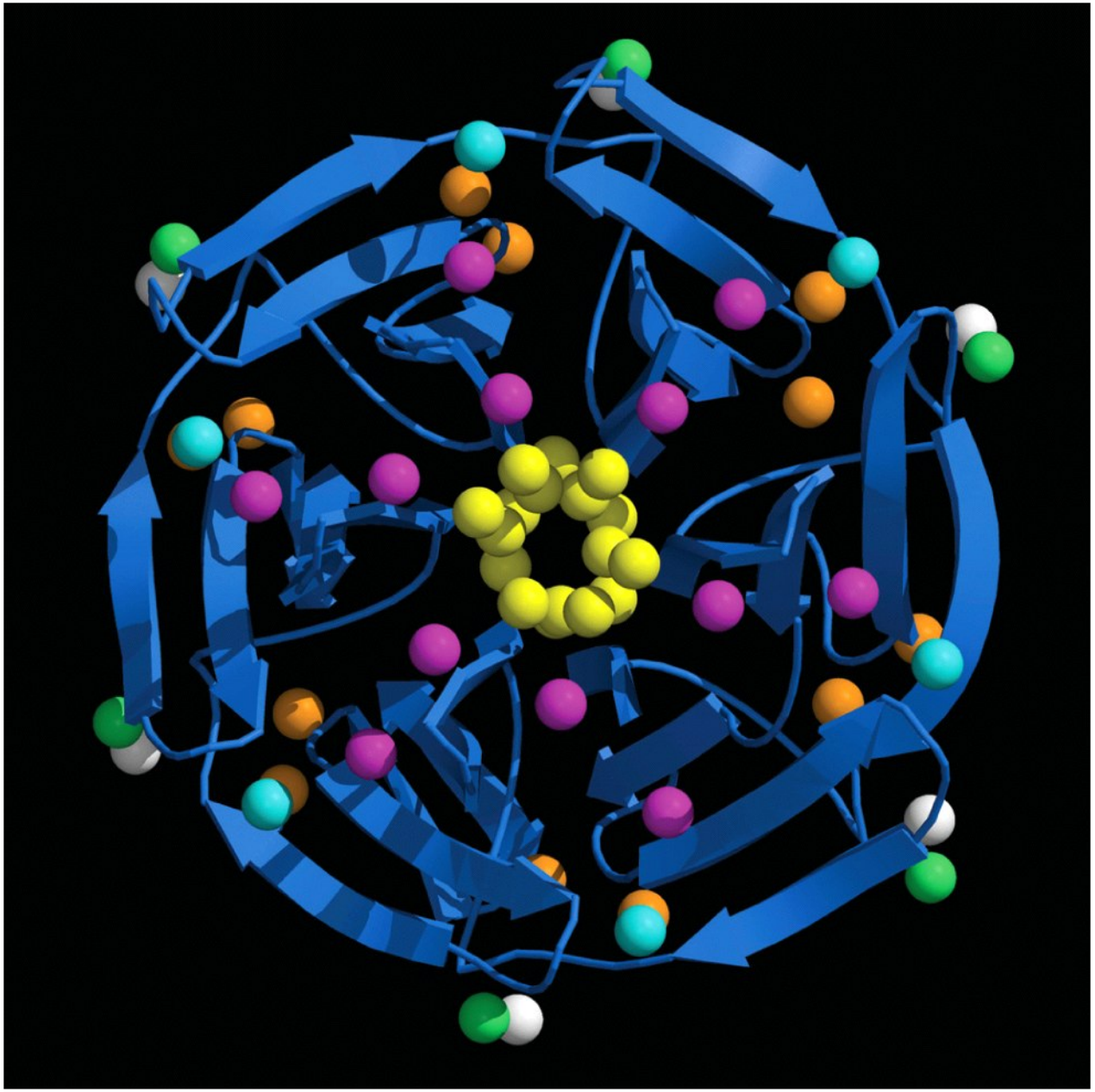
Lodish • Berk • Kaiser • Krieger • Scott • Bretscher •Ploegh • Matsudaira

• MOLECULAR CELL BIOLOGY

• SIXTH EDITION

• CHAPTER 3

• Protein Structure and Function



Human signaling protein keapl

Ribbon diagram

Conformations

Structural proteins

Scaffold protein

Transport protein

Regulatory protein

enzymes

Functional classes:

Structural proteins

Transport proteins

Regulatory proteins

Motor proteins

Different conformation = different function

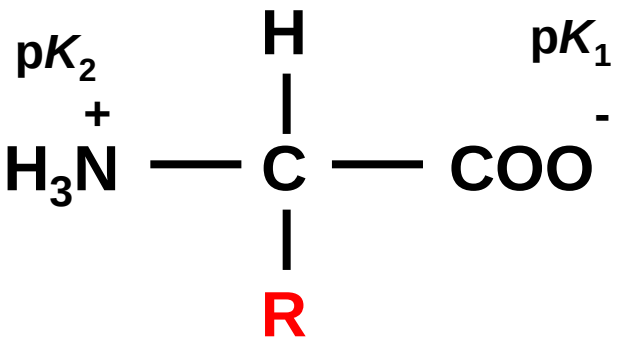
Hierarchical structure of proteins

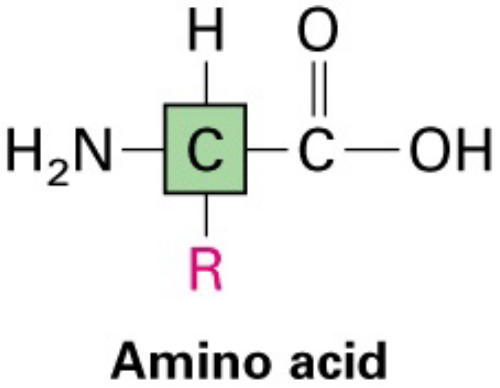
Proteins are single, unbranched chains of amino acid monomers

There are 20 different amino acids; All amino acids have the same general structure but the side chain (R group) of each is different

A protein's amino acid sequence determines its three-dimensional structure (conformation)

In turn, a protein's structure determines the function of that protein

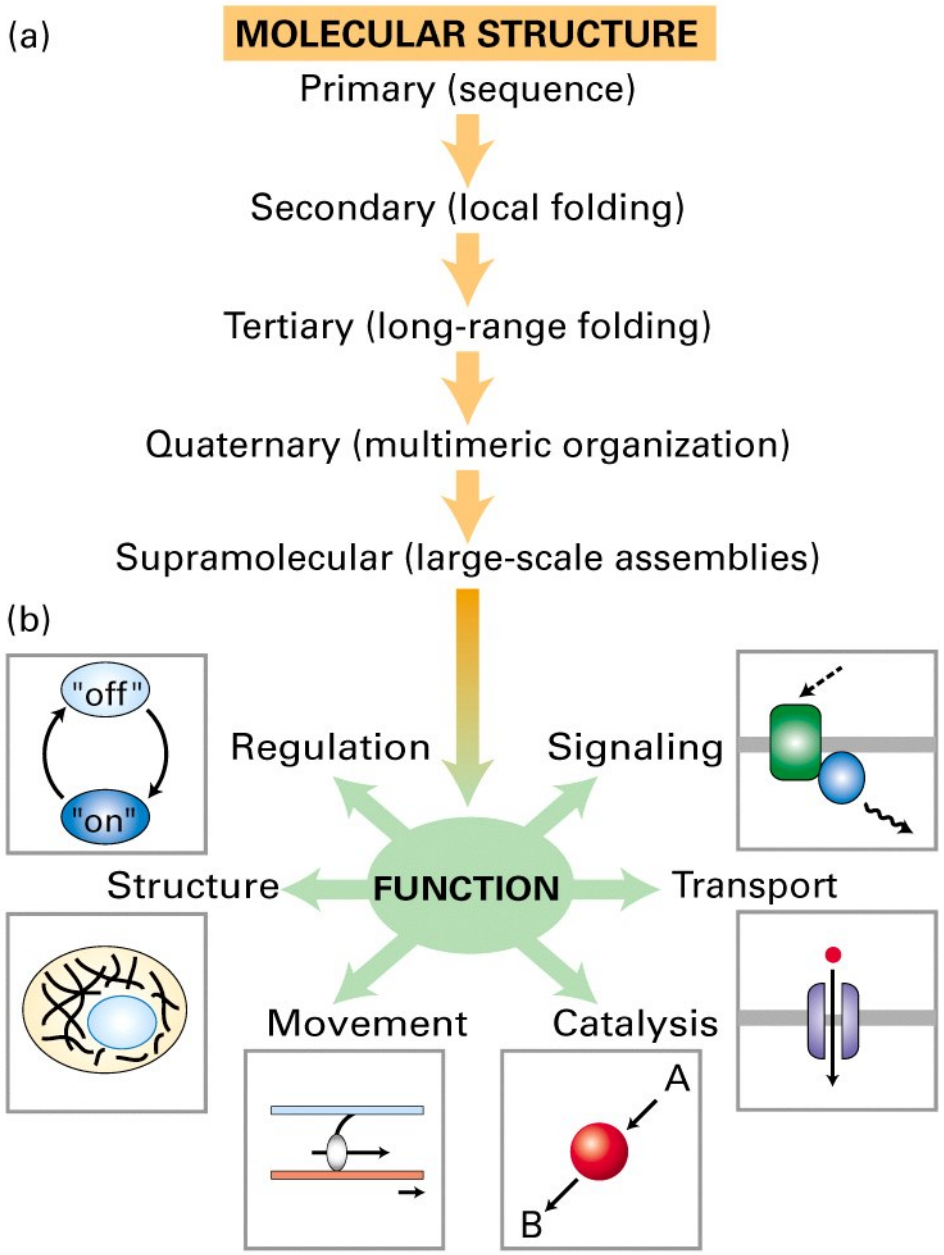




Amino acid

Four levels of structure determine the shape of proteins

- Primary: the linear sequence of amino acids
- Secondary: the localized organization of parts of a polypeptide chain (e.g., the α helix or β sheet)
- Tertiary: the overall, three-dimensional arrangement of the polypeptide chain
- Quaternary: the association of two or more polypeptides into a multi-subunit complex



Four levels of protein hierarchy

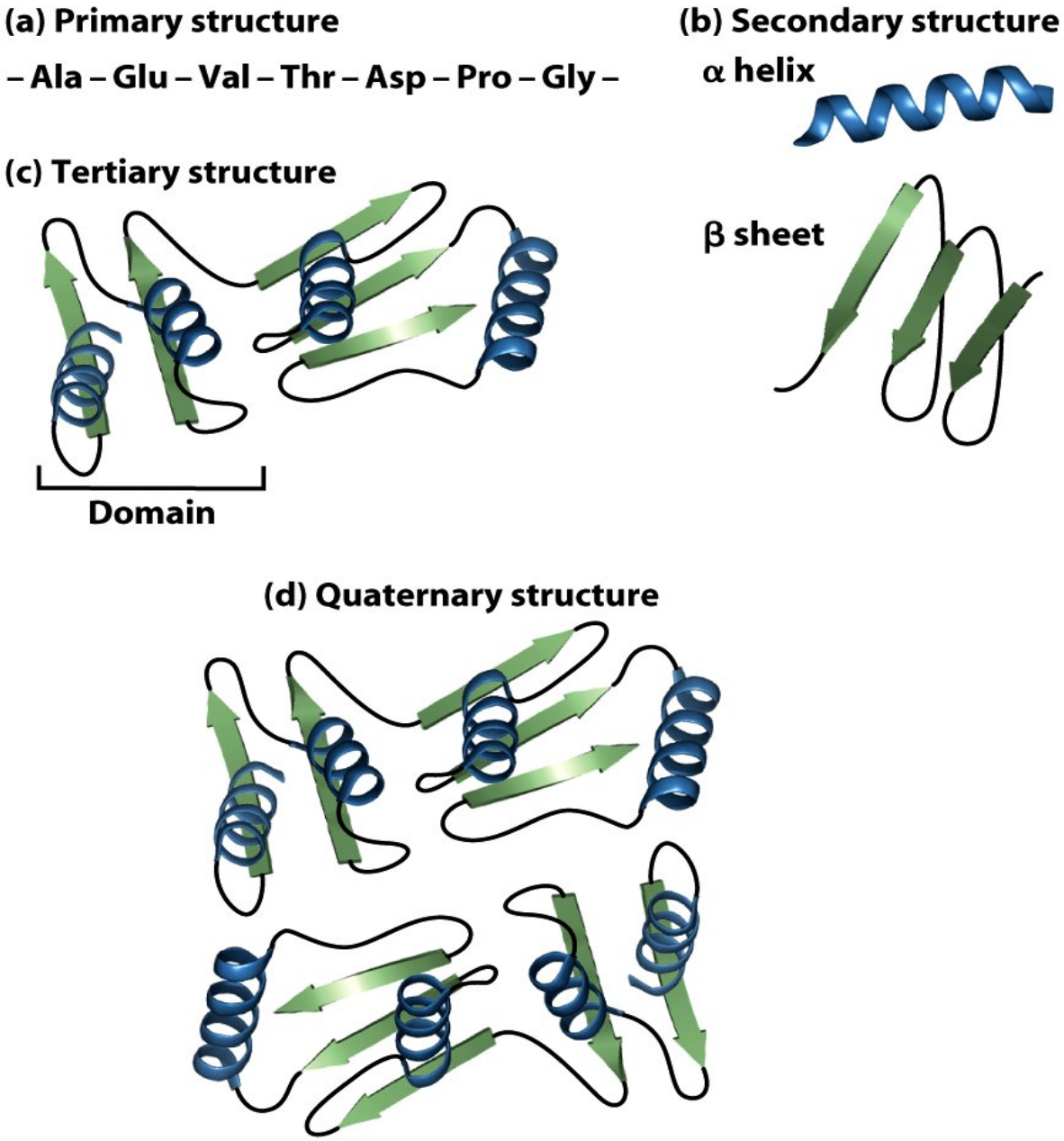


Figure 3-2
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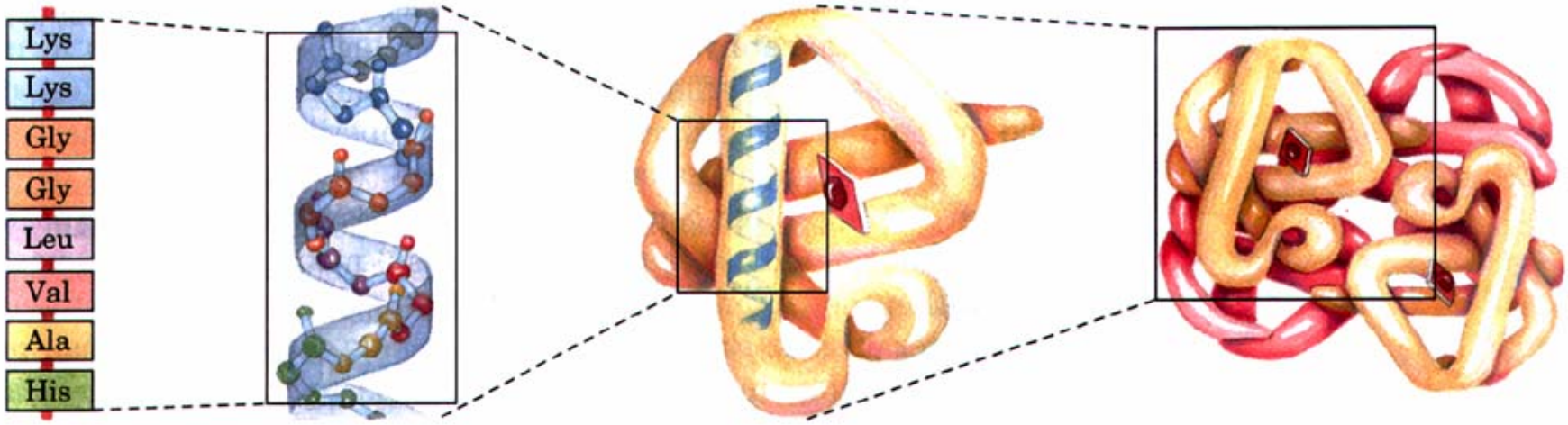
Amino acid → 2 amino acids → peptide → polypeptide



1º 2º 3º 4º

一級primary 二級secondary 三級tertiary 四級quaternary

Nelson & Cox (2000) Lehninger Principles of Biochemistry

The primary structure of a protein is its liner arrangement of amino acid

Peptide bond - linkage between amino acids is a secondary amide bond

Formed by condensation of the α-carboxyl of one amino acid with the α-amino of another amino acid (loss of H_2O molecule)

Primary structure - linear sequence of amino acids in a polypeptide or protein

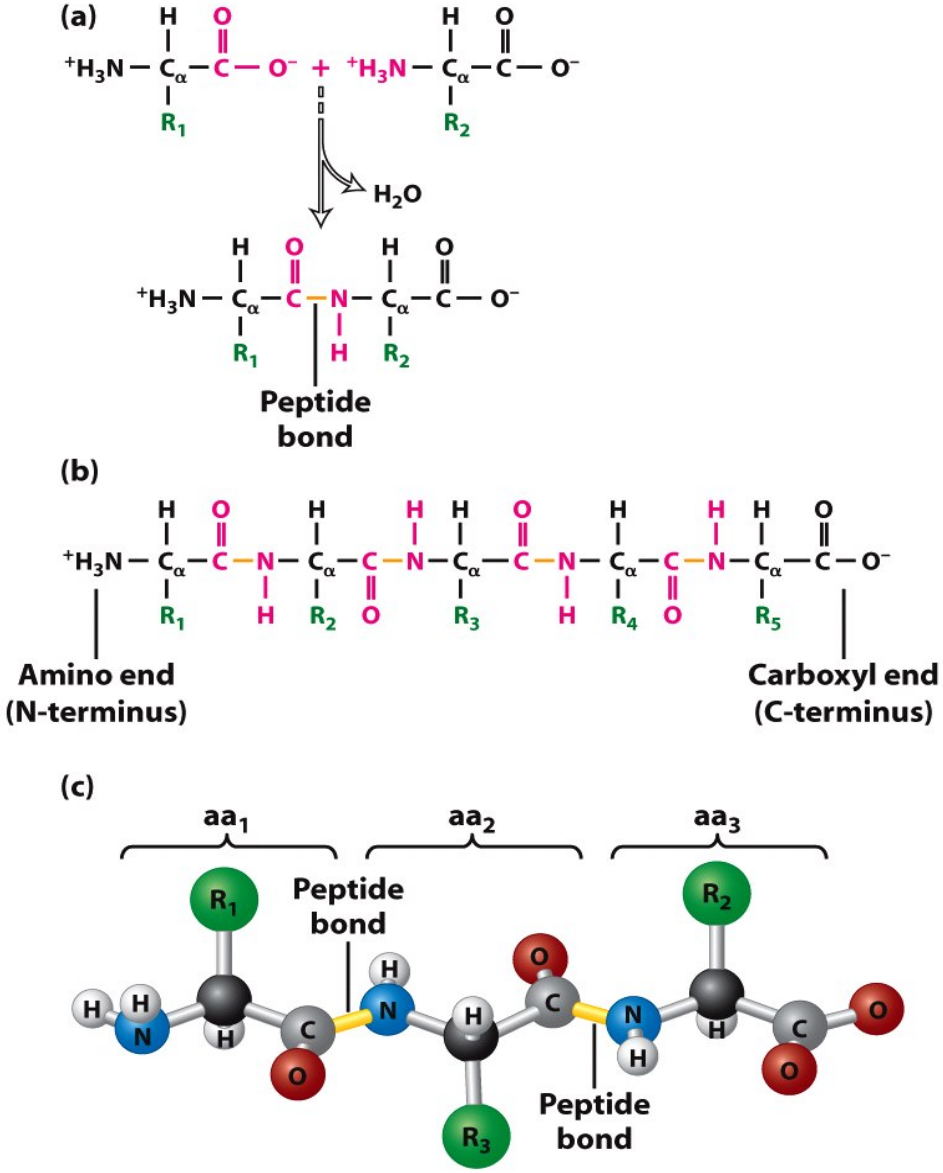


Figure 3-3
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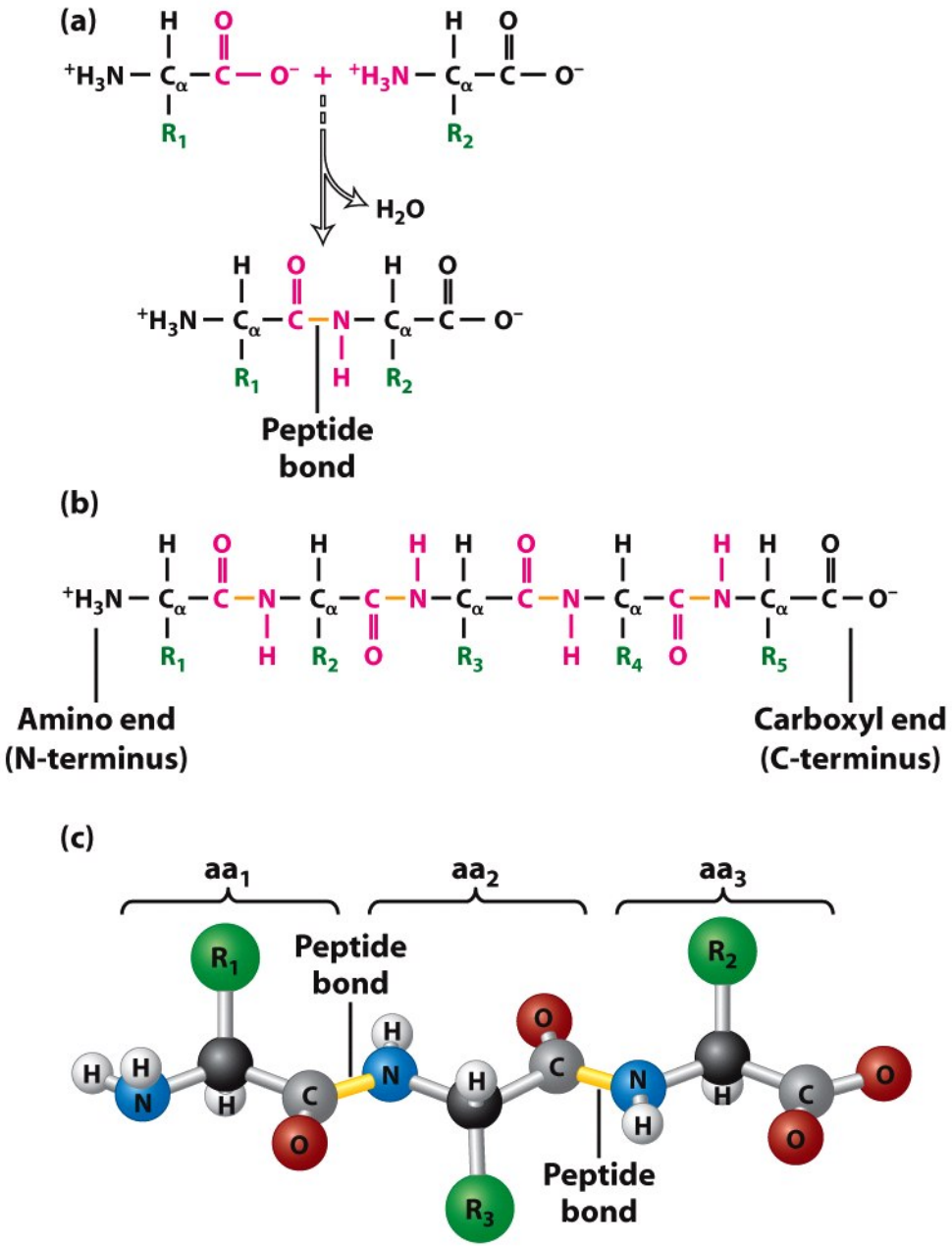

Figure 3-3
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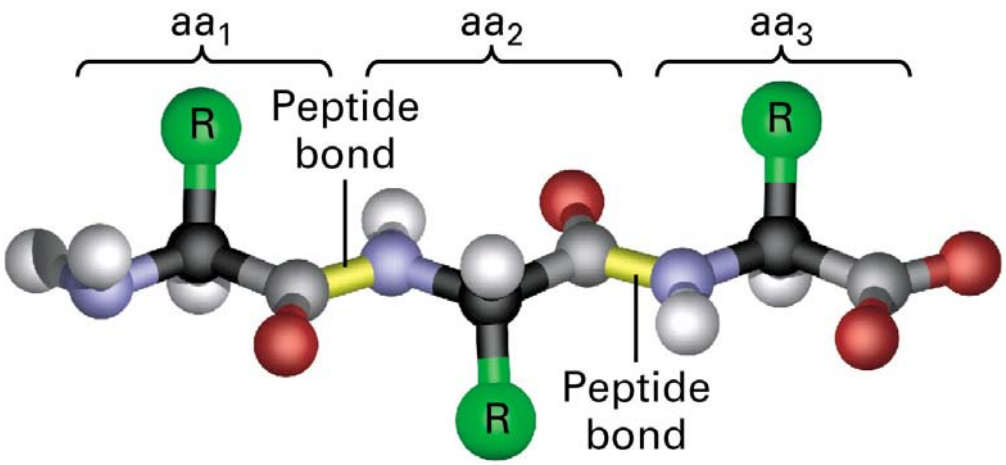
The backbone of protein (polypeptide)



N-terminal

C-terminal

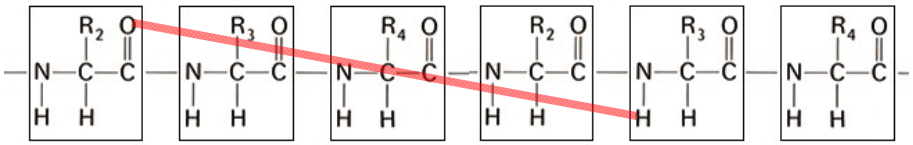
N-C-C-N-C-C-N-C-C-N-C-C

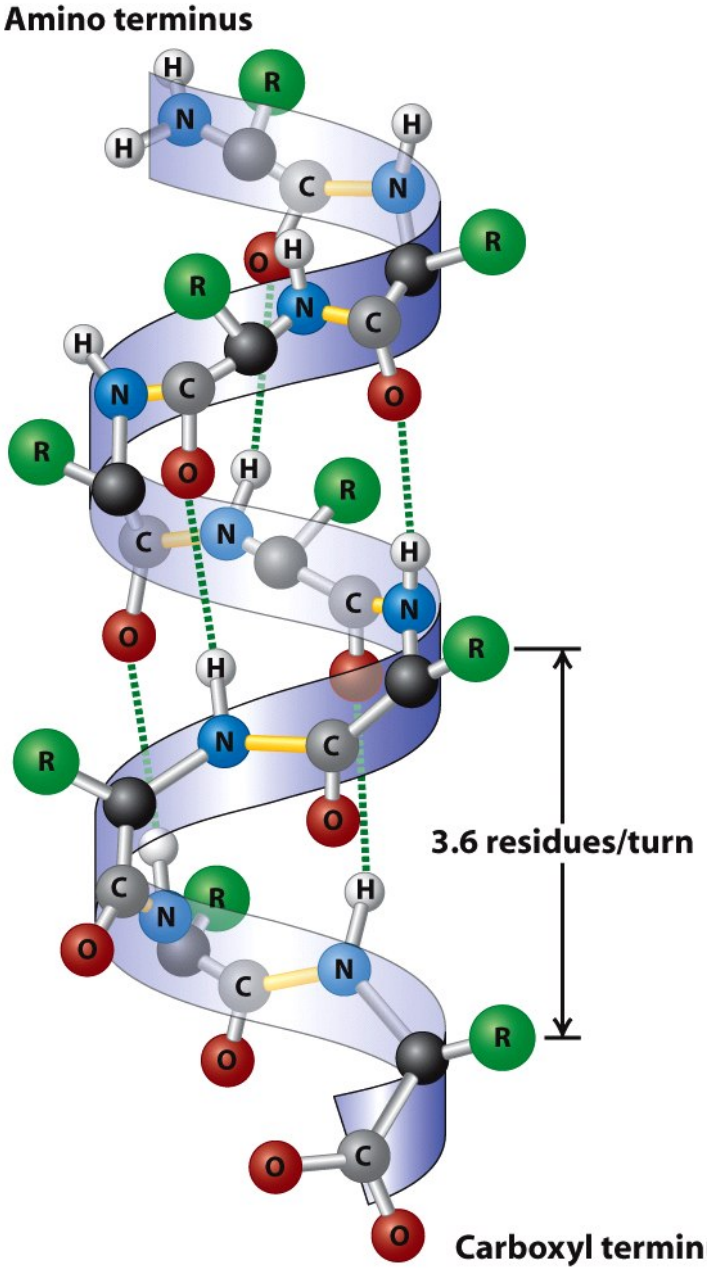
Unit (單位)

Peptide bond (胜鍵)

Secondary structure: the α helix

Secondary structure are the core elements of protein architecture





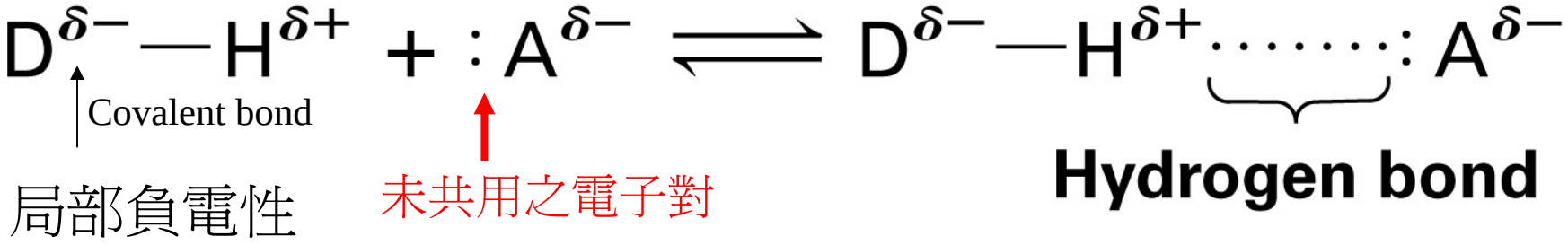
每 3.6 胺基酸繞一圈，每圈 5.4 Å 高

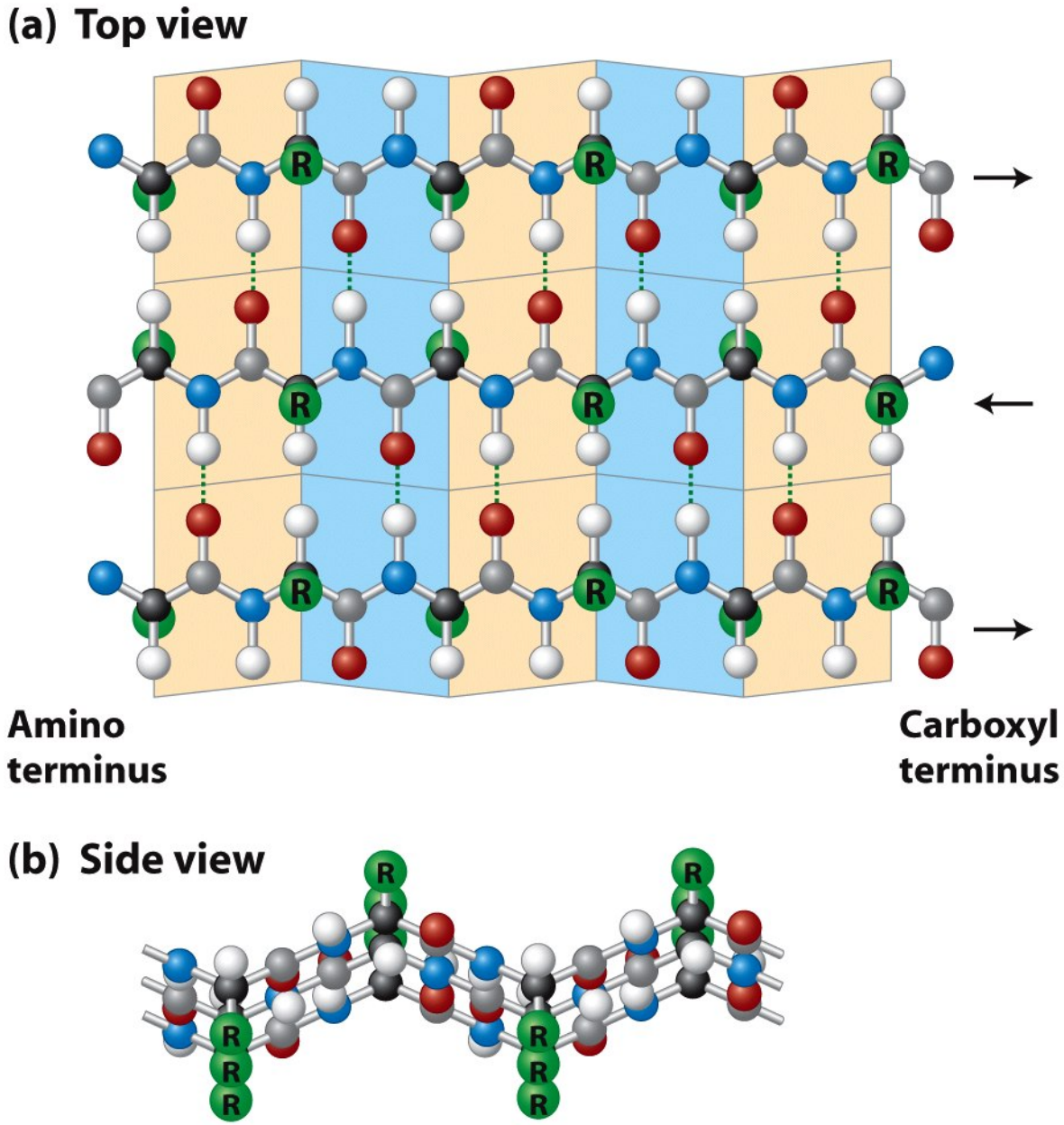
Carbonyl (C=O) 與下游 H-N- 生成 氫鍵

Hydrogen bonds determine water solubility of uncharged molecules

Intra-molecular

Inter-molecular





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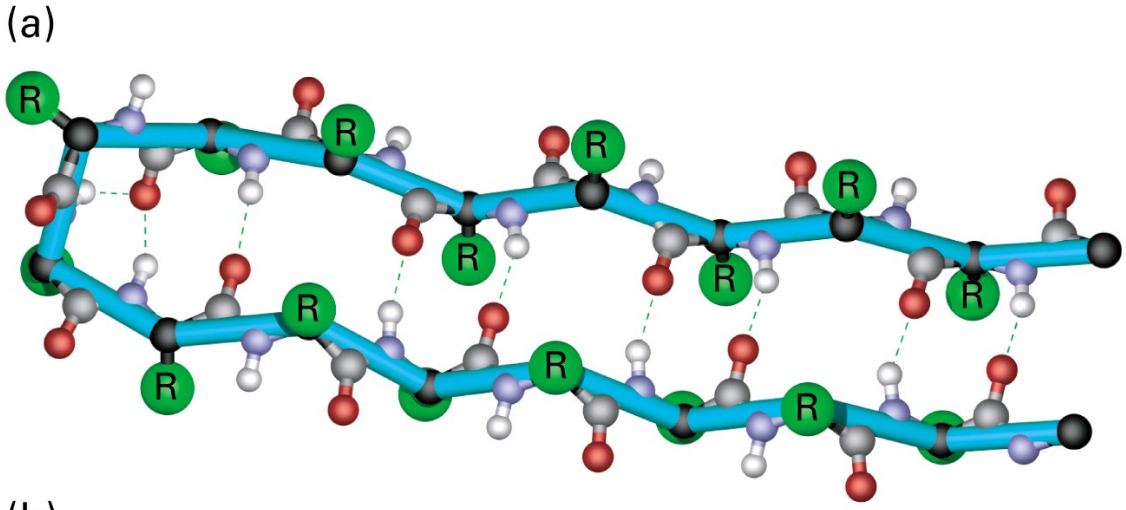
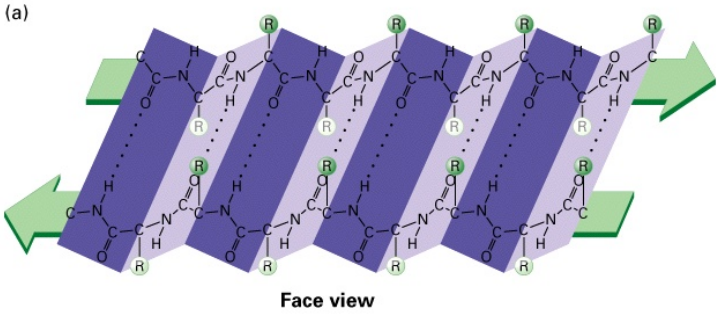
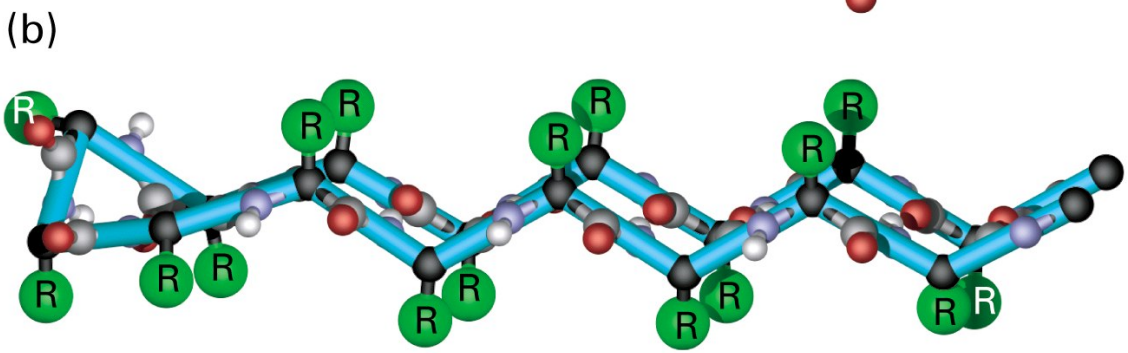
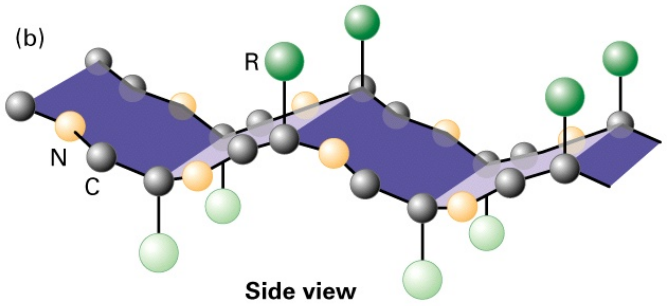
Secondary structure: the beta sheet









