

# Regulation of Protein Function

*It's what makes the cell!*

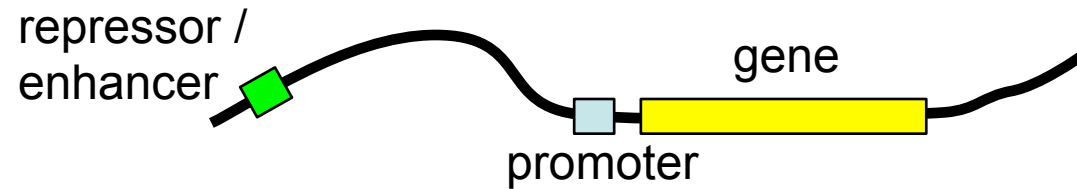
# Regulation of Protein Function

*It's what makes the cell!*

- Transcriptional control
- Translational control
- Protein quantity (concentration)
- Protein lifetime
- Spatial targeting/co-localization
- Binding of effector molecules  
(noncovalent modification)
- Covalent modification
- pH and redox environment

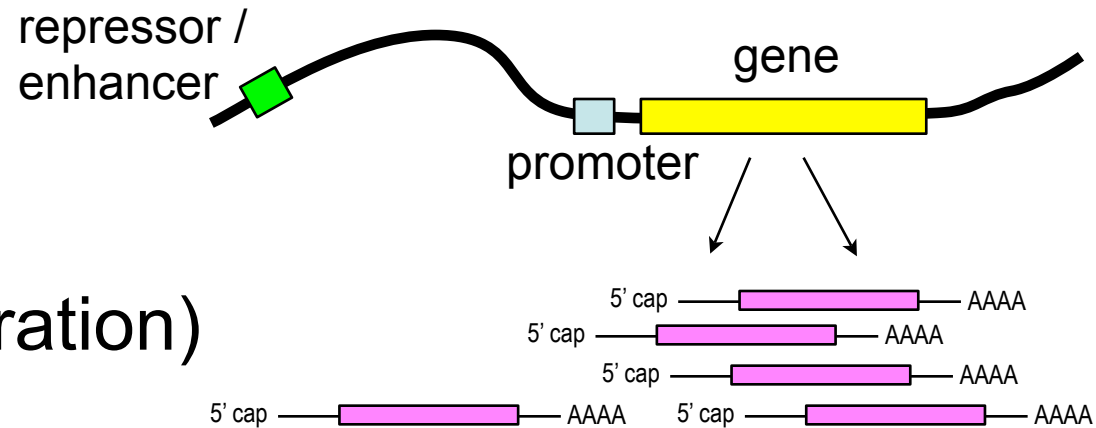
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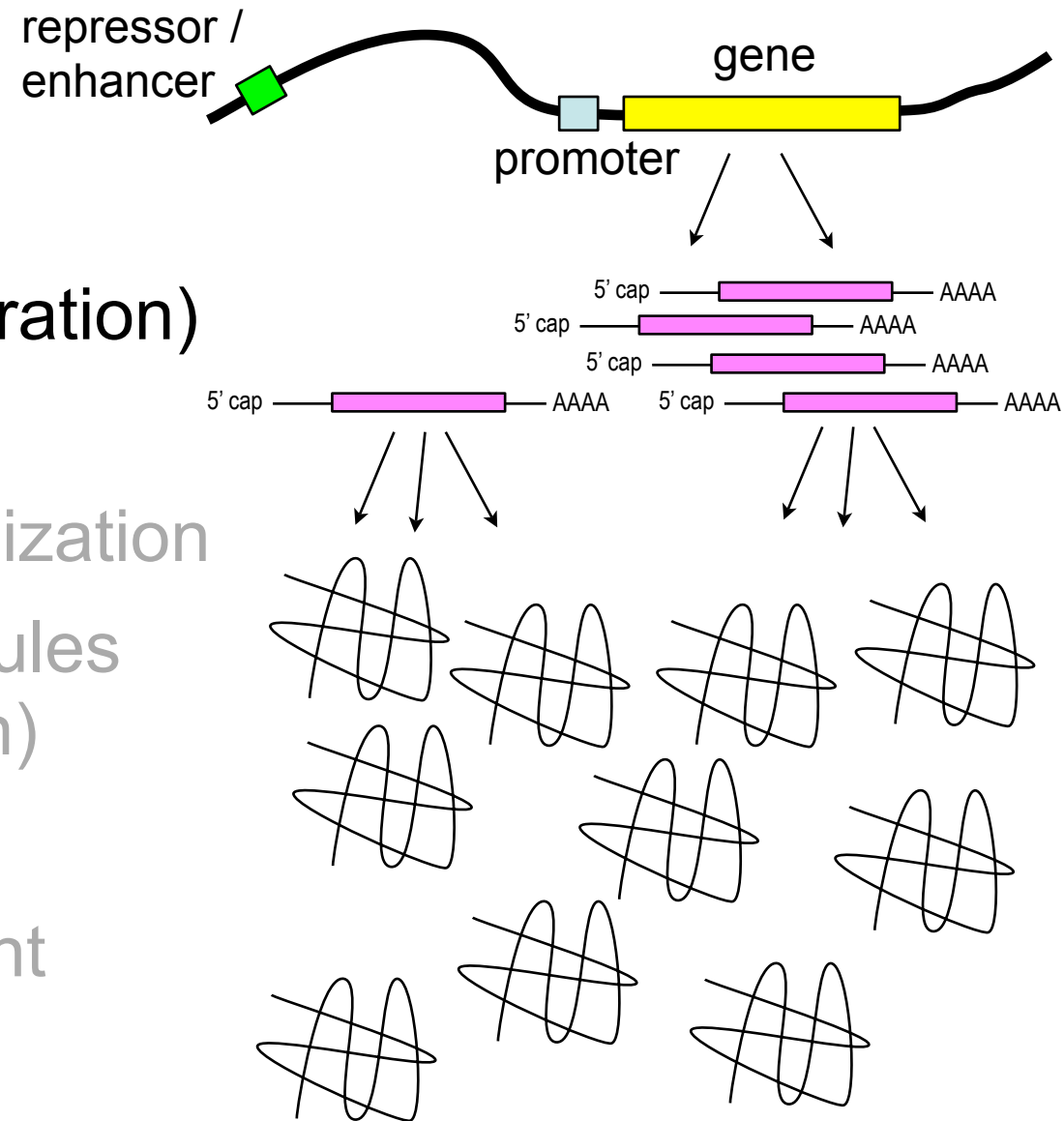
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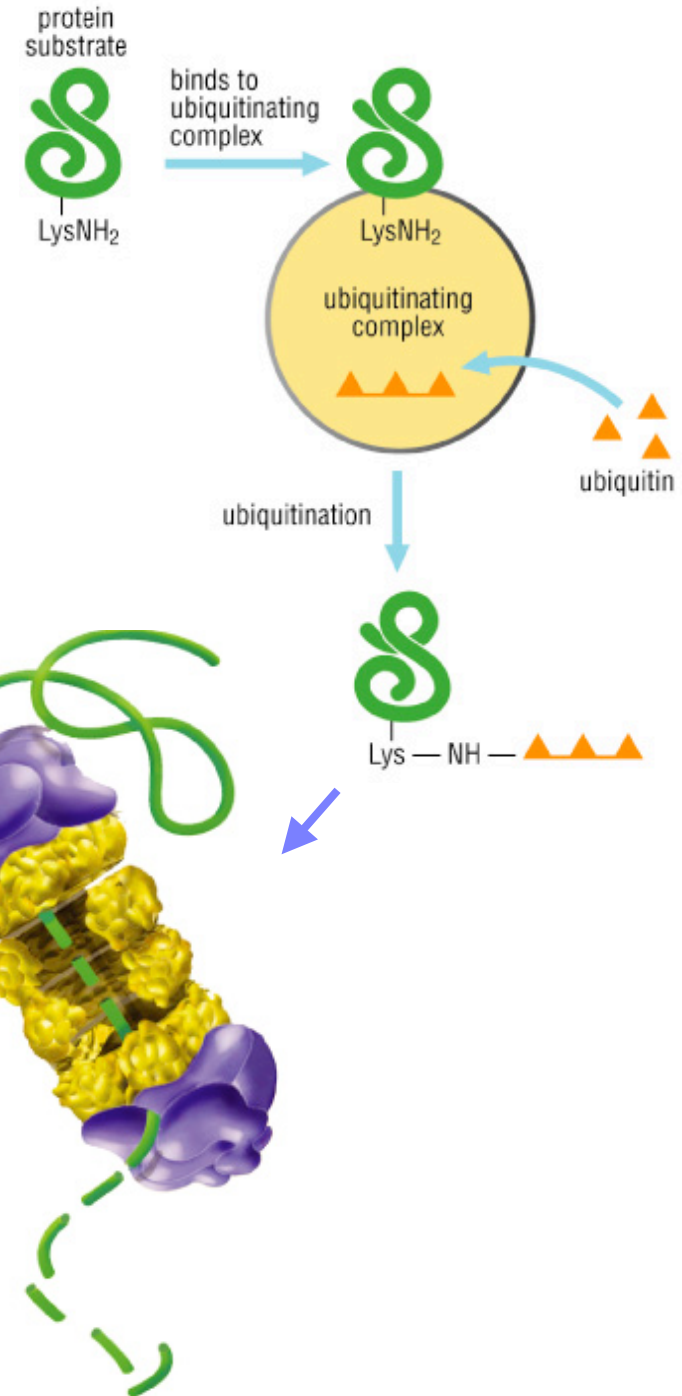
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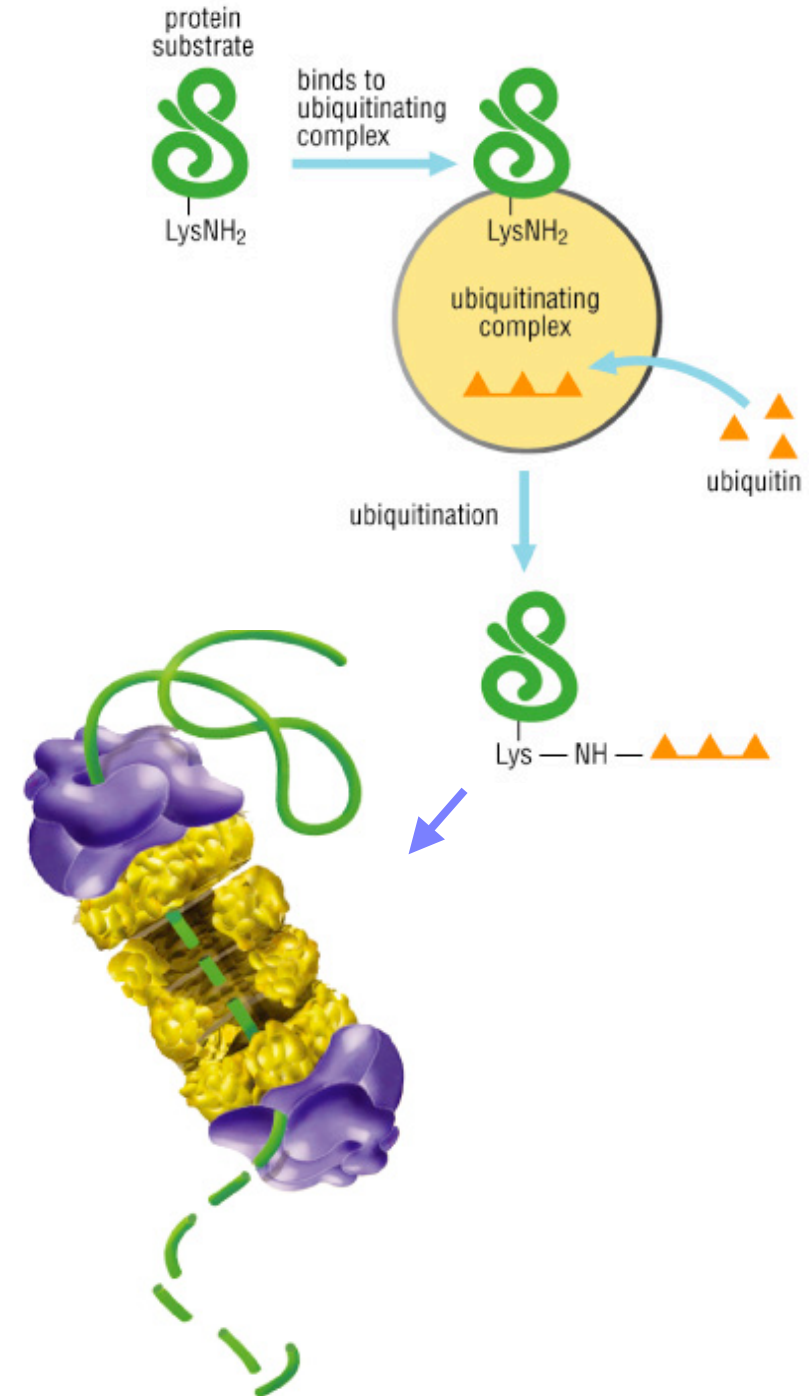
# Regulation of Protein Function

