Lecture 4:
Amino Acids

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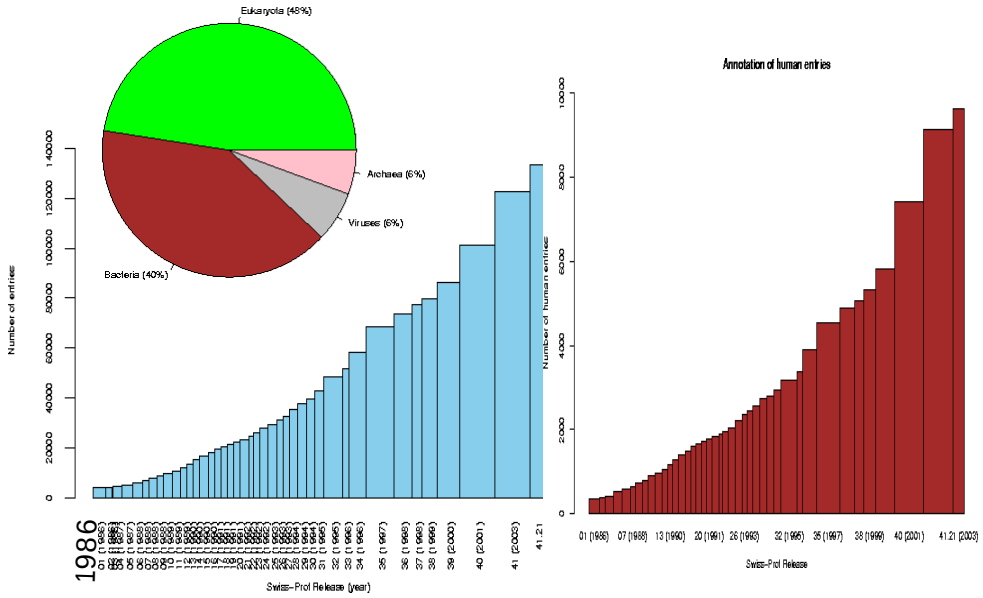
Swiss-prot database: How many proteins? From where?



Use http://us.expasy.org to get to swiss-prot database

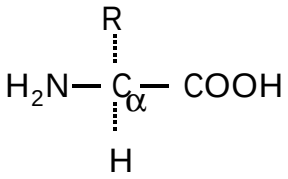
Proteins are the "workhorses" of the cell

Enzymes - catalyze chemical reactions
Regulatory proteins - control physiological function
Transport proteins - move substances around/between cells
Storage proteins - provide a reservoir for a substance
Motor proteins - endow cells with capability of movement
Structural proteins - create and maintain biological structure
Protective proteins -active role in cell defense or protection
Exotic proteins - have specialized adaptive functions

And all this with just 20 amino acids and some post-translational modifications!

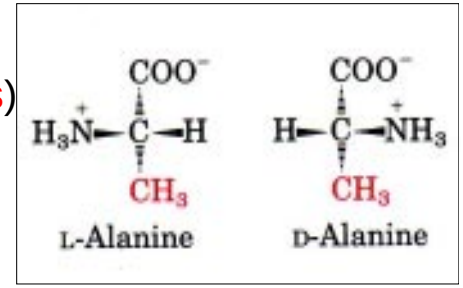
Amino Acids

- Proteins are polymers of amino acids (AAs)
- There are 20 standard AAs
- Four features of AAs structure
  - alpha carbon (H attached)
  - a carboxyl group (acidic)
  - an amino group (basic)
  - R group (variable)
- The R group differentiates 19 of the 20 AAs (Pro is the exception)



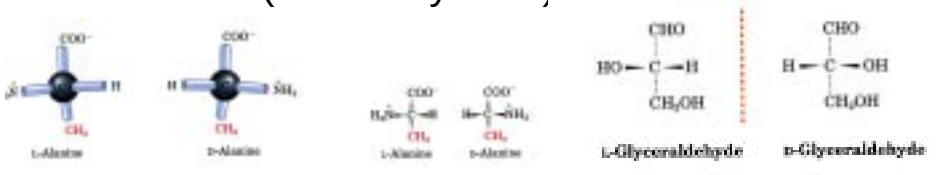
Enantiomers

- AAs are chiral (assymetric; 4 substituents attached to the Cα, (except Gly);
- AAs have two possible stereoisomers (enantiomers) that are mirror images of one another and cannot be superimposed on one another;
- Enantiomers are physically and chemically indistinguishable by most techniques.



