative
m_{ij} (or m_{p_j}, m_{q_j}, m_{r_j})	statistical coefficient of each term of the partition function
k	site affinity constant
k_j	site affinity constant for class j
k_{MA}, k_{MH}	site affinity constant for binding M-A and M-H, respectively
b_A	coefficient for cooperativity A...A
b_H	coefficient for cooperativity H...H
J_j	binary generating function for class j
$\boldsymbol{J}_j$	column or row vector representing generating function J_j
$\boldsymbol{\Gamma}_j$	diagonal matrix representing cooperativity function Γ_j
$\boldsymbol{J}_{\gamma_j}$	column or row vector $\boldsymbol{J}_j$ modified by $\boldsymbol{\Gamma}_j$
$\boldsymbol{O}_{i'}$, $\boldsymbol{O}_{(i-1)'}$, $\boldsymbol{O}_{(i_t-i)'}$	power vector operators
$\boldsymbol{O}_i$, $\boldsymbol{O}_{(i-1)}$	power tensor operators
X	M, A, H as receptor
Y	M, A, H as ligand
$\boldsymbol{L}_l$ (or $\boldsymbol{L}_{1,2}$ or $\boldsymbol{L}_{1,2,3}$, etc.)	tensor matrix
$\{b_j\}$,$\{q_j\}$,$\{r_j\}$,$\{i_j\}$	element of vector J_j
$\{b_j \cdots, q_j \cdots, r_j \cdots\}$	element of tensor $L_{j \cdots j \cdots j \cdots}$