The Fundamental Problem With Enzyme Inhibition Theory

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Abstract

The failure of modern enzyme inhibition drug theory stems from the derivation of the inhibition equations. These equations do not recognize the difference between the chemical equilibrium, that defines the formation of the enzyme-inhibitor complex, and the effect an inhibitor has when it binds to the enzyme. Chemical equilibrium can only be used to determine the concentration of components found in a sample, but the traditional inhibition equations define inhibition with the enzyme-inhibitor binding equilibrium, destroying their usefulness in characterizing inhibition. This problem stems from the form the inhibition term takes in these equations. The traditional Inhibition term, when it was derived, was left in an arrangement that obscures its only function as a chemical equilibrium term. However, rearranging the inhibition term to establish its link to common binding curves allows us to analyse how this inhibition term affects the classic inhibition equations, pointing the way to correcting and simplifying the modeling of drug interactions. Here we will look at the derivations of biological binding curves and enzyme inhibition equations noting the forms chemical equilibrium terms take in biological equations. This will demonstrate the traditional inhibition term is just an inverted binding curve and thus a chemical equilibrium, which provides no information about the effect produced by the formation of the enzyme-inhibitor complex. From this analysis we will look at the simple alternative for these inhibition equations that clearly

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distinguishes between the chemical equilibrium and the effect inhibitor binding produces resulting in a single equation that defines inhibition and activation.

Chemical equilibriums in biological studies

The use of binding curves is ubiquitous in the biological study of molecular interactions (Hulme & Trevethick, 2010) and can be represented with the general form of equation (1).

Eq 1

$$\frac{[X]}{[X] + K_x}$$

This universal prevalence stems from their ability to be adapted to describe many interactions such as ligands binding with receptors (K_d), substrate binding with enzymes (K_M), and inhibitors binding to receptors or enzymes (IC_{50}). The adaptability of binding curves has also led to their use in defining macro effects associated with medicine (EC50) and toxicology (LD50). However, the derivation of these equations follows the same path and demonstrates that they are all just a simplified chemical equilibrium.

When deriving the equation for a binding curve we are referring to two molecules coming together to form a complex, and this is usually defined using a chemical equilibrium (Equation 2). In this example, we will be defining the binding of a ligand (L) and its receptor (R).

Eq 2

$$K_d = \frac{[R][L]}{[RL]}$$

Here the chemical equilibrium is defined with a dissociation constant (K_d) which describes the relationship between the concentrations of the ligand, receptor, and receptor-ligand complex (RL) at equilibrium. To determine these equilibrium concentrations would generally require a quadratic equation where the concentration of the ligand and receptor must be adjusted to account for the formation of the complex. However, in biological studies, while we may be able to get a signal relating to the formation of the complex, we usually are limited by the inability to isolate and quantify the receptor.

The convenience of binding curves comes from their ability to define association based on the concentration of the ligand alone. To derive the binding curve, we must start by defining the percent binding based on the concentration of the receptor-ligand complex and total receptor concentration (equation 3)

Eq 3

$$\%\ binding = \frac{[RL]}{[R]_{total}}$$

Next, we want to define what the total receptor concentration represents, which is the sum of the free receptor and receptor found in the complex with the ligand (equation 4).

Eq 4

$$[R]_{total} = [R] + [RL]$$

To remove the free receptor concentration, we can rearrange the chemical equilibrium (equation 2) to define free enzyme in terms of the binding constant, ligand, and receptor-ligand complex (equation 5).

Eq 5

$$[R] = \frac{K_d[RL]}{[L]}$$

By inserting this into the equation we used to define total receptor concentration (equation 4) we remove the free receptor concentration from the equation (equation 6).

Eq 6

$$[R]_{total} = \frac{K_d[RL]}{[L]} + [RL]$$

This can then be inserted into the equation defining the percent binding (Equation 3) to remove the total receptor concentration (equation 7).

Eq 7

$$\%\ binding = \frac{[RL]}{\frac{K_d[RL]}{[L]} + [RL]}$$

This allows us to simplify by dividing out the complex (equation 8).

