Advanced Cell Biology. Lecture 13

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Outline

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- Announcements
- How proteins work
- How proteins are controlled

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- How proteins work
- How proteins are controlled

Lab 4

- Go to the Web site and download assignment
- Join one of three review teams, participate in preparing presentation about one of three papers of DNA discovery
- Prepare questions to two other teams
- At the end of lab, everybody will evaluate the each team expertise

Enzymes

- Enzymes, or organic catalysts, transform the ligand after binding
- All enzymes are highly specific, e.g., to 3D conformation (accept D-glucose and ignore L-glucose etc.)
- Enzymes are often work in groups: there are tandems, chains and even pathways

How lysozyme works

- Main function—non-specific immune reaction; hydrolysis of bacterial cell wall polysaccharides (actually, peptidoglycans)
- Two amino acids works: Glu 35 and Asp 52
- First contributes proton to oxygen “bridge” and polarize water molecule, second attaches to C_1 atom

How lysozyme works

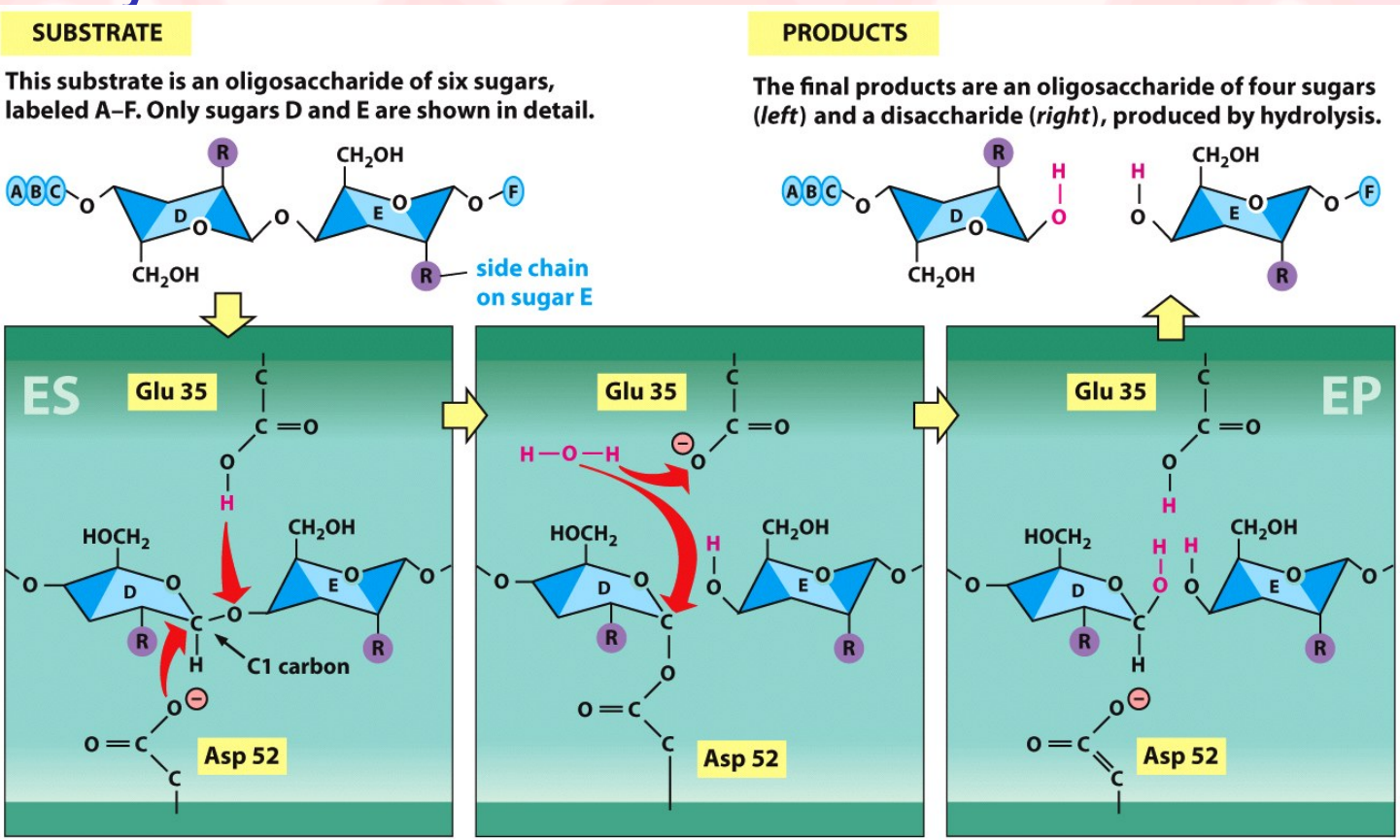


Figure 4-31 Essential Cell Biology 3/e (© Garland Science 2010)

