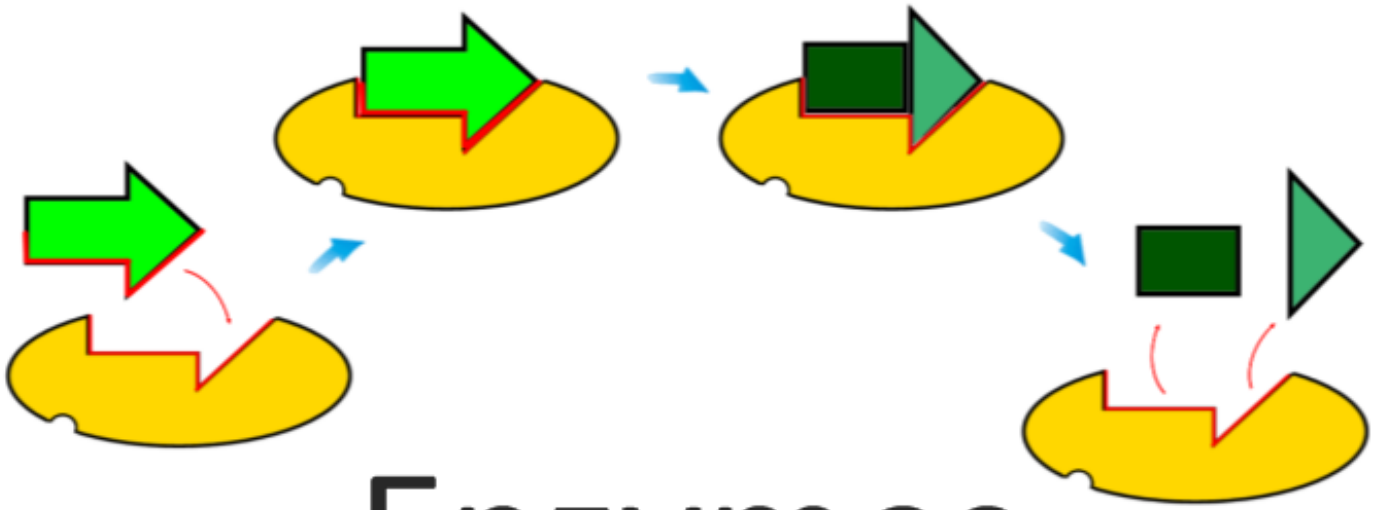
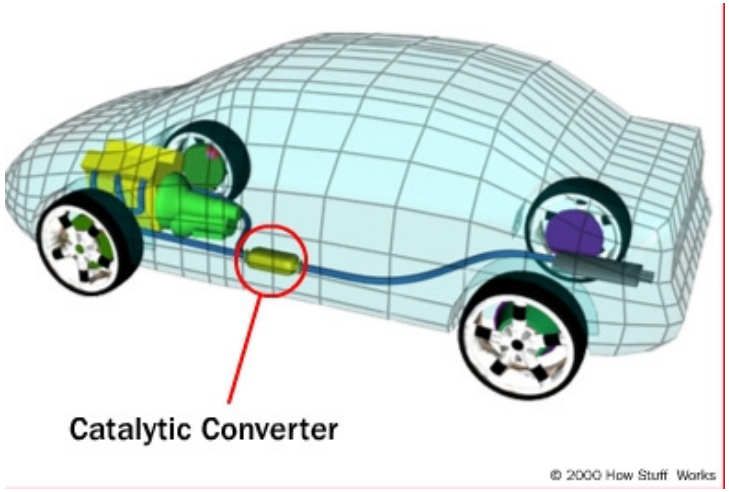


Enzymes

(Core and C2/7.6)

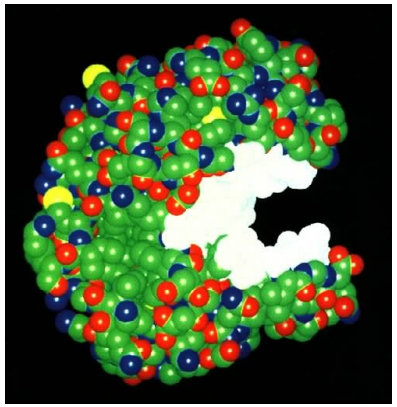
First…Catalysts

- Manganese dioxide (a black powder) will catalyze the breakdown of hydrogen peroxide.
- Car exhaust pipes use catalytic converters help convert carbon monoxide (CO) and unburned hydrocarbons (HC), and oxides of nitrogen (NOx) to produce carbon dioxide (CO_2), nitrogen (N_2), and water (H_2O).
- Some catalysts are very expensive e.g. platinum in a catalytic converter.
- The lead in leaded gas will bind with and “poison” the catalyst in a catalytic converter.



What is an Enzyme?

- A catalyst that accelerates a biological reactions in a cell
- Most are tertiary or quaternary, globular proteins
- Lowers the activation energy of a reaction→increases the rate of reaction



- May contain prosthetic groups or cofactors such as metal ions or organic compounds (vitamins)
- Only changes the rate of reaction. They do not change the equilibrium or end products.
- Specific to one particular reaction (substrate / reactant→ products)

What is an Enzyme? Animations

- http://highered.mcgraw-hill.com/sites/0072495855/student_view0/chapter2/animation__how_enzymes_work.html

- http://www.northland.cc.mn.us/biology/biology1111/animations/enzyme.swf

What is an Enzyme? (cont'd)

- The particular shape determines which chemical reaction the enzyme can speed up
- In speeding up the reaction, the enzyme combines temporarily with the substance(s) it is acting on
- Present in very small amounts due to high molecular activity
- Enzymes are not permanently changed nor "used up" in the process of catalyzing a reaction
- Activity is lost if denatured

Classification of Enzymes

- **Generally end in *–ase***
- **Identifies a reacting substance**

  sucrase – reacts sucrose

  lipase - reacts lipid
- **Describes function of enzyme**

  oxidase – catalyzes oxidation

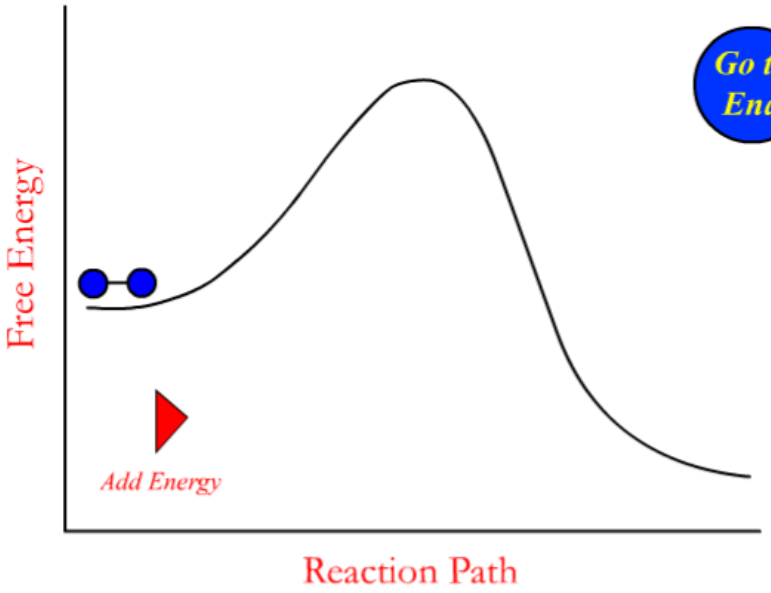
  hydrolase – catalyzes hydrolysis
- **Common names of digestion enzymes still use *–in***

  pepsin (protein→peptides), trypsin (hydrolyzes protein)

Classification of Enzymes

Class	Catalyzes
• **Oxidoreductoases**	**oxidation-reduction**
oxidases - oxidize ,reductases – reduce	
• **Transferases**	**transfer group of atoms**
transaminases – transfer amino groups	
kinases – transfer phosphate groups	
• **Hydrolases**	**hydrolysis**
proteases - hydrolyze peptide bonds	
lipases – hydrolyze lipid ester bonds	
• **Lyases**	**add/remove atoms from double bonds**
carboxylases – add CO_2	
hydrolases – add H_2O	
• **Isomerases**	**rearrange atoms**
• **Ligases**	**combine molecules using ATP**

Enzymes lower the activation energy of a reaction.

