

## **Analysis of Enzyme Kinetics in Invertebrates**

- Derivation of the Michaelis-Menten Relation
- Experimental Use of the Michaelis-Menten Equation
- Enzyme Kinetics Experiment

## Derivation of the Michaelis-Menten Relation

### I. Background

A. The M-M relation describes how the rate of a “reaction” depends on the amount of a “substance” that is transformed.

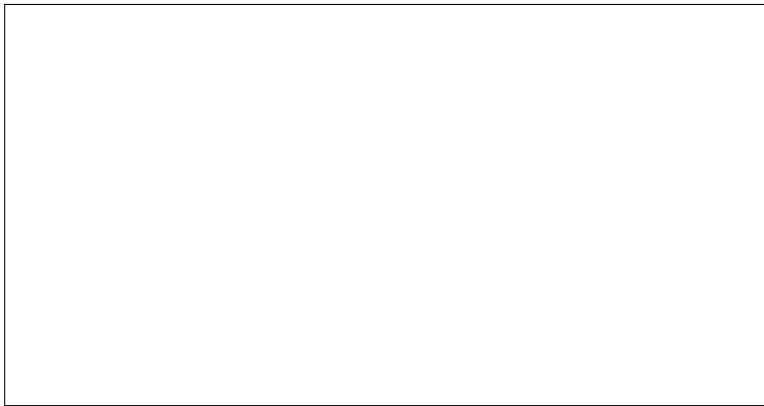
B. Classical example: enzyme and substrate.

The transformed substance is sucrose. The reaction is the creation of glucose and fructose from sucrose.

C. “Reaction” and “substrate” need not be chemical

### II. Chemical Equations

Two compounds ( $A$  and  $B$ ) combine at rate  $k_1$  to form compound  $C$ . Compound  $C$  dissociates at rate  $k_2$ . What is the chemical equation?



### III. Mathematical representation of chemical dynamics

A. Convert to **rates of change** for  $A$ ,  $B$ , and  $C$

B. Integrate rates to get amounts of  $A$ ,  $B$ , and  $C$  at different times

C.

$$\frac{dA}{dt} = -k_1AB + k_2C$$

$$\frac{dB}{dt} = -k_1AB + k_2C$$

$$\frac{dC}{dt} = k_1AB - k_2C$$

D. Steady-state (equilibrium) of the system:

*All of the above rates equal 0*

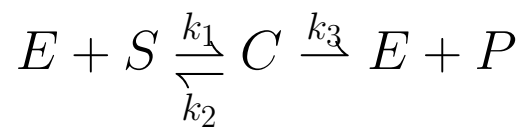
E. Mass Action: random “encounter” of compounds  $A$  and  $B$  is proportional to the product of concentrations of  $A$  and  $B$

F. Stoichiometry:

$$\underbrace{2A + B \xrightleftharpoons[k_2]{k_1} C}_{\text{chemical}} \implies \underbrace{\frac{dC}{dt} = k_1 A^2 B - k_2 C}_{\text{mathematical}} \quad (1)$$

#### IV. Enzyme Kinetics and Michaelis-Menten


A. Chemical equation



where  $E$ =enzyme,  $S$ =substrate,  $C$ =complex, and  $P$ =product.

## B. Mathematical Equation

How many rate equations? What symbols? What equations?



### C. Simplify: Apply Conservation of Mass

let  $r = E + C =$  total number of molecules of E  
in a unit volume

so,  $E = r - C$

This means we don't need an explicit rate equation  
for  $E$ .

New equations after substituting equation for  $E$ :

$$\frac{dS}{dt} = -k_1 S(r - C) + k_2 C$$

$$\frac{dC}{dt} = k_1 S(r - C) - (k_2 + k_3)C$$

$$\frac{dP}{dt} = k_3 C$$

But,  $dS/dt$  and  $dC/dt$  do not depend on  $P$ , so  $P$   
can be solved later once we know  $C$ .

### D. Simplify: Quasi-steady-state for $C$

1. Assume

$$\frac{dC}{dt} \approx 0$$

This is a quasi-steady-state, since  $dS/dt \neq 0$ . We are assuming that the intervals between observations is long enough that  $C$  reaches steady-state between observations.

2. Solve for  $C$  at quasi-steady-state

$$0 = k_1 r S - (k_1 S + (k_2 + k_3)) C$$

implies

$$C = \frac{k_1 r S}{(k_2 + k_3) + k_1 S}$$

3. Re-write  $S$  rate equation

$$\begin{aligned} \frac{dS}{dt} &= -k_1 r S + k_1 S C + k_2 C \\ &= \boxed{-k_1 S(r - C)} + k_2 C \\ &= \boxed{-(k_2 + k_3) C} + k_2 C \\ &= -k_3 C \end{aligned}$$

Notice the substitution of the boxed material. Why are these two quantities equal?

4. Substitute  $C$  in  $dS/dt$

$$\begin{aligned} \frac{dS}{dt} &= -k_3 \left[ \frac{k_1 r S}{(k_2 + k_3) + k_1 S} \right] \\ &= -\frac{k_3 r S}{\left(\frac{k_2 + k_3}{k_1}\right) + S} \end{aligned}$$

E. What about  $P$ , the product?

1.

$$\begin{aligned}\frac{dP}{dt} &= k_3 C = -\frac{dS}{dt} \\ &= \frac{k_3 r S}{\left(\frac{k_2 + k_3}{k_1}\right) + S}\end{aligned}$$

2. What is the shape of the curve relating  $dP/dt$  and  $S$ ?

3. Strip away complicated parameters:

$$\frac{dP}{dt} = v \boxed{\frac{S}{u + S}}$$

Call the box  $W$ . What is the graph of  $W$ ?

Cases to consider:

i. If  $S = 0$ , then  $W =$

ii. If  $S \ll u$ ,  $W =$

iii. If  $S \gg u$ ,  $W =$

Sketch the graph of  $dP/dt$  in the axes below.

F. Interpret the constants biochemically.

1.  $v = k_3r$ . In words,  $v$  is the maximum rate of product ( $P$ ) formation. Call it  $V_{\max}$ , ( $V$  for “velocity”).

What are the units of  $V_{\max}$ ?

Units of  $V_{\max} =$

2.

$$u = \frac{k_2 + k_3}{k_1}$$

So far, we have:

$$\frac{dP}{dt} = \frac{V_{\max}S}{u + S}$$

What are the units of  $u$ ?

Units of  $u =$

Do the overall units of  $dP/dt$  check out?

To give an intuitive interpretation of  $u$ , we will use a CMT (**C**lever **M**athematical **T**rick) and re-arrange the equation above for  $dP/dt$  (algebra steps left for student):

$$\frac{u}{S} = \frac{V_{\max} - dP/dt}{dP/dt}$$

Suppose  $S = u$ , then  $u/S = 1$  and

$$\frac{dP}{dt} = V_{\max} - dP/dt$$

or  $2\frac{dP}{dt} = V_{\max}$

or  $\frac{dP}{dt} = \frac{1}{2}V_{\max}$

In words, when  $S = u$ , the rate of product formation is *one-half the maximum rate of product formation*. So,  $u$  is the  $\frac{1}{2}$ -saturation constant. Also known as the *Michaelis-Menten constant* =  $K_m$ .

G. Finally, we're done. The Michaelis-Menten equation that relates the rate of product formation to the substrate concentration is:

$$\frac{dP}{dt} = \frac{V_{\max}S}{K_m + S}$$

And has the graph: