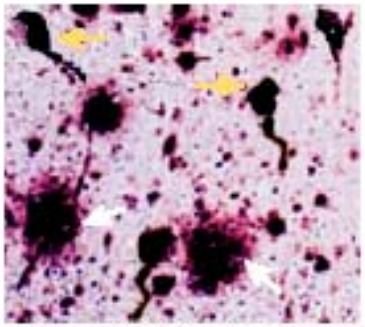
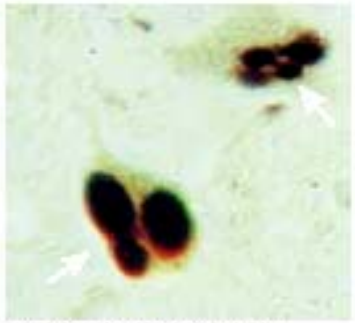
ESTRUCTURA Y FUNCION DE LAS PROTEÍNAS

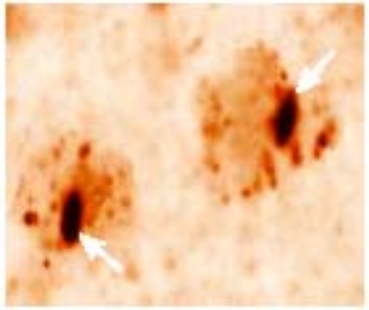
ENFERMEDADES NEURODESGENERATIVAS
FORMACION DE AGREGADOS MOLECULARES



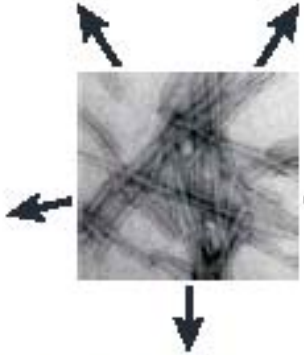
Alzheimer's plaques and tangles

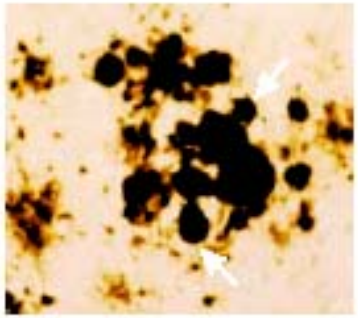


Parkinson's Lewy bodies

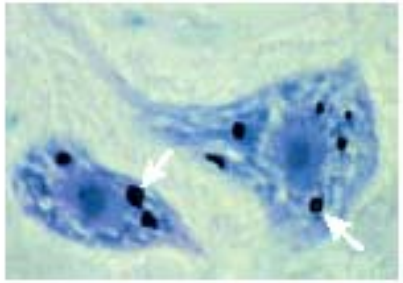


Huntington's intranuclear inclusions





Prion amyloid plaques

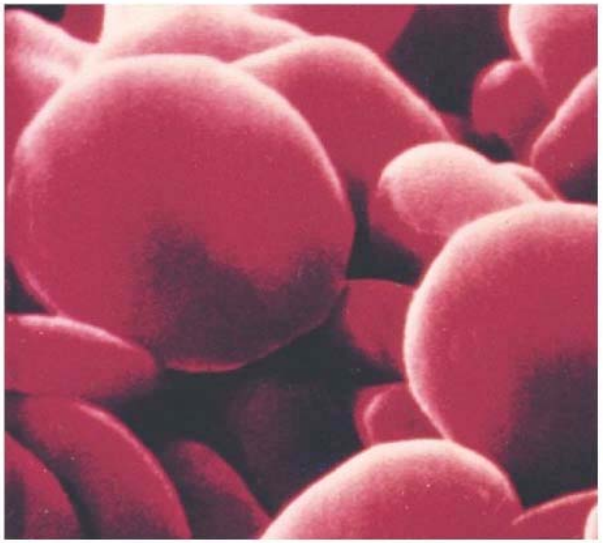


Amyotrophic lateral sclerosis aggregates

¿Qué proteínas conoce?

Función de las proteínas

(a)



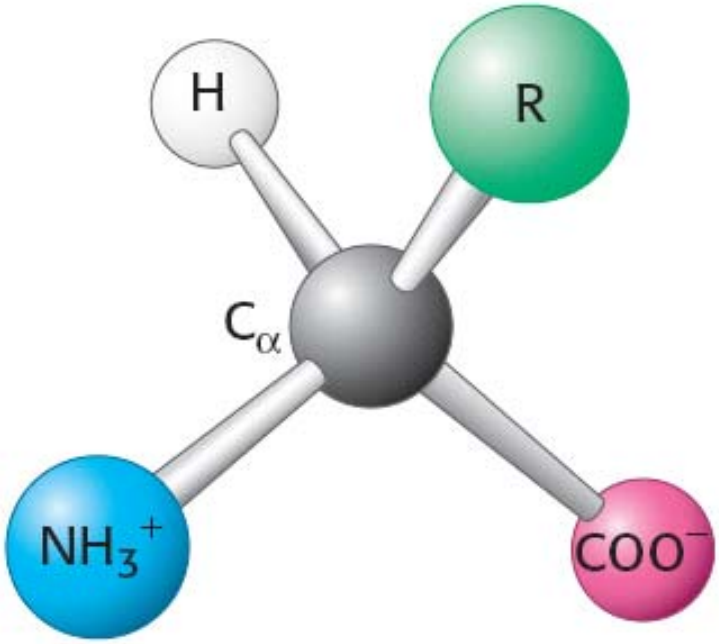
(b)

(c)

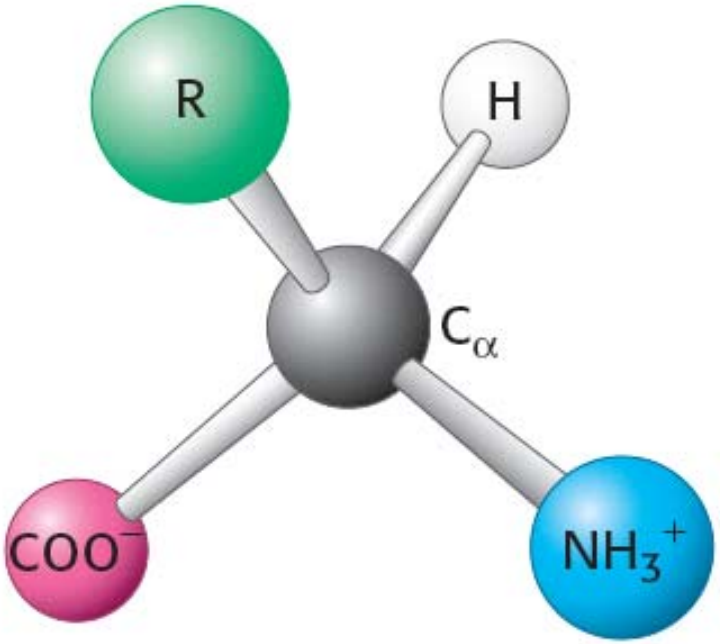
¿Qué componentes químicos forman una proteína?

ESTRUCTURA Y PROPIEDADES DE LOS AMINOÁCIDOS

ESTEREOISOMERIA:
En las proteínas se encuentran sólo aminoácidos L



L isomer



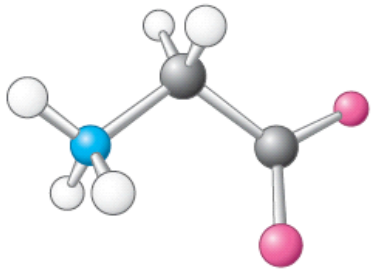
D isomer

ESTRUCTURAS IONICAS

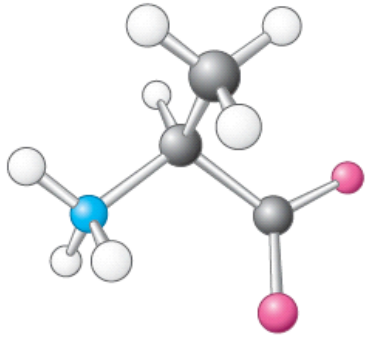
Los grupos carboxilo y amino se ionizan

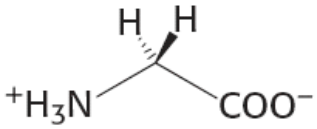
¿a qué pH?

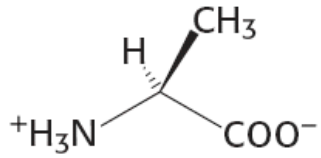
Glycine
(Gly, G)

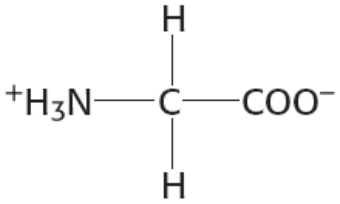


Alanine
(Ala, A)

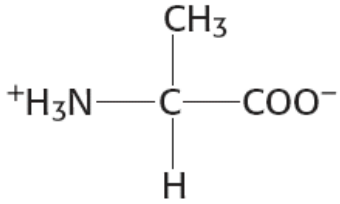






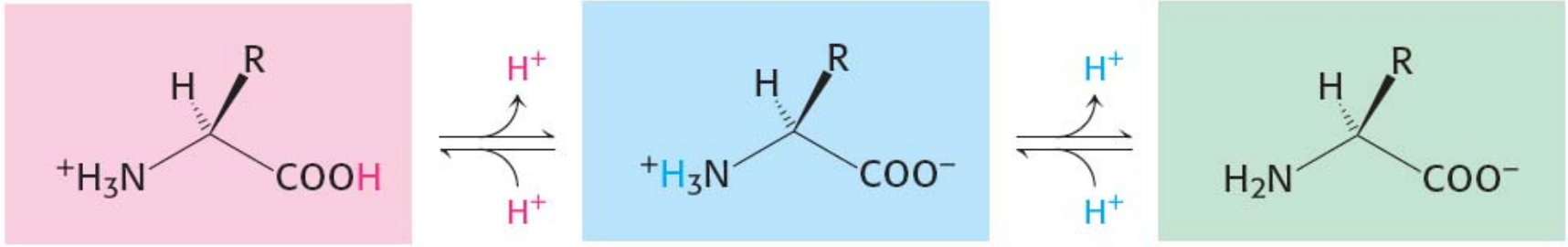


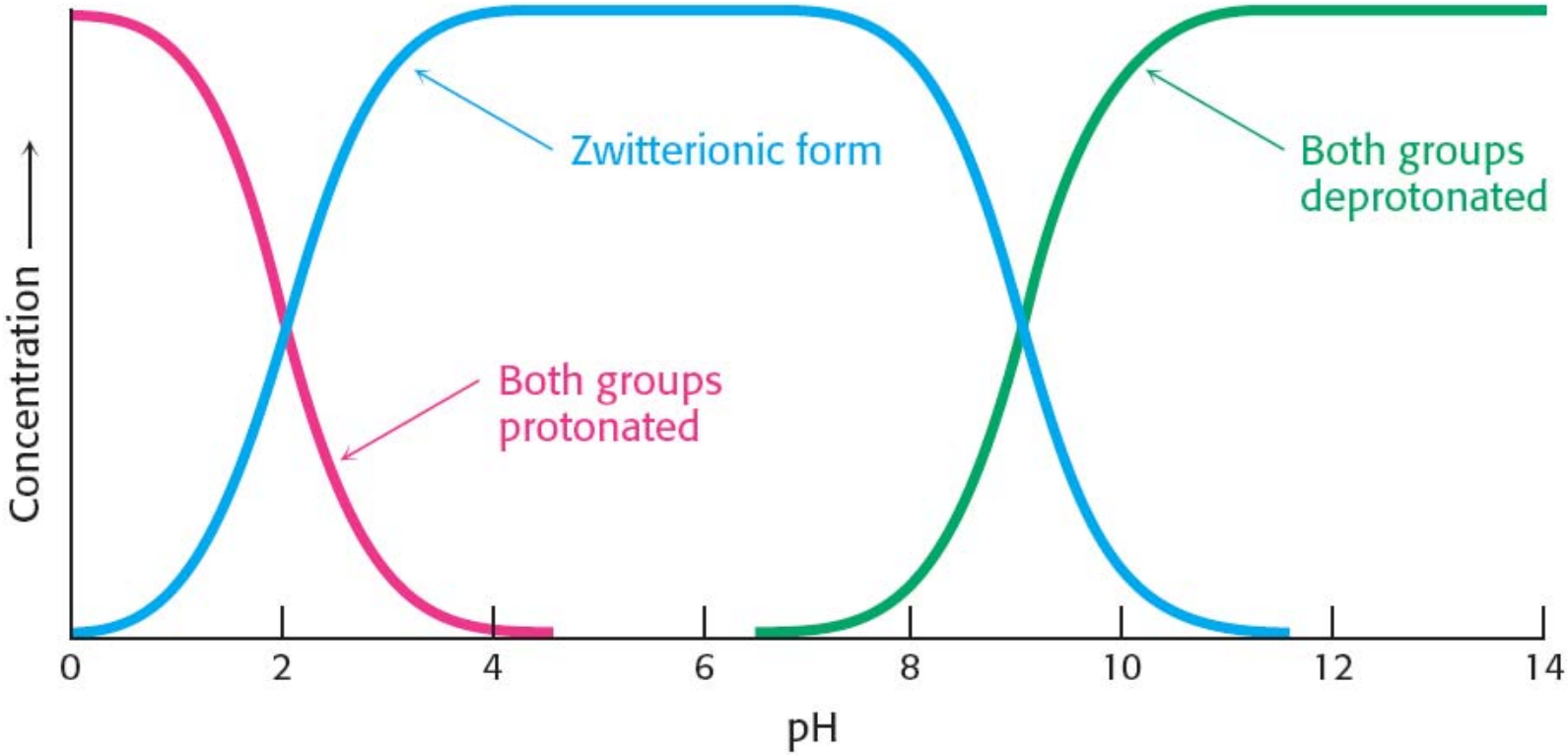
Glycine
(Gly, G)



Alanine
(Ala, A)

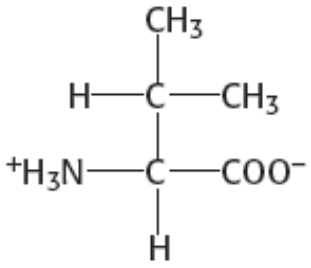
EQUILIBRIO ÁCIDO-BASE



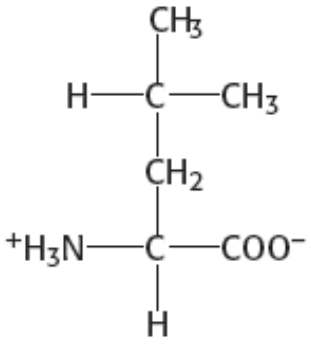


ESTRUCTURA DE LAS CADENAS LATERALES

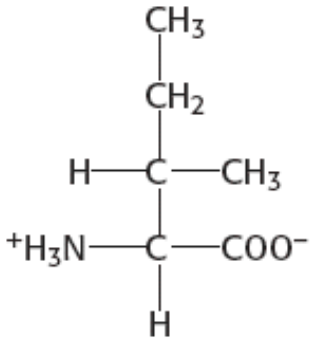
ALIFÁTICOS



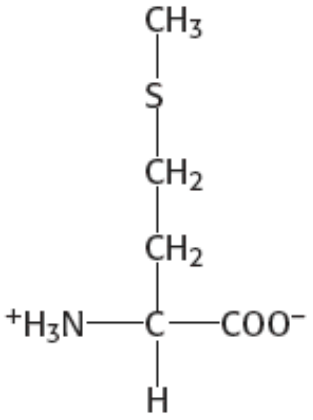
Valine
(Val, V)



Leucine
(Leu, L)

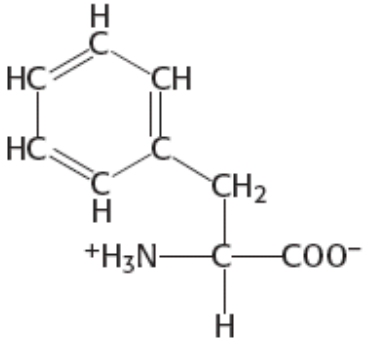


Isoleucine
(Ile, I)

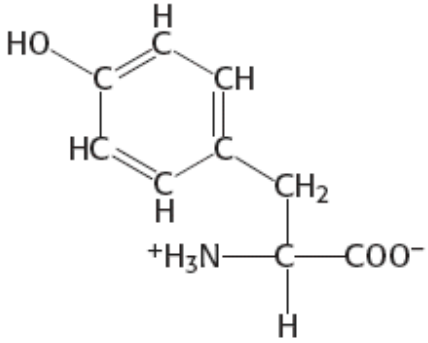


Methionine
(Met, M)

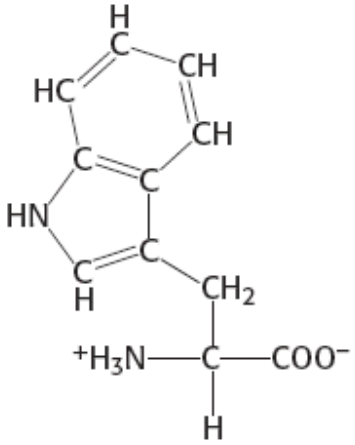
AROMÁTICOS



Phenylalanine
(Phe, F)

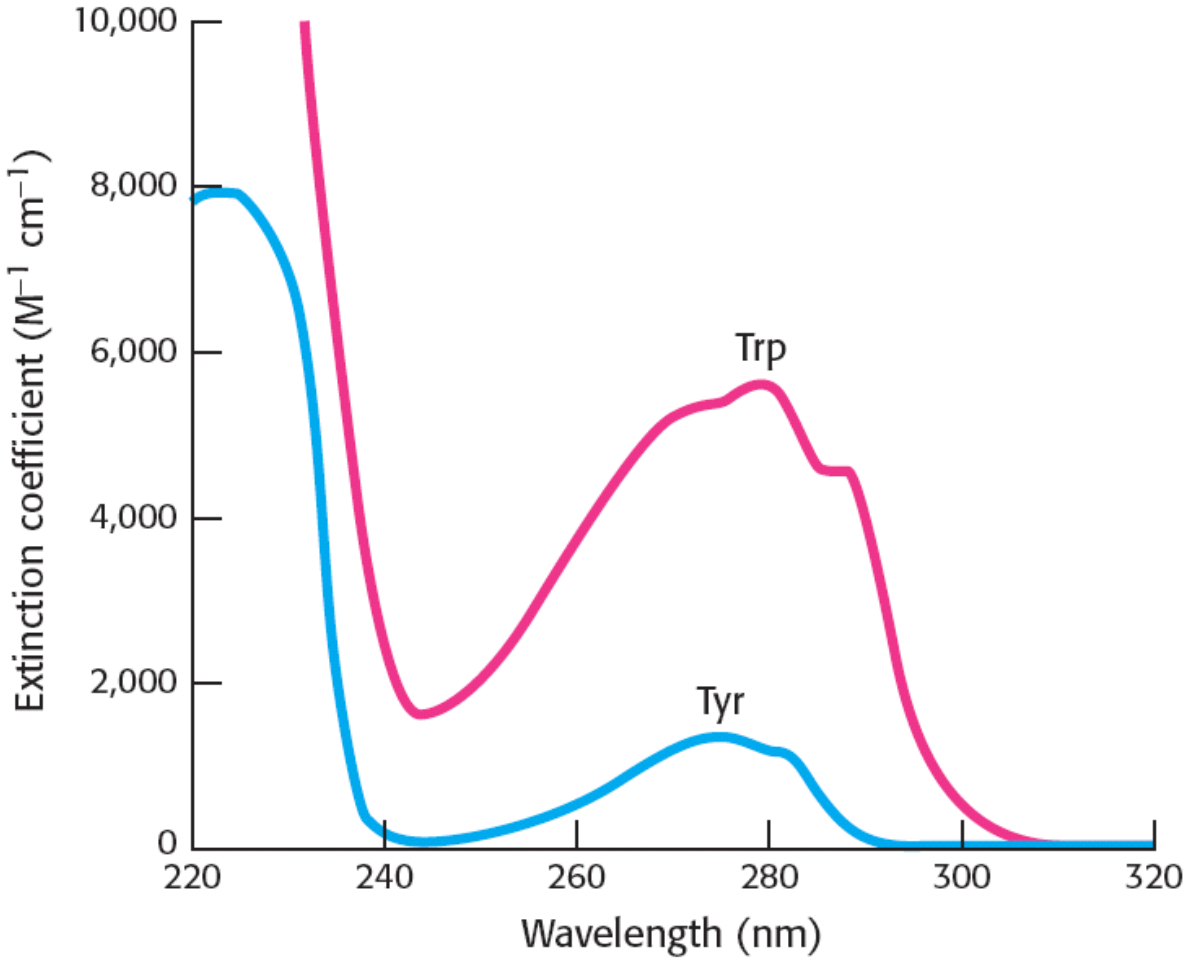


Tyrosine
(Tyr, Y)



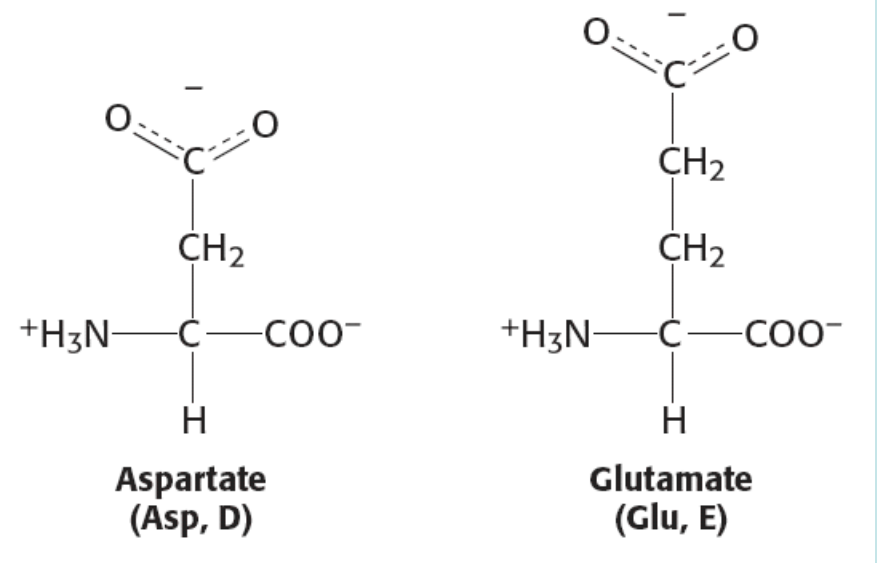
Tryptophan
(Trp, W)

ABSORCION DE LUZ UV:
grupos químicos aromáticos absorben luz uv

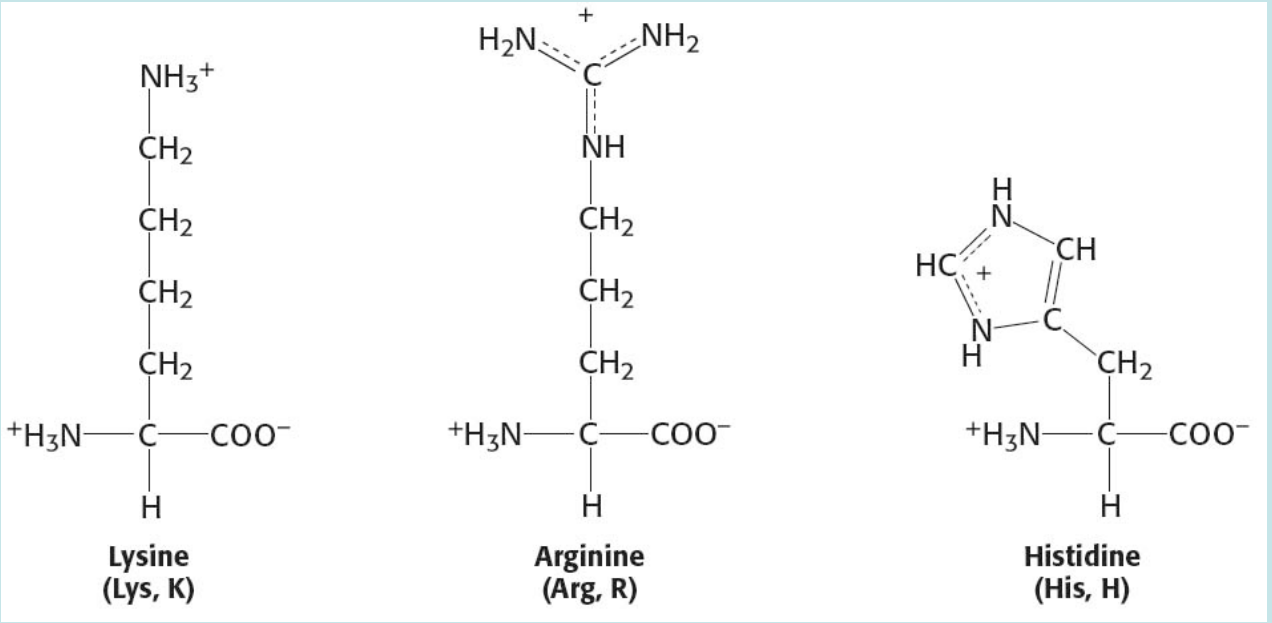


ESTRUCTURA DE LAS CADENAS LATERALES

NEGATIVOS

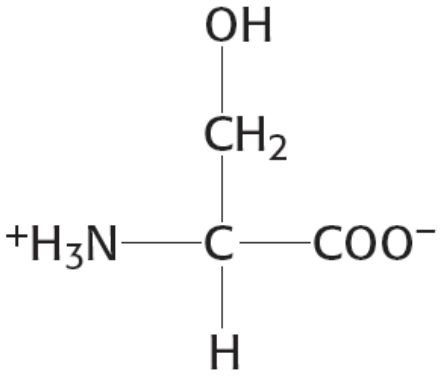


POSITIVOS

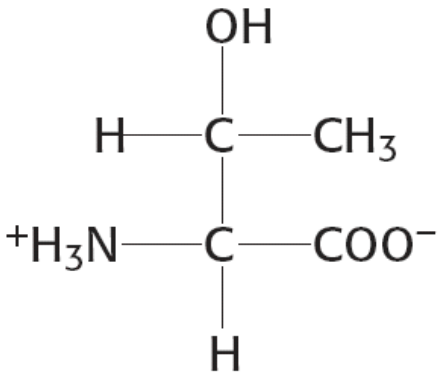


ESTRUCTURA DE LAS CADENAS LATERALES

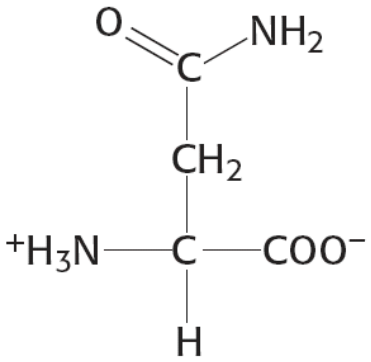
POLARES



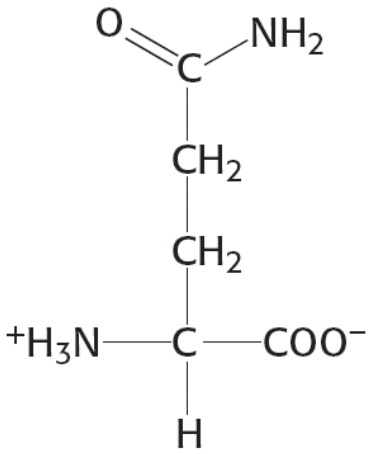
Serine
(Ser, S)



Threonine
(Thr, T)

