Cooperativity in Dimerizing Systems

Comparison of Epidermal Growth Factor an Estrogen Receptors

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May 2016

Abstract

By eight o'clock everything was ready, and we were on the other side of the river. We jumped into the stage, the driver cracked his whip, and we bowled away and left "the States" behind us. It was a superb summer morning, and all the landscape was brilliant with sunshine. There was a freshness and breeziness, too, and an exhilarating sense of emancipation from all sorts of cares and responsibilities, that almost made us feel that the years we had spent in the close, hot city, toiling and slaving, had been wasted and thrown away. We were spinning along through Kansas, and in the course of an hour and a half we were fairly abroad on the great Plains. Just here the land was rolling–a grand sweep of regular elevations and depressions as far as the eye could reach–like the stately heave and swell of the ocean's bosom after a storm. And everywhere were cornfields, accenting with squares of deeper green, this limitless expanse of grassy land. But presently this sea upon dry ground was to lose its "rolling" character and stretch away for seven hundred miles as level as a floor!

Acknowledgments

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for Megan

Contents

1 Introduction

1.1 Ligand Binding and Cooperativity

The complex biochemical processes that give rise to life depend on the recognition of one molecule by another: a hormone binding to its receptor, an enzyme binding its substrate, a transcription factor binding DNA, etc. A receptor recognizes its ligand, conventionally the smaller molecule, through non-covalent interactions comprising electrostatic forces, van der Waals forces, and the hydrophobic effect. The degree of recognition or *affinity* of the receptor for its ligand is determined by the strength and number of non-covalent interactions between them, and those in turn are determined by the structure and chemical composition of the molecules, e.g., the type and spatial configuration of amino acids at the binding site of a receptor protein.

Analyses of ligand-receptor systems focus on affinity as the system's characteristic property and quantify it with the equilibrium constant of the binding reaction. A larger association constant = higher affinity, assoc constant also relates to range of concentrations or ligand binding interval, in class systems this is rouhgly 2 log units and we will see how this varies... This model is derived in appenix A.1

Differences in the magnitude of affinity are why a given receptor binds one ligand versus another. For example, an estrogen receptor will bind the hormone estrogen and effect a cellular change, but that same receptor will be unaffected by a another hormone such as insulin. This is because the structure and composition of the binding site on the receptor are complementary to those of the ligand and enable the formation of sufficient non-covalent interactions. Estrogen and insulin differ significantly in structure and composition (one is a steroid and the other a peptide) hence insulin cannot interact favor-

ably with the binding site on the estrogen receptor. The converse is also true that a ligand will have a greater affinity if it can participate in additional or stronger interactions. This applies to receptors as well, where structural and compositional differences of binding sites account for differences in affinity. These phenomena have important implications when recalling a protein has conformational flexibility and can take on structural changes.
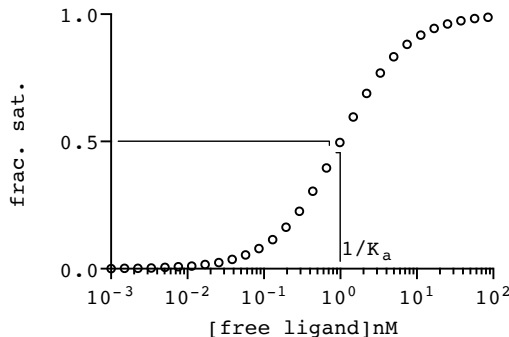

Figure 1: Example of a theoretical binding isotherm for a receptor with a single site with association constant $K_a = 1$ nm^{-1}. The fractional saturation of the receptor (the concentration of ligand bound to the receptor vs. the total concentration of receptor) is plotted against the concentration of free ligand in the system. When the

Consider a receptor with multiple binding sites for the same ligand. If the sites are structurally and compositionally the same, then we expect them to have the same affinity. However, if the structure changes, so will the nature of the interactions with the ligand and subseqently the affinity. In some receptors, a ligand can induce conformational changes when it binds and consequently modify affinity at another site. Depending on whether the affinity increases or decreases, the system is said to exhibit positive or negative cooperativity.*

Cooperativity functions as a modulator of biochemical processes by shrinking or expanding the range of concentrations over which a receptor will bind its ligand. The classic example of this is the binding of oxygen by hemoglobin. Positive cooperativity in that system narrows the binding interval for oxygen

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*Cooperativity can be further classified as homotropic if the affinity is changed for an identical ligand (as explained) or heterotropic if the affinity is changed for a different ligand. In this work, cooperativity will always refer to homotropic cooperativity.

