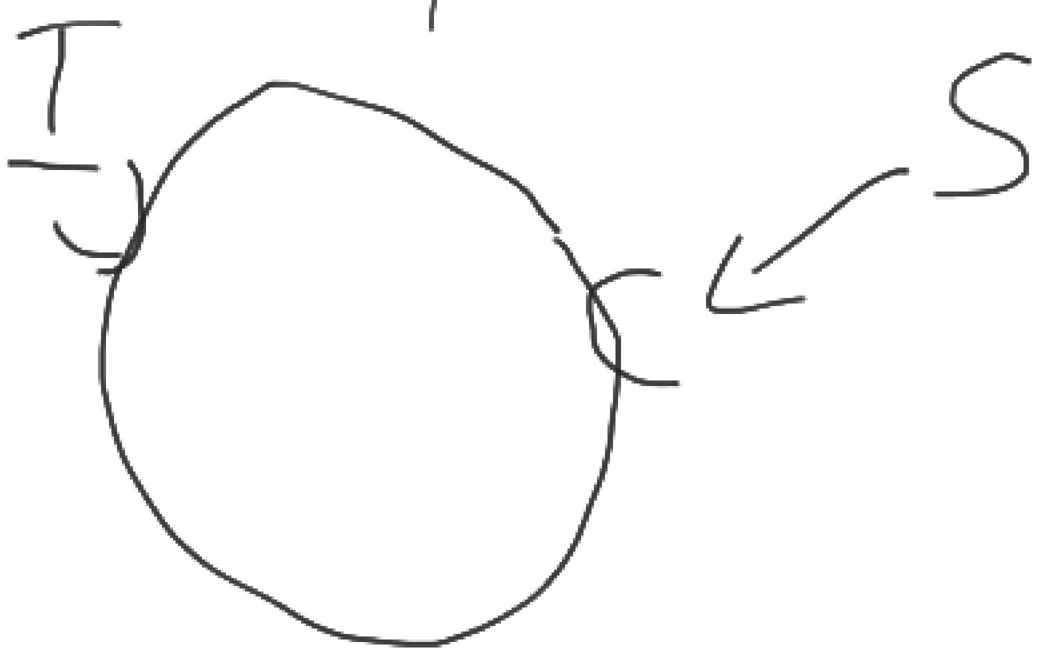
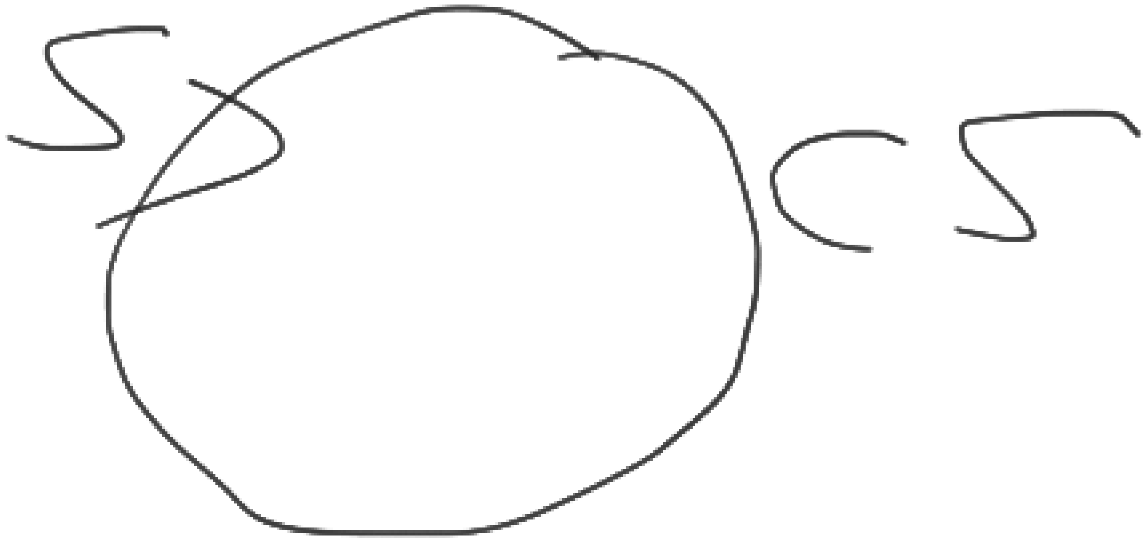
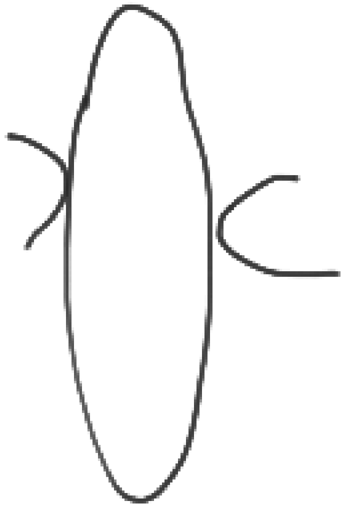
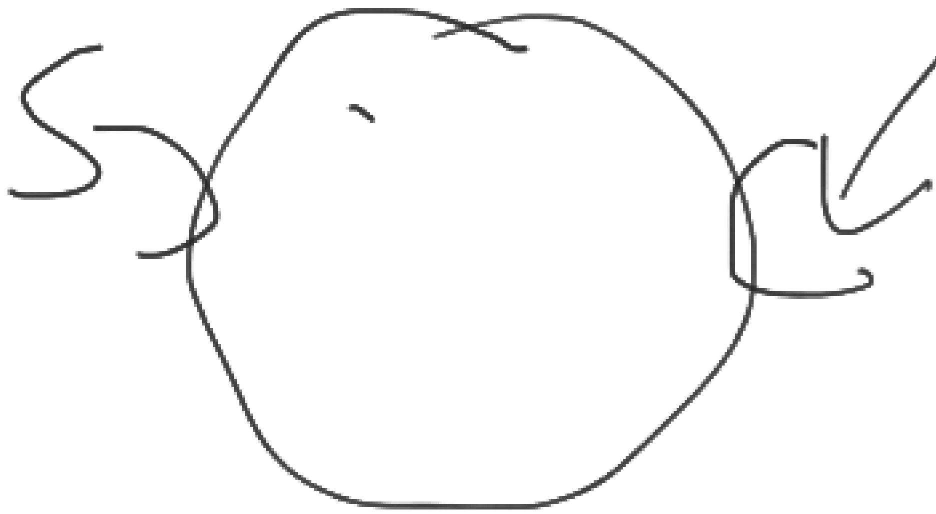
Cooperatividad

