

Assessment of Cooperativity in Self-Assembly

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Abstract: A method has been proposed to assess cooperativity in self-assembly processes. The method is based on a clear distinction between intermolecular and intramolecular processes which are compared with the corresponding reference reactions. It has been applied to two classical cases, namely the self-assembly of helicates and of porphyrin ladders, by using data previously published by the groups of Lehn and Anderson, respectively. Contrarily to the conclusions of the authors, pointing out self-assembly processes driven by positive cooperativity, the method here presented indicates in both cases the absence of cooperative effects. The methods previously used to assess cooperativity, in particular Scatchard plot and/or Hill plot, are criticized as being inappropriate for self-assembly, because they are pertinent to a specific case only, namely the intermolecular binding of a monovalent ligand L to a multivalent receptor M, a case very different from self-assembly which involves both inter- and intramolecular interactions. The present method underscores the fact that positive cooperativity in artificial self-assembling systems is probably much more rare than it was previously thought.

Introduction

Cooperativity takes place when the binding of one ligand influences the binding strength of a macromolecule toward a subsequent ligand (or ligands). This effect, which is the basis of enzyme control and many other vital biological processes,¹ has been rigorously defined in the case of multiple intermolecular binding of a monovalent ligand to a polyvalent macromolecule.^{1,2} On the contrary, cooperativity, in the case of intramolecular binding of a polyvalent ligand, has not been adequately defined, and much confusion emerges from the literature, especially in the field of supramolecular chemistry where multiple intramolecular interactions play a key role. This is particularly true in the case of self-assembly,³ which in recent years has developed as one of the central themes of supramolecular chemistry. In fact, it has been demonstrated that formation of defined linear oligomers by self-assembly is not possible,⁴ and thus intramolecular binding appears as a necessary prerequisite for self-assembly in solution.^{5,6} Needless to say, cooperativity could play a crucial role in its success.

Along with artificial self-assembling systems, polyvalent intramolecular interactions, possibly involving cooperativity, are widespread in biological systems, but only recently, as the importance of interactions involving multiple proteins and ligands has begun to be unraveled, their study has become a new focus of inquiry in molecular biochemistry.⁷

Here, a critical examination of the meaning of cooperativity in the case of multiple intramolecular binding and a method for the assessment of positive cooperativity in self-assembly and other polyvalent intramolecular processes are presented.

Theory and Discussion

Background. Before facing the problem of cooperativity in the case of multiple intramolecular binding, it is useful to briefly review some concepts regarding multiple intermolecular binding of a monovalent ligand to a polyvalent macromolecule.²

Consider two binding sites, $-A$ and $-B$, each capable of reacting with the other only in a reversible addition reaction

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- (2) (a) Connors, K. A. *Binding Constants: The Measurement of Molecular Complex Stability*; Wiley: New York, 1987; Chapter 2. (b) Perlmutter-Hayman, B. *Acc. Chem. Res.* **1986**, *19*, 90–96.

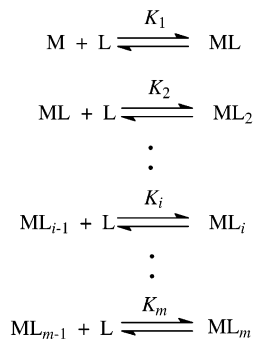
- (3) For recent reviews on self-assembly, see: (a) Lehn, J.-M. *Supramolecular Chemistry: Concepts and Perspectives*; VCH Publishers: Weinheim, 1995. (b) Whitesides, G. M.; Simanek, E. E.; Mathias, J. P.; Seto, C. T.; Chin, D. N.; Mammen, M.; Gordon, D. M. *Acc. Chem. Res.* **1995**, *28*, 37–44. (c) Lawrence, D. S.; Jiang, T.; Levett, M. *Chem. Rev.* **1995**, *95*, 2229–2260. (d) Philp, D.; Stoddart, J. F. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1154–1196. (e) *Comprehensive Supramolecular Chemistry*; Atwood, J. L., Davies, J. E. D., MacNicol, D. D., Vögtle, F., Sauvage, J.-P., Hosseini, M. W., Eds.; Pergamon: Oxford, 1996; Vol. 9. (f) Zeng, F. W.; Zimmerman, S. C. *Chem. Rev.* **1997**, *97*, 1681–1712. (g) Linton, B.; Hamilton, A. D. *Chem. Rev.* **1997**, *97*, 1669–1680. (h) Conn, M. M.; Rebek, J. *Chem. Rev.* **1997**, *97*, 1647–1668. (i) Fujita, M. *Chem. Soc. Rev.* **1998**, *27*, 417–425. (j) Caulder, D. L.; Raymond, K. N. *J. Chem. Soc., Dalton Trans.* **1999**, 1185–1200. (k) Leininger, S.; Olenyuk, B.; Stang, P. J. *Chem. Rev.* **2000**, *100*, 853–908. (l) Lindoy, L. F.; Atkinson, I. *Self-Assembly in Supramolecular Systems (Monographs in Supramolecular Chemistry)*; Stoddart, J. F., Ed.; The Royal Society of Chemistry: Cambridge, U.K., 2000. (m) *Molecular Self-Assembly (Structure and Bonding)*; Fujita, M., Ed.; Springer: Heidelberg, 2000; Vol. 96. (n) Swiegers, G. F.; Malefetsse, T. J. *Chem.—Eur. J.* **2001**, *7*, 3636–3643. (o) Albrecht, M. *Chem. Rev.* **2001**, *101*, 3457–3497. (p) Rowan, S. J.; Cantrill, S. J.; Cousins, G. R. L.; Sanders, J. K. M.; Stoddart, J. F. *Angew. Chem., Int. Ed.* **2002**, *41*, 898–952. (q) Seidel, S. R.; Stang, P. J. *Acc. Chem. Res.* **2002**, *35*, 972–983. (r) *Supramolecular Chemistry and Self-Assembly*, Special Issue. *Science* **2002**, *295*, 2395–2421.
- (4) Ercolani, G. *J. Chem. Soc., Chem. Commun.* **2001**, 1416–1417.
- (5) Ercolani, G. *J. Phys. Chem. B* **1998**, *102*, 5699–5703.
- (6) Ercolani, G. *J. Phys. Chem. B* **2003**, *107*, 5052–5057.
- (7) Mammen, M.; Choi, S.-K.; Whitesides, G. M. *Angew. Chem., Int. Ed.* **1998**, *37*, 2754–2794.

characterized by the microscopic intermolecular equilibrium constant K_{inter} (eq 1).



