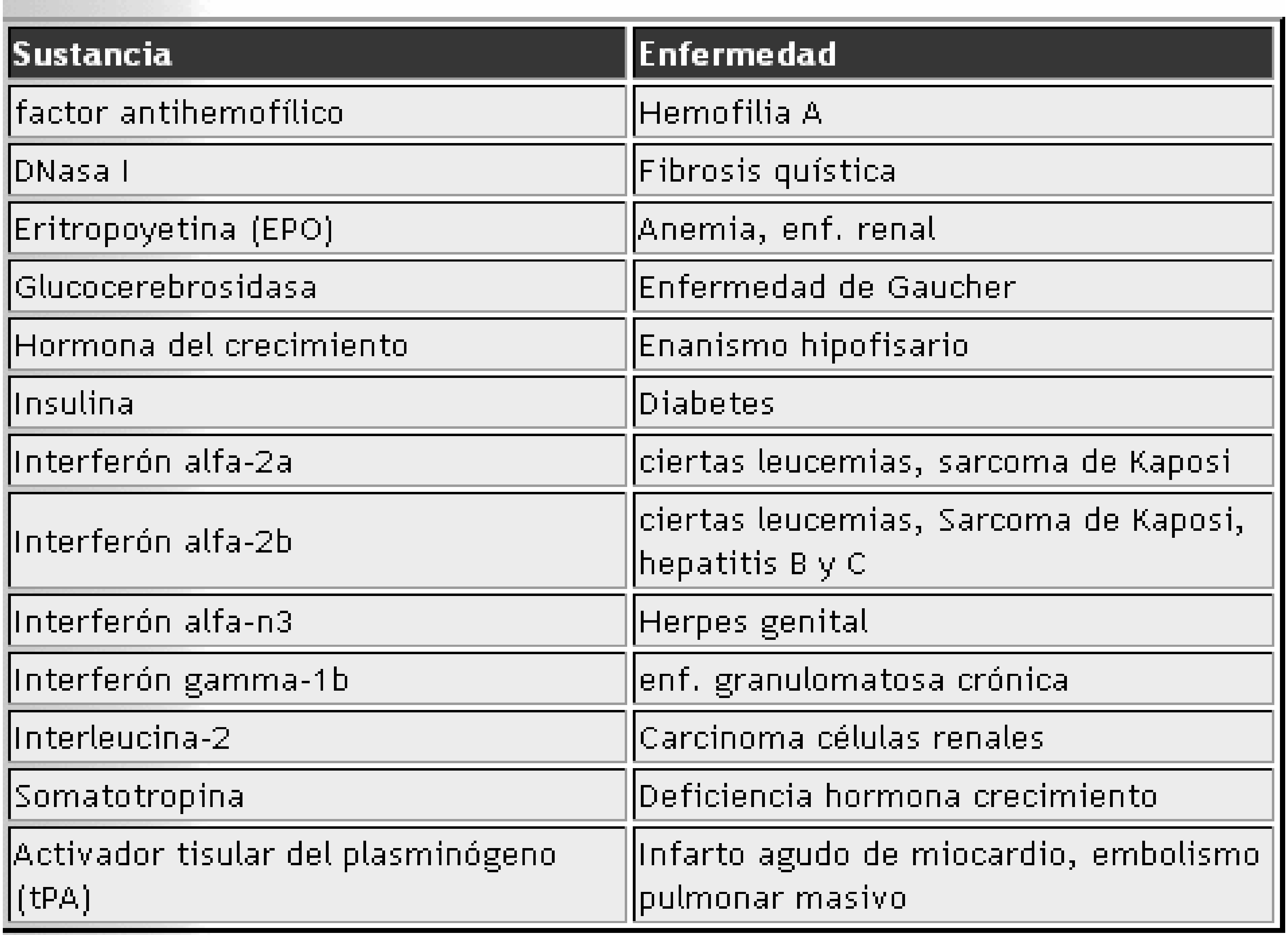
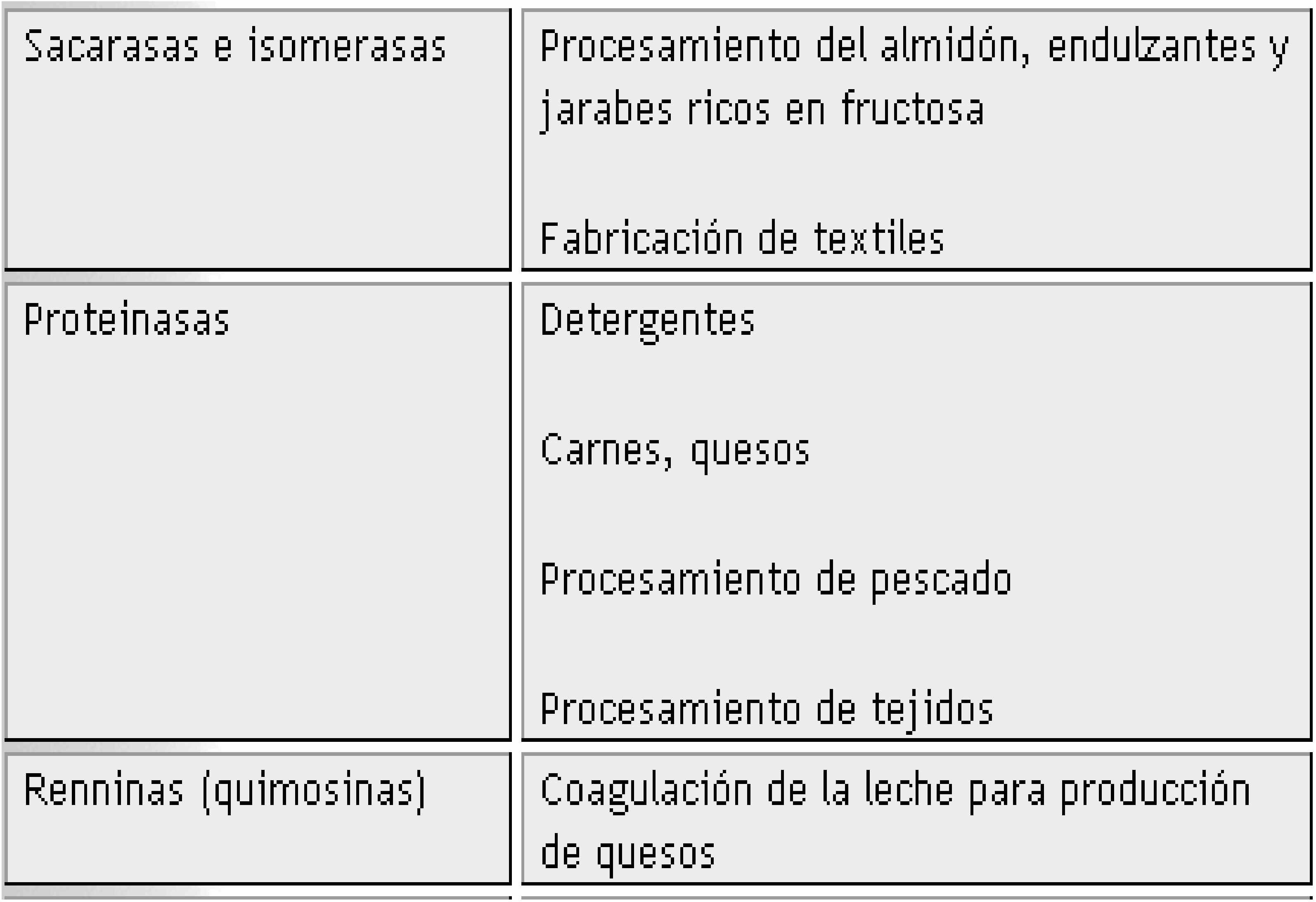
# PROTEINAS Y PEPTIDOS TERAPEUTICOS RECOMBINANTES



| Sustancia | Enfermedad |
|---|---|
| factor antihemofílico | Hemofilia A |
| DNasa I | Fibrosis quística |
| Eritropoyetina (EPO) | Anemia, enf. renal |
| Glucocerebrosidasa | Enfermedad de Gaucher |
| Hormona del crecimiento | Enanismo hipofisario |
| Insulina | Diabetes |
| Interferón alfa-2a | ciertas leucemias, sarcoma de Kaposi |
| Interferón alfa-2b | ciertas leucemias, Sarcoma de Kaposi, hepatitis B y C |
| Interferón alfa-n3 | Herpes genital |
| Interferón gamma-1b | enf. granulomatosa crónica |
| Interleucina-2 | Carcinoma células renales |
| Somatotropina | Deficiencia hormona crecimiento |
| Activador tisular del plasminógeno (tPA) | Infarto agudo de miocardio, embolismo pulmonar masivo |