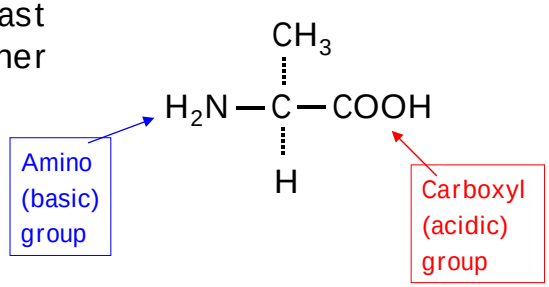
Characteristics of AAs

- AAs can be either D- or L- enantiomers (except Gly);
- Proteins are made from L-AAs; Not known why;
- D-AAs are found in bacterial cell walls and antibiotics (relatively rare):



Acid-Base Properties of Amino Acids

- All amino acids have at least two ionizable protons (other than R);

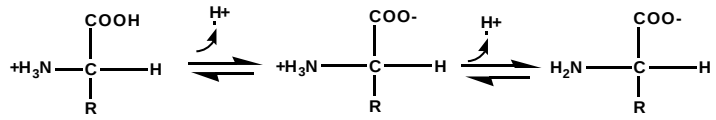


- pK_a value for:
  - amino group ~ 10
  - carboxyl groups ~ 2
  - Some R groups are ionizable

Non-ionic form of ALANINE

Zwitterions

AAs that lack charged R groups at neutral pH



Cationic Form (< pH 2)
Net (+) charge

Neutral Zwitterion Form

Anionic Form (> pH 8.5)
Net (-) charge

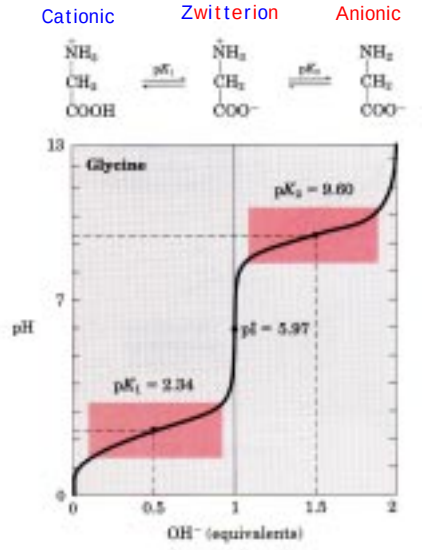
Carboxyl is deprotonated
Amino group is protonated
Charge = 0 (isoelectric pH, pI)

Gly Titration (R group -H)

- Similar to the titration of a monoprotic acid;
- Isoelectric point (pI) where net charge = 0; thus, no migration in an electric field;

$$pI = \frac{(pK_1 + pK_2)}{2}$$

- pI lies midway between the two pKa values that indicate the protonation & deprotonation of the isoionic form.



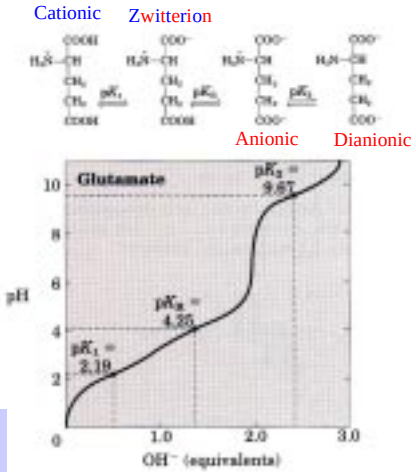
Glu Titration (R group: $-(CH_2)_2-COO^-$)

Glutamic acid = Glutamate

$$pI = \frac{(pK_1 + pK_2)}{2}$$

$$= \frac{(2.19 + 4.25)}{2}$$

$$= 3.22$$



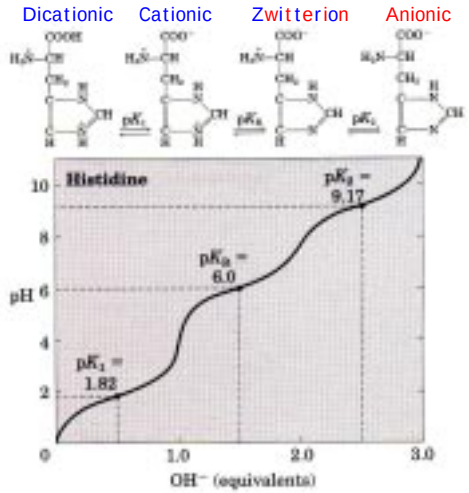
What would be the physiological state?