Now, consider the interaction between a substrate M having m identical and independent binding sites $-B$, and a ligand L with a unique binding site $-A$. A series of stepwise equilibria can be written (Scheme 1).

Scheme 1



It is useful to remark that the various equilibria, apart from the number of ligand molecules already bound to the substrate, are virtually identical and expressed, therefore, by equilibrium constants having the same dimensions, namely (concentration)⁻¹. On purely statistical grounds, it is easy to show that eq 2 holds

$$K_i = K_{\text{inter}} (m - i + 1)/i \quad (2)$$

so that

$$\frac{K_{i+1}}{K_i} = \frac{i(m - i)}{(i + 1)(m - i + 1)} \quad (3)$$

The ratio K_{i+1}/K_i provides a criterion to assess cooperativity: if the equality in eq 3 is satisfied, the binding is *noncooperative or statistical*; if the binding of a ligand favors the binding of a subsequent ligand, K_{i+1}/K_i is higher than it would be for statistical binding, then we speak of *positive cooperativity*; on the contrary, when the binding of a ligand impedes the binding of a subsequent ligand, then K_{i+1}/K_i is lower than the value predicted by eq 3, *negative cooperativity* having taken place. There are other equivalent tests to assess cooperativity, mainly graphical ones, based on the calculation of the *occupancy* r , that is to say the average number of occupied sites of M. It is easy to show that the occupancy is given by eq 4

$$r = \frac{\sum_{i=1}^m i\beta_i[L]^i}{1 + \sum_{i=1}^m \beta_i[L]^i} \quad (4)$$

where β_i are the overall binding constants ($\beta_i = \prod_j^i K_j$) and $[L]$ is the free ligand concentration. If the binding is statistical, i.e., if eq 2 holds, it can be demonstrated that eq 4 reduces to the

binding isotherm shown in eq 5,

$$r = \frac{mK_{\text{inter}}[L]}{1 + K_{\text{inter}}[L]} \quad (5)$$

that can be put in the linear form known as the Scatchard equation^{2,8}

$$\frac{r}{[L]} = -K_{\text{inter}}r + mK_{\text{inter}} \quad (6)$$

or in the linear form known as the Hill equation^{2,9}

$$\log\left(\frac{r}{m-r}\right) = \log[L] + \log K_{\text{inter}} \quad (7)$$

Since all of the eqs 5–7 describe noncooperative behavior, plots that deviate from the above equations are diagnostic for cooperativity. Positive or negative deviations from the binding isotherm (eq 5), which indicate positive or negative cooperativity, respectively, are not always recognizable, unless an independent evaluation of K_{inter} from monofunctional model compounds is available. A plot of $r/[L]$ as a function of r (Scatchard plot) is a much better diagnostic because it presents, respectively, a concave downward curve, a straight line, and a concave upward curve for positive cooperativity, noncooperativity, and negative cooperativity. Plots of $\log[r/(m-r)]$ vs $\log[L]$ (Hill plots) are also widely applied as aids in the study of cooperative binding; apart from the case of noncooperativity that is evidenced by a straight line of unit slope, cooperativity manifests itself as two lines of unit slope connected by an S-shaped curve. The value of the slope in the central region of the curve is called the Hill coefficient (n_H). Values of $n_H > 1$ and < 1 are diagnostic for positive cooperativity and negative cooperativity, respectively. Since n_H can vary between 0 and m , it provides a quantitative measure of cooperativity.

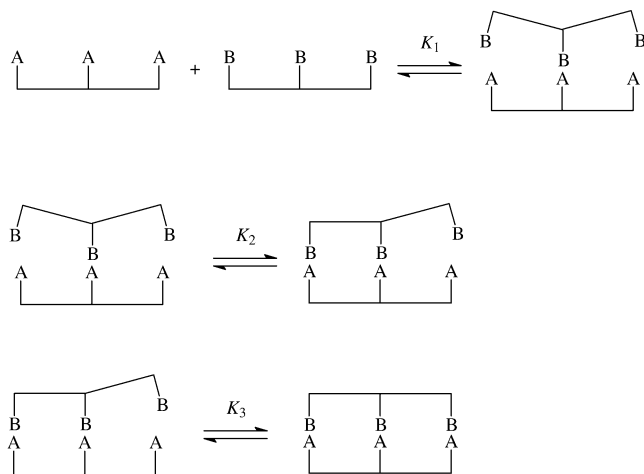
Cooperativity in Self-Assembly. “Self-assembly” is a broad term that encompasses a number of diverse subjects ranging from the noncovalent association of organic molecules in solution to the growth of semiconductor quantum dots on solid substrates.^{3r} Lehn in his seminal book on supramolecular chemistry defined supramolecular self-assembly as “the spontaneous association of either a few or many components resulting in the generation of either discrete oligomolecular supermolecules or extended polymolecular assemblies such as molecular layers, films, membranes, etc.”.^{3a} In the present work, the term self-assembly refers to the first of the two alternatives indicated by Lehn, namely the spontaneous generation in solution of a well-defined, discrete supramolecular architecture from a given set of components under thermodynamic equilibration. To speak of cooperativity in self-assembly, the formed complex must be polycyclic with a clear pattern of identical cyclic substructures. An example will suffice: consider two ligands with three complementary binding sites $-A$ and $-B$. Among the possible pathways for self-assembly, consider that reported in Scheme 2.

