Water

- High Boiling Point
- High Specific Heat (Heat Capacity)
- Very polar – "universal solvent"
- Density solid < Density of Liquid
- Each H_2O can make 4 H-bonds
- Permanent Dipole (b/c of shape and bond angles)

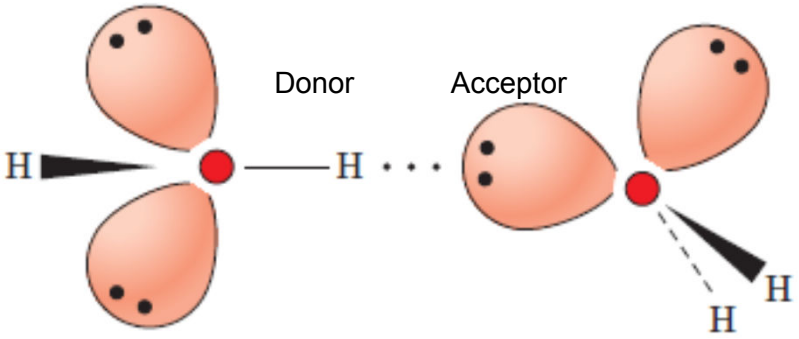


Figure 2-2 | A hydrogen bond in water.

H-Bonding

- Angle (linear = strongest)
- Distance (between donor and acceptor)
- Partial Charges on Participants
- Dielectric Constant (**E**)

(a measure of a solvent's ability to shield charges)

$$F = \frac{kq_1q_2}{\mathbf{E}r^2}$$

Polar: **E**>15

Apolar: **E**<15

Bonds Strengths: Ionic > H-bond > Dipole/Dipole > London Dispersion

Acid/Base Chemistry

Weak Acid Dissociation:

$HA \leftrightarrow H^+ + A^-$

$HB \leftrightarrow H^+ + B^-$

Dissociation Constant $\mathbf{K_a} = \frac{[H^+][A^-]}{[HA]}$

larger K_a = stronger weak acid
smaller pKa = stronger weak acid

Henderson-Hasselbach Equation:

$$pH = pK + \log\frac{[A^-]}{[HA]}$$

$pH < pK_a$ $[A^-] < [HA]$
$pH = pK_a$ $[A^-] = [HA]$
$pH > pK_a$ $[A^-] > [HA]$

Can rearrange this eqn to solve for the ratio of deprotonated to protonated:

$pH - pKa = \log[A^-]/[HA]$

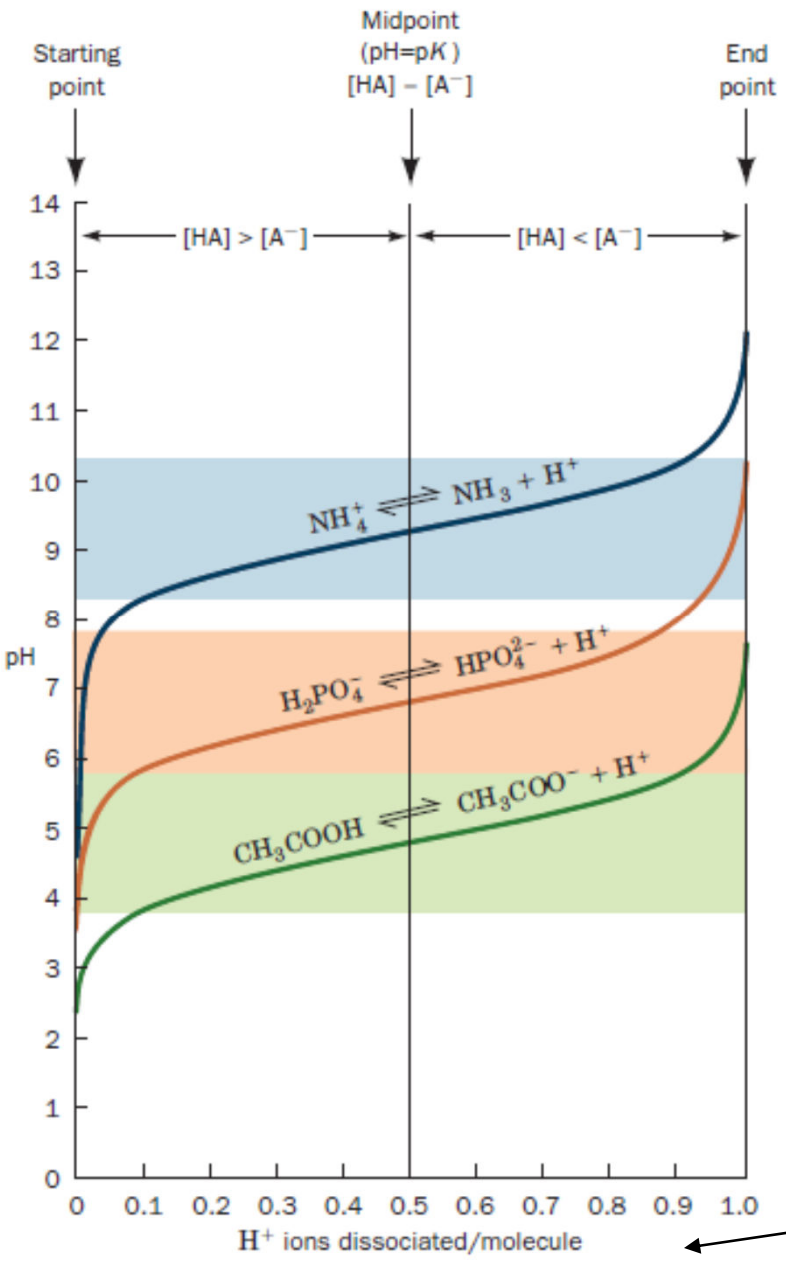
$pH - pKa = 10\text{^}[A-]/[HA]$

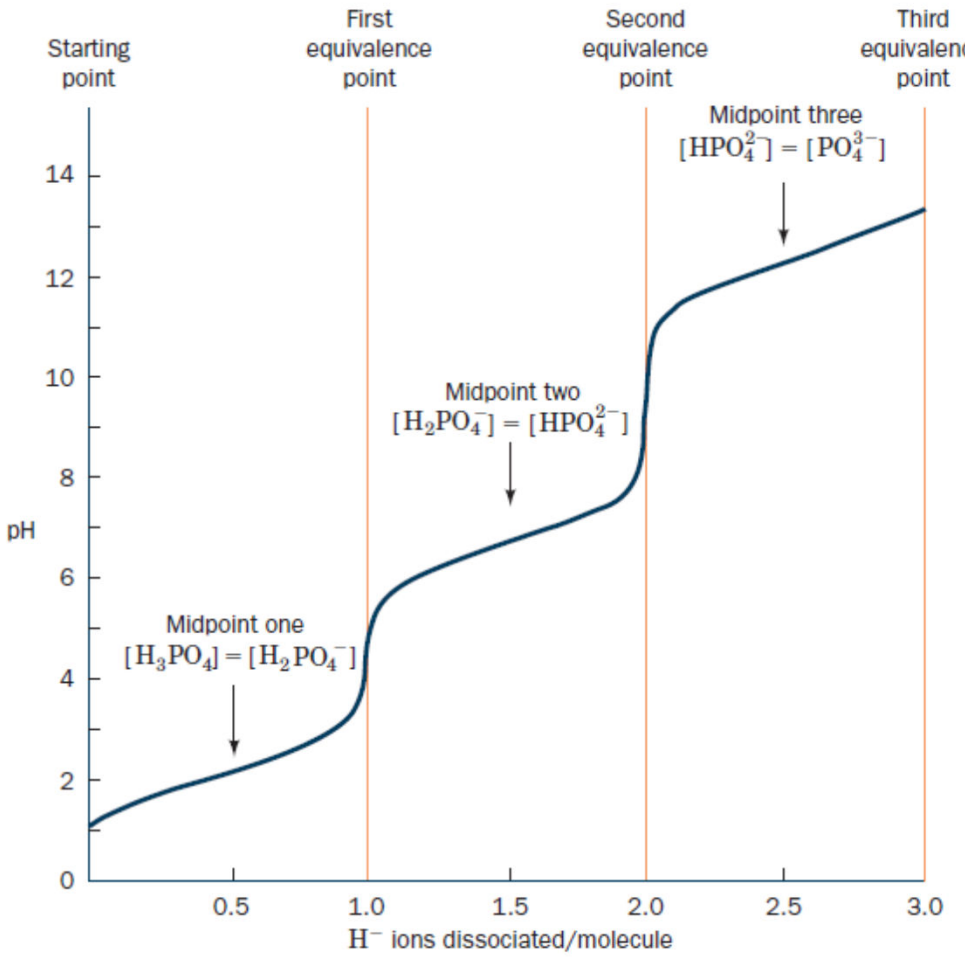
Buffering (80%) occurs +/- 1 pH unit from pKa

