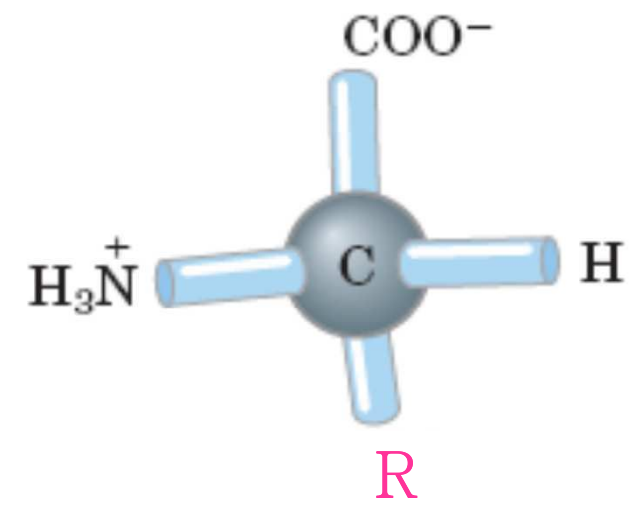
Le proteine

- catalitiche (enzimi)

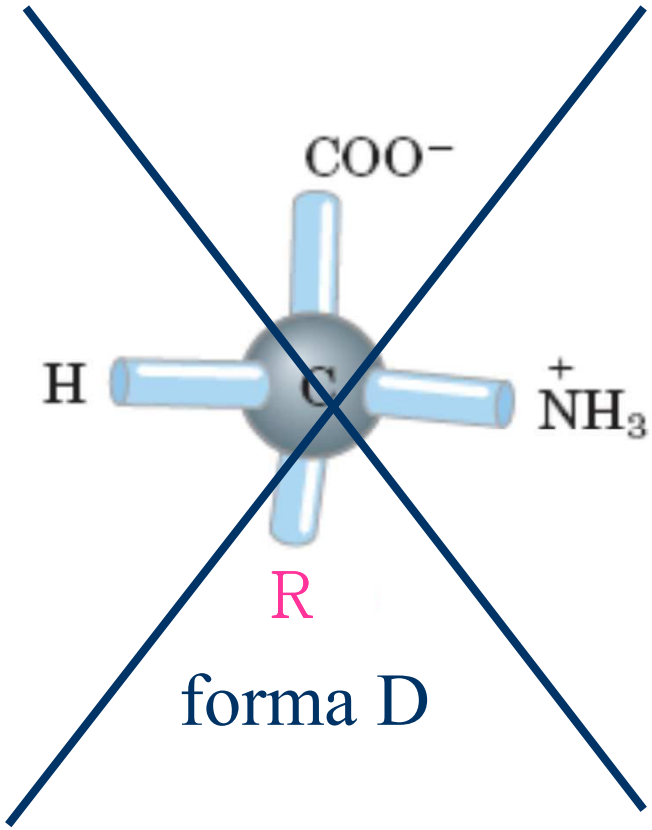
- di trasporto (solubili e di membrana)
- di difesa (anticorpi)
- strutturali

- di riserva (latte, albume)
- di regolazione
- recettori (solubili e di membrana)
- ad azione tossica (veleno di serpente)

Le proteine sono costituite da L-aminoacidi

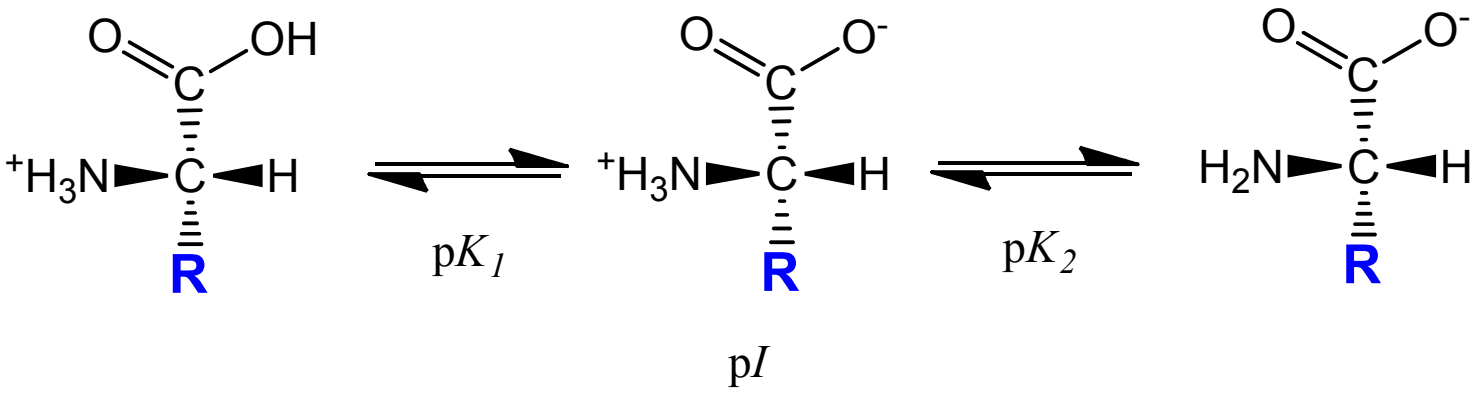


forma L



forma D

Il punto isoelettrico (p*I*)

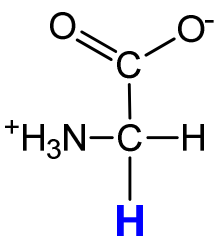


$$pI = \frac{pK_1 + pK_2}{2}$$

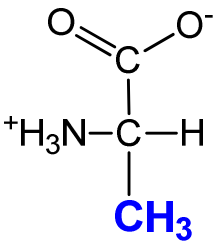
Ad esempio, il pI per l'alanina (R = CH_3):

$$pI = \frac{pK_1 + pK_2}{2} = \frac{2.35 + 9.87}{2} = 6.11$$

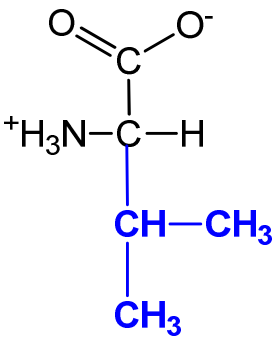
Aminoacidi con residuo idrofobico o apolare.



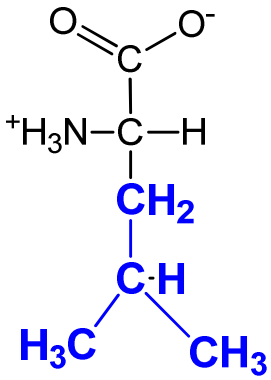
Glicina, Gly, G



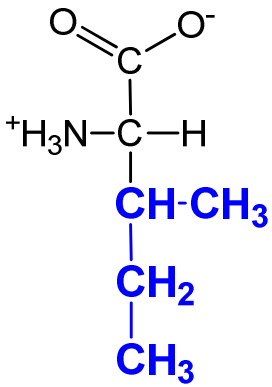
Alanina, Ala, A



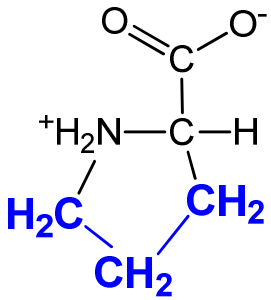
Valina, Val, V



Leucina, Leu, L

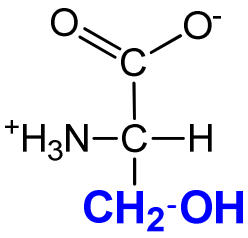


Isoleucina, Ile, I

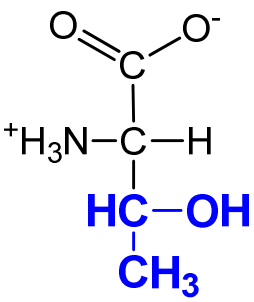


Prolina, Pro, P

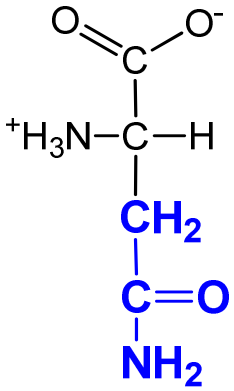
Aminoacidi con residuo idrofilico o polare. p*I* intorno a 5.6



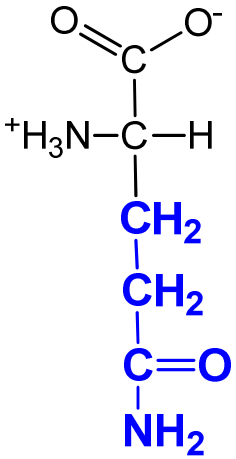
Serina, Ser, S



Treonina, Thr, T

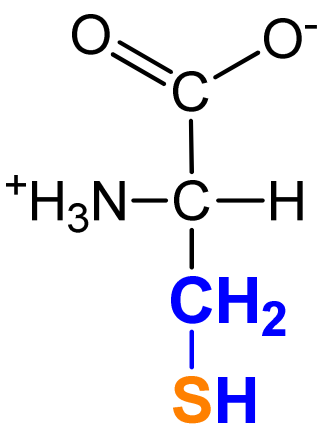


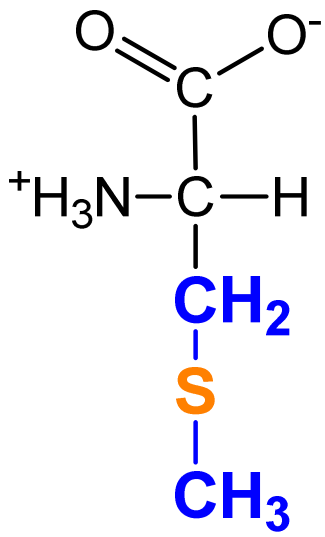
Asparagina, Asn, N



Glutammina, Gln, Q

Aminoacidi contenenti zolfo





Cisteina, Cys, C

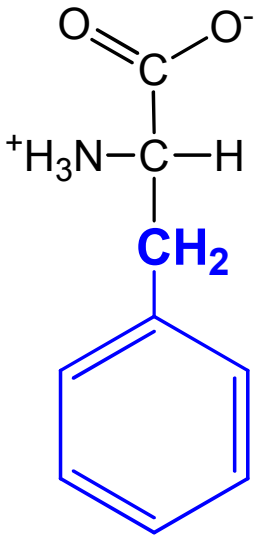
R polare

pK_R=*8.2*

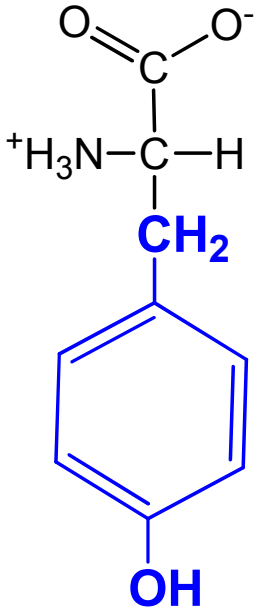
Metionina, Met, M

R apolare

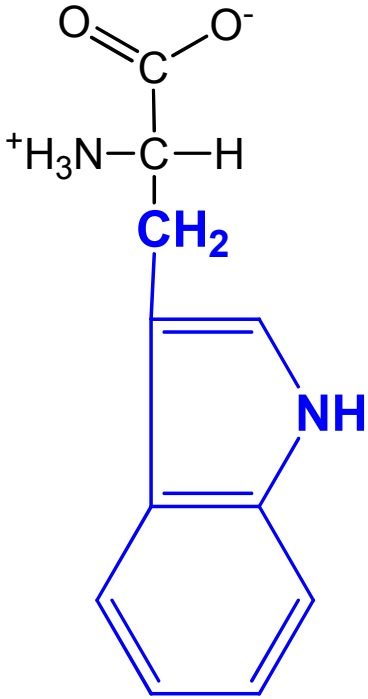
Aminoacidi aromatici. p*I* intorno a 5.0



Fenilalanina, Phe, F



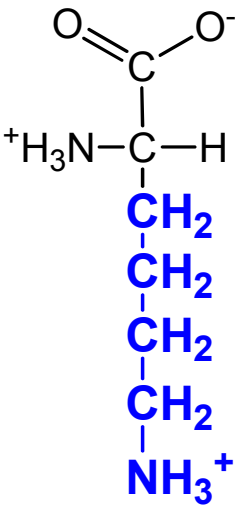
Tirosina, Tyr, Y

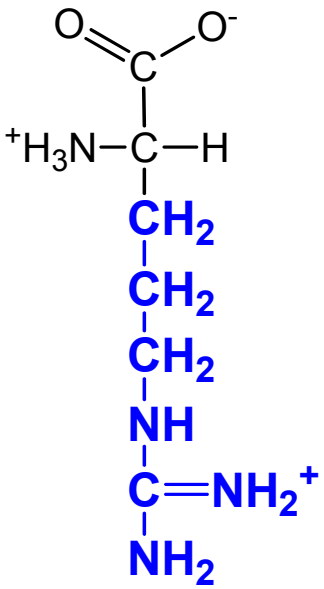


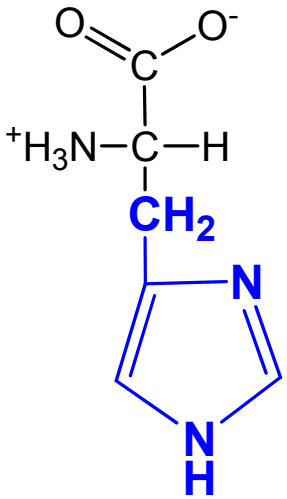
Triptofano, Trp, W

$pK_R = 10.07$

Aminoacidi con residuo R carico positivamente a pH fisiologico (aminoacidi basici)

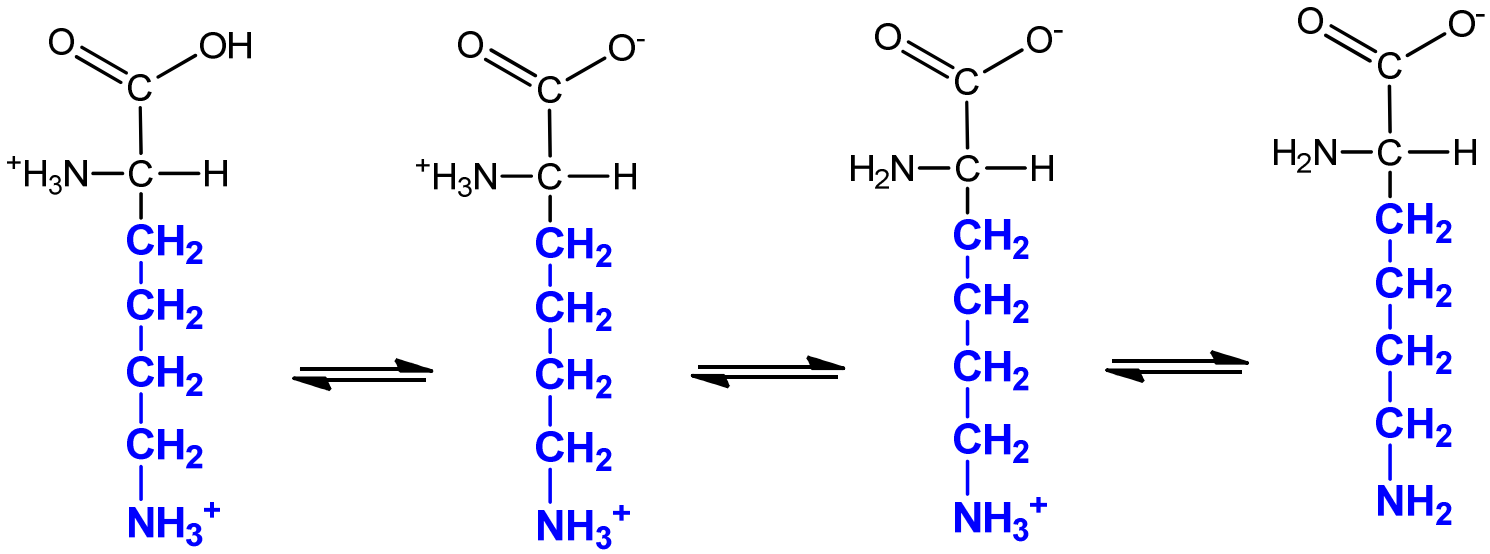






	Lisina, Lys, K	Arginina, Arg, R	Istidina, His, H
pK_R:	10.53	12.48	6.00

Lisina



pK 2.18 8.95 10.53

pI = 9.74

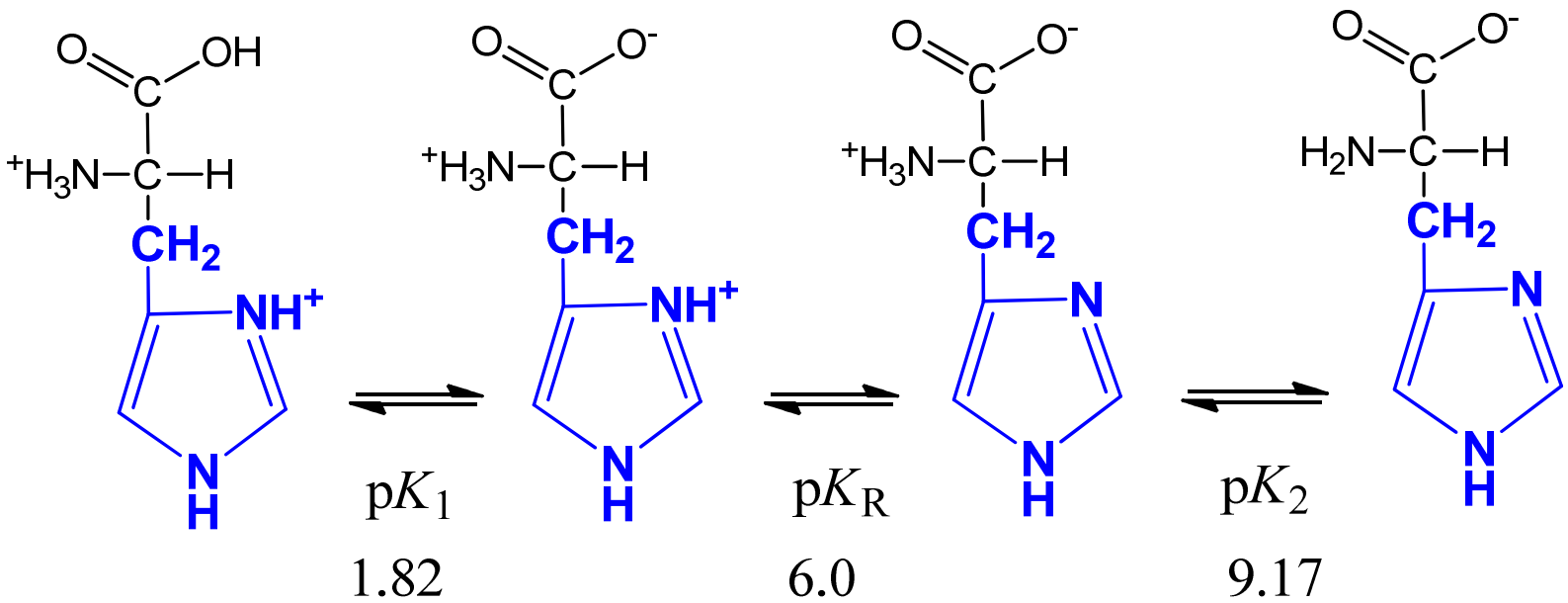
Cariche +2 +1 0 -1

pH del mezzo in cui si trova al 100% nella forma disegnata:

< 1.18 5.58 9.74 > 11.53

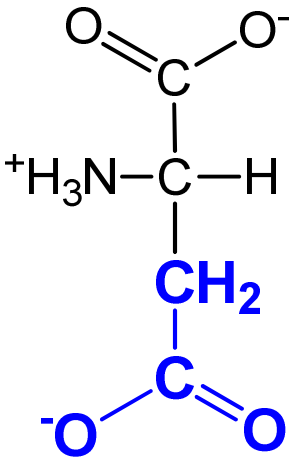
pH del mezzo in cui è al 50% nella forma a sx e 50% nella forma a dx:

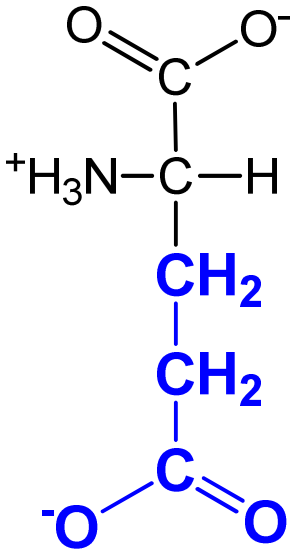
2.18 8.95 10.53



$$pI = \frac{pK_R + pK_2}{2} = \frac{6.0 + 9.17}{2} = 7.58$$

Aminoacidi con gruppo R carico negativamente
Aminoacidi acidi





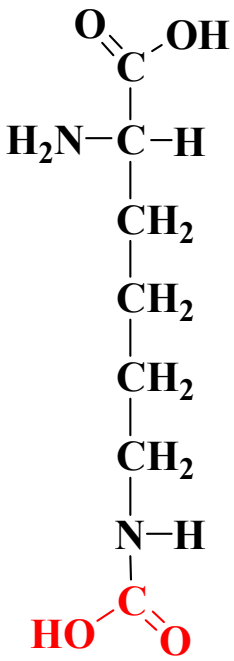
Aspartato, Asp, D

Glutammato, Glu, E

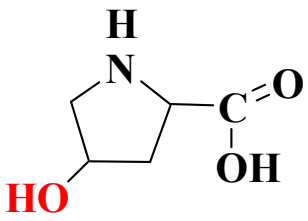
pK_R: 3.65

4.25

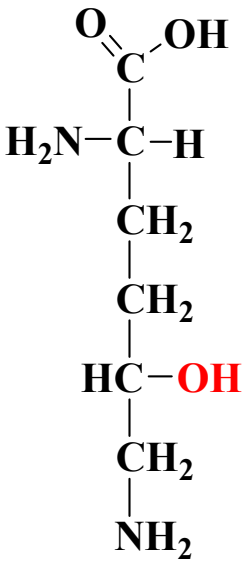
Aminoacidi proteici modificati



carbossilisina

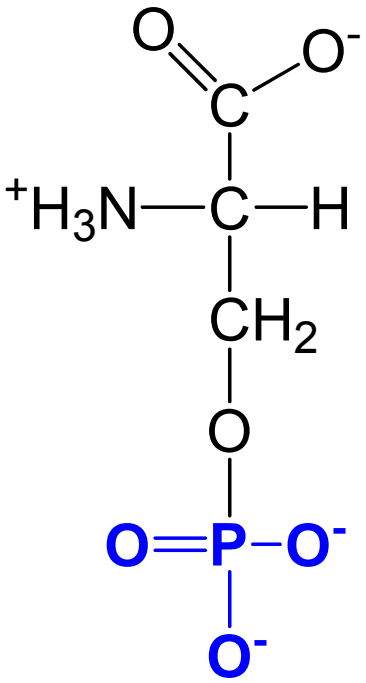


4-idrossiprolina

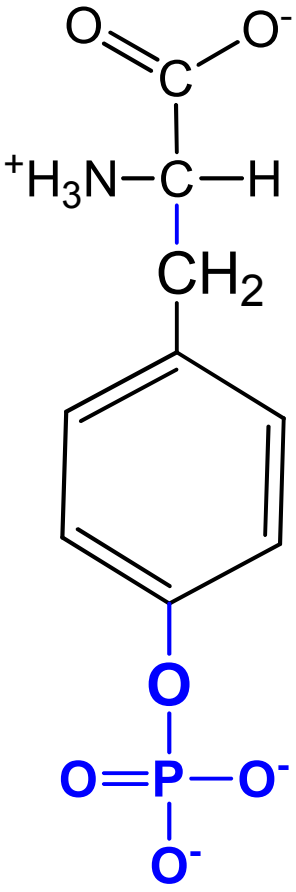


5-idrossilisina

Aminoacidi modificati per fosforilazione



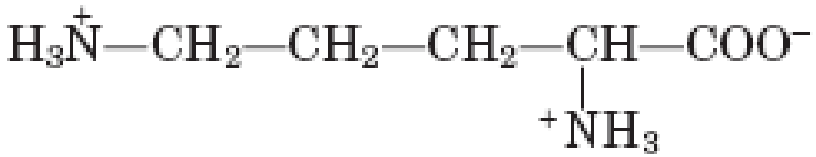
Fosfoserina



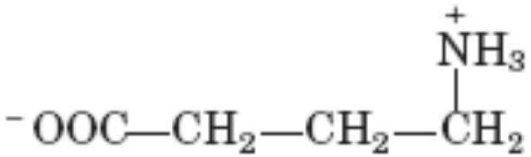
Fosfotirosina

Aminoacidi non proteici

Esistono circa 300 aminoacidi che non fanno parte di proteine



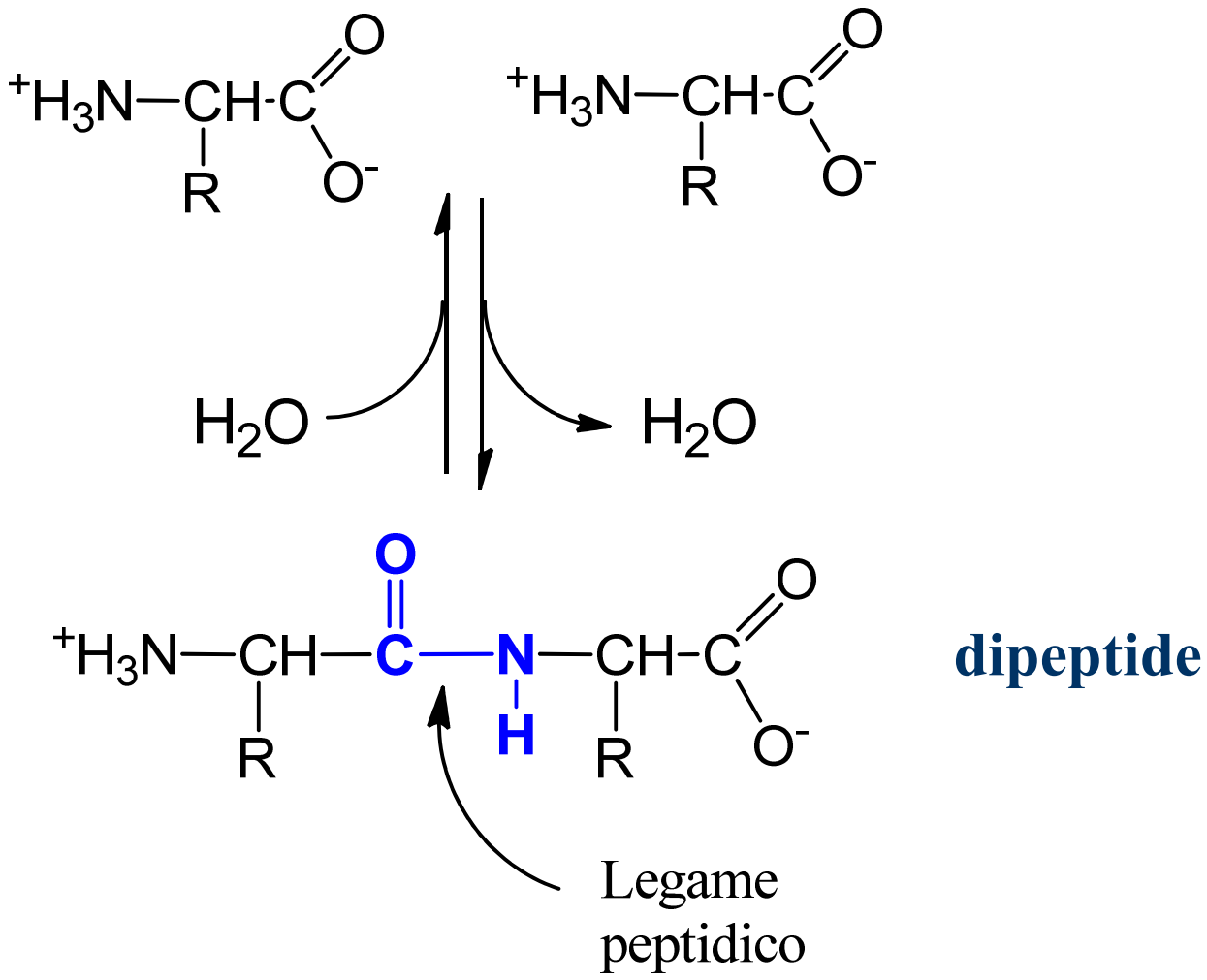
Ornitina



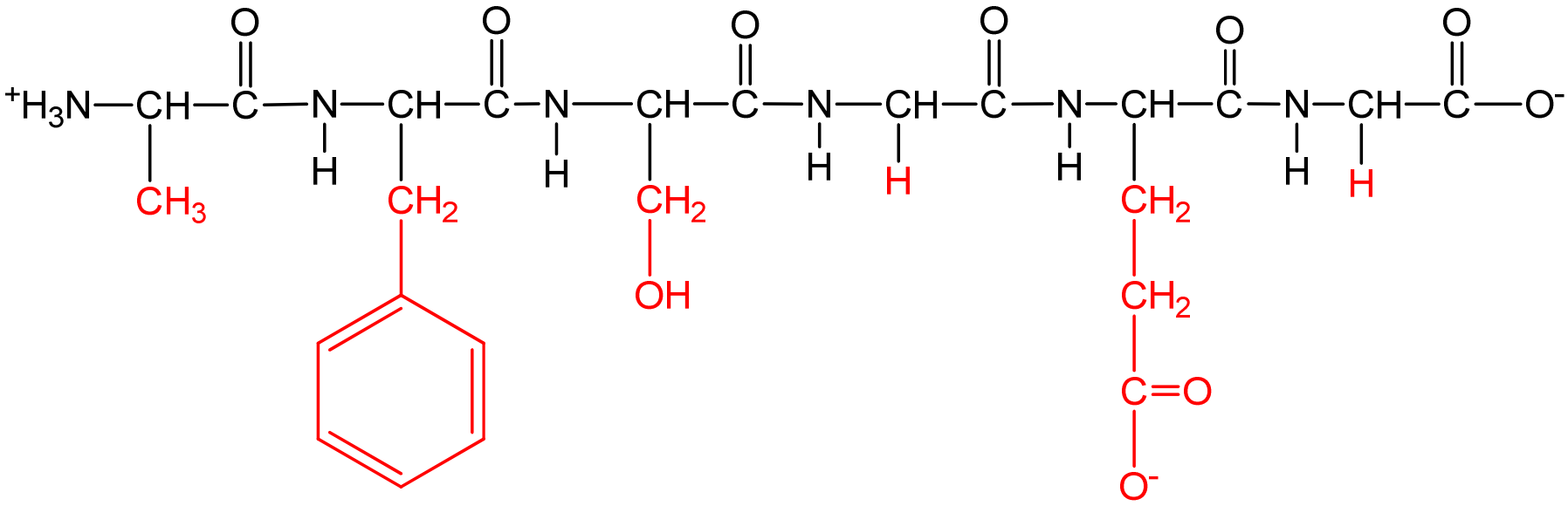
Acido γ-aminobutirrico

Struttura delle proteine (polipeptidi)

Il legame peptidico



dipeptide

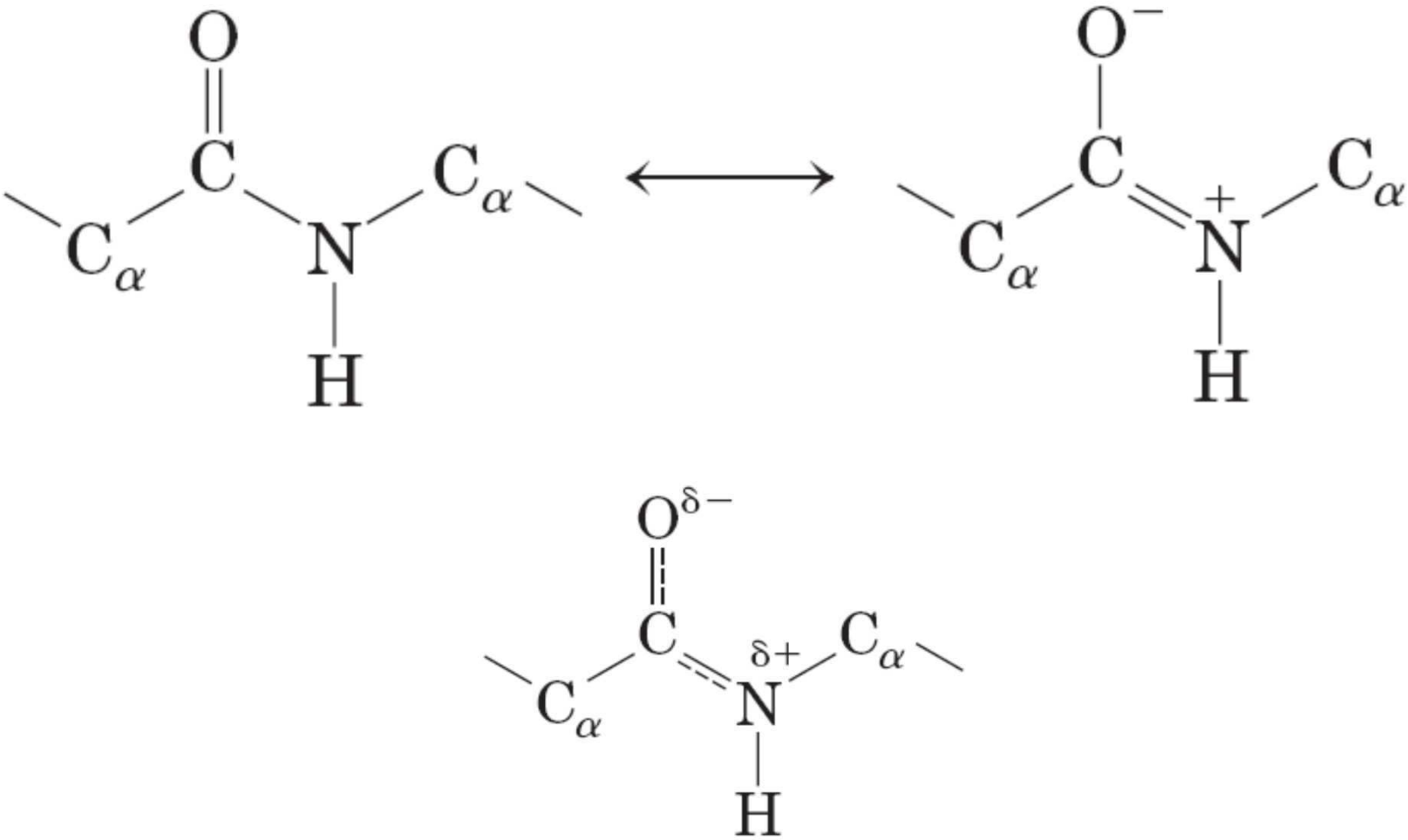


Estremità aminoterminale

Estremità carbossiterminale

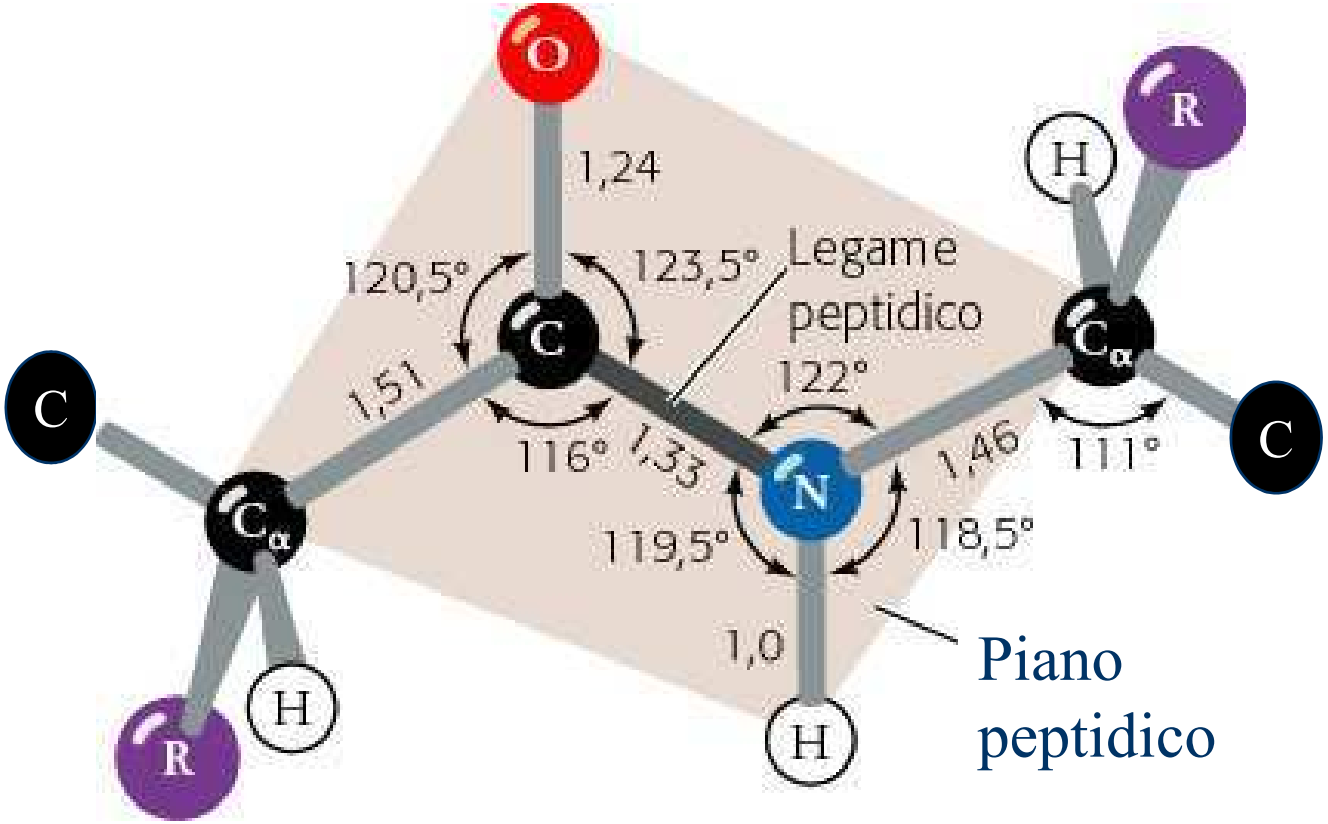
Le proteine sono costituite da una o più catene polipeptidiche

Struttura del legame peptidico

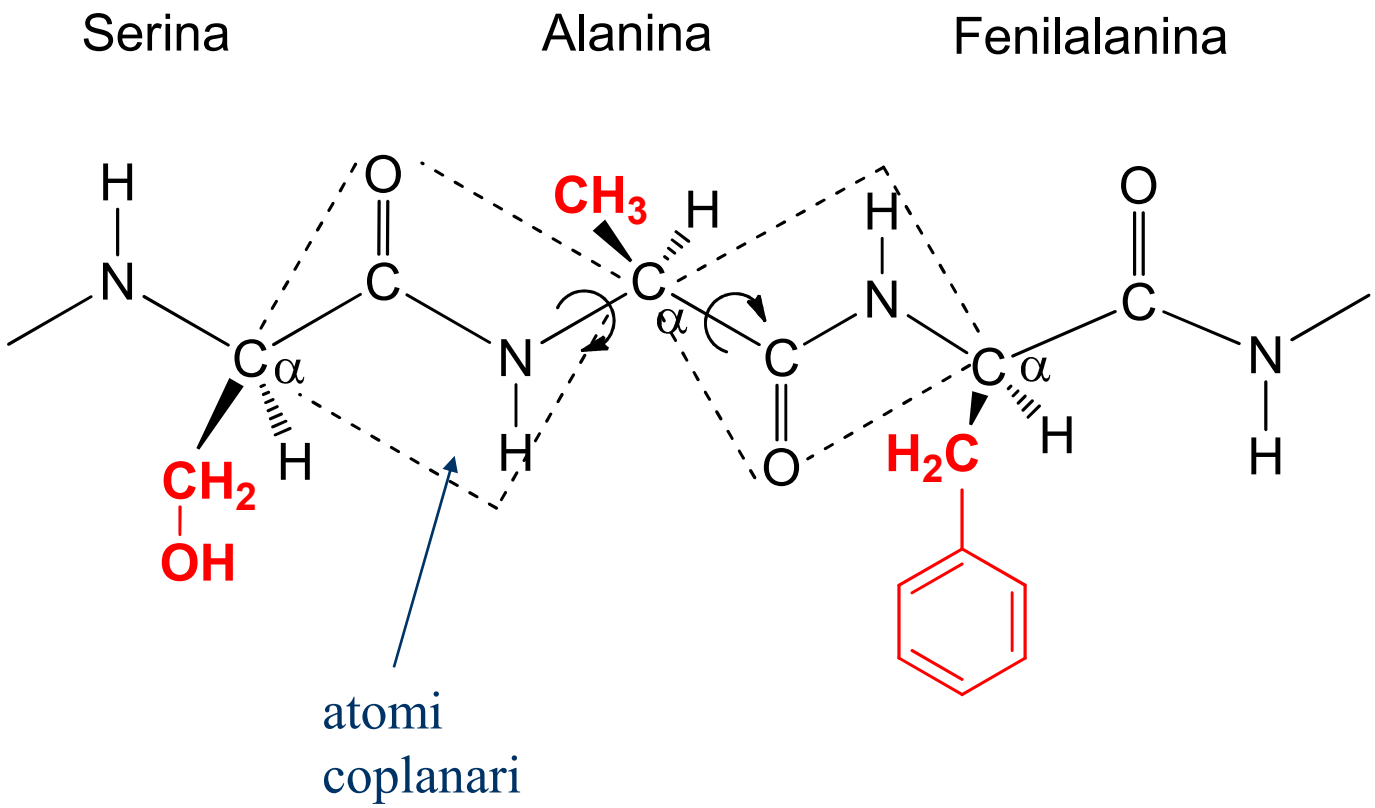


ibrido di risonanza con caratteristiche di un dipolo

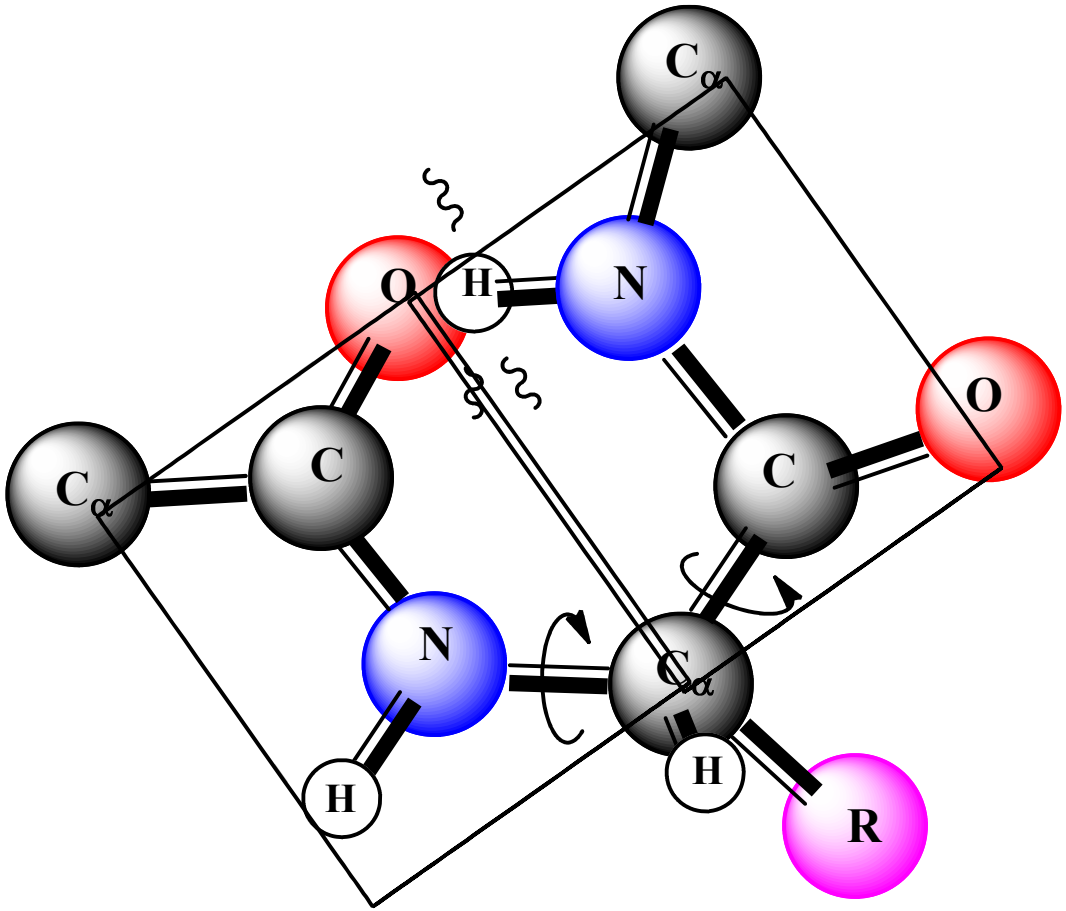
Il legame peptidico è più corto di un legame semplice: confronta C-N con C_α-N

