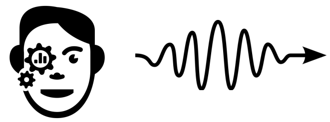
The Key Mathematical Flaw in the Protein-Ligand Binding Expression



"The only true wisdom is in knowing you know nothing."

− Socrates

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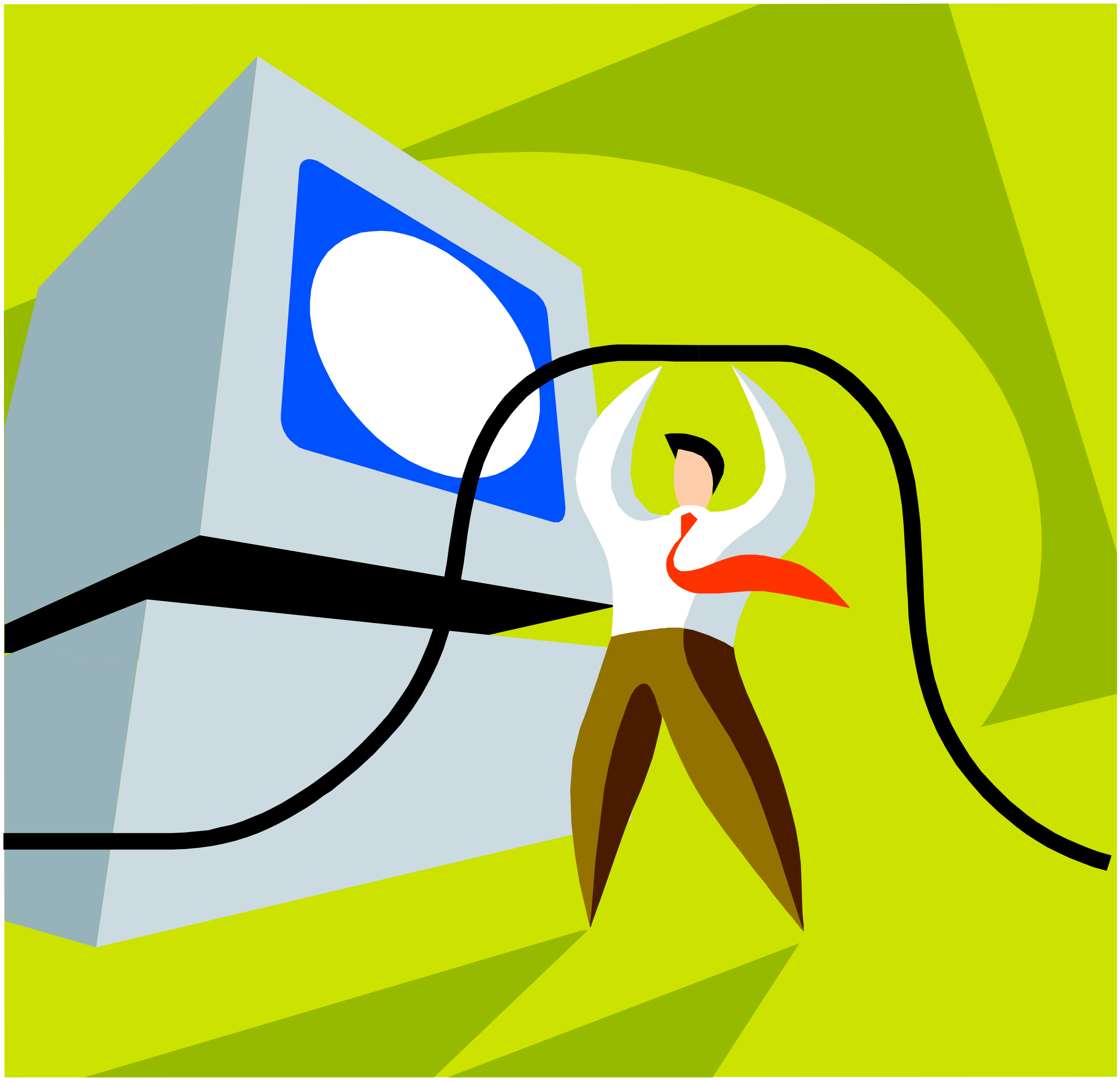

