ENZYME INHIBITION
Competitive
and
Non-competitive

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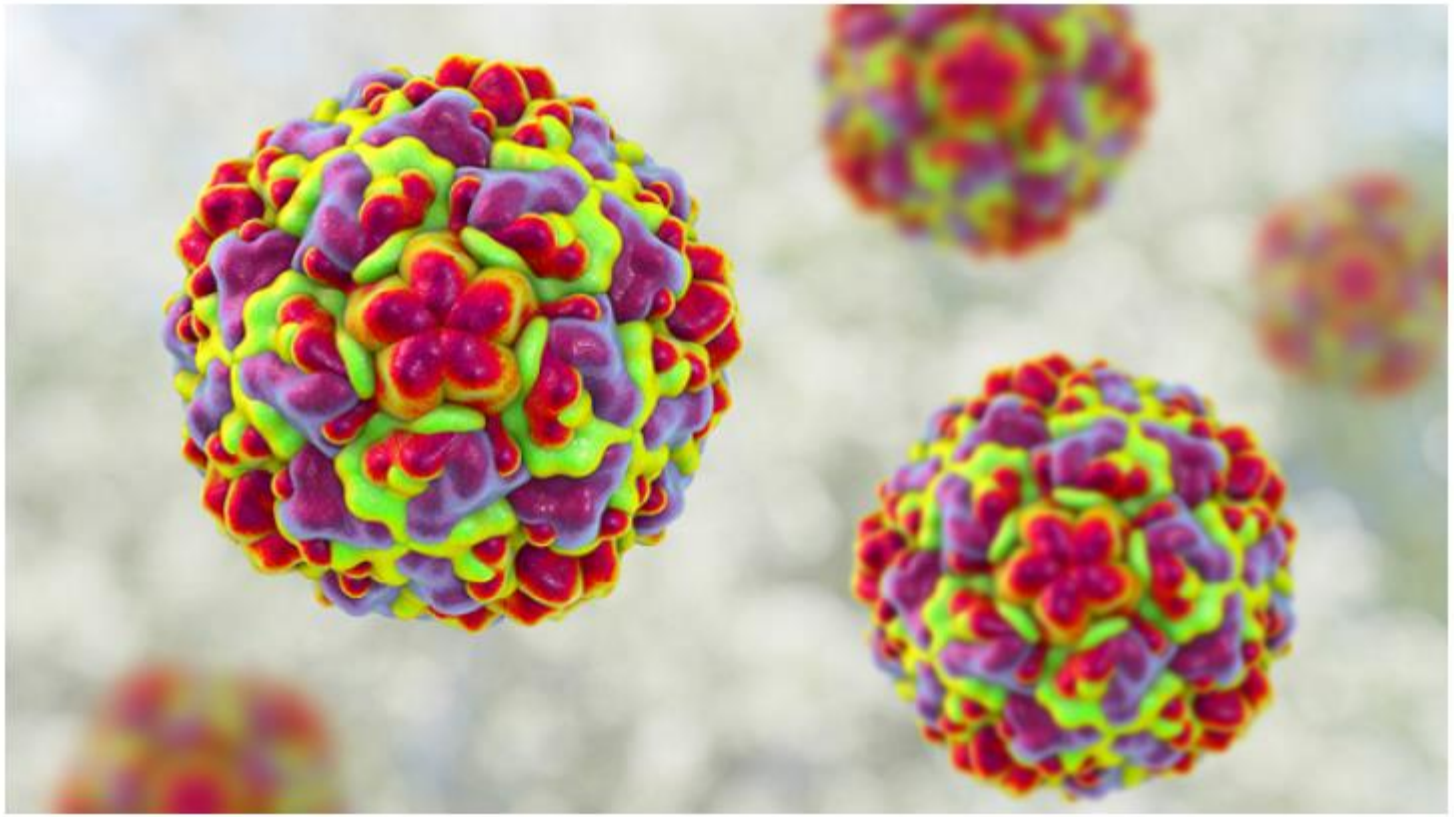
TheScientist
EXPLORING LIFE, INSPIRING INNOVATION

Researchers Develop a Drug Against the Common Cold

In an in vitro study, the compound completely blocked the replication of rhinoviruses.

Catherine Offord
May 15, 2018



ISTOCK, DR_MICROBE

Researchers have developed a compound that blocks the replication of rhinoviruses, the pathogens behind most cases of the common cold. The in vitro study, published yesterday (May 14) in Nature Chemistry, could provide the first step to developing a treatment for a condition that affects the average adult two to three times a year.

“The common cold is an inconvenience for most of us, but can cause serious complications in people with conditions like asthma and [chronic obstructive pulmonary disease],” study coauthor Ed Tate of Imperial College London says in a statement. “A drug like this could be extremely beneficial if given early in infection.”
Despite being so widespread, the common cold has consistently eluded effective medical treatment, both because of the vast number of viruses that cause it—more than 150 strains of rhinovirus infect humans—and because these pathogens are particularly fast-evolving.

