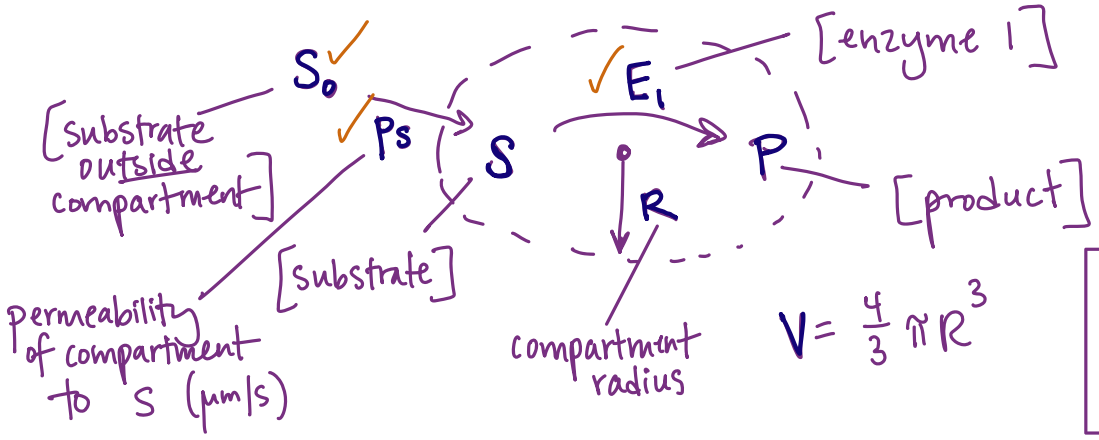
COMPARTMENTALIZATION! Is it useful? We'll use a carboxysome-inspired simple model. Q: what size compartment maximizes product formation?

Michaelis-Menten recap: $\frac{dP}{dt} = k_{cat} E \frac{S}{K_M + S}$

I. ONE REACTION — in a compartment



Inspiration: carbonic anhydrase

✓ $k_{cat}^{(1)} \approx 5 \times 10^4$ 1/s

✓ $K_M^{(1)} \approx 1 \times 10^4$ μM

✓ $P_S \approx 90$ μm/s

✓ = fixed, "known" quantities

GOAL: figure out the compartment size that maximizes product formation rate.

$$\frac{dP}{dt} = k_{cat}^{(1)} E_1 \frac{S}{K_M^{(1)} + S}$$

but we don't know S ∴

How can we figure out S? How will it depend on R?

Let's use what we know about steady-state:

$$V \cdot \frac{S}{dt} = 0 = \text{flux in} \cdot \text{surface area} - \text{rate of } S \to i \cdot \text{volume}$$

$$\left[\frac{\#}{\mu m^2 \cdot s}\right] = \left[\frac{\mu m}{s}\right]\left[\frac{\#}{\mu m^3}\right]$$

$J = P \Delta C$

S | S_0

$$0 = P_S(S_0 - S) \cdot 4\pi R^2 - k_{cat}^{(1)} E_1 \frac{S}{K_M^{(1)} + S} \cdot \frac{4}{3}\pi R^3$$

Algebra: (goal: quadratic eqn)

define $R = \frac{3 P_S}{k_{cat}^{(1)}}$ $\quad \frac{[\mu m/s]}{[1/s]}$

$$\frac{3 P_S}{k_{cat}^{(1)}}(S_0 - S)(K_M^{(1)} + S) = E_1 S R$$

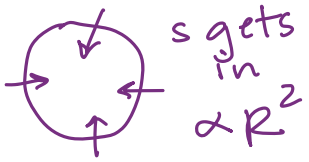
$$S_0 K_M^{(1)} - K_M^{(1)} S + S_0 S - S^2 = E_1 S \frac{R}{R}$$

$$\frac{-S^2}{A} + \underbrace{\left(S_0 - K_M^{(1)} - E_1 \frac{R}{R}\right)}_{B} S + \underbrace{S_0 K_M^{(1)}}_{C} = 0$$

$$S(R) = \frac{1}{2}\left(S_0 - K_M^{(1)} - E_1\frac{R}{R}\right) \pm \frac{1}{2}\sqrt{\left(S_0 - K_M^{(1)} - E_1\frac{R}{R}\right)^2 + 4S_0K_M^{(1)}}$$

