

Chem 452 – Lecture 6

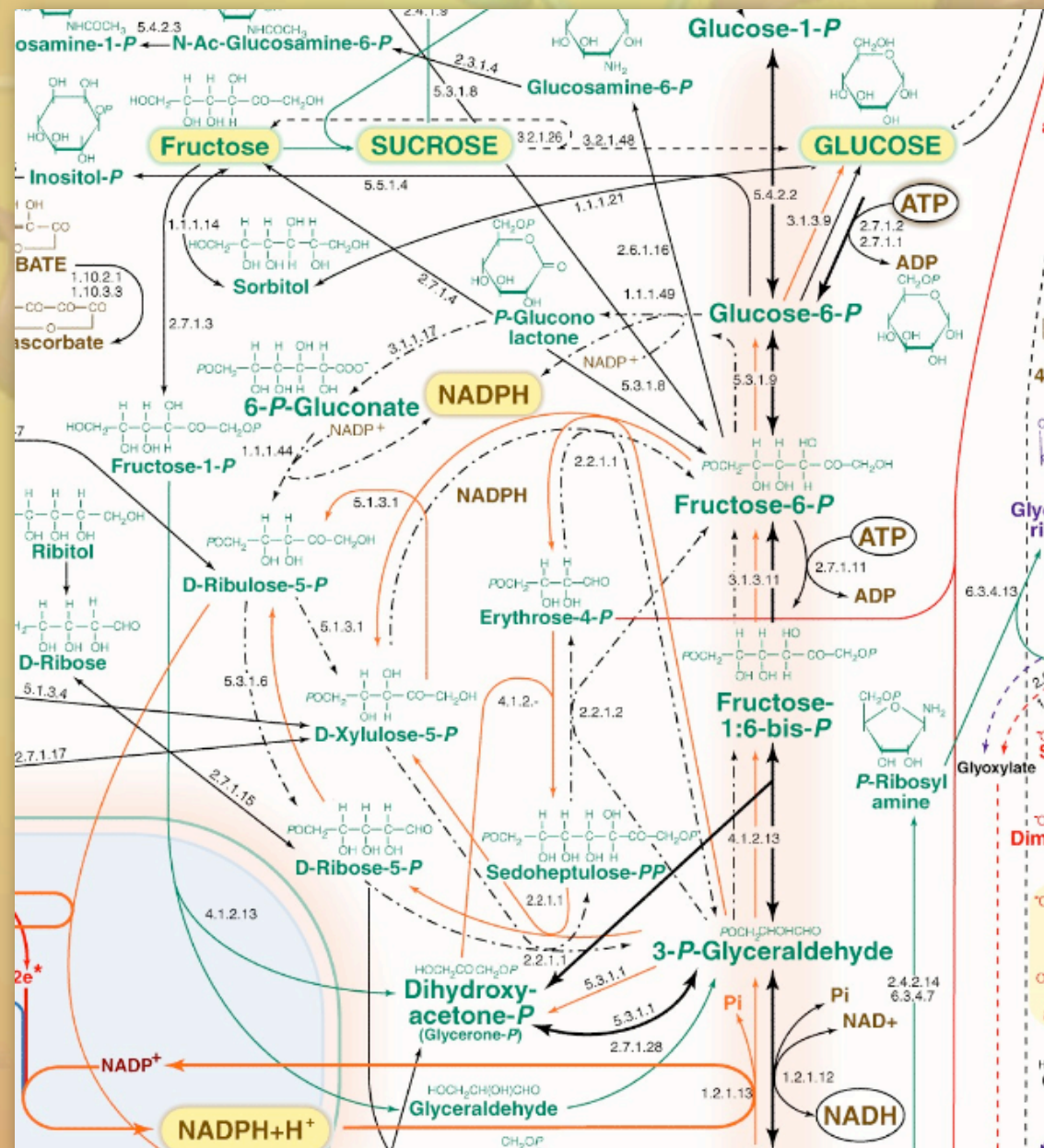
Regulatory Strategies

111031

Living cells contain thousands of metabolites linked to one another by a dizzying array of chemical reactions. These reactions link one metabolite to another and collectively are arranged into metabolic pathways, which crisscross and intersect to form a large interconnected network. Each reaction is catalyzed by one or more enzymes and many of these enzymes play a large role in controlling the flow of material through the network. In this lecture we will focus on some of the strategies used to regulate enzyme activity, and consequently, metabolic processes.

Introduction

- ✦ Metabolism comprises a vast network of interconnecting metabolic pathways.

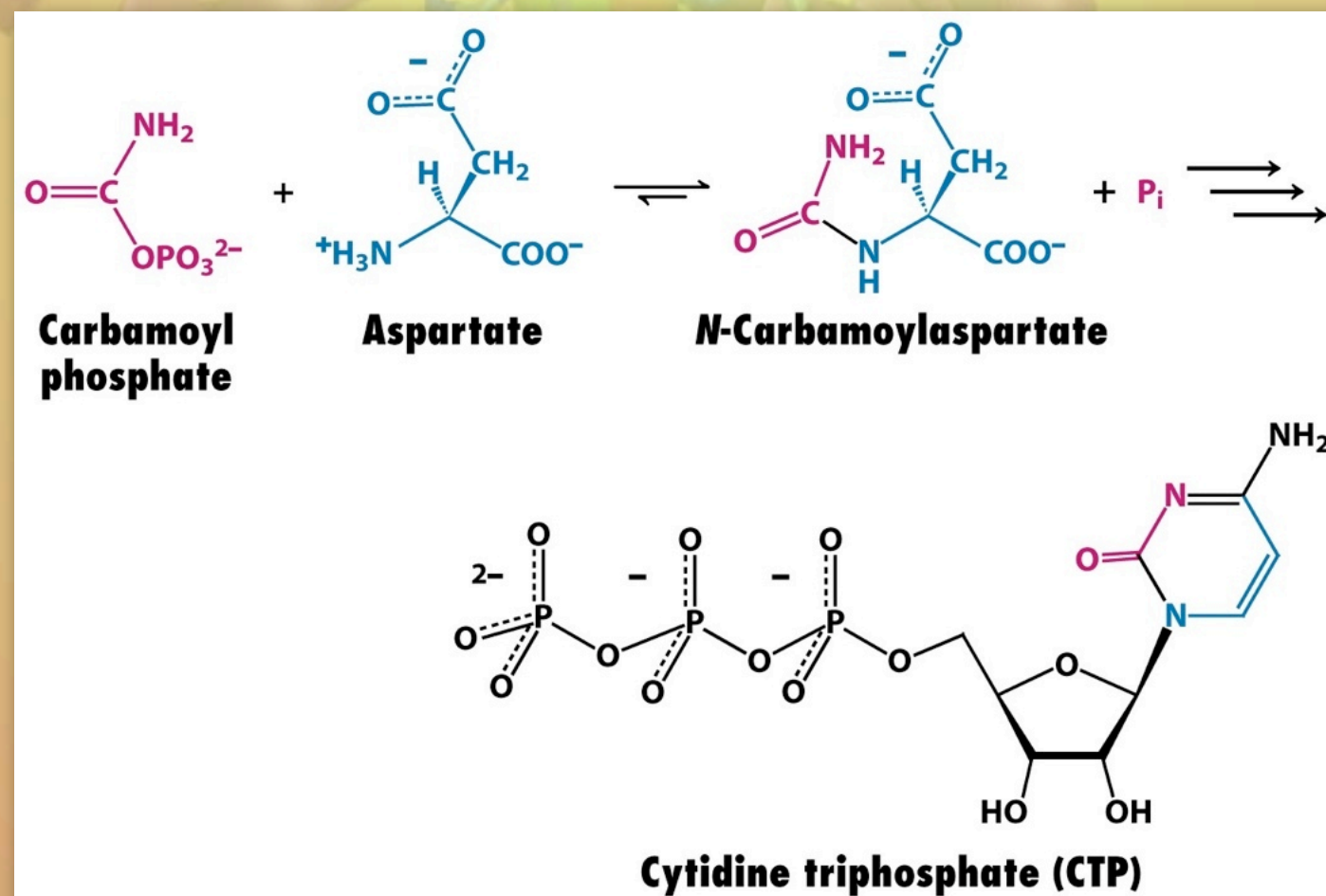


Introduction

- ✦ One of the primary strategies for regulating metabolism is to regulate the activity of some of the key enzymes in this network.
- ✦ There are several mechanisms used to do this:
 - **Allosteric Control**
 - **Multiple Forms of Enzymes (Isozymes)**
 - **Reversible Covalent Modifications**
 - **Proteolytic Activation**
 - **Controlling the level of Enzyme Present**