The constant K_1 is an intermolecular constant having dimensions mol⁻¹ L, whereas the constants K_2 and K_3 are dimensionless intramolecular constants. The case illustrated in the previous

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(9) (a) Hill, A. V. *J. Physiol. (London)* **1910**, *40*, 4. (b) Hill, A. V. *Biochem. J.* **1913**, *7*, 471.

Scheme 2



section suggests that, to assess cooperativity, only virtually identical processes described by equilibrium constants having the same dimensions should be compared. Therefore intermolecular and intramolecular processes should be considered as forming two distinct groups within which cooperativity is assessed independently. In the example illustrated in Scheme 2, since there is only one intermolecular interaction, the processes relevant to cooperativity are those intramolecular. Thus if the closure of the first ring facilitates the closure of the virtually identical second ring, there is positive cooperativity. If, for the sake of simplicity, we consider the bond between $-A$ and $-B$ as nonfreely rotatable so as to ignore the contribution of the internal symmetry number, the first cyclization is statistically favored by a symmetry factor 2,¹⁰ whereas the second is statistically disfavored by the same factor; thus positive cooperativity would require $K_3/K_2 > 1/4$. In most cases, however, it is difficult, if not impossible, to extract the relevant stepwise equilibrium constants, whereas the overall self-assembly constant is generally more accessible experimentally. It would be useful, therefore, to devise a method to assess cooperativity that is based on the overall constant and on data available from simple reference compounds.

Let us consider two building blocks, L, having l binding sites $-A$, and M, having m binding sites $-B$. The two building blocks are predisposed to yield the assembly S, whose molecular formula is $L_{pm}M_{pl}$, where p is a coefficient needed to account for the stoichiometry of L and M within S. The overall self-assembly equilibrium is shown in eq 8, and a cartoon showing the self-assembly of a cube is reported, by way of illustration, in Figure 1.



The number of molecules in the assembly, N , is equal to $pl + pm$, and the number of bonds joining them, B , is equal to plm . To join pmL and pIM molecules, $N - 1$ intermolecular bonds are required. Because the assembly of S requires in total B bonds, the number of intramolecular bonds is $B - N + 1$.

Let us consider the reference reactions for the formation of the intermolecular and intramolecular bonds, respectively. The intermolecular reference reaction is that occurring between monofunctional reactants, as reported in eq 1, which allows the evaluation of K_{inter} . The intramolecular reference reaction

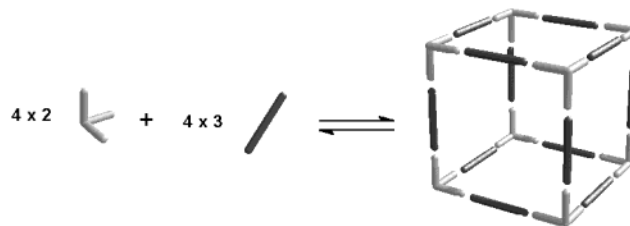
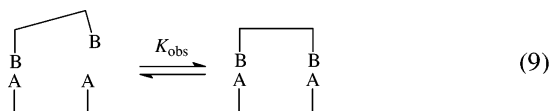


Figure 1. Self-assembly of a cube by two predisposed building blocks. In the example shown, $p = 4$, $m = 2$, and $l = 3$.

consists of the ring closure process yielding the cyclic substructure characteristic of the assembly. For example, in the case of the self-assembly process reported in Scheme 2, the intramolecular reference reaction would be that reported in eq 9.



The microscopic (statistically corrected) intramolecular constant K_{intra} is given by $K_{\text{obs}}/\sigma_{\text{intra}}$, where σ_{intra} is the symmetry factor of the reaction.¹⁰ In the present case, $\sigma_{\text{intra}} = 1/2$. Having defined the microscopic intermolecular and intramolecular constants, we can evaluate the statistical or noncooperative self-assembly equilibrium constant K_S by eq 10.

$$K_S = \sigma_{\text{sa}} K_{\text{inter}}^{N-1} K_{\text{intra}}^{B-N+1} \quad (10)$$

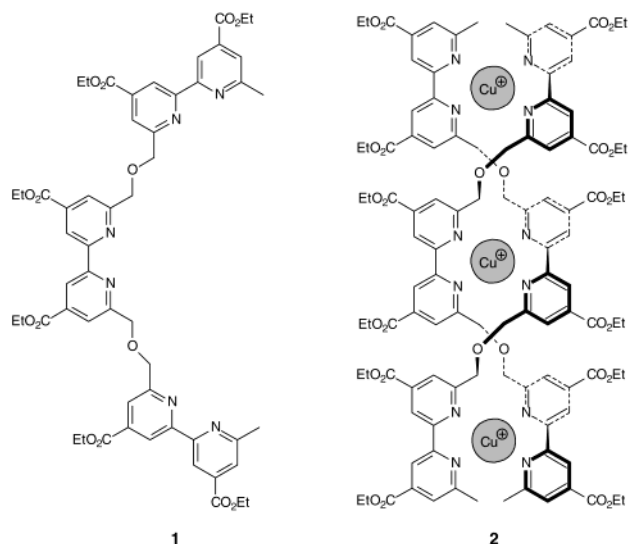
where σ_{sa} , equal to $\sigma_L^{pm}\sigma_M^{pl}/\sigma_S$, is the symmetry factor of the self-assembly equilibrium.¹⁰ An additional factor of 2 multiplying the right-hand side of eq 10 should be considered if the assembly S is chiral and the reagents are achiral. This correction would account for the entropy of mixing the two enantiomers (ref 10, p 177).

