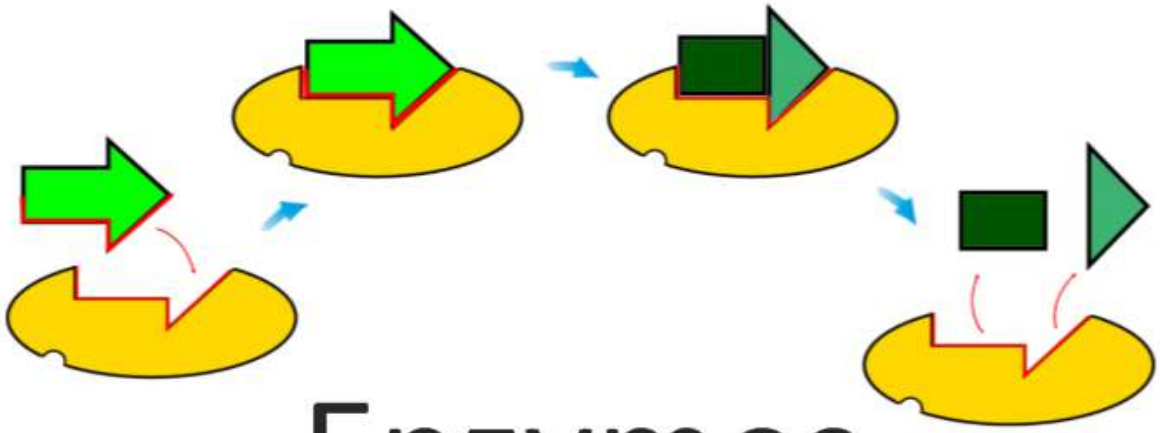


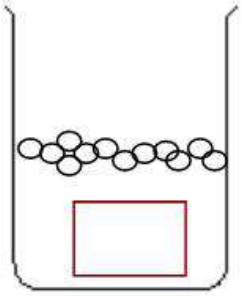
# Enzymes

## (Core and C2/7.6)

Stephen Taylor
Bandung International School

# Factors affecting enzme activity investigations

Decomposition of $H_2O_2$ by catalase < in liver / in potato



## Design

| Levels/marks | Aspect 1 | Aspect 2 | Aspect 3 |
|---|---|---|---|
| | Defining the problem and selecting variables | Controlling variables | Developing a method for collection of data |
| **Complete/2** | Formulates a focused problem/research question and identifies the relevant variables. | Designs a method for the effective control of the variables. | Develops a method that allows for the collection of sufficient relevant data. |

## Data collection and processing

| Levels/marks | Aspect 1 | Aspect 2 | Aspect 3 |
|---|---|---|---|
| | Recording raw data | Processing raw data | Presenting processed data |
| **Complete/2** | Records appropriate quantitative and associated qualitative raw data, including units and uncertainties where relevant. | Processes the quantitative raw data correctly. | Presents processed data appropriately and, where relevant, includes errors and uncertainties. |

## Conclusion and evaluation

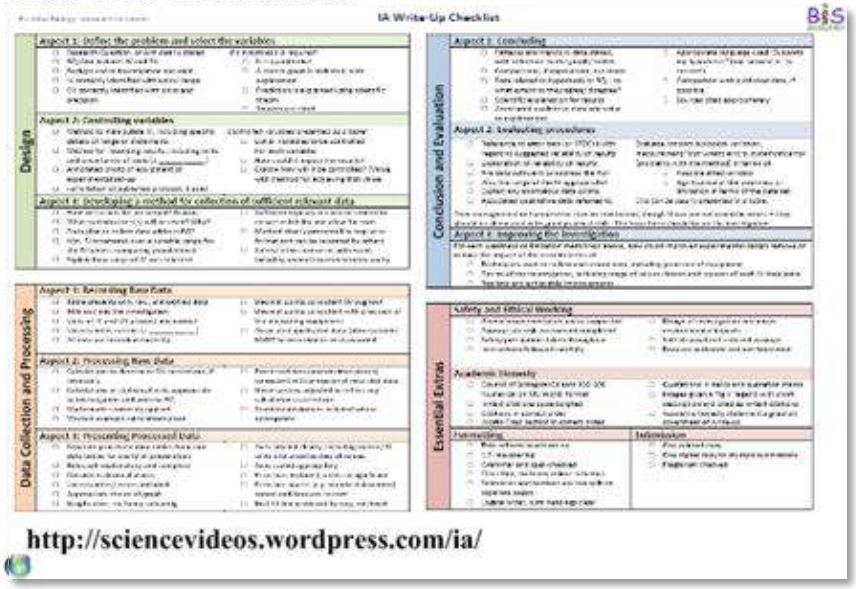
| Levels/marks | Aspect 1 | Aspect 2 | Aspect 3 |
|---|---|---|---|
| | Concluding | Evaluating procedure(s) | Improving the investigation |
| **Complete/2** | States a conclusion, with justification, based on a reasonable interpretation of the data. | Evaluates weaknesses and limitations. | Suggests realistic improvements in respect of identified weaknesses and limitations. |

Timeline:

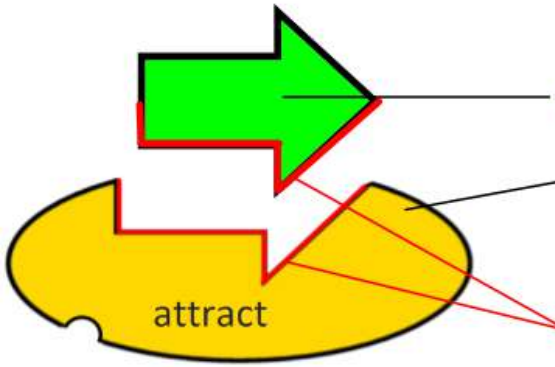
1. Double period planning and testing
2. Double period data collection
3. Single period data processing

Due: One week later, printed & turnitin

Use the checklist:



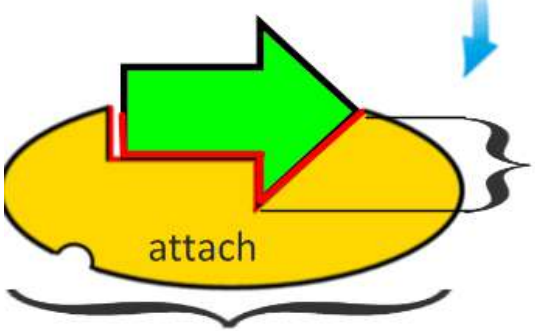
# Enzymes, substrates and active sites




Substrate: reactant in a biochemical reaction.

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

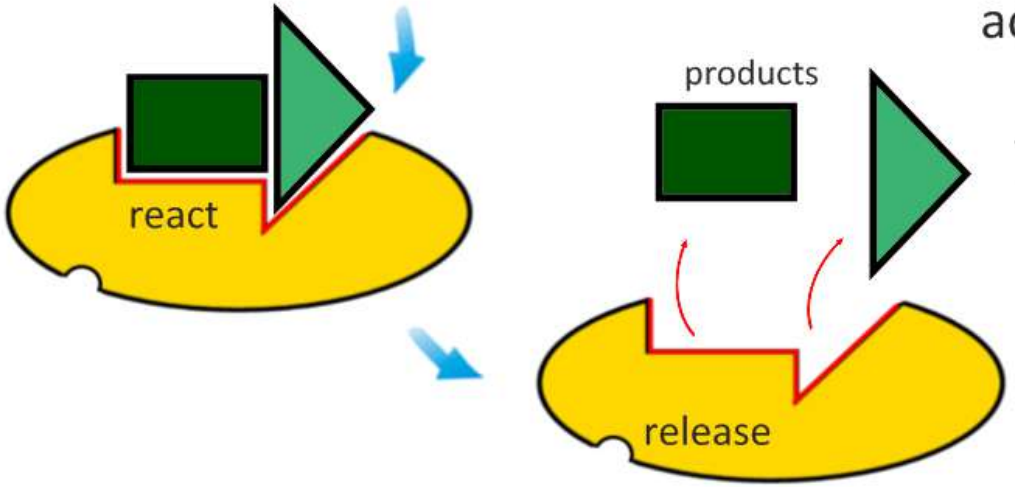
Polar regions of amino acids attract substrate and active site of the enzyme




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

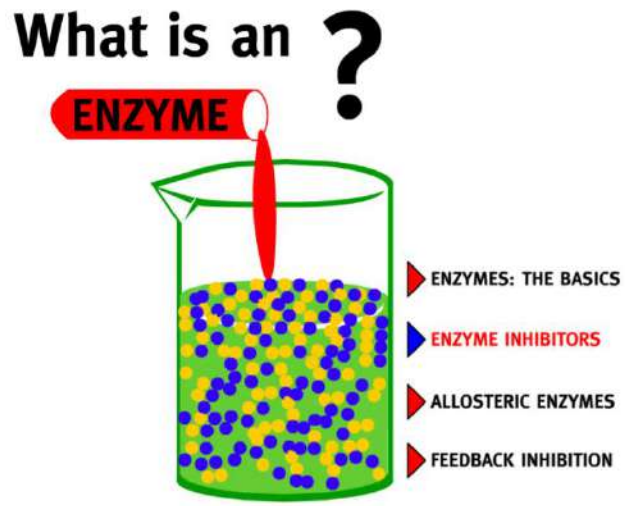
enzyme-substrate complex

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

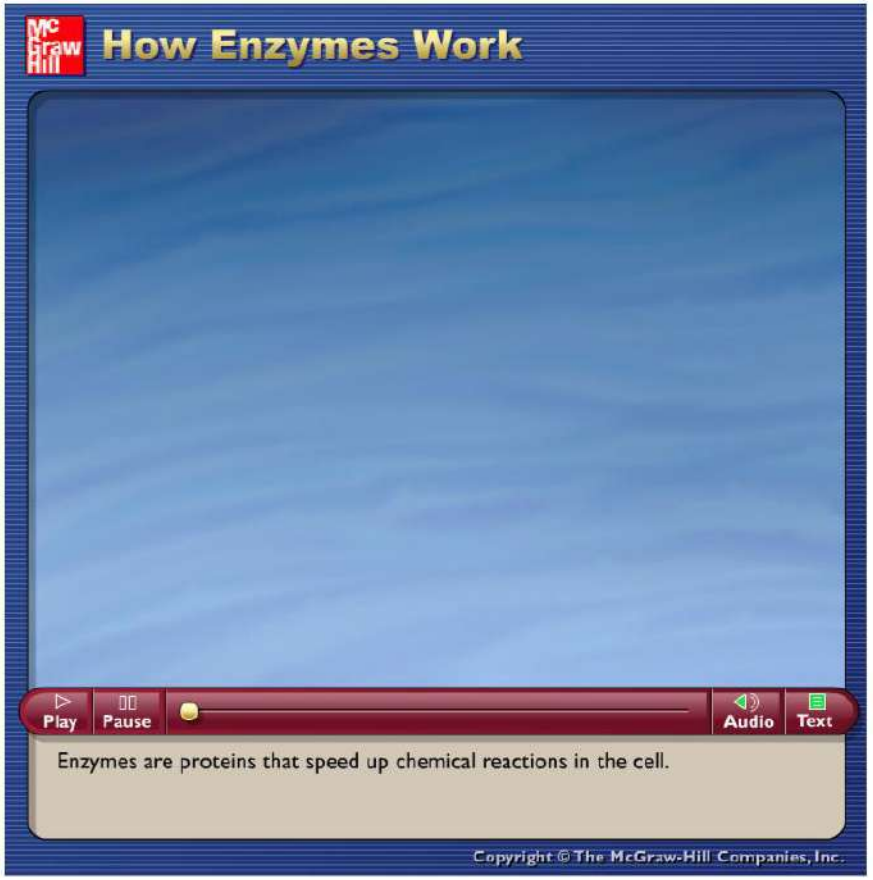



The products are released and the enzyme is used again.

