Laboratory 11

Enzyme Kinetics

Properties of Enzymes

Enzymes

- mostly proteins; some are RNAs (Ribozymes).

Active site

- Binds substrate, forming an enzyme-substrate complex (ES).
- ES is converted to enzyme-product (EP) which subsequently dissociates in enzyme and product.

Specificity

- Highly specific, catalyzes one type of reaction.

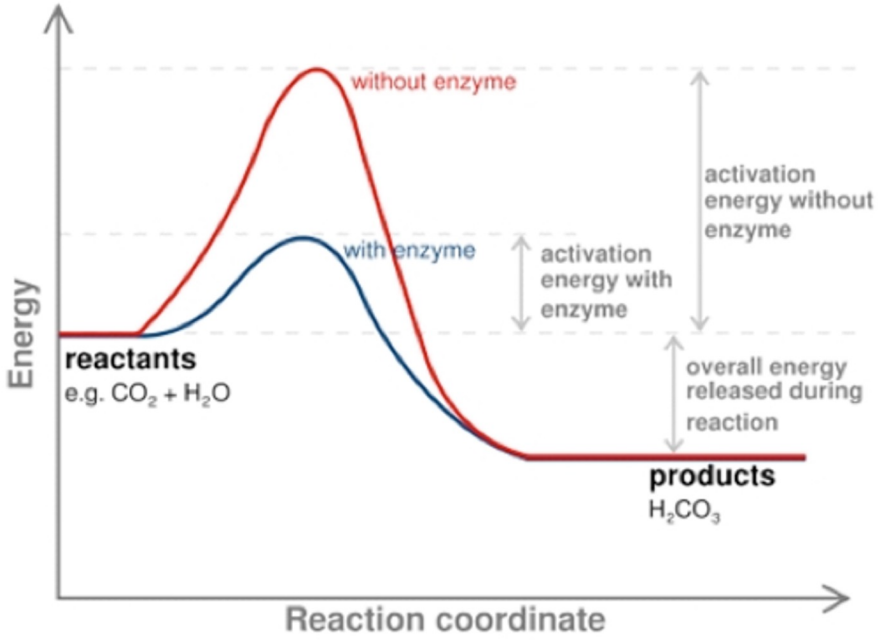
Cofactors

- Non protein: metal ions (Fe^{2+}, Zn^{2+} for α-amylase2 Ca^{2+}).
- Organic molecules (coenzymes): NAD^{+}, FAD, Coenzyme A.

Regulation

- By activators and inhibitors to respond to what the cell needs.

How do Enzymes work?



Enzymes reduce the activation energy of a reaction, but not the free energy of the overall reaction (energy of reactions minus energy of products)

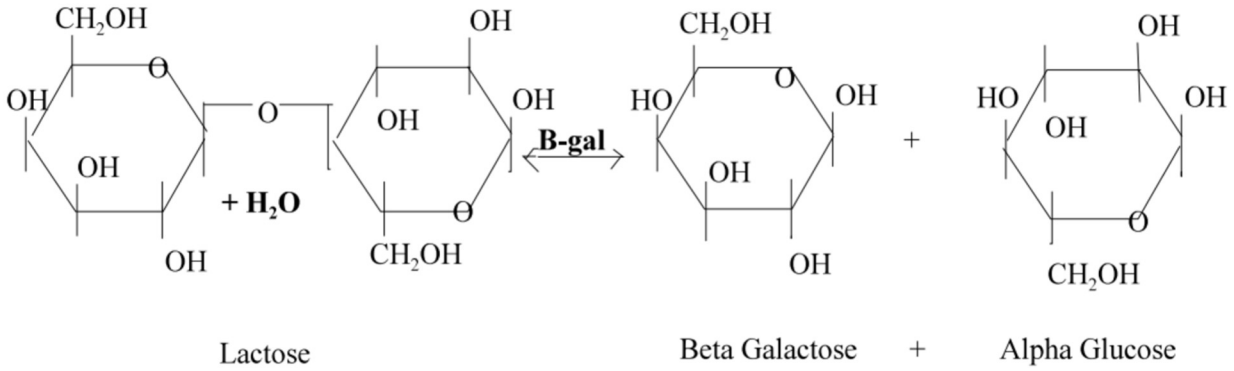
Example: reaction of carbon dioxide and water
Upon addition of carbon dioxide to water it will undergo hydrolysis to form carbonic acid

Enzyme Kinetics

We will determine kinetic parameters of **beta-galactosidase** using the **lactose analogue** orhto-nitrophenil β-D-galactopyranoside **ONPG** as substrate

