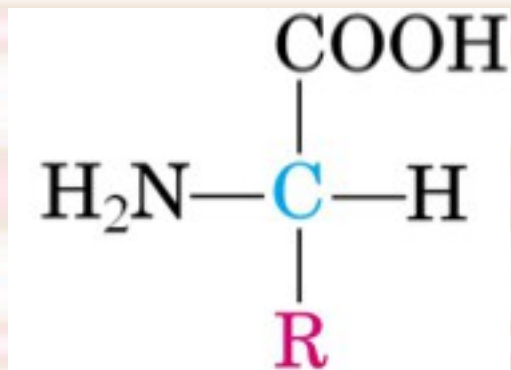


Biomolecules: amino acids

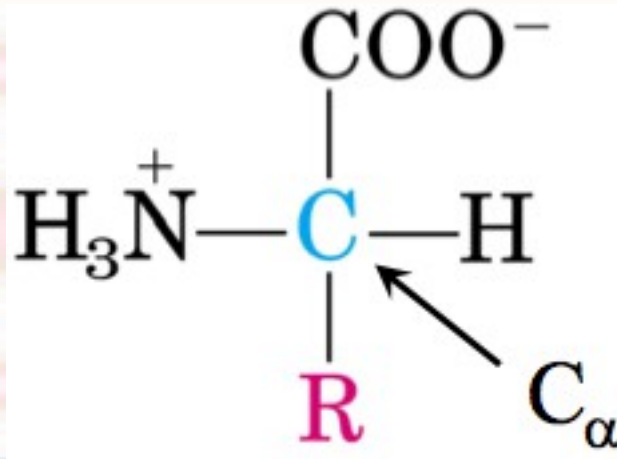
## *Amino acids*

- ◆ Amino acids are the building blocks of proteins
- ◆ They are also part of hormones, neurotransmitters and metabolic intermediates
- ◆ There are 20 different amino acids in nature, but they all share a common scaffold:
  - One central carbon atom hybridized sp<sup>3</sup>
  - One amino group
  - One carboxylic group
  - One hydrogen
  - One side chain, different per each aa



## *Amino acids*

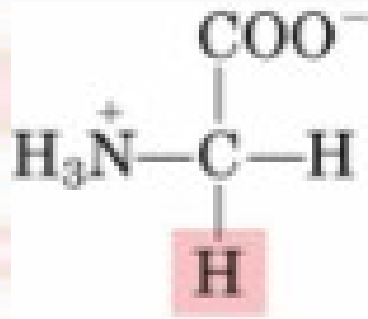
- ◆ All amino acids are non-volatile solids at RT and have very high boiling points (200-300°C)
- ◆ They are very soluble in water, but not in apolar solvents
- ◆ They have acid constants higher than general carboxylic acids ( $K_a=10^{-2} - 10^{-3}$  M)
- ◆ They have basic constants lower than those of generic amines ( $K_b= 10^{-4}$  M)
- ◆ All these characteristics can be explained assuming that the aa in solution has a dipolar structure, called zwitterion



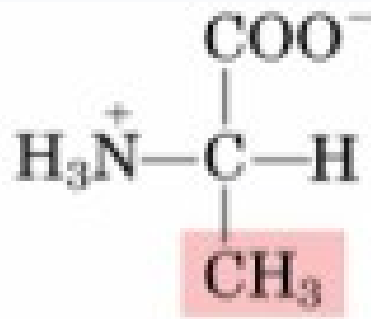
## *Amino acids*

- ◆ Mammals need to assume with the diet 10 out of 20 aa, since they are not able to synthesize them:
- ◆ Cysteine, Phenylalanine, Histidine, Isoleucine, Lysine, Leucine, Methionine, Threonine, Valine and Tryptophan
- ◆ Depending on side chains characteristics, we can classify:
  - Apolar side chains (Gly, Ala, Val, Pro, Leu, Met, Ile)
  - Aromatic side chains (Phe, Tyr, Trp)
  - Uncharged polar side chains (Ser, Thr, Cys, Asn, Gln)
  - Positively charged side chains (His, Lys, Arg)
  - Negatively charged side chains (Glu, Asp)
- ◆ Their names can be shortened in a 3-letters code and in a 1-letter code

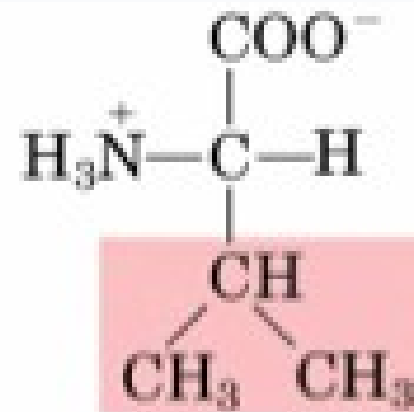
## *Apolar amino acids*



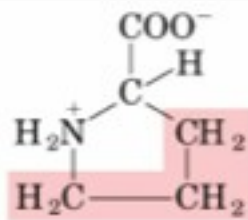
Glycine (Gly, G)



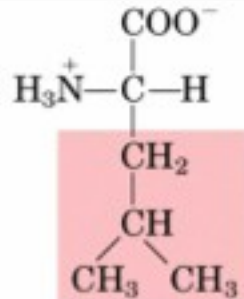
Alanine (Ala, A)



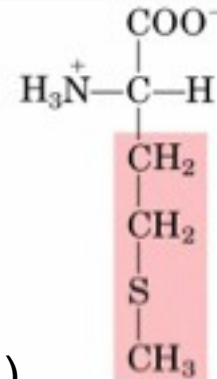
Valine (Val, V)



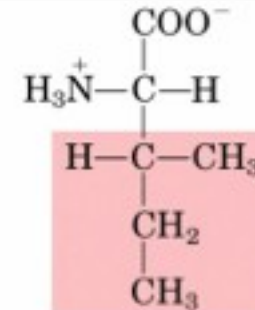
Proline (Pro, P)



Leucine (Leu, L)

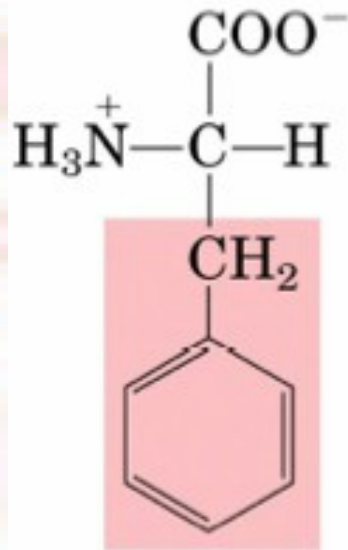


Methionine (Met, M)

