Métodos de Detección y Análisis de Proteínas

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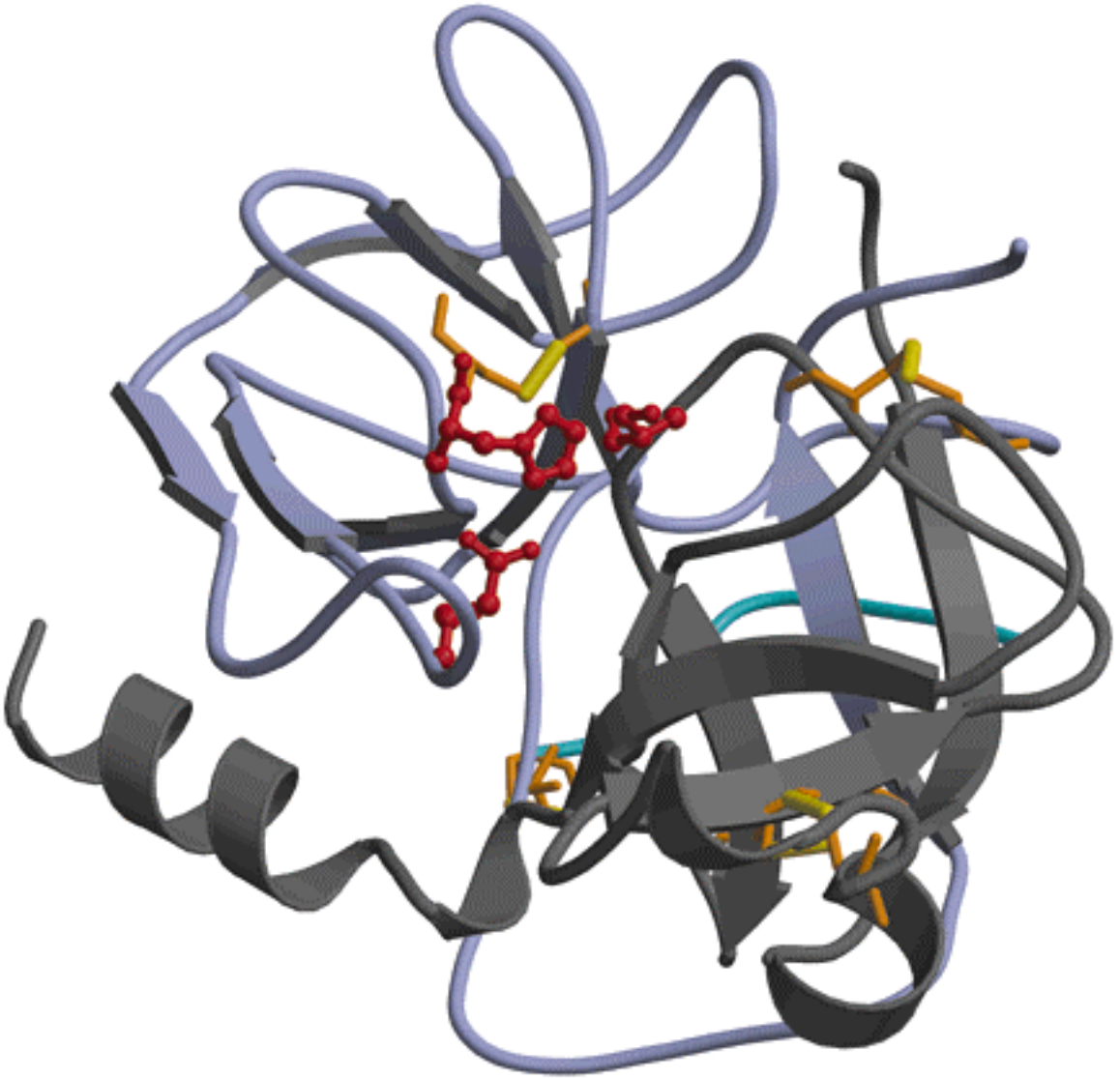
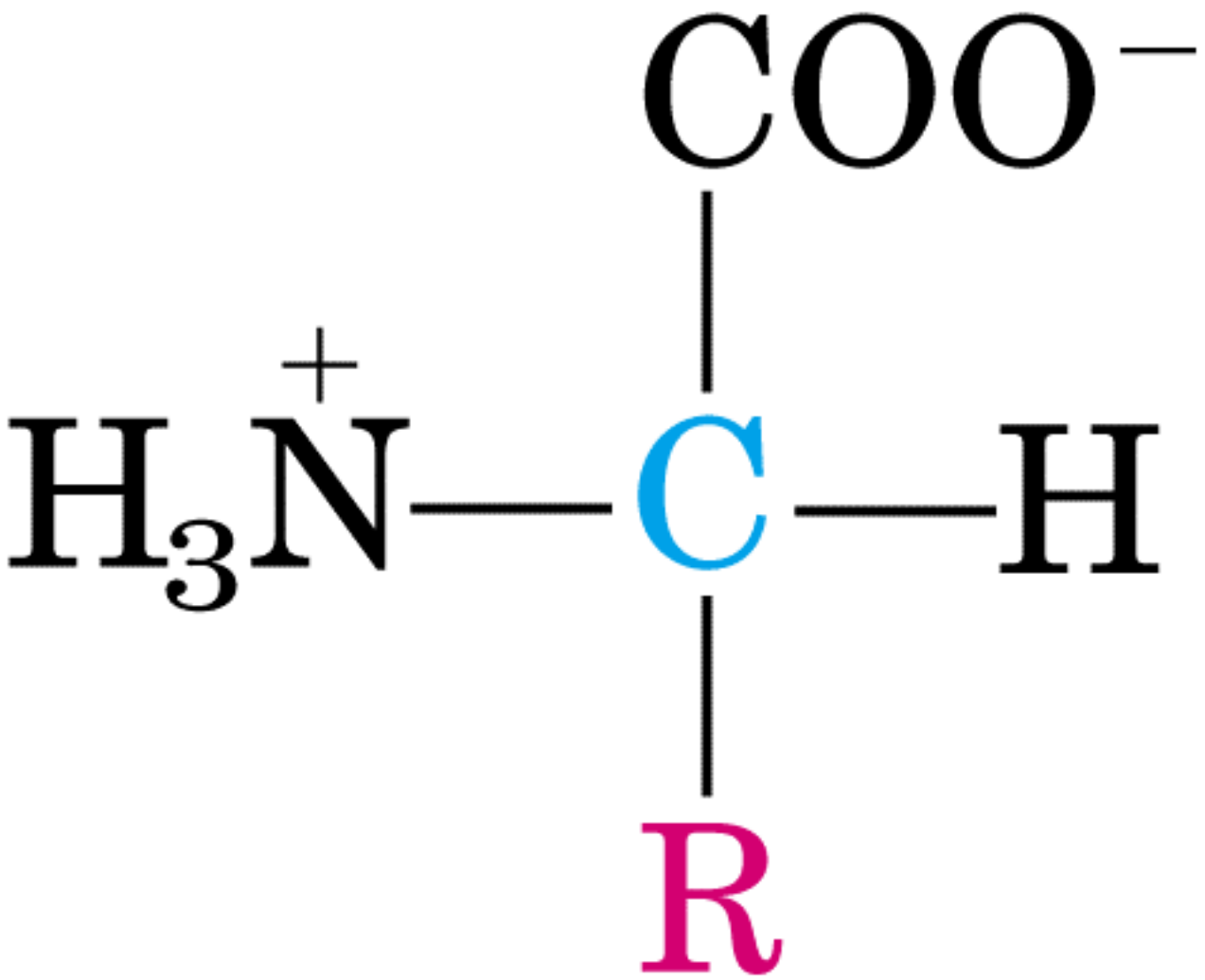
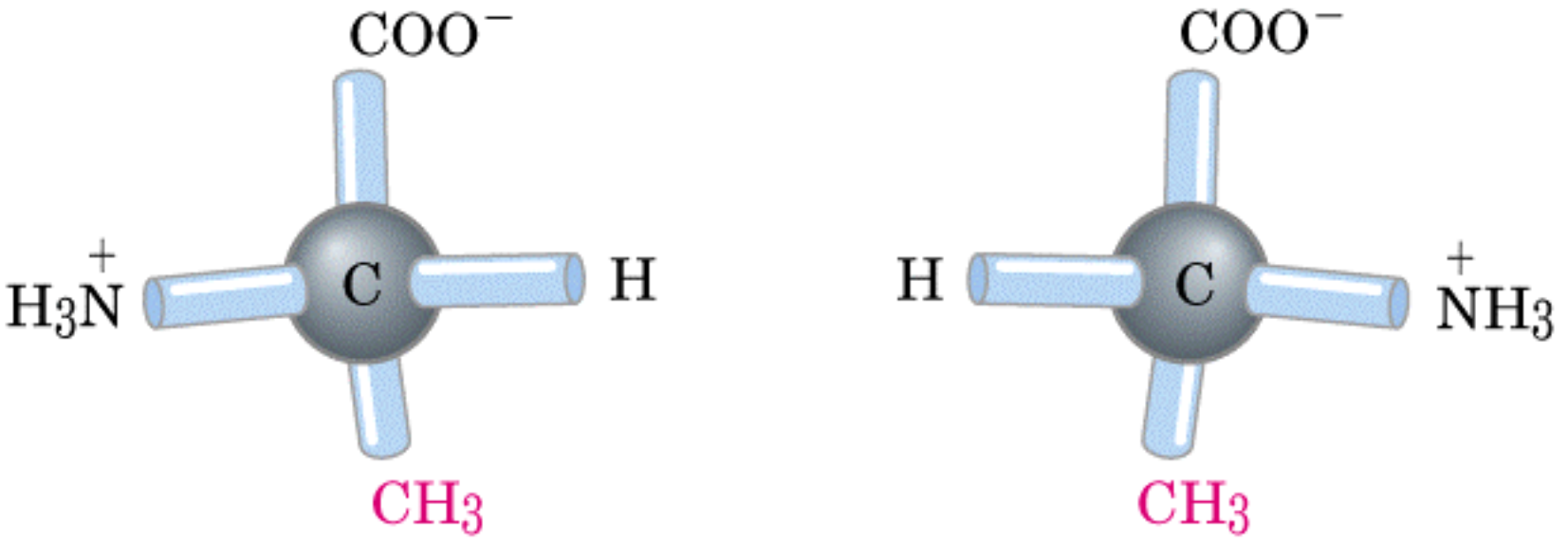
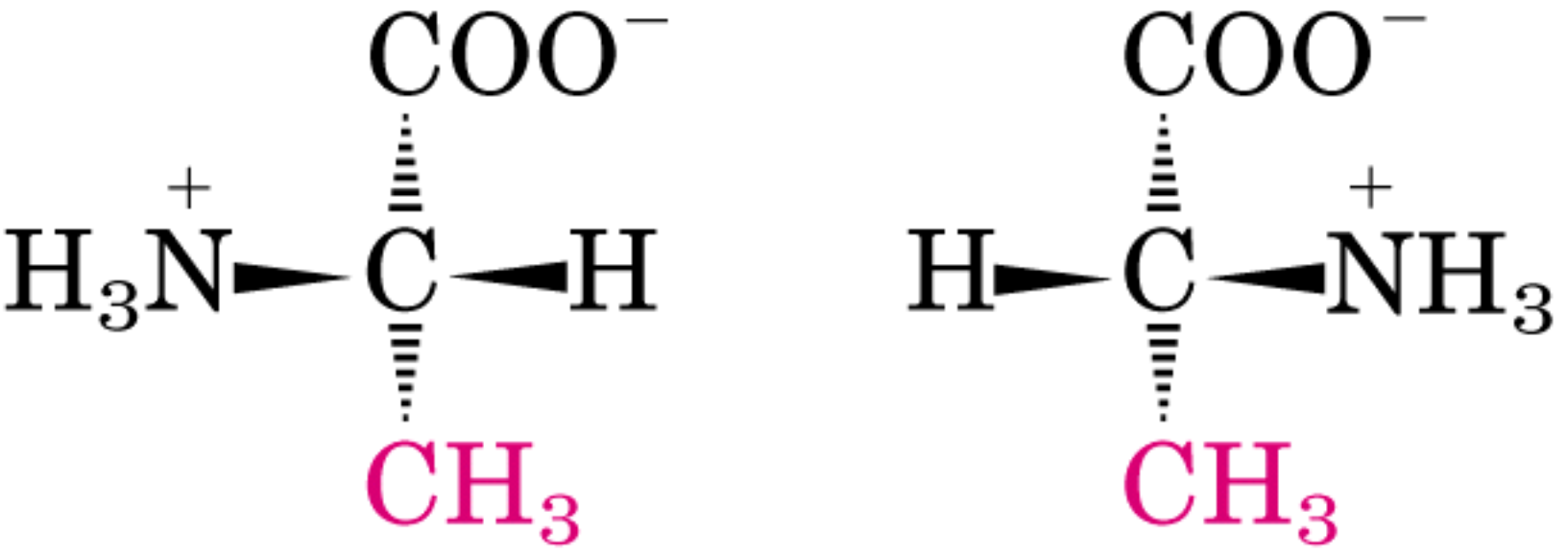