↑ only keep + cuz no negative [ ] allowed

$$S(R) = \frac{1}{2}\left(S_0 - K_M^{(1)} - E_1\frac{R}{R}\right) + \frac{1}{2}\sqrt{\left(S_0 - K_M^{(1)} - E_1\frac{R}{R}\right)^2 + 4S_0K_M^{(1)}}$$



consumption of $S \propto R^3$

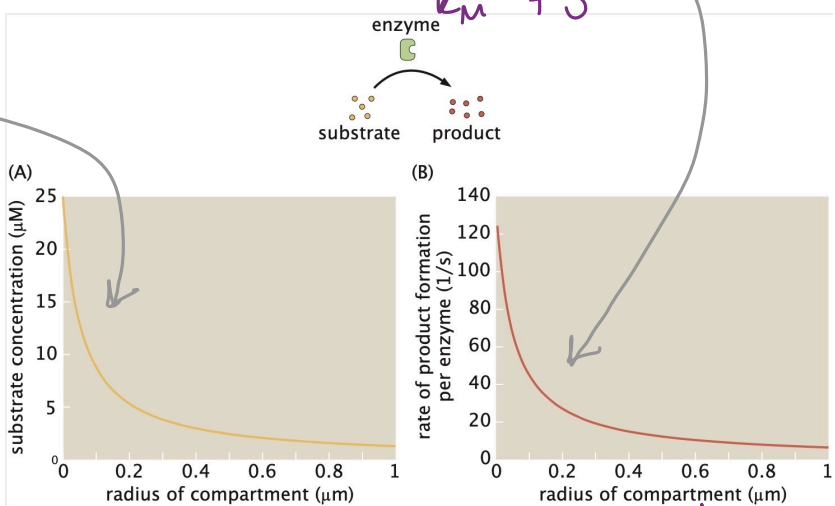
Now we have $S(R)$, we can calculate $\frac{dP}{dt}$ ☺

$$\frac{dP}{dt} = k_{cat}^{(1)} E_1 \frac{S(R)}{K_M^{(1)} + S(R)}$$ plug in!

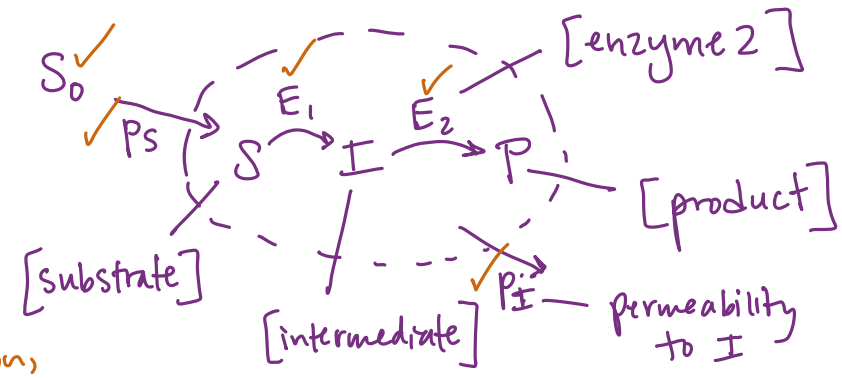
$$\text{production rate per enzyme} = \frac{dP}{dt}\frac{1}{E_1} = k_{cat}^{(1)}\frac{S}{K_M^{(1)} + S}$$

compartment E_1 E_1 E_1 ~

no benefit to compartmentalizing E_1 ☺



II. TWO REACTIONS – in a compartment



✓ = known, fixed

carbonic anhydrase "E_1"

✓ $k_{cat}^{(1)} \approx 5\times10^4$ 1/s

✓ $K_M^{(1)} \approx 1\times10^4$ μM

RuBisCO "E_2"

✓ $k_{cat}^{(2)} \approx 3$ 1/s

✓ $K_M^{(2)} \approx 50$ μM

$$\frac{dI}{dt} = k_{cat}^{(1)} E_1 \frac{S(R)}{K_M^{(1)} + S(R)}$$ We know $S(R)$ ✓

$$\frac{dP}{dt} = k_{cat}^{(2)} E_2 \frac{I(R)}{K_M^{(2)} + I(R)}$$ What's $I(R)$?

