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Section 1. Conceptual Frameworks for Dose-Response Curves: Classical Binary Binding Equilibria

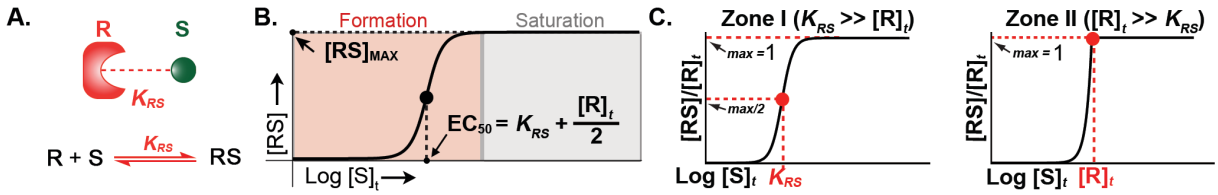


Figure S1. (A) Pictorial and mathematical depiction of a simple, binary binding interaction, where S is the substrate and R is the receptor. **(B)** Plot of a binary sigmoidal dose-response curve showing two critical points: the maximum [RS] value ($[RS]_{max}$) and the $[S]_t$ value that yields half-maximal [RS] formation (EC_{50}). The EC_{50} can be determined by the simple expression shown(1). **(C)** A zone-based framework to understand the behavior of binary dose-response curves. Zone I: When the K_{RS} is the dominant parameter (K_{RS} >> $[R]_t$), the EC_{50} = K_{RS}. Zone II: When the concentration is the dominant parameter ($[R]_t$ >> K_{RS}), the position of the saturation point is equal to the concentration.

In order to develop a conceptual framework for ternary complex equilibria we needed to understand what exactly a "conceptual framework" was. To do this we, we did an extensive search of the literature to compile the theoretical results that have led to the complete understanding of binary complex sigmoidal dose-response curves (Figure S1). In this manner we hoped to not only understand the theoretical requirements to developing a "conceptual framework" for ternary complex bell-shaped curves but also build that framework on concepts analogous to those used in binary binding equilibria.

In general, binary binding equilibria treat the amount of binding between a receptor (R) and a substrate (S) where the relative energetic favorability of binding is described by the dissociation constant, K_{RS} (Figure S1A). The formation of the complex (RS) generally correlates with some biological event and as a result the data curves of the most interest plot the amount of complex (or alternatively the % biological response) versus the concentration of substrate, $[S]_t$ (Figure S1B). These s-shaped curves have been conceptualized in terms of two critical points: the EC_{50} (Effective Concentration 50%, or concentration of ligand (dose) which elicits a 50% maximal binding) and a saturating height, $[RS]_{max}$ (Figure S1B). It has been shown that the EC_{50} is equal to $K_{RS} + [R]_t/2$ whereas $[RS]_{max}$ is equal to $[R]_t$ (1, 2).

The relative values of the two measurable binary binding parameters – K_{RS} and $[R]_t$ – allow for binary systems to be divided into two zones of experimental conditions (Figure S1C). When a system has a "dominant" dissociation constant (K_{RS} >> $[R]_t$), it is classified as Zone I (also known as Langmuir-Hill conditions), and EC_{50} = K_{RS}. When a system has a "dominant" concentration ($[R]_t$ >> K_{RS}), it is classified as Zone II (also known as saturating conditions), and the position of the inflection point of the curve (EC_{100}, or $[S]_{t,max}$) is equal to $[R]_t$. These zones are the two possible limiting cases in a binary equilibrium, and derivation of simple expressions defining them is contingent on the existence of an exact analytical solution (1). In the sections that follow we: (1) Derive a set of exact analytical solutions for non-cooperative and cooperative ternary complex equilibria and (2) Use these exact analytical solutions to develop a conceptual framework based on "critical points" analogous to EC_{50} and $[RS]_{max}$ for binary binding curves.

Section 2. Proof that Cooperative Ternary Equilibria is Algebraically Unsolvable

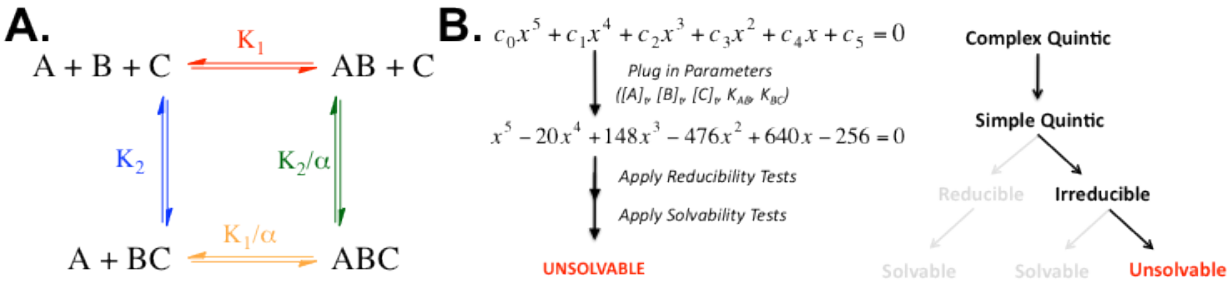


Figure S2. (A) The Cooperative Ternary Complex Equilibrium. **(B)** Unsolvability proof. A specific example was obtained by substituting parameter values into the [ABC] quintic (eq S13). Second, reducibility and solvability tests were applied to show that this example was algebraically unsolvable. Thus, a specific counter-example was used to prove the general unsolvability of [ABC].

The conservation of mass equations:

$$[A]_t = [A] + [AB] + [ABC], \tag{S1}$$

$$[B]_t = [B] + [AB] + [BC] + [ABC], \tag{S2}$$

and

$$[C]_t = [C] + [BC] + [ABC]. \tag{S3}$$

as well as equations derived from the law of mass action:

$$K_{AB} = \frac{[A][B]}{[AB]}, \tag{S4}$$

$$K_{BC} = \frac{[B][C]}{[BC]}, \tag{S5}$$

and

$$\frac{K_{AB}K_{BC}}{\alpha} = \frac{[A][B][C]}{[ABC]}, \tag{S6}$$

describe the cooperative ternary complex model shown in Figure S2.A. Attempting to solve this system of equations for any solution concentration ([A], [B], [C], [AB], [BC], or [ABC]) as a function of measurable parameters (K_{AB}, K_{BC}, $[A]_t$, $[B]_t$, and $[C]_t$) and cooperativity (α) leads to large quintic polynomials (not shown). In the following proof (Figure S2B), we identify a specific case for which these expressions are unsolvable, demonstrating that the cooperative ternary complex model is not generally analytically solvable for [ABC] in terms of $[B]_t$.

To obtain the quintic equation in terms of ABC, we first rearrange eqs S4-6, solving for [AB], [BC], and [B], respectively:

$$[AB] = \frac{[A][B]}{K_{AB}}, \tag{S7}$$

$$[BC] = \frac{[B][C]}{K_{BC}}, \tag{S8}$$

and

$$[B] = \frac{K_{AB}K_{BC}[ABC]}{\alpha[A][C]}. \tag{S9}$$

By substituting these equations into eqs S1-S3, we obtain

$$[\mathrm{A}]_t = [\mathrm{A}] + \frac{K_{BC}[\mathrm{ABC}]}{\alpha[\mathrm{C}]} + [\mathrm{ABC}], \tag{S10}$$

$$[\mathrm{B}]_t = \frac{K_{AB}K_{BC}[\mathrm{ABC}]}{\alpha[\mathrm{A}][\mathrm{C}]} + \frac{K_{BC}[\mathrm{ABC}]}{\alpha[\mathrm{C}]} + \frac{K_{AB}[\mathrm{ABC}]}{\alpha[\mathrm{A}]} + [\mathrm{ABC}], \tag{S11}$$

and

$$[\mathrm{C}]_t = [\mathrm{C}] + \frac{K_{AB}[\mathrm{ABC}]}{\alpha[\mathrm{A}]} + [\mathrm{ABC}]. \tag{S12}$$

By solving eqs S10 and S12 for [A] and [C], respectively, and then substituting these into eq S11 and solving for [ABC], we obtain the following quintic polynomial:

$$\frac{1}{\alpha}\left(\begin{array}{l}
[\mathrm{ABC}]^5(\alpha-1)\alpha^2 - [\mathrm{ABC}]^4\alpha\left(\begin{array}{l}\alpha^2(2[\mathrm{A}]_t+[\mathrm{B}]_t+2[\mathrm{C}]_t)+\\ 2\alpha(K_{AB}+K_{BC}-[\mathrm{A}]_t-[\mathrm{C}]_t)-\\ 2(K_{AB}+K_{BC})\end{array}\right)+\\
[\mathrm{ABC}]^3\left(\begin{array}{l}\alpha^3\left(\begin{array}{l}[\mathrm{A}]_t^2+\\ 2[\mathrm{A}]_t([\mathrm{B}]_t+2[\mathrm{C}]_t)+\\ [\mathrm{C}]_t(2[\mathrm{B}]_t+[\mathrm{C}]_t)\end{array}\right)+\\
\alpha^2\left(\begin{array}{l}-[\mathrm{A}]_t([\mathrm{B}]_t+3[\mathrm{C}]_t-2K_{AB}-3K_{BC})+\\ K_{\mathrm{BC}}([\mathrm{B}]_t+2[\mathrm{C}]_t+K_{AB})-\\ [\mathrm{A}]_t^2+[\mathrm{B}]_t^2-[\mathrm{B}]_t[\mathrm{C}]_t-[\mathrm{C}]_t^2+\\ [\mathrm{B}]_tK_{AB}+3[\mathrm{C}]_tK_{AB}\end{array}\right)+\\
\alpha\left(\begin{array}{l}K_{BC}^2-\\ 2K_{BC}([\mathrm{A}]_t+[\mathrm{C}]_t+K_{AB})+\\ K_{AB}(K_{AB}-2([\mathrm{A}]_t+[\mathrm{C}]_t))\end{array}\right)-(K_{AB}-K_{BC})^2\end{array}\right)-\\
[\mathrm{ABC}]^2\alpha\left(\begin{array}{l}\alpha^2([\mathrm{B}]_t[\mathrm{C}]_t^2+2[\mathrm{A}]_t[\mathrm{C}]_t(2[\mathrm{B}]_t+[\mathrm{C}]_t)+[\mathrm{A}]_t^2([\mathrm{B}]_t+2[\mathrm{C}]_t))-\\
\left(\begin{array}{l}[\mathrm{A}]_t([\mathrm{B}]_t+[\mathrm{C}]_t)+\\ [\mathrm{C}]_t([\mathrm{C}]_t-[\mathrm{B}]_t-K_{AB})\end{array}\right)K_{AB}-\left(\begin{array}{l}[\mathrm{A}]_t^2+[\mathrm{C}]_t([\mathrm{B}]_t+K_{AB})+\\ [\mathrm{A}]_t([\mathrm{C}]_t-[\mathrm{B}]_t+K_{AB})\end{array}\right)K_{BC}+\\
\alpha\left(\begin{array}{l}-[\mathrm{A}]_t^2([\mathrm{B}]_t+[\mathrm{C}]_t-K_{BC})+\\ [\mathrm{A}]_t\left(\begin{array}{l}[\mathrm{B}]_t^2+\\ [\mathrm{B}]_t(K_{AB}+K_{BC}-2[\mathrm{C}]_t)-\\ [\mathrm{C}]_t^2+3[\mathrm{C}]_t(K_{AB}+K_{BC})+\\ K_{AB}K_{BC}\end{array}\right)+\\
[\mathrm{C}]_t\left(\begin{array}{l}[\mathrm{B}]_t^2+\\ [\mathrm{B}]_t(K_{AB}+K_{BC}-[\mathrm{C}]_t)+\\ K_{AB}([\mathrm{C}]_t+K_{BC})\end{array}\right)\\
[\mathrm{A}]_tK_{BC}^2\end{array}\right)+\\
[\mathrm{ABC}]\alpha^2[\mathrm{A}]_t[\mathrm{C}]_t\left(\begin{array}{l}[\mathrm{B}]_t^2+\\ [\mathrm{B}]_t(2\alpha[\mathrm{C}]_t+K_{AB}+K_{BC}-[\mathrm{C}]_t)+\\ [\mathrm{A}]_t((2\alpha-1)[\mathrm{B}]_t+\alpha[\mathrm{C}]_t+K_{BC})+\\ K_{AB}([\mathrm{C}]_t+K_{BC})\end{array}\right)-\alpha^3[\mathrm{A}]_t^2[\mathrm{B}]_t[\mathrm{C}]_t^2
\end{array}\right)=0. \tag{S13}$$

A polynomial f of the form

$$f(X) = a_0 + a_1X \ldots a_{n-1}X^{n-1} + a_nX^n, \quad \textbf{(S14)}$$

such that $n = 5$ is insoluble if it can be shown to be irreducible and have exactly three real roots. A specific case of the [ABC] quintic polynomial (eq S13) – where $[A]_t$, $[B]_t$, $[C]_t$, K_{AB}, K_{BC}, and α are given the integer values 2, 1, 1, 1, 1, and 2, respectively – will be tested for these properties. The substituted polynomial

$$2[ABC]^5 - 20[ABC]^4 + 74[ABC]^3 - 119[ABC]^2 + 80[ABC] - 16 \quad \textbf{(S15)}$$

can be converted into a monic polynomial by first multiplying by $a_n^{n-1} = 2^4$ and then substituting $[ABC]/a_n = [ABC]/2$ for [ABC]. The resulting monic quintic polynomial is

$$[ABC]^5 - 20[ABC]^4 + 148[ABC]^3 - 476[ABC]^2 + 640[ABC] - 256. \quad \textbf{(S16)}$$

It has been shown that a polynomial $f(X)$ is irreducible over the set of integers ($\mathbb{Z}$) when $P(f) > 2n$, where n is the degree of polynomial f, and

$$P(f) = \tilde{o}\#\{m \in Z \mid f(n) = \pm p\} \quad \textbf{(S17)}$$

such that p is a prime.(3) The arguments -29, -23,-13, -9, -7, -5, 1, 11, 19, 21, and 25 yield values for eq S16 of -38685473, -14100659, -1356689, -342733, 143651, -49481, 37, 14407, 724879, 1368377, and 3983869, respectively, and each of these values is a prime. Thus, it follows that eq S16 is irreducible over $\mathbb{Z}$. Gauss's Lemma states that if a polynomial in $\mathbb{Z}[X]$, irreducible over $\mathbb{Z}$, is considered in $\mathbb{Q}$ (where $\mathbb{Q}$ is the set of rational numbers), it will be irreducible over $\mathbb{Q}[X]$(4). Thus, eq S16 is irreducible over $\mathbb{Q}$.

For a monic, irreducible polynomial of degree p (where p is a prime) that has coefficients in $\mathbb{Q}$, it has been shown that the Galois group of the polynomial is equivalent to the symmetric group S_p when the polynomial has exactly two zeros in $\mathbb{C} \setminus \mathbb{R}$, where $\mathbb{C}$ is the set of complex numbers and $\mathbb{R}$ is the set of real numbers.(4) Because the symmetric group S_5 is insoluble, any quintic with exactly three real roots must be insoluble. The roots of eq S16 are 0.671, 2.410, 3.037, 6.941 - 1.979i, and 6.941 + 1.979i. Thus, eq S16 is insoluble by radicals and the symbolic eq S13, of which eq S16 is a case, must be insoluble by radicals as well.

Section 3. Algebraic Solutions for Maximum Values in Cooperative Ternary Equilibria

A. Solution for [B] at $[ABC]_{max}$

Rearranging eqs S1 and S6 provides expressions for [A] and [C] in terms of the variables [ABC] and [B]:

$$[A] = \frac{[A]_t - [ABC]}{1 + [B]/K_{AB}} = \frac{K_{AB}([A]_t - [ABC])}{K_{AB} + [B]} \tag{S18}$$

and

$$[C] = \frac{[C]_t - [ABC]}{1 + [B]/K_{BC}} = \frac{K_{BC}([C]_t - [ABC])}{K_{BC} + [B]}. \tag{S19}$$

Substitution of eqs S18-19 into eq S6 and solving for [ABC] provides a ratio between first-order terms,

$$[ABC] = \frac{\alpha([A]_t - [ABC])[B]([C]_t - [ABC])}{(K_{AB} + [B])(K_{BC} + [B])}, \tag{S20}$$

and rearranging this expression affords a quadratic in [ABC]:

$$[ABC]^2 - [ABC]\left(\frac{[A]_t + [C]_t + (K_{AB} + [B])(K_{BC} + [B])}{\alpha[B]}\right) + [A]_t[C]_t = 0. \tag{S21}$$

Partial differentiation of eq S21 with respect to [B] then provides

$$2[ABC]\frac{d[ABC]}{d[B]} - \left([A]_t + [C]_t + \frac{(K_{AB} + [B])(K_{BC} + [B])}{\alpha[B]}\right)\frac{d[ABC]}{d[B]} + [ABC]\frac{d\left(\frac{(K_{AB} + [B])(K_{BC} + [B])}{\alpha[B]}\right)}{d[B]} = 0. \tag{S22}$$

At $[ABC]_{max}$, $d[ABC]/d[B] = 0$, and this equation simplifies to

$$\frac{d\left(\frac{(K_{AB} + [B]_{max})(K_{BC} + [B]_{max})}{\alpha[B]_{max}}\right)}{d[B]_{max}} = 0, \tag{S23}$$

since [ABC] is, by definition, greater than zero. Because this equation only applied at the maximum value of the ternary complex ($[ABC]_{max}$), the expression for [B] derived below only applies at this maximum, and is termed $[B]_{max}$. Differentiation of eq S23 yields

$$-\frac{K_{AB}}{[B]_{max}^2}(K_{BC} + [B]_{max}) + \left(\frac{K_{AB}}{[B]_{max}} + 1\right) = 0, \tag{S24}$$

which can be rearranged to

$$-\frac{K_{AB}K_{BC}}{[B]_{max}^2} - \frac{K_{AB}}{[B]_{max}} + \frac{K_{AB}}{[B]_{max}} + 1 = 0, \tag{S25}$$

then

$$1 = \frac{K_{AB}K_{BC}}{[B]_{max}^2}, \tag{S26}$$

which simplifies to

$$[B]_{max} = \sqrt{K_{AB}K_{BC}}. \quad \textbf{(S27)}$$

This simple expression, which relates $[B]_{max}$ – the concentration of unbound B at which [ABC] is maximized – to equilibrium dissociation constants K_{AB} and K_{BC}, was first derived by Perelson and coworkers in 1981(5).

B. Solution for $[B]_t$ at $[ABC]_{max}$

Subtracting eq S2 from eqs S1 and S3 yields

$$[A]_t - [B]_t = [A] - [B] - \frac{[B][C]}{K_{BC}}, \quad \textbf{(S28)}$$

and

$$[C]_t - [B]_t = [C] - [B] - \frac{[A][B]}{K_{AB}}, \quad \textbf{(S29)}$$

respectively. Substituting eq S27 into eqsS28 and S29, then provides

$$[A]_t - [B]_{t,max} = [A] - \sqrt{K_{AB}K_{BC}} - [C]\frac{\sqrt{K_{AB}}}{\sqrt{K_{BC}}} \quad \textbf{(S30)}$$

and

$$[C]_t - [B]_{t,max} = [C] - \sqrt{K_{AB}K_{BC}} - [A]\frac{\sqrt{K_{BC}}}{\sqrt{K_{AB}}}, \quad \textbf{(S31)}$$

respectively. Because [B] is equivalent to the root of $K_{AB}{\cdot}K_{BC}$, only at the ternary complex maximum, the expression for $[B]_t$ derived below represents the $[B]_t$ value at which the ternary complex is highest; we refer to this value as $[B]_{t,\max}$. Rearranging eq S30 to

$$\frac{\sqrt{K_{BC}}}{\sqrt{K_{AB}}}\left([A]_t - [B]_{t,max}\right) = -[C] - K_{BC} + [A]\frac{\sqrt{K_{BC}}}{\sqrt{K_{AB}}} \quad \textbf{(S32)}$$

and adding this to eq S31 enables cancellation of [A] and [C] terms, resulting in

$$\frac{\sqrt{K_{BC}}}{\sqrt{K_{AB}}}[A]_t - \frac{\sqrt{K_{BC}}}{\sqrt{K_{AB}}}[B]_{t,max} + [C]_t - [B]_{t,max} = -K_{BC} - \sqrt{K_{AB}K_{BC}}. \quad \textbf{(S33)}$$

Solving for $[B]_{t,\max}$ provides an expression as a function of $[A]_t$, $[C]_t$, K_{AB}, and K_{BC}:

$$[B]_{t,max} = \frac{\sqrt{K_{BC}}}{\sqrt{K_{AB}} + \sqrt{K_{BC}}}\left([A]_t + K_{AB}\right) + \frac{\sqrt{K_{AB}}}{\sqrt{K_{AB}} + \sqrt{K_{BC}}}\left([C]_t + K_{BC}\right). \quad \textbf{(S34)}$$

Notably, this expression lacks the cooperativity term (α), indicating that $[B]_{t,\max}$ retains the same value even in highly cooperative systems. Different forms of this equation have also been reported recently (*28, 52*).

The form of eq S34 is a weighted average of the parameter sums associated with the binding of A to B ($[A]_t + K_{AB}$) and B to C ($[C]_t + K_{BC}$). Therefore, these parameters sums each must occur on alternate sides of $[B]_{t,\max}$ (Figure S3). This implies that each binding event can be associated with one side of the ternary complex curve. This "separability" will be further explored in later sections. Note that because the ABC ternary complex is symmetric, we arbitrarily define $([A]_t + K_{AB}) \leq ([C]_t + K_{BC})$.

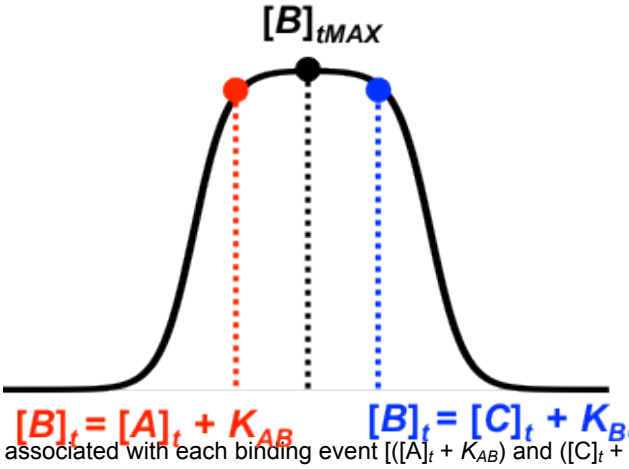


Figure S3. The *parameter sums* associated with each binding event [($[A]_t + K_{AB}$) and ($[C]_t + K_{BC}$)] must occur on either side of $[B]_{t,max}$. This implies that each binding event can be "resolved" to separate sides of the curve.