Positive cooperativity, noncooperativity, and negative cooperativity would be evidenced by an experimental K_S value greater, equal, and lower, respectively, than that predicted by eq 10. In the example shown in Scheme 2, eq 10 yields $K_S = 2 K_{\text{inter}}K_{\text{intra}}^2$. Considering the symmetry factors of the equilibria in Scheme 2, it is easy to show that $K_1 = 2K_{\text{inter}}$ (remember that we have considered the bond between $-A$ and $-B$ nonfreely rotatable), $K_2 = 2K_{\text{intra}}$, and $K_3 = K_{\text{intra}}/2$. Since $K_S = K_1K_2K_3$, it follows that eq 10 is consistent with our previous criterion for self-assembly based on the ratio K_3/K_2 ; indeed if $K_3 > K_{\text{intra}}/2$, then $K_3/K_2 > 1/4$ and $K_S > 2K_{\text{inter}}K_{\text{intra}}^2$.

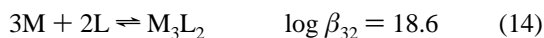
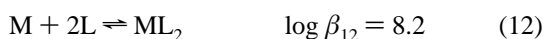
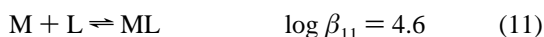
(10) The symmetry factor of an equilibrium is easily calculated on the basis of the symmetry number of the species participating in the process. The symmetry number of a molecule, σ , is defined as the total number of independent permutations of identical atoms or groups in a molecule that can be arrived at by simple rotations of the entire molecule or by rotations about freely rotating single bonds within the molecule. In practice, the symmetry number is found by multiplying the symmetries of the independent axes. These axes may be of two types, external or internal. External axes generate an identical arrangement of atoms by rigid rotation of the molecule as a whole; internal axes do so by rotations arounds bonds within the molecule. The symmetry number affects the entropy of the molecule by a factor $-R \ln \sigma$. Therefore, for a generic equilibrium $aA + bB \rightleftharpoons cC$, if σ_A , σ_B , and σ_C are the symmetry numbers of A, B, and C, respectively, the effect of their symmetry on the equilibrium constant is given by the factor $\sigma_A^a\sigma_B^b/\sigma_C^c$. Lowry, T. H.; Richardson, K. S. *Mechanism and Theory in Organic Chemistry*, 3rd ed.; Harper & Row: New York, 1987; pp 175–177.

In the following, we will apply the method illustrated here to the self-assembly of helicates and double-strand porphyrin ladders, two classical cases which have been previously investigated quantitatively.

Self-Assembly of Helicates. Among the most fascinating assemblies that have been reported in the literature, the helicates, first due to the ingenuity of Lehn and co-workers, occupy a preeminent position.^{3a,11} They also represent one of the few examples in which cooperativity has been investigated in detail. Lehn and Pfeil carried out an analysis of the binding of Cu^I ions to the tris-bipyridine ligand **1** and concluded that the assembly of the resulting trihelicate **2** is a self-organization process displaying positive cooperativity.¹²

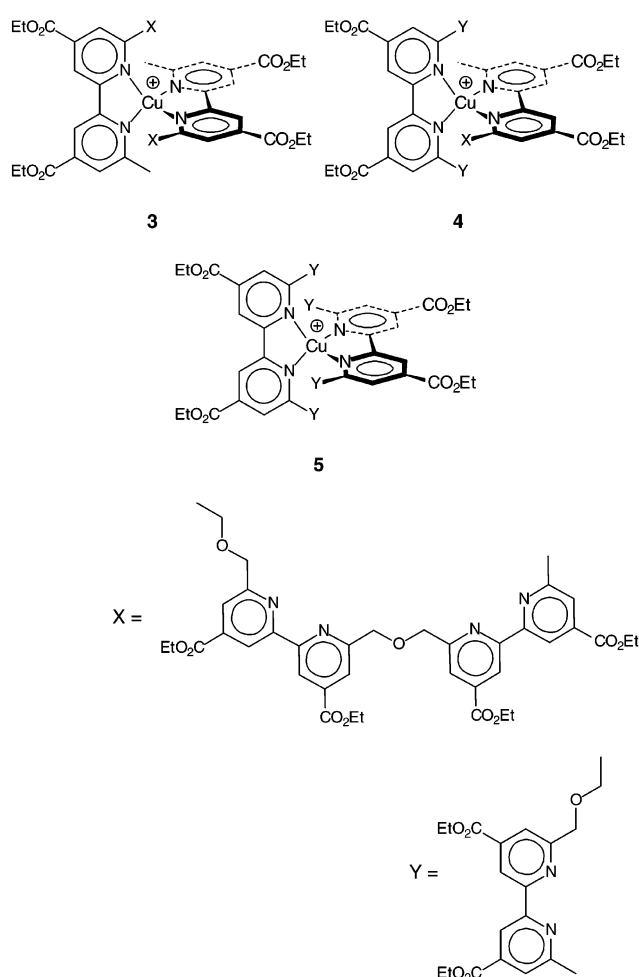


Spectrophotometric titration data were analyzed in terms of four equilibria (eqs 11–14 in which M = Cu⁺ and L = **1**) whose stability constants were obtained with an estimated error on log β_{ij} of $\pm 10\%$.¹³

