Cooperativity

Multivalent interactions in human biology

REVIEWS

Polyvalent Interactions in Biological Systems: Implications for Design and Use of Multivalent Ligands and Inhibitors**

Mathai Mammen, Seok-Ki Choi, and George M. Whitesides*

Angew. Chem. Int. Ed. **1998**, *37*, 2754 – 2794

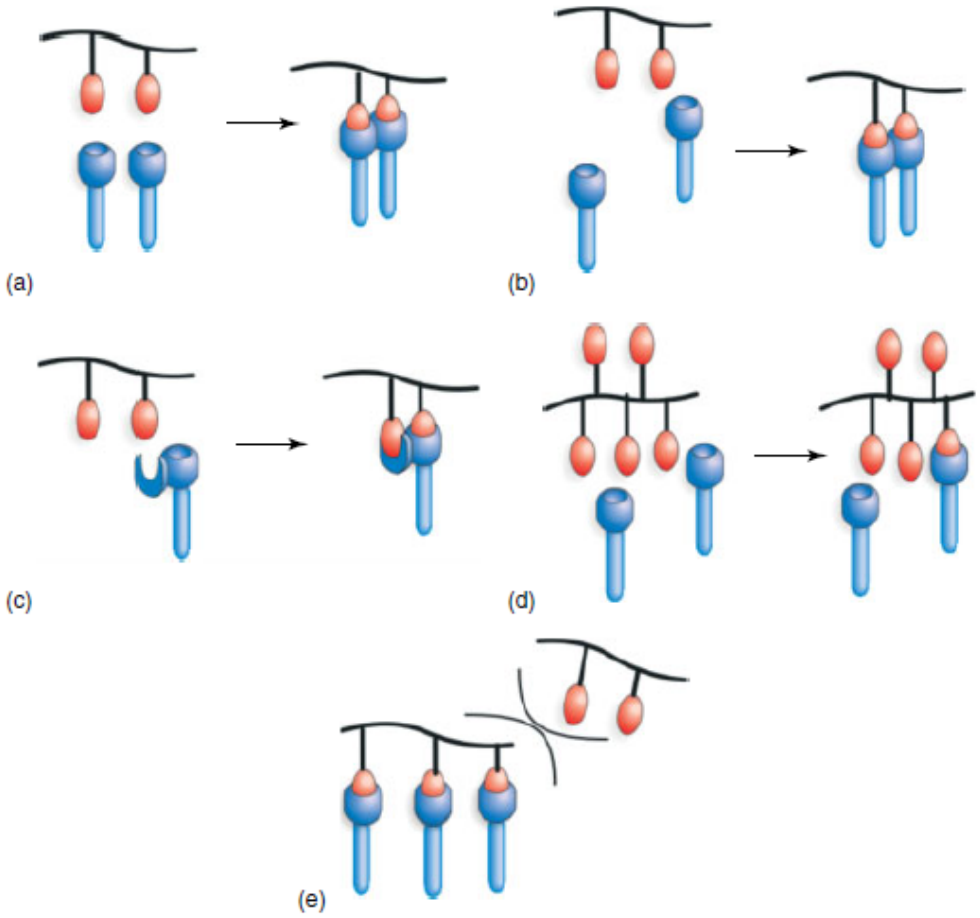

Figure 1 Mechanisms by which multivalent ligands can interact with cell-surface receptors. (a) Multivalent ligands can bind oligomeric receptors by occupying multiple binding sites (chelate effect). (b) Multivalent ligands can cause receptors to cluster on the cell surface. This can lead to activation of signaling pathways. (c) Multivalent ligands can occupy primary and secondary binding sites on a receptor. (d) Multivalent ligands display higher local concentrations of binding epitopes, which can result in higher apparent affinities. (e) The steric bulk of the multivalent ligand precludes further interactions with ligands.

2.1. Adhesion of a Virus to the Surface of a Cell: Influenza and Bronchial Epithelial Cells

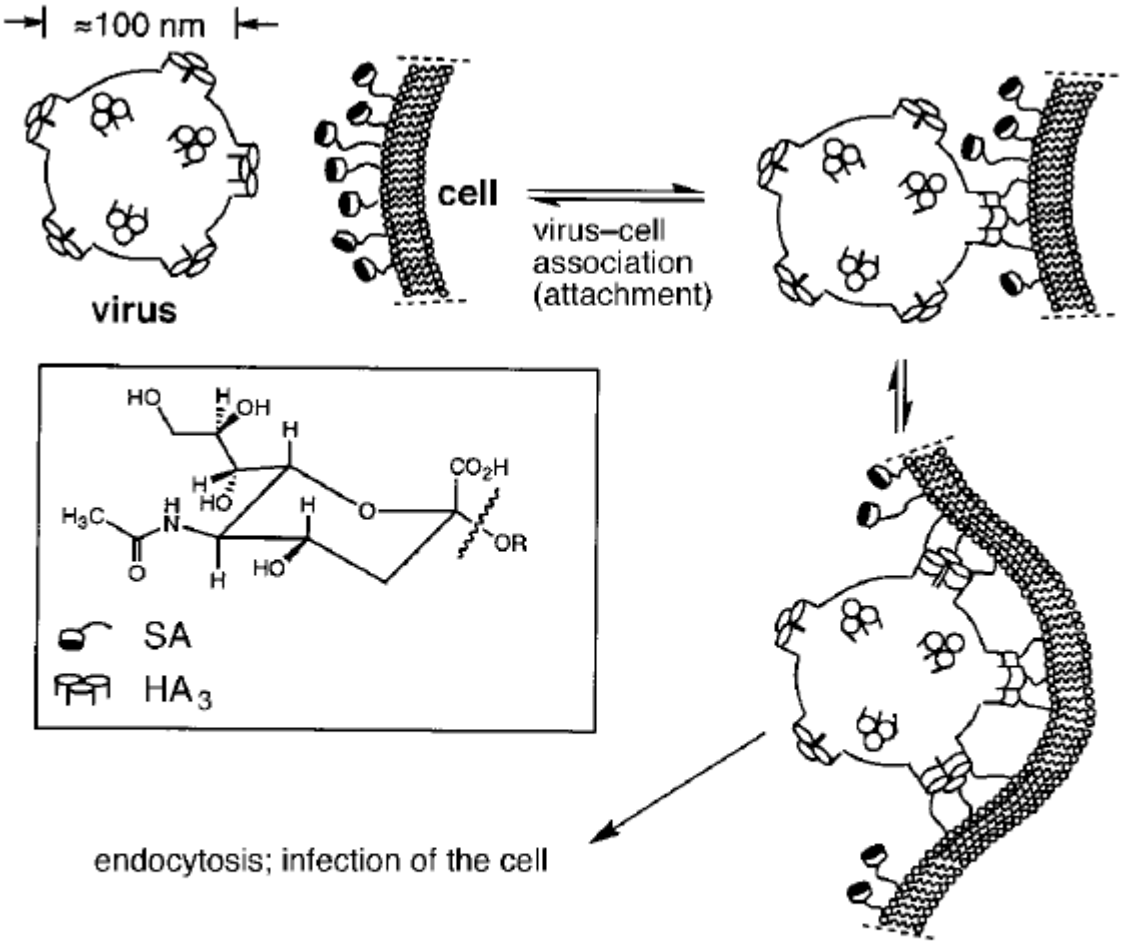

Figure 1. The influenza virus attaches to cells by interaction of trimeric hemagglutinin (HA_3, shown as protruding cylinders on the virus) with sialic acid (SA, shown as caps on the cell). Only a few of the hemagglutinin trimers and SA groups are represented; neither is to scale.

2.3. Binding of Cells to Other Cells: Neutrophil and Arterial Endothelial Cells

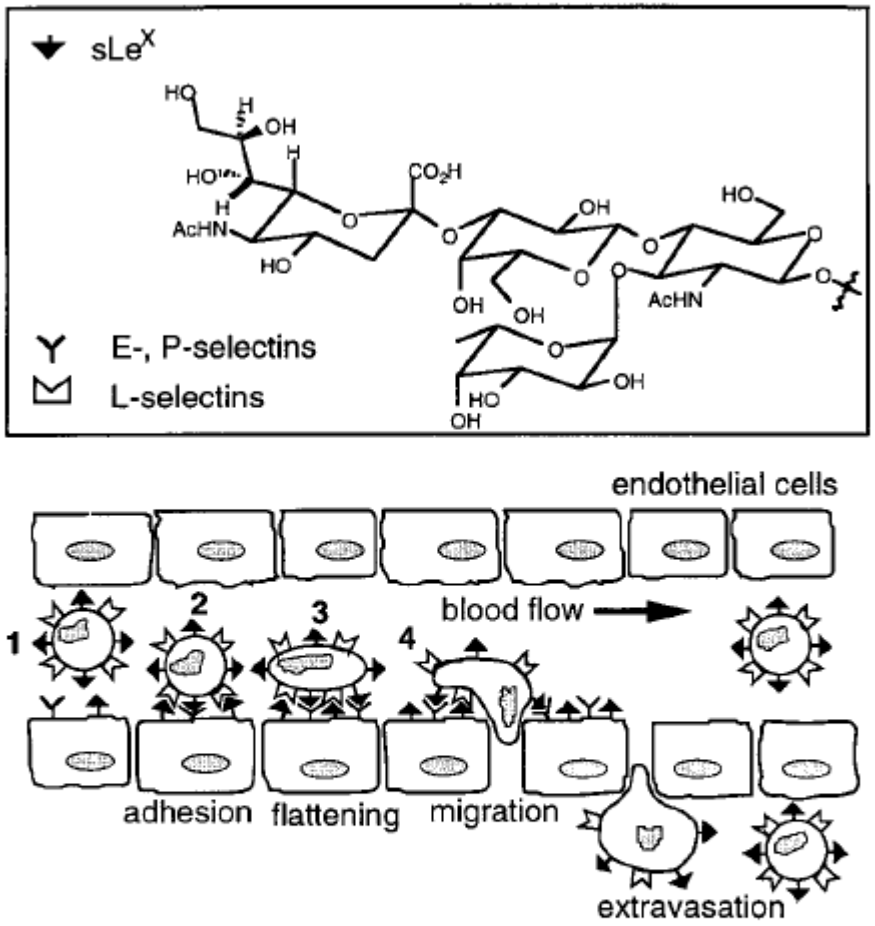

Figure 3. Injury results in expression and display of E- and P-selectins on the surface of nearby endothelial cells. Neutrophils (**1**) are attracted to this site (**2**). The neutrophil interacts polyvalently (through sLe^X moieties) with E- and P-selectins. In addition, L-selectins, expressed on the surface of the neutrophil, interact with sLe^X moieties present on the endothelial cell. Neutrophils change their shape upon attachment (**3**). Further adhesion mediated by integrins leads to extravasation (**4**).

2.5. Binding of Polyvalent Molecules to Polyvalent Molecules: Binding of Transcription Factors to Multiple Sites on DNA

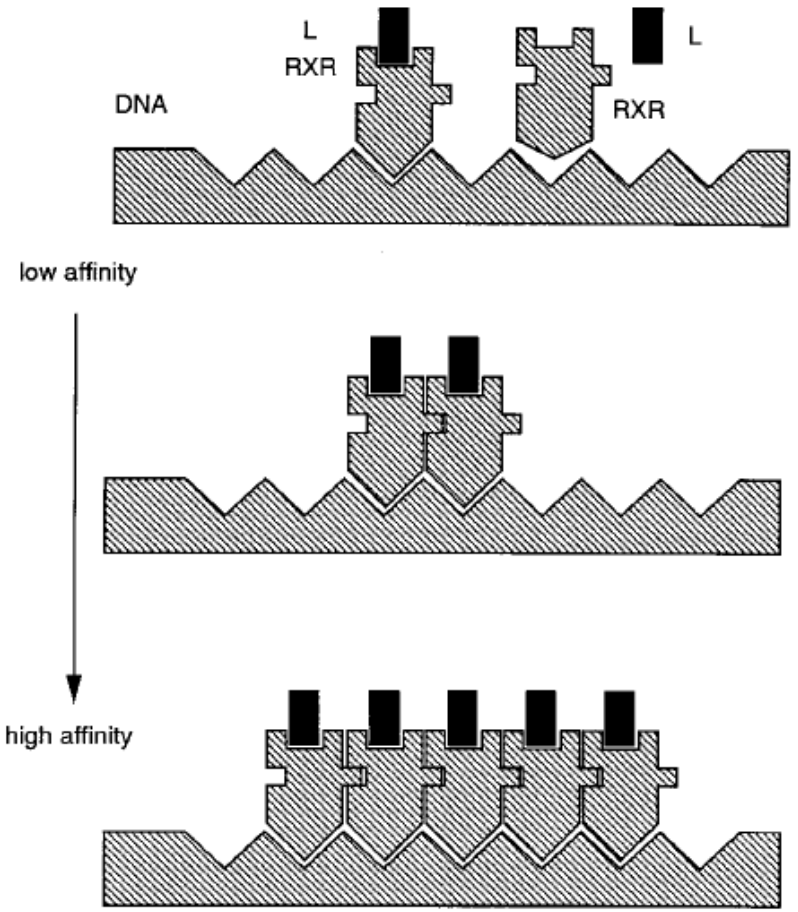

Figure 5. Binding of transcription factors to multiple sites on DNA. Top: The complex of monomeric retinoid X receptor (RXR) and ligand (L), RXR-L, binds with low affinity to the cellular retinol-binding protein II element (CRBP-II) on DNA. Middle: The dimeric complex $(RXR\text{-}L)_2$ has higher affinity than the monomeric complex. Bottom: The pentameric complex $(RXR\text{-}L)_5$ has very high affinity for DNA.