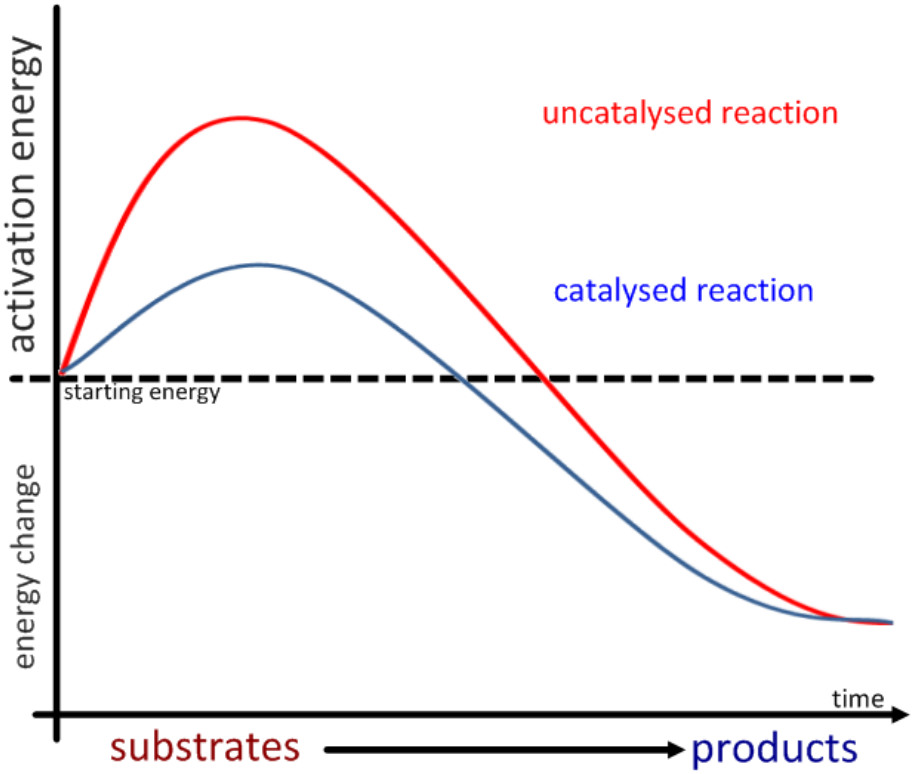


Go to End

http://www.stolaf.edu/people/giannini/flashanimat/enzymes/transition%20state.swf

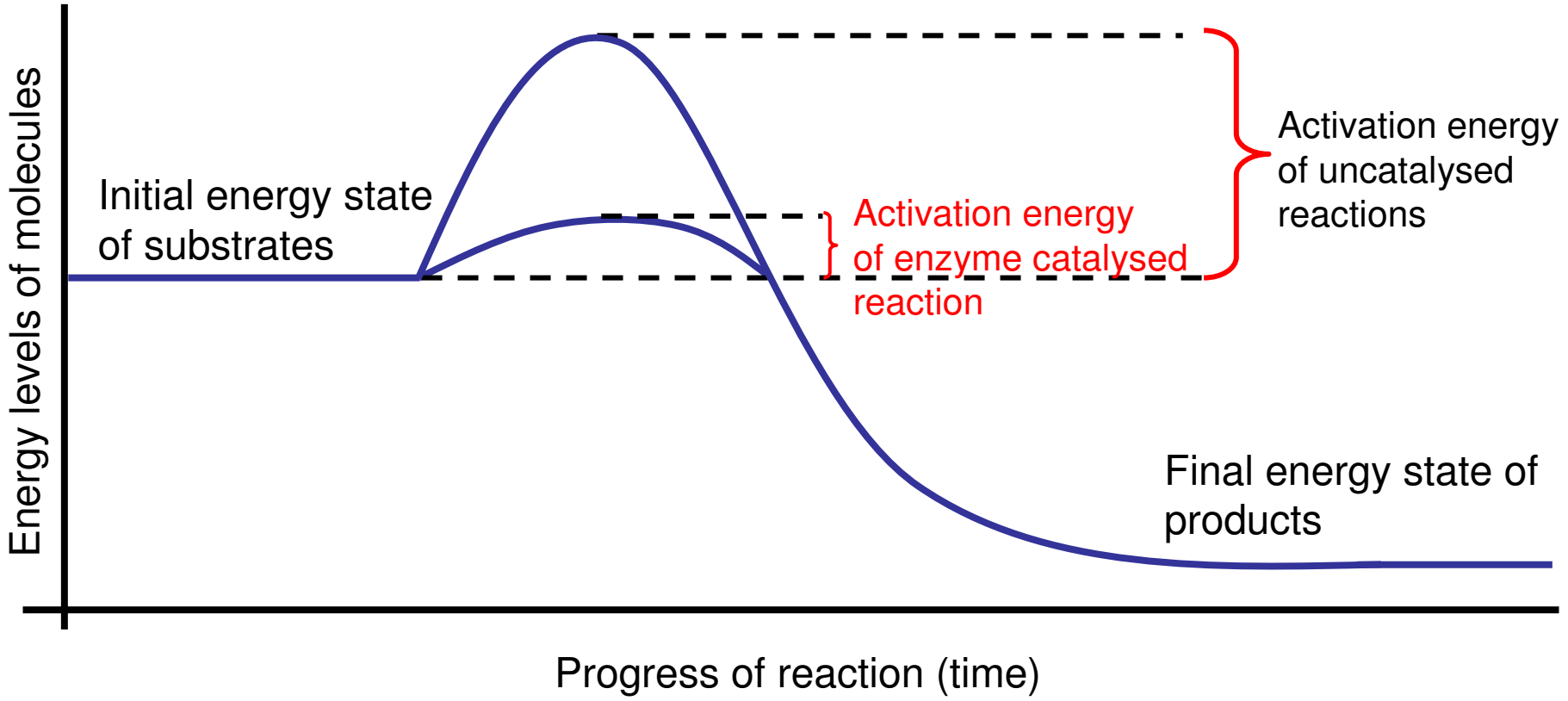
Activation energy is the amount of energy that must be put into a reaction to make it occur.

An enzyme stresses the bonds in the substrate(s), reducing the activation energy required for a reaction to occur.

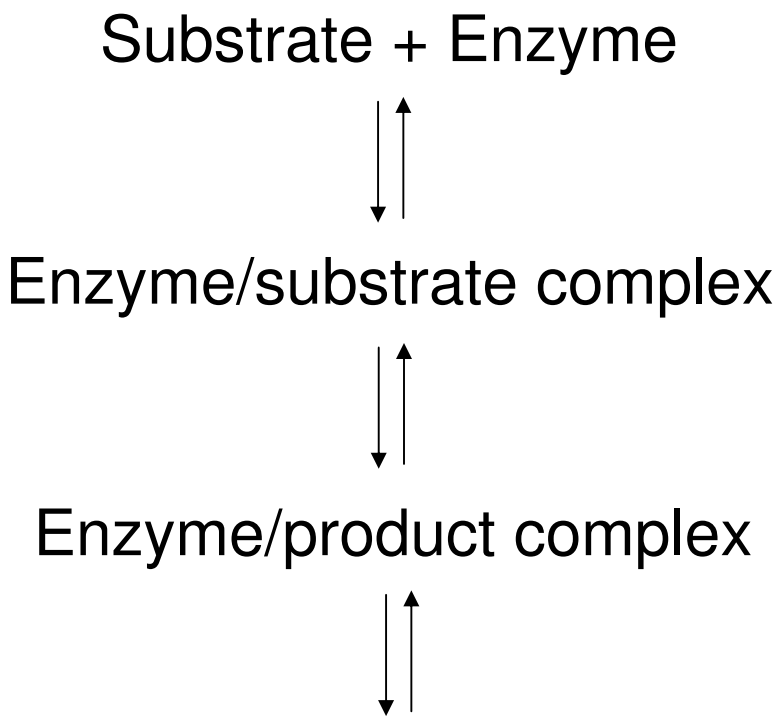


- http://www.stolaf.edu/people/giannini/biological%20animations.html

Enzymes lower the activation energy of a reaction



Enzymes lower activation energy by forming an enzyme/substrate complex

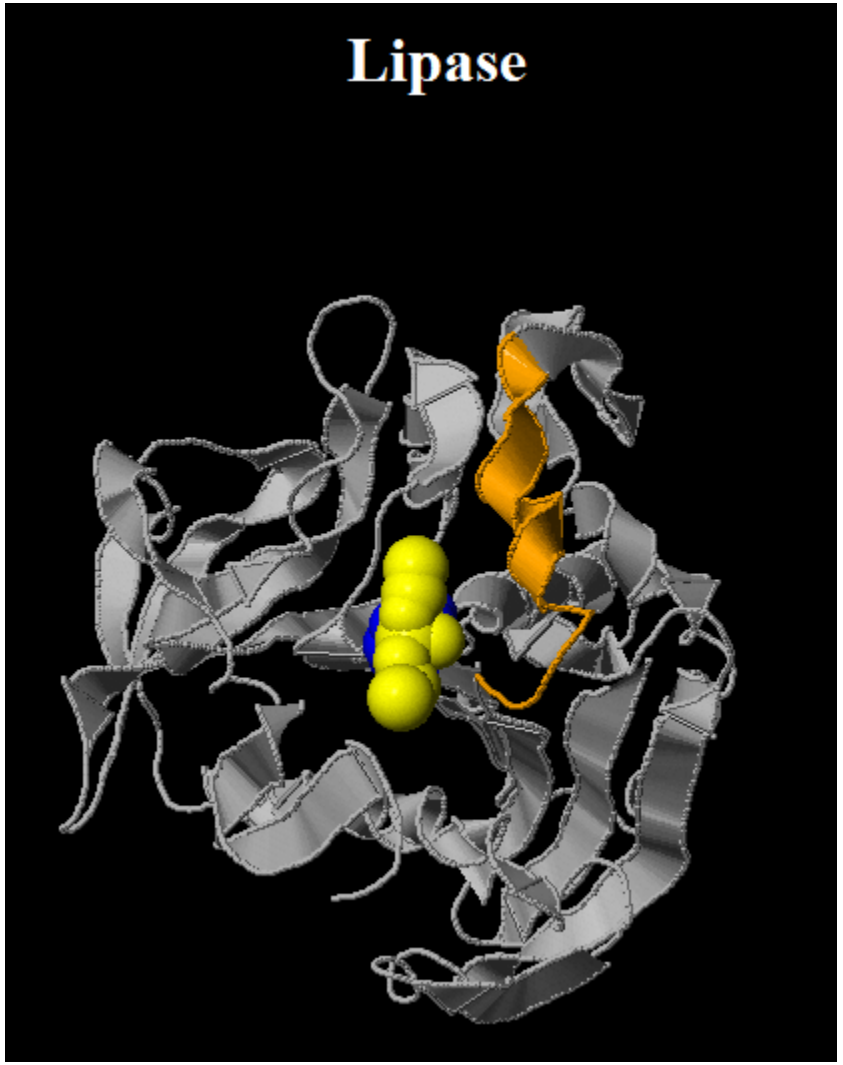


Examples of Enzymes

- Salivary Amylase
  - is an enzyme that catalyses the breakdown of into sugars. Amylase is present in human saliva, where it begins the chemical process of digestion. Food that contains much starch but little sugar, such as rice and potato, taste slightly sweet as they are chewed because amylase turns some of their starch into sugar in the mouth.
  - The pancreas also makes amylase (alpha amylase) to hydrolyse dietary starch into disaccharides and trisaccharides which are converted by other enzymes to glucose to supply the body with energy.

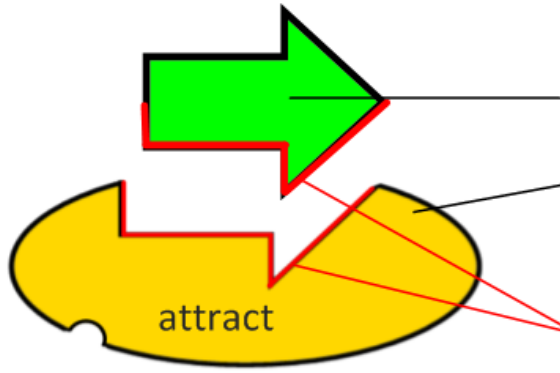
Enzymes are globular proteins

- Active site has a specific shape due to tertiary structure of protein.
- A change in shape of the protein affects shape of active site and the function of the enzyme.



Click to link to jmol interactive representation courtesy of University of Arizona

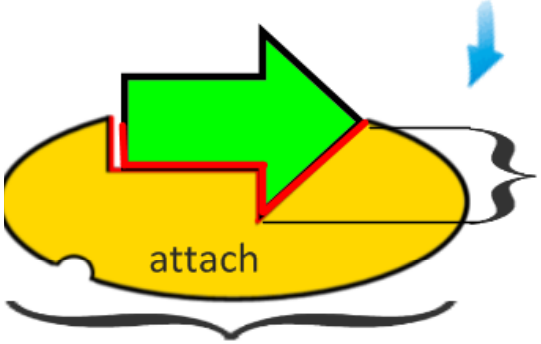
Enzymes, substrates and active sites



Substrate: reactant in a biochemical reaction.

Enzyme: globular protein which acts as a catalyst for biochemical reactions.

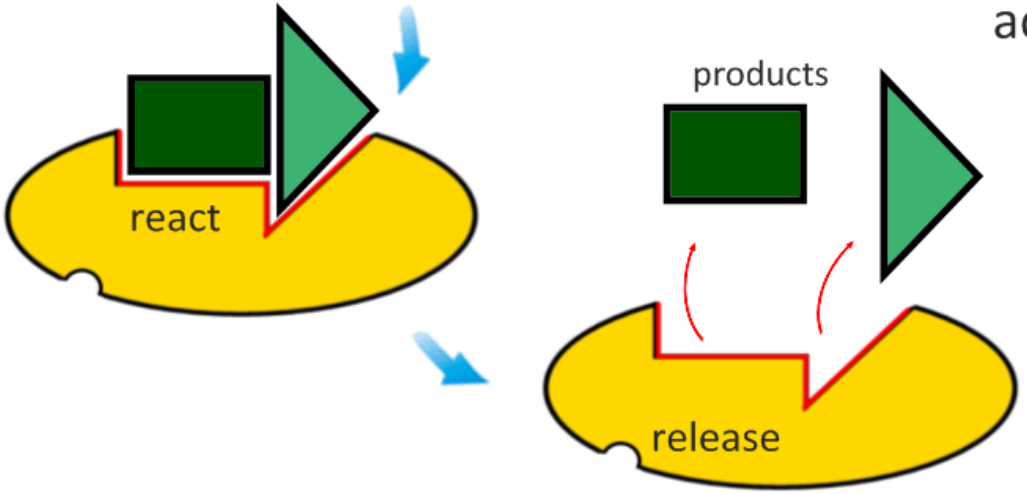
Polar regions of amino acids attract substrate and active site of the enzyme



Active Site: region on the surface of an enzyme to which substrates bind and which catalyses the reaction.

