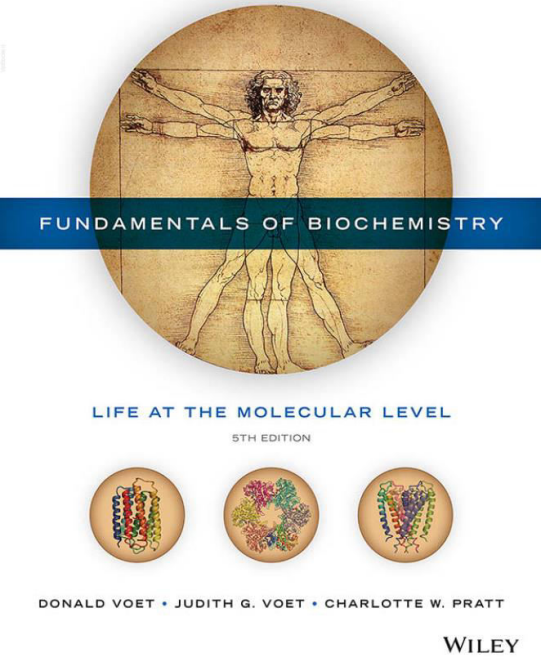
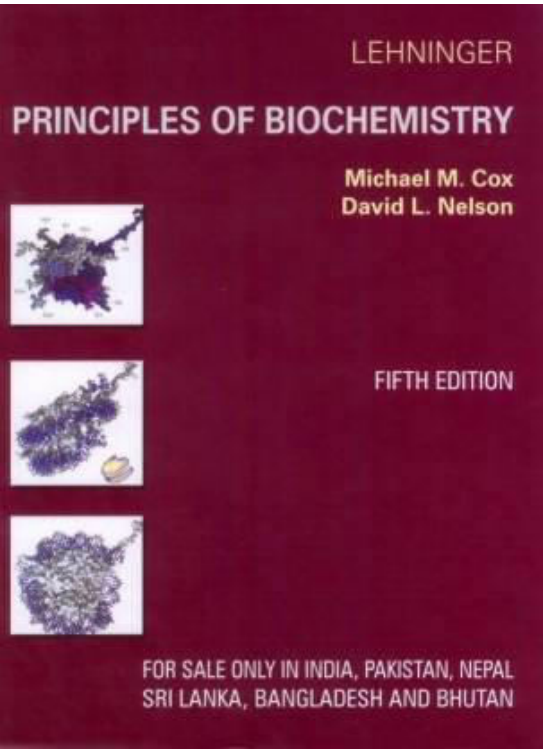


Control of Enzyme Activity



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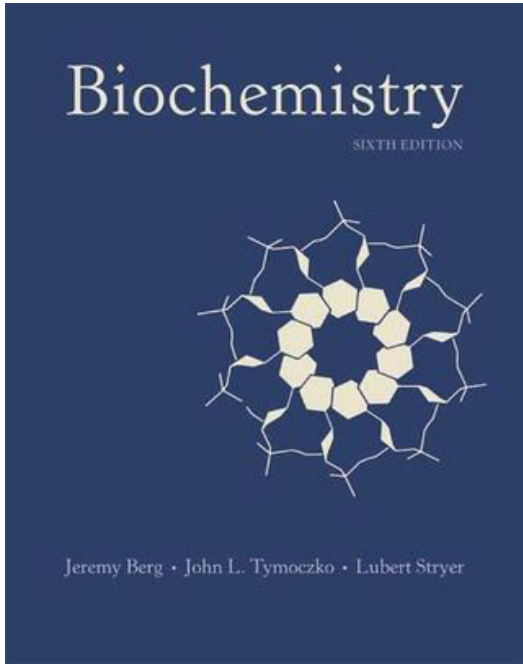


An organism must be able to control the catalytic activities of its component enzymes so that it can coordinate its numerous metabolic processes.

There are **two ways** that this may occur:

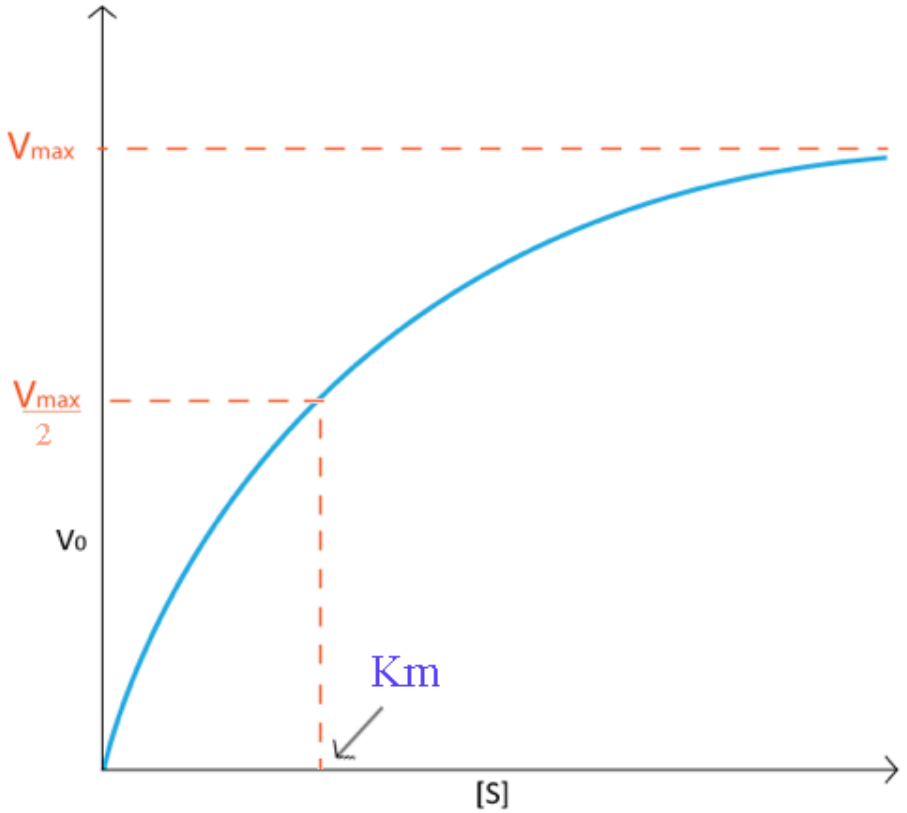
1. **Control of enzyme availability:** The amount of a given enzyme in a cell depends on both its rate of synthesis and its rate of degradation.

2. **Control of enzyme activity:** An enzyme's activity can be inhibited by the **accumulation of product** and by the **presence of other types of inhibitors.** **E**nzyme's catalytic activity can be modulated-either negatively or positively-through **structural alterations** that influence the enzyme's substrate-binding affinity or turnover number.