2

so that it is efficiently transported from higher concentrations in the lungs to lower concentrations in tissues. Systems exhibiting positive cooperativity approach a switch-like state where the sites on the receptor In systems exhibiting negative cooperativity, the ligand binding interval is expanded, could be considered a damping effect

We will see that it is imoprtants in signaling systems, but really what we need are appropriate models to study it

Negative cooperativity plays as role as we will see in some receptor systems — response damped

For receptor proteins involved in cell signaling, modulation is an important component. Variations in affinity under different conditions. Dimerizing systems, blach blach

Dimerization, protein-protein interactions can be treated as a ligand-binding

We will see that EGFR exhibits negative coop and estrogen receptor exhibits pos coop but it also is a dimerizing system

Models to study cooperativity

This can be extended across multiple ligands or sites...and other disclaimers

2   Model for a Dimerizing Receptor System

2.1   Schematic Representation

Symbol	Species
L	free ligand
R	free receptor monomer
RL	occupied receptor monomer
(RR)	free receptor dimer
(RR)L	singly-occupied receptor dimer
(RR)LL	fully-occupied receptor dimer

Table 1: Description of species in model shown in Fig. 2

$$
\begin{array}{ccc}
\text{R + R} & \xrightarrow{\;D_{20}\;} & \text{(RR)} \\[1em]
\Big\updownarrow K_{11} & & \Big\updownarrow K_{21} \\[1em]
\text{R + RL} & \xrightarrow{\;D_{21}\;} & \text{(RR)L} \\[1em]
\Big\updownarrow K_{11} & & \Big\updownarrow K_{22} \\[1em]
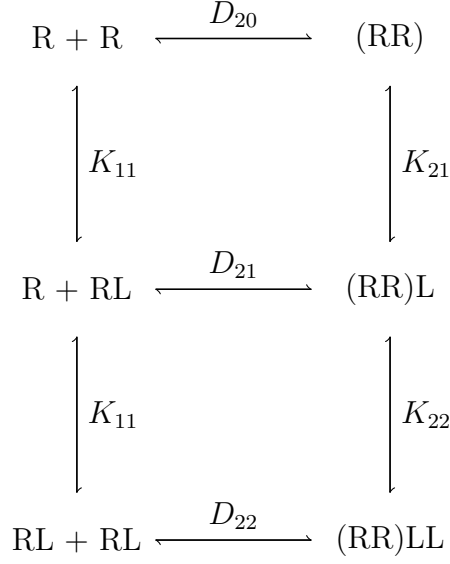\text{RL + RL} & \xrightarrow{\;D_{22}\;} & \text{(RR)LL}
\end{array}
$$

Figure 2: Model proposed by Wyman and Gill for a receptor system that dimerizes.[3]

2.2 Fractional Saturation Equation

We can construct the equation for the fractional saturation for the system described in Fig. ?? expressing the species in terms of their equilibrium constants as catalogued in Table 3

$$
\bar{\nu} = \frac{[\text{RL}] + [\text{(RR)L}] + 2[\text{(RR)LL}]}{[\text{R}] + [\text{RL}] + 2[\text{(RR)}] + 2[\text{(RR)L}] + 2[\text{(RR)LL}]} \tag{2.1}
$$

$$
\bar{\nu} = \frac{K_{11}[\text{L}] + K_{21}D_{20}[\text{R}][\text{L}] + 2K_{21}K_{22}D_{20}[\text{R}][\text{L}]^2}{1 + K_{11}[\text{L}] + 2D_{20}[\text{R}] + 2K_{21}D_{20}[\text{R}][\text{L}] + 2K_{21}K_{22}D_{20}[\text{R}][\text{L}]^2} \tag{2.2}
$$

2.3 *p-bar* Notation

To continue our analysis it will be useful to introduce the following notation:

Association Constant	Reaction
K_{11}	$R + L \rightleftharpoons RL$
K_{21}	$(RR) + L \rightleftharpoons (RR)L$
K_{22}	$(RR)L + L \rightleftharpoons (RR)LL$
D_{20}	$R + R \rightleftharpoons (RR)$
D_{21}	$R + RL \rightleftharpoons (RR)L$
D_{22}	$RL + RL \rightleftharpoons (RR)LL$