# ENZIMAS RECOMBINANTES Y ACTIVIDAD PRODUCTIVA 1



| ENZIMA | ACTIVIDAD PRODUCTIVA |
|---|---|
| Sacarasas e isomerasas | Procesamiento del almidón, endulzantes y jarabes ricos en fructosa<br>Fabricación de textiles |
| Proteinasas | Detergentes<br>Carnes, quesos<br>Procesamiento de pescado<br>Procesamiento de tejidos |
| Renninas (quimosinas) | Coagulación de la leche para producción de quesos |

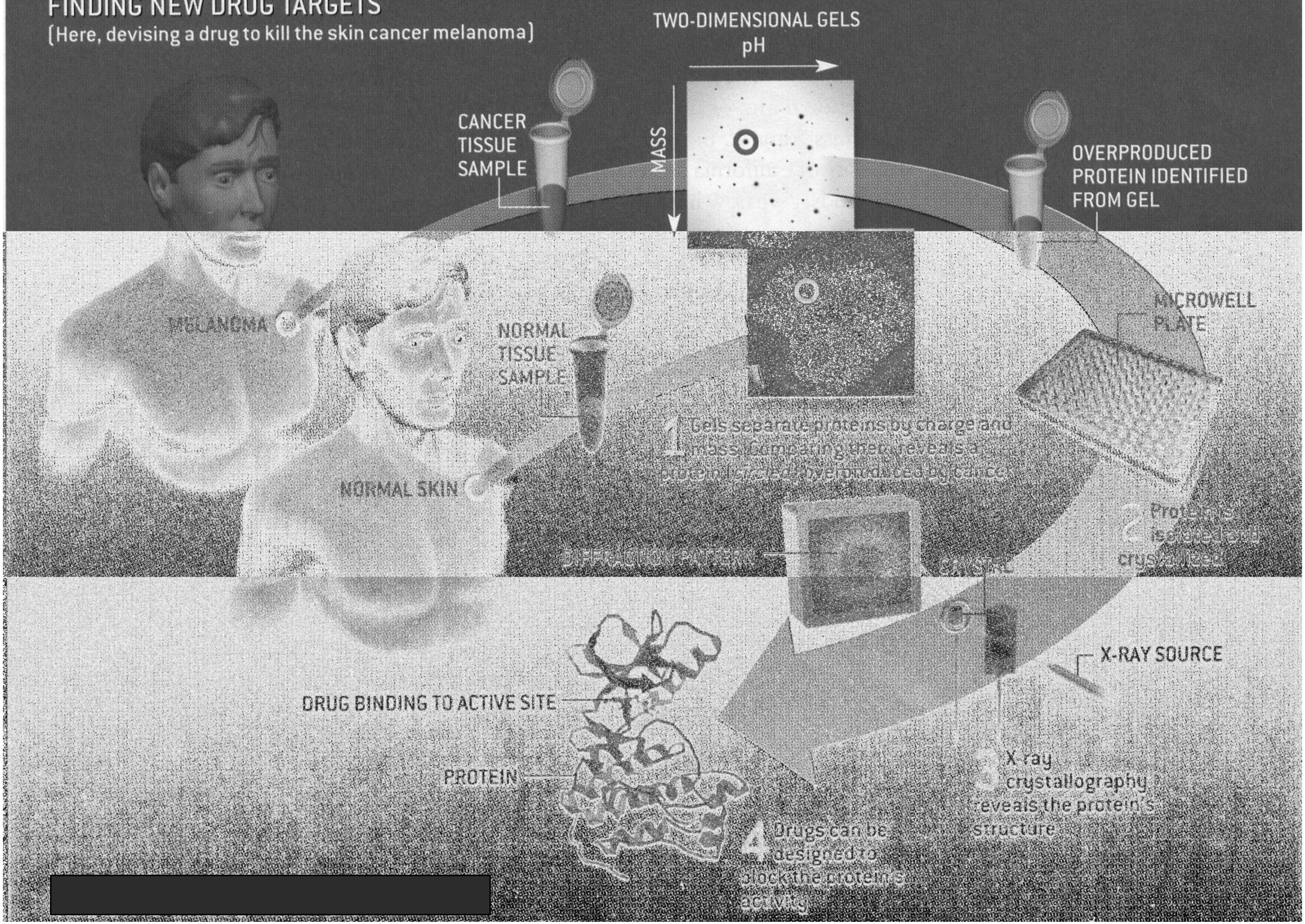
# ENZIMAS RECOMBINANTES Y ACTIVIDAD PRODUCTIVA 2

| ENZIMA | ACTIVIDAD PRODUCTIVA |
|---|---|
| Lipasas | Detergentes<br>Procesamiento de pieles<br>Saborizantes<br>Procesamiento de carne y queso |
| Celulasas | Producción de zumos de frutas<br>Producción de olivas<br>Modificación de granos y fibras<br>“Envejecimiento” de prendas vaqueras |

# HOW PROTEOMICS CAN HELP DRUG DEVELOPMENT

## FINDING NEW DRUG TARGETS

(Here, devising a drug to kill the skin cancer melanoma)


TWO-DIMENSIONAL GELS
pH
MASS
CANCER TISSUE SAMPLE
OVERPRODUCED PROTEIN IDENTIFIED FROM GEL
MELANOMA
NORMAL TISSUE SAMPLE
NORMAL SKIN
MICROWELL PLATE
DIFFRACTION PATTERN
CRYSTAL
X-RAY SOURCE
DRUG BINDING TO ACTIVE SITE
PROTEIN

