Molecular Recognition

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# Allosteric, Chelate, and Interannular Cooperativity: A Mise au Point

*Gianfranco Ercolani* and Luca Schiaffino*

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## 1. Introduction

Cooperativity is a key mechanism that regulates the behavior of complex molecular systems in biology and supramolecular chemistry.[1,2] There are, however, some basic issues concerning the definition and assessment of different types of cooperativity that must be addressed if we are to achieve a truly quantitative understanding of the phenomenon.

Recently Whitty,[3] as well as Hunter and Anderson,[4] delineated two types of cooperativity, namely allosteric and chelate cooperativity. The former phenomenon arises from the interplay of two or more intermolecular binding interactions, as exemplified by the binding of oxygen to hemoglobin,[5] and the latter arises from the presence of one or more intramolecular binding interactions, that is, as a consequence of the chelate effect (multivalency). However, while allosteric cooperativity is well recognized, the assessment of chelate cooperativity is still unsatisfactory because of a number of pitfalls that have not been adequately highlighted. To remedy this problem, we report an analysis that consistently assesses chelate cooperativity and introduces, by necessity, a third type of cooperativity, namely interannular cooperativity, which arises from the interplay of two or more intramolecular binding interactions.

A system comprising a collection of identical interactions displays cooperativity when its behavior as a whole is different from the expected behavior based on the properties of isolated individual interactions. Thus, to meaningfully assess cooperativity, the following three-step procedure appears mandatory: 1) evaluation of the single isolated interaction (the reference);[6] 2) development of a model (the noncooperative model) where each interaction of the system behaves independently of the others, that is, it behaves as the reference; 3) testing of the behavior of the real system against that predicted by the noncooperative model: any deviation, positive or negative, of the real system is by definition the unambiguous mark of cooperativity. We will analyze the three types of cooperativity by following the above three-step procedure.

## 2. Allosteric Cooperativity

Allosteric (intersite) cooperativity is the type of cooperativity that is best understood.[2a,7] Accordingly, we will only briefly consider the archetypal case that involves the binding of a monovalent ligand B to a divalent receptor AA (Figure 1). This treatment is instructive because it clearly

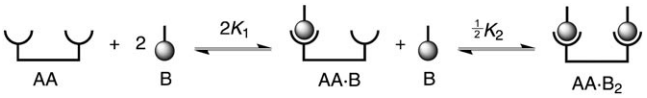


***Figure 1.*** Binding of a monovalent ligand B to a divalent receptor AA.

illustrates the steps necessary to assess cooperativity. The equilibria in Figure 1 are expressed by the two microscopic association constants $K_1$ and $K_2$ multiplied by the statistical factors 2 and $^1/_2$, respectively.[8,9] The reference constant $K$ can be evaluated by studying the binding of the monovalent ligand B to a monovalent model of the receptor A, or alternatively, by directly taking the value of the constant $K_1$ as the reference constant. Thus, in the absence of cooperativity, $K_2 = K_1 = K$. Cooperativity can be quantified by an interaction parameter (or cooperativity factor) that is given by the ratio of the overall experimental constant to the hypothetical overall noncooperative constant $\alpha = K_1K_2/K^2$. The factor $\alpha$ will be larger than 1 in the case of positive cooperativity, equal to 1 in the case of noncooperativity, and smaller than 1 in the case of negative cooperativity. It is worth remarking that a cooperativity factor is a ratio of two overall equilibrium constants that have the same units; the constant in the numerator is affected by cooperative interactions and the constant in the denominator is the reference. The cooperativity factor can be viewed as the equilibrium constant for the conversion of the hypothetical complex with noninteracting sites (noncooperative) into the complex with interacting sites (cooperative; Figure 2). The cooperative interaction may be

[*] Prof. G. Ercolani, Dr. L. Schiaffino
Dipartimento di Scienze e Tecnologie Chimiche
Università di Roma Tor Vergata
Via della Ricerca Scientifica
Fax: (+39) 0672594328
E-mail: ercolani@uniroma2.it

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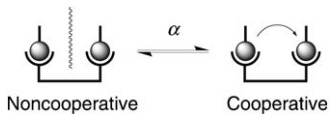


***Figure 2.*** A cooperativity factor can be viewed as the equilibrium constant for the conversion of the hypothetical complex with non-interacting sites (noncooperative) into the complex with interacting sites (cooperative).

any one of the possible interactions (electrostatic, steric, conformational, etc.).