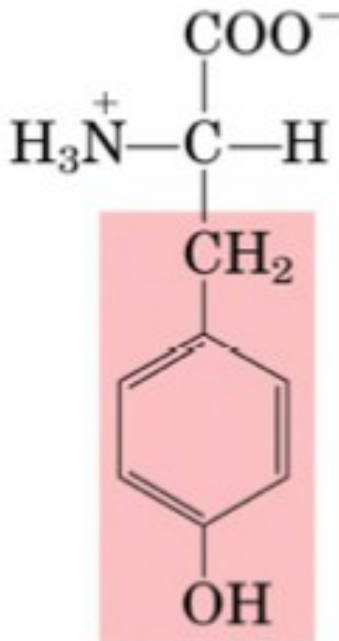


Isoleucine (Ile, I)

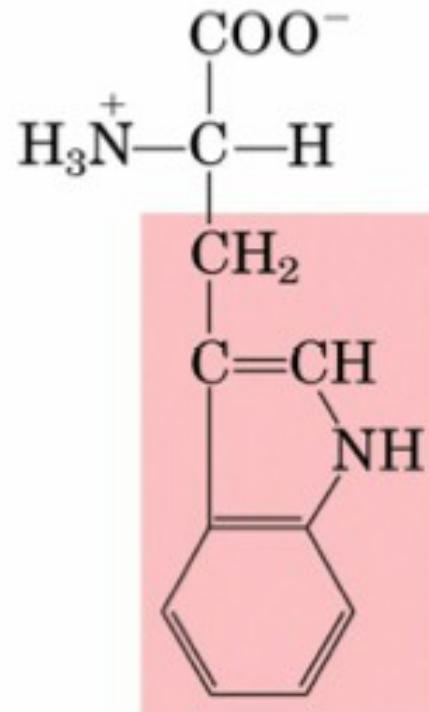
## *Aromatic amino acids*



Phenylalanine (Phe, F)

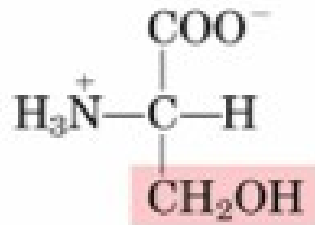


Tyrosine (Tyr, Y)

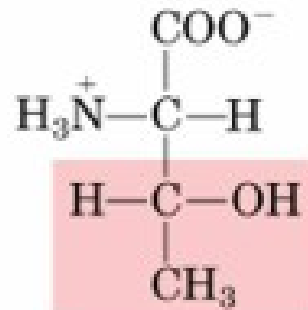


Tryptophan (Trp, W)

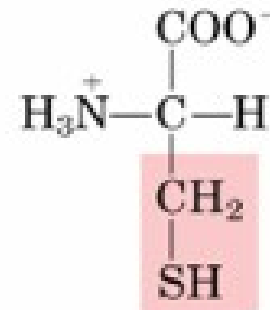
## *Polar amino acids*



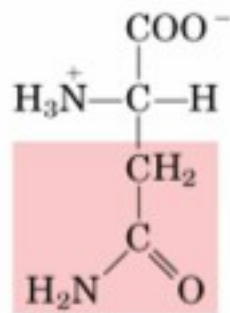
Serine (Ser, S)



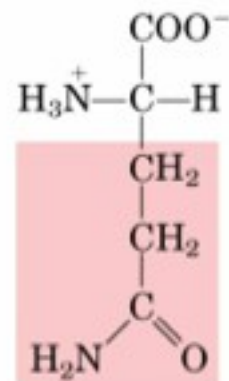
Threonine (Thr, T)



Cysteine (Cys, C)

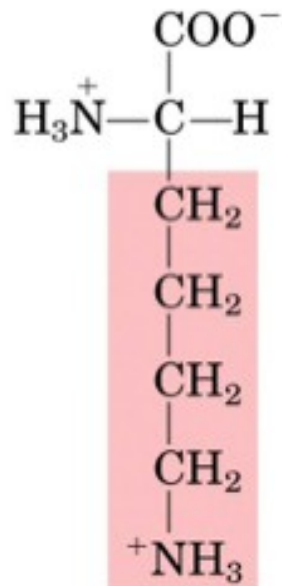


Asparagine (Asn, N)

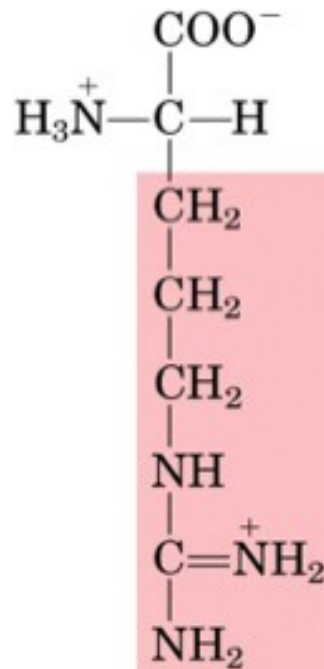


Glutamine (Gln, Q)

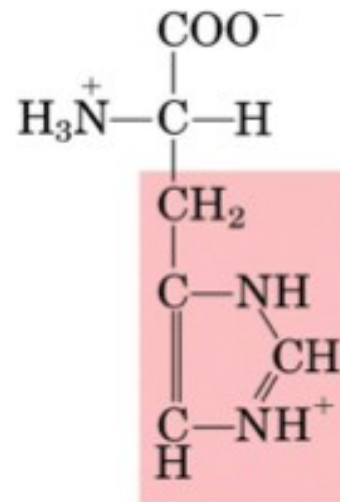
## Basic amino acids



Lysine (Lys, K)



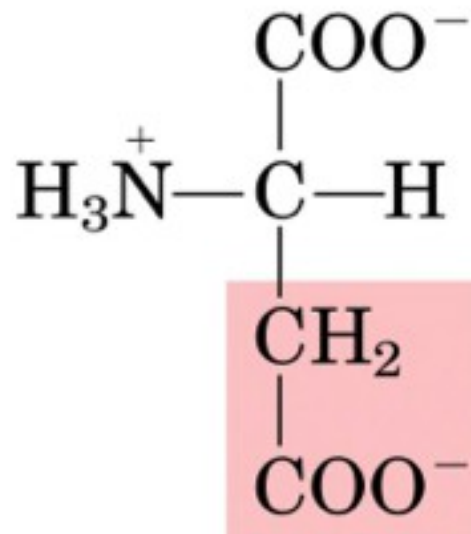
Arginine (Arg, R)



