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Global and local metric geometry of ligand binding thermodynamics

(linkage/equilibrium/macromolecule)

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ABSTRACT **An abstract metric representation of ligand binding thermodynamics reveals the basic connections between the classical thermodynamic theory of binding and linkage [Wyman, J. (1964) *Adv. Protein Chem.* 19, 223–286] and its site-specific formulation [Di Cera, E. (1990) *Biophys. Chem.* 37, 147–164]. These two approaches are limit cases of a general metric formulation of binding and linkage that also includes a number of intermediate "mixed" representations.**

It has been shown by Weinhold (1–3) that a simple thermodynamic system of a fixed scale, containing c independent chemical components and ν distinct phases, can be associated with an abstract Euclidean space, $\mathbb{R}^t$, whose dimension, t, is equal to the number of degrees of freedom of the system as fixed by the Gibbs phase rule $t = c - \nu + 2$. The (nonorthogonal) basis set $\{|\mathfrak{R}_j\rangle\} \in \mathbb{R}^t$ is uniquely defined in terms of the association

$$dR_j \leftrightarrow |\mathfrak{R}_j\rangle \qquad [1]$$

by which the differential of each field, R_j, is mapped into a ket in $\mathbb{R}^t$. Each field, R_j, is defined, in the Griffiths–Wheeler sense (4), as the derivative

$$R_j = \partial U(\{X_j\})/\partial X_j, \qquad [2]$$

where $U(\{X_j\})$ is the internal energy of the system and $\{X_j\}$ is the set of extensities conjugate to $\{R_j\}$. The metric of $\mathbb{R}^t$ is determined by the properties of the Gram matrix, **G**, whose elements

$$g_{ij} = \langle\mathfrak{R}_i|\mathfrak{R}_j\rangle \equiv \partial R_i/\partial X_j \qquad [3]$$

express the mutual projections of the kets and are associated with the fluctuations of the fields. The first two laws of thermodynamics imply that **G** is symmetric and positive definite; i.e.,

$$\langle\mathfrak{R}_i|\mathfrak{R}_j\rangle = \langle\mathfrak{R}_j|\mathfrak{R}_i\rangle \qquad [4]$$

because $U(\{X_j\})$ has an exact differential (first law) and

$$\langle\mathfrak{R}_i|\mathfrak{R}_i\rangle > 0 \qquad [5]$$

because $U(\{X_j\})$ has an extremal value at equilibrium (second law). The nonsingularity of **G** ensures that the dimension of $\mathbb{R}^t$ actually equals the number of degrees of freedom of the system and that the inverse Gram matrix $\mathbf{G}^{-1}$, with elements $\langle X_i|X_j\rangle = \partial X_i/\partial R_j$, exists and is itself symmetric and positive definite. The connection of the foregoing abstract metric treatment with the properties of real systems is to be seen in the fundamental properties of the matrix **G**, as well as in the association embodied by Eq. **3**.

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In the case of a macromolecular system at constant temperature and pressure, which we are particularly interested in here, the dimension of the Euclidean space is set by the number of components, or ligands, that interfere with each other using the macromolecule as a transducer. The macromolecule is used as a scaling factor for all t extensities, $\{X_j\}$, that under the assumption of mass law binding reflect the amount of each of the ligands bound to the macromolecule (5, 6). The fields $\{R_j\}$ represent the chemical potential of each ligand. The Gram matrix contains all partial derivatives involving chemical potentials and number of ligands bound to the macromolecule and its properties reflect linkage and stability relationships for these partial derivatives (7). A complete metrization of ligand binding thermodynamics can thus be achieved by substituting for $\{R_j\}$ and $\{X_j\}$ the relevant quantities of interest. This metric will be referred to as the *global* metric of the system, since it encapsulates binding and linkage arising from the mutual interference among different ligands. Nested within this global picture is a different description of ligand binding and linkage effects that involves phenomena occurring *locally* at the level of individual sites of the macromolecule.