## 3. Chelate Cooperativity

To assess chelate cooperativity, we consider the simple case involving the binding of a divalent ligand BB to a divalent receptor AA. The ligand is present in a large excess relative to the receptor so that complexes involving more than one receptor molecule can be neglected, and $\alpha = 1$ to exclude allosteric cooperativity (Figure 3). Under the given conditions, there are only four possible states for the receptor: free AA, the partially bound 1:1 open complex *o*-AA·BB, the fully bound 1:1 cyclic complex *c*-AA·BB, and the 1:2 complex AA·$(BB)_2$. The intramolecular binding interaction $K_{intra}$ is expressed as the product $^1/_2 K\,EM$, where $^1/_2$ is the statistical factor for the cyclization process,[8,9] $K$ is the microscopic intermolecular constant that expresses the strength of the binding interaction, and $EM$, which is the microscopic effective molarity (in units of mol $L^{-1}$),[10,11] is a parameter that accounts for the ease of the intramolecular process. For the sake of discussion, we show in Figure 4 the speciation profiles for the equilibria shown in Figure 3 in the absence ($K_{intra} = 0$), and in the presence of the chelate interaction, for example, $K_{intra} = 25$. Analogous plots have been previously reported.[4] When Figure 4b is compared with Figure 4a, it appears that the presence of the chelate interaction leads to a sharp decrease of the partially bound intermediate complex *o*-AA·BB to favor the fully bound cyclic complex *c*-AA·BB. This feature is characteristic of cooperative systems that, in the most extreme cases, are represented by an "all-or-none" process that involves the complete depletion of intermediate states. However, although the overall binding tends to an "all-or-none" process, the speciation profile of the chelate complex *c*-AA·BB is bell-shaped, thus suggesting that the intramolecular process can be better regarded as "none-all-none". This behavior is due to the fact that the cyclic complex is disfavored with respect to the unbound receptor at low concentrations, whereas the cyclic complex must compete with the fully bound 1:2 open complex at high concentrations. Indeed, the concentration of the cyclic complex depends linearly on ligand concentration [Equation (1)], whereas that of the fully bound open complex depends on the square of the ligand concentration [Equation (2)]; the cyclic complex is favored at low concentrations, but the fully bound open complex overwhelms the cyclic one when the concentration is increased. When the right-hand sides of Equations (1) and (2) are equal, we obtain Equation (3), which shows the ligand concentration at which the switch between the fully bound open complex and the cyclic complex occurs.

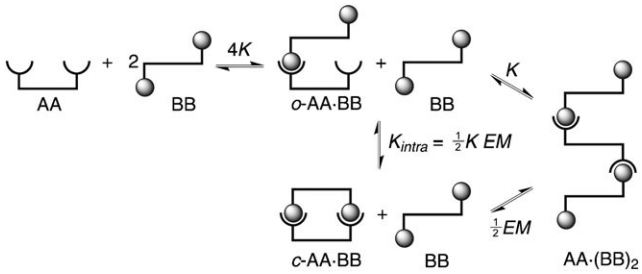


***Figure 3.*** Binding of a divalent ligand BB to a divalent receptor AA, assuming $[BB]_0 \gg [AA]_0$ and $\alpha = 1$.

*Gianfranco Ercolani obtained his PhD in 1979 from the University of Rome Sapienza, and is now Full Professor of Organic Chemistry at the University of Rome Tor Vergata. He has broad research interests in physical organic chemistry, computational chemistry, and supramolecular chemistry, and his current research interests include cooperativity mechanisms, asymmetric catalysis, and chiral amplification.*

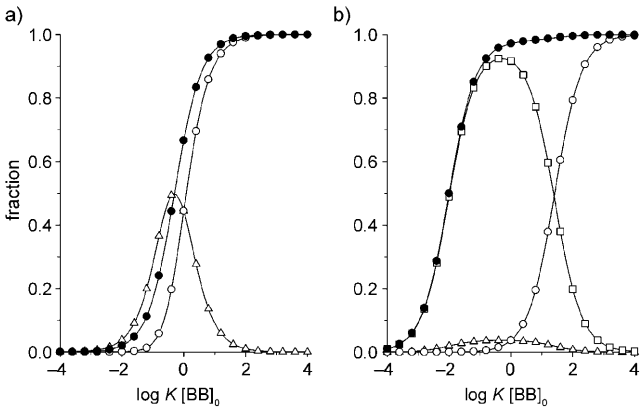


***Figure 4.*** Speciation profiles for the equilibria shown in Figure 3: a) in the absence of the chelate interaction ($K_{intra} = 0$); b) in the presence of the chelate interaction, for example, $K_{intra} = 25$, corresponding to $KEM = 50$. The concentration scale on the abscissa is normalized by multiplying by $K$. Symbols represent fractions of *o*-AA·BB (△); AA·$(BB)_2$ (○); *c*-AA·BB (□); total fraction of occupied binding sites of the receptor AA (●).