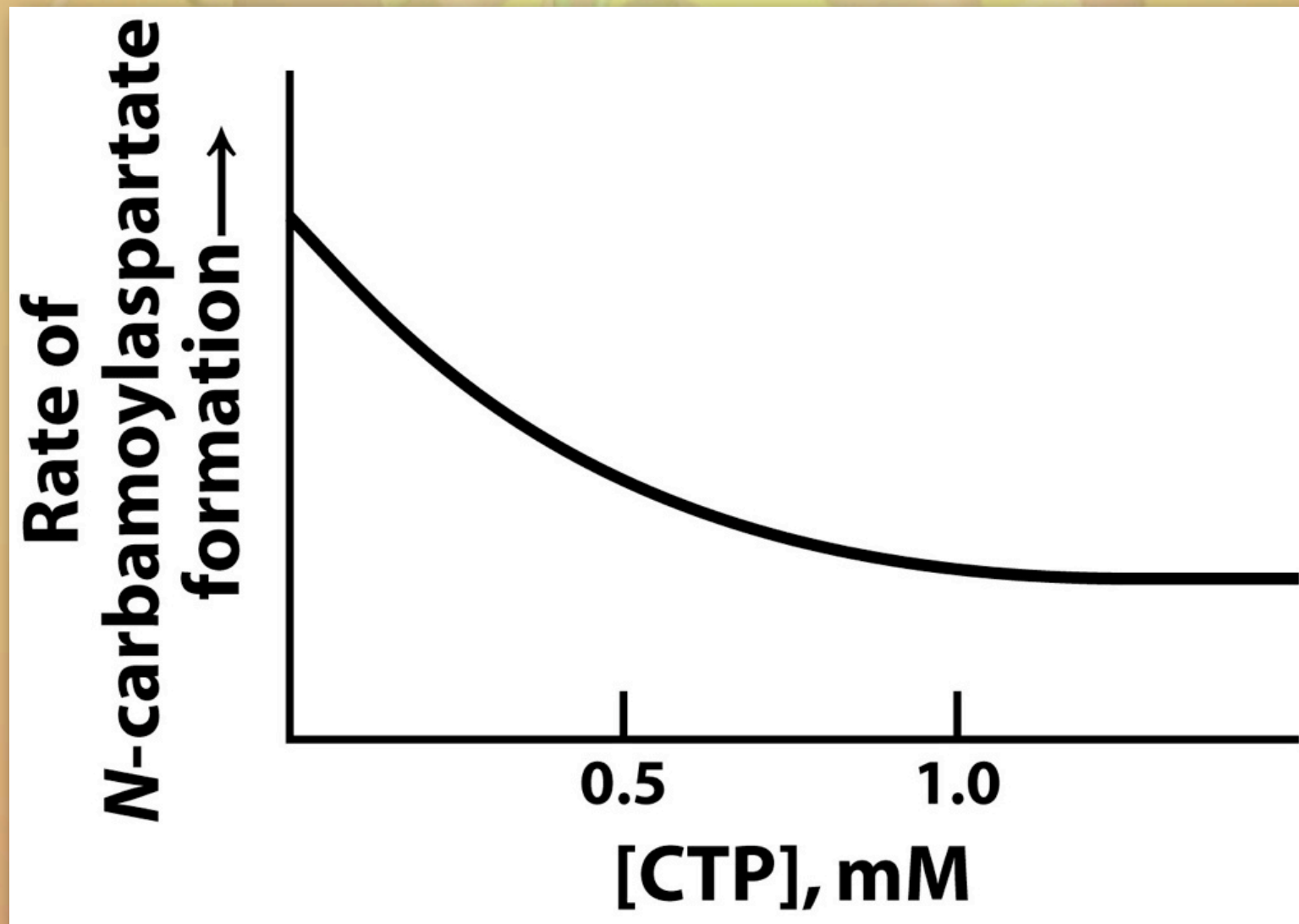
ATCase is Allosterically Regulated

- ♦ Aspartate transcarbamoylase (ATCase) provides an good example of allosteric control.
- ATCase catalyzes the first reaction in the pathway leading to the synthesis of the nucleotide cytidine triphosphate



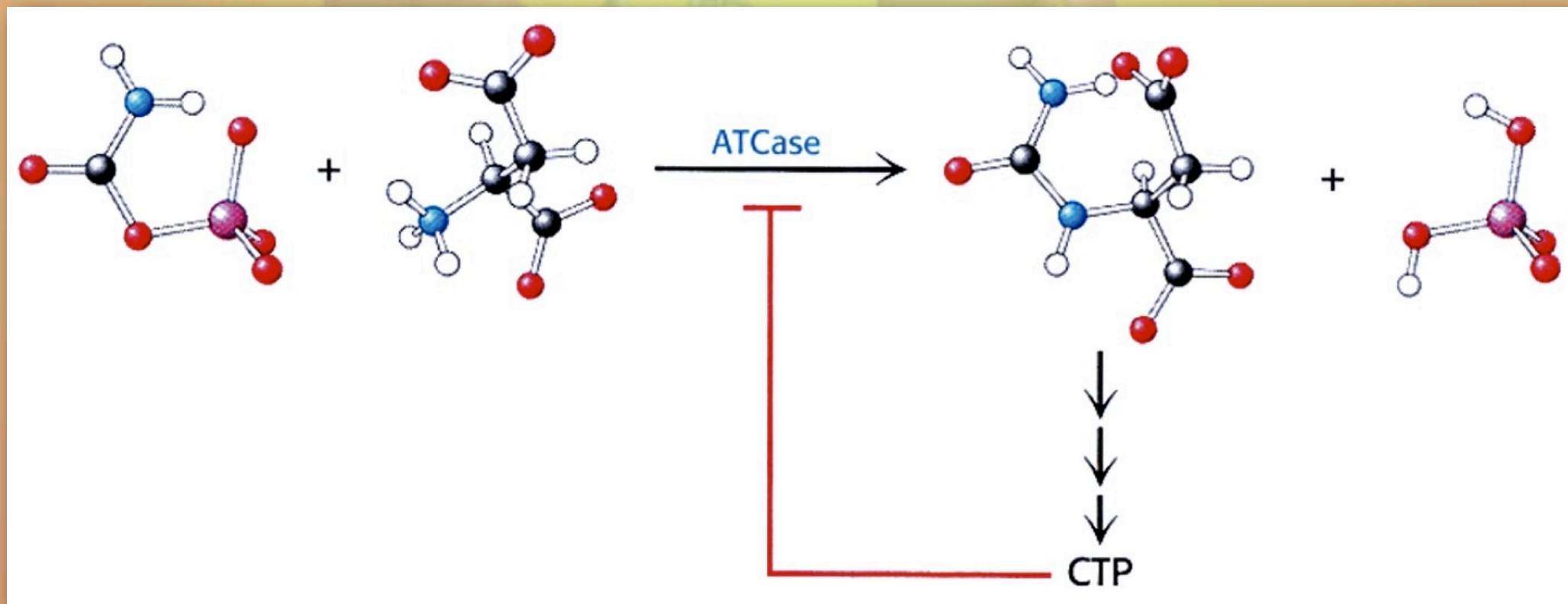
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- ♦ ATCase is inhibited by the end-product of this pathway (CTP).



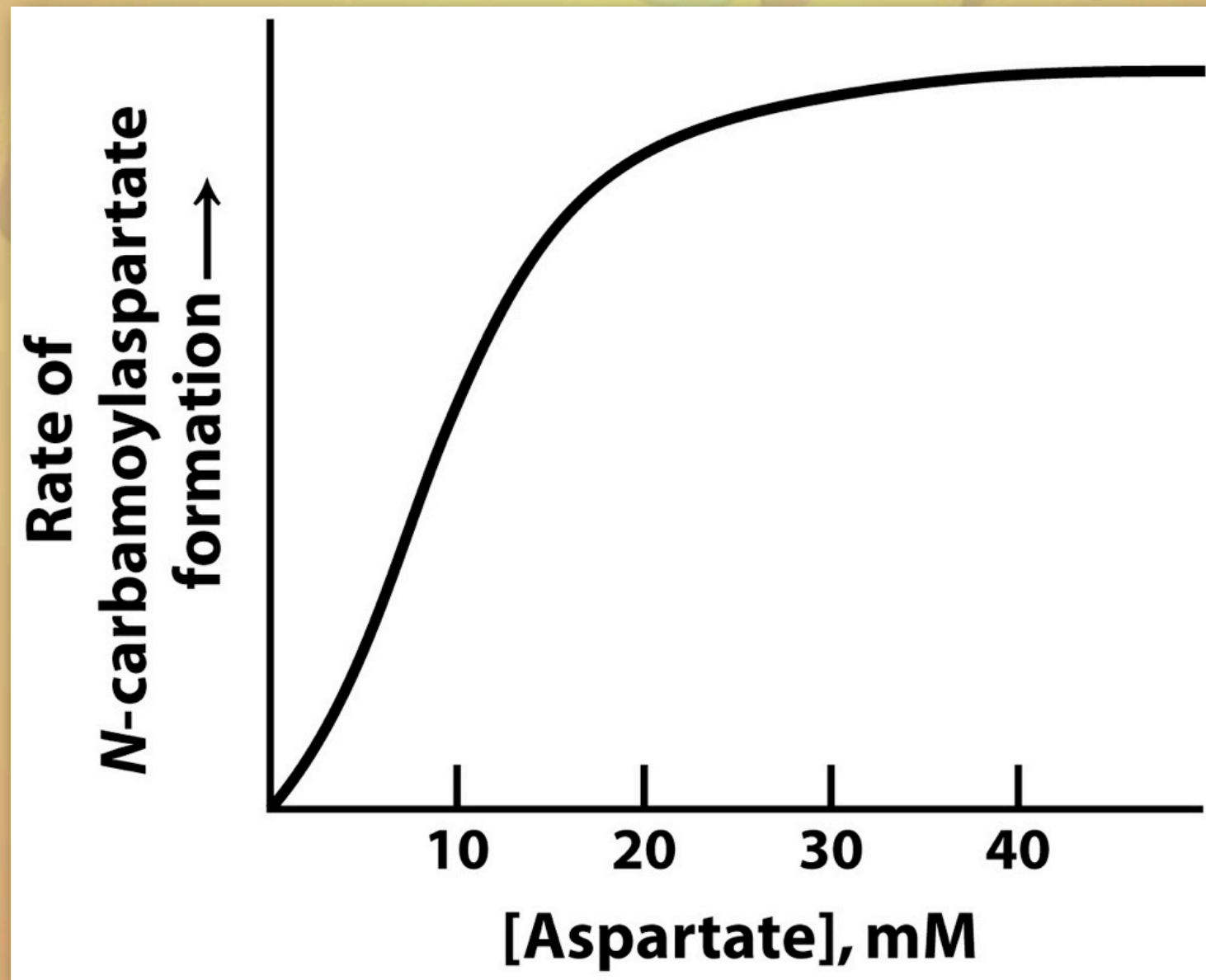
ATCase is Allosterically Regulated

- ♦ ATCase is inhibited by the end-product of this pathway (CTP).
 - This is an example of **feedback inhibition**.
 - This is a common strategy used to regulate biosynthetic pathways.



