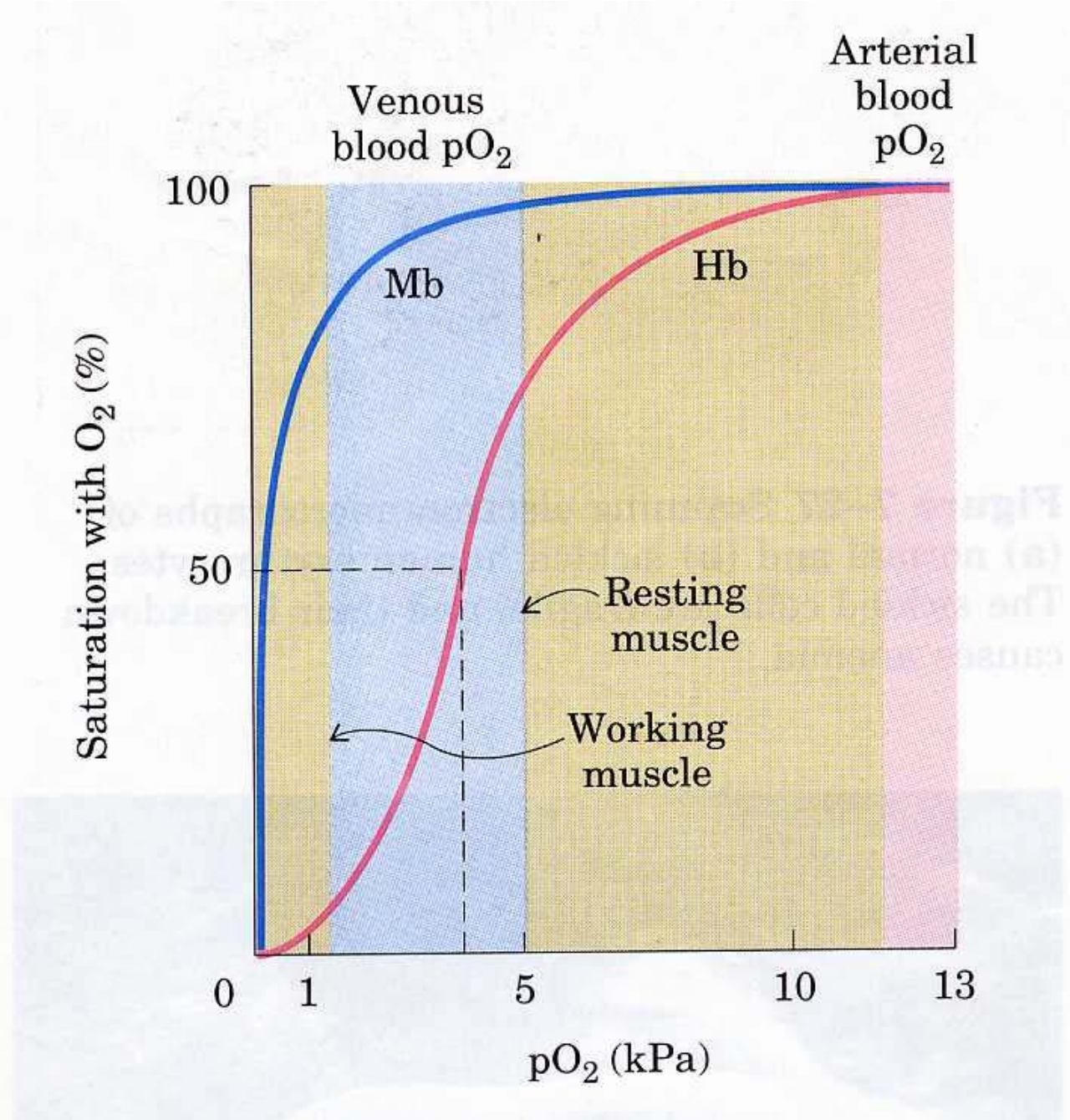
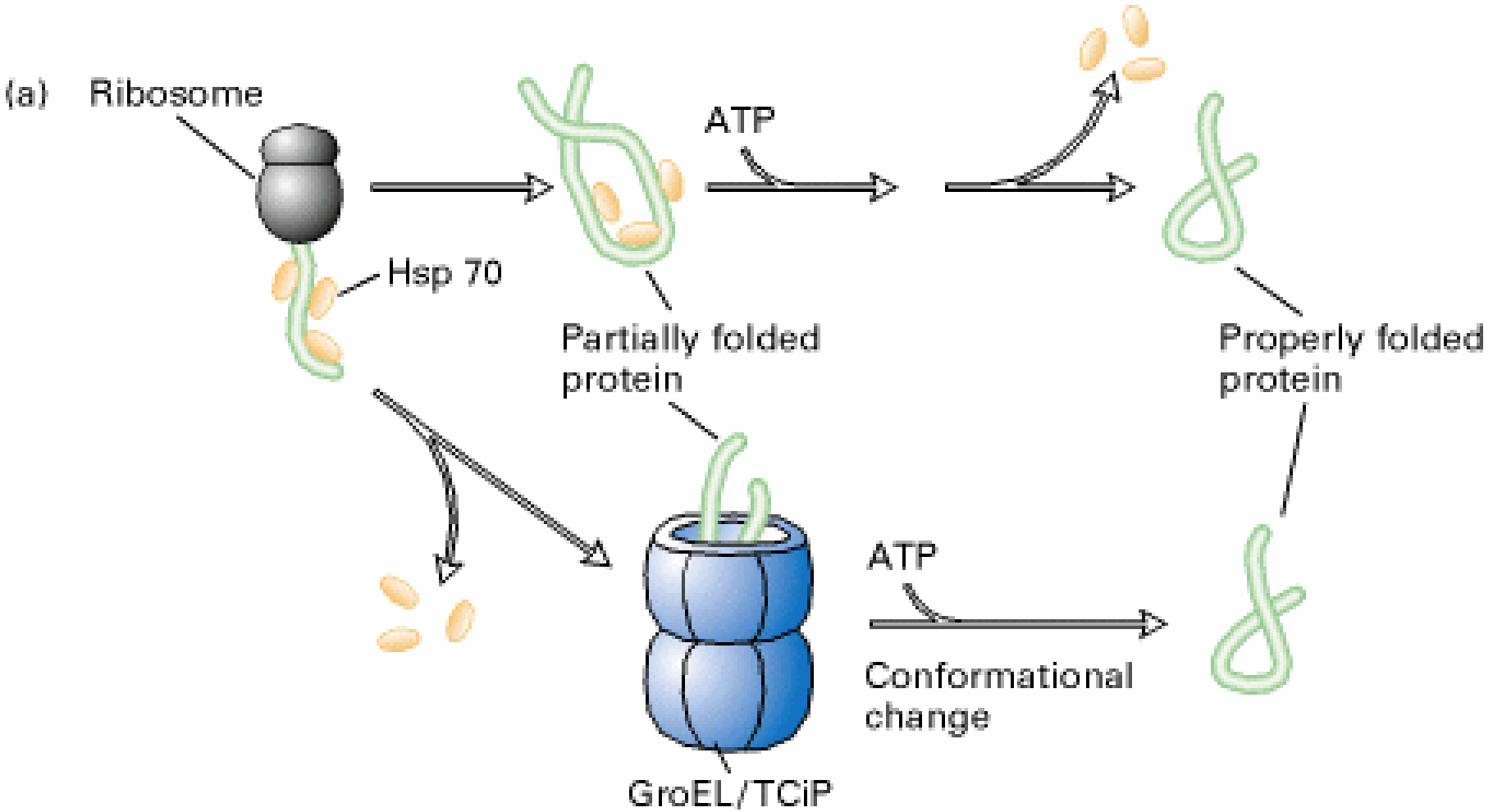

Arterial blood pO_2
Venous blood pO_2
100
50
Saturation with O_2 (%)
Mb
Hb
Resting muscle
Working muscle
0
1
5
10
13
pO_2 (kPa)


(a) Ribosome
Hsp 70
ATP
Partially folded protein
Properly folded protein
ATP
Conformational change
GroEL/TCiP

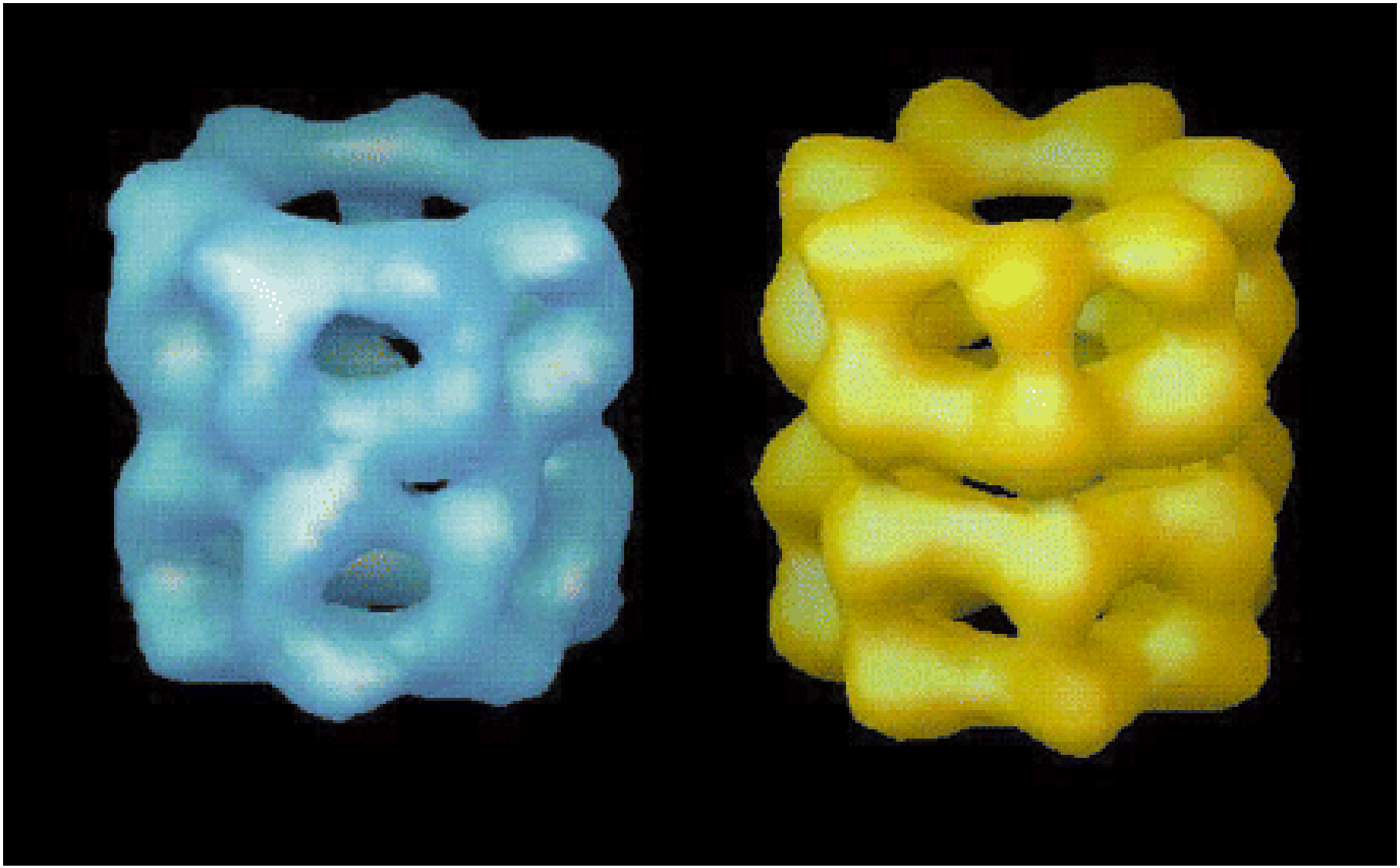


Table 6.1 Industrial enzymes and their commercial uses.

