

II Equilibrium binding and cooperativity

In the previous Section we considered Michaelis-Menten kinetics. We found that the traditional form of the Michaelis-Menten equation [I.6] is derived by assuming a quasi-steady state in which the concentration of enzyme-substrate complex is fairly constant over time. Additionally we had to assume that initially the substrate is in excess. In this Section, we first will take a step back and focus on the steady state behavior of reversible reactions and introduce the concept of multiple binding sites. Initially we will consider multiple binding sites that are independently binding substrates. However for most protein complexes the binding of substrates is not independent. For example, after binding the first substrate molecule the binding probability of the second substrate is affected. This phenomenon is called cooperativity.

In the previous section it was assumed that one substrate molecule binds to one enzyme molecule. In biological reactions however proteins often bind multiple substrates. Assume a protein has n binding sites for a substrate. P_j denotes the protein bound to j substrate molecules S . The reactions describing this process are:



where $j = 1, 2, \dots, n$.

The time-evolution of the concentration of unbound protein P_0 is ($j=1$):

$$\frac{d[P_0]}{dt} = -k_{+1}[P_0][S] + k_{-1}[P_1] \quad [\text{II.2}]$$

where k_{+1} and k_{-1} are the forward and backward rate constants of [II.1] for $j=1$. The association and dissociation constants are defined as:

$$\begin{aligned} K_a &= \frac{k_{+1}}{k_{-1}} \\ K_d &= \frac{k_{-1}}{k_{+1}} = \frac{1}{K_a} \end{aligned} \quad [\text{II.3}]$$

In steady state, $d[P_0]/dt = 0$:

$$K_a = \frac{[P_1]}{[P_o][S]} \quad [11.4]$$

To characterize all n reactions, we introduce the n association constants K_j , $j = 1, 2, \dots, n$.

$$K_j = \frac{[P_j]}{[P_{j-1}][S]} \quad [11.5]$$

It is experimentally difficult to measure $[P_j]$, a more convenient quantity is the average number r ($0 < r < n$) of substrates bound to the protein. Because there are j substrates bound to P_j , r is given by:

$$r = \frac{[P_1] + 2[P_2] + 3[P_3] + \dots + n[P_n]}{[P_o] + [P_1] + [P_2] + \dots + [P_n]} \quad [11.6]$$

combining [11.5] and [11.6] gives Adair's equation:

$$r = \frac{K_1[S] + 2K_1K_2[S]^2 + 3K_1K_2K_3[S]^3 + \dots + nK_1K_2\dots K_n[S]^n}{1 + K_1[S] + K_1K_2[S]^2 + \dots + K_1K_2\dots K_n[S]^n} \quad [11.7]$$

Note that $0 < r < n$, one often uses the normalized form, called the saturation function $Y = r/n$ ($0 < Y < 1$).

Identical and independent binding sites

For now let's assume we have n identical binding sites and that binding at a given site is independent of the state of binding of all other sites. The rate constants k_+ and k_- characterize the binding and unbinding rates respectively. In steady state, [11.2] can now be written as:

$$0 = -nk_+[P_o][S] + k_-[P_1] \quad [11.8]$$

The factor n takes into account that there are n possible binding sites available for binding the first substrate. On the other hand there is only one possibility to loose a substrate going from state P_1 to P_o . Similarly for $j=2$ we can deduce:

$$0 = -(n-1)k_+[P_1][S] + 2k_-[P_2] \quad [11.9]$$

because there are (n-1) possibilities to add a substrate and only 2 possibilities to remove a substrate. If the intrinsic association constant K is defined as:

$$K \equiv \frac{k_+}{k_-} \quad \text{[II.10]}$$

we find that $K_1 = nK$ and $K_2 = (n-1)K/2$. In general, one can write:

$$K_j = \frac{(n-j+1)K}{j} \quad \text{[II.11]}$$

for $j = 1, 2, \dots, n$. By substituting [II.11] in [II.7] an explicit equation for r as a function of K , n , and $[S]$ is found. We will not go through the details of the derivation. If you are interested, see for example Bisswanger (2002, p. 11-16). The final result is elegantly simple:

$$r = \frac{nK[S]}{1+K[S]} \quad \text{[II.12]}$$

Note that the mathematical form of this equation is very similar to Michaelis-Menten kinetics. However this result is a steady-state (equilibrium) property while Michaelis-Menten equation is not. Equation [II.12] can also be derived in a more hand waving manner. As the n binding sites are identical and independent, it is not important to view them as clustered in one protein. If $[F]$ is the concentration of free binding site and $[B]$ the concentration of bound sites in steady state, then the association constant for this equilibrium is given by:

$$K = \frac{[B]}{[F][S]} \quad \text{[II.13]}$$

The total number of sites is: $n[P]=[F]+[B]$, this combined with [II.13] gives:

$$r = \frac{[B]}{[P]} = \frac{nK[S]}{1+K[S]} \quad \text{[II.14]}$$

Non-identical and independent binding sites

Now consider the case in which the binding sites are non-identical. Each binding site family (with n_j binding sites) is characterized by its own association constant K_j . At low concentrations first the binding sites with the high affinities will be occupied, the lower affinity binding site will only be occupied at larger $[S]$. As the binding site are

independent the binding equation [II.12] holds for each binding site family and r is just the sum of the different individual processes:

$$r = \frac{n_1 K_1 [S]}{1 + K_1 [S]} + \frac{n_2 K_2 [S]}{1 + K_2 [S]} + \dots + \frac{n_m K_m [S]}{1 + K_m [S]} \quad [\text{II.15}]$$

Identical and interacting binding sites

In the following discussion we will confine ourselves to two binding sites ($n=2$). First, let us assume that both binding sites are identical. In this case we only have to consider three states for the protein-substrate complex: no substrate bound, one substrate molecule bound, and two substrate molecules bound. The rate constants k_+ and k_- characterize the transitions between the unbound and single-bound state, and k_+^* and k_-^* the transitions between single-bound and double-bound states. The intrinsic association constants are defined by: $K = k_+/k_-$ and $K^* = k_+^*/k_-^*$. Analogous to [II.10] and [II.11] we find:

$$\begin{aligned} K_1 &= 2K \\ K_2 &= \frac{1}{2}K^* \end{aligned} \quad [\text{II.16}]$$

By using Adair's equation [II.7] we find:

$$r = \frac{2K[S] + 2KK^*[S]^2}{1 + 2K[S] + KK^*[S]^2} \quad [\text{II.17}]$$

The saturation function $Y = r/n$ is:

$$Y = \frac{K[S] + KK^*[S]^2}{1 + 2K[S] + KK^*[S]^2} \quad [\text{II.18}]$$

For $K=K^*$ we recover the hyperbolic (Michaelis-Menten like) equation [II.12]:

$$\tilde{Y} = \frac{K[S]}{1 + K[S]} \quad [\text{II.19}]$$

Let's compare the functional forms of [II.18] and [II.19] in more detail. The difference between the two functions is:

$$Y - \tilde{Y} = \frac{(K^* - K)K[S]^2}{(1 + K[S])(1 + 2K[S] + KK^*[S]^2)} \quad [\text{II.20}]$$

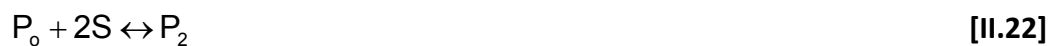
Positive cooperativity is often defined as $Y - \tilde{Y} > 0$, and negative cooperativity as $Y - \tilde{Y} < 0$. In other words, positive cooperativity occurs when the affinity of binding a second ligand is larger than binding the first ligand ($K^* > K$). For negative cooperativity the binding affinity for the second ligand is smaller than for the first ($K^* < K$).

Another, often used, definition for cooperativity is sigmoidality (from 'S shaped'). For a sigmoidal curve the second derivative should change sign. Let's introduce the dimensionless variables $\beta = K^*/K$ and $x = K[S]$:

$$\begin{aligned} Y &= \frac{x(1 + \beta x)}{1 + 2x + \beta x^2} \\ \frac{dY}{dx} &= \frac{1 + 2x\beta + \beta x^2}{(1 + 2x + \beta x^2)^2} \\ \frac{d^2Y}{dx^2} &= 2 \frac{\beta - 2 - \beta x[3 + 3x\beta + \beta x^2]}{(1 + 2x + \beta x^2)^3} \end{aligned} \quad [\text{II.21}]$$

The second derivative can only change sign if $\beta > 2$. Note that this definition yields a different criterion for cooperativity. According to the first definition a reaction is cooperative for $\beta > 1$, whereas according to the second definition $\beta > 2$. During the rest of the course we will use the first definition.

