REGULATION BY EFFECTOR AND ALLOSTERY

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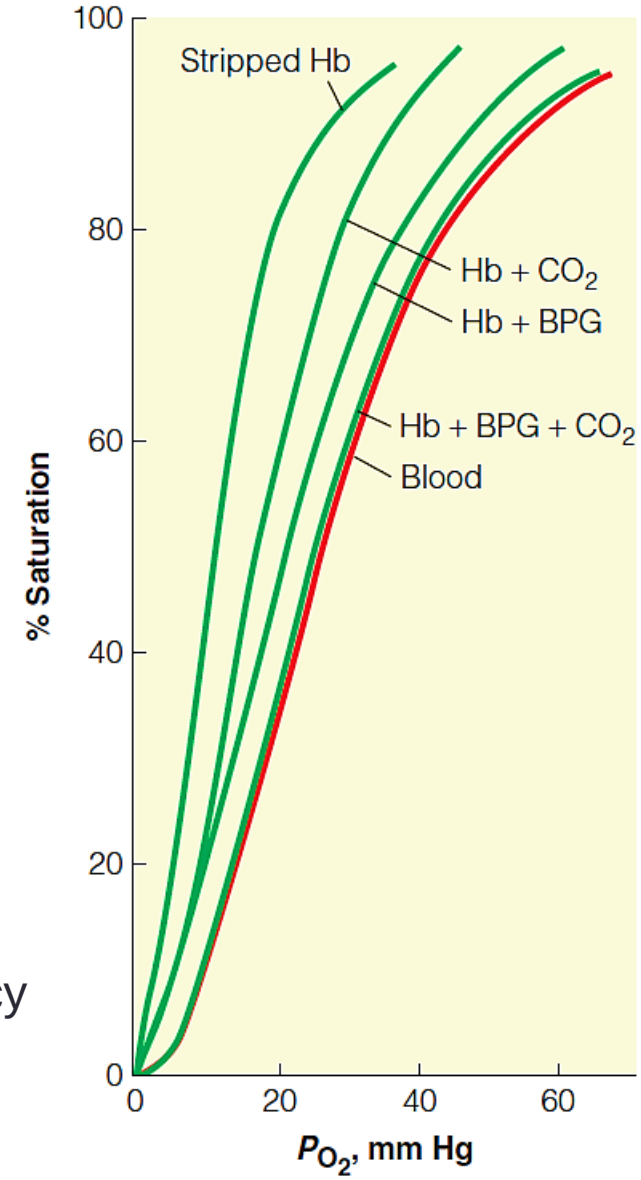
SUSTC

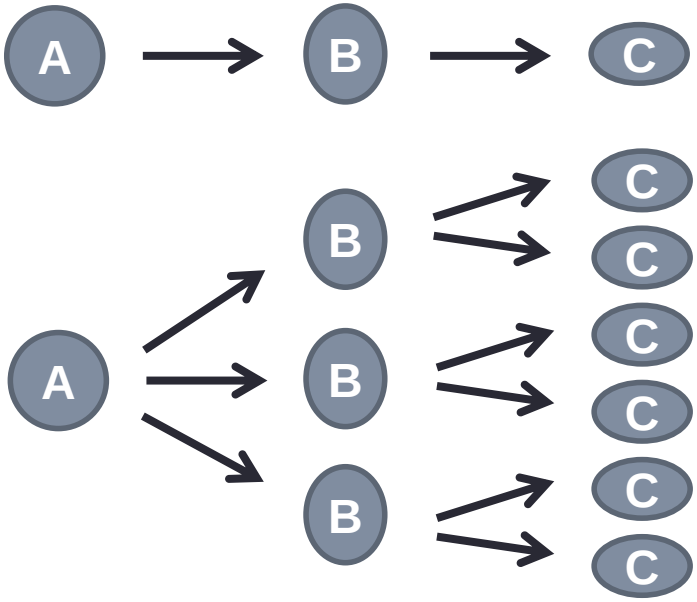
Effector

- The activity of proteins is controlled is by binding regulatory molecules, termed effector ligands or effectors
- Effectors can be as small as a proton or as large as another protein
  - pH-induced conformational changes, such as Bohr effect of Hb

Hb's O2 binding efficiency modulated by effectors



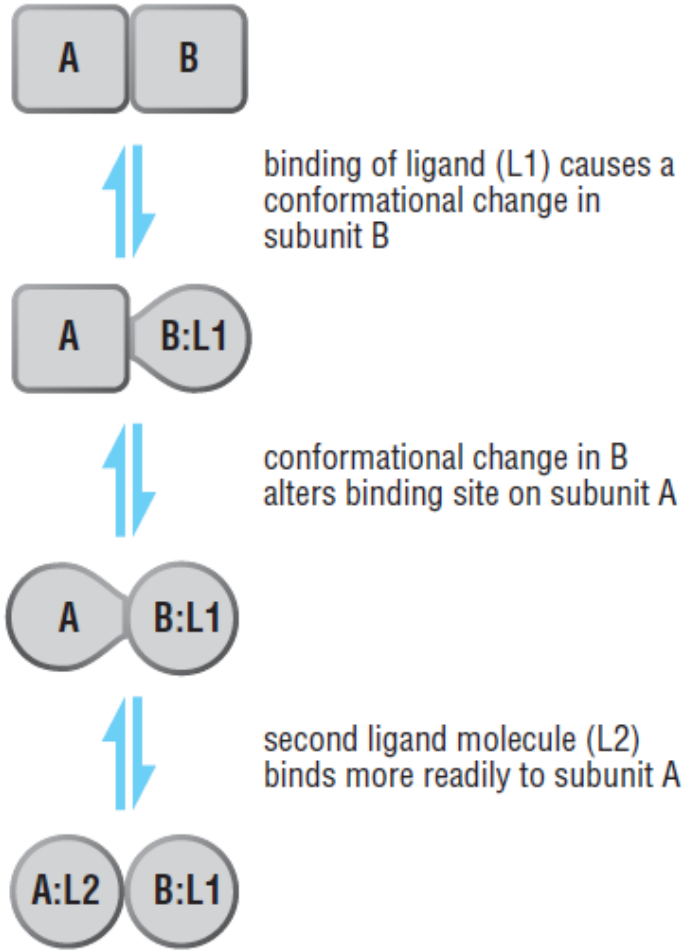
Effector amplifies biological effects via cooperativity