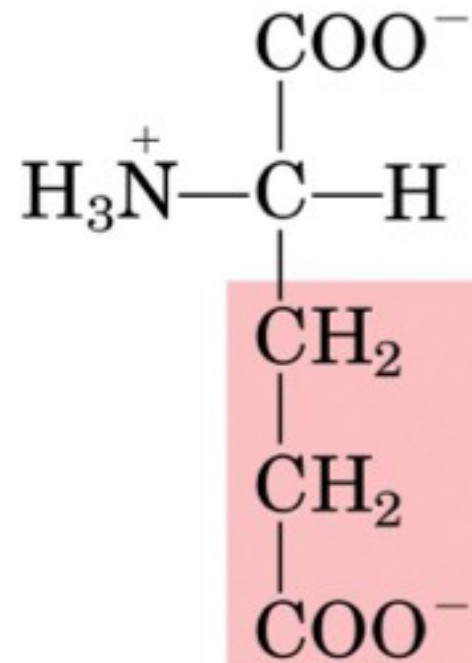
Histidine (His, H)



## *Acidic amino acids*

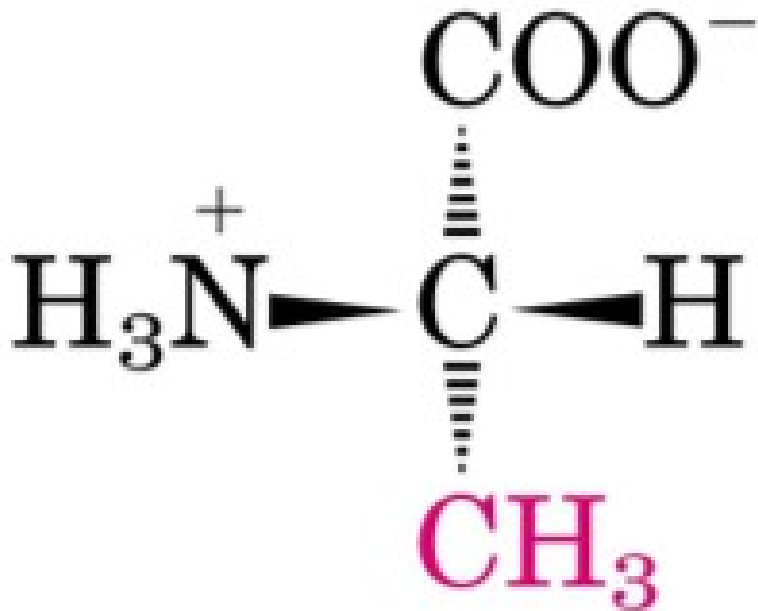


Aspartic acid (Asp, D)

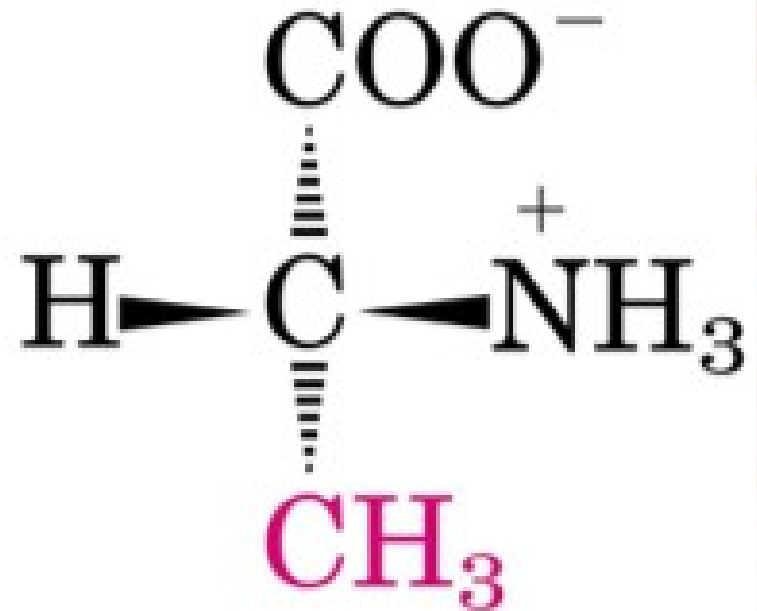


Glutamic acid (Glu, E)

*All amino acids but Gly are chiral*



L-  
Alanine



D-  
Alanine

In living organism there will only be L-amino acids incorporated into proteins

A few bacteria can synthesize D-aa, but they need a special enzyme

## Properties of amino acids: acid-base reactions

	$pK_{\text{COOH}}$	$pK_{\text{NH}_3^+}$	$pK_{\text{R}}$	pI
GLY	2.3	9.6	-	6.0
ALA	2.3	9.7	-	6.0
VAL	2.3	9.6	-	6.0
PRO	2.0	10.6	-	6.3
LEU	2.4	9.7	-	6.0
MET	2.3	9.2	-	5.8
ILE	2.4	9.7	-	6.1
PHE	1.8	9.1	-	5.5
TYR	2.2	9.1	10.1	5.7
TRP	2.4	9.4	-	5.9
SER	2.2	9.2	-	5.7
THR	2.6	10.4	-	6.5
CYS	1.8	10.8	8.3	5.0
ASN	2.0	8.8	-	5.4
GLN	2.2	9.1	-	5.7
LYS	2.2	9.0	10.5	9.8
ARG	2.2	9.0	12.5	10.8
HIS	1.8	9.2	6.0	7.6
ASP	2.1	9.8	3.9	3.0
GLU	2.2	9.7	4.3	3.2

$pK_a$  ranges from 1.8 and 2.4  
 $pK_b$  ranges from 9.0 and 10.8

$pK_{\text{R}}$  refers to the properties of the charged side chains

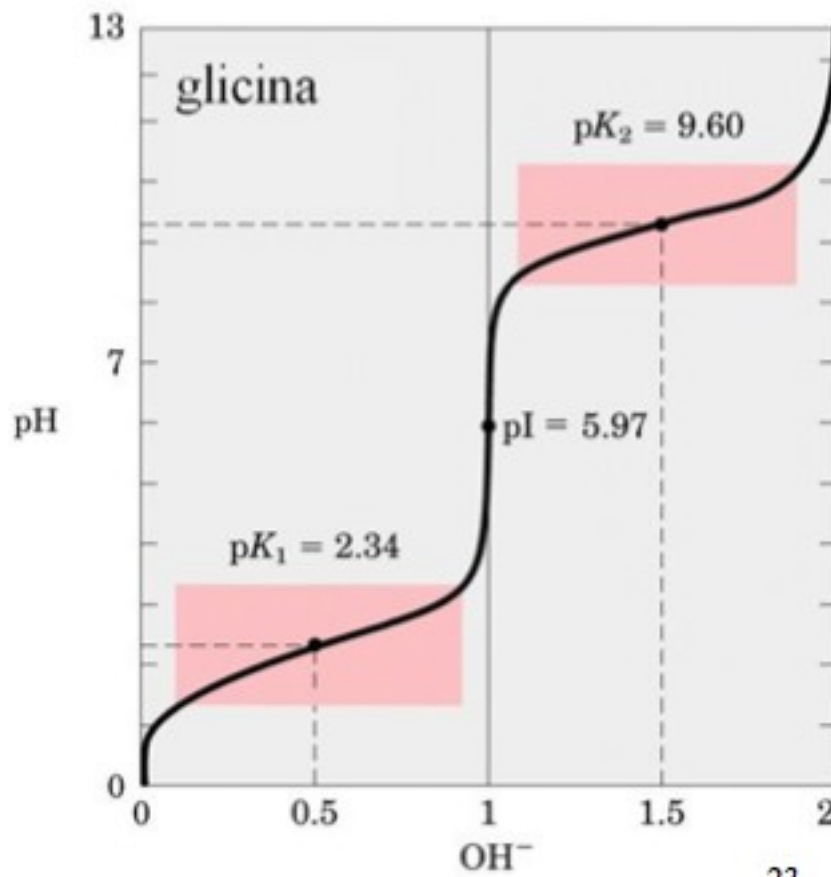
pI is the isoelectric point: the pH at which the aa has reached neutrality (no net charge)