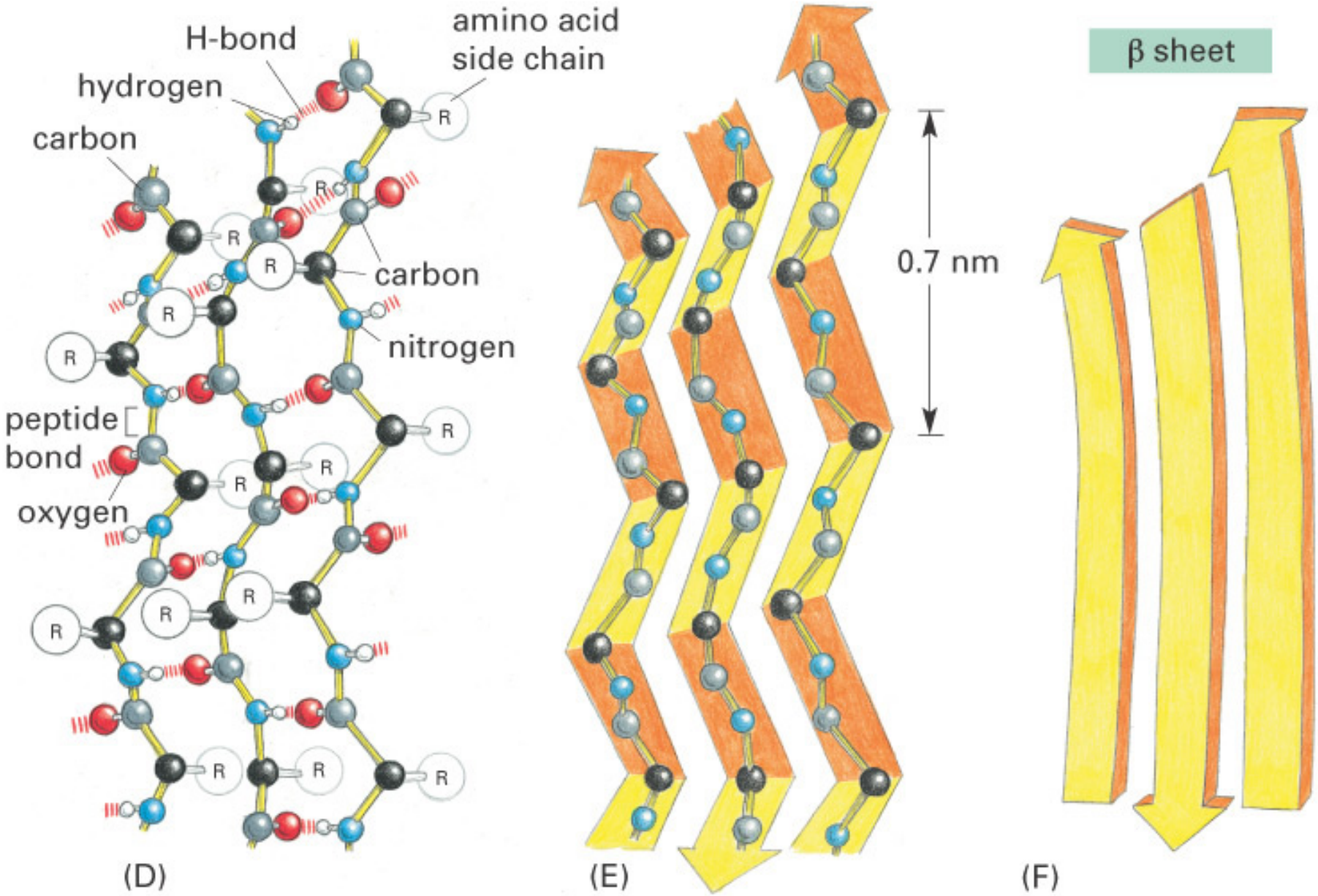
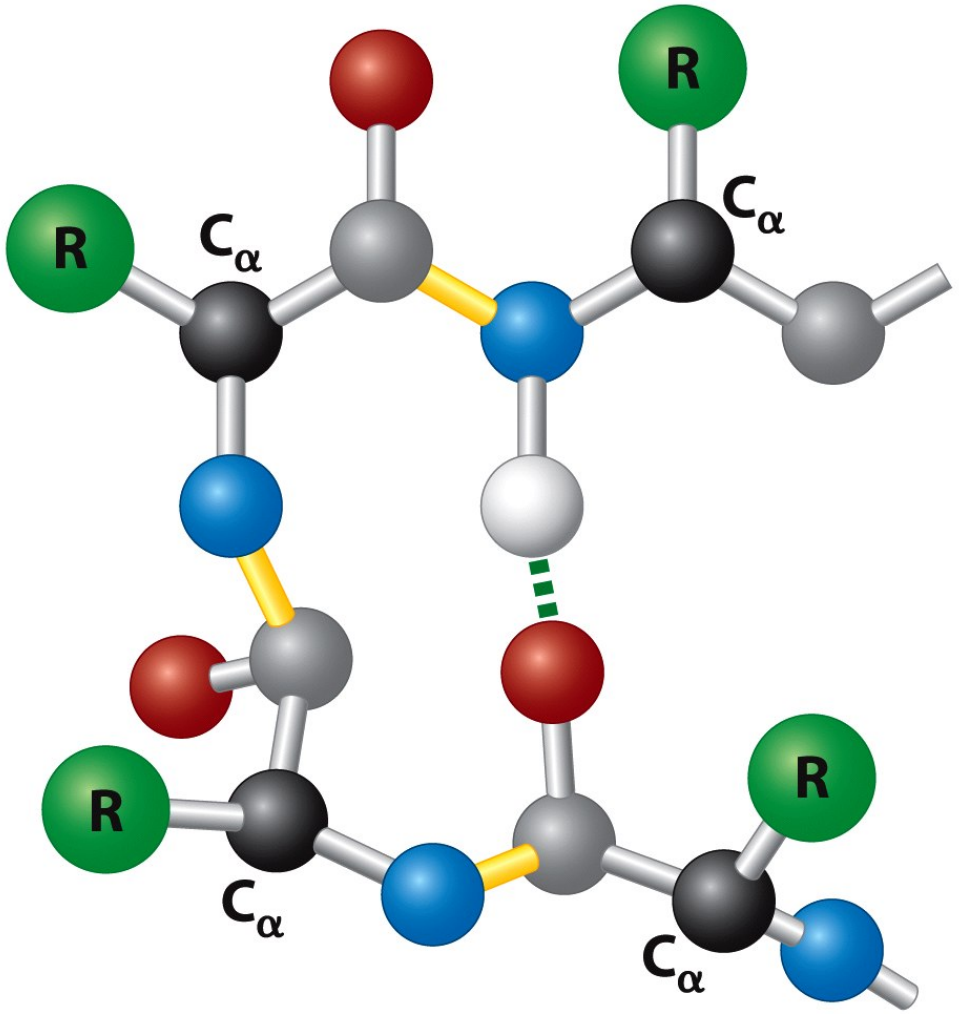


Figure 4-10 part 2 of 2 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Structure of a β turn

1. A short U-shaped beta turn
2. Four residue
3. H-bond stable
4. Proline and glycine present



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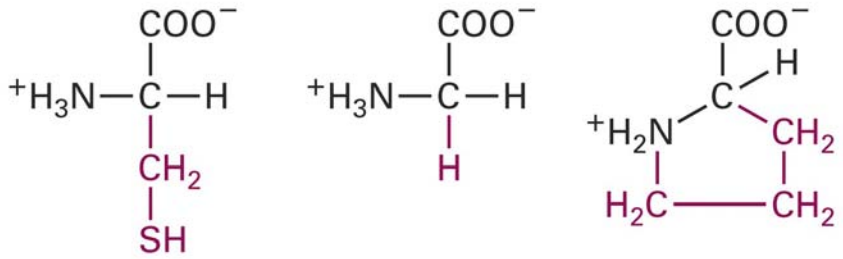
SPECIAL AMINO ACIDS



Cysteine (Cys or C) **Glycine (Gly or G)** **Proline (Pro or P)**

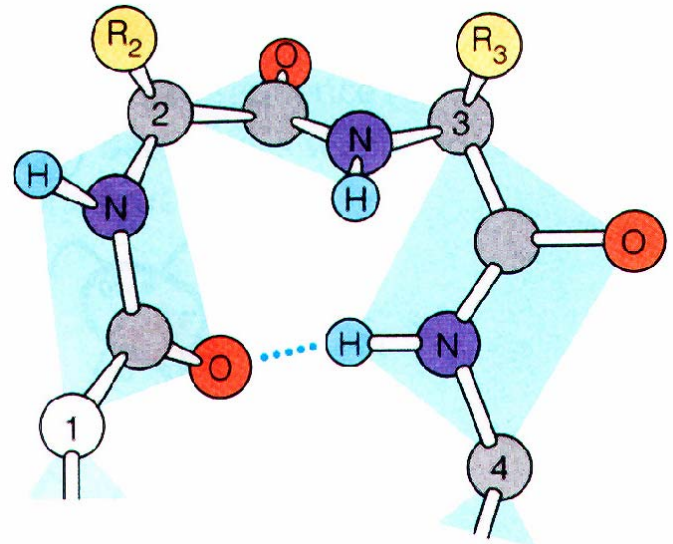
Reverse Turns: β turn, γ turn

It also related with H-bond

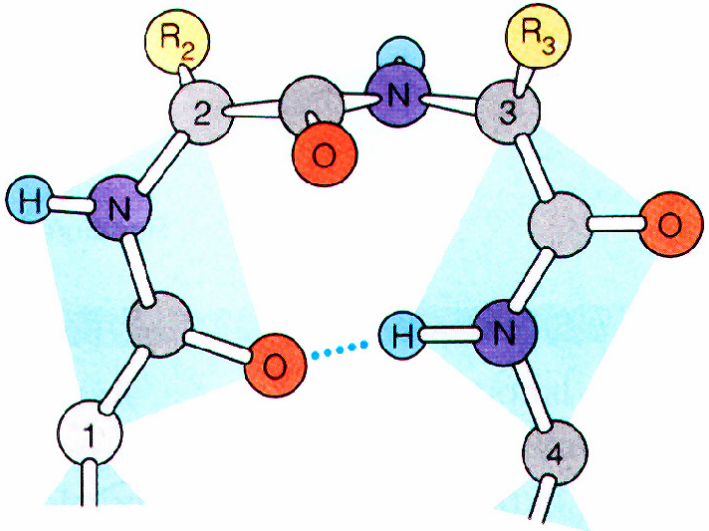
β turn

三個胺基酸夾一氫鍵

R 在同一側

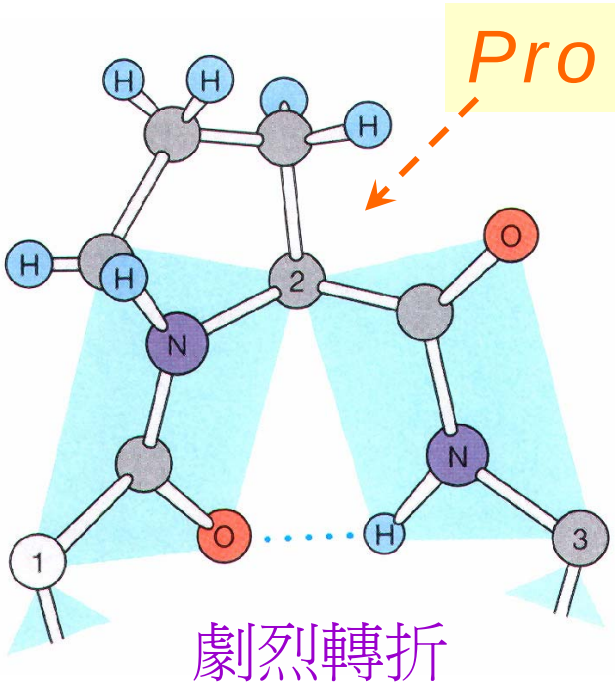


R 在相對側

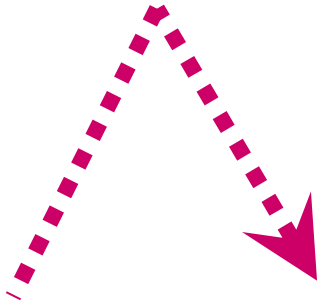


γ turn

兩個胺基酸夾一氫鍵



劇烈轉折



Mathews et al (2000) Biochemistry (3e) p.181

Oil drop model of protein folding

Hydrophobic nonpolar
Hydrophilic polar

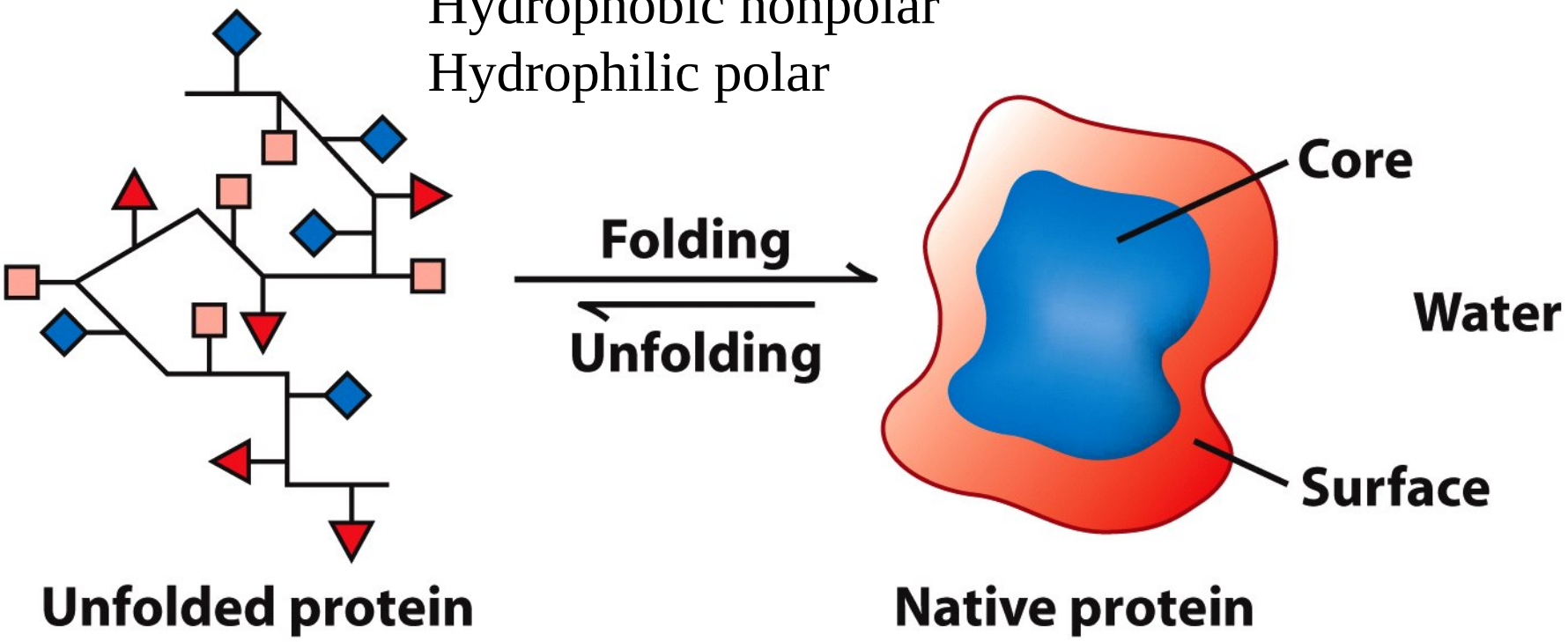


Figure 3-7
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Hydrophobic interact with hydrophobic
Hydrophilic interact with hydrophilic

Uncharged hydrophilic polar side chains are found on both the surface and inner core of protein

Integral membrane protein
Globular protein

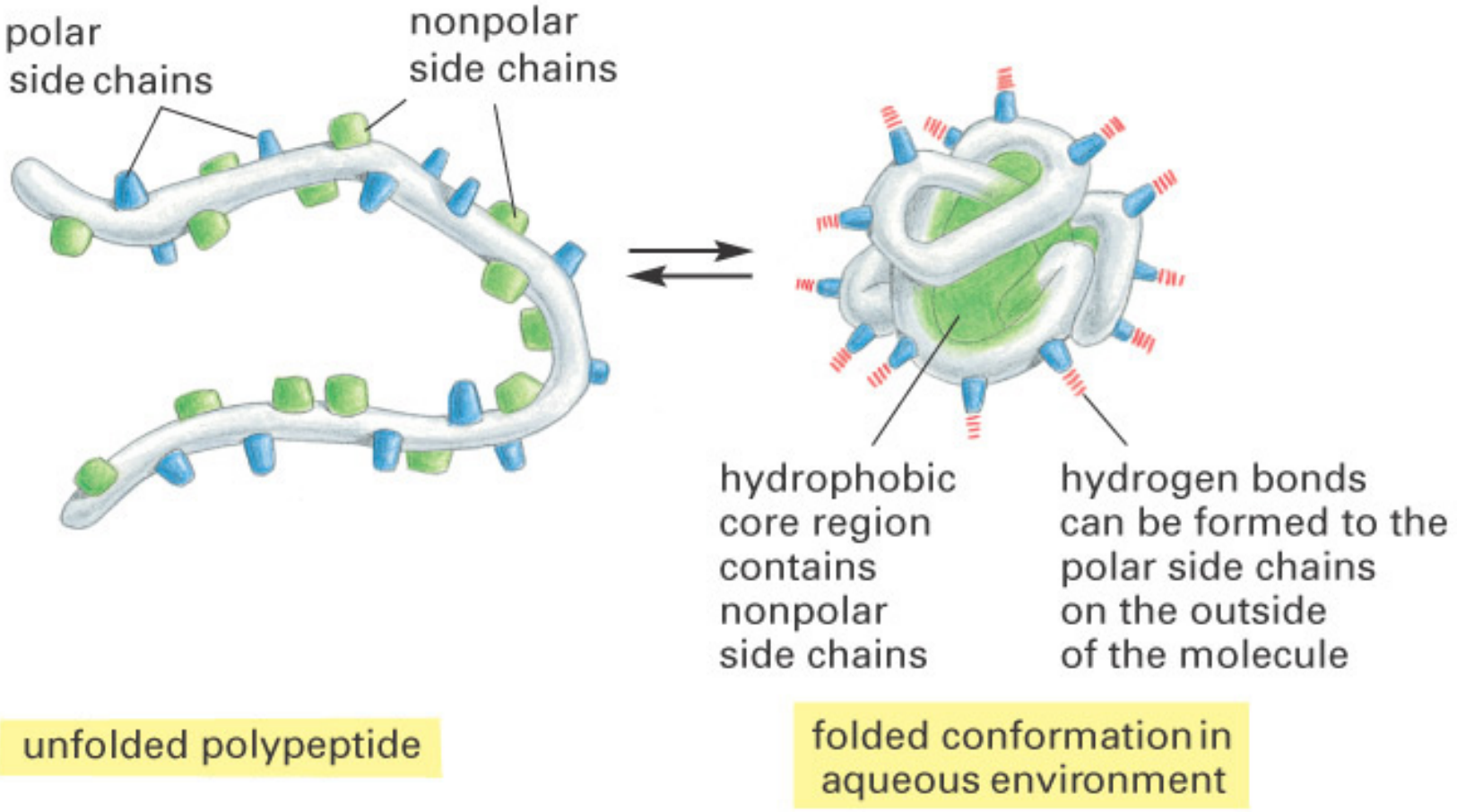


Figure 4-5 Essential Cell Biology, 2/e. (© 2004 Garland Science)

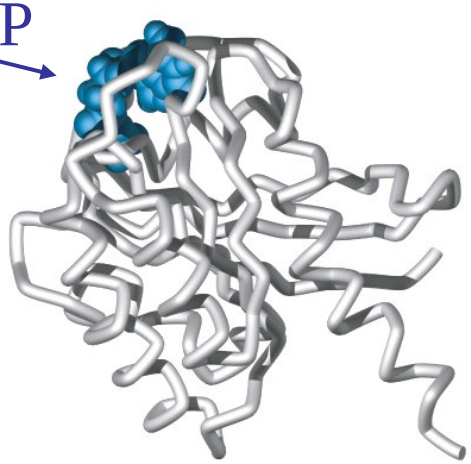
Overall folding of a polypeptide chain yields its tertiary structure

Different way of depicting the conformation of proteins convey different types of information

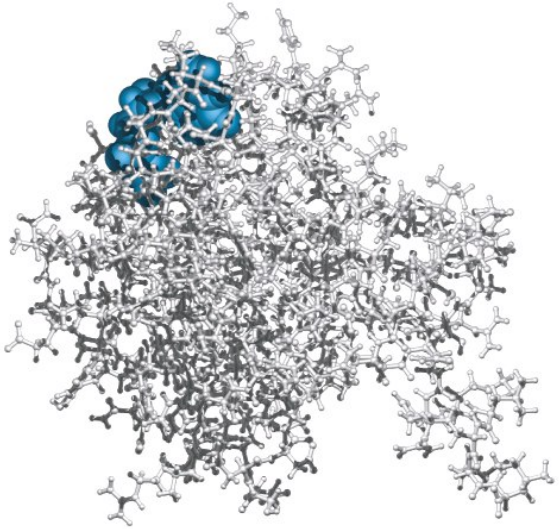
Ras

(a) C_α backbone trace

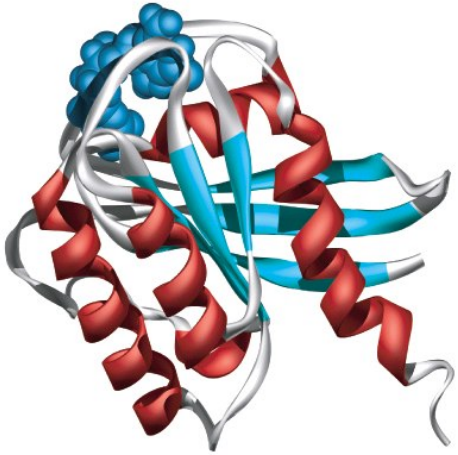
GDP



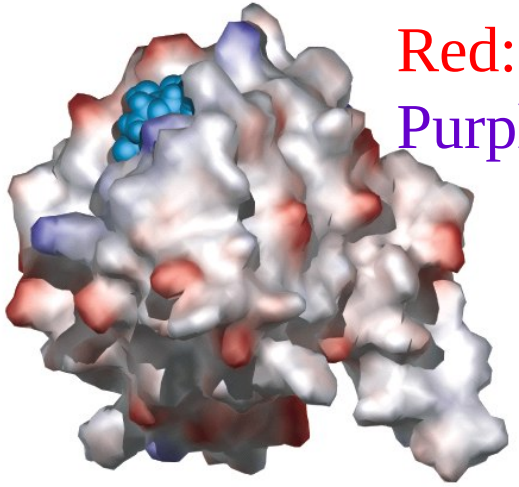
(b) Ball and stick 球與棒



(c) Ribbons 帶狀



(d) Solvent-accessible surface 溶劑親水表面



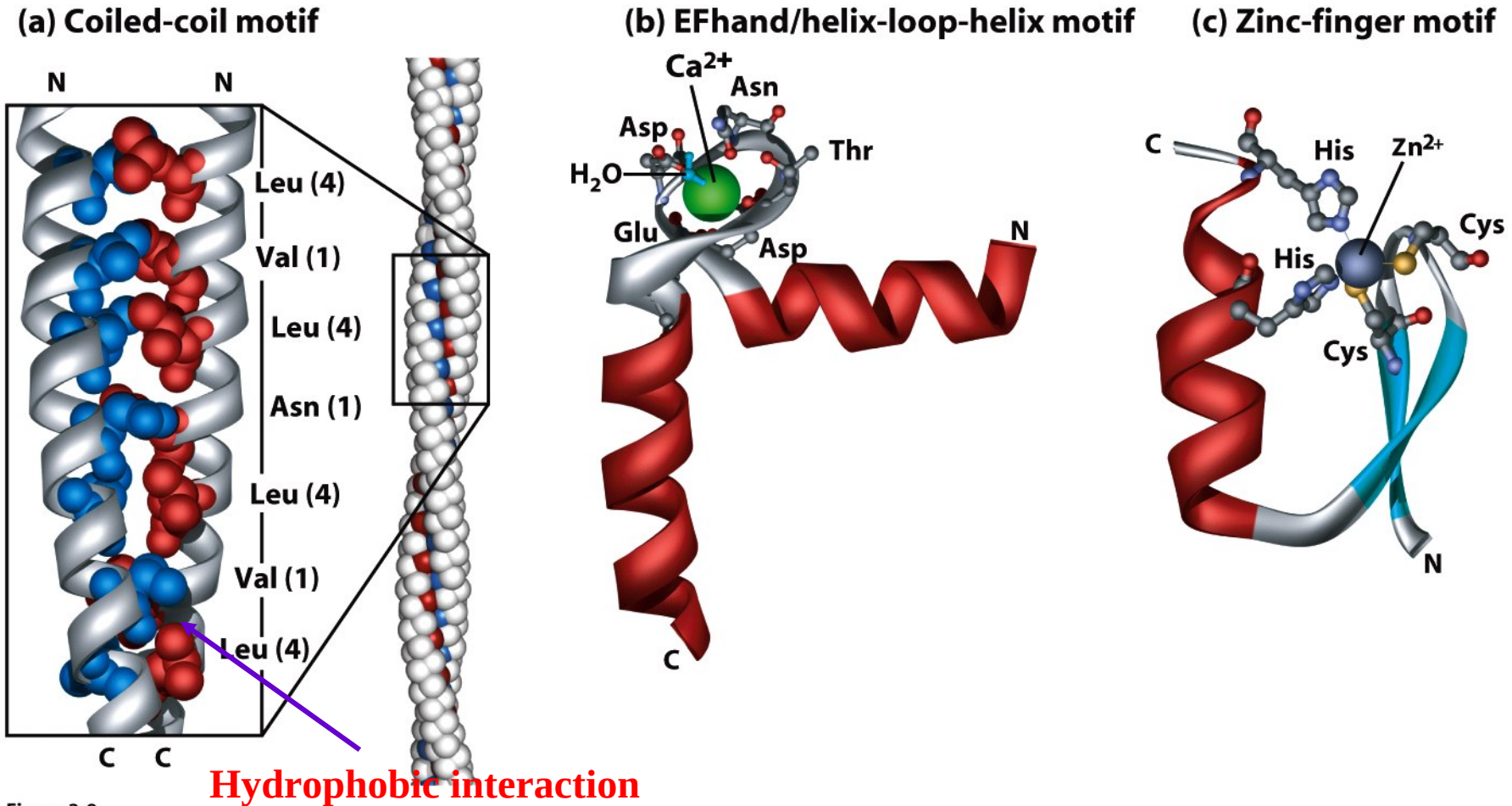
Red: negative charge

Purple: positive charge

Different graphical representations of the same protein

Motifs are regular combination of secondary structures

Motif: particular combinations of secondary structures, it build up the tertiary structure of a protein; super-secondary strucure and 2-5 secondary structure



