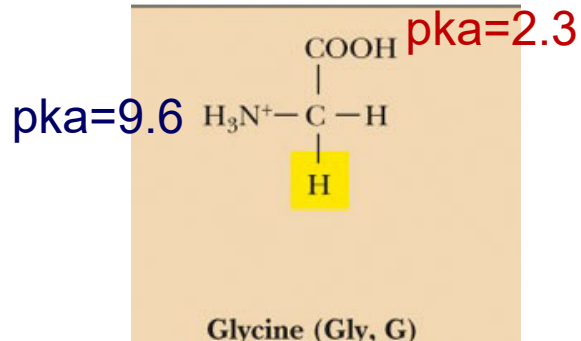


Example

What is the pH of a **glycine** solution if αNH_3^+ is $1/3$ dissociated?



$$\bullet \text{ pH} = \text{pK}_{a1} + \log \frac{[\text{Base}]}{[\text{Acid}]}$$

$$\begin{aligned} \text{pH} &= \text{pK}_a + \log \left(\frac{[\text{Gly}^-]}{[\text{Gly}^0]} \right) \\ &= 9.6 + \log(1/2) \\ &= 9.3 \end{aligned}$$

Example

- a) What is the pH of a 0.3M solution of **Leucine hydrochloride** (fully protonated form, H_2A^+) solution ?
- b) What is the pH of a 0.3M **sodium Leucinate** (fully deprotonated form, A^-) (?)
- c) What is the pH of a 0.3M solution of **isoelectric Leucine**?

a)

$\text{pK}_a=2.4$ (pK_a of αCOOH) & $\text{pK}_a=9.6$ (pK_a of αNH_3^+)

$$\text{K}_a=10^{-\text{pK}_a}=10^{-2.4}=3.98\times 10^{-3}$$

Assume only a small amount of H_2A^+ dissociated



$0.3-x$

x

x

$$\text{K}_a = \frac{[\text{HA}^0][\text{H}_3\text{O}^+]}{[\text{H}_2\text{A}^+]}$$

$$=x^2/0.3-x$$

$$X=3.45\times 10^{-2}$$

$$\text{pH}=-\log(X=3.45\times 10^{-2})$$

$$\text{pH}=1.46$$

b)



Assume only a small amount of A^- protonated

$\text{pK}_a=9.6$ (pK_a of αNH_3^+), $\text{K}_b=14-9.6=4.4$

$$\text{K}_b=10^{-\text{pK}_b}=10^{-4.4}=3.98\times 10^{-5}$$

$$\text{Then pH}=14-2.46=11.54$$

$$\text{K}_b = \frac{[\text{HA}^0][\text{OH}^-]}{[\text{A}^-]}$$

$$=x^2/0.3-x$$

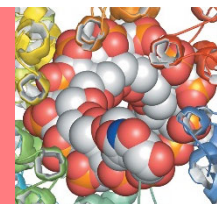
$$X=3.46\times 10^{-3}=[\text{OH}^-]$$

$$\text{pOH}=2.46$$

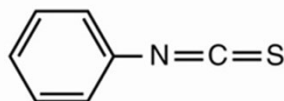
c)

$$\text{isoelectric Leucine}= 2.4+9.6/2=6.0$$

4.3 What Reactions Do Amino Acids Undergo?

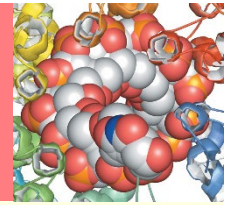


- A number of reactions of amino acids are noteworthy because they are essential to the **degradation, sequencing, and chemical synthesis of peptides and protein.**
- **Carboxyl** groups form **amides & esters**
- **Amino** groups form **Schiff bases** and **amides**



- **Edman reagent (phenylisothiocyanate)** reacts with the α -amino group of an amino acid or peptide to produce a phenylthiohydantoin (**PTH**) derivative.

Edman's reagent



PITC(phenylisothiocyanate)
Edman's reagent

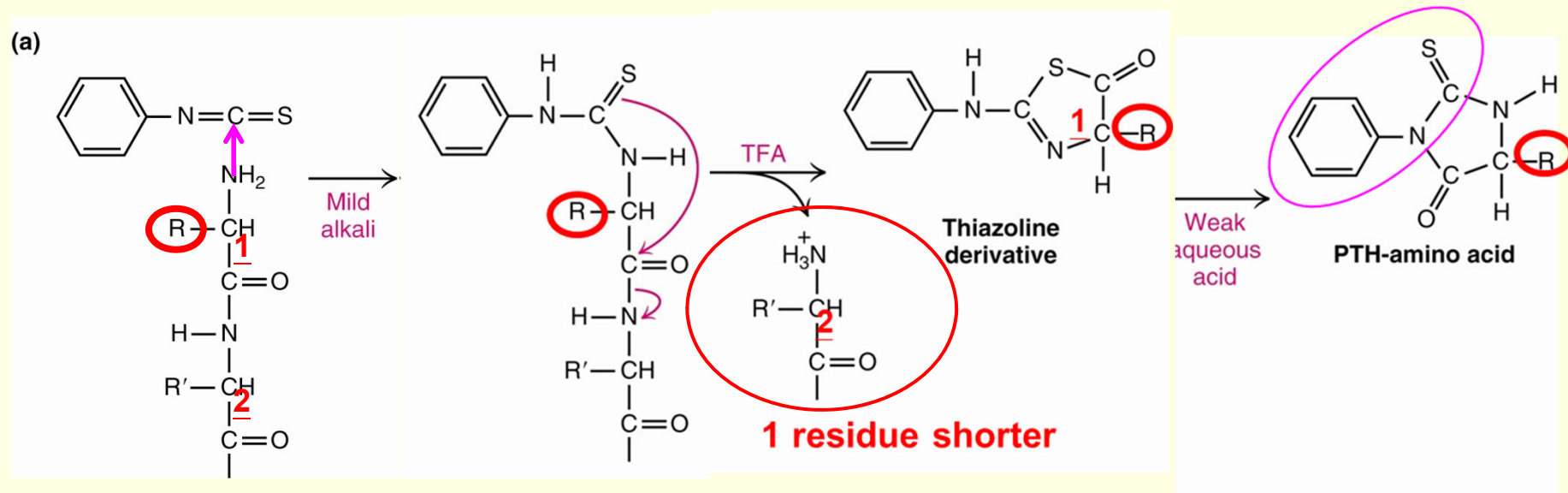
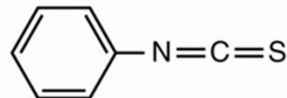
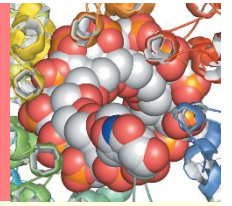


Figure 4.8 (a) **Edman's reagent** reacts with the **N-terminal amino acid** of a peptide or protein to form a **cyclic thiazoline derivative** that reacts in weak aqueous acid to form a **PTH-amino acid**.

4.3 What Reactions Do Amino Acids Undergo?



- **Side chains(R)** show unique reactivity.
Few reactions are specific to a single kind of side chain.
- **Cys** residues can form **disulfides**.
In protein disulfide linkage stabilize protein structure.