1 Gels separate proteins by charge and mass. Comparing them reveals a protein (*circled*) overproduced by cancer.

2 Protein is isolated and crystallized

3 X-ray crystallography reveals the protein's structure

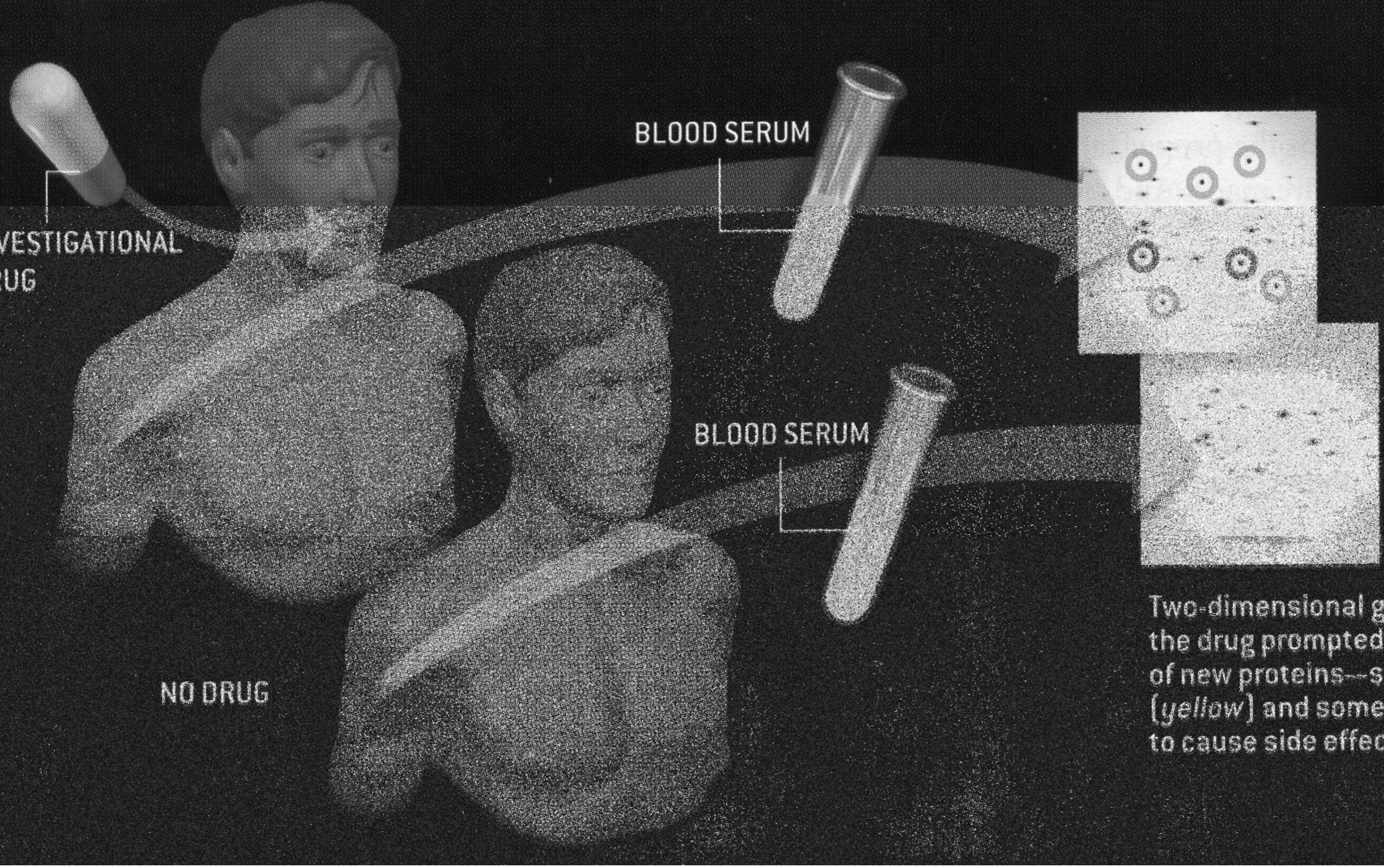
4 Drugs can be designed to block the protein's activity

[redacted]

