



# BCH2333 Biochemistry FINAL EXAM

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**'RAISING MARKS, RAISING MONEY, RAISING ROOFS'**

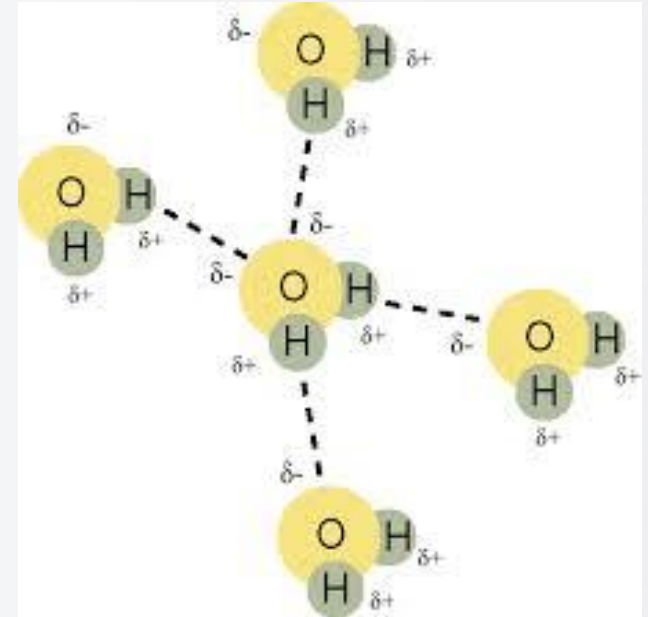
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# Overview

- Mezl
  - Water
  - Buffers
  - Carbohydrates
  - Nucleic acids
- Figeys
  - Amino acids
  - Proteins
- Bennett
  - Enzyme kinetics
  - Enzymes
  - Lipids

- Sun - generates energy
  - Nuclear reaction
- Plants - traps energy
  - REDUCE
  - $\text{Energy} + \text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{carbon compound} + \text{O}_2$
- Animals - releases energy
  - OXIDIZE
  - $\text{Carbon compound} + \text{O}_2 \rightarrow \text{CO}_2 + \text{H}_2\text{O} + \text{energy (ATP/NADPH)}$

- Hydrogen bonds
  - Length
  - Melting point
  - Boiling point
  - Heat of vaporation
- Catabolism vs. anabolism
- Hydrophobicity vs. hydrophilicity
- Hydration



- Coulomb's Law

$$F = \frac{kq_1q_2}{Dr^2}$$

- D: capacity of a solvent to separate and stabilize charged molecules
  - ↑ H bonds
  - ↑ dipole moment

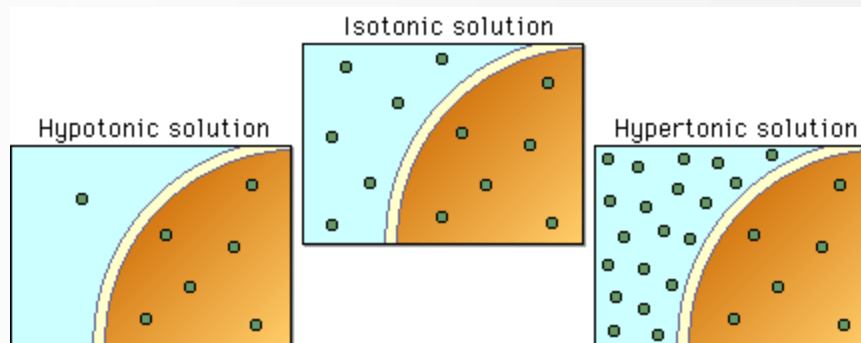


- Osmosis and Diffusion

- $\pi = CRT$

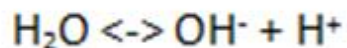
- $\pi$ : osmotic pressure
- $R$ : 0.082 L atm/kM
- $T$ : temperature (K)
- $C$ : concentration (molar, molal, osmolar)

- Hypertonic vs. hypotonic



# Water

## Dissociation of water



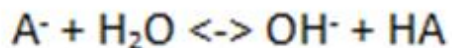
$$K_d = [\text{H}^+][\text{OH}^-] = 10^{-14}$$

## Dissociation of acid



$$K_a = \frac{[\text{H}][\text{A}]}{[\text{HA}]}$$

## Dissociation of base



$$K_b = \frac{[\text{OH}][\text{HA}]}{[\text{A}]}$$

## Other Equations

$$\text{pH} = -\log[\text{H}^+]$$

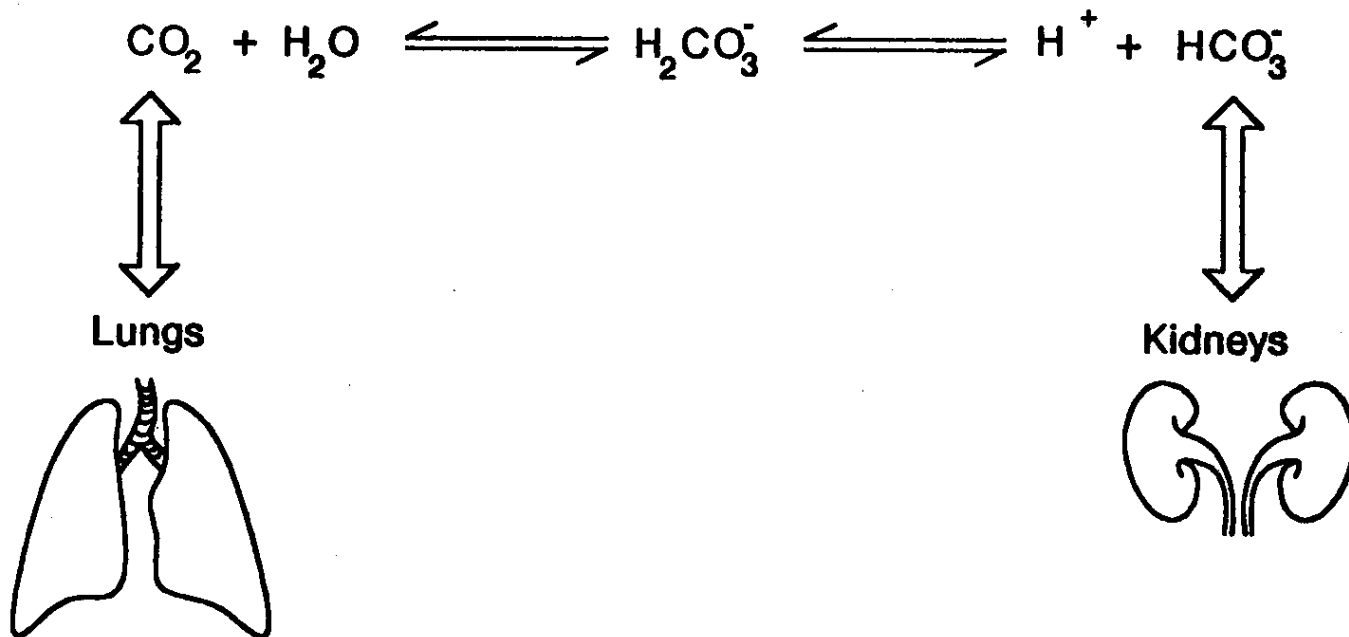
$$\text{pOH} = -\log[\text{OH}^-]$$

$$\text{pOH} + \text{pH} = 14$$

$$\text{pH} = \text{pK}_a + \log \frac{[\text{A}]}{[\text{HA}]}$$

- Buffers

- pKa close to pH you want to work with
- Concentration that is sufficiently high





# Water Review Questions

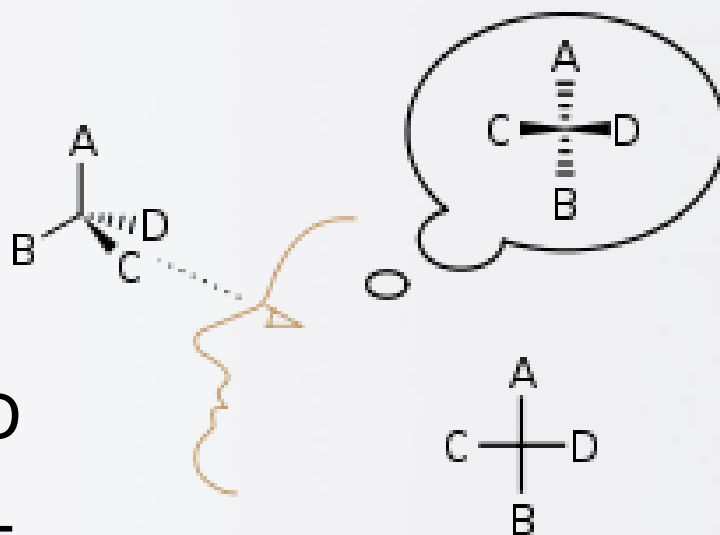
- Consider  $O_2$  and  $NaCl$ . Which one will dissolve in water? Explain.
- What is the osmotic pressure created by a 1M protein solution? Height of a theoretical column?
- A 1L solution contains a 10 mM weak acid that has been adjusted to pH 7. The  $pK_a$  of the weak acid is 7. You add 4 milliequivalents of base. What is the pH?

- Isomers!

- Structural isomers
- Stereoisomers
- Conformational isomers
- Geometric isomers

- Fischer Projection

- D vs. L
  - 'A' = oxidized
  - If 'D' is functional group: D
  - If 'C' is functional group: L



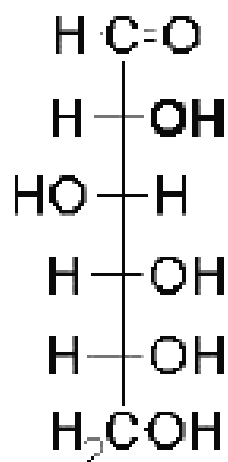
- Definitions

- Chiral
- Prochiral
- Racemic mixture
- Enantiomers
- Diastereoisomers
- Epimers
- Monosaccharides
- Oligosaccharides
- Polysaccharides
  - Homopolymer
  - Heteropolymer

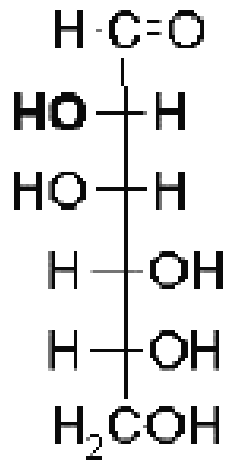
- Nomenclature

- “-ose”: sugar
- ‘aldose’: aldehyde
- ‘ketose’: ketone
- D vs. L sugar: last chiral center
- Triose: 3C, tetrose: 4C, pentose: 5C, hexose: 6C
- hemi-acetal vs. hemi-ketal
- Pyranose: six atom ring
- Furanose: five atom ring
- Anomeric carbon: C1, becomes chiral
- “-uronic acid”: oxidized C6
- Alditols: reduced sugar at ketone/aldehyde
- Deoxy-sugar: hydroxyl group is replaced by a hydrogen

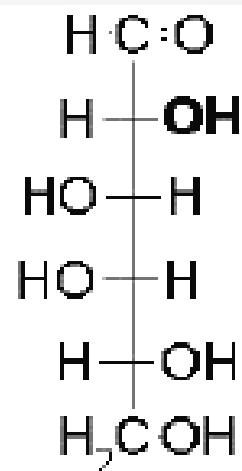
- Our Friends the SUGARS



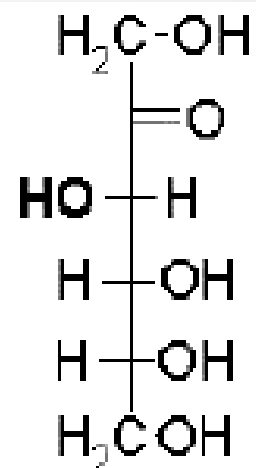
D-Glucose



D-Mannose



D-Galactose

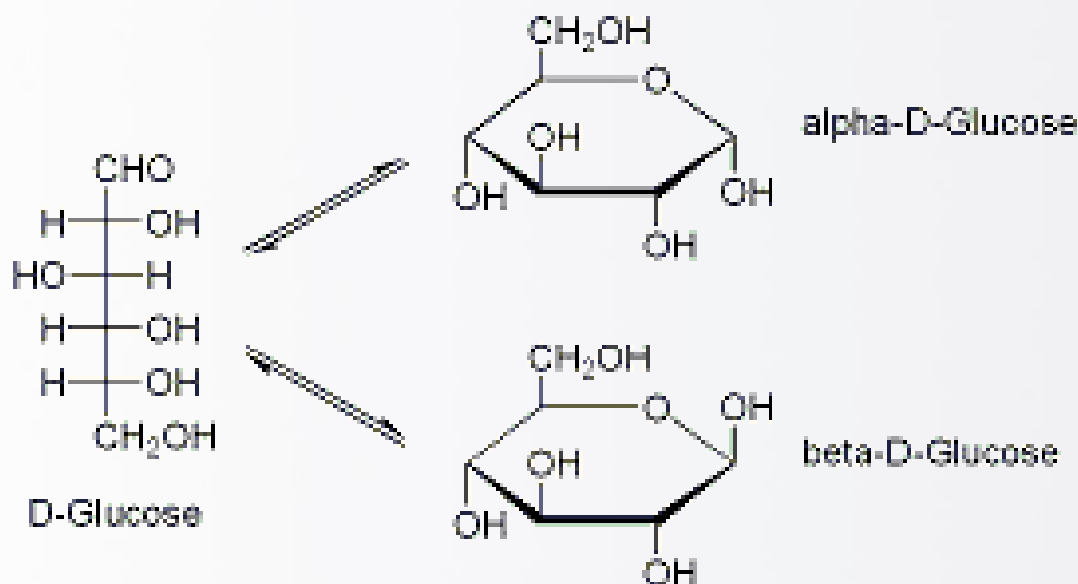


D-Fructose



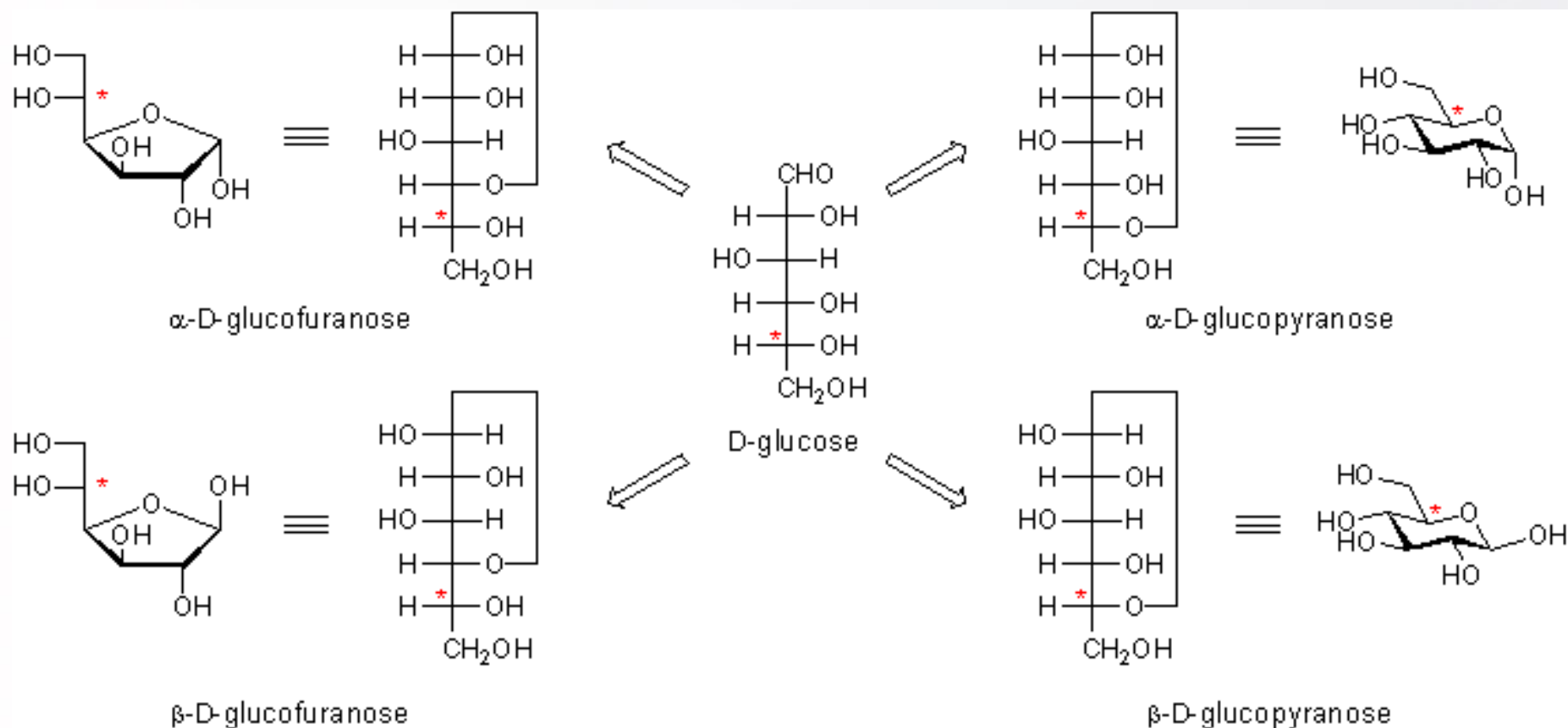
- Mutorotation

- Reference carbon (RC): defines the ring as D or L
- Alpha: C1 hydroxyl has SAME orientation as RC
- Beta: C1 hydroxyl has OPPOSITE orientation as RC



- [http://www.chem.ucla.edu/harding/ec\\_tutorials/tutorial43.pdf](http://www.chem.ucla.edu/harding/ec_tutorials/tutorial43.pdf)

- One more thing...



- Chemistry

- Oxidation

- Chemical: Fehling's test (copper)

- Positive = free aldehyde or ketone; REDUCING sugar

- Enzymatic:

- Glucose oxidase: Glucose to glucono-lactone
      - Oxidation of primary alcohol (C6): “-uronic acid”

- Reduction

- Alditols; only exist in linear form

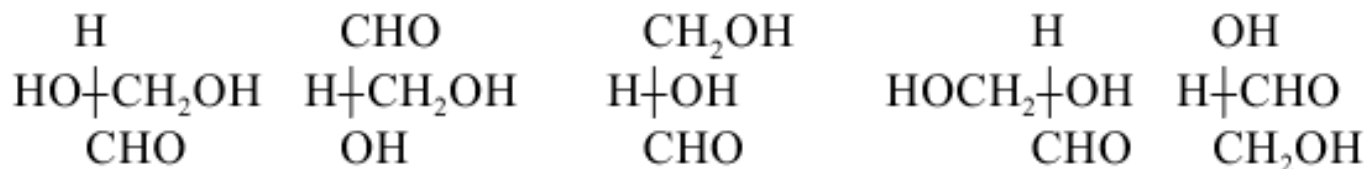
- Esterification: Alcohol + acid  $\rightarrow$  ester

- Group addition

- Amino group, sulfo group, amino acetic acid

# Sugars Review Questions

- D or L?

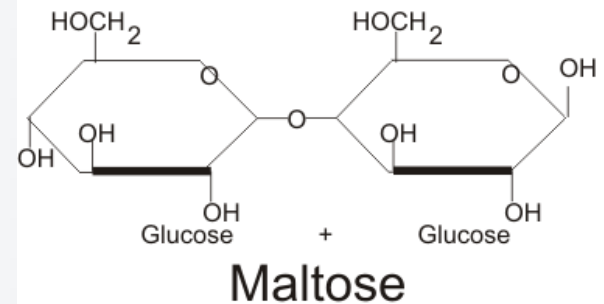
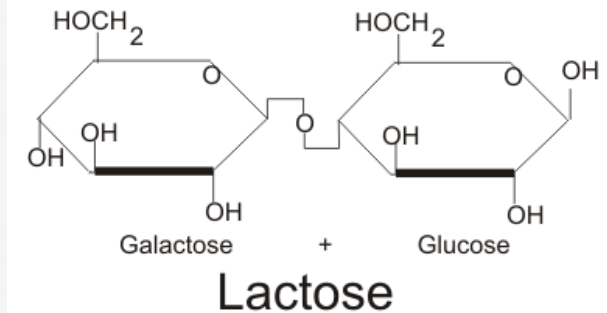
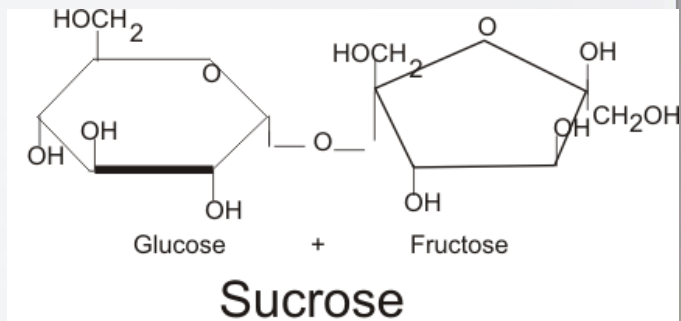


- Draw and name the 5 compounds which exist in a solution of fructose.
- For this sugar...
  - D or L?
  - Pyranose or furanose?
  - Alpha or beta?
  - Hexose or pentose?
  - Do we know its name?



# Carbohydrates

- Glycosidic bonds
  - Alpha-1, beta-2 bond
    - Sucrose =  $\alpha$ -glucose +  $\beta$ -fructose
    - Negative Fehling test
  - Beta 1-4 bond
    - Lactose = galactose + glucose
  - Alpha 1-4 bond
    - Maltose =  $\alpha$ -glucose +  $\alpha$ -glucose

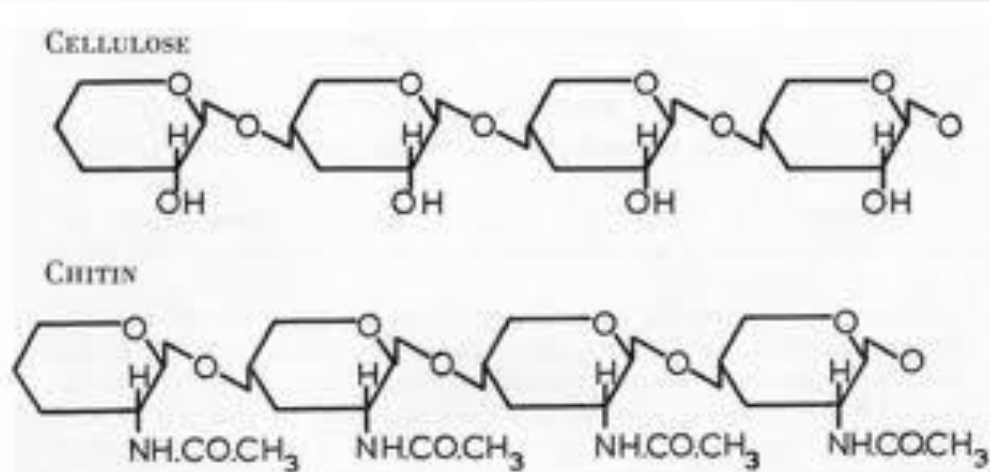




- Energy stores
  - Starch
    - Amylose
      - Glucose,  $\alpha$  1-4 bonds
      - Helix, 6 residues/turn, left-handed
    - Amylopectin
      - Glucose,  $\alpha$  1-4 bonds with branching  $\alpha$  1-6 bonds (24-30 res)
  - Glycogen
    - Glucose,  $\alpha$  1-4 bonds with branching  $\alpha$  1-6 bonds (8-12 res)

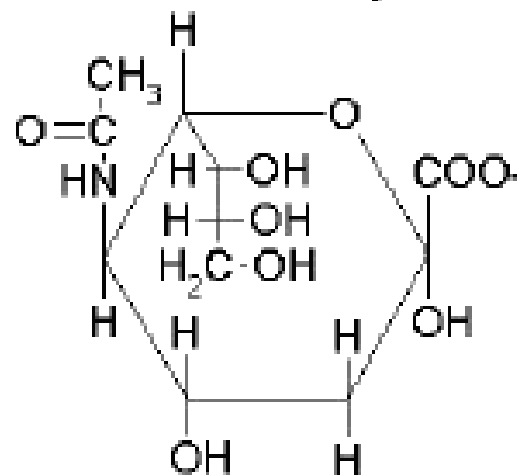
# Carbohydrates

- Structural Polysaccharides
  - Cellulose
    - Glucose,  $\beta$  1-4 bonds, strong H-bonds
  - Chitin
    - N-acetyl-glucosamine polymer
    - Aldose: glucose with N-acetyl group on C2 ,  $\beta$  1-4



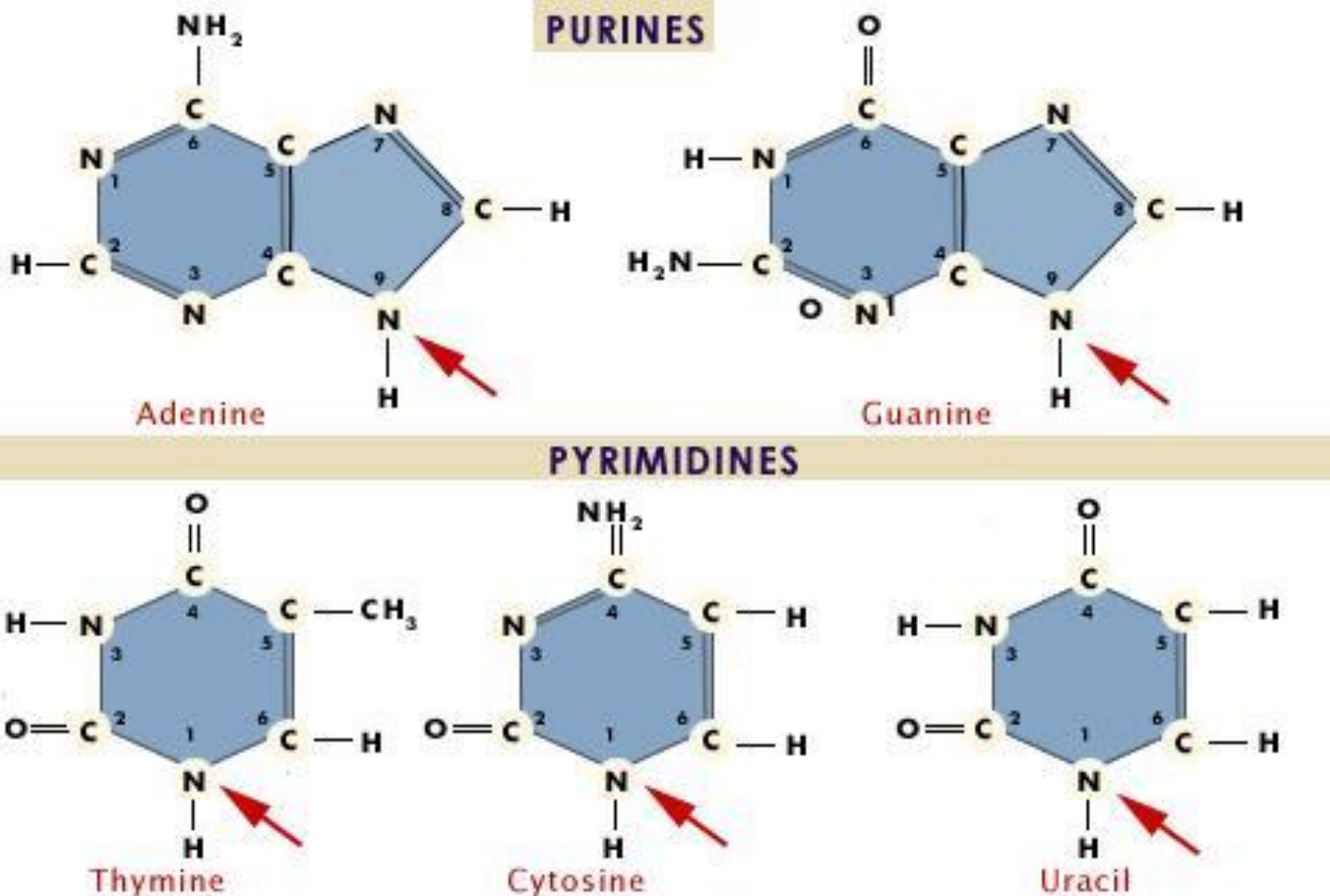
# Carbohydrate Review Questions

- Which bonds and sugars are present in amylopectin, cellulose, and chitin?
- Describe this sugar.
  - D or L?
  - Alpha or beta?
  - Furanose or pyranose?
  - Would it react to Fehling's?
  - Numerical name? (# of Cs)
  - Can you find the prochiral carbon? Deoxygenated carbon? Anomeric carbon? What is the substituent called? Can you name D or L for each carbon?