Reactions of Amino Acids

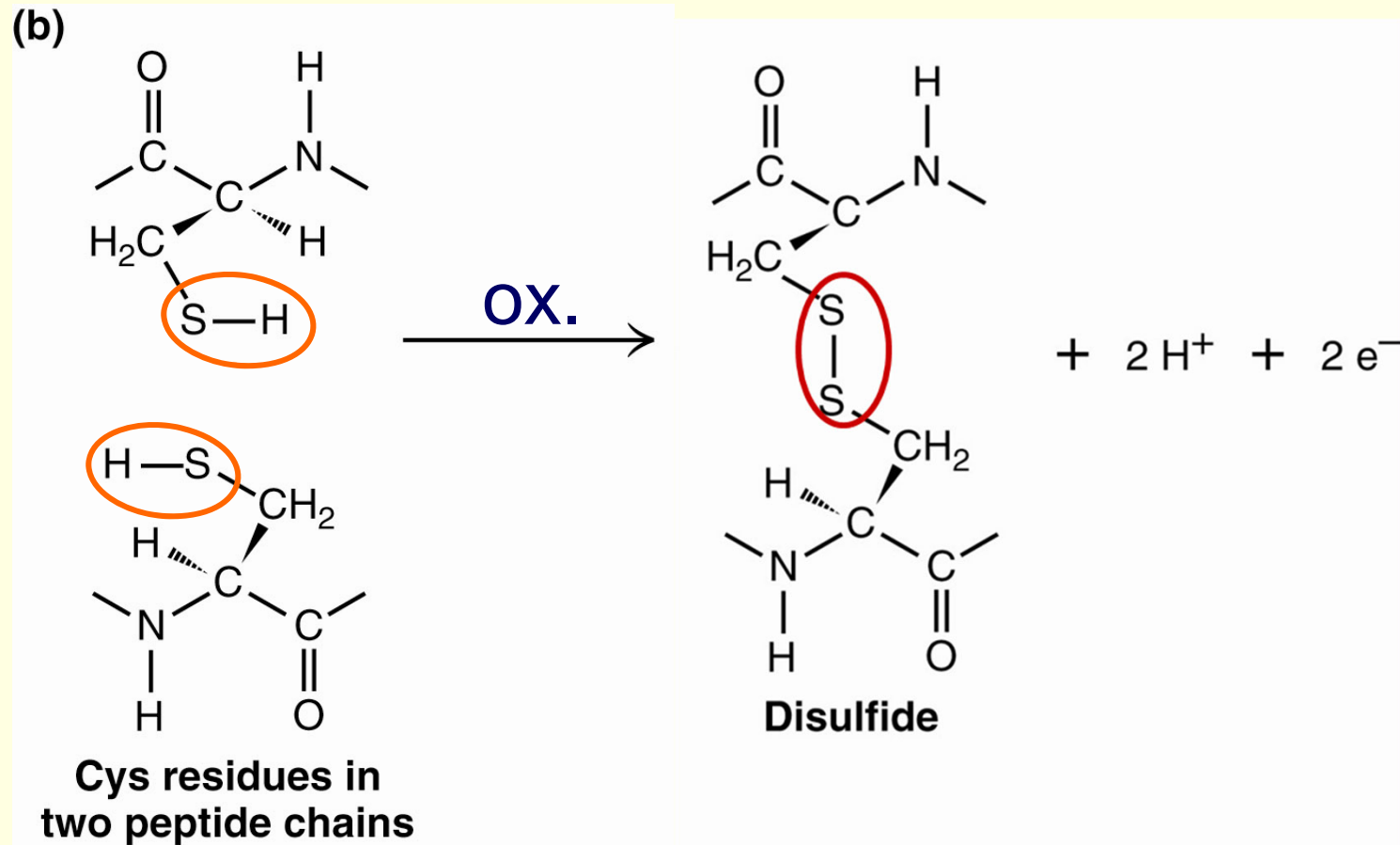
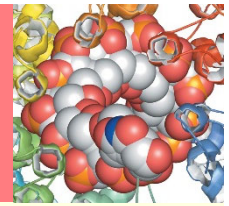
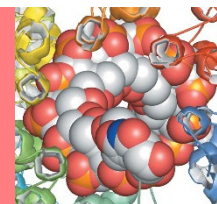


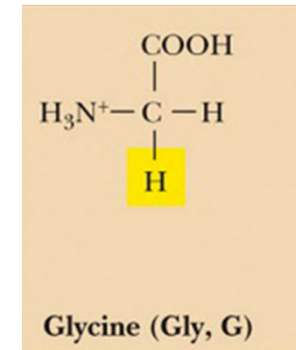
Figure 4.8

(b) Cysteine residues react with each other to form **disulfides**. The disulfide linkages that **stabilize** protein structure.

4.4a Amino Acids are Chiral Molecules



- Amino Acids are **Chiral** Molecules
- The α -carbon is “**asymmetric**” or “**chiral**” (from the Greek *cheir*, meaning “hand”)
- **Two** possible **configurations** for the α -carbon constitute nonsuperimposable **mirror image** isomers, or **enantiomers**
- Enantiomeric molecules display a special property called **optical activity**—the ability to rotate the plane of polarization of plane-polarized light.
- **Clockwise** rotation of incident light is referred to as **Dextrorotatory (D)** behavior
- **Counterclockwise** rotation is called **Levorotatory (L)** behavior.
- **(+)** for **Dextrorotatory** compounds
- **(-)** for **Levorotatory** compounds



All amino acids but **glycine** are **chiral**

L-amino acids predominate in nature

D,L-nomenclature is based on D- and L-glyceraldehyde

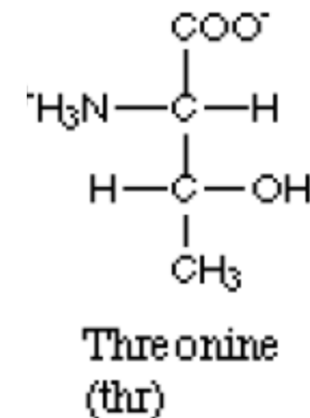
R,S-nomenclature system is superior,

Proposed in 1956 by Robert Cahn, Sir Christopher Ingold and Vladimir Prelog

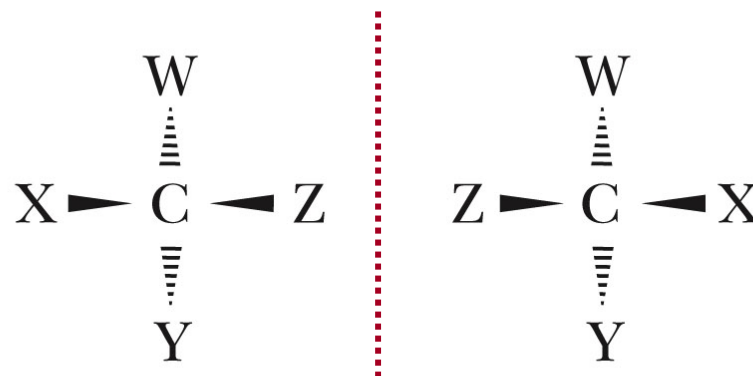
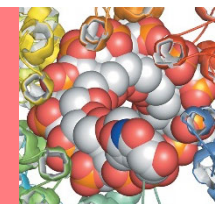
Isoleucine and **threonine**

(with **two** chiral centers) can be named
Unambiguously L-Threonine (2S,3R)