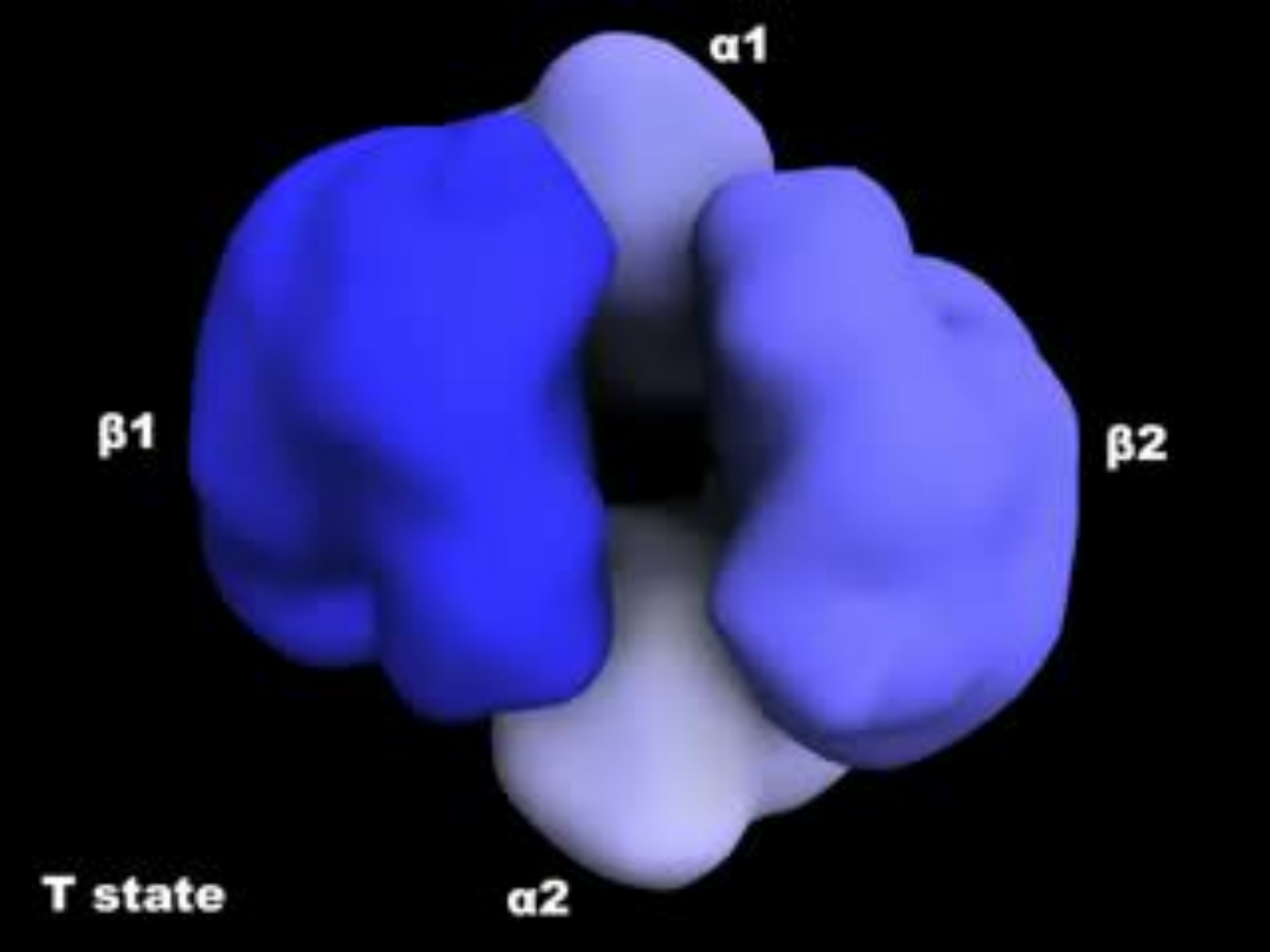


- Rapid and massive biological response is hard to achieve with a linear system
- Amplification allows a single molecule to regulate many copies of a target protein or pathway
- Signal amplification can be achieved by covalent modification or by cooperativity

Cooperative binding by effectors

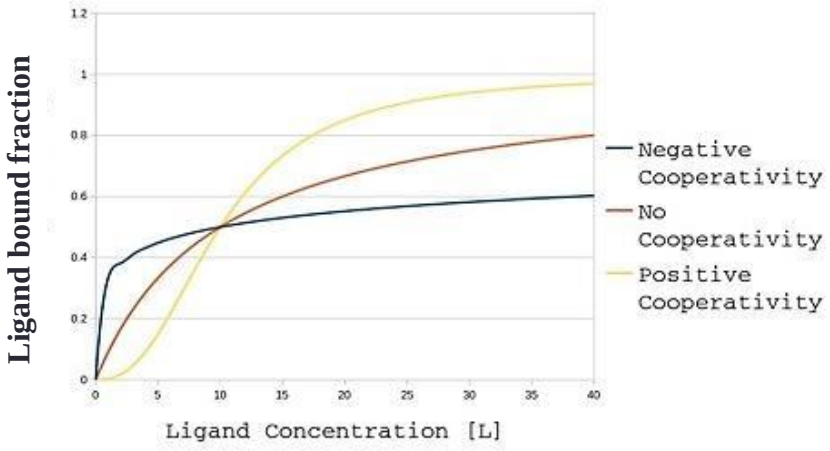
- Cooperativity is only present in oligomeric proteins
  - Multiple effector ligand binding sites
- Cooperative binding
  - Cooperativity between binding sites for the same ligand on a protein
  - Depending on the ligand binding induced a conformational change of the protein




