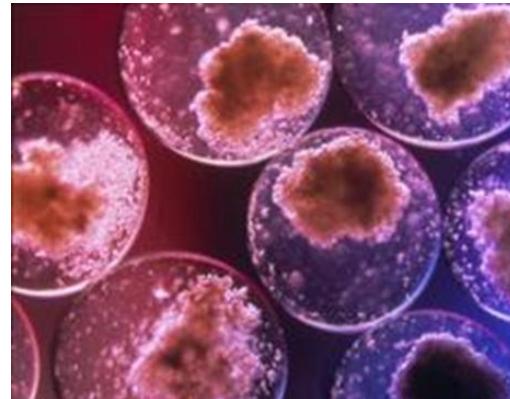
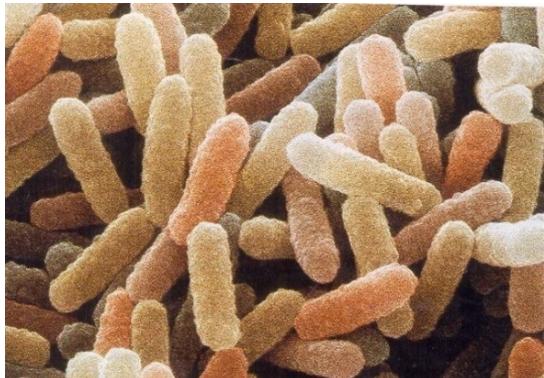


# Chemistry and introduction to biochemistry

## Macromolecules in living organisms



Aminoacids and sugars

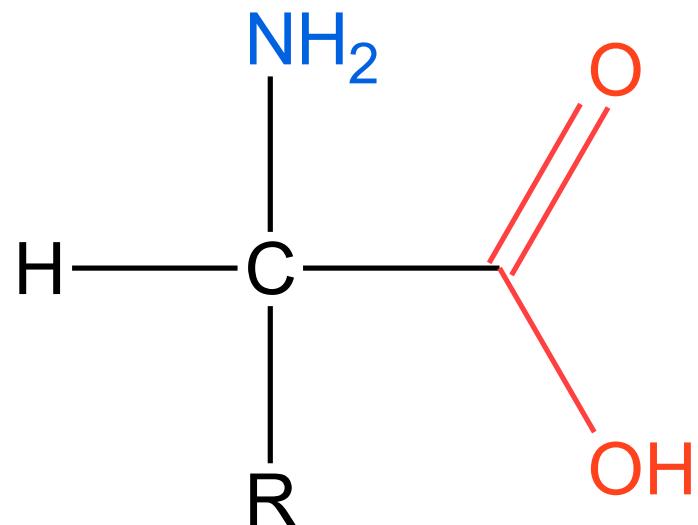
Sugars and polysaccharides

Lipids and membranes

Nucleotides and nucleic acids

# Chemico-physical properties of aminoacids

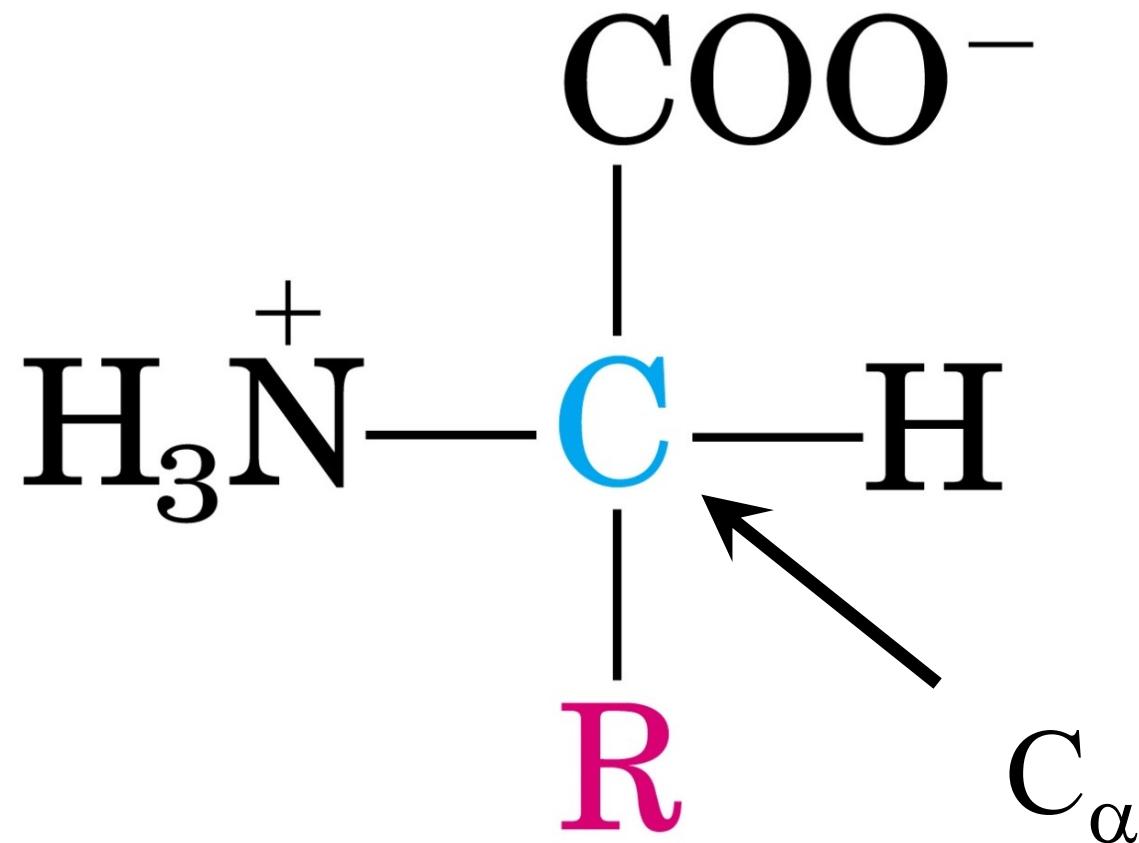
- Protein building blocks, also found in hormones and neurotransmitters
- They have an **aminic** and a **carboxyl** group



Some of their properties are in contrast with the common behavior of these groups

- a) At difference from aliphatic amines ( $R-NH_2$ ) and carboxylic acids ( $R-COOH$ ), aminoacids are **non volatile crystalline solids with high melting points (200-300°C)**.
- b) **Insoluble in apolar solvents** (benzene, ether, etc) and are **soluble in water**
- c) Their water solutions behave as **sloution** of compounds with **high dipole moment**
- d) Their **acidic constants** ( $R-COOH$ ) **are higher** with respect to carboxylic acids ( $K_A \approx 10^{-2} M$ ) and their **basic constants are lower** with respect to aliphatic amines ( $K_B \approx 10^{-4} M$ )

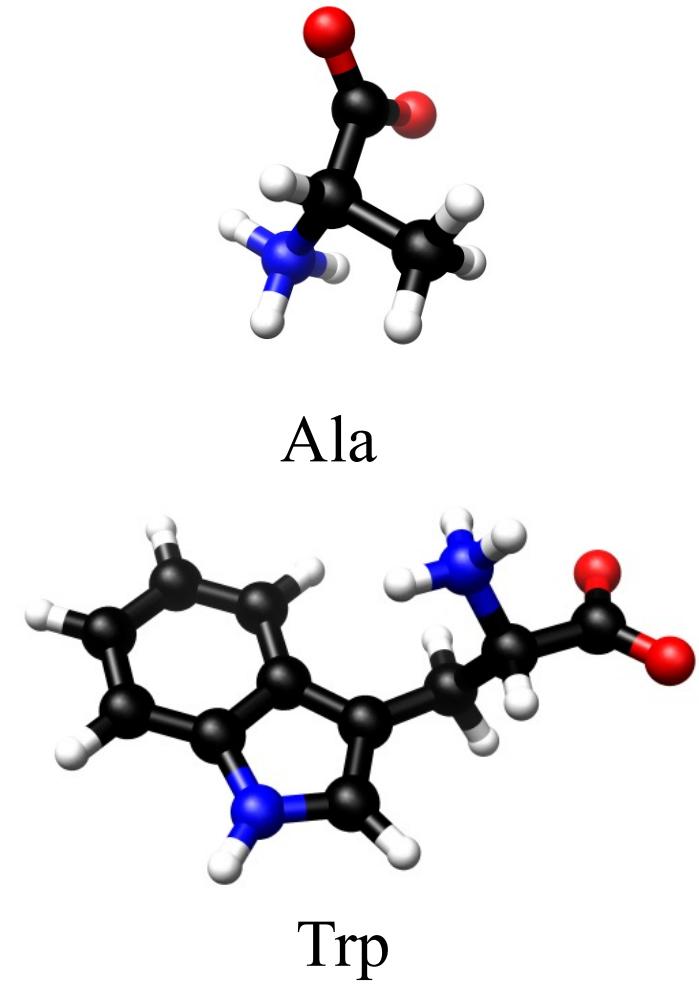
These properties indicate that in solution we have this structure:



Amino acids are **dipolar ions** or **zwitterions**

# NOMENCLATURE

ALANINE	ALA	A
CYSTEINE *	CYS	C
ASPARTIC ACID	ASP	D
GLUTAMMICO ACIDO	GLU	E
PHENILALANINE *	PHE	F
GLYCINA	GLY	G
HISTIDINE *	HIS	H
ISOLEUCINE *	ILE	I
LYSINA *	LYS	K
LEUCINE *	LEU	L
METHIONINE *	MET	M
ASPARAGINE	ASN	N
PROLINE	PRO	P
GLUTAMINE	GLN	Q
ARGININE	ARG	R
SERINE	SER	S
THREONINE *	THR	T
VALINE *	VAL	V
THRIPTOPHANE *	TRP	W
TYROSINE	TYR	Y



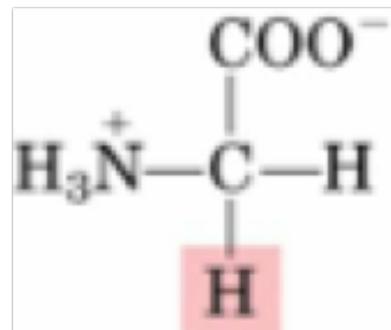
\* essential, must be present in the diet.

## ***Amino acids***

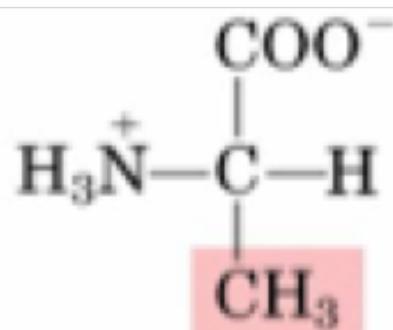
- ▶ Mammals need to assume with the diet 10 out of 20 aa, since they are not able to synthesize them:
- ▶ Cysteine, Phenylalanine, Histidine, Isoleucine, Lysine, Leucine, Methionine, Threonine, Valine and Tryptophan
- ▶ Depending on side chains characteristics, we can classify:
  - Apolar side chains (Gly, Ala, Val, Pro, Leu, Met, Ile)
  - Aromatic side chains (Phe, Tyr, Trp)
  - Uncharged polar side chains (Ser, Thr, Cys, Ans, 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

NB. Structures at pH 7

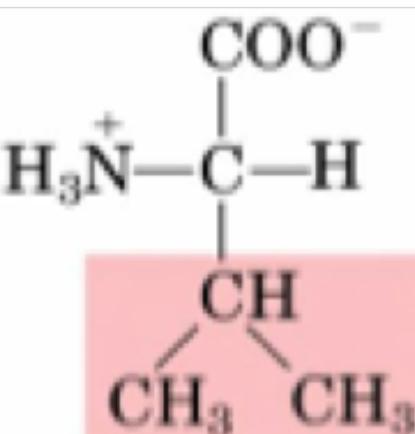
### *Apolar amino acids*



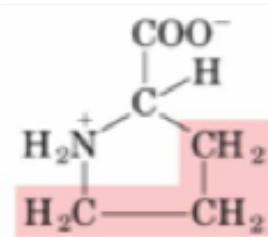
Glycine (Gly, G)



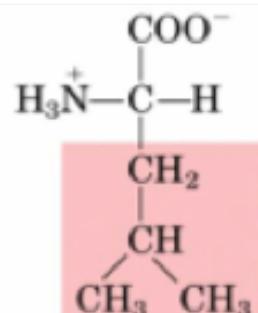
Alanine (Ala, A)



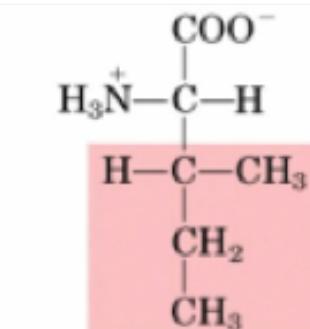
Valine (Val, V)



Proline (Pro, P)



Leucine (Leu, L)

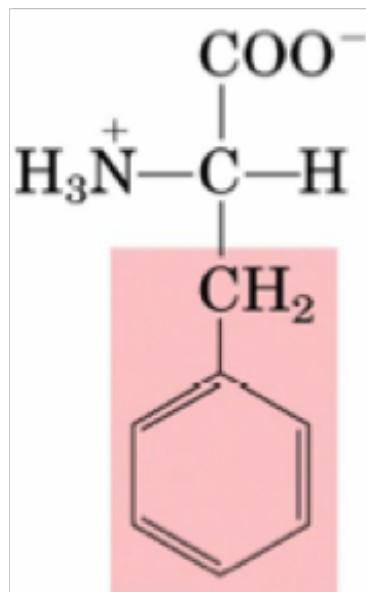


Isoleucine (Ile, I)

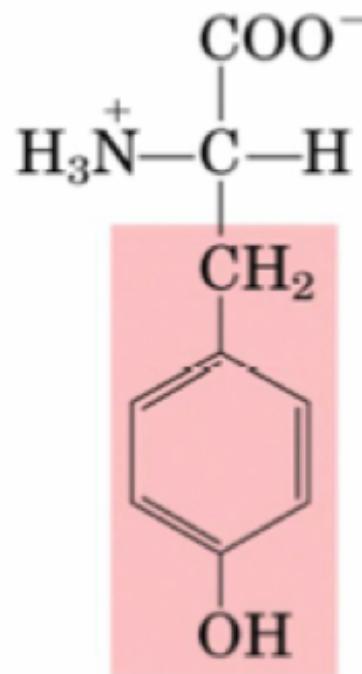


Methionine (Met, M)

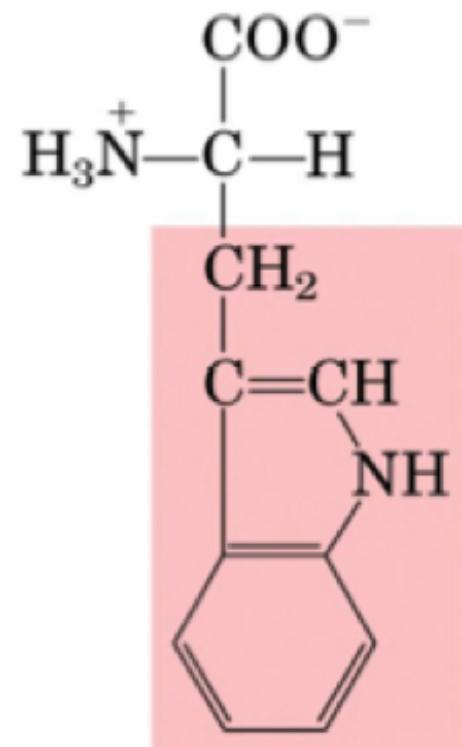
## *Aromatic amino acids*



Phenylalanine (Phe, F)

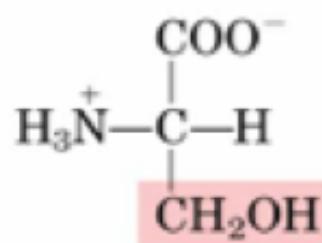


Tyrosine (Tyr, Y)

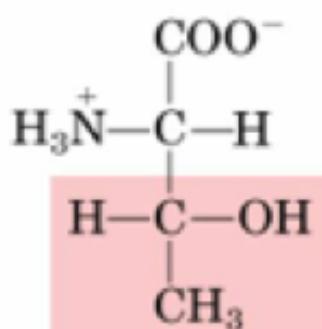


Tryptophan (Trp, W)

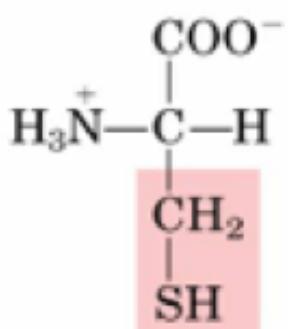
## *Polar amino acids*



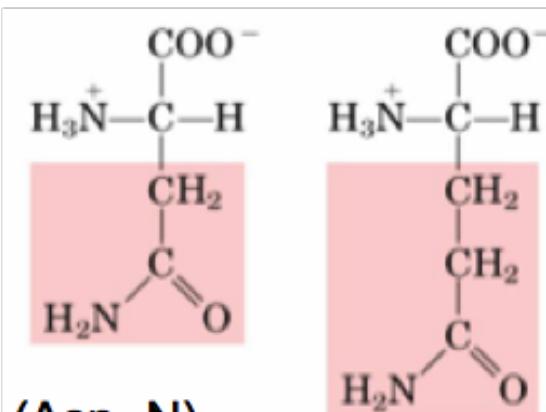
Serine (Ser, S)



Threonine (Thr, T)



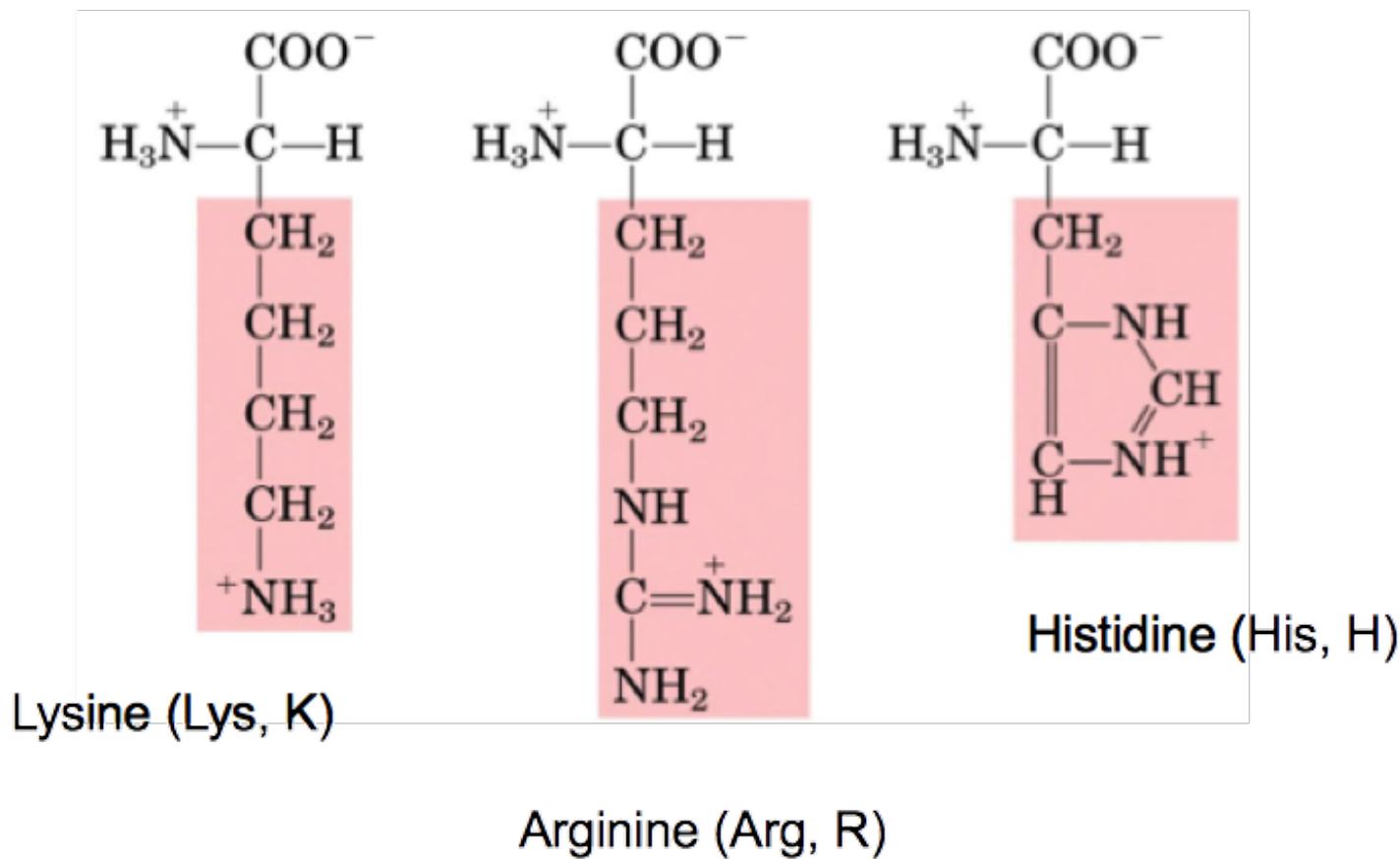
Cysteine (Cys, C)



