

Cooperativity in Dimerizing Systems

Comparison of Epidermal Growth Factor and Estrogen Receptors

Michael C. Fitzgerald

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

Lorem ipsum dolor sit amet, consectetur adipiscing elit. Etiam lobortis facilisis sem. Nullam nec mi et neque pharetra sollicitudin. Praesent imperdiet mi nec ante. Donec ullamcorper, felis non sodales commodo, lectus velit ultrices augue, a dignissim nibh lectus placerat pede. Vivamus nunc nunc, molestie ut, ultricies vel, semper in, velit. Ut porttitor. Praesent in sapien. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Duis fringilla tristique neque. Sed interdum libero ut metus. Pellentesque placerat. Nam rutrum augue a leo. Morbi sed elit sit amet ante lobortis sollicitudin. Praesent blandit blandit mauris. Praesent lectus tellus, aliquet aliquam, luctus a, egestas a, turpis. Mauris lacinia lorem sit amet ipsum. Nunc quis urna dictum turpis accumsan semper.

for Megan

Contents

1	Introduction	1
1.1	Ligand Binding and Cooperativity	1
2	Definitions	3
3	Models	3
4	Data	7
	Bibliography	10
	Appendices	A1
A	Derivations	A1
A.1	One-Site Receptor	A1
A.2	Multi-Site Receptors	A1
A.3	Criteria for Cooperativity	A2
B	R Code	A4

1 Introduction

AUTHOR'S NOTE: The following section contains what I hope is the essential background to get an unfamiliar reader up to speed and provide context on the topic of ligand-binding and cooperativity as it pertains to this work. Many of the nuances will be detailed in later sections and supported with models. Several treatises are available on this topic, and the reader is directed to them if more in-depth background is desired [klotz, wyman gil].

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 roughly 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.

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 subsequently 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 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 important 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, black black

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

We will see that EGFR exhibits negative coop and estrogen receptor exhibits

*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.

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 Definitions

The following notation will be used in the construction and analysis of models.

L = free ligand

R = free receptor monomer

RL = bound receptor monomer

(RR) = free receptor dimer

(RR)L = singly bound receptor dimer

(RR)LL = fully bound receptor dimer

3 Models

Lorem ipsum dolor sit amet, consectetur adipiscing elit. Etiam lobortis facilisis sem. Nullam nec mi et neque pharetra sollicitudin. Praesent imperdiet mi nec ante. Donec ullamcorper, felis non sodales commodo, lectus velit ultrices augue, a dignissim nibh lectus placerat pede. Vivamus nunc nunc, molestie ut, ultricies vel, semper in, velit. Ut porttitor. Praesent in sapien. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Duis fringilla tristique neque. Sed interdum libero ut metus. Pellentesque placerat. Nam rutrum augue a leo. Morbi sed elit sit amet ante lobortis sollicitudin. Praesent blandit blandit mauris. Praesent lectus tellus, aliquet aliquam, luctus a, egestas a, turpis. Mauris lacinia lorem sit amet ipsum. Nunc quis urna dictum turpis accumsan semper. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Etiam

lobortis facilisis sem. Nullam nec mi et neque pharetra sollicitudin. Praesent imperdiet mi nec ante. Donec ullamcorper, felis non sodales commodo, lectus velit ultrices augue, a dignissim nibh lectus placerat pede. Vivamus nunc nunc, molestie ut, ultricies vel, semper in, velit. Ut porttitor. Praesent in sapien. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Duis fringilla tristique neque. Sed interdum libero ut metus. Pellentesque placerat. Nam rutrum augue a leo. Morbi sed elit sit amet ante lobortis sollicitudin. Praesent blandit blandit mauris. Praesent lectus tellus, aliquet aliquam, luctus a, egestas a, turpis. Mauris lacinia lorem sit amet ipsum. Nunc quis urna dictum turpis accumsan semper. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Etiam lobortis facilisis sem. Nullam nec mi et neque pharetra sollicitudin. Praesent imperdiet mi nec ante. Donec ullamcorper, felis non sodales commodo, lectus velit ultrices augue, a dignissim nibh lectus placerat pede. Vivamus nunc nunc, molestie ut, ultricies vel, semper in, velit. Ut porttitor. Praesent in sapien. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Duis fringilla tristique neque. Sed interdum libero ut metus. Pellentesque placerat. Nam rutrum augue a leo. Morbi sed elit sit amet ante lobortis sollicitudin. Praesent blandit blandit mauris. Praesent lectus tellus, aliquet aliquam, luctus a, egestas a, turpis. Mauris lacinia lorem sit amet ipsum. Nunc quis urna dictum turpis accumsan semper. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Etiam lobortis facilisis sem. Nullam nec mi et neque pharetra sollicitudin. Praesent imperdiet mi nec ante. Donec ullamcorper, felis non sodales commodo, lectus velit ultrices augue, a dignissim nibh lectus placerat pede. Vivamus nunc nunc, molestie ut, ultricies vel, semper in, velit. Ut porttitor. Praesent in sapien. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Duis fringilla tristique neque. Sed interdum libero ut metus. Pellentesque placerat. Nam rutrum augue a leo. Morbi sed elit sit amet ante lobortis sollicitudin.

