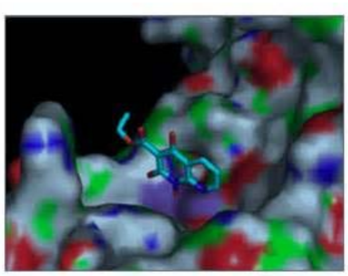
Protein Function

Pratt and Cornely

Structure and Function

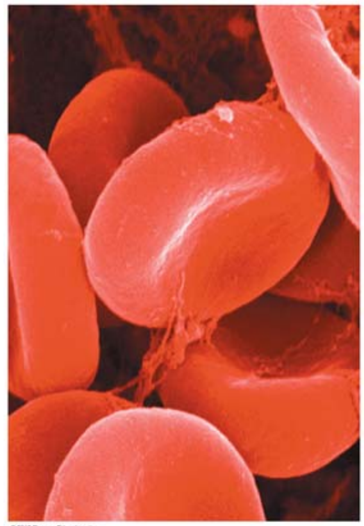
- Structure
  - Intermolecular Forces
  - Steric interactions
  - Molecular Recognition
  - BINDING!



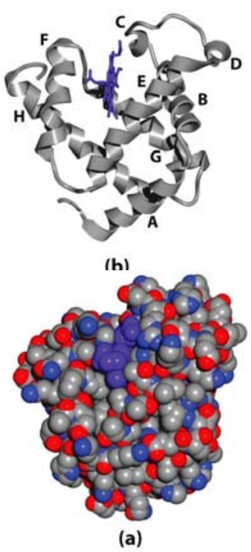
- Function
  - Transport Structure
  - Motor
  - Catalysis
  - Immunity
  - Regulation
  - Signaling

Case Study: Hemoglobin

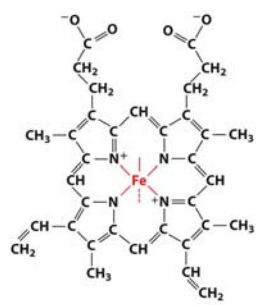
- Myoglobin
  - Oxygen storage
- Hemoglobin
  - Oxygen delivery



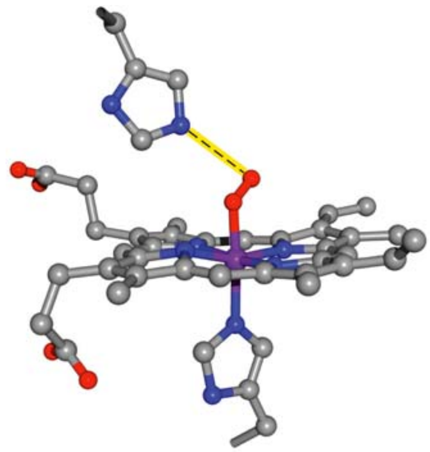
Structure of Myoglobin



- Noncovalent binding
- Hydrophobic pocket
- His F8 (proximal)
- His E7 (distal)



Reversible Oxygen Binding



- Tight hydrophobic pocket
- Fe^{+2} easily oxidized outside of globin
- Hydrophobic pocket limits Fe^{+3} formation

Binding Constants and Curves

- From point of view of dissociation:

$$MbO_2 \rightleftharpoons Mb + O_2$$

$$K_D = [Mb]\,[O_2]/[MbO_2] \text{ and}$$

fractional saturation $Y = [MbO_2]/([Mb] + [MbO_2])$

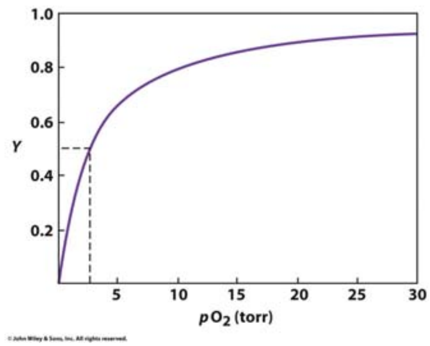
- Rearrange to give

$$Y = \frac{[O_2]}{K_D + [O_2]} = \frac{pO_2}{K_D + pO_2}$$

- *The amount of oxygen bound (Y) is a* ***hyperbolic*** *function of the amount of oxygen present and the affinity of myoglobin for oxygen (K_D)*