What is an enzyme?

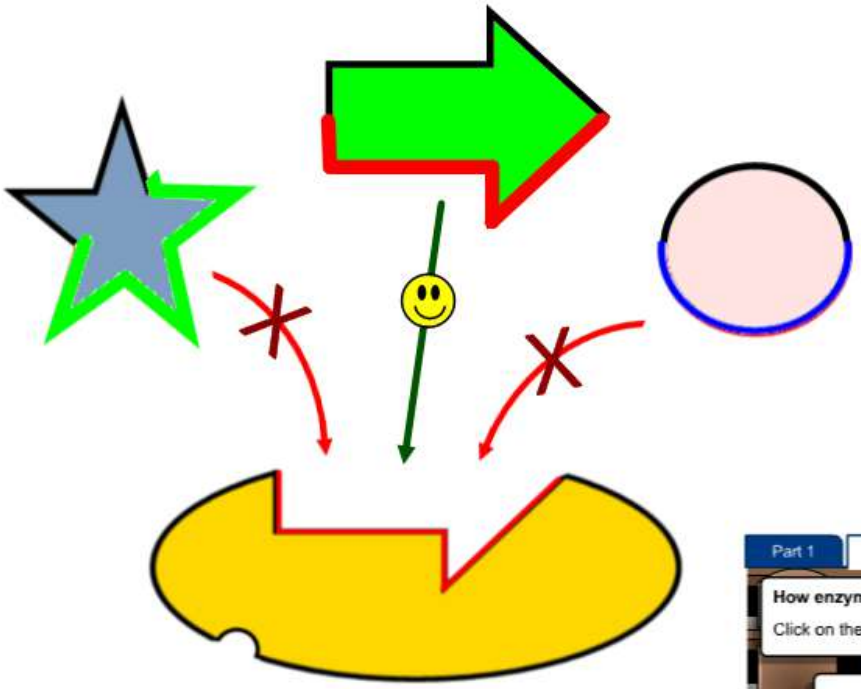



http://www.northland.cc.mn.us/biology/biology1111/animations/enzyme.swf




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

# Enzymes are specific to their substrates



enzyme substrate

## The Lock-and-Key hypothesis:

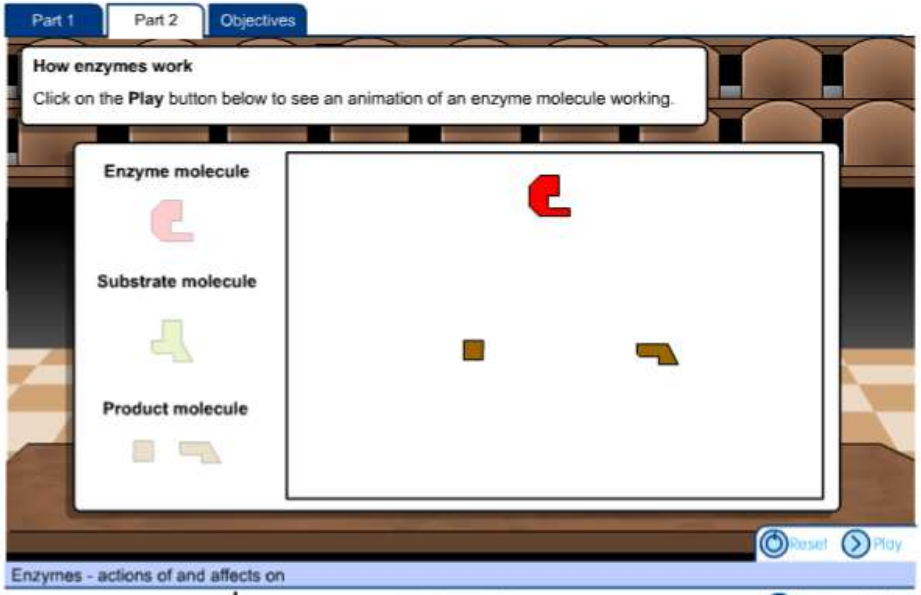
The substrate and the active site match each other in two ways:

### Structurally

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

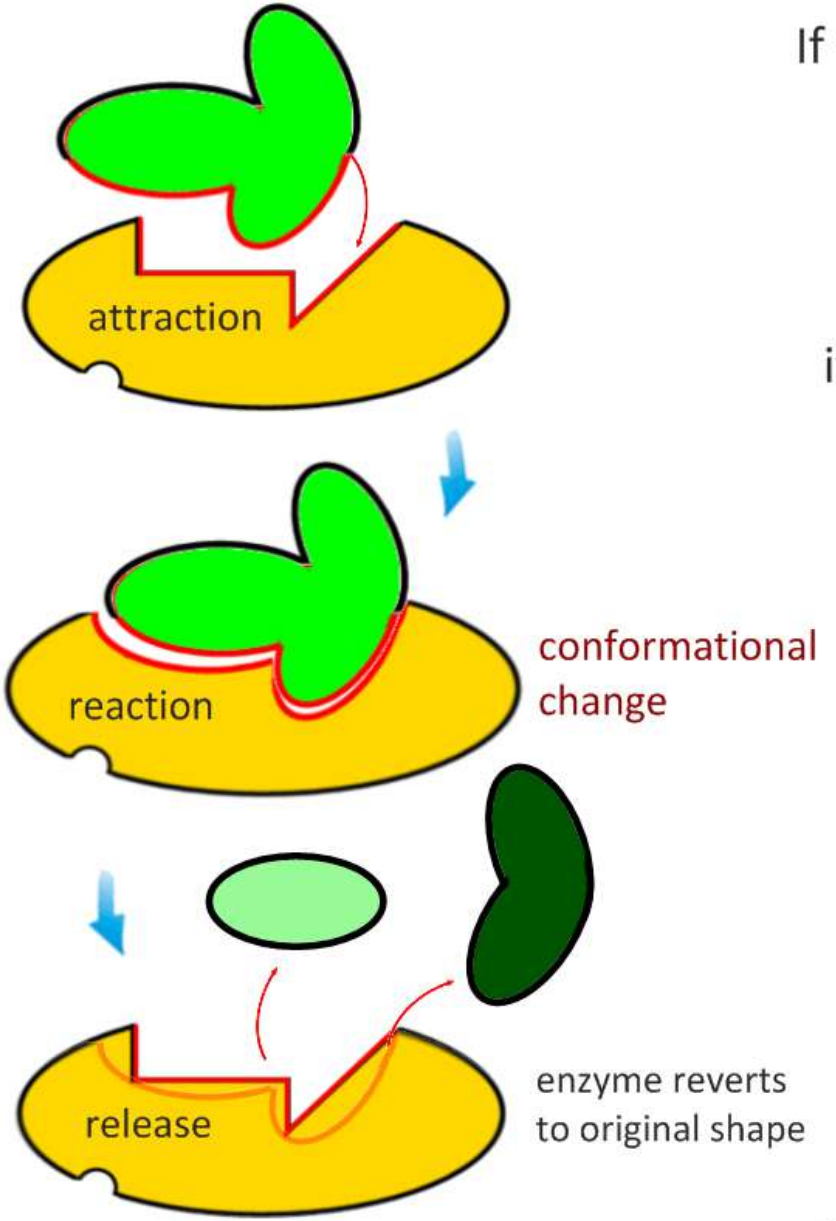
### Chemically

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

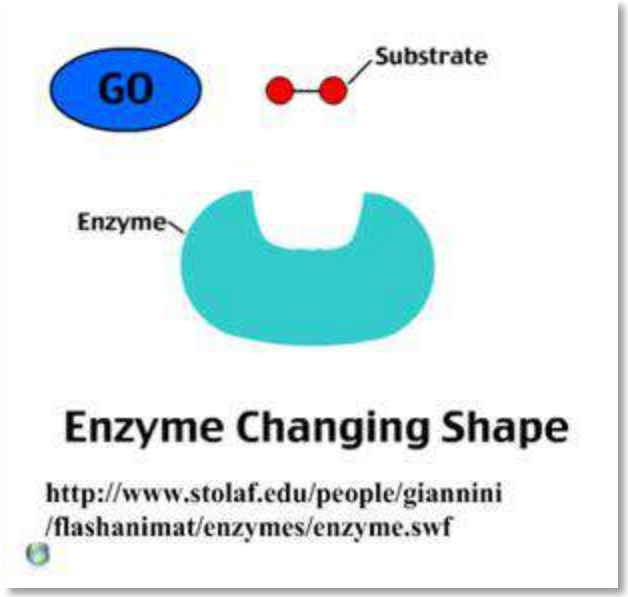



If the lock-and-key model were true, one enzyme would only catalyase one reaction. In actuality, some enzymes can catalyse multiple reactions.

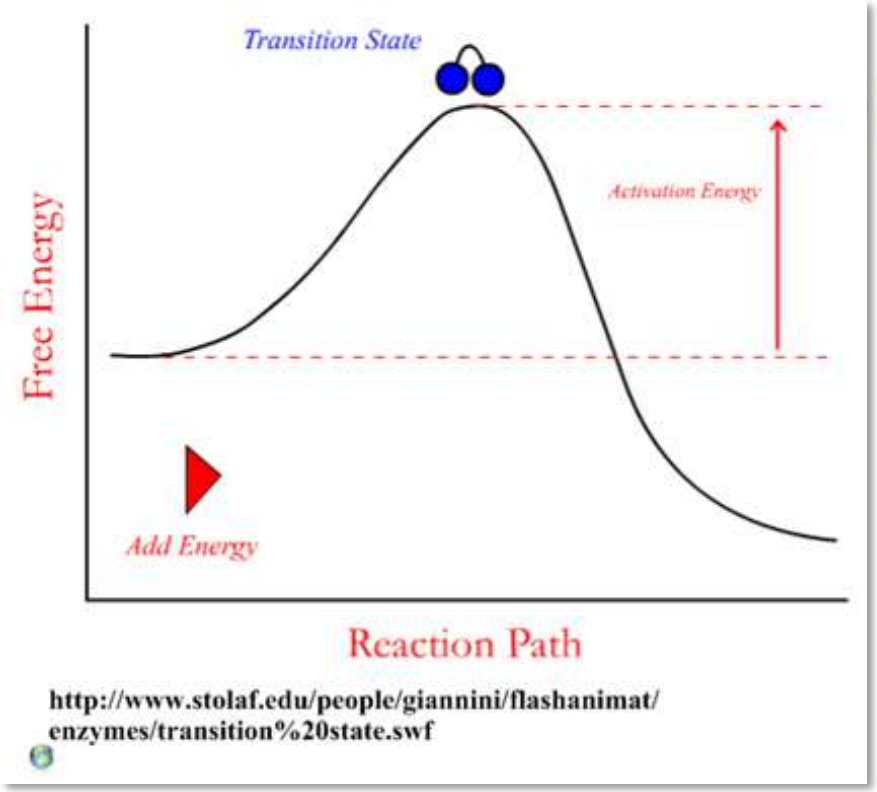
As the substrate approaches the enzyme, it induces a conformational change in the active site - it changes shape to fit the substrate.

