The "No Ligand Depletion" Assumption Is Unnecessary and Can Be Misleading

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Discovery on Target, 2013

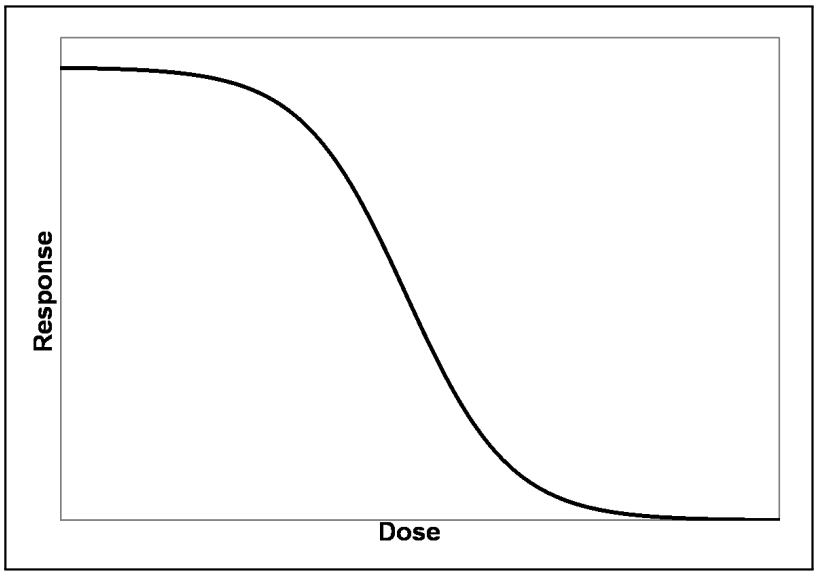
GPCR-Based Drug Discovery

Boston, Massachusetts, USA
24-25 September 2013

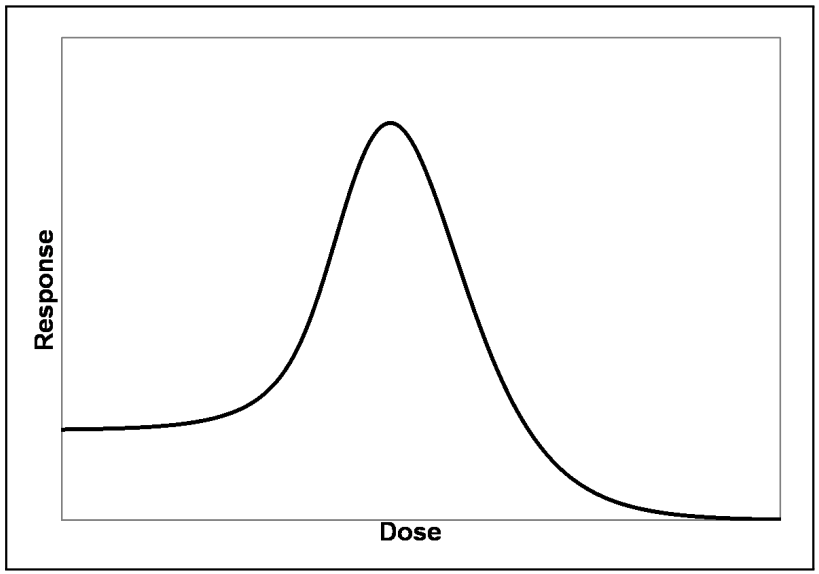
Equilibrium Calculation in Receptor Pharmacology

Equilibrium Calculation in Receptor Pharmacology

My Initiation Problem: A "Bumpy" Response Curve



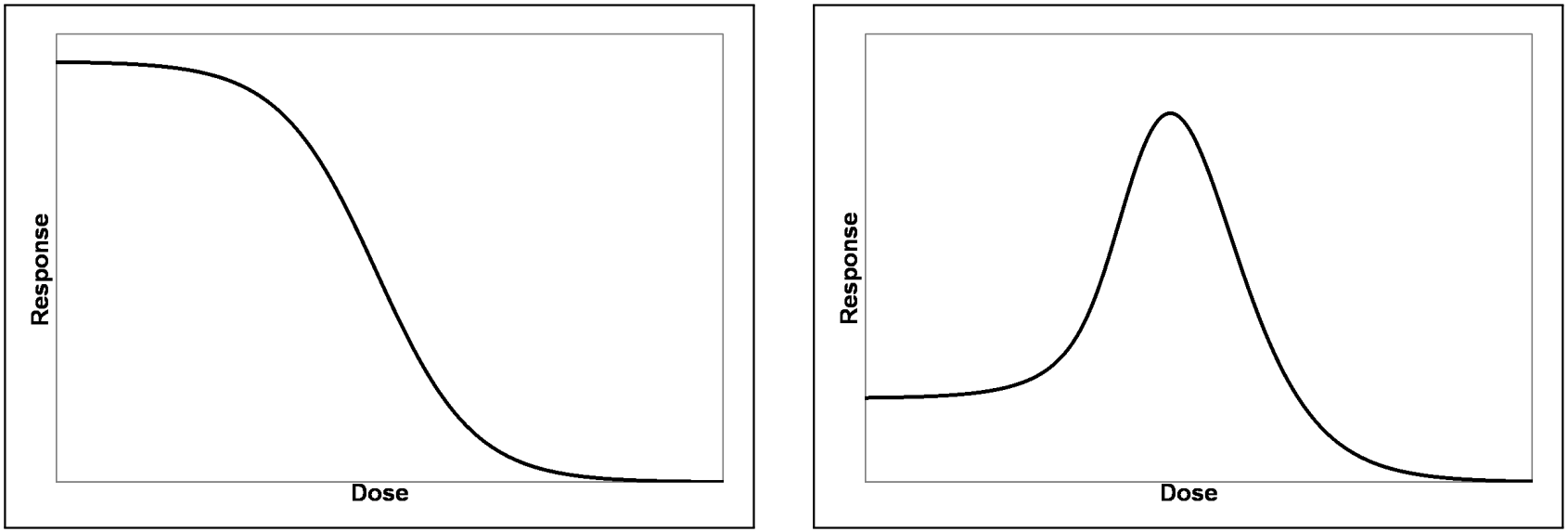
Sigmoid response curves are expected and what usual formulas give.



