Chapter 2 – Enzyme Kinetics

2.1 The Law of Mass Action

chemical species : $C_1, \dots, C_m$

concentrations : $c_1, \dots, c_m$

unit moles $/m^3$ = mol$/m^3$

Law of Mass Action

A chemical reaction proceeds at a rate proportional to the concentrations of participating reactants. The constant of proportionality is called the <u>rate constant</u>.

$$\lambda_1 C_1 + \dots \lambda_m C_m \underset{k_b}{\overset{k_f}{\rightleftharpoons}} \nu_1 C_1 + \dots + \nu_m C_m$$

Forward rate : $k_f c_1^{\lambda_1} \dots c_m^{\lambda_m}$

k_f, k_b - rate constants

Reverse rate $k_b c_1^{\nu_1} \dots c_m^{\nu_m}$

Example

$$A + B \underset{k_b}{\overset{k_f}{\rightleftharpoons}} C$$

A—B

$$\frac{da}{dt} = -k_f ab + k_b c$$

$$\frac{db}{dt} = -k_f ab + k_b c$$

$$\frac{dc}{dt} = +k_f ab - k_b c$$

$b + c = \text{constant}$, $a + c = \text{constant}$.

$\frac{d}{dt}(b+c) = 0$ $\frac{d}{dt}(a+c) = 0$

$$mA + nB \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C$$

$$\frac{da}{dt} = -mk_1 a^m b^n + mk_{-1} c$$

$$\frac{db}{dt} = -nk_1 a^m b^n + nk_{-1} c$$

$$\frac{dc}{dt} = k_1 a^m b^n - k_{-1} c$$

Mass conservation

$a + mc = \text{constant}$, $b + nc = \text{constant}$

$\frac{d}{dt}(a+mc) = 0$ $\frac{d}{dt}(b+nc) = 0$

2.2 Michaelis-Menten kinetics

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

$$C = S\text{-}E$$

$$\frac{ds}{dt} = -k_1 se + k_{-1} c \qquad s(0) = s_0$$

$$\frac{dc}{dt} = k_1 se - k_{-1} c - k_2 c \qquad c(0) = 0$$

$$\frac{de}{dt} = -k_1 se + k_{-1} c + k_2 c \qquad e(0) = e_0 \ll s_0$$

$$\left.\frac{dp}{dt} = k_2 c \right\} \text{decouples} \qquad p(0) = 0$$

Mass conservation $\frac{d}{dt}(e+c) = 0 \Rightarrow \boxed{e(t) = e_0 - c(t)}$

$$\boxed{\begin{aligned} \frac{ds}{dt} &= -k_1(e_0 - c)s + k_{-1} c \\ \frac{dc}{dt} &= k_1(e_0 - c)s - (k_{-1} + k_2) c \end{aligned}}$$

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

$$\frac{ds}{dt} = -k_1(e_0 - c)s + k_{-1}c \qquad s(0) = s_0$$

$$\frac{dc}{dt} = k_1(e_0 - c)s - (k_{-1} + k_2)c \qquad c(0) = 0$$

$$t = \frac{\tau}{k_1 e_0}, \qquad s = s_0 u, \qquad c = e_0 v$$

$$\frac{d}{dt} = \frac{d\tau}{dt}\frac{d}{d\tau} = k_1 e_0 \frac{d}{d\tau}$$

$$\cancel{k_1}\cancel{e_0}\cancel{s_0}\frac{du}{d\tau} = -\cancel{k_1}(\overset{1}{\cancel{e_0}} - \cancel{e_0}v)\cancel{s_0}u + \frac{k_{-1}\cancel{e_0}}{k_1 s_0}v$$

$$\cancel{k_1}\frac{e_0}{s_0}\cdot\cancel{e_0}\frac{dv}{d\tau} = \cancel{k_1}(\overset{1}{\cancel{e_0}} - \cancel{e_0}v)\cancel{s_0}u - \frac{(k_{-1} + k_2)}{k_1 s_0}\cancel{e_0}v$$

$$K = \frac{k_{-1} + k_2}{k_1 s_0}, \qquad \lambda = \frac{k_2}{k_1 s_0}, \qquad \varepsilon = \frac{e_0}{s_0} \ll 1$$

$$\boxed{\begin{aligned} \frac{du}{d\tau} &= -u + (u + K - \lambda)v \qquad & u(0) = 1 \\ \varepsilon\frac{dv}{d\tau} &= u - (u + K)v \qquad & v(0) = 0 \end{aligned}}$$

