LECTURE 2

* How do we model biochemical reactions that happen in a cell?

- From Chemistry we know that reactions can be described by using the LAW OF MASS ACTION:

In Chemistry: $A + B \underset{k_2}{\overset{k_1}{\rightleftharpoons}} C$

$\Downarrow$

The notation says that chemicals A and B must interact in the ratio 1:1 to form product C. The reaction is reversible and it occurs at a rate that depends on constants k_1 (formation of C) and k_2 (decomposition of C into A, B again)

LoMA: $A + B \underset{k_2}{\overset{k_1}{\rightleftharpoons}} C \iff \begin{cases} \frac{d[C]}{dt} = k_1[A][B] - k_2[C] \\ \frac{d[A]}{dt} = k_2[C] - k_1[A][B] \end{cases}$

(concentrations)

The LoMA is a "model template" to represent a reaction as a system of ODEs It relates the rate of accumulation of the product $\left(\frac{d[C]}{dt}\right)$ or of the reactants A, B (e.g., $\frac{d[A]}{dt}$) to the concentrations of the chemicals [A], [B], and [C] The coefficients k_1, k_2 are "rate constants" and depend on (1) shape and size of the molecules and (2) the temperature of the mixture

In most of the cases the reverse reaction $A + B \leftarrow C$ is much slower than the forward reaction $A + B \rightarrow C$ $\Rightarrow$ In the LoMA: $k_1 >> k_2$

$\Downarrow$

$$\frac{d[C]}{dt} \cong k_1[A][B]$$

This highlights one feature of the LoMA, i.e., it is LINEAR:

If the concentration of either of the two reactants is doubled then the reaction rate doubles too!

$$[A]_0,\ [B]_0 \Rightarrow \frac{d[C]_0}{dt} \cong k_1 [A]_0 [B]_0$$

$$[A]_1 = 2[A]_0,\ [B_1] = [B]_0 \Rightarrow \frac{d[C]_1}{dt} \cong 2\frac{d[C]_0}{dt}$$

Also, there is an interesting relationship between reaction rates when the reaction involves chemicals of the same species:

$$A + A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} C$$

LoMA ⇕

$$\frac{d[C]}{dt} = k_1 [A]^2 - k_2 [C]$$

$$\frac{d[A]}{dt} = \underbrace{2k_2 [C]}_{\text{there is a "2" because for each unit of C two units of A are released}} - \underbrace{2k_1 [A]^2}_{\text{there is a "2" because for each unit of C that is formed, two units of A are consumed}}$$

Hence: $\frac{d[A]}{dt} = -2\frac{d[C]}{dt}$ ⇒ The reaction rate for the reactant in the reverse reaction is twice the rate for the product in the forward reaction

It happens because of the principle of conservation:

$$x \triangleq [A] + 2[C]$$

$$\frac{dx}{dt} = \frac{d[A]}{dt} + 2\frac{d[C]}{dt} = -2\frac{d[C]}{dt} + 2\frac{d[C]}{dt} = 0$$

⇓

The concentration of C is always half the concentration of A

The LoMA predicts that at equilibrium (i.e., when the concentrations of reactants and product do not change anymore) one has:

(a) $[A] + [B] \underset{k_2}{\overset{k_1}{\rightleftharpoons}} [C]$

At equilibrium $\frac{d[C]_{eq}}{dt} = 0 \Rightarrow k_1 [A]_{eq} [B]_{eq} - k_2 [C]_{eq} = 0 \Rightarrow$

$$\frac{[A]_{eq}[B]_{eq}}{[C]_{eq}} = \frac{k_2}{k_1} = \text{constant}$$

$k_{eq} \triangleq \frac{k_2}{k_1}$ - equilibrium constant - It describes the relative preference for the chemicals to be in the combined state C compared to the dissociated state

$k_{eq} >> 1 \Leftrightarrow k_2 >> k_1 \Leftrightarrow$ The preference is for the dissociated state

$k_{eq} << 1 \Leftrightarrow k_1 >> k_2 \Leftrightarrow$ The preference is for the combined state

Note this:

$$\frac{d[A]}{dt} + \frac{d[C]}{dt} = \left(-k_1[A][B] + k_2[C]\right) + \left(k_1[A][B] - k_2[C]\right) = 0 \Rightarrow$$

