Ctrl & Comp in Bio Sys - Westlake Univ., Fall 2025

Lecture 4: Diversity of bioregulation by time scale separation and Adaptation biomachines

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Lecturer: Fangzhou Xiao *Scribe: Jiacheng Wei + Chengqian Li + Xia Yao*

Contents

1 The explanation of chemical reaction network equation

At first, let us review how we describe a system of chemical reaction network (CRN). According to our lecture, we have equation below:

$$\dot{\vec{x}} = \frac{dx}{dt} = \Gamma \Lambda_k x^{\alpha}$$

But this equation looks confusing because it lacks an explanation of each character. Let us write this equation in a more understandable form:

$$\dot{\vec{x}} = \frac{d\vec{x}}{dt} = \Gamma \Lambda_k \vec{x}^{\alpha}$$

Here, $\dot{\vec{x}}$ means derivative of x with respect to t, so it is equal to dx / dt. And x is called by me the **concentration vector of biomolecules** because it represents every concentration of biomolecules we consider. It can be written as $[x_1, x_2 ..... x_n]^T$. So $\dot{\vec{x}}$ means the conception change of every biomolecule we consider, it can be written as $[dx_1/dt, dx_2/dt ..... dx_n/dt]^T$.

For Γ, it is the uuppercase form of gamma (γ), it is called **the matrix of stoichiometric number change** by me. To understand this, we must first figure out the definition of "stoichiometic number chnange". For a chemical reaction:

$$4x_1 + x_2 = 2x_1 + 3x_2$$

The coefficient in front of the variable x_1 and x_2 is called the stoichiometric number. To make a difference of the coefficient in front of these reactants and products, let us use $_1$ to represent the coefficient "4" in front of reactant x_1, use $_2$ to represent coefficient "1" in front of reactant x_2. Similarly, we can also use $_1$ and $_2$ to represent cofficient in front of products x_1 and x_2. We notice the x_1 and X_2 appear in both reactants and products, so we can then definite another physical quantity γ, for γ_1, it equals to $\alpha_1 - \beta_1$, for γ_2, it equals to $\alpha_2 - \beta_2$. The γ shown here is "stoichiometic number chnange". For every variable, or biomolecules we consider, they have a unique γ. And when $\gamma < 0$, that means after this reaction happens, the quantity of this biomolecule will decrease. For example, for biomolecule x_1, after one single reaction happens, it will consume $4x_1$ biomolecules and generates 2, so it consumes $2x_1$ in total. And for $\gamma > 0$, that means after this reaction happens, the quantity of this biomolecule will increase. Notably, for many biochemical reactions or even chemical reactions that occurred in tubes or reaction kettles, the molecules will rarely appear on both both reactant side and the product side, because they will be invited. One example is alkaline hydrogen oxygen fuel cell, the positive pole half reaction is:

$$2H_2 - 4e^- + 4OH^- = 4H_2O$$

and the negative pole half reaction is:

$$O_2 + 4e^- + 2H_2O = 4OH^-$$

when we consider the whole reaction of this battery, we just simply combine this 2 half reactions together, and it becomes:

$$2H_2 + O_2 + 2H_2O = 4H_2O$$

For water, it appears in both reactant side and product side, we can divide $2H_2O$ from both side, so we get:

$$2H_2 + O_2 = 2H_2O$$

but we can also save the divided 2 water molecules in both sides, because they have practical significance, it means 2 water molecules is consumed in negative pole, and 4 water molecules generated in positive pole. For water, its α equals to 4, its β equals 2, and its γ equals 4-2=2.

So, for "the matrix of stoichiometric number change", it is the matrix formed by γ, in other words, every element in this matrix is γ.

So how do these γ arrange? We first need to definite the chemical reaction network (CRN). Now this CRN can be written as:

$$\alpha_{11}x_1 + \alpha_{12}x_2 + ... + \alpha_{1n}x_n = \beta_{11}x_1 + \beta_{12}x_2 + \beta_{1n}x_n$$
$$\alpha_{21}x_1 + \alpha_{22}x_2 + ... + \alpha_{2n}x_n = \beta_{21}x_1 + \beta_{22}x_2 + \beta_{2n}x_n$$
$$.....$$
$$\alpha_{m1}x_1 + \alpha_{m2}x_2 + ... + \alpha_{mn}x_n = \beta_{m1}x_1 + \beta_{m2}x_2 + \beta_{mn}x_n$$

Here, M and N are positive integers.