$\varepsilon \ll 1$, typically $\varepsilon \sim 10^{-6}$.

$$\frac{du}{d\tau} = -u + (u + k - \lambda v) \qquad u(0) = 1$$

$$\varepsilon \frac{dv}{d\tau} = u - (u+k)v \qquad v(0) = 0$$

Tempting: set $\varepsilon = 0 \Rightarrow v = \dfrac{u}{u+k}$

Inconsistent with I.C.s : $v(0) = 0 \neq \dfrac{u(0)}{u(0)+k} = \dfrac{1}{1+k}$

Singular perturbation investigation

INNER SOLUTION: $\sigma = \dfrac{\tau}{\varepsilon}$ $\left(\dfrac{d}{d\tau} = \dfrac{d\sigma}{d\tau}\dfrac{d}{d\sigma} = \dfrac{1}{\varepsilon}\dfrac{d}{d\sigma} \right)$

$$u(\tau,\varepsilon) = \hat{u}(\sigma,\varepsilon) = \hat{u}_0(\sigma) + \varepsilon \hat{u}_1(\sigma) + \dots$$
$$v(\tau,\varepsilon) = \hat{v}(\sigma,\varepsilon) = \hat{v}_0(\sigma) + \varepsilon \hat{v}_1(\sigma) + \dots$$

$$\cancel{\frac{1}{\varepsilon}}\left(\frac{d\hat{u}_0}{d\sigma} + \varepsilon \frac{d\hat{u}_1}{d\sigma} + \dots \right) = -\varepsilon(\hat{u}_0 + \varepsilon \hat{u}_1 + \dots)$$
$$+ \varepsilon(\hat{u}_0 + \varepsilon \hat{u}_1 + \dots k - \lambda)(\hat{v}_0 + \varepsilon \hat{v}_1 + \dots)$$

$$\cancel{\varepsilon} \cdot \cancel{\frac{1}{\varepsilon}}\left(\frac{d\hat{v}_0}{d\sigma} + \varepsilon \frac{d\hat{v}_1}{d\sigma} + \dots \right) = (\hat{u}_0 + \varepsilon \hat{u}_1 + \dots)$$
$$- (\hat{u}_0 + \varepsilon \hat{u}_1 + \dots + k)(\hat{v}_0 + \varepsilon \hat{v}_1 + \dots)$$

Terms $O(1)$ $\quad \dfrac{d\hat{u}_0}{d\sigma} = 0 \Rightarrow \hat{u}_0 = \text{constant} = 1$ by ICs.

$$\frac{d\hat{v}_0}{d\sigma} = \hat{u}_0 - (\hat{u}_0 + k)\hat{v}_0$$

$$\frac{d\hat{v}_0}{d\sigma} = 1-(1+k)\hat{v}_0$$

$$\frac{d}{d\sigma}\left(\hat{v}_0 e^{(1+k)\sigma}\right) = e^{(1+k)\sigma}$$

$$\hat{v}_0(\sigma) e^{(1+k)\sigma} - \underbrace{\hat{v}_0(0)}_{=0} e^{0} = \frac{1}{1+k}\left(e^{(1+k)\sigma} - 1\right)$$

$$\boxed{\hat{v}_0 = \frac{1-e^{-(1+k)\sigma}}{1+k} \qquad \hat{u}_0 = 1 \qquad \text{INNER}}$$

OUTER SOLUTION

$$u(\tau,\varepsilon) = u_0(\tau) + \varepsilon u_1(\tau) + \cdots$$
$$v(\tau,\varepsilon) = v_0(\tau) + \varepsilon v_1(\tau) + \cdots$$

$$\left(\frac{du_0}{d\tau} + \varepsilon\frac{du_1}{d\tau} + \cdots\right) = -\left(u_0 + \varepsilon u_1 + \cdots\right) + \left(u_0 + \varepsilon u_1 + \cdots k - \lambda\right)\left(v_0 + \varepsilon v_1 + \cdots\right)$$

$$\varepsilon\left(\frac{dv_0}{d\tau} + \varepsilon\frac{dv_1}{d\tau} + \cdots\right) = \left(u_0 + \varepsilon u_1 + \cdots\right) - \left(u_0 + \varepsilon u_1 + \cdots + k\right)\left(v_0 + \varepsilon v_1 + \cdots\right)$$