Eq 8

$$\%\ binding = \frac{1}{\frac{K_d}{[L]} + 1}$$

Followed by multiplying by the ligand concentration (equation 9).

Eq 9

$$\% \, binding = \frac{[L]}{K_d + [L]}$$

This gets us to the familiar binding curve (equation 1) and shows the direct link between the constant used in the binding curves and the more complex chemical equilibrium. Therefore, the constants found in binding curves are the same constants used in the chemical equilibrium, with the caveat that binding curves are only valid when the concentration of the ligand is not significantly affected by the formation of the receptor-ligand complex.

Binding curves as simplified chemical equilibriums are easily adapted to describe many biological interactions simply by redefining the subject of study.

Michaelis Menten equation

For enzyme kinetics, the derivation of the Michaelis Menten equation (1913) follows a similar pattern except the association of the substrate with the enzyme results in a catalytic transformation of the substrate to product (equation 10). This is defined as the reaction rate (v), which is equal to the catalytic rate constant (k_{cat}), multiplied by the concentration of the enzyme-substrate complex (ES).

Eq 10

$$v = k_{cat}[ES]$$

Like in the binding curve derivation above, where we defined the forms, the receptor is found in (equation 4) we must also define the enzyme forms (equation 11).

Eq 11

$$[E]_{total} = [E] + [ES]$$

Next, we want to remove the free enzyme concentration with the chemical equilibrium (equation 12)

Eq 12

$$[E] = \frac{K_M[ES]}{[S]}$$

Inserting this into the equation (11) we get equation (13)

Eq 13

$$[E]_{total} = \frac{K_M[ES]}{[S]} + [ES]$$

To combine this equation with our rate equation, we first want to solve for the enzyme-substrate complex by factoring out the term for the complex concentration (equation 14).

Eq 14

$$[E]_{total} = \left(\frac{K_M}{[S]} + 1\right)[ES]$$

Followed by dividing by the term containing the substrate affinity constant (equation 15)

Eq 15

$$[ES] = \frac{[E]_{total}}{\left(\frac{K_M}{[S]} + 1\right)}$$

With the complex defined, we can insert it into the rate equation (equation 10) to remove the complex concentration from the equation (equation 16).

Eq 16

$$v = k_{cat} \frac{[E]_{total}}{\left(\frac{K_M}{[S]} + 1\right)}$$

We can now combine the total enzyme concentration with the catalytic rate to get the maximum catalytic rate (V_{max}), the value we would get if all the enzymes were converting substrate to product at the same time (equation 17).

Eq 17

$$v = V_{max} \frac{1}{\left(\frac{K_M}{[S]} + 1\right)}$$

We follow up by multiplying by the substrate concentration, giving us the Michaelis Menten equation which is composed of the maximum catalytic rate multiplied by a binding curve (equation 18).

Eq 18

$$v = V_{max} \frac{[S]}{K_M + [S]}$$

So, as you can see most molecular interactions in biology are defined by binding curves which are chemical equilibriums. However, as you can see with the Michaelis Menten equation, a binding curve is not sufficient for describing the catalytic activity of an enzyme and must be multiplied by the maximum catalytic rate to provide any useful information about the enzymatic activity. A binding curve just like a chemical equilibrium only describes the propensity of molecules to come together to form a complex and this underlies the problem with inhibition equations.

Traditional inhibition term

Traditional inhibition equations use the inhibition term (equation 19) to define inhibition.

Eq 19

$$1 + \frac{[I]}{K_i}$$

In this term, you can clearly see the inhibitor's chemical equilibrium constant, but it lacks the structure of a binding curve so obscures the relationship between this term and binding curves. A partial clue is present above in the derivation of the ligand-receptor binding curve (equations 8 & 9) and the Michaelis Menten equation (equations 17 & 18) where before multiplying by the ligand or substrate we have a very similar term in the denominator of the equations (equation 20).