For the first row of the matrix, it represents the first reaction of CRN written above, it is:

$$[\gamma_{11}, \gamma_{12}.....\gamma_{1n}]$$

The second row of the matrix is:

$$[\gamma_{21}, \gamma_{22}.....\gamma_{2n}]$$

The last row, which is the number m row, is:

$$[\gamma_{m1}, \gamma_{m2}.....\gamma_{mn}]$$

For Λ_k, it is called **diagonal matrix of reaction rate constant**. In linear algebra, we often use Λ to represent a diagonal matrix. The diagonal matrix means every element don't locate in diagonal line is zero. In other words, for element a_{ij} in matrix $A_{m\times n}$, where $1 \leqslant i \leqslant m$, $1 \leqslant j \leqslant n$, unless i=j, $a_{ij} = 0$. But that doesn't mean when i=j, $a_{ij} \neq 0$. Another question is what is "reaction rate constant", that means for a reaction $4x_1 + x_2 = 2x_1 + 3x_2$, according to the Law of mass action the reaction rate equals to $v = k \times x_1^4 \times x_2$, the k shown here is "reaction rate constant". So, for this diagonal matrix, it can be written as k_1, k_2.....k_n located on the diagonal line.

The last one in this equation needed to explain is $\vec{x}^{\alpha}$, I call it **mass action expression vector**. For every element in this vector, its form is:

$$x_{i1}^{\alpha_{i1}} + x_{i2}^{\alpha_{i2}} + ... + x_{in}^{\alpha_{in}}$$

with $1 \leqslant i \leqslant m$.

This so-called "mass action expression vector" is formed by mass action expression of each reaction in CRN. And the first reaction occupies the first row of this vector, the second reaction occupies the second row of this vector..... In other words, if the reaction "$\alpha_{11}x_1 + \alpha_{12}x_2 + ... + \alpha_{1n}x_n = \beta_{11}x_1 + \beta_{12}x_2 + \beta_{1n}x_n$" is represented by "reaction 1",

the mass action expression of reaction 1 is $x_{11}^{\alpha_{11}} + x_{12}^{\alpha_{12}} + ... + x_{1n}^{\alpha_{1n}}$. If the mass action expression of reaction 1 is represented by "M1", the "mass action expression vector" can be written as:

$$[M_1, M_2.....M_m]^T$$

That is all the characters participated in the equation describing the chemical reaction network (CRN). And now I will briefly introduce how can we get this equation. Now we only consider there is one reaction in the CRN, the CRN can be described by:

$$\alpha_1 x_1 + \alpha_2 x_2 = \beta_1 x_1 + \beta_2 x_2$$

What does $d\vec{x}/dt$ mean here? It is $[dx_1/dt, dx_2/dt]^T$, so we solve these two elements of this vector separately. For dx_1/dt, it equals:

$$dx_1/dt = \gamma_1 \times \nu_1 = \gamma_1 \times k_1 \times x_2^{\alpha_1} \times x_2^{\alpha_2}$$

The first "=" above comes from the relationship between molecule changing rate and reaction rate. Considering in 1 second, the reaction happens N times, and the γ represents when there is 1 reaction happens, the molecules change (increase/decrease) is $\gamma = \alpha - \beta$, the molecule changes is $\gamma \times N$. Notably, the γ can indicate whether molecules increase or decrease, as we discussed above. The second "=" is the Law of mass action.

When the reaction number increases to m, the total molecule number increase to n, the equation of chemical reaction network becomes:

$$\dot{x} = \frac{d\vec{x}}{dt} = \Gamma \Lambda_k \vec{x}^{\alpha}$$

2 Time Scale Separation

What is the meaning of "Time Scale Separation"? I think it is the thought to consider those "fast reaction" and "slow reaction". The meaning of "fast reaction" is that it can become chemical equilibrium quickly. For example, we consider the enzyme catalysis process:

$$E + S <=> [k_1][k_{-1}]ES -> [k_{cat}]E + P$$

Here we consider the first step (binding reaction). In this step, Enzyme (E) binds substance (S) and becomes complex ES, and ES can dissociate into E and S. In other words, this step is reversible. For any reversible chemical reaction, we can use reaction equilibrium constant K (uppercase) to describe how thorough is the reaction, which is a thermodynamic parameter. And there is also reaction rate constant k (lowercase) to describe how fast can the reaction go, which is a kinetic parameter and obey the Law of Mass Action.

$$K = \frac{k_1}{k_{-1}} = \frac{c(E) \times c(S)}{c(ES)}$$

For the dissociation constant K_d, it describes how easy the complex ES can dissociate, which is the reciprocal of K.

$$K_d = \frac{1}{K}$$

The second step (catalytic reaction) is the complex ES becomes E and product (P). This process is considered as irreversible, so there is only one reaction rate constant called K_{cat} representing the k of catalysis.