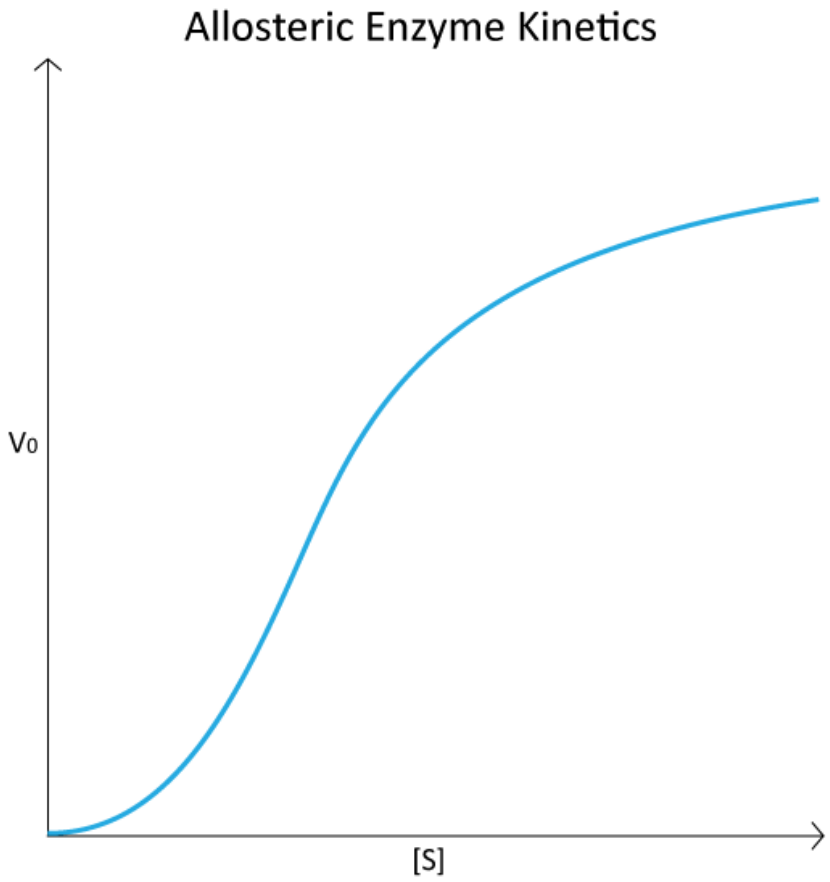
    I. **Allosteric effectors**
    II. **Product Inhibition or Feedback Inhibition**
    III. **Covalent modification (Phosphorylation/dephosphorylation)**

Allosteric regulation

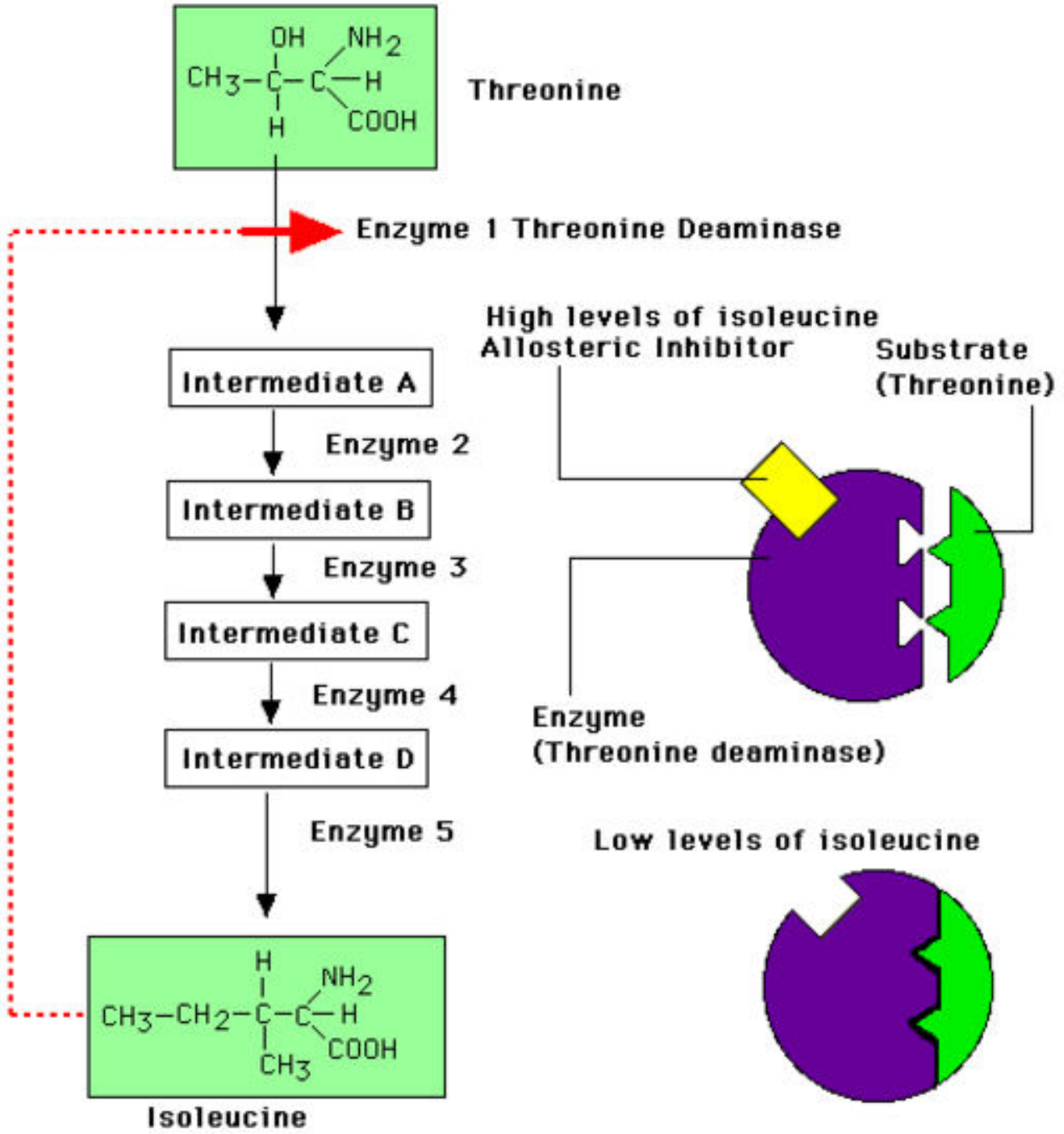
Substrate Does Not Change Enzyme Binding of Substrate