Table 2: Description of constant in model shown in Fig. 2

Species		Reaction
$[(RR)]$	$=$	$D_{20}[R]^2$
$[RL]$	$=$	$K_{11}[R][L]$
$[(RR)L]$	$=$	$K_{21}[(RR)][L] \equiv K_{21}D_{20}[R]^2[L]$
$[(RR)LL]$	$=$	$K_{22}[(RR)L][L] \equiv K_{21}K_{20}D_{20}[R]^2[L]^2$

Table 3: Table of species

$$\bar{p}_n \bar{\nu}_n \tag{2.3}$$

where $\bar{p}_n$ is the fractional population of the n-mer species, and $\bar{\nu}_n$ is the fractional saturation of that species. In the case of a dimerizing system, the apparent fractional saturation of the system can be decomposed into the fractional saturations of the monomer and dimer populations:

$$\bar{\nu}_{\text{sys}} = \bar{p}_1 \bar{\nu}_1 + \bar{p}_2 \bar{\nu}_2 \quad \text{(dimerizing receptor system)} \tag{2.4}$$

where $\bar{p}_1$ is the fraction of the population of receptors that is in monomeric form, i.e., $\frac{\text{monomeric species}}{\text{all species}}$, and $\bar{\nu}_1$ is fractional saturation of the monomeric receptor. Likewise, $\bar{p}_2$ is the fraction that is in dimeric form and $\bar{\nu}_2$ is the fractional saturation of the dimeric receptor. This form is easily extended to higher order aggregating systems:

$$\bar{\nu}_{\text{sys}} = \bar{p}_1 \bar{\nu}_1 + \bar{p}_2 \bar{\nu}_2 + \cdots + \bar{p}_n \bar{\nu}_n \quad \text{(general aggregating system)} \tag{2.5}$$

5

This form is particularly useful when the equation for $\bar{\nu}_n$ takes on one the canonical forms derived in the appendix because it allows us to set strict criteria for cooperativity. We can show that the fractional saturation describing the dimerizing system, equation 2.2, takes on this form.

First we construct $\bar{p}_1$ and $\bar{p}_2$ using the species in Table 3:

$$\bar{p}_1 = \frac{[\text{R}] + [\text{RL}]}{[\text{R}] + [\text{RL}] + 2[(\text{RR})] + 2[(\text{RR})\text{L}] + 2[(\text{RR})\text{LL}]}$$

(2.6)

$$= \frac{1 + K_{11}[\text{L}]}{1 + K_{11}[\text{L}] + 2D_{20}[\text{R}](1 + K_{21}[\text{L}](1 + K_{22}[\text{L}]))}$$

$$\bar{p}_2 = \frac{2[\text{RR}] + 2[\text{RRL}] + 2[\text{RRLL}]}{[\text{R}] + [\text{RL}] + 2[(\text{RR})] + 2[(\text{RR})\text{L}] + 2[(\text{RR})\text{LL}]}$$

(2.7)

$$= \frac{2D_{20}[\text{R}](1 + K_{21}[\text{L}](1 + K_{22}[\text{L}]))}{1 + K_{11}[\text{L}] + 2D_{20}[\text{R}](1 + K_{21}[\text{L}](1 + K_{22}[\text{L}]))}$$

$$\bar{\nu}_1 = \frac{[\text{RL}]}{[\text{R}] + [\text{RL}]}$$

(2.8)

$$= \frac{K_{11}[\text{L}]}{1 + K_{11}[\text{L}]}$$

$$\bar{\nu}_2 = \frac{[(\text{RR})\text{L}] + 2(\text{RR})\text{LL}}{2[(\text{RR})] + 2[(\text{RR})\text{L}] + 2[(\text{RR})\text{LL}]}$$

$$= \frac{K_{21}D_{20}[\text{L}][\text{R}]^2 + 2K_{21}K_{22}D_{20}[\text{L}]^2[\text{R}]^2}{2D_{20}[\text{R}]^2 + 2K_{21}D_{20}[\text{L}][\text{R}]^2 + 2K_{21}K_{22}D_{20}[\text{L}]^2[\text{R}]^2}$$

(2.9)

$$= \left(\frac{1}{2}\right)\frac{K_1[\text{L}] + 2K_1K_2[\text{L}]^2}{1 + K_1[\text{L}] + K_1K_2[\text{L}]^2}$$

