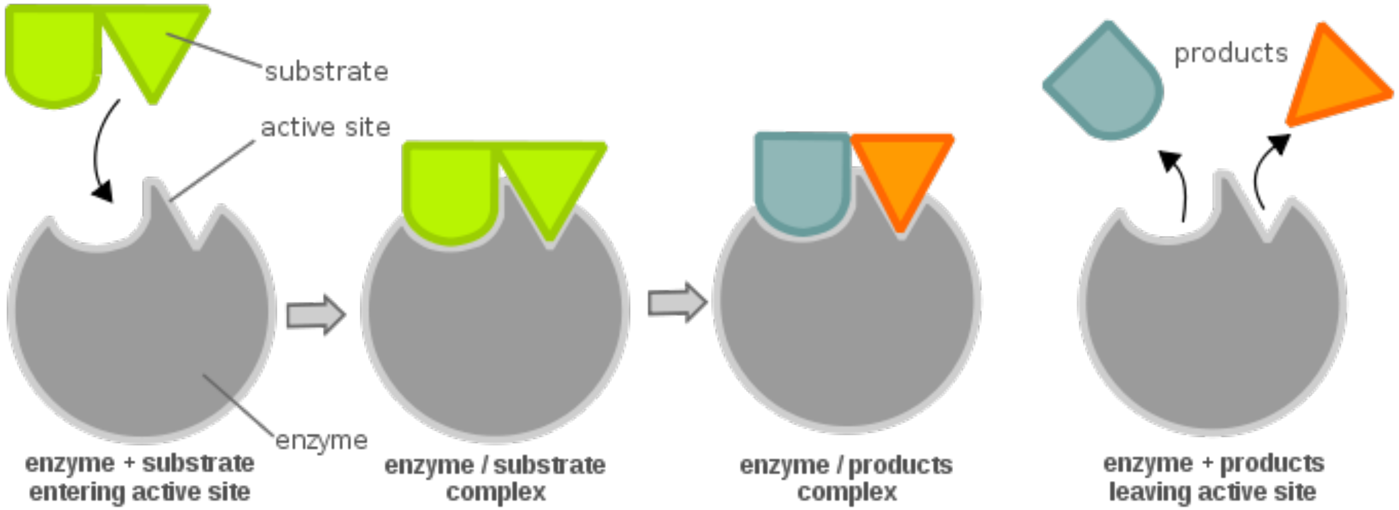
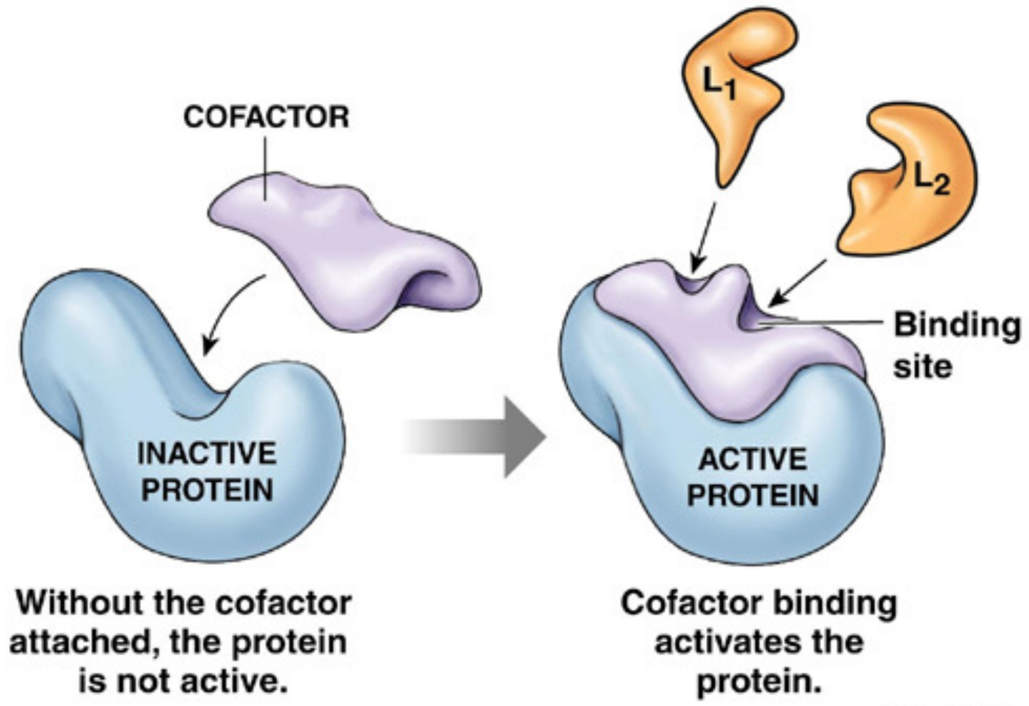
Lesson 6
Enzyme kinetics