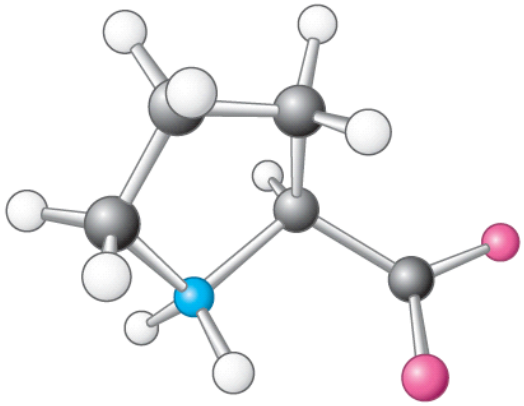


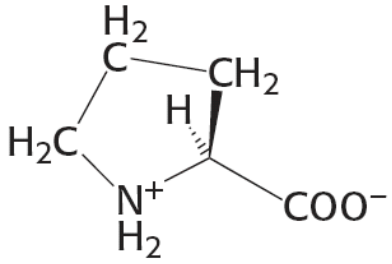
Asparagine
(Asn, N)



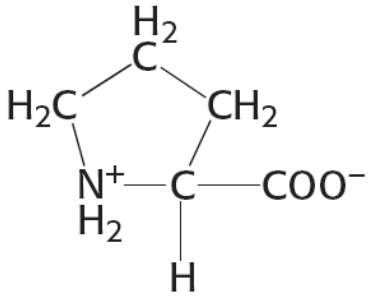
Glutamine
(Gln, Q)

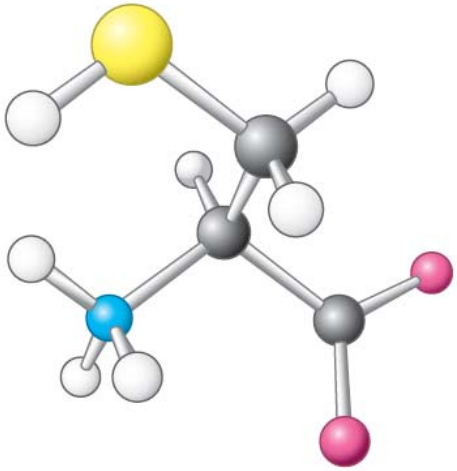
ESTRUCTURA DE LAS CADENAS LATERALES

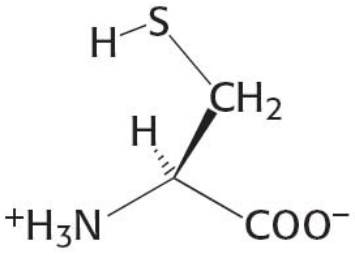




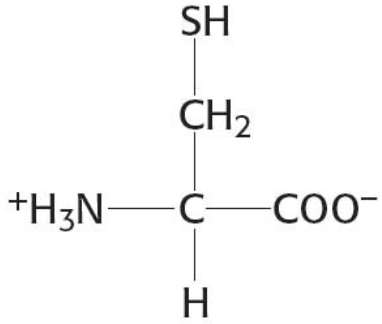
Proline
(Pro, P)



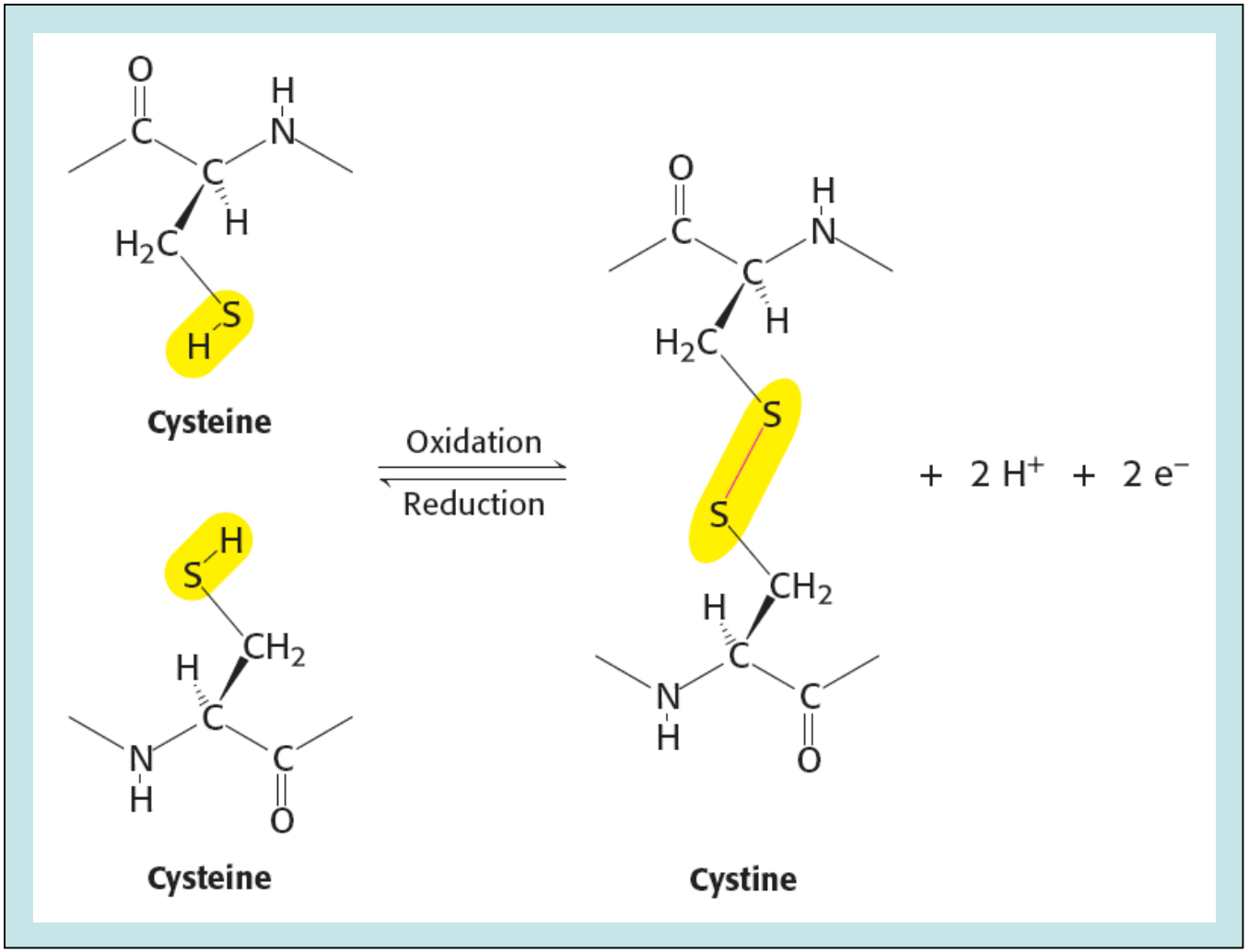




Cysteine
(Cys, C)

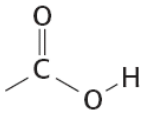
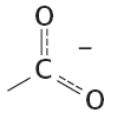
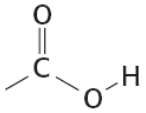
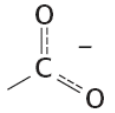
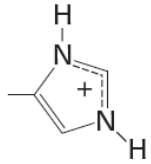
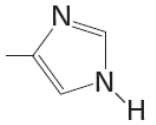
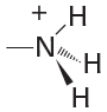
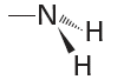
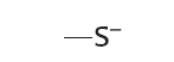
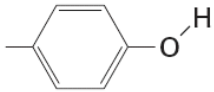
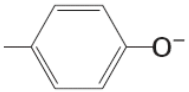
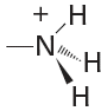
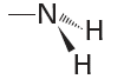
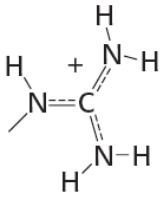
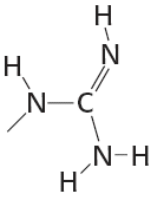


OXIDACION DE CISTEÍNA



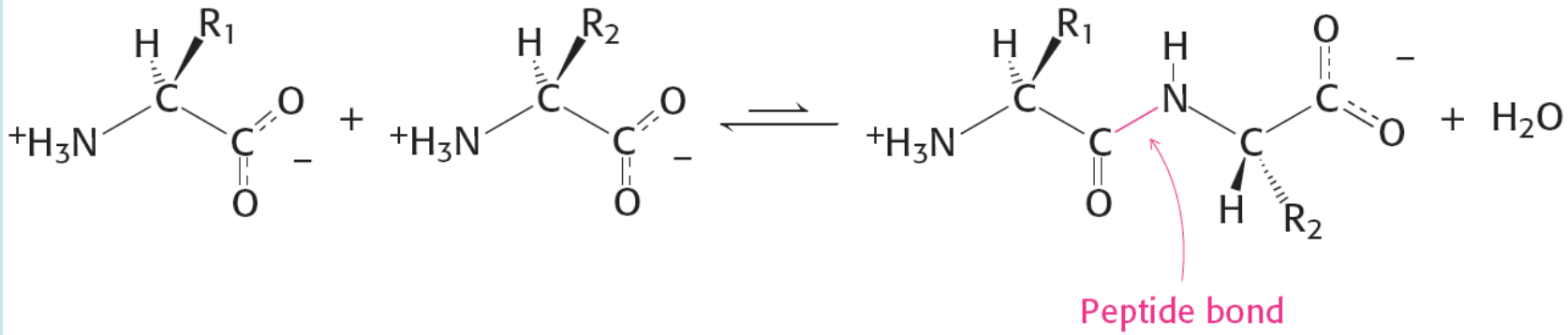
ESTRUCTURA DE LAS CADENAS LATERALES

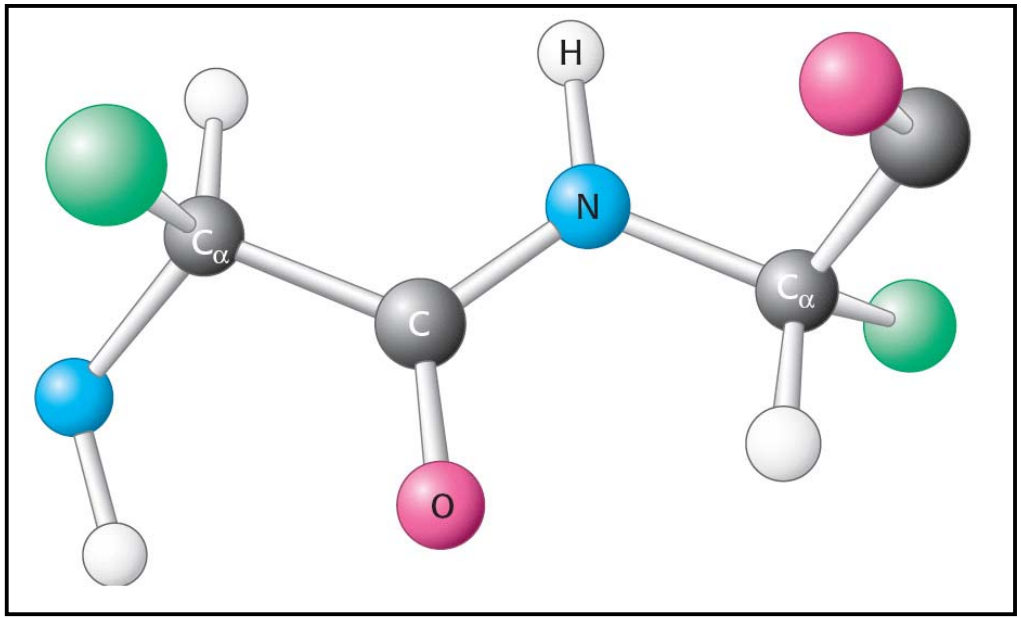
pK DE LAS CADENAS LATERALES

Group	Acid	⇌	Base	Typical pK_a*
Terminal α-carboxyl group		⇌		3.1
Aspartic acid Glutamic acid		⇌		4.1
Histidine		⇌		6.0
Terminal α-amino group		⇌		8.0
Cysteine		⇌		8.3
Tyrosine		⇌		10.9
Lysine		⇌		10.8
Arginine		⇌		12.5

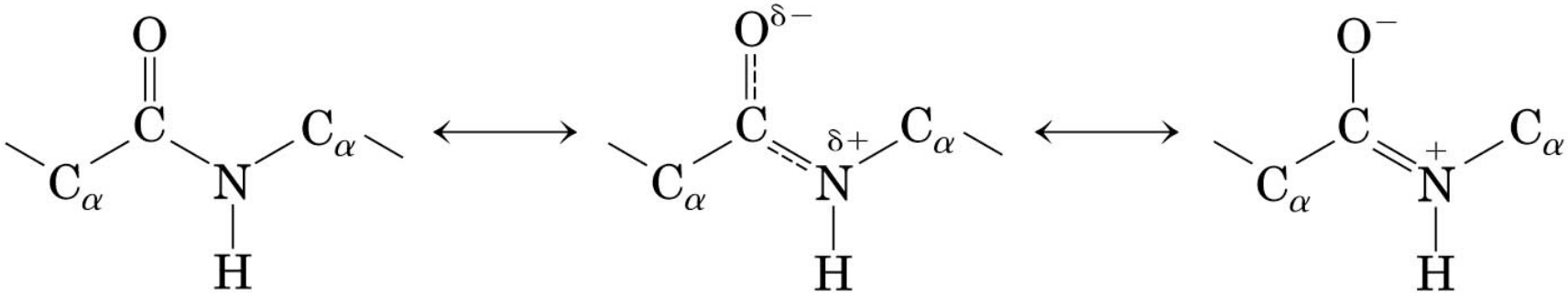
¿Qué reacciones químicas pueden ocurrir entre los aminoácidos?

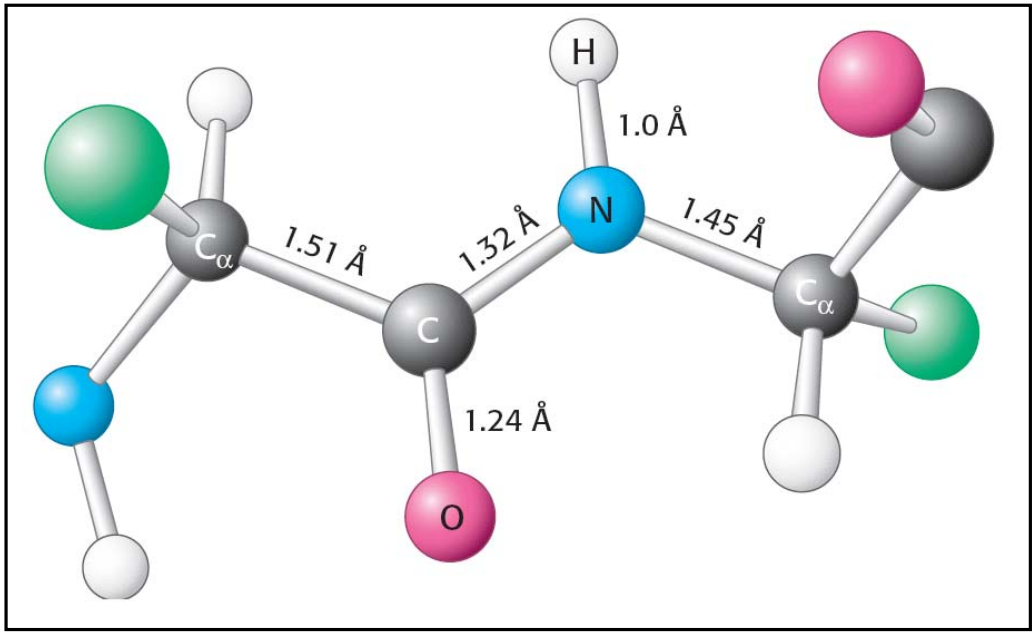
FORMACION DEL ENLACE PEPTIDICO



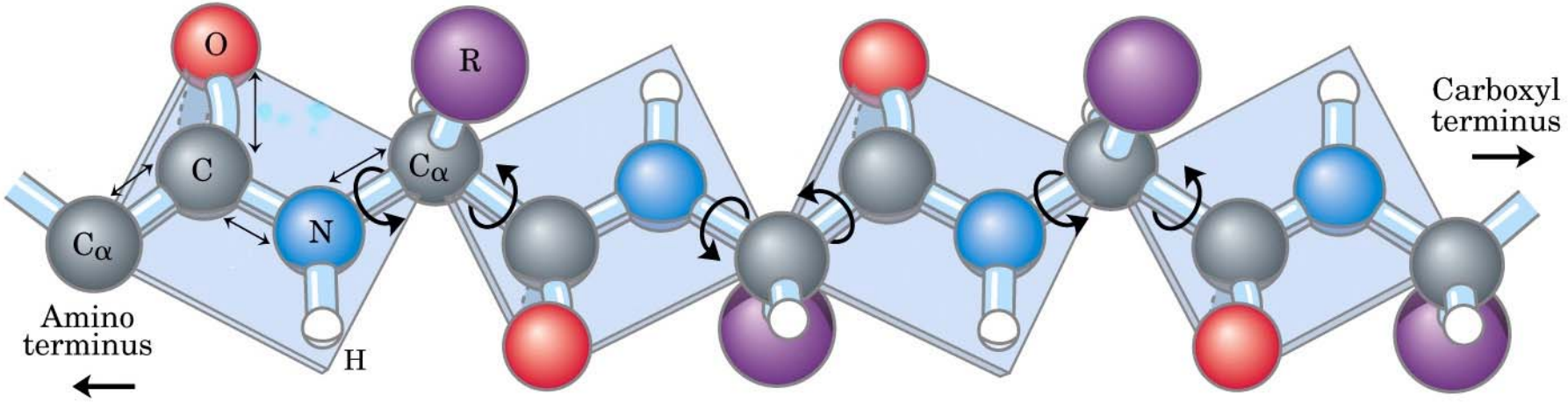


El enlace peptídico tiene carácter de doble enlace y una rotación restringida



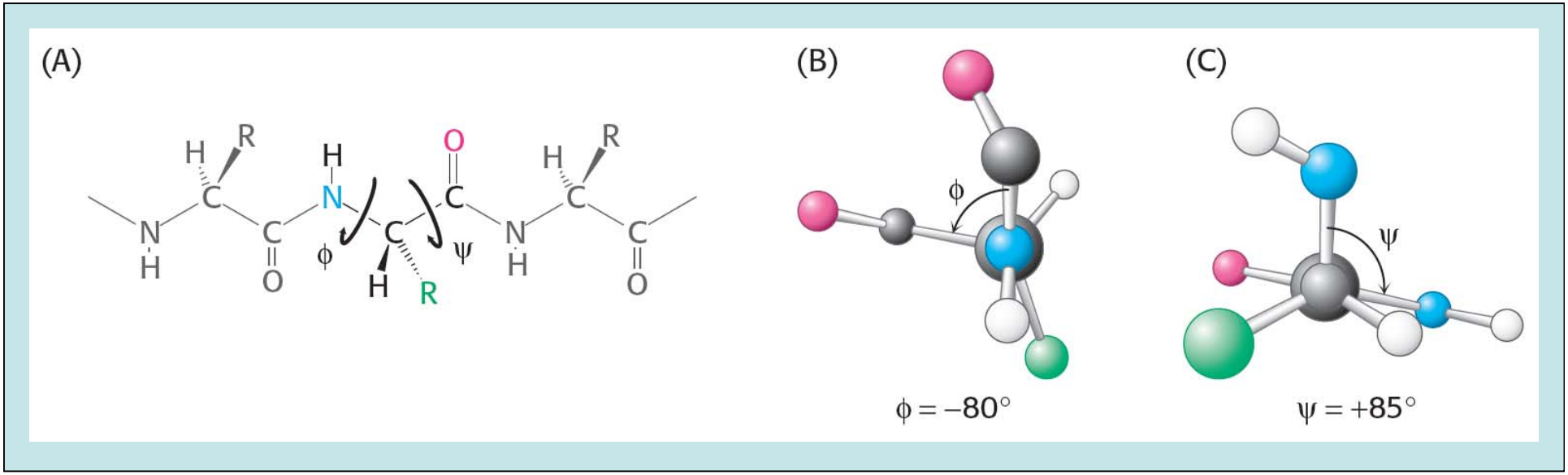


El enlace petídico tiene una geometría plana



Sólo existe libre rotación en torno al Cα

Otra visión de la libre rotación en torno al Cα: ángulos de Ramachandran



Visualización de las rotaciones del enlace peptídico

Abrir programa **Mage**

Archivo E3 ScSt kinimage 1.

ESTRUCTURA DE LAS PROTEINAS

ESTRUCTURA:
PRIMARIA
SECUNDARIA
TERCIARIA
CUATERNARIA

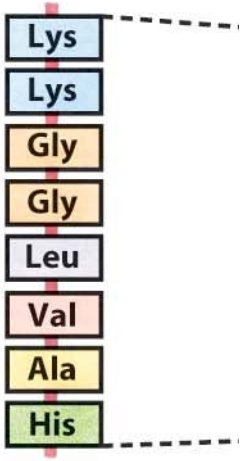
¿QUE IMPORTANCIA TIENE LA SECUENCIA DE AMINOACIDOS EN LA FUNCIÓN DE UNA PROTEÍNA?

Primary structure

Secondary structure

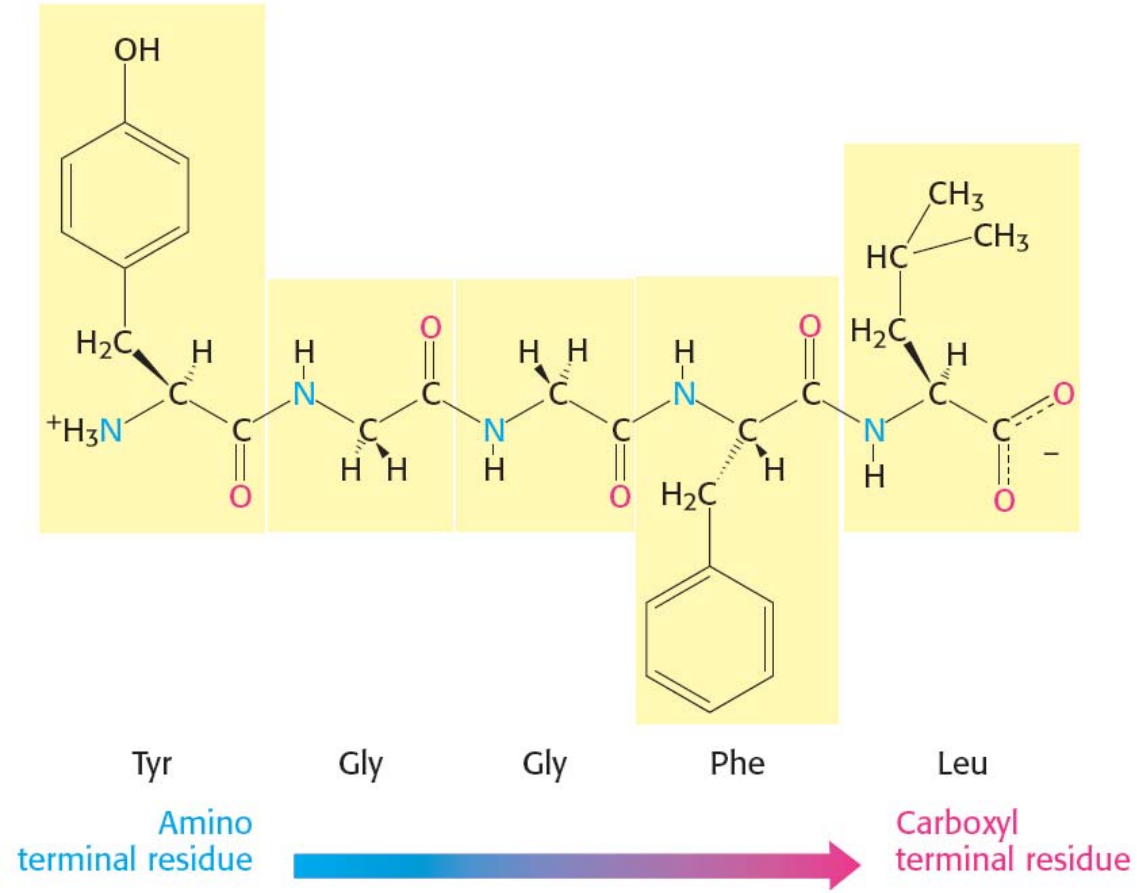
Tertiary structure

Quaternary structure



Amino acid residues

ESTRUCTURA PRIMARIA:
determina todos los niveles superiores de organización y la función biológica de una proteína



¿CUAL ES EL LIMITE EN EL NUMERO DE AMINOACIDOS DE UNA CADENA PEPTIDICA?

table 5–2

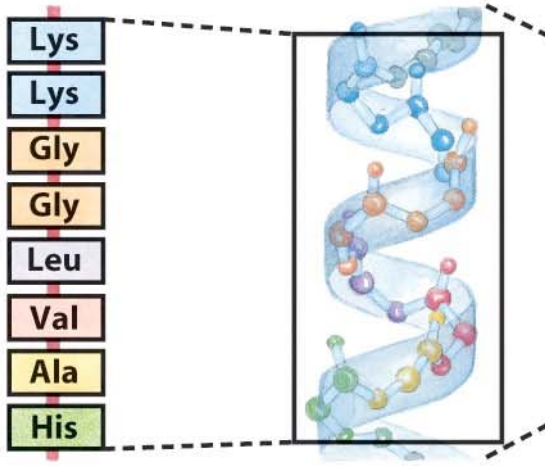
Molecular Data on Some Proteins

	Molecular weight	Number of residues	Number of polypeptide chains
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	609	1
Hexokinase (yeast)	102,000	972	2
RNA polymerase (*E. coli*)	450,000	4,158	5
Apolipoprotein B (human)	513,000	4,536	1
Glutamine synthetase (*E. coli*)	619,000	5,628	12
Titin (human)	2,993,000	26,926	1

LA RESTRICCION EN LAS ROTACIONES DEL ENLACE PEPTIDICO FAVORECE ALGUNAS CONFORMACIONES DE LA CADENA PEPTIDICA

ESTRUCTURA SECUNDARIA

Primary structure | **Secondary structure** | **Tertiary structure** | **Quaternary structure**