Ej de más de 1 sitio para unión de C



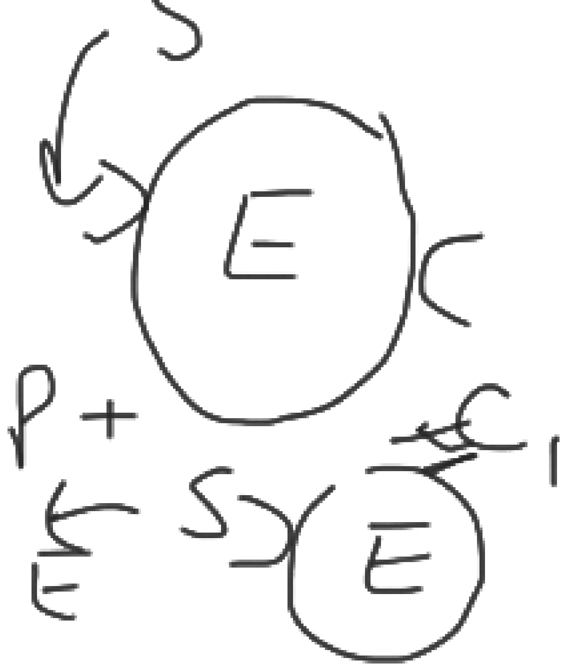






S en más o menos fácil pegar el 2º sustrato

Ejemplo que sirve para analizar coop. o no coop, ayudan.