Enzyme	Industrial use
α-Amylase	Beer making, alcohol production
Aminoacylase	Preparation of L-amino acids
Bromelain	Meat tenderizer, juice clarification
Catalase	Antioxidant in prepared foods
Cellulase	Alcohol and glucose production
Ficin	Meat tenderizer, juice clarification
Glucoamylase	Beer making, alcohol production
Glucose isomerase	Manufacture of high fructose syrups
Glucose oxidase	Antioxidant in prepared foods
Invertase	Sucrose inversion
Lactase	Whey utilization, lactose hydrolysis
Lipase	Cheese making, preparation of flavorings
Papain	Meat tenderizer, juice clarification
Pectinase	Clarifying fruit juices, alcohol production
Protease	Detergent, alcohol production
Rennet	Cheese making

Table 6.2 Properties of T4 lysozyme and six engineered variants.

Enzyme	Amino acid position							No. of -S-S-	Relative activity (%)	T_m (°C)
	3	9	21	54	97	142	164			
wt[a]	Ile	Ile	Thr	Cys	Cys	Thr	Leu	0	100	41.9
pwt	Ile	Ile	Thr	Thr	Ala	Thr	Leu	0	100	41.9
A	Cys	Ile	Thr	Thr	Cys	Thr	Leu	1	96	46.7
B	Ile	Cys	Thr	Thr	Ala	Thr	Cys	1	106	48.3
C	Ile	Ile	Cys	Thr	Ala	Cys	Leu	1	0	52.9
D	Cys	Cys	Thr	Thr	Cys	Thr	Cys	2	95	57.6
E	Ile	Cys	Cys	Thr	Ala	Cys	Cys	2	0	58.9
F	Cys	Cys	Cys	Thr	Cys	Cys	Cys	3	0	65.5

[a] wt, Wild-type T4 lysozyme; pwt, pseudo-wild-type enzyme; A through F, six engineered cysteine variants; -S-S-, disulfide bonds; T_m, "melting" temperature (a measure of thermostability).

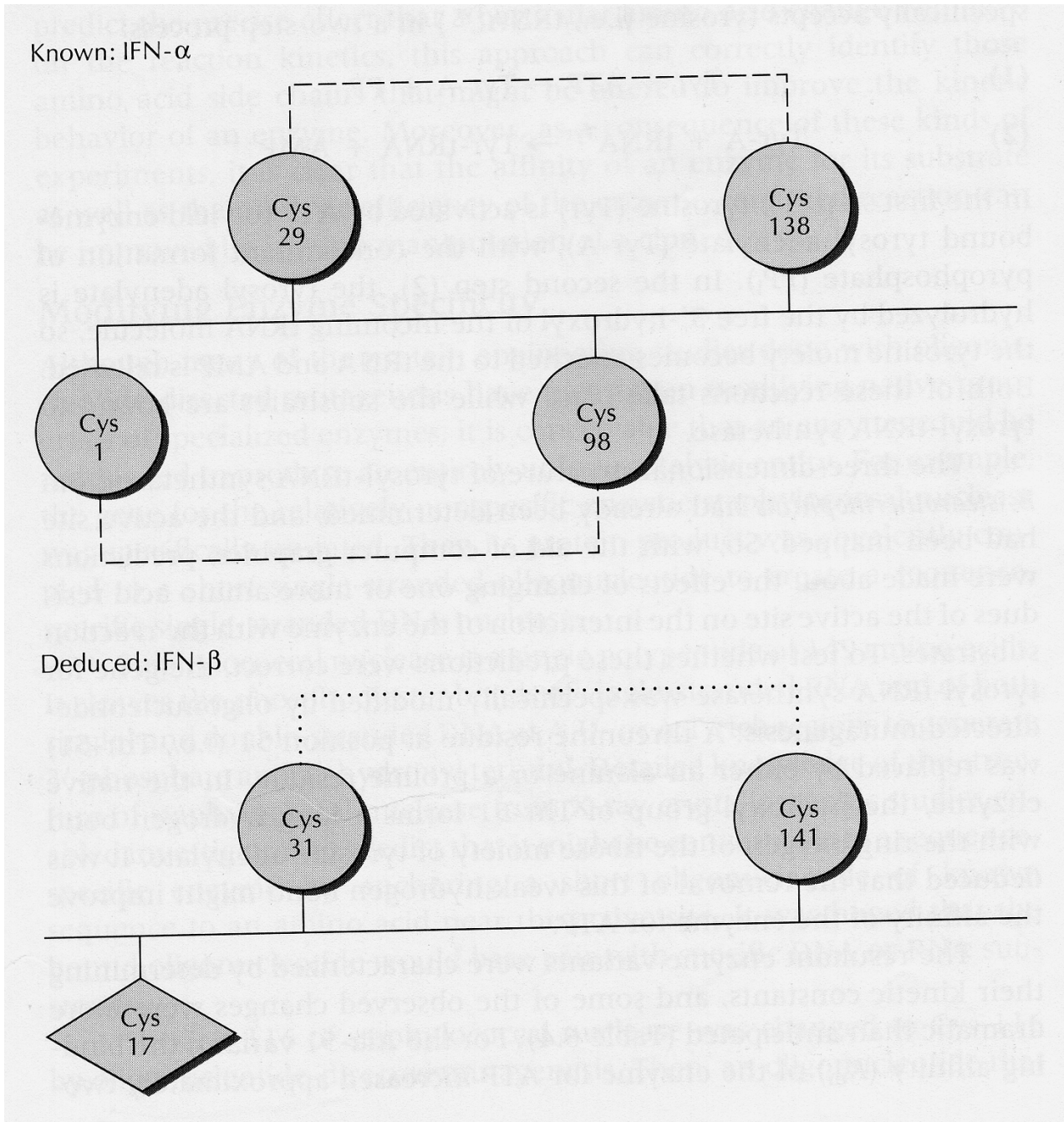
Adapted from Matsumura et al. 1989. *Nature* **342**:291–293.

Table 6.3 Stability at 100°C of the yeast enzyme triosephosphate isomerase and its engineered derivatives.[a]

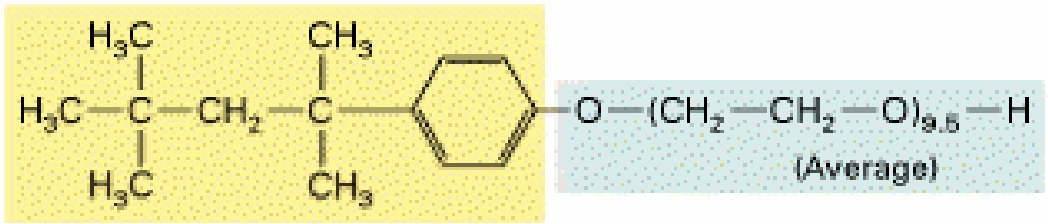
Enzyme	Amino acid position 14	Amino acid position 78	Half-life (min)
Wild-type	Asn	Asn	13
Variant A	Asn	Thr	17
Variant B	Asn	Ile	16
Variant C	Thr	Ile	25
Variant D	Asp	Asn	11

[a] Enzyme stability is expressed as the half-life, or rate of enzyme inactivation, at 100°C. A longer half-life indicates a more stable enzyme.

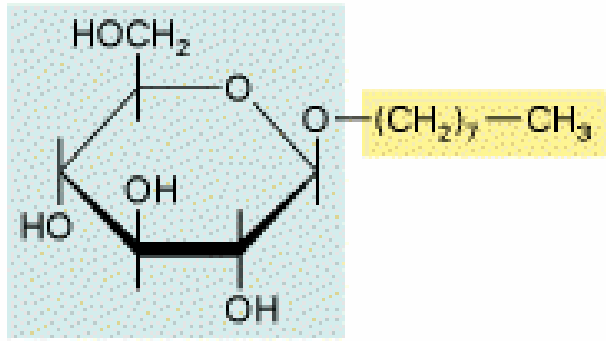
Adapted from Ahern et al. 1987. *Proc. Natl. Acad. Sci. USA* **84:**675–679.


Known: IFN-α
Cys 29
Cys 138
Cys 1
Cys 98
Deduced: IFN-β
Cys 31
Cys 141
Cys 17

Nonionic detergents

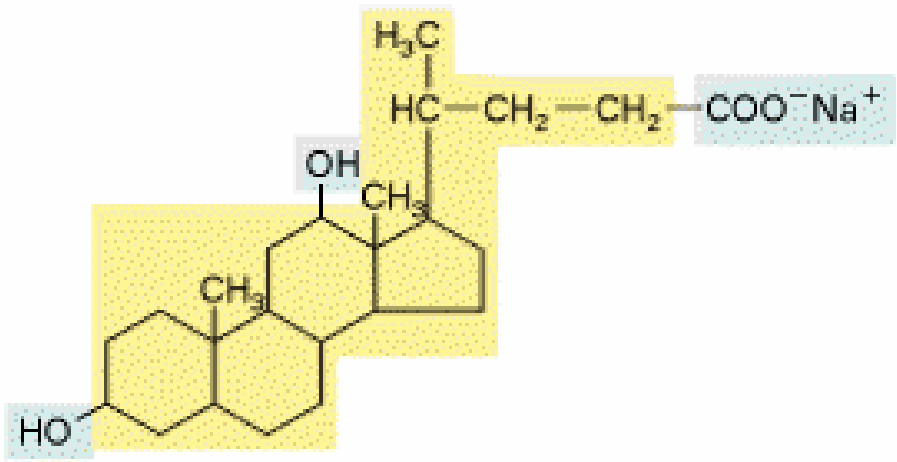


Triton X-100
(polyoxyethylene(9.5)*p*-*t*-octylphenol)

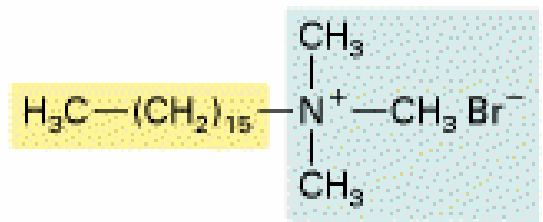


Octylglucoside
(octyl-β-D-glucopyranoside)