- Transcriptional control
- Translational control
- Protein quantity (concentration)
- Protein lifetime
  - Intrinsic stability
  - Tagged for destruction
    - (poly-)Ubiquitination
    - Targeted to proteasome
      - Cleaved to short polypeptides



# Regulation of Protein Function

- Ubiquitination
  - multi-enzyme pathway
    - ubiquitin ligase adds to a near N-term Lys
  - How targeted?
    - phosphorylation
    - hydroxylation
    - N-term aa identity
      - Protective
        - » Met, Ser, Thr, Ala, Val, Cys, Gly, Pro
- Why?
  - Quality, temporal control



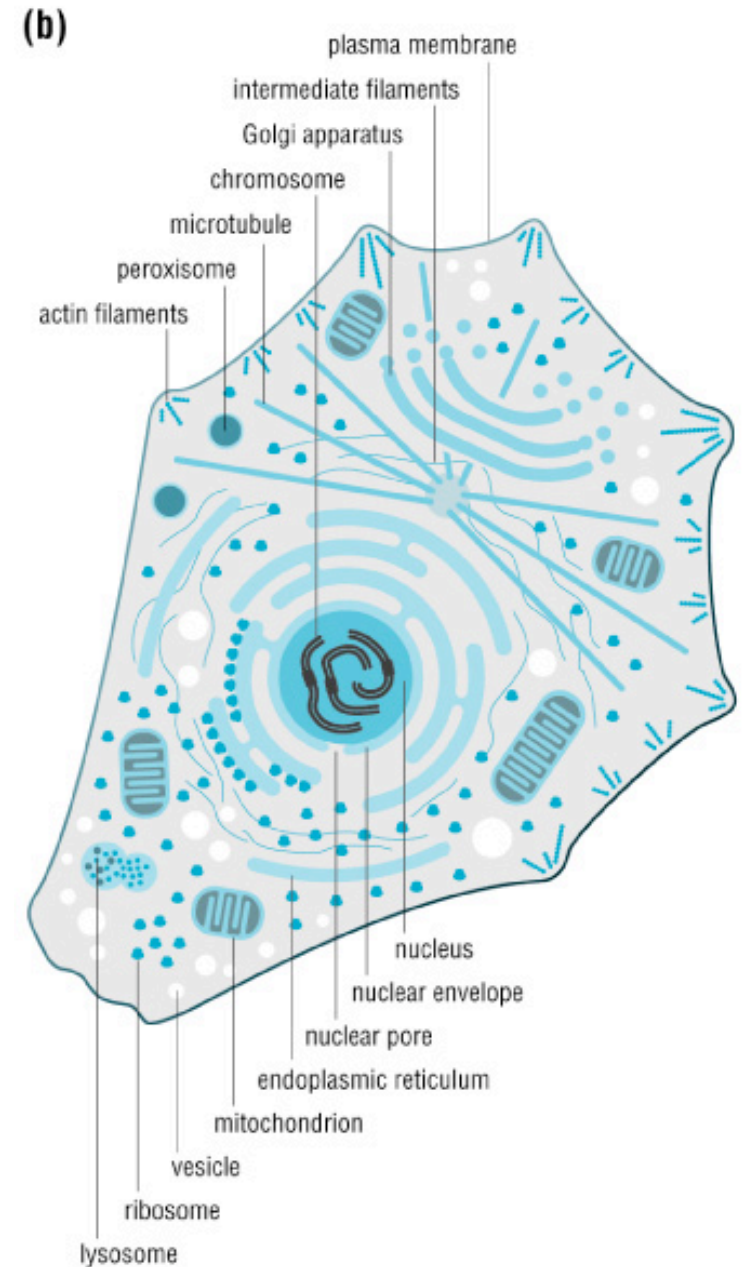
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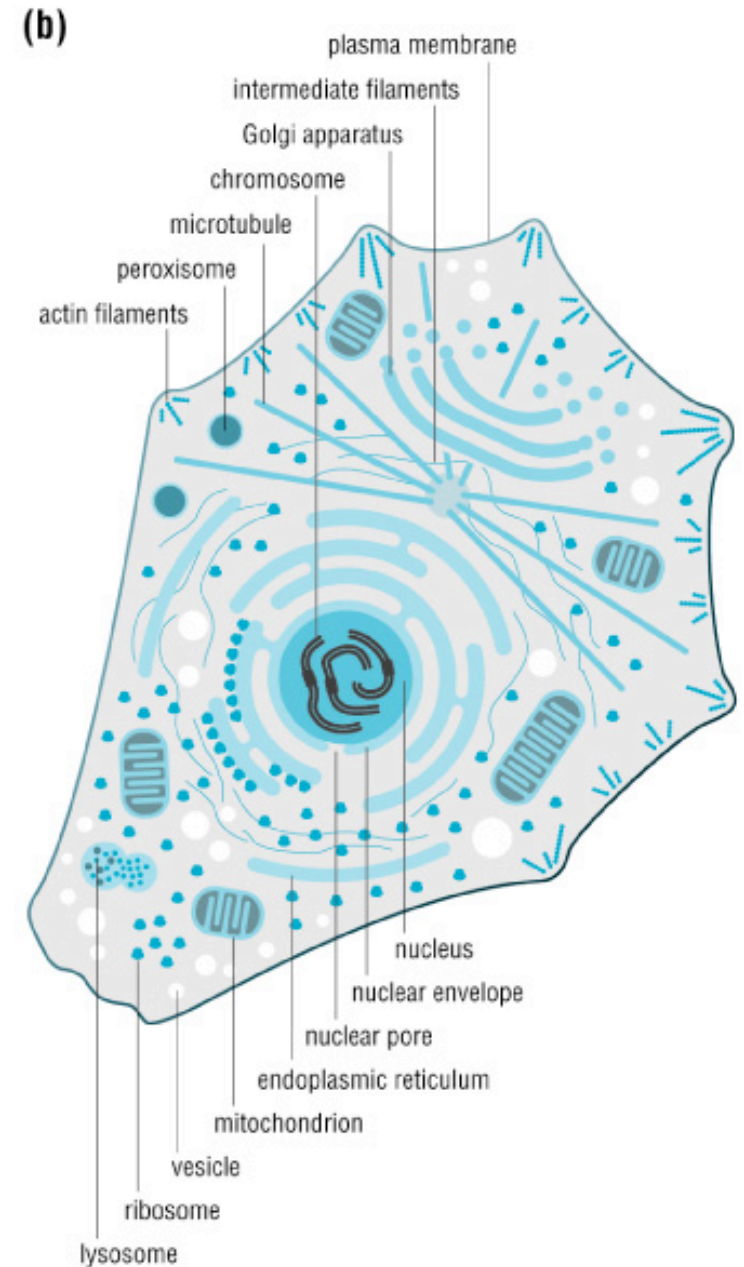
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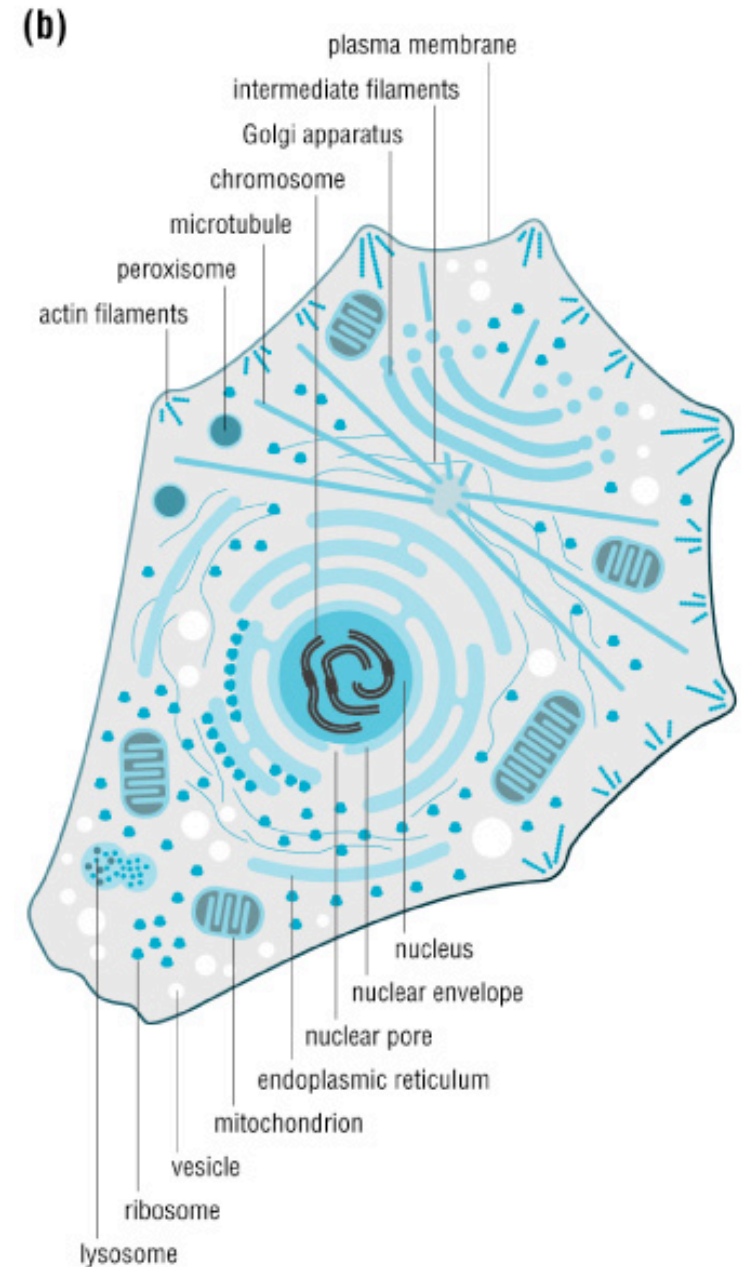
# Regulation of Protein Function