Lysozyme I movie

Lysozyme II movie

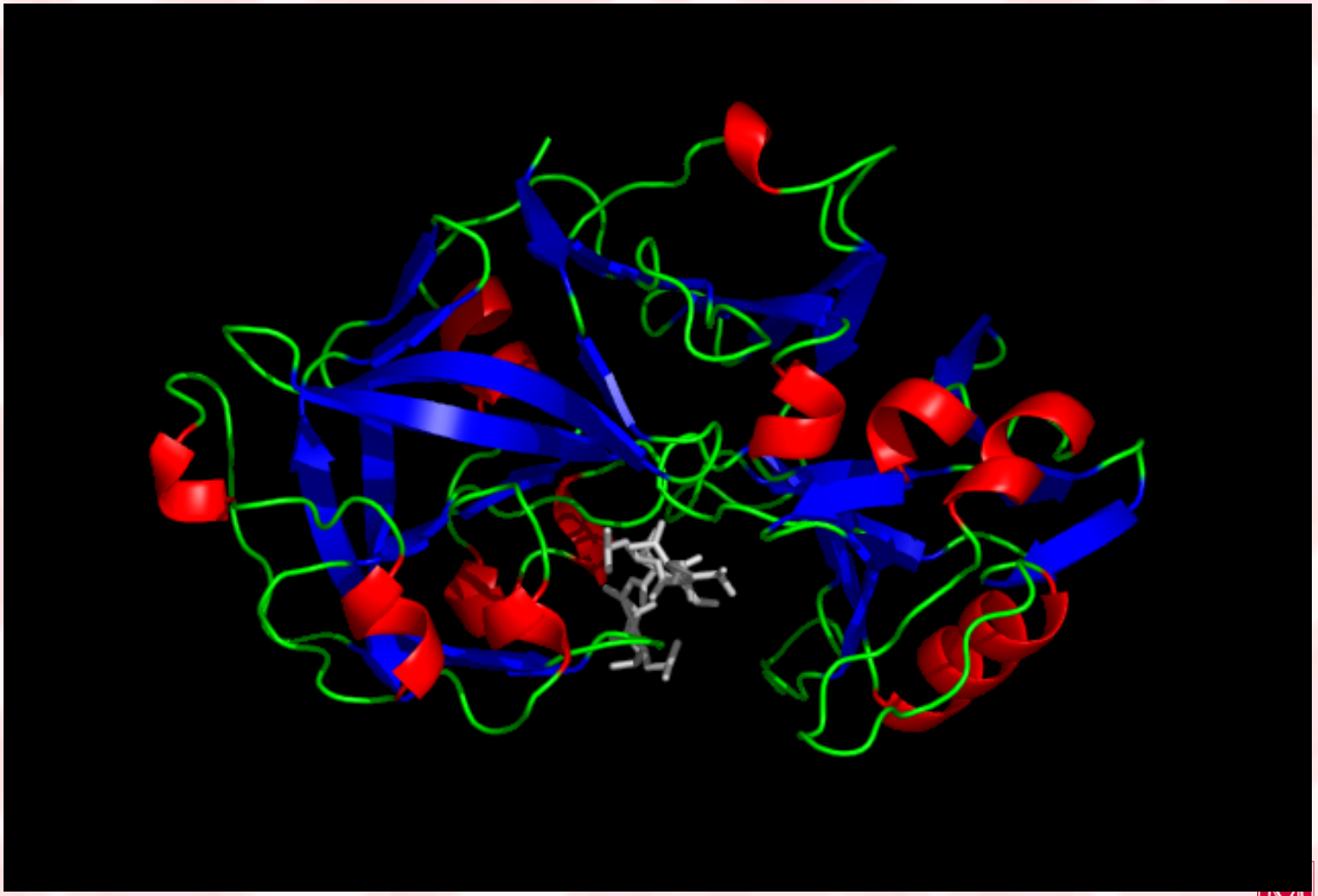
Sites

- Transition site is a place where ligand will be modified
- Active site is a place on enzyme which does main chemical job