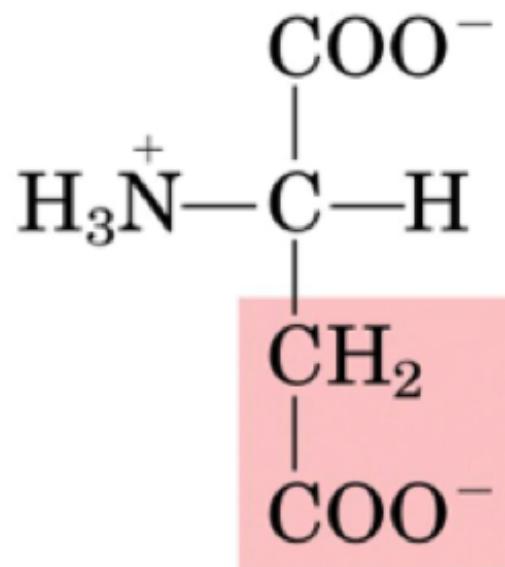
Asparagine (Asn, N)

Glutamine (Gln, Q)

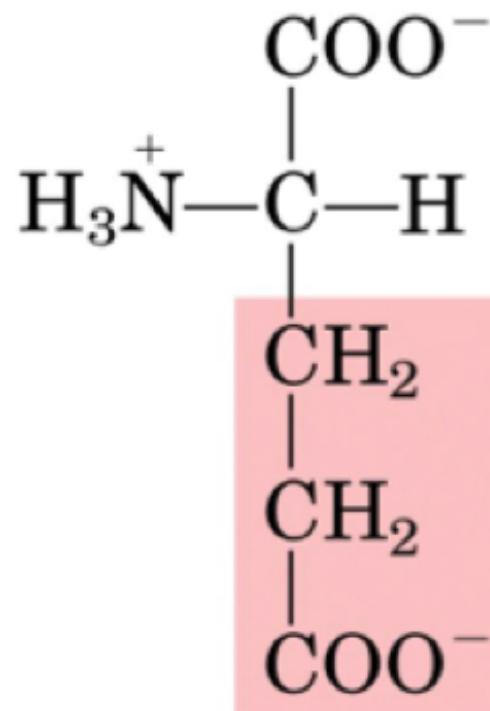
## *Basic amino acids*



## *Acidic amino acids*

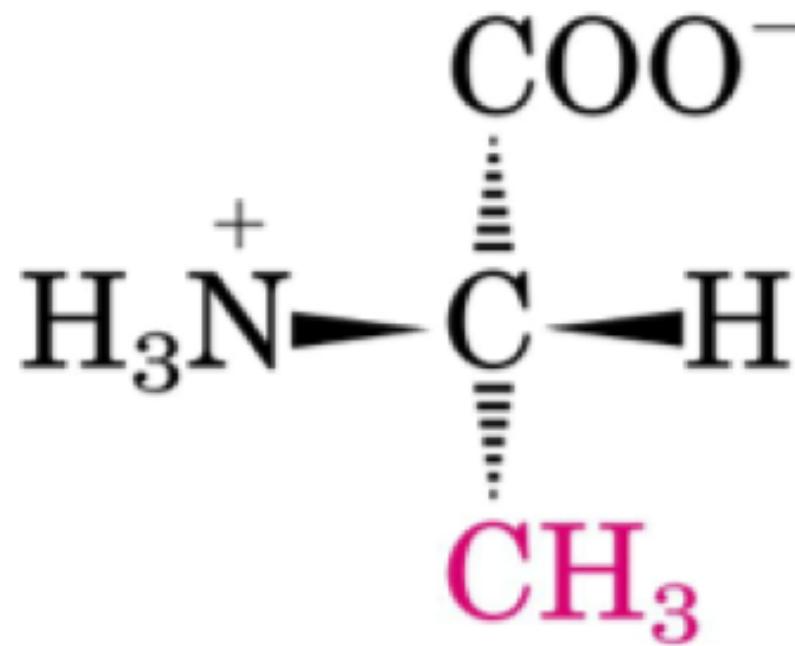


Aspartic acid (Asp, D)

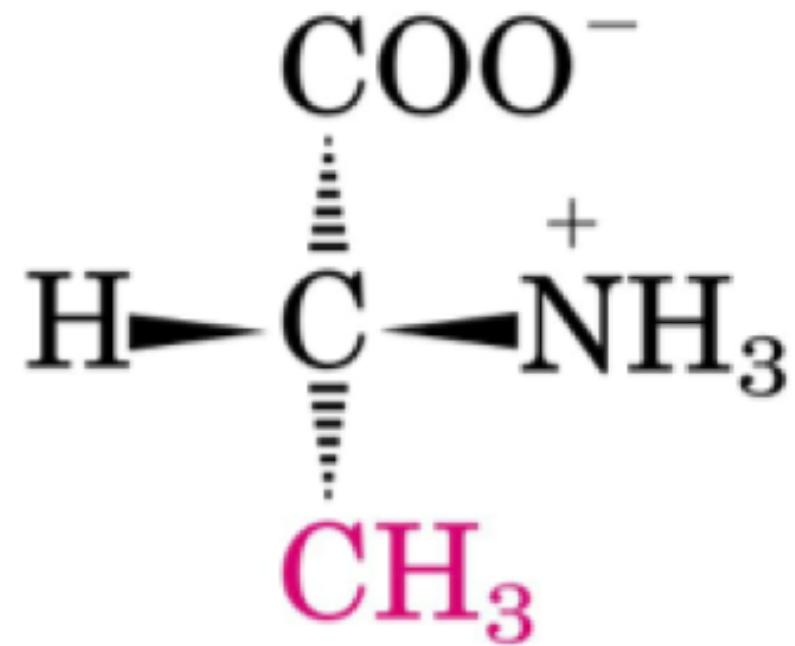


Glutamic acid (Glu, E)

*All amino acids but Gly are chiral*



L-Alanine



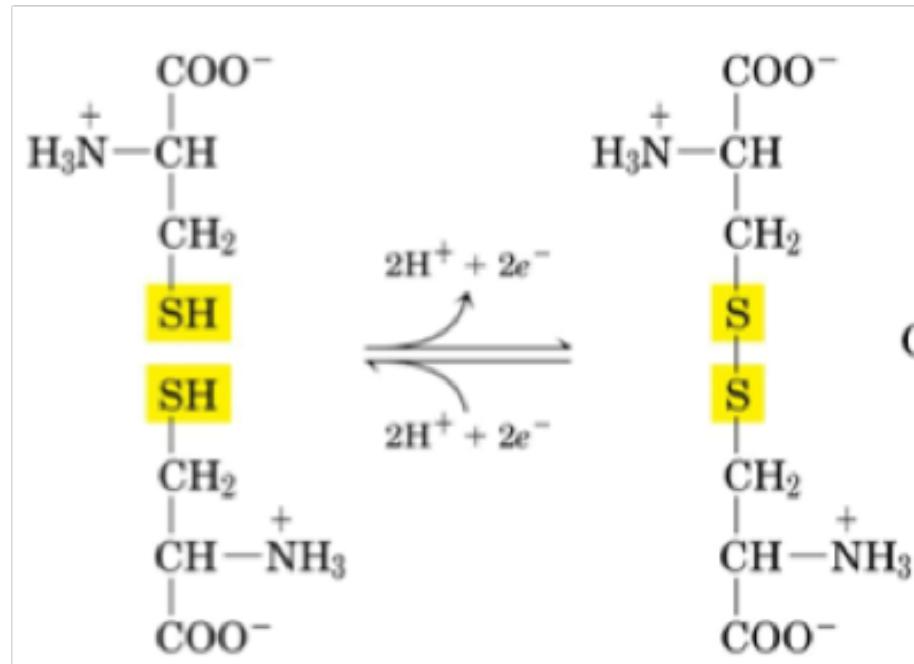
D-Alanine

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

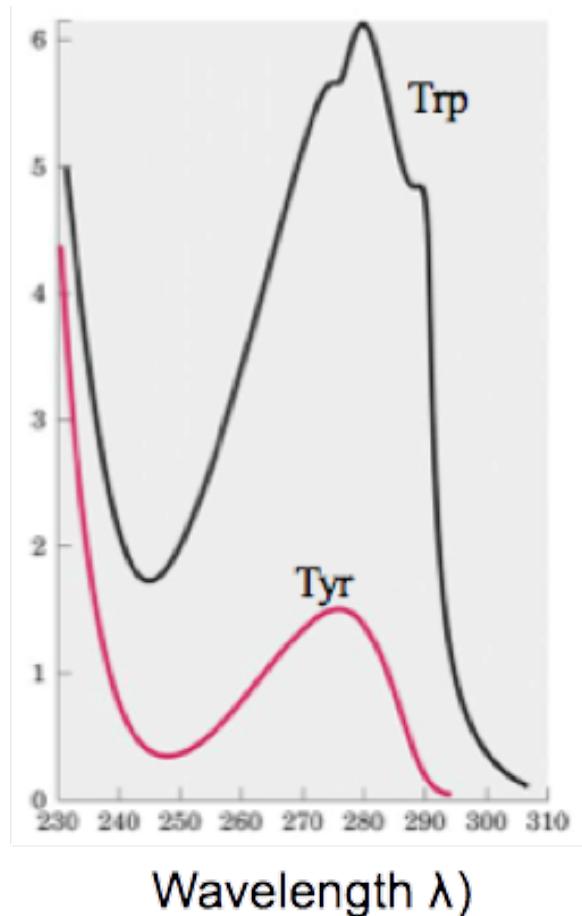
A few bacteria can synthesize D-aa, but they need a special enzyme

## *Properties of 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*



- ◆ 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

## Acid-base equilibria

	pK <sub>COOH</sub>	pK <sub>NH3+</sub>	pK <sub>R</sub>	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

## ACID-BASE PROPERTIES

pKa ranges from 1.8 and 2.4

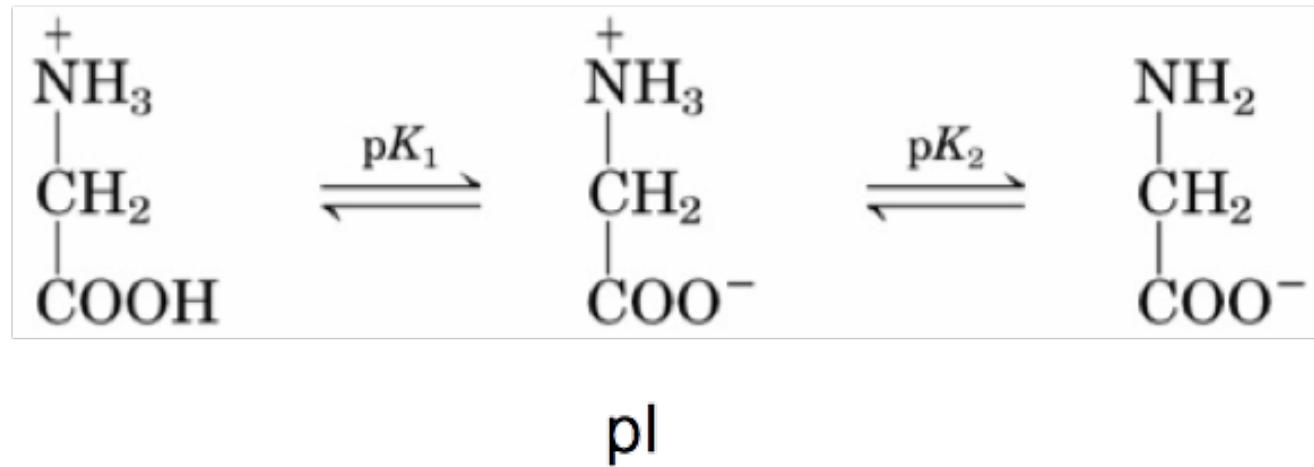
pKb ranges from 9.0 and 10.8

pK<sub>R</sub> 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 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 (cation)
- ◆ At  $\text{pH} < \text{pI} \rightarrow$  the aa has a net charge of +1 (anion)
- ◆ At  $\text{pH} = \text{pI} \rightarrow$  there is the zwitterion, with a net charge of 0



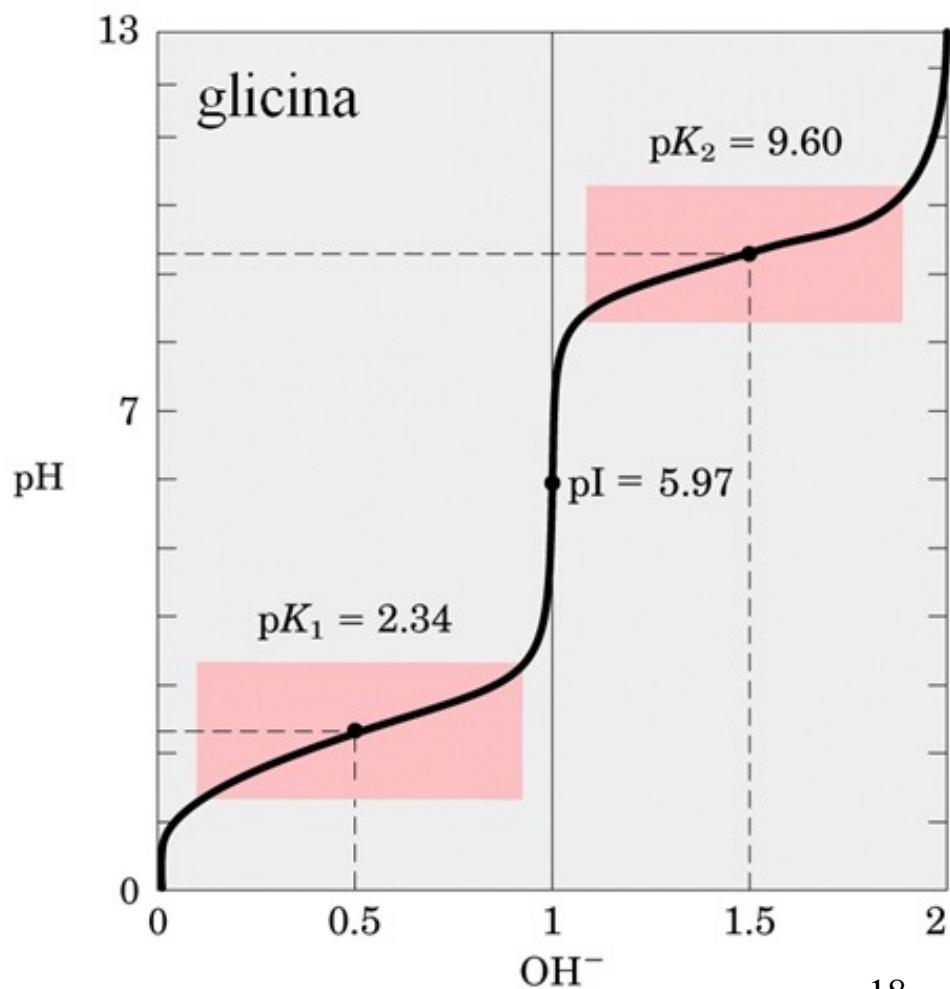
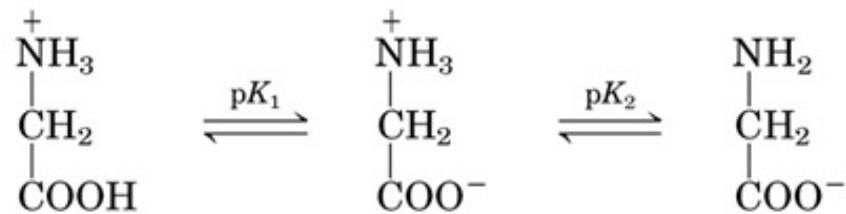
# Effect of substituents on pK

ACID	FORMULA	pK
acetic	$\text{CH}_3\text{—COOH}$	4.75
chloroacetic	$\text{ClCH}_2\text{—COOH}$	2.87
dichloroacetic	$\text{Cl}_2\text{CH—COOH}$	1.48
aminoacetic (glycine)	$^+\text{H}_3\text{N—CH}_2\text{—COOH}$	2.35

H. Gutfreund, Enzyme physical properties, Wiley Interscience, 1972, London

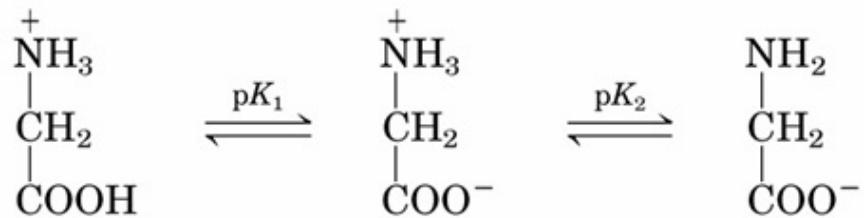
	pK <sub>COOH</sub>	pK <sub>NH3+</sub>	pK <sub>R</sub>	pI
GLY	2.3	9.6	-	6.0
ALA	2.3	9.7	-	6.0
VAL	2.3	9.6	-	6.0
PRO	2.0	10.6	-	6.3
LEU	2.4	9.7	-	6.0
MET	2.3	9.2	-	5.8
ILE	2.4	9.7	-	6.1
PHE	1.8	9.1	-	5.5
TYR	2.2	9.1	10.1	5.7
TRP	2.4	9.4	-	5.9
SER	2.2	9.2	-	5.7
THR	2.6	10.4	-	6.5
CYS	1.8	10.8	8.3	5.0
ASN	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

## Il pH o punto isoelettrico



## The isoelectric point (or pH)

It is the pH with the highest concentration of the zwitterionic species..