When we use the principle of "Time Scale Separation", we consider the binding reaction as fast reaction, and the catalytic reaction as slow reaction. That means we can consider the binding reaction has reached chemical equilibrium. Notably, this opinion (fast reaction has reached chemical equilibrium) is used by Michaelis and Menten in 1913 to explain enzyme kinetics. And in 1925, G.E.Briggs and James B.S.Haldane use steady-state approximation (SSA) to explain enzyme kinetics, and SSA is considered as a better model in biochemistry textbook. In SSA, it no longer considers the binding reaction reaches equilibrium, it considers the concentration of ES complex is not changed, in other words, it has $\frac{dc(ES)}{dt} = 0$, so it satisfies the following equation:

$$k_1 \times E \times S = k_{-1} \times ES + k_{cat} \times ES$$

But in the following part, we use "Time Scale Separation", that means we should remember the binding reaction is in equilibrium, and this equation is true in any case.

$$K = \frac{E \times S}{ES}$$

$$K = \frac{E \times S}{C}$$

Here, C and ES has the same meaning, they are the concentration of enzyme-substance complex. **Here, I am not sure why Fangzhou thinks here he uses Quasi-steady state assumption (QSSA), I think QSSA means $\frac{dc(ES)}{dt}0$, but it changes very slow, much slower than the changes of S, in other words, $\frac{dc(ES)}{dt} \ll \frac{dc(S)}{dt}$.**

With this background knowledge, **let us start to understand Fangzhou's regime thoery!** Let us start with a 2-dimension graph, the x-axis is S_{tot}, which means it is the total substance concentration. For an enzyme kinetics assay, it is the final substance concentration you add

in the tube. The y-axis is the E_{tot}, which means the total concentration of enzyme. The graph can be divided into three regimes, each regime has distinct biological meaning.

Interestingly, the term "regime" carries profound religious connotations, originally referring to the specific parishes overseen by missionaries from different churches during the Middle Ages. This is similar to the word "dogma" in the central dogma—which initially denoted doctrines in the Bible. Likewise, "transcript" originally referred to the copying of religious scriptures; "translation" initially meant translating religious texts, such as rendering written Latin religious works into spoken English; and "canonical" derives from the religious term "canon," meaning authoritative texts or norms.

So why this graph divides the whole space as three regimes? Fangzhou pointed out the restrictions of every regime, that is like the version of the Bible used by missionaries in this parish (regime).

In regime 1, the restriction is $E_{tot} = E, S_{tot} = S$. The biological meaning of this regime is the binding affinity of enzyme and substance is low, there is very little ES complex in the solution. Because $E_{tot} = E$ and $E_{tot} = E + C$, E represents free enzyme that doesn't bind substance, C represents ES complex, as described above. So we can get $E \gg C$ and $E_{tot} \gg C$. Similarly, we can get $S \gg C$ and $S_{tot} \gg C$. Now we focus on the equilibrium equation $K_d = \frac{E \times S}{C}$, we can get $\frac{S}{K_d} = \frac{C}{E}$. And because we have $E_{tot} = E, S_{tot} = S$ in regime 1, we can get $\frac{S_{tot}}{K_d} = \frac{C}{E_{tot}}$. And because we previously proved $E_{tot} \gg C$, we have $\frac{S_{tot}}{K_d} = \frac{C}{E_{tot}} \ll 1$, that is $S_{tot} \ll K_d$. Similarly, we can also get $E_{tot} \ll K_d$. So, we know when we set the restriction $E_{tot} = E, S_{tot} = S$, it equals to $S_{tot} \ll K_d$ and $E_{tot} \ll K_d$, and it occupies the region surrounded by x-axis, y-axis, $x = K_d$ and $y = K_d$. In my figure, I set K_d as 1.