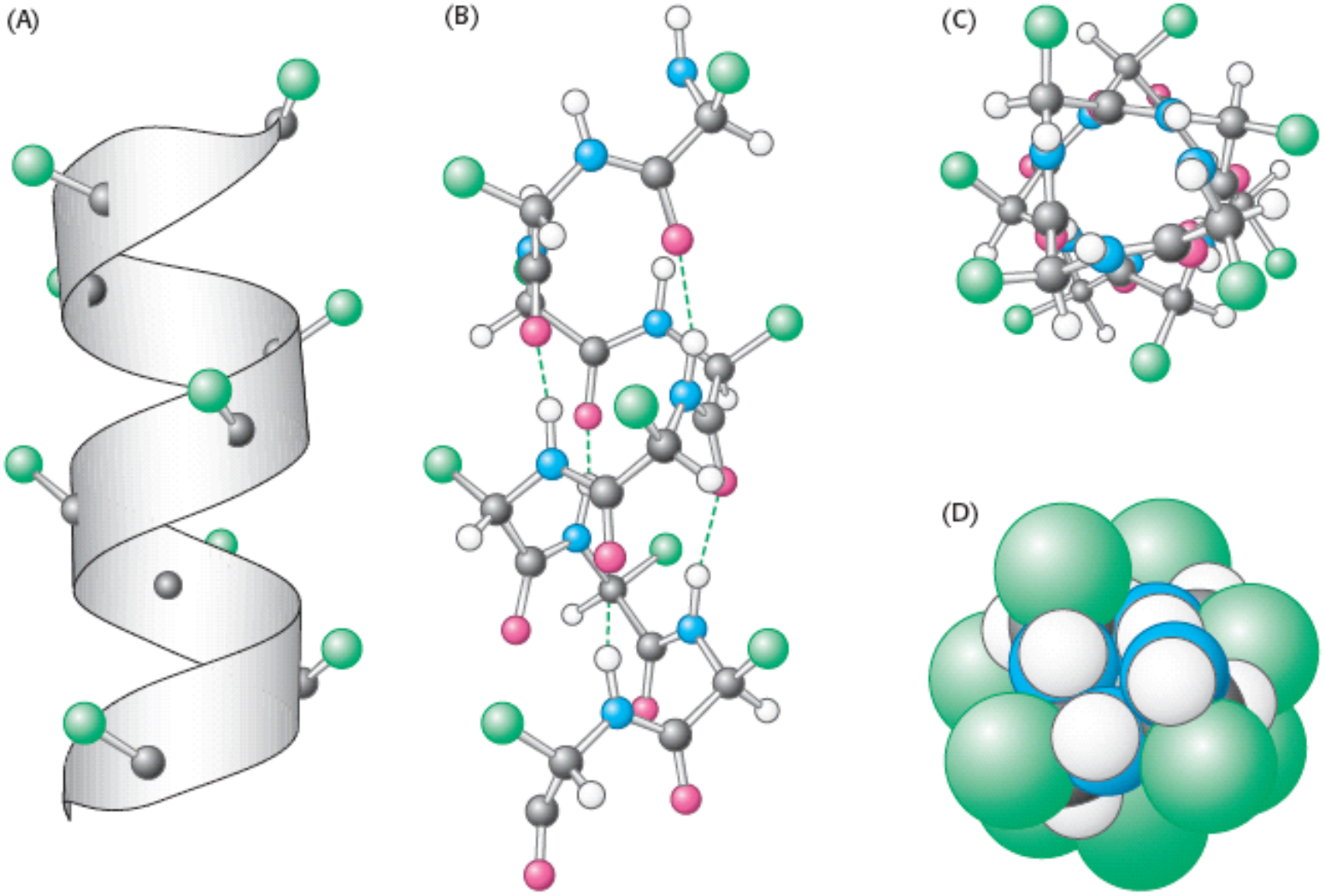


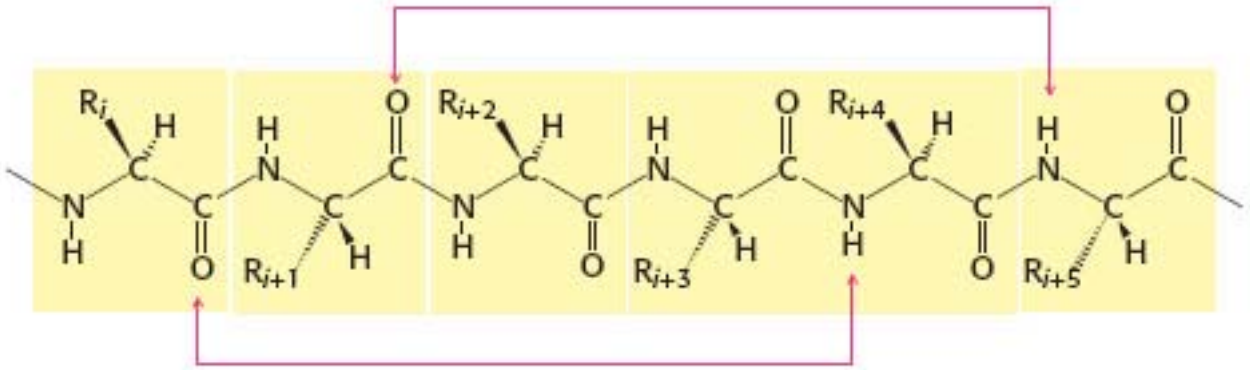
Amino acid residues

α Helix

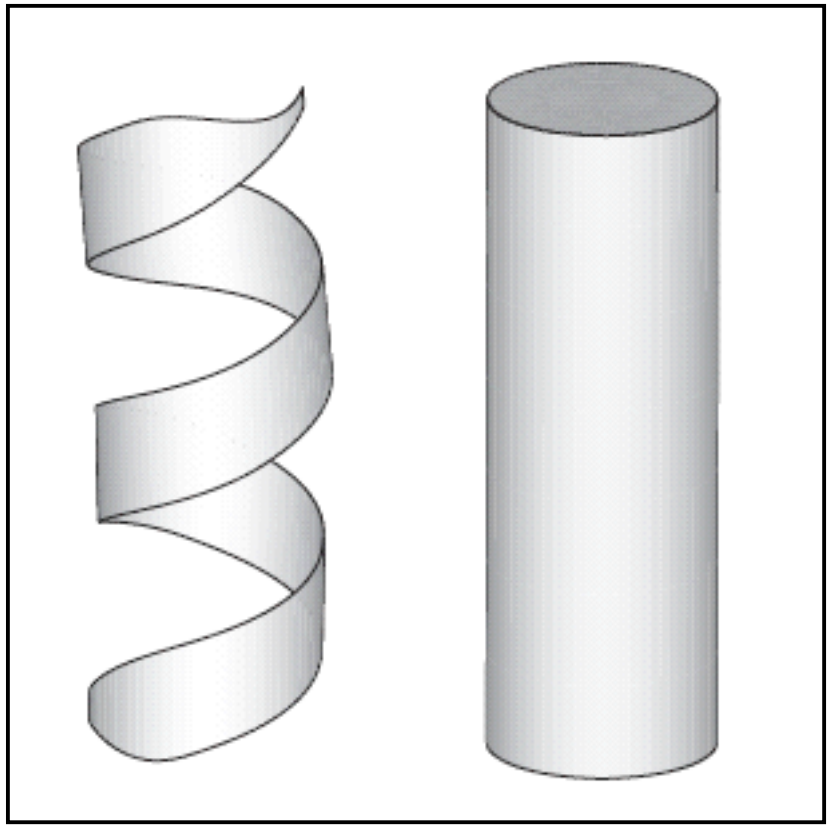
ESTRUCTURA SECUNDARIA

Hélice α: se estabiliza por puentes de H entre los átomos de enlaces peptídicos cercanos



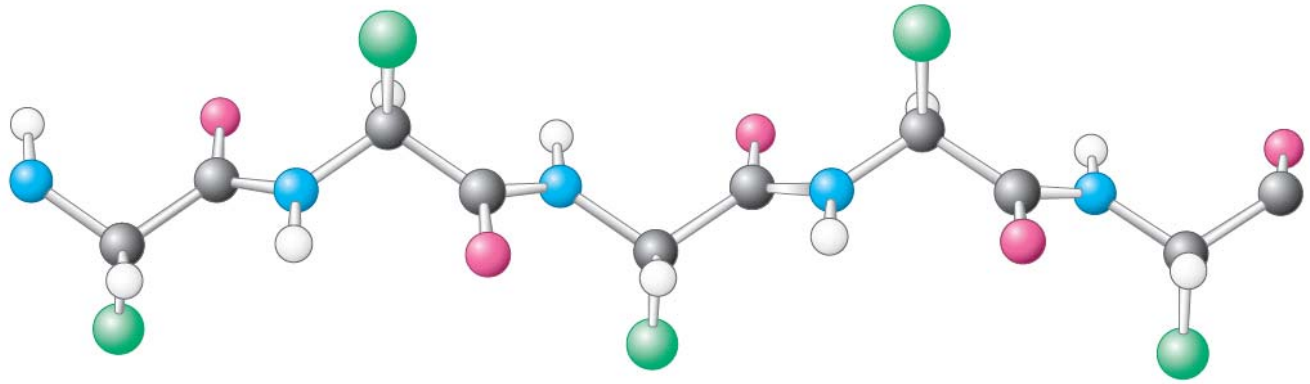


Otras representaciones de la α-hélice



ESTRUCTURA SECUNDARIA

Estructura β: es una forma extendida de la cadena polipeptídica

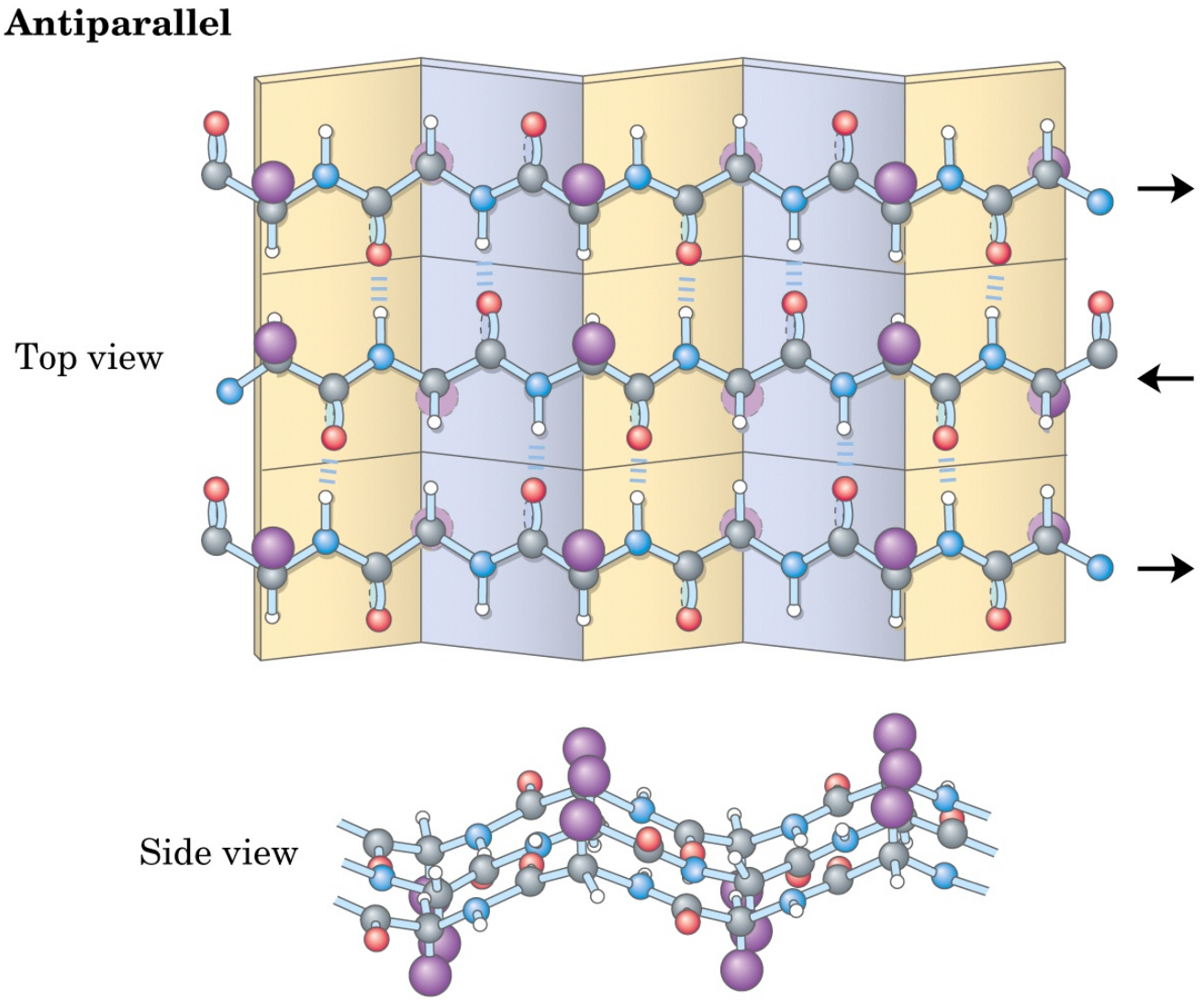


ESTRUCTURA SECUNDARIA

Estas formas extendidas se asocian formando láminas β

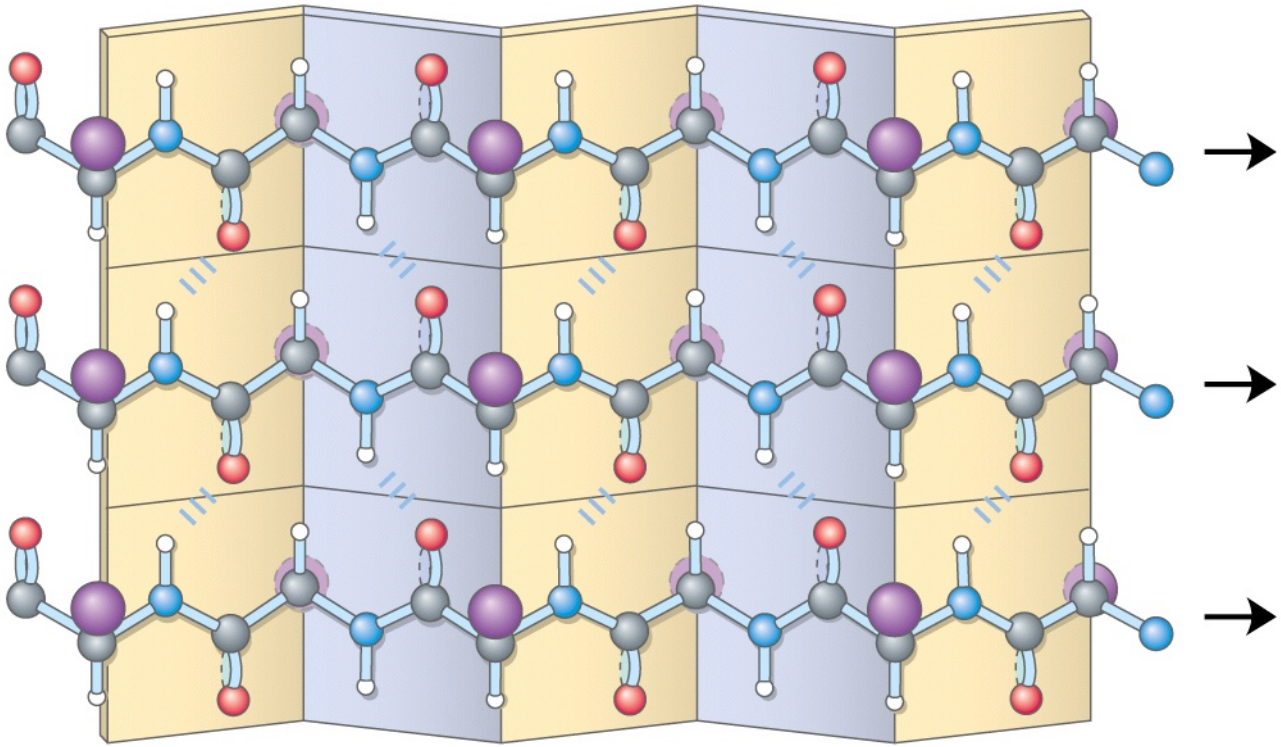
ESTRUCTURA SECUNDARIA

Las láminas β se estabilizan por puentes de H entre los átomos de enlaces peptídicos de segmentos β distintos

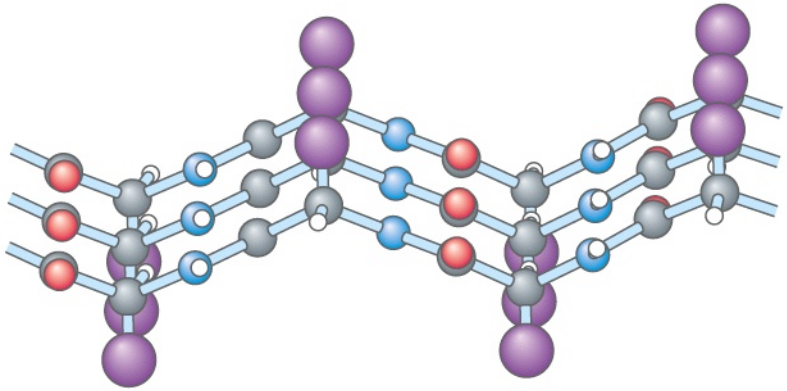


(b) Parallel

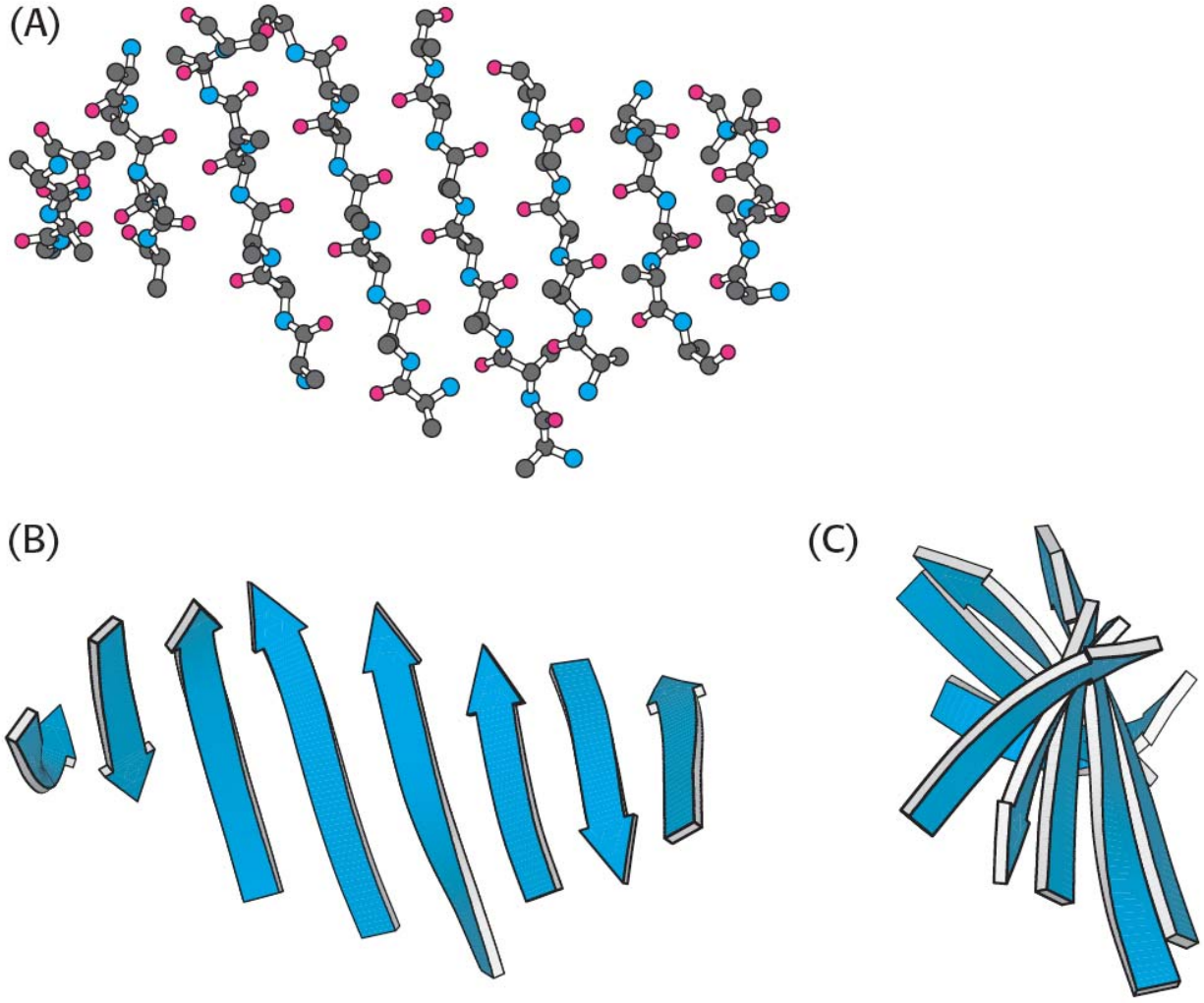
Top view



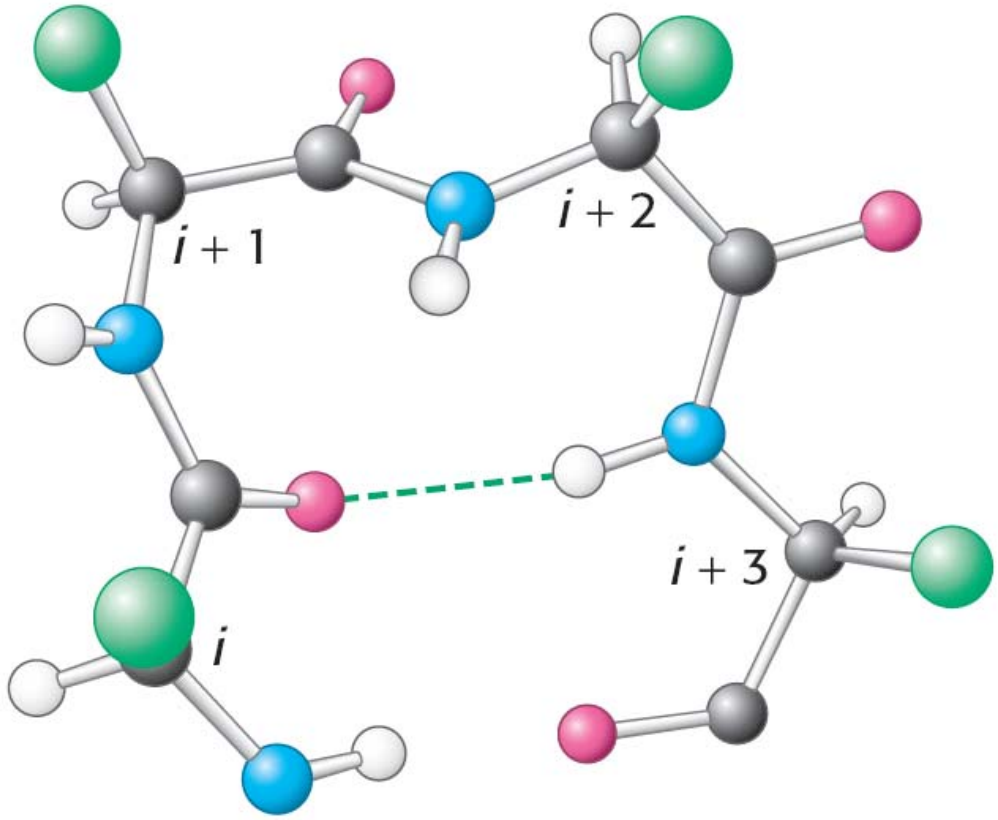
Side view



Otra representación de las láminas β



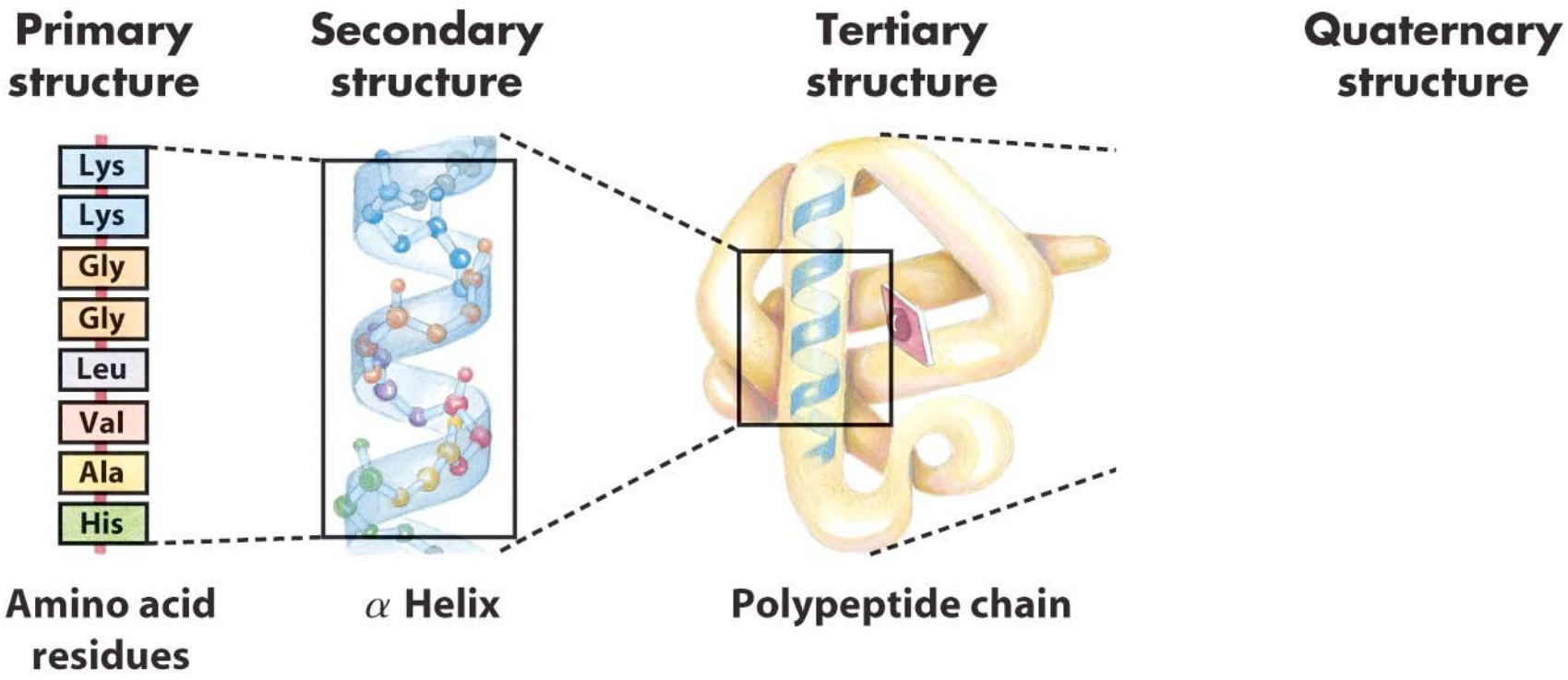
ESTRUCTURA SECUNDARIA
Vueltas (loops): conectan hélices α o segmentos β



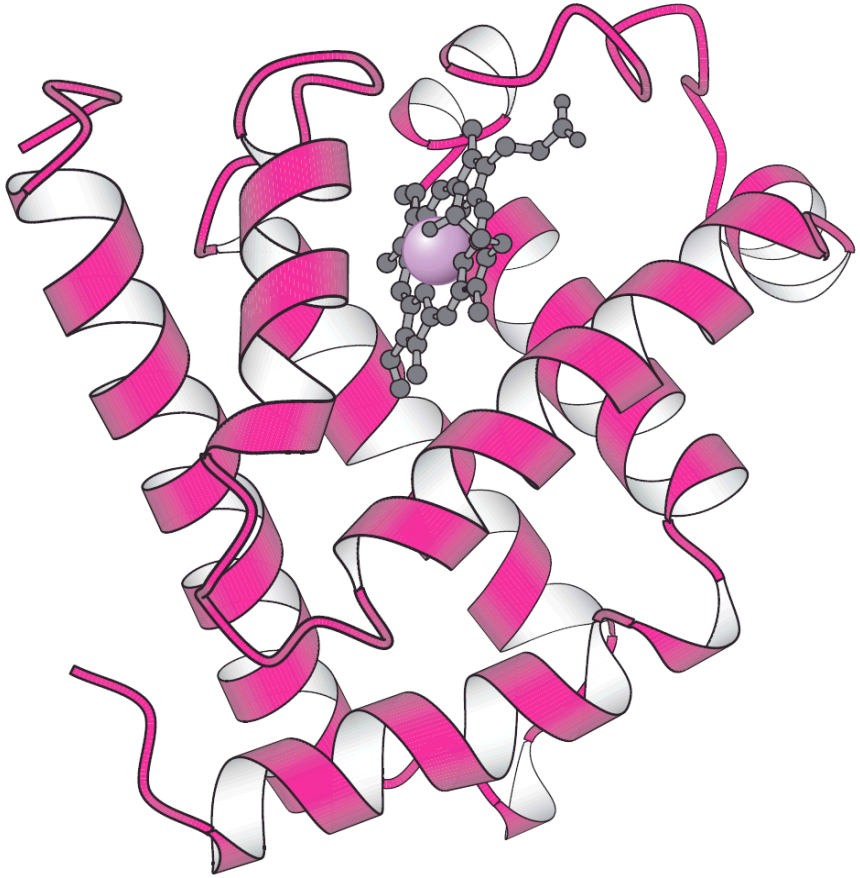
Visualización de las estructuras secundarias

Abrir programa **Mage**

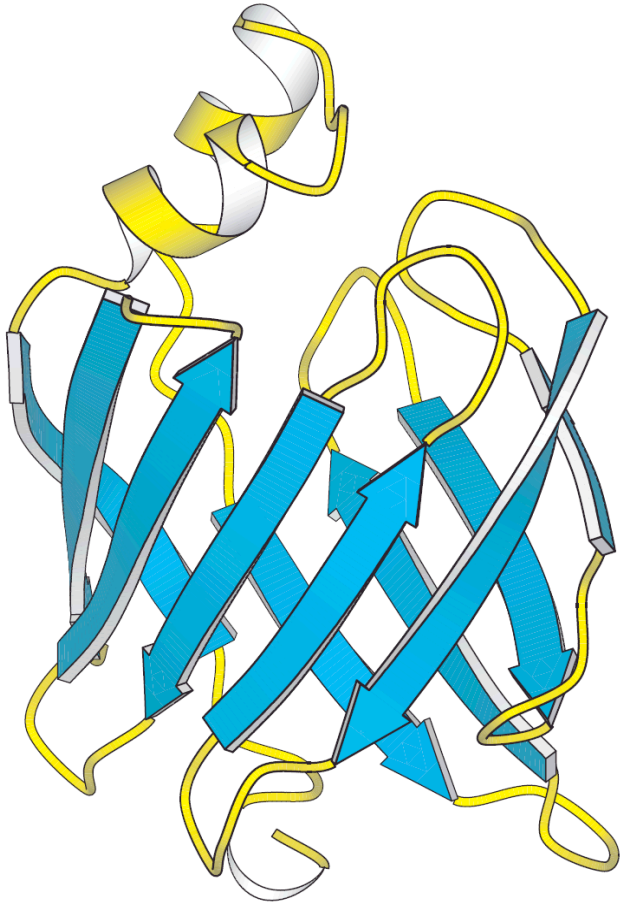
Archivo: EO3 ScStc, kinimage 2, 3, 4, 5.