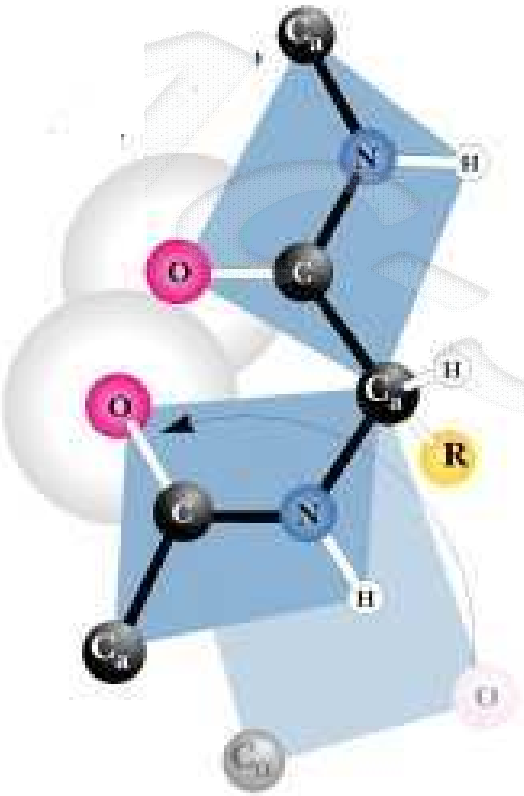


Il legame peptidico

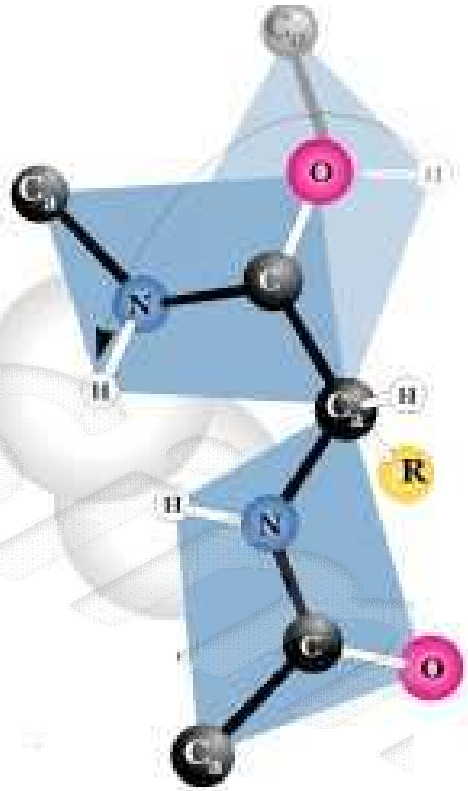


Alcune disposizioni spaziali dei piani peptidici non sono consentite

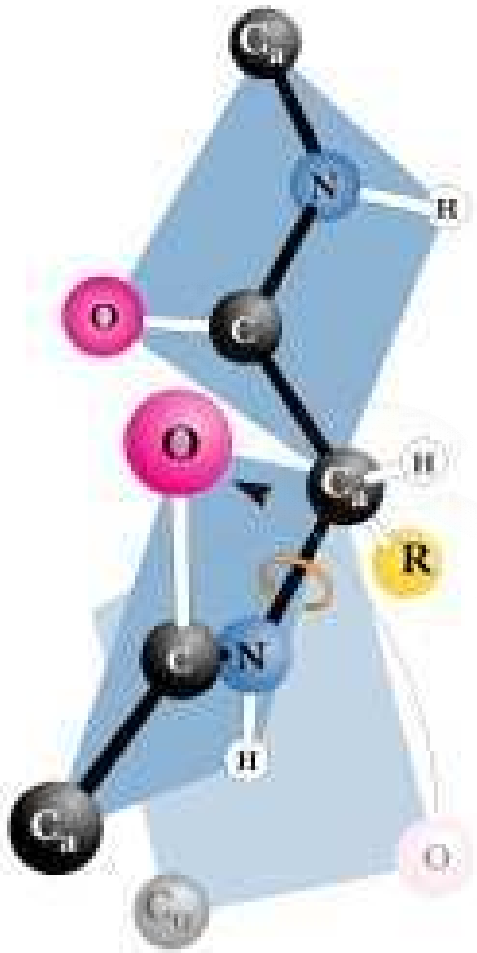




$\phi = 0 \quad \psi = 180$

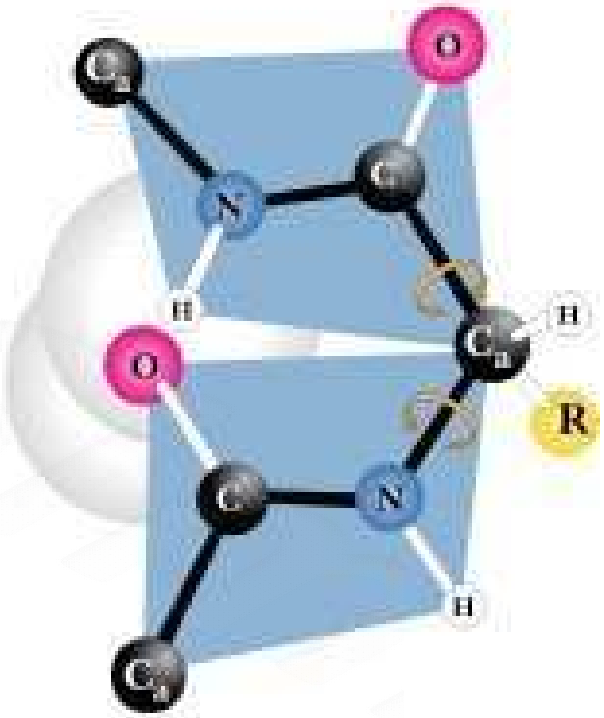


$\phi = 180 \quad \psi = 0$



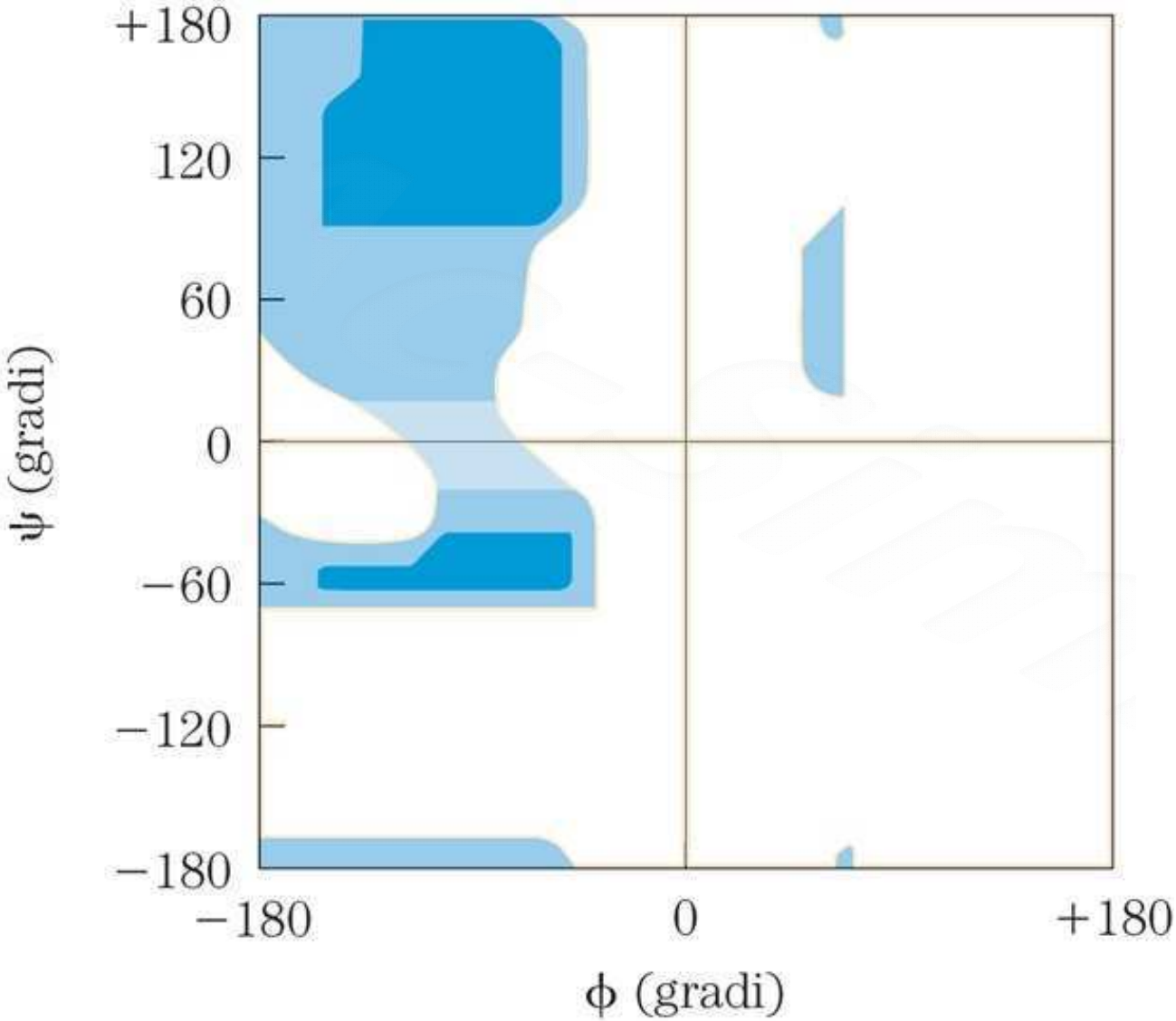
$\phi = -60 \quad \psi = 180$

Un'ulteriore rotazione di ϕ di 120 sposta il più lontano possibile dalla catena laterale l'ingombrante gruppo carbonilico

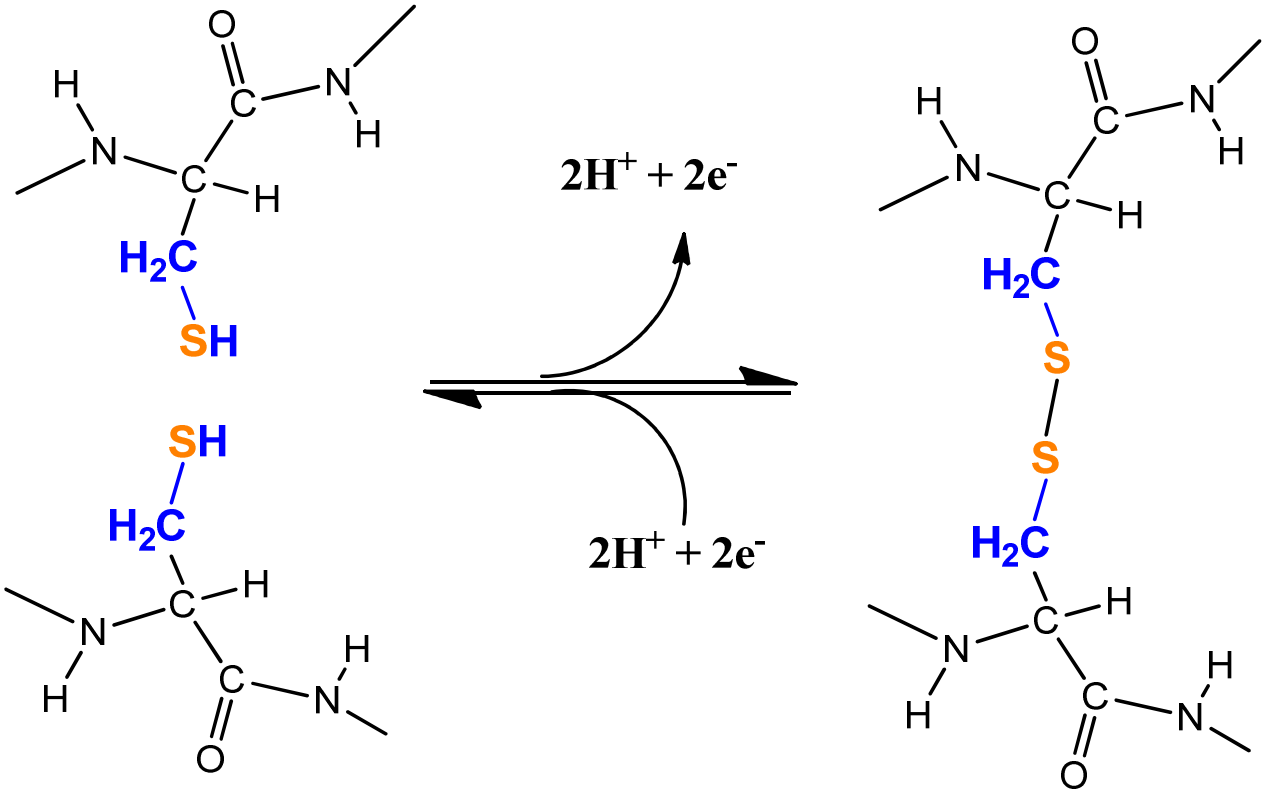


$\phi = 0 \quad \psi = 0$

Grafico di Ramachandran



Formazione di un ponte disolfuro



Due cisteine affiancate
intracatena o intercatena

ponte disolfuro

La struttura delle proteine

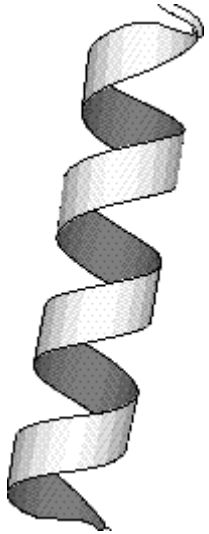
- Ogni proteina ha una struttura tridimensionale (**nativa**) funzionale a un preciso ruolo biochimico e biologico

- La struttura dipende dalla composizione e dalla sequenza aminoacidica

- La **struttura nativa** è stabilizzata da numerose interazioni deboli: legami idrogeno (proteine fibrose); legami idrogeno, interazioni idrofobiche, interazioni ioniche e più raramente da ponti disolfuro (proteine globulari). I ponti disolfuro possono essere presenti anche tra catene polipeptidiche sia di proteine fibrose sia globulari.

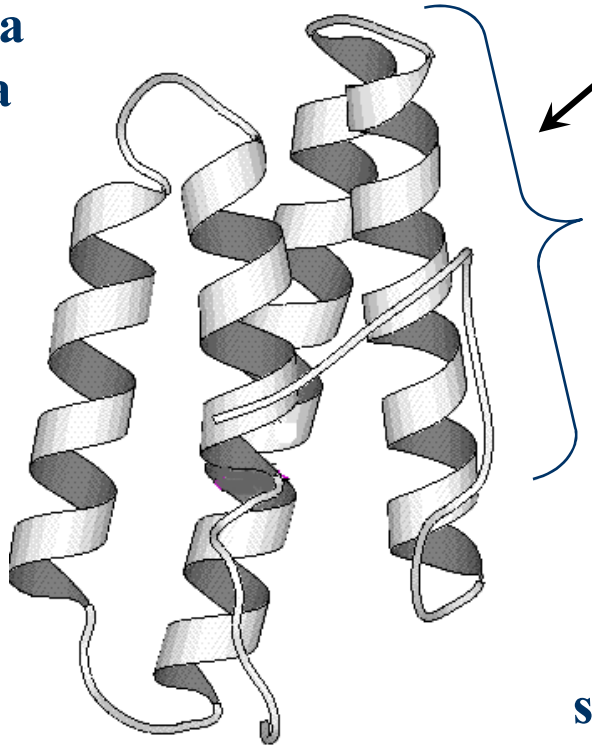
LIVELLI ORGANIZZATIVI DELLE PROTEINE

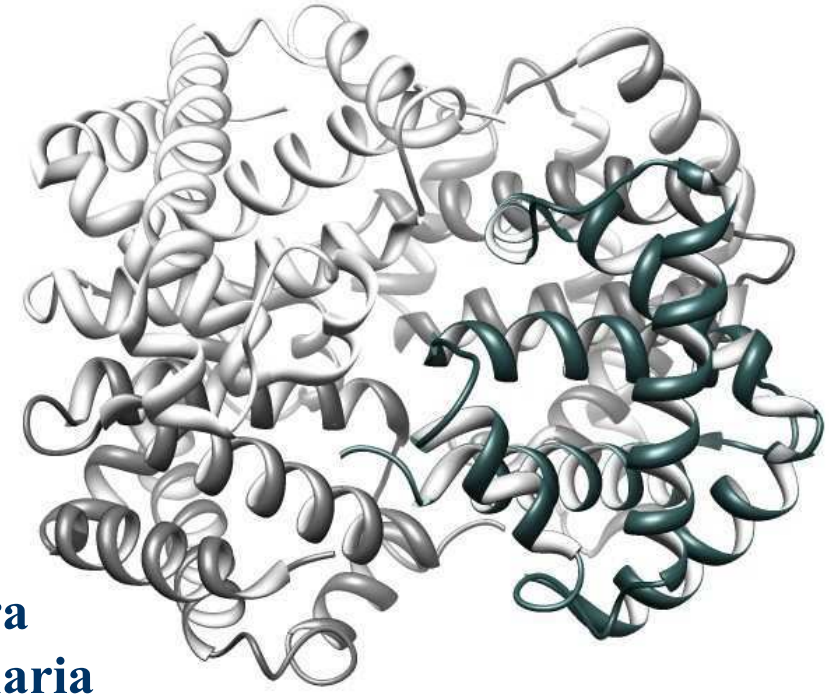
struttura primaria
Asp-Ala-Met-Phe-Gly-Gly-Pro-.....



struttura secondaria

struttura terziaria





struttura quaternaria

Esempio di struttura primaria *

```
MIPFLLMVAF ADTVLQVSAQ SQPSCDSKVY CQGKLLHVVE MSRIFNDSKT 50
FVELKMINDE QTTLENFDNF LRDTNHKRTR ADLMKFVSDN FKQENEFESW 100
TPTDFTDNPT LLSRIEDKTI RQFAQDLVKI WPTLARKVKK EVLDYPEHYS 150
LLPVDNGFII PGGRFTEFYY WDSYWIVEGL LLSDMHETVR GMLDNFLSIV 200
EKYGFIPNGA RVFYLNRSQP PLLTLMVSLY VSATNDMEWL AKNIRTIDTE 250
LRFWLNNRLV DVVKDGIVYK LAQYNSNSGS PRPESYYEDV TTASVFSDER 300
DKAELYMDLK SAAESGWDFS SRWIVDEYGG TRGNLSALHT RRIIPVDLNA 350
FLCQAFQKLS EFYQTLGDYP NATFWSKLVK IWQHSIEMVH YNRDDGIWYD 400
WDNELSQHRR MFFPSNFAPL WSETFDSRNA EILGEMAAEY FITQNMMDYH 450
GGIPTSLSHT GEQWDYPNAW PPMQSIIVMG LDKSGSYRAK QLARELARRW 500
VKANLIGFRQ TGEMFEKYNV EVPGQNGGGG EYVVQSGFGW TNGVVLEFIN 550
QFFTT    555 aa
```

*** Le sequenze non vanno imparate a memoria, ma sono depositate in banche dati proteiche**

banche dati

Esempio: sequenza parziale nota
WDSYWIVEGLLLSDMHETVR

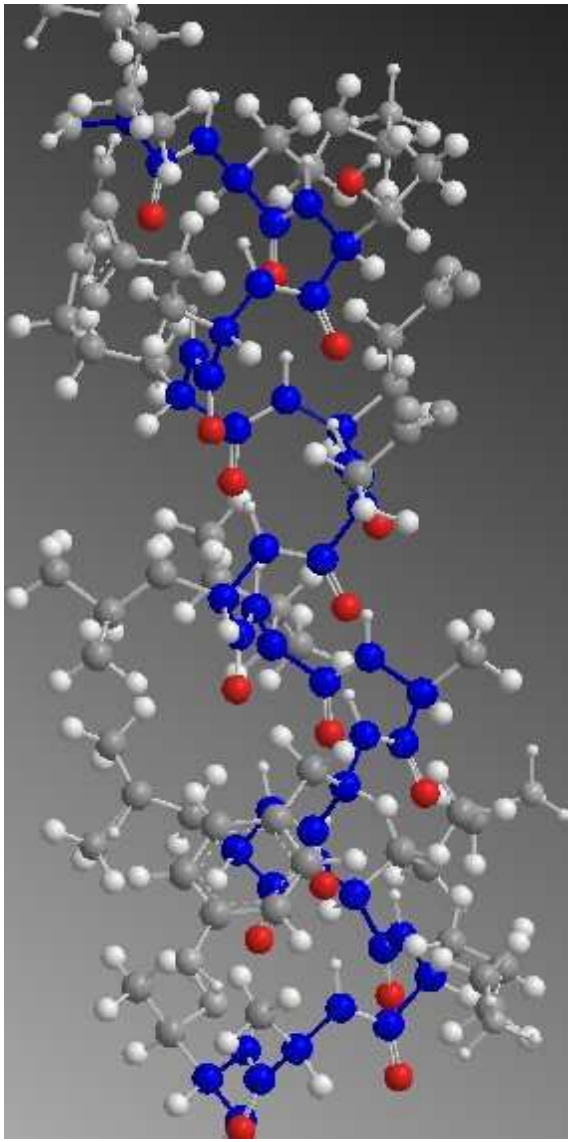
⇩

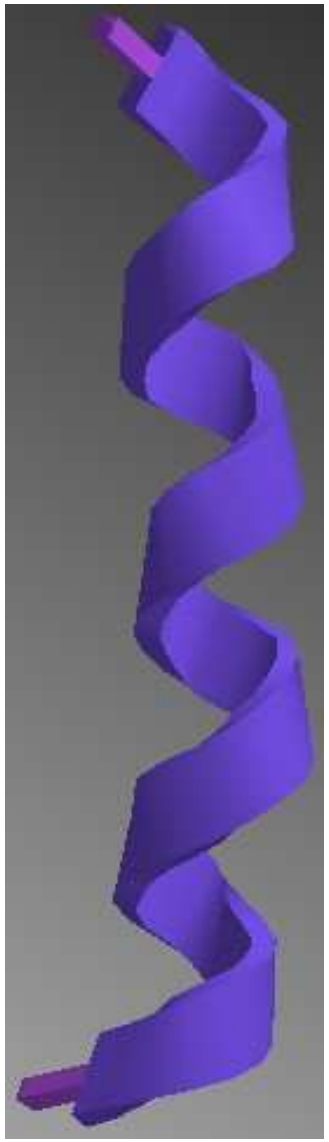
ricerca sequenze (internet)