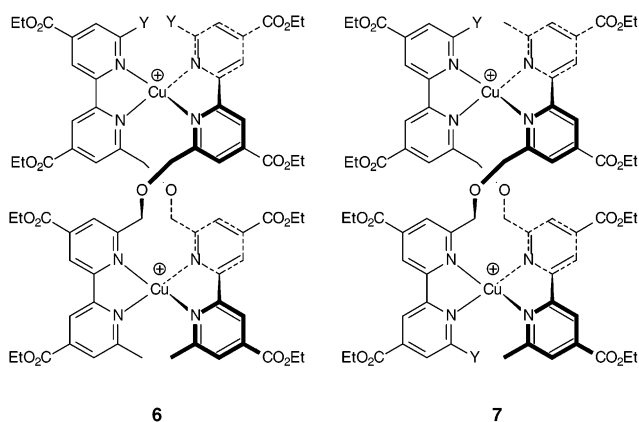


It is interesting to apply the approach illustrated in the previous section to the above data to disclose the possible cooperative behavior in the formation of trihelicate **2**. Unfortunately, the above data do not refer to simple model systems; however, since eq 10 is valid also for preassembly equilibria, we can extract from the intermediate β_{ij} values the necessary information. We consider as the reference intermolecular process the simultaneous binding of a Cu⁺ ion to two bipyridine ligands. An estimate of the constant K_{inter} can be obtained from the constant β_{12} by considering the ML₂ species, **3–5**, that are formed in equilibrium 12.

- (11) Constable, E. In *Comprehensive Supramolecular Chemistry*; Atwood, J. L., Davies, J. E. D., MacNicol, D. D., Vögtle F., Sauvage, J.-P., Hosseini, M. W., Eds.; Pergamon: Oxford, 1996; Vol. 9, pp 213–252.
 (12) Pfeil, A.; Lehn, J.-M. *J. Chem. Soc., Chem. Commun.* **1992**, 838–840.
 (13) Although in the original paper (ref 12) β_{ij} values were distractedly associated with stepwise binding constants, it is evident from the context that they refer to overall binding constants.



The complex **3** (point group C_2) has symmetry number 2, but since it is chiral, this effect is compensated by the entropy of mixing of the enantiomers, thus considering that the ligand **1** (point group C_{2v}) has symmetry number 2, the equilibrium constant for the formation of **3** is $4K_{\text{inter}}$. The complex **4** (point group C_s) has symmetry number 1 and is achiral; thus the equilibrium constant for its formation is $4K_{\text{inter}}$. The complex **5** (point group D_{2d}) has symmetry number 4 and is achiral; thus it forms with a constant K_{inter} . From this analysis, it can be concluded that $\beta_{12} = 9K_{\text{inter}}$, from which $\log K_{\text{inter}} = 7.25$. An estimate of the constant K_{intra} can be obtained from β_{22} . Considering the equilibrium 13 in detail, the M_2L_2 species **6** and **7** can form.



We have deliberately ignored the formation of the M_2L_2 species in which the two Cu^+ ions occupy the first and the third binding site of the two ligands because this species would involve the formation of a larger ring that is entropically disfavored according to the Jacobson–Söckmayer theory.¹⁴ Both complex **6** and **7** are chiral (point group C_2) and have symmetry number 2; thus according to eq 10, their formation, respectively, occurs with a constant $4K_{inter}K_{intra}$. Therefore, $\beta_{22} = 8K_{inter}K_{intra}$, from which $\log K_{intra} = 5.35$. Now let us consider the formation of the fully formed assembly **2**; since it is chiral (point group D_2) and has symmetry number 4, application of eq 10 yields $K_S = \beta_{32} = 2K_{inter}K_{intra}^2$. With the obtained values of K_{inter} and K_{intra} , we can calculate the statistical or noncooperative value of $\log \beta_{32}$ as 18.25. Since the experimental value of $\log \beta_{32}$ is 18.6 with an estimated error of $\pm 10\%$, we can conclude that *the self-assembly of the trihelicate 2 is noncooperative within the experimental errors.*

But why did Pfeil and Lehn reach opposite conclusions? Their analysis was based on three tests, namely eq 3, the Scatchard plot, and the Hill plot, but all of these tests are valid indicators of cooperative behavior only if, in the absence of cooperativity, all the stepwise equilibrium constants follow eq 2. This requires, *inter alia*, that all the stepwise constants have the same dimensions and refer to virtually identical intermolecular processes (Scheme 1). It is evident that this condition is not realized in an assembly process where part of the stepwise constants are intramolecular. The conclusion is that all of the three tests used by Pfeil and Lehn to assess cooperativity in the self-assembly of the trihelicate **2** are without theoretical foundation.

To see the question in more detail, let us extract from eqs 12–14 the following stepwise equilibrium constants



By applying eq 3 with $m = 3$ (number of binding sites for the metal ion), Pfeil and Lehn concluded that since $K_3/K_2 > 1/3$, this is sufficient for indicating positive cooperativity.¹⁵ This conclusion is not correct for two reasons. The first and more fundamental one is that the application of eq 3 with $m = 3$ requires the existence of a process leading to ML_2 virtually identical to those described by eqs 15 and 16 and whose constant K_1 would yield a K_2/K_1 value consistent with eq 3. In fact, this process does not exist, because the process leading to ML_2 (eq 12) is radically different from the previous ones, even as far as the dimensions of the constant K_1 (equal to β_{12}) are concerned. This implies that the statistical value $K_3/K_2 = 1/3$ is erroneous. The second reason is that even if this statistical value were correct, the experimental errors in the constants are so large that one cannot conclude that $K_3/K_2 > 1/3$. Indeed passing to the logarithms, one obtains

$$\log(K_3/K_2) = \log \beta_{12} + \log \beta_{32} - 2 \log \beta_{22} \quad (17)$$

Even considering an error on the $\log \beta_{ij}$ of ± 0.1 , much lower than the estimated $\pm 10\%$, from the theory of error propagation, one obtains that $\log(K_3/K_2) = -0.2 \pm 0.4$ that is not greater, within the experimental error, than $\log(1/3) = -0.48$.