Best Buffering is at pKa, because here $[A^-] = [HA]$

Buffering capacity is the ability of a buffer to resist changes in pH, is dependent on the concentration of buffer and pH of solution

pH = pKa at half equivalence point

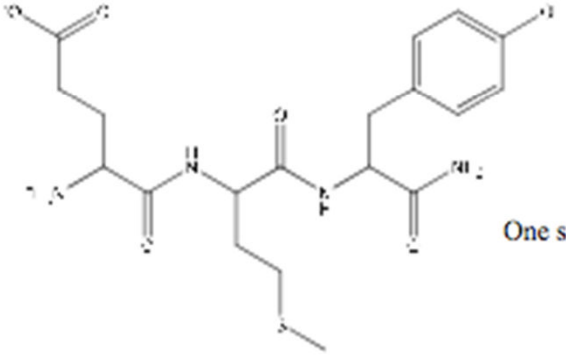




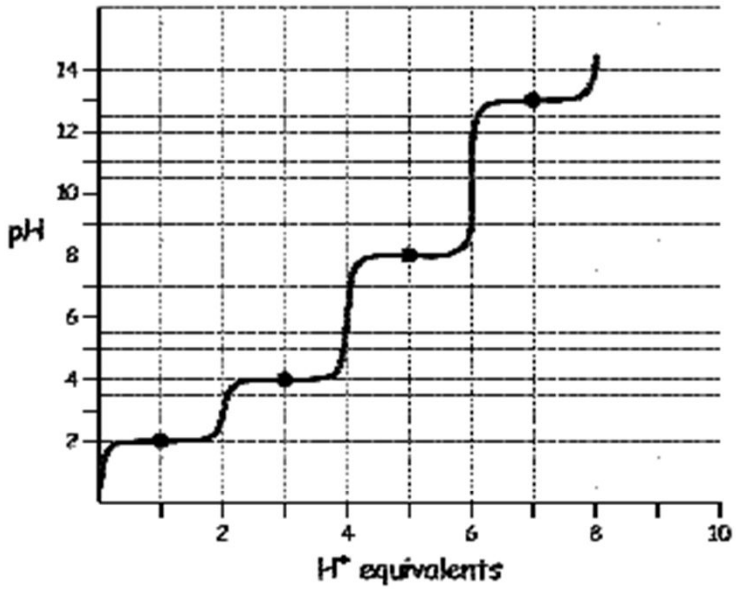
Can also be OH^- equivalents

(need 1 mole of OH- equivalents per ionizable group)

24. (17 pts) After taking this class, you decide you love biochemistry so much that you want to study it more in graduate school. So you end up being a TA for a class like 153A. On the first exam you grade a titration curve problem. The question asks the students to draw a pH titration curve for the following peptide, and to label the axes of the curve.



One student gives this drawing:

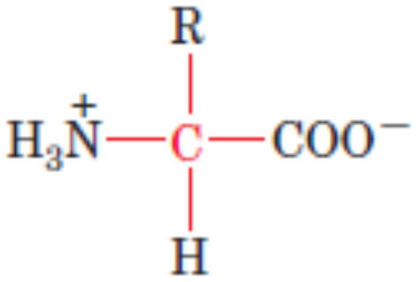


a. What errors in the drawing would cause you to deduct points? List each error. (1 error or type of error per line; you may not need all of the lines.) If there are no errors, list 'none.'

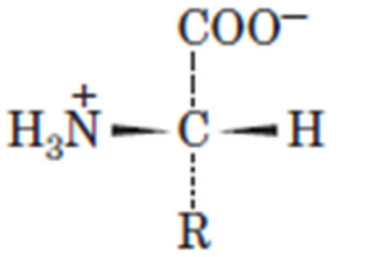
b. The exam also asks the students to calculate the net (average) charge of this peptide at pH 7. What is the correct answer? (Show your work.)

Amino Acids

- Usually found as a **zwitterion**



- L-stereochemistry
  Amino group on left
  Carbon 1 (carboxy) on top



L-α-Amino acid

Amino Acid pKa's

- Carboxyl groups pK_a ~ 2.0
- NH_3 (N-termini) pK_a ~ 9.5

Inductive Effect

- e^- withdrawing effect
- Lowers pKa

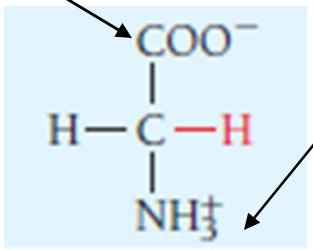
Electrostatic Effect

- Charge effect
  - Molecules prefer a net neutral charge
- Can raise and lower pKa's

pKa is 2.35

Lower than pKa in acetic acid (CH_3-COOH) because N is withdrawing electrons

Glycine

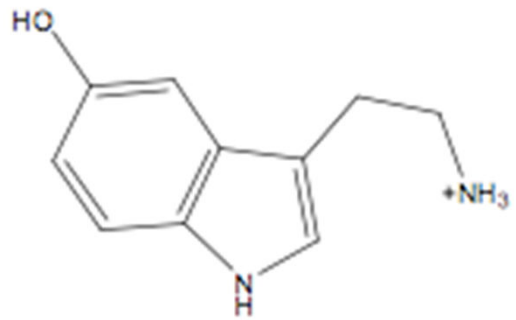


pKa is 9.78.

Higher than NH_3 in ethylamine (CH_3-CH_2-NH_3^+) because COO- is withdrawing electrons

Higher than NH_3 in O-methyl glycine (NH_3-CH_2-C-OCH_3) to increase range ⇓ O that glycine has net 0 charge

12. (10 points) Serotonin is a broad-activity neurotransmitter, well-known for its ability to improve one's mood. Serotonin is synthesized from tryptophan. The structure of serotonin at physiological pH is shown to the right.



Consider how the structural differences between serotonin and tryptophan would likely affect the pKa of the amino group.

a. List any structural differences between serotonin and tryptophan that affect the amino group's pKa.

b. *How* do these differences affect the pKa? Separate out the individual effects or factors caused by the difference(s) is part **a**, choose the most significant, and complete the provided sentences. (Fill in each individual factor, selecting from the list below; fill in the causative structural difference; circle whether each factor raises or lowers the pKa of serotonin's or tryptophan's amino group. You may not need to complete both of the provided sentences.)

hydrophobic effect	steric hindrance
inductive effect	electrostatic effect
hydrogen bonding	

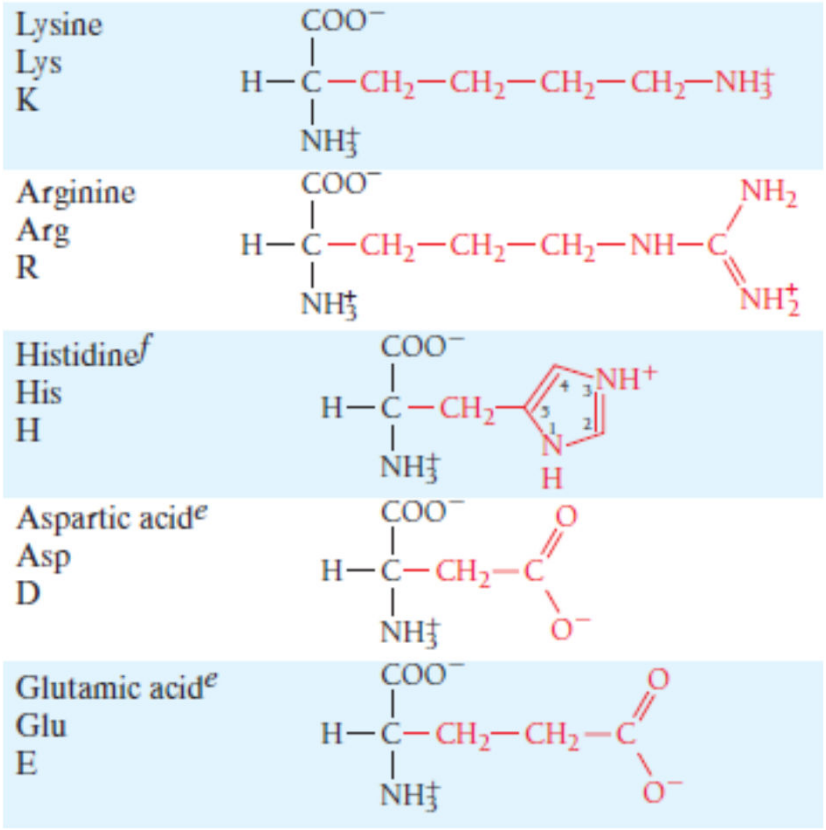
c. The net result of these effects would likely be a(n) _____ pKa for the serotonin amino group, as compared to that of tryptophan.

dramatically lower	somewhat higher
somewhat lower	dramatically higher
unchanged	

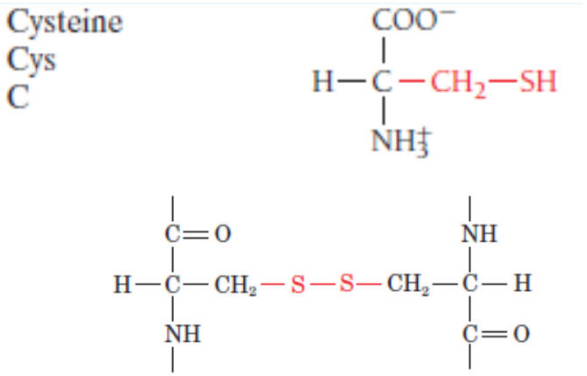
Amino Acids

Can form an Ion Pair at pH 7:

Asp, Glu, Arg, Lys, His (sometimes)



Can Disulfide Bond at pH 7:



