A Note on the *y*-intercept in Ligand-binding Equations

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This brief note aims to clarify some confusion that has arisen with regards to the reason why it 'works' to take the y-intercept at a value of $x = 1$ in the ligand-binding equations that we have dealt with in Chem4411 Lab #10.

Preliminaries

We begin with the central ligand-binding equation that is used in this work, which can be found on pg 146 of the Fa2012 lab manual (see the notes posted to the Collab site for a derivation of this relationship):

$$\frac{[P]_T}{Y} = \frac{1}{nk}\left[\frac{1}{1-Y}\right] + \frac{[L]_T}{n} = \frac{1}{nk}\left(\frac{[L]_T}{[L]}\right) + \frac{[L]_T}{n} \quad (1)$$

where $[P]_T$ is the total concentration of protein P, $[L]_T$ is the total concentration of ligand L, Y is the fractional saturation (see below), n is the number of ligand-binding sites per protein monomer (always an integer ≥ 1), and k is the intrinsic (microscopic) association constant (*i.e.*, $k = [P{\cdot}L]/[P][L]$).

Also, a few more preliminaries as far as notations and conventions go: Note that $[L]$ is the *free* ligand concentration, so the amount of *bound* ligand is simply $[L]_T - [L]$; similarly, the concentration of ligand-bound protein is $[P]_T - [P]$ (P is free protein). Note that the fractional saturation (Y) is, by definition, the concentration of bound ligand divided by the total ligand concentration:

$$Y = \frac{[L]_T - [L]}{[L]_T} = 1 - \frac{[L]}{[L]_T} \quad (2)$$

Y has the following properties: *(i)* Y is maximal (=1) when $[L] = 0$, *i.e.* **no** free ligand, all in the bound state; *(ii)* Y is minimal (=0) when $[L] \equiv [L]_T$, *i.e.* **all** free ligand, none in the bound state.

The fractional saturation Y is also given by the following key relation (from the derivation document I provided; this is a rearrangement of Eq 1):

$$Y = \frac{nk\,[L]\,([P]_T/[L]_T)}{1 + k\,[L]} \quad (3)$$

This Eq 3 can be 'linearized' (a fancy way of stating "*algebraically rearranged so as to be in the form of a linear equation*", $y = mx + b$); indeed, linearization of this Eq 3 yields Eq 1 above. Finally, a relationship that will be useful is that $1/(1-Y) = [L]_T/[L]$ (rearrangement of Eq 2).

The y-intercept & its enigma

Note that Eq 1 is of the general form $y = mx + b$, where $y = \frac{[P]_T}{Y}$, $m = \frac{1}{nk}$, $x = \left[\frac{1}{1-Y}\right]$, and $b = \frac{[L]_T}{n}$. Say we are, for whatever reason, interested in calculating the y-intercept of the above line (see Fig 1 for an example of such plots). By definition, this value — the y-intercept — occurs when the product $mx = 0$ (typically we think of this as the point where $x = 0$, though really it's just the point where $mx = 0$ and we cross the y-axis). Therefore, we can simply think of the intercept as the value somewhere along the x-axis, wherever that may be, where $y = b$, and this in turn leads us to conclude (from Eq 1) that the y-intercept $= [L]_T/n$. This provides a good 'operational' definition of the y-intercept, which, again, we can compute as being $[L]_T/n$.

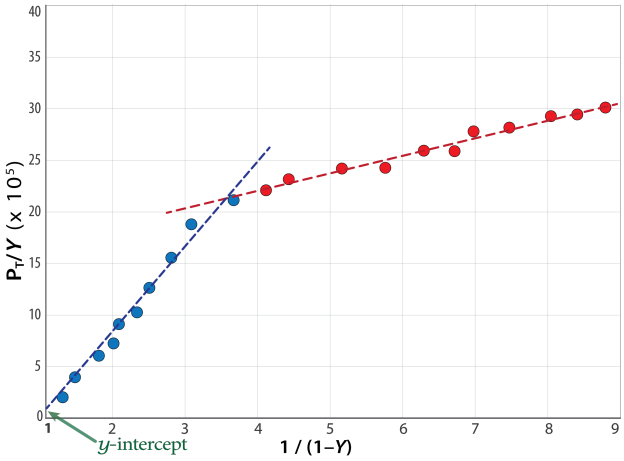

Figure 1: A sample ligand-binding curve, such as shown by Sohl & Splittgerber (1991), *J Chem Ed, 68(3)* 262-264. Note the two linear regimes: a higher-slope region at lower values of $[1/(1-Y)]$ and a lower-slope linear region at higher values of the abscissa.

