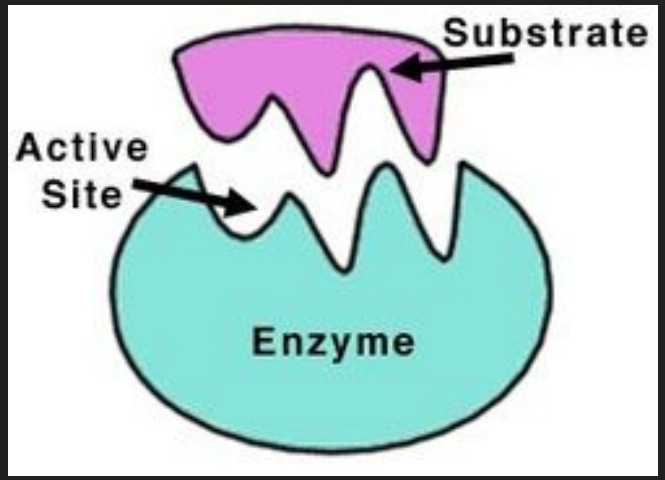
Enzymes 8.1

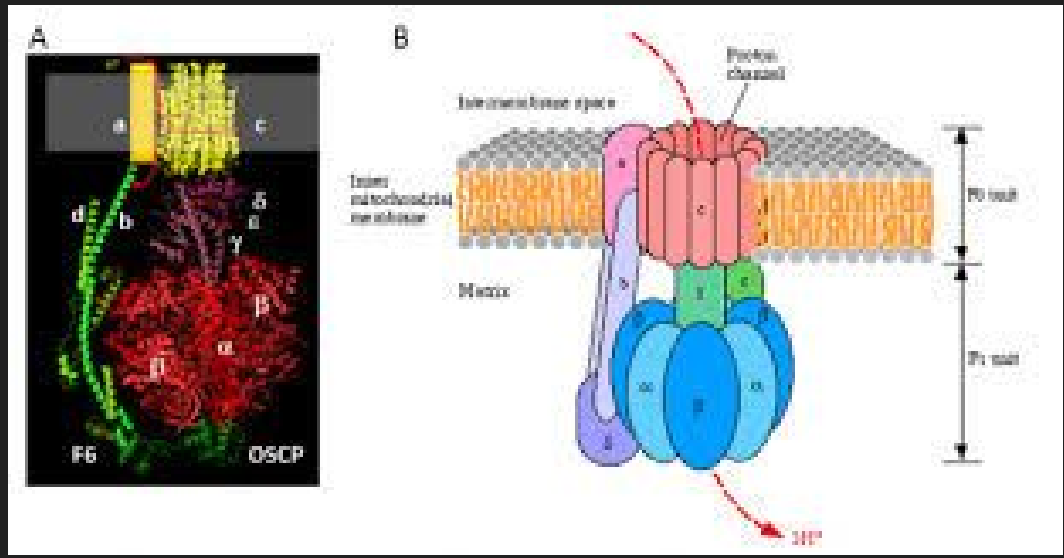
MONDAY Review (SL 2.5)

-Enzymes are catalysts: bring about biochemical reactions!

-Optimal environment: affected by pH, temp, and substrate concentration

-Active site to which specific substrate binds





Understandings:

- Metabolic pathways consist of chains and cycles of enzyme-catalysed reactions
- Enzymes lower the activation energy of the chemical reactions that they catalyse
- Enzyme inhibitors can be competitive or non-competitive
- Metabolic pathways can be controlled by end-product inhibition

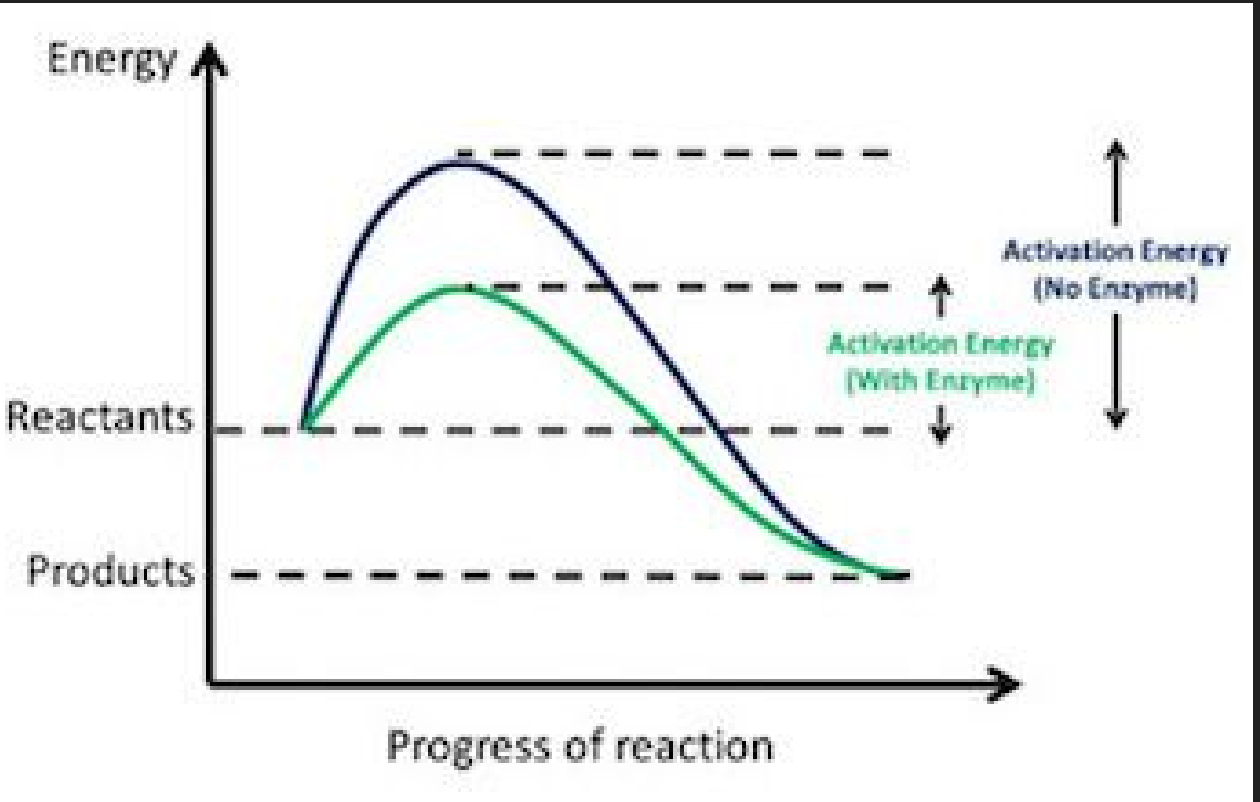
Applications:

- End-product inhibition of the pathway that converts threonine to isoleucine
- Use of databases to identify potential new anti-malarial drugs

Skills:

- Calculating and plotting rates of reaction from raw experimental results
- Distinguishing different types of inhibition from graphs at specified substrate concentration

8.1 U2: Enzymes lower the activation energy of the chemical reactions they catalyze



Activation energy = substrate→ product

Energy breaks bonds in substrate (lactose → glucose)

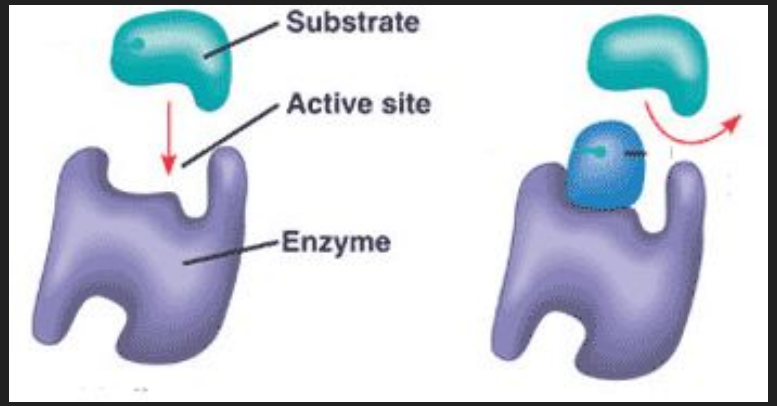
Enzyme is unchanged and can keep working!

8.1U 3: Enzyme inhibitors can be competitive or noncompetitive

Competitive:

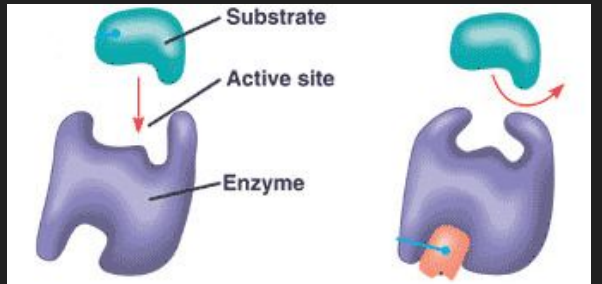
binds to activation site and substrate can't bind

http://www.kscience.co.uk/animations/anim_2.htm



Non-competitive:

binds to enzyme, not necessarily on activation site and changes shape so substrate can't bind



8.1S1 Distinguishing different types of inhibition from graphs at specified substrate concentration