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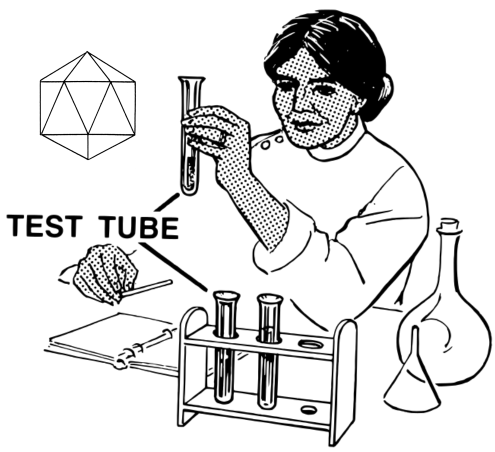
✓ ABSTRACT:

The dissociation of a **protein-ligand complex** (PL) can be represented by the equilibrium reaction PL ⇌ P + L, with the equilibrium relationship defined by **the dissociation constant** K such that $K = \frac{[P]\,[L]}{[PL]}$. In this equation $[P] = [P]_T - [PL]$ and $[L] = [L]_T - [PL]$, where $[P]_T$ and $[L]_T$ represent the initial total concentrations of the protein and ligand, respectively. **Case1:** If we substitute $[P]_T - [PL]$ for [P] and $[L]_T - [PL]$ for [L], then equilibrium relationship becomes $K = \frac{([P]_T - [PL])\,([L]_T - [PL])}{[PL]}$. From this, it follows that $[PL] = \frac{[P]\,[L]_T}{K + [P]}$. **Case2:** If we substitute $[L]_T - [PL]$ for [L], $[P]_T - [PL]$ for [P], and $[P]_T - [P]$ for [PL], the equilibrium relationship becomes $K = \frac{([P]_T - [PL])\,([L]_T - [PL])}{[P]_T - [P])}$. From this it follows that $K - [L] = K\,F_{FP} - F_{BP}\,[L]$ (which is an incorrect result). **Conclusion:** To avoid obtaining incorrect results, substitutions for ' [PL] ' should not be used in conjunction with substitutions for ' [L] ' and ' [P] '.



A protein in solution can exist in two forms: bound and unbound. Depending on the protein's affinity for the ligand, a portion of the protein may bind to the ligand while the rest remains unbound. If the binding between the protein and ligand is reversible, a chemical equilibrium is established between the bound and unbound states, represented by the reaction:

P (protein) + L (ligand) ⇌ PL (protein-ligand complex)

The dissociation constant for this equilibrium is:

$$K = \frac{[P]\,[L]}{[PL]}$$

In this equation, $[P] = [P]_T - [PL]$ and $[L] = [L]_T - [PL]$, where $[P]_T$ and $[L]_T$ represent the initial total concentrations of the protein and ligand, respectively. The **dissociation constant** K is a key measure of a protein's affinity for its ligand. It indicates the concentration of the protein needed to achieve a significant level of interaction with the ligand. Specifically, when the protein concentration equals K, 50% of the ligand will be bound in the protein-ligand complex, and the remaining 50% will be free "[L]". This is true when the protein is present in excess relative to the ligand. Generally, for effective ligand binding, proteins should have a K value of 1×10^{-6} M or lower. Smaller K values indicate stronger binding affinity, while higher K values suggest weaker binding.