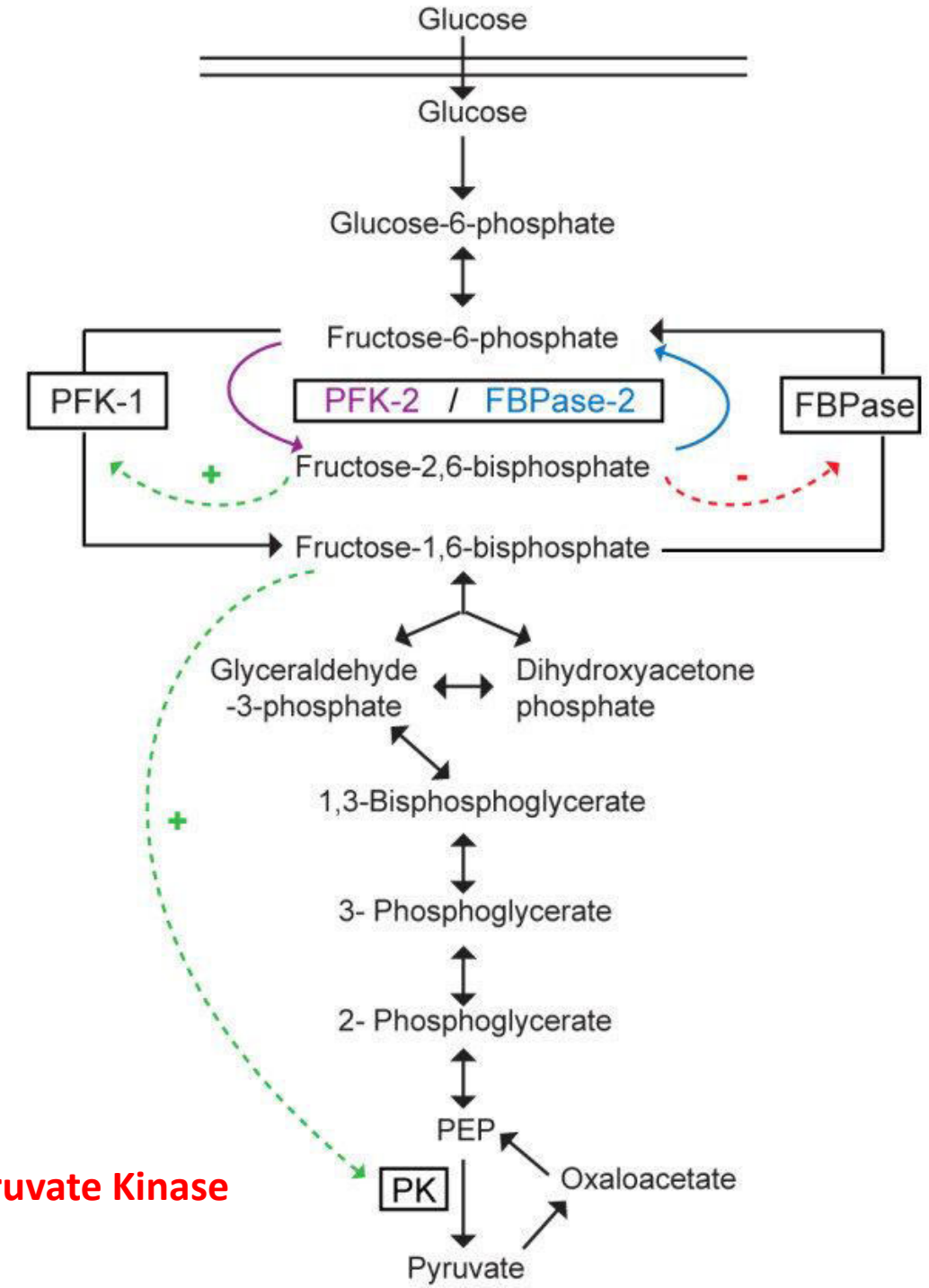
Substrate Does Change Enzyme Binding of Substrate