$$S + E \underset{k'_1}{\overset{k_1}{\rightleftharpoons}} C_1 \xrightarrow{k_2} P + E$$

$$C_1 + S \underset{k'_3}{\overset{k_3}{\rightleftharpoons}} C_2 \xrightarrow{k_4} P + C_1$$

$$S + E \underset{k'_1}{\overset{k_1}{\rightleftharpoons}} C_1 \xrightarrow{k_2} E + P \quad ; \quad C_1 + S \underset{k'_3}{\overset{k_3}{\rightleftharpoons}} C_2 \xrightarrow{k_4} P + C_1$$

$$\frac{d[S]}{dt} = -k_1[S][E] + k'_1[C_1] - k_3[C_1][S] + k'_3[C_2]$$

$$\frac{d[E]}{dt} = -k_1[S][E] + k'_1[C_1] + k_2[C_1]$$

$$\frac{d[C_2]}{dt} = k_3[C_1][S] - k'_3[C_2] - k_4[C_2]$$

$$\frac{d[C_1]}{dt} = k_1[S][E] - k'_1[C_1] - k_2[C_1] - k_3[C_1][S] + k'_3[C_2] + k_4[C_2]$$

$$\frac{d[E]}{dt} + \frac{d[C_1]}{dt} + \frac{d[C_2]}{dt} = 0 \Rightarrow [E] + [C_1] + [C_2] = e_0 = \text{const}$$

enzima total

$$[E] = e_0 - [C_1] - [C_2]$$

Me quedo con 3 ecuaciones y 3 incógnitas

Adimensionalización

$\sigma = \frac{[S]}{s_0}$, $x_1 = \frac{[C_1]}{e_0}$, $x_2 = \frac{[C_2]}{e_0}$, donde $S_0 = [S](t=0)$

$\tau = k_1 e_0 t$

$S + E \xrightarrow{k_1} C_1$

$\frac{d[S]}{dt} = \underbrace{-k_1 [E]}_{\simeq -k_1 e_0} [S]$; $\frac{d[E]}{dt} = \underbrace{-k_1 [S]}_{\simeq k_1 S_0} [E]$

1/tiempo 1/tiempo

Si $e_0 \ll S_0 \Rightarrow k_1 e_0 \ll k_1 S_0$

$\frac{1}{k_1 e_0} \gg \frac{1}{k_1 S_0}$

Significa que mido el tiempo en unidades de $\frac{1}{k_1 e_0}$

Definiendo $\epsilon = e_0/S_0 \ll 1$ las 3 ecuac^s para $[S]$, $[C_1]$, $[C_2]$ quedan en forma adimensional:

$$\frac{d\sigma}{d\tau} = -\sigma(1-x_1-x_2) + \frac{k_1'}{k_1 S_0}x_1 - \frac{k_3}{k_1}\sigma x_1 + \frac{k_3'}{k_1 S_0}x_2$$

$$\epsilon\frac{dx_1}{d\tau} = \sigma(1-x_1-x_2) - \frac{k_3}{k_1}\sigma x_1 - \frac{k_1'+k_2}{k_1 S_0}x_1 + \frac{k_4+k_3'}{k_1 S_0}x_2 \to \simeq 0$$

$$\epsilon\frac{dx_2}{d\tau} = \frac{k_3}{k_1}\sigma x_1 - \frac{k_4+k_3'}{k_1 S_0}x_2$$

$\simeq 0$

Ec^s dif. lineales si σ = const $\to$ exp disminuyen en el tiempo en la escala $\sim \epsilon \to$ van a constante

De la 2^a luego a

$$x_2 \simeq \frac{(k_3/k_1)\sigma x_1}{\frac{k_4+k_3'}{k_1 S_0}}$$

$\to$ metiéndolo en parte derecha de $\epsilon\frac{dx_1}{d\tau} = 0 \Rightarrow$

$$\frac{[C_1]}{e_0} = x_1 = \frac{\sigma \frac{k_4 + k'_3}{k_3 S_0}}{\sigma^2 + \frac{k_4 + k'_3}{k_3 S_0}\sigma + \frac{k'_1 + k_2}{k_1 S_0} \cdot \frac{k_4 + k'_3}{k_3 S_0}} \to 0 \; ; \quad C_1 \xrightarrow{k_2} P + E$$

$$; \quad C_2 \xrightarrow{k_4} P + C_1$$

$$\frac{[C_2]}{e_0} = x_2 = \frac{\sigma^2}{\sigma^2 + \frac{k_4 + k'_3}{k_3 S_0}\sigma + \frac{k'_1 + k_2}{k_1 S_0} \cdot \frac{k_4 + k'_3}{k_3 S_0}}$$