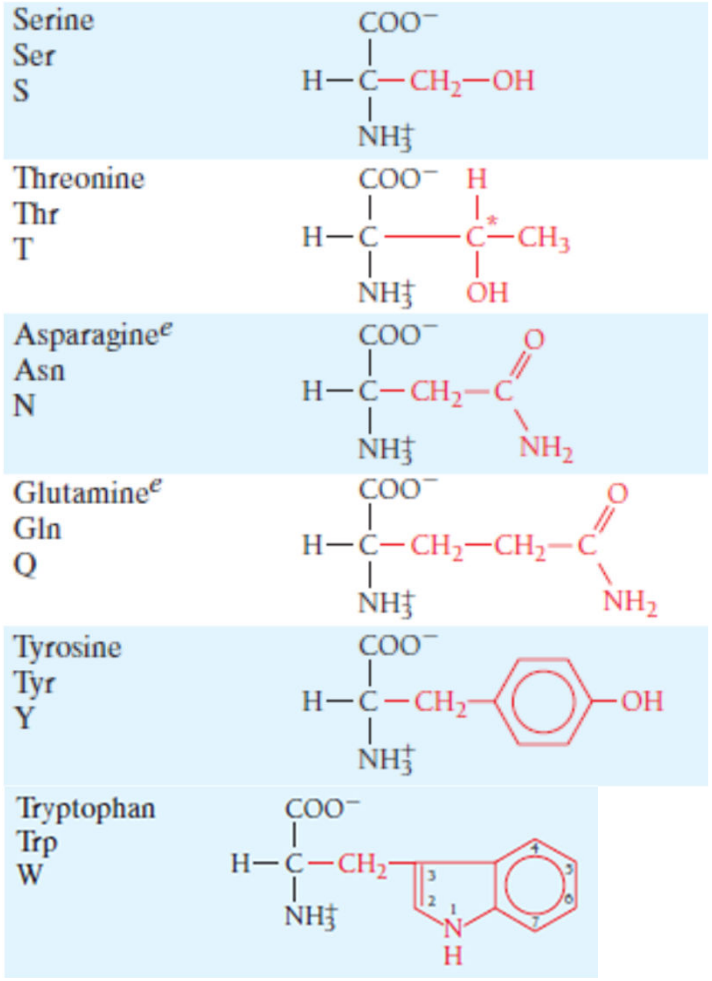
Can participate in Van der Waals contacts at pH 7:

ALL 20!

Amino Acids

Can H-bond at pH 7:

Asp, Glu, Arg, Lys, Ser, Thr, Asn, Gln, Tyr, His, Trp



Can Ionize (gain or lose a proton):

Charged (Arg, Lys, Asp, Glu, His)

Alcohols (Ser, Thr, Tyr)

Cys

*only side chains with groups that can gain or lose protons can ionize.

Note that amino acids with NH_2 groups (Asn, Gln) are NOT IONIZABLE!

Isoelectric Point (pI)

- Net charge on protein/aa is 0

$$pI = \tfrac{1}{2}(pK_i + pK_j)$$

To solve these problems, make a table with pH ranges that are the pKas. Then figure out the charge on each ionizable group at the given pH. One of these pH ranges will sum to 0. These are the two pKas to plug into the pI equation.

pH	NH_3^+ (pKa 8)	Arg (pKa 12.5)	Tyr OH (pK 10)	Σ
< 8	+1	+1	0	+2
8-10	0	+1	0	+1
10-12.5	0	+1	-1	0
>12.5	0	0	-1	-1

pI = (10+12.5)/2 = 11.25

Henderson-Hasselbach Eqn can be used to determine net charge

$$10^{pH-pKa} = [A^-]/[HA]$$

Example: A protein has three ionizable groups (NH_3 at N-termini, Arg, Tyr)

To find the net charge at pH 7,

$[NH_2]/[NH_3^+] = 10^{(7-8)} = 10^{-1} = 1/10$ 10 out of 11 have a +1 charge = 90%

$[O^-]/[OH] = 10^{(7-10)} = 10^{-3} = 1/1000$ 1 out of 1001 have a -1 charge = 0.099%

$[NH2]/[NH3+] = 10^{(7-12.5)} = 10^{-5.5}$ = 100% in NH_3^+ form

Add the percentages of each species (paying attention to the sign/charge)

+0.9 -0.0009 +1 = +1.9

We can then say that that most molecules have a charge of +2, a few are +1

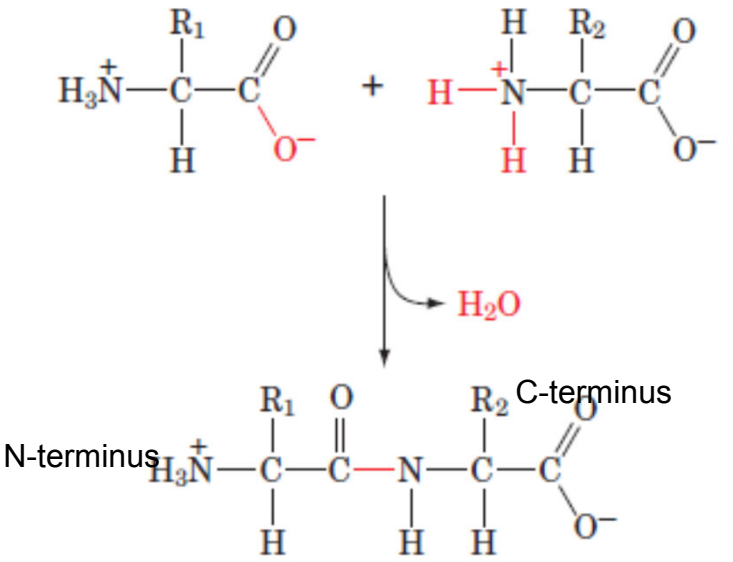
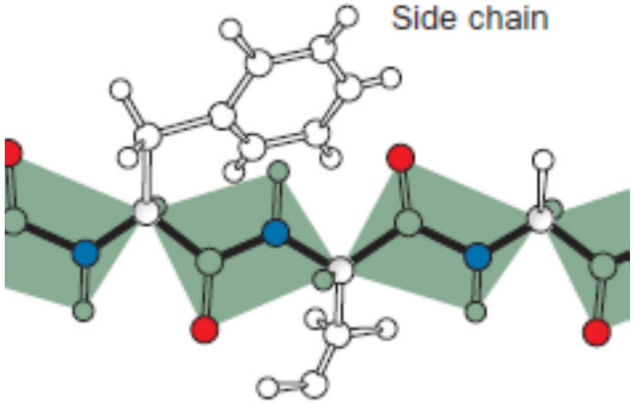


Figure 4-3 | Condensation of two amino acids.

Condensation Reaction eliminates water, forming a **peptide bond** that joins two amino acids

Peptide Bond:

- Has partial double bond character
- Is planar
- Φ (N to Cα)
  - no Φ at N-terminal
- Ψ (Cα to Carbonyl C)
  - no Ψ at C-terminal



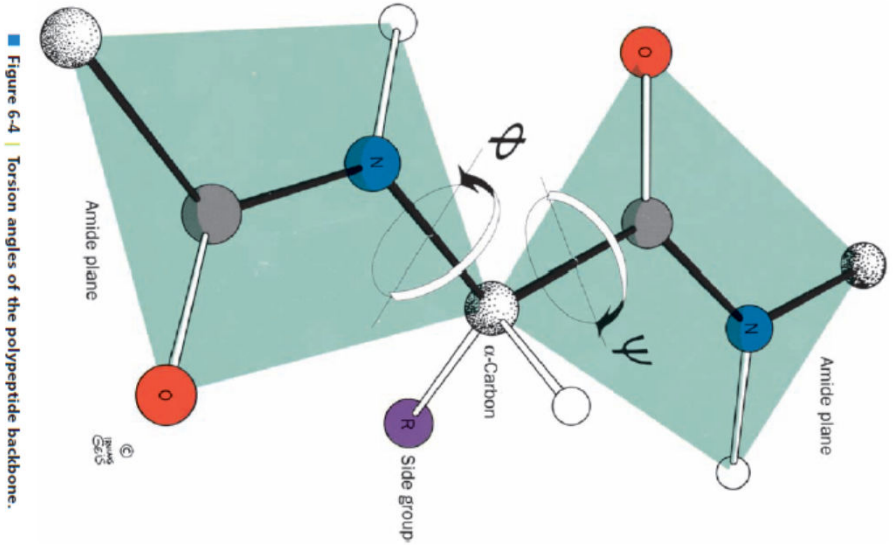
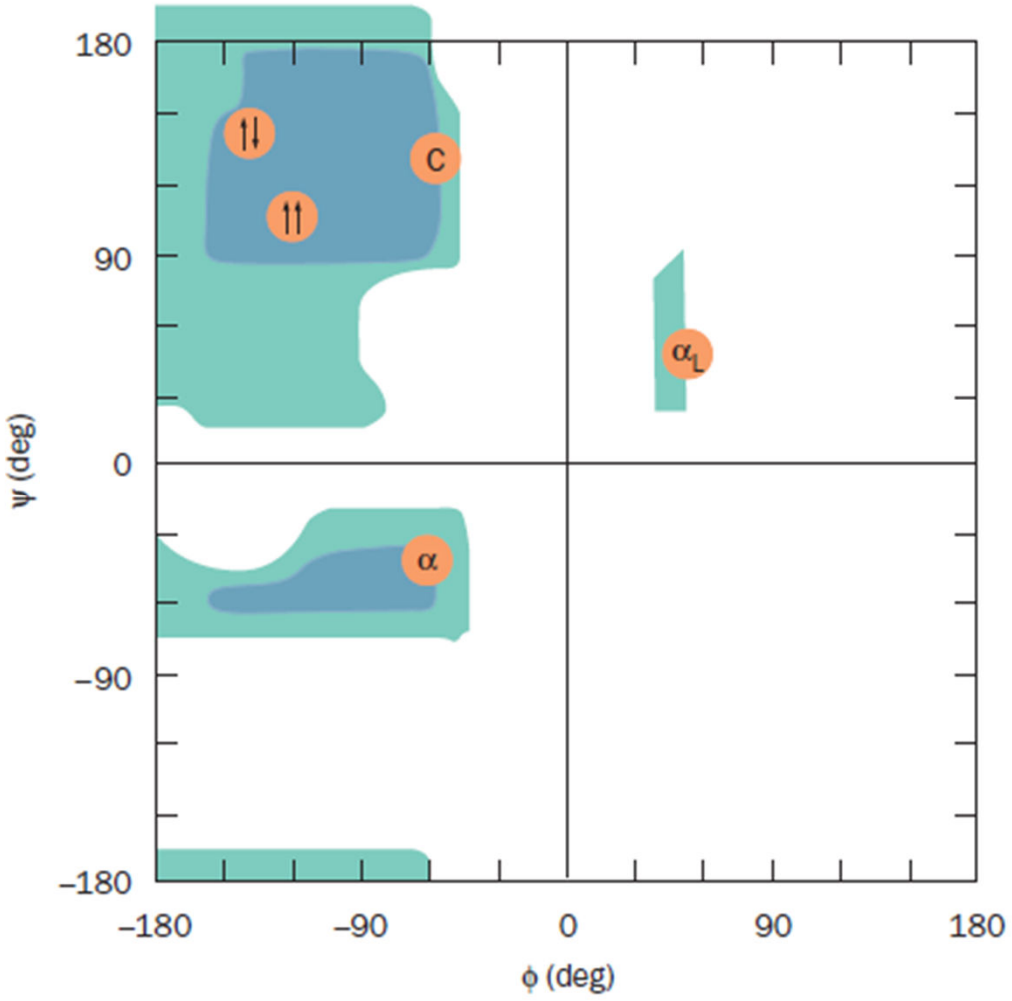