C. Solution for $[ABC]_{max}$

Substituting eq S27 into eq S21 provides

$$[ABC]_{max}^2 - [ABC]_{max}\left(\frac{[A]_t + [C]_t + \left(K_{AB} + \sqrt{K_{AB}K_{BC}}\right)\left(K_{BC} + \sqrt{K_{AB}K_{BC}}\right)}{\alpha\sqrt{K_{AB}K_{BC}}}\right) + [A]_t[C]_t = 0, \quad \textbf{(S35)}$$

which can be simplified to yield

$$[ABC]_{max}^2 - [ABC]_{max}\left([A]_t + [C]_t + \frac{\left(\sqrt{K_{AB}} + \sqrt{K_{BC}}\right)^2}{\alpha}\right) + [A]_t[C]_t = 0. \quad \textbf{(S36)}$$

An expression for $[ABC]_{max}$ – the concentration of ternary complex when $[B]_t = [B]_{t,\max}$ – can easily be derived from this equation using the general quadratic formula:

$$[ABC]_{max} = \frac{[A]_t + [C]_t + \frac{\left(\sqrt{K_{AB}} + \sqrt{K_{BC}}\right)^2}{\alpha} - \sqrt{\left([A]_t + [C]_t + \frac{\left(\sqrt{K_{AB}} + \sqrt{K_{BC}}\right)^2}{\alpha}\right)^2 - 4[A]_t[C]_t}}{2}. \quad \textbf{(S37)}$$

It is worth noting that this relationship depends on cooperativity (α), unlike the position of the maximum ($[B]_{t,\max}$, eq S34). This observation is consistent with prior observations by Perelson, Whitty, and Whitesides in related systems (5, 6). Different forms of this equation have also been reported recently (7, 8).

D. Ratiometric model for the physical behavior of bell-shaped curve

Equations S7 and S8 provide a semi-quantitative, phenomenological explanation for autoinhibition, or the "prozone effect", in ternary equilibria (Figure S4A). Multiplying eq S7 by eq S8 yields

$$[AB][BC] = \frac{[A][B]^2[C]}{K_{AB}K_{BC}}, \quad \textbf{(S38)}$$

and replacing [B] with eq S27 for $[B]_{max}$ yields

$$[AB][BC] = \frac{[A]\sqrt{K_{AB}K_{BC}}^{2}[C]}{K_{AB}K_{BC}} = [A][C]. \quad \textbf{(S39)}$$

This equation implies that the product of the binary species ([AB]·[BC]) and the free species ([A]·[C]) are equal when $[B]_t = [B]_{t,max}$. In phenomenological terms, the value of $[B]_{t,max}$ can be understood as that at which the activities of free and binary solution species are in balance (Figure S4B). Perturbation of the system through the addition of B, for example, will induce consumption of A and C and the decomposition of ternary complex into constituent binary and free species. Overall, then, [AB]·[BC] will increase while [A]·[C] decreases, corresponding to the "autoinhibitory," or left, portion of the curve. The left region of the binding curve can be understood in similar terms.

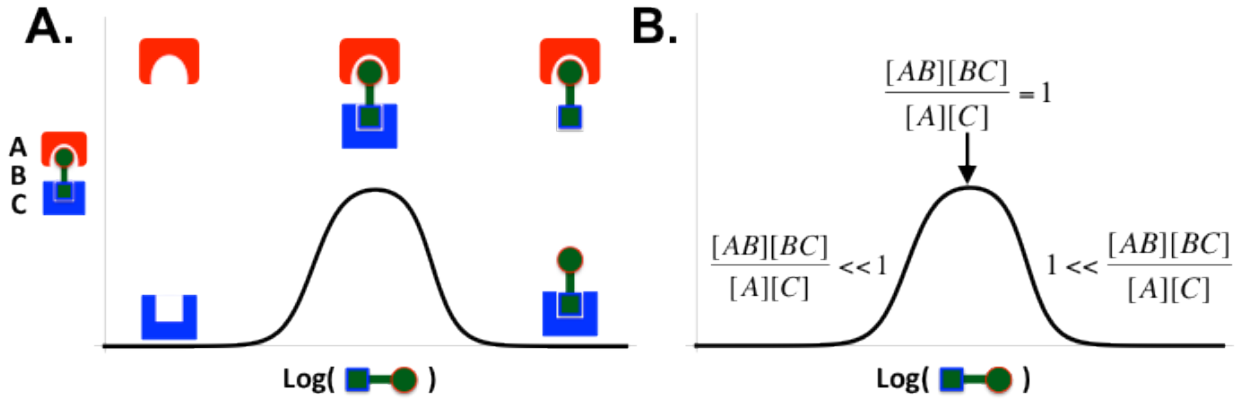


Figure S4. (A) The bell-shaped dose response of ternary complex equilibria can be conceptually understood as representing a balance between two extremes: too little B results in no ternary complex formation (far left) and too much B out-competes ternary complex formation (far right). Thus, an intermediate amount of B achieves maximal ABC formation. **(B)** This conceptual model finds quantitative grounding in the form of the ratio of the binary to free species at equilibrium. In this section we show that when this ratio equals unity (and thus the "activities" of the free and binary species are balanced), maximal [ABC] forms.

Section 4. A Complete Analytical Solution to the Non-Cooperative Ternary Equilibrium Model

A. Solutions for [A] AND [C]

Replacing α with unity in eq S6 reflects the special case in which the ABC complex forms without cooperativity; that is, when the A–B and B–C binding events are completely independent of one another. This substitution simplifies eq S13 into a quartic equation. Because a general solution exists for quartic polynomials (9), it follows that a general solution must also exist relating [ABC] to measurable parameters for non-cooperative, ternary equilibria.

Because the form of the general quartic equation is very complex, we sought alternative means to simplify the solution for [ABC]. Replacing [AB], [BC], and [ABC] in eqs S1-3 using eqs S4-S6, and substituting α with unity, we obtained the following conservation of mass equations in terms of the free concentrations of A, B and C:

$$[\mathrm{A}]_t = [\mathrm{A}] + \frac{[\mathrm{A}][\mathrm{B}]}{K_{AB}} + \frac{[\mathrm{A}][\mathrm{B}][\mathrm{C}]}{K_{AB}K_{BC}}, \tag{S40}$$

$$[\mathrm{B}]_t = [\mathrm{B}] + \frac{[\mathrm{A}][\mathrm{B}]}{K_{AB}} + \frac{[\mathrm{B}][\mathrm{C}]}{K_{BC}} + \frac{[\mathrm{A}][\mathrm{B}][\mathrm{C}]}{K_{AB}K_{BC}}, \tag{S41}$$

and

$$[\mathrm{C}]_t = [\mathrm{C}] + \frac{[\mathrm{B}][\mathrm{C}]}{K_{BC}} + \frac{[\mathrm{A}][\mathrm{B}][\mathrm{C}]}{K_{AB}K_{BC}}. \tag{S42}$$

Rearranging and factoring the right-hand side of eqs S40-41 makes clear that the contribution of [B] and [C] to both equations is exactly the same:

$$[\mathrm{A}]_t - [\mathrm{A}] = \frac{[\mathrm{A}]}{K_{AB}} \cdot [\mathrm{B}] \cdot \left(1 + \frac{[\mathrm{C}]}{K_{BC}}\right). \tag{S43}$$

and

$$[\mathrm{B}]_t = \left(1 + \frac{[\mathrm{A}]}{K_{AB}}\right) \cdot [\mathrm{B}] \cdot \left(1 + \frac{[\mathrm{C}]}{K_{BC}}\right). \tag{S44}$$

Combining eqs S43 and S44 using the $[\mathrm{B}] \cdot \left(1 + \frac{[\mathrm{C}]}{K_{BC}}\right)$ terms results in a 2nd order polynomial in the concentration of unbound A ([A]),

$$0 = [\mathrm{A}]^2 - [\mathrm{A}]\left([\mathrm{B}]_t + K_{AB} - [\mathrm{A}]_t\right) - [\mathrm{A}]_t K_{AB}. \tag{S45}$$

This can be solved using the quadratic formula to yield

$$[\mathrm{A}] = \frac{[\mathrm{A}]_t - [\mathrm{B}]_t - K_{AB} \pm \sqrt{\left([\mathrm{B}]_t + K_{AB} - [\mathrm{A}]_t\right)^2 + 4[\mathrm{A}]_t K_{AB}}}{2}, \tag{S46}$$

and then rearranged to

$$[\mathrm{A}] = [\mathrm{A}]_t - \frac{[\mathrm{A}]_t + [\mathrm{B}]_t + K_{AB} \pm \sqrt{\left([\mathrm{A}]_t + [\mathrm{B}]_t + K_{AB}\right)^2 - 4[\mathrm{A}]_t[\mathrm{B}]_t}}{2}. \tag{S47}$$

Only one form of the radical gives physically reasonable results,

$$[\mathrm{A}] = [\mathrm{A}]_t - \frac{[\mathrm{A}]_t + [\mathrm{B}]_t + K_{AB} - \sqrt{\left([\mathrm{A}]_t + [\mathrm{B}]_t + K_{AB}\right)^2 - 4[\mathrm{A}]_t[\mathrm{B}]_t}}{2}, \tag{S48}$$

and this is identical in form to the exact expression for [A] in binary A–B equilibria. One can rationalize this behavior for noncooperative ternary equilibria because A–B and B–C binding events are completely independent of one another, such that the presence of C should not affect A–B binding and vice-versa.

In an analogous manner, the equivalent equation for the equilibrium concentration of unbound C can be derived:

$$[\mathrm{C}] = [\mathrm{C}]_t - \frac{[\mathrm{B}]_t + [\mathrm{C}]_t + K_{BC} - \sqrt{\left([\mathrm{B}]_t + [\mathrm{C}]_t + K_{BC}\right)^2 - 4[\mathrm{B}]_t[\mathrm{C}]_t}}{2}. \quad \textbf{(S49)}$$

B. Solution for [B]

Solving eq S44 for [B] provides

$$[\mathrm{B}] = \frac{[\mathrm{B}]_t}{\left(1 + {[\mathrm{A}]}/{K_{AB}}\right)\left(1 + {[\mathrm{C}]}/{K_{BC}}\right)}, \quad \textbf{(S50)}$$

which can be rearranged to

$$[\mathrm{B}] = \frac{K_{AB}K_{BC}[\mathrm{B}]_t}{(K_{AB} + [\mathrm{A}])(K_{BC} + [\mathrm{C}])}. \quad \textbf{(S51)}$$

Substituting [A] using eq S48 provides

$$[\mathrm{B}] = \frac{K_{AB}K_{BC}[\mathrm{B}]_t}{\left(K_{AB} + [\mathrm{A}]_t - \frac{[\mathrm{A}]_t + [\mathrm{B}]_t + K_{AB} - \sqrt{\left([\mathrm{A}]_t + [\mathrm{B}]_t + K_{AB}\right)^2 - 4[\mathrm{A}]_t[\mathrm{B}]_t}}{2}\right)(K_{BC} + [\mathrm{C}])}. \quad \textbf{(S52)}$$

Rearranging this equation yields the following expression for [B]:

$$[\mathrm{B}] = \frac{2K_{AB}K_{BC}[\mathrm{B}]_t}{\left([\mathrm{A}]_t - [\mathrm{B}]_t + K_{AB} + \sqrt{\left([\mathrm{A}]_t - [\mathrm{B}]_t + K_{AB}\right)^2 + 4[\mathrm{B}]_t K_{AB}}\right)(K_{BC} + [\mathrm{C}])}. \quad \textbf{(S53)}$$

The conjugate of the radical term can be used to rationalize the denominator,

$$[\mathrm{B}] = \frac{2K_{AB}K_{BC}[\mathrm{B}]_t}{\left([\mathrm{A}]_t - [\mathrm{B}]_t + K_{AB} + \sqrt{\left([\mathrm{A}]_t - [\mathrm{B}]_t + K_{AB}\right)^2 + 4[\mathrm{B}]_t K_{AB}}\right)(K_{BC} + [\mathrm{C}])} \times \frac{[\mathrm{A}]_t - [\mathrm{B}]_t + K_{AB} - \sqrt{\left([\mathrm{A}]_t - [\mathrm{B}]_t + K_{AB}\right)^2 + 4[\mathrm{B}]_t K_{AB}}}{[\mathrm{A}]_t - [\mathrm{B}]_t + K_{AB} - \sqrt{\left([\mathrm{A}]_t - [\mathrm{B}]_t + K_{AB}\right)^2 + 4[\mathrm{B}]_t K_{AB}}}, \quad \textbf{(S54)}$$

and further simplification and rearrangement yields

$$[\mathrm{B}] = \frac{K_{BC}\left([\mathrm{B}]_t - [\mathrm{A}]_t - K_{AB} + \sqrt{\left([\mathrm{A}]_t - [\mathrm{B}]_t + K_{AB}\right)^2 + 4[\mathrm{B}]_t K_{AB}}\right)}{2\left(K_{BC} + [\mathrm{C}]\right)}. \quad \textbf{(S55)}$$

The portion of the equation originating from the [A] term becomes identical to the expression for the equilibrium concentration of free [B] in a binary A–B equilibrium:

$$[\mathrm{B}] = \frac{K_{BC}\left([\mathrm{B}]_t - \frac{[\mathrm{A}]_t + [\mathrm{B}]_t + K_{AB} - \sqrt{\left([\mathrm{A}]_t + [\mathrm{B}]_t + K_{AB}\right)^2 - 4[\mathrm{A}]_t[\mathrm{B}]_t}}{2}\right)}{\left(K_{BC} + [\mathrm{C}]\right)}. \quad \textbf{(S56)}$$

After repeating this same procedure (analogous to eqs S52 through S57) for the $\left(K_{BC} + [\mathrm{C}]_t\right)$ term in eq S51, the concentration of free B reduces to the following expression:

$$[\mathrm{B}] = \frac{\left([\mathrm{B}]_t - \frac{[\mathrm{A}]_t + [\mathrm{B}]_t + K_{AB} - \sqrt{\left([\mathrm{A}]_t + [\mathrm{B}]_t + K_{AB}\right)^2 - 4[\mathrm{A}]_t[\mathrm{B}]_t}}{2}\right)}{[\mathrm{B}]_t} \times \frac{\left([\mathrm{B}]_t - \frac{[\mathrm{B}]_t + [\mathrm{C}]_t + K_{BC} - \sqrt{\left([\mathrm{B}]_t + [\mathrm{C}]_t + K_{BC}\right)^2 - 4[\mathrm{B}]_t[\mathrm{C}]_t}}{2}\right)}{[\mathrm{B}]_t}. \quad \textbf{(S57)}$$

C. Solutions for [AB], [BC], AND [ABC]

We can now solve for the concentrations of binary and ternary complexes at equilibrium in terms of measurable parameters by replacing [A], [B] and [C] in eqs S7-9 with the solutions derived above.

An expression for [AB],

$$[\mathrm{AB}] = \frac{[\mathrm{A}][\mathrm{B}]}{K_{AB}}, \quad \textbf{(S7)}$$

can be obtained by multiplication of eqs S48 and S57, divided by K_{AB}, which yields

$$[\mathrm{AB}] = \frac{\left(\frac{[\mathrm{A}]_t + [\mathrm{B}]_t + K_{AB} - \sqrt{\left([\mathrm{A}]_t + [\mathrm{B}]_t + K_{AB}\right)^2 - 4[\mathrm{A}]_t[\mathrm{B}]_t}}{2}\right)\left([\mathrm{B}]_t - \frac{[\mathrm{B}]_t + [\mathrm{C}]_t + K_{BC} - \sqrt{\left([\mathrm{B}]_t + [\mathrm{C}]_t + K_{BC}\right)^2 - 4[\mathrm{B}]_t[\mathrm{C}]_t}}{2}\right)}{[\mathrm{B}]_t} \quad \textbf{(S58)}$$

after simplification. An expression for [BC],

$$[\mathrm{BC}] = \frac{[\mathrm{B}][\mathrm{C}]}{K_{BC}} \quad \textbf{(S8)}$$

can be obtained in terms of measurable parameters by multiplying eqs S57 by S49, dividing by K_{BC}, and simplifying to yield

$$[\mathrm{BC}] = \frac{\left([\mathrm{B}]_t - \frac{[\mathrm{A}]_t + [\mathrm{B}]_t + K_{AB} - \sqrt{\left([\mathrm{A}]_t + [\mathrm{B}]_t + K_{AB}\right)^2 - 4[\mathrm{A}]_t[\mathrm{B}]_t}}{2}\right)\left(\frac{[\mathrm{B}]_t + [\mathrm{C}]_t + K_{BC} - \sqrt{\left([\mathrm{B}]_t + [\mathrm{C}]_t + K_{BC}\right)^2 - 4[\mathrm{B}]_t[\mathrm{C}]_t}}{2}\right)}{[\mathrm{B}]_t} \quad \textbf{(S59)}$$

after simplification. Finally, to obtain an expression for the ternary complex,

$$[\mathrm{ABC}] = \frac{[\mathrm{A}][\mathrm{B}][\mathrm{C}]}{K_{AB}K_{BC}}, \quad \textbf{(S60)}$$

as a function of measurable parameters, the product of eqs S48, S57, and S49 can be divided by $(K_{AB}K_{BC})$, yielding

$$[\mathrm{ABC}] = \frac{\left(\frac{[\mathrm{A}]_t + [\mathrm{B}]_t + K_{AB} - \sqrt{([\mathrm{A}]_t + [\mathrm{B}]_t + K_{AB})^2 - 4[\mathrm{A}]_t[\mathrm{B}]_t}}{2}\right)\left(\frac{[\mathrm{B}]_t + [\mathrm{C}]_t + K_{BC} - \sqrt{([\mathrm{B}]_t + [\mathrm{C}]_t + K_{BC})^2 - 4[\mathrm{B}]_t[\mathrm{C}]_t}}{2}\right)}{[\mathrm{B}]_t} \quad \textbf{(S61)}$$

after simplification.

D. Definition of ϕ_{AB}, ϕ_{BC}, Q_1, and Q_2

Each of the two quadratic roots in the numerator of eq S61 exclusively contain terms corresponding to either A–B or B–C binding interactions, and are given the names ϕ_{AB} and ϕ_{BC}, respectively:

$$\phi_{AB} = \frac{[\mathrm{A}]_t + [\mathrm{B}]_t + K_{AB} - \sqrt{([\mathrm{A}]_t + [\mathrm{B}]_t + K_{AB})^2 - 4[\mathrm{A}]_t[\mathrm{B}]_t}}{2} \quad \textbf{(S62)}$$

and

$$\phi_{BC} = \frac{[\mathrm{B}]_t + [\mathrm{C}]_t + K_{BC} - \sqrt{([\mathrm{B}]_t + [\mathrm{C}]_t + K_{BC})^2 - 4[\mathrm{B}]_t[\mathrm{C}]_t}}{2}. \quad \textbf{(S63)}$$

The concentrations of unbound A and C ([A] and [C]) can also be defined as

$$[\mathrm{A}] = [\mathrm{A}]_t - \phi_{AB} \quad \textbf{(S64)}$$

and

$$[\mathrm{C}] = [\mathrm{C}]_t - \phi_{BC}. \quad \textbf{(S65)}$$

Equations S62–S63 (defining ϕ_{AB} and ϕ_{BC}) and eqs S64–S65 are identical to expressions for [AB], [BC], [A], and [C] in binary binding equilibria, respectively. In this context, ϕ_{AB} and ϕ_{BC} in non-cooperative ternary equilibria can be understood physically as representing the total amount of A or C present in the bound state, or

$$\phi_{AB} = [\mathrm{AB}] + [\mathrm{ABC}] \quad \textbf{(S66)}$$

and

$$\phi_{BC} = [\mathrm{BC}] + [\mathrm{ABC}]. \quad \textbf{(S67)}$$

In this light, the equations for [B], [AB], [BC] and [ABC] can be viewed as joint independent probabilities. Thus, expressing eq S61 in terms of ϕ_{AB} and ϕ_{BC} and normalizing by $[\mathrm{A}]_t$ provides

$$\frac{[\mathrm{ABC}]}{[\mathrm{A}]_t} = \frac{\phi_{AB}}{[\mathrm{A}]_t} \times \frac{\phi_{BC}}{[\mathrm{B}]_t} = \frac{[\mathrm{AB}] + [\mathrm{ABC}]}{[\mathrm{A}]_t} \times \frac{[\mathrm{BC}] + [\mathrm{ABC}]}{[\mathrm{B}]_t}, \quad \textbf{(S68)}$$

such that the fraction of A engaged in ternary complex $([\mathrm{ABC}]/[\mathrm{A}]_t)$ equals the fraction of A bound to B $(\phi_{AB}/[\mathrm{A}]_t)$ multiplied by the fraction of B bound to C $(\phi_{BC}/[\mathrm{B}]_t)$. Stated differently, the probability that both B *and* C are bound to A $([\mathrm{ABC}]/[\mathrm{A}]_t)$ is equal to the product of the probabilities that B is bound to A $(\phi_{AB}/[\mathrm{A}]_t)$ and that B is bound to C $(\phi_{BC}/[\mathrm{B}]_t)$.

The fraction of A that is bound to *only* B $\left([\mathrm{AB}]/[\mathrm{A}]_t\right)$ is equal to the product of the probabilities that B is bound to A $\left(\phi_{AB}/[\mathrm{A}]_t\right)$ and that B is <u>not</u> bound to C $\left(\left([\mathrm{B}]_t-\phi_{BC}\right)/[\mathrm{B}]_t\right)$, or

$$\frac{[\mathrm{AB}]}{[\mathrm{A}]_t}=\frac{\phi_{AB}}{[\mathrm{A}]_t}\times\frac{[\mathrm{B}]_t-\phi_{BC}}{[\mathrm{B}]_t}=\frac{[\mathrm{AB}]+[\mathrm{ABC}]}{[\mathrm{A}]_t}\times\frac{[\mathrm{B}]+[\mathrm{AB}]}{[\mathrm{B}]_t}. \qquad \textbf{(S69)}$$

Defining each of the fractions containing ϕ in eq S68 as Q_1 and Q_2 enables a fully normalized treatment of [ABC] and constituent binary interactions:

$$\frac{[\mathrm{ABC}]}{[\mathrm{A}]_t}=\frac{\phi_{AB}}{[\mathrm{A}]_t}\times\frac{\phi_{BC}}{[\mathrm{B}]_t}=\mathrm{Q}_1\times\mathrm{Q}_2. \qquad \textbf{(S70)}$$

Section 5. Understanding Non-Cooperative Equilibria

A. The Resolvability assumption

For non-cooperative ternary equilibria, the concentration of ABC equals the product of two quadratic roots, as outlined above. We can further simplify this mathematical relationship by applying two limiting assumptions that are frequently satisfied in experimental and physiological systems: "resolvability" and "dominance." The following section details how these limiting assumptions simplify eq S61 in terms of critical points ($[B]_{t,max}$, $[ABC]_{max}$, TF_{50}, and TI_{50}).

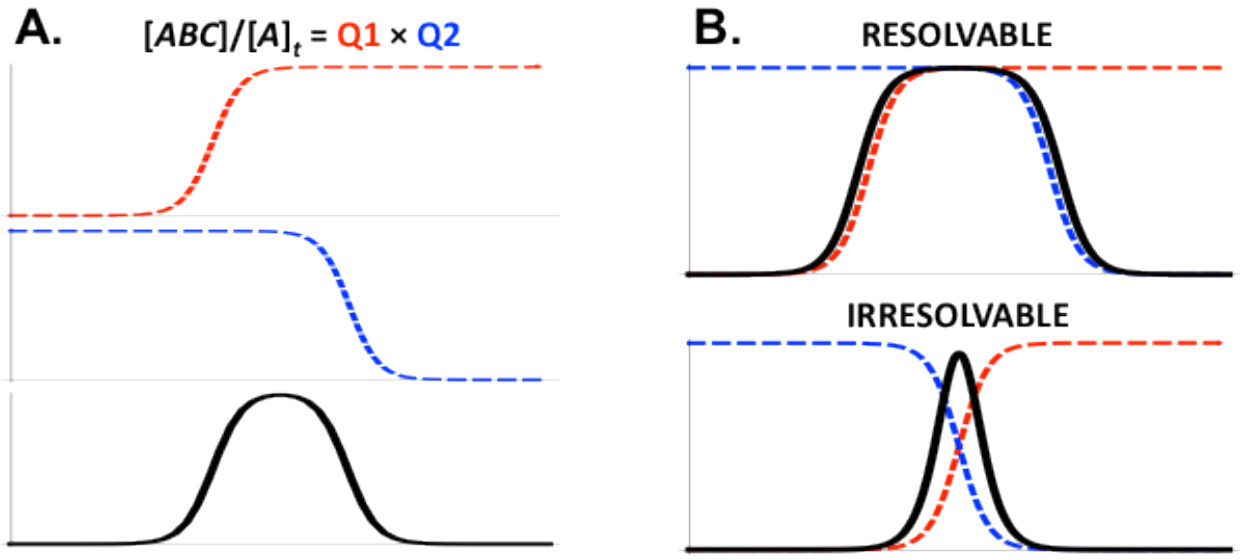


Figure S5. (A) We define "resolvable conditions" as satisfied when the higher plateaus of Q_1 and Q_2 overlap at $[B]_{t,max}$. **(B)** The *parameter sums* associated with each binding event $[([A]_t + K_{AB})$ and $([C]_t + K_{BC})]$ must occur on either side of $[B]_{t,max}$. This implies that the binding events can be "resolved" to separate sides of the curve.