$$[c\text{-AA}\cdot\text{BB}] = 2K^2\,EM[\text{AA}][\text{BB}] \quad (1)$$

$$[\text{AA}(\text{BB})_2] = 4K^2[\text{AA}][\text{BB}]^2 \quad (2)$$

$$[\text{BB}]_{switch} = EM/2 \quad (3)$$

It appears that $EM$ is the threshold concentration of ligand binding groups B above which the intramolecular process loses the competition with the intermolecular one.[10] Thus, the advantage provided by the chelate interaction is dissipated at high concentrations. The conclusion is that chelate cooperativity, in contrast with allosteric cooperativity, depends on ligand concentration.[12,13] This fact has been previously overlooked, thus leading to improper assessments of chelate cooperativity. For example, it has been advocated that chelate cooperativity manifests itself when the intramolecular constant $K_{intra}$ is larger than $4K$.[3,14] This comparison is meaningless because the intramolecular constant is dimensionless whereas the intermolecular constant is expressed in units of $mol^{-1}L$. Similarly, with reference to the binding of a divalent asymmetric ligand AB to a complementary receptor (Figure 5), some authors argued that if the Gibbs free energy of binding of the species AB is more favorable than the sum of the free energies of binding for the individual parts A and B, then positive cooperativity occurs.[15,16] The absence of chelate cooperativity would require that the observed Gibbs binding energies of the two molecules A and B are additive in the molecule AB, so that $\Delta G_{AB}{}^0 = \Delta G_A{}^0 + \Delta G_B{}^0$. However, as pointed out by Jencks,[17] there is no basis for this assumption: the addition of Gibbs energies is equivalent to the multiplication of binding constants and, if $K_A$, $K_B$, and $K_{AB}$ are measured in units of $mol^{-1}L$, the equation $K_{AB}(mol^{-1}L) = K_A K_B\ (mol^{-1}L)^2$ is meaningless. Jencks also pointed out that the correct way to address the problem of additivity of binding energies is to add a connection Gibbs energy that represents the change in the probability of binding that results from the connection of A and B in AB. This connection Gibbs energy is in fact the free energy associated with the $EM$, that is, $\Delta G_{EM}{}^0$. Accordingly $\Delta G_{AB}{}^0 = \Delta G_A{}^0 + \Delta G_B{}^0 + \Delta G_{EM}{}^0$, which, when translated into binding constants, gives a dimensionally correct equation $K_{AB}(mol^{-1}L) = K_A K_B\, EM(mol^{-1}L)$. In light of this consideration, it is tempting to assume that in the absence of chelate cooperativity $EM = 1\ mol\,L^{-1}$, whereas $EM > 1\ mol\,L^{-1}$ and $EM < 1\ mol\,L^{-1}$ would indicate positive and negative cooperativity, respectively. However this assumption is inconsistent because, since the $EM$ has units of concentration, its numerical value depends on the choice of standard state. Indeed, a consistent cooperativity factor, which is the ratio of two equilibrium constants with the same units, must be dimensionless. Moreover, as pointed out above, chelate cooperativity must depend on ligand concentration and not solely on a constant parameter, as is the case for the $EM$.

Other authors advocated the product $KEM$ as a measure of chelate cooperativity.[4] This conclusion is inconsistent for several reasons: 1) the product $KEM$, apart from the statistical factor $^1/_2$, coincides with the equilibrium constant for the intramolecular binding interaction $K_{intra}$. A single binding interaction cannot be a measure of cooperativity because, by definition, cooperativity is a collective property that arises from the interplay of two or more binding interactions; 2) a cooperativity factor is a ratio of two equilibrium constants, the constant in the numerator is the constant affected by cooperative interactions and the constant in the denominator is the reference. It is evident that the product $KEM$ does not satisfy this requirement. 3) Chelate cooperativity must depend on ligand concentration, whereas $KEM$ is independent of ligand concentration; 4) a consistent cooperativity factor must tend to 1 in the absence of cooperativity, whereas $KEM$ tends to zero. We have previously shown that the product $KEM$ is related to the maximum yield of a cyclic or multicyclic assembly,[10a] but this relation has no bearing on chelate cooperativity.