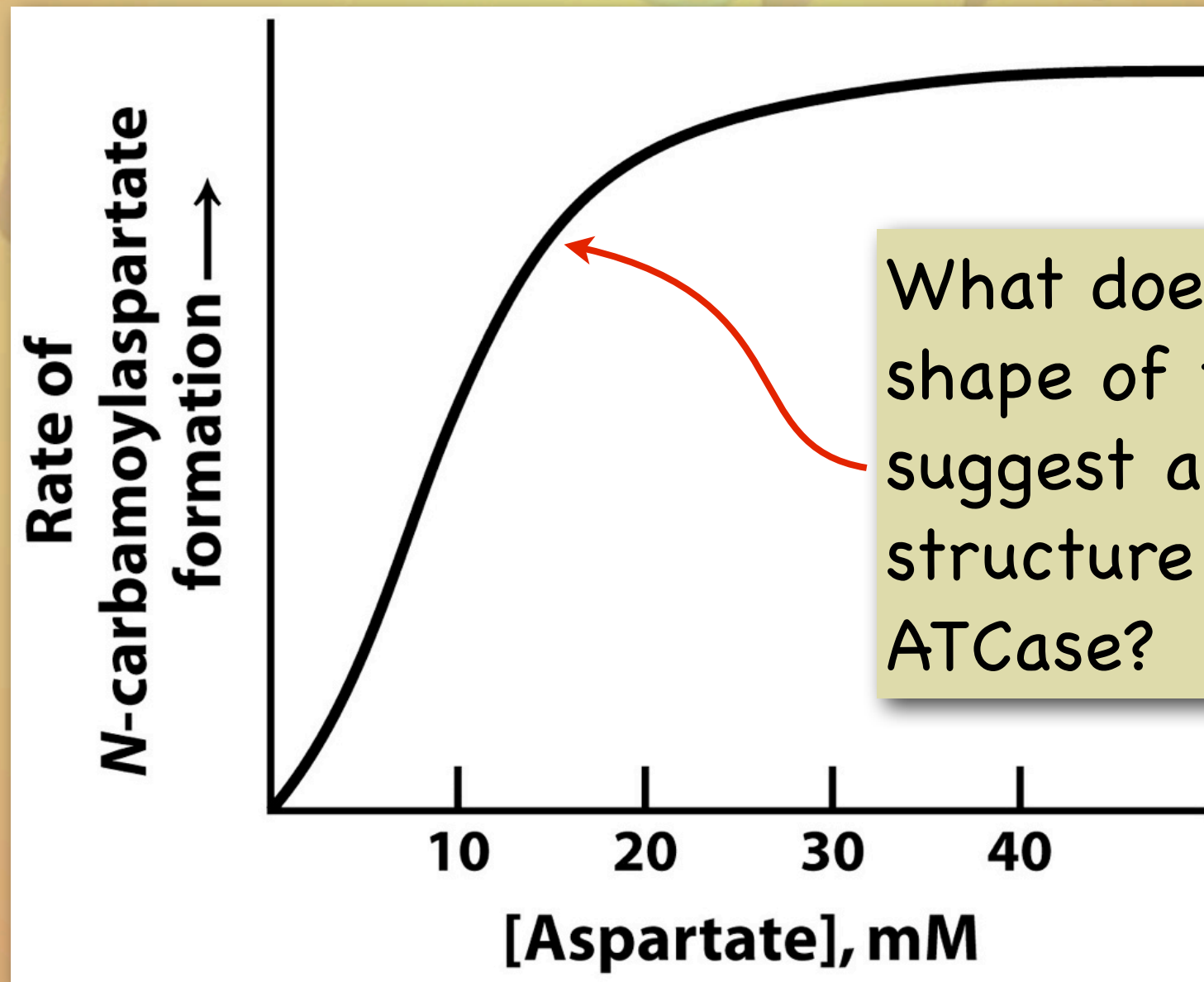
ATCase is Allosterically Regulated

- ✦ Most allosterically regulated enzymes do not obey Michaelis-Menten kinetics.



ATCase is Allosterically Regulated

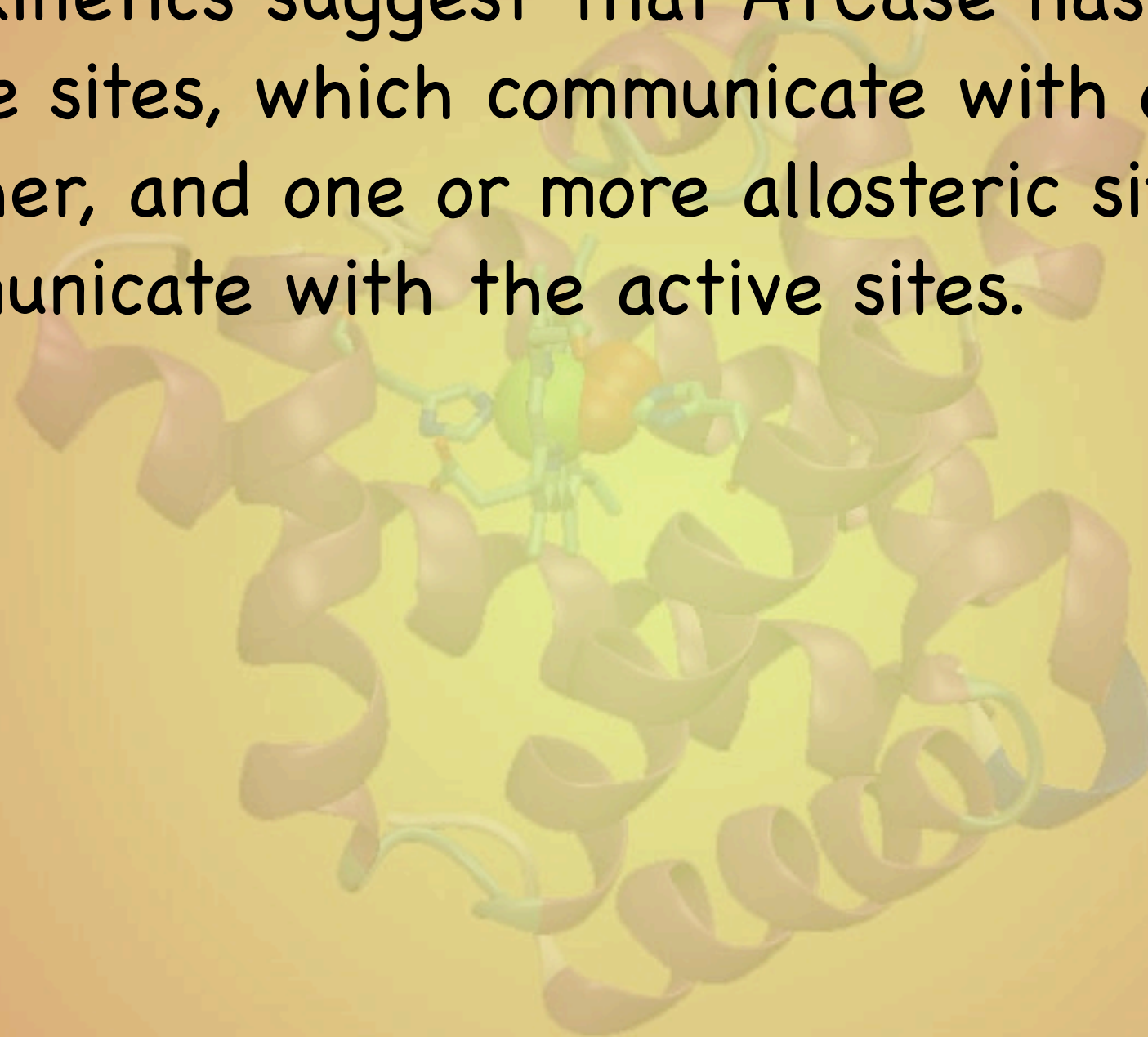
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What does the shape of this curve suggest about the structure of ATCase?

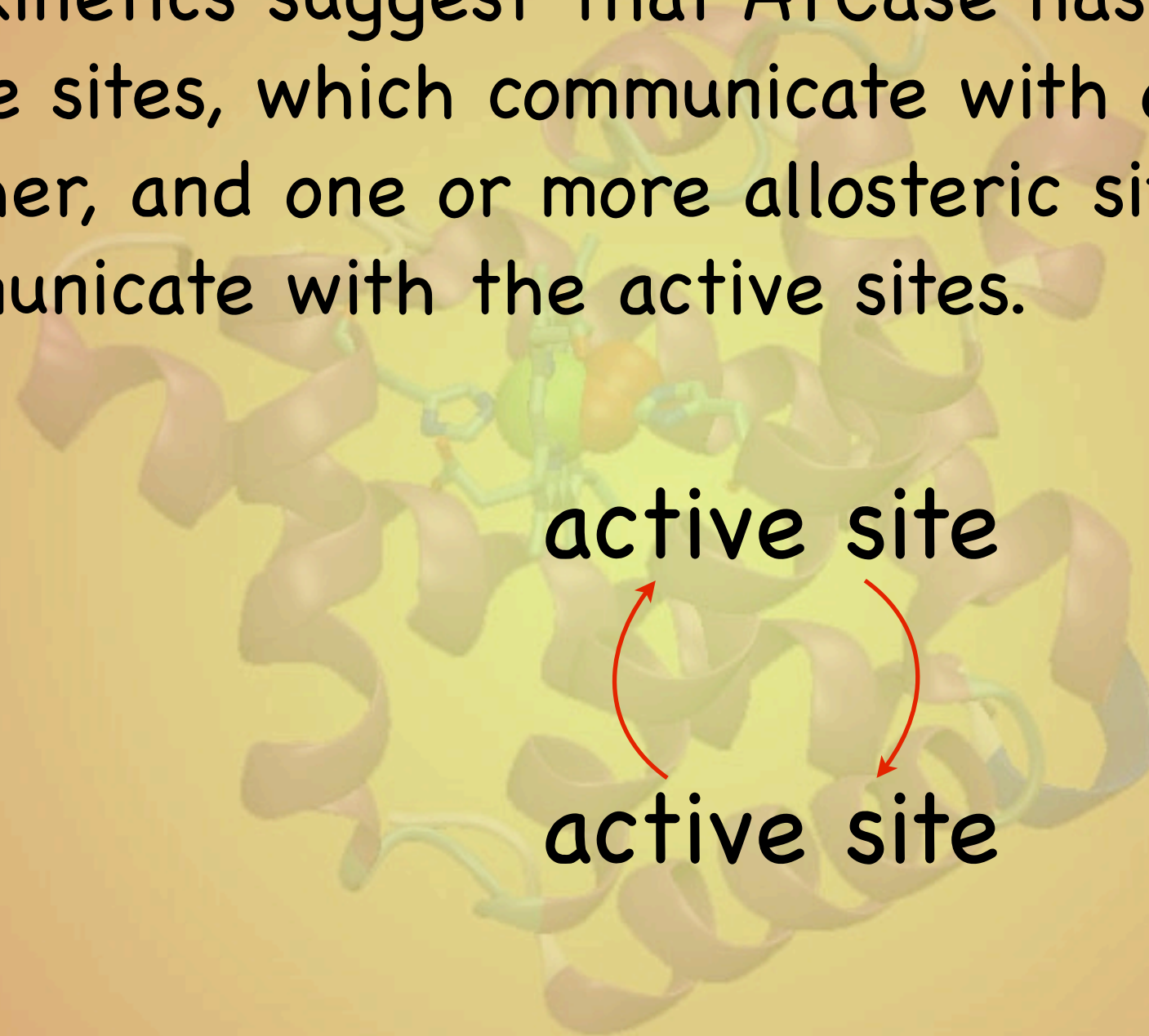
ATCase is Allosterically Regulated

- ✦ The kinetics suggest that ATCase has multiple active sites, which communicate with one another, and one or more allosteric sites, which communicate with the active sites.