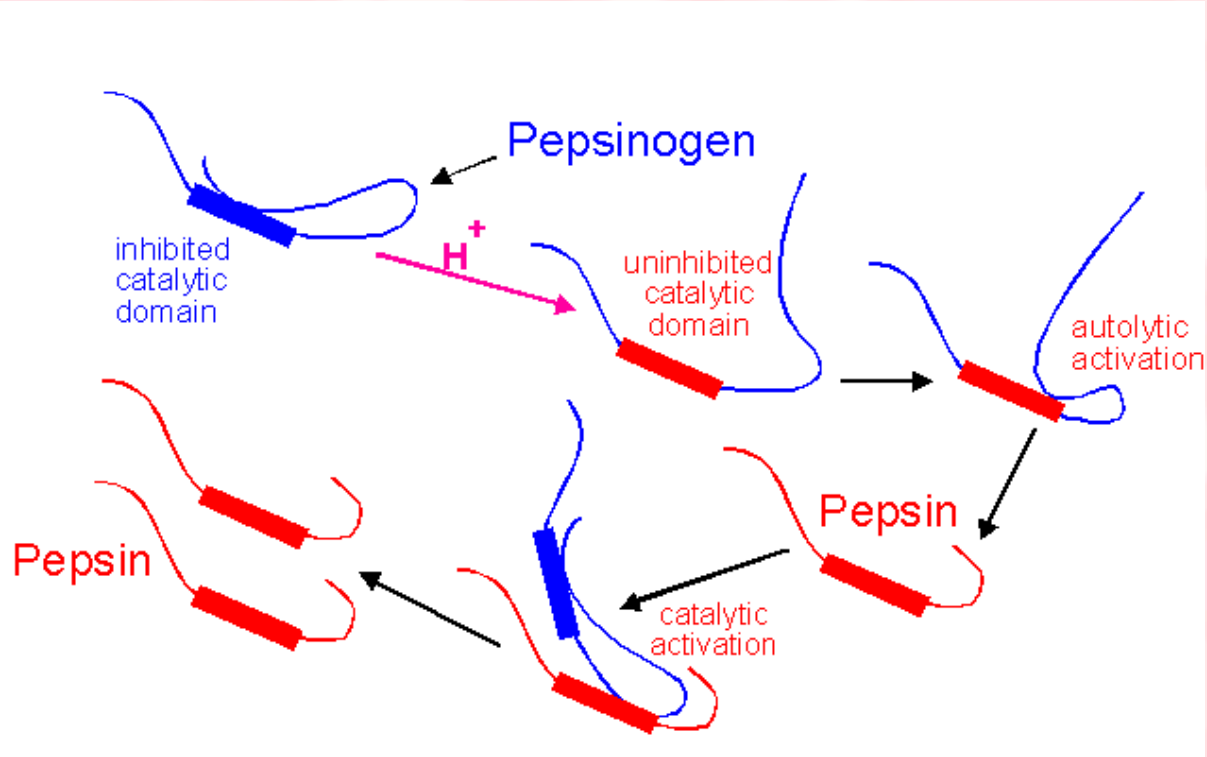
Why pepsin did not digest itself?

- Pepsin catalyze destruction of peptide bonds
- It works only on specific transition sites: cleaving aromatic amino acids like phenylalanine, tryptophan, and tyrosine from N-terminal
- It is poor of these amino acids, but still degrades and needs the constant supply of inactive pepsinogen

Pepsin



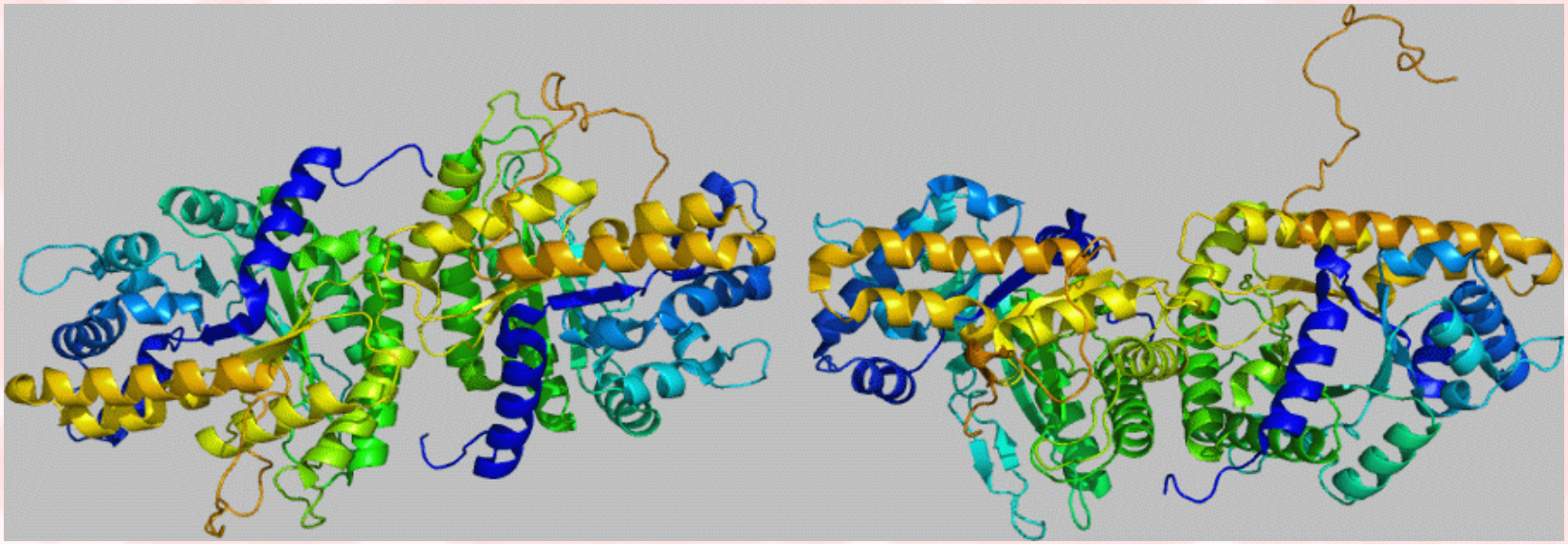
Activation of pepsinogen



Inhibition

- Many recessive mutations inhibit different enzymes
- For example, error in aldolase B gene (which split the fructose molecule) results in HFI (hereditory fructose intolerance), the disease which is often fatal: unprocessed fructose trap phosphates and block many other metabolic reactions
- Many drugs **reversely** inhibit enzymes