$O(1)$:
$$\frac{du_0}{d\tau} = -u_0 + (u_0 + k - \lambda)v_0$$

$$0 = u_0 - (u_0+k)v_0 \Rightarrow \boxed{v_0 = \frac{u_0}{u_0+k}}$$

$$\Rightarrow \boxed{\frac{du_0}{d\tau} = -\frac{\lambda u_0}{u_0+k}}$$

Matching conditions:

$$\lim_{\sigma \to \infty} \tilde{u}_0 = \lim_{\tau \to 0} u_0 = 1$$

$$\lim_{\sigma \to \infty} \tilde{v}_0 = \lim_{\tau \to 0} v_0 = \frac{1}{1+k}$$

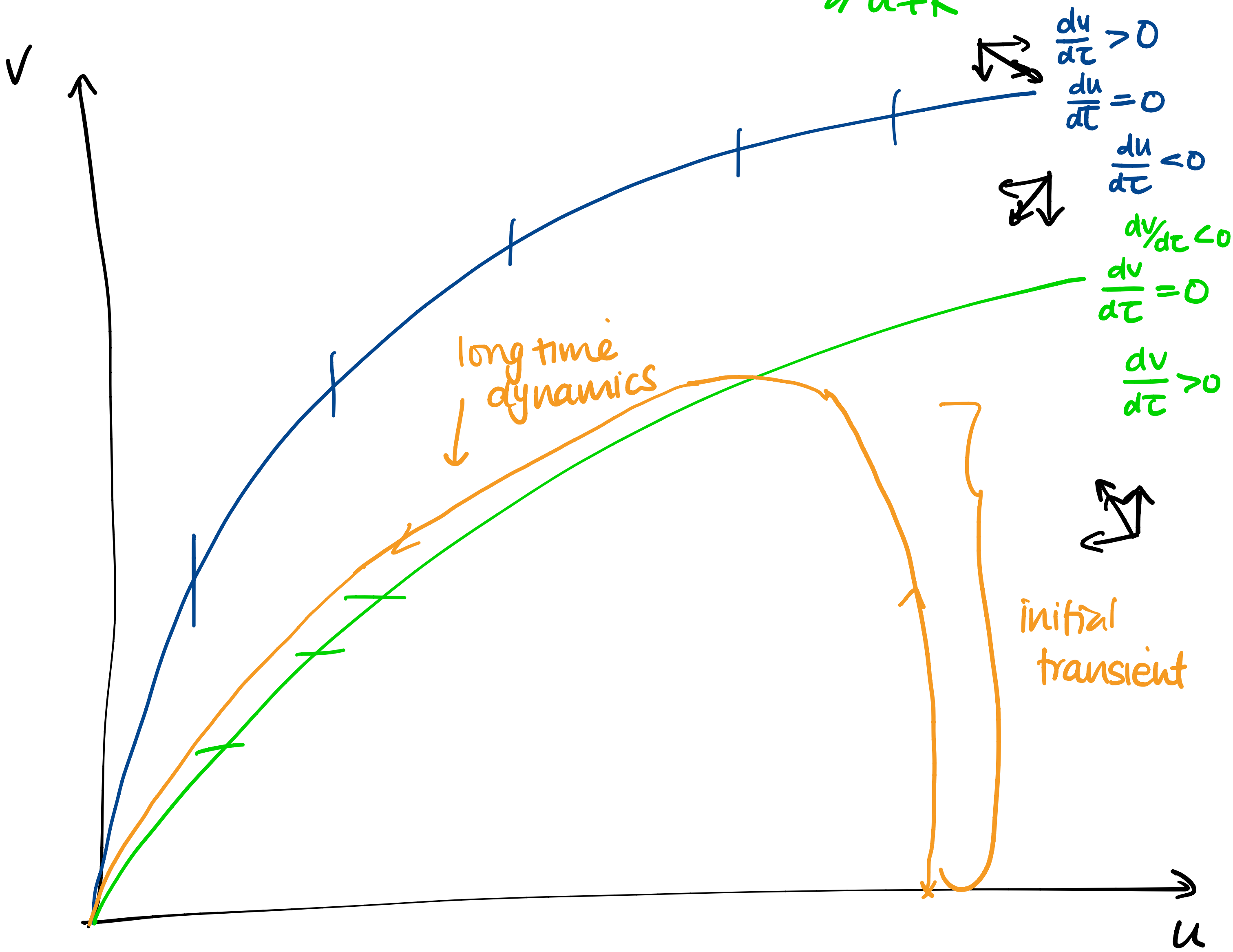
$$\frac{du}{d\tau} = -u + (u+k-\lambda)v$$