Instead of trying to target the viruses themselves, a team led by researchers at Imperial focused its sights on a human enzyme that the viruses hijack to assemble their protein coats after invading cells. Blocking this enzyme with a small-molecule inhibitor, the team found, completely prevented the protein coat from being formed, and halted viral replication without killing cells.

“Because the inhibitor targets proteins that are common to most types of rhinovirus, it would likely have a broad range of activity,” Peter Barlow, an immunologist and spokesperson for the British Society for Immunology who was not involved in the work, tells Newsweek. “While this study was conducted entirely in vitro . . . it shows great promise in terms of eventually developing a drug treatment to combat the effects of this virus in patients.”

Speaking to The Guardian, study coauthor Roberto Solari of Imperial emphasizes that much more research is needed for this to become a reality. “We haven’t done any animal studies, and we obviously haven’t done any studies in humans, so I can’t tell you formally what the animal toxicity of this compound is,” he says. “There is a still a long way before this becomes a medicine.”

Science News *from research organizations*

A near-universal way to measure enzyme inhibition

Technique could offer new approach to drug discovery

Date: March 1, 2018

Source: McGill University

Summary: Researchers have invented a new technique for measuring how quickly drugs interact with their molecular targets. The discovery provides scientists with a new way to investigate the effectiveness of drug candidates that might otherwise have been overlooked.

Researchers at McGill University have invented a new technique for measuring how quickly drugs interact with their molecular targets. The discovery provides scientists with a new way to investigate the effectiveness of drug candidates that might otherwise have been overlooked.

The new method centres on the principle of enzyme inhibition. Countless pharmaceuticals, ranging from antibiotics to chemotherapy drugs, work by blocking the action of enzymes, and the search for new enzyme-inhibiting substances remains a major focus of drug development.

In a paper published in *Nature Communications*, the McGill team, led by chemistry professors Nicolas Moitessier and Anthony Mittermaier, demonstrate the use of isothermal titration calorimetry (ITC) to measure the heat generated by enzyme activity and thereby the rates at which inhibitor substances blocked that activity.

"One key difference between ITC and other methods is that ITC measures the rate of reaction directly," Mittermaier explains.

Existing methods for measuring enzyme activity look at that activity indirectly, by measuring changes in concentration caused by enzymatic catalysis as a function of time. These measurements often depend on special reagents that change colour or fluorescence when acted on by the enzyme, and require a unique test to be developed for each enzyme being studied.

Because ITC measures the production of heat - a near-universal feature of chemical reactions - it can be applied to just about any enzyme.

"ITC is as close as you can get to a universal enzyme test," Mittermaier says.

In addition to its generality, the ITC method gives a direct read-out of enzyme activity because it detects heat flow in real time. By providing a direct window on the reaction, ITC offers researchers a better insight into the mechanisms by which enzyme inhibition proceeds. It is usually very challenging, and sometimes next to impossible, to obtain this information from conventional assays.

The real-time nature of ITC is particularly promising for researchers investigating covalent inhibitors. These strongly binding molecules have potential as long-acting drugs but had previously fallen out of favour in drug development due to toxicity concerns. The insight ITC offers into the relationship between an inhibitor's molecular structure and how it reacts with its target will support renewed interest in covalent inhibitors and facilitate the work of developing them into drugs that are both highly effective and safe.

Inhibitors

- Inhibitors are chemicals that reduce the rate of enzymic reactions
- Usually specific and work at low concentrations
- Block the enzyme but they do not usually destroy it
- Many drugs and poisons are inhibitors of enzymes in the nervous system.

The effect of enzyme inhibition

- **Irreversible inhibitors:**
  Combine with the functional groups of the amino acids in the active site, irreversibly

  Examples: nerve gases and pesticides, containing organophosphorus, combine with serine residues in the enzyme acetylcholine esterase.

The effect of enzyme inhibition

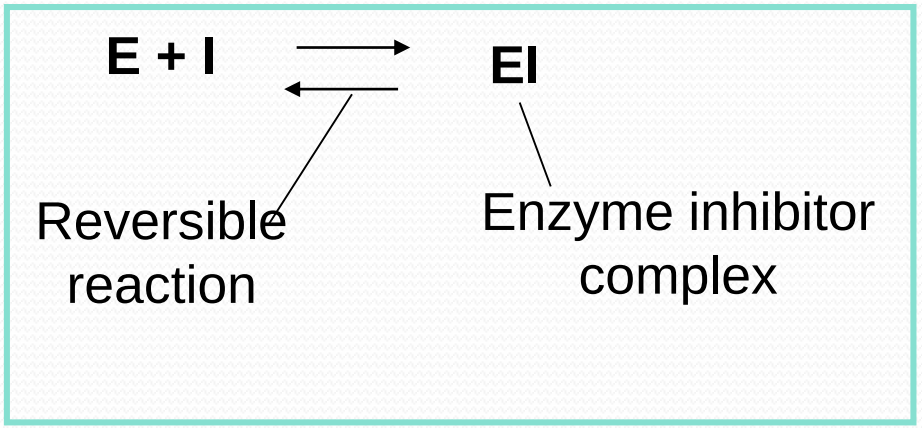
- **Reversible inhibitors:** Can be washed out of the solution of enzyme by dialysis.