- Spatial targeting/co-localization
  - More common in eukaryotes, as there are more compartments
    - in an organelle
    - attached to a membrane
    - attached to cytoskeleton
    - associated with above...
  - May allow eukaryotes to “do more with less”
  - Achieved/signaled by
    - localization sequences
    - post-translational modification
    - Binding to scaffold / membrane



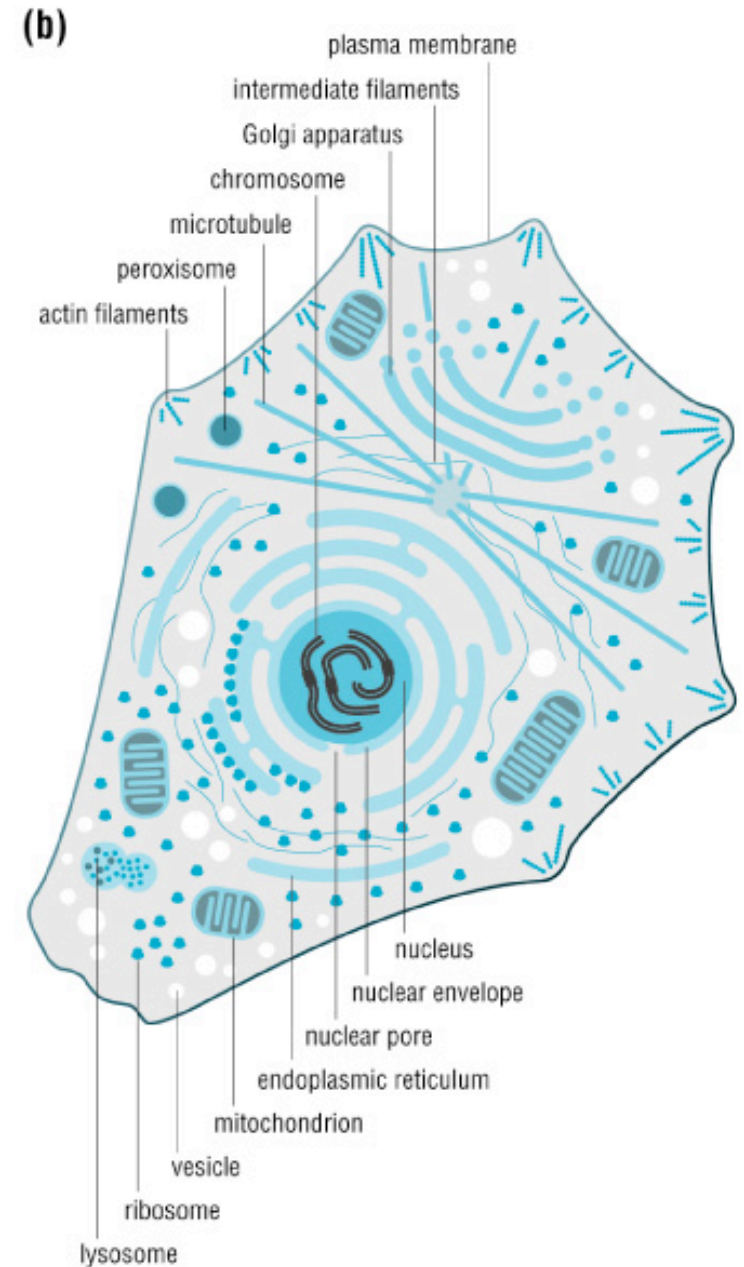
# Regulation of Protein Function

- **Localization signals** (sequences)
  - Endoplasmic reticulum (KDEL)
    - targets to ER
    - and ultimately to plasma membrane
  - Nuclear localization (KRKR)
    - targets to nucleus
  - Others
    - extracellular secretion
    - mitochondrial import
  - Can be N-terminal, C-terminal, or even internal sequences in the protein
- post-translational modification
- Binding to scaffold / membrane



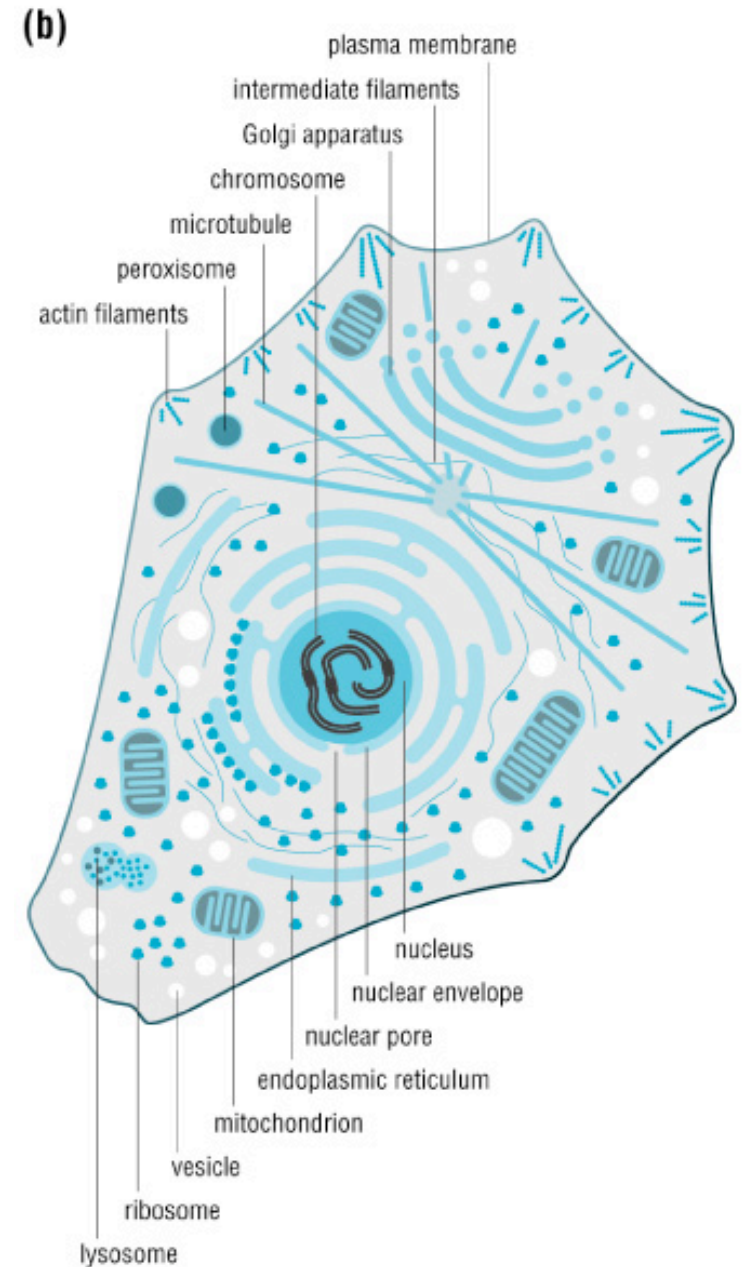
# Regulation of Protein Function

- localization signals (sequences)
- Post-translational modification
  - differ from intrinsic localization signals in that they **regulatable**.
  - Phosphorylation of Ser, Tyr, Thr
    - by protein kinases
    - oft-used in signaling
- Binding to scaffold / membrane