Generalities

- Enzymes are biological catalysts
- Very specific, targeting only one defined reacting species (substrate)
- Enzymes are proteins
- Some have a nonprotein part called cofactor
- Cofactors can be
  - metal ions (Mg^{++})
  - Organic molecules (coenzymes)
- Only one region of the enzyme is responsible for substrate interaction (active site)



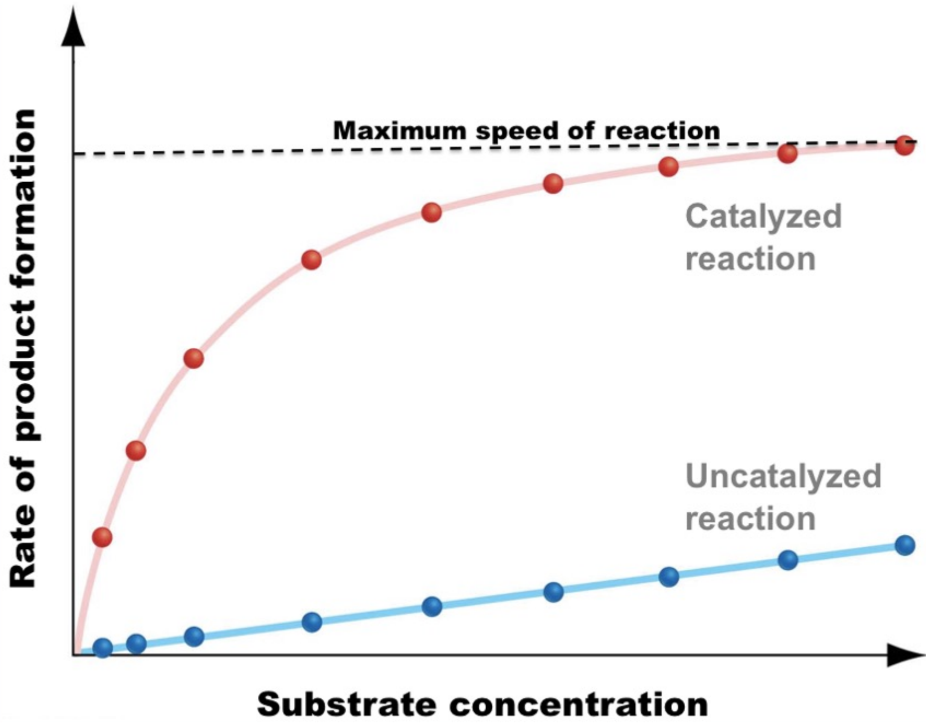
Enzyme classification

- Enzymes names begin with EC followed by 4 numbers separated by dots
  - EC 2.7.4.4
- First number → which of the 6 major enzyme classes the molecules belongs to
- Other numbers → enzyme sub-classes
- Names are indicative of the action performed