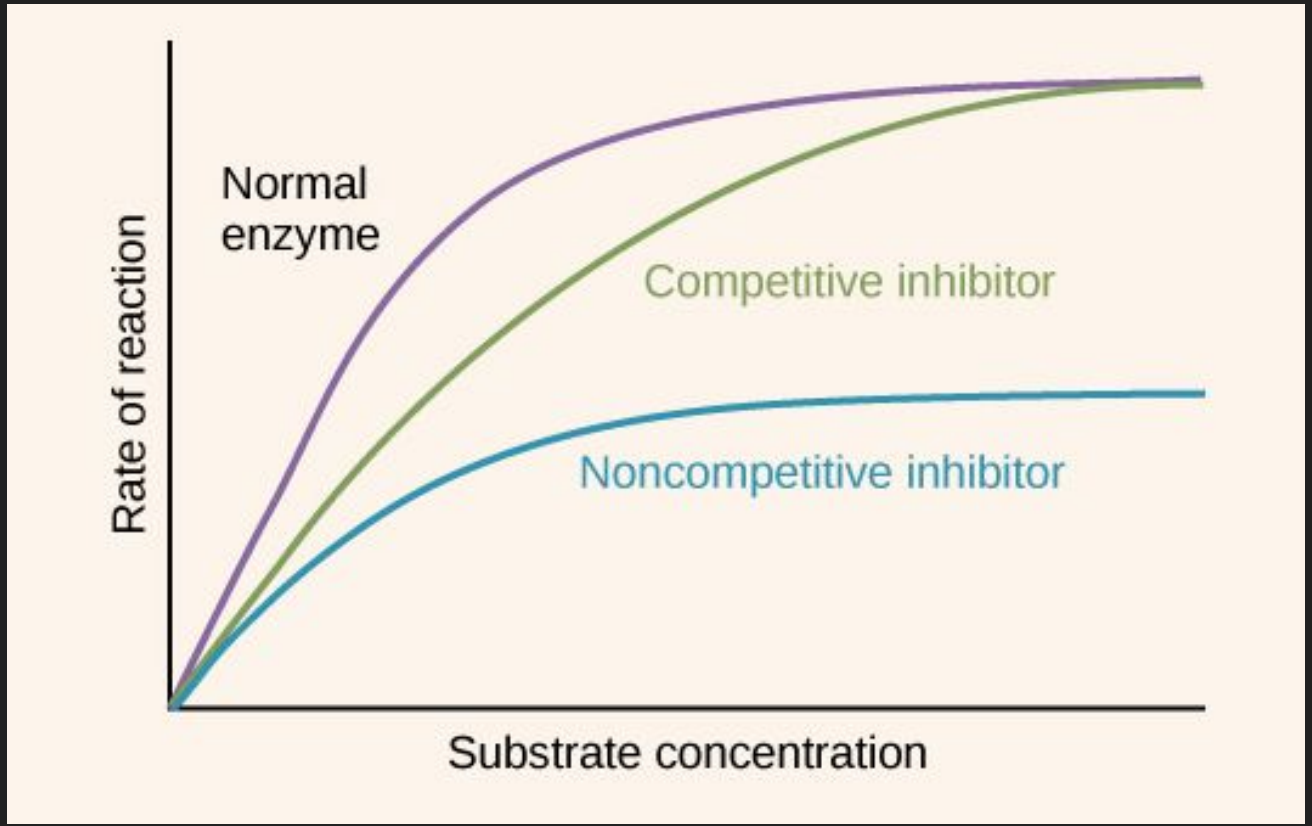
Simulation 1: normal conditions, low substrate concentration : 20 seconds, 8 pieces of cereal

Simulation 2: normal conditions, high substrate concentration :20 seconds, 35 pieces of cereal

Simulation 3: competitive inhibition, place a cup over some of your sample, 20 seconds, 35 pieces of cereal

Simulation 4: noncompetitive inhibition, one person tapes their thumbs to their hand and can only use their fingers, the other person can compete normally: 20 seconds, 35 pieces

8.1S1 Distinguishing different types of inhibition from graphs at specified substrate concentration



Real Life examples

Penicillin : competitive inhibition

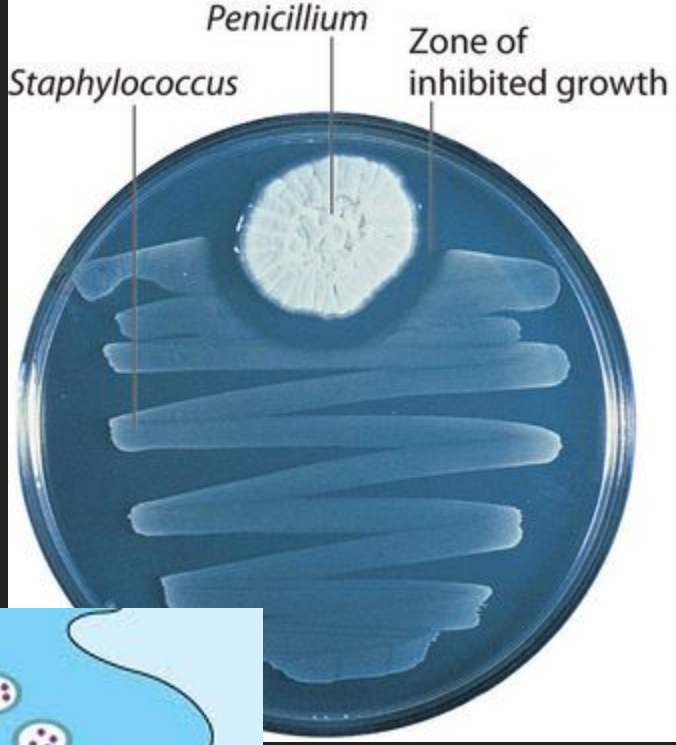
Transpepitase: builds cell walls

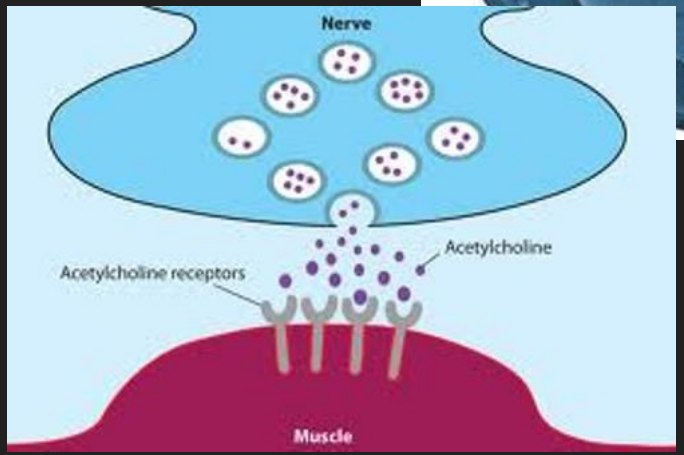
Binds to active sight, causes permeability

Acetylcholine: neurotransmitter involved in muscle activation

Acetylcholinesterase: enzymes to promote release in brain

Succynl choline: used in anesthesia to relax muscles of patients





TUESDAY 8.1U2: Calculate rates of reaction from raw experimental results

Salivary amylase - enzyme in spit that breaks down starch and sugar

The human body is normally around 98.7 degrees F and 37 degrees F. Most people's mouths have a pH of 7, while the stomach has a pH of 3.

