Ligand depletion *in vivo* modulates the dynamic range and cooperativity of signal transduction

Nicolas Le Novère, EMBL-EBI
Dose-response is the most general measurement in biomedical sciences
How general is a dose-response?
Calmodulin, the memory switch

State transitions of calmodulin
State transitions of calmodulin
Calmodulin is ultra-sensitive

PNAS, 103: 13968-13973
Origins of cooperativity: Bohr

Bohr C (1903) Theoretische behandlung der quantitativen verhältnisse bei der sauerstoff aufnahme des hämoglobins Zentralbl Physiol 17: 682
The possible effects of the aggregation of the molecules of haemoglobin on its dissociation curves. By A. V. Hill.

In a previous communication Barcroft and I gave evidence which seemed to us to prove conclusively that dialysed haemoglobin consists simply of molecules containing each one atom of iron. The molecular weight is therefore Hb = 16,660. These experiments have not been published yet, but I shall assume the results.

Other observers (Reid, Ronaf, Hufner and Gansaer) working on different solutions have obtained divergent results. The method used by all of them was the direct estimation of the osmotic pressure, by means of a membrane permeable to salts, but not to haemoglobin. The method involves a relatively large error, because the quantity measured is small. It is doubtful however whether this can explain the discordant results.

Our work led me to believe that the divergence between the results of different observers was due to an aggregation of the haemoglobin molecules by the salts present in the solution, a consequent lowering of the number of molecules, and an increase in the average molecular weight as observed by the osmotic pressure method. To test this hypothesis I have applied it to several of the dissociation curves obtained by Barcroft and Cameron with haemoglobin in solutions of various salts, and with haemoglobin prepared by Bohr's method.

The equation for the reaction would be:

\[ \text{Hb} + O_2 \rightleftharpoons \text{HbO}_2, \]
\[ \text{Hb}_n + nO_2 \rightleftharpoons \text{Hb}_n\text{O}_2n, \]

where \( \text{Hb}_n \) represents the aggregate of \( n \) molecules of Hb. I have supposed that in every solution there are many different sized aggregates, corresponding to many values of \( n \).

If there were in the solution only Hb and \( \text{Hb}_n \) the dissociation curve would be:

\[ y = \lambda \left( \frac{K_x}{1 + K_x} \right) + (100 - \lambda) \left( \frac{K_x}{1 + K_x} \right) \]

where \( \lambda \) is the fraction of \( \text{Hb}_n \), \( (100 - \lambda) \) is the fraction of Hb, \( K' \) is the equilibrium constant of the reaction \( \text{Hb}_2 + 2O_2 \rightleftharpoons \text{Hb}_2\text{O}_4 \) and \( K \) that of \( \text{Hb} + O_2 \rightleftharpoons \text{HbO}_2 \); \( K \) has the value 1.25 (Barcroft and Roberts).
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\[
y = \lambda \frac{Kx^n}{1 + Kx^n} + (100 - \lambda) \frac{Kx^n}{1 + Kx^n},
\]

where \(\lambda\)\% is as Hb\(_n\), (100 - \(\lambda\))\% as Hb, \(K\) is the equilibrium constant of the reaction \(\text{Hb} + 2O_2 \rightleftharpoons \text{Hb}_2O_2\), and \(K\) that of \(\text{Hb} + O_2 \rightleftharpoons \text{Hb}O_2\); \(K\) has the value 125 (Barcroft and Roberts).
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\[ y = \lambda \frac{K'x}{1 + K'x} + (100 - \lambda) \frac{Kx}{1 + Kx} \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdOTS

where \( \lambda \) is as Hb, \( (100 - \lambda) \) as Hb, \( K' \) is the equilibrium constant of the reaction \( \text{Hb} + O_2 \rightleftharpoons \text{HbO}_2 \), and \( K \) that of \( \text{Hb} + O_2 \rightleftharpoons \text{HbO}_2 \). \( K \) has the value 125 (Barcroft and Roberts).
Hill equation

\[
\bar{Y} = \frac{K^n[X]^n}{1 + K^n[X]^n}
\]

Hill plot

\[
\log \frac{\bar{Y}}{1 - \bar{Y}} = n \log K + n \log[x]
\]

Effect increases in function of the signal to the power of \(n\):
\(n>1\), ultra-sensitive
\(n<1\), infra-sensitive

BUT cooperativity of ligand, not of binding sites: unique affinity
THE HEMOGLOBIN SYSTEM.