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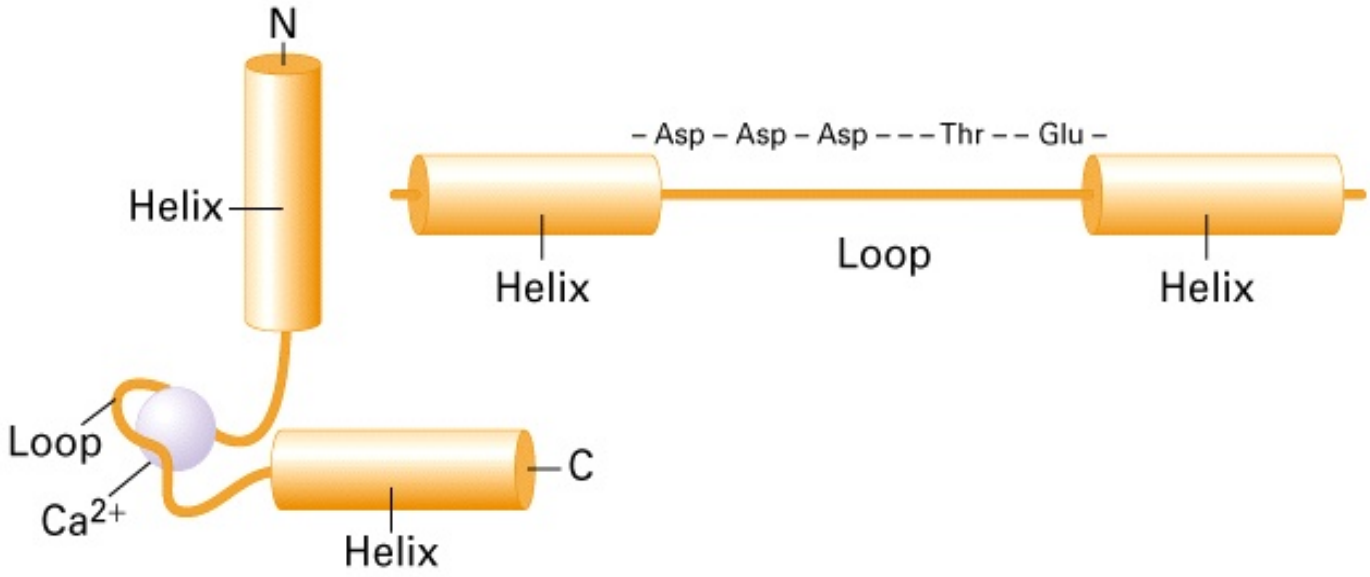
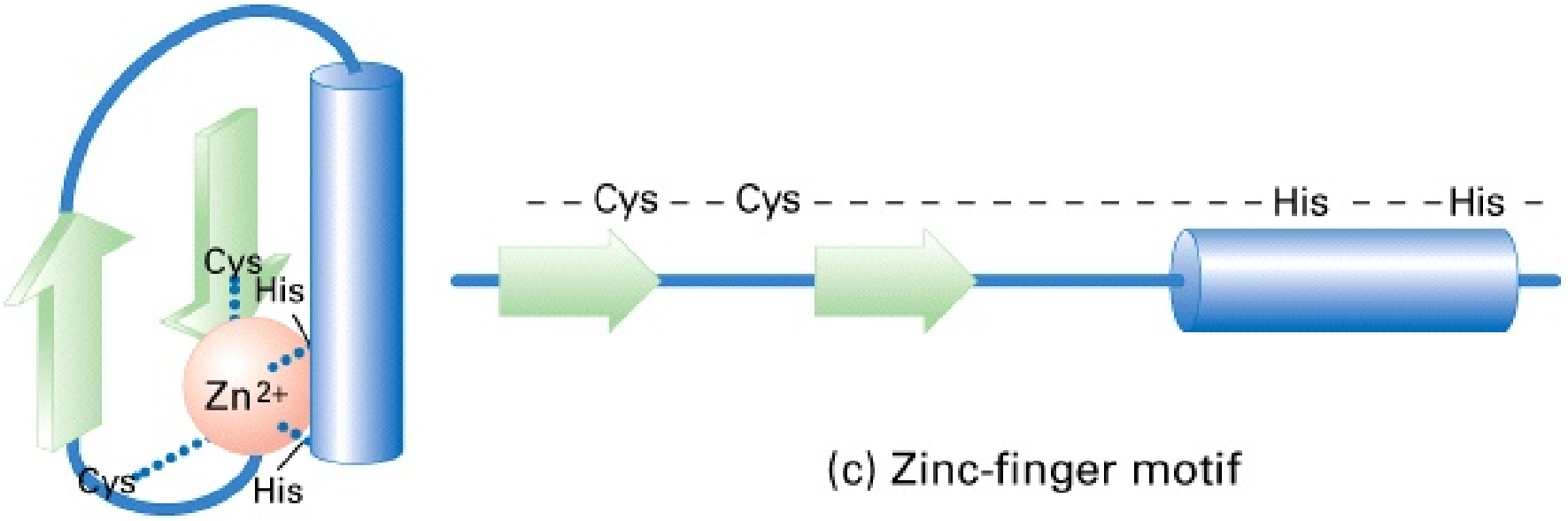
(b) Helix–loop–helix motif



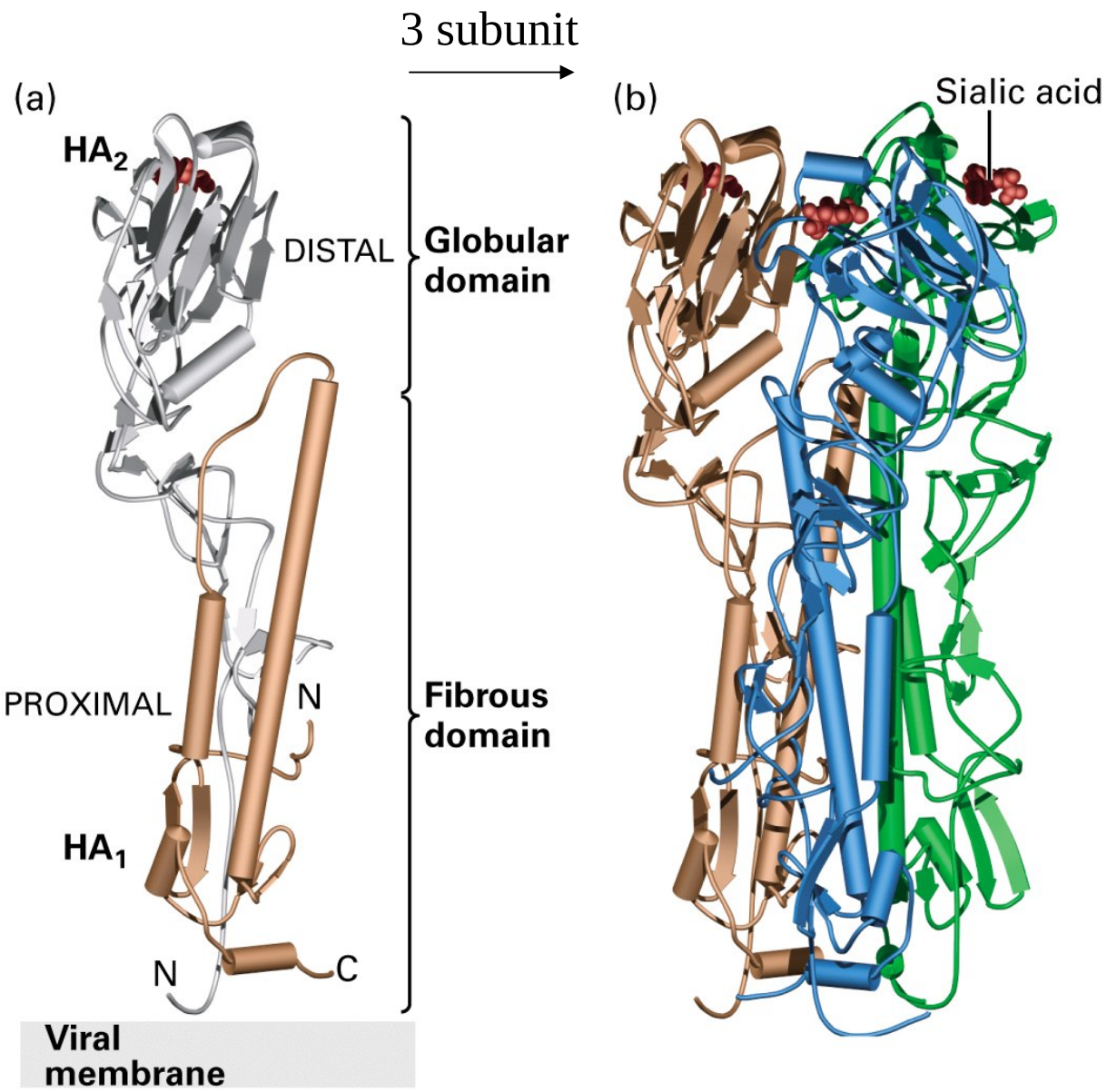


(c) Zinc-finger motif

Structural and functional domains are modules of tertiary structure

Domain

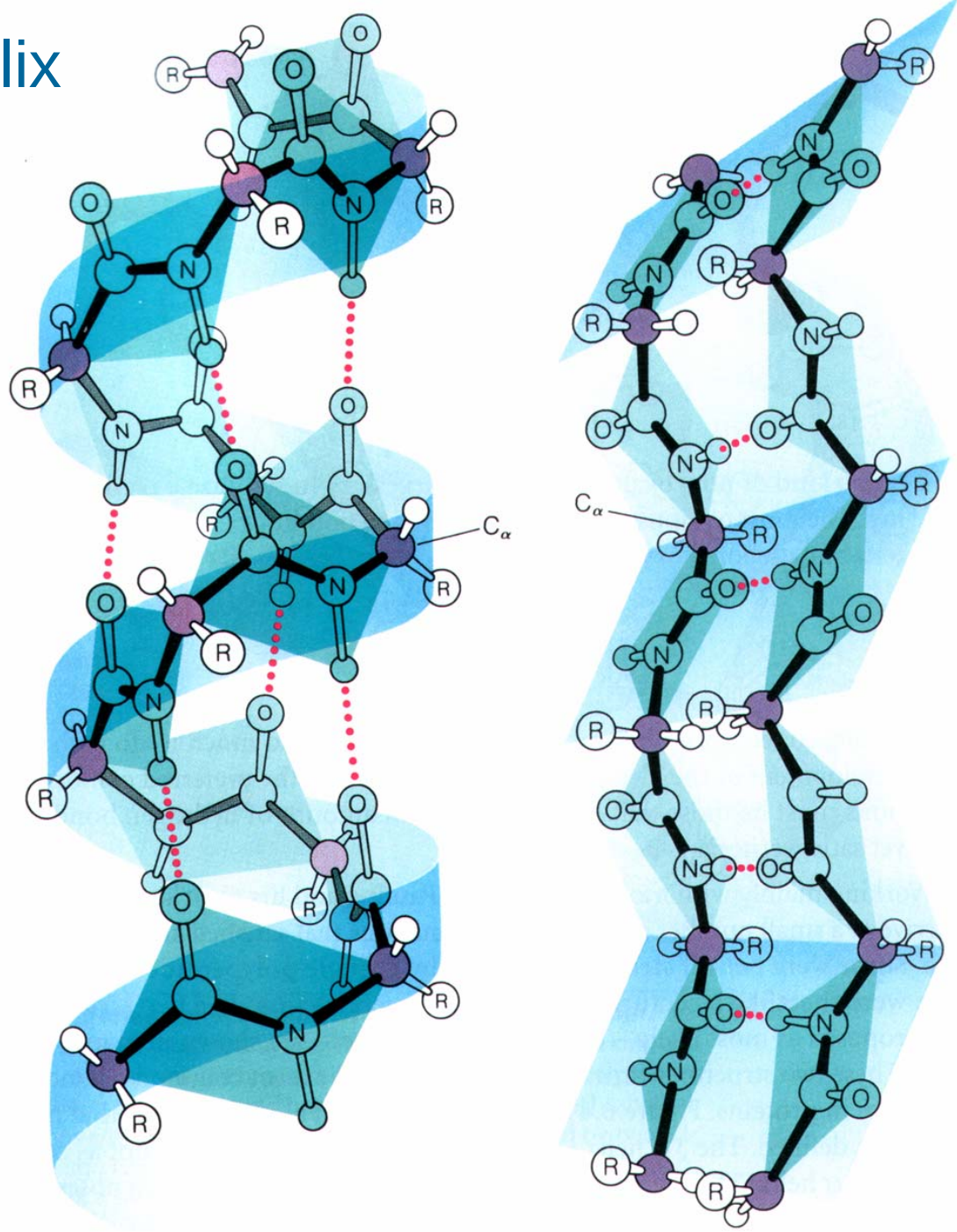
(a) Tertiary structure
(b) Quaternary structure



Hemagglutinin(流行性感冒表面蛋白質-血細胞凝集素)

α helix

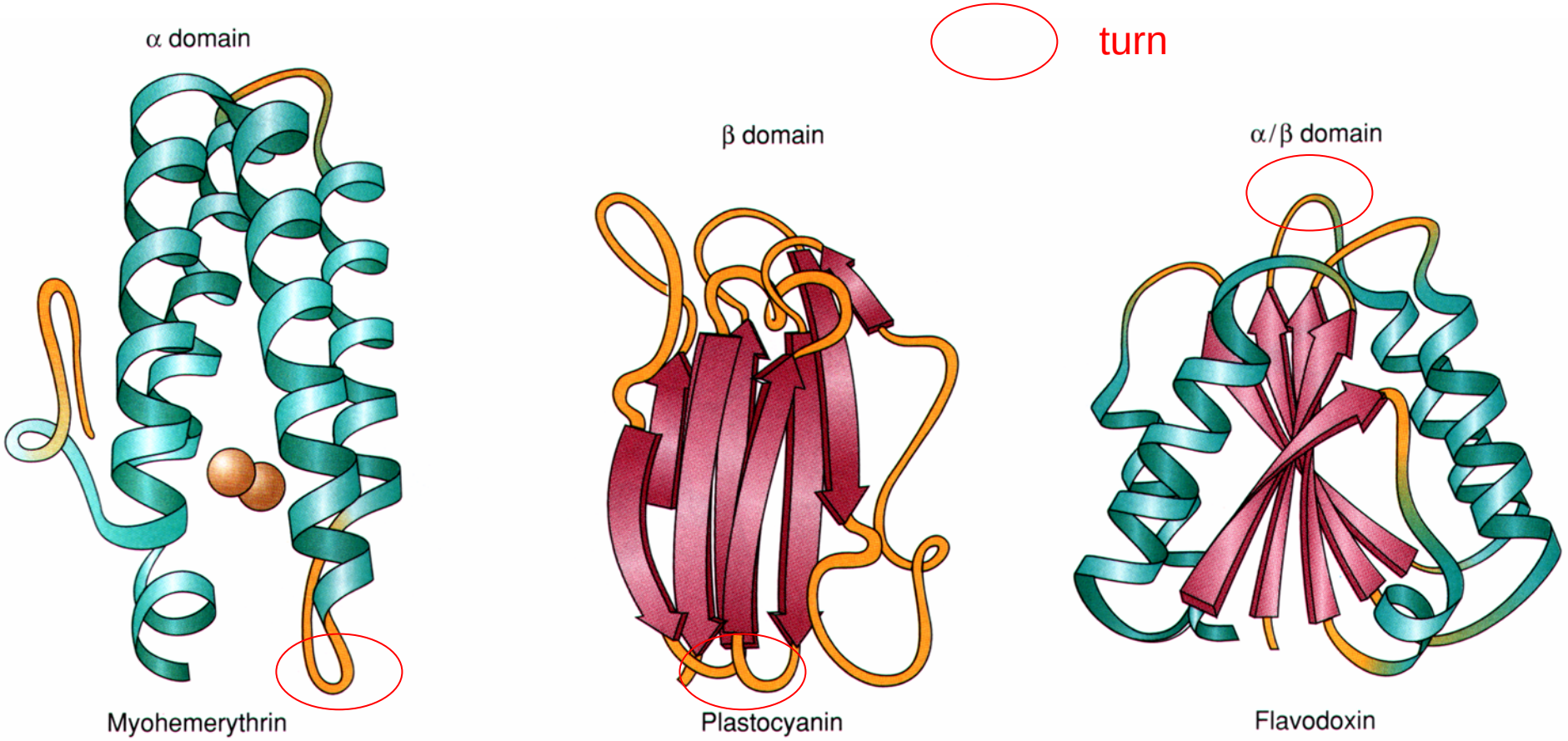
β sheet



兩者都由
H-bond組成

Mathews et al (2000) Biochemistry (3e) p.164

Secondary structure produced Tertiary structure



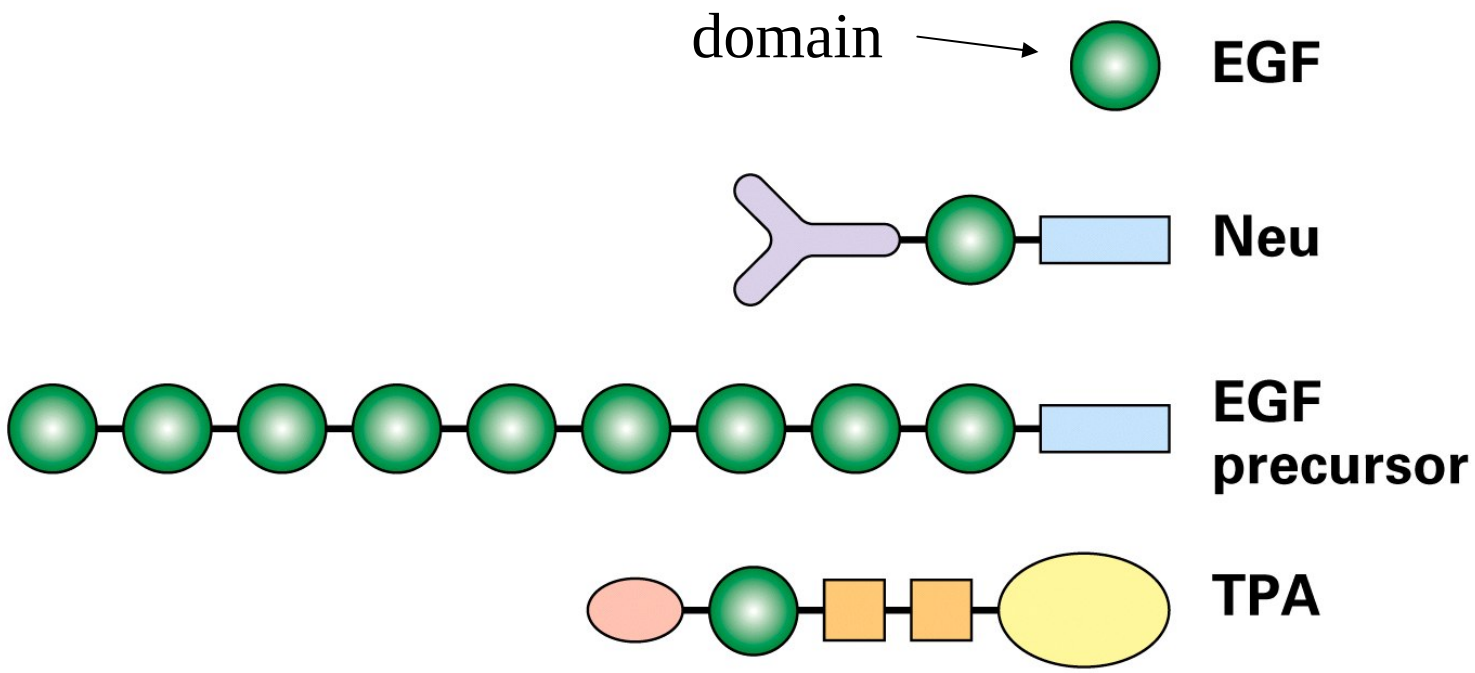
all α helices

all β sheets

helices + sheets

Kleinsmith & Kish (1995) Principles of Cell and Molecular Biology (2e) p.26

Structural and functional domains are modules of tertiary structure



Tissue plasminogen activator

Various proteins illustrating their modular nature

Epidermal growth factor (EGF) is generated by proteolytic cleavage of a precursor protein.

These proteins also contain other widely distributed domains indicated by shape and color

Quaternary structure

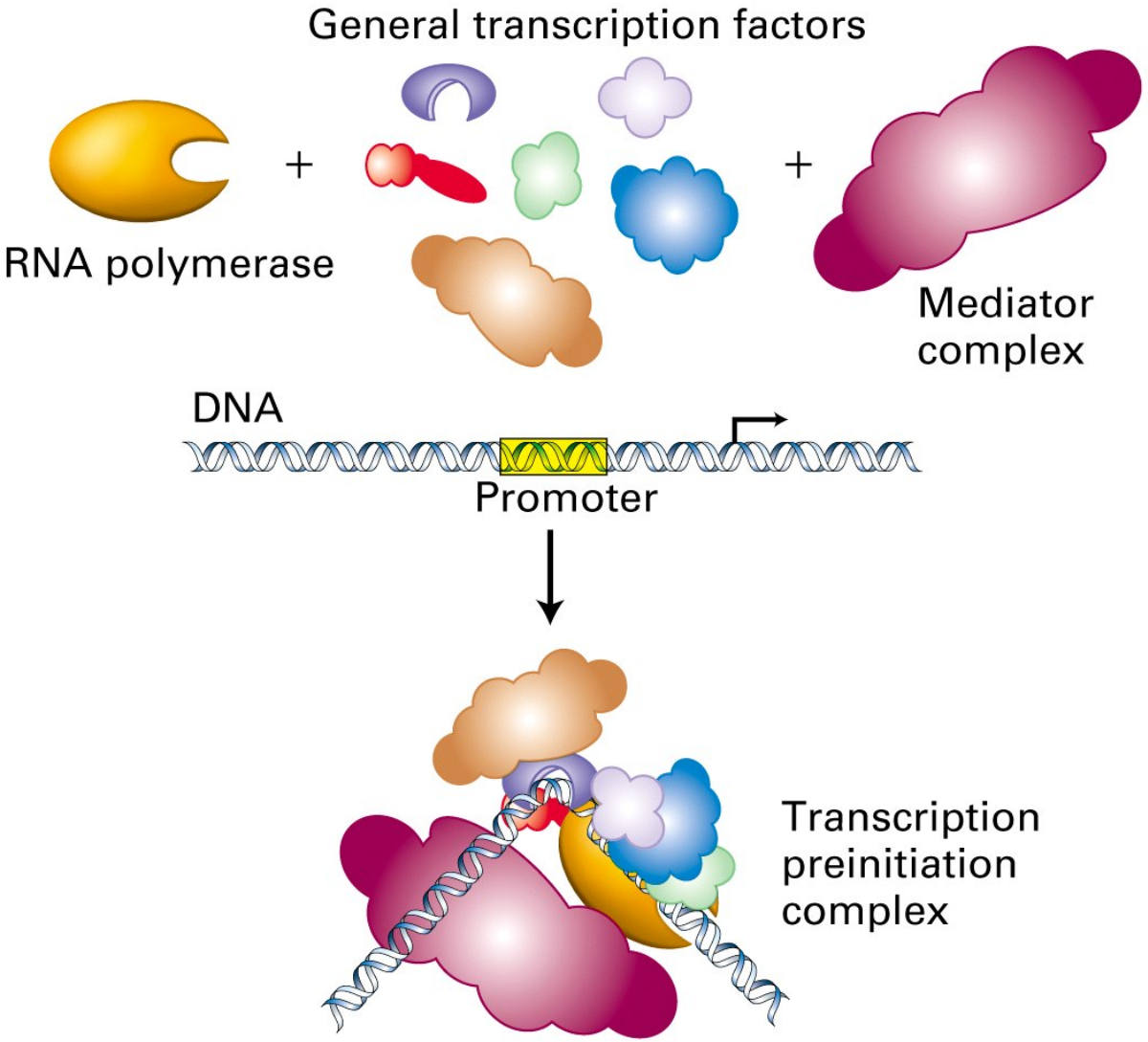
Two or more polypeptides or subunit → multimeric protein
Quaternary structure: a fourth level of structural organization; it describes the number and relative positions of subunits in multimeric protein.
The highest level of protein structure is the association of protein into macromolecular assemblies.

Folding, modification, and degradation of proteins

- A newly synthesized polypeptide chain must undergo folding and often chemical modification to generate the final protein
- All molecules of any protein species adopt a single conformation (the native state), which is the most stably folded form of the molecule

Proteins associate into multimeric structures and macromolecular assemblies

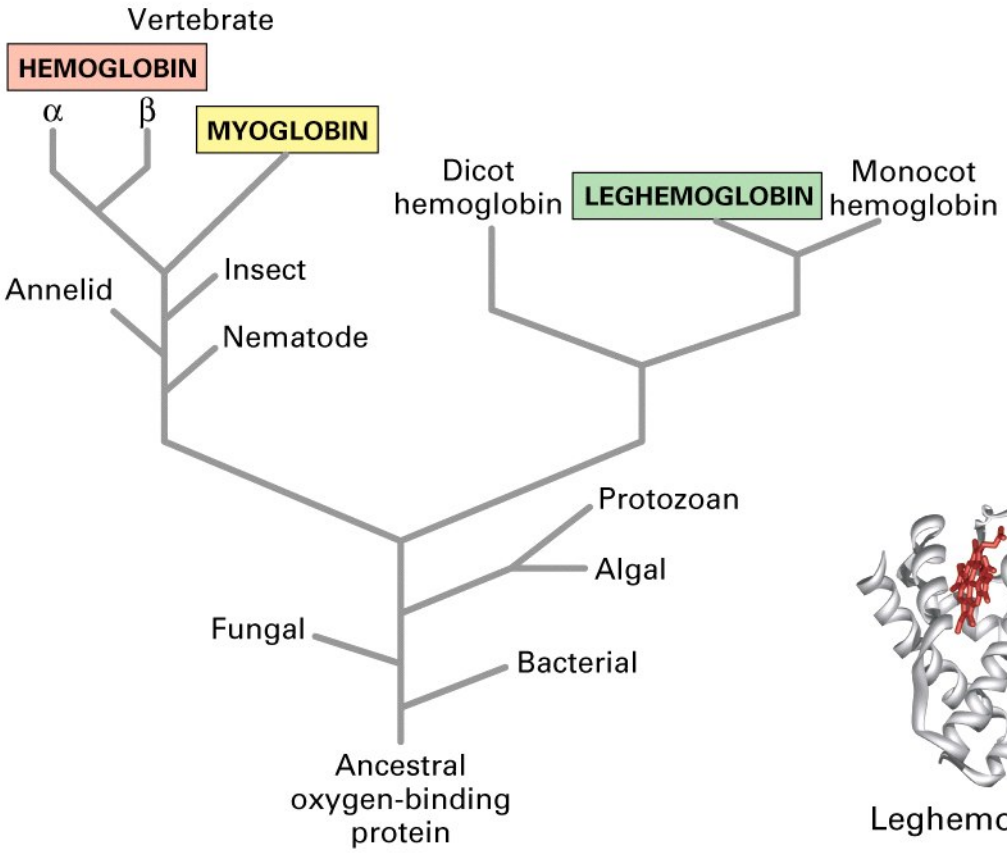
The mRNA transcription-initiation machinery

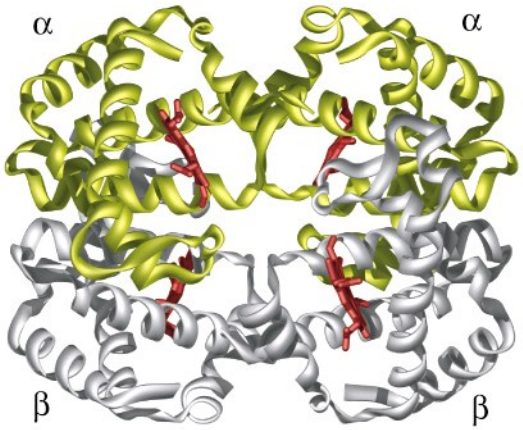


Members of protein families have a common evolutionary ancestor

Sequence homology suggests functional and evolutionary relationships between proteins

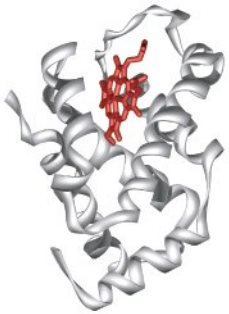
Homology: have a common ancestry are referred to as homologs. It is similarity in their sequence or structure.



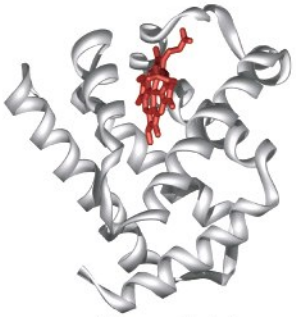


Hemoglobin

Leghemoglobin



β subunit of hemoglobin



Myoglobin

Similarity homology homologs

Different amino acid sequence ----> different conformation -→ different function

High sequence similarity about >50 % : related structure or function

Family and superfamily
Family protein about >30% amino acid sequence similarity

Folding, modification, and degradation of proteins

The information for protein folding is encoded in the sequence
Conformational folding can denature to polypeptides

Planar peptide bonds limit the shapes into which protein can fold

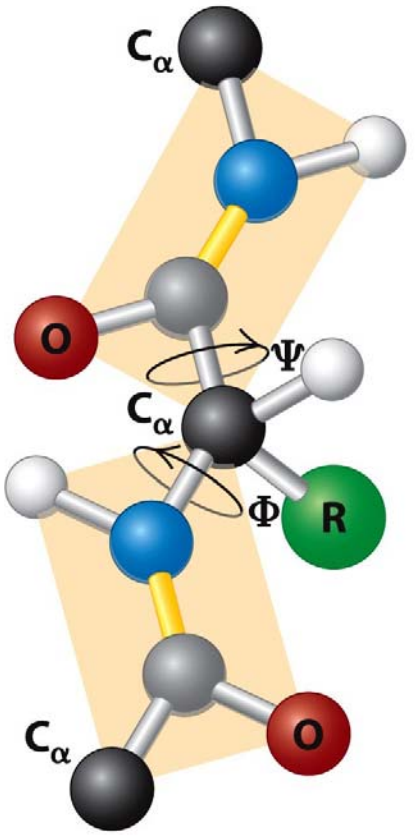


Figure 3-14
Molecular Cell Biology, Sixth Edition

Information directing a protein's folding is encoded in its amino acids sequence

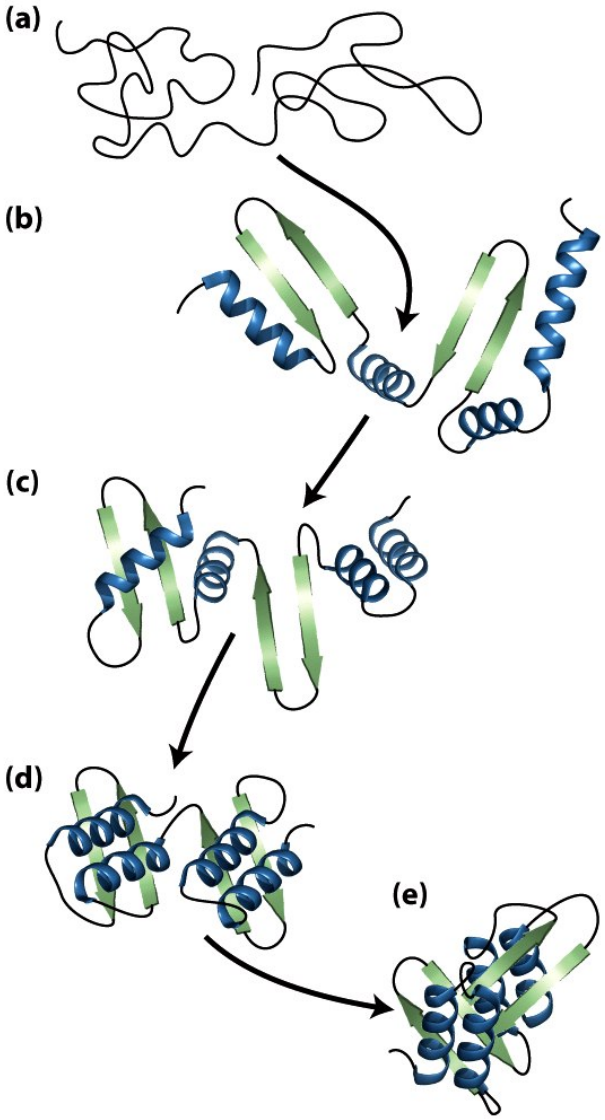


Figure 3-15
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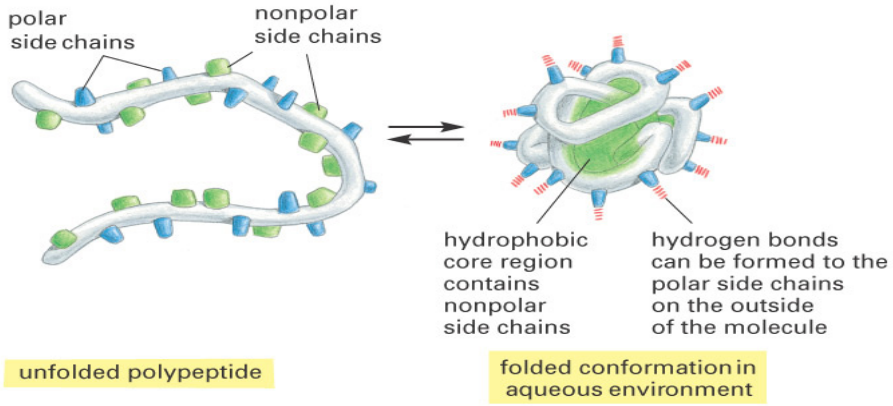

Figure 4-5 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Denature or denaturation : disrupt noncolavent interaction

Only colavent bond: disulfide bond need beta-mercaptoethanol

Folding of protein in vivo is promoted by chaperones

HSP: heat shock protein

SBD

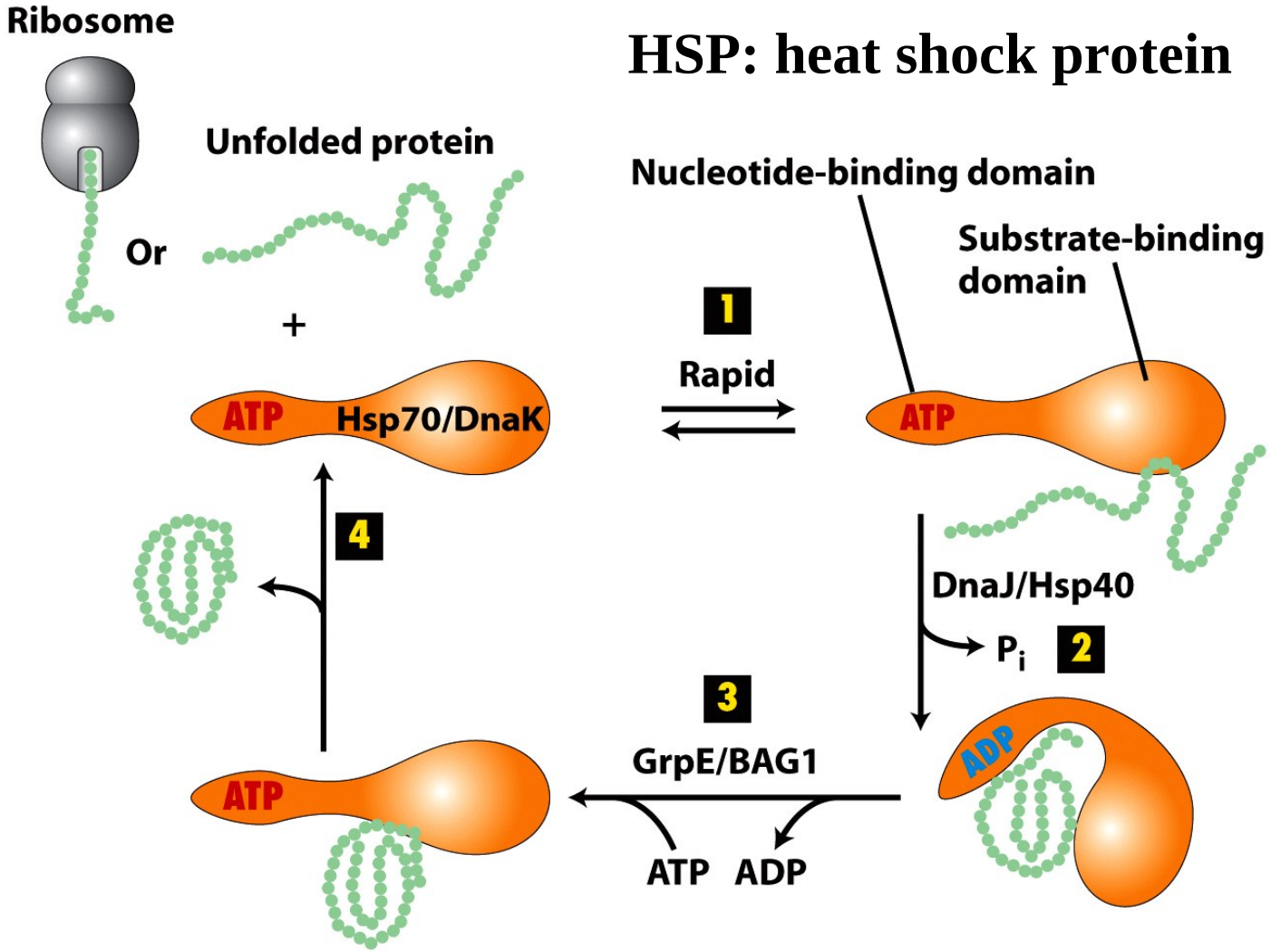

Figure 3-16
Molecular Cell Biology, Sixth Edition

Chaperone-mediate protein folding

Molecular chaperone

Members of Hsp70 family (homologs)

- DnaK (bacteria)
- Hsp70 (cytosol, mitochondrial of eukaryotic cells)
- BiP (endoplasmic reticulum)

Co-chaperone : Hsp 40/DnaJ

Protein folding from primary to final

Primary structure dictates final structure but most proteins cannot assume final conformation without help.