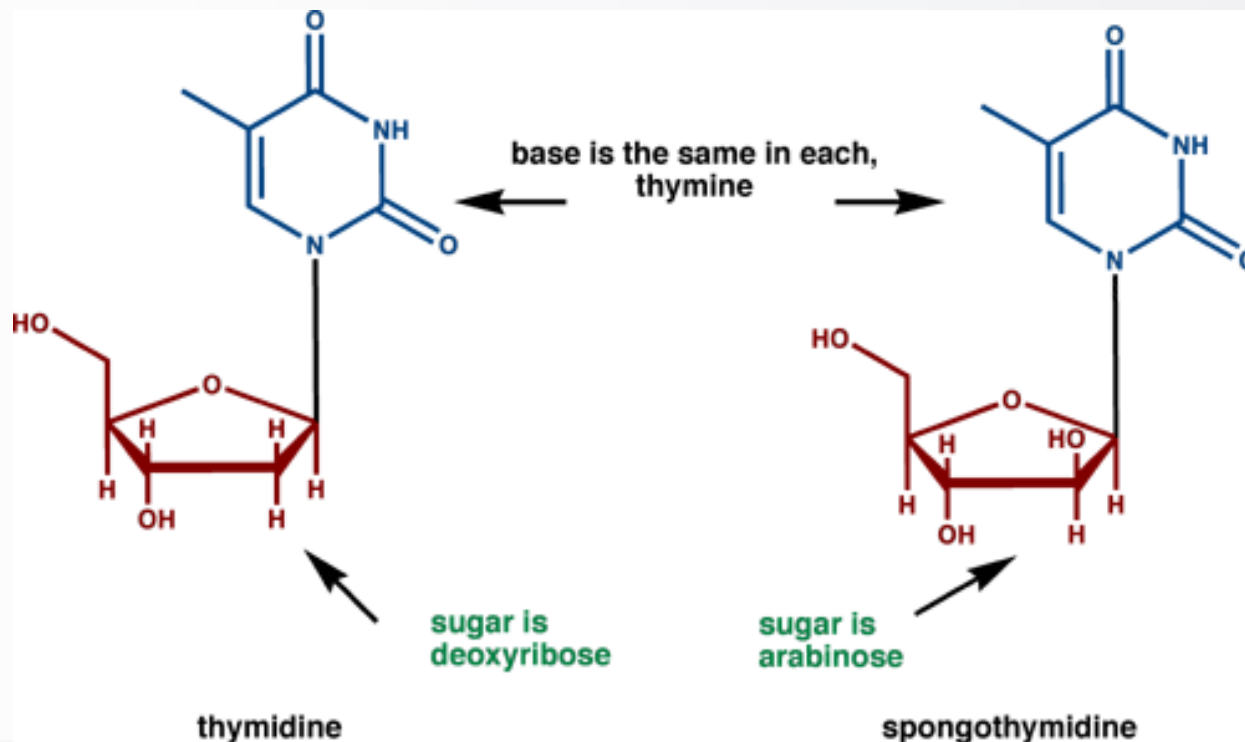
# Nucleic Acids

- Nitrogenous bases



# Nucleic Acids

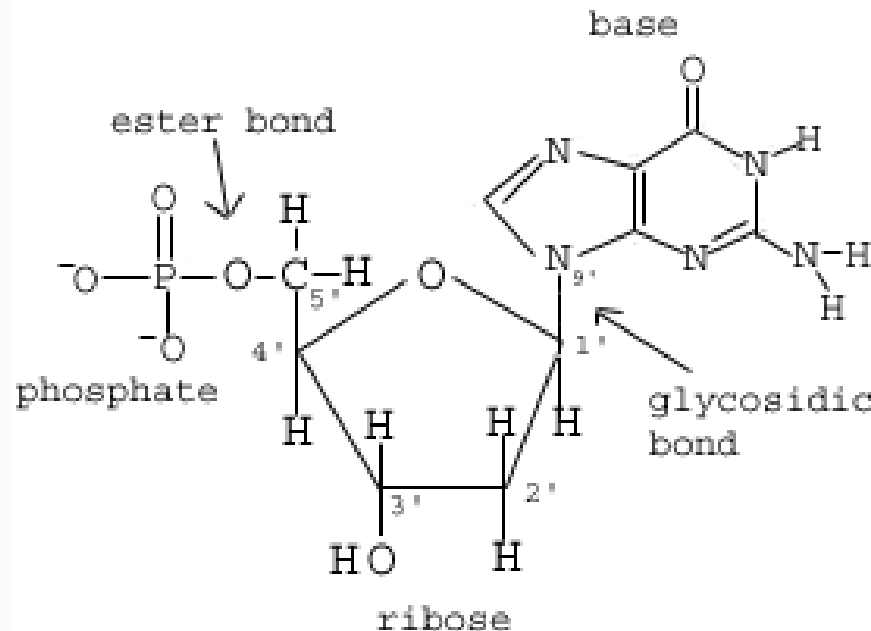
- Sugar
  - $\beta$ -D-Ribose (RNA),  $\beta$ -D-2-deoxyribose
- Nucleoside: sugar + base





# Nucleic Acids

- Nucleotides:
  - nucleosides esterified with phosphate
    - ATP - phosphate-phosphate bonds
    - cyclic AMP - phosphate bonded to 5' and 3' of ribose



# Nucleic Acids

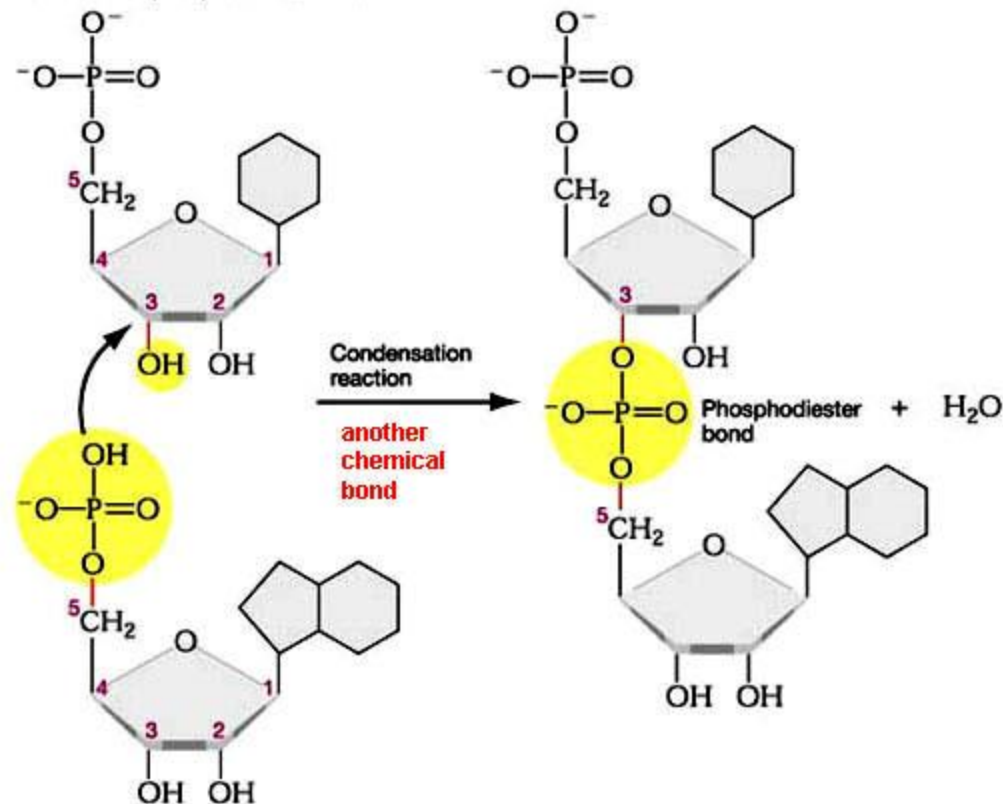
- Naming

	A	G	C	U	T
Base:	Adenine	Guanine	Cytosine	Uracil	Thymine
Nucleoside:	Adenosine	Guanosine	Cytidine	Uridine	---
Deoxynucleoside:	Deoxyadenosine	Deoxyguanosine	Deoxycytidine	---	Thymidine
Nucleotide:	Adenosine monophosphate (AMP)	Guanosine monophosphate (GMP)	Cytidine monophosphate (CMP)	Uridine monophosphate (UMP)	---
Deoxynucleotide:	Deoxyadenosine monophosphate (dAMP)	Deoxyguanosine monophosphate (dGMP)	Deoxycytidine monophosphate (dCMP)	---	Thymidine monophosphate (TMP)

# Nucleic Acids

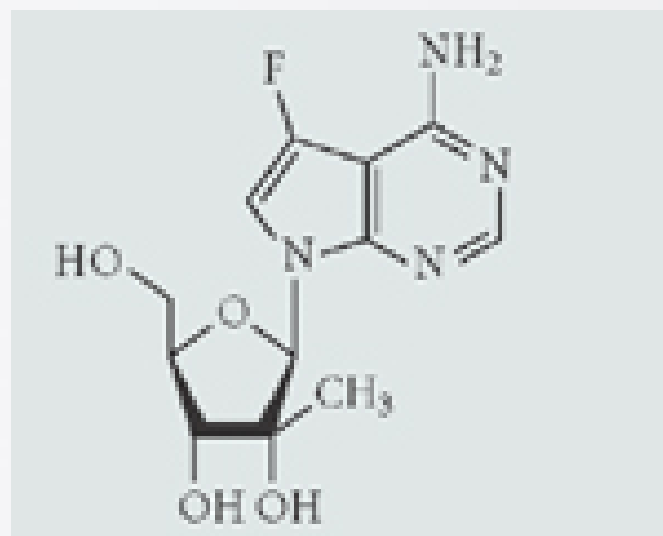
- 5'-3' Phosphodiester Bond

Formation of phosphodiester bond



# Nucleic Acids

- Tips for naming unusual nucleosides
  - Name the base ring (purine/pyrimidine?)
  - Name the sugar, how is it unique?
  - Deoxygenated groups? Which carbons?
  - Unusual substituents? Carbon number and orientation (D or L)



- Structure of DNA

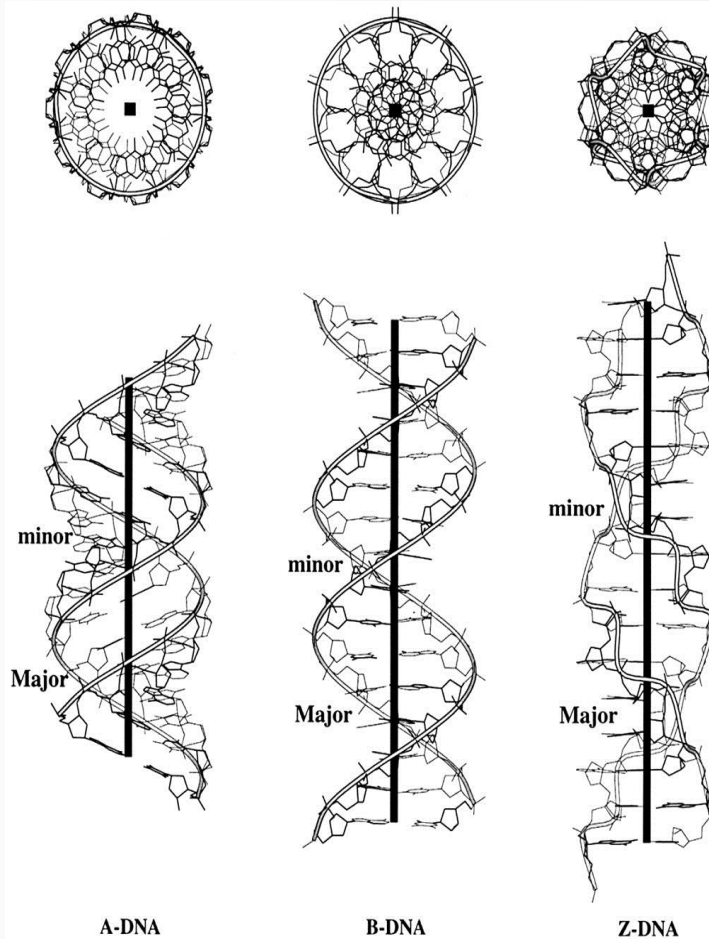
- Double stranded, antiparallel
- Helix: hydrophilic backbone, hydrophobic bases
- Right handed, 10 base/turn, 2nm diameter, 3.4nm height of one turn
- Two grooves; major (water) and minor
- Stability: depends on G-C (3 H bonds)
- Van der Waals forces: stacking of bases



# Nucleic Acids

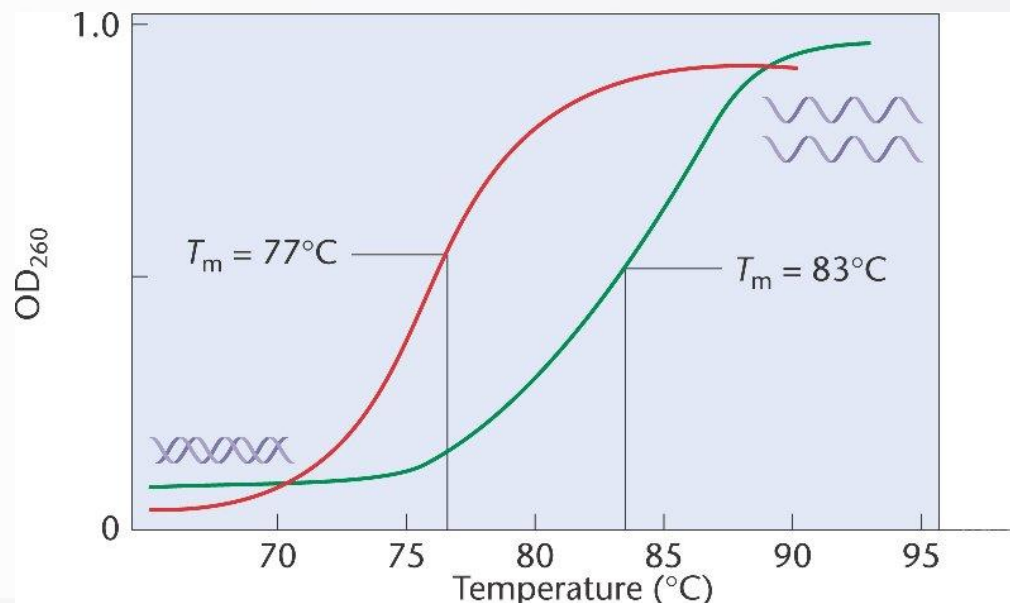
Name	BDNA	Watson Crick	Z DNA
	Most stable structure at reduced humidity (less water)	Helix	Structure obtained at high salt with some -C-G- containing sequences
Aspect	More compact - like pushing down on both ends, fatter Grooves are smaller	Reference structure	Thinner Backbone zigzags
Type of helix	Right handed	Right handed	Left handed
Grooves	Two grooves - No water to fill in large groove	Two grooves	One groove - Axis is outside base pairs - Second groove is filled in by base pairs
Importance	Structure of DNA-RNA hybrids, sporulating gram positive bacteria	Basic structure of DNA	Some small regions in chromosomes
Base pairs per turn	11	10	12
Length per base pair	Less	3.4Å	More
Diameter	Larger	2nm	smaller

- Different types of DNA



# Nucleic Acids

- Denaturation of DNA -> Reassociation
  - Breaking of base-base interaction
  - Hyperchromic shift: 30-40% increase in absorption due to decreased hydrogen bonding
  - Melting temperature: one half of the max hyperchromic shift



- Supercoiling of DNA
  - Extra twists compared to Watson Crick
    - Positive supercoil: twist is right handed (same as helix) - overwinding
    - Negative supercoil: twist is left handed - unwinding
  - Linkage number: number of twists in DNA + number of coils
    - Normal: 1 turn every 10 base pairs
    - Humans are 5% undercoiled

# Nucleic Acids

- DNA packaging
  - Chromatin:
    - DNA + equal mass histones + RNA + small amount of non-histone regulatory proteins
  - Histones:
    - 7-8 basic proteins (lysine, arginine)
  - Nucleosome:
    - 2 left-handed turns of DNA around 4 histones
    - One nucleosome every 200 bp
  - Chromosome:
    - Coiling of nucleosome chains



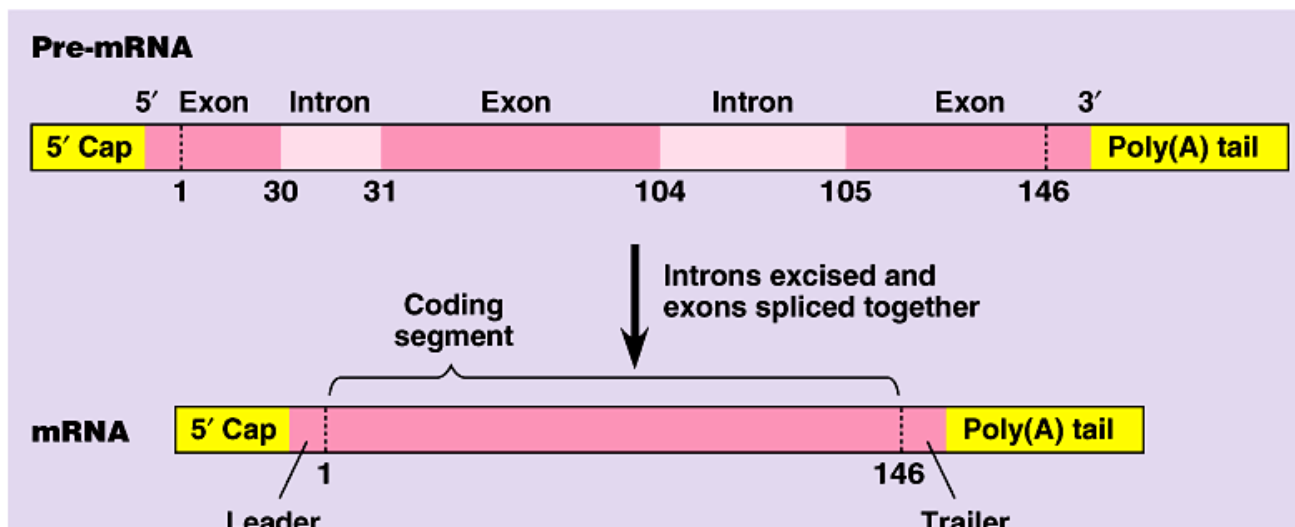
# Nucleic Acids

- Human genetic material
  - $3 \times 10^9$  base pairs, 25,000 genes (per set)
  - 23 pairs of chromosomes
  - 17,000 bp genome in mitochondria
    - Bacterial
    - Inherited from mother

## • Types of RNA

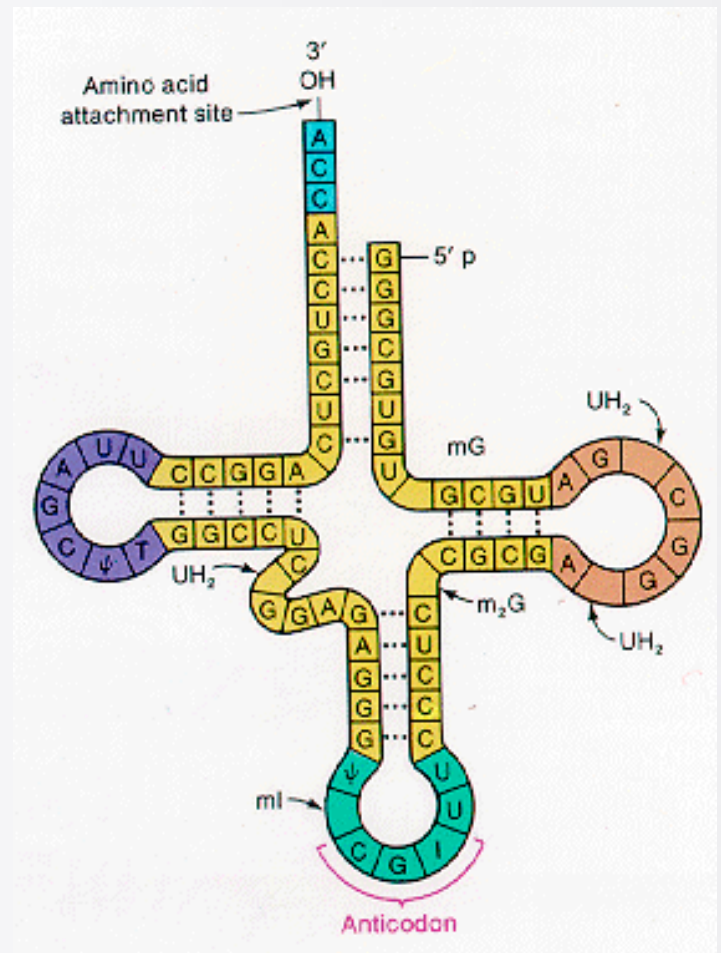
RNA	Ribosomal rRNAs	Transfer tRNAs	Messenger mRNAs
Role	Structural Constituent of ribosome	Catalytic Transfer amino acids into protein	Informational Bring the information for a protein into the cytoplasm
% of total RNA	82% - dominant - Structural component of the ribosome - RNA used as a building material for the enzyme	16%	2% - tiny amounts
# of different types/molecules	4	40 - more types than amino acids - redundancy	10,000-30,000
Size	28S, 18S Used for markers of integrity	TINY	
Distinguishing feature	Abundance Only 4 species	Small size "strange bases"	5' cap, 3' tail Many species

- mRNA modifications
  - Splice introns out of mRNA
  - 5' cap - 7 methylguanosine
  - Poly (A) tail at 3' end
  - AUG can be spliced off ('start' signal)