Primary structure
Secondary structure
Tertiary structure
Quaternary structure
Lys
Lys
Gly
Gly
Leu
Val
Ala
His
Amino acid residues
α Helix
Polypeptide chain

ESTRUCTURA TERCIARIA: distribución espacial de los distintos segmentos de una proteína



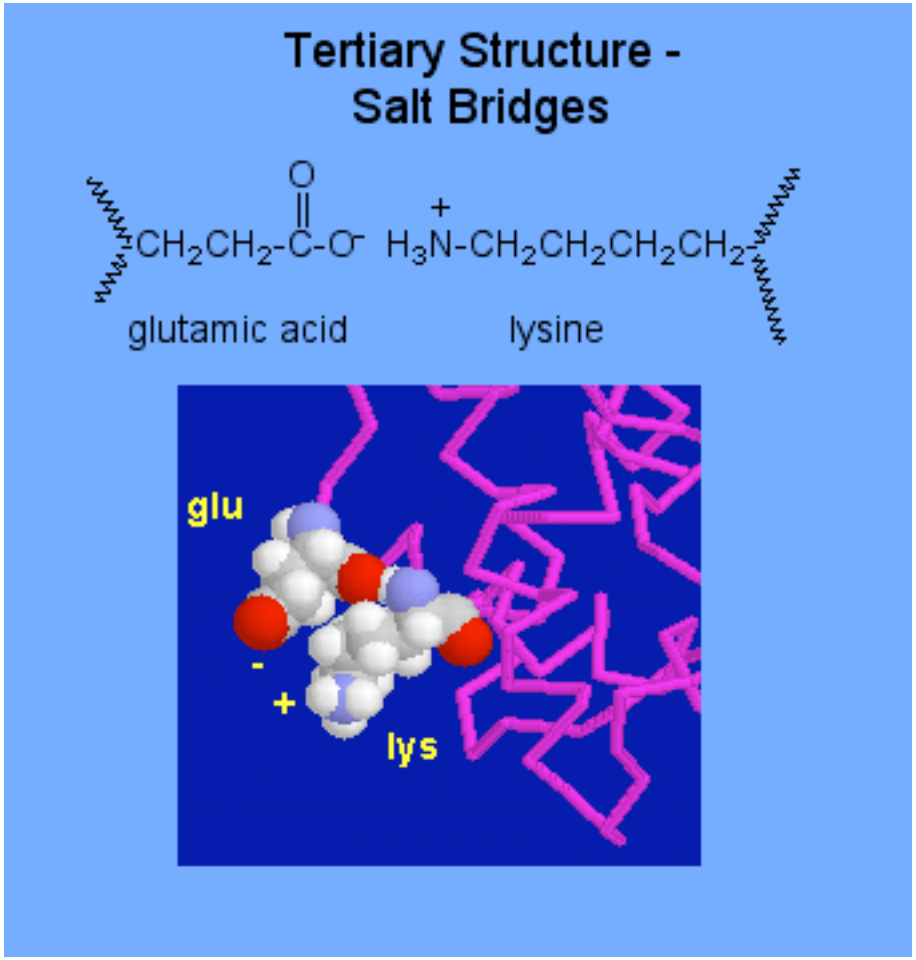
Mioglobina



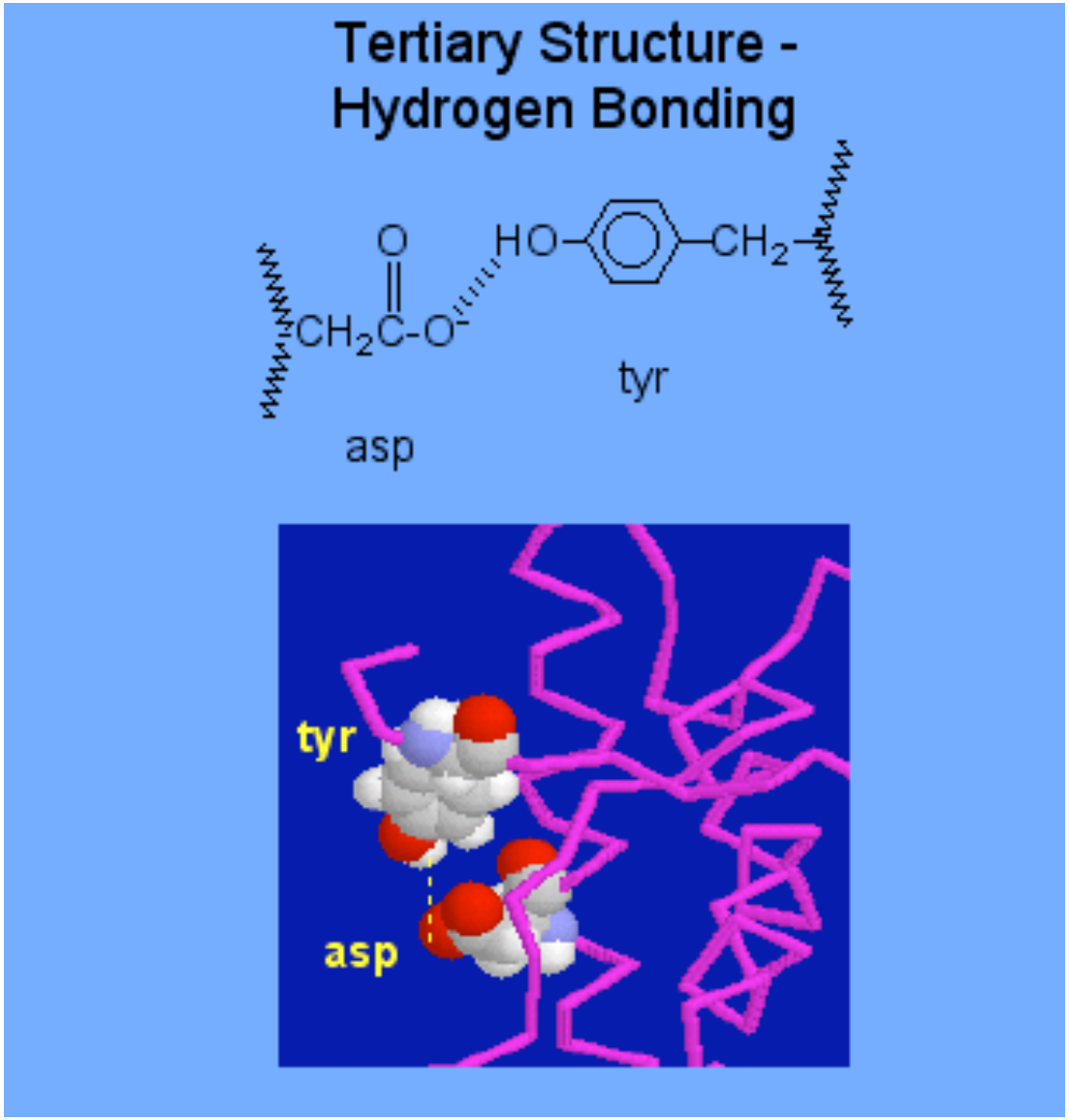
proteína ligante de ac. grasos

Interacciones químicas que estabilizan la estructura terciaria de las proteínas

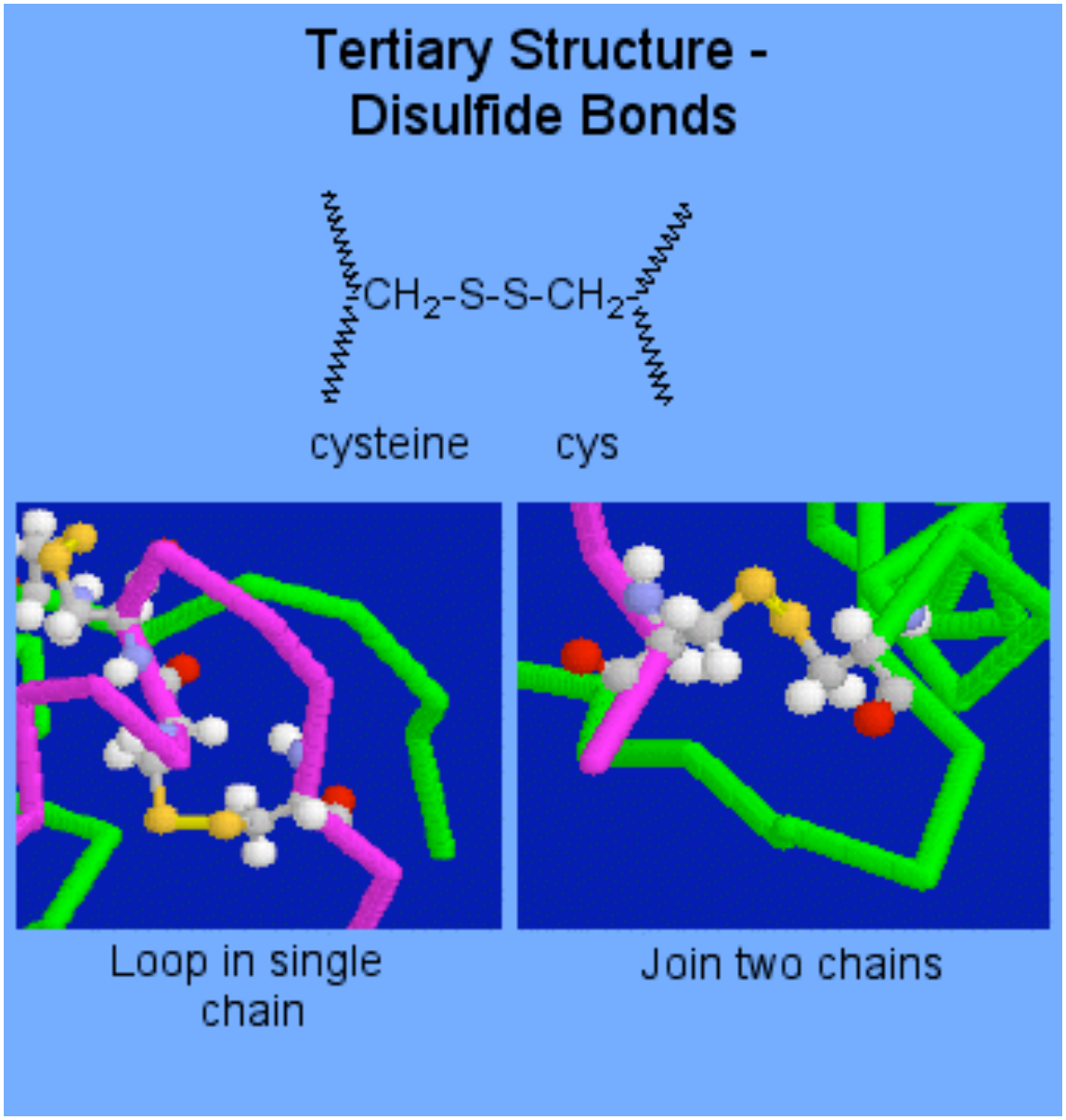
- Interacciones iónicas o electrostáticas (salinas)



- Interacciones puentes de hidrógeno

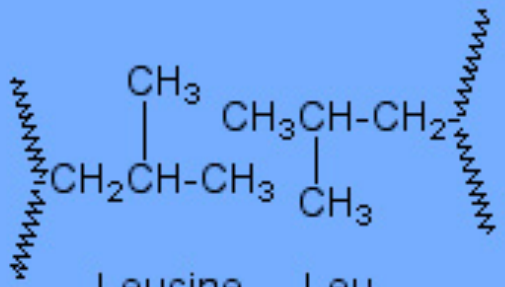


- Interacciones puentes disulfuro

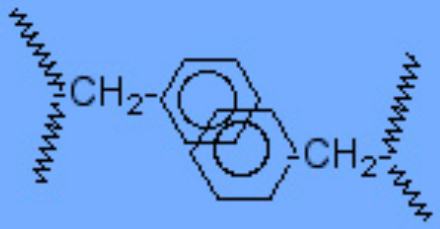


- Interacciones hidrofóbicas

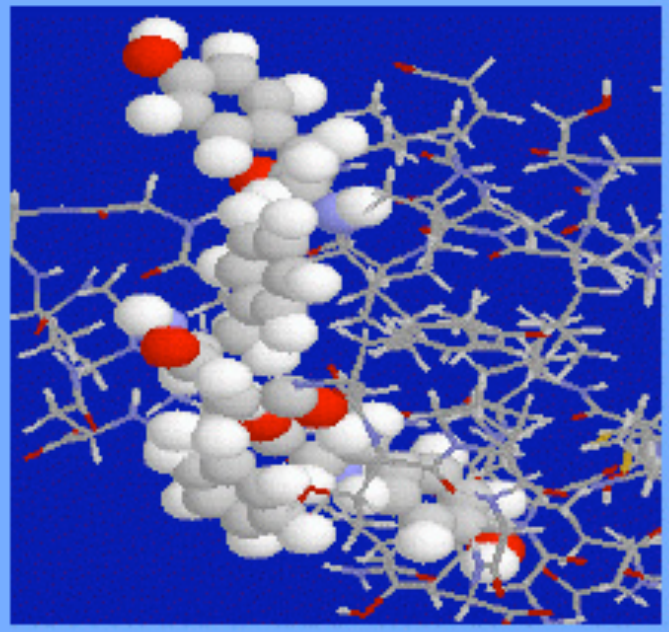
Tertiary Structure - Hydrophobic Interactions



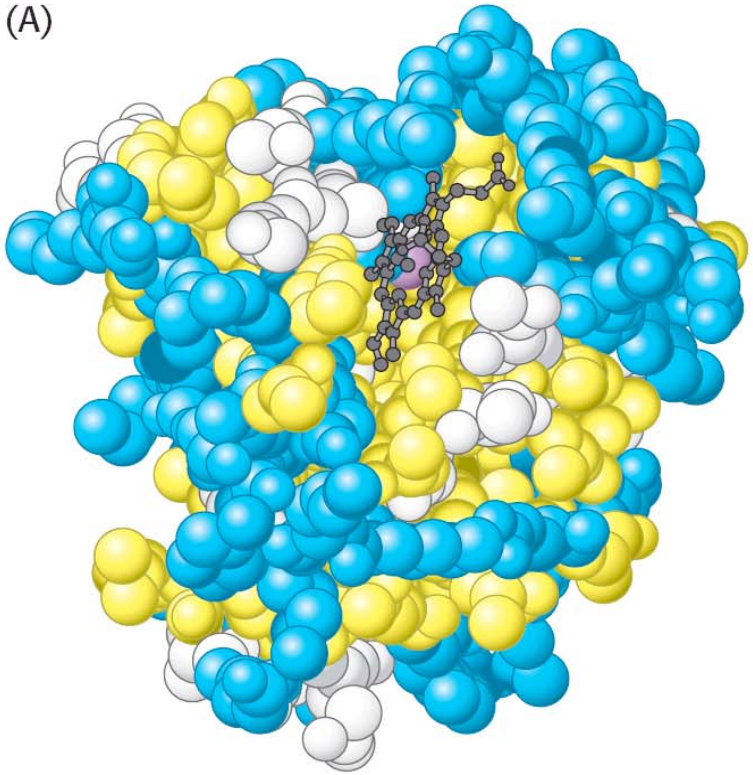
Leucine Leu



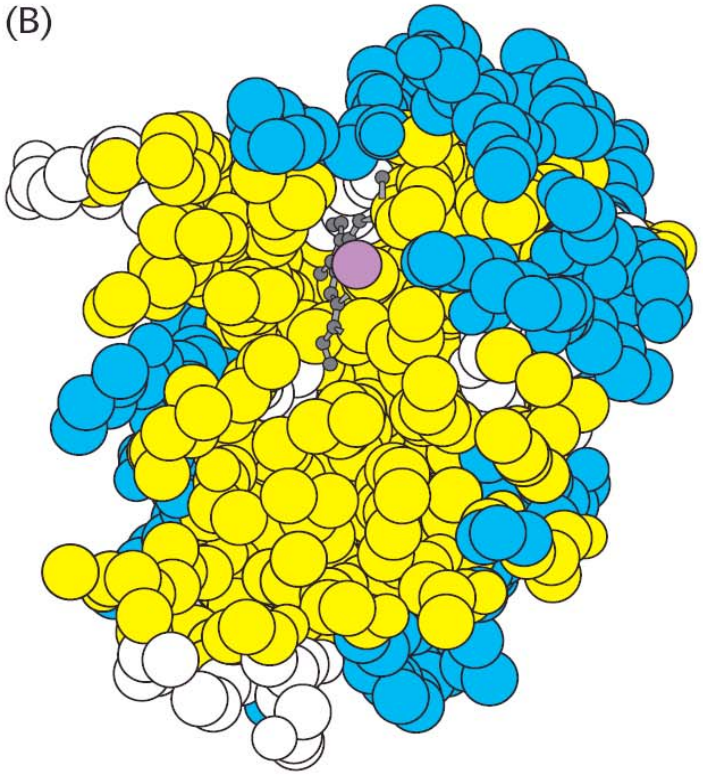
Phenyalanine Phe



En las proteínas citoplasmáticas, los aminoácidos apolares (amarillo) se localizan preferentemente hacia el interior de la proteína



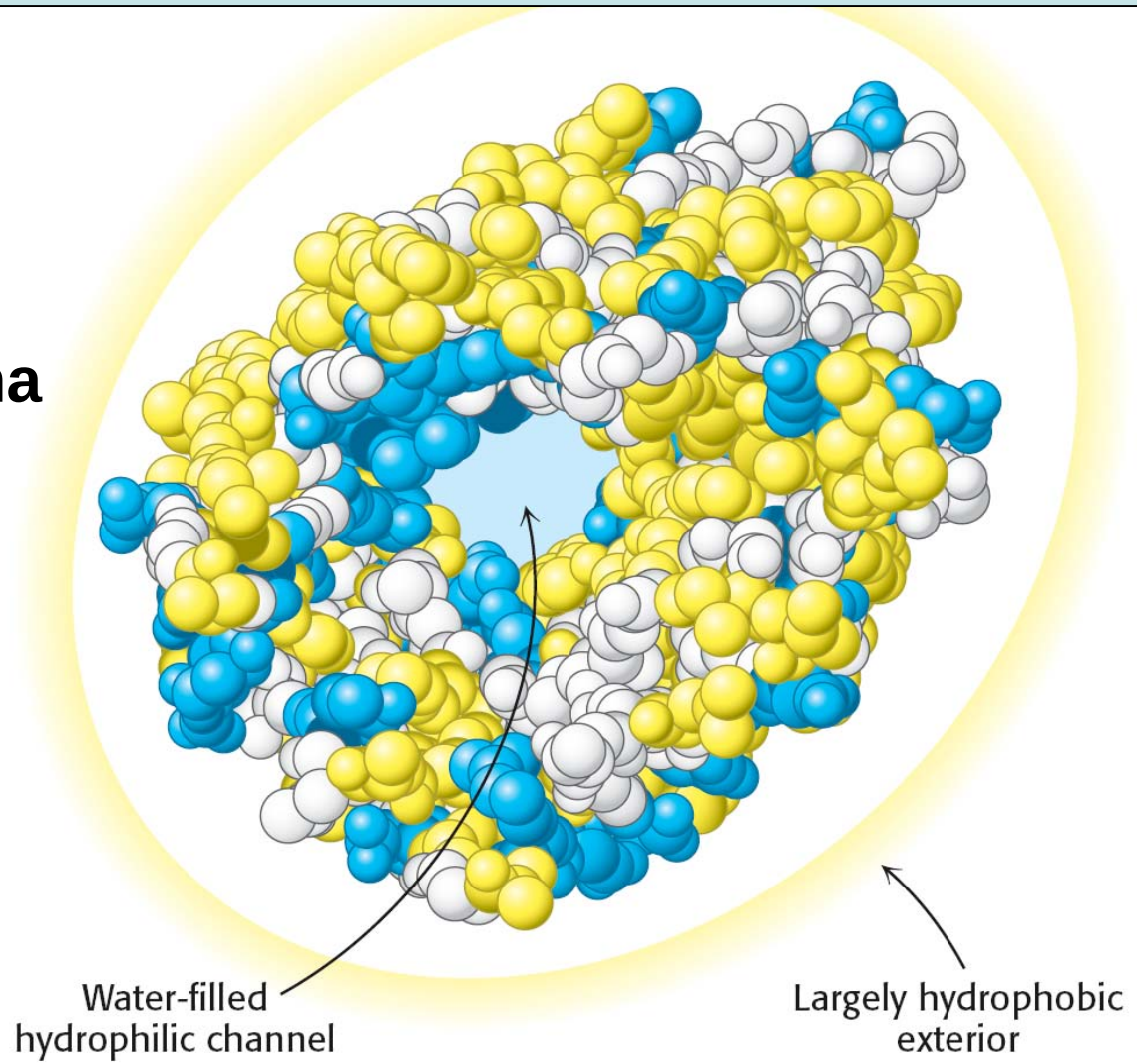
mioglobina



Sección transversal de la mioglobina

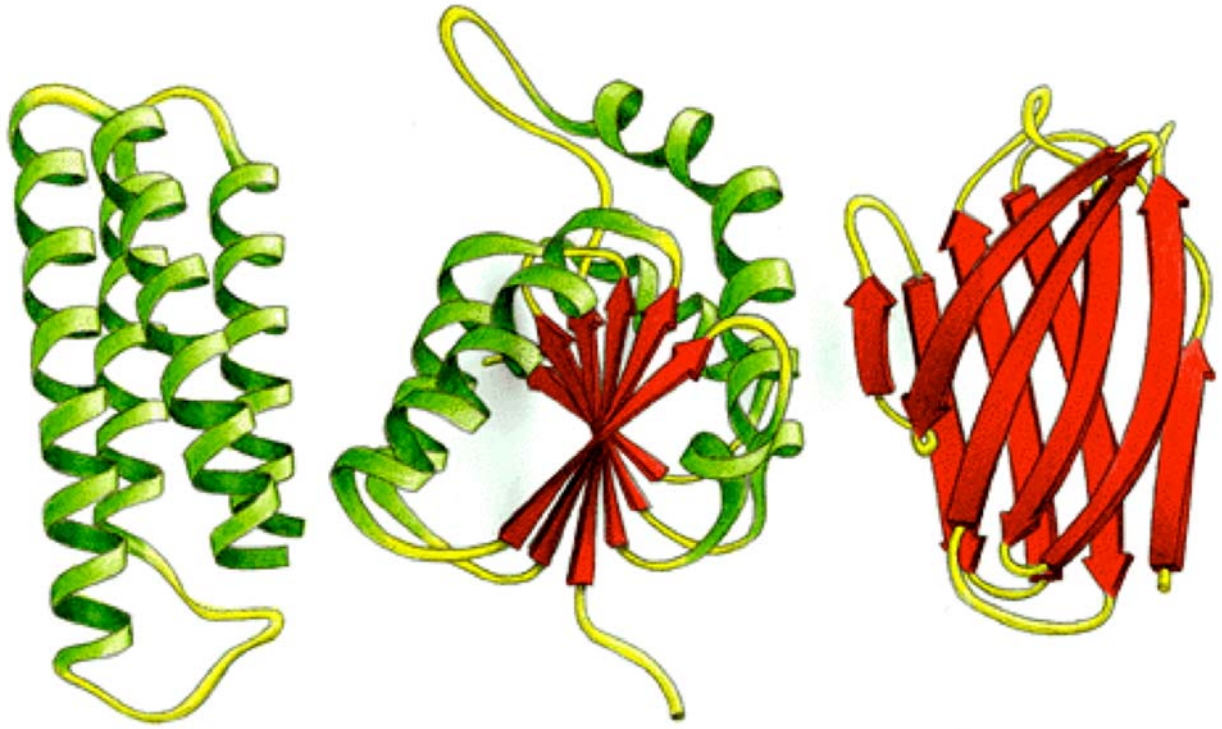
En las proteínas de membranas, los aminoácidos polares (azul) se localizan preferentemente hacia el interior de la proteína

Porina, de la membrana externa de las bacterias

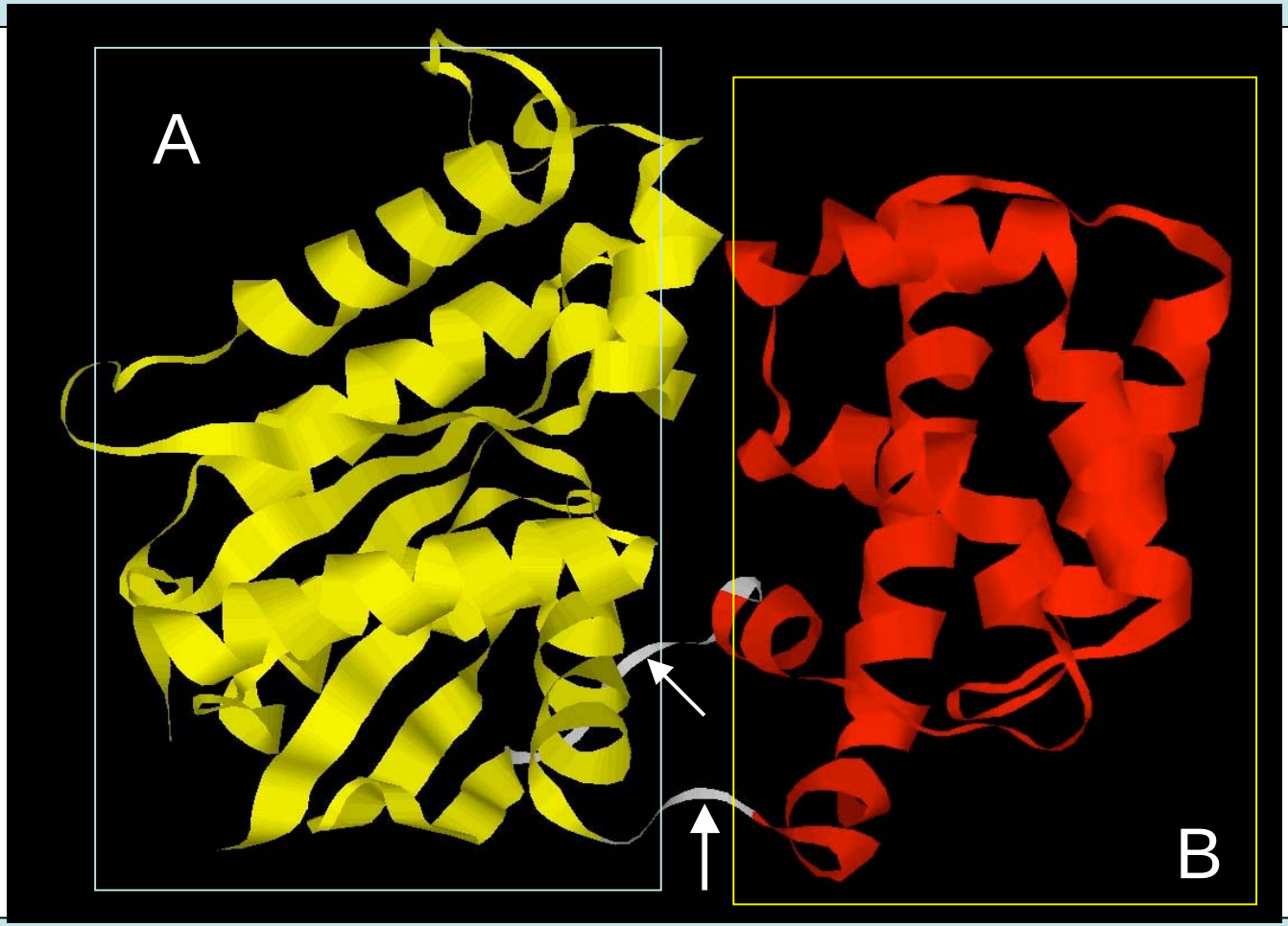


PLIEGUES ESTRUCTURALES:

Existen ciertos pliegues conservados en diferentes proteínas. Estos pliegues pueden formar parte de DOMINIOS estructurales



Proteína G, subunidad Giα: dos dominios independientes unidos por conectores (flechas)
A: dominio ras (amarillo)



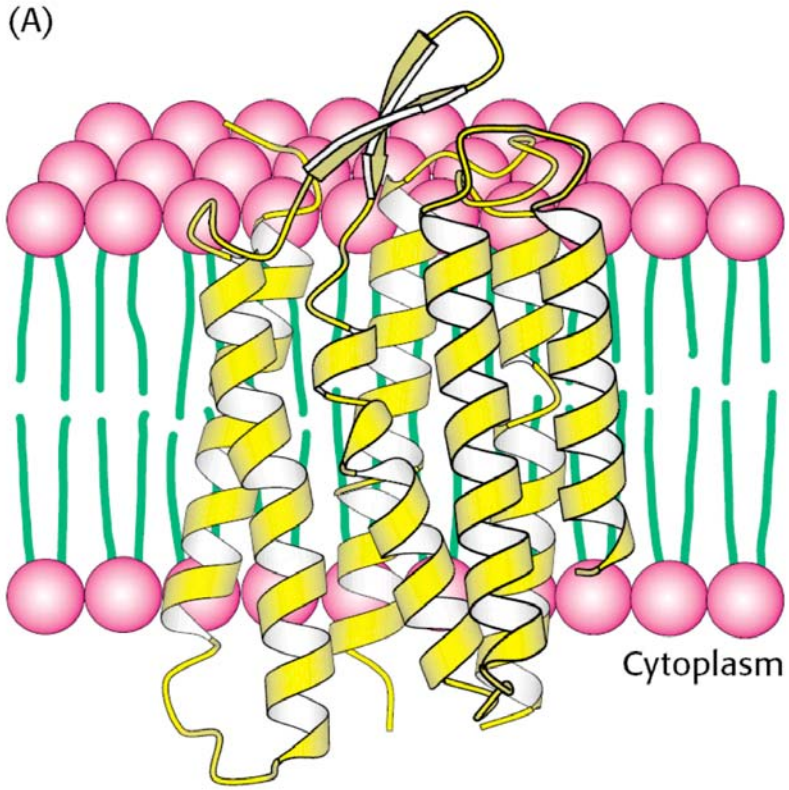
B: dominio helicoidal (rojo)

Visualización de DOMINIOS en la subunidad α de la proteína Gi

Abrir programa rasmol

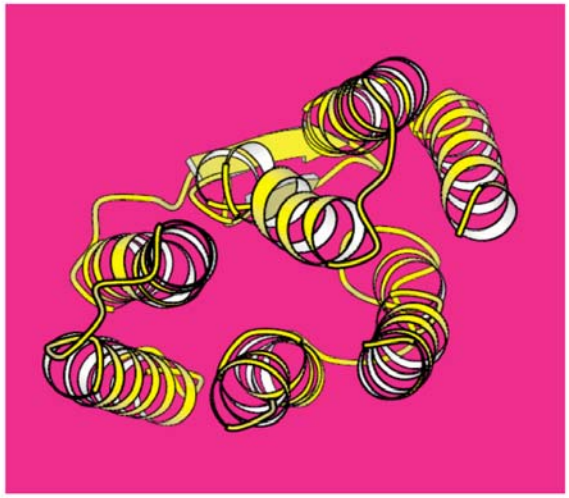
Archivo Gi

Bacteriorodopsina (involucrada en la trasmición de fotones)



(B)

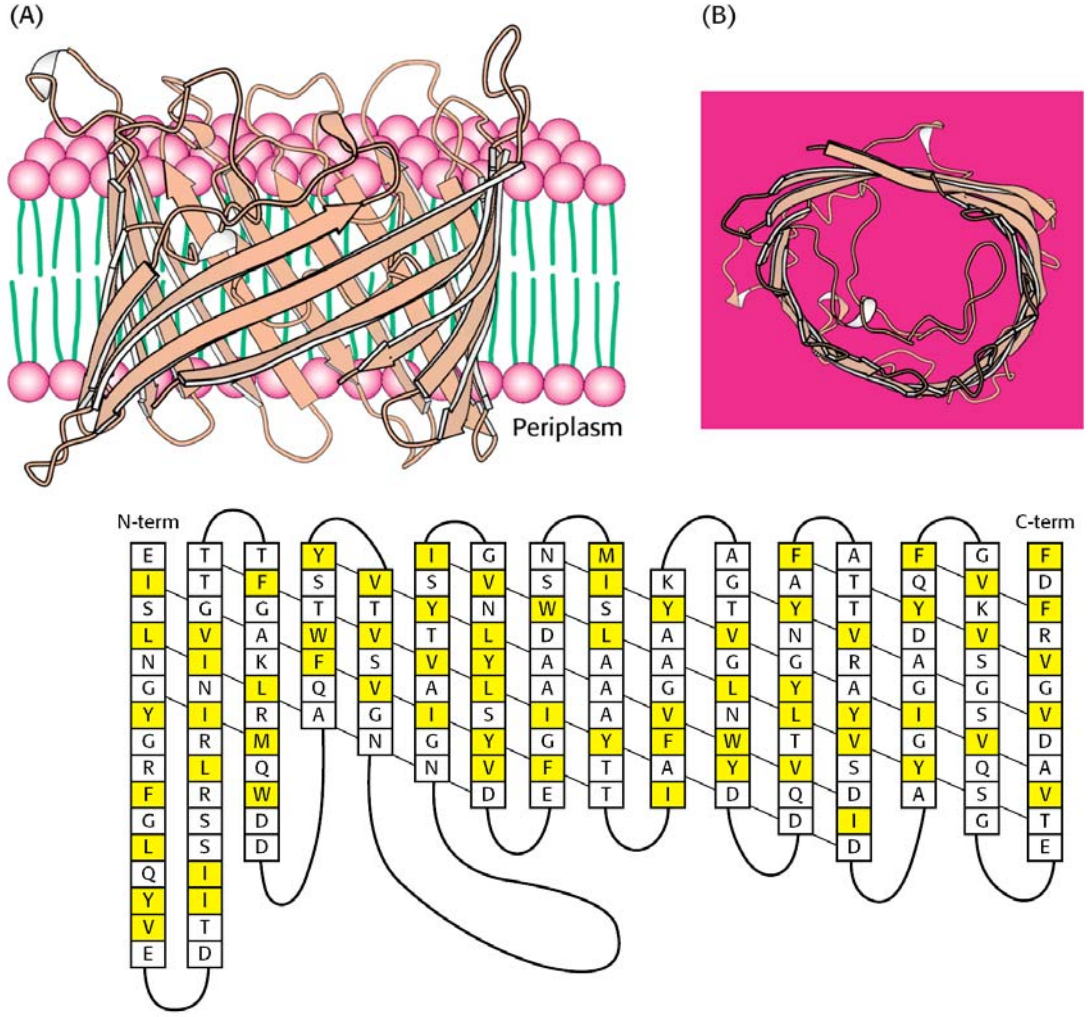
Vista desde el citoplasma



A Q I T G R P E W I W L A L G T A L M G L G T L Y F L V K G M G V S D P D A K K F Y A I T T L V P A
I A F T M Y L S M L L G Y G L T M V P F G G E Q N P I Y W A R Y A D W L F T T P L L L L D L A L L V
D A D Q G T I L A L V G A D G I M I G T G L V G A L T K V Y S Y R F V W W A I S T A A M L Y I L Y V
L F F G F T S K A E S M R P E V A S T F K V L R N V T V V L W S A Y V V V W L I G S E G A G I V P L
N I E T L L F M V L D V S A K V G F G L I L L R S R A I F G E A E A P E P S A D G A A A T S

Secuencia de aminoácidos de la bacteriorodopsina (en amarillo las hélices α)

Porina (proteína que permite el paso de moléculas hidrofílicas a través de la membrana)

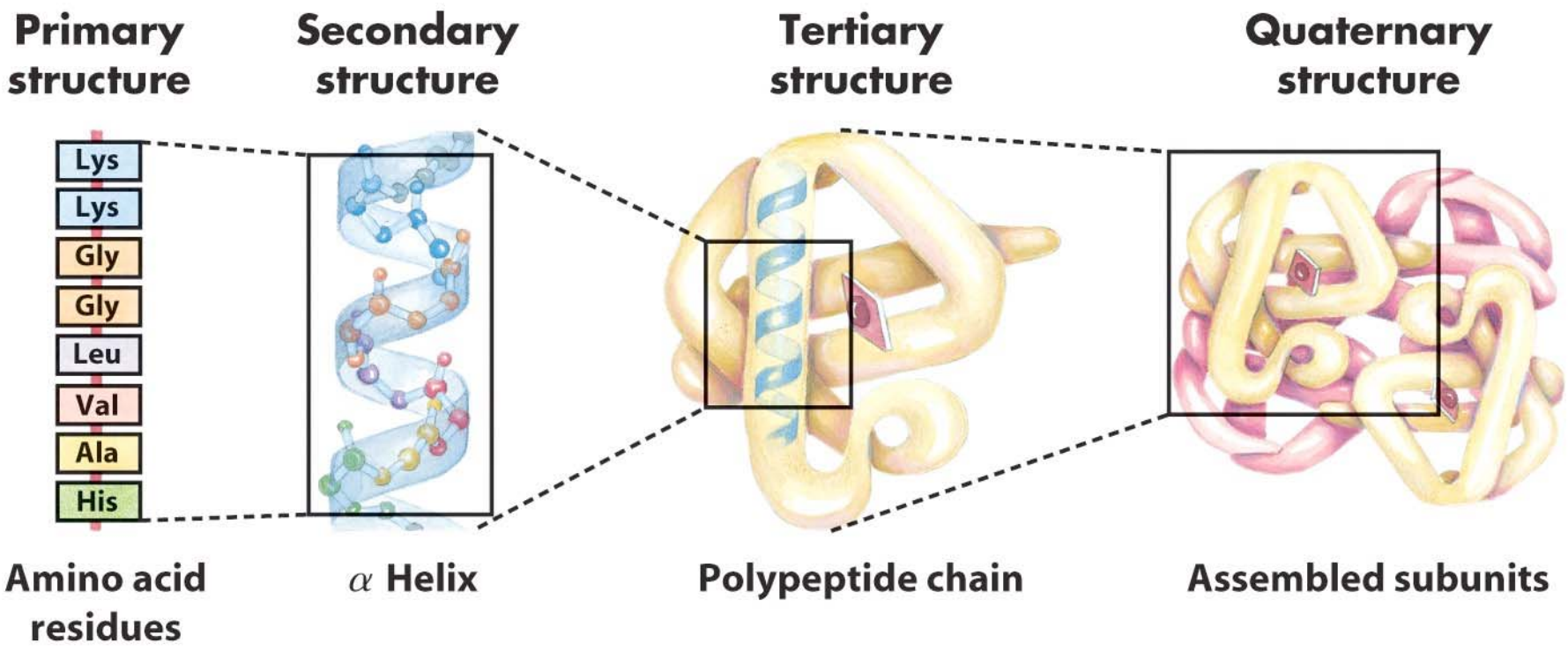


Estructura secundaria de la porina (los aminoácidos hidrofóbicos se orientan hacia los lípidos de la membrana)

Distribución de las cadenas laterales en la estructura terciaria

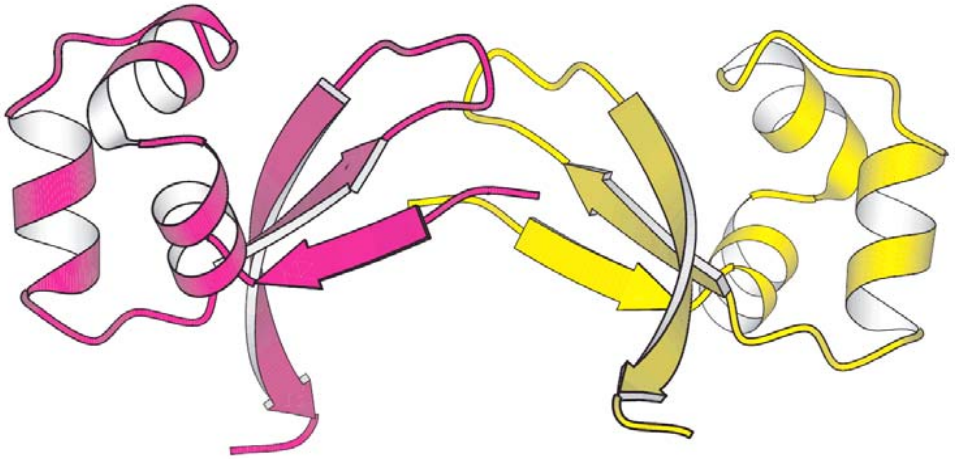
Abrir programa Mage

Archivo: E Basic, kinimage 3

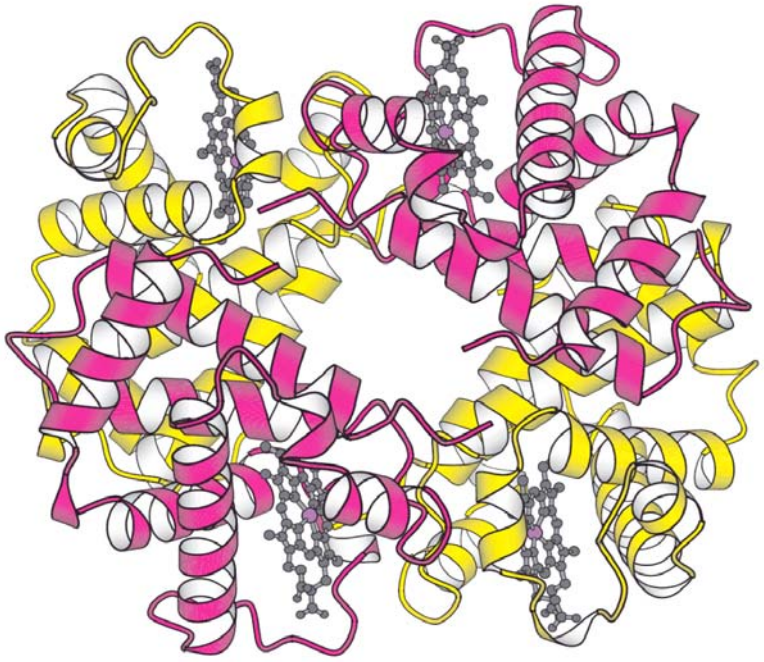

Primary structure
Secondary structure
Tertiary structure
Quaternary structure
Lys
Lys
Gly
Gly
Leu
Val
Ala
His
Amino acid residues
α Helix
Polypeptide chain
Assembled subunits

ESTRUCTURA CUATERNARIA

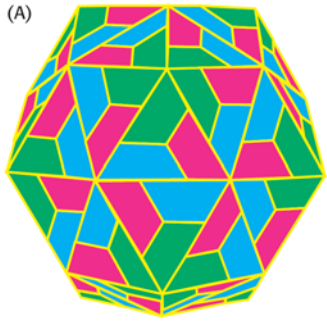
Dímero
TBP

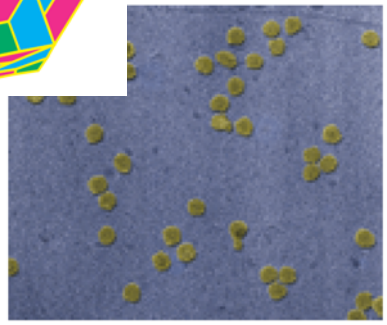


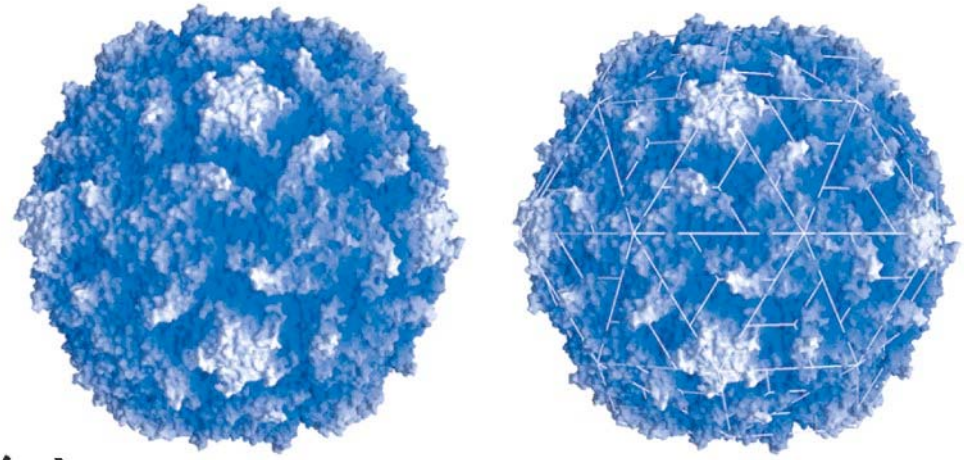
Tetrámero
Hemoglobina



Oligómero
virus

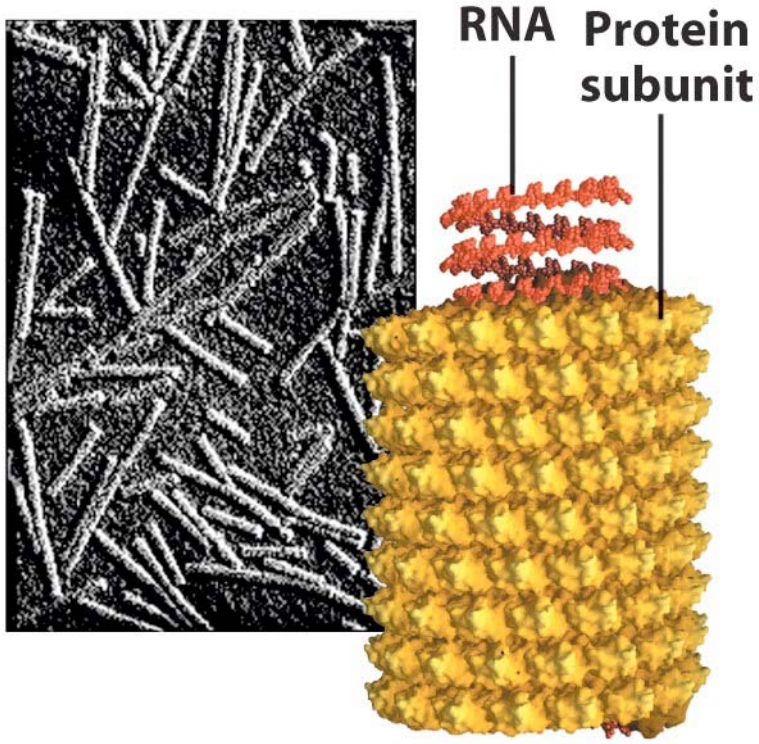






(a)

virus de la polio



(b)

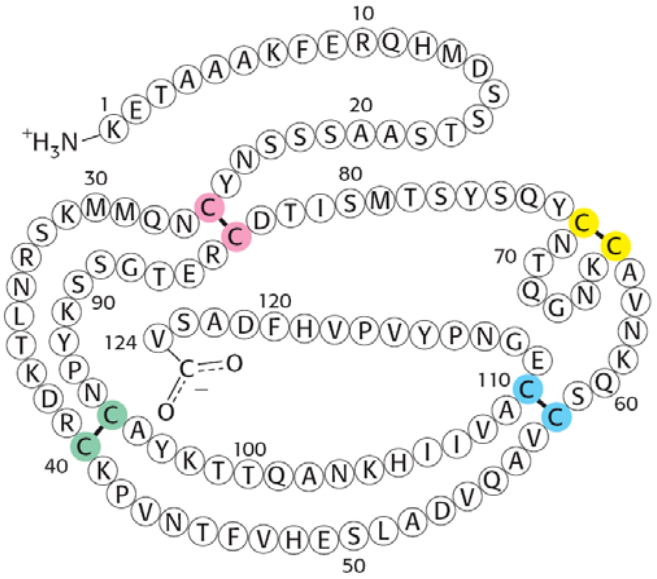
virus del mosaico del tabaco

RELACIÓN ENTRE LA ESTRUCTURA Y LA FUNCIÓN EN LAS PROTEÍNAS

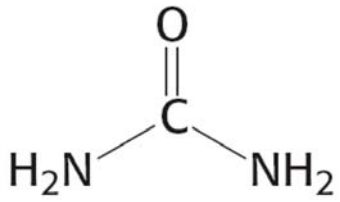
IMPORTANCIA DEL PLEGAMIENTO EN LA FUNCIÓN DE LAS PROTEÍNAS

¿Qué interacciones mantienen la estructura terciaria de una proteína?

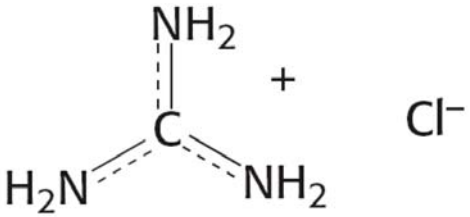
¿Cómo se puede alterar la estructura terciaria?



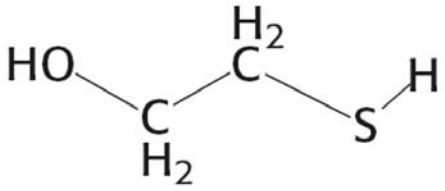
Bovine ribonuclease



Urea



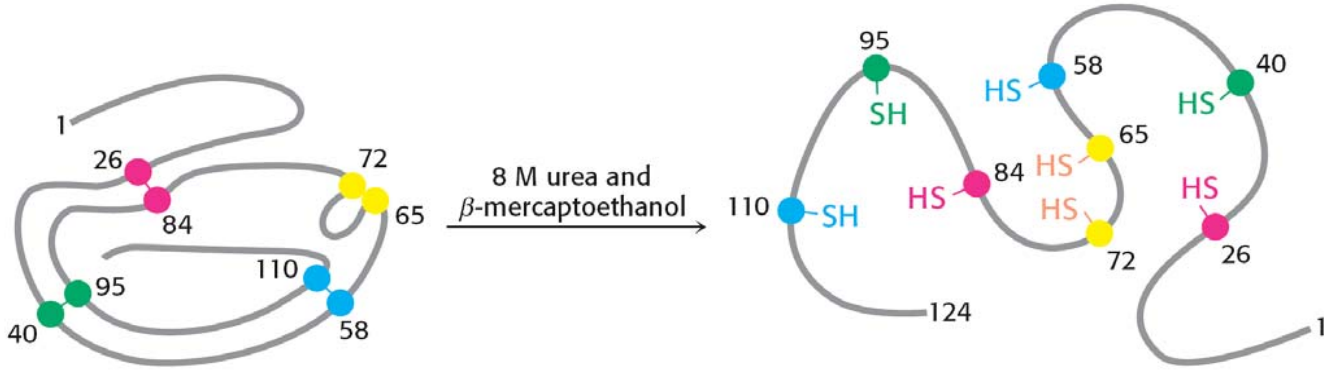
Guanidinium chloride



β-Mercaptoethanol

¿Qué interacciones se afectan al tratar una proteína con urea o con cloruro de guanidina?

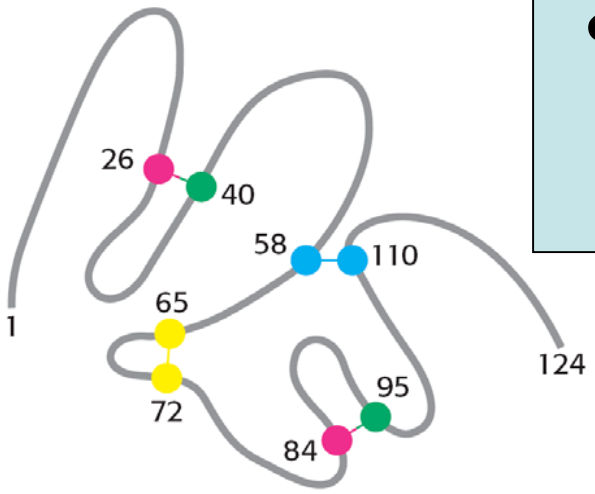
¿Qué efecto tiene el β-mercapto etanol en las proteínas?



Native ribonuclease

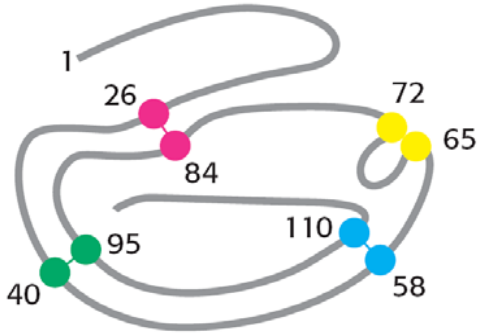
Denatured reduced ribonuclease

¿Se puede recuperar la estructura y/o la función de una proteína que se ha desnaturado?