Fever?

Hypothermia?

Does it work once swallowed into the stomach? (pH of 3)

Alkalosis? Occurs when body contains too many bases

Is your saliva more effective at digesting than your lab partners?

Design your own experiment

Pick an environmental change to test

Potatoes are made of starch → can be broken down by salivary amylase in sugar

Use potato solution to test out optimum conditions for salivary amylase

Decide on 4 different variations to create in your question

MAKE PREDICTIONS

Example: 10 degrees, 20 degrees, 30 degrees, 70 degrees

Design 2 trials for each variation for a total of 8 test tubes, not including the controls. We will do the controls today:

How to tell if enzyme is working?

Enzyme = saliva (amylase)

Substrate = potato (starch)

Indicator: Benedicts solution to test for presence of sugar

Potato breaks down into sugar

Blue → Red when sugar is present (enzyme worked)

Under your 4 different variations, use benedict's to test for presence of sugar

Control Procedures

Control Procedure:

1. Put ½ a pipet of potato solution into 4 test tubes
2. Set 2 test tubes in one beaker and label with tape " water"
3. Set 2 test tubes in one beaker and label with tape " saliva"
4. Spit 4 mL of your own saliva into one of remaining test tubes. Add 1 mL of *warm* water.
5. Fill the other test tube with 5mL of warmish water.
6. Pipet saliva into each of the 2 test tubes containing potato. Stir vigorously.
7. Pipet water into the other 2 test tubes containing potato. Stir vigorously.
8. Add one drop of Benedict's reagent to all four test tubes.
9. Label your tubes with tape
10. Put in beaker half full of water.
11. Put in hot water bath until color change occurs (4-10 minutes: record the time!)
12.

Wednesday

GOALS

1. Graph the color change over time in your four variations to show rates of reactions (8.1S2)
2. ***Clean up after yourself!!!!!!!***

PROCEDURE

1. Barely cover the bottom of each of the 8 beakers in potato solution.
2. Label your test tubes according to your experimental design (2 replications for each variation)
3. Spit into all test tubes, enough so potato: spit is about 1:1
4. If doing pH, add variations at this point (vinegar or baking soda)
5. Add a whole pipet of Benedict’s reagent to all test tubes.
6. Put in beaker half full of water.
7. ** If you are doing temperature change, place samples in water baths according to your 4 variations. Let sit for 4 minutes and then put in water bath
8. Put in hot water bath to start Benedict's test if not doing temp
9. **Record the specific color of each tube every minute for each test tube for 10 minutes.**

Challenge: by changing just one letter at a time, get from 'TREAD' to 'BLINK'. All intermediates must be real English words.

