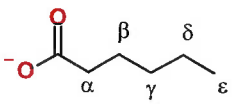
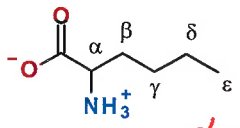
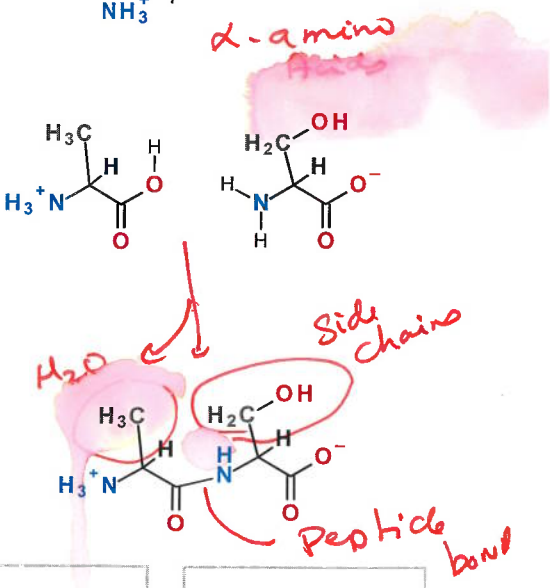
Lecture 5: Amino Acids:

A. Structure and Properties:





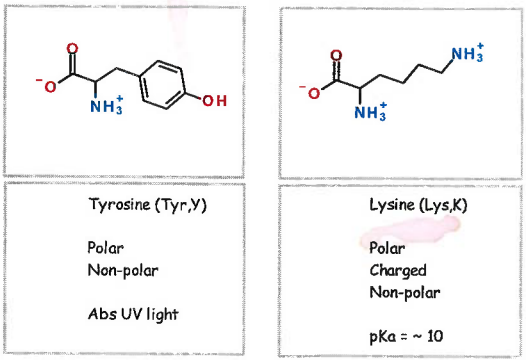
- An amino acid is a carboxylic acid with an amino group. The first carbon after the carbonyl carbon is the α-carbon. Most biological amino acids are α-amino acids because the amino group is attached to the α-carbon.
- The "mainchain" or "backbone" atoms (N, C_α, H_α, C=O) are the same in each of the 20 commonly found amino acids.
- The sidechain atoms are unique to each amino acid and give rise to the unique properties of that amino acid.
- The sidechain atoms are designated with Greek letters, based on the nomenclature for carboxylic acids.
- The pK_a of the carboxylate is ~2.0 and that of the amino group is ~9.0. Sidechain ionizable groups are also found on some amino acids, see next page.
- Amino acids are joined together to form linear polymers by the formation of a **peptide bond** between the carboxyl of one amino acids and the amino group of the next. This reaction releases water and is thus **dehydration** reaction. It is also referred to as a **condensation** reaction because two amino acids are condensed into one.
- The peptide bond can be broken by the addition of water, a reaction called **hydrolysis** *(hydro-lysis)*.



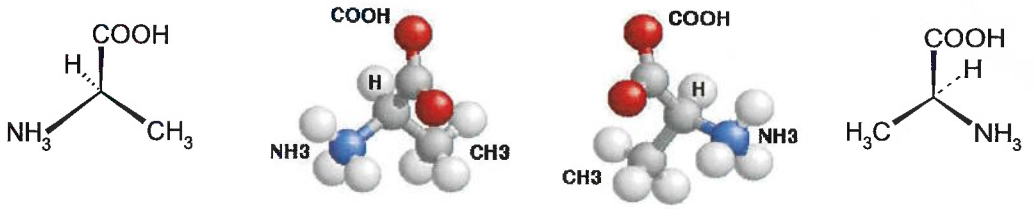
Expectations:

- Full name of each (20) amino acid+Se-Methionine
- 3 Letter & 1 letter code
- Structure of each amino acid
- Properties of the side chains:
  - i) Ionization of groups (pK_a)
  - ii) H-bonding capability
  - iii) Functional groups (polar/nonpolar)
- UV absorbance, calculation of protein concentration.