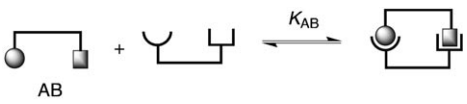


***Figure 5.*** Binding of a divalent asymmetric ligand AB to a complementary receptor.

What is, then, the correct way to assess chelate cooperativity? In fact, there are two related, but distinct, chelate cooperativity factors. The first factor is related to the overall binding (the sum of intermolecular and intramolecular binding) with respect to the intermolecular binding, and the second factor is related to only the intramolecular binding with respect to the intermolecular binding. In both cases, the intermolecular binding (Figure 3 with $EM = 0\ mol\,L^{-1}$) is used as a reference. In the absence of chelate cooperativity, the overall constant for the fully bound receptor is $4K^2$. To assess chelate cooperativity for the overall binding, we consider the total concentration of the fully saturated receptor $C_{SR}$, which is given by Equation (4).

$$C_{SR} = [AA(BB)_2] + [c - AA \cdot BB] = [AA(BB)_2]\left(1 + \frac{EM}{2[BB]}\right) \qquad (4)$$

We then consider the overall constant in the presence of chelate cooperativity as given by $4KK_{app}$, where $K_{app}$, which is defined by Equation (5), is the apparent constant for the formation of the fully saturated receptor starting from the partially bound open complex.

$$K_{app} = \frac{C_{SR}}{[o - AA \cdot BB][BB]} = K\left(1 + \frac{EM}{2[BB]}\right) \qquad (5)$$

The cooperativity factor $\beta'$ for the overall binding will be given by the ratio of the overall constant $4KK_{app}$ in the presence of the chelate interaction and the reference constant $4K^2$ [Eq. (6)].

$$\beta' = 1 + \frac{EM}{2[BB]} \qquad (6)$$

The factor $\beta'$ satisfies all the requisites for a chelate cooperativity factor: it is derived from a ratio of two equilibrium constants that have the same units, it depends on ligand concentration, and tends to 1 in the absence of chelate cooperativity, that is, when either the $EM$ tends to zero or the ligand concentration tends to infinity. The factor $\beta'$ is never smaller than 1, thus the chelate cooperativity can only be positive as far as the overall binding is concerned. The fact that $\beta'$ is larger at low ligand concentrations depends on the fact that the 1:1 cyclic complex $c$-AA·BB is increasingly more

  

stable than the fully bound 1:2 complex AA·$(BB)_2$ as the dilution is increased.

The term $EM/2$[BB] in the right-hand side of Equation (6) is the contribution of the intramolecular process to the cooperativity factor $\beta'$. The cooperativity factor $\beta$ reflects the intramolecular binding alone with respect to the intermolecular binding [Eq. (7)].

$$\beta = \frac{EM}{2[\mathrm{BB}]} \tag{7}$$

The factor $\beta$ also represents the apparent equilibrium constant for the conversion of the complex AA·$(BB)_2$ into the cyclic complex *c*-AA·BB (Figure 6). The value of $\beta$ depends

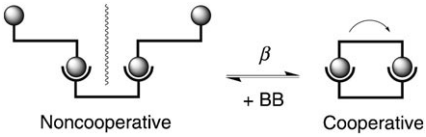


***Figure 6.*** The cooperativity factor $\beta$ represents the apparent equilibrium constant (dependent on ligand concentration) for the conversion of the complex AA·$(BB)_2$ (noncooperative) into the chelate complex *c*-AA·BB (cooperative).

on the ligand concentration: if the concentration of the divalent ligand is equal to $EM/2$, the chelate interaction is noncooperative ($\beta = 1$). Positive and negative chelate cooperativity require [BB] $< EM/2$ (i.e., $\beta > 1$) and [BB] $> EM/2$ (i.e., $\beta < 1$), respectively. Obviously, the higher the value of $EM$, the larger the concentration range over which the chelate effect displays positive cooperativity. As shown in Figure 4b, as the ligand concentration is increased, the initially assembled cyclic complex undergoes disassembly. The process of disassembly is due to a decrease in chelate cooperativity, which changes from positive to negative cooperativity when [BB] $= EM/2$.