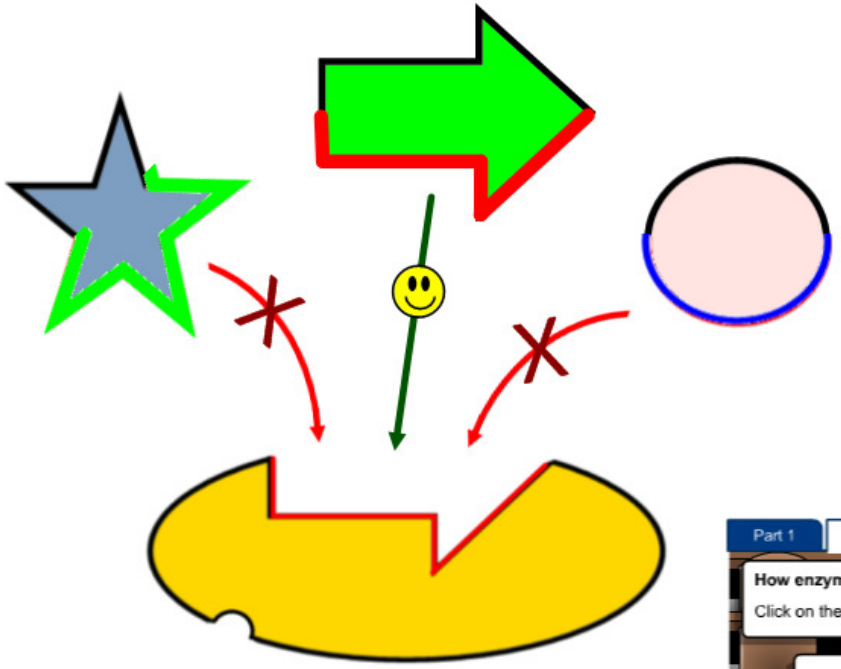
enzyme-substrate complex

Once a substrate has been locked into the active site, the reaction is catalysed.



The products are released and the enzyme is used again.

Enzymes are specific to their substrates



enzyme substrate

The Lock-and-Key hypothesis:

The substrate and the active site match each other in two ways:

Structurally

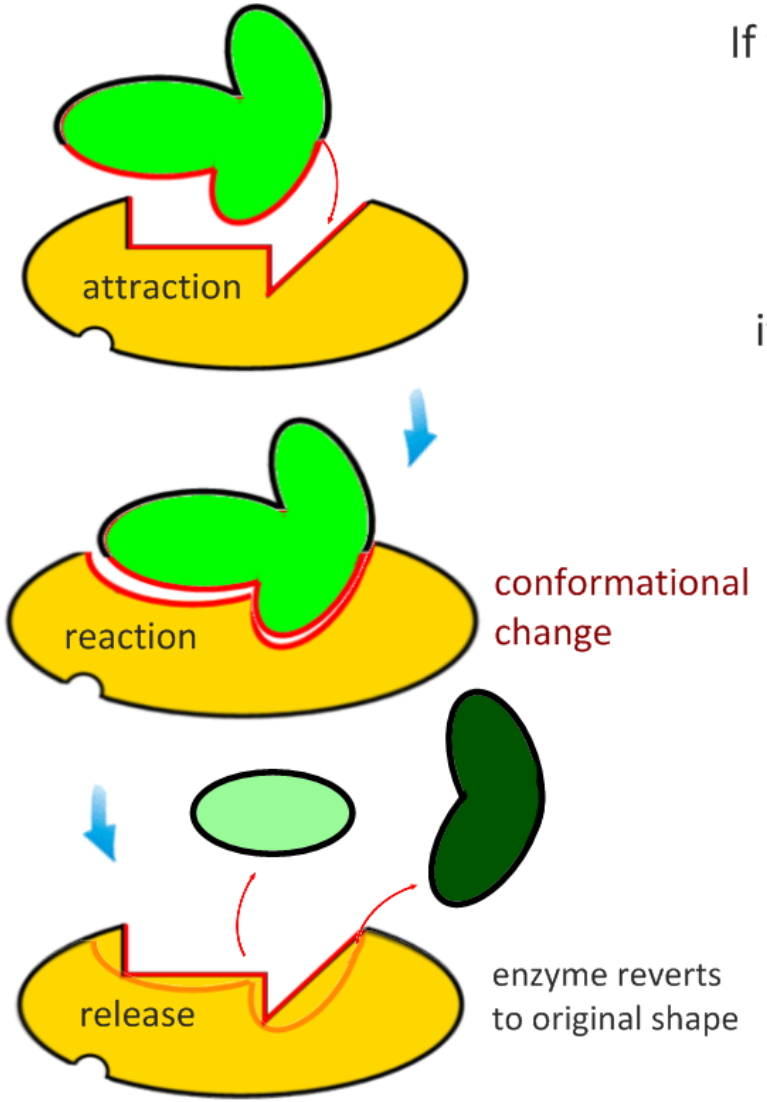
The 3D structured of the active site is specific to the substrate. Substrates that don't fit, won't react.

Chemically

Substrates that are not chemically attracted to the active site won't be able to react.

source unknown

The induced-fit model better explains enzyme activity

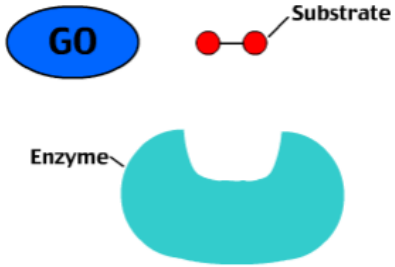


If the lock-and-key model were true, one enzyme would only catalyase one reaction. In actuality, some enzymes can catalyse multiple reactions.

As the substrate approaches the enzyme, it induces a conformational change in the active site - it changes shape to fit the substrate.

This stresses the substrate, reducing the activation energy of the reaction.

3d-inducedfit.mov



Enzyme Changing Shape

http://www.stolaf.edu/people/giannini/flashanimat/enzymes/enzyme.swf

- http://www.stolaf.edu/people/giannini/biological%20anamations.html

Denaturation

Enzymes are globular proteins.
Their structure can be altered by changes in pH or temperature - if the shape of the active site is changed considerably, they will not function.

http://www.biotopics.co.uk/other/andnat.html

Denaturation is changing the structure of a protein (enzyme) so that it cannot carry out its function.

High temperatures cause denaturation as the extra energy leads to increased vibration, breaking intra-molecular bonds.

Changes in pH cause denaturation as hydrogen bonds are broken.

Both methods result in an altered 3D structure of the active site, and this change is irreversible.

- http://highered.mcgraw-hill.com/sites/0072943696/student_view0/chapter2/animation__protein_denaturation.html

Factors affecting enzyme activity:

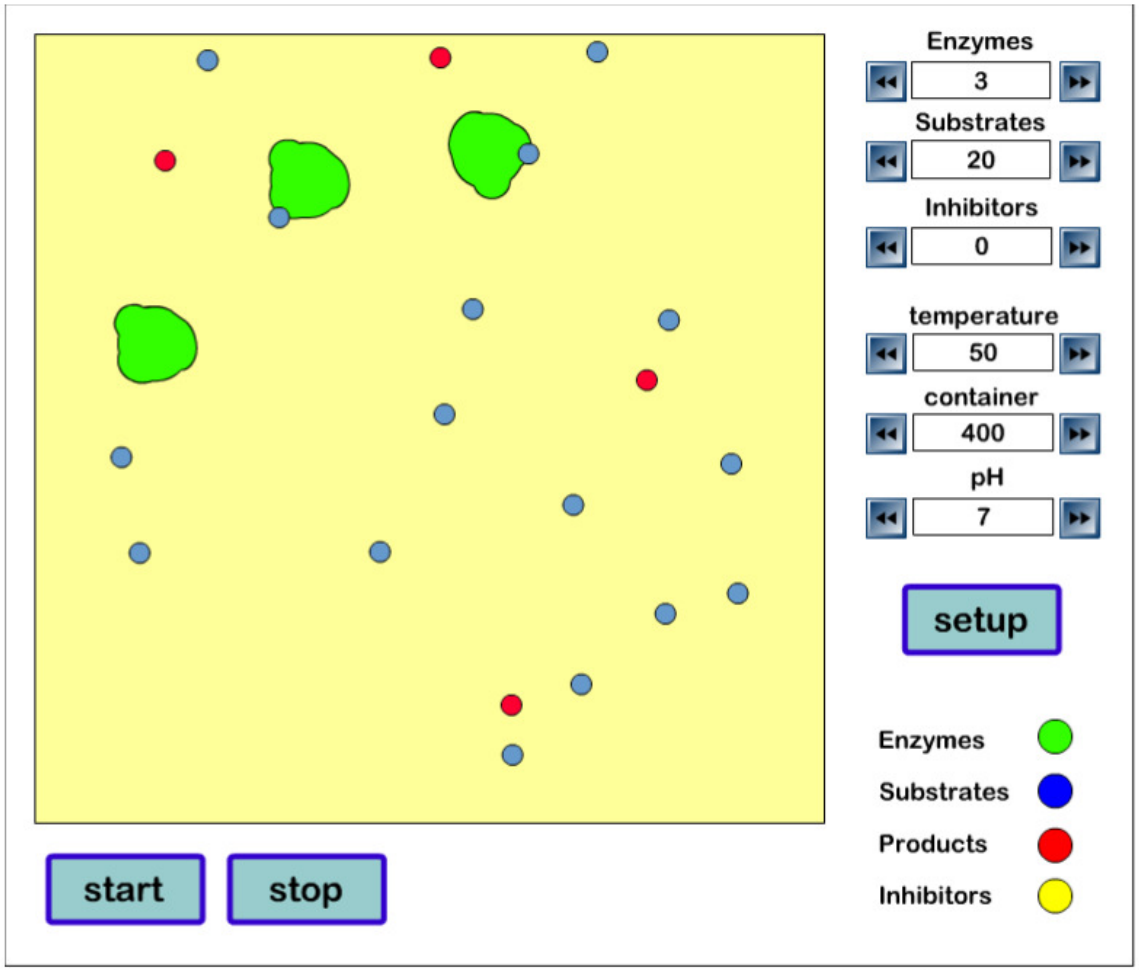
Use this animation to the following factors affect enzyme activity:

temperature

pH

substrate concentration

When you have finished this, complete the notes on *enzyme activity.*

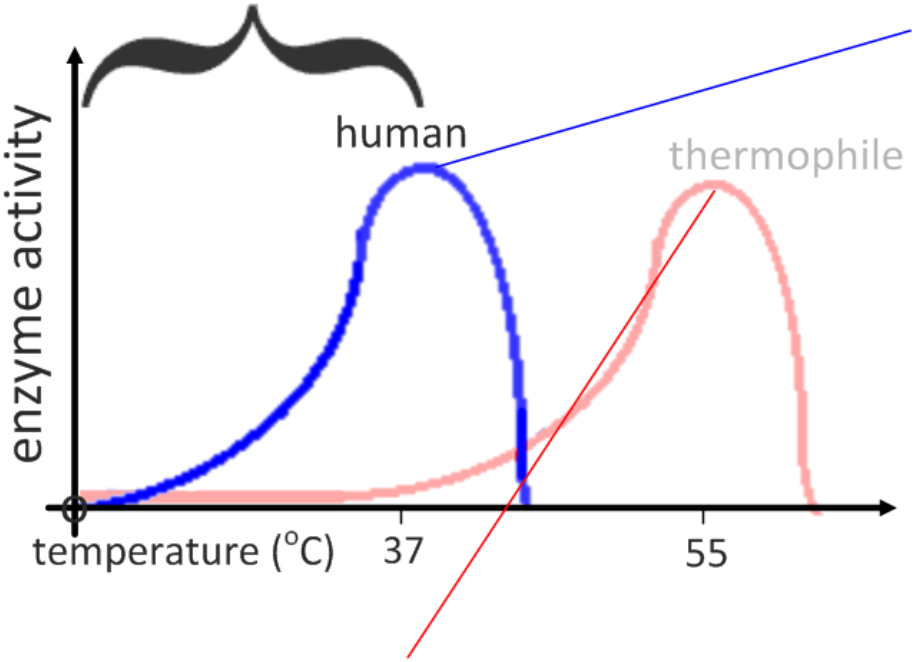


http://www.kscience.co.uk/animations/model.swf

- http://www.kscience.co.uk/animations/model.swf

The Effect of Temperature on Enzyme Activity

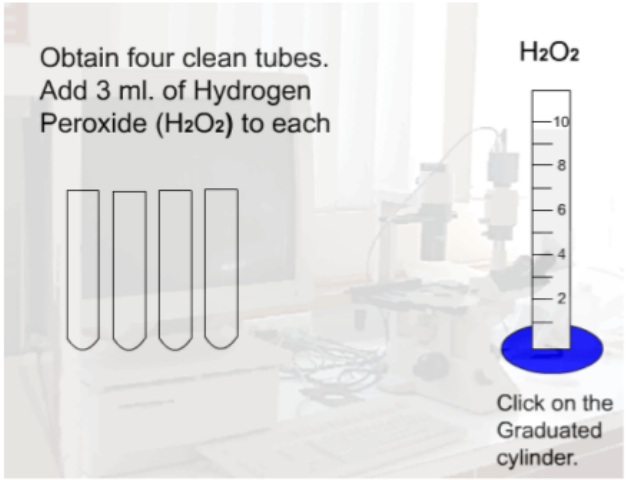
As temperature increases, rate of reaction increases as molecules have more energy, move faster and therefore collide and react more frequently.