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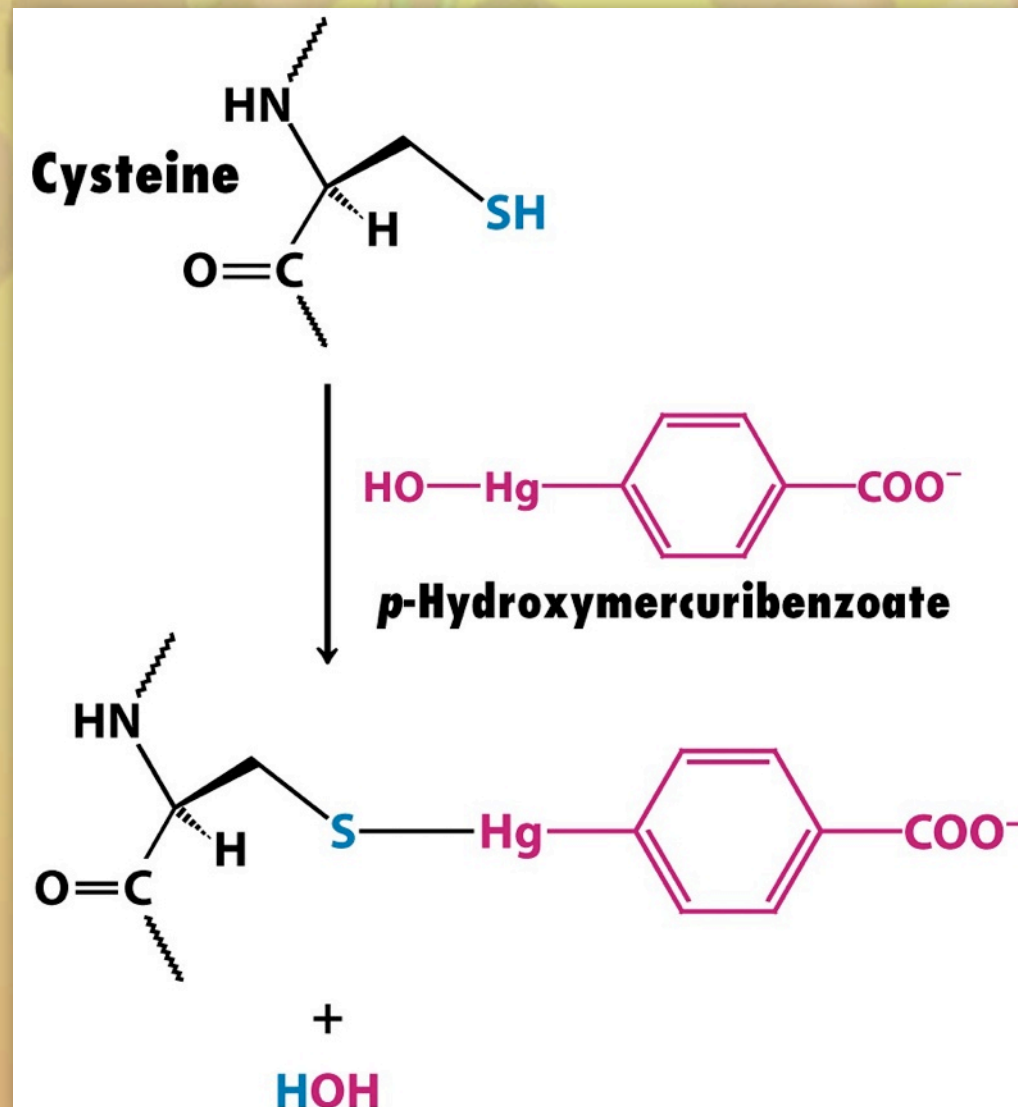
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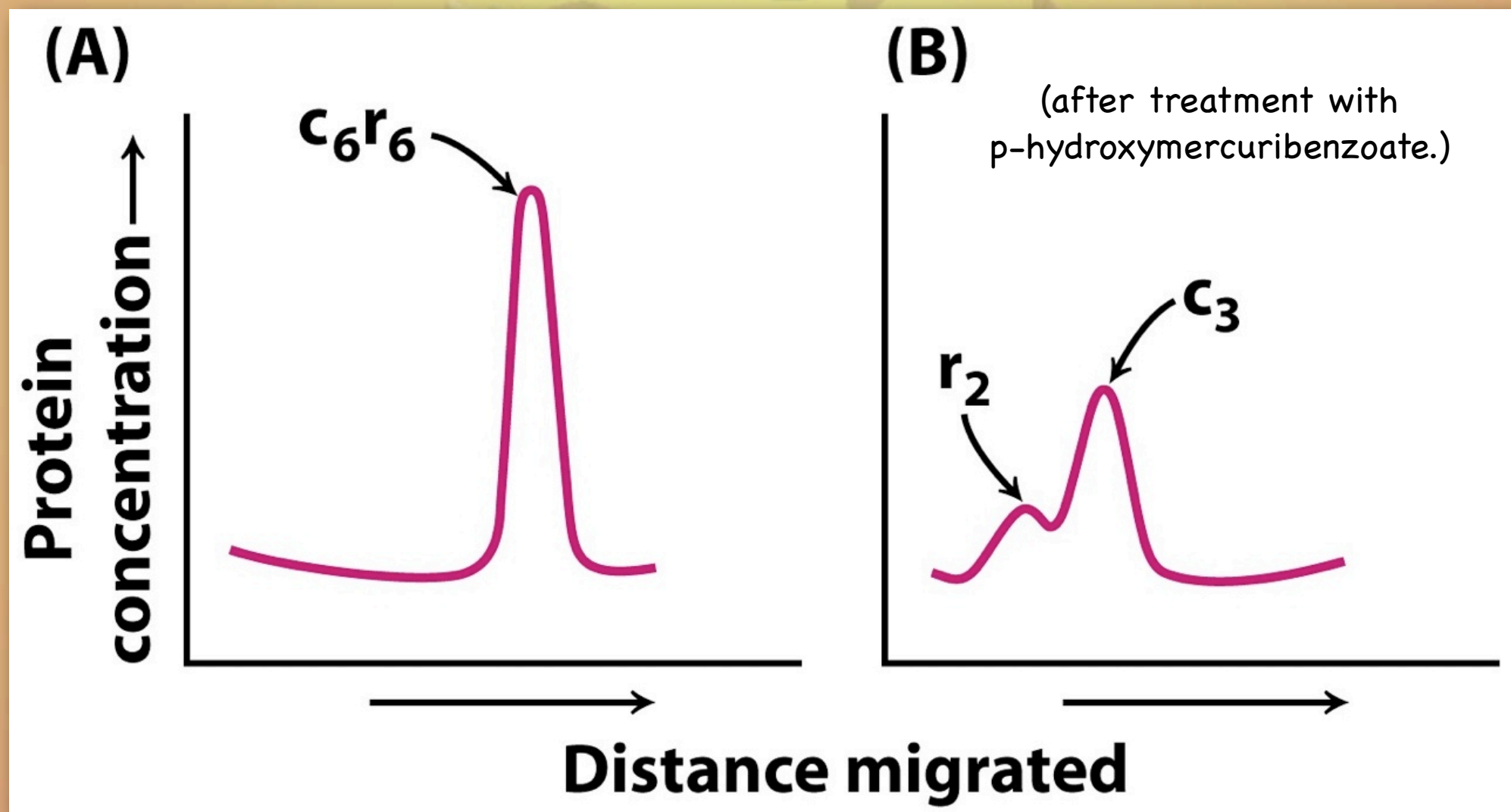
ATCase is Allosterically Regulated

- ♦ ATCase can be separated into individual subunits by reacting it with p-hydroxymercuribenzoate.



ATCase is Allosterically Regulated

- ✦ The subunits can then be separated by ultracentrifugation.



ATCase is Allosterically Regulated

- ✦ The subunit arrangement was determined by the ultracentrifugation studies:
 - There are 2 types of subunits (c and r)
 - There are 6 of each type (c_6r_6)
 - The c subunits form trimers (c_3)
 - The r subunits form dimers (r_2)

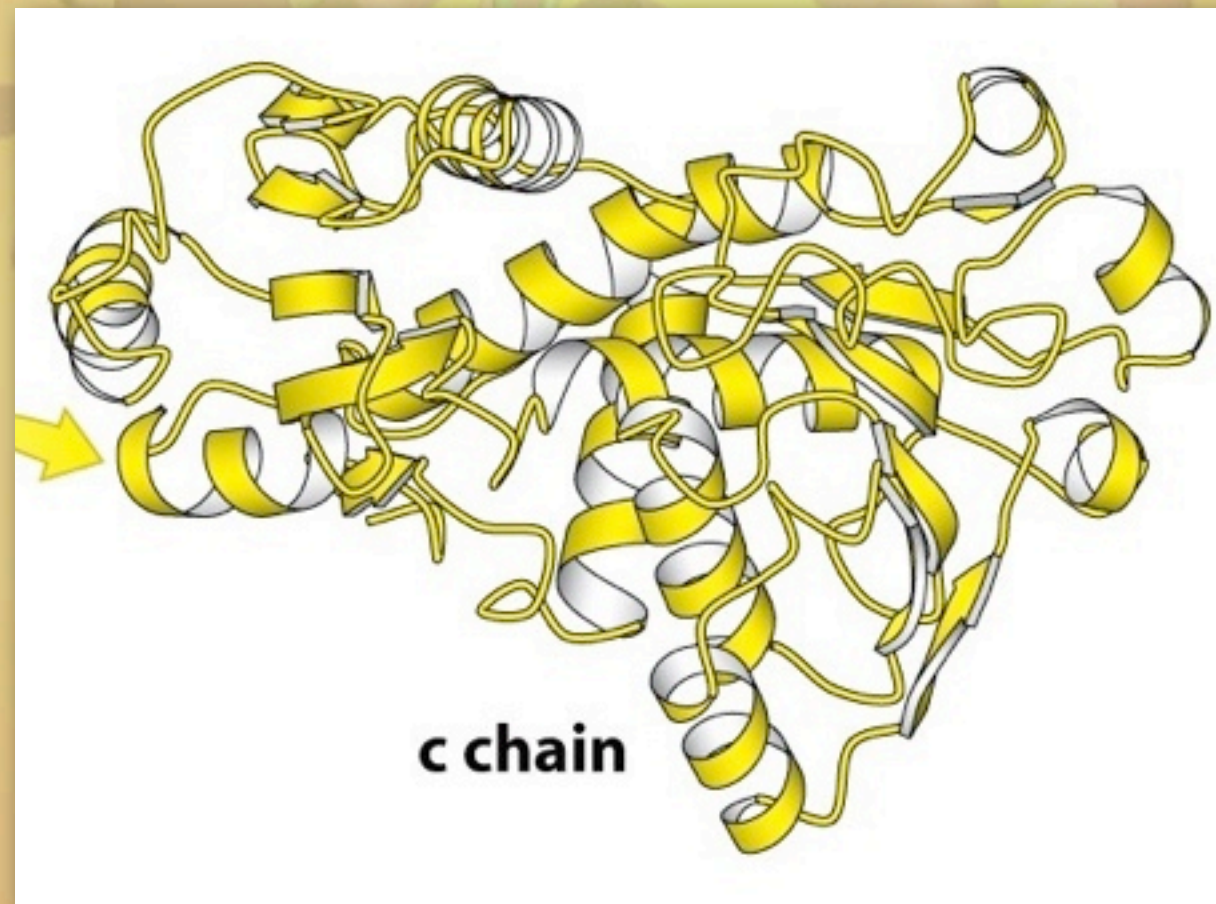
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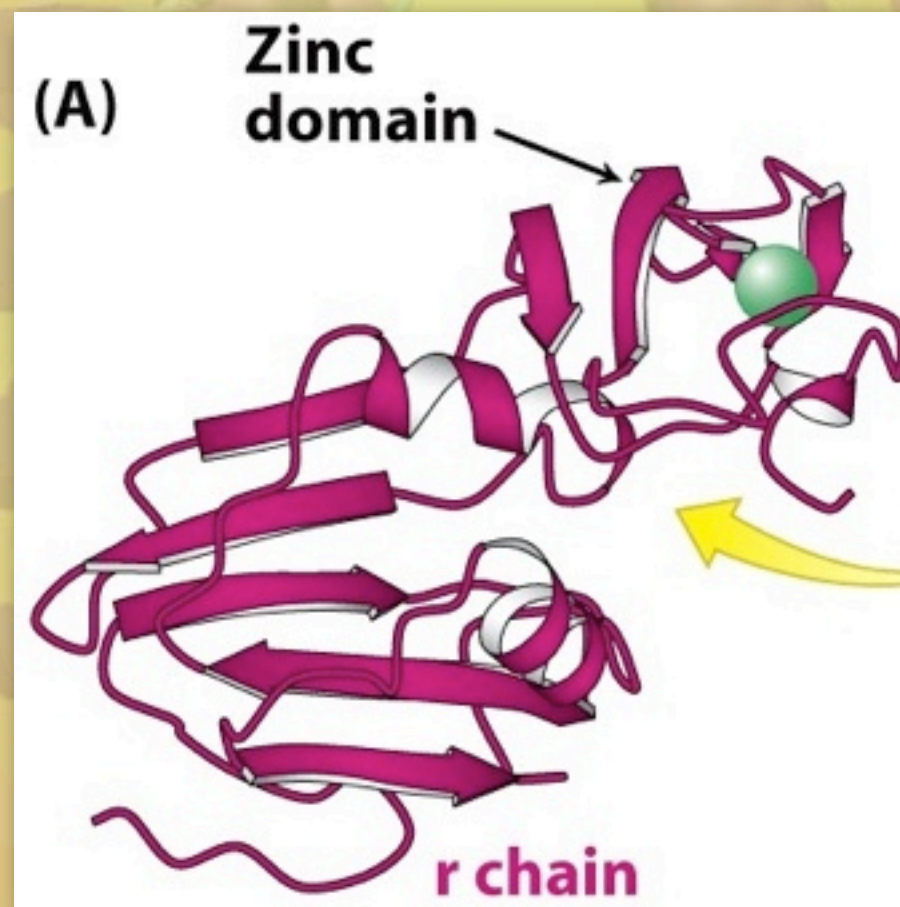
- ✦ The larger, **c** subunits (34 kDa each) are the **catalytic** subunits and contain the active sites.