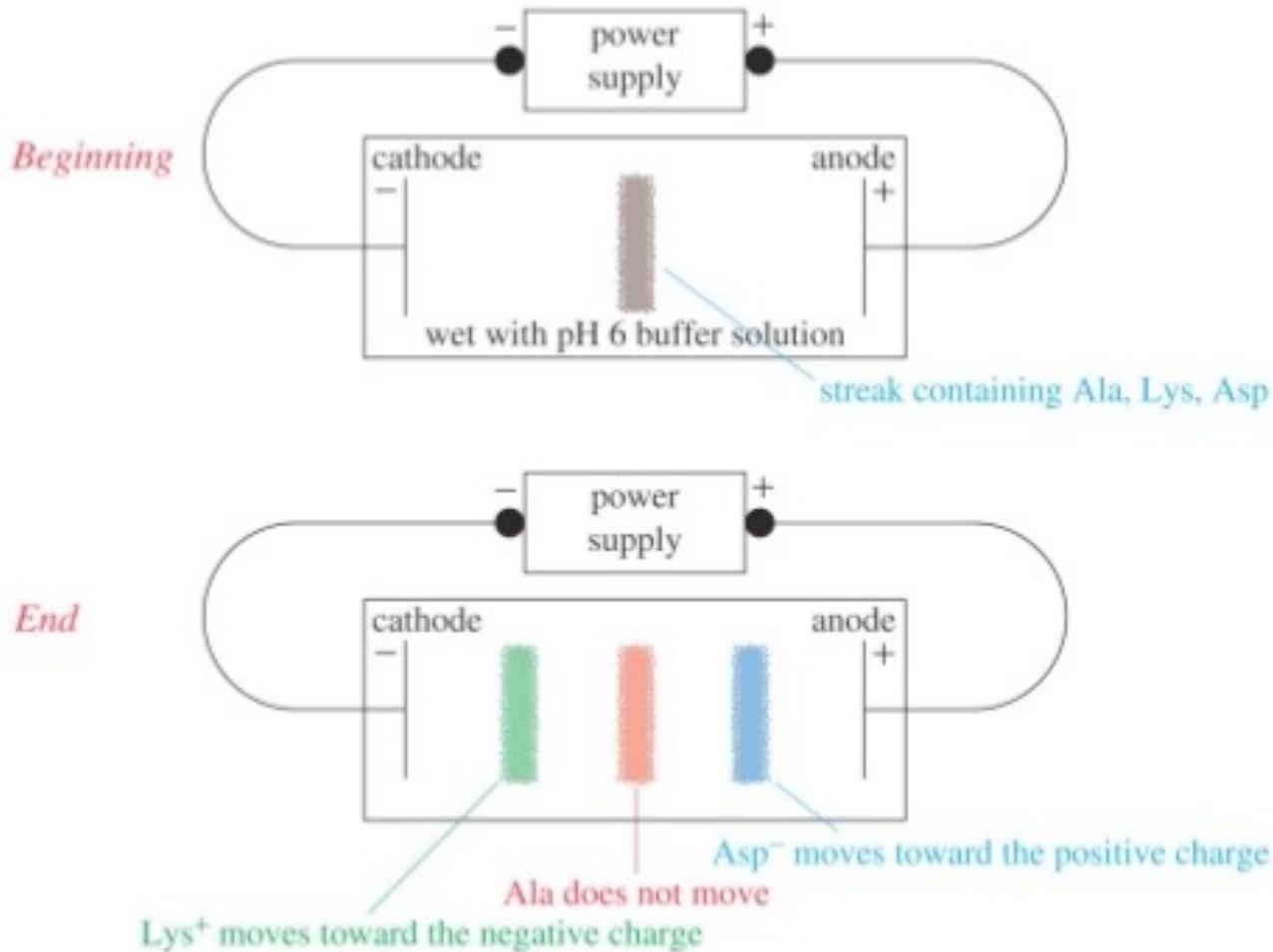


$\text{pH} < \text{pI} \rightarrow \text{cation}$

$\text{pH} = \text{pI} \rightarrow \text{zwitterion}$

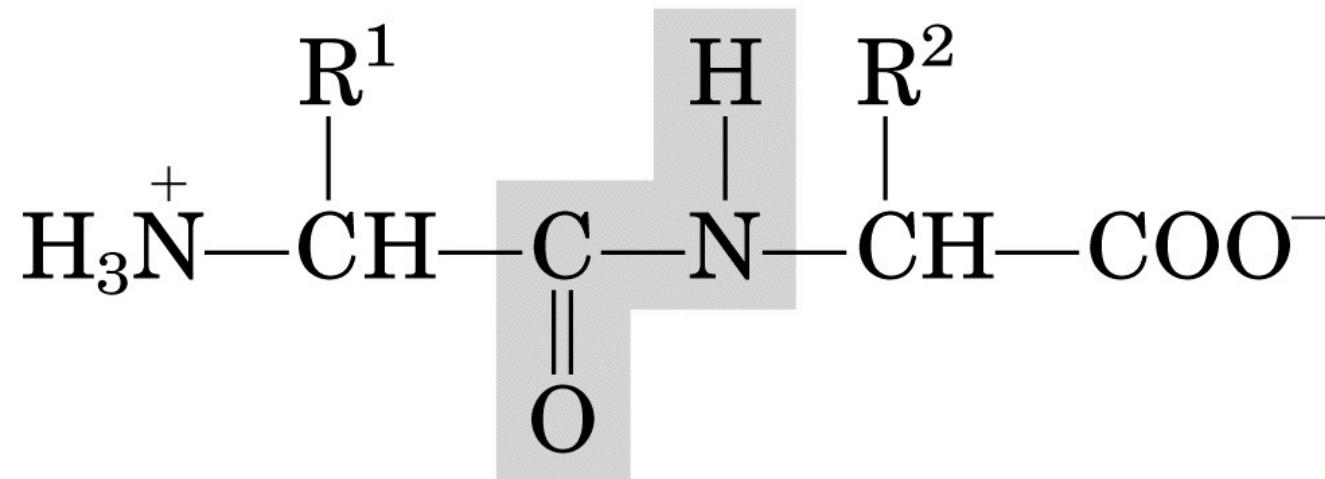
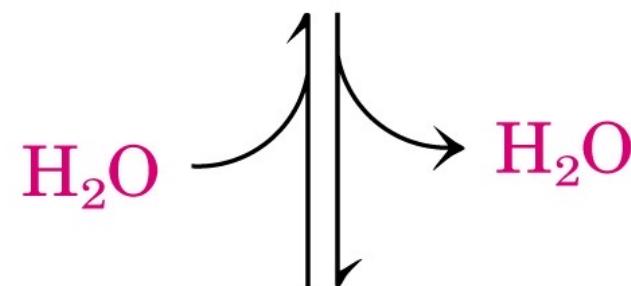
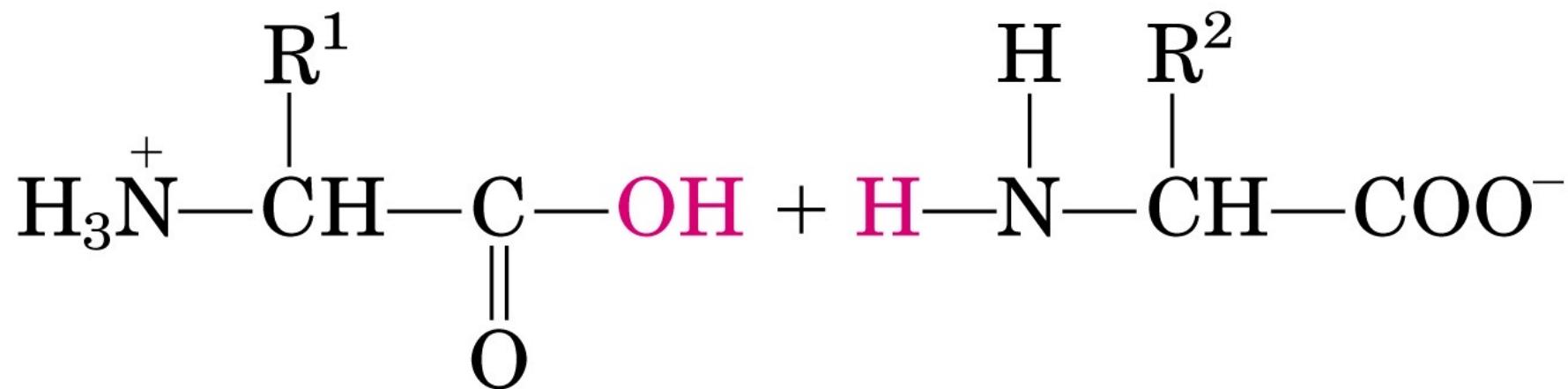
$\text{pH} > \text{pI} \rightarrow \text{anion}$

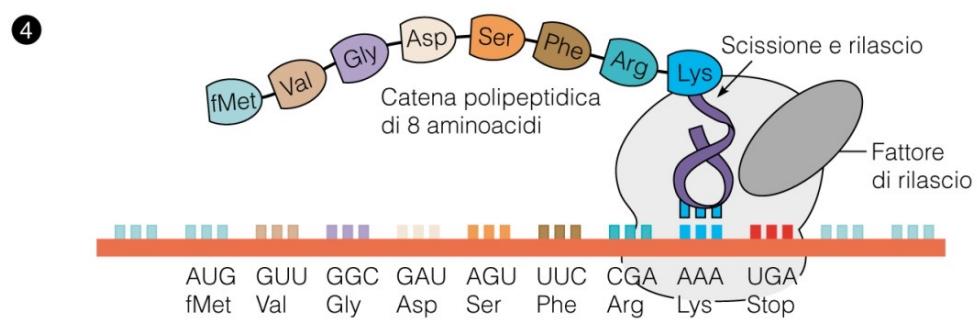
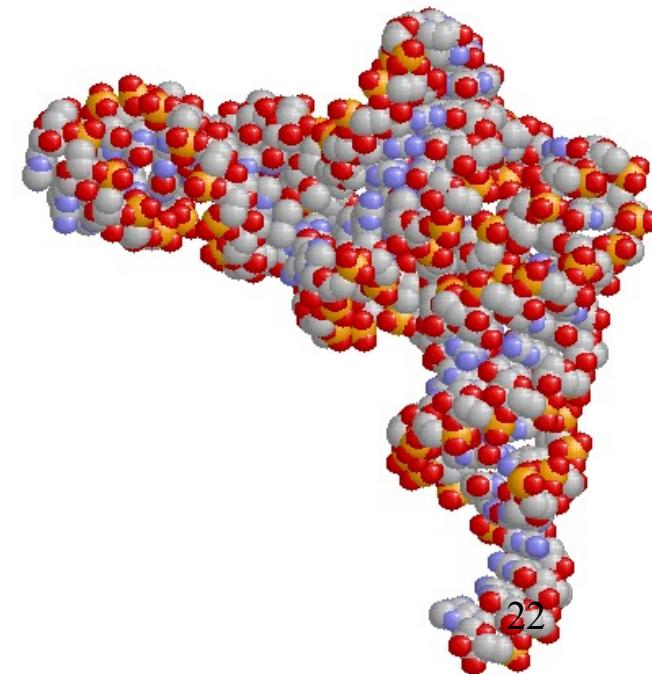
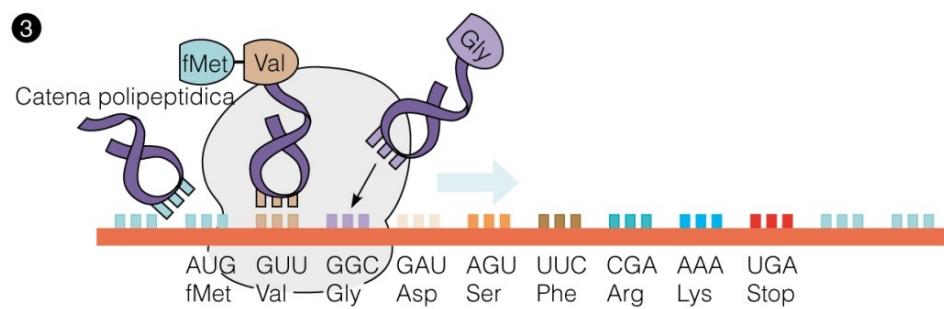
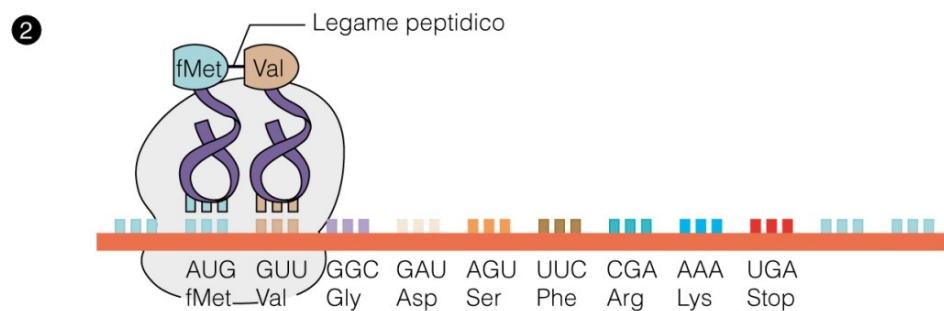
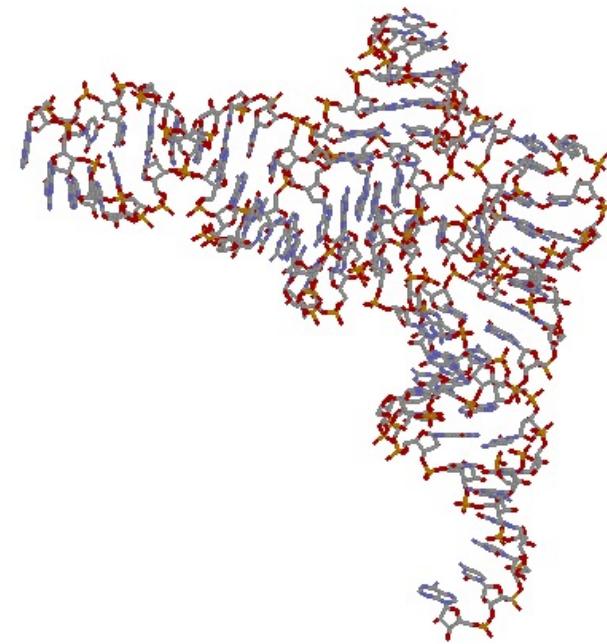
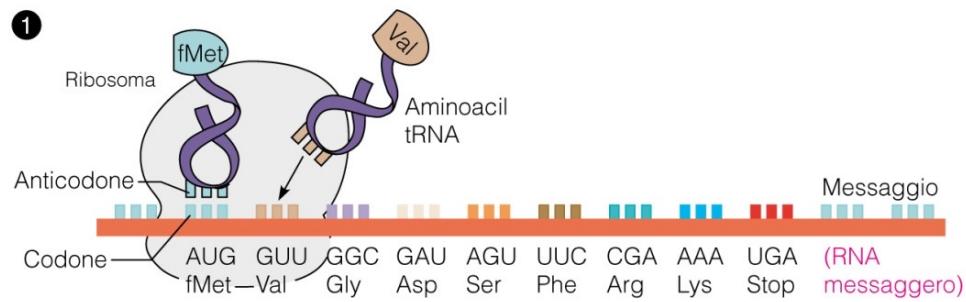
# Electrophoresis



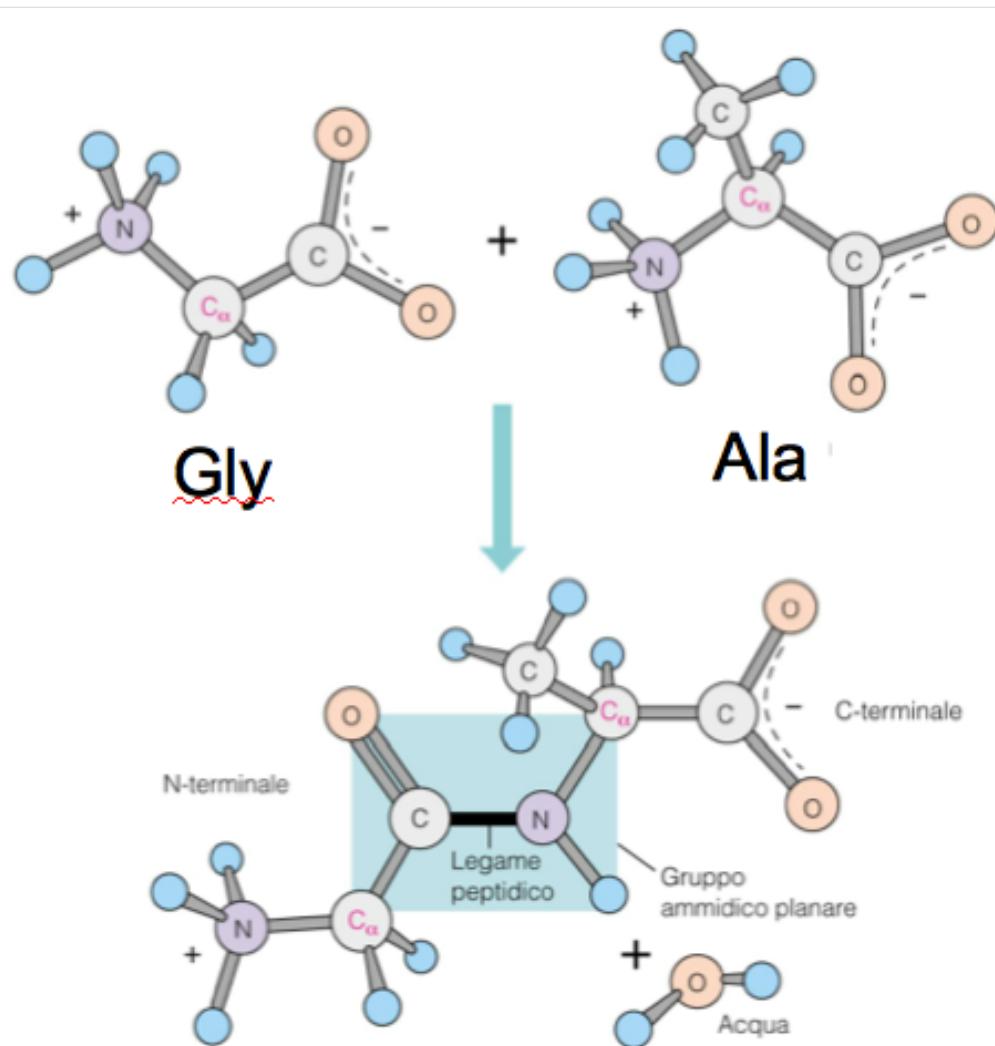
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## Peptide bond.





## Polymerization of aa



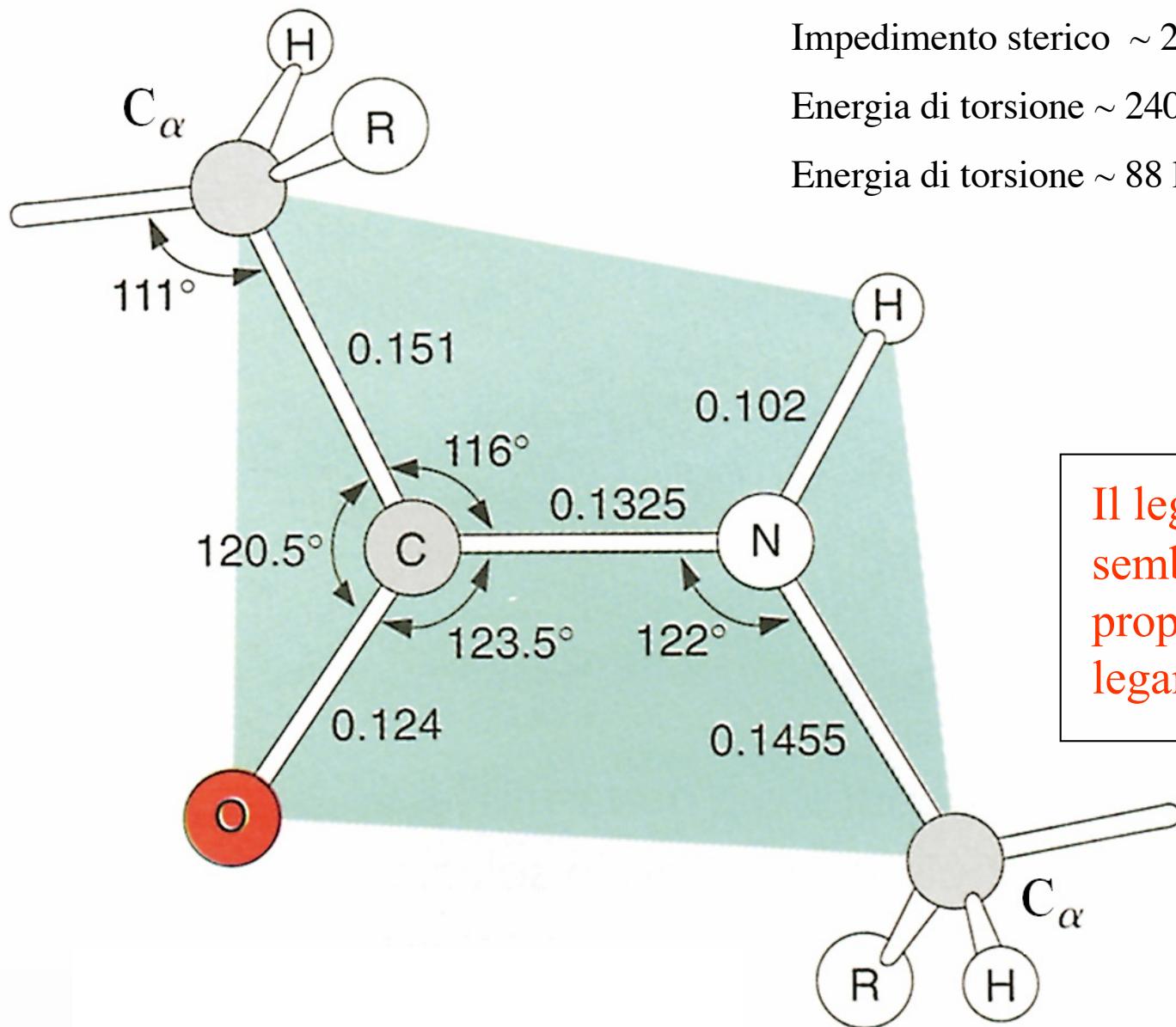
- The peptide bond has 40% character of double bond
- 6 atoms lie in the plane of the bond
- There is a partial negative charge on the carbonyl O and a partial positive charge on the amino N
- All the peptide bonds in proteins are in the *trans* configurations

Energia di torsione  $\sim 12 \text{ kJ mol}^{-1}$  (etano)