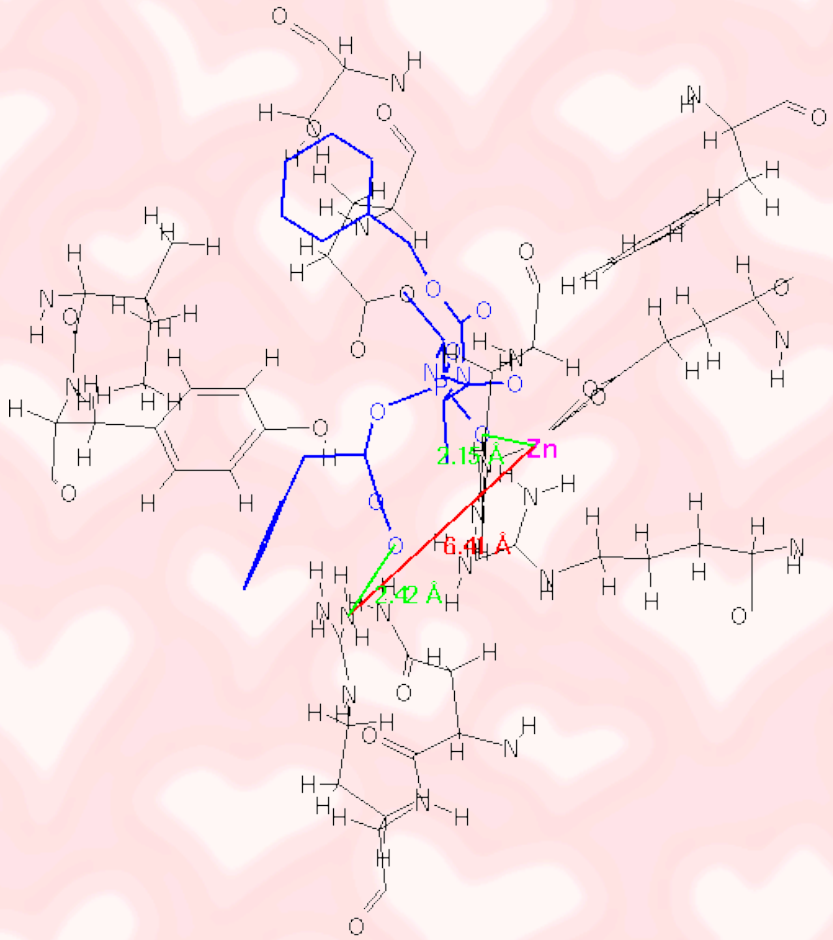
Aldolase B



Binding of small molecules

- Small molecules bring additional functions to proteins
- Carotenoid retinal binds to rhodopsin (accepts ν); porphyrine heme binds to hemoglobin (accepts O_2/CO_2)
- Carboxypeptidase binds Zn atoms