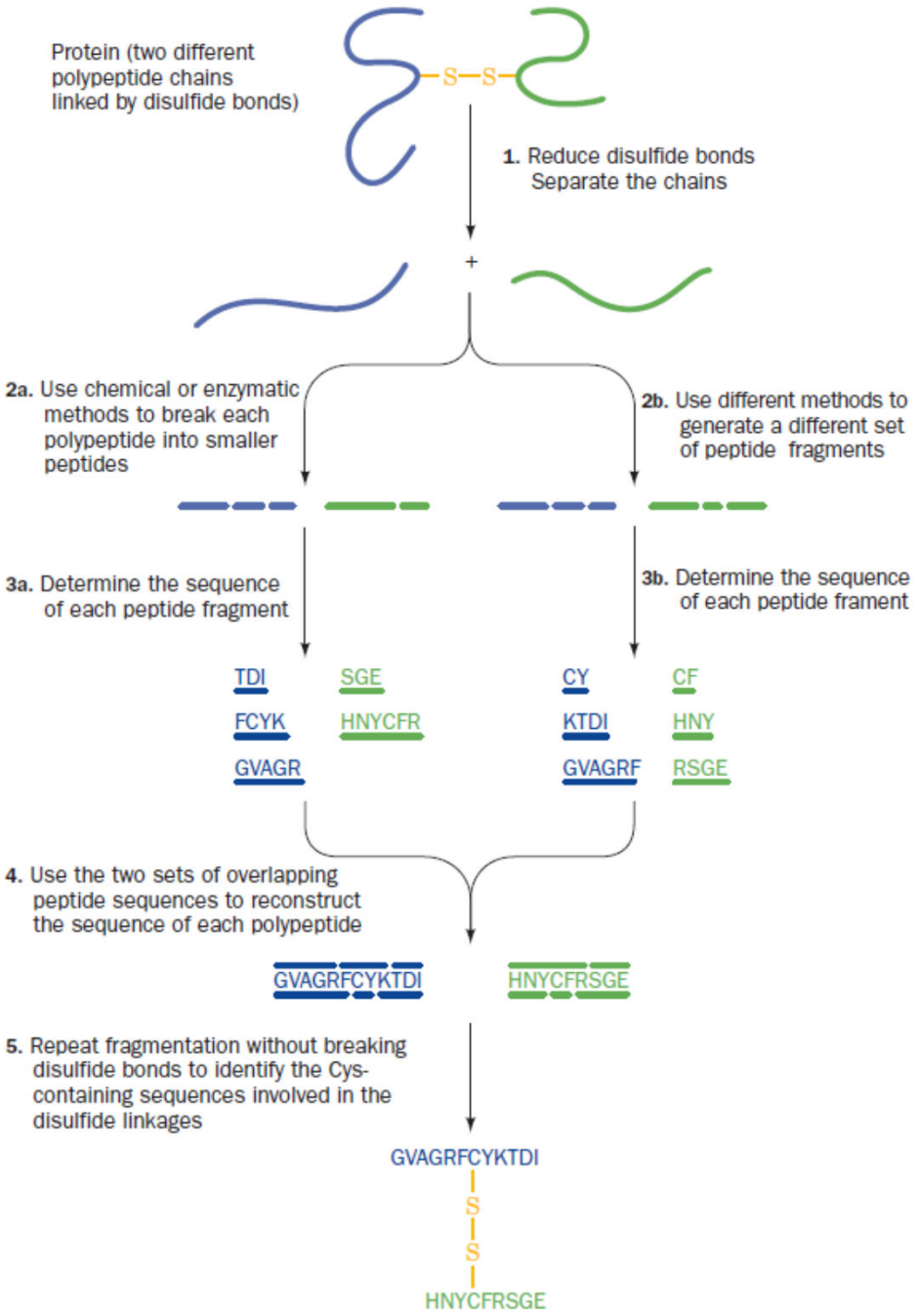
Figure 6-4 | Torsion angles of the polypeptide backbone.

Conformations of the Protein Backbone (Φ, Ψ) are limited by STERIC CLASHES



Ramachandran Plot:

plots the allowed phi/psi conformations



■ **Figure 5-12 | Overview of protein sequencing.**

1. Determine # of peptide chains present
   - Count # N-termini
   - DNFB or dansyl chloride react w/ N-terminus
   - hydrolyze all peptide bonds (acid treatment)
   - isolate and ID N-terminal aminos
   - Problem: reaction at Lys or other 1° amines
2. Separate Chains
   may need to reduce disulfides/ block with IAA
3. Fragment polypeptides
   - Enzymatically (endopeptidase) or chemically (CNBr) – these specifically cleave
4. Sequence Fragments
   1. Edman degradation
      - Edman's reagent adds to N-terminal under basic conditions, switch to acidic conditions and cut off N-terminal residue, ID this residue, repeat
   2. Mass spec
5. Reconstruct sequence
   - this required fragmenting in different places to get overlapping segments

Multiple sequence alignments

Sequence Identity = fraction of positions that are the **same** amino acids

Sequence similarity = fraction of positions with the **same or similar** amino acids
(conservative substitutions)

Homologs

- **Orthologs** = proteins of same function but in different organisms

- **Paralogs** = related sequences of slightly different function (same organism)
thought to arise by gene duplication

Conserved and similar positions are probably important for structure/function

Rate of protein mutation is related to the ability of the protein to accommodate the mutation

Alpha helices

Rise = 5.4Å (per repeat)
3.6 amino acids per repeat
1.5Å rise per amino acid

H-bonding in backbone stabilizes structure
C=O of i H-bonds to i+4

Small electric dipole
N-termini has free amide groups (+)
C-termini has free carbonyls (-)

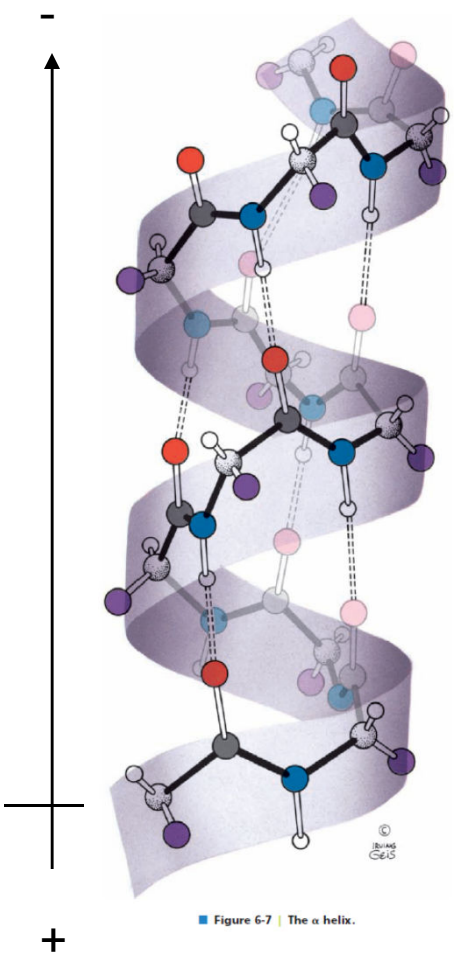
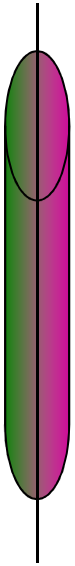


Figure 6-7 | The α helix.