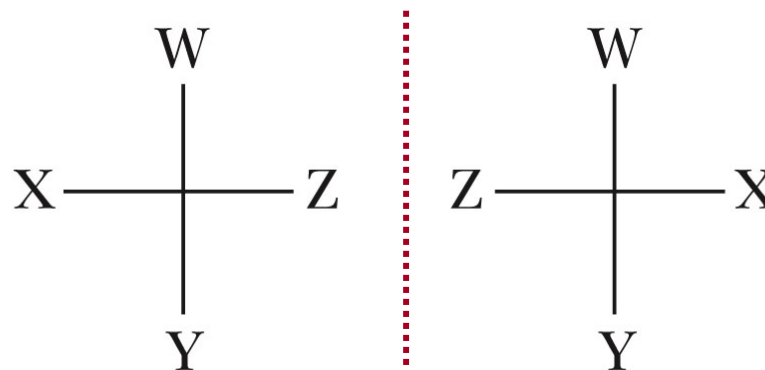
Enantiomeric isomers



4.4b Chiral Molecules Are Described by the D,L and (R,S) Naming Conventions

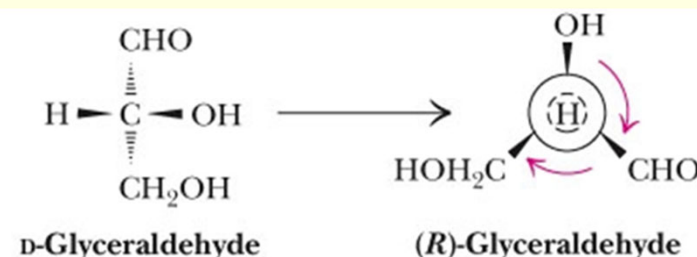
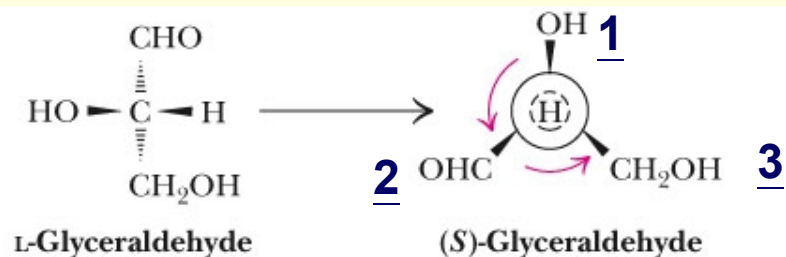
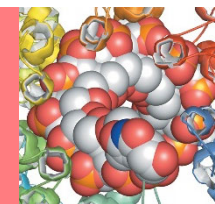


Perspective drawing

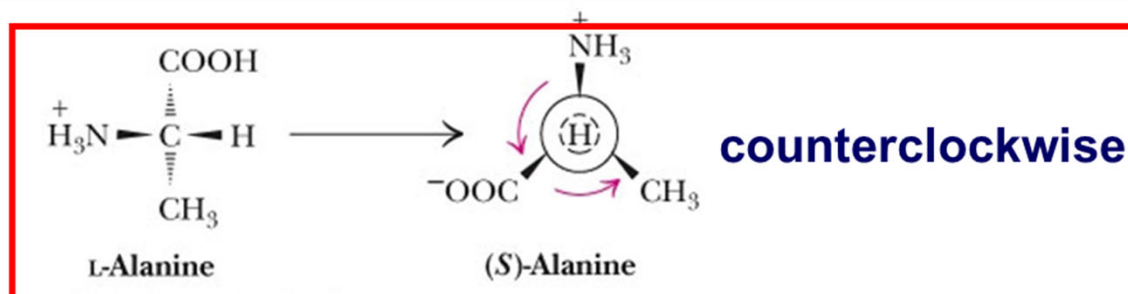


Fischer projections

Rules for Description of **Chiral** Centers in the **(R,S)** System



L-amino acids predominate in nature



▲ The assignment of (R) and (S) notation for glyceraldehyde and L-alanine.

Naming a chiral center in the **(R,S)** system is accomplished by **viewing the molecule from the chiral center** to the atom with the lowest priority.

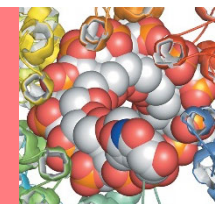
R=D is from the Latin *rectus* meaning “**right**”

S = L is from the Latin *sinistrus* meaning “**left**”

The priorities of the functional groups are:

SH > OH > NH₂ > COOH > CHO > CH₂OH > CH₃

Discovery of Optically Active Molecules and Determination of Absolute Configuration



a **six**-carbon sugar

4 asymmetric centers

each **C** could be either of **2** configurations,
glucose conceivably could exist in any one of **16**
possible isomeric structures.

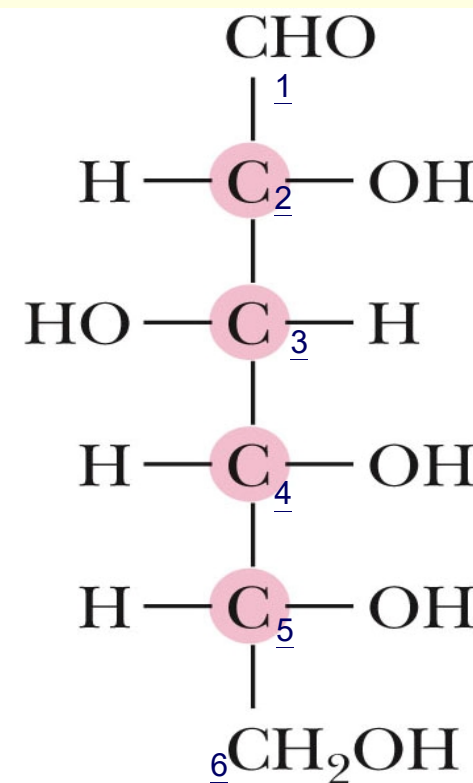
It took three years to complete the solution of an elaborate chemical and logical puzzle.

1891, Fischer had reduced his puzzle to a choice between **two** enantiomeric structures.

Methods for determining *absolute* configuration were not yet available, so Fischer made a simple guess.

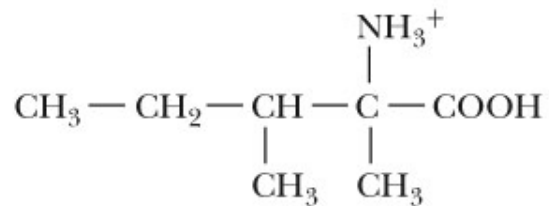
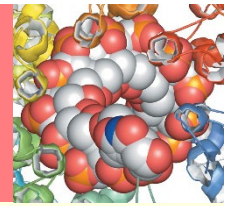
Nobel Prize in chemistry in 1902.

Fischer's proposed structure was confirmed by J. M. Bijvoet in 1951 (by **X-ray** diffraction).

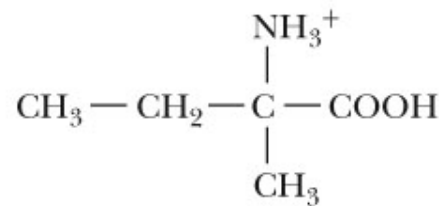


**The absolute
configuration of
(+)-glucose “D”
(2R,3S,4R,5R)**

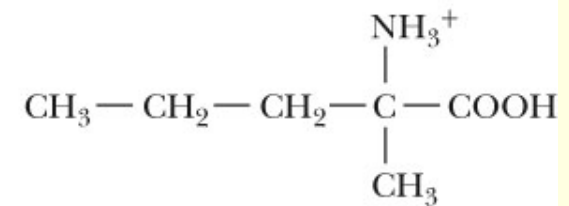
The Murchison Meteorite – Discovery of Extraterrestrial **Handedness**



2-Amino-2,3-dimethylpentanoic acid*



Isovaline



α -Methylnorvaline

▲ Amino acids found in the Murchison meteorite.