"Bumpy" response curves can occur and cannot be simulated with usual formulas.

Solving the "Bump Problem"

Solved the actual math problem, not a convenient substitute.

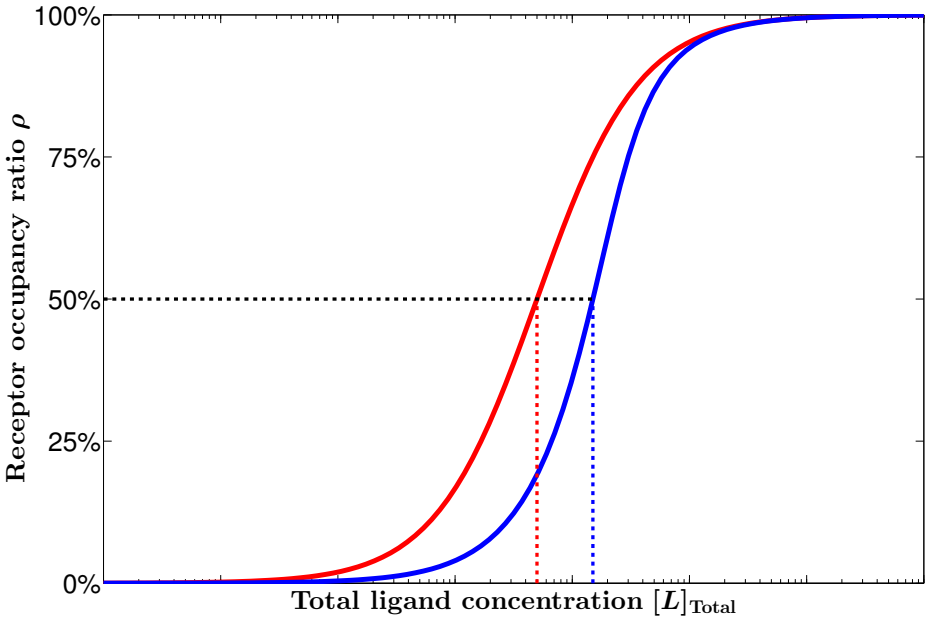


Developed and developing methods and algorithms to

- Simulate and explain bumpy response;
- Simulate responses (sigmoid or not), without manual derivation of formulas and assumptions this requires.

Equilibrium Calculation in Receptor Pharmacology

Current Practices – Illustrated with Trivial Receptor-Ligand Interaction



$$R + L \rightleftharpoons RL$$

"No Ligand Depletion" assumed

$$[R]_{\text{Total}} \ll [L]_{\text{Total}} \qquad [L]_{\text{Equil}} = [L]_{\text{Total}}$$

$$\rho = \frac{[RL]_{\text{Equil}}}{[R]_{\text{Total}}} = \frac{[L]_{\text{Total}}}{K_d + [L]_{\text{Total}}}$$