It is interesting to generalize these results to the case of the binding of an *n*-valent ligand $^n$B to an *n*-valent receptor $^n$A to form a 1:1 multicyclic complex *c*-$^n$A·$^n$B, the constituent rings of which have identical structure so that their $EM$ values are also identical. It is assumed that the ligand is present in a large excess relative to the receptor and that $\alpha = 1$. Owing to the effect of chelate cooperativity, we can ignore the presence of intermediate states and only consider the unbound and fully bound species (Figure 7a). For the sake of illustration, the mixture of the 1:*n* fully bound open complexes in the case $n = 3$ is shown in Figure 7b. Equation (7) can be easily generalized to the multivalent case to produce Equation (8), where $\beta$ is the apparent constant for the equilibrium shown in Figure 8.

$$\beta = \frac{2}{n^n}\left(\frac{EM}{[{}^n\mathrm{B}]}\right)^{n-1} \tag{8}$$

The factor $\beta$ depends on a statistical factor multiplied by the ratio of the $EM$ to the ligand concentration, raised to the degree of cyclicity of the assembly. It is evident that although

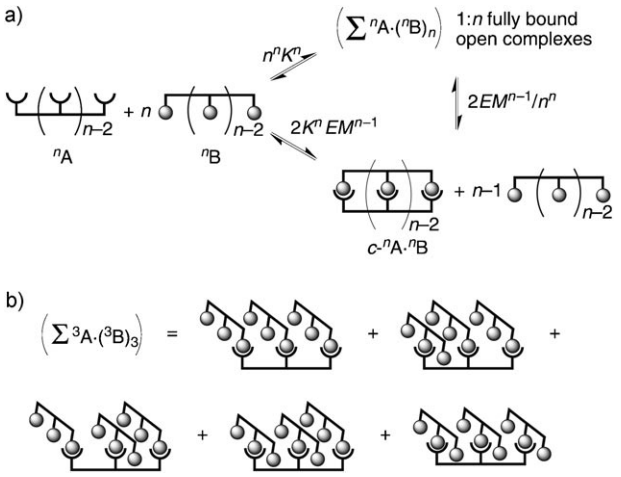


***Figure 7.*** a) Binding of an *n*-valent ligand $^n$B to an *n*-valent receptor $^n$A, assuming $[{}^n\mathrm{B}]_0 \gg [{}^n\mathrm{A}]_0$ and $\alpha = 1$. Justifications for the expressions for the equilibrium constants are in the Supporting Information. b) Mixture of the 1:*n* fully bound open complexes in the case $n = 3$.

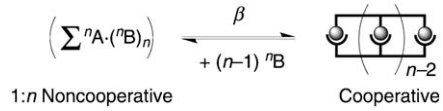


***Figure 8.*** The cooperativity factor $\beta$ represents the apparent equilibrium constant (dependent on ligand concentration) for the conversion of the mixture of complexes $^n$A·$(^n\mathrm{B})_n$ (noncooperative) into the chelate complex *c*-$^n$A·$^n$B (cooperative).

the $EM$ is not by itself a measure of chelate cooperativity, it is the key structural parameter on which $\beta$ is dependent.

As suggested in Equation (8), as the degree of cyclicity is increased, chelate cooperativity becomes more and more sensitive to ligand concentration when $\beta \approx 1$; that is, when $[{}^n\mathrm{B}] \approx (2/n^n)^{1/(n-1)}\ EM$. This behavior is clearly illustrated by the speciation profiles of the chelate complex *c*-$^n$A·$^n$B calculated for the cases $KEM = 50$ and $n = 2$, 3, 4, and 10 (Figure S1 in the Supporting Information). The profiles show that disassembly (or denaturation) of the chelate complex *c*-$^n$A·$^n$B becomes sharper and sharper as the value of $n$ increases because of an increasingly sharp drop of chelate cooperativity. This drop is accompanied by a complementary sharp increase of the fractional amount of the 1:*n* fully bound open complexes. The apparent cooperativity of the process of denaturation is in fact due to dissipation of chelate cooperativity caused by the increasing ligand concentration.

Of course, denaturation can also be carried out by addition of a monovalent ligand B to the preformed chelate complex *c*-$^n$A·$^n$B (see the Supporting Information), but this denaturation procedure does not change the physical basis of the phenomenon.

## 4. Interannular Cooperativity

Interannular cooperativity arises from the interplay of two or more intramolecular binding interactions. To illustrate the

  

phenomenon, we consider a tetravalent receptor $^4$A in which one pair of binding sites can freely rotate with respect to the other pair (Figure 9). We assume that a divalent ligand BB is

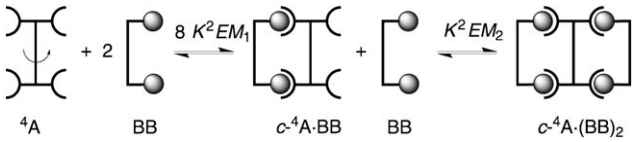


***Figure 9.*** Binding of a divalent ligand BB to a tetravalent receptor $^4$A with a free internal rotation, assuming $[BB]_0 \gg [^4A]_0$ and $\alpha = 1$.