↑ assuming that A and C are involved only in reaction (a)

$\Rightarrow [A] + [C] = \text{constant}$

Let us call: $A_0 \triangleq [A] + [C]$ - Then we have:

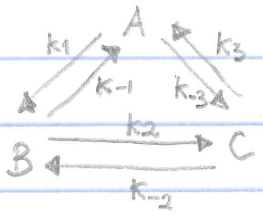
$$\frac{[A]_{eq}[B]_{eq}}{[C]_{eq}} = k_{eq} \Rightarrow [C]_{eq} k_{eq} = (A_0 - [C]_{eq})[B]_{eq} \Rightarrow [C]_{eq} = A_0 \frac{[B]_{eq}}{k_{eq} + [B]_{eq}}$$

Hence, at equilibrium we can set a direct relationship between $[C]_{eq}$ and $[B]_{eq}$

In particular: $[B]_{eq} = k_{eq} \Rightarrow [C]_{eq} = \frac{A_0}{2} \Rightarrow$ Half of reactant A has been converted in C

The LoMA is the "building block" we use to model more complex chemical reactions that may occur in a cell:

Ex:



$$\frac{d[A]}{dt} = k_{-1}[B] + k_3[C] - (k_1 + k_{-3})[A]$$

$$\frac{d[B]}{dt} = k_1[A] + k_{-2}[C] - (k_2 + k_{-1})[B]$$

$$\frac{d[C]}{dt} = k_2[B] + k_{-3}[A] - (k_{-2} + k_3)[C]$$

This example allows us to study the implications of LoMA when the reactions form a loop:

At equilibrium:

$$(k_1 + k_{-3})[A]_{eq} = k_{-1}[B]_{eq} + k_3[C]_{eq}$$

$$(k_2 + k_{-1})[B]_{eq} = k_1[A]_{eq} + k_{-2}[C]_{eq}$$

$$(k_3 + k_{-2})[C]_{eq} = k_2[B]_{eq} + k_{-3}[A]_{eq}$$

These relationships are linearly dependent (the sum of any two gives the

third one) ⇒ The problem has a degree of freedom, i.e., one among $[A]_{eq}$, $[B]_{eq}$, and $[C]_{eq}$ must be known in order to determine the other two

⇓

This reflects the fact that the model focuses on having the net concentrations in equilibrium but it does not look at whether each reaction is in equilibrium

Ex: $(k_1 + k_{-3})[A]_{eq} = k_{-1}[B]_{eq} + k_3[C]_{eq}$ - It says the net amount of A transformed in B or C must equalize the amount of A produced from B and C but it doesn't say that the amount of A transformed in B must equalize the amount of A produced from B

⇓

This latter condition is what happens at equilibrium and it reflects a state of energetic equilibrium (thermodynamic equilibrium). Hence we must correct the model by adding the constraints:

$$\left.\begin{aligned} k_1[A]_{eq} &= k_{-1}[B]_{eq} \\ k_2[B]_{eq} &= k_{-2}[C]_{eq} \\ k_3[C]_{eq} &= k_{-3}[A]_{eq} \end{aligned}\right\} \Rightarrow \begin{aligned} k_{-1}/k_1 &= [A]_{eq}/[B]_{eq} \\ k_{-2}/k_2 &= [B]_{eq}/[C]_{eq} \\ k_{-3}/k_3 &= [C]_{eq}/[A]_{eq} \end{aligned}$$

these are equilibrium constants

The constraints above can be boiled down to the constraint:

$$k_{eq1} \triangleq \frac{k_{-1}}{k_1}$$

$$k_{eq2} \triangleq \frac{k_{-2}}{k_2}$$

$$k_{eq3} \triangleq \frac{k_{-3}}{k_3}$$

$$\boxed{k_{eq1} \cdot k_{eq2} \cdot k_{eq3} = 1}$$

Summarizing, LoMA is an appealing tool to model biochemical reactions but: (1) it builds linear relationships and biochemical reactions are nonlinear

(2) it may need additional constraints in case of detailed balance

(3) it assumes that both reactants and products are changed during the reaction while biochemical reactions often involve CATALYSTS

⇓

We need to bear in mind these limitations and use LoMA as a building block in more sophisticated modeling architectures that compensate for these limitations