COO⁻
H3N+
C
H
R

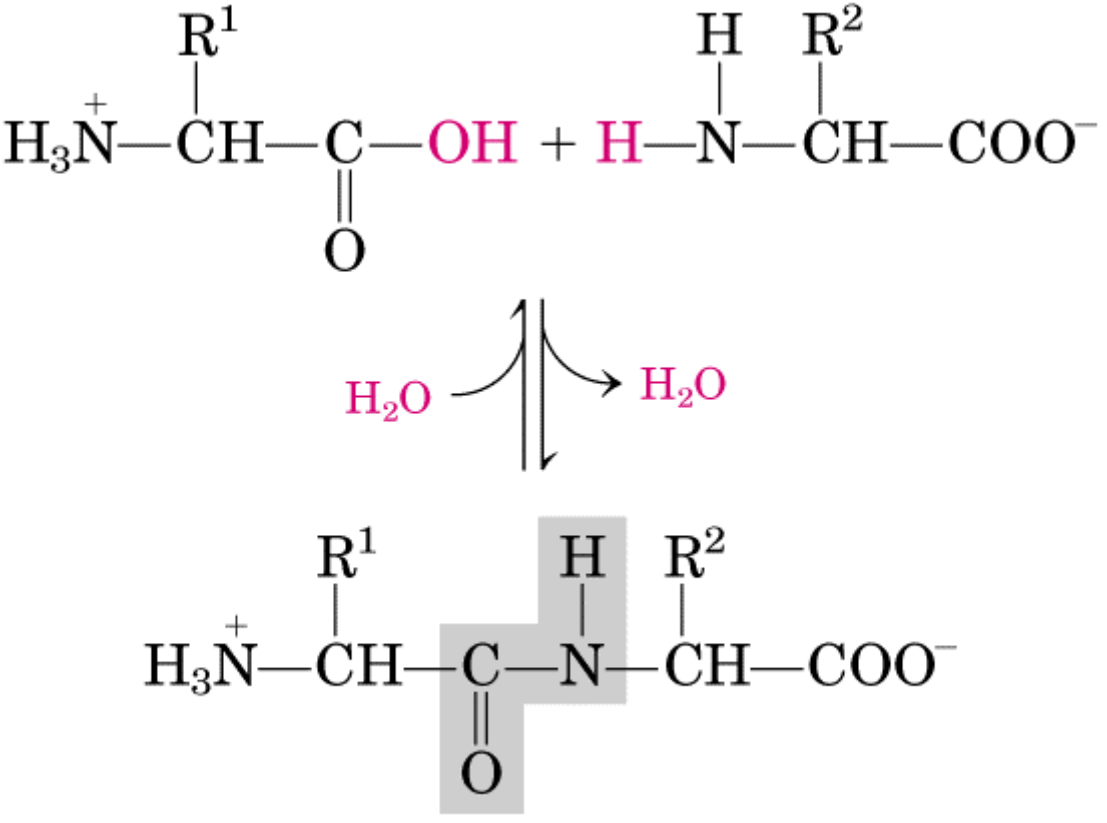


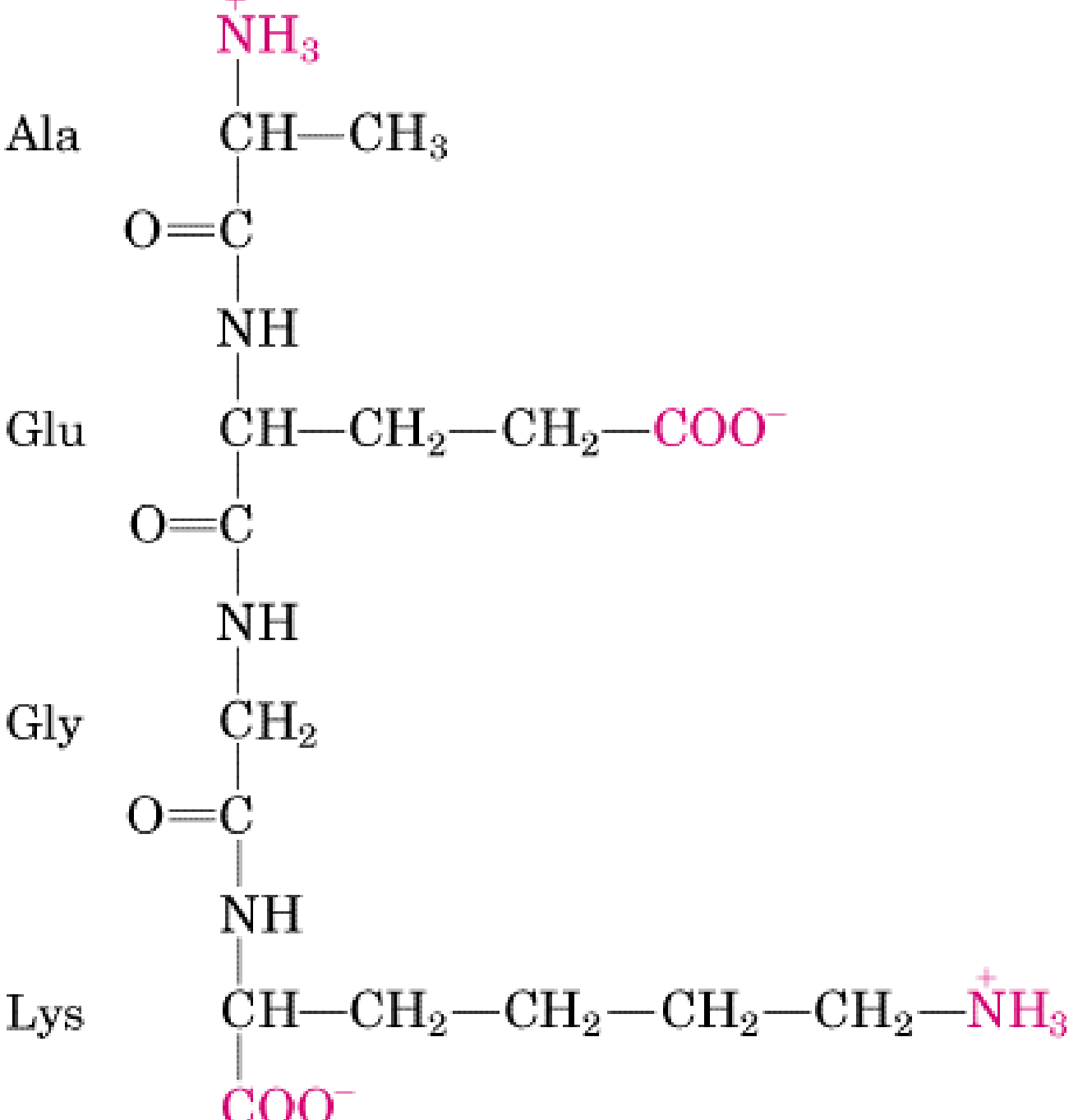
(a)

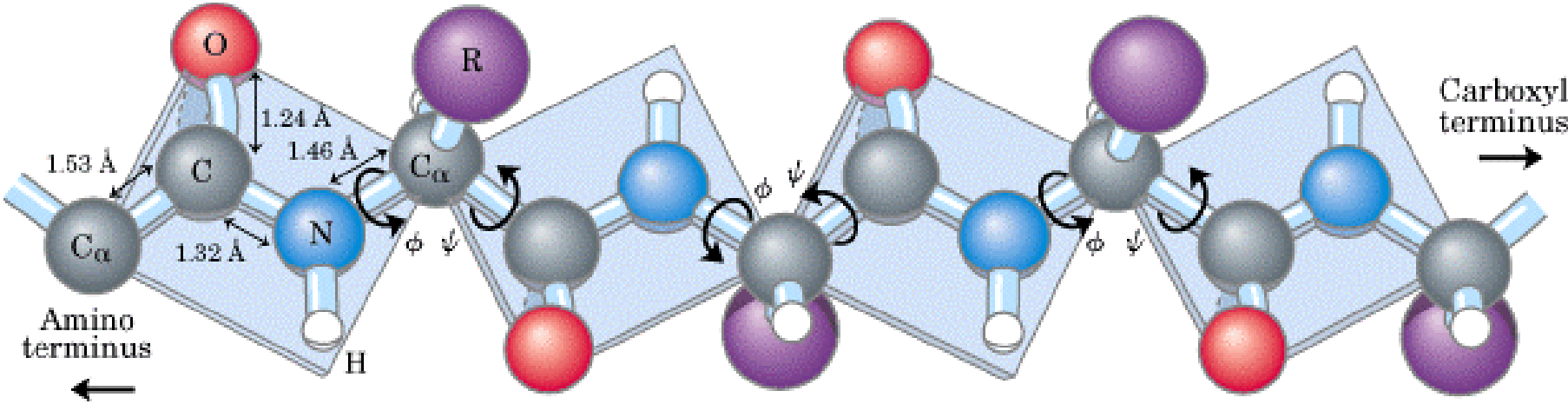


L-Alanine D-Alanine

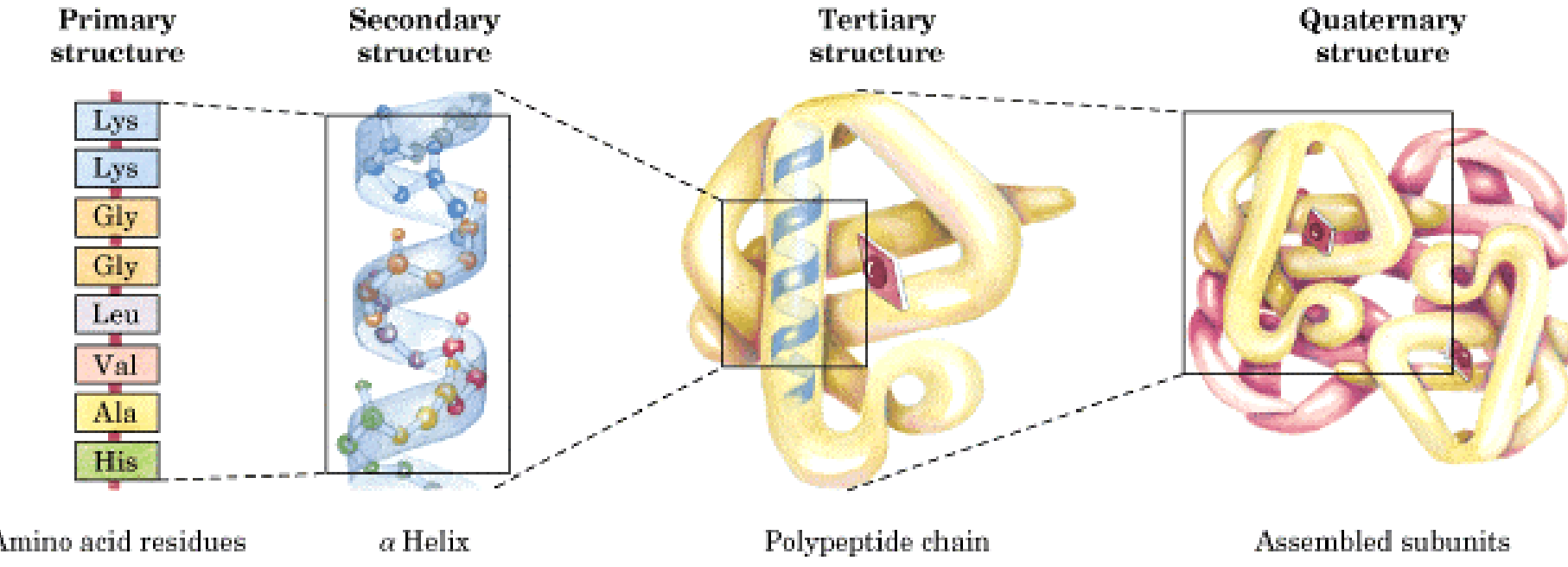
(b)

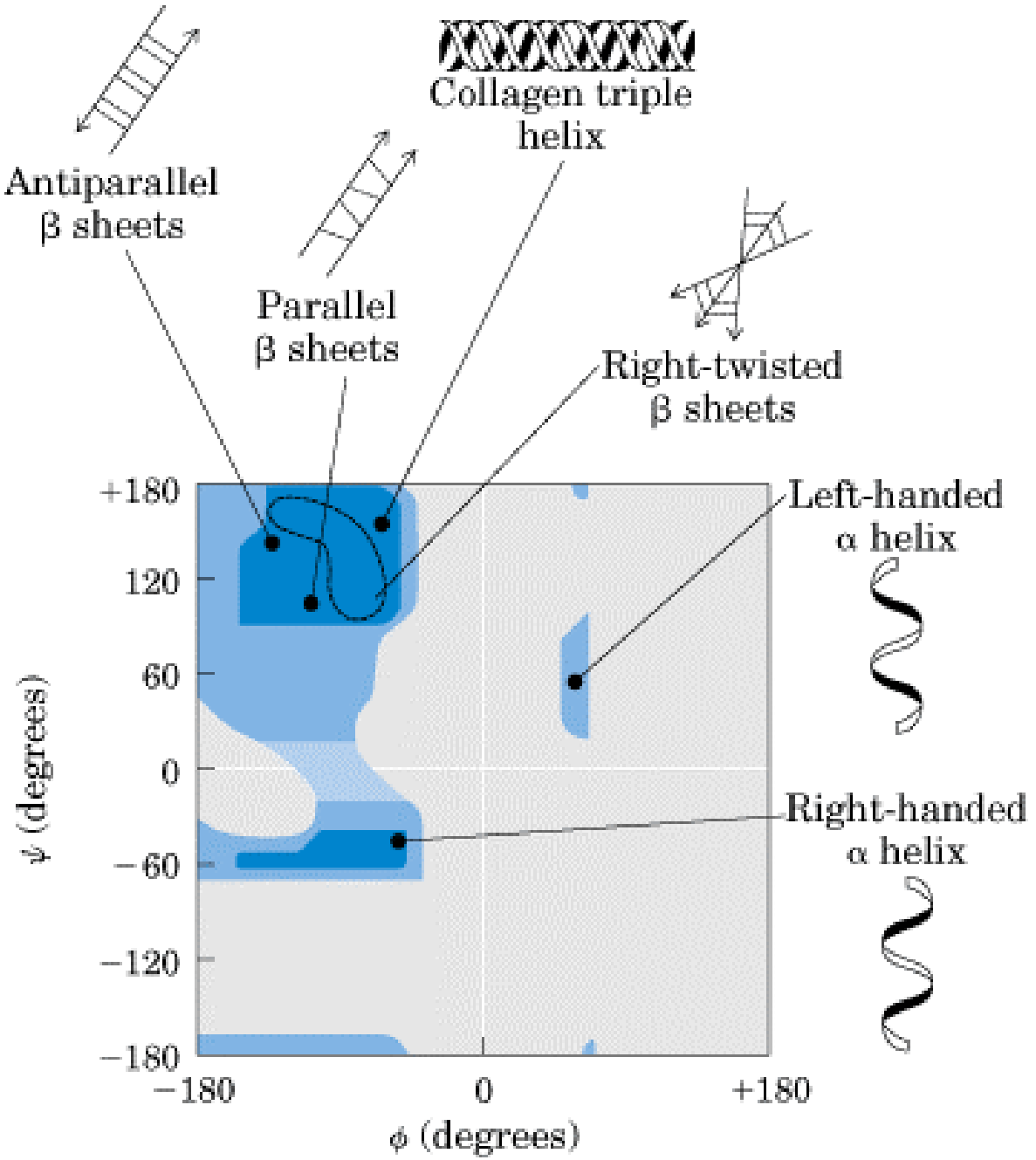

R1
H3N+—CH—C—OH + H—N—CH—COO−
O
H
R2
H2O
H2O
R1
H
R2
H3N+—CH—C—N—CH—COO−
O


NH3+
Ala
CH—CH3
O=C
NH
Glu
CH—CH2—CH2—COO−
O=C
NH
Gly
CH2
O=C
NH
Lys
CH—CH2—CH2—CH2—CH2—NH3+
COO−

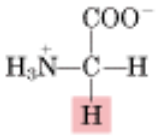

O
R
1.24 Å
1.46 Å
1.53 Å
C
C_α
C_α
N
1.32 Å
H
ϕ
ψ
ϕ
ψ
ϕ
ψ
Amino terminus
Carboxyl terminus

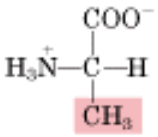
(b)

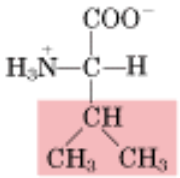

Primary structure
Secondary structure
Tertiary structure
Quaternary structure
Lys
Lys
Gly
Gly
Leu
Val
Ala
His
Amino acid residues
α Helix
Polypeptide chain
Assembled subunits

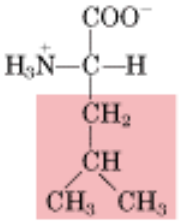

Collagen triple helix
Antiparallel β sheets
Parallel β sheets
Right-twisted β sheets
Left-handed α helix
Right-handed α helix
+180
120
60
0
−60
−120
−180
ψ (degrees)
−180
0
+180
ϕ (degrees)