α1
β1
β2
T state
α2

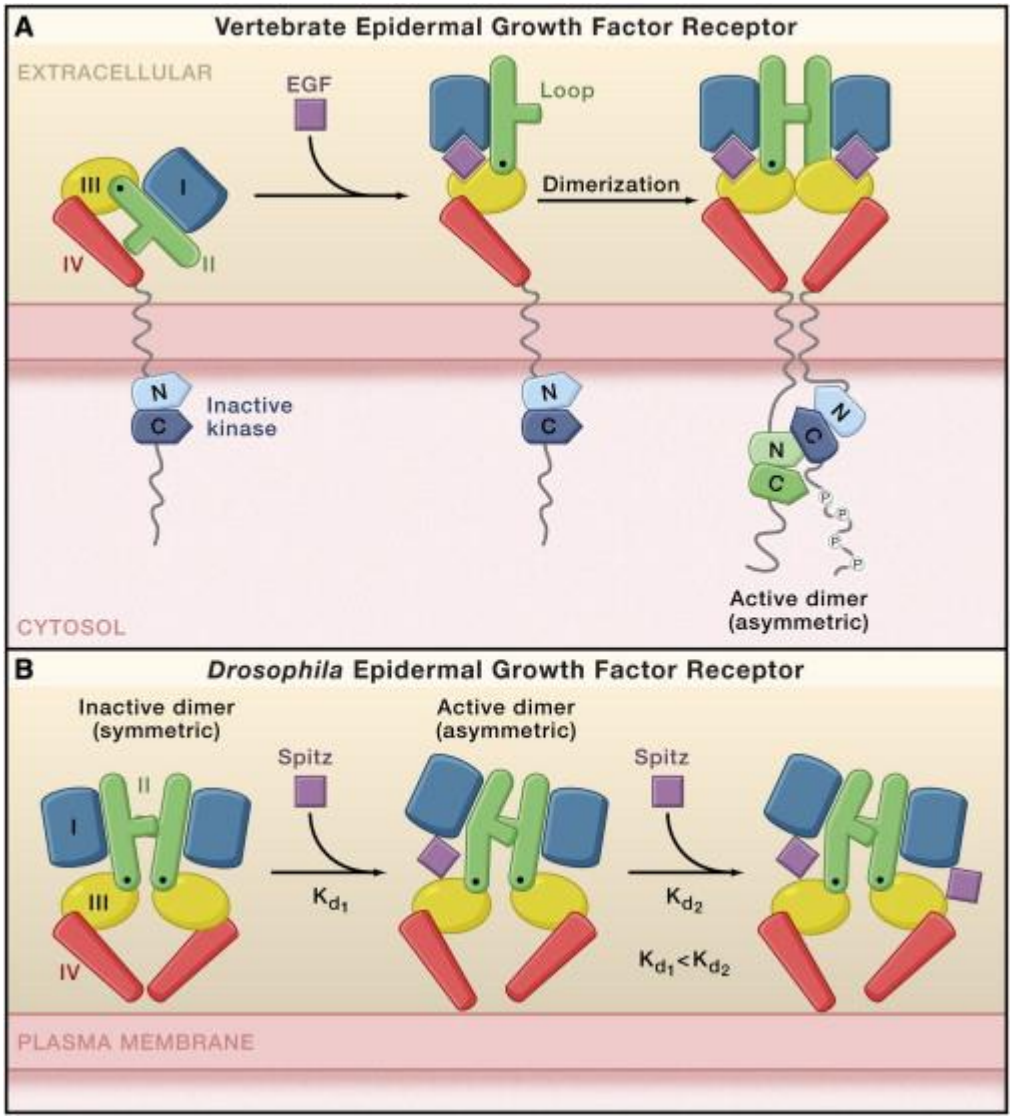
Cooperativity can be positive or negative

- Positive cooperativity
  - Binding of one molecule of a ligand to a protein makes it easier for a second molecule of that ligand to bind
  - Protein activation
- Negative cooperativity
  - Binding of the second molecule is more difficult
  - Protein inhibition