Multivalent interactions in supramolecular chemistry

A Trivalent System from Vancomycin·D-Ala-D-Ala with Higher Affinity Than Avidin·Biotin

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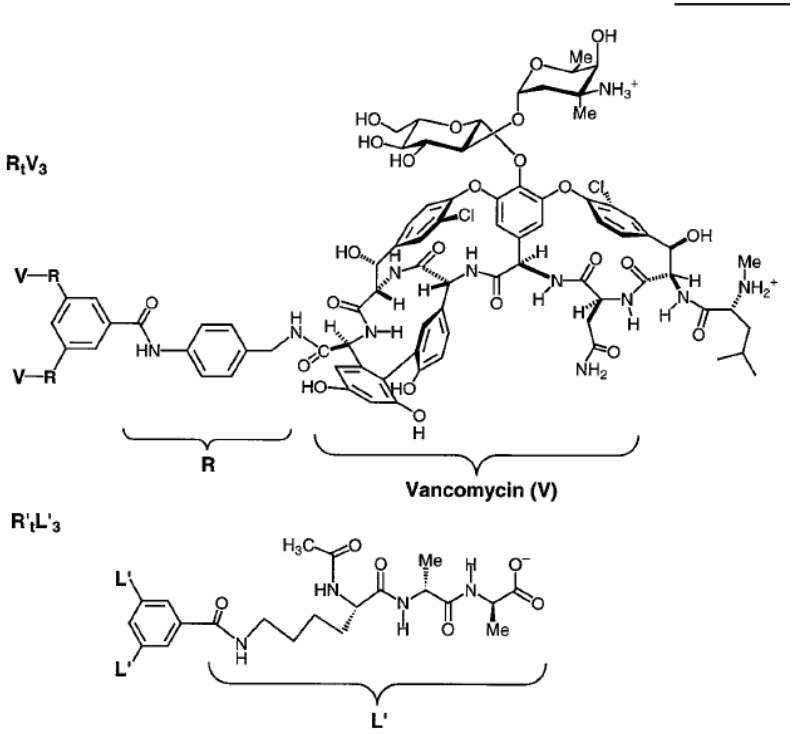


Scheme 1. Structures of the trivalent derivatives of vancomycin, R_tV_3, and of DADA, $R'_tL'_3$.

dissociation constant (K_d) $\approx 4 \times 10^{-17} \pm 1 \times 10^{-17}$ M.

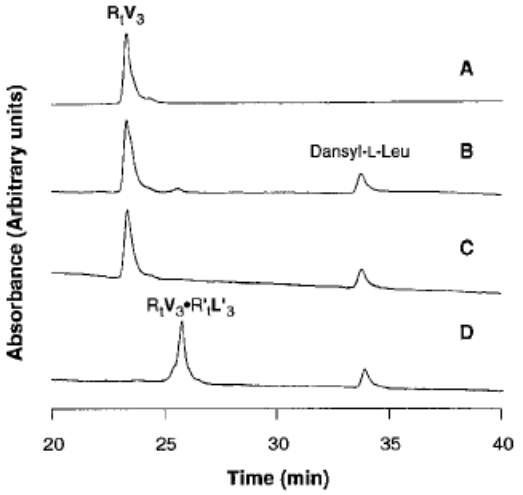
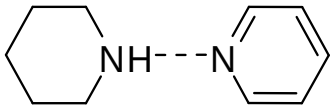


Fig. 1. HPLC of aliquots of samples that contained (**A**) R_tV_3 (4.5 μM); (**B**) R_tV_3 (4.5 μM); (**C**) R_tV_3 (4.5 μM) + **L** (19.1 mM); and (**D**) R_tV_3 (4.5 μM) + $R'_tL'_3$ (4.5 μM). Dansyl-L-Leu (10 μM) was introduced into each sample except (A) as an internal standard. All analyses were carried out under the same conditions, with a Rainin (Woburn, Massachusetts) analytical reverse-phase C18 column, linear eluting gradient from 85% solvent A [0.1% trifluoroacetic acid (TFA) in water] and 15% solvent B (0.1% TFA in acetonitrile) to 70% A and 30% B, over 45 min. The absorbance was monitored at 280 nm wavelength.

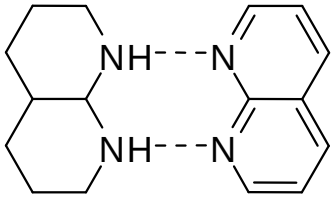
Additivity (?)

In order to obtain a strong recognition between the host and the guest using weak non-covalent interaction, multiple interactions must be used.



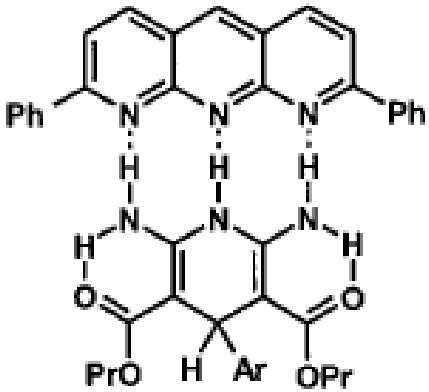
$K_{ass} = 25\ M^{-1}$

$\Delta G = -7.9\ kJ\ mol^{-1}$



$K_{ass} = 6.4 \times 10^{3}\ M^{-1}$

$\Delta G = -21.6\ kJ\ mol^{-1}$

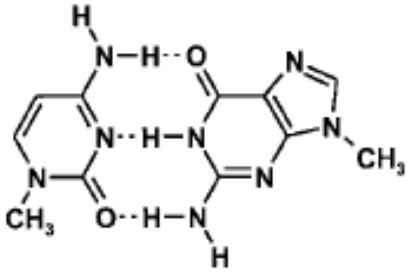


$K_{ass} = 1.5 \times 10^{6}\ M^{-1}$

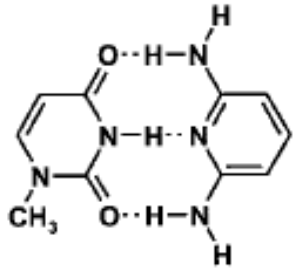
$\Delta G = -35.3\ kJ\ mol^{-1}$

Additivity (?)

In some cases however, binding constant are much lower: H-bond acceptors and donors are also charge centers!



$K_{ass} = 10^4\ M^{-1}$



$K_{ass} = 10^2\ M^{-1}$

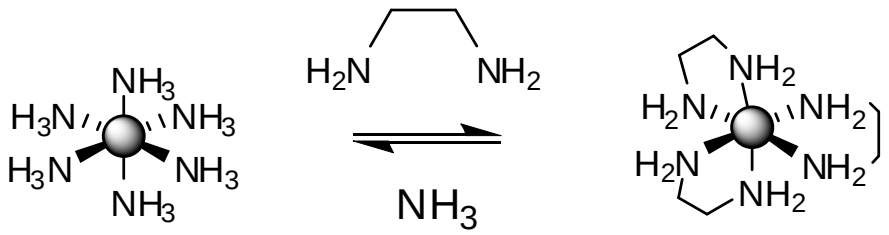
attractive secondary interaction

repulsive secondary interaction

Each H-bond contributes with 7.8 kJ mol^{-1}, each secondary interaction with ± 2.9 kJ mol^{-1}

Chelate effect

Host with multiple binding sites results in more stable complexes than multiple unidentate ligand (chelate cooperativity)



M = Ni^{2+}

log K = 8.76

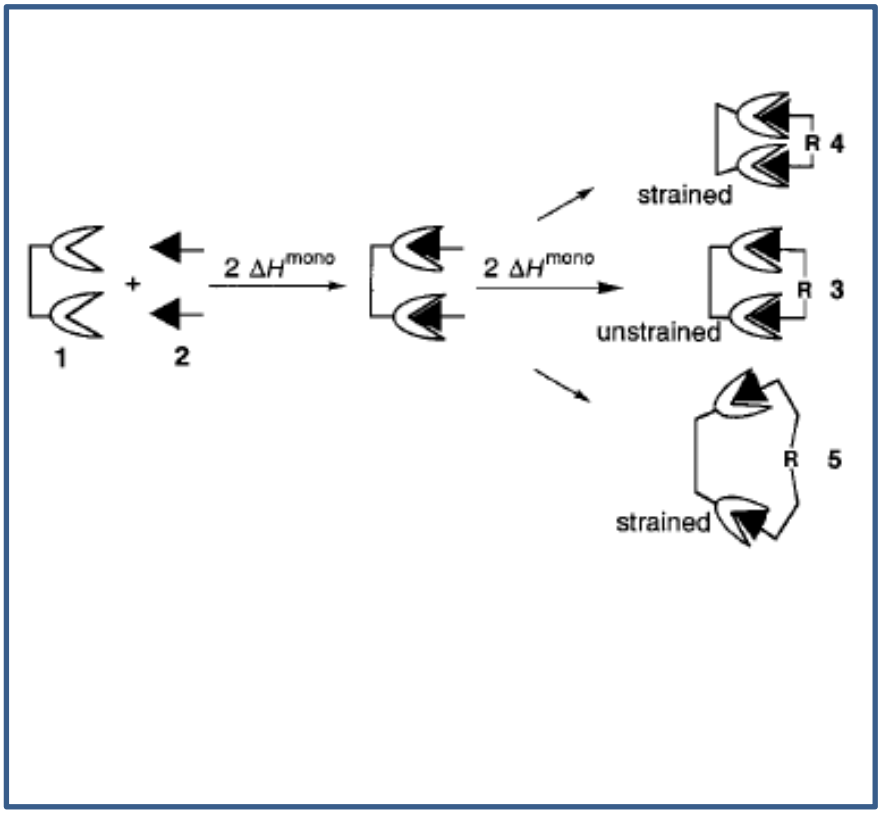
$\Delta H° = -29\ kJ\ mol^{-1}$

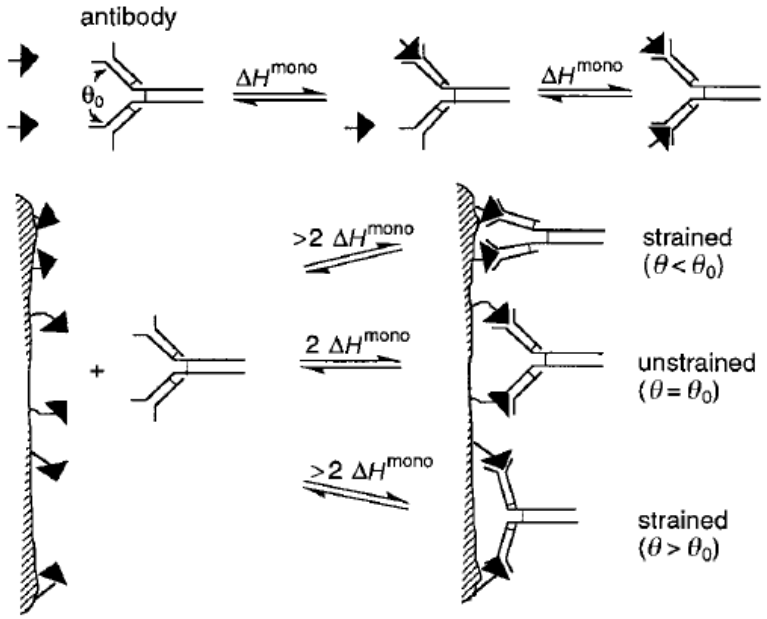
$-T\Delta S° = -25\ kJ\ mol^{-1}$

1. Greater basicity of primary amines
2. Weaker solvatation of primary amines
3. Decreased repulsive interaction between binding sites
4. Steric interactions and strain in the complex

1. Conformational changes
2. Greater number of free species

Enthalpy



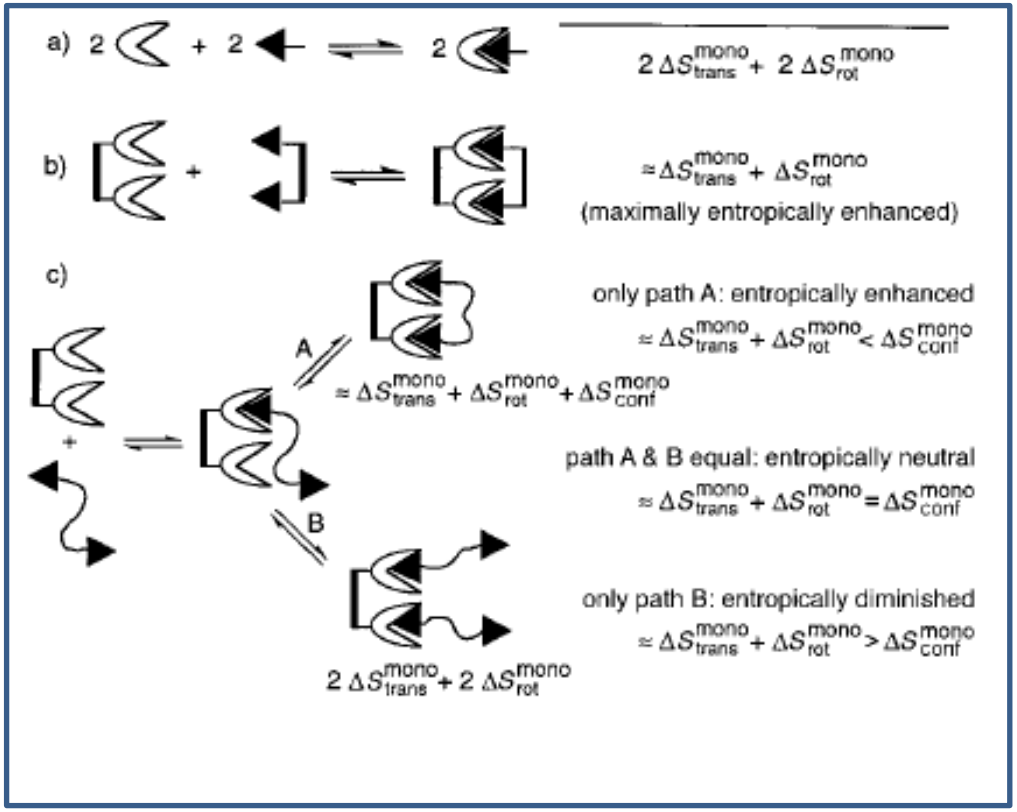


Positive cooperativity is due to entropic and enthalpic contributions to binding.

Enthalpy: secondary functional groups interactions, conformational changes, ring strain, polarization of the interacting groups

Entropy

$$\Delta S_N^{\text{poly}} = \Delta S_{\text{trans},N}^{\text{poly}} + \Delta S_{\text{rot},N}^{\text{poly}} + \Delta S_{\text{conf},N}^{\text{poly}} + \Delta S_{\text{H}_2\text{O},N}^{\text{poly}}$$



Case I: $\Delta S_{\text{conf}} = 0$

Case II: $\Delta S_{\text{conf}} \neq 0$

Positive cooperativity is due to entropic and enthalpic contributions to binding.