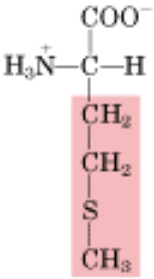
Nonpolar, aliphatic R groups

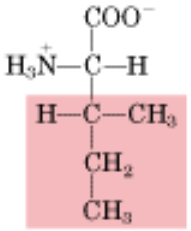

Glycine


Alanine

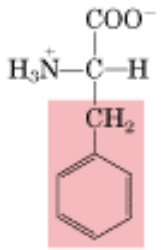

Valine

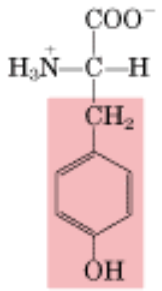

Leucine

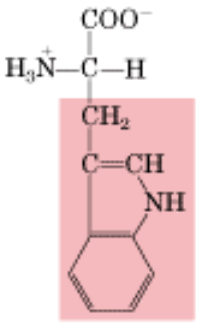

Methionine


Isoleucine

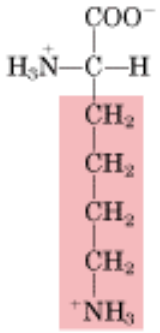
Aromatic R groups

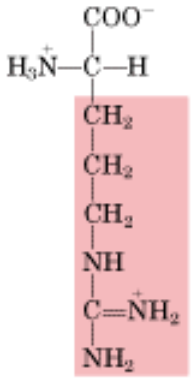

Phenylalanine

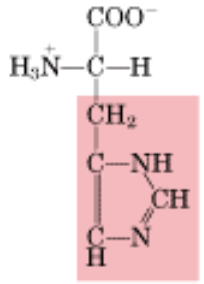

Tyrosine


Tryptophan

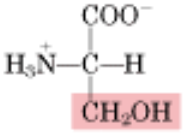
Positively charged R groups

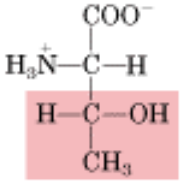

Lysine

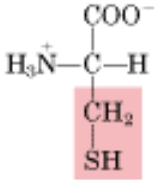

Arginine

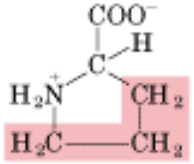

Histidine

Polar, uncharged R groups

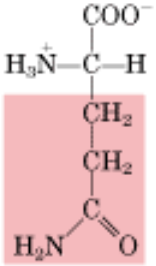

Serine


Threonine


Cysteine

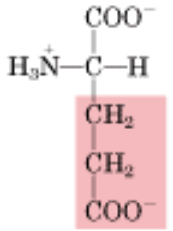

Proline

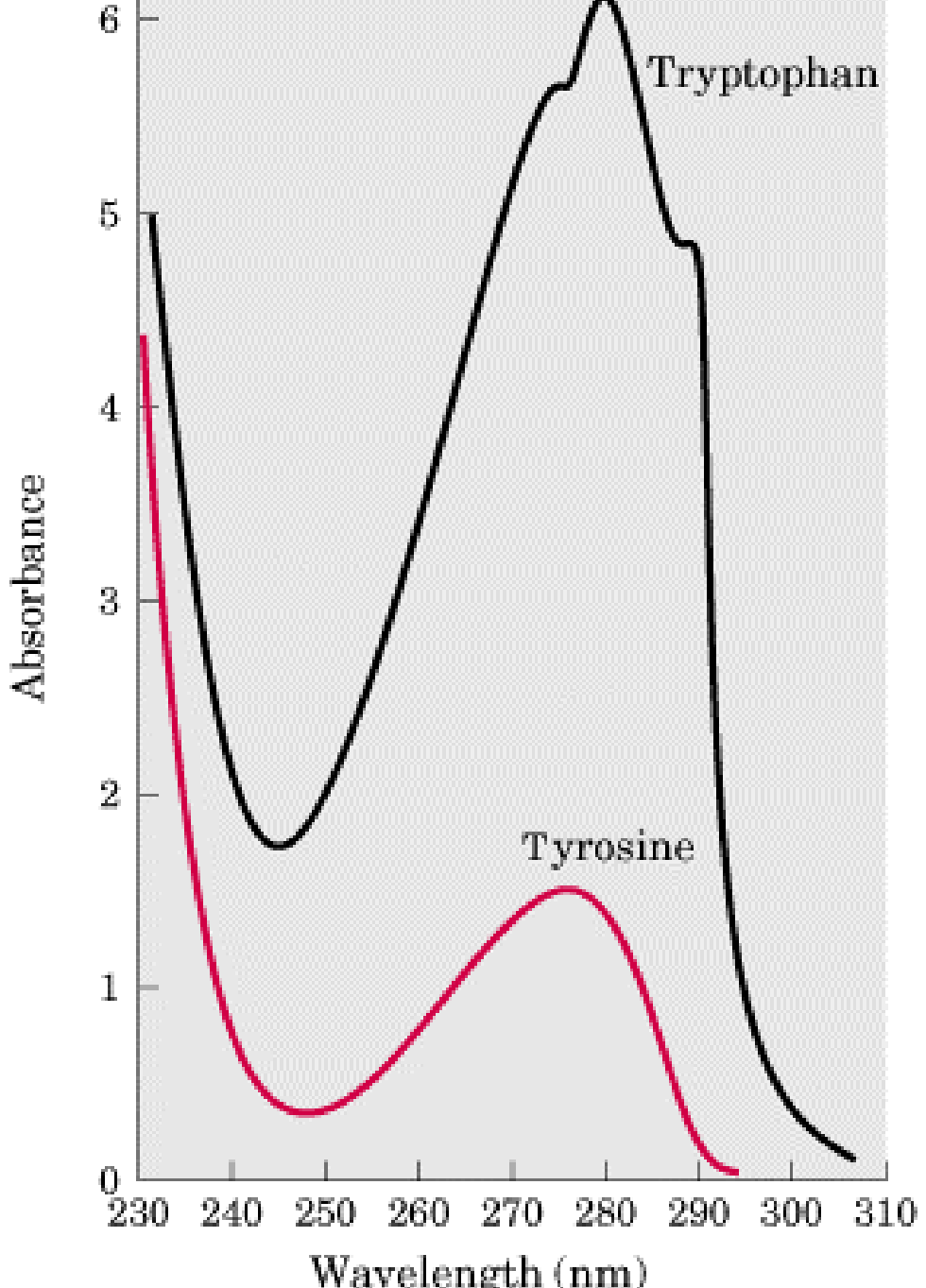

Asparagine

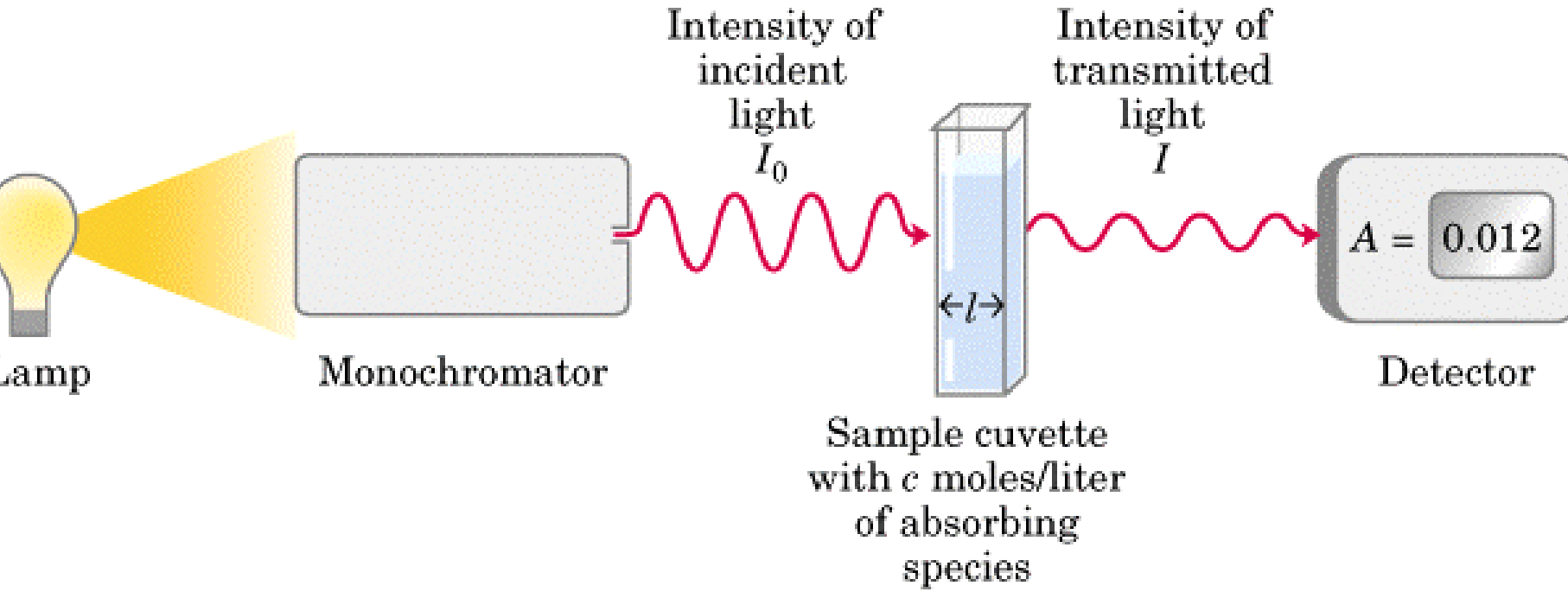

Glutamine

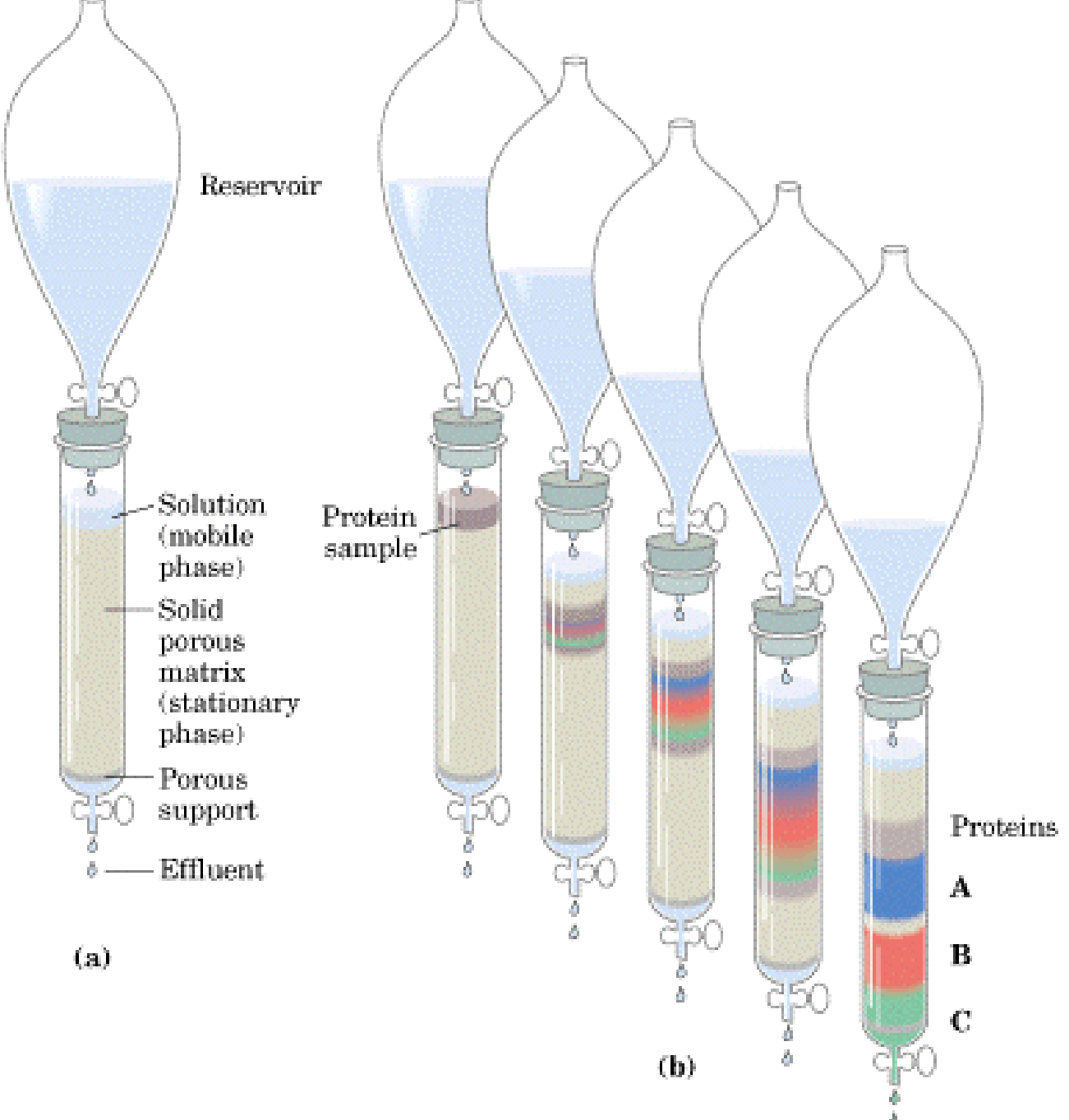
Negatively charged R groups

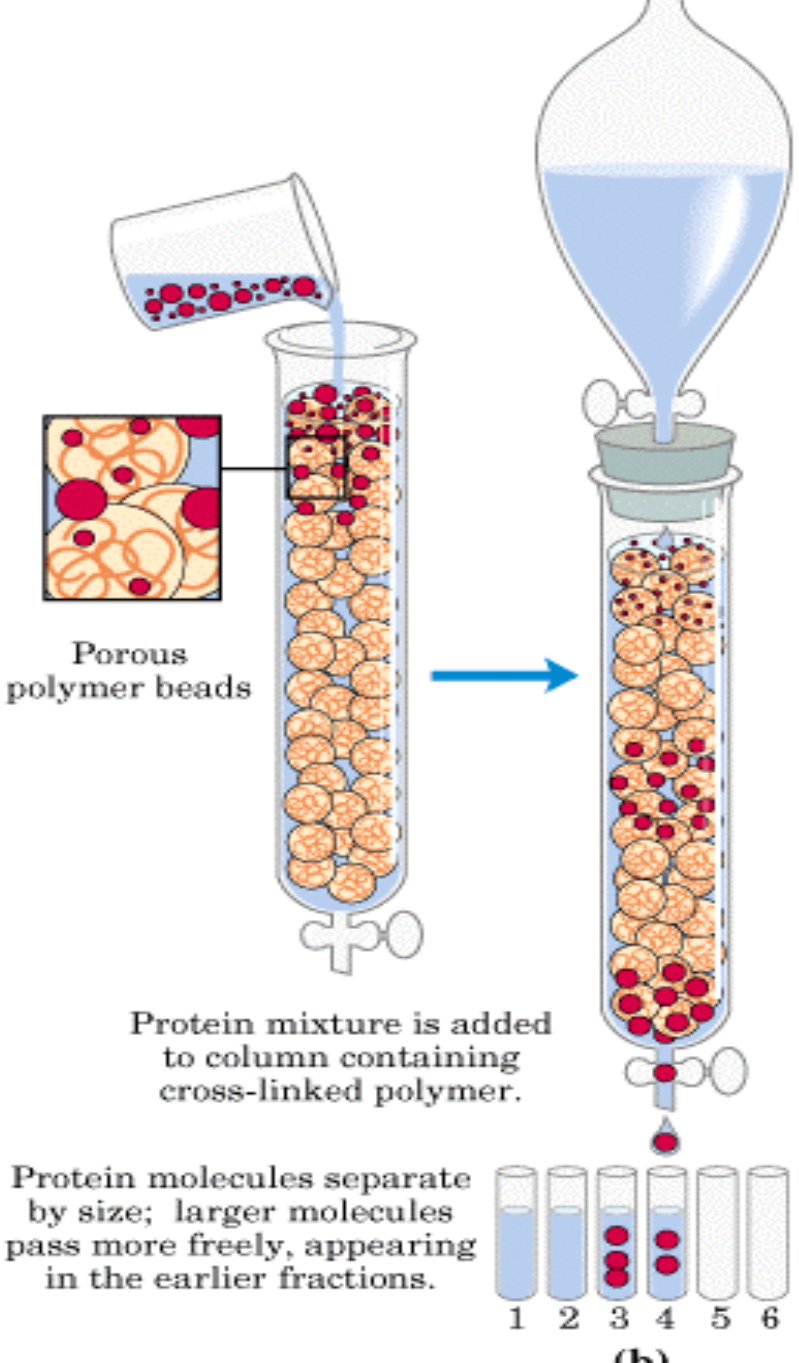

Aspartate

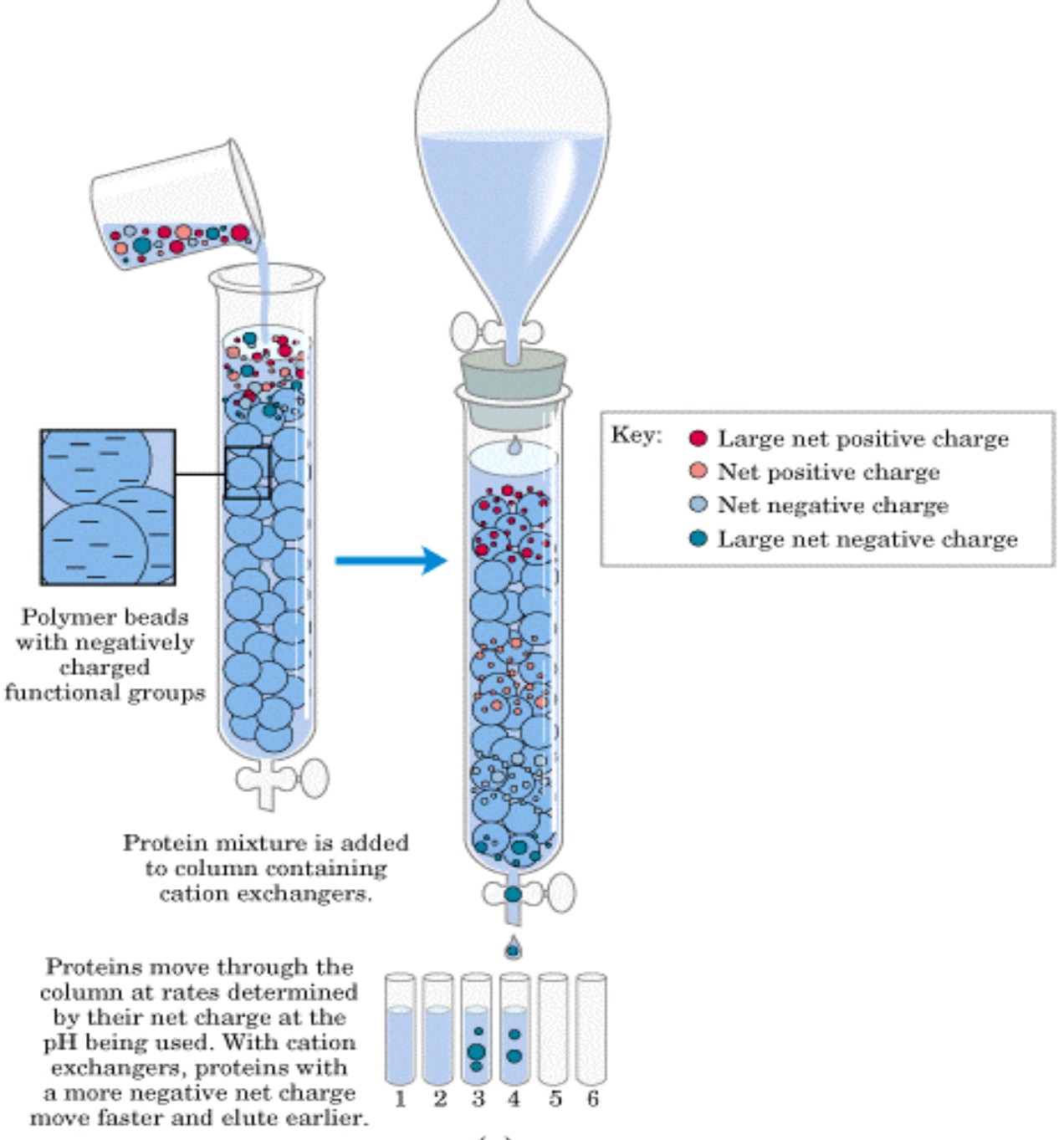

Glutamate

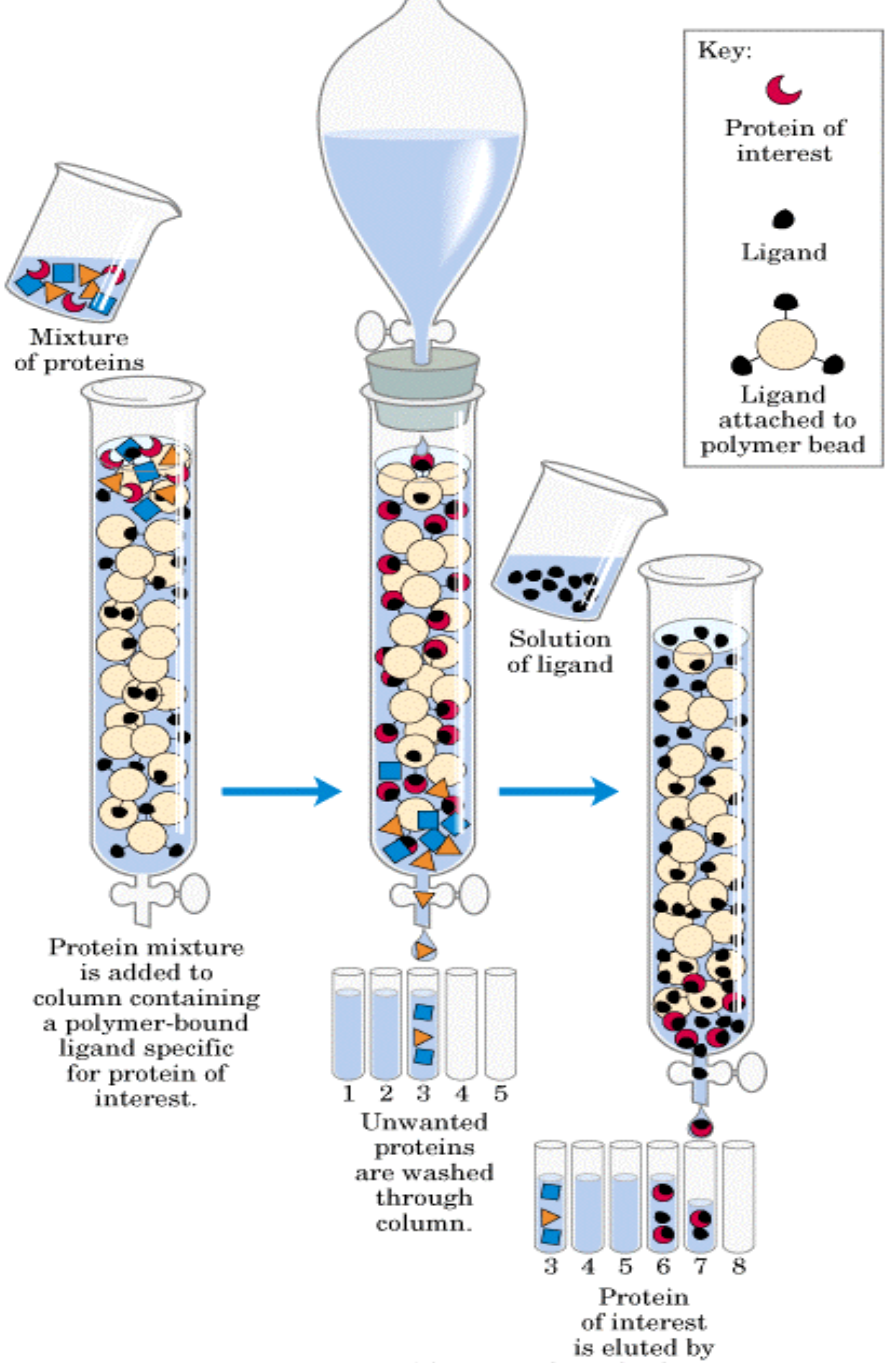

Absorbance
0
1
2
3
4
5
6
230
240
250
260
270
280
290
300
310
Wavelength (nm)
Tryptophan
Tyrosine

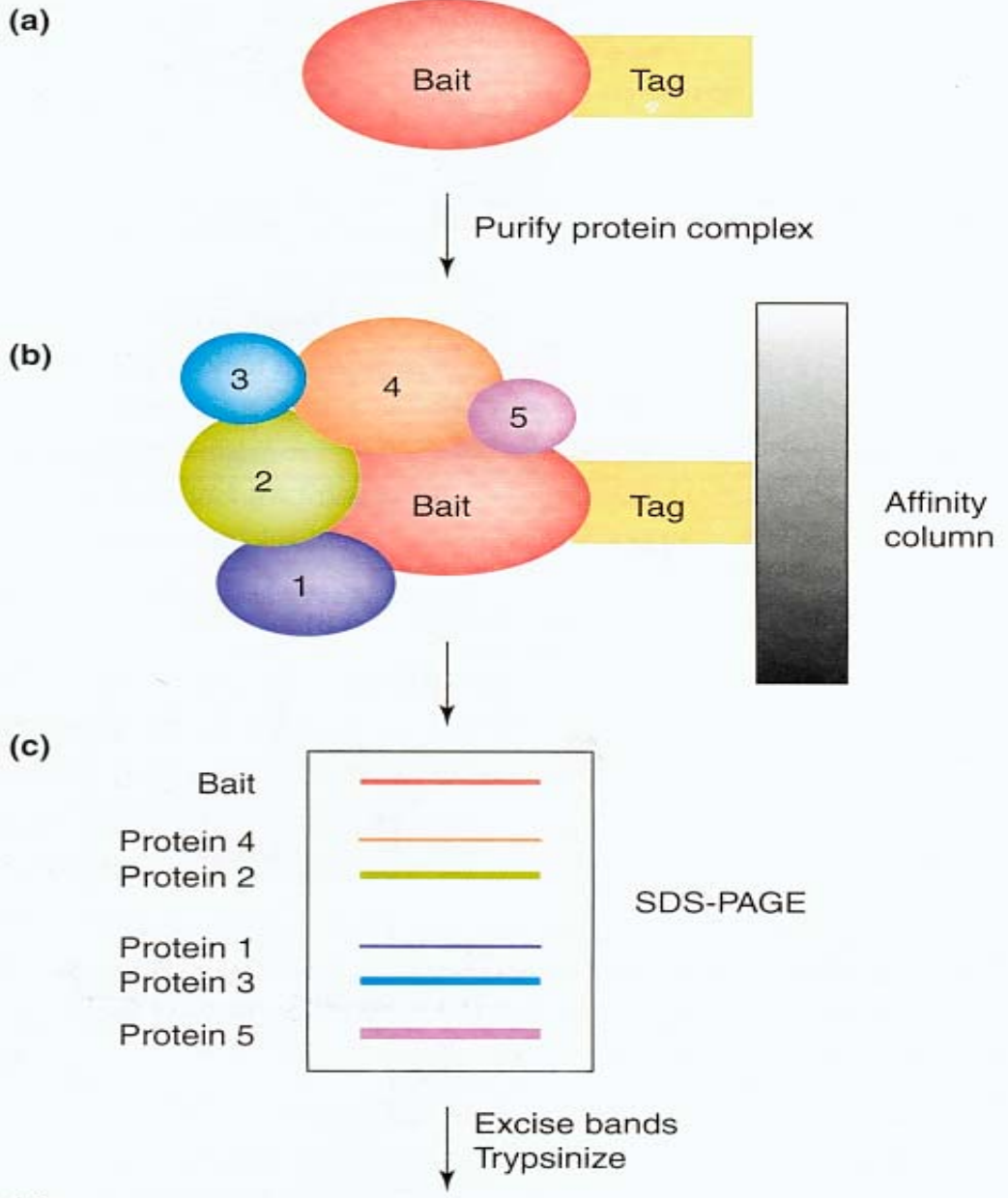

Intensity of incident light I_0
Intensity of transmitted light I
$A = 0.012$
$\leftarrow l \rightarrow$
Lamp
Monochromator
Sample cuvette with c moles/liter of absorbing species
Detector


Reservoir
Solution (mobile phase)
Solid porous matrix (stationary phase)
Porous support
Effluent
(a)
Protein sample
Proteins
A
B
C
(b)


Porous
polymer beads
Protein mixture is added
to column containing
cross-linked polymer.
Protein molecules separate
by size; larger molecules
pass more freely, appearing