[View Live Figure](#)

ATCase is Allosterically Regulated

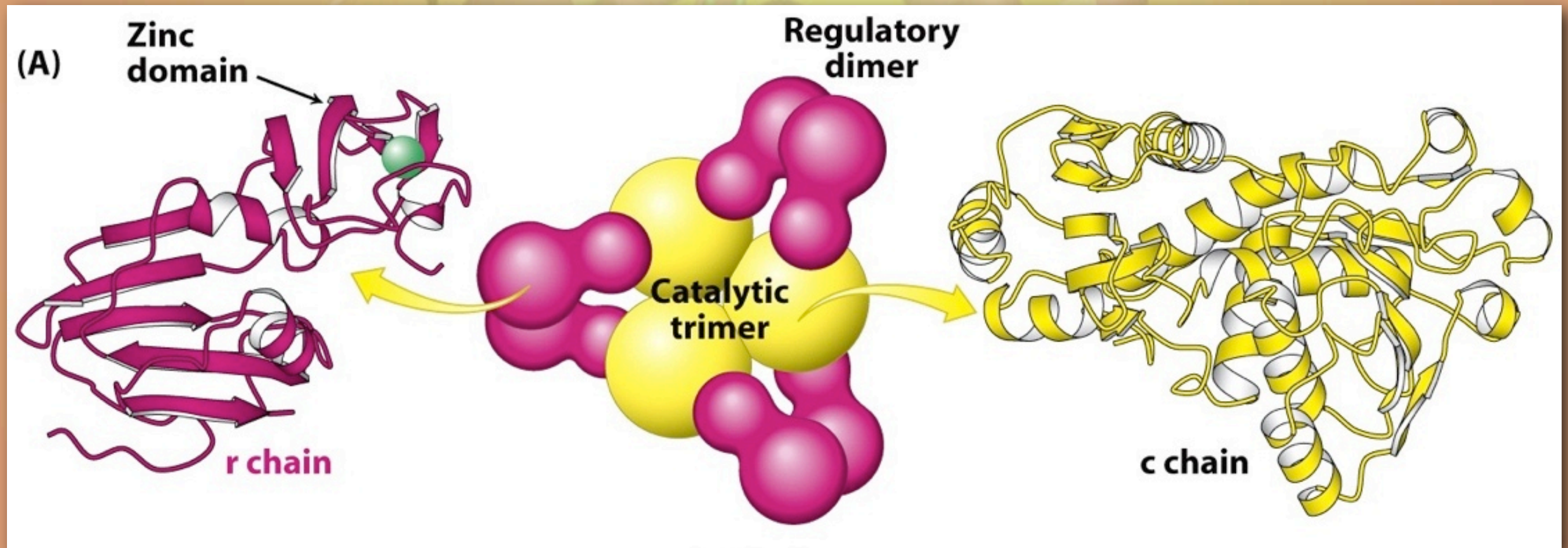
- ✦ The smaller r subunits (17 kDa each) are the **regulatory** subunits and contain the allosteric sites



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ATCase is Allosterically Regulated

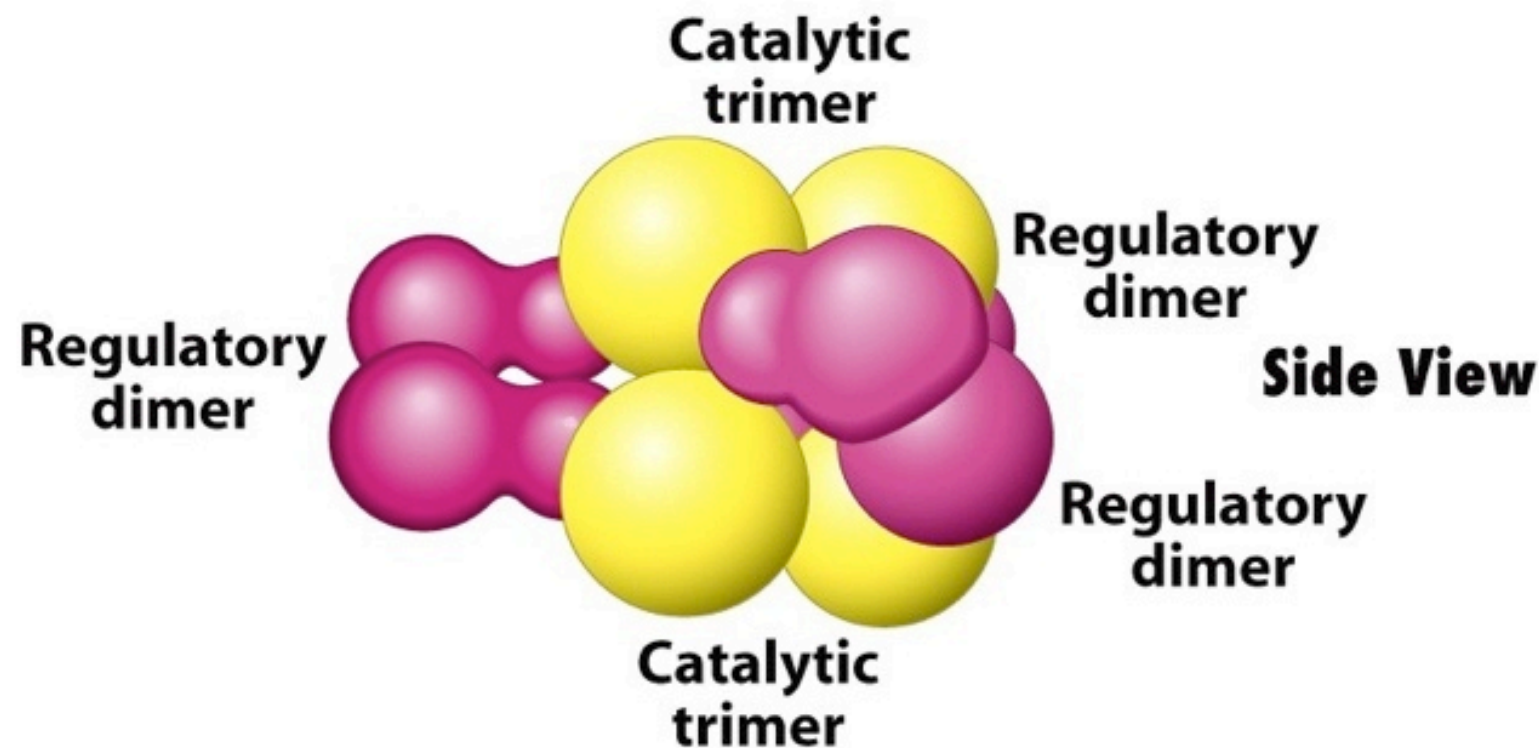
- ✦ The three dimers of *r* subunits bridge the two trimers of *c* subunits.



