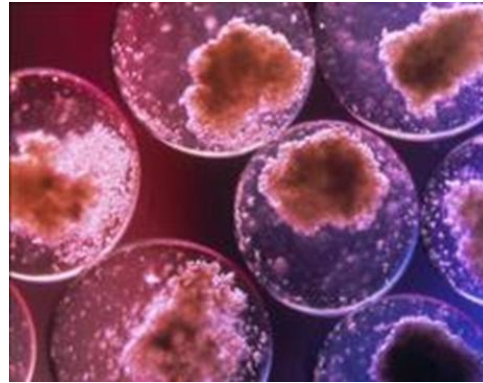
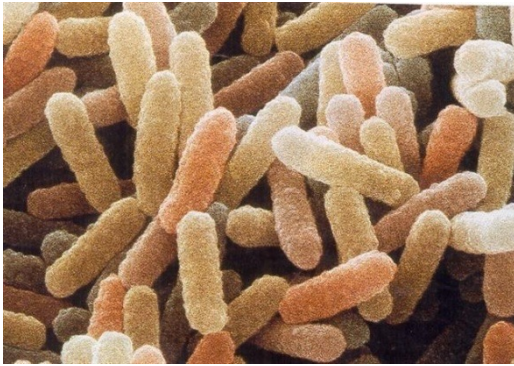




Corso di Chimica e Propedeutica Biochimica

Le molecole degli organismi viventi



Amminoacidi e proteine

Zuccheri e polisaccaridi

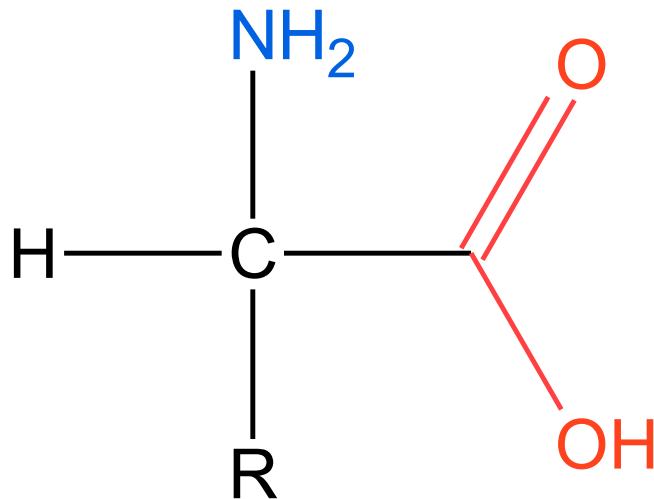
Lipidi e membrane

Nucleotidi e acidi nucleici



Proprietà chimico-fisiche degli amminoacidi

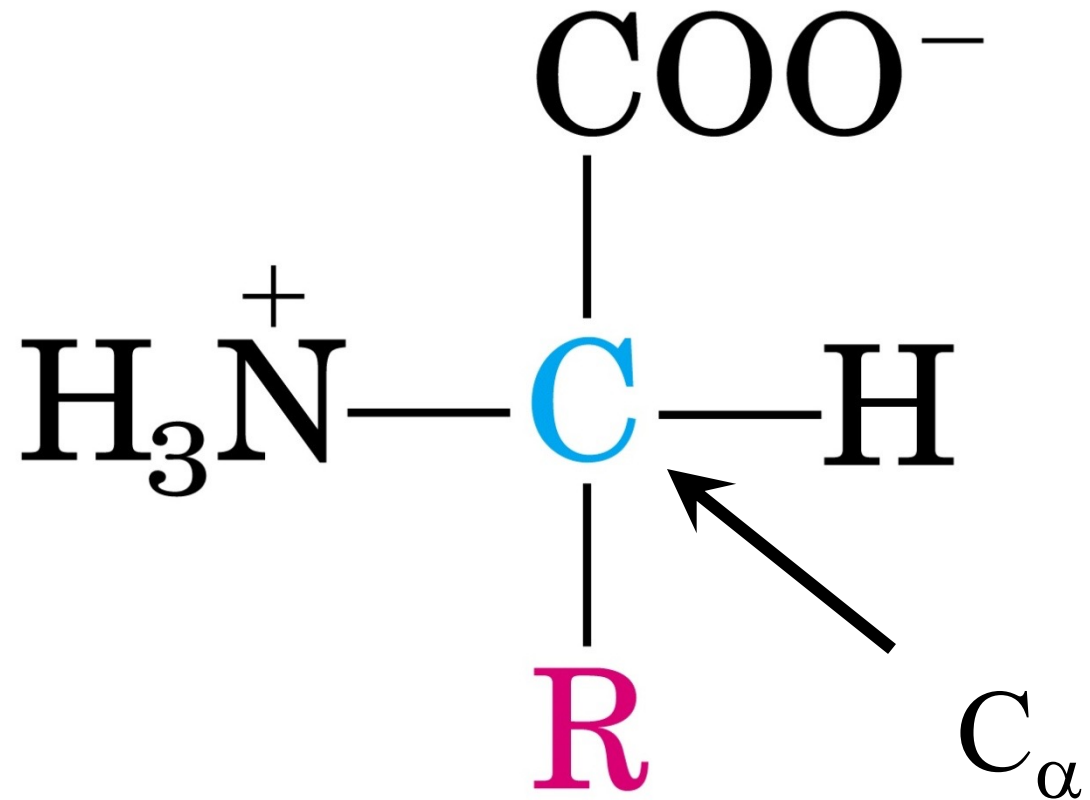
- Sono i componenti delle proteine ma anche di ormoni e neurotrasmettitori (L-Glu, L-Asp, L-Cys...)
- Anche se si dice comunemente che gli amminoacidi contengono un gruppo **amminico** ed un gruppo **carbossilico**



Alcune loro proprietà, sia chimiche che fisiche, non concordano con questa struttura:

- a) A differenza delle ammine alifatiche ($R-NH_2$) e degli acidi carbossilici ($R-COOH$), gli amminoacidi **sono solidi cristallini non volatili che fondono a temperature elevate ($200-300^\circ C$)**.
- b) Sono **insolubili nei solventi apolari** (benzene, etere, etc) e sono invece **solubili in acqua**
- c) Le loro soluzioni acquose si comportano come soluzioni di sostanze ad **alto momento dipolare**
- d) Le **costanti acidità** ($R-COOH$) sono **più alte** rispetto agli acidi carbossilici semplici ($K_A \approx 10^{-2} M$) e quelle di **basicità più basse** rispetto alle ammine alifatiche ($K_B \approx 10^{-4} M$)

Tutte queste proprietà concordano con una struttura ionica dipolare del tipo:



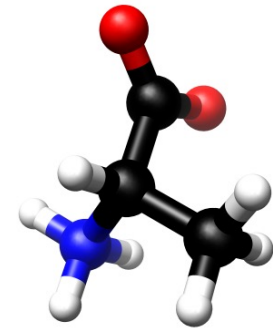
Ovvero gli amminoacidi sono **ioni dipolari** o **zwitterioni**

Amino acids

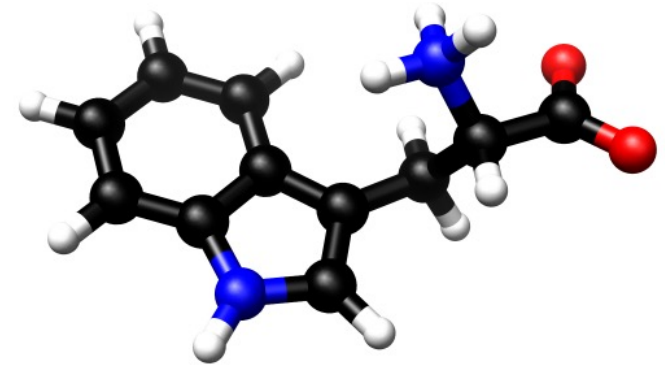
- ▶ Mammals need to assume with the diet 10 out of 20 aa, since they are not able to synthesize them:
- ▶ Cysteine, Phenylalanine, Histidine, Isoleucine, Lysine, Leucine, Methionine, Threonine, Valine and Tryptophan
- ▶ Depending on side chains characteristics, we can classify:
 - Apolar side chains (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

NOMENCLATURA

ALANINA		ALA	A
CISTEINA	*	CYS	C
ACIDO ASPARTICO		ASP	D
ACIDO GLUTAMMICO		GLU	E
FENILALANINA	*	PHE	F
GLICINA		GLY	G
ISTIDINA	*	HIS	H
ISOLEUCINA	*	ILE	I
LISINA	*	LYS	K
LEUCINA	*	LEU	L
METIONINA	*	MET	M
ASPARAGINA		ASN	N
PROLINA		PRO	P
GLUTAMMINA		GLN	Q
ARGININA		ARG	R
SERINA		SER	S
TREONINA	*	THR	T
VALINA	*	VAL	V
TRIPTOFANO	*	TRP	W
TIROSINA		TYR	Y



Ala

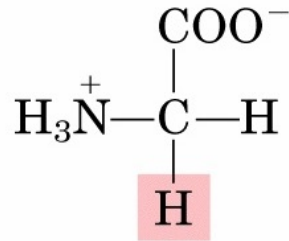


Trp

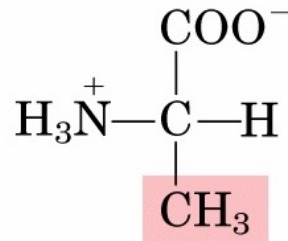
* essenziali, ovvero devono essere assunti con la dieta.