Impedimento sterico  $\sim 24 \text{ kJ mol}^{-1}$  (butano)

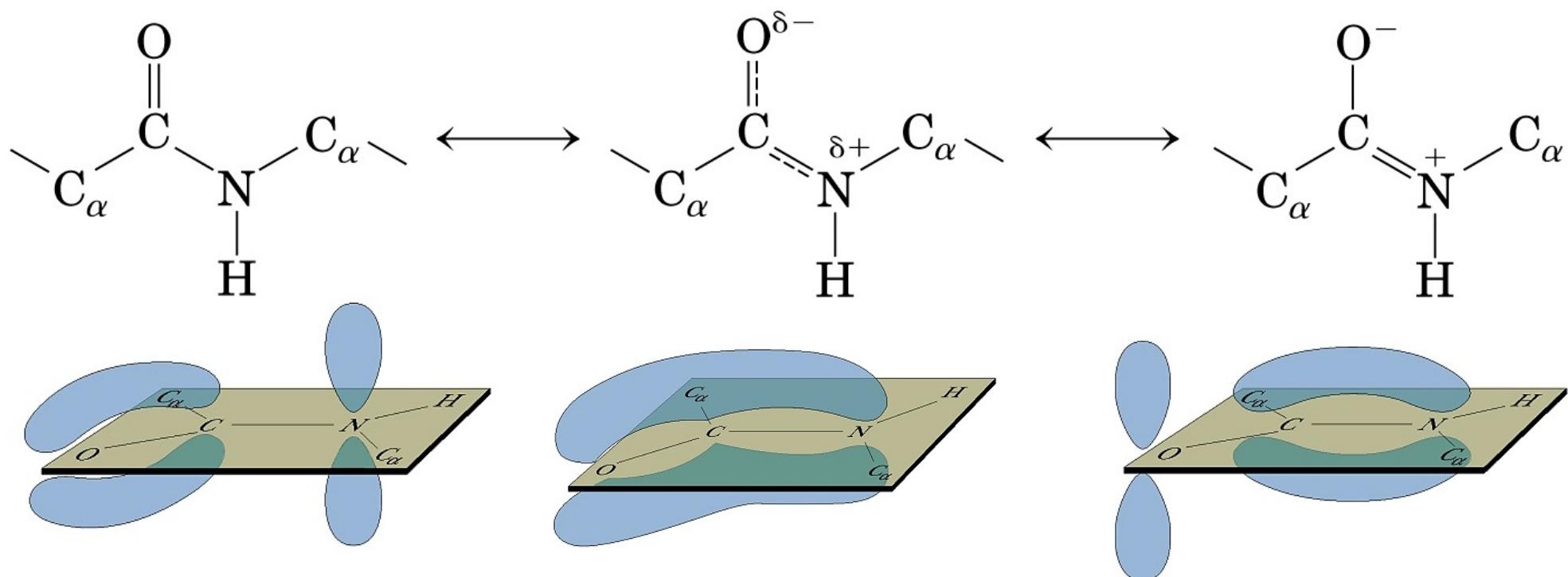
Energia di torsione  $\sim 240 \text{ kJ mol}^{-1}$  (2-butene)

Energia di torsione  $\sim 88 \text{ kJ mol}^{-1}$  (legame peptidico)



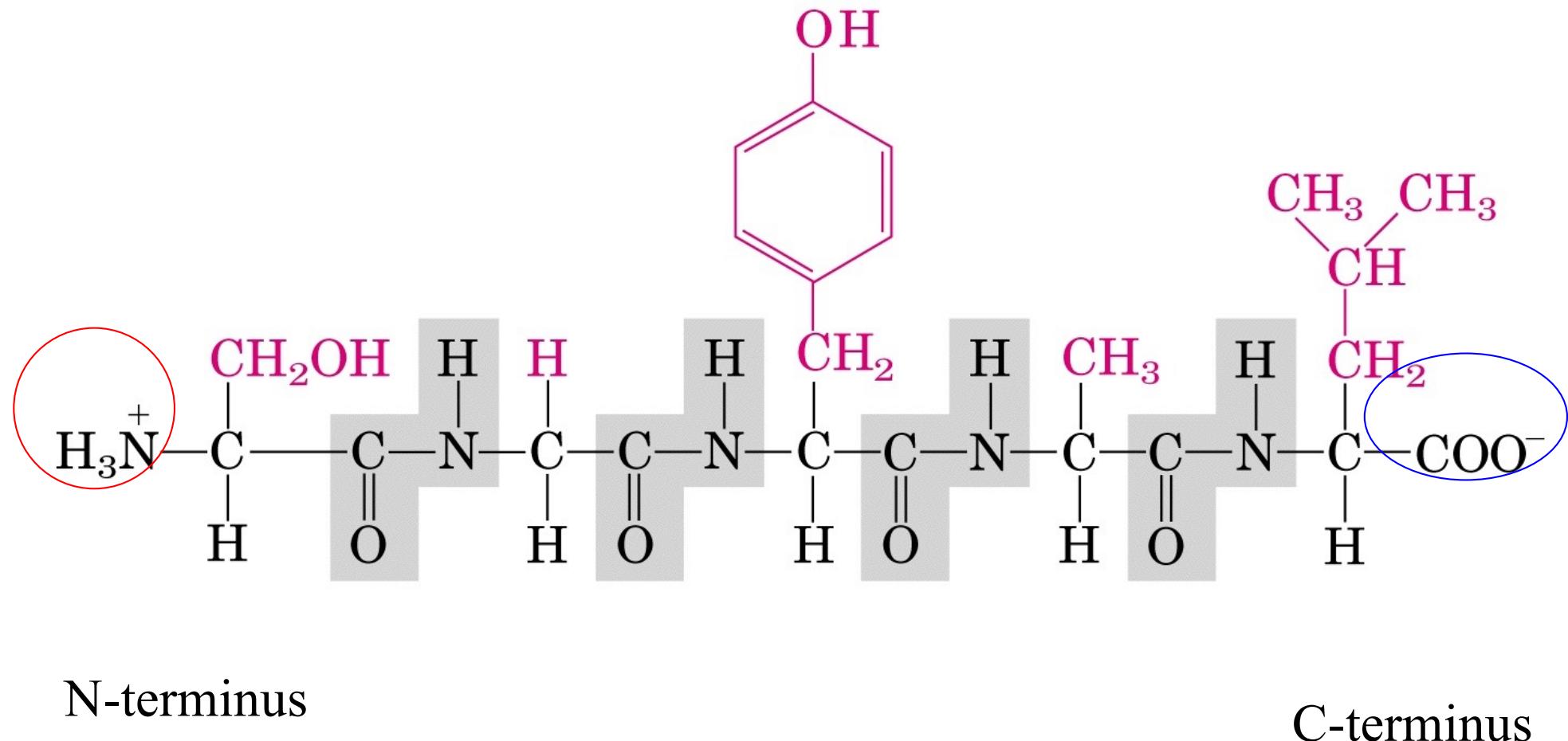
Il legame peptidico  
sembra non avere le  
proprietà strutturali di un  
legame semplice

The peptide bond has a partial double bond character. The carbonyl oxygen atom has a partial negative charge and the amide nitrogen has a partial positive charge. This generates an electric dipole in the hybrid. Virtually all peptide bonds in proteins are present in the trans configuration.



Resonance hybrid

The polypeptide chain (protein) has a direction.



N-terminus

C-terminus

# The sequence of a protein, maltoporin.

N-term residue



VAL ASP PHE HIS GLY TYR ALA ARG SER GLY ILE GLY TRP THR GLY SER GLY GLY GLU GLN 20  
GLN CYS PHE GLN THR THR GLY ALA GLN SER LYS TYR ARG LEU GLY ASN GLU CYS GLU THR 40  
TYR ALA GLU LEU LYS LEU GLY GLN GLU VAL TRP LYS GLU GLY ASP LYS SER PHE TYR PHE 60  
ASP THR ASN VAL ALA TYR SER VAL ALA GLN GLN ASN ASP TRP GLU ALA THR ASP PRO ALA 80  
PHE ARG GLU ALA ASN VAL GLN GLY LYS ASN LEU ILE GLU TRP LEU PRO GLY SER THR ILE 100  
TRP ALA GLY LYS ARG PHE TYR GLN ARG HIS ASP VAL HIS MET ILE ASP PHE TYR TYR TRP 120  
ASP ILE SER GLY PRO GLY ALA GLY LEU GLU ASN ILE ASP VAL GLY PHE GLY LYS LEU SER 140  
LEU ALA ALA THR ARG SER SER GLU ALA GLY GLY SER SER SER PHE ALA SER ASN ASN ILE 160  
TYR ASP TYR THR ASN GLU THR ALA ASN ASP VAL PHE ASP VAL ARG LEU ALA GLN MET GLU 180  
ILE ASN PRO GLY GLY THR LEU GLU LEU GLY VAL ASP TYR GLY ARG ALA ASN LEU ARG ASP 200  
ASN TYR ARG LEU VAL ASP GLY ALA SER LYS ASP GLY TRP LEU PHE THR ALA GLU HIS THR 220  
GLN SER VAL LEU LYS GLY PHE ASN LYS PHE VAL VAL GLN TYR ALA THR ASP SER MET THR 240  
SER GLN GLY LYS GLY LEU SER GLN GLY SER GLY VAL ALA PHE ASP ASN GLU LYS PHE ALA 260  
TYR ASN ILE ASN ASN ASN GLY HIS MET LEU ARG ILE LEU ASP HIS GLY ALA ILE SER MET 280  
GLY ASP ASN TRP ASP MET MET TYR VAL GLY MET TYR GLN ASP ILE ASN TRP ASP ASN ASP 300  
ASN GLY THR LYS TRP TRP THR VAL GLY ILE ARG PRO MET TYR LYS TRP THR PRO ILE MET 320  
SER THR VAL MET GLU ILE GLY TYR ASP ASN VAL GLU SER GLN ARG THR GLY ASP LYS ASN 340  
ASN GLN TYR LYS ILE THR LEU ALA GLN GLN TRP GLN ALA GLY ASP SER ILE TRP SER ARG 360  
PRO ALA ILE ARG VAL PHE ALA THR TYR ALA LYS TRP ASP GLU LYS TRP GLY TYR ASP TYR 380  
THR GLY ASN ALA ASP ASN ASN ALA ASN PHE GLY LYS ALA VAL PRO ALA ASP PHE ASN GLY 400  
GLY SER PHE GLY ARG GLY ASP SER ASP GLU TRP THR PHE GLY ALA GLN MET GLU ILE TRP 420  
TRP 421



C-term residue

**Table 6–1** Molecular data on some proteins

	Molecular weight	Number of residues	Number of polypeptide chains
Insulin (bovine)	5,733	51	2
Cytochrome c (human)	13,000	104	1
Ribonuclease A (bovine pancreas)	13,700	124	1
Lysozyme (egg white)	13,930	129	1
Myoglobin (equine heart)	16,890	153	1
Chymotrypsin (bovine pancreas)	21,600	241	3
Chymotrypsinogen (bovine)	22,000	245	1
Hemoglobin (human)	64,500	574	4
Serum albumin (human)	68,500	~550	1
Hexokinase (yeast)	102,000	~800	2
Immunoglobulin G (human)	145,000	~1,320	4
RNA polymerase ( <i>E. coli</i> )	450,000	~4,100	5
Apolipoprotein B (human)	513,000	4,536	1
Glutamate dehydrogenase (bovine liver)	1,000,000	~8,300	~40

# Type of conjugated protein:

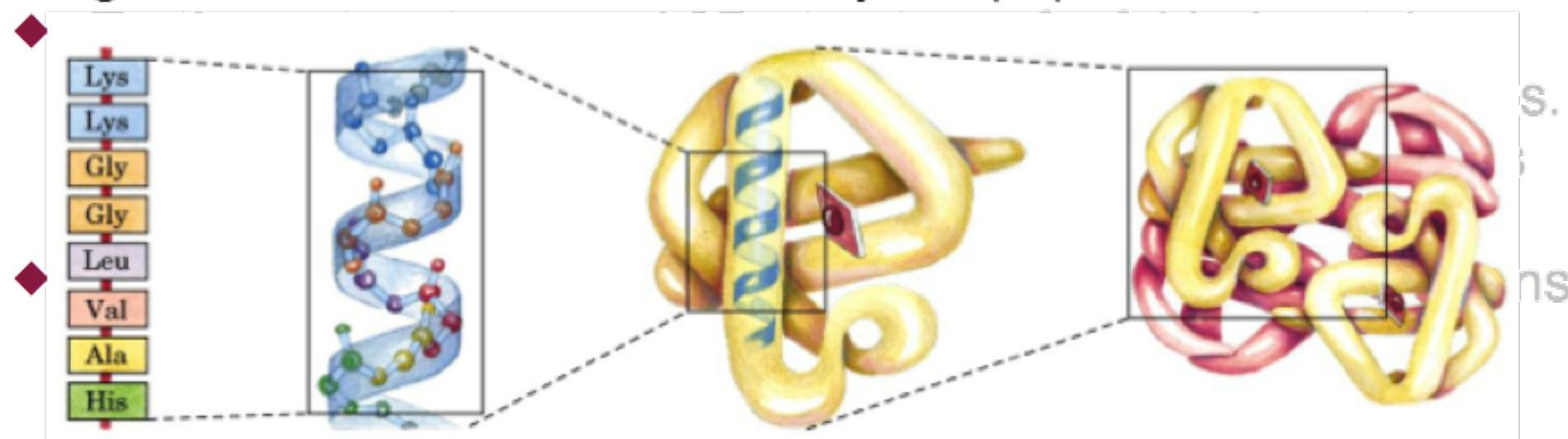
- **Nucleoproteins:** Contains nucleic acids, nitrogen, and phosphorus. Present in chromosomes and in all living forms as a combination of protein with either RNA or DNA.
- **Glycoprotein:** bone (osseomucoid); tendons (tendomucoid); cartilage (chondromucoid).
- **Phosphoproteins:** milk (casein); egg yolk (ovovitellin). Phosphoric acid joined in ester linkage to protein.
- **Lipoproteins:** serum lipoprotein; brain, nerve tissues, milk, and eggs. Water-soluble protein conjugated with lipids.
- **Chromoproteins:** hemoglobin; myoglobin; flavoproteins; respiratory pigments; cytochromes, colored proteins.

## Isoelectric Points of Several Common Proteins

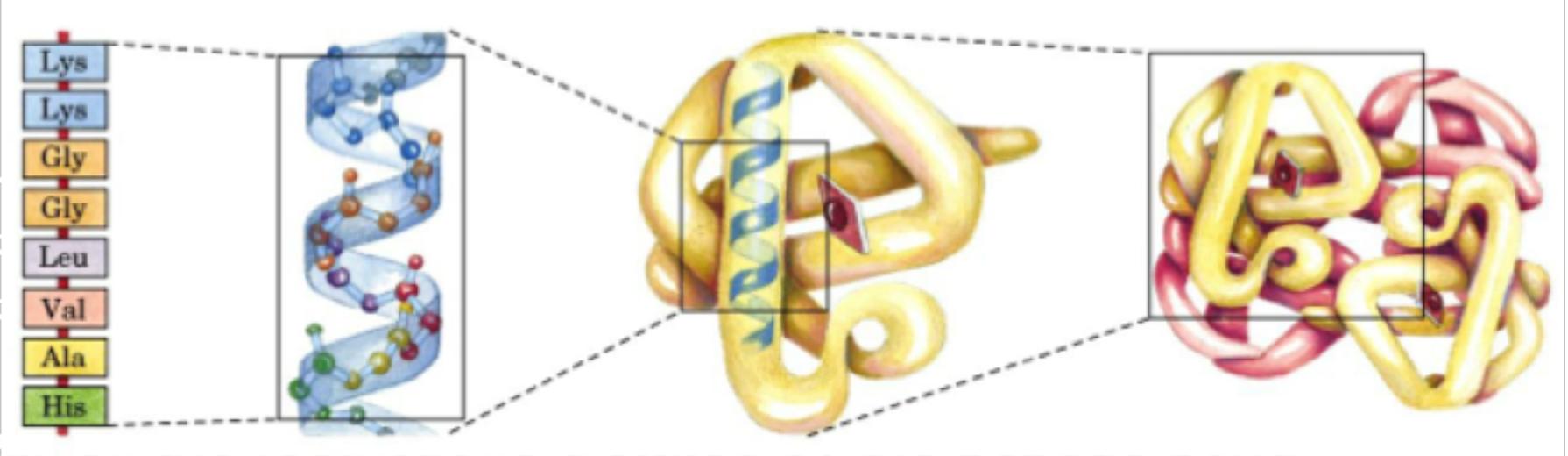
Protein	pI
Pepsin	<1.0
Ovalbumin (hen)	4.6
Serum albumin (human)	4.9
Tropomyosin	5.1
Insulin (bovine)	5.4
Fibrinogen (human)	5.8
$\gamma$ -Globulin (human)	6.6
Collagen	6.6
Myoglobin (horse)	7.0
Hemoglobin (human)	7.1
Ribonuclease A (bovine)	9.4
Cytochrome <i>c</i> (horse)	10.6
Histone (bovine)	10.8
Lysozyme (hen)	11.0
Salmine (salmon)	12.1

## *Hierarchical organization of proteins*

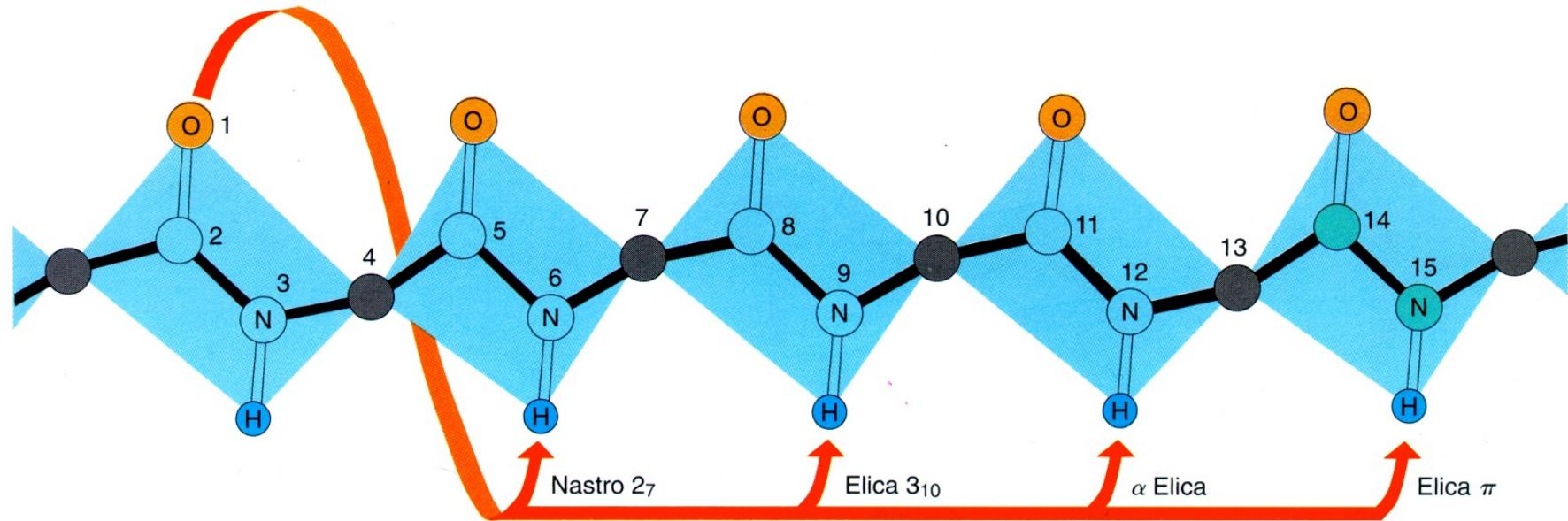
- ◆ **Primary structure:** the linear sequence of aa bound by peptide bonds
- ◆ **Secondary structures:** regular organization of aa in the space, stabilized by hydrogen bonds among CO and NH of several peptide bonds. It can either be periodic (alpha-helix, beta-sheet) or random. Not all the possible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