Eq 20

$$\frac{K_d}{[L]} + 1$$

The presence of equation (20) in the denominator is not a coincidence as this is an alternative way of expressing a binding curve (equations 8 & 17) and as such hints at the true nature of the inhibition term (equation 19).

By rearranging the inhibition term (equation 19), we can show how it corresponds to a binding curve. To start we will multiply one by the inhibitor binding constant to get a common denominator (equation 21).

Eq 21

$$\frac{K_i + [I]}{K_i}$$

Next, we will add zero to the denominator in the form of the inhibitor concentration minus itself (equation 22).

Eq 22

$$\frac{K_i + [I]}{K_i + [I] - [I]}$$

We will then divide by the term in the numerator, the inhibitor binding constant plus the inhibitor concentration (equation 23).

Eq 23

$$\frac{\frac{K_i + [I]}{K_i + [I]}}{\frac{K_i + [I] - [I]}{K_i + [I]}}$$

This then reduces to the simpler expression (equation 24).

Eq 24

$$\frac{1}{1 - \frac{[I]}{K_i + [I]}}$$

This form clearly shows that the inhibition term is simply an inverted binding curve that is being subtracted from one. The subtraction of the binding curve from one indicates that this term also defines the fraction of the enzyme population that is not bound by the inhibitor.

This has many implications for the modeling of enzyme inhibitors as the inhibition term has been used to define classical forms of inhibition based on how it is inserted in the Michaelis Menten equation. The main implication is that this term is simply a chemical equilibrium so can only be used to determine the tendency for the inhibitor and enzyme to form a complex and provides no further information on what the inhibitor may do to the enzyme. Nonetheless, knowing this, the

question is, how have the classic inhibition equations survived so long and why are they still being used?

Classic inhibition equations were divided up based on their effect on the enzymatic activity. As we saw above the Michaelis Menten equation is a combination of a binding curve and the rate of catalytic turnover. Inhibition was observed to affect either of these properties or both at the same time. As such, derivations based on the same principles used to produce the binding curves above produced three base equations.

Noncompetitive inhibition equation

The noncompetitive inhibition equation (equation 25) was derived to describe changes affecting the reaction rate and as such was defined by the V_{MAX} being divided by the inhibition term (equation 26).

Eq 25

$$v = V_{max}\frac{[S]}{(K_M + [S])\left(1 + \frac{[I]}{K_i}\right)}$$

Eq 26

$$\frac{V_{max}}{\left(1 + \frac{[I]}{K_i}\right)}$$

This is the best possible outcome, that could be expected from the incorporation of the inhibition term (equation 19) into the Michaelis Menten equation, because in this case, substitution with the rearranged form of the inhibition term (equation 24, 27), produces a term that perfectly describes

the shut down of the enzyme's catalytic activity as the inhibitor-enzyme complex is formed (equation 28, 29).

Eq 27

$$\frac{V_{max}}{\left(\frac{1}{1-\frac{[I]}{K_i+[I]}}\right)}$$

Eq 28

$$V_{max}\left(1-\frac{[I]}{[I]+K_i}\right)$$

Eq 29

$$V_{max}-V_{max}\frac{[I]}{[I]+K_i}$$

The inhibition term in this format allows the correction of this term so it can be used to distinguish between the binding curve and the effect the inhibitor has on the enzyme (equation 30).

Eq 30

$$V_{max}-\Delta V_{max}\frac{[I]}{[I]+K_i}$$

With a simple delta term, we now can define the effect of inhibitor binding as something other than complete inhibition (equation 31), such as partial inhibition or even activation.

Eq 31

$$\Delta V_{max}=(V_{max}-V_{max2})$$

Competitive inhibition equation

The competitive inhibition equation (equation 32) was derived to describe changes affecting the chemical equilibrium for enzyme-substrate complex formation. As such, the derivation produces an inhibition term that multiplies into the K_M (equation 33).