The effect of enzyme inhibition

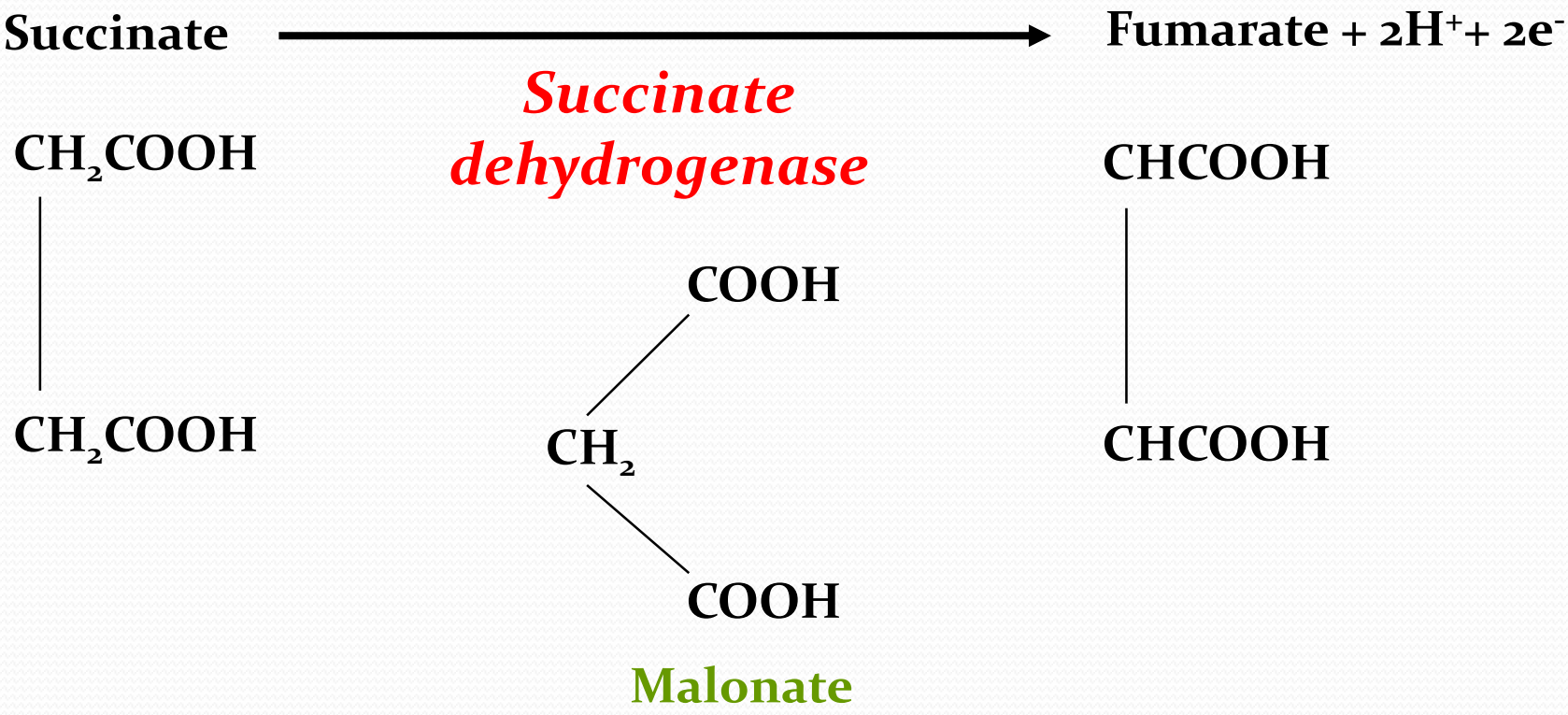
1. **Competitive:** These compete with the substrate molecules for the active site