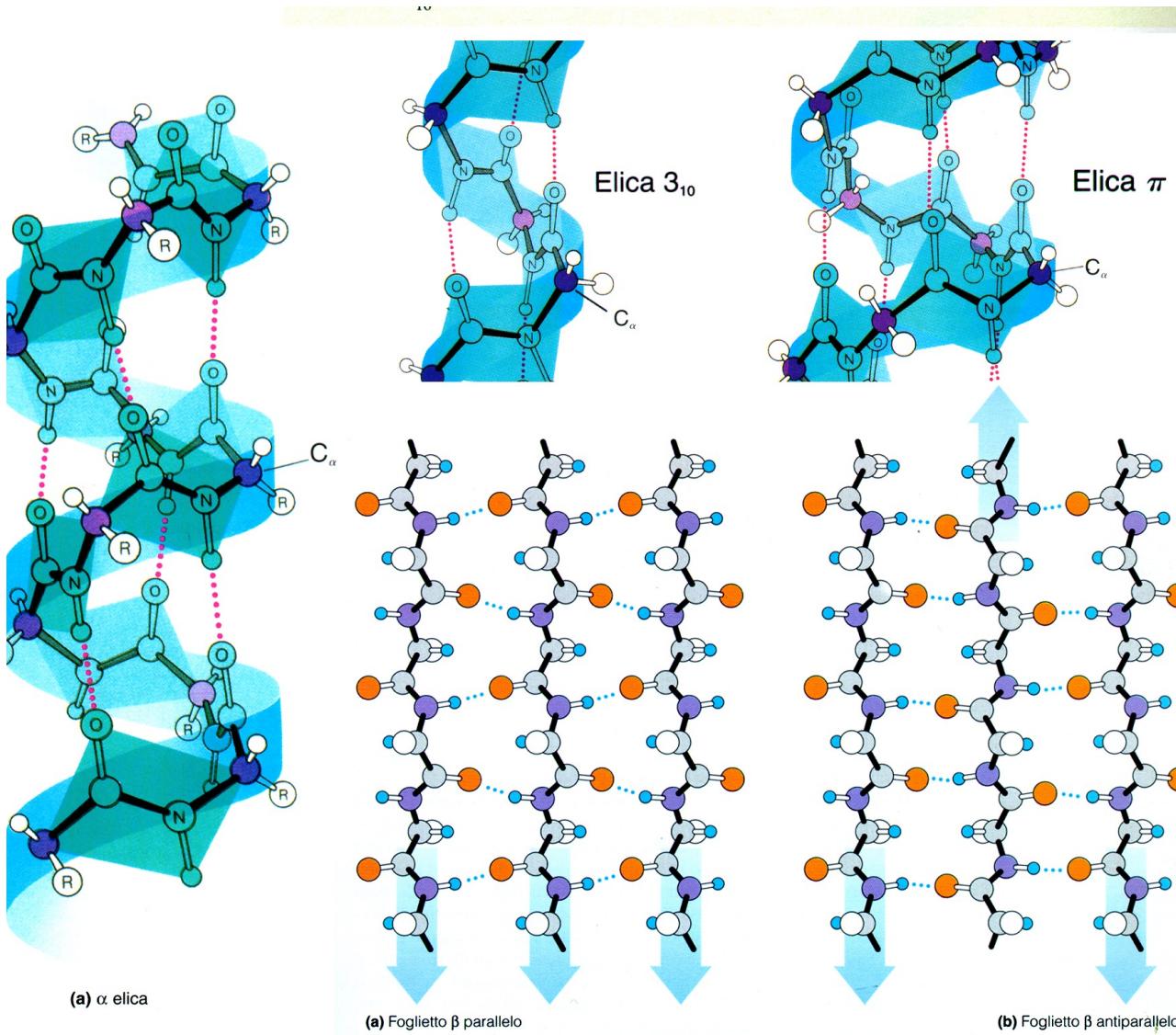


## *Hierarchical organization of proteins*

- ◆ 
- ◆ **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)

## Secondary structure

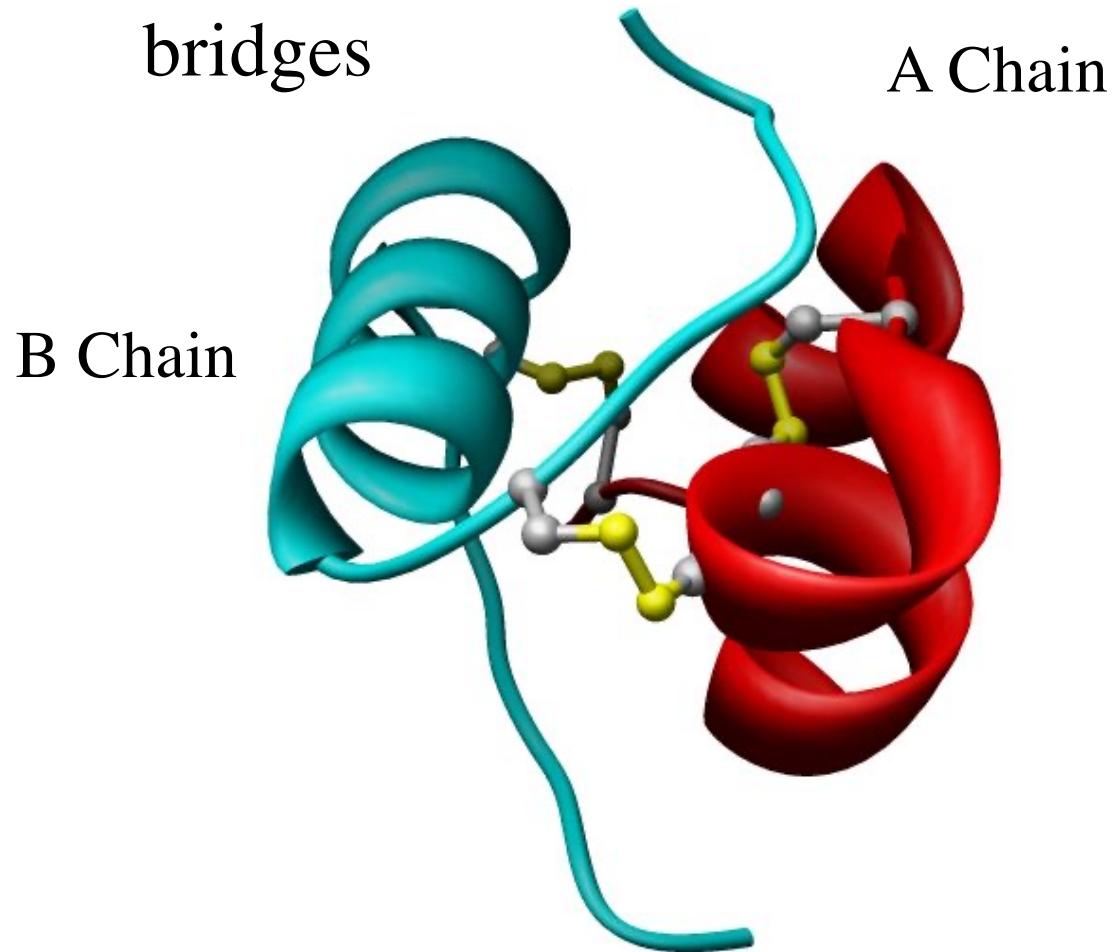




Secondary structure  
 $\alpha$  Helix  
 $\beta$  Sheet

# Insulin

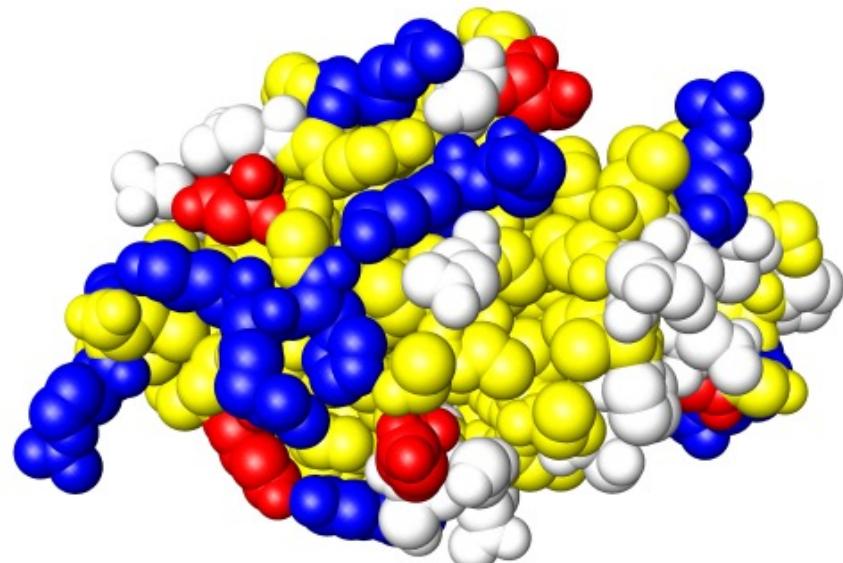
## Role of disulphide bridges



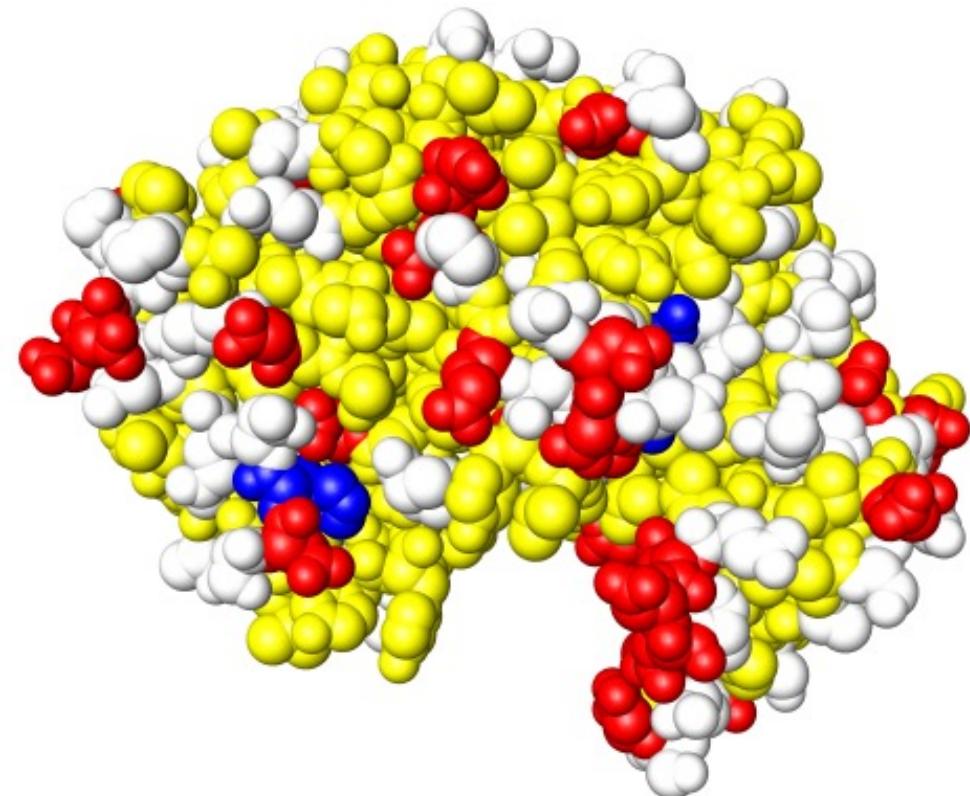
A chain		B chain
$^+ \text{NH}_3$		$^+ \text{NH}_3$
Gly		Phe
Ile		Val
Val		Asn
Gln		Gln
5 Gln		5 His
Cys		Leu
S	—	Gly
S	—	Ser
10 Ala		10 His
Ser		Leu
Val		Val
Cys		Leu
Ser		Val
Leu		Glu
Tyr		Ala
Gln		15 Leu
15 Leu		Tyr
Glu		Leu
Asn		Val
Tyr		20 Cys
20 Cys	—	20 Gly
Asn		Glu
COO <sup>-</sup>		Arg
		Gly
		Phe
		25 Phe
		Tyr
		Thr
		Pro
		Lys
		30 Ala
		COO <sup>-</sup>

## Tertiary structure

Lysozyme (*Gallus gallus*) pI = 11

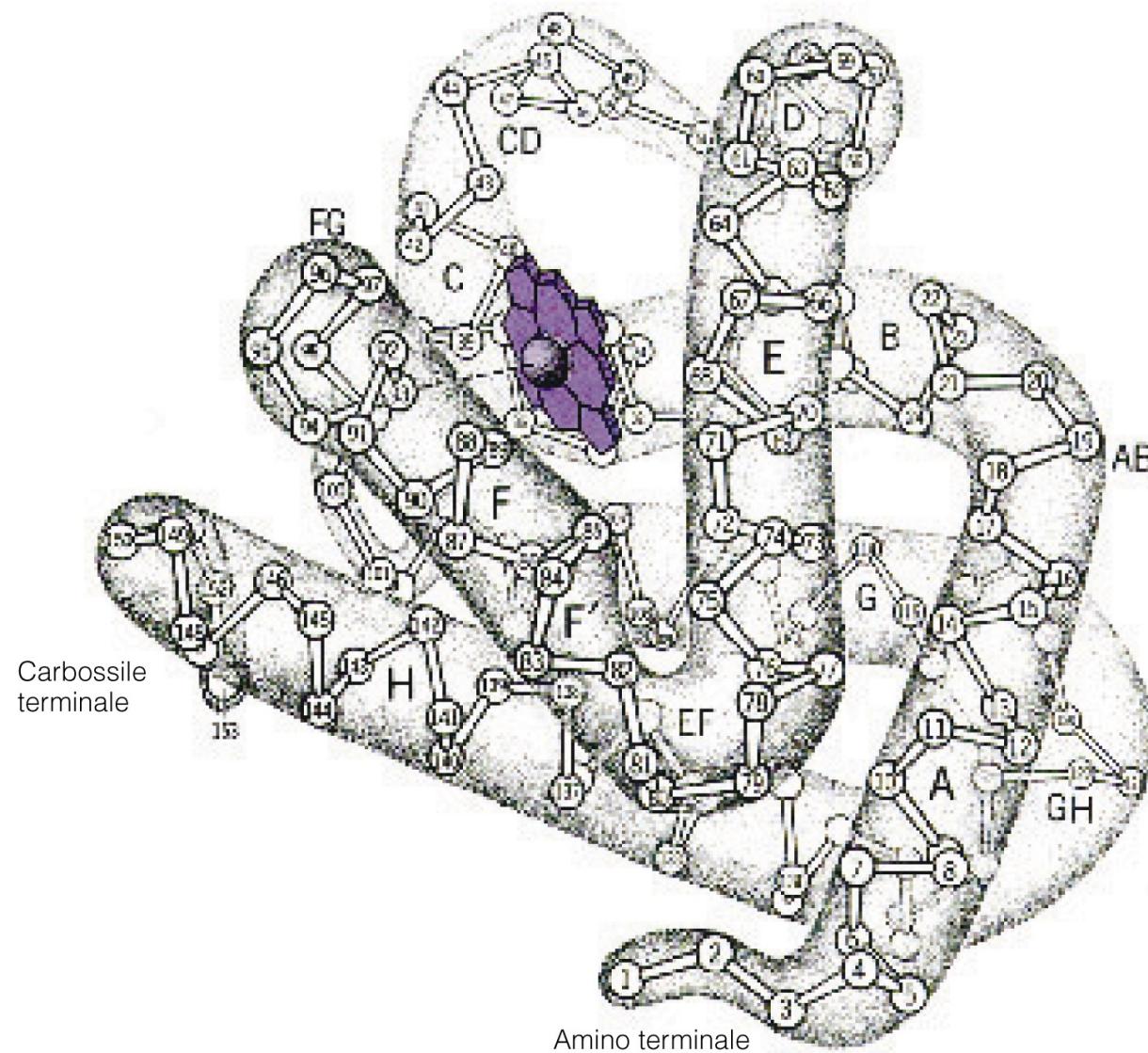


Pepsin (*Sus scrofa*) pI = 1

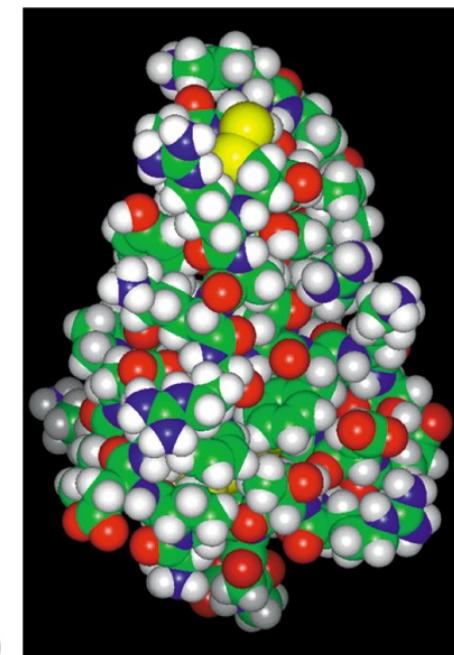
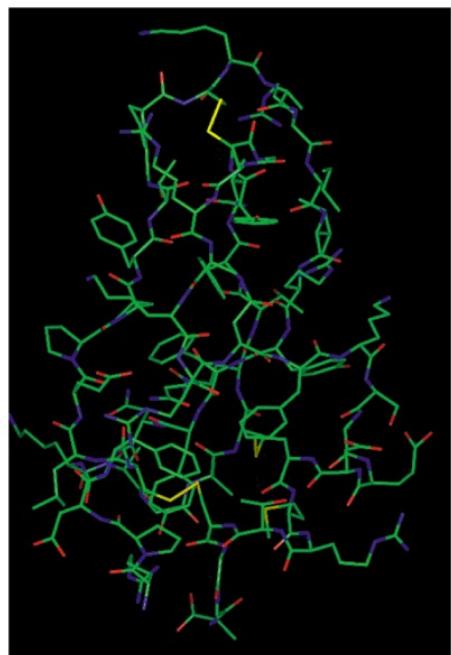


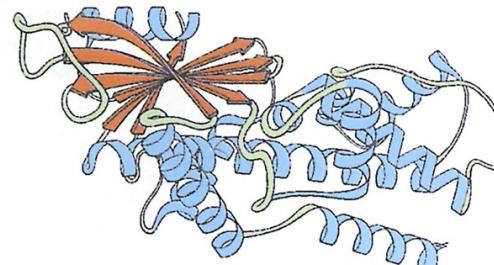
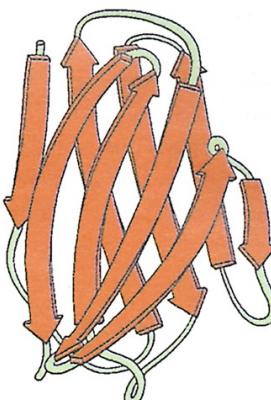
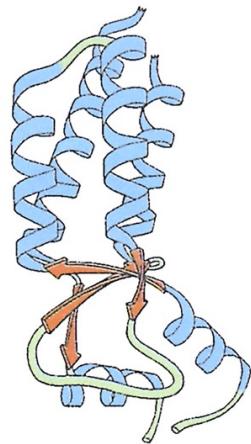
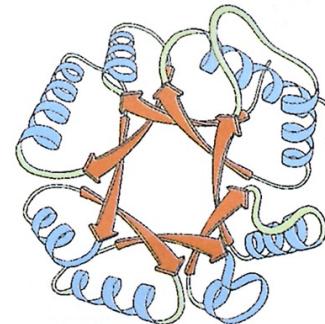
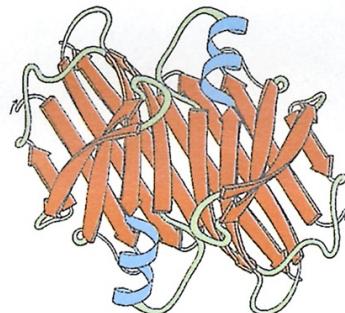
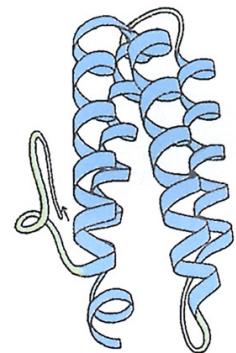
acidic = red; basic = blue; polar = white; hydrophobic = yellow

