# Decomposing Complex Cooperative Ligand Binding into Simple Components: Connections between Microscopic and Macroscopic models.

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submitted to *J. Phys. Chem. B.*

January 5, 2004

## Supporting Information

## Details of the Derivation of Cooperativity Measure $\Xi$

Here we investigate how the slope at the inflection point of decoupled binding curves can be used as a measure of cooperativity. We consider two limiting cases. The maximum cooperativity is achieved if either none or all $N$ ligands bind, i.e. only two states exist: no ligands bound or all ligands bound.

$$\langle X \rangle = \frac{N e^{-\beta(G^{\mathbf{k}} - N\mu_{\mathrm{L}})}}{1 + e^{-\beta(G^{\mathbf{k}} - N\mu_{\mathrm{L}})}} \tag{1}$$

The first and second derivative of eq 1 are given in eq 2 and 3, respectively.

$$\frac{\partial \langle X \rangle}{\partial \mu_{\mathrm{L}}} = \frac{\beta N^2 e^{-\beta(G^{\mathbf{k}} - N\mu_{\mathrm{L}})}}{(1 + e^{-\beta(G^{\mathbf{k}} - N\mu_{\mathrm{L}})})^2} \tag{2}$$

$$\frac{\partial^2 \langle X \rangle}{\partial {\mu_{\mathrm{L}}}^2} = \frac{\beta^2 N^3 e^{-\beta(G^{\mathbf{k}} - N\mu_{\mathrm{L}})}(1 - e^{-\beta(G^{\mathbf{k}} - N\mu_{\mathrm{L}})})}{(1 + e^{-\beta(G^{\mathbf{k}} - N\mu_{\mathrm{L}})})^3} \tag{3}$$

From eq 3 the inflection point $\mu_{\mathrm{I}}$ of eq 1 is given by eq 4.

$$\frac{\partial^2 \langle X \rangle}{\partial {\mu_{\mathrm{I}}}^2} = 0 : \qquad \mu_{\mathrm{L}} = \frac{G^{\mathbf{k}}}{N} \tag{4}$$

The slope at the inflection point is consequently given by eq 5

$$\frac{\partial \langle X \rangle(\mu_{\mathrm{I}})}{\partial \mu_{\mathrm{L}}} = \frac{\beta N^2}{4} \tag{5}$$

Eq 5 gives the maximum possible slope for a binding curve of $N$ ligands and one receptor.

On the other extreme is a non-cooperative system of $N$ non-interacting equivalent sites. The total binding curve of such a system is given by eq 6

$$\langle X \rangle = N \frac{e^{-\beta(G_i^{\mathrm{o}} - \mu_{\mathrm{L}})}}{1 + e^{-\beta(G^{\mathrm{o}} - \mu_{\mathrm{L}})}} \tag{6}$$

The first and the second derivative of eq 6 are given by eqs 7 and 8.

$$\frac{\partial \langle X \rangle}{\partial \mu_{\mathrm{L}}} = N \frac{\beta e^{-\beta(G^{\mathrm{o}} - \mu_{\mathrm{L}})}}{(1 + e^{-\beta(G^{\mathrm{o}} - \mu_{\mathrm{L}})})^2} \tag{7}$$

$$\frac{\partial^2 \langle X \rangle}{\partial {\mu_{\mathrm{L}}}^2} = N \frac{\beta^2 e^{-\beta(G^{\mathrm{o}} - \mu_{\mathrm{L}})}(1 - e^{-\beta(G^{\mathrm{o}} - \mu_{\mathrm{L}})})}{(1 + e^{-\beta(G^{\mathrm{o}} - \mu_{\mathrm{L}})})^3} \tag{8}$$

The slope at the inflection point is then given by eq 9.

$$\frac{\partial \langle X \rangle(\mu_{\mathrm{I}})}{\partial \mu_{\mathrm{L}}} = \frac{\beta N}{4} \tag{9}$$

The combination of eq 5 and eq 5 suggests the following cooperativity measure $\Xi$:

$$\Xi = \frac{4}{\beta N} \frac{\partial \langle X \rangle (\mu_{\mathrm{I}})}{\partial \mu_{\mathrm{L}}} \bigg|_{at\ the\ inflection\ point} \tag{10}$$