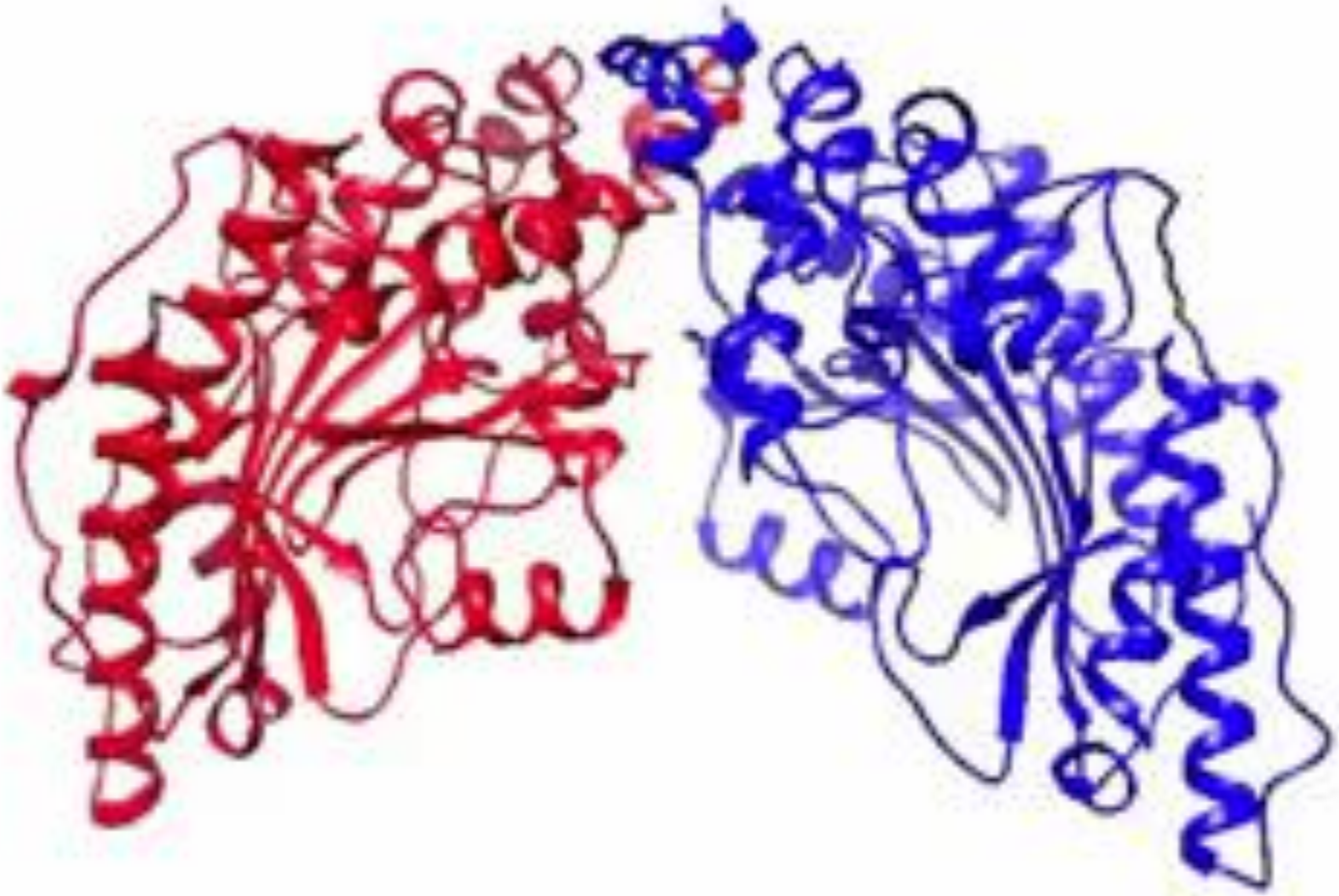
Binding curve

EGFR and negative cooperativity



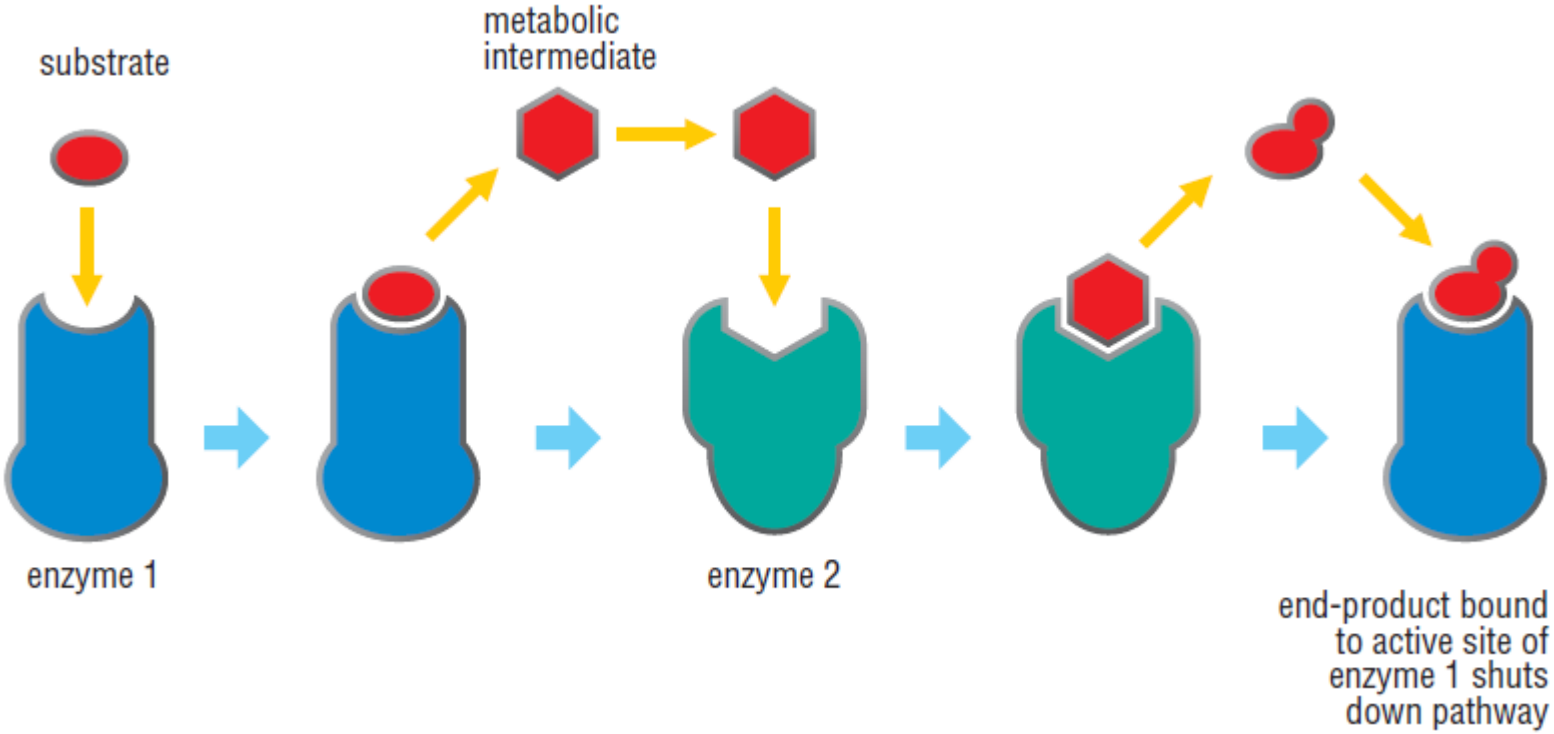
Negative cooperative binding is usually asymmetric

Rabbit muscle creatine kinase and negative cooperativity



Enzyme activities highly depend on effectors

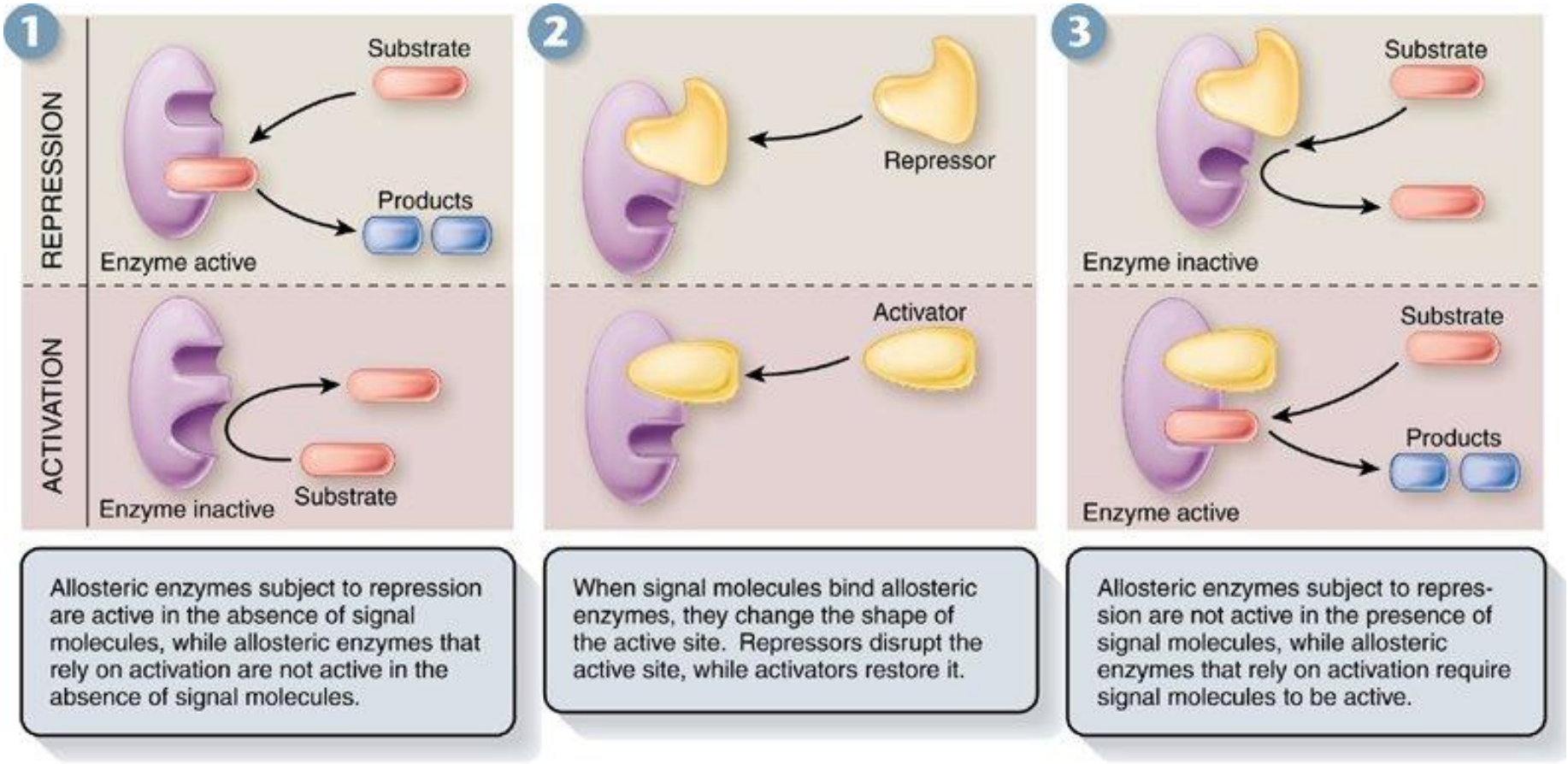
- Effector ligand binds to active sites of enzymes instead of substrates, which is a common mechanism for enzyme inhibition
- Feedback-inhibition (negative feedback)
  - Many metabolic enzymes are inhibited by their own product or by the product of an enzyme downstream from them in the same metabolic pathway