in the earlier fractions.
1 2 3 4 5 6
(b)


Key:
Large net positive charge
Net positive charge
Net negative charge
Large net negative charge
Polymer beads with negatively charged functional groups
Protein mixture is added to column containing cation exchangers.
Proteins move through the column at rates determined by their net charge at the pH being used. With cation exchangers, proteins with a more negative net charge move faster and elute earlier.
1
2
3
4
5
6


Key:
Protein of interest
Ligand
Ligand attached to polymer bead
Mixture of proteins
Protein mixture is added to column containing a polymer-bound ligand specific for protein of interest.
1 2 3 4 5
Unwanted proteins are washed through column.
Solution of ligand
3 4 5 6 7 8
Protein of interest is eluted by


(a)
Bait
Tag
Purify protein complex
(b)
3
4
5
2
Bait
Tag
1
Affinity column
(c)
Bait
Protein 4
Protein 2
Protein 1
Protein 3
Protein 5
SDS-PAGE
Excise bands
Trypsinize
(d)
Identification of peptides by
mass spectrometry and database searches

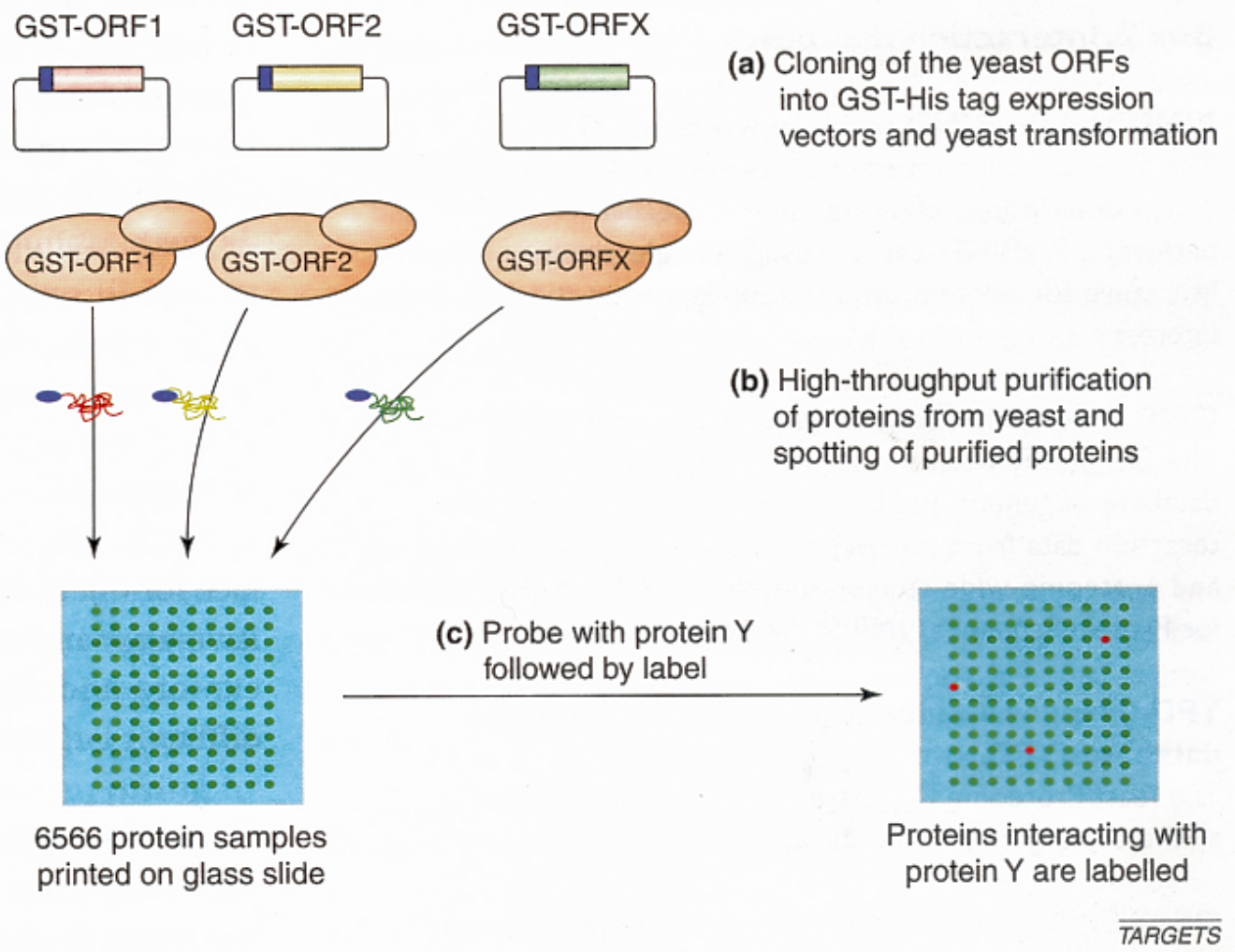
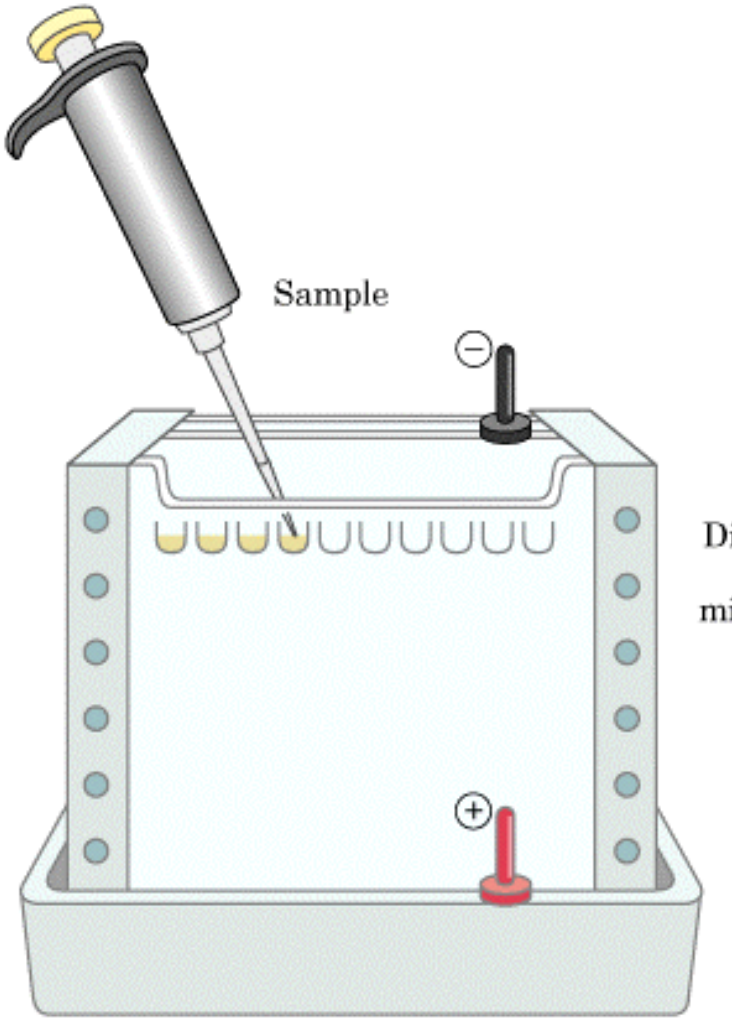
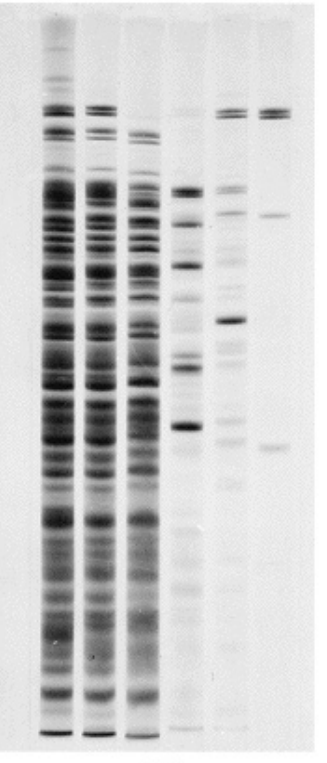


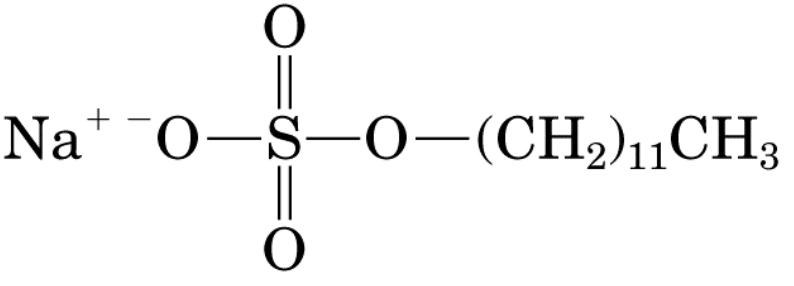
Figure 3. Identification of protein interactions on proteome chips. **(a)** Open reading frames (ORFs) encoding all bait proteins are cloned into expression vectors as fusions to glutathione-S-transferase (GST) and a polyhistidine tag, expressed in yeast and **(b)** purified by means of GST affinity chromatography. The purified proteins are printed onto nickel-coated glass slides to yield the final proteome chip. **(c)** A protein Y under investigation is incubated on the chip, followed by labelled antibodies directed against the protein Y. The identity of interacting proteins is determined by their position on the proteome chip.