$$\begin{array}{ccc}
R + R & \xleftarrow{D_{20}} & (RR) \\
\downarrow K_{11} & & \downarrow K_{21} \\
R + RL & \xleftarrow{D_{21}} & (RR)L \\
\downarrow K_{11} & & \downarrow K_{22} \\
RL + RL & \xleftarrow{D_{22}} & (RR)LL
\end{array}$$

Figure 1: this is a nugget model proposed by Wyman and Gill.³

Praesent blandit blandit mauris. Praesent lectus tellus, aliquet aliquam, luctus a, egestas a, turpis. Mauris lacinia lorem sit amet ipsum. Nunc quis urna dictum turpis accumsan semper.

The lazy brown dog jumped over the office chairs fulfilling the prophecy.^{1,2} Lorem ipsum dolor sit amet, consectetur adipiscing elit. Etiam lobortis facilisis sem. Nullam nec mi et neque pharetra sollicitudin. Praesent imperdiet mi nec ante. Donec ullamcorper, felis non sodales commodo, lectus velit ultrices augue, a dignissim nibh lectus placerat pede. Vivamus nunc nunc, molestie ut, ultricies vel, semper in, velit. Ut porttitor. Praesent in sapien. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Duis fringilla tristique neque. Sed interdum libero ut metus. Pellentesque placerat. Nam rutrum augue a leo. Morbi sed elit sit amet ante lobortis sollicitudin. Praesent blandit blandit mauris. Praesent lectus tellus, aliquet aliquam, luctus a, egestas a, turpis. Mauris lacinia lorem sit amet ipsum. Nunc quis urna dictum turpis accumsan semper. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Etiam lobortis facilisis sem. Nullam nec mi et neque pharetra sollicitudin. Praesent imperdiet mi nec ante. Donec ullamcorper, felis non sodales commodo, lectus velit ultrices augue, a dignissim nibh lectus placerat pede. Vivamus nunc nunc, molestie ut, ultricies vel, semper in, velit. Ut porttitor. Prae-