Carboxypeptidase active site with zinc



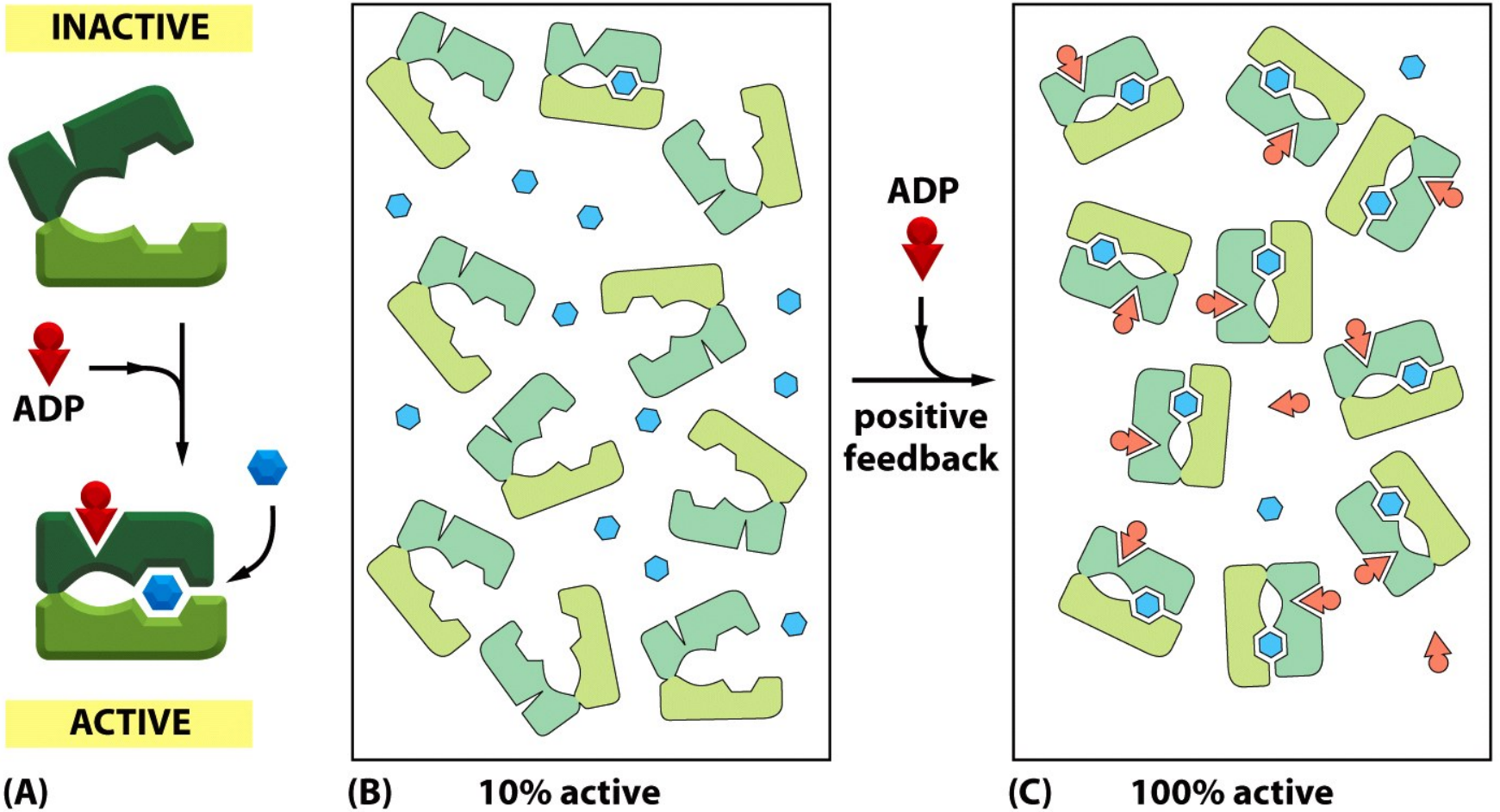
Feedback inhibition

- Feedback inhibition occurs when later product in a pathway suppresses earlier stage of this pathway
- This is a negative regulation
- There are also positive regulation processes: ADP acts as a positive regulator in oxidative pathways

Allosteric enzymes

- Many proteins have at leas two binding sites: for substrate and for regulatory molecules
- Depending on the regulatory state, proteins may change conformation
- *Allosteric* proteins have more than one stable conformation (each with different activity)

Allosteric protein with positive feedback



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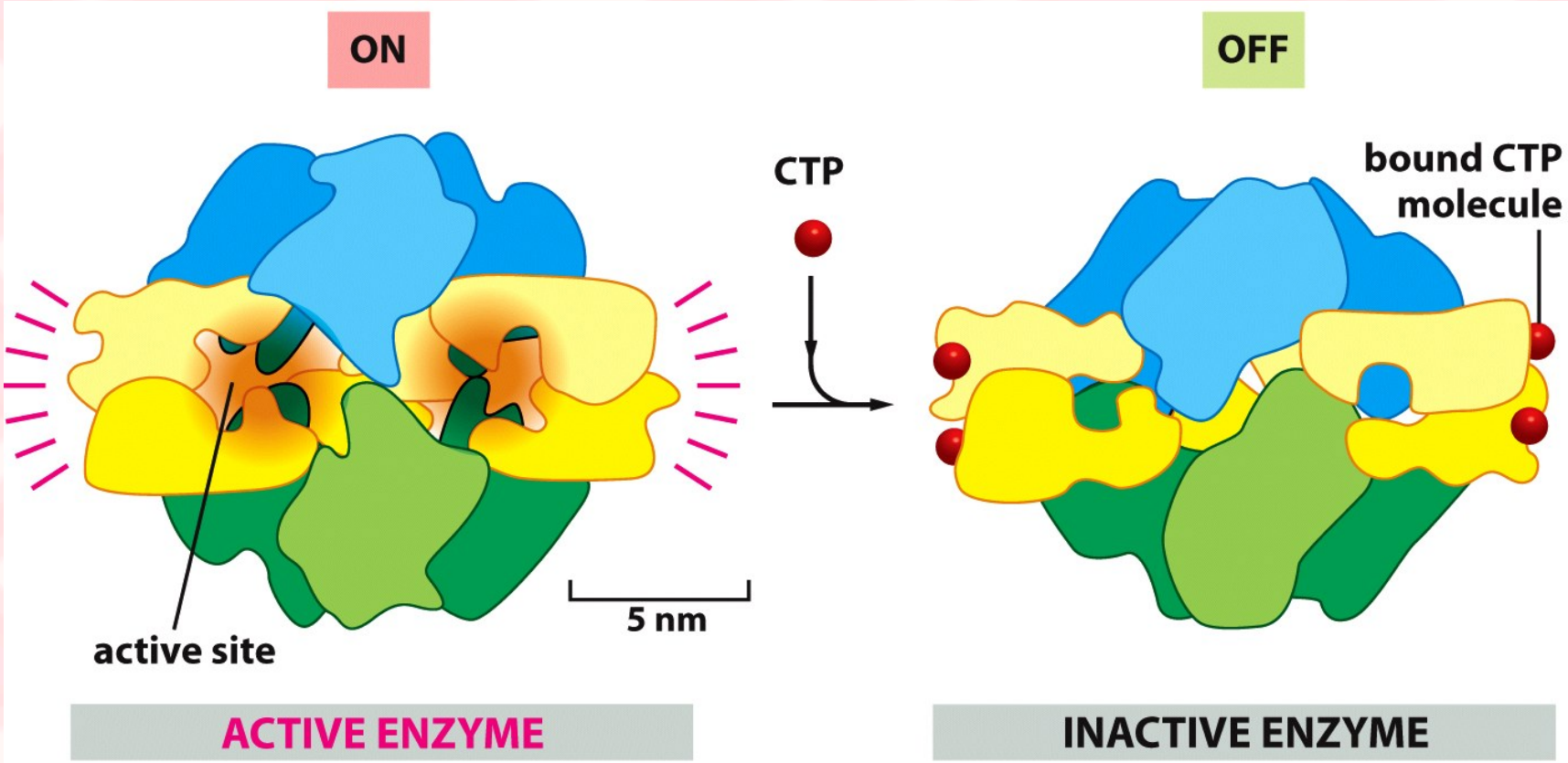
Allosteric enzyme aspartate transcarbamoylase with negative feedback from CTP