In fact, the correct statistical value of $\log(K_3/K_2)$ can be obtained by substituting in eq 17 the statistical expressions obtained by us: $\beta_{12} = 9K_{inter}$, $\beta_{22} = 8K_{inter}K_{intra}$, and $\beta_{32} = 2K_{inter}K_{intra}^2$, yielding a value of -0.55 , that again is not lower than $\log(K_3/K_2)$ within the experimental error.

Pfeil and Lehn also reported a Scatchard plot with an impressive downward curvature that was interpreted in terms of positive cooperativity, but is it appropriate to use the Scatchard plot (as well as the Hill plot) in self-assembly? A curvature in the Scatchard plot is significant only if it represents a deviation from the noncooperative linear behavior expressed by eq 6. But eq 6 can be obtained only if the stepwise constants obey eq 2. We have shown that this statistical behavior cannot be followed in self-assembly because it is not even dimensionally correct; thus the use of the Scatchard plot in this context is deceptive because it will always show a fake curvature, devoid of any meaning, whatever the values of the stepwise constants. The same arguments apply to the Hill plot as well, because the noncooperative linear Hill equation (eq 7) also depends on the statistical behavior expressed by eq 2.

The present remarks also apply to the self-assembly of silver(I) trihelicates¹⁶ as well as to a recent claim of positive cooperativity in the self-assembly of trimetallic lanthanide helicates.¹⁷

Self-Assembly of Porphyrin Ladders. In an excellent paper by Anderson and Taylor, a careful study was reported of the self-assembly of a series of zinc porphyrin oligomers, from the monomer through to the hexamer, with a bidentate ligand (DABCO), to form stable ladder complexes as shown in Scheme 3.¹⁸

Experimental equilibrium constants K_S obtained in both toluene and chloroform are reported in Table 1.

The equilibrium in Scheme 3 with $n = 1$ can be taken as reference to estimate the constant K_{inter} . Considering that the symmetry factor of the reaction in this case is 1, $K_S = K_{inter}^2$, from which $\log K_{inter} = 4.85 \pm 0.02$ in toluene and 4.35 ± 0.02 in chloroform. Application of eq 10 to the equilibria shown in Scheme 3 with $n > 1$ gives the following general formula

$$K_S = 2^n K_{inter}^{n+1} K_{intra}^{n-1} \quad (18)$$

The case $n = 2$ can be taken as reference for the intramolecular reaction and used to estimate $\log K_{intra}$ (4.85 ± 0.16 in toluene and 4.62 ± 0.16 in chloroform). Knowing K_{inter} and K_{intra} , the statistical constants can be calculated by eq 18 in all the other cases; the results are reported in Table 1 together with the corresponding errors calculated by the theory of error propagation. The accordance between experimental and statistical $\log K_S$ values is extraordinarily good. Overall these data

(14) (a) Jacobson, H.; Söckmayer, W. H. *J. Chem. Phys.* **1950**, *18*, 1600–1606. (b) Ercolani, G.; Mandolini, L.; Mencarelli, P.; Roelens, S. *J. Am. Chem. Soc.* **1993**, *115*, 3901–3908. (c) This conclusion is valid as long as the smaller ring in **6** and **7** is devoid of significant strain factors that in principle could also arise from electrostatic repulsions between the two Cu^+ ions.

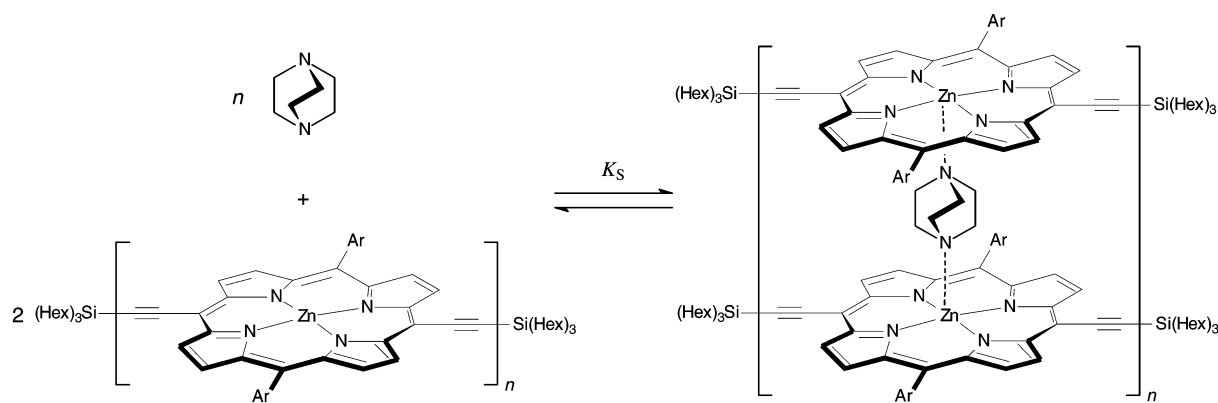
(15) Actually in the original paper (ref 12) the constants K_3 and K_2 are indicated as K_4 and K_3 , respectively. However we have preferred to use K_3 and K_2 for the sake of consistency with the indexes in eq 3.

(16) Garrett, T. M.; Koert, U.; Lehn, J.-M. *J. Phys. Org. Chem.* **1992**, *5*, 529–532.

(17) Floquet, S.; Ouali, N.; Bocquet, B.; Bernardinelli, G.; Imbert, D.; Bünzli, J.-C. G.; Hopfgartner, G.; Piguet, C. *Chem.–Eur. J.* **2003**, *9*, 1860–1875.