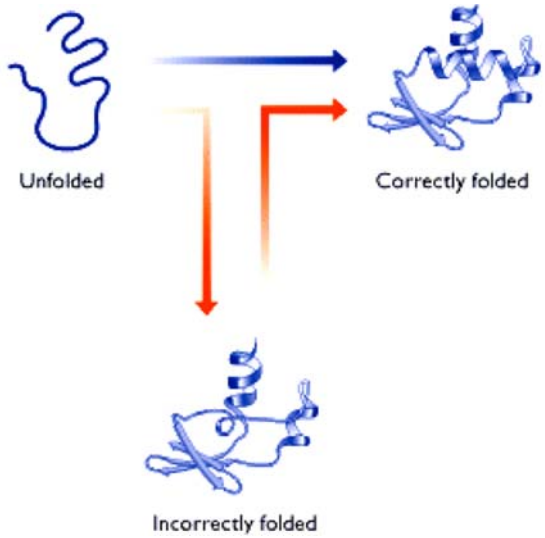
Scrambled ribonuclease

Trace of
β-mercaptoethanol



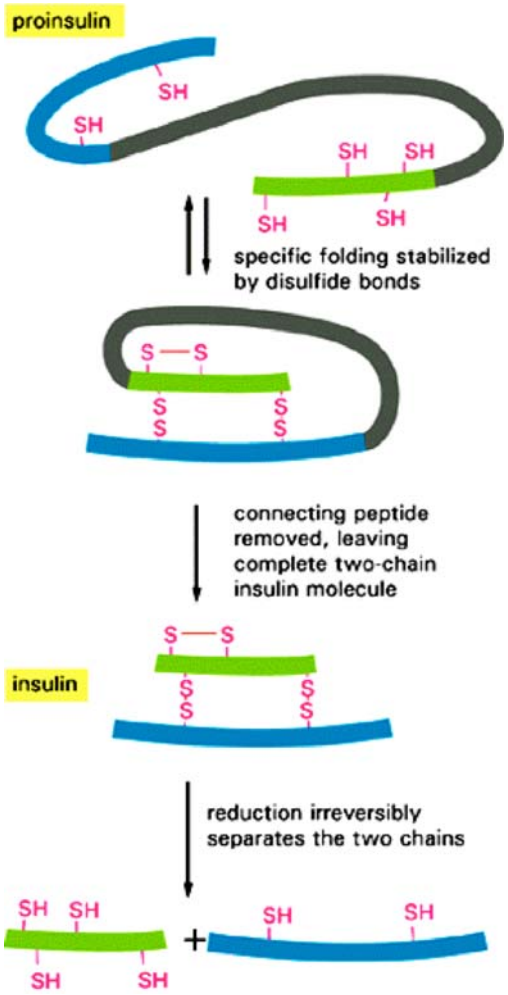
Native ribonuclease

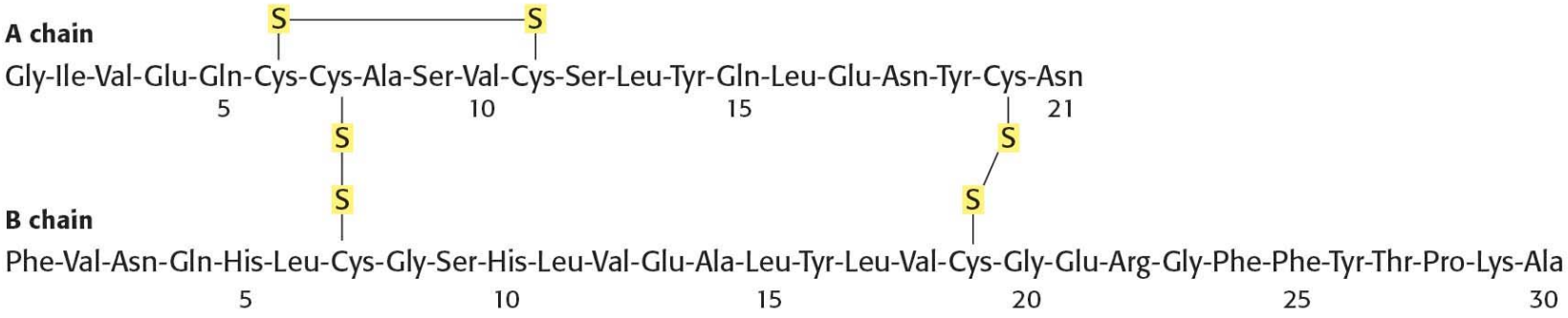
¿Son estables los intermediarios del plegamiento?



INSULINA.

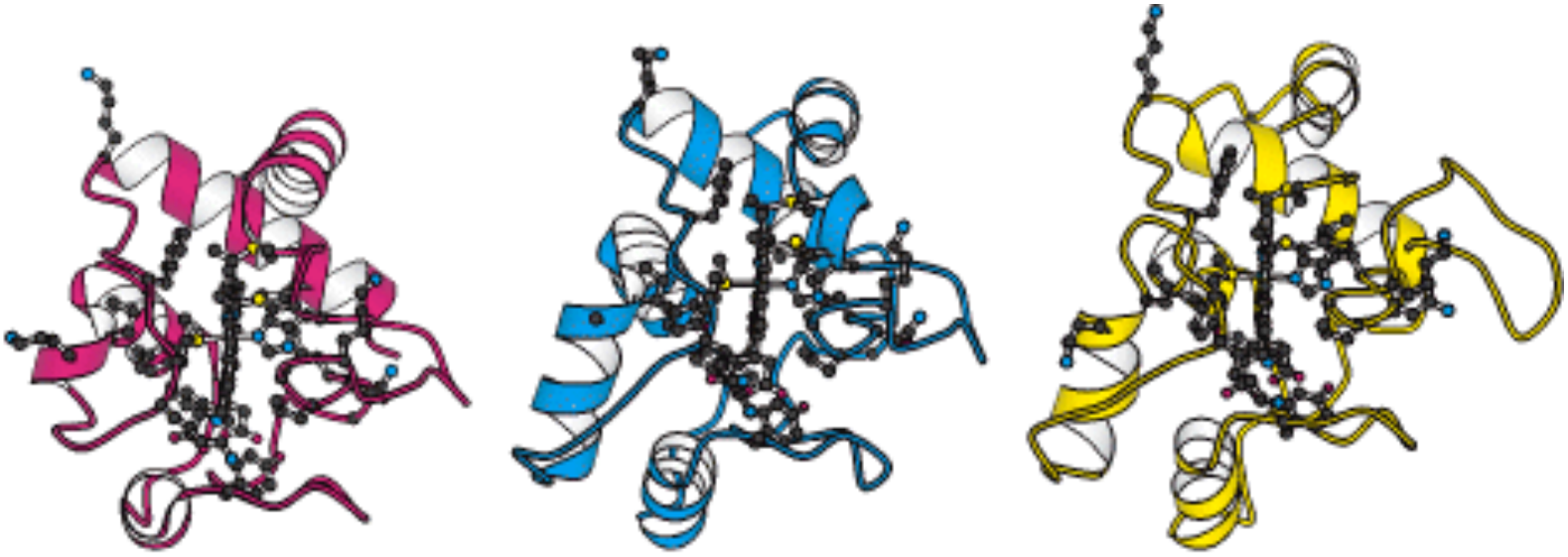
Las cadenas aisladasde la insulina madura son incapaces de generar la insulina nativa cuando se elimina el agente reductor del medio.
¿Qué conclusión puede obtener Ud. de este experimento?





En distintos organismos, más que la secuencia, se conserva la estructura (evolución).

Citocromo C

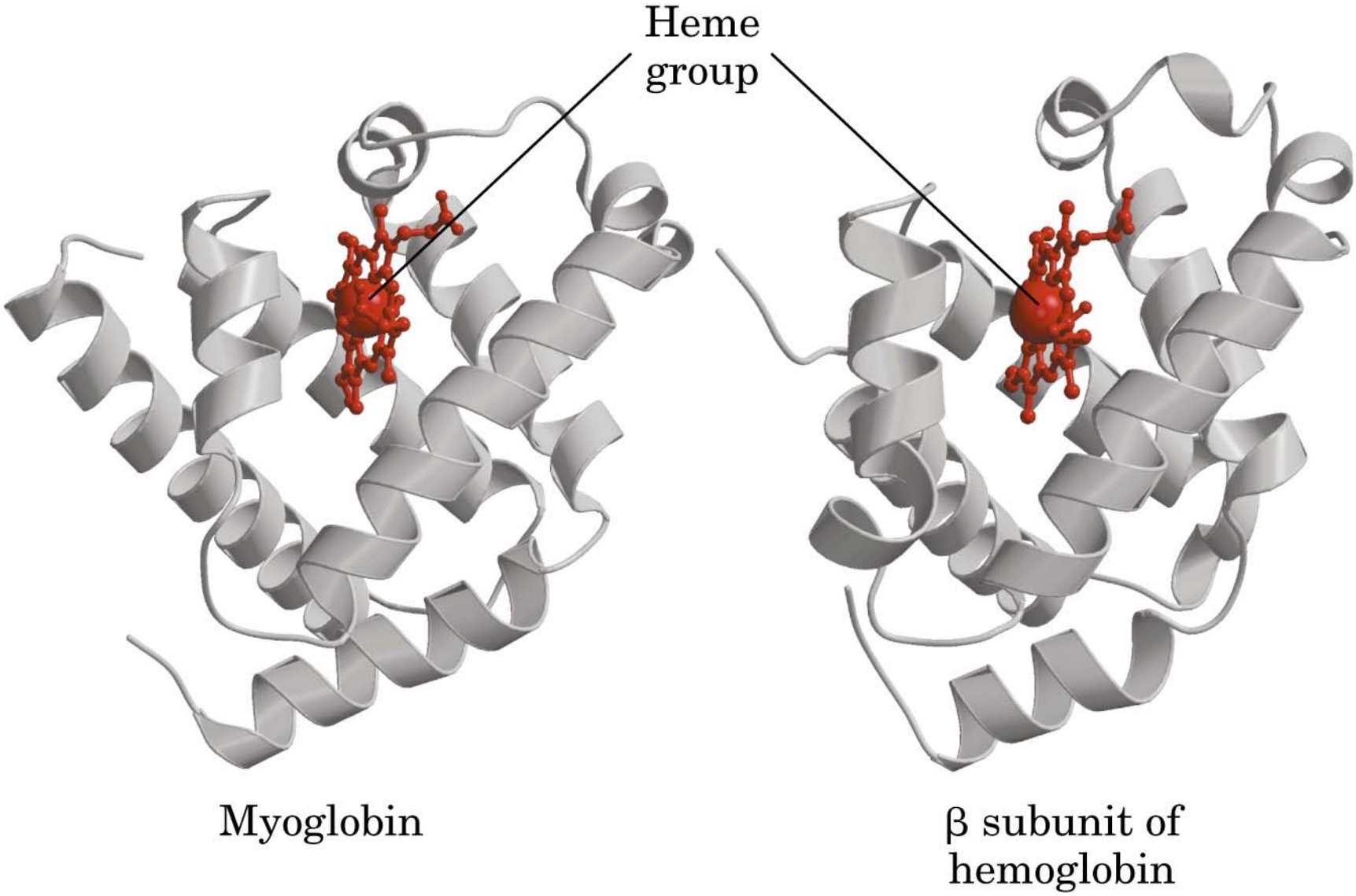


26 de 104 aminoácidos se conservan en todos los citocromos

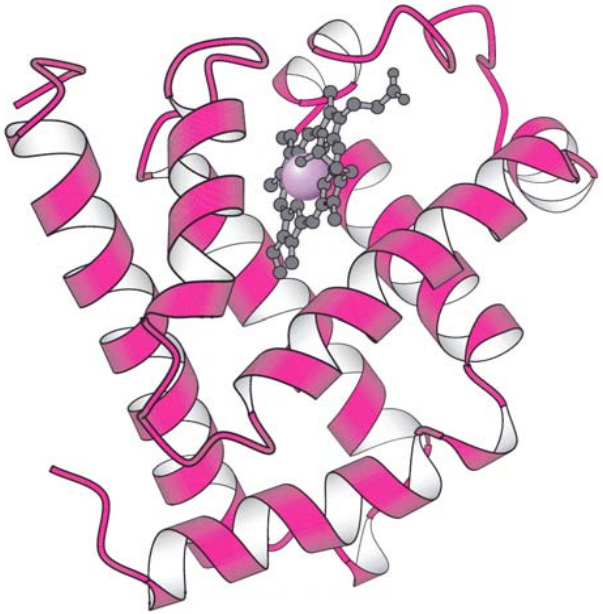
La estructura de una proteína determina su función

CAMBIOS EN LA ESTRUCTURA DE UNA PROTEÍNA PUEDEN SIGNIFICAR GRANDES DIFERENCIAS EN LA FUNCIÓN

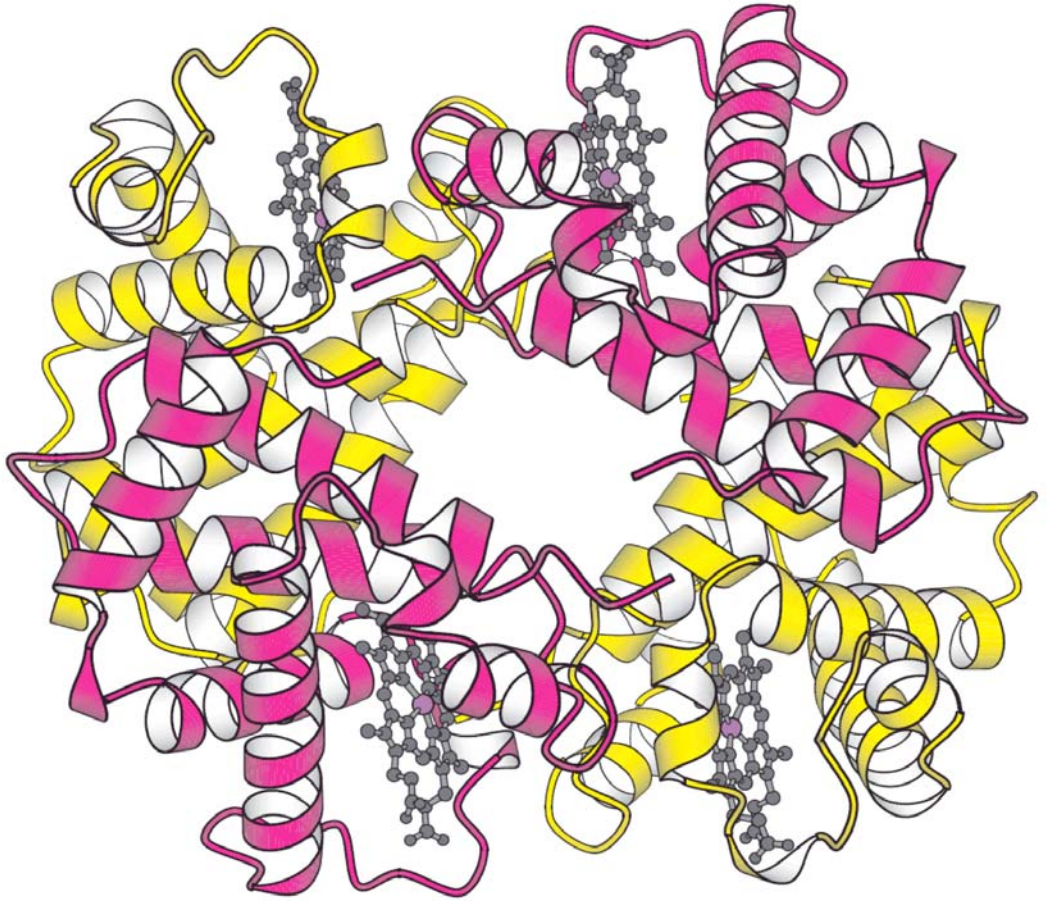
Las Hb y Mb tienen estructuras conservadas



sin embargo sus funciones son diferentes:¿por qué razón?



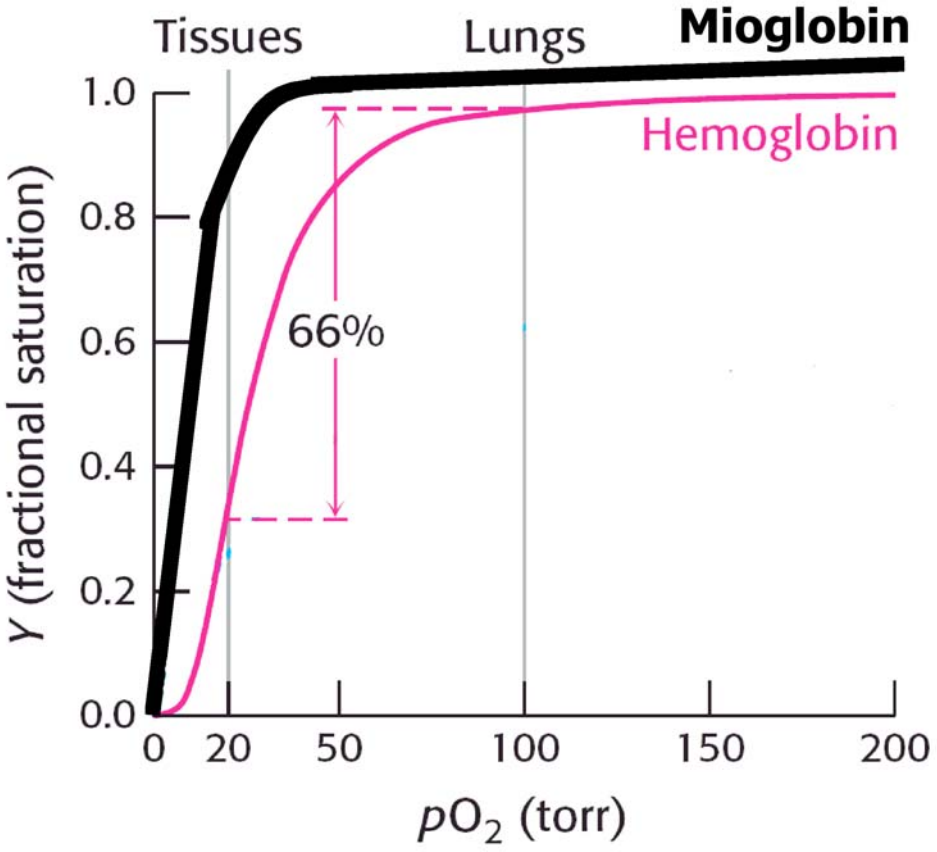
mioglobina: monomero



Hemoglobina: tetrámero

SATURACIÓN CON O_2 DE LA HEMOGLOBINA Y MIOGLOBINA

¿Cómo se explican las curvas de saturación diferentes?



$$MbO_2 \rightarrow Mb + O_2$$

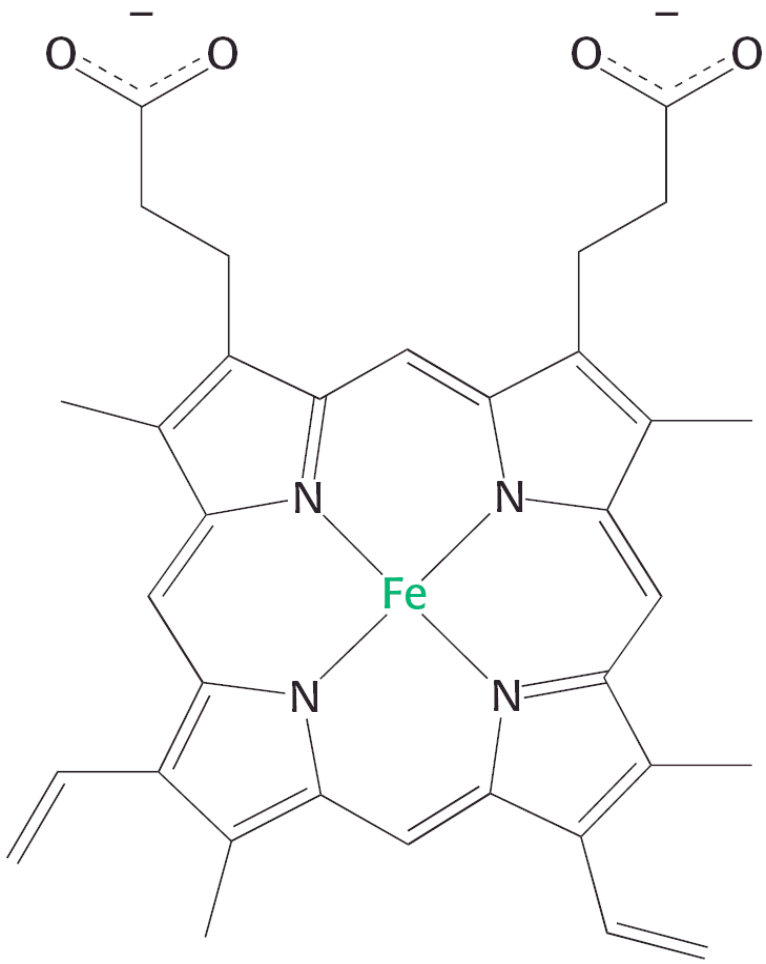
$$K = \frac{[Mb]\,[O_2]}{[MbO_2]} \qquad Y = \frac{pO_2}{pO_2 + K}$$

$$(HbO_2)^n \rightarrow nHb + nO2$$

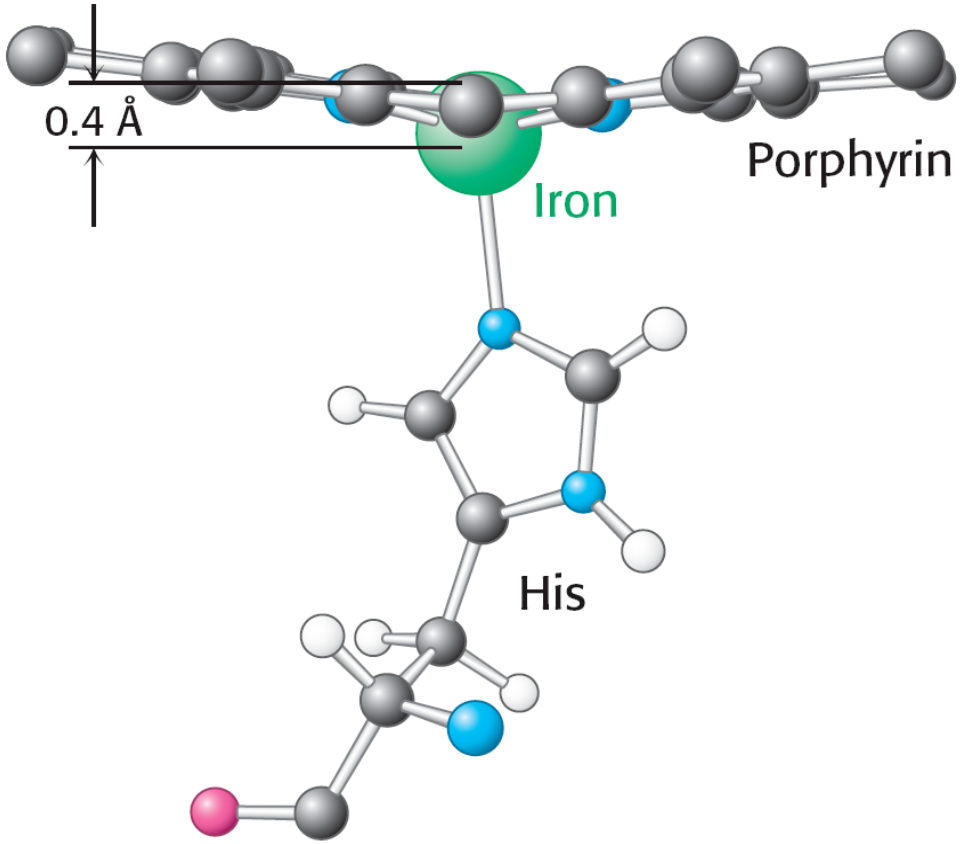
$$Y = \frac{(pO2)^n}{(pO2)^n + (P_{50})^n}$$

$$\log \frac{Y}{1 - Y} = n \log O_2 - n \log P_{50}$$

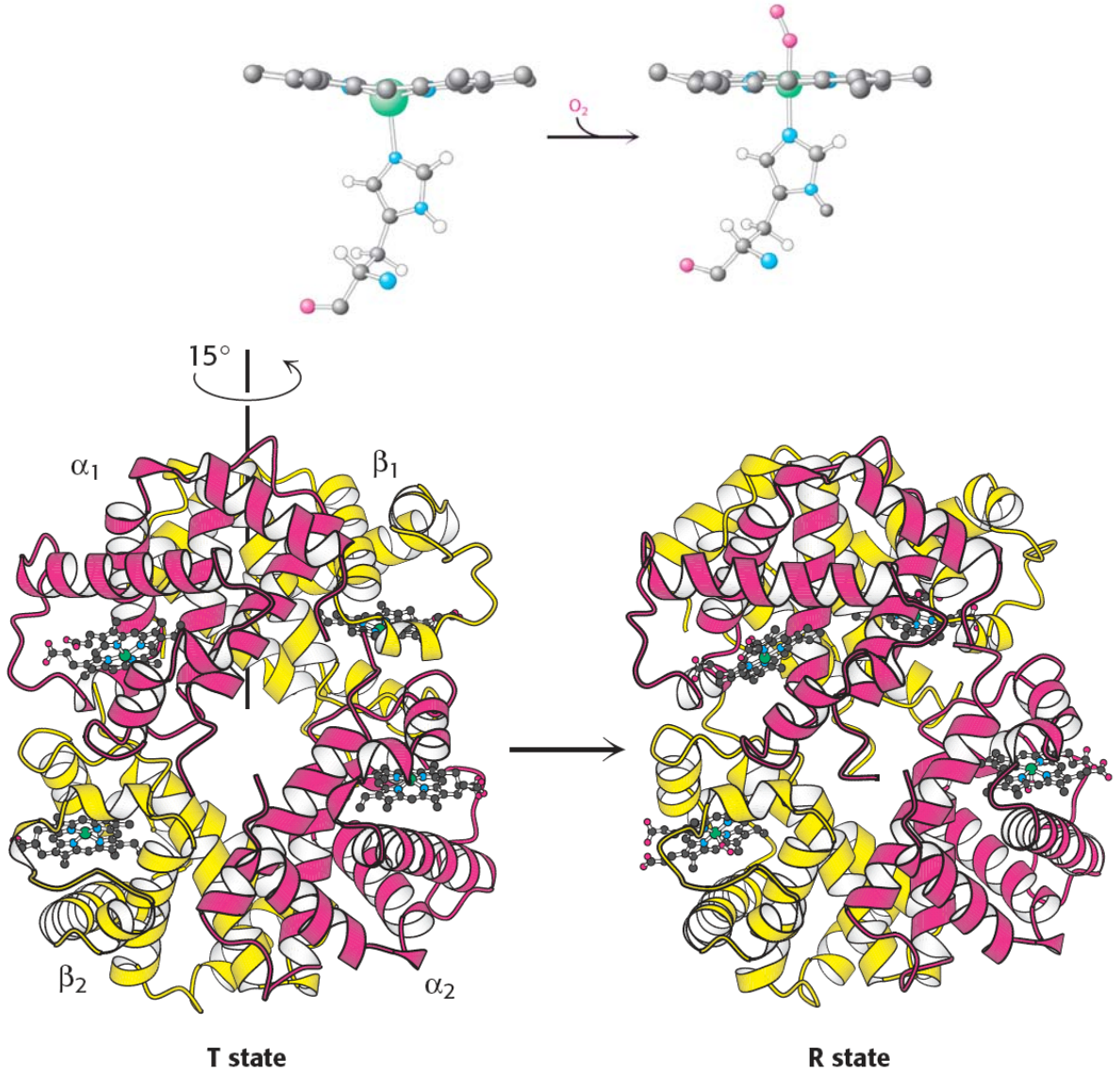
ESTRUCTURA DEL GRUPO HEMO



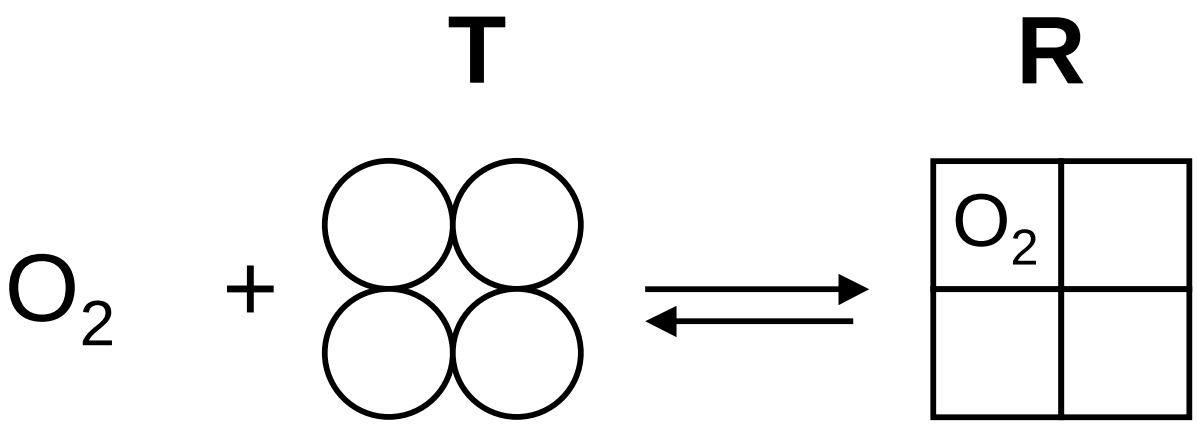
Heme
(Fe-protoporphyrin IX)