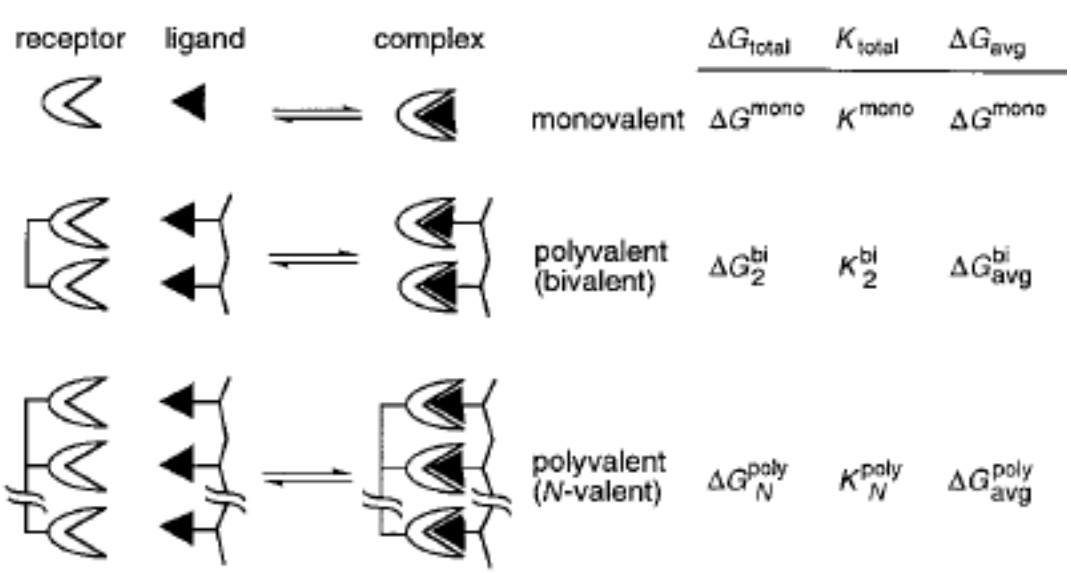
Entropy: loss of motion of the molecule, including internal rotation and vibrations

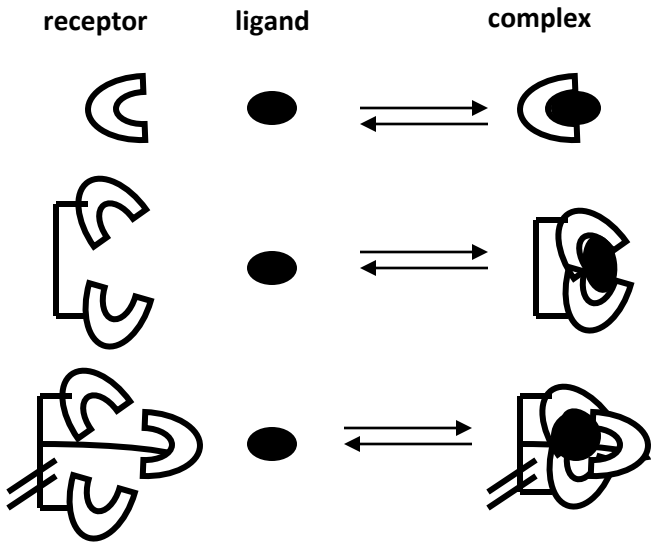
(contribution already paid for in connecting together the recognition elements)

Interestingly, entropy and enthalpy can have partly compensating effects on the affinity of polyvalent interactions: Whereas conformational flexibility increases the conformational entropic cost of association, the same flexibility increases the likelihood that all ligand–receptor interactions can occur without energetic strain. This loss in conformational entropy on association of a polyvalent ligand with a polyvalent receptor has been notoriously difficult to quantitate.

Multiple interactions in binding: definitions

(reference: *Angew. Chem. Int. Ed.* **1998**, *37*, 2754 – 2794)





multiple binding sites: receptor interacts with a multivalent ligand

multiple binding sites: receptor interacts with a monovalent ligand

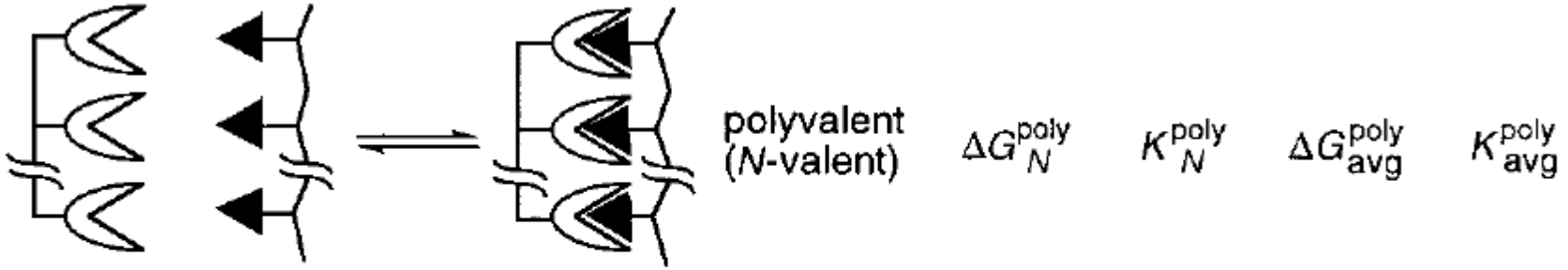


Figure 6. Proposed nomenclature for the polyvalent interactions; relationships between free energies of binding (ΔG) and inhibition constants (K_i) for both monovalent and polyvalent systems.

The average free energy of interaction, ΔG_{avg}^{poly}, between a single ligand moiety and a single receptor moiety in the polyvalent interaction shown in Figure 6 is equal to $\Delta G_N^{poly}/N$ [Eq. (3)]. A monovalent ligand–receptor interaction occurs

$$\Delta G_{avg}^{poly} = \Delta G_N^{poly}/N \tag{3}$$

$$\Delta G = -RT\ln(K) \tag{4}$$

$$K_N^{poly} = (K_{avg}^{poly})^N \tag{5}$$

Measuring cooperativity: α value

The average free energy of interaction between a ligand moiety and receptor moiety in a polyvalent interaction (ΔG^{poly}_{avg}) can be greater than, equal to, or less than the free energy in the analogous monovalent interaction [ΔG^{mono}; Eqs. (6)–(8)]. Following accepted nomenclature in biochem-

$$\Delta G^{poly}_{avg} = \alpha \Delta G^{mono} \tag{6}$$

$$N\Delta G^{poly}_{avg} = \Delta G^{poly}_{N} = \alpha N \Delta G^{mono} \tag{7}$$

$$K^{poly}_{N} = (K^{poly}_{avg})^{N} = (K^{mono})^{\alpha N} \tag{8a}$$

!! this is a wrong comparison !!

$$\alpha = \frac{\lg(K^{poly}_{N})}{\lg(K^{mono})^{N}} \tag{8b}$$

α=degree of cooperativity

α>1: positive cooperativity (synergistic)

α=1: noncooperative (additive)

α<1: negative cooperativity (interfering)

in all cases overall binding constants increase!

when talking about cooperativity (in binding) we often consider this one as the typical situation.

On the contrary this is a rare situation!

most of the available examples are characterized by α<1

Don't be fooled by the overall strength of binding which is always larger:

$$\Delta G_N^{poly} = \Delta G^{mono} - RT \ln(\beta)$$

$$\beta = K_N^{poly} / K^{mono}$$

Case study

Helicate Self-organisation: Positive Cooperativity in the Self-assembly of Double-helical Metal Complexes

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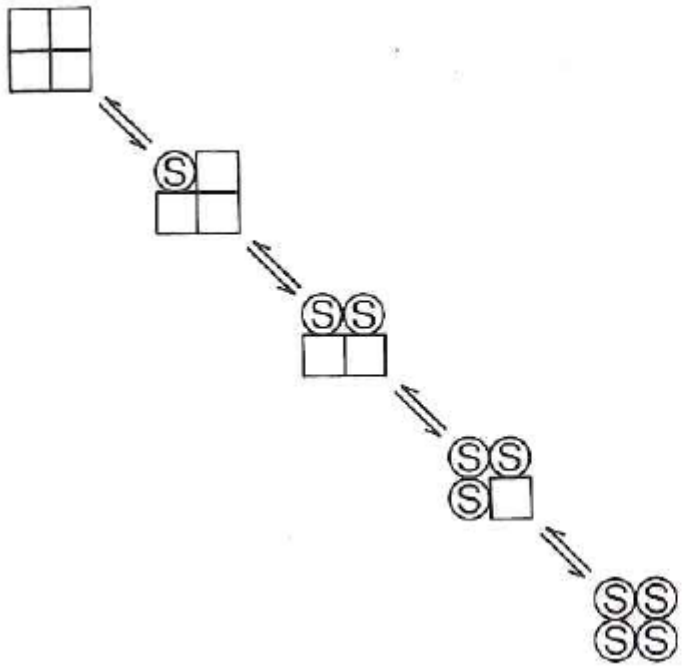
Analysis of the binding of Cu^{I} ions to the tris-bipyridine ligand **3** indicates that the assembly of the resulting trihelicate **1** is a self-organisation process displaying positive cooperativity.

see PDF

1. Hill equation

imagine an enzyme with *n* binding sites for *n* substrates



Koshland, Némethy and Filmer

Figure 10.5 The KNF model for the binding of ligands to a tetrameric protein.

We have

$$E + nS \rightleftharpoons ES_n \tag{10.1}$$

and

$$K = \frac{[E][S]^n}{[ES_n]} \tag{10.2}$$

The degree of saturation Y is given by

$$Y = \frac{[ES_n]}{[E]_0} \tag{10.3}$$

Equations 10.2 and 10.4 may be manipulated to give

$$\log \frac{Y}{1 - Y} = n \log[S] - \log K \tag{10.5}$$

A similar equation called the Hill plot (equation 10.6) is found to describe satisfactorily the binding of ligands to allosteric proteins in the region of 50% saturation (10 to 90%) (Figure 10.7).

Hill plot

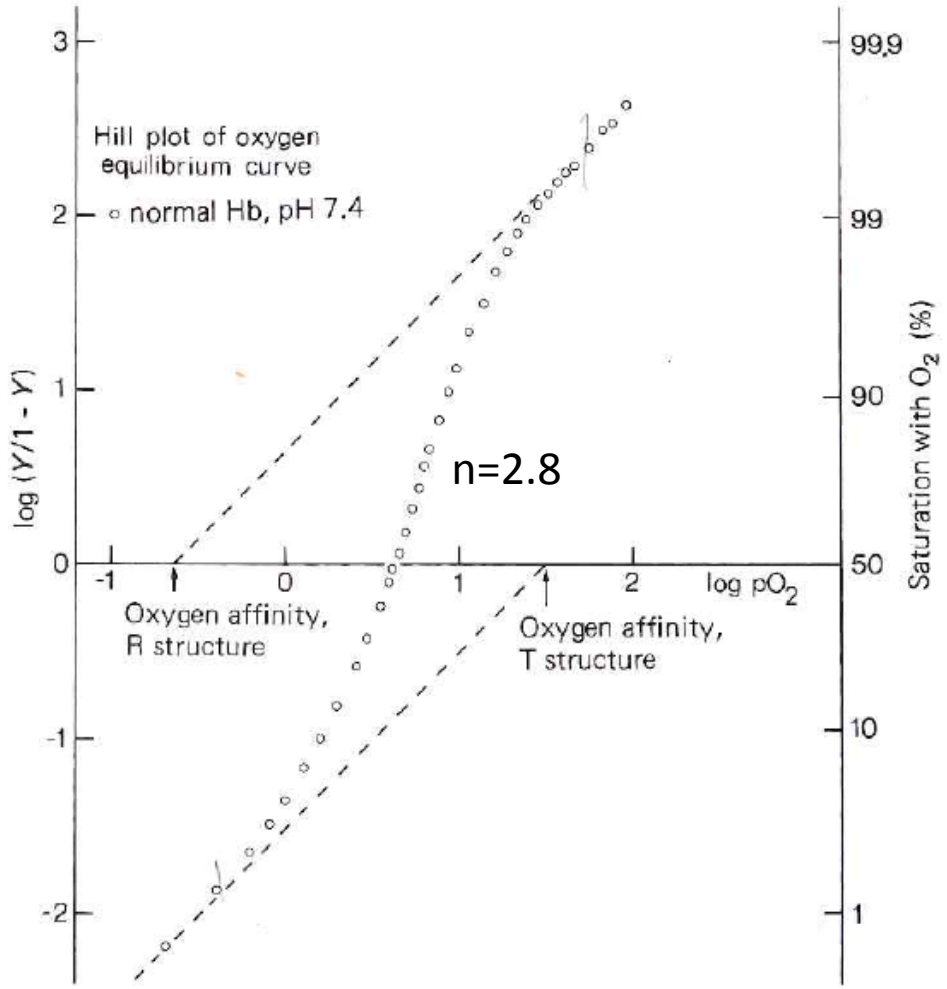


Figure 10.7 A Hill plot of the oxygen-binding curve of hemoglobin. [From J. V. Kilmartin, K. Imai, and R. T. Jones, in *Erythrocyte structure and function*, Alan R. Liss, 21 (1975).]

the slope in the region of 50% saturation is called the Hill constant

n>1: positive cooperativity

n=1: no cooperativity

n<1: negativecooperativity

2. Scatchard plot

1. The single binding site

The binding of a ligand to a single site on a protein is described by the following equations:

$$PL \underset{}{\overset{K_L}{\rightleftharpoons}} P + L \tag{6.21}$$

$$K_L = \frac{[P][L]}{[PL]} \tag{6.22}$$

where [P] and [L] are the concentrations of the unbound protein and ligand.

In terms of the total protein concentration $[P]_0$,

$$[PL] = \frac{[P]_0[L]}{[L] + K_L} \tag{6.23}$$

Equation 6.23 is in the same form as the Michaelis-Menten equation, and may be manipulated in the same way. A good strategy in plotting the data is to use the equivalent of the Eadie plot:

$$[PL] = [P]_0 - K_L \frac{[PL]}{[L]} \tag{6.24}$$

A plot of [PL] against [PL]/[L] gives K_L.

Equation 6.24 cannot be used directly with spectroscopic data since [PL] is not known. However, because [PL] is usually directly proportional to the change in the spectroscopic signal being observed, we have

$$\Delta F = \Delta F_{max} - K_L \frac{\Delta F}{[L]} \tag{6.25}$$

where ΔF is the change in spectroscopic signal when [L] is added to the protein solution. A plot of ΔF against $\Delta F/[L]$ gives K_L and ΔF_{max}, the change in signal when all the protein is converted into complex.