[View Live Figure](#)

ATCase is Allosterically Regulated

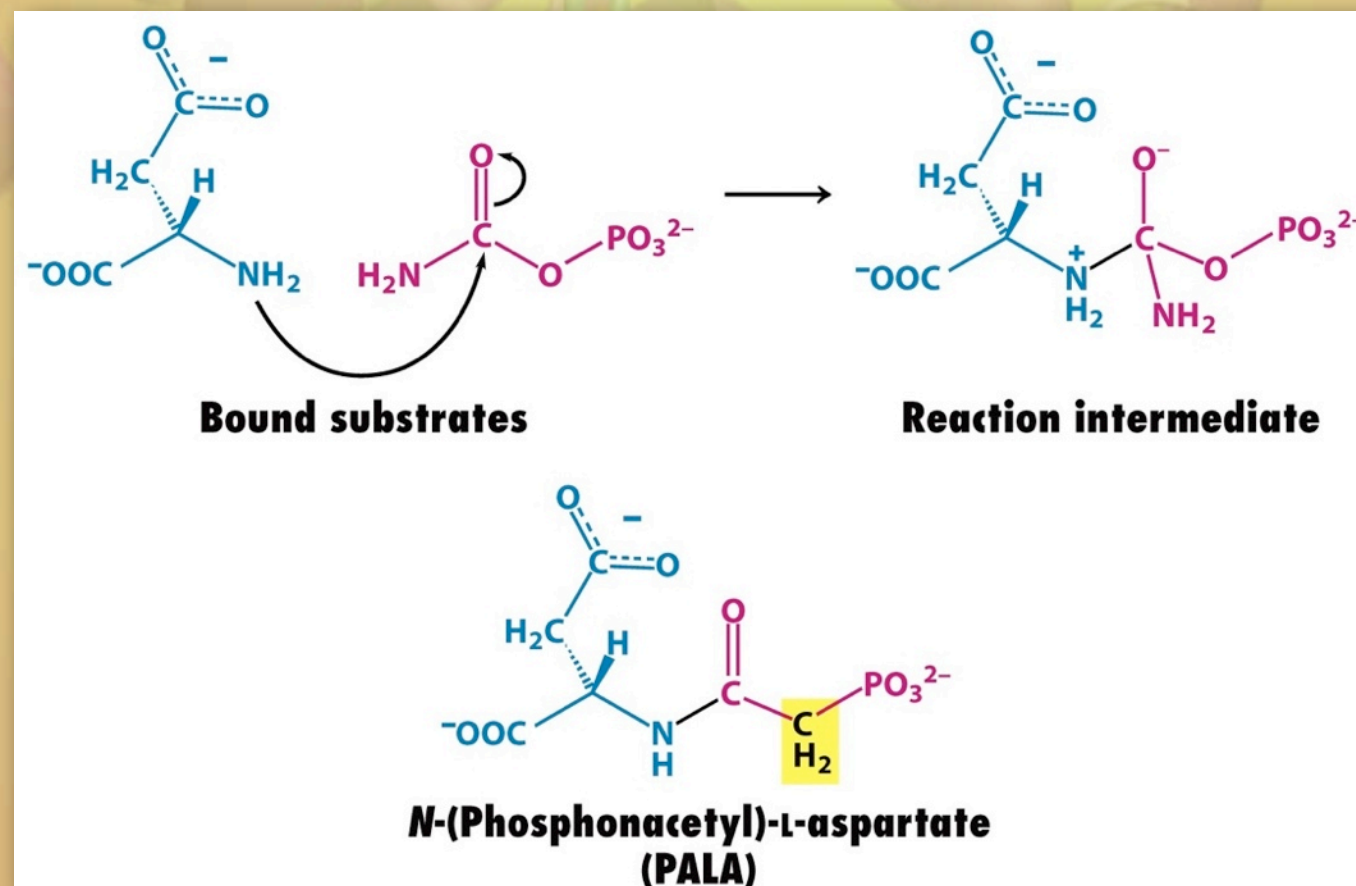
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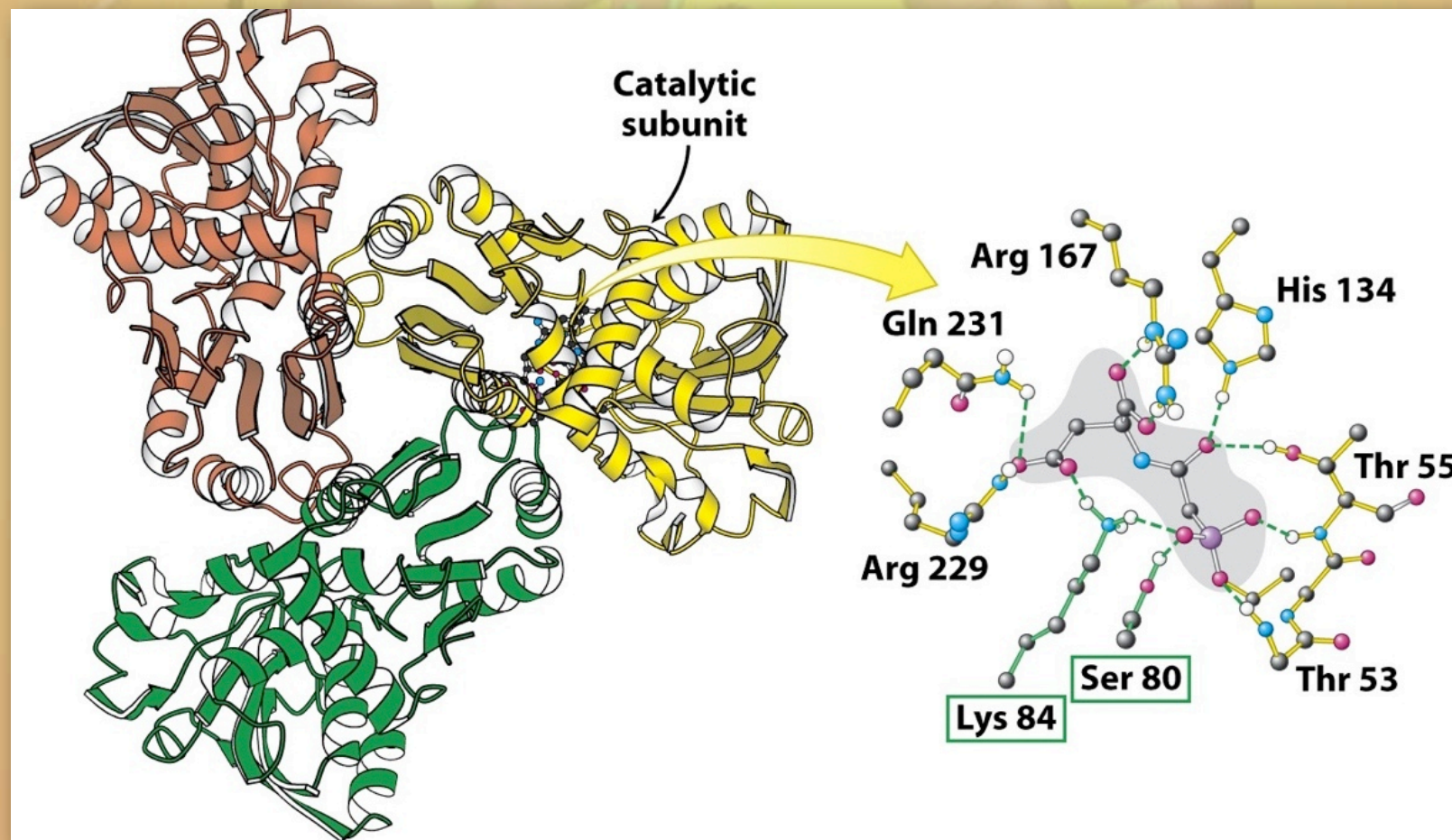
ATCase is Allosterically Regulated

- ✦ A substrate analogue, N-phosphoacetyl-L-aspartate (PALA), was used to locate the active sites.



ATCase is Allosterically Regulated

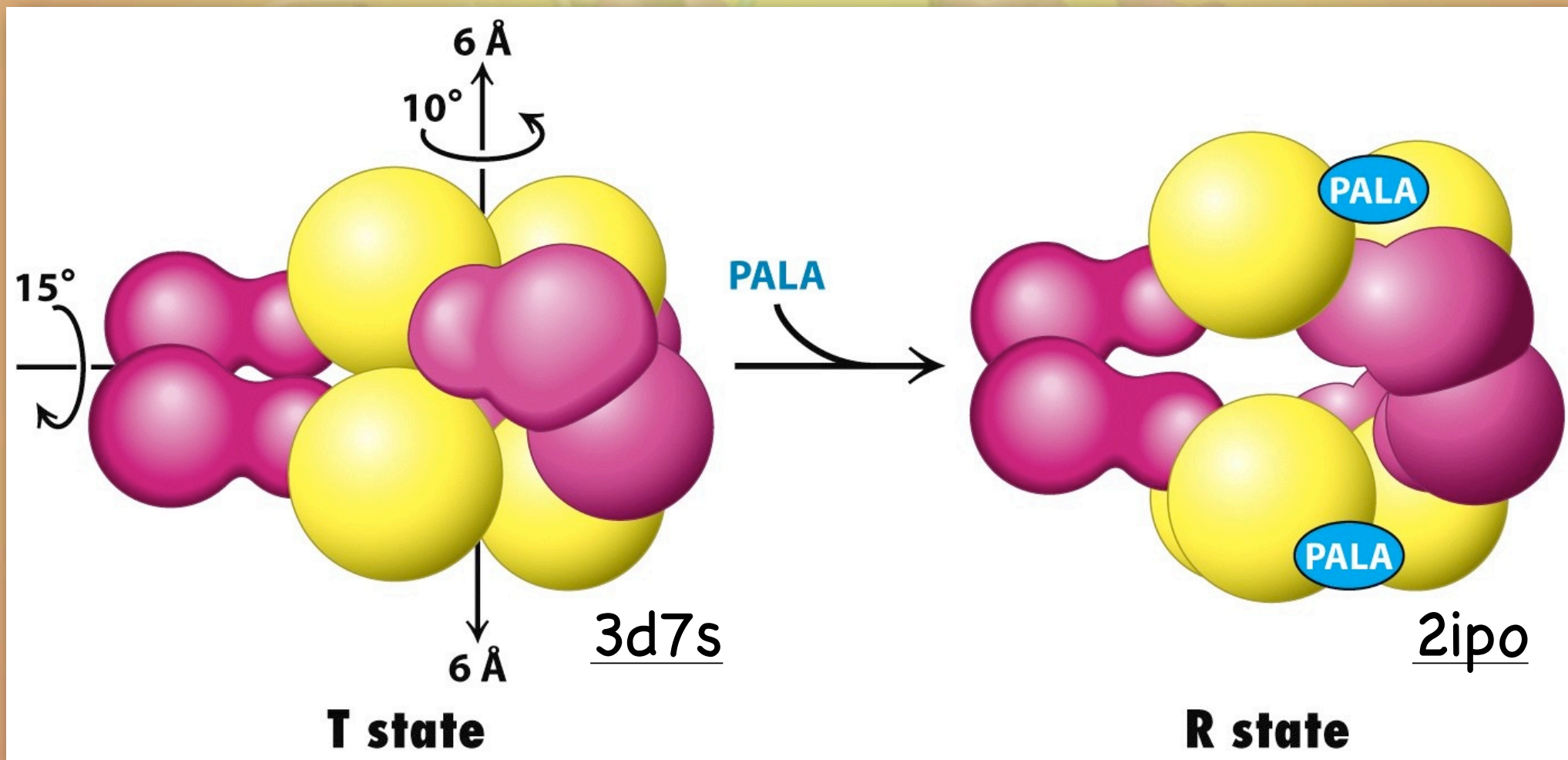
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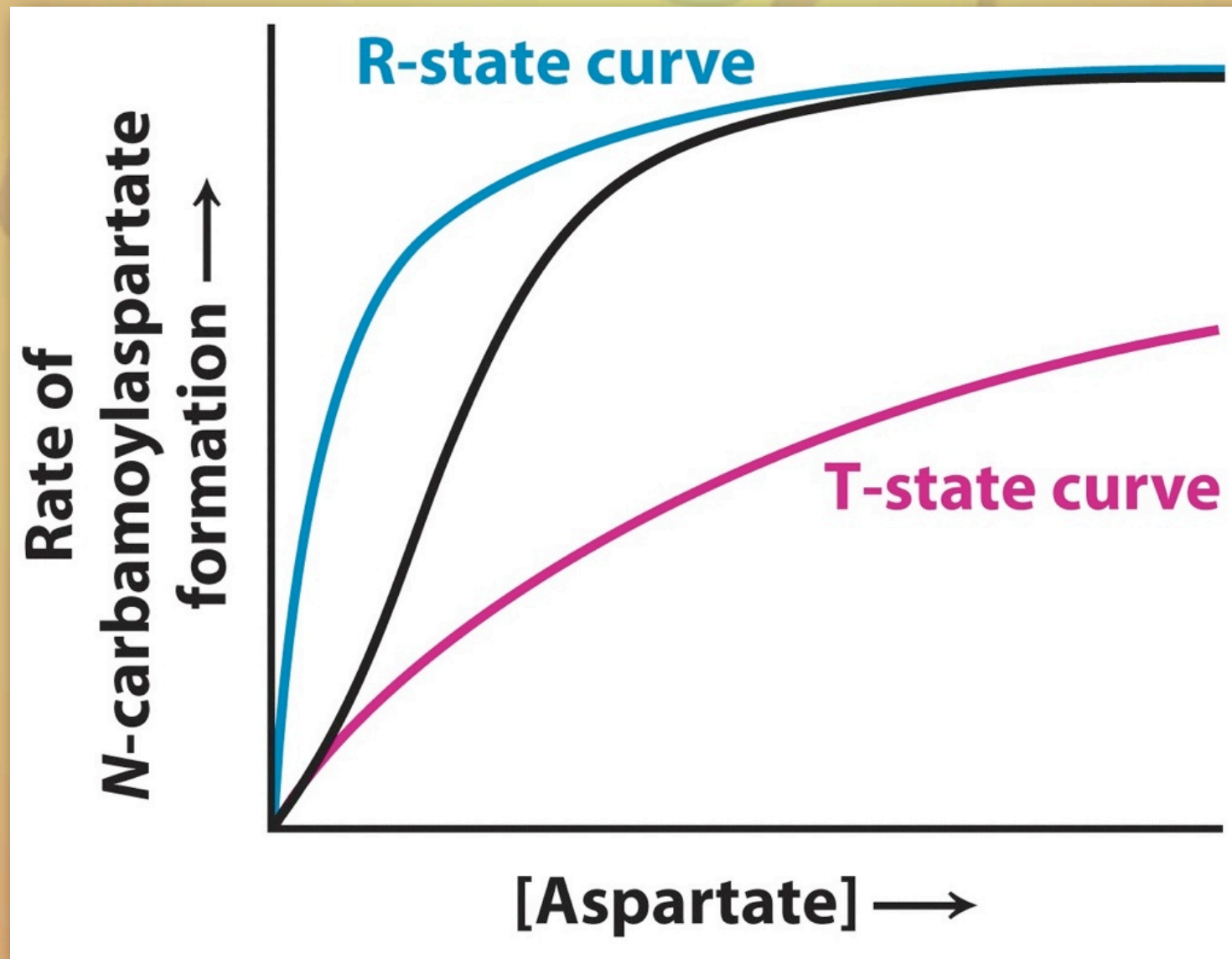
ATCase is Allosterically Regulated