Crunching through the algebra, it's clear that $\bar{p}_1\bar{\nu}_1 + \bar{p}_2\bar{\nu}_2 = \bar{\nu}_{sys}$.

$$\bar{p}_1\bar{\nu}_1 = \frac{[\text{R}] + [\text{RL}]}{[\text{R}] + [\text{RL}] + 2[(\text{RR})] + 2[(\text{RR})\text{L}] + 2[(\text{RR})\text{LL}]}$$

$$\times \frac{[\text{RL}]}{[\text{R}] + [\text{RL}]} \tag{2.10}$$

$$= \frac{[\text{RL}]}{[\text{R}] + [\text{RL}] + 2[(\text{RR})] + 2[(\text{RR})\text{L}] + 2[(\text{RR})\text{LL}]}$$

$$\bar{p}_2\bar{\nu}_2 = \frac{2[\text{RR}] + 2[\text{RRL}] + 2[\text{RRLL}]}{[\text{R}] + [\text{RL}] + 2[(\text{RR})] + 2[(\text{RR})\text{L}] + 2[(\text{RR})\text{LL}]}$$

$$\times \frac{[(\text{RR})\text{L}] + 2(\text{RR})\text{LL}}{2[(\text{RR})] + 2[(\text{RR})\text{L}] + 2[(\text{RR})\text{LL}]} \tag{2.11}$$

$$= \frac{[(\text{RR})\text{L}] + 2(\text{RR})\text{LL}}{[\text{R}] + [\text{RL}] + 2[(\text{RR})] + 2[(\text{RR})\text{L}] + 2[(\text{RR})\text{LL}]}$$

$$\bar{p}_1\bar{\nu}_1 + \bar{p}_2\bar{\nu}_2 = \frac{[\text{RL}] + [(\text{RR})\text{L}] + 2(\text{RR})\text{LL}}{[\text{R}] + [\text{RL}] + 2[(\text{RR})] + 2[(\text{RR})\text{L}] + 2[(\text{RR})\text{LL}]} \tag{2.12}$$

References

1. Notides, A. C. *et al.* Positive cooperativity of the estrogen receptor. *PNAS* **78,** 4926–4930 (1981).

2. Brandt, M. E. & Vickery, L. E. Cooperativity and Dimerization of Recombinant Human Estrogen Receptor Hormone-binding Domain. *J. Biol. Chem.* **272,** 4843–4849 (1997).

3. Wyman, J. & Gill, S. J. *Binding and Linkage: Functional Chemistry of Biological Macromolecules* 354 pp. (University Science Books, 1990).

4. Rohatgi, A. *WebPlotDigitizer* version 3.9. 2015. <http://arohatgi.info/WebPlotDigitizer>.

Appendix A Derivations

A.1 One-Site Receptor

Start with the ligand binding reaction

$$R + L \rightleftharpoons RL \qquad (A.1.1)$$

and define the equilibrium association constant

$$K_1 = \frac{[RL]}{[R][L]} \quad \equiv \quad [RL] = K_1[R][L] \qquad (A.1.2)$$

define the fractional saturation $\bar{\nu}$

$$\bar{\nu} = \frac{[\text{bound ligand}]}{[\text{total receptor}]} = \frac{[RL]}{[R]+[RL]} \qquad (A.1.3)$$

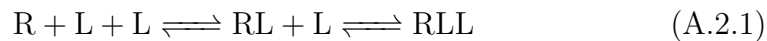use A.1.2 to substitute [RL] and factor out [R] to get

$$\bar{\nu} = \frac{K_1[L]}{1 + K_1[L]} \qquad (A.1.4)$$

A.2 Multi-Site Receptors

Two-Site

For a two-site receptor, extend the one-site binding reaction with an additional ligand binding step

$$R + L + L \rightleftharpoons RL + L \rightleftharpoons RLL \qquad (A.2.1)$$

and define the second step equilibrium association constant

$$K_1 = \frac{[RL]}{[R][L]} \quad \text{and} \quad K_2 = \frac{[RLL]}{[RL][L]} \tag{A.2.2}$$

add the new species to the fractional saturation definition including a factor of 2 (1 mole of RLL has 2 equivalents of L)

$$\bar{\nu} = \frac{[RL] + 2[RLL]}{[R] + [RL] + [RLL]} \tag{A.2.3}$$

substitute [RL] and [RLL] using A.2.2, factor out [R], and normalize with a factor of $\frac{1}{n}$, where $n =$ the number of binding sites, in this case: 2 (this is required so that $\bar{\nu}$ takes on values between 0 and 1)