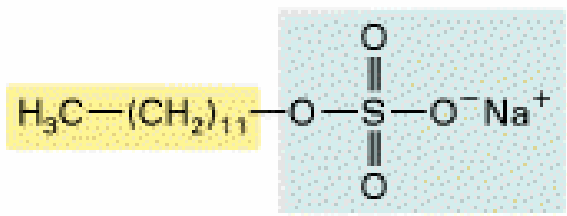
Ionic detergents



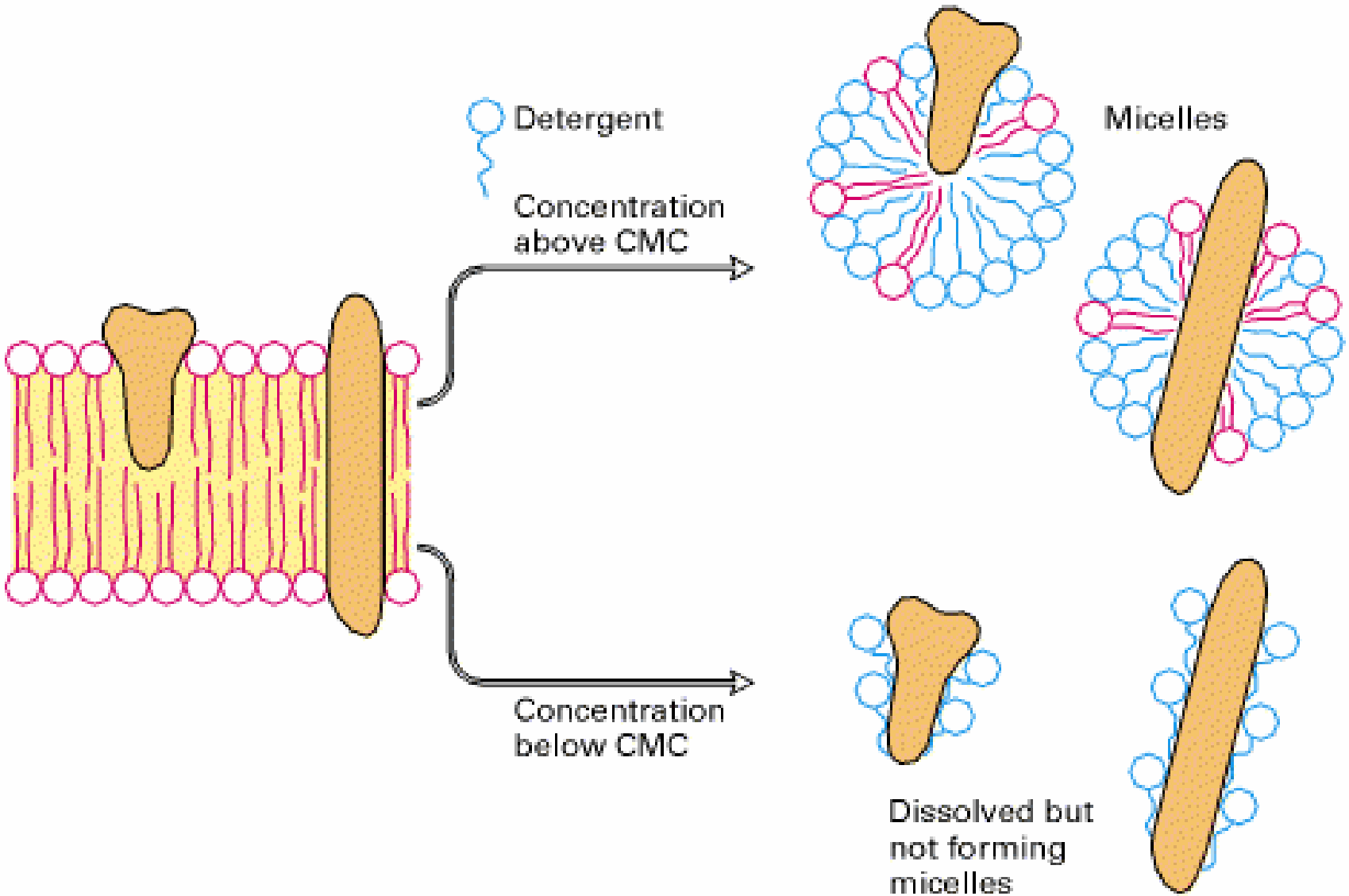
Sodium deoxycholate

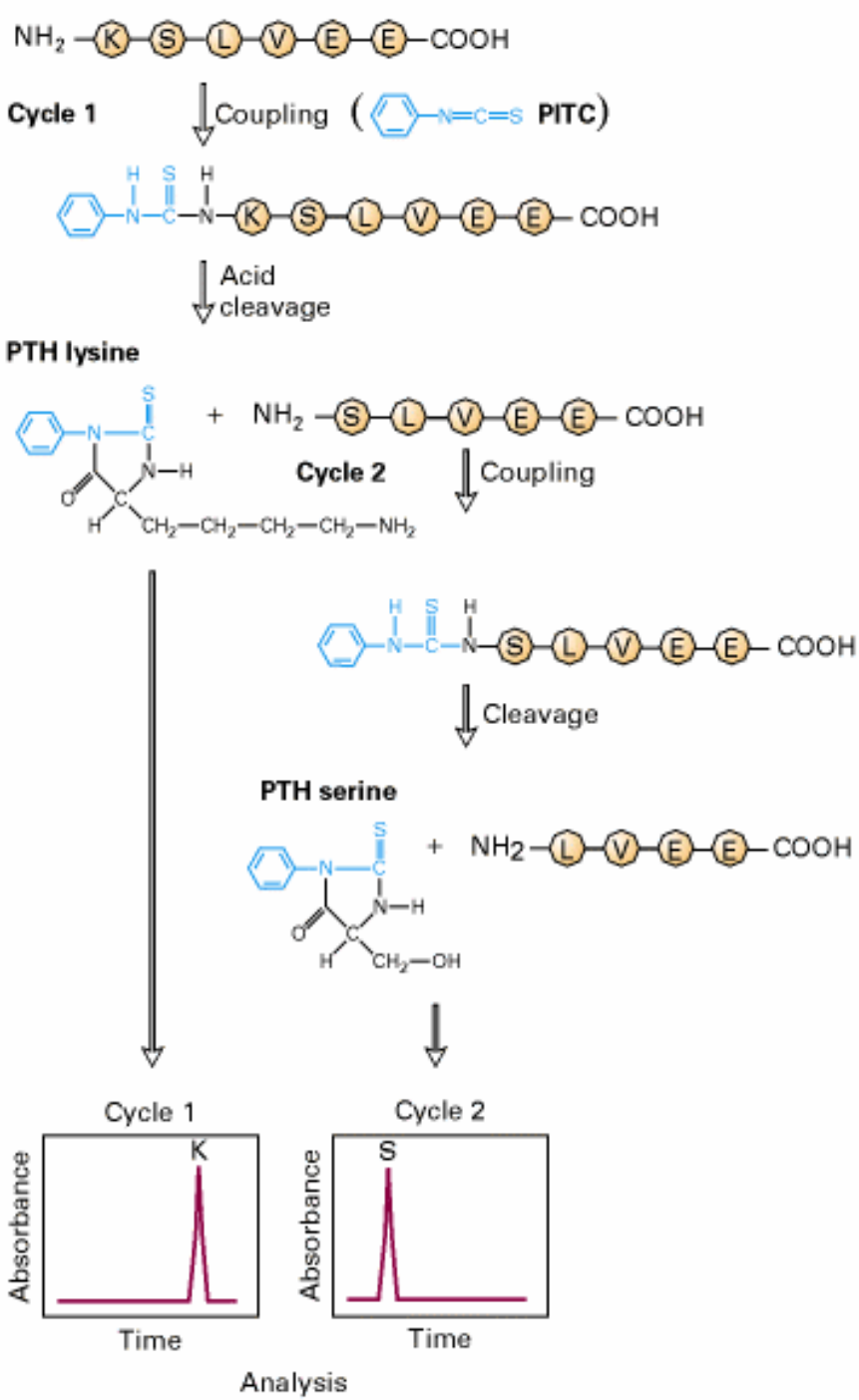


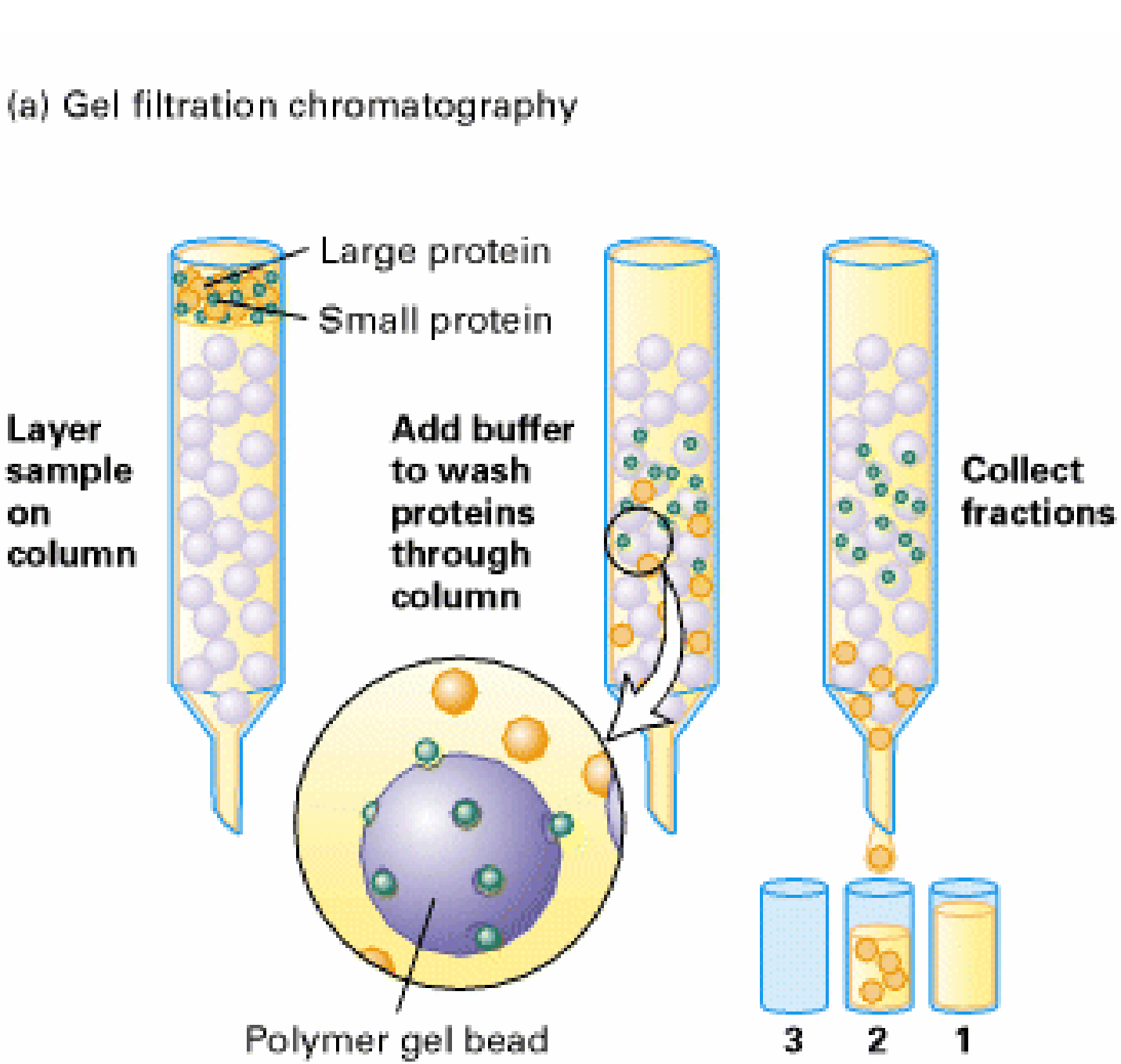
Cetyltrimethylammonium bromide

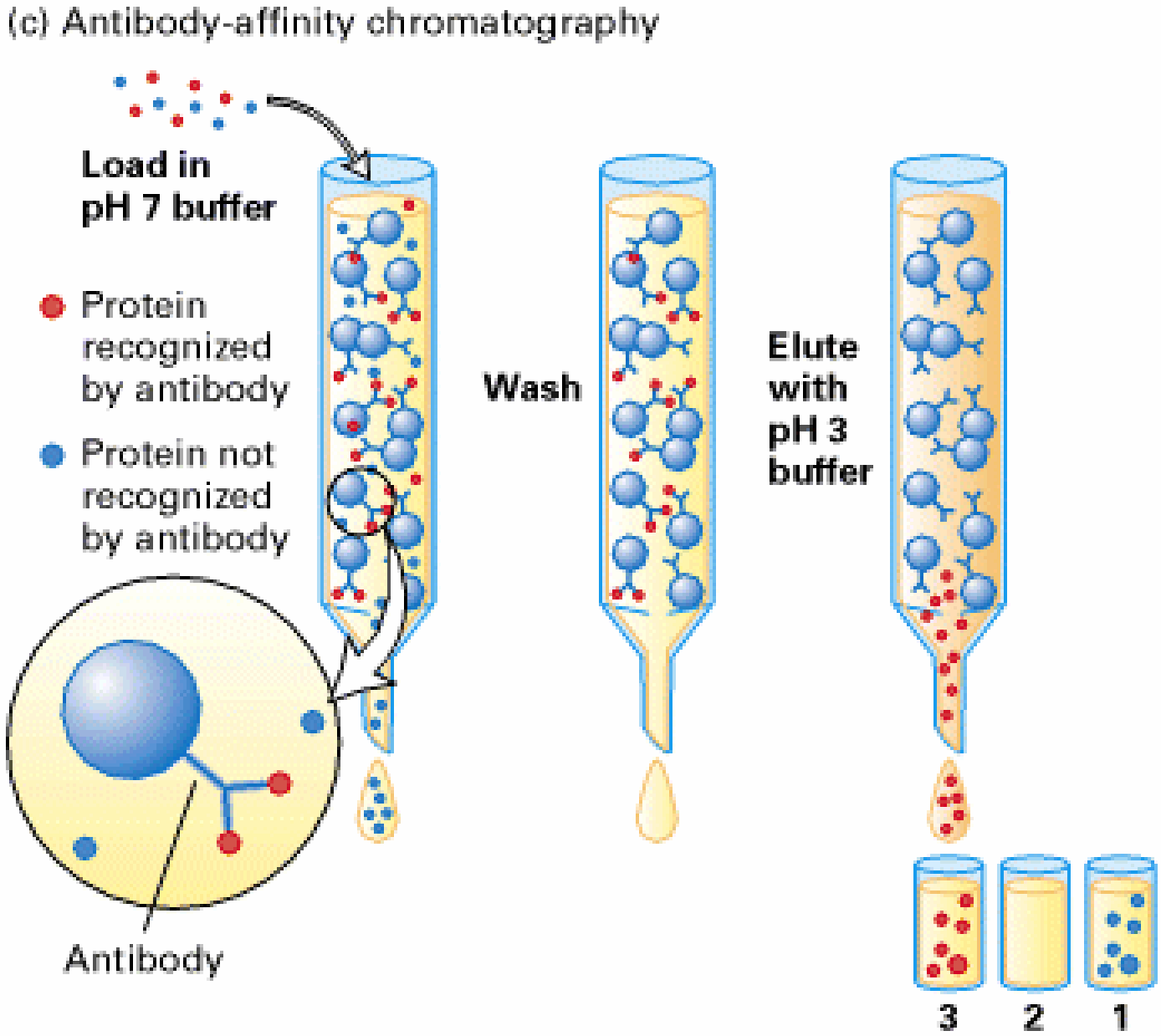


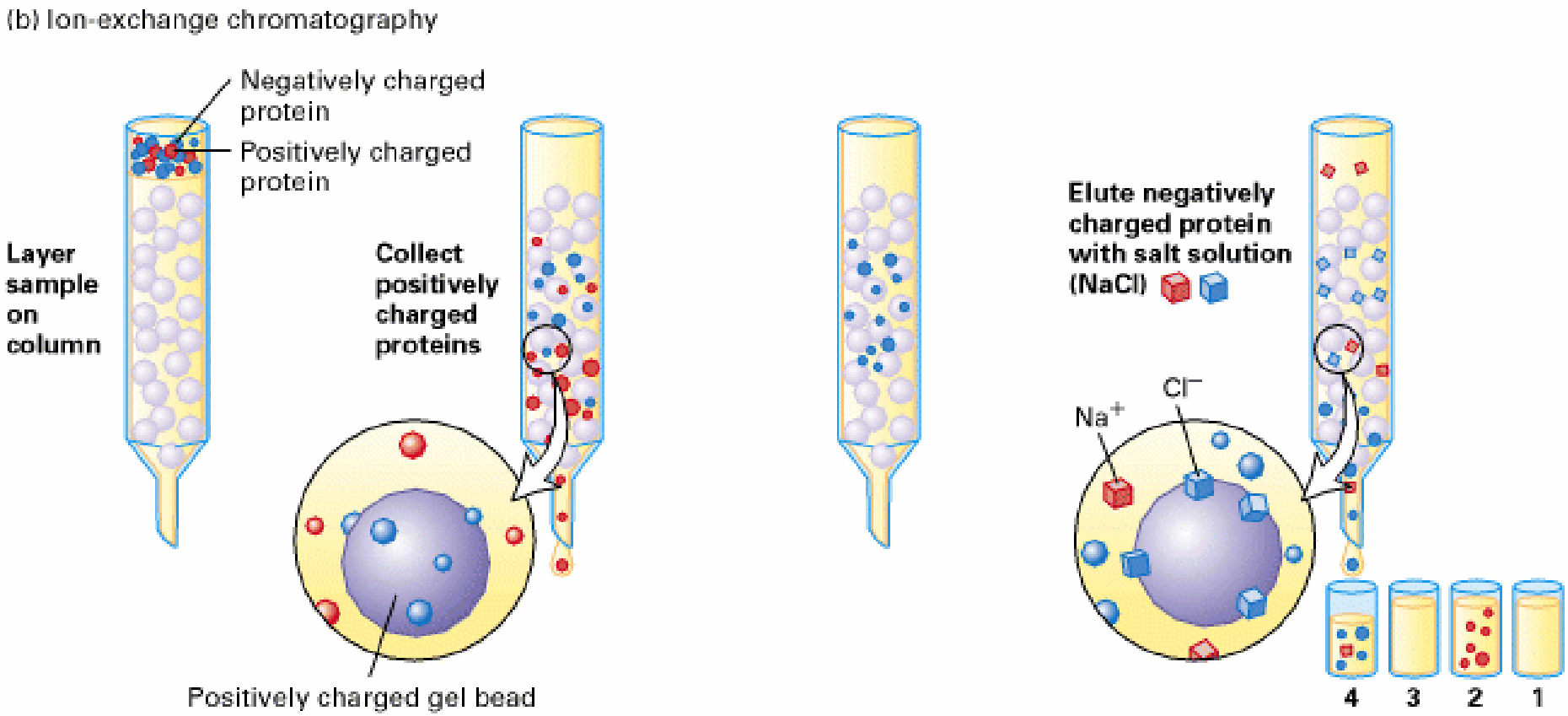
Sodium dodecylsulfate (SDS)

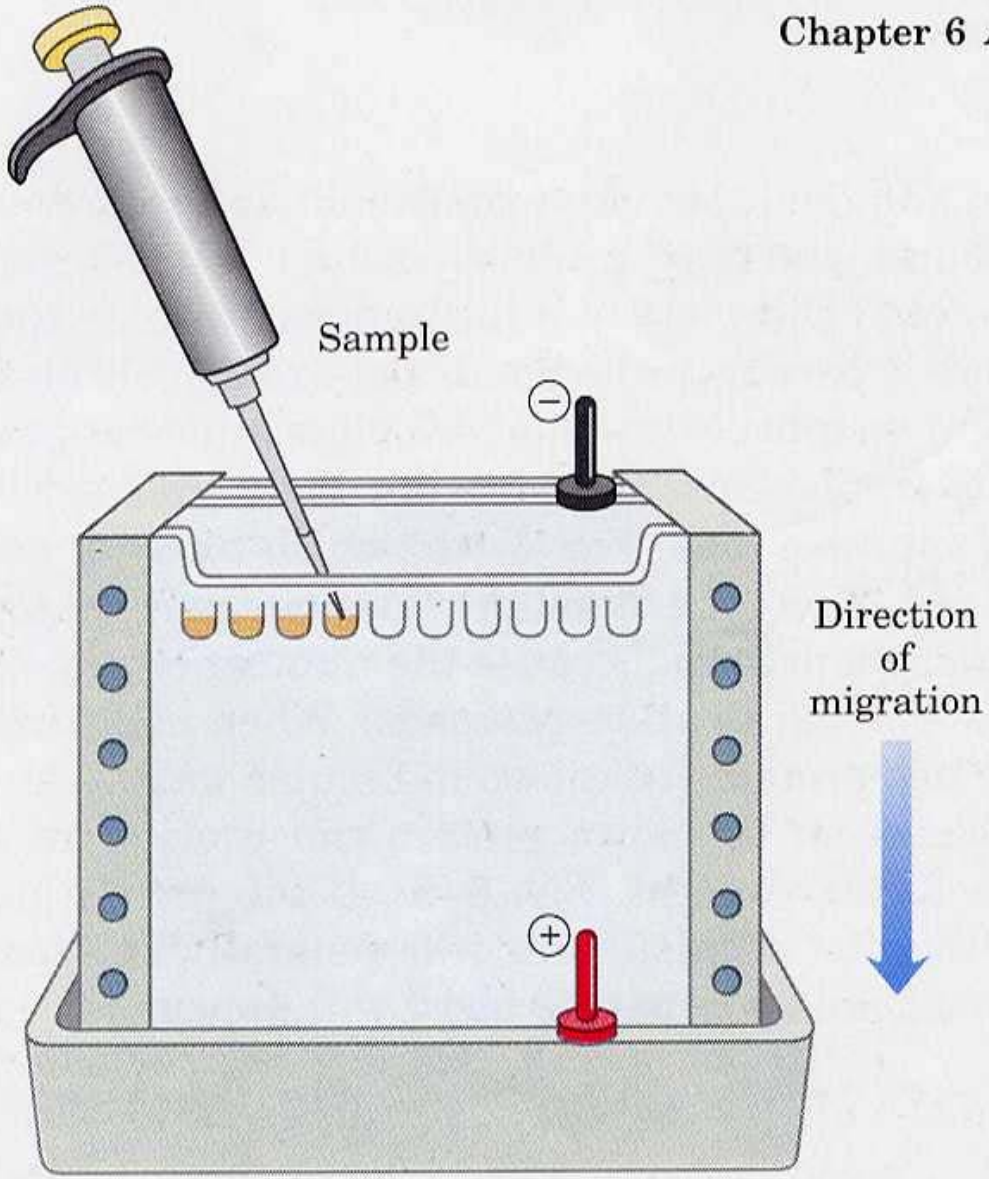

Detergent
Concentration above CMC
Micelles
Concentration below CMC