Chaperones provide this help.

Chaperones are necessary

Few proteins can achieve their active conformation unaided. (it must need help)

During stress proteins unfold and need to reassemble. Note that chaperones are also called heat shock proteins (Hsp).

Protein complexes may require help from a chaperone to form; other complexes may require help to be broken down.

Chaperonin mediated protein folding

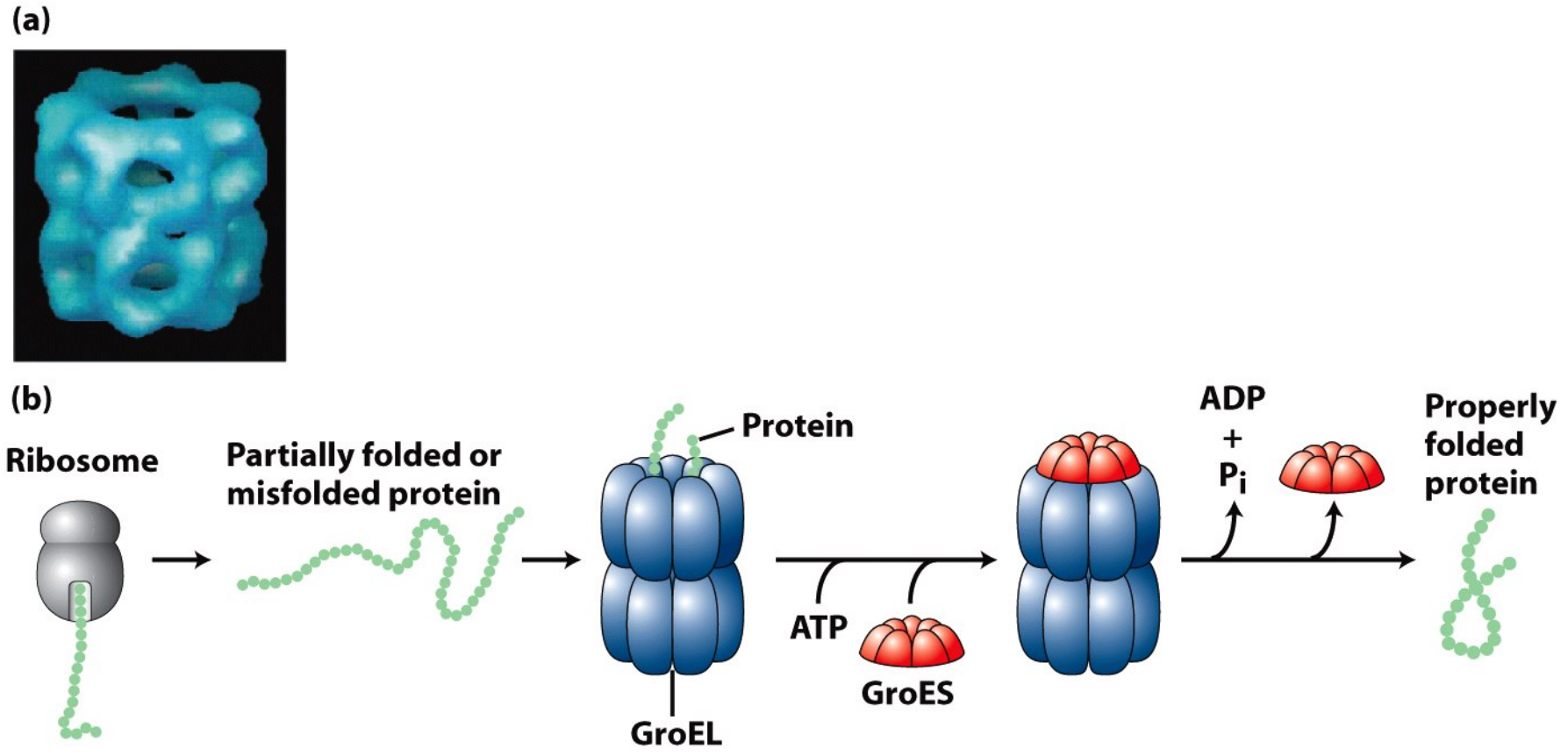

Figure 3-17
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GroEL/Hsp60 system (Chaperonin)

Constitutively expressed and increased in response to stress

One of the key chaperone systems for most cytosolic proteins

Note that it is also important in protein translocation and degradation

GroEL is chaperone (Hsp60)

GroES is regulatory protein

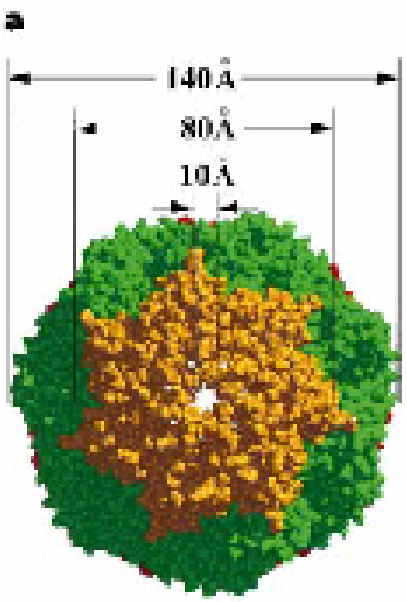
Structure

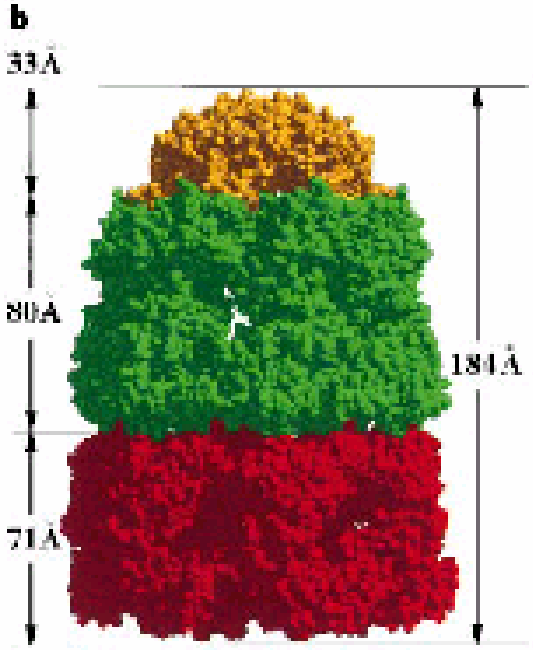
- GroEL 14 x 57 kDa (2 rings of 7)
- GroES 7 x 10 kDa

inner surface is hydrophobic interact with hydrophobic region of polypeptides

note that native (folded) proteins do not bind

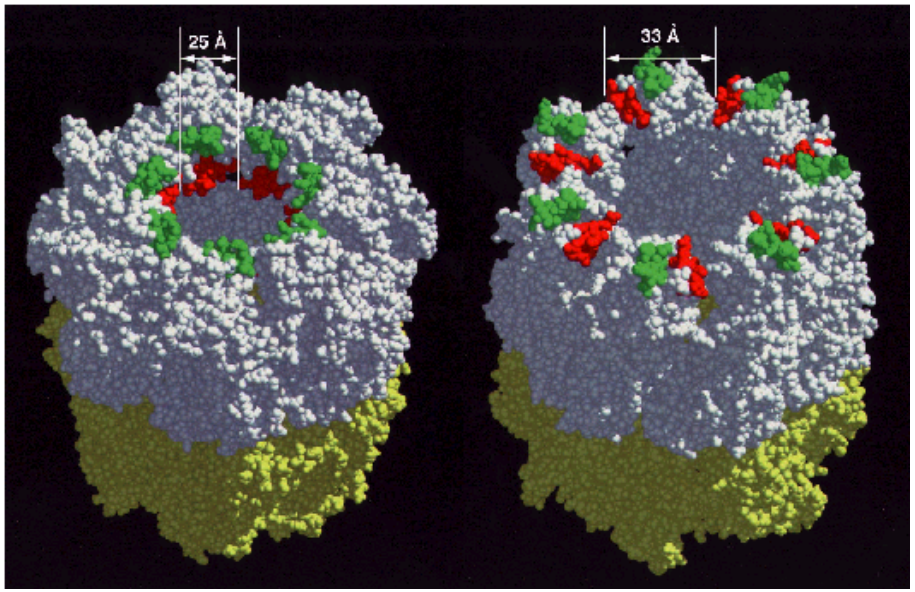
GroES binds to GroEL





GroES: ;dome-shaped; heptameric ring of 10 kDa subunits (7)

GroEL: cylindrical (圓柱形) structure ;
two heptameric rings of ~57 kDa subunits (7)



Two families of molecular chaperone for protein folding:

DnaK/DnaJ/GrpE (or hsp70) family: bind to growing polypeptide chains while they are being synthesized by ribosomes and prevent premature folding (co-translational)

Chaperonin family (GroE chaperonin): assist correct folding at a later stage (post-translational)

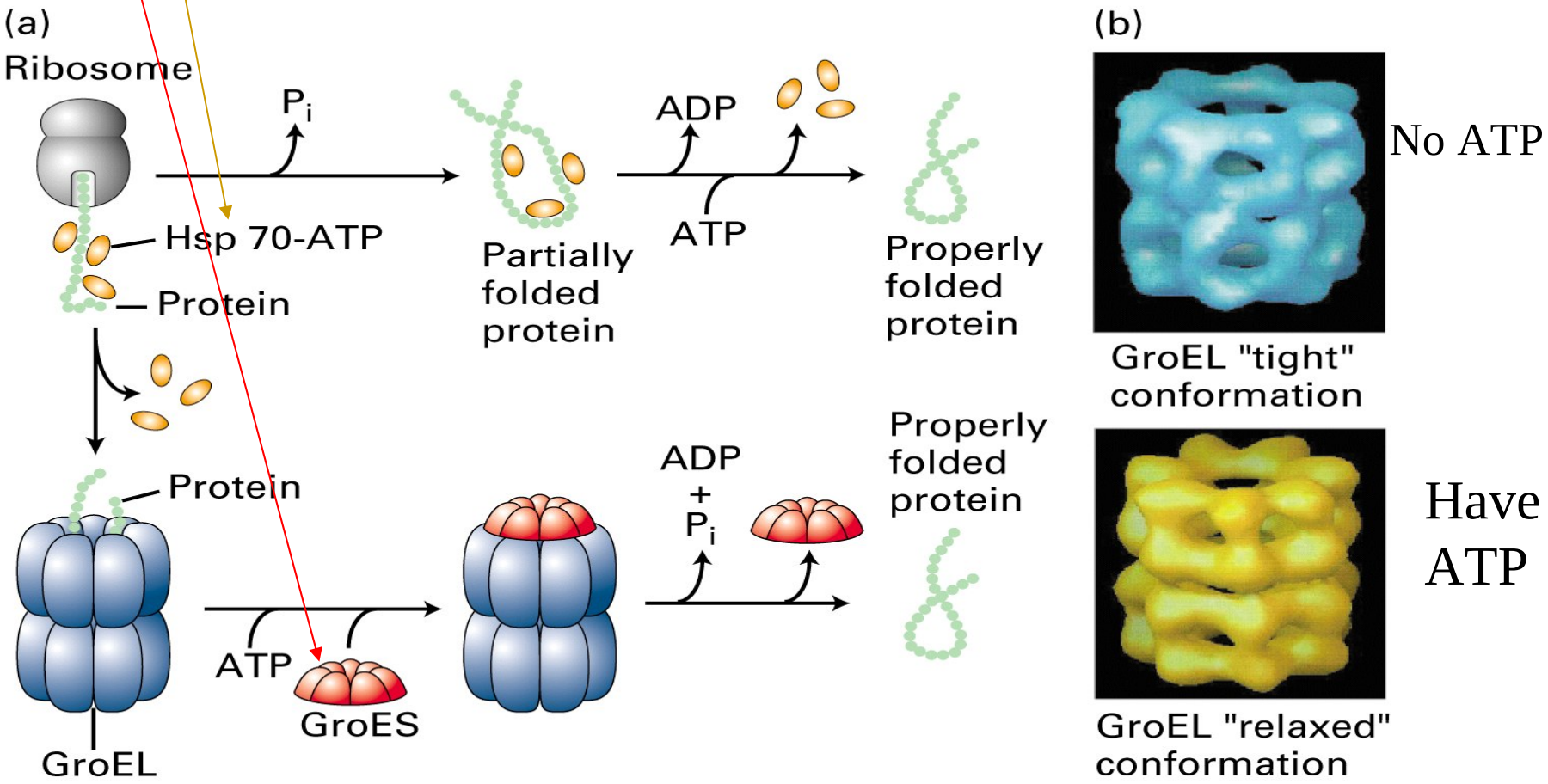
- Molecular chaperons
  - Binds to unfolded and partially folded proteins to prevent in proper association of exposed hydrophobic patches (bind to hydrophobic part)
  - Assist folding of larger multidomain proteins
  - Heat shock proteins (rate of their syntheses increases with at elevated temperature)
  - Hsp70 – monomeric 70 kDa proteins
    - Binds to newly synthesised protein peptide emerging from the ribosome
  - Hsp90 proteins – involved in the folding of proteins participating in signal transduction (steroid hormon receptors etc).

Chaperonins – large multisubunit proteins

Folding of protein in vivo is promoted by chaperones

Molecular chaperones: bind and stabilize unforlded or partly folded proteins, preventing these proteins from aggreating and being degraded

Chaperonin: directly facilitate the folding of proteins



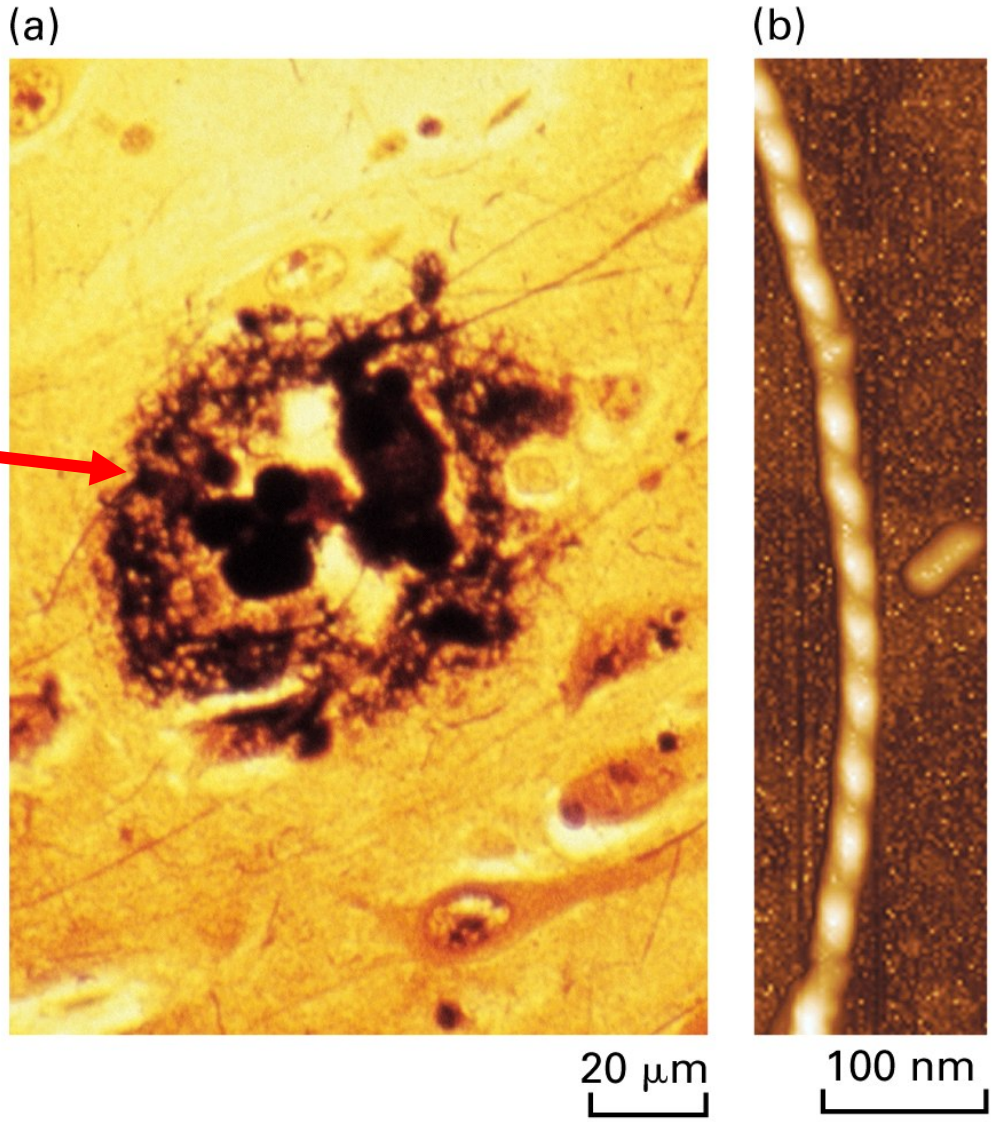
Alternatively folded proteins are implicated in slowly developing diseases

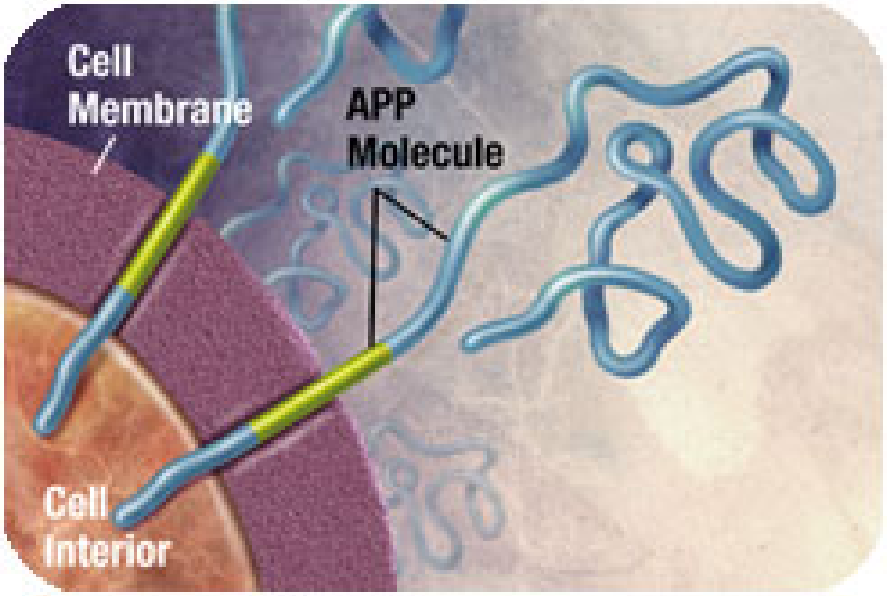
Misfolding protein

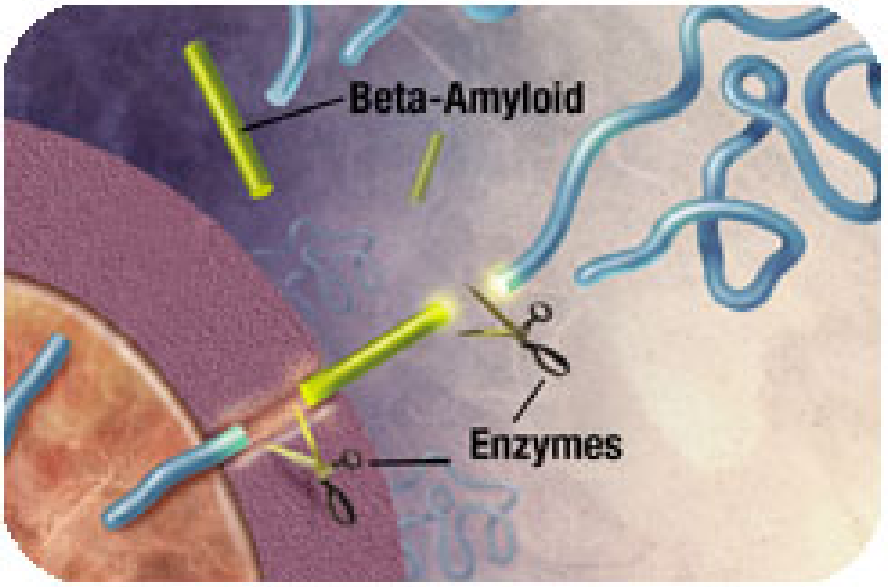
Insoluble plaques composed of amyloid protein from unknown mechanism of proteolysis of the amyloid precursor protein.

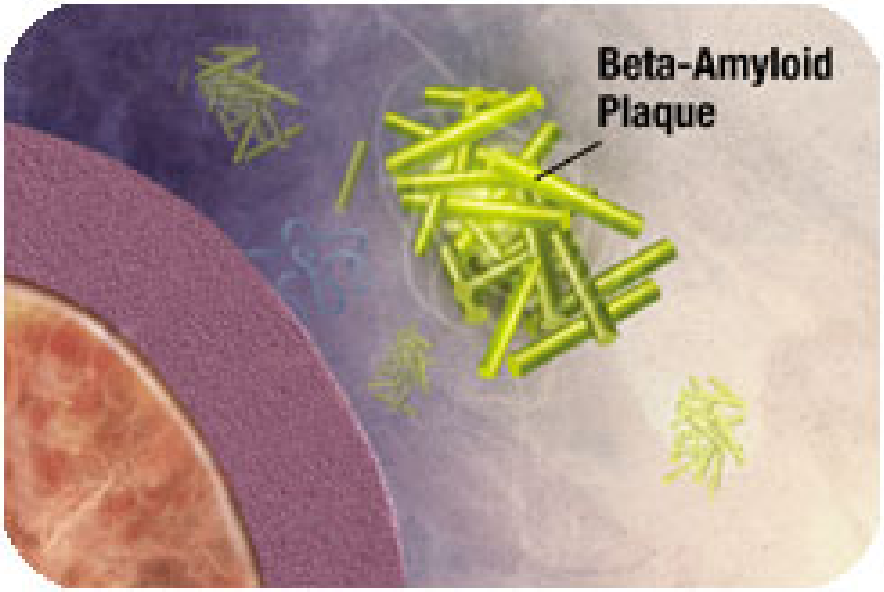
α-helix $\rightarrow$ beta sheet

Alzheimer's disease







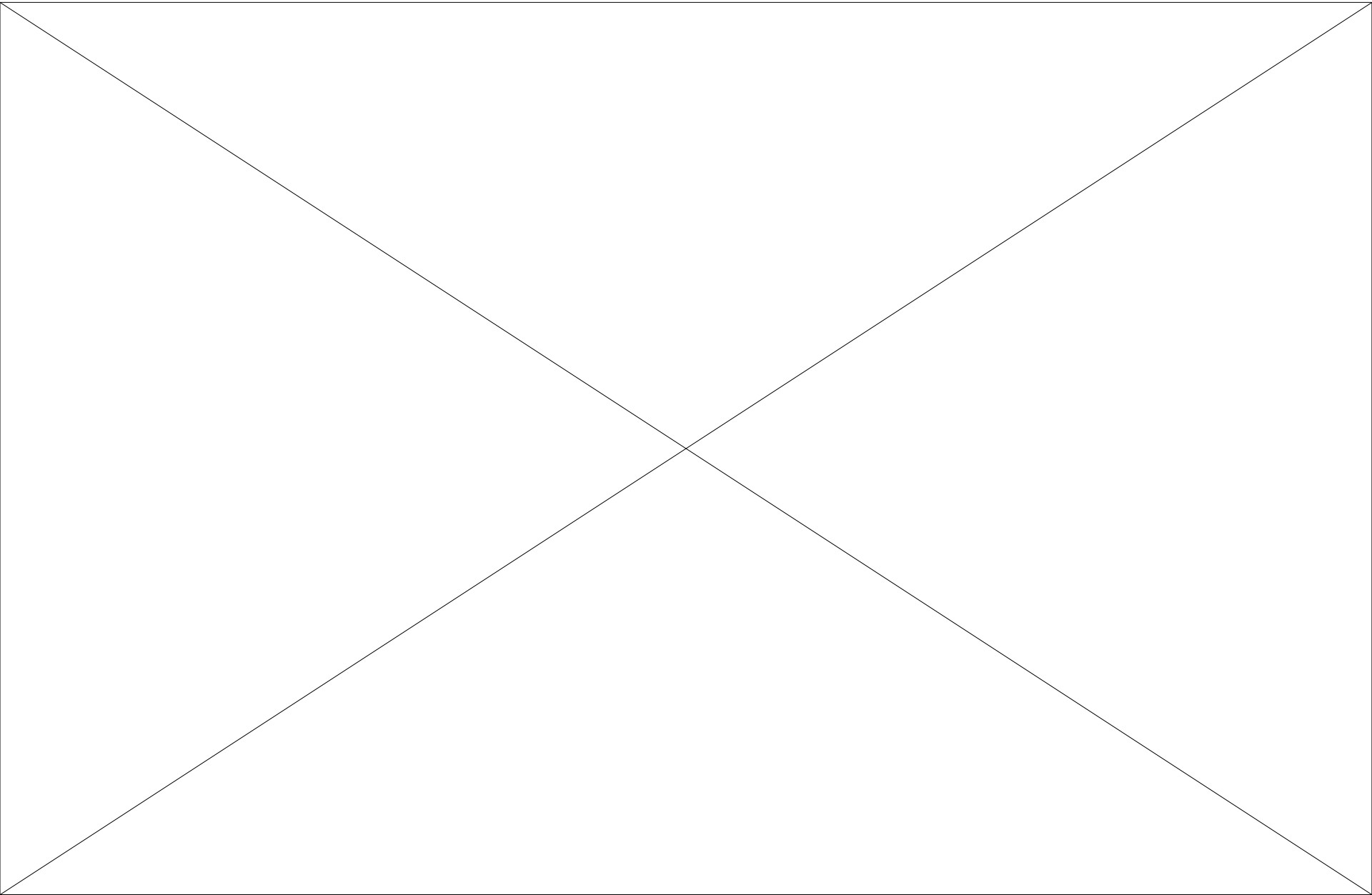


Beta-amyloid Plaques

Amyloid precursor protein (APP) is the precursor to amyloid plaque.

1. APP sticks through the neuron membrane.
2. Enzymes cut the APP into fragments of protein, including beta-amyloid.
3. Beta-amyloid fragments come together in clumps to form plaques.

Protein folding



Specific and affinity of protein-ligand binding depend on molecular complementarity

Ligand: the molecule to which a protein binds is often called it

Specificity: the ability of molecular and molecular interaction

Affinity: tightness or strength of binding

Kd: affinity usually use dissociation constant = 1/Keq

Ligand binding site: both specificity and affinity of a protein for a ligand depend on the structure

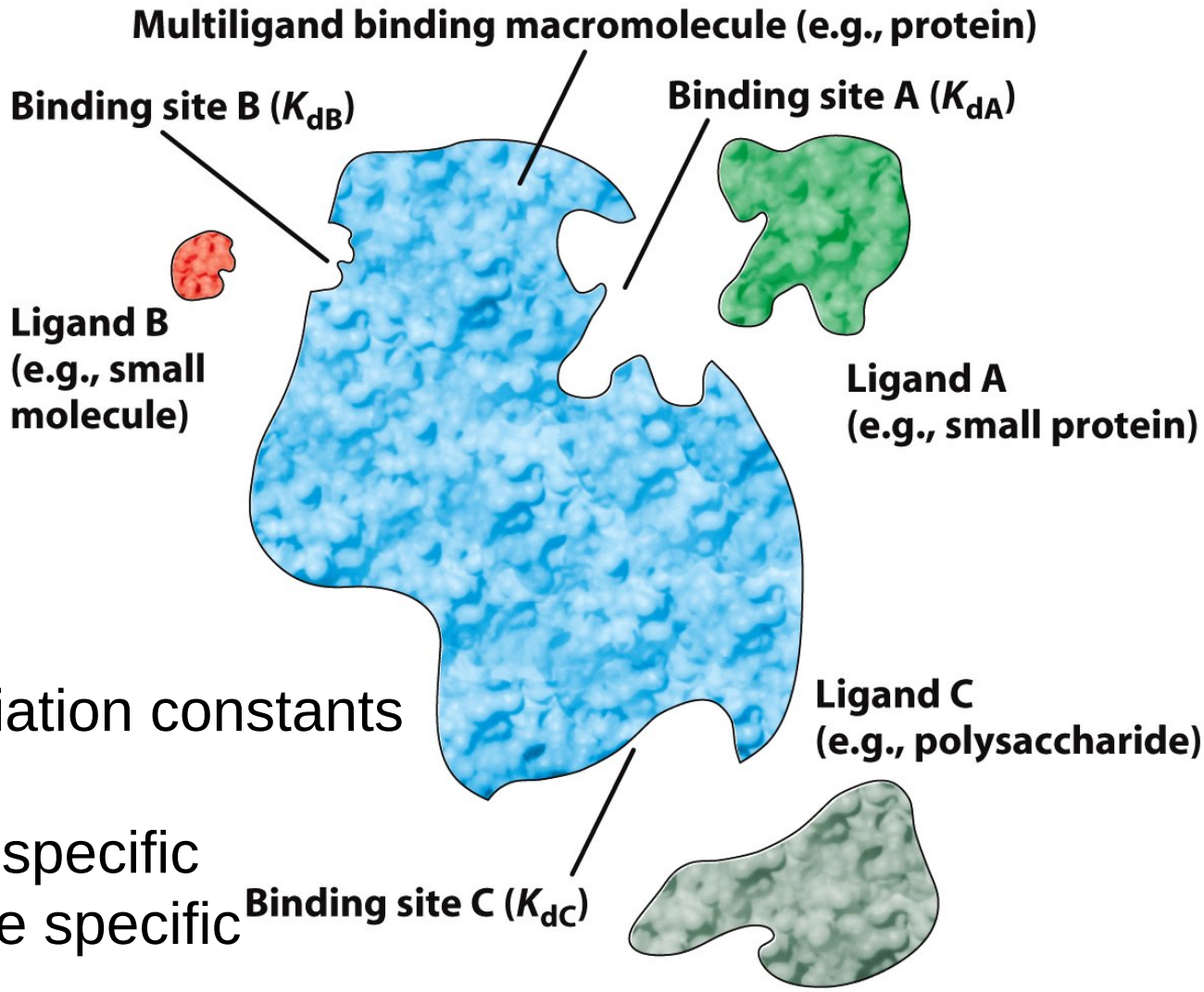
$$A + B \underset{Kr}{\overset{Kf}{\longleftrightarrow}} C$$

$$Keq = \frac{Kf}{Kr}$$

$$P + L \longleftrightarrow C$$

$$Kd = \frac{[P][L]}{[C]}$$

Dissociation constants of binding reactions reflect the affinity of interacting molecules



Kd: dissociation constants

Kd ↑ non-specific

↓ more specific

Figure 2-24
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$R + L \Leftrightarrow RL$

$$K_D = \frac{[R][L]}{[RL]} = \frac{K\ off}{K\ on}$$

Given $[R_T] = [R]+[RL]$

[RL]/RT= the fraction of receptors that have bond ligand

Derive the following equation:

$$\frac{[RL]}{R_T} = \frac{1}{1 + K_D/[L]}$$

Kd: dissociation constant of receptor-ligand complex; ↓ complex more good

RL →response

RT: total receptor number

Dissociation Constant (Kd): is the free ligand conc at which 50% of receptor is occupied.

Kd represents affinity of ligand binding to receptor (1 affinity).

