Amino Acids

1. General Structure of Amino Acids

- General formula of α-amino (carboxylic) acids $H_2N - C^{\alpha}H(R) - COOH$

- Amphipathic; Zwitterions : $^{+}H_3N - C^{\alpha}H(R) - COO^{-}$

- ***L***-α-amino acids vs. ***D***-α-amino acids : $^{+}H_3N - C^{\alpha}(R) - H$ $\quad$ $H - C^{\alpha}(R) - NH_3^{+}$
  (Levorotatory / Dextrorotatory)

- Isomers, Stereoisomers (configuration), Enantiomers, Diastereoisomers

- *RS* system : Priority of 4 groups (R *rectus* - right handed, clockwise; S *sinister* - left handed)

(-H < $-CH_3$ < $-C_6H_6$ < CH_2OH < -CHO < - COOH < -COOR < $-NH_2$ < -OH< -OR < -SH)

2. The common R-groups - the "alphabet of life" (GAVLIPFYWMCSTHKRDENQ)
Neutral - nonpolar (aliphatic, aromatic); neutral - polar; acidic; basic

R =		
	Hydrogen	(Gly)
	Aliphatic	(Ala, Val, Leu, Ile, Pro)
	Aromatic	(Phe, Tyr, Trp)
	Sulfur Containing	(Met, Cys)
	Alcohol Containing	(Ser, Thr)
	Basic R Groups	(His, Lys, Arg)
	Acidic R Groups	(Asp, Glu)
	Amides	(Asn, Gln)

3. Uncommon amino acids and their derivatives
- D-alaine (bacterial cell walls)
- L-ornithine (urea cycle, polyamine synthesis)
- Homoserine
- GABA (γ-amino butyric acid)
- Histamine, Adrenaline, Serotonin, Thyroxine

4. Modified amino acids
- occurs after incorporated into protein
- phosphorylation of Ser, Thr, Tyr
- γ-carboxyglutamic acid in prothrombin--binds Ca^{++}
- 4-hydroxyproline and 5-hydroxylysine in collagen

5. Ionization / Titration properties of amino acids ; pKa 's

$^{+}H_3N$ - CHR - COOH : R (Glu, Asp) ~ 4
~9-10 ~2 R (His) ~ 6 ; R (Lys) ~ 10.5 ; R (Arg) ~ 12.5
R (Cys) ~ 8.3 ; R (Tyr) ~ 10.1

- Isoelectric point (pI)
    - pH at which there is no net charge, electrically neutral
    - amino acids with ionizable carboxyl side chains (+1 0 -1 -2)
        pI= average of pKas of the two carboxyl groups (pI = (pK_1 + pK_2) / 2)
    - amino acids with N containing ionizable groups (+2 +1 0 -1)
        pI = average of pKas of the N groups (pI = (pK_2 + pK_3) / 2)

6. Peptide Bonds - Proteins are linear polymers of a.a. residues linked by "peptide bonds."
    - Reaction: a.a.R1 + a.a.R2 = dipeptide (R1-R2) + water
        ΔG of this reaction is +10 kJ/mol ; proteins are metastable
        (acid hydrolysis (6N HCl) / proteases
    - Resonance structures result in planar amide group
    - Peptides (dipeptide, tripeptide, etc. ..... polypeptide - proteins)
    - Primary Structure : $^{+}H_3N$ - GVLAADEMLLKFYEE - COO^{-}
        N-terminus C-terminus
    - Animo acids --> amino acid residues (Glycyl-valyl-leucyl-alanyl-alanyl-aspartyl- etc.)
    - Blocking groups: N-terminus (formyl- , acetyl-); C-terminus (amide)

7. Small Peptides of Physiological Interest
    - Glutathione (GSH or γGlu-Cys-Gly)
        - scavenger for oxidizing agents (2 GSH = GS-SG + 2 H)
    - Enkephalins - Tyr-Gly-Gly-Phe-Leu (or Met)
        - natural brain analgesics - have structural similarity to opiates
    - Oxytocin and Vasopressin - Nonapeptides
    - Aspartame (Asp-Phe-methyl ester)
        - "NutraSweet" - ~200x sweeter than sugar
        - Concern about Phe and oxidation of methanol
            - Phenylketonuria (PKU) - accumulation of phenylpyruvate

8. Protein Purification Techniques: chromatography / fractional precipitation
    - Column chromatography (fractions / eluate)
        - Ion-exchange chromatography
            proteins have charges
            bind to charged column matrix depending on their charge
            anionic--negatively charged: phosphcellulose, heparin sepharose, S-sepharose
            cationic--positively charged: DEAE, Q-sepharose
            elute from column based on charge and displacement by salt or pH
        - Affinity Chromatography
            column matrix has a ligand that specifically binds a protein
                ATP-agarose
            specialty affinity columns for binding recombinant proteins with "tags"
                6XHis added at N or C terminus--binds Ni++ column; many others
        - High Performance Liquid Chromatography (HPLC)
            gravity flow very slow--depends on size and amount of liquid at the top
            HPLC used high pressure to force liquid through
            special matrixes and columns
            fast and sometimes better resolution

- Size Exclusion (Gel Filtration)
    separates on the basis of size, not charge
    porous beads--think of golf balls
    small molecules go into the holes and get trapped temporarily
    large molecules are too large to enter the holes and pass on by
    exclusion size--depends on the size of the holes
    how long the molecules get trapped determines elution order
        large out first > medium > small out last
    choose the size of matrix for the separation needed
- Electrophoresis
    - SDS PAGE (Sodium dodecyl sulfate - Polyacrylamide gel electrophoresis)
        binding SDS causes all proteins to have neg. charge ~ charge/size ratio
        separate by size since all proteins have similar charge to mass ratio
    - Nucleic Acids (Phosphate backbone - neg. charge /nucleotide)

9. Amino Acid Analysis (A.A. Composition of proteins) (~ 1 picomole)
    - use 6M HCl to hydrolyze protein into amino acids (Glx, Asx, Trp)
    - treat with PITC (phenylisothiocyanate) at pH 9
    - separate with HPLC; meas. abs 254nm

10. Protein Sequencing :
    - Edman degradation
    - Proteases (Mass spec for sequencing)

11. Databases - Nucleic acid sequences / Protein sequences
    - NCBI (Medline, GenBank, Entrez, Blast)
    - Comparing sequences (function / evolution) (MACAW)
        Conservative changes - change in aa preserves the character
            Asp ⇒ Glu / Lys ⇒ Arg / Tyr ⇒ Phe / Thr ⇒ Ser
        Non-conservative changes - change in aa alters the character
            Lys ⇒ Gln / Phe ⇒ Ser / Met ⇒ Asp

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Useful Web sites:

Molecular Models for Biochemistry at CMU (Carnegie Mellon)
http://info.bio.cmu.edu/Courses/BiochemMols/BCMolecules.html

National Center for Biotechnology Information / (GenBank, Blast, Entrez)
http://www.ncbi.nlm.nih.gov/