$$\frac{d[P]}{dt} = k_2 [C_1] + k_4 [C_2] = k_2 e_0 x_1 + k_4 e_0 x_2$$

Alta coop positiva

$$S + E \underset{k'_1}{\overset{k_1}{\rightleftharpoons}} C_1 \; ; \quad S + C_1 \underset{k'_3}{\overset{k_3}{\rightleftharpoons}} C_2 \qquad k_1 \ll k_3$$

$$2S + E \to C_2$$

$$k_1 \to 0 \; ; \quad k_3 \to \infty \qquad k_1 k_3 \to \text{finito}$$

$$\frac{k_4 + k'_3}{k_3 S_0} \ll 1 \; ; \quad \left(\frac{k'_1 + k_2}{k_1 S_0} \gg 1\right) \to \text{Muy rápido de } C_1$$

$$e_0 X_2 = [C_2] \cong e_0 \frac{[S]^2}{[S]^2 + \frac{k'_1 + k_2}{k_1} \cdot \frac{k_4 + k_3}{k_3}}$$

Si tengo el

$$2S + E \underset{k'}{\overset{k}{\rightleftharpoons}} C$$

$$\frac{d[C]}{dt} = k[S]^2[E] - k'[C]$$

$$\frac{d[E]}{dt} = -k[S]^2[E] + k'[C]$$

$$\rightarrow [E] + [C] = e_0$$

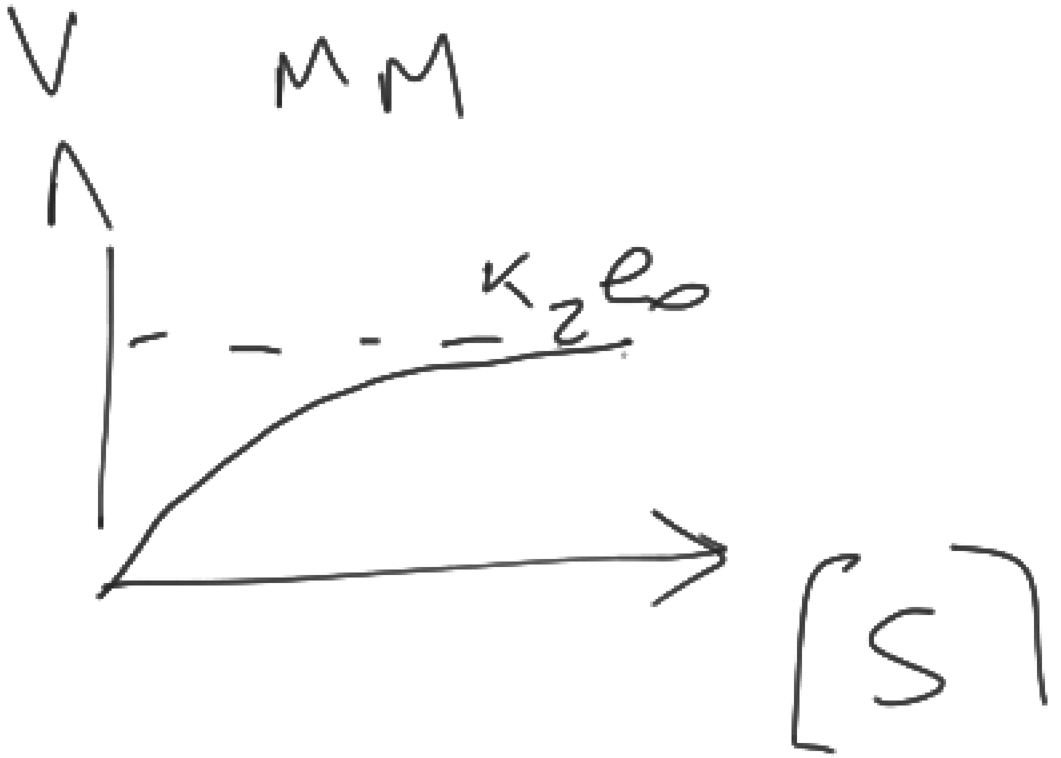
equilibrio $\rightarrow k'[C] = k[S]^2(e_0 - [C])$