In regime 2, the restriction is $E_{tot} = E, S_{tot} = C$, we need to figure out where regime 2 located in 2D graph, And the answer is $E_{tot} \gg K_d$ and $E_{tot} \gg S_{tot}$. Here I will get the proof process. Because $E_{tot} = E$ and $E_{tot} = E + C$, we have $E_{tot} \gg C$. And because $S_{tot} = C$, we prove $E_{tot} \gg S_{tot}$. Then is the other border. Because $S_{tot} = S + C$ and $S_{tot} = C$, we get $C \gg S$. And because of the equilibrium equation $K_d = \frac{E \times S}{C}$, we can get $\frac{E}{K_d} = \frac{C}{S}$. Because we get $C \gg S$ before, we have $\frac{E}{K_d} \gg 1$, that is $E \gg K_d$, and that becomes $E_{tot} \gg K_d$. So we get two borders: $E_{tot} \gg K_d$ and $E_{tot} \gg S_{tot}$. That means the y-axis, $y = K_d$ and $x = y$ is the border of regime 2, as my figure shows. The regime 2 shows there are many enzyme, so almost all substance is binding with enzyme, and there is still many free enzyme left. In other words, in this tube, the dominant component is free enzyme, then the less one is enzyme-substance complex, and there is very little free substance here.

In regime 3, the restriction is $E_{tot} = C$ and $S_{tot} = S$. This restriction equals $S_{tot} \gg K_d$ and $S_{tot} \gg E_{tot}$. The proof process is the same as regime 2, just changes character E to character S. And in regime 3, the biological meaning is there are many substance in the tube, and almost all enzyme is binding with substance. The concentration of component is $S \gg ES \gg E$, and in regime 2 is $E \gg ES \gg S$.

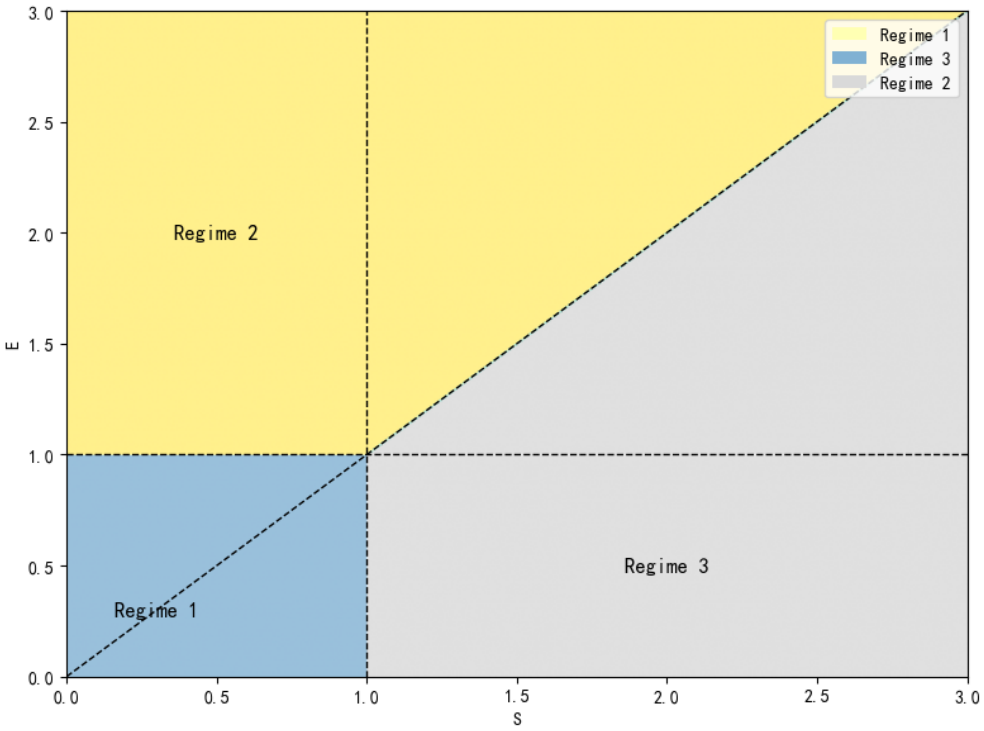

Figure 1 Three Regimes

So, we can draw the graph with 3 regimes now! It is as in Figure 1.

3 “LEGO” of Bioregulation

After we analyzed the 3 regimes, we can try to find what behaviors will have in this enzyme kinetic system. **In our class, Fangzhou gives us 3 situations, or so-called "LEGO". They are saturation, bottleneck and ultrasensitivity.** Now let us discuss each of them!