# Nucleic Acids

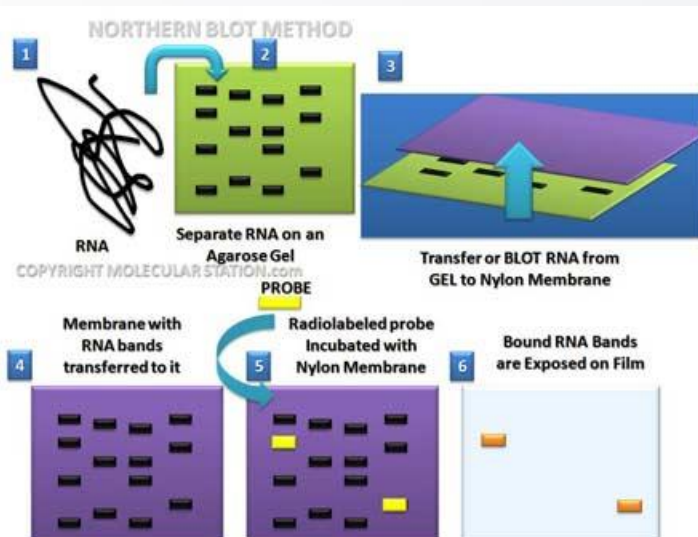
- tRNA structure
  - 80 nucleotides
  - 3D L-shaped structure
  - 3' end
    - CCA tail
    - Binds amino acids
  - Anticodon region
  - Homologous/isoaccepting





# Nucleic Acids

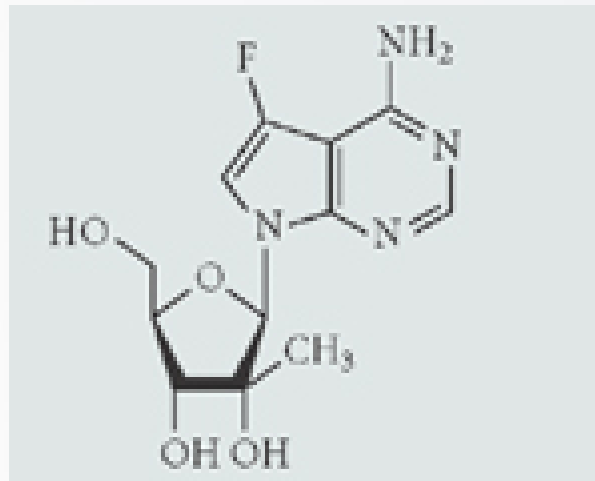
- Nucleic Acid Methods
  - Preparation of DNA/RNA: restriction enzymes
  - Amount: absorbance at 260nm
  - Size: electrophoresis
  - Blotting and hybridize: southern vs. northern





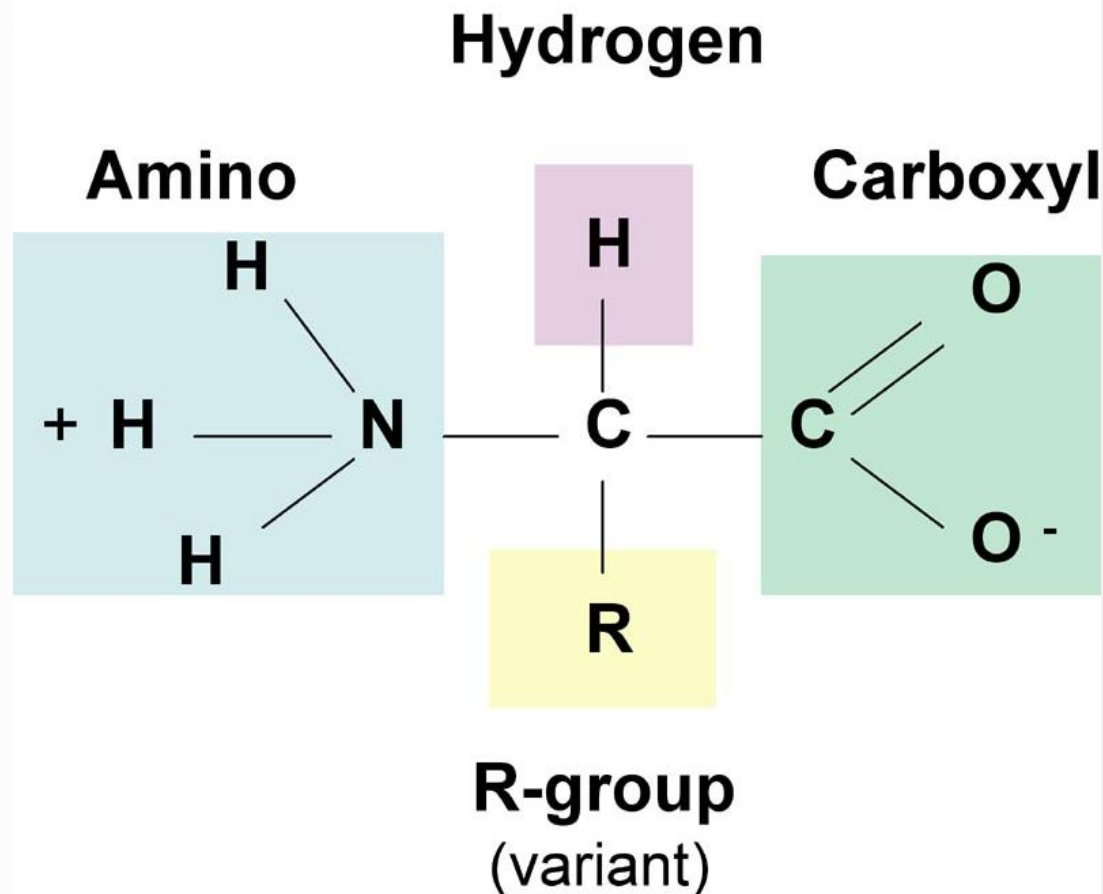
# Nucleic Acid Review Questions

- Draw a G-C nucleotide pair to scale.
- Draw the structure of Watson-Crick DNA. How are other DNA types different? What extra coiling occurs in humans?
- Describe this nucleotide



# Proteins

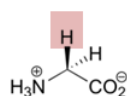
- Amino Acid Structure



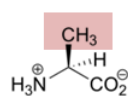
# Proteins

## • Introducing... the amino acids!

### Nonpolar, aliphatic side groups



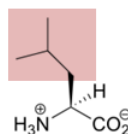
Glycine  
Gly, G



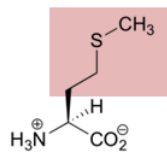
Alanine  
Ala, A



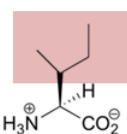
Valine  
Val, V



Leucine  
Leu, L

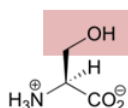


Methionine  
Met, M

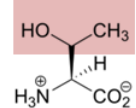


Isoleucine  
Ile, I

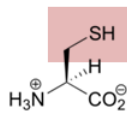
### Polar, uncharged side groups



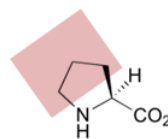
Serine  
Ser, S



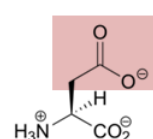
Threonine  
Thr, T



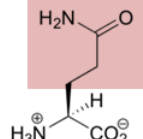
Cysteine  
Cys, C



Proline  
Pro, P

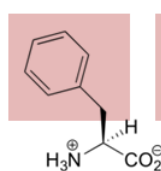


Aspartate  
Asp, D

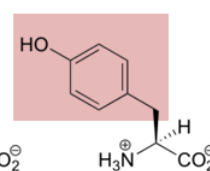


Glutamine  
Gln, Q

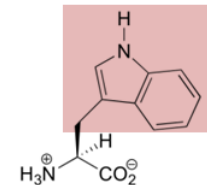
### Aromatic side groups



Phenylalanine  
Phe, F

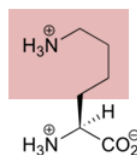


Tyrosine  
Tyr, Y

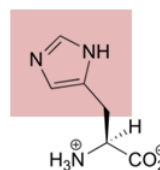


Tryptophan  
Trp, W

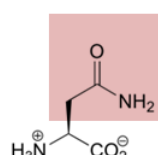
### Positively charged side groups



Lysine  
Lys, K

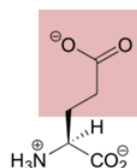


Histidine  
His, H

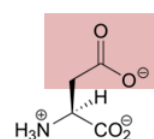


Asparagine  
Asn, N

### Negatively charged side groups



Glutamate  
Glu, E

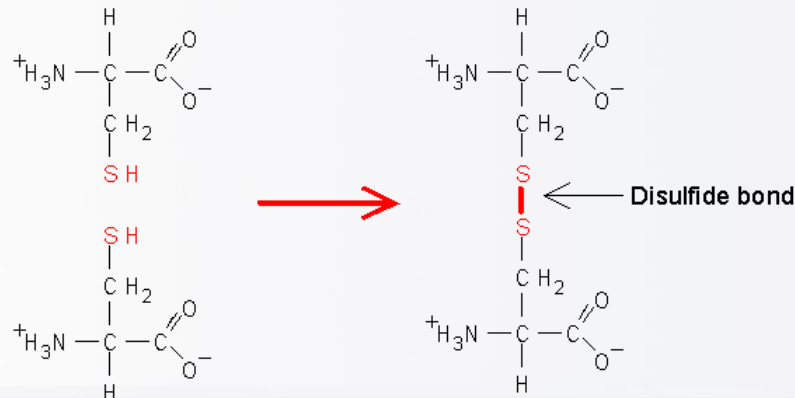


Aspartate  
Asp, D

# Proteins

- Non-polar side chains
  - Aliphatic (G, A, L, I, V)
    - Very hydrophobic, inside proteins
  - Sulfur-containing (M)
    - Most hydrophobic!
    - First amino acid in protein chain - inside
  - Cyclic (P)
    - Conformational rigidity, structural disruptor
    - Turns or bends of a protein
  - Aromatic (W, F)
    - Ultraviolet light

- Uncharged, polar side chains
  - Hydroxyl (S, T, Y)
    - Hydrogen bonding; pKa 10.46
  - Amide (N, Q)
    - Hydrogen bonds
  - Sulfur containing (C)
    - Disulphide bonds - oxidation of 2 thiol groups





- Charged, polar side chains
  - Basic (L, R, H)
    - Positively charged when pH is BELOW their pKa
      - Lysine: amino group, pKa 10.5
      - Arginine: guanidino group, pKa 12.5
      - Histidine: imidazol group, pKa 6.0
  - Acidic (D, E)
    - Negatively charged when pH is ABOVE their pKa
      - Aspartic acid: pKa 3.9
      - Glutamic acid: pKa 4.1

# Proteins

- Essential

- Isoleucine (I, Ile)
- Leucine (L, Leu)
- Lysine (K, Lys)
- Methionine (M, Met)
- Phenylalanine (F, Phe)
- Threonine (T, Thr)
- Tryptophan (W, Trp)
- Valine (V, Val)

- During rapid growth

- Arginine (R, Argy)\*
- Histidine (H, His)\*

- Nonessential

- Alanine (A, Ala)
- Asparagine (N, Asn)
- Aspartic acid (D, Asp)
- Cysteine (C, Cys)
- Glutamic acid (E, Glu)
- Glutamine (Q, Gln)
- Glycine (G, Gly)
- Proline (P, Pro)
- Serine (S, Ser)
- Tyrosine (Y, Tyr)

# Proteins

- Chirality
  - Optical activity
  - Enzymatic: L only

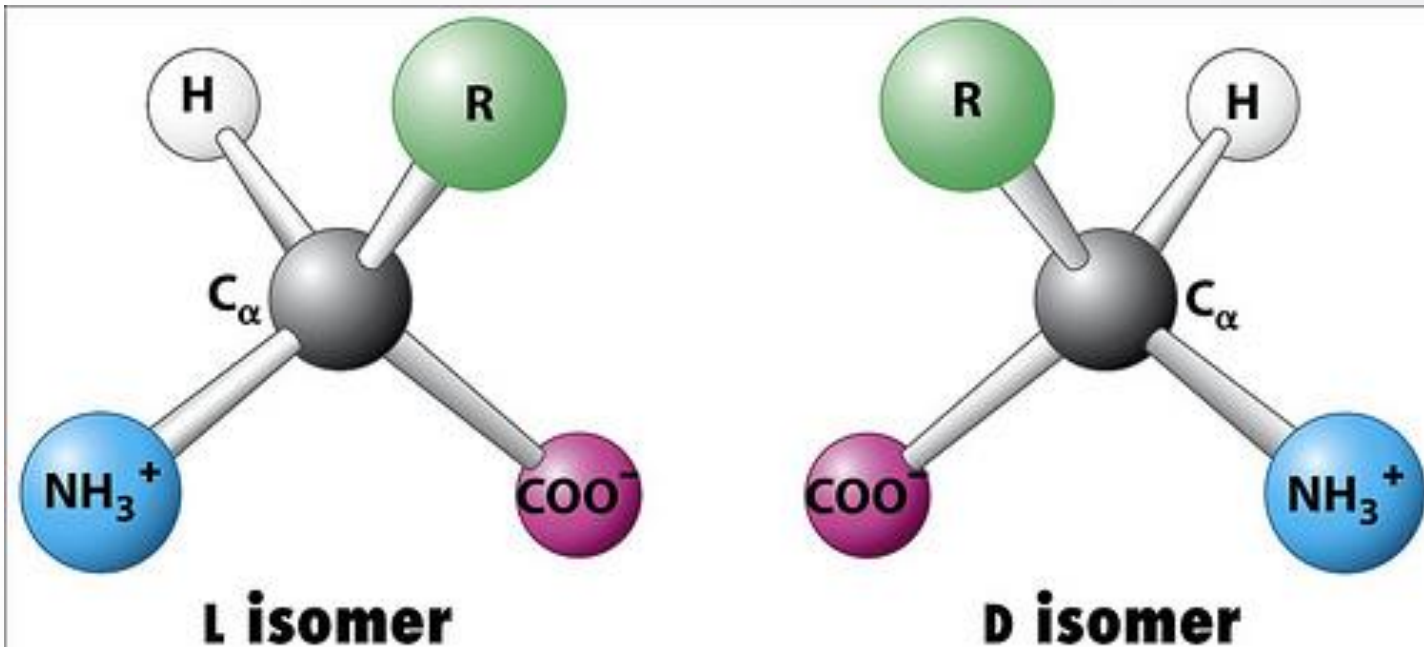
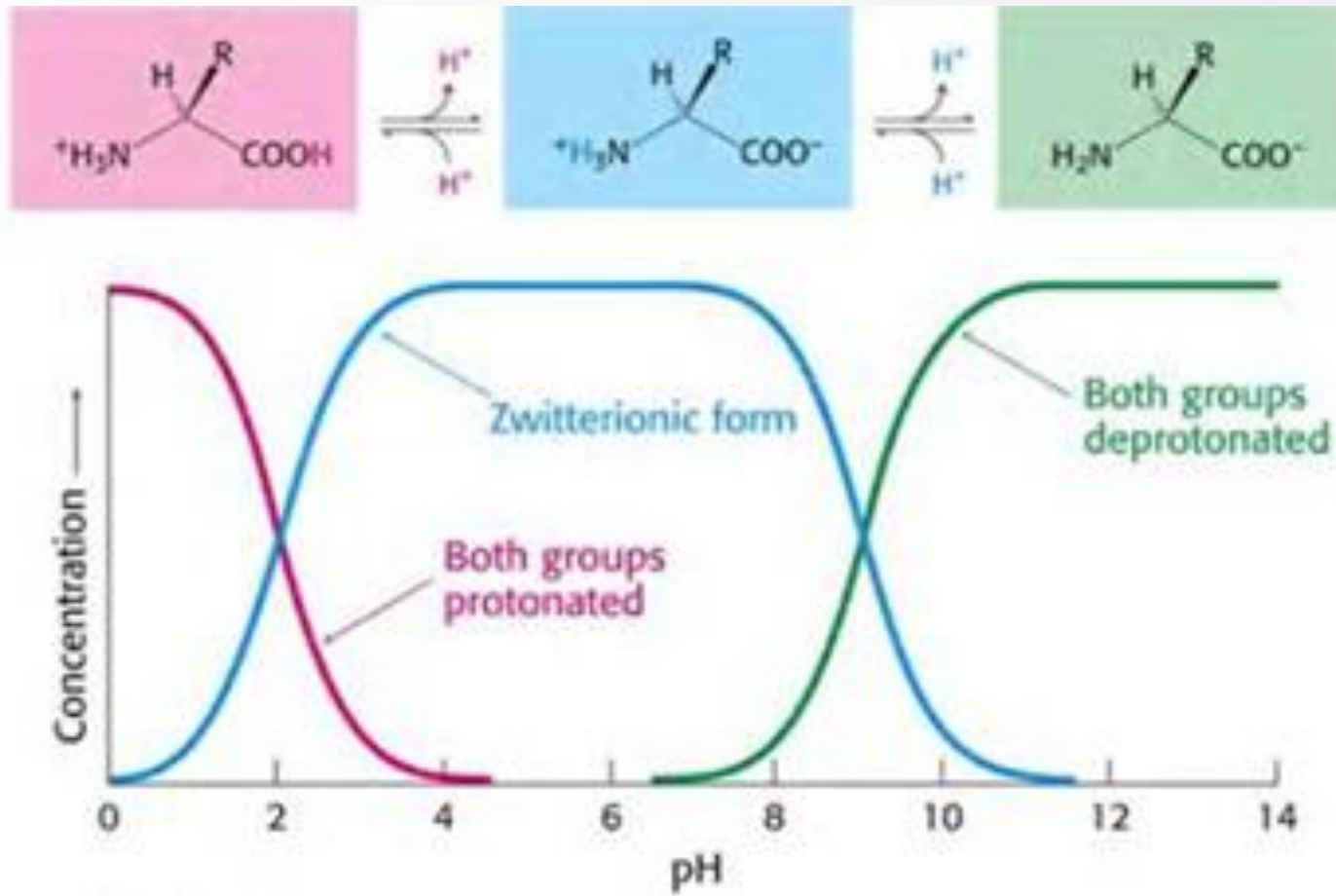


Figure 2-4  
Biochemistry, Sixth Edition  
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# Proteins

- Zwitterion





- Titrations

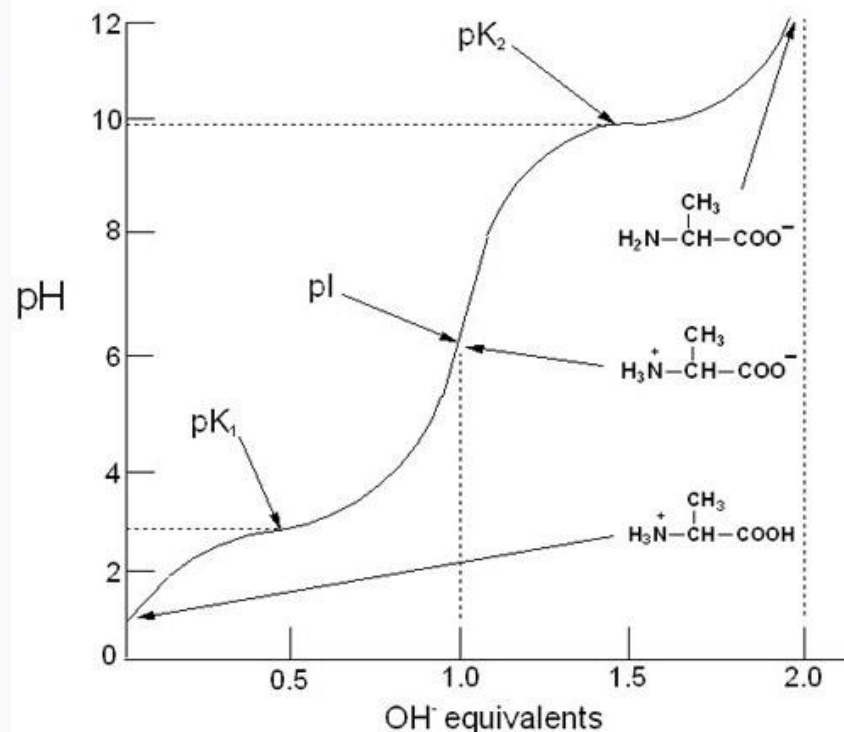
- Isoelectric point: the pH where net charge = 0

- Average of  $pK_1$  and  $pK_2$ \*

- $pK_1 \sim 2$

- $pK_2 \sim 10$

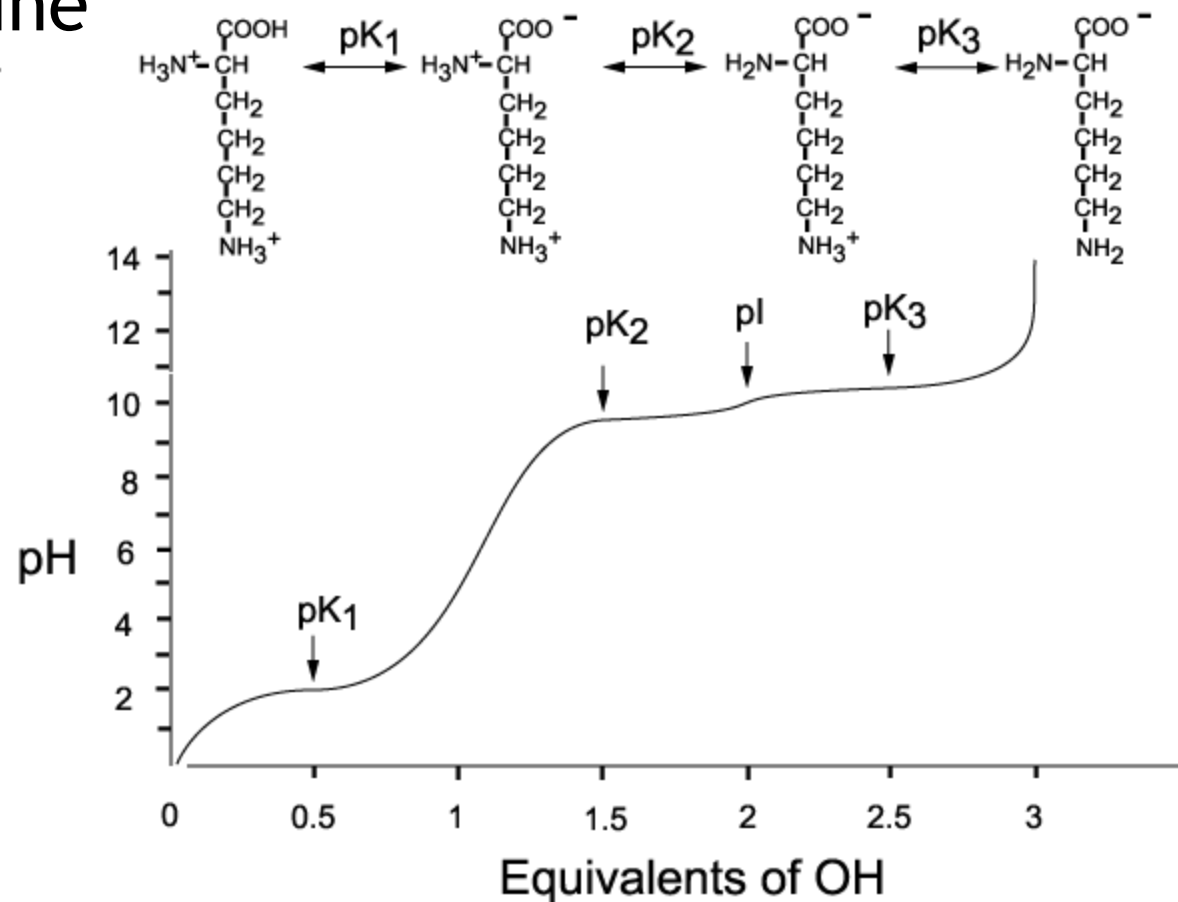
- E.g. alanine



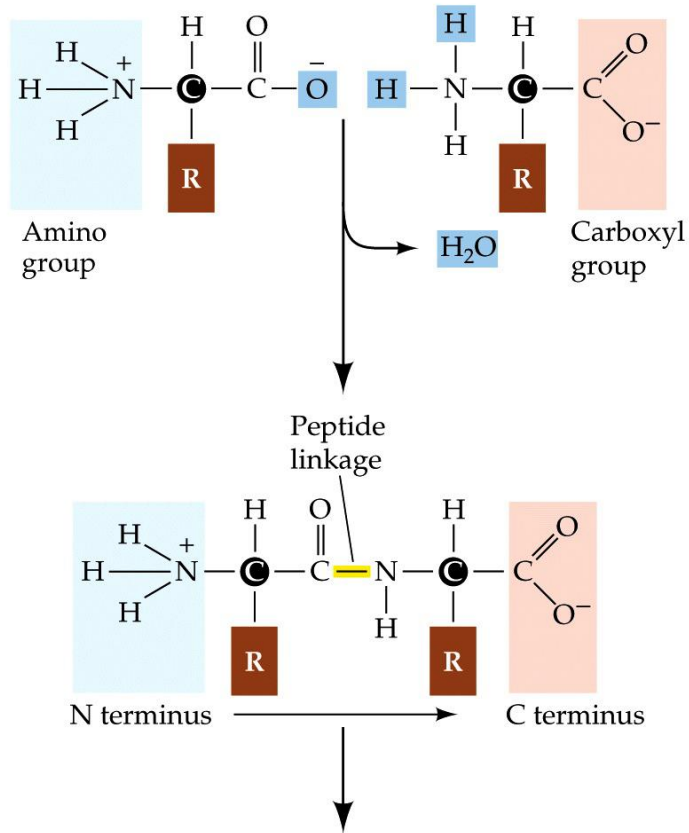


# Proteins

- Titrations of acidic/basic amino acids
  - E.g. lysine



## • Peptide bond



© 2001 Sinauer Associates, Inc.