Global and Local Forms of the Gibbs–Duhem Equation

Since only t vectors can be linearly independent in $\mathbb{R}^t$, then for an arbitrary ket $|\mathcal{H}\rangle \in \mathbb{R}^t$ one necessarily has

$$|\mathcal{H}\rangle = \sum_{j=1}^{t} c_j|\mathfrak{R}_j\rangle, \qquad [6]$$

where c_j is the jth component of $|\mathcal{H}\rangle$. There are infinite such kets that can be constructed in $\mathbb{R}^t$, but one of them is particularly interesting as it corresponds to the negative of the reference field, $-|\mathfrak{R}_0\rangle$, whose conjugate extensity, X_0, has been used to scale all other extensities $\{X_j\}$. When the reference field is substituted in Eq. **6** an abstract metric form of the Gibbs–Duhem equation is obtained; i.e.,

$$-|\mathfrak{R}_0\rangle = \sum_{j=1}^{t} X_j|\mathfrak{R}_j\rangle, \qquad [7]$$

where it is understood that each $X_j = -\partial R_0/\partial R_j$ is expressed in X_0 units. As pointed out by Weinhold (2), the Gibbs–Duhem equation has a simple geometric interpretation as the "impossibility of finding $t + 1$ linearly independent vectors in a t-dimensional space." There are, however, additional aspects that need to be stressed in the case of macromolecular systems. First, one has to recognize that the thermodynamic picture cast in terms of the Gibbs–Duhem equation is only one of infinite alternative ways of characterizing the thermodynamic properties of the system. In fact, any arbitrary ket $|\mathcal{H}\rangle \in \mathbb{R}^t$, of which $-|\mathfrak{R}_0\rangle$ is a particular one, can be associated with the differential of a potential that is a function of the

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fields $\{R_j\}$. Second, one can think of the Gibbs–Duhem equation as a way of characterizing the overall behavior of the basis set $\{|\Re_j\rangle\} \in \mathbb{R}^t$, with $-|\Re_0\rangle$ being a resulting vector over the manifold spanned by the basis set $\{|\Re_j\rangle\}$. Such an interpretation can be extended to any ket $|\Re_j\rangle$ that can be regarded as a resulting vector over the subspace $\mathbb{R}^{r(j)}$ spanned by $r(j)$ linearly independent kets. This means that any component $|\Re_j\rangle$ of the basis set $\{|\Re_j\rangle\}$ can give rise to a *local* Gibbs–Duhem equation in $\mathbb{R}^{r(j)}$; i.e.,

$$|\Re_j\rangle = \sum_{i=1}^{r(j)} c_{ji}|\Re_{ji}\rangle, \qquad [8]$$

where the component c values are expressed in X_j units. The basis set $\{|\Re_{ji}\rangle\}$ forms a submetric isomorphic with the positive-definite metric in $\mathbb{R}^t$. Substitution of Eq. **8** into Eq. **7** yields

$$|\mathcal{L}\rangle = \sum_{j=1}^{t} \sum_{i=1}^{r(j)} X_j c_{ji}|\Re_{ji}\rangle, \qquad [9]$$

where $|\mathcal{L}\rangle \equiv -|\Re_0\rangle$ is the differential of a potential associated with the Gibbs–Duhem equation and $r(j)$ is the dimension of the manifold associated with the ket $|\Re_j\rangle$. Equivalence between Eqs. **7** and **9** leads to the operator representation

$$\partial/\partial R_j = \sum_{i=1}^{r(j)} \partial/\partial R_{ji} = \nabla_j, \qquad [10]$$

whereby the derivative $\partial/\partial R_j$ in the global picture can be given a local interpretation as the "gradient" computed over the subspace $\mathbb{R}^{r(j)}$, so that the jth component of $|\mathcal{L}\rangle$ in $\mathbb{R}^t$ is expressed in terms of the components of $|\Re_j\rangle$ in $\mathbb{R}^{r(j)}$. Hence,

$$\sum_{i=1}^{r(j)} c_{ji} = 1. \qquad [11]$$

Introduction of the new quantities $X_{ji} \equiv X_j c_{ji}$, which are scaled only with respect to the reference component X_0, leads to a generalized local form of the Gibbs–Duhem equation as follows

$$|\mathcal{L}\rangle = \sum_{j=1}^{t} \sum_{i=1}^{r(j)} X_{ji}|\Re_{ji}\rangle. \qquad [12]$$