(18) Taylor, P. N.; Anderson, H. L. *J. Am. Chem. Soc.* **1999**, *121*, 11538–11545.

Scheme 3

**Table 1.** Experimental and Calculated $\log K_S$ Values in Toluene and Chloroform for Porphyrin Oligomers and DABCO

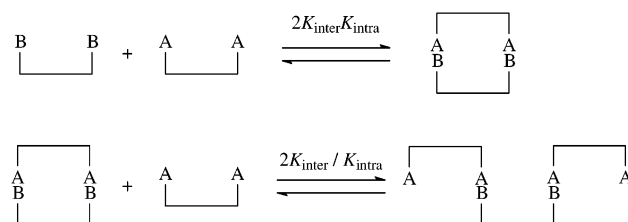
n	$(\log K_S)_{\text{exp}}^a$ in PhMe	$(\log K_S)_{\text{calc}}^b$ in PhMe	$(\log K_S)_{\text{exp}}^a$ in CHCl_3	$(\log K_S)_{\text{calc}}^b$ in CHCl_3
1	9.69 ± 0.04		8.69 ± 0.04	
2	20.0 ± 0.1		18.3 ± 0.1	
3	29.7 ± 0.2	30.0 ± 0.4	27.2 ± 0.2	27.5 ± 0.4
4	39.9 ± 0.2	40.0 ± 0.6	37.2 ± 0.2	36.8 ± 0.6
5	50.3 ± 0.3	50.0 ± 0.8	46.5 ± 0.3	46.1 ± 0.8
6	60.5 ± 0.3	60.0 ± 0.9	56.5 ± 0.3	55.4 ± 0.9

^a Data from ref 18. ^b This work.

indicate that *the self-assembly of porphyrin ladders also occurs without cooperative effects.*

Anderson and Taylor, on the contrary, sustained that positive cooperativity has taken place. The reasons for this conclusion are in part due to the fact that they consider the chelate effect a cooperative effect. Indeed they wrote “The difference between self-assembly and simple coordination is that self-assembly processes involve multiple interactions operating in one or more closed loops; the interaction that closes the loop becomes intramolecular, resulting in cooperativity through the chelate effect. Nonchelated multicomponent complexes, in which the interactions do not form a closed loop, do not benefit by this chelative cooperativity”.¹⁸ This terminology, shared, to say the truth, by several other authors,¹⁹ is in our opinion a source of much confusion and merits discussion in some detail. It is well-known that intramolecular reactions can be strongly advantaged with respect to the corresponding intermolecular processes. This effect is quantitatively expressed by the effective molarity (EM) which is defined as the ratio $K_{\text{intra}}/K_{\text{inter}}$.²⁰ According to the classical analysis of Page and Jencks,²¹ this advantage is mainly entropic and is due to the significant losses of translational plus rotational entropy that occur in an intermolecular process with respect to the corresponding intramolecular one;²² they estimated that this advantage can be translated into a maximum EM value of 10^8 mol L^{-1} . We agree with Anderson and Taylor that the

Scheme 4



EM is a major driving force for self-assembly; indeed we ourselves proposed a model for self-assembly of monocyclic⁵ and polycyclic⁶ supermolecules pointing out that the driving force depends on the product $K_{\text{inter}}\text{EM}$ and on the degree of cyclicity of the assembly. However we strongly argue against the definition of this effect as a cooperative effect, for the simple reason that, involving a comparison of an intramolecular constant with an intermolecular one, it is concentration dependent. A simple example will clarify this point; consider the titration of a substrate M having two binding site $-B$, with a ligand L having two binding sites $-A$. The equilibria in Scheme 4 can be envisaged

Consider the second equilibrium in Scheme 4; if we indicate with ML the cyclic species and with ML_2 the acyclic one and express the concentration of the free ligand L in terms of the concentration of the binding sites $-A$ so that $[\text{L}] = [-A]/2$, it is easy to show that $[\text{ML}_2]/[\text{ML}] = [-A]/\text{EM}$. In other words, when the concentration of binding sites is lower than the EM, the cyclic species is favored over the acyclic one, whereas the opposite occurs when the concentration of binding sites is greater than the EM. Therefore, we are left with the unpleasant result that an intramolecular process could display positive or negative cooperativity depending on the concentration. The same criticism can be raised to a definition of cooperativity in intramolecular processes given by Whitesides et al.⁷ as well as, more recently, by Calderone and Williams.^{19b} According to these authors, if the Gibbs' free energy of binding of a species $A-B$ is more favorable than the sum of the free energies of binding for the individual parts $-A$ and $-B$, there is positive cooperativity; on the contrary, if binding of the linked ligand is less favorable than binding the unlinked ligands, there is negative cooperativity. When translated in terms of equilibrium constants, this definition is equivalent in saying that intramolecular processes are positively cooperative if $\text{EM} > 1 \text{ mol L}^{-1}$, whereas they are negatively cooperative if $\text{EM} < 1 \text{ mol L}^{-1}$. It is apparent that fixing the value $\text{EM} = 1 \text{ mol L}^{-1}$ as the border

(19) For recent examples, see: (a) ref 7. (b) Calderone, C. T.; Williams, D. H. *J. Am. Chem. Soc.* **2001**, *123*, 6262–6267. (c) Tobey, S. L.; Anslyn, E. V. *J. Am. Chem. Soc.* **2003**, *125*, 10963–10970.

(20) For reviews on the concept of EM and its application to ring closure reactions, see: (a) Kirby, A. J. *Adv. Phys. Org. Chem.* **1980**, *17*, 183–278. (b) Mandolini, L. *Adv. Phys. Org. Chem.* **1986**, *22*, 1–111. (c) Galli, C.; Mandolini, L. *Eur. J. Org. Chem.* **2000**, 3117–3125.