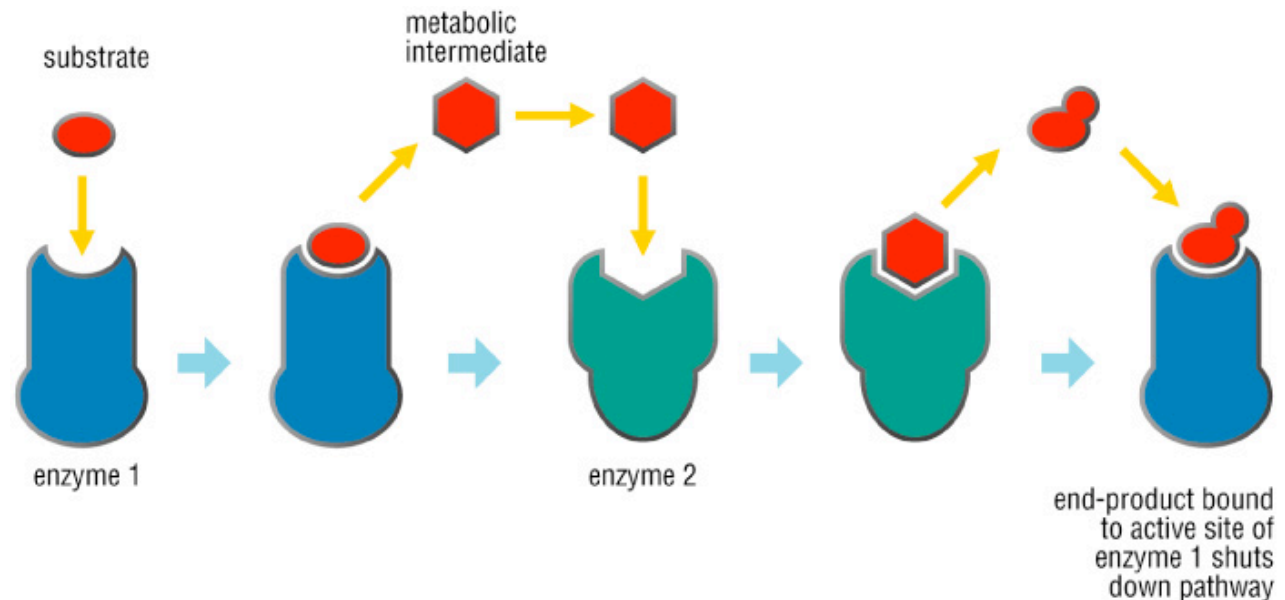
# Regulation of Protein Function

- localization signals (sequences)
- post-translational modification
- Binding to scaffold / membrane
  - Lipid anchoring
    - covalently attach N-/C-terminus to a lipid
    - localizes soluble protein near membrane
    - membrane structure can further localize such a protein
  - Scaffold typically has a recognition domain
    - SH3 domain binds Pro-rich seqs
    - SH2 domain binds phosphorylated Tyr
    - membrane lipids bind PH domain



# Regulation of Protein Function

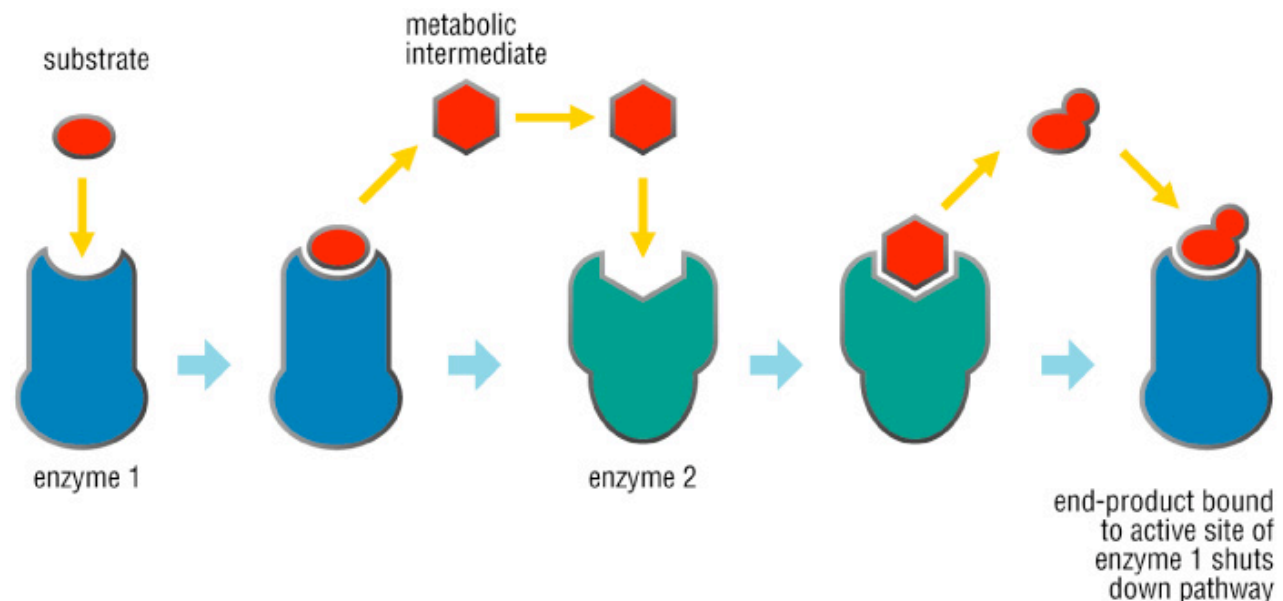
Probably the most well-recognized form of enzyme modulation (inhibitor and activators)



# Regulation of Protein Function

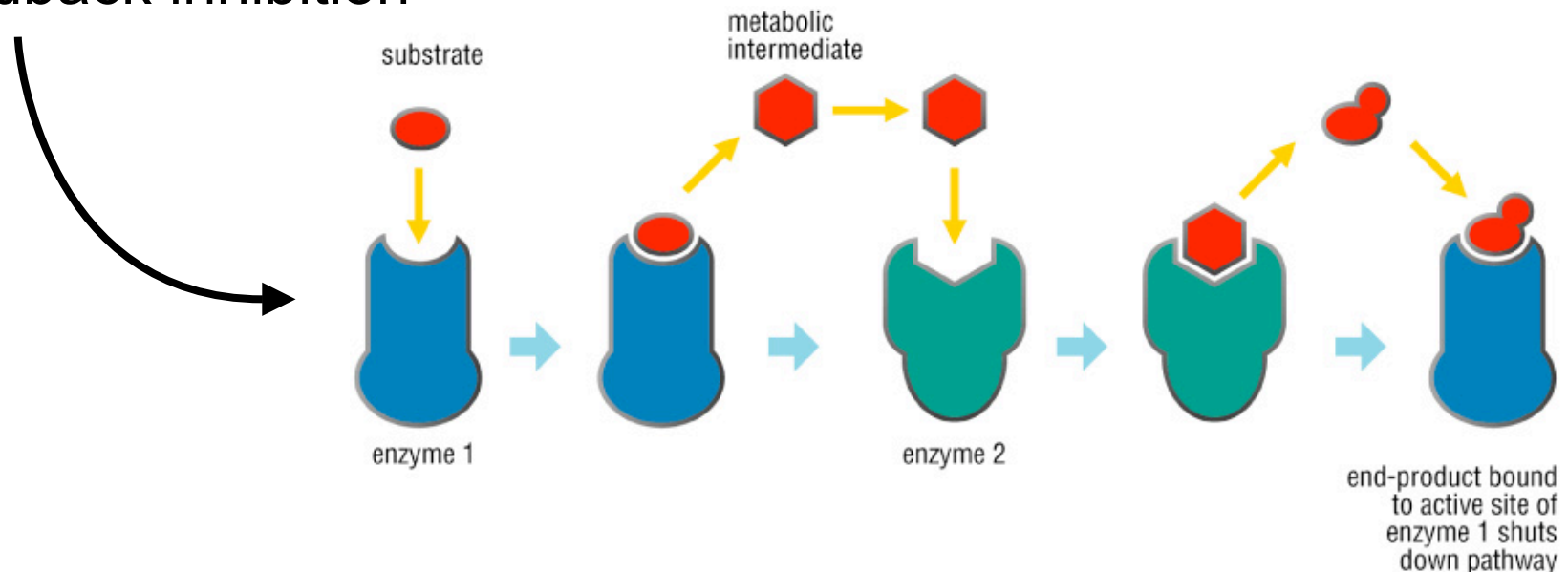
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- Protein quantity (concentration)
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# Regulation of Protein Function

- Binding of effector molecules (noncovalent modification)
  - Competitive binding
    - binds at active site, displacing substrate or other effector molecule
  - Noncompetitive binding
    - allostery - action at a distance (eg. effects  $k_{cat}$ )
  - Common:
    - Feedback inhibition





# Regulation of Protein Function

- **Binding of effector molecules** (noncovalent modification)
  - Cooperativity between binding sites for the same ligand, in which binding at one site affects affinity at the other