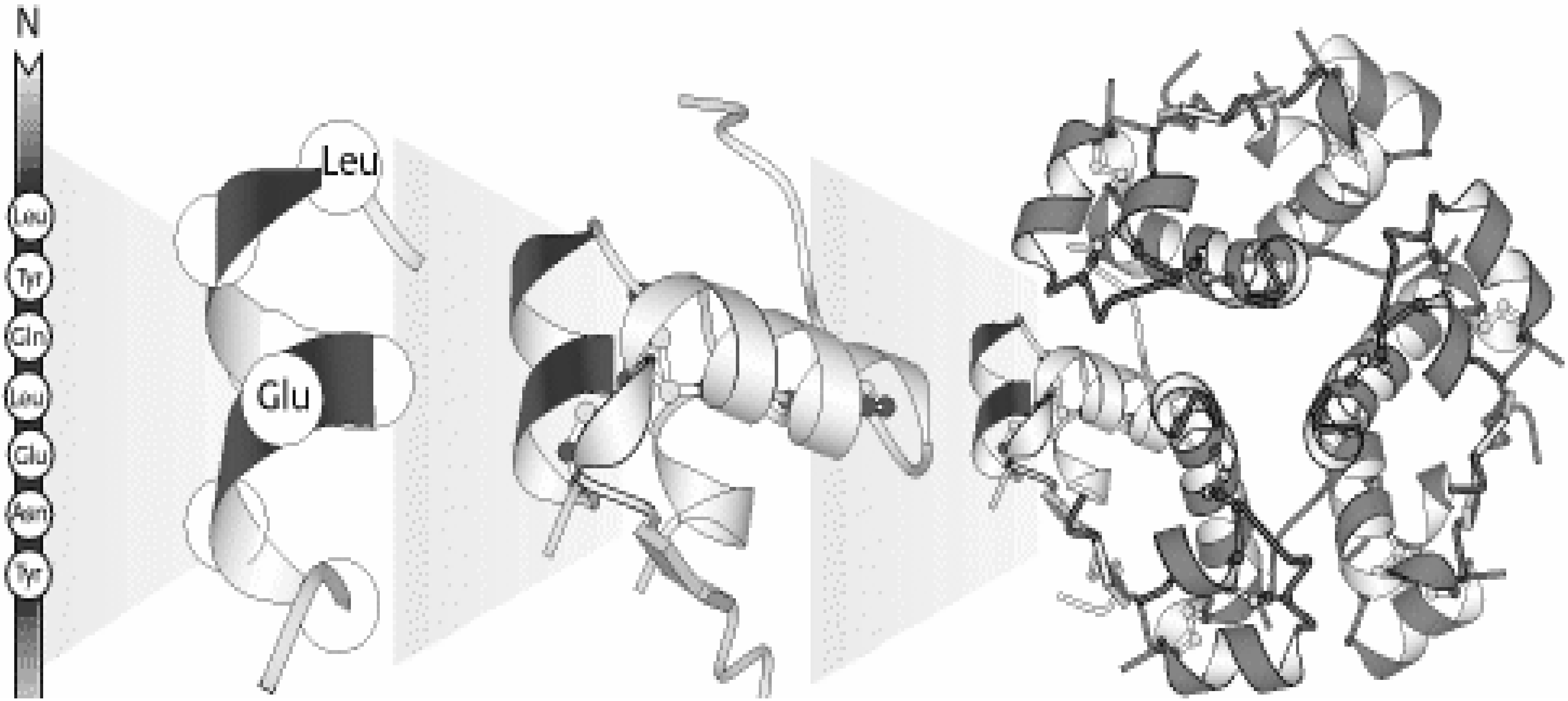
activity

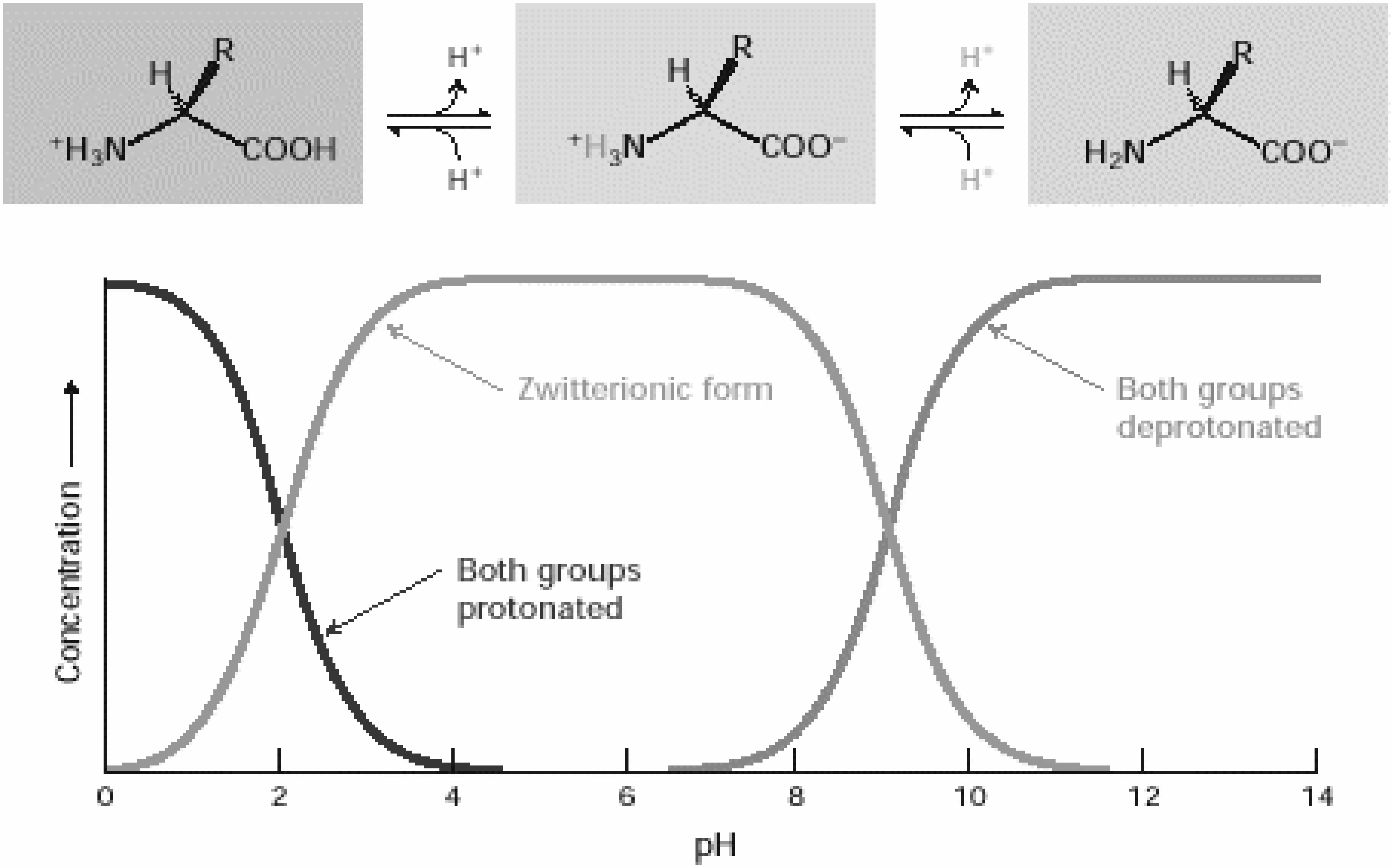
## AVOIDING DRUGS WITH SIDE EFFECTS

(Here, determining whether an investigational drug prompts production of possibly harmful proteins)


INVESTIGATIONAL DRUG
BLOOD SERUM
BLOOD SERUM
NO DRUG

Two-dimensional gels show that the drug prompted the production of new proteins—some innocuous (*yellow*) and some with potential to cause side effects (*red*)


N
Leu
Tyr
Gln
Leu
Glu
Asn
Tyr
Leu
Glu



$^+H_3N$–CH(R)–COOH
$H^+$
$H^+$
$^+H_3N$–CH(R)–$COO^-$
$H^+$
$H^+$
$H_2N$–CH(R)–$COO^-$
Concentration
Zwitterionic form
Both groups deprotonated
Both groups protonated
0
2
4
6
8
10
12
14
pH

## Table 6–2 Amino acid composition of two proteins

| | Number of residues per molecule of protein | |
|---|---|---|
| Amino acid | Human cytochrome *c* | Bovine chymotrypsinogen |
| Ala | 6 | 22 |
| Arg | 2 | 4 |
| Asn | 5 | 15 |
| Asp | 3 | 8 |
| Cys | 2 | 10 |
| Gln | 2 | 10 |
| Glu | 8 | 5 |
| Gly | 13 | 23 |
| His | 3 | 2 |
| Ile | 8 | 10 |
| Leu | 6 | 19 |
| Lys | 18 | 14 |
| Met | 3 | 2 |
| Phe | 3 | 6 |
| Pro | 4 | 9 |
| Ser | 2 | 28 |
| Thr | 7 | 23 |
| Trp | 1 | 8 |
| Tyr | 5 | 4 |
| Val | 3 | 23 |
| Total | 104 | 245 |

## Table 6–1 Molecular data on some proteins

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

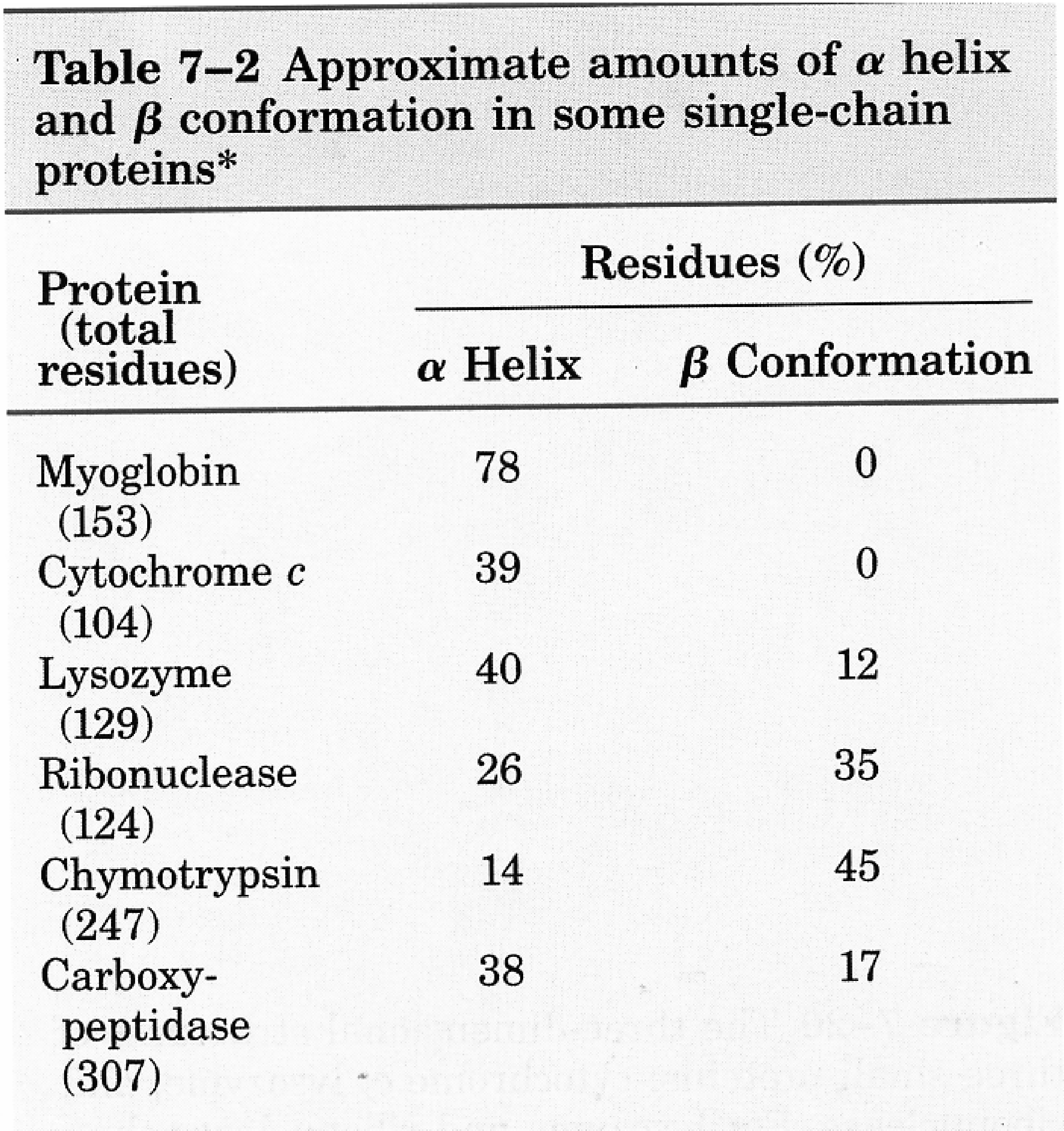
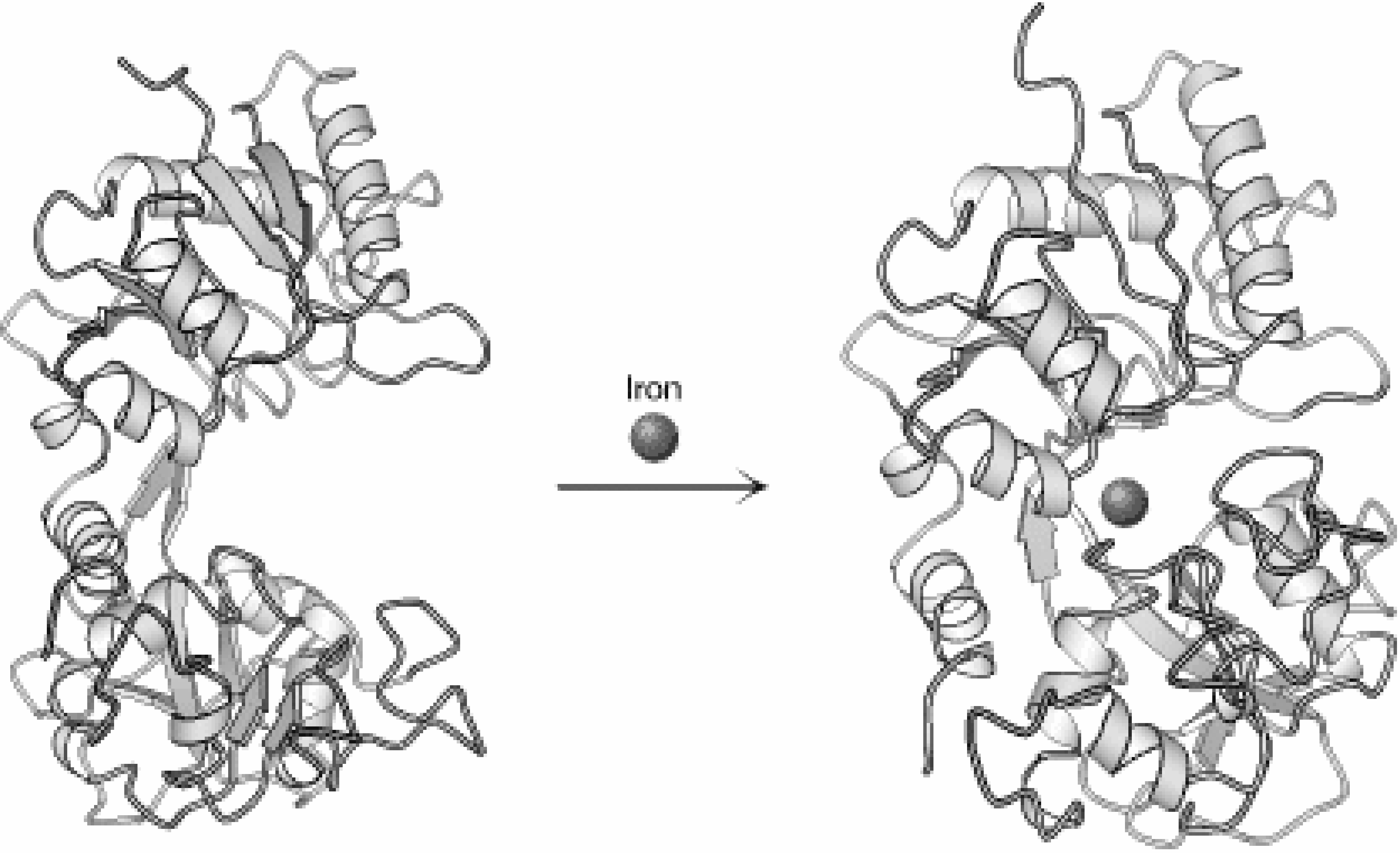


