Enzymes

Properties

- Nearly all enzymes are proteins, although a few catalytically active RNA molecules have been identified.
- Enzyme catalyzed reactions usually take place under relatively mild conditions (temperatures well below $100^{o}C$, atmospheric pressure and neutral pH) as compared with the corresponding chemical reactions.
- Enzymes are catalysts that increase the rate of a chemical reaction without being changed themselves in the process.

Properties

- Enzymes are highly specific with respect to the substrates on which they act and the products that they form.
- Enzyme activity can be regulated, varying in response to the concentration of substrates or other molecules.
- They function under strict conditions of temperature and pH in the body.

Coenzymes and Prosthetic Groups

- Many enzymes require the presence of small, non-protein units or cofactors to carry out their particular reaction.
- Cofactors may be either one or more inorganic ions, such as Zn^{2+} or Fe^{2+} or a complex organic molecule called a coenzyme.
- A metal or coenzyme that is covalently attached to the enzyme is called a prosthetic group (heme in hemoglobin).
- Some coenzymes, such as NAD^{+}, are bound and released by the enzyme during its catalytic cycle and in effect function as co-substrates. Many coenzymes are derived from vitamin precursors.

Holo enzyme and Apo enzyme

- A complete catalytically-active enzyme together with its coenzyme or metal ion is called a holo enzyme.

- The protein part of the enzyme on its own without its cofactor is termed an apo enzyme.

Isoenzymes

- Isoenzymes are different forms of an enzyme which catalyze the same reaction, but which exhibit different physical or kinetic properties, such as isoelectric point, pH optimum, substrate affinity or effect of inhibitors.
- Different isoenzyme forms of a given enzyme are usually derived from different genes and often occur in different tissues of the body.

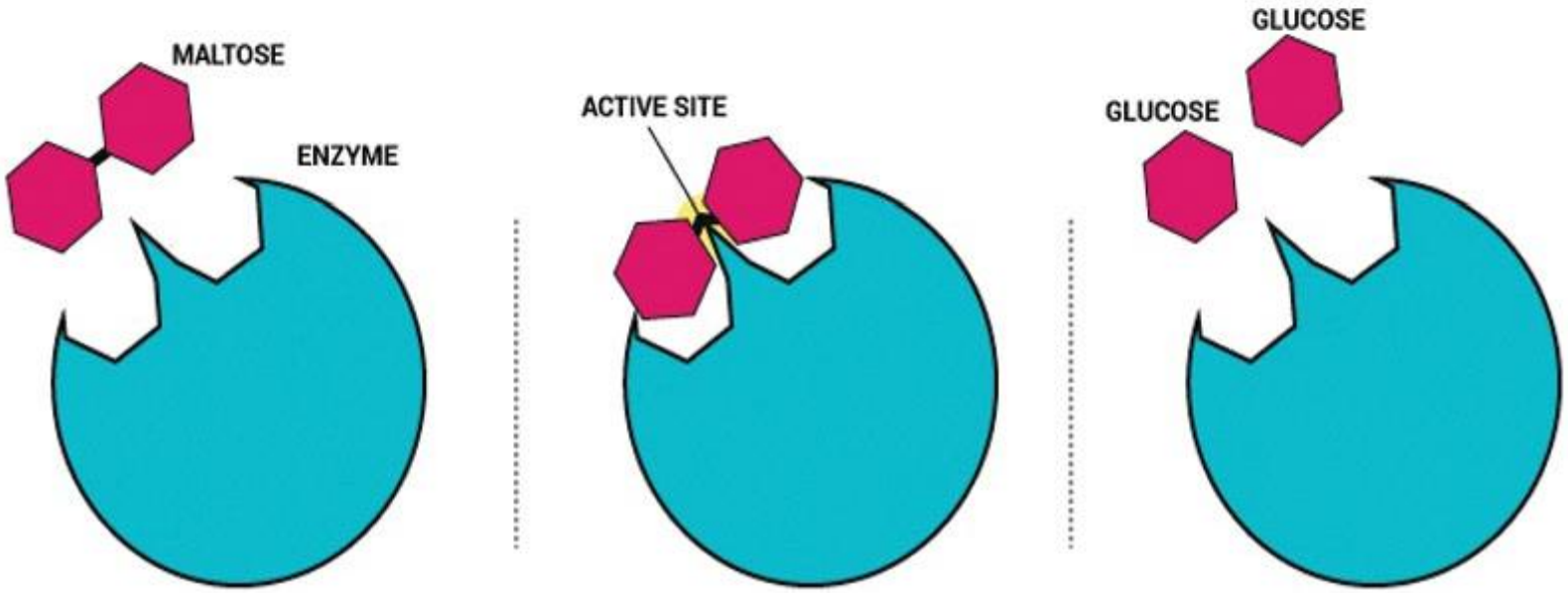
Isoenzymes

- An example of an enzyme which has different isoenzyme forms is lactate dehydrogenase (LDH) which catalyzes the reversible conversion of pyruvate into lactate in the presence of the coenzyme NADH.
- LDH is a tetramer of two different types of subunits, called H and M, which have small differences in amino acid sequence. Two subunits can combine randomly with each other, forming five isoenzymes that have the compositions H_4, H_3M, H_2M_2, HM_3 and M_4. The five isoenzymes can be resolved electrophoretically.

Active sites

- The active site of an enzyme is the region that binds the substrate and converts it into product.
- It is usually a relatively small part of whole enzyme molecule and is a three-dimensional entity formed by amino acid residues that can lie far apart in the linear polypeptide chain.
- The active site is often a cleft or crevice on the surface of the enzyme that forms a predominantly nonpolar environment which enhances the binding of the substrate.
- The substrate(s) is bound in the active site by multiple weak forces (electrostatic interactions, hydrogen bonds, van der Waals bonds, hydrophobic interactions; and in some cases by reversible covalent bonds.

ENZYMES



Nomenclature

- Many enzymes are named by adding the suffix ‘-ase’ to the name of their substrate.
- Example. Urease is the enzyme that catalyzes the hydrolysis of urea, and fructose-1,6-bisphosphatase hydrolyzes fructose-1,6-bisphosphate.
- However, other enzymes, such as trypsin and chymotrypsin, have names that do not denote their substrate.
- Some enzymes have several alternative names.
- To rationalize enzyme names, a system of enzyme nomenclature has been internationally agreed.
- International Union of Biochemistry and Molecular Biology (IUBMB)

Nomenclature

- This system places all enzymes into one of six major classes based on the type of reaction catalyzed. Each enzyme is then uniquely identified with a four-digit classification number.
- Example: Trypsin has the Enzyme Commission (EC) number 3.4.21.4, where

the first number (3) denotes that it is a hydrolase

the second number (4) that it is a protease that hydrolyzes peptide bonds

the third number (21) that it is a serine protease with a critical serine residue at the active site, and

the fourth number (4) indicates that it was the fourth enzyme to be assigned to this class.

- For comparison, chymotrypsin has the EC number 3.4.21.1, and elastase 3.4.21.36.

Classification

Oxidoreductases

- Catalyze oxidation-reduction reactions where electrons are transferred.
- These electrons are usually in the form of hydride ions or hydrogen atoms.
- The most common name used is a dehydrogenase and sometimes reductase is used.
- An oxidase is referred to when the oxygen atom is the acceptor.

Transferases

- Catalyze group transfer reactions.
- The transfer occurs from one molecule that will be the donor to another molecule that will be the acceptor.
- Most of the time, the donor is a cofactor that is charged with the group about to be transferred.
- Example: Hexokinase used in glycolysis.

Classification

Hydrolases

- Catalyze reactions that involve hydrolysis.
- It usually involves the transfer of functional groups to water.
- When the hydrolase acts on amide, glycosyl, peptide, ester, or other bonds, they not only catalyze the hydrolytic removal of a group from the substrate but also a transfer of the group to an acceptor compound
- For example: Chymotrypsin.

Lyases

- Catalyze reactions where functional groups are added to break double bonds in molecules or the reverse where double bonds are formed by the removal of functional groups.
- For example: Fructose bisphosphate aldolase used in converting fructose 1,6-bisphospate to G3P and DHAP by cutting C-C bond.