Number	*Classification*	*Biochemical Properties*
1	Oxidoreduc-tases	Act on many chemical groupings to add or remove hydrogen atoms
2	Transferases	Transfer functional groups between donor and acceptor molecules. Kinases are specialized transferases that regulate metabolism by transferring phosphate from ATP to other molecules
3	Hydrolases	Add water across a bond, hydrolyzing it
4	Lyases	Add water, ammonia, or carbon dioxide across double bonds, or remove these elements to produce double bonds
5	Isomerases	Carry out many kinds of isomerization: L to D isomerizations, mutase reactions (shifts of chemical groups), and others
6	Ligases	Catalyze reactions in which 2 chemical groups are joined (or ligated) with the use of energy from ATP

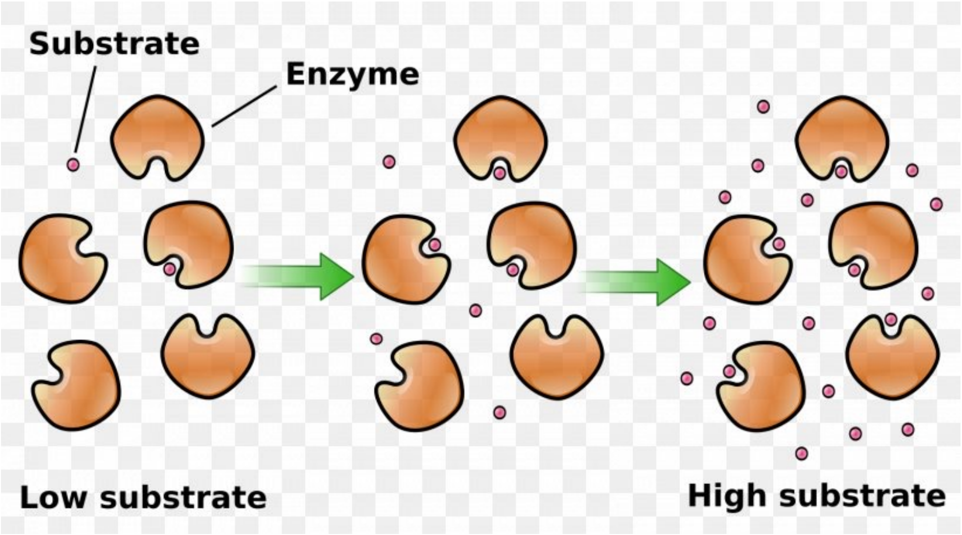
Enzyme kinetics

- Enzyme assay → Experiment to determine the enzyme's catalytic activity
- Plot of reaction rate (V) vs. [substrate = S] in the absence (blue) and presence (red) of enzyme
- Note:
  - In both cases, at low [S] both curves are approximately linear
    - first-order kinetics
  - At equal, intermediate [S], V in enzyme-catalyzed is substantially higher that in uncatalyzed reaction
  - In uncatalyzed reactions V continue to increase with [S] while in enzyme-catalyzed reactions it reaches an asymptotic value V_{max}
    - V becomes independent of [S] → zero-order kinetics