**Table 7–2 Approximate amounts of $\alpha$ helix and $\beta$ conformation in some single-chain proteins***

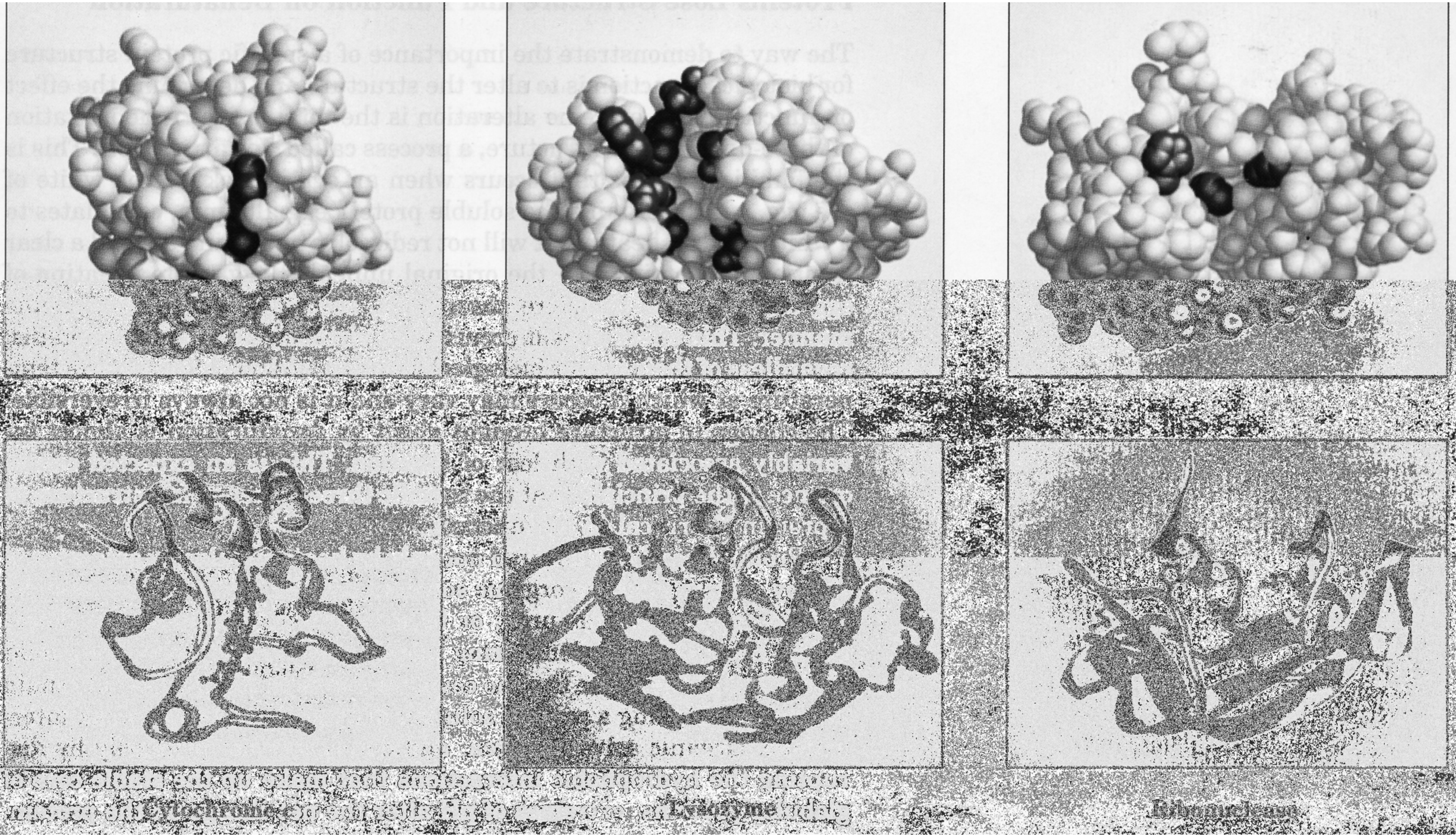
| Protein (total residues) | Residues (%) | |
|---|---|---|
| | $\alpha$ Helix | $\beta$ Conformation |
| Myoglobin (153) | 78 | 0 |
| Cytochrome *c* (104) | 39 | 0 |
| Lysozyme (129) | 40 | 12 |
| Ribonuclease (124) | 26 | 35 |
| Chymotrypsin (247) | 14 | 45 |
| Carboxypeptidase (307) | 38 | 17 |