where the normalization factor is chosen so that

for non-cooperative binding: $\Xi = 1$

and for fully cooperative binding: $1 < \Xi \leq N$

***Cooperativity in the system of two interacting sites.*** The total binding curve is

$$\langle X \rangle = \frac{e^{-\beta(G_1^{\mathrm{o}} - \mu_{\mathrm{L}})} + e^{-\beta(G_2^{\mathrm{o}} - \mu_{\mathrm{L}})} + 2e^{-\beta(G_1^{\mathrm{o}} + G_2^{\mathrm{o}} + W - 2\mu_{\mathrm{L}})}}{1 + e^{-\beta(G_1^{\mathrm{o}} - \mu_{\mathrm{L}})} + e^{-\beta(G_2^{\mathrm{o}} - \mu_{\mathrm{L}})} + e^{-\beta(G_1^{\mathrm{o}} + G_2^{\mathrm{o}} + W - 2\mu_{\mathrm{L}})}} \tag{11}$$

which can be rewritten with

$$\begin{aligned} A &= e^{-\beta G_1^{\mathrm{o}}} + e^{-\beta G_2^{\mathrm{o}}} \\ B &= e^{-\beta(G_1^{\mathrm{o}} + G_2^{\mathrm{o}} + W)} \\ \lambda &= e^{\beta \mu_{\mathrm{L}}} \end{aligned} \tag{12}$$

Total binding curve

$$\langle X \rangle = \frac{A\lambda + 2B\lambda^2}{1 + A\lambda + B\lambda^2} \tag{13}$$

First derivative of the Total binding curve is

$$\frac{\partial \langle X \rangle}{\partial \mu_{\mathrm{L}}} = \frac{\beta\lambda(A + 4B\lambda + AB\lambda^2)}{(1 + A\lambda + B\lambda^2)^2} \tag{14}$$

Second derivative of the Total binding curve is

$$\frac{\partial^2 \langle X \rangle}{\partial {\mu_{\mathrm{L}}}^2} = \frac{\beta^2\lambda(1 - B\lambda^2)(A - B^2\lambda + 8B\lambda + AB\lambda^2)}{(1 + A\lambda + B\lambda^2)^3} \tag{15}$$

The total binding curve has only one inflection point for cooperative binding curves

$$\mu_{\mathrm{I}} = -\frac{1}{2} \ln B = \frac{1}{2}\beta(G_1^{\mathrm{o}} + G_2^{\mathrm{o}} - W) \Longrightarrow \lambda_{\mathrm{I}} = \frac{1}{\sqrt{B}} \tag{16}$$

The slope of the binding curve eq 13 at its inflection point is

$$\frac{\partial \langle X \rangle}{\partial \mu_{\mathrm{L}}} \bigg|_{\lambda_{\mathrm{I}}} = \frac{\beta\lambda_{\mathrm{I}}(A + 4B\lambda_{\mathrm{I}} + AB\lambda_{\mathrm{I}}^2)}{(1 + A\lambda_{\mathrm{I}} + B\lambda_{\mathrm{I}}^2)^2} = \frac{\beta(4B + 2A\sqrt{B})}{4B + 4A\sqrt{B} + A^2} \tag{17}$$

The cooperativity measure is then given by

$$\Xi = 2\frac{4B + 2A\sqrt{B}}{4B + 4A\sqrt{B} + A^2} \tag{18}$$

***Relation of $\Xi$ to the Hill coefficient.*** For two sites one can show that $\Xi$ is exactly equivalent to the Hill coefficient. Note, however, that this statement is generally not valid for $N > 2$ as seen from the examples in the current work. Let $Y$ be saturation of the molecule, i.e.

$$Y = \frac{1}{N}\langle X \rangle \tag{19}$$

and consider only two interacting sites. The Hill coefficient $n_{Hill}$ is defined as slope of the $Y/(1-Y)$ curve when the receptor is half saturated.

$$n_{Hill} = \frac{\partial \frac{Y}{1-Y}}{\partial \mu_{\mathrm{L}}} \quad at \quad \mu_{\mathrm{I}} = -\frac{1}{2}\ln B \rightarrow \lambda_{\mathrm{I}} = \frac{1}{\sqrt{B}} \tag{20}$$

From eq 11 one can find that

$$\frac{Y}{1-Y} = \frac{A\lambda + 2B\lambda^2}{2 + A\lambda} \tag{21}$$

$$\frac{\partial \frac{Y}{1-Y}}{\partial \mu_{\mathrm{L}}} = \frac{2\lambda(A + 4B\lambda + AB\lambda^2)}{(2 + A\lambda)^2} \tag{22}$$

$$n_{Hill} = \frac{\partial \frac{Y}{1-Y}}{\partial \mu_{\mathrm{L}}}\Big|_{\mu_{\mathrm{I}}} \tag{23}$$