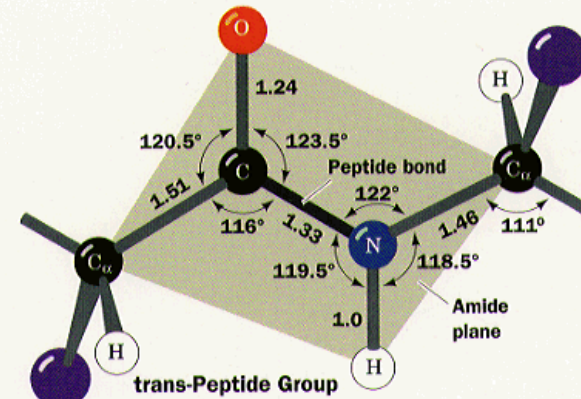


Figure 7-1. The trans-peptide group.

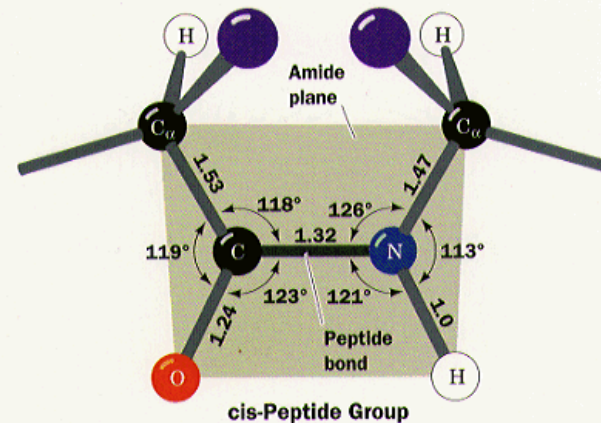
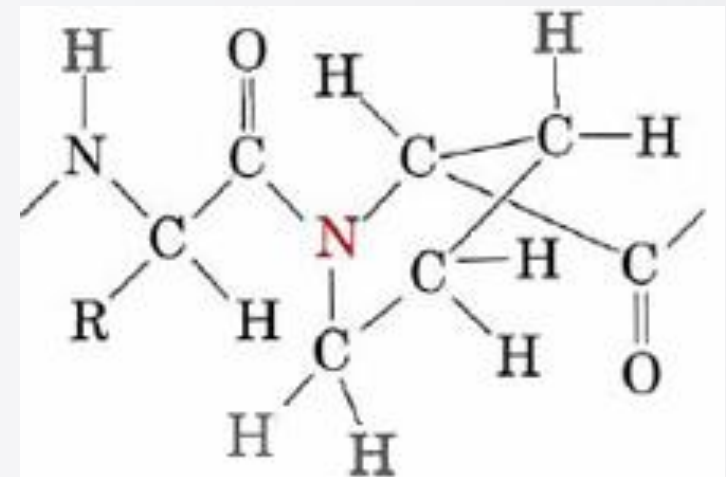
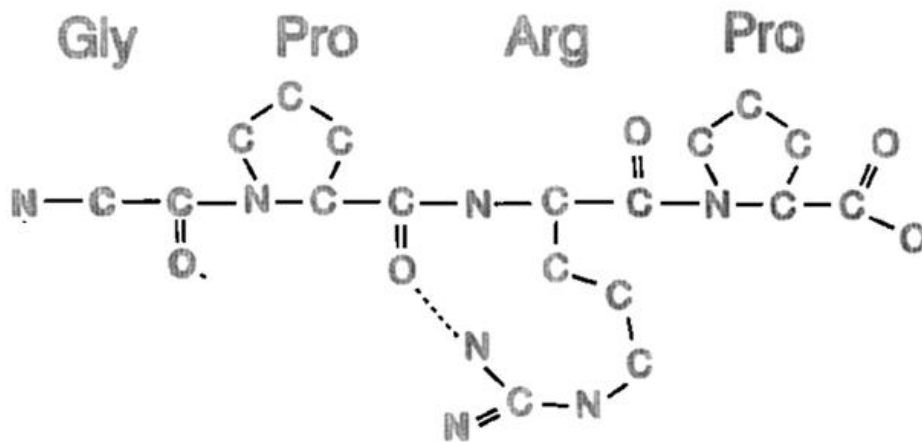


Figure 7-2. The cis-peptide group.

# Proteins

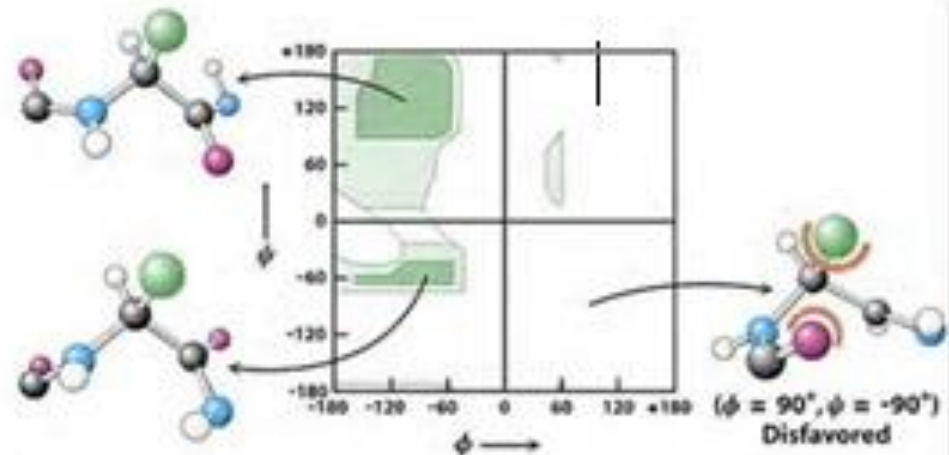
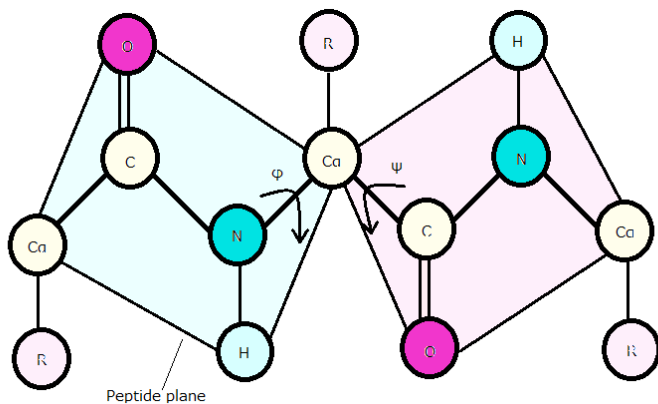
- Primary Structure
  - Chain from amino end to carboxyl end



- Secondary Structure

- Torsion angles - Ramachandran diagram

- $\Phi$  (phi) angle -  $C\alpha$ -N bond
- $\Psi$  (psi) angle -  $C\alpha$ -C bond
  - Beta pleated sheet:  $\Phi = -119$  and  $\Psi = 113$
  - Right-alpha helix :  $\Phi = -57$ ,  $\Psi = -47$
  - Left-alpha helix:  $\Phi = 57$ ,  $\Psi = 47$





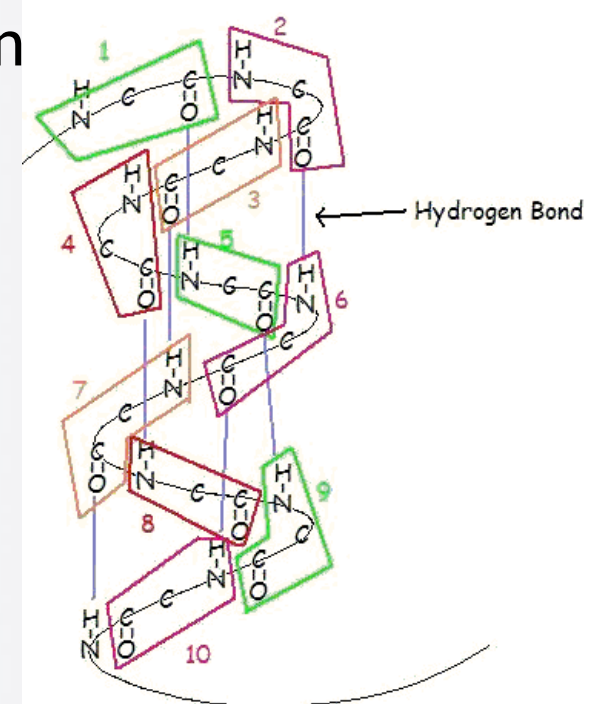
- Secondary Structure

- Helices

- Rise (r) - height of one amino acid
    - Helical pitch (p) - height of helix per turn
    - Number (n) of amino acids per turn
    - Number (N) of atoms per turn

- Right-handed alpha-helix

- $3.6_{13}$ 
      - 3.6 amino acids/turn, 13 atoms/turn
      - 0.54 nm pitch ( $3.6 \times 0.15\text{nm}$ )
    - Hydrogen bonds: n to n+4
    - Torsion angles:  $\Phi = -57$ ,  $\Psi = -47$





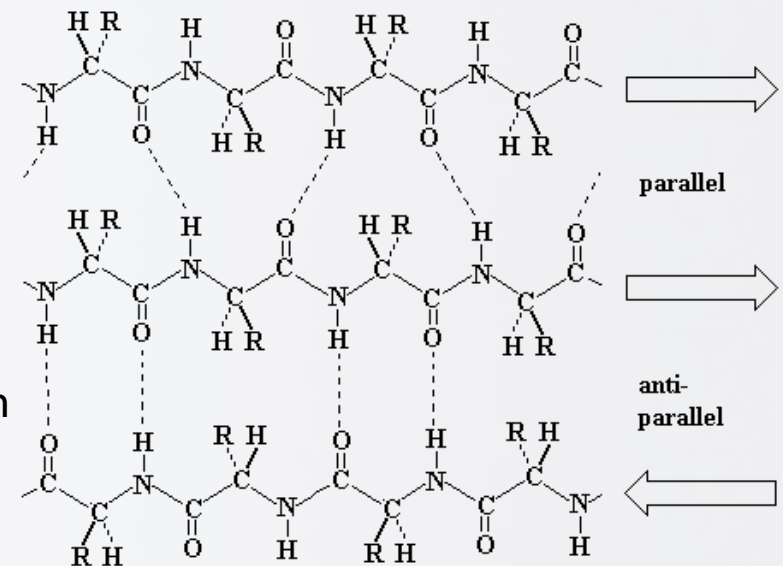
- Secondary Structure

- Pleated sheets

- Chains align side by side, R groups above/below
    - Torsion angles:  $\Phi = -119$  and  $\Psi = 113$
    - Hydrogen bonds
    - Antiparallel or parallel

- Hairpin turn

- Uses 4 amino acids
    - H-bond between 1<sup>st</sup> and 4<sup>th</sup>
    - 2<sup>nd</sup> = proline, 3<sup>rd</sup> = glycine

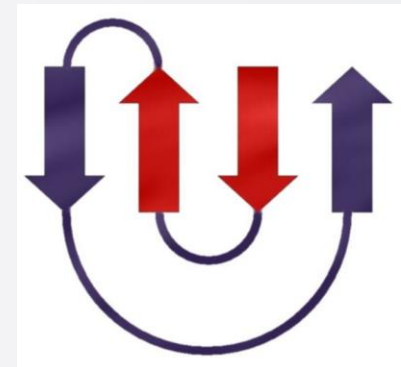
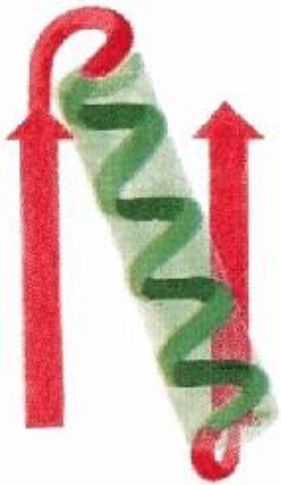


- Tertiary Structure

- Motifs = Super secondary structure

- Combined secondary structures

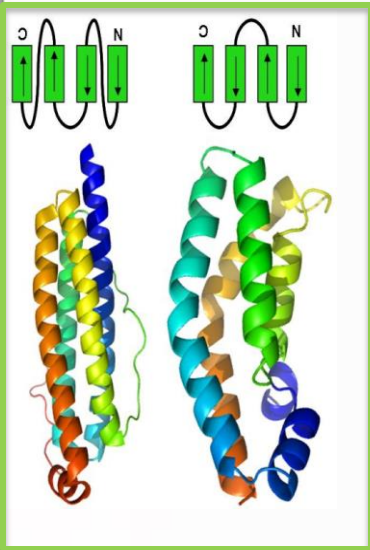
- Beta alpha beta motif
      - Beta hairpin motif
      - Alpha alpha motif
      - Greek key motif



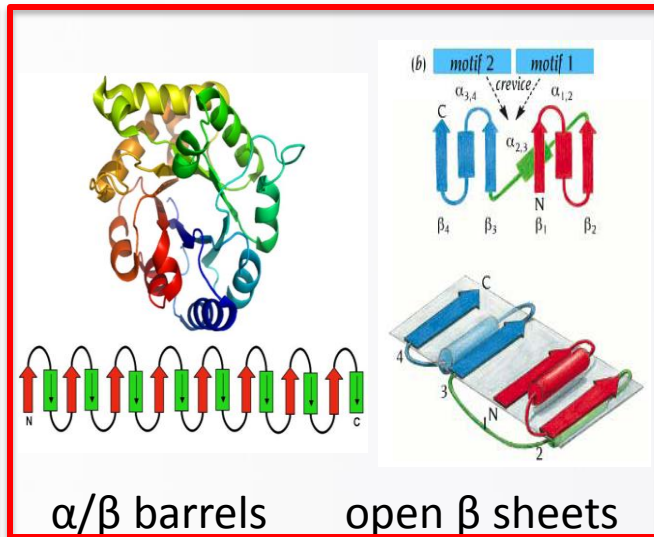
- Tertiary Structure

- Fold: Groups of motifs
- Domain: globular proteins, specific functions

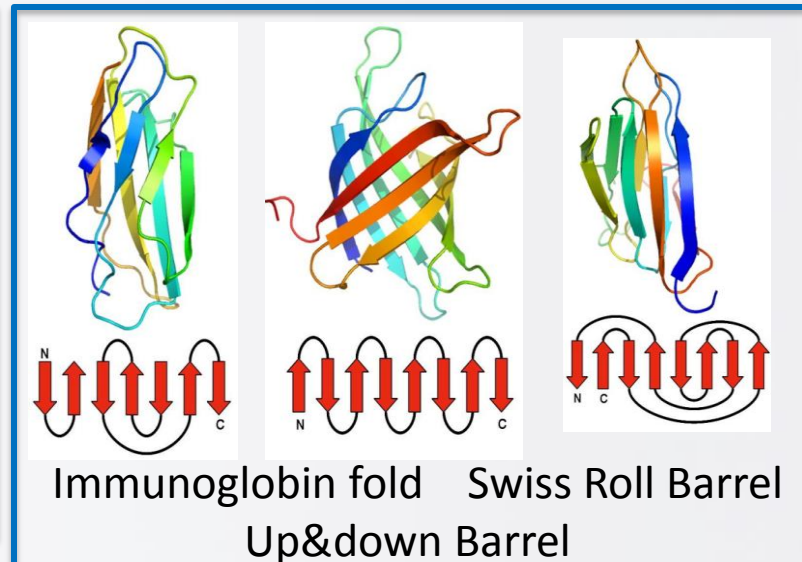
Alpha Domain



Alpha/beta Domain



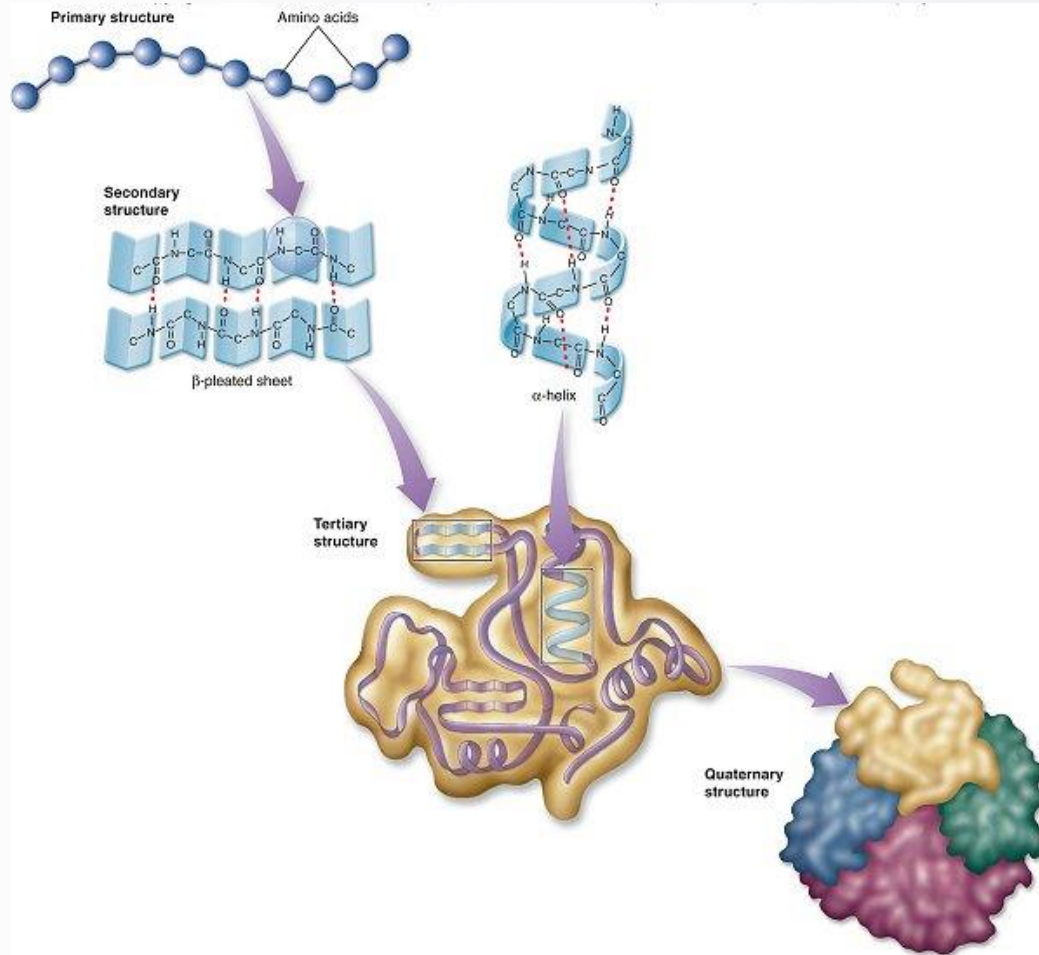
Beta Domain



- Quaternary structure
  - Protein composed of  $>1$  polypeptide chain
  - Advantages
    - Change faulty subunit
    - 3D positioning of enzymes
    - Multiple functions
  - Subunit interaction
    - Multisubunit protein: identical / non-identical chains
    - Protomers: identical subunits of oligometric enzyme
      - Haemoglobin



- Protein Structure Overview





- Fibrous Proteins

- $\alpha$ -keratin

- Skin, nails, hair, horns, feathers, beak claws

- Structure

- Right handed helix, rich in Cys, Glu, Gln, Ser

- Forces

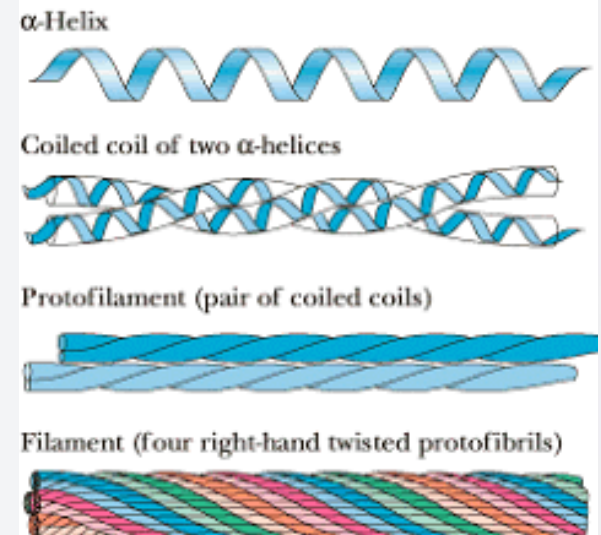
- Hydrophobic interactions

- » Repetitive sequence abcdefg

- » a and d are nonpolar

- Disulphide bonds

- » Rich in serine



- Fibrous Proteins

- Collagen

- Skin, teeth, cartilage, tendons, ligaments

- Structure

- Composed of 1/3 glycine, 1/5 proline, 1/5 4-hydroxyproline
    - Repeating triplets of Gly-X-Y
    - Three left-handed  $3.3_{10}$  helices form a superhelix
    - Hydrogen bonds between glycine and proline

1. Alpha chain



2. Procollagen



3. Tropocollagen



4. Packing of Tropocollagen molecules into FIBRILS



- Stabilization forces

- 1. Hydrophobic forces ( $>40\text{kJ/mol}$ )
  - Kyte-Doolittle Scale (high number = hydrophobic)
  - Hopp-Woods Scale (high number = hydrophilic)
- 2. Hydrogen bonds ( $12\text{-}30\text{kJ/mol}$ )
  - Occurs between: R group/R group, R group/water, R group/backbone atoms, backbone atoms/water
- 3. Electrostatic forces ( $12\text{-}20\text{kJ/mol}$ )
  - Ionic interactions: salt bridge
  - Dipole-dipole interactions ( $0.1\text{-}4\text{kJ/mol}$ )
- 4. Disulfide bonds

# Proteins

- Analysis
  - In silico analysis
  - Concentration
  - Molecular mass
  - Purification
  - Sequencing
  - Secondary structure determination
  - Tertiary / quaternary structure determination

# Proteins

- In silico analysis
  - Primary structure needed
  - Potential functions of protein
    - Translate DNA into protein
    - Compare sequence to database
    - Find repeats, motifs, domains
    - Prediction of secondary, tertiary, quaternary



- Quantification of Concentration
  - Bradford colorimetric assay
    - Coomassie Brilliant Blue binds to hydrophobic residue
    - Standard curve needed, absorb at 465-565nm
  - Ninhydrin assay
    - Oxidizes amino acids to ammonia, aldehyde,  $\text{CO}_2$
    - Ammonia reacts with hydrindantin to form purple
    - Standard curve needed, absorb at 570nm
  - Ultraviolet spectroscopy
    - R groups of Trp, Tyr, and Phe absorb light at 280nm
    - $A_{280} = \epsilon CL$

# Proteins

- Molecular mass determination

- Ultracentrifugation

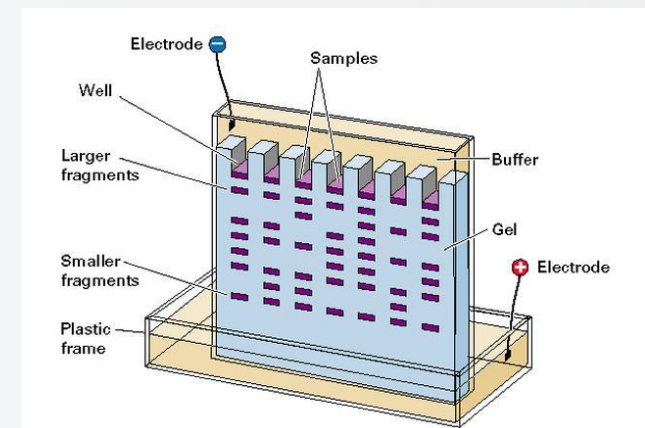
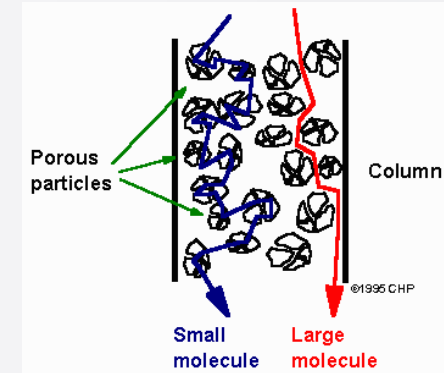
- Velocity sedimentation
- Equilibrium sedimentation