However, a mathematical difficulty arises here: Why is it valid to take the y-intercept as the value of y at $x = 1$? As noted above, the fractional saturation Y ranges from 0 (all free ligand, none bound to P) to 1 (no free ligand, all bound to P). This means that the abscissa of our graph of $[P]_T/Y$ *versus* $[1/(1-Y)]$ (Fig 1) spans from $x = 1$ (as $Y \to 0$) to ∞ (as $Y \to 1$). Now, in the $y = mx + b$ way of viewing such a graph, at least two conceptual difficulties arise:

1. Most seriously, note that as $x \to 1$ (*i.e.*, $Y \to 0$) the value of the ordinate (y) explodes as the quotient $\frac{[P]_T}{Y}$ grows to ∞; in mathematical terms, this is a *singularity*.

2. It may seem that substitution of $x = 1$ into our working equation (Eq 1) to obtain the y-intercept would yield a value of $\frac{1}{nk} + \frac{[L]_T}{n}$, rather than the $\frac{[L]_T}{n}$ that was originally claimed.

So, what gives?

The solution

The above difficulties stem from viewing the y-intercept as being strictly the y-value **at** $x = 1$. This is not a valid view of the world because of the singularity ('*discontinuity*') at exactly $x = 1$. Instead, to calculate the intercept properly we must really consider the value of $y(x)$ as $x \to 1$, which corresponds to $Y \to 0$ (no bound ligand), or in other words to the mathematical limit as $[L] \to \infty$ (for which we can also see that, necessarily, $[L]_T \to \infty$, and so $\frac{[L]_T}{[L]} \to 1$ as desired [see Eq 1]). Therefore, let us take the following limit:

$$\lim_{\frac{[L]_T}{nk[L]} \to 0} \frac{[P]_T}{Y}, \text{ which equals} \tag{4}$$

$$\lim_{\frac{[L]_T}{nk[L]} \to 0} \left(\frac{1}{nk} \frac{[L]_T}{[L]} + \frac{[L]_T}{n} \right). \tag{5}$$

But wait, why is the above limit taken as the independent variable $[L]_T/nk[L] \to \boldsymbol{0}$ rather than unity? To see this, note that for any specific case we wish to consider (say a given ligand-binding experiment), $[L]_T$ is **constant**, n and k are also **constants** (which we are interested in determining, actually), and $[L]$ is the only free/independent parameter. Therefore it is valid to proceed, numerically, by taking the limit as $[L] \to \infty$ (note that $[L] \simeq [L]_T$ as $[L] \to \infty$, since $[L]$ can never exceed the total $[L]_T$), which corresponds to the $x \to 1$ (or $Y \to 0$) limit that interests us.

Notice something about the above limit: To solve it most easily we can view the **entire** product mx as that quantity which tends to 0 to give us the y-intercept (indeed, when $mx = 0$, $y = b$, which is the intercept). Substituting the equivalency from Eq 1 into Eq 4 (shown in blue in Eq 5), we see that the value of this limit — *i.e.*, the y-intercept — is the $\frac{[L]_T}{n}$ that was originally claimed.

Another path to the same conclusion is to substitute the complete definitions of all the expressions into Eq 4 and then consider the limit in full detail:

$$\lim_{\frac{[L]_T}{nk[L]} \to 0} \frac{[P]_T}{\frac{nk[L]\left(\frac{[P]_T}{[L]_T}\right)}{1+k[L]}} \tag{6}$$

The numerator is from Eq 4 and the denominator is simply the formula for Y as expressed in Eq 3. Though this Eq 6 limit may look formidable, the argument of the limit can be algebraically simplified as follows:

$$\frac{[P]_T}{\frac{nk[L]\left(\frac{[P]_T}{[L]_T}\right)}{1+k[L]}} \iff \frac{[P]_T\,(1+k[L])}{[P]_T \cdot n \cdot k \cdot [L]/[L]_T} \iff \tag{7}$$

$$\frac{[L]_T + k[L][L]_T}{n \cdot k \cdot [L]} \iff \tag{8}$$

$$\frac{[L]_T}{n \cdot k \cdot [L]} + \frac{[L]_T}{n} \tag{9}$$

Now, substituting the final expression above (Eq 9) into the original limit (Eq 6),

$$\lim_{\frac{[L]_T}{nk[L]} \to 0} \left(\frac{[L]_T}{n \cdot k \cdot [L]} + \frac{[L]_T}{n} \right), \tag{10}$$

we see that we recover Eq 5, where the first term vanishes to 0 and the y-intercept equals $\frac{[L]_T}{n}$.

Conclusion

The main lesson here is the following: Because of the singularity at $x = 1$ in the linear forms of the ligand-binding equations, one must invoke the concept of the *limit* to understand why the y-intercept is $[L]_T/n$ as the abscissa x (that is, $[1/(1-Y)]$) approaches unity.