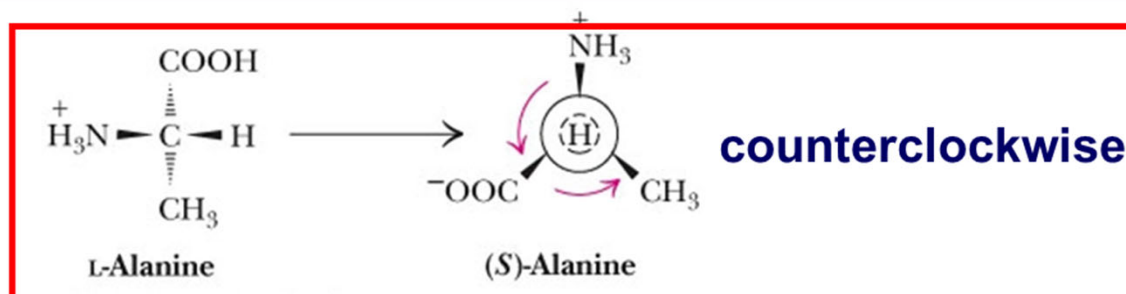
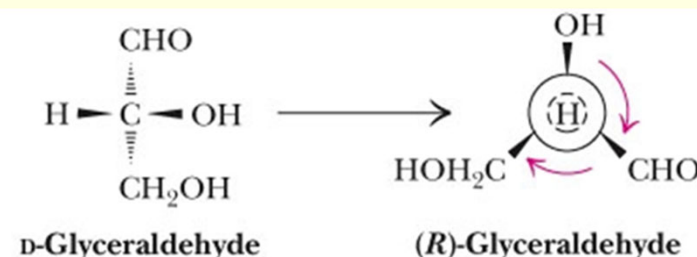
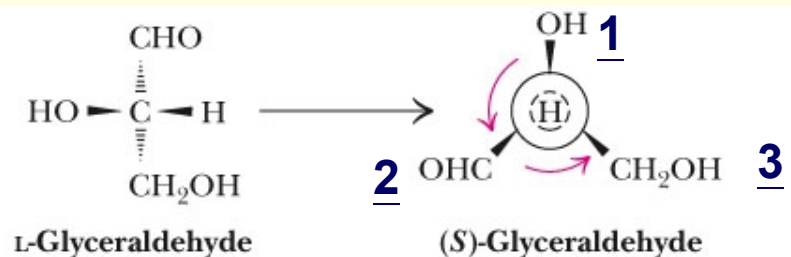
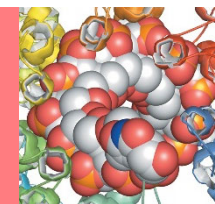
Why do **L**-amino acids predominate in biological systems?

What process might have selected **L-amino acids** over their D- counterparts?

The meteorite found near Murchison, Australia may provide answers. Certain amino acids found in the meteorite have been found to have **L-enantiomeric** excesses of 2% to 9%.

This may be the first demonstration that a **natural L-enantiomer** enrichment occurs in certain cosmological environments.

Rules for Description of **Chiral Centers** in the **(R,S)** System



▲ The assignment of (*R*) and (*S*) notation for glyceraldehyde and L-alanine.

Naming a chiral center in the (**R,S**) system is accomplished by **viewing the molecule from the chiral center** to the atom with the lowest priority.

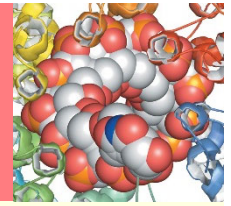
R=D is from the Latin *rectus* meaning “**right**”

S = L is from the Latin *sinistrus* meaning “**left**”

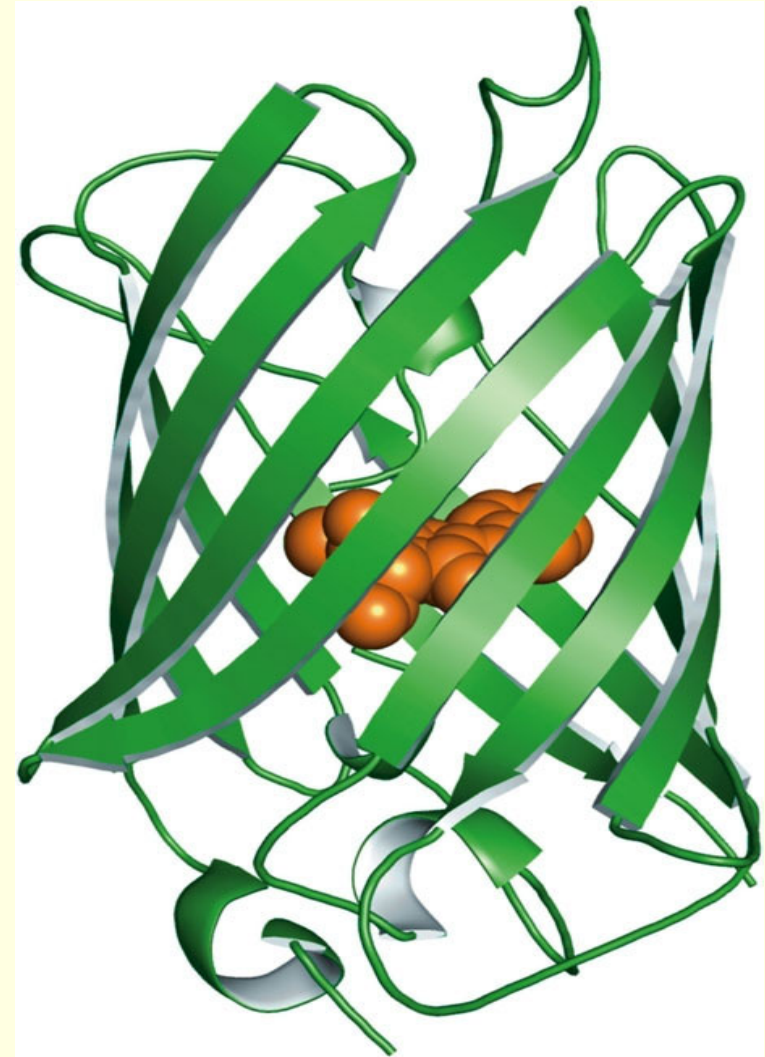
The priorities of the functional groups are:

SH > OH > NH₂ > COOH > CHO > CH₂OH > CH₃

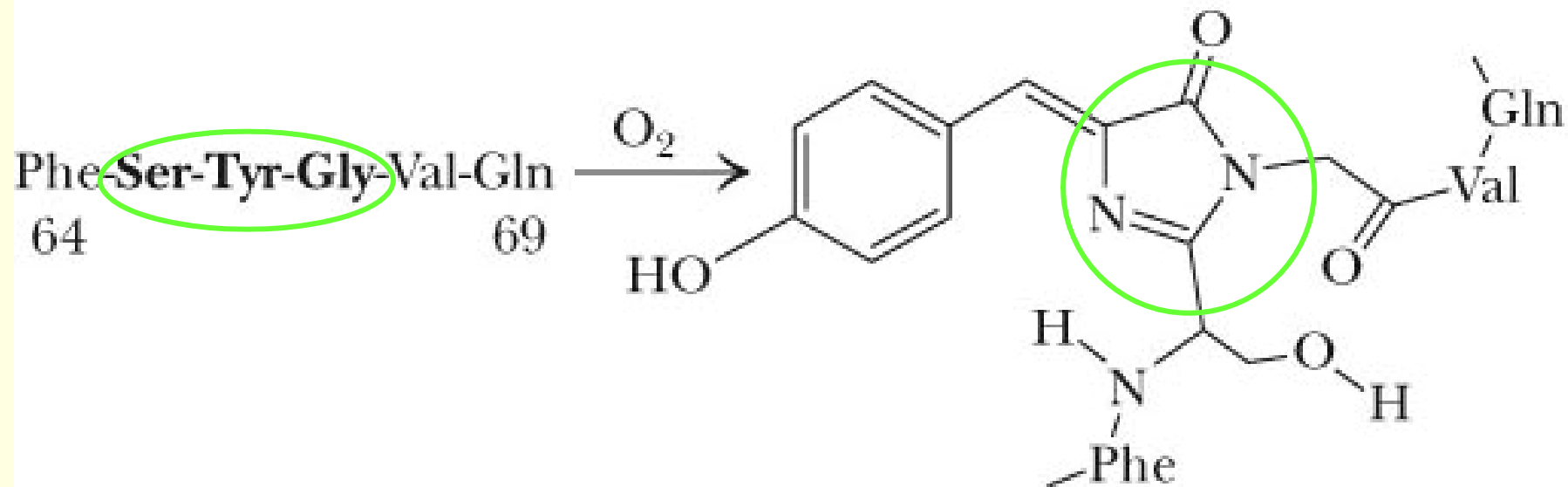
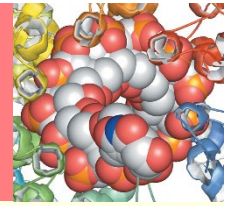
Green Fluorescent Protein (GFP)



