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ALLOSTERIC ENZYMES AND THEIR KINETICS

BY

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ZOOLOGY: SEM- III, PAPER- C7T: FUNDAMENTALS OF BIOCHEMISTRY, UNIT 5: ENZYMES

- Allosteric enzymes are enzymes that change their conformational ensemble upon binding of an effector (allosteric modulator) which results in an apparent change in binding affinity at a different ligand binding site.

- This "action at a distance" through binding of one ligand affecting the binding of another at a distinctly different site, is the essence of the allosteric concept.

- Allostery plays a crucial role in many fundamental biological processes, including but not limited to cell signaling and the regulation of metabolism.

- Allosteric regulation (or allosteric control) is the regulation of a protein by binding an effector molecule at a site other than the enzyme's active site. The site to which the effector binds is termed the **allosteric site**.

- Allosteric sites allow effectors to bind to the protein, often resulting in a conformational change involving protein dynamics.

- Effectors that enhance the protein's activity are referred to as allosteric activators, whereas those that decrease the protein's activity are called allosteric inhibitors.

- Allosteric regulations are a natural example of control loops, such as feedback from downstream products or feed forward from upstream substrates.

- Long-range allostery is especially important in cell signaling.

- Allosteric regulation is also particularly important in the cell's ability to adjust enzyme activity.

- Enzymes without coupled domains/subunits display normal Michaelis-Menten kinetics, whereas most allosteric enzymes have multiple coupled domains/subunits and show cooperative binding.

- Allosteric enzymes displaying a sigmoidal dependence on the concentration of their substrates in positively cooperative systems.

- This allows most allosteric enzymes to greatly vary catalytic output in response to small changes in effector concentration.

- Effector molecules, which may be the substrate itself (homotropic effectors) or some other small molecule (heterotropic effector), may cause the enzyme to become more active or less active by redistributing the ensemble between the higher affinity and lower affinity states.

- The binding sites for heterotropic effectors, called allosteric sites, are usually separate from the active site yet thermodynamically coupled.

- **Allosteric Database** provides a central resource for the display, search and analysis of the structure, function and related annotation for allosteric molecules, including allosteric enzymes and their modulators.

- Each enzyme is annotated with detailed description of allostery, biological process and related diseases, and each modulator with binding affinity, physicochemical properties and therapeutic area.

Kinetic properties:

- Hemoglobin, though not an enzyme, is the best example of an allosteric protein molecule.

- The *E. coli* enzyme aspartate carbamoyltransferase (ATCase) has become another good example of allosteric regulation. Other prominent examples of allosteric enzymes in metabolic pathways are glycogen phosphorylase, phosphofructokinase, glutamine synthetase.

- The kinetic properties of allosteric enzymes are often explained in terms of a conformational change between a

low-activity, low-affinity "tense" or T state and a high-activity, high-affinity "relaxed" or R state. These structurally distinct enzyme forms have been shown to exist in several known allosteric enzymes.

- However, the molecular basis for conversion between the two states is not well understood. Two main models have been proposed to describe this mechanism:

- The "concerted model" of Monod, Wyman, and Changeux, and

- The "sequential model" of Koshland, Nemethy, and Filmer.

- In the **concerted model**, the protein is thought to have two “all-or-none” global states. This model is supported by positive cooperativity where binding of one ligand increases the ability of the enzyme to bind to more ligands.

The model is not supported by negative cooperativity where losing one ligand makes it easier for the enzyme to lose more.

- In the **sequential model**, there are many different global conformational/energy states. Binding of one ligand changes the enzyme so it can bind more ligands more easily, i.e. every time it binds a ligand it wants to bind another one.

- Neither model fully explains allosteric binding, however. The recent combined use of physical techniques (for example, x-ray crystallography and solution small angle x-ray scattering or SAXS) and genetic techniques (site-directed mutagenesis or SDM) may improve our understanding of allostery.

Properties of Allosteric Enzymes:

- Allosteric or Regulatory enzymes have multiple subunits (Quaternary Structure) and multiple active sites.

- Allosteric enzymes have active and inactive shapes differing in 3D structure.

- Allosteric enzymes often have multiple inhibitor or activator binding sites involved in switching between active and inactive shapes.

- Allosteric enzymes have characteristic “S”-shaped curve for reaction rate vs. substrate concentration. This is because the substrate binding is “Cooperative.”

