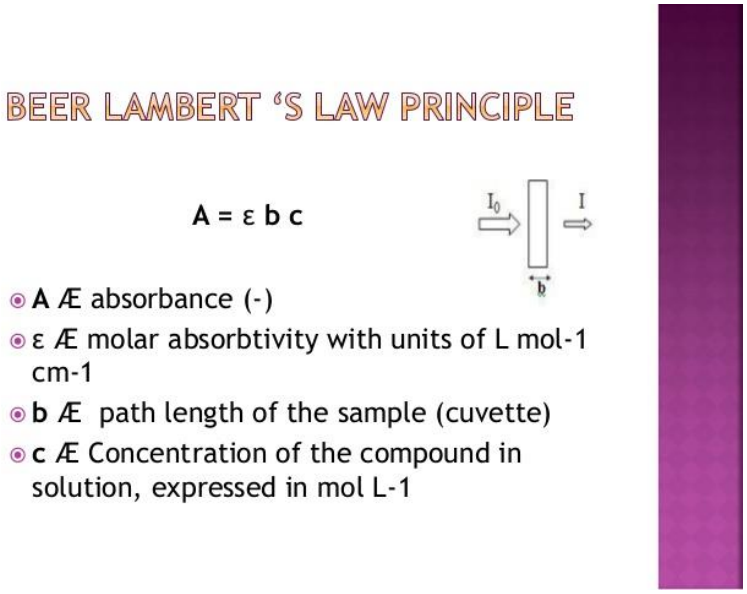
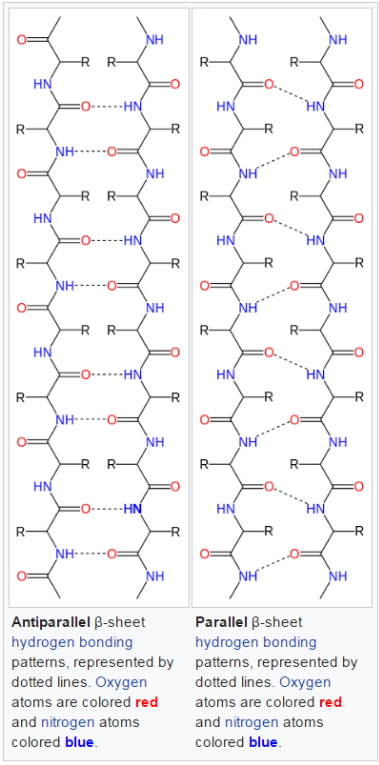
Amino Acids and Proteins Skillz

1. Structures: Know how to draw all the amino acids. Non-polar & Aromatic are hydrophobic. Polar and charged are hydrophilic. If you can't draw them, or at least know the side chains, you are probably fucked. So start here. Note: The following mnemonics don't use the 1 letter abbrev.
    a. Non- polar: GAP-VILM
        i. Glycine, alanine, proline, valine isoleucine, Leucine, methionine
    b. Polar: CATS-G
        i. Cysteine, Asparagine, Threonine, Serine, Glutamine
    c. Non-polar Aromatic: TTP (one letter abbreviation in reverse alphabetical order **Y**oung **W**ild & **F**ree)
        i. Tyrosine(Y), Tryptophan(W), Phenylalanine(F)
    d. Charged: HAL from GA
        i. Histidine, Arginine, Lysine (positive)
        ii. Glutamic acid, aspartic acid (negative)(also called glutamate and aspartate)
2. UV absorption
    a. Corresponds to the absorbance of energy equivalent that is required to promote molecular outer electrons into a higher energy molecular orbital. From HOMO to LUMO. Highest Occupied Molecular Orbital to Lowest Unoccupied Molecular Orbital.