Dissolved but not forming micelles


NH2 K S L V E E COOH
Cycle 1
Coupling
PITC
COOH
Acid cleavage
PTH lysine
+
NH2 S L V E E COOH
Cycle 2
Coupling
COOH
Cleavage
PTH serine
+
NH2 L V E E COOH
Cycle 1
Cycle 2
Absorbance
K
S
Time
Analysis

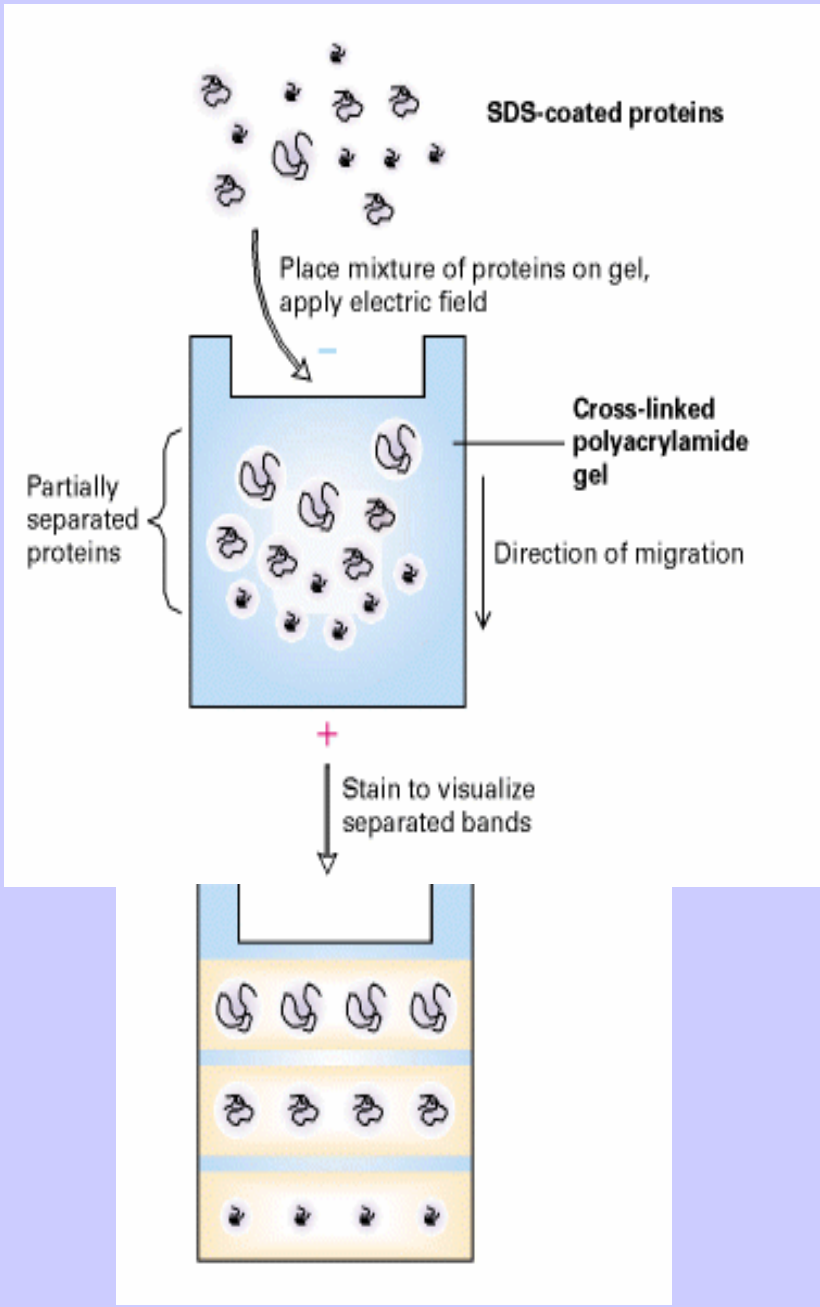

(a) Gel filtration chromatography
Large protein
Small protein
Layer sample on column
Add buffer to wash proteins through column
Collect fractions
Polymer gel bead
3
2
1