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

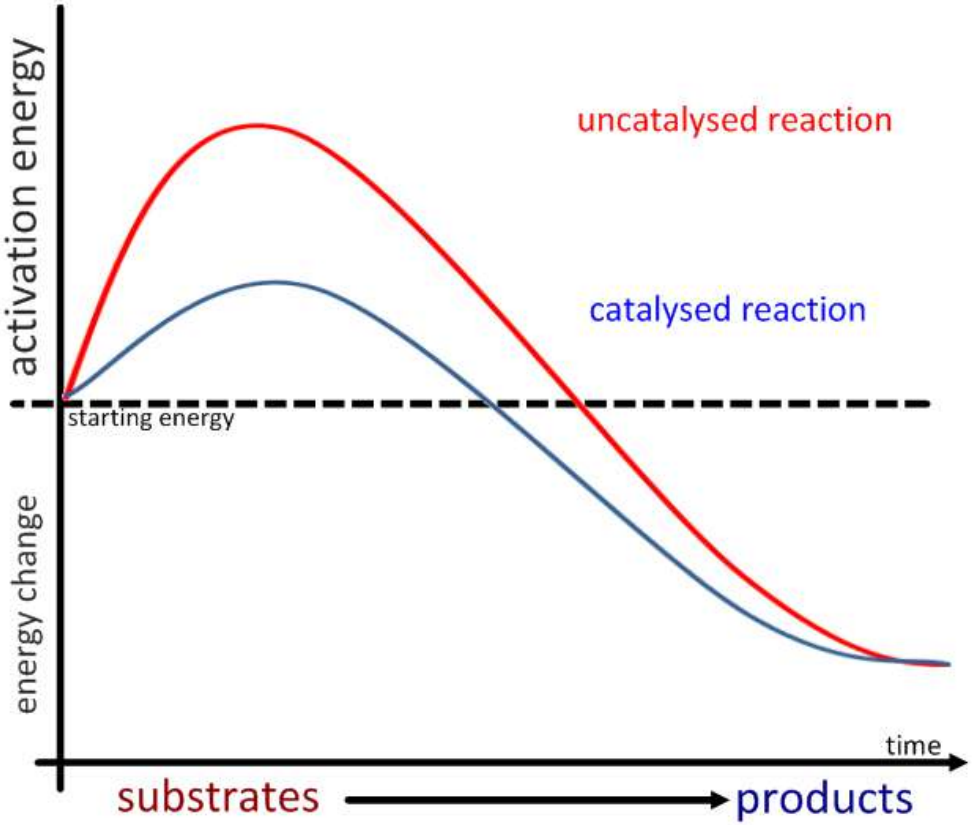
3d-inducedfit.mov



# Enzymes lower the activation energy of a reaction.



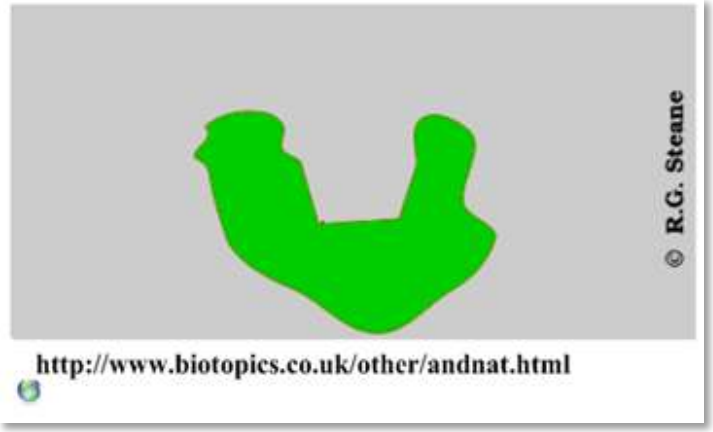

Activation energy is the amount of energy that must be put into a reaction to make it occur.




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

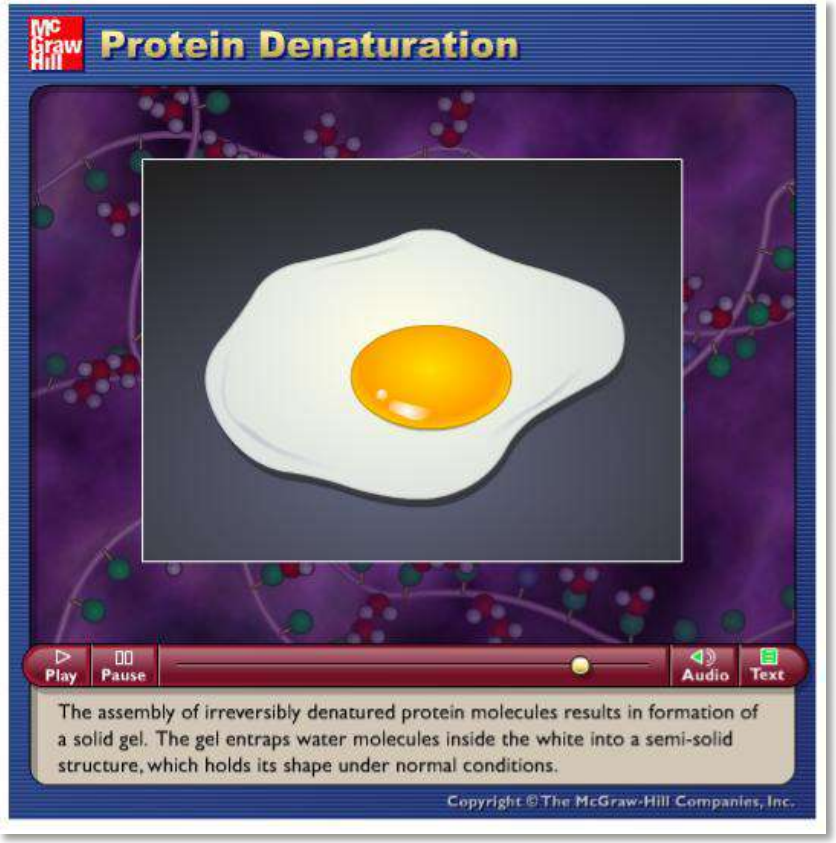
# Denaturation

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:

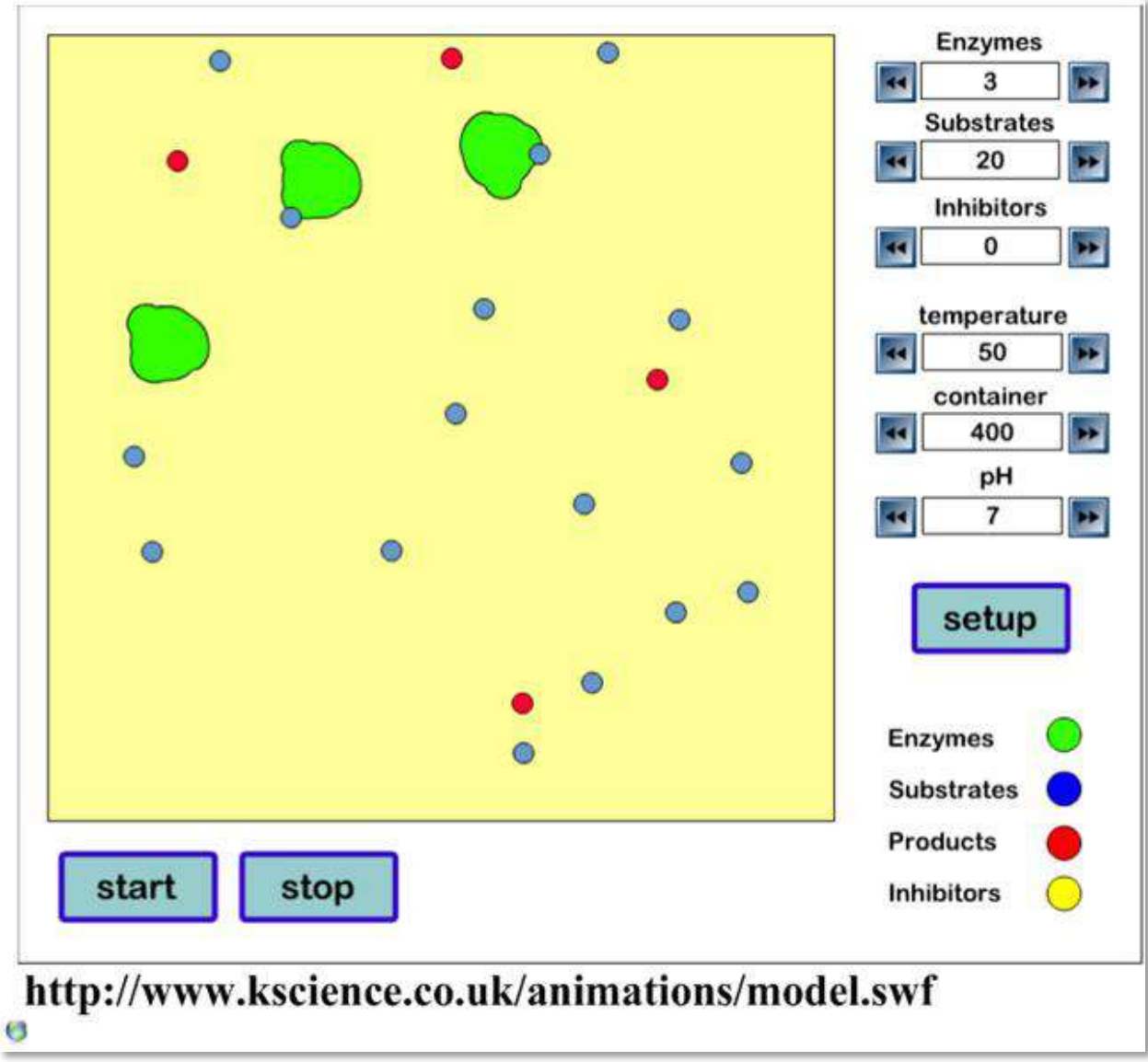
temperature

pH

substrate concentration

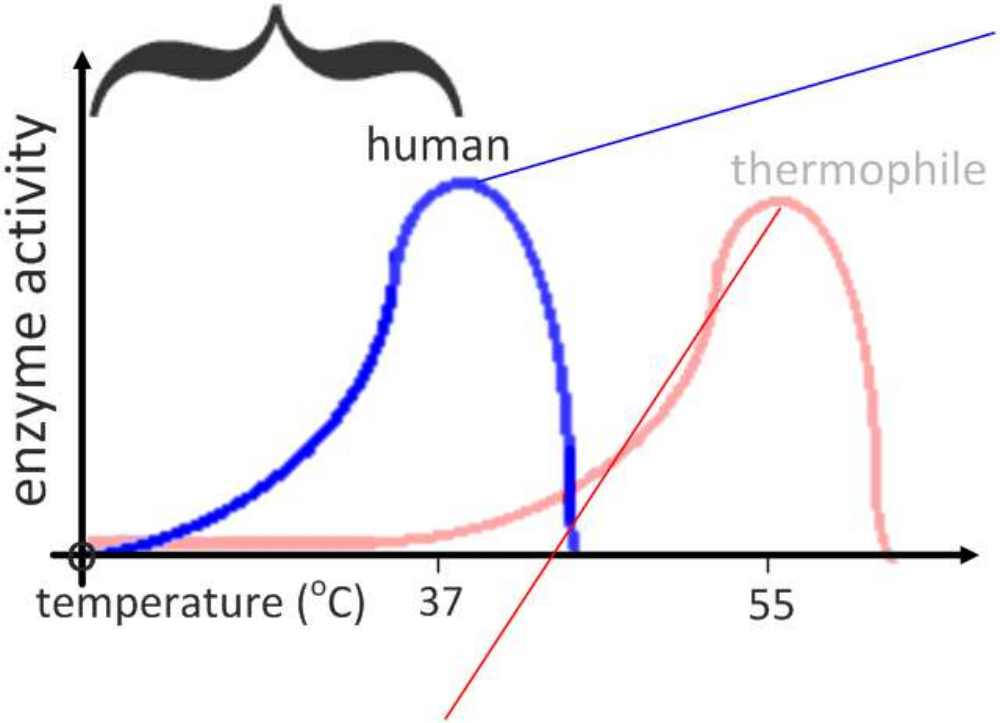
When you have finished this, complete the notes on