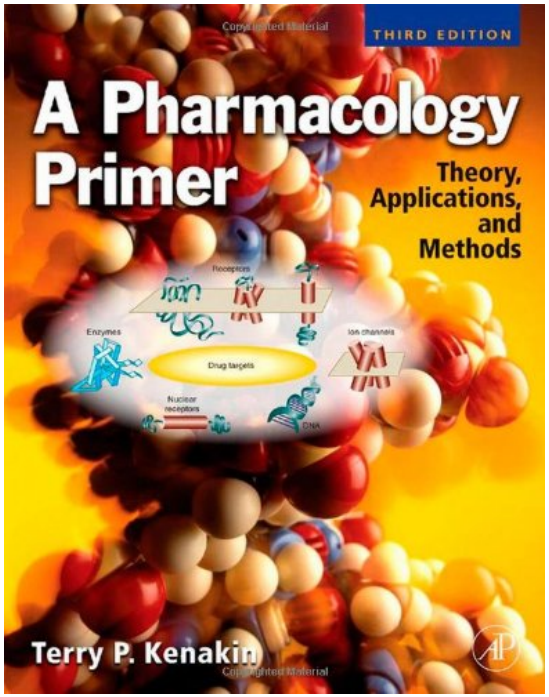
$$K_{d,\text{apparent}} = K_d$$

"No Ligand Depletion" not assumed

$$\rho = \frac{[RL]_{\text{Equil}}}{[R]_{\text{Total}}} = \cdots$$

$$K_{d,\text{apparent}} = K_d + \frac{[R]_{\text{Total}}}{2}$$

More Examples



Issues with Forcibly Derived Formulas

- $[R]_{\text{Total}} \ll [L]_{\text{Total}}$ is not mathematically sound.
  Operational wisdom: $[R]_{\text{Total}} < 10\%$ of $[L]_{\text{Total}}$.
- Not always applicable experimentally,
  e.g. in miniaturized assays.
- Need not be applicable *in vivo*.
- New formula derivation needed for every new biochemical interaction system.
- Only possible with *"receptor-centric"* models.
  (Unique species in role of receptor contained in all complex species.)

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Equilibrium Calculation in Receptor Pharmacology

"No Ligand Depletion" Assumption Discarded for Competitive Antagonism

$$R + A \rightleftharpoons RA$$
$$+$$
$$B$$
$$\Updownarrow$$
$$RB$$

Miniaturized Receptor Binding Assays: Complications Arising from Ligand Depletion
Clare M. Scaramellini Carter, Juliet R. Leighton-Davies and Steven J. Charlton
J Biomol Screen 2007; 12; 255 originally published online Jan 26, 2007

"No Ligand Depletion" Assumption Partially Discarded for Allosteric Modulation

$$\begin{array}{ccc} R + A & \rightleftharpoons & RA \\ + & & + \\ B & & B \\ \Updownarrow & & \Updownarrow \\ RB + A & \rightleftharpoons & RAB \end{array}$$

"No Ligand Depletion" discarded for A, maintained for B

$$[B]_{\text{Equil}} = [B]_{\text{Total}}$$

0022-3565/08/3253-927–934$20.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

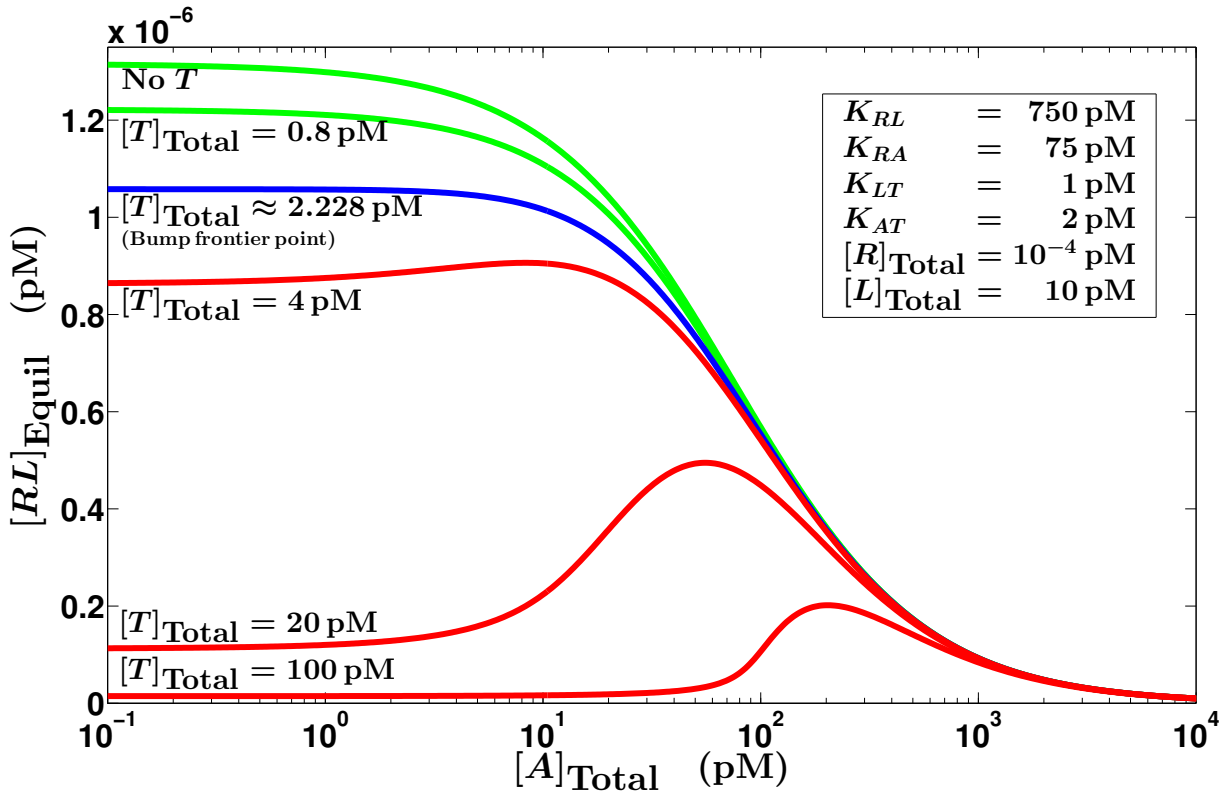
JPET 325:927–934, 2008

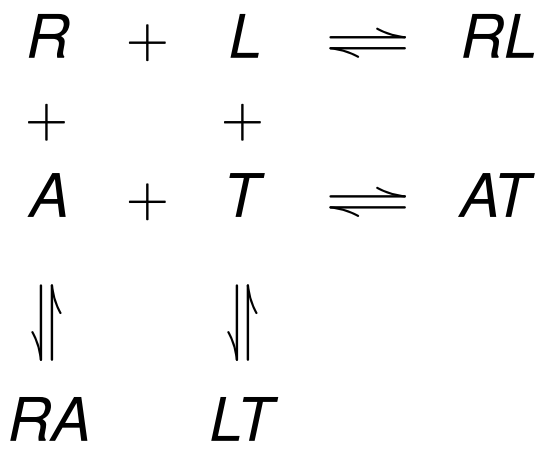
The Impact of Orthosteric Radioligand Depletion on the Quantification of Allosteric Modulator Interactions