Amphipathic helix = half hydrophobic, half hydrophilic
Helical wheel projections can show this

5 factors influencing helix stability

1. Intrinsic propensity of amino acids (Ala likes to be in helices)
2. Interactions between R-groups (ionic interactions)
3. Bulkiness of adjacent R groups (Phe, Trp)
4. Occurrence of Pro/Gly (destabilize helices)
   - Pro is not very flexible and causes helix kinks,
     Pro cannot H-bond because its N is missing an H
   - Gly is **very** flexible)
5. Interactions with ends of helix and R groups
   - (Arg at C-terminal ends)

Beta-sheet/strand

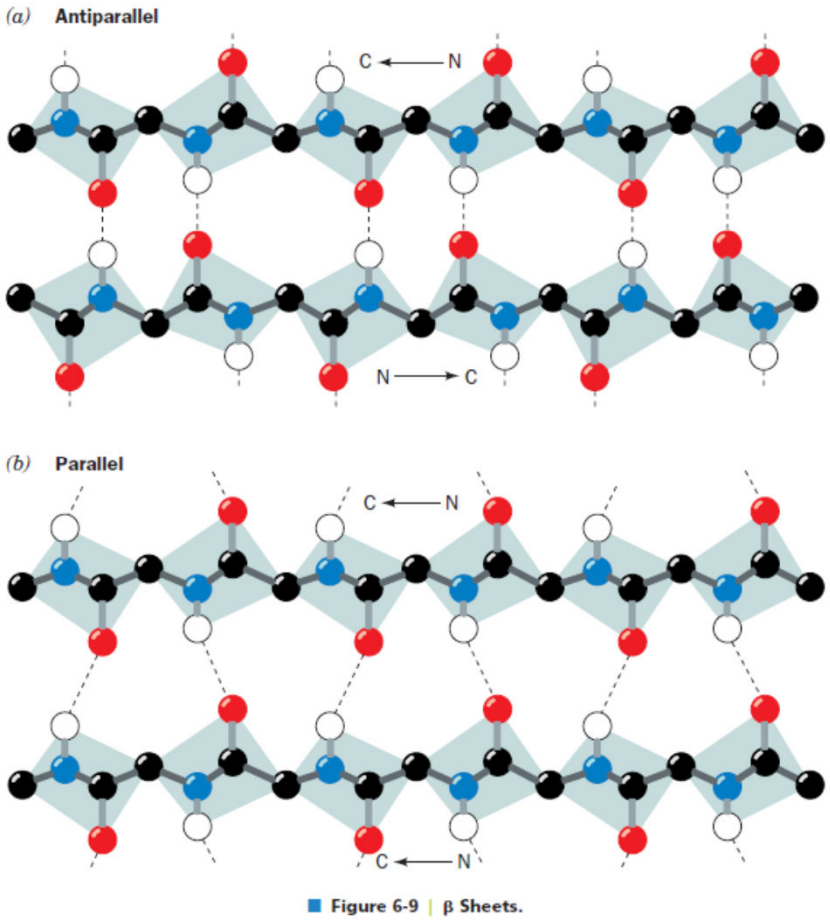


Figure 6-9 | β Sheets.

Antiparallel

- 7Å rise
- 2 amino acids per repeat
- H-bonds are linear

Parallel

- 6.5Å rise
- 2 amino acids per repeat
- H-bonds are slanted

Do not see fully extended (phi = 180^{o}, psi = 180^{o}) because then R groups will start to interfere with protein backbone

Sheets are in non-continuous regions

Beta-turns

- 4 amino acids, Pro/Gly common
- H-bond b/t C=0 of amino acid 1 and NH of amino acid 4

Stabilizing Interactions in Proteins

1º covalent peptide bond

2º: H-bonding (backbone N-H· · · · O)

Electrostatic Ion Pairs

Steric compatibility

Van der Waals

Hydrophobic Effect

3º: 1+2 and disulfide bonds

4º: same as 3º

Hydrophobic Effect

-Maximizing the entropy of water

-Water is ordered around nonpolar substances. It forms a shell, motion is restricted and entropy is lower

-Proteins have a hydrophobic core and a more hydrophilic surface.

-This drives protein folding because the protein becomes more ordered but the water becomes **less** ordered

Carbohydrates $(CH_2O)_n$

Fisher Projections

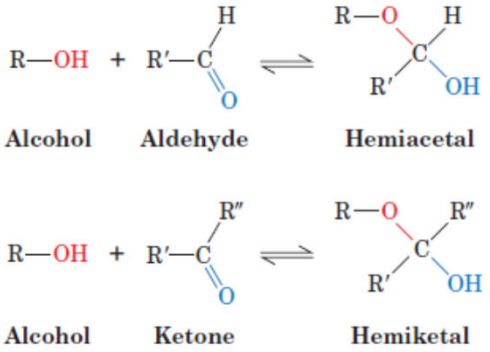
- If the OH on the last chiral carbon is on the right, sugar is D
- If OH on the last chiral carbon is on the left, sugar is L

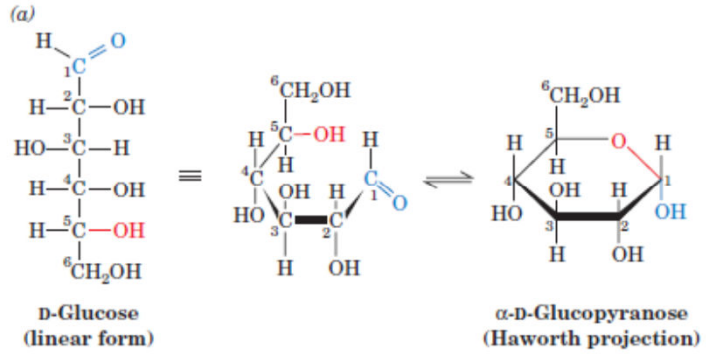
Steroisomers

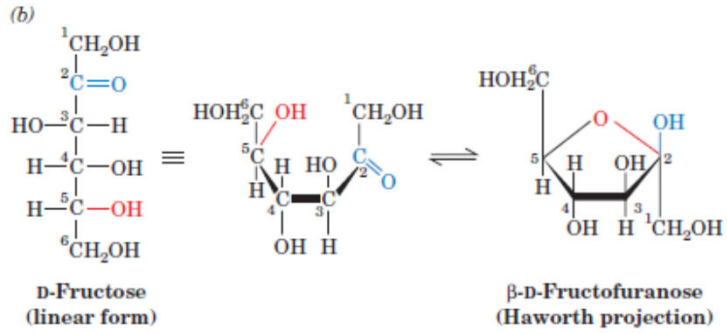
- Number of conf's possible = 2^n (n=# chiral centers)
- Epimer = sugars that differ at 1 stereocenter
  - Glucose and Galactose are epimers at C4

Sugars cyclize

- Anomeric carbon
  - Has 2 bonds to oxygen
- Alpha anomer = OH on **opposite** side of ring as C6
- Beta anomer = OH on same side of ring as C6



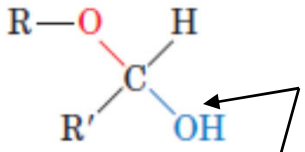




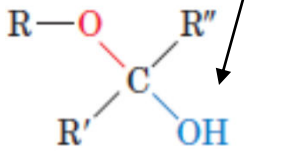
Reducing Sugars:

•Can reduce Cu^{++} to Cu^{+}, sugar gets oxidized

•Requires the sugar to be linear so that carbonyl is accessible (but remember that cyclic sugars can open up and then be reducing)



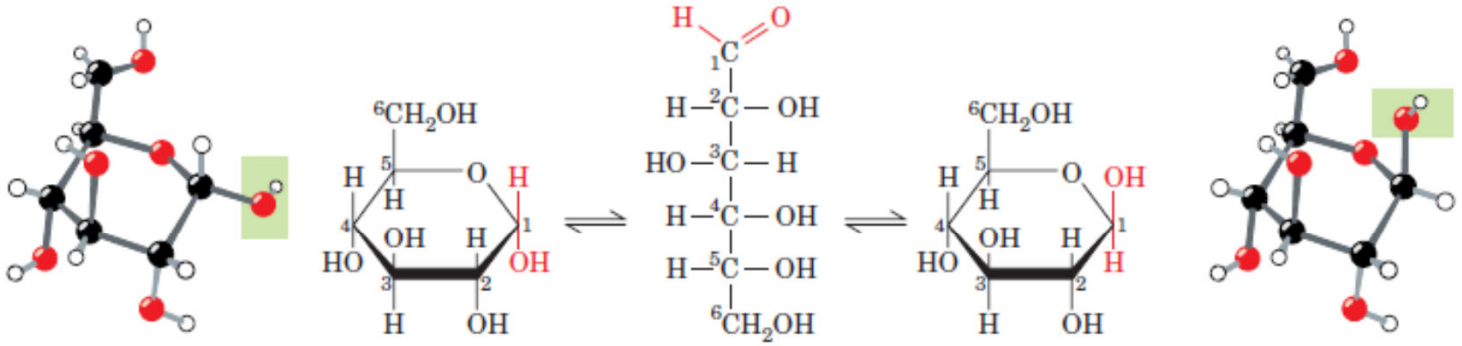
Hemiacetal



Hemiketal

These are reducing because an OH is attached to the anomeric carbon

If the OH was "OR" (a glycosidic bond) then the sugar could not open up and would **not** be reducing



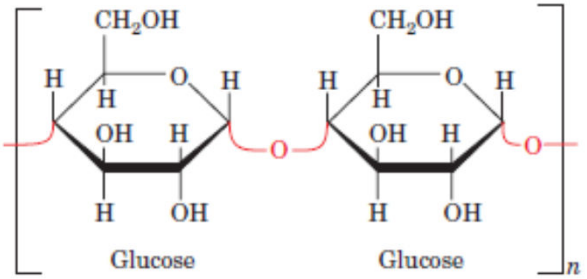
■ Figure 8-4 | α and β anomers.