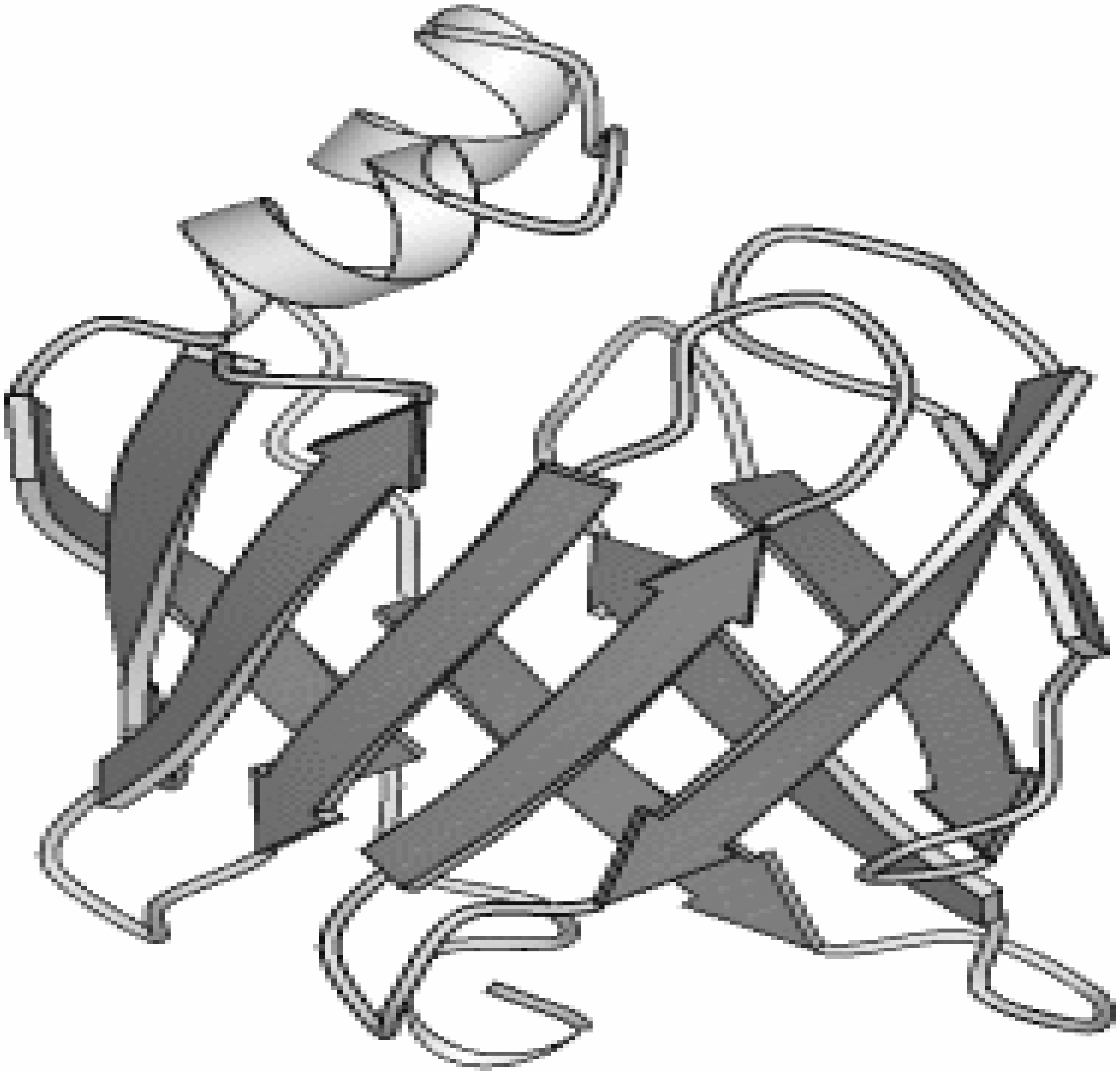

Iron

## Table 6–3 Conjugated proteins

| Class | Prosthetic group | Example |
|---|---|---|
| Lipoproteins | Lipids | $\beta_1$-Lipoprotein of blood |
| Glycoproteins | Carbohydrates | Immunoglobulin G |
| Phosphoproteins | Phosphate groups | Casein of milk |
| Hemoproteins | Heme (iron porphyrin) | Hemoglobin |
| Flavoproteins | Flavin nucleotides | Succinate dehydrogenase |
| Metalloproteins | Iron | Ferritin |
|  | Zinc | Alcohol dehydrogenase |
|  | Calcium | Calmodulin |
|  | Molybdenum | Dinitrogenase |
|  | Copper | Plastocyanin |

# Modelos Espaciales de Proteínas. Esfera y Cinta

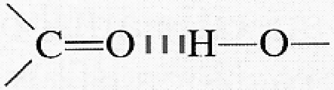
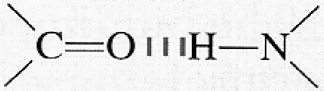
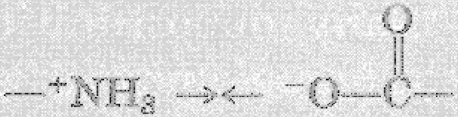
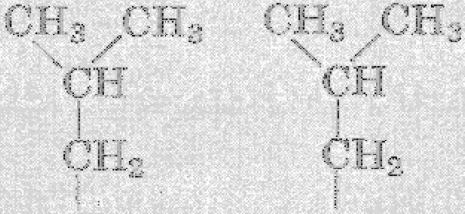