Vimesh A. Avlani, David J. McLoughlin, Patrick M. Sexton, and Arthur Christopoulos

Equilibrium Calculation in Receptor Pharmacology

Receptor-Ligand-Antagonist-Trap Model





R : Receptor

L : Ligand

A : Antagonist

T : Trap

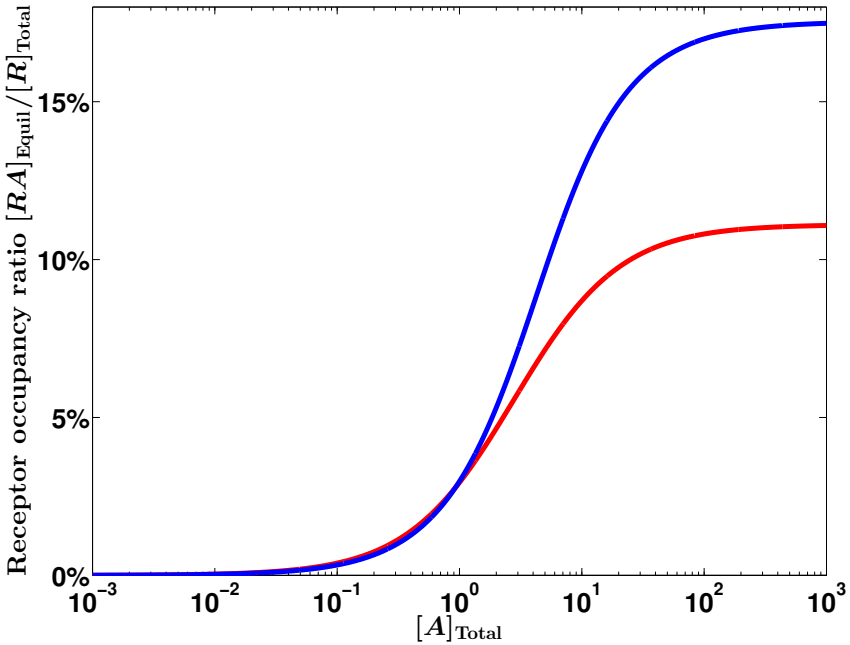
- Actually solving the math problem gives the “bump” feature.
- Solving the usual substitute only gives sigmoid curves.

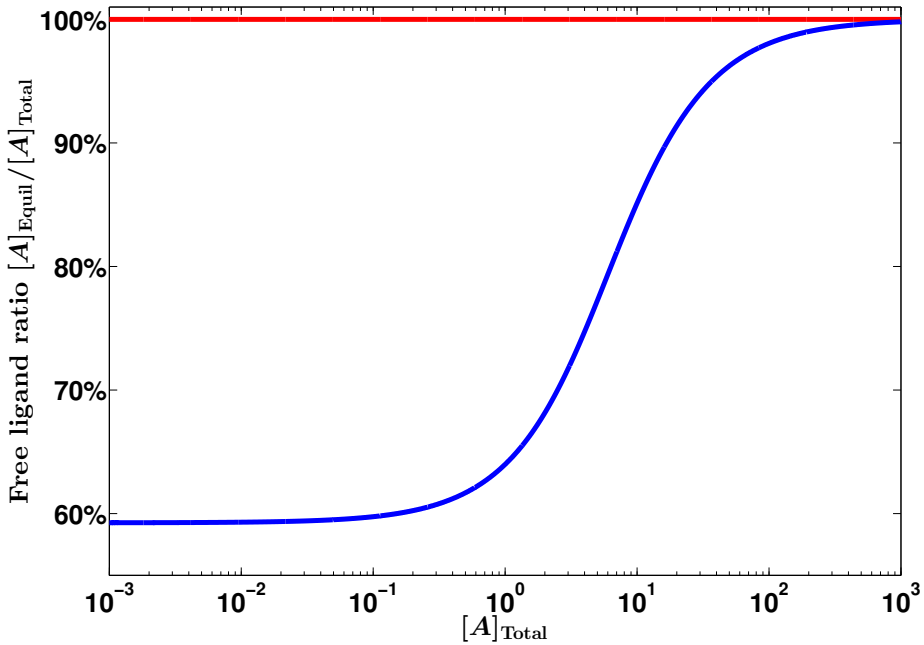
Equilibrium Calculation in Receptor Pharmacology

Allosteric Ternary Complex Model

$$
\begin{array}{ccccc}
R & + & A & \rightleftharpoons & RA \\
+ & & & & + \\
B & & & & B \\
\Downarrow & & & & \Downarrow \\
RB & + & A & \rightleftharpoons & RAB
\end{array}
$$

Allosteric Ternary Complex Model





$K_{\text{d},A} = 5\,\text{pM} \quad K_{\text{d},B} = 1\,\text{pM} \quad \alpha = 2 \quad [B]_{\text{T}} = 4\,\text{pM}$

“No Ligand Depletion” assumed

“No Ligand Depletion” not assumed

$[R]_{\text{T}} = 2\,\text{pM}$

The “No Ligand Depletion” method can be substantially inaccurate.