*enzyme activity.*



# The Effect of Temperature on Enzyme Activity

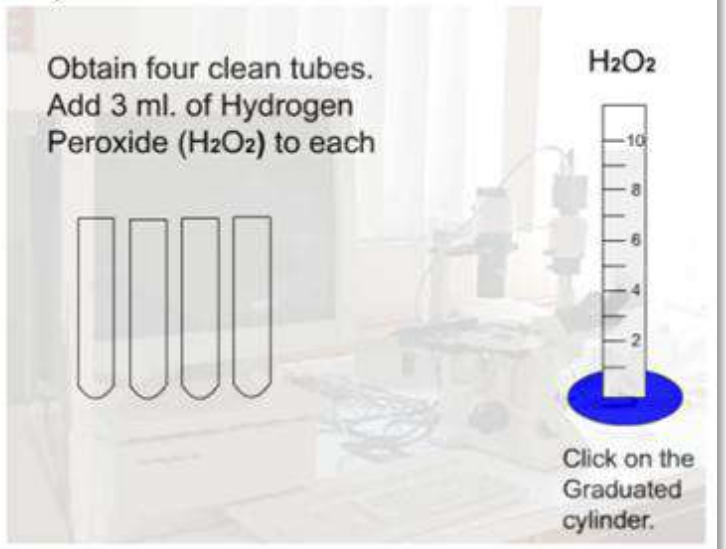
As temperature increases, rate of reaction increases as molecules have more energy, move faster and therefore collide and react more frequently.




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

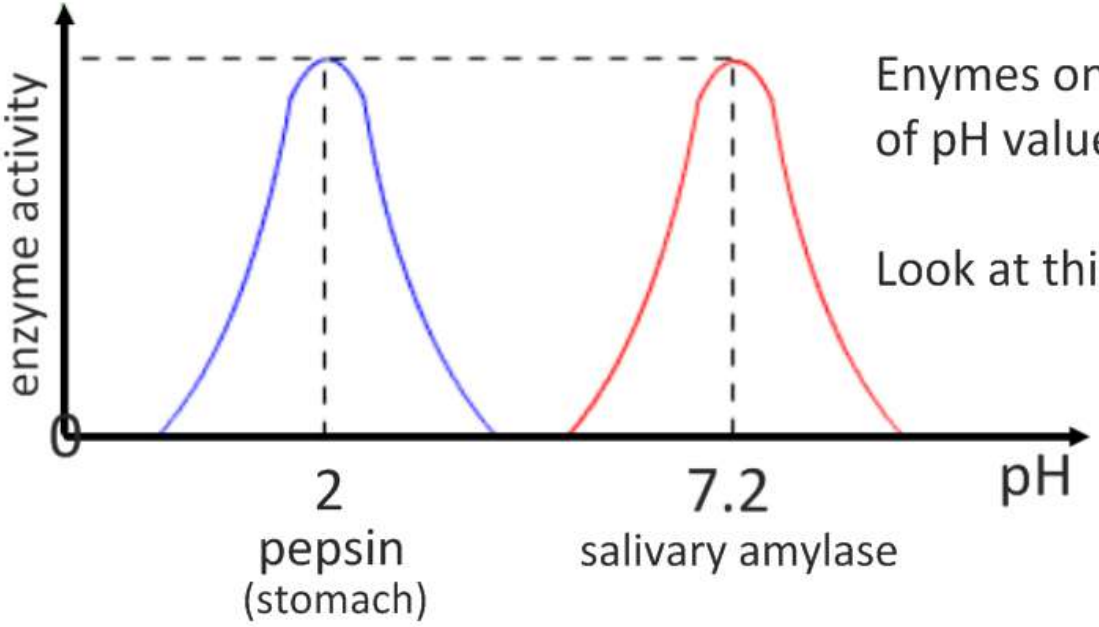
A thermophile, such as bacteria at deep-sea vents, is an organism that is able to withstand much higher temperatures before its enzymes denature.

## Try this virtual lab:




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

# The Effect of pH on Enzyme Activity.



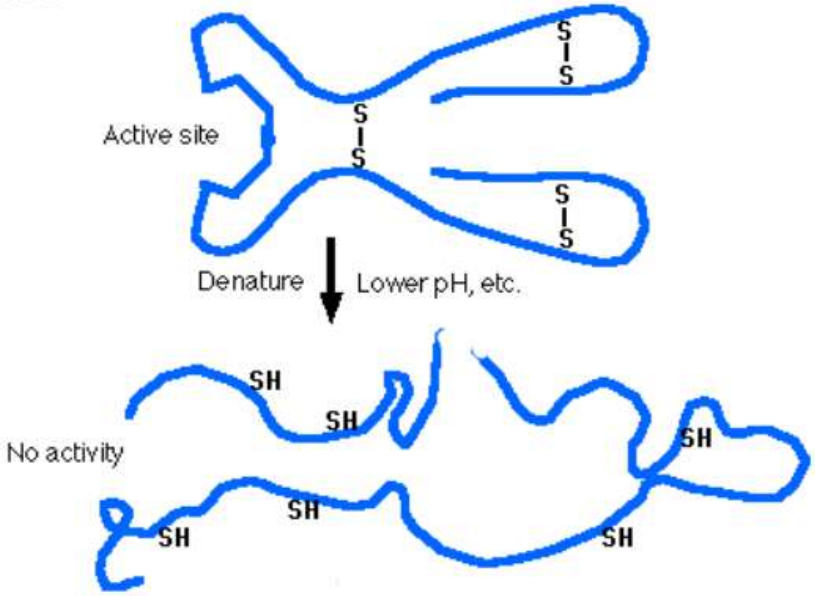

Enymes only operate within a narrow range of pH values. This is called an optimum pH.

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.

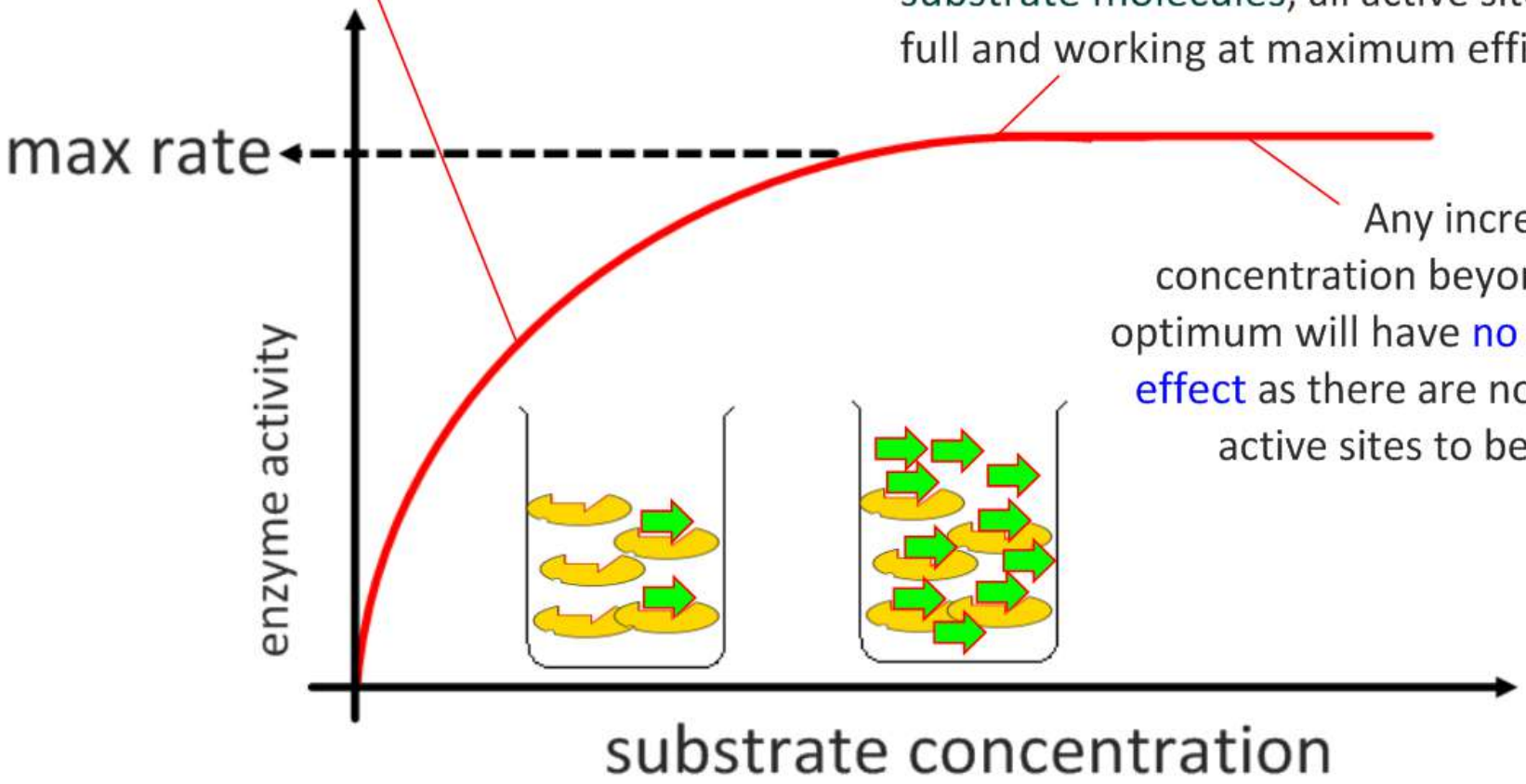



http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/D/Denaturing.gif

# 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.

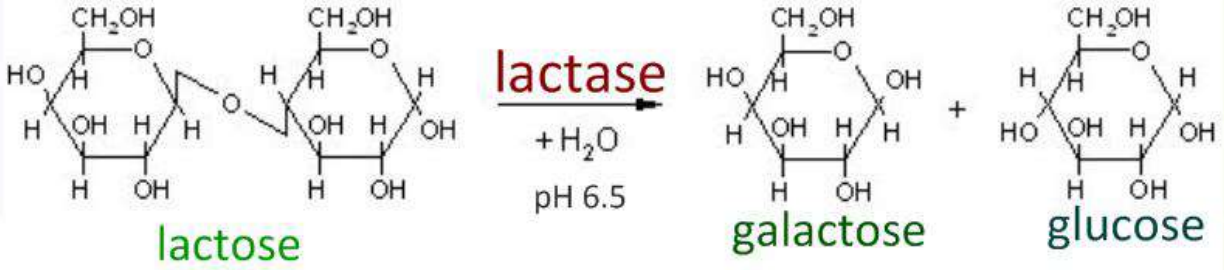



Any increase in concentration beyond the optimum will have no added effect as there are no extra active sites to be used.