✓ **CASE 1:**

Using the equilibrium relationship $K = \frac{[P]\,[L]}{[PL]}$ and substituting,

$[P]_T - [PL]$ for [P]

$[L]_T - [PL]$ for [L] Gives:

$$K = \frac{([P]_T - [PL])([L]_T - [PL])}{[PL]}$$

$$K\,[PL] = [P]_T\,[L]_T - [P]_T\,[PL] - [PL]\,[L]_T + [PL]^2$$

Dividing throughout by [PL] gives:

$$K = \frac{[P]_T\,[L]_T}{[PL]} - [P]_T - [L]_T + [PL]$$

But

$$[P]_T = [PL] + [P]$$

And, therefore:

$$K = \frac{[P]_T\,[L]_T}{[PL]} - [P] - [L]_T$$

$$K = \frac{[P]_T\,[L]_T}{[PL]} - [L]_T - [P]$$

$$K = [L]_T\left(\frac{[P]_T}{[PL]} - 1\right) - [P]$$

From this it follows that:

$$K + [P] = \frac{[P]\,[L]_T}{[PL]}$$

Rearranging:

$$[PL] = \frac{[P]\,[L]_T}{K + [P]} \qquad \ldots\ldots [1]$$

DISCUSSION:

This describes a rectangular hyperbola with key properties:

- **Saturation:** When $[P] \gg K$, [PL] approaches $[L]_T$
- **Half-saturation:** When [P] = K, $[PL] = \frac{[L]_T}{2}$. This means the dissociation constant equals the free protein concentration needed for 50% of the ligand to be bound.
- **Linearity:** When $[P] \ll K$, [PL] is roughly proportional to [P] with a slope of $\frac{[L]_T}{K}$.

CASE 2:

Using the equilibrium relationship $K = \frac{[P]\,[L]}{[PL]}$ and substituting,

$[P]_T$ – [PL] for [P]

$[L]_T$ – [PL] for [L]

$[P]_T$ – [P] for [PL] Gives:

$$K = \frac{([P]_T - [PL])\,([L]_T - [PL])}{([P]_T - [P])}$$

$$K([P]_T - [P]) = ([P]_T - [PL])([L]_T - [PL])$$

$$K[P]_T - K[P] = [P]_T[L]_T - [P]_T[PL] - [PL][L]_T + [PL]^2$$

Rearranging:

$$K[P]_T - [P]_T[L]_T + [P]_T[PL] = -[PL][L]_T + [PL]^2 + K[P]$$

$$[P]_T(K - [L]_T + [PL]) = [PL](-[L]_T + [PL]) + K[P]$$

Further, if we substitute:

$$[L]_T = [PL] + [L]$$

Then we get:

$$[P]_T(K - [PL] - [L] + [PL]) = [PL](-[PL] - [L] + [PL]) + K[P]$$

$$[P]_T(K - [L]) = -[PL][L] + K[P]$$

Which is the same as:

$$[P]_T(K - [L]) = K[P] - [PL][L]$$

$$K - [L] = K\frac{[P]}{[P]_T} - \frac{[PL]}{[P]_T}[L]$$

Labeling $\frac{[P]}{[P]_T}$ as F_{FP} (fraction of free protein) and $\frac{[PL]}{[P]_T}$ as F_{BP} (fraction of bound protein), the above expression can be rewritten as:

$$K - [L] = K\ F_{FP} - F_{BP}\ [L] \qquad \text{….. [2]}$$

DISCUSSION:

- If $F_{FP} = F_{BP} = 1$, then the left-hand side (LHS) equals the right-hand side (RHS), making Equation (2) true.
- If $F_{FP} = F_{BP} \neq 1$, then the left-hand side (LHS) does not equal the right-hand side (RHS), rendering Equation (2) invalid.
- Let's verify the condition "$F_{FP} = F_{BP} = 1$."