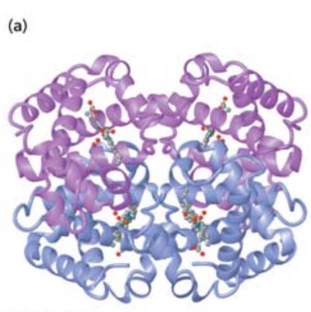
Apply to Myoglobin

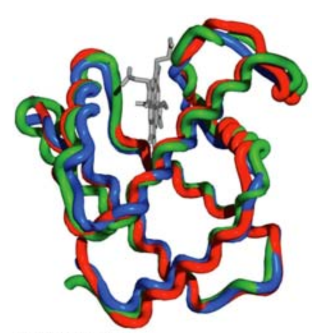
- Y = fraction bound (level of saturation)
- Hyperbolic curve
- K_D is determined at 1/2Y
- Binding is half maximal when the oxygen pressure is equal to the dissociation constant
- Myoglobin is half saturated at 2.8 torr O_2
- In tissue, pO_2 ~ 30 torr; myoglobin is nearly saturated



Structure of Hemoglobin

- Oligomer of four units resembling Mb
- $\alpha_2\beta_2$ tetramer
- Treated as dimer of $\alpha\beta$ units

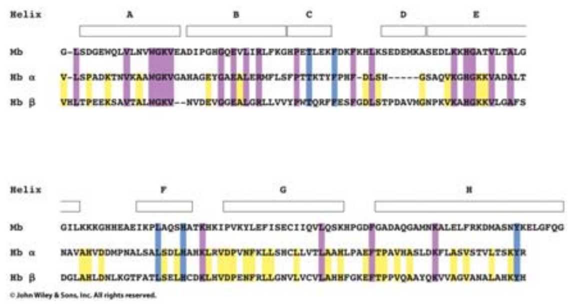




α Hb
βHb
Mb

Structural vs Sequence Homology

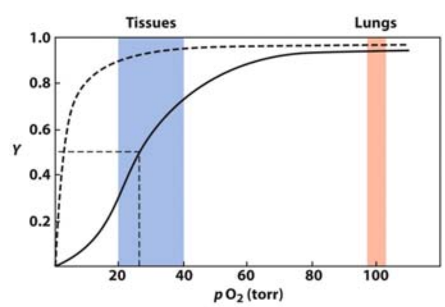
- 18% sequence homology
- Invariant residues
- Conservatively substituted
- Variable positions



Purple: Invariant across all vertebrates
Blue: Identical in human myoglobin and hemoglobin
Yellow: Identical in human α and β chains

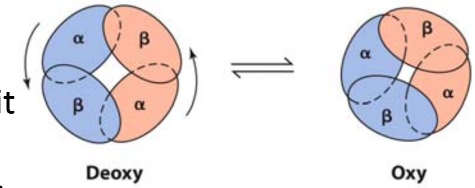
Binding Curve of Hemoglobin

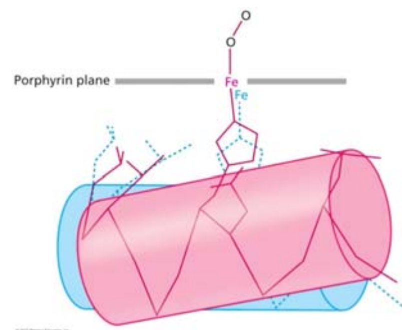
- Hemoglobin is half-saturated at 26 torr O_2
- Hemoglobin has less affinity for oxygen
- Hb saturated in lungs
- When it reaches tissues, oxygen is released
- Steep in important region
- But why sigmoidal?



Cooperativity

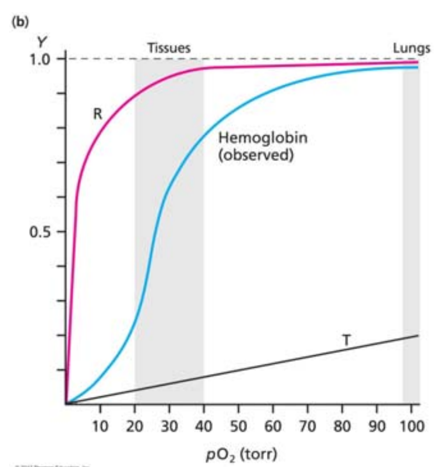
- Binding of oxygen changes shape of subunit
- Shape of subunit affects shapes of other subunits
  - Oxygen-bound unit causes other subunits to become relaxed
  - Other subunits bind oxygen more favorably
  - The rich become richer
  - Cooperative binding