Eq 32

$$v = V_{max}\frac{[S]}{K_M\left(1+\frac{[I]}{K_i}\right)+[S]}$$

Eq 33

$$K_M\left(1+\frac{[I]}{K_i}\right)$$

This is the worst possible outcome for the inhibitory effect on the enzyme-substrate equilibrium term, because in this case, insertion of the rearranged form of the inhibition term (equation 34), produces a term that shows the substrate affinity is being divided by the fraction of the enzyme population not bound by the inhibitor (equation 35).

Eq 34

$$K_M\left(\frac{1}{1-\frac{[I]}{K_i+[I]}}\right)$$

Eq 35

$$\frac{K_M}{1-\frac{[I]}{K_i+[I]}}$$

In the traditional form (equation 33) the substrate affinity is multiplied by the equation of a straight line, which produces the characteristic linear increase in the substrate affinity constants value associated with this equation. This linear change being associated with the inversion of the inhibitor's binding curve is not surprising as inversion of the Michaelis Menten equation has been used to linearize its binding curve and simplify the determination of its constants for many years (Lineweaver–Burk 1953).

However, there are several potential reasons this equation has been accepted for so long. The primary reason probably relates to it being proposed during a time when graphing data by hand was the only way to analyse the data (Henri 1903; Cornish-Bowden et al., 2014). This, combined with the ability to spear almost any data set with a straight line, especially when there are only three or four points on the line, probably also played a role. Additionally, the increase in the substrate affinity constants value associated with this equation is inhibitory in nature as larger binding constants in relation to binding curves mean less affinity, as more substrate in this case would be needed to produce binding.

Another puzzling thing associated with this equation is inherent in its name. The competitive inhibition equation implies there is competition between the substrate and the inhibitor for the catalytic site of the enzyme. If an inhibitor prevented the substrate from binding to the active site, the enzyme would be shut off, which is already covered mathematically by the noncompetitive inhibition equation. Changes in substrate affinity should reflect changes in the binding interaction between the substrate and the enzyme like the princess and the pea story. An inhibitor that changes the substrate affinity would only reduce the propensity of the substrate to form a complex with the enzyme and this should be a one-to-one relationship.

The active site blockade assumption comes from the derivation of the competitive inhibition equation, where the definition of the enzyme states used in this derivation include the enzyme inhibitor complex (equation 36) rather than the enzyme inhibitor substrate complex (equation 37).

Eq 36

$$[E]_{total} = [E] + [EI] + [ES]$$

Eq 37

$$[E]_{total} = [E] + [EIS] + [ES]$$

While using the enzyme-inhibitor complex (equation 36) leads to the traditional competitive inhibition equation with the inverted inhibitor binding curve, using the enzyme-inhibitor-substrate complex (equation 37) leads to a preferable equation that can relate changes in substrate affinity directly to the formation of the enzyme-inhibitor-substrate complex.

Changes in substrate affinity

To start with, the rate equation used to generate this equation must include, product formation from both the enzyme-substrate complex and the enzyme-inhibitor-substrate complex, as catalysis may occur with both complexes (equation 38).

Eq 38

$$v = k_{cat}[ES] + k_{cat}[EIS]$$

The next step is to multiply the rate equation by one but in this case, one will be the total enzyme divided by its parts (equations 39 & 40).

Eq 39

$$v = k_{cat}[ES] + k_{cat}[EIS] \times \frac{[E]_{total}}{[E] + [ES] + [EIS]}$$

Eq 40

$$v = \frac{[E]_{total}(k_{cat}[ES] + k_{cat}[EIS])}{[E] + [ES] + [EIS]}$$

From here we will start to insert our chemical equilibriums for the enzyme-inhibitor-substrate complex (equations 41 & 42)

Eq 41

$$[EIS] = \frac{[I][ES]}{K_i}$$

Eq 42

$$v = \frac{[E]_{total}\left(k_{cat}[ES] + k_{cat}\frac{[I][ES]}{K_i}\right)}{[E] + [ES] + \frac{[I][ES]}{K_i}}$$

We can follow this up by inserting the chemical equilibriums for the enzyme-substrate complex (equation 43 &44)

Eq 43

$$[ES] = \frac{[E][S]}{K_M}$$

Eq 44

$$v = \frac{[E]_{total}\left(k_{cat}\frac{[E][S]}{K_M} + k_{cat}\frac{[E][S][I]}{K_M K_i}\right)}{[E] + \frac{[E][S]}{K_M} + \frac{[E][S][I]}{K_M K_i}}$$

Tidying the equation up a little we can see that free enzyme is in all the terms so we can divide it by itself removing it from the expression (equations 45 & 46).