Above the optimum temperature, further increase in temperature leads to denaturation of the enzyme. The active site is changed and so loses function.

A thermophile, such as bacteria at deep-sea vents, is an organism that is able to withstand much higher temperatures before its enzymes denature.

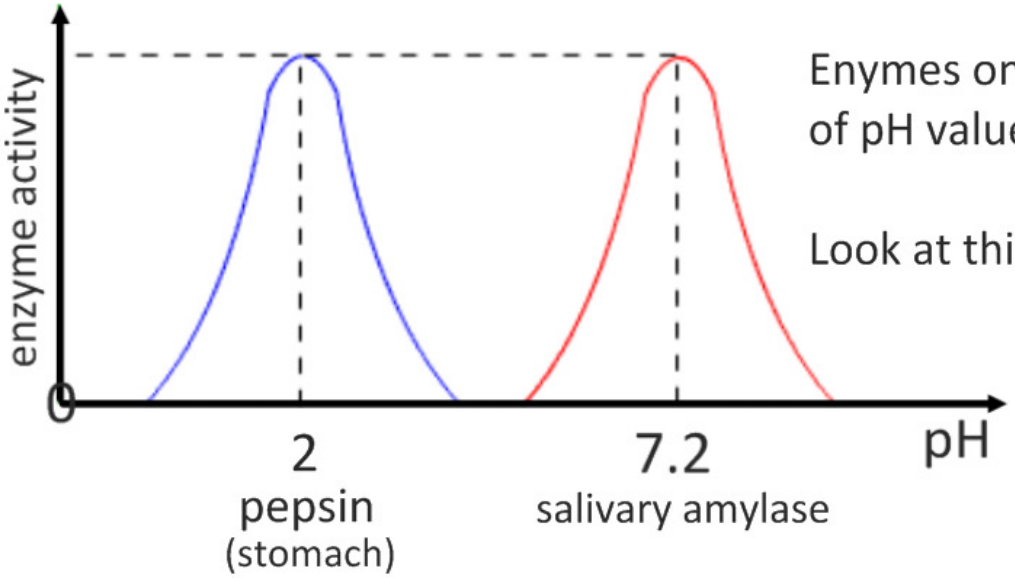
Try this virtual lab:



http://bioweb.wku.edu/courses/Biol120/Web/enzyme2.asp

- http://bioweb.wku.edu/courses/Biol120/Web/enzyme2.asp

The Effect of pH on Enzyme Activity.



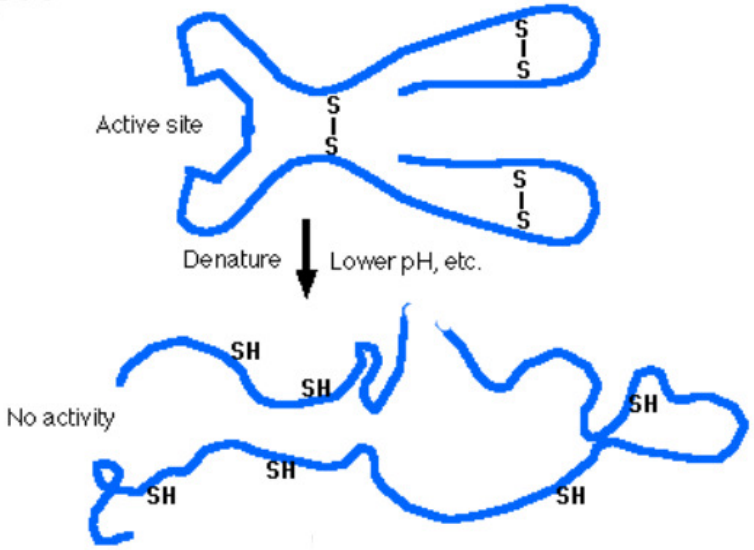
Enymes only operate within a narrow range of pH values. This is called an optimum pH.

Look at this example of two digestive enzymes.

If there is a deviation from the optimum pH, the hydrogen bonds between amino acids in the structure of the enzyme are broken.

This results in the loss of the shape of the active site of the enzyme, so it does not function.

This is usually a permanent change.



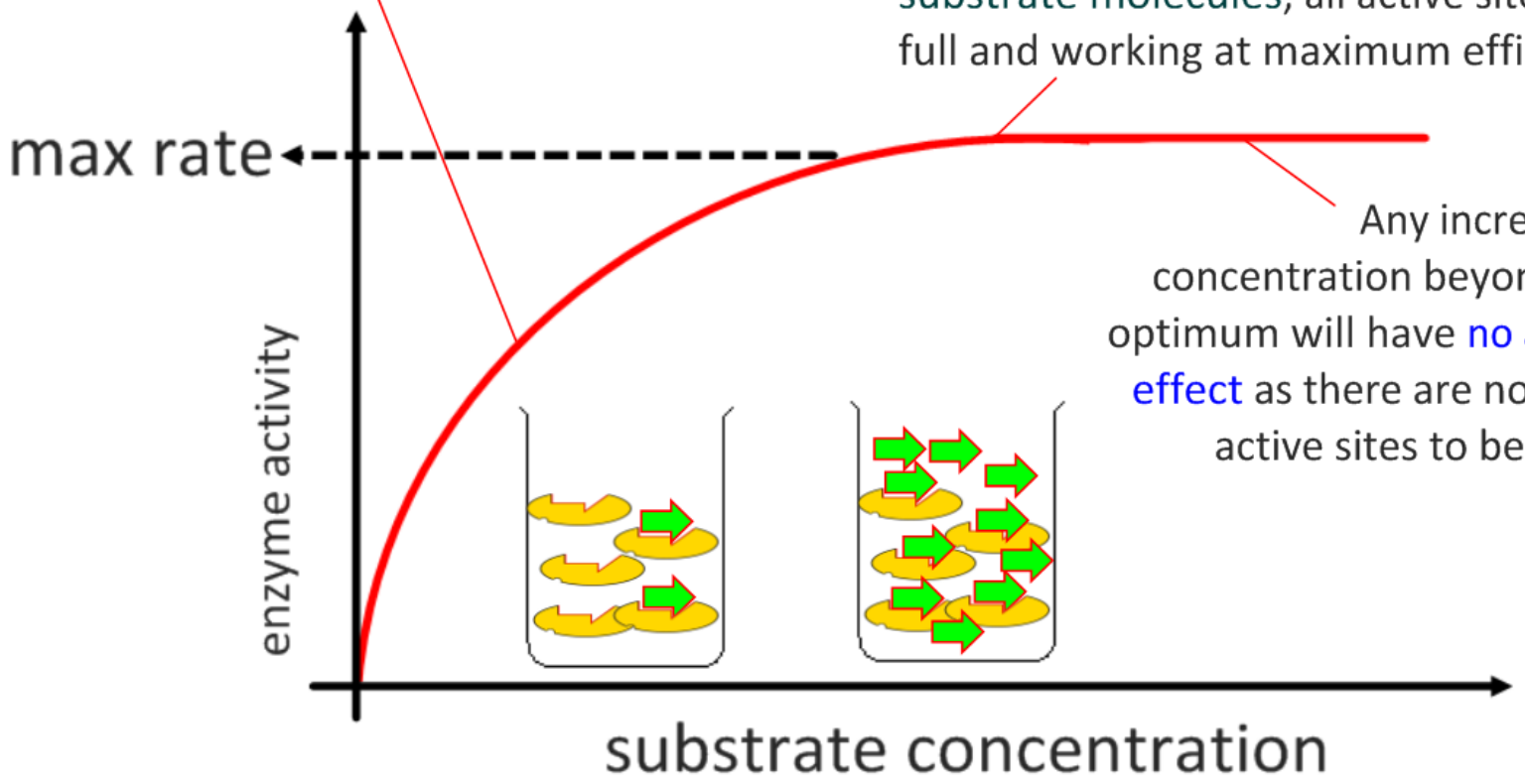
http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/D/Denaturing.gif

- pH affects the formation of hydrogen bonds and sulphur bridges in proteins and so affects shape.

The Effect of Substrate Concentration on Enzyme Activity

Increasing substrate concentration increases the rate of reaction.

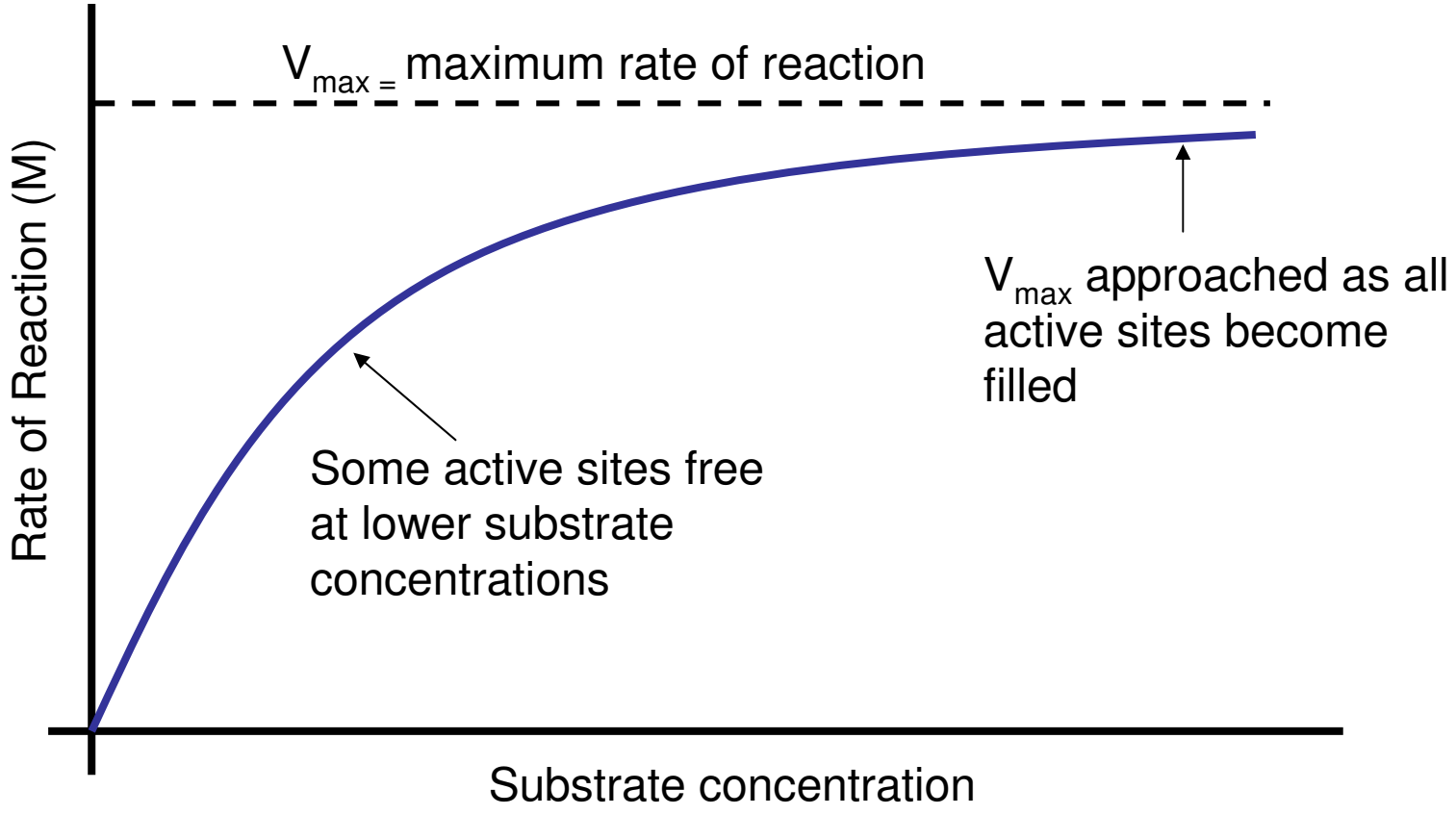
At the optimum concentration of substrate molecules, all active sites are full and working at maximum efficiency.