Cytochrome c
Lysozyme
Ribonuclease

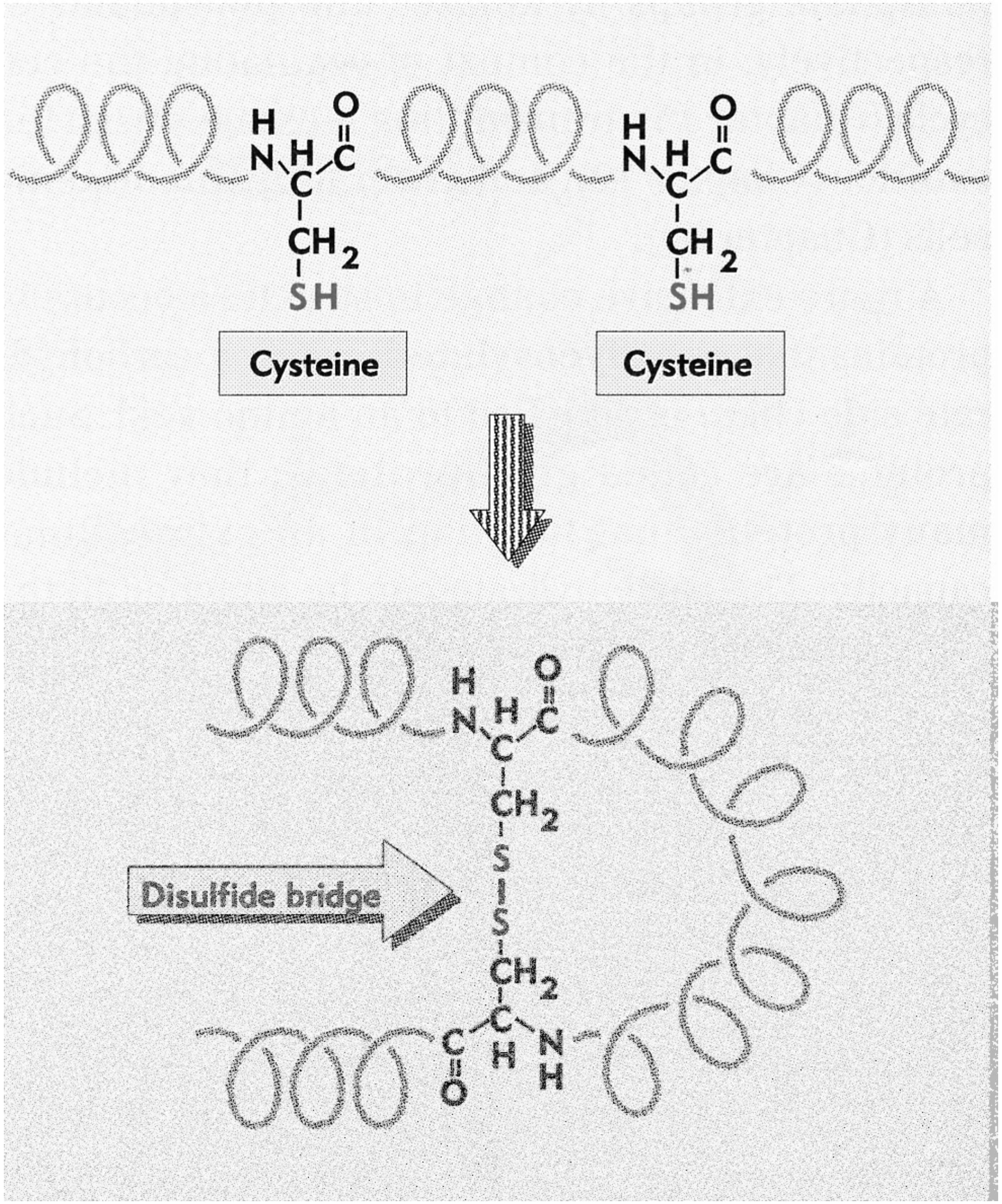


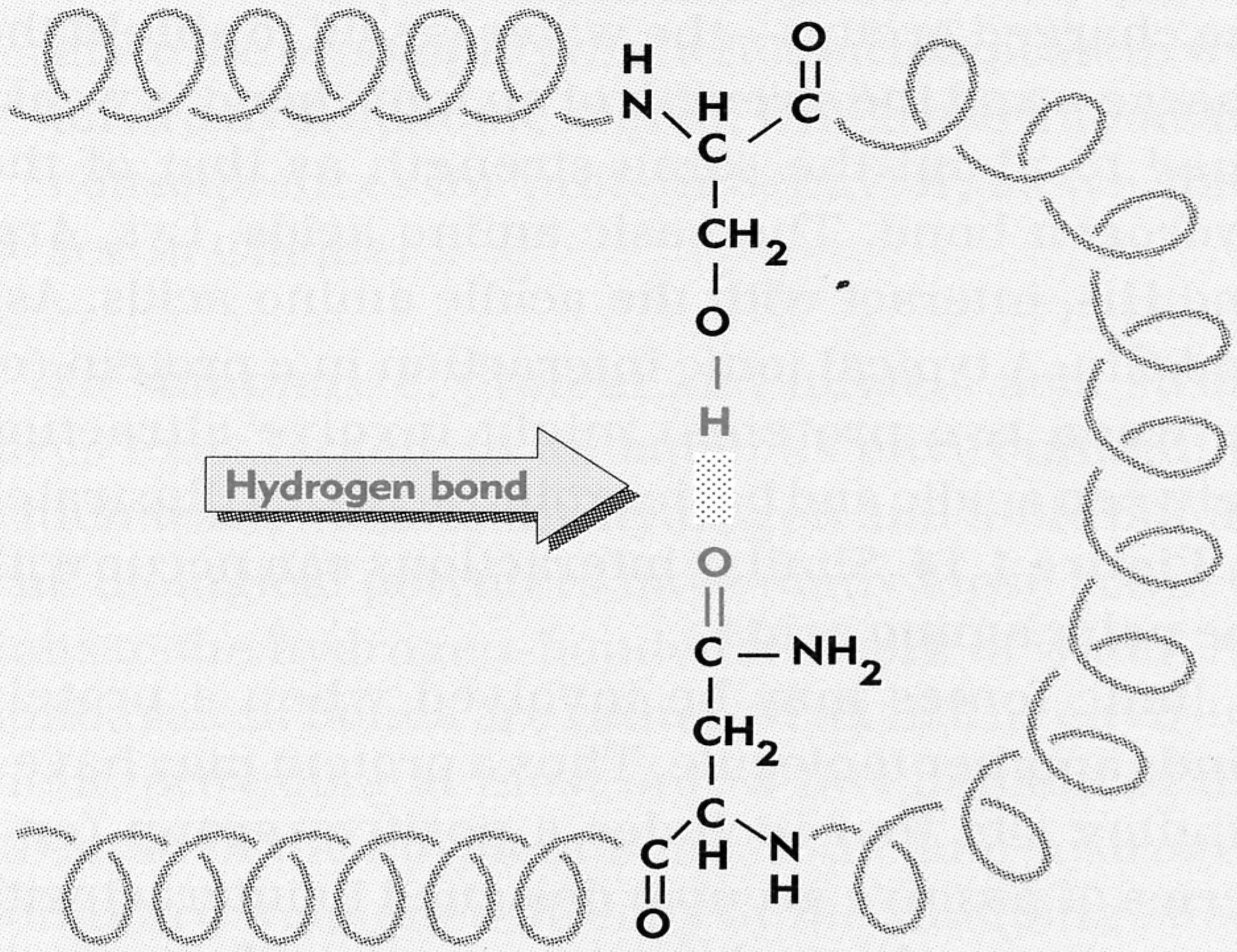
# Plegamiento y Estructura Terciaria

“Para el plegamiento apropiado y la formación correcta de los posibles enlaces disulfuro de una molécula proteica, no se requiere una mayor o especial información, que la ya contenida en la secuencia de amino-ácidos”

**Table 4–4 Four weak interactions among biomolecules in aqueous solvent**

| Weak interaction | | Stabilization energy (kJ/mol) |
|---|---|---|
| Hydrogen bonds | | |
| Between neutral groups |  | 8–21 |
| Between peptide bonds |  | 8–21 |
| Ionic interactions | | |
| Attraction |  $-{}^{+}NH_3 \rightarrow\leftarrow {}^{-}O-\overset{\overset{O}{\Vert}}{C}-$ | 42 |
| Repulsion | $-{}^{+}NH_3 \longleftrightarrow H_3N^{+}-$ | ≈ −21 |
| Hydrophobic interactions |  | 4–8 |
| van der Waals interactions | Any two atoms in close proximity | 4 |