Now consider the limit for which intermediate states can be neglected. In this example, that would mean that single-bound states are very unlikely. The effective reaction would be:



The saturation function is now:

$$Y = \frac{K[S]^2}{1 + K[S]^2} \quad [\text{II.23}]$$

where $K = [P_2]/([P_o][S]^2)$ is the association constant of reaction [II.22]. Note that in this case the units of K are $(M)^{-2}$. This limit was first considered by Hill who proposed a

graphical way to represent equations such as [II.23]. In a Hill plot one plots $\ln[Y/(1-Y)]$ versus $\ln[S]$. The slope of this graph is called the Hill number which in this case equals

2. The Hill number is often used as an estimation of the number of binding sites of a protein. However one should be very careful as [II.23] involves a major assumption (no intermediate states). Let's calculate the Hill number n_H for the case [II.21] in which intermediate states are allowed:

$$n_H = \frac{d}{d(\ln[S])} \ln \left[\frac{Y}{1-Y} \right] = x \frac{d}{dx} \ln \left[\frac{Y}{1-Y} \right] = 1 + \frac{(\beta-1)x}{(1+x)(1+\beta x)} \quad [\text{II.24}]$$

The Hill number is plotted in Fig. 3 as a function of x at different values of β . The Hill number only approaches 2 for very large β and small x .

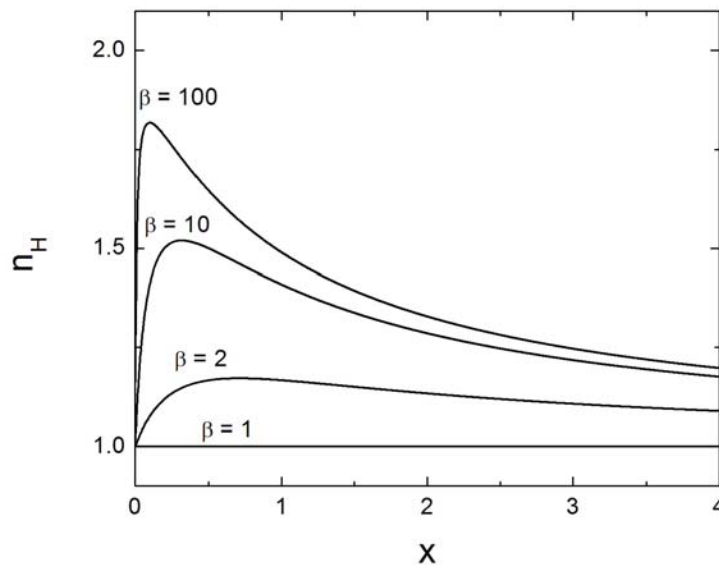


Figure 3. The Hill number as a function of the dimensionless concentration at different values of β for a protein with two identical interacting binding sites. The mathematical form is given by equation [II.24].