Sugars can mutarotate (interconversion of α/β anomers) as long as the sugar is reducing

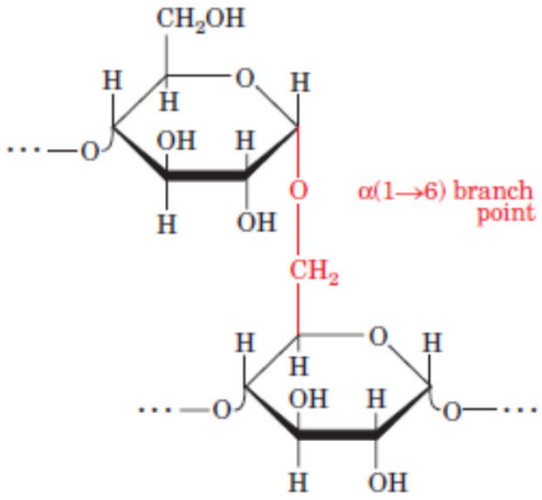
α-Linked Sugars

- **Loose (flexible), highly hydrated, helical, granular, branched, rings in chair conf**

- **Glycogen**
  - Glucose in α1-4 (linear) and α1-6 (branched) linkages
  - One reducing end, many non-reducing ends
  - Chain grows by adding to non-reducing ends
- **Starch**
  - Amylose (α1-4 glucoses, linear) winds in among a mesh of amylopectin
  - Amylopectin (branched)
  - Many non-reducing ends, few reducing ends



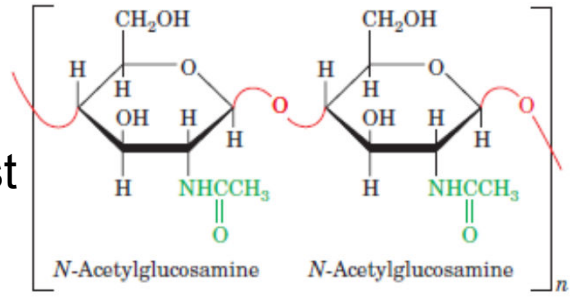
α-Amylose



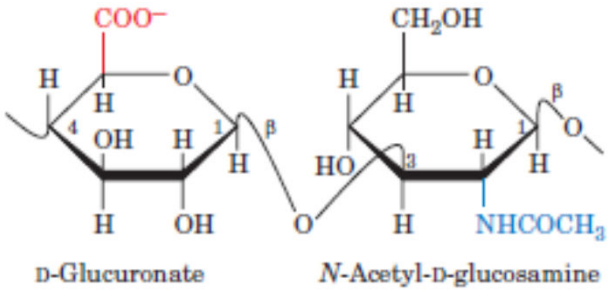
Amylopectin

β-Linked Sugars

- Extended, Fibrous, Extensive H-bonding, Rigid, Rings in Chair conformation, Provide Support & Lubrication
- Cellulose
  - Glucose with β1-4 linkages
  - Extended chains, very close packing, not very much hydration = rigid fibers that are hard to digest
- Chitin
  - β1-4 linked N-acetylglucosamine
- Peptidoglycan
  - Chains of alternating N-acetylglucosamine and N-acetyl muramic acid
  - Combined with peptides
  - Rigid mesh-like shell around bacteria
- Glycosaminoglycans
  - Alternating sugar with amino-sugar, β1-3 linkages
  - Negatively charged
  - Shock absorbers, highly hydrated
  - Ex: Heparin



Chitin

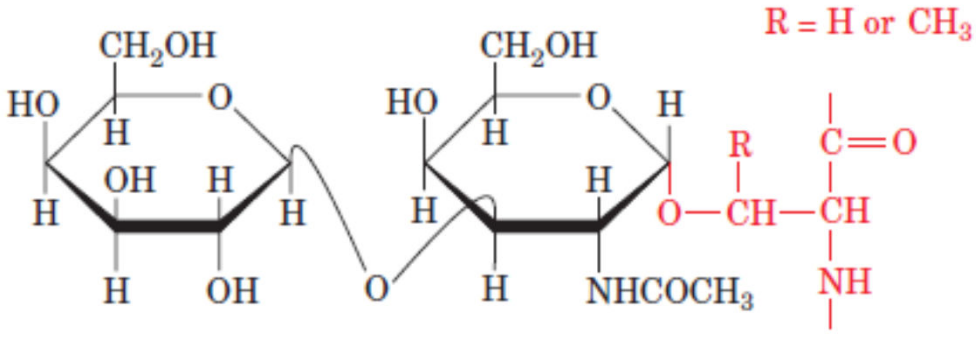


Hyaluronate

Glycoproteins

- N-linked = attached to Asn
  - Attached **during synthesis**
- O-linked = attached to Ser/Thr
  - Attached **after folding**

- Microheterogeneity = diversity in sequence of attached sugar
- Glycoforms = different patterns of glycosylation



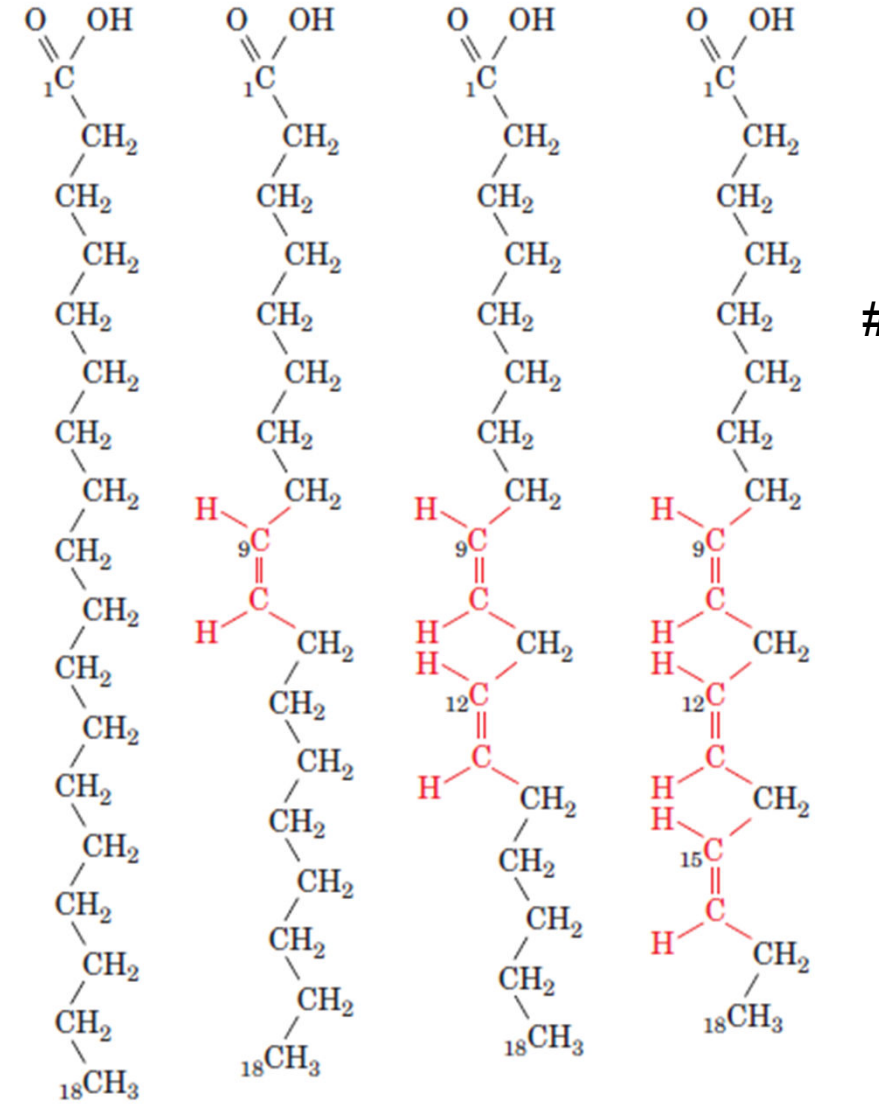
β-Galactosyl-(1→3)-α-*N*-acetylgalactosaminyl-Ser/Thr

Lipids

- Functions:
  - Energy storage (triacylglycerols)
  - Membranes (structural)
  - Signalling
    - Intracellular (sphingolipids, phosphotidylinositol)
    - Intercellular
    - Intertissue (steroid hormones)
    - Interorganism (pheromones, volatile plant lipids)
  - Insulation
  - Light Absorption
  - Nutrition
  - Electron Carriers (CoQ)
  - Enzyme cofactors
  - Antioxidants

Fatty Acids

- COOH at one end, 4-36 carbons
  - (even # of carbons only)
- Lipid oxidation releases energy
  - Lipids are VERY reduced so they can be oxidized more than sugars
  - Not hydrated. Means more energy per unit weight
  - 6x the amount of energy as sugars
- Melting Points
  - Higher as chains get longer
    (more Van der Waals contacts)
  - Lower as # of double bonds increases
    (more kinks = worse packing)