VI. THE OXYGEN DISSOCIATION CURVE OF HEMOGLOBIN.*

By G. S. ADAIR.

With the Collaboration of A. V. Bock and H. Field, Jr.

(From the Medical Laboratories of the Massachusetts General Hospital, Boston.)

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Adair (1925) J Biol Chem 63: 529

\[
\bar{Y} = \frac{1}{n} \frac{K_1[x] + 2K_2[x]^2 + 3K_3[x]^3 + 4K_4[x]^4}{1 + K_1[x] + K_2[x]^2 + K_3[x]^3 + K_4[x]^4}
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Fig. 2. Test of formula (6). Curve drawn from 6 experimental points from Table IV.
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\]

Imai (1973) Biochemistry 12: 798-808
Adair-Klotz model applied to Calmodulin


\[
\bar{Y} = \frac{1}{n} \frac{K_1[Ca] + 2K_1K_2[Ca]^2 + 3K_1K_2K_3[Ca]^3 + 4K_1K_2K_3K_4[Ca]^4}{1 + K_1[Ca] + K_1K_2[Ca]^2 + K_1K_2K_3[Ca]^3 + K_1K_2K_3K_4[Ca]^4}
\]

*J Mol Biol*, 12: 88-118
Modulation of thermal equilibria $\neq$ induced-fit

Transition State

$L = \frac{[T_0]}{[R_0]}$  
$c = \frac{K^R}{K^T}$

"inactive" = T  
"active" = R
Concerted transitions $\neq$ sequential model
\[ \alpha = \frac{[x]}{K^R} \]

\[ \bar{Y} = \frac{\alpha (1 + \alpha)^{n-1} + L c \alpha (1 + c \alpha)^{n-1}}{(1 + \alpha)^n + L (1 + c \alpha)^n} \]
Monod-Wyman-Changeux model

\[ \alpha = \frac{[x]}{K^R} \]

\[ \bar{Y} = \frac{\alpha(1 + \alpha)^{n-1} + Lc\alpha(1 + c\alpha)^{n-1}}{(1 + \alpha)^n + L(1 + c\alpha)^n} \]

\[ \bar{R} = \frac{(1 + \alpha)^n}{(1 + \alpha)^n + L(1 + c\alpha)^n} \]
“Hill” Plot for MWC model
Concerted transition

Allosteric model of Calmodulin function

fraction of occupied binding sites ($\bar{Y}$)

obtained with 25 $\mu$M CaM
Calcium dose-response on 25 μM Calmodulin

fraction of occupied binding sites (Y)
Calcium dose-response on 0.1 μM Calmodulin

fraction of occupied binding sites (Y)
Calcium dose-response on 0.1 μM Calmodulin

fraction of occupied binding sites (Y)

What is ligand depletion?
What is ligand depletion?

Chemistry (mass-action law)

\[ f(\text{free ligand}) = \]
What is ligand depletion?

**Chemistry (mass-action law)**

\[ \text{free ligand} = f(\text{free ligand}) \]

**Cellular signalling**

\[ \text{total signal} = f(\text{total signal} + \text{free ligand}) \]
What is ligand depletion?

**Chemistry (mass-action law)**

\[
\text{total signal} = f \left( \text{free ligand} \right)
\]

If

\[
	ext{ligand} + \text{cell} \ll K_d
\]

\[
= f \left( \text{total signal} \right)
\]

**Cellular signalling**
This is generally not the case in signalling: Concentrations of sensors are in micromolar range, as are the dissociation constants.