Eq 45

$$v = \frac{[E]_{total} k_{cat} \frac{[E][S]}{K_M} + [E]_{total} k_{cat} \frac{[E][S][I]}{K_M K_i}}{[E] + \frac{[E][S]}{K_M} + \frac{[E][S][I]}{K_M K_i}}$$

Eq 46

$$v = \frac{[E]_{total} k_{cat} \frac{[S]}{K_M} + [E]_{total} k_{cat} \frac{[S][I]}{K_M K_i}}{1 + \frac{[S]}{K_M} + \frac{[S][I]}{K_M K_i}}$$

Next, we can recognize the total enzyme concentration multiplied by the catalytic rate is the V_{max} (equation 47) and we can then multiply all the terms by the KM (equation 48).

Eq 47

$$v = \frac{V_{max} \frac{[S]}{K_M} + V_{max} \frac{[S][I]}{K_M K_i}}{1 + \frac{[S]}{K_M} + \frac{[S][I]}{K_M K_i}}$$

Eq 48

$$v = \frac{V_{max}[S] + V_{max} \frac{[S][I]}{K_i}}{K_M + [S] + \frac{[S][I]}{K_i}}$$

From here we can factor out our traditional inhibition term (equation 19) to produce an equation that looks like one of the classical inhibition equations (equation 49).

Eq 49

$$v = \frac{V_{max}[S]\left(1 + \frac{[I]}{K_i}\right)}{K_M + [S]\left(1 + \frac{[I]}{K_i}\right)}$$

When we divide by the inhibition term, we end up with the KM being divided by the inhibition term (equation 50).

Eq 50

$$v = \frac{V_{max}[S]}{\frac{K_M}{\left(1 + \frac{[I]}{K_i}\right)} + [S]}$$

Inserting the rearranged form of the inhibition term, we have an equation (equations 51 & 52) that parallels the form of the noncompetitive equation (equation 29).

Eq 51

$$v = \frac{V_{max}[S]}{\frac{K_M}{\left(\frac{1}{1 - \frac{[I]}{K_i + [I]}}\right)} + [S]}$$

Eq 52

$$v = \frac{V_{max}[S]}{K_M - K_M\frac{[I]}{[I] + K_i} + [S]}$$

This equation implies that the substrate binding constant can only shrink as the inhibitor binds to the enzyme making it only useful for describing theoretical activation pushing the K_M to zero. This, just like in the traditional inhibition equations is due to the equation's lack of differentiation between the chemical equilibrium and the effect the inhibitor binding produces. To fix this we can modify the second K_M akin to what was done with the noncompetitive equation (equations 30 & 31),

by inserting a delta term that allows us to define changes in the substrate binding equilibrium according to what is observed experimentally (equation 53).

Eq 53

$$v = \frac{V_{max}[S]}{K_M - \Delta K_M \frac{[I]}{[I] + K_i} + [S]}$$

Mixed inhibition

The mixed inhibition equation (equation 54) was derived to describe changes affecting the chemical equilibrium and the catalytic rate at the same time.