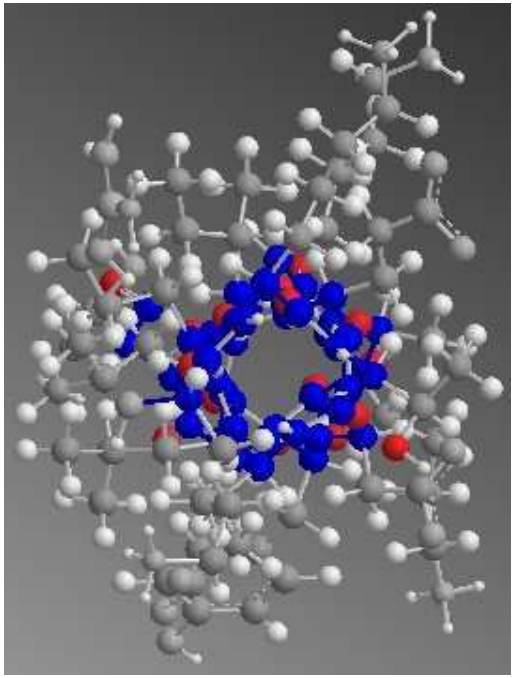
⇩

Identificazione della proteina

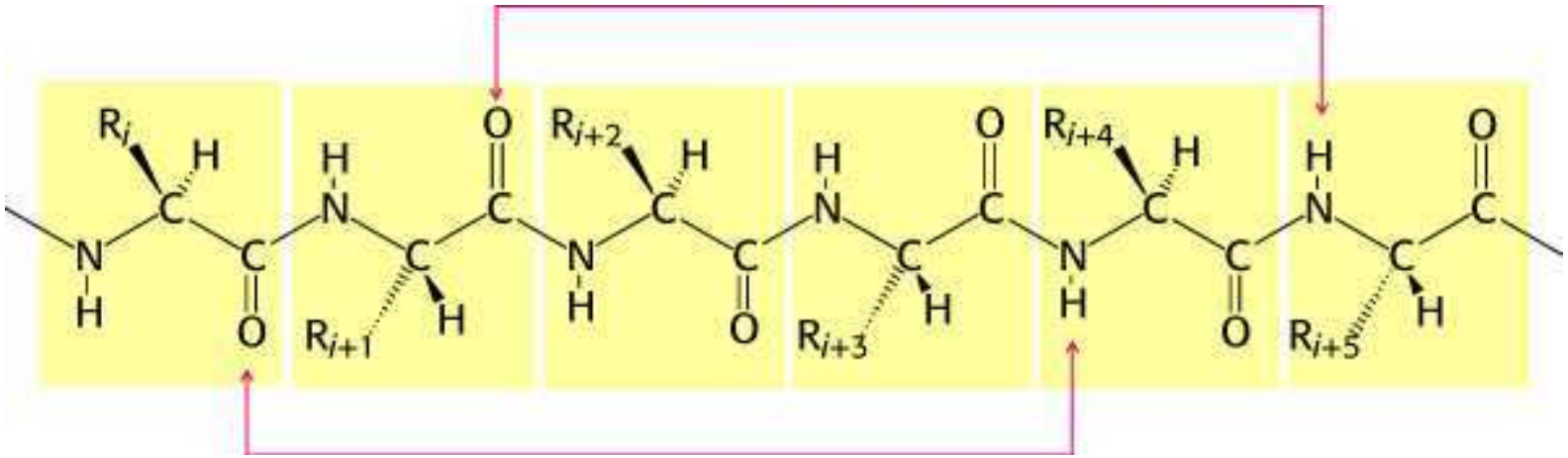
Struttura secondaria. L'α-elica

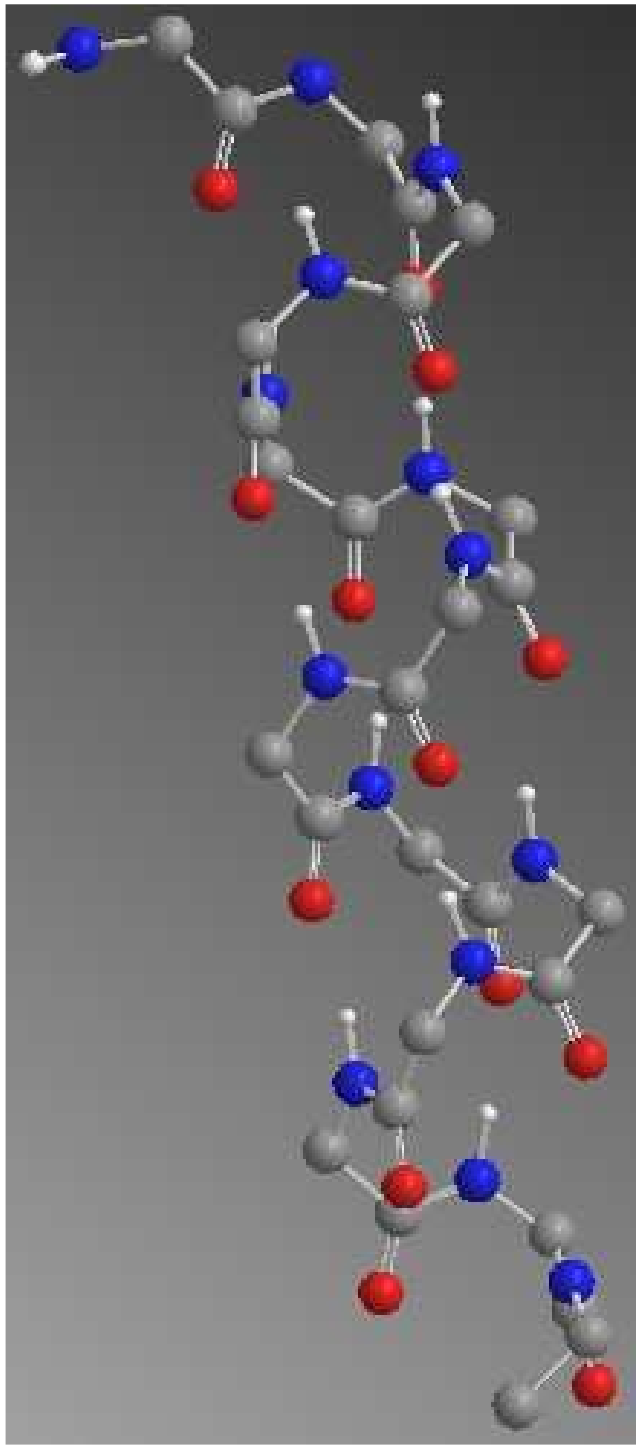


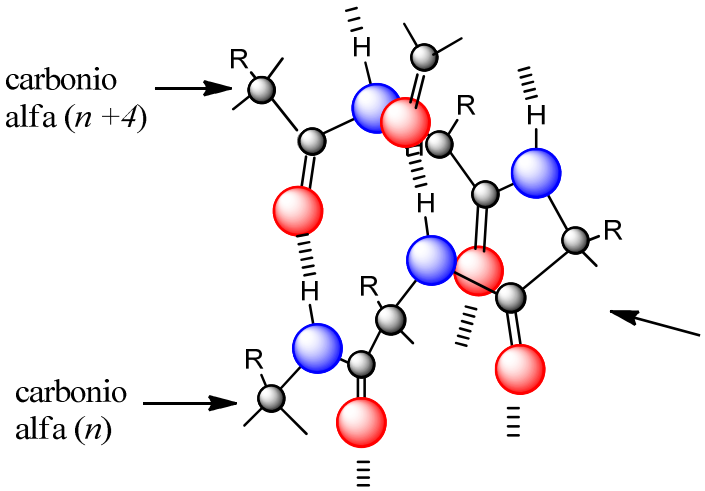




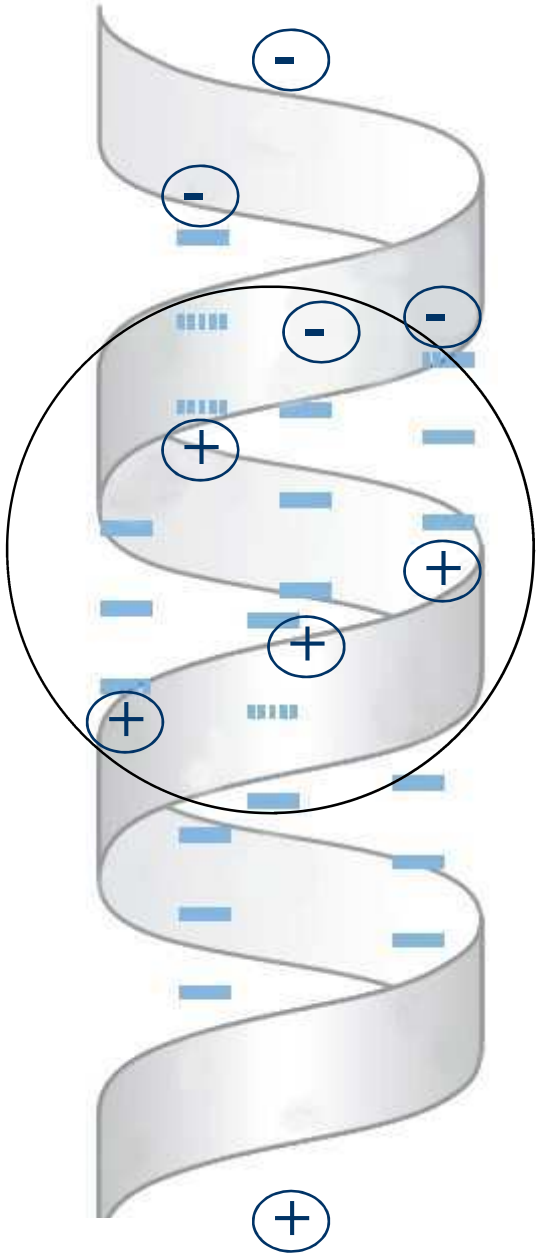
L'α-elica è stabilizzata solo da ponti idrogeno tra l'ossigeno carbonilico di un aminoacido e l'idrogeno amidico del quarto aminoacido successivo



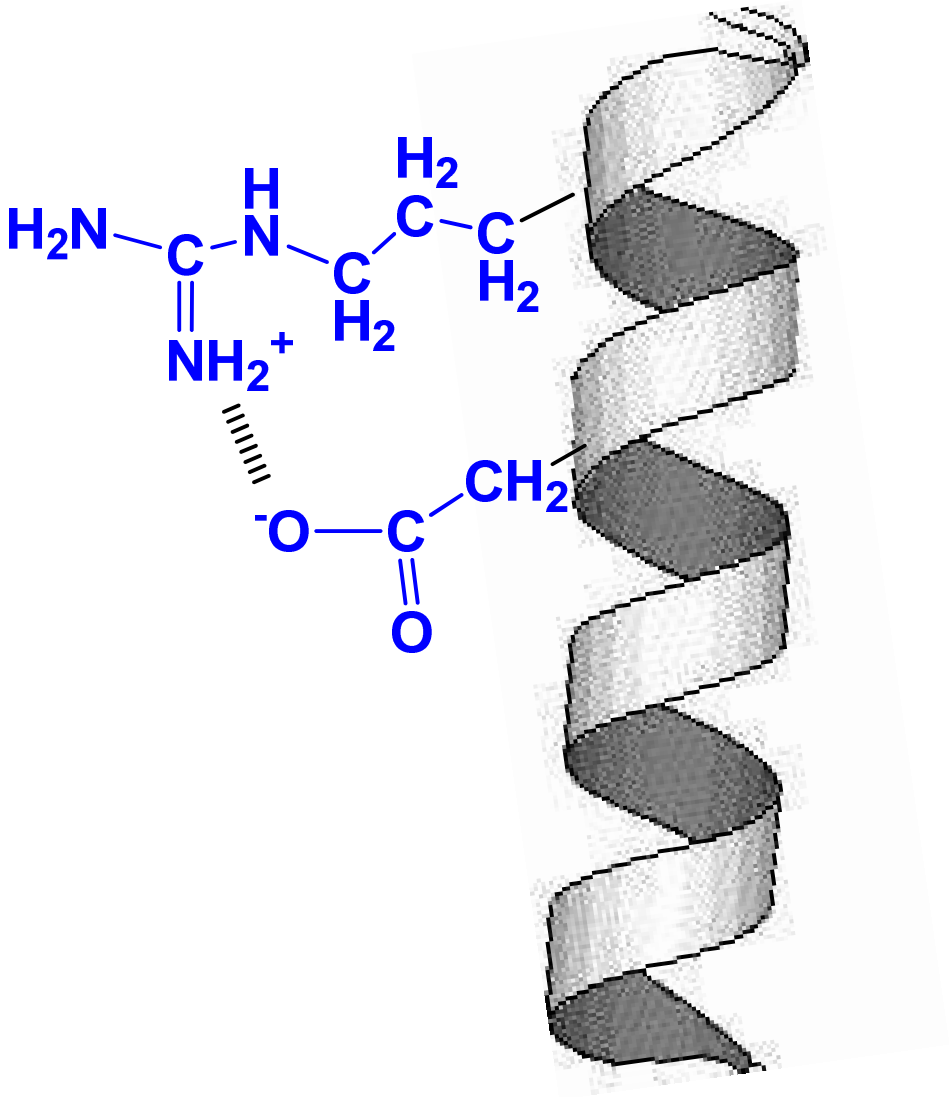




distanza del
ponte idrogeno = 13 atomi

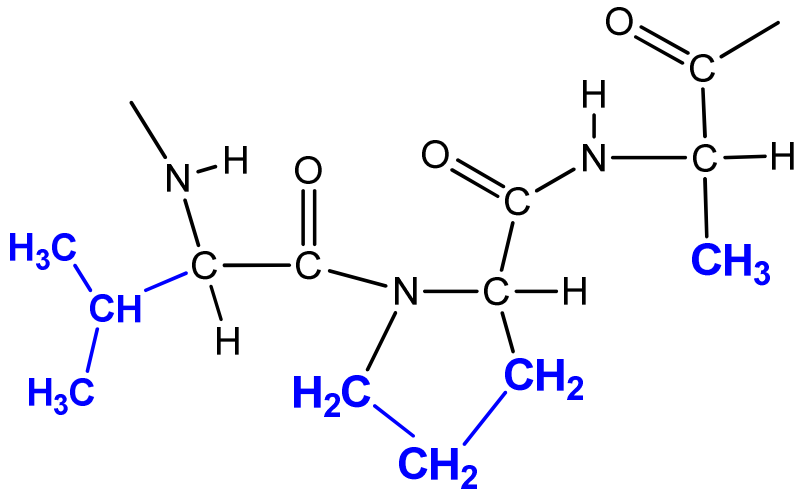


Possibili interazioni tra gruppi R nell'alfa elica



Fattori che destabilizzano l'alfa elica

- vicinanza di aminoacidi con la stessa carica o con gruppo R ingombrante
- presenza all'estremità dell'elica di aminoacidi con la stessa carica di quella dell'estremità
- presenza di prolina



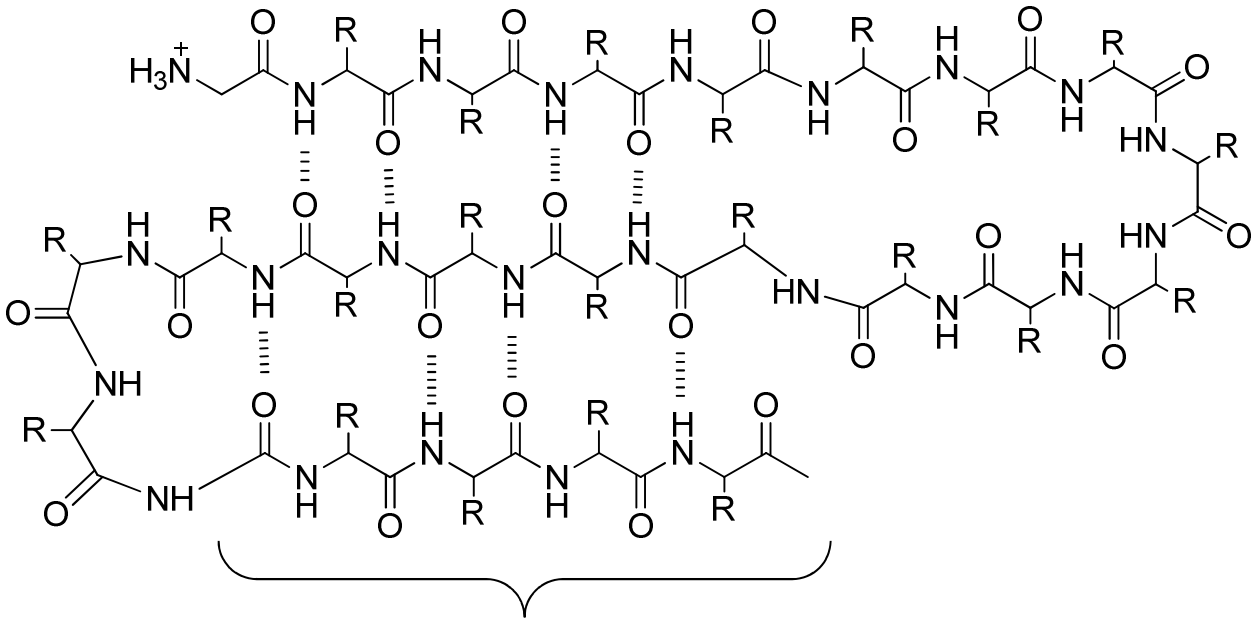
Prolina

1. manca l'idrogeno sull' azoto per il legame con l'ossigeno carbonilico
2. il gruppo R è legato all'azoto impedendo la rotazione del legame N-C_α

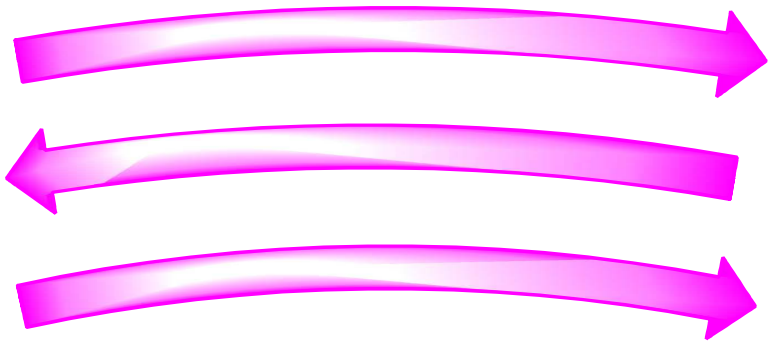
Altre conformazioni periodiche

- foglietto beta
- poliprolina: 3 AA per giro
- elica 3_{10} : legame H (giro) ogni 3 AA (10 atomi)
- elica π : legame H ogni 5 AA (16 atomi)