## Properties of amino acids: acid-base reactions

The previous table has been obtained by titrating each amino acid with a strong base

Here is shown the titration curve for Glycine

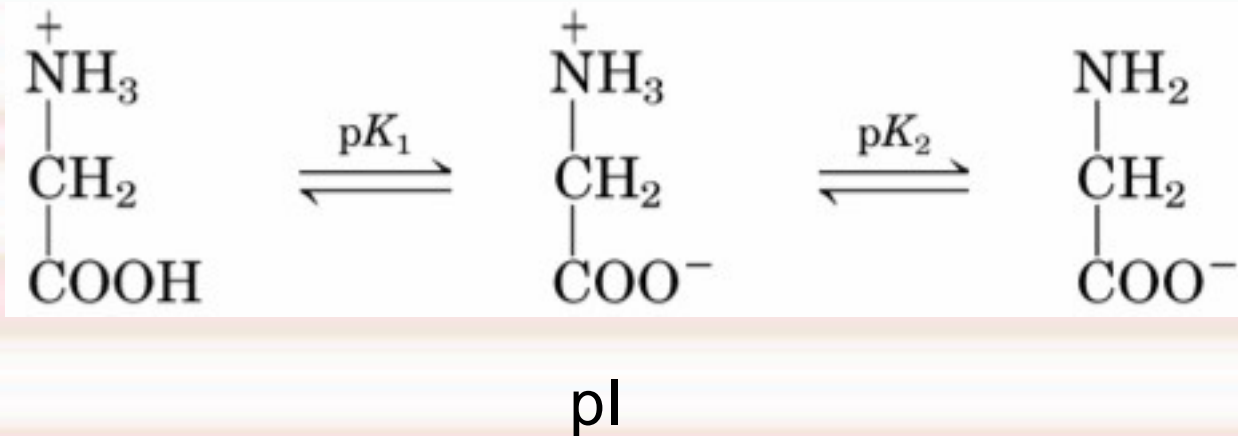
There are two areas where the curve resembles that of a buffer (pK<sub>1</sub> and pK<sub>2</sub>) and there is only one neutralization point (pI)



$$pI = \frac{pK_1 + pK_2}{2} = \frac{2.34 + 9.60}{2} = 5.97$$

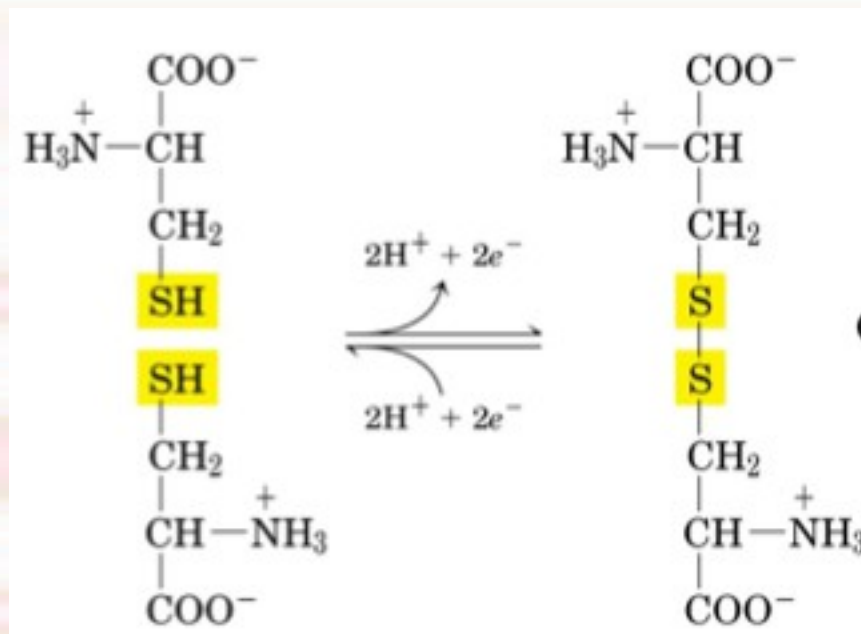
## Properties of the aa: acid-base reactions

- ◆ The ionizability of an aa is pH dependent:
- ◆ At  $\text{pH} > \text{pI} \rightarrow$  the aa has a net charge of -1 (anion)
- ◆ At  $\text{pH} < \text{pI} \rightarrow$  the aa has a net charge of +1 (cation)
- ◆ At  $\text{pH} = \text{pI} \rightarrow$  there is the zwitterion, with a net charge of 0

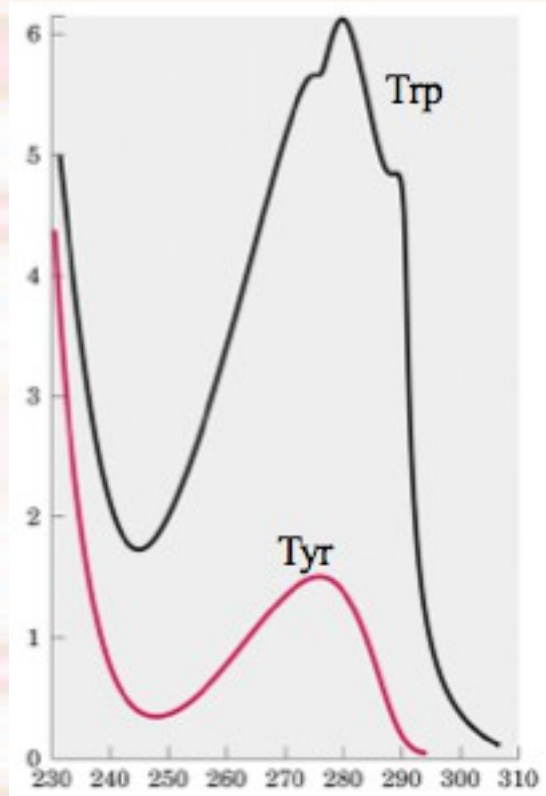


## *Properties of aa: redox reactions*

- ◆ Cysteine has a reactive -SH group which can shuttle electrons and protons to acceptor proteins
- ◆ It can also react with a second Cys to form a covalent bond: the disulfide bond
- ◆ Disulfide bonds usually occurs in extracellular proteins, given that the extracellular environment is highly oxidative