The inhibitor's action is proportional to its concentration

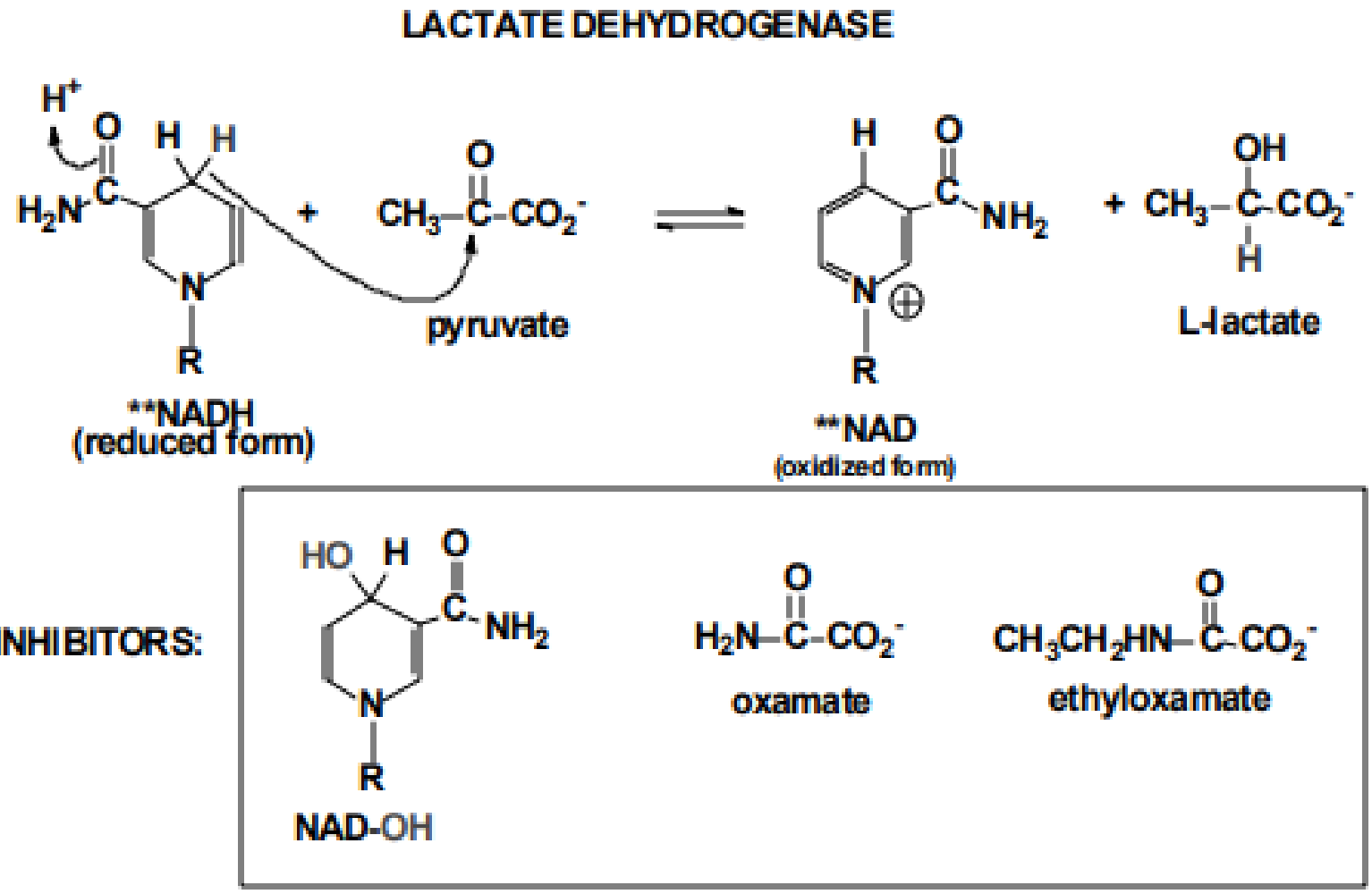
Resembles the substrate's structure closely.