Classification

Isomerases

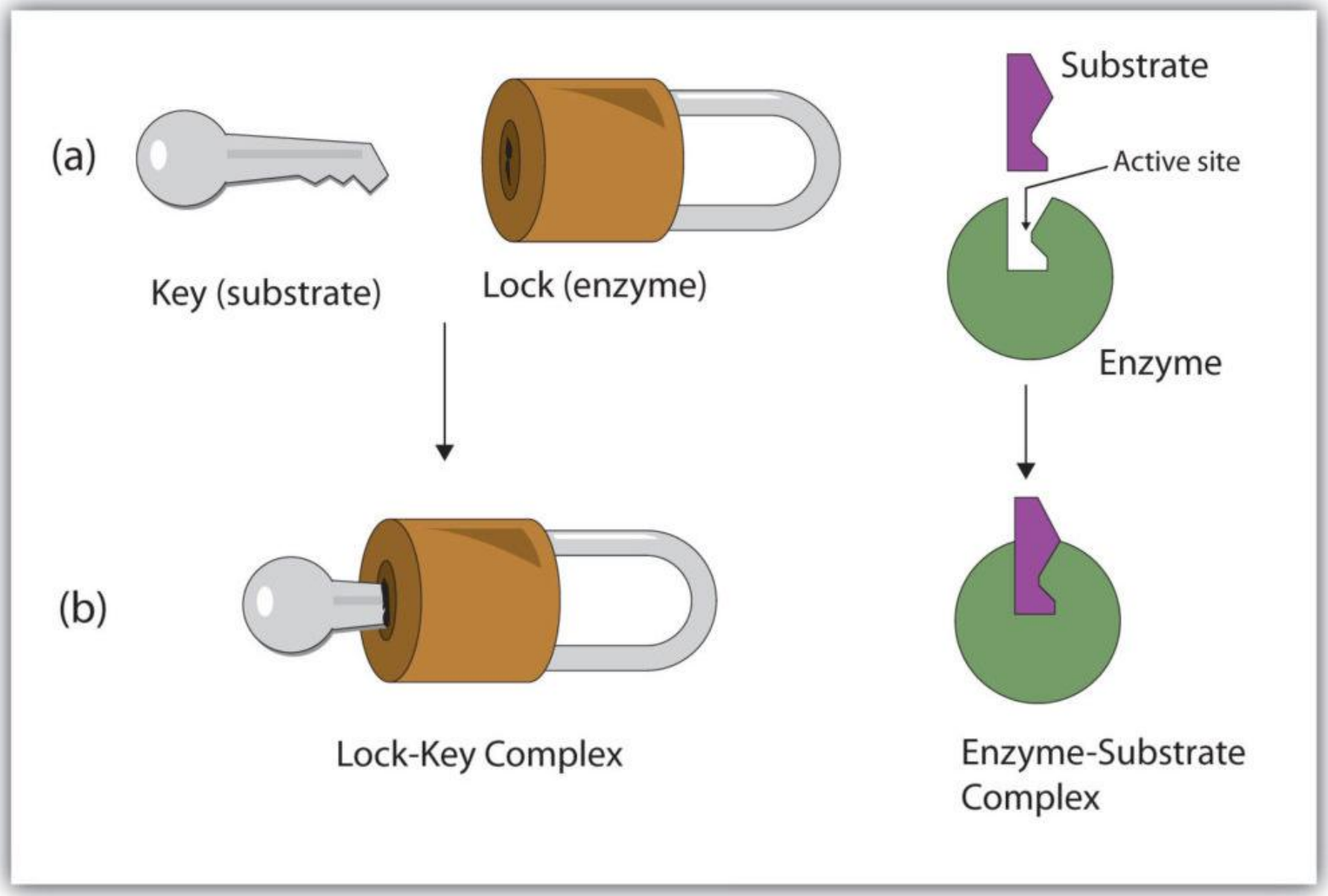
- Catalyze reactions that transfer functional groups within a molecule so that isomeric forms are produced.
- These enzymes allow for structural or geometric changes within a compound.
- For example: phosphoglucose isomerase for converting glucose 6-phosphate to fructose 6-phosphate. Moving chemical group inside same substrate.

Ligases

- They are involved in catalysis where two substrates are ligated and the formation of carbon-carbon, carbon-sulfide, carbon-nitrogen, and carbon-oxygen bonds due to condensation reactions.
- These reactions are coupled to the cleavage of ATP.

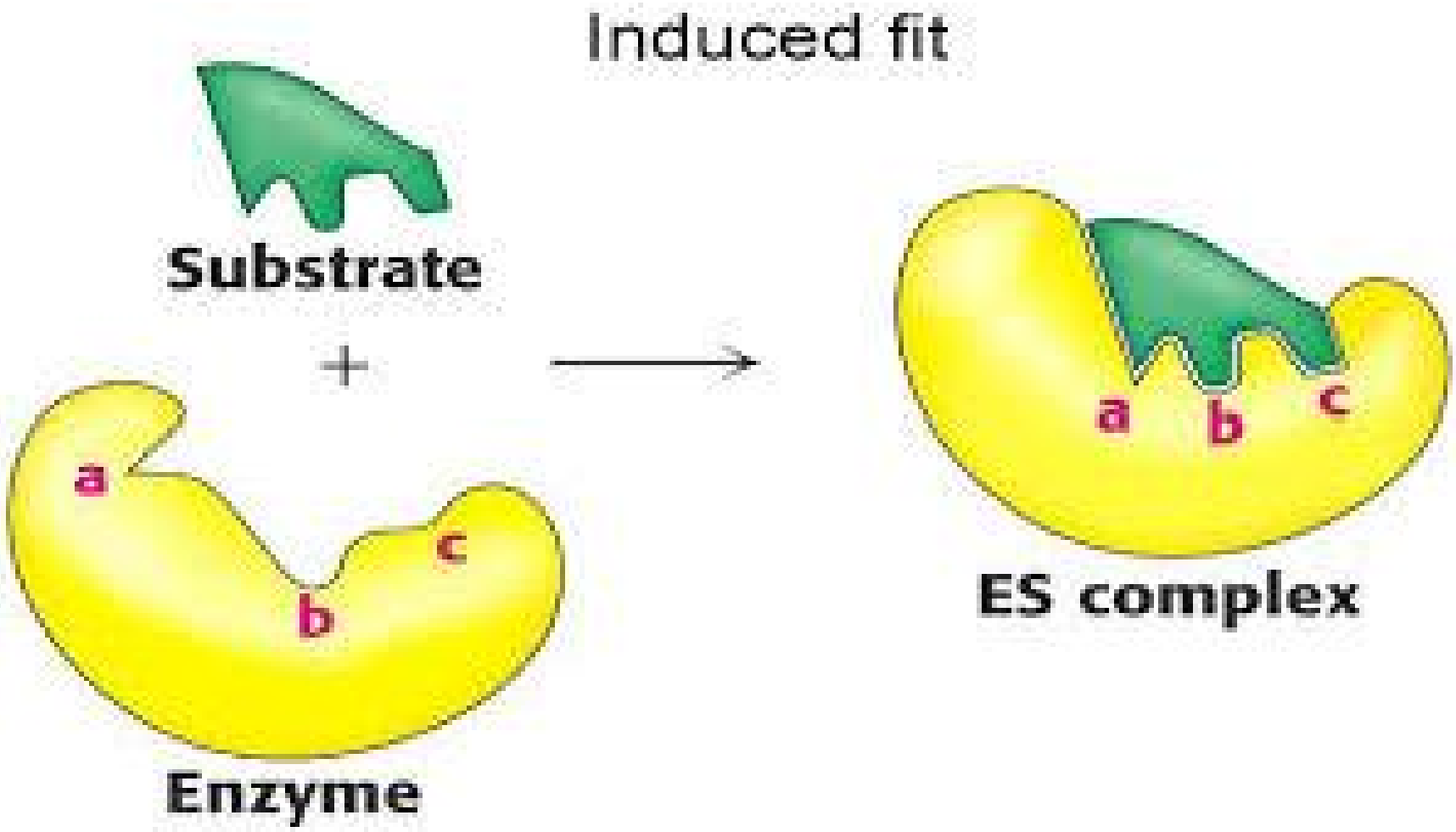
Substrate Enzyme binding models

- **The Lock and Key Model**
- In the lock-and-key model proposed was proposed by Emil Fischer in 1894.
- According to the model, the shape of the substrate and the active site of the enzyme are thought to fit together like a key into its lock.
- The two shapes are considered as rigid and fixed, and perfectly complement each other when brought together in the right alignment.


(a)
Key (substrate)
Lock (enzyme)
Substrate
Active site
Enzyme
(b)
Lock-Key Complex
Enzyme-Substrate Complex

Substrate Enzyme binding models

- **The Induced Fit Model**
- In the induced-fit model was proposed by Daniel E. Koshland, Jr., in 1958.
- It states that the binding of substrate induces a conformational change in the active site of the enzyme.
- In addition, the enzyme may distort the substrate, forcing it into a conformation similar to that of the transition state.
- For example, the binding of glucose to hexokinase induces a conformational change in the structure of the enzyme such that the active site assumes a shape that is complementary to the substrate (glucose) only after it has bound to the enzyme.


Induced fit
Substrate
+
a
b
c
Enzyme
a
b
c
ES complex

For the enzyme catalyzed reaction:

E + S --k_1--> ES complex --kcat--> E + P

..... <--k_{-1}--

$V_0 = k_{cat}\,[ES]$. (2)

$[ES] = [E]_{total}$.
So $V_{max} = k_{cat}\,[E]_{total}$. (3)

Rate of formation of ES = $k_1[E][S]$.
Rate of consumption of ES = $k_{-1}[ES] + k_{cat}\,[ES]$.
So in the steady state, $k_{-1}[ES] + k_{cat}\,[ES] = k_1[E][S]$. (4)

$(k_{-1} + k_{cat})\,[ES] = k_1[E][S]$,
and $(k_{-1} + k_{cat})/k_1 = [E][S]/[ES]$. (5)

To simplify (5), first group the kinetic constants by defining them as K_m :

$K_m = (k_{-1} + k_{cat})/k_1$ (6)

and then express [E] in terms of [ES] and [E]total:

$[E] = [E]_{total} - [ES]$ (7)

Substitute (6) and (7) into (5):

$Km = ([E]_{total} - [ES])\ [S]/[ES]$ (8)

Solve (8) for [ES]: First multiply both sides by [ES]:

$[ES]\ K_m = [E]_{total}\ [S] - [ES][S]$

Then collect terms containing [ES] on the left:

$[ES]\ K_m + [ES][S] = [E]_{total}\ [S]$

Factor [ES] from the left-hand terms:

$[ES](K_m + [S]) = [E]_{total}\ [S]$

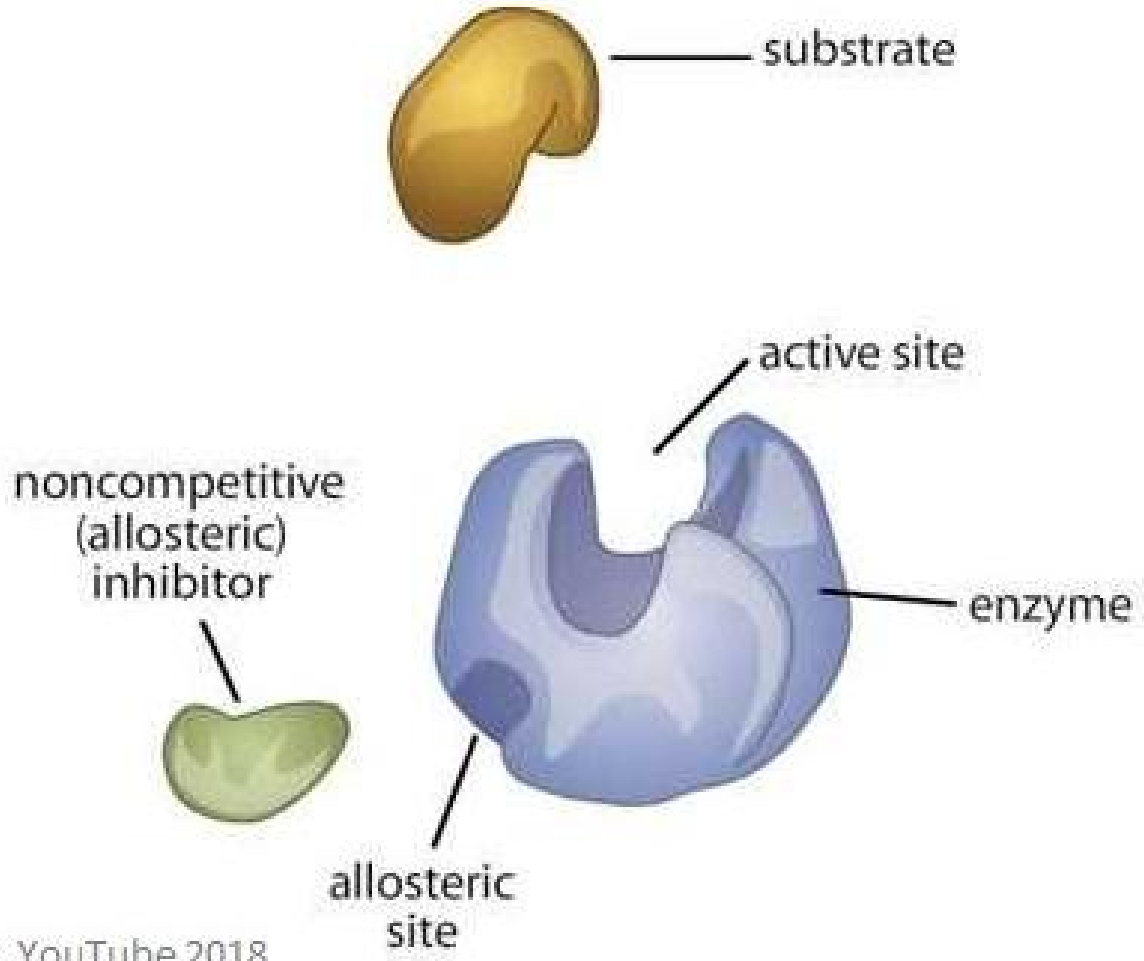
and finally, divide both sides by $(K_m + [S])$:

$[ES] = [E]_{total}\ [S]/(K_m + [S])$ (9)

Substitute (9) into (2): $V_0 = k_{cat} [E]_{total} [S]/(K_m + [S])$ (10)

Recalling (3), substitute V_{max} into (10) for $k_{cat} [E]_{total}$:

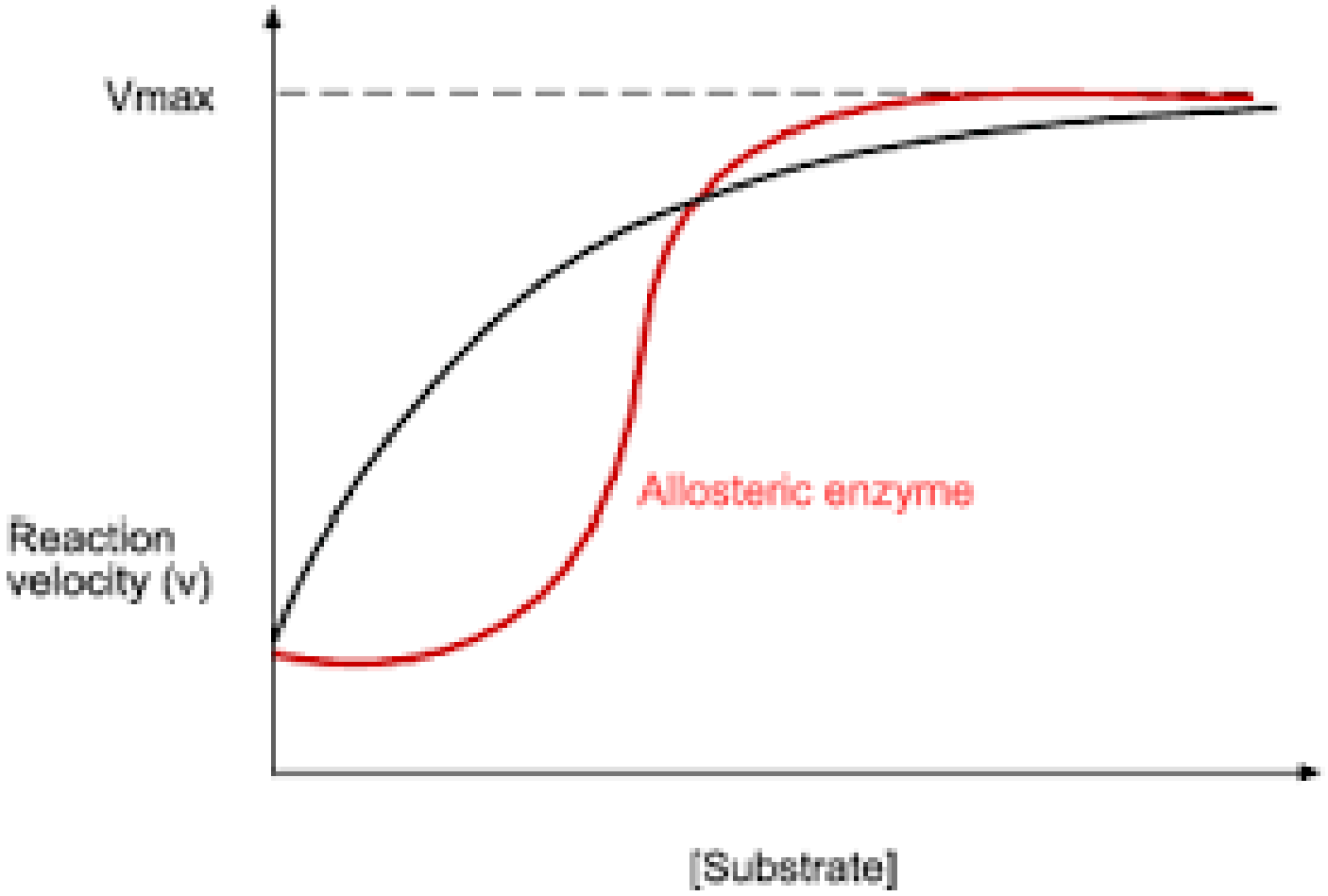
$V_0 = V_{max} [S]/(K_m + [S])$ (11)