- A jellyfish (*Aequorea victoria*) native to the northwest Pacific Ocean contains a **green fluorescent protein**.
- Provide a **defense** mechanism for jellyfish
- GFP** is a naturally fluorescent protein.
- The light-transducing capability of **GFP** is the result of a reaction between 3 amino acids (**serine, tyrosine, and glycine**) in the protein itself.
- To form the **pigment** complex—termed a **chromophore**.
- No** enzymes are required; the reaction is **autocatalytic**.
- Genetic engineering techniques can be used to “**tag**” virtually any protein, structure, or organelle in a cell.
- The **GFP chromophore** lies in the center of a β -barrel protein structure.

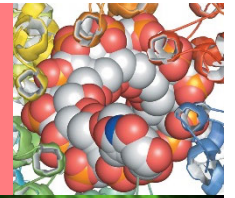


Green Fluorescent Protein



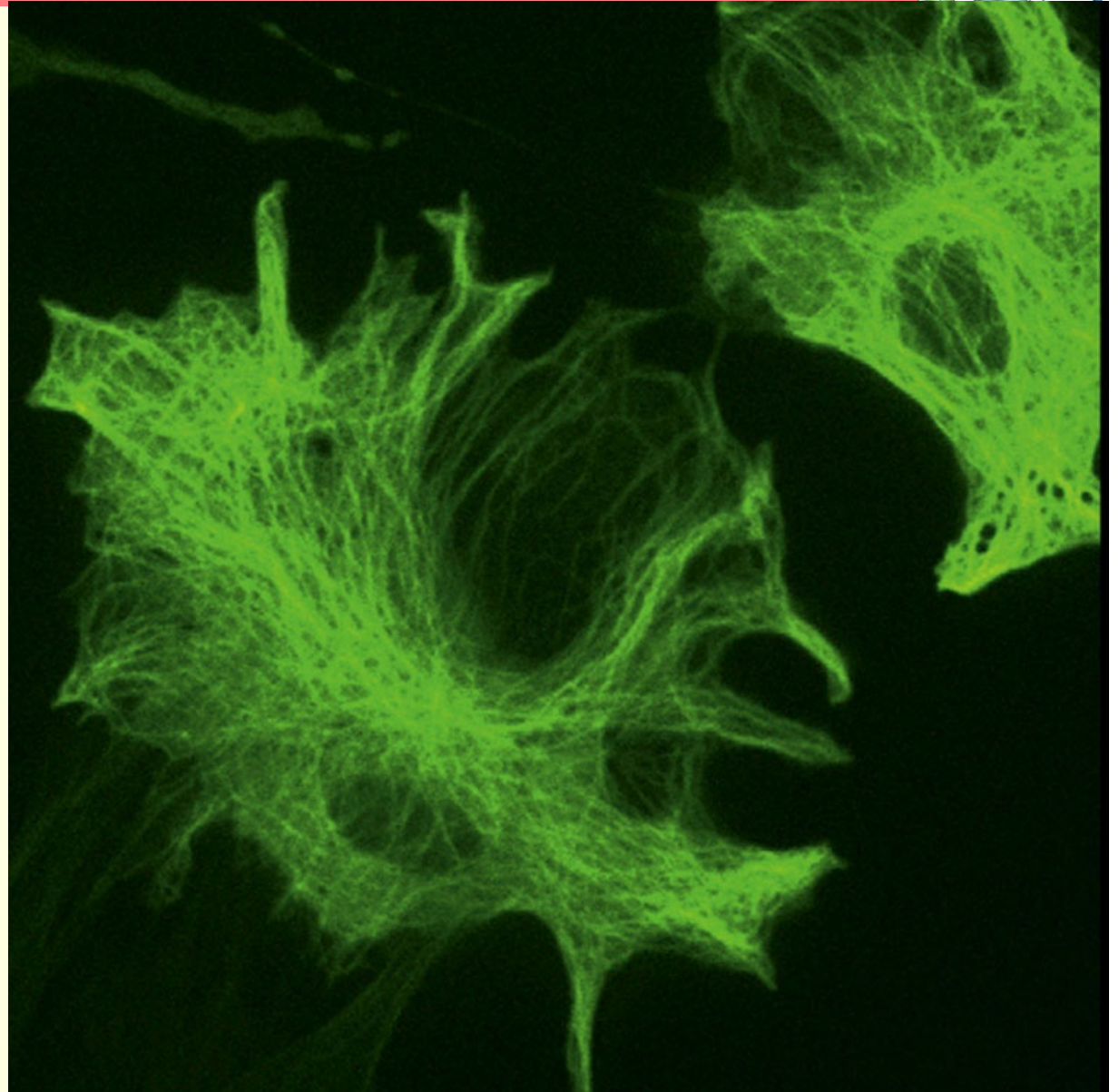
The prosthetic group of GFP is an oxidative product of the sequence –FSYGVQ–.