Allosteric Inhibitiors



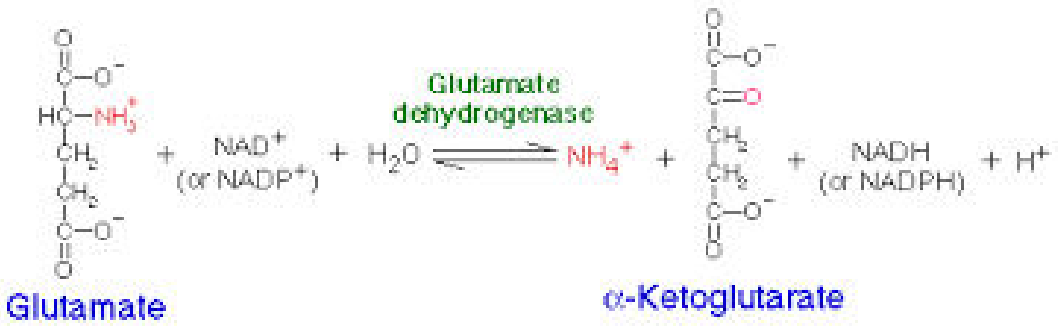
Allosteric Activators

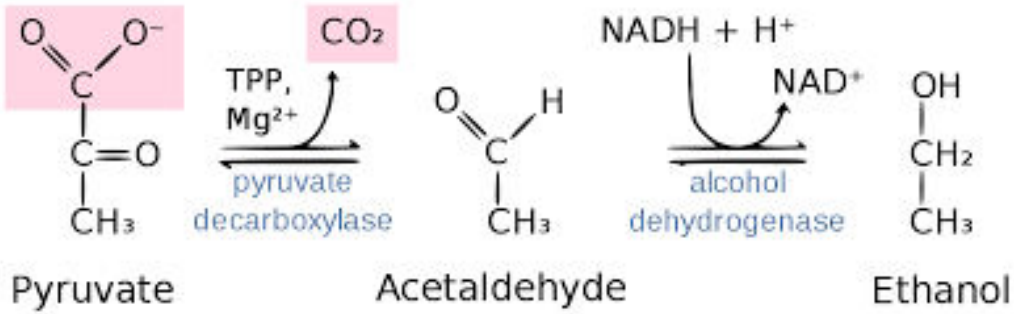


Pyruvate Kinase

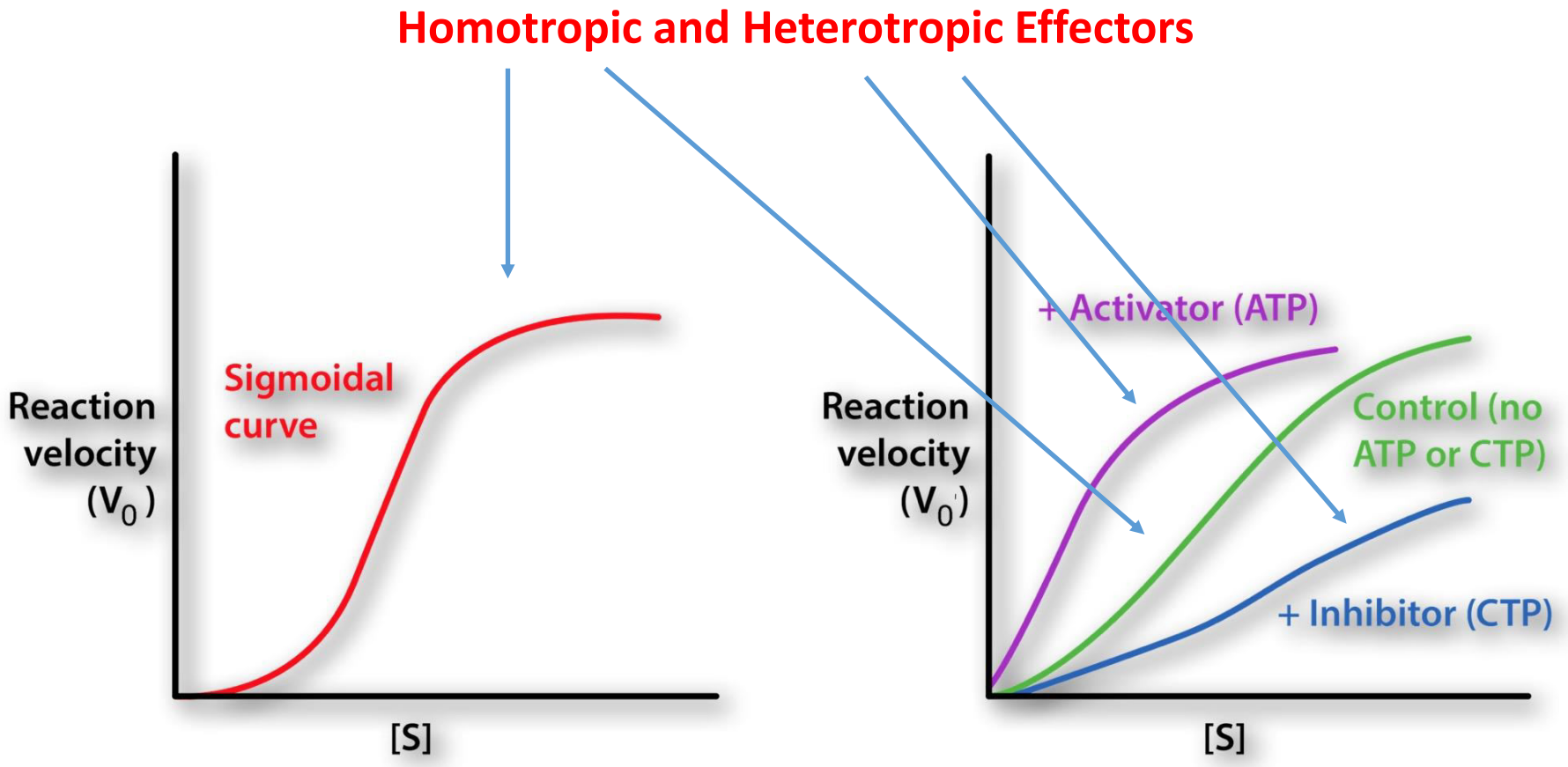
Allosteric modulation

Enzyme	Activators	Inhibitors
Glutamate Dehydrogenase	ADP	ATP, NADH
Hexokinase	ADP	Glucose 6 Phosphate and ATP
Pyruvate Decarboxylase	Acetyl CoA	ADP



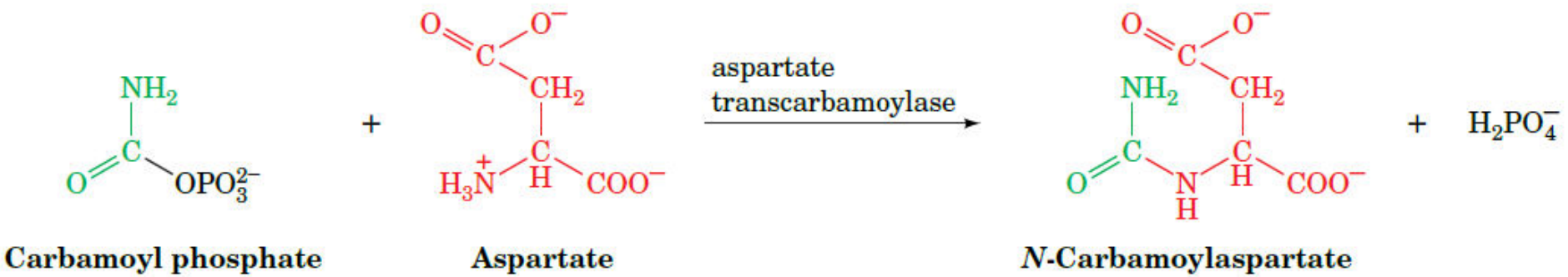


Homotropic and Heterotropic Effectors



Allosteric Control

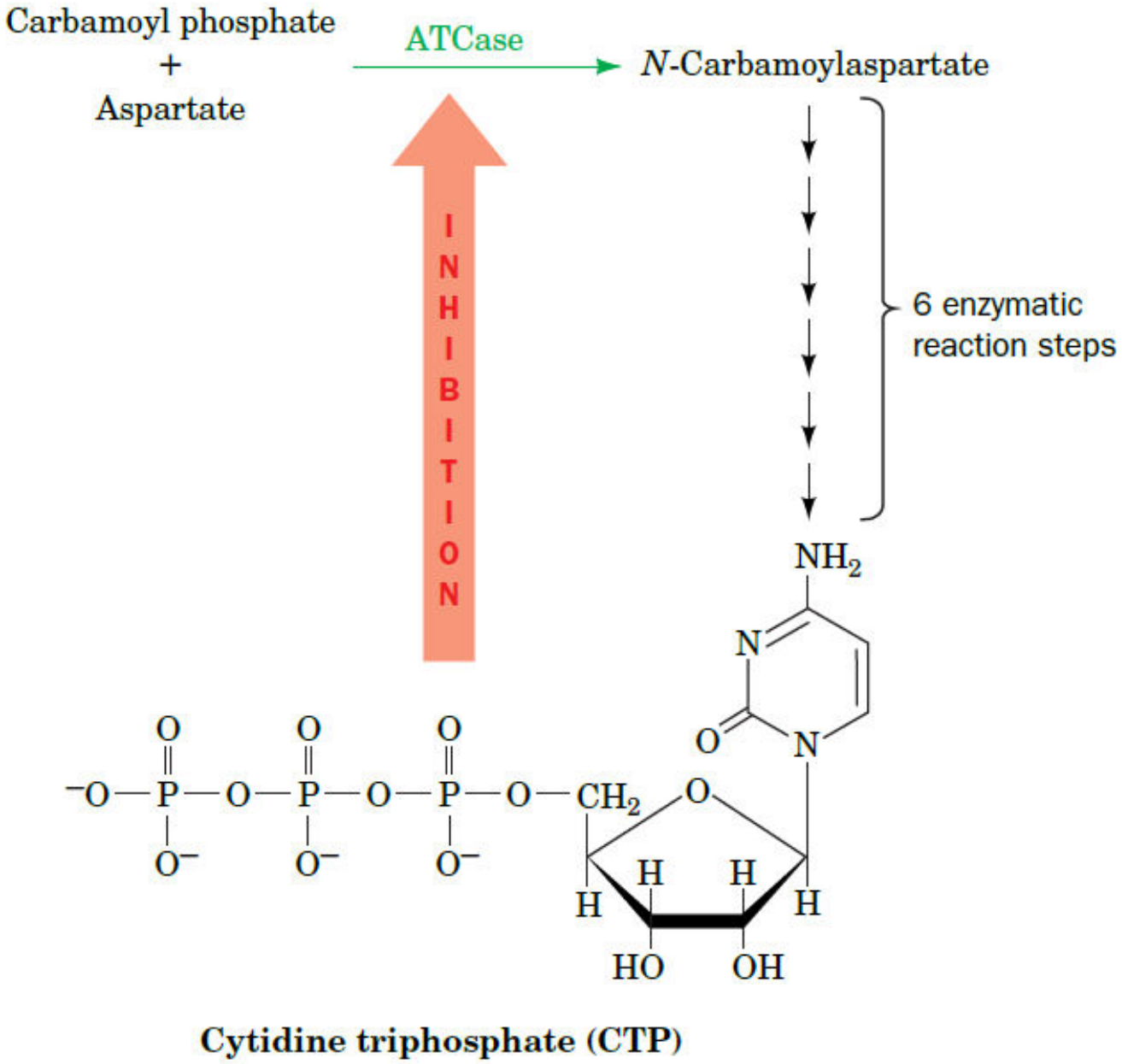
Aspartate trans-carbamoylase (ATCase)