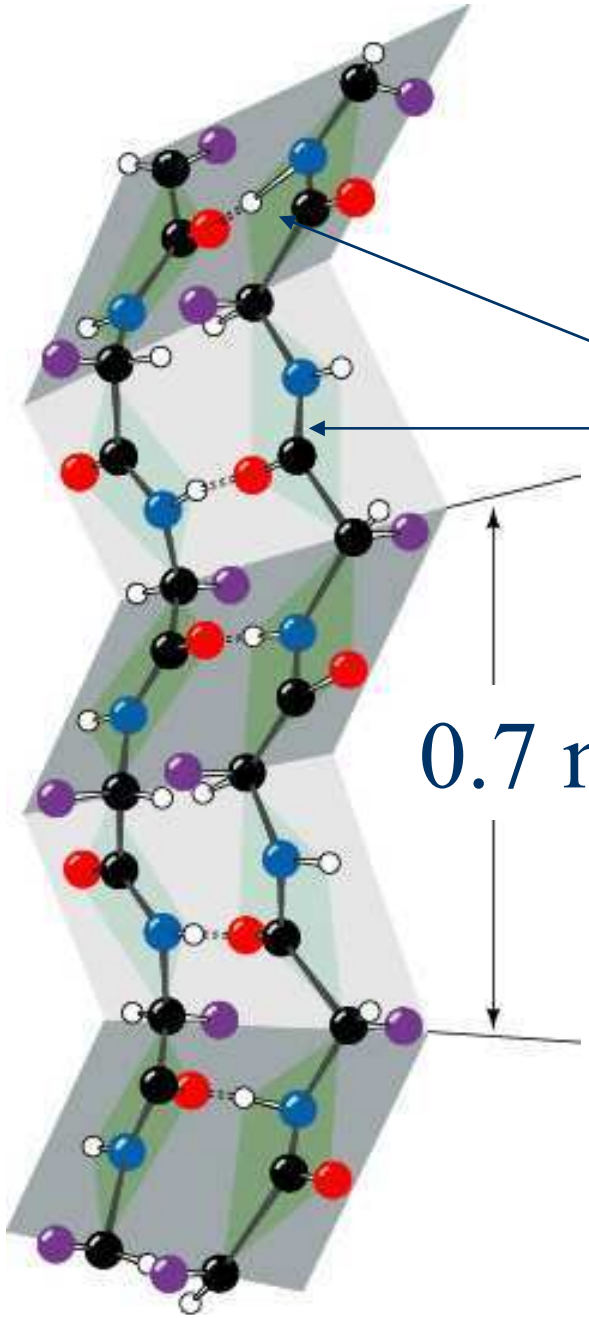
Il foglietto beta

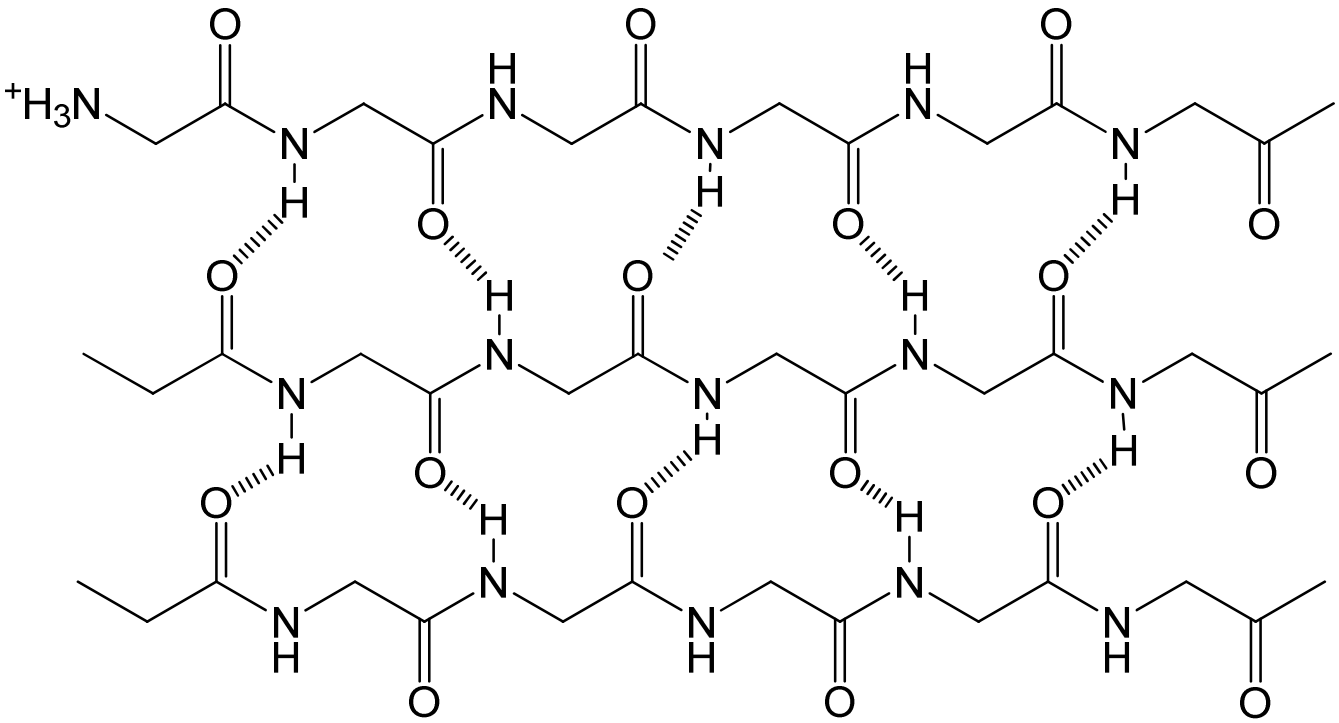


Foglietto beta antiparallelo

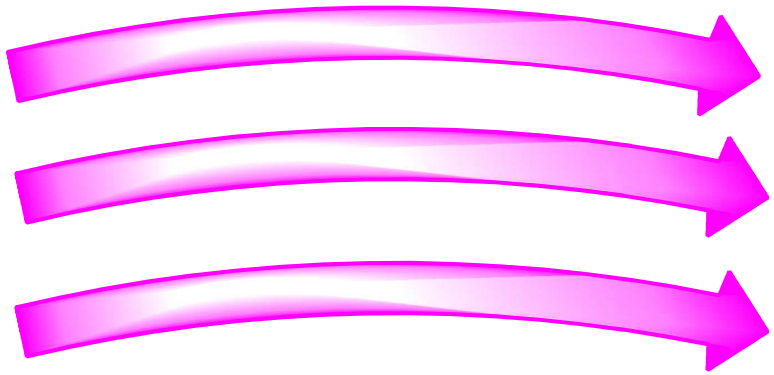


Il foglietto beta





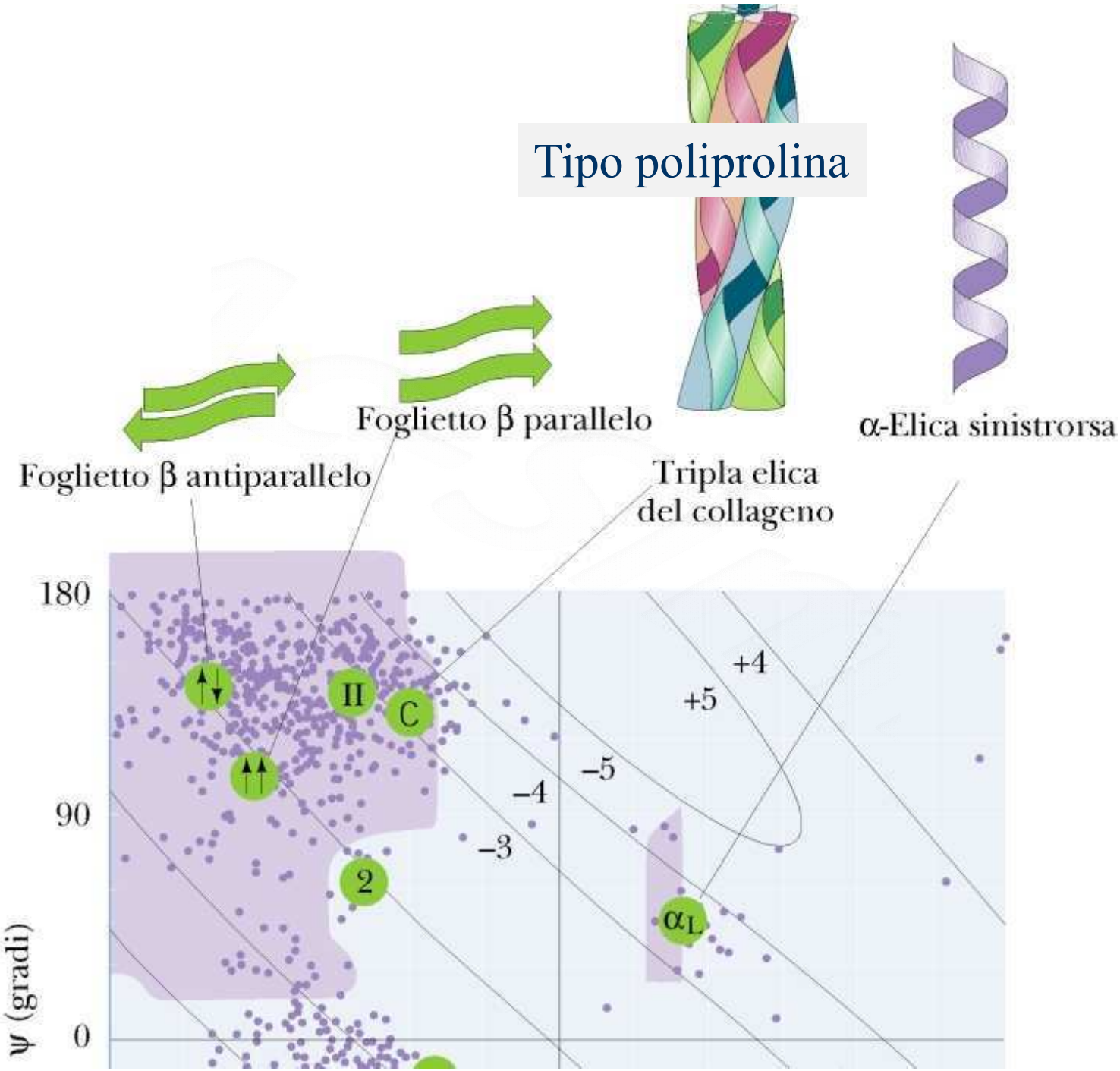
Foglietto beta parallelo

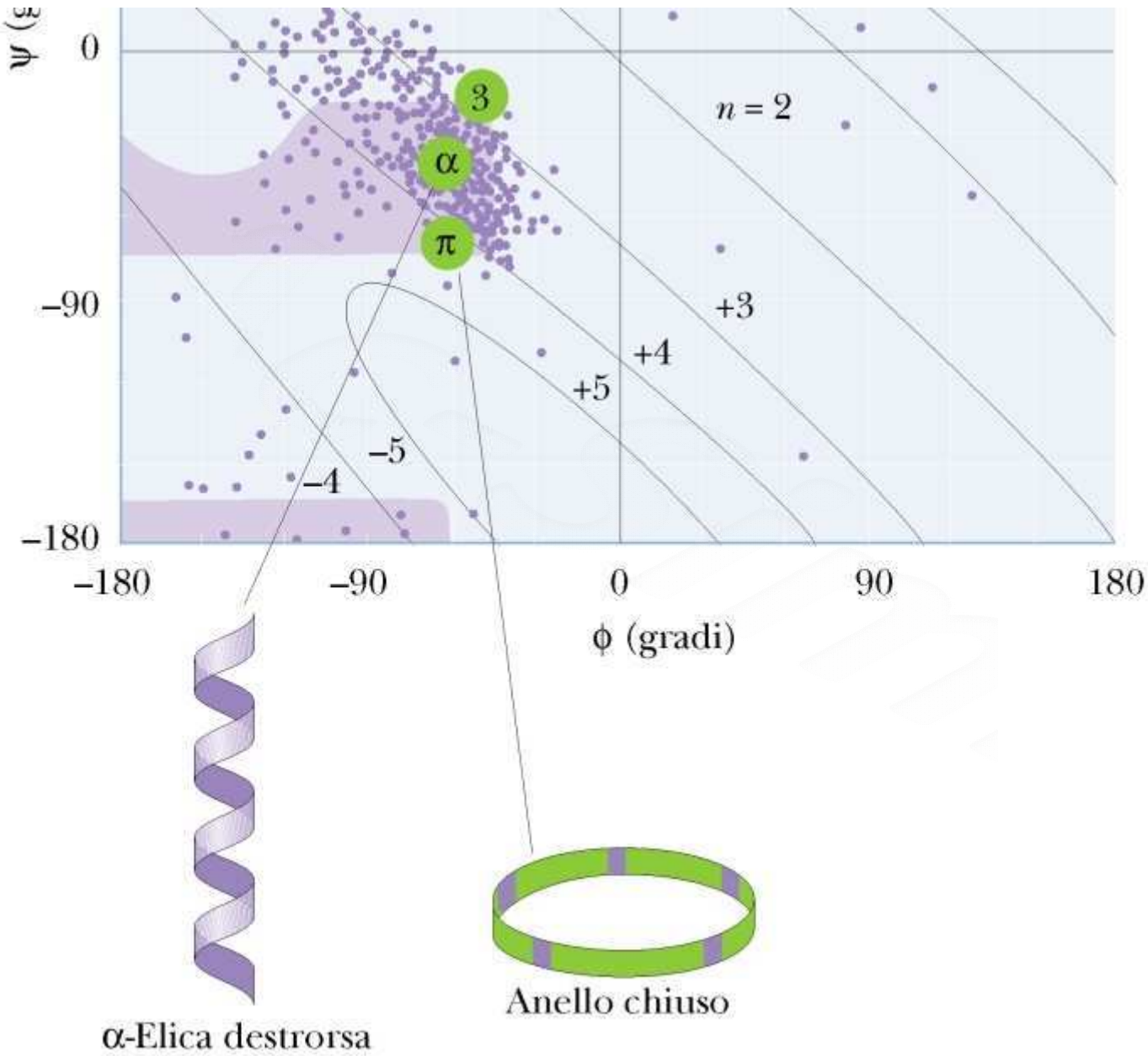


Curiosità

- Il bozzolo del baco da seta è filato in 3 giorni ed è costituito un solo filamento lungo da 300 a 900 metri (30-100 mg).

- La seta del ragno è molto elastica: può allungarsi fino a 20 volte prima di spezzarsi (acciaio solo 8%)


Tipo poliprolina
Foglietto β parallelo
α-Elica sinistrorsa
Foglietto β antiparallelo
Tripla elica
del collageno
180
90
0
ψ (gradi)
II
C
2
αL
+4
+5
–5
–4
–3


ψ (gradi)
0
−90
−180
−180
−90
0
90
180
ϕ (gradi)
3
α
π
n = 2
+3
+4
+5
−5
−4
α-Elica destrorsa
Anello chiuso

La struttura terziaria delle proteine

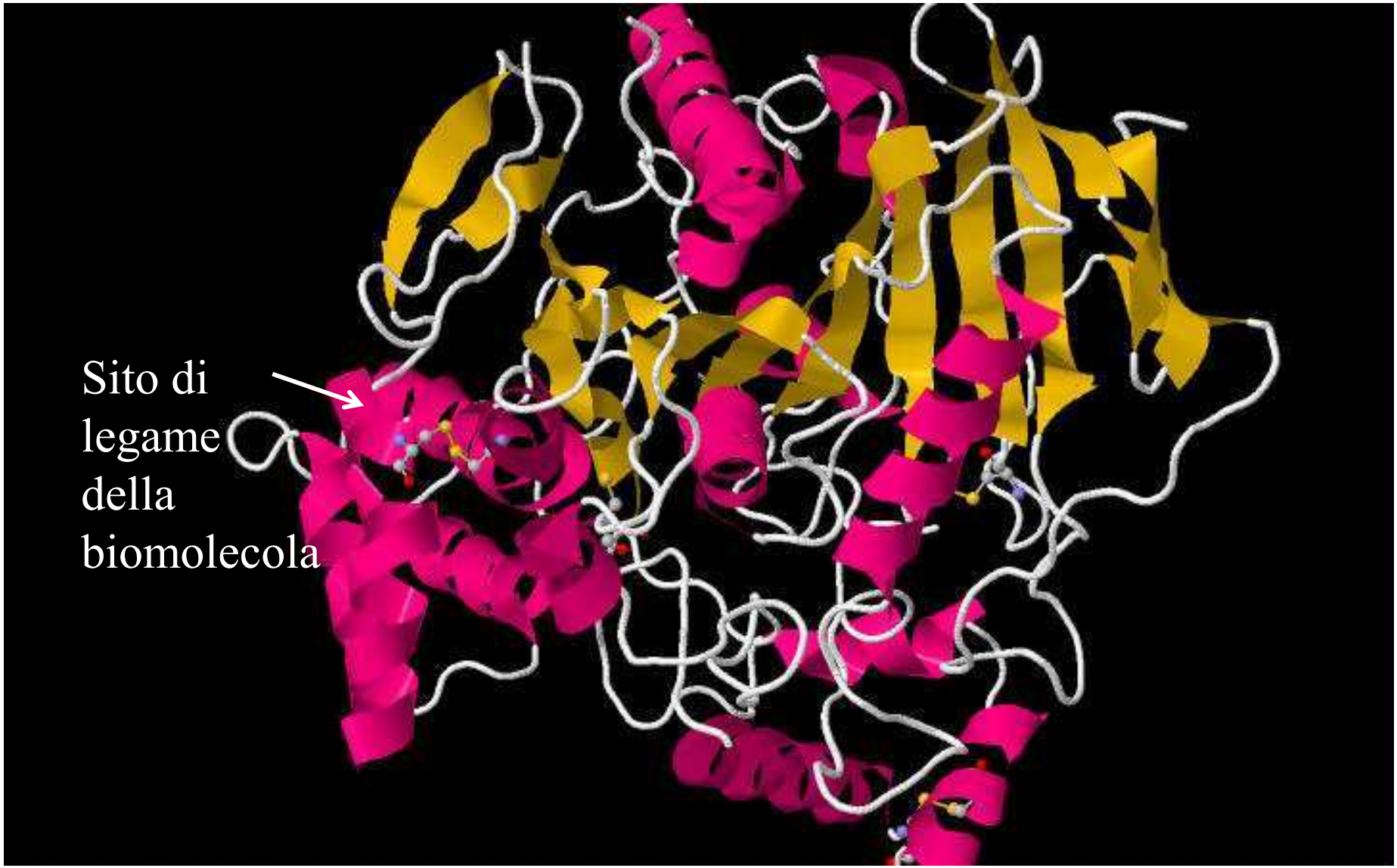
Ingombro di una proteina di 64.000 Da (580 aa)

struttura beta = 200 x 0.5 nm

alfa elica = 90 x 1.1 nm

terziaria globulare
= 10 x 6 nm

La struttura terziaria delle proteine

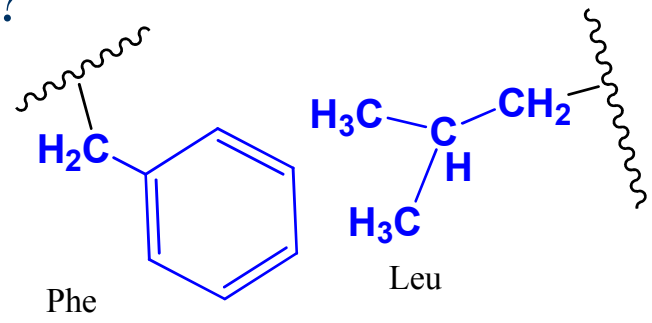


La struttura tridimensionale di tipo terziario contiene quasi sempre tratti (in genere 8-20 aminoacidi) in struttura regolare (secondaria) separati da segmenti cosiddetti in struttura *random*. Va tenuto presente, tuttavia, che la disposizione dei segmenti *random* non è per nulla casuale.

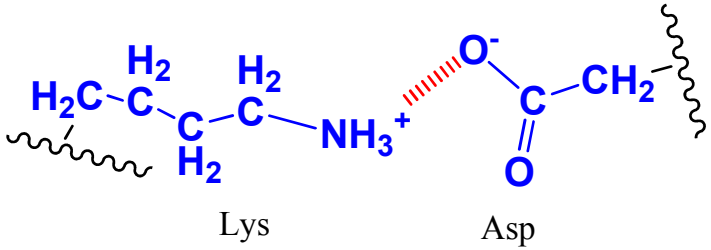
I ponti disolfuro sono in genere presenti in proteine che esplicano la loro funzione fuori dalla cellula (es. anticorpi, proteasi)

Quali sono le interazioni che mantengono una struttura terziaria in una precisa configurazione spaziale?

