Exercises on enzyme kinetics

1 - You have isolated two versions of the same enzyme, a wild type and a mutant differing from the wild type at a single amino acid. Working carefully but expeditiously, you then establish the following kinetic characteristics of the enzymes.

	Maximum velocity	K_M
Wild type	100 μmol/min	10 mM
Mutant	1 μmol/min	0.1 mM

(a) With the assumption that the reaction occurs in two steps in which k_{-1} is much larger than k_2, which enzyme has the higher affinity for substrate?

K_M is a measure of affinity only if k_2 is rate limiting, which is the case here. Therefore, the lower K_M means higher affinity. The mutant enzyme has a higher affinity.

(b) What is the initial velocity of the reaction catalyzed by the wild-type enzyme when the substrate concentration is 10 mM?

50 m mol minute^{-1}. 10 mM is K_M, and K_M yields ½ Vmax . Vmax is 100 m mol minute^{-1}, and so. . .

(c) Which enzyme alters the equilibrium more in the direction of product?

Enzymes do not alter the equilibrium of the reaction.

2 - In the conversion of A into D in the following biochemical pathway, enzymes E_A , E_B , and E_C have the K_M values indicated under each enzyme. If all of the substrates and products are present at a concentration of 10^{-4} M and the enzymes have approximately the same Vmax , which step will be rate limiting and why?

$$\mathrm{A} \underset{E_A}{\rightleftharpoons} \mathrm{B} \underset{E_B}{\rightleftharpoons} \mathrm{C} \underset{E_C}{\rightleftharpoons} \mathrm{D}$$

$$K_M = \quad 10^{-2}\,\mathrm{M} \qquad 10^{-4}\,\mathrm{M} \qquad 10^{-4}\,\mathrm{M}$$

The first step will be the rate-limiting step. Enzymes E_B and E_C are operating at ½ Vmax , whereas the K_M for enzyme E A is greater than the substrate concentration. E_A would be operating at approximately 10^{-2} Vmax.

3 - For an enzyme that follows simple Michaelis–Menten kinetics, what is the value of Vmax if V0 is equal to 1 mmol/minute at 10 K_M?

11 m mol minute^{-1}

4 – We obtain the following results of reaction speeds for different concentrations of substrate. The concentration of enzyme is the same in all conditions.

	Reaction speed (µmol/s)	Substrate concentration (mM)
Experiment 1	0.54	0.01
Experiment 2	4.93	0.1
Experiment 3	27.2	1
Experiment 4	45.25	5
Experiment 5	49.3	10
Experiment 6	53.76	100
Experiment 7	54.24	1000
Experiment 8	54.29	10000

(a) Find the value of the maximum reaction speed.

It is the value indicated in blue, after increasing substrate concentration reaction speed remains the same, which indicates that the reaction cannot go any faster. In this situation, all enzyme molecules are binding substrate and contributing actively to the reaction.

(b) Find the value of the Michaelis constant (K_M).

It is the value indicated in orange, which is the concentration of substrate at which the speed of the reaction is 50% of the maximum speed. You can see that at [S] = 1; the reaction speed is half of the maximum reaction speed.

(c) Calculate the reaction speed when the substrate concentration is 3.5 mM.

$$54.3 \cdot \frac{3500}{3500+1000} = 42.23 \text{ µmol/s}$$

(d) Explain how maximum speed can be improved.

By increasing the concentration of enzyme in the system, $V_{max} = k_2 \cdot [E]_{total}$. K_2 is a constant and cannot be changed, but the total amount of enzyme in the system is an experimental condition that can be changed.

5 - We obtain the following results of substrate produced in one minute for different concentrations of substrate. The concentration of enzyme is the same in all conditions.

	Product produced (µmol/min)	Substrate concentration (mM)
Experiment 1	4.22	0.001
Experiment 2	37.19	0.01
Experiment 3	169.03	0.1
Experiment 4	261.88	1
Experiment 5	277.1	10
Experiment 6	278.7	100
Experiment 7	278.8	1000

(a) Find the value of the maximum reaction speed.

It is the value indicated in blue, after increasing substrate concentration reaction speed remains the same, which indicates that the reaction cannot go any faster. In this situation, all enzyme molecules are binding substrate and contributing actively to the reaction.

(b) Find the value of the Michaelis constant (K_M).

$$V = Vmax \cdot \frac{[S]}{[S] + KM} \rightarrow KM = \left(Vmax \cdot \frac{[S]}{V}\right) - [S] \rightarrow KM = 64.6\ \mu M$$

(c) Calculate the reaction speed when the substrate concentration is 0.35 mM.

$$278.8 \cdot \frac{350}{350+64.6} = 235.4\ \mu\text{mol/min}$$

6 - We obtain the following results of reaction speeds for different concentrations of substrate. The concentration of enzyme is the same in all conditions.

	Reaction speed (µmol/s)	Substrate concentration (mM)
Experiment 1	3.8	0.1
Experiment 2	11.09	0.5
Experiment 3	14.6	1

Find the value of the maximum reaction speed and the Michaelis constant.

$$V = Vmax \cdot \frac{[S]}{[S] + KM} \rightarrow KM = \left(Vmax \cdot \frac{[S]}{V}\right) - [S] \rightarrow$$

$$\left(Vmax \cdot \frac{[S]1}{V1}\right) - [S]1 = \left(Vmax \cdot \frac{[S]2}{V2}\right) - [S]2 \rightarrow$$

$$Vmax = \frac{[S]1 - [S]2}{\frac{[S]1}{V1} - \frac{[S]2}{V2}} \rightarrow Vmax = 21.3\ \mu mol/s$$

$$V = Vmax \cdot \frac{[S]}{[S] + KM} \rightarrow KM = \left(Vmax \cdot \frac{[S]}{V}\right) - [S] \rightarrow KM = 460\ \mu M$$

7 - We obtain the following results of reaction speeds for different concentrations of substrate and enzyme. These are the results we obtain:

	Reaction speed (µmol/s)	Substrate concentration (mM)	Enzyme concentration (µM)
Experiment 1	33.83	0.5	10
Experiment 2	61.02	1.0	10
Experiment 3	16.91	0.5	5

(a) Find the value of the k2 constant.

$$V = k2 \cdot [E] \cdot \frac{[S]}{[S] + KM} \rightarrow KM = \left(\frac{k2 \cdot [E] \cdot [S]}{V}\right) - [S] \rightarrow$$

$$\left(\frac{k2 \cdot [E]1 \cdot [S]1}{V1}\right) - [S]1 = \left(\frac{k2 \cdot [E]2 \cdot [S]2}{V2}\right) - [S]2 \rightarrow$$

$$k2 = \frac{[S]1 - [S]2}{\frac{[E]1 \cdot [S]1 \cdot V2 - [E]2 \cdot [S]2 \cdot V1}{V1 \cdot V2}} \rightarrow k2 = 31.09\ 1/s$$

(b) Find the value of the Michaelis constant.

$$V = k2 \cdot [E] \cdot \frac{[S]}{[S] + KM} \rightarrow KM = \left(\frac{k2 \cdot [E] \cdot [S]}{V}\right) - [S] \rightarrow KM = 4100\ \mu M$$

(c) Find the value of the maximum reaction speed for each experimental condition.

$$Vmax = k2 \cdot [E] \rightarrow Vmax1 = Vmax2 = k2 \cdot 10 = 310.9\ \mu M/s$$

$$Vmax3 = k2 \cdot 5 = 155.4\ \mu M/s$$