His Titration

- Only AA with physiological buffering power;
- $pI = \frac{(6.0 + 9.17)}{2}$

  $= 7.59$

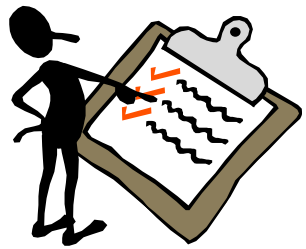
Charged R groups are extremely important in the structure and function of proteins.



AA Classsification

20 common amino acids: differ in *size, shape, charge, hydrogen bonding capability, hydrophobicity & chemical reactivity*

- Non-polar (hydrophobic)
- Charged polar
  - Negative
  - Positive
- Uncharged polar



A	Alanine	**A**lanine
V	Valine	**V**aline
L	Leucine	**L**eucine
I	Isoleucine	**I**soleucine
M	Methionine	**M**ethionine
F	Phenylalanine	**F**enylalanine
P	Proline	**P**roline
W	Tryptophan	No easy way!
G	Glycine	**G**lycine
S	Serine	**S**erine
N	Asparagine	asparagi**N**e
T	Threonine	**T**hreonine
Y	Tyrosine	t**Y**rosine
Q	Glutamine	**Q**-tamine
C	Cysteine	**C**ysteine
H	Histidine	**H**istidine
D	Aspartic Acid	asparDic acid
E	Glutamic Acid	gluE-tamic acid
K	Lysine	hij**K** -lysine
R	Arginine	**R**ginine

You need to know:

Structure of A.A.

pKa's of C, N, R groups

Name, 3 letter & 1 letter code

Chemical nature of side groups

hydrophobic

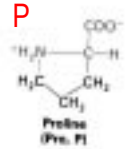
polar uncharged

charged (acid, basic)

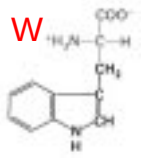
Non-polar Amino Acids (hydrophobic)

Amino acid	Three-letter code	One-letter code	R group
Glycine	Gly	G	-H
Alanine	Ala	A	-CH3
Valine	Val	V	-CH(CH3)2
Leucine	Leu	L	-CH2-CH(CH3)2
Isoleucine	Ile	I	-CH(CH3)-CH2-CH3
Methionine	Met	M	-CH2-CH2-S-CH3
Proline	Pro	P	See below
Phenylalanine	Phe	F	-CH2-C6H5
Tryptophan	Trp	W	See below

P

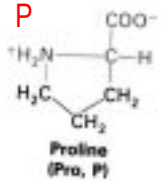


W

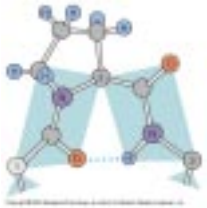


Proline;Pro;P

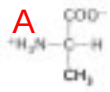
P



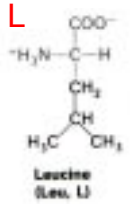
- Side chain bonded to Cα and N;
- Imposes rigid restraints on the rotation of the N-Cα peptide bond;
- Dramatically influences protein structure;
- Proline specifies bends and turns in polypeptide chains.