(21) (a) Page, M. I.; Jencks, W. P. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 1678–1683. (b) Page, M. I. *Chem. Soc. Rev.* **1973**, *2*, 295–323.

(22) There are however different views underscoring the role of enthalpic effects. For example, see: (a) ref 19b. (b) Bruce, T. C.; Lightstone, F. C. *Acc. Chem. Res.* **1999**, *32*, 127–136.

for cooperative or anticooperative behavior is absolutely arbitrary. In fact, cooperative or anticooperative behavior depends on the concentration of binding sites with respect to the EM, whatever the value of the latter. The conclusion is that any process involving at least one intramolecular interaction could be carried at a concentration sufficiently low to display positive cooperativity. It follows that the definition of the chelate effect in terms of cooperativity is ambiguous, to say the least, and should be rejected. To avoid these ambiguities, we reiterate that cooperativity must be assessed by a comparison among homogeneous processes.

Having clarified this point, it is obvious that effects such as an all-or-nothing two-state assembly, sigmoidal binding curves, and narcissistic self-sorting, all observed by Anderson and Taylor, are only evidence of a large stability of the assembly due to a high value of both the product $K_{\text{inter}}K_{\text{EM}}$ and the degree of cyclicity of the assembly⁶ but not of positive cooperativity. Other evidences for positive cooperativity, according to Anderson and Taylor, are the large Hill coefficients and the linear dependence of the Gibbs free energy for ladder formation vs the number of rungs. In the previous section, we have already commented upon the lack of meaning of the Hill plot in self-assembly. As to the fact that adding each new rung to the ladder decreases the free energy by the same amount, it is not evidence of positive cooperativity but, on the contrary, of its absence. Indeed, with a little manipulation of eq 18, eq 19 is obtained

$$-\Delta G^\circ = RT \ln K_S = nRT \ln(2K_{\text{inter}} K_{\text{intra}}) - RT \ln(K_{\text{intra}}/K_{\text{inter}}) \quad (19)$$

that shows the meaning of the slope and the intercept in the plot obtained by Anderson and Taylor. The intercepts of the plots in toluene and chloroform are close to zero simply because K_{inter} and K_{intra} have very similar numerical values. According to Anderson and Taylor, the intercepts correspond to entropies of ladder initiation; in our interpretations, they correspond to the free energy associated with the EM for the closure of the cyclic substructure of the assembly. According to the classical view of Page and Jencks, this contribution is purely entropic only in the absence of ring strain.²¹

Generalization of the results provided by eq 19 suggests a practical method to assess cooperativity in those cases in which the degree of cyclicity of the assembly can be systematically increased, as in double strand helicates or ladders. Indeed, a plot of $-\Delta G^\circ$ against the number of cyclic substructures of the assembly is expected to be linear with a positive slope in the absence of cooperativity, whereas positive or negative deviation from the linearity would be evidence of positive or negative cooperativity, respectively. For example, it has been reported that ladders based on hydrogen-bonded dimerization of oligo-2-aminopyridines display linear free energy plots in both CDCl_3 and cyclohexane.²³ This would indicate the absence of coopera-

tive effects. On the contrary, studies of the nucleic acids (A)₁₇ and (U)₁₇ have shown that a certain number of base pairs are required to form before double helix assembly will occur. The double helix nucleation structure is believed to consist of three to four base pairs. In terms of its free energy, the formation of a base pair is unfavorable. However, additional base pairs formed after nucleation make a large and negative contribution to the overall free energy of double helix formation. Thus, a plot of $-\Delta G^\circ$ for the self-assembly of the DNA double helix against the number of base pairs formed displays a large positive deviation from linearity indicating positive cooperativity (ref 3d and references therein cited).

Conclusions

In summary, it has been pointed out that, to assess cooperativity, only virtually identical processes described by equilibrium constants having the same dimensions should be compared. Therefore intermolecular and intramolecular processes should be considered as forming two distinct groups within which cooperativity is assessed independently. On the basis of this view, a method has been proposed to evaluate the statistical stability constant of the assembly. The method is based on the knowledge of the following parameters: (i) the reference constant for the intermolecular processes, K_{inter} ; (ii) the reference constant for the intramolecular processes, K_{intra} ; (iii) the number of intermolecular and intramolecular interactions required to build the assembly (easily obtainable from the number of molecules in the assembly, N , and the number of bonds joining them, B); (iv) the symmetry factor of the self-assembly reaction. The statistical stability constant of the assembly is then compared with the experimental one; if the latter exceeds the former, there is positive cooperativity, whereas if the opposite occurs, negative cooperativity has taken place. The method has been applied to two classical cases, namely the self-assembly of helicates and of porphyrin ladders, by using data previously published by the groups of Lehn¹² and Anderson,¹⁸ respectively. Contrarily to the conclusions of the authors, pointing out self-assembly processes driven by positive cooperativity, the method presented here indicates in both cases the absence of cooperative effects. The methods previously used to assess cooperativity, in particular, the Scatchard plot and/or Hill plot, are criticized as being inappropriate for self-assembly, because they are pertinent to a specific case only, namely, the intermolecular binding of a monovalent ligand L to a multivalent receptor M , a case very different from self-assembly which involves both inter- and intramolecular interactions.

The rather diffuse usage of the term cooperativity to indicate the advantage of intramolecular reactions with respect to the corresponding intermolecular ones is criticized on the basis that this effect is concentration dependent and can easily be reverted upon changing the concentration of binding groups.

The cases examined in the light of the present method suggest that positive cooperativity in artificial self-assembling systems is probably much more rare than it was previously thought.

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