NB. Tutte le strutture riportate sono quelle prevalenti a pH 7

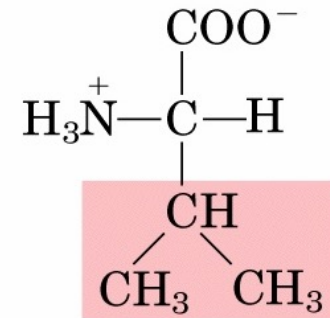
Apolari, gruppi R alifatici



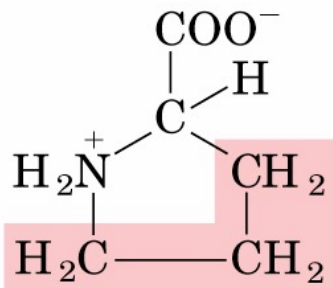
Glicina



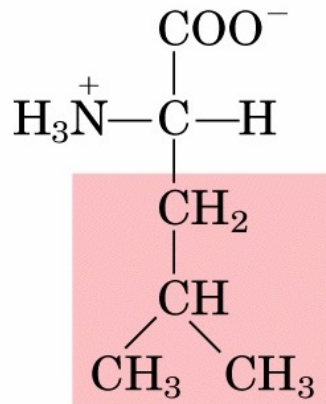
Alanina



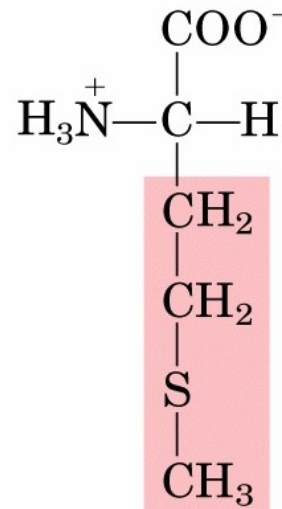
Valina



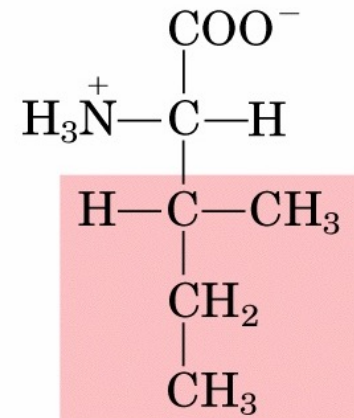
Prolina



Leucina

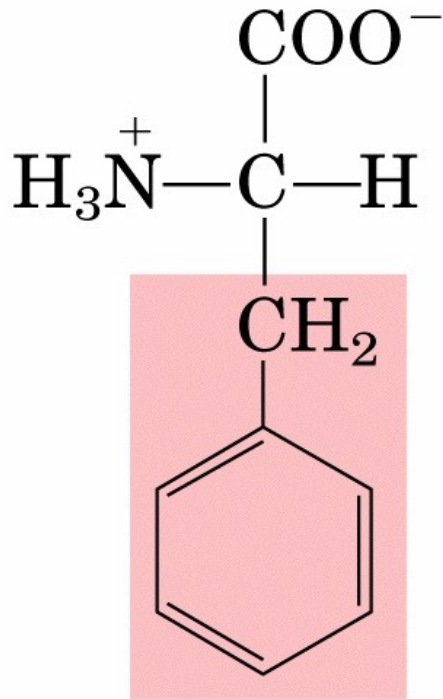


Metionina

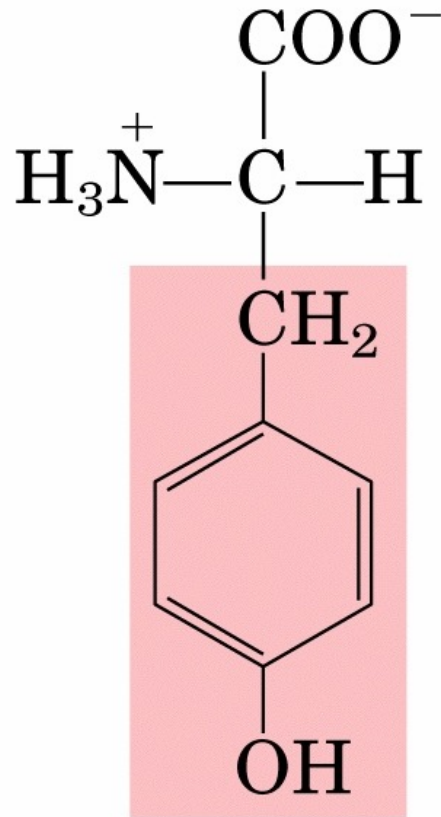


Isoleucina

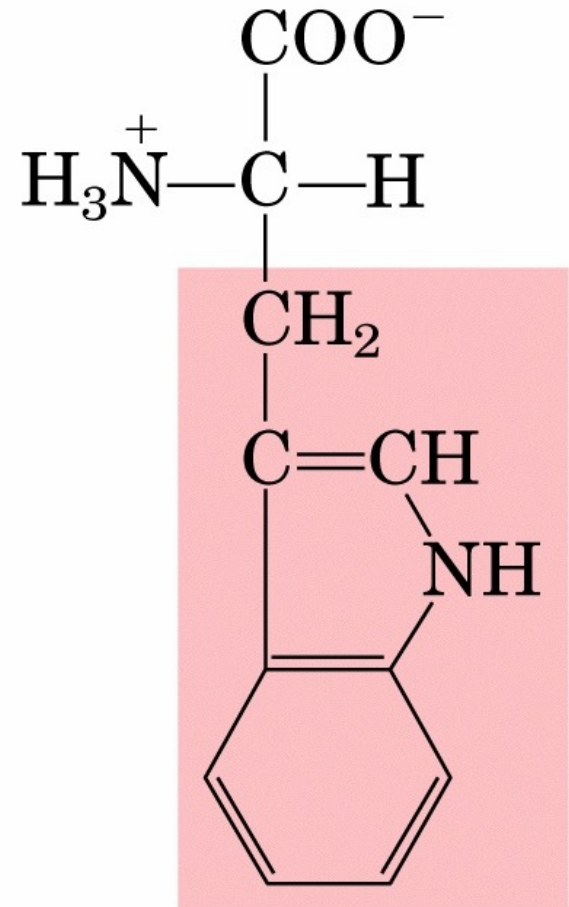
Gruppi R Aromatici



Fenilalanina

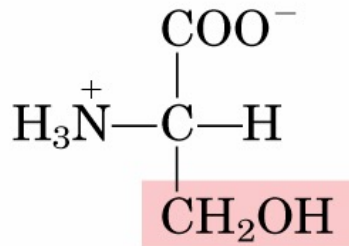


Tirosina

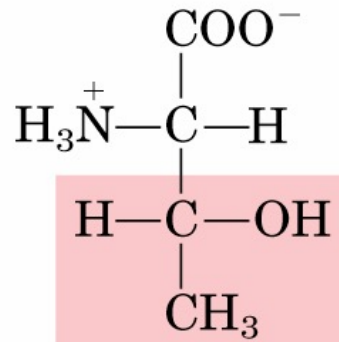


Triptofano

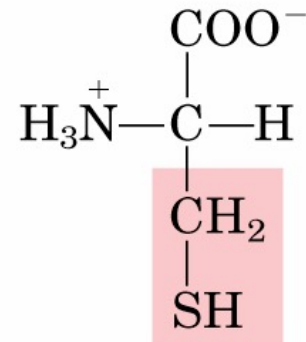
Gruppi R polari non carichi



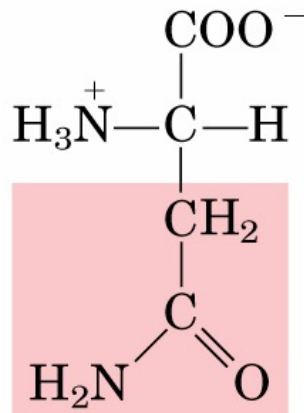
Serina



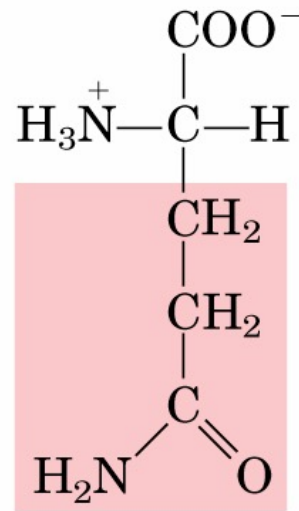
Treonina



Cisteina

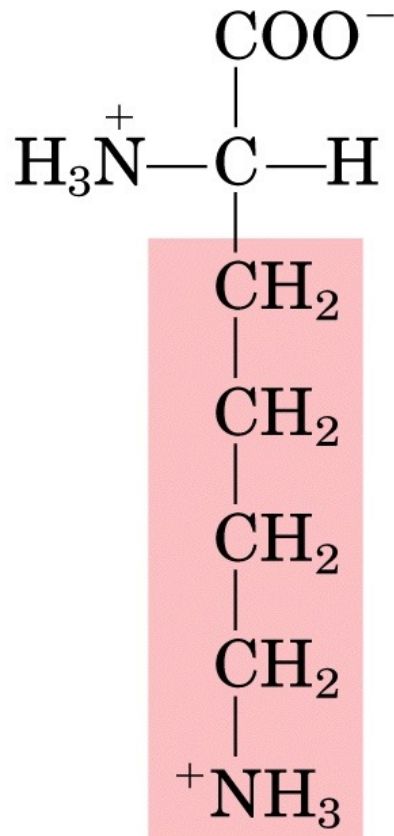


Asparagina

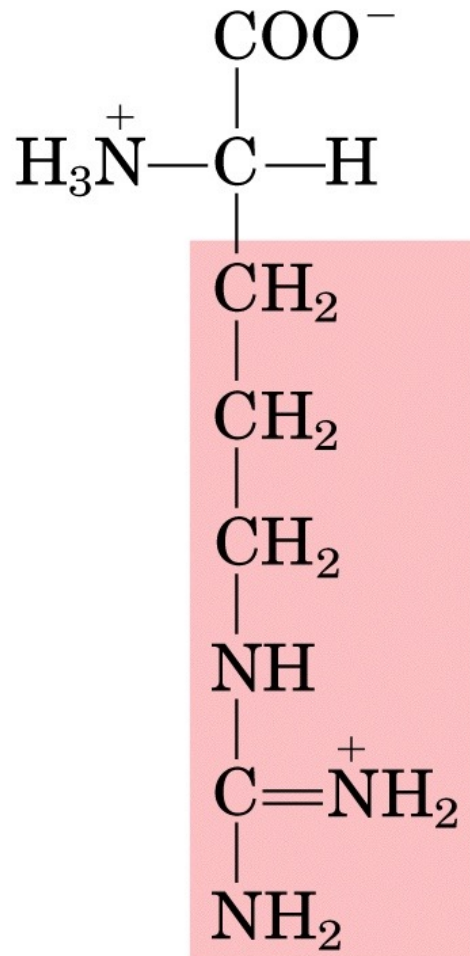


Glutammina

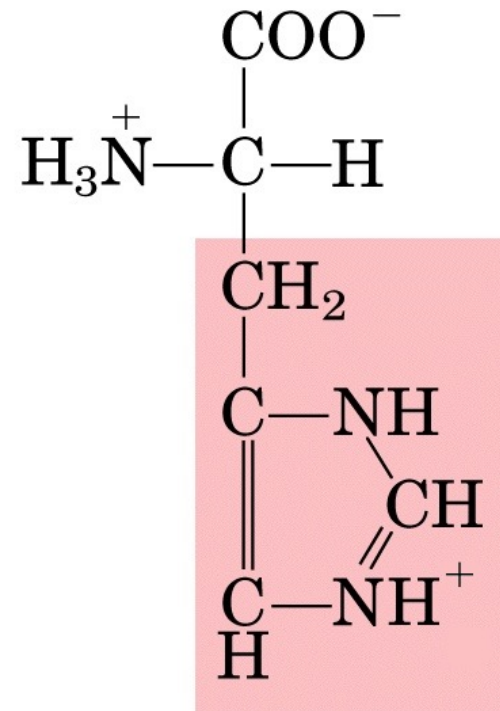
Gruppi R carichi positivamente



Lisina

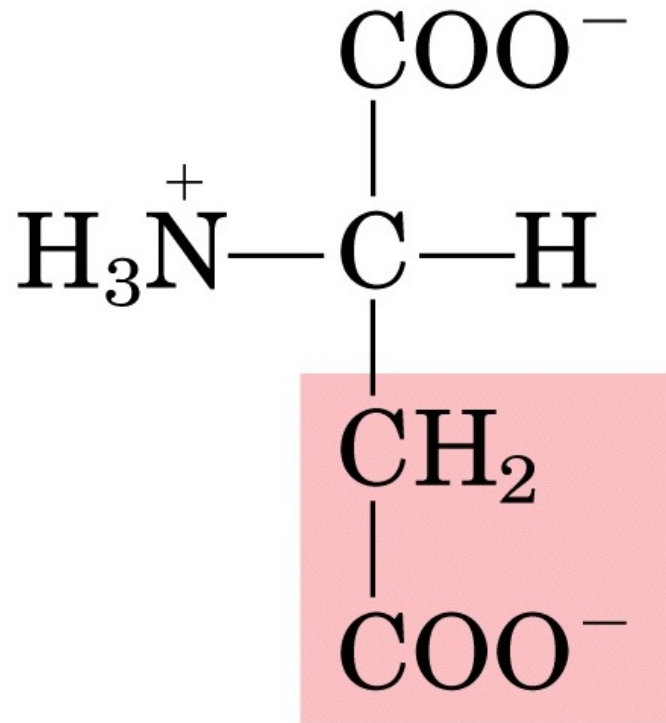


Arginina

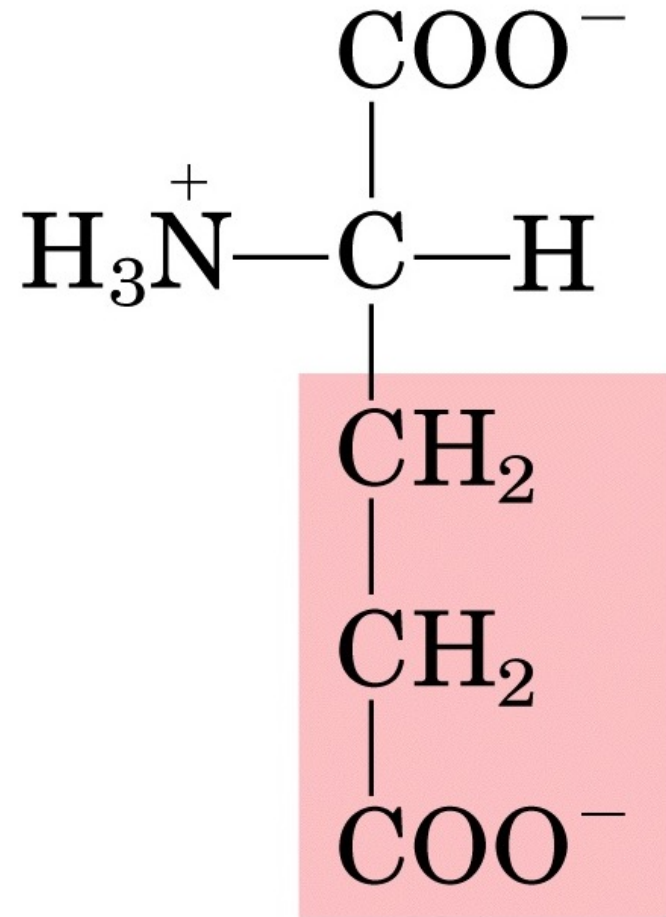


Istidina

Gruppi R carichi negativamente

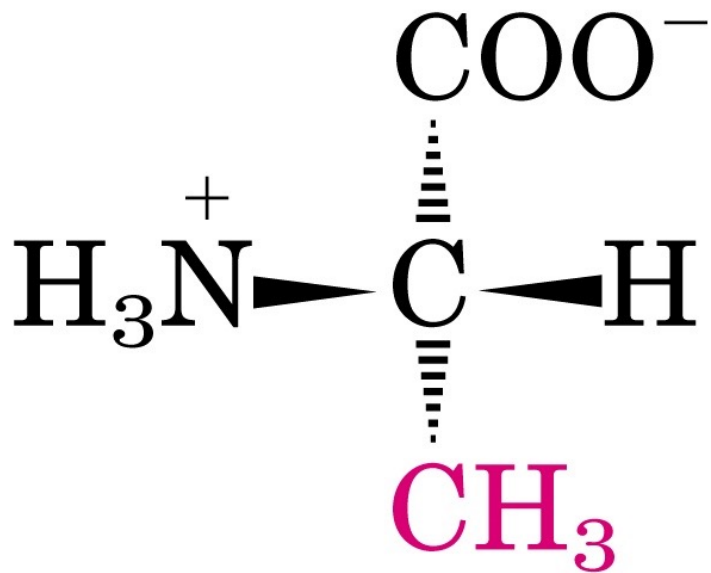


Aspartato

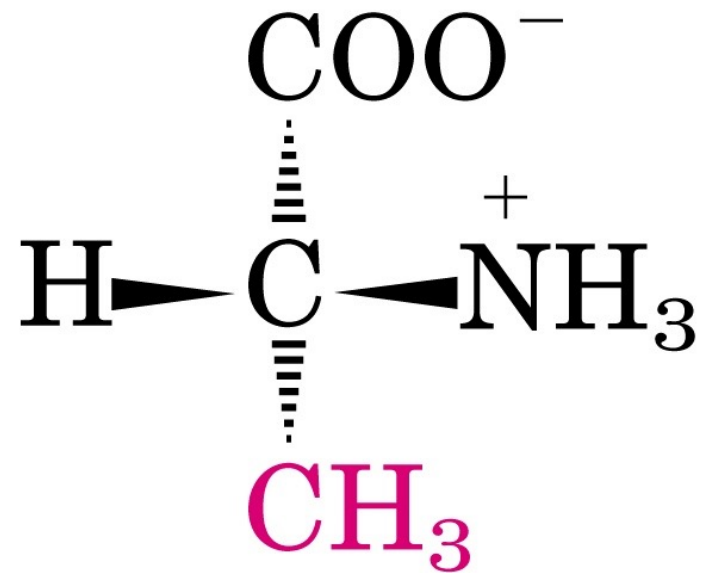


Glutammato

Gli amminoacidi sono **chirali** (tranne Gly) con soltanto la **serie L** presente nelle proteine



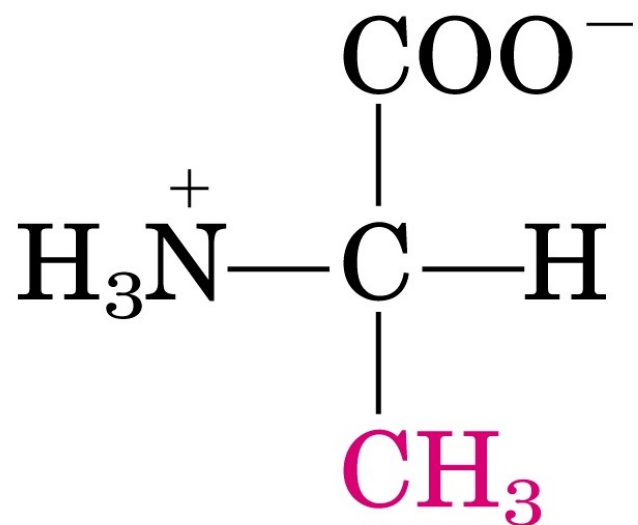
L-alanina



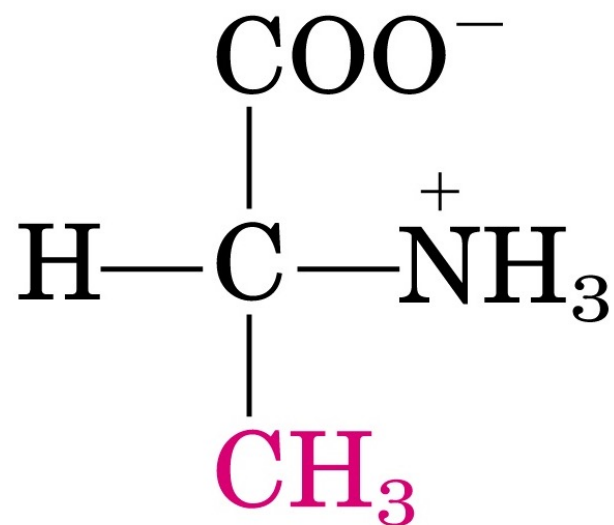
D-alanina

Ponendo il C_α nel piano del foglio, le **freccie in grassetto** si dipartono dal piano del foglio verso l'osservatore, quelle **tratteggiate** escono dal piano del foglio allontanandosi dall'osservatore.