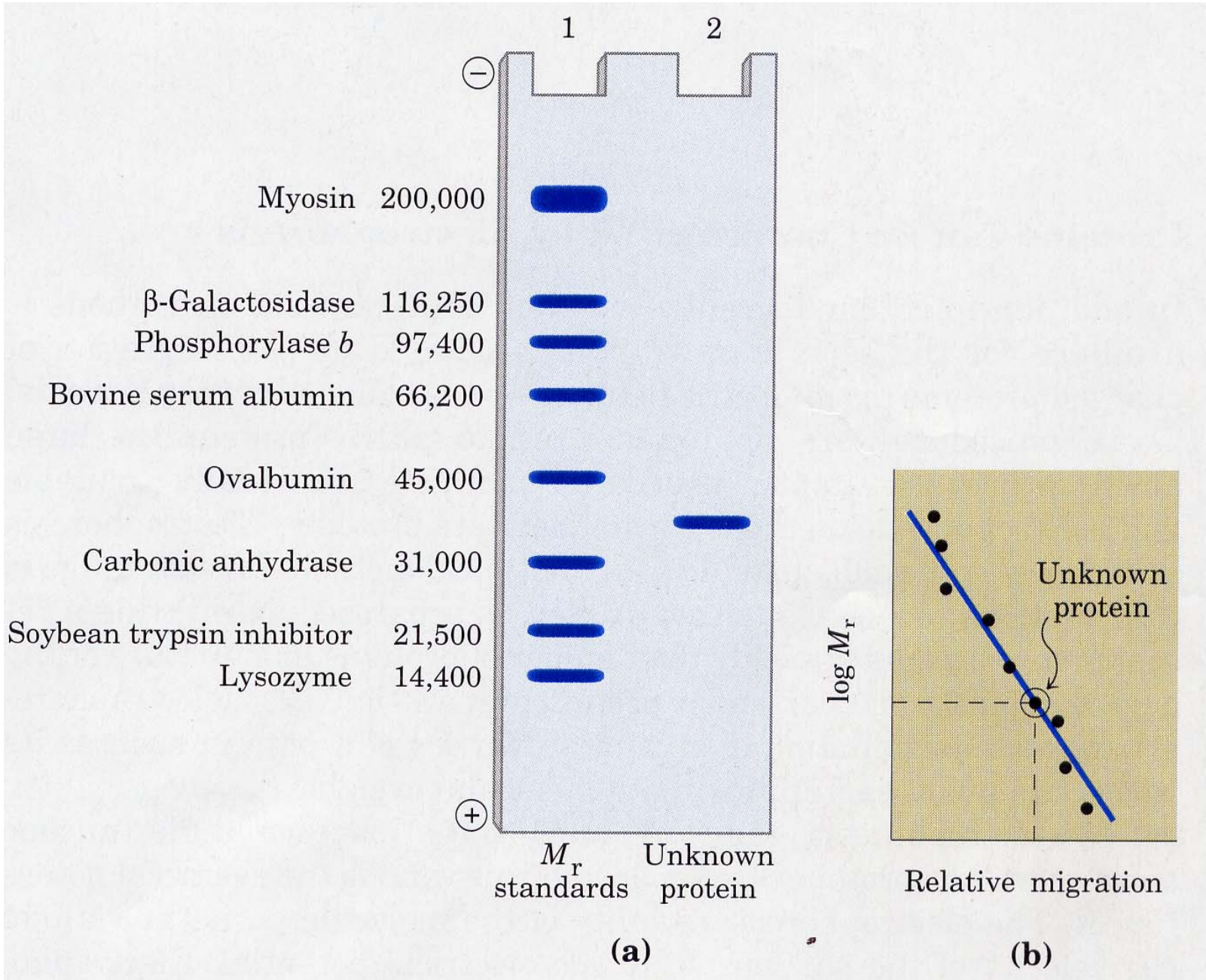

(c) Antibody-affinity chromatography
Load in pH 7 buffer
Protein recognized by antibody
Protein not recognized by antibody
Wash
Elute with pH 3 buffer
Antibody
3
2
1


(b) Ion-exchange chromatography
Negatively charged protein
Positively charged protein
Layer sample on column
Collect positively charged proteins
Positively charged gel bead
Elute negatively charged protein with salt solution (NaCl)
Na+
Cl−
4
3
2
1



(a)


SDS-coated proteins
Place mixture of proteins on gel, apply electric field
−
Cross-linked polyacrylamide gel
Partially separated proteins
Direction of migration
+
Stain to visualize separated bands


1
2
Myosin 200,000
β-Galactosidase 116,250
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)

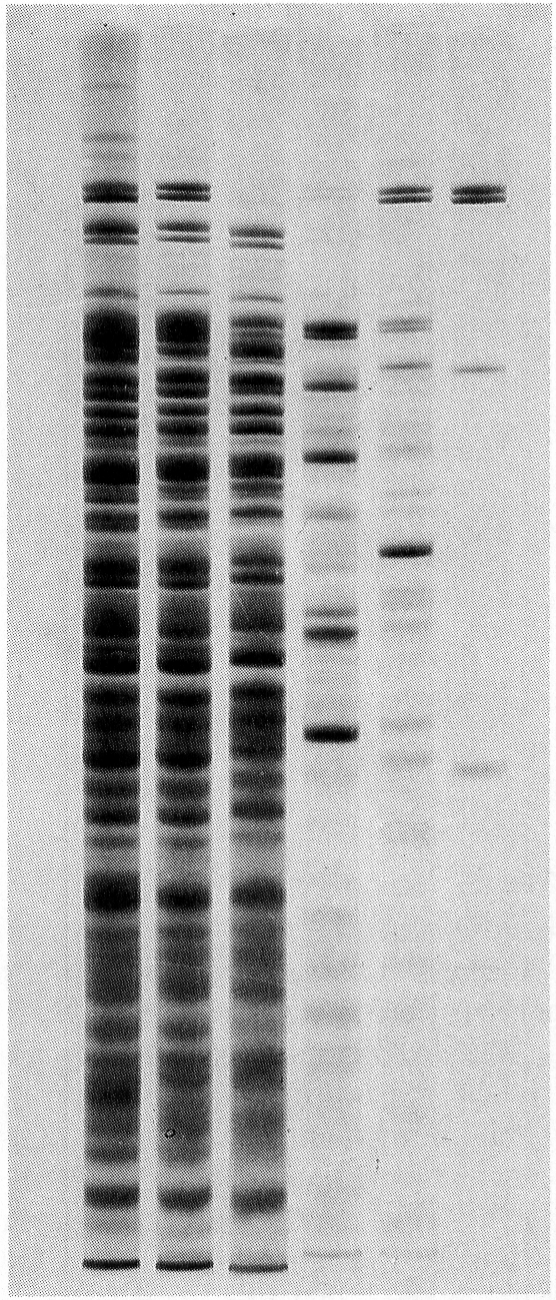
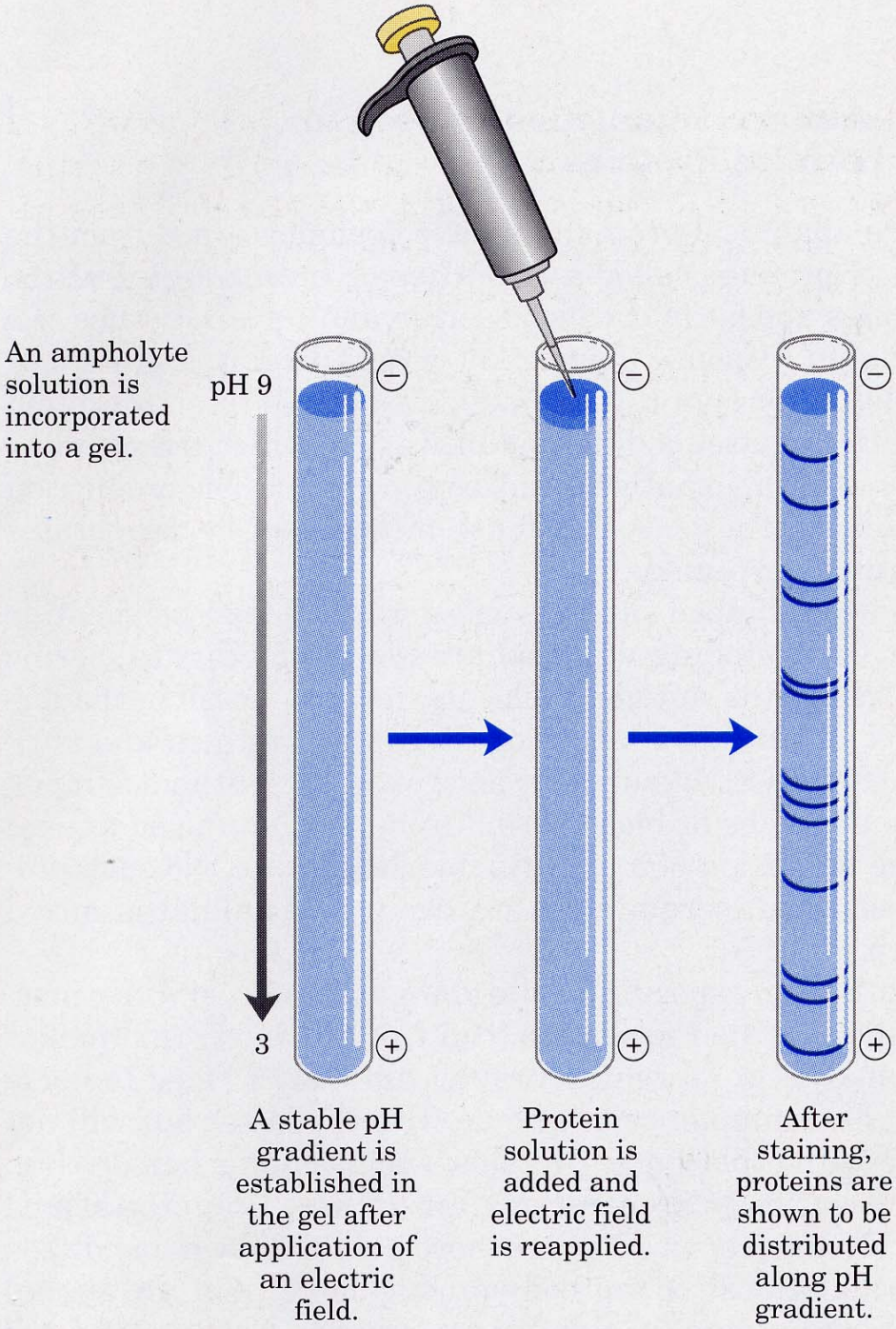
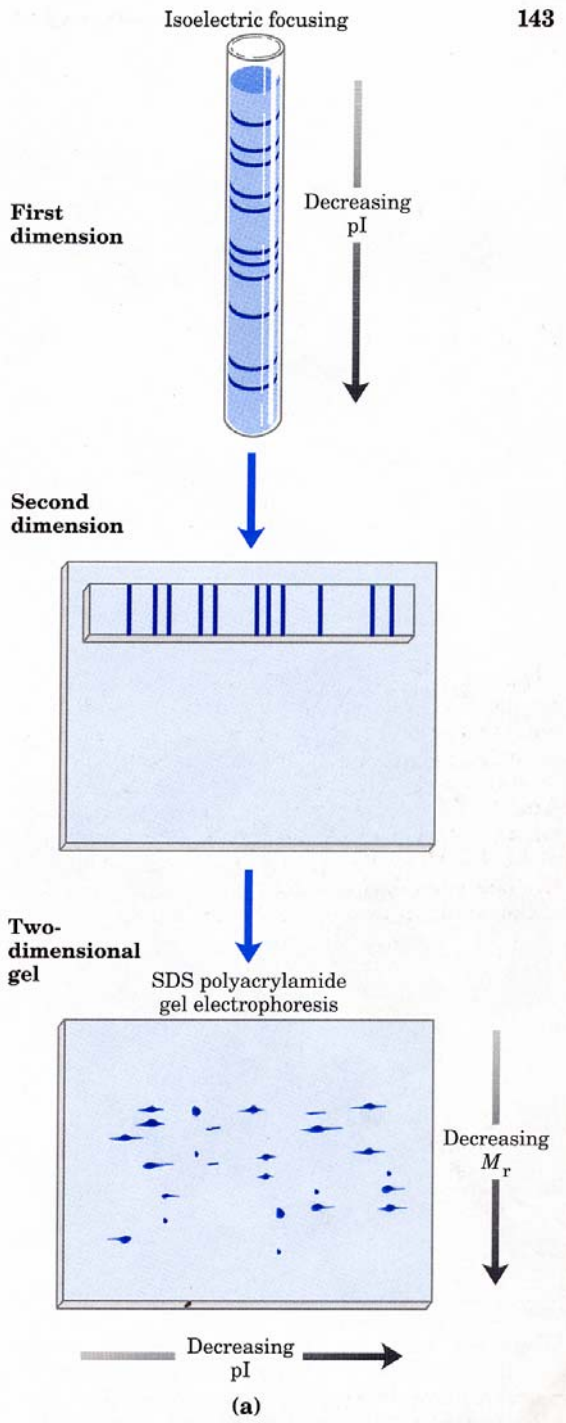