Eq 54

$$v = V_{max} \frac{[S]}{K_M \left(1 + \frac{[I]}{K_{is}}\right) + [S]\left(1 + \frac{[I]}{K_{ii}}\right)}$$

This equation is a combination of the competitive and noncompetitive equations, but it is derived under the assumption that the binding of the inhibitor can only affect the substrate affinity by binding to the enzyme alone, and changes in catalytic rate are produced by binding to the enzyme-substrate complex. Hence, there are two inhibition constants as the inhibitor would have a different affinity for the enzyme versus the enzyme-substrate complex. Once again this is an artifact of the failure to distinguish between the chemical binding equilibrium and the effect the inhibitor produces when it binds to the enzyme. It is absurd to believe that a single binding interaction would be unable to affect the chemical equilibrium and catalytic rate at the same time, but when we divide by the second inhibition term to apply the inhibition terms to the V_{max} and K_M (equation 55) this is exactly what is implied.

Eq 55

$$v = \frac{V_{max}}{\left(1 + \frac{[I]}{K_{ii}}\right)} \frac{[S]}{\frac{K_M}{\left(1 + \frac{[I]}{K_{ii}}\right)}\left(1 + \frac{[I]}{K_{is}}\right) + [S]}$$

This is because if the inhibitor binding terms (K_{ii} & K_{is}) equal each other, their effect on the substrate affinity cancels out, reverting the mixed inhibition equation to the noncompetitive equation (equation 25). This has led to the claim that the noncompetitive inhibition equation is just a special form of the mixed inhibition equation.

Changes in catalytic rate and substrate affinity

To fix this problem we could simply insert, the terms we used to define changes in substrate affinity (equation 53) and catalytic rate (equation 30), into the Michaelis Menten equation. However, this equation can also be derived in the traditional way. To start with, we need to define the rate equation in the same way it was defined for the Michaelis Menten equation (10). We will also define the total enzyme states using the enzyme-inhibitor-substrate complex as we did when deriving the effect on substrate affinity (equation 37). This time we will start by rearranging our enzyme states to define the concentration of free enzyme. To do this we will start by inserting the inhibitor binding equilibrium (equation 41) to produce equation (56).

Eq 56

$$[E]_{tot} = [E] + [ES] + \frac{[ES][I]}{k_i}$$

Next, we will insert the enzyme-substrate equilibrium terms (equation 43) to remove the enzyme-substrate complex (equation 57).

Eq 57

$$[E]_{tot} = [E] + \frac{[E][S]}{K_M} + \frac{\frac{[E][S]}{K_M}[I]}{k_i}$$

From here we can factor out the enzyme-substrate equilibrium term to reveal the classic inhibition term (equation 58).

Eq 58

$$[E]_{tot} = [E] + \frac{[E][S]}{K_M}\left(1 + \frac{[I]}{k_i}\right)$$

Next, we can factor out the free enzyme (equation 59).

Eq 59

$$[E]_{tot} = [E]\left(1 + \frac{[S]}{K_M}\left(1 + \frac{[I]}{k_i}\right)\right)$$

Following this we will rearrange to define the concentration of the free enzyme (equation 60)

Eq 60

$$[E] = \frac{[E]_{tot}}{\left(1 + \frac{[S]}{K_M}\left(1 + \frac{[I]}{k_i}\right)\right)}$$

Now we will redefine the rate equation (equation 10) by replacing the concentration of the enzyme-substrate complex with the chemical equilibrium (equation 43) to give us equation (61).

Eq 61

$$v = k_{cat}\frac{[E][S]}{K_M}$$

This allows us to substitute equation (60) into the rate equation (61), removing the free enzyme concentration (equation 62).

Eq 62

$$v = k_{cat}\frac{[E]_{tot}[S]}{K_M\left(1+\frac{[S]}{K_M}\left(1+\frac{[I]}{k_i}\right)\right)}$$

We can now tidy it up a little by combining the catalytic rate and total enzyme concentration to produce the V_{max} and multiply the bottom by the K_M (equation 63).

Eq 63

$$v = \frac{[S]}{K_M+[S]\left(1+\frac{[I]}{k_i}\right)}V_{max}$$

This gets us to a form where we can now divide by the traditional inhibition term to apply it to the K_M and V_{max} (equation 64).