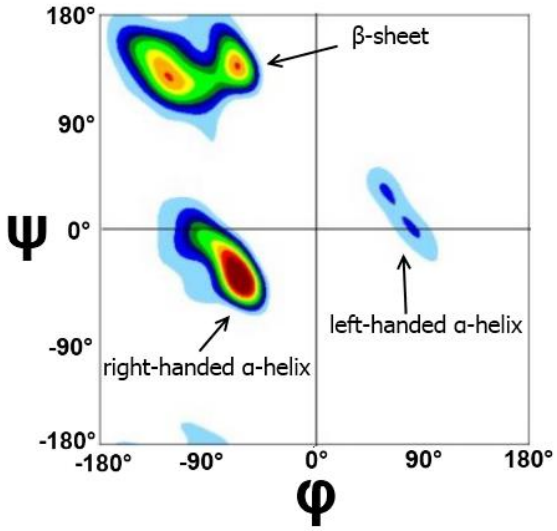
    b. absorptivity = extinction coefficient
    c. All proteins absorb UV energy at 254nm (amide bond)
    d. Tryptophan absorbs 5000 $M^{-1}cm^{-1}$ at 280nm
    e. Tyrosine absorbs 1000 $M^{-1}cm^{-1}$ at 280 nm
    f. Phenylalanine absorbs 250 $M^{-1}cm^{-1}$ at 280nm
    g. Ex: If peptide A contains 3 tyrosine and 1 tryptophan, and peptide B contains 1 tyrosine and 3 tryptophan, then peptide B would absorb the most light (5000+5000+5000+250) at 280nm. So peptide B would have a greater extinction coefficient.
3. Protein Structure

a. Primary – Order of amino acids Ex: Gly-Pro-Cys-Try
b. Secondary – alpha helix or beta pleated sheet
    i. Alpha Helix – 3.6 residues per turn. Hydrogen bonding between peptide N-H and O=C of 4th AA on N-terminal side.
    ii. Beta Pleated Sheet
    iii. 
Antiparallel β-sheet hydrogen bonding patterns, represented by dotted lines. Oxygen atoms are colored **red** and nitrogen atoms colored **blue**.

Parallel β-sheet hydrogen bonding patterns, represented by dotted lines. Oxygen atoms are colored **red** and nitrogen atoms colored **blue**.
    iv. Beta-turn - C=O H-bonding of 1stresidue with amide nitrogen of 4th. Pro, Gly common for 2 and 3 in sequence.
c. Tertiary – overall structure of <u>single</u> protein. Disulfide bonds formed by oxidation of side chain of cysteine affect the tertiary structure.
d. Quaternary Structure – arrangement of <u>multiple</u> proteins in a complex.

4. Peptide/Amide bond
    a. A Delocalization
        i. Basic nitrogen's are double bonded in a ring. Non-basic nitrogen's are bonded to at least 1 hydrogen
        ii. Hindered Rotation – no rotation of omega angle between peptides.

b. Ramachandran Plot

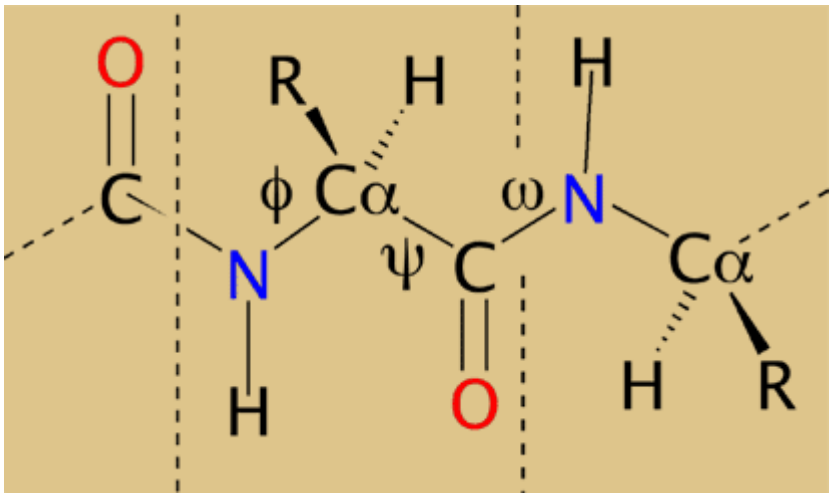


i.

ii. Know which angle is on which axis and that they both span from -180 to +180.

iii. Phi angles are the degrees of rotation between the nitrogen and alpha carbon (carbon that's connected to the carbonyl carbon). Psi angles are the degrees of rotation between the carbonyl carbon and the alpha carbon.

iv.

v. 

vi. Omega (w) are the degrees of rotation between the carbonyl carbon and the nitrogen. No rotation around omega, so not involved in Ram-a-man-dran plot.