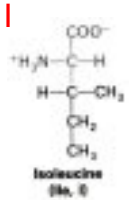
A



L



I



Simple Aliphatics

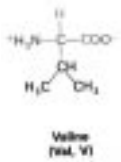
Alanine;Ala;A

Valine;Val;V

Leucine;Leu;L

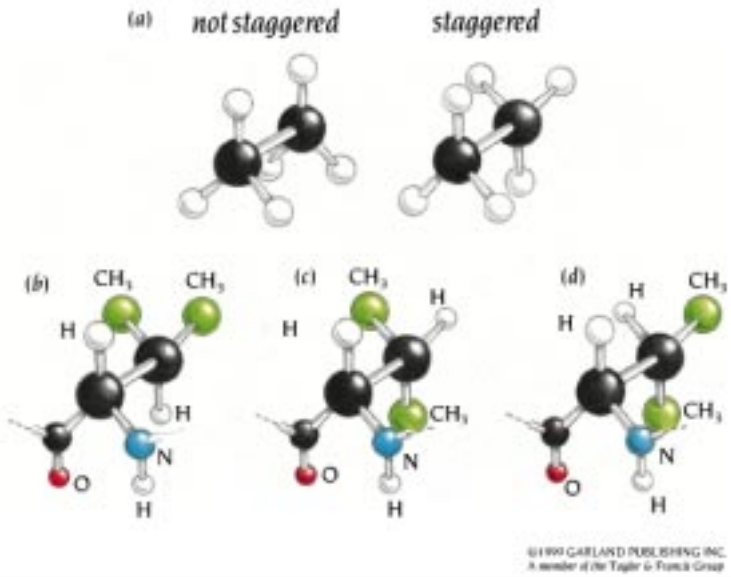
Isoleucine;Ile;I

V



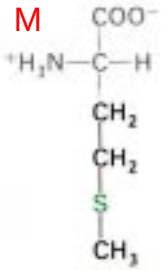
- Branched Chain Amino Acids
- Only alkyl chains (i.e. C's and H's);
- Hydrophobic (repel H_2O); Form part of hydrophobic core
- Although no reactivity they promote protein folding (Ala, Leu --> α-helix; Val, Ile --> β-sheet);
- Ala: best substitution for minimal structural and functional impact;
- Staggard rotamer conformation.

Staggered vs. not-staggered



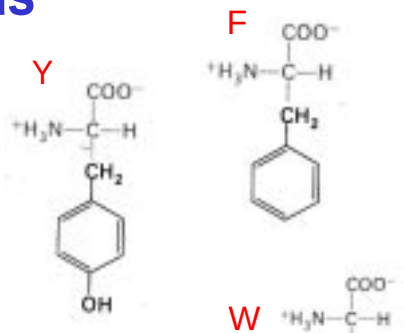
Methionine

- Thioether sidechain
- Little chemical reactivity
- Special in that it is first amino acid in a polypeptide chain

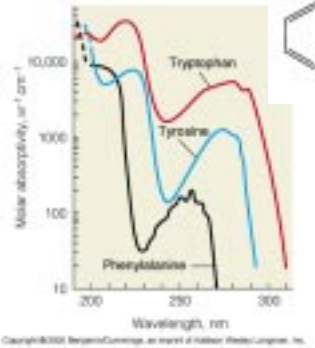


Aromatic Amino Acids

Phenylalanine;Phe;F
Tyrosine;Tyr;Y
Tryptophan;Trp;W

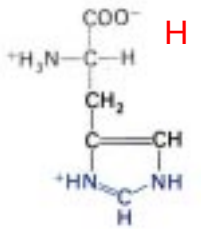


- Less abundant (especially Trp);
- Phe most abundant
- Conjugated π electron clouds confer aromatic character;
- Have ultraviolet; can be used to quantitate proteins (absorption at 280 nm)
- Presence of hydrophilic groups (Tyr O & Trp NH) permits partial solution exposure on protein surface
- Tyr can ionize at high pH (pKa = 10.5)--therefore its has polar characteristics



Charged Polar Amino Acids

Amino acid	Three-letter code	One-letter code	R group
Lysine	Lys	K	-CH2-CH2-CH2-CH2-NH2
Arginine	Arg	R	-CH2-CH2-CH2-NH-C(NH2)2
Histidine	His	H	-See below
Aspartic acid	Asp	D	-CH2-CO-OH
Glutamic acid	Glu	E	-CH2-CH2-CO-OH



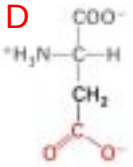
Acidic Residues

Glutamic Acid;Glu;E

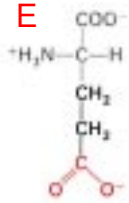
Aspartic Acid;Asp;D

- Differ by one methylene unit;
- Deprotonated at neutral pH (pKa = 3.9-4.1);
- Can serve as nucleophiles in chemical reactions;
- Love to bind metal ions, especially Ca^{++} & Mg^{++};
- H-bond donors and acceptors;
- Chemically reactive (form ester linkages that can be reduced);
- Impart electrostatic properties to proteins: charge charge interactions with substrates, other proteins, etc.