As illustrated in Figure S5, under conditions where plateaus of Q_1 and Q_2 overlap at $[B]_{t,max}$, each constituent binary binding event can be mathematically and conceptually associated with a different side of the curve. Such conditions are termed "resolvable". More specifically, the Q_1 term is identical to a normalized A–B binding curve at increasing concentrations of B. At high concentrations of B total, this function increases and plateaus at a value of 1, which indicates quantitative/complete binding of B to A (Figure S5A). The higher plateau of the Q_2 term occurs at low concentrations of B total (Figure S5A).

For the purposes of this discussion, we assume a system is resolvable if the parameter sums differ by more than one order of magnitude. Under these conditions, the plateau regions of Q_1 and Q_2 overlap to a sufficient extent to treat the A–B and B–C binding events independently, as illustrated in Figure S5. Under such conditions, [ABC] predominantly reflects the behavior of Q_1 for values of $[B]_t$ less than $[B]_{t,max}$, and it reflects Q_2 at values greater than $[B]_{t,max}$. Resolvable systems can be envisioned to behave physically as depicted in Figure S6, such that full saturation of the limiting terminal species (A in this discussion) occurs at a value of $[B]_t$ lower than those at which autoinhibition is observed. As can be seen in Figure S6A, the maximal ternary complex plateau can be viewed as a state with saturation of one side of the complex (A saturated with B). After this point, any additional B added will compete with AB for binding to C. As long as the majority of C is not bound, this competition is negligible. Once B begins to saturate C, however,

this competition becomes pronounced as excess free B prevents the formation of ternary complex.

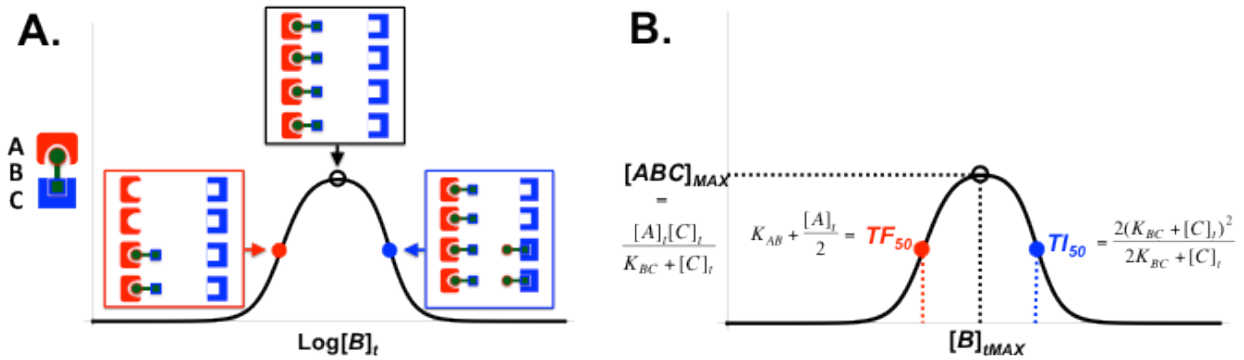


Figure S6. (**A**) When the plateaus of the Q_1 and Q_2 overlap at $[B]_{t,max}$, the binding events can be resolved to separate sides of the non-cooperative ternary complex curve. TF_{50} represents half-saturation of the A side of the ternary complex, $[B]_{t,max}$ represents saturation of the same side, and TI_{50} represents half-saturation of the C side of the ternary complex. (**B**) Summary of equations describing the critical features of resolvable curves derived in this section.

$[ABC]_{max}$ Under Resolvable conditions

Under resolvable conditions, $[ABC]_{max}$ simplifies to the product of the y-axis values of the plateau regions of ϕ_{AB} and Q_2 (Figure 5A). The former of these plateaus is obtained as the limit of ϕ_{AB} at infinity,

$$\lim_{[B]_t \to \infty} \phi_{AB} = \lim_{[B]_t \to \infty} \frac{[A]_t + [B]_t + K_{AB} - \sqrt{([A]_t + [B]_t + K_{AB})^2 - 4[A]_t[B]_t}}{2} = [A]_t. \quad \textbf{(S71)}$$

An expression for the y-axis value of the higher Q_2 plateau is obtained by recognizing that the ϕ_{BC} term in Q_2 is equivalent to [BC] in a binary B–C equilibrium, or

$$[BC]^{binary} = \phi_{BC} = \frac{[B][C]}{K_{BC}}. \quad \textbf{(S72)}$$

In a B–C binary system (i.e., no A species is present), the total concentrations of both species can be represented as

$$[B]_t^{binary} = [B] + [BC]^{binary} \quad \textbf{(S73)}$$

and

$$[C]_t^{binary} = [C] + [BC]^{binary}. \quad \textbf{(S74)}$$

Equation S72 can be substituted into the above two equations to yield

$$[B]_t^{binary} = [B] + \frac{[B][C]}{K_{BC}} \quad \textbf{(S75)}$$

and

$$[C]_t^{binary} = [C] + \frac{[B][C]}{K_{BC}}. \quad \textbf{(S76)}$$

As $[B]_t$ approaches 0 (i.e., where the Q_2 plateau occurs), [B] and [BC] must also approach 0 (eq S73). Therefore, the free concentration of [C] approaches the total concentration of $[C]_t$ as the amount bound to B ($[BC]^{binary}$) also approaches zero in eq S74. Stated alternatively,

$$\lim_{[B]_t \to 0} [C]_t = [C], \quad \textbf{(S77)}$$

and thus

$$[C]_{t,[B]_t \to 0} = [C] \quad \textbf{(S78)}$$

in the higher plateau of Q_2. Substitution of eq S78 into eq S75 and solving for the free concentration of B yields

$$[B]_{[B]_t \to 0} = \frac{[B]_t}{1 + [C]_t / K_{BC}}. \quad \textbf{(S79)}$$

Substituting eqs S78 and S79 into eq S72 yields

$$[BC]^{binary}_{[B]_t \to 0} = \phi_{BC,[B]_t \to 0} = \frac{\frac{[B]_t}{1 + [C]_t / K_{BC}}[C]_t}{K_{BC}} = \frac{[B]_t [C]_t}{K_{BC} + [C]_t}, \quad \textbf{(S80)}$$

which is an expression for ϕ_{BC} in the higher plateau of Q_2. Thus,

$$\lim_{[B]_t \to 0} Q_2 = \lim_{[B]_t \to 0} \frac{\phi_{BC,[B]_t \to 0}}{[B]_t} = \frac{\frac{[B]_t [C]_t}{K_{BC} + [C]_t}}{[B]_t} = \frac{[C]_t}{K_{BC} + [C]_t}. \quad \textbf{(S81)}$$

As stated above, the value of $[ABC]^R_{max}$ equals the product of the y-axis values of the higher plateaus of ϕ_{AB} and Q_2, which have been obtained for each of these functions at the limits of infinity and 0, respectively. Thus,

$$[ABC]^R_{max} = \lim_{[B]_t \to \infty} \phi_{AB} \bullet \lim_{[B]_t \to 0} Q_2 = \frac{[A]_t [C]_t}{K_{BC} + [C]_t}, \quad \textbf{(S82)}$$

where the R superscript denotes resolvable conditions.

TF_{50} Under Resolvable conditions

Under resolvable conditions, the value of the TF^R_{50} is equal to the value of $[B]_t$ where ϕ_{AB} is half maximal. Because ϕ_{AB} can be defined using the system of equations describing binary equilibria, we use a description of the A–B binary half-maximum point to derive TF^R_{50}. Fractional binding in binary A–B binding equilibria is

$$\frac{[A]}{[A]^{binary}_t} = \frac{[A]}{[A] + [AB]} = \frac{[A]}{[A] + \frac{[A][B]}{K_{AB}}} = \frac{K_{AB}}{K_{AB} + [B]}. \quad \textbf{(S83)}$$

To obtain the TF^R_{50}, we must convert the free concentration of B to the measurable parameter $[B]_t$. Because [AB] is known to be exactly $[A]_t/2$ at the half-maximum, we can substitute the free concentration of B with $[B]_t - [AB] = [B]_t - [A]_t/2$, resulting in

$$\frac{[A]}{[A]^{binary}_t} = \frac{K_{AB}}{K_{AB} + [B]} = \frac{K_{AB}}{K_{AB} + [B]_t - \frac{[A]_t}{2}} = \frac{1}{2}. \quad \textbf{(S84)}$$

Solving the last equivalence in eq S84 for $[B]_t$, we arrive at

$$TF^R_{50} = K_{AB} + \frac{[A]_t}{2}. \quad \textbf{(S85)}$$

TI_{50} Under Resolvable conditions

The TI^R_{50} concentration, being solely defined by the parameters associated with Q_2, can be expressed in terms analogous to binary B–C binding equilibria, where the fractional amount of binding $([BC]/[B]_t)$ will be half maximal at half the Q_2 maximum (the higher plateau),

$$\frac{1}{2}\underset{[B]_t \to 0}{Limit}\, Q_2 = \frac{1}{2}\frac{[C]_t}{K_{BC} + [C]_t}. \quad \textbf{(S86)}$$

Thus,

$$\frac{[BC]}{[B]_t} = \frac{1}{2}\frac{[C]_t}{K_{BC} + [C]_t} \quad \textbf{(S87)}$$

at the TI_{50}^{R}. Solving this for [BC] yields

$$[BC]^{1/2\max} = \frac{1}{2}\frac{[C]_t[B]_t}{K_{BC} + [C]_t}. \quad \textbf{(S88)}$$

Utilizing the conservation of mass equation for a B–C binary equilibrium ($[B]_t$ = [B] + [BC]) and the mass action equation (eq S8), the fractional amount of B–C binding,

$$\frac{[BC]}{[B]_t} = \frac{[BC]}{[B] + [BC]} = \frac{[B][C]/K_{BC}}{[B] + [B][C]/K_{BC}} = \frac{[C]}{K_{BC} + [C]}. \quad \textbf{(S89)}$$

In a B–C binary system, $[C]_t$ = [BC] + [C]. Using this equivalence, we can replace the [C] term in the above expression and substitute eq S88 for [BC]. This yields

$$\frac{[C]}{K_{BC} + [C]} = \frac{[C]_t - [BC]}{K_{BC} + [C]_t - [BC]} = \frac{[C]_t - \frac{1}{2}\frac{[C]_t[B]_t}{K_{BC} + [C]_t}}{K_{BC} + [C]_t - \frac{1}{2}\frac{[C]_t[B]_t}{K_{BC} + [C]_t}}. \quad \textbf{(S90)}$$

Both the latter expression above and eq S87 are equivalent to the fractional amount of B–C binding ($[BC]/[B]_t$) at the half maximal point. Setting these two expressions equal yields

$$\frac{1}{2}\frac{[C]_t}{K_{BC} + [C]_t} = \frac{[C]_t - \frac{1}{2}\frac{[C]_t[B]_t}{K_{BC} + [C]_t}}{K_{BC} + [C]_t - \frac{1}{2}\frac{[C]_t[B]_t}{K_{BC} + [C]_t}}. \quad \textbf{(S91)}$$

This equation can be simplified by multiplying both sides by 2 and the right side of equation by 2/2,

$$\frac{[C]_t}{K_{BC} + [C]_t} = \frac{4[C]_t - 2\frac{[C]_t[B]_t}{K_{BC} + [C]_t}}{2K_{BC} + 2[C]_t - \frac{[C]_t[B]_t}{K_{BC} + [C]_t}}. \quad \textbf{(S92)}$$

By consolidating fractions in the numerator and denominator of the right hand side of the equation, we obtain

$$\frac{[C]_t}{K_{BC} + [C]_t} = \frac{\frac{4[C]_t(K_{BC} + [C]_t) - 2[C]_t[B]_t}{K_{BC} + [C]_t}}{\frac{2(K_{BC} + [C]_t)^2 - [C]_t[B]_t}{K_{BC} + [C]_t}}. \quad \textbf{(S93)}$$

Cancelling out the $K_{BC} + [C]_t$ terms from the numerator and denominator yields

$$\frac{[C]_t}{K_{BC} + [C]_t} = \frac{4[C]_t(K_{BC} + [C]_t) - 2[C]_t[B]_t}{2(K_{BC} + [C]_t)^2 - [C]_t[B]_t}. \quad \textbf{(S94)}$$

Cancelling out $[C]_t$ on both sides of the equation,

$$\frac{1}{K_{BC}+[\mathrm{C}]_t}=\frac{4\left(K_{BC}+[\mathrm{C}]_t\right)-2[\mathrm{B}]_t}{2\left(K_{BC}+[\mathrm{C}]_t\right)^2-[\mathrm{C}]_t[\mathrm{B}]_t}, \tag{S95}$$

cross-multiplying,

$$2\left(K_{BC}+[\mathrm{C}]_t\right)^2-[\mathrm{C}]_t[\mathrm{B}]_t=4\left(K_{BC}+[\mathrm{C}]_t\right)^2-2[\mathrm{B}]_t\left(K_{BC}+[\mathrm{C}]_t\right), \tag{S96}$$

combining like terms,

$$-[\mathrm{C}]_t[\mathrm{B}]_t=2\left(K_{BC}+[\mathrm{C}]_t\right)^2-2[\mathrm{B}]_t\left(K_{BC}+[\mathrm{C}]_t\right), \tag{S97}$$

and rearranging provides

$$2[\mathrm{B}]_t\left(K_{BC}+[\mathrm{C}]_t\right)-[\mathrm{C}]_t[\mathrm{B}]_t=2\left(K_{BC}+[\mathrm{C}]_t\right)^2. \tag{S98}$$

Solving the above equation for $[\mathrm{B}]_t$ then yields

$$\mathrm{TI}_{50}^{\mathrm{R}}=\frac{2\left(K_{BC}+[\mathrm{C}]_t\right)^2}{2K_{BC}+[\mathrm{C}]_t}. \tag{S99}$$

In an irresolvable system, these expressions for $\mathrm{TF}_{50}^{\mathrm{R}}$ and $\mathrm{TI}_{50}^{\mathrm{R}}$ will over- and underestimate, respectively, the true values, such that

$$\mathrm{TF}_{50}\le K_{AB}+\frac{[\mathrm{A}]_t}{2} \tag{S100}$$

and

$$\mathrm{TI}_{50}\ge\frac{2(K_{BC}+[\mathrm{C}]_t)^2}{2K_{BC}+[\mathrm{C}]_t}. \tag{S101}$$

These expressions are also related to the parameters sums, which frame $[\mathrm{B}]_{t,max}$,(Figure S3):

$$K_{AB}+[\mathrm{A}]_t\le[\mathrm{B}]_{t,max}\le K_{BC}+[\mathrm{C}]_t. \tag{S102}$$

Based on the form of eqs S100-102, the following expression ranking must hold:

$$\mathrm{TF}_{50}\le K_{AB}+\frac{[\mathrm{A}]_t}{2}<K_{AB}+[\mathrm{A}]_t\le[\mathrm{B}]_{t,max}\le K_{BC}+[\mathrm{C}]_t<\frac{2(K_{BC}+[\mathrm{C}]_t)^2}{2K_{BC}+[\mathrm{C}]_t}\le\mathrm{TI}_{50}. \tag{S103}$$

Thus, the parameter sums are bounded by the $\mathrm{TF}_{50}^{\mathrm{R}}$, $\mathrm{TI}_{50}^{\mathrm{R}}$, and $[\mathrm{B}]_{t,max}$ (Figure S7), or

$$\mathrm{TF}_{50}\le K_{AB}+[\mathrm{A}]_t\le[\mathrm{B}]_{t,max}\le K_{BC}+[\mathrm{C}]_t\le\mathrm{TI}_{50}. \tag{S104}$$

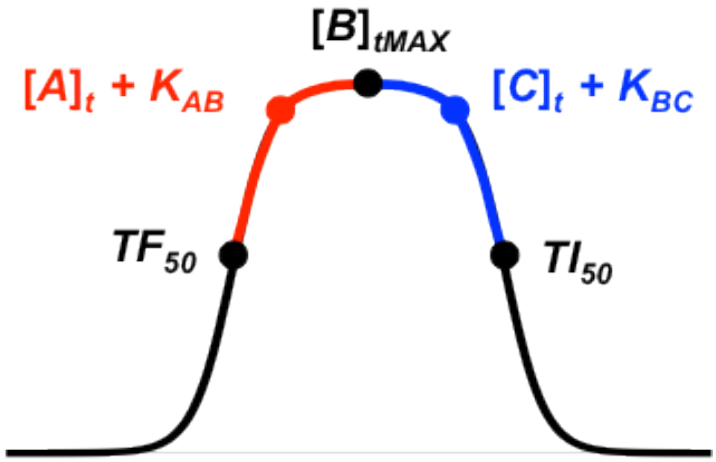


Figure S7. The A–B *parameter sums* ($[\mathrm{A}]_t + K_{AB}$) are bounded by the TF_{50} and $[\mathrm{B}]_{t,max}$; the B–C *parameter* sums ($[\mathrm{C}]_t + K_{BC}$)] are bounded by $[\mathrm{B}]_{t,max}$ and the TI_{50}.

Under resolvable conditions, the parameter sums can provide simple approximations for the TI_{50} and TF_{50},

$$TF_{50} = K_{AB} + \frac{[A]_t}{2} \approx K_{AB} + [A]_t \quad \textbf{(S105)}$$

and

$$TI_{50} = \frac{2(K_{BC} + [C]_t)^2}{2K_{BC} + [C]_t} \approx K_{BC} + [C]_t. \quad \textbf{(S106)}$$

These estimates can be used to understand non-cooperative curves when dominance (see next section) breaks down.

B. Dominance and the Quadrant Model

The behavior of quadratic roots of the kind found in eq S61 can be simplified when either measurable parameter (e.g., $[A]_t$ or K_{AB}) is present in large excess over the other (*31*). For example, in binary equilibria (Figure S8A), when $[A]_t << K_{AB}$, the value of $[A]_t$ at half-maximal complex formation (i.e., the EC_{50}) approximates the binding constant (Figure S8BI). Indeed, consideration of such limiting behavior forms the conceptual basis of many mathematical models used in enzyme kinetics and pharmacology (e.g., Michealis-Menton, Hill). When $[A]_t >> K_{AB}$, on the other hand, the saturation point can be used to determine the stoichiometry of a complex (Figure S8BII). In general, when one parameter is greater than the other by more than an order of magnitude, the larger parameter tends to dominate the behavior of ϕ_{AB}, giving rise to the binding curves in Figure S8B.

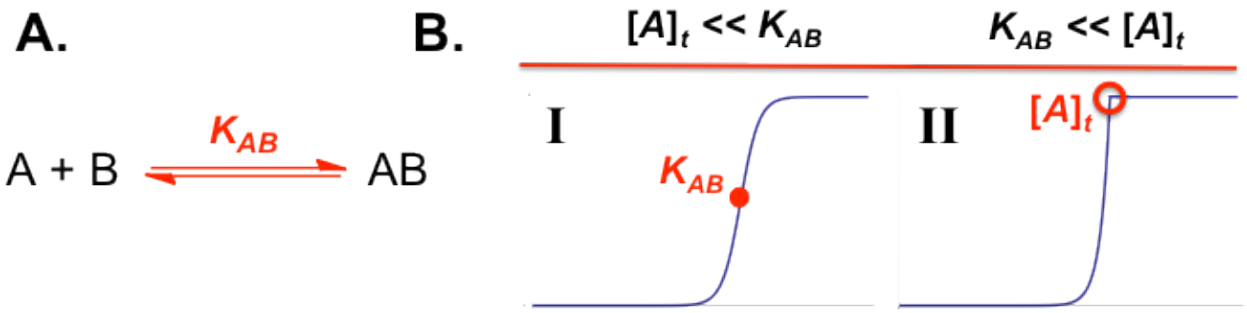


Figure S8. Physical Framework for Binary Binding Equilibria (A) General equilibrium scheme describing binary binding equilibria. **(B)** The shape of binary binding curves can be understood in terms of the larger binding parameters K_{AB} or $[A]_t$. When K_{AB} is dominant (I) it approximates the position of the half-saturation point (closed circle) of the curve, as is assumed in Langmuir and Hill models. When $[A]_t$ is dominant (II) it approximates the saturation point (open circle) of the curve. The latter conditions have been used classically to determine binding stoichiometry.

The assumption of "dominance" applied to ternary complex formation gives rise to 4 limiting scenarios (Figure S9), which can be arrayed in quadrants as follows: $[A]_t << K_{AB}$, $[C]_t << K_{BC}$ (Quadrant I); $K_{AB} << [A]_t$, $[C]_t << K_{BC}$ (Quadrant II); $[A]_t << K_{AB}$, $K_{BC} << [C]_t$ (Quadrant III); and $K_{AB} << [A]_t$, $K_{BC} << [C]_t$ (Quadrant IV). Expressions for the TF_{50} and TI_{50} correspond to values of $[B]_t$ leading to half-maximal ternary complex concentration on the left and right sides of binding curves, respectively. Note that, by convention, we assign A and its respective K_d to be the terminal component for which the quantity $K_{AB} + [A]_t$ is less than or equal to the quantity $K_{BC} + [C]_t$. Under both irresolvable conditions in general and resolvable conditions in particular, the critical values describing non-cooperative curves are summarized in Table S1.

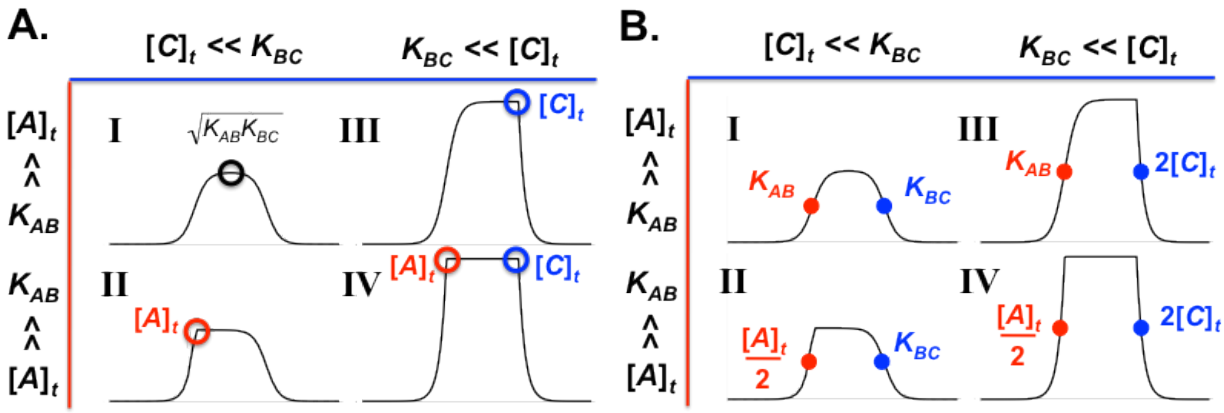


Figure S9. A. The equations for $[B]_{t,max}$ (open circles) are simple functions of the dominant parameters, which are insensitive to the presence or absence of resolvability. **B.** The equations for the TF_{50} and TI_{50} (closed circles) are simple functions of the dominant parameters when both the assumptions of dominance and resolvability are made.