SDS-PAGE

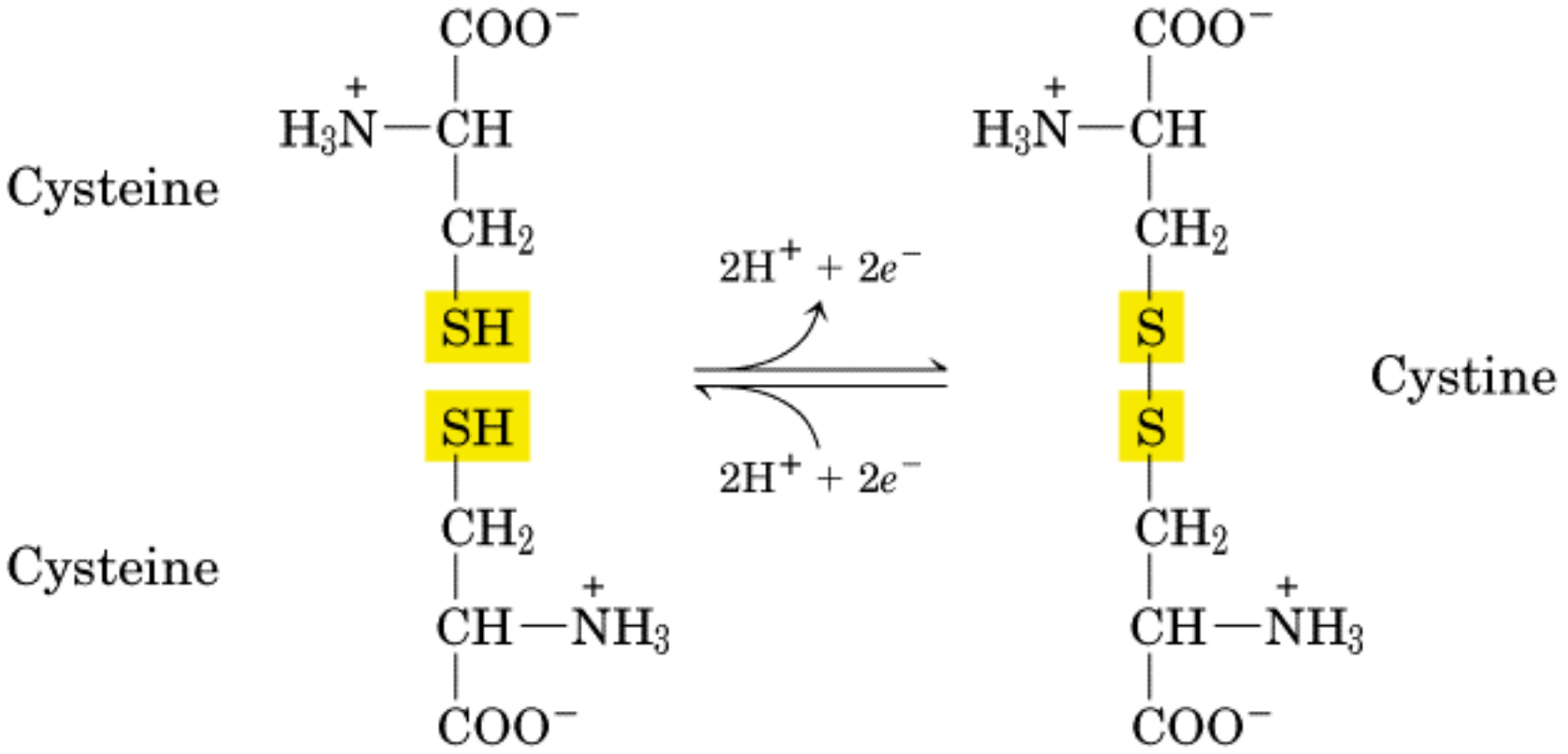


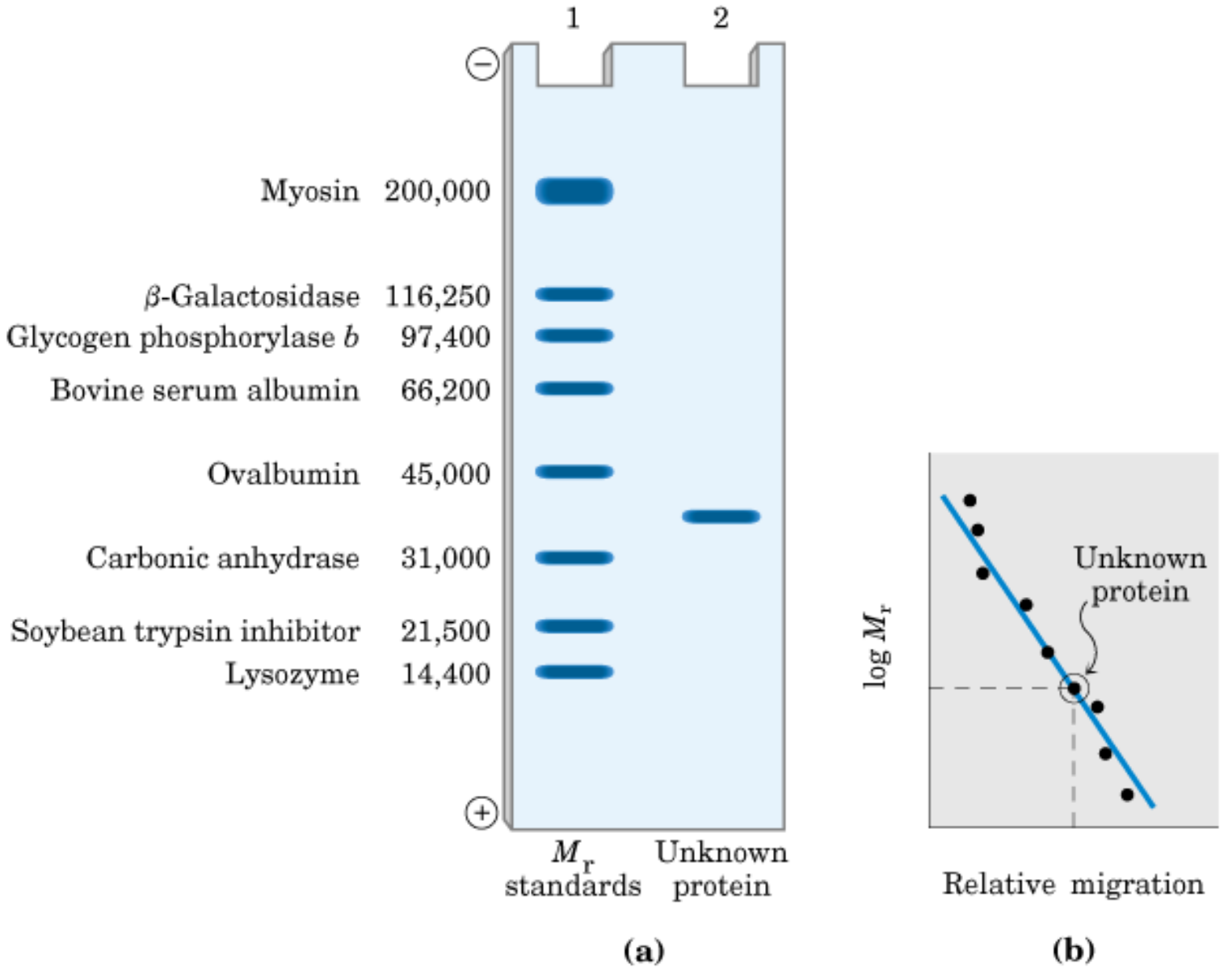
(a)





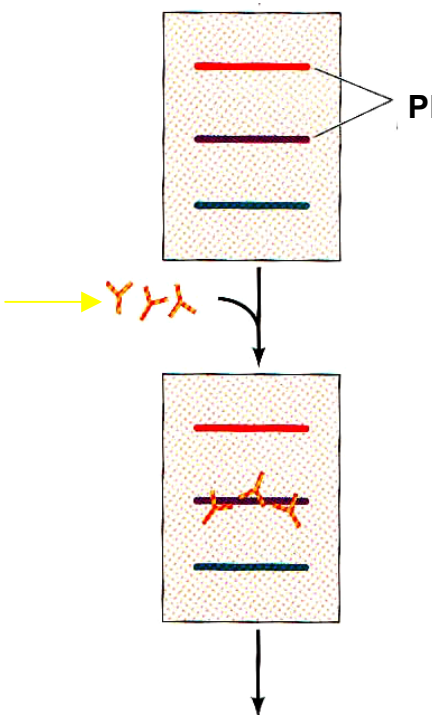
Sodium dodecyl sulfate (SDS)


Cysteine
Cysteine
Cystine
2H+ + 2e−
2H+ + 2e−


1
2
Myosin 200,000
β-Galactosidase 116,250
Glycogen phosphorylase b 97,400
Bovine serum albumin 66,200
Ovalbumin 45,000
Carbonic anhydrase 31,000
Soybean trypsin inhibitor 21,500
Lysozyme 14,400
M r standards
Unknown protein
(a)
log M r
Unknown protein
Relative migration
(b)

WESTERN BLOT

3. INCUBACION CON EL ANTICUERPO PRIMARIO
4. INCUBACION CON UN ANTICUERPO SECUNDARIO ACOPLADO A UNA ENZIMA (FOSFATASA ALCALINA O PEROXIDASA)
5. DETECCION



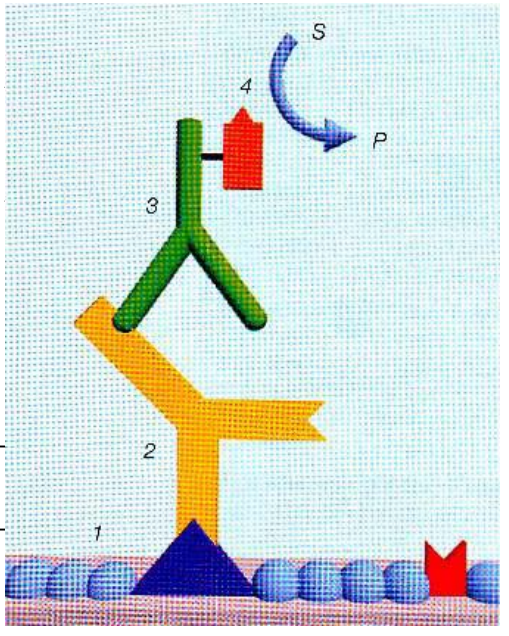
PROTEINAS

ENZIMA (FA/PEROXIDASA)

ANTICUERPO SECUNDARIO

ANTICUERPO PRIMARIO

PROTEINAS INMOBILIZADAS

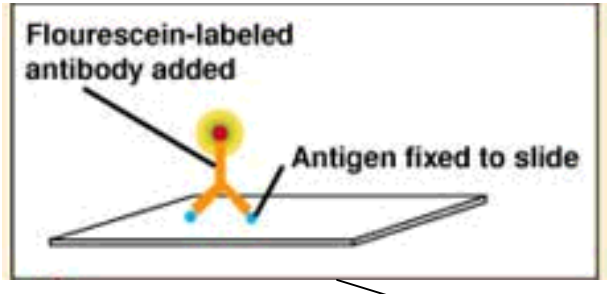


- **INMUNOHISTOQUIMICA/INMUNOFLUORESCENCIA**

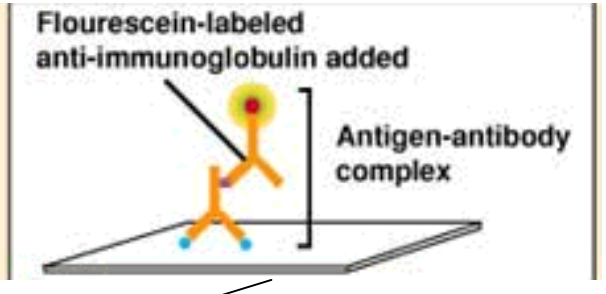
  DETECTA LA PRESENCIA DE UNA PROTEINA EN UNA CELULA/TEJIDO/ORGANISMO PERMITIENDO CONOCER SU EXPRESION ESPACIAL