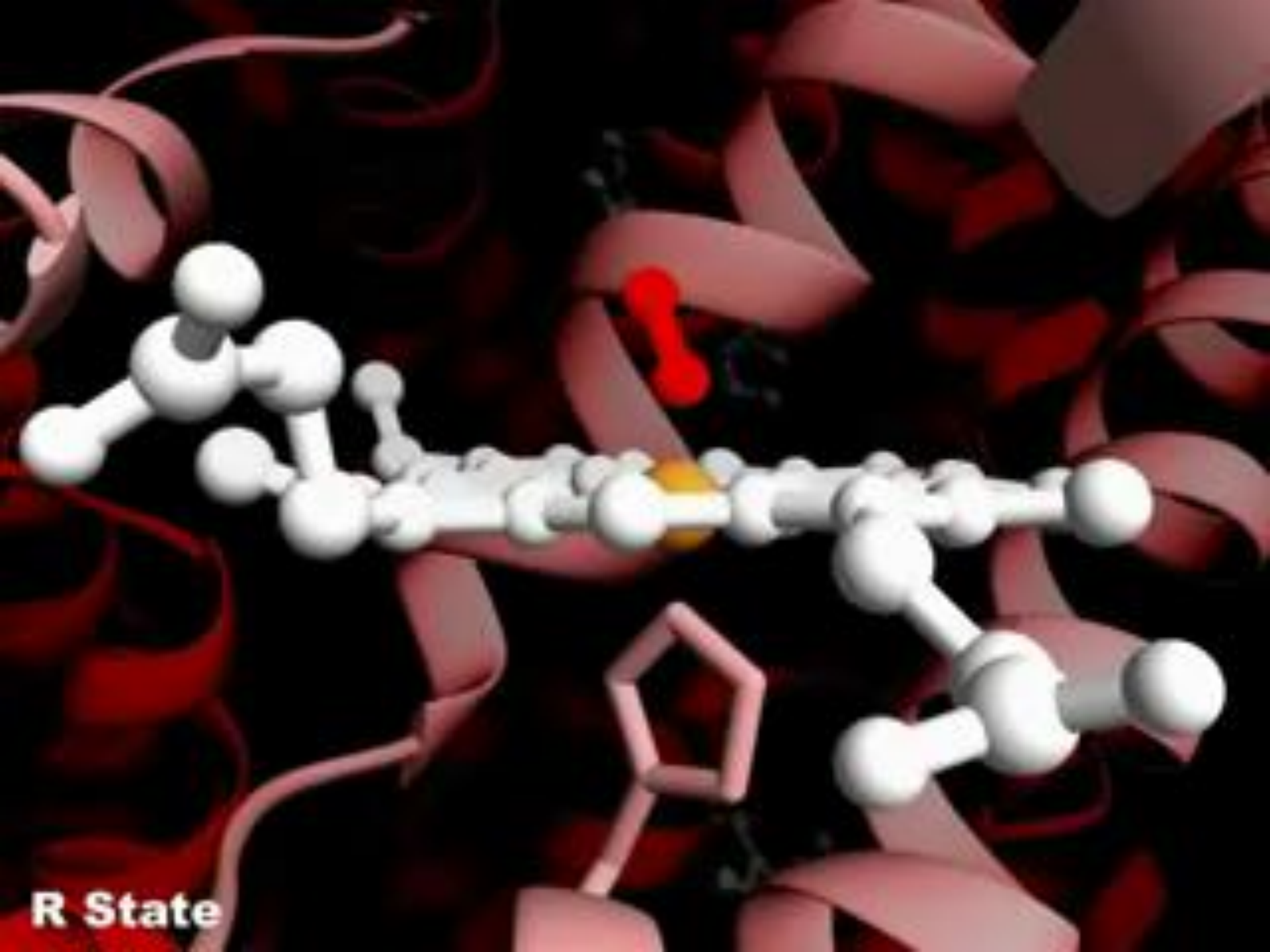


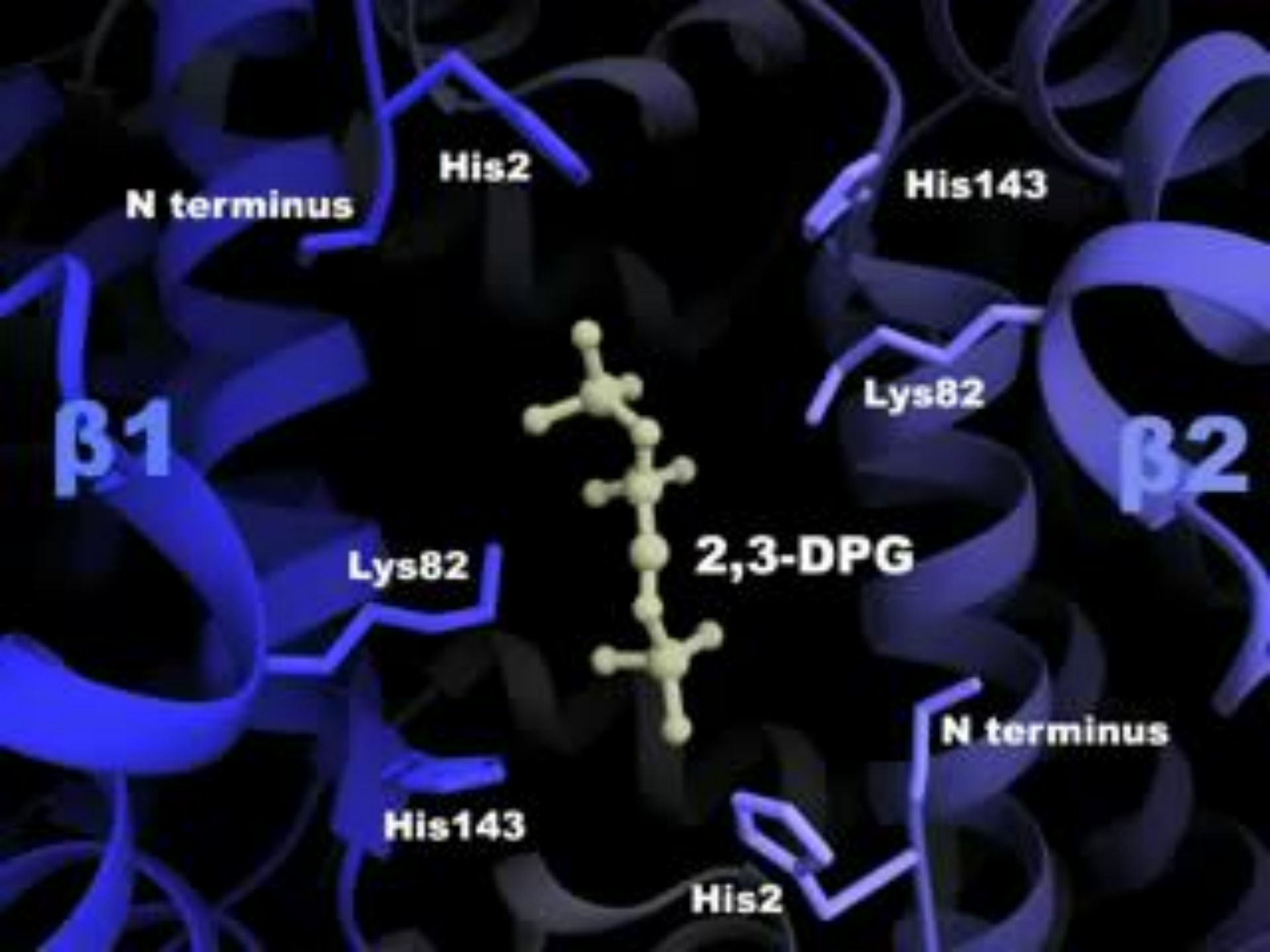
Effector and allostery

- Some effectors can cause conformational changes at distant sites
  - Binding at a site distinct from the enzyme's catalytic site or from the site through which the protein's function is mediated
  - The most common type of regulatory effector
- Allostery ("another structure" in Greek)
  - Allosteric protein
  - Allosteric effector
    - Allosteric activator: stabilizes the more active form
    - Allosteric inhibitor: stabilizes the less active form
- Allosteric effector can be another protein or small molecule
  - Monomeric protein (allosteric protein) is often regulated by another protein (allosteric effector)
  - Small ligands (allosteric effector) regulate oligomeric protein (allosteric protein)