# 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.

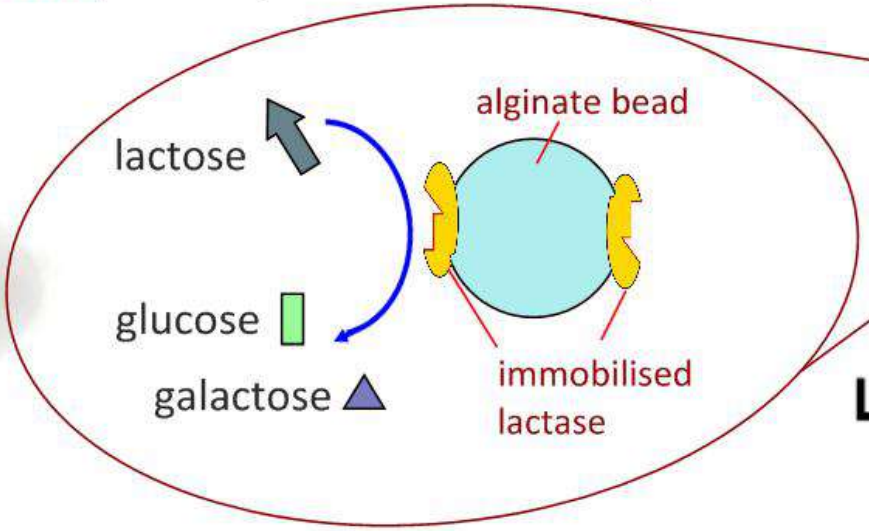
Lactose-free milk is made by different methods:

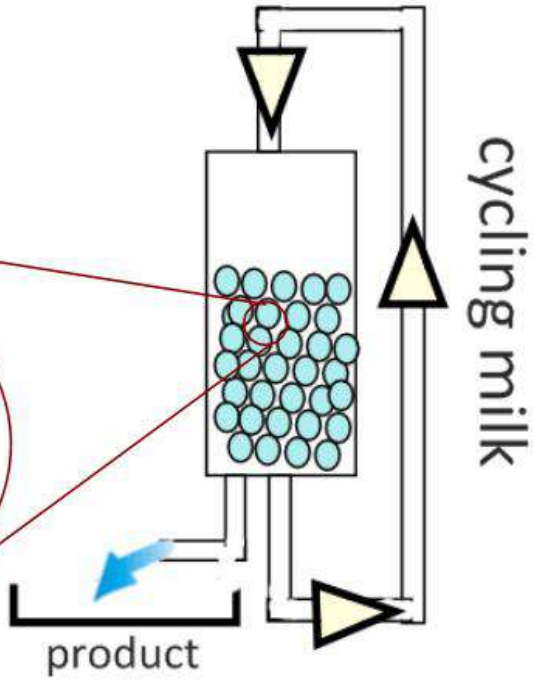
a. Add lactase to milk
   (lower quality and wasteful of lactase)

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

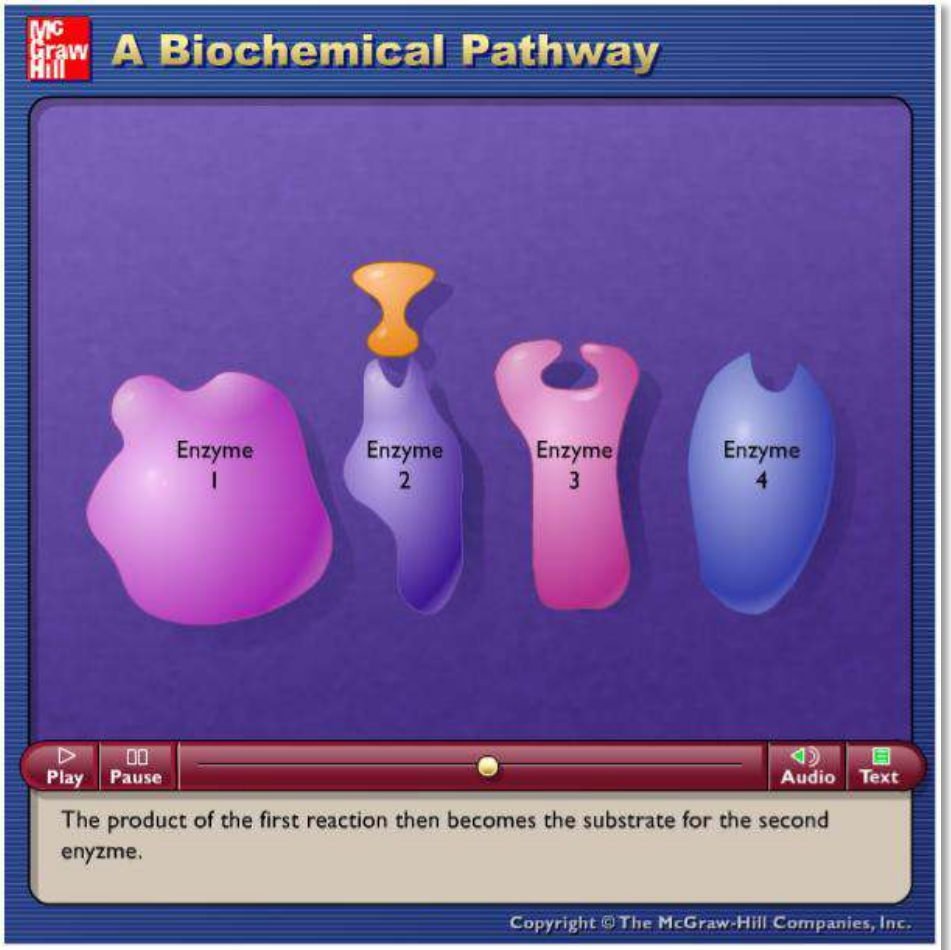
Metabolic pathways* are chains or cycles of enzyme-catalysed reactions.
The product of one reaction is a reactant in the next.

TREAD initial substrate

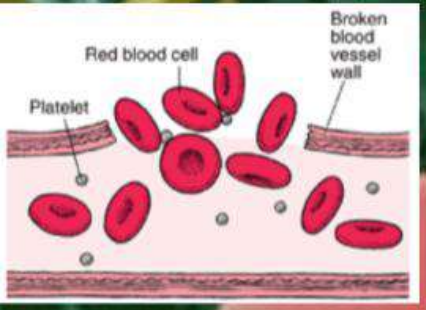
BREAD
BREED
BLEED
BLEND
BLIND
intermediates

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




Platelet/ Cell Damage

clotting factors

Thrombin

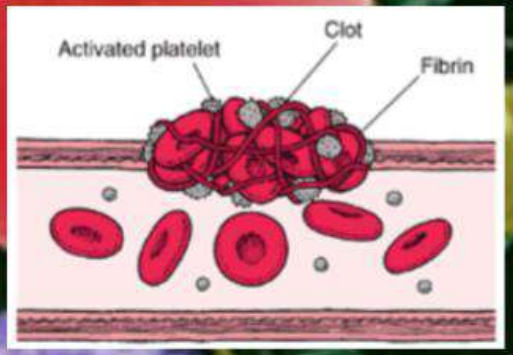
Fibrinogen (soluble)

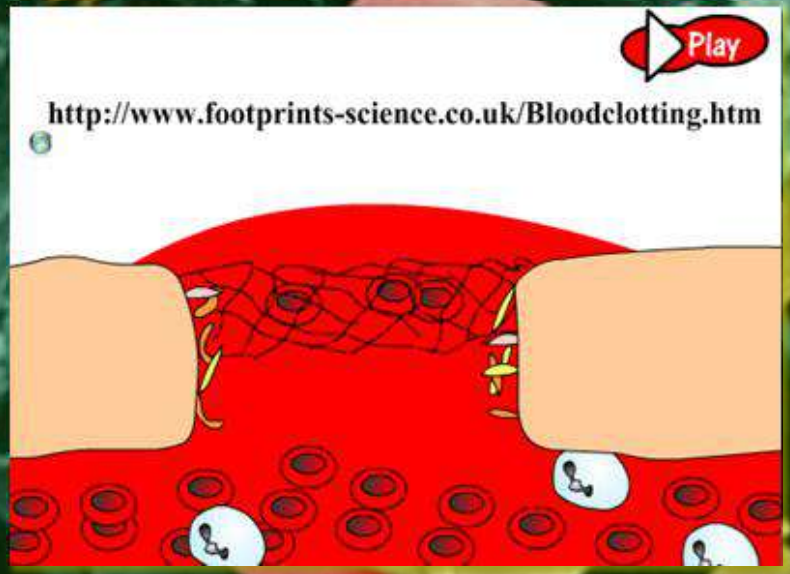
Fibrin (fibrous)

Captures Erythrocytes

Clot

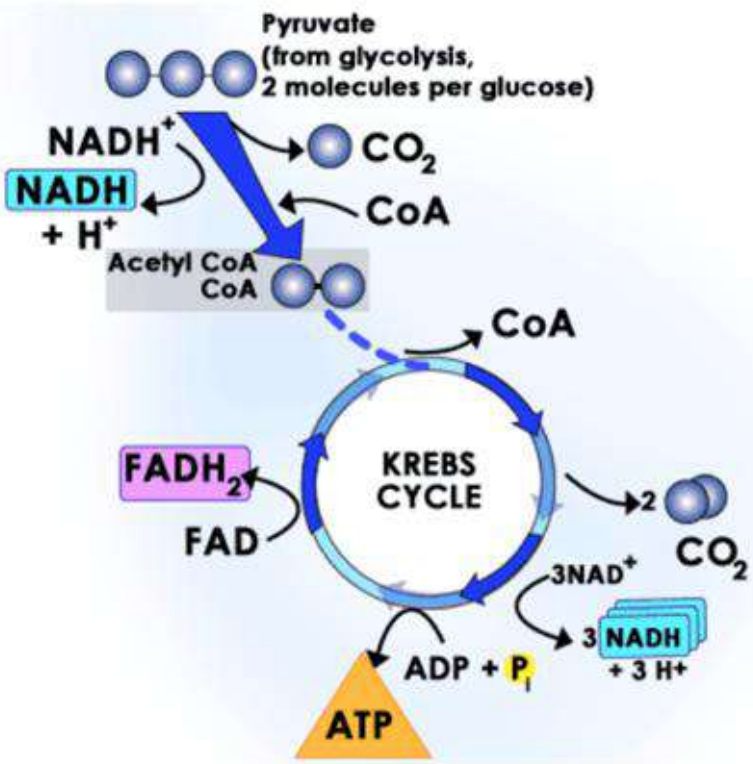
Blood clotting is an example of a metabolic pathway: a chain of biochemical reactions