LA UNIÓN DE O_2 PROVOCA CAMBIOS EN LA ESTRUCTURA DE LA Hb



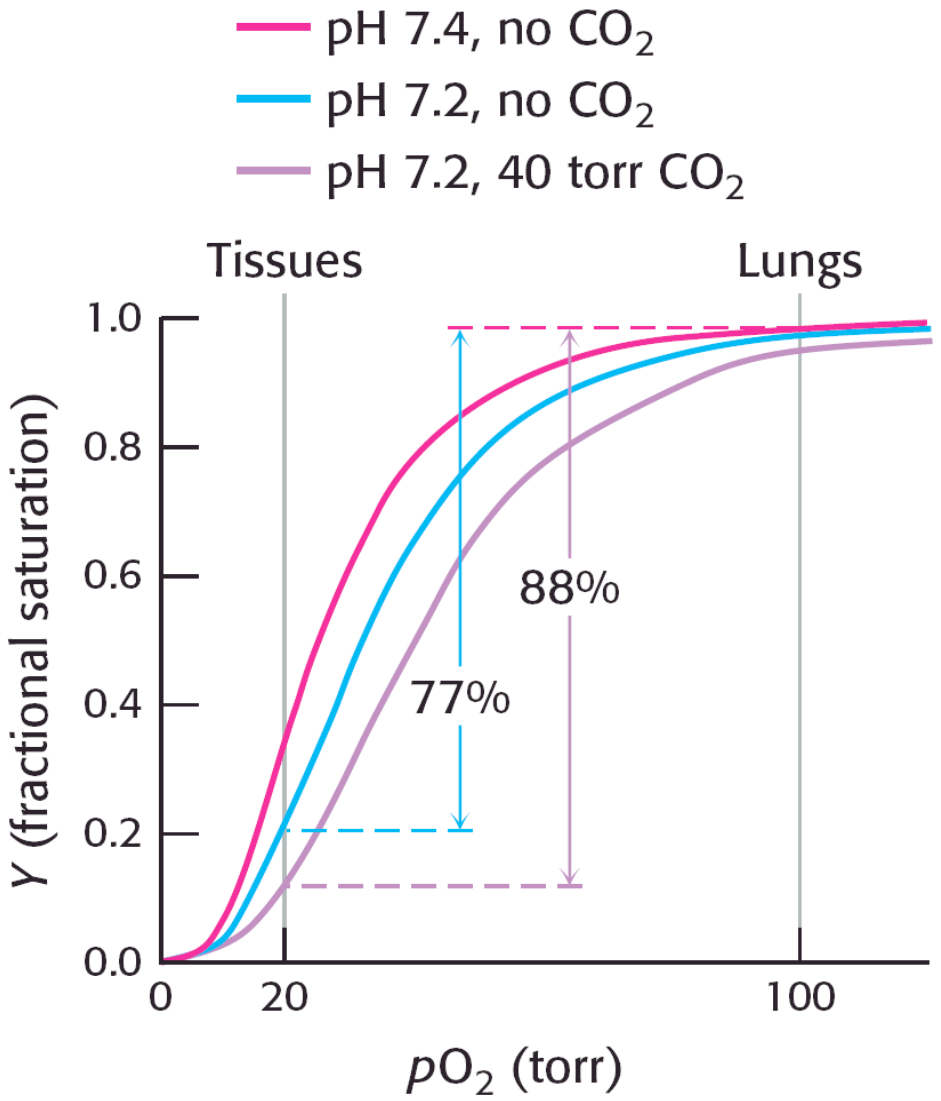
Modelo concertado: Jacques Monod



$$L = \frac{[T]}{[R]}$$

T: estado de baja afinidad por el oxígeno
R: estado de alta afinidad por el oxígeno

EL pH Y EL CO_2 AFECTAN LA SATURACIÓN DE LA Hb



EN LOS SERES HUMANOS EXISTEN VARIOR TIPOS DE Hb

EMBRIONARIAS

Hb GOWER1	$\zeta_2 \varepsilon_2$
Hb GOWER2	$\alpha_2 \varepsilon_2$
Hb PORTLAND	$\zeta_2 \gamma_2$

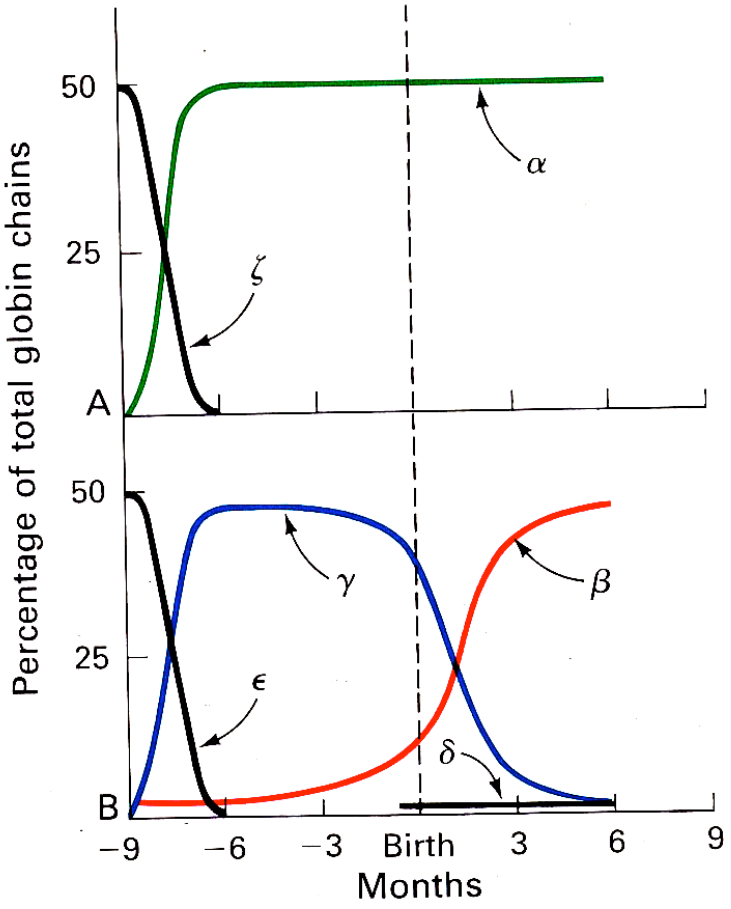
FETAL

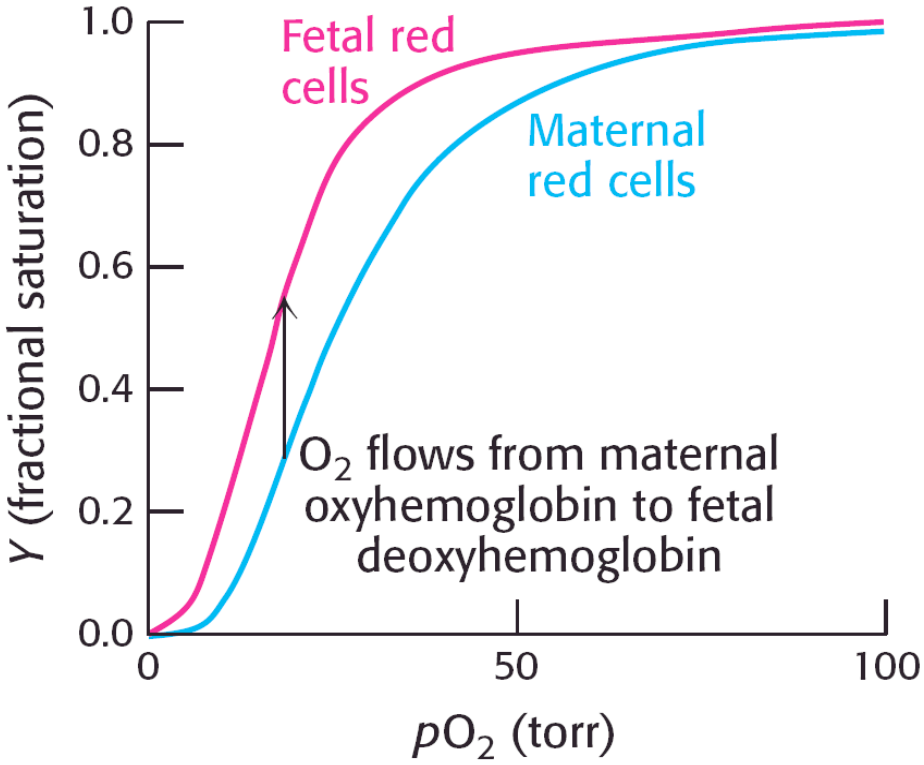
Hb F	$\alpha_2 \gamma_2$

ADULTAS

Hb A	$\alpha_2 \beta_2$
Hb A	$\alpha_2 \delta_2$

LAS DIFERENTES GLOBINAS DE HUMANOS SE EXPRESAN Y SE COMPORTAN DE MANERA DIFERENTE





Abrir programa rasmol

Archivo Hemoglobina.pdb

LA FUNCIÓN DE LAS PROTEÍNAS SE PUEDE ALTERAR POR MODIFICACIONES DE LA ESTRUCTURA

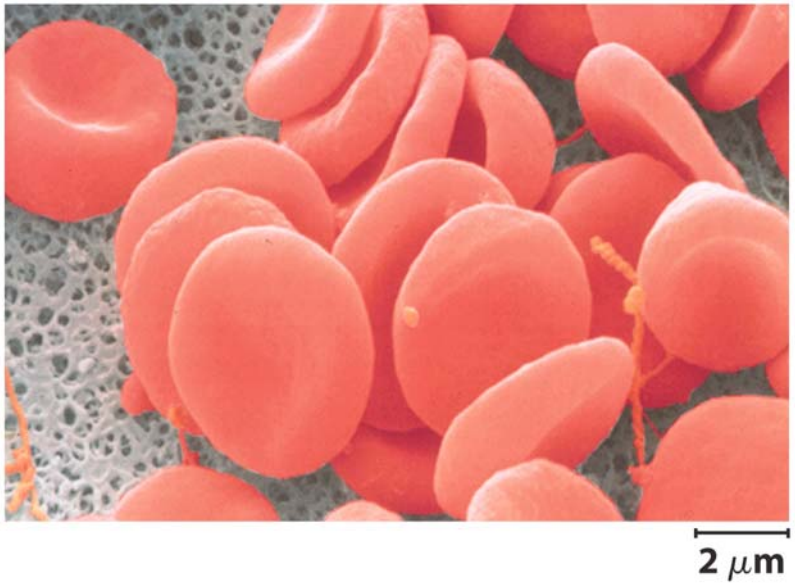
HEMOGLOBINAS MUTANTES

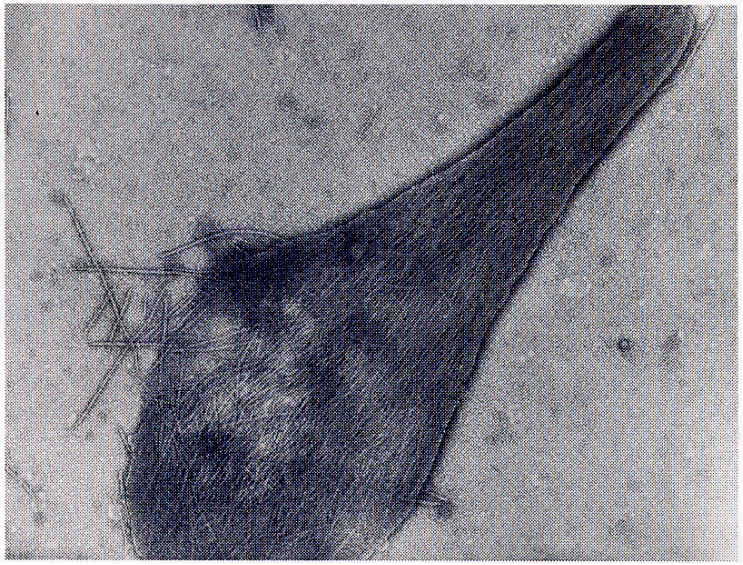
TIPO	MUTACIÓN	EFECTO
Hammersmith	F42βS CD1	debilita unión de hemo
Bibba	L136αP H19	rompe hélice α
Yakima	D99βH G1	rompe puente H que estabiliza forma T
Kansas	N102βT G4	rompe puente H que estabiliza forma R
S	E6βV	polimerización de la hemoglobina (lisis de eritrocitos)

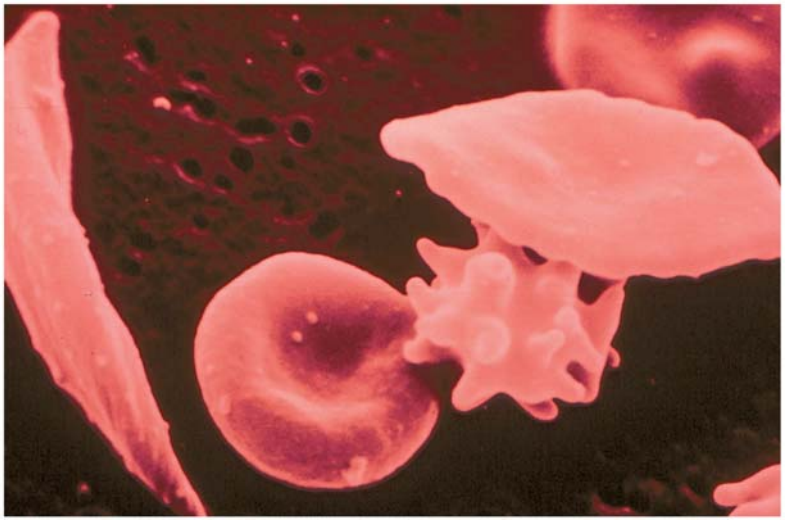
ALGUNAS MUTACIONES PROVOCAN AGREGADOS MOLECULARES QUE PUEDEN SER PATOLÓGICOS

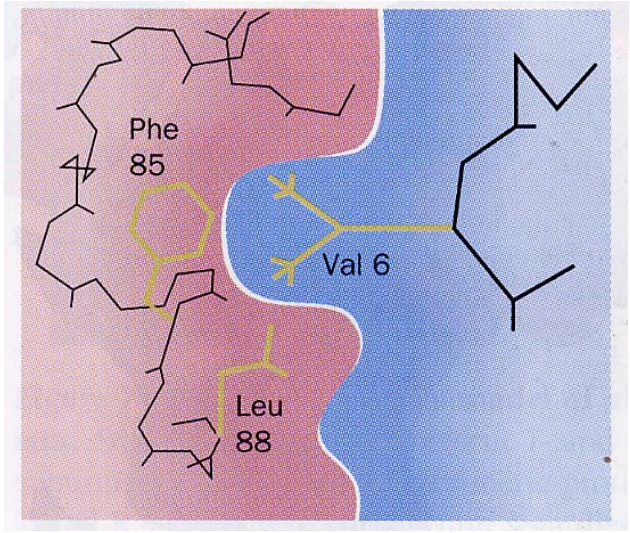
ANEMIA FALCIFORME
ENFERMEDADES NEURODESGENERATIVAS

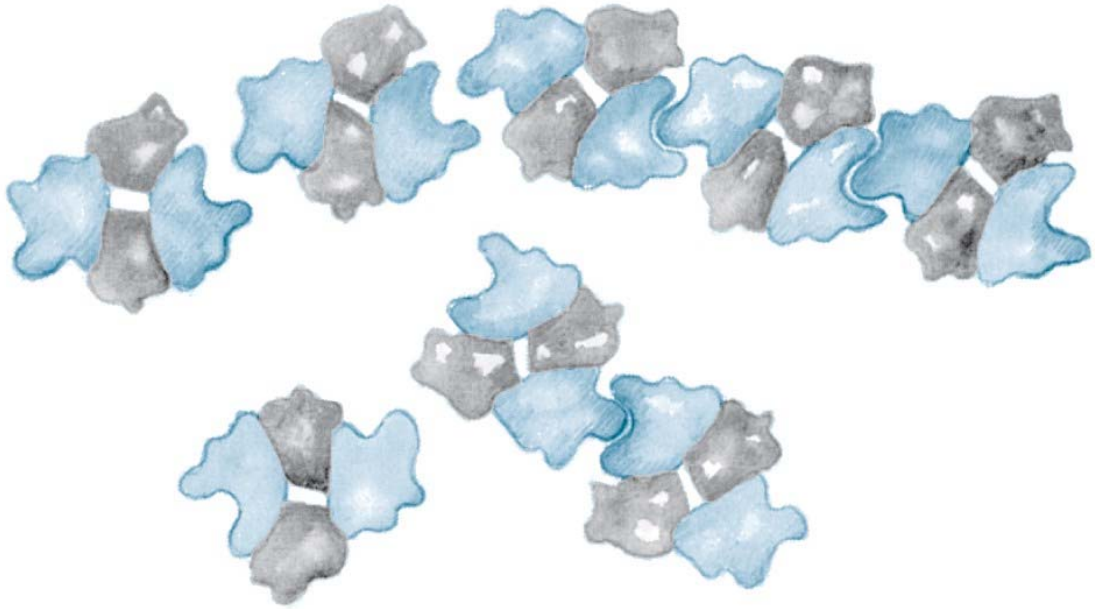
HEMOGLOBINA S: ANEMIA FALCIFORME

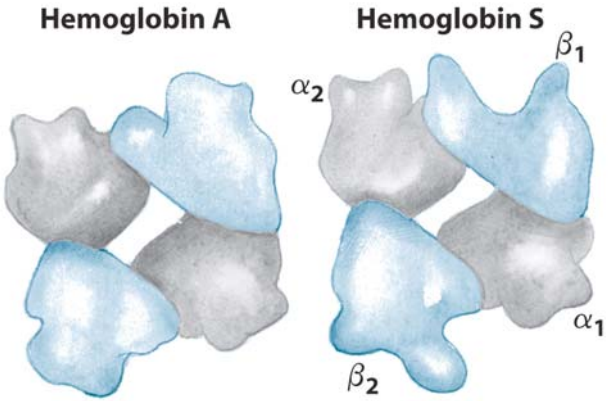




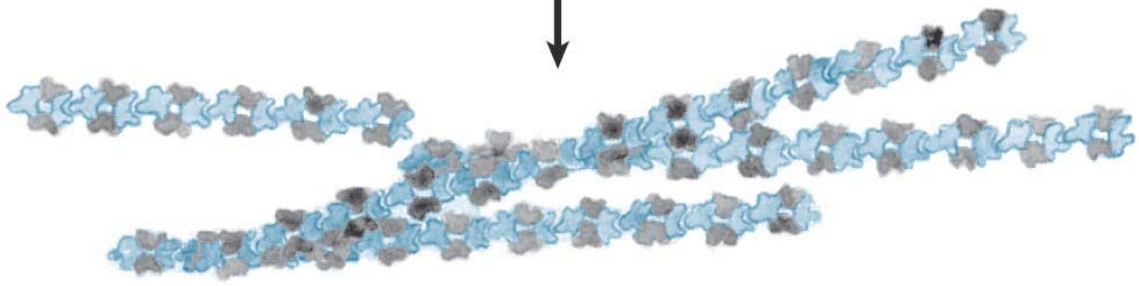




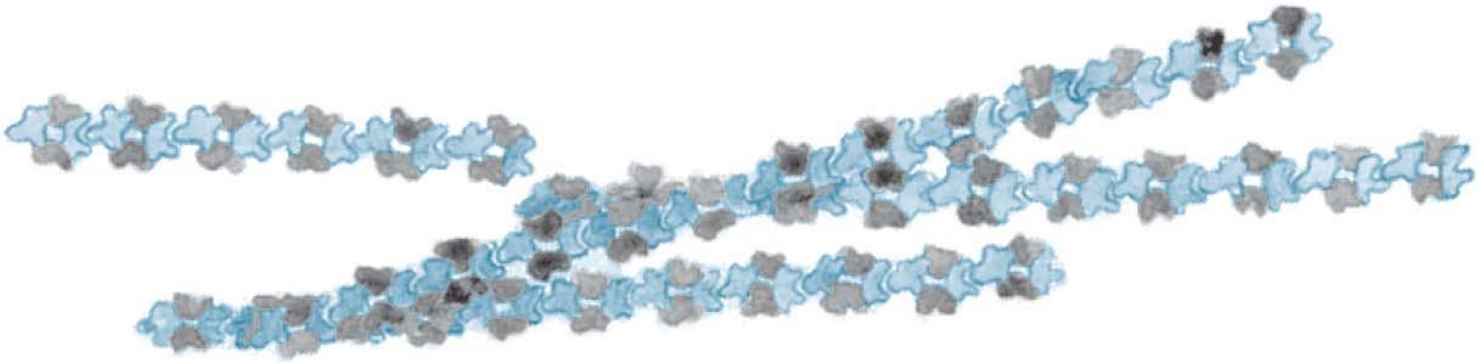




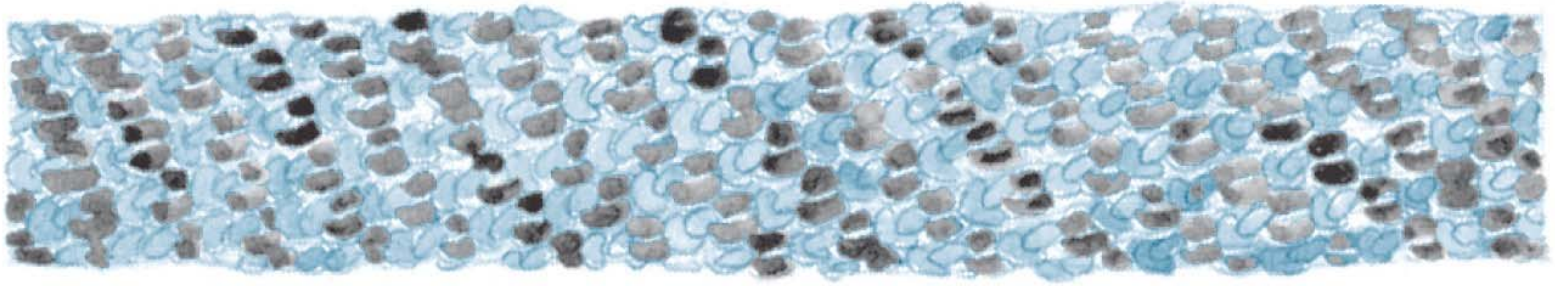
Interaction between molecules



Strand formation

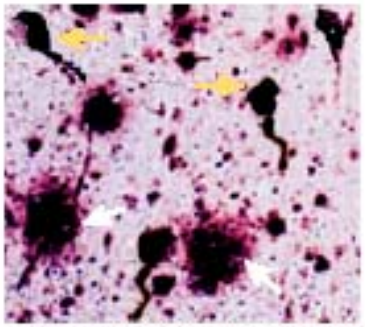


Strand formation

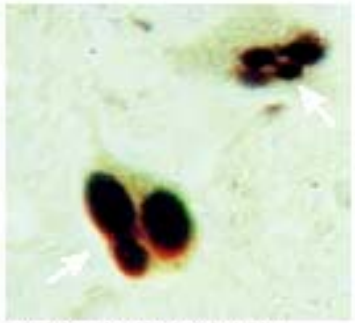


Alignment and crystallization
(fiber formation)

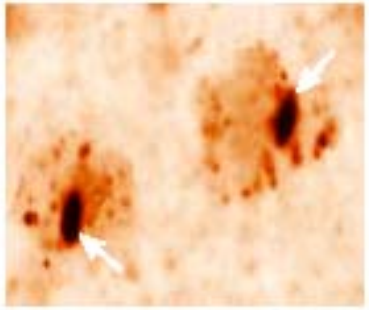
ENFERMEDADES NEURODESGENERATIVAS
FORMACION DE AGREGADOS MOLECULARES



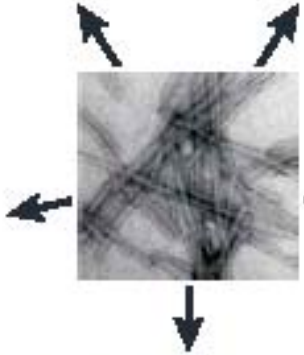
Alzheimer's plaques and tangles

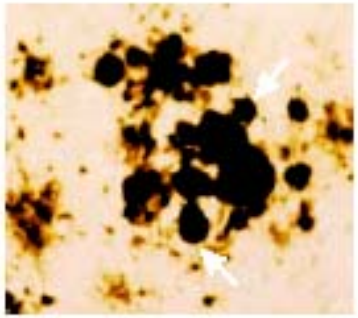


Parkinson's Lewy bodies

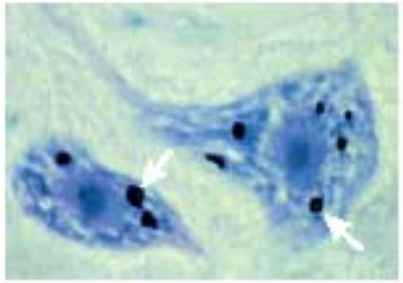


Huntington's intranuclear inclusions





Prion amyloid plaques

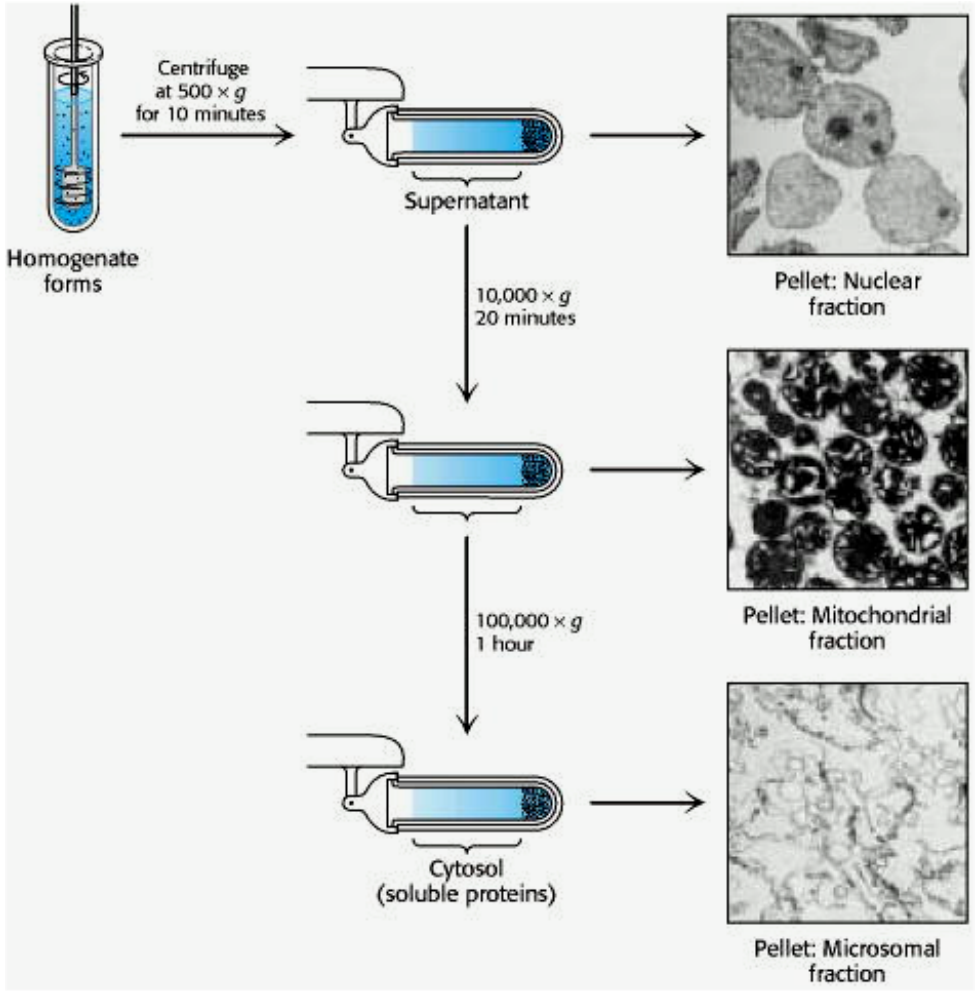


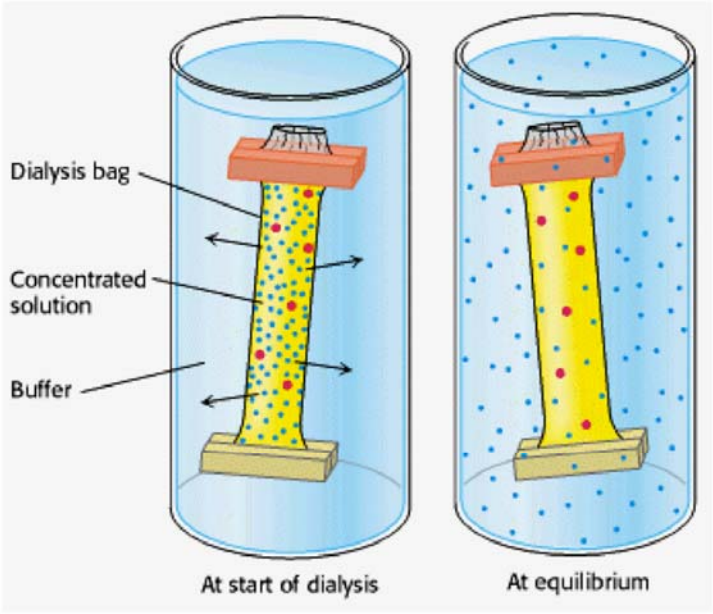
Amyotrophic lateral sclerosis aggregates

