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)

**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)
  \[ F = \frac{kq_1q_2}{E r^2} \]
  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 $K_a = \frac{[H^+][A^-]}{[HA]}$

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

**Henderson-Hasselbach Equation:**

\[ pH = pK_a + \log \left( \frac{[A^-]}{[HA]} \right) \]

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

\[ pH < pK_a \quad [A^-] < [HA] \]
\[ pH = pK_a \quad [A^-] = [HA] \]
\[ pH > pK_a \quad [A^-] > [HA] \]

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

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
$\text{pH} = \text{pK}_a$ at half equivalence point

Can also be $\text{OH}^-$ equivalents

(need 1 mole of $\text{OH}^-$ equivalents per ionizable group)
Oxidation Numbers

\[ \text{OX} \# = v_e^- - (l_p + \text{shared } e^-) \]

Valence electrons

Lone pairs

Shared Electrons:
- C-H bond, carbon gets e⁻
- C-C bond, e⁻ are split evenly
- C-O bond, oxygen gets e⁻
Amino Acids

• Usually found as a zwitterion

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

\[ \text{L-\alpha-Amino acid} \]
Amino Acid pKα’s

- Carboxyl groups pKα ~ 2.0
- NH₃ (N-termini) pKα ~ 9.5

**Inductive Effect**
- e⁻ withdrawing effect
- Lowers pKα

**Electrostatic Effect**
- Charge effect
  - Molecules prefer a net neutral charge
  - Can raise and lower pKα’s

PkJα is 2.35
Lower than pKα in acetic acid (CH₃-COOH) because N is withdrawing electrons

Glycine

PkJα is 9.78.
Higher than NH₃ in ethylamine (CH₃-CH₂-NH₃⁺) because COO⁻ is withdrawing electrons

Higher than NH₃ in O-methyl glycine (NH₃-CH₂-C-OCH₃) to increase range that glycine has net 0 charge
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

\[ pI = \frac{1}{2}(pK_i + pK_j) \]

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.

<table>
<thead>
<tr>
<th>pH</th>
<th>NH\textsubscript{3}\textsuperscript{+} (pK\textsubscript{a} 8)</th>
<th>Arg (pK\textsubscript{a} 12.5)</th>
<th>Tyr OH (pK 10)</th>
<th>Σ</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 8</td>
<td>+1</td>
<td>+1</td>
<td>0</td>
<td>+2</td>
</tr>
<tr>
<td>8-10</td>
<td>0</td>
<td>+1</td>
<td>0</td>
<td>+1</td>
</tr>
<tr>
<td>10-12.5</td>
<td>0</td>
<td>+1</td>
<td>-1</td>
<td>0</td>
</tr>
<tr>
<td>&gt;12.5</td>
<td>0</td>
<td>0</td>
<td>-1</td>
<td>-1</td>
</tr>
</tbody>
</table>

\[ pI = (10 + 12.5)/2 = 11.25 \]
Henderson-Hasselbach Eqn can be used to determine net charge

\[ 10^{\text{pH}-\text{pK}_a} = \frac{[A^-]}{[HA]} \]

**Example:** A protein has three ionizable groups (NH₃ at N-termini, Arg, Tyr)

To find the net charge at pH 7,

\[ \frac{[\text{NH}_2]}{[\text{NH}_3^+]} = 10^{(7-8)} = 10^{-1} = 1/10 \quad 10 \text{ out of } 11 \text{ have a } +1 \text{ charge} = 90\% \]

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

\[ \frac{[\text{NH}_2]}{[\text{NH}_3^+]} = 10^{(7-12.5)} = 10^{-5.5} = 100\% \text{ in } \text{NH}_3^+ \text{ form} \]

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

**Peptide Bond:**
- Has partial double bond character
- Is planar
- $\Phi$ (N to $C_\alpha$)
  - no $\Phi$ at N-terminal
- $\Psi$ ($C_\alpha$ to Carbonyl C)
  - no $\Psi$ at C-terminal
Conformations of the Protein Backbone ($\Phi$, $\Psi$) are limited by STERIC CLASHES

Ramachandran Plot:
plots the allowed phi/psi conformations
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
Sequencing Proteins with Mass Spec

- **Bottom-Up Proteomics**
  - Fragment protein (e.g. enzymatically) and separate fragments
  - Ionize fragments, trap in the spectrometer, and measure m/z
  - Select one m/z peak and fragment (e.g. by collision)
  - Measure m/z of the smaller fragments and use a database to match the peaks to known sequences

- **Top-Down Proteomics**
  - Ionize *whole* protein(s), trap in the spectrometer, and measure m/z
  - Use the instrument to select one m/z peak and fragment the protein
  - (e.g. by collision)
  - Measure m/z ratios of the fragments and use a database to match the peaks to known sequences
  - OR Select a peak and fragment again, then match to sequence

- **Shotgun Proteomics**
  - Used to separate and sequence a MIXTURE of different proteins
Tools to Measure Protein Structure