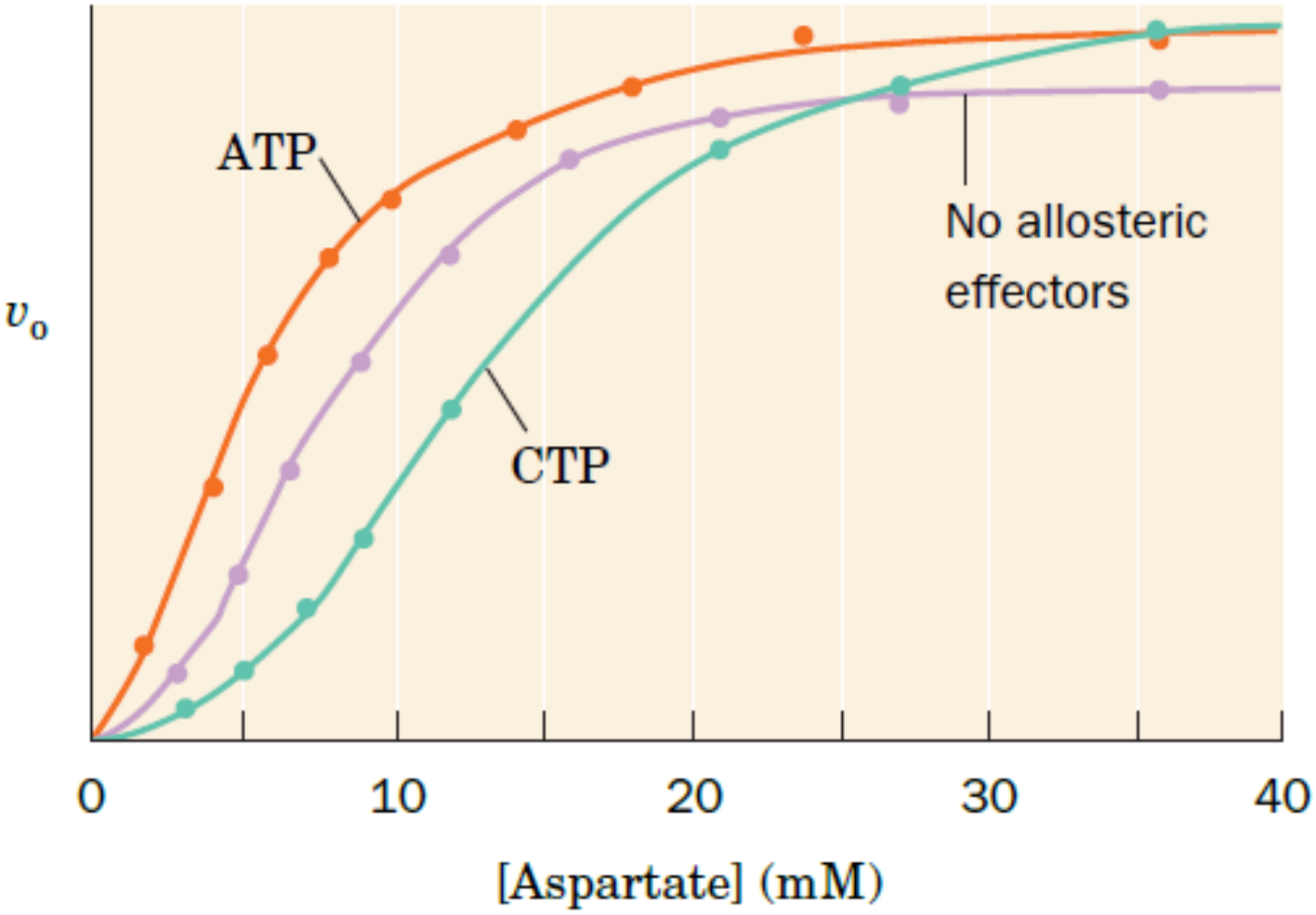


- This reaction is the first step for the **biosynthesis of pyrimidines**.
- The allosteric behavior of *E. coli* ATCase has been investigated by **John Gerhart and Howard Schachman**, who demonstrated that both of its substrates bind cooperatively to the enzyme.

Feedback inhibition

CTP, which is a product of the pyrimidine biosynthetic pathway, is an example of a feedback inhibitor, since it inhibits an earlier step in its own biosynthesis.





0.4 mM CTP (an inhibitor)
2.0 mM ATP (an activator)

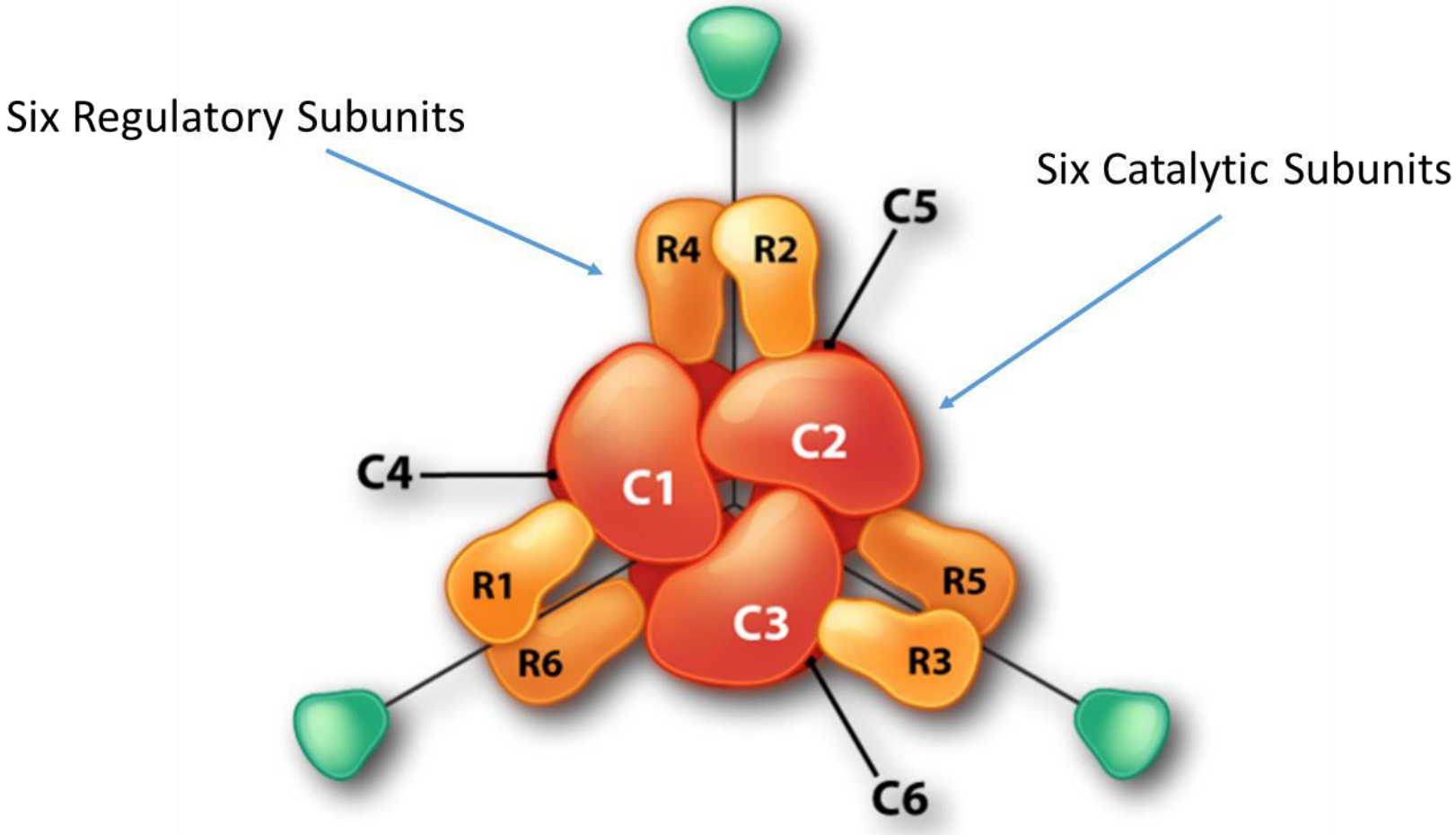
- The Vo versus [S] curve for ATCase is **sigmoidal**, rather than hyperbolic as it is in enzymes that follow the Michaelis-Menten model.
- ATCase is **allosterically inhibited** by cytidine triphosphate (CTP), a pyrimidine nucleotide, and is **allosterically activated** by adenosine triphosphate (ATP), a purine nucleotide.
- **Cooperative substrate binding.**