**Chemistry (mass-action law)**

\[ f(\text{free ligand}) = f(\text{total signal}) = f(\text{ligand} + \text{sensor}) \]

**Cellular signalling**

\[ \text{if } \text{ligand} + \text{sensor} \ll K_d \]

What is ligand depletion?
Dose-response depends on Calmodulin concentration

\[ [\text{CaM}] = 10^{-7} \text{ M} \]

no ligand depletion

\[ [\text{CaM}] = 1.8 \times 10^{-6} \text{ M} \]

\[ [\text{CaM}] = 13.8 \times 10^{-6} \text{ M} \]

\[ [\text{CaM}] = 28 \times 10^{-6} \text{ M} \]

\[ [\text{CaM}] = 40 \times 10^{-6} \text{ M} \]

\[ [\text{Ca}^{2+}]_{\text{tot}} \]

\( \bar{R} \) & \( \bar{Y} \)

Fractional occupancy

Fractional activation

\( \bar{R}' \) & \( \bar{Y}' \)
Dose-response depends on Calmodulin concentration

\[ [\text{CaM}] = 1.8 \times 10^{-6} \text{ M} \]
rat spleen

\[ [\text{CaM}] = 13.8 \times 10^{-6} \text{ M} \]
bovine hypothalamus

\[ [\text{CaM}] = 10^{-7} \text{ M} \]
no ligand depletion

\[ [\text{CaM}] = 28 \times 10^{-6} \text{ M} \]
bovine caudate nucleus

\[ [\text{CaM}] = 40 \times 10^{-6} \text{ M} \]
published dose-response experiments

Fractional occupancy
Fractional activation
Ligand-depletion modifies sensitivity

\[ [\text{CaM}] = 1.8 \times 10^{-6} \text{ M} \]
rat spleen
\(~1 \mu\text{M} \text{ to } 45 \mu\text{M}\)

\[ [\text{CaM}] = 10^{-7} \text{ M} \]
no ligand depletion
\(~100 \text{ nM} \text{ to } 25 \mu\text{M}\)

\[ [\text{CaM}] = 28 \times 10^{-6} \text{ M} \]
bovine caudate nucleus
\(~5 \mu\text{M} \text{ to } 120 \mu\text{M}\)
But we cannot build a large $[\text{Ca}^{2+}]$ in neurons ...

\[ [\text{CaM}] = 1.8 \times 10^{-6} \text{ M} \]
rat spleen

\[ [\text{CaM}] = 10^{-7} \text{ M} \]
no ligand depletion

<table>
<thead>
<tr>
<th>Tissue</th>
<th>$[\text{CaM}]$</th>
<th>Fractional occupancy</th>
<th>Fractional activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat spleen</td>
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Published dose-response experiments

Concentration span of a calcium spike in neurons

$[\text{Ca}^{2+}]_{\text{tot}}$
Evaluating cooperativity: Hill Plot?

\[ \bar{Y} = \frac{K^n[X]^n}{1 + K^n[X]^n} \]

\[ \log \frac{\bar{Y}}{1 - \bar{Y}} = n \log K + n \log[x] \]

Effect increases in function of the signal to the power of \( n \):
- \( n > 1 \), ultra-sensitive
- \( n < 1 \), infra-sensitive

BUT cooperativity of ligand, not of binding sites: unique affinity
Hill number not suitable for state function

L=10

L=1000

L=100000
Hill number not suitable for state function
Hill number not suitable for state function

\[ \log \left[ \frac{R}{1-R} \right] \& \log \left[ \frac{Y}{1-Y} \right] \]
Hill number not suitable for state function

The maximum value of $n_H$ does not depend on the free energy of conformational transition and does correspond to the maximal cooperativity.
Hill number not suitable for state function

\[ n_H \] is significantly lower than 1 for many values of \( \alpha \), which is usually associated to negative cooperativity. One needs to normalise.
\[ \bar{R} = \frac{(1 + \alpha)^N}{L(1 + c\alpha)^N + (1 + \alpha)^N} \]

can be rearranged as:

\[ \bar{R} = \frac{1}{1 + L \left( \frac{1 + c\alpha}{1 + \alpha} \right)^N} \]
If we define the relative stabilisation of the T state by the ligand as

\[ \Omega = \frac{1 + c\alpha}{1 + \alpha} \]

then

\[ \bar{R} = \frac{1}{1 + L\Omega^N} \]
\[ \bar{R} = \frac{(1 + \alpha)^N}{L(1 + c\alpha)^N + (1 + \alpha)^N} \]