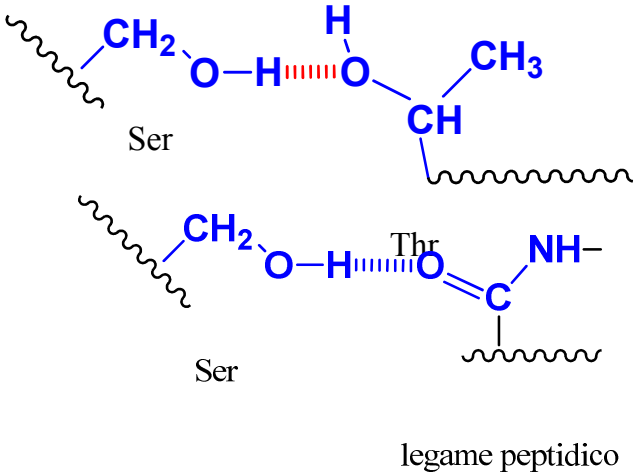
1. Interazioni idrofobiche
   - interno



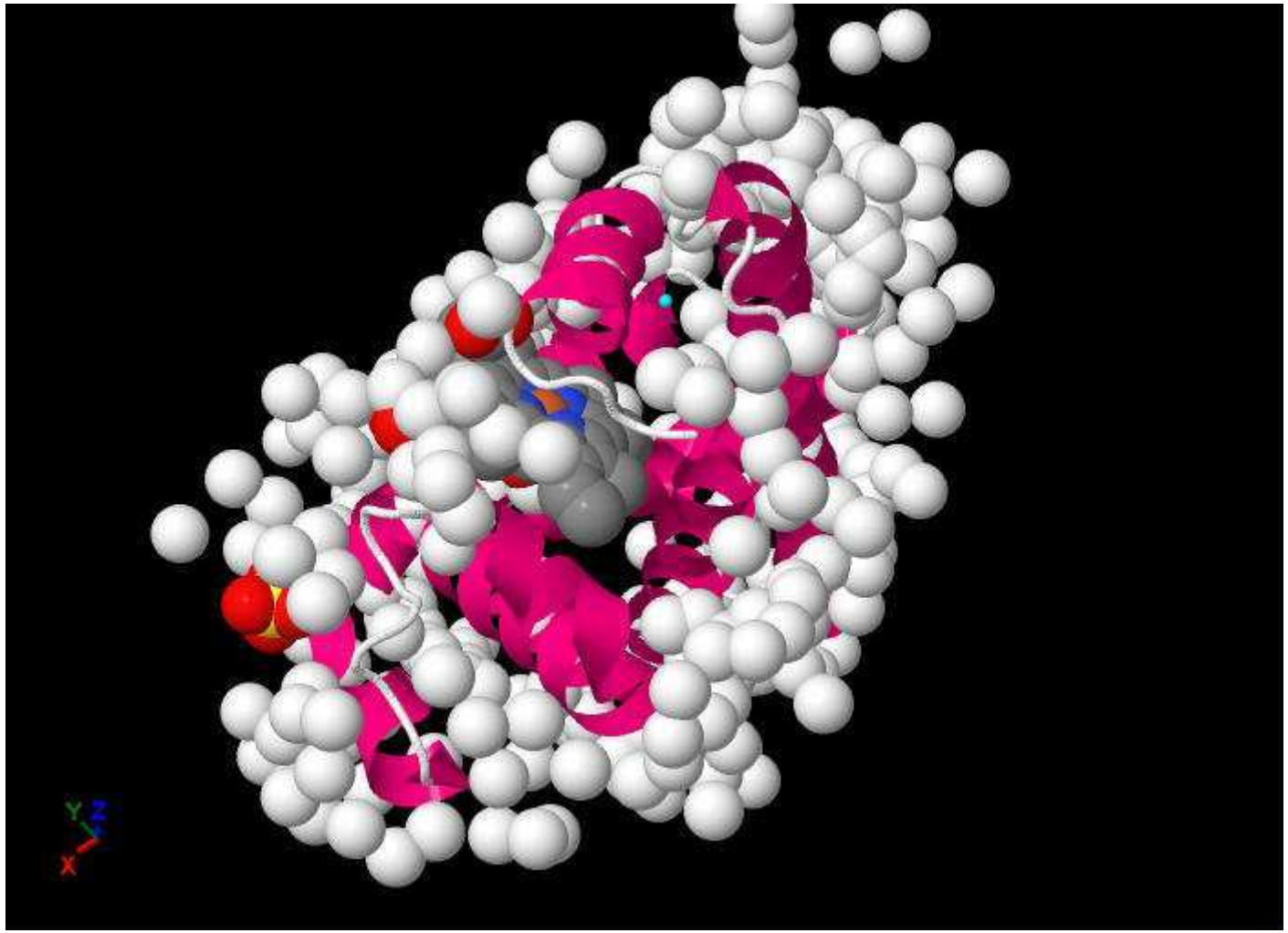
2. Interazioni ioniche
   - interno e superficie



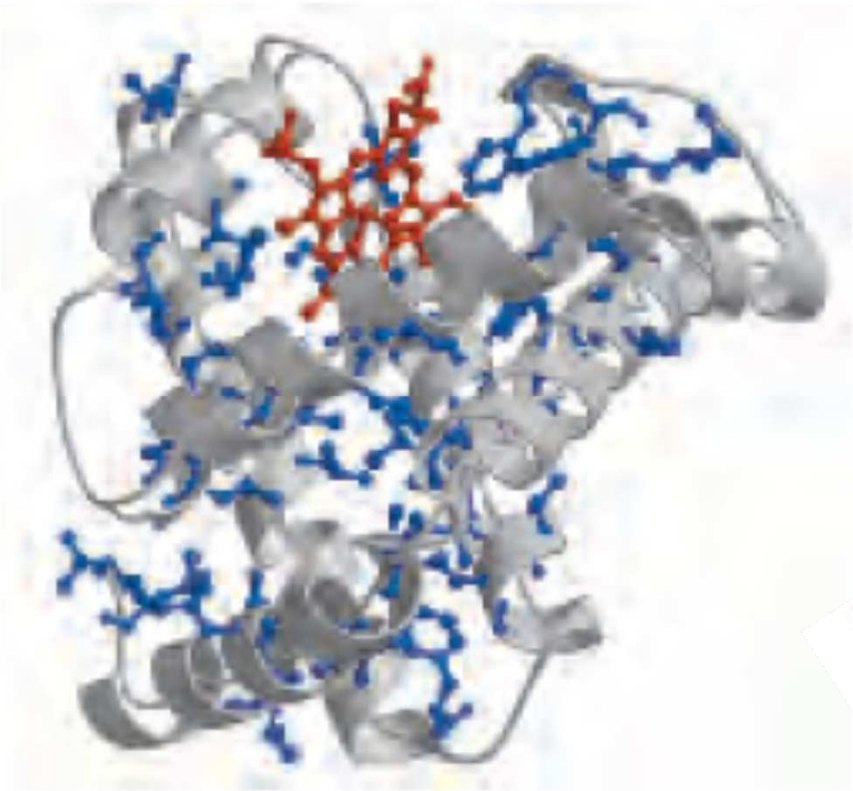
3. Legami idrogeno
   - interno e superficie
   molti con il solvente

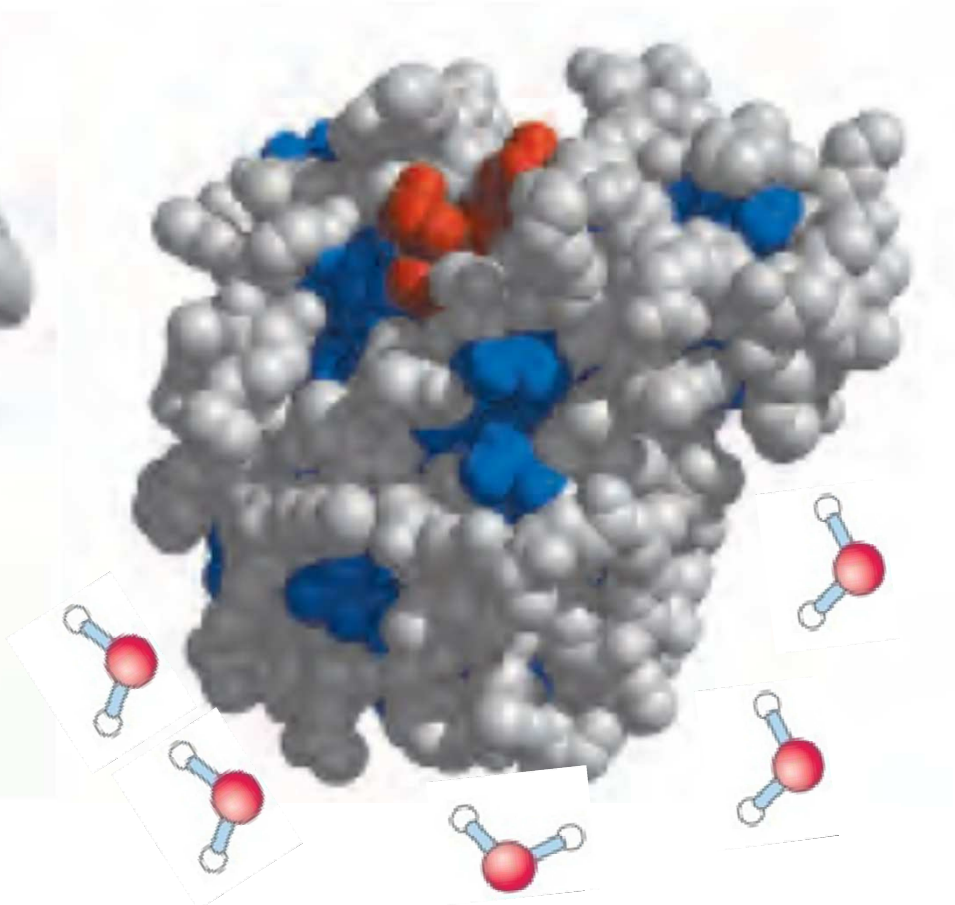


velo d’acqua intorno alle proteine

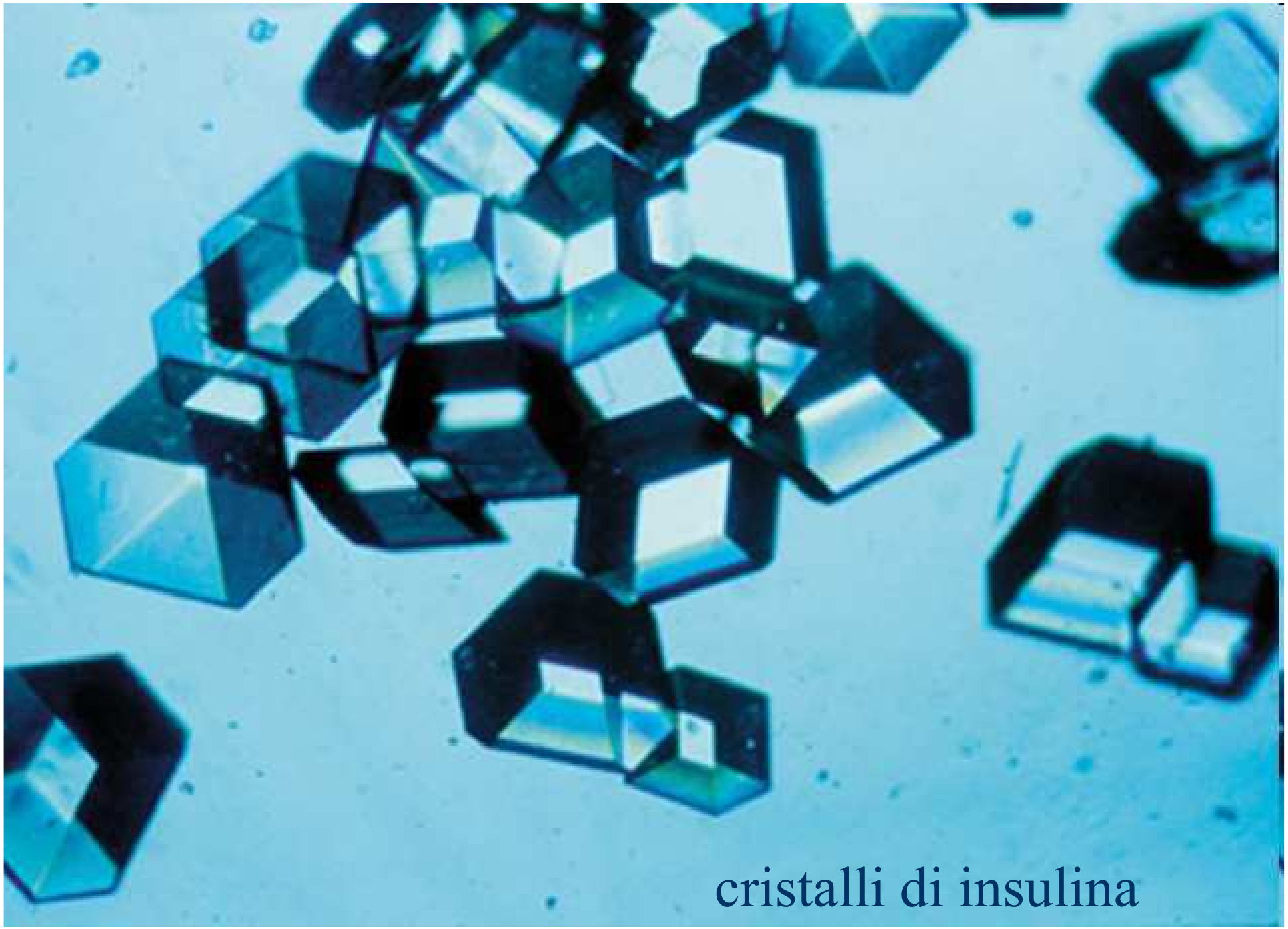


In una struttura terziaria gli aminoacidi idrofobici sono principalmente all'interno





I gruppi polari sono rivolti all'esterno dove c'è acqua



cristalli di insulina

Struttura quaternaria: proteine costituite da più catene polipeptidiche

- proteine fibrose
  - collagene
- proteine globulari
  - proteine di trasporto (emoglobina)
  - proteine di membrana (carrier)
  - enzimi

Complessi sovramolecolari

Complessi multienzimatici (aggregati di enzimi che compiono una serie concatenata di reazioni)

Complessi strutturali filamentosi (microfilamenti e micro-tubuli di actina e tubulina)

Ribosomi

Virus

Il collagene

1. **Proteina animale più abbondante (25-35% delle proteine totali di un organismo superiore)**

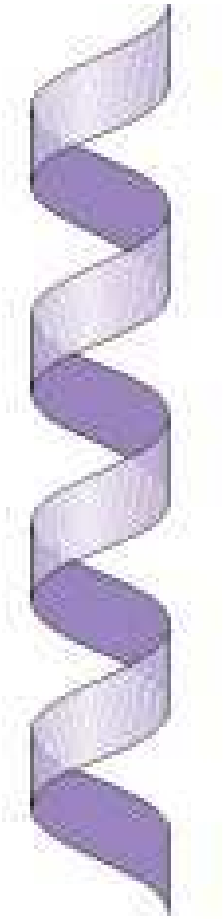
2. **L'unità fondamentale è una triplice elica destrorsa**

3. **Ogni elica ha la struttura di una poliprolina sinistrorsa**

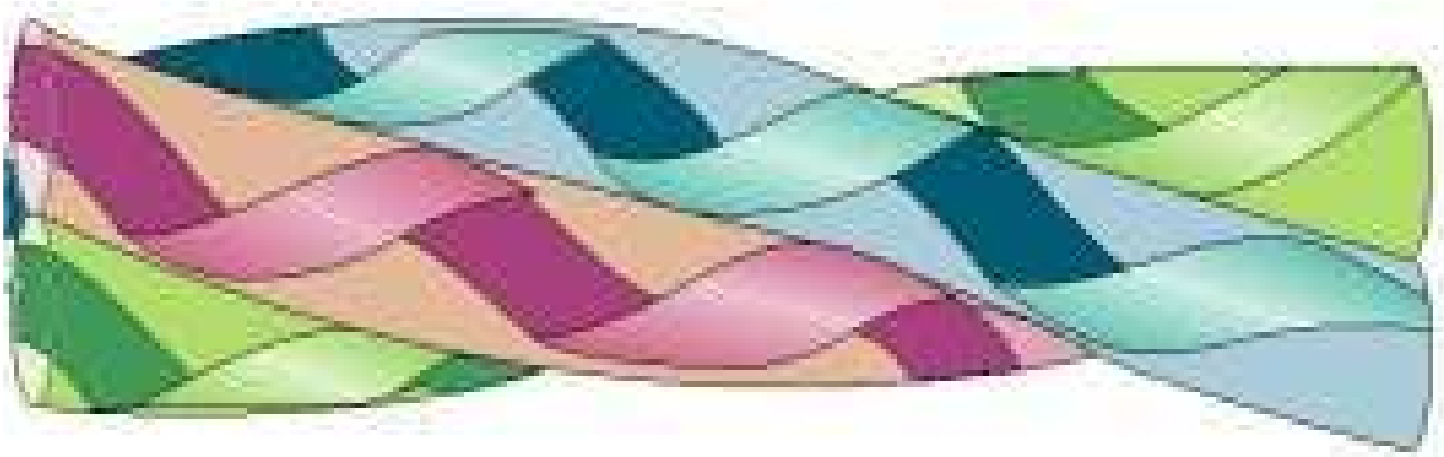
4. **Contiene aminoacidi modificati (idrossiprolina e idrossilisina**

5. **La biosintesi di queste modificazioni richiede acido ascorbico (vitamina C)**

Elica
Sinistrorsa



destrorso
verso l'alto
da sinistra a destra



Struttura primaria del collagene.

-Gly-Pro-Arg-Gly-Pro-Hyp-Gly-Ser-Ala-

-Gly-Ser-Hyp-Gly-Lys-Asp-Gly-Leu-Asn-

-Gly-Leu-Hyp-Gly-Pro-Ile-Gly-Hyp*-Hyp-

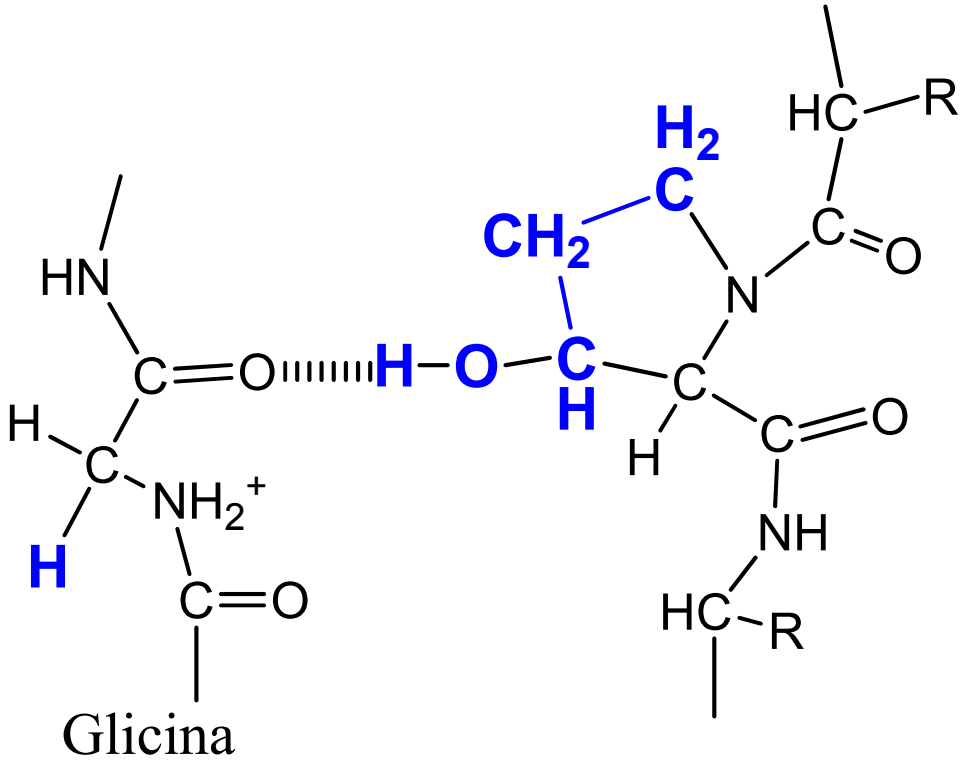
-Gly-Pro-Arg-Gly-Arg-Thr-Gly-Asp-Ala-

-Gly-Pro-Ala-Gly-Pro-Hyp-Gly-Pro-Hyp-

-Gly-Pro-Hyp-Gly-Pro-Hyp-Gly-Pro-Pro-

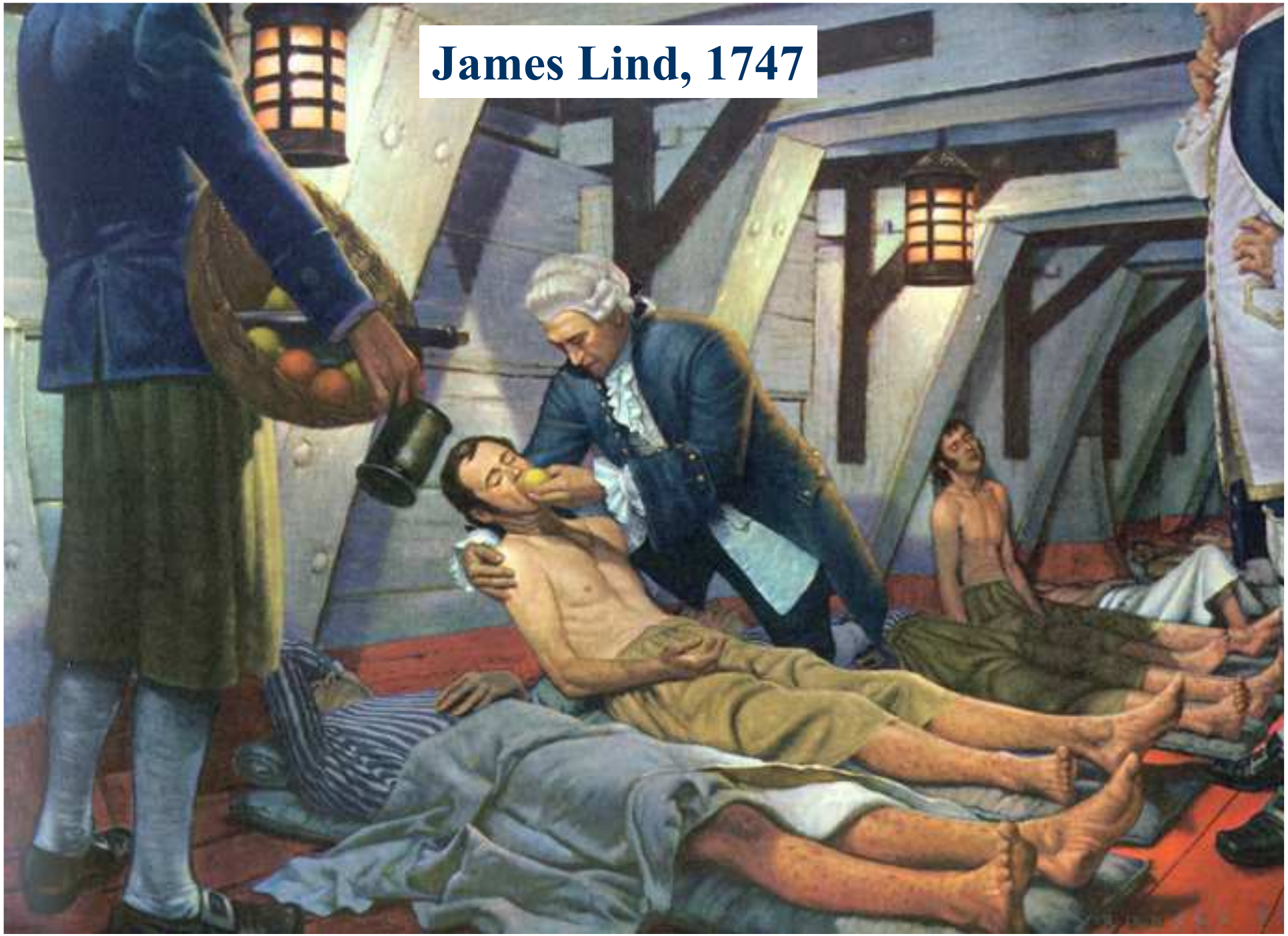
Hyp* 4-idrossiprolina
Hyp 3-idrossiprolina