Allosteric Changes Alter ATCase's Substrate-Binding Sites

- *E. coli* ATCase (300 kD) has the subunit composition **c6r6**, where c and r represent its catalytic and regulatory subunits.

- The X-ray structure of ATCase, determined by William Lipscomb, reveals that the **catalytic subunits are arranged as two trimers (c3)** in complex with **three regulatory dimers (r2)**.

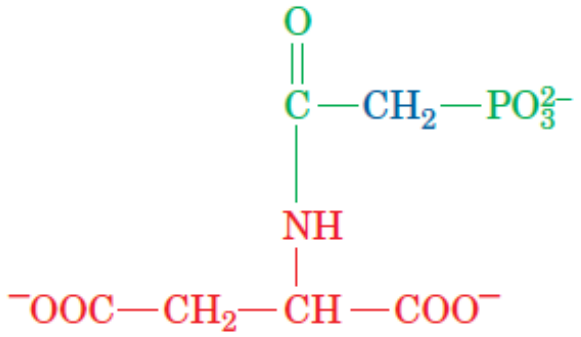
- Each **regulatory dimer joins two catalytic subunits in different c3 trimers**.


Six Regulatory Subunits
Six Catalytic Subunits
R4
R2
C5
C4
C1
C2
R1
C3
R5
R6
R3
C6

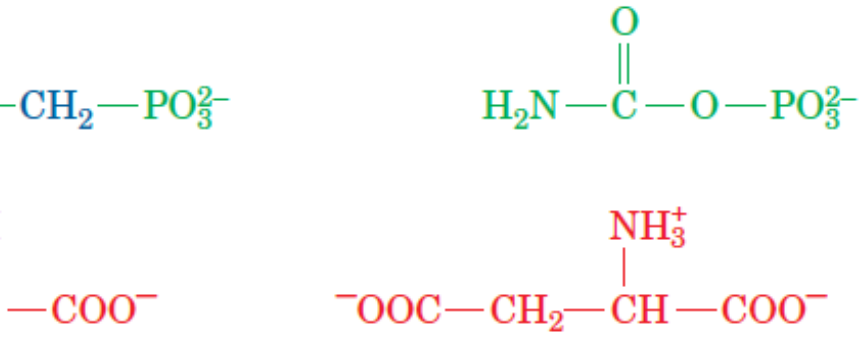
- The **isolated catalytic trimers are catalytically active**, have a maximum catalytic rate greater than that of intact ATCase, exhibit a **noncooperative (hyperbolic) substrate saturation curve**, and are **unaffected by the presence of ATP or CTP**.

- The **isolated regulatory dimers bind the allosteric effectors but are devoid of enzymatic activity**.

- Evidently, the **regulatory subunits allosterically reduce the activity of the catalytic subunits** in the intact enzyme.