Notazione di Fischer

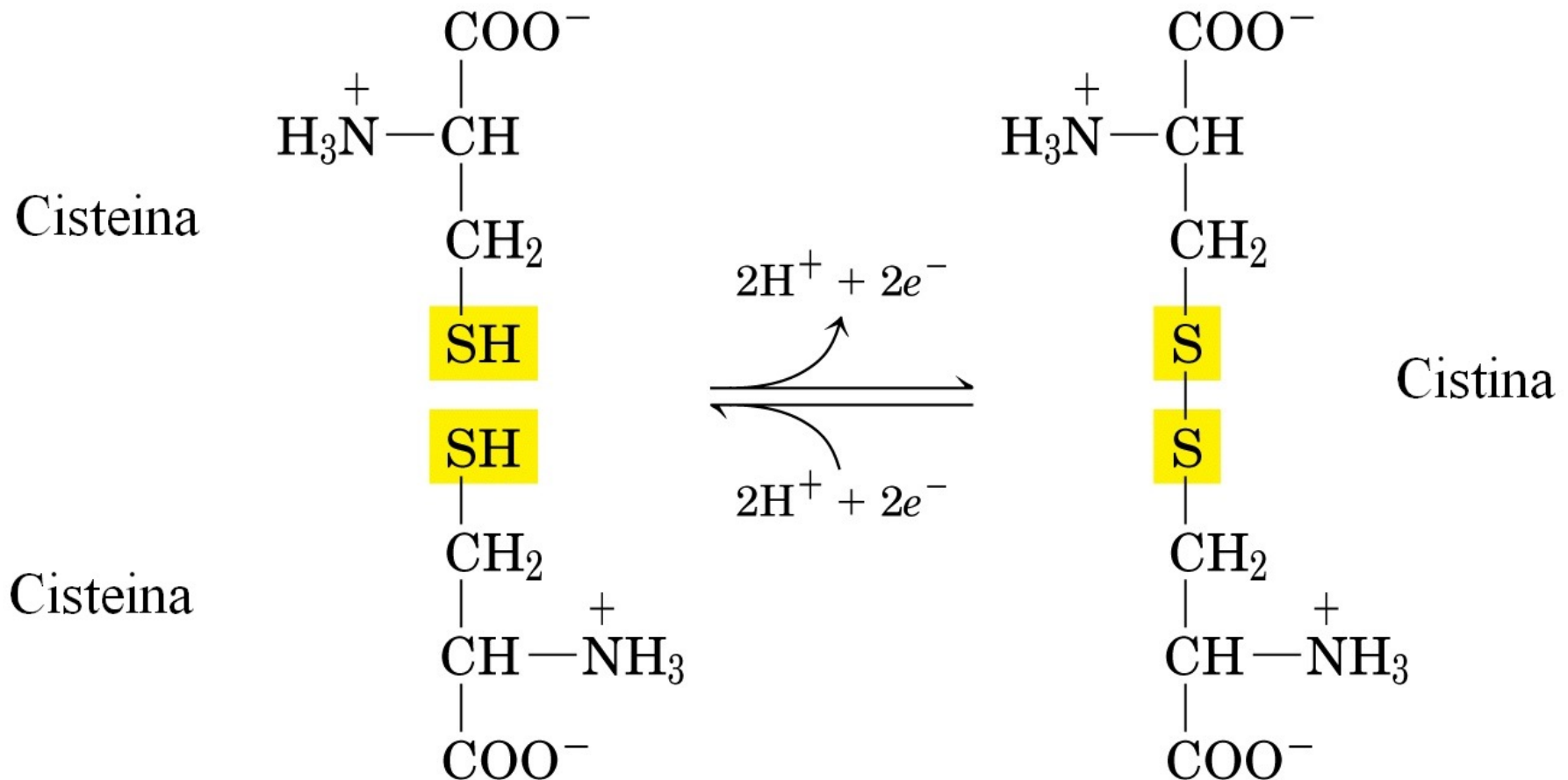


L-alanina

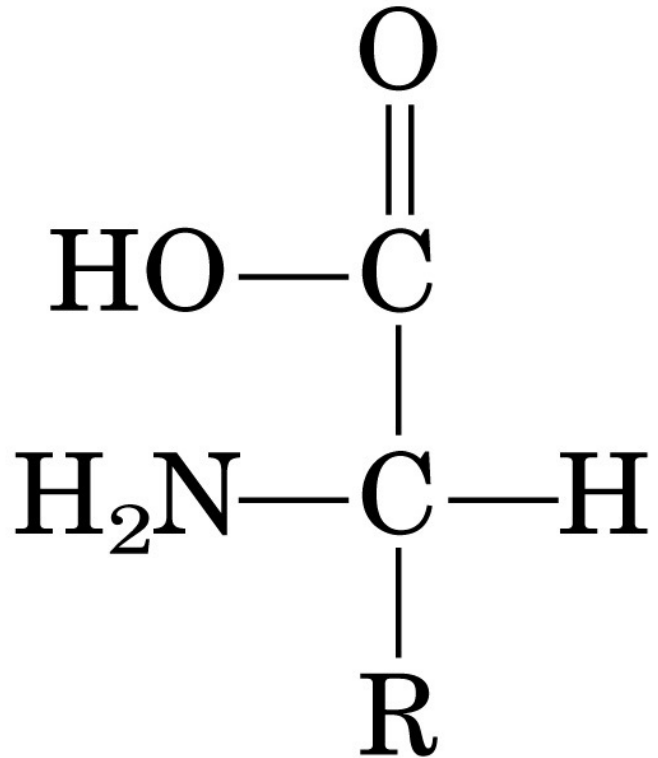


D-alanina

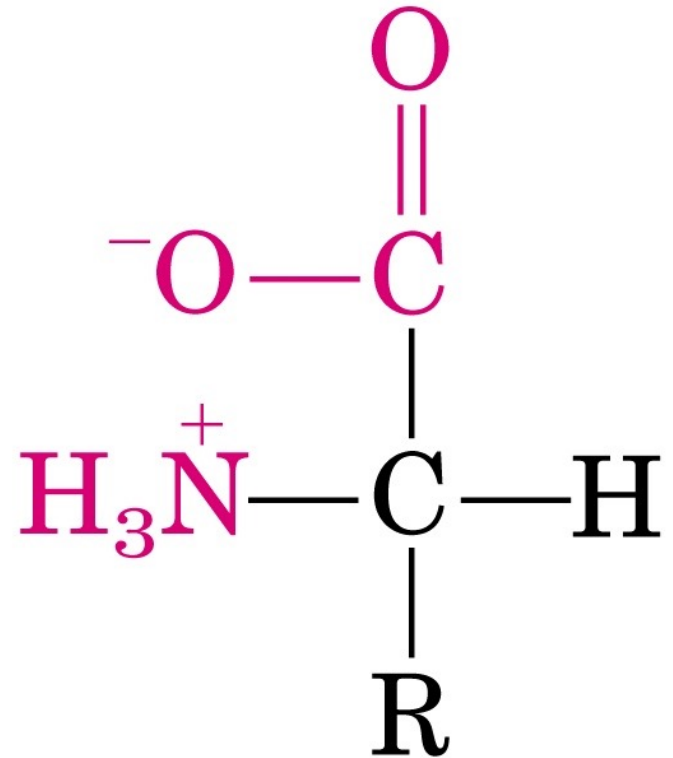
Formazione di ponti disolfuro



Proprietà acido-basiche



forma non
ionica

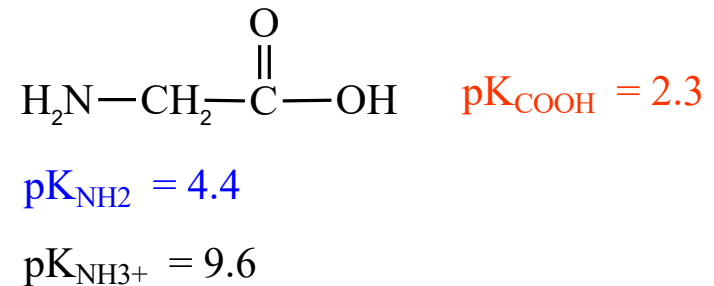
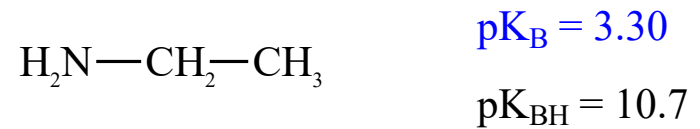


forma
zwitterionica

PROPRIETA' ACIDO-BASICHE

	pK _{COOH}	pK _{NH3+}	pK _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

Le **costanti di acidità** (COOH) sono **più alte** (pK ≈ 2.2) rispetto agli acidi carbossilici semplici e **quelle di basicità** (NH₂) **più basse** (pK ≈ 4.4) rispetto alle ammine alifatiche semplici

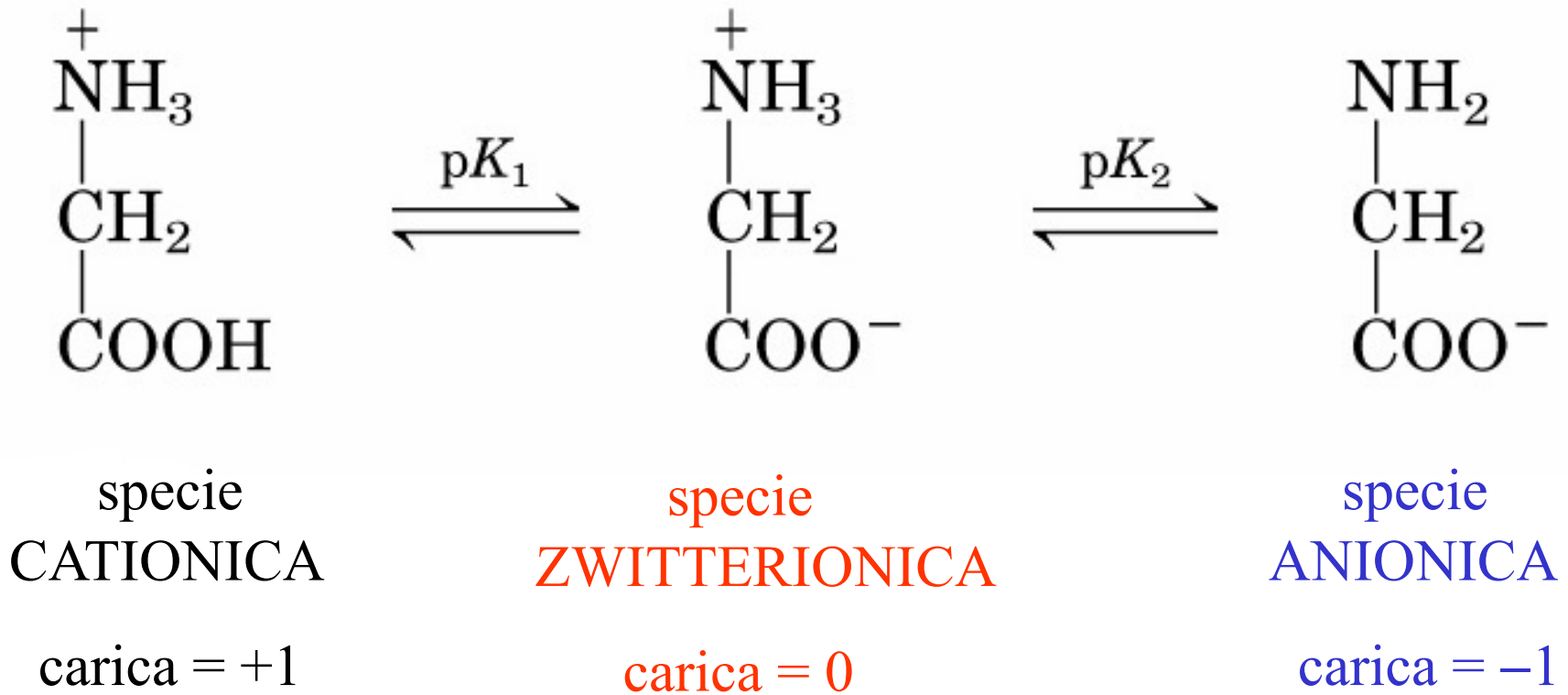


Effetto dei sostituenti sulle costanti di dissociazione di alcuni acidi.

ACIDO	FORMULA	pK
acetico	$\text{CH}_3\text{-COOH}$	4.75
cloroacetico	$\text{ClCH}_2\text{-COOH}$	2.87
dicloroacetico	$\text{Cl}_2\text{CH-COOH}$	1.48
amminoacetico (glicina)	$^+\text{H}_3\text{N-CH}_2\text{-COOH}$	2.35

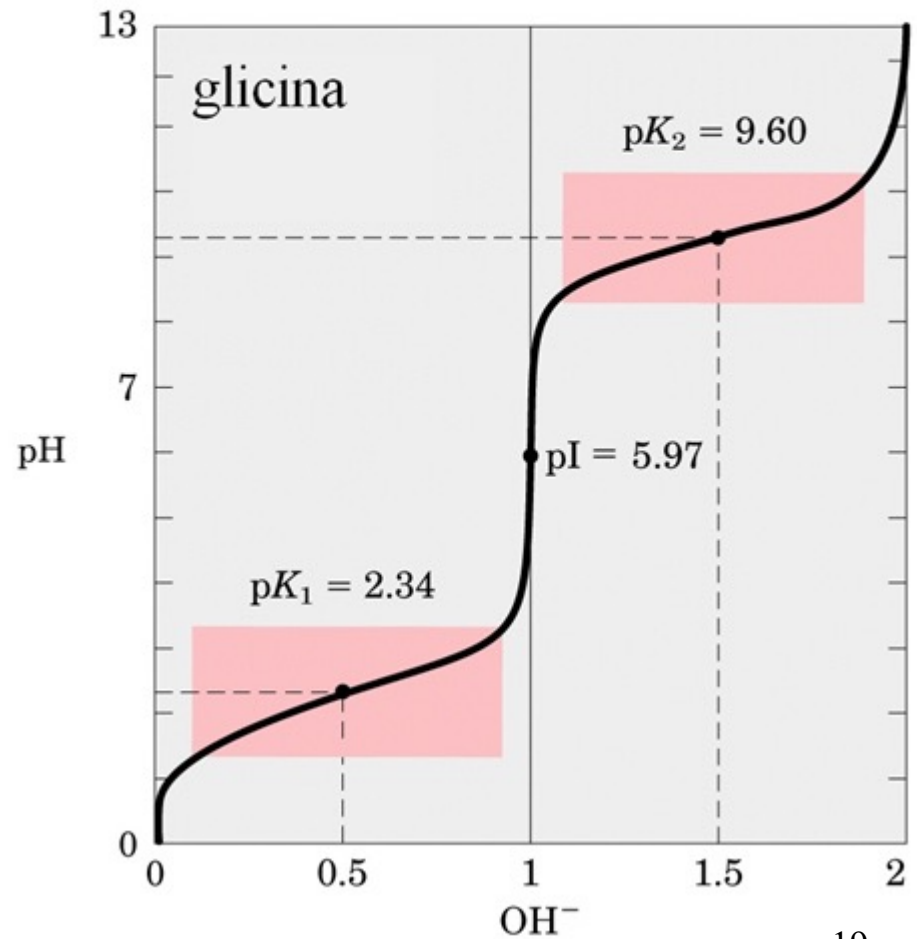
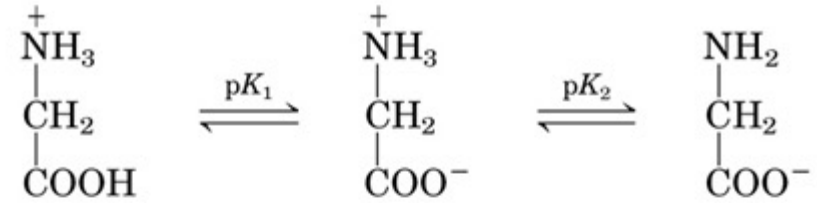
H. Gutfreund, Enzyme physical properties, Wiley Interscience, 1972, London

Il grado di ionizzazione di un amminoacido è pH dipendente



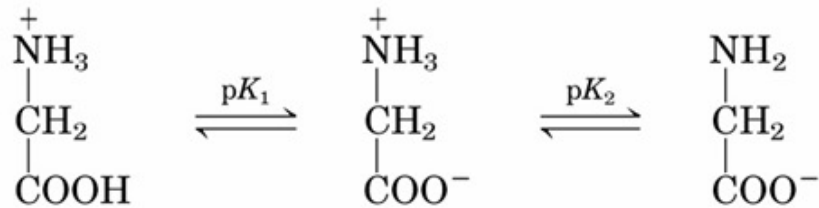
	pK _{COOH}	pK _{NH3+}	pK _R	pI
GLY	2.3	9.6	-	6.0
ALA	2.3	9.7	-	6.0
VAL	2.3	9.6	-	6.0
PRO	2.0	10.6	-	6.3
LEU	2.4	9.7	-	6.0
MET	2.3	9.2	-	5.8
ILE	2.4	9.7	-	6.1
PHE	1.8	9.1	-	5.5
TYR	2.2	9.1	10.1	5.7
TRP	2.4	9.4	-	5.9
SER	2.2	9.2	-	5.7
THR	2.6	10.4	-	6.5
CYS	1.8	10.8	8.3	5.0
ASN	2.0	8.8	-	5.4
GLN	2.2	9.1	-	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

Il pH o punto isoelettrico



Il punto o pH isoelettrico

Il punto o pH isoelettrico è quel valore di pH a cui è massima la concentrazione della specie zwitterionica.

