

## The Core Mathematical Error of PROTEIN LIGAND BINDING Expression

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**Abstract:** The dissociation of a protein – ligand complex (PL) can be described by a simple equilibrium reaction:  $PL \rightleftharpoons P + L$  the corresponding equilibrium relationship is defined

$$K [PL] = [P] [L] \quad (K = \text{dissociation constant})$$

In this equation  $[P] = [P]_T - [PL]$  and  $[L] = [L]_T - [PL]$  where  $[P]_T$  and  $[L]_T$  are the initial total concentrations of the protein and ligand, respectively.

**Case1:** If we substitute

$[L]_T - [PL]$  for  $[L]$  and  $[P]_T - [PL]$  for  $[P]$ , then equilibrium relationship becomes

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

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  $[PL]$ , then equilibrium relationship becomes

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

From this it follows that

$$K - [L] = K F_{FP} - F_{BP} [L] \quad (\text{wrong result})$$

**Conclusion:** Substitution for ' [PL] ' along with the substitutions for ' [L] ' and ' [P] ' should be avoided in order to prevent the occurrence of wrong result.

**Keywords:** Protein –ligand binding, protein - ligand complex, equilibrium reaction, dissociation constant.

A protein in solution exists in two forms: bound and unbound. Depending on a specific protein's affinity for ligand, a proportion of the protein may become bound to ligands, with the remainder being unbound. If the protein ligand binding is reversible, then a chemical equilibrium will exist between the bound and unbound states, such that:



The dissociation constant for this reaction is,

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

In this equation  $[P] = [P]_T - [PL]$  and  $[L] = [L]_T - [PL]$  where  $[P]_T$  and  $[L]_T$  are the initial total concentrations of the protein and ligand, respectively. The dissociation constant  $K$  is a useful way to present the affinity of a protein for its ligand. This is because the number  $K$  quickly tells us the concentration of protein that is required to yield a significant amount of interaction with the target ligand. Specifically, when protein concentration equals  $K$ , the 50% of the target ligand will exist in the protein ligand complex and 50% of the ligand will remain in the free form  $[L]$ . (This holds true under conditions where protein is present in excess relative to ligand). Typically, proteins must display a  $\mathbf{K \leq 1 \times 10^{-6} M}$  for the interaction with their target ligand. When considering the  $K$  for proteins, smaller numbers mean better binding. The higher the  $K$  value the protein does not bind well to the ligand.

### CASE 1:

Using the equilibrium relationship  $K [PL] = [L] [P]$  and substituting,

$$[L]_T - [PL] \text{ for } [L]$$

$$[P]_T - [PL] \text{ for } [P] \text{ Gives:}$$

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

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

Dividing throughout by [PL] gives:

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

But

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

And, therefore,

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

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

From this it follows that

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

Rearranging

$$[PL] = \frac{[P] [L]_T}{K + [P]} \quad \dots (1)$$

## DISCUSSION

This defines a rectangular hyperbola with several important regional properties:

- **Saturation:** when  $[P] \gg K$ ,  $[PL]$  asymptotically approaches  $[L]_T$ .
- **Half-saturation:** when  $[P] = K$ ,  $[PL] = \frac{[L]_T}{2}$  – in other word, the dissociation constant is equal to the (free) protein concentration needed to ensure that 50% of the ligand will be bounded.
- **Linearity:** when  $[P] \ll K$ ,  $[PL]$  is ~ proportional to  $[P]$  with slope =  $\frac{[L]_T}{K}$ .

## CASE 2:

Using the equilibrium relationship  $K [PL] = [L] [P]$  and substituting,

$$[P]_T - [P] \text{ for } [PL]$$

$$[L]_T - [PL] \text{ for } [L]$$

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

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

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

Rearranging

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

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

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:

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

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

Labeling  $\frac{[P]}{[P]_T}$  as  $F_{FP}$  (fraction of free protein) and  $\frac{[PL]}{[P]_T}$  as  $F_{BP}$  (fraction of bound protein) then above

expression turn into

$$K - [L] = K F_{FP} - F_{BP} [L] \quad \dots (2)$$

## DISCUSSION

- If  $F_{FP} = F_{BP} = 1$ , then the LHS = RHS, and the Eq. (2) is true.
- If  $F_{FP} = F_{BP} \neq 1$ , then the LHS  $\neq$  RHS, and the Eq. (2) is invalid.

Let us now check the validity of the condition

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

As per the **protein conservation law**,

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

From this it follows that

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

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

$$1 = 2$$

The condition  $F_{FP} = F_{BP} = 1$  cannot be achieved, since  $1 \neq 2$ .

In fact, the only way it can happen that  $K - [L] = K - [L]$  is if both  $F_{FP} = F_{BP} = 1$ . Since  $F_{FP} = F_{BP} \neq 1$ , Eq. (2) does not therefore hold well.

## CASE NOTES

### CASE 1

If we substitute

$[L]_{T-} - [PL]$  for  $[L]$  and  $[P]_{T-} - [PL]$  for  $[P]$ , then equilibrium relationship

$K [PL] = [L] [P]$  becomes

$$K [PL] = ([L]_{T-} - [PL]) ([P]_{T-} - [PL])$$

From this it follows that

$$[PL] = \frac{[P] [L]_{T-}}{K + [P]}$$

### CASE 2

If we substitute

$[L]_{T-} - [PL]$  for  $[L]$ ,

$[P]_{T-} - [PL]$  for  $[P]$  and  $[P]_{T-} - [P]$  for  $[PL]$ , then equilibrium relationship

$K [PL] = [L] [P]$  becomes

$$K ([P]_{T-} - [P]) = ([L]_{T-} - [PL]) ([P]_{T-} - [PL])$$

From this it follows that

$$K - [L] = K F_{FP} - F_{BP} [L] \text{ (wrong result)}$$

## CONCLUSION

Substitution for ' $[PL]$ ' along with the substitutions for ' $[L]$ ' and ' $[P]$ ' should be avoided in order to prevent the occurrence of wrong result.

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