2. Multiple binding sites

a. Identical

If there are n identical noninteracting sites on the protein, equation 6.24 may be modified to the *Scatchard plot*.

$$\nu = n - K_L \frac{\nu}{[L]} \tag{6.26}$$

where ν is the number of moles of ligand bound per mole of protein. The stoichiometry n and K_L are obtained from the plot of ν against $\nu/[L]$.

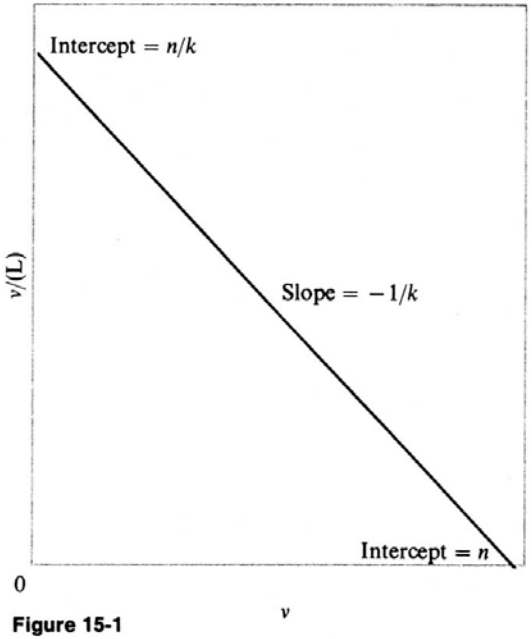


Figure 15-1

Scatchard plot for identical, independent binding sites.

b. Nonidentical

If there are two classes of sites, one weak and the other strong, the Scatchard plot will be biphasic and composed of the sum of two different Scatchard plots. The determination of the values of K_L from such plots is satisfactory only when they differ by at least a factor of 10.

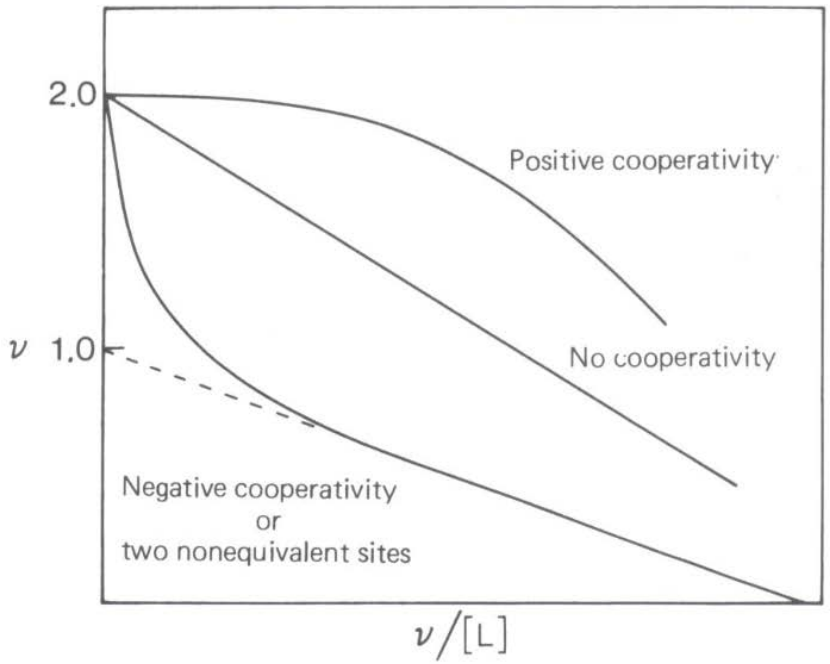
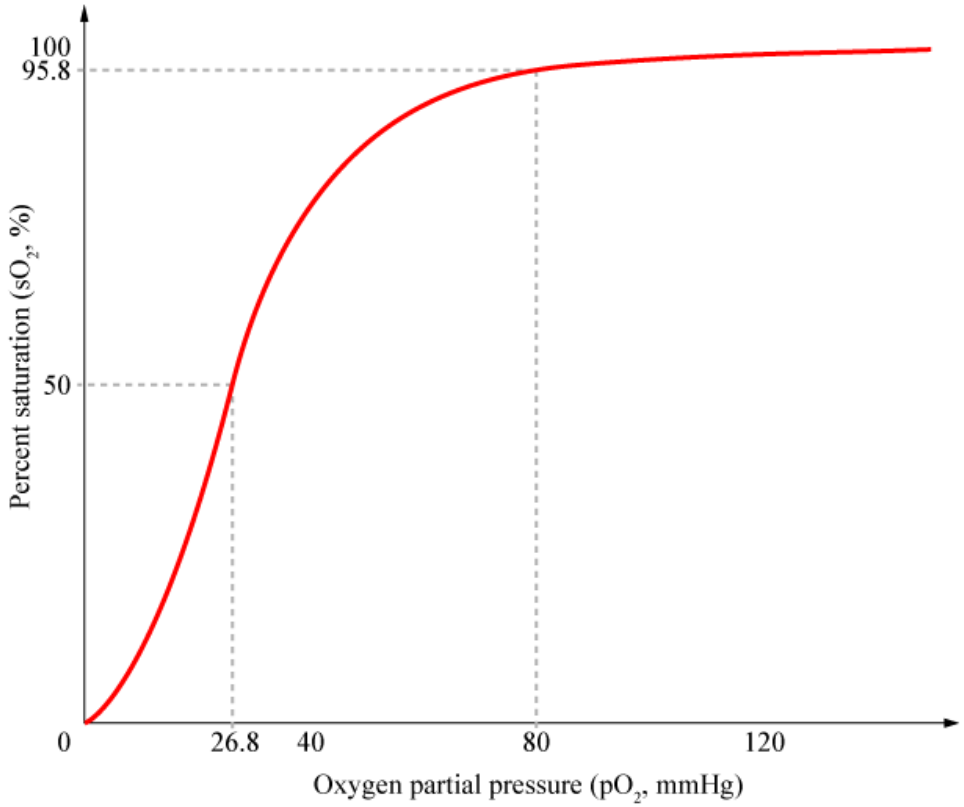


Figure 10.10 Plots of stoichiometry ν against $\nu/[L]$ for the binding of ligand (L) to a dimeric protein.

3. Binding curve

a sigmoidal isotherm is indicative of cooperativity



But is Lehn's conclusion correct ?

large negative entropy. This may be due to the pronounced organisation of the components that takes place in the process.

The present results indicate that the helicate formation process is driven to completion by positive cooperativity. Helicate formation is thus a true self-organisation process, along the lines discussed above.

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ARTICLES
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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.

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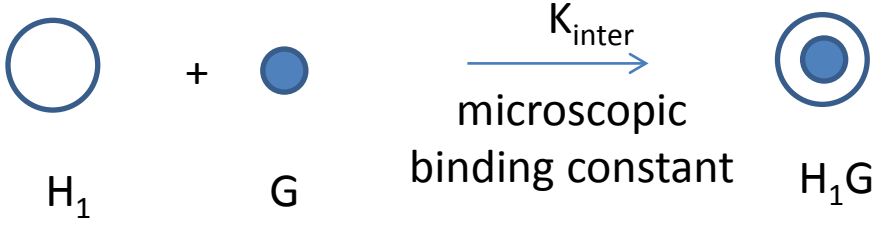
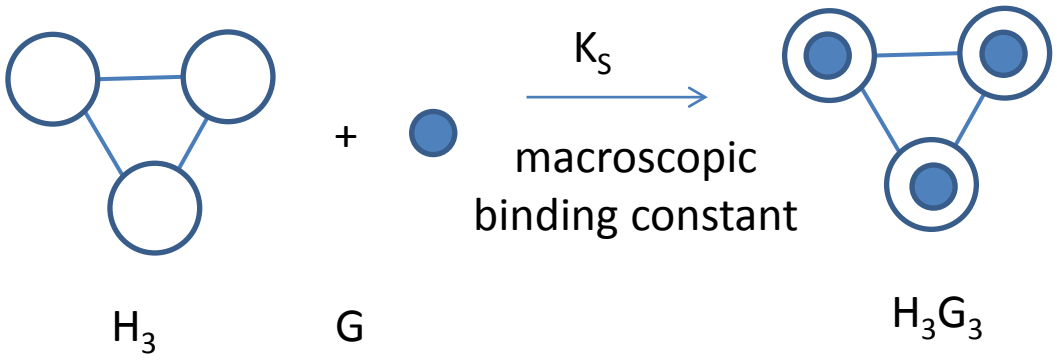
How are the microscopic constant, K_{inter}, and macroscopic constant, K_S, related ?



$$K_{inter} = \frac{H_1G}{H_1 \times G}$$



$$K_S = \frac{H_3G_3}{H_3 \times G^3}$$

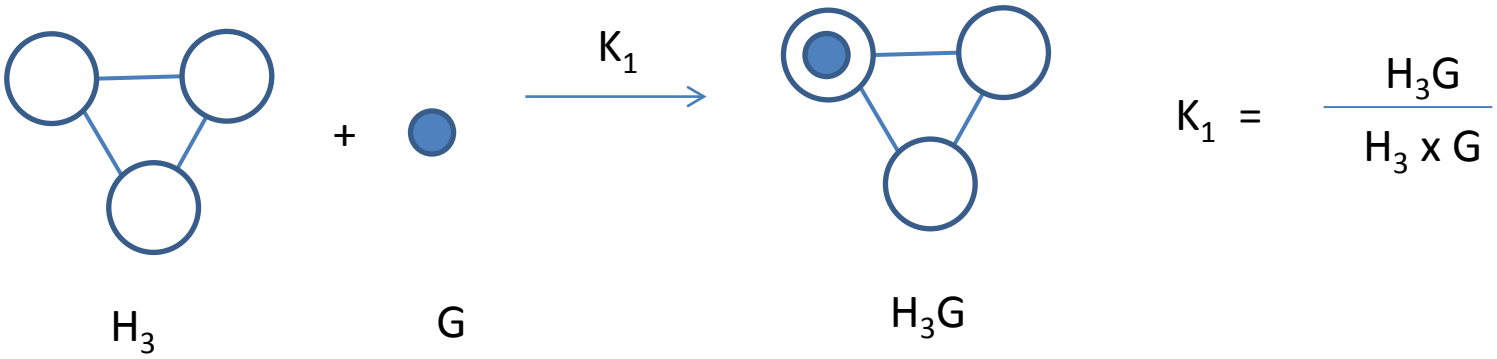
in case of non-cooperativity (indipendent binding sites) we can anticipate that

$$K_S = K_{inter}K_{inter}K_{inter} = K_{inter}^3$$

but what about the individual binding steps ?

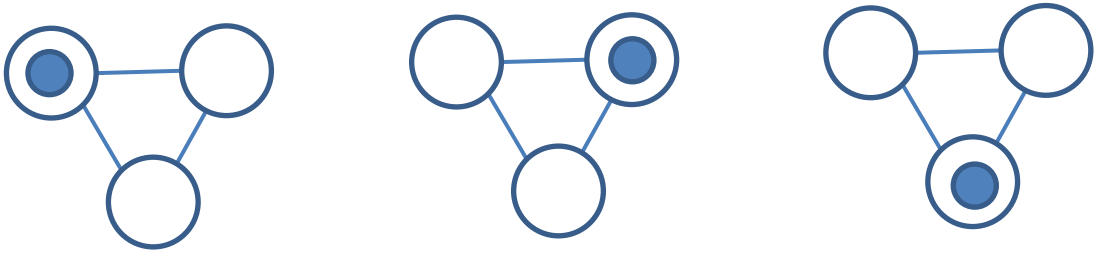
K_S is the statistical, or noncooperative, self-assembly equilibrium constant

1. complexation of the first guest



What is the relation between K_1 and K_{inter} ?

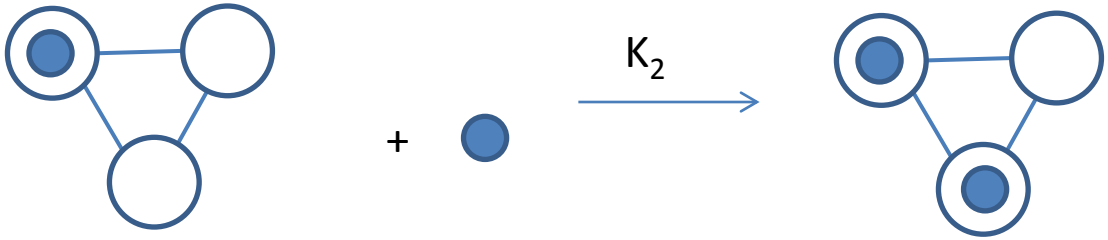
complex H_3G
has 3 sites for binding G,
which are all identical



thus $K_1 = 3K_{inter}$

it's like having a 3-fold higher concentration of H_1

2. complexation of the second guest

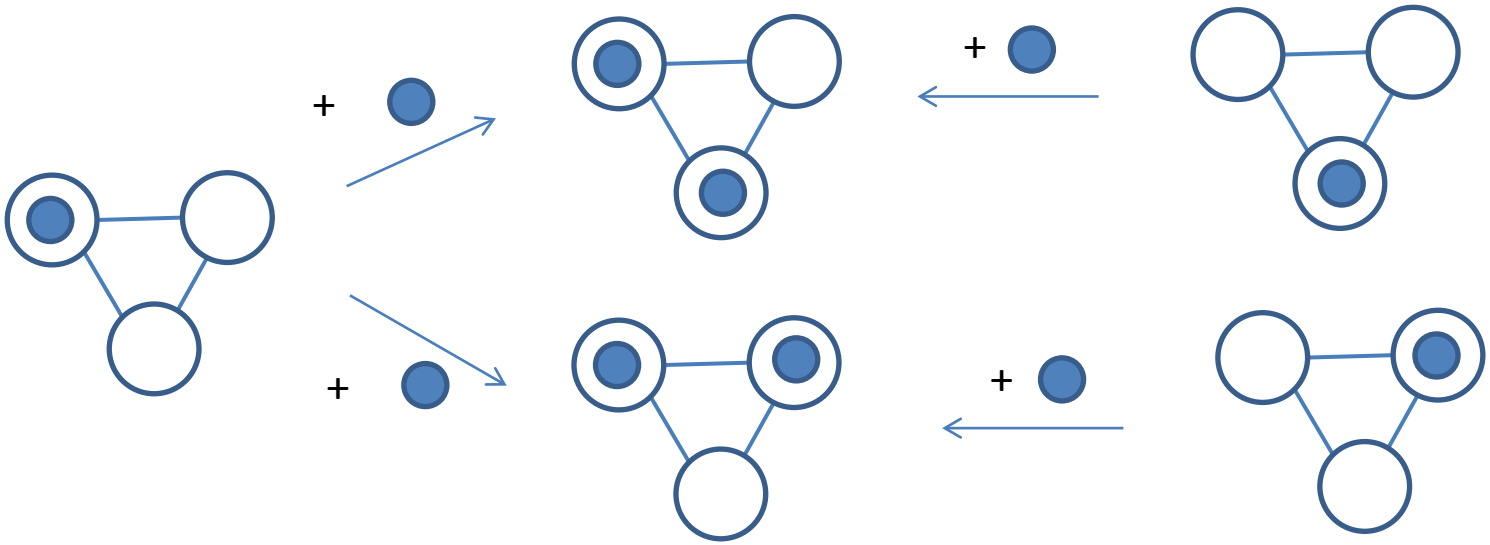


$K_2 = \frac{H_3G_2}{H_3G \times G}$

H_3G G H_3G_2

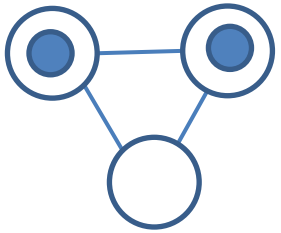
What is the relation between K_2 and K_{inter} ?