## Properties of aa: spectroscopic behaviour

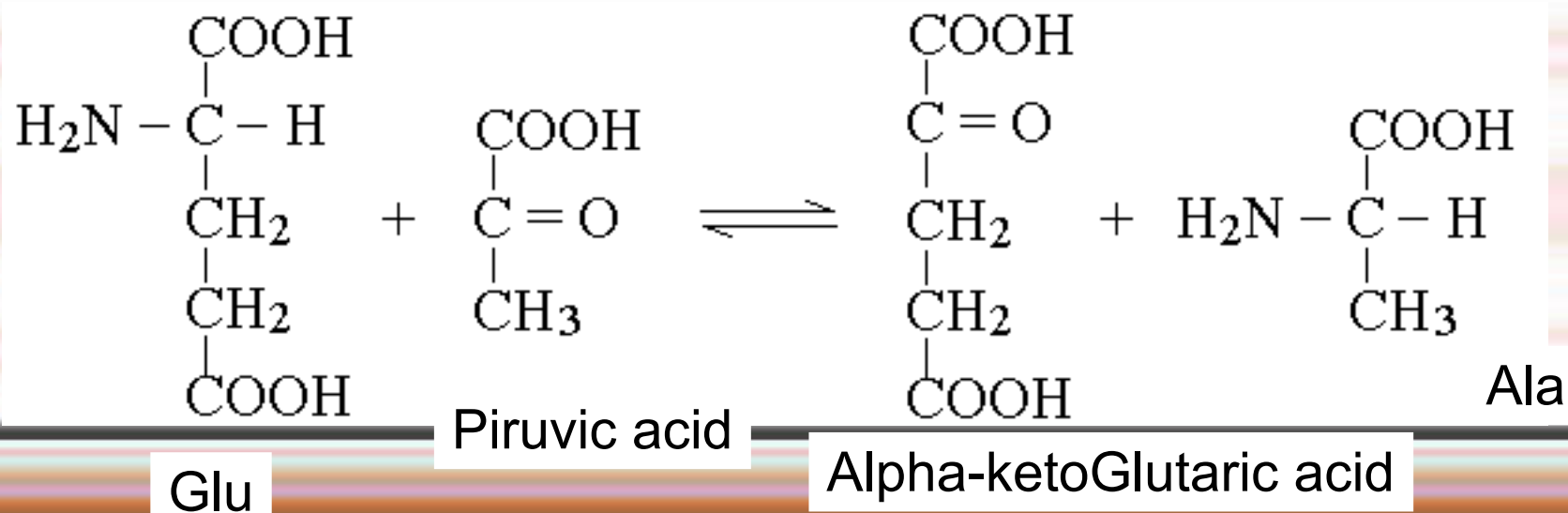


Wavelength  $\lambda$ )

- ◆ Aromatic side chains can absorb UV light and can be used to calculate protein quantity in solution
- ◆ The total absorbance of a sample is directly proportional to the number of Trp and/or Tyr and hence to the concentration of a given protein

## Reaction of aa: transamination

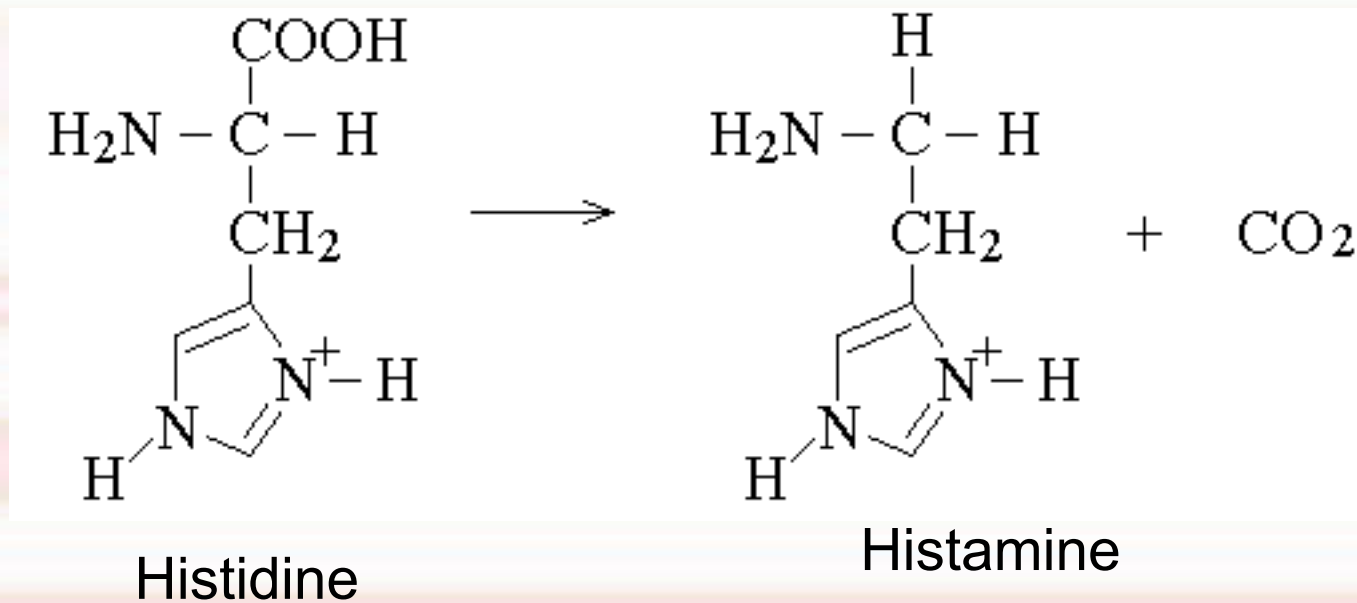
- ◆ Aa can exchange amino and ketonic groups: this reaction is reversible and called transamination
- ◆ it is usually performed inside the cytoplasm
- ◆ One aa can react with an alpha-ketoacid to give an alpha-ketoacid and an amino acid
- ◆ Many of those enzymes work in the liver and the level of transaminase in the blood will reveal a pathological state of this organ