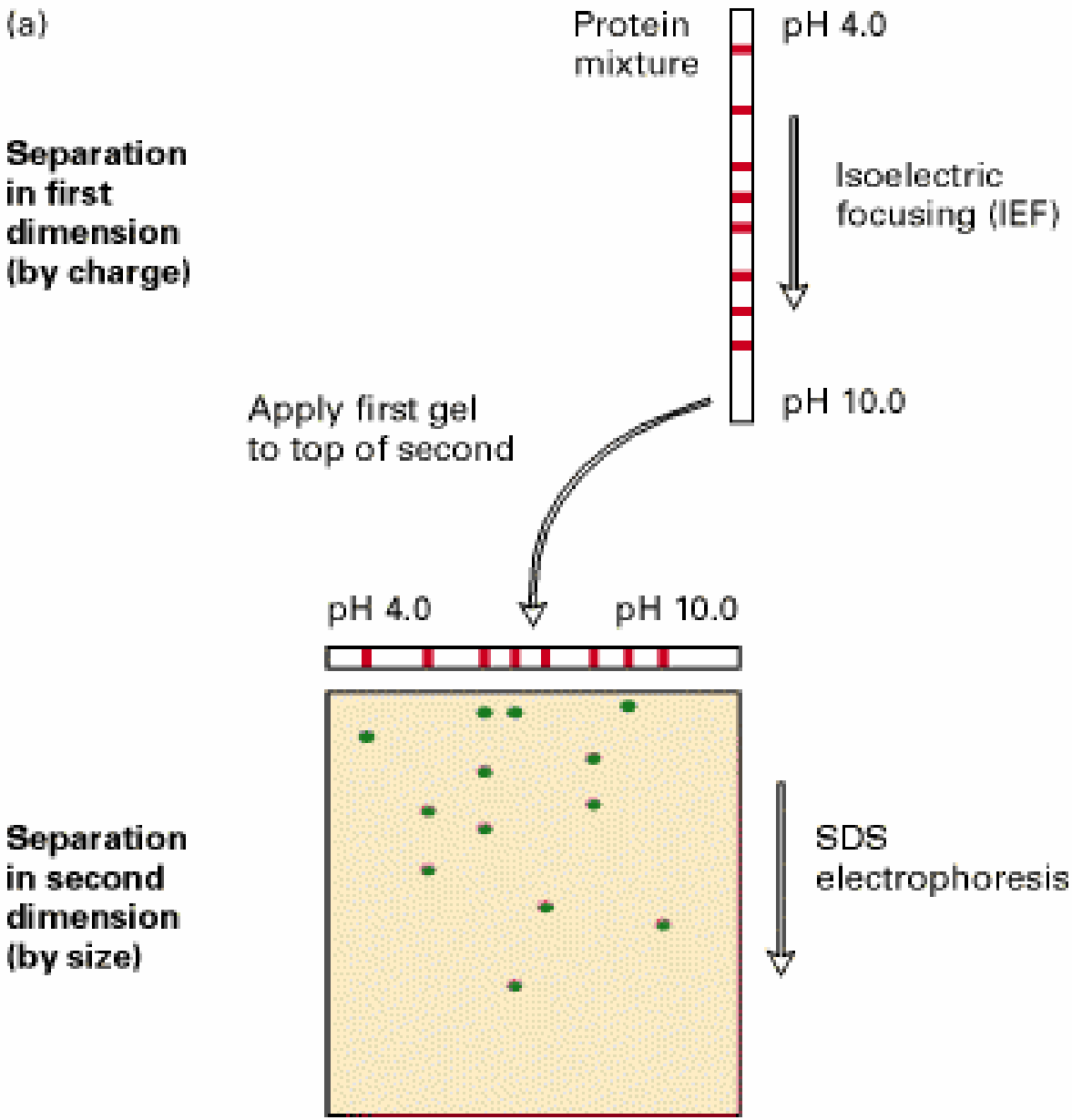
Table 6–5 The isoelectric points of some proteins

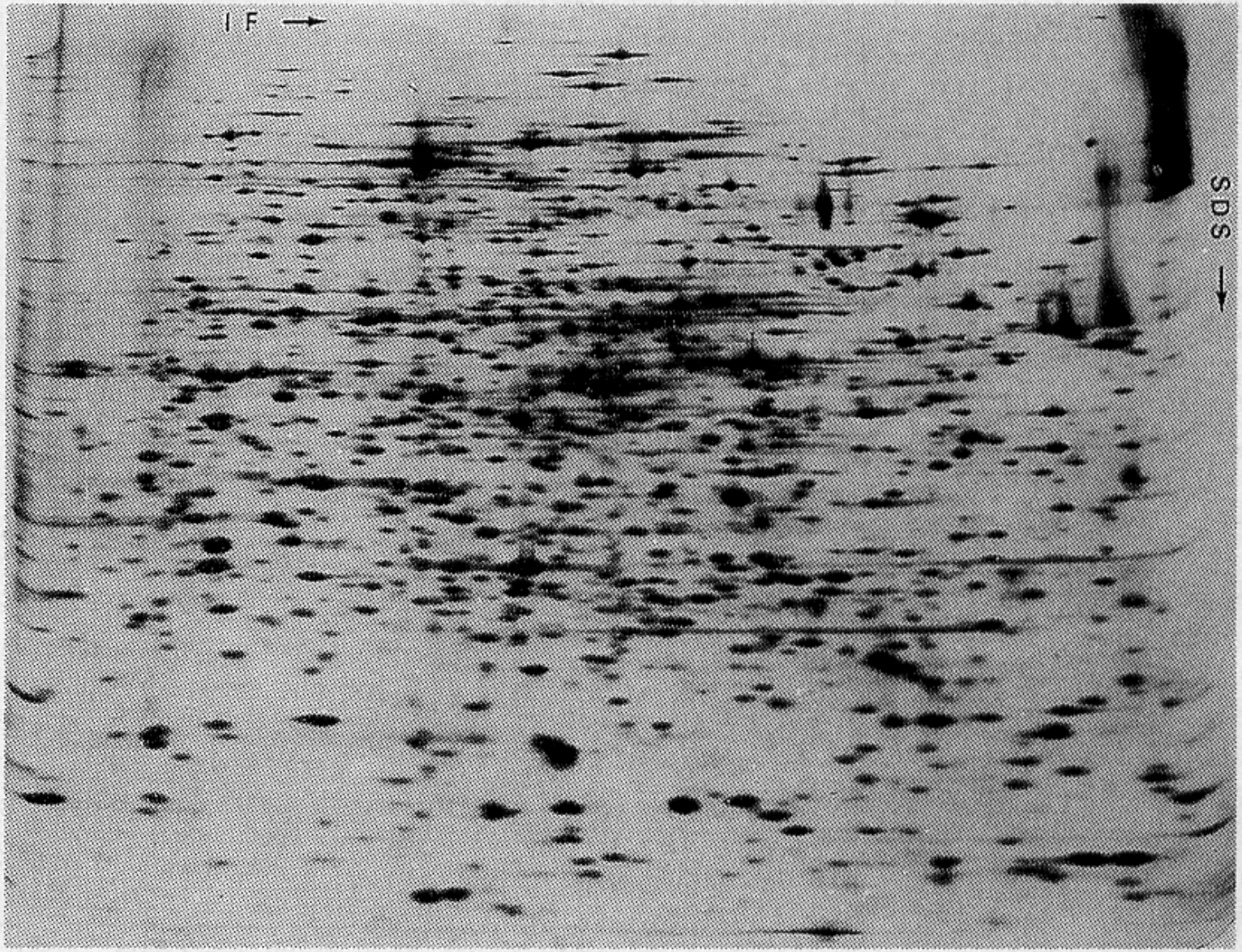
	pI
Pepsin	~1.0
Egg albumin	4.6
Serum albumin	4.9
Urease	5.0
β-Lactoglobulin	5.2
Hemoglobin	6.8
Myoglobin	7.0
Chymotrypsinogen	9.5
Cytochrome *c*	10.7
Lysozyme	11.0


An ampholyte solution is incorporated into a gel.
pH 9
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.