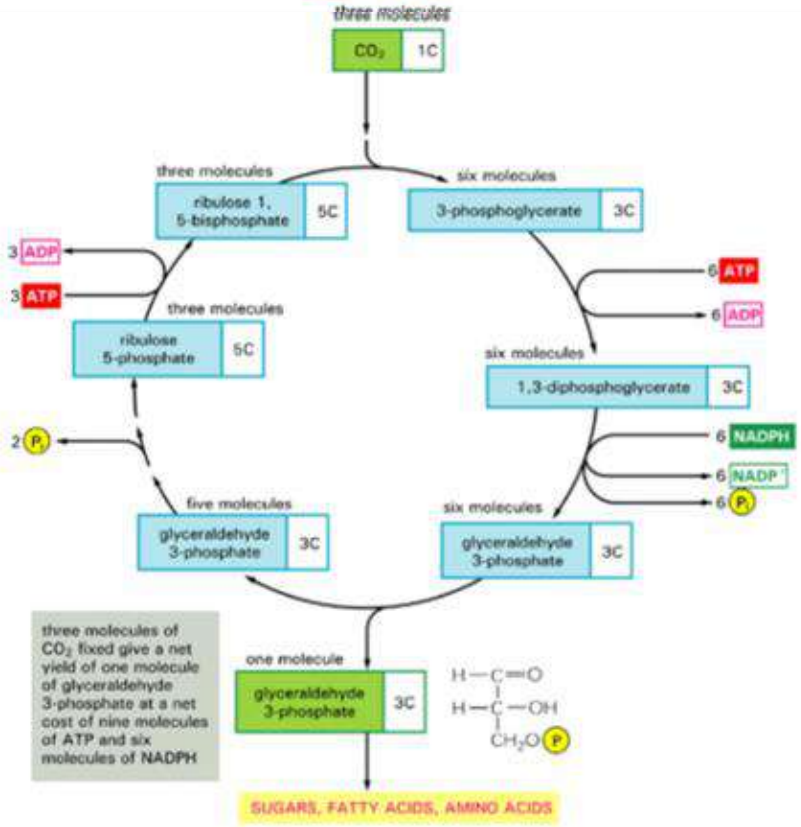
The Krebs Cycle (cell respiration) and Calvin Cycle (photosynthesis) are examples of enzyme-catalysed, cyclical metabolic pathways.

## Krebs




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

## Calvin

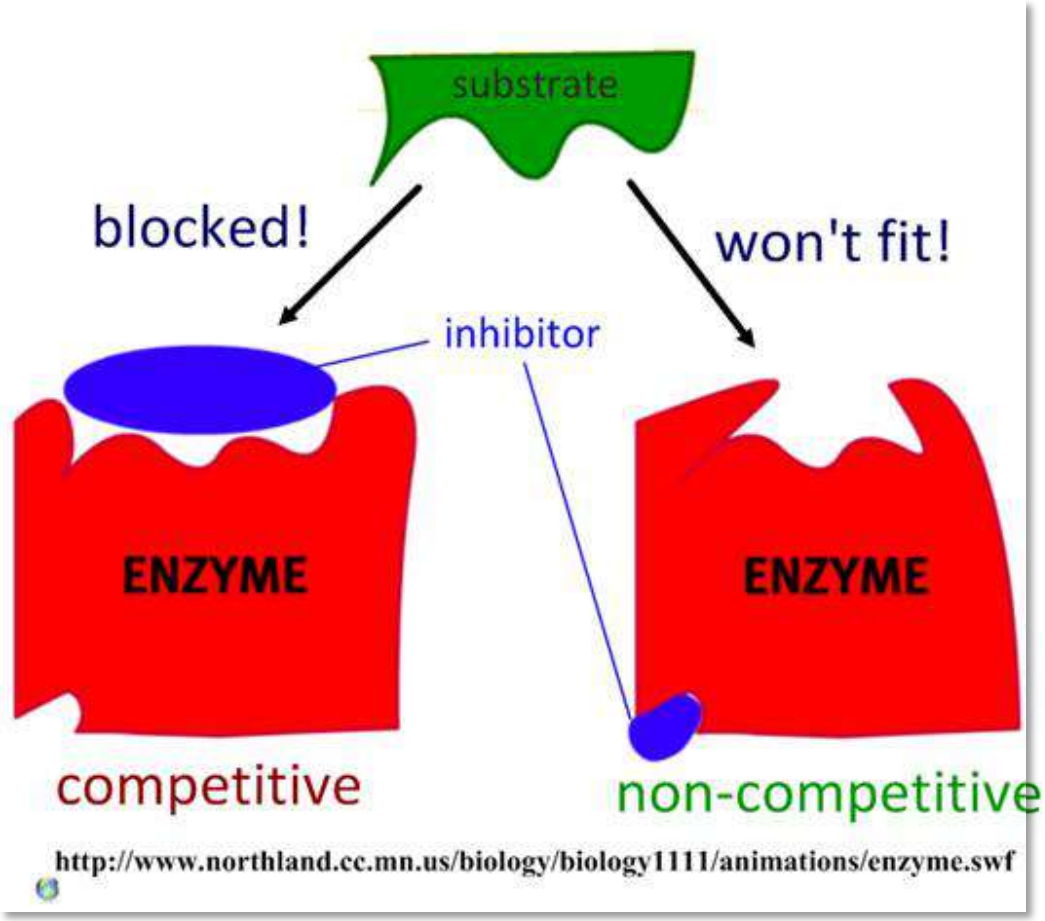



http://library.thinkquest.org/C004535/calvin_cycle.html

# Enzymes can be inhibited by other molecules.

Inhibition can be competitive or non-competitive.

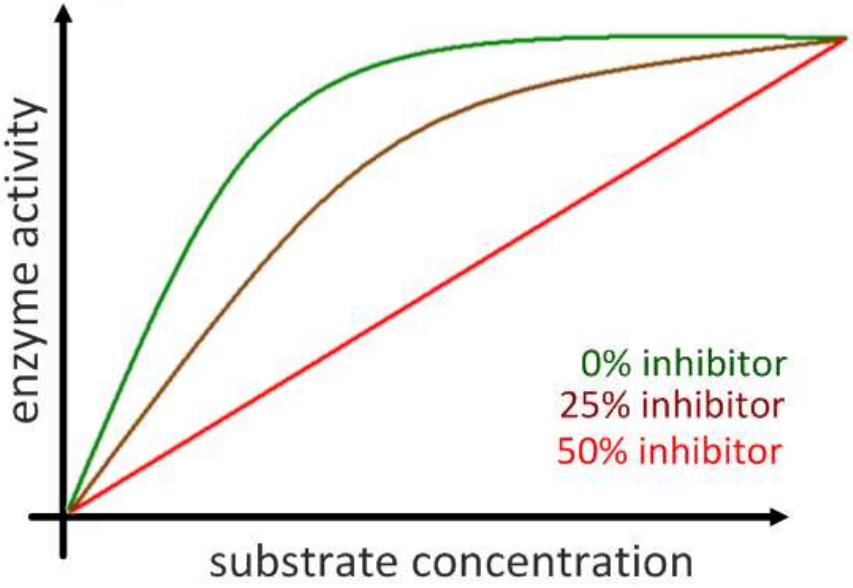
inhibitor fits the active site and prevents the substrate from entering



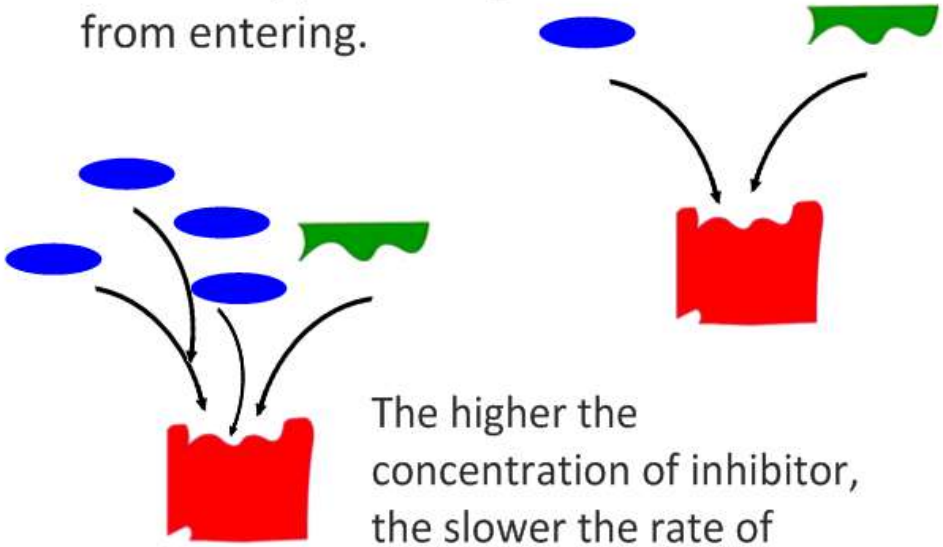

inhibitor fits into an allosteric site*, causing a conformational change in the active site: the substrate cannot attach to react

*'other' site

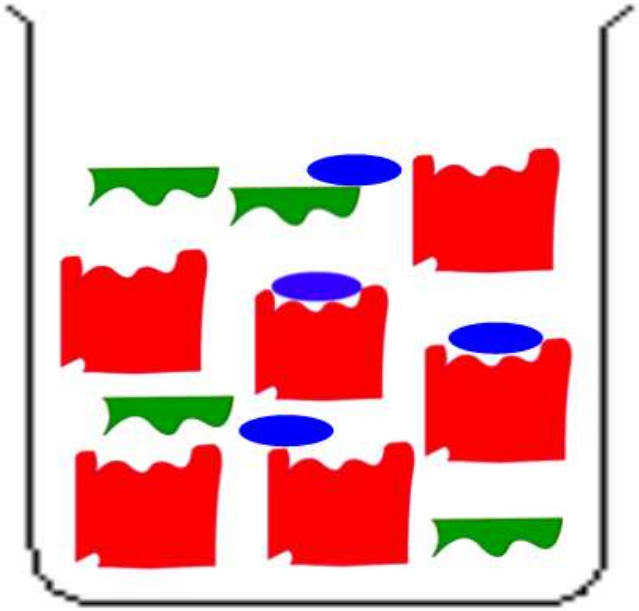
# Competitive Inhibition




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



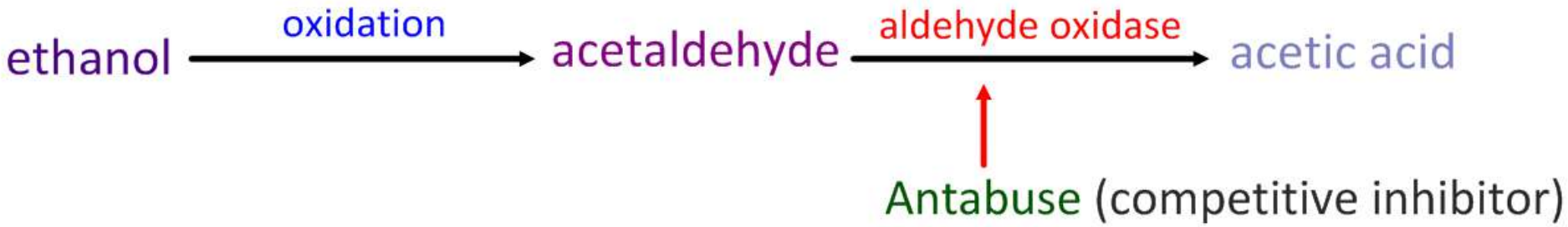
The higher the concentration of inhibitor, the slower the rate of reaction.