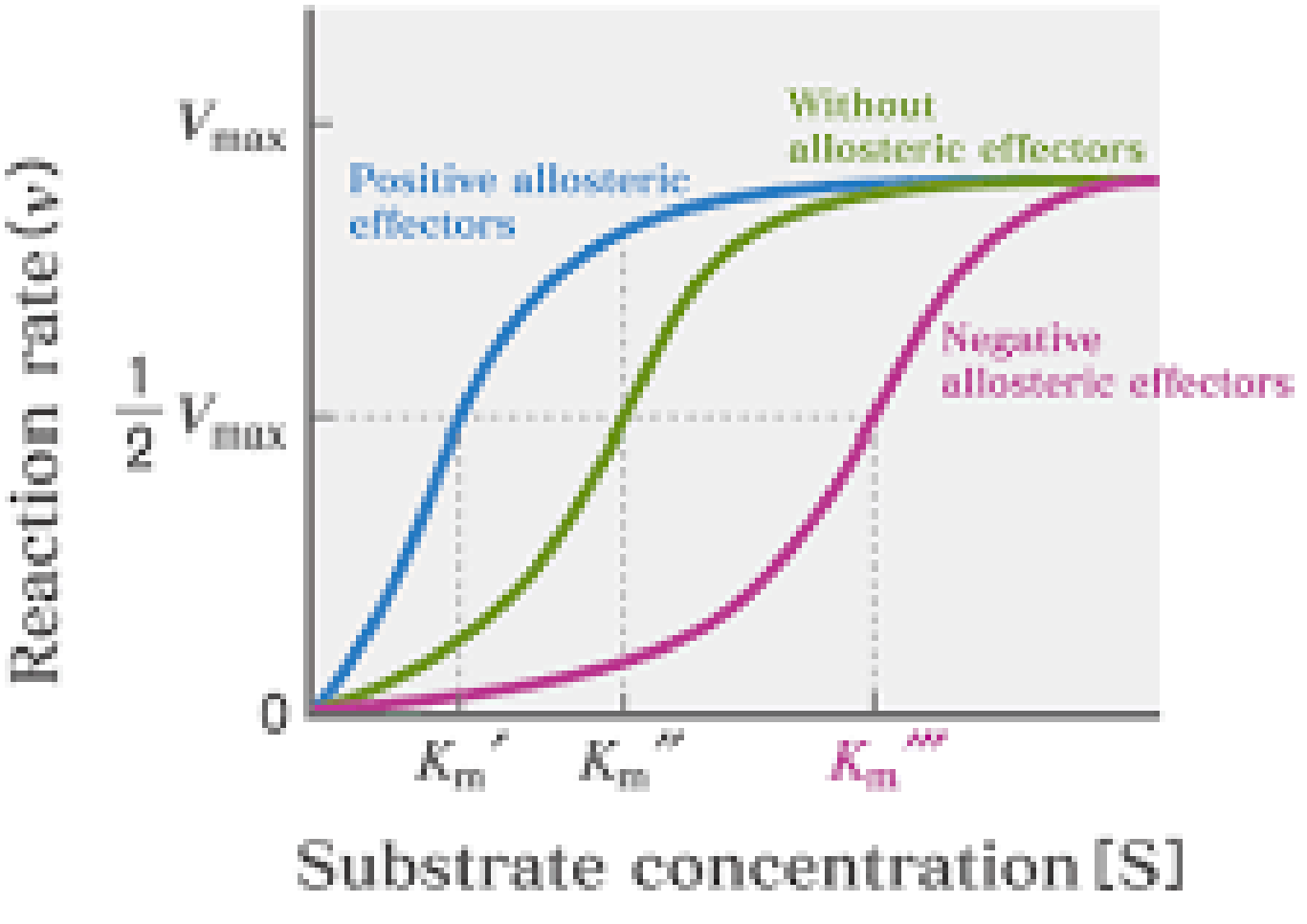


worldethq, YouTube 2018

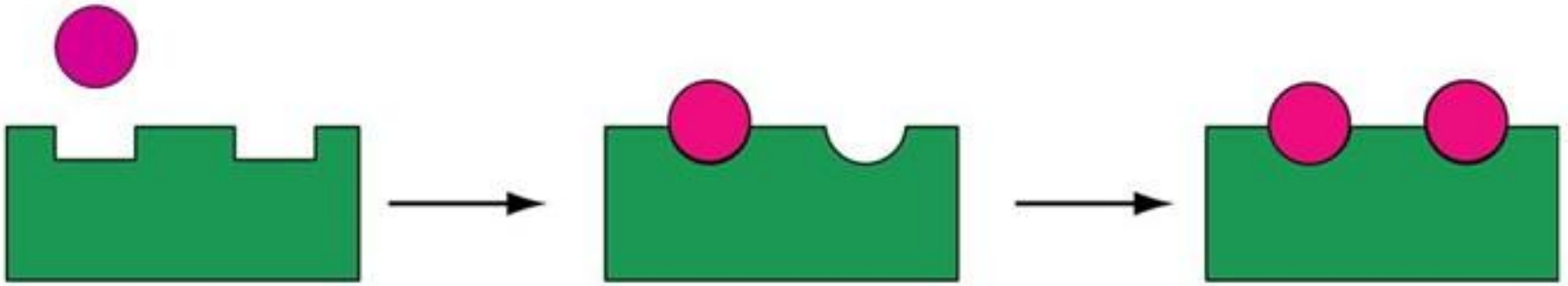
Allosteric Enzymes

- Allosteric enzymes are enzymes which have an additional site for an effector to bind to, as well as the active site
- Efforts regulate the activity of the enzyme – they can either activate or inhibit
- Allosteric enzymes are larger and more complex than normal enzymes
- They are regulated through homotropic regulation or heterotropic regulation

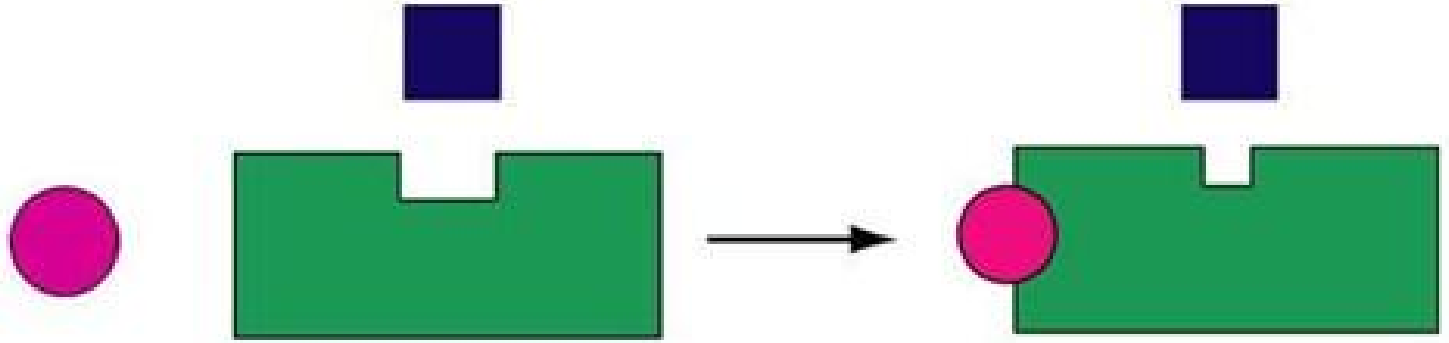

Vmax
Allosteric enzyme
Reaction velocity (v)
[Substrate]