- **Circular dichroism (CD) (2º Structure)**
  - Measures amide absorption of circularly polarized UV light
  - Ellipticity is the difference in absorption of left-handed and right-handed circularly polarized light
  - Different secondary structures show different ellipticity
- **2D NMR (3º Structure)**
  - Reveals distances between molecules
- **X-ray Crystallography (3º Structure)**
  - Crystals of protein diffract x-rays. Diffraction data can be processed to produce an electron density map.
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 (-)

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-sheets/strands

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

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

Do not see fully extended ($\phi = 180^\circ$, $\psi = 180^\circ$) 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

1° covalent peptide bond

2°: H-bonding (backbone N-H····O)
   - Electrostatic Ion Pairs
   - Steric compatibility
   - Van der Waals
   - Hydrophobic Effect

3°: 1+2 and disulfide bonds

4°: same as 3°

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 \((\text{CH}_2\text{O})_n\)

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 conformations 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 \textbf{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).

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.

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 glucose, linear) winds in among a mesh of amylopectin
  – Amylopectin (branched)
  – Many non-reducing ends, few reducing ends
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
  – Proteoglycans are proteins + glycosaminoglycans
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)
18:2(\(\begin{array}{c}9,12\end{array}\)) or (\(\omega\)-6) or (n-6)

- Double bonds are \textbf{cis}!
- Double bonds occur every 3 carbons
- We cannot synthesize \(\omega\)-6 or \(\omega\)-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

<table>
<thead>
<tr>
<th>Name of $X$</th>
<th>$\text{OH}$</th>
<th>Formula of $X$</th>
<th>Name of Phospholipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>$-H$</td>
<td></td>
<td>Phosphatic acid</td>
</tr>
<tr>
<td>Ethanolamine</td>
<td>$-\text{CH}_2\text{CH}_2\text{NH}_3^+$</td>
<td></td>
<td>Phosphatidylethanolamine</td>
</tr>
<tr>
<td>Choline</td>
<td>$-\text{CH}_2\text{CH}_2\text{N(CH}_3)_2$</td>
<td></td>
<td>Phosphatidylcholine (lecithin)</td>
</tr>
<tr>
<td>Serine</td>
<td>$-\text{CH}_2\text{CH}({\text{NH}_3})\text{COO}^-\text{CH}_2\text{OH}$</td>
<td></td>
<td>Phosphatidylserine</td>
</tr>
</tbody>
</table>

**myo-Inositol**

Glycerol: $-\text{CH}_2\text{CH}({\text{OH}})\text{CH}_2\text{OH}$

Phosphatidylinositol

**Phosphatidylglycerol**

$-\text{CH}_2\text{CH}({\text{OH}})\text{CH}_2\text{O}^-\text{P}\text{O}_3^-\text{CH}_2\text{OH}$

Diphosphatidylglycerol (cardiolipin)

---

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

- Sphingosine + FA = ceramide

- Sphigomyelins = ceramides with phosphocholine or phosphoethanolamine

- Sphingophospholipids (charged)
Glycosphingolipids

• Cerebrosides
  (monosaccharide attached to ceramide, uncharged)

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

• Slightly amphipathic because of –OH
• Fused planar rings
Membranes

- Amphipathic conical lipids form micelles in water
- Amphipathic cylindrical lipids form bilayers in water
- Membrane fluidity affected by:
  - Temp (higher temp = more fluid)
  - Chain length
  - Composition
  - Cholesterol %
- longer, saturated = stiffer
- shorter unsaturated = more fluid
- Lipids diffuse readily within one leaflet of bilayer (lateral diffusion)
- Lipids do not diffuse (flip) to other leaflet very often (transverse diffusion)
- Membrane composition varies between organisms, within cells, and between leaflets
- Specialized enzymes catalyze lipid “flipping” and transport
- FRAP = Fluorescence Recovery After Photobleaching
  - Can be used to measure membrane diffusion/fluidity
Membrane Proteins

• Integral (span membrane)
  – Hydropathy plots can predict which regions may be in membrane
  – α-helix bundles, β-barrels
    • These structures maximize hydrogen bonding of protein backbone
  – Backbone H-bonding must be satisfied within protein, no H-bonding groups exposed to lipid
  – Large aromatics (Phe, Tyr, Trp) flank interfacial region
Membrane Proteins

- **Lipid-linked**
  - Prenylation (CXXY motif)
    - Attachment of isoprenes to protein
      - 3 isoprenes added = farnesyl
      - 4 isoprenes added = geranylgeranyl
    - These are in inner leaflet
  - Fatty-acylated
    - 14:0 attached to N-terminal (amide linkage)
    - 16:0 attached to a Cys (thioester linkage)
    - These are in the inner leaflet
  - GPI (glycosylphosphatidylinositol) anchors
    - These are in the outer leaflet

- **Peripheral**
Lipid Rafts

- Membrane microdomains
- Thicker, stiffer part of membrane
- Enriched in:
  - sphingolipids (longer chain fatty acids, saturated)
  - Cholesterol
- GPI-linked proteins and doubly acetylated proteins are found in rafts
- Prenylated proteins are not in rafts
- There is a “membrane skeleton” that limits the movement of proteins and lipid