- Moreover, the binding of first substrate at first active site stimulates active shapes, and promotes binding of second substrate.

- A modulator is a metabolite, when bound to the allosteric site of an enzyme, alters its kinetic characteristics. The modulators for allosteric enzyme may be either stimulatory or inhibitory.

- A stimulator is often the substrate itself. The regulatory enzymes for which substrate and modulator are identical are called homotropic.

- When the modulator has a structure different then the substrate, the enzyme is called heterotropic.

- Some enzymes have more than one modulators.

- The allosteric enzymes also have one or more regulatory or aliosteric sites for binding the modulator.

- Enzymes with several modulators generally have different specific binding sites for each as shown below:

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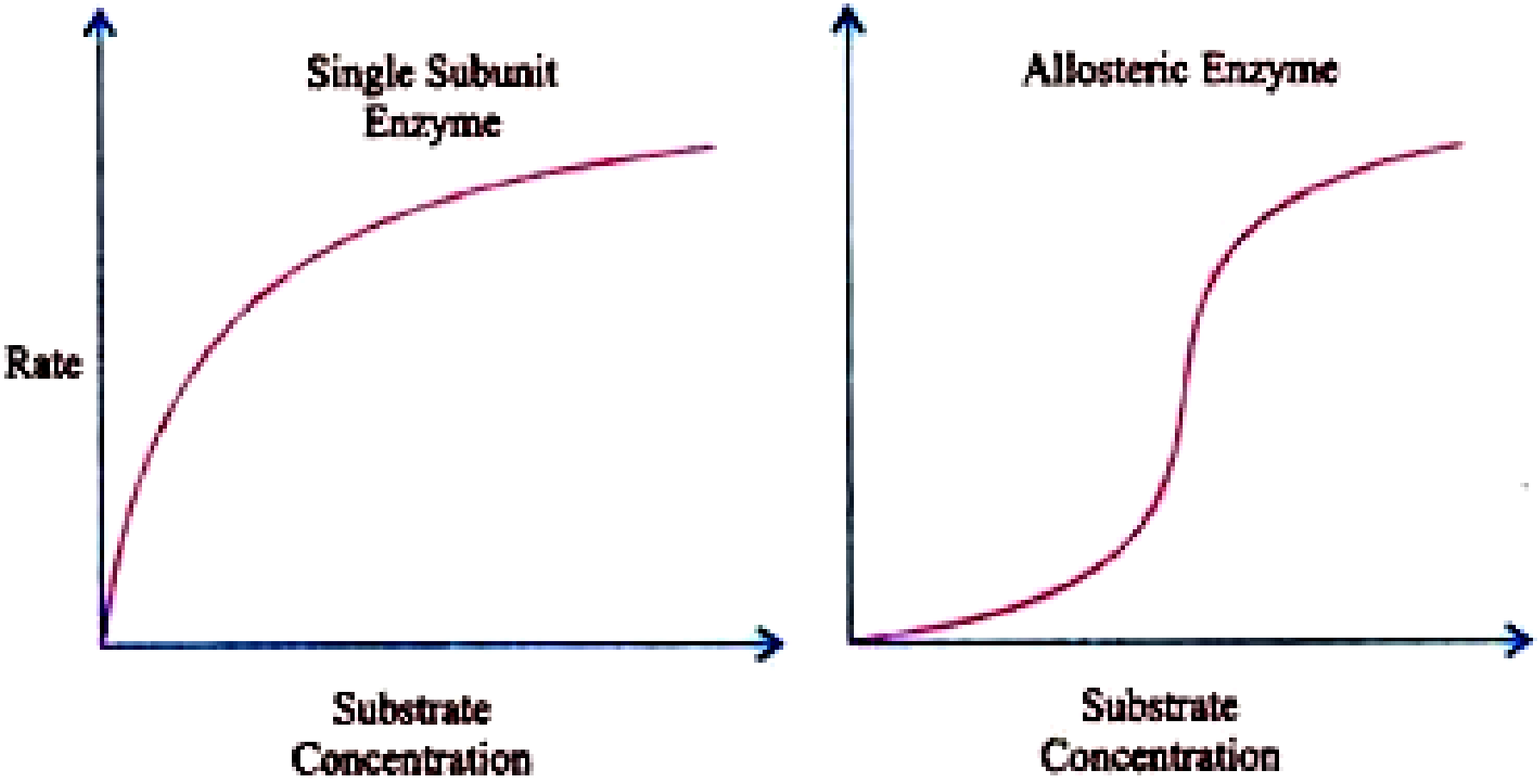
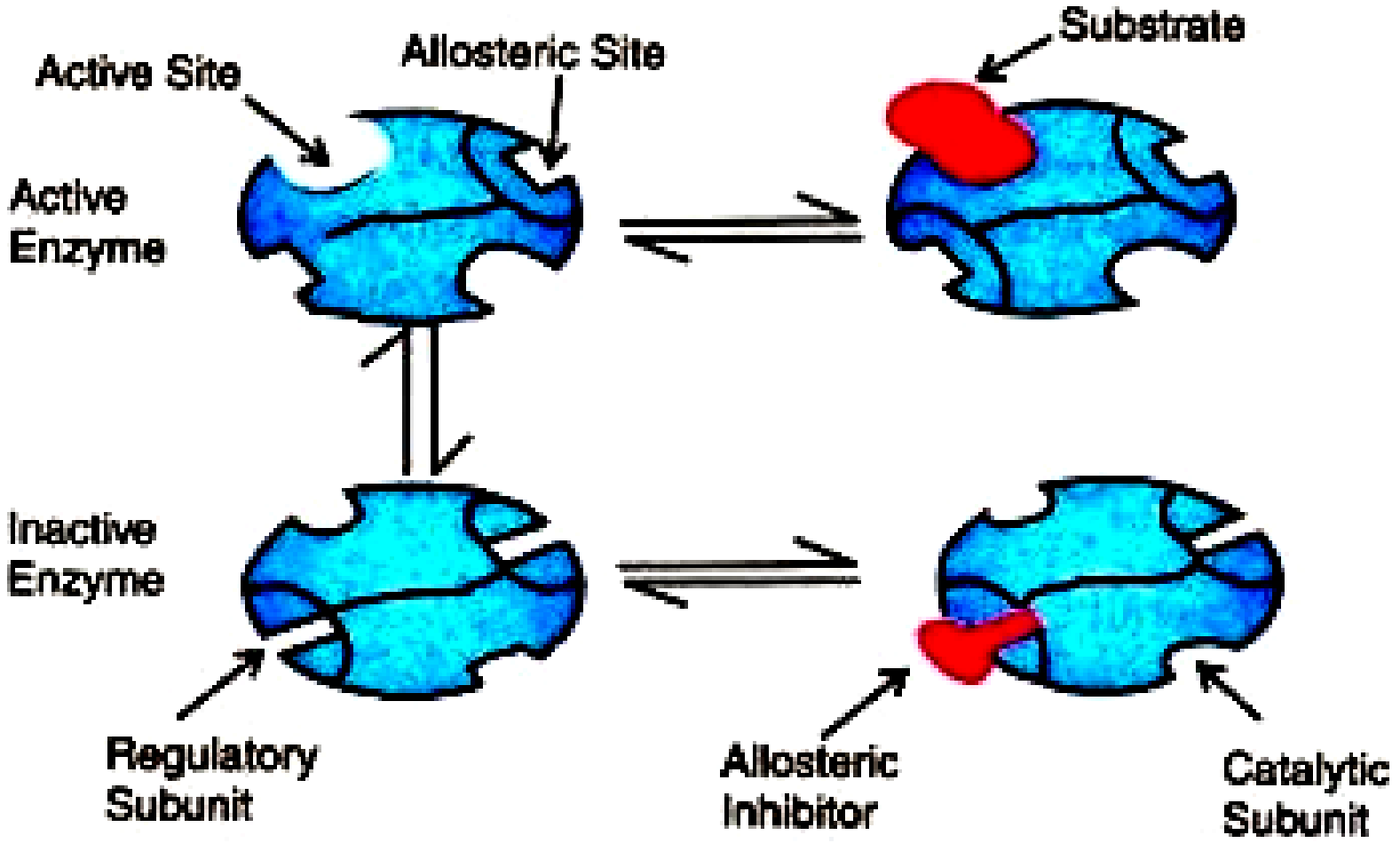


- The sigmoid curve is given by homo-tropic enzymes in which the substrate also serve as a positive (stimulator) modulator.

- Curve for the non-regulatory enzymes is hyperbolic, as also predicted by the Michaelis-Menten equation, whereas allosteric enzymes do not show Michaelis- Menten relationship because their kinetic behaviour is greatly altered by variation in the concentration of modulators.