- Positive cooperativity

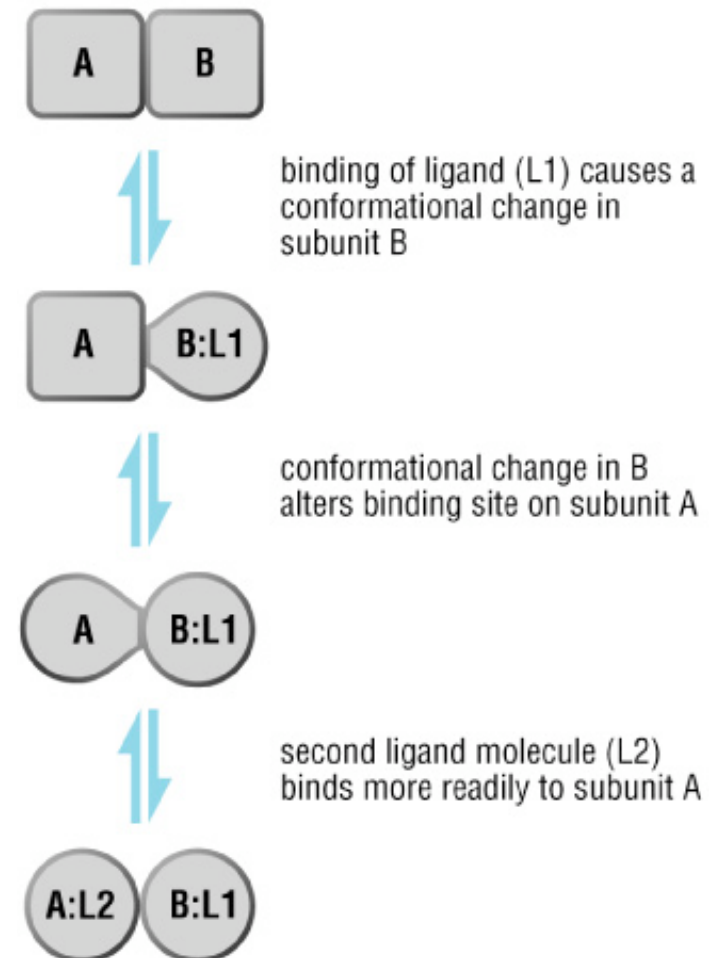
- Binding at one site makes binding at the second site *stronger*

- Negative cooperativity

- Binding at one site makes binding at the second site *weaker*

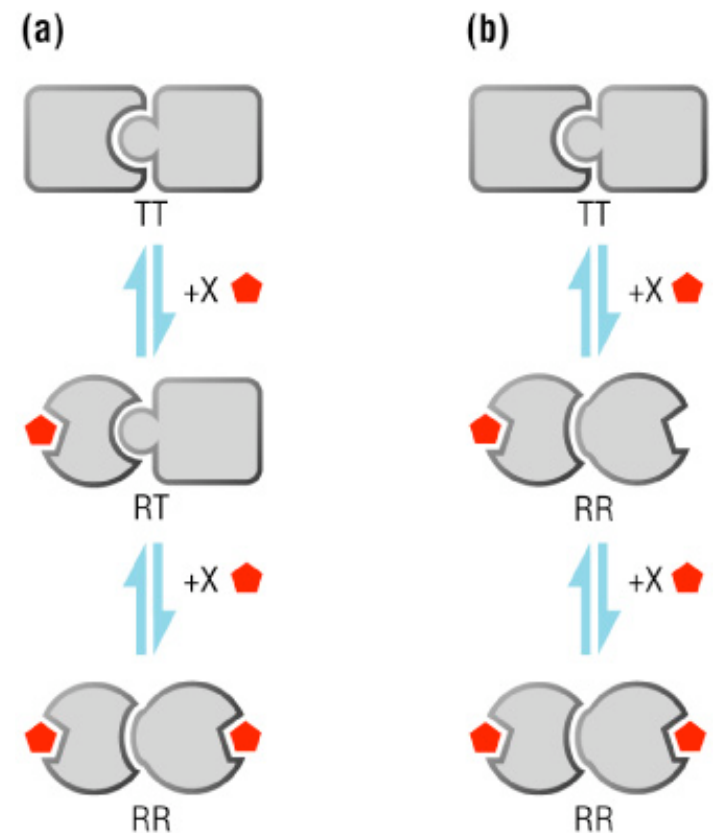
- Reflects flexibility in structure - binding at one site distorts the other

- “Cooperativity is only present in oligomeric proteins, where there are  $\geq 2$  subunits, each with a binding site for the ligand”



# Regulation of Protein Function

- **Binding of effector molecules** (noncovalent modification)
  - Allostery - action at a distance
- Allosteric activator
  - Binding at one site makes binding at the second site *stronger*
- Allosteric inhibitor
  - Binding at one site makes binding at the second site *weaker*
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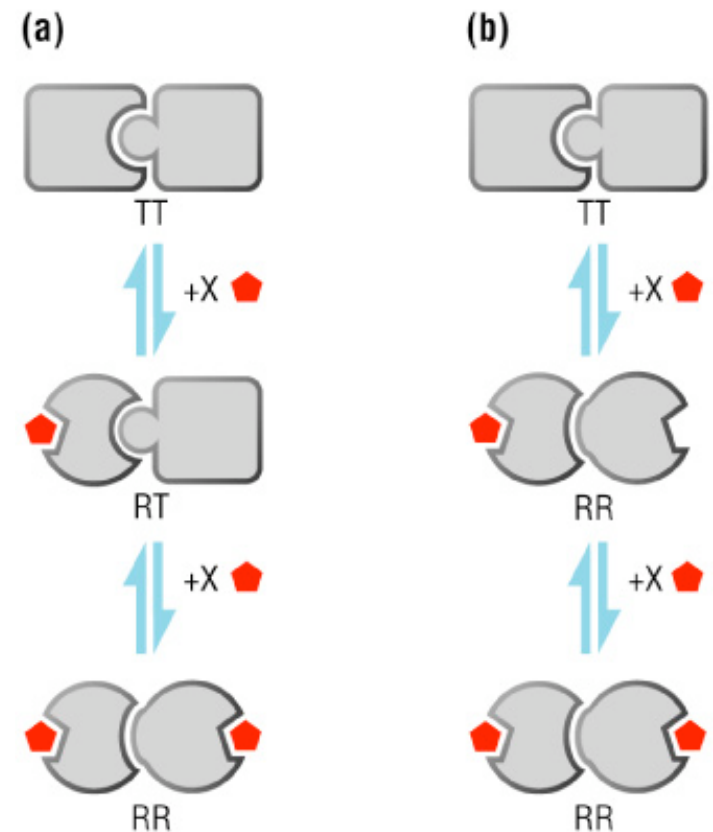


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- **Binding of effector molecules** (noncovalent modification)
  - Allostery - action at a distance

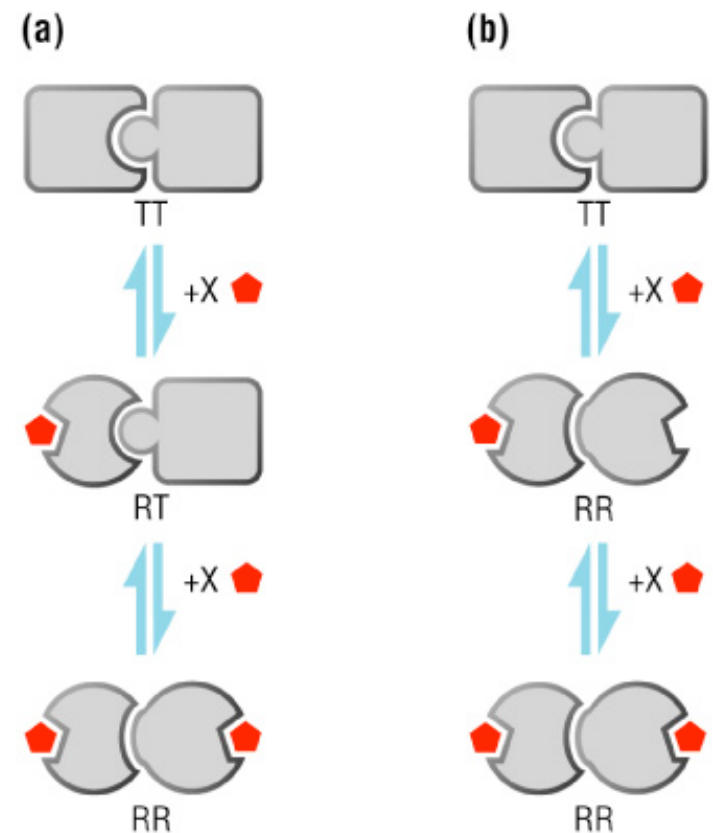
Advertisement: Chem 728

- Allosteric activator
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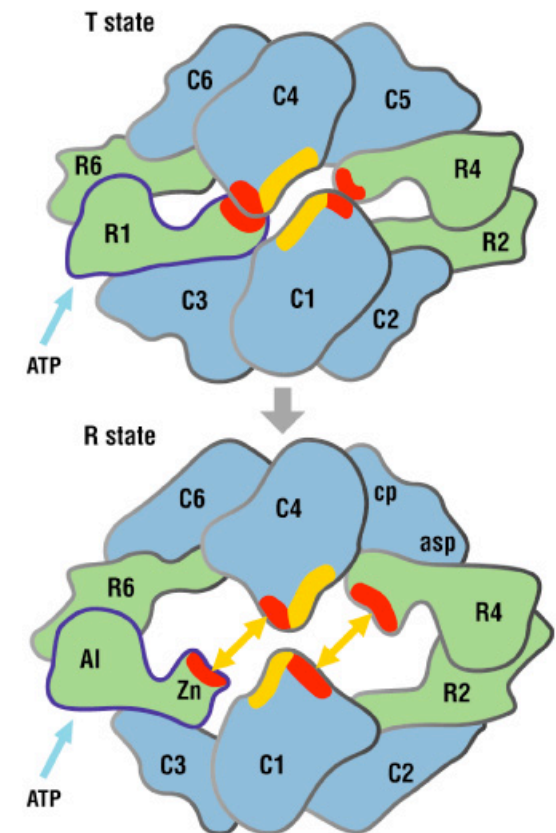
# Regulation of Protein Function

- **Allostery**
  - Effector ligand can be a small molecule or another protein
- Hemoglobin / O<sub>2</sub>
  - O<sub>2</sub> binding to one subunit activates remaining subunits



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- Aspartate transcarbamoylase
  - ATP binding triggers change that opens active site for substrate binding