- The **substrate** binding site is on the catalytic subunit – often referred to as the **C subunit**. The effector binding site is on the **regulatory subunit** – often referred to as the **R subunit**.
- This interaction between all of the subunits can be expressed using the **Hill coefficient**. When **n=1**, there will be **no interaction** between the subunits in the enzyme. The **larger the Hill coefficient** the **stronger the interactions** between all of the subunits in the enzyme.
- When an effector binds to an enzyme, it is called **cooperative binding**.



Rolf Matthews 2018



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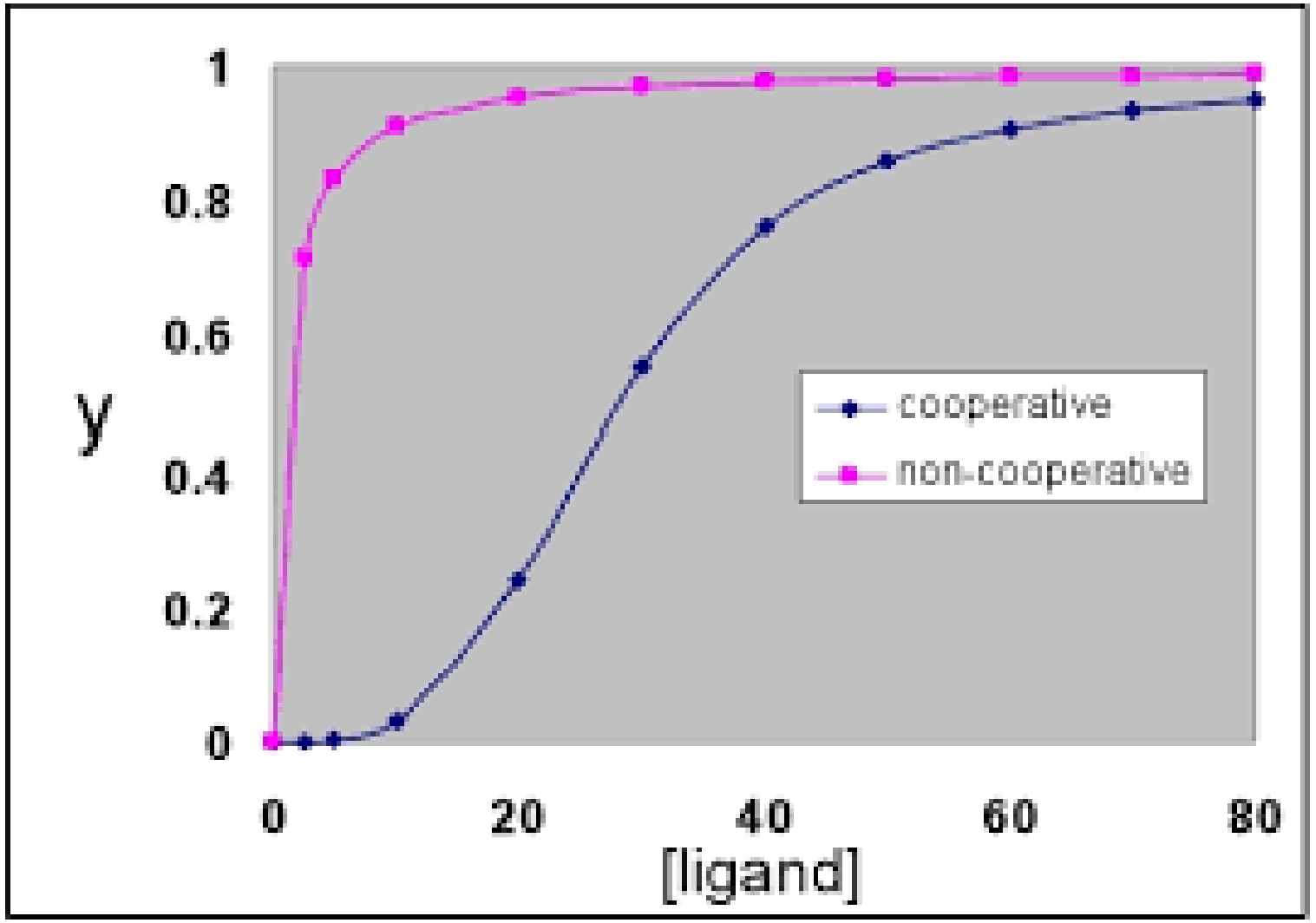
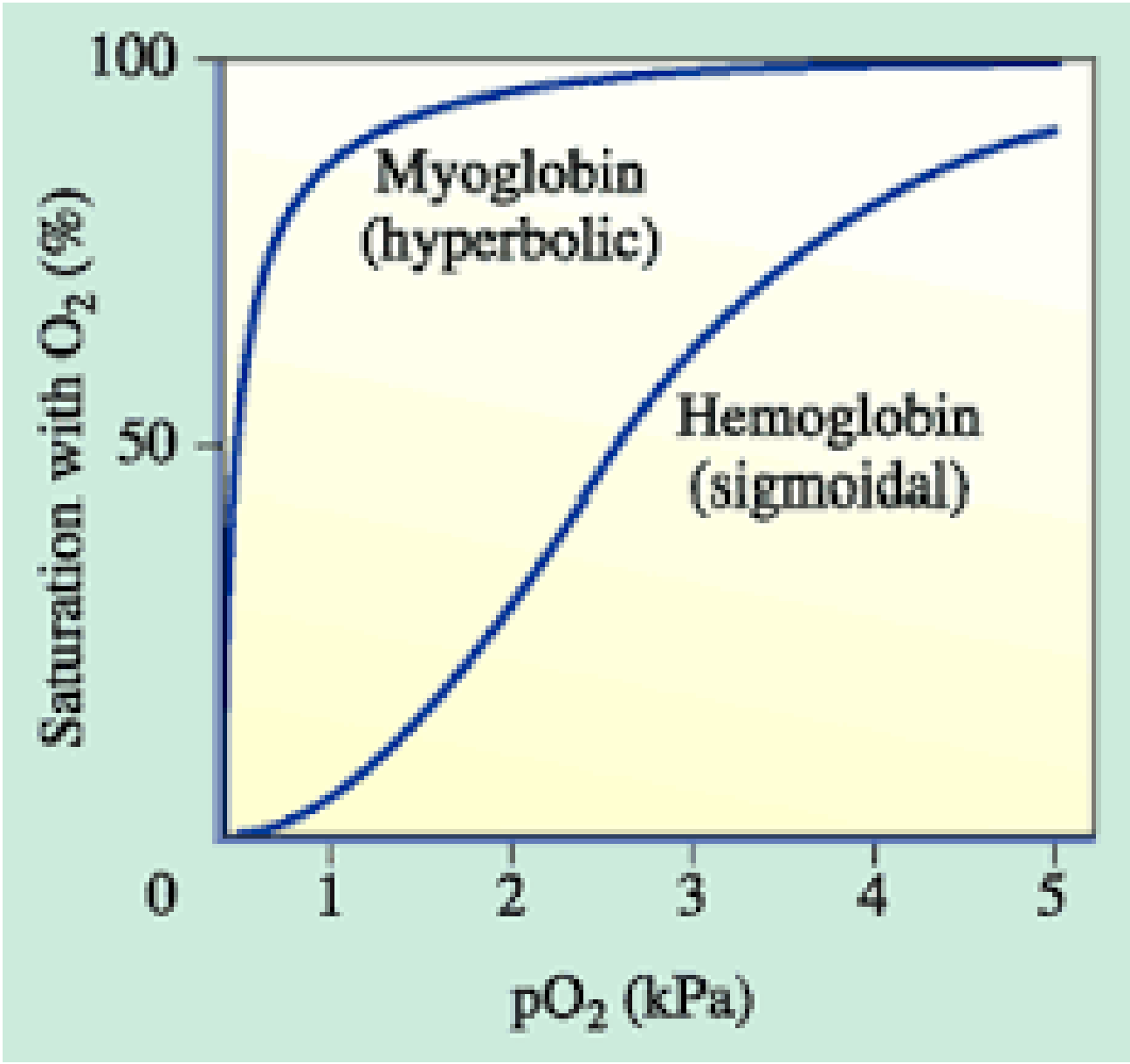
Homotropic Regulation