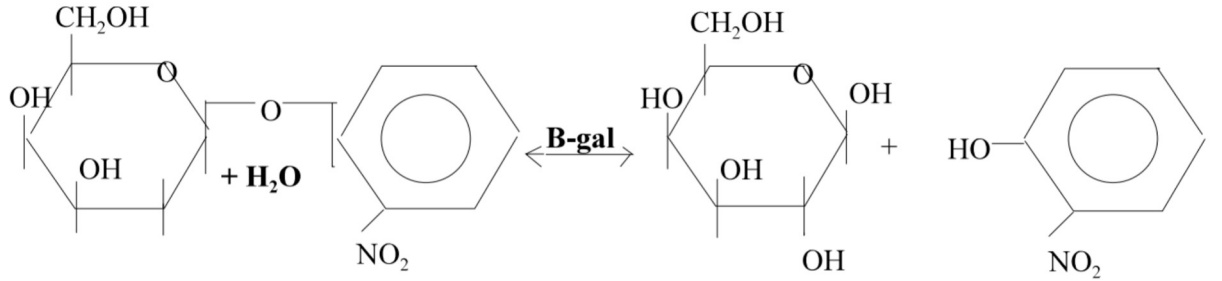
Task: Determine K_m and V_{max}

Enzymatic reaction



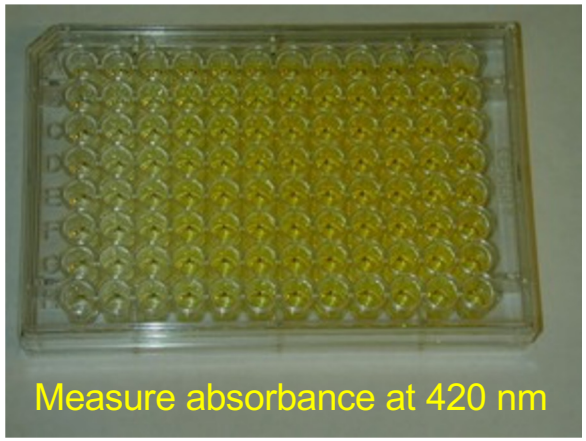
Lactose Beta Galactose + Alpha Glucose

natural substrate



ONPG (colorless) Beta Galactose **ONP** (yellow)

lactose analog



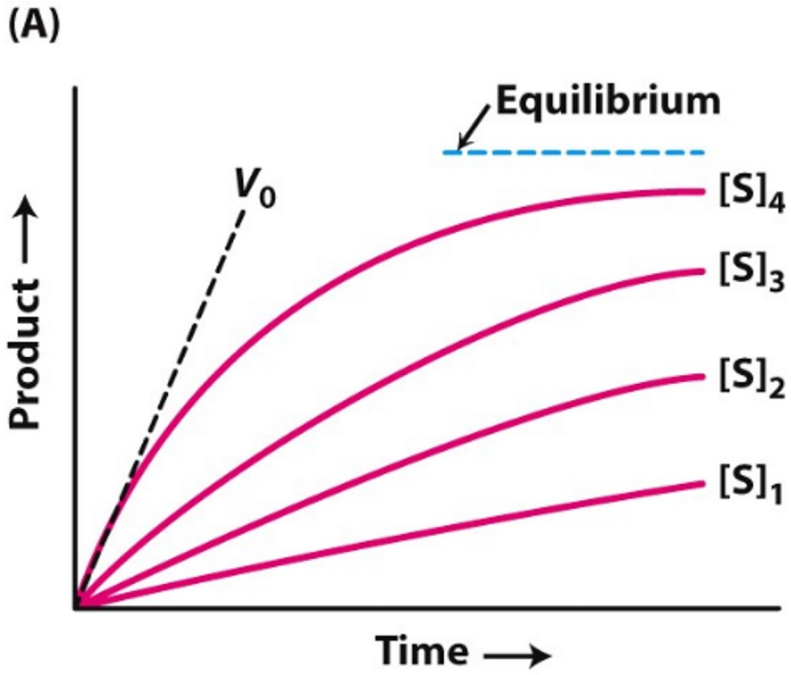
Experiments

Effect of substrate concentration on the activity of beta-galactosidase

Determine initial velocity (V_0) values for each concentration of ONPG +/- IPTG inhibitor (substrate analogue)

Create Michaelis-Menten and Lineweaver–Burk plots

Determining Initial Velocity (Vo)



The **initial velocity** (V_0) for each substrate concentration is determined from the **slope** of the curve at the beginning of the reaction.