# Myoglobin



# Ways to depict protein structures



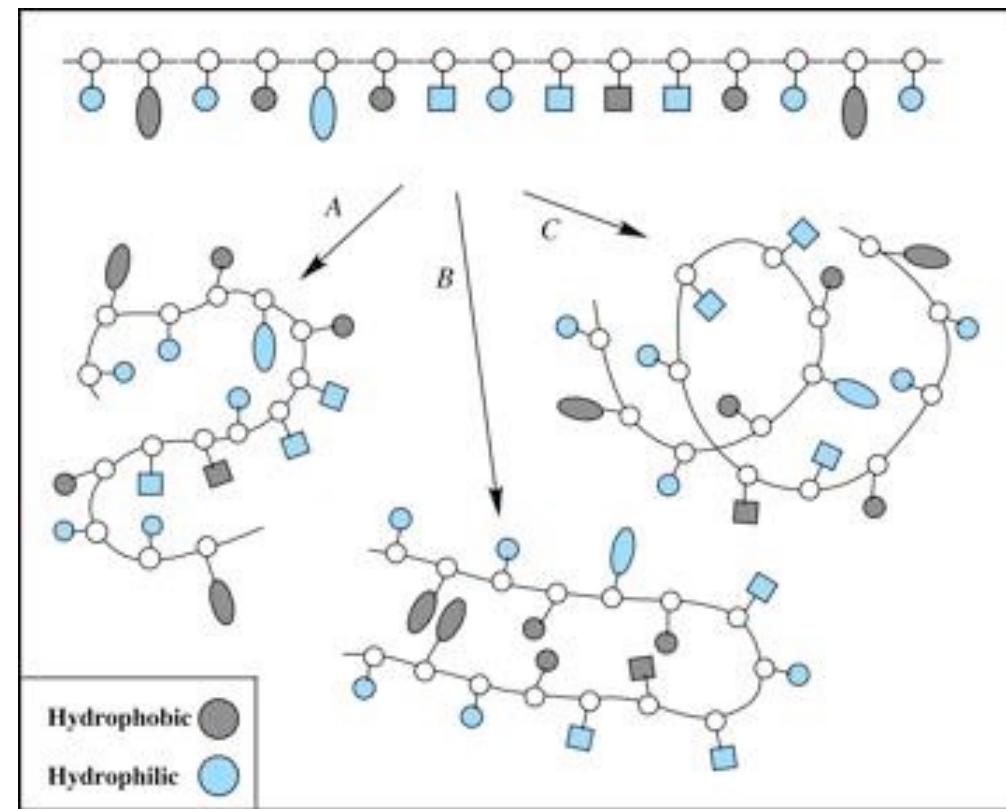
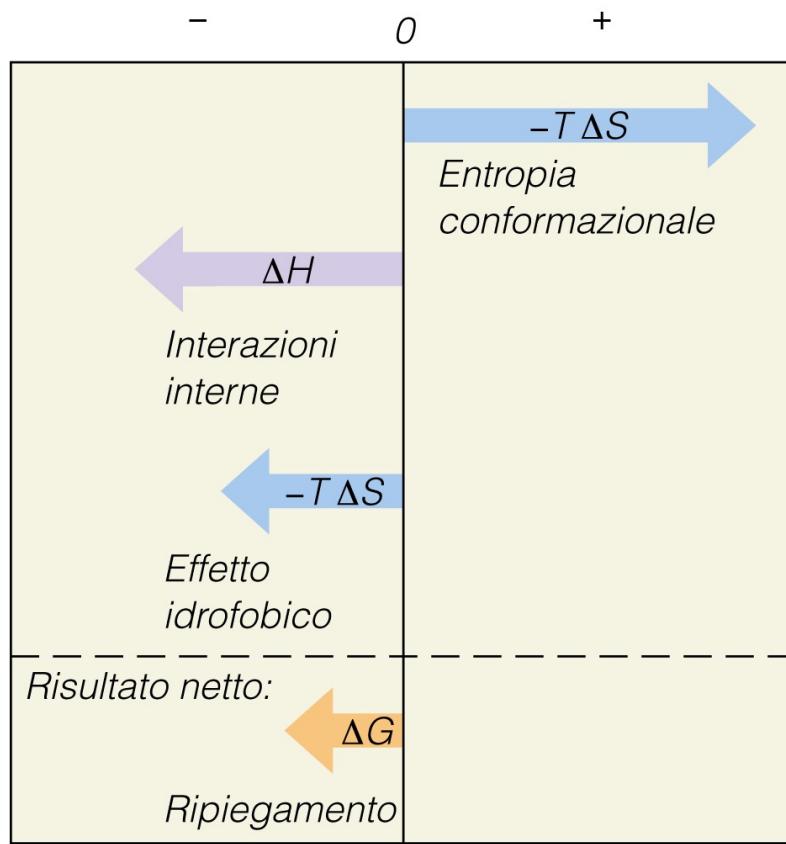


(a) Prevalentemente ad  $\alpha$  elica

(b) Prevalentemente a foglietto  $\beta$

(c) Struttura mista ad  $\alpha$  elica e a foglietto  $\beta$

# Protein folding



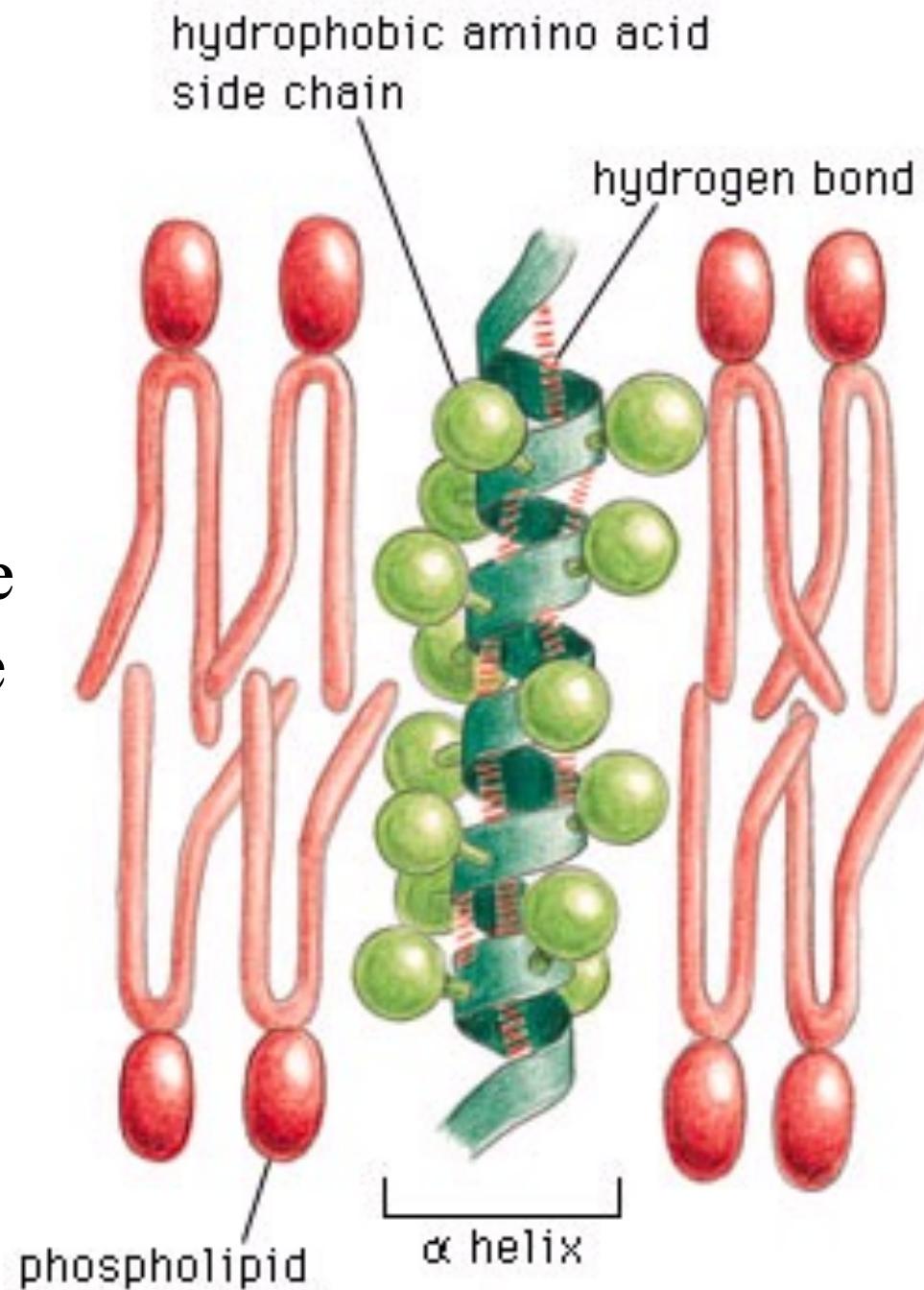
## **Membrane Proteins**

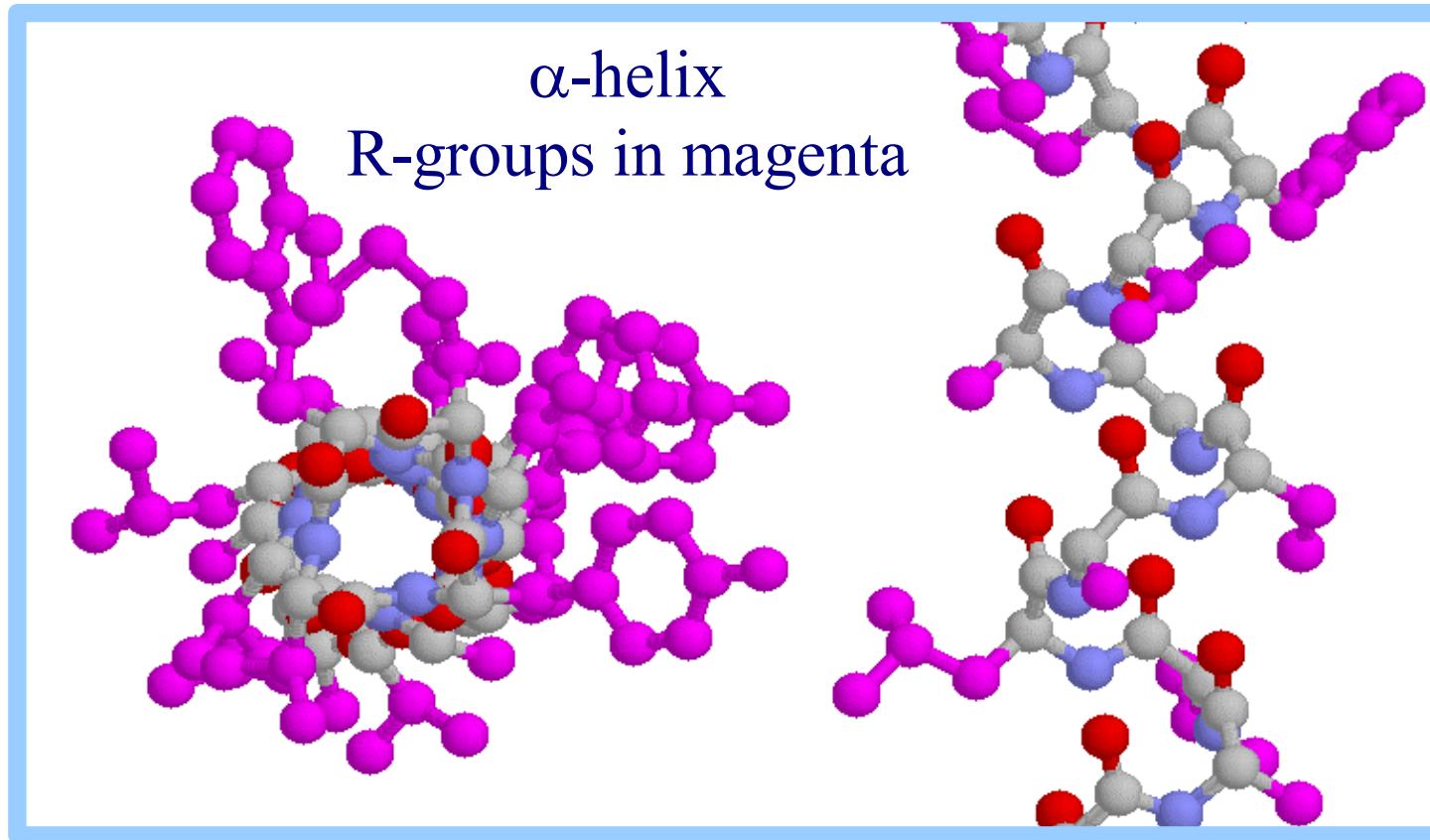
20-30% of the total open reading frames are integral membrane proteins

Membrane proteins are targets for >40% of FDA approved drugs  
G-protein coupled receptors, channels, transporters, receptors, proteases (RIP), etc

There are ~1.5% of deposited structures (555 unique)  
membrane protein structures

The peptide bonds are hydrogen bonded to each other in the interior while the hydrophobic amino acid side chains contact the lipid chains.





In an  $\alpha$ -helix, amino acid R-groups protrude out from the helically coiled polypeptide backbone.

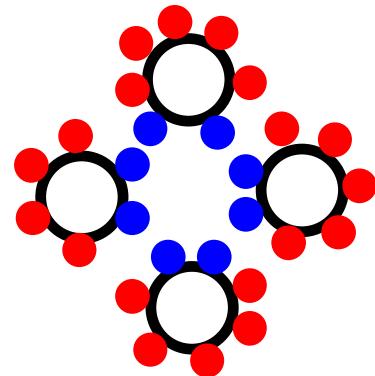
The largely hydrophobic R-groups of a membrane-spanning  $\alpha$ -helix contact the hydrophobic membrane core, while the more polar peptide backbone is buried.

Colors: C N O R-group (H atoms not shown).

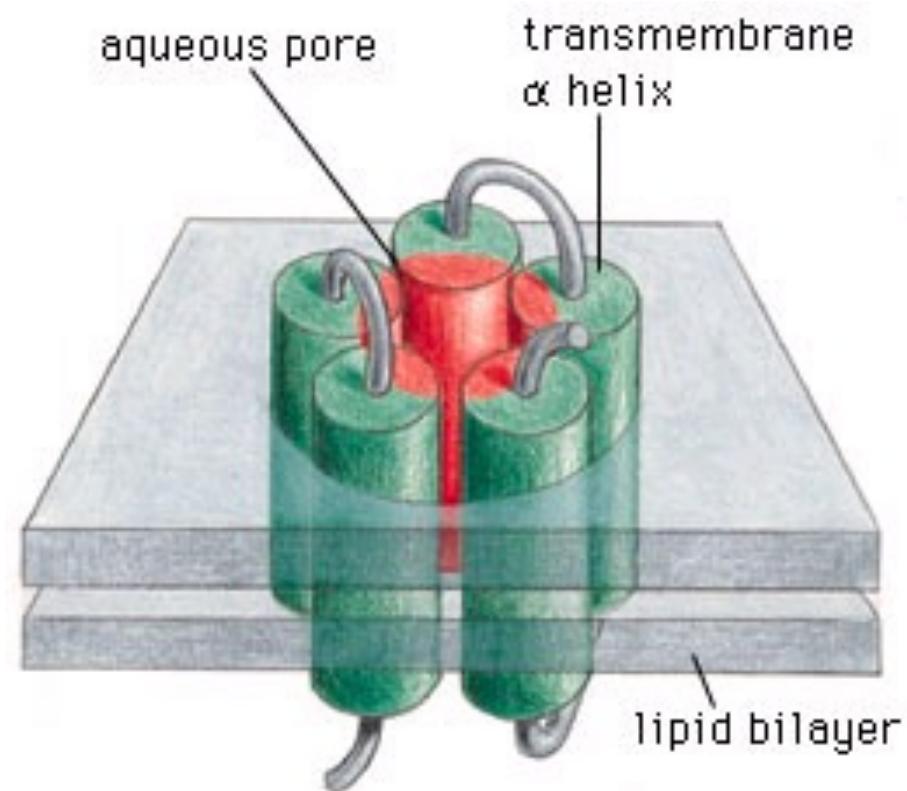
**Since a 20-amino acid  $\alpha$ -helix just spans a lipid bilayer, one could use Hydropathy plots to search for 20-amino acid stretches of hydrophobic amino acids in the primary sequence of a protein to identify membrane proteins in open reading frames.**

Except →

Simplified helical wheel diagram of four  $\alpha$ -helices lining the lumen of an ion channel.



- Polar amino acid R-group
- Non-polar amino acid R-group



©1998 GARLAND PUBLISHING

An  $\alpha$ -helix lining a **water-filled channel** might have polar amino acid R-groups facing the lumen, & non-polar R-groups facing lipids or other hydrophobic  $\alpha$ -helices.

Such **mixed polarity** would prevent detection by a hydropathy plot.

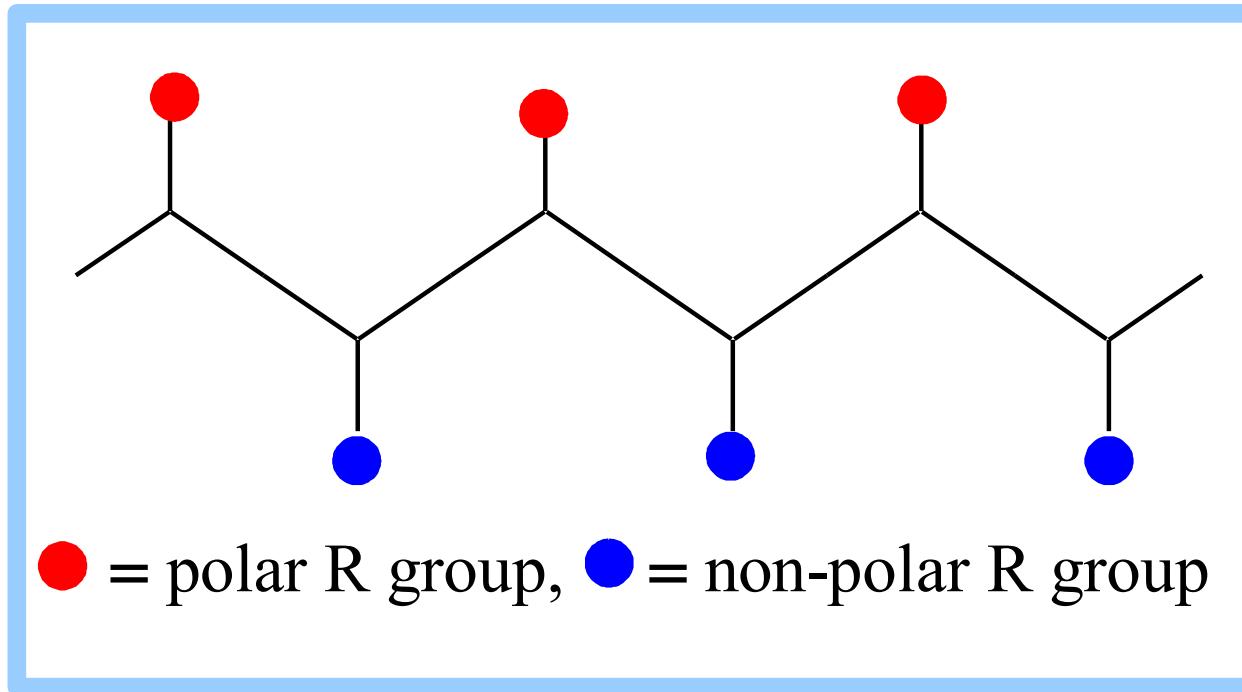
# Porin is a $\beta$ -barrel membrane protein

Porins reside in bacterial outer membrane, have instead  $\beta$  barrel structures.

A  $\beta$  barrel are  $\beta$  sheets rolled up to form a cylindrical pore.

$\beta$  barrels are less versatile since they can only form wide pores,  $\sim 20$  Å in radius.