- Size exclusion chromatography

- Beads with pores, use with molecules of known mw

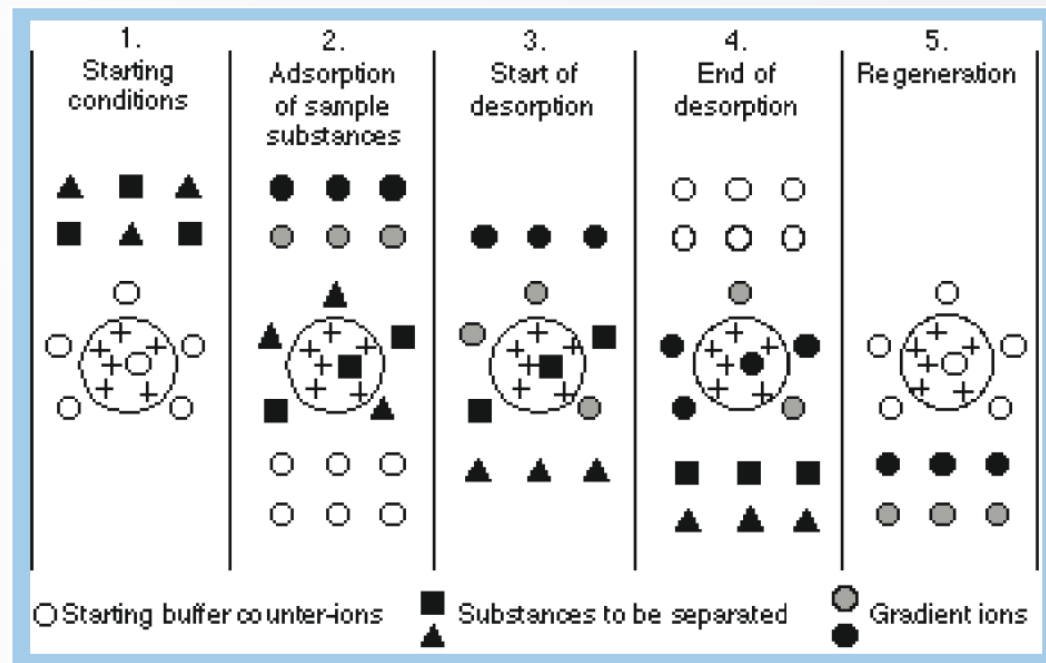
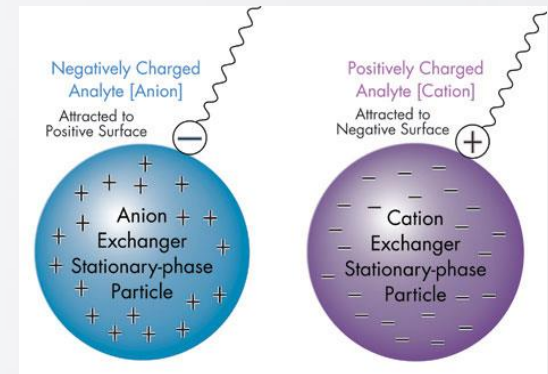
- Gel electrophoresis

- Porous polyacrylamide gel
- SDS assigns negative charge



- Purification

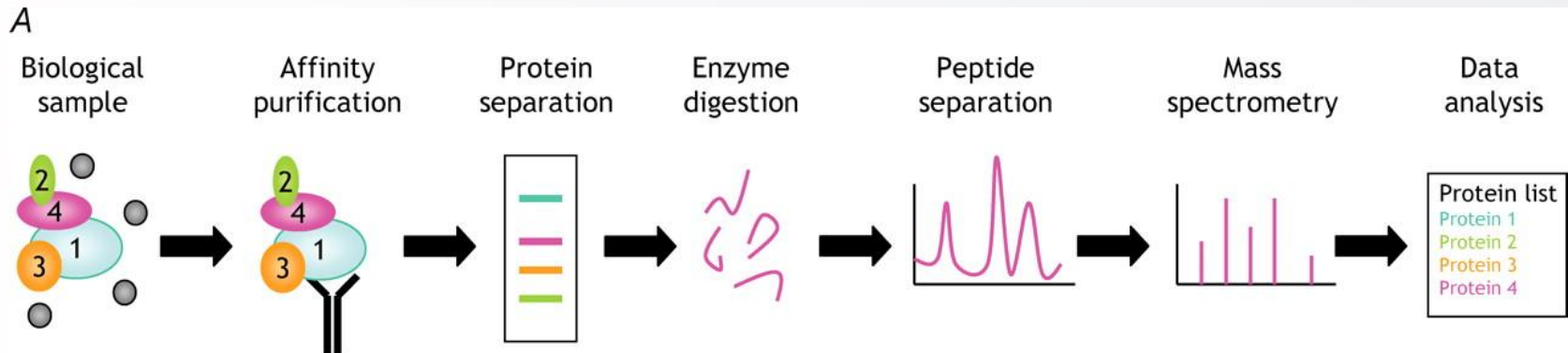
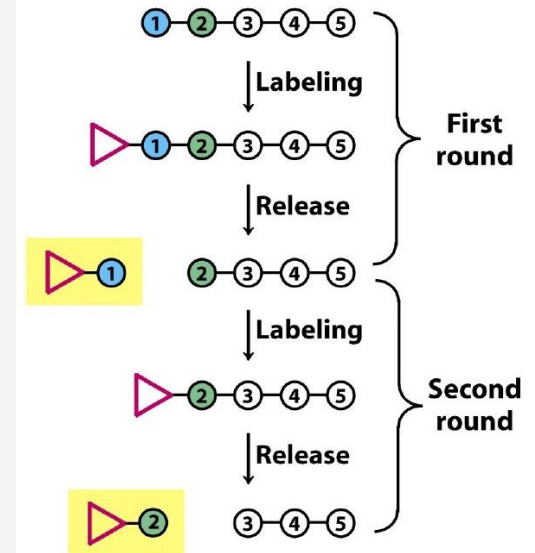
- Ion exchange chromatography
  - Stationary phase - charged matrix
  - Cation vs anion exchanger



# Proteins

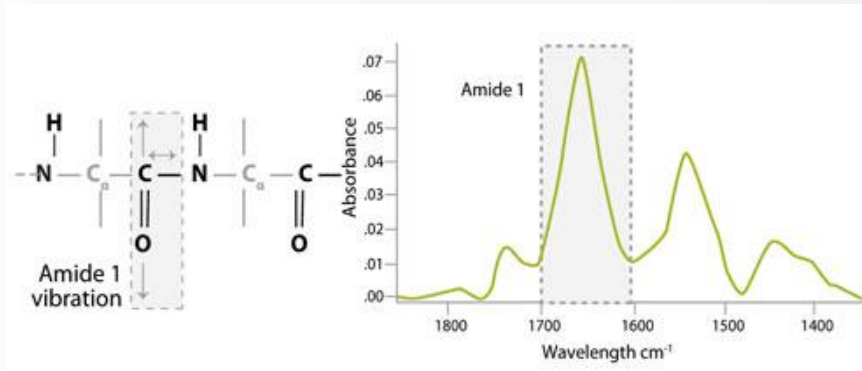
- Protein Sequencing

- Edman degradation
  - Small chains only
- Mass spectrometry
  - Mass-to-charge ratio ( $m/z$ )
  - Protein cleaved by endopeptidases
  - Charge acquired through protonation of lys, arg



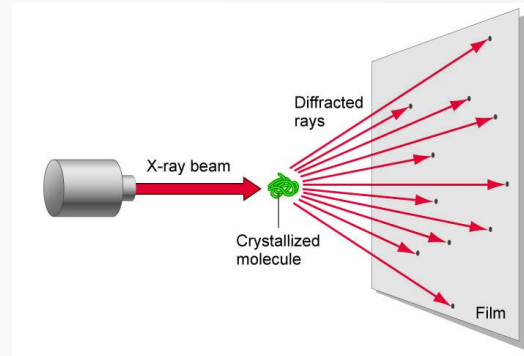


- Secondary Structure Determination
  - Circular dichroism spectroscopy
    - Absorption of circular polarized light (right or left)
  - Infrared spectroscopy
    - Molecular vibration of molecules
    - Look for Amide I peak
      - Alpha helix - 1650 peak; Beta sheet - 1640 peak



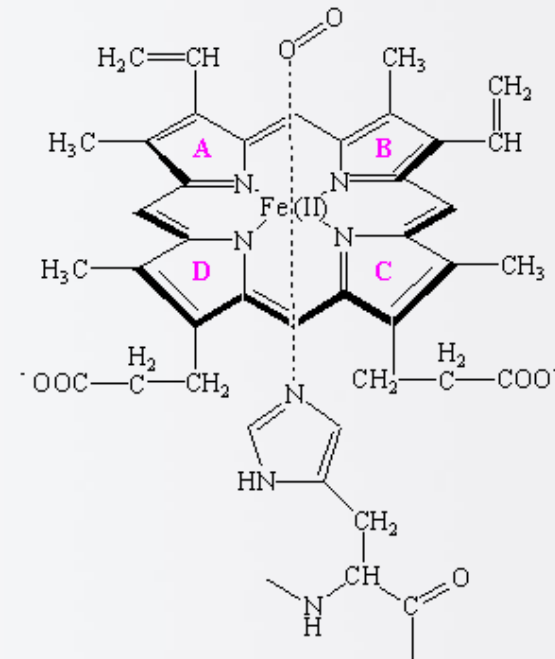
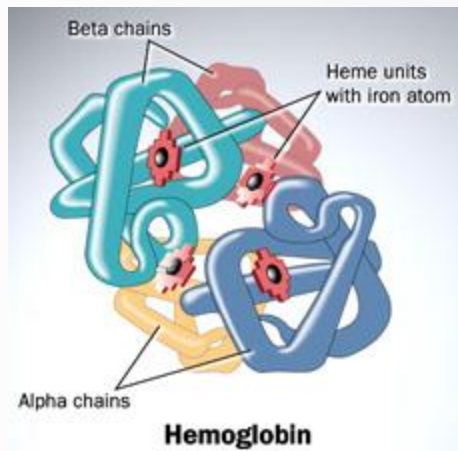


- Tertiary and quaternary structure
  - X-ray diffraction
    - Crystal formation - very difficult
    - Bombard crystal with x-rays, get specific pattern
  - 2D NMR
    - Takes multiple 2D pictures and overlays them
    - In solution, no need to crystalize



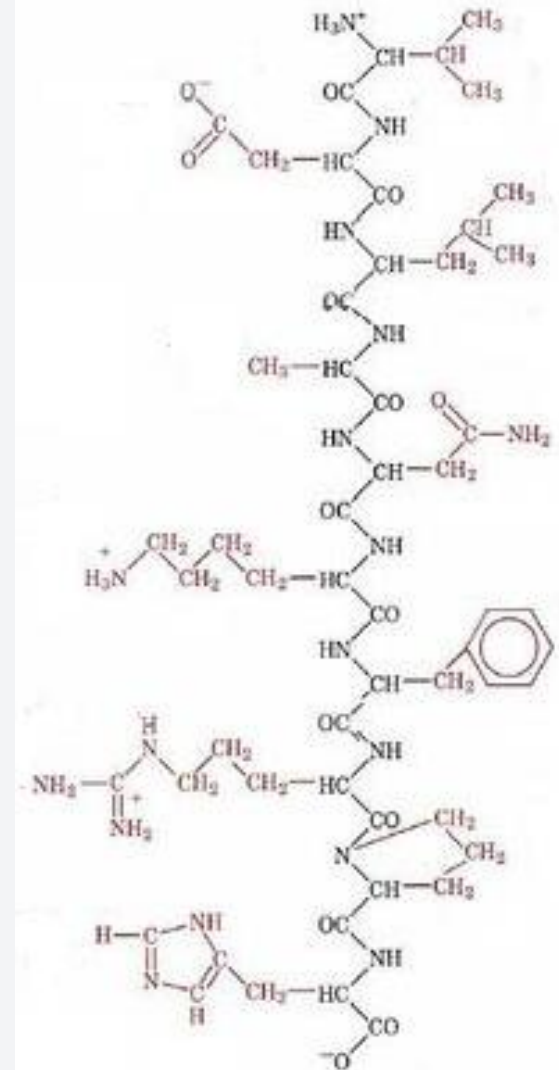
- Haemoglobin

- Heterotetramer: 2  $\alpha$ -subunits ; 2  $\beta$ -subunits
  - Globin fold: 8 helices
  - Heme group: 4 pyrrole groups with methane bridges, histidine, atom of O<sub>2</sub>
- Relaxed vs. tense form



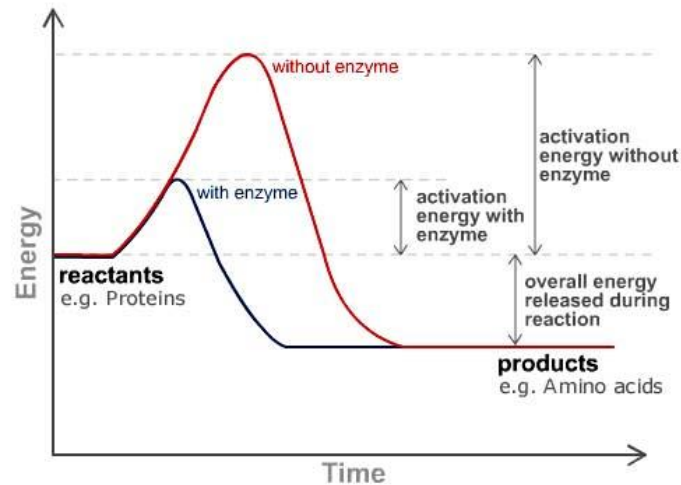
# Proteins Review Questions

- Draw a titration curve for aspartic acid with the corresponding structures.
- What is the amino acid sequence of this protein?
- Draw one complete turn of an alpha helix.



# Enzymes

- Enzymes vs. chemical catalysis
  - Higher reaction rates
  - Milder reaction conditions
  - Greater reaction specificity
  - Capacity for control



# Enzymes

- Catalytic activity depends on protein shape
- Some require additional components
  - Cofactors: non-protein molecules (metals)
  - Coenzymes: organic or metallorganic
  - Prosthetic: cofactor/coenzyme is covalently bound
    - Apoenzyme: enzyme lacking its prosthetic
    - Holoenzyme: enzyme with its prosthetic
  - Isoenzyme: different enzyme catalyses same reaction



# Enzymes

- ES complex
  - $E + S \rightleftharpoons ES \rightleftharpoons E + P$
  - Substrate: substance that is transformed
  - Active site: part of enzyme where rxn occurs
    - Binding site: binds substrate
    - Catalytic site: transformation of substrate to product

- Reaction Equilibrium

- Large negative  $\Delta G$  indicates favourable reaction
  - $\Delta G = -RT \ln K$
  - Depends on temperature and equilibrium constant

- Reaction Rates

- First order

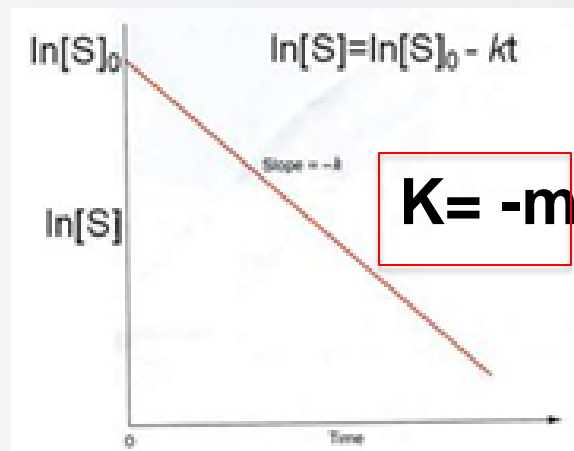
- $V = k[S]$
- Rate depends on concentration only
- $k$  has units of  $[s^{-1}]$

- Second order

- $V = K[S_1][S_2]$
- Rate depends on conc of two compounds
- $K$  has units of  $[M^{-1}s^{-1}]$

# Enzymes

- Determining rate constant
  - Take  $\ln$  of  $[S]$
  - Graph  $\ln[S]$  vs. time
    - $y=mx+b$  becomes  $\ln[S] = \ln[S]_0 - kt$



- Determining half life
  - Substitute  $[S]$  for  $[S]_0/2$  (substrate half life) in the equation  $\ln[S] = \ln[S]_0 - kt$

$$\frac{\ln\left(\frac{[S]_0}{2}\right)}{[S]_0} = -kt_{1/2}$$

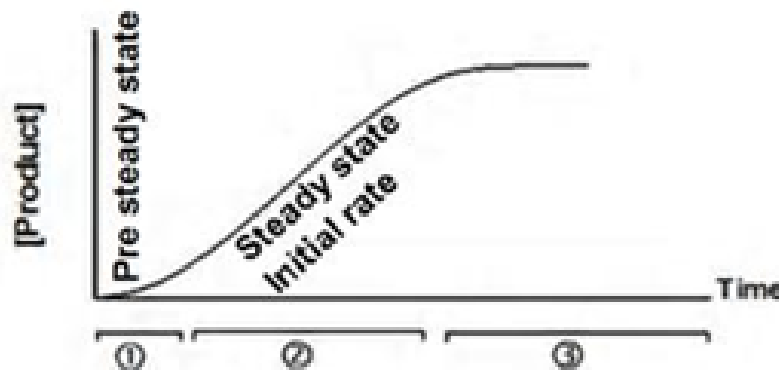
$$t_{1/2} = \frac{\ln 2}{k}$$

- Half life only depends on  $k$
- Rate depends on  $[S]$ ... but  $[S]$  changes over time

# Enzymes

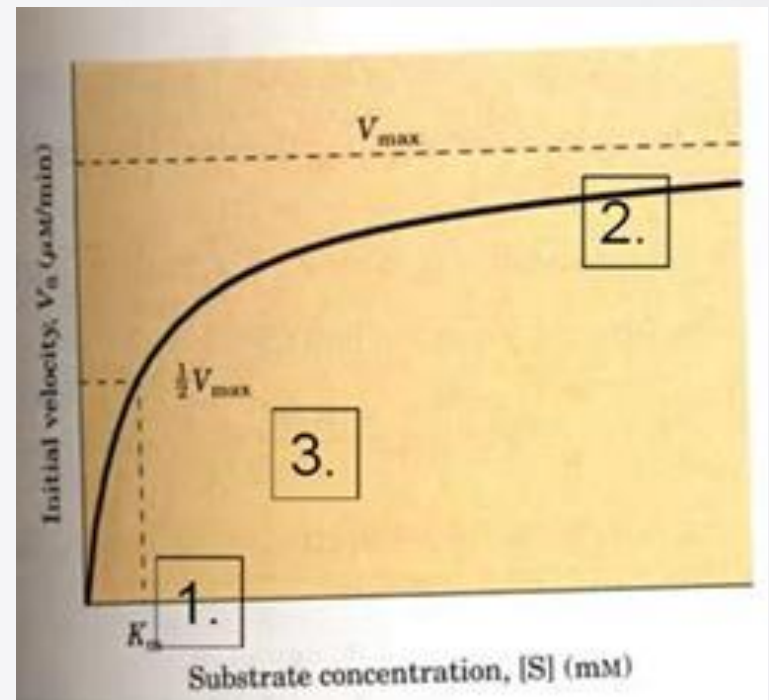
- **Initial Rates**

1. Rate of product accumulation increases over time
  - Enzyme and substrate bind
2.  $[P]$  increases linearly with time while  $[S]$  is considered constant
  - Steady state phase = INITIAL VELOCITY
  - Concentration of enzyme-substrate complex doesn't change appreciably
3. Substrate is depleted, curve levels off and concentration of product reaches a plateau



# Enzyme

- Michaelis constant:  $K_m$ 
    - The  $[S]$  where the reaction is in equilibrium
1. At low  $[S]$ , enzyme is in uncombined form (E), increasing  $[S]$  increases  $V_o$  significantly
  2. At higher  $[S]$ , less free enzyme,  $V_o$  increases get smaller until it plateaus at  $V_{max}$
  3.  $\frac{1}{2} V_{max} = K_m$





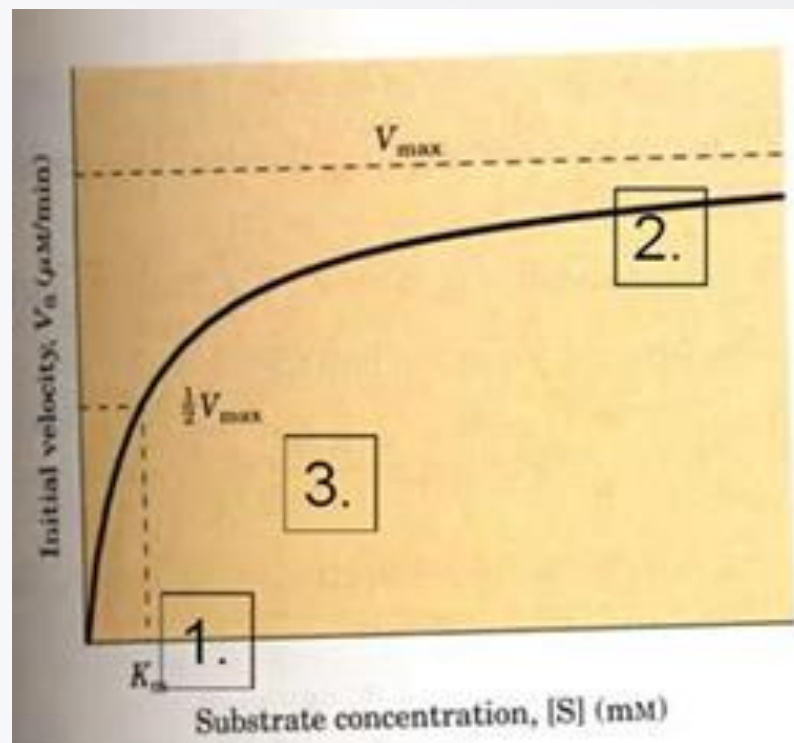
# Enzymes



$$V_o = \frac{V_{max}[S]}{K_m + [S]}$$

$$K_m = \frac{(k_1 + k_2)}{k_1}$$

$$K_s = \frac{k_{-1}}{k_1}$$



$$K_m = [S] \text{ when } V_o = 1/2V_{max}$$

- **Lineweaver-Burk Equation**

- To determine  $K_m$  and  $V_{max}$

1. Calculate  $1/[S]$  and  $1/V_o$

2. Graph LWB

- $X = 1/[S]$

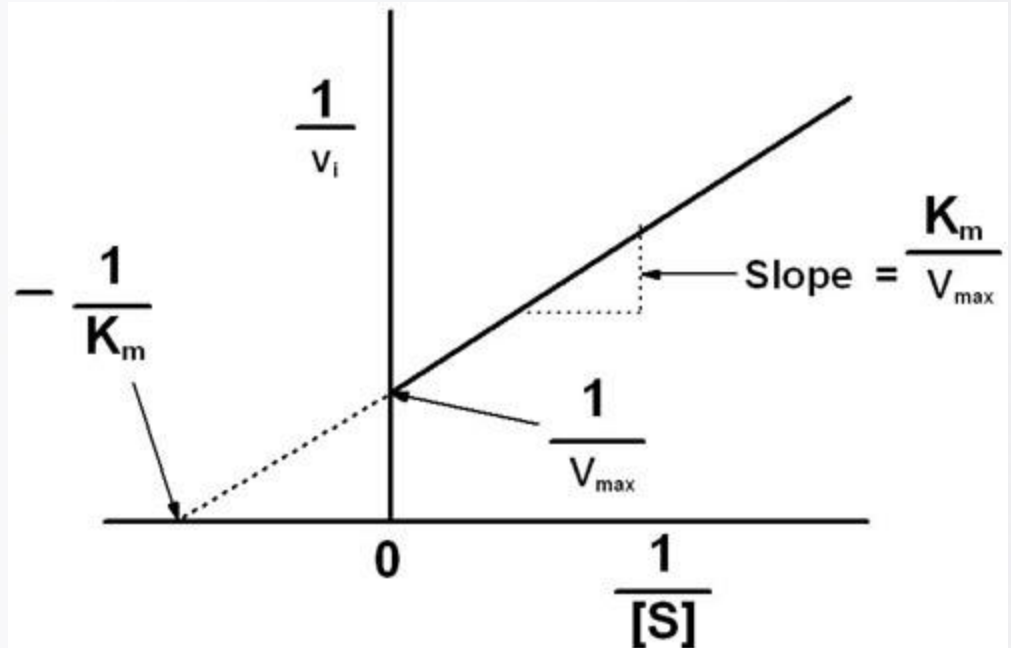
- $Y = 1/V_o$

3. Calculate  $K_m$

- X-int =  $-1/K_m$

4. Calculate  $V_{max}$

- Y-int =  $1/V_{max}$



# Enzymes

- Inhibition

- A substance can alter the activity of an enzyme by combining with it in a way that it influences the binding of substrate and/or its turnover number

- Competitive inhibition
- Uncompetitive inhibition
- Mixed inhibition

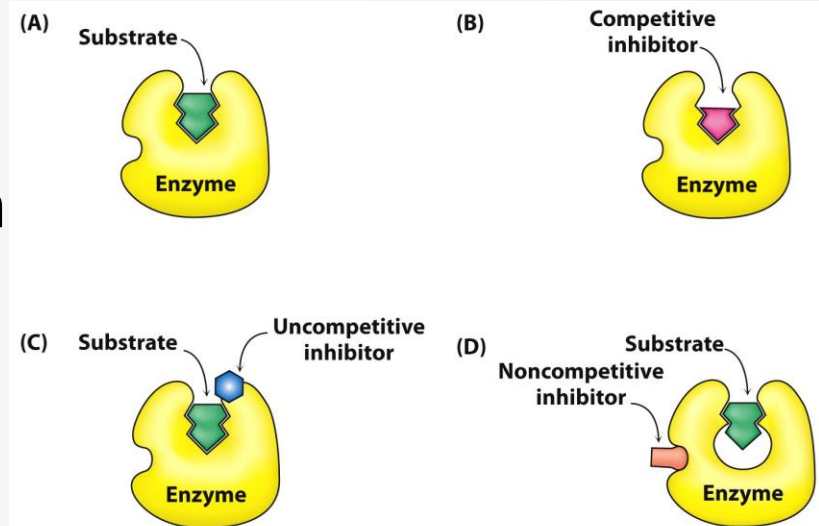
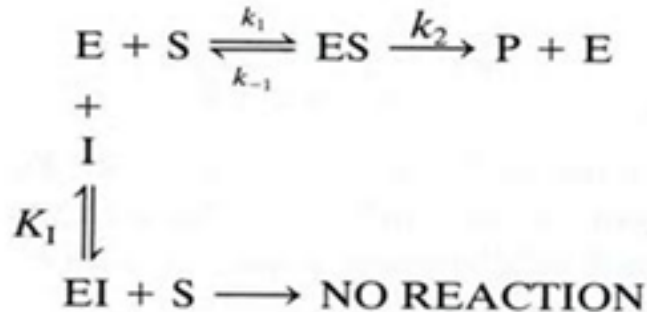