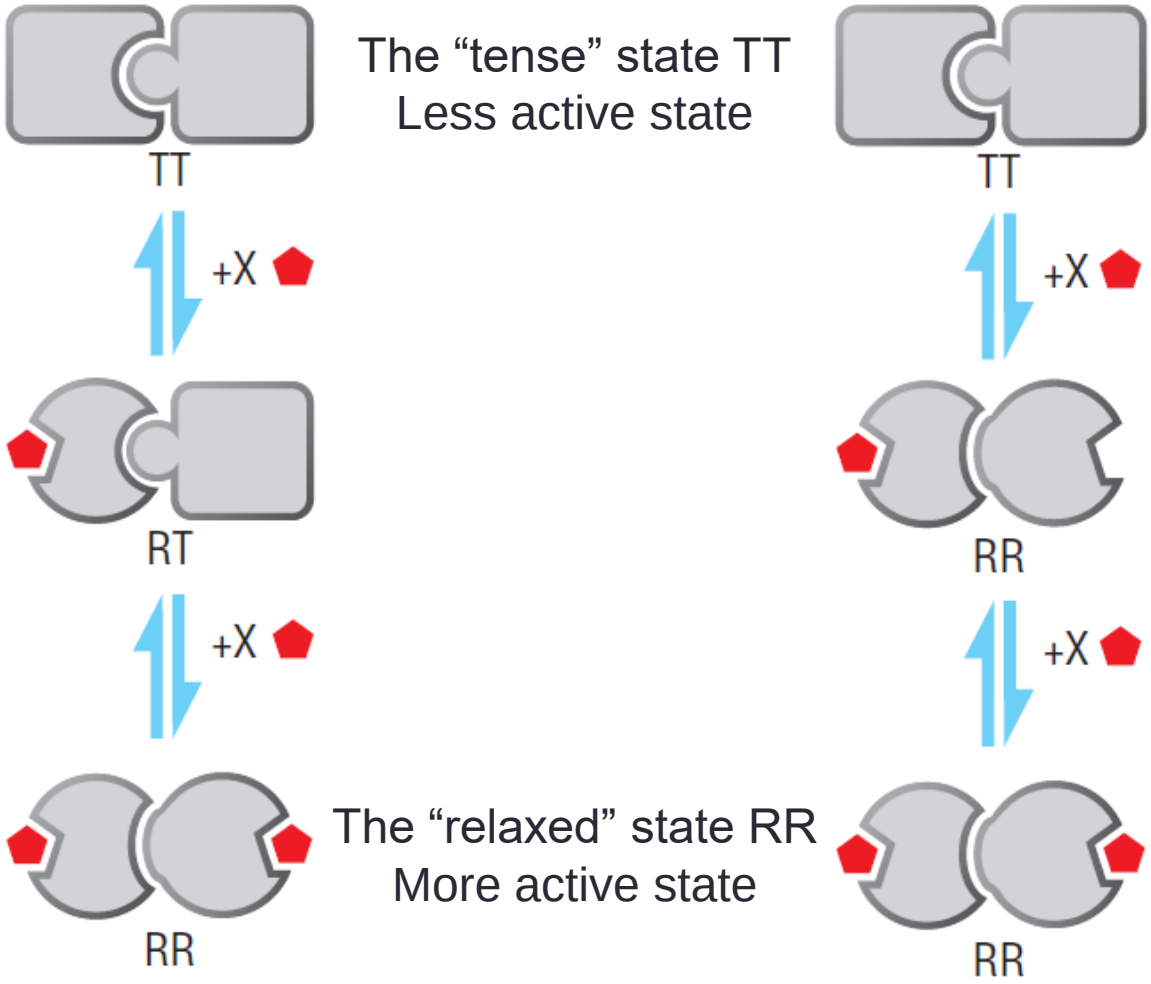
Enzyme and allosteric regulation




R State


His2
N terminus
His143
Lys82
β1
β2
2,3-DPG
Lys82
N terminus
His143
His2

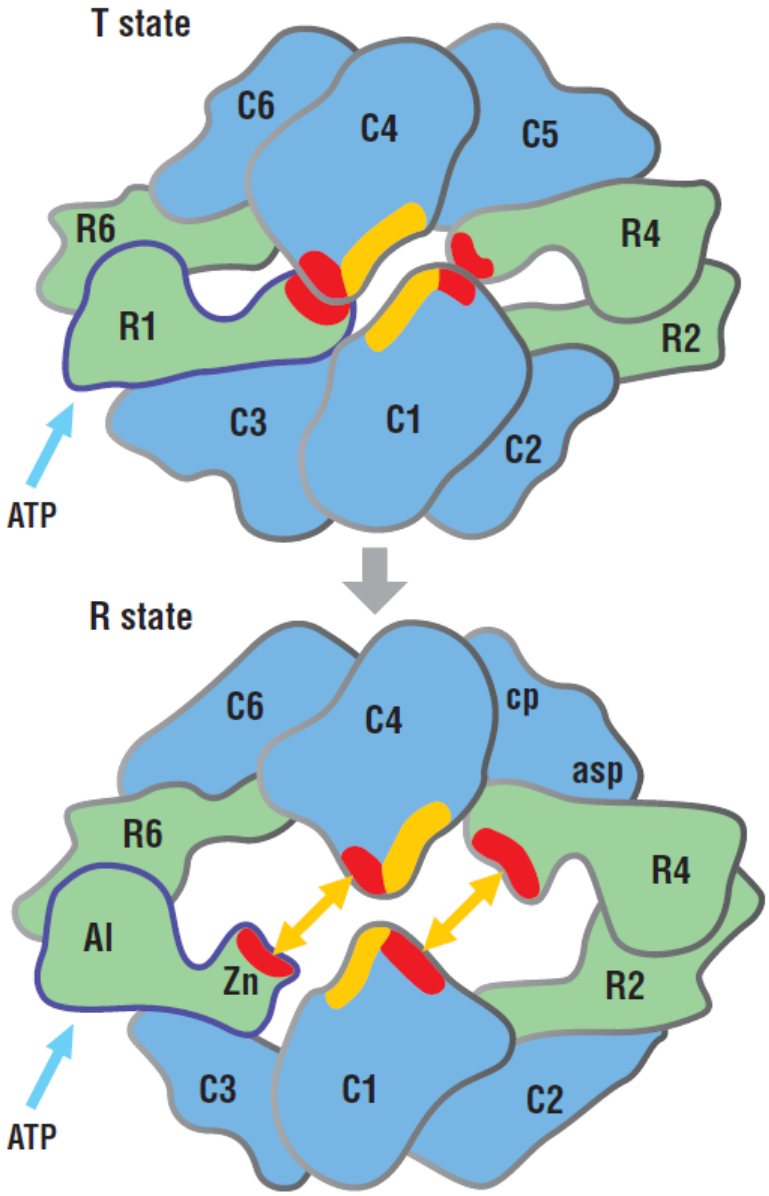
Allosteric regulation



Sequential conformational change

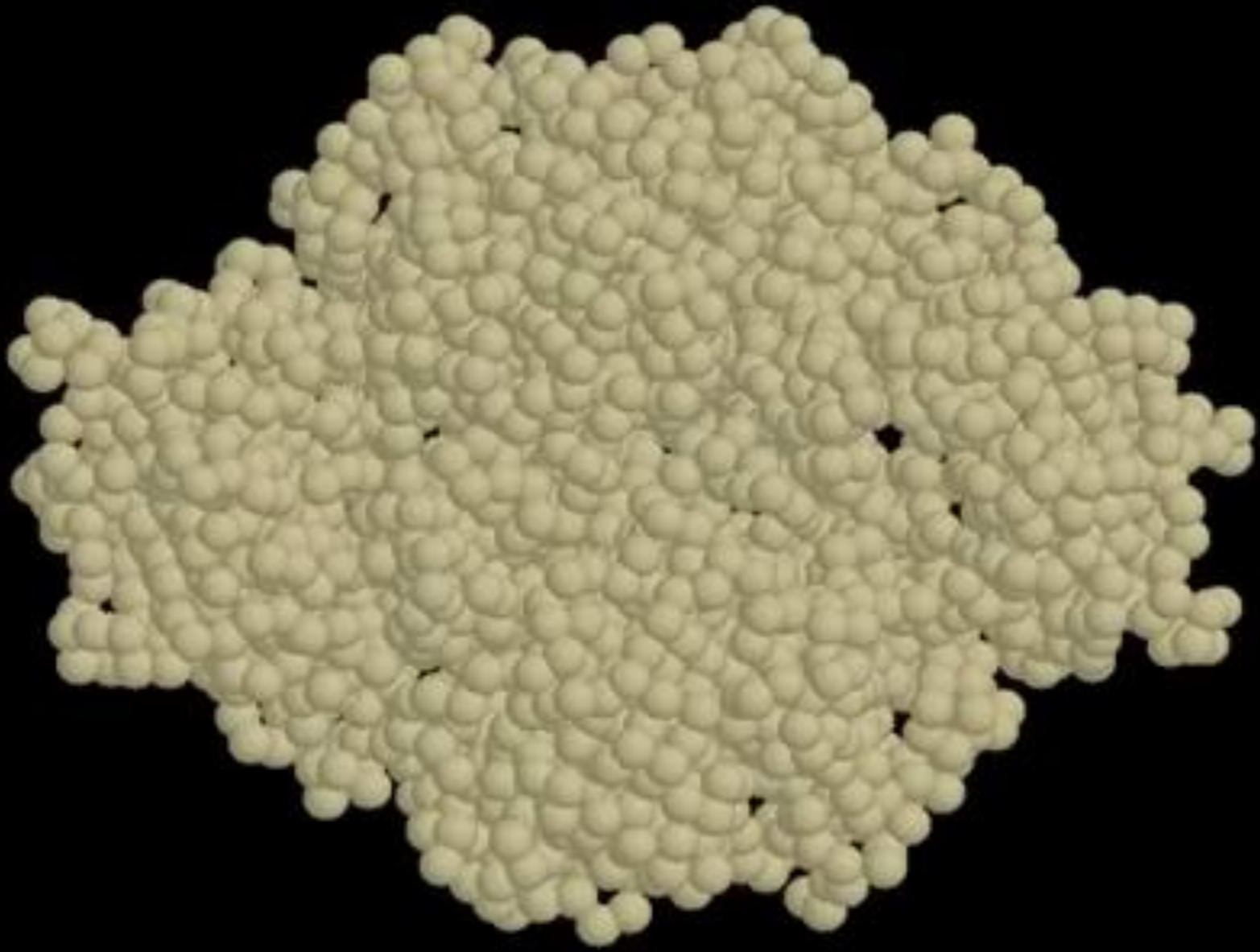
Preexisting conformational equilibrium

ATCase (aspartate transcarbamoylase)



- ATCase catalyzes the formation of N-carbamoyl aspartate (pyrimidine synthesis from carbamoyl phosphate and L-aspartate).
- Allosteric inhibitor: cytidine triphosphate (CTP), the end-product of pyrimidine synthesis
- Allosteric activator: ATP, the end-product of purine biosynthesis
- CTP is a feedback inhibitor and shuts down ATCase when pyrimidine levels are high; ATP activates the enzyme when purine levels are high and pyrimidines are needed to pair with them to make nucleic acids.

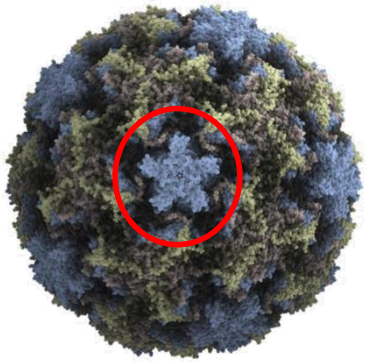
In hetero-oligomeric enzymes, the regulatory site is often located on a different subunit from the active site

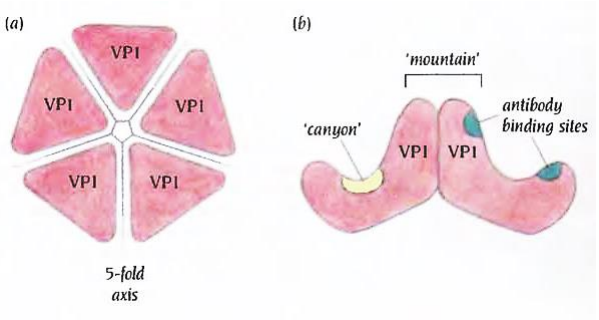


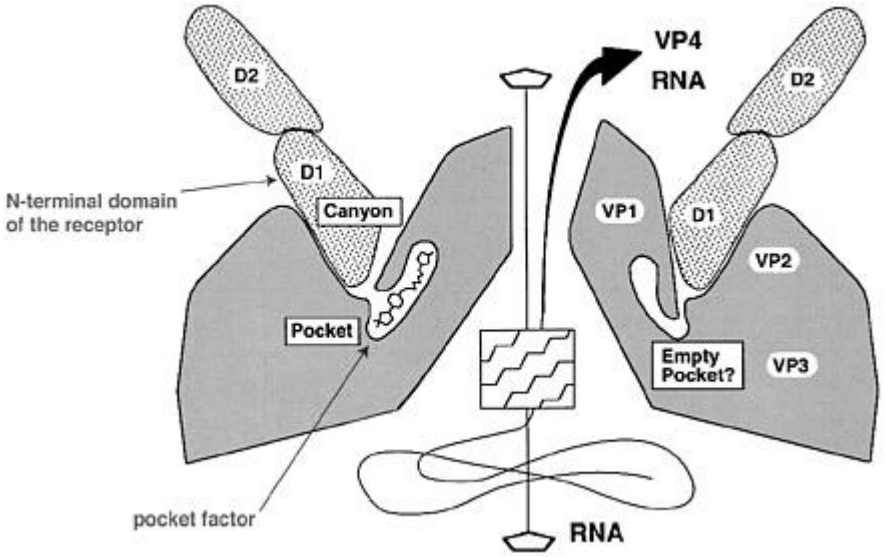
Disruption of function without disrupting the active site or ligand-binding site

- Disruption of a protein
  - Drug
  - Mutation
- Introduction of disruption into any part of the protein may lead to activity lost that depends on the propagation of a conformational change
  - The binding of drug directly to the active site or functional ligand-binding site, or a mutation at these sites
  - Drugs or mutations may also disrupt a protein's function by binding elsewhere and interfering with the conformational transitions necessary for function