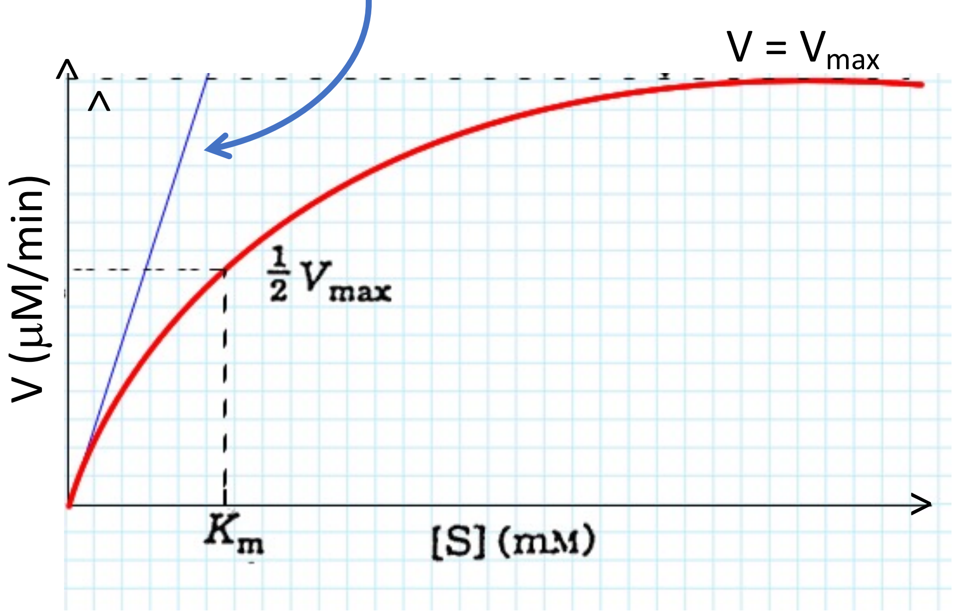
Enzyme kinetics

- V_{max} corresponds to a specific condition called enzyme saturation



All enzyme molecules are part of a enzyme-substrate complex and no free enzyme molecules are available to accommodate additional substrate molecules

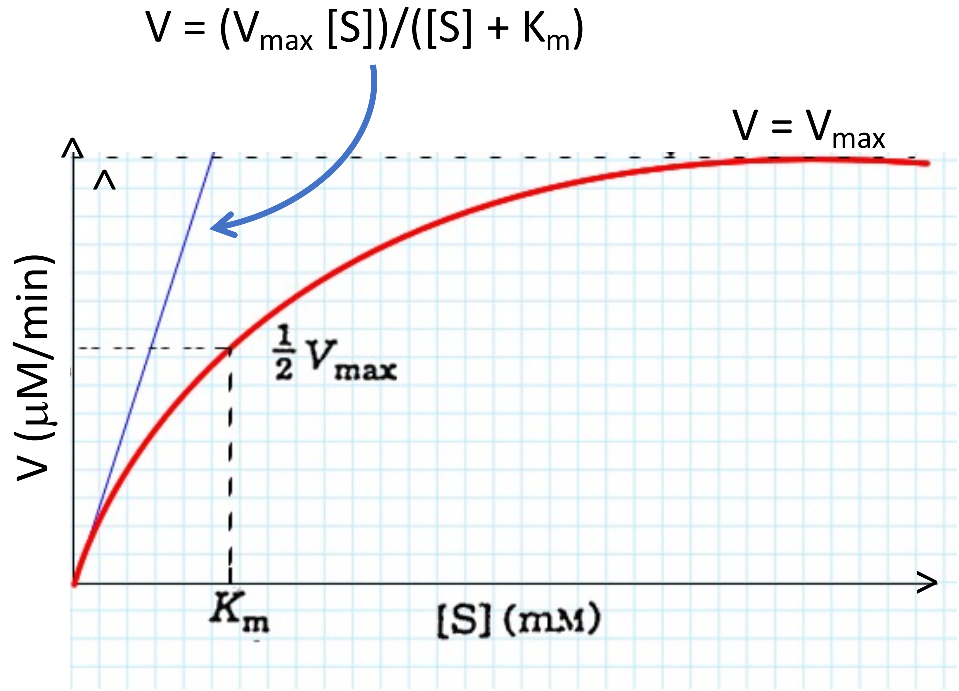
$V = (V_{max} [S])/([S] + K_m)$



The Michaelis-Menten equation

- Three further important points on the graph in the first-order kinetics region
  - The point at which V_0 = ½ Vmax
  - [S] at which V_0 = ½ Vmax = K_m [in mol/L or M]
    - K_m = Michaelis-Menten constant → rough measure of the affinity of the enzyme for the substrate
      - K_m varies in a wide range for different enzymes
  - In this region, the behavior of V = f[S] is described by the so-called Michaelis-Menten equation