Flash card examples →



B. Chirality & Optical Activity: In all amino acids (except glycine) the α-carbon is chiral. In some amino acids, additional chiral centers are present. These are chiral centers because all four groups attached to the carbon are different. This means that the mirror images of these compounds cannot be superimposed. The two mirror images are called ***enantiomers***.

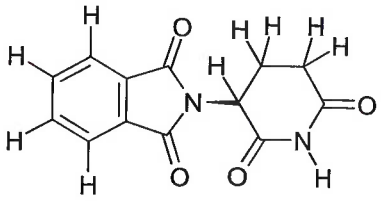


Enantiomers have the following attributes:

- *Identical* physical properties (except rotation of polarized light).
- Markedly *different* biological properties.
- Most common amino acids have an S configuration. An older, but very much used, notation is D and L. This notation is based on the chirality of a reference compound and **all amino acids that are found in proteins that are ribosomal in origin are L.**

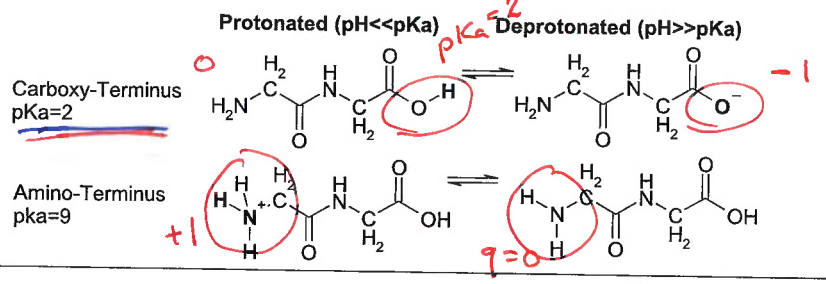
Importance of chirality in Biology: Usually only one enantiomer is active in biological systems. As indicated above, only L-amino acids are used to make proteins on the ribosome. Amino acids of the other enantiomer (D) are generally harmless. This is not always the case for other compounds with chiral centers.

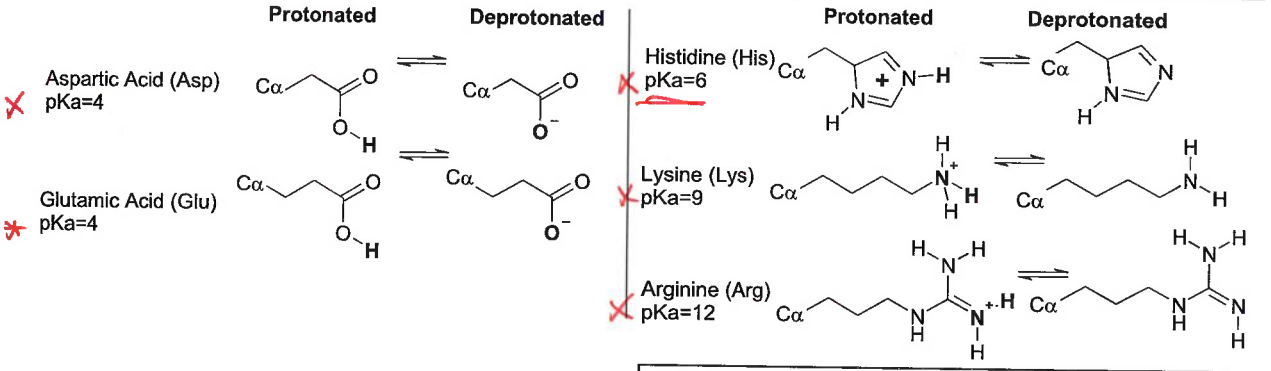
Thalidomide. This drug was prescribed as a sedative in the late 50s and early 60s. It was withdrawn because it causes birth defects by interfering with the development of the baby (**teratogens**). This activity is associated with only one enantiomer. The other enantiomer is safe.