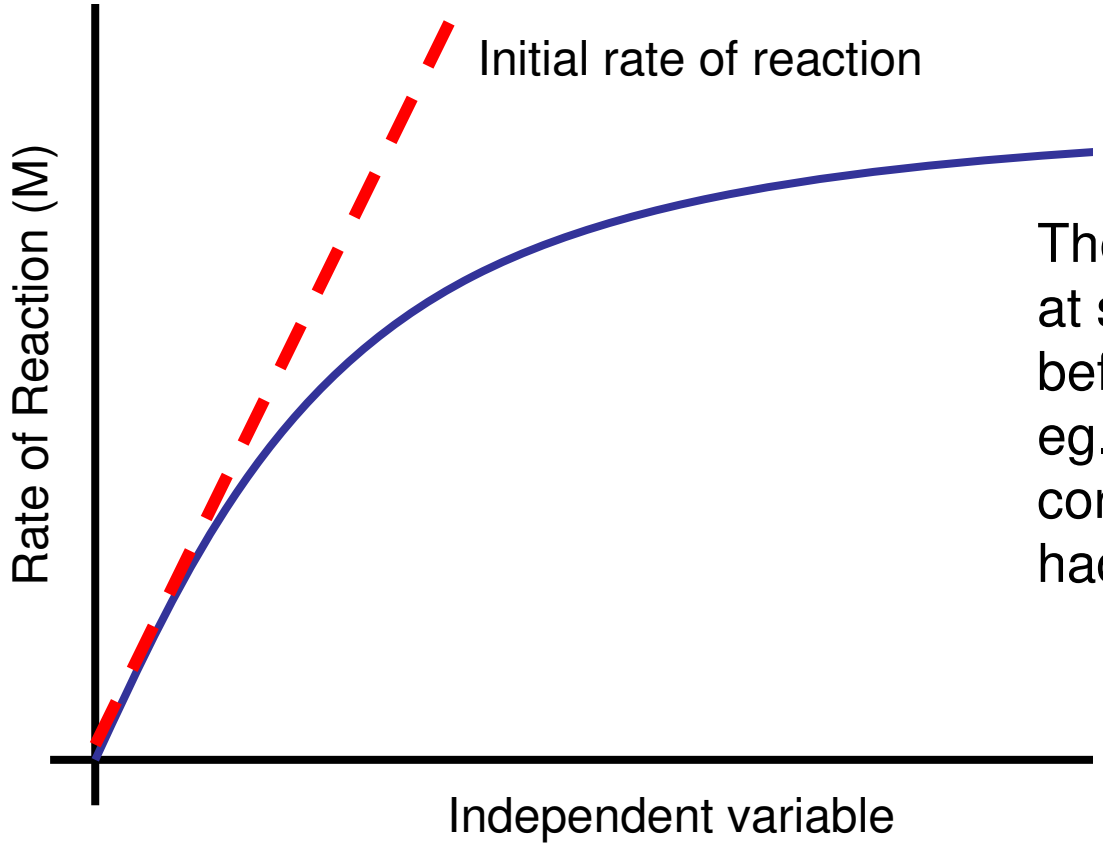
Any increase in concentration beyond the optimum will have no added effect as there are no extra active sites to be used.

Characteristics of enzymes

- Rate of enzyme action is dependent on number of substrate molecules present



Why do scientists measure the initial rate of reaction of enzyme-catalysed reactions?

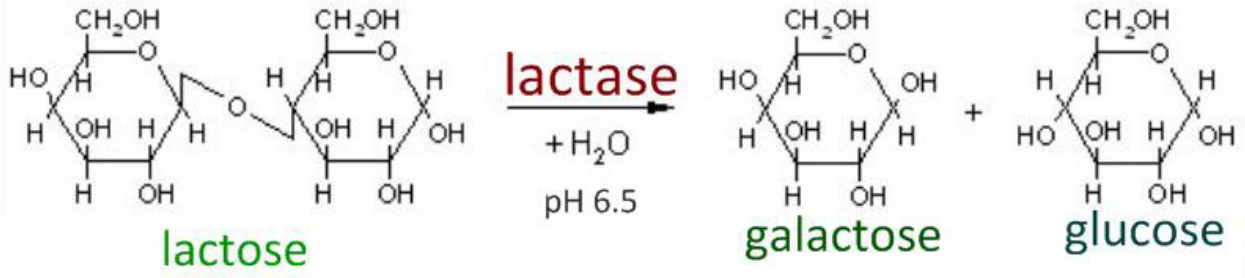


They measure rate at start of reaction before any factors, eg. substrate concentration, have had time to change.

Lactose Intolerance

Lactose (milk sugar) can cause allergies in some people.

This is often because they are unable to produce the enzyme lactase in sufficient quantities.



http://en.wikipedia.org/wiki/Lactase

http://www.superlaugh.com/dan/lactose.htm

Most people produce less lactase as they get older - after all, we don't live off milk once we have been weaned. In some regions, such as Europe, a mutation has allowed lactase production to continue into adulthood. This mutation is not present in people who are lactose intolerant.

Global estimates of lactose intolerance:

http://en.wikipedia.org/wiki/Lactose_intolerance

How can we cope with lactose intolerance?

1. Take a lactase supplement

   These are produced industrially using the *Aspergillus niger* fungus (also used to make other enzymes).

2. Drink lactose-free milk

   Milk is treated with lactase (produced by *A. niger*) and essentially 'pre-digested' before being packaged.

   Lactose-free milk is made by different methods:

   a. Add lactase to milk

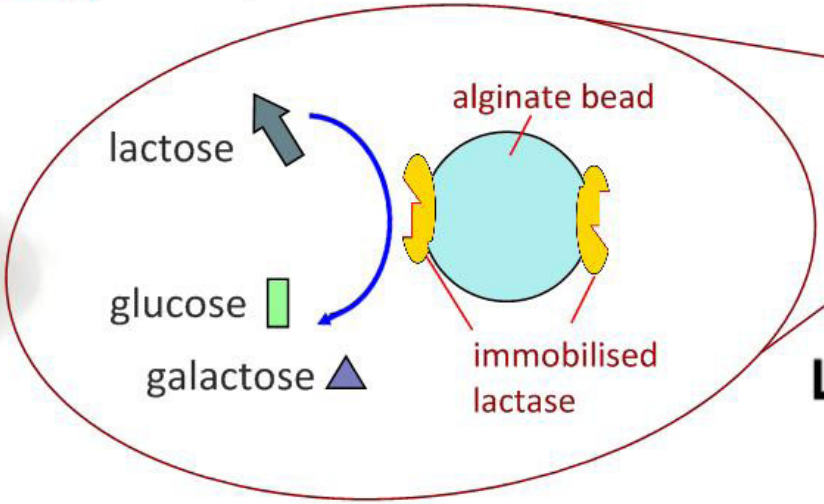
      (lower quality and wasteful of lactase)

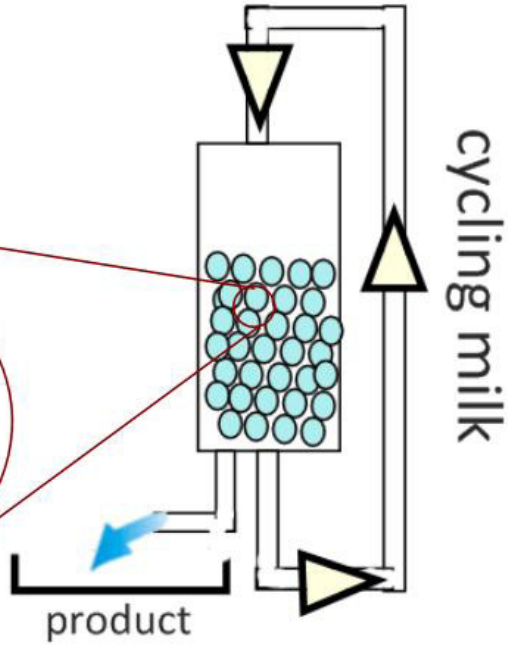
   b. Run milk through apparatus with immobilised lactase

      (uses alginate beads, no enzyme in final product)

Aspergillus niger

http://129.215.156.68/Images/asexual.htm





Challenge: by changing just one letter at a time, get from 'Tread' to 'Blink'. All intermediates must be real (English) words.

TREAD

_____

_____

_____

_____

_____

BLINK

Metabolic pathways* are chains or cycles of enzyme-catalysed reactions.
The product of one reaction is a reactant in the next.

TREAD initial substrate

BREAD

BREED

BLEED — intermediates

BLEND

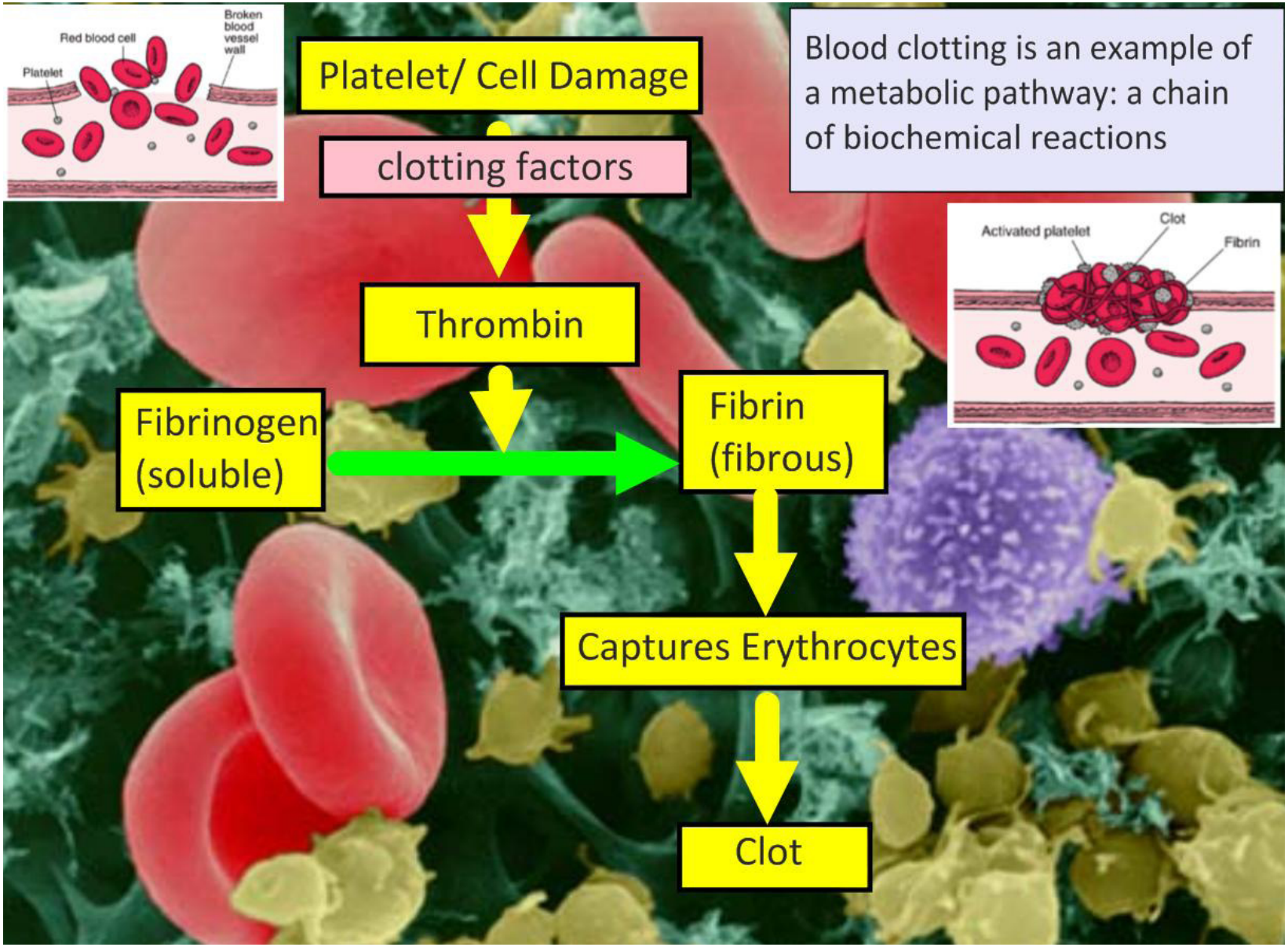
BLIND

BLINK end-product

*or *biochemical pathways*

http://highered.mcgraw-hill.com/olcweb/cgi/pluginpop.cgi?it=swf::535::535::/sites/dl/free/0072437316/120070/bio09.swf::A%20Biochemical%20Pathway