- A **homotropic allosteric effector** is a substrate for the enzyme, as well as a regulatory molecule – the prefix ‘homo’ refers to them being the **same**. They are usually **activators** of the enzyme. The below image shows a homotropic allosteric effector.
- Example of homotropic allosteric effector is **oxygen** effector of haemoglobin in human body.

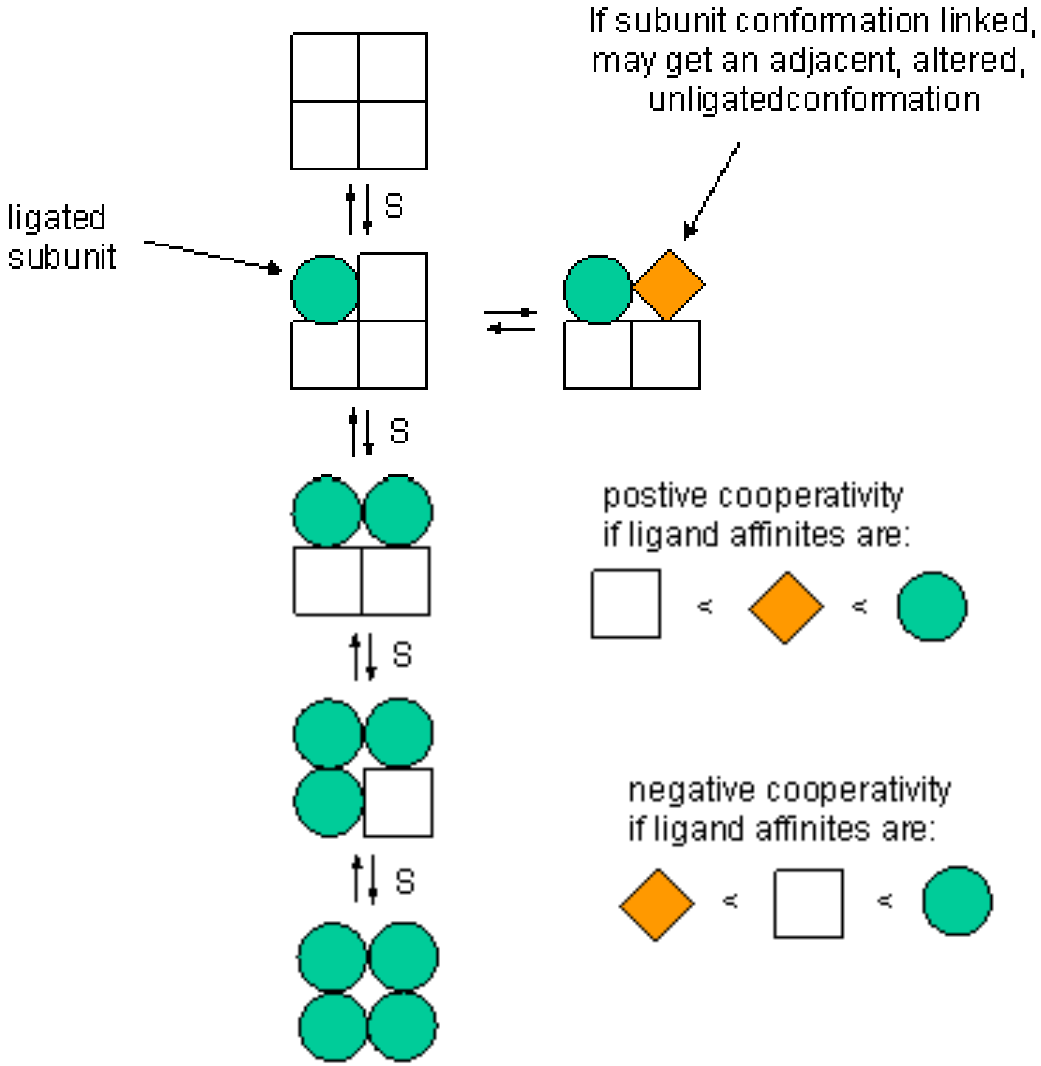
Heterotropic Regulation

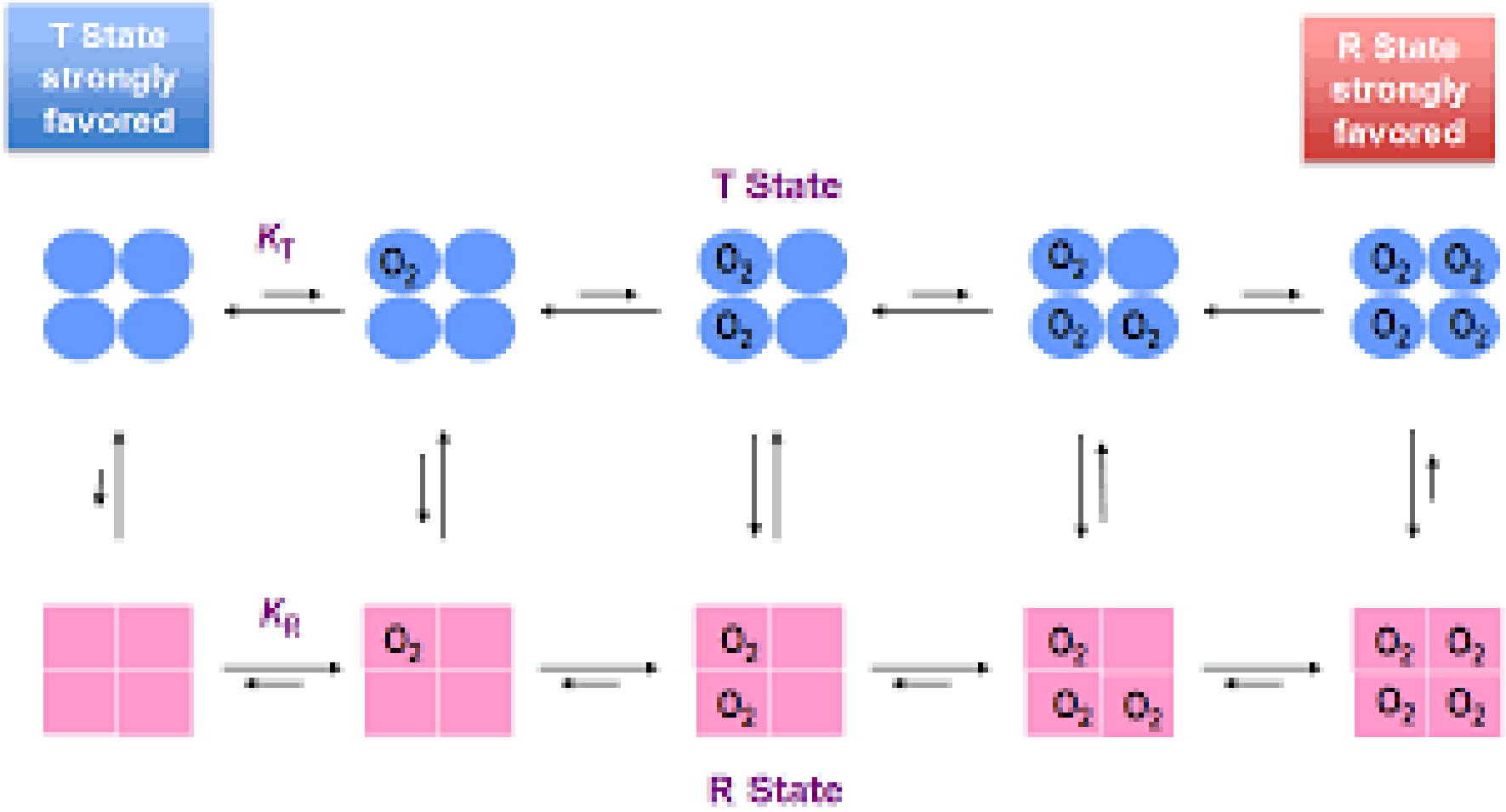
- A **heterotropic allosteric effector** is a regulatory molecule which is **not also the substrate** for the enzyme. It can either **activate or inhibit** the enzyme it binds to. The below image shows a heterotropic allosteric effector.
- Example of homotropic allosteric effector is **carbon dioxide** effector of haemoglobin in human body.

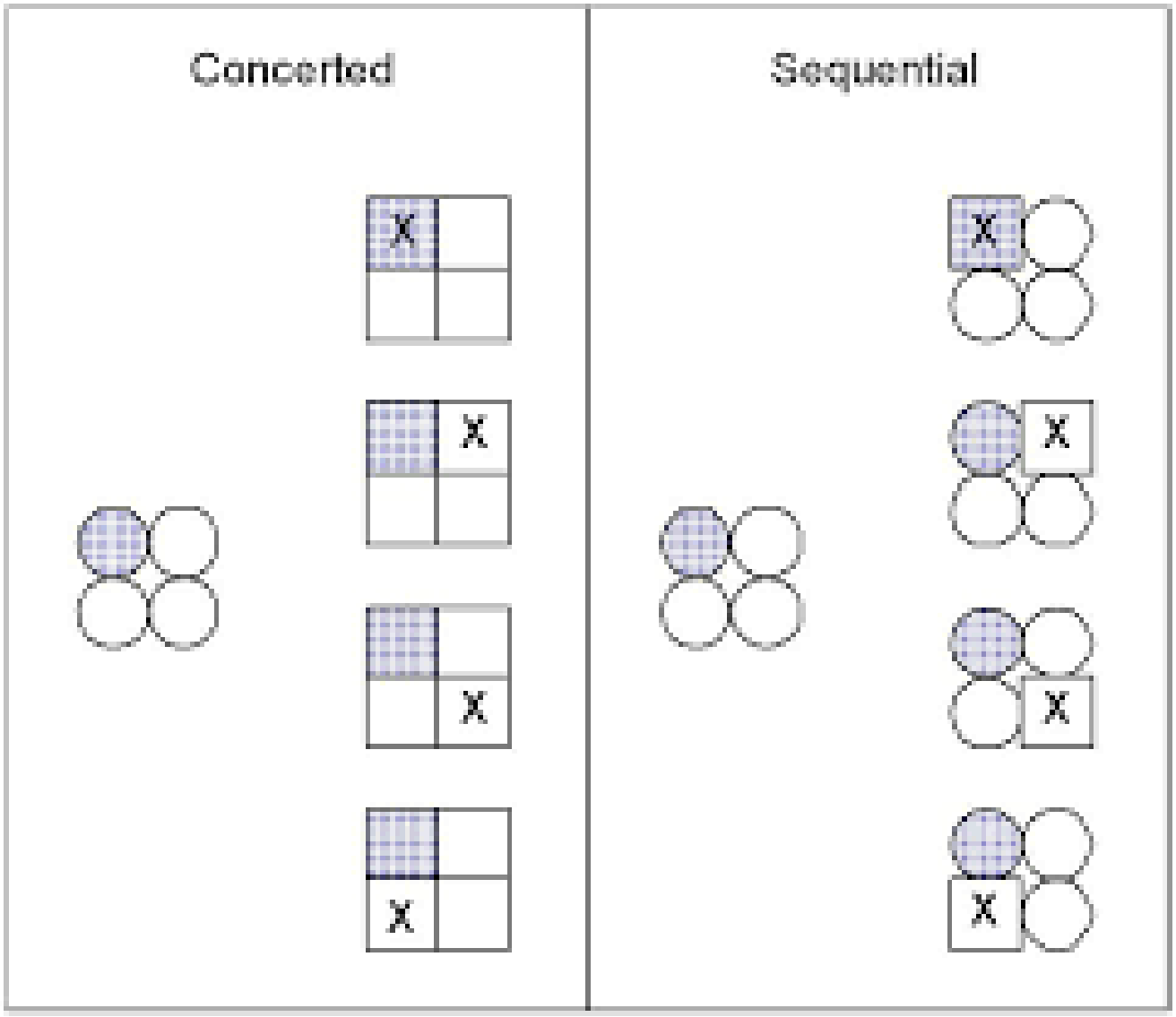

1
0.8
0.6
0.4
0.2
0
y
0
20
40
60
80
[ligand]
cooperative
non-cooperative

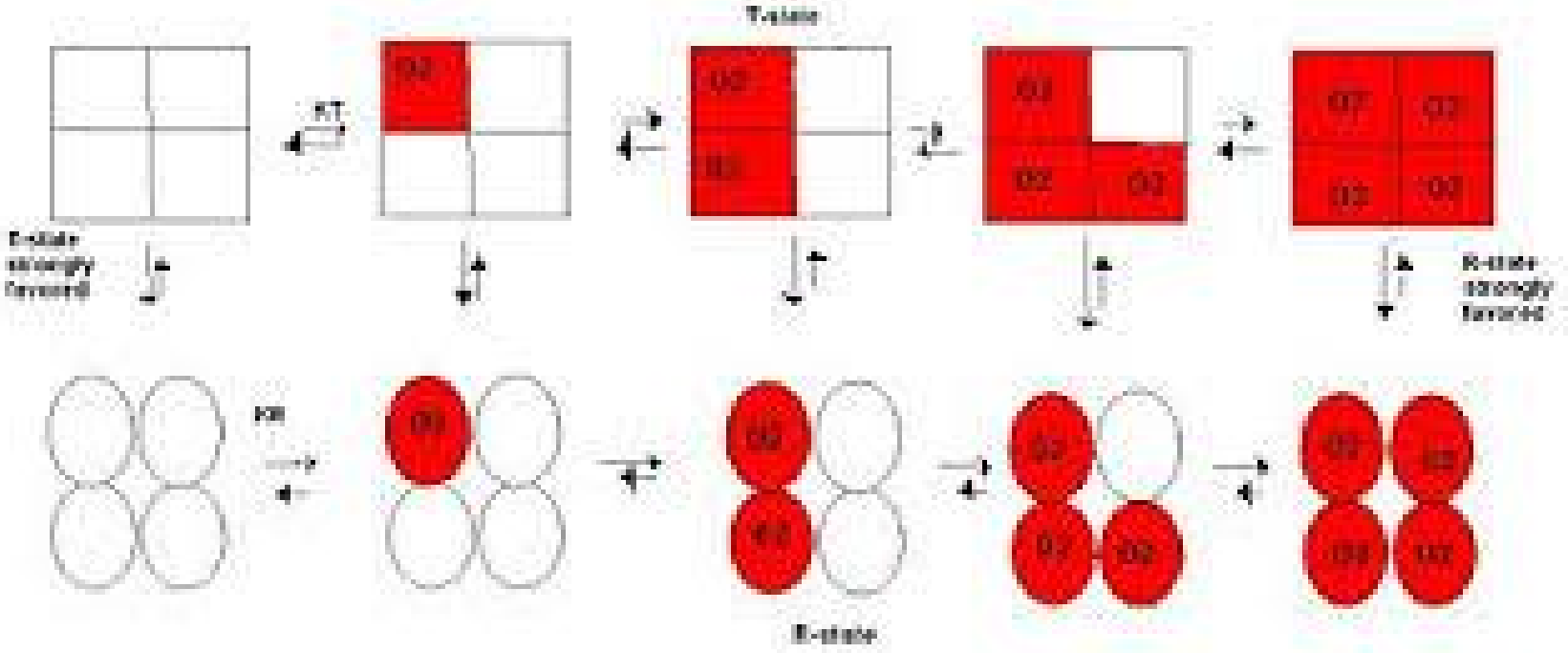

100
50
0
Saturation with O_2 (%)
Myoglobin
(hyperbolic)
Hemoglobin
(sigmoidal)
1
2
3
4
5
pO_2 (kPa)