$$= \frac{2(2A\sqrt{B} + 4B)}{4B + 4A\sqrt{B} + A^2} \tag{24}$$

From comparison with eq 18 one see that

$$n_{Hill} = \Xi \; for \; N = 2. \tag{25}$$

**Adair equation re-written using microscopic constants.**

For the sake of simplicity, we restrict the discussion to the N=4 ligand binding sites. We choose N=4 mainly due to the importance of the hemoglobin test case. The total ligand binding curve of a molecule that can bind $N$ ligands of the same type is given by:

$$\mathrm{R} + 4\,\mathrm{L} \overset{\overline{K}_1}{\rightleftharpoons} \mathrm{RL} + 3\,\mathrm{L} \overset{\overline{K}_2}{\rightleftharpoons} \mathrm{RL}_2 + 2\,\mathrm{L} \overset{\overline{K}_3}{\rightleftharpoons} \mathrm{RL}_3 + \mathrm{L} \overset{\overline{K}_4}{\rightleftharpoons} \mathrm{RL}_4 \tag{26}$$

The species $\mathrm{RL}_i$ is the macrostate of the receptor with $i$ ligands bound. The total binding curve $\langle X \rangle$ is in general described by eq 27.

$$\langle X \rangle = \frac{\mathfrak{a}_1[\mathrm{L}] + 2\,\mathfrak{a}_2[\mathrm{L}]^2 + 3\,\mathfrak{a}_3[\mathrm{L}]^3 + 4\,\mathfrak{a}_4[\mathrm{L}]^4}{1 + \mathfrak{a}_1[\mathrm{L}] + \mathfrak{a}_2[\mathrm{L}]^2 + \mathfrak{a}_3[\mathrm{L}]^3 + \mathfrak{a}_4[\mathrm{L}]^4} \tag{27}$$

In terms of macroscopic binding constants, we have

$$\begin{aligned} \mathfrak{a}_1 &= \overline{K}_1 \\ \mathfrak{a}_2 &= \overline{K}_1\overline{K}_2 \\ \mathfrak{a}_3 &= \overline{K}_1\overline{K}_2\overline{K}_3 \\ \mathfrak{a}_4 &= \overline{K}_1\overline{K}_2\overline{K}_3\overline{K}_4 \end{aligned} \tag{28}$$

In terms of microscopic binding constants, we have

$$\begin{aligned} \mathfrak{a}_1 &= k_{1000}^{0000} + k_{0100}^{0000} + k_{0010}^{0000} + k_{0001}^{0000} \\ \mathfrak{a}_2 &= k_{1000}^{0000}k_{1100}^{1000} + k_{1000}^{0000}k_{1010}^{1000} + k_{1000}^{0000}k_{1001}^{1000} + k_{0100}^{0000}k_{0110}^{0100} + k_{0100}^{0000}k_{0101}^{0100} + k_{0010}^{0000}k_{0011}^{0010} \\ \mathfrak{a}_3 &= k_{0001}^{0000}k_{0011}^{0001}k_{0111}^{0011} + k_{0001}^{0000}k_{0011}^{0001}k_{1011}^{0011} + k_{0001}^{0000}k_{0001}^{0101}k_{1101}^{0101} + k_{1000}^{0000}k_{1100}^{1000}k_{1110}^{1100} \\ \mathfrak{a}_4 &= k_{1000}^{0000}k_{1100}^{1000}k_{1110}^{1100}k_{1111}^{1110} \end{aligned} \tag{29}$$

Different combinations of microscopic constants can yield the same coefficients $\mathfrak{a}_i$.

Adair[1–3] developed a model in which it is assumed that all of the ligands bind with the same affinity to the same macrostate, i. e., $k_1 = k_{1000}^{0000} = k_{0100}^{0000}...$, $k_2 = k_{1100}^{1000} = k_{1010}^{1000} = ...$, etc. This assumption implies that all binding sites are equivalent. The coefficients in eq 27 are then given by

$$\begin{aligned} \mathfrak{a}_1 &= 4k_1 \\ \mathfrak{a}_2 &= 6k_1k_2 \\ \mathfrak{a}_3 &= 4k_1k_2k_3 \\ \mathfrak{a}_4 &= k_1k_2k_3k_4 \end{aligned} \tag{30}$$

Comparing eq 28 with eq 30, one finds

$$\begin{aligned} k_1 &= \tfrac{1}{4}\overline{K}_1 \\ k_2 &= \tfrac{2}{3}\overline{K}_2 \\ k_3 &= \tfrac{3}{2}\overline{K}_3 \\ k_4 &= 4\overline{K}_4 \end{aligned} \tag{31}$$

The Adair model is equivalent to eq 27 considering eq 31. Thus, the Adair model with $N$ binding sites can always fit a binding curve of $N$th degree. However, from a good fit to the Adair equation one can *not conclude* that binding constants for binding of the $i$th ligand are all equal.

# References

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