complex H_3G has 2 sites available for G (x2) , but each complex can be formed in 2 ways(/2)



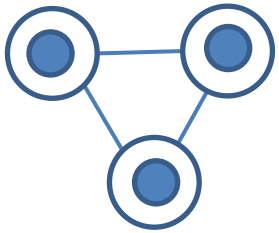
thus $K_2 = 2K_{inter}/2 = K_{inter}$

3. complexation of the third guest



+

K_3

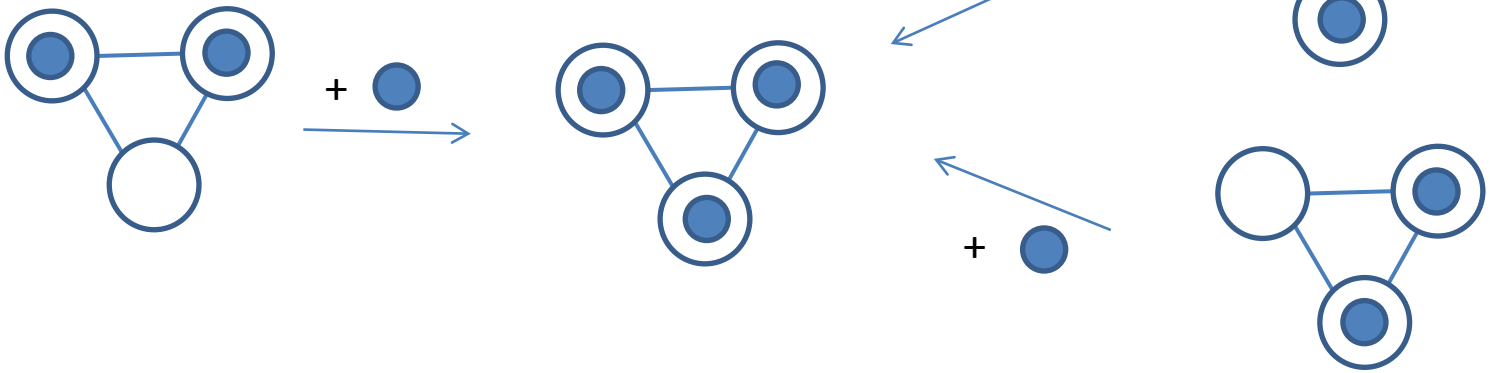


$K_3 = \frac{H_3G_3}{H_3G_2 \times G}$

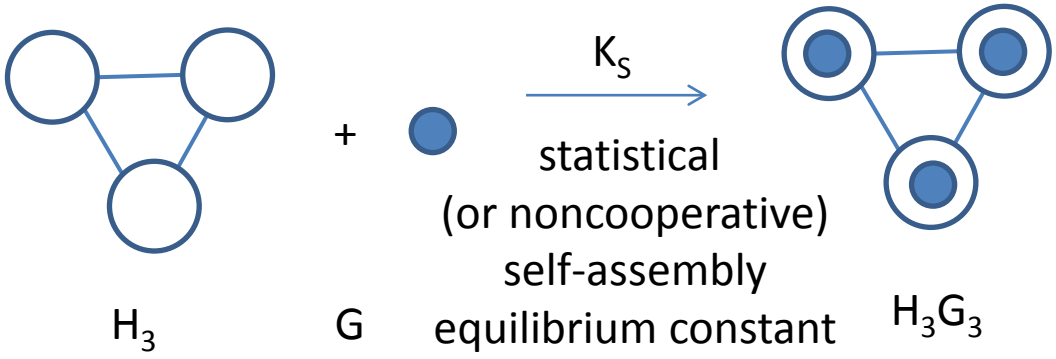
H_3G_2 G H_3G_3

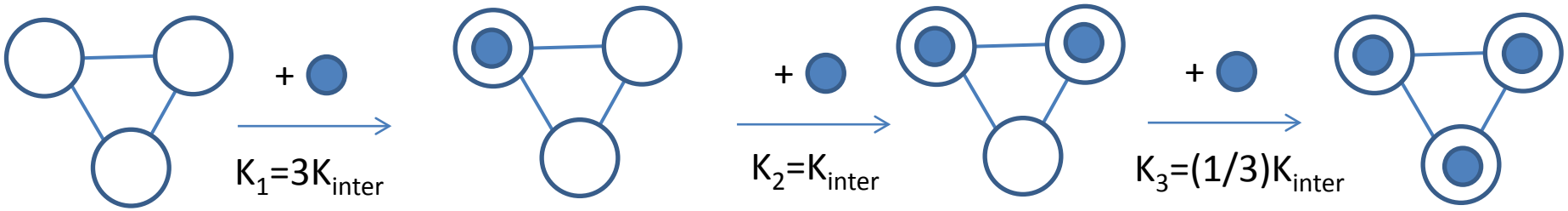
What is the relation between K_3 and K_{inter} ?

complex H_3G_2 has 1 site available for G (x1) , but the final complex can be formed in 3 ways(/3)



thus $K_3 = 1/3\ K_{inter}$

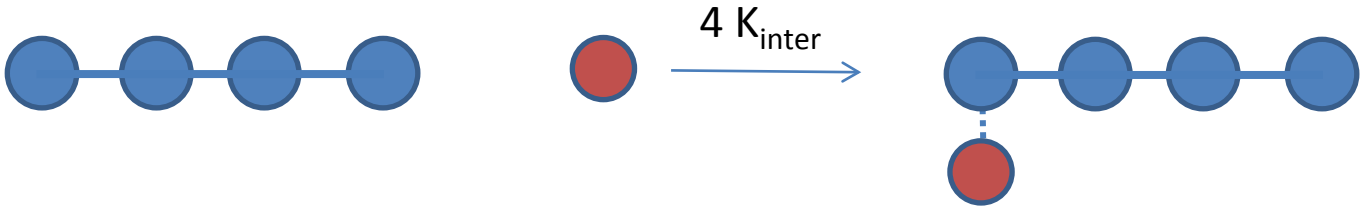




$$K_S = K_1\,K_2\,K_3 = (3K_{inter})(K_{inter})(1/3K_{inter}) = K_{inter}^{\,3}$$

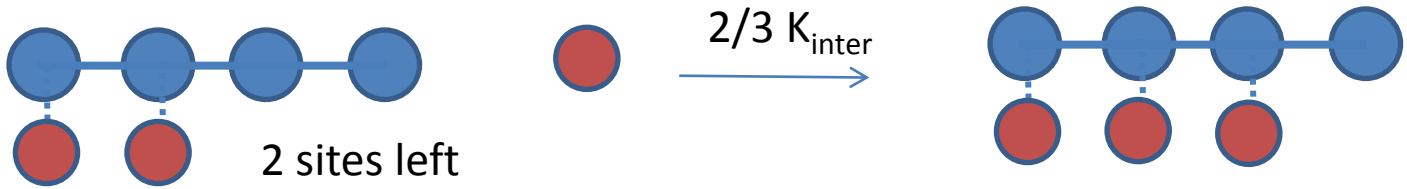
$K_i = K_{inter}(m-i+1)/i$

m: number of identical and indipendent binding sites



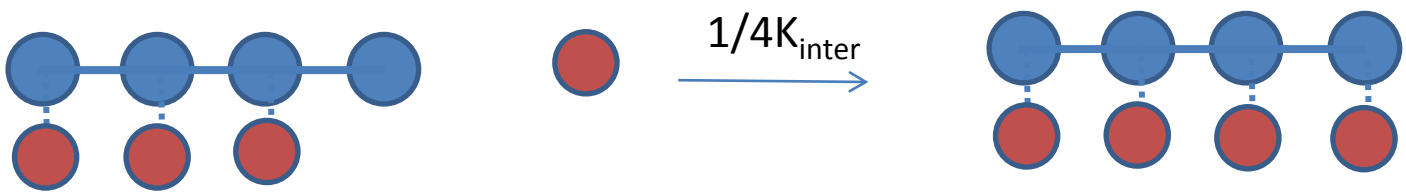
3 sites left

but each complex can be formed in 2 ways



2 sites left

but each complex can be formed in 3 ways



1 sites left

but final complex can be formed in 4 ways

this criterium is used to evaluate cooperativity
and is at the basis of the Hill equation and Scatchard plot

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

positive cooperativity: $K_{i+1} > K_i$

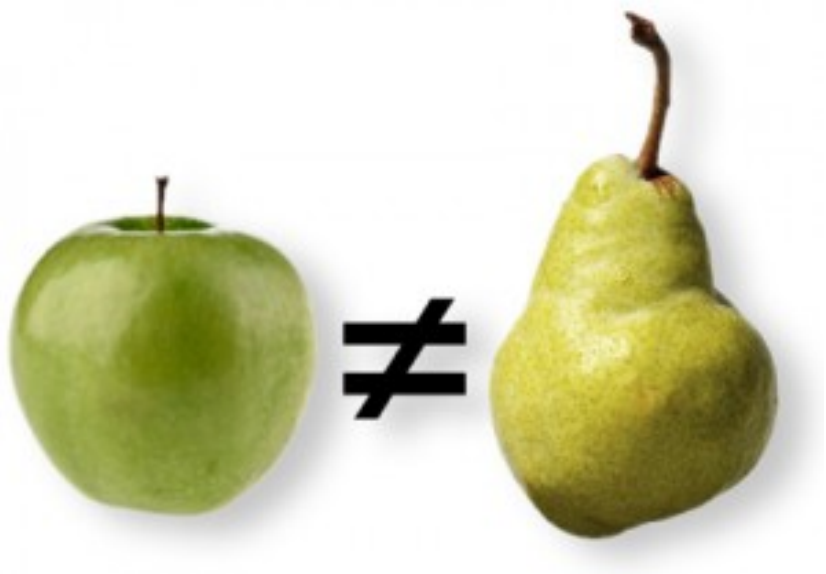
no cooperativity: $K_{i+1} = K_i$

negative cooperativity: $K_{i+1} < K_i$

however, it is only valid when comparing the same binding events

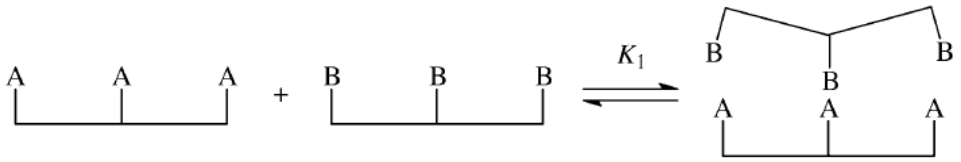
to assess cooperativity, only virtually identical processes described by equilibrium constants having the same dimensions should be compared.

to assess cooperativity, only virtually identical processes described by equilibrium constants having the same dimensions should be compared.



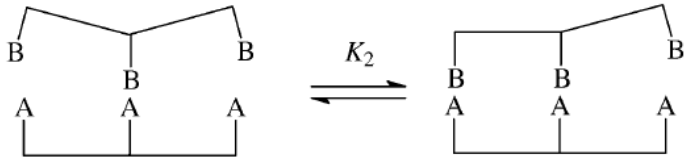
EXAMPLE

inter



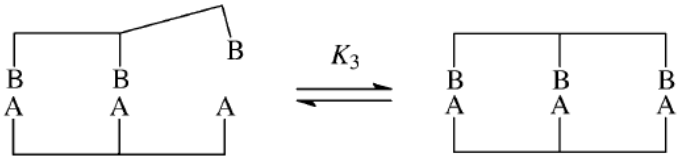
$K_1 = 2\,K_{inter}$
(rigid system)

intra



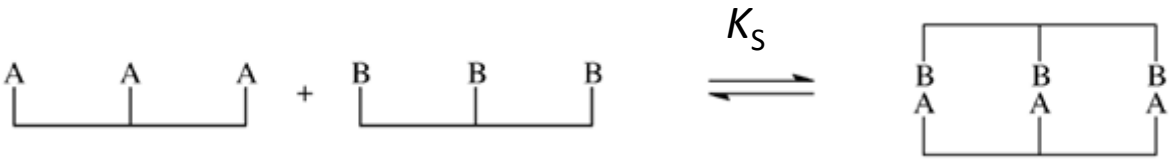
$K_2 = 2\,K_{intra,2}$

intra



$K_3 = 1/2\,K_{intra,3}$

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 or, in other words, $K_{intra,3} > K_{intra,2}$ (or $K_{intra,3}/K_{intra,2} > 1$)
This implies $K_3/K_2 > 1/4$