TREAD

_____

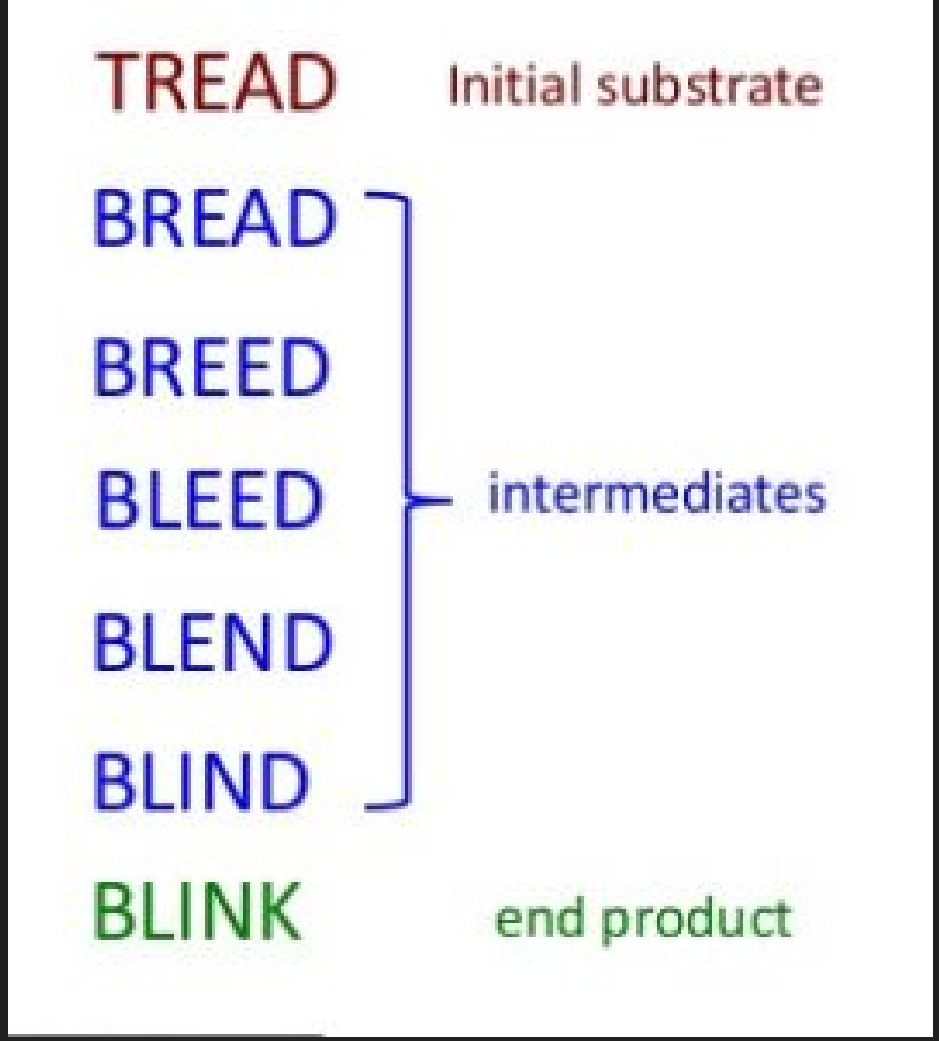
_____

_____

_____

_____

BLINK

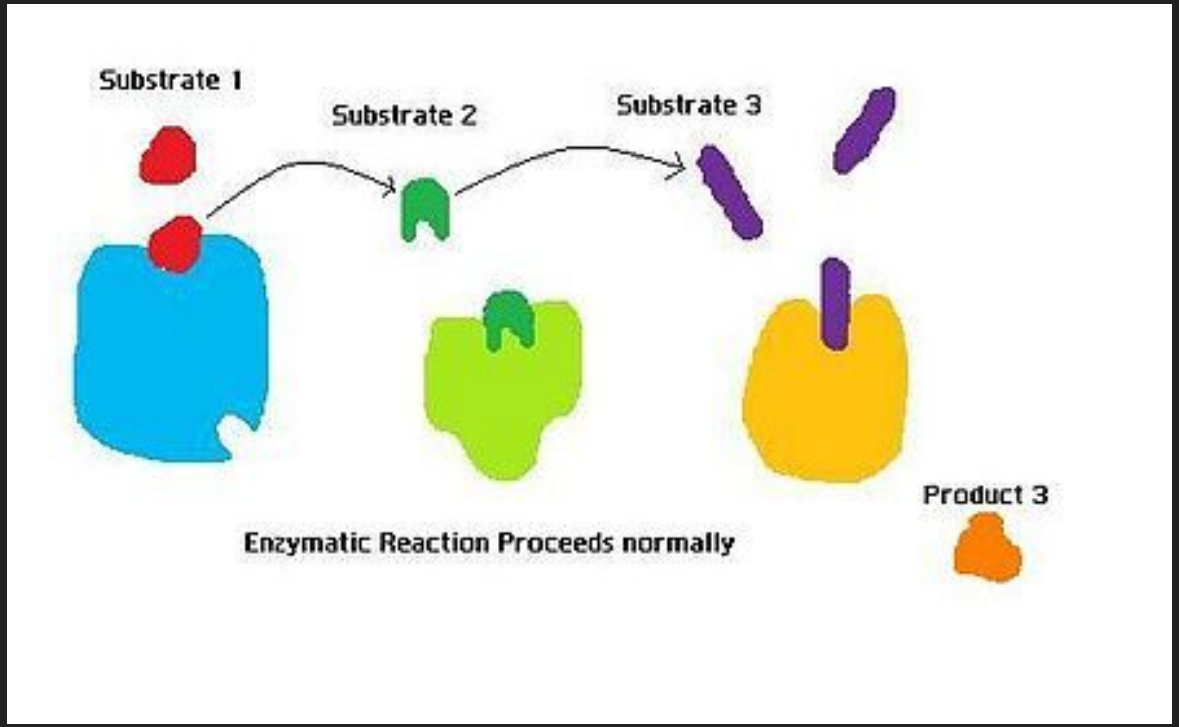


Enzymes lower activation energy by promoting chemical changes in small steps.

8.1U1 Metabolic pathways consist of chains and cycles of enzyme-catalysed reaction