Ponti idrogeno inter-catena
Nessun ponte idrogeno intra-catena

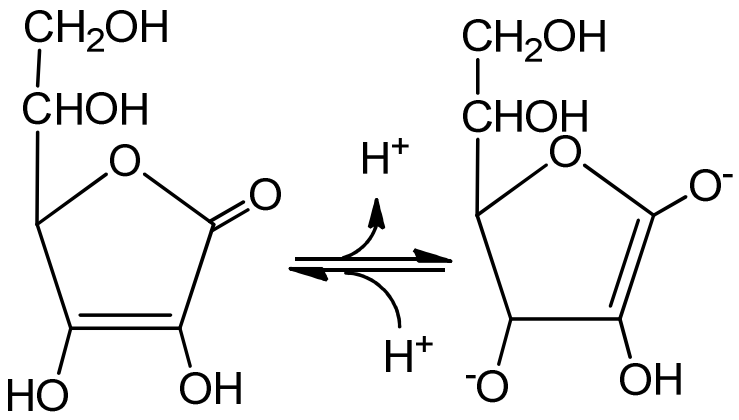


Carenza di vitamina C

Scorbuto


James Lind, 1747

Acido ascorbico

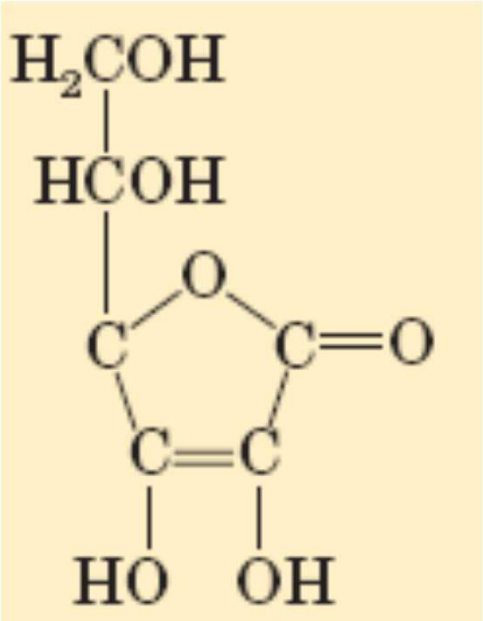


Sintetizzato da glucosio attraverso 8 tappe enzimatiche;
Uomo, scimmie, criceto e alcuni pipistrelli mancano dell'ultimo enzima

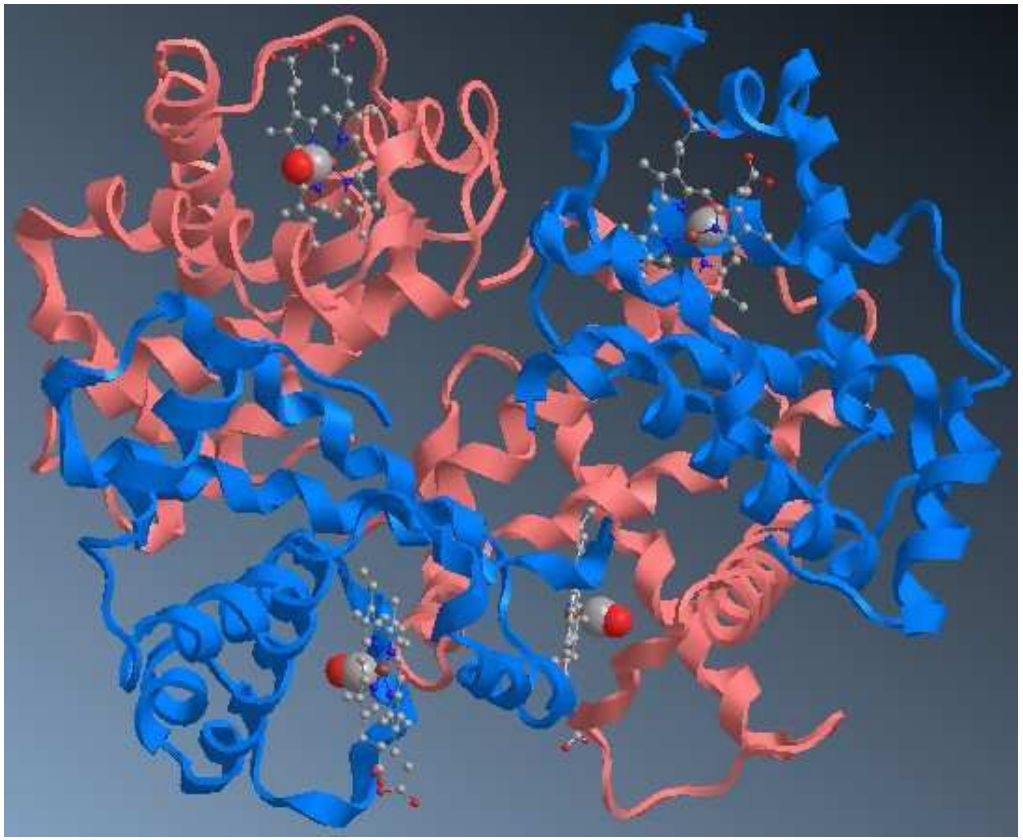
prolil-idrossilasi

PROLINA ⟹ IDROSSIPROLINA

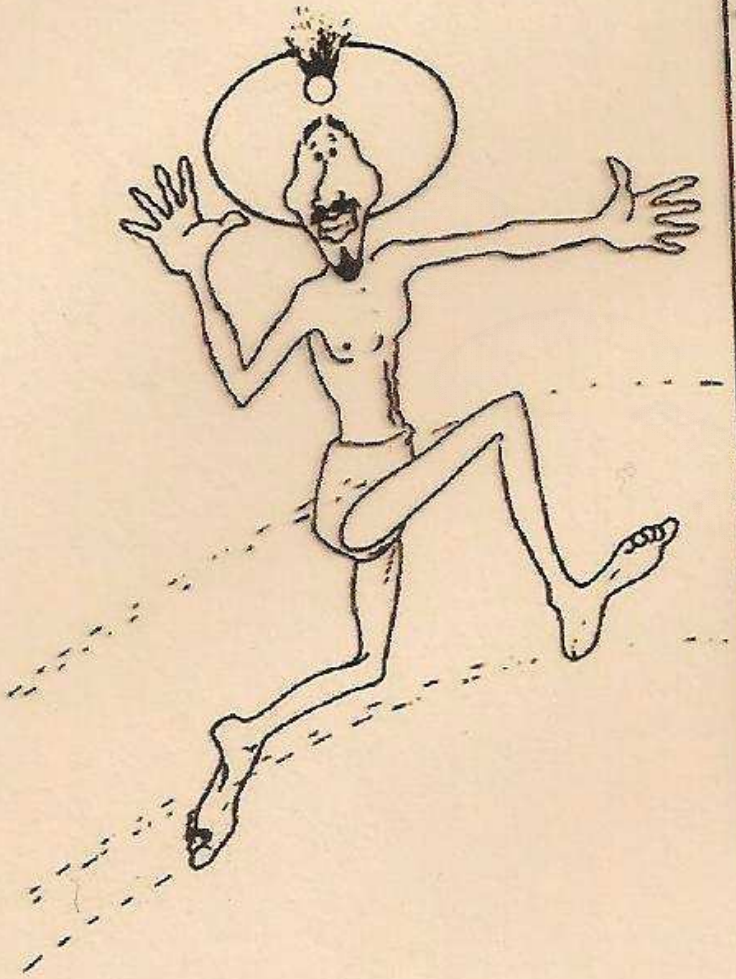
cofattore
VITAMINA C
(acido ascorbico)



Struttura quaternaria: proteine globulari



Questa proteina è un tetramero composto da 4 catene polipeptidiche ognuna delle quali genera una subunità, in questo caso uguali a 2 a 2.



THE
FLEA-PIT
THEATRE

PROUDLY PRESENTS

THE GREAT
PROTEIN-O

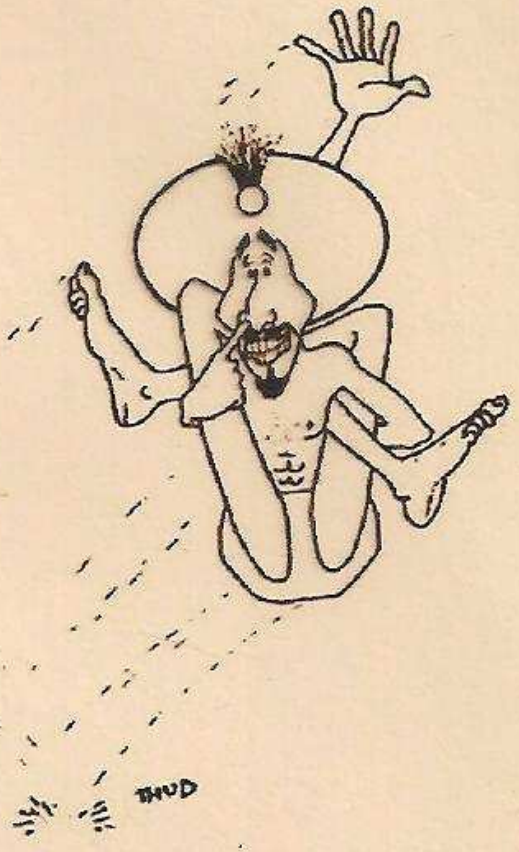
SEE HIS
AMAZING
SPLIT-SECOND
LEAP

FROM "FULLY-EXTENDED"
TO "TIGHTLY-COILED"
(Twice nightly)

"I DON'T KNOW HOW HE DOES IT!" LEVINTHAL, 1966

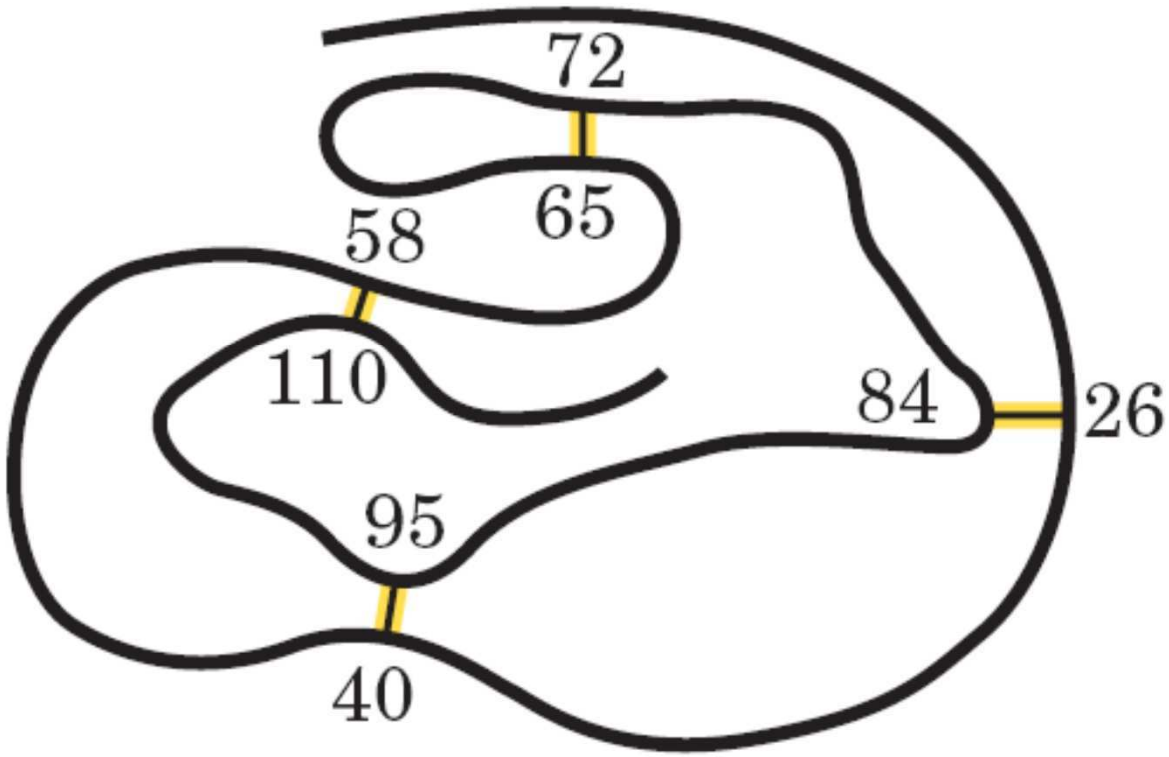
"SPECTACULAR" THE GUARDIAN

PRODUCED BY TAB FOSTER CO. LTD.

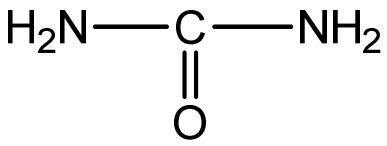


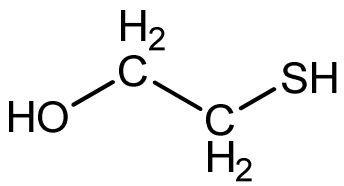
- La struttura tridimensionale (nativa) dipende dalla sequenza aminoacidica ossia dalla struttura primaria

Denaturazione della ribonucleasi

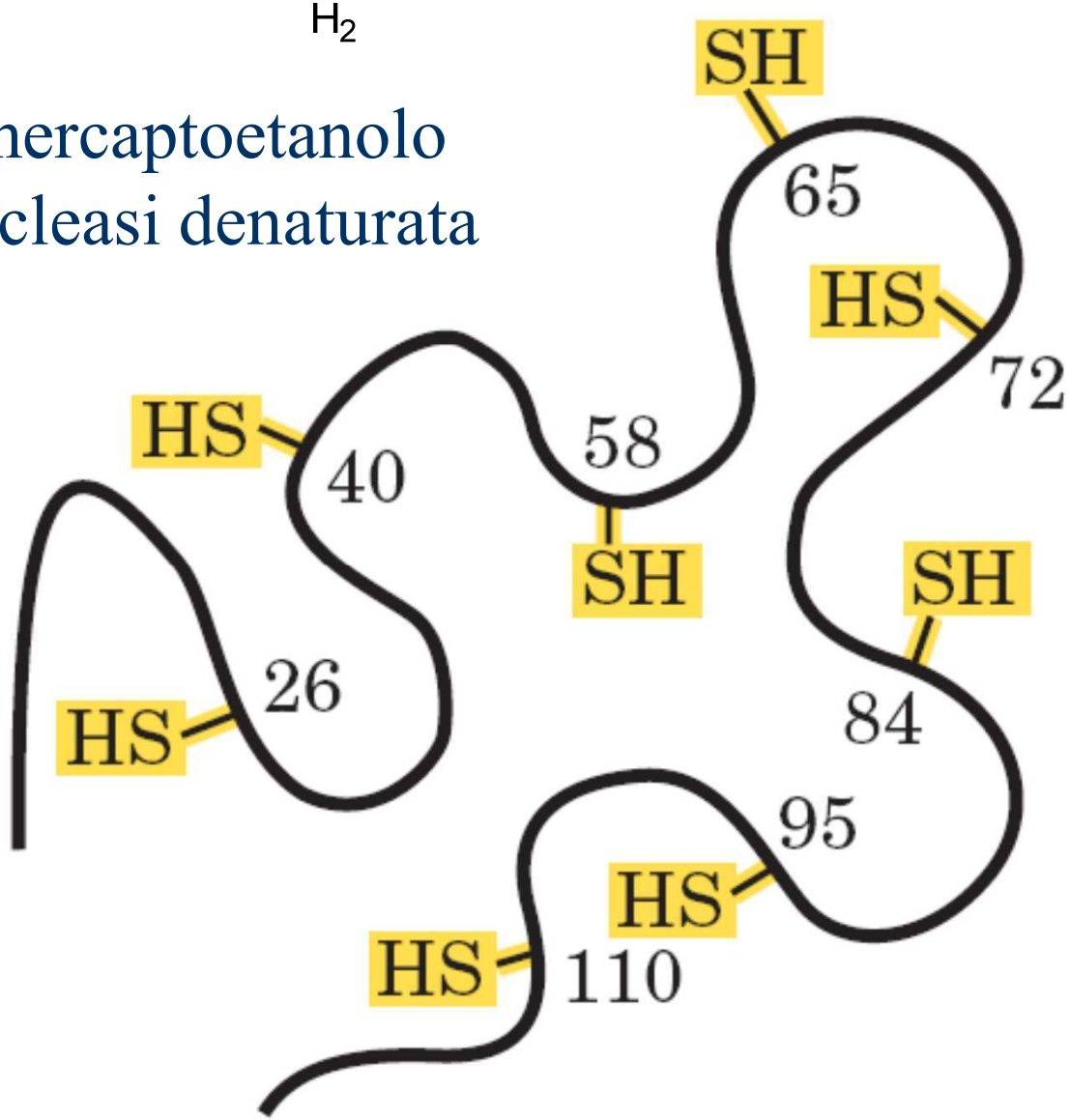


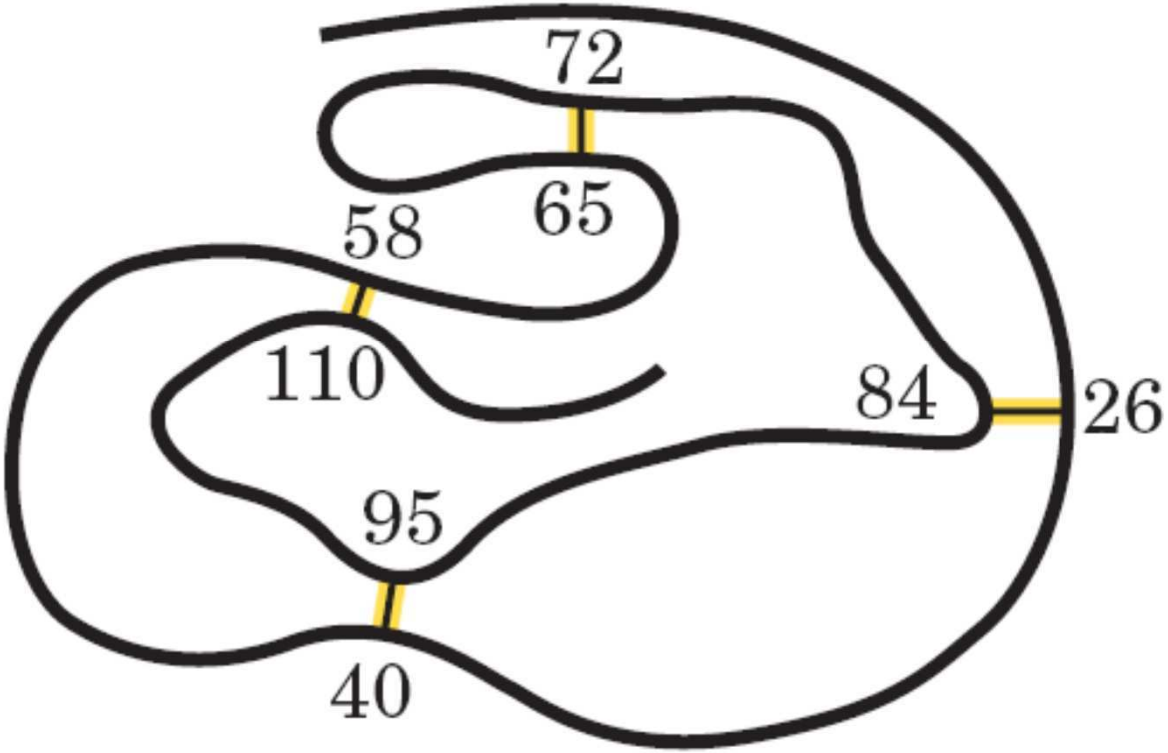
Ribonucleasi attiva





Urea + mercaptoetanolo
= ribonucleasi denaturata





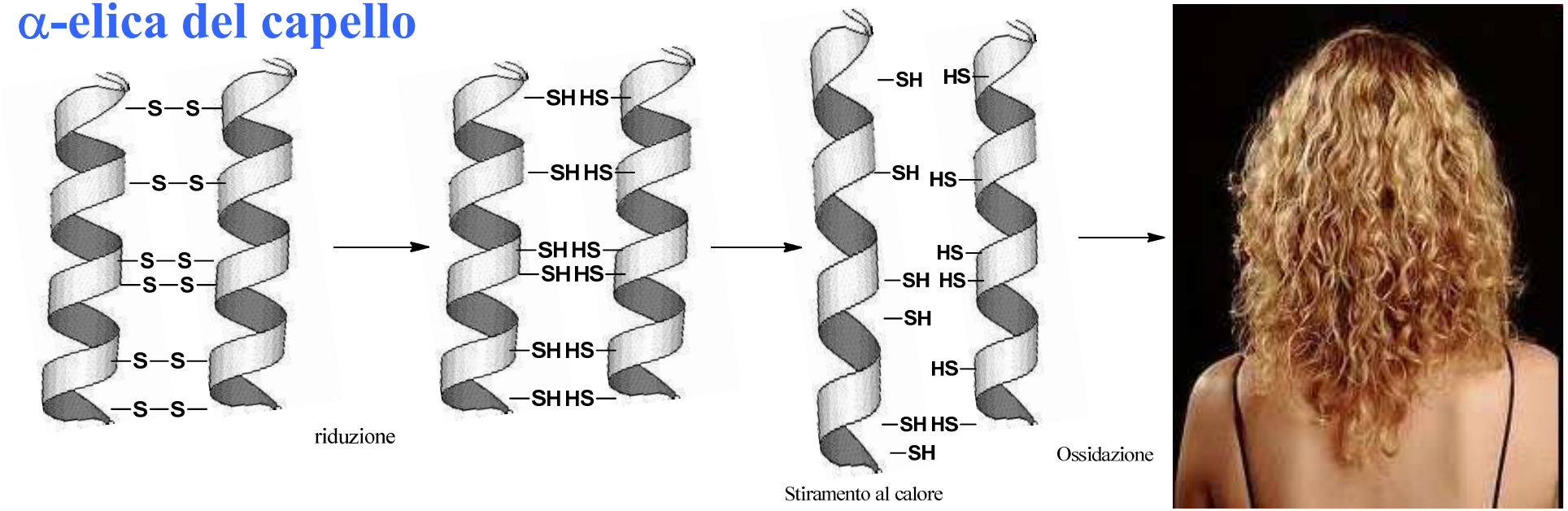
vengono rimossi prima l'urea e poi il mercaptoetanolo:
la ribonucleasi si riattiva in pochi secondi