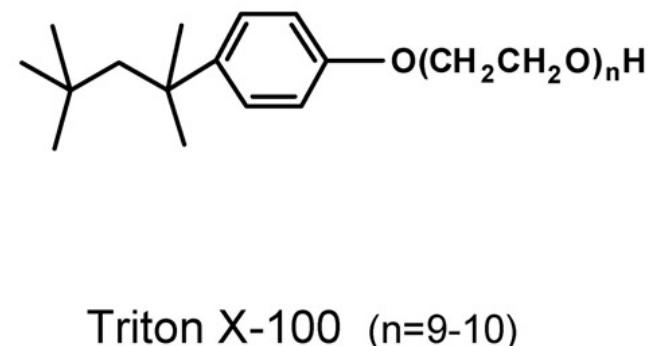
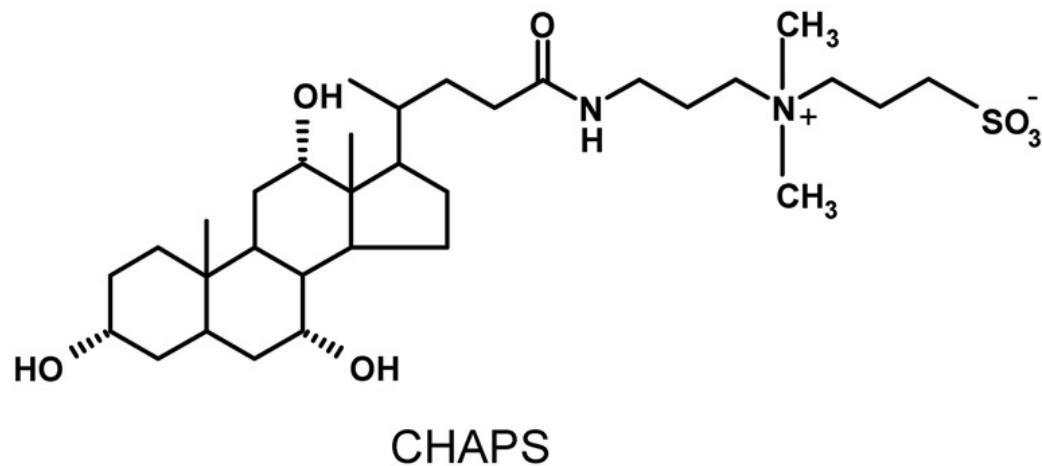
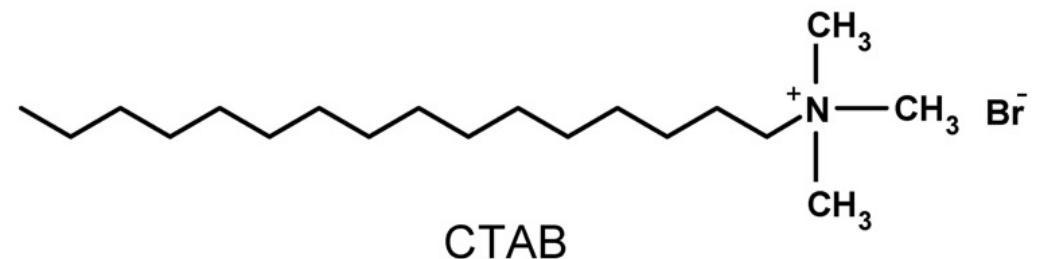
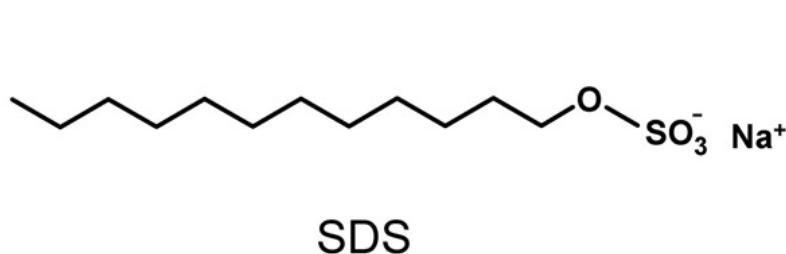


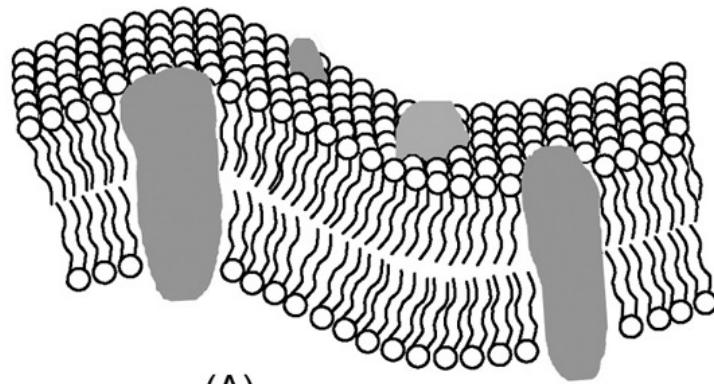
In a  $\beta$ -strand, amino acid R-groups alternately point above & below the strand.

Much of porin primary structure consists of  
**alternating polar & non-polar amino acids.**

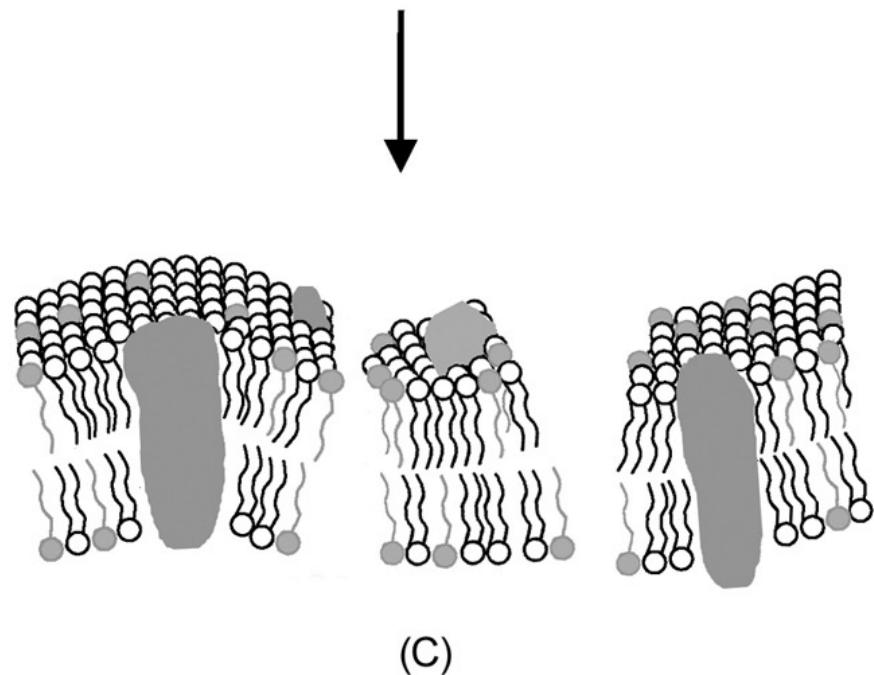
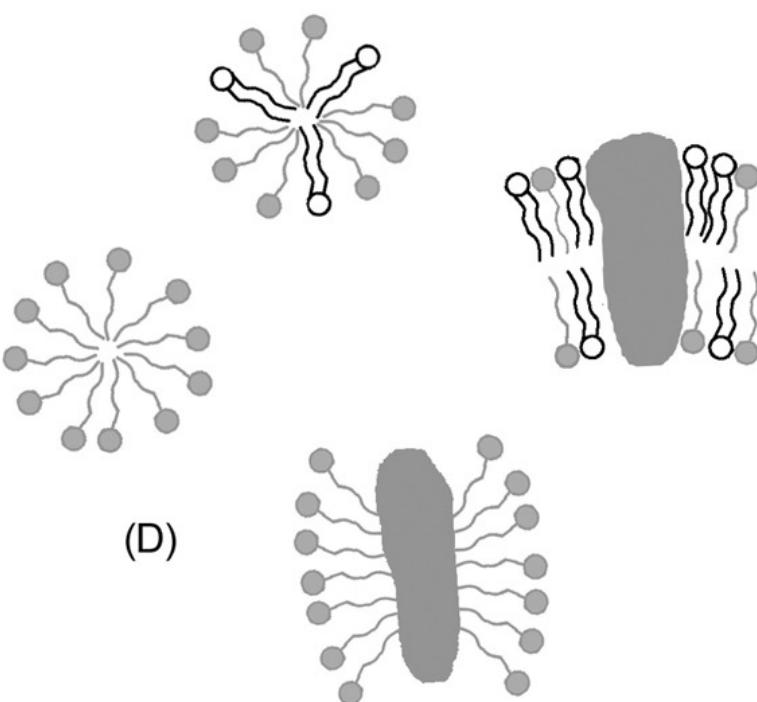
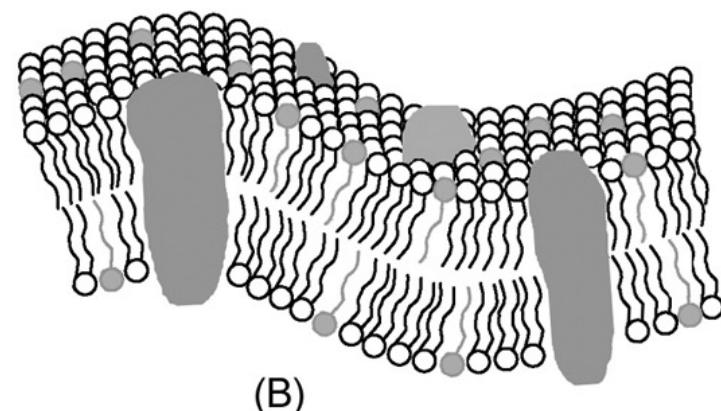
- Polar residues face the aqueous lumen.
- Non-polar residues are in contact with membrane lipids.

# Working with Membrane Proteins





+ Detergent

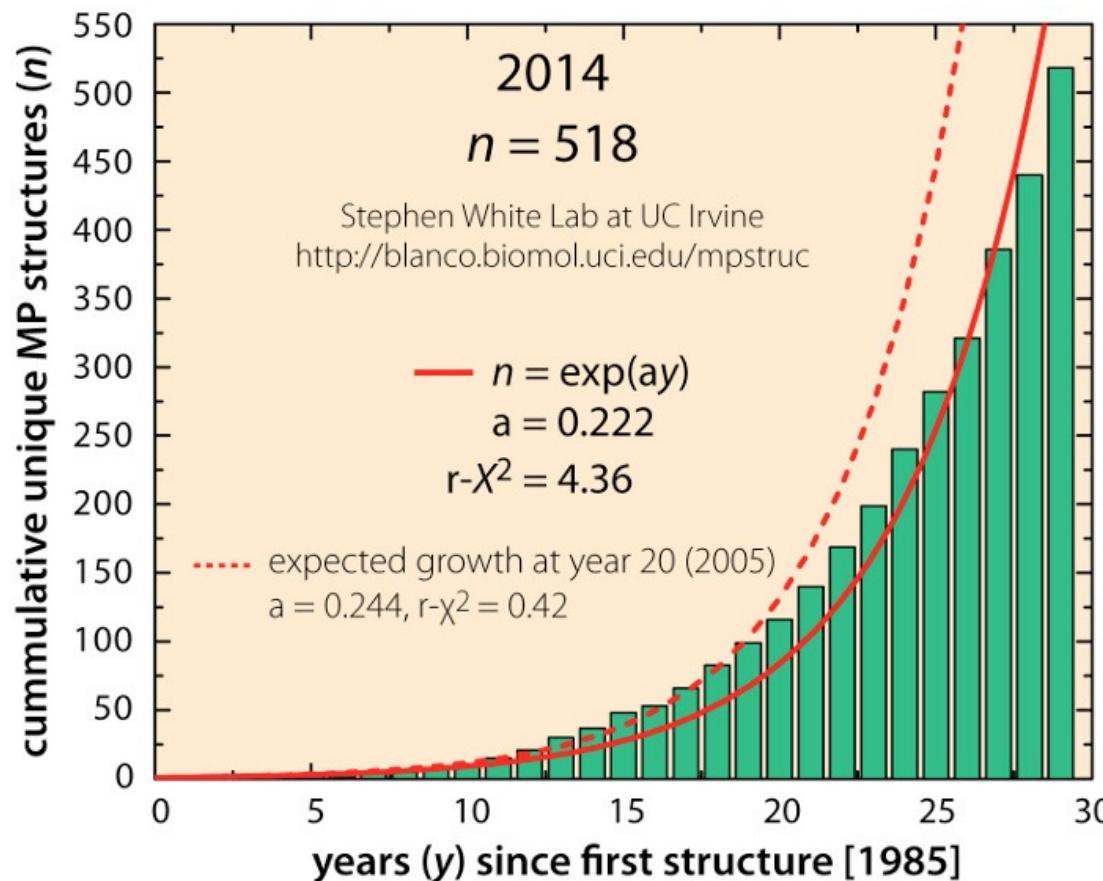


# Membrane Protein Structure Determination

~30% of the entire proteome (prokaryotic and eukaryotic)

~50% of all commercial drugs are against membrane protein targets

~1.5% of deposited structures (555 unique)



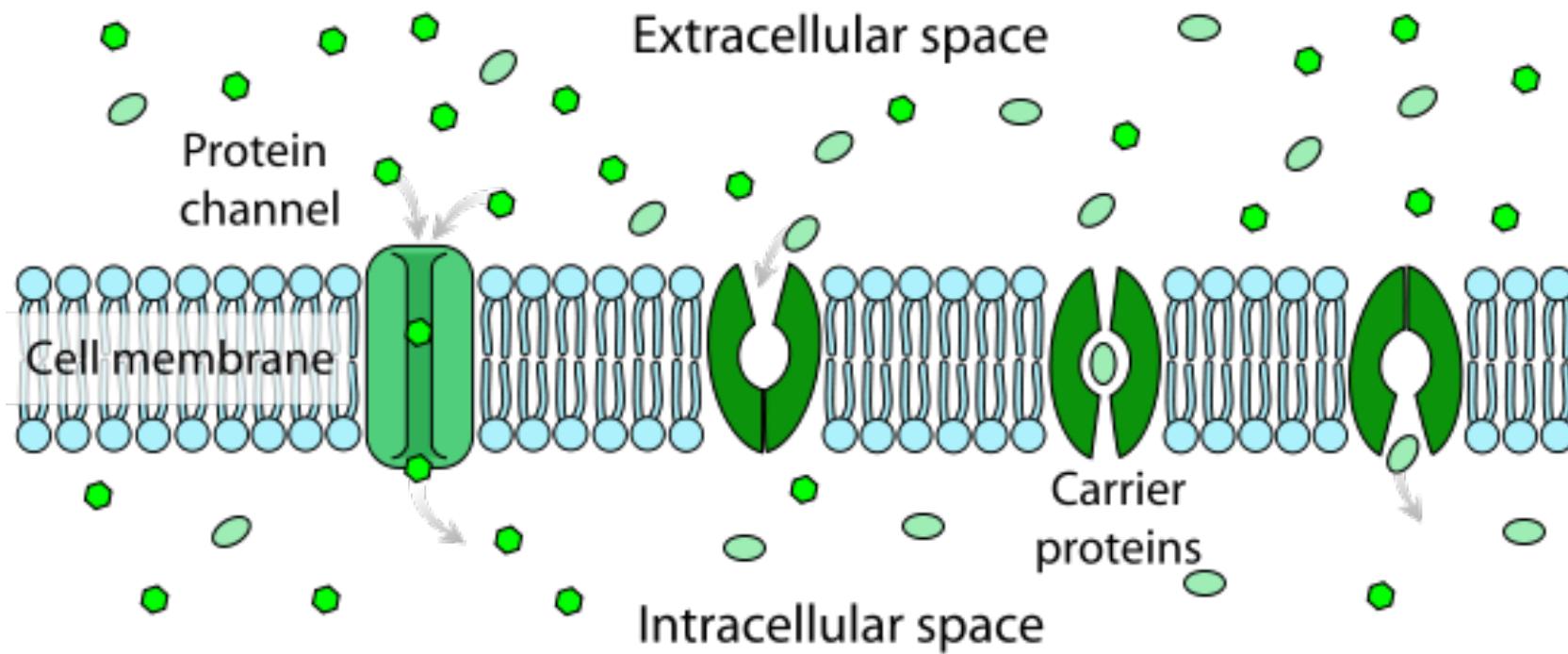
# Integral Membrane Proteins

Channels / Transporters

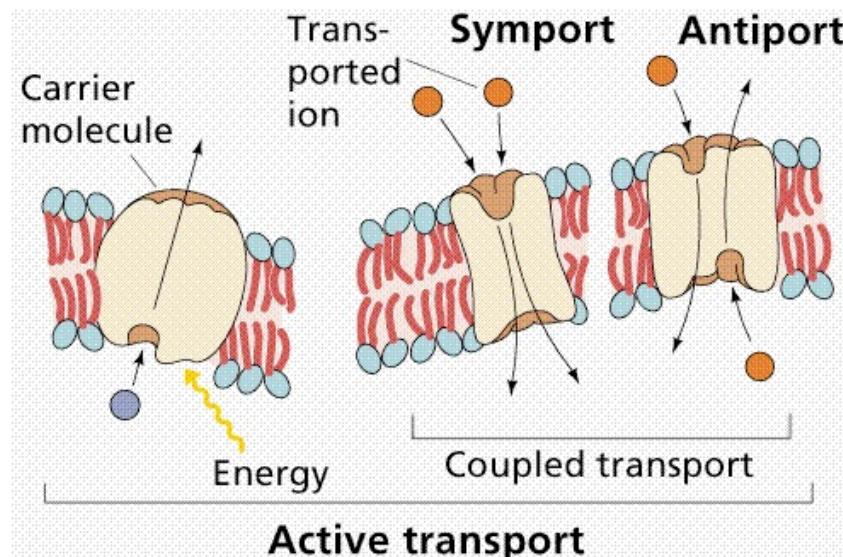
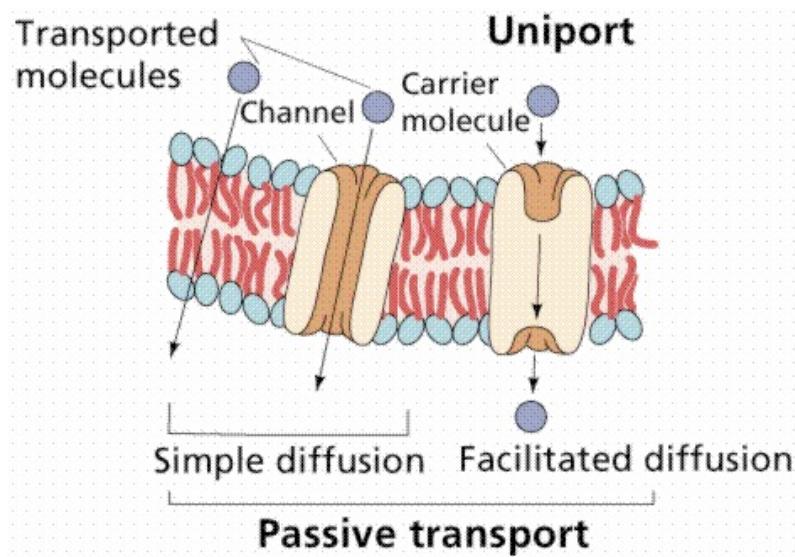
Receptors

Enzymes

# Molecular Physiology of Membrane transport

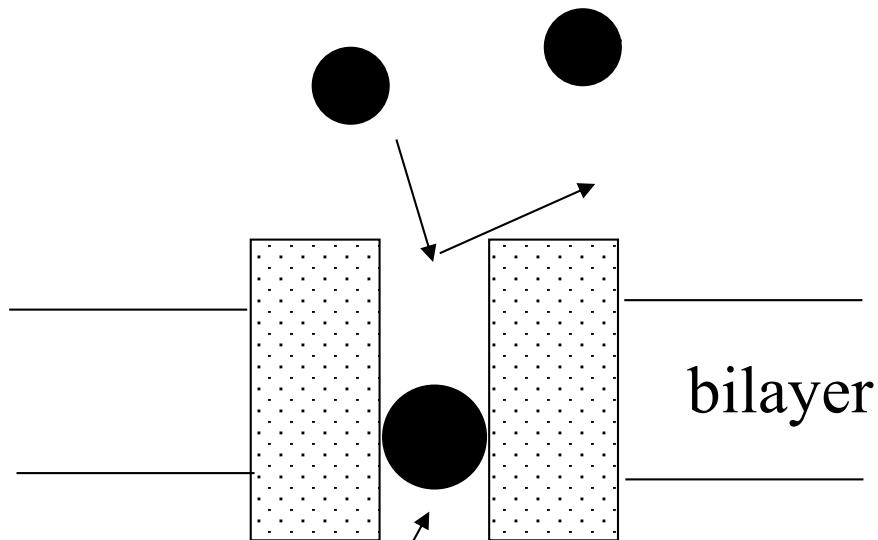


# Channels / Transporters

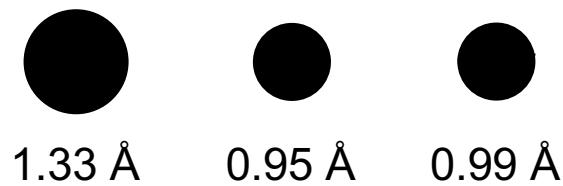


Images from Purves et al., Life: The Science of Biology, 4th Edition, by Sinauer Associates ([www.sinauer.com](http://www.sinauer.com)) and WH Freeman ([www.whfreeman.com](http://www.whfreeman.com))