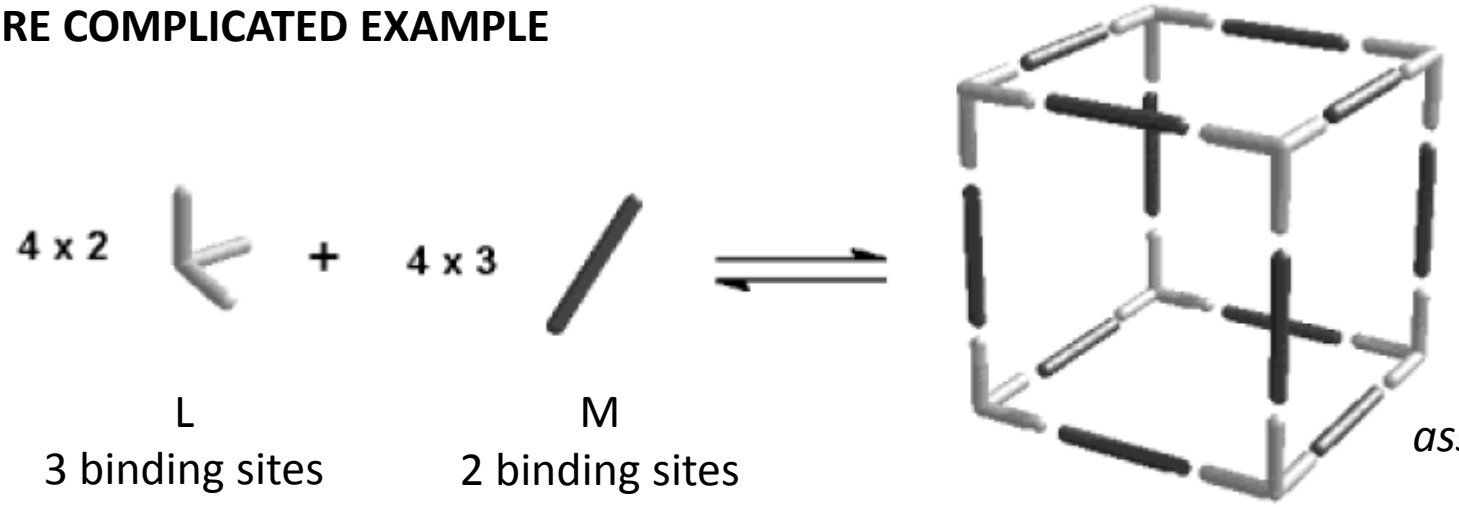


$K_S = K_1\ K_2\ K_3 = (2K_{inter})(2K_{intra})(1/2K_{intra}) = 2\ K_{inter}\ K_{intra}^2$

in case of positive cooperativity : $K_3 / K_2 > 1/4$

and thus $K_{obs} > K_S$ ($= 2\ K_{inter}\ K_{intra}^2$)

A MORE COMPLICATED EXAMPLE



L
3 binding sites

M
2 binding sites

assembly S

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

l = # binding sites on L
m = # binding sites on M
p = factor to correct for stoichiometry

assembly S has stoichiometry: L_8M_{12} (or $L_{pl}M_{pm}$)

N: number of molecules in assembly S = *pl* + *pm* = 20

B: number of bonds = *plm* = 24

To form the assembly 19 (*N*-1) *inter*molecular bonds are required (defined by K_{inter})

The amount of *intra*molecular bonds is given by *B*-*N*+1 (defined by K_{intra})

statistical factor determined by the symmetry

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

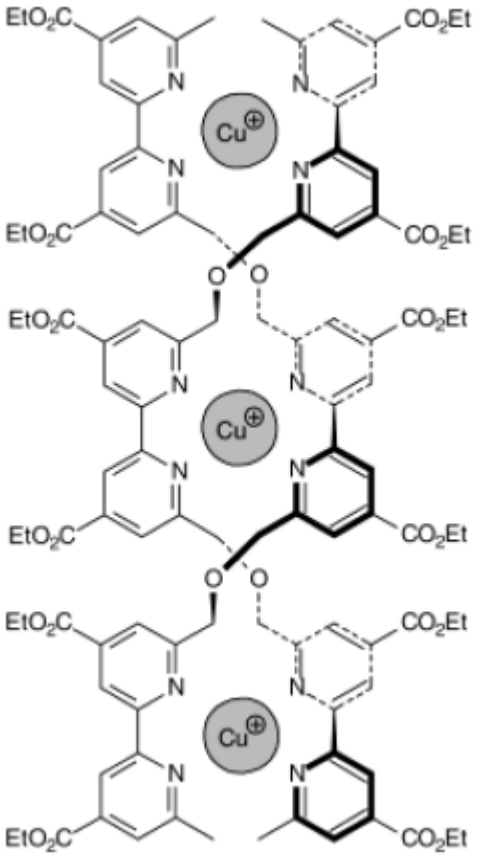
where σ_{sa}, equal to $\sigma_L^{pm}\sigma_M^{pl}/\sigma_S$, is the symmetry factor of the self-assembly equilibrium.[10] An additional factor of 2 multiply-

positive cooperativity: $K_{obs} > K_S$

no cooperativity: $K_{obs} = K_S$

negative cooperativity: $K_{obs} < K_S$

What about Lehn's example ?



$$M + L \rightleftharpoons ML \qquad \log \beta_{11} = 4.6 \qquad (11)$$

$$M + 2L \rightleftharpoons ML_2 \qquad \log \beta_{12} = 8.2 \qquad (12)$$

$$2M + 2L \rightleftharpoons M_2L_2 \qquad \log \beta_{22} = 13.5 \qquad (13)$$

$$3M + 2L \rightleftharpoons M_3L_2 \qquad \log \beta_{32} = 18.6 \qquad (14)$$

Positive cooperativity is a thermodynamically clearly defined feature that may be revealed by several criteria or tests.[10] A multiequilibria system presents positive cooperativity if the ratio $K_{m+1}:K_m$ is higher than the value calculated from eqn. (5); it is non-cooperative (statistical) if the ratio is the same as this value and has negative cooperativity if it is smaller.

$$\frac{K_{m+1}}{K_m} = \frac{m(t-m)}{(m+1)(t-m+1)} \qquad (5)$$

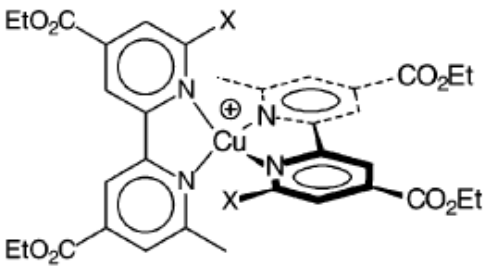
With the β_{ml} values shown above, $K_4 > K_3/3$, which is sufficient for indicating positive cooperativity. There are a

So is this true?

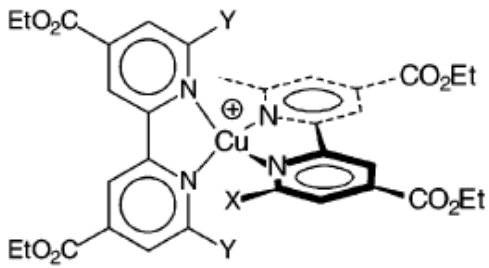
We (*i.e.* Ercolani) consider as the reference intermolecular process the simultaneous binding of a Cu^+ ion to two bipyridine ligands.

An estimate of the constant K_{inter} can be obtained from the constant β_{12} by considering the ML_2 species, **3**-**5**, that are formed in equilibrium 12.

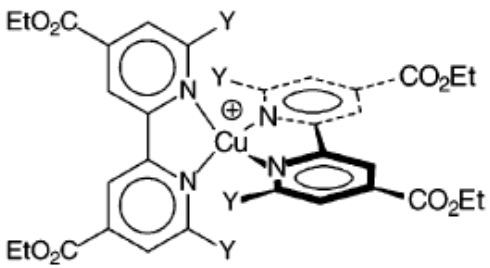
$$M + 2L \rightleftharpoons ML_2 \qquad \log \beta_{12} = 8.2 \qquad (12)$$



3



4



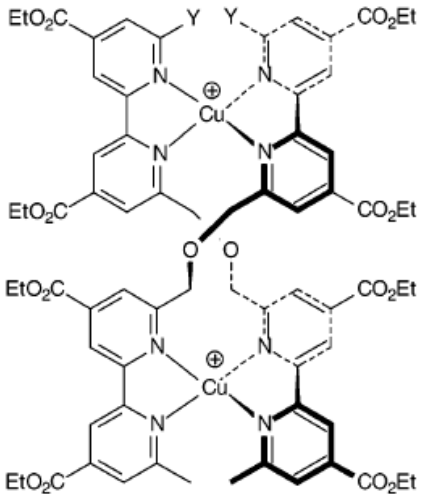
5

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 $4K_{inter}$.
The complex **4** (point group *Cs*) has symmetry number 1 and is achiral; thus the equilibrium constant for its formation is 4 K_{inter}.
The complex **5** (point group *D*2*d*) has symmetry number 4 and is achiral; thus it forms with a constant K_{inter}.

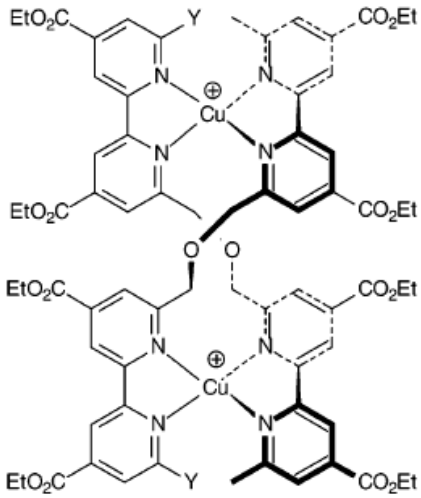
From this analysis, it can be concluded that $\beta_{12} = 9K_{inter}$, and thus $\log K_{inter} = 7.25$.

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.

$$2M + 2L \rightleftharpoons M_2L_2 \qquad \log \beta_{22} = 13.5 \qquad (13)$$



6



7

Both complexes **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 $4K_{inter}K_{intra}$.

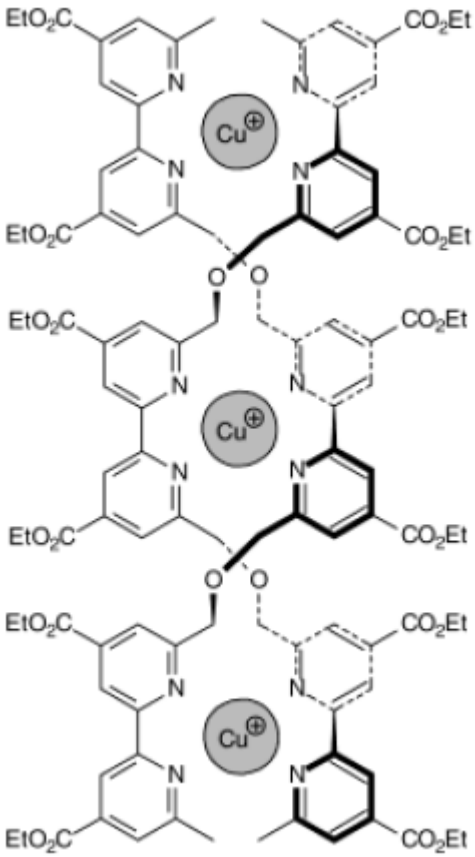
Therefore, $\beta_{22} = 8K_{inter}K_{intra}$

from which $\log K_{intra} = 5.35$.
(remember that K_{inter} is known)

PS We (*i.e.* Ercolani) 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-Sockmayer theory.

Now let's have a look at the final assembly.

$$3M + 2L \rightleftharpoons M_3L_2 \qquad \log \beta_{32} = 18.6 \qquad (14)$$



since it is chiral (point group D_2) and has symmetry number 4, application of eq 10 yields

$K_S = \beta_{32} = 2K_{inter}K_{intra}^2$

Having determined that log K_{inter} = 7.25 and log K_{intra} = 5.35

it follows that log K_S = 18.25

Since the experimental value of log β_{32} (= K_{obs}) = 18.6 with an estimated error of 10%, we (*i.e.* Ercolani) can conclude that

the self-assembly of the trihelicate **2** *is noncooperative within the experimental errors.*

Essays

Molecular Recognition

DOI: 10.1002/anie.200902490

What is Cooperativity?

Christopher A. Hunter and Harry L. Anderson**

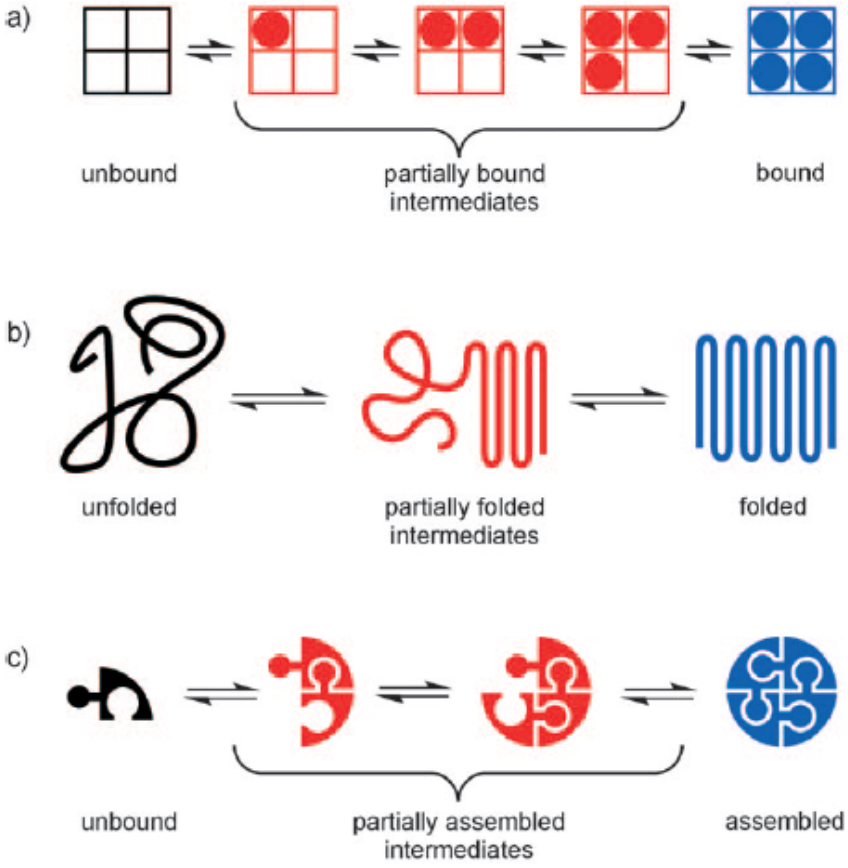
allosteric cooperativity · chelate cooperativity · cooperative effects · self-assembly · supramolecular chemistry

Dedicated to Professor Jean-Marie Lehn on the occasion of his 70th birthday

1. Introduction: It's All or Nothing

Angew. Chem. Int. Ed. **2009**, *48*, 7488 – 7499

It’s all or nothing



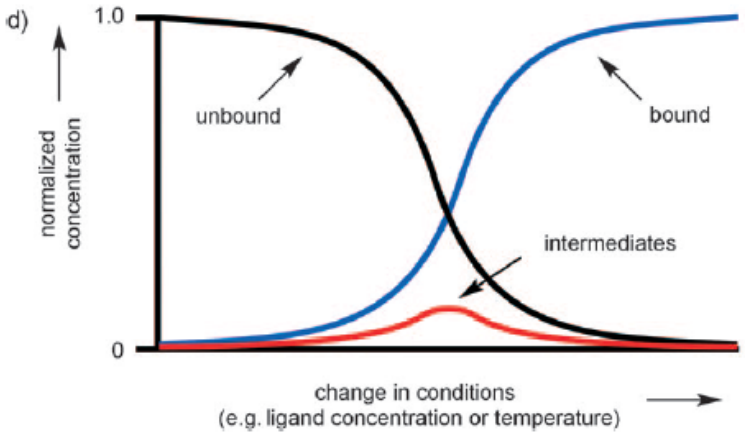


Figure 1. Representation of processes that display positive cooperativity: a) hemoglobin binding oxygen, b) protein folding, and c) supramolecular self-assembly. d) Speciation profiles. Positive cooperativity leads to a low peak concentration of intermediates and a sharp transition from unbound to bound.