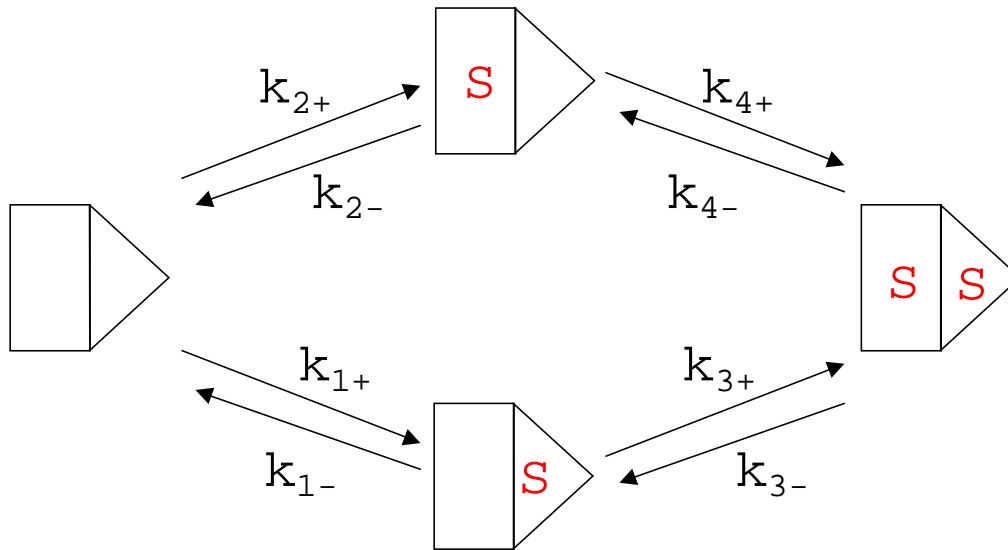


Figure 4. Two independent interacting binding sites.

Non-identical and interacting binding sites

How would the analysis above change if the two binding sites are non-identical? The ligand binding to the two binding sites is now characterized by the rate constants $k_{\pm 1}$, $k_{\pm 2}$, $k_{\pm 3}$, and $k_{\pm 4}$ (Fig. 4) and the four intrinsic association constants $K_j = k_{+j}/k_{-j}$ ($j=1,2,3,4$). In this case there are four states of the protein-ligand complex: nothing bound, site 1 bound, site 2 bound, and two sites bound. The principle of detailed balance (thermodynamic equilibrium) does not allow any net fluxes between states. Therefore:

$$K_1 = \frac{[P_1]}{[P_o][S]}; K_2 = \frac{[P'_1]}{[P_o][S]}; K_3 = \frac{[P_2]}{[P_1][S]}; K_4 = \frac{[P_2]}{[P'_1][S]} \quad [\text{II.25}]$$

Rewriting (31) gives:

$$K_1 K_3 = K_2 K_4 \quad [\text{II.26}]$$

The saturation function is given by:

$$Y = \frac{1}{2} \frac{[P'_1] + [P_1] + 2[P_2]}{[P_o] + [P_1] + [P'_1] + [P_2]} = \frac{[P_o][S](K_1 + K_2) + 2[P_1][S]K_3}{[P_o] + [S]([P_o](K_1 + K_2) + [P_1][S]K_3)} \Rightarrow$$

$$Y = \frac{K_1[S] + K_2[S] + 2K_1K_3[S]^2}{1 + K_1[S] + K_2[S] + K_1K_3[S]^2} \quad [\text{II.27}]$$

Note that [II.27] is independent of K_4 as expected because of the detailed balance equation [II.26].

If we define

$$\begin{aligned} J &= \frac{1}{2}(K_1 + K_2) \\ J^* &= \frac{2K_1K_3}{(K_1 + K_2)} \\ x' &= J[S] \\ \beta' &= \frac{J^*}{J} \end{aligned} \quad \text{[II.28]}$$

The saturation function can be written in the same form as for the identical interacting binding sites:

$$Y = \frac{x'(1 + x'\beta')}{1 + 2x' + \beta'x'^2} \quad \text{[II.29]}$$

In the limit $K_1=K_2$ we find $x=x'$ and $\beta=\beta'$ (identical interacting sites). In the limit $K_1=K_3$ and $K_2=K_4$ we recover the independent binding case:

$$Y = \frac{K_1[S]}{1 + K_1[S]} + \frac{K_2[S]}{1 + K_2[S]} \quad \text{[II.30]}$$

In the case we can write β' as:

$$\beta' = \frac{4K_1K_2}{(K_1 + K_2)^2} = \frac{4K_1K_2}{4K_1K_2 + (K_1 - K_2)^2} \quad \text{[II.31]}$$

Note that $\beta' = 1$ for identical sites and $\beta' < 1$ for non-identical sites. This implies that binding curves exhibiting negative cooperativity could arise from a protein that has independent binding sites or from a protein that has two interacting sites in which the second binding event is less likely than the first.

Further reading on enzyme kinetics and cooperativity

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