Each ligand has its own specific affinity to the receptor. This can be used to define a new drug or confirm a receptor.

Specific and affinity of protein-ligand binding depend on molecular complementarity

Molecular complementarity: High affinity and specific interaction to take place, the shape and chemical surface of binding site must be complementary to ligand moleculae
Antibody
Antigen
CDR: complementarity-determining regions

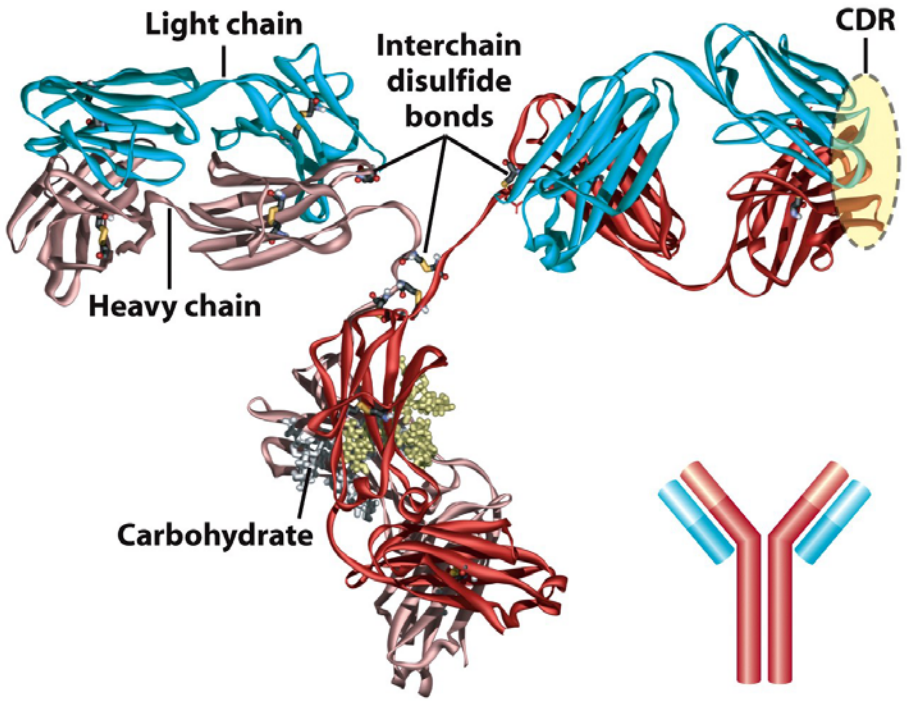

Figure 3-19a
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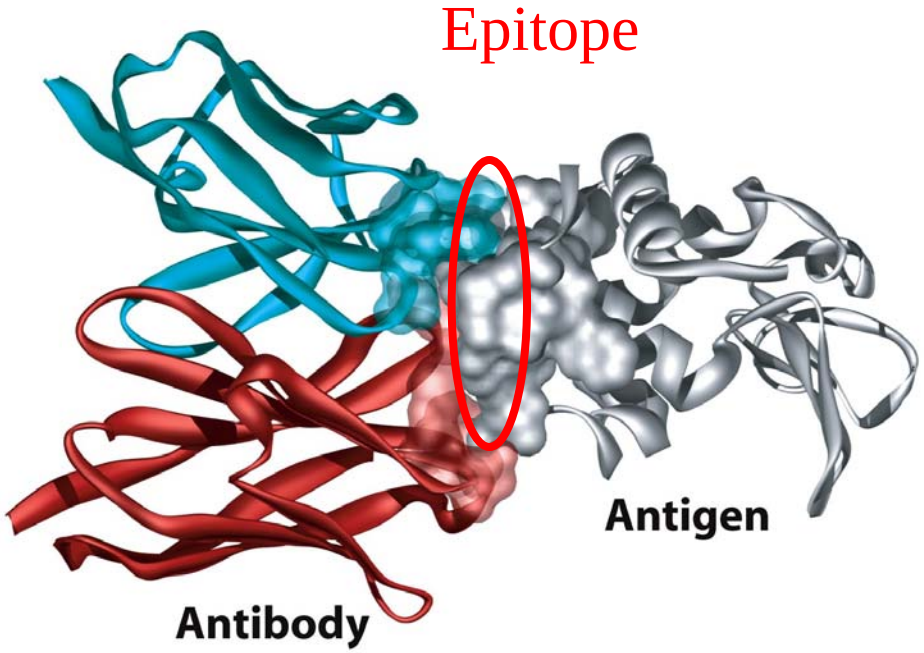

Figure 3-19b
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affinity and specificity

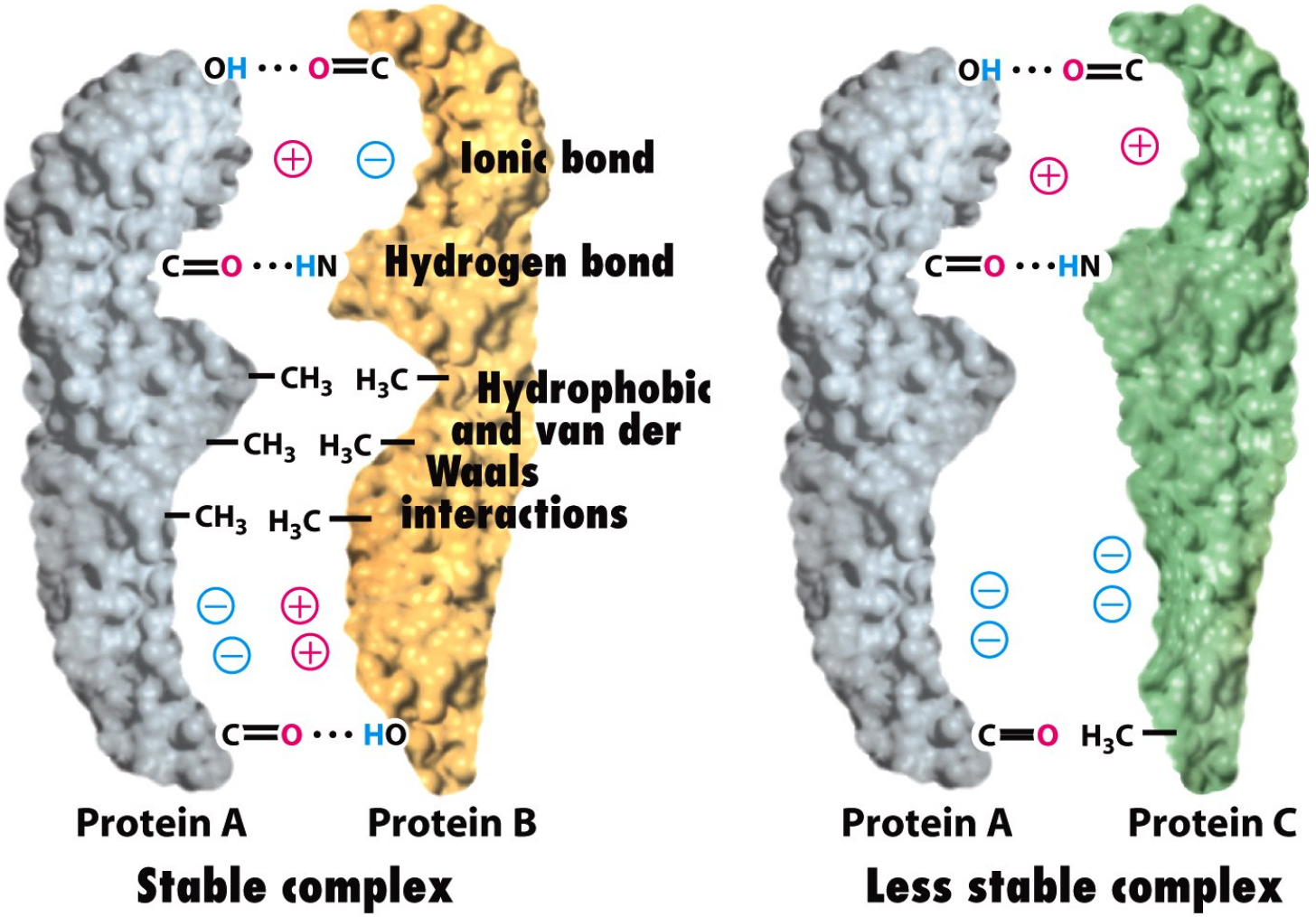

Figure 2-12
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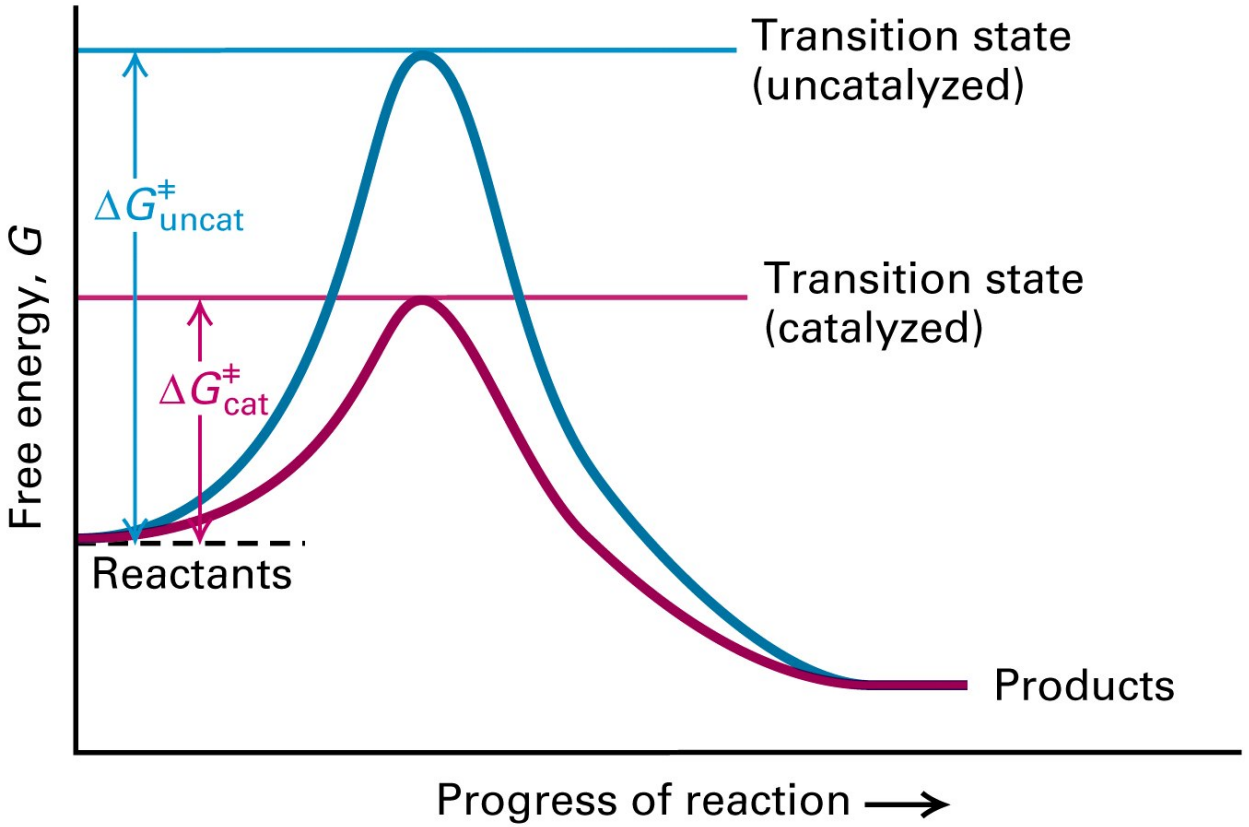
Enzyme are highly efficient and specific catalysts



A reaction will take place spontaneously only if the total G of the products is less than that of reactants.

All chemical reactions→high energy transition state→ rate of reaction is inversely to G → So need enzyme for catalysts

Active site of the enzyme trypsin

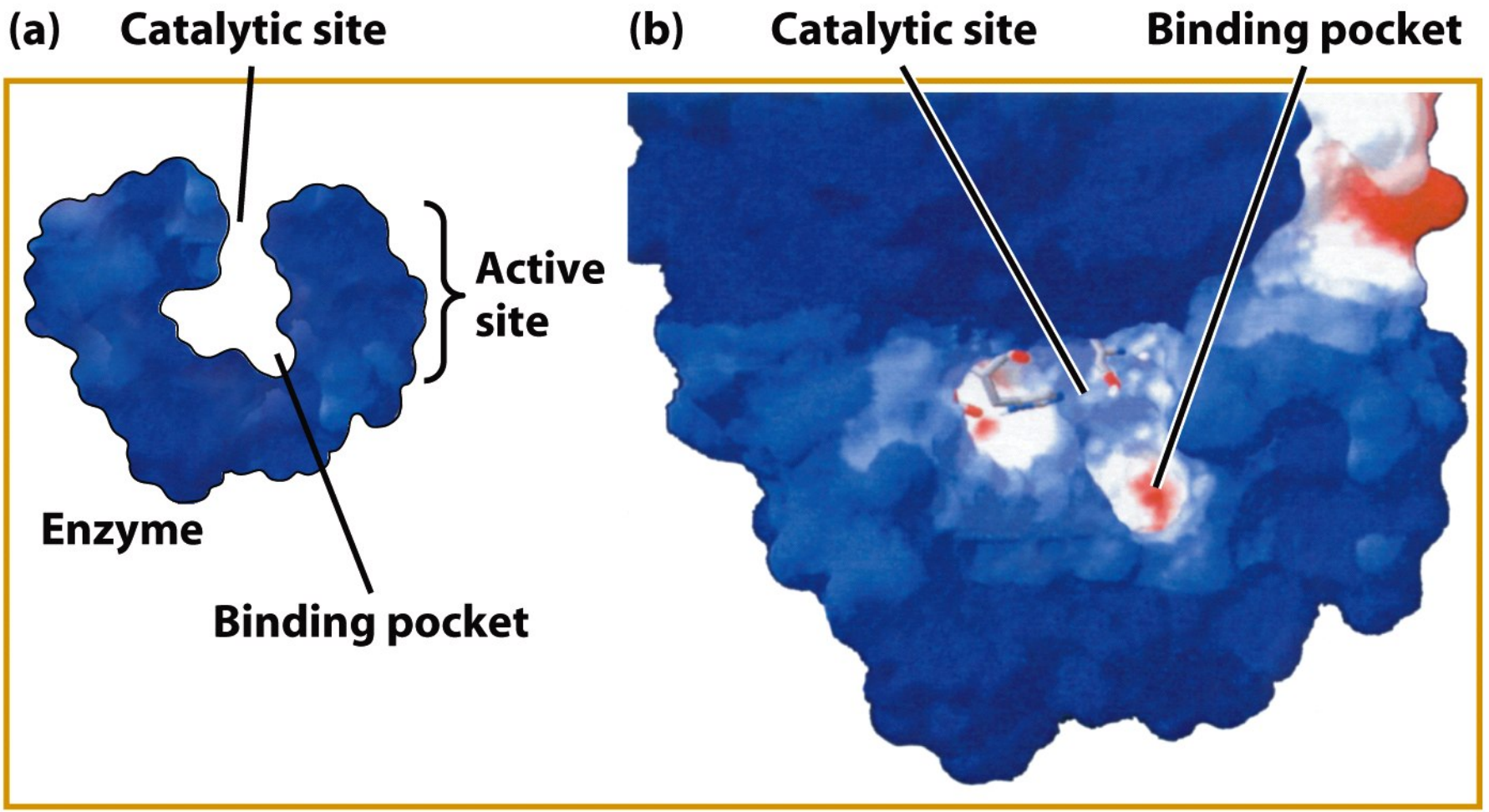


Figure 3-21
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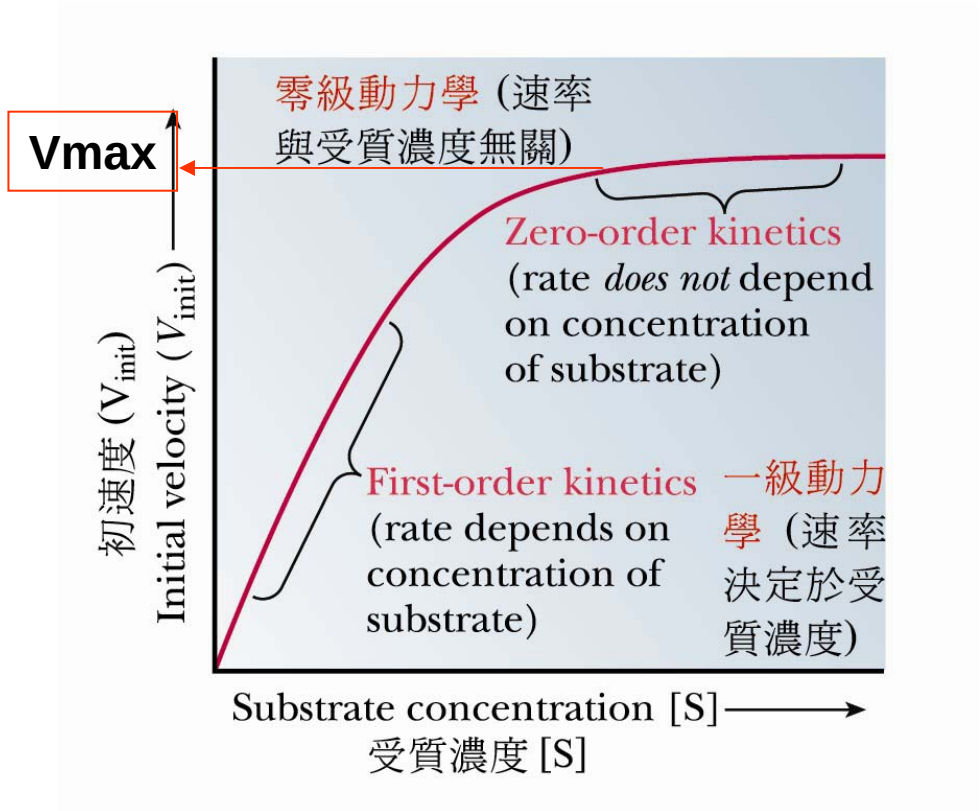
Highly efficient and specific catalysts
An enzyme active site binds substrates and carries out catalysis
Active site: specific and chemical reaction site

Michaelis-Menten equation:



在足夠的基質濃度下，一定量的酵素所能催化的最高反應速率，即爲其 Vmax 。
要讓一個酵素達致其 Vmax，就要把基質量調至最高濃度。

$$V = \frac{V_{max}[S]}{K_M + [S]}$$

若酵素的 *Km* 越低，則表示它要接近 Vmax 所需的基質濃度越低。

若某一酵素有數種基質，各有不同的 *Km*，則 ***Km*** 越低的基質，表示它與酵素的親和力越大，催化反應愈容易進行。

Km 與 [S] 一樣是濃度單位 (mM 或 mM)。

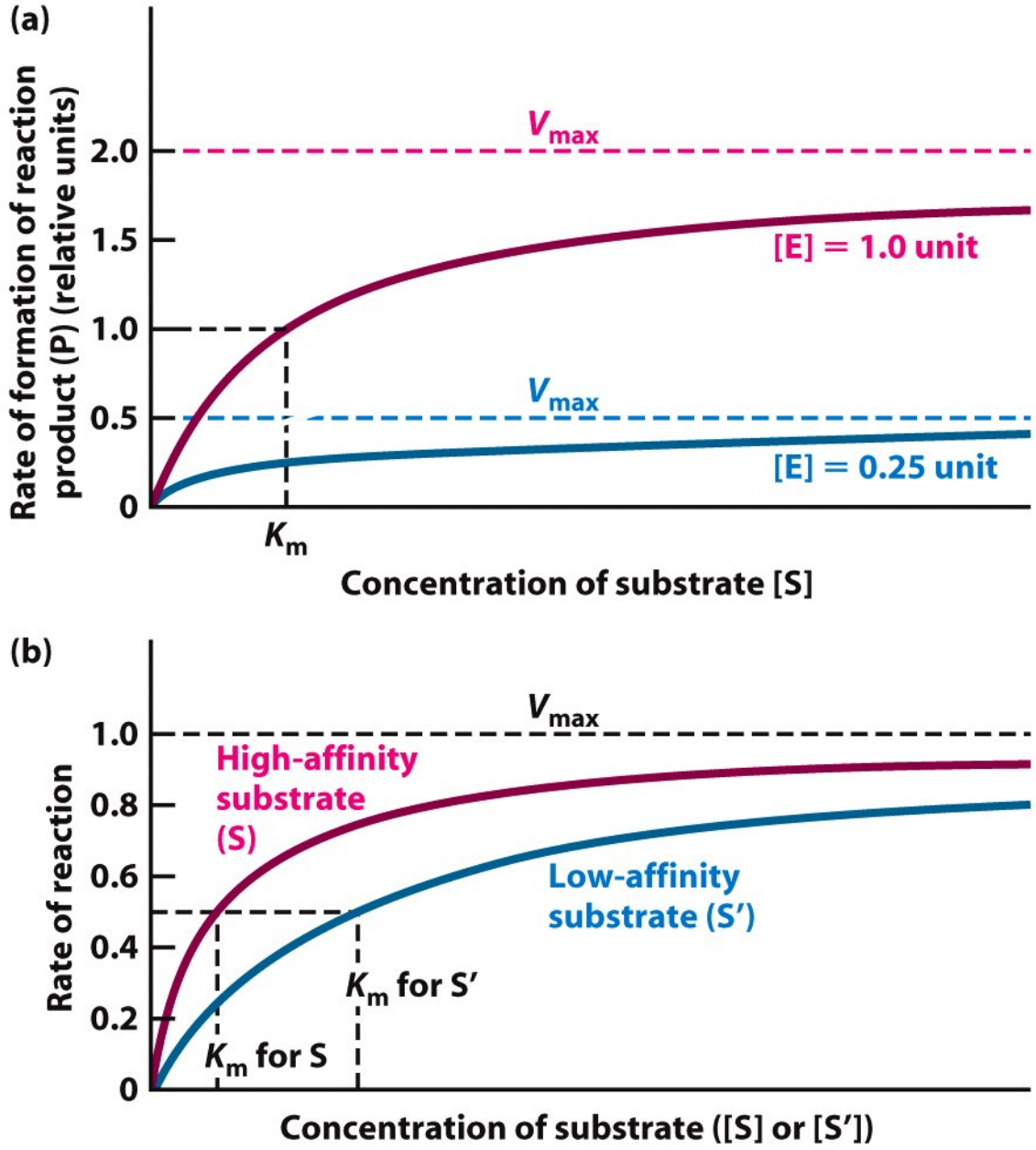


Figure 3-22
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Enzyme can enhance reaction

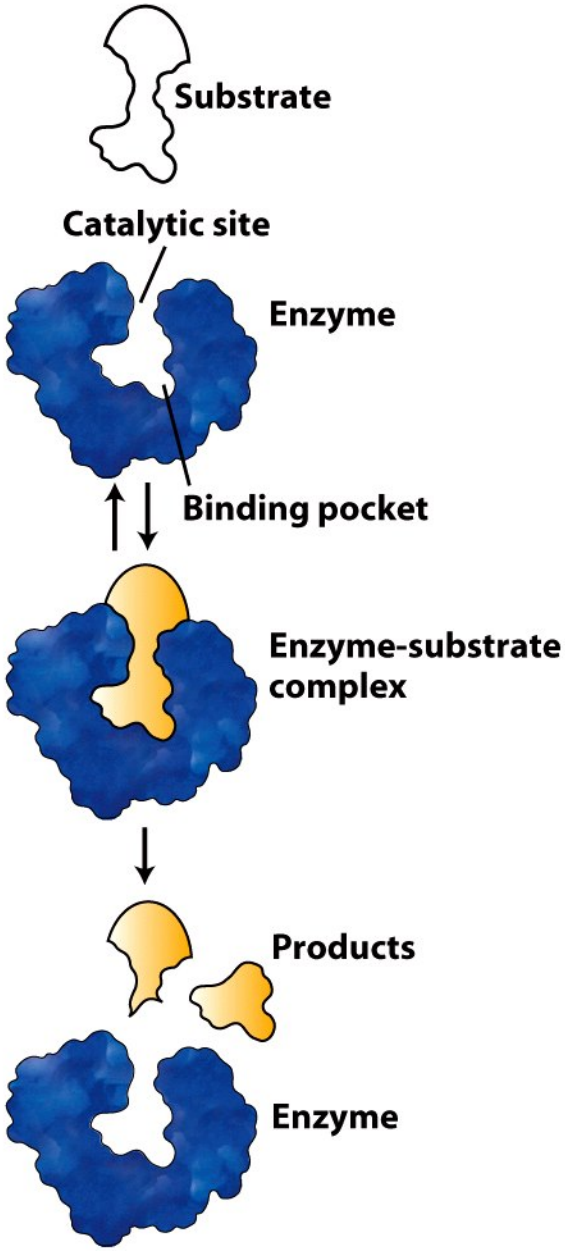

Figure 3-23
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No enzyme

E: enzyme

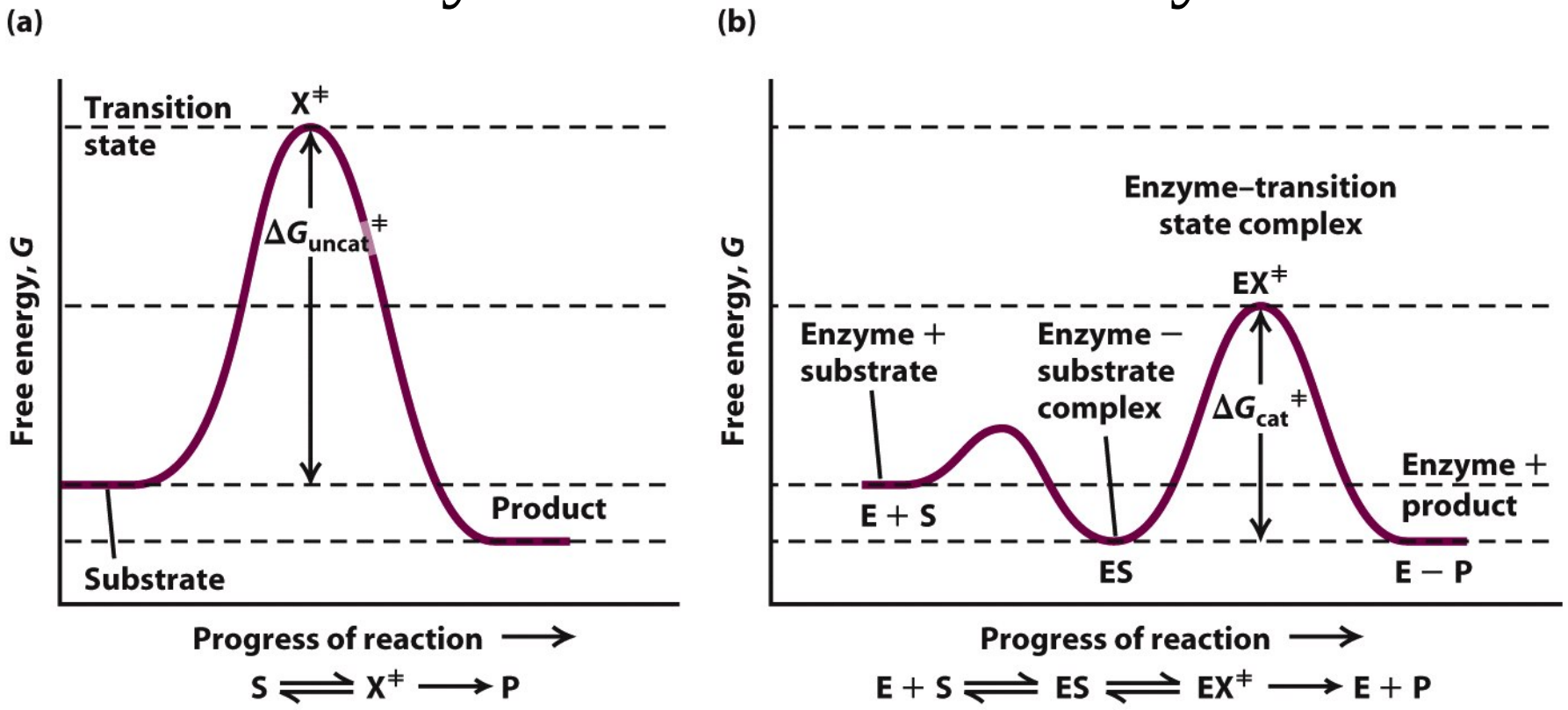

Figure 3-24
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Substrate binding in the active site of protease

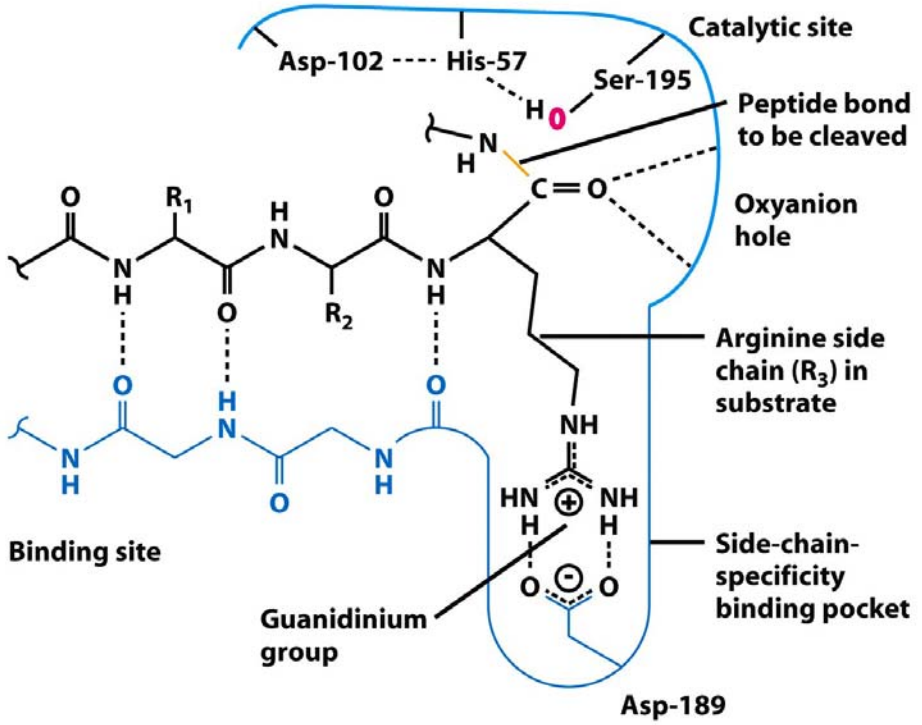

Figure 3-25a
Molecular Cell Biology, Sixth Edition

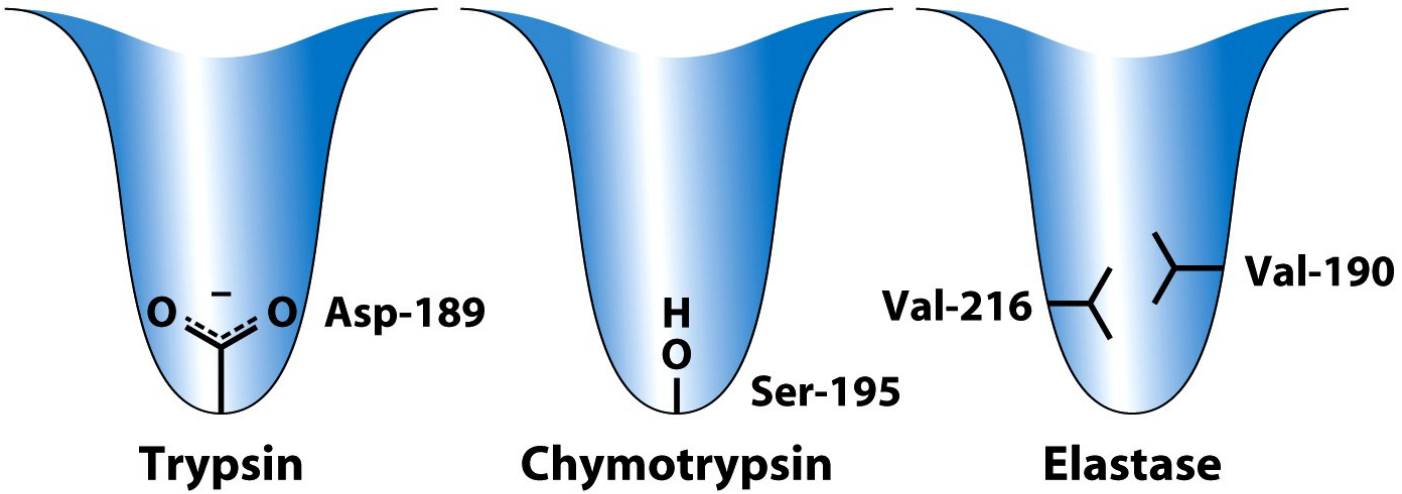

Figure 3-25b
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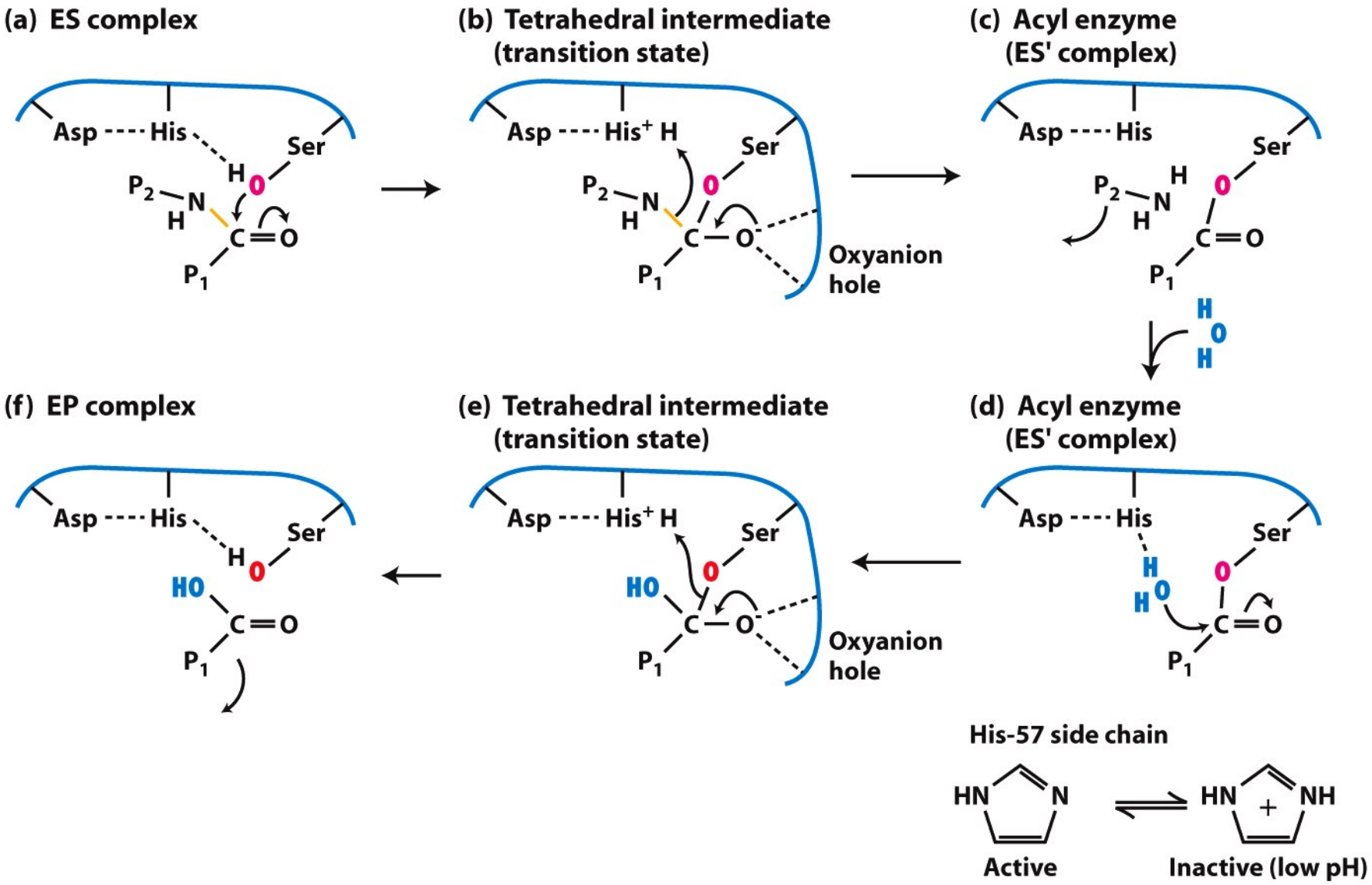