***N*-(Phosphonacetyl)-L-aspartate (PALA)**

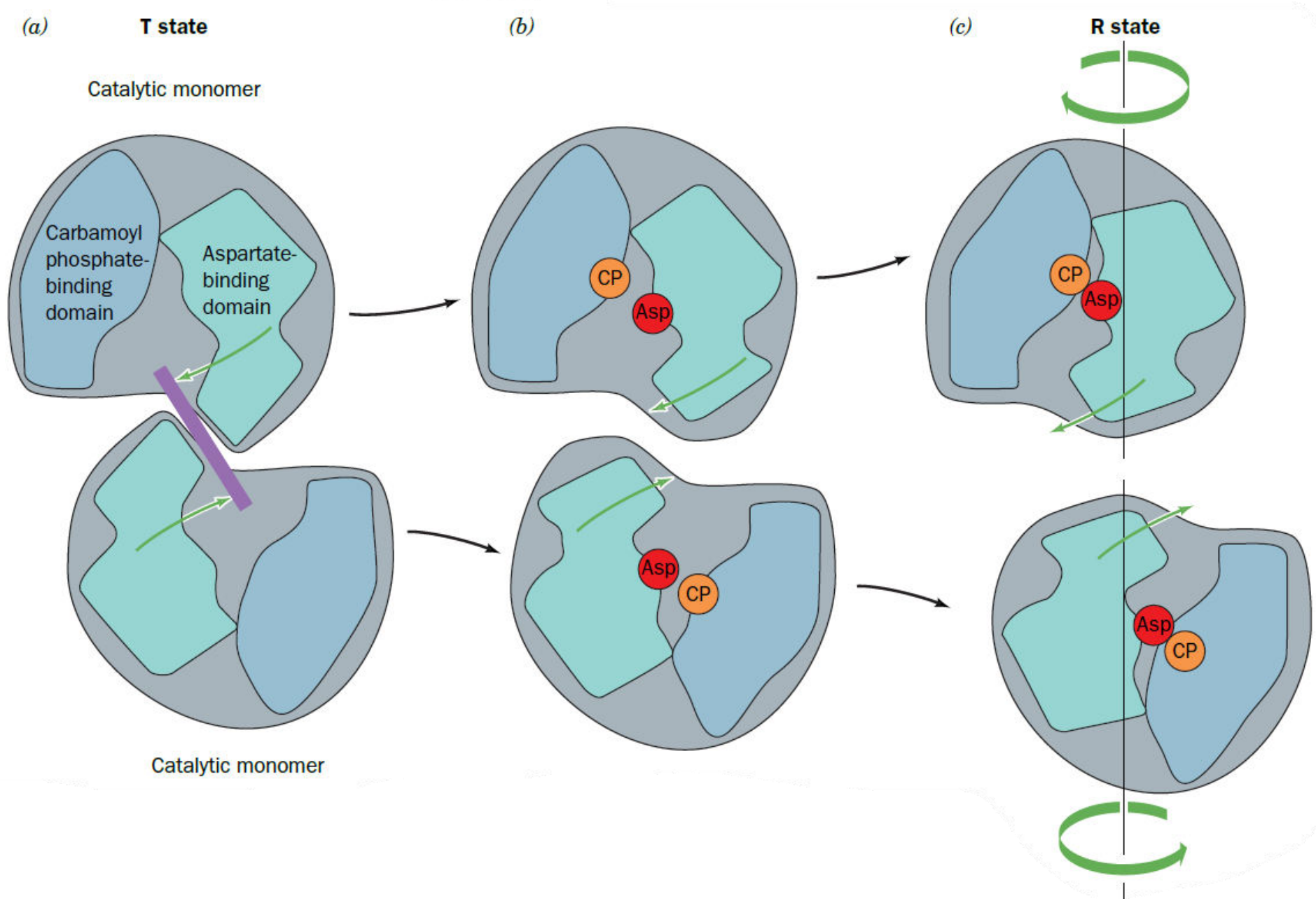


Carbamoyl phosphate
+
Aspartate

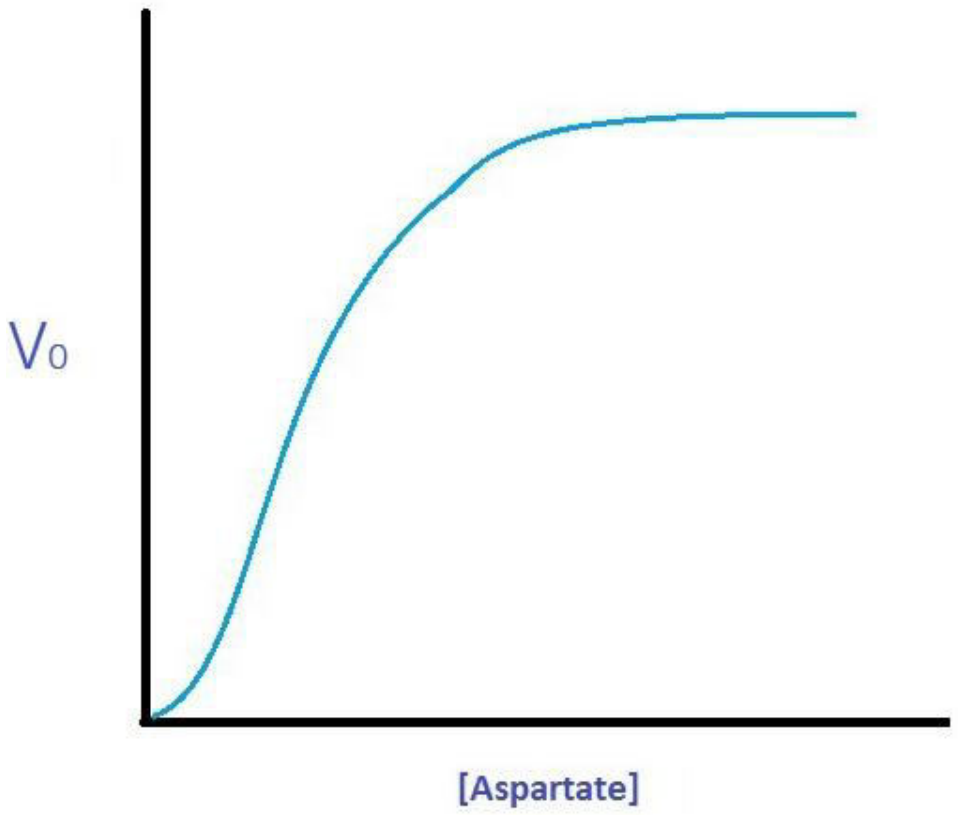
- X-Ray structures have been determined for the T-state **ATCase-CTP complex** and the R-state **ATCase-PALA** complex.

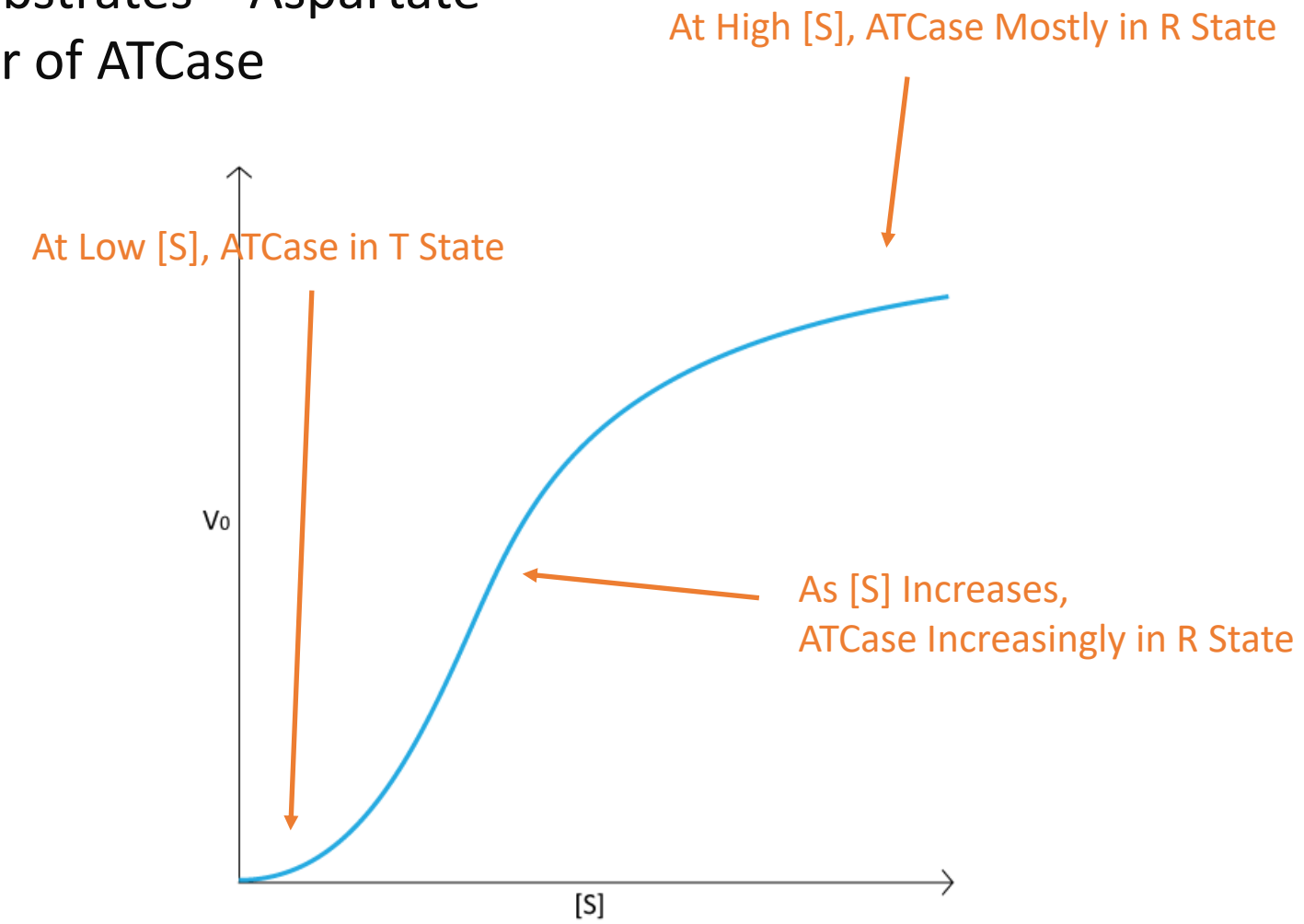
- Structural studies reveal that in the T → R transition, the enzyme's catalytic trimers separate along the molecular threefold axis by ~11 Å and reorient around this axis relative to each other by 12°.

- In addition, the regulatory dimers rotate clockwise by 15° around their twofold axes and separate by ~4 Å along the threefold axis.