INMUNOFLUORESCENCIA

A) DIRECTA



B) INDIRECTA



INCUBACION

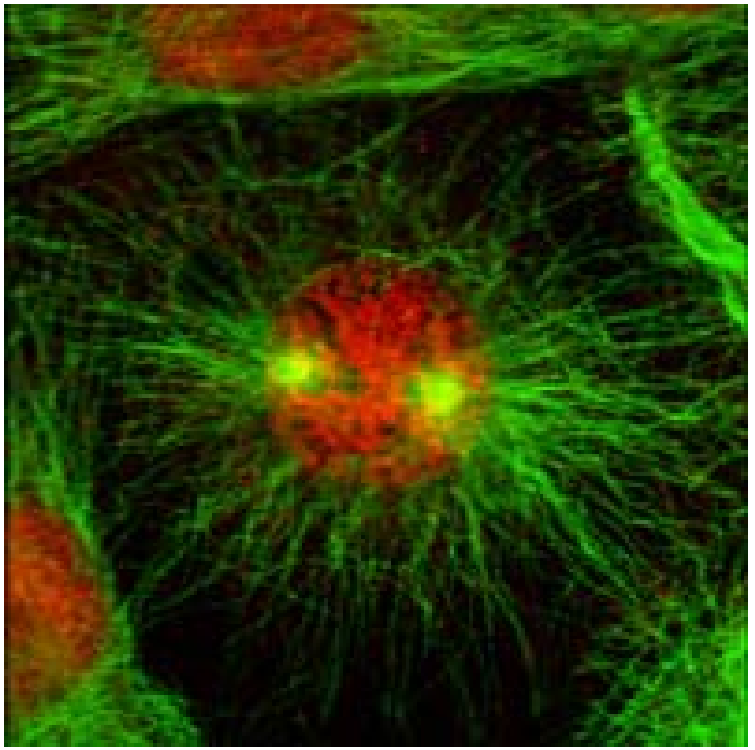
↓

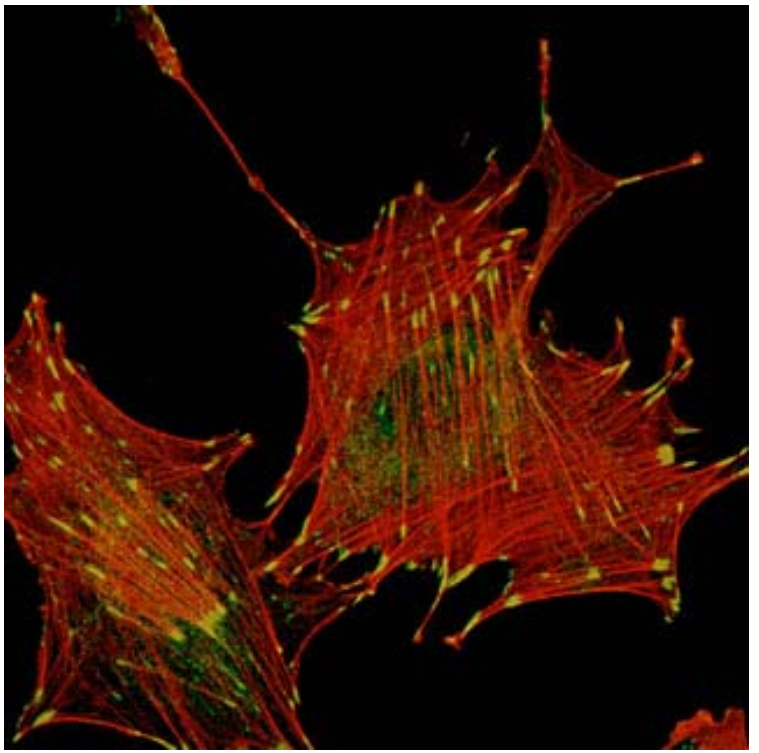
LAVADO

↓

VISUALIZACION EN MICROSCOPIO

INMUNOFLUORESCENCIA





ANTICUERPO PRIMARIO

ANTI-TUBULINA **ANTI-ACTINA**

ANTICUERPO SECUNDARIO

FLOURESCEINA **RODAMINA**

table 5–6

The Isoelectric Points of Some Proteins

Protein	pI
Pepsin	~1.0
Egg albumin	4.6
Serum albumin	4.9
Urease	5.0
β-Lactoglobulin	5.2
Hemoglobin	6.8
Myoglobin	7.0
Chymotrypsinogen	9.5
Cytochrome *c*	10.7
Lysozyme	11.0

table 4–5

The pH Scale

$[H^+]$ (M)	pH	$[OH^-]$ (M)	pOH*
10^0 (1)	0	10^{-14}	14
10^{-1}	1	10^{-13}	13
10^{-2}	2	10^{-12}	12
10^{-3}	3	10^{-11}	11
10^{-4}	4	10^{-10}	10
10^{-5}	5	10^{-9}	9
10^{-6}	6	10^{-8}	8
10^{-7}	7	10^{-7}	7
10^{-8}	8	10^{-6}	6
10^{-9}	9	10^{-5}	5
10^{-10}	10	10^{-4}	4
10^{-11}	11	10^{-3}	3
10^{-12}	12	10^{-2}	2
10^{-13}	13	10^{-1}	1
10^{-14}	14	10^0 (1)	0

*The expression pOH is sometimes used to describe the basicity, or OH^- concentration, of a solution; pOH is defined by the expression $pOH = -\log [OH^-]$, which is analogous to the expression for pH. Note that in all cases, pH + pOH = 14.

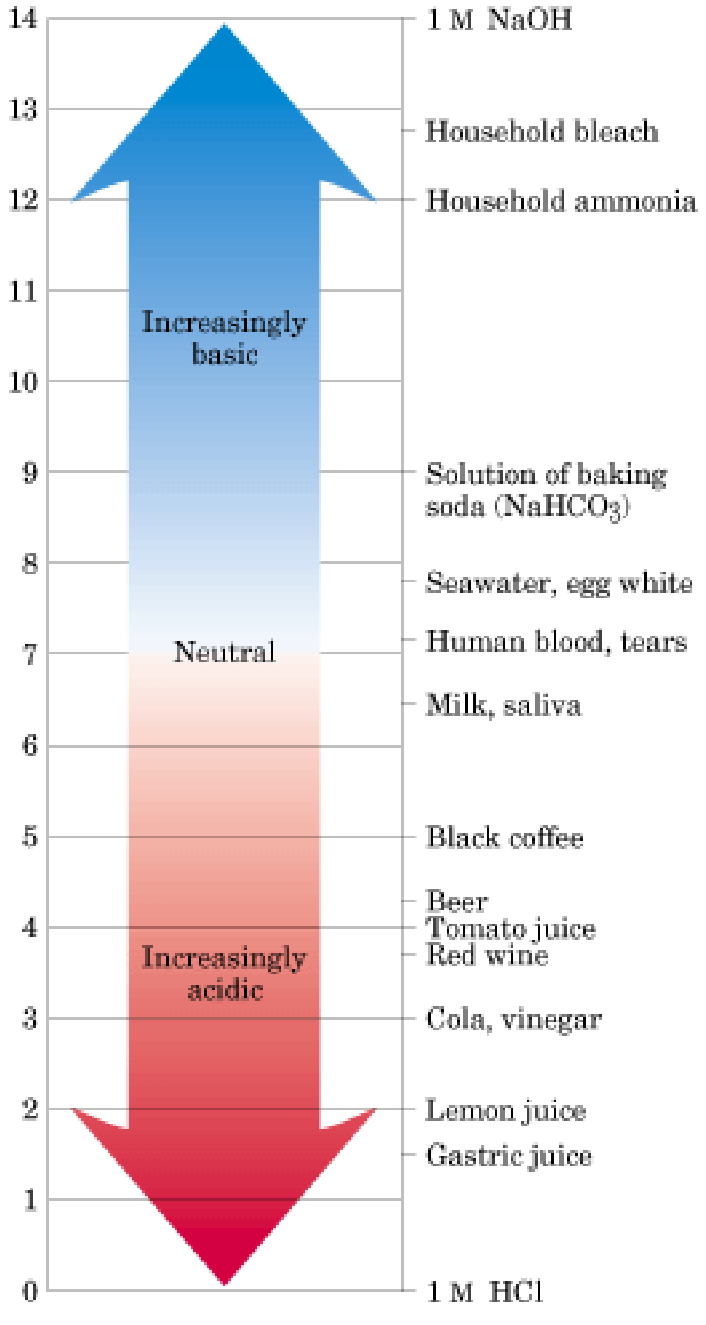


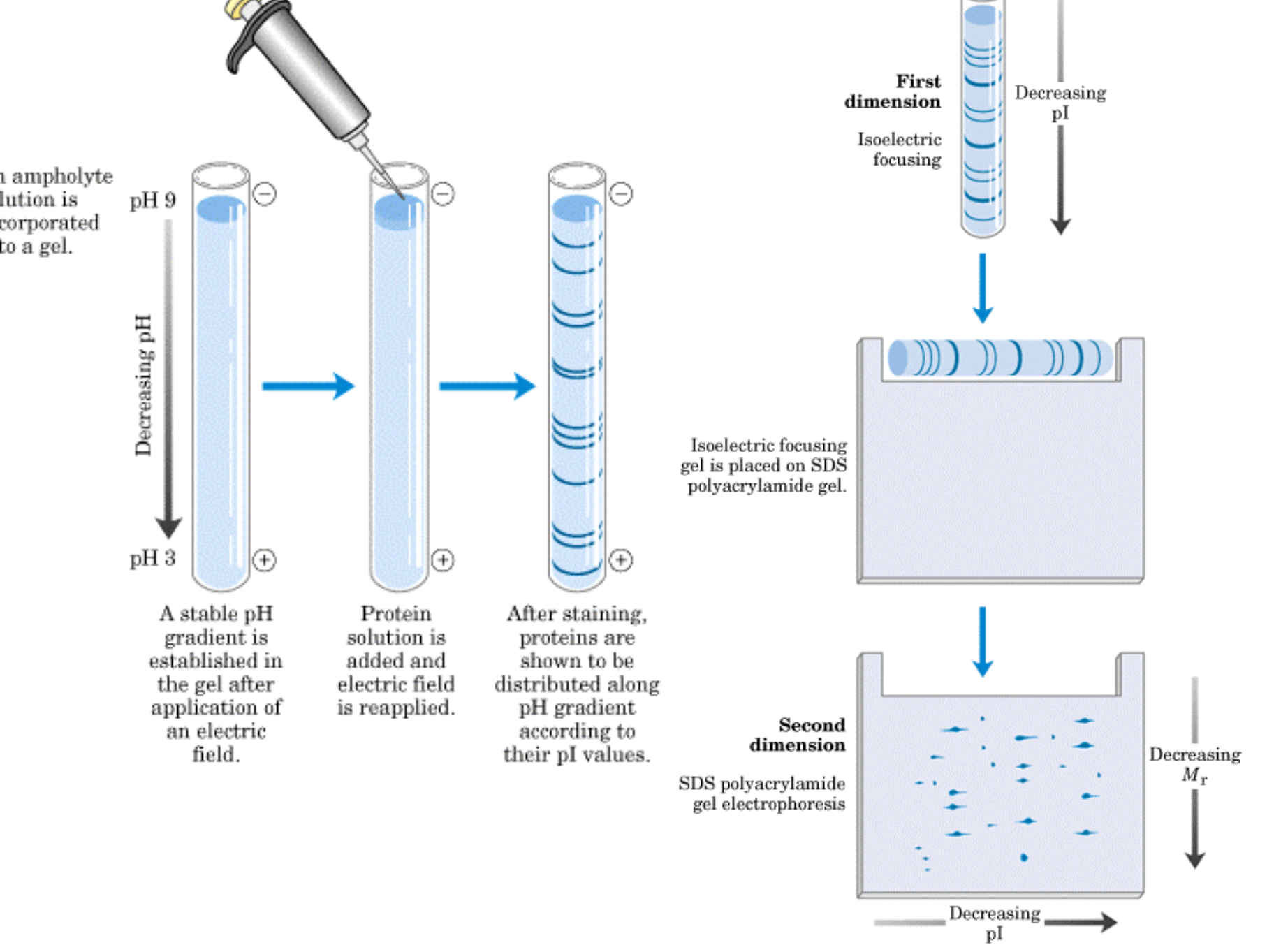
table 5–1

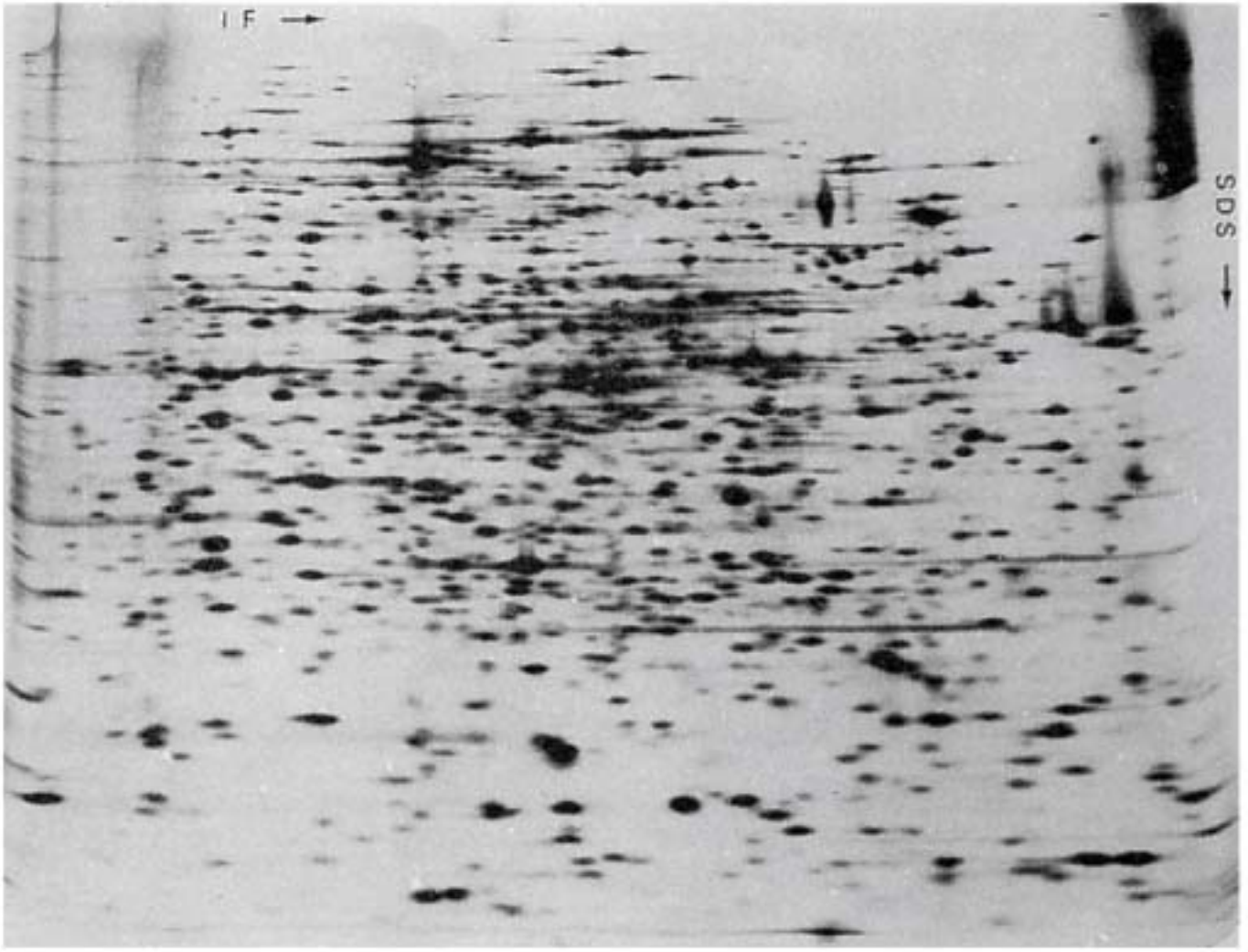
Properties and Conventions Associated with the Standard Amino Acids

Amino acid	Abbreviated names		M_r	pK_a values: pK_1 (—COOH)	pK_2 ($—NH_3^+$)	pK_R (R group)	pI	Hydropathy index*	Occurrence in proteins (%)†
Nonpolar, aliphatic R groups									
Glycine	Gly	G	75	2.34	9.60		5.97	−0.4	7.2
Alanine	Ala	A	89	2.34	9.69		6.01	1.8	7.8
Valine	Val	V	117	2.32	9.62		5.97	4.2	6.6
Leucine	Leu	L	131	2.36	9.60		5.98	3.8	9.1
Isoleucine	Ile	I	131	2.36	9.68		6.02	4.5	5.3
Methionine	Met	M	149	2.28	9.21		5.74	1.9	2.3
Aromatic R groups									
Phenylalanine	Phe	F	165	1.83	9.13		5.48	2.8	3.9
Tyrosine	Tyr	Y	181	2.20	9.11	10.07	5.66	−1.3	3.2
Tryptophan	Trp	W	204	2.38	9.39		5.89	−0.9	1.4
Polar, uncharged R groups									
Serine	Ser	S	105	2.21	9.15		5.68	−0.8	6.8
Proline	Pro	P	115	1.99	10.96		6.48	1.6	5.2
Threonine	Thr	T	119	2.11	9.62		5.87	−0.7	5.9
Cysteine	Cys	C	121	1.96	10.28	8.18	5.07	2.5	1.9
Asparagine	Asn	N	132	2.02	8.80		5.41	−3.5	4.3
Glutamine	Gln	Q	146	2.17	9.13		5.65	−3.5	4.2
Positively charged R groups									
Lysine	Lys	K	146	2.18	8.95	10.53	9.74	−3.9	5.9
Histidine	His	H	155	1.82	9.17	6.00	7.59	−3.2	2.3
Arginine	Arg	R	174	2.17	9.04	12.48	10.76	−4.5	5.1
Negatively charged R groups									
Aspartate	Asp	D	133	1.88	9.60	3.65	2.77	−3.5	5.3
Glutamate	Glu	E	147	2.19	9.67	4.25	3.22	−3.5	6.3

*A scale combining hydrophobicity and hydrophilicity of R groups; it can be used to measure the tendency of an amino acid to seek an aqueous environment (− values) or a hydrophobic environment (+ values). See Chapter 12. From Kyte, J. & Doolittle, R.F. (1982) *J. Mol. Biol.* **157**, 105 – 132.