Table S1. Critical values for non-cooperative ternary equlibria assuming dominance without resolvability as well as both dominance and resolvability.

DOMINANCE ASSUMPTION ALONE:

	$[B]_{t,max}$	$[ABC]_{max}$	TF_{50}	TI_{50}
I	$\boxed{\sqrt{K_{AB}K_{BC}}}$	$\frac{[A]_t[C]_t}{\left(\sqrt{K_{AB}}+\sqrt{K_{BC}}\right)^2}$	$\frac{1}{2}\left(K_{AB}+4\sqrt{K_{AB}K_{BC}}+K_{BC}-\left(\sqrt{K_{AB}}+\sqrt{K_{BC}}\right)\sqrt{K_{AB}+6\sqrt{K_{AB}K_{BC}}+K_{BC}}\right)$	$\frac{1}{2}\left(K_{AB}+4\sqrt{K_{AB}K_{BC}}+K_{BC}+\left(\sqrt{K_{AB}}+\sqrt{K_{BC}}\right)\sqrt{K_{AB}+6\sqrt{K_{AB}K_{BC}}+K_{BC}}\right)$
II	$[A]_t$	$\frac{[A]_t[C]_t}{[A]_t+K_{BC}}$	$\frac{[A]_tK_{BC}}{[A]_t+2K_{BC}}$	$2[A]_t+K_{BC}$
III	$[C]_t$	$\frac{[A]_t[C]_t}{[C]_t+K_{AB}}$	$\frac{[C]_tK_{AB}}{[C]_t+2K_{AB}}$	$2[C]_t+K_{AB}$
IV	$[A]_t \rightarrow [C]_t$	$[A]_t$	$\frac{[A]_t}{2}$	$2[C]_t$

BOTH DOMINANCE AND RESOLVABILITY ASSUMPTIONS:

	$[B]_{t,max}$	$[ABC]_{max}$	TF_{50}	TI_{50}
I	$\sqrt{K_{AB}K_{BC}}$	$\frac{[A]_t[C]_t}{K_{BC}}$	K_{AB}	K_{BC}
II	$[A]_t$	$\frac{[A]_t[C]_t}{K_{BC}}$	$\frac{[A]_t}{2}$	K_{BC}
III	$[C]_t$	$[A]_t$	K_{AB}	$2[C]_t$
IV	$[A]_t \rightarrow [C]_t$	$[A]_t$	$\frac{[A]_t}{2}$	$2[C]_t$

Inspection of Table S1 reveals that the $[B]_{t,max}$ equations are simple and unaffected by the presence or absence of resolvability (Figure S9A). Expressions for TF_{50} and TI_{50} values, on the other hand, are quite complex under conditions of dominance alone, and simplify on application of resolvability conditions (Figure S9B). The error associated with each of these assumptions (dominance alone and both dominance/resolvability) are derived below.

Derivation Of Critical Parameter Derivations Assuming Dominance Only

The critical points for each quadrant were obtained by applying the limits associated with each quadrant to the general equations for $[B]_{t,max}$, $[ABC]_{max}$, the TF_{50}, and the TI_{50}, as follows.

$[B]_{t,max}$ Under Dominant Conditions

Starting from the general equation for $[B]_{t,max}$,

$$[B]_{t,max} = \frac{\sqrt{K_{BC}}}{\sqrt{K_{AB}}+\sqrt{K_{BC}}}([A]_t + K_{AB}) + \frac{\sqrt{K_{AB}}}{\sqrt{K_{AB}}+\sqrt{K_{BC}}}([C]_t + K_{BC}), \quad \textbf{(S34)}$$

application of each dominance assumption yields simplified forms, as follows. For **Quadrant I**,

$$\lim_{\substack{[A]_t \to 0 \\ [C]_t \to 0}} \frac{\sqrt{K_{BC}}}{\sqrt{K_{AB}}+\sqrt{K_{BC}}}([A]_t + K_{AB}) + \frac{\sqrt{K_{AB}}}{\sqrt{K_{AB}}+\sqrt{K_{BC}}}([C]_t + K_{BC}) = \sqrt{K_{AB}K_{BC}}. \quad \textbf{(S107)}$$

For **Quadrant II**,

$$\lim_{\substack{K_{AB} \to 0 \\ [C]_t \to 0}} \frac{\sqrt{K_{BC}}}{\sqrt{K_{AB}}+\sqrt{K_{BC}}}([A]_t + K_{AB}) + \frac{\sqrt{K_{AB}}}{\sqrt{K_{AB}}+\sqrt{K_{BC}}}([C]_t + K_{BC}) = [A]_t. \quad \textbf{(S108)}$$

For **Quadrant III**,

$$\lim_{\substack{[A]_t \to 0 \\ K_{BC} \to 0}} \frac{\sqrt{K_{BC}}}{\sqrt{K_{AB}}+\sqrt{K_{BC}}}([A]_t + K_{AB}) + \frac{\sqrt{K_{AB}}}{\sqrt{K_{AB}}+\sqrt{K_{BC}}}([C]_t + K_{BC}) = [C]_t. \quad \textbf{(S109)}$$

Note that in Quadrants II and III, $[B]_{t,max} = [A]_t$ and $[B]_{t,max} = [C]_t$, respectively (Figure S9BII-III). In **Quadrant IV**, the maximum is an indeterminate average of $[A]_t$ and $[C]_t$:

$$\lim_{\substack{K_{AB} \to 0 \\ K_{BC} \to 0}} \frac{\sqrt{K_{BC}}}{\sqrt{K_{AB}}+\sqrt{K_{BC}}}([A]_t + K_{AB}) + \frac{\sqrt{K_{AB}}}{\sqrt{K_{AB}}+\sqrt{K_{BC}}}([C]_t + K_{BC}) = \frac{0}{0}[A]_t + \frac{0}{0}[C]_t. \quad \textbf{(S110)}$$

Because the Quadrant IV assumptions provide no insight into the values of K_{AB} and K_{BC}, $[B]_{t,max}$ can occur anywhere between $[A]_t$ and $[C]_t$, as shown in the following analysis:

$$\lim_{K_{AB} \to 0} \frac{\sqrt{K_{BC}}}{\sqrt{K_{AB}}+\sqrt{K_{BC}}}([A]_t + K_{AB}) + \frac{\sqrt{K_{AB}}}{\sqrt{K_{AB}}+\sqrt{K_{BC}}}([C]_t + K_{BC}) = [A]_t, \quad \textbf{(S111)}$$

and

$$\lim_{K_{BC} \to 0} \frac{\sqrt{K_{BC}}}{\sqrt{K_{AB}}+\sqrt{K_{BC}}}([A]_t + K_{AB}) + \frac{\sqrt{K_{AB}}}{\sqrt{K_{AB}}+\sqrt{K_{BC}}}([C]_t + K_{BC}) = [C]_t. \quad \textbf{(S112)}$$

$[ABC]_{max}$ Under Dominant conditions

Quadrant I. Starting from the general equation for $[ABC]_{max}$ in non-cooperative systems,

$$[ABC]_{max}^2 - [ABC]_{max}\left([A]_t + [C]_t + \left(\sqrt{K_{AB}}+\sqrt{K_{BC}}\right)^2\right) + [A]_t[C]_t = 0, \quad \textbf{(S113)}$$

for Quadrant I systems that the height of $[ABC]_{max} << [A]_t$ (this is based on the TPF, discussed in Section 5), and therefore, that the $[ABC]^2_{max}$ term in eq S113 can be disregarded, resulting in

$$-[ABC]_{max}\left([A]_t + [C]_t + \left(\sqrt{K_{AB}} + \sqrt{K_{BC}}\right)^2\right) + [A]_t[C]_t = 0. \quad \textbf{(S114)}$$

K_d values are much greater than concentrations, $[A]_t$ and $[C]_t$, are expected to make little contribution to the $[ABC]_{max}$ coefficient, and can be removed, resulting in

$$-[ABC]_{max}\left(\left(\sqrt{K_{AB}} + \sqrt{K_{BC}}\right)^2\right) + [A]_t[C]_t = 0. \quad \textbf{(S115)}$$

Solving this for $[ABC]_{max}$ yields

$$[ABC]_{max} = \frac{[A]_t[C]_t}{\left(\sqrt{K_{AB}} + \sqrt{K_{BC}}\right)^2}. \quad \textbf{(S116)}$$

Quadrant II. Based on TPF (see Section 5), the assumption that $[ABC]_{max} << [A]_t$ also holds for Quadrant II. Thus, we begin with eq S114,

$$-[ABC]_{max}\left([A]_t + [C]_t + \left(\sqrt{K_{AB}} + \sqrt{K_{BC}}\right)^2\right) + [A]_t[C]_t = 0, \quad \textbf{(S114)}$$

and make the additional assumption in Quadrant II that K_{AB} and $[C]_t$ make minimal contributions to the coefficient of the $[ABC]_{max}$ term, yielding

$$-[ABC]_{max}\left([A]_t + K_{BC}\right) + [A]_t[C]_t = 0. \quad \textbf{(S117)}$$

Solving this equation for $[ABC]_{max}$ affords

$$[ABC]_{max} = \frac{[A]_t[C]_t}{[A]_t + K_{BC}}. \quad \textbf{(S118)}$$

Quadrant III. Given the Quadrant III assumption that $[A]_t << [C]_t$, and because $[ABC]_{max}$ must be less than or equal to $[A]_t$ (the limiting reagent) by the law of mass action, the $[ABC]^2_{max}$ cannot appreciably contribute to the left hand side of eq S113, again affording

$$-[ABC]_{max}\left([A]_t + [C]_t + \left(\sqrt{K_{AB}} + \sqrt{K_{BC}}\right)^2\right) + [A]_t[C]_t = 0. \quad \textbf{(S114)}$$

Furthermore, based on the Quadrant III assumptions, K_{BC} and $[A]_t$ should make minimal contribution to the coefficient of the $[ABC]_{max}$ term, which yields

$$-[ABC]_{max}\left([C]_t + K_{AB}\right) + [A]_t[C]_t = 0. \quad \textbf{(S119)}$$

and solving this equation for $[ABC]_{max}$ yields

$$[ABC]_{max} = \frac{[A]_t[C]_t}{[C]_t + K_{AB}}. \quad \textbf{(S120)}$$

Quadrant IV. In this quadrant, the concentration of $[ABC]_{max}$ can be comparable to $[A]_t$ and $[C]_t$, so the $[ABC]^2_{max}$ term must be maintained. The binding constants, however, do not appreciably contribute to the coefficient of the $[ABC]_{max}$ term under the assumptions of Quadrant IV, simplifying eq S113 to

$$[ABC]^2_{max} - [ABC]_{max}\left([A]_t + [C]_t\right) + [A]_t[C]_t = 0. \quad \textbf{(S121)}$$

Factoring this provides

$$\left([ABC]_{max} - [A]_t\right)\left([ABC]_{max} - [C]_t\right) = 0. \quad \textbf{(S122)}$$

The only physically reasonable solution for $[ABC]_{max}$ in this equation is the concentration of limiting reagent, which is $[A]_t$ by convention; thus

$$[ABC]_{max} = [A]_t. \tag{S123}$$

TF_{50} and TI_{50} Under Dominant conditions

To obtain equations for the TF_{50} and TI_{50} for each quadrant, we first set $\alpha = 1$ and solved eq S13 for $[B]_t$ to obtain an equation for $[B]_t$ as a function of [ABC]. By solving this equation with the quadratic formula, we obtained an expression for the left hand side of the curve (the subtracted root, containing the TF_{50}) and the right hand side of the curve (the summed root, containing the TI_{50}):

$$[B]_t = \frac{-[ABC]^4 - [A]_t^2[C]_t^2 + [ABC]^3\left([A]_t + [C]_t - K_{AB} + K_{BC}\right)}{2[ABC]\left([ABC] - [A]_t\right)\left([ABC] - [C]_t\right)} + \frac{[ABC][A]_t[C]_t\left([A]_t + [C]_t + K_{AB} + K_{BC}\right) - 2[ABC]^2\left([C]_t K_{AB} + [A]_t\left([C]_t + K_{BC}\right)\right)}{2[ABC]\left([ABC] - [A]_t\right)\left([ABC] - [C]_t\right)} \pm \frac{\sqrt{\begin{pmatrix} -[ABC]^4 - [A]_t^2[C]_t^2 + [ABC]^3\left([A]_t + [C]_t + K_{AB} + K_{BC}\right) + \\ [ABC][A]_t[C]_t\left([A]_t + [C]_t + K_{AB} + K_{BC}\right) - \\ 2[ABC]^2\left([C]_t K_{AB} + [A]_t\left([C]_t + K_{BC}\right)\right) \end{pmatrix}^2 - 4\left([ABC]\begin{pmatrix} -[C]_t\left([A]_t + K_{AB}\right) + \\ [ABC]\left([C]_t + K_{BC}\right) \end{pmatrix}\begin{pmatrix} [ABC]\left([A]_t + K_{AB}\right) - \\ [A]_t\left([C]_t + K_{BC}\right) \end{pmatrix}\right)\times [ABC]\left([ABC] - [A]_t\right)\left([ABC] - [C]_t\right)}}{2[ABC]\left([ABC] - [A]_t\right)\left([ABC] - [C]_t\right)} \tag{S124}$$

To simplify these equations using the dominance assumptions, we first substituted the quadrant-specific $[ABC]_{max}$ value (see Table S1) divided by two (to specify the half-maximal point) and then took the limit of these results as the non-dominant parameters approach zero.

Quadrant I.

$$TF_{50}^{QI} = \lim_{\substack{[ABC]\to\frac{[A]_t[C]_t}{2\left(\sqrt{K_{AB}}+\sqrt{K_{BC}}\right)^2} \\ [A]_t\to 0 \\ [C]_t\to 0}} \text{Eq S124(-)} \tag{S125}$$

After simplification, the limits of eq S124 taken above yield

$$TF_{50}^{QI} = \frac{1}{2}\left(K_{AB} + 4\sqrt{K_{AB}K_{BC}} + K_{BC} - \left(\sqrt{K_{AB}} + \sqrt{K_{BC}}\right)\sqrt{K_{AB} + 6\sqrt{K_{AB}K_{BC}} + K_{BC}}\right) \tag{S126}$$

$$TI_{50}^{QI} = \lim_{\substack{[ABC]\to\frac{[A]_t[C]_t}{2\left(\sqrt{K_{AB}}+\sqrt{K_{BC}}\right)^2} \\ [A]_t\to 0 \\ [C]_t\to 0}} \text{Eq S124(+)} \tag{S127}$$

After simplification, the limits of eq S124 taken above yield

$$TI_{50}^{QI} = \frac{1}{2}\left(K_{AB} + 4\sqrt{K_{AB}K_{BC}} + K_{BC} - \left(\sqrt{K_{AB}} + \sqrt{K_{BC}}\right)\sqrt{K_{AB} + 6\sqrt{K_{AB}K_{BC}} + K_{BC}}\right) \tag{S128}$$

Quadrant II.

$$TF_{50}^{QII} = \lim_{\substack{[ABC]\to\frac{[A]_t[C]_t}{2([A]_t+K_{BC})} \\ K_{AB}\to 0 \\ [C]_t\to 0}} \text{Eq S124(-)} \tag{S129}$$

$$TF_{50}^{QII} = \frac{[A]_t K_{BC}}{[A]_t + 2K_{BC}} \tag{S130}$$

$$TI_{50}^{QII} = \lim_{\substack{[ABC]\to\frac{[A]_t[C]_t}{2([A]_t+K_{BC})} \\ K_{AB}\to 0 \\ [C]_t\to 0}} \text{Eq S124(+)} \tag{S131}$$

$$TI_{50}^{QII} = 2[A]_t + K_{BC} \tag{S132}$$

Quadrant III.

$$TF_{50}^{QIII} = \lim_{\substack{[ABC]\to\frac{[A]_t[C]_t}{2([C]_t+K_{AB})} \\ [A]_t\to 0 \\ K_{BC}\to 0}} \text{Eq S124(-)} \tag{S133}$$

$$TF_{50}^{QIII} = \frac{[C]_t K_{AB}}{[C]_t + 2K_{AB}} \tag{S134}$$

$$TI_{50}^{QIII} = \lim_{\substack{[ABC]\to\frac{[A]_t[C]_t}{2([C]_t+K_{AB})} \\ [A]_t\to 0 \\ K_{BC}\to 0}} \text{Eq S124(+)} \tag{S135}$$

$$TI_{50}^{QIII} = 2[C]_t + K_{AB} \tag{S136}$$

Quadrant IV.

$$TF_{50}^{QIV} = \lim_{\substack{[ABC]\to\frac{[A]_t}{2} \\ K_{AB}\to 0 \\ K_{BC}\to 0}} \text{Eq S124(-)} \tag{S137}$$

$$TF_{50}^{QIV} = \frac{[A]_t}{2} \tag{S138}$$

$$TI_{50}^{QIV} = \lim_{\substack{[ABC]\to\frac{[A]_t}{2} \\ K_{AB}\to 0 \\ K_{BC}\to 0}} \text{Eq S124(+)} \tag{S139}$$

$$TI_{50}^{QIV} = 2[C]_t \tag{S140}$$

C. Combining Dominance and Resolvability Assumptions

Derivation Of Critical Points Assuming Both Dominance And Resolvability

The half-maximal critical points when a system is resolvable (Figure S6B, eqs S85 and S99) can be further simplified when dominance assumptions are made. When K_{AB} >> $[A]_t$ (Quadrants I and III),

$$TF_{50}^{K_{AB}>>[A]_t} = \lim_{[A]_t \to 0} K_{AB} + \frac{[A]_t}{2} = K_{AB}. \quad \textbf{(S141)}$$

When K_{AB} << $[A]_t$, as in Quadrants II and IV,

$$TF_{50}^{K_{AB}<<[A]_t} = \lim_{K_{AB} \to 0} K_{AB} + \frac{[A]_t}{2} = \frac{[A]_t}{2}. \quad \textbf{(S142)}$$

For the TI_{50}s under dominant and resolvable conditions, when K_{BC} >> $[C]_t$ (Quadrants I and II),

$$TI_{50}^{K_{BC}>>[C]_t} = \lim_{[C]_t \to 0} \frac{2(K_{BC} + [C]_t)^2}{2K_{BC} + [C]_t} = K_{BC}. \quad \textbf{(S143)}$$

Finally, when K_{BC} << $[C]_t$ (Quadrants III and IV),

$$TI_{50}^{K_{BC}<<[C]_t} = \lim_{K_{BC} \to 0} \frac{2(K_{BC} + [C]_t)^2}{2K_{BC} + [C]_t} = 2[C]_t. \quad \textbf{(S144)}$$

Conceptual Framework In Terms Of Dominant Points

Combining both dominance and resolvability assumptions yields the simplified framework shown in Figure S10, which combines expressions for half-maximal points with simplified $[B]_{t,max}$ expressions to understand each side of the curve in terms of its dominant critical parameter. Interestingly, each quadrant directly parallels the how binary equilibria are understood, such that dominant parameters alone can be used to infer critical values. When binding constants are dominant, they represent the half-maximum, either the TF_{50} or TI_{50}. When concentrations are dominant, they represent the maximum, which can be an inflection point. Though Quadrant I does not have a dominant concentration, its maximum can be expressed as a simple function of its binding constants..

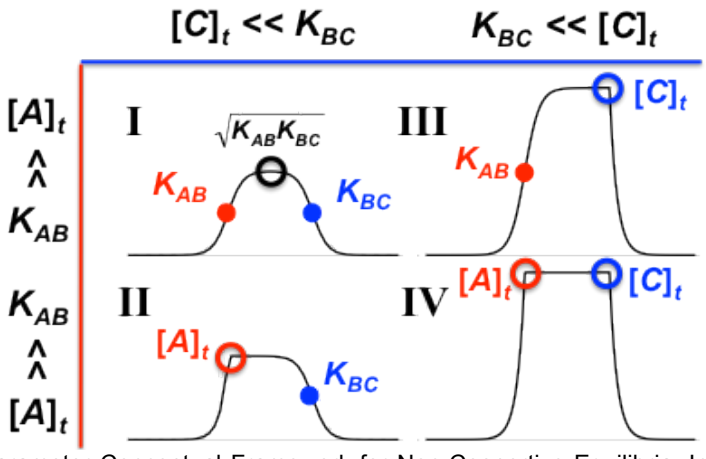


Figure S10. Dominant Parameter Conceptual Framework for Non-Coopertive Equilibria. In non-cooperative ternary complex equilibria, the K_d's approximate the TF_{50} and/or TF_{50}'s and the concentrations template the $[B]_{t,max}$ point(s).

D. Error In The Noncooperative Framework When The Resolvability Assumption Does Not Hold

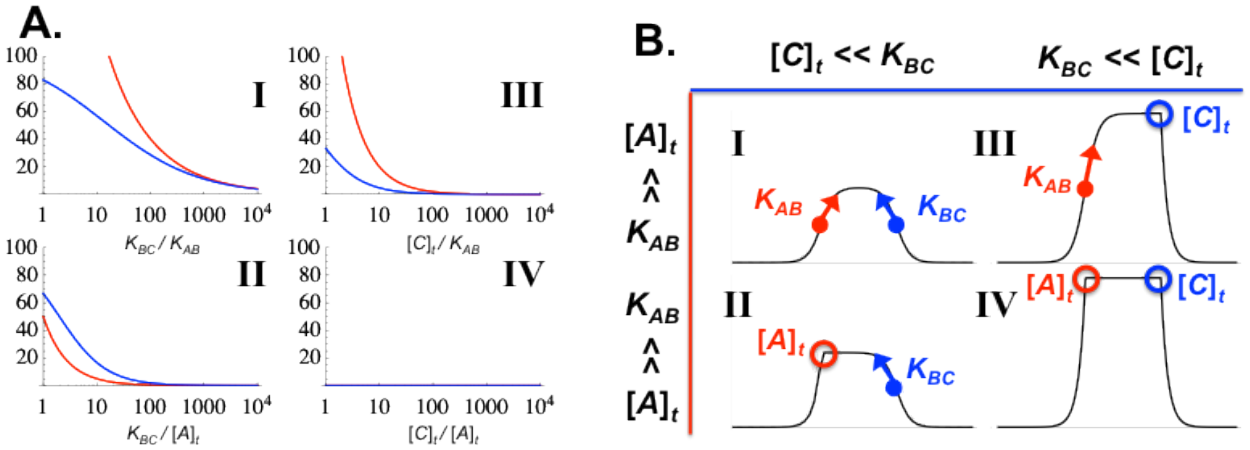


Figure S11. **(A)** Percent error of TF_{50} (red) and TI_{50} (blue) approximations from Figure S10 as a function of the ratio of the dominant parameters (here equivalent to the ratio of the parameter sums) for each Quadrant. **(B)** A physical picture of how resolvability breakdown affects the roles of the dominant parameters in the noncooperative framework (Figure S10): dominant K_d's coalesce into the maximum while dominant concentrations remain at the maximum.

Presented below is a general treatment of the error in utilizing the expressions derived assuming both dominance and resolvability (Figure S10, Table S1) when the resolvability assumption does not hold. Figure S11A, which plots the results of this analysis, shows that the largest resolvability-based error occurs when binding constants are dominant, whereas the least error occurs when concentrations are dominant. For this reason, Quadrant I conditions appear to require a difference of approximately two orders of magnitude to assure resolvability, Quadrants II and III conditions require one order of magnitude, and Quadrant IV is always resolvable.