As a system approaches the limit of strong positive cooperativity, only the extreme states are significantly populated.

Such systems can exhibit “all-or-nothing” behavior in two senses:

1) **At the molecular level:** any individual molecule is likely to be fully bound or fully unbound; it spends little time in intermediate states.

2) **At the macroscopic level**: the behavior of the ensemble is characterized by a population switch from mainly free to mainly bound over a small change in conditions. Under most conditions, one state predominates, and this leads to the sigmoidal binding isotherms and sharp melting transitions that are the classical signatures of cooperativity, as illustrated by the binding of oxygen to hemoglobin and the denaturation of lysozyme in Figure 2.

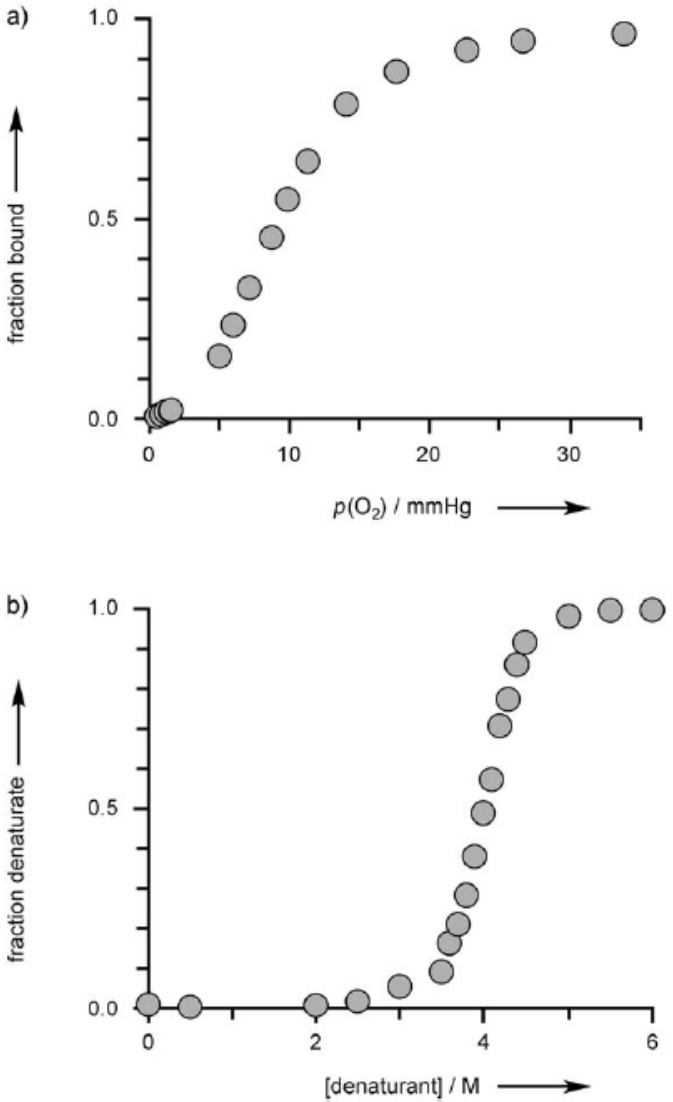


Figure 2. Experimentally observed isotherms for a) oxygen binding by hemoglobin as a function of oxygen concentration[10] and b) the denaturation of lysozyme as a function of guanidine hydrochloride concentration.[11]

Cooperativity

allosteric cooperativity:

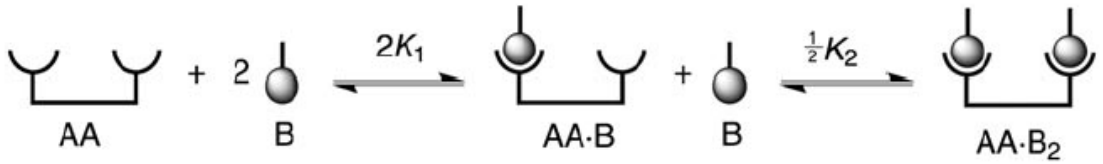


Figure 1. Binding of a monovalent ligand B to a divalent receptor AA.

chelate cooperativity:

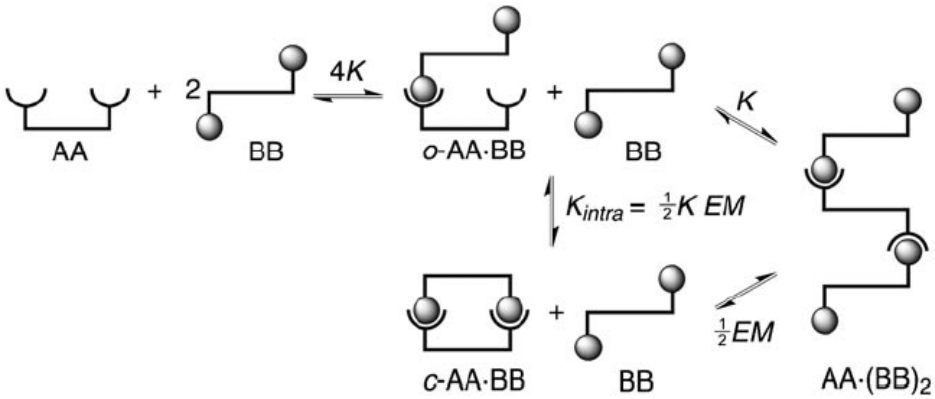
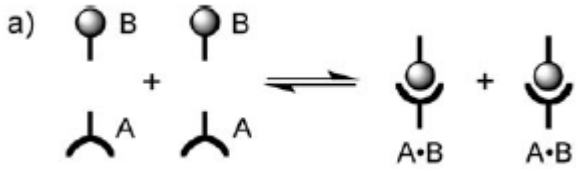


Figure 3. Binding of a divalent ligand BB to a divalent receptor AA, assuming $[BB]_0 \gg [AA]_0$ and $\alpha = 1$.

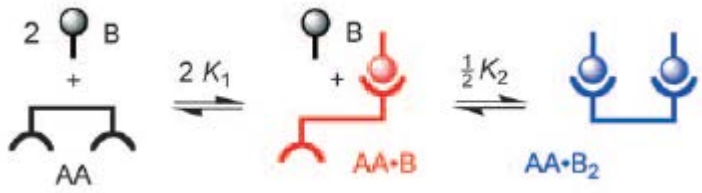
The reference system



We start by considering a system where there can be no cooperativity because there is only one interaction. The complex between a receptor with one binding site (**A**) and a ligand with one binding site (**B**) is our reference point for assessing other scenarios. This simple two-state equilibrium is characterized by the association constant *K* [Eq. (1)], where [**A**·**B**] and [**A**] are the concentrations of bound and free receptor, and [**B**] is the concentration of free ligand.

$$K = \frac{[\mathrm{A} \cdot \mathrm{B}]}{[\mathrm{A}]\ [\mathrm{B}]}$$

Allosteric Ligand Binding (*e.g.* hemoglobin)



The equilibria are characterized by two microscopic association constants K_1 and K_2, which are defined by Equations (2) and (3).

$$2\,K_1 = \frac{[\mathrm{AA}\cdot\mathrm{B}]}{[\mathrm{AA}]\,[\mathrm{B}]}$$

$$\tfrac{1}{2}K_2 = \frac{[\mathrm{AA}\cdot\mathrm{B}_2]}{[\mathrm{AA}\cdot\mathrm{B}]\,[\mathrm{B}]}$$

At the molecular level, the cooperativity of the system is described by the interaction parameter α, which is defined by Equation (4).

$$\alpha = \frac{K_2}{K_1}$$

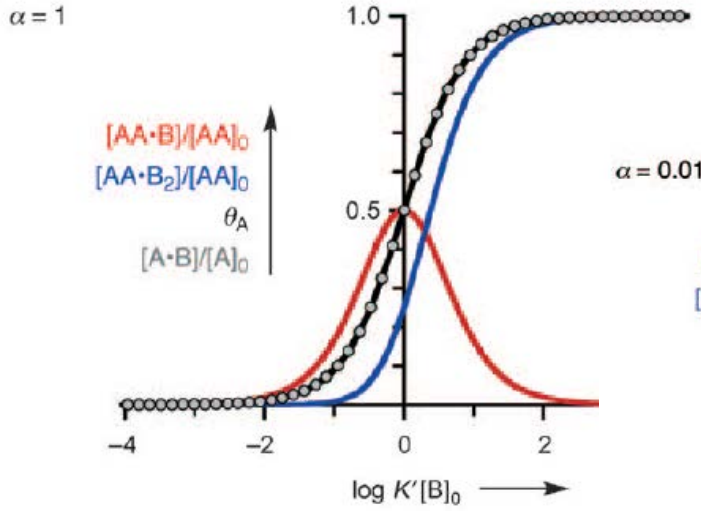
In the absence of cooperativity, the microscopic association constants are identical to the value for the corresponding reference receptor with one binding site, that is, $K_1 = K_2 = K$ and $\alpha = 1$.

At the molecular level

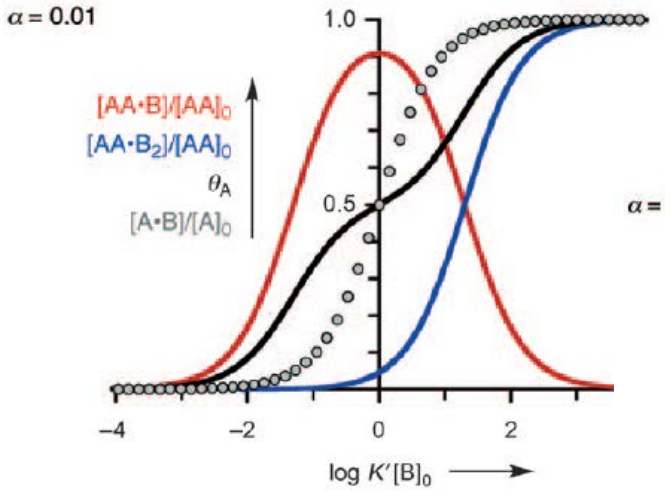
Under a given set of conditions, the total fraction of receptor sites that are bound to ligand is defined as the **binding-site occupancy** of the receptor θ_A, which is given by Equation (5), where $[AA]_0$ is the total receptor concentration (free and bound)

$$\theta_A = \frac{\frac{1}{2}[AA \cdot B] + [AA \cdot B_2]}{[AA]_0} \qquad (5)$$

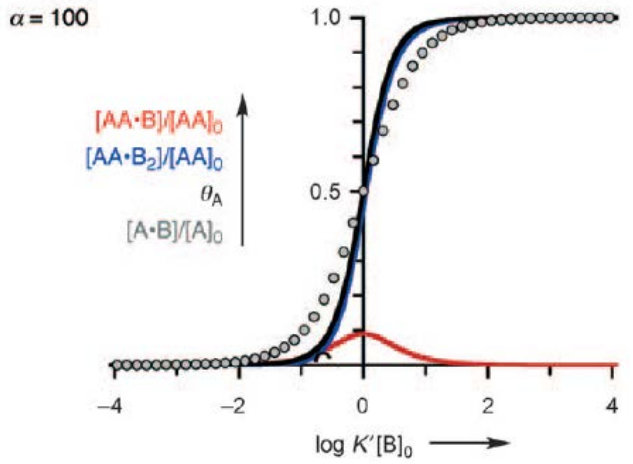
No cooperativity (α=1)



Negative cooperativity (α=0.01)



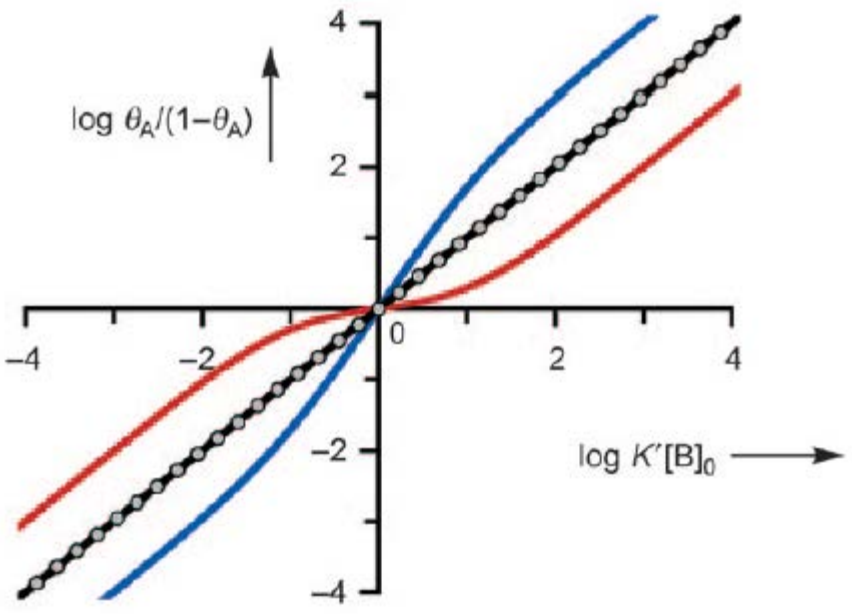
Positive cooperativity (α=100)



The concentration scales in the plots are normalized by the apparent association constant per site K', where 1/K' is the concentration at θ_A=50%.