Equilibrium Calculation in Receptor Pharmacology

Summary

Calculating Equilibrium in Receptor Pharmacology

- Customary methods
  - Assume “No Ligand Depletion”
  - Manually derive explicit formulas
  - Reduced accuracy
  - Typically sigmoid curves
- Developed and developing better methods
  - Mathematics and algorithms to actually solve equations for equilibrium

Equilibrium Calculation in Receptor Pharmacology

More Shots at the "No Ligand Depletion" Assumption

Biochimica et Biophysica Acta, 632 (1980) 464—469
© Elsevier/North-Holland Biomedical Press

COMPETITIVE BINDING STUDIES WITH MULTIPLE SITES
EFFECTS ARISING FROM DEPLETION OF THE FREE RADIOLIGAND

J.W. WELLS, N.J.M. BIRDSALL, A.S.V. BURGEN and E.C. HULME

0026-895X/87/060603-07$02.00/0
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MOLECULAR PHARMACOLOGY, 31:603–609

Ligand Dissociation Constants From Competition Binding Assays: Errors Associated with Ligand Depletion

AVRAM GOLDSTEIN and RONALD W. BARRETT

Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 6654–6658, October 1987
Biochemistry

An accurate method for determination of receptor–ligand and enzyme–inhibitor dissociation constants from displacement curves

AMNON HOROVITZ AND ALEXANDER LEVITZKI

ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS
Vol. 284, No. 1, January, pp. 26–29, 1991

A Simple Method for Calculating the Dissociation Constant of a Receptor (or Enzyme)·Unlabeled Ligand Complex from Radioligand Displacement Measurements

Robert L. Martin, Franco Renosto, and Irwin H. Segel

0026-895X/95/061197-07$3.00/0
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MOLECULAR PHARMACOLOGY, 47:1197–1203 (1995).

Interpretation of Binding Curves Obtained with High Receptor Concentrations: Practical Aid for Computer Analysis

STÉPHANE SWILLENS

Miniaturized Receptor Binding Assays: Complications Arising from Ligand Depletion
Clare M. Scaramellini Carter, Juliet R. Leighton-Davies and Steven J. Charlton
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Mol Pharmacol 73:718–726, 2008

Cell-Based and Biochemical Structure-Activity Analyses of Analogs of the Microtubule Stabilizer Dictyostatin

Brianne S. Raccor, Andreas Vogt, Rachel P. Sikorski, Charitha Madiraju, Raghavan Balachandran, Kia Montgomery, Youseung Shin, Yoshikazu Fukui, Won-Hyuk Jung, Dennis P. Curran, and Billy W. Day

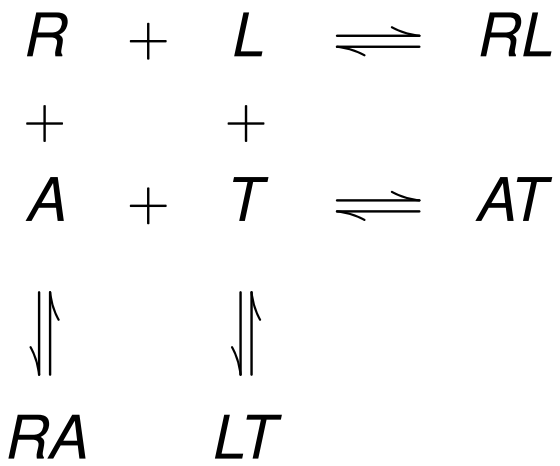
Journal of Neuroscience Methods 188 (2010) 32–38

Concentration of receptor and ligand revisited in a modified receptor binding protocol for high-affinity radioligands: [^{3}H]Spiperone binding to D_2 and D_3 dopamine receptors

Juan Zhen, Tamara Antonio, Aloke K. Dutta, Maarten E.A. Reith

Receptor-Ligand-Antagonist-Trap Model

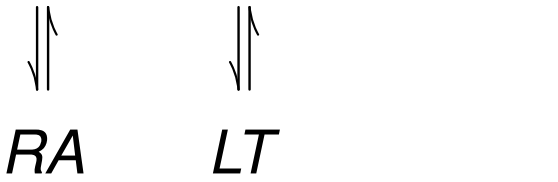
Polynomial System for Equilibrium State (with Association Constants)



$$
\begin{aligned}
[R] + K_{RL}[R][L] + K_{RA}[R][A] &= [R]_{\text{Total}} \\
[L] + K_{RL}[R][L] + K_{LT}[L][T] &= [L]_{\text{Total}} \\
[T] + K_{AT}[A][T] + K_{LT}[L][T] &= [T]_{\text{Total}} \\
[A] + K_{AT}[A][T] + K_{RA}[R][A] &= [A]_{\text{Total}}
\end{aligned}
$$