Mechanisms of allosteric effect.

Mechanism of Action of Allosteric Enzymes:

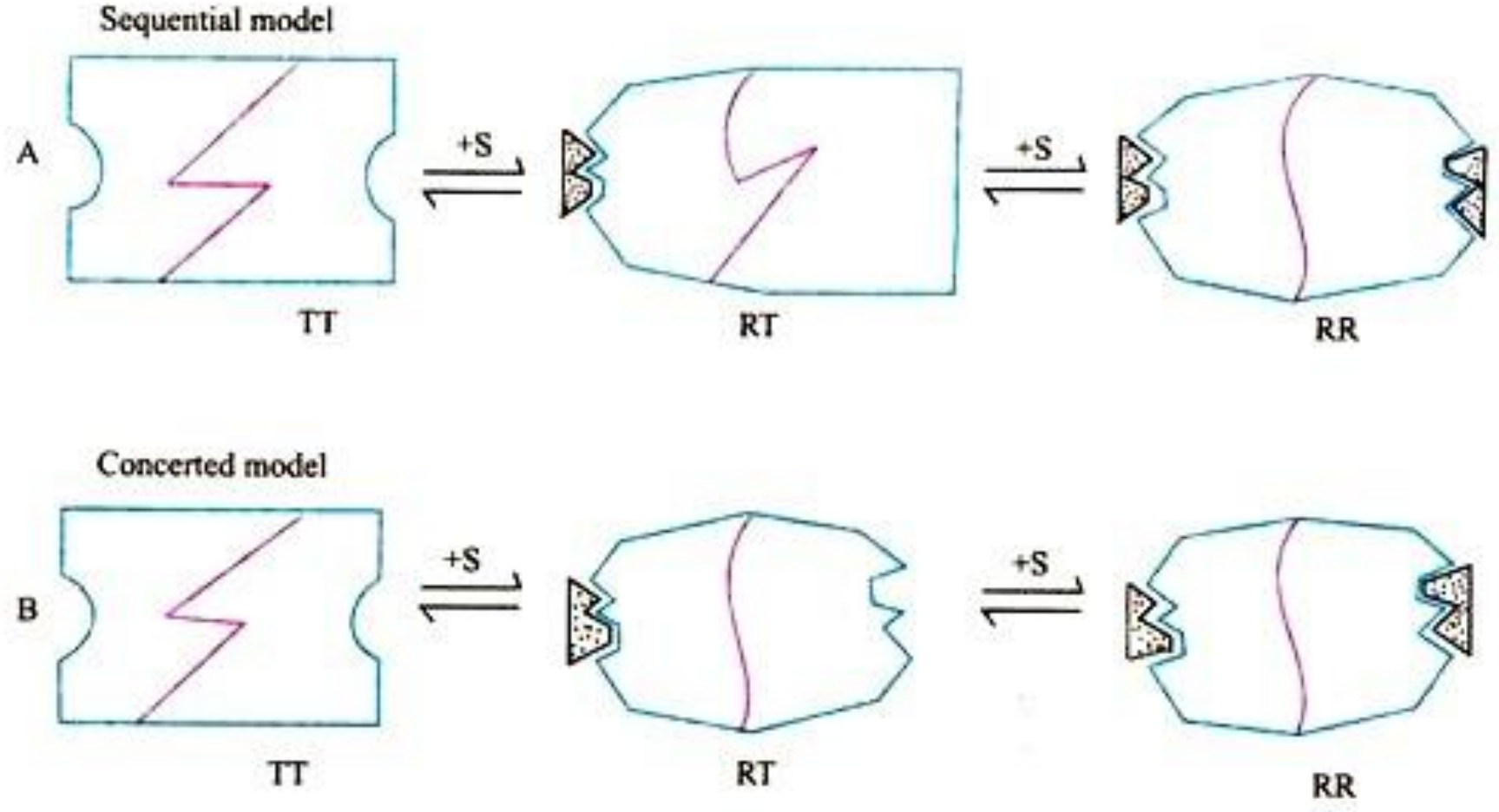
Two general models for the inter-conversion of inactive and active forms of allosteric enzymes have been proposed:

(a) Simple sequential model:

This model was proposed by Koshland Jr. in the year 1966. According to this theory, the aliosteric enzyme can exist in only two conformational changes individually. If we consider

an allosteric enzyme consisting of two identical subunits, each containing an active site as shown below:

The T (tense) form has low affinity and the R (relaxed) form has high affinity for substrate. In this model, the binding of substrate to one of the subunits induces a T → R transition in that subunit but not in the other subunits.