present in a large excess relative to the receptor so that complexes involving more than one receptor molecule can be neglected, but not in such a large excess so that disassembly of the bicyclic complex $c$-$^4$A·$(BB)_2$ occurs. Moreover, we assume that $\alpha = 1$ in order to exclude allosteric cooperativity. The first ligand molecule BB binds to the receptor to form a ring that hampers the internal rotation. The binding of the second BB molecule is then much easier because the entropy loss involved in freezing the internal rotation has been already satisfied by the binding of the first molecule. Since the closure of the first ring facilitates the closure of the virtually identical second ring, there is positive cooperativity. However this type of cooperativity is not due to an increase of the affinity of the binding sites of the receptor but to an increase of the *EM* of the second ring with respect to that of the first ring. Clear-cut examples of this type of cooperativity are the double-wheel octapyridyl receptor reported by Shinkai and co-workers,[2b,18] and the bisporphyrin tetrapyridyl receptor reported by Wilson and Anderson[19] (Figure 10). Shinkai's receptor consists of two porphyrin "wheels", each of which bears four pyridinyl binding sites connected by a cerium "axle", so that the wheels can rotate relative to each other. Simultaneous binding by hydrogen bonding of a first ditopic ligand, such as (1*R*,2*R*)-cyclohexane-1,2-dicarboxylic acid, to both the wheels suppresses their internal rotational freedom so that the successive ligands are bound more efficiently. Anderson's receptor consists of two porphyrins, each of which bears two pyridinyl binding sites and can freely rotate about a buta-

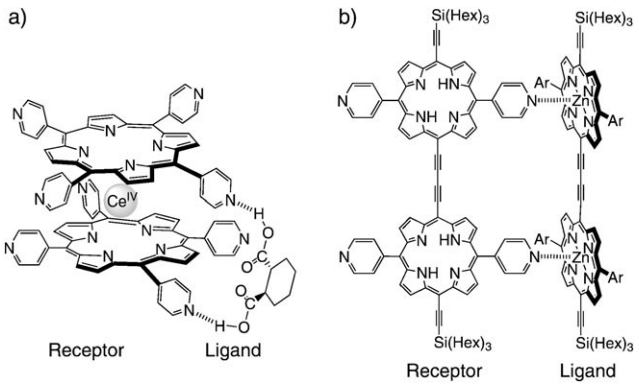


***Figure 10.*** Receptors formed from corresponding bidentate ligands reported by a) Shinkai and co-workers and b) Wilson and Anderson. Hex = hexyl.

diynyl axle. Simultaneous binding by metal coordination of a bis(zinc porphyrin) ligand makes coordination of a second ligand molecule easier because of reduced torsional motion. Of course, the freezing of torsional motion is just one of the possible mechanisms of interannular cooperativity, other mechanisms can involve either attractive or repulsive interligand interactions.

The equilibria in Figure 9 are expressed in terms of a statistical factor, a microscopic intermolecular constant $K$, and the microscopic effective molarities $EM_1$ and $EM_2$. The reference constant $K$ can be evaluated by studying the binding of the monovalent ligand B to a monovalent receptor model A, while the reference *EM* value can be evaluated by studying the binding of the ligand BB to a divalent receptor model AA (Figure 11). In the absence of interannular coop-

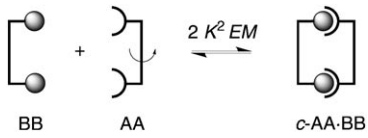


***Figure 11.*** Association of the ligand BB to a divalent receptor model AA for the evaluation of the reference *EM* value.

erativity, $EM_2 = EM_1 = EM$. With reference to Figure 9, interannular cooperativity can be quantified by a cooperativity factor that is given by the ratio of the overall experimental constant to the hypothetical overall noncooperative constant, $\gamma = EM_1EM_2/EM^2$.

To correctly evaluate the reference *EM* value, it is important to exclude the presence of allosteric cooperativity ($\alpha = 1$), or to quantify its importance if $\alpha \neq 1$, by studying the association of the monovalent ligand B to the divalent receptor model AA (Figure 1). Indeed, should the presence of an allosteric factor be ignored, the evaluation of the reference *EM* value would be affected, with the result that allosteric cooperativity would go either unnoticed or misinterpreted as interannular cooperativity.

Since the concepts of interannular and chelate cooperativity are subtle and could be confused, it is worth noting that: 1) while chelate cooperativity arises from the mere presence of one or more independent intramolecular binding interactions, interannular cooperativity depends on the interplay of two or more such interactions; 2) interannular cooperativity necessarily implies the presence of chelate cooperativity, but the opposite is not true; 3) interannular cooperativity, in contrast with chelate cooperativity, is independent of ligand concentration.