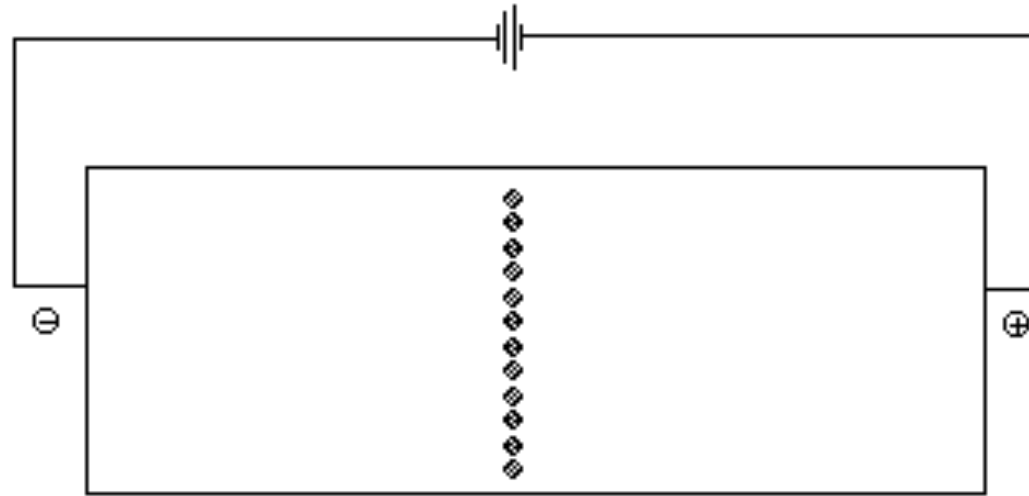


$\text{pH} < \text{pI} \rightarrow$ catione

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

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

ELETTROFORESI



Il pH o punto isoelettrico

	pK _{COOH}	pK _{NH3+}	pK _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

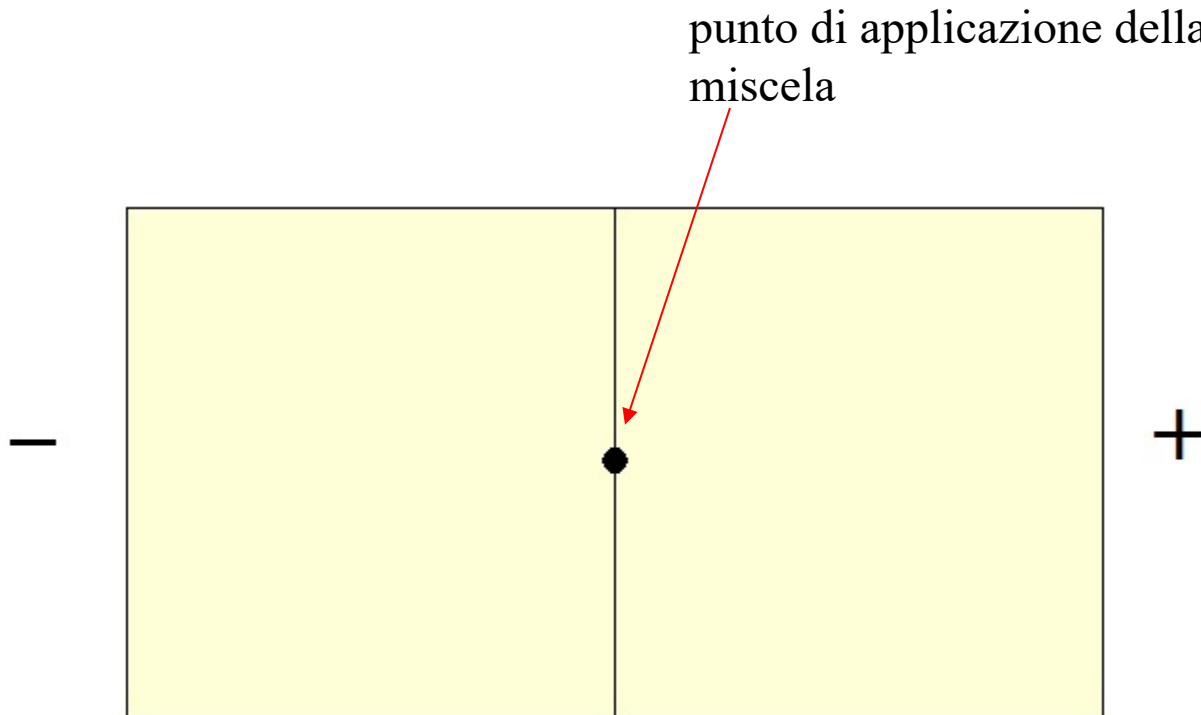
pH < pI → catione

pH = pI → zwitterione

pH > pI → anione

Un esempio

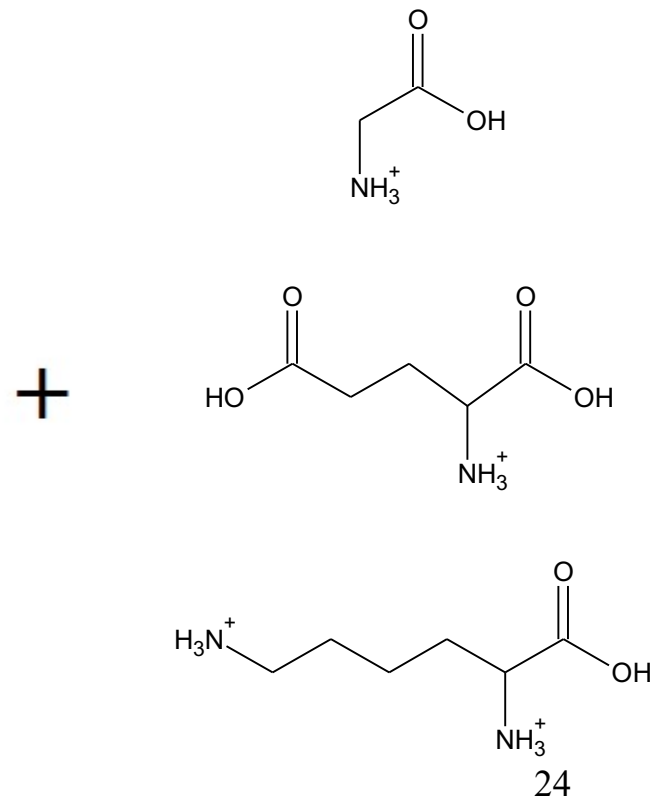
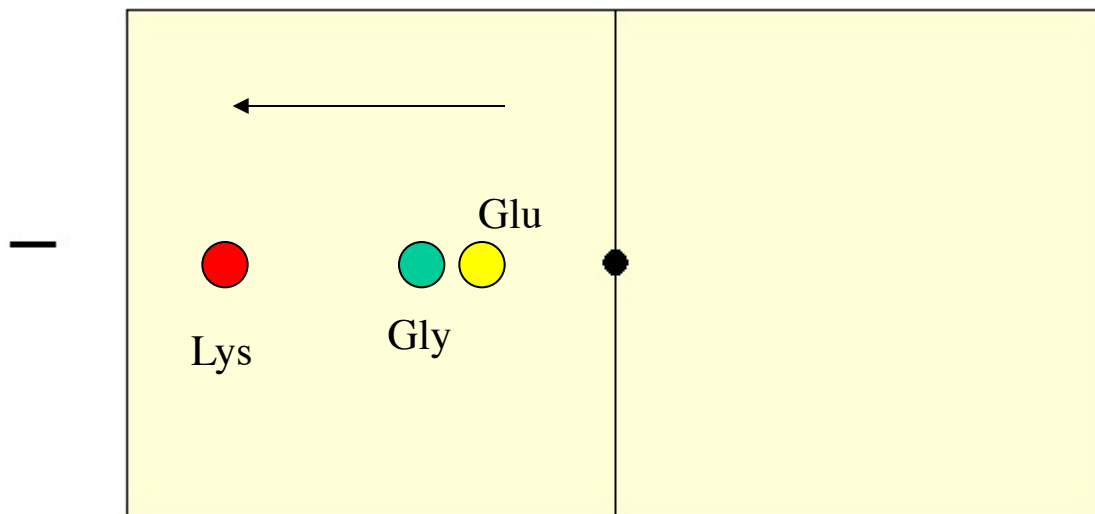
Si abbia una miscela di 3 amminoacidi : Gly ($pI=5.97$), Lys ($pI=9.74$) e Glu (3.22). Se l'elettroforesi su carta e' allestita a pH 1, 6 e 11. Si indichi in ciascun caso se sia possibile separare la miscela.



Si abbia una miscela di 3 amminoacidi : Gly (pI=5.97), Lys (pI=9.74) e Glu (3.22). Se l'elettroforesi su carta e' allestita a pH 1, 6 e 11. Si indichi in ciascun caso se sia possibile separare la miscela.

pH=1

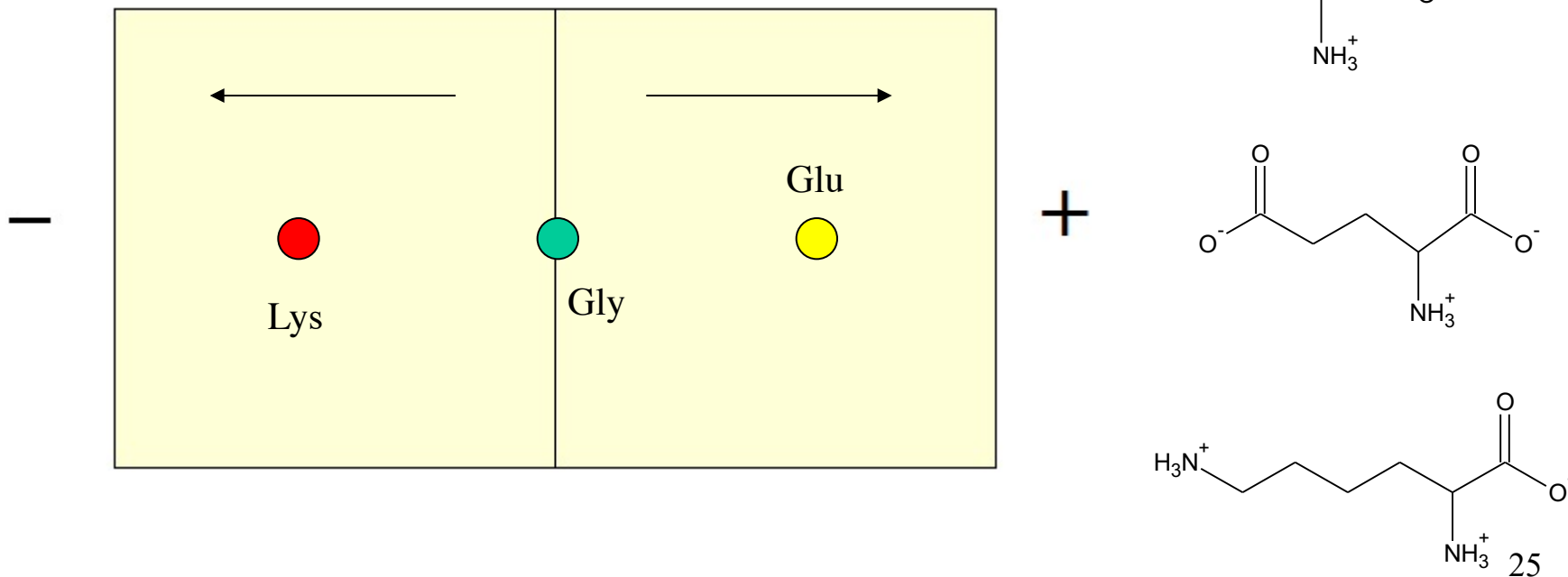
In ogni caso $\text{pH} < \text{pI}$ e gli amminoacidi si troveranno in forma cationica



Si abbia una miscela di 3 amminoacidi : Gly ($pI=5.97$), Lys ($pI=9.74$) e Glu (3.22). Se l'elettroforesi su carta e' allestita a pH 1, 6 e 11. Si indichi in ciascun caso se sia possibile separare la miscela.

pH=6

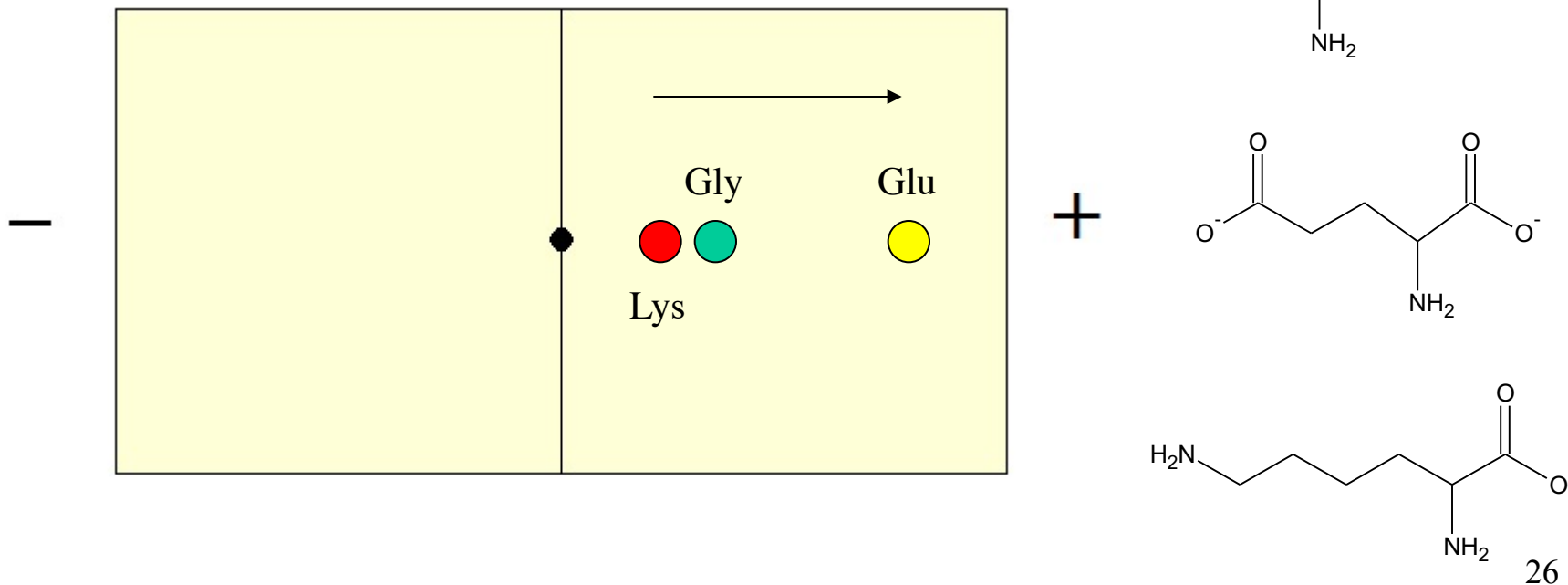
Gly non migra ($pH=pI$), Glu è in forma anionica ($pH>pI$) e Lys è in forma cationica ($pH<pI$)



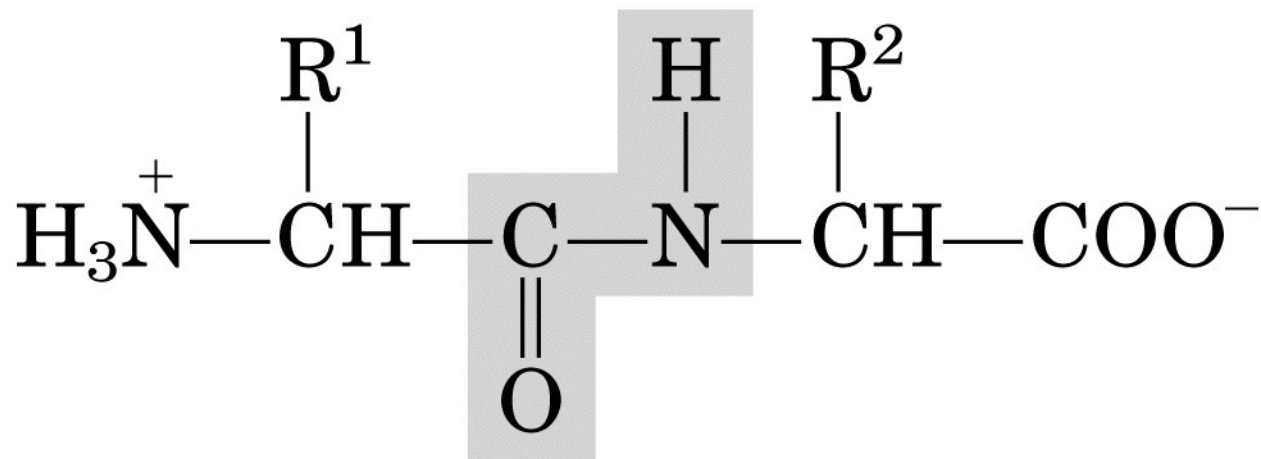
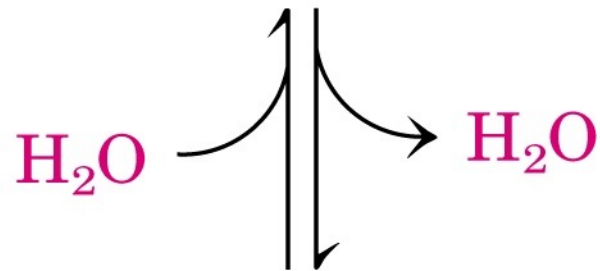
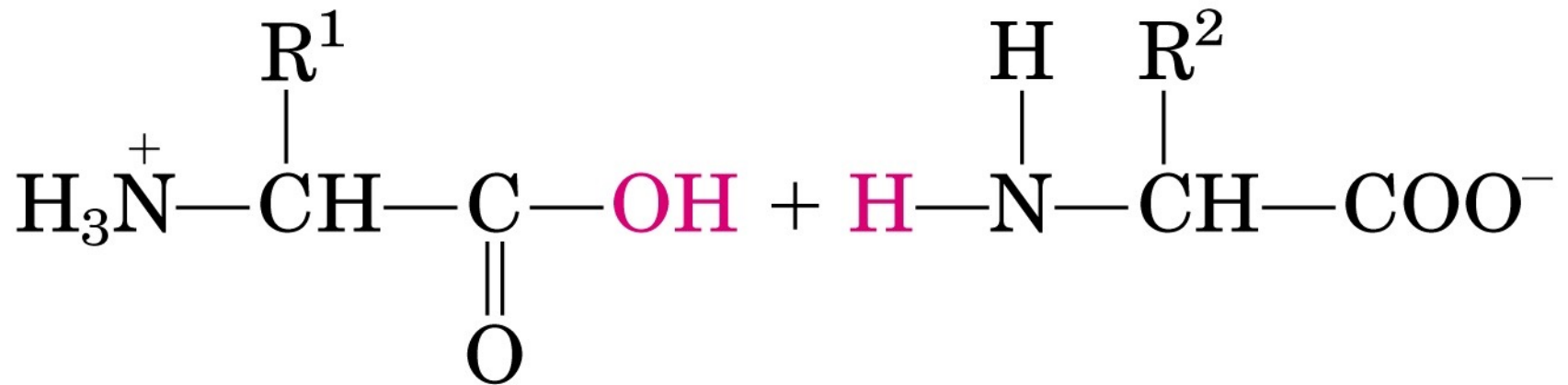
Si abbia una miscela di 3 amminoacidi : Gly (pI=5.97), Lys (pI=9.74) e Glu (3.22). Se l'elettroforesi su carta e' allestita a pH 1, 6 e 11. Si indichi in ciascun caso se sia possibile separare la miscela.