Stearic acid Oleic acid Linoleic acid α-Linolenic acid

18:0 18:1(Δ^9) 18:2($\Delta^{9,12}$) 18:3(ω-3)

18:2($\Delta^{9,12}$) or (ω-6) or (n-6)

Carbons

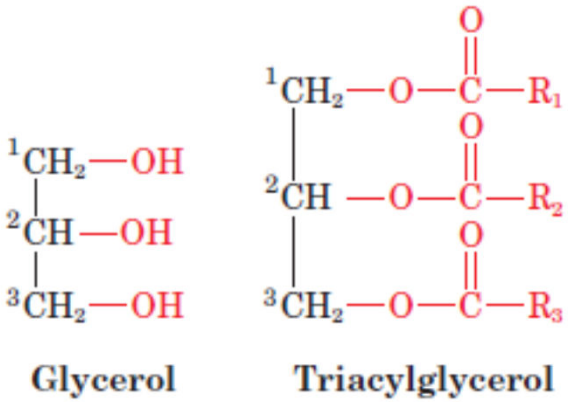
double bonds

Delta name:
name starting carbon of the double bond from COOH end

ωname:
name starting carbon of the double bond from methyl end

- Double bonds are **cis**!
- Double bonds occur every 3 carbons
- We cannot synthesize ω-6 or ω-3 FA, need these from diet

Triacylglycerols



- Energy storage, thermal insulation
- Naming

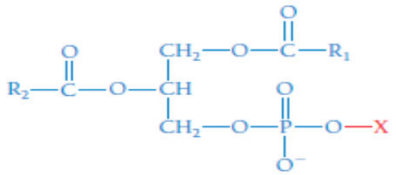
For ex: 1-palmitoyl-2-stearoyl-3-___oyl glycerol

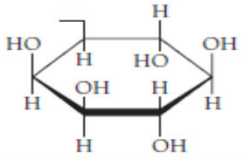
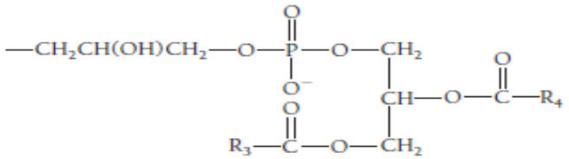
Glycerol-based Lipids:

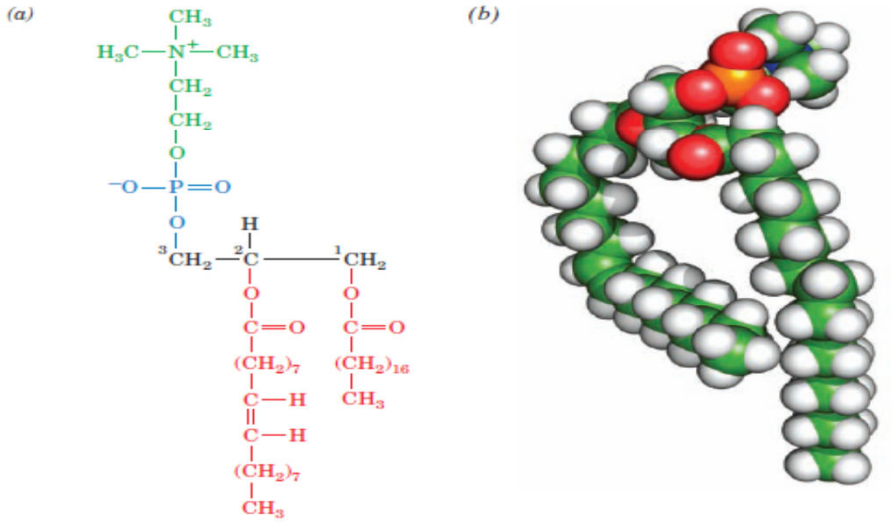
Glycerophospholipids = glycerol + FA + phosphate + group attached to phosphate

Glyceroglycolipids = glycerol + FA+ oligosaccharide

Table 9-2 The Common Classes of Glycerophospholipids



Name of X—OH	Formula of —X	Name of Phospholipid
Water	—H	Phosphatidic acid
Ethanolamine	$-CH_2CH_2NH_3^+$	Phosphatidylethanolamine
Choline	$-CH_2CH_2N(CH_3)_3^+$	Phosphatidylcholine (lecithin)
Serine	$-CH_2CH(NH_3^+)COO^-$	Phosphatidylserine
myo-Inositol		Phosphatidylinositol
Glycerol	$-CH_2CH(OH)CH_2OH$	Phosphatidylglycerol
Phosphatidylglycerol		Diphosphatidylglycerol (cardiolipin)



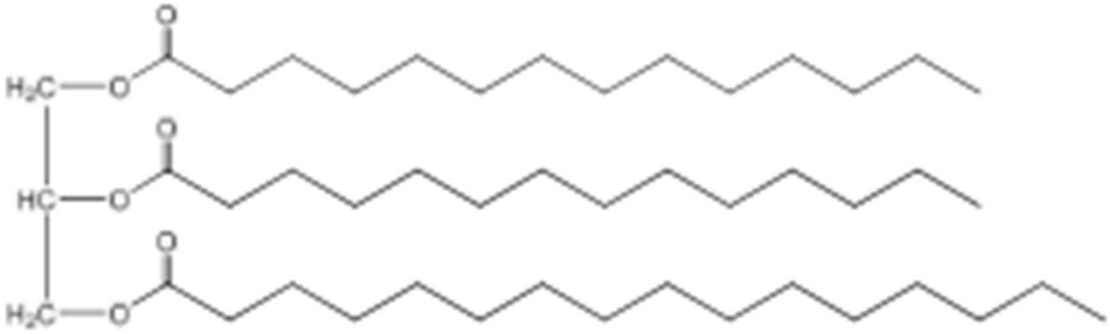
1-Stearoyl-2-oleoyl-3-phosphatidylcholine

Figure 9-4 | **The glycerophospholipid 1-stearoyl-2-oleoyl-3-phosphatidylcholine.**

26. (46 pts) Like the breakdown of glucose, the breakdown of lipids provides energy for the cell.

    a. In what form are lipids stored in the body? (Name the lipid class.)

    b. A specific example of a lipid is shown below. Name this lipid.



Sphingolipids

- Sphingosine + FA = ceramide
- Sphigomyelins = ceramides with phosphocholine or phosphoethanolamine
- Sphingophospholipids (charged)

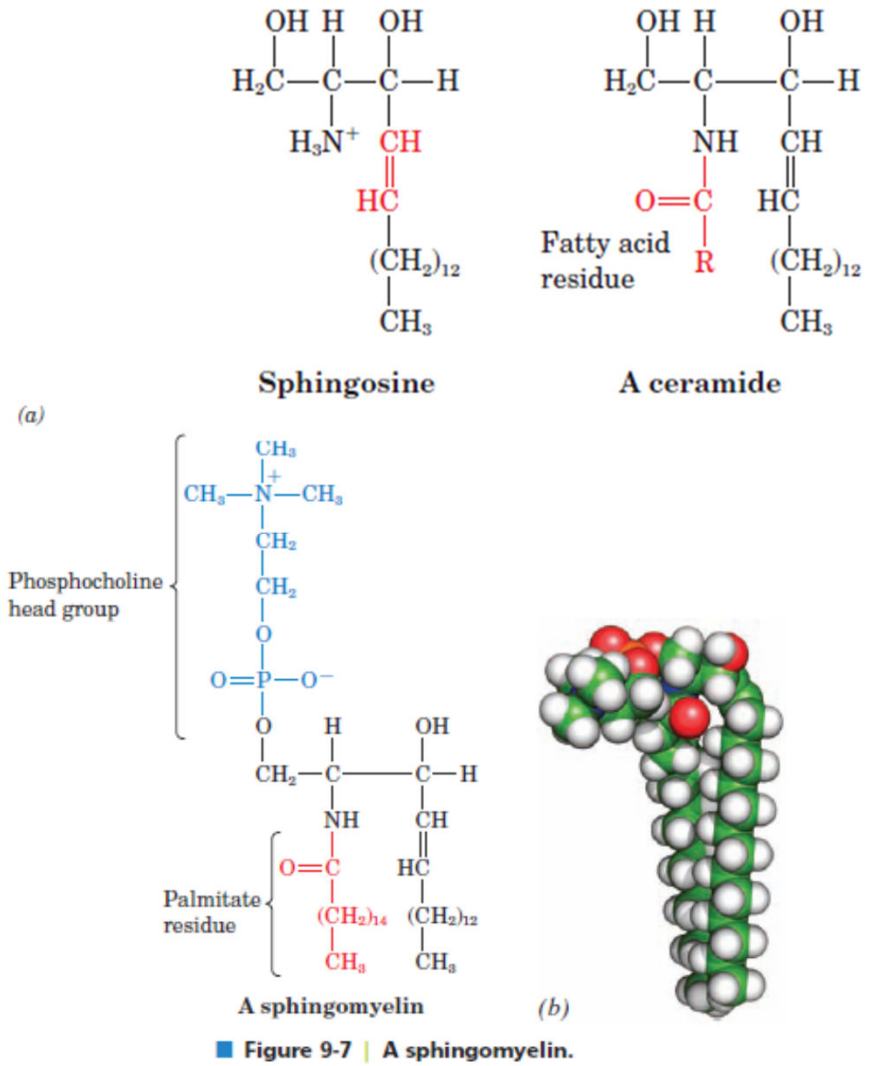


Figure 9-7 | A sphingomyelin.