The behavior of the dominant parameters under increasingly non-resolvable conditions is described in Figure S11B. Dominant concentrations are not affected by the resolvability assumption and consistently approximate $[B]_{t,max}$. Dominant K_d's, however, begin to coalesce into the maximum as the conditions become increasingly irresolvable. This can be understood in terms of the bounds of the parameter sums, which have been shown to lie between the $[B]_{t,max}$ and the TF_{50} or TI_{50} (Figure S7).

The relative error associated with the physical picture in Figure S10 when the resolvability assumption does not hold can be calculated by dividing the difference between the irresolvable and resolvable TF_{50} or TI_{50} from Table S1 by the irresolvable TF_{50} or TI_{50} (here the irresolvable values represent the "true" values, when only dominance is assumed):

$$TF_{50}^{R}\ \text{Error} = \frac{\left|TF_{50}(\text{Dominance}) - TF_{50}(\text{Dominance} + \text{Resolvability})\right|}{TF_{50}(\text{Dominance})} \qquad \textbf{(S145)}$$

and

$$TI_{50}^{R}\ \text{Error} = \frac{\left|TI_{50}(\text{Dominance}) - TI_{50}(\text{Dominance} + \text{Resolvability})\right|}{TI_{50}(\text{Dominance})}. \qquad \textbf{(S146)}$$

As detailed below, these equations were rearranged to define the relative error as a function of the ratio of larger dominant term (K_{BC} or $[C]_t$ by convention) over the smaller dominant term (K_{AB} or $[A]_t$ by convention).

Quadrant I.

The Quadrant I error associated with resolvability can be defined as

$$\mathrm{TF}_{50}^{\mathrm{R}}\ \mathrm{Error} = \frac{K_{AB} - \frac{1}{2}\left(K_{AB} + 4\sqrt{K_{AB}K_{BC}} + K_{BC} - \left(\sqrt{K_{AB}} + \sqrt{K_{BC}}\right)\sqrt{K_{AB} + 6\sqrt{K_{AB}K_{BC}} + K_{BC}}\right)}{\frac{1}{2}\left(K_{AB} + 4\sqrt{K_{AB}K_{BC}} + K_{BC} - \left(\sqrt{K_{AB}} + \sqrt{K_{BC}}\right)\sqrt{K_{AB} + 6\sqrt{K_{AB}K_{BC}} + K_{BC}}\right)}. \quad \textbf{(S147)}$$

Subtracting terms in the numerator and multiplying both the numerator and the denominator by $1/K_{AB}$ yields

$$\mathrm{TF}_{50}^{\mathrm{R}}\ \mathrm{Error} = \frac{\left(K_{AB} - 4\sqrt{K_{AB}K_{BC}} - K_{BC} + \left(\sqrt{K_{AB}} + \sqrt{K_{BC}}\right)\sqrt{K_{AB} + 6\sqrt{K_{AB}K_{BC}} + K_{BC}}\right)}{\left(K_{AB} + 4\sqrt{K_{AB}K_{BC}} + K_{BC} - \left(\sqrt{K_{AB}} + \sqrt{K_{BC}}\right)\sqrt{K_{AB} + 6\sqrt{K_{AB}K_{BC}} + K_{BC}}\right)} \times \frac{1/K_{AB}}{1/K_{AB}}. \quad \textbf{(S148)}$$

By distributing this fraction, we were able to obtain the relative error for the TF_{50} in Quadrant I in terms of the ratio of K_{BC}/K_{AB},

$$\mathrm{TF}_{50}^{\mathrm{R}}\ \mathrm{Error} = \frac{\left(1 - 4\sqrt{\frac{K_{BC}}{K_{AB}}} - \frac{K_{BC}}{K_{AB}} + \left(1 + \sqrt{\frac{K_{BC}}{K_{AB}}}\right)\sqrt{1 + 6\sqrt{\frac{K_{BC}}{K_{AB}}} + \frac{K_{BC}}{K_{AB}}}\right)}{\left(1 + 4\sqrt{\frac{K_{BC}}{K_{AB}}} + \frac{K_{BC}}{K_{AB}} - \left(1 + \sqrt{\frac{K_{BC}}{K_{AB}}}\right)\sqrt{1 + 6\sqrt{\frac{K_{BC}}{K_{AB}}} + \frac{K_{BC}}{K_{AB}}}\right)}. \quad \textbf{(S149)}$$

In an exactly analogous manner, we were able to obtain the error in the TI_{50} of Quadrant I, defined as

$$\mathrm{TI}_{50}^{\mathrm{R}}\ \mathrm{Error} = \frac{\frac{1}{2}\left(K_{AB} + 4\sqrt{K_{AB}K_{BC}} + K_{BC} + \left(\sqrt{K_{AB}} + \sqrt{K_{BC}}\right)\sqrt{K_{AB} + 6\sqrt{K_{AB}K_{BC}} + K_{BC}}\right) - K_{BC}}{\frac{1}{2}\left(K_{AB} + 4\sqrt{K_{AB}K_{BC}} + K_{BC} + \left(\sqrt{K_{AB}} + \sqrt{K_{BC}}\right)\sqrt{K_{AB} + 6\sqrt{K_{AB}K_{BC}} + K_{BC}}\right)}, \quad \textbf{(S150)}$$

which can be rearranged to

$$\mathrm{TI}_{50}^{\mathrm{R}}\ \mathrm{Error} = \frac{\left(1 + 4\sqrt{\frac{K_{BC}}{K_{AB}}} - \frac{K_{BC}}{K_{AB}} + \left(1 + \sqrt{\frac{K_{BC}}{K_{AB}}}\right)\sqrt{1 + 6\sqrt{\frac{K_{BC}}{K_{AB}}} + \frac{K_{BC}}{K_{AB}}}\right)}{\left(1 + 4\sqrt{\frac{K_{BC}}{K_{AB}}} + \frac{K_{BC}}{K_{AB}} + \left(1 + \sqrt{\frac{K_{BC}}{K_{AB}}}\right)\sqrt{1 + 6\sqrt{\frac{K_{BC}}{K_{AB}}} + \frac{K_{BC}}{K_{AB}}}\right)}. \quad \textbf{(S151)}$$

Quadrant II.

The error associated with resolvability in Quadrant II can be simplified to a simple expression in terms of $K_{BC}/[A]_t$ for both the TF_{50},

$$\mathrm{TF}_{50}^{\mathrm{R}}\ \mathrm{Error} = \frac{\frac{[A]_t}{2} - \frac{[A]_t K_{BC}}{[A]_t + 2K_{BC}}}{\frac{[A]_t K_{BC}}{[A]_t + 2K_{BC}}} = \frac{[A]_t\left([A]_t + 2K_{BC}\right) - 2[A]_t K_{BC}}{2[A]_t K_{BC}} = \frac{[A]_t}{2K_{BC}} = \frac{1}{2K_{BC}/[A]_t}, \quad \textbf{(S152)}$$

as well as the TI_{50},

$$TI_{50}^{R}\ \text{Error} = \frac{2[A]_t + K_{BC} - K_{BC}}{2[A]_t + K_{BC}} = \frac{2[A]_t}{2[A]_t + K_{BC}} = \frac{2}{2 + K_{BC}/[A]_t}. \quad \textbf{(S153)}$$

Quadrant III.

The resolvability-associated error in Quadrant III can be expressed in terms of the ratio of the dominant parameters ($[C]_t/K_{AB}$) for both the TF_{50},

$$TF_{50}^{R}\ \text{Error} = \frac{K_{AB} - \dfrac{[C]_t K_{AB}}{[C]_t + 2K_{AB}}}{\dfrac{[C]_t K_{AB}}{[C]_t + 2K_{AB}}} = \frac{K_{AB}\left([C]_t + 2K_{AB}\right) - 2[C]_t K_{AB}}{2[C]_t K_{AB}} = \frac{2K_{AB}}{[C]_t} = \frac{2}{2[C]_t/K_{AB}}, \quad \textbf{(S154)}$$

and the TI_{50},

$$TI_{50}^{R}\ \text{Error} = \frac{2[C]_t + K_{AB} - 2[C]_t}{2[C]_t + K_{AB}} = \frac{K_{AB}}{2[C]_t + K_{AB}} = \frac{1}{2[C]_t/K_{AB} + 1}. \quad \textbf{(S155)}$$

Quadrant IV.

The error associated with the resolvability assumption in Quadrant IV systems is zero for both the TF_{50},

$$TF_{50}^{R}\ \text{Error} = \frac{\dfrac{[A]_t}{2} - \dfrac{[A]_t}{2}}{\dfrac{[A]_t}{2}} = 0, \quad \textbf{(S156)}$$

and the TI_{50},

$$TI_{50}^{R}\ \text{Error} = \frac{2[C]_t - 2[C]_t}{2[C]_t} = 0. \quad \textbf{(S157)}$$

E. Error in the Noncooperative Framework When the Dominance Assumption Does Not Hold

As detailed below, Figure S12A plots the general expressions for the error associated with using the simple critical parameter expressions derived using both the dominance and resolvability assumptions (Figure S10, Table S1) when the dominance assumption does not hold. In all quadrants, a difference between a K_d and concentration only has to be an order of magnitude for the dominance assumption to remain valid, and the error associated using the Figure S10 framework when dominance is not present is less pronounced than when resolvability assumptions are not satisfied.

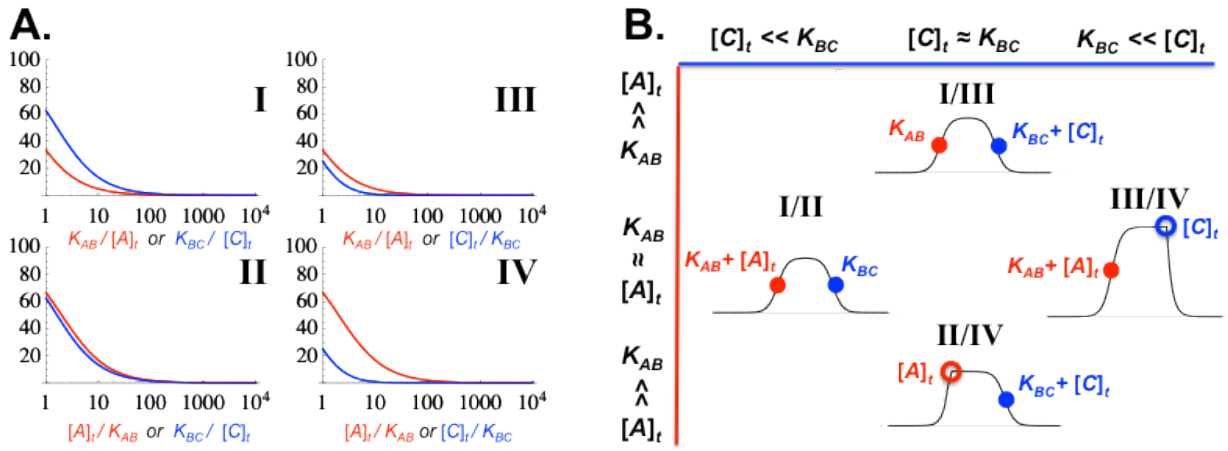


Figure S12. (A) Percent error of TF_{50} (red) and TI_{50} (blue) approximations from Figure S10 as a function of the ratio of the parameters associated with each binding event. **(B)** A physical picture of how the breakdown of dominance affects the roles of the dominant parameters in the noncooperative framework (Figure S10): for each side of the curve where dominance breaks down, the TF_{50} or TI_{50} can be approximated via the parameter sum rather than a particular dominant parameter.

The behavior of non-cooperative curves under non-dominant conditions can be qualitatively described as being between quadrants (Figure S12B). For example, when the parameters for the B-C binding event are similar ($K_{BC} \approx [C]_t$), but the binding constant for the A–B binding event is dominant ($K_{AB} >> [A]_t$), the conditions of the system are between Quadrants I and III. When there is no dominance for one binding event/side of the curve, then its respective TF_{50}'s or TI_{50}'s can be approximated by the parameter sum (see resolvable section above).

The error associated with using the expressions in Figure S10 when the dominance assumption does not hold can be defined by dividing the difference between the resolvable TF_{50} or TI_{50} obtained from Table S1 and the respective "purely resolvable" expression from Figure S6B divided by that same respective "purely resolvable" TF_{50} or TI_{50}. Alternatively stated,

$$TF_{50}^{D}\ \text{Error} = \frac{\left|TF_{50}(\text{Resolvability}) - TF_{50}(\text{Dominance} + \text{Resolvability})\right|}{TF_{50}(\text{Resolvability})} \quad \textbf{(S158)}$$

and

$$TI_{50}^{D}\ \text{Error} = \frac{\left|TI_{50}(\text{Resolvability}) - TI_{50}(\text{Dominance} + \text{Resolvability})\right|}{TI_{50}(\text{Resolvability})}. \quad \textbf{(S159)}$$

These error expressions equations can be analyzed with respect to relative dominance of parameters by rearranging them to define the relative error as a function of the ratio of the larger term in the parameter sum (e.g., for the TF_{50}: $[A]_t$ or K_{AB}) over the smaller term in the parameter sum. This was done for both possibilities for TF_{50} and TI_{50}; these general error treatments are presented below.

Quadrant I & III TF_{50}^{D} (assumes $K_{AB} >> [A]_t$):

$$TF_{50}^{D}\ \text{Error} = \frac{(K_{AB} + [A]_t/2) - K_{AB}}{K_{AB} + [A]_t/2} = \frac{[A]_t/2}{K_{AB} + [A]_t/2} = \frac{1}{2K_{AB}/[A]_t + 1}. \quad \textbf{(S160)}$$

Quadrant II & IV TF_{50}^{D} (assumes $[A]_t >> K_{AB}$):

$$TF_{50}^{D}\ \text{Error} = \frac{(K_{AB} + [A]_t/2) - [A]_t/2}{K_{AB} + [A]_t/2} = \frac{K_{AB}}{K_{AB} + [A]_t/2} = \frac{2}{2 + [A]_t/K_{AB}}. \quad \textbf{(S161)}$$

Quadrant I & II TI_{50}^{D} (assumes $K_{BC} >> [C]_t$):

$$TI_{50}^{D}\ \text{Error} = \frac{\dfrac{2(K_{BC} + [C]_t)^2}{2K_{BC} + [C]_t} - K_{BC}}{\dfrac{2(K_{BC} + [C]_t)^2}{2K_{BC} + [C]_t}} = \frac{2(K_{BC} + [C]_t)^2 - K_{BC}(2K_{BC} + [C]_t)}{2(K_{BC} + [C]_t)^2}$$

$$= \frac{3K_{BC}[C]_t + 2[C]_t^2}{2K_{BC}^2 + 4K_{BC}[C]_t + 2[C]_t^2} = \frac{3\dfrac{K_{BC}}{[C]_t} + 2}{2\left(\dfrac{K_{BC}}{[C]_t}\right)^2 + 4\dfrac{K_{BC}}{[C]_t} + 2}. \quad \textbf{(S162)}$$

Quadrant III & IV TI_{50}^{D} (assumes $[C]_t >> K_{BC}$):

$$TI_{50}^{D}\ \text{Error} = \frac{\dfrac{2(K_{BC} + [C]_t)^2}{2K_{BC} + [C]_t} - 2[C]_t}{\dfrac{2(K_{BC} + [C]_t)^2}{2K_{BC} + [C]_t}} = \frac{2(K_{BC} + [C]_t)^2 - 2[C]_t(2K_{BC} + [C]_t)}{2(K_{BC} + [C]_t)^2}$$

$$= \frac{2K_{BC}^2}{2K_{BC}^2 + 4K_{BC}[C]_t + 2[C]_t^2} = \frac{2}{2 + 4\dfrac{[C]_t}{K_{BC}} + 2\left(\dfrac{[C]_t}{K_{BC}}\right)^2}. \quad \textbf{(S163)}$$

F. When Both Dominance and Resolvability Assumptions Cannot be Made

When neither the dominance nor resolvability assumptions can be applied to a system, the shape of non-cooperative curves cannot be described using simple rules based on the roles of the parameters. Under these circumstances, all that can be known is that the parameter sums occur between the TF_{50} and TI_{50} and $[B]_{t,max}$:

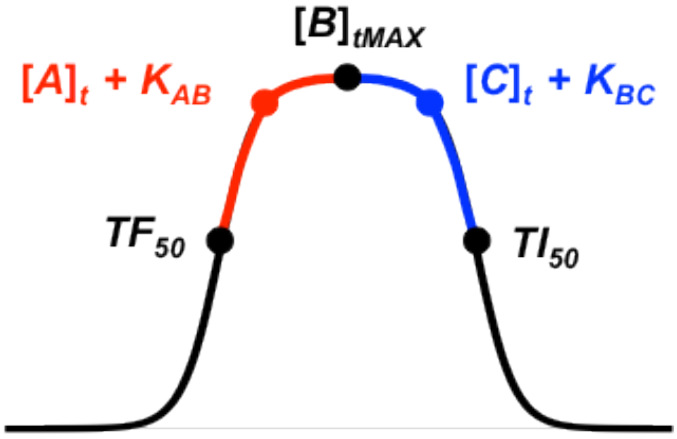


Figure S7. See above.

Because non-dominant, non-resolvable cases cannot be simply conceptualized, it becomes necessary to use the complete equation for non-cooperative curves (eq S61) to describe their behavior. Fortunately, conditions where there is no resolvability and no dominance in a system's paramters equate to circumstances where all the parameters approximately equal ($K_{AB} \approx [A]_t \approx K_{BC} \approx [C]_t$). Statistically, such circumstances are unlikely, and for most literature analyzed herein both the resolvability and dominance assumptions hold, at least approximately.

Section 6. Cooperative Ternary Systems

A. **Cooperative Quadrant Overview**

As summarized and then derived below we have determined general simple expressions for the critical points $[ABC]_{max}$, $[B]_{t,max}$, TF_{50} and TI_{50} under cooperative conditions. These expressions are very useful because the capture the essential information contained in ternary complex bell-shaped curve: the height ($[ABC]_{max}$), the position($[B]_{t,max}$), and the width(TF_{50} and TI_{50}).

Simplifying these expressions for the 4 different sets of relative conditions of a system allows one to understand these expressions, which elaborate the non-cooperative intuitive framework, derived above. Overall we reduce 4 very complex equations to 16 very simple equations divided into 4 sets of system conditions (quadrants) and 2 cooperative regimes ($\alpha > \alpha_{crit}$, $\alpha < \alpha_{crit}$).

Table S2. Critical values for cooperative ternary equilibria assuming dominance without resolvability.

WIDTH PERTURBATION REGIME ($\alpha > \alpha_{crit}$):

Q	$[B]_{t,max}$	$[ABC]_{max}$	TF_{50}	TI_{50}
I	$\sqrt{K_{AB}K_{BC}}$	$\frac{\alpha[A]_t[C]_t}{\alpha[X]_t + K_{BC}}$	$\frac{[L]_t}{2} + \frac{K_{AB}}{\alpha / \alpha_{crit}}$	$\frac{\alpha}{\alpha_{crit}} K_{BC}$
II	$[A]_t$	$\frac{\alpha[A]_t[C]_t}{\alpha[X]_t + K_{BC}}$	$\frac{[A]_t}{2}$ when $A \equiv L$; use Eq. S181 when $C \equiv L$	$\frac{\alpha}{\alpha_{crit}} K_{BC}$
III	$[C]_t$	$\frac{\alpha[A]_t[C]_t}{\alpha[C]_t + K_{weak}}$	$\frac{[A]_t}{2} + \frac{K_{AB}}{\alpha}$	$[C]_t(1+\alpha)$
IV	$[A]_t \rightarrow [C]_t$	$[A]_t$	$\frac{[A]_t}{2} + \frac{K_{AB}}{\alpha}$	$[C]_t(1+\alpha)$

HEIGHT PERTURBATION REGIME ($\alpha > \alpha_{crit}$):

Q	$[B]_{t,max}$	$[ABC]_{max}$	TF_{50}	TI_{50}
I	$\sqrt{K_{AB}K_{BC}}$	$\frac{\alpha[A]_t[C]_t}{K_{BC}}$	K_{AB}	K_{BC}
II	$[A]_t$	$\frac{\alpha[A]_t[C]_t}{K_{BC}}$	$\frac{[A]_t}{2}$	K_{BC}
III	$[C]_t$	$\frac{\alpha[A]_t[C]_t}{K_{weak}}$	$\frac{[C]_t}{4}\left(1+\frac{2\sqrt{K_{AB}}}{\sqrt{K_{AB}}+\sqrt{K_{BC}}} - \sqrt{1+\frac{4\sqrt{K_{AB}K_{BC}}}{\left(\sqrt{K_{AB}}+\sqrt{K_{BC}}\right)^2}}\right)$	$\frac{[C]_t}{4}\left(1+\frac{2\sqrt{K_{AB}}}{\sqrt{K_{AB}}+\sqrt{K_{BC}}} + \sqrt{1+\frac{4\sqrt{K_{AB}K_{BC}}}{\left(\sqrt{K_{AB}}+\sqrt{K_{BC}}\right)^2}}\right)$
IV	$[A]_t \rightarrow [C]_t$	$\frac{\alpha[A]_t[C]_t}{K_{weak}}$	$\frac{[C]_t}{4}\left(1+\frac{2\sqrt{K_{AB}}}{\sqrt{K_{AB}}+\sqrt{K_{BC}}} - \sqrt{1+\frac{4\sqrt{K_{AB}K_{BC}}}{\left(\sqrt{K_{AB}}+\sqrt{K_{BC}}\right)^2}}\right)$	$\frac{[C]_t}{4}\left(1+\frac{2\sqrt{K_{AB}}}{\sqrt{K_{AB}}+\sqrt{K_{BC}}} + \sqrt{1+\frac{4\sqrt{K_{AB}K_{BC}}}{\left(\sqrt{K_{AB}}+\sqrt{K_{BC}}\right)^2}}\right)$

Quadrant I Overview

When α = 1 (Orange curves, Figure S13A–D), Quadrant I systems do not form appreciable ternary complex because $\alpha < \alpha_{crit}$. With negative and positive cooperativity, the height scales linearly with cooperativity while the curve shape remains unchanged (red, cyan, green and blue curve, Figure S13A–D). With sufficient positive cooperativity ($\alpha > \alpha_{crit}$), however, the height of the cure begins to plateau (Figure S13B and C) and the width begins to scale with cooperativity (Figure S13B and D). The TF_{50} will inversely scale with cooperativity until $[L]_t/2$ is approached .