Figure 3-26
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pH dependence of enzyme activity

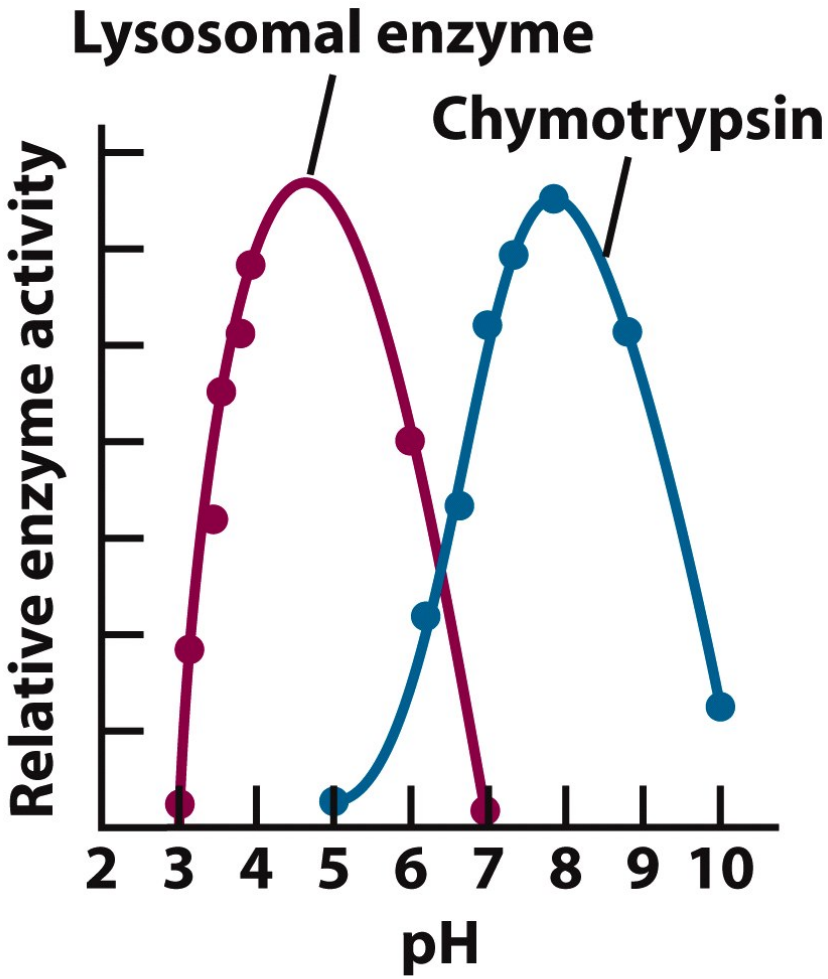

Figure 3-27
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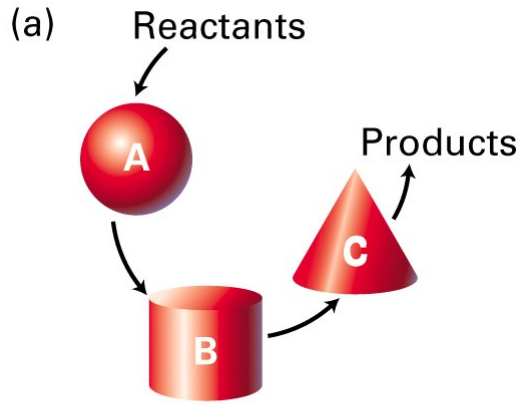
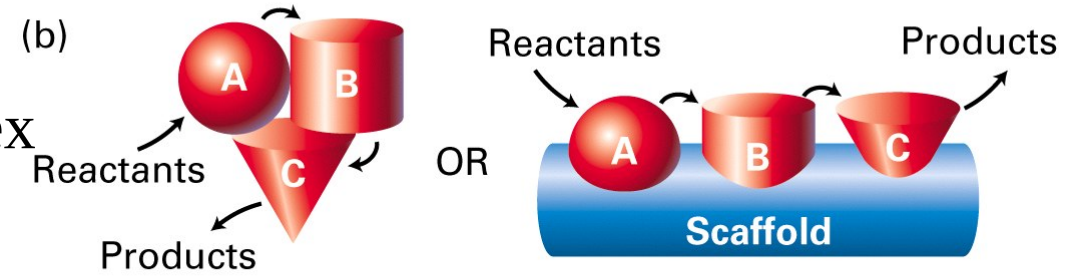
Enzyme inhibitor

Enzymes in a common pathway are often physically associated with one another

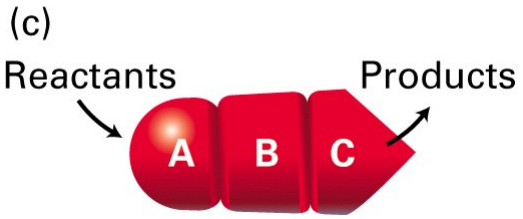


Diffusion → very slow



Complex subunit

Association



Integration of different catalytic activities in a single protein

Evolution of multifunctional enzyme

Enzyme called molecular motors convert energy into motion

Molecular motors (motor protein): generate the forces necessary for many cellular movements, cells depend on specialized enzymes.

Mechanochemical enzyme

Regulation protein function I: PROTEIN DEGRADATION

Synthesis
degradation

Regulation protein fuction II: Noncovalent and covalent modification

Ubiquitin marks cytosolic proteins for degradation in proteasomes

Degradation of protein

1. Lysosome: primarily toward ***extracellular*** protein and aged or defective organelles of the cells. *Membrane organelles.*
2. Proteasomes: **Ubiquitin dependent**; for intracellular unfolding, aged protein.
   1. control native cytosolic protein
   2. misfolded in the course of their synthesis in the ER

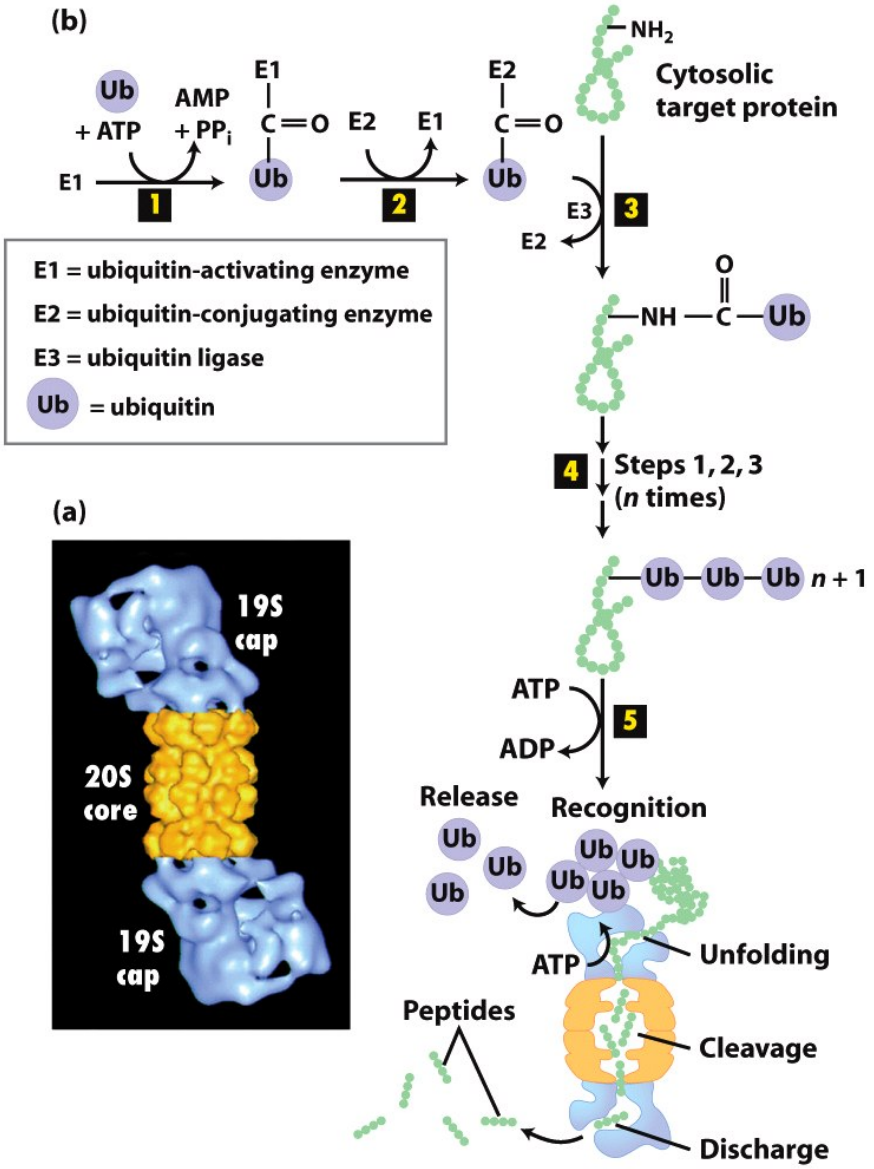


Figure 3-29
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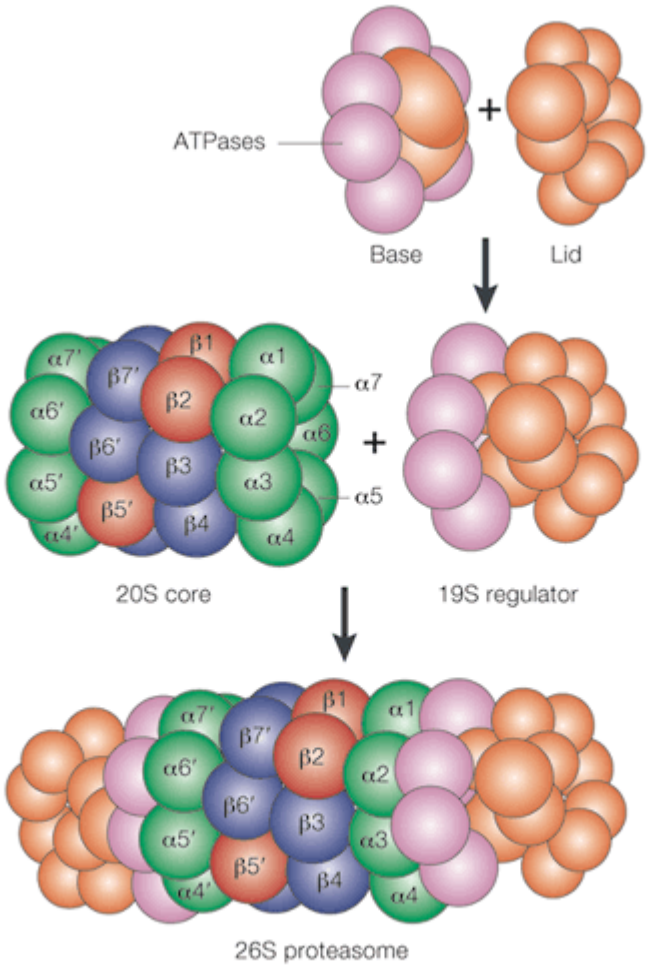
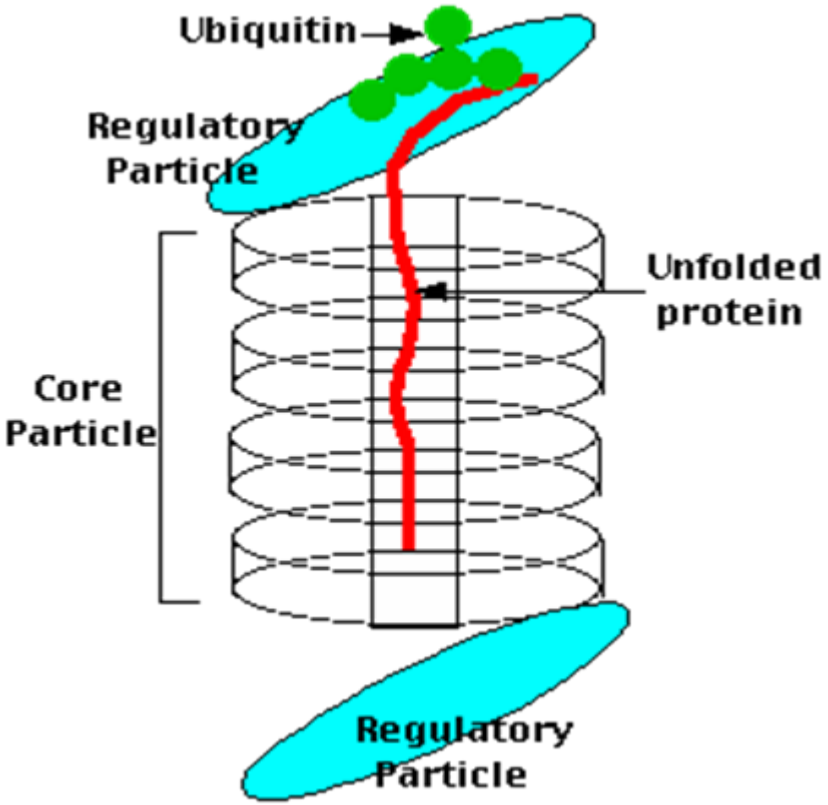
THE PROTEASOME





Nature Reviews | Molecular Cell Biology

UBIQUITIN

76 Amino Acid polypeptide

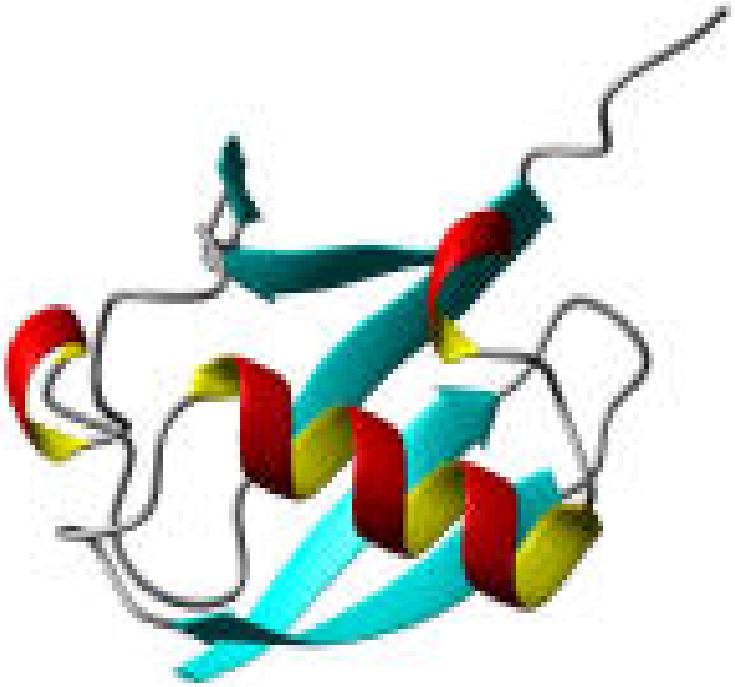
3 Amino acid differences between yeast and human homologues

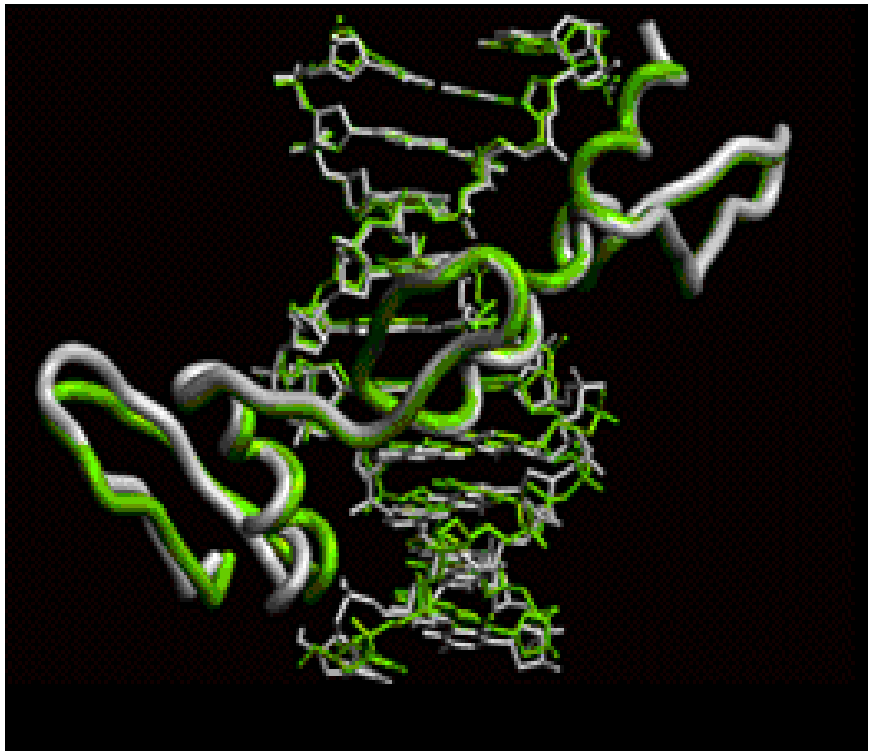
C-Terminal Gly residue is activated via an ATP to form a thiol ester

Found only in eukaryotic organisms and is not found in either eubacteria or archaebacteria.

Among eukaryotes, ubiquitin is highly conserved, meaning that the amino acid sequence does not differ much when very different organisms are compared.

Ub is a heat-stable protein that folds up into a compact globular structure.





Degradation of a Protein Via the Ubiquitin-Proteasome Involves Two Successive Steps

1. Covalent attachment of multiple ubiquitin molecules to a protein substrate.

2. Degradation of the tagged protein by the 26s proteasome.
(ubiquitin is recycled)

Ubiquitination: In general, multiple ubiquitin units are arranged in polyubiquitin chains linked via Lys_{48} of ubiquitin, targeting the protein for degradation

Ubiquitin Conjugation: A 3 Step Mechanism

Ubiquitin (Ub) activating enzyme E_1	High energy thiol ester is formed between C-terminal Gly of ubiqutin and a Cys in the E_1 active site (ATP/AMP)
Ubiquitin conjugating enzymes E_2	Ub is transferred to a Cys of E_2 forming a new thiol ester
Ubiquitin ligase E_3	Ub forms isopeptide bond between C-terminal Gly of Ub and ε -amino group of Lys on a target protein

Functional design of proteins

Protein function generally involves conformational changes

Proteins are designed to bind **a range of molecules** (ligands)

- Binding is characterized by two properties: **affinity** and **specificity**

Antibodies exhibit precise ligand-binding specificity

Enzymes are highly efficient and specific catalysts

- An enzyme's active site binds substrates and carries out catalysis

affinity and specificity

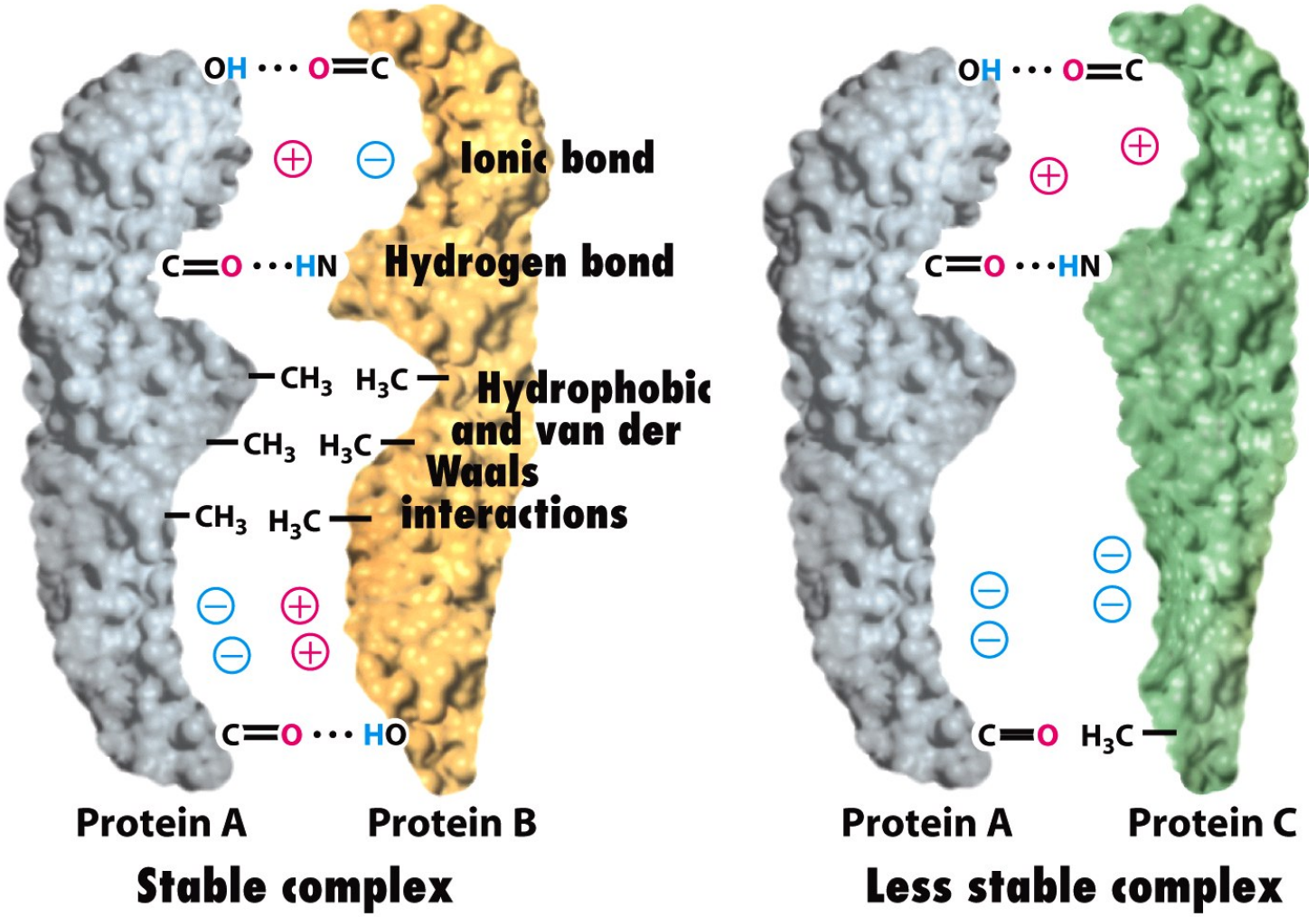


Figure 2-12
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Regulation protein fuction II: Noncovalent and covalent modification

Mechanisms that regulate protein function

- Allosteric transitions
  - Release of catalytic subunits, active ⌦ inactive states, cooperative binding of ligands
- Phosphorylation ⌦ dephosphorylation
- Proteolytic activation
- Compartmentalization

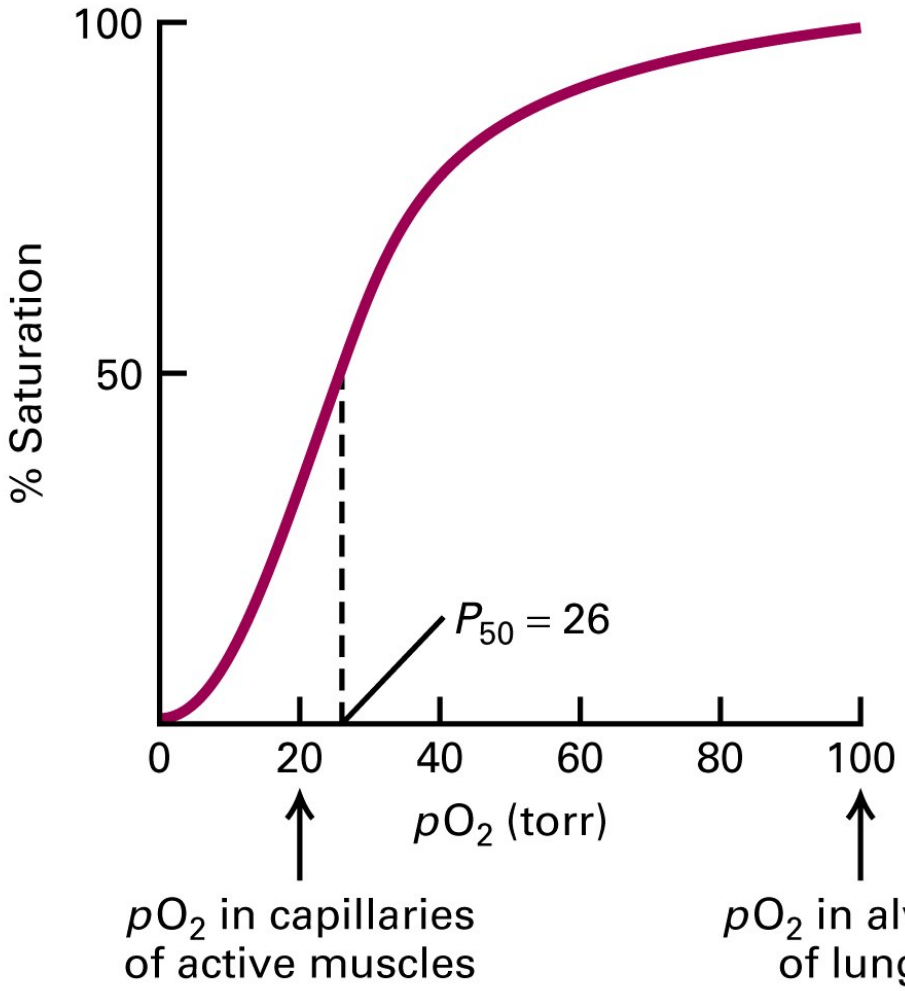
Noncovalent binding permit allosteric, or cooperative, regulation of protein

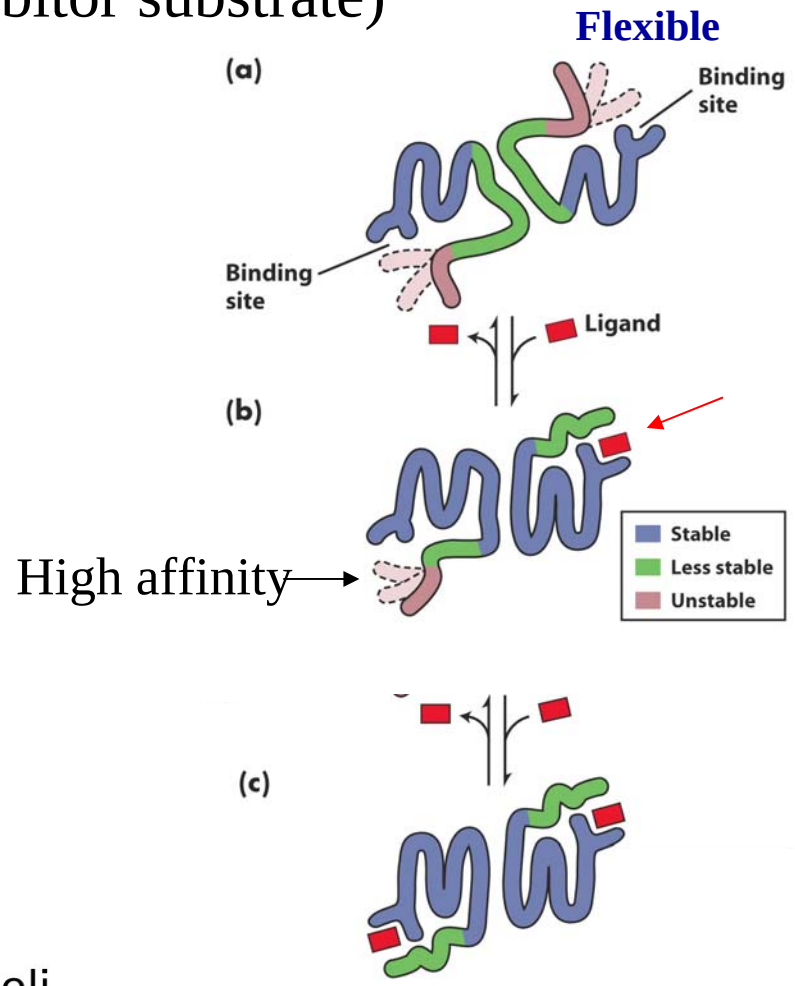
Allostery: other shape, change protein 3 or 4 structure
Allosteric protein
Allosteric effector
Allosteric binding site
Cooperativity

Factor bind to → protein A site (noncovalent)→ change protein structure → affect other binding site (activity site) → allosteric effect ; when factor = protein, also called allosteric protein; its binding site also celled allosteric binding site

異位性調節

Allostey: any change in a protein's 3 or 4 structure or both induced by the binding of a ligand (activator, inhibitor substrate)





結合一個後→讓另一個更容易結合 **(postive regulation)**

釋放一個後→讓另一個結合力下降→更容易釋放

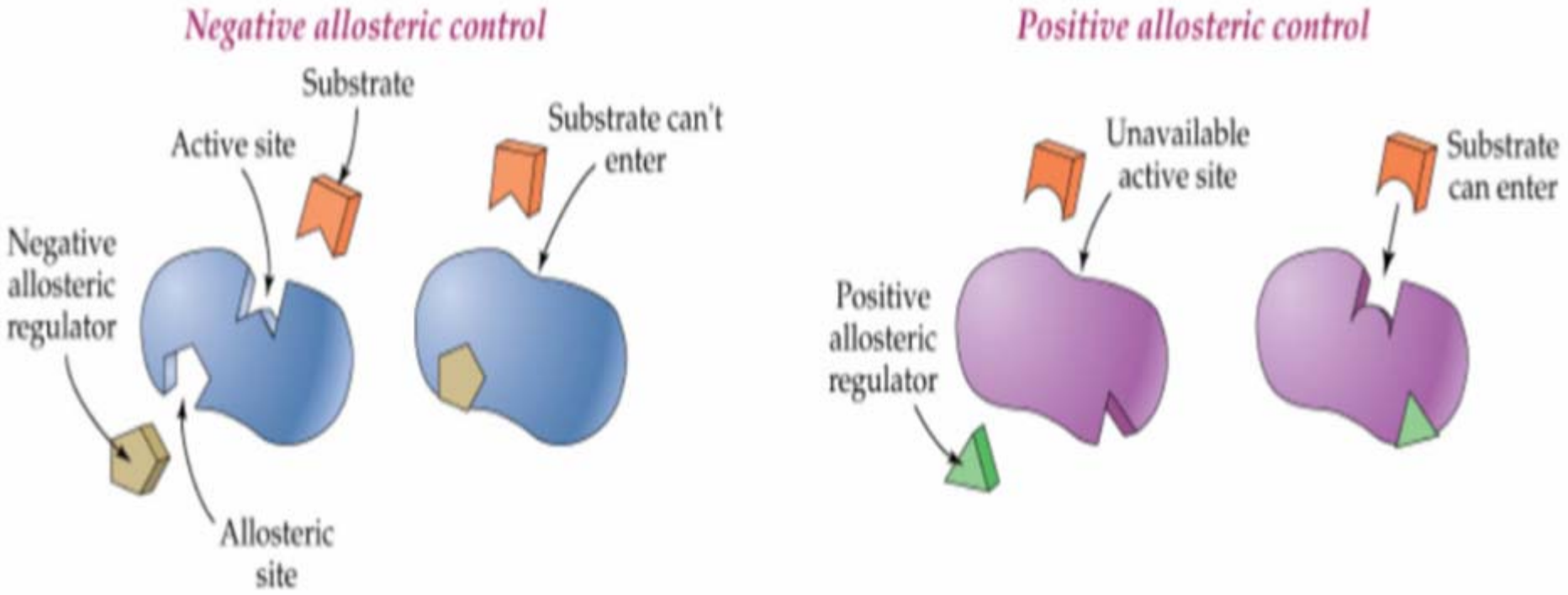
Allosteric protein – a protein in which the binding of a ligand to one site affects the binding properties of another site on the same protein (also called **induced fit model**). The conformational changes induced by the **modulator(s)** interconvert more-active and less-active forms of the protein.