- ✦ The binding of PALA produces a large conformational change in the enzyme.



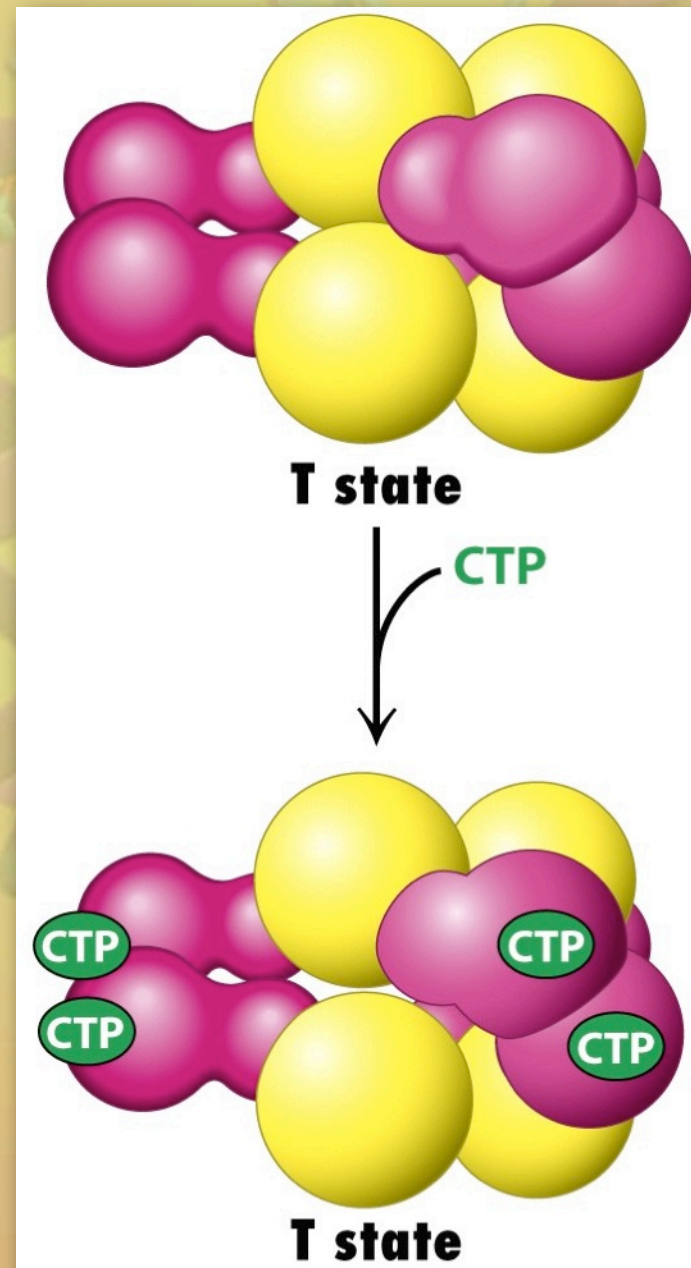
ATCase is Allosterically Regulated

- ✦ The kinetics can be interpreted in the same way used to interpret the O_2 binding to hemoglobin.



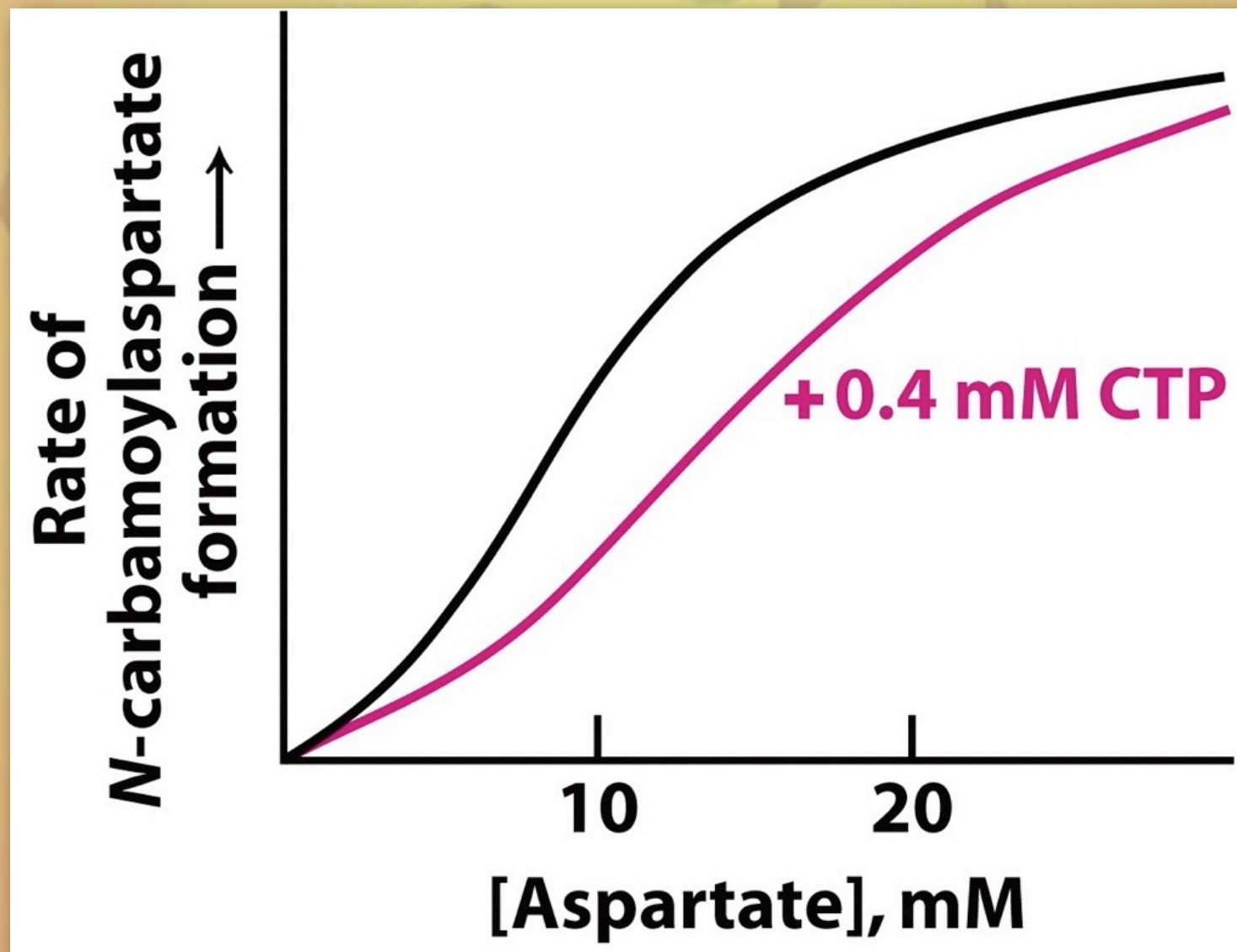
ATCase is Allosterically Regulated

- ✦ Cytidine triphosphate (CTP) binds to the allosteric sites and stabilizes the T-state of the enzyme.