At the macroscopic level

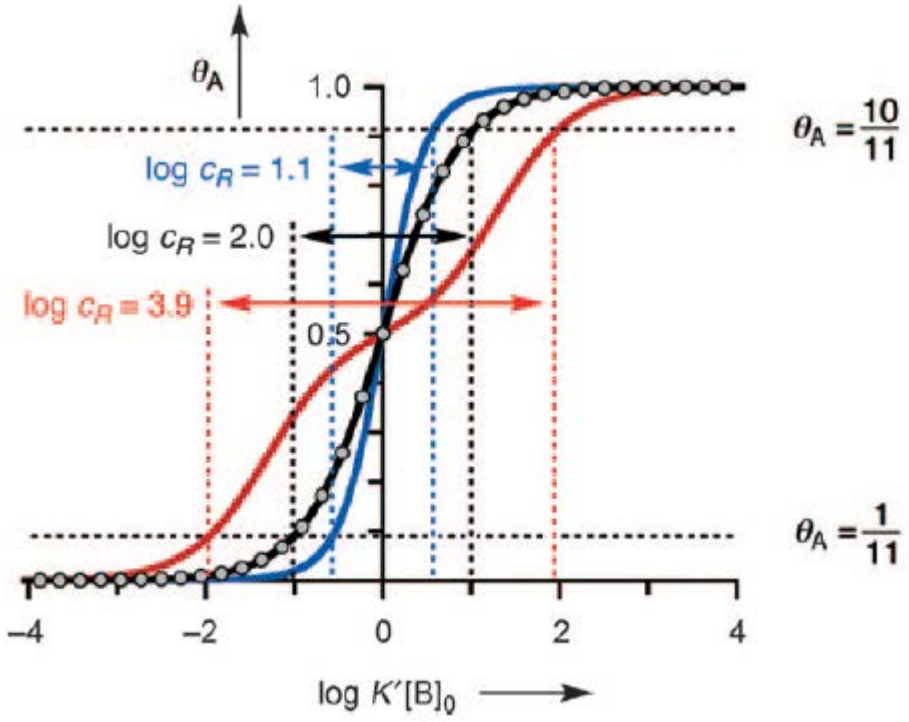
At the macroscopic level, cooperativity in allosteric systems is usually characterized by plotting log $\{\theta_A/(1-\theta_A)\}$ versus log $[B]_0$ in a Hill plot.
The Hill coefficient n_H is the slope of this plot measured at 50% saturation, that is, at log log $\{\theta_A/(1-\theta_A)\}$ =0.



The macroscopic behavior of systems of this type can also be characterized by **the switching window** c_R [Eq. (6)],

$$c_R = \frac{[B]_0 \text{ at } \theta_A = 10/11}{[B]_0 \text{ at } \theta_A = 1/11} \qquad (6)$$

which is the factorial increase in ligand concentration required to change the bound/free receptor ratio from 1:10 to 10:1 (Figure 5b).



In other words, c_R is a measure of the sharpness of the bound–free transition.

chelate cooperativity:

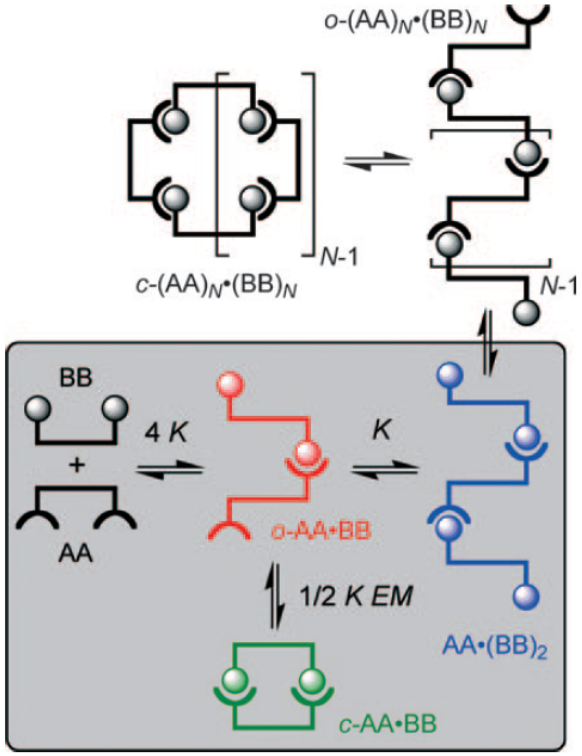
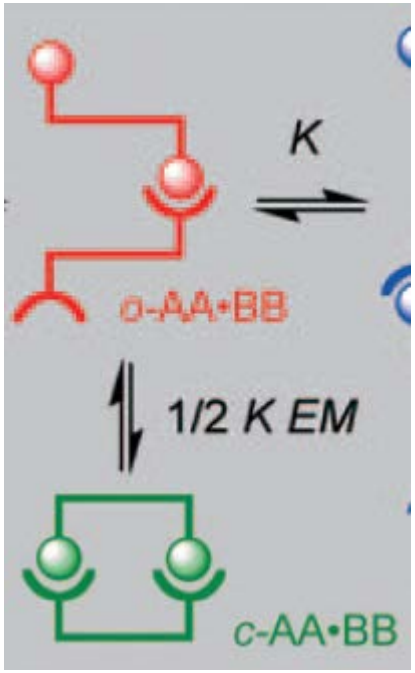


Figure 9. A two-site receptor (AA) that interacts with a divalent ligand (BB), assuming $\alpha = 1$.[12] Many states are possible for this system, but if $[BB]_0 \gg [AA]_0$, only the species inside the box are populated.

If the ligand is present in a large excess relative to the receptor, then we can ignore complexes that involve more than one receptor, because they will not be significantly populated. Under these conditions, there are only four states for the receptor (highlighted in the box in Figure 9): free **AA**, two 1:1 complexes (the partially bound open intermediate ***o*-AA·BB** and the fully bound cyclic complex ***c*-AA·BB**), and the 2:1 complex **AA**·(**BB**)$_2$. Here we limit ourselves to the scenario where α =1 and there is no allosteric cooperativity.

At the molecular level, the key feature that defines the properties of this system is the intramolecular binding interaction that leads to the cyclic 1:1 complex c-AA·BB.
This interaction is described using **the effective molarity** (EM) as defined in Equation (7).



$$\frac{1}{2}K\,EM = \frac{[c\text{-AA} \cdot \text{BB}]}{[o\text{-AA} \cdot \text{BB}]} \qquad (7)$$

As implied by this equation, the ratio of the open and closed 1:1 complexes is independent of the ligand concentration.
The product ***K EM*** determines the extent to which the cyclic complex is populated, and is the **key molecular parameter** that defines the cooperativity of self-assembled systems.

For complexes where the binding sites are identical, there is a statistical factor (K_σ=2), which accounts for the difference in binding site concentration between a monovalent ligand (B) and a divalent ligand (BB). Some authors incorporate the statistical factor into the value of EM.

***K EM* << 1** (Figure 10 a). Under these conditions, the partially bound intermediate is more stable than the cyclic complex. The system is unaffected by the presence of the cyclic complex, and the behavior is identical to that found for monovalent ligands (compare Figure 4b and Figure 10a).

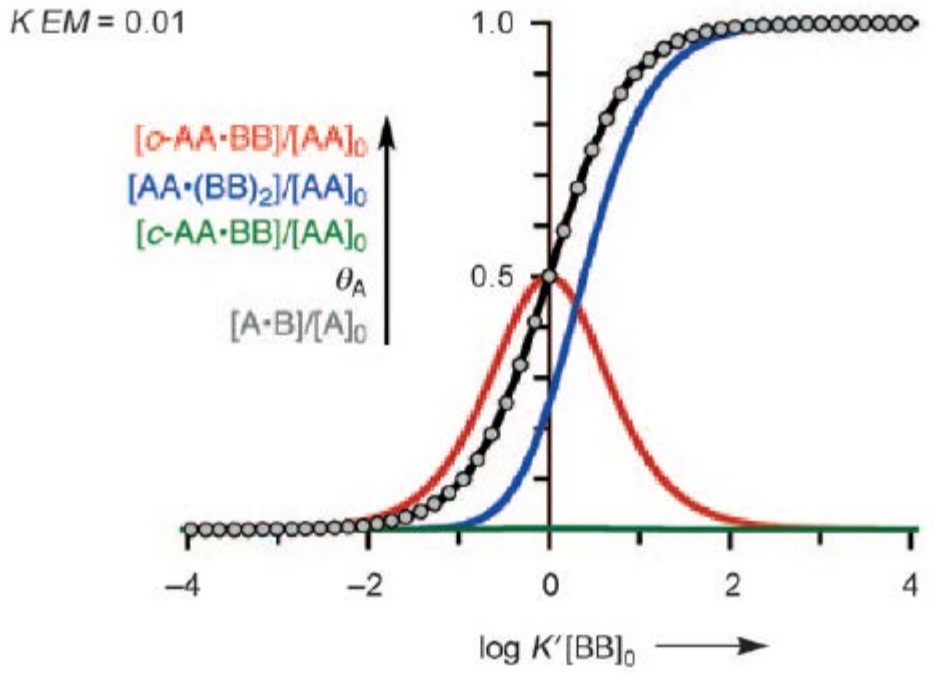


Figure 10. Speciation profiles for the equilibria shown in Figure 9 (cyclic complex *c*-AA·BB in green, open intermediate *o*-AA·BB in red, 2:1 complex AA·$(BB)_2$ in blue, and total binding-site occupancy θ_A in black). a) $KEM = 0.01$ and b) $KEM = 100$. In both cases, $\alpha = 1$. The speciation profile for the system with one intermolecular interaction is shown for reference (gray dots).[14, 27]

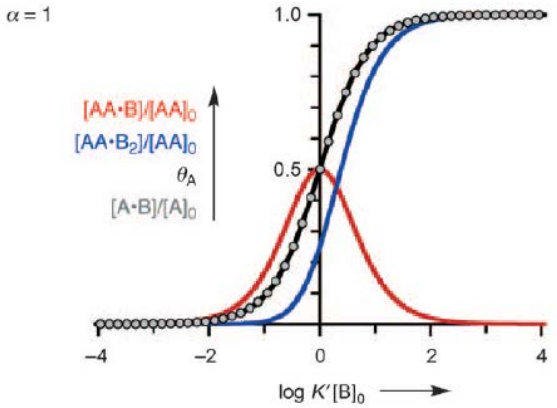
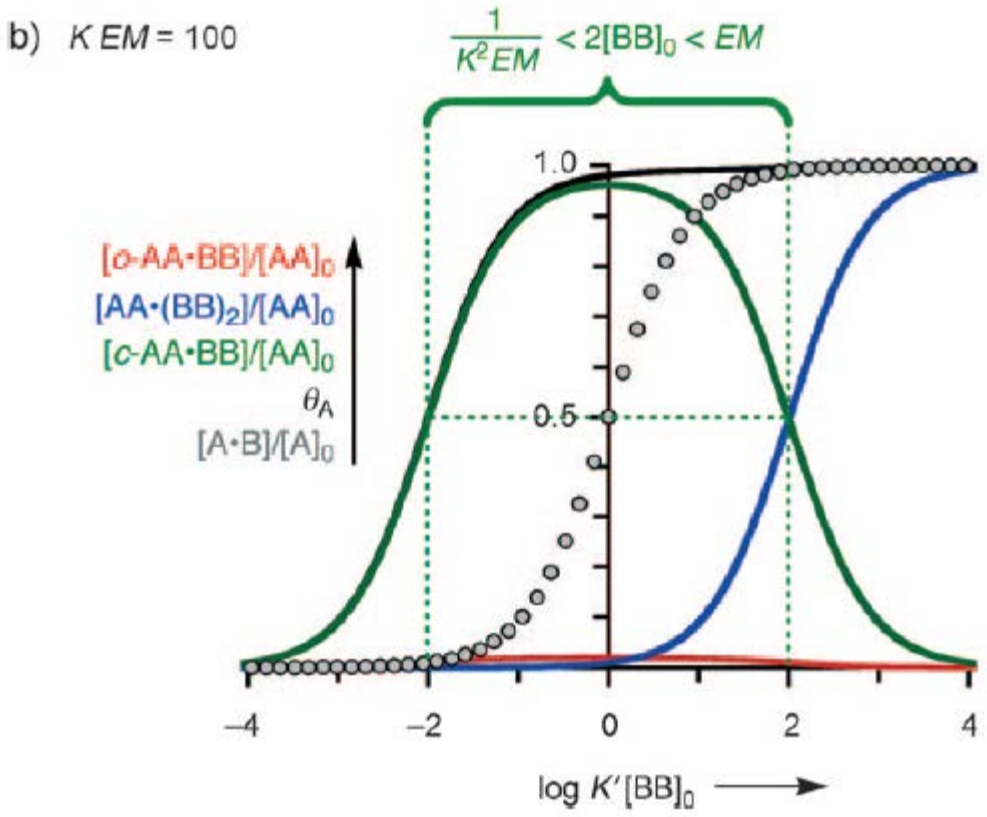


Figure 4b

K EM >>1 (Figure 10b). In this case, the cyclic complex is more stable than the partially bound intermediate, and **c-AA·BB** is the major species over a wide concentration range. The open partially bound intermediate **o-AA·BB** is barely populated, and formation of the 2:1 complex **AA·(BB)**$_2$ is suppressed compared to the situation with the corresponding monovalent ligands.
The cyclic 1:1 complex **c-AA·BB** opens to form the 2:1 complex **AA·(BB)**$_2$ only when 2[**BB**]$_0$>EM. In other words, *EM defines the concentration at which simple monovalent intermolecular interactions compete with cooperative intramolecular ones*.



Cooperative assembly of the complex is driven by the difference in strength between the intermolecular and intramolecular interactions, and is a consequence of the molecular architecture.
This phenomenon gives rise to the chelate effect, so we call it ***chelate cooperativity***.
This is the type of cooperativity exhibited in the folding of proteins and supramolecular self-assembly (Figure 1b, c).