sent in sapien. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Duis fringilla tristique neque. Sed interdum libero ut metus. Pellentesque placerat. Nam rutrum augue a leo. Morbi sed elit sit amet ante lobortis sollicitudin. Praesent blandit blandit mauris. Praesent lectus tellus, aliquet aliquam, luctus a, egestas a, turpis. Mauris lacinia lorem sit amet ipsum. Nunc quis urna dictum turpis accumsan semper. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Etiam lobortis facilisis sem. Nullam nec mi et neque pharetra sollicitudin. Praesent imperdiet mi nec ante. Donec ullamcorper, felis non sodales commodo, lectus velit ultrices augue, a dignissim nibh lectus placerat pede. Vivamus nunc nunc, molestie ut, ultricies vel, semper in, velit. Ut porttitor. Praesent in sapien. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Duis fringilla tristique neque. Sed interdum libero ut metus. Pellentesque placerat. Nam rutrum augue a leo. Morbi sed elit sit amet ante lobortis sollicitudin. Praesent blandit blandit mauris. Praesent lectus tellus, aliquet aliquam, luctus a, egestas a, turpis. Mauris lacinia lorem sit amet ipsum. Nunc quis urna dictum turpis accumsan semper. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Etiam lobortis facilisis sem. Nullam nec mi et neque pharetra sollicitudin. Praesent imperdiet mi nec ante. Donec ullamcorper, felis non sodales commodo, lectus velit ultrices augue, a dignissim nibh lectus placerat pede. Vivamus nunc nunc, molestie ut, ultricies vel, semper in, velit. Ut porttitor. Praesent in sapien. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Duis fringilla tristique neque. Sed interdum libero ut metus. Pellentesque placerat. Nam rutrum augue a leo. Morbi sed elit sit amet ante lobortis sollicitudin. Praesent blandit blandit mauris. Praesent lectus tellus, aliquet aliquam, luctus a, egestas a, turpis. Mauris lacinia lorem sit amet ipsum. Nunc quis urna dictum turpis accumsan semper. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Etiam lobortis facilisis sem. Nullam nec mi et neque pharetra sollicitudin. Praesent imperdiet mi nec ante. Donec ullamcorper, felis non sodales commodo, lectus velit ultrices augue, a dignissim nibh lectus placerat pede. Vivamus nunc nunc, molestie ut, ultricies vel, semper in, velit. Ut porttitor. Praesent in sapien. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Duis fringilla tristique neque. Sed interdum libero ut metus. Pellentesque placerat. Nam rutrum augue a leo. Morbi sed elit sit amet ante lobortis sollicitudin. Praesent blandit blandit mauris. Praesent lectus tellus, aliquet aliquam, luctus a, egestas a, turpis. Mauris lacinia lorem sit amet ipsum. Nunc quis urna dictum turpis accumsan semper.

4 Data

Lorem ipsum dolor sit amet, consectetur adipiscing elit. Etiam lobortis facilisis sem. Nullam nec mi et neque pharetra sollicitudin. Praesent imperdiet mi nec ante. Donec ullamcorper, felis non sodales commodo, lectus velit ultrices augue, a dignissim nibh lectus placerat pede. Vivamus nunc nunc, molestie ut, ultricies vel, semper in, velit. Ut porttitor. Praesent in sapien. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Duis fringilla tristique neque. Sed interdum libero ut metus. Pellentesque placerat. Nam rutrum augue a leo. Morbi sed elit sit amet ante lobortis sollicitudin. Praesent blandit blandit mauris. Praesent lectus tellus, aliquet aliquam, luctus a, egestas a, turpis. Mauris lacinia lorem sit amet ipsum. Nunc quis urna dictum turpis accumsan semper. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Etiam lobortis facilisis sem. Nullam nec mi et neque pharetra sollicitudin. Praesent imperdiet mi nec ante. Donec ullamcorper, felis non sodales commodo, lectus velit ultrices augue, a dignissim nibh lectus placerat pede. Vivamus nunc nunc, molestie ut, ultricies vel, semper in, velit. Ut porttitor. Praesent in sapien. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Duis fringilla tristique neque. Sed interdum libero ut metus. Pellentesque placerat. Nam rutrum augue a leo. Morbi sed elit sit amet ante lobortis sollicitudin. Praesent blandit blandit mauris. Praesent lectus tellus, aliquet aliquam, luctus a, egestas a, turpis. Mauris lacinia lorem sit amet ipsum. Nunc quis urna dictum turpis accumsan semper. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Etiam lobortis facilisis sem. Nullam nec mi et neque pharetra sollicitudin. Praesent imperdiet mi nec ante. Donec ullamcorper, felis non sodales commodo, lectus velit ultrices augue, a dignissim nibh lectus placerat pede. Vivamus nunc nunc, molestie ut, ultricies