Receptor-Ligand-Antagonist-Trap Model

Polynomial System for Equilibrium State, Reformulated as a Fixed-Point Equation



$$[R] = \frac{[R]_{\text{Total}}}{1 + K_{RL}[L] + K_{RA}[A]}$$

$$[L] = \frac{[L]_{\text{Total}}}{1 + K_{RL}[R] + K_{LT}[T]}$$

$$[T] = \frac{[T]_{\text{Total}}}{1 + K_{AT}[A] + K_{LT}[L]}$$

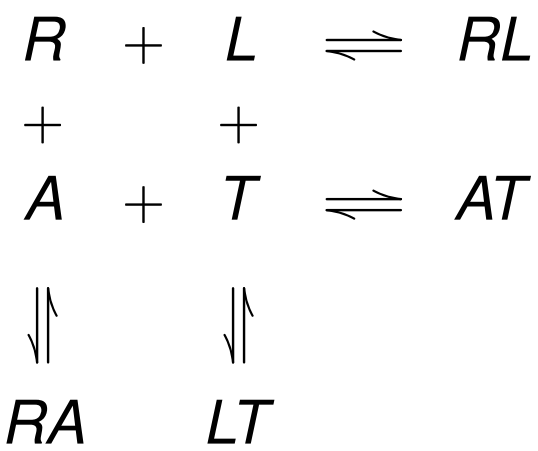
$$[A] = \frac{[A]_{\text{Total}}}{1 + K_{AT}[T] + K_{RA}[R]}$$

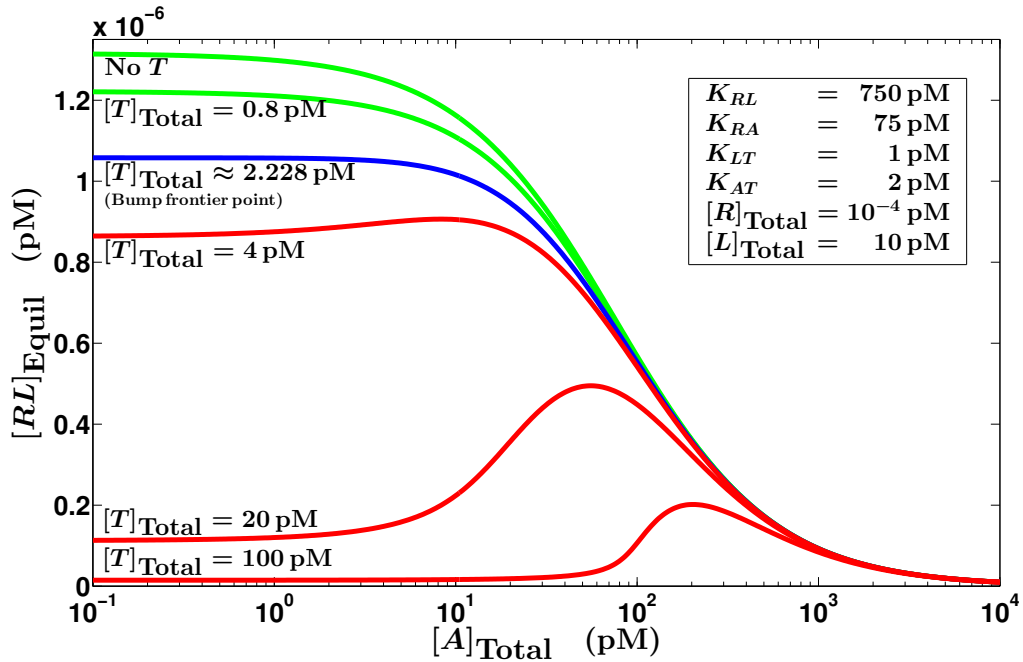
Verboten

$[L] = [L]_{\text{Total}}$ and $[A] = [A]_{\text{Total}}$
in right-hand side of equations

Receptor-Ligand-Antagonist-Trap Model

Polynomial System for Equilibrium State, Solved by Fixed-Point Iterations





Gory details

ELSEVIER

Available online at www.sciencedirect.com
ScienceDirect
Journal of Theoretical Biology 244 (2007) 478–488

Journal of Theoretical Biology
www.elsevier.com/locate/yjtbi

Monotonicity of interleukin-1 receptor–ligand binding with respect to antagonist in the presence of decoy receptor

Gilles Gnacadja , Alex Shoshitaishvili , Michael J. Gresser , Brian Varnum , David Balaban , Mark Durst , Chris Vezina , Yu Li

Gorier details

MATHEMATICAL METHODS IN THE APPLIED SCIENCES
Math. Meth. Appl. Sci. 2007; **30**:201–211
Published online 23 October 2006 in Wiley InterScience (www.interscience.wiley.com) DOI: 10.1002/mma.782
MOS subject classification: 47 H 10; 74 G 25; 74 G 30; 74 G 15; 92 C 40

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Fixed points of order-reversing maps in $\mathbb{R}^n_{>0}$ and chemical equilibrium