$$\varepsilon \frac{dv}{d\tau} = u - (u+k)v$$

Null clines

$v = \dfrac{u}{u+k-\lambda}$ u n.c.

$v = \dfrac{u}{u+k}$ v n.c.



Pseudo steady state hypothesis

eg. Michaelis-Menten equations

$$\frac{du}{dt} = -\frac{\lambda u}{u+k} \quad \text{with} \quad u(0)=1, \quad V = \frac{u}{u+k}$$

Defn When the time derivative is fast ie

$$\varepsilon \frac{dv}{d\tau} = g(u,v) \qquad \varepsilon \ll 1, \quad g(u,v) \sim O(1)$$

taking the temporal dynamics to be trivial ie

$$\frac{dv}{d\tau} \simeq 0$$

is known as the pseudo steady state hypothesis

$$S + E \rightarrow C$$

$$C \rightarrow P$$

$$C \rightarrow S + E$$

2.3 More complex systems

Allosteric enzymes

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C_1 \xrightarrow{k_2} P + E$$

S–E

$$S + C_1 \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} C_2 \xrightarrow{k_4} C_1 + P$$

E–S
|
S

$$\frac{ds}{dt} = -k_1 s e + k_{-1} c_1 - k_3 s c_1 + k_{-3} c_2 \qquad s(0) = s_0$$

$$\frac{de}{dt} = -k_1 s e + (k_{-1} + k_2) c_1 \qquad e(0) = e_0$$

$$\frac{dc_1}{dt} = k_1 s e - (k_{-1} + k_2) c_1 - k_3 s c_1 + (k_{-3} + k_4) c_2 \qquad c_1(0) = 0$$

$$\frac{dc_2}{dt} = k_3 s c_1 - (k_{-3} + k_4) c_2 \qquad c_2(0) = 0$$

$$\frac{dP}{dt} = k_2 c_1 + k_4 c_2 \qquad P(0) = 0$$

Mass conservation: $\frac{d}{dt}(e + c_1 + c_2) = 0$

$$\Rightarrow \quad e(t) + c_1(t) + c_2(t) = e_0$$

multiple enzymes

$$E + X \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E_1 \qquad E_1 + S \xrightarrow{k_3} P + Q + E$$

inactive   active E-X

$$E_1 + X \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E_2$$

inactive

X
|
E - X

$$\phi \xrightarrow{k_0} X \xrightarrow{k_4} \phi$$

$$e(t) + e_1(t) + e_2(t) = \text{constant}$$

$$\frac{d}{dt}(e + e_1 + e_2) = 0$$

: check few ODE system.

$$\frac{d}{dt}(x + e_1 + e_2) = k_0 - k_4 x$$

Generally,

$$\frac{du}{d\tau} = f(u, v_1, \ldots, v_n)$$

$$\varepsilon_i \frac{dv_i}{dt} = g_i(u, v_1, \ldots, v_n) \qquad i = 1, \ldots, n$$

After an initial transient, $g_i(u, v_1, \ldots, v_n) \approx 0$

$$\Rightarrow \quad \frac{du}{d\tau} = f(u, v_1(u), \ldots, v_n(u))$$

$$\text{s.t.} \quad g_i(u, v_1, \ldots, v_n) = 0 \qquad i = 1, \ldots, n$$

$v_i(u)$ are the roots of these equations.

<u>Pseudo steady state hypothesis</u>.