The consequence of Eq. **12** is that the metric constructed in terms of the *global* basis set $\{|\Re_j\rangle\} \in \mathbb{R}^t$ can be mapped 1:1 into the metric constructed in terms of the *local* basis set $\{|\Re_{ji}\rangle\} \in \mathbb{R}^\Sigma$, where $\Sigma = r(1) + r(2) + \ldots r(t)$ (8, 9). The two representations are isomorphic and differ solely for the dimensionality of the manifold to which they are associated. The higher dimensionality of $\mathbb{R}^\Sigma$ arises from the fact that each ket of $\mathbb{R}^t$ is now expressed *locally* in terms of its components in the subspace $\mathbb{R}^{r(j)}$. The possibility of accessing the information stored at the local level is translated into an increased number of linearly independent vectors in the abstract hyperspace associated with the thermodynamic system under consideration. The classical form of the Gibbs–Duhem equation is obtained from this new, local description, when the independent vectors $|\Re_{ji}\rangle$ of each of the subspaces $\mathbb{R}^{r(j)}$ are expressed in terms of their resulting, global vectors $|\Re_j\rangle$. In the global description each vector $|\Re_j\rangle$ can be given a simple geometric representation as the vector drawn from the origin of the basis set in $\mathbb{R}^{r(j)}$ to the center of mass of the subspace. In fact, if the "masses" $c_{j1}, c_{j2}, \ldots, c_{jr(j)}$ in Eq. **8** are located at $|\Re_{j1}\rangle, |\Re_{j2}\rangle, \ldots, |\Re_{jr(j)}\rangle$ in the subspace $\mathbb{R}^{r(j)}$, then $|\Re_j\rangle$ is the coordinate of the center of mass of the subspace. Consequently, for any vector $|\Re_j\rangle \in \mathbb{R}^t$ one necessarily has

$$|\Re_{ji}\rangle_{min} \leq |\Re_j\rangle \leq |\Re_{ji}\rangle_{max}, \qquad [13]$$

whereby the ket $|\Re_j\rangle$ is bounded between the smallest and largest kets in the subspace $\mathbb{R}^{r(j)}$.

Metrization of Local (Site-Specific) Binding and Linkage Effects

The construction of a local metric as discussed above from a classical, global treatment of equilibrium thermodynamics stems from the possibility of defining new independent thermodynamic fields that can be accessed in the experimental approach to the system under consideration. Such a possibility is particularly relevant for a system composed of a biological macromolecule and a number of ligands that interact with it. In the global treatment of this system the ket $-|\Re_0\rangle$ associated with the differential of the chemical potential of the macromolecule is expressed in terms of its components X_j in the basis set $\{|\Re_j\rangle\} \in \mathbb{R}^t$, where each field R_j equals the chemical potential of ligand j. Let us now take one of these ligands, say j, and consider it as the reference component of an abstract subspace whose basis set is associated with $r(j)$ "ligands." What do these $r(j)$ ligands represent? For the basis set of the subspace to have a physical meaning, the $r(j)$ ligands must be associated with measurable quantities that can be distinguished from one another, just as in the case of the t ligands entering the definition of the global form of the Gibbs–Duhem equation. Consequently, ligand j has as many ligands in the abstract subspace $\mathbb{R}^{r(j)}$ as there are sites of the macromolecule where it can bind, provided one can follow each site-specific binding reaction separately. When this is the case, as recently shown in a number of biochemical systems (9), then the number of sites for ligand j sets the dimension of a local subspace where $|\Re_j\rangle$ parallels $|\Re_0\rangle$ in the global picture. The fields of this local description must reflect the properties of each individual X_j-binding site of the macromolecule and, by analogy with the basis set in the global description, they must be defined in the absence of any other field. There is one, and only one, such quantity, that is, the association constant K_{ji} between ligand X_j and its ith binding site when all the other sites are unligated (8, 9). This quantity reflects the properties of site i alone so that the field $|\Re_j\rangle$ can be cast in terms of a basis set $\{|\Re_{ji}\rangle\}$ associated with the logarithm of each site-specific association constant. Metrization of the subspace therefore hinges upon the association

$$dR_{ji} = d\ln K_{ji} \leftrightarrow |\Re_{ji}\rangle \qquad [14]$$

that parallels Eq. **1** in the global picture. The term X_{ji} conjugate to the field $|\Re_{ji}\rangle$ gives the probability of the ith site for ligand j being ligated. The ratio X_{ji}/X_j, where X_j is the number of ligands j bound to the macromolecule, represents the "mass" located at $|\Re_{ji}\rangle$ in the subspace.