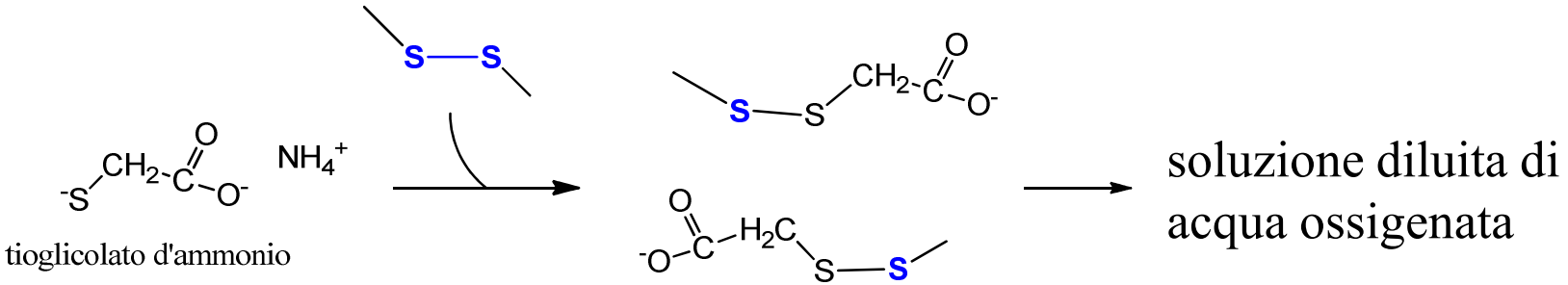
Le proteine possono perdere la struttura nativa (denaturazione) per effetto di:

- **Temperatura**: legami idrogeno
- **pH**: ponti salini, legami idrogeno
- **Solventi organici**: interazioni idrofobiche
- **Urea**: interazioni polari
- **Detergenti**: interazioni idrofobiche
- **Reagenti tiolici**: ponti disolfuro

La biochimica della permanente

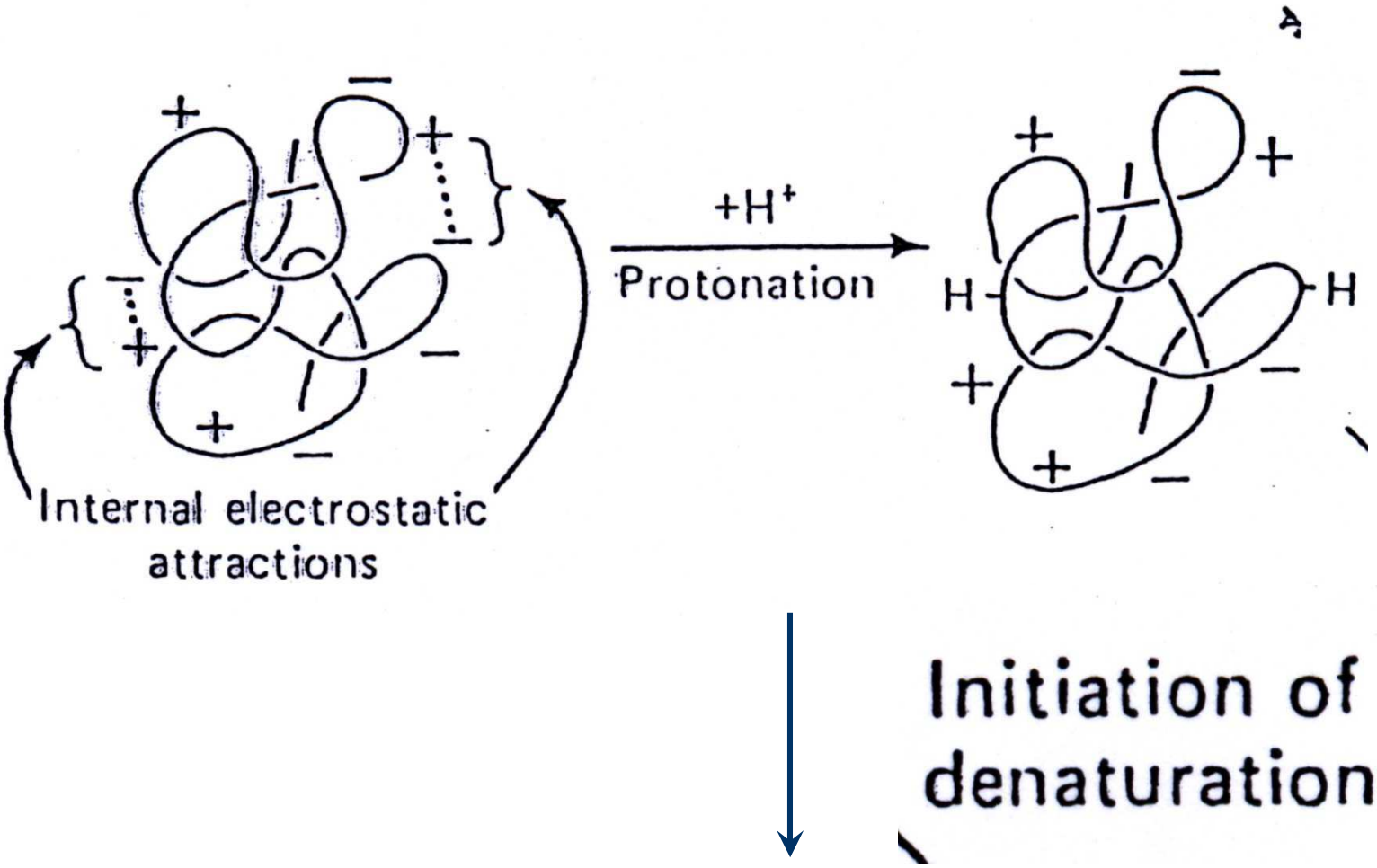
α-elica del capello

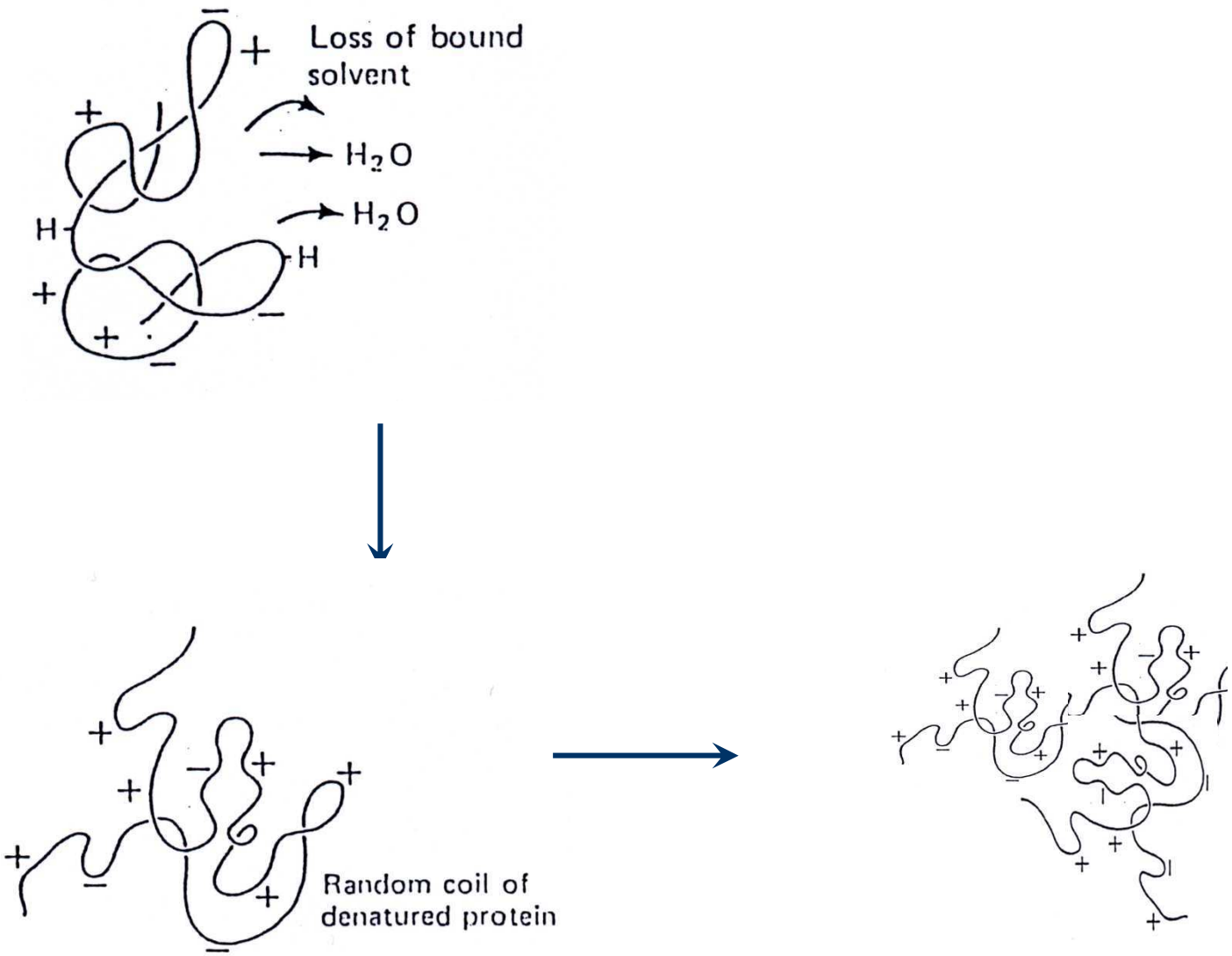




soluzione diluita di acqua ossigenata

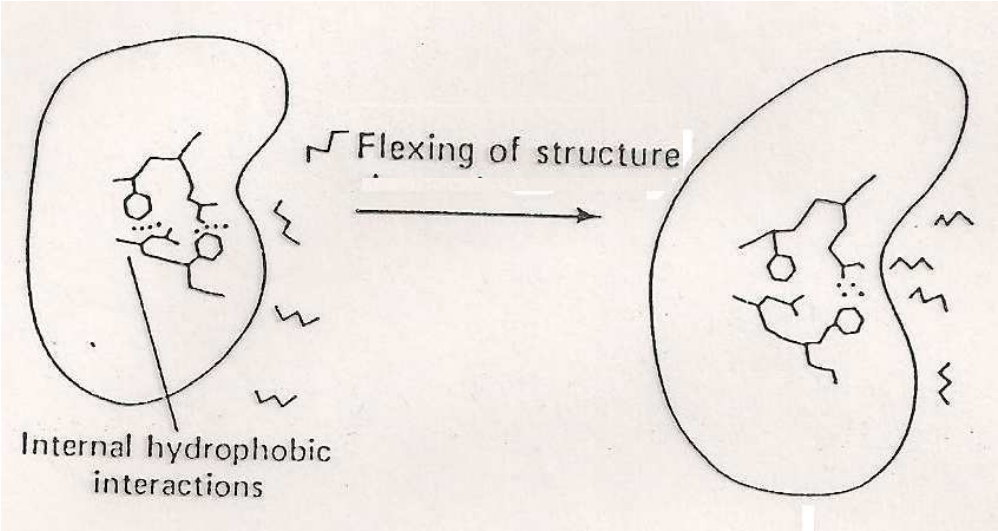
Variazioni di pH possono essere denaturanti



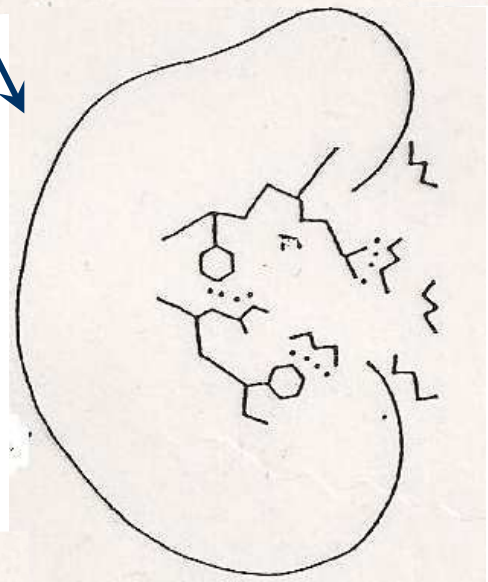


Protein aggregation and precipitation

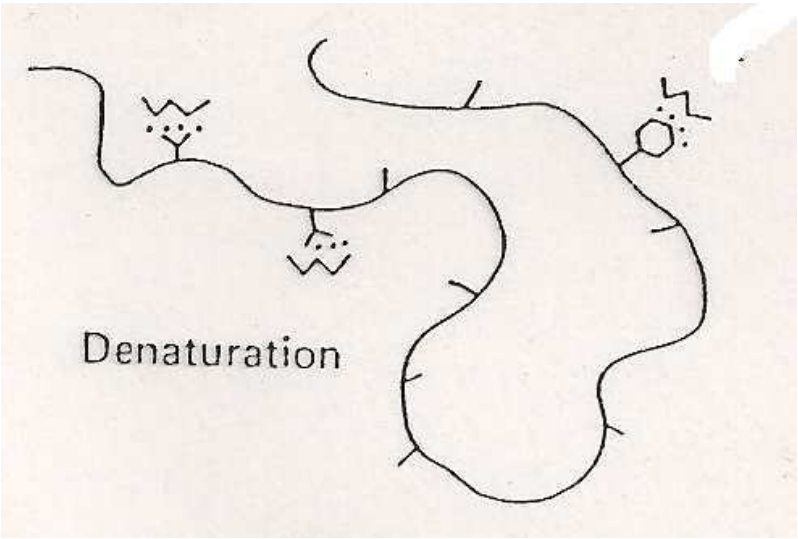
I solventi organici di natura idrofobica possono essere denaturanti

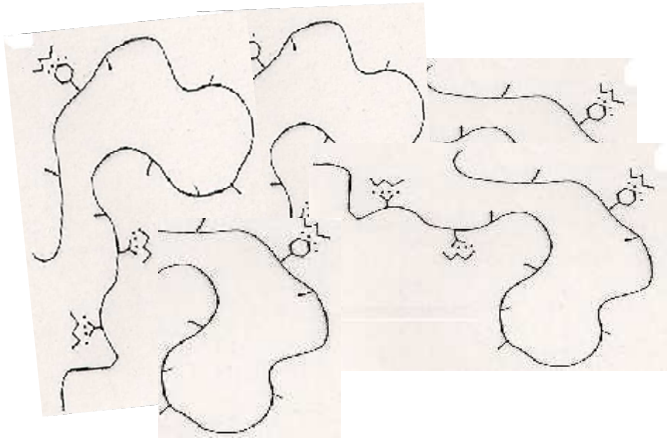


Organic solvent molecules (⌇) interact with hydrophobic residues



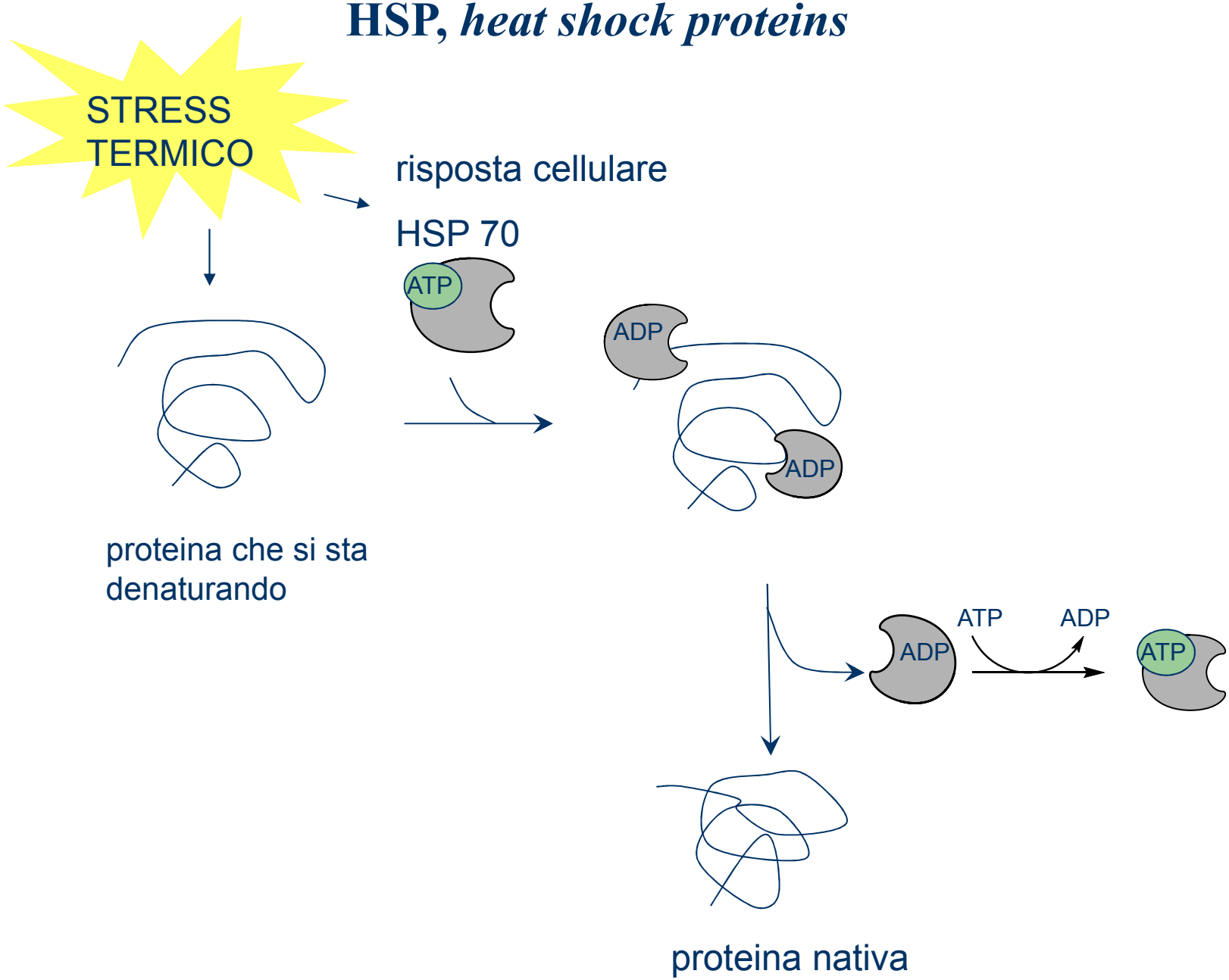
Disruption of internal hydrophobic interactions



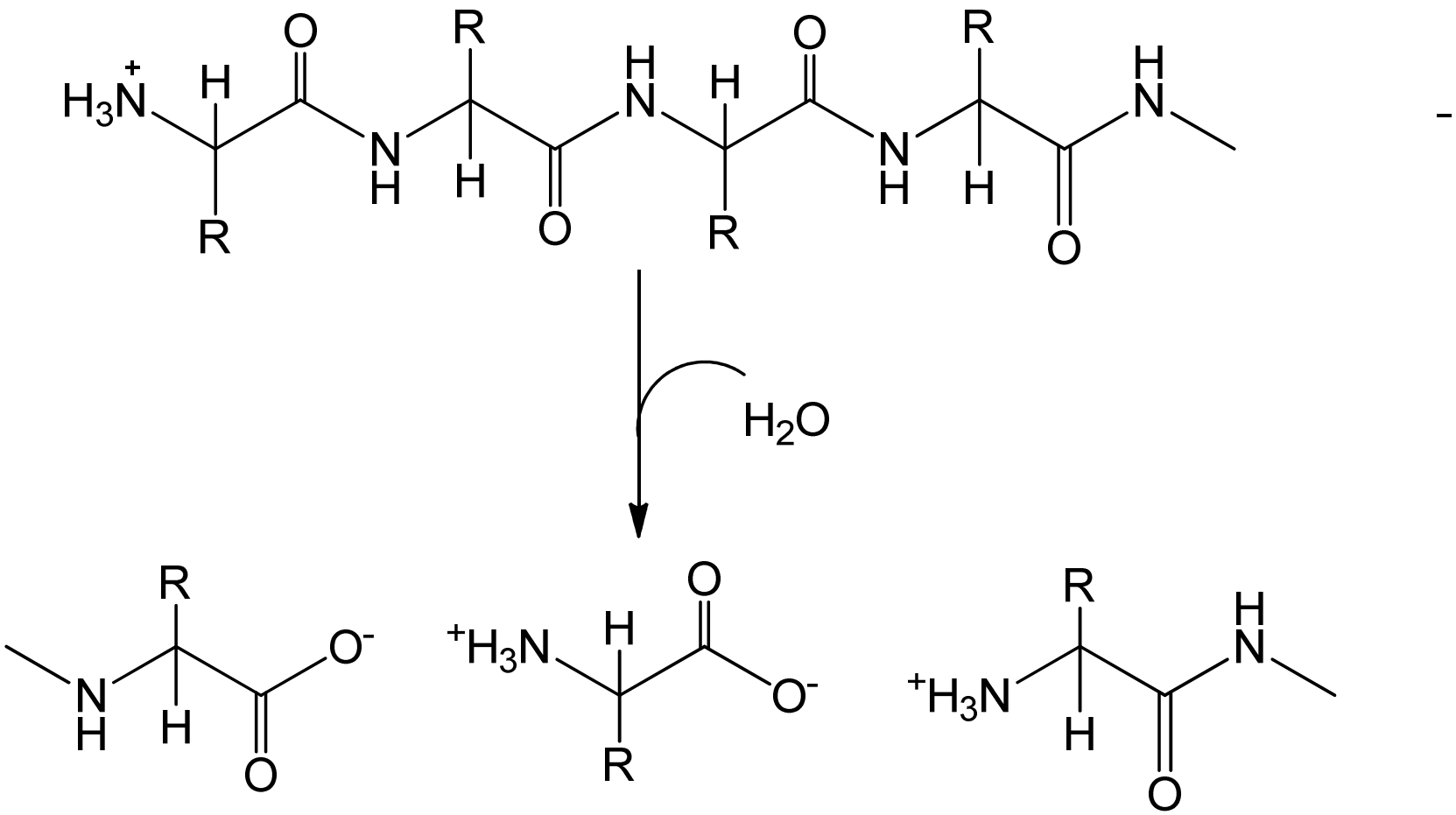


Possibly protein aggregation and precipitation

HSP, *heat shock proteins*



Degradazione



La struttura primaria è andata perduta

Degradazione

In chimica: a caldo (160°C) in HCl 6N

In biochimica: a T ambiente o corporea, mediata da enzimi detti proteasi (reazione di idrolisi)

- **nei processi digestivi extracellulari per l'assorbimento degli aminoacidi** (tripsina, chimotripsina, aminopeptidasi)
- nel turnover delle proteine
- nella proteolisi controllata (ormoni, zimogeni, fattori proteici)
- nei meccanismi di aggressione cellulare (virus, tossine proteiche, etc.) e di difesa