The effect of enzyme inhibition

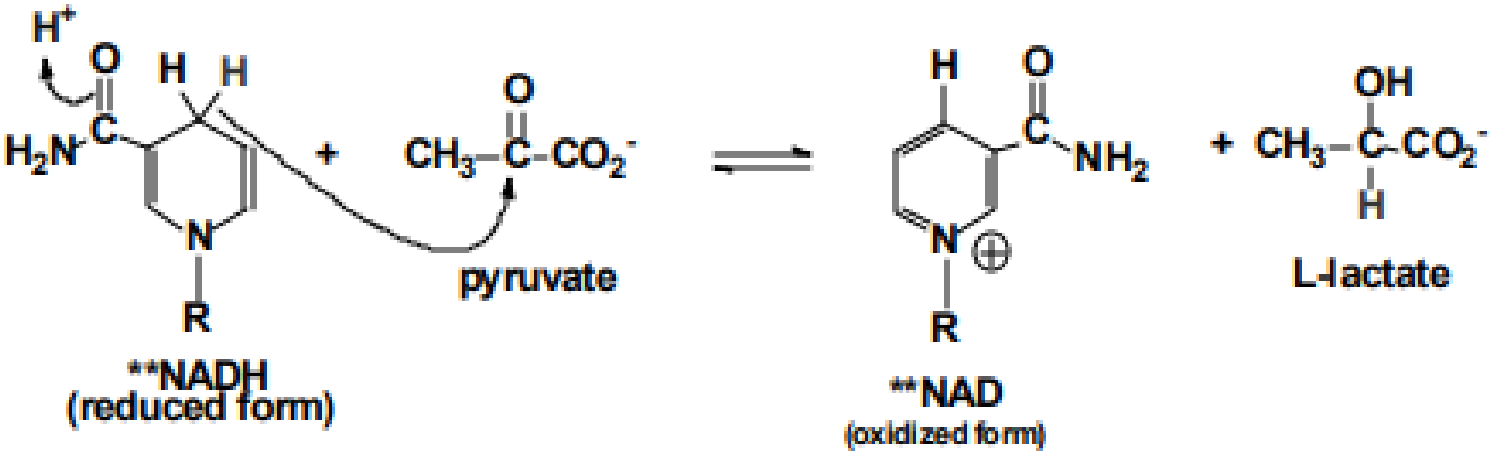


Examples: Competitive and Noncompetitive Inhibition



For multisubstrate rxns, the type of inhibition depends upon the substrate that is varied in the inhibition experiment!

LACTATE DEHYDROGENASE



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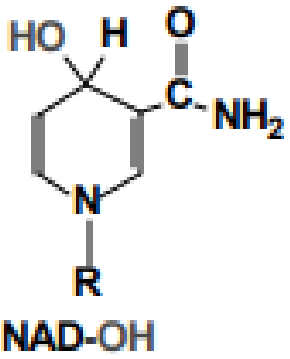


What substrate does this inhibitor resemble?

NADH

The effect of enzyme inhibition

2. **Non-competitive:** Not influenced by the concentration of the substrate.
   Inhibits by binding irreversibly to the enzyme but **not at the active site**

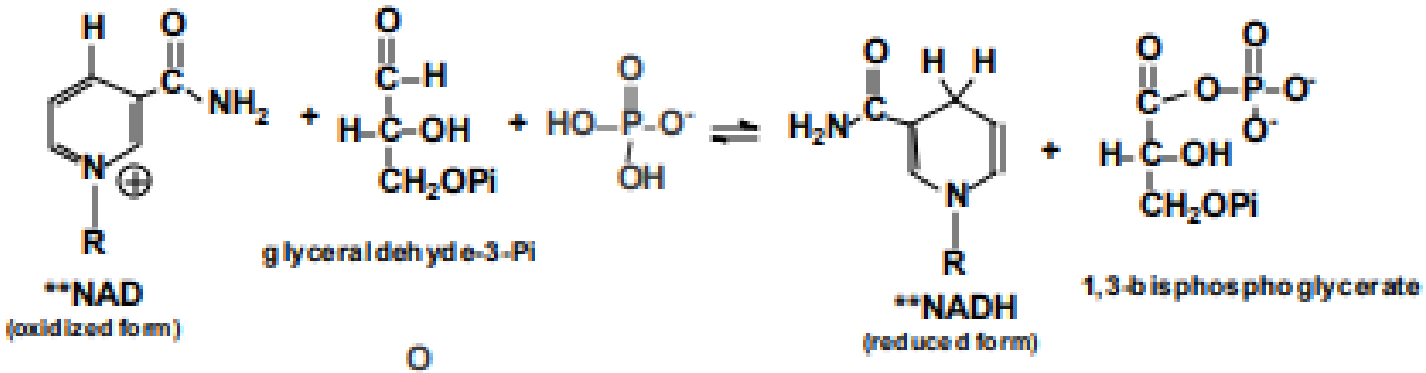
   Examples

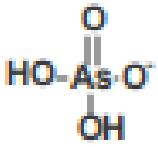
- **Cyanide** combines with the iron in the enzymes cytochrome oxidase
- Heavy metals, **Ag** or **Hg**, combine with **–SH** groups. These can be removed by using a chelating agent such as EDTA.

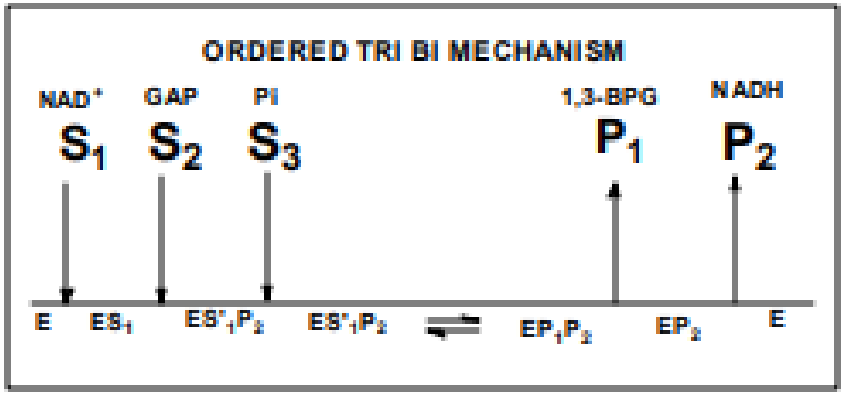
Example: Uncompetitive Inhibition

This type of inhibition requires that one or more substrates bind to E before the inhibitor can bind

GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE



INHIBITOR: 

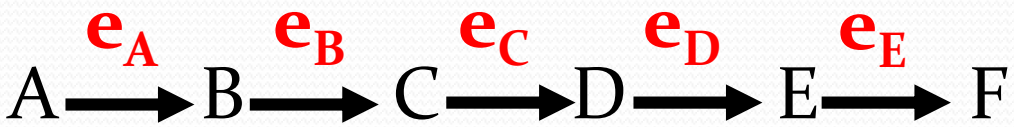


Applications of inhibitors

- **Negative feedback**: end point or end product inhibition
- **Poisons** snake bite, plant alkaloids and nerve gases
- **Medicine** antibiotics, sulphonamides, sedatives and stimulants.

Enzyme pathways

Cell processes (e.g. respiration or photosynthesis) consist of series of pathways controlled by enzymes

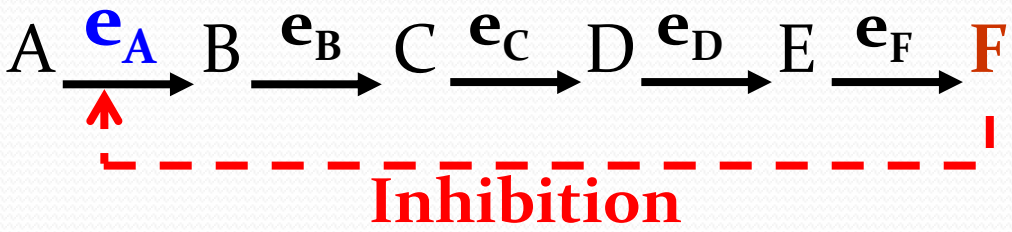


Each step is controlled by a different enzyme ($\mathbf{e_A}$, $\mathbf{e_B}$, $\mathbf{e_C}$ etc)

Possible because of enzyme specificity.

End point inhibition

- The first step (controlled by e_A) is often controlled by the end product (**F**)
- Therefore **negative feedback** is possible



- The end products are controlling their own rate of production
- There is no build up of intermediates (B, C, D and E).