Gilles Gnacadja

Allosteric Ternary Complex Model

Polynomial System for Equilibrium State (with Association Constants)

$$\begin{array}{ccccc} R & + & A & \rightleftharpoons & RA \\ + & & & & + \\ B & & & & B \\ \Downarrow & & & & \Downarrow \\ RB & + & A & \rightleftharpoons & RAB \end{array}$$

$$\begin{aligned} [R] + K_{RA}[R][A] + K_{RB}[R][B] + \alpha K_{RA}K_{RB}[R][A][B] &= [R]_{\text{Total}} \\ [A] + K_{RA}[R][A] + \alpha K_{RA}K_{RB}[R][A][B] &= [A]_{\text{Total}} \\ [B] + K_{RB}[R][B] + \alpha K_{RA}K_{RB}[R][A][B] &= [B]_{\text{Total}} \end{aligned}$$

Allosteric Ternary Complex Model

Polynomial System for Equilibrium State, Reformulated as a Fixed-Point Equation

$$\begin{array}{ccccc} R & + & A & \rightleftharpoons & RA \\ + & & & & + \\ B & & & & B \\ \Downarrow & & & & \Downarrow \\ RB & + & A & \rightleftharpoons & RAB \end{array}$$

$$[R] = \frac{[R]_{\text{Total}}}{1 + K_{RA}[A] + K_{RB}[B] + \alpha K_{RA} K_{RB}[A][B]}$$

$$[A] = \frac{[A]_{\text{Total}}}{1 + K_{RA}[R] + \alpha K_{RA} K_{RB}[R][B]}$$

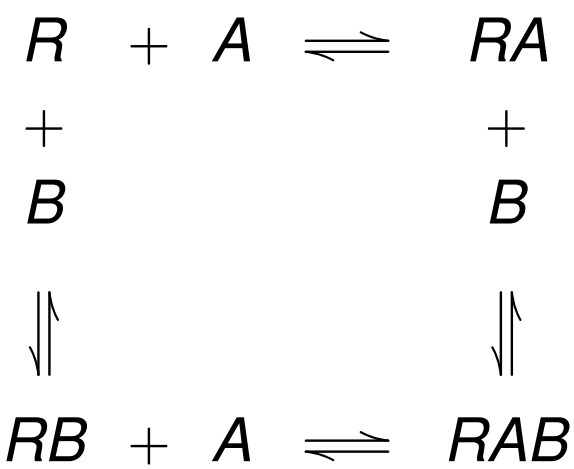
$$[B] = \frac{[B]_{\text{Total}}}{1 + K_{RB}[R] + \alpha K_{RA} K_{RB}[R][A]}$$

Verboten

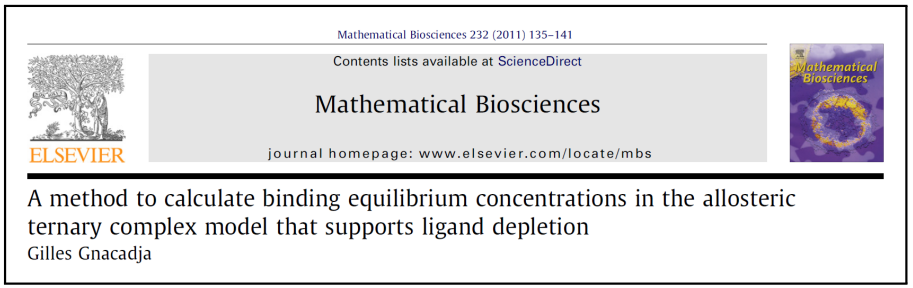
$[A] = [A]_{\text{Total}}$ and $[B] = [B]_{\text{Total}}$
in right-hand side of equations