  - In the absence of structural information, one should not automatically assume that any mutation that disrupts a protein's function must be in the active site or that any molecule that inhibits function does so

Binding of anti-viral compounds to the rhinovirus coat protein







- The binding blocks entry of the virus into host cells
- The binding does not happen in the site on the virus that binds the cell-surface virus receptor
- They bind to an unrelated site where they stabilize the structure of the coat protein
- The binding prevents the structural rearrangements required for receptor-mediated virus entry into the cell

Understanding of protein allostery provides new thinks in drug design

Binding of gene regulatory proteins to DNA

- Binding of gene regulatory proteins to DNA is often controlled by ligand-induced conformational changes
- Gene expression is regulated by activators and repressors
- Activators and repressors are under the control of specific regulatory ligands
  - Co-activators and co-repressors
    - Small molecules
    - Metal ions
    - proteins
  - Their binding determines whether or not the activator or repressor can bind to DNA

DtxR and Fe^{2+}

- Expression of the gene encoding diphtheria toxin is under the control of a specific repressor, DtxR
- Binding of DtxR to its operator sequence is controlled in turn by the concentration of Fe2+ in the bacterial cell
- Iron acts as a co-repressor by binding to DtxR and inducing a conformational change that allows the DNA-recognition motif in the repressor to fit into the major groove of DNA
- The absence of bound iron, the repressor adopts a conformation in which DNA binding is sterically blocked

Allostery provides another level of regulation in protein function control