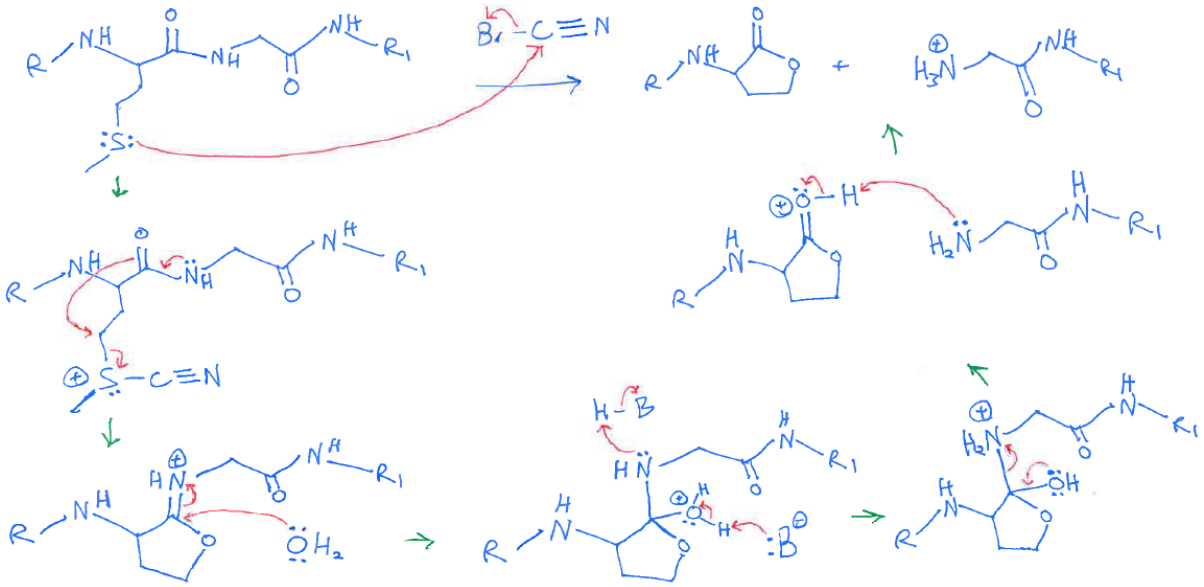
5. Protein Folding
   a. Hydrophobic effect – causes hydrophobic groups to gather together in the presence of water or other polar solvent. Hydrophobic amino acids will cluster together toward the inside of the protein to avoid water, which affects folding.
   b. Stabilizing interactions
      i. Alpha helix and Beta sheets are held together by hydrogen bonding.
      ii. Ionic bonds aka salt bridges can be formed between amino acids that have charged side chains. HAL from GA – Histidine, Arginine, Lysine, Glutamic acid,

Aspartic acid. One possible trick, the side chain on histidine has a pka of around 6 so if the question asked which could participate in ionic bonding at a pH of 7, it would be all of these charged amino acids except histidine, because its side chain would already be deprotonated and no longer charged.

iii. Disulfide bonds are between cysteine amino acids, they form through oxidation.

