Cooperativity
Multivalent interactions in human biology

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

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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 (HA₃, 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 sLeX moieties) with E- and P-selectins. In addition, L-selectins, expressed on the surface of the neutrophil, interact with sLeX 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

![Diagram of DNA binding](image)

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-L), has higher affinity than the monomeric complex. Bottom: The pentameric complex (RXR-L), 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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Fig. 1. HPLC of aliquots of samples that contained (A) R,V₃ (4.5 μM); (B) R,V₅ (4.5 μM); (C) R,V₃ (4.5 μM) + L (19.1 mM); and (D) R,V₅ (4.5 μM) + L',L' (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.

Scheme 1. Structures of the trivalent derivatives of vancomycin, R,V₃, and of dADA, R',L'.

dissociation constant (Kᵢ) ≈ 4 × 10⁻¹⁷ ± 1 × 10⁻¹⁷ M.
Multivalent interactions in supramolecular chemistry

Additivity (?)

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

\[ K_{\text{ass}} = 25 \, \text{M}^{-1} \quad \Delta G = -7.9 \, \text{kJ mol}^{-1} \]

\[ K_{\text{ass}} = 6.4 \times 10^3 \, \text{M}^{-1} \quad \Delta G = -21.6 \, \text{kJ mol}^{-1} \]

\[ K_{\text{ass}} = 1.5 \times 10^6 \, \text{M}^{-1} \quad \Delta G = -35.3 \, \text{kJ mol}^{-1} \]
Additivity (?)

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

\[
K_{ass} = 10^4 \text{ M}^{-1}
\]

\[
K_{ass} = 10^2 \text{ M}^{-1}
\]

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)

\[ M = \text{Ni}^{2+} \]
\[ \text{log } K = 8.76 \]

\[ \Delta H^\circ = -29 \text{ kJ mol}^{-1} \]
\[ -T\Delta S^\circ = -25 \text{ kJ mol}^{-1} \]

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
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.
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


- **Monovalent** binding sites:
  - Receptor interacts with a monovalent ligand
  - Reaction: $\Delta G_{\text{total}}^\text{mono} = K_{\text{mono}}^\text{mono}$

- **Polivalent** (bivalent) binding sites:
  - Receptor interacts with a multivalent ligand
  - Reaction: $\Delta G_{\text{total}}^\text{bi} = K_{\text{bi}}^\text{bi}$

- **Polyvalent** (N-valent) binding sites:
  - Receptor interacts with a multivalent ligand
  - Reaction: $\Delta G_{\text{total}}^\text{poly} = K_{\text{poly}}^\text{poly}$

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**Multiple binding sites:**
- Receptor interacts with a multivalent ligand
- Receptor interacts with a monovalent ligand
Figure 6. Proposed nomenclature for the polyvalent interactions; relationships between free energies of binding ($\Delta G$) and inhibition constants ($K_i$) for both monovalent and polyvalent systems.

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

\[
\Delta G_{\text{avg}}^{\text{poly}} = \frac{\Delta G_N^{\text{poly}}}{N} \tag{3}
\]

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

\[
K_N^{\text{poly}} = (K_{\text{avg}}^{\text{poly}})^N \tag{5}
\]
Measuring cooperativity: $\alpha$ value

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

$$\Delta G_{\text{avg}}^{\text{poly}} = \alpha \Delta G_{\text{mono}}^{\text{mono}}$$  \hspace{1cm} (6)

$$N \Delta G_{\text{avg}}^{\text{poly}} = \Delta G_{N}^{\text{poly}} = \alpha N \Delta G_{\text{mono}}^{\text{mono}}$$  \hspace{1cm} (7)

$$K_{N}^{\text{poly}} = (K_{\text{avg}}^{\text{poly}})^N = (K_{\text{mono}}^{\text{mono}})^{\alpha N}$$  \hspace{1cm} (8a)

$$\alpha = \frac{\lg(K_{N}^{\text{poly}})}{\lg((K_{\text{mono}}^{\text{mono}})^N)}$$  \hspace{1cm} (8b)

!! this is a wrong comparison !!
$\alpha =$ degree of cooperativity

- $\alpha > 1$: positive cooperativity (synergistic)
- $\alpha = 1$: noncooperative (additive)
- $\alpha < 1$: 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 $\alpha < 1$

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

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

$$\beta = K_N^{\text{poly}} / K_N^{\text{mono}}$$
Helicate Self-organisation: Positive Cooperativity in the Self-assembly of Double-helical Metal Complexes