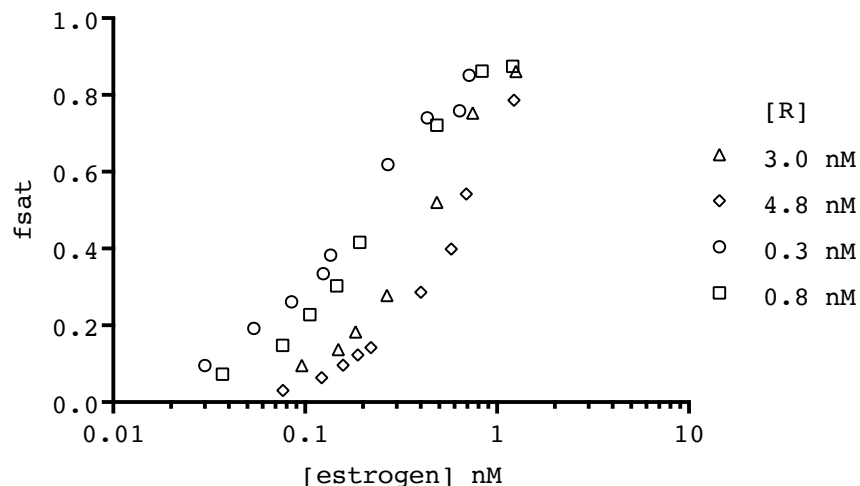


Figure 2: this is a nooj model.¹

vel, semper in, velit. Ut porttitor. Praesent in sapien. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Duis fringilla tristique neque. Sed interdum libero ut metus. Pellentesque placerat. Nam rutrum augue a leo. Morbi sed elit sit amet ante lobortis sollicitudin. Praesent blandit blandit mauris. Praesent lectus tellus, aliquet aliquam, luctus a, egestas a, turpis. Mauris lacinia lorem sit amet ipsum. Nunc quis urna dictum turpis accumsan semper. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Etiam lobortis facilisis sem. Nullam nec mi et neque pharetra sollicitudin. Praesent imperdiet mi nec ante. Donec ullamcorper, felis non sodales commodo, lectus velit ultrices augue, a dignissim nibh lectus placerat pede. Vivamus nunc nunc, molestie ut, ultricies vel, semper in, velit. Ut porttitor. Praesent in sapien. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Duis fringilla tristique neque. Sed interdum libero ut metus. Pellentesque placerat. Nam rutrum augue a leo. Morbi sed elit sit amet ante lobortis sollicitudin. Praesent blandit blandit mauris. Praesent lectus tellus, aliquet aliquam, luctus a, egestas a, turpis. Mauris lacinia lorem sit amet ipsum. Nunc quis urna dictum turpis accumsan semper. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Etiam lobortis facilisis sem. Nullam nec mi et neque pharetra sollicitudin. Praesent imperdiet mi nec ante. Donec ullamcorper, felis non sodales commodo, lectus velit ultrices augue, a dignissim nibh lectus placerat pede. Vivamus nunc nunc, molestie ut, ultricies vel, semper in, velit.

Ut porttitor. Praesent in sapien. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Duis fringilla tristique neque. Sed interdum libero ut metus. Pellentesque placerat. Nam rutrum augue a leo. Morbi sed elit sit amet ante lobortis sollicitudin. Praesent blandit blandit mauris. Praesent lectus tellus, aliquet aliquam, luctus a, egestas a, turpis. Mauris lacinia lorem sit amet ipsum. Nunc quis urna dictum turpis accumsan semper. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Etiam lobortis facilisis sem. Nullam nec mi et neque pharetra sollicitudin. Praesent imperdiet mi nec ante. Donec ullamcorper, felis non sodales commodo, lectus velit ultrices augue, a dignissim nibh lectus placerat pede. Vivamus nunc nunc, molestie ut, ultricies vel, semper in, velit. Ut porttitor. Praesent in sapien. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Duis fringilla tristique neque. Sed interdum libero ut metus. Pellentesque placerat. Nam rutrum augue a leo. Morbi sed elit sit amet ante lobortis sollicitudin. Praesent blandit blandit mauris. Praesent lectus tellus, aliquet aliquam, luctus a, egestas a, turpis. Mauris lacinia lorem sit amet ipsum. Nunc quis urna dictum turpis accumsan semper. Data was extracted using the web application WebPlotDigitizer.⁴

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



and define the equilibrium association constant

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

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

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

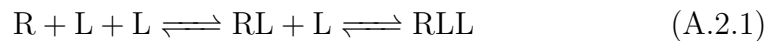
use A.1.2 to substitute $[RL]$ and factor out $[R]$ to get