For saturation and bottleneck, they appear in the 2D graph, whose x-axis is S_{tot} and y-axis is C_{tot}, or call it ES_{tot}.

Let us talk about saturation now: imagine a line crossing regime 1 and regime 2, that means we don't change the total enzyme concentration, and increase the concentration of total substance. That is like we are doing a substance tritation assay to draw the Michaelis-Menten function. For this assay, the x-axis is the concentration of substance, that is the same as our figure, the S_{tot}. And the y-axis is the reaction rate, which has the relationship $v = k_{cat} \times C$ or $v = k_{cat} \times c(ES)$, that is the same as our y-axis, too.

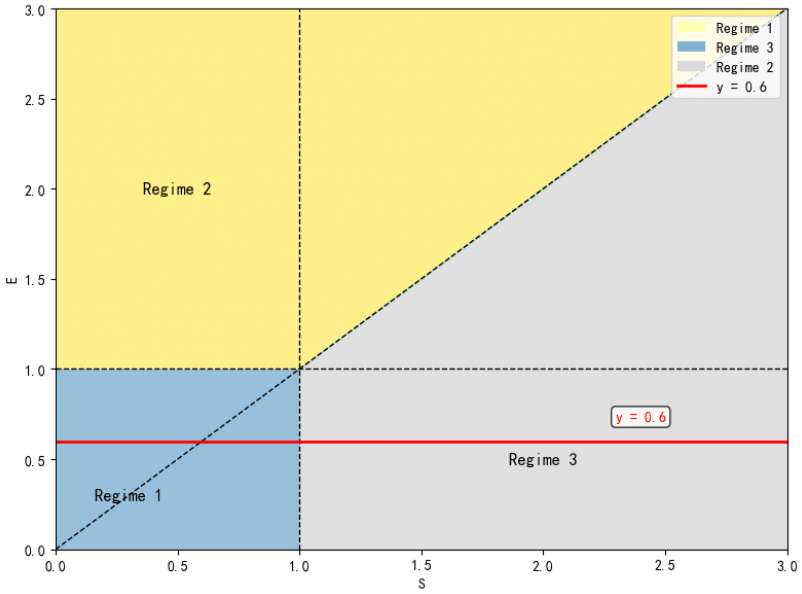

Figure 2

In regime 1, according to the equilibrium equation $K_d = \frac{E \times S}{C}$, we can get $C = \frac{E \times S}{K_d}$. Because in regime 1, we have $E_{tot} = E, S_{tot} = S$, so we get the equation of C:

$$C = \frac{E_{tot} \times S_{tot}}{K_d}$$

Because K_d and E_{tot} is constant now, because we just move along a line parallel to x-axis, the concentration of total enzyme is the same, and the K_d can't change when we choose a

pair of special enzyme and substance. So the equation of C is at the same form of $y = kx$, which is positive proportional function, and the slope of C is $\frac{E_{tot}}{K_d}$.

In regime 3, the restriction is $E_{tot} = C$, so the equation of C is:

$$C = E_{tot}$$

Because E_{tot} is a constant, C becomes constant now.

To link these two equation of C, we can use Hill function. The form of Hill function is as below.

$$\theta = \frac{E}{E + C}$$

The θ here is called Hill coefficient, which is proposed by Archibald Vivian Hill. The Hill coefficient is well-known by the explanation of the binding of oxygen and hemoglobin. For our enzyme catalysis process, the concentration of ES complex can be described as below.

$$C = (E + C) \times \frac{C}{E + C} = E_{tot} \times \frac{C}{E + C} = E_{tot} \times (1 - \theta)$$

Now we call $1 - \theta$ as φ, and it can be written as below.

$$\varphi = 1 - \theta = \frac{C}{E + C} = \frac{\frac{E \times S}{K_d}}{E + \frac{E \times S}{K_d}} = \frac{\frac{S}{K_d}}{1 + \frac{S}{K_d}}$$

So, the C can be written as below.

$$C = E_{tot} \times (1 - \theta) = E_{tot} \times \varphi = \frac{\frac{E_{tot} \times S}{K_d}}{1 + \frac{S}{K_d}}$$

Because in regime 1 and 3, we alaways have $S = S_{tot}$, so the equation can be written as below.

$$C = \frac{\frac{E_{tot} \times S}{K_d}}{1 + \frac{S}{K_d}} = \frac{\frac{E_{tot} \times S_{tot}}{K_d}}{1 + \frac{S_{tot}}{K_d}}$$