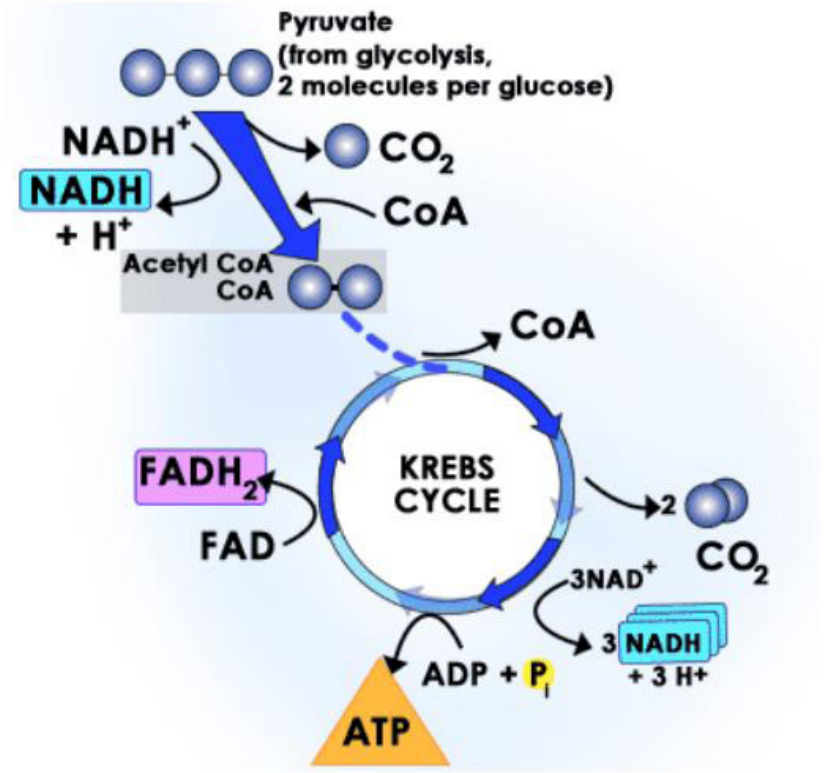
- http://highered.mcgraw-hill.com/sites/0072943696/student_view0/chapter2/animation__a_biochemical_pathway.html



- http://www.footprints-science.co.uk/Bloodclotting.htm

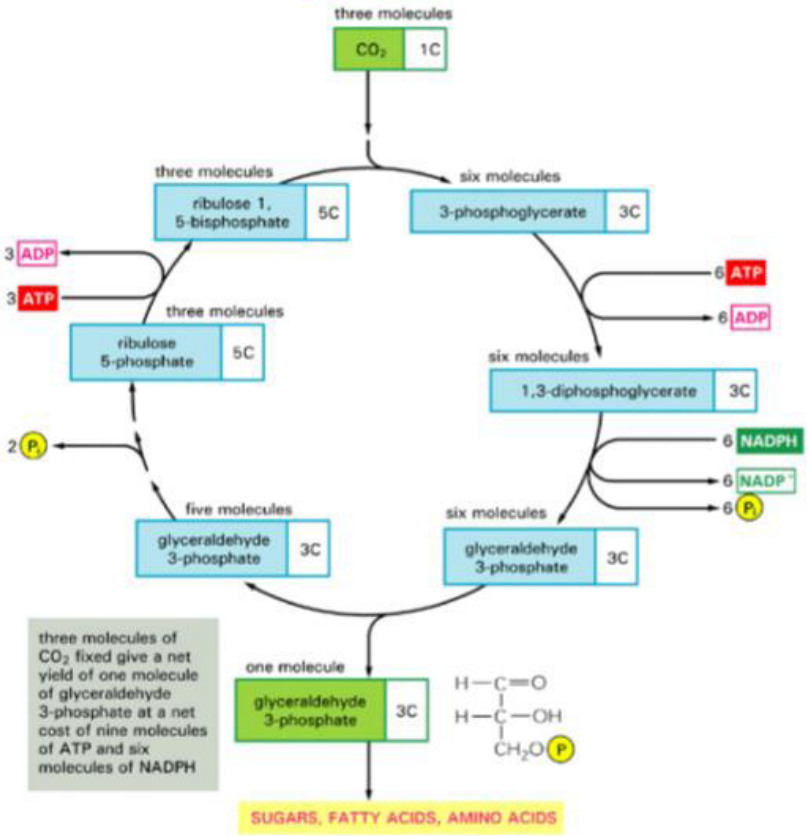
The Krebs Cycle (cell respiration) and Calvin Cycle (photosynthesis) are examples of enzyme-catalysed, cyclical metabolic pathways.

Krebs



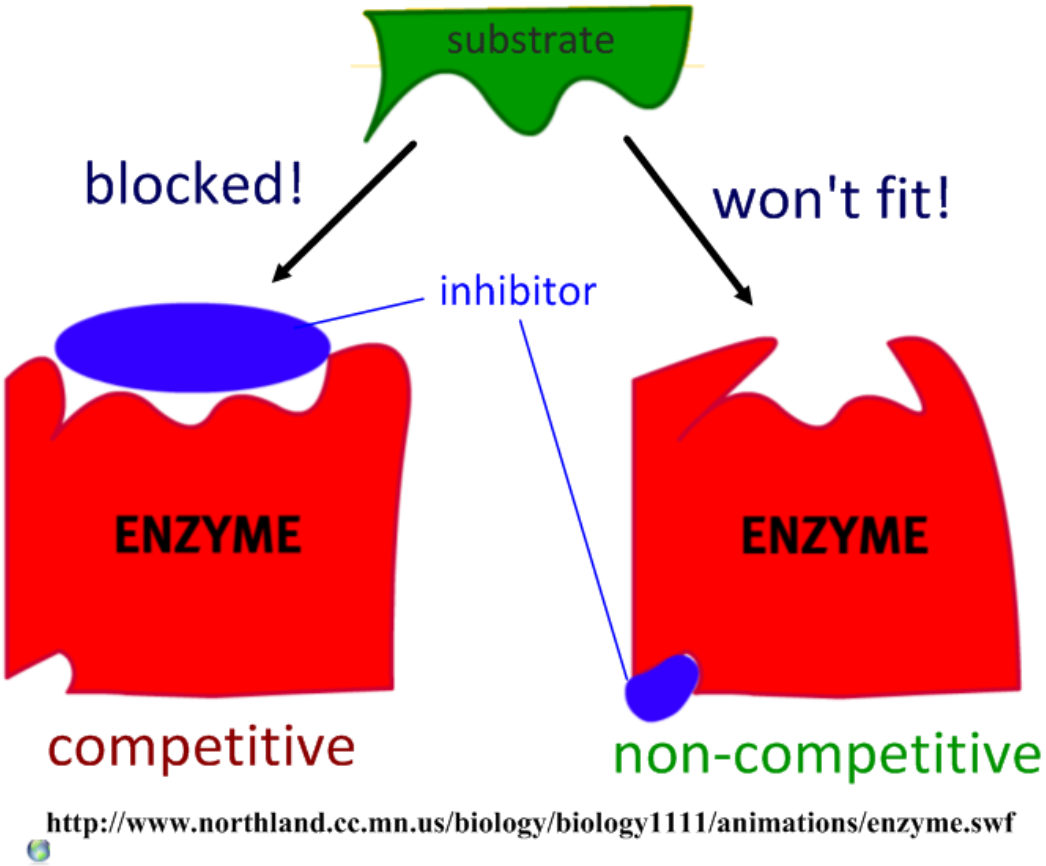
http://www.sparknotes.com/health/carbohydrates/section3.rhtml

Calvin



http://library.thinkquest.org/C004535/calvin_cycle.html

inhibitor fits the active site and prevents the substrate from entering

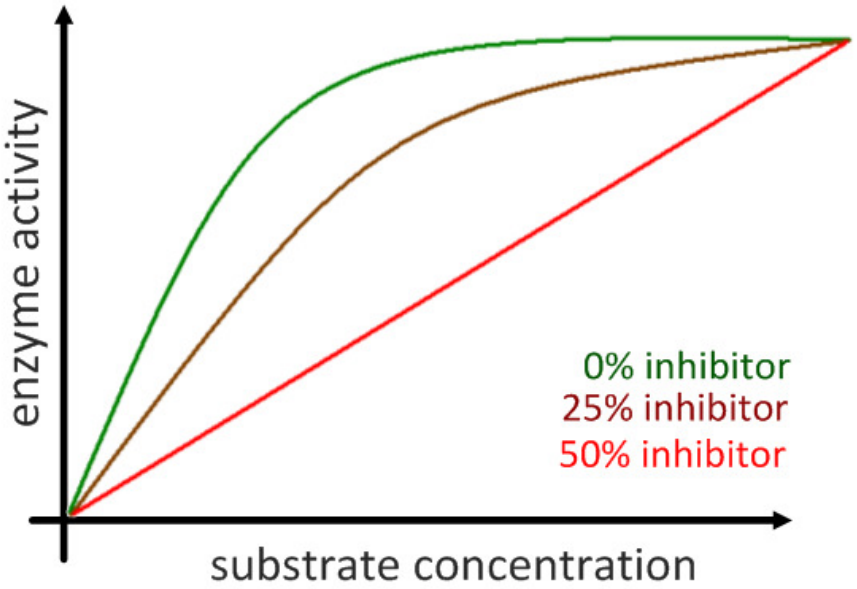


inhibitor fits into an allosteric site*, causing a conformational change in the active site: the substrate cannot attach to react