Eq 64

$$v = \frac{[S]}{\frac{K_M}{\left(1+\frac{[I]}{k_i}\right)}+[S]}\frac{V_{max}}{\left(1+\frac{[I]}{k_i}\right)}$$

From here we just need to rearrange the inhibition term into a form displaying the inhibitor's binding curve (equations 21-24, 27-29 & 51, 52) producing equation (65).

Eq 65

$$v = \frac{[S]}{K_M - K_M\frac{[I]}{k_i+[I]}+[S]}V_{max} - V_{max}\frac{[I]}{k_i+[I]}$$

This gets us to an equation that clearly displays the inhibitor binding curve which allows a single inhibitor binding interaction to affect substrate affinity and the reaction rate. However, this equation also only defines inhibition with the inhibitor's chemical equilibrium for binding the enzyme, so must be modified to distinguish the effect from the binding equilibrium (equation 66).

Eq 66

$$v = \frac{[S]}{K_M - \Delta K_M \frac{[I]}{k_i + [I]} + [S]} V_{max} - \Delta V_{max} \frac{[I]}{k_i + [I]}$$

Conclusions

The use of the traditional inhibition term (equation 19) represents a failure to distinguish between the chemical equilibrium for enzyme-inhibitor binding and the effect the inhibitor produces when it binds to the enzyme. This problem has persisted for more than a century confusing and complicating the study of molecular interactions in biological systems. Equation (66) resolves the issue by clearly separating the chemical equilibrium from the effect. This equation has been present in the literature for many years (Fontes et al., 2000; Walsh et al., 2007), providing a superior unified equation for the modeling of inhibitory and activator effects on enzymatic activity. The lack of adaption of this equation into biological studies and drug development is most likely attributable to the failure to recognize the fundamental flaw associated with the traditional inhibition term. However, with the recognition of the failure to distinguish between the chemical equilibrium and inhibitor effect associated with the traditional inhibition equations, equation (66) shifts from being a better model for these molecular interactions to the correct model.

With over a century of theoretical development without recognition of this problem, the field of enzyme kinetics has expanded in artificial complexity and dwindled in relevance. The artificial complexity arises from a desire to fix the failings of the classic inhibition equations by deriving more equations to fill in the gaps. The huge landscape of equations developed to describe inhibitory effects, many of them derived from individual biological systems, did not provide significant enough insight to be of interest to many beyond the researchers deriving them, and in the end, the field reverted to the dominance of the classic inhibition equations and IC50 values.

The biological sciences are in a renaissance period with the emergence of the Omics fields, big data, and AI, but conspicuously missing is the study of modifiers of enzyme kinetic activity. While attempts have been made to standardize and establish databases compiling enzymatic information (Prešern & Goličnik, 2023), only the Braunschweig Enzyme Database (BRENDA) and the System for the Analysis of Biochemical Pathways—Reaction Kinetics (SABIO-RK), contain information relating to enzyme inhibitors, and this data is limited to the classic inhibition equations and ic50 values. This provides the impression that all enzymes conform to Michaelis Menten's kinetics, stifling and confusing the study of biological systems, which ultimately hinders the development of therapeutics. As the performance of AI relies on the quality of the data it is trained on, it is unlikely that the agents being trained on Omics databases will be able to provide much more insight into diseases, as the biological databases they are being trained on, can be thought of as blueprints that lack significant biological context. Drawing a statistical correlation to expression levels or mutational prevalence in a population has been used for many years and in diseases like Alzheimer's disease has in the end led to articles linking the disease to most processes in the cell (Morgan et al., 2022). However, equation (66) while specific for enzymes that follow Michaelis Menten kinetics provides a simple intuitive interpretation of biological interactions (Walsh et al., 2007; Walsh, 2014). Additionally, it can act as an expandable framework for assessing more

complicated interactions (Walsh, 2012; Walsh 2019; Walsh & Blain 2020). This adaptability provides more context to biological systems, facilitating the generation of new mechanistic theories, which have the potential to impact the search for cures for diseases like Alzheimer's disease (Walsh, 2023).

For more information on these topics please follow my YouTube channel Fractured Biochemistry.

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