When in regime 1, we have $S_{tot} \ll K_d$, so we get $\frac{S_{tot}}{K_d} \ll 1$, we can ignore the $\frac{S_{tot}}{K_d}$ in the denominator, the equation changes to this form.

$$C = \frac{\frac{E_{tot} \times S_{tot}}{K_d}}{1} = \frac{E_{tot} \times S_{tot}}{K_d}$$

When in regime 3, we have $S_{tot} \gg K_d$, so we get $\frac{S_{tot}}{K_d} \gg 1$, we can ignore the "1" in the denominator, the equation changes to this form.

$$C = \frac{\frac{E_{tot} \times S_{tot}}{K_d}}{\frac{S_{tot}}{K_d}} = E_{tot}$$

So, this equation can link both regime 1 and regime 3, let us review the equation described C again.

$$C = E_{tot} \times \varphi = \frac{\frac{E_{tot} \times S_{tot}}{K_d}}{1 + \frac{S_{tot}}{K_d}}$$

So when crossing regime 1 and regime 3, in other words, when the total concentration of substance crossing K_d, the concentration of C is changing smoothly. In the language of mathematics, the left limit of $S_{tot} = K_d$ is equal to the right limit.

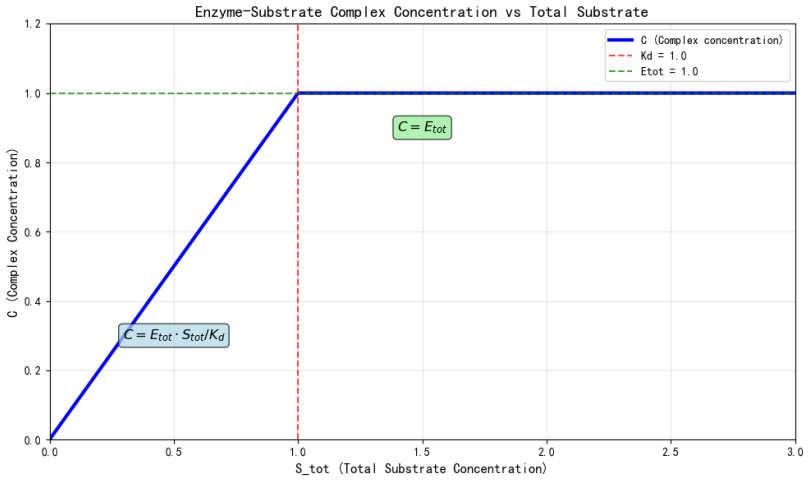

Figure 3

Then let us talk about another behavior, or another "LEGO". It is called "**bottleneck**". Bottleneck appears when the concentration of total substance moves along a line parallel with x-axis and crosses regime 2 and regime 3. That means the total concentration of enzyme is relatively high (higher than K_d). And the concentration of total substance increases from relatively low ($S_{tot} \ll K_d$) to relatively high ($S_{tot} \gg E_{tot} \gg K_d$). When

in regime 2, the restriction is $E_{tot} = E$ and $S_{tot} = C$. So, the concentration of C is very obvious.

$$C = S_{tot}$$

And in regime 3, the restriction is $E_{tot} = C$, so the equation of C is:

$$C = E_{tot}$$

To link these two situation, the concentration of C can be written as below.

$$C = min(S_{tot}, E_{tot})$$

This form looks like the concentration of C is controlled by the smaller one of the total concentration of enzyme or substance.