Existence of a local metric greatly expands the dimensionality of the abstract metric space while keeping the type of metric unaltered. The dimension of the Euclidean space associated with the system under consideration is equal to the number of ligands, in the case of the global picture, and increases to the total number of sites of the macromolecule in the local description. Therefore, associated with Eq. **12** is the Gram matrix $\mathbf{G}^\Sigma$ whose elements

$$g_{im,jn} = \langle\Re_{im}|\Re_{jn}\rangle \qquad [15]$$

express the mutual projections of the kets and are associated with the fluctuations of the site-specific fields. Again, from the first two laws of thermodynamics it follows that $\mathbf{G}^\Sigma$ is symmetric and positive definite; i.e.,

$$\langle\Re_{im}|\Re_{jn}\rangle = \langle\Re_{jn}|\Re_{im}\rangle \qquad [16]$$

and

$$\langle \Re_{im} | \Re_{im} \rangle > 0 \qquad [17]$$

while the nonsingularity of $\mathbf{G}^{\Sigma}$ ensures that the dimension of $\mathbb{R}^{\Sigma}$ actually equals the total number of ligand binding sites of the macromolecule, as well as the fact that the inverse Gramian $(\mathbf{G}^{\Sigma})^{-1}$ exists and is itself symmetric and positive definite (9).

Mixed Representations

From the operator equivalence in Eq. **10** it follows that there are 2^t possible representations of the Gibbs–Duhem equation, which can be obtained by in turn substituting each field $|\Re_j\rangle$ with the local basis set $\{|\Re_{ji}\rangle\}$. Each of these representations gives rise to a different Gram matrix. The choice of a particular representation is solely dictated by our ability to access the information stored at the local level for each particular ligand. When only the global information can be accessed, we are dealing with the "classical" description of binding and linkage effects cast in terms of the global fields $|\Re_j\rangle$ associated with the chemical potentials of the t ligands present (5, 6). On the other hand, when each binding reaction can be followed at the local, site-specific level then we deal with a higher-dimensional abstract metric space where each coordinate is associated with the association constant of each site (8, 9). Between these two limiting representations there are a number of "mixed" representations where some binding and linkage effects are treated globally and some locally. In this case we deal with the interplay between global and local properties of the system involving two or more ligands.

Consider the case of two ligands, each having two binding sites for the macromolecule. The possible Gibbs–Duhem representations are

$$|\mathcal{L}\rangle = X_1|\Re_1\rangle + X_2|\Re_2\rangle \qquad [18a]$$

$$|\mathcal{L}\rangle = X_{11}|\Re_{11}\rangle + X_{12}|\Re_{12}\rangle + X_2|\Re_2\rangle \qquad [18b]$$

$$|\mathcal{L}\rangle = X_1|\Re_1\rangle + X_{21}|\Re_{21}\rangle + X_{22}|\Re_{22}\rangle \qquad [18c]$$

$$|\mathcal{L}\rangle = X_{11}|\Re_{11}\rangle + X_{12}|\Re_{12}\rangle + X_{21}|\Re_{21}\rangle + X_{22}|\Re_{22}\rangle, \qquad [18d]$$

where X_1 and X_2 are the number of ligands 1 and 2 bound to the macromolecule, X_{11} and X_{12} are the probabilities of ligand 1 binding to its sites 1 and 2, and similarly X_{21} and X_{22} are the probabilities of ligand 2 binding to its sites 1 and 2. It also follows from Eqs. **11** and **12** that $X_{11} + X_{12} = X_1$ and $X_{21} + X_{22} = X_2$. The first representation, Eq. **18a**, is the classical global description based on the chemical potentials of ligands 1 and 2. The last representation, Eq. **18d**, is the local description based on the site-specific association constants of the four sites. Two mixed representations, Eq. **18b** and **18c**, show the interplay between ligand chemical potentials and site-specific association constants. The Gram matrices

$$\mathbf{G}^2 = \begin{pmatrix} \langle \Re_1|\Re_1\rangle & \langle \Re_1|\Re_2\rangle \\ \langle \Re_2|\Re_1\rangle & \langle \Re_2|\Re_2\rangle \end{pmatrix} = \left(\begin{array}{c:c} g_{11} & g_{12} \\ \hdashline g_{21} & g_{22} \end{array}\right) \qquad [19a]$$