$$V = (V_{max} [S])/([S] + K_m)$$



At low [S], [S] << K_m → $V = (V_{max} [S])/K_m$

At high [S], [S] >> K_m → $V = V_{max}$

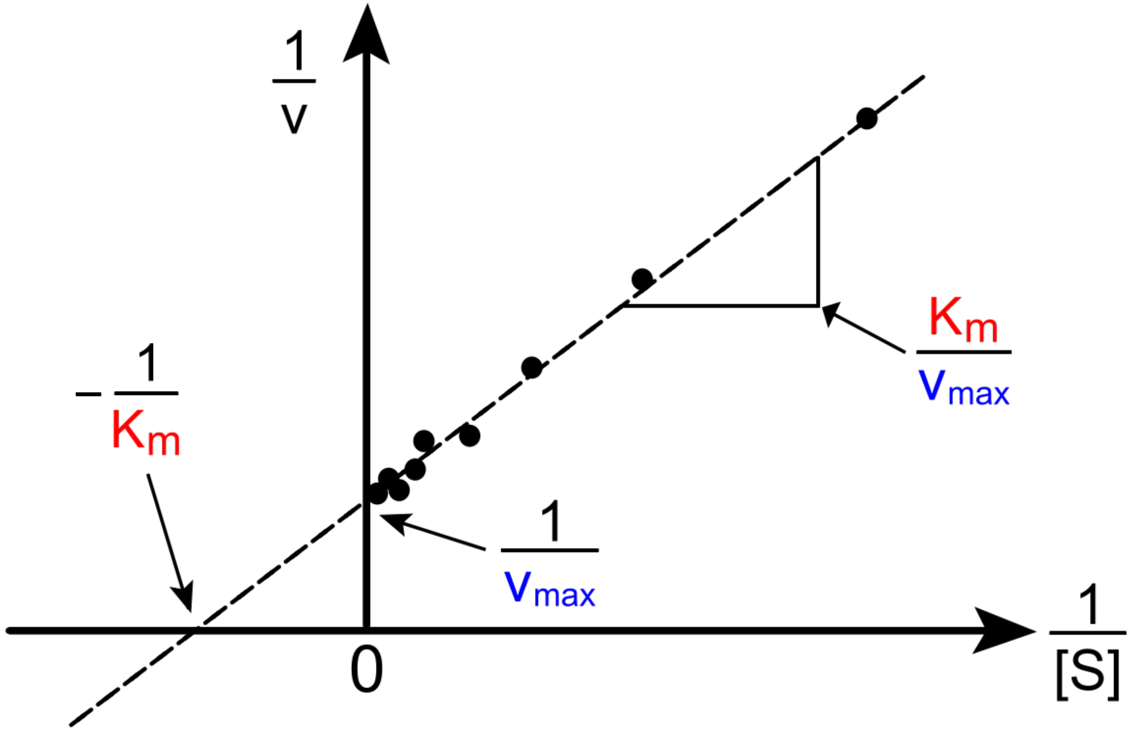
At [S] = K_m → $V = ½ V_{max}$

The Lineweaver-Burk plot

- K_m and V_{max} can be easily found by data fitting with a spreadsheet
  - Yet, a Lineaweaver-Burk (or double-reciprocal plot) is still exceedingly common

$1/V = [(K_m/V_{max}) \times 1/[S]] + 1/V_{max}$

- Main problem with LB plots
  - Overemphasizes low [S] velocities
  - Gives more an order of magnitude of K_m and V_{max} than an accurate estimate of values