$$\bar{\nu} = \left(\frac{1}{2}\right) \frac{K_1[L] + 2K_1K_2[L]^2}{1 + K_1[L] + K_1K_2[L]^2} \tag{A.2.4}$$

n-Sites

The above approach can be used to derive the binding equation for n binding sites

$$\bar{\nu} = \left(\frac{1}{n}\right) \frac{K_1[L] + 2K_1K_2[L]^2 + \cdots + nK_1K_2\cdots K_n[L]^n}{1 + K_1[L] + K_1K_2[L]^2 + \cdots + K_1K_2\cdots K_n[L]^n} \tag{A.2.5}$$

A.3 Formalizing Cooperativity

Cooperativity in two-site sytems

As discussed in the introduction, homotropic cooperativity is a change in affinity at an otherwise identical binding site on the same receptor upon binding of the same ligand. We can develop a formal definition of cooperativity using binding equations. The simplest system in which cooperativity can arise is a receptor with two binding sites. It is useful to first consider a system in which there are two one-site receptors in equal proportion.[3]

$$2\bar{\nu} = \frac{K_a[\text{L}]}{1 + K_a[\text{L}]} + \frac{K_b[\text{L}]}{1 + K_b[\text{L}]} \qquad (\text{A.3.1})$$

We can view this as a being equivalent to a receptor with two non-interacting (cooperativity prohibited) sites with affinities K_a and K_b. There is no restriction on whether the affinities are equal or not. Combining their separate fractional saturation equations by cross-multiplying yields

$$2\bar{\nu} = \frac{(K_a + K_b)[\text{L}] + 2K_aK_b[\text{L}]^2}{1 + (K_a + K_b)[\text{L}] + K_aK_b[\text{L}]^2} \qquad (\text{A.3.2})$$

We choose to assign $(K_a + K_b) = K_1$ and $K_aK_b = K_1K_2$ so that we can recover the fractional saturation equation for a receptor with two sites A.2.4.

If the two sites on the receptor are indeed equivalent, i.e.:

$$K_a = K_b = k \qquad (\text{A.3.3})$$

then

$$K_1 = 2k \quad \text{and} \quad K_1K_2 = k^2 \implies K_2 = \frac{k}{2} \qquad (\text{A.3.4})$$

This establishes the relationship between K_1 and K_2 when the sites have the same affinity:

$$K_1 = 4K_2 \qquad (\text{A.3.5})$$

If binding data for a receptor that has two sites with invariant affinity is fit with this model, then we would expect $K_1 \approx 4K_2$ within some error related to the error in the data.

If the data were fit and $K_1 > 4K_2$ or $K_1 < 4K_2$ (again, given a margin of error), this would indicate negative or positive cooperativity, respectively.

Cooperativity in n-site systems

$$n\bar{\nu} = \frac{K_1[\text{L}]}{1 + K_1[\text{L}]} + \frac{K_2[\text{L}]}{1 + K_2[\text{L}]} + \cdots + \frac{K_n[\text{L}]}{1 + K_n[\text{L}]} \tag{A.3.6}$$

This is the numerator:

$$\sum_{i=1}^{n} K_i \prod_{j=1}^{n} (1 + K_{j \neq i}) \tag{A.3.7}$$

This is the denominator:

$$\prod_{i=1}^{n} (1 + K_n) \tag{A.3.8}$$

If all K's are equal

$$(1 + k)^n \tag{A.3.9}$$

This will expand to a polynomial of order n

In the above example $n = 2$

We are interested in the groupings of K's when the above is expanded for n sites.

$$(1 + k)^n = 1 + \sum_{i=1}^{n} c_i k^i \tag{A.3.10}$$

Where

$$c_i = \frac{n!}{i!(n - i)!} \tag{A.3.11}$$

$$c_i k^i = K_1 K_2 \cdots K_i \tag{A.3.12}$$

$$K_i = \frac{c_i k^i}{c_{i-1} k^{i-1}} = \left(\frac{n - i + 1}{i} \right) k \tag{A.3.13}$$

A4

The equation for K_i can be used to determine relationships between values of the expected association constants for a multi-site receptor assuming equal affinity at all sites, and deviations from those expected values can be used to determine cooperativity.

Appendix B   R Code

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