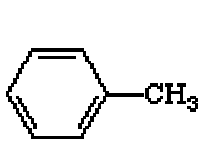
†Average occurrence in over 1150 proteins. From Doolittle, R.F. (1989) Redundancies in protein sequences. In *Prediction of Protein Structure and the Principles of Protein Conformation* (Fasman, G.D., ed) Plenum Press, NY, pp. 599–623.


n ampholyte
lution is
corporated
to a gel.
pH 9
Decreasing pH
pH 3
A stable pH gradient is established in the gel after application of an electric field.
Protein solution is added and electric field is reapplied.
After staining, proteins are shown to be distributed along pH gradient according to their pI values.
First dimension
Isoelectric focusing
Decreasing pI
Isoelectric focusing gel is placed on SDS polyacrylamide gel.
Second dimension
SDS polyacrylamide gel electrophoresis
Decreasing M_r
Decreasing pI

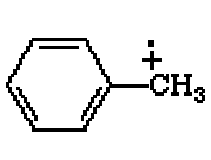


(b)

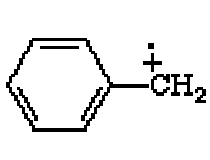
Espectrometría de masas (MS)



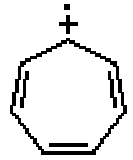
Tolueno



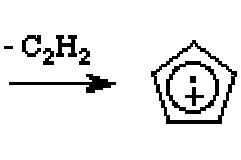
m+



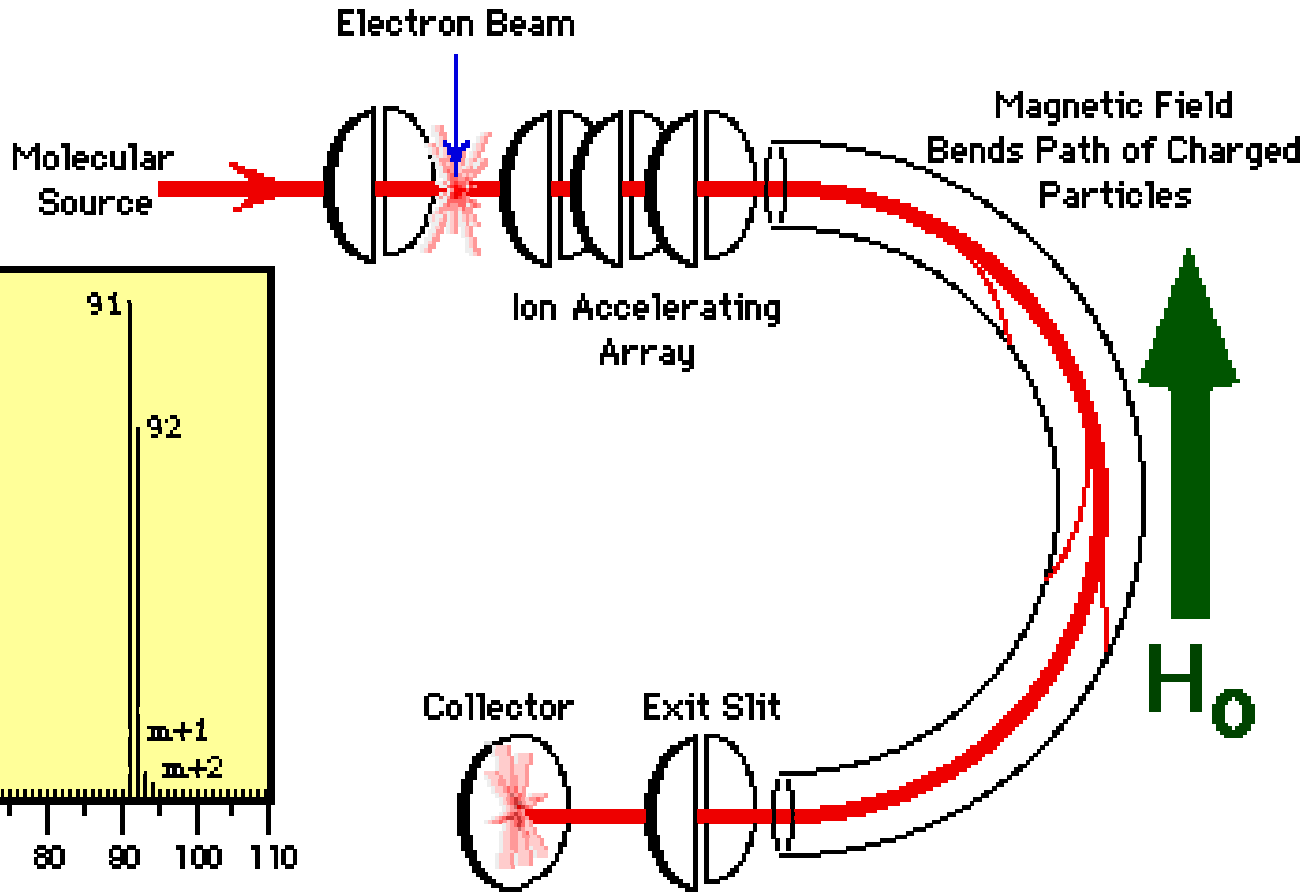
m-1

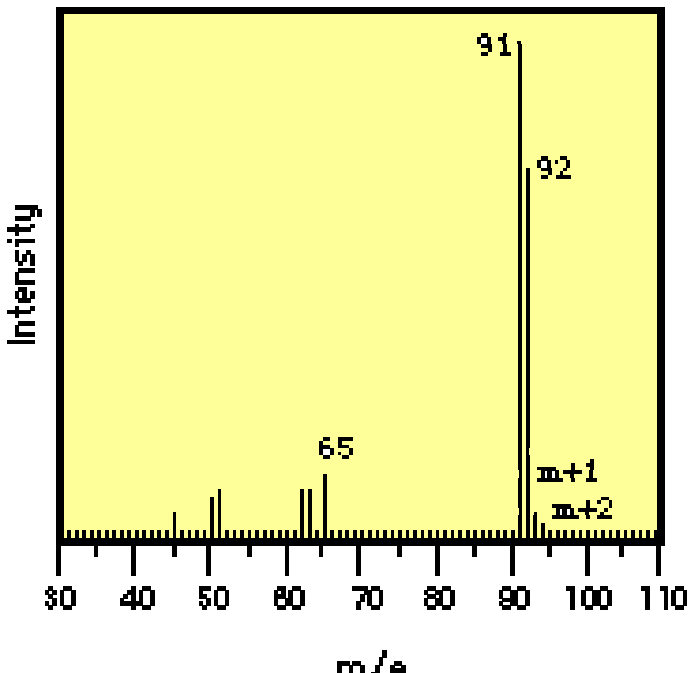


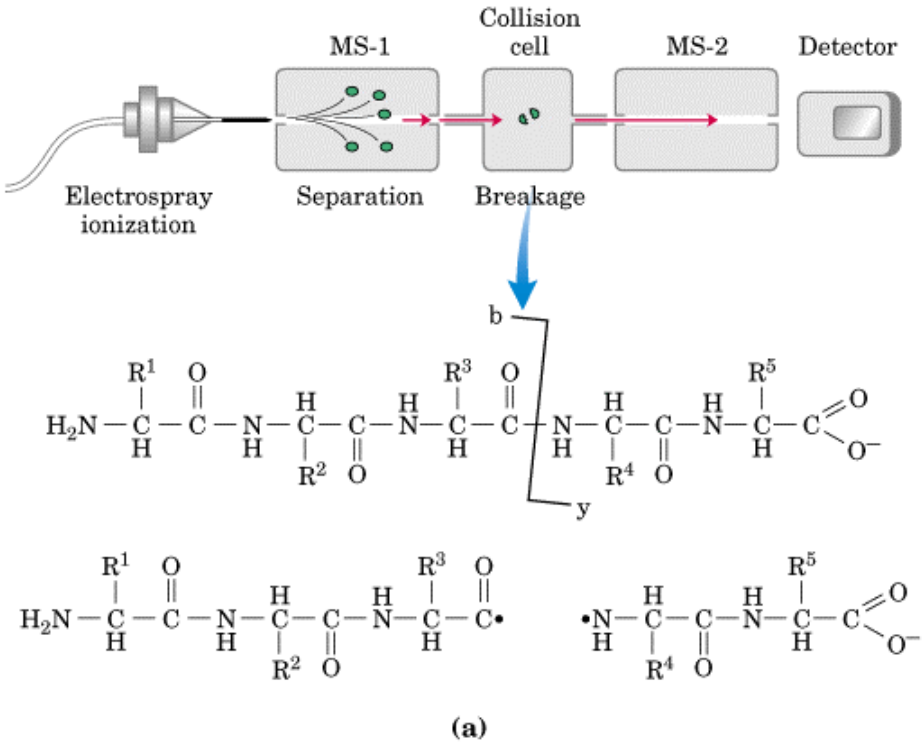
Tropylium Ion



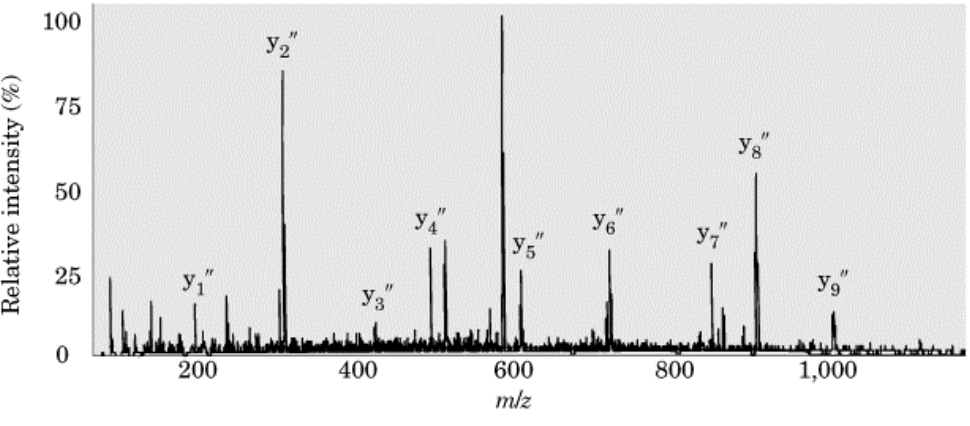
m/e = 6






Collision
cell
MS-1
MS-2
Detector
Electrospray
ionization
Separation
Breakage
b
y

(a)


Relative intensity (%)
100
75
50
25
0
200
400
600
800
1,000
m/z
y2″
y1″
y3″
y4″
y5″
y6″
y7″
y8″
y9″

(b)


ProteinProspector
v 4.0.5
Proteomics tools for mining sequence databases in conjunction with Mass Spectrometry experiments.
ProteinProspector Asia Pacific
ProteinProspector London
This server uses the IBM AIX version of Protein Prospector
Administrative Resources
Instructions
Administering ProteinProspector
Installing ProteinProspector
Windows NT/2000 (Intel) Version
AIX Version
User's Manual
Frequently Asked Questions - UCSF
Frequently Asked Questions - Local Copy
Known Bugs
Current Bug Listing - UCSF
Bug Listing - Local Copy
(known at release of this version)
ProteinProspector Revision History
ProteinProspector Automation Guidance
Useful Tables
Mutation Mass Shifts
Dipeptide Masses
Trypsin Autolysis Products
Mass Spectrometry
Mass Spectrometry on the Internet
MSLinks
i-mass
For questions/comments send email to: pbaker@cgl.ucsf.edu
ProteinProspector Tools
MS-Fit
MS-Tag
MS-Seq
MS-Pattern
MS-Homology
MS-NonSpecif
MS-Digest
MS-Product
MS-Comp
MS-Isotope
DB-Stat
MS-Bridge
MS-Fit Batch
MS-Fit Web Batch
MS-Tag Batch
MS-Tag Web Batch
Sequence Database Search Programs
MS-Fit (search with peptide-mass fingerprinting data from MS)
MS-Tag (search with fragment-ion tag data from MS/MS)
MS-Seq (search with sequence tag data from MS/MS)
MS-Pattern (search with Edman microsequence / peptide MS data)
MS-Homology (homology based searches)
MS-Bridge (linked peptide search of MS data)
MS-Bridge Upload (linked peptide search of MS data with file upload facility)
MS-NonSpecific (find peptides with non-specific cleavages)
MS-Fit Batch (MS-Fit batch searching for licensees)
MS-Fit Web Batch (MS-Fit batch searching for web site users)
MS-Tag Batch (MS-Tag batch searching for licensees)
MS-Tag Web Batch (MS-Tag batch searching for web site users)
Peptide / Protein MS Utility Programs
MS-Digest (peptide masses from enzymatic digestion of protein)
MS-Product (fragment ion masses for peptide)
MS-Product Upload (fragment ion masses for peptide with file upload facility)
MS-Comp (AA compositions fitting parent or fragment mass and immonium ion
MS-Isotope (isotope patterns of peptides and organic molecules)
FASTA Database Manipulation/Information Tools
DB-Stat (Database statistics)
Credits: These programs were developed in the UCSF Mass Spectrometry Facility, which is directed by Dr. Alma Burlingame, Professor of Chemistry and Pharmaceutical Chemistry at UCSF.
Protein Prospector publication to cite:
Clauser K. R., Baker P. R. and Burlingame A. L., Role of accurate mass measurement (+/- 10 ppm) in protein identification strategies employing MS or MS/MS and database searching. Analytical Chemistry, Vol. 71, 14, 2871- (1999)
Last Modified: 5/10/2002 pbaker@cgl.ucsf.edu
Return to UMass Medical School: Proteomic Mass Spectrometry Lab

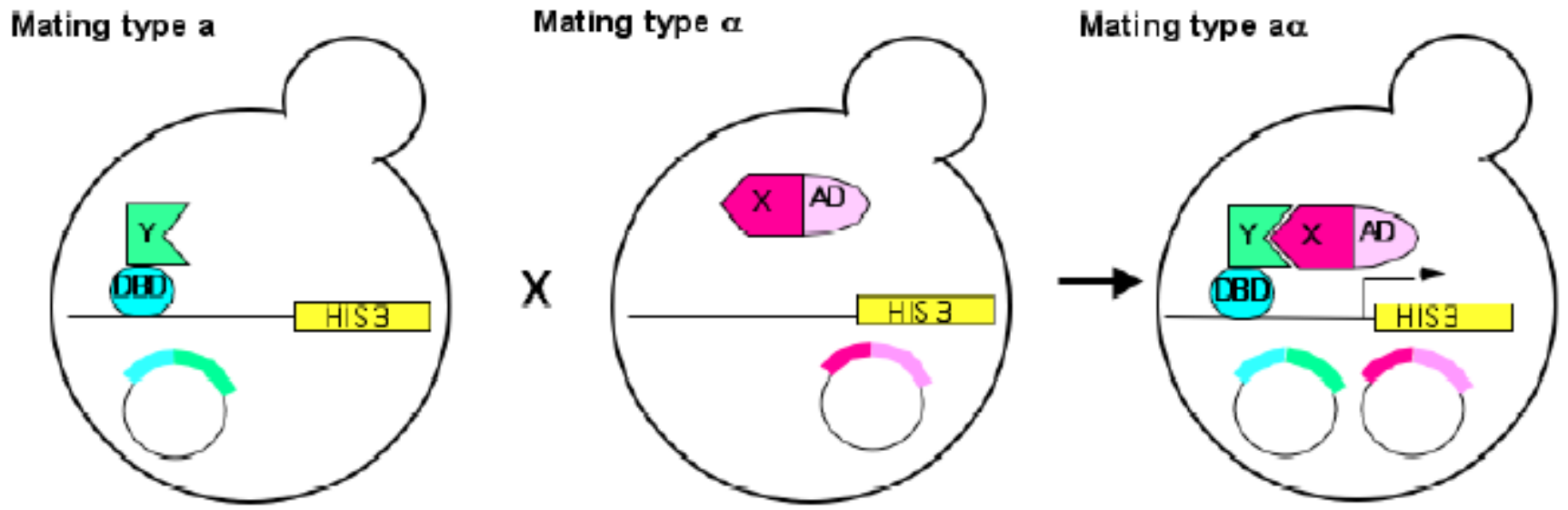


Figure 1: Classical Yeast Two-Hybrid System. A protein of interest Y is expressed in yeast as a fusion to a DNA-binding domain (DBD, "bait"; circles denote expression plasmids). Another protein of interest X is fused to a transcriptional activation domain (AD, "prey"). The two yeast strains are mated to combine the two fusion proteins in one cell. If proteins Y and X interact in the resulting diploids cells, they reconstitute a transcription factor which activates a reporter gene (here: HIS3) and therefore allows the cell to growth on selective media (here: media lacking histidine).