For instance, with regard to (3), one must remember that a catalyst is a compound that facilitates the reaction (e.g., it accelerates the reaction by lowering the free energy of activation of the reaction) but it is not changed by the reaction. Enzymes are catalysts and are involved in a number of biochemical reactions in the cells

- Deviations of enzyme reactions from the LoMA
  - enzymes are not changed by the reaction
  - enzymes limit the effects of the concentration of reactants on the reaction rate ⇒ NONLINEARITY

- Solution: The enzyme (E) reaction to convert a reactant S (substrate) into a product P is modeled as a two-stage process with an intermediate product:

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C \xrightarrow{k_2} P + E$$

C: complex later broken into P and E

Note that there is no reverse reaction from P+E to C

⇓

We assume that product P is continuosly removed thus preventing the reverse reaction to occur

Hence the "model template" is:

$e \triangleq [E]$

$s \triangleq [S]$

$c \triangleq [C]$

$p \triangleq [P]$

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C \xrightarrow{k_2} P + E$$

$$\begin{cases} \frac{dc}{dt} = k_1 se - k_2 c - k_{-1} c \\ \frac{ds}{dt} = k_{-1} c - k_1 se \\ \frac{dp}{dt} = k_2 c \\ \frac{de}{dt} = k_{-1} c - k_1 se + k_2 c \end{cases}$$

Note this: $\frac{dc}{dt} + \frac{de}{dt} = 0 \Rightarrow [C] + [E] = e_0 - \text{constant}$

This means that we are assuring that the total amount of enzyme does not change over time

Let us focus on two central equations in this model:

$\frac{ds}{dt} = k_{-1} c - k_1 se$

It tells the rate at which the substrate is consumed

$\frac{dp}{dt} = k_2 c$

It tells the rate at which the product is formed $\Rightarrow \frac{dp}{dt} = V$ velocity of the reaction

To study the rate of formation of the product, it is often appropriate to assume that S and C are in instantaneous equilibrium; i.e.:

$$\boxed{k_{-1} c = k_1 s e}$$

Hence: $k_{-1} c = k_1 s e \iff s e = \underbrace{\frac{k_{-1}}{k_1}}_{K_{eq_1}} c \iff e = K_{eq_1} \frac{c}{s} \iff$ (K_{eq_1} – equilibrium constant)

$\iff$ ($\uparrow$ $e + c = e_0$)

$$\iff e_0 - c = K_{eq_1} \frac{c}{s} \iff c = \frac{s e_0}{K_{eq_1} + s} \quad (a)$$

Let us now plug (a) into the equation for the reaction velocity:

$$v = \frac{dp}{dt} = k_2 c = k_2 e_0 \frac{s}{K_{eq_1} + s} \Rightarrow$$ The velocity is limited by the enzyme

At the beginning: $s \gg K_{eq_1} \Rightarrow v \tilde{=} k_2 e_0 \triangleq V_{max}$

If $K_{eq_1} \gg s$: $v \tilde{=} \frac{k_2 e_0}{K_{eq_1}} s = \frac{V_{max}}{K_{eq_1}} s$ – The relationship is approximately linear in S (as for the LoMA)

As the substrate becomes scarce: $s \tilde{=} 0 \Rightarrow v \tilde{=} 0$

To better understand: Plot $v = V_{max} \frac{s}{K_{eq_1} + s}$ for various sets (V_{max}, K_{eq_1}) of your choice and $s = 0 : 0.05 : 3.0$

Let us elaborate more on the assumption: $k_{-1}c = k_1 se$

- Is it enough?

$k_{-1}c = k_1 se \nearrow \quad \frac{ds}{dt} = 0$ - It cannot be true all the time otherwise there would be no product formed

$\Downarrow \quad \frac{dc}{dt} = -k_2 c$ - It is inconsistent with (a)

$\Downarrow$

We need further assumptions!

AA #1) $k_2 << k_{-1}$ - This implies: $\frac{dc}{dt} \cong k_1 se - k_{-1}c$ [C] varies at about the same rate as [S]

AA #2) The speed of consumption of S does not follow the LoMA

- So how do we estimate ds/dt ?