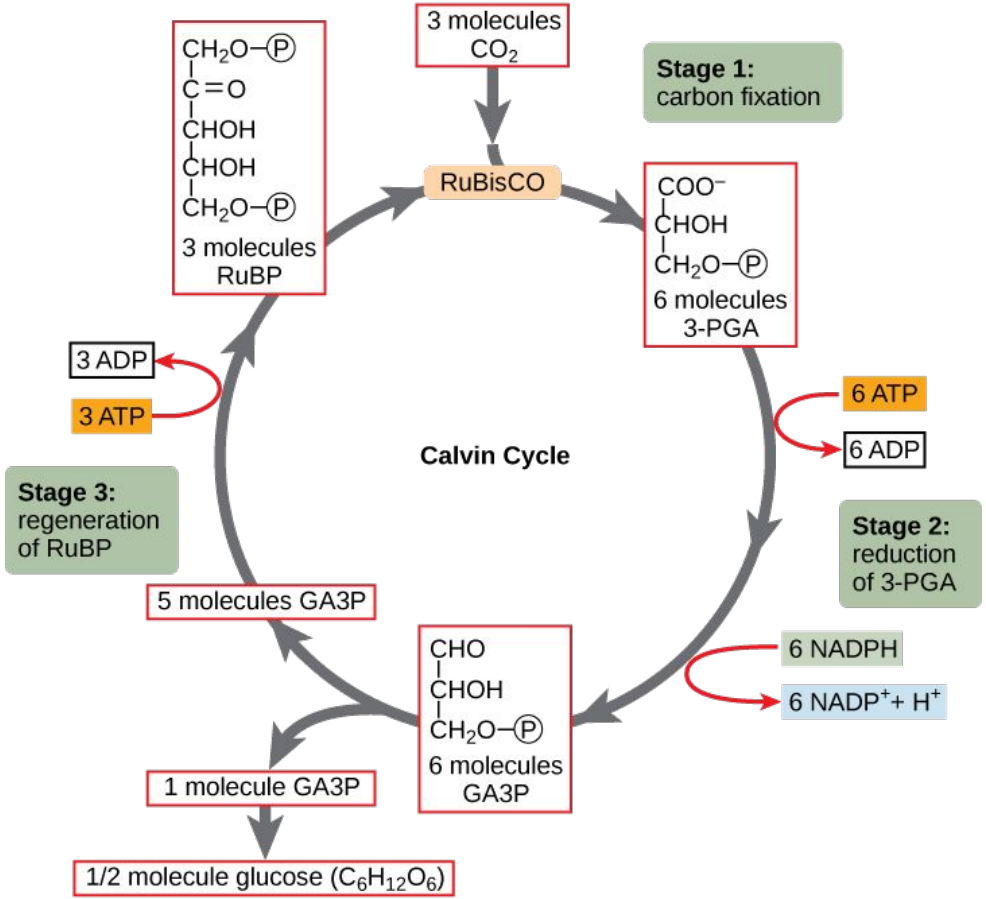
Chemical reactions can't occur in one jump

Products of first reaction are substrates in next reaction



Occurs in cycles or chains

Products of first reaction are substrates in next reaction



8.1U4 Metabolic pathways can be controlled by end-product inhibition

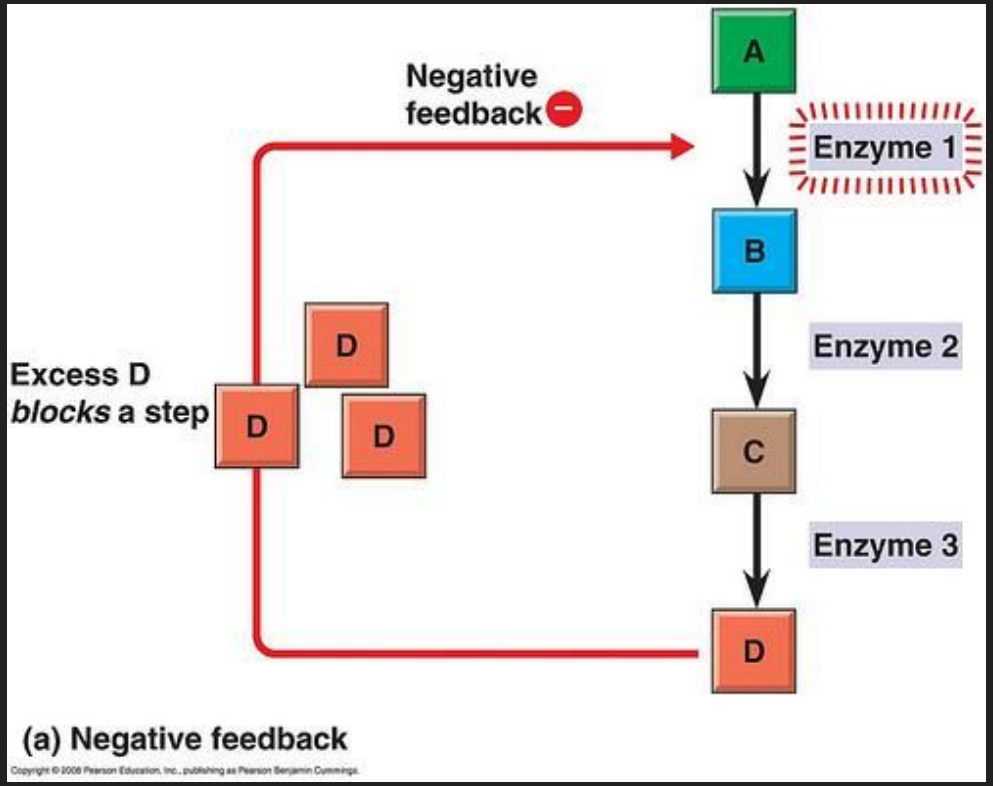
Product of chain reaction acts as inhibitor to first enzyme in reaction chain

Product binds to allosteric site (not on active site)