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- **Allostery**

- Effector ligand can be a small molecule or another protein

- Hemoglobin / O<sub>2</sub>

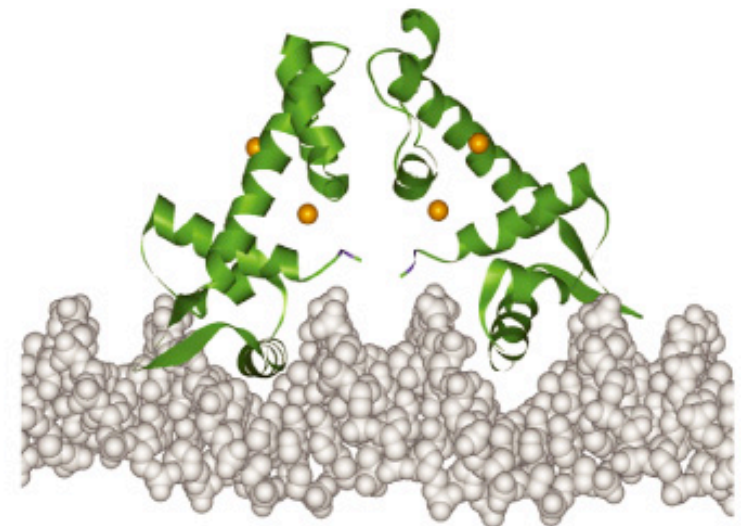
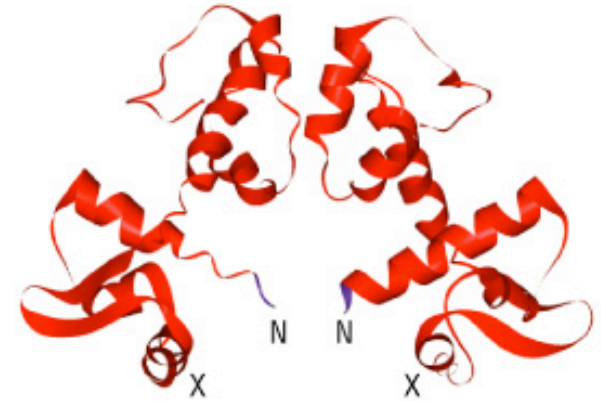
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- Aspartate transcarbamylase

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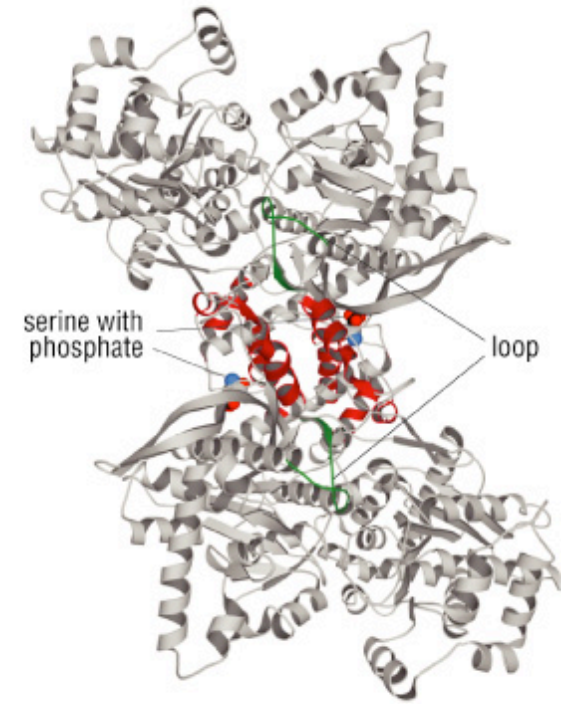
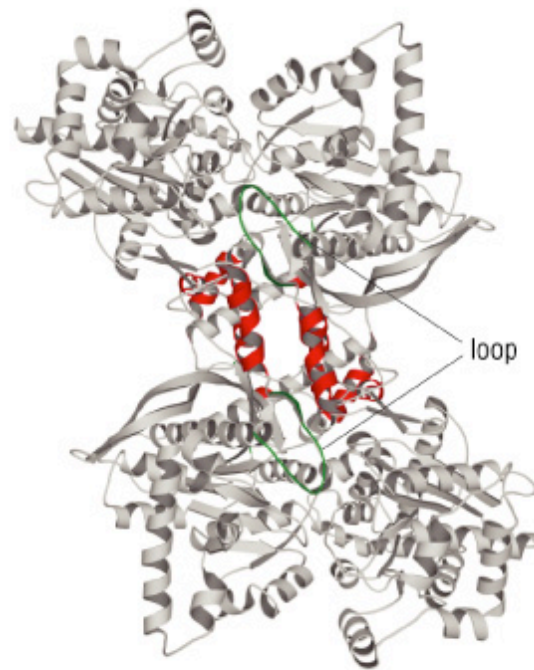
- DtxR repressor

- Binding of Fe<sup>2+</sup> alters spacing of major groove reading heads to allow proper fit in two consecutive major grooves



# Regulation of Protein Function

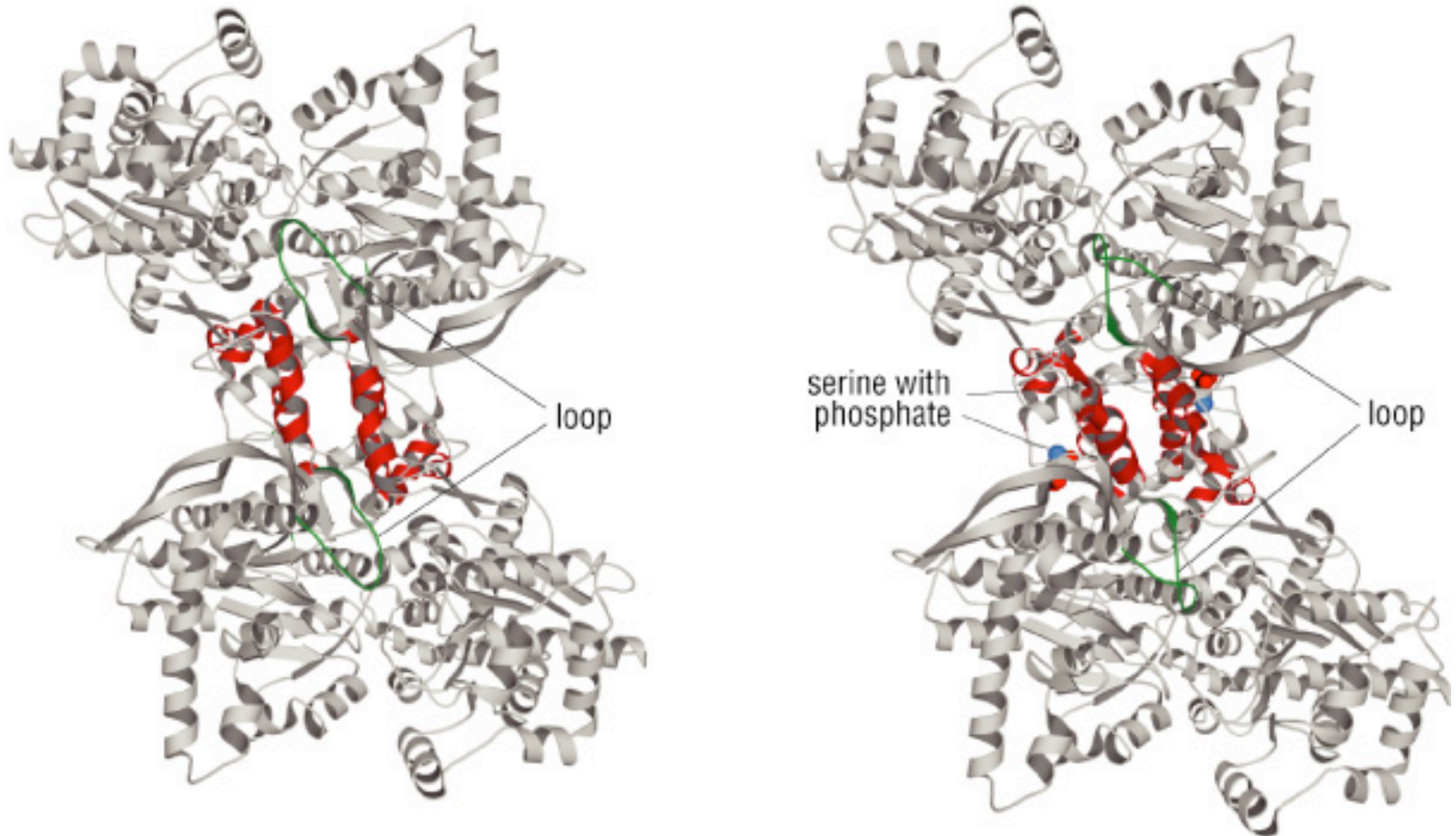
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- Translational control
- Protein quantity (mRNA stability)
- Protein lifetime
- Spatial targeting
- Binding of effectors (noncovalent modification)
- **Covalent modification**
- pH and redox environment



Phosphorylation of Ser14 induces movement of a loop that prior to phosphorylation is blocking the active site

Glycogen phosphorylase

# Regulation of Protein Function



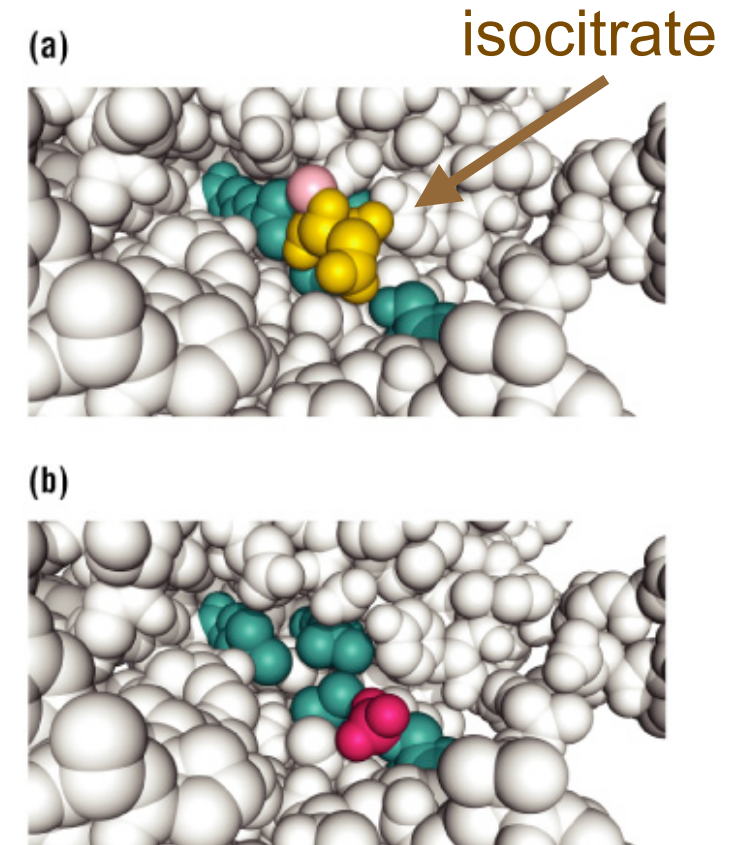
Glycogen phosphorylase



# Regulation of Protein Function

- **Phosphorylation of Ser/Tyr hydroxyl** (and His/Asp)
  - Added by protein kinases
  - Removed by protein phosphatases
  - Controlled reversibility
- Adds a double negative charge to a polar, but uncharged amino acid
  - Adds electrostatic repulsion / attraction
  - Adds new H-bonding potential
  - Adds potential recognition site for binding of a second protein
    - eg. SH2 domains bind P-Tyr