$$\mathbf{G}^3 = \begin{pmatrix} \langle \Re_{11}|\Re_{11}\rangle & \langle \Re_{11}|\Re_{12}\rangle & \langle \Re_{11}|\Re_2\rangle \\ \langle \Re_{12}|\Re_{11}\rangle & \langle \Re_{12}|\Re_{12}\rangle & \langle \Re_{12}|\Re_2\rangle \\ \langle \Re_2|\Re_{11}\rangle & \langle \Re_2|\Re_{12}\rangle & \langle \Re_2|\Re_2\rangle \end{pmatrix} = \left(\begin{array}{c:c} \mathbf{G}_{11} & \mathbf{C}_{12} \\ \hdashline \mathbf{C}_{21} & g_{22} \end{array}\right) \qquad [19b]$$

$$\mathbf{G}^3 = \begin{pmatrix} \langle \Re_1|\Re_1\rangle & \langle \Re_1|\Re_{21}\rangle & \langle \Re_1|\Re_{22}\rangle \\ \langle \Re_{21}|\Re_1\rangle & \langle \Re_{21}|\Re_{21}\rangle & \langle \Re_{21}|\Re_{22}\rangle \\ \langle \Re_{22}|\Re_1\rangle & \langle \Re_{22}|\Re_{21}\rangle & \langle \Re_{22}|\Re_{22}\rangle \end{pmatrix} = \left(\begin{array}{c:c} g_{11} & \mathbf{C}_{12} \\ \hdashline \mathbf{C}_{21} & \mathbf{G}_{22} \end{array}\right) \qquad [19c]$$

$$\mathbf{G}^4 = \begin{bmatrix} \langle \Re_{11}|\Re_{11}\rangle & \langle \Re_{11}|\Re_{12}\rangle & \langle \Re_{11}|\Re_{21}\rangle & \langle \Re_{11}|\Re_{22}\rangle \\ \langle \Re_{12}|\Re_{11}\rangle & \langle \Re_{12}|\Re_{12}\rangle & \langle \Re_{12}|\Re_{21}\rangle & \langle \Re_{11}|\Re_{22}\rangle \\ \langle \Re_{21}|\Re_{11}\rangle & \langle \Re_{21}|\Re_{12}\rangle & \langle \Re_{21}|\Re_{21}\rangle & \langle \Re_{21}|\Re_{22}\rangle \\ \langle \Re_{22}|\Re_{11}\rangle & \langle \Re_{22}|\Re_{12}\rangle & \langle \Re_{22}|\Re_{21}\rangle & \langle \Re_{22}|\Re_{22}\rangle \end{bmatrix}$$

$$= \left(\begin{array}{c:c} \mathbf{G}_{11} & \mathbf{G}_{12} \\ \hdashline \mathbf{G}_{21} & \mathbf{G}_{22} \end{array}\right) \qquad [19d]$$

are associated with each representation. The elements of the matrix $\mathbf{G}^4$ can be partitioned into four matrices. The diagonal matrices correspond to the Gram matrices of the two subspaces spanned by the site-specific fields coupled to the two ligands. The off-diagonal matrices reflect linkage properties between the two subspaces. The remaining three Gram matrices in Eq. **19a–c** can be partitioned in an analogous way. In general, each diagonal matrix will always be associated with the Gram matrix of a subspace, and each off-diagonal matrix will reflect linkage between subspaces. In the case of the global representation each subspace is one-dimensional and the Gram matrix associated with it is simply the diagonal element of the Gram matrix of the whole t-dimensional hyperspace. In the case of a mixed representation, one-dimensional subspaces linked to global fields coexist with multidimensional subspaces spanned by the local fields. In general, for a system of t ligands there are a total of 2^t possible representations. One of them is t-dimensional and global, one is Σ-dimensional and local, and $2^t - 2$ are mixed and with a dimensionality bounded from t to Σ.