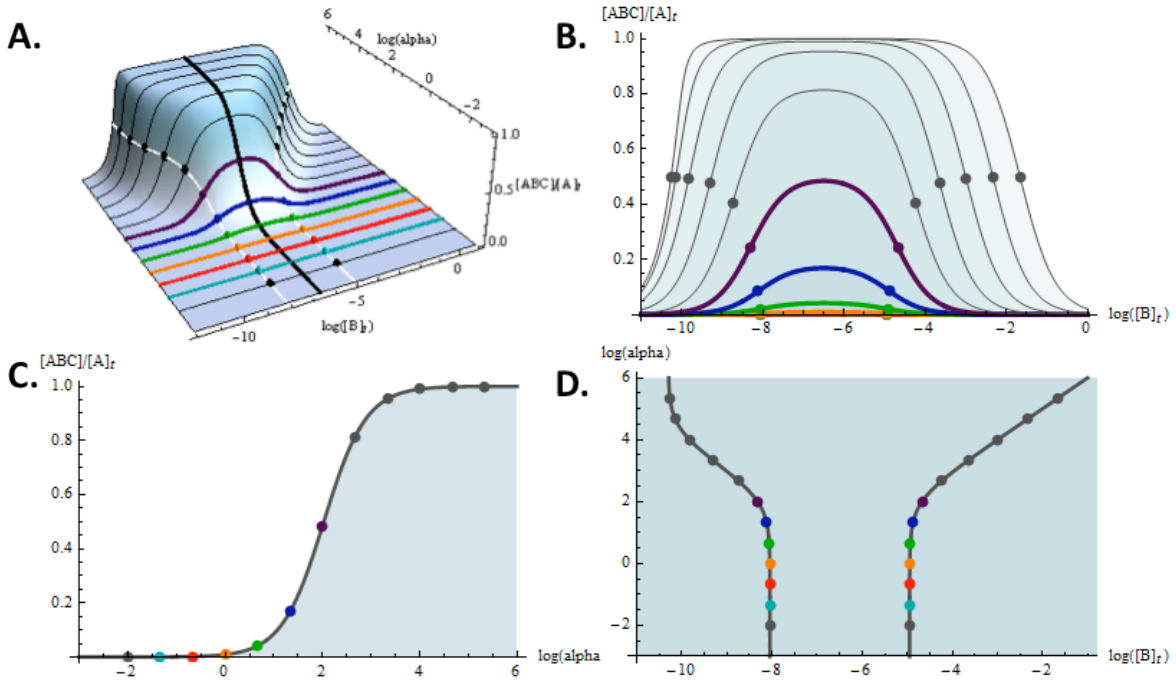


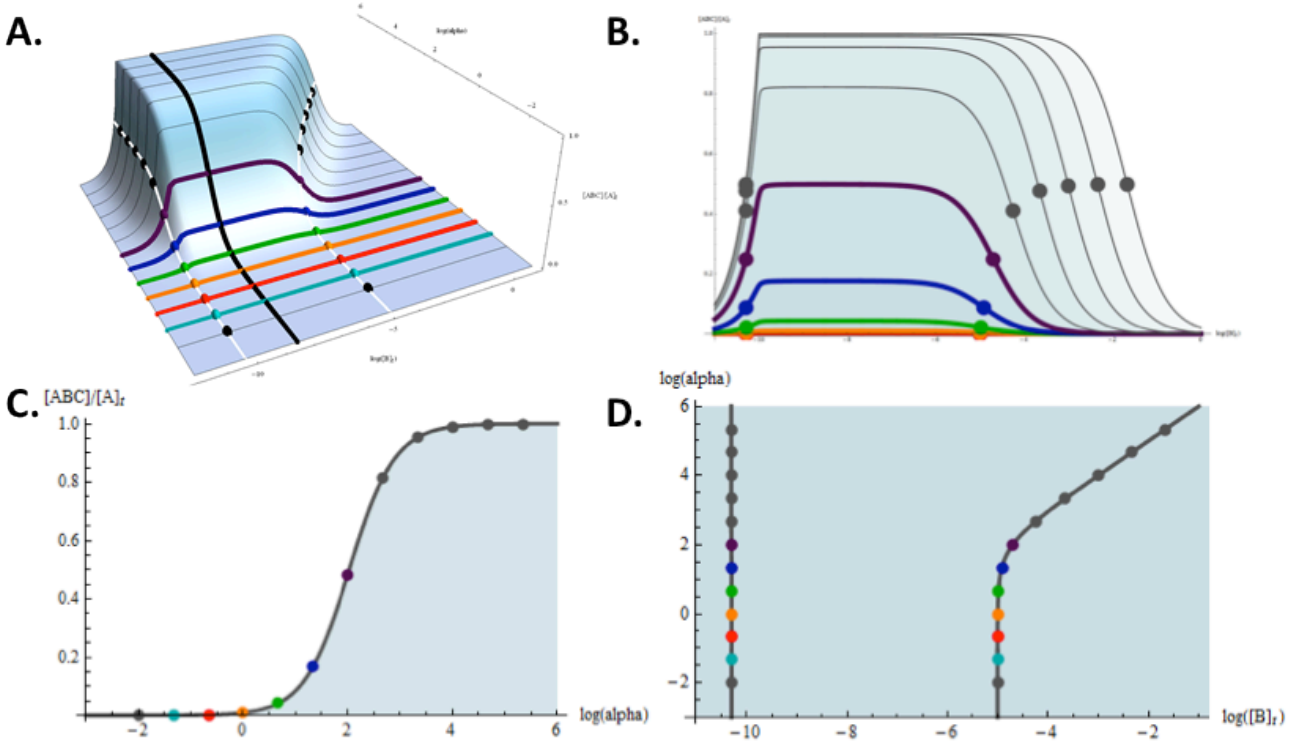
Figure S13. (A) Additional dimension (z-axis) of cooperativity (α) overlaid on top of non-cooperative Quadrant I (orange line) curve (i.e. $[ABC]_{max}$ (y-axis) vs $[B]_t$ (x-axis)). **(B)** The front view of (A) shows the dual width/height effect of cooperativity and the transition between them (when $\alpha \approx \alpha_{crit}$). **(C)** The side view of (A) isolates the height effect of cooperativity in terms of the TPF. **(D)** The top view of (A) isolates the width effect of cooperativity in terms of TF_{50} and TI_{50}. (A assumed to be the limiting reagent in this simulation)

Table S3. Critical values for cooperative ternary equlibria in Quadrant I.

$[B]_{t,max}$	$[ABC]_{max}$	TF_{50}	TI_{50}
WIDTH PERTURBATION REGIME ($\alpha > \alpha_{crit}$):			
$\sqrt{K_{AB}K_{BC}}$	$\frac{\alpha[A]_t[C]_t}{\alpha[X]_t + K_{BC}}$	$\frac{[L]_t}{2} + \frac{K_{AB}}{\alpha / \alpha_{crit}}$	$\frac{\alpha}{\alpha_{crit}} K_{BC}$
HEIGHT PERTURBATION REGIME ($\alpha < \alpha_{crit}$):			
$\sqrt{K_{AB}K_{BC}}$	$\frac{\alpha[A]_t[C]_t}{K_{BC}}$	K_{AB}	K_{BC}
NON-COOPERATIVE ($\alpha = 1$):			
$\sqrt{K_{AB}K_{BC}}$	$\frac{\alpha[A]_t[C]_t}{K_{BC}}$	K_{AB}	K_{BC}

Quadrant II Overview

When α = 1 (Orange curves, Figure S14A–D), Quadrant II systems do not form appreciable ternary complex because $\alpha < \alpha_{crit}$. With negative and positive cooperativity, the height scales linearly with cooperativity while the curve shape remains unchanged (red, cyan, green and blue curves, Figure S14A–D). With sufficient positive cooperativity (once $\alpha > \alpha_{crit}$), however, the height of the cure begins to plateau (Figure S14B and C) and the width begins to scale with cooperativity (Figure S14B and D). If $[L]_t = [A]_t$, then the TF_{50} is a constant throughout (shown). If $[L]_t = [C]_t$, then the TF_{50} will inversely scale with cooperativity until $[C]_t/2$ is approached (not shown).



Fig, S14. (A) Additional dimension (z-axis) of cooperativity (α) overlaid on top of non-cooperative Quadrant II (orange line) curve (i.e. $[ABC]_{max}$ (y-axis) vs $[B]_t$ (x-axis)). **(B)** The front view of (A) shows the dual width/height effect of cooperativity and the transition between them (when $\alpha \approx \alpha_{crit}$). **(C)** The side view of (A) isolates the height effect of cooperativity in terms of the TPF. **(D)** The top view of (A) isolates the width effect of cooperativity in terms of TF_{50} and TI_{50}. (A assumed to be the limiting reagent in this simulation)

Table S4. Critical values for cooperative ternary equilibria in Quadrant II.

$[B]_{t,max}$	$[ABC]_{max}$	TF_{50}	TI_{50}
		WIDTH PERTURBATION REGIME ($\alpha > \alpha_{crit}$):	
$[A]_t$	$\frac{\alpha[A]_t[C]_t}{\alpha[X]_t + K_{BC}}$	$\frac{[A]_t}{2}$ when A ≡ L; use Eq. S181 when C ≡ L	$\frac{\alpha}{\alpha_{crit}} K_{BC}$
		HEIGHT PERTURBATION REGIME ($\alpha < \alpha_{crit}$):	
$[A]_t$	$\frac{\alpha[A]_t[C]_t}{K_{BC}}$	$\frac{[A]_t}{2}$	K_{BC}
		NON-COOPERATIVE ($\alpha = 1$):	
$\sqrt{K_{AB}K_{BC}}$	$\frac{\alpha[A]_t[C]_t}{K_{BC}}$	$\frac{[A]_t}{2}$	K_{BC}

Quadrant III Overview

When α = 1 (orange curves, Figure S15A–D), Quadrant III systems form near quantitative ternary complex since α > α_{crit}. With positive cooperativity, the height does not change appreciably while the TI_{50} scales linearly with α and the TF_{50} scales inversely with α until $[A]_t/2$ is approached (green, blue, and purple curves; Figure S15A–D). With negative cooperativity, however, the width and height both decrease until α < α_{crit}, at which point the TF_{50} and TI_{50} plateau at S200 while the height scales linearly with cooperativity (red and cyan curves Figure S15A–D).

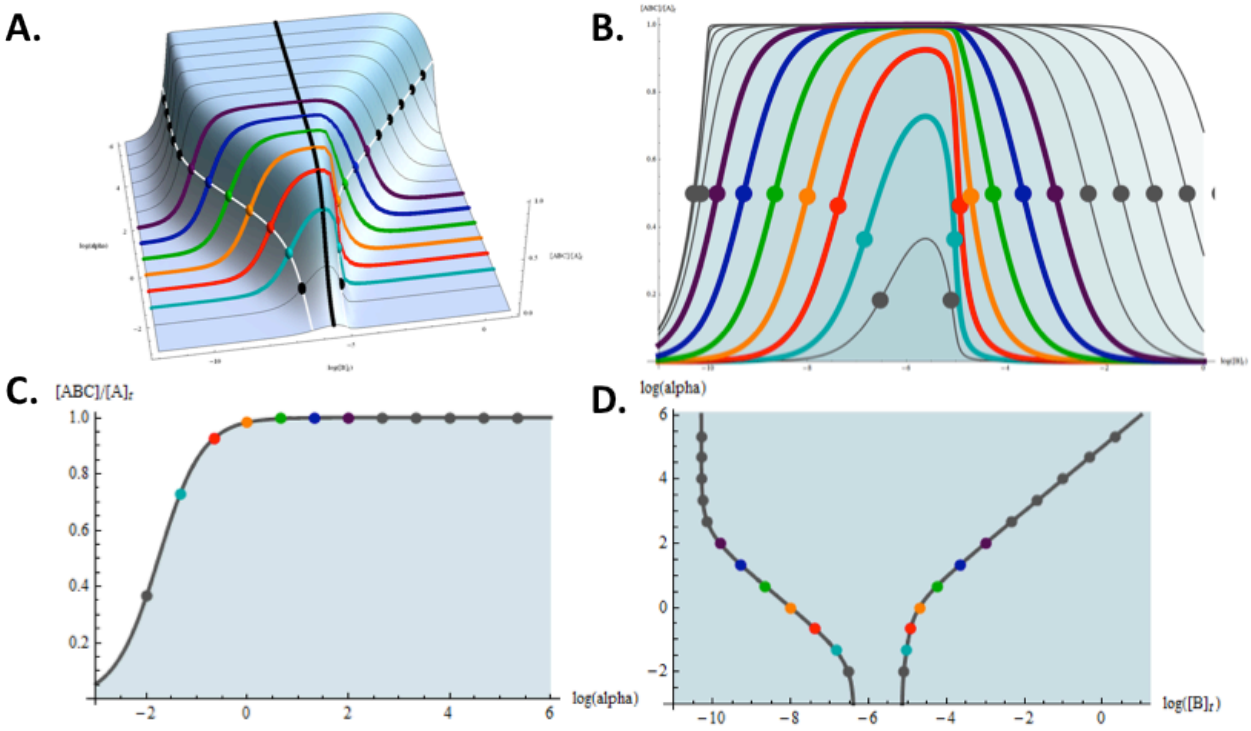


Figure S15. (A) Additional dimension (z-axis) of cooperativity (α) overlaid on top of non-cooperative Quadrant III (orange line) curve (i.e. $[ABC]_{max}$ (y-axis) vs $[B]_t$(x-axis)). **(B)** The front view of (A) shows the dual width/ height effect of cooperativity and the transition between them (when α ≈ α_{crit}). **(C)** The side view of (A) isolates the height effect of cooperativity in terms of the TPF. **(D)** The top view of (A) isolates the width effect of cooperativity in terms of TF_{50} and TI_{50}. (A is the limiting reagent in this quadrant)

Table S5. Critical values for Cooperative ternary equilibria in Quadrant III.

$[B]_{t,max}$	$[ABC]_{max}$	TF_{50}	TI_{50}
		WIDTH PERTURBATION REGIME (α > α_{crit}):	
$[C]_t$	$\frac{\alpha[A]_t[C]_t}{\alpha[C]_t + K_{weak}}$	$\frac{[A]_t}{2} + \frac{K_{AB}}{\alpha}$	$[C]_t(1+\alpha)$
		NON-COOPERATIVE (α = 1):	
$[C]_t$	$\frac{\alpha[A]_t[C]_t}{\alpha[C]_t + K_{weak}}$	K_{AB}	$2[C]_t$
		HEIGHT PERTURBATION REGIME (α < α_{crit}):	
$[C]_t$	$\frac{\alpha[A]_t[C]_t}{K_{weak}}$	$\frac{[C]_t}{4}\left(1+\frac{2\sqrt{K_{AB}}}{\sqrt{K_{AB}}+\sqrt{K_{BC}}}-\sqrt{1+\frac{4\sqrt{K_{AB}K_{BC}}}{(\sqrt{K_{AB}}+\sqrt{K_{BC}})^2}}\right)$	$\frac{[C]_t}{4}\left(1+\frac{2\sqrt{K_{AB}}}{\sqrt{K_{AB}}+\sqrt{K_{BC}}}+\sqrt{1+\frac{4\sqrt{K_{AB}K_{BC}}}{(\sqrt{K_{AB}}+\sqrt{K_{BC}})^2}}\right)$

Quadrant IV Overview

When α = 1 (orange curves, Figure S16A–D), Quadrant IV systems form quantitative ternary complex. With positive cooperativity, the height and TF_{50} do not change while the TI_{50} scales linearly with α (green, blue, and purple curves; Figure S16A–D). With negative cooperativity, however, the width and height both decrease until $\alpha < \alpha_{crit}$, at which the TF_{50} and TI_{50} plateau at S200 while the height scales linearly with cooperativity (red and cyan curves; Figure S16A–D).

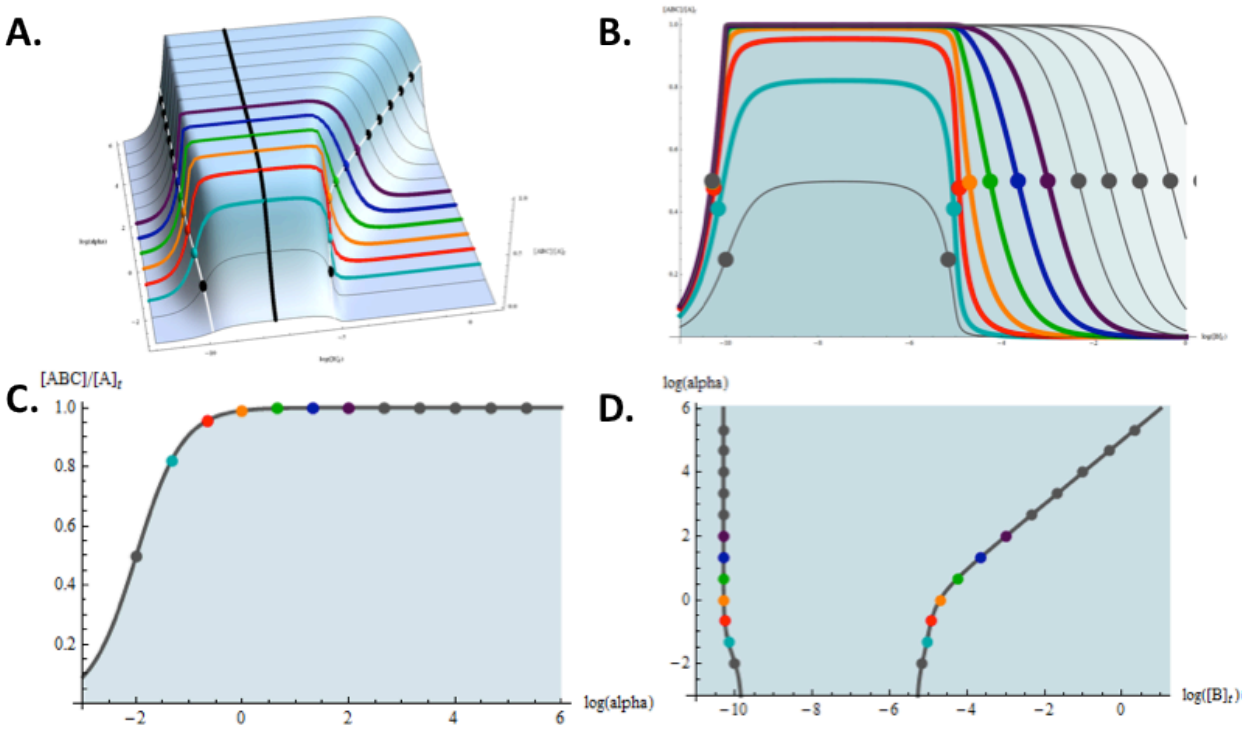


Figure S16. (A) Additional dimension (z-axis) of cooperativity (α) overlaid on top of non-cooperative Quadrant IV (orange line) curve (i.e. $[ABC]_{max}$ (y-axis) vs $[B]_t$(x-axis)). **(B)** The front view of (A) shows the dual width/ height effect of cooperativity and the transition between them (when $\alpha \approx \alpha_{crit}$). **(C)** The side view of (A) isolates the height effect of cooperativity in terms of the TPF. **(D)** The top view of (A) isolates the width effect of cooperativity in terms of TF_{50} and TI_{50}. (A is the limiting reagent in this quadrant)

Table S6. Critical values for Cooperative ternary equilibria in Quadrant IV.

$[B]_{t,max}$	$[ABC]_{max}$	TF_{50}	TI_{50}
WIDTH PERTURBATION REGIME (α > αcrit):			
$[A]_t \rightarrow [C]_t$	$[A]_t$	$\frac{[A]_t}{2}$	$[C]_t(1+\alpha)$
NON-COOPERATIVE (α = 1):			
$[A]_t \rightarrow [C]_t$	$[A]_t$	$\frac{[A]_t}{2}$	$2[C]_t$
HEIGHT PERTURBATION REGIME (α < αcrit):			
$[A]_t \rightarrow [C]_t$	$\frac{\alpha[A]_t[C]_t}{K_{weak}}$	$\frac{[C]_t}{4}\left(1+\frac{2\sqrt{K_{AB}}}{\sqrt{K_{AB}}+\sqrt{K_{BC}}}-\sqrt{1+\frac{4\sqrt{K_{AB}K_{BC}}}{(\sqrt{K_{AB}}+\sqrt{K_{BC}})^2}}\right)$	$\frac{[C]_t}{4}\left(1+\frac{2\sqrt{K_{AB}}}{\sqrt{K_{AB}}+\sqrt{K_{BC}}}+\sqrt{1+\frac{4\sqrt{K_{AB}K_{BC}}}{(\sqrt{K_{AB}}+\sqrt{K_{BC}})^2}}\right)$

B. General Solution to Cooperative Ternary Equilibria

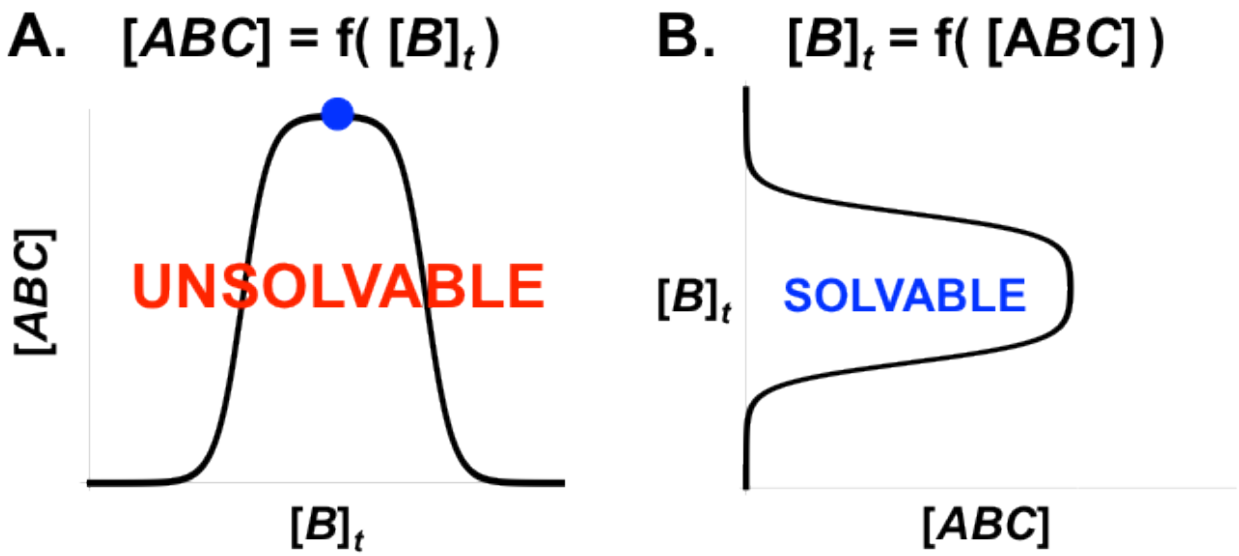


Figure S17. Algebraic solvability depends on the "direction" of a given function. (**A)** When defined as a function of $[B]_t$, [ABC] is algebraically unsolvable, and its value can only be defined generally at $[B]_{t,max}$ (blue dot). (**B)** Because $[B]_t$ can be expressed as a function of [ABC], and we can also express [ABC] at any point as a fraction of $[ABC]_{max}$, we can frame an analytical solution for cooperative ternary equilibria "backwards" (i.e., for $[B]_t$ as a function of $[ABC]_{max}$).

Though we have demonstrated that the cooperative ternary complex cannot directly be solved for algebraically (Figure S17A), the unsolvable cooperative [ABC] quintic polynomial eq S13 can be rearranged by collecting the $[B]_t$ terms, yielding a quadratic polynomial in $[B]_t$, which can be solved to yield

$$[B]_t = \frac{1}{2}\left(\frac{[A]_t + [C]_t - K_{AB} - K_{BC} + \frac{\alpha([ABC]-[A]_t)([ABC]-[C]_t)}{[ABC]} + \frac{[A]_t(K_{BC}-K_{AB})}{\alpha([ABC]-[A]_t)} + \frac{[C]_t(K_{AB}-K_{BC})}{\alpha([ABC]-[C]_t)} \pm \left(\frac{\alpha}{[ABC]} + \frac{1}{[A]_t-[ABC]} + \frac{1}{[C]_t-[ABC]}\right)\sqrt{\begin{array}{l}(\alpha([ABC]-[A]_t)([ABC]-[C]_t)-[ABC]K_{AB})^2 - \\ 2[ABC]K_{BC}(\alpha([ABC]-[A]_t)([ABC]-[C]_t)+[ABC]K_{AB})+ \\ [ABC]^2K_{BC}{}^2\end{array}}}{\alpha}\right). \quad \textbf{(S164)}$$

Thus, the inverse case, where $[B]_t$ is a function of [ABC], is soluble, because algebraic insolubility is inherently directional (Figure S17). Each root of eq S164 reflects an expression for one side of the cooperative bell-shaped curve; the subtracted form represents the left side of the curve and the summed form the right side. Because we know the range of [ABC]s generally for every ternary complex curve (0 to $[ABC]_{max}$), we can also obtain a $[B]_t$ value for each point of the curve. By obtaining a value for $[ABC]_{max}$ in a given curve, dividing it by two, and inserting it into eq S164, the TF_{50} and TI_{50} can be obtained for any cooperative curve. Of course, this method will also yield the value of any fractional $[ABC]_{max}$ value, and is the method used to create the Excel sheet associated with this paper.