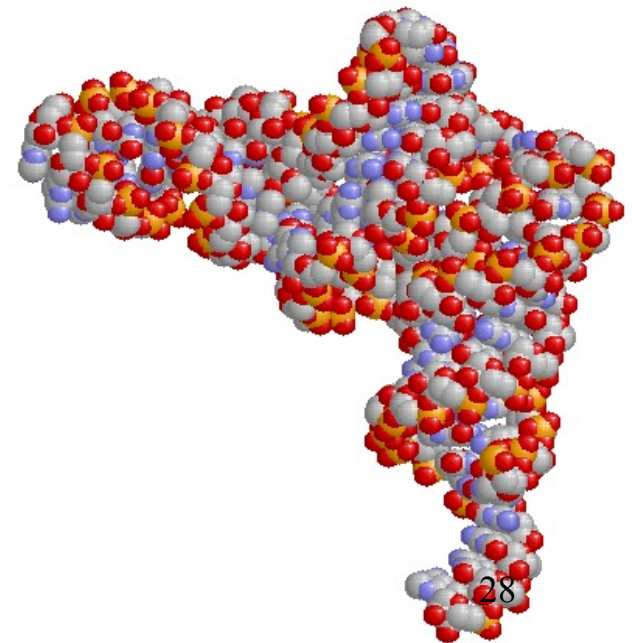
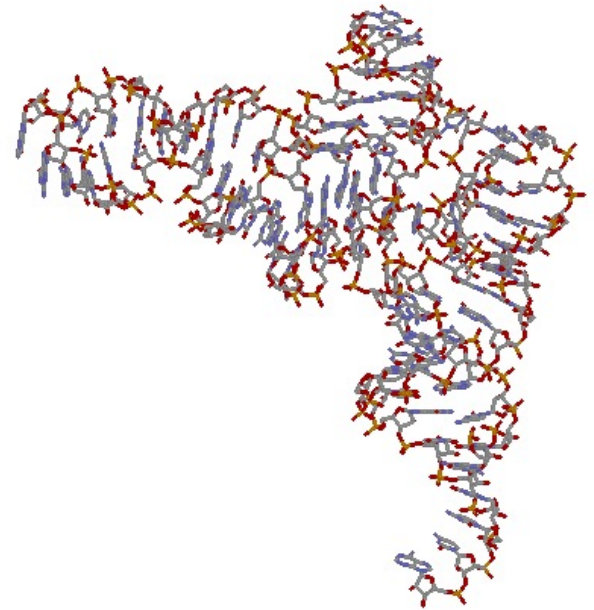
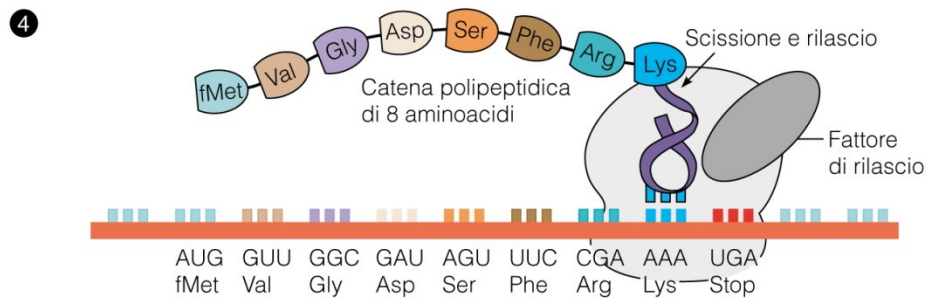
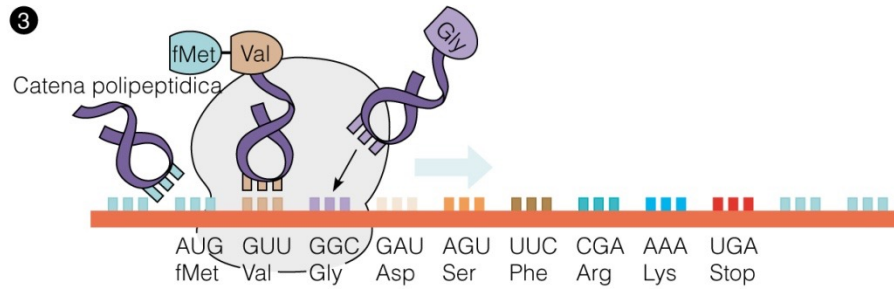
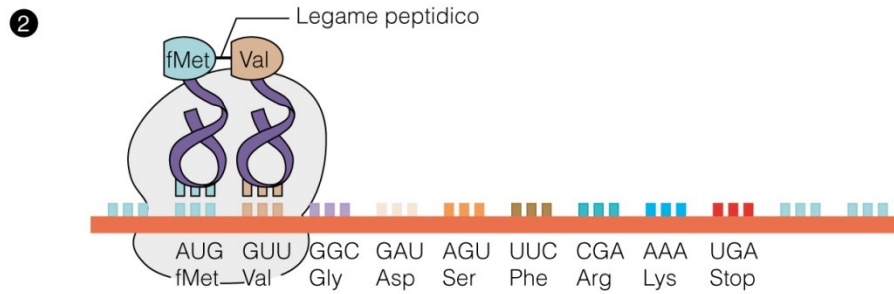
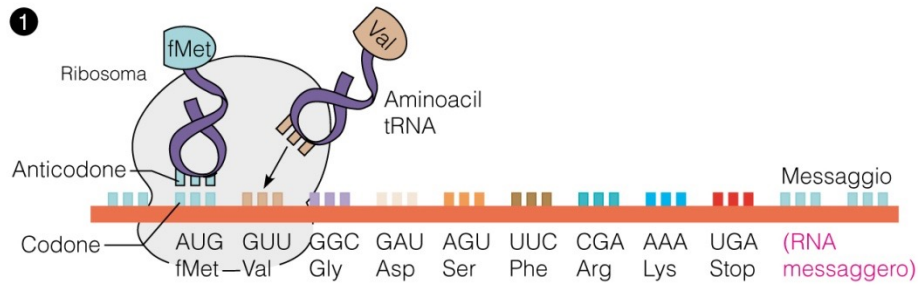
pH=11

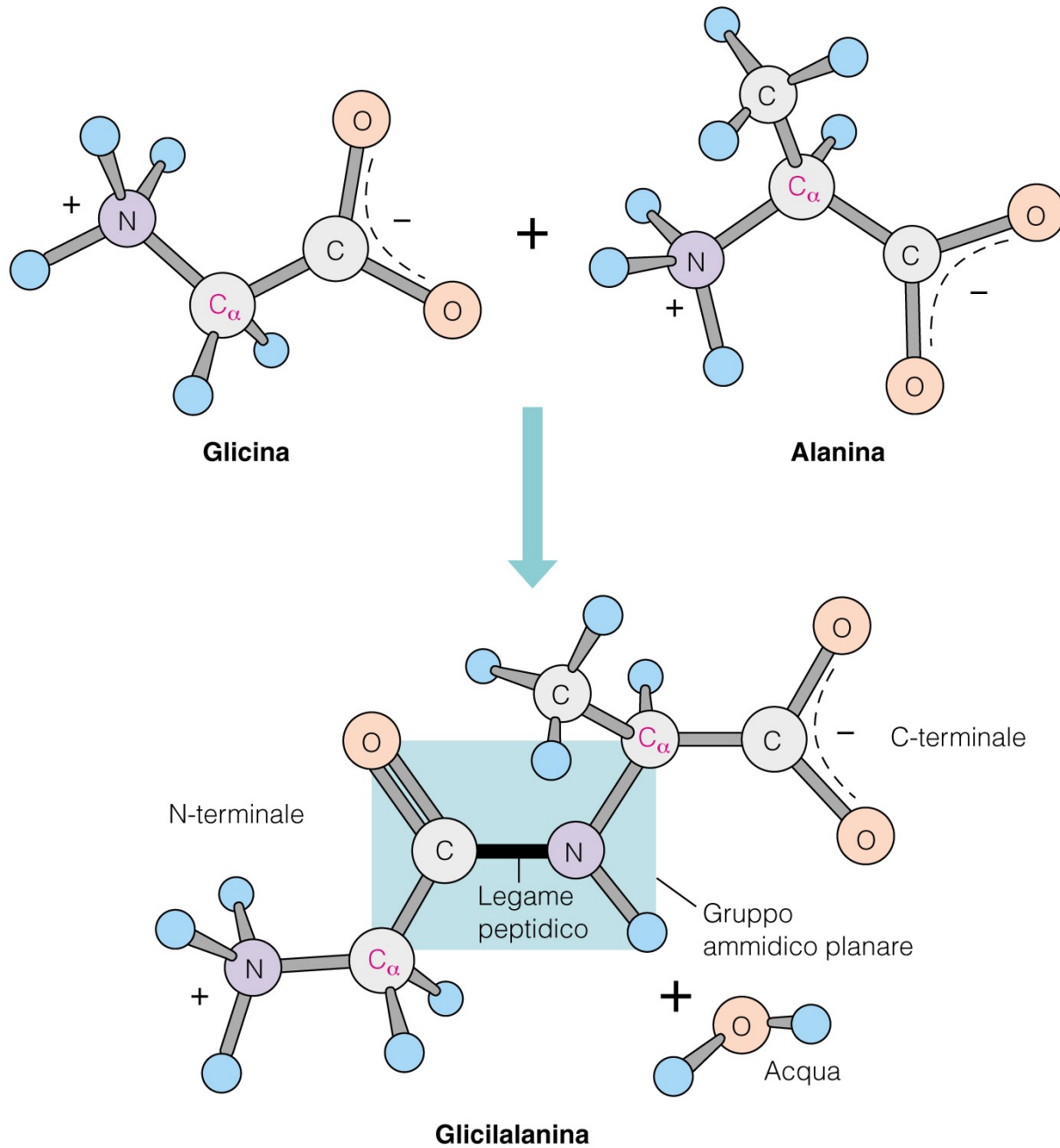
In ogni caso $\text{pH} > \text{pI}$ e gli amminoacidi si troveranno in forma anionica



Il legame peptidico





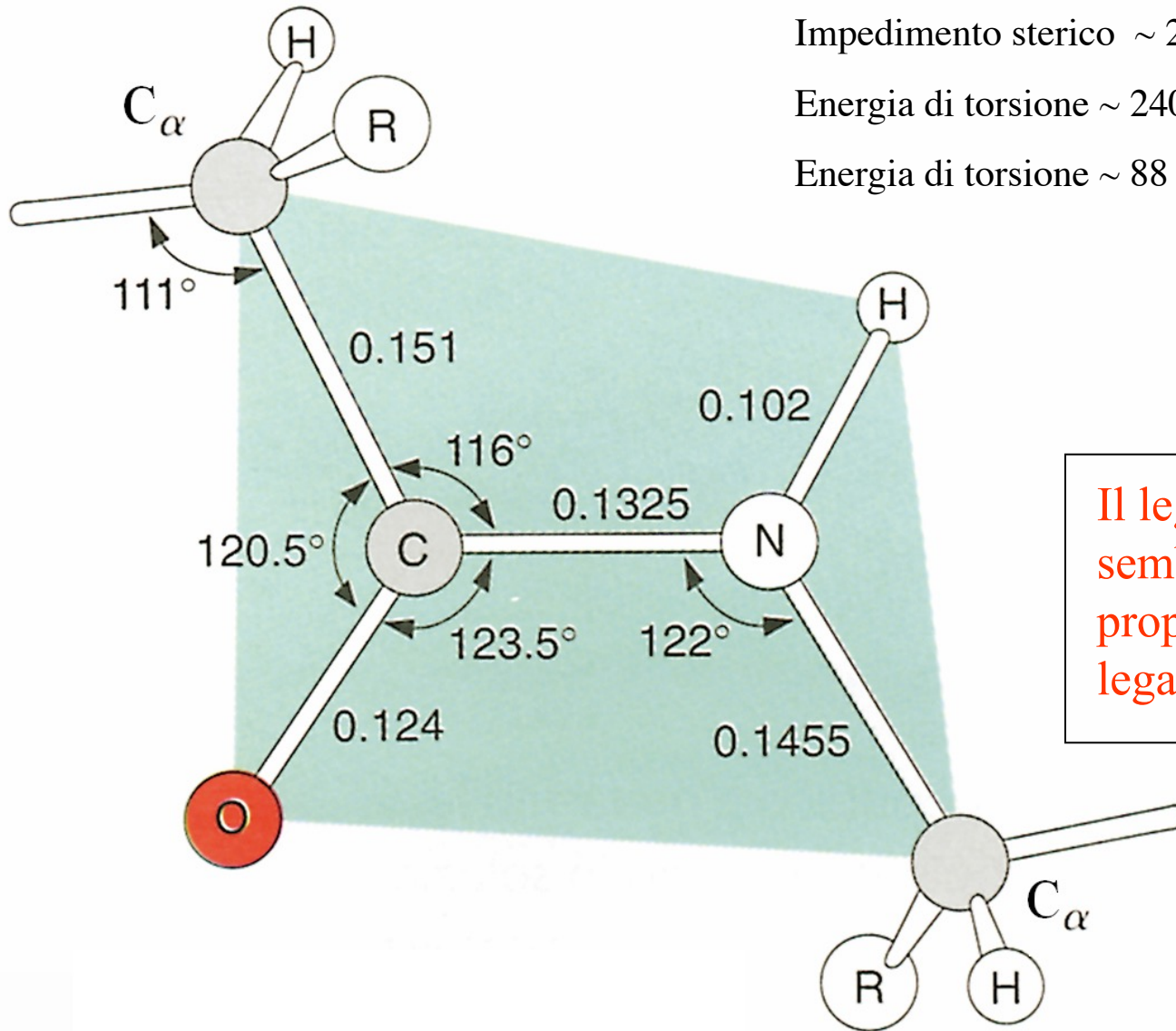


Energia di torsione $\sim 12 \text{ kJ mol}^{-1}$ (etano)

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)

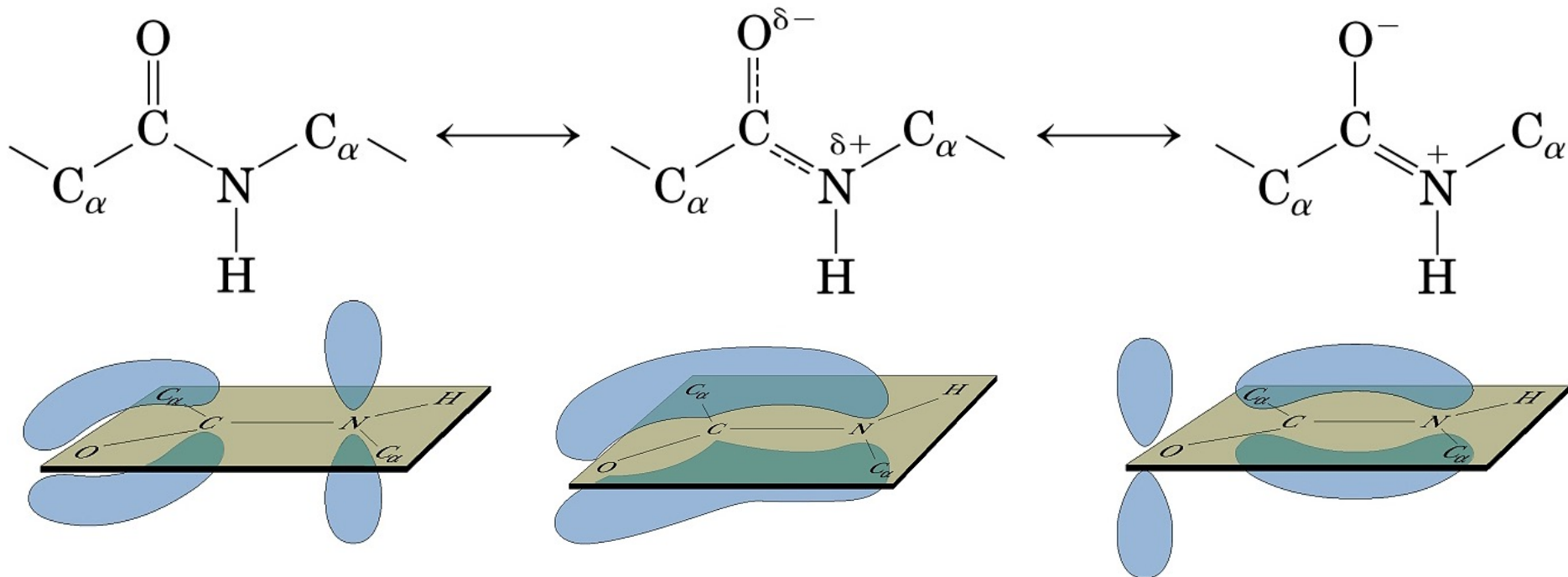


Il legame peptidico sembra non avere le proprietà strutturali di un legame semplice

Il legame peptidico ha **parziale carattere di doppio legame**

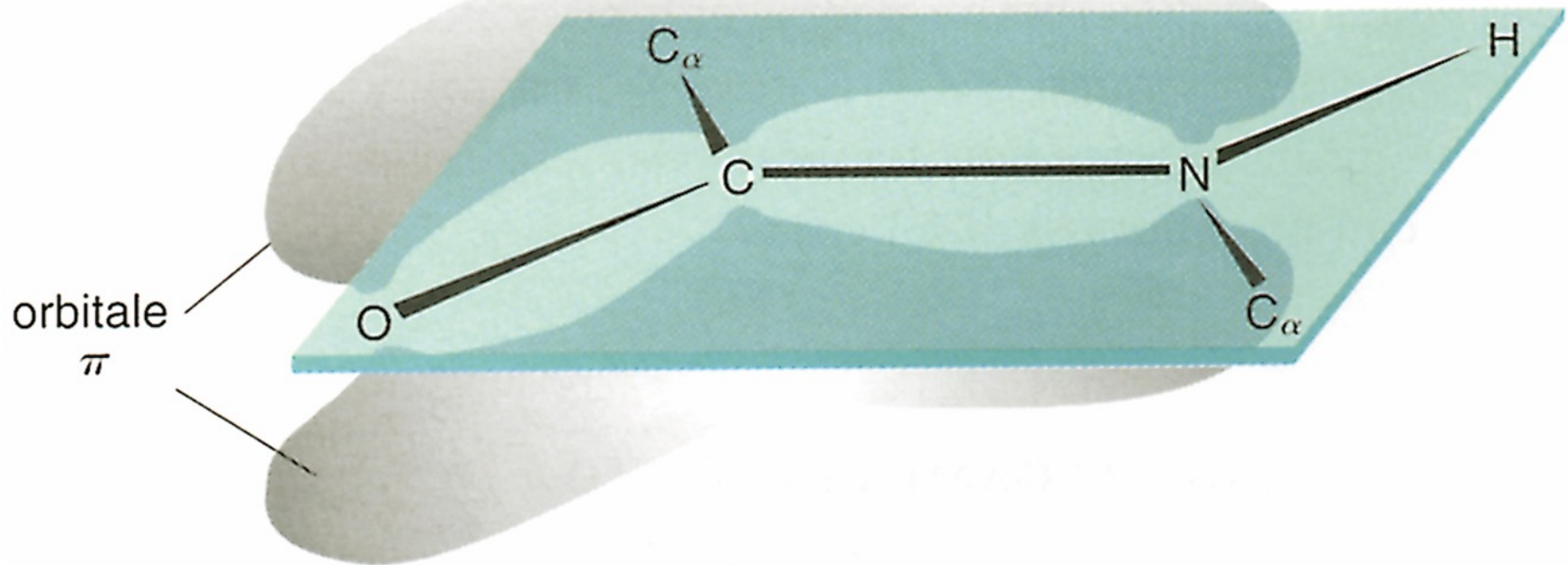
L'atomo di ossigeno carbonilico presenta una carica negativa parziale e l'azoto ammidico una parziale carica positiva. Si genera così un dipolo elettrico **nell'ibrido di risonanza**.