6. Chromatography
    a. Gel filtration
        i. Molecules are pushed by an electric field through a gel with small pores. Smaller molecules can travel through these small pores easier. Therefore small molecules travel further.
    b. Ion-exchange chromatography with pH gradient
        i. Anion exchange - Anions are negatively charged. To separate them, the gel resin must be positively charged. Start with a high pH buffer and then use decreasing pH buffers. Eventually each anion will be protonated (charge becomes neutral) and elute. Anion with the highest PI will elute first, lowest PI will elute last.
        ii. Cation exchange - cations are positively charged. To separate them, the gel resin must be negatively charged. Start with a low pH buffer and then use increasing pH buffers. Eventually each cation will be deprotonated (charge becomes neutral) and elute. Cations with the lowest PI will elute first, highest PI will elute last.
    c. SDS-PAGE
        i. SDS causes proteins to denature and disassociate from each other. Intrinsic charge of protein is masked. Each protein is now negatively charged relative it its size. The similar mass to charge ratio essentially takes the charge out of the equation. They all travel toward the anode (positive) but now they are all attracted in the same amount. Now the separation is all due to size. As the protein-sds molecules travel through the small pores of the gel, bigger molecules have a harder time fitting through. Therefore the smaller molecules travel further.
    d. Affinity Chromatography
        i. Method of separating biochemical mixtures based on a highly specific interaction such as between antigen and antibody, enzyme and substrate, or receptor and ligand.
        ii. Wessel test definition: A DNA fragment was attached to a support and used to isolate a **specific** RNA. This is an example of <u>Affinity Chromatography</u>.
7. Solid Phase Synthesis – synthesized from C terminus to N terminus (opposite of nature) Memorize a-e for fill in the blank.
    a. C-terminus attached to bead via ester linkage
    b. Fmoc or tBoc protect amino terminus
    c. Deprotect N-terminus (Multiple mechanisms, Fuck em)
    d. Activate with DCC

    e. HF or TFA deprotection and cleavage from bead
8. Sequencing
    a. Peptide laddering – Determining the sequence
    b. LC-MS/MS (pending)
        i. Molecular cations
        ii. B-series
        iii. Y-series
        iv. Mechanism of fragmentation
    c. Edman degradation – reagent is phenylisothiocyanate (PITC)
    d. Endopeptidases
        i. Trypsin – Cleaves after(c-terminal) Arginine(R) and Lysine(K), unless followed by proline.
        ii. Chymotrypsin – Cleaves after aromatic amino acids Tyrosine(Y), Tryptophan(W), Phenylalanine(F), unless followed by proline.
    e. Cyanogen Bromide – cleaves after Methionine(M).
        i. Mechanism on next page
    f. Disulfide reduction – memorize these for fill in blank
        i. Dithiothreitol (DTT) is used to reduce disulfide bonds.
        ii. Iodoacetamide is used to react with the resulting thiols.
        iii. Penyl isothiocyanate reacts with the N-terminal amino acid



CYANOGEN BROMIDE CLEAVAGE ON METHIONINE

* H–B is generic proton donor, $:B^{\ominus}$ is generic proton acceptor.

- The pKa values and the isoelectronic point, pI, are given below for the 20 α-amino acids.
- pKa_1= α-carboxyl group, pKa_2 = α-ammonium ion, and pKa_3 = side chain group.

Amino acid	pKa_1	pKa_2	pKa_3	pI
Glycine	2.34	9.60	---	5.97
Alanine	2.34	9.69	---	6.00
Valine	2.32	9.62	---	5.96
Leucine	2.36	9.60	---	5.98
Isoleucine	2.36	9.60	---	6.02
Methionine	2.28	9.21	---	5.74
Proline	1.99	10.60	---	6.30
Phenylalanine	1.83	9.13	---	5.48
Tryptophan	2.83	9.39	---	5.89
Asparagine	2.02	8.80	---	5.41
Glutamine	2.17	9.13	---	5.65
Serine	2.21	9.15	---	5.68
Threonine	2.09	9.10	---	5.60
Tyrosine	2.20	9.11	---	5.66
Cysteine	1.96	8.18	---	5.07
Aspartic acid	1.88	9.60	3.65	2.77
Glutamic acid	2.19	9.67	4.25	3.22
Lysine	2.18	8.95	10.53	9.74
Arginine	2.17	9.04	12.48	10.76
Histidine	1.82	9.17	6.00	7.59