Allosteric Ternary Complex Model

Polynomial System for Equilibrium State, Solved by Special Reduction



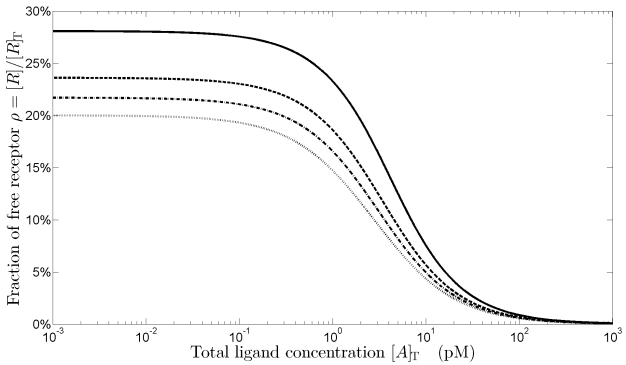
The details

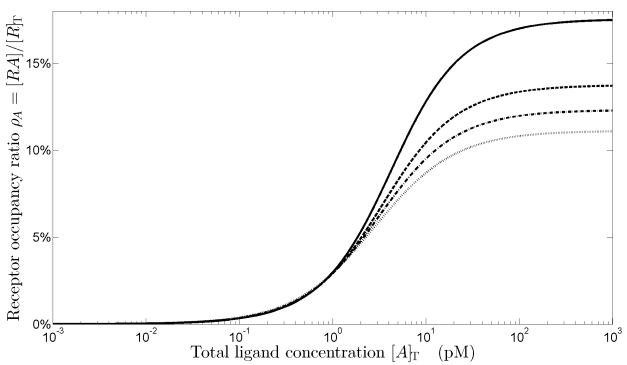


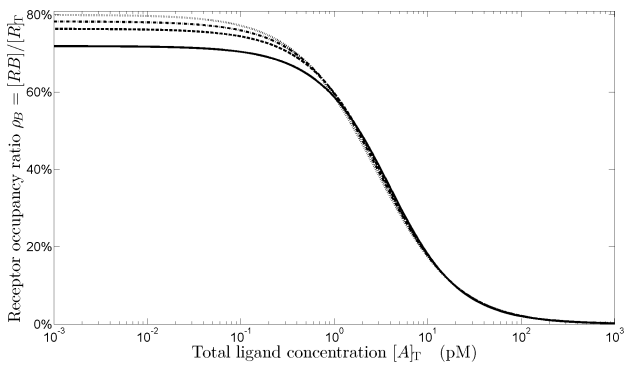
Method not fully an instance of approach advocated:

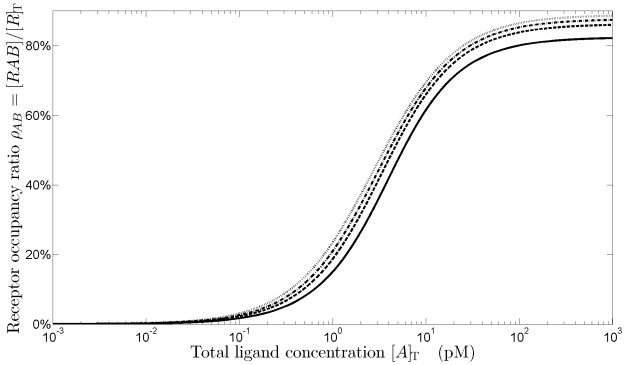
- ✔ “No Ligand Depletion” assumption eliminated;
- ✘ Manual labor changed, not eliminated (but done once and for all);
- ✔ ATCM pervasive, could not await comprehensive algorithm.

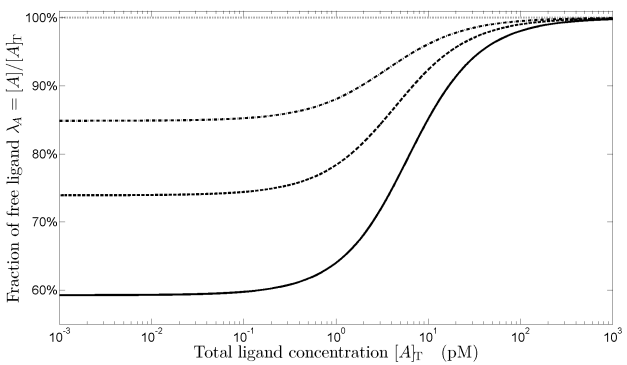
Usual Method Can Be Substantially Inaccurate

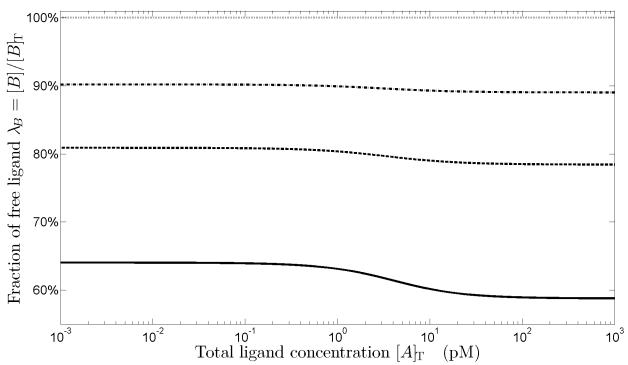












$K_{d,A} = 5$ pM
$K_{d,B} = 1$ pM
$\alpha = 2$
$[B]_T = 4$ pM

Usual method; independent of $[R]_T$
Method presented; $[R]_T = 0.5$ pM
Method presented; $[R]_T = 1$ pM
Method presented; $[R]_T = 2$ pM

Extended Summary

Pharmacology = Pharmacokinetics (PK) + Pharmacodynamics (PD)
- Applicable math: dynamical systems (differential equations), etc.
- Discovery-stage Receptor Pharmacology is neither PK nor PD.

Pharmacology = **"Pharmacostatics" (PS)** + PK + PD
- Applicable math: **polynomial equations**, dynamical systems, etc.

Customary practice is to derive explicit formulas at all cost:
- Manual labor for every new model;
- Reduced accuracy;
- Typically sigmoidal response curves;
- Sometimes models are simply abandoned.

Relevant polynomial equations possess nice distinctive mathematical properties. Developed and developing algorithms to actually solve them with a priori assurance of success.

Goal: Enable scientists to study pharmacostatics of biochemical models that would otherwise be fudged or discarded.

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- Applicable math: **polynomial equations**, dynamical systems, etc.

Customary practice is to derive explicit formulas at all cost:
- Manual labor for every new model;
- Reduced accuracy;
- Typically sigmoidal response curves;
- Sometimes models are simply abandoned.

Relevant polynomial equations possess nice distinctive mathematical properties. Developed and developing algorithms to actually solve them with a priori assurance of success.

Goal: Enable scientists to study pharmacostatics of biochemical models that would otherwise be fudged or discarded.