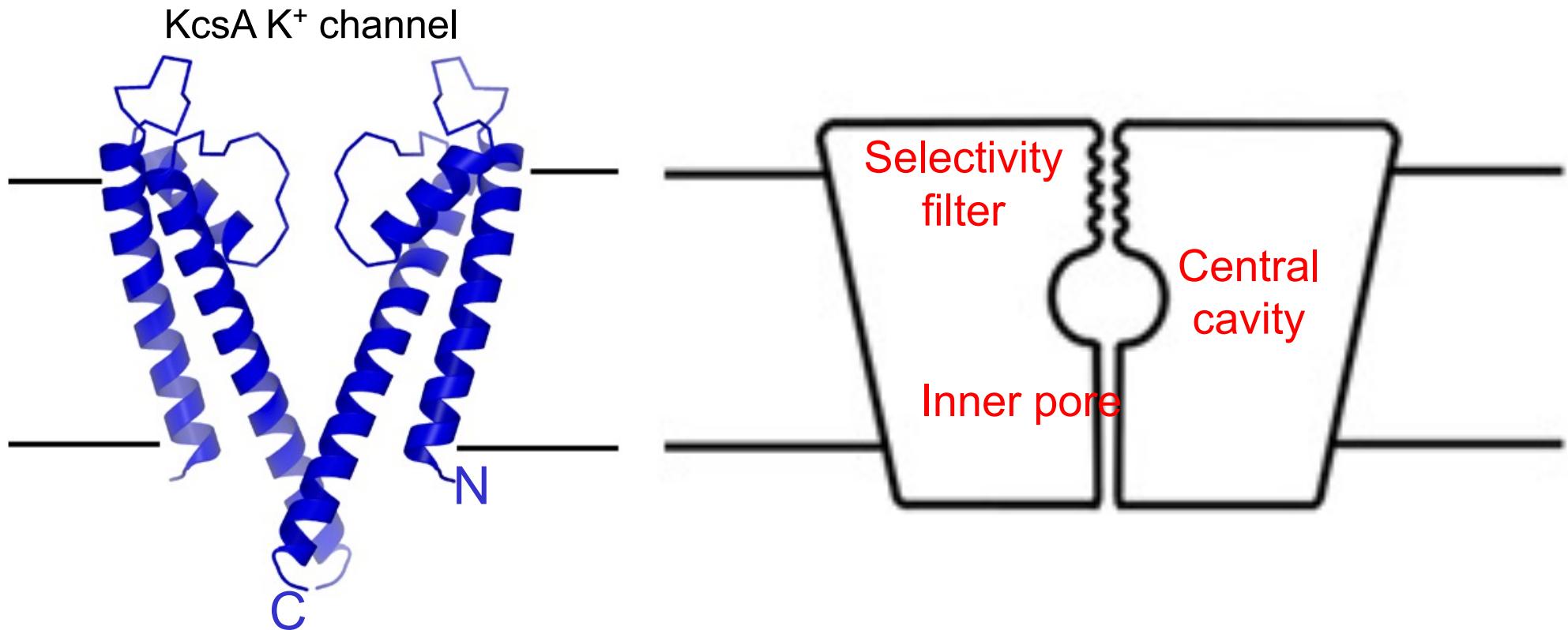
High selectivity and high throughput



bilayer



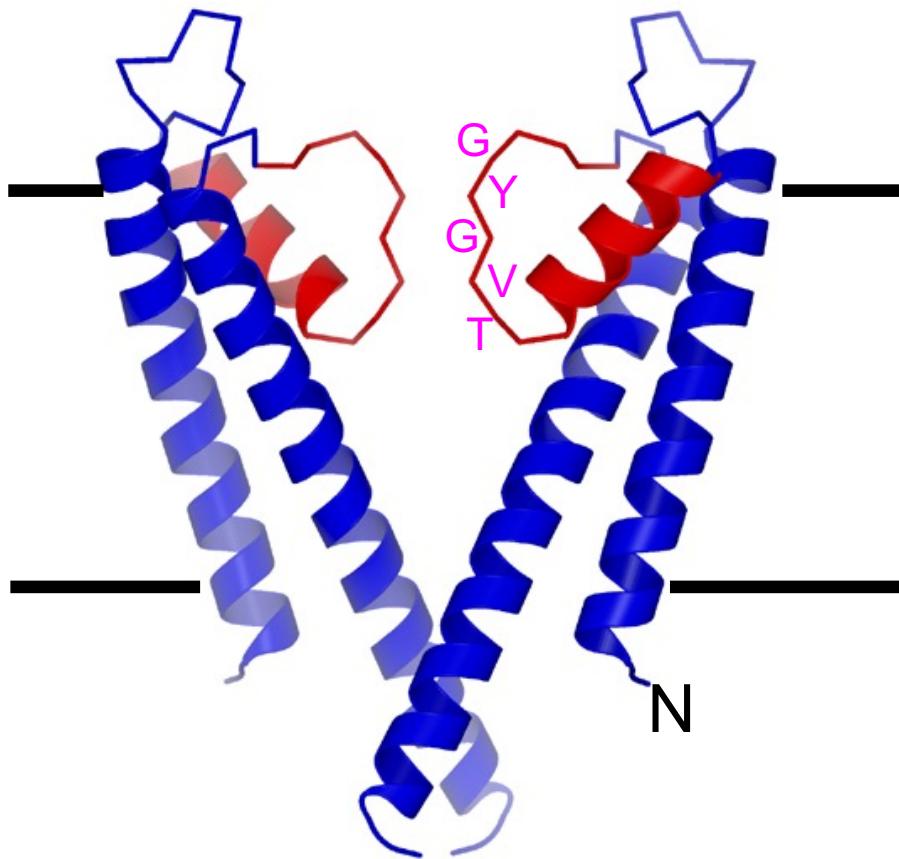
# KcsA $\text{K}^+$ channel is a model pore domain



Zhou Y, Morais-Cabral J, Kaufman A, MacKinnon, R. *Nature*, 414, 2001

Doyle,D.A., Morais-Cabral J., Pfuetzner,R.A., Kuo,A., Gulbis,J.M., Cohen,S.L., Chait,B.T., MacKinnon,R. *Science*, 280, 1998

# Sequence conservation at the selectivity filter



KcsA

ALWWSVETAT**TVGYGDLYP**

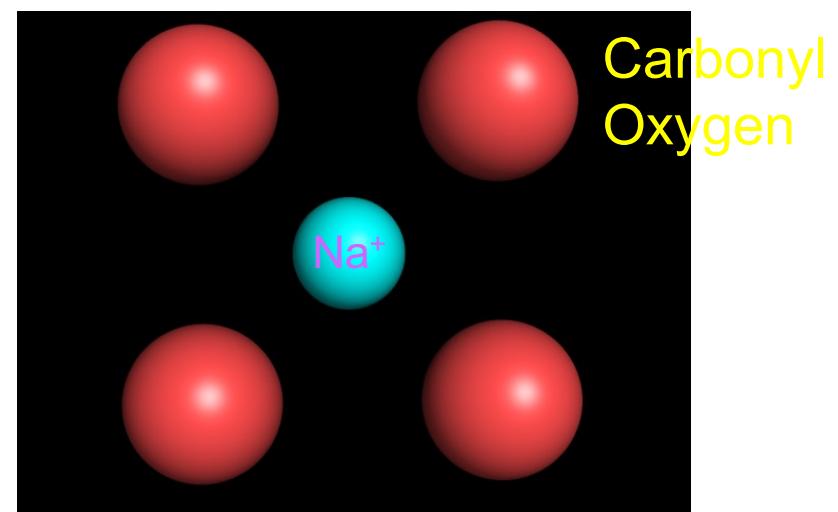
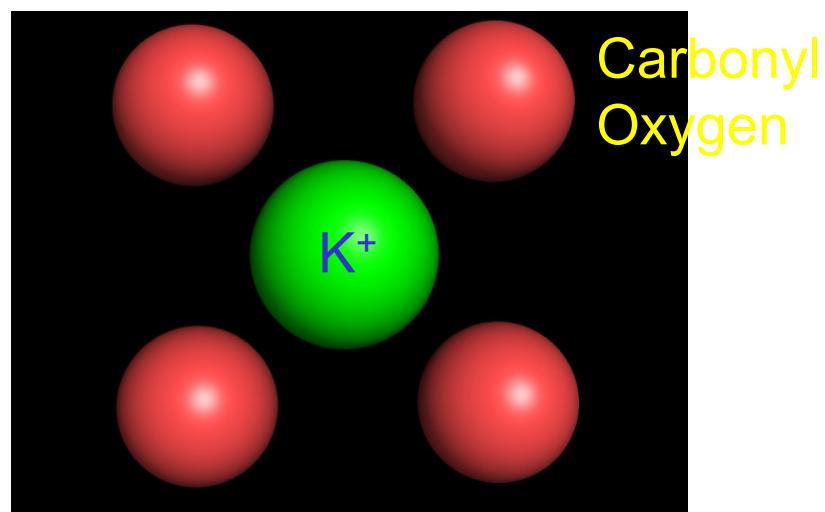
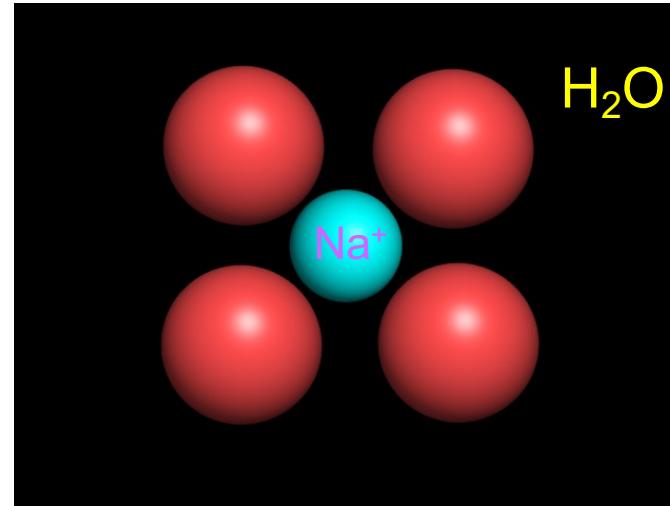
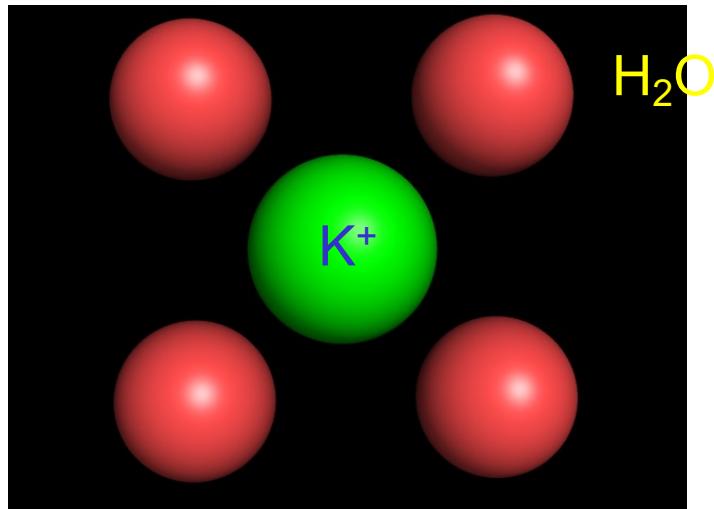
KvAP

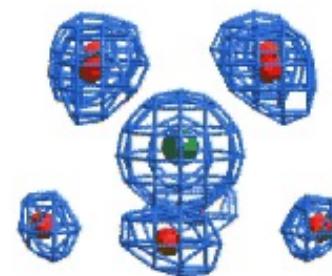
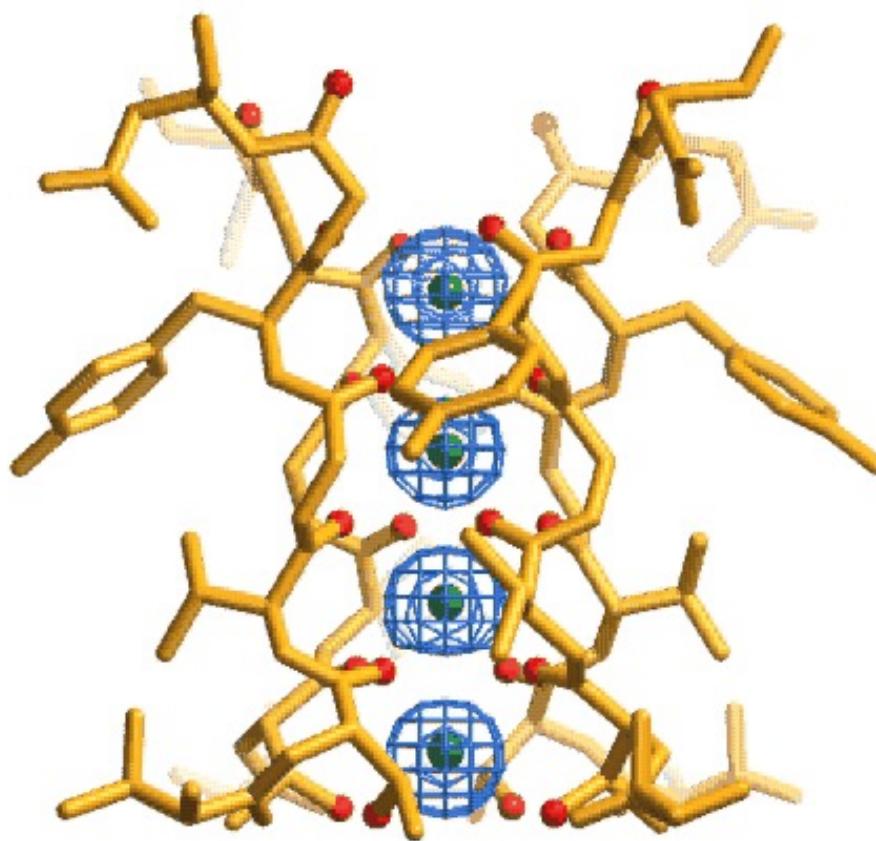
ALWWAVVTAT**TVGYGDVVP**

Fly Kv

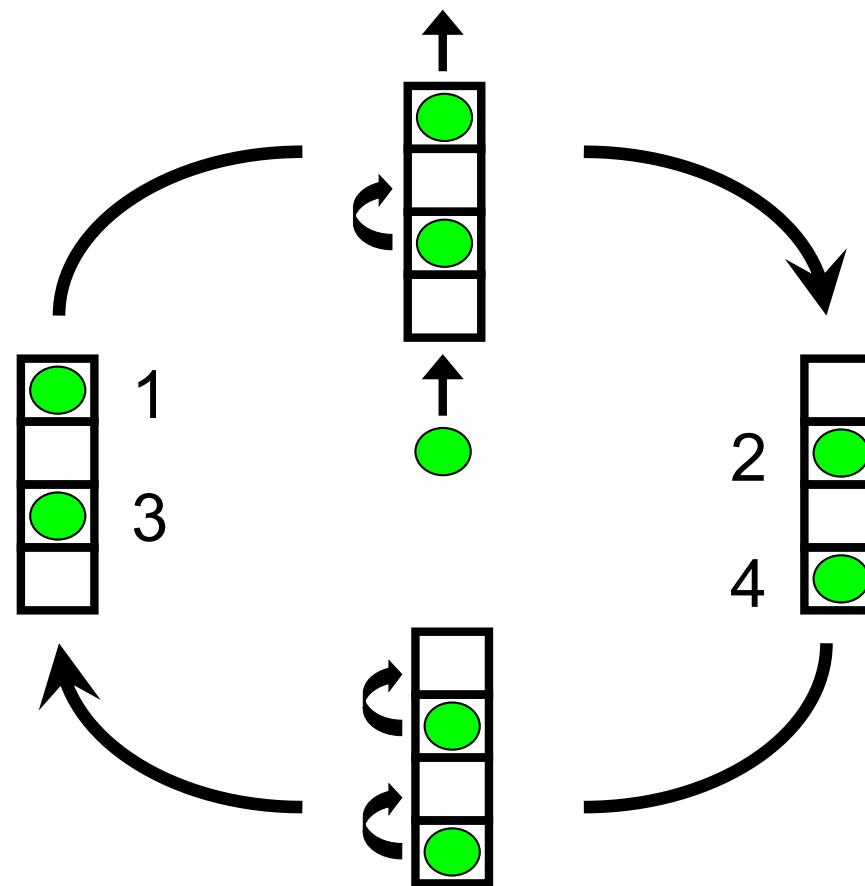
AFWWAVVTMT**TVGYGDMTP**

$K^+$  channel selectivity filter mimics the hydration shell of a  $K^+$  ion

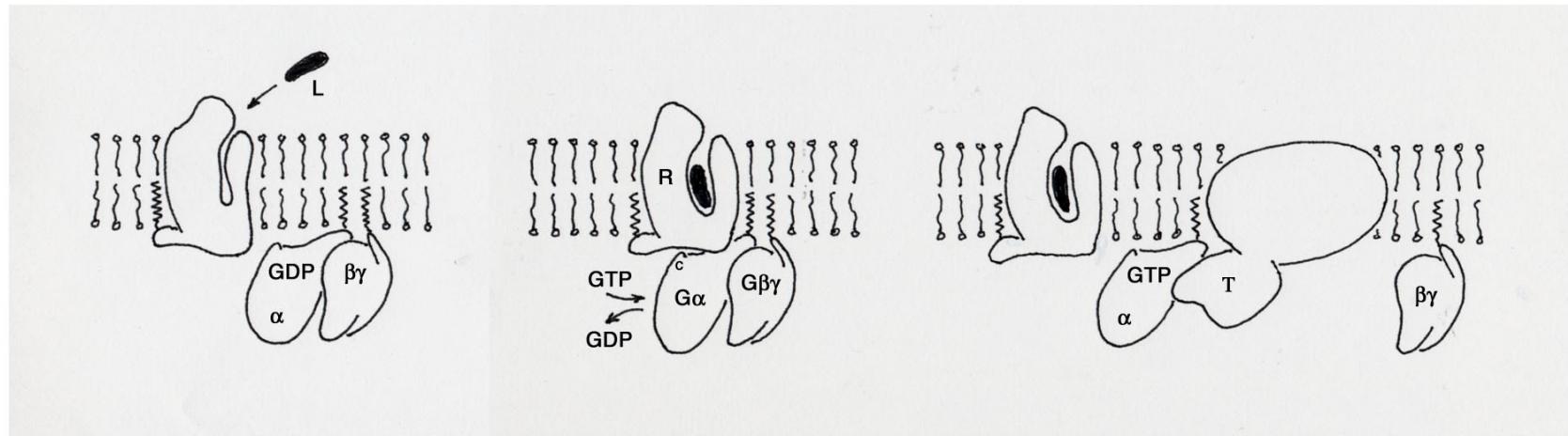




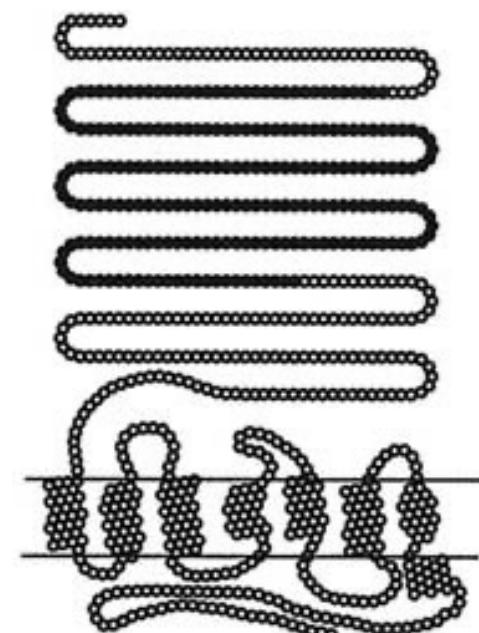
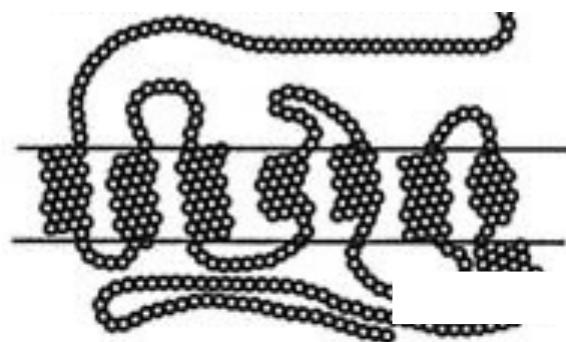
# A simple through-put cycle



# Receptors – G Protein Coupled Receptors (GPCRs)



- Photons
- Peptides
- Hormones
- Amino Acids
- Small molecules



# Structural Studies on GPCRs