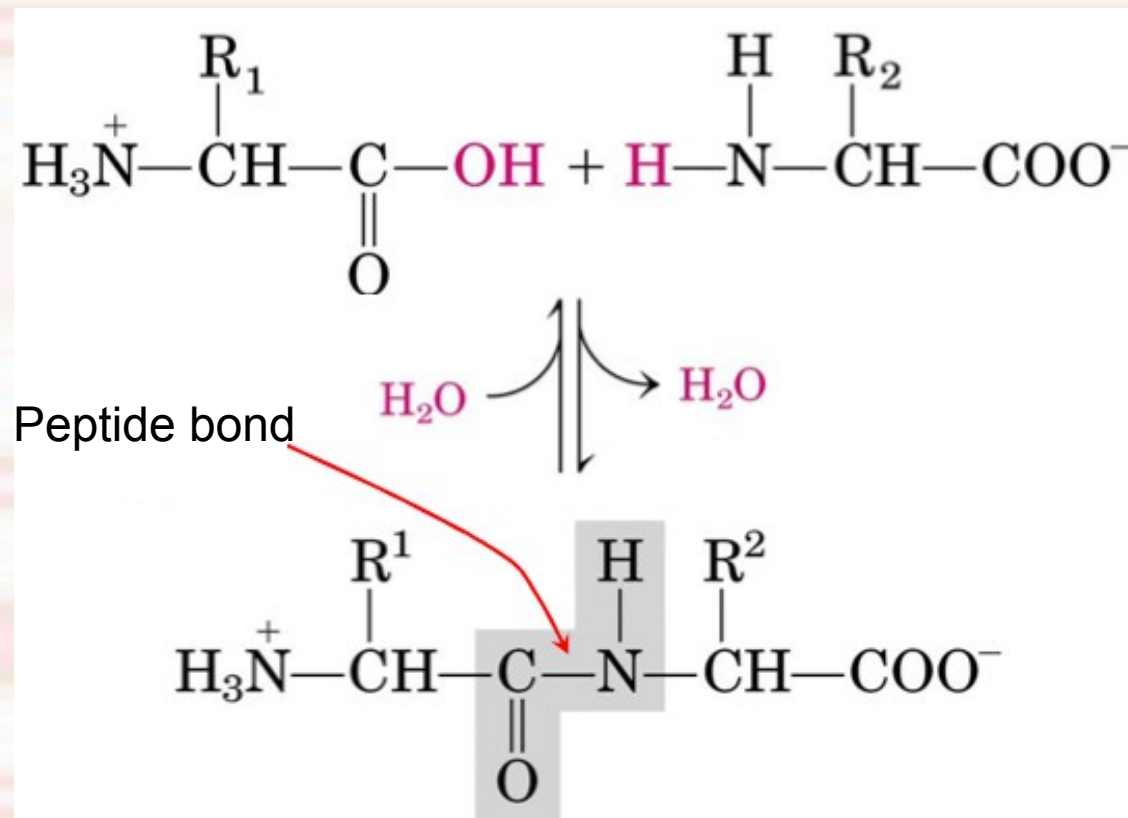
## *Reaction of aa: decarboxylation*

- ◆ This reaction will lead to the formation of  $\text{CO}_2$  and the amine
- ◆ It is catalysed by amine decarboxylases
- ◆ some of the amines produced could be hormones or active metabolites



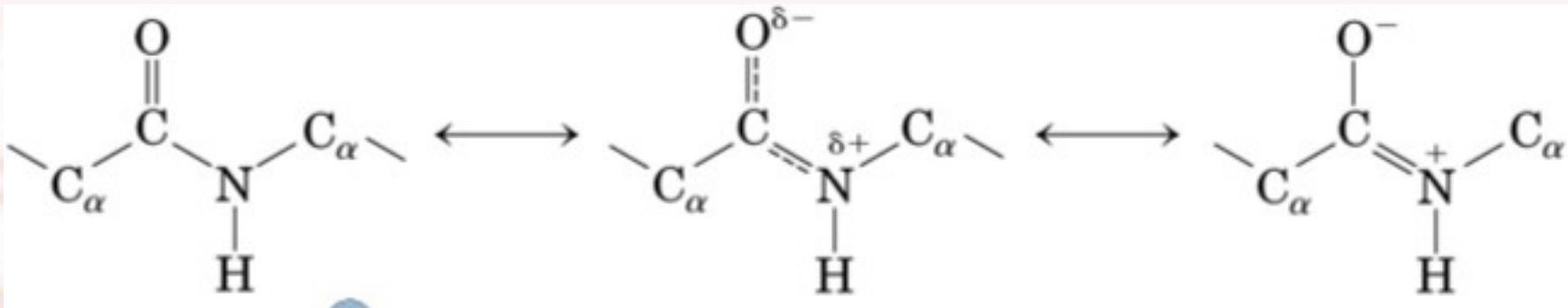
## *Polymerization of aa*

- ◆ The acid group of one aa will react with the amino group of the following residue in a condensation reaction leading to a dipeptide