D



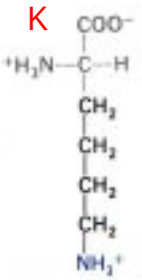
E



Basic Residues

Lysine;Lys;K and Arginine;Arg,R

K

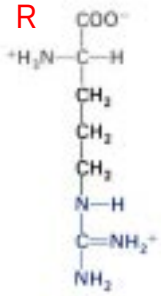


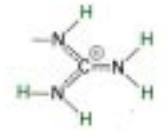
- Positively charged at neutral pH; lysine has a pKa ~ 10.5; arg pKa ~12);

  Likes protein surfaces, electrostatic interactions, especially phosphates (nucleic acids);

  Lysines are chemically reactive, NH_2 is a good nucleophile.
- Guanidinium group of arginine has three resonance forms;

R



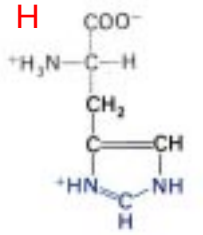


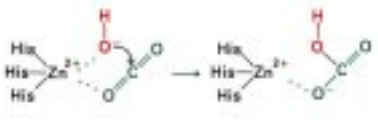
Hydrogen donor groups of arginine

Basic Residue Histidine;His;H

- Only standard amino acid with side chain pKa near neutral pH (pKa ~ 6.0)
- Two resonance forms;
- Non-protonated N is a good nucleophile loves metals especially Zn;
- Histidine containing peptides are impor biological buffers
- Involved in the active site of enzymes: can both donate and accept protons (general acid and base);
- The protonation/deprotonation reaction readily occurs at pH 7.0;
- Histidine is also aromatic, uses this with substrates

H

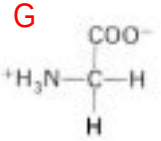




Uncharged Polar Amino Acids

Amino acid	Three-letter code	One-letter code	R group
Serine	Ser	S	-CH2-OH
Threonine	Thr	T	-CH(OH)-CH3
Asparagine	Asn	N	-CH2-CO-NH2
Glutamine	Gln	Q	-CH2-CH2-CO-NH2
Tyrosine	Tyr	Y	-CH2-C6H4-OH
Cysteine	Cys	C	-CH2-SH
Glycine	Gly	G	-H

G



Glycine;Gly;G

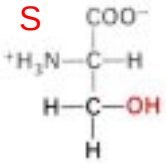
- The simplest and smallest amino acid (no side chain);
- Not asymmetric (no D-L);
- Can also be considered as a non-polar;
- Three unique properties:
  - Used where backbones closely approach each other
  - Can assume conformations forbidden to others
  - More flexible than others

Hydroxyl Residues

Serine;Ser;S

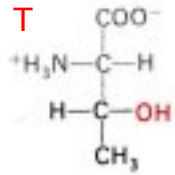
Threonine;Thr;T

S

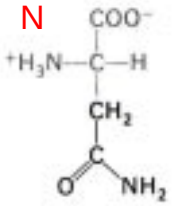


- Chemical reactivity of ethanol (pKa = 14-15);
  - Thus, proton not readily given up, BUT can be activated nonetheless;
- Polarity is contributed by hydroxyl. OH provides H-bonds to solvent and ligand;
- Thr has an additional asymmetric carbon;
- Important in protein surface hydration.

T



N

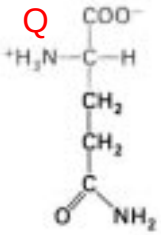


The Amides

Asparagine;Asn;N

Glutamine;Gln;Q

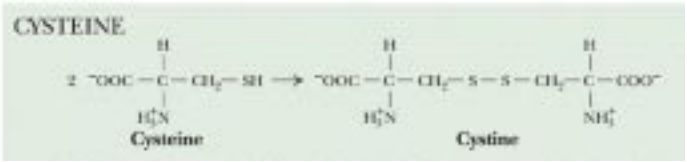
Q



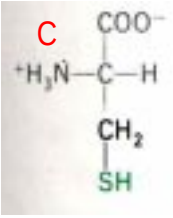
- Hydrogen bonding characteristics resembling the peptide backbone;
- Similar to acid forms;
- Chemically labile at extremes of pH;
- Most likely on protein surfaces

Cysteine;Cys;C

- Most chemically reactive of all amino acid
  - susceptible to oxidation;
  - pKa = 8.5;
- Disulfide bond (cystine) stabilizes long range interactions;
- Adds stability to 3D structure.