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Analysis of the binding of CuI 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
We have

\[ E + nS \rightleftharpoons ES_n \]  \hspace{1cm} (10.1)

and

\[ K = \frac{[E][S]^n}{[ES_n]} \]  \hspace{1cm} (10.2)

The degree of saturation \( Y \) is given by

\[ Y = \frac{[ES_n]}{[E]_0} \]  \hspace{1cm} (10.3)

Equations 10.2 and 10.4 may be manipulated to give

\[ \log \frac{Y}{1 - Y} = n \log[S] - \log K \]  \hspace{1cm} (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

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

- $n > 1$: positive cooperativity
- $n = 1$: no cooperativity
- $n < 1$: negative cooperativity

**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).]
1. The single binding site

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

\[ \text{PL} \xrightarrow{k_l} P + L \]  

\[ K_L = \frac{[P][L]}{[PL]} \]  

(6.21)  

(6.22)  

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

In terms of the total protein concentration \([P]_0\),

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

(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:

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

(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

\[ \Delta F = \Delta F_{\text{max}} - K_L \frac{\Delta F}{[L]} \]  

(6.25)  

where \(\Delta F\) is the change in spectroscopic signal when [L] is added to the protein solution. A plot of \(\Delta F\) against \(\Delta F/[L]\) gives \(K_L\) and \(\Delta F_{\text{max}}\), 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.

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

(6.26)

where $\nu$ is the number of moles of ligand bound per mole of protein. The stoichiometry $n$ and $K_L$ are obtained from the plot of $\nu$ 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 $v$ against $v/[L]$ 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.
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.
Kobs = KinterKinterKinter = Kinter^3

but what about the individual binding steps?
1. complexation of the first guest

\[
K_{\text{obs,1}} = \frac{H_3G}{H_3 \times G}
\]

What is the relation between \(K_{\text{obs,1}}\) and \(K_{\text{inter}}\)?

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

thus \(K_{\text{obs,1}} = 3K_{\text{inter}}\)
2. complexation of the second guest

\[ K_{\text{obs,2}} = \frac{H_3G_2}{H_3G \times G} \]

What is the relation between \( K_{\text{obs,2}} \) and \( K_{\text{inter}} \)?

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

Thus \( K_{\text{obs,2}} = \frac{2K_{\text{inter}}}{2} = K_{\text{inter}} \)
3. complexation of the third guest

$$K_{obs,3} = \frac{H_3G_3}{H_3G_2 \times G}$$

What is the relation between $K_{obs,3}$ and $K_{inter}$?

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

$$K_{obs,3} = \frac{1}{3} K_{inter}$$
$K_{obs} = K_{obs,1} K_{obs,2} K_{obs,3} = (3K_{inter})(K_{inter})(1/3K_{inter}) = K_{inter}^3$

$K_{obs} = \frac{H_3G_3}{H_3 \times G^3}$
\[ K_i = K_{\text{inter}} \frac{(m-i+1)}{i} \]

\( m \): number of identical and independent binding sites

- 4 sites left
  - \[ 4K_{\text{inter}} \]
  - but each complex can be formed in 2 ways

- 3 sites left
  - \[ \frac{3}{2} K_{\text{inter}} \]
  - but each complex can be formed in 2 ways

- 2 sites left
  - \[ \frac{2}{3} K_{\text{inter}} \]
  - but each complex can be formed in 3 ways

- 1 site left
  - \[ \frac{1}{4} K_{\text{inter}} \]
  - 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.
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 $(N-1)$ intermolecular bonds are required (defined by $K_{\text{inter}}$)

The amount of intramolecular bonds is given by $B-N+1$ (defined by $K_{\text{intra}}$)
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, $K_{\text{intra},3} > K_{\text{intra},2}$ (or $K_{\text{intra},3}/K_{\text{intra},2} > 1$) This implies $K_3/K_2 > 1/4$
\[ K_S = \sigma_{sa} K_{\text{inter}}^{N-1} K_{\text{intra}}^{B-N+1} \]  \hspace{1cm} (10)

where \( \sigma_{sa} \), 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: \( K_{\text{obs}} > K_S \)

no cooperativity: \( K_{\text{obs}} = K_S \)

negative cooperativity: \( K_{\text{obs}} < K_S \)
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


see PDF
Cooperativity

allosteric cooperativity:

![Diagram of allosteric cooperativity](image1)

chelate cooperativity:

![Diagram of chelate cooperativity](image2)

interannular cooperativity:

![Diagram of interannular cooperativity](image3)