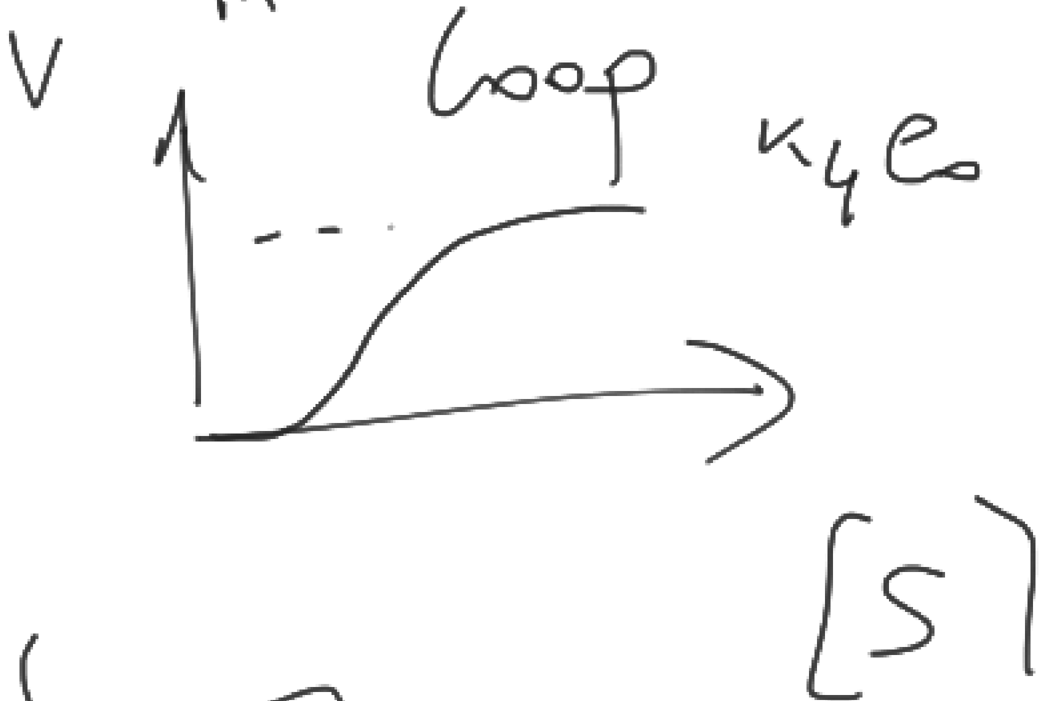
$$\rightarrow (k' + k[S]^2)[C] = k[S]^2$$

$$[C] = \frac{[S]^2}{[S]^2 + k'/k}$$

MM $\quad [C] = e_0 \dfrac{[S]}{[S]+k_M} \rightarrow V \simeq k_2 [C]$

Ahora $\quad [C] = e_0 \dfrac{[S]^2}{[S]^2+\tilde{K}_M^2} \rightarrow V \simeq k_4 [C]$





Si hay más sitios
mucha loop

$\rightarrow nS + E \rightleftharpoons C \; ; \; V \sim \dfrac{[S]^n}{[S]^n + (K_M)^n}$