Yellow fluorescent protein



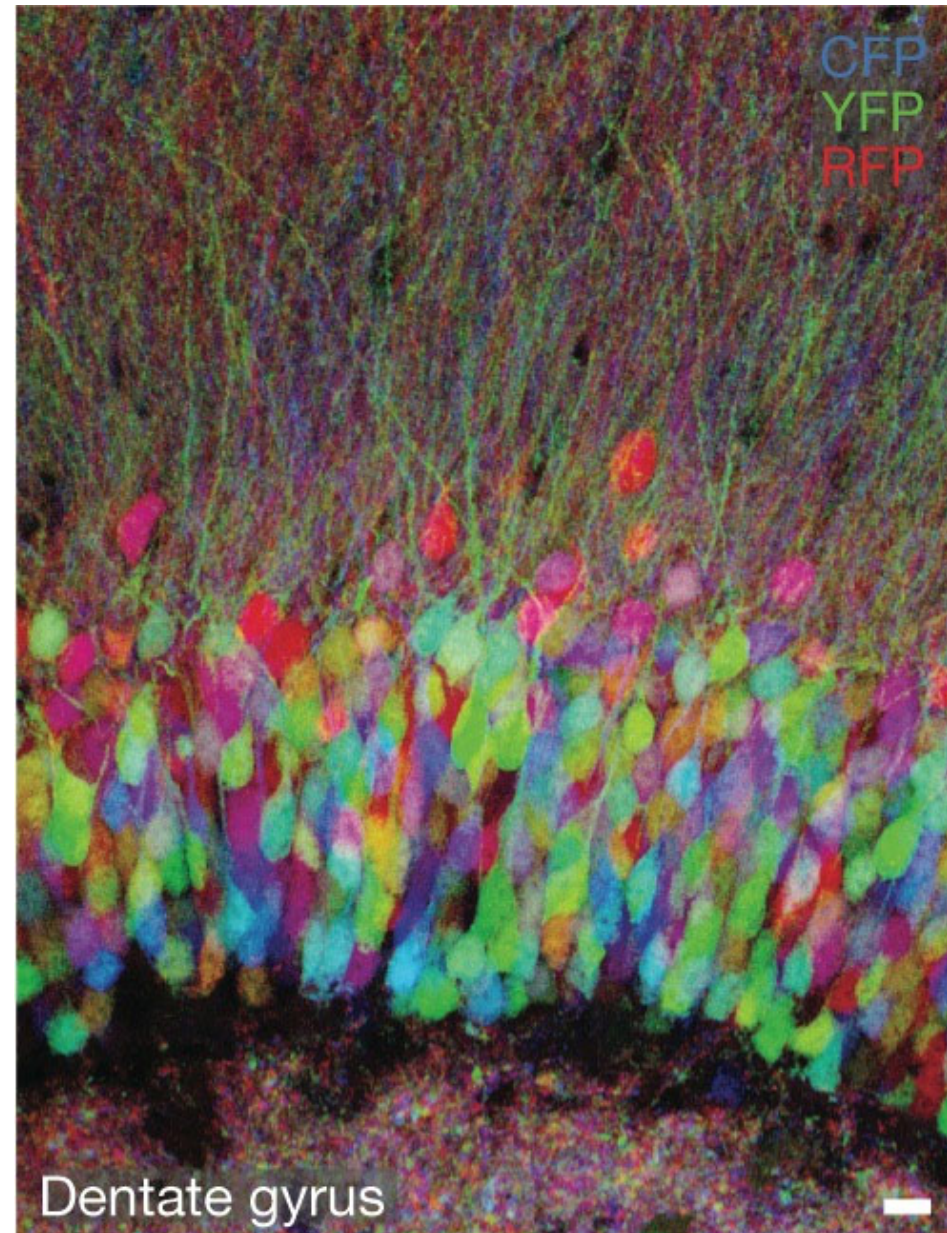
- Amino acid substitutions in GFP can **tune** the color of emitted light.

- African green monkey kidney cells expressing **yellow fluorescent protein (YFP)** fused to α -tubulin, a cytoskeletal protein.

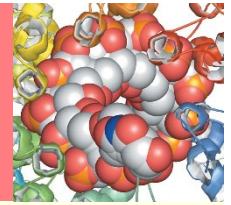


Expression of combinations of **3 different fluorescent proteins (CFP/YFP/RFP)** in a mouse brain produces ten different colorations of neurons. Individual neurons in a mouse brain appear in different colors in a fluorescence microscope.

This “**Brainbow**” method enables many distinct cells within a brain circuit to be viewed at one time. This technique may facilitate analysis of **neuronal circuits** on a large scale.

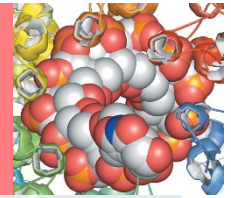


4.5a Phenylalanine, Tyrosine, and Tryptophan Absorb Ultraviolet Light



- All amino acids absorb at **infrared** wavelengths (780nm -1 mm)
- Only **Phe, Tyr, and Trp** absorb **UV** (100-400 nm)
- Absorbance at **280 nm** is a good diagnostic device for amino acids
- The aromatic amino acids also exhibit relatively weak **fluorescence**
- **Tryptophan** can exhibit weak fluorescence
Phosphorescence—a relatively long-lived emission of light.
- These **fluorescence and phosphorescence** properties are especially useful in the study of protein structure and dynamics

Spectroscopic Properties



Phe, Tyr, and Trp

UV spectra of the aromatic amino acids at pH 6.

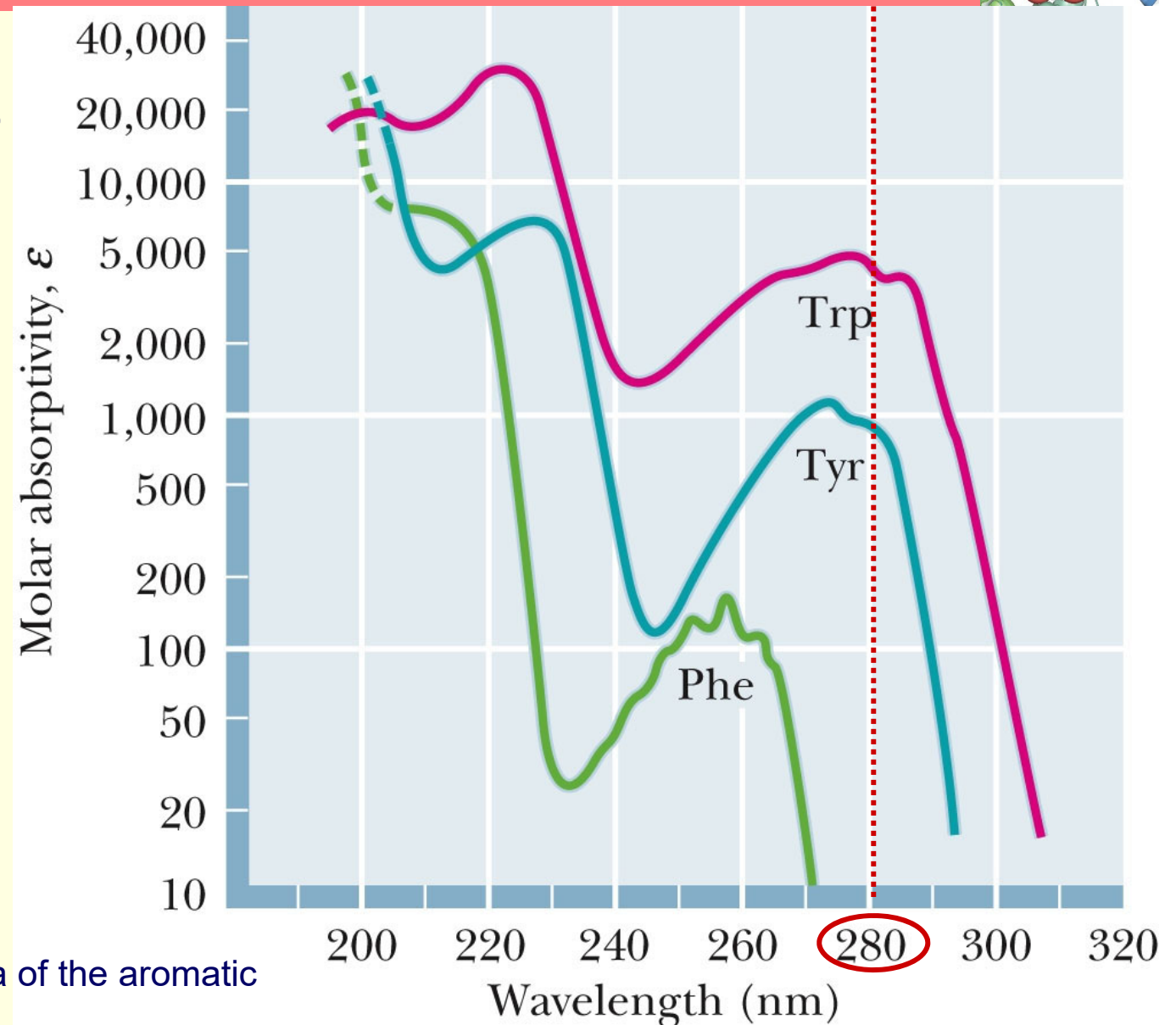
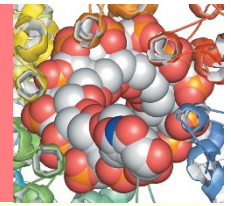


Figure 4.10 The UV spectra of the aromatic amino acids at pH 6.

4.5b Amino Acids can be Characterized by Nuclear Magnetic Resonance (NMR)



- **NMR spectra** are characteristic of each residue in a protein, and high resolution NMR measurements can be used to elucidate 3D structures of proteins
- All isotopes that contain an **odd** number of protons and/ or neutrons have an **intrinsic magnetic moment**
- Apply a very **strong magnetic field**, the energy level will be different because of the direction of the magnetic field and the energy difference.
- The most commonly studied nuclei are ^1H and ^{13}C

分子環境受到相鄰原子磁場的影響，相互遮蔽，因而有特殊的共振頻率，分佈於低-高磁場區
遮蔽效應越大的，共振頻率降低；高場區(Upfield)。

Spectroscopic Properties

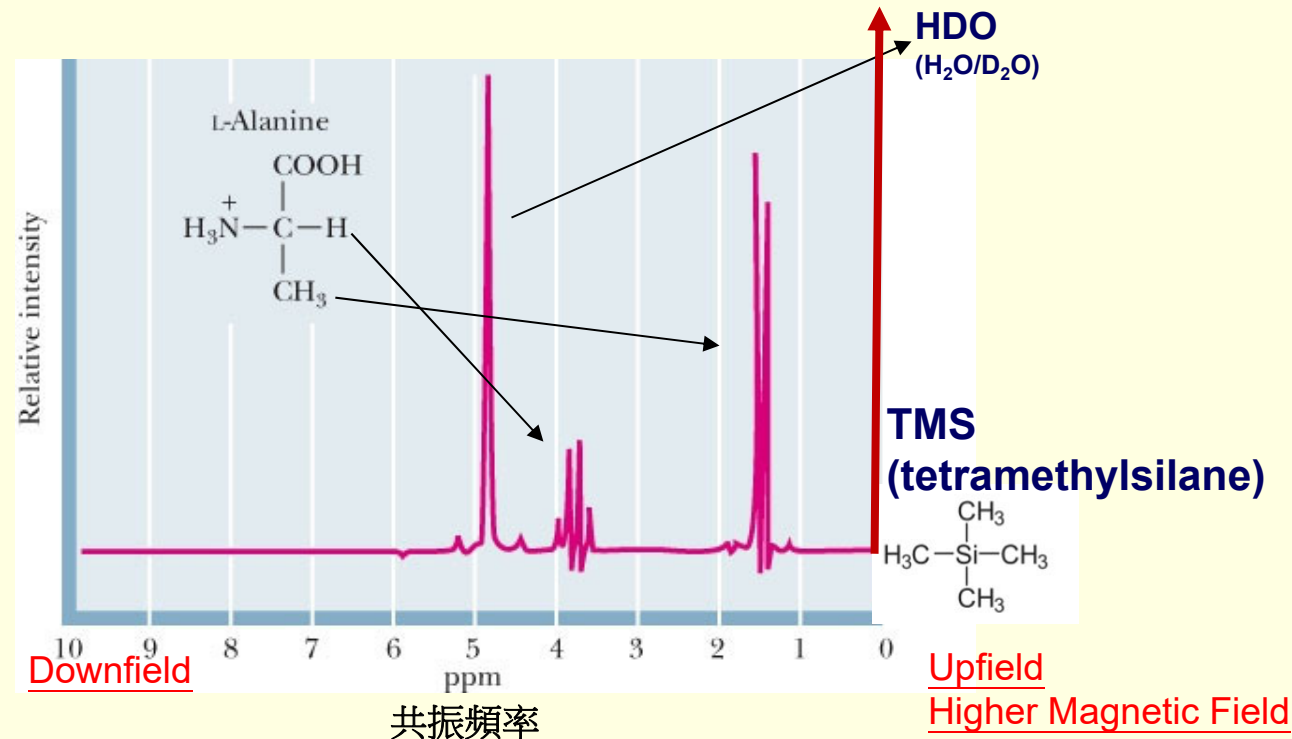
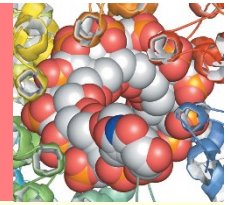


Figure 4.11 **Proton NMR** spectra of several amino acids.

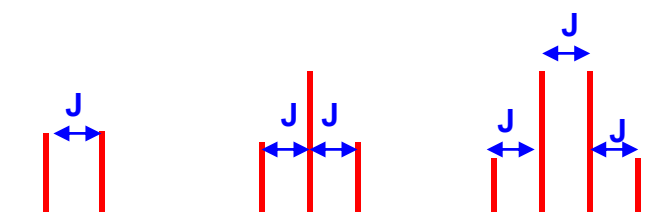
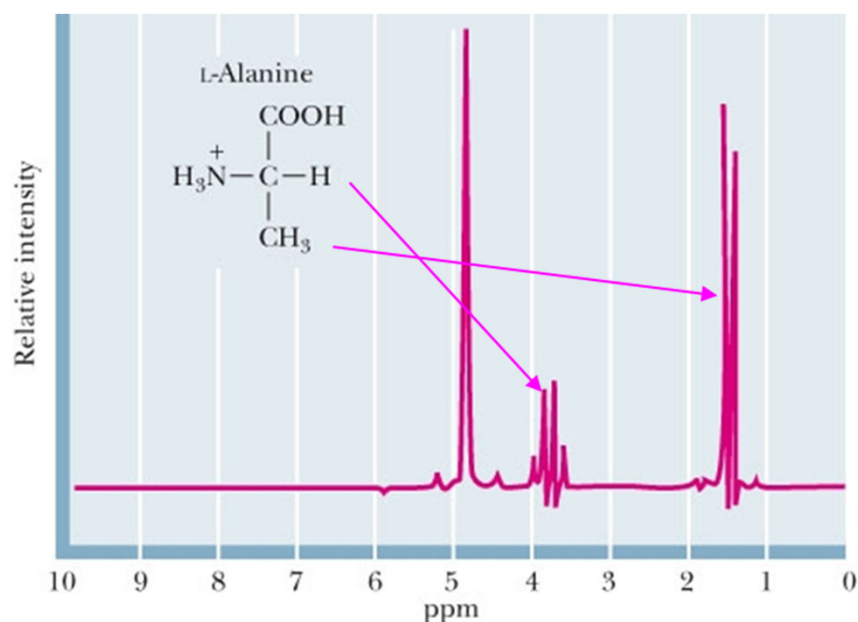
- **TMS (tetramethylsilane) as 0 ppm**
- The **chemical shift** (ppm) of amino acid protons depends on their particular chemical environment
- The magnitude of the **coupling constants** between protons on adjacent carbons depends in some cases on the ionization state of the amino acid.

Coupling constant

The magnitude of the **coupling constants** between protons on adjacent carbons depends in some cases on the ionization state of the amino acid.

Two signals from the different hydrogens are **split** into close groupings of two or more **resonances**.

Compounds having different sets of hydrogen atoms bonded to adjacent carbon atoms. The signal splitting in proton spectra is usually small, and is designated as **J** (referred to as the **coupling constant**).



doublet
(2 equal
intensity
signals),

triplet
(3 signals)
(1:2:1)

quartet
(4 signals)
(1:3:3:1).

Spectroscopic Properties

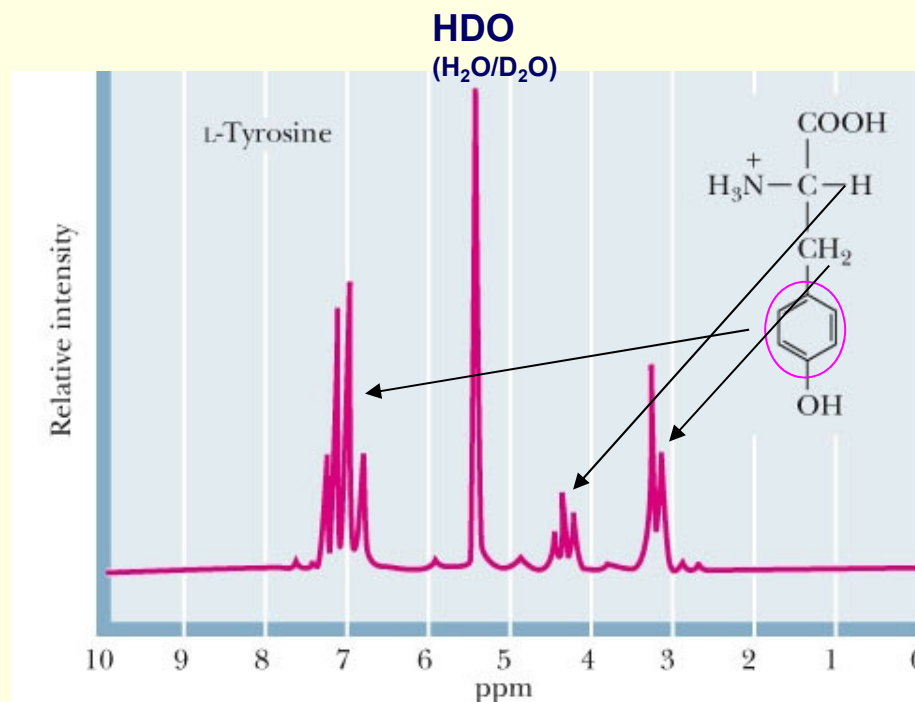
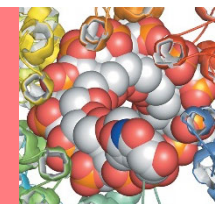
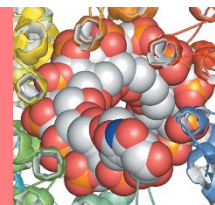


Figure 4.11 **Proton NMR** spectra of several amino acids. TMS (tetramethylsilane) as 0ppm

- The **chemical shift** (ppm) of amino acid protons depends on their **particular chemical environment**
- The magnitude of the **coupling constants** between protons on adjacent carbons depends in some cases on the ionization state of the amino acid.

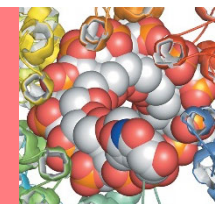


超高磁場生物用核磁共振儀(BioNMR850)

BioNMR core facility in NTHU-LS

生科院 B1

Spectroscopic Properties



^{13}C NMR chemical shifts

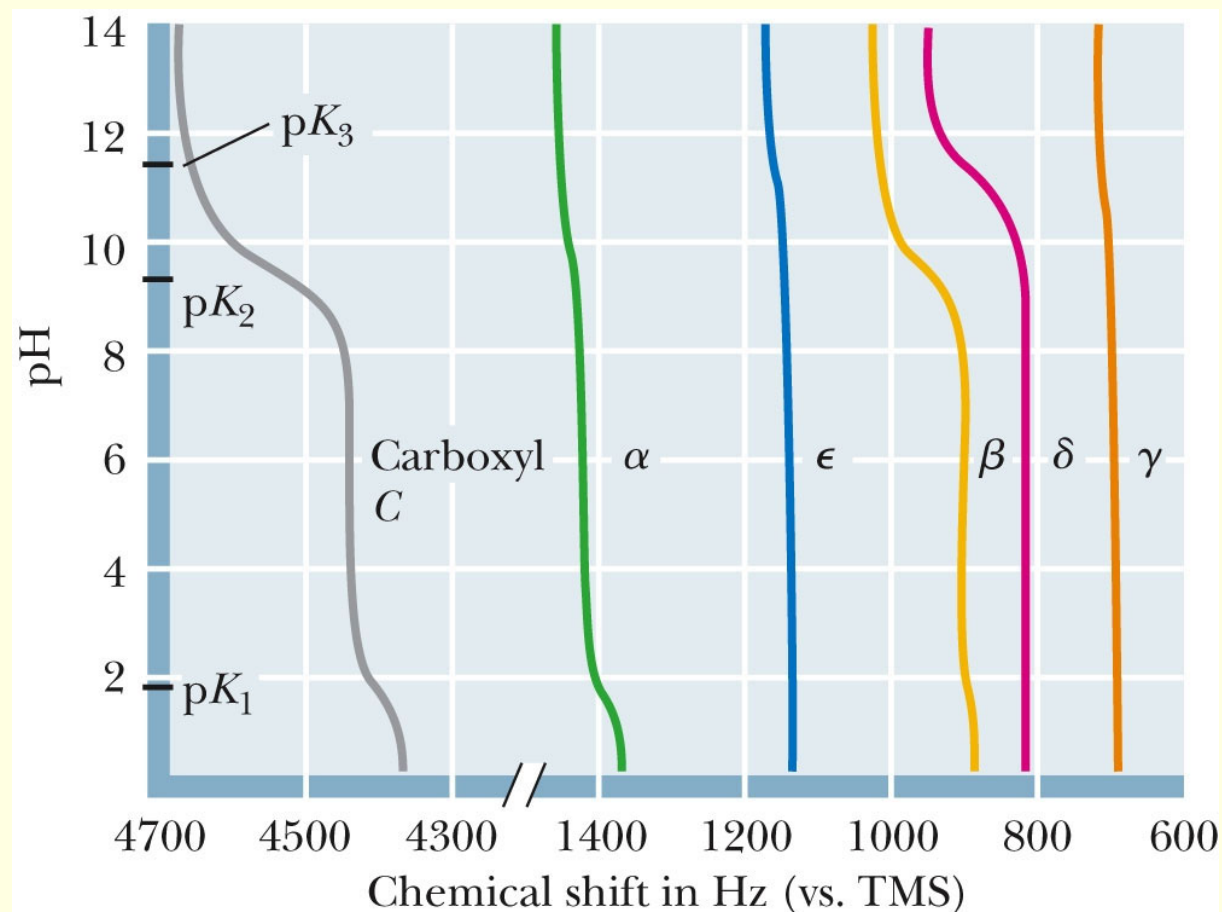
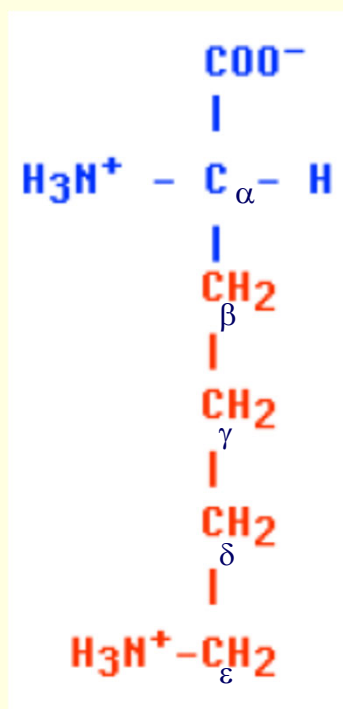


Figure 4.12 A plot of chemical shifts versus pH for the carbons of lysine.