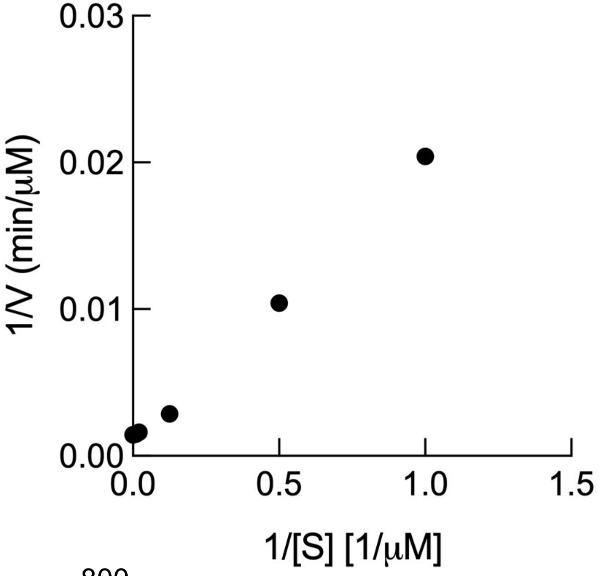
Quiz time

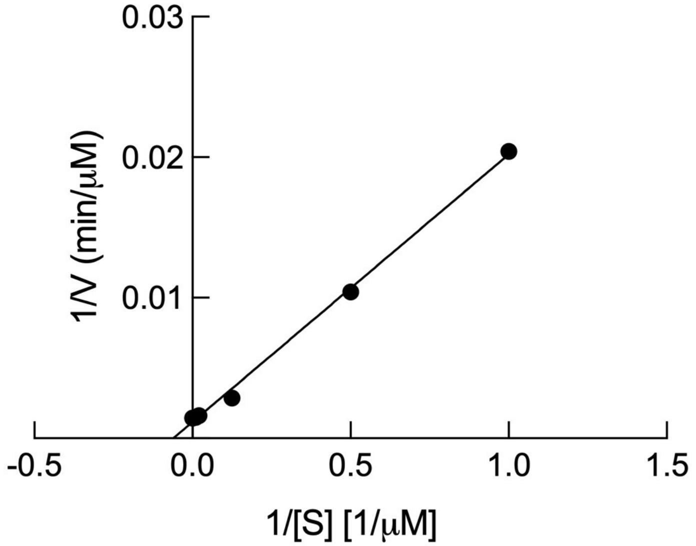
- An enzymatic assay yielded the following data set:
- Q1. Determine V_{max} and K_m

[S] [μM]	V (μM/min)
1	49
2	96
8	349
50	621
100	676
1000	698
5000	699

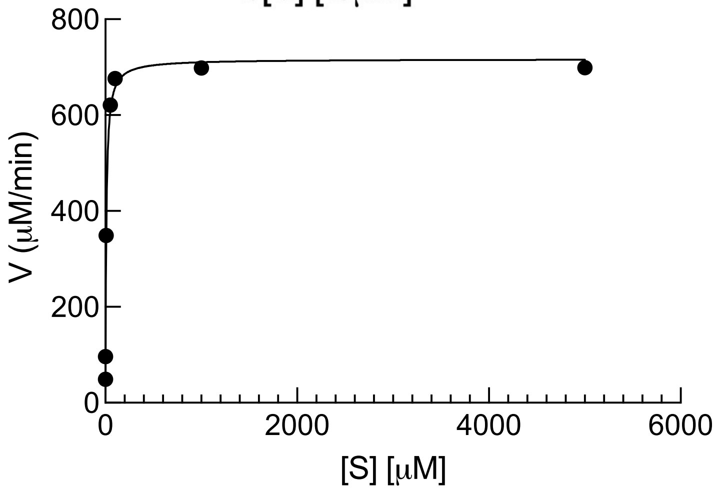
Quiz time

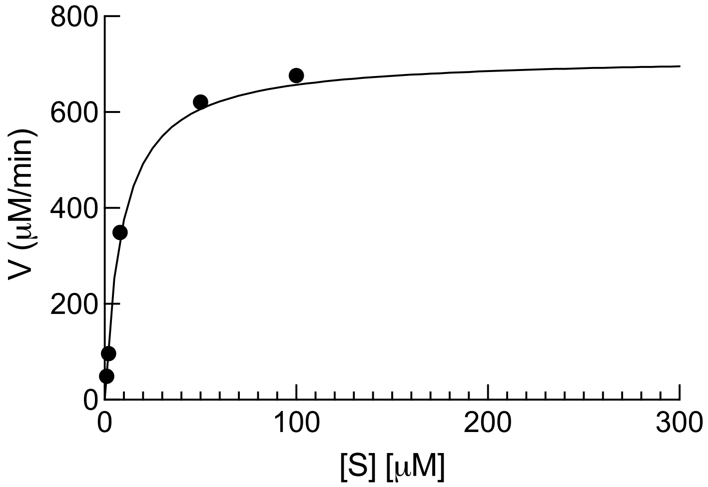
- R1.