Figure 8.14  
Biochemistry, Seventh Edition  
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# Enzymes

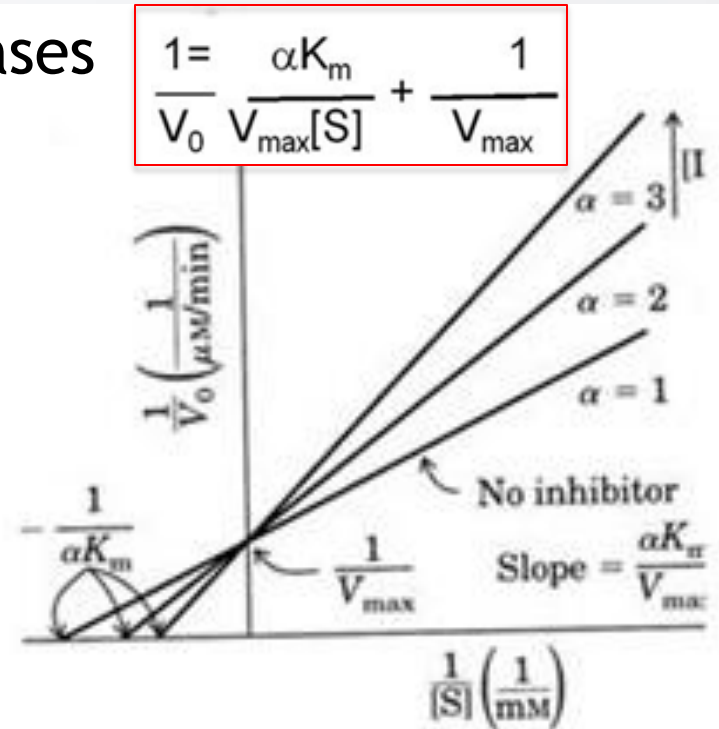
- Competitive inhibition

- Competes directly with substrate at active site
- Inhibition can be overcome by using a high [S]
  - Vmax unchanged, Km increases



$$V_0 = \frac{V_{\max} [\text{S}]}{\alpha K_m + [\text{S}]}$$

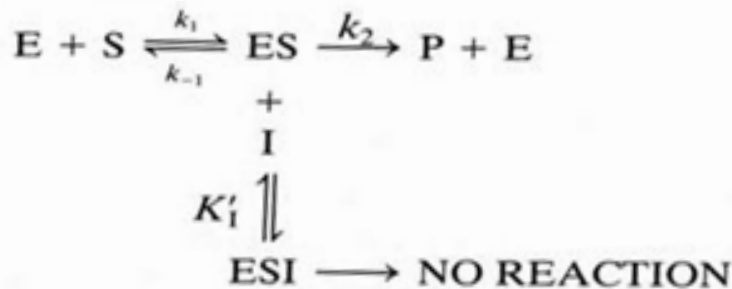
$$\alpha = 1 + \frac{[\text{I}]}{K_I} \quad \text{and} \quad K_I = \frac{[\text{E}][\text{I}]}{[\text{EI}]}$$





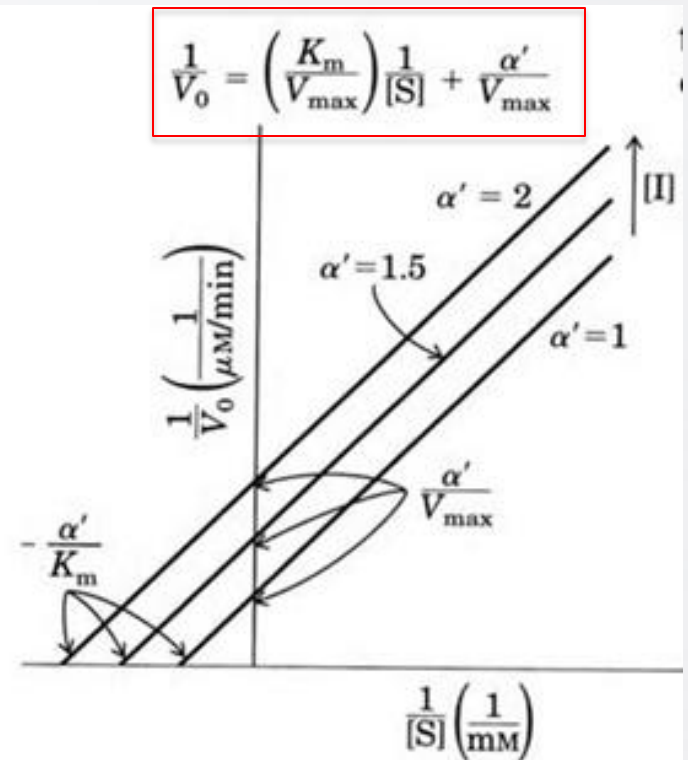
- Uncompetitive inhibition

- Inhibitor binds to ES but not E, not at active site
- Effects cannot be overcome by increasing [S]
  - Lowers Vmax, lowers Km



$$V_0 = \frac{V_{\max} [S]}{K_m + \alpha' [S]}$$

$$\alpha' = 1 + \frac{[I]}{K'_I} \quad \text{and} \quad K'_I = \frac{[ES][I]}{[ESI]}$$





# Enzymes

## • Mixed inhibition

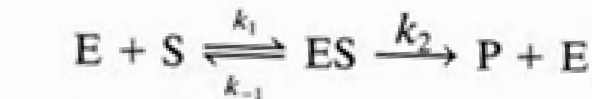
- Binds to either E or ES, not at active site

- Lowers Vmax, lowers Km

- Noncompetitive inhibition

- $\alpha = \alpha'$ : equal affinity for E and ES

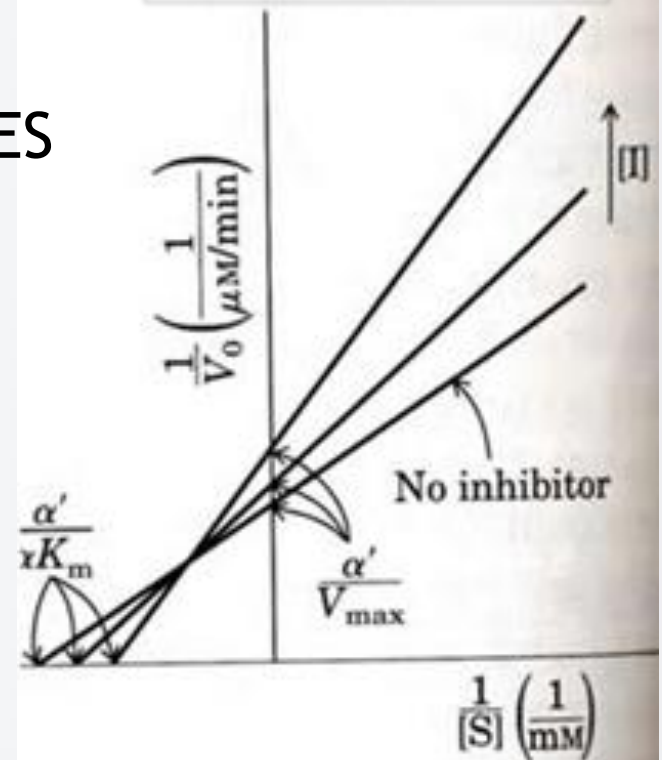
- Lowers Vmax, Km unchanged



ESI  $\longrightarrow$  NO REACTION

$$V_0 = \frac{V_{\max} [S]}{\alpha K_m + \alpha' [S]}$$

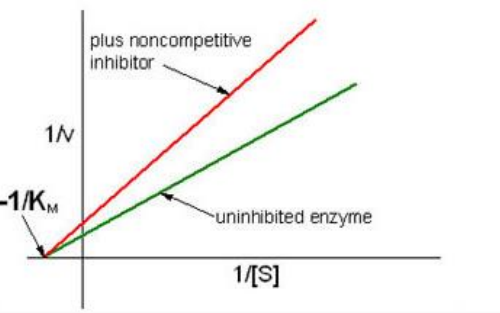
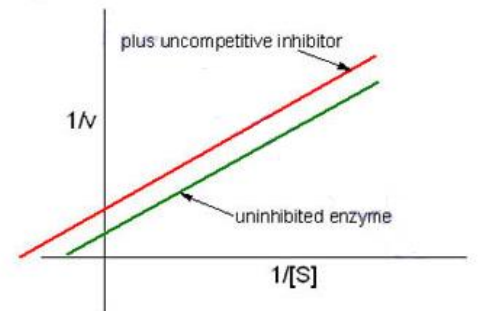
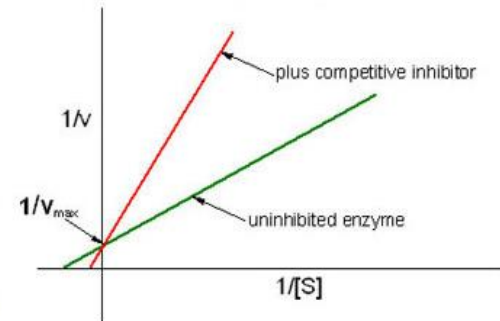
$$\frac{1}{V_0} = \left( \frac{\alpha K_m}{V_{\max}} \right) \frac{1}{[S]} + \frac{\alpha'}{V_{\max}}$$



# Enzymes

## • Summary: Qualitative

Inhibition	$V_{max}$	$K_m$
Competitive	No change	Increases
Uncompetitive	Decreases	Decreases
Mixed (do not intersect at y axis)	Decreases	Decreases
Noncompetitive (mixed where $\alpha = \alpha'$ )	Decreases	No change



- Summary: Quantitative

	MM equation	x-int	y-int	K <sub>i</sub>
No inhibition	$V_o = \frac{V_{\max} [S]}{K_m + [S]}$	$= \frac{-1}{K_m}$	$= \frac{1}{V_{\max}}$	-----
Competitive	$V_o = \frac{V_{\max} [S]}{\alpha K_m + [S]}$	$= \frac{-1}{\alpha K_m}$	$= \frac{1}{V_{\max}}$	$K_I = \frac{[E][I]}{[EI]}$
Uncompetitive	$V_o = \frac{V_{\max} [S]}{K_m + \alpha' [S]}$	$= \frac{-\alpha'}{K_m}$	$= \frac{\alpha'}{V_{\max}}$	$K_I = \frac{[ES][I]}{[ESI]}$
Mixed	$V_o = \frac{V_{\max} [S]}{\alpha K_m + \alpha' [S]}$	$= \frac{-\alpha'}{\alpha K_m}$	$= \frac{\alpha'}{V_{\max}}$	$K_I \text{ and } K_I'$

# Enzyme Review Questions

- Calculate the 1<sup>st</sup> order rate and half life of this enzyme.

Time (min)	[Sucrose] M
0	0.5011
30	0.4511
60	0.4038
90	0.3626
130	0.3148
180	0.2674

- This table gives rates of an enzyme 1) in the absence of an inhibitor or 2) in the presence of an inhibitor (10mM). What type of inhibition is this?

[S] (mM)	(1) $v_o$ ( $\mu M \cdot s^{-1}$ )	(2) $v_o$ ( $\mu M \cdot s^{-1}$ )
1	2.5	1.17
2	4.0	2.10
5	6.3	4.00
10	7.6	5.7
20	9.0	7.2



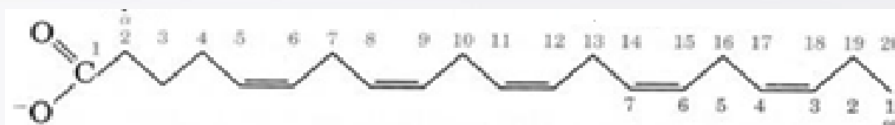
- Fatty acids
  - Carboxylic acids with long chain hydrocarbon side groups
    - Tail: aliphatic, 4-36C
    - Head: polar
  - Very low oxidation state



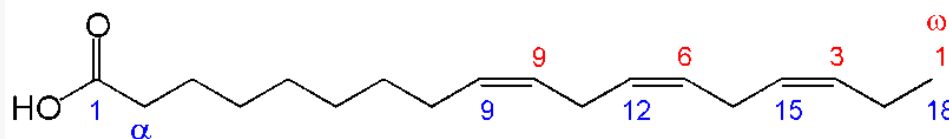


## • Naming

- C1: carboxyl carbon
- C2: alpha carbon
- X:Y $\Delta^{a,b,c}$



20:5( $\Delta^{5,8,11,14,17}$ ) Eicosapentaenoic acid (EPA),



18:3(n-3) alpha-linolenic acid

- X: number of carbons in the chain
- Y: number of double bonds
- a, b, c: location of double bond relative to C1
  - a represents the first carbon in the double bond
  - One number for every double bond present
- X:Y $\omega^N$  or X:Y(n-N)
  - C1 is methyl carbon at the end of the chain
  - N: position of the double bond relative to the  $\omega$  carbon

- Common names to know

Symbol <sup>a</sup>	Common Name	Systematic Name
<i>Saturated fatty acids</i>		
12:0	Lauric acid	Dodecanoic acid
14:0	Myristic acid	Tetradecanoic acid
16:0	Palmitic acid	Hexadecanoic acid
18:0	Stearic acid	Octadecanoic acid
20:0	Arachidic acid	Eicosanoic acid
22:0	Behenic acid	Docosanoic acid
24:0	Lignoceric acid	Tetracosanoic acid
<i>Unsaturated fatty acids (all double bonds are cis)</i>		
16:1n 7	Palmitoleic acid	9-Hexadecenoic acid
18:1n 9	Oleic acid	9-Octadecenoic acid
18:2n 6	Linoleic acid	9,12-Octadecadienoic acid
18:3n 3	α-Linolenic acid	9,12,15-Octadecatrienoic acid
18:3n 6	γ-Linolenic acid	6,9,12-Octadecatrienoic acid
20:4n 4	Arachidonic acid	5,8,11,14-Eicosatetraenoic acid
20:5n 3	EPA	5,8,11,14,17-Eicosapentaenoic acid
22:6n 3	DHA	4,7,10,13,16,19-Docosahexenoic acid
24:1n 9	Nervonic acid	15-Tetracosenoic acid

- Properties

- Length

- Increasing length decreases melting point
    - Normal: 12-24 C long, even numbers (most are 16,18)
      - Concatenation: enzymatic addition via acetyl-Co-A

- Saturated fats

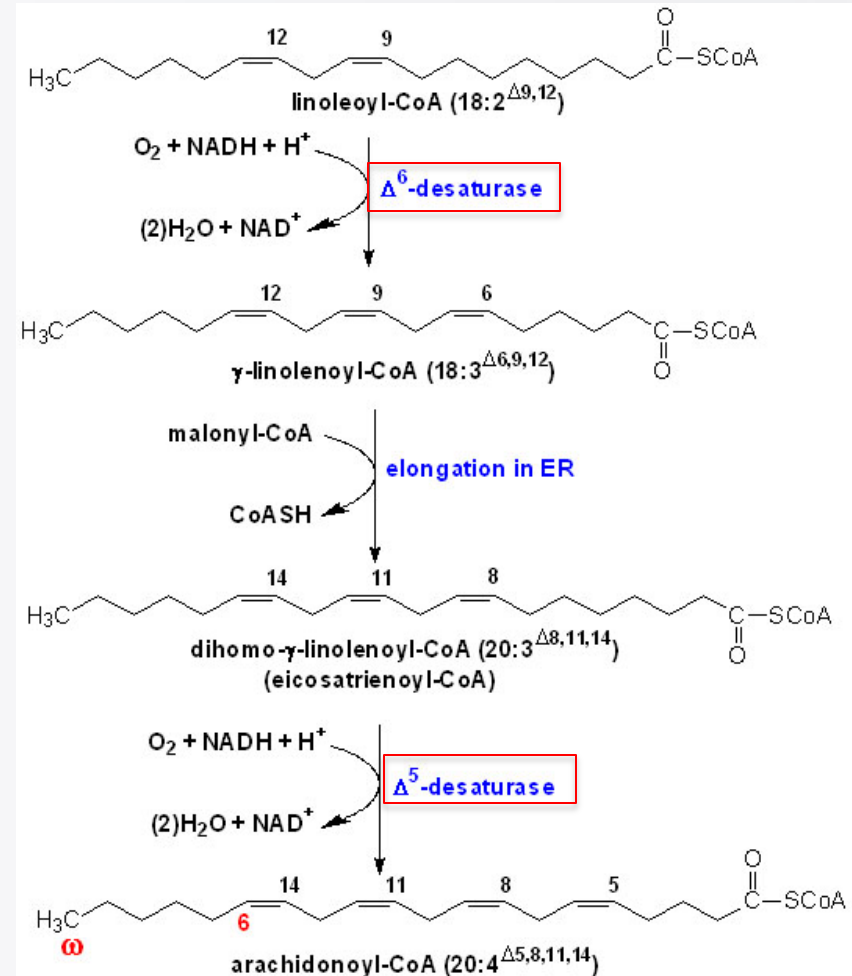
- Highly flexible - wide range of conformations
    - Packed into crystalline arrays

- Polyunsaturated fatty acids

- Flexibility restricted: cis conformation 30° bend
    - Melting points decrease with degrees of unsaturation

- Essential Fatty Acids

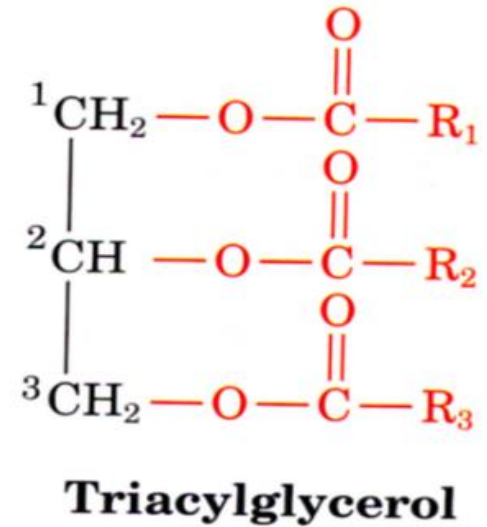
- Omega-3 and omega-6
  - Omega-6: meat
  - Omega-3: fish, olive oils
- Alpha-linolenic acid
- Arachidonic acid - cats





- Triacylglycerol

- Fatty acid triesters of glycerol
- Energy reserves in animals
  - Highly reduced, anhydrous
- Simple vs. mixed
- Nomenclature
  - 1-fatty acyl-2-fatty acyl-3-fatty acyl-glycerol
    - E.g. linoleic -> linoleoyl, stearic -> stearyl



- Adipocytes

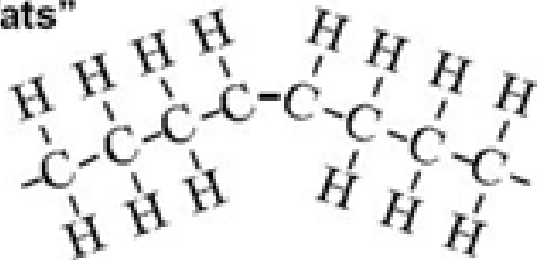
- Energy store, Thermal insulation, Buoyancy



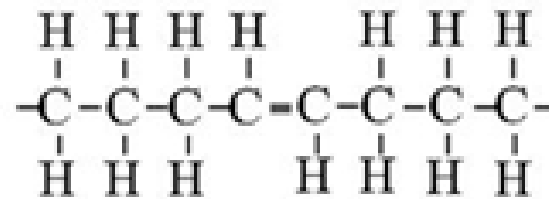
- Trans Fats

- Spoiling - oxidative cleavage of double bond
- Hydrogenation increases stability
  - Convert cis double bond to single bonds
  - Increases melting point
  - Also creates trans double bonds

"fats"



Cis configuration - bent molecule

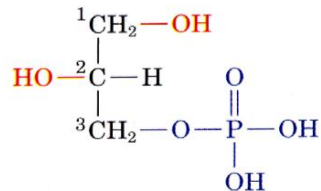


Trans configuration - straight molecule

- Five major classes of lipids
  1. Glycerophospholipids
    - Plasmalogen
    - Platelet activating factors
  2. Galactolipids and sulfolipids
  3. Archeal tetraether lipids
  4. Sphingolipids
    - Sphingomyelins
    - Glycosphingolipids
    - Gangliosides
  5. Sterols
    - Cholesterol

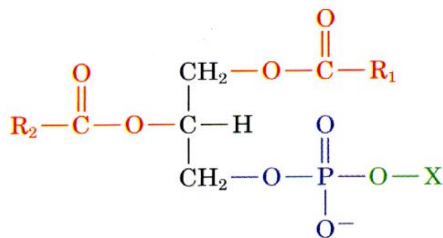
- Glycerophospholipids
  - Ester linked, “phosphotidyl-X”
    - C1 is prochiral, Sn3 is phosphate group
    - Sn1 = saturated, Sn2 = unsaturated; 16-20C

(a)



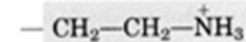
*sn*-Glycerol-3-phosphate

(b)

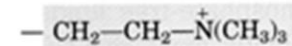


Glycerophospholipid

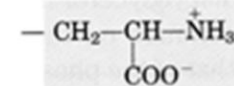
Ethanolamine



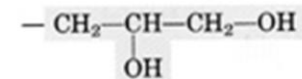
Choline



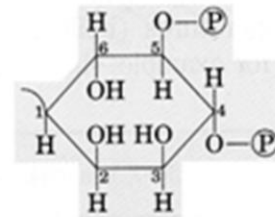
Serine



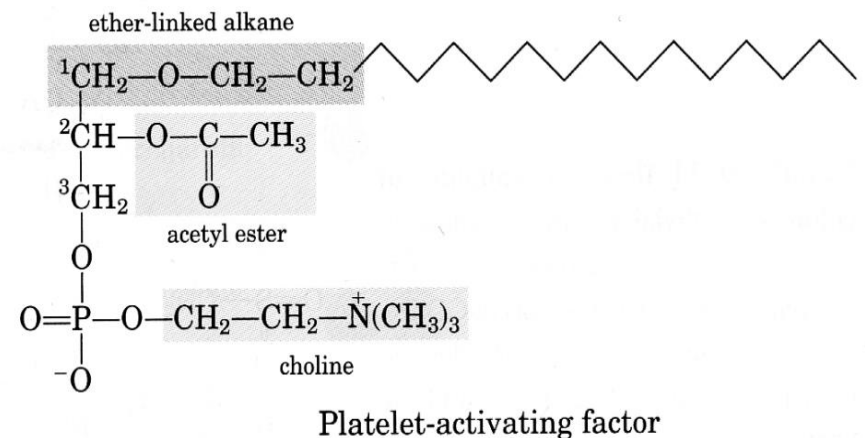
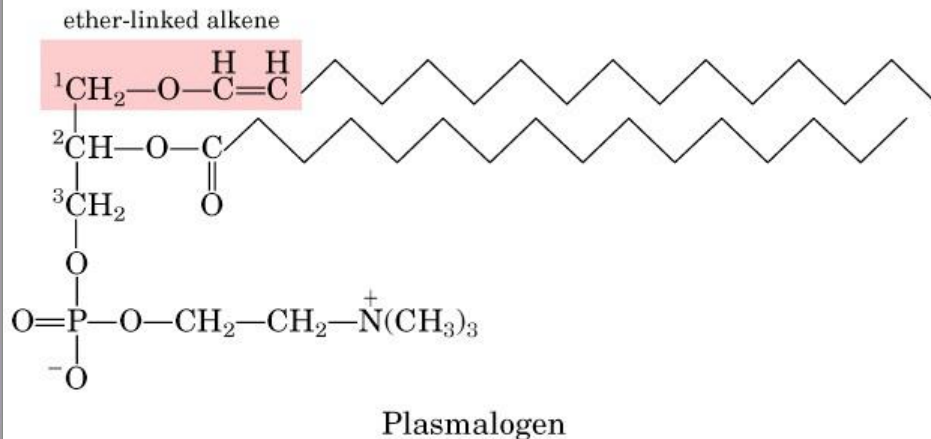
Glycerol



*myo*-Inositol 4,5-bisphosphate



- Glycerophospholipids
  - Ether linked
    - Plasmalogens: neurons and brain
      - Ether linked alkene; a,b-unsaturated ether linkage
    - Platelet activating factors
      - Ether linked alkane at sn1; acyl acetate at sn2