Kinetic models of allosteric enzymes (A) ; Simple sequential model (B) Concerted or symmetry model.

(b) Concerted or Symmetry Model:

This model was proposed by Jacques Monod and his colleagues in 1965. According to them, an allosteric enzyme can exist in still two conformations, active and relaxed or inactive form.

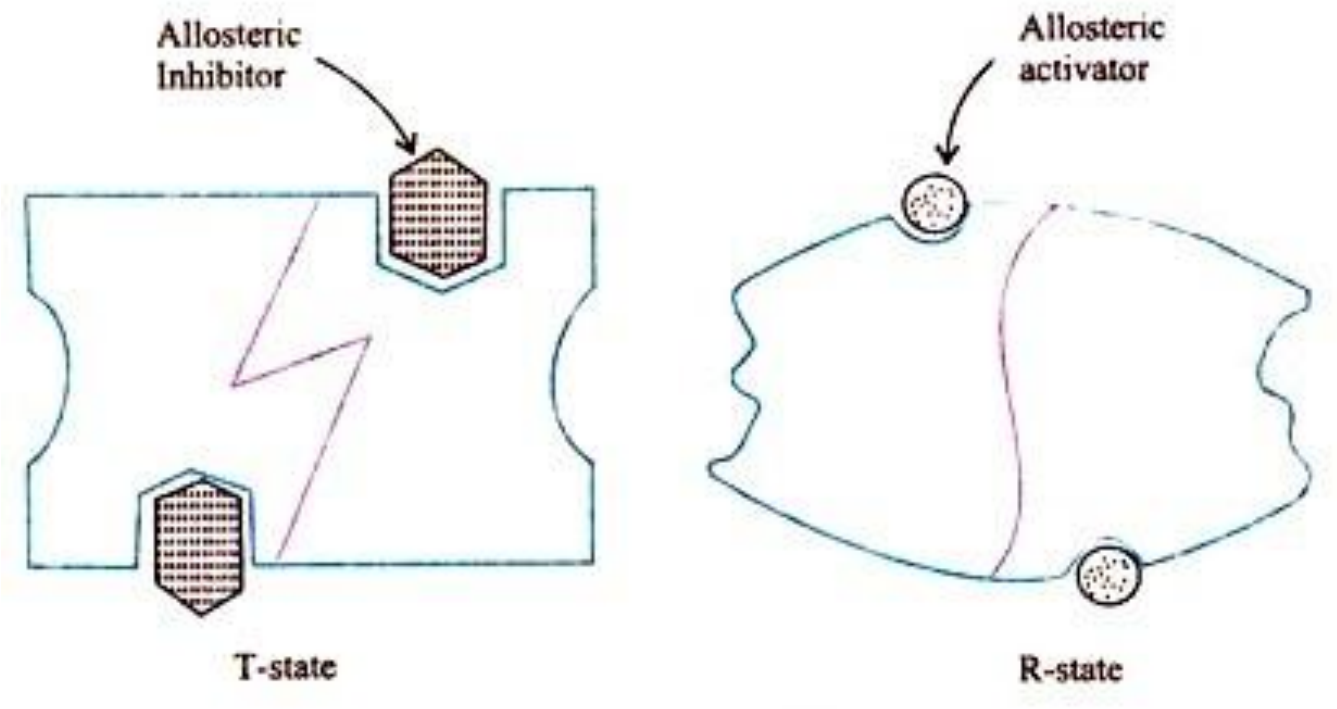
All subunits are either in the active form or all are in inactive form. Every substrate molecule that binds with enzyme increases the probability of transition from the inactive to the

active site. The effect of allosteric activators and inhibitors can be explained quite easily by this model.

An allosteric inhibitor binds preferably to the T form whereas an allosteric activator binds to the R form. An allosteric inhibitor shifts The R → T conformational equilibrium towards T. Whereas an allosteric activator shifts it toward R.

The result is that an allosteric activator increases the binding to substrate of the enzyme, whereas an allosteric inhibitor

decreases substrate binding. Symmetry is conserved in this model but not in the sequential model.



Effect of activator and inhibitor on substrate binding.

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THANK YOU

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