(a)
T state
(b)
(c)
R state
Catalytic monomer
Carbamoyl phosphate-binding domain
Aspartate-binding domain
CP
Asp
Asp
CP
CP
Asp
Asp
CP
Catalytic monomer

- ATCase is affected by one of its substrates – Aspartate
- Aspartate is a Homotropic Effector of ATCase





Binding of Aspartate by ATCase favors the R-State so additional Substrate Binding is favored

The Binding of Allosteric Modifiers Causes Structural Changes in ATCase

- Both the **inhibitor CTP** and the **activator ATP** bind to the same site on the outer edge of the regulatory subunit, about 60 Å away from the nearest catalytic site.

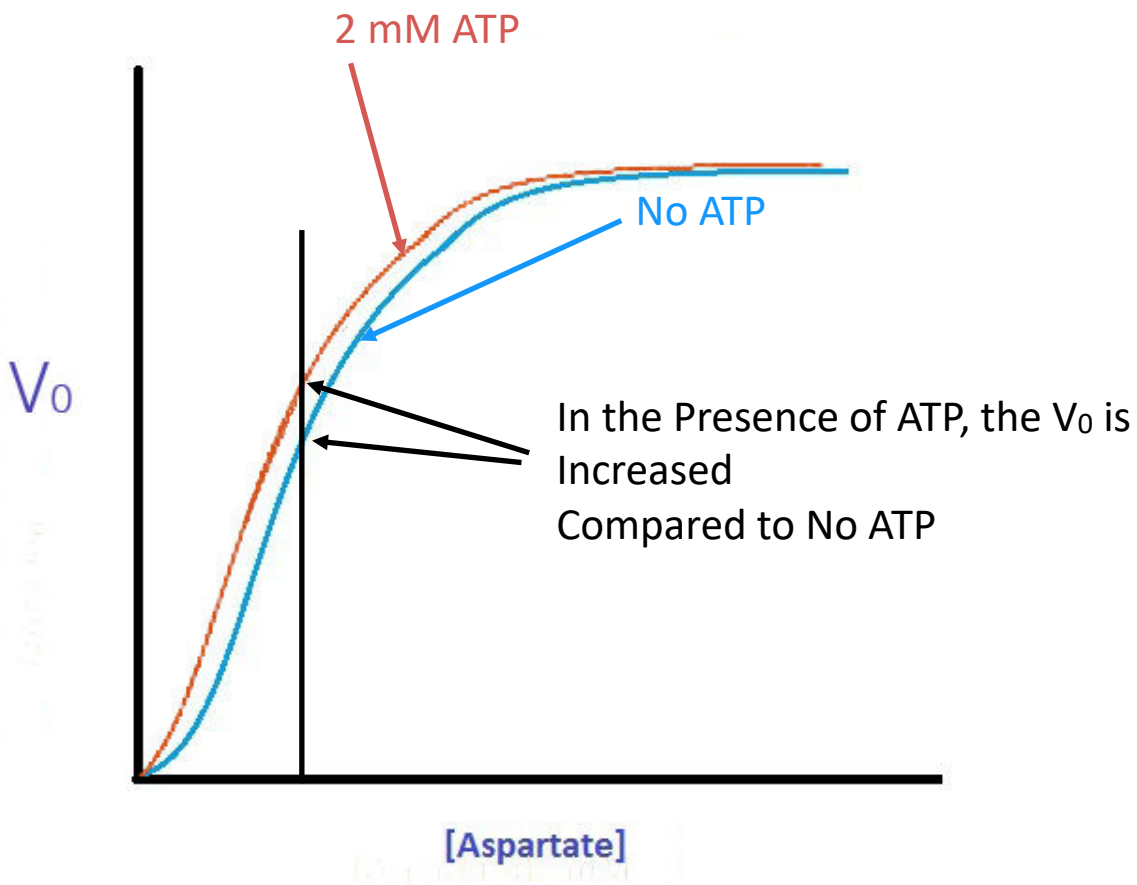
- CTP binds preferentially to the T state, increasing its stability, whereas ATP binds preferentially to the R state, increasing its stability.

- When CTP binds to R-state ATCase, it induces a contraction in the regulatory dimer that causes the catalytic trimers to come together by 0.5 Å (become more T-like; that is, less active).

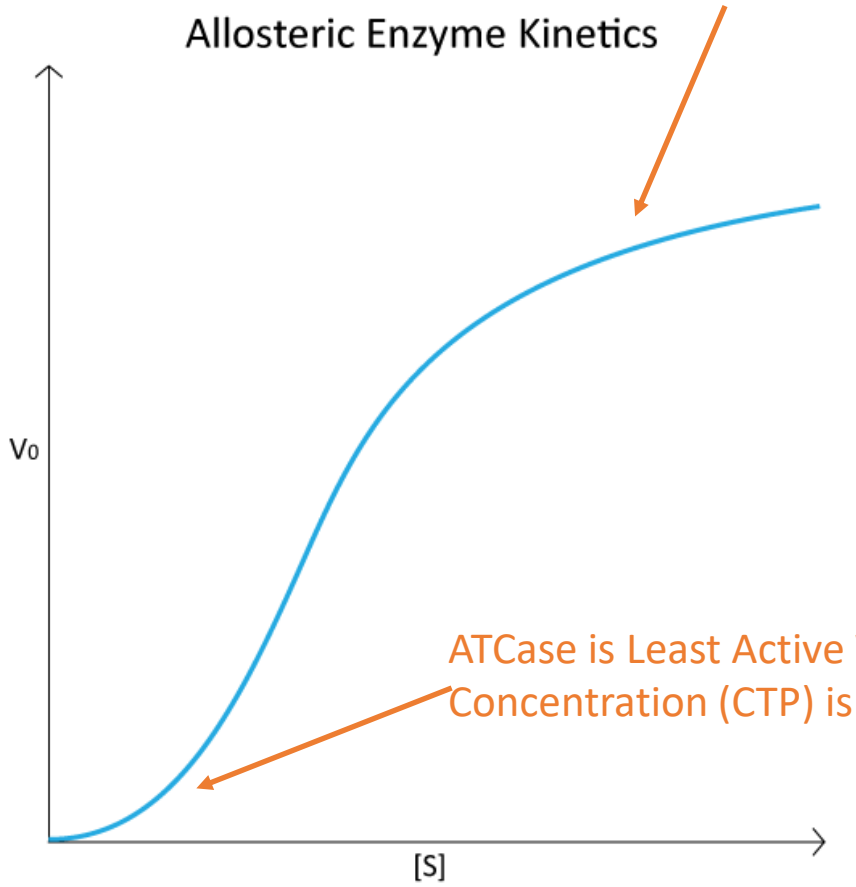
- ATP has essentially opposite effects when binding to the T-state enzyme: It causes the catalytic trimers to move apart by 0.4 Å (become more R-like; that is, more active).

Allosteric Control of ATCase

ATP Activates ATCase (Converts to R State)



Thus, ATCase is Most Active When Energy (ATP) is High and When Pyrimidines are Low in Concentration Relative to Purines



Aspartate is a Substrate,
but Neither ATP nor CTP is.
All Affect the Enzyme

ATP and CTP Bind Regulatory Sites
ATP Favors R State
CTP Favors T State

Six Regulatory Subunits - R1 to R6

Six Catalytic Subunits - C1 to C6

Aspartate Binds to
Catalytic Subunits
Favors R State