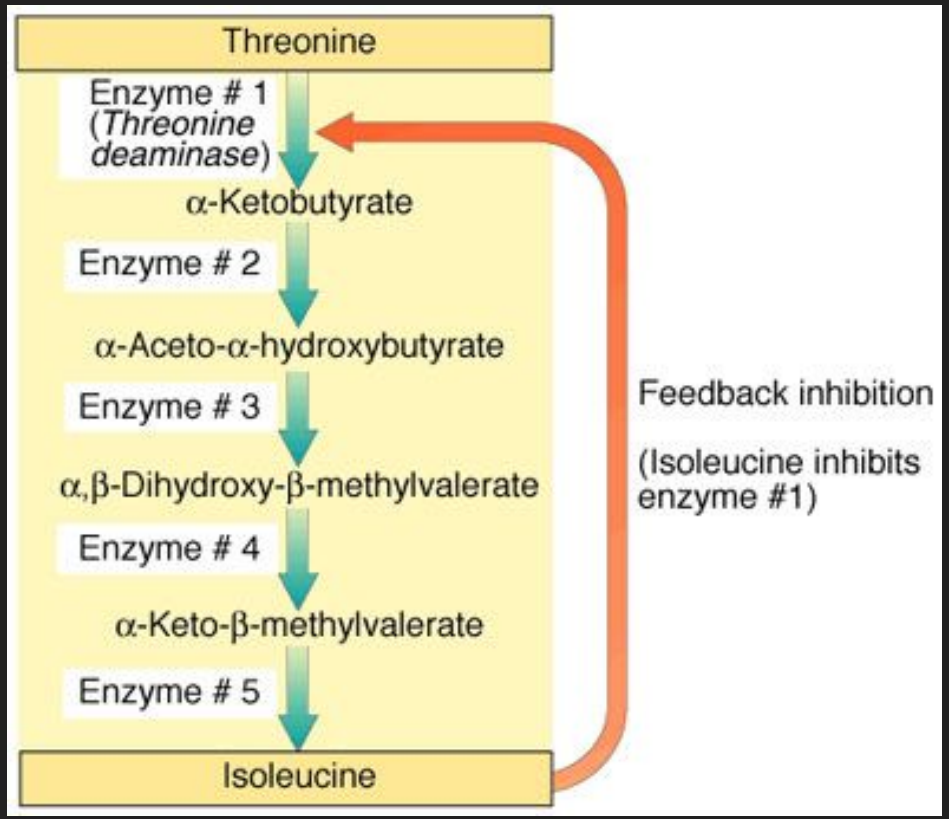
Prevents reaction from starting again

Useful to regulate homeostasis

http://highered.mheducation.com/sites/0072943696/student_view0/chapter2/animation__feedback_inhibition_of_biochemical_pathways.html



8.1A1: End-product inhibition of the pathway that converts threonine to isoleucine



Threonine (amino acid) enters a chain reaction of enzymes

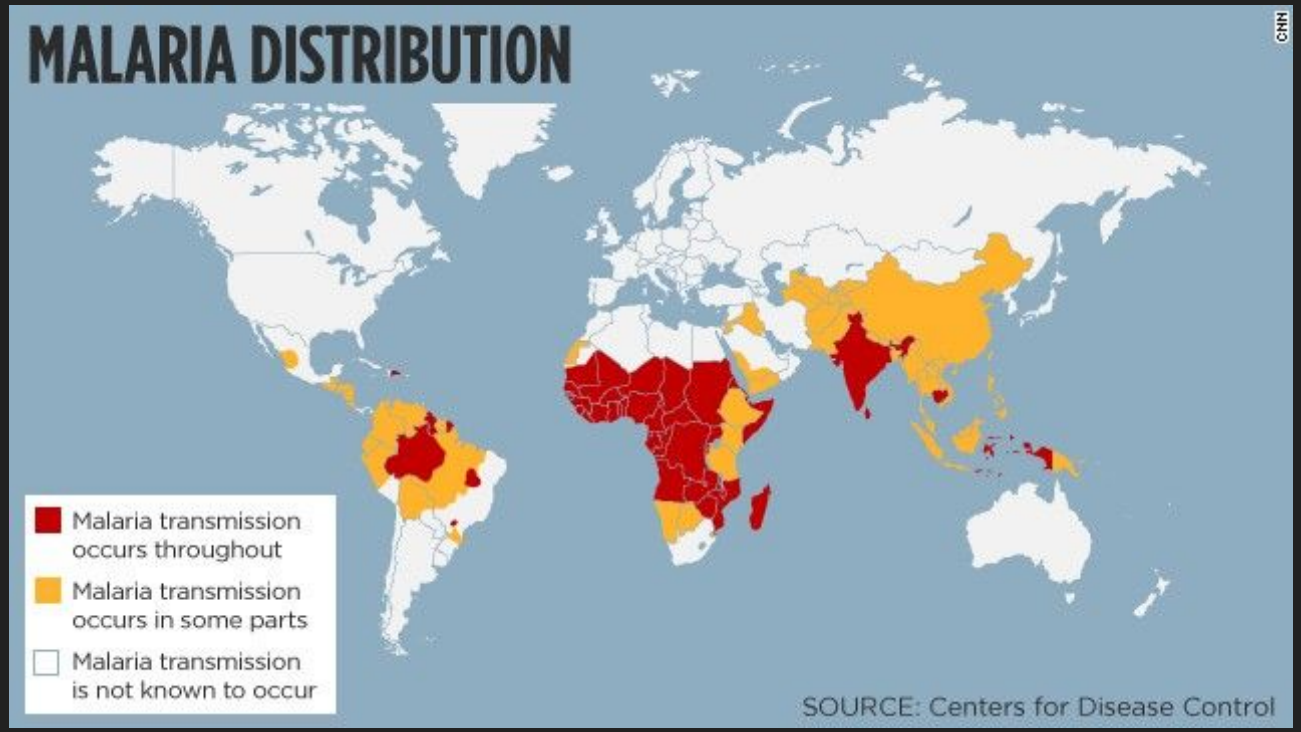
Product (Isoleucine) acts as inhibitor

8.1A2: Use of databases to identify potential new antimalarial drugs

Malaria is caused by mosquitoes that transmit a protozoan into your blood.

