Review of: "Unlocking Complexity: The Versatility of Substrate Modulation Equations in Enzyme Analysis"

Alessandro Pesaresi[1]

1 Italian National Research Council

Potential competing interests: No potential competing interests to declare.

I have two main concerns about this work.

1. Allosteric Modulation and Michaelis-Menten Model

An enzyme whose activity is allosterically modulated by its substrate, by definition, does not obey the Michaelis-Menten model. Therefore, defining the substrate affinity in terms of the Michaelis constant (K_m) for such an enzyme is incorrect. Instead, it should be referred to as a "half-saturation concentration."

This is not just a matter of nomenclature; in the second paragraph, the author states:

> *Both the substrate modulation equation and the nonessential activator equation are based on the notion that the enzyme has a normal catalytic rate that is altered by the binding of an activator or a secondary substrate.*

When considering simulations, the "normal catalytic rate" can be assumed to be the rate obtained when either K_{ss} *is set to* ∞ *or b is set to 1. However, in a real case, what does the term "normal rate" indicate when the substrate itself causes activation? How should one practically cope with a versus [S] plot in this context? Depending on the circumstances, not only* K_m *is not defined but even the half-saturation can turn into a problematic concept. Which, in my opinion, requires closer attention (see point 2).*

2. Equilibrium Constants and Catalytic Rates

Examining Equation 13 reveals a potentially critical flaw: while both K_m *and* K_{ss} *represent affinities, they are fundamentally different types of constants.* K_{ss} *is a true equilibrium constant, whereas* K_m *depends on both the substrate dissociation constant (*K_s*) and* k_{cat} *according to the well-known equation* $K_m = (k_{-1}+k_{cat})/k_1$.

If the binding of to the allosteric site alters k_{cat}*, it would indirectly affect* K_m *as well. This means we would have two different enzyme forms, one with* K_{m1} *and* k_{cat1}*, and the other with* K_{m2} *and* k_{cat2}.

To account for these complications and achieve a thorough analytical description of the versus [S] relationship, the equations would likely need to include quadratic terms in [S]. This addition would significantly increase their complexity and potentially limit their practical use. While the simpler equations formulated by the author are certainly more practical, we must ask: at what cost?

To clarify this point, the author might generate computed initial velocities and try to fit them with his model. This would also provide a valuable example of how to treat experimental data.

Conclusion

While the effort to develop new equations for steady-state kinetics with greater accuracy is commendable, I believe that, as educators and members of the biochemistry community, we should shift our focus from the outdated method of initial velocities -and the associated need to derive equations- to the more modern approach of computer-assisted global fitting of full reaction time courses.

Direct simulations of enzymatic reactions greatly simplify the study of mechanisms that deviate from the Michaelis-Menten model. For an example of the power of this analytical method, I recommend reviewing the comprehensive mechanism resolution of butyrylcholinesterase by Jure Stojan (Molecules, 22(8), 1248). See this link for the data analysis.

Nevertheless, upon receiving a satisfactory resolution to the aforementioned criticism, in scenarios where, for any reason, the conventional method remains the preferred choice, these equations might help analyze the intricate kinetics of substrate-modulated enzymes.

Minor Issues

1. ***Equation 2 Misleading**: In Equation 1, >1 implies activation and 0< <1 implies inhibition. According to Equation 2 however, any >0 implies activation. Either Equation 2 needs reformulation, or the text should explain its significance.*

2. ***Notation of Equilibrium Constants**: To avoid confusion, equilibrium and affinity constants should be denoted with capital letters (e.g., K_{ss} instead of k_{ss}, K_m instead of k_m).*

3. ***Schematic Example Missing**: The paper discusses enzymes with allosteric sites triggered by substrates but does not provide a schematic example. This omission leaves readers unclear about the activation mechanism. Specifically, should we assume an enzyme with a rigid structure where both the active and allosteric sites are permanently formed and accessible by substrate, or does the binding of S to the active site induce the allosteric site? In the latter case, only two E – S equilibria can exist: + → and + → S. In the former, the + → ∗ equilibrium should also be considered (where ∗ represents the substrate bound only to the allosteric site).*

4. ***Partial Model Consideration****: Equations 1 and 22-23 (etc.) align with a mechanism where the allosteric site is induced by . However, the existence of a constitutive allosteric site is also a probable possibility. An explicit comment on the partial nature of this model, accommodating only one of the two possibilities, is recommended.*
5. ***Figure 1 Caption****: The caption of Figure 1 should explicitly state what the black, blue, and green (or red) traces represent.*