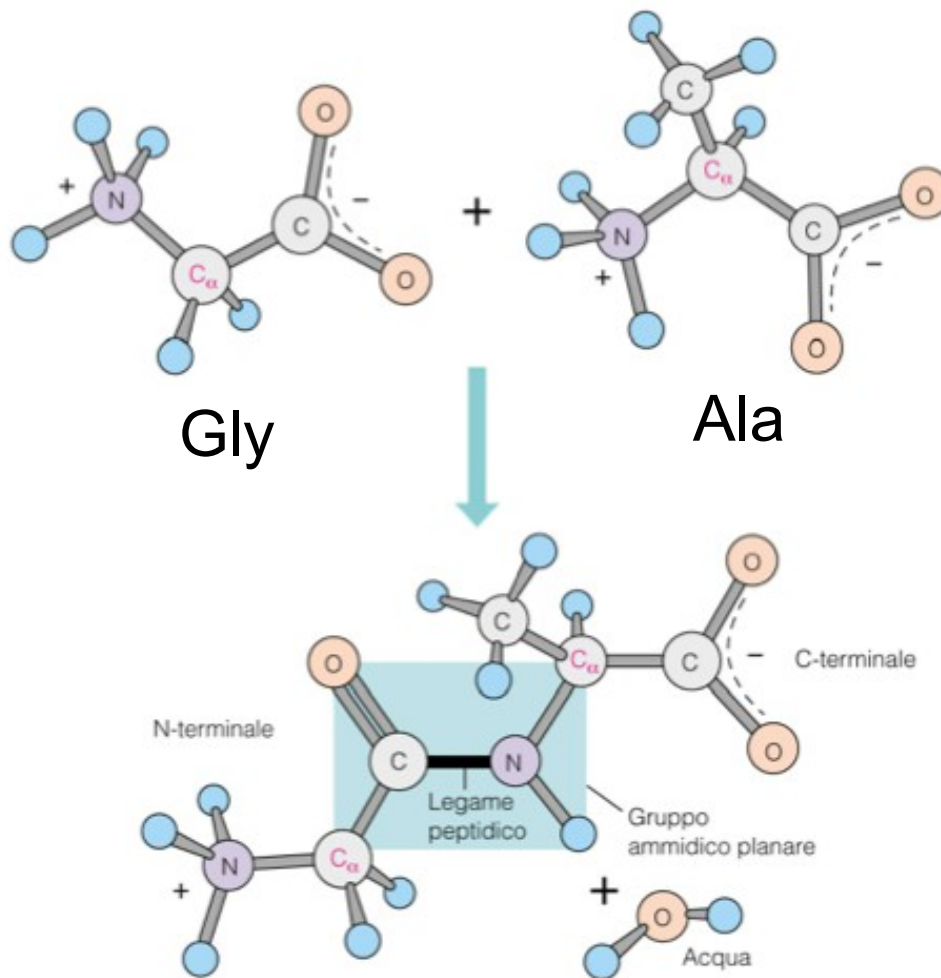
## Polymerization of aa

- ◆ This reaction, which would normally give rise to an amide bond, lead to the formation of a special bond: the peptide bond
- ◆ Its length is shorter than a single bond and longer than a double bond
- ◆ its energy is in between that of a single and of a double bond



There is resonance due to the particular electronic configuration of the bond → rigidity of the molecule and *trans* configuration of the bond

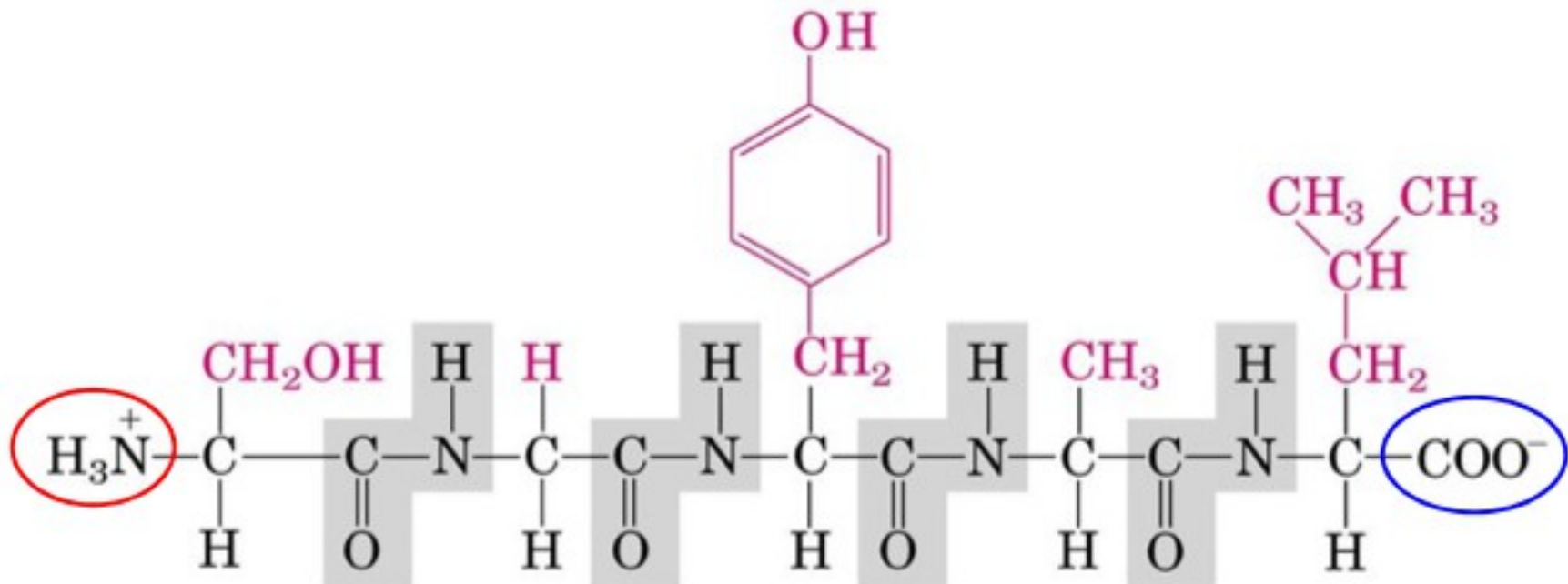
## Polymerization of aa



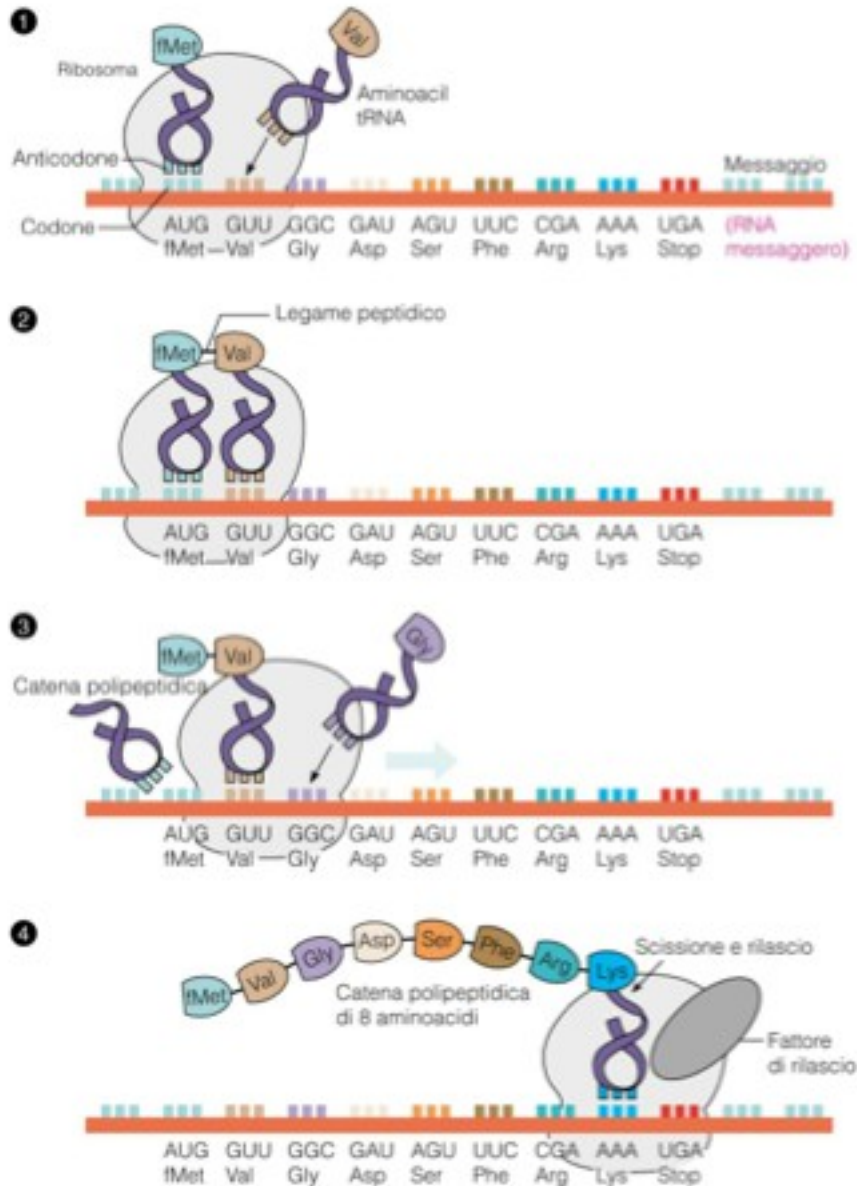
- The peptide bond has 40% character of double bond
- 6 atoms lie in the plane of the bond
- There is a partial negative charge on the carbonyl O and a partial positive charge on the amino N
- All the peptide bonds in proteins are in the *trans* configurations