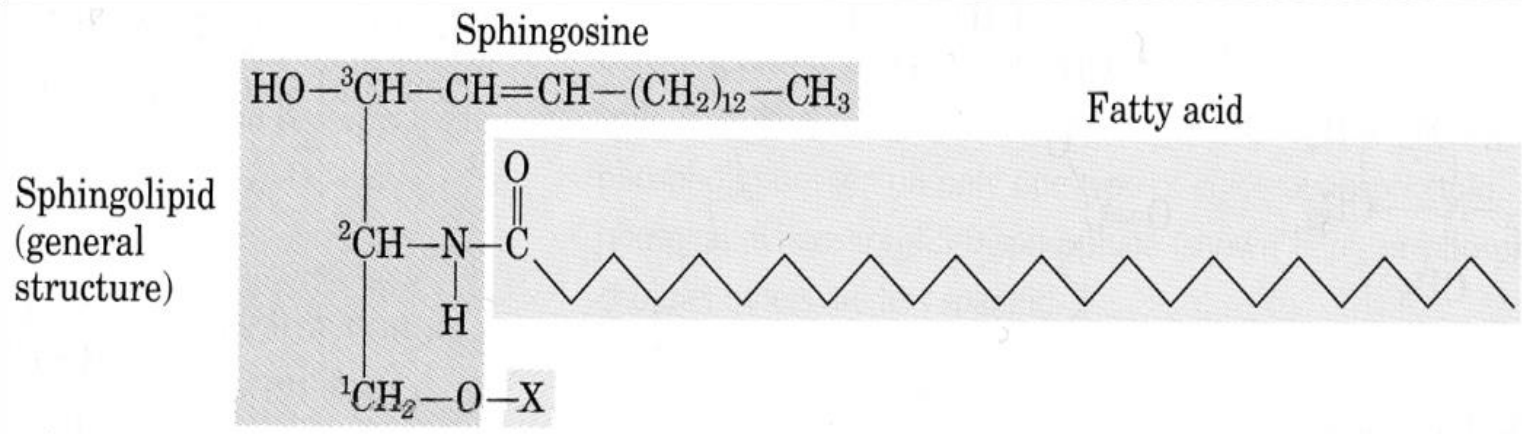


- Spingolipids

- Structure

- Spingosine - a long-chain amino alcohol
- A long chain fatty acid
- A polar head group (glycosidic/phosphodiester)

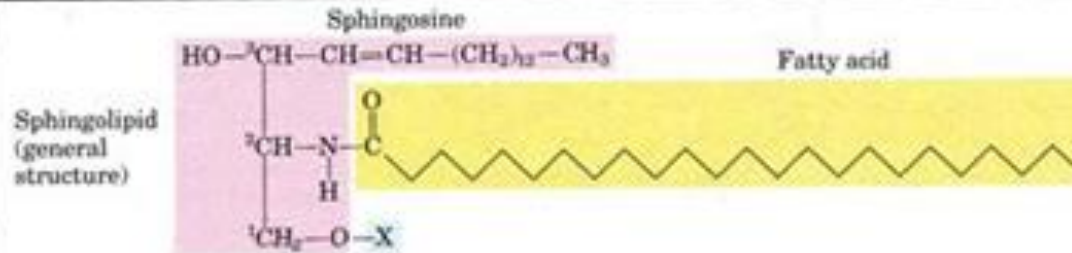
- Nomenclature: 1 is x-group

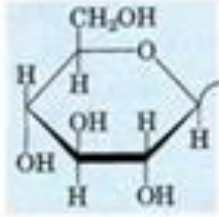






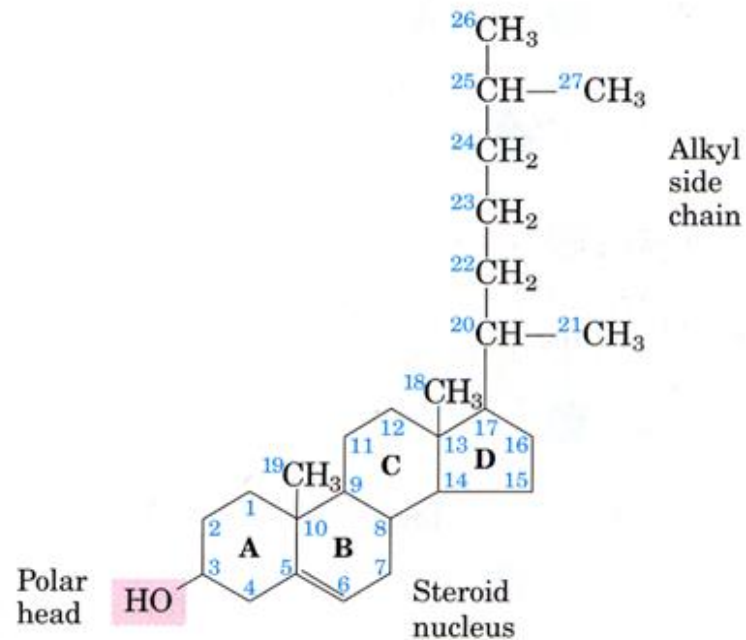
- Spingolipid classes
  - Sphingomyelins
    - X = phosphocholine or phosphoethanolamine
    - Myelin sheaths
  - Glycosphingolipids
    - X = sugar, no phosphate
    - Outer face of plasma membrane
  - Gangliosides
    - X = complex oligosaccharide with sialic acid
    - Cell-surface membranes, brain lipids

# Lipids



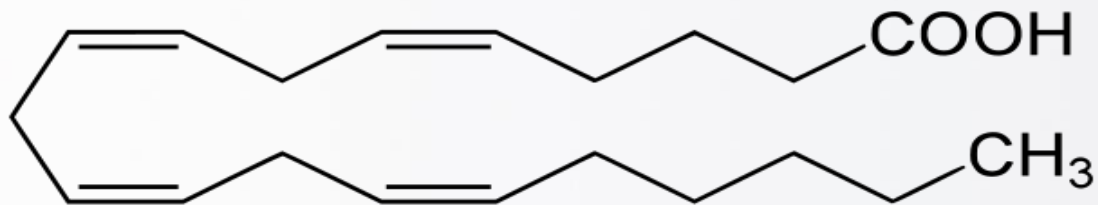
Name of sphingolipid	Name of X—O	Formula of X
Ceramide	—	—H
Sphingomyelin	Phosphocholine	$\begin{array}{c} \text{O} \\    \\ -\text{P}-\text{O}-\text{CH}_2-\text{CH}_2-\text{N}^+(\text{CH}_3)_3 \\   \\ \text{O}^- \end{array}$
Neutral glycolipids Glucosylcerebroside	Glucose	
Lactosylceramide (a globoside)	Di-, tri-, or tetrasaccharide	
Ganglioside GM2	Complex oligosaccharide	

- Sterols
  - Cholesterol
    - Stability to animal plasma membranes
    - Borders lipid rafts

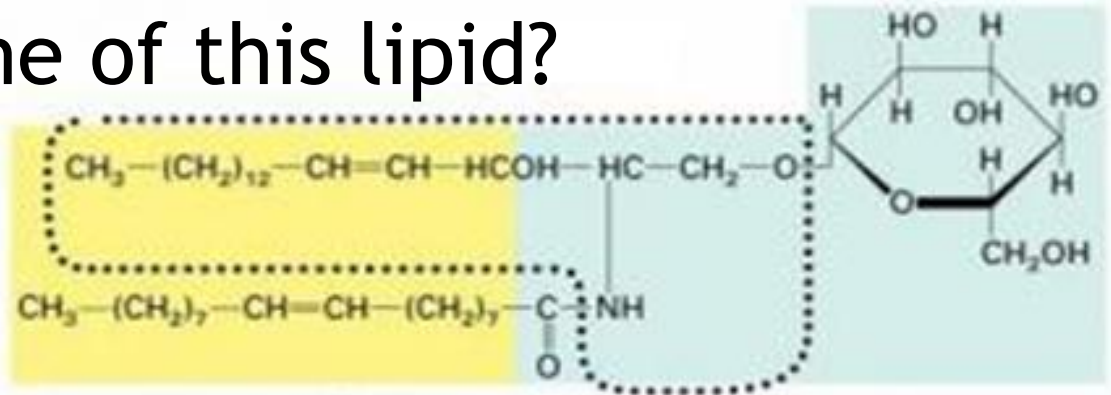


# Lipid Structure Review Questions

- Give the symbol, common name, and systemic name of this lipid.



- Draw the two major ether-linked glycerophospholipids.
- What is the name of this lipid?





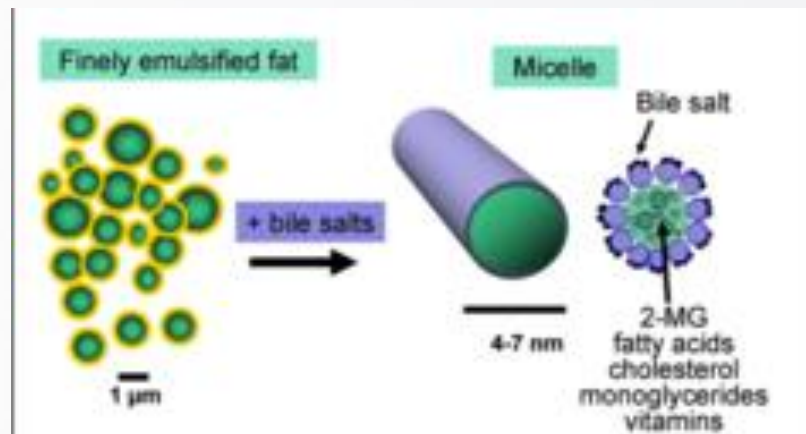
- Digestion
  1. Fat globules to Micelles
  2. Triacylglycerols to fatty acids
  3. Intestinal Mucosa absorbs fatty acids
  4. Chylomicrons packs fatty acids
  5. Transport via lymphatic and circulatory
  6. Triacylglycerols to Fatty Acids
  7. Fatty acids enter Cells
  8. Fatty acids are Oxidized



- Digestion

- Fat globules (insoluble fat) to Micelles

- Fat digestion takes place at lipid-water interfaces
    - Emulsion - bile salts (taurocholic acid)
      - Synthesized: cholesterol, stored: gall bladder, released into: small intestine
    - Churning - peristaltic movement

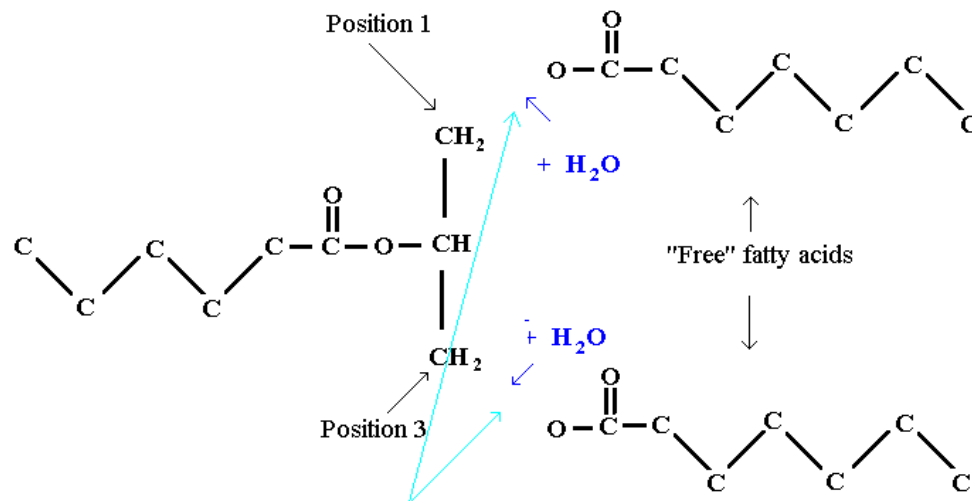


- Digestion

- Triacylglycerols to fatty acids

- Pancreatic lipase (triacylglycerol lipase) catalyzes hydrolysis of triacylglycerols at position 1 and 3

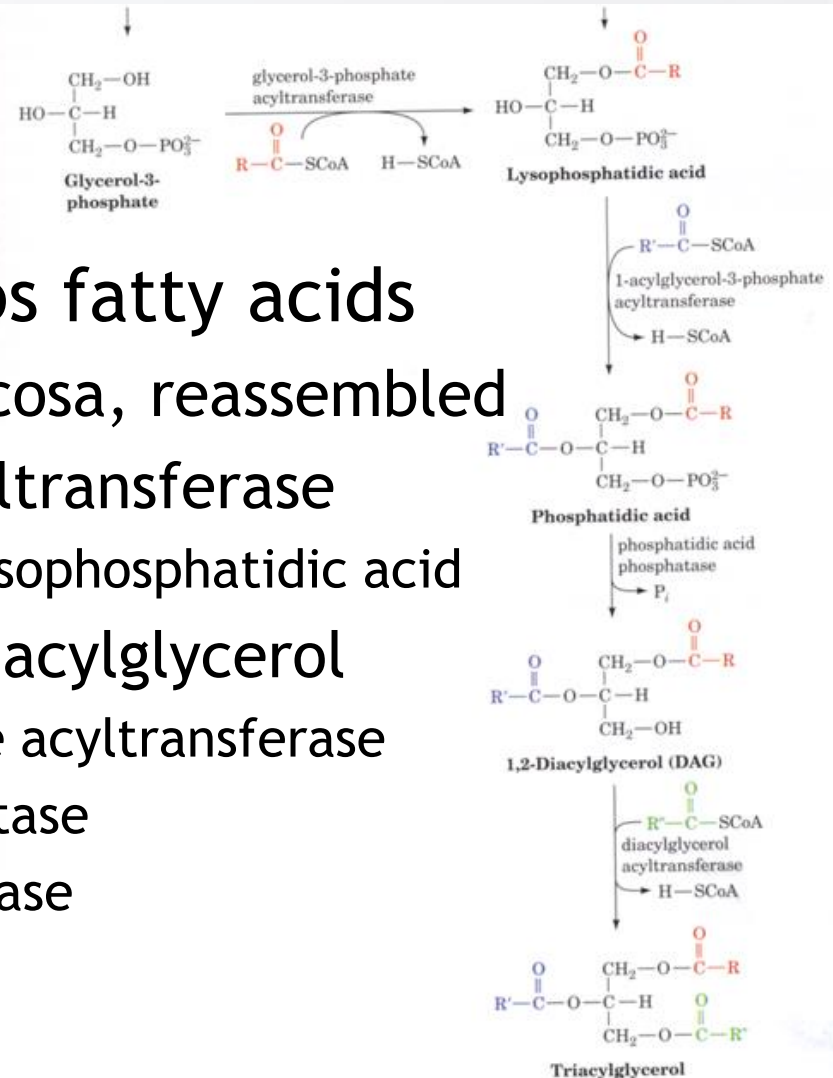
- Forms 1,2-diacylglycerol and 2-acylglycerols



Pancreatic lipase is an enzyme that breaks the bonds between glycerol and the fatty acids at positions 1 and 3, liberating the 2 fatty acids.

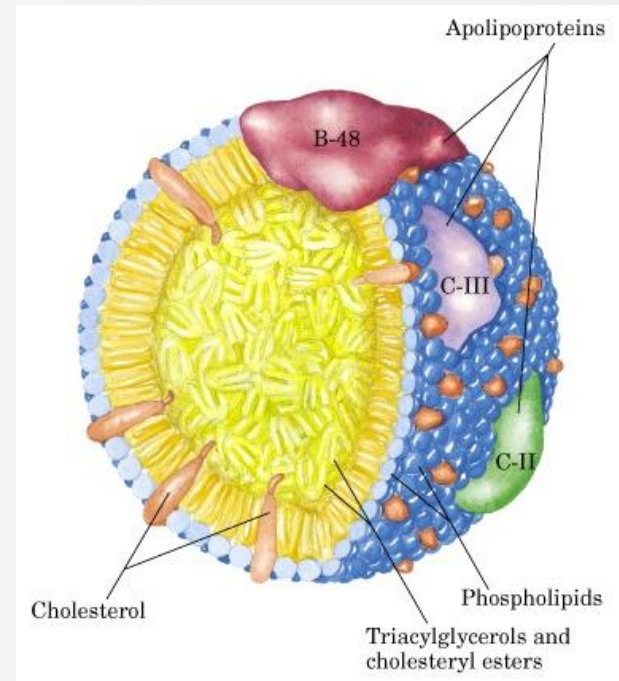
## • Digestion

- Intestinal mucosa absorbs fatty acids
  - Broken down to cross mucosa, reassembled
  - Glycerol-3-phosphate acyltransferase
    - Glycerol-3-phosphate to lysophosphatidic acid
  - Lysophosphatidic acid to triacylglycerol
    - 1-acylglycerol-3-phosphate acyltransferase
    - Phosphatidic acid phosphatase
    - Diacylglycerol acyltransferase



- Digestion

- Chylomicrons pack fatty acids
  - Triacylglycerols, cholesterol, apolipoproteins are incorporated into water-soluble chylomicrons
    - Surface = phospholipids
    - Interior = triacylglycerols (87%)
- Transport via lymphatic and circulatory systems

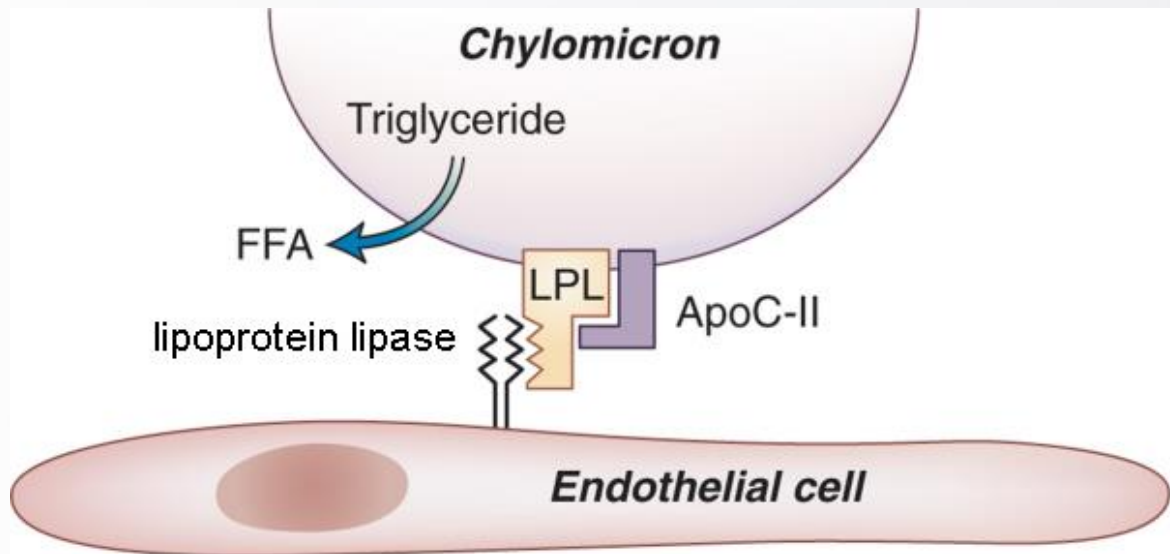




- **Digestion**

- **Triacylglycerols to Fatty Acids**

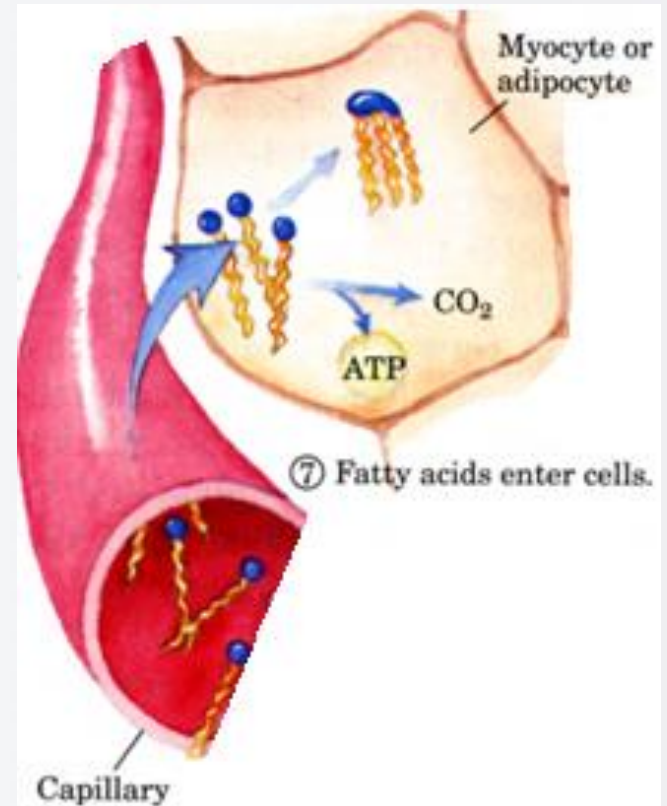
- Lipoprotein lipase - capillary endothelial cell
    - Activated by apoC-II on chylomicron
    - Converts triacylglycerols to fatty acids and glycerol



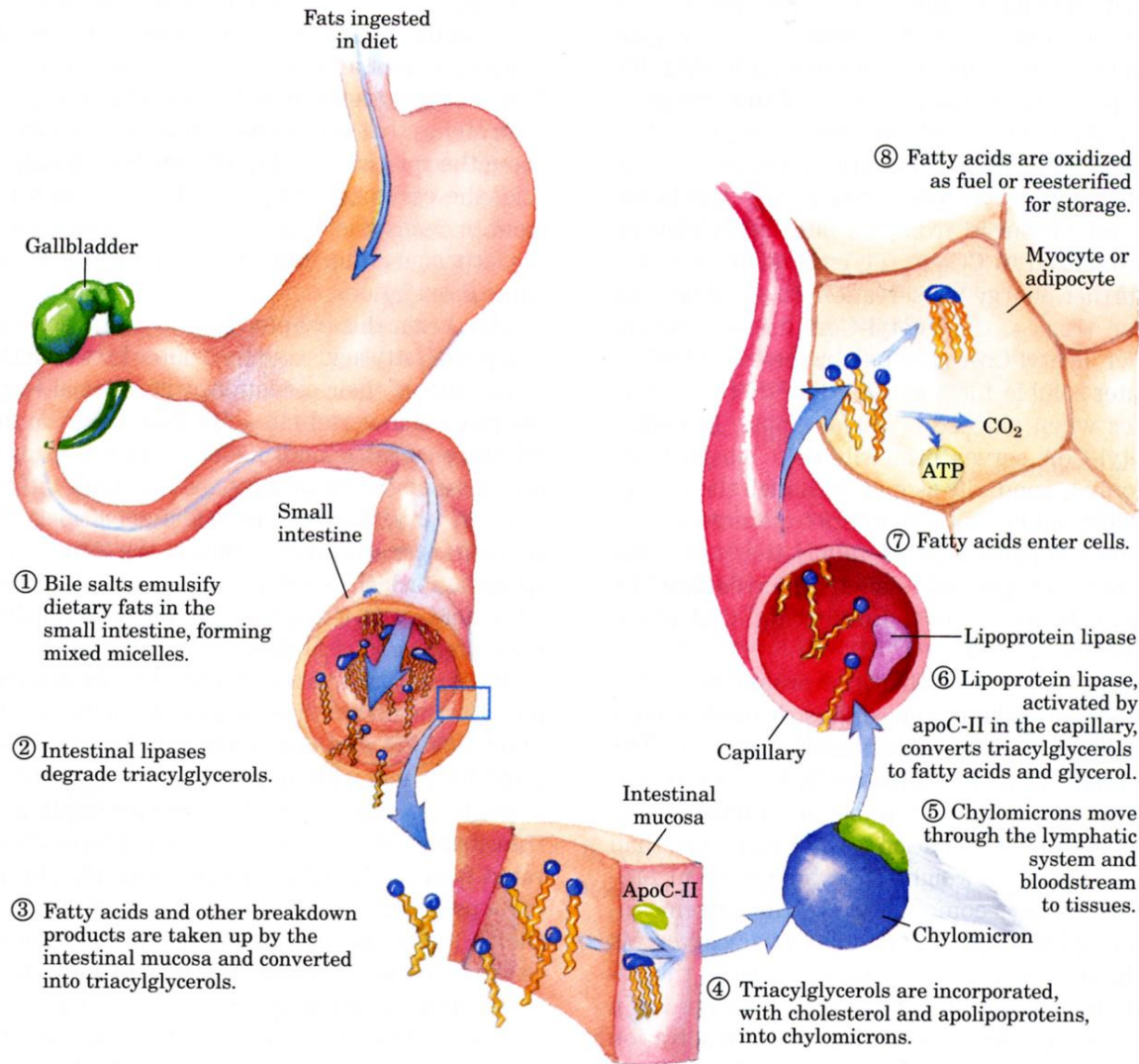


- Digestion

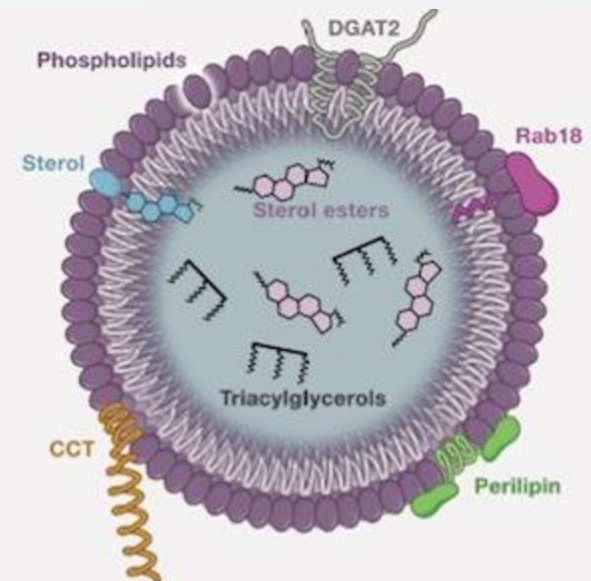
- Fatty acids enter Cells
  - Lipid soluble and small
  - Can diffuse through capillary membrane into cell
- Fatty acids are Oxidized or Stored
  - Adipocyte - lipid storage
  - Myocyte - lipid oxidation