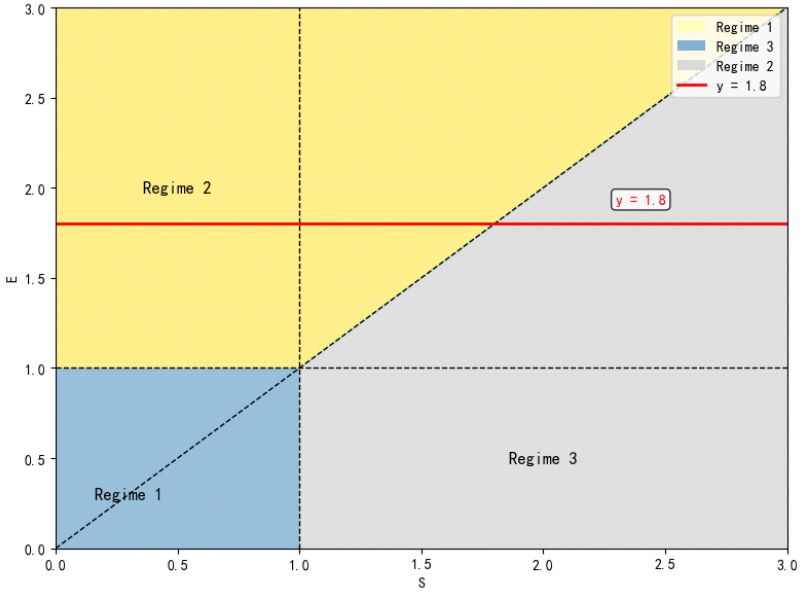

Figure 4

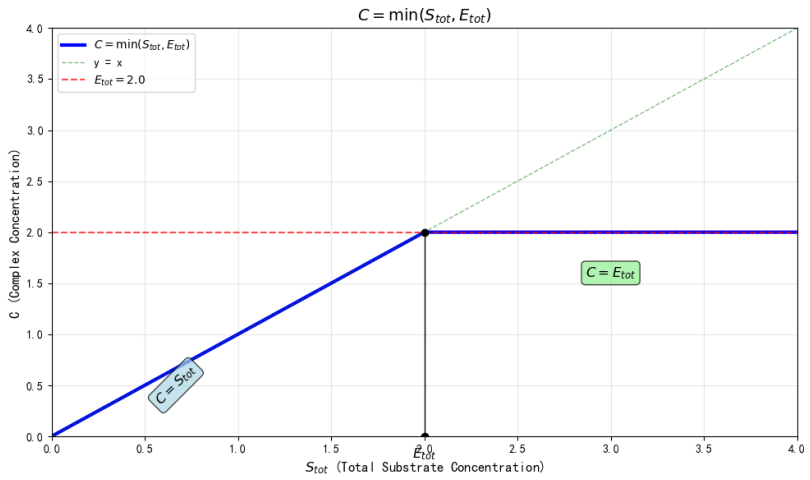

Figure 5

The third behavior or "LEGO" appears in the $E_{tot} - S_{tot}$ graph, which means the x-axis is S_{tot} and the y-axis is E_{tot}. The third behavior is called "ultrasensitivity". we continues to imagine a line parallel to x-axis crossing regime 2 and 3, in regime 2, the restriction is $E_{tot} = E, S_{tot} = C$, so the concentration of enzyme is as below.

$$E = E_{tot}$$

In regime 3, the restriction becomes $S = S_{tot}$ and $E_{tot} = C$. So the concentration of enzyme is no longer equals E_{tot}. Considering the chemical equilibrium equation, the form can switch as below.

$$K_d = \frac{E \times S}{C} = \frac{E \times S_{tot}}{E_{tot}}$$

Here, we use $S = S_{tot}$ and $E_{tot} = C$ to change the E and C in the equation. So the concentration of enzyme is as below.

$$E = \frac{E_{tot} \times K_d}{S_{tot}}$$

So, if we consider the left limit and right limit when $S_{tot} = E_{tot}$, we will find they are different. Why we consider the border is $S_{tot} = E_{tot}$ here? That is because the border of regime 2 and 3 is no longer $S_{tot} = K_d$, but it is $S_{tot} = E_{tot}$.

For the left limit, it is as below.

$$\lim_{S_{tot} \to E_{tot}^{+}} E = E_{tot}$$

For the right limit, it is as below.

$$\lim_{S_{tot} \to E_{tot}^{-}} E = \frac{E_{tot}^2}{K_d}$$

So, there is a gap when S_{tot} is crossing the border of regime 2 and regime 3. And we can also notice when E_{tot} is bigger, the gap will become bigger. That is because the length of the gap equals to:

$$length = \frac{E_{tot}^2}{K_d} - E_{tot} = E_{tot}(\frac{E_{tot}}{K_d} - 1) = E_{tot} \times \frac{E_{tot} - K_d}{K_d}$$

When $E_{tot} - K_d$ becomes bigger, the length of the gap will become bigger, the ultrasensitivity will become much more obvious!