Cysteine
Cysteine
Disulfide bridge

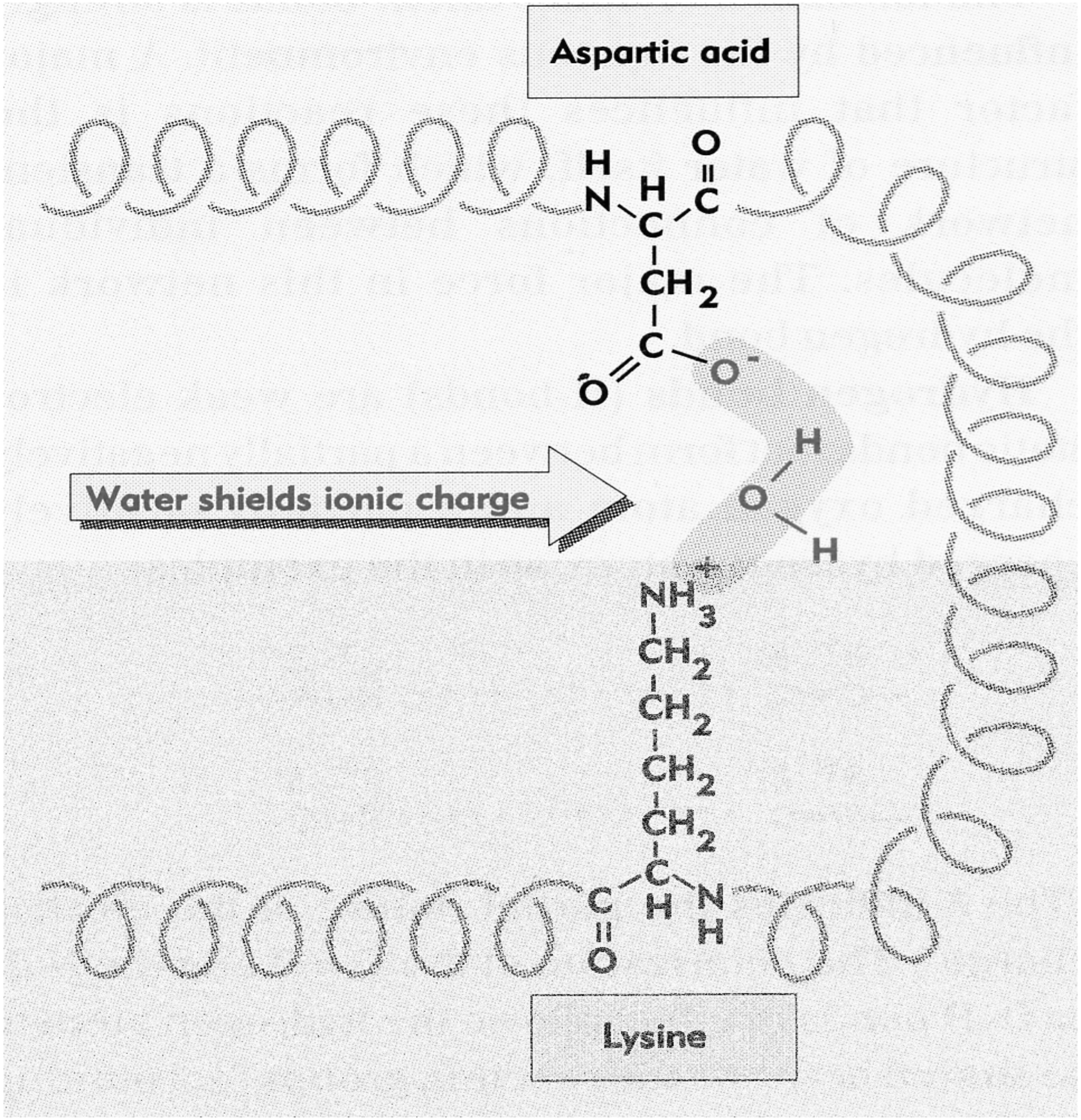


**Serine**

**Hydrogen bond**

**Asparagine**

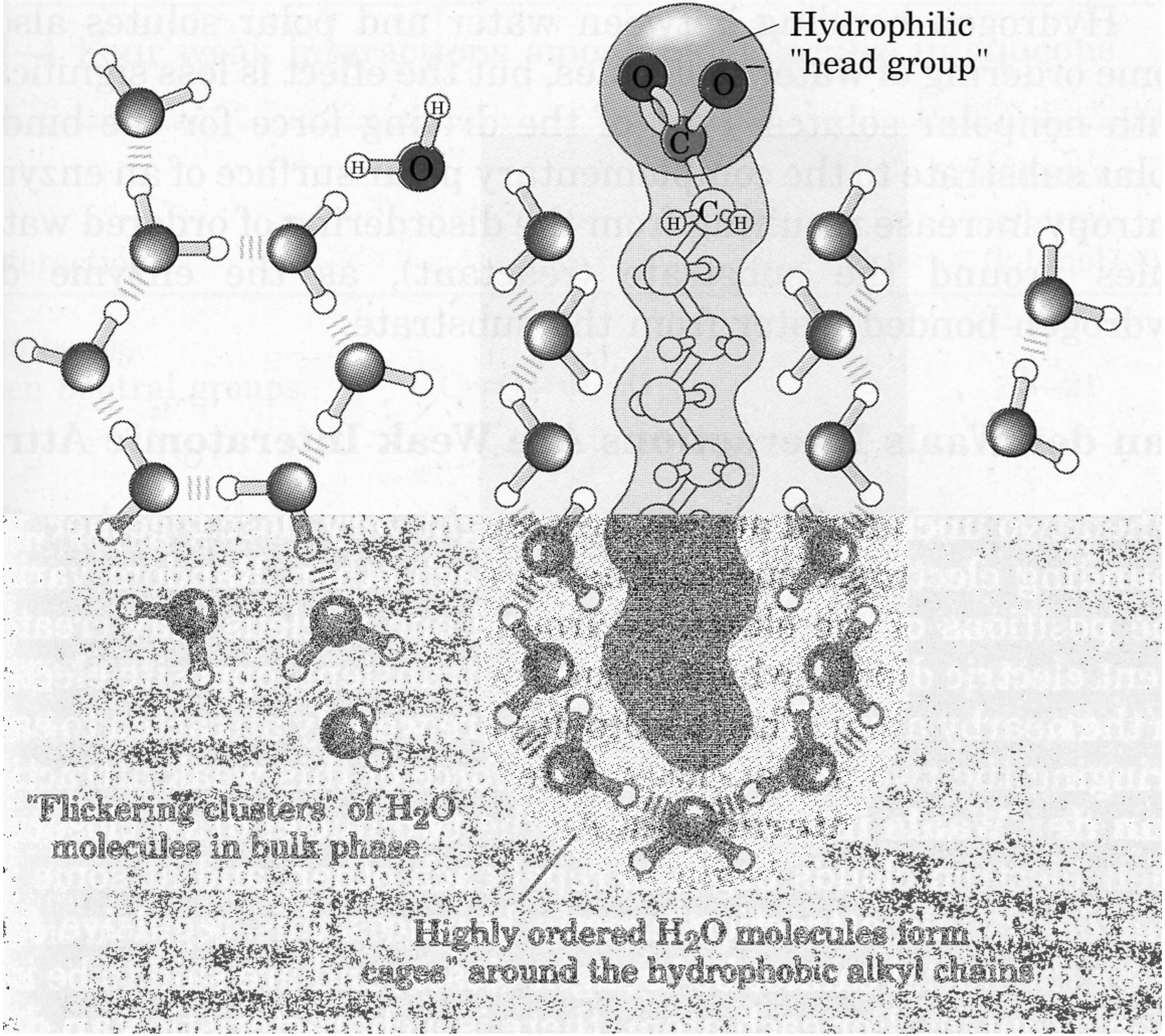
H
N
H
C
O
C
$CH_2$
O
H
O
C
$NH_2$
$CH_2$
C
C
H
N
H
O

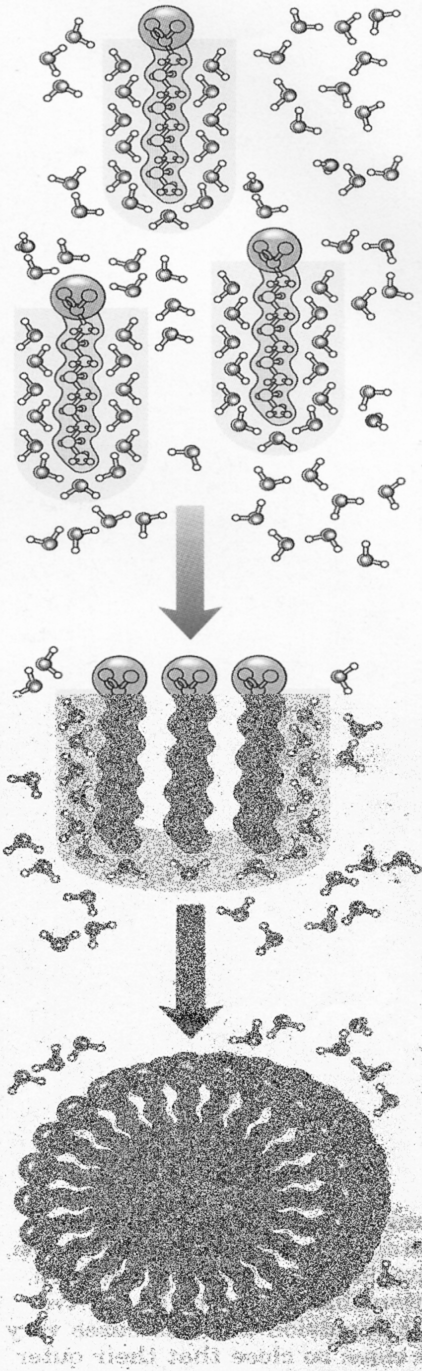

H
N
H
C
C
O
CH2
C
O
O-
H
O
H
+
NH3
CH2
CH2
CH2
CH2
C
H
N
H
C
O

**Aspartic acid**

Water shields ionic charge

**Lysine**


Hydrophilic
"head group"
O
O
C
H
H
O
C
H
H
"Flickering clusters" of $H_2O$ molecules in bulk phase
Highly ordered $H_2O$ molecules form "cages" around the hydrophobic alkyl chains



**Dispersion of lipids in $H_2O$**

Each lipid molecule forces surrounding $H_2O$ molecules to become highly ordered.

**Clusters of lipid molecules**

Only lipid portions at the edge of the cluster force the ordering of water. Fewer $H_2O$ molecules are ordered, and entropy is increased.

**Micelles**

All hydrophobic groups are sequestered from water; no highly ordered shell of $H_2O$ molecules is present, and entropy is increased.

(b)