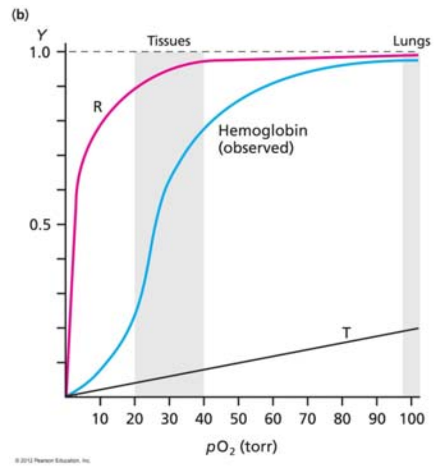
Conformational Equilibrium

- Deoxy state is unfavorable for binding (tense)
- R ⇀↽ T
- Relaxed state binds oxygen well (low half-saturation)
- Tense state binds oxygen poorly
- Equilibrium lies toward Tense state



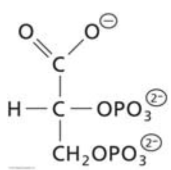
Observed Curve

- At low $[O_2]$, curve looks like T
- At high $[O_2]$, curve looks like R
- Slope is steep in the range of tissue $[O_2]$
- Small change in $[O_2]$ leads to great change in binding affinity



Why is Tight Favored?

- **Allosteric protein: "Other space"**
- 2,3-bisphosphoglycerate binds in central cavity, but only to T
- Pushes equilibrium of all subunits toward the "Tight" conformation
- No 2,3-BPG: Hemoglobin's oxygen affinity is too high
- Fetal Hb doesn't bind BPG; greater oxygen affinity than adult



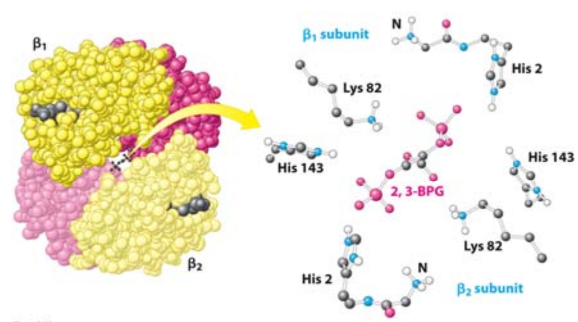


Figure 9.10
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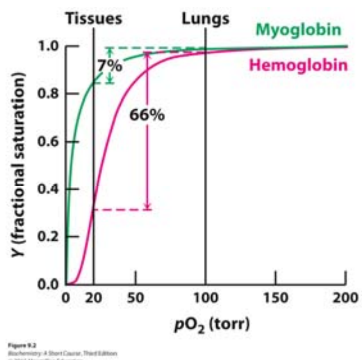
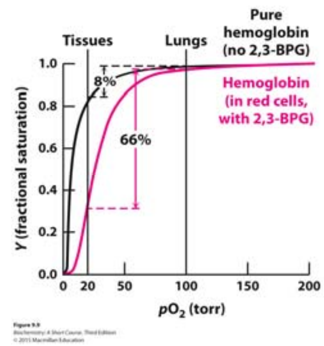
Binding Curve Shift

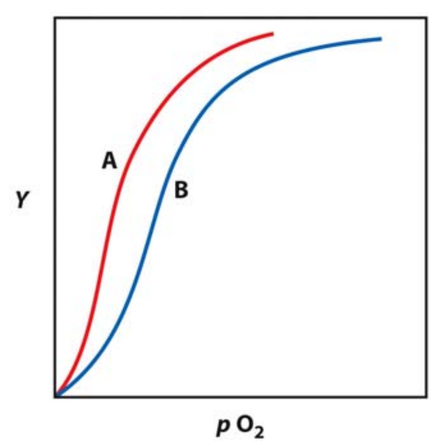
- Pure hemoglobin similar to myoglobin in binding
- 2,3-BPG causes the binding curve to shift to the right





Problem 25

- Fetal Hb is an $\alpha_2\gamma_2$ protein. At birth, adult Hb is produced so that by 6 months, 98% of the baby's Hb is adult. In the graph, which is the binding curve for fetal Hb? What is the physiological purpose?