Example: Phosphofructokinase and ATP

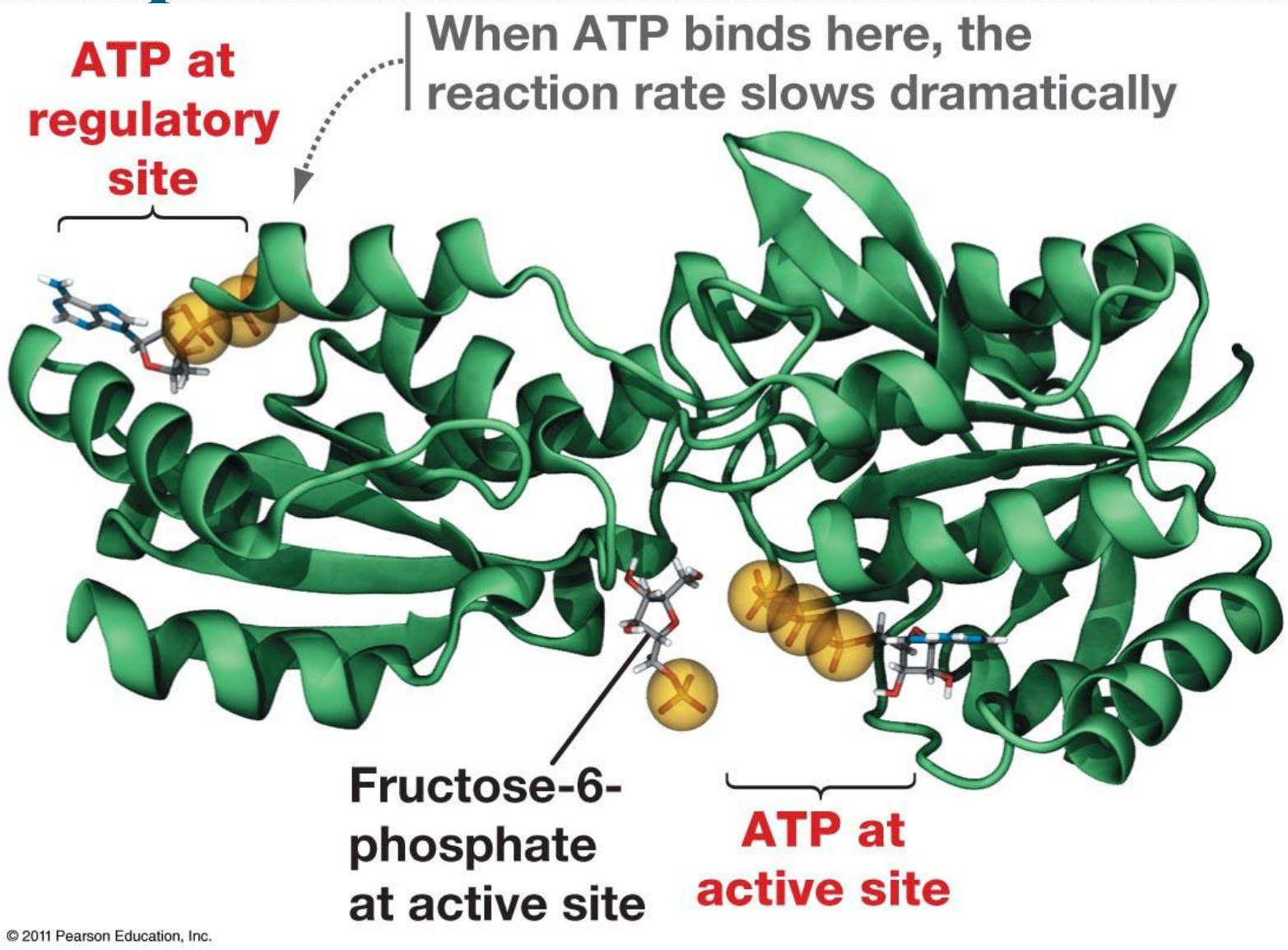
Substrate: Fructose-6-phosphate

Reaction

fructose-6-phosphate + ATP → fructose-1,6-bisphosphate + ADP

Phosphofructokinase

Phosphofructokinase

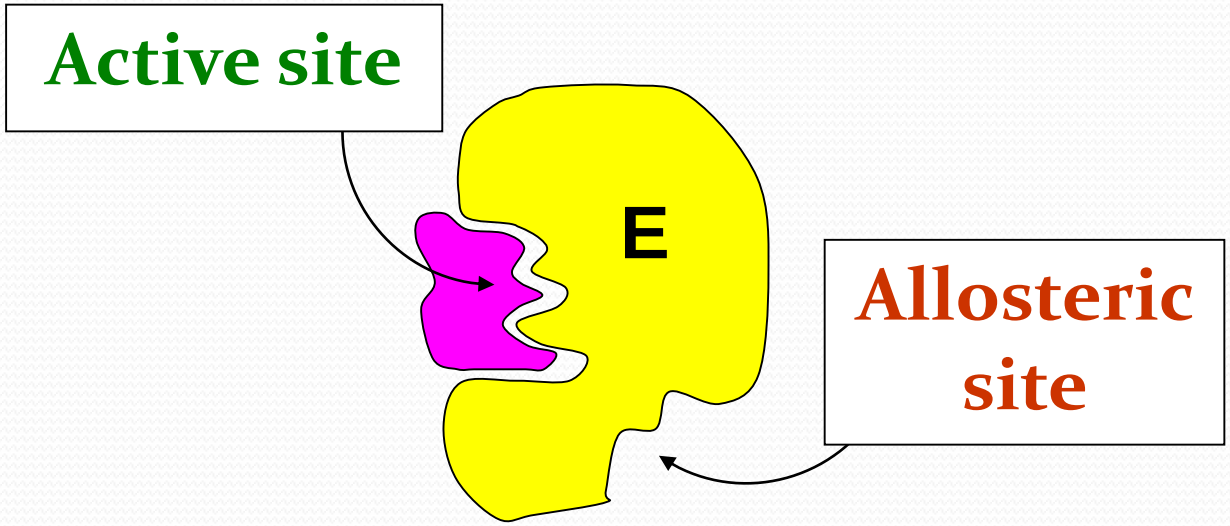


ATP is the end point

- This reaction near the beginning of the respiration pathway in cells
- The **end product** of respiration is **ATP**
- **If there is a lot of ATP** in the cell this enzyme is inhibited
- Respiration slows down and less ATP is produced
- As ATP is used up the inhibition stops and the reaction speeds up again.

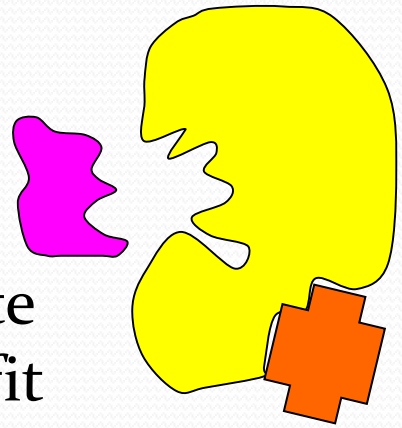
The switch: Allosteric inhibition

Allosteric means "**other site**"



Switching off

- These enzymes have **two receptor sites**
- One site fits the substrate like other enzymes
- The other site fits an inhibitor molecule.