Glycosphingolipids

•Cerebrosides
(monosaccharide attached to ceramide, uncharged)

•Gangliosides
(oligosaccharide attached to ceramide, charged, at least one sialic acid attached to sugars

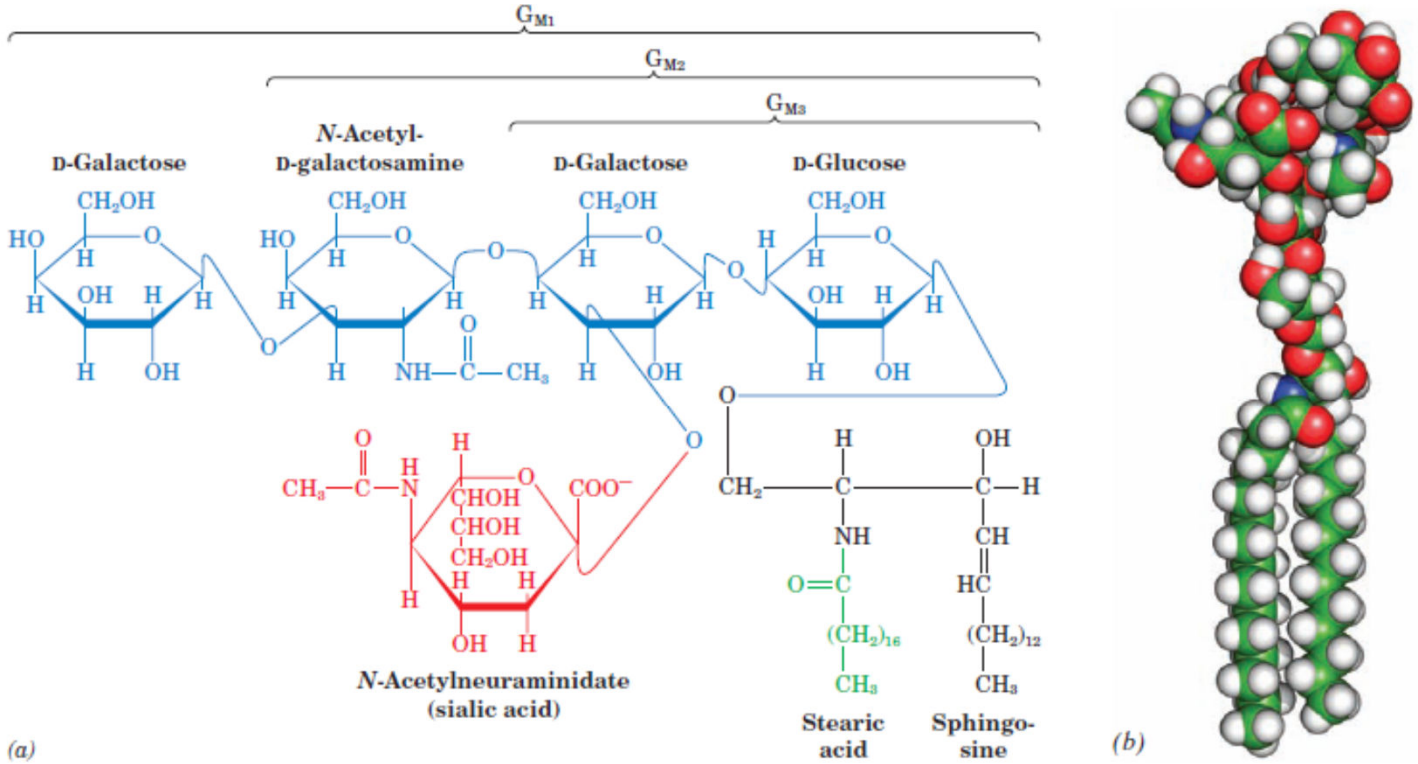
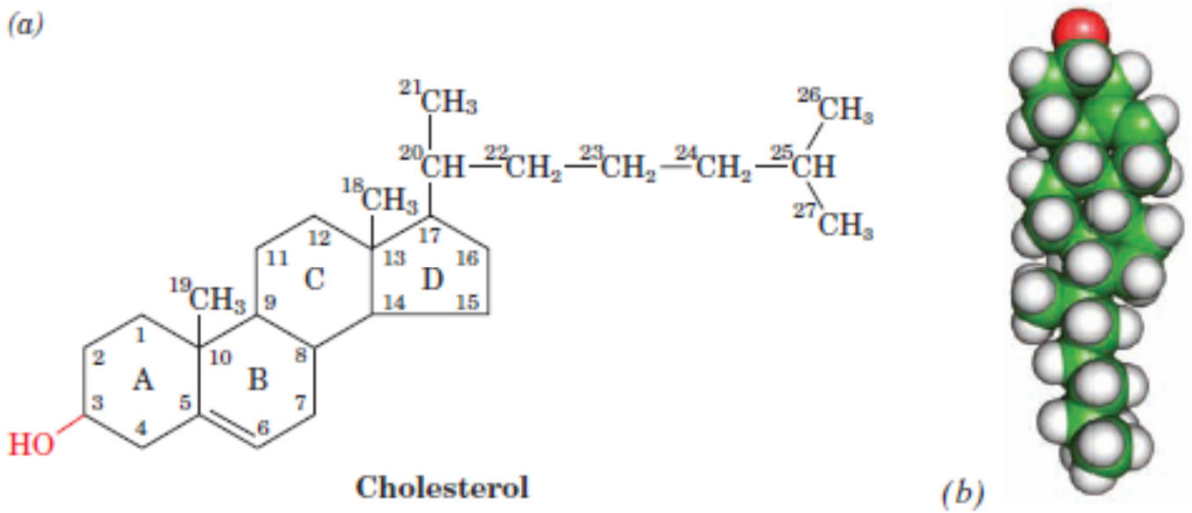


Figure 9-9 | Gangliosides.

Sterols

- Slightly amphipathic because of –OH
- Fused planar rings



■ Figure 9-10 | Cholesterol.

3. Which of the following statements is correct?

Water has a high dielectric constant and is able to dissolve polar substances well.
Water has a low dielectic constant and is able to dissolve polar substances well.
Water has a high dielectric constant and is able to dissolve apolar substances well.
Water has a low dielectric constant and is able to dissolve apolar substances well.

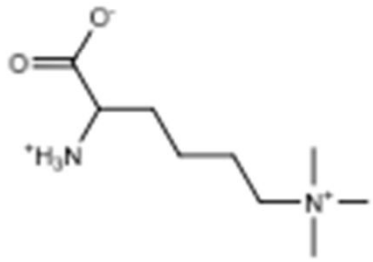
21. (10 pts) For each of the following amino acid pairs, name the most likely interaction that would occur between their side chains:

g. Asn and Glu
h. Arg and Glu
i. Met and Phe
j. Ala and Ser
k. Tyr and Thr

8. (8) Below are segments of the sequences of cytochrome C from human and wheat germ. These sequences correspond to the C-terminal 25 residues of the two proteins. Note that residue 'X' in the wheat germ sequence refers to trimethyllysine, the structure of which is shown.

```
MIFVGIKKKEERADLIAYLKKATNE  (human)
MVFPGLXKPQDRADLIAYLKKATSS  (wheat germ)
```

Trimethyllysine:



a. (5) Which of the following values best describes the *identity* of the two sequence fragments? Which best describes their *similarity*?

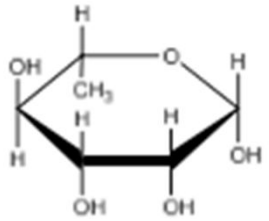
A. 25%	E. 65%
B. 35%	F. 75%
C. 45%	G. 85%
D. 55%	H. 95%

b. (3) These sequences are (choose all that apply):

A. orthologous
B. paralogous
C. homologous
D. homogeneous

10. (4) If you could pull a 30 Å α-helix into an extended, antiparallel β-strand conformation, how long would it become? Show your work.

11. (15) Pectins are polysaccharide components of plant cell walls. While naturally found in plant-derived foods, they are also used as thickening agents in processed foods. The hexose rhamnose, shown below, is a minor component of pectins:



Rhamnose

a. (2) True or False? Rhamnose is a ketose.

b. (2) True or False? Rhamnose is a glycoside.

c. (2) True or False? Rhamnose is mutarotatory.

d. (2) Which anomer of rhamnose is depicted above?

e. (4) Draw the Fisher projection of rhamnose.

f. (3) Rhamnose is the 6-deoxy form of which sugar?

4. (4 pts) Multiple Choice. Which one of the following statements is correct?
    a. The melting point of linoleic acid is higher than that of oleic acid.
    b. Peripheral membrane proteins can be identified by stretches of hydrophobic amino acids in their sequence.
    c. A membrane's fluidity increases with increasing saturation of its lipids.
    d. Phosphatidylcholines are a type of membrane glycerolipids.
    e. None of the above.

15. (8 points) The hot springs of Yellowstone National Park teem with algae, bacteria, and archaea that are able to grow at temperatures close to 100°C. These organisms are called 'thermophiles' because of their ability to thrive in hot temperatures. Considering *only* the environmental temperature, rank the following fatty acids in order of their likely abundance in the plasma membranes of thermophilic bacteria:

stearic acid
lauric acid
oleic acid
18:3, ω-3
20:0
16:1 (Δ^9)