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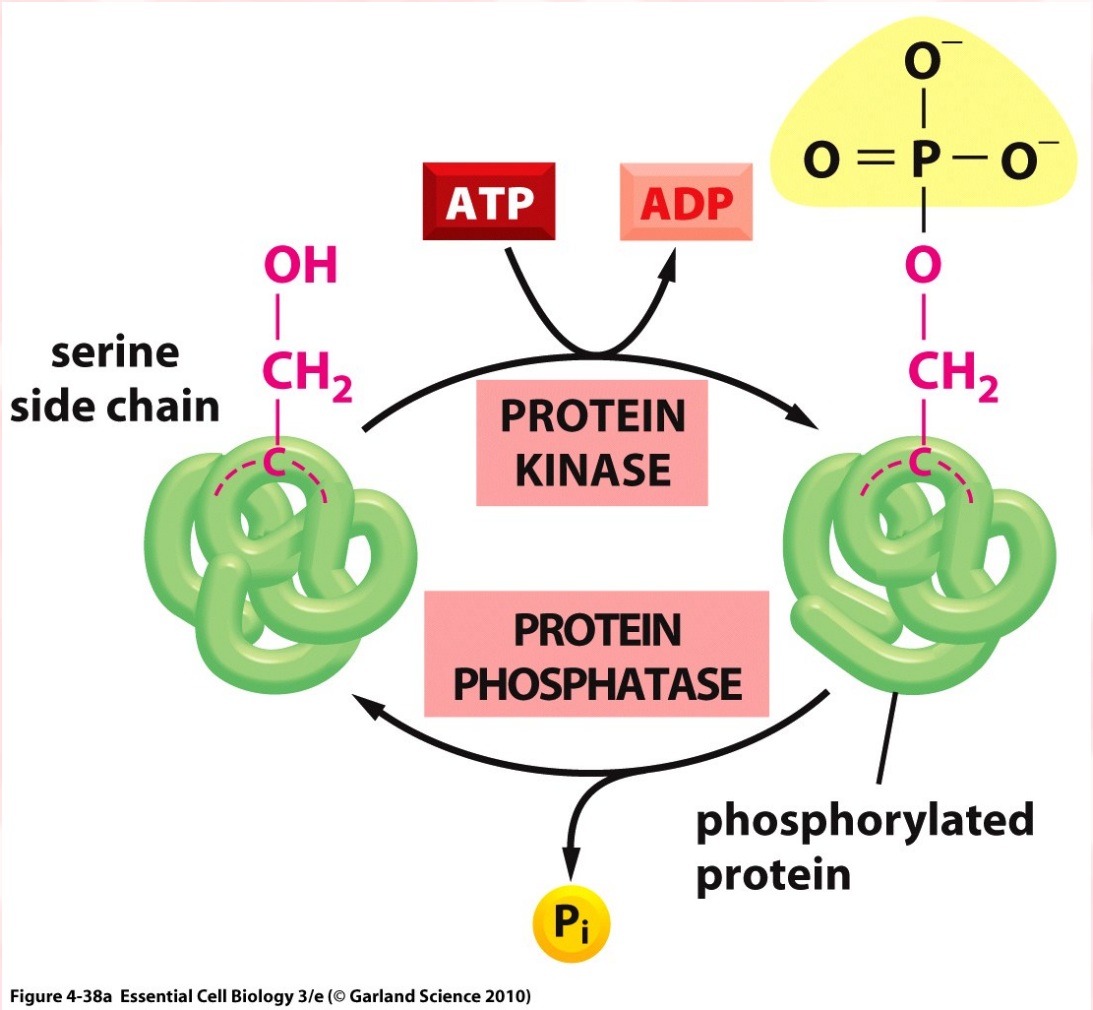
Protein phosphorylation