Virtualmente tutti i legami peptidici nelle proteine sono presenti nella configurazione *trans*.



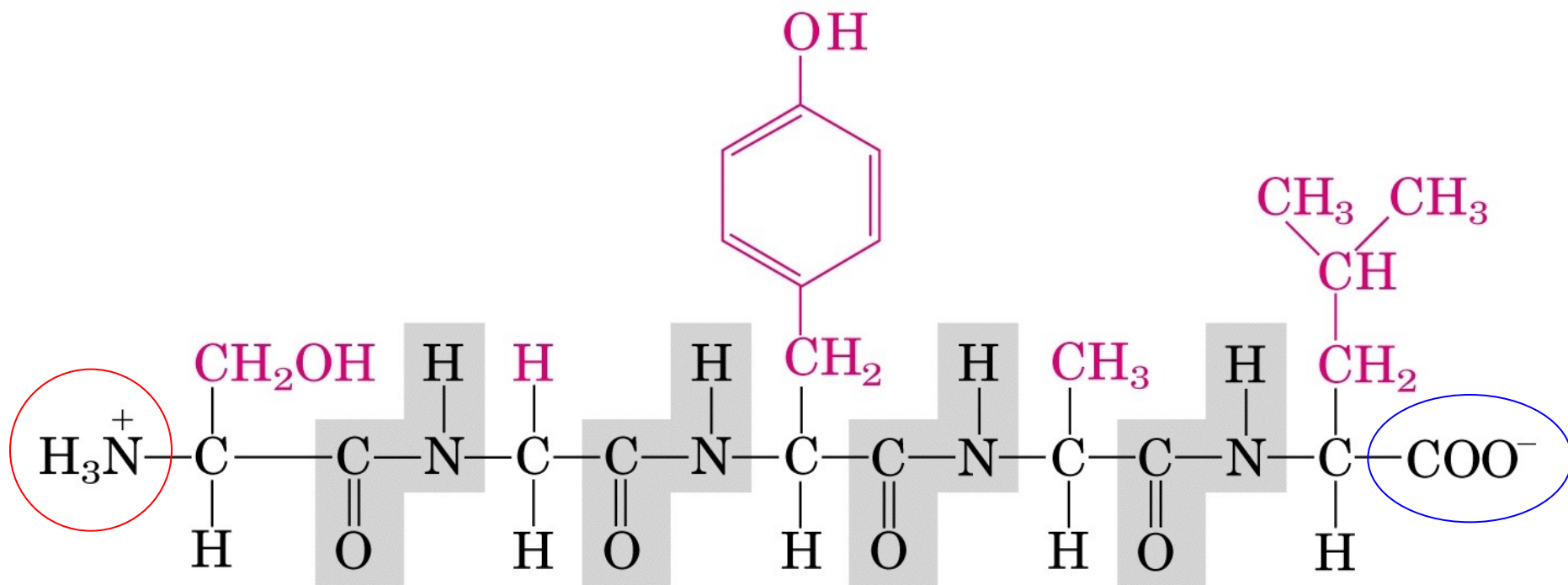
ibrido di risonanza

Parziale carattere di doppio legame del legame peptidico



- parziale carattere di doppio legame : ~ 40%
- 6 atomi giacciono su di un piano
- virtualmente tutti i legami peptidici nelle proteine sono presenti nella configurazione *trans*.

La catena polipeptidica presenta una direzionalità



Estremità
N-terminale

Estremità
C-terminale

La maltoporina

residuo N-terminale



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



residuo C-terminale

Dati molecolari di alcune proteine

	peso molecolare	numero di residui	numero di catene polipeptidiche
citocromo c (umano)	13 000	104	1
ribonucleasi A (pancreas bovino)	13 700	124	1
lisozima (albume)	13 930	129	1
mioglobina (cuore equino)	16 890	153	1
chimotripsina (pancreas bovino)	21 600	241	3
chimotripsinogeno (bovino)	22 000	245	1
emoglobina (umana)	64 500	574	4
albumina serica (umana)	68 500	609	1
esochinasi (lievito)	102 000	972	2
RNA polimerasi (E. coli)	450 000	4 158	5
apolipoproteina B (umana)	513 000	4 536	1
glutammina sintetasi (E. coli)	619 000	5 628	12
titina (umana)	2 993 000	26 926	1

Le proteine possono essere classificate in **semplici** e **coniugate**

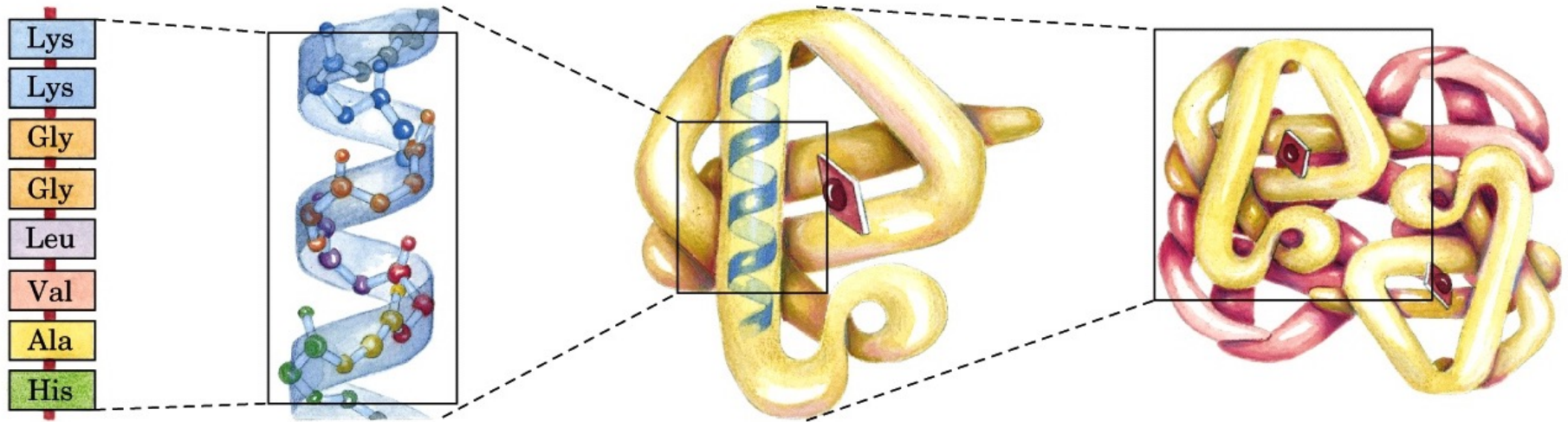
Proteine coniugate

classe	gruppo prostetico	esempio
lipoproteine	lipidi	β_1 -lipoproteina del sangue
glicoproteine	carboidrati	immunoglobulina G
fosfoproteine	fosfato	caseina del latte
emoproteine	eme (ferro-porfirina)	emoglobina
flavoproteine	nucleotidi flavinici	succinato deidrogenasi
metalloproteine	ferro	ferritina
	zinco	alcol deidrogenasi
	calcio	calmodulina
	molibdeno	dinitrogenasi
	rame	plastocianina

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
γ -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

I livelli di organizzazione strutturale delle proteine



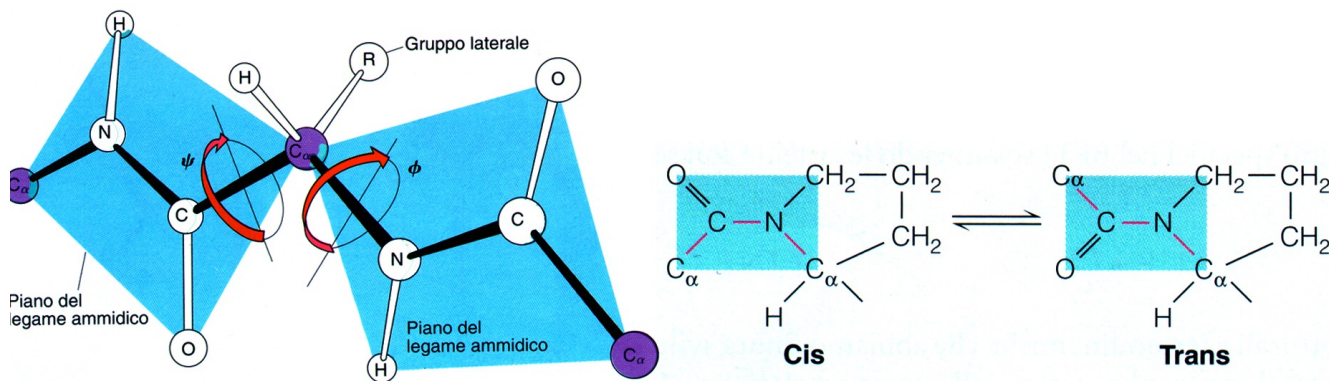
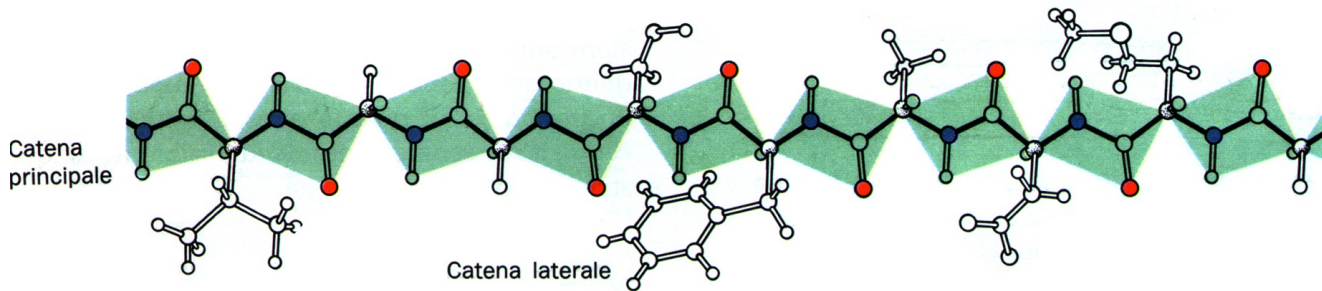
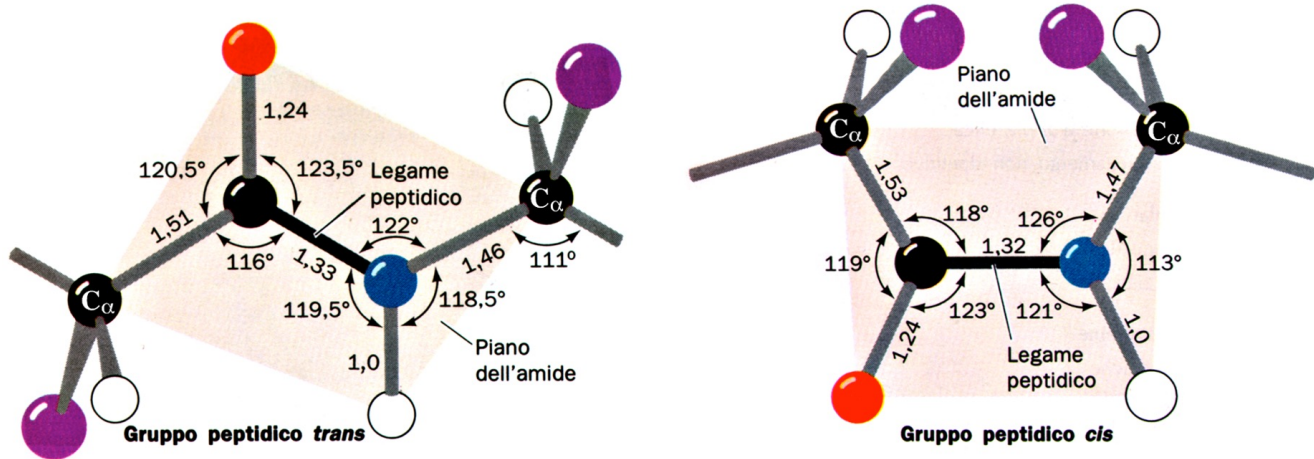
struttura primaria: la sequenza degli amminoacidi

struttura secondaria: organizzazione regolare e ricorrente dei peptidi nello spazio o conformazione locale di un polipeptide

struttura terziaria: struttura tridimensionale di una catena polipeptidica

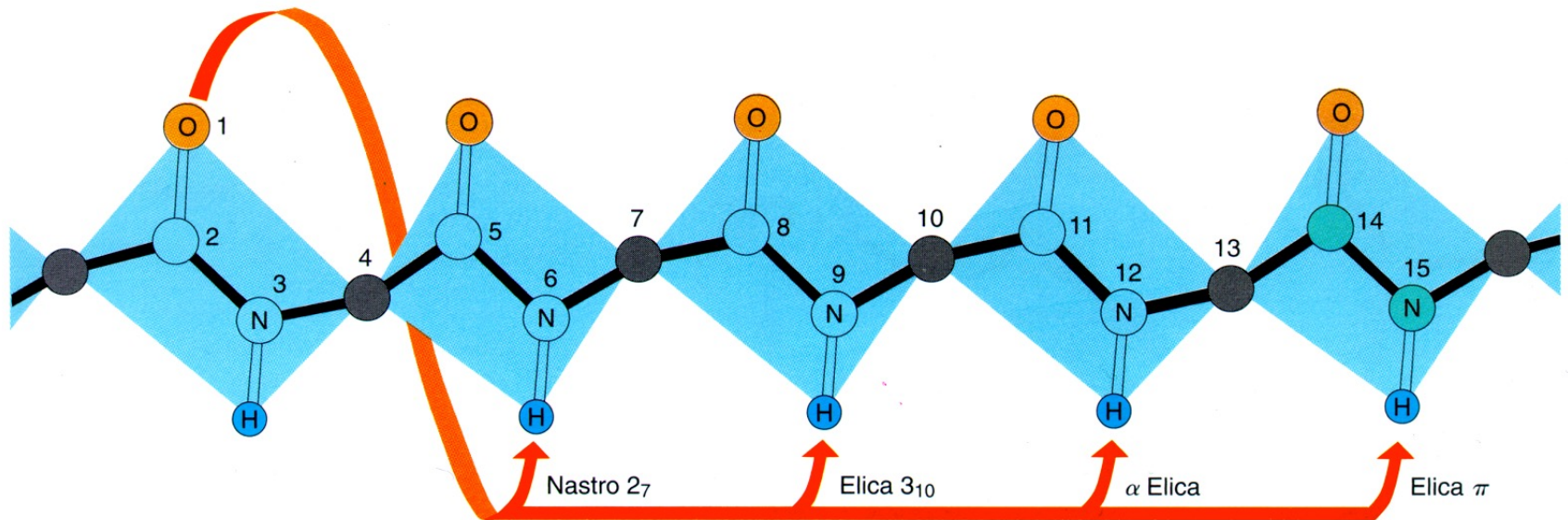
struttura quaternaria: organizzazione strutturale di più catene polipeptidiche

Ciascun livello è studiato con metodi sperimentali diversi



**Il legame peptidico ha configurazione trans.
La catena polipeptidica può ruotare attorno ai legami ϕ e ψ .**

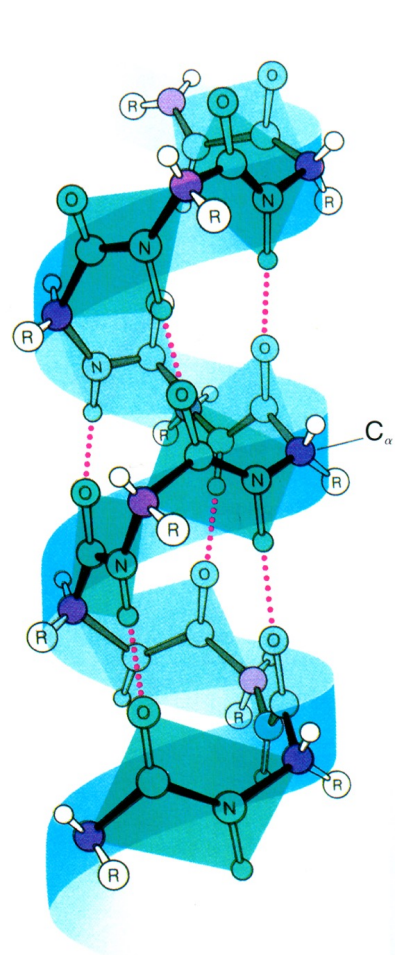
La struttura secondaria



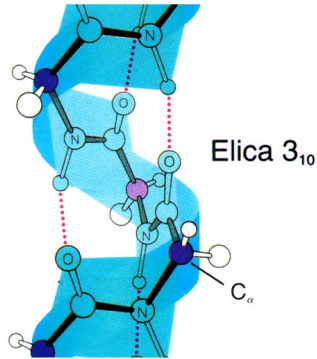
^aIl legame idrogeno è da una catena polipeptidica a un'altra.