The modulators for allosteric proteins may be either **inhibitors** or **activators**

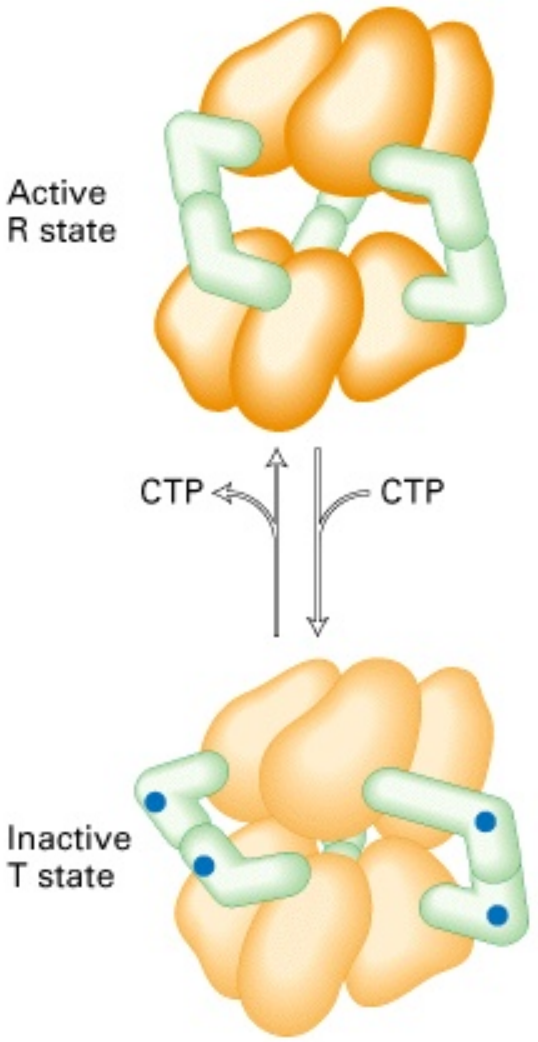
allos --- other
stereos --- solid or shape
Homotropic interaction --- liagnd = modulator
Heterotropic interaction --- ligand ≠ modulator

O_2 --- as both a normal ligand and an activating homotropic modulator for Hb

<u>Allosteric control:</u> either an activator or inhibitor acts on a portion of the enzyme <u>other than the active site</u> to regulate enzyme function.



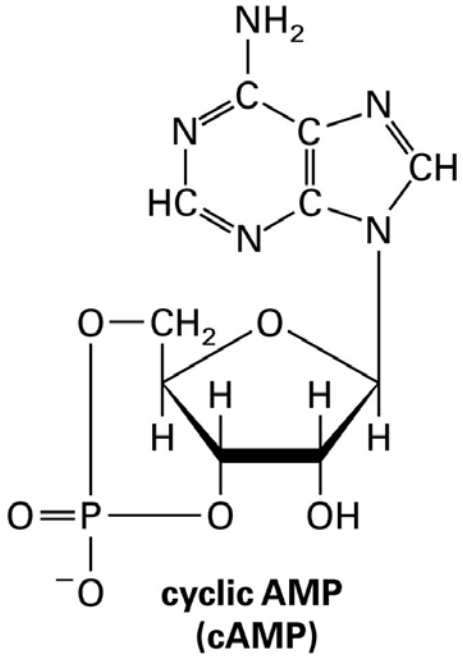
Allosteric transition between active and inactive states

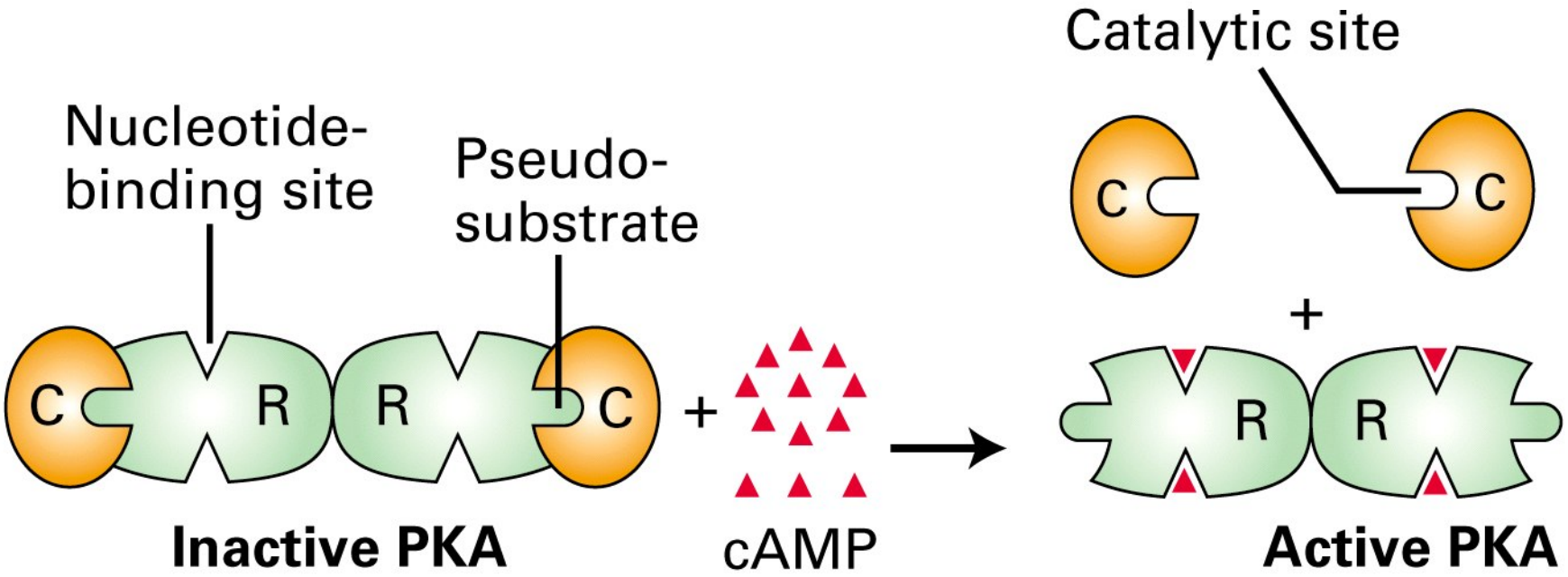


Allosteric release of catalytic subunits

Ligand-indced activation of protein kinase

Ligand binding can induce allosteric release of catalytic subunits or transition to a state with different activity

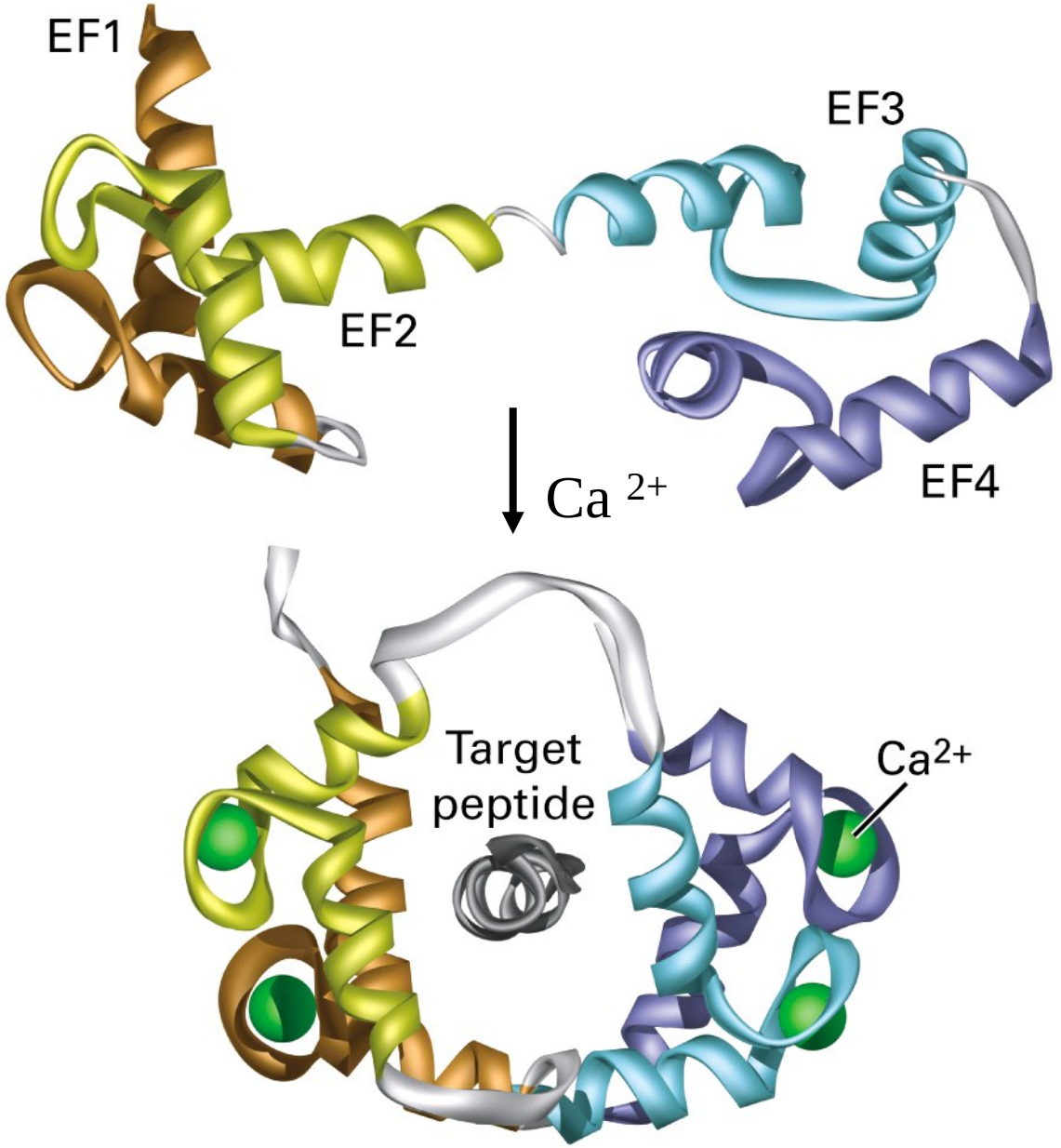




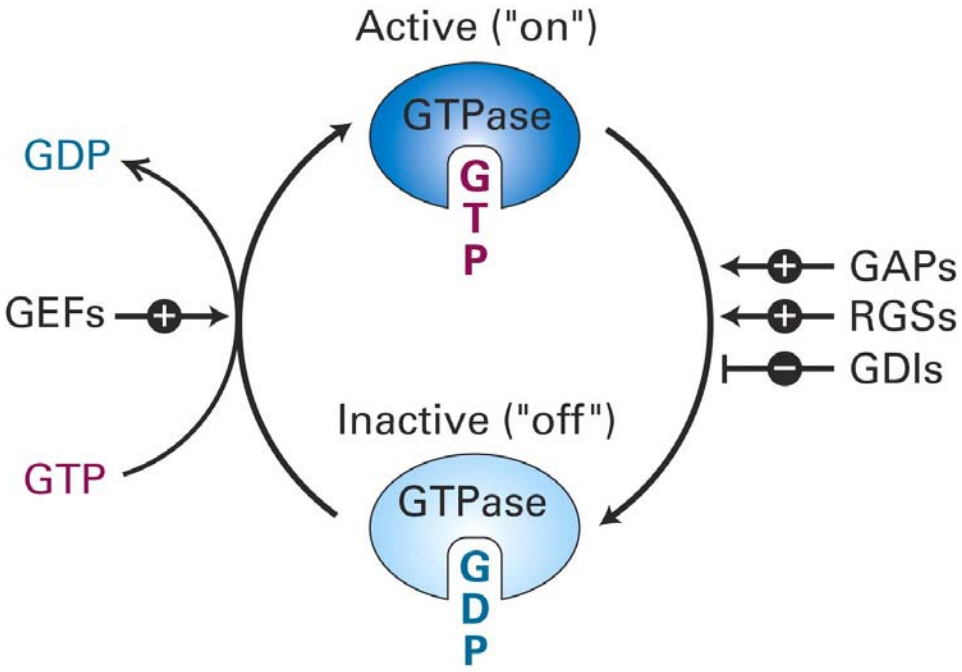
Switch mediated by Ca^{2+}/calmodulin

In normal condition:
- cytosolic calcium is low 10^{-7} M by ER or pump.

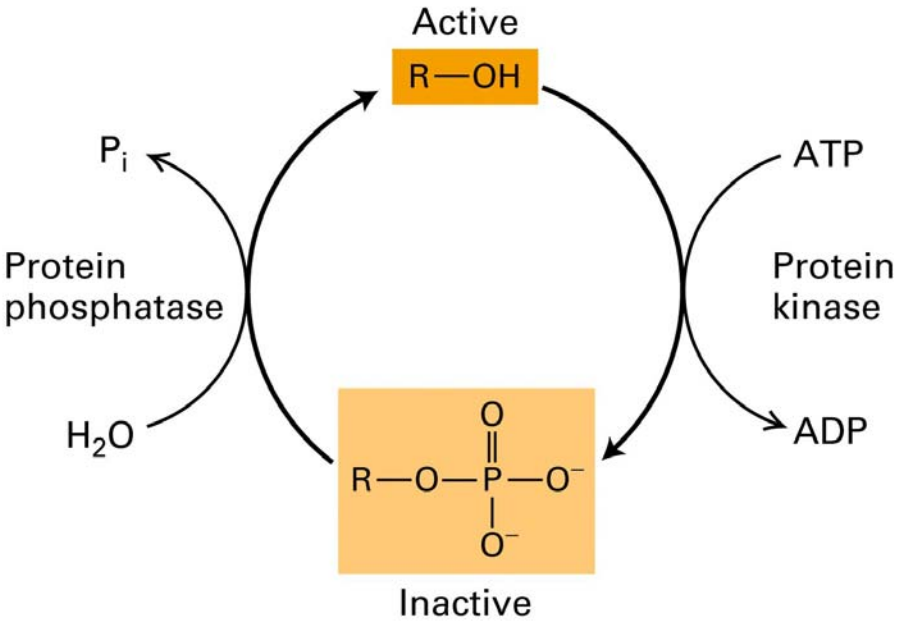
ER release calcium to
- 10-100 fold → sense
- calmodulin →
- conformal change →
- regulated other protein or molecule



Cycling of GTPase switch proteins between the active and inactive forms

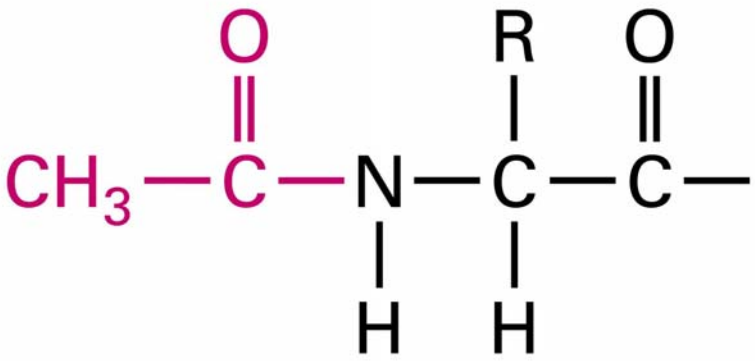


Regulation of protein activity by kinase/phosphatase switch



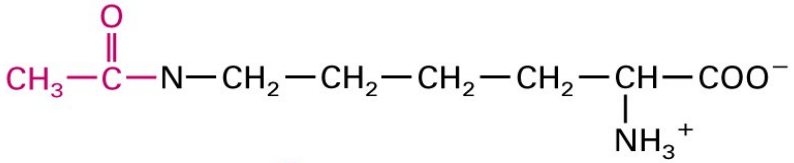
Many proteins undergo chemical modification of amino acids residues

20 amino acid → chemical modification →100 up
Acetylation: about 80% chemical modification
Phosphorylation: serine, threonine, tyrosine
Glycosylation
hydroxylation
Methylation
carboxylation

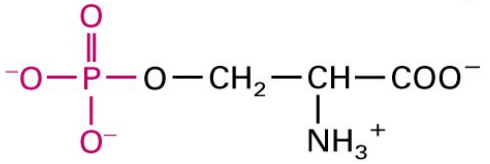


Acetylated N-terminus

Acetyl lysine

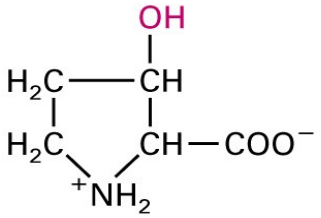


Phosphoserine



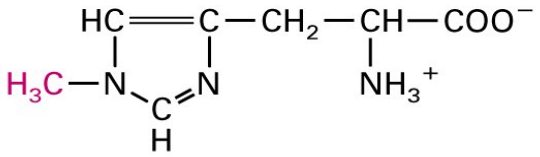
4

3-Hydroxyproline



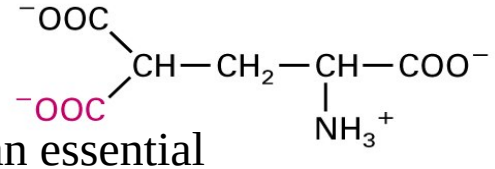
Mainly in collagen

3-Methylhistidine



Mainly in actin

γ-Carboxyglutamate



Mainly in prothrombin, an essential blood clotting factor

Purifying, detecting, and characterizing proteins

A protein must be purified to determine its structure and mechanism of action

Molecules, including proteins, can be separated from other molecules based on differences in physical and chemical properties

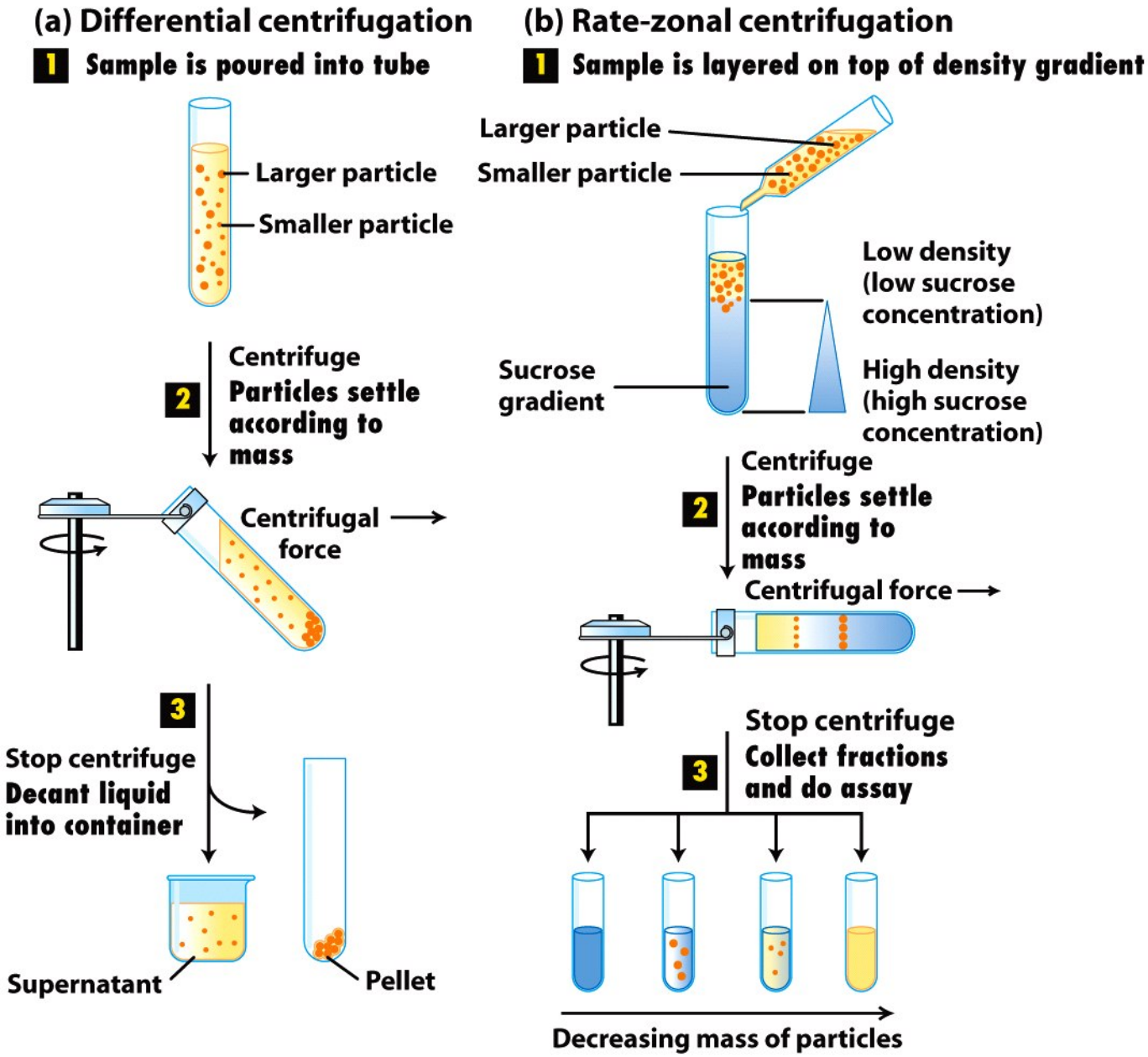


Figure 3-34
Molecular Cell Biology, Sixth Edition

SDS-polyacrylaminde gel electrophoresis (SDS-PAGE)

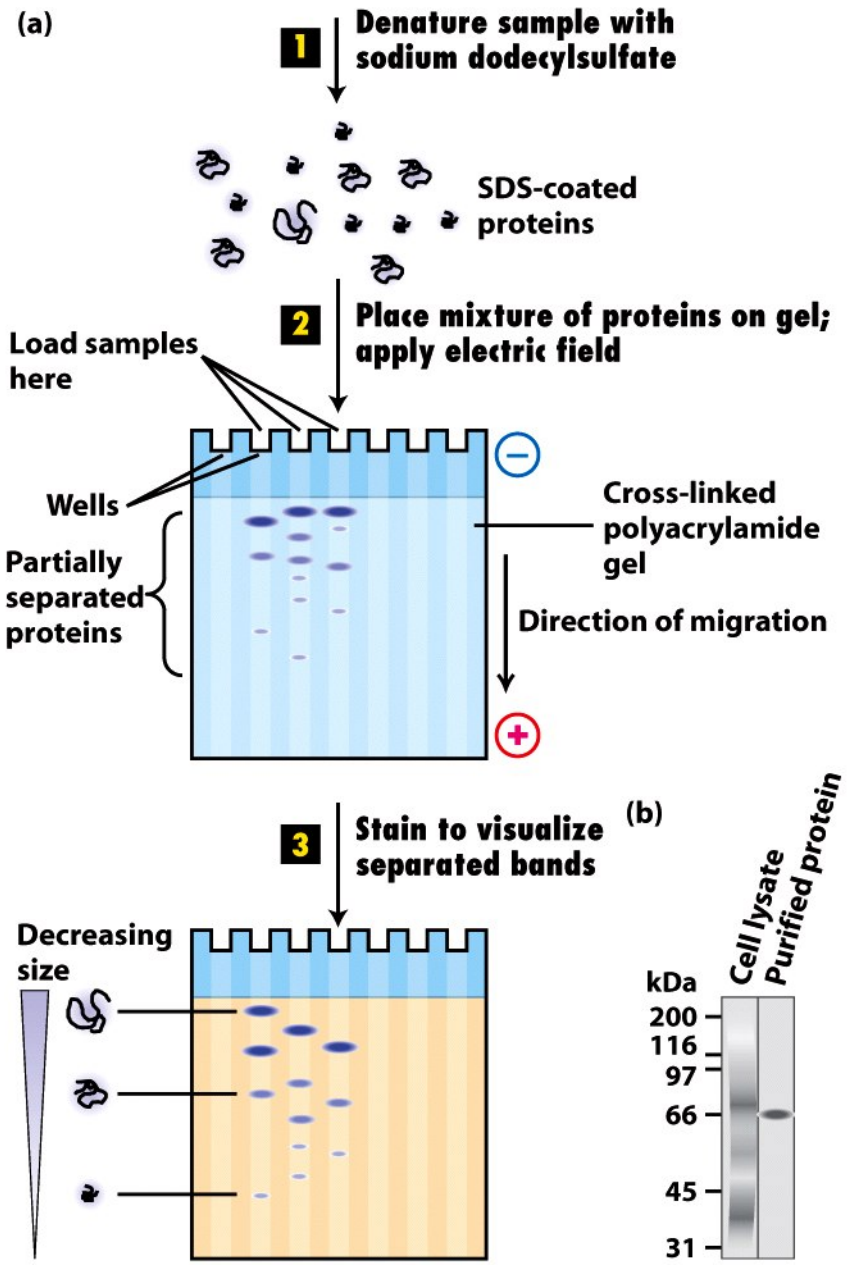


Figure 3-35
Molecular Cell Biology, Sixth Edition

Two-dimensional electrophoresis

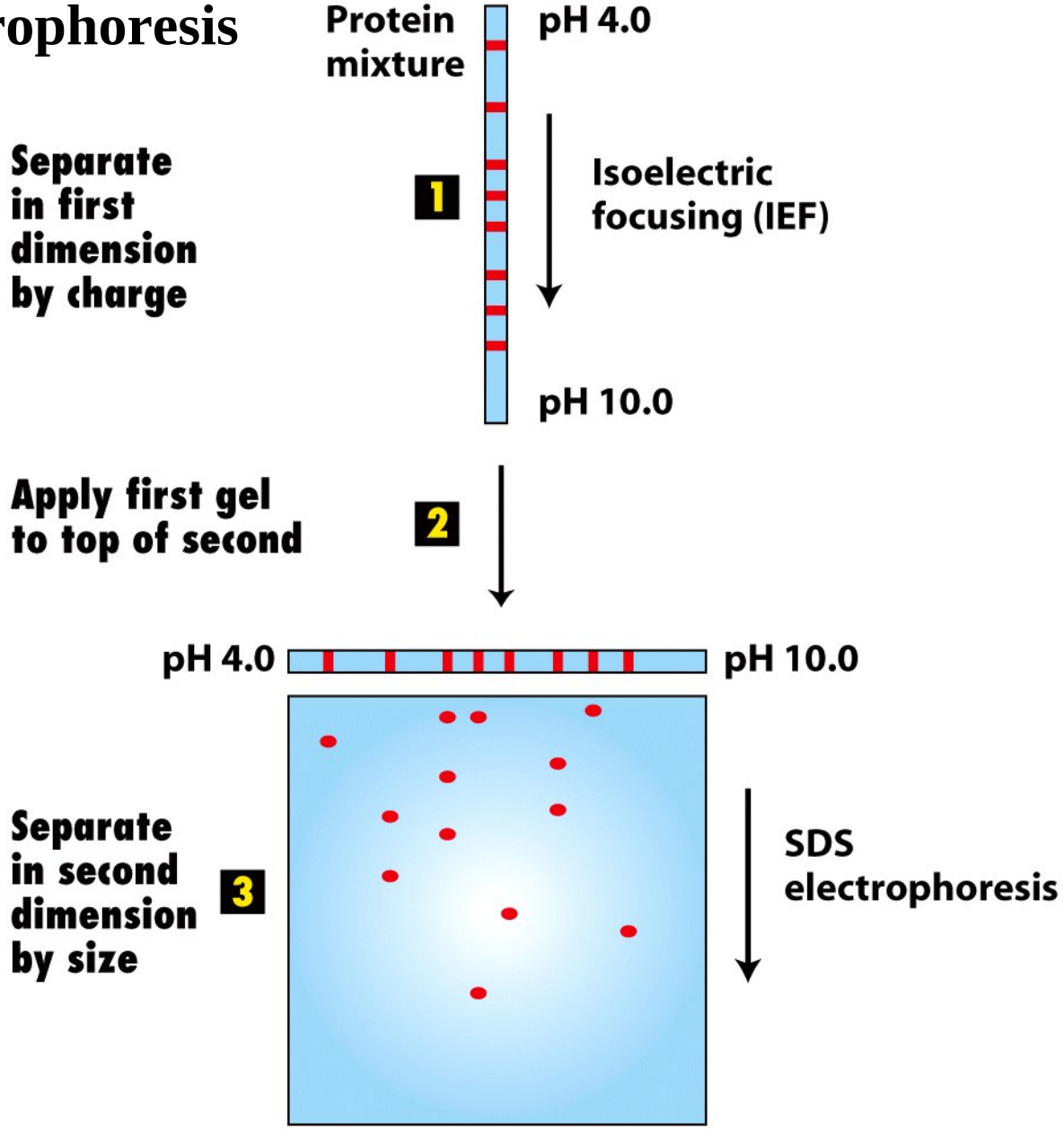


Figure 3-36a
Molecular Cell Biology, Sixth Edition

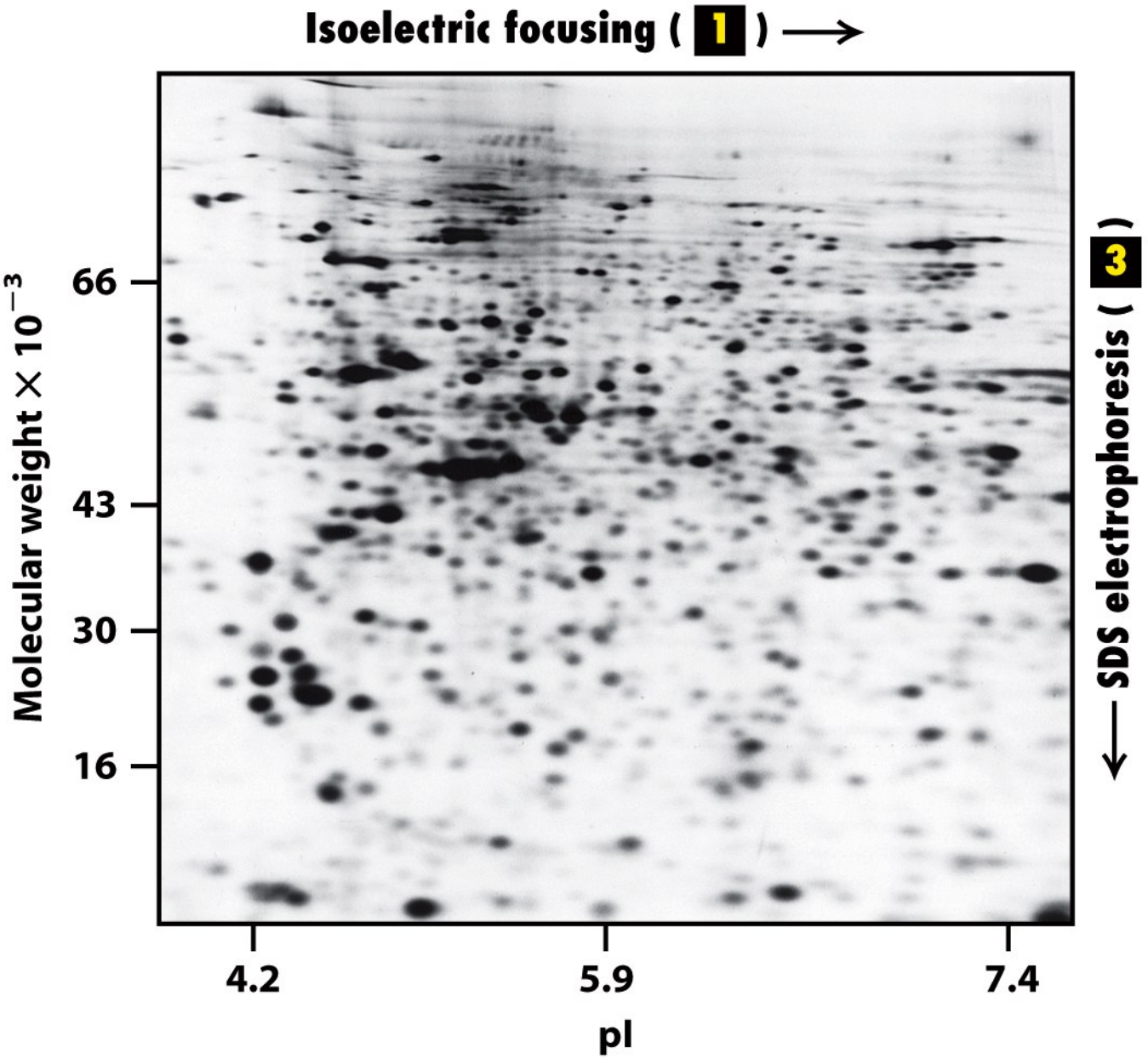


Figure 3-36b
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Gel filtration chromatography