C. Ionization Properties of Amino Acids.

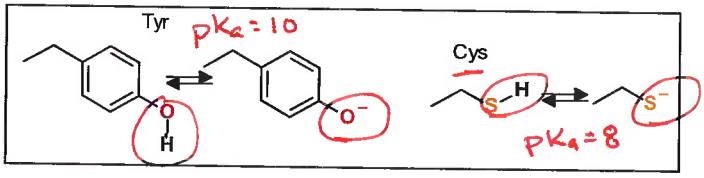
Ionizable Amino Acids/Protonated - deprotonated forms



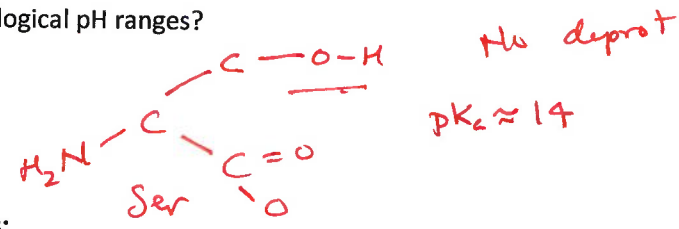


Other sidechain ionizations that are typically less important for function:

- Tyr-OH pK_a=10, Cys-SH, pK_a=8.



** Which groups don't ionize at physiological pH ranges?



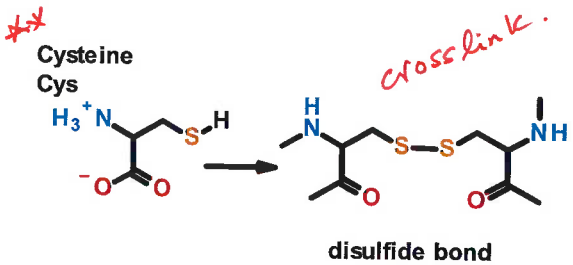
Amino acids with unique properties:

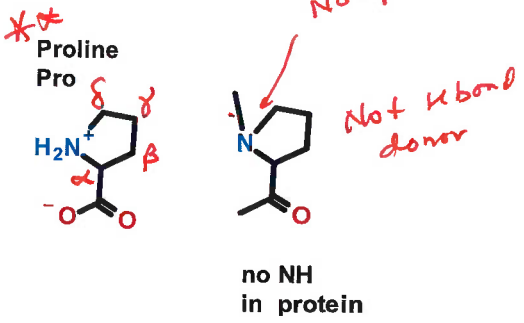
Cysteine:

- Forms disulfide bond by oxidation of the sulfur – crosslink stabilizes proteins.

Proline:

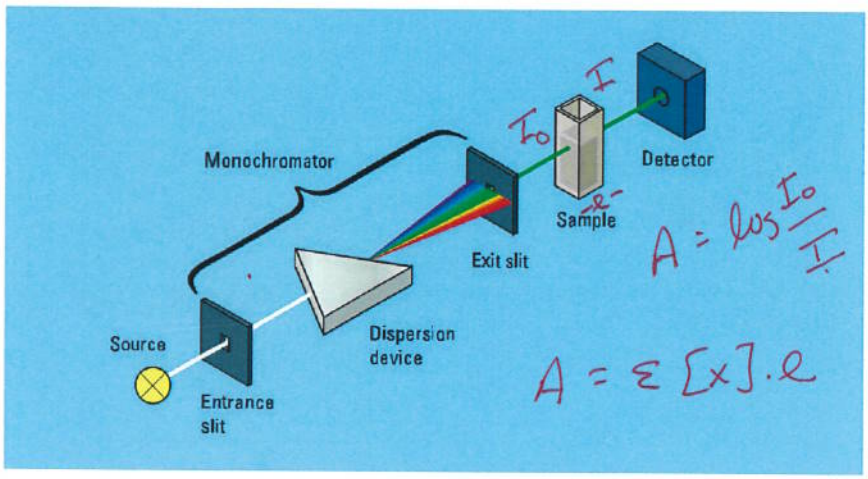
- No H-bond donor when incorporated into proteins.
- Ring reduces flexibility of sidechain and the mainchain atoms.