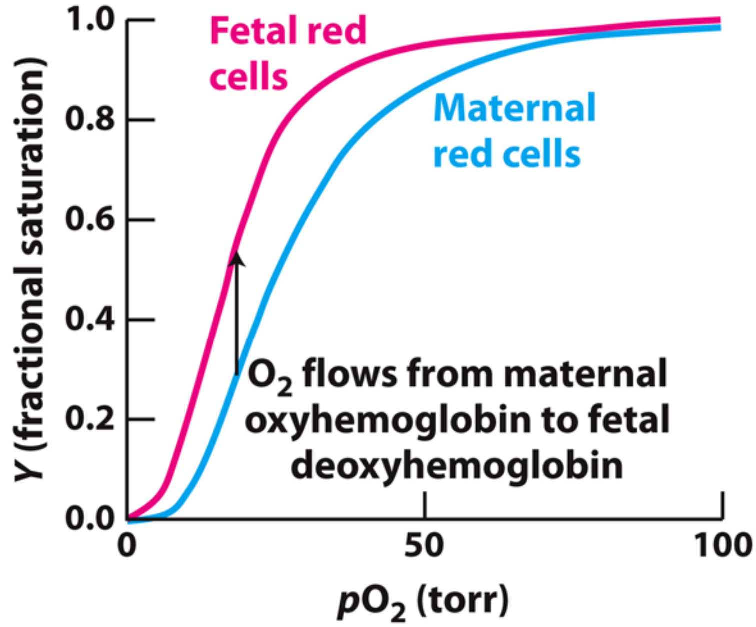


Figure 9.11
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Bohr Effect

- pH also affects oxygen binding
- Lower pH leads to protonation of protein
- Ion pairs form in central cavity that stabilize the deoxy (unbound, tense) form

$Hb{\cdot}H^+ + O_2 \rightleftharpoons Hb{\cdot}O_2 + H^+$

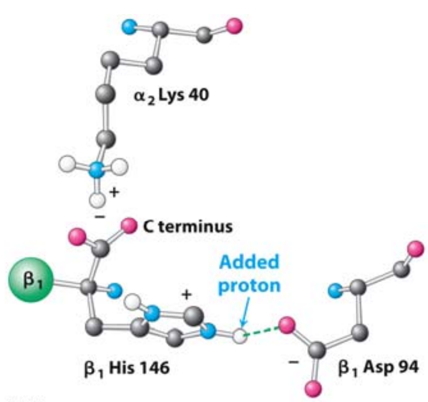

Figure 9.15
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Bohr Effect

- Steep slope in tissue [O_2] range leads to big impact from small pH change
- Curve shifted to the right: less binding
- Bohr effect leads to significantly greater release of oxygen from hemoglobin when pH is lower

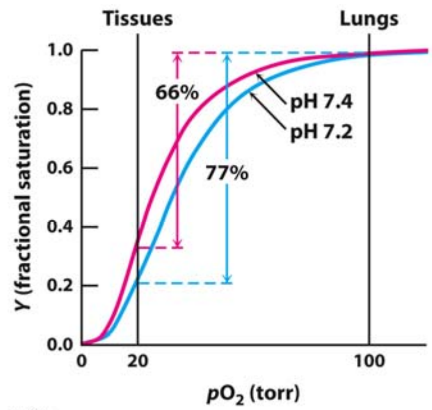

Figure 9.14
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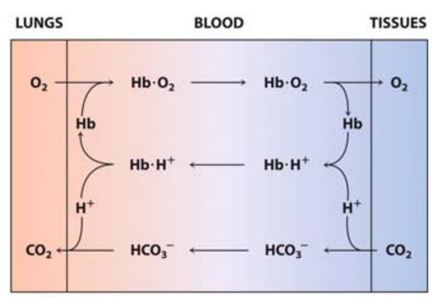
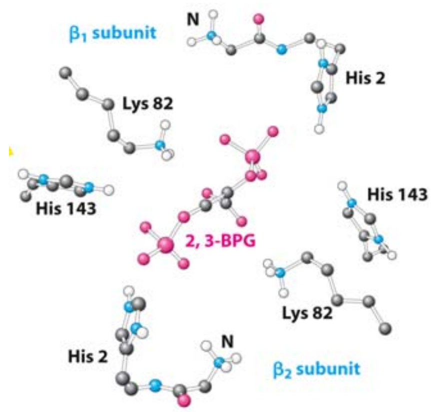
Physiology

- In tissue, CO_2 produced
- Raises H^+ concentration
- Hemoglobin has decrease oxygen affinity in tissue compared to lungs
- Protons shuttled to lungs on Hb



Problem 31

- Propose a few explanations of how a K→N mutation of a residue in the central cavity could lead to a mutant Hb with greater oxygen binding affinity.