- Majority of proteins are controlled through *phosphorylation*: attaching a phosphate group to one of side chains (e.g., –OH)
- *Protein kinase* catalyzes phosphorylation
- *Protein phosphataze* catalyzes *dephosphorylation*

Phosphorylation



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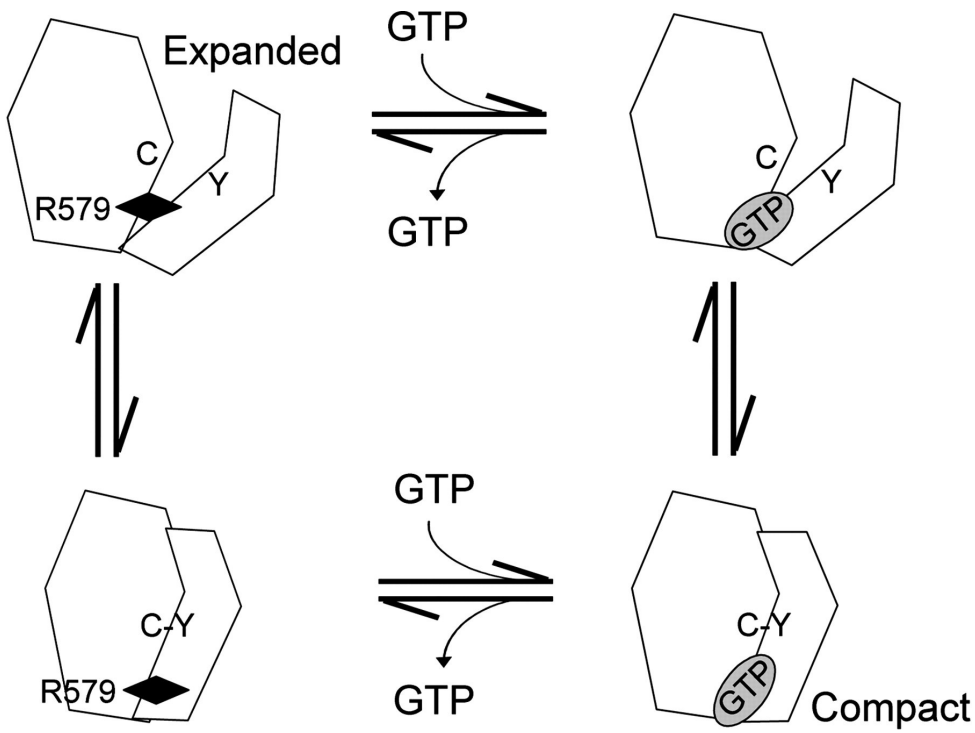
GTP binding

- Instead of phosphate group, some proteins may bind *GTP*, guanosine triphosphate
- Attached GTP releases phosphate and turn in GDP; at the same moment, protein changes its conformation to inactive. The process is reversible.
- Bacterial elongation factor EF-Tu beating transport RNA while activated (with GTP); after GTP hydrolysis it releases tRNA

Model of allosteric inhibition of transglutaminase 2 by GTP.