Again, let's use steady-state:

$$\frac{dI}{dt} \cdot V = 0 = \begin{pmatrix}\text{rate of} \\ S \to I\end{pmatrix} - \begin{pmatrix}\text{rate of} \\ I \to P\end{pmatrix} V - \begin{matrix}\text{flux of} \\ I \text{ out}\end{matrix} \cdot \text{surface area}$$

$$0 = \left(k_{cat}^{(1)} E_1 \frac{S}{K_M^{(1)} + S} - k_{cat}^{(2)} E_2 \frac{I}{K_M^{(2)} + I}\right) \frac{4}{3}\pi R^3 - P_I \underbrace{\left(I - I_{out}\right)}_{=0} 4\pi R^2$$

algebra to solve for I

define $\bar{S} = \frac{k_{cat}^{(1)} E_1 S}{K_M' + S}$ (solved above)

define $B = \frac{3P_i}{R} K_M^{(2)} + k_{cat}^{(2)} E_2 - \bar{S}$

$$I(R) = \frac{-BR}{6P_I} + \frac{R}{6P_I}\sqrt{B_2^2 + \frac{12P_i}{R}\bar{S}K_M^{(2)}}$$

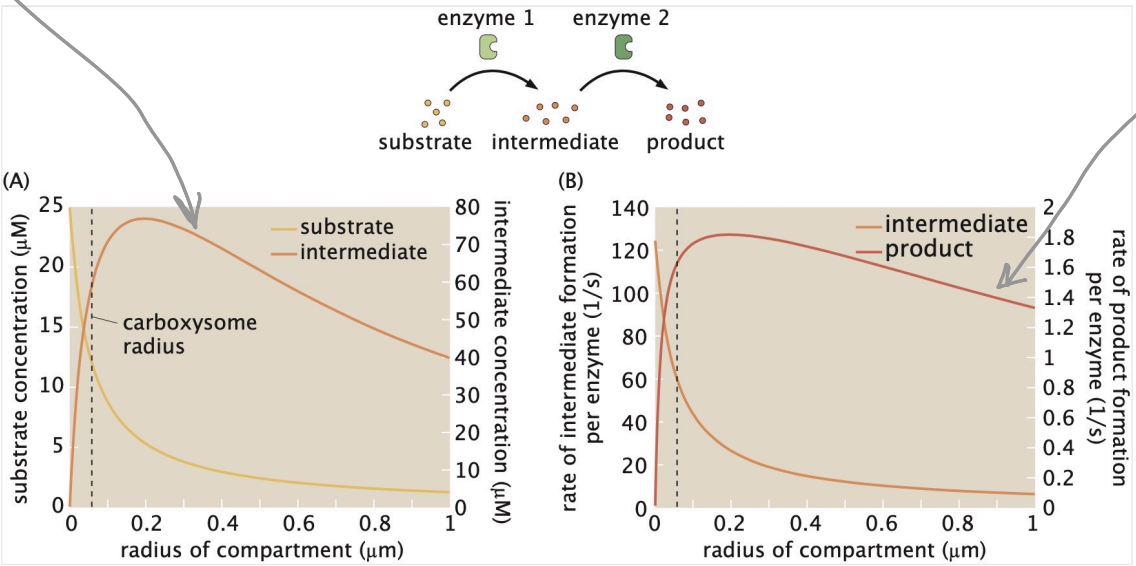
$$I(R) = \frac{-R}{6P_I}\left(\frac{3P_I}{R}K_M^{(2)} + k_{cat}^{(2)}E_2 - \bar{S}\right) + \frac{R}{6P_I}\sqrt{\left(\frac{3P_I}{R}K_M^{(2)} + k_{cat}^{(2)}E_2 - \bar{S}\right)^2 + \frac{12P_I}{R}\bar{S}K_M^{(2)}}$$

Now we have I, so we can get $\frac{dP}{dt}$ ☺

rate of product formation per enzyme $= \frac{dP}{dt}\frac{1}{E_2} = k_{cat}^{(2)} \frac{I}{K_M^{(2)} + I}$ ← plug in

Intuition: too big, no s gets in ... too small, all I escapes

* yes, there's an optimal compartment size! Compartmentalization helps for 2 sequential reactions.



Parameters used for plots (mostly from Hinzpeter et al.)

$S_0 = 25\ \mu M$

$P_s = 90\ \mu m/s$

$P_i = 18\ \mu m/s$

carbonic anhydrase dehydration

$e_1 = 1000\ \mu M$

$k_{cat}^{(1)} = 5 \times 10^4\ 1/s$

$K_M^{(1)} = 1 \times 10^4\ \mu M$

RuBisCO

$e_2 = 3200\ \mu M$

$k_{cat}^{(2)} = 3\ 1/s$

$K_M^{(2)} = 50\ \mu M$

range of R:

(0.001 μm : 0.001 μm : 1 μm)

★ w/ these parameters, $R_{optimal} \approx 180\ nm$

★ actual size of carboxysome radius $\approx 60\ nm$