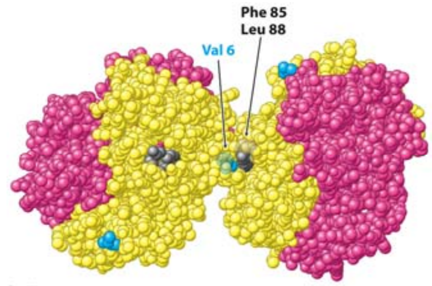
Problem 31

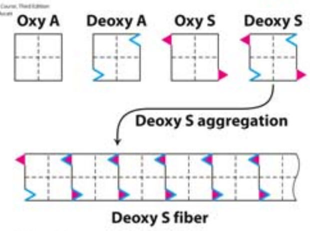
- Propose a few explanations of how a K→N mutation of a residue in the central cavity could lead to a mutant Hb with greater oxygen binding affinity.

- It might change the conformation of the F-helix such that His F8 binds oxygen better
- Since the central cavity is less +, BPG might bind worse, favoring R
- It might destabilize ion pairs that normally stabilize the T state

Pathology

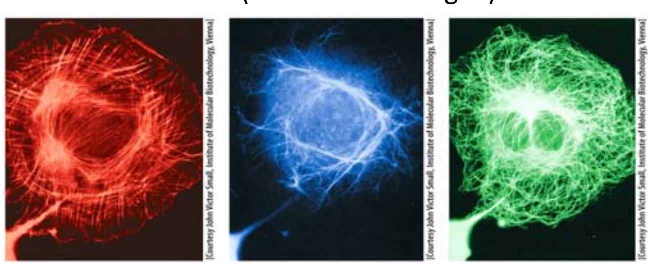
Figure 9.18
Biochemistry: A Short Course, Third Edition
Eye of Science/Science Source



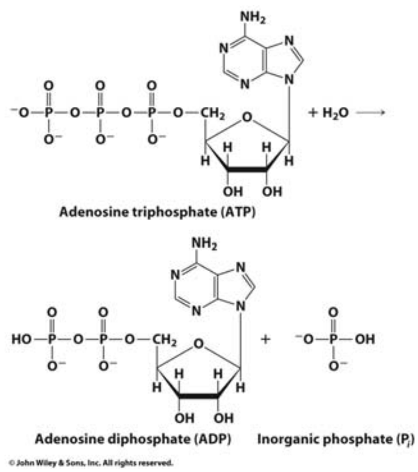


Structural proteins

- Associated with motors and motion
  - globular subunits, NTP binding
  - Microfilaments (actin)
  - Microtubules (tubulin)
- Exclusively Structural
  - coiled-coil, triple helix
  - Intermediate filaments (keratin and collagen)



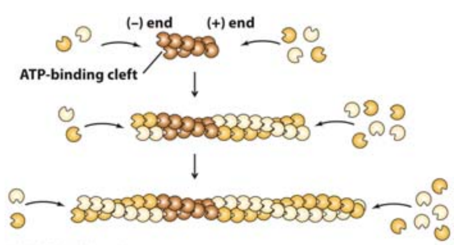
G-actin



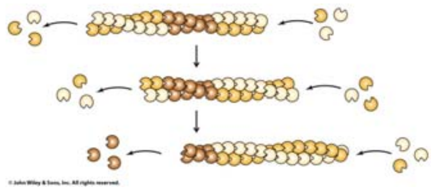
- Globular monomer
- Binds Atp
- Only hydrolyzes ATP when in polymerized filament, F-actin

F-actin

- ATP hydrolysis makes polymerization favorable
- Directional: ATP binding ends line up to give (-)-end Grow faster at (+)-end



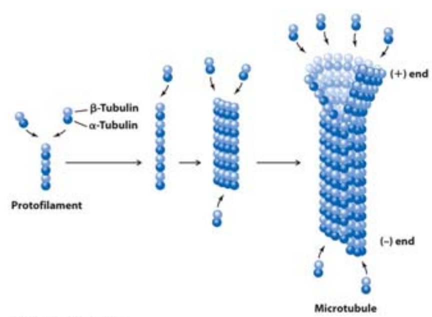
Actin Function



- Recruiting of actin to certain areas of cell by extracellular signal
  - Cell shape change
  - movement

