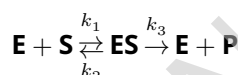


Enzyme Kinetics

7% of total						
Question	7.1	7.2	7.3	7.4	7.5	Total
Points	3	4	2	8	17	34
Score						

The Michaelis–Menten (MM) mechanism was introduced in 1913 to describe the kinetics of enzyme catalysis. In this mechanism, enzyme **E** catalyzes the conversion of substrate **S** to the product **P**:



The initial rate for an enzymatic reaction following the MM mechanism is usually given as:

$$v_0 = \frac{v_{\max}[\mathbf{S}]_0}{[\mathbf{S}]_0 + K_M} \quad (1)$$

when the initial concentration of **E** is much lower than the initial concentration of **S** ($[\mathbf{E}]_0 \ll [\mathbf{S}]_0$). The Michaelis constant is defined as $K_M = \frac{k_2 + k_3}{k_1}$. The initial rate can also be expressed as the product of the relative flow j and $[\mathbf{E}]_0$:

$$v_0 = j[\mathbf{E}]_0 \quad (2)$$

Note: Questions 7.1 and 7.2 can have one, multiple, or no correct answer(s).

7.1 **Choose** the correct alternative form(s) of the initial rate (v_0) expressions (1) and (2) **on the answer sheet**. $[\mathbf{ES}]_{\max}$ is the maximum concentration of the **ES** complex. 3pt

7.2 **Choose** the pair(s) of axes (y vs. x) **on the answer sheet** that are expected to give a linear plot. 4pt

Many enzymes catalyze multi- rather than single-substrate transformations. However, if the concentration of one of the substrates is much higher than that of the other substrate or it is kept constant, the MM kinetics is also valid. Here we will look at *two independent enzymatic systems* that follow the MM kinetics.

Enzymatic System I

Enzyme **E** converts substrates **A** and **B** to products **P_A** and **P_B**. At rapid pre-equilibrium between the free enzyme and all enzyme-substrate complexes, the following v_0 equation applies:

$$v_0 = \frac{k[\mathbf{E}]_0[\mathbf{A}]_0[\mathbf{B}]_0}{(K + [\mathbf{A}]_0)(K + [\mathbf{B}]_0)} \quad (3)$$

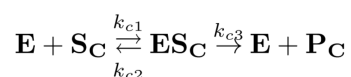
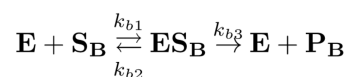
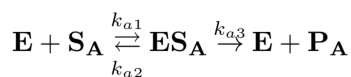
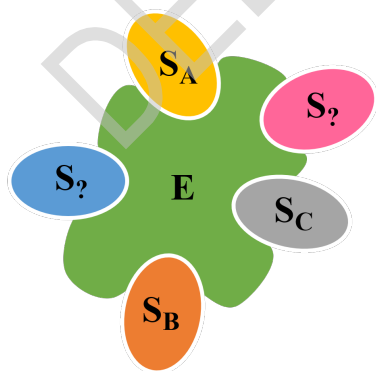
k is the rate constant of one of the reactions. The same equilibrium constant K characterizes the dissociation of either substrate from the corresponding active site of **E**.

7.3 **Show** that equation (3) takes the MM form (1) if the concentration of substrate **B** is maintained at a constant value c_0 . **Give** the expression for v_{\max} in this case. 2pt

7.4 **Propose** a kinetic scheme for the Enzymatic System I consistent with equation (3), showing all the intermediates and products. **Indicate** the reaction with a rate constant k . 8pt

Enzymatic System II

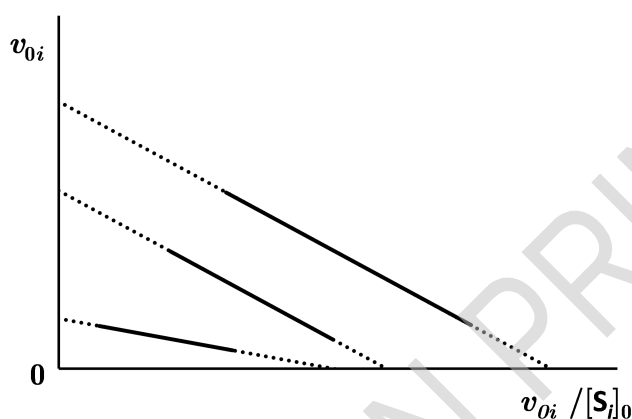
Enzyme **E** has five active sites, each of which is specific to one of the substrates **S_A**, **S_B**, or **S_C** that are selectively transformed to products **P_A**, **P_B**, or **P_C**, respectively. There is at least one active site for each substrate. Each active site is independent of the others.



For **E**, it is known that:

1. The affinity for **S_C** is higher than for **S_B**.

2. The plot of v_{0i} vs $v_{0i}/[S_i]_0$, known as Eadie-Hofstee plot, for S_A , S_B , S_C , with rate given per active site (v_{0i}), is shown below, but the scale and the legend are omitted.



3. When **E** is saturated with S_A , S_B , S_C , the catalytic turnover number (TON) for S_C per site is 10200 min^{-1} , and 2023 P_A , P_B , P_C , product molecules in total are synthesized per second. At the same time, no more than $5.94 \cdot 10^6$ molecules of P_A and P_B are detected to form per hour.

4. At equimolar concentrations of S_A , S_B , S_C , which are at least 1000 times lower than corresponding K_M values, the P_A , P_B , P_C , concentrations become proportional to the catalytic efficiency ($\varepsilon_i = \frac{k_{i3}}{K_{M,i}}$), and their ratio is 3 : 2 : 5, respectively.

5. Two ES_i complexes have equal rate constants for dissociation to **E** and S_i . The activation barrier for the reaction of ES_C into the initial compounds is 1266 J mol^{-1} higher than for the reaction into the final products. Assume that the pre-exponential factors are equal for both reactions and $T = 25 \text{ }^\circ\text{C}$.

6. For $E + S_j$ reaction: $k_{c1} = 1.57 \cdot 10^7 \text{ M}^{-1}\text{s}^{-1}$ and $k_{a1} = k_{b1}$.

7.5 **Fill in** the table **on the answer sheet** and **provide** your calculations. 17pt

Hints:

- Use information from 1. and 2. to find the relation between $K_{M,A}$, $K_{M,B}$ and $K_{M,C}$ ($<$, $>$, $=$).
- Information from 3. and 5. allows you to complete the first column (*number of active sites for each substrate*) and the last row (*all the constants for substrate S_C*) of the table. Check that the sum of active sites is equal to 5.