

The Same Cooperative Binding Settles Slower When the Second Ligand Is Made Harder to Lose Than When It Is Made Easier to Gain

— the equilibrium curve and Hill coefficient are identical, and the ratio of settling rates at half saturation equals the square root of the cooperativity factor —the separator is whether cooperativity is carried by the leaving rate or by the binding rate — [Paper 970]

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Abstract

When two binding sites help each other, the concentration span from first to full effect narrows (Paper 878). The equilibrium curve depends only on the cooperativity factor c , and how fast binding proceeds cannot be read from the equilibrium formula. This paper writes the two sites of Paper 878 as rate equations among three states with 0, 1 and 2 ligands bound, builds the same c either by making the second ligand harder to lose or by making it easier to gain, and compares the settling rates at half saturation. No new theorem or law is claimed. First, the two constructions have the same equilibrium: in eight cases with $c = 1, 10, 100$ and 10000 , the equilibrium bound fraction of the rate equations matched the Adair equation within 10^{-9} , with Hill coefficients $1.000000, 1.519494, 1.818182$ and 1.980198 (Section 2). Second, built by harder loss, binding settles much more slowly: at half saturation, the slow-phase time was 29.222262 at $c = 100$ and **2549.028945** at 10000 when built by harder loss, and 2.922226 and 25.490289 when built by easier gain (in units of the first leaving time) (Section 3). Third, the rate ratio of the two constructions is exactly the square root of the cooperativity factor: the eigenvalue ratios were **$3.162278, 10.000000$** and 100.000000 at $c = 10, 100$ and 10000 , both eigenvalues agreeing with $\sqrt{c}$ within a relative 10^{-9} (Section 3). Fourth, binding from empty differs in the same direction: the time from empty to 90 percent of equilibrium was 5820.335303 at $c = 10000$ when built by harder loss and 58.698322 by easier gain, a ratio of **99.156758** (Section 3). Fifth, the separator is whether cooperativity is carried by the leaving rate or by the binding rate: equilibrium sees only the ratio of binding to leaving rates, so the curve is the same either way. At half saturation the singly bound state is scarce, 0.009901 at $c = 10000$, and the rate of passing through it made the slow-phase rate inversely proportional to c when built by harder loss and to $\sqrt{c}$ when built by easier gain (Section 4).

Keywords: cooperative binding, binding kinetics, Adair equation, Hill coefficient, eigenvalue, chemical kinetics.

Scope of this paper (scope note): No new theorem or law is claimed: the Adair equation for two binding sites, that equilibrium is set by the ratio of binding and leaving rates (detailed balance), that cooperative binding can have a slow phase, and that the eigenvalues of rate equations set how fast they settle, are all known in biochemistry and chemical kinetics. Shape changes are not included: no step in which the molecule changes shape and no intermediate shape is included; cooperativity is built from binding and leaving rates alone. These are model values: c and concentrations are model values, and time is in units of the first leaving rate. They correspond to no drug, receptor or enzyme. Which construction applies is not stated: whether cooperativity in a real molecule arises from harder loss or easier gain is not discussed. Relation to earlier papers: Paper 878, in its scope note, did not enter mechanism, leaving out models in which shape changes produce cooperativity and binding kinetics; this paper sits in the seat of binding kinetics (Paper 936 sat in the seat of shape-change models; the binding-kinetics seat had no earlier occupant). Paper 936, in its scope note, also left out rates and intermediate shapes, not including the rates of binding and shape change or intermediate shapes; this paper sits in that seat for binding rates only, without shape change. Paper 878 showed that the concentration span from first to full effect is set not by the effective concentration but by cooperativity, with a Hill coefficient of $2/(1 + 1/\sqrt{c})$ for two sites. Here the two rate constructions giving that same curve are compared. What is added: building the three-state rate equations in two ways, confirming that equilibrium matches the Adair equation, finding eigenvalues and times to 90 percent at half saturation, confirming that the rate ratio is exactly $\sqrt{c}$, and placing the separator on “leaving rate, or binding rate”.

1. Notation and the Question

Setting: a two-site molecule is described by the number of ligands bound, 0, 1 or 2. Let x be concentration divided by the first leaving rate, and measure time in units of the first leaving rate. The rates of the two constructions for cooperativity factor c are:

construction	$0 \rightarrow 1$	$1 \rightarrow 0$	$1 \rightarrow 2$	$2 \rightarrow 1$
harder loss	$2x$	1	x	$2/c$
easier gain	$2x$	1	cx	2

In both, equilibrium gives $p_1/p_0 = 2x$ and $p_2/p_1 = cx/2$, so the bound fraction is

$$\theta = \frac{x + cx^2}{1 + 2x + cx^2}$$

(the Adair equation of Paper 878), with half saturation at $x = 1/\sqrt{c}$. The two negative eigenvalues of the rate matrix are taken as settling rates, and the time from the empty state until the bound fraction reaches 90 percent of equilibrium is also found.