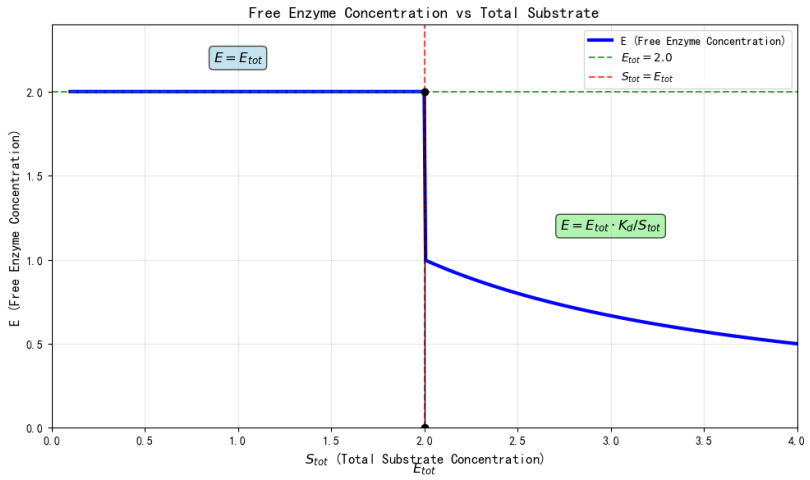

Figure 6

For ultrasensitivity, there is an example given in our class: imagine the binding of a kind of ligand and receptor is very tight (almost irreversible), that means when c(ligand) is bigger than c(receptor), there is almost no free receptor, almost all receptor is binding with ligand. And now the free ligands start to degrade, for example, they are ubiquitinated protein inhibitor. So when the total concentration of ligand crosses the border of $S_{tot} = E_{tot}$, here S_{tot} means the total concentration of ligand and E_{tot} means the total concentration of receptor, the concentration of receptor will undergo a very shape increase from almost zero to almost E_{tot} (the total concentration of receptor). This is just like the substance

or inhibitor of enzyme with Hill coefficient larger than 1, for example, the figure shown below.

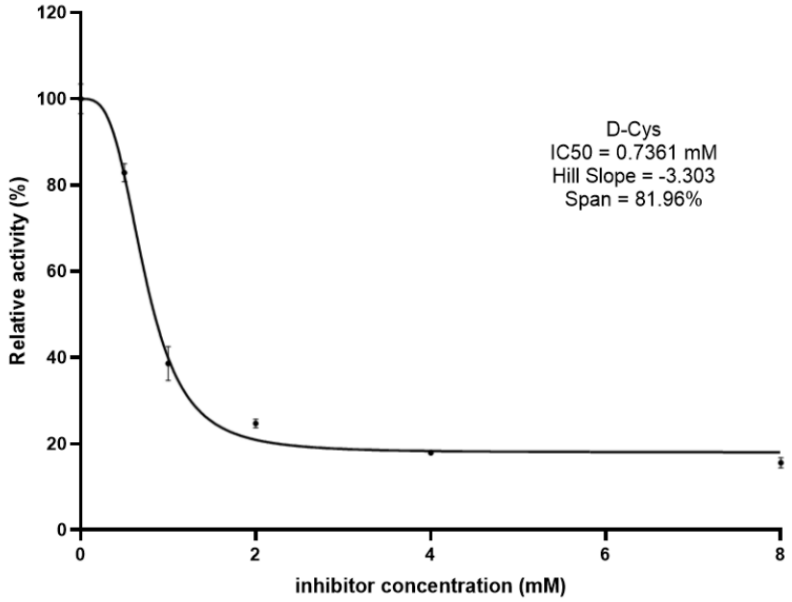

Figure 7

4 Adaptation Biomachines

In the section, Fangzhou told us a way to control the system, avoiding being disturbed. This well-known and widely-used control method is Proportion Integration Differentiation (PID). We can describe PID controller as the equation below.

$$u(t) = K_p \times y(t) + K_I \times \int_0^t y(t)\,dt + K_D \times \frac{dy(t)}{dt}$$

Here, u(t) is the output of a PID controller, and y(t) is the disturbance. The PID controller contains three parts: the present, the past, and the future.

$K_p \times y(t)$ represents the present, it is to give a force in the opposite direction.

And $K_I \times \int_0^t y(t)\,dt$ presents the past, because it is the integral over all time in the past. It is used to control the steady-state error. The steady-state error means the error is relatively small and will not grow when time goes. But if we allow this small error to accumulate, the system will become far from steady state over time.

And the $K_D \times \frac{dy(t)}{dt}$ represents the future, because it contains the derivative of y(t), it can reveal the change of y(t). This item can prevent the system from falling into shock, or control the over regulation caused by the first two items.