Global and Local Linkage Effects

The interplay between global and local representations is best understood mathematically through Eq. **10**. The global linkage relationship (5, 6)

$$\partial X_i/\partial R_j = \partial X_j/\partial R_i \qquad [20a]$$

can be given a local interpretation using the gradient operator ∇. The linkage between ligand i and the sites binding ligand j is embodied by the relationship

$$\nabla_j X_i = \partial X_j/\partial R_i. \qquad [20b]$$

Since $X_i = X_{i1} + X_{i2} = \ldots X_{ir(i)}$, then the left-hand side of Eq. **18b** simply states that the sum of all elements of the submatrix $\mathbf{G}^{-1}_{ij}$ of $(\mathbf{G}^{\Sigma})^{-1}$ equals the global derivative on the right-hand side. Therefore, a partial derivative expressing linkage in the global sense corresponds to the sum of the elements of a local matrix. Similarly, for the linkage between ligand j and the sites of ligand i one has

$$\partial X_i/\partial R_j = \nabla_i X_j \qquad [20c]$$

and finally

$$\nabla_j X_i = \nabla_i X_j \qquad [20d]$$

expresses linkage between the two sets of sites.

The generality of Eq. **10** can be exploited further to derive other linkage relationships. From the symmetry properties of the Gram matrix of the subspace associated with the global field $|\Re_j\rangle$ it follows, for any two local fields $|\Re_{jm}\rangle$ and $|\Re_{jn}\rangle$, that

$$\langle\Re_{jm}|\Re_{jn}\rangle = \langle\Re_{jn}|\Re_{jm}\rangle. \quad [21]$$

Hence, from the symmetry properties of the inverse Gram matrix of this subspace it must follow that (8, 9)

$$\partial X_{jm}/\partial R_{jn} = \partial X_{jn}/\partial R_{jm}. \quad [22]$$

Summing over the index m on both sides of Eq. **22** yields

$$\partial X_j/\partial R_{jn} = \nabla_j X_{jn} = \partial X_{jn}/\partial R_j, \quad [23]$$

which is an interesting linkage relationship in the subspace $\mathbb{R}^{r(j)}$ involving the gradient and the basis vector $|\Re_{ji}\rangle$. The effect of the local field R_{jn} on the global quantity X_j equals the effect of the gradient, or else the global field R_j, on the local quantity X_{jn}. Summing now over n in both sides of Eq. **23** yields

$$\nabla_j X_j = \partial X_j/\partial R_j = g_{jj}^{-1} > 0, \quad [24]$$

which is the effect of the global field on its conjugate extensity or else the binding capacity of the ligand (7). We have thus expressed the binding capacity of a ligand as the sum of the elements of the inverse Gram matrix of the subspace spanned by the local fields associated with the ligand chemical potential. This sum must be positive as a consequence of the second law. It also follows from Eq. **23** that the sum of the elements of the nth row, or equivalently of the nth column, of the inverse Gram matrix of the subspace gives the binding capacity of the nth site and, consequently, the binding capacity of the ligand equals the sum of the site-specific binding capacities (8, 9). The linkage relationship given in Eq. **23** allows for a definition of the binding capacity of site n as the change of the number of ligands bound to the macromolecule, X_j, due to a change in the site-specific association constant K_{jn} of that site. Such a change is equivalent to the sum of the changes of each site-specific binding isotherm, X_{j1}, X_{j2}, . . . , $X_{jr(j)}$ due to a change of K_{jn}, or else to the sum of the changes of X_{jn} due to changes of each of the association constants K_{j1}, K_{j2}, . . . , $K_{jr(j)}$.

Conclusion

Application of the foregoing abstract metric considerations to real systems is straightforward once the partition function of the system, related as it is to the potential associated with the Gibbs–Duhem equation, is cast in terms of the relevant global and local quantities, such as ligand chemical potentials and site-specific association constants. Elementary transformations in the abstract metric space map into corresponding transformations of the partition function. The whole information stored in the partition function can be explored by suitable "contractions" of the abstract metric space that correspond to sampling linearly independent sets of site-specific configurations of the system (9). The "complexity" of such a geometric interpretation of binding and linkage effects is only apparent. Indeed this approach reveals in a rather general and straightforward way the basic aspects that dominate global and local binding effects at equilibrium. It also points out that a "continuous" transition exists between the classical theory of binding and linkage (5, 6) and the recently developed theory of site-specific binding phenomena (8, 9). Both theories can now be interpreted as limit cases of the general approach taken here where the transition from the global to the local metric is traced using intermediate "mixed" representations.

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