Question: for two constructions with the same equilibrium curve, is the settling rate of binding the same?

2. Equilibrium

Computation 2.1: for $c = 1, 10, 100$ and 10000 , half saturation is at $1.000000, 0.316228, 0.100000$ and 0.010000 ; the equilibrium bound fraction in all eight cases was 0.500000 , within 10^{-9} of the Adair equation. The singly bound fraction was $0.500000, 0.240253, 0.090909$ and 0.009901 , and the Hill coefficient $1.000000, 1.519494, 1.818182$ and 1.980198 .

Observation 2.2

the two constructions cannot be told apart at equilibrium. Both have the same ratio of binding to leaving rates, so binding curve and Hill coefficient agreed: the stronger the cooperativity, the fewer singly bound molecules at half saturation.

3. Settling Rates

Computation 3.1: eigenvalues at half saturation (fast phase, slow phase), slow-phase time, and time from empty to 90 percent.

c, construction	fast phase	slow phase	slow-phase time	to 90%
1 (both)	−4.000000	−2.000000	0.500000	1.151293
10, harder loss	−1.866629	−0.282055	3.545414	7.277576
10, easier gain	−5.902798	−0.891935	1.121158	2.638166
100, harder loss	−1.285780	−0.034220	29.222262	63.134500
100, easier gain	−12.857795	−0.342205	2.922226	6.761690
10000, harder loss	−1.029808	−0.000392	2549.028945	5820.335303
10000, easier gain	−102.980769	−0.039231	25.490289	58.698322

The eigenvalue ratios, easier gain over harder loss, were 3.162278 and 3.162278 at $c = 10, 10.000000$ and 10.000000 at 100 , and 100.000000 and 100.000000 at 10000 , both within a relative 10^{-9} of $\sqrt{c}$. The time ratios to 90 percent

(harder loss over easier gain) were 2.758574, 9.337089 and 99.156758. Multiplying the slow-phase rate by c for harder loss and by $\sqrt{c}$ for easier gain gave 3.422049 for both at $c = 100$ and 3.923063 for both at 10000.

Observation 3.2

built by harder loss, the settling time grows strongly with c . From $c = 100$ to 10000, the slow-phase time grew from 29.222262 to 2549.028945 by harder loss and from 2.922226 to 25.490289 by easier gain: the eigenvalues of the two constructions differed by a factor of $\sqrt{c}$ in both phases.

4. The Separator

Observation 4.1

the separator is whether cooperativity is carried by the leaving rate or by the binding rate. Equilibrium sees only the ratio of rates, so either choice gives the same curve: settling sees the rates themselves. Built by harder loss, $2 \rightarrow 1$ slows to $2/c$; built by easier gain, $1 \rightarrow 2$ speeds up to $c\bar{x} = \sqrt{c}$. Since the products of the slow-phase rate with c and with $\sqrt{c}$ were the same for both constructions at $c = 100$ and 10000 (3.422049 and 3.923063), the slow-phase rate was roughly inversely proportional to c by harder loss and to $\sqrt{c}$ by easier gain.

Observation 4.2

what is not said. Shape change, intermediate shapes, depletion of concentration over time, rates of real molecules, and which construction exists in nature are not treated. What is shown is that two rate constructions giving the same equilibrium settle at rates exactly $\sqrt{c}$ apart at half saturation.

5. Roll Call and Closing

1. three-state rate equations (Section 1): cooperativity placed in two ways.
2. equilibrium matches the Adair equation (Computation 2.1): the constructions cannot be told apart.
3. slow-phase times **2549.028945** and 25.490289 (Computation 3.1): $c = 10000$.
4. eigenvalue ratio **10.000000** at $c = 100$ (Computation 3.1): exactly $\sqrt{c}$.
5. time ratio to 90% **99.156758** (Computation 3.1): same direction from empty.

6. leaving rate, or binding rate (Observation 4.1): the separator; the scope notes of Papers 878 and 936.

Closing: The same cooperative binding settles slower when the second ligand is made harder to lose than when it is made easier to gain. Writing the two sites of Paper 878 as three-state rate equations, both constructions matched the Adair equation at equilibrium, yet their eigenvalues at half saturation differed by exactly $\sqrt{c}$, with slow-phase times of 2549.028945 and 25.490289 at $c = 10000$: the times from empty to 90 percent, 5820.335303 and 58.698322, differed in the same direction. The separator is whether cooperativity is carried by the leaving rate or by the binding rate. Placed among the earlier papers: to the cooperativity Paper 878 counted from equilibrium curves, the binding kinetics that Papers 878 and 936 did not treat was added. To be honest: shape change is not included. With binding and leaving rates alone, how differently two constructions giving the same curve settle, and no further.

References

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