C. Understanding the Height of the Cooperative Ternary Complex Curves

For ternary systems in which autoinhibition begins at concentrations of $[B]_t$ below those at which the limiting terminal species is fully saturated, a high proportion of A or C will not participate in ternary complex, even at the curve maximum ($[B]_t = [B]_{t,\max}$). The fraction of the limiting reagent that participates in ternary complex at $[B]_{t,\max}$ for a given set of starting parameters is expressed by the ratio $[ABC]_{max}/[L]_t$, termed the ternary partition fraction, or TPF, where $[L]_t$ is the concentration of the *l*imiting terminal reagent (A or C). Similarly, $[X]_t$ represents the non-limiting, or e*x*cess terminal reagent (A or C). Because many of the cooperative ternary complex expressions rely on knowing at system's TPF, we have derived simple expressions to calculate it, both approximately and exactly.

Derivation of an Approximate TPF Expression

In order to understand what parameters have the greatest effect on the TPF, we first replace the terms in the $[ABC]_{max}$ second-order polynomial,

$$[ABC]_{max}^2 - [ABC]_{max}\left([A]_t + [C]_t + \frac{\left(\sqrt{K_{AB}} + \sqrt{K_{BC}}\right)^2}{\alpha}\right) + [A]_t[C]_t = 0, \quad \textbf{(S36)}$$

with relative concentration and binding constants (each of which is equivalent in Eq S36),

$$[ABC]_{max}^2 - [ABC]_{max}\left([X]_t + [L]_t + \frac{\left(\sqrt{K_{strong}} + \sqrt{K_{weak}}\right)^2}{\alpha}\right) + [X]_t[L]_t = 0, \quad \textbf{(S165)}$$

where K_{strong} and K_{weak} represent the smaller and larger binding constants, respectively. Assuming that $K_{weak} >> K_{strong}$ and that $[X]_t >> [L]_t$ simplifies the above equation to

$$[ABC]_{max}^2 - [ABC]_{max}\left([X]_t + \frac{K_{weak}}{\alpha}\right) + [X]_t[L]_t = 0. \quad \textbf{(S166)}$$

Recognizing that $[ABC]_{max}$ cannot be greater than $[L]_t$, and assuming that $[X]_t >> [L]_t$, the squared $[ABC]_{max}$ term can be dropped, simplifying the equation to

$$-[ABC]_{max}\left([X]_t + \frac{K_{weak}}{\alpha}\right) + [X]_t[L]_t = 0. \quad \textbf{(S167)}$$

Solving this for $[ABC]_{max}$ yields

$$[ABC]_{max} = \frac{[L]_t[X]_t}{[X]_t + K_{weak}/\alpha}. \quad \textbf{(S168)}$$

Dividing both sides by $[L]_t$ and rearranging gives

$$TPF \equiv \frac{[ABC]_{max}}{[L]_t} = \frac{\alpha}{\alpha + K_{weak}/[X]_t}, \quad \textbf{(S169)}$$

which shows the relationship between the ternary partition function and the parameters α, $[X]_t$, and K_{weak}. This equation implies that the TPF is maximal when $\alpha > K_{weak}/[X]_t$, minimal when $\alpha < K_{weak}/[X]_t$, and equal to ½ when $\alpha = K_{weak}/[X]_t$, as shown in Figure S18. Thus, cooperativity and the excess reagent are positively associated with the fractional saturation, whereas the larger binding constant (K_{weak}) is negatively correlated with the fractional saturation. This provides a simple means for understanding the TPF in non-cooperative systems ($\alpha = 1$),

where the TPF will be maximal only when $[X]_t >> K_{weak}$ (e.g., Quadrants III and IV) and minimal when $[X]_t << K_{weak}$ (e.g., Quadrants I and II). Cooperativity, then, must be less than 1 to decrease the TPF in QIII/QIV systems and much greater than 1 to increase the TPF of QI/II systems.

Notably, the simpler form of eq S169 is quite an accurate approximation of the general relationship between α and $[ABC]_{max}$; based on the expansion of $\left(\sqrt{K_{AB}}+\sqrt{K_{BC}}\right)^2$ to $K_{AB}+2\sqrt{K_{AB}K_{BC}}+K_{BC}$, its value can only range between K_{weak} (when $K_{weak} >> K_{strong}$) and $4K_{weak}$ (when $K_{weak} = K_{strong}$). Similarly, the concentration estimate will be accurate to within a factor of 2 because the actual value of $[X]_t - [L]_t/2$ can only range from $[X]_t$ (when $[X]_t >> [L]_t$) to $[X]_t/2$ (when $[X]_t = [L]_t$). Therefore, even when the simplifying assumptions applied to eq S174 do not hold, the estimate provided by eq S169 cannot be in error by more than one order of magnitude. To demonstrate this, the maximum error cases are all derived independently in Section 6E, below.

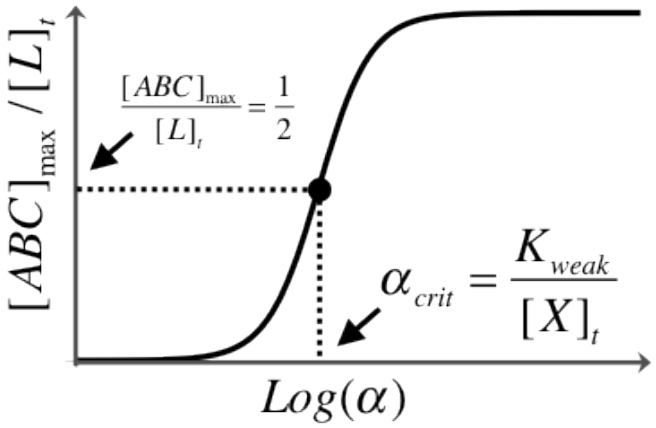


Figure S18. The height ($[ABC]_{max}$) of cooperative ternary complex bell-shaped curves can be conceptually understood using α_{crit} which defines the cooperativity at which $[ABC]_{max}$ is half maximal. Additionally, this term divides ternary complex behavior into two regimes where when $\alpha < \alpha_{crit}$ $[ABC]_{max}$ scales linearly with α and when $\alpha > \alpha_{crit}$ $[ABC]_{max}$ does not change greatly with α.

In general the effects of cooperativity can be understood in terms of the expression $\alpha_{crit} = K_{weak}/[X]_t$, where we define when $\alpha < \alpha_{crit}$ changes in cooperativity scale linearly with $[ABC]_{max}$ and when $\alpha > \alpha_{crit}$ the $[ABC]_{max}$ begins to plateau and cooperativity has very little effect on $[ABC]_{max}$. This simplification relies on their being both an excess reagent and weaker binding constant. We treat the error in eq 169 when these assumptions do not hold, and derive an exact expression for the α_{crit} in the next section.

Derivation of an Exact α_{crit} Expression

Equation S36 solved for $[ABC]_{max}$,

$$[ABC]_{max} = \frac{[A]_t + [C]_t + \frac{\left(\sqrt{K_{AB}}+\sqrt{K_{BC}}\right)^2}{\alpha} - \sqrt{\left([A]_t + [C]_t + \frac{\left(\sqrt{K_{AB}}+\sqrt{K_{BC}}\right)^2}{\alpha}\right)^2 - 4[A]_t[C]_t}}{2}, \quad \textbf{(S37)}$$

is similar to the form of the general solution for a two-component reversible binding interaction, such as

$$[\mathrm{RS}] = \frac{[\mathrm{R}]_t + [\mathrm{S}]_t + K_d - \sqrt{([\mathrm{R}]_t + [\mathrm{S}]_t + K_d)^2 - 4[\mathrm{R}]_t[\mathrm{S}]_t}}{2}, \quad \textbf{(S170)}$$

where R and S represent the *r*eceptor and *s*ubstrate. $[\mathrm{A}]_t$ and $[\mathrm{C}]_t$ in eq S37 correspond to $[\mathrm{R}]_t$ and $[\mathrm{S}]_t$, respectively, and the binary K_d corresponds to

$$\frac{\left(\sqrt{K_{AB}} + \sqrt{K_{BC}}\right)^2}{\alpha} \quad \textbf{(S171)}$$

in eq S37. As demonstrated in eqs S83-S85, at the half-maximal (or half-saturation) point in binary binding (i.e., $[\mathrm{RS}]/[\mathrm{R}]_t$),

$$[\mathrm{S}]_t = K_d + \frac{[\mathrm{R}]_t}{2}, \quad \textbf{(S85)}$$

which assumes that $[\mathrm{R}]_t$ is the limiting reagent. Collecting all the terms gives

$$\frac{[\mathrm{S}]_t - ([\mathrm{R}]_t/2)}{K_d} = 1, \quad \textbf{(S172)}$$

which holds for a binary binding system at the half-maximal point. The corresponding ratio for eq S37 replaces the K_d in the above equation with eq S171 and $[\mathrm{R}]_t$ and $[\mathrm{S}]_t$ with $[\mathrm{L}]_t$ and $[\mathrm{X}]_t$, respectively, resulting in

$$\frac{\alpha([\mathrm{X}]_t - [\mathrm{L}]_t/2)}{\left(\sqrt{K_{AB}} + \sqrt{K_{BC}}\right)^2} = 1. \quad \textbf{(S173)}$$

Because of the correspondence between eqs S37 and S170, the above equation is true when $[\mathrm{ABC}]_{max}/[\mathrm{L}]_t = 0.5$, just as Eq S172 is true when $[\mathrm{RS}]/[\mathrm{R}]_t = 0.5$. Thus, Eq S169 can be rewritten using this exact ratio,

$$\alpha_{crit} \equiv \frac{\left(\sqrt{K_{AB}} + \sqrt{K_{BC}}\right)^2}{([\mathrm{X}]_t - [\mathrm{L}]_t/2)} \quad \textbf{(S174)}$$

and this represents a general expression for the α_{crit} in that it does not require assumptions concerning the relative values of the concentrations or binding constants. If such assumptions are made, this equation reduces to eq S169.

D. Understanding the Width of the Cooperative Ternary Complex Curves

Calculating the TF_{50} the TI_{50} when $\alpha > \alpha_{crit}$

General TF_{50} Expression

The TPF approaches 1 when the product of α and $[X]_t$ is greater than K_{weak}. Thus, excess of either terminal species (Quadrants III/IV) or the product of α and $[X]_t$ (Quadrants I/II with high cooperativity) will result in a curve that is conceptually similar to Quadrants III and IV. In these quadrants, for all $[B]_t < [B]_{t,\max}$, nearly all bound limiting reagent is in ternary complex (we define limiting reagent as A in eqs S175–179 below, in order to relate them to the original system of equations, eqs S1–6). The ternary complex outcompetes the binary complex either because there is such an excess of the $[C]_t$ species (which would be equal to $[X]_t$ in these cases) or because cooperativity is so high that it partitions all bound species into ternary rather than binary complexes. Thus, [ABC] >> [AB] for $\alpha > \alpha_{crit}$ systems, and [AB] can be removed from eqs S1–6, yielding

$$[A]_t = [A] + [ABC], \quad \textbf{(S175)}$$

$$[B]_t = [B] + [BC] + [ABC], \quad \textbf{(S176)}$$

$$[C]_t = [C] + [BC] + [ABC], \quad \textbf{(S177)}$$

$$K_{BC} = \frac{[B][C]}{[BC]}, \quad \textbf{(S178)}$$

and

$$\frac{K_{AB}K_{BC}}{\alpha} = \frac{[A][B][C]}{[ABC]}. \quad \textbf{(S179)}$$

This system of equations can first be solved for $[B]_t$ and simplified by substituting $[L]_t/2$ for [ABC], which specifies the $[B]_t$ at which curve is half-maximal since $[ABC]_{max}$ approaches the limiting reagent, $[L]_t$, as the TPF nears 1. This simplifies the solution to

$$TF_{50}^{\alpha>\alpha_{crit}} = \frac{[L]_t}{2} + \frac{K_{Lim}}{\alpha} + \frac{K_{Lim}K_{XS}}{\alpha\left([X]_t - {[L]_t}/{2}\right) - K_{Lim}}. \quad \textbf{(S180)}$$

where K_{Lim} and K_{XS} are the binding constants associated with the limiting reagent and excess reagents, respectively. When the $\alpha > \alpha_{crit}$, then eq S173 must be greater than 1. Thus, the $\alpha\left([X]_t - {[L]_t}/{2}\right)$ term is greater than K_{Lim} and eq S180 can be simplified to

$$TF_{50}^{\alpha>\alpha_{crit}} = \frac{[L]_t}{2} + \frac{K_{Lim}}{\alpha} + \frac{K_{Lim}K_{XS}}{\alpha\left([X]_t - {[L]_t}/{2}\right)}. \quad \textbf{(S181)}$$

As α increases, the second and third terms will approach 0, and $\frac{[L]_t}{2}$ is the TF_{50} limit as $\alpha \to \infty$.

General TI_{50} Expression

When the $\alpha > \alpha_{crit}$, the TI_{50} must be greater than both K_{AB} and K_{BC} because 1) in systems where the largest parameter is a concentration (Quadrant III/IV-type systems), the TI_{50} is defined

by this concentration and it will be larger than either of the K_ds and 2) in systems where the largest parameter is a K_d, it will require pronounced cooperativity to achieve a high TPF (eq S169), which will widen the curve to the point where the cooperative TI_{50} is greater than the K_d that defined the TI_{50} in the non-cooperative curve. Because the TI_{50} occurs well beyond both K_ds, little free terminal species occur, as all A and C are bound into binary or ternary complexes. Thus, free terminal species can be ignored in eqs S1–6, resulting in the following system of equations

$$[A]_t = [AB] + [ABC], \tag{S182}$$

$$[B]_t = [B] + [AB] + [BC] + [ABC], \tag{S183}$$

$$[C]_t = [BC] + [ABC], \tag{S184}$$

and

$$\frac{1}{\alpha} = \frac{[AB][BC]}{[B][ABC]}. \tag{S185}$$

By solving these equations for $[B]_t$ in terms of measurable parameters and substituting $[L]_t/2$ to define $[B]_t$ as the half-maximal point, we obtain

$$TI_{50}^{\alpha>\alpha_{crit}} = \alpha\left([X]_t - \frac{[L]_t}{2}\right) + \left([X]_t + \frac{[L]_t}{2}\right). \tag{S186}$$

Quadrant I/II Simplification

In Quadrant I, eq S181 can be simplified to

$$TF_{50}^{\alpha>\alpha_{crit},QI/II} = \frac{[L]_t}{2} + \frac{K_{Lim}K_{Xs}}{\alpha([X]_t - [L]_t/2)} \tag{S187}$$

based on the fact that, under these conditions, $K_{Xs}/([X]_t\text{-}[L]_t/2) >> 1$. Additionally given the fact in this Quadrants $\alpha_{crit} \approx K_{weak}/([X]_t - [L]_t/2)$ and K_{weak} is equivalent to K_{BC}, we can simplify eq S187 to

$$TF_{50}^{\alpha>\alpha_{crit},QI/II} = \frac{[L]_t}{2} + \frac{K_{AB}}{\alpha / \alpha_{crit}}. \tag{S188}$$

Quadrant II simplification is more complicated. If A is the limiting reagent, the TF_{50} is equivalent to $[A]_t/2$, regardless of the amount of cooperativity. If C is the limiting reagent, then eq 181 must be used.

In Quadrants I and II, eq S186 can be simplified to

$$TI_{50}^{\alpha>\alpha_{crit},QI/II} = \alpha\left([X]_t - \frac{[L]_t}{2}\right) \tag{S189}$$

based on the fact that under these conditions $\alpha >> 1$. Additionally, given the fact in these Quadrants $\alpha_{crit} \approx K_{weak}/([X]_t - [L]_t/2)$ and K_{weak} is equivalent to K_{BC}, we can simplify eq S189 to

$$TI_{50}^{\alpha>\alpha_{crit},QI/II} = \frac{\alpha}{\alpha_{crit}}K_{BC}. \tag{S190}$$

Quadrant III/IV Simplification

Equation S181 can be simplified based on Quadrant III/IV assumptions that $[X]_t$ is at least one order of magnitude greater than either K_d or $[L]_t$. This implies that the last term must be at least one order of magnitude smaller than the second term. Additionally it is known that $[X]_t$ is equivalent to $[C]_t$, and by extension that $[L]_t$ is equivalent to $[A]_t$. Thus, eq S181 simplifies to

$$\mathrm{TF}_{50}^{\alpha>\alpha_{crit},\mathrm{QIII/IV}} = \frac{[\mathrm{A}]_t}{2} + \frac{K_{AB}}{\alpha}. \quad \textbf{(S191)}$$

In Quadrants III and IV, $[X]_t$ is $[C]_t$ and it is known that $[C]_t >> [A]_t$; thus, eq S186 simplifies to

$$\mathrm{TI}_{50}^{\alpha>\alpha_{crit},\mathrm{QIII/IV}} = [\mathrm{C}]_t\,(1+\alpha). \quad \textbf{(S192)}$$

Calculating the TF_{50} the TI_{50} when $\alpha < \alpha_{crit}$

As $\alpha \to 0$, the system approximates a competition equilibrium, where little [ABC] forms relative to free and binary species. In this system, the laws of mass action are the same as in the ternary complex model,

$$K_{AB} = \frac{[\mathrm{A}][\mathrm{B}]}{[\mathrm{AB}]}, \quad \textbf{(S4)}$$

$$K_{BC} = \frac{[\mathrm{B}][\mathrm{C}]}{[\mathrm{BC}]}, \quad \textbf{(S5)}$$

and

$$\frac{K_{AB}K_{BC}}{\alpha} = \frac{[\mathrm{A}][\mathrm{B}][\mathrm{C}]}{[\mathrm{ABC}]}, \quad \textbf{(S6)}$$

but the [ABC] term is removed from the conservation of mass equations,

$$[\mathrm{A}]_t = [\mathrm{A}] + [\mathrm{AB}], \quad \textbf{(S193)}$$

$$[\mathrm{B}]_t = [\mathrm{B}] + [\mathrm{AB}] + [\mathrm{BC}], \quad \textbf{(S194)}$$

and

$$[\mathrm{C}]_t = [\mathrm{C}] + [\mathrm{BC}]. \quad \textbf{(S195)}$$

Solving this system of equations for $[B]_t$ yields

$$[\mathrm{B}]_t = -\frac{1}{2[\mathrm{ABC}]\alpha^2[\mathrm{A}]_t[\mathrm{C}]_t}\left(\begin{array}{l} -[\mathrm{ABC}]\alpha^2[\mathrm{A}]_t{}^2[\mathrm{C}]_t - [\mathrm{ABC}]\alpha^2[\mathrm{A}]_t[\mathrm{C}]_t{}^2 - \alpha^3[\mathrm{A}]_t{}^2[\mathrm{C}]_t{}^2 + \\ [\mathrm{ABC}]^2\alpha[\mathrm{A}]_tK_{AB} - [\mathrm{ABC}]^2\alpha[\mathrm{C}]_tK_{AB} + [\mathrm{ABC}]\alpha^2[\mathrm{A}]_t[\mathrm{C}]_tK_{AB} - \\ [\mathrm{ABC}]^2\alpha[\mathrm{A}]_tK_{BC} + [\mathrm{ABC}]^2\alpha[\mathrm{C}]_tK_{BC} + [\mathrm{ABC}]\alpha^2[\mathrm{A}]_t[\mathrm{C}]_tK_{BC} \pm \\ \alpha\left([\mathrm{ABC}][\mathrm{A}]_t + [\mathrm{ABC}][\mathrm{C}]_t + \alpha[\mathrm{A}]_t[\mathrm{C}]_t\right)\sqrt{\begin{array}{l}\alpha^2[\mathrm{A}]_t{}^2[\mathrm{C}]_t{}^2 - 2[\mathrm{ABC}]\alpha[\mathrm{A}]_t[\mathrm{C}]_tK_{AB} + \\ [\mathrm{ABC}]^2K_{AB}{}^2 - 2[\mathrm{ABC}]\alpha[\mathrm{A}]_t[\mathrm{C}]_tK_{BC} - \\ 2[\mathrm{ABC}]^2K_{AB}K_{BC} + [\mathrm{ABC}]^2K_{BC}{}^2\end{array}} \end{array}\right). \quad \textbf{(S196)}$$

In order to constrain $[B]_t$ as a half maximal point, [ABC] must be substituted for an $[ABC]_{max}/2$ expression. Because $\alpha \to 0$ is a limiting condition, it can be used to simplify $[ABC]_{max}$ prior to substitution. As α approaches zero in the quadratic polynomial defining the magnitude of the maximum,

$$[\mathrm{ABC}]_{max}^2 - [\mathrm{ABC}]_{max}\left([\mathrm{A}]_t + [\mathrm{C}]_t + \frac{\left(\sqrt{K_{AB}} + \sqrt{K_{BC}}\right)^2}{\alpha}\right) + [\mathrm{A}]_t[\mathrm{C}]_t = 0, \quad \textbf{(S36)}$$

the $[ABC]_{max}^2$ term approaches zero most rapidly and can be ignored. In addition, the $[A]_t$ and $[C]_t$ terms contribute negligibly to the coefficient of $[ABC]_{max}$, simplifying the equation to

$$[\mathrm{ABC}]_{max}\left(\frac{\left(\sqrt{K_{AB}} + \sqrt{K_{BC}}\right)^2}{\alpha}\right) + [\mathrm{A}]_t[\mathrm{C}]_t = 0. \quad \textbf{(S197)}$$

$[ABC]_{max}$ can then be approximated in the $\alpha \to 0$ limit as

$$[ABC]_{max} = \frac{\alpha [A]_t [C]_t}{\left(\sqrt{K_{AB}} + \sqrt{K_{BC}}\right)^2} \tag{S198}$$

This $[ABC]_{max}$ expression divided by 2 can then be substituted into eq S196. After the limit of this expression is taken as $\alpha \to 0$, the result is

$$[B]_t^{\text{half-max}} = \frac{1}{4}\left(\begin{array}{l} 2[B]_{t,max} + [A]_t + [C]_t + 2\left(K_{AB} + K_{BC} + 3\sqrt{K_{AB}K_{BC}}\right) \pm \\ \left([A]_t + [C]_t + 2\left(\sqrt{K_{AB}} + \sqrt{K_{BC}}\right)^2\right)\sqrt{1 + \frac{4\sqrt{K_{AB}K_{BC}}}{(\sqrt{K_{AB}} + \sqrt{K_{BC}})^2}} \end{array} \right), \tag{S199}$$

where the difference is the TF_{50}, the sum is the TI_{50}, and $[B]_{t,max}$ is Eq 34. This can be simplified based on quadrant assumptions as shown below. In order to examine all the relationships present in this equation, $[B]_{t,max}$ should be replaced with the eq 34 expression. After rearranging, this yields

$$\begin{aligned} [B]_t^{\text{half-max}} = {} & [A]_t\left(\frac{1}{4} + \frac{\sqrt{K_{BC}}}{2\left(\sqrt{K_{AB}} + \sqrt{K_{BC}}\right)}\right) + [C]_t\left(\frac{1}{4} + \frac{\sqrt{K_{AB}}}{2\left(\sqrt{K_{AB}} + \sqrt{K_{BC}}\right)}\right) + \frac{1}{2}\left(\sqrt{K_{AB}} + \sqrt{K_{BC}}\right)^2 + \sqrt{K_{AB}K_{BC}} \pm \\ & \frac{1}{4}\left([A]_t + [C]_t + 2\left(\sqrt{K_{AB}} + \sqrt{K_{BC}}\right)^2\right)\sqrt{1 + \frac{4\sqrt{K_{AB}K_{BC}}}{\left(\sqrt{K_{AB}} + \sqrt{K_{BC}}\right)^2}}. \end{aligned} \tag{S200}$$

<u>Quadrant I Simplification</u>

The first two terms in eq S200 can be eliminated because they are multiplied by the non-dominant concentrations. In addition, the third term can be approximated as K_{BC}, assuming resolvability. Among the terms following the ± symbol, $[A]_t$ and $[C]_t$ can be ignored, and $\left(\sqrt{K_{AB}} + \sqrt{K_{BC}}\right)^2$ can be approximated as K_{BC}, again assuming resolvability. Thus, the simplified form of the half-maximal critical points using Quadrant I assumptions is

$$[B]_t^{\text{half-max}} = \sqrt{K_{AB}K_{BC}} + \frac{1}{2}K_{BC}\left(1 \pm \sqrt{1 + \frac{4K_{AB}}{\sqrt{K_{AB}K_{BC}}}}\right), \tag{S201}$$

where the difference is the TF_{50} and the sum the TI_{50}.