Note this: $\frac{ds}{dt} + \frac{dc}{dt} = \frac{d}{dt}(s+c) = -k_2 c \approx 0$

In light of AA#1 we can say that the total quantity s+c varies at such slow pace compared to the other quantities that one may assume S and C be in an instantaneous equilibrium

$$\frac{d}{dt}\left(s+c\right) = -k_2 c$$

$$\frac{d}{dt}\left(s + \frac{e_0 s}{k_{eq_1}+s}\right) = \frac{ds}{dt} + e_0 \frac{ds}{dt}\frac{1}{k_{eq_1}+s} - \frac{ds}{dt}\frac{e_0 s}{(k_{eq_1}+s)^2} =$$

$$= \frac{ds}{dt}\left(1 + \frac{e_0}{k_{eq_1}+s} - \frac{e_0 s}{(k_{eq_1}+s)^2}\right) =$$

$$= \frac{ds}{dt}\left(1 + \frac{k_{eq_1} e_0}{(k_{eq_1}+s)^2}\right)$$

Hence: $\frac{ds}{dt} = -k_2 \left(1 + \frac{k_{eq_1} e_0}{(k_{eq_1}+s)^2}\right)^{-1} c = -k_2 e_0 \frac{s(s+k_{eq_1})}{k_{eq_1} e_0 + (s+k_{eq_1})^2}$

Let us recap: • We had to model the enzyme reactions while taking into account the differences between this type of reactions and those obeying to the LoMA

• The key aspects of our solution were (1) introducing an intermediate stage and (2) assuming that complex C and substrate S are in instantaneous equilibrium

Another way to solve the problem is keeping (1) and replacing (2) with:
(2b) The rate of formation and breakdown of C must be almost equal

⇓

It means that complex C has reached a quasi-steady-state condition - This has a precise mathematical formulation:

$$\text{Quasi-steady-state condition} \iff \frac{dc}{dt} \cong 0$$

With this condition we have:

$$\frac{dc}{dt} = 0 \iff k_2 c = k_1 s e - k_{-1} c$$

Hence: $\frac{ds}{dt} = -k_2 c = -\frac{dp}{dt}$ ⇒ Substrate is consumed at the same rate product is formed

Also, because $e + c = e_0$ - constant, we have:

$$k_2 c = k_1 s e - k_{-1} c \iff k_2 c = k_1 s (e_0 - c) - k_{-1} c \iff$$

$$(k_{-1} + k_2) c + k_1 s c = k_1 s e_0 \iff c = \frac{s e_0}{s + \frac{k_{-1} + k_2}{k_1}}$$

Let us call: $k_m \triangleq \frac{k_{-1}+k_2}{k_1}$ $V_{max} \triangleq k_2 e_0$ - Hence:

$$V = \frac{dP}{dt} = \frac{k_2 s e_0}{s+k_m} \iff \boxed{V = \frac{s V_{max}}{s+k_m}}$$

Michaelis - Menten Law

Note the (formal) similarity between the expression of the velocity with the quasi-steady-state (QSS) approximation and the expression with the equilibrium approximation, i.e., we just have k_m instead of K_{eq_1} However:

$$k_m \neq K_{eq_1}$$

However, if the assumptions of the equilibrium approximation are satisfied, i.e., if $k_{-1} >> k_2$, then $k_m \cong K_{eq_1}$ and the two solutions give similar results.

Note two things:

- $s = k_m \Rightarrow V = V_{max}/2$ k_m has a physiological meaning

- $\frac{ds}{dt} = -V = -V_{max}\frac{s}{s+k_m}$ We can procede analytically and obtain:

Integration by parts

$$\frac{ds}{dt} = -V_{max}\frac{s}{s+k_m} \iff \frac{s+k_m}{s}ds = -V_{max}\,dt \iff$$

$$ds + k_m\frac{1}{s}ds = -V_{max}\,dt \iff \int_{s_0}^{s(t)} ds + \int_{s_0}^{s(t)} \frac{k_m}{s} ds = -V_{max}\int_0^t dt$$

$$\iff s(t) - s_0 + k_m(\ln s - \ln s_0) = -V_{max}t$$

$$t = \frac{1}{V_{max}}\left((s_0 - s) + k_m \ln\left(\frac{s_0}{s}\right)\right)$$ - We can use this formula to solve the inverse problem, i.e., "knowing s_0 and s, to determine the time t necessary to bring the concentration of the substrate to s"