From **Protein Structure and Function**  
by Gregory A Petsko and Dagmar Ringe



Isocitrate dehydrogenase  
phosphorylation of Ser 113 adds  
charge to substrate binding site

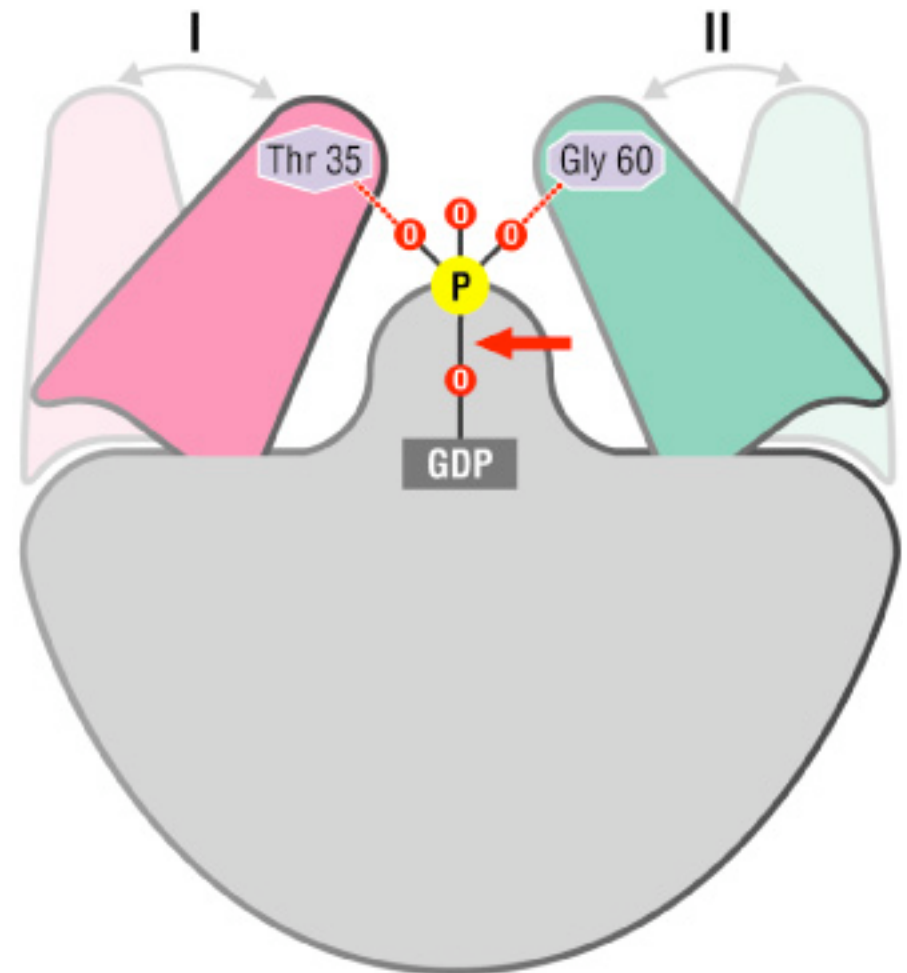
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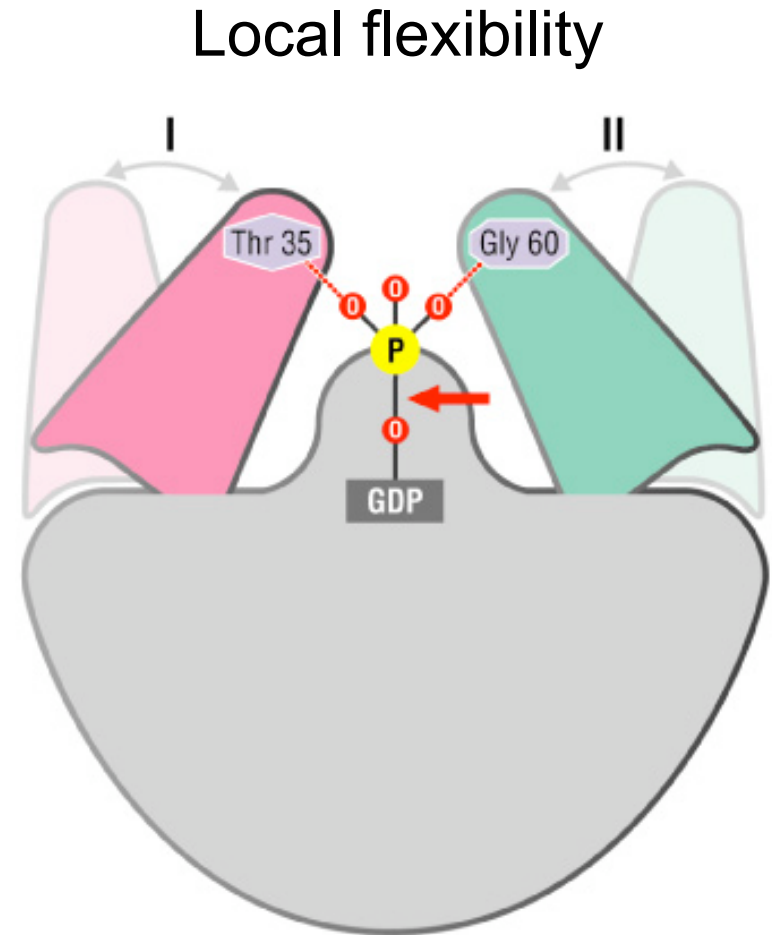
- Protein switches based on nucleotide hydrolysis
- G-proteins
  - GTP bound presents a  $\gamma$ -phosphate
  - GDP bound removes the  $\gamma$ -phosphate
  - GTP hydrolysis switches from first state to second
  - Different proteins bind to the two states
- Motor proteins
  - Same idea, but with ATP

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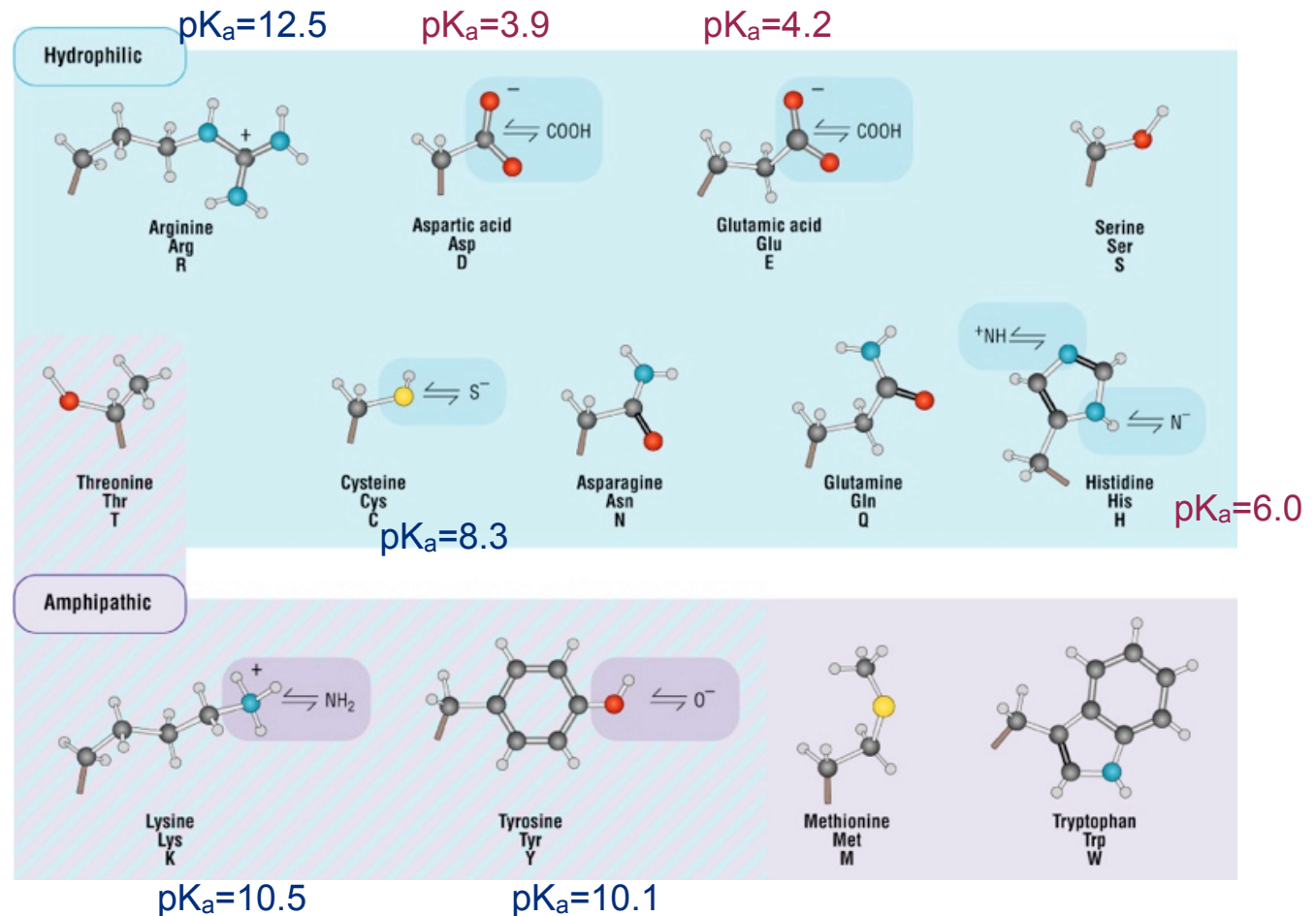
# Regulation of Protein Function