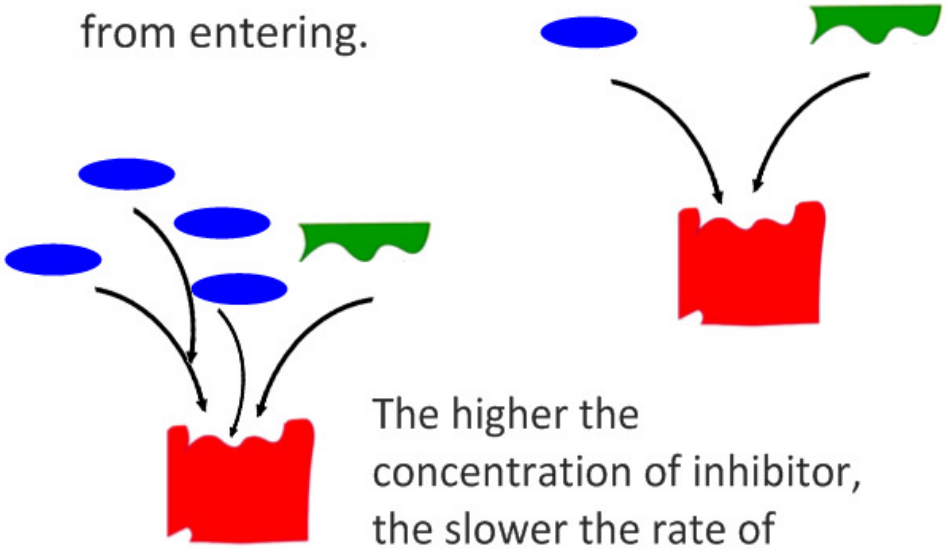
*'other' site

- http://www.northland.cc.mn.us/biology/biology1111/animations/enzyme.swf

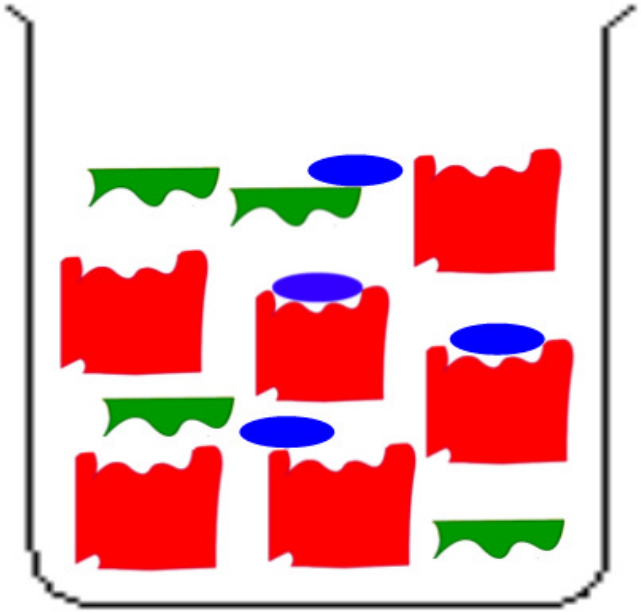
Competitive Inhibition



A competitive inhibitor blocks the active site, preventing the substrate from entering.



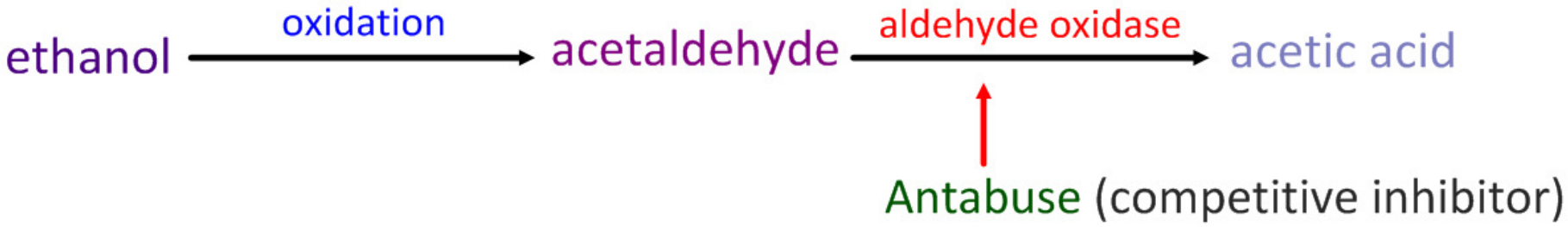
The higher the concentration of inhibitor, the slower the rate of reaction.



Even with competitive inhibition, the same maximum rate of reaction will be achieved if more substrate is added - because we have not changed the number of enzymes available.

Overcoming alcoholism: an example of competitive inhibition

Normal metabolism of ethanol (alcohol):



Antabuse (disulfiram) competes with the aldehyde oxidase and prevents the acetaldehyde from being converted to acetic acid.

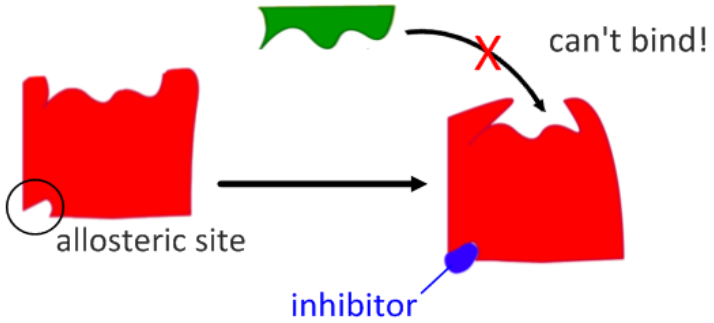
A build up of acetaldehyde follows, resulting in a strong feeling of nausea and other strong hangover symptoms - a good deterrent from drinking.

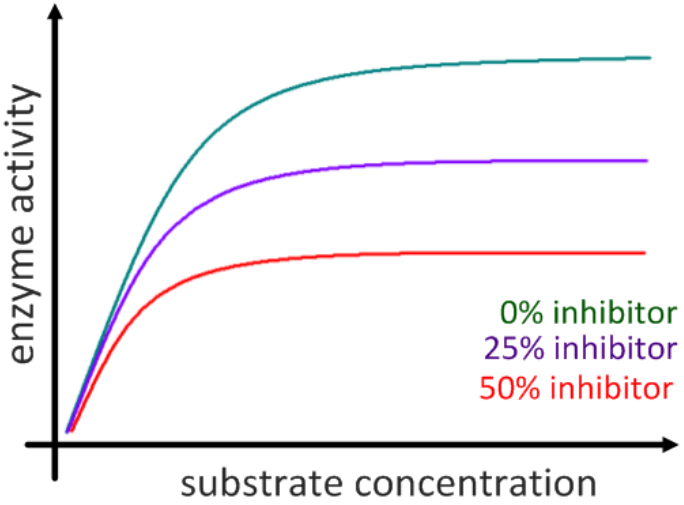
Antabuse is administered as a daily pill, so its efficacy relies on the patient's own motivation - if they stop taking it, they can drink again.

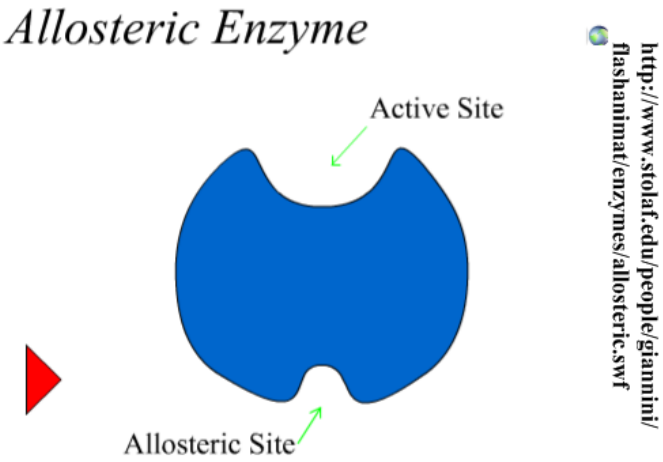
Image: 'Glass of wine'
www.flickr.com/photos/12191709@N00/92783024

Non-Competitive Inhibition

Non-competitive inhibitors bind to an allosteric (other) site on the enzyme. The active site is altered and the substrate cannot attach and react.







As concentration of inhibitor increases, the rate of reaction decreases.
This is because there are fewer functional active sites available for reaction.

The maximum rate of reaction is also reduced - with fewer functional active sites, the enzyme has reduced ability to process the substrates, even if substrate concentration is increased.

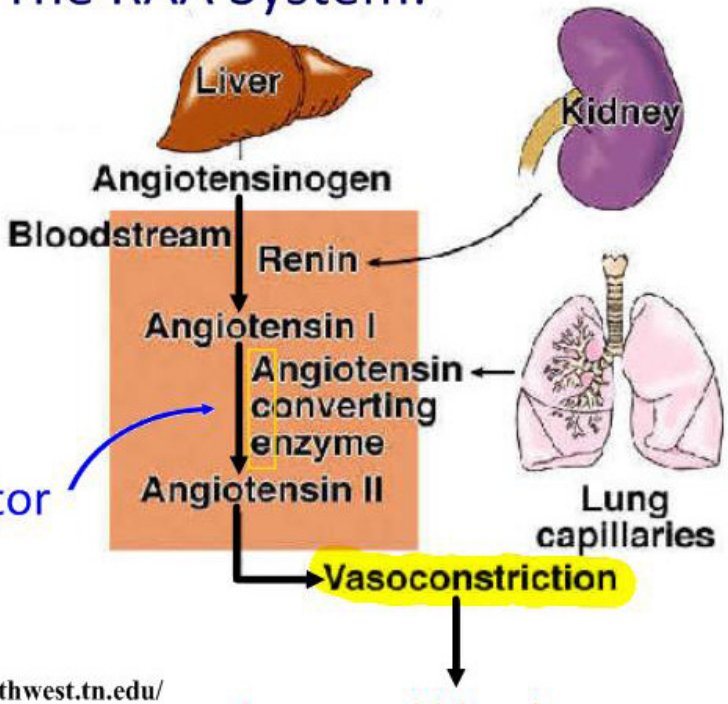
- http://www.stolaf.edu/people/giannini/flashanimat/enzymes/allosteric.swf

ACE Inhibitors: Helping Control Blood Pressure

The RAA system causes *vasoconstriction* (tightening of blood vessels) when blood pressure drops (such as after heavy bleeding).

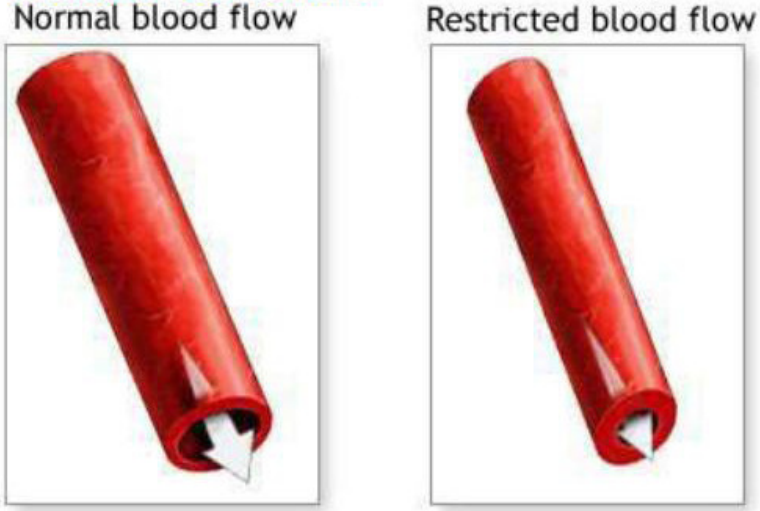
In people with hypertension or heart failure, the action of angiotensin II can make their problem worse.

The RAA System:



http://faculty.southwest.tn.edu/rburkett/A&P2%20u28.jpg

Vasoconstriction:



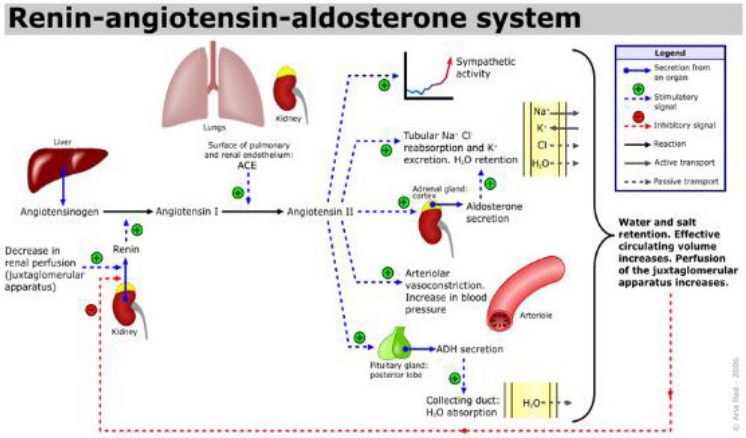
http://www.nlm.nih.gov/medlineplus/ency/images/ency/fullsize/8983.jpg

A.D.A.M.