^bStericamente permesse ma non osservate nelle proteine.

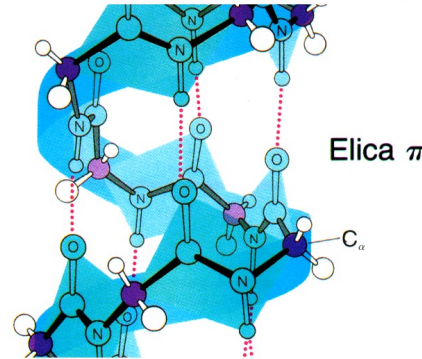
Tipo di struttura	Residui/ giro	Passo per residuo (nm)	Numero di atomi per ansa chiusa da un legame idrogeno	ϕ (°)	ψ (°)
Foglietto β antiparallelo	2.0	0.34	— ^a	-139	+135
Foglietto β parallelo	2.0	0.32	— ^a	-119	+113
3_{10} elica	3.0	0.20	10	-49	-26
α elica (3.6_{13})	3.6	0.15	13	-57	-47
π elica (4.4_{16}) ^b	4.4	0.12	16	-57	-70



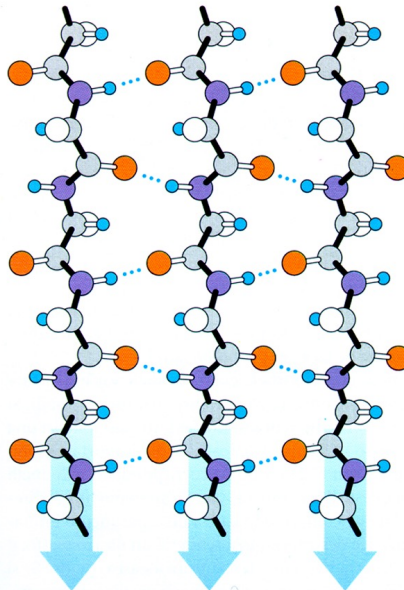
(a) α elica



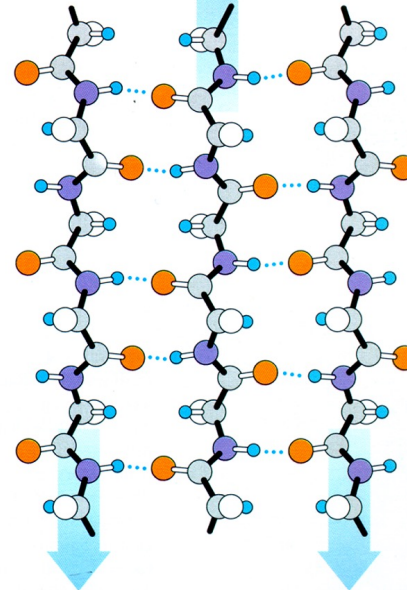
Elica 3_{10}



Elica π



(a) Foglietto β parallelo



(b) Foglietto β antiparallelo

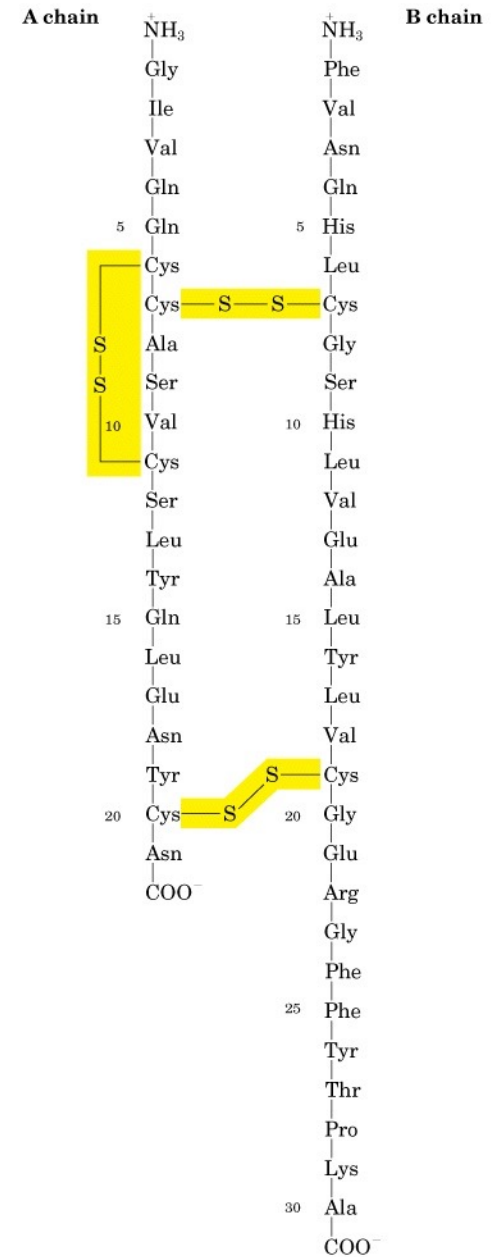
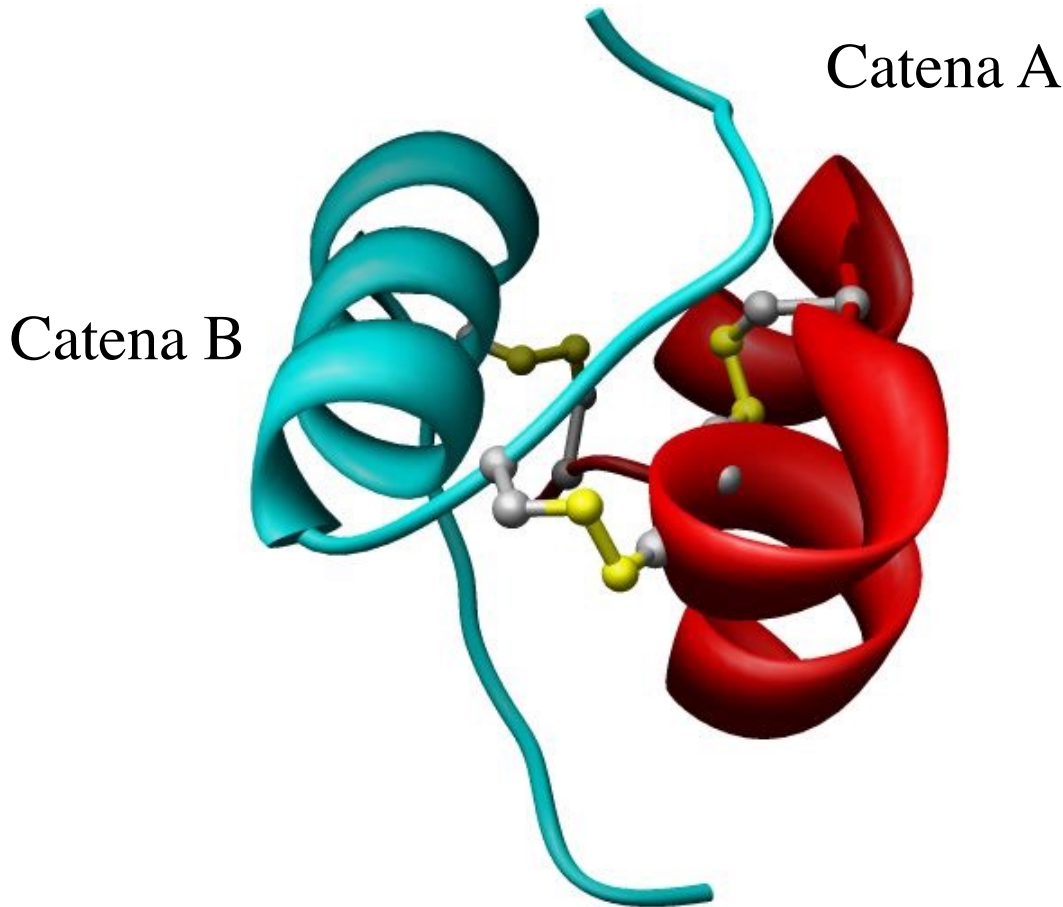
Due tipi di foglietto

Struttura secondaria

α elica

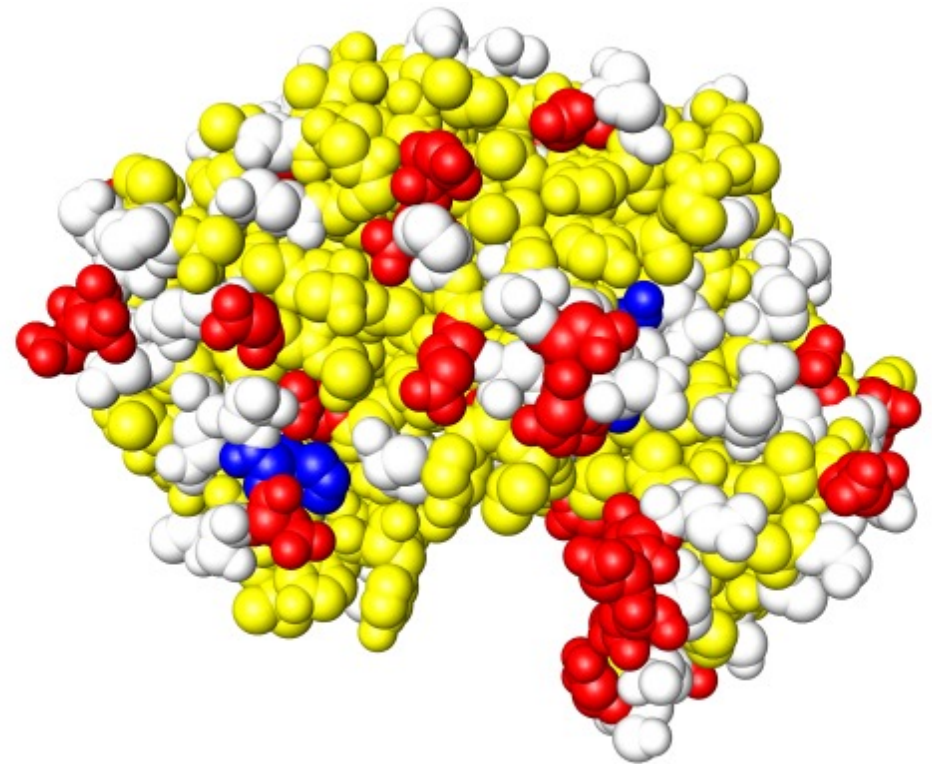
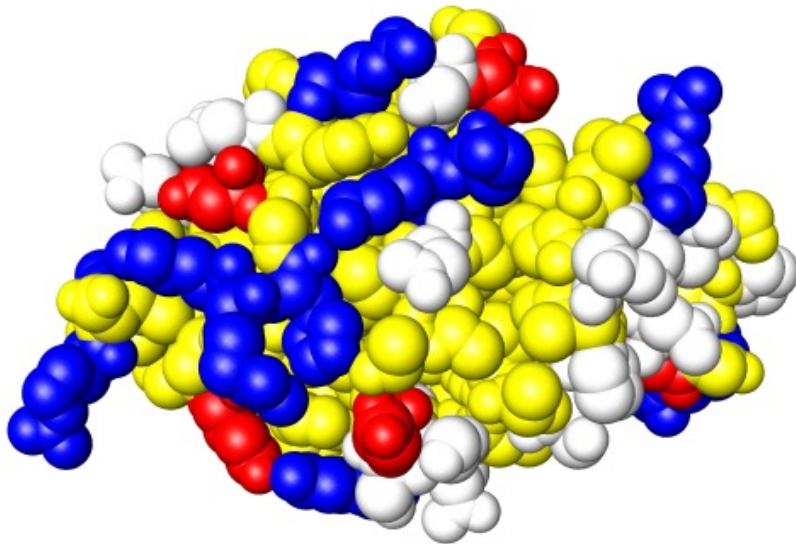
β foglietto

L'insulina



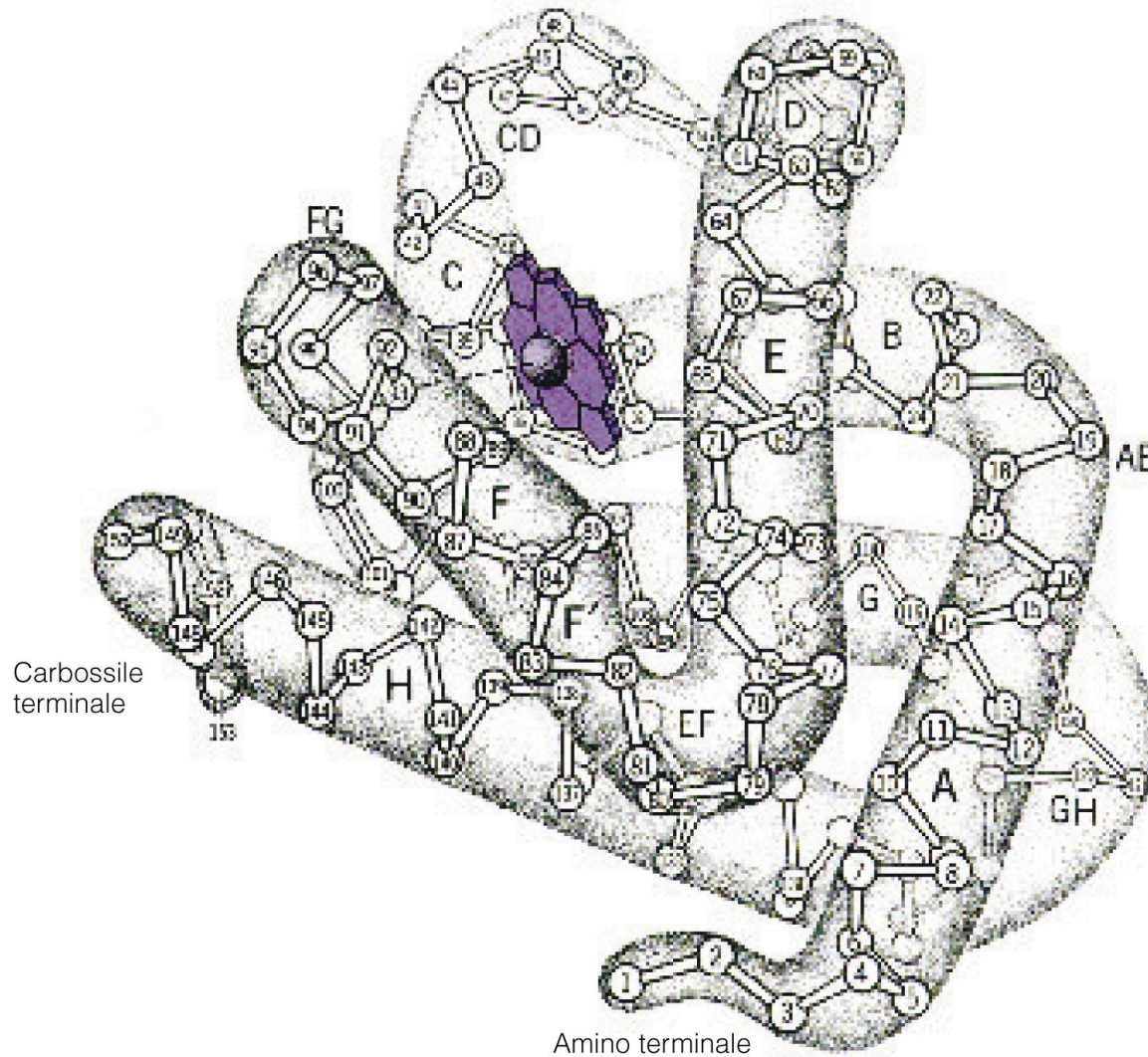
Lisozima (*Gallus gallus*) pI = 11

Pepsina (*Sus scropha*) pI = 1

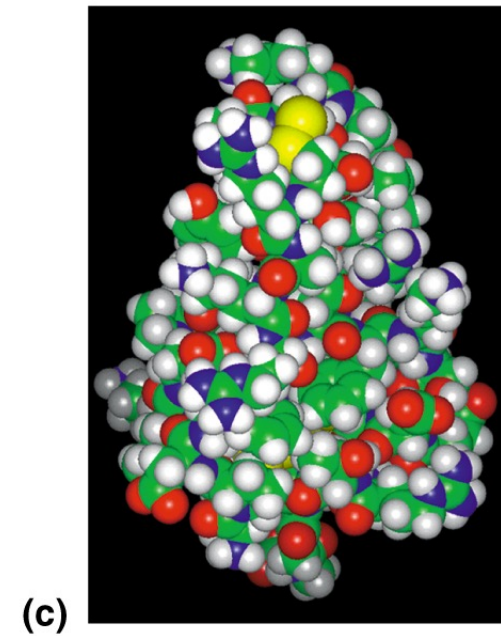
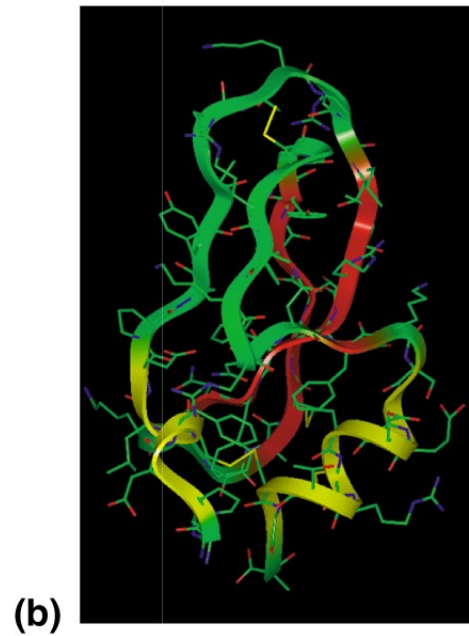
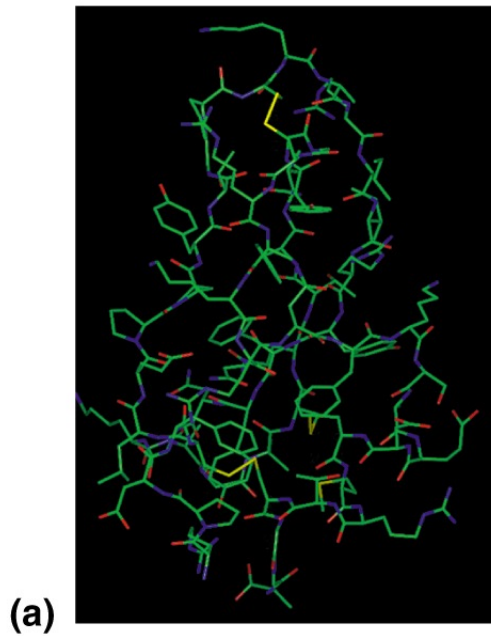