Microtubules

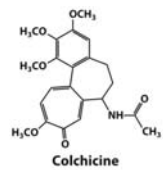
- Similarities: built from globular protein subunits, GTP binding, directional growth
- Differences: stronger tube



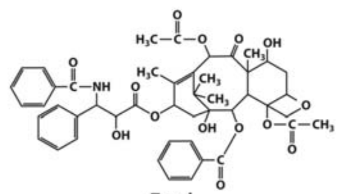
Drugs Target Microtubules

- Colchicine—binds polymerization site
  - depolymerization
- Taxol—only binds to polymerized tubulin
  - blocks depolymerization

Problem 42: Why do both of these drugs block cell division?



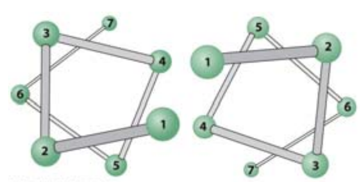
Colchicine

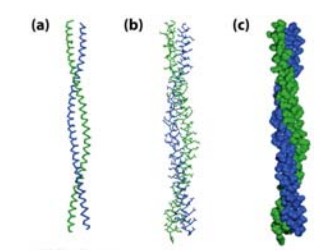


Taxol

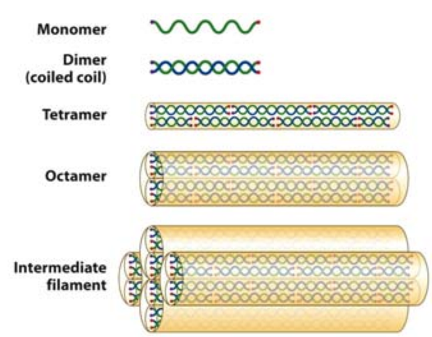
Keratin

- Coiled-Coil



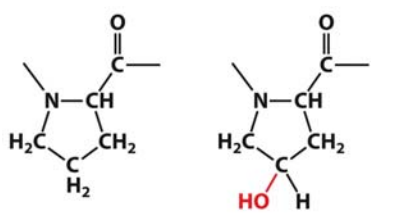


Problem 52: "Hard" collagens (hair, claws) have a high sulfur content, but "soft" keratins (skin) have lower sulfur content. Explain.

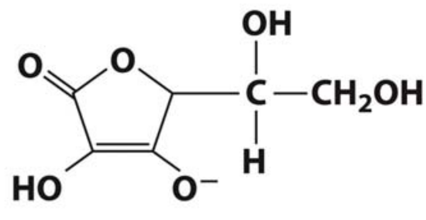


Collagen

- Repeating Gly-Pro-Hyp units
- Vitamin C, scurvy



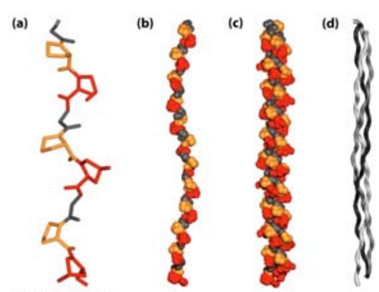
Proline Hydroxyproline (Hyp)

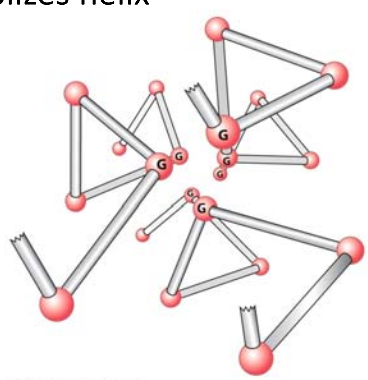


Ascorbate (vitamin C)

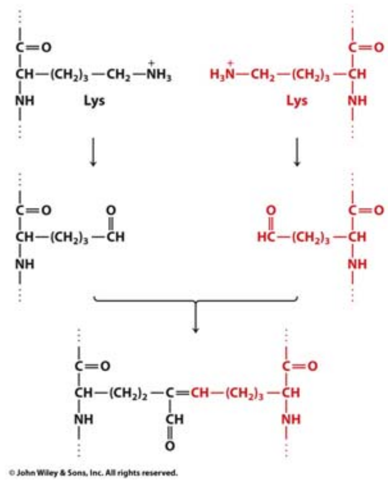
Collagen: Triple Helix

- Pro, Hyp force left handed turn
- Gly packs in middle (sterics)
- Backbone H-bonding stablizes helix