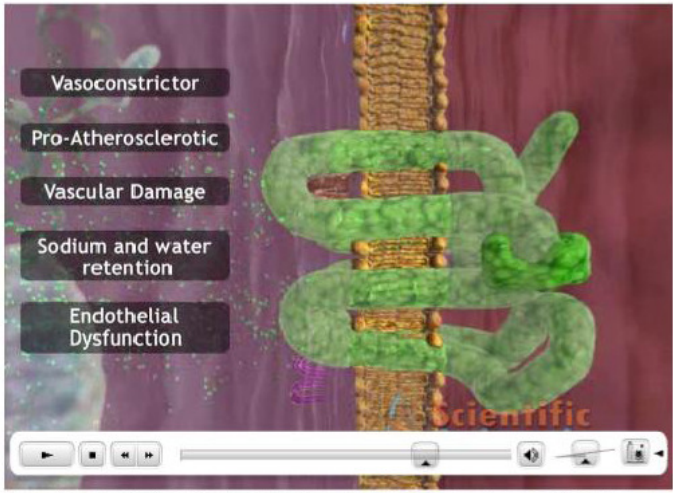
ACE Inhibitors are medications that inhibit Angiotensin Converting Enzymes - they prevent increased blood pressure.

They are non-competitive and reversible.

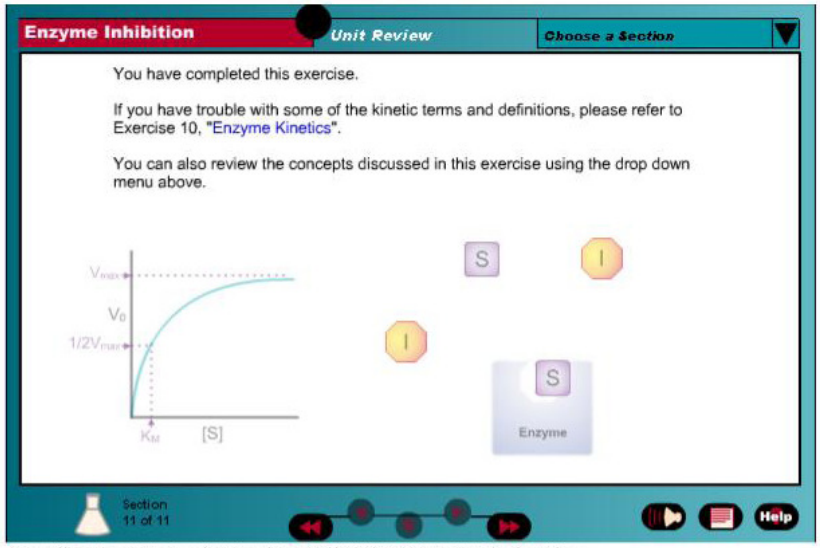
More ACE-Inhibitor resources:



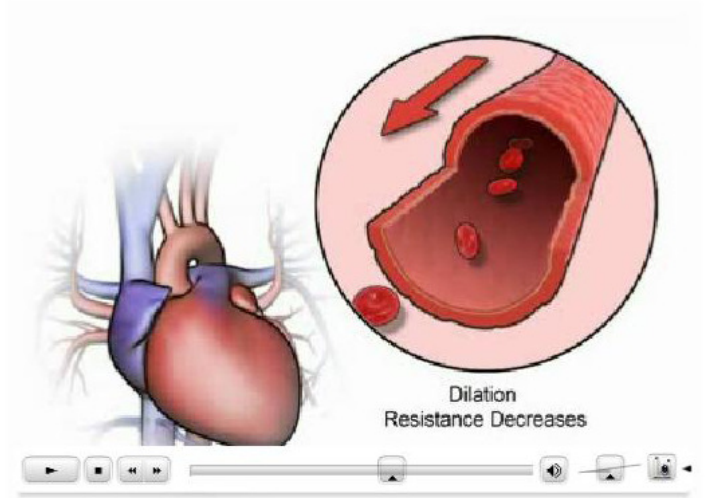
http://en.wikipedia.org/wiki/Renin-angiotensin_system



http://www.scientificanimations.com/cs-pharmacology-moa-video1.html



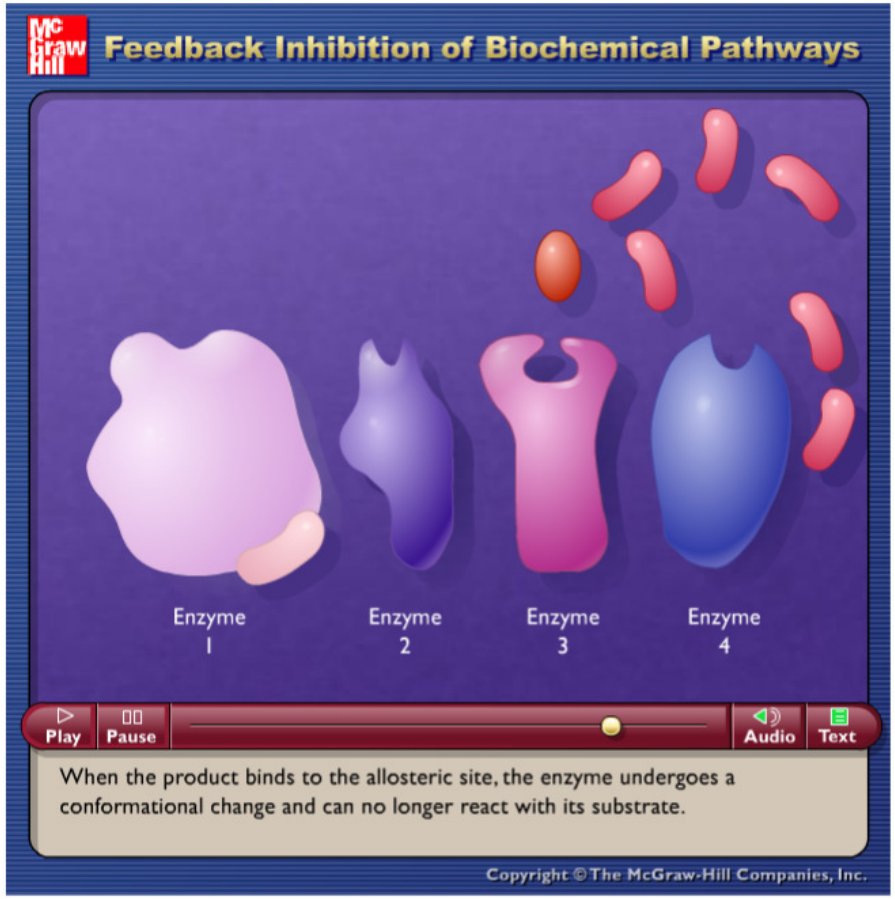
http://www.wiley.com/college/pratt/0471393878/student/animations/enzyme_inhibition/index.html



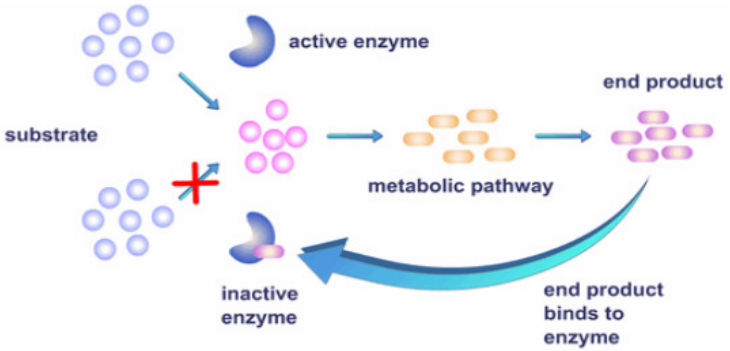
http://www.heartfailurematters.org/EN/Animation/Pages/animation_7.aspx

- http://en.wikipedia.org/wiki/Renin-angiotensin_system
- http://www.wiley.com/college/pratt/0471393878/student/animations/enzyme_inhibition/index.html
- http://www.heartfailurematters.org/EN/Animation/Pages/animation_7.aspx

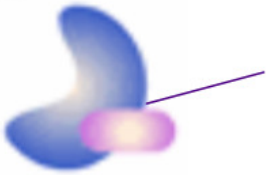
End-product inhibition prevents a large build-up of products



http://highered.mcgraw-hill.com/olcweb/cgi/pluginpop.cgi?it=swf::535::535::/sites/dl/free/0072437316/120070/bio10.swf::Feedback%20Inhibition%20of%20Biochemical%20Pathways



http://scholar.hw.ac.uk/site/biology/topic13.asp?outline=



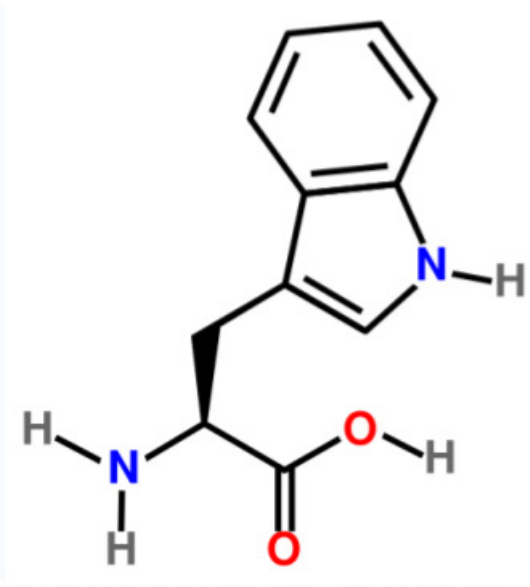
Allosteric site: place where end product binds on the enzyme (not active site)

Causes conformational change (locking) of active site - this is temporary.

Example of
Negative Feedback Control

- http://highered.mcgraw-hill.com/olcweb/cgi/pluginpop.cgi?it=swf::535::535::/sites/dl/free/0072437316/120070/bio10.swf::

Tryptophan: an example of end-product (feedback) inhibition



http://en.wikipedia.org/wiki/Tryptophan

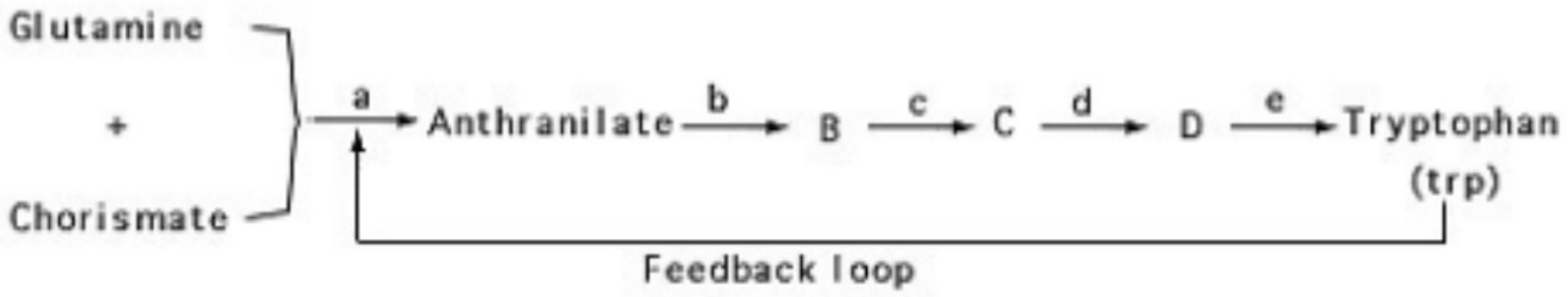
Tryptophan is an essential amino acid
(we can't produce it, so have to get it in our diet).

E. coli bacteria can produce this enzyme when needed. If they are in a tryptophan-rich medium or have produced a high level of tryptophan, it will act as an end-product inhibitor - preventing further production of itself. This helps the cell conserve energy - it is not wasted on excess production.

When tryptophan levels decrease, inhibition ends and the metabolic pathway resumes.

E. coli

(SEM - fc)



http://www.textbookofbacteriology.net/regulation.html

E. coli from: http://www.thebacteriabusters.com/E_coli_O157H7.jpg