Isoelectric focusing
First dimension
Decreasing pI
Second dimension
Two-dimensional gel
SDS polyacrylamide gel electrophoresis
Decreasing M_r
Decreasing pI

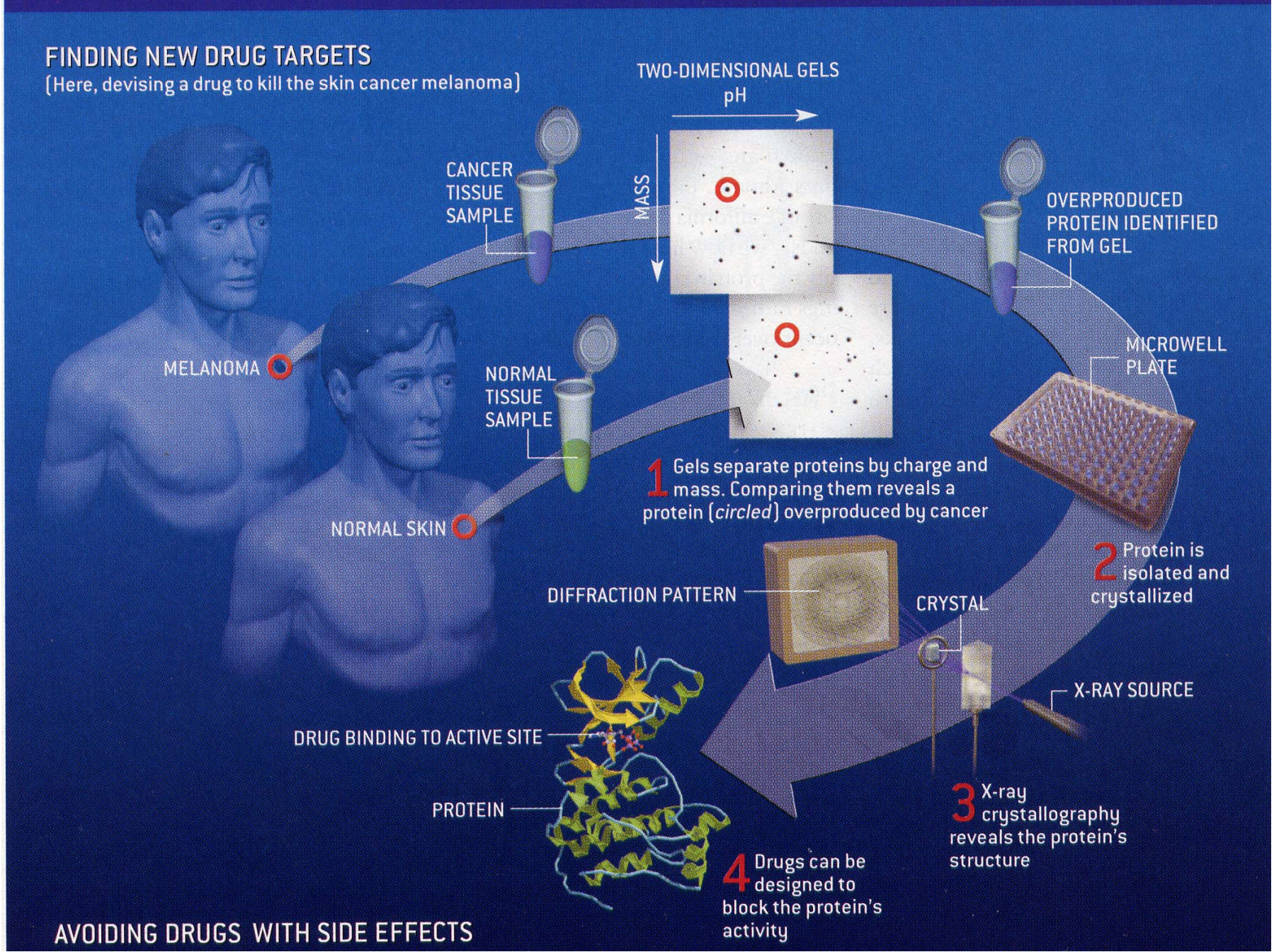
(a)

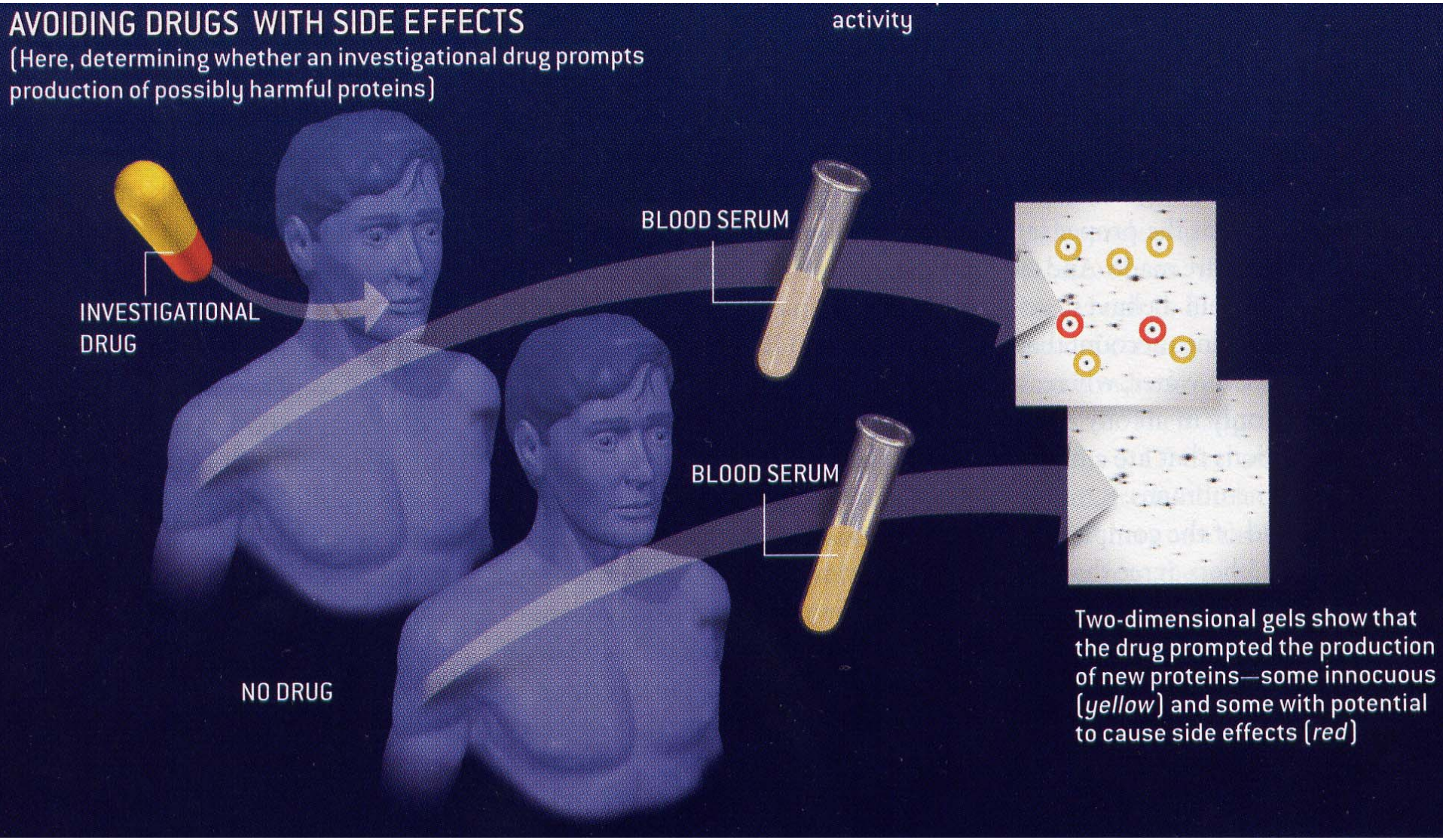

(a)
Protein mixture
pH 4.0
Separation in first dimension (by charge)
Isoelectric focusing (IEF)
pH 10.0
Apply first gel to top of second
pH 4.0
pH 10.0
Separation in second dimension (by size)
SDS electrophoresis

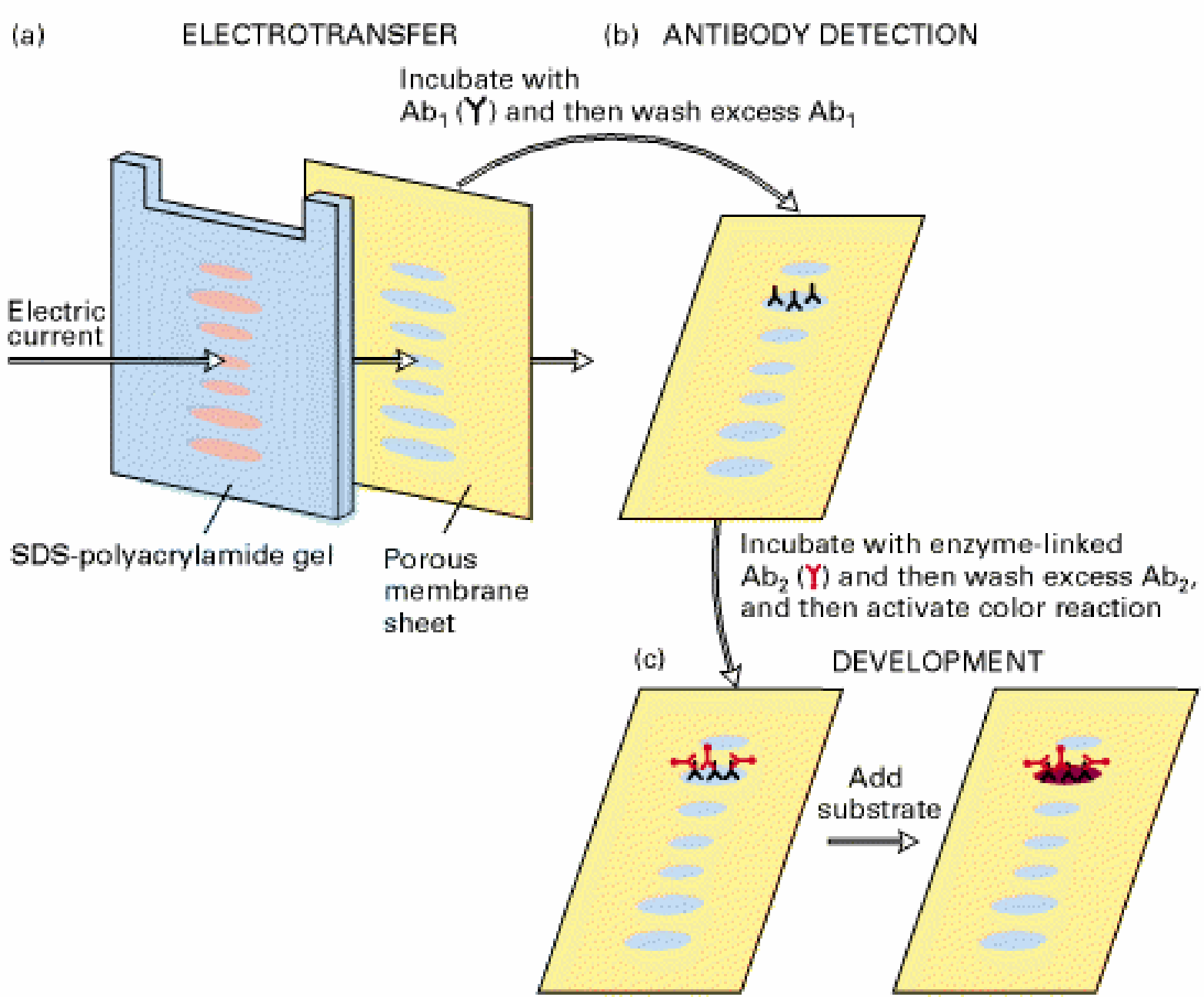


(b)