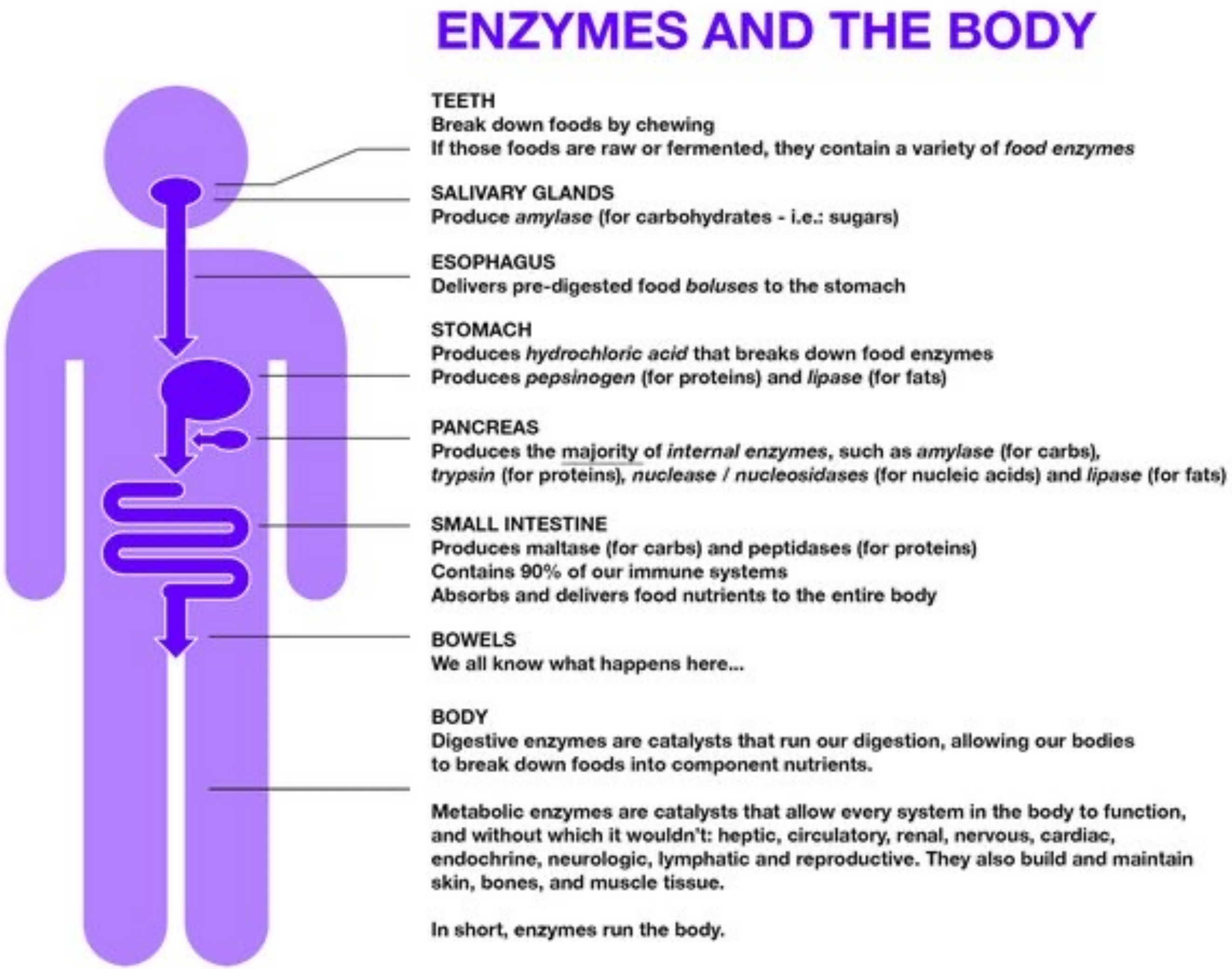
V_{max}	863 μM/min
K_m	16.4 μM
R^2	0.9977