Michaelis-Menten Plot

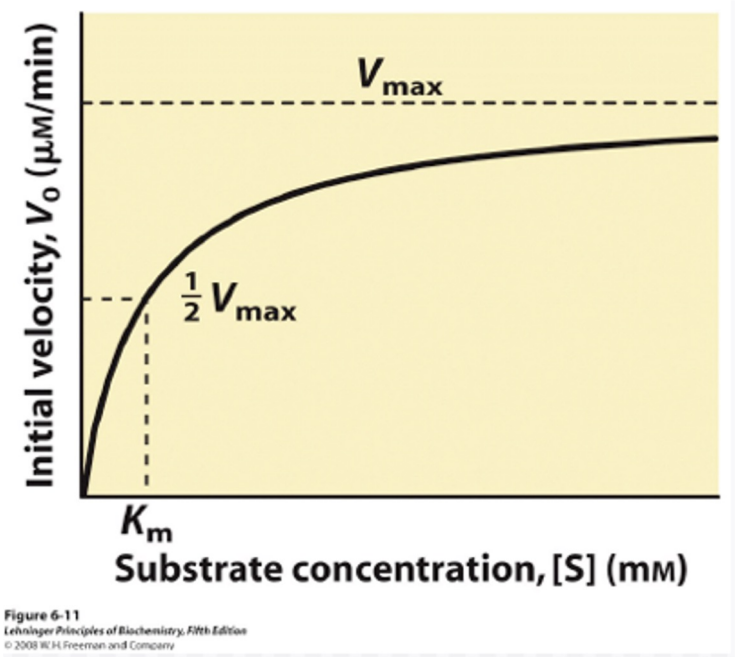
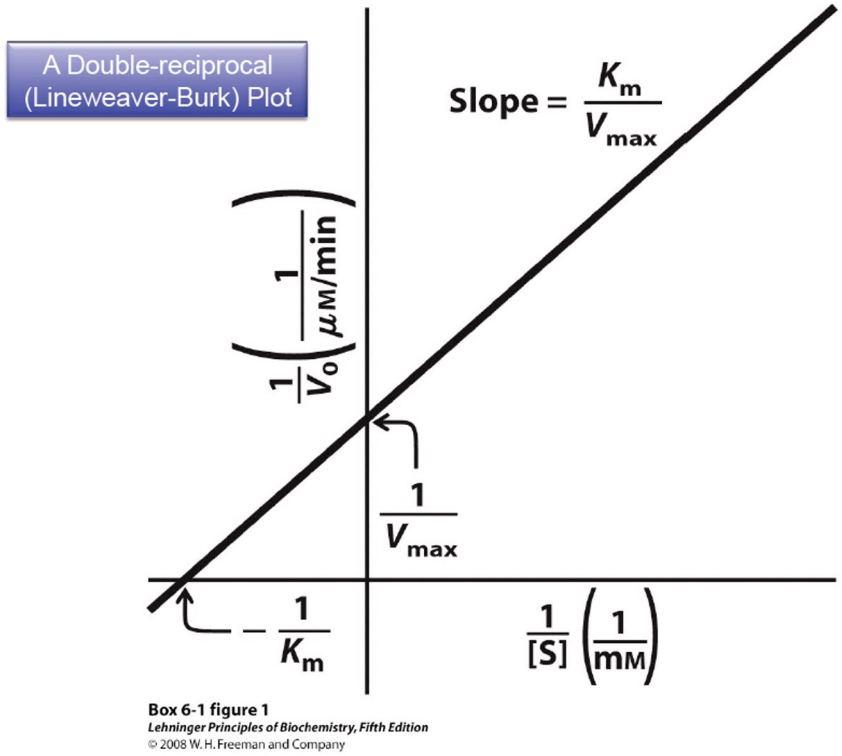


Figure 6-11
Lehninger Principles of Biochemistry, Fifth Edition

Michaelis constant (Km)
- reflects the **affinity** of an enzyme for a **substrate**
- equal to the **[S]** at which the reaction velocity is ½ **Vmax**
- small Km: high affinity
- large Km: low affinity

due to the upward gradual slope you obtain a hyperbolic curve (approximation of Vmax and Km)
-> plot $1/v_0$ versus 1/[S] (Lineweaver-Burk Plot) to determine Vmax and Km

Lineweaver-Burk Plot



Box 6-1 figure 1
Lehninger Principles of Biochemistry, Fifth Edition

Effect of Enzyme Inhibition on Km and Vmax