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.

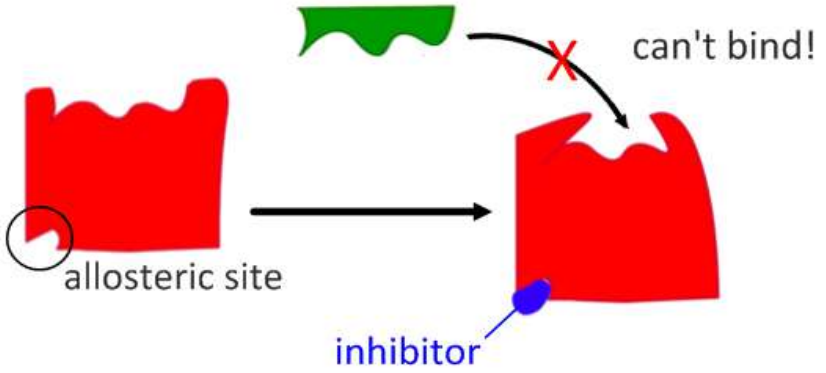
A build up of acetaldehyde follows, resulting in a strong feeling of nausea and other strong hangover symptoms - a good deterrent from drinking.

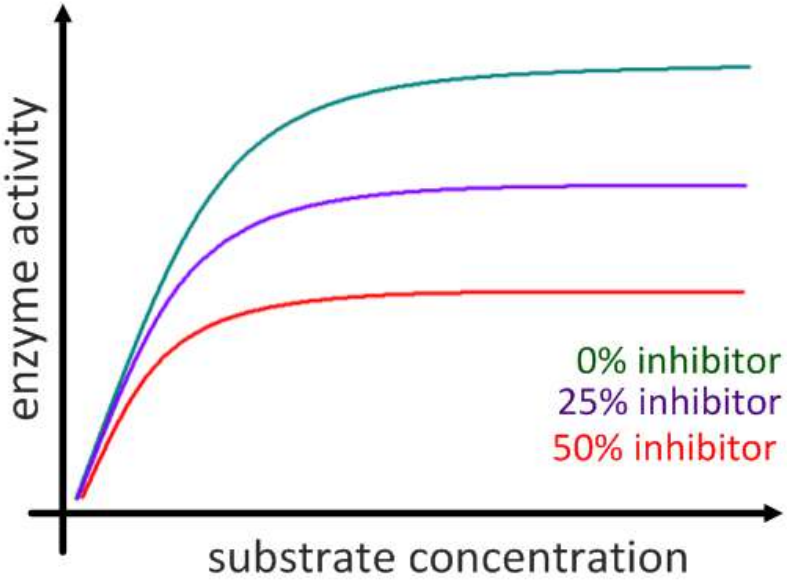
Antabuse is administered as a daily pill, so its efficacy relies on the patient's own motivation - if they stop taking it, they can drink again.

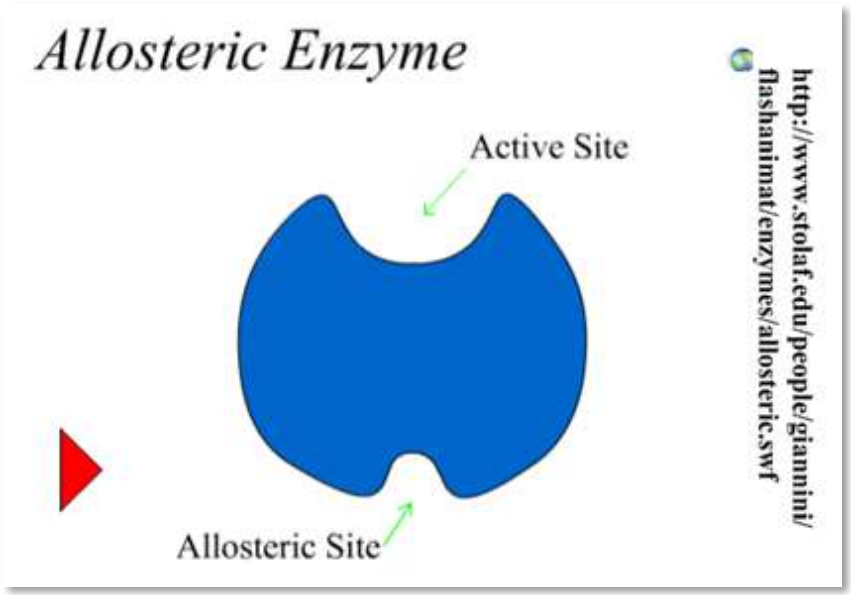
Image: 'Glass of wine'
www.flickr.com/photos/12191709@N00/92783024

# 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.

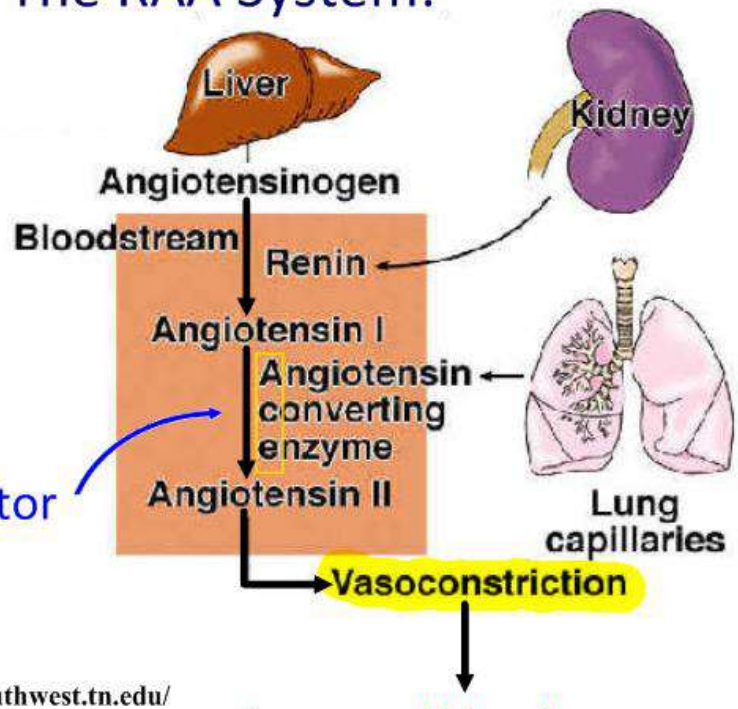
The maximum rate of reaction is also reduced - with fewer functional active sites, the enzyme has reduced ability to process the substrates, even if substrate concentration is increased.

# 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

increased blood pressure

## Vasoconstriction:

Normal blood flow

Restricted blood flow

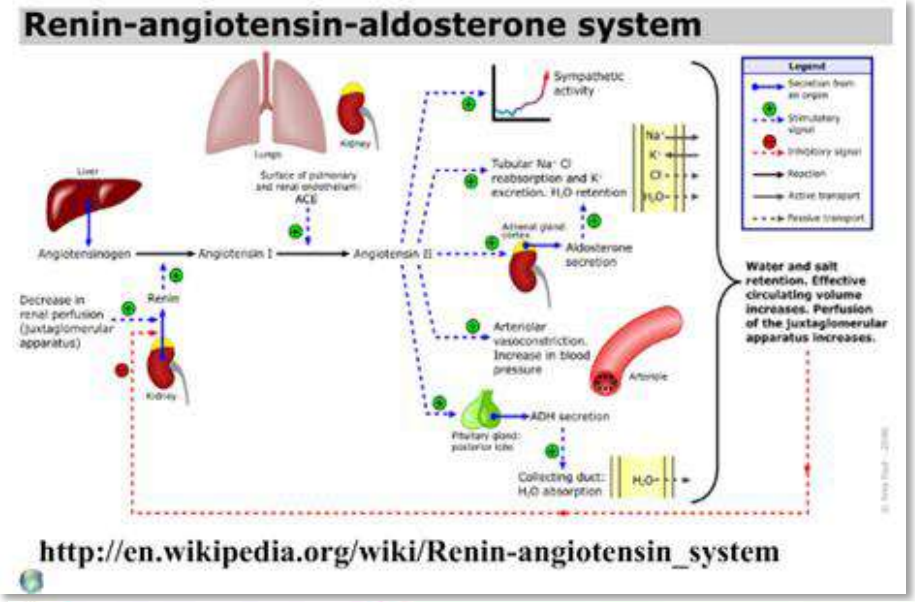
http://www.nlm.nih.gov/medlineplus/ency/images/ency/fullsize/8983.jpg

A.D.A.M.

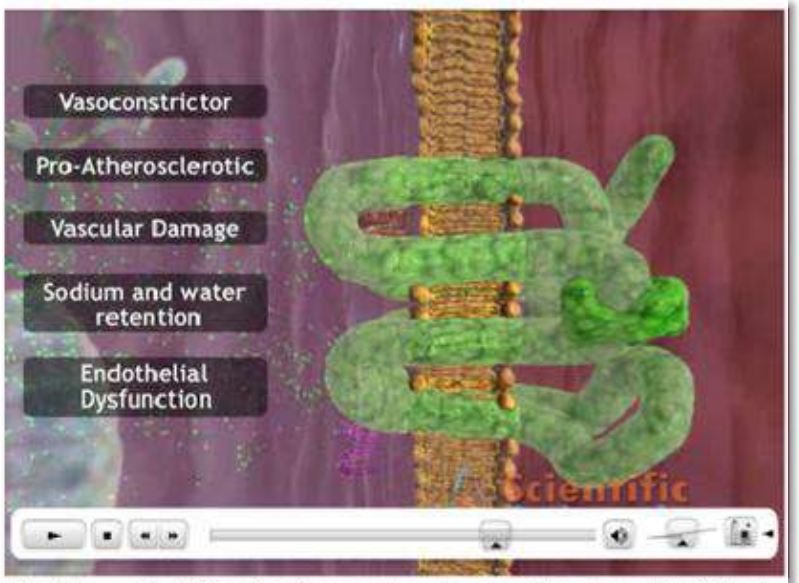
ACE Inhibitors are medications that inhibit Angiotensin Converting Enzymes - they prevent increased blood pressure.

They are non-competitive and reversible.