- G-proteins (GTPases)
  - Conserved sequence motifs
    - P-loop: binds  $\alpha, \beta$ -phosphate
      - $GX_4GKS/T$
    - Switch I
      - $DX_nT$
    - Switch II
      - $GX_2G$
    - Guanine base-binding region
      - $N/TKXD$
- Motor proteins (ATPases)
  - P-loop:  $GX_4GKS/T$
  - Switch I:  $NX_2SSR$
  - Switch II:  $DX_2G$
  - Adenine base-binding region
    - $RXRP$



# Regulation of Protein Function

- Transcriptional
- Translational control
- Protein quantity
- Protein lifetime
- Spatial targeting
- Binding of effectors (e.g., allosteric regulation, phosphorylation, etc.)
- Covalent modification
  - pH and redox environment
    - protonation/deprotonation changes local charge
    - redox state change changes charge / coordination
    - redox change favors/disfavors disulfide bond

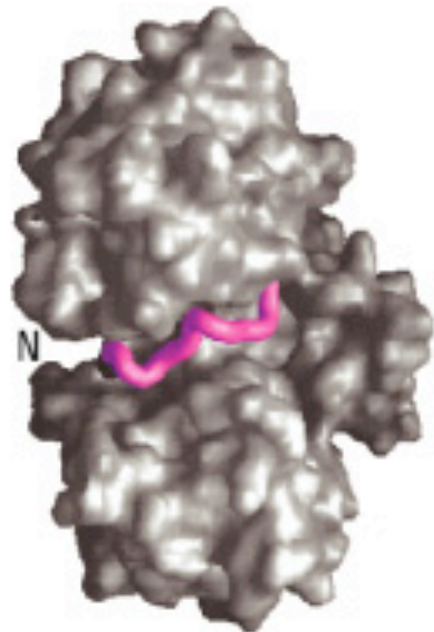


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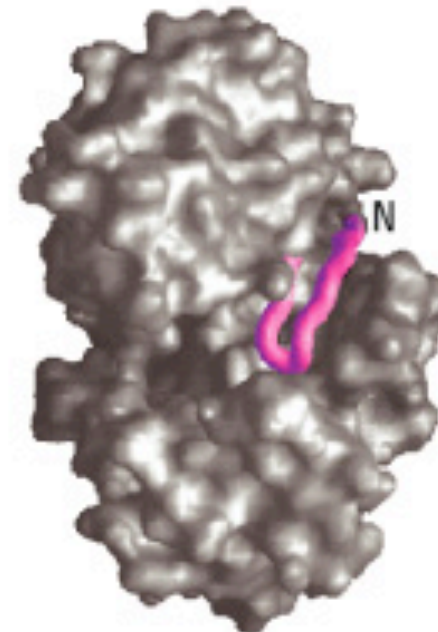
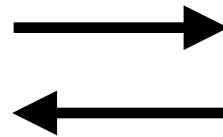
- pH and redox environment
  - protonation/deprotonation changes local charge
    - cathepsin is activated in the endosome

N-terminus binds in  
substrate binding site

substrate binding site open  
catalytic residues protonated



neutral pH



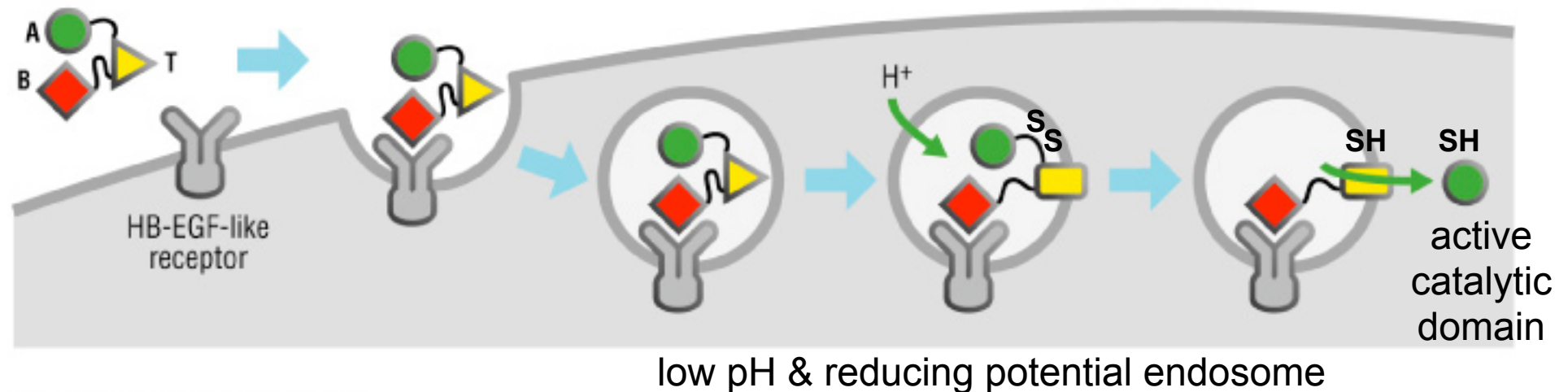
low pH

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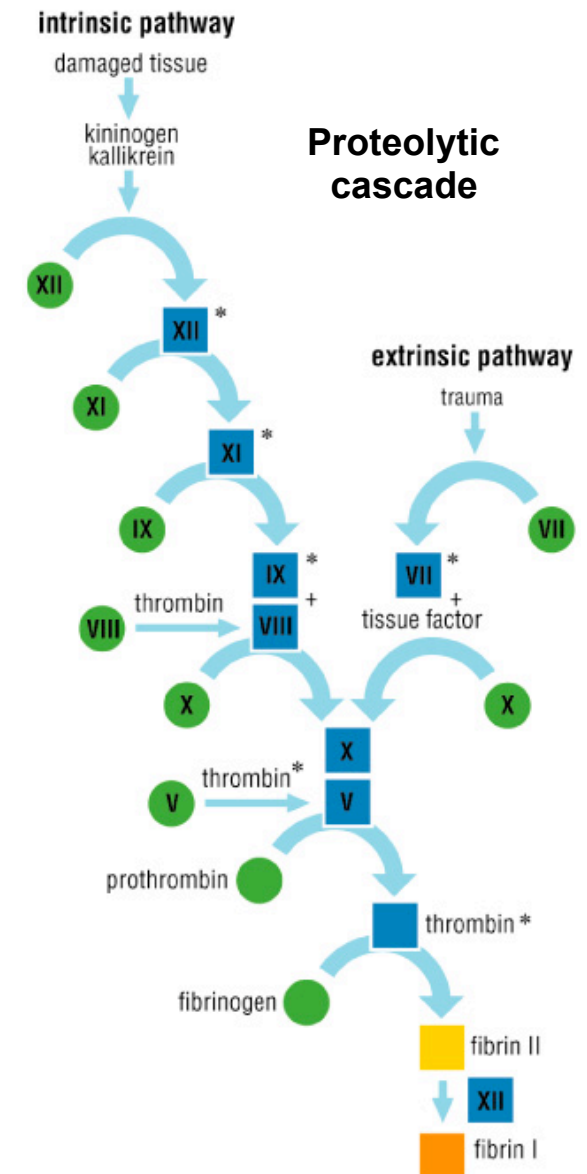
diphtheria toxin



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- Covalent modification - **proteolysis**
  - trypsinogen -> trypsin
  - plasminogen -> plasmin
  - prothrombin -> thrombin
- pH and redox environment

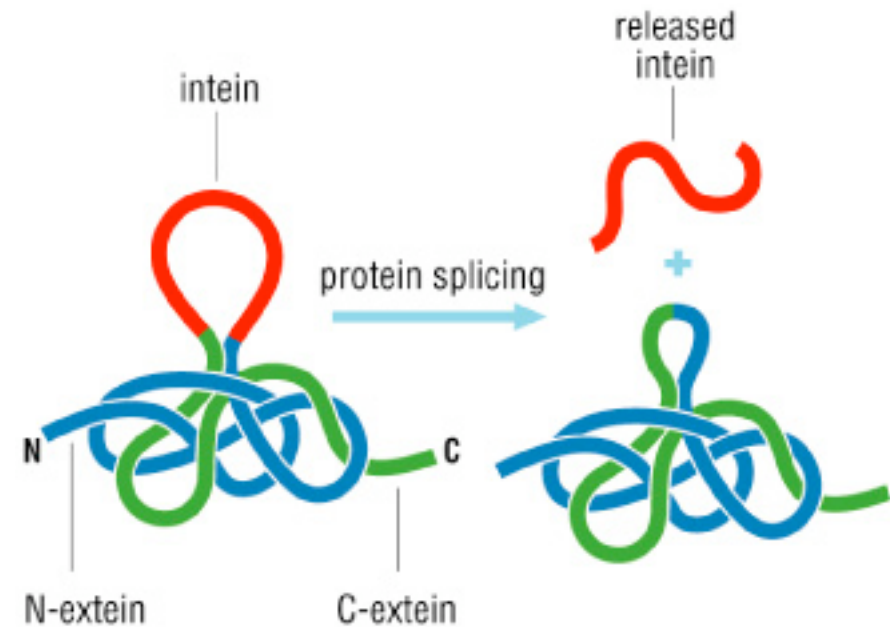
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- Spatial targeting/co-localization
- Binding of effector molecules (e.g., phosphorylation)
- Covalent modification - **splicing**
  - nomenclature similar to that of nucleic acids
    - exon/intron      extein/intein
  - one step, so does not require ATP
- pH and redox environment



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