Begg G E et al. PNAS 2006;103:19683-19688

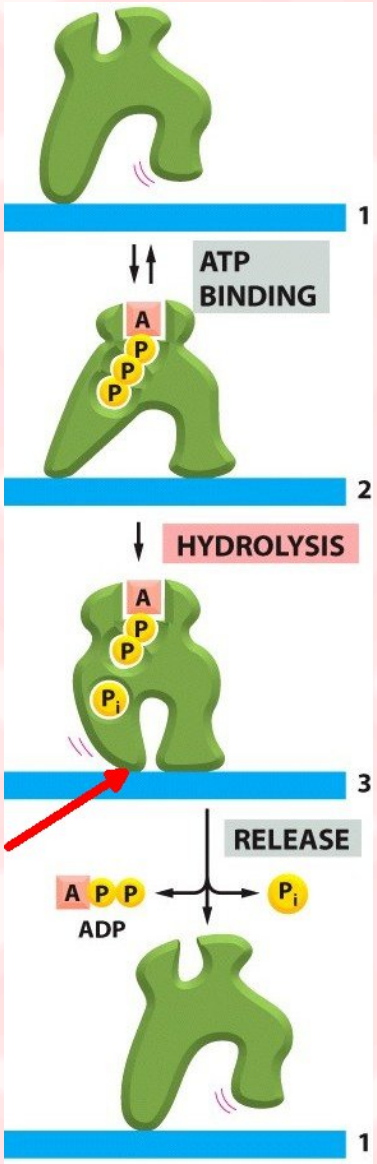
PNAS

EF-Tu movie

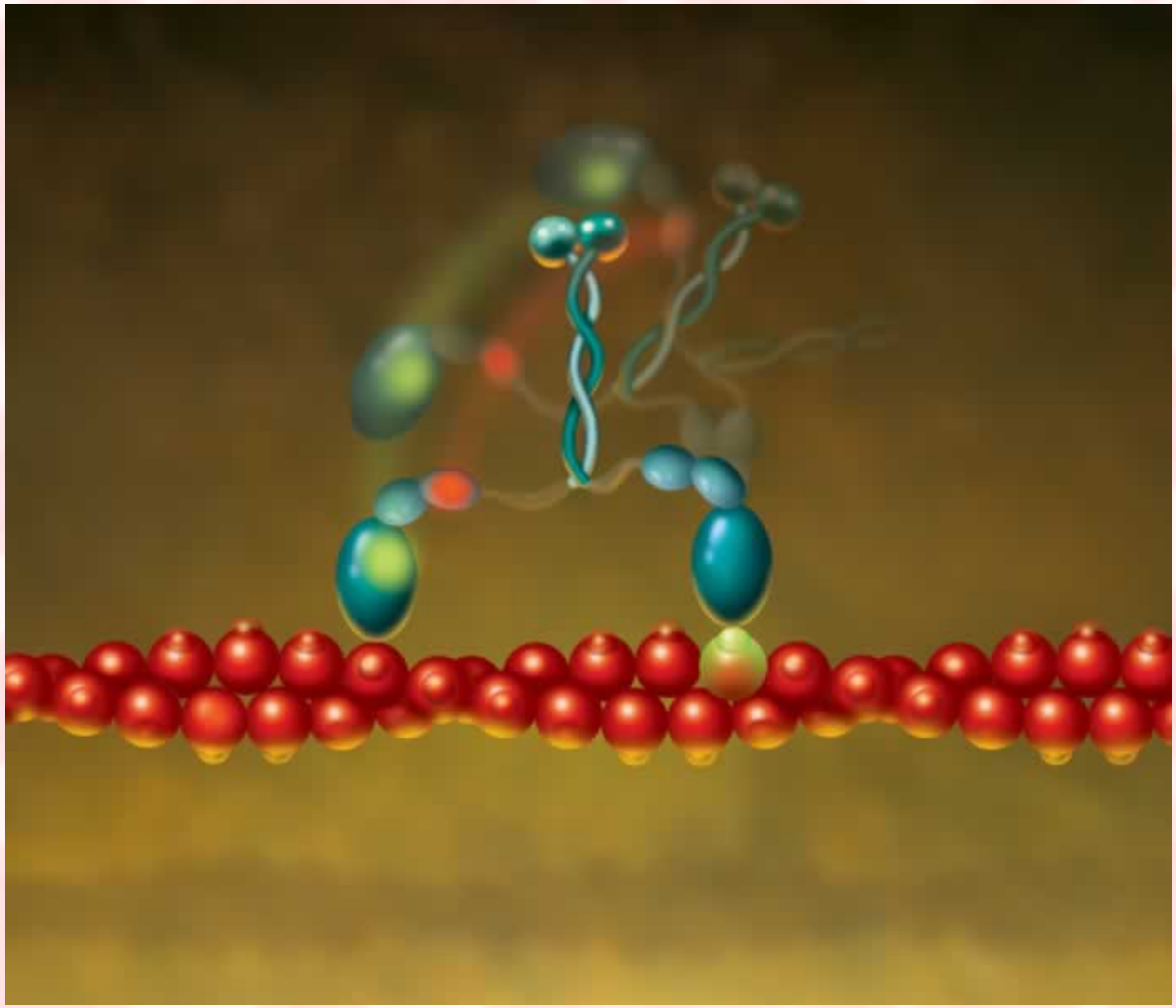
Allosteric motor proteins

- Changes of conformation allow some proteins to “walk” along the surfaces
- As an example, *myosin* runs along *actin* filaments in muscle cells
- Motor proteins are ATP/GTP-binding: ATP (or GTP) provides both change in conformation of motor proteins
- Hydrolysis of ATP/GTP makes reverse reaction practically impossible (only ATP synthetases may reconstruct the ATP): therefore, they walk directionally

Protein walking



Myosin



Final question (1 point)

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How many amino acids are in the active site of lysozyme?

Summary

- Almost all proteins bind to other molecules (ligands)
- Enzymes convert ligands
- Some other proteins use ligands as additions to their active sites

For Further Reading

A. Shipunov.
Advanced Cell Biology [Electronic resource].
2011—onwards.
Mode of access: `http://ashipunov.info/shipunov/school/biol_250`

B. Alberts et al.
Essential Cell Biology. 3rd edition.
Garland Science, 2009.
Chapter 4: pages 141–155.