## 5. Stability of an Assembly

The overall equilibrium constant $K_{sa}$ for the formation of an assembly starting from its constituent building blocks often depends on a plurality of intermolecular and intramolecular interactions, thus it is very useful to establish theoretical models that allow the prediction of the stability of an assembly on the basis of the knowledge of the single

  

elementary interactions. By taking into account the various types of cooperativity discussed so far, a master equation for the stability of an assembly can be formulated [Eq. (9)] that, depending on the specific case at hand, gives rise to a number of theoretical models.

$$K_{sa} = \alpha\gamma K_\sigma K^b EM^c \quad (9)$$

The parameters that appear in Equation (9) have the following meanings: $\alpha$ and $\gamma$ are cooperativity factors that account for overall allosteric and interannular cooperativity, respectively; $K_\sigma$ is the statistical factor of the assembly process and can be easily evaluated on the basis of the symmetry numbers of the assembly and of its constituent building blocks;[8,9] $K$ and $EM$ are the key reference parameters, the first parameter corresponds to the strength of the single binding interaction and the second, in the case of a cyclic or multicyclic assembly, corresponds to the ease of formation of the reference cyclic structure; these parameters can be obtained experimentally by suitable models; $b$ is the number of binding interactions that join the building blocks together; and $c$ is the degree of cyclicity of the assembly given by $b-i+1$, where $i$ is the number of building blocks.[9,10a,13] On the basis of the values of these parameters several models can result:

1) The noncooperative model ($\alpha=\gamma=1$, $c=0$). This model applies to assemblies that involve only intermolecular interactions without any allosteric effect. The occupation of the various binding sites of the receptor is dictated only by statistics. This model is the reference for spotting the presence of allosteric effects in real systems, and also applies to the formation of a given oligomer in isodesmic polymerizations. This process is exemplified by a monomer A–B that undergoes a reversible polymerization in which all of the stepwise association constants are identical and equal to $K$. The formation constant of each oligomer $(A–B)_i$ is given by Equation (9) in which $\alpha=\gamma=1$, $c=0$, $K_\sigma=1$, and $b=i-1$.[10a,20]

2) The allosteric cooperative model ($\alpha\neq1$, $\gamma=1$, $c=0$). This model is typical of cooperative systems, such as hemoglobin, which involve only intermolecular interactions. This model also applies to the reversible formation of oligomers under the condition of different stepwise association constants, as in the case of nucleation-growth polymerizations.[20]

3) The chelate cooperative model ($\alpha=\gamma=1$, $c>0$). This model applies to cyclic and multicyclic assemblies in which the constituent cyclic units are identical. Every cyclic or multicyclic assembly benefits from chelate cooperativity that measures the stability of the assembly with respect to the corresponding fully saturated open receptor. Chelate cooperativity, in contrast with allosteric and interannular cooperativity, is concentration-dependent, and decreases on increasing the concentration of the ligand up to change from favorable ($\beta>1$) to unfavorable ($\beta<1$), thus promoting disassembly (denaturation) at high ligand concentrations. The chelate cooperative model depends on two reference parameters, $K$ and $EM$, that allow the calculation of the hypothetical chelate cooperative constant of the assembly by using Equation (9), assuming $\alpha=\gamma=1$. The experimental stability constants of a number of assemblies of different topologies, that is, helicates,[21] ladders,[22] and $D_{3h}$[23] and $D_{4h}$[24] symmetrical cages, are consistent with those calculated by the chelate cooperative model, thus all these cases confirm the absence of other cooperative effects.[9,10a,13] This result makes the chelate cooperative model a powerful instrument for predicting self-assembly behavior.

4) The allosteric–chelate cooperative model ($\alpha\neq1$, $\gamma=1$, $c>0$). In this model, both allosteric and chelate cooperativity are taken into account. The factor $\alpha$ can be evaluated by studying the interaction of the receptor with a monovalent ligand. Alternatively, provided that interannular cooperativity can be excluded, $\alpha$ can be obtained as the ratio of the experimental self-assembly constant to that of the chelate cooperative model. A further approach consists in defining a theoretical function for the factor $\alpha$. This approach has been pioneered by Piguet and co-workers for investigating the stability of self-assembled polynuclear complexes such as metallohelicates.[2e] Their model, which is dubbed the "extended site binding model", can be considered within the framework of the allosteric-chelate cooperative model with the factor $\alpha$ given by Equation (10), where $\Delta E_k^{M,M}$ accounts for intermetallic interactions arising from coulombic effects between metal ions in solution, and $\Delta E_l^{L,L}$ accounts for the fact that ligands have different binding affinities for successive attachments to the same metal ion.

$$\alpha = \prod_k \exp(-\Delta E_k^{M,M}/RT) \prod_l \exp(-\Delta E_l^{L,L}/RT) \quad (10)$$

Often, however, the opposing contributions of similar magnitudes brought by coulombic and solvation effects within the homocomponent interactions $\Delta E_k^{M,M}$ and $\Delta E_l^{L,L}$ produce negligible allosteric cooperative effects (slightly positive or negative),[25] thus justifying, in many cases, the use of the simple chelate cooperative model.