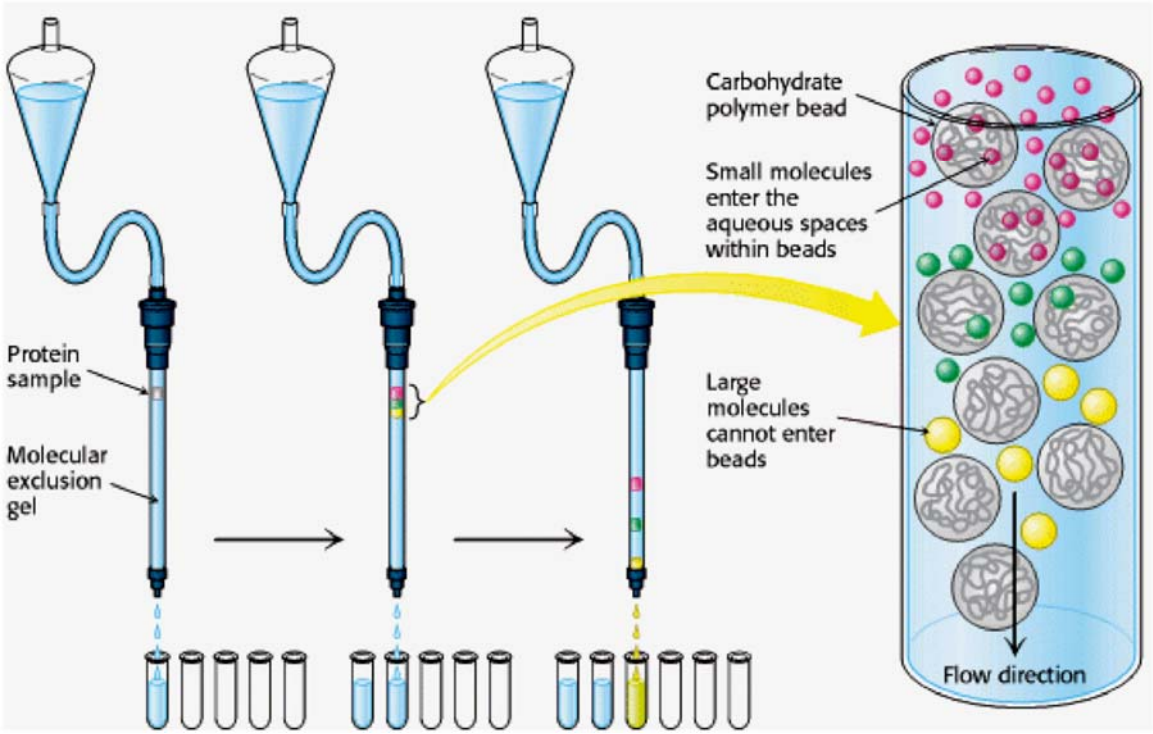
PROTEINAS INVOLUCRADAS EN ENFERMEDADES NEURODESGENERATIVAS

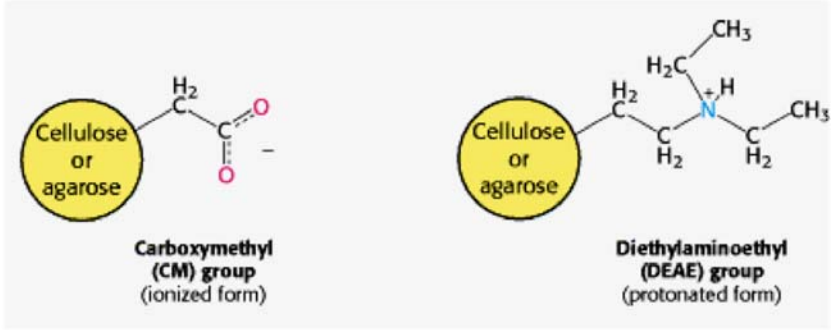
Enfermedad	rasgos clínicos	proteína	localización
Alzheimer	**Demencia**	**amiloide β y tau(τ)**	**extracelular citoplasm.**
Encefalopatía Espongiforme Trasmisible	**Demencia, Ataxia insomnio**	**proteína prión**	**extracelular**
Parkinson	**Desórdenes de movimiento**	**α-sinucleina**	**citoplasma**
Esclerosis Lateral Amiotrófica	**Desórdenes de movimiento**	**superóxido dismutasa**	**citoplasma**

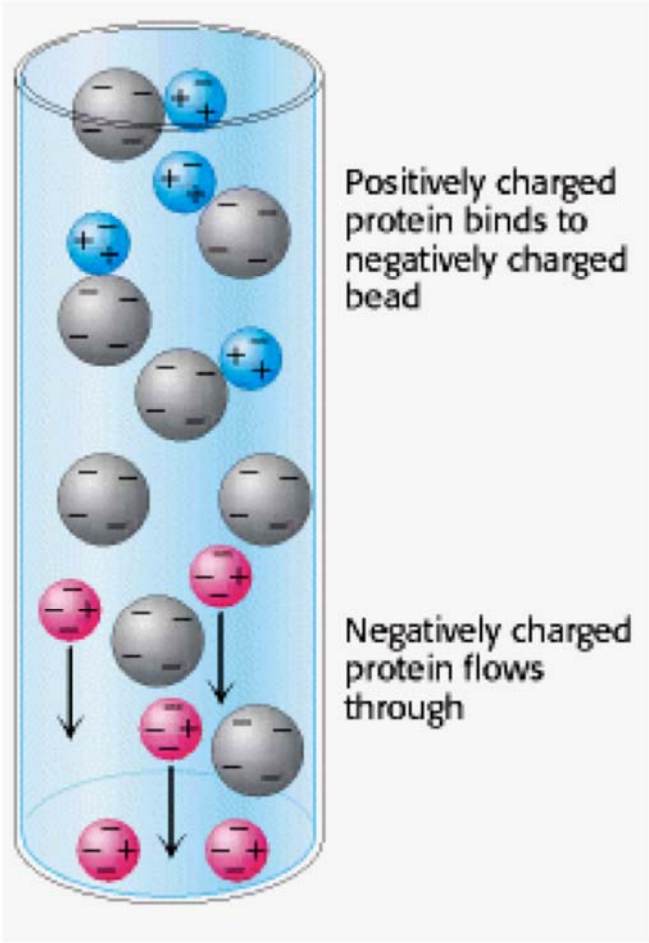
FIN

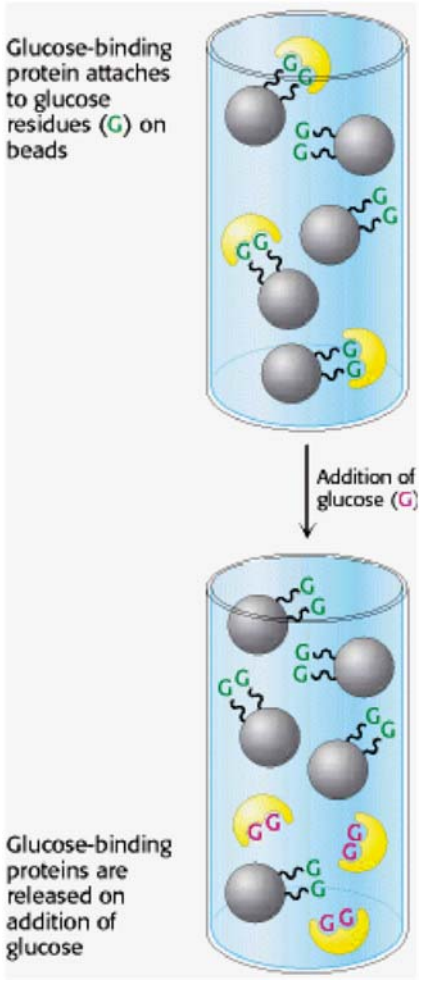

Centrifuge
at 500 × g
for 10 minutes
Homogenate
forms
Supernatant
Pellet: Nuclear
fraction
10,000 × g
20 minutes
Pellet: Mitochondrial
fraction
100,000 × g
1 hour
Cytosol
(soluble proteins)
Pellet: Microsomal
fraction

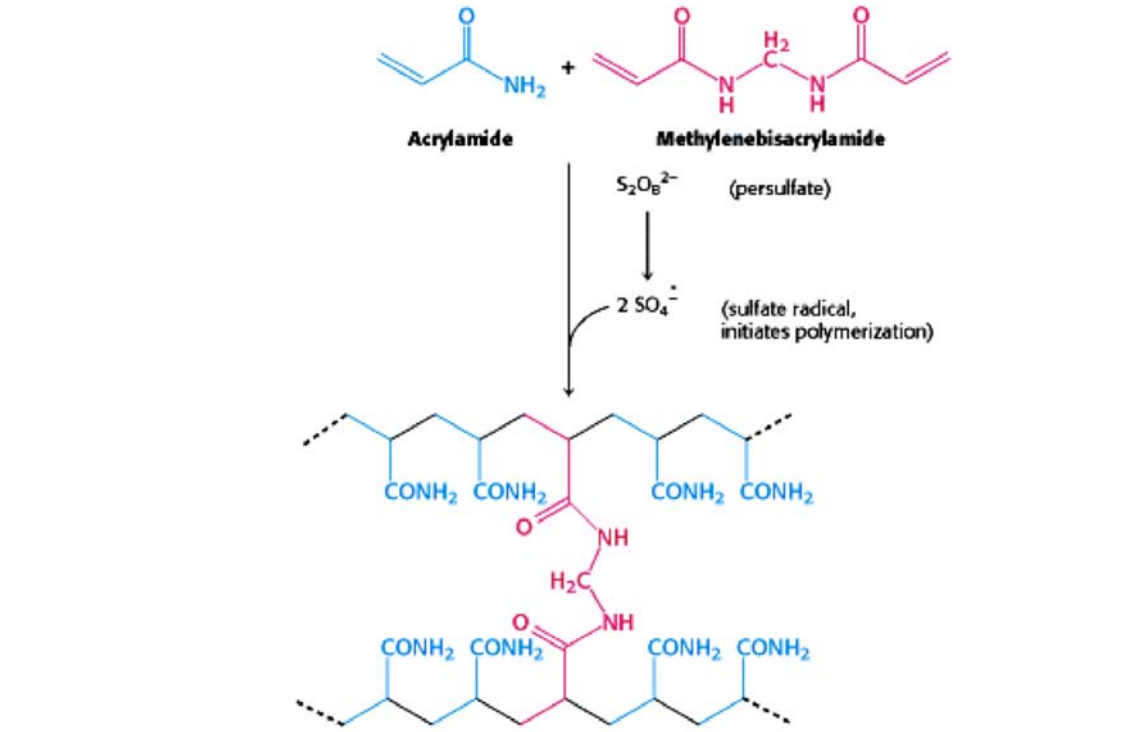

Dialysis bag
Concentrated solution
Buffer
At start of dialysis
At equilibrium

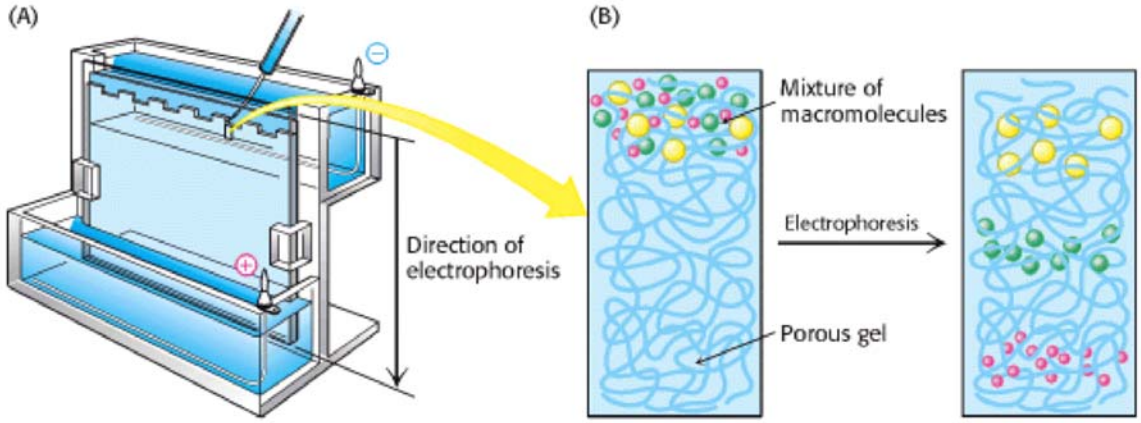

Protein sample
Molecular exclusion gel
Carbohydrate polymer bead
Small molecules enter the aqueous spaces within beads
Large molecules cannot enter beads
Flow direction


Cellulose or agarose
Carboxymethyl (CM) group
(ionized form)
Cellulose or agarose
Diethylaminoethyl (DEAE) group
(protonated form)

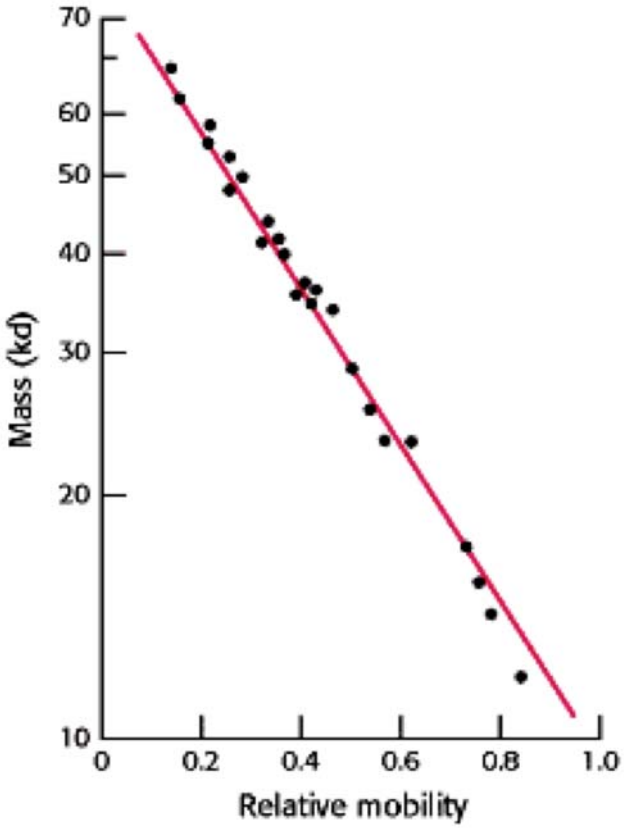

Positively charged protein binds to negatively charged bead
Negatively charged protein flows through

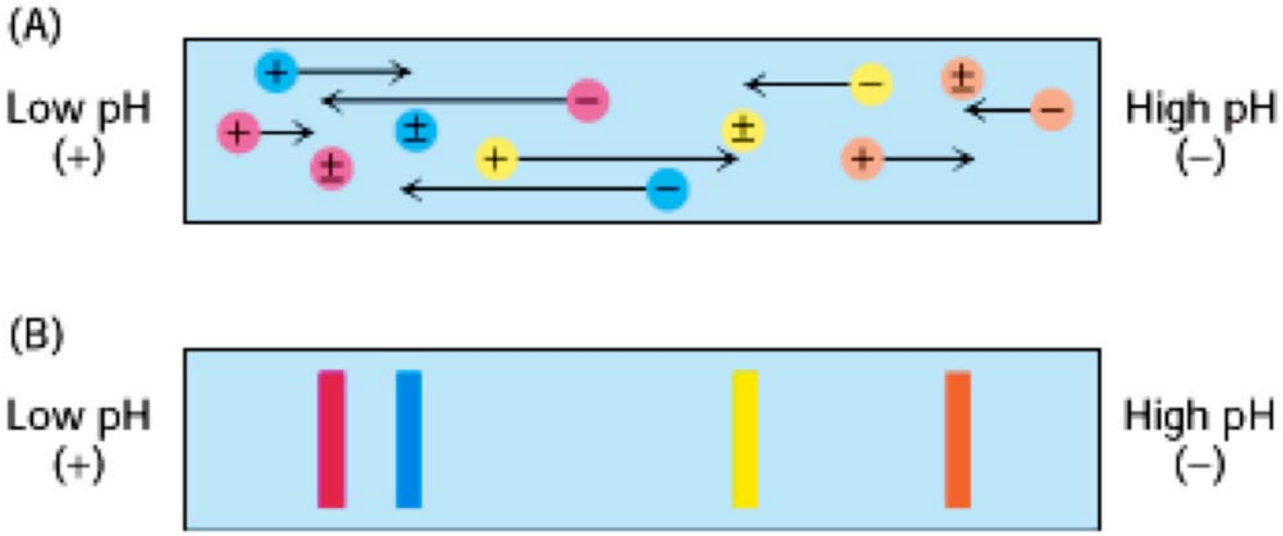

Glucose-binding protein attaches to glucose residues (G) on beads
Addition of glucose (G)
Glucose-binding proteins are released on addition of glucose


Acrylamide
Methylenebisacrylamide
$S_2O_8^{2-}$ (persulfate)
$2\ SO_4^{\bullet-}$
(sulfate radical, initiates polymerization)


(A)
(B)
Direction of electrophoresis
Mixture of macromolecules
Electrophoresis
Porous gel


kD
70
60
40
30
25
19


Mass (kd)
70
60
50
40
30
20
10
0
0.2
0.4
0.6
0.8
1.0
Relative mobility


(A)
Low pH
(+)
High pH
(–)
(B)
Low pH
(+)
High pH
(–)

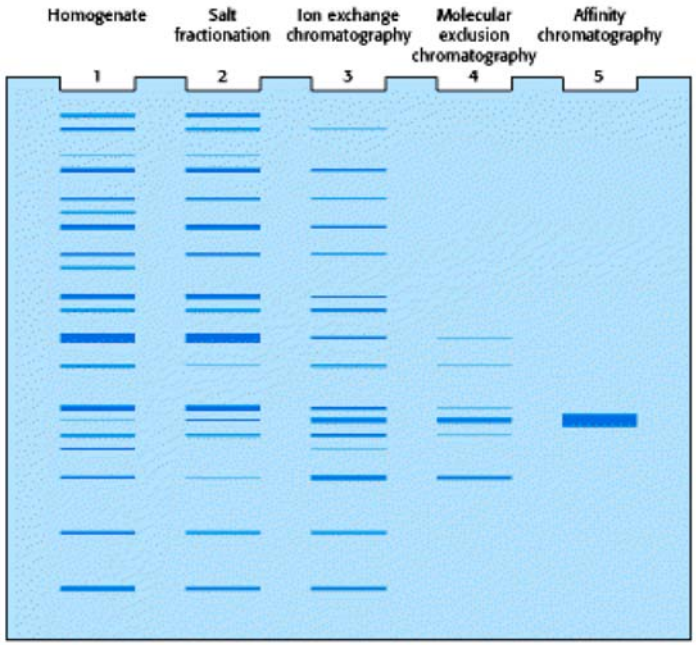
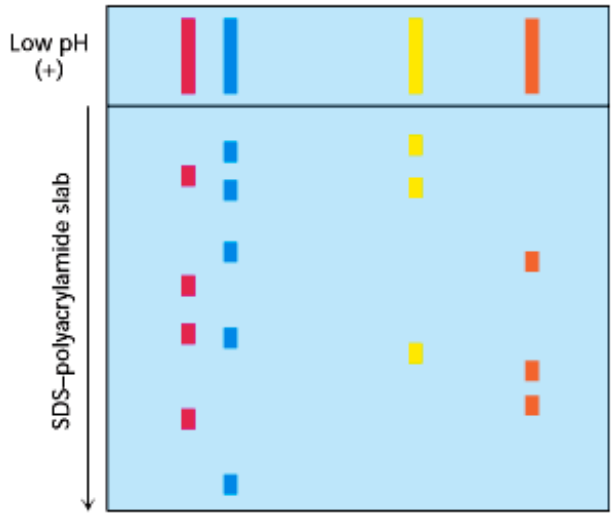
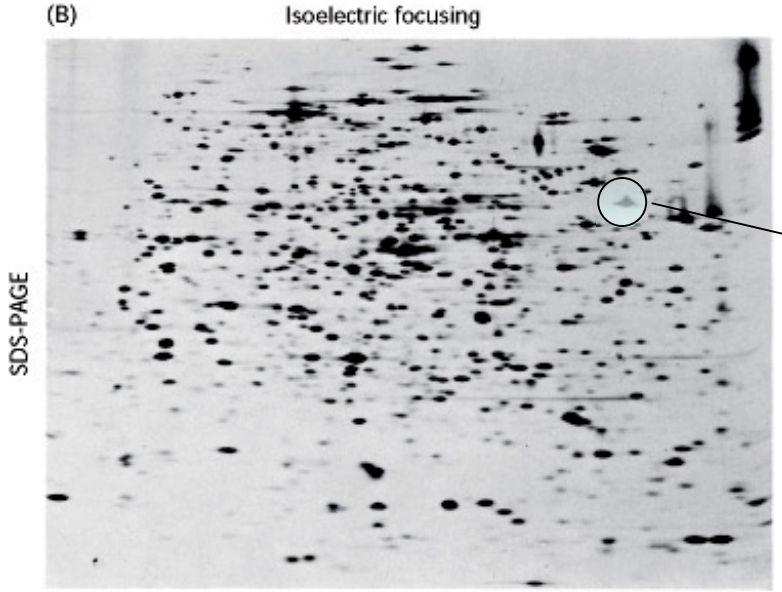


Table 4.1. Quantification of a purification protocol for a fictitious protein

Step	Total protein (mg)	Total activity (units)	Specific activity, (units mg^{-1}	Yield (%)	Purification level
Homogenization	15,000	150,000	10	100	1
Salt fractionation	4,600	138,000	30	92	3
Ion-exchange chromatography	1,278	115,500	90	77	9
Molecular exclusion chromatography	68.8	75,000	1,100	50	110
Affinity chromatography	1.75	52,500	30,000	35	3,000





Espectrometria
De masa

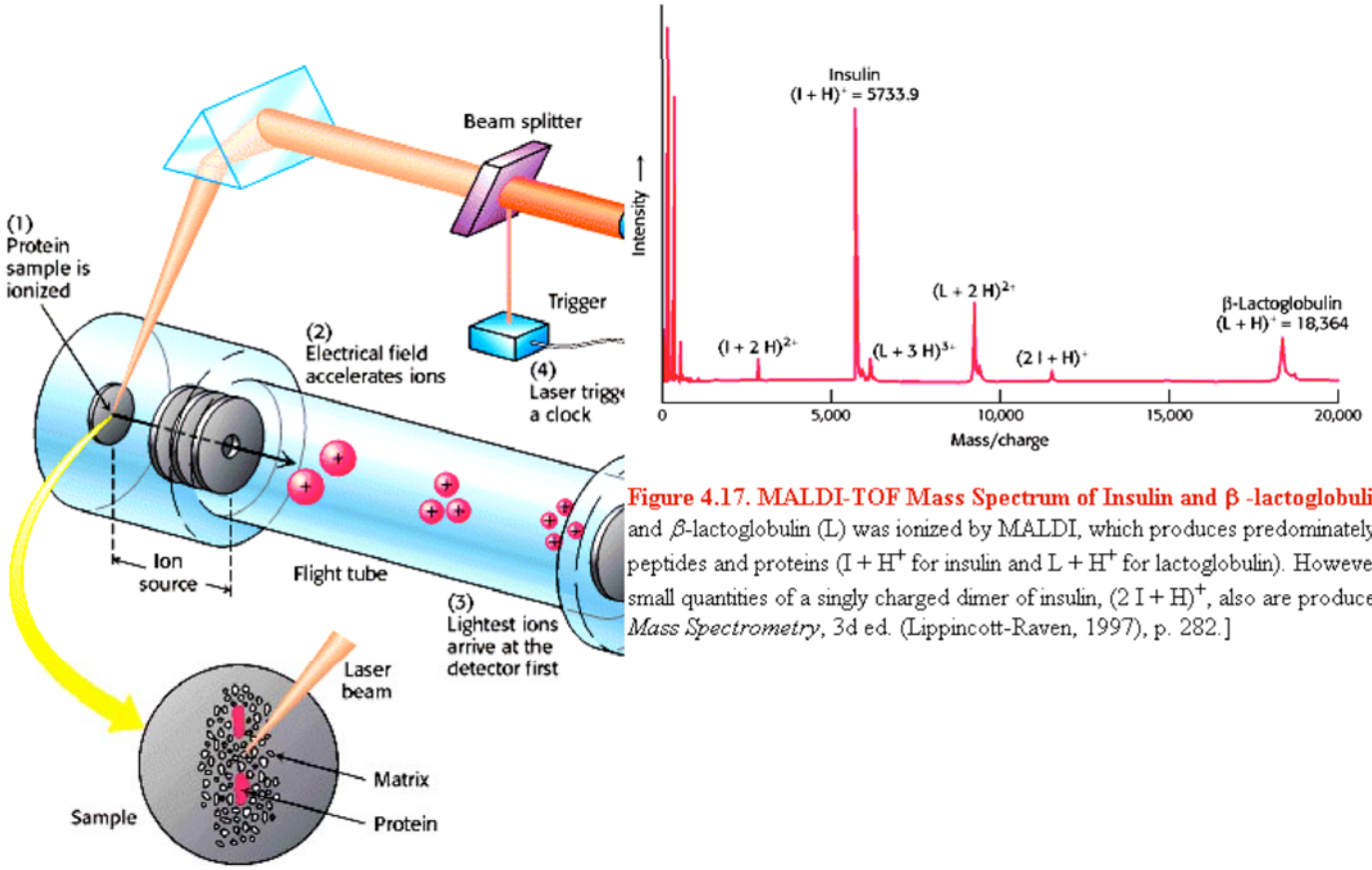


Figure 4.17. MALDI-TOF Mass Spectrum of Insulin and β -lactoglobulin. A mixture of 5 pmol each of insulin (I) and β-lactoglobulin (L) was ionized by MALDI, which produces predominately singly charged molecular ions from peptides and proteins ($I + H^+$ for insulin and $L + H^+$ for lactoglobulin). However, molecules with multiple charges as well as small quantities of a singly charged dimer of insulin, $(2\,I + H)^+$, also are produced. [After J. T. Watson, *Introduction to Mass Spectrometry*, 3d ed. (Lippincott-Raven, 1997), p. 282.]

Figure 4.16. MALDI-TOF Mass Spectrometry. (1) The protein sample, embedded in an appropriate matrix, is ionized by the application of a laser beam. (2) An electrical field accelerates the ions formed through the flight tube toward the detector. (3) The lightest ions arrive first. (4) The ionizing laser pulse also triggers a clock that measures the time of flight (TOF) for the ions. [After J. T. Watson, *Introduction to Mass Spectrometry*, 3d ed. (Lippincott-Raven, 1997), p. 279.]