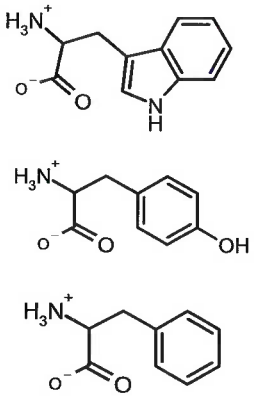




UV Absorption Properties of Amino Acids

Three aromatic amino acids absorb light in the ultraviolet range (UV).





The extinction coefficients (or molar absorption coefficients) of these amino acids are:

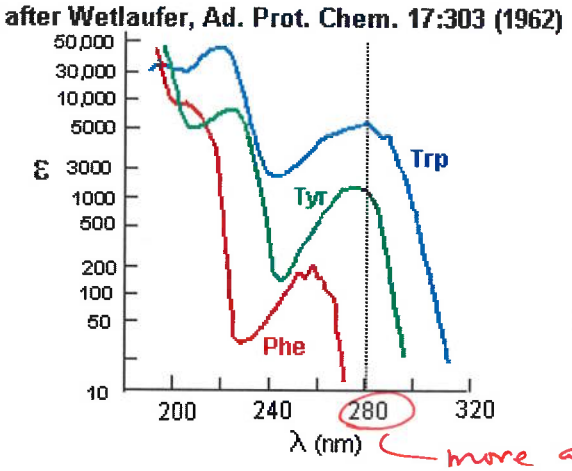
Amino acid	Extinction Coefficient $\varepsilon(\lambda_{MAX})$
Trp	5,500 $M^{-1}cm^{-1}$ (280 nm)
Tyr	1,490 $M^{-1}cm^{-1}$ (274 nm~280nm)
Phe	~0 $M^{-1}cm^{-1}$ (280 nm)

The amount of light absorbed by a solution of concentration [X] is given by the Beer-Lambert Law:

$$A = \log \frac{I_0}{I} = \varepsilon [X] l$$

where

- A is the absorbance of the sample;
- I_0 is the intensity of the incident light;
- I is the intensity of the light that leaves the sample.
- ε is the molar extinction coefficient at a specific wavelength, *e.g.* at λ_{max};
- [X] is the concentration of the absorbing species
- *l* is the path length (usually 1 cm).



Therefore, given an extinction coefficient it is possible to measure the concentration of a protein.

Calculation of molar extinction coefficients: If a molecule contains a mixture of N different chromophores, the molar extinction coefficient can generally be calculated as the sum of the molar extinction coefficient for each absorbing group in the protein:

$$\varepsilon_{\text{Protein}} = \sum_{i=1}^{N} \varepsilon_i = n_{Tyr} \cdot \varepsilon_{Tyr} + n_{Trp} \cdot \varepsilon_{Trp}$$

Therefore, the molar extinction coefficient for a protein can be calculated from its amino acid composition.

Example:

i) A protein has two Tryptophan (Trp) residues and one Tyrosine (Tyr) what is its extinction coefficient?

$$\varepsilon^{280}_{\text{Protein}} = (1)(1490) + 2(5500) = 12490\ M^{-1}cm^{-1}$$

ii) The absorption of a solution of the protein is 0.5 with a path length of 1 cm. What is the concentration of the protein?

$\ell = 1$

$$A = \varepsilon [x] \cdot \ell$$

$$[x] = \frac{A}{\varepsilon \ell}$$

$= 0.5 / (12490 \cdot 1)$

4×10^{-5} M.