  According to the protein conservation law:

  $$[P]_T = [PL] + [P]$$

  From this, we get:

  $$1 = F_{BP} + F_{FP}$$

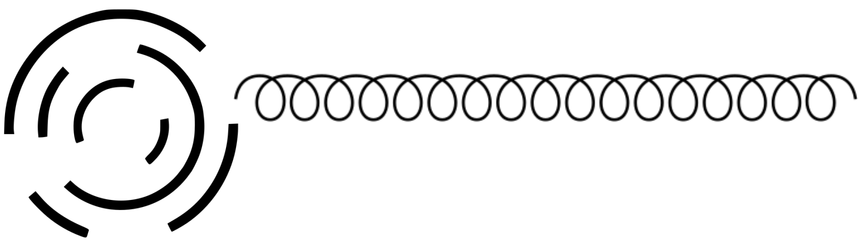
  If we assume $F_{BP} = F_{FP} = 1$, we get:

  $$1 = 2$$

  This shows that the condition $F_{FP} = F_{BP} = 1$ is impossible, since 1 is not equal to 2.

  In fact, the only way it can happen that $K - [L] = K - [L]$ is if both $F_{FP} = F_{BP} = 1$.

  Since $F_{FP} = F_{BP} \neq 1$, Equation (2) is not valid.



✓ CONCLUSION:

In Case 1, the substitutions correctly lead to:

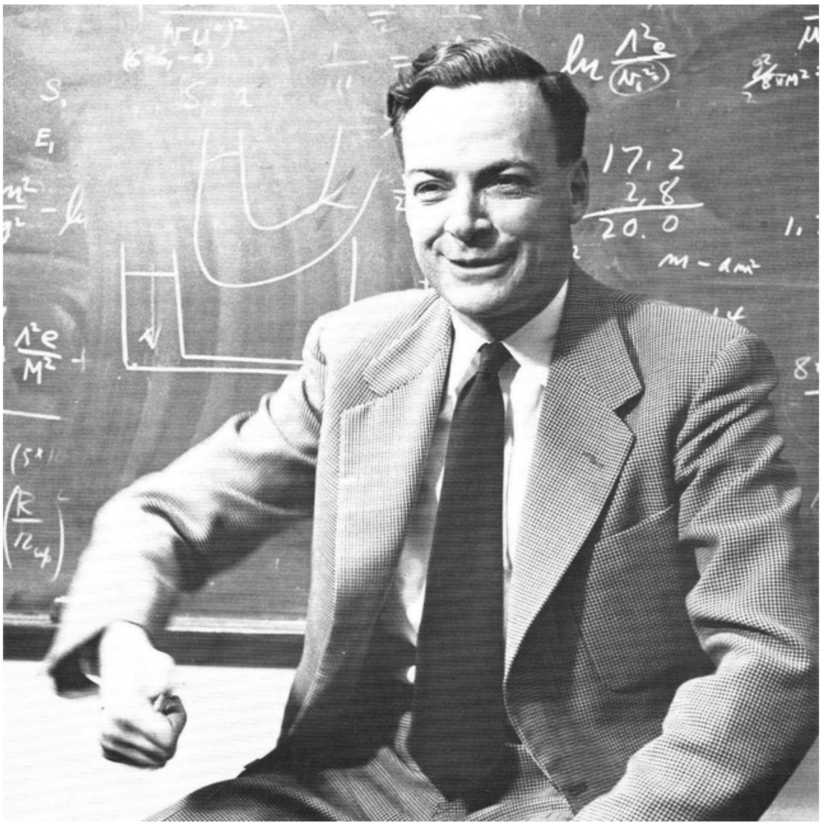
$$[PL] = \frac{[P]\,[L]_T}{K + [P]}$$

In Case 2, the substitutions produce an incorrect result:

$$K - [L] = K\,F_{FP} - F_{BP}\,[L]$$

Therefore, Case 1 is correct, while Case 2 is not. Substituting [PL] along with substitutions for [L] and [P] should be avoided to prevent incorrect results.

"I can live with doubt and uncertainty. I think it's much more interesting to live not knowing than to have answers which might be wrong."



— Richard P. Feynman