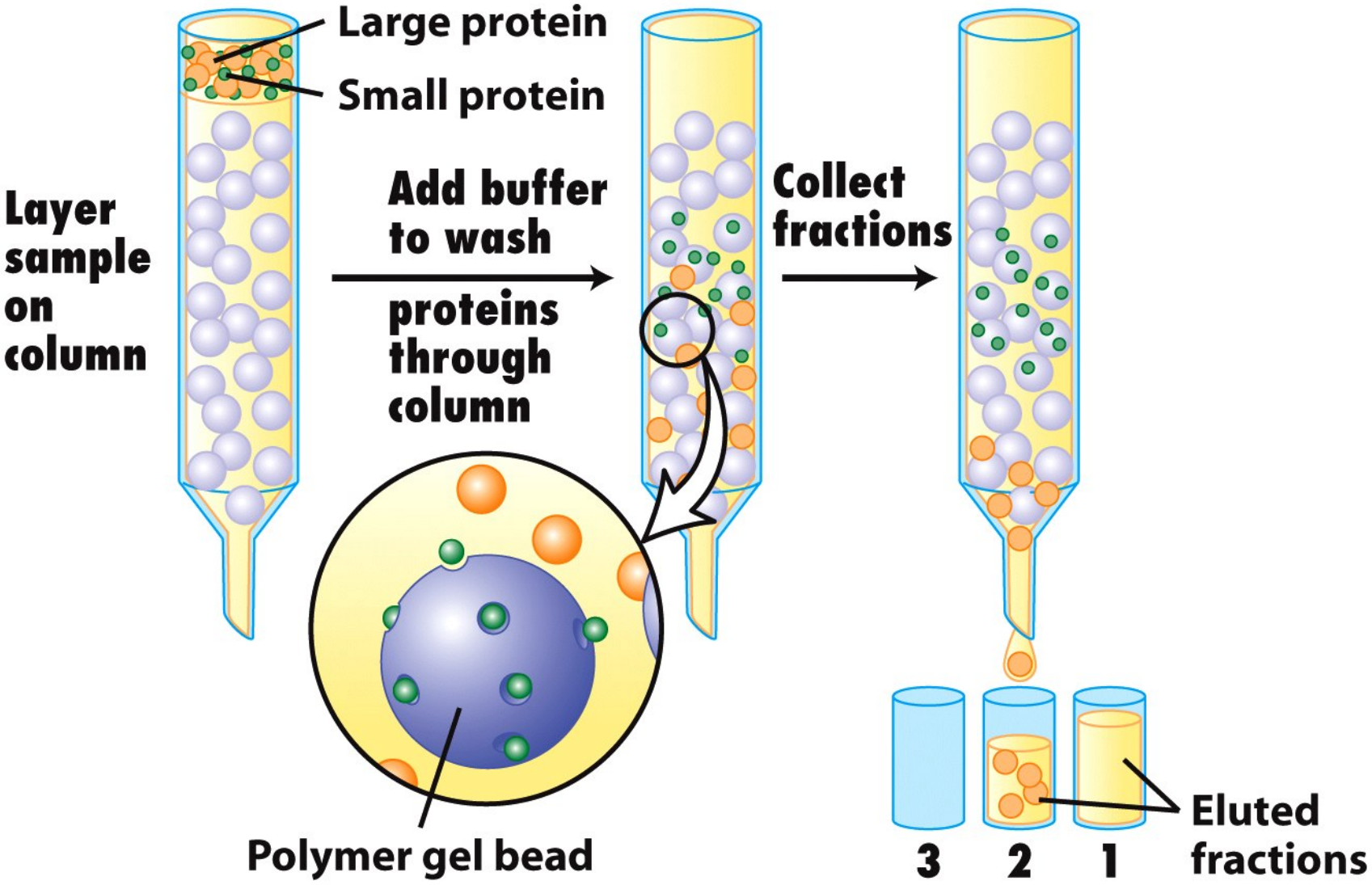

Figure 3-37a
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Ion-exchange chromatography

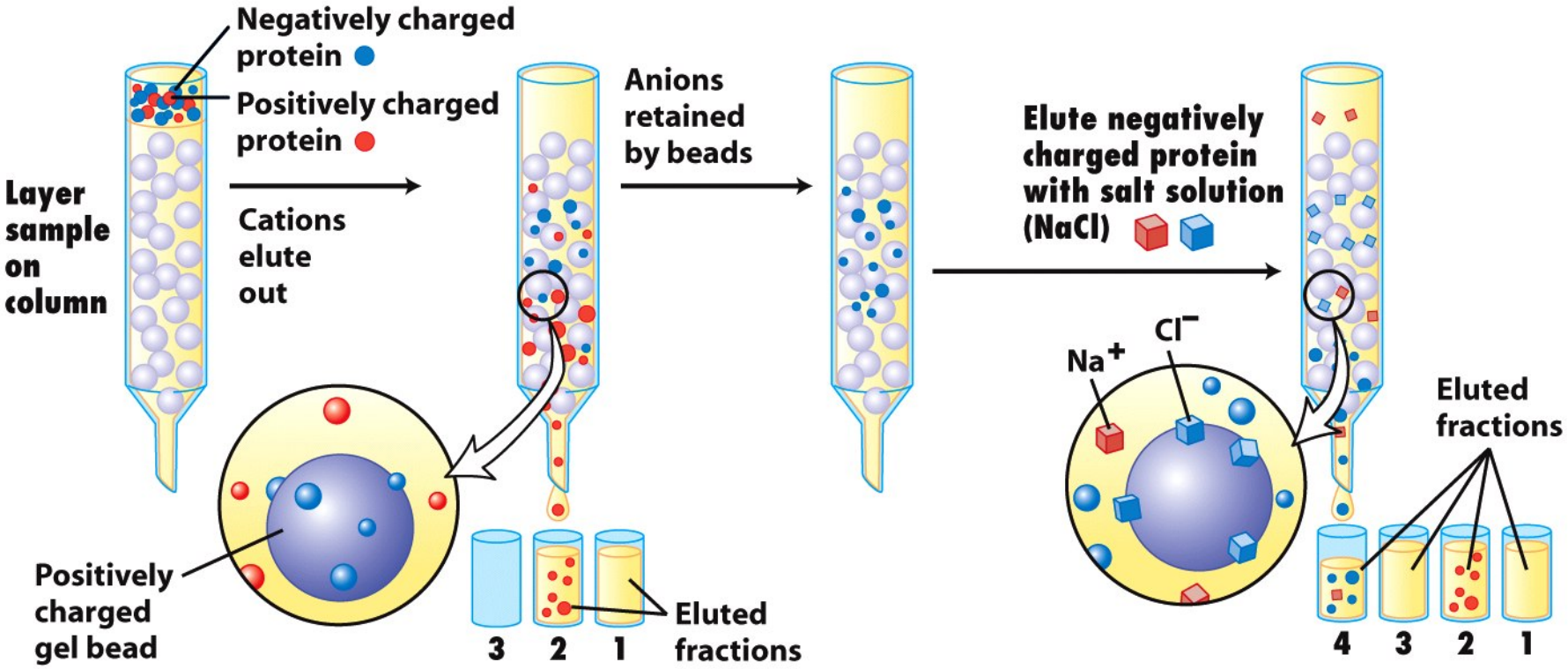

Figure 3-37b
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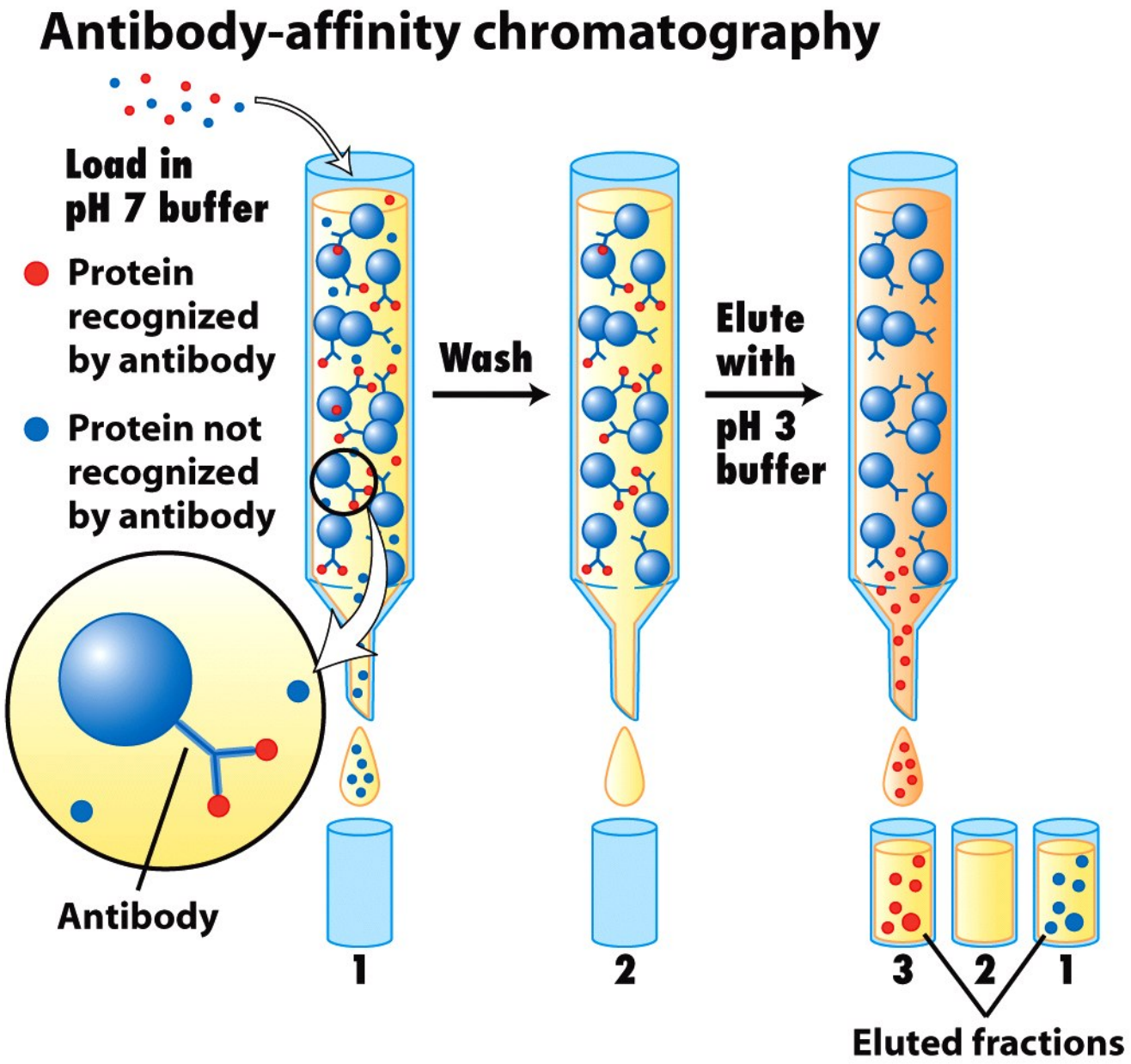


Figure 3-37c
Molecular Cell Biology, Sixth Edition

immunoblotting

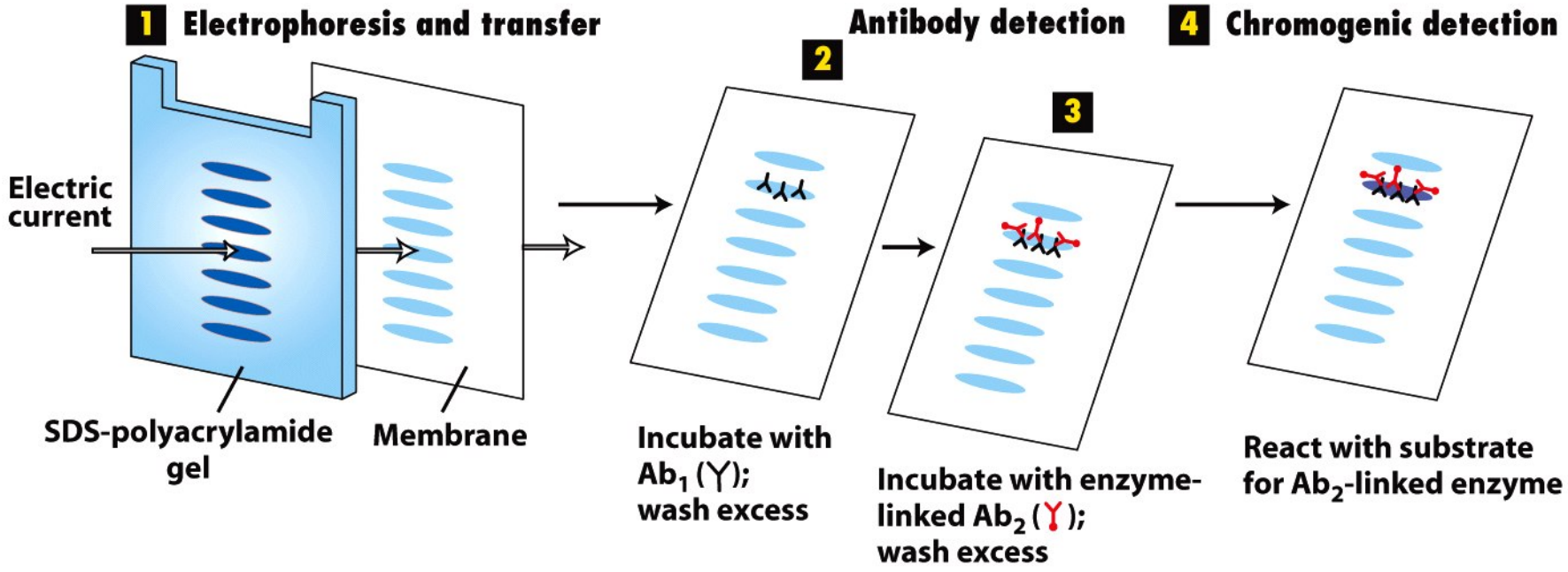


Figure 3-38
Molecular Cell Biology, Sixth Edition

TABLE 3-1 Radioisotopes Commonly Used in Biological Research

ISOTOPE	HALF-LIFE
Phosphorus-32	14.3 days
Iodine-125	60.4 days
Sulfur-35	87.5 days
Tritium (hydrogen-3)	12.4 years
Carbon-14	5730.4 years

Table 3-1
Molecular Cell Biology, Sixth Edition

Pulse-chase exp

脈搏　補捉

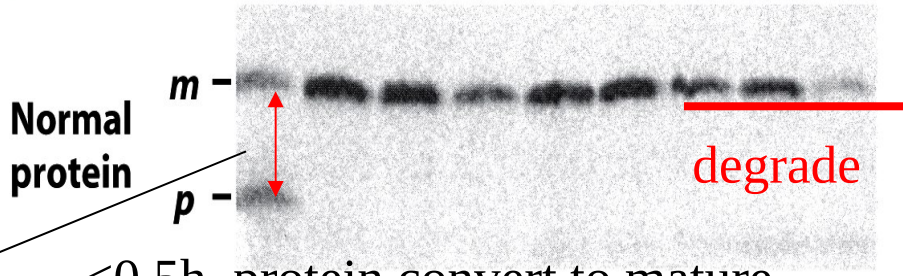
To investigate the fate of a specific newly synthesized protein

Cell + isotope for 0.5h
↓ wash
Different time point
↓
Immunoprecipitation
↓
Specific protein
↓
SDS-PAGE
↓

Low density lipoprotein receptor

(a)

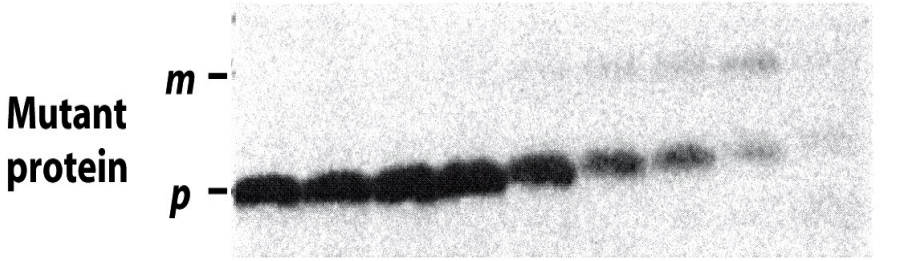
Pulse (h)	0.5								
Chase (h)	0	.5	1	2	4	6	8	12	24



<0.5h, protein convert to mature

PTM Glyco..

(b)



Precursor protein (*p*) converted to mature protein (*m*) by posttranslational carbohydrate addition

Figure 3-39
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Mass spectrometry can determine the mass and sequence of proteins

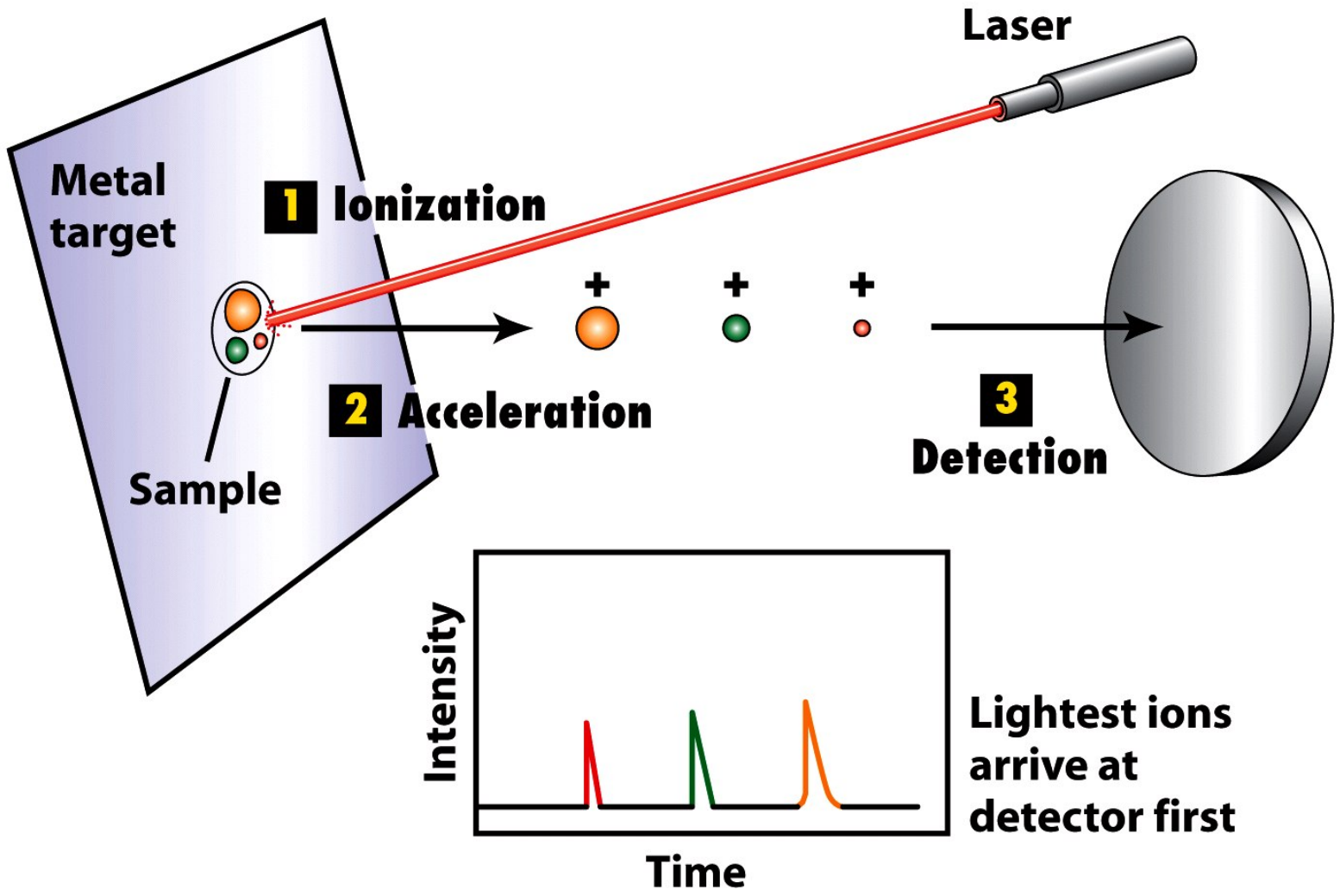


Figure 3-40
Molecular Cell Biology, Sixth Edition

MALDI-TOF

Mass spectrometry can determine the mass and sequence of proteins

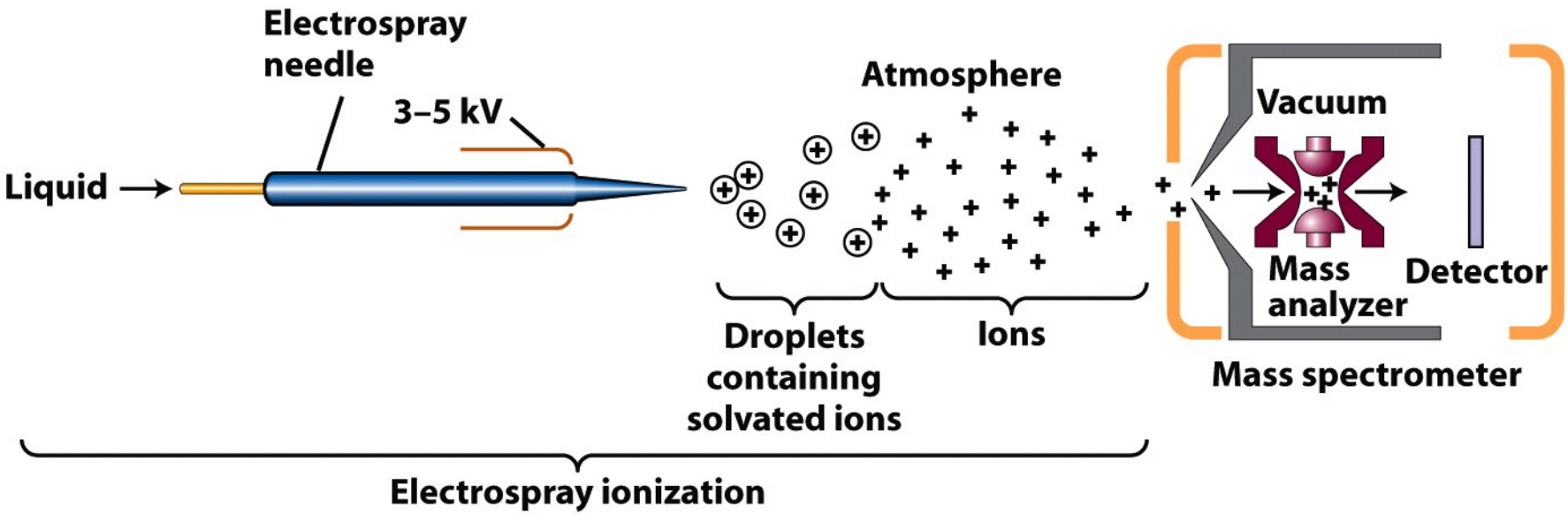


Figure 3-41a
Molecular Cell Biology, Sixth Edition

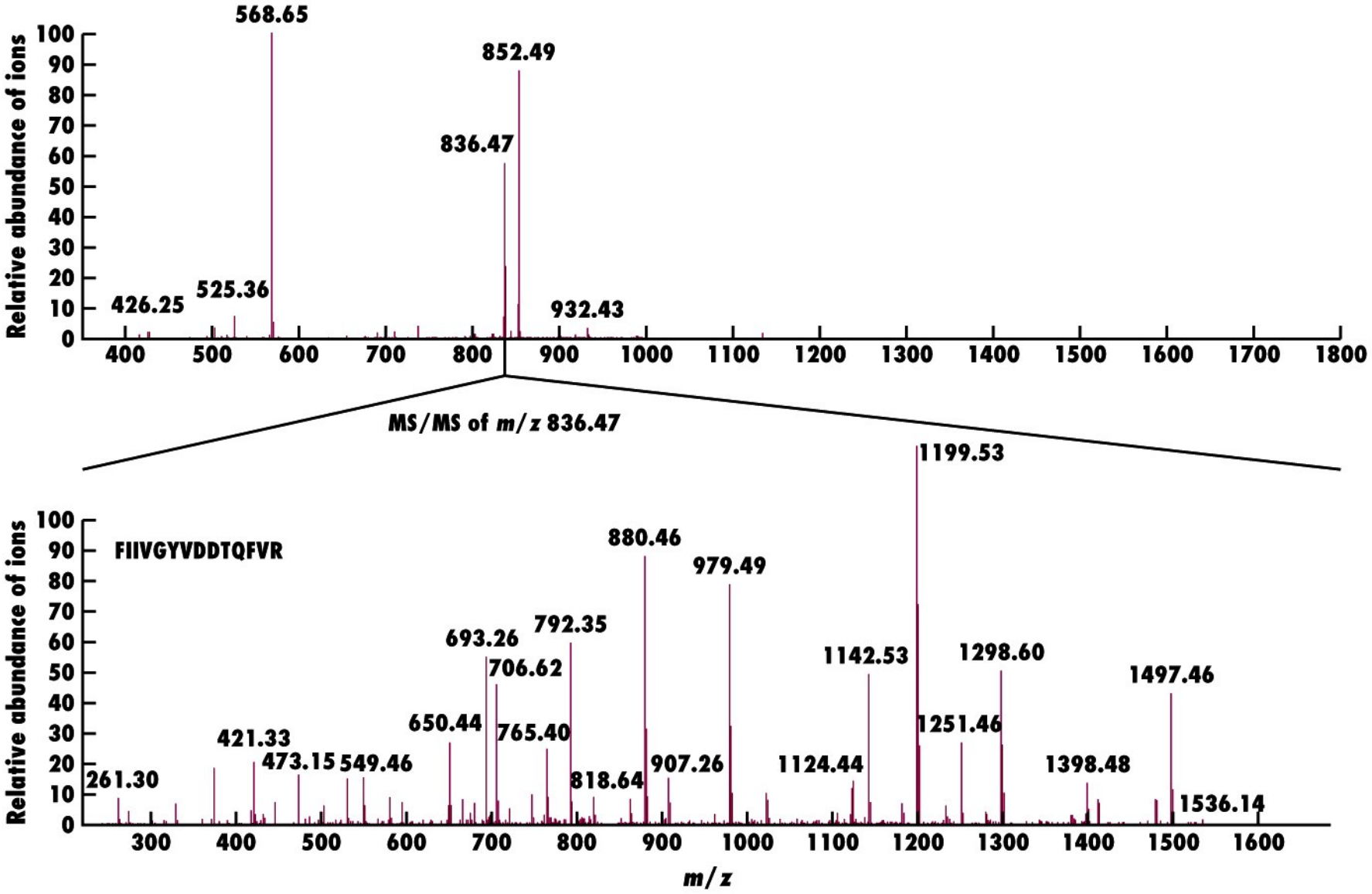


Figure 3-41b
Molecular Cell Biology, Sixth Edition

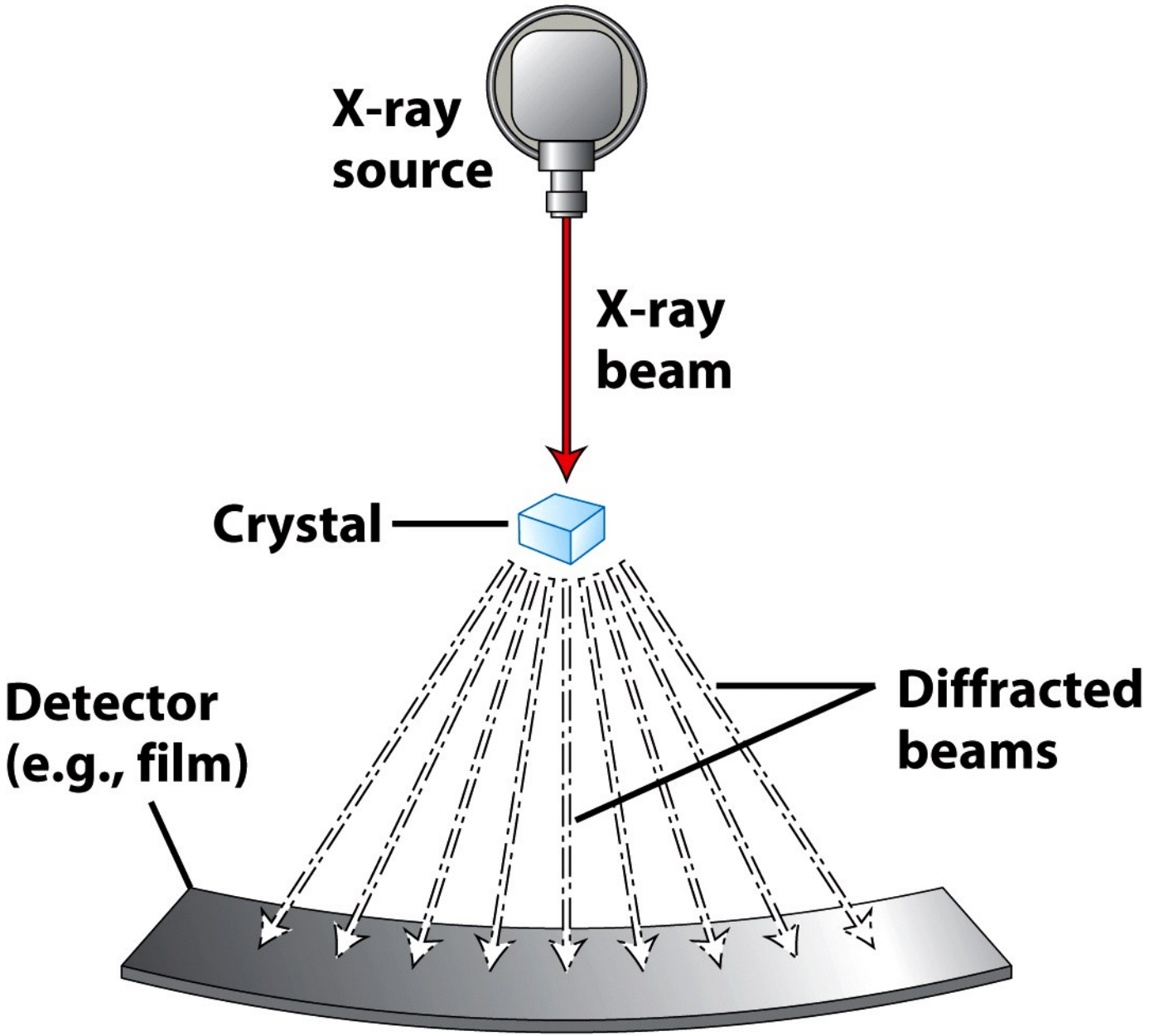
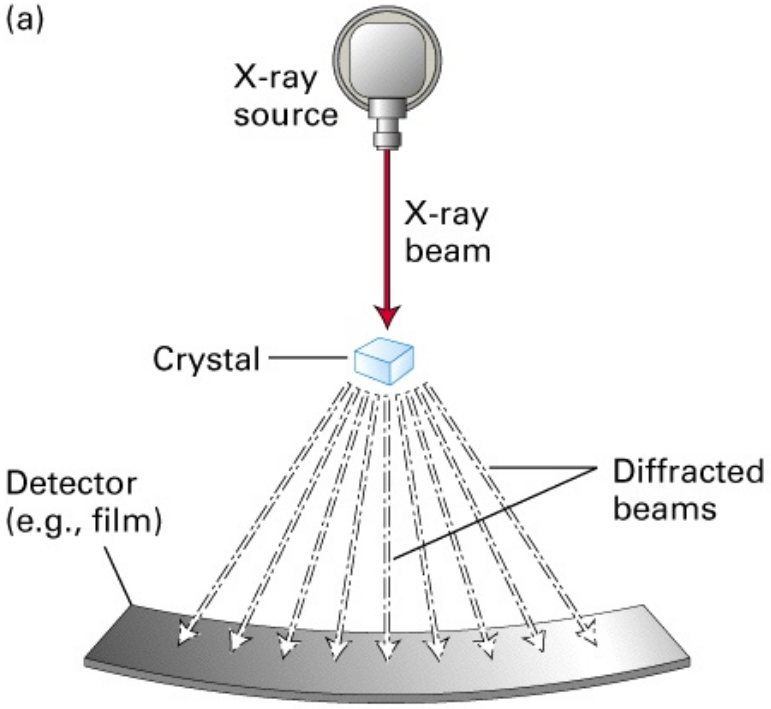
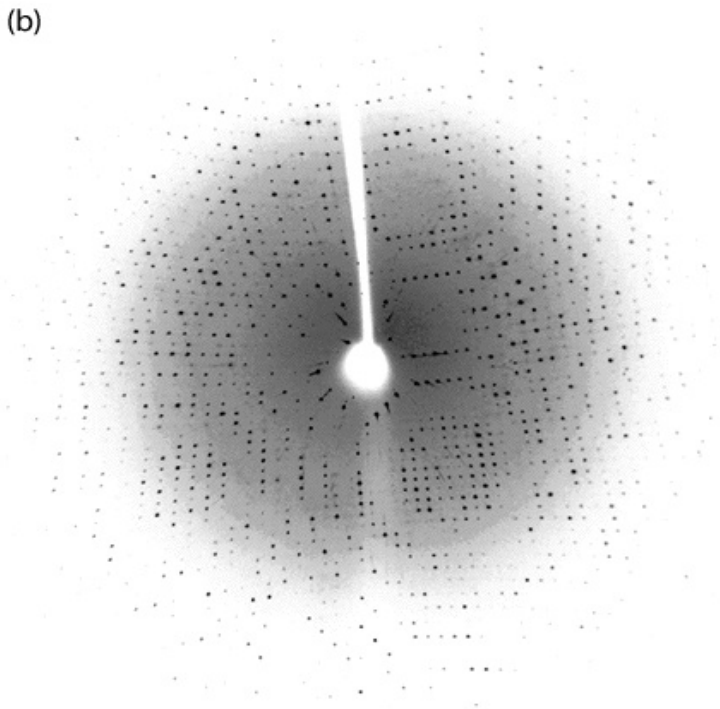


Figure 3-42a
Molecular Cell Biology, Sixth Edition

X-ray crystallography is used to determine protein structure





Other techniques such as cryoelectron microscopy and NMR spectroscopy may be used to solve the structures of certain types of proteins

Figure 3-49

Advance technique in mass spectrometry are critical to proteomic analysis