With the Michaelis-Menten equation:

$$\frac{ds}{dt} = -V_{max}\frac{s}{s + k_m} \quad (*)$$

one may ask: "what would happen if we artificially alter the rate of the substrate?" This manipulation can be modeled by introducing an input function $u(t)$ in $(*)$

$$\frac{ds}{dt} = -V_{max}\frac{s}{s+k_m} + u(t)$$

Ex: $u(t) = \begin{cases} \lambda & t>0 \\ 0 & t\leq 0 \end{cases}$

$$\frac{ds}{dt} = -V_{max}\frac{s}{s+k_m} + u(t) \iff \frac{ds}{dt} = -V_{max}\frac{s}{s+k_m} + \lambda$$

(assuming that we consider $t>0$)

$$\iff \frac{ds}{dt} = \frac{(\lambda - V_{max})s + \lambda k_m}{s + k_m} \iff dt = \frac{s + k_m}{\lambda k_m + (\lambda - V_{max})s}\,ds \quad (**)$$

(integration by parts)

While the solution to $(**)$ can be computed numerically, it can be determined the value that s asymptotically tends to. In general, as $t \longrightarrow +\infty$ we expect that s converges to a steady state value

Hence we expect:

$$\lim_{t\to+\infty} \frac{ds}{dt} = 0 \iff s_\infty \triangleq \lim_{t\to+\infty} s(t) \quad -V_{max}\frac{s_\infty}{s_\infty + k_m} + \lambda = 0$$

$$\iff s_\infty = \frac{k_m \lambda}{-\lambda + V_{max}}$$

And, because $s_\infty \geq 0$ (concentration cannot be negative), we must impose: $V_{max} > \lambda$ in order to have a bounded solution

Ex: $u(t) = \alpha e^{-\beta t}$

$$\frac{ds}{dt} = -V_{max}\frac{s}{s+k_m} + \alpha e^{-\beta t} \iff \frac{ds}{dt} = \frac{(\alpha e^{-\beta t} - V_{max})s + k_m \alpha e^{-\beta t}}{s+k_m}$$

The asymptotic value s_∞ is:

$$\frac{-V_{max}\, s_\infty}{s_\infty + k_m} = 0 \iff s_\infty = 0$$

However in this case $s(t)$ will have a non-monotonic behavior. In fact, the condition for maxima and minima brings:

$$(\alpha e^{-\beta \hat{t}} - V_{max})\hat{s} + k_m \alpha e^{-\beta \hat{t}} = 0 \qquad \hat{s} \triangleq s(\hat{t})$$

$$\alpha e^{-\beta \hat{t}}(\hat{s} + k_m) = \hat{s} V_{max} \iff e^{-\beta \hat{t}} = \frac{\hat{s} V_{max}}{\alpha(\hat{s}+k_m)} \iff \hat{t} = -\frac{1}{\beta} \ln\left(\frac{\hat{s} V_{max}}{\alpha(\hat{s}+k_m)}\right)$$

The response of s to $u(t)$ under the quasi-steady-state approximation can be computed in Simulink - Similarly the global effects of $u(t)$ on the full system (e.g., on the formation of the product p) can be predicted by running a numerical simulation

- Interactive Presentation in Simulink
(if time allows... regardless, check the example on Husky CT)

To summarize:

- In case of enzyme reactions we can develop a useful model by using the LoMA in a two-step process and adding constraints
- A convenient formula for the velocity of the reaction can be obtained by using one of two approximations (equilibrium versus quasi-steady state) and expressed as a function of [S]
- The QSS approximation is appealing because it provides a simplified evolution model for [S] which can be used to estimate the effects of exogenous input functions on the enzyme reaction

REFERENCE:

Text book (volume 1): chapter 1, sec. 1.1 - 1.3 - 1.4 - 1.4.1 - 1.4.2 (in part)
Enderle - Bronzino "Introduction to Biomedical Engineering", 3rd Ed., Academic Press, 2010 - chapter 8, sec. 8.1 - 8.2 - 8.3 - 8.3.1

A copy of the chapter of this additional book is available on Husky CT. Please download it