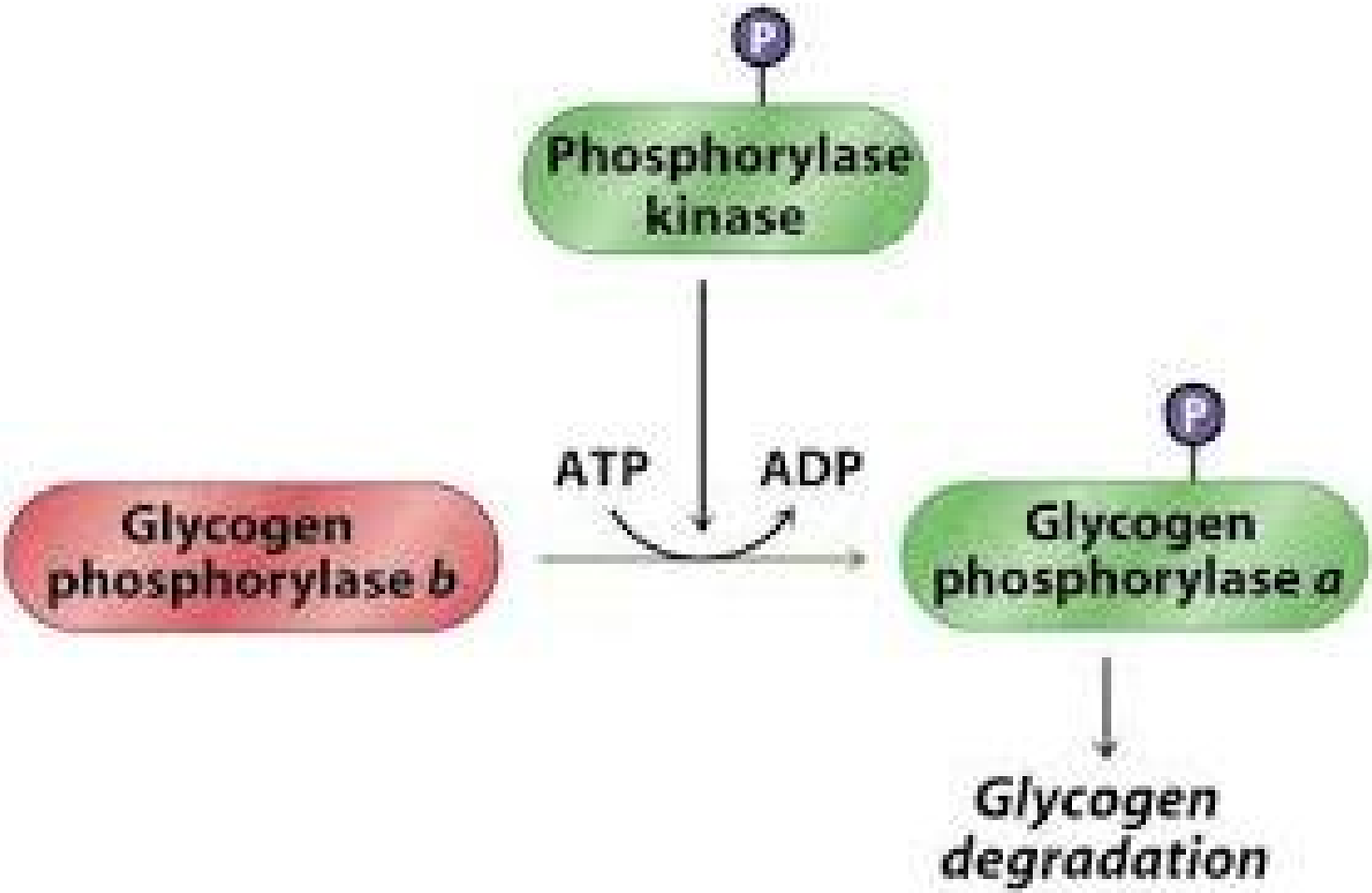
KNF Model – Sequential Model

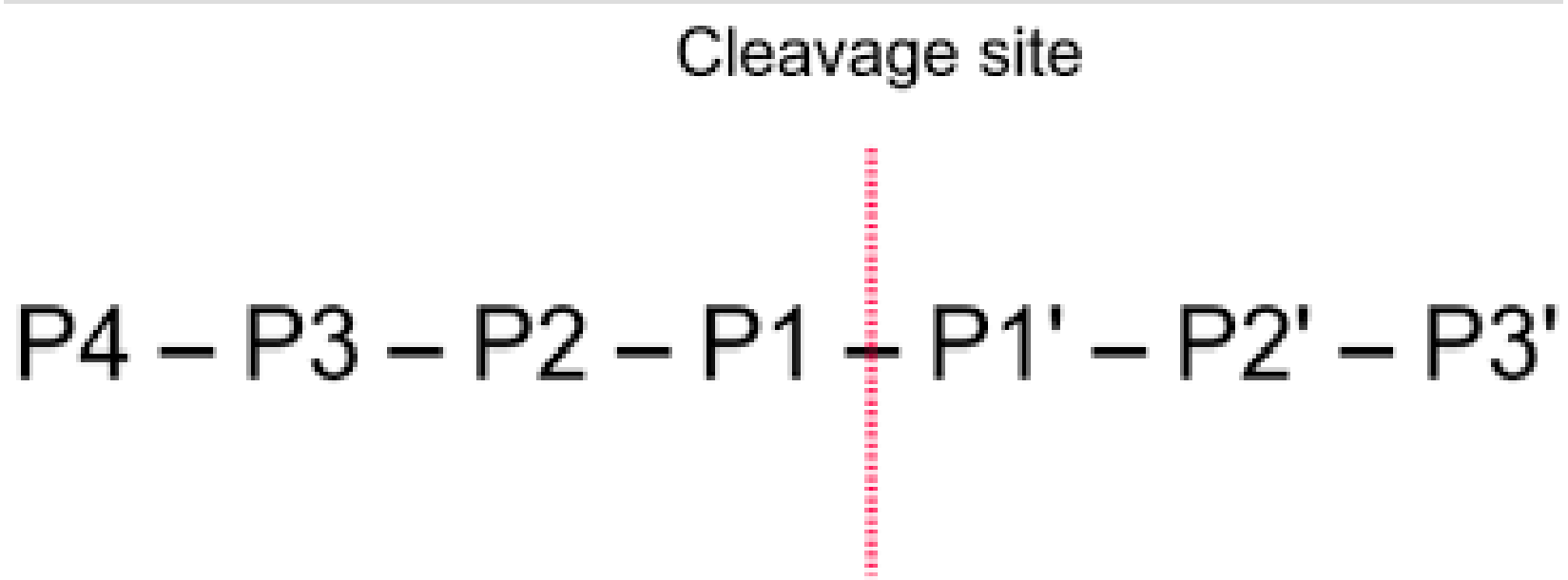


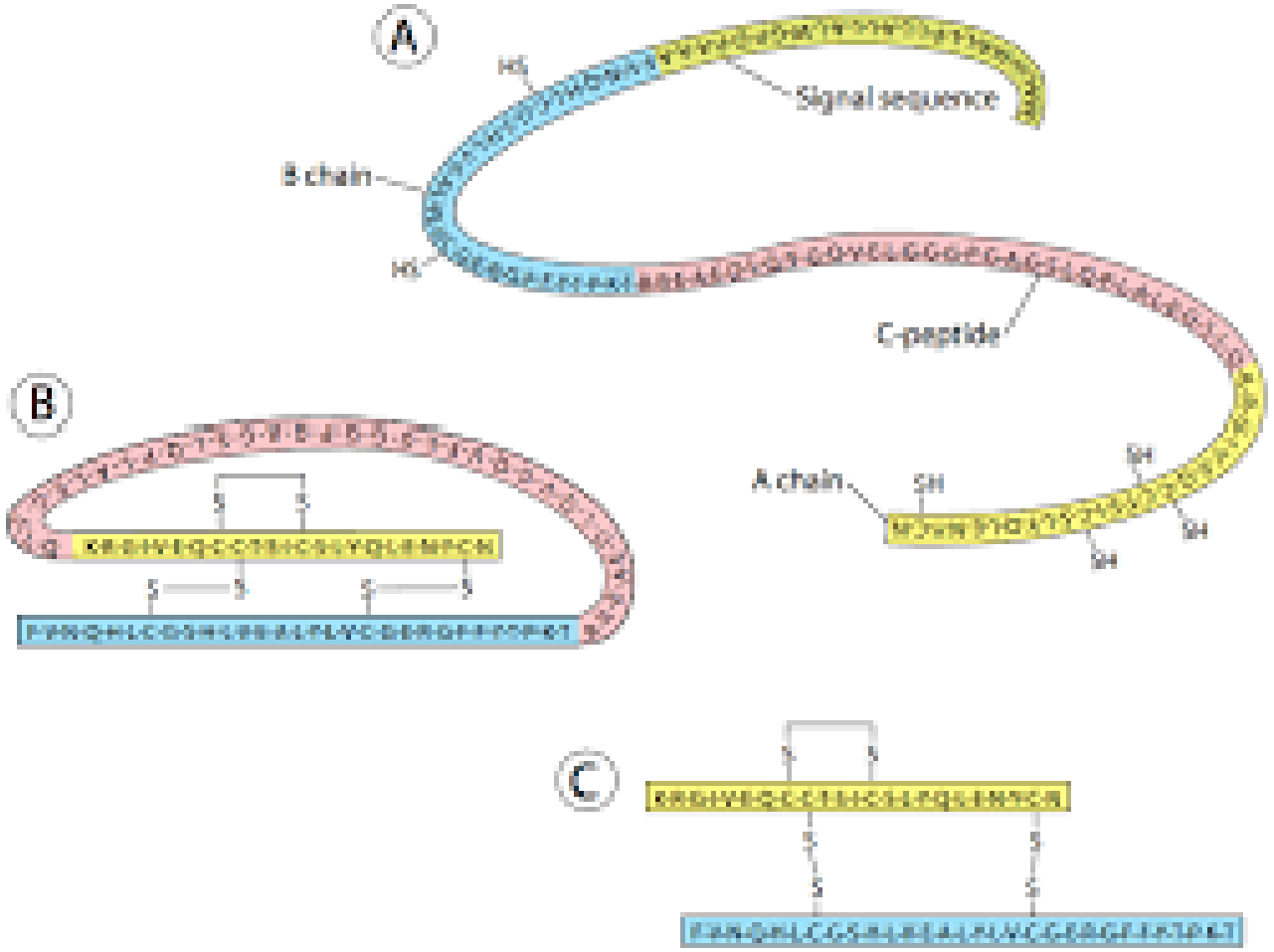

T State strongly favored
R State strongly favored
T State
K_T
O_2
K_R
R State

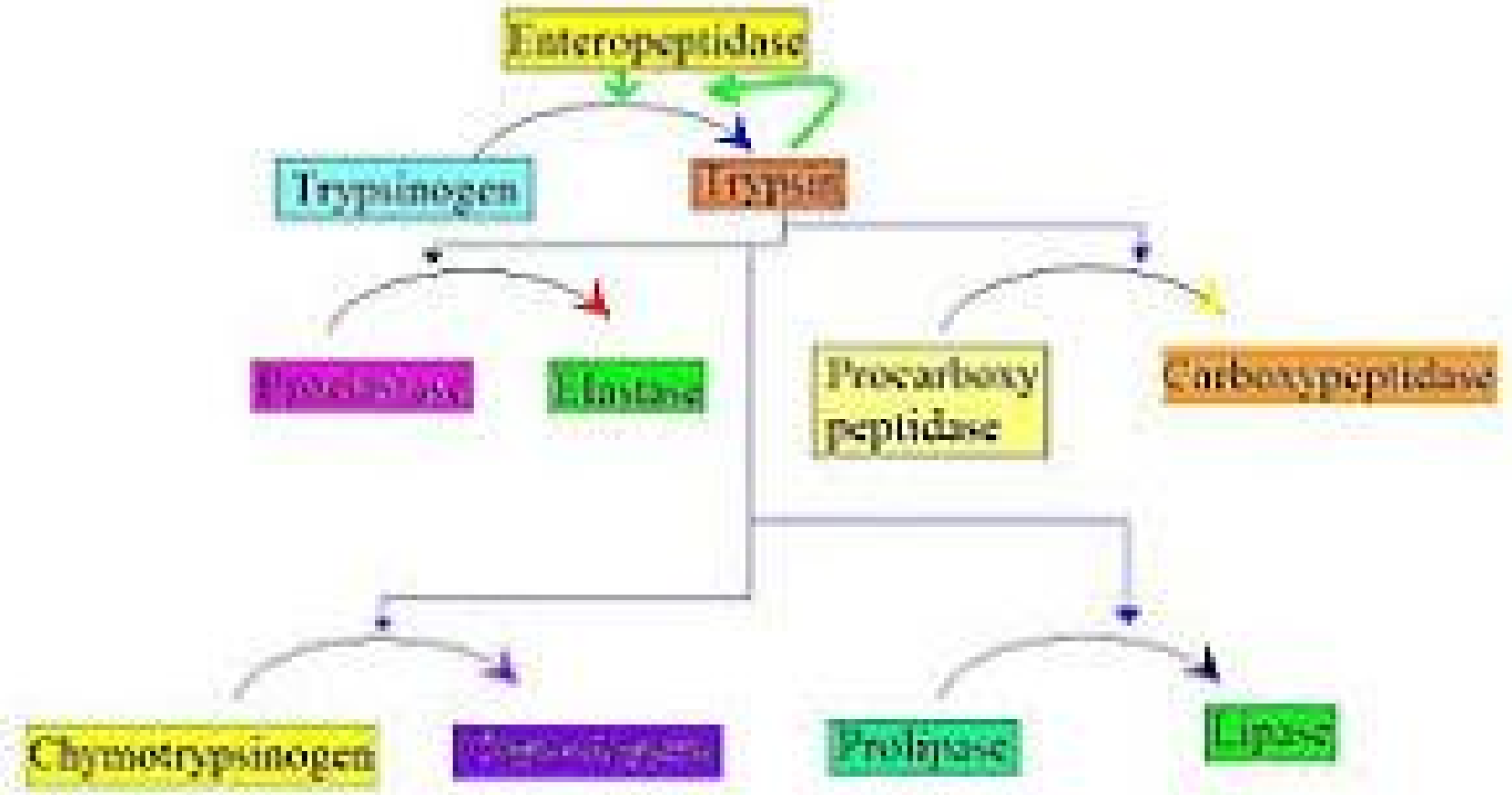

Concerted
Sequential
X
X
X
X
X
X
X
X

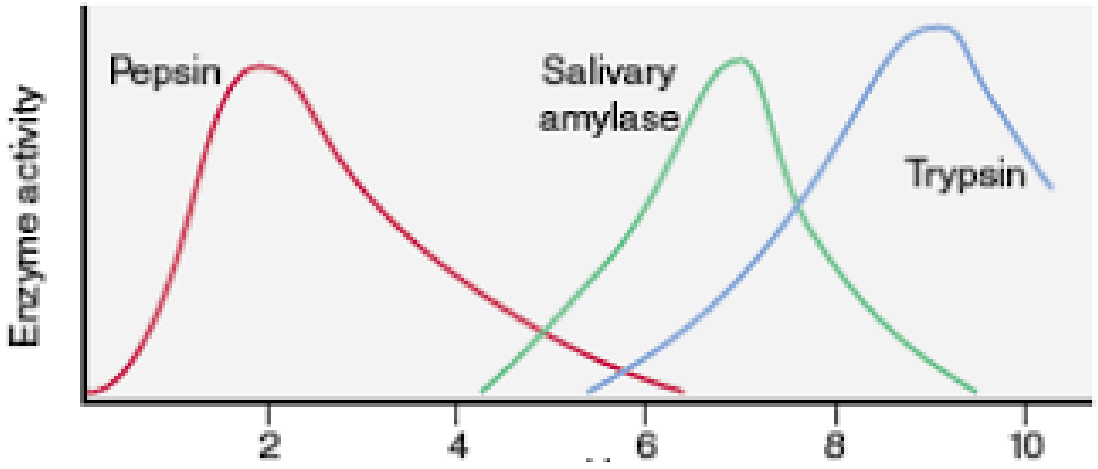


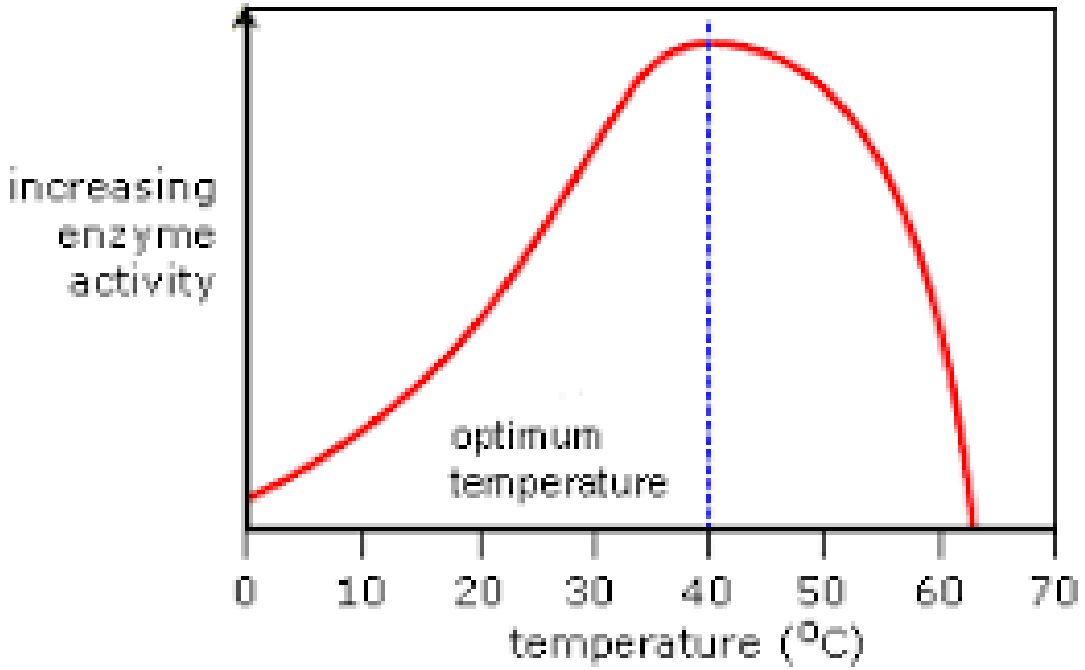

P
Phosphorylase kinase
ATP
ADP
Glycogen phosphorylase b
P
Glycogen phosphorylase a
Glycogen degradation


Cleavage site
P4 – P3 – P2 – P1 – P1' – P2' – P3'


A
Signal sequence
B chain
C-peptide
A chain
B
C


Enteropeptidase
Trypsinogen
Trypsin
Elastase
Procarboxy peptidase
Carboxypeptidase
Chymotrypsinogen
Prolipase
Lipase


Enzyme activity
Pepsin
Salivary amylase
Trypsin
2
4
6
8
10


increasing enzyme activity
optimum temperature
0
10
20
30
40
50
60
70
temperature (°C)