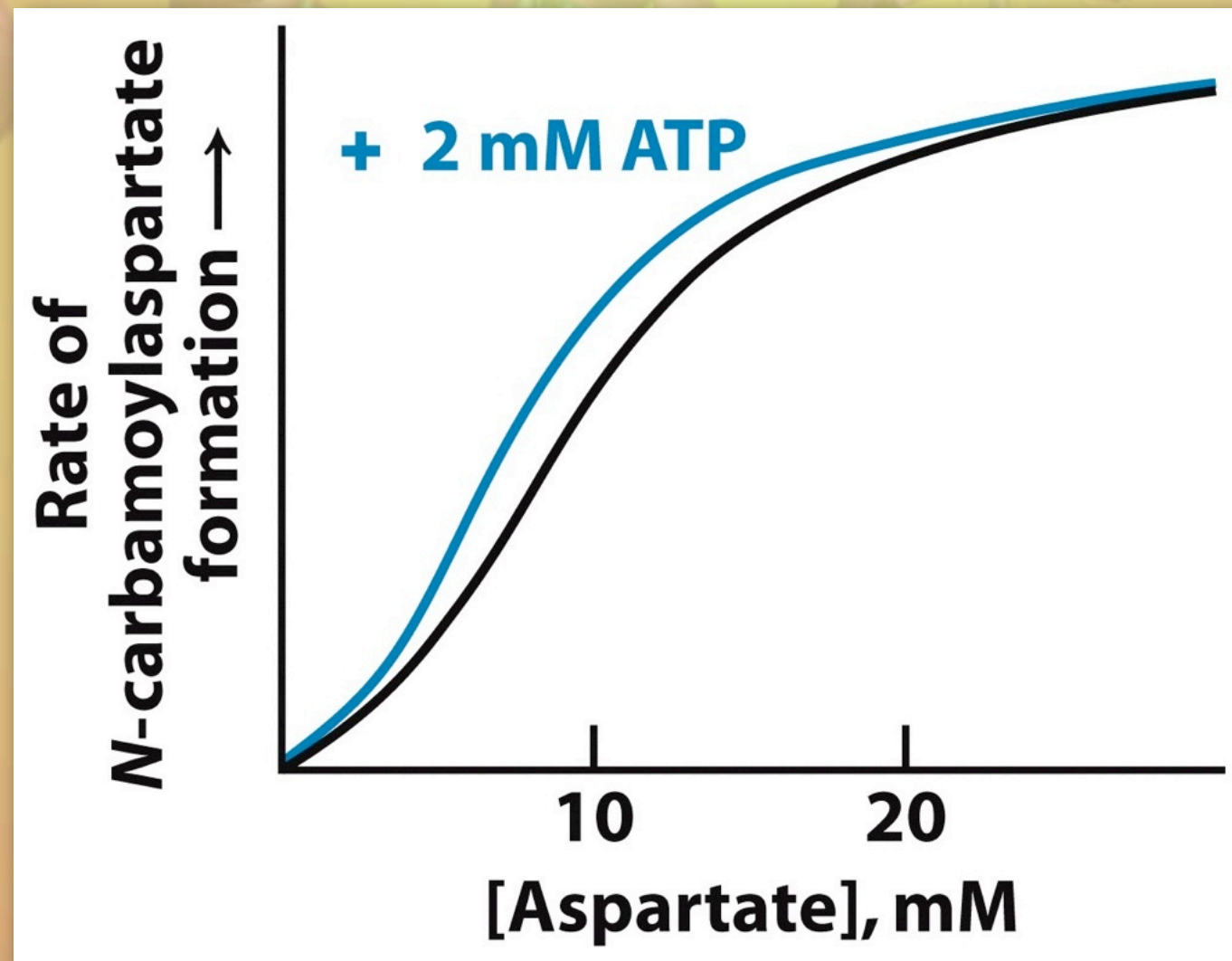
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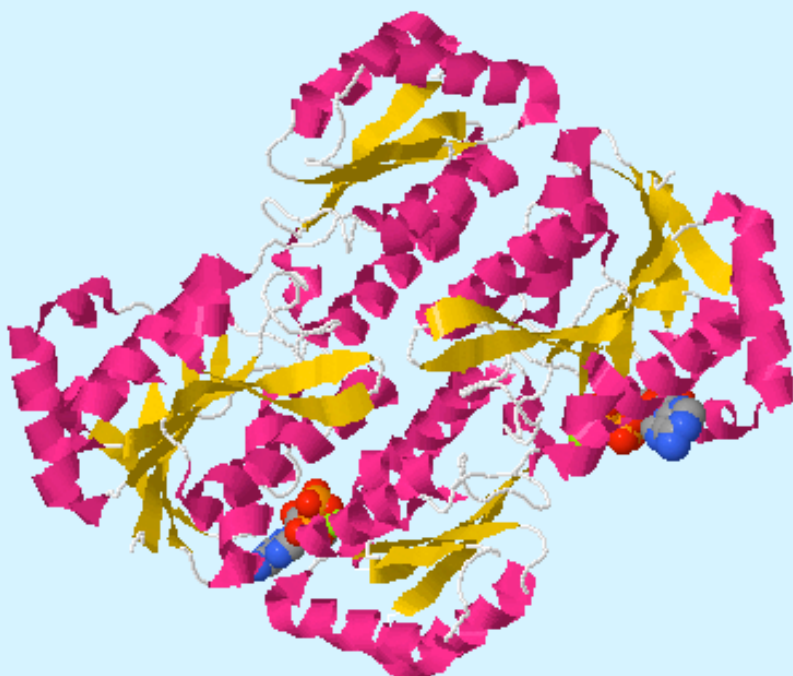
ATCase is Allosterically Regulated

- ✦ Adenosine triphosphate (ATP) also an **allosteric effector** for ATCase.
 - It enhances rather than inhibits the ATCase activity.



Phosphofructokinase is also Allosterically Regulated

Protein Structure - Phosphofructokinase (1PFK)



Jmol

-
- ☒ Product: Fructose-1,6-bisphosphate
- ☒ Product: ADP
- ☒ Allosteric activator: ADP

Tertiary Structure

- The 3-dimensional fold
-

Primary Structure

- Sequence of 320 amino acid residues
- [Show sequence](#)
-

Secondary Structure

- Periodic structures involving the backbone
-
- ☒ show all
- ☐ α -helix
- ☐ β -sheet

Quaternary Structure

- Association of multiple polypeptides
-
-

LDH Has Multiple Isozymes

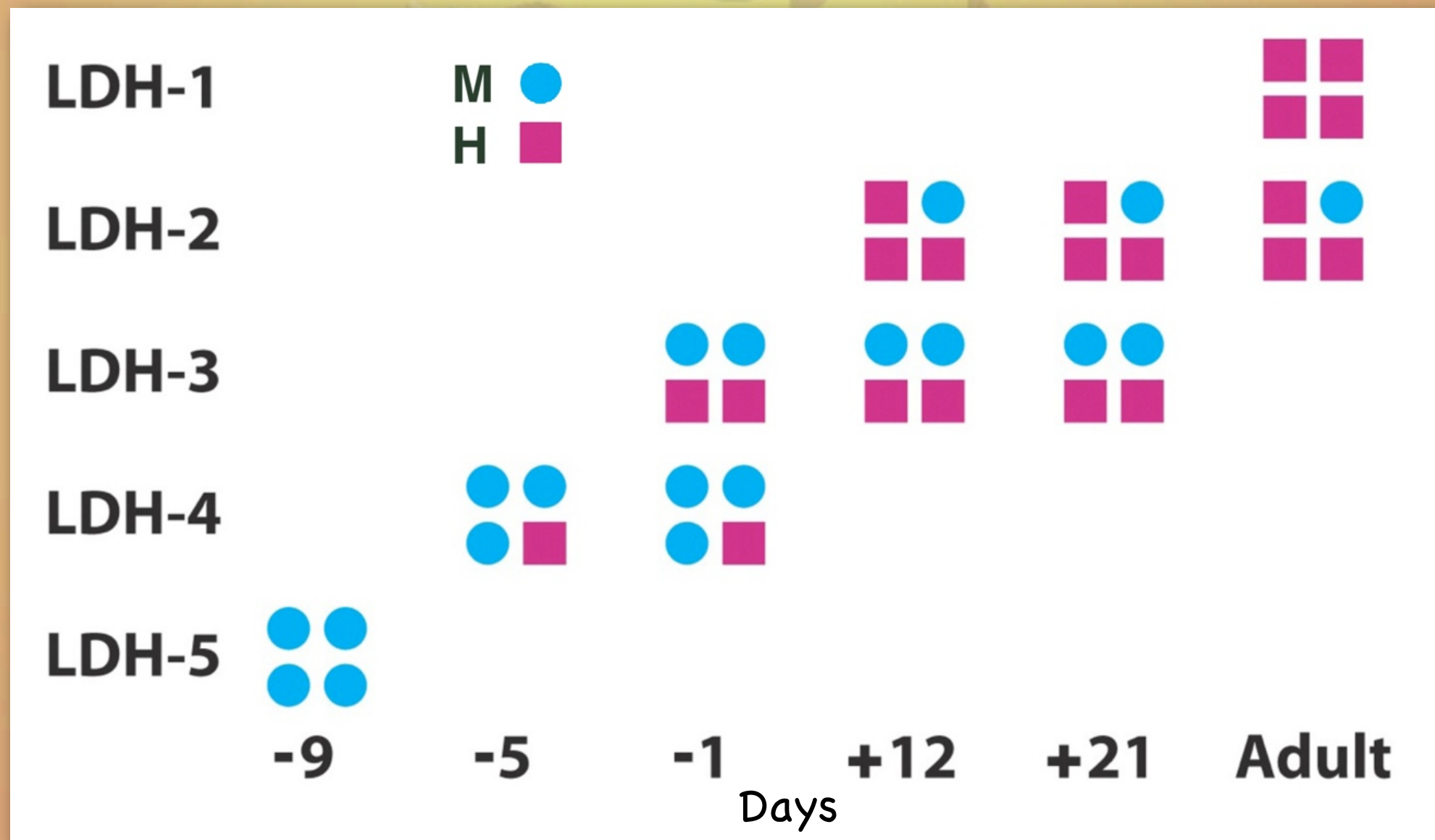
- ✦ Another strategy for regulating enzyme activity is to produce multiple forms of an enzyme.
- ✦ These multiple forms are called **isozymes**.
 - They are homologous proteins with different amino acid sequences
 - They usually display different K_M values with respect to the substrate.
- ✦ **Lactate Dehydrogenase (LDH)** provides a good example.

LDH Has Multiple Isozymes

- ♦ There are two isozymes of LDH
 - M (for skeletal muscle)
 - H (for heart muscle)
 - Has lower K_M than M isozyme
 - Is allosterically inhibited by pyruvate
- ♦ LDH is tetrameric
 - H and M subunits can be combined in different ratios:
 - H_4
 - H_3M
 - H_2M_2
 - HM_3
 - M_4




































LDH Has Multiple Isozymes

- ✦ Altering the ratio of the two isozymes of LDH is used to modify LDH activity during the life of a rat.



LDH Has Multiple Isozymes

- ✦ It is also used to modify the activities of LDH in different tissues.

	Heart	Kidney	Red blood cell	Brain	Leukocyte	Muscle	Liver
H_4							
H_3M							
H_2M_2							
HM_3							
M_4							

Next up

- ✦ Regulatory Strategies, con'd (Chapter 10)
 - Reversible covalent modifications
 - Proteolytic activation of proenzymes