5) The interannular–chelate cooperative model ($\alpha=1$, $\gamma\neq1$, $c>0$). This model takes into account both interannular and chelate cooperativity. Provided that allosteric cooperativity can be excluded a priori or by the study of the interaction of the receptor with a monovalent ligand, the factor $\gamma$ can be obtained as the ratio of the experimental self-assembly constant to that of the chelate cooperative model. Typical assemblies to which this model can be applied are those of Shinkai and Anderson (Figure 10).

6) The allosteric–interannular–chelate cooperative model ($\alpha\neq1$, $\gamma\neq1$, $c>0$). This is the most general model, in which all the possible types of cooperativity are taken into account. To separate the product $\alpha\gamma$ into its components, the factor $\alpha$ must be evaluated separately by studying the interaction of the receptor with a monovalent ligand. At present there are no clear-cut examples in which all of the three types of cooperativity have been evidenced and quantified.

In conclusion, how should cooperativity in self-assembly be assessed? We have mentioned that three types of cooperativity should be considered: allosteric cooperativity ($\alpha$), chelate cooperativity ($\beta$), and interannular cooperativity ($\gamma$). While the presence of chelate cooperativity is immedi-

  

ately evident on the basis of the cyclic or multicyclic nature of the assembly, allosteric and interannular cooperativity need reference models or traditional plots to be spotted (binding isotherms, Scatchard plots, and Hill plots).[7] In any case, Equation (9) provides the conceptual framework to quantitatively assess cooperativity in self-assembly. The concepts illustrated here should pave the way for further advances related to the theoretical prediction of the key cooperative factors $\alpha$, $\beta$, and $\gamma$. The model for $\alpha$ proposed by Piguet and co-workers is a first step in this direction,[2e,25] hopefully other steps will follow.

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[6] This procedure is strictly valid for homotropic cooperativity (cooperative binding of more molecules of the same ligand). In the case of heterotropic cooperativity (cooperative binding of different ligand molecules), more than a single reference interaction must be defined. For a recent example of heterotropic cooperativity, see: A. B. C. Deutman, C. Monnereau, M. Moalin, R. G. E. Coumans, N. Veling, M. Coenen, J. M. M. Smits, R. de Gelder, J. A. A. W. Elemans, G. Ercolani, R. J. M. Nolte, A. E. Rowan, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 10471–10476.

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[8] For a generic equilibrium $a\,A + b\,B \rightleftarrows c\,C$, if $\sigma_A$, $\sigma_B$, and $\sigma_C$ are the symmetry numbers of A, B, and C, respectively, the statistical value of the equilibrium constant is given by the factor $K_\sigma = (\sigma_A)^a(\sigma_B)^b/(\sigma_C)^c$. The symmetry number of a molecule is defined as the total number of independent permutations of identical atoms or groups in a molecule that can be arrived at by simple rotations of the entire molecule or by rotations about single bonds within the molecule. If a species participating in the equilibrium is chiral and present as racemic mixture, the symmetry number must be multiplied by $^1/_2$ to account for the entropy of mixing of the two enantiomers. Alternatively, the statistical value of an equilibrium constant can be evaluated as the ratio of the number of microspecies of products to that of reactants. For a recent discussion on statistical factors, see Ref. [9] and references therein.

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[11] Often the symbol *EM* is used to indicate the effective molarity, which is the product of the microscopic effective molarity by the statistical factor of the cyclization process. Following the example of Hunter and Anderson (Ref. [4]), we use here the symbol *EM* for the microscopic effective molarity.

[12] One of us has argued against the general tendency to consider the chelate effect as a cooperative effect, on the basis that the driving force provided by the chelate effect, in contrast with allosteric cooperativity, is concentration-dependent (Ref. [13]). For many chemists, this argument is not sufficiently strong to exclude the chelate effect from the sources of cooperativity (see, for example, Refs. [3] and [4]). In fact we have no difficulty to acknowledge this widespread opinion and to embrace the concept of chelate cooperativity, provided, however, that its concentration dependence is recognized.

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