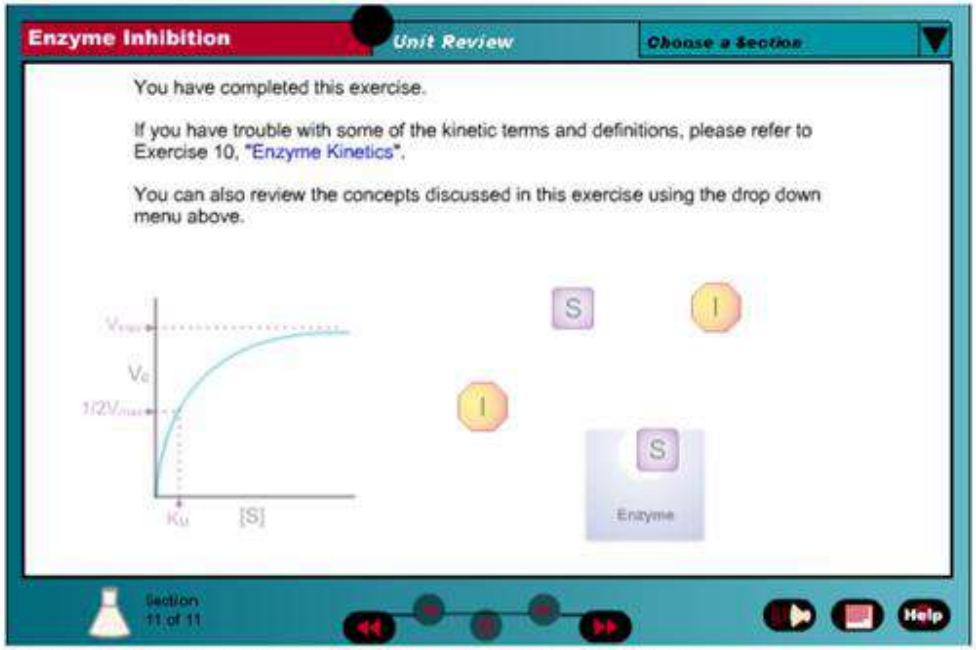
# More ACE-Inhibitor resources:



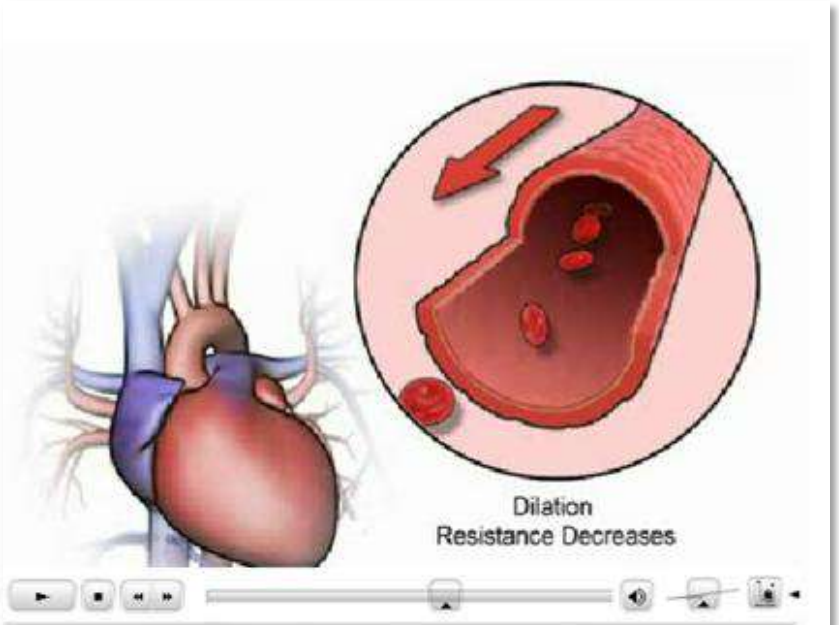

http://en.wikipedia.org/wiki/Renin-angiotensin_system




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

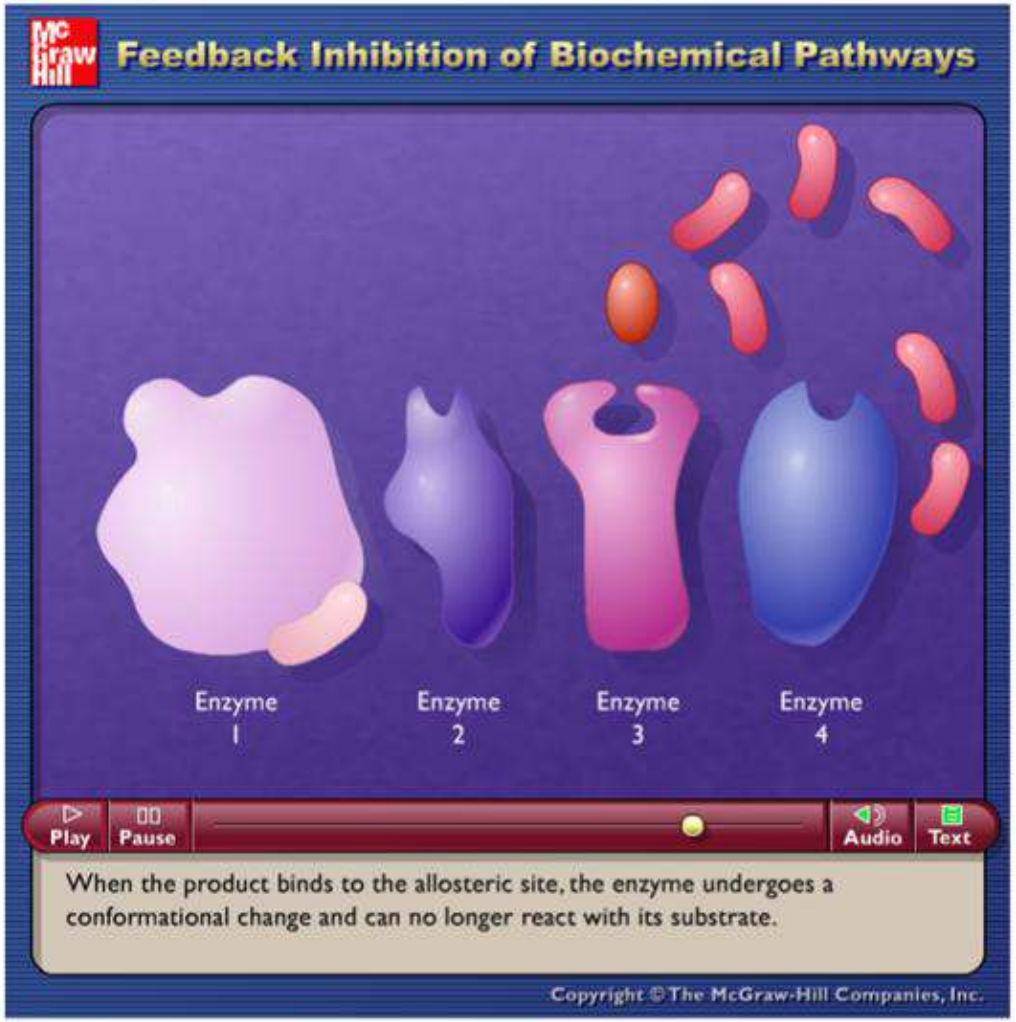



http://www.wiley.com/college/pratt/0471393878/student/animations/enzyme_inhibition/index.html

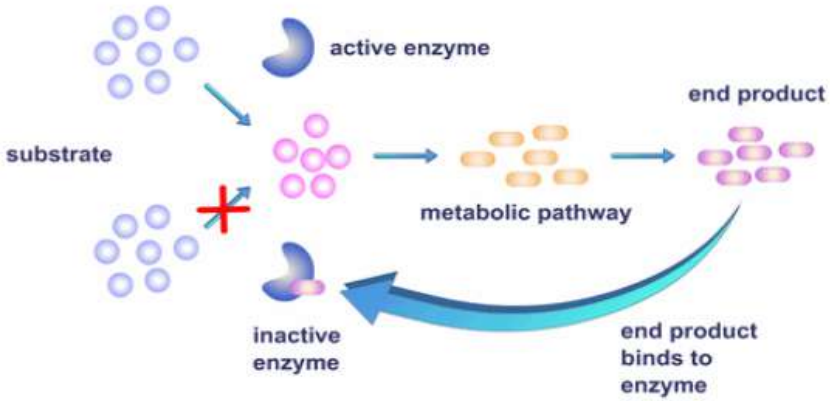



http://www.heartfailurematters.org/EN/Animation/Pages/animation_7.aspx

# 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



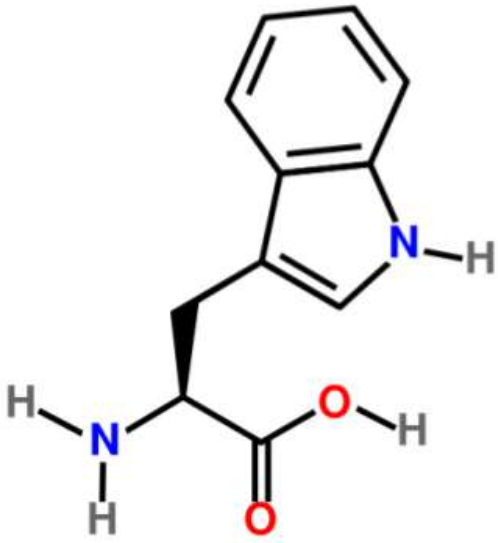

http://scholar.hw.ac.uk/site/biology/topic13.asp?outline=

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

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




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

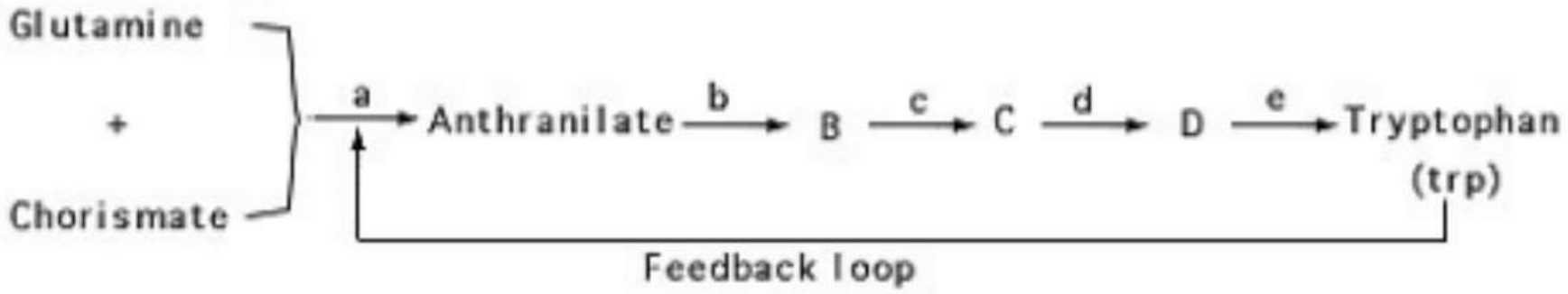
Tryptophan is an essential amino acid
(we can't produce it, so have to get it in our diet).

*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.

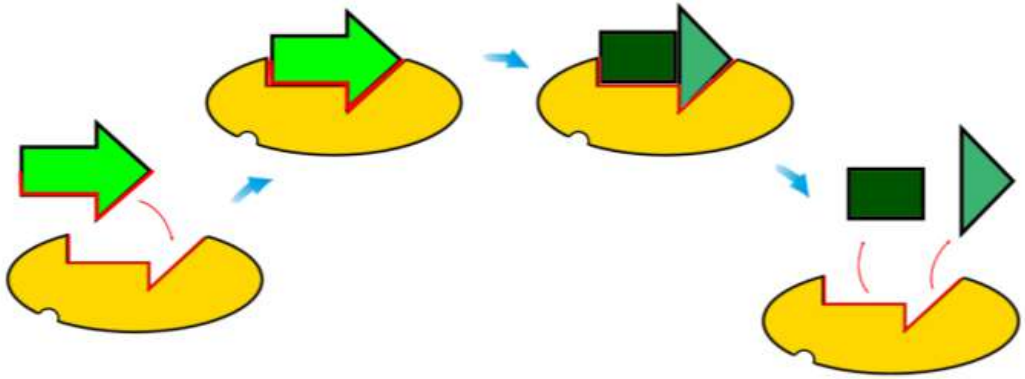
*E. coli*

(SEM - fc)




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

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



For more resources and animations visit:

http://sciencevideos.wordpress.com