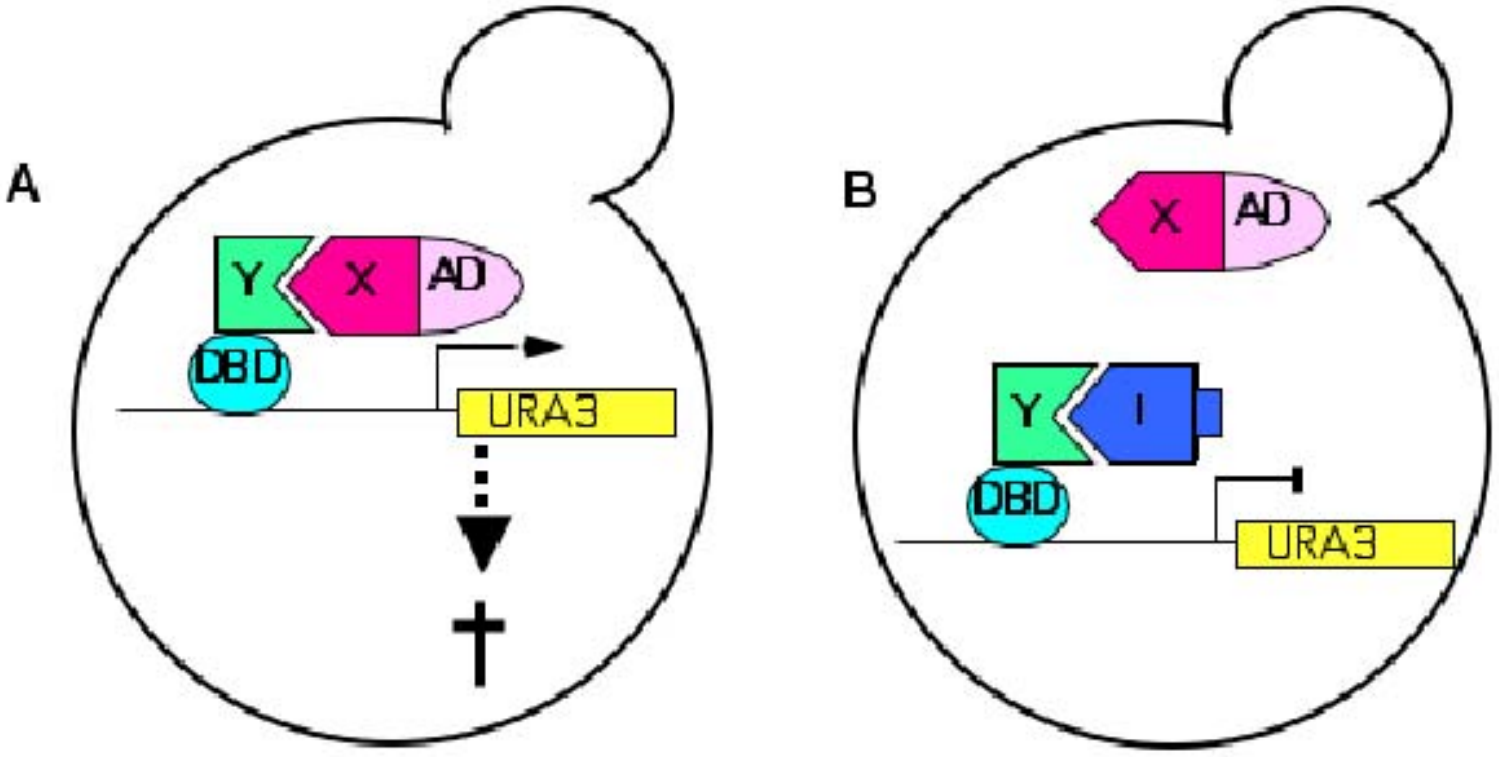
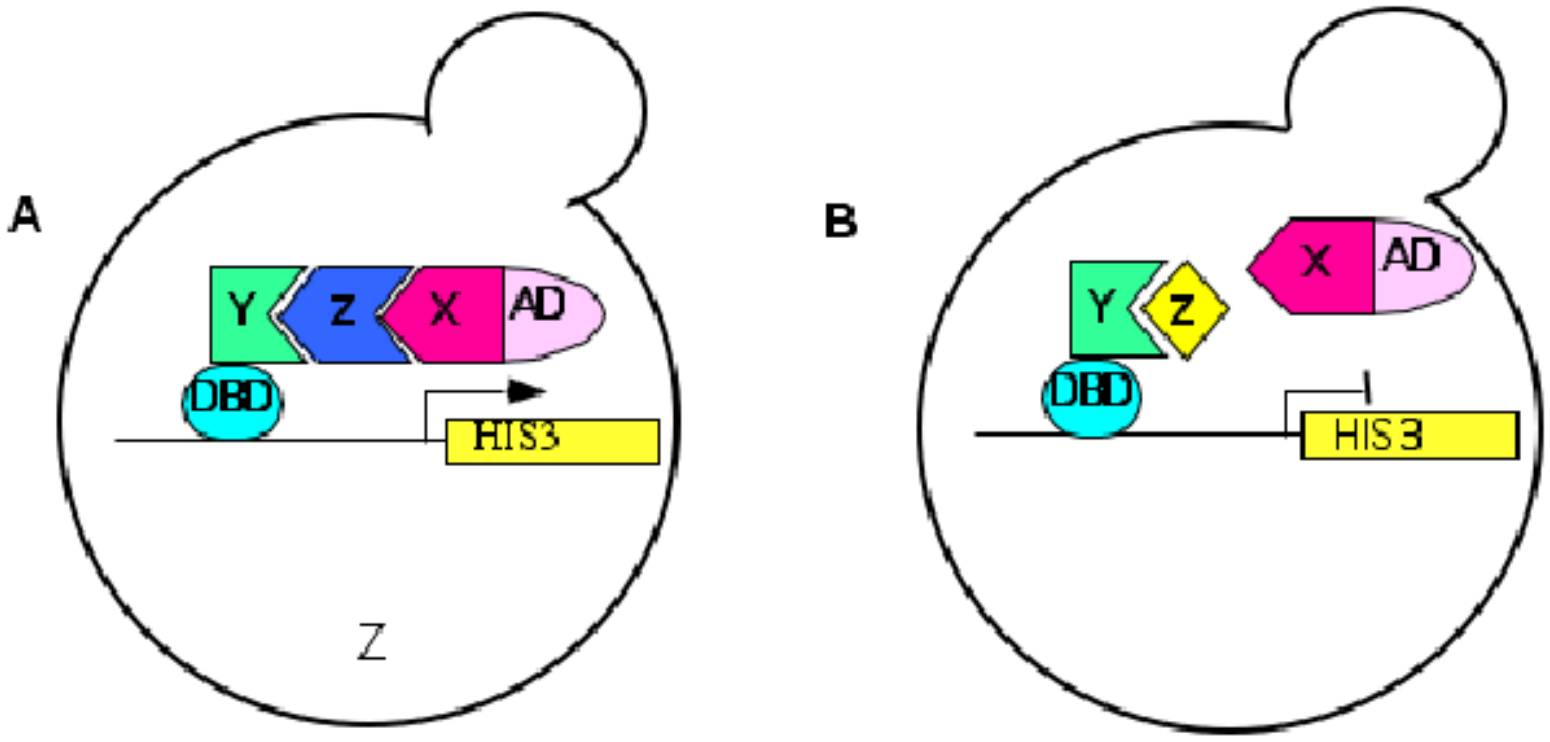
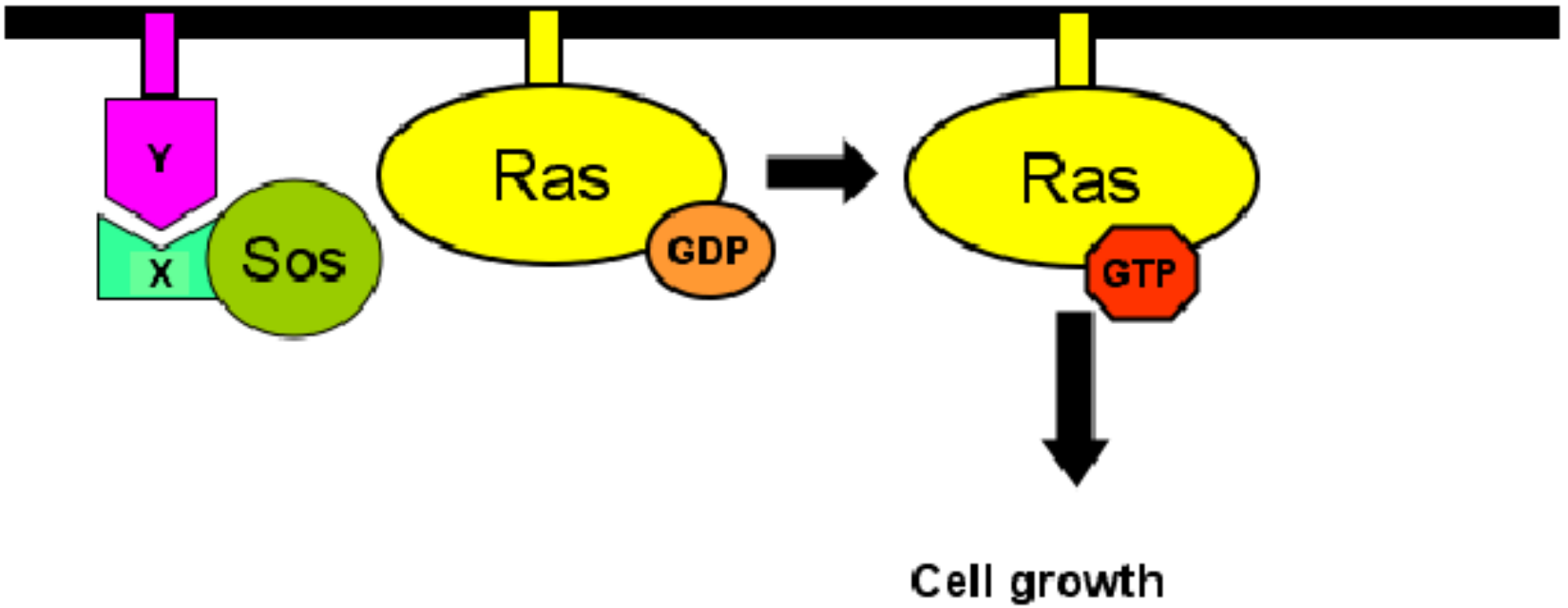


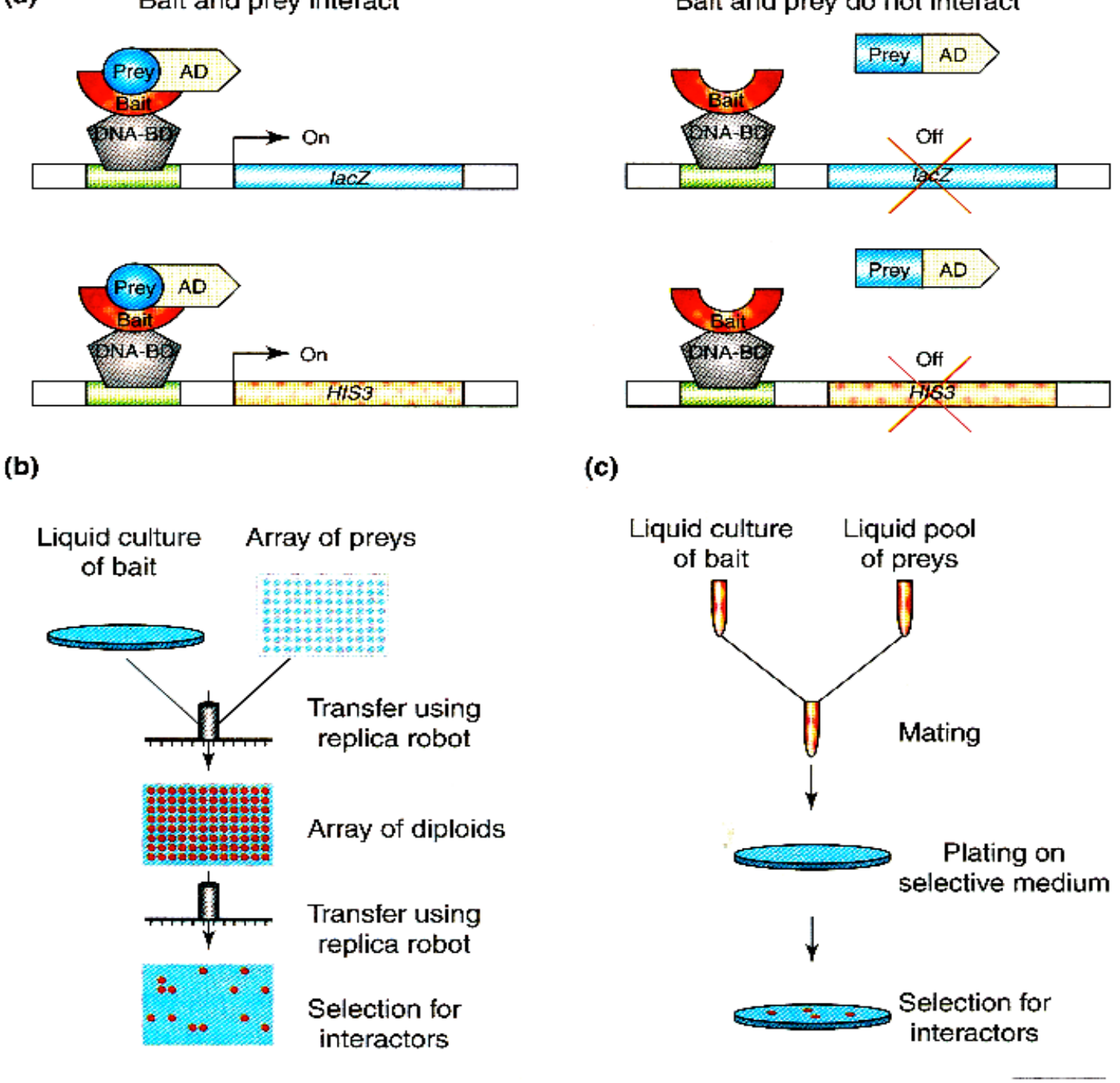
Figure 2. Reverse Two-Hybrid System. The interaction of bait and prey in the yeast cell is lethal (A), selecting for yeasts were the interaction is disrupted (B). Selection can be induced by the addition of FOA (5-fluoro-orotic acid) which is converted to the toxic compound 5-fluorouracil by the URA3 gene product.



gure 3. Three-Hybrid System. A third protein (Z) is expressed along with the DBD and AD fusion
xpression of the reporter gene is used to select for interactions that occur only in the presence of this prote
). This third protein alternatively can prevent the formation of a two-hybrid complex (B). Alternatively, Z m
a hybrid RNA molecule with part of the sequence binding to Y and the other to X ("RNA three-hybrid").



gure 4. Sos Recruitment System. Protein X is fused to a human Ras guanyl exchange factor, So
tative interaction membrane protein Y (or localized to the membrane by a myristoylation tag) is c
pressed. Interaction between X and Y recruits Sos to the membrane, where it stimulates guanyl nucleotic
change on Ras. GTP-bound Ras stimulates cell growth.


Bait and prey interact
Bait and prey do not interact
Prey
AD
Bait
DNA-BD
On
Off
lacZ
HIS3
(b)
Liquid culture of bait
Array of preys
Transfer using replica robot
Array of diploids
Transfer using replica robot
Selection for interactors
(c)
Liquid culture of bait
Liquid pool of preys
Mating
Plating on selective medium
Selection for interactors

Artefactos del sistema de doble híbrido

Falsos positivos:

1. Si un factor de transcripción es usado como cebo
2. Por interacciones inespecíficas
3. Si el cebo o la presa afectan la viabilidad celular
4. Eventos aleatorios de origen desconocido

Falsos negativos:

1. Impedimento estérico entre las proteínas de fusión previene la función del reporter
2. Inestabilidad de las proteínas de fusión
3. Falla en la localización subcelular relevante para la actividad del reportero

Identificación de los Artefactos:

1. Confirmar la reproducibilidad de la interacción
2. Uso de múltiples reporteros
3. Uso de métodos independientes para determinar especificidad

Método	Ventajas	Desventajas
oble híbrido *vivo*	Simple, barato y sensible	Falsos positivo
spectrometría de Masas *vivo*	Detecta complejos proteicos	Caro, sofistica y lento (*)
hips de proteínas *vitro*	Condiciones definidas	potencialmente no fisiológico (*

) Requiere purificación de proteínas

Referencias

- Lehninger, A. (1994) Principles of Biochemistry. 3rd ed. http://www.bioinfo.org.cn/book/biochemistry/

- Mathews & van Holde (1999). Biochemistry http://www.aw-bc.com/mathews/

Vollert & Uetz: The Two-Hybrid System