V_{max}	717 μM/min
K_m	9.13 μM
R^2	0.9945

(Some) Enzymes and the body



Enzyme	Secreted by	Function
Salivary Amylase (Ptyalin)	Salivary Glands	Converts starch to maltose
Renin	Stomach	Converts milk proteins to peptides
Pepsin	Stomach	Converts other proteins to peptides
Gastric Amylase	Stomach	Converts starch to maltose
Gastric Lipase	Stomach	Converts butter fat into fatty acids and glycerol
Trypsin	Pancreas	Converts proteins to peptides
Chymotrypsin	Pancreas	Converts proteins to peptides
Steapsin (Pancreatic Lipase)	Pancrease	Converts fats into fatty acids and glycerol
Carboxypolypeptidase	Pancreas	Converts peptides into amino acid.
Pancreatic Amylase	Pancreas	Converts starch to maltose
Entirokinase	Small Intestine	entirokinase activates trypsinogen to tryspsin.
Eripsin	Small Intestine	Converts polypeptides to amino acids.
Maltase	Small Intestine	Digests Maltose to glucose.
Sucrase	Small Intestine	Digests sucrose into glucose and fructose.
Lactase	Small Intestine	Digests lactose into glucose and galactose.

Where the money is: enzymes and industry

Common uses of enzymes in industry include:

Detergents contain proteases and lipases to help breakdown protein and fat stains

Enzymes are used to breakdown the starch in grains into **biofuels** that can be combusted

In the **textiles** industry enzymes help in the processing of fibres, e.g. polishing cloth to make it appear more shiny

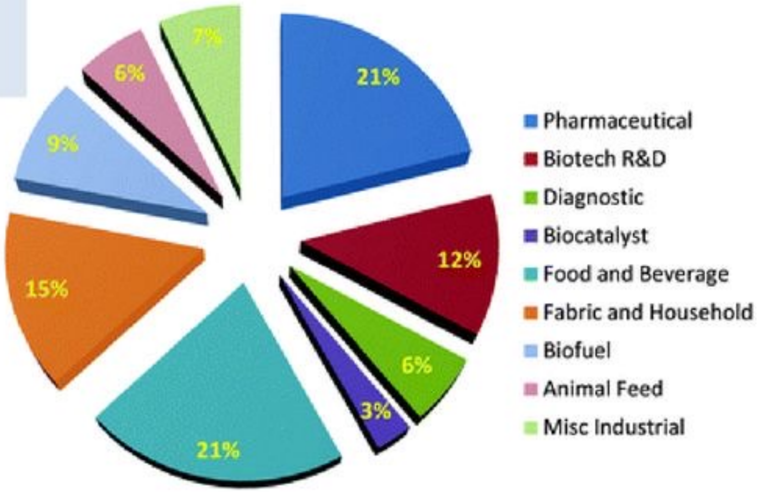
In the **brewing** industry enzymes help a number of processes including the clarification of the beer

In **Medicine & Biotechnology** enzymes are widely used in everything from diagnostic tests tests to contact lens cleaners to cutting DNA in genetic engineering.

Enzymes are widely used in the **food** industry, e.g.

- fruit juice, pectin to increase the juice yield from fruit
- Fructose is used as a sweetener, it is converted from glucose by isomerase
- Rennin is used to help in cheese production

Paper production uses enzymes to helping in the pulping of wood



Estimated total 2010 market value: $5.8B

The global market for enzymes in industrial applications should grow from **$6.4 billion** in 2021 to $8.7 billion by 2026, at compound annual growth rate (CAGR) of 6.3% for the period of 2021-2026.