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

A.2 Multi-Site Receptors

Two-Site

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



and define the second step equilibrium association constant

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

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

$$\bar{\nu} = \frac{[\text{RL}] + 2[\text{RLL}]}{[\text{R}] + [\text{RL}] + [\text{RLL}]} \quad (\text{A.2.3})$$

substitute $[\text{RL}]$ and $[\text{RLL}]$ using A.2.2, factor out $[\text{R}]$, 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)

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

***n*-Sites**

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

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

A.3 Criteria for Cooperativity

Cooperativity in two-site systems

Consider a system in which there are two one-site receptors in equal proportion

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

combining their separate fractional saturation equations by cross-multiplying yields

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

substituting this with the assignments $(K_a + K_b) = K_1$ and $K_aK_b = K_1K_2$ returns the binding equation for a receptor with two sites

If the two receptors have equivalent receptor sites, i.e., $K_a = K_b = k$, then $K_1 = 2k$

Appendix B R Code

Lorem ipsum dolor sit amet, consectetur adipiscing elit. Etiam lobortis facilisis sem. Nullam nec mi et neque pharetra sollicitudin. Praesent imperdiet mi nec ante. Donec ullamcorper, felis non sodales commodo, lectus velit ultrices augue, a dignissim nibh lectus placerat pede. Vivamus nunc nunc, molestie ut, ultricies vel, semper in, velit. Ut porttitor. Praesent in sapien. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Duis fringilla tristique neque. Sed interdum libero ut metus. Pellentesque placerat. Nam rutrum augue a leo. Morbi sed elit sit amet ante lobortis sollicitudin. Praesent blandit blandit mauris. Praesent lectus tellus, aliquet aliquam, luctus a, egestas a, turpis. Mauris lacinia lorem sit amet ipsum. Nunc quis urna dictum turpis accumsan semper. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Etiam lobortis facilisis sem. Nullam nec mi et neque pharetra sollicitudin. Praesent imperdiet mi nec ante. Donec ullamcorper, felis non sodales commodo, lectus velit ultrices augue, a dignissim nibh lectus placerat pede. Vivamus nunc nunc, molestie ut, ultricies vel, semper in, velit. Ut porttitor. Praesent in sapien. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Duis fringilla tristique neque. Sed interdum libero ut metus. Pellentesque placerat. Nam rutrum augue a leo. Morbi sed elit sit amet ante lobortis sollicitudin. Praesent blandit blandit mauris. Praesent lectus tellus, aliquet aliquam, luctus a, egestas a, turpis. Mauris lacinia lorem sit amet ipsum. Nunc quis urna dictum turpis accumsan semper. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Etiam lobortis facilisis sem. Nullam nec mi et neque pharetra sollicitudin. Praesent imperdiet mi nec ante. Donec ullamcorper, felis non sodales commodo, lectus velit ultrices augue, a dignissim nibh lectus placerat pede. Vivamus nunc nunc, molestie ut, ultricies

vel, semper in, velit. Ut porttitor. Praesent in sapien. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Duis fringilla tristique neque. Sed interdum libero ut metus. Pellentesque placerat. Nam rutrum augue a leo. Morbi sed elit sit amet ante lobortis sollicitudin. Praesent blandit blandit mauris. Praesent lectus tellus, aliquet aliquam, luctus a, egestas a, turpis. Mauris lacinia lorem sit amet ipsum. Nunc quis urna dictum turpis accumsan semper. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Etiam lobortis facilisis sem. Nullam nec mi et neque pharetra sollicitudin. Praesent imperdiet mi nec ante. Donec ullamcorper, felis non sodales commodo, lectus velit ultrices augue, a dignissim nibh lectus placerat pede. Vivamus nunc nunc, molestie ut, ultricies vel, semper in, velit. Ut porttitor. Praesent in sapien. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Duis fringilla tristique neque. Sed interdum libero ut metus. Pellentesque placerat. Nam rutrum augue a leo. Morbi sed elit sit amet ante lobortis sollicitudin. Praesent blandit blandit mauris. Praesent lectus tellus, aliquet aliquam, luctus a, egestas a, turpis. Mauris lacinia lorem sit amet ipsum. Nunc quis urna dictum turpis accumsan semper.