# Lipids



- Storage lipids
  - Stored in adipocytes as lipid droplets
    - Core: sterol esters (cholesterols)
    - Bulk: Triacylglycerols
    - Outside: single leaflet of phospholipids
  - Restricted enzyme access

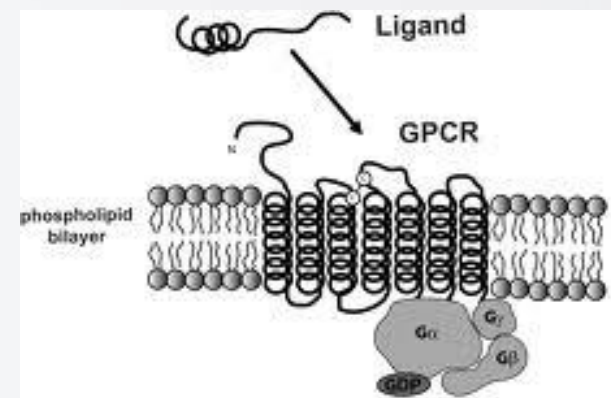




- Storage Lipids Mobilization
  1. Epinephrine and glucagon secreted
  2. E and G bind to GPCR
  3. Gs-protein activated
  4. PKA phosphorylates lipase
  5. Hydrolysis of lipids
  6. Fatty acids released into bloodstream
  7. Fatty acid uptake by tissue

- Storage Lipid Mobilization

- Epinephrine and glucagon secreted
  - Adrenal medulla releases E and G in response to low blood glucose
- Epinephrine and glucagon bind to GPCR
  - Found on surface of adipocytes
  - 7-transmembrane spanning protein
  - GPCR bound to Gs protein

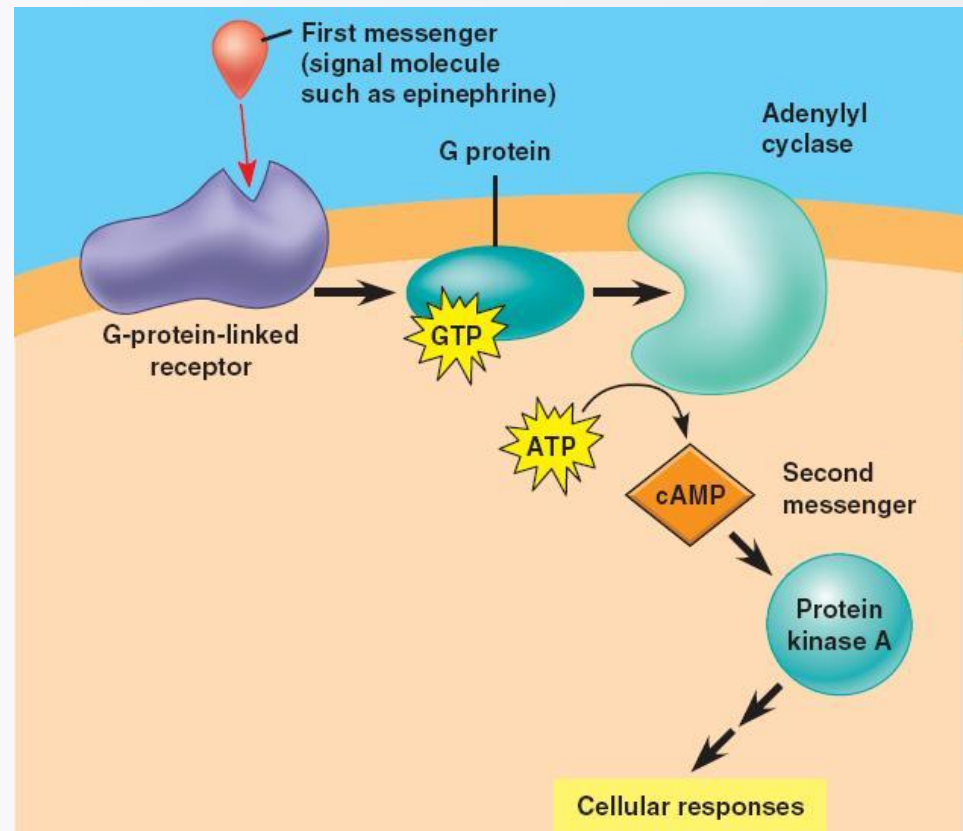




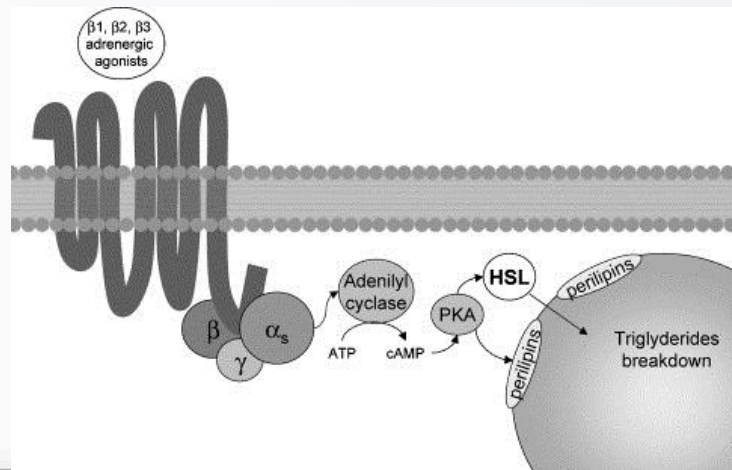
- Storage Lipid Mobilization

- Gs-protein activated

- GPCR
    - Gs-protein
    - Adenylyl cyclase
    - cAMP
    - PKA

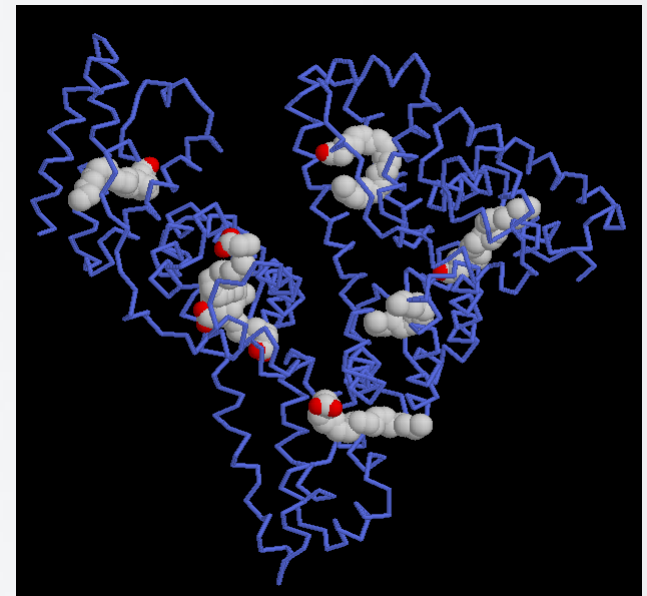
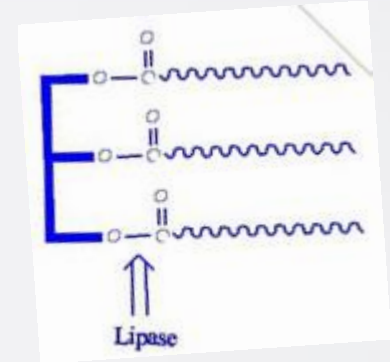


- Storage Lipid Mobilization
  - PKA phosphorylates targets
    - Phosphorylates Peripilins
      - Protein responsible for protecting lipid droplet
      - Allows phosphorylated hormone-sensitive lipase into droplet
    - Phosphorylates Hormone Sensitive Lipase - active
      - Hydrolyses triacylglycerides to fatty acids and glycerol

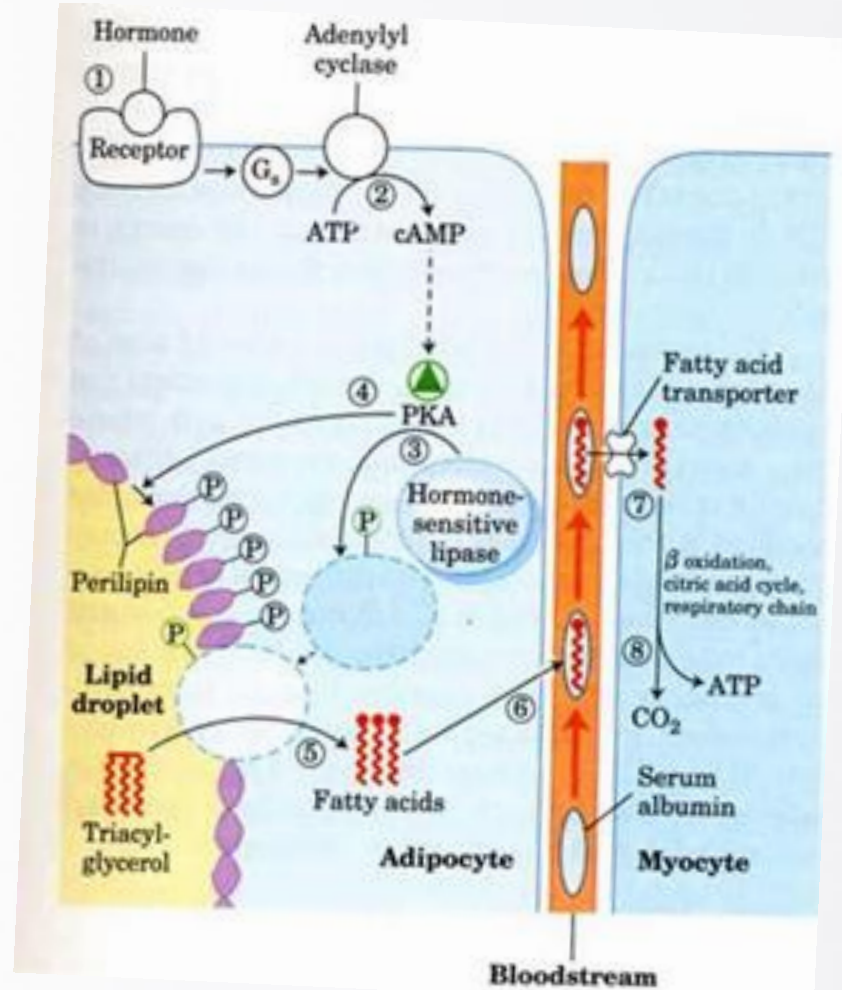


- Storage Lipid Mobilization

- Hydrolysis of lipids
  - Fatty acids released into cytosol
- Fatty acids released into bloodstream
  - Bind to protein serum albumin
- Fatty acid uptake by tissue



- Storage Lipid Mobilization



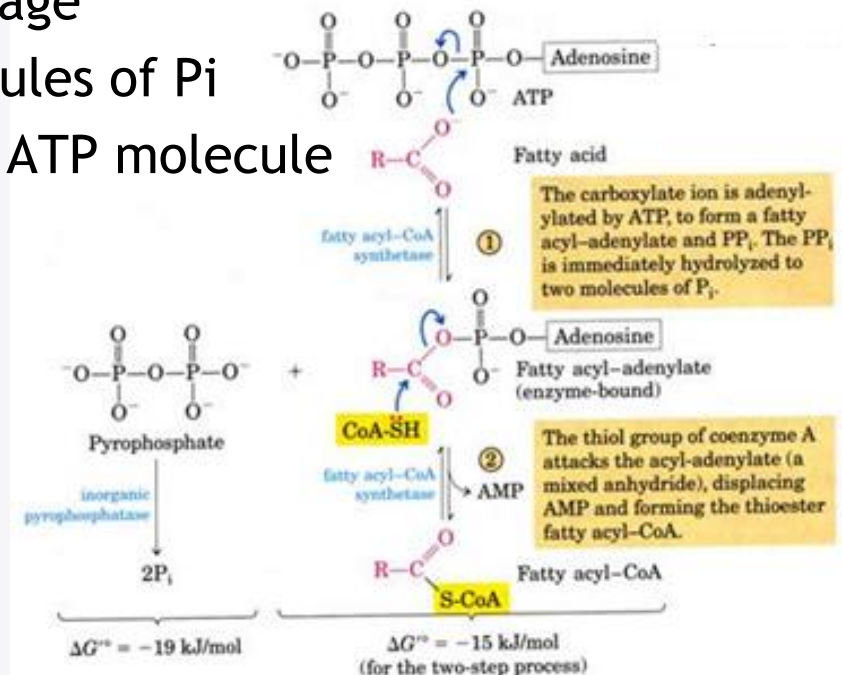


## • Fatty Acid -> Mitochondria

### - Priming

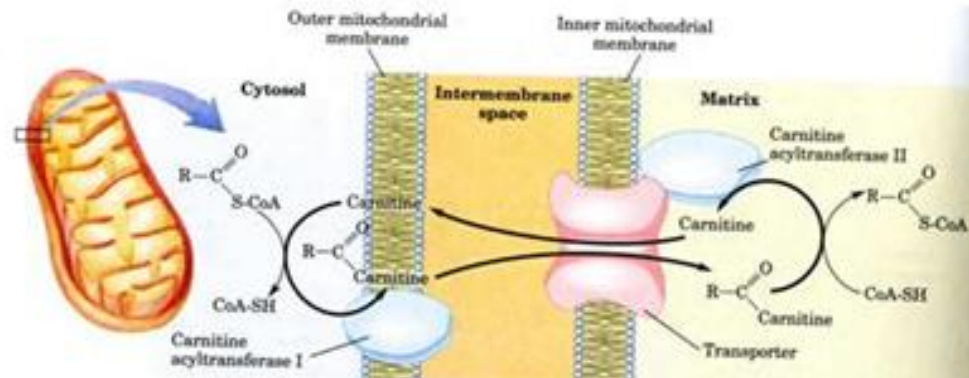
#### • Acylation - fatty acyl-CoA

- Thiokinases - Thioester linkage
- PPi hydrolyzed to two molecules of Pi
  - » 2 equivalents of ATP, 1 ATP molecule

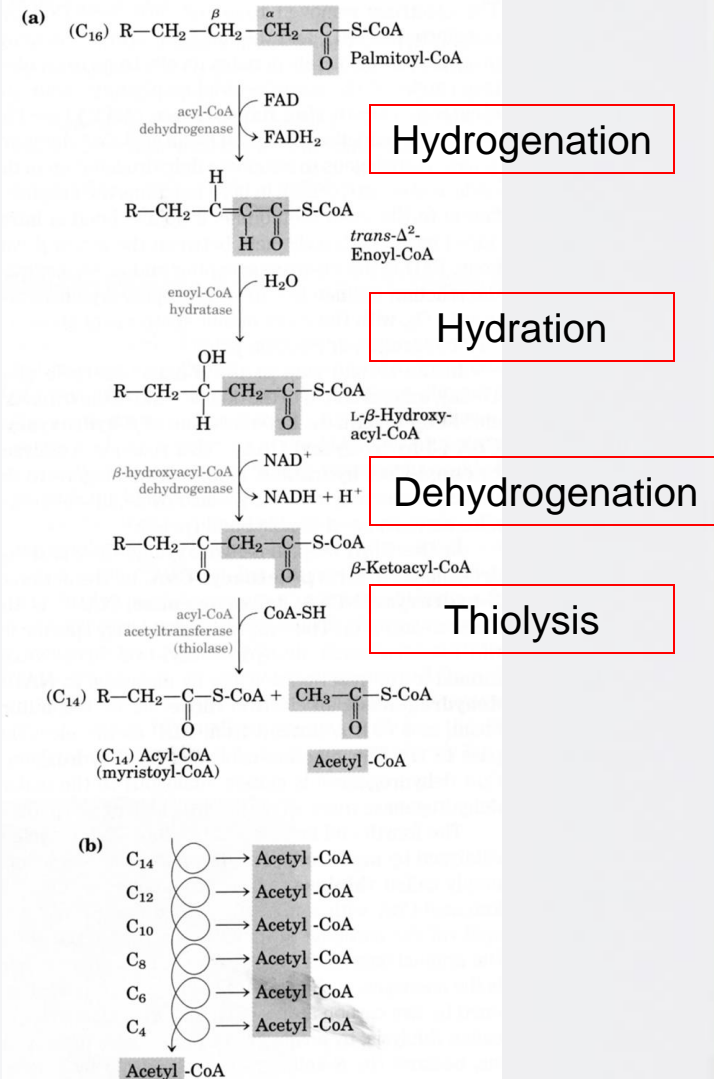




- Fatty Acid -> Mitochondria
  - Transport = Carnitine shuttle
    - Acyl group of cytosolic acyl-CoA transferred to carnitine, releasing CoA
    - Acyl-carnitine -> mitochondrial matrix
    - Acyl group transferred to mitochondrial CoA
    - Carnitine returned to cytosol



- Fatty Acid Oxidation
  - Beta oxidation
    - Hydrogenation
    - Hydration
    - Dehydrogenation
    - Thiolysis
  - Citric acid cycle
  - Electron transport chain



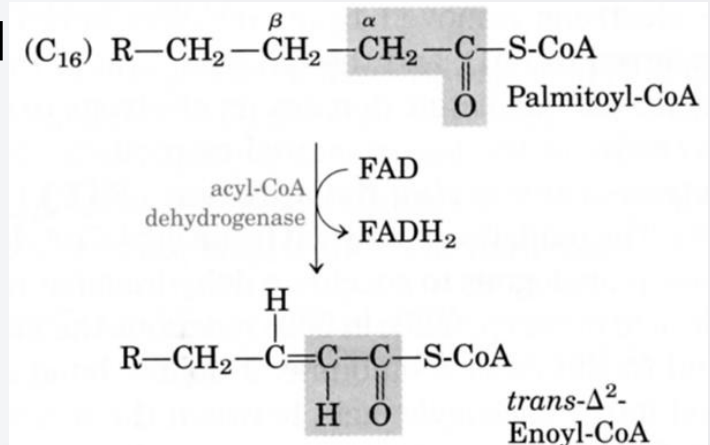
- Fatty Acid Oxidation

- Beta oxidation

- Starts from carboxyl end of fatty acyl chain
- N carbons  $\rightarrow$   $N/2 - 1$  cycles  $\rightarrow$   $N/2$  acetyl-CoA

1. Dehydrogenation

- Different enzyme used for each length of fatty acid chain
- Flavoenzyme acyl-CoA dehydrogenase
- Transient trans- $\alpha,\beta$  double bond
- Generates 1  $\text{FADH}_2$  per cycle

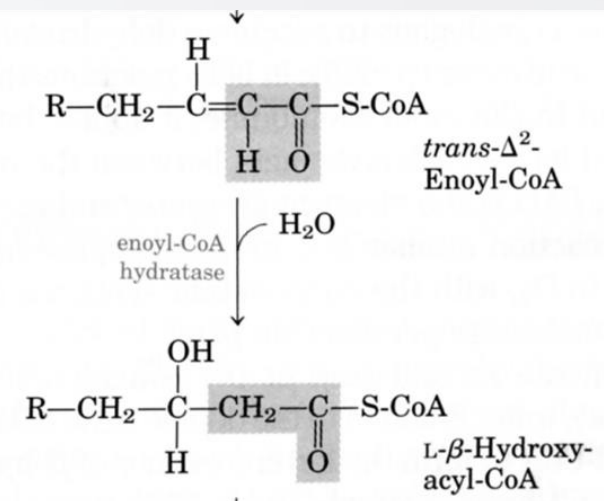


- Fatty Acid Oxidation

- Beta oxidation

- 2. Hydration

- Enoyl-CoA hydratase (EH) hydrates double bond
      - Forms 3-L-hydroxyacyl-CoA



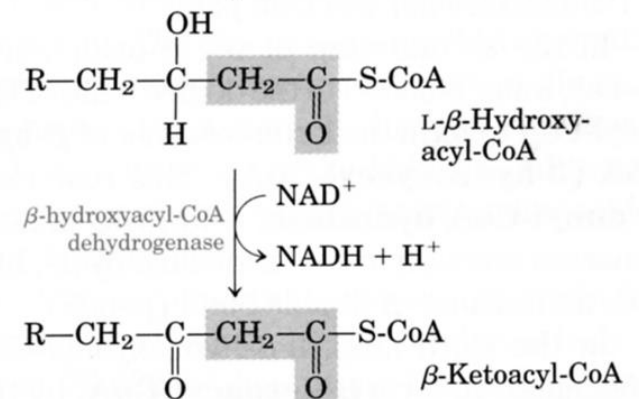


- Fatty Acid Oxidation

- Beta oxidation

- 3. Dehydrogenation

- 3-L-hydroxyacyl-CoA dehydrogenase (HAD)
      - NAD<sup>+</sup> dehydrogenation of β-hydroxyacyl
      - Forms corresponding β-ketoacyl-CoA
      - NAD<sup>+</sup> is electron acceptor, NADH goes to ETC

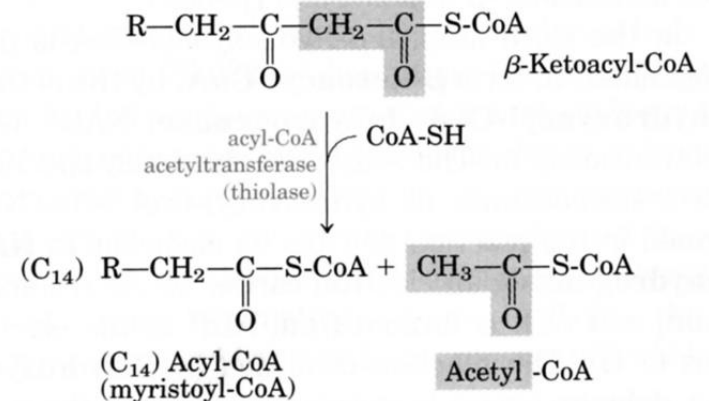


- Fatty Acid Oxidation

- Beta oxidation

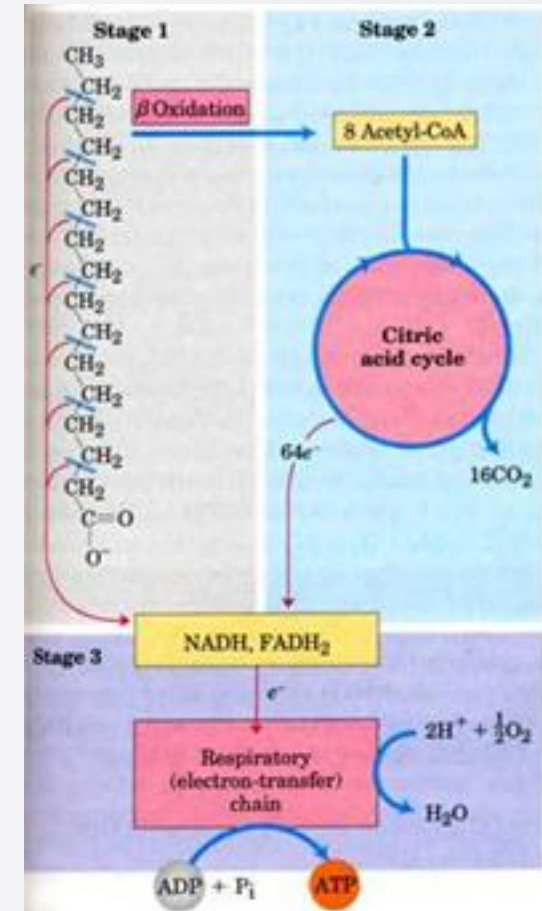
- 4. Thiolysis

- C $\alpha$ -C $\beta$  cleavage of  $\beta$ -Ketoacyl-CoA
    - Forms acetyl-CoA and new acyl-CoA with two less C



- Fatty Acid Oxidation

- Beta oxidation
  - Acetyl-CoA is converted to  $\text{CO}_2$
- Citric acid cycle
  - Electrons ( $\text{NADH}$ ,  $\text{FADH}_2$ ) pass to  $\text{O}_2$
  - Oxidative phosphorylation - ATP



- Fatty Acid Oxidation
  - Calculating ATP
    - Beta oxidation yields:
      - $(N/2)$  molecules acetyl-CoA
      - $(N/2 - 1)$  NADH
      - $(N/2 - 1)$   $\text{FADH}_2$
      - Loss of 2 equivalents of ATP
    - Citric acid cycle of  $N/2$  acetyl-CoA
      - $(N/2) \times 3$  NADH
      - $(N/2)$   $\text{FADH}_2$
      - $(N/2)$  GTP - ATP equivalents
    - Electron Transport Chain
      - NADH generates 3 ATP
      - $\text{FADH}_2$  generates 2 ATP





# Lipid Metabolism Review Questions

- Describe the major steps of lipid digestion
- Compare structure and function of a chylomicron and a lipid droplet
- What is the lipid mobilization signaling pathway (agonist, receptors, secondary messengers, and targets)?
- What are the 4 major steps in beta oxidation?
- What is the net ATP yield of stearic acid?