$$V = V_{max} \frac{[S]^n}{[S]^n + (K_M)^n}$$ ec de Hill

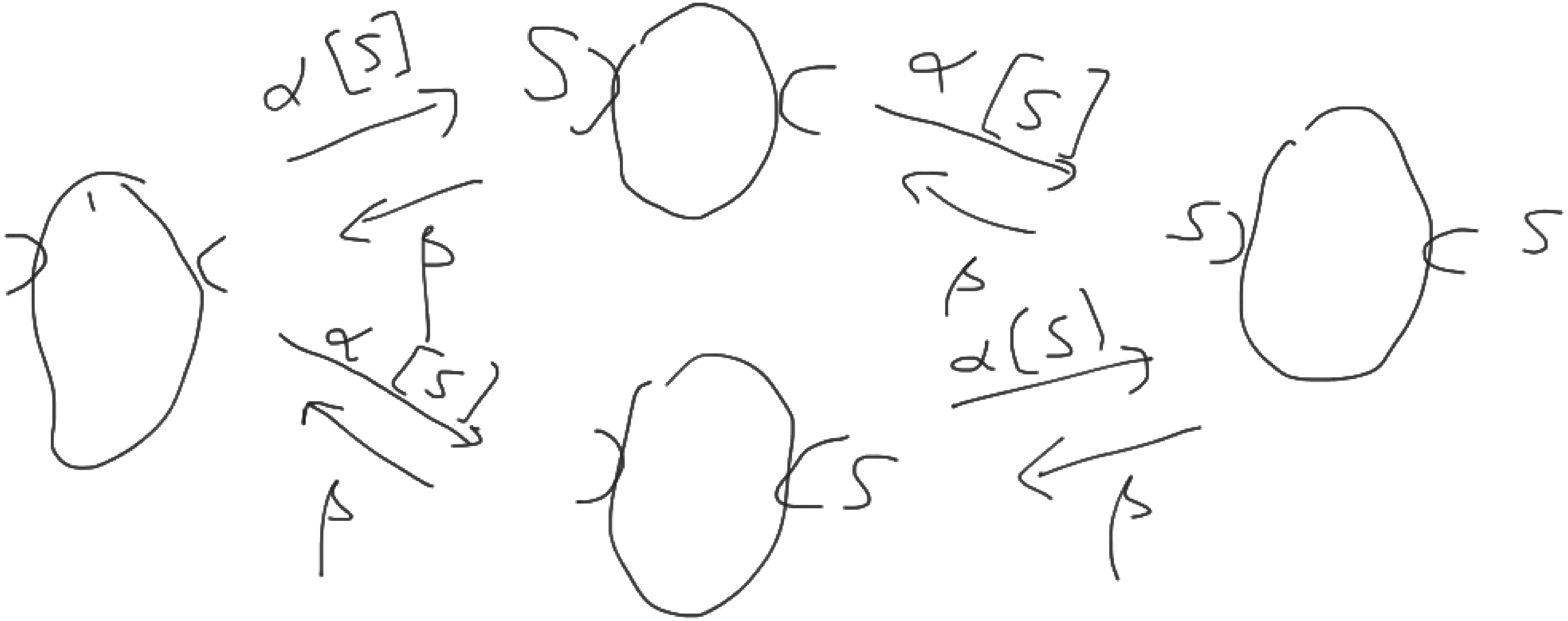
Gráficos de Hill

$$n \log [S] = \log \left(\frac{V}{V_{max} - V} \right) + n \log (K_M)$$

$$\log [S] = \frac{1}{n} \log \left(\frac{V}{V_{max} - V} \right) + \log (K_M)$$

Gráfico de Hill

$[S]$ vs $\frac{V}{V_{max} - V}$ en gráfico logaritmo



$$E \underset{k'_1}{\overset{k_1[S]}{\rightleftharpoons}} C_1 \underset{k'_3}{\overset{k_3[S]}{\rightleftharpoons}} C_2$$