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We assume that the free energy of conformational change spread over all the subunits (symmetrical protein)

\[ \lambda = \sqrt[N]{L} \]
If we define the relative stabilisation of the T state by the ligand as
\[ \Omega = \frac{1 + c\alpha}{1 + \alpha} \]
then
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We assume that the free energy of conformational change spread over all the subunits (symmetrical protein)

\[ \lambda = \sqrt[N]{L} \]

State function for an equivalent monomer
\[ \bar{R}^* = \frac{1}{1 + \lambda \Omega} \]
Equivalent monomer for calmodulin

\[ \bar{R} \text{ & } \bar{Y} \]

\[ \bar{R}^* \text{ & } \bar{Y}^* \]
New index of cooperativity

\[ \bar{R} = \frac{1}{1 + L\Omega^N} \]

\[ \bar{R}^* = \frac{1}{1 + \lambda\Omega} \]
New index of cooperativity

\[ R = \frac{1}{1 + L\Omega^N} \]

\[ \frac{dR}{d\alpha} = \frac{N L\Omega^{N-1}(1 - c)}{(1 + L\Omega^N)^2(1 + \alpha)^2} \]

\[ R^* = \frac{1}{1 + \lambda\Omega} \]

\[ \frac{dR^*}{d\alpha} = \frac{\lambda(1 - c)}{(1 + \lambda\Omega)^2(1 + \alpha)^2} \]
New index of cooperativity

\[ \bar{R} = \frac{1}{1 + L\Omega^N} \]

\[ \bar{R}^* = \frac{1}{1 + \lambda\Omega} \]

\[ \frac{d\bar{R}}{d\alpha} = \frac{NL\Omega^{N-1}(1 - c)}{(1 + L\Omega^N)^2(1 + \alpha)^2} \]

\[ \frac{d\bar{R}^*}{d\alpha} = \frac{\lambda(1 - c)}{(1 + \lambda\Omega)^2(1 + \alpha)^2} \]

\[ \nu = \frac{d\bar{R}/d\alpha}{d\bar{R}^*/d\alpha} = \frac{N(1 + \lambda\Omega)^2(\lambda\Omega)^{N-1}}{(1 + (\lambda\Omega)^N)^2} \]

\[ \nu \text{ is insensitive too ligand depletion!} \]
New index of cooperativity

\[ \bar{R} = \frac{1}{1 + L\Omega^N} \]

\[ \bar{R}^* = \frac{1}{1 + \lambda\Omega} \]

\[ \frac{d\bar{R}}{d\alpha} = \frac{NL\Omega^{N-1}(1 - c)}{(1 + L\Omega^N)^2(1 + \alpha)^2} \]

\[ \frac{d\bar{R}^*}{d\alpha} = \frac{\lambda(1 - c)}{(1 + \lambda\Omega)^2(1 + \alpha)^2} \]

\[ \nu = \frac{d\bar{R}}{d\bar{R}^*/d\alpha} = \frac{N(1 + \lambda\Omega)^2(\lambda\Omega)^{N-1}}{(1 + (\lambda\Omega)^N)^2} \]

effective \( \nu = \frac{d\bar{R}'}{d\alpha} \)
Ligand-depletion decreases effective cooperativity.
Highly cooperative: bacterial flagellar motor
Concerted behaviour of bacterial flagellar coupling energy between subunits

\[ E_J = 3kT \]

\[ E_J = 4kT \]

Ligand-depletion increases dynamic range
Ligand-depletion increases dynamic range
Ligand depletion explains different reports


cooperativity = 2.5

cooperativity = 10.3
How general is a dose-response?
A “dose-response” cannot be reused directly!
Dose-responses are the basic characterisations of “systems”, but also at the core of pharmacological treatments. Here we show that:

- A “dose-response” cannot be reused directly in models of signalling systems. Instead one needs to build “mechanistic” models and run parameter-fitting approaches.
- Ligand depletion decreases the effective cooperativity of transducers *in situ*.
- Ligand depletion increases the dynamic range.
- Modifying the concentration of the sensor may be a powerful way to quickly adapt to a new environment, and switch from a measurement mode to a detection mode.
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