Enzyme Kinetics: How they do it

(R1) Formation of Enzyme-Substrate complex: \[ E + S \rightleftharpoons ES \]
(R2) Formation of Product (i.e. reaction): \[ ES \rightarrow E + P \]
(R3) 'Desorption' (decoupling/unbinding) of product is usually instantaneous, hence lumped into step (R2).

Enzymatic reaction: \[ E + S \xrightarrow{k_{tf} \over k_{tb}} ES \xrightarrow{k_2} E + P \]

Looks and sounds familiar??

heterog. catalysis: \[ A + S \xrightarrow{k_{ads} \over k_{des}} AS \xrightarrow{k_{des}} S + P \]

Let's look at the kinetics:

Hence, rate of product formation:

But we don’t know the concentration of the enzyme-substrate complex [ES]!
Michaelis-Menten Kinetics

However, since the time-dependent enzyme concentration is also difficult to measure, we replace this by: \([E] = [E]_0 - [ES]\) (remember: it’s a catalyst!)

\[
[ES] = \frac{k_{1f}}{k_{1b}} \frac{[E][S]}{[E]_0 - [ES]} = \frac{k_{1f}}{k_{1b}} \frac{[E][S]}{[E]_0 - [ES]}
\]

Substitute into \(r_p\): \(r_p = k_2 \times \frac{[ES]}{[E]_0}[S]

If we now define two more quantities...

...we can re-write this as:

\[
\frac{r_p}{K_m + [S]} = \frac{v_{max} [S]}{K_m + [S]}
\]

Michaelis constant

maximum ‘velocity’

(this is really a rate, but then... those biologists!)
Effect of Substrate Concentration

Look familiar, sound familiar??  => compare to Langmuir-Hinshelwood Kinetics!
Lineweaver-Burk Plot

In principle, one could determine $K_m$ and $v_{\text{max}}$ from the previous plot. However, since $v_{\text{max}}$ is an asymptote, this would be very imprecise...

**Hence:** linearize the plot!

(...sound again familiar??)

'Lineweaver-Burk' plot: double reciprocal plot of 'velocity' vs $[S]$

**Problem:**
Taking inverse of measured data changes the statistical error and weight.
Alternative: by re-arranging the MM equation, we get

**Advantage:**
No

**Disadvantage:**
Both

Other plots have been suggested, but nowadays often a nonlinear fitting routine to the MM expression is used.
Let's go back to the reaction mechanism again. What if we don’t assume ESA, but rather assume PSSA for ES? **Do it!**

Enzymatic reaction: \[ E + S \xrightarrow{k_{if}} ES \xrightarrow{k_{ib}} E + P \]

PSSA for [ES]:

Replacing again the (time-dependent) [E] by: \[ [E] = [E]_0 - [ES] \]

Substitute into \( r_P \): \[ r_P = k_2 [ES] = \]
Enzyme Kinetics: Example

You are evaluating a 4.0 mg/ml solution of an enzyme (\(M = 40,000\) Da) by measuring the initial rates ("velocities", \(v_o\)) at several initial substrate concentrations \([A]\).

a) Determine \(K_m\) and \(V_{max}\).

b) What is \(k_r\) for the (product forming) reaction?

<table>
<thead>
<tr>
<th>[A] mM</th>
<th>(v_o) mM/min</th>
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<tr>
<td>0.25</td>
<td>0.060</td>
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<tr>
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<tr>
<td>12</td>
<td>0.430</td>
</tr>
<tr>
<td>20</td>
<td>0.460</td>
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</tbody>
</table>
Remember Catalyst Deactivation?

- Enzymes are highly specialized and hence very sensitive...
- they are hence prone to deactivation (even more so than inorganic catalysts!)
- **denaturation** is an important form of enzyme deactivation

Denaturation is a consequence of the importance of the spatial structure of enzymes for their function.

![RNAse A](image)
Effect of Temperature

Enzyme denature at high temperatures.

Two opposing effects of T-increases on enzyme reactions:
‘Arrhenius vs Denaturation’!

For most enzymes, reaction rate plateaus slightly above body temperature (37° C) (exception: extremophiles).

Denaturation is an activated process, which follows an Arrhenius-type kinetics. The activation energy for enzyme reactions is typically ~ 50 kJ/mol, while activation energies for deactivation vary from 150-500 kJ/mol (mostly ~300 kJ/mol).

What does this mean for temperature increases in enzyme reaction?
(Consider the effect of a T-rise from 30°C - 40°C on the kinetic rate constants.)
Effect of pH

Each enzyme exhibits peak activity at narrow pH range (pH optimum).

At this pH:

- R-groups have proper charge
- tertiary structure is correct

pH optimum reflects the pH of the body fluid in which the enzyme is found.

If pH changed reaction rate decreases
Quick Learning Check

Sucrase functions at a temperature of 37°C and a pH of 6.2. Determine the effect of the following on its rate of reaction:

(1) no change   (2) increase   (3) decrease

A. Changing the pH to 4
B. Running the reaction at 70°C
C. Increasing the concentration of sucrose
D. Increasing the concentration of sucrase