$k_1 = 2\alpha$ $\qquad$ $k_3 = \alpha$

$k'_1 = \beta$ $\qquad$ $k'_3 = 2\beta$

No tomamos límite $k_1 \to 0$, $k_3 \to \infty$

$k_1 k_3 \to$ finito

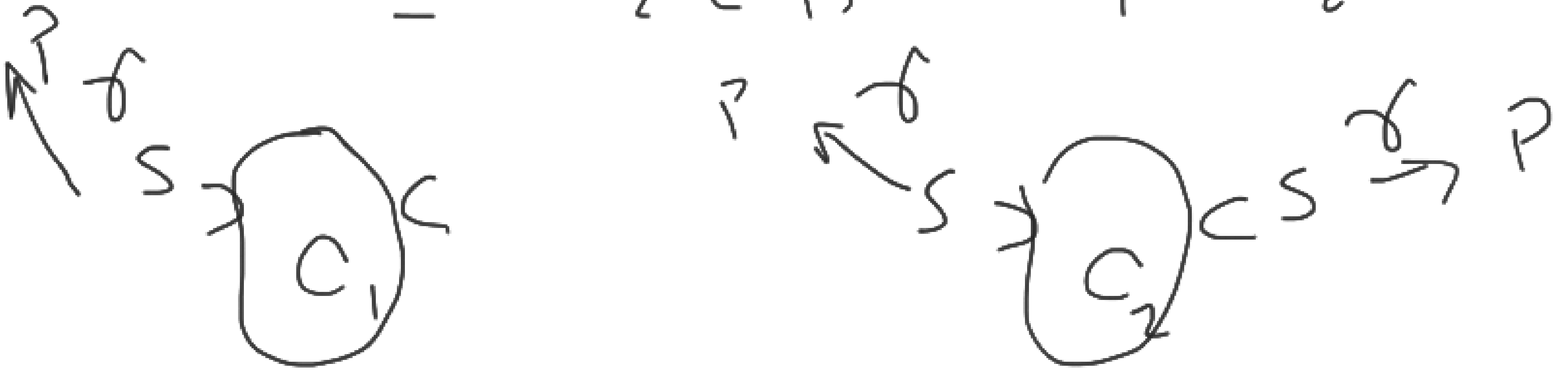
Si no que usamos

$k_1 = 2\alpha$; $k_3 = \alpha$; $k'_1 = \beta$; $k'_3 = 2\beta$

$k_1 = 2k_3$; $k'_1 = k'_3/2$

Aprox variedad, adiabática

$$V = k_2[C_1] + k_4[C_2]$$



$C_1 \xrightarrow{k_2} P + E$

$k_2 = \gamma$

$C_2 \xrightarrow{k_4} P + C_1$

$k_4 = 2\gamma \Rightarrow k_4 = 2k_2$

Escribiendo las transiciones en términos de 3 constantes: k_2, k_3, k'_1

$$v = k_2[C_1] + k_4[C_2] = k_2[C_1] + 2k_2(C_2)$$

Se obtiene (en aprox cuantitativa)

2 sitios de ligadura

enzima total

$$v = \frac{2 e_0 k_2 [S]}{[S] + \frac{k_2 + k'_1}{k_3}}$$

En el caso de hemoglobina y mioglobina lo que se compara es la fracción de sitios ocupados. Por qué me interesa la fracción de sitios ocupados

Si la tasa de generación de prod a partir de 1 sitio ocupado es indep de si el otro está ocupado o no tenemos en el caso de 2 sitios

$$v = k_2[C_1] + 2k_2[C_2] = 2k_2 e_0 \underbrace{\left[\frac{[C_1]}{2e_0} + \frac{2[C_2]}{2e_0}\right]}_{\text{fracción de sitios ocupados}}$$

En el caso de mioglobina y hemoglobina y de las figuras → fracción de sitios de ocupados.

Mioglobina
$$O_2 + Mb \underset{k_-}{\overset{k_+}{\rightleftharpoons}} MbO_2$$

$$[Mb]_T = \text{const} = [Mb] + [MbO_2]$$

$$\Rightarrow [MbO_2] = [Mb]_T \frac{[O_2]}{[O_2] + \frac{k_-}{k_+}} \quad \frac{k_-}{k_+} = K_d$$

$$\frac{[MbO_2]}{[Mb]_T} = Y = \frac{[O_2]}{[O_2] + K_d}$$

4b : H_j = hemoglobina con j O_2 ligados = con j sitios ocupados con O_2.

$j = 0, 1, 2, 3, 4$

$$O_2 + H_{j-1} \underset{k_{-j}}{\overset{k_j}{\rightleftharpoons}} H_j \qquad 1 \leq j \leq 4$$

$$\frac{k_{-j}}{k_{+j}}$$

Mucha coop

$$4O_2 + H_0 \underset{\tilde{k}'}{\overset{\tilde{k}}{\rightleftharpoons}} H_4$$

$[Hb]_T = [H_0] + [H_4]$; eq: $[H_4] = [Hb]_T \dfrac{[O_2]^4}{[O_2]^4 + (\tilde{K}_d)^4}$

$4[H_4]$ = conc. de sitios ocupados

$4[Hb]_T$ = conc de sitios disponíveis

$$Y = \frac{4[H_4]}{4[Hb]_T} = \frac{[H_4]}{[Hb]_T} = \frac{[O_2]^4}{[O_2]^4 + (\tilde{K}_d)^4}$$

Sistema envolve H_1, H_2, H_3

$$\Rightarrow \quad Y = \frac{[H_1] + 2[H_2] + 3[H_3] + 4[H_4]}{4[Hb]_T}$$

$$[O_2][H_{j-1}]K_j = [H_j]K_{-j} \qquad 1 \le j \le 4$$

$$[H_b]_T = \sum_{j=0}^{4} [H_j] \Rightarrow$$ se encuentra

$$\alpha_0 = 1$$

$$Y = \frac{\sum_{j=0}^{4} j\,\alpha_j\,[O_2]^j}{4\sum_{j=0}^{4} \alpha_j\,[O_2]^j} \qquad \alpha_j = \prod_{i=1}^{j} \frac{k_{+i}}{k_{-i}}$$