Attacks red blood cells

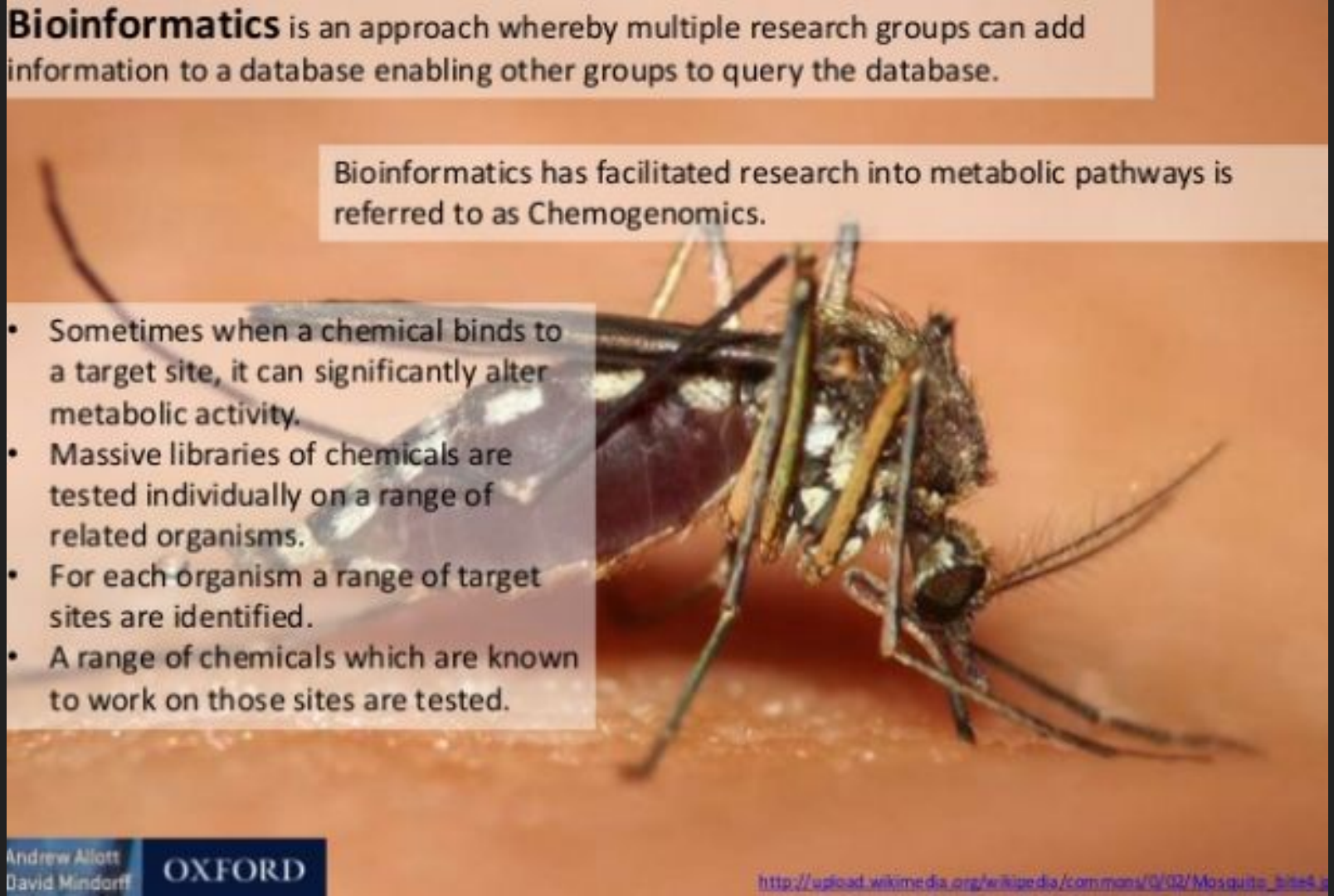
Often fatal



Bioinformatics is an approach whereby multiple research groups can add information to a database enabling other groups to query the database.

Bioinformatics has facilitated research into metabolic pathways is referred to as Chemogenomics.

- Sometimes when a chemical binds to a target site, it can significantly alter metabolic activity.
- Massive libraries of chemicals are tested individually on a range of related organisms.
- For each organism a range of target sites are identified.
- A range of chemicals which are known to work on those sites are tested.



Andrew Allott
David Mindorff
OXFORD

Increasing drug resistance to anti-malarial drugs has lead to the use of Bioinformatics and Chemogenomics to try and identify new drugs.

- In one study, approx. 300,000 chemicals were screened against a chloroquine-sensitive 3D7 strain and the chloroquine-resistant K1 strain of P. falciparum.
- Other related and unrelated organisms, including human cell lines, were also screened.
- (19) new chemicals that inhibit the enzymes normally targeted by anti-malarial drugs were identified
- Additionally (15) chemicals that bind to malarial proteins were identified - this can help in the location of P. falciparum
- These results indicate possible new directions for drug research.

As bioinformatics involves the use of huge databases this approach only became practical with the development of modern computing and the internet.

Andrew Alloti
David Mindorff
OXFORD

Can you explain this graph?