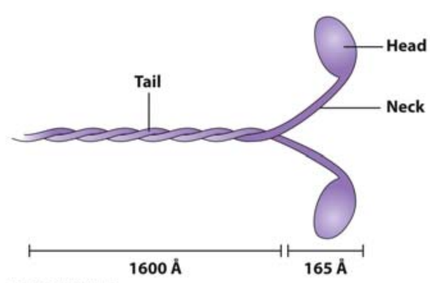


Collagen; Crosslinking

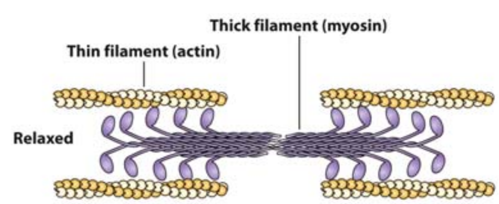


- Post-translation modification
- Aldol condensation
- High tensile strength
- Connective tissue: cartilage, ligaments, tendons
  - Collagen gives strength
  - Hydrated polysaccharides give resilience

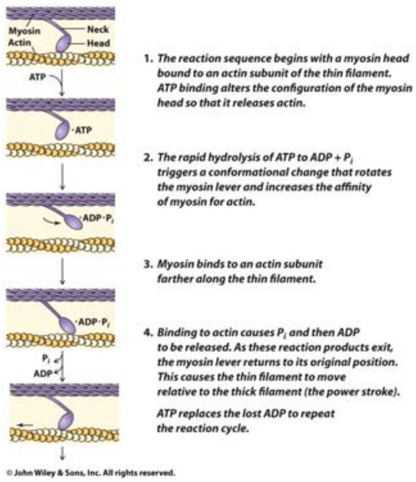
Motors: Myosin Structure



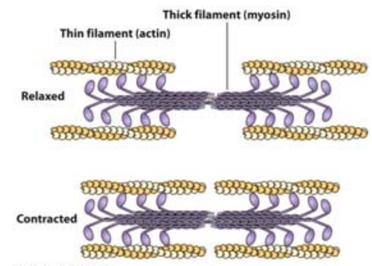
- ATP binding site in head
- Reinforced neck
- Tail forms thick filaments
- Actin forms thin filament



Myosin Mechanism

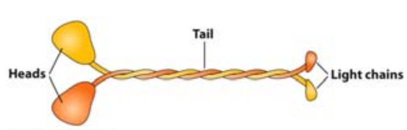


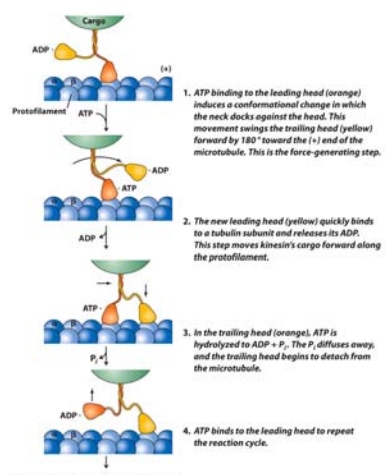
- “Hop”
- Nonprocessive process of many molecules acting separately with overall contraction



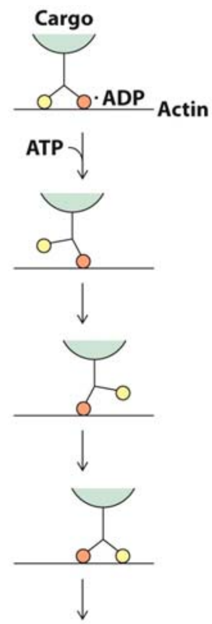
Kinesin

- Transformation of chemical energy of ATP into mechanical energy
- Processive mechanism
- Allows carrying of vesicles





Problem 72: Myosin type V is a two-headed myosin that operates as a transport motor to move its cargo along an actin filament. Its mechanism is similar to muscle myosin, but it acts processively. Based on the starting point provided in the figure to the right, propose a mechanism, starting with entry of ATP. How does each step of ATP hydrolysis reaciton correspond to a conformational change in myosin V?



Problem 34:

- Check the appropriate blanks

	Exclusively structural	Motor proteins	Not motor, but undergo structural change	Contain NTP binding sites
Actin				
Tubulin				
Keratin				
Collagen				
Myosin				
kinesin				