Llamando $\bar{K}_i = \dfrac{k_{-i}}{k_{+i}}$; $\bar{K}_1 = 45.9$; $\bar{K}_2 = 23.9$

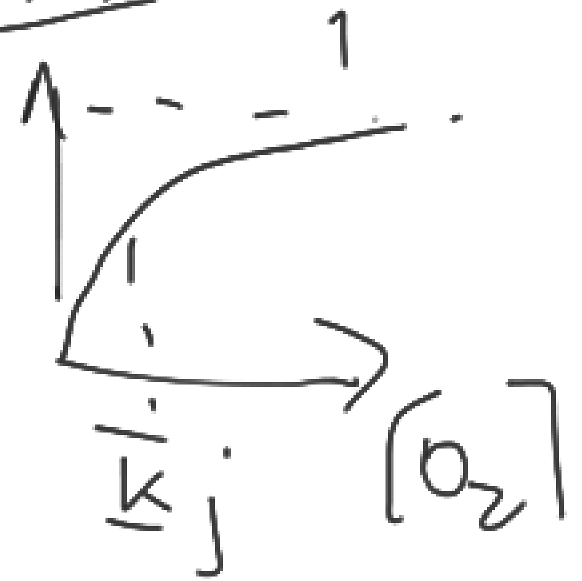
$\bar{K}_3 = 243.1$ $\bar{K}_4 = 1.52$

en mm de Hg

$$O_2 + H_{j-1} \underset{k_{-j}}{\overset{k_{+j}}{\rightleftharpoons}} H_j$$

$$k_{+j} \cdot [O_2]\left([H_{j-1}] + [H_j] - [H_j]\right) = k_{-j}[H_j]$$

$$[H_j] = \frac{k_{+j}[O_2]\left([H_{j-1}] + [H_j]\right)}{k_{+j}[O_2] + k_{-j}}$$



$$[H_j] = \frac{[O_2]\left([H_{j-1}] + [H_j]\right)}{[O_2] + \underbrace{k_{-j}/k_{+j}}_{= K_j}}$$

Modelo MWC

Supone que la enzima puede estar en el estado tenso, T, o en el estado relajado, R.

Modelo más sencillo → 2 sitios de ligadura, sitios de ligadura son indep entre sí

R_j = molécula en estado relajado con j sitios ocupados por sustratos

T_j = molécula en estado tenso con j sitios ocupados

$$\bar{K}_2 = \frac{[R_0]}{[T_0]}$$

$$R_0 \underset{k'_1}{\overset{2k_1[S]}{\rightleftharpoons}} R_1 \underset{2k'_1}{\overset{k_1[S]}{\rightleftharpoons}} R_2$$

$$R_0 \underset{k'_2}{\overset{k_2}{\rightleftharpoons}} T_0$$

$$T_0 \underset{k'_3}{\overset{2k_3[S]}{\rightleftharpoons}} T_1 \underset{2k'_3}{\overset{k_3[S]}{\rightleftharpoons}} T_2$$

$$Y = \frac{[R_1] + 2[R_2] + [T_1] + 2[T_2]}{2\left([R_0] + [R_1] + [R_2] + [T_0] + [T_1] + [T_2]\right)}$$

$$\bar{K}_1 = \frac{k'_1}{k_1} \; ; \; \bar{K}_3 = \frac{k'_3}{k_3}$$

Equilibrio de las reacciones → calcular fracción de sitios ocupados

$$y = \frac{S\bar{K}_1^{-1}\left(1 + S\bar{K}_1^{-1}\right) + \bar{K}_2^{-1}\left(S\bar{K}_3^{-1}\left(1 + S\bar{K}_3^{-1}\right)\right)}{\left(1 + S\bar{K}_1^{-1}\right)^2 + \bar{K}_2^{-1}\left(1 + S\bar{K}_3^{-1}\right)^2}$$

Dependiendo de la relación entre $\bar{K}_3^{-1}$ y $\bar{K}_1^{-1}$

↙ MM

↘ Coop