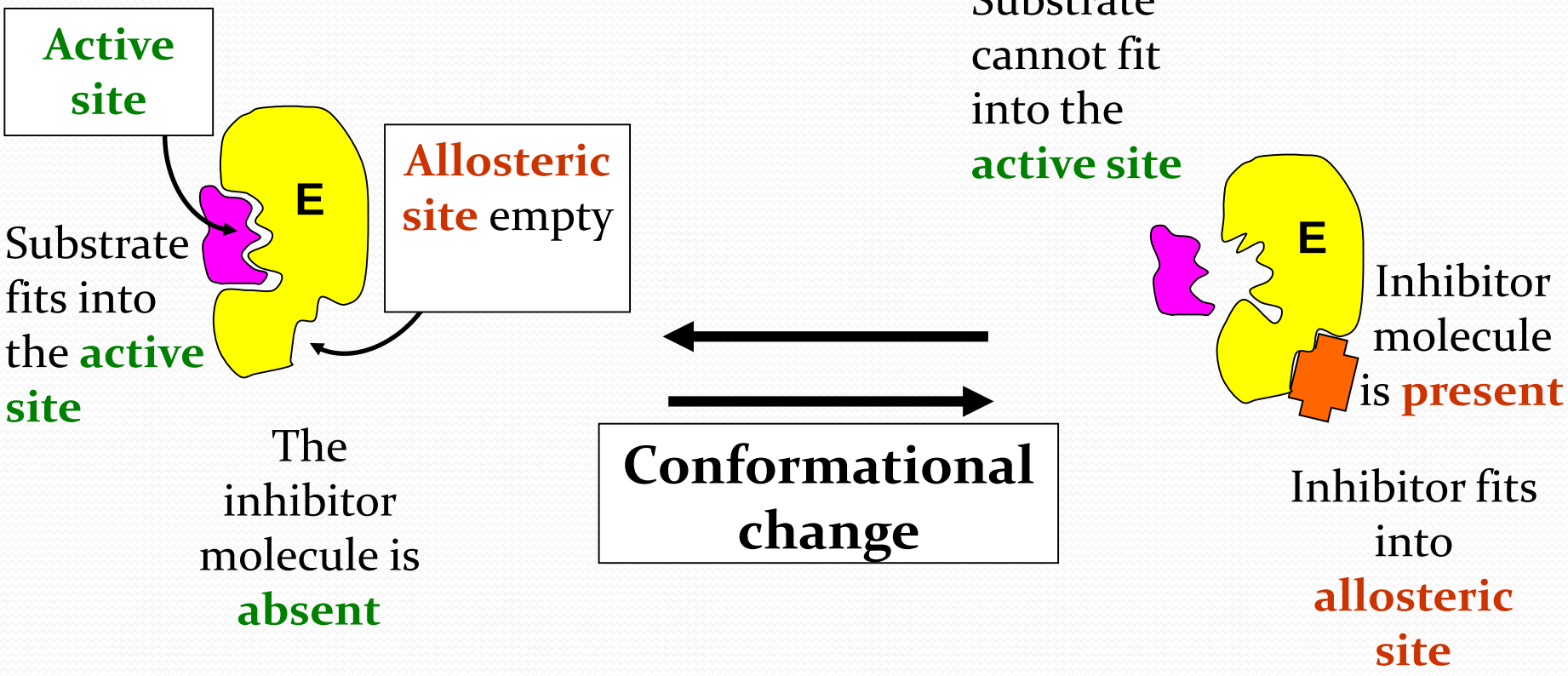


Inhibitor molecule

Substrate cannot fit into the **active site**

Inhibitor fits into **allosteric site**

This allosteric site switches the enzyme on and off



A change in shape

- Inhibitor is present
  Fits into its site
  Conformational change in the enzyme molecule
- The enzyme's molecular shape changes
- The **active site** of the substrate changes
- The substrate cannot bind with the enzyme.

Negative feedback is achieved

- The reaction slows down
- **Not** competitive inhibition but it is reversible
- Inhibitor concentration diminishes
  Enzyme's conformation changes back to its active form
- The reaction speeds up again.

Summary

Competitive	Non-competitive
Inhibits the enzyme activity	Inhibits the enzyme activity
Increasing the inhibitor concentration increases the effect	Changing the concentration of inhibitor does not influence the effect
Inhibitor competes with the substrate at the active site (similar molecule)	Inhibitor does not bind at the active site
Active site stays the same	Active site may change shape (e.g. allosteric inhibition)