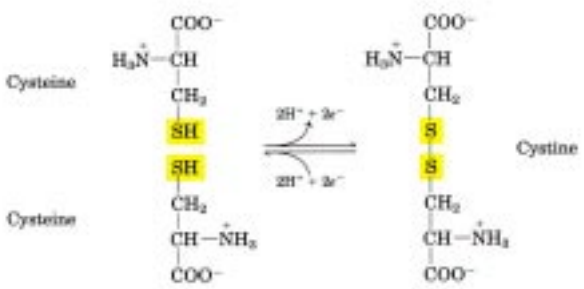


C



Disulfide Bond

Formed by oxidation of the thiol groups from two cysteines



Amino Acid Properties

Amino acids with ionizable R groups are biologically active

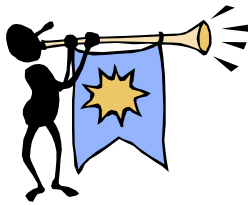
Garrett/Grisham, Biochemistry with a Human Focus
Table 4.1

Table 4.1 pK_a Values of Common Amino Acids

Amino Acid	α-COOH pK_a	α-NH_3^+ pK_a	R group pK_a
Alanine	2.4	9.7	
Arginine	2.2	9.0	12.5
Asparagine	2.0	8.8	
Aspartic acid	2.1	9.8	3.9
Cysteine	1.7	10.8	8.3
Glutamic acid	2.2	9.7	4.3
Glutamine	2.2	9.1	
Glycine	2.3	9.6	
Histidine	1.8	9.2	6.0
Isoleucine	2.4	9.7	
Leucine	2.4	9.6	
Lysine	2.2	9.0	10.5
Methionine	2.3	9.2	
Phenylalanine	1.8	9.1	
Proline	2.1	10.6	
Serine	2.2	9.2	~13
Threonine	2.6	10.4	~13
Tryptophan	2.4	9.4	
Tyrosine	2.2	9.1	10.1
Valine	2.3	9.6	

Essential AAs

- Can not be biosynthesized;
- Must be obtained from diet



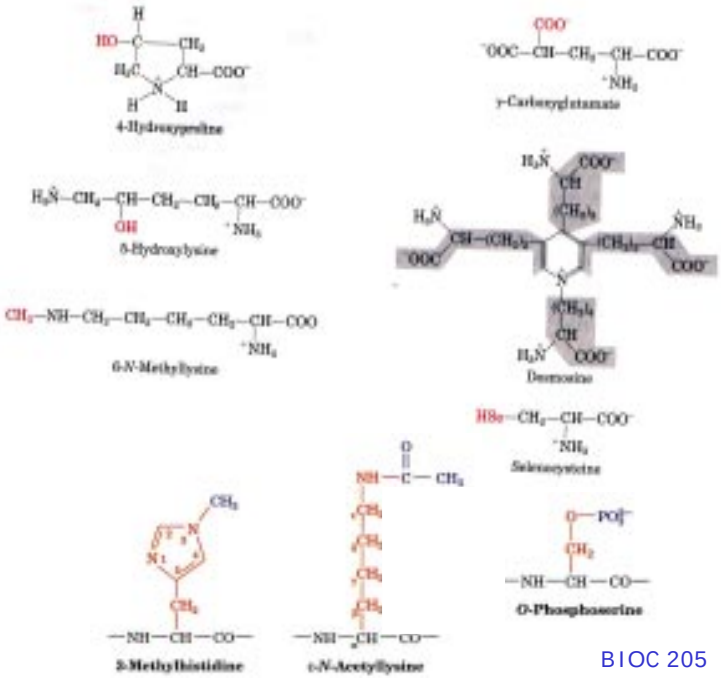
- Arg
- His
- Ile
- Lys
- Val
- Met
- Phe
- Thr
- Trp
- Leu

*Essential during growth of juveniles

PVT TIM HALL

Uncommon Amino Acids & Amino Acid Derivatives

- 4-hydroxyproline
- 5-hydroxylysine
- o-phosphoserine
- phosphotyrosine
- γ-carboxyglutama
- 3-methylhistidine
- ε-N-acetyllysine



Review

What are the four features of AA structure?

What are the pKa values for:

- amino groups?
- carboxyl groups?
- which R groups are ionizable? What are their pKas?

What is a zwitterion?

How do you calculate the pI of an AA?

What are the structures of the 20 AAs?

How are the 20 AAs classified? Where would you find hydrophobic AAs in a protein? Where would charged groups be found?

Thinking ahead: do you think that there are many free hydrogen bond donors or acceptors in the interior of a protein?

What is an essential AA?