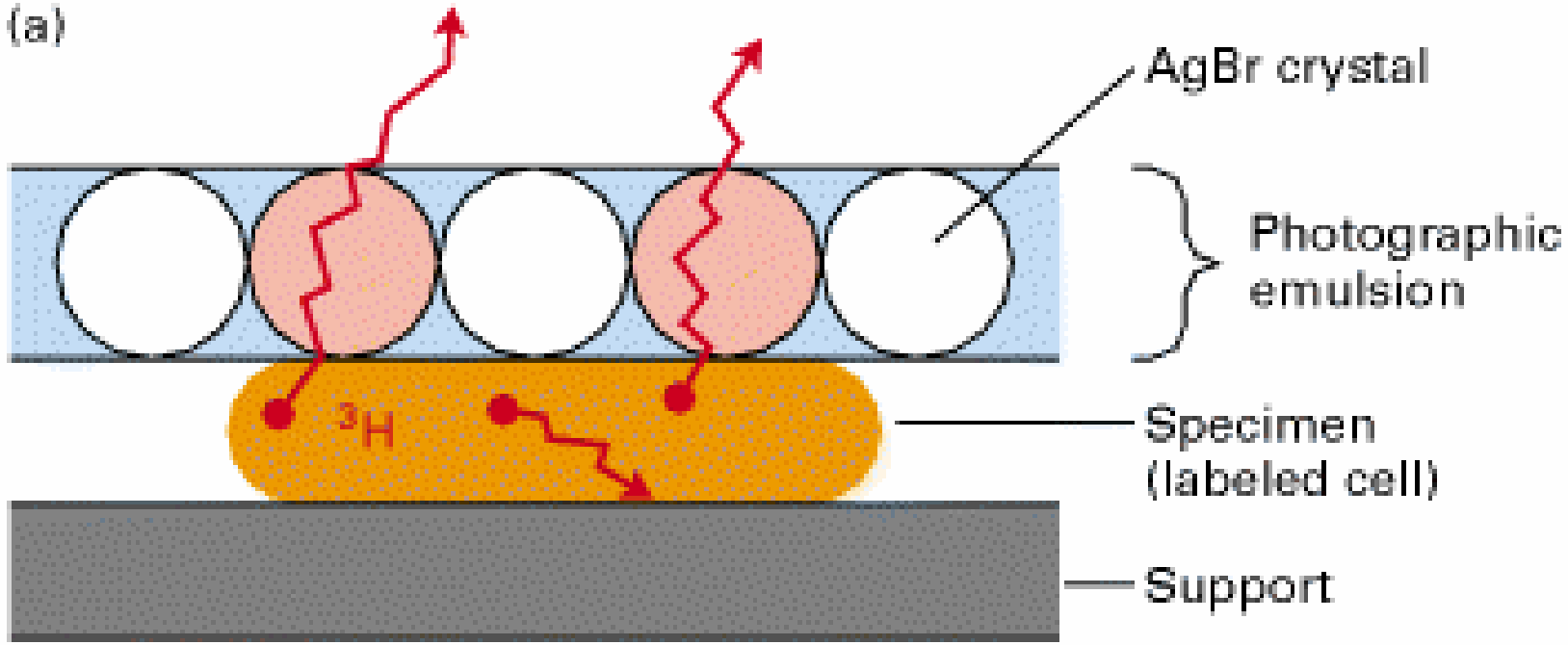
HOW PROTEOMICS CAN HELP DRUG DEVELOPMENT

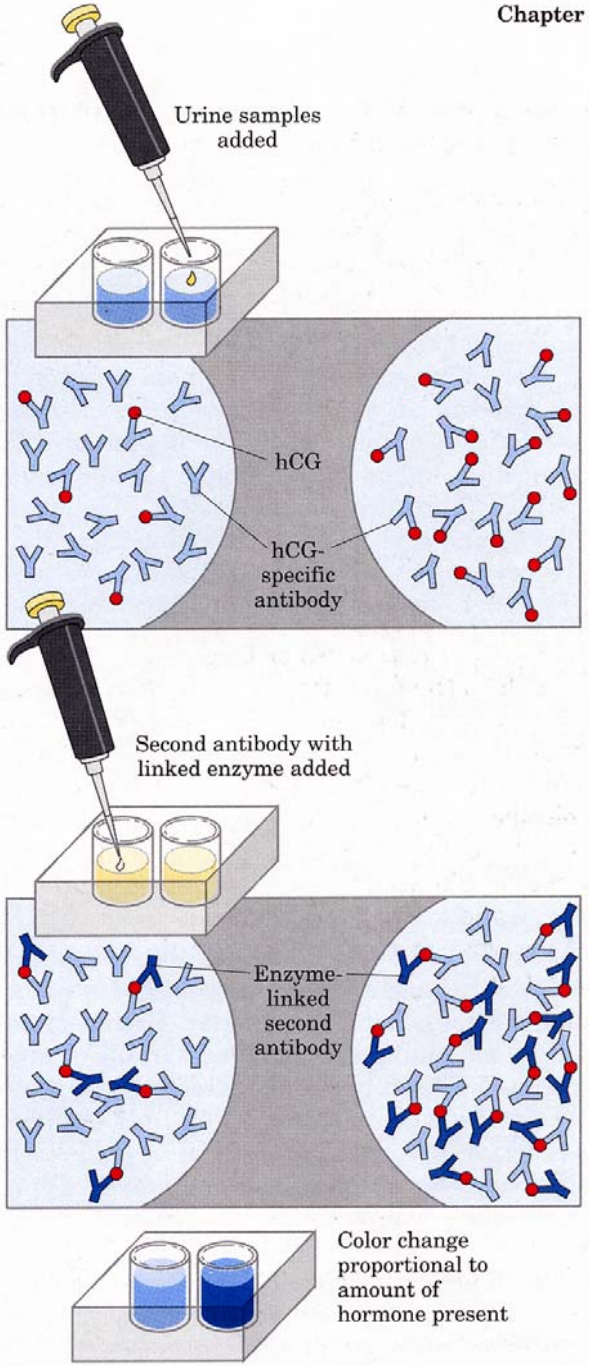



AVOIDING DRUGS WITH SIDE EFFECTS
(Here, determining whether an investigational drug prompts production of possibly harmful proteins)
activity
INVESTIGATIONAL DRUG
BLOOD SERUM
BLOOD SERUM
NO DRUG
Two-dimensional gels show that the drug prompted the production of new proteins—some innocuous (*yellow*) and some with potential to cause side effects (*red*)

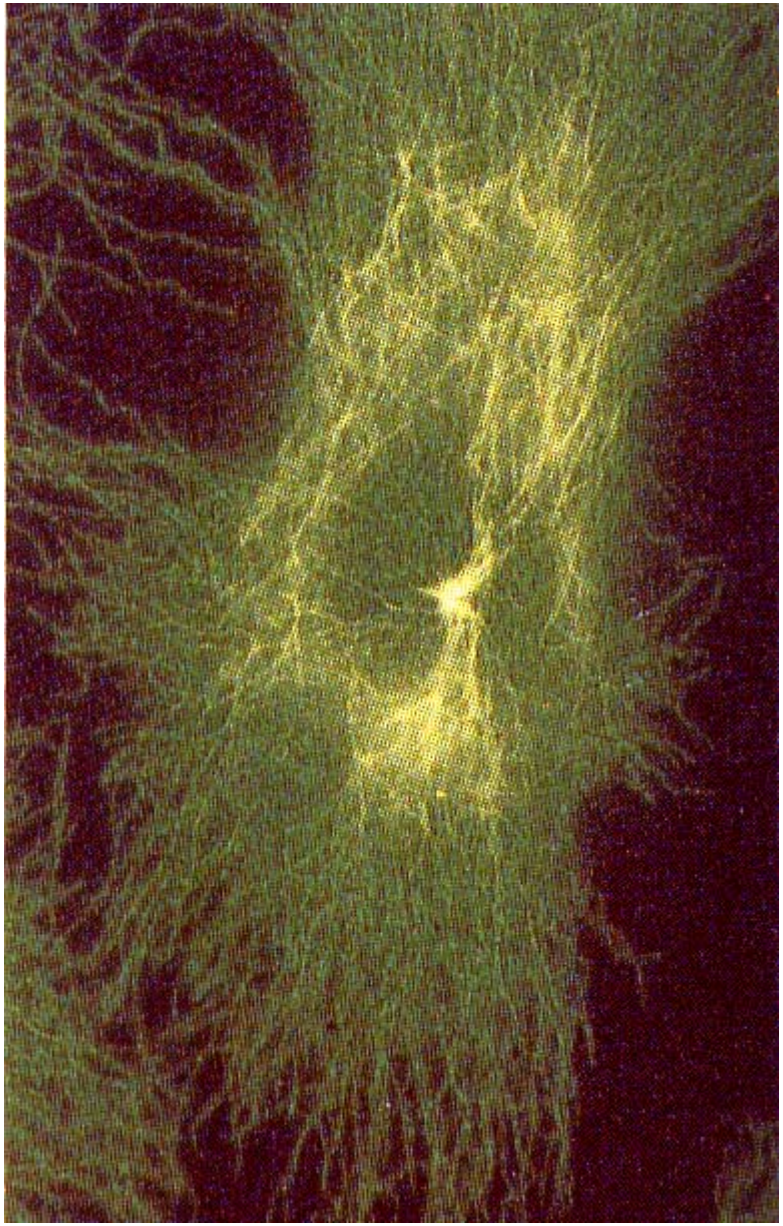

(a) ELECTROTRANSFER
(b) ANTIBODY DETECTION
Incubate with
Ab1 (Y) and then wash excess Ab1
Electric
current
SDS-polyacrylamide gel
Porous
membrane
sheet
Incubate with enzyme-linked
Ab2 (Y) and then wash excess Ab2,
and then activate color reaction
(c) DEVELOPMENT
Add
substrate

Isotope	Half-Life	Energy of Emitted Particle (MeV)*	Specific Activity of Labeled Compounds (mCi/mmol)†
Tritium (hydrogen-3)	12.35 years	0.0186	10^2–10^5
Carbon-14	5730 years	0.156	1–10^2
Phosphorus-32	14.3 days	1.709	10–10^5
Phosphorus-33	25.5 days	0.248	10–10^4
Sulfur-35	87.5 days	0.167	1–10^6
Iodine-125	60 days	0.035	10^2–10^4
Iodine-131	8.07 days	0.806	10^2–10^4


(a)
AgBr crystal
Photographic emulsion
^3H
Specimen (labeled cell)
Support


Urine samples added
hCG
hCG-specific antibody
Second antibody with linked enzyme added
Enzyme-linked second antibody
Color change proportional to amount of hormone present

(a)



SECUENCIA (+/-300)

ESTAQUEERAUNANIÑADECERAPERONOERA
UNANIÑADECERAERAUNAGAVILLAPARADAE
NLAERAPERONOERAUNAGAVILLASINOLAFLO
RTIERNADELAMARAVILLATAMPOCOERALAFL
ORSINOQUEERAUNRAYITODESOLPARADOEN
LA VIDRIERANOERAUNRAYITODESOLSIQUIER
AUNAPAJITADENTRODEMISOJITOSERAALLEG
UENSEAMIRARCOMOHEPERDIDOENTERAENE
NESTELAGRIMÓNMIPASCUAVERDADERA

ESTA QUE ERA UNA NIÑA DE CERA
PERO NO ERA UNA NIÑA DE CERA,
ERA UNA GAVILLA PARADA EN LA ERA,
PERO NO ERA UNA GAVILLA
SINO LA FLOR TIERNA DE LA MARAVILLA,
TAMPOCO ERA LA FLOR, SINO QUE ERA
UN RAYITO DE SOL PARADO EN LA VIDRIERA,
NO ERA UN RAYITO DE SOL SIQUIERA
UNA PAJITA DENTRO DE MIS OJITOS ERA,
ALLEGUENSE A MIRAR COMO HE PERDIDO ENTERA
EN ESTE LAGRIMÓN MI PASCUA VERDADERA

ESTA QUE UNA NIÑA DE CPERO NO UNA NIÑA DE CUNA GAVILLA PARADA EN LA, PERO NO UNA GAVILLA SINO LA FLOR TIERNA DE LA MARAVILLA, TAMPOCO LA FLOR, SINO QUE UN RAYITO DE SOL PARADO EN LA VIDRINO UN RAYITO DE SOL SIQUIUNA PAJITA DENTRO DE MIS OJITOS, ALLEGUENSE A MIRAR COMO HE PERDIDO ENTEN ESTE LAGRIMÓN MI PASCUA VERDAD.