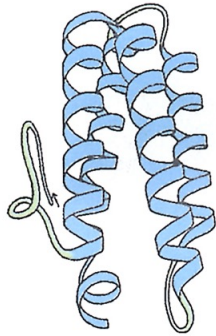
Codice colori: acidi = rosso; basici = blu; polari = bianco; idrofobici = giallo

Struttura della mioglobina

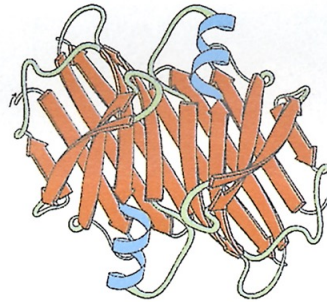


Rappresentazioni di strutture proteiche

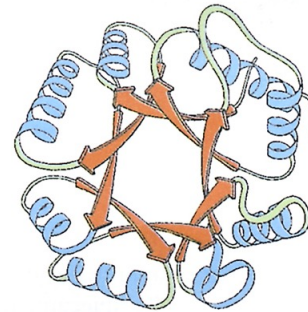




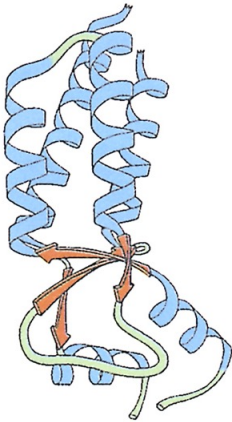
Mioemeritina



Prealbumina

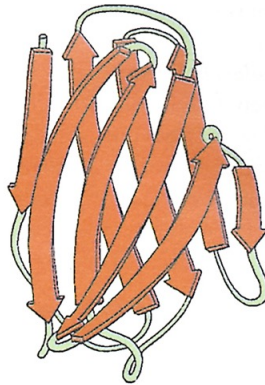


Piruvato chinasi, dominio 1



Proteina del capsido del
mosaico del tabacco

(a) Prevalentemente
ad α elica



Immunoglobulina, dominio V_2

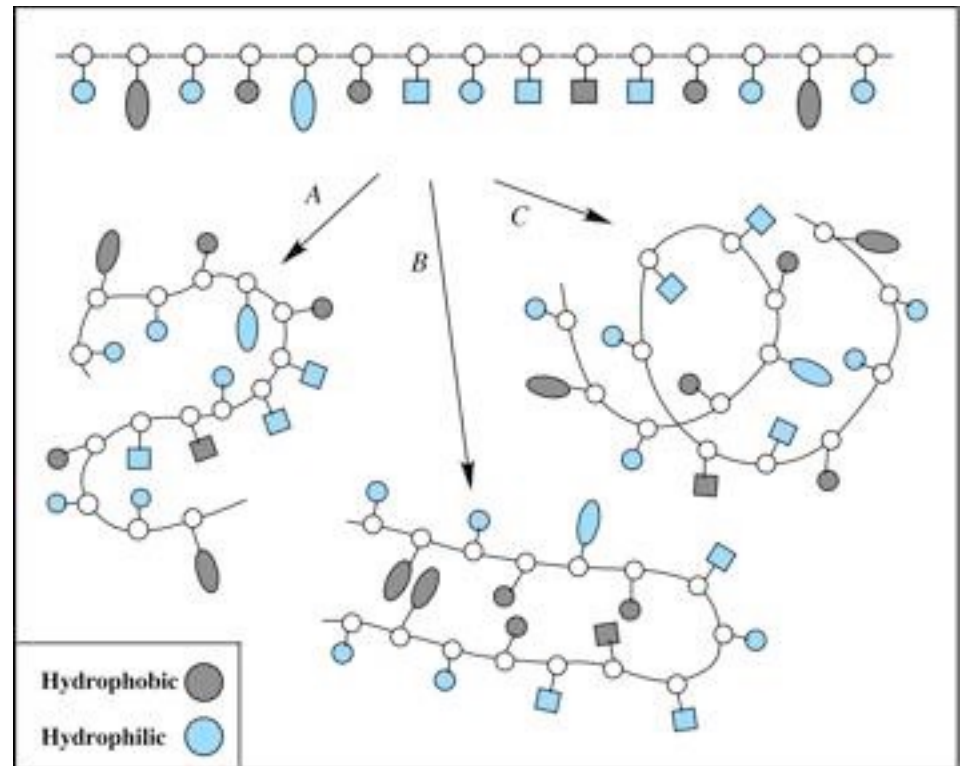
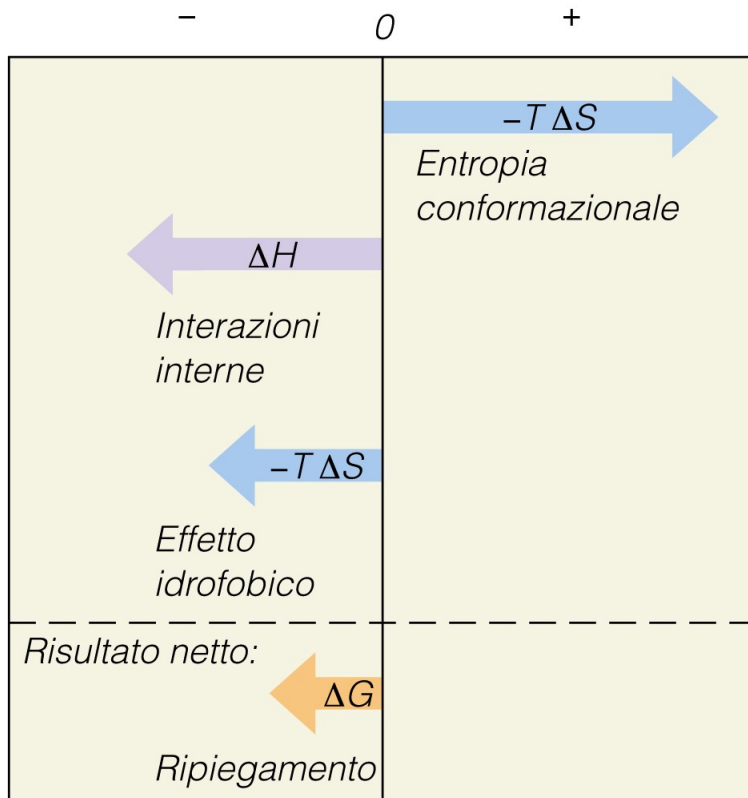
(b) Prevalentemente
a foglietto β



Esochinasi, dominio 2

(c) Struttura mista ad α elica
e a foglietto β

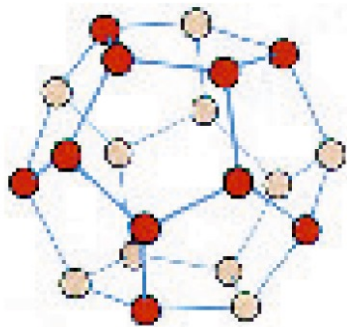
Il “folding” delle proteine



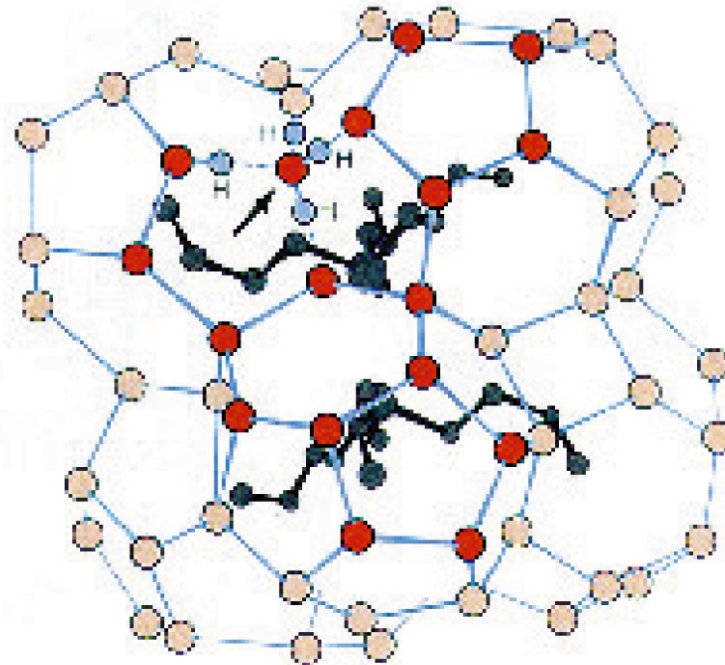
Effetto idrofobico

L'effetto idrofobico:

la dispersione in acqua di un composto idrofobico porta ad una diminuzione di entropia

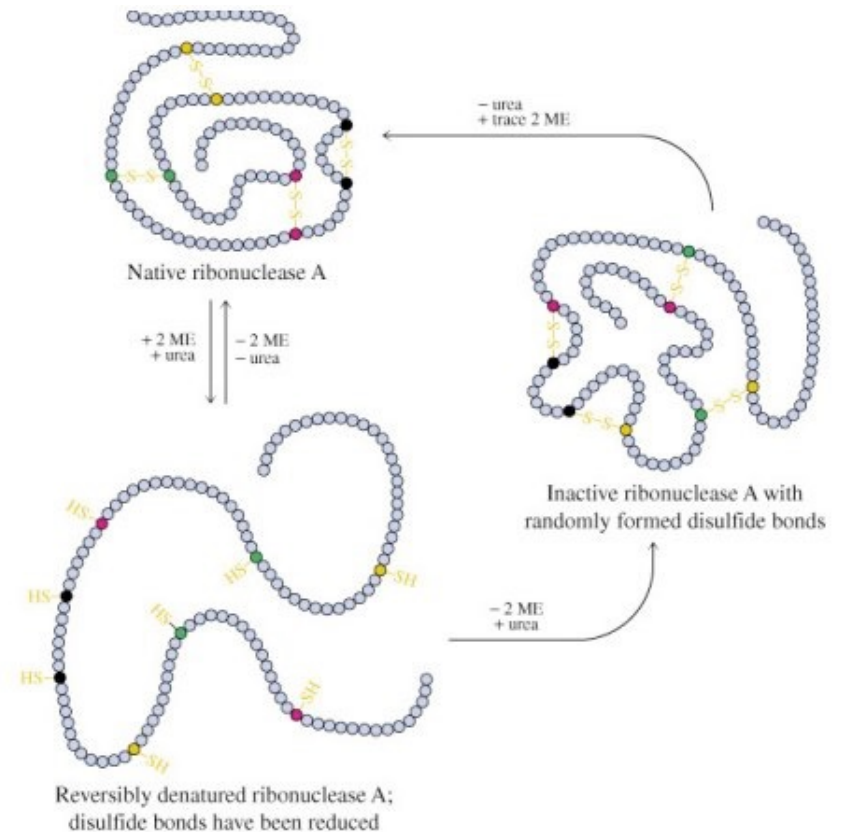
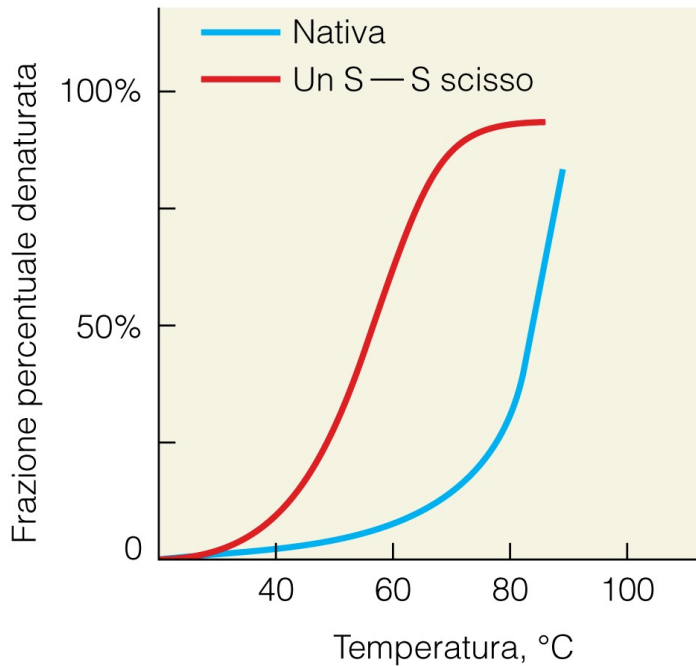


(a) L'unità base della struttura a clatrato

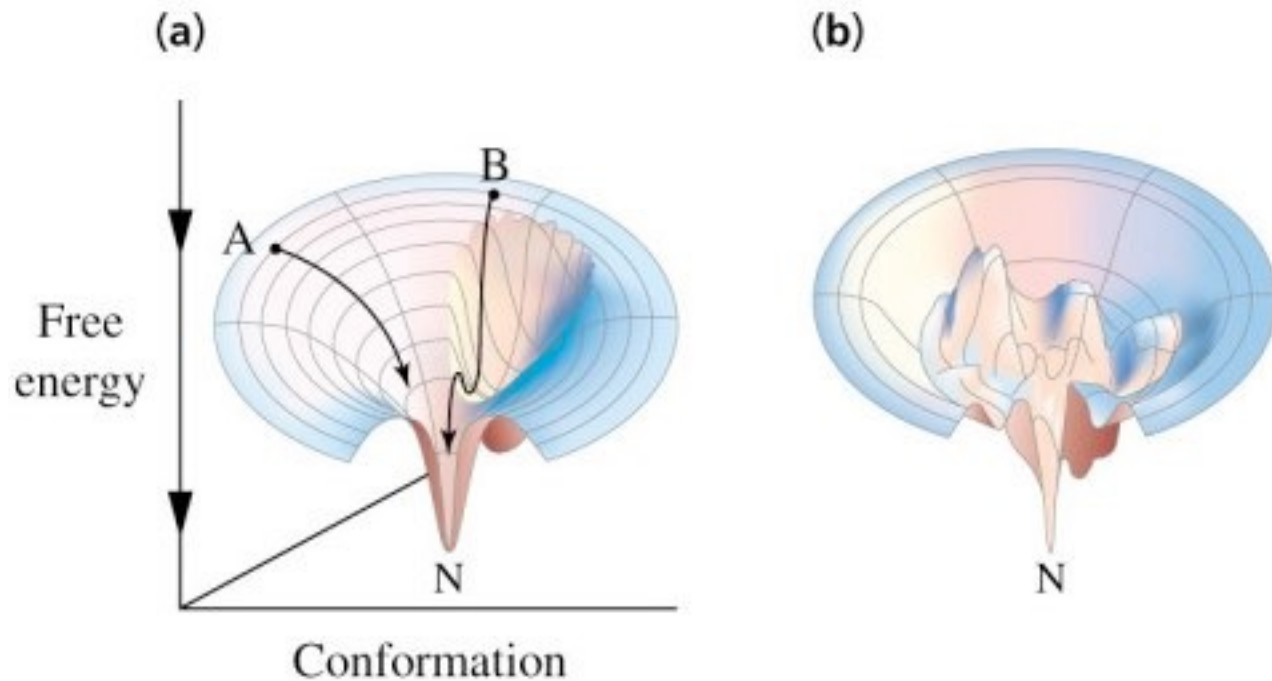


(b) La struttura a clatrato attorno agli idrocarburi

Il folding è un processo spontaneo e reversibile



Le proteine esplorano uno spazio limitato durante il folding.



* Anfinsen's paradox is simply that given the size of the conformation space available to a protein of any significant size, finding a particular conformation starting from any other conformation could take longer than the age of the Universe.

* Consider a polypeptide with 100 amino acid residues, and two equally possible conformations per residue

The number of possible conformations is then of the order of 10^{130}

Assume an interconversion time for conformations of the order of 10^{-12} seconds (which is too short by several orders of magnitude). Then, it will take 10^{10} years to sample all the conformations, proceeding randomly

* However, numerous examples exist of proteins folding in times of the order of a second or less.