<u>Quadrant II Simplification</u>

Assuming that $K_{BC} >> K_{AB}$, the second term of eq S200 multiplied by $[A]_t$ becomes ½, whereas the second term multiplied by $[C]_t$ becomes 0. All $\left(\sqrt{K_{AB}} + \sqrt{K_{BC}}\right)^2$ terms can be approximated as K_{BC}, which implies that the second term under the radical can be neglected. Finally, the $\sqrt{K_{AB}K_{BC}}$ term can be eliminated. This results in

$$[B]_t^{\text{half-max}} = [A]_t\left(\frac{1}{4} + \frac{1}{2}\right) + \frac{[C]_t}{4} + \frac{1}{2}\sqrt{K_{BC}}^2 \pm \left(\frac{[A]_t}{4} + \frac{[C]_t}{4} + \frac{1}{2}\sqrt{K_{BC}}^2\right)\sqrt{1}, \tag{S202}$$

which simplifies to

$$TF_{50}^{\alpha < \alpha_{crit}, \text{QII}} = \frac{[A]_t}{2} \tag{S203}$$

and

$$TI_{50}^{\alpha<\alpha_{crit},QII} = [A]_t + \frac{[C]_t}{2} + K_{BC}. \quad \textbf{(S204)}$$

SQuadrant III and IV Simplification

In both Quadrants III and IV, $[C]_t$ is the excess reagent. Applying this assumption to eq S200 allows for the elimination of the first $[A]_t$ term as well as the third and fourth terms, since these will all be smaller than the second $[C]_t$ term. In addition, the $[A]_t$ and $\left(\sqrt{K_{AB}} + \sqrt{K_{BC}}\right)^2$ can be dropped from the coefficient of the radical for the same reason. This simplifies to

$$[B]_t^{\text{half-max}} = \frac{[C]_t}{4}\left(1 + \frac{2\sqrt{K_{AB}}}{\sqrt{K_{AB}} + \sqrt{K_{BC}}} \pm \sqrt{1 + \frac{4\sqrt{K_{AB}K_{BC}}}{(\sqrt{K_{AB}} + \sqrt{K_{BC}})^2}}\right), \quad \textbf{(S205)}$$

where the difference represents the TF_{50} and the sum the TI_{50}.

E. Maxium Error in TPF Approximation

Below we derive the expressions used calculate the maximum error for the TPF approximation above, which relate $[ABC]_{max}/[L]_t$ to $\alpha[X]_t/K_{weak}$ for all relative relationships of K_{weak} and K_{strong} and $[X]_t$ and $[L]_t$. In this manner we are able to generally describe the behavior of the TPF and show that the the maximal error associated with the $\alpha[X]_t/K_{weak}$ approximation is around a factor of 4–8. As can be seen in Figure S19 break down in the assumption that $K_{weak} >> K_{strong}$ leads to a small translation in the curve to the right (Figure S19, dashed purple curve), while breakdown in the assumption that $[X]_t >> [L]_t$ leads to a small change in the curve shape (Figure S19, dashed brown curve). Breakdown in both assumptions leads the both of these changes simultaneously (Figure S19, dotted green curve).

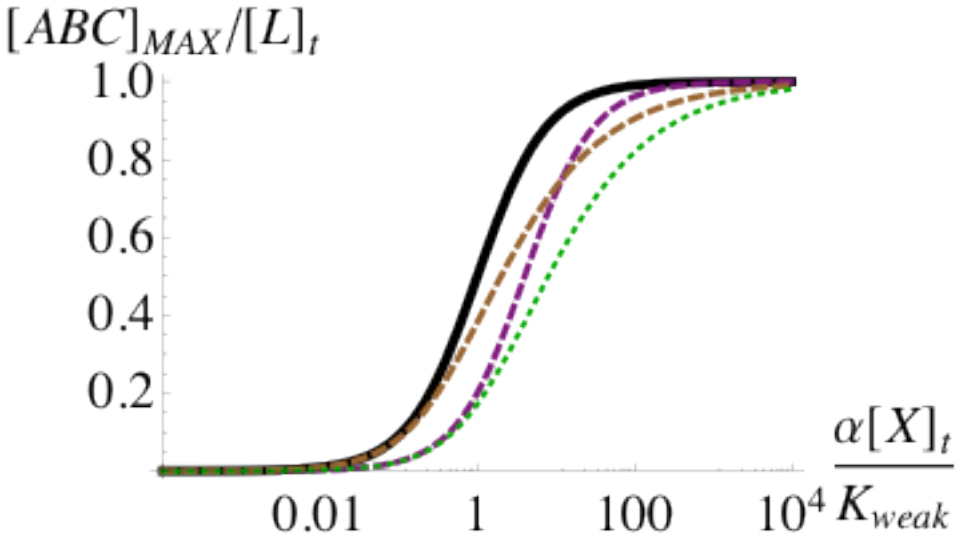


Figure S19. Comparison of Simplified TPF expression (assuming $K_{weak} >> K_{strong}$ and $[X]_t >> [L]_t$,) overlaid with "maximum error" TPF curves derived when $K_{wea} = K_{strong}$ (dashed purple curve), when $[X]_t = [L]_t$, (dashed brown curve), when $K_{wea} = K_{strong}$ and $[X]_t = [L]_t$, (dotted green curve). Overall the maximum error of the TPF approximation derived above is around a factor of 4.

As shown in Section 6, assuming $K_{weak} >> K_{strong}$ and $[X]_t >> [L]_t$,

$$\frac{[ABC]_{max}}{[L]_t} = \frac{\alpha[X]_t/K_{weak}}{\alpha[X]_t/K_{weak}+1} \quad \textbf{(S169)}$$

Maximum Error in $K_{weak} >> K_{strong}$ Assumption

Second, assuming $K_{weak} = K_{strong}$ and $[X]_t >> [L]_t$,

$$[ABC]_{max}^2 - [ABC]_{max}\left([X]_t + [L]_t + \frac{\left(\sqrt{K_{strong}} + \sqrt{K_{weak}}\right)^2}{\alpha}\right) + [X]_t[L]_t = 0 \quad \textbf{(S165)}$$

simplifies to

$$-[ABC]_{max}\left([X]_t + \frac{4K_{weak}}{\alpha}\right) + [X]_t[L]_t = 0 \quad \textbf{(S206)}$$

because the squared term must be less than $[L]_t$, which is less than $[X]_t$. Solving for $[ABC]_{max}$ yields

$$[\mathrm{ABC}]_{max} = \frac{[\mathrm{L}]_t[\mathrm{X}]_t}{[\mathrm{X}]_t + 4K_{weak}/\alpha}. \quad \textbf{(S207)}$$

Dividing both sides by $[\mathrm{L}]_t$ and rearranging gives

$$\frac{[\mathrm{ABC}]_{max}}{[\mathrm{L}]_t} = \frac{\alpha[\mathrm{X}]_t/K_{weak}}{\alpha[\mathrm{X}]_t/K_{weak} + 4}. \quad \textbf{(S208)}$$

Maximum Error in $[X]_t >> [L]_t$ Assumption:

Third, assuming $K_{weak} >> K_{strong}$ and $[\mathrm{X}]_t = [\mathrm{L}]_t$,

$$[\mathrm{ABC}]_{max}^2 - [\mathrm{ABC}]_{max}\left([\mathrm{X}]_t + [\mathrm{L}]_t + \frac{\left(\sqrt{K_{strong}} + \sqrt{K_{weak}}\right)^2}{\alpha}\right) + [\mathrm{X}]_t[\mathrm{L}]_t = 0 \quad \textbf{(S165)}$$

simplifies to

$$[\mathrm{ABC}]_{max}^2 - [\mathrm{ABC}]_{max}\left(2[\mathrm{X}]_t + \frac{K_{weak}}{\alpha}\right) + [\mathrm{X}]_t^2 = 0. \quad \textbf{(S209)}$$

Solving this for $[\mathrm{ABC}]_{max}$ gives

$$[\mathrm{ABC}]_{max} = \frac{2[\mathrm{X}]_t + K_{weak}/\alpha - \sqrt{(2[\mathrm{X}]_t + K_{weak}/\alpha)^2 - 4[\mathrm{X}]_t^2}}{2}. \quad \textbf{(S210)}$$

Dividing both sides by $[\mathrm{X}]_t$ (which is equivalent to $[\mathrm{L}]_t$) and rearranging affords

$$\frac{[\mathrm{ABC}]_{max}}{[\mathrm{X}]_t} = \frac{2 + (\alpha[\mathrm{X}]_t/K_{weak})^{-1} - \sqrt{\left(2 + (\alpha[\mathrm{X}]_t/K_{weak})^{-1}\right)^2 - 4}}{2}. \quad \textbf{(S211)}$$

Because $[\mathrm{X}]_t = [\mathrm{L}]_t$,

$$\frac{[\mathrm{ABC}]_{max}}{[\mathrm{L}]_t} = \frac{2 + (\alpha[\mathrm{X}]_t/K_{weak})^{-1} - \sqrt{\left(2 + (\alpha[\mathrm{X}]_t/K_{weak})^{-1}\right)^2 - 4}}{2}. \quad \textbf{(S212)}$$

Maximum Simultaneous Error in $K_{weak} >> K_{strong}$ and $[X]_t >> [L]_t$ Assumptions:

Fourth, assuming $K_{weak} = K_{strong}$ and $[\mathrm{X}]_t = [\mathrm{L}]_t$,

$$[\mathrm{ABC}]_{max}^2 - [\mathrm{ABC}]_{max}\left([\mathrm{X}]_t + [\mathrm{L}]_t + \frac{\left(\sqrt{K_{strong}} + \sqrt{K_{weak}}\right)^2}{\alpha}\right) + [\mathrm{X}]_t[\mathrm{L}]_t = 0 \quad \textbf{(S165)}$$

simplifies to

$$[\mathrm{ABC}]_{max}^2 - [\mathrm{ABC}]_{max}\left(2[\mathrm{X}]_t + \frac{4K_{weak}}{\alpha}\right) + [\mathrm{X}]_t^2 = 0. \quad \textbf{(S213)}$$

Solving this for $[\mathrm{ABC}]_{max}$ gives

$$[\mathrm{ABC}]_{max} = \frac{2[\mathrm{X}]_t + 4K_{weak}/\alpha - \sqrt{(2[\mathrm{X}]_t + 4K_{weak}/\alpha)^2 - 4[\mathrm{X}]_t^2}}{2}. \quad \textbf{(S214)}$$

Dividing both sides by $[\mathrm{X}]_t$ and rearranging yields

$$\frac{[\mathrm{ABC}]_{max}}{[\mathrm{X}]_t} = \frac{2 + 4(\alpha[\mathrm{X}]_t/K_{weak})^{-1} - \sqrt{\left(2 + 4(\alpha[\mathrm{X}]_t/K_{weak})^{-1}\right)^2 - 4}}{2}. \quad \textbf{(S215)}$$

Recognizing that $[\mathrm{X}]_t = [\mathrm{L}]_t$, the above equation becomes

$$\frac{[ABC]_{max}}{[L]_t} = \frac{2 + 4\left(\alpha[X]_t / K_{weak}\right)^{-1} - \sqrt{\left(2 + 4\left(\alpha[X]_t / K_{weak}\right)^{-1}\right)^2 - 4}}{2}. \quad \textbf{(S216)}$$

F. Error in TF_{50} and TI_{50} Approximations at α_{crit}

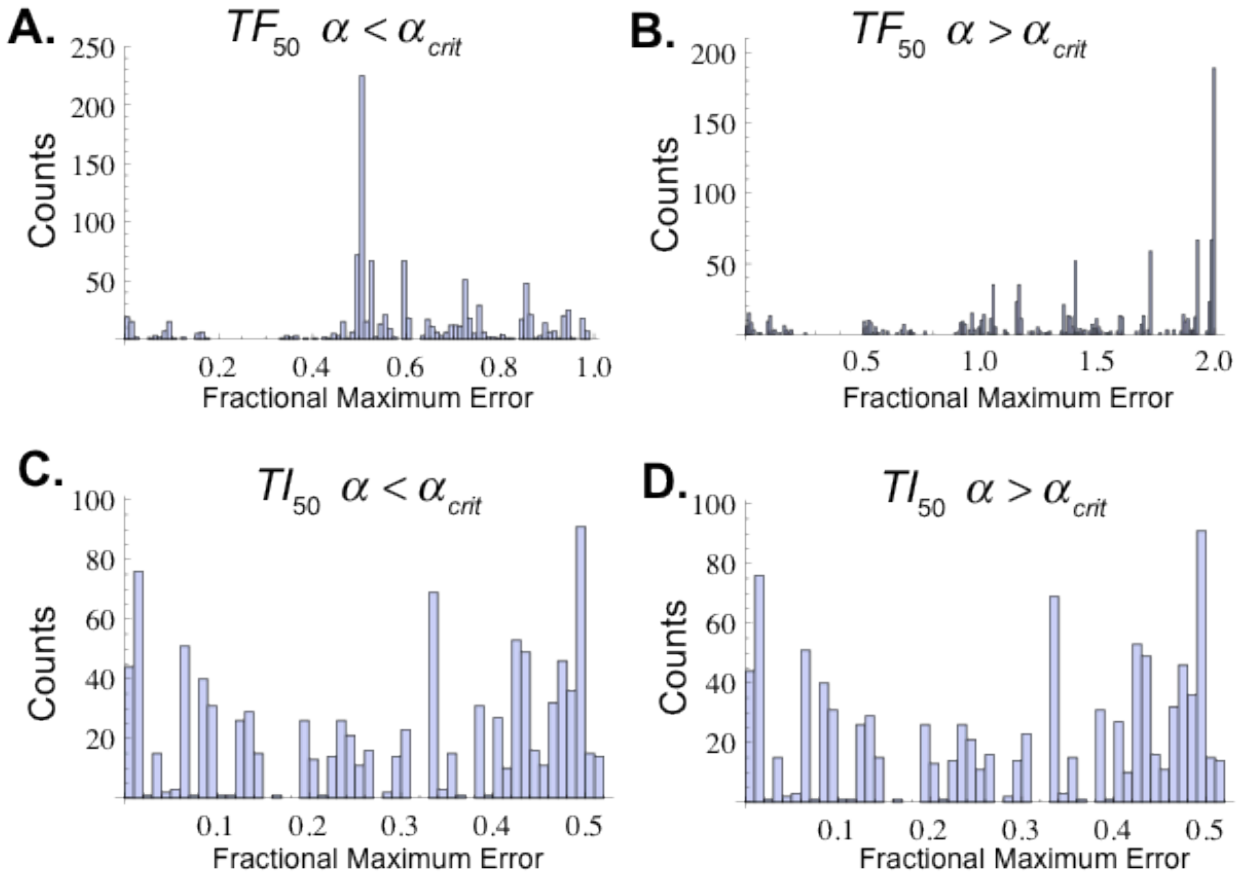


Figure S20. Histrogram presentation of numerically calculated maximal error across all physically reasonable concentration and dissociation constant parameter space (nanomolar to milimolar) for **(A)** Eq S200(-) (TF_{50} $\alpha << \alpha_{crit}$), **(B)** Eq S181 (TF_{50} $\alpha >> \alpha_{crit}$), **(C)** Eq S200(+) (TI_{50} $\alpha << \alpha_{crit}$), and **(D)** Eq S186 (TI_{50} $\alpha >> \alpha_{crit}$).

In order to characterize the error for the above and below α_{crit} TF_{50} and TI_{50} expressions, we estimated the relative error defined by calculating

$$TF_{50}^{\alpha=\alpha_{crit}}\ \text{Error} = \frac{\left|TF_{50}\left(\text{Exact@}\alpha_{crit}\right) - TF_{50}\left(\textit{Approximate@}\alpha_{crit}\right)\right|}{TF_{50}\left(\text{Exact@}\alpha_{crit}\right)} \quad \textbf{(S217)}$$

and

$$TI_{50}^{\alpha=\alpha_{crit}}\ \text{Error} = \frac{\left|TI_{50}\left(\text{Exact@}\alpha_{crit}\right) - TI_{50}\left(\textit{Approximate@}\alpha_{crit}\right)\right|}{TI_{50}\left(\text{Exact@}\alpha_{crit}\right)} \quad \textbf{(S218)}$$

when $\alpha = \alpha_{crit}$ (due to the fact these expressions were derived assuming $\alpha >> \alpha_{crit}$ or $\alpha << \alpha_{crit}$).

Unfortunately, analytical expressions or error analogous to the ones used for the non-cooperative TF_{50} and TI_{50}s proved intractable. As an alternative, we numerically calculated the error associated with all biologically reasonable parameter space (nM to mM concentration and dissociation constant values) using the Mathematica 7 software package. The lists of errors for

$TF_{50}^{\alpha<\alpha_{crit}}$, $TF_{50}^{\alpha>\alpha_{crit}}$, $TI_{50}^{\alpha<\alpha_{crit}}$, and $TI_{50}^{\alpha>\alpha_{crit}}$ was organized into histograms, where the x-axis represents the magnitude of error calculated (grouped in 0.01 (or 1% error) bins) and the y-axis represents the relative frequency with which this error in that bin was calculated (Figure S20). The $[A]_t$ values tested were 10^{-9}, 10^{-8}, 10^{-7}, and 10^{-6}; the $[C]_t$ values were 10^{-5}, 10^{-4}, 10^{-3}, and 10^{-2}. These were chosen to examine reasonable parameters without confusing the limiting and excess reagents, which were assumed to be $[A]_t$ and $[C]_t$, respectively. As shown in Figure S20, the maximal possible error encountered for $TF_{50}^{\alpha<\alpha_{crit}}$, $TF_{50}^{\alpha>\alpha_{crit}}$, $TI_{50}^{\alpha<\alpha_{crit}}$, and $TI_{50}^{\alpha>\alpha_{crit}}$ was 100%, 200%, 50%, and 60%, respectively.

Section 7: Flowchart Describing the Use of Our Model

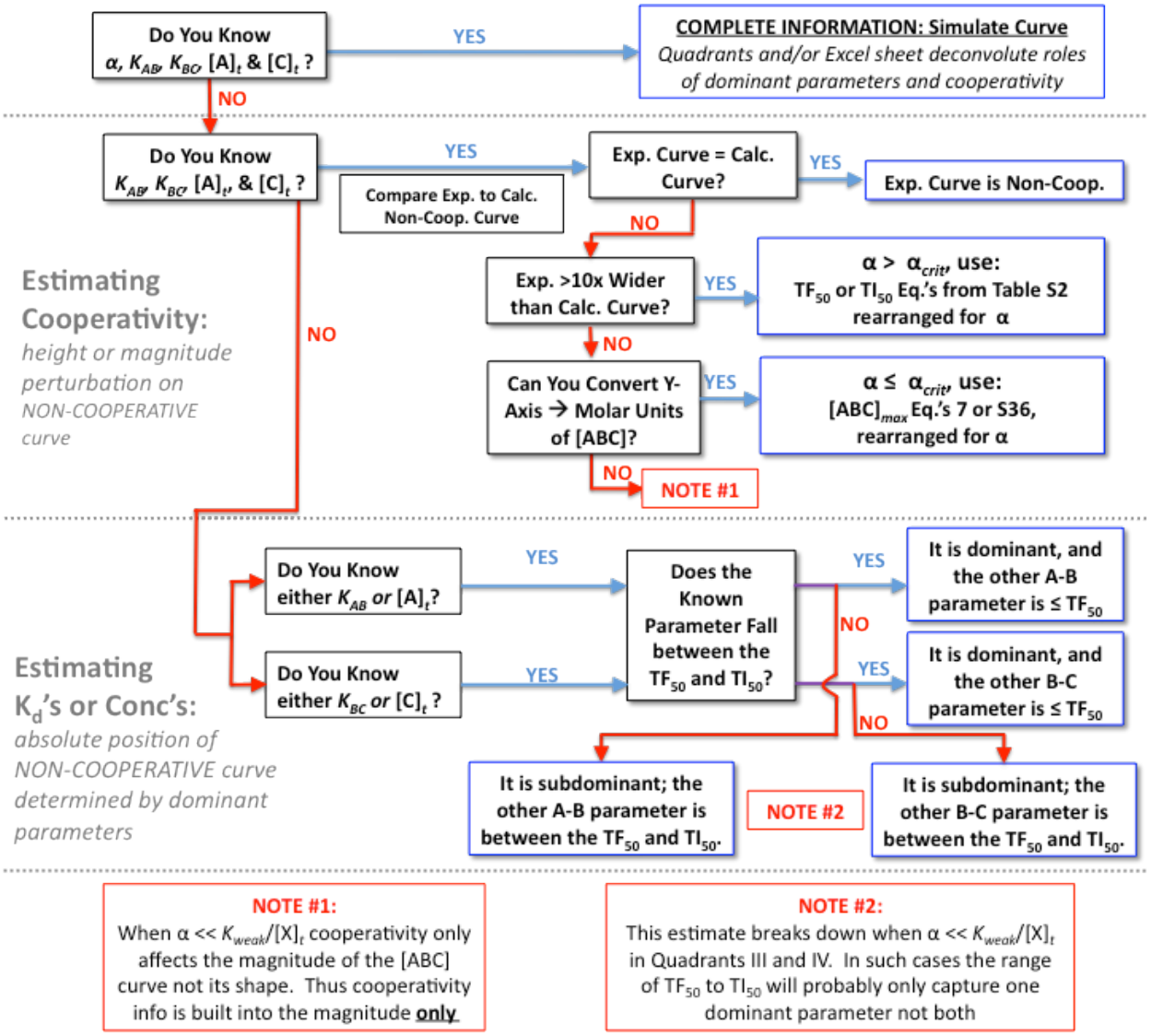


Figure S21. Flow chart to guide investigators in the application of the mathematical models and conceptual framework presented in this manuscript to their experimental data. Readers should start at the upper left hand corner where, if complete information is known, the included Excel file can be used to simulate ternary complex curves with full annotation of our framework. If some parameters are unknown, the remaining portion of the flow chart guides estimation of those parameters using deductive reasoning based on comparison of our models and experimental data.

Section 8: Supplemental References

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