## Polymerization of aa

- The condensation reaction always attaches the  $\text{-COOH}$  of the  $\text{aa}_i$  to the  $\text{-NH}_2$  of  $\text{aa}_{i+1}$
- The polypeptide chain has a polarity: it will start with a free amino group and it will end with a free carboxylic group
- These are called N-terminal and C-terminal ends



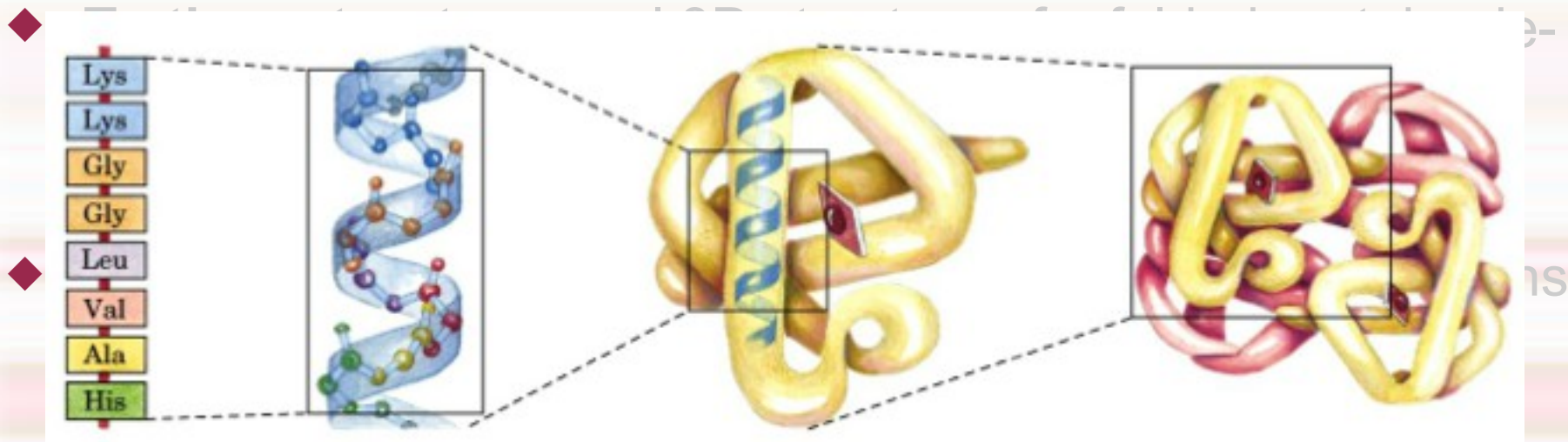
## *In vivo polymerization: translation*



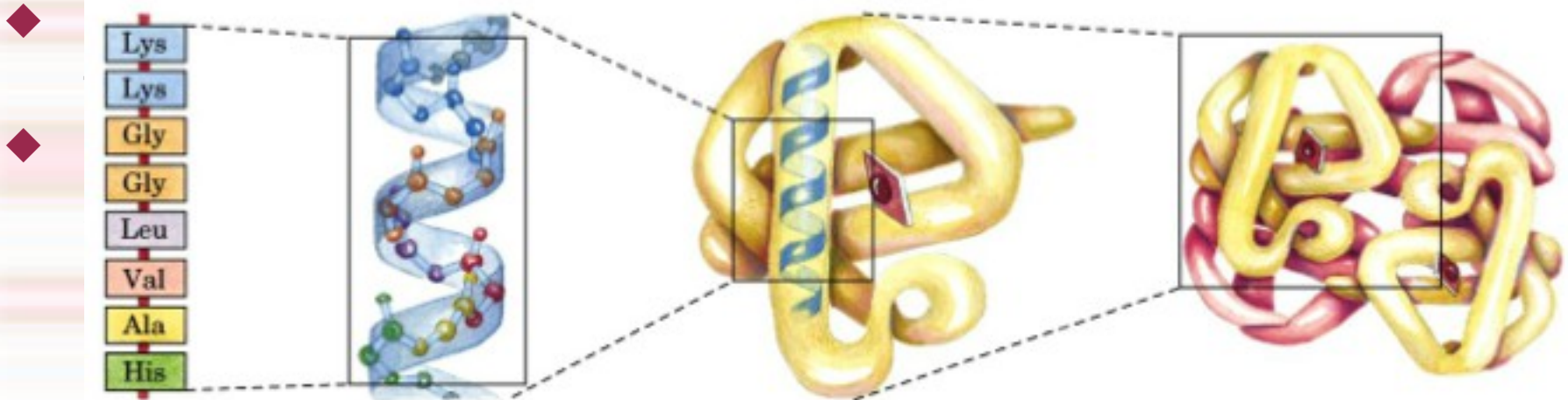
- ◆ Polypeptide chains and proteins are synthesized by the ribosomes, which read the mRNA and charge the proper aa-tRNA

## *Hierarchical organization of proteins*

- ◆ **Primary structure:** the linear sequence of aa bound by peptide bonds
- ◆ **Secondary structures:** regular organization of aa in the space, stabilized by hydrogen bonds among CO and NH of several peptide bonds. It can either be periodic (alpha-helix, beta-sheet) or random. Not all the possible fold are allowed given the restrictions dictated by the peptide bond



## *Hierarchical organization of proteins*



- ◆ **Tertiary structure:** real 3D structure of a folded protein, derived from the assembly of secondary structure elements. These are stabilized by polar and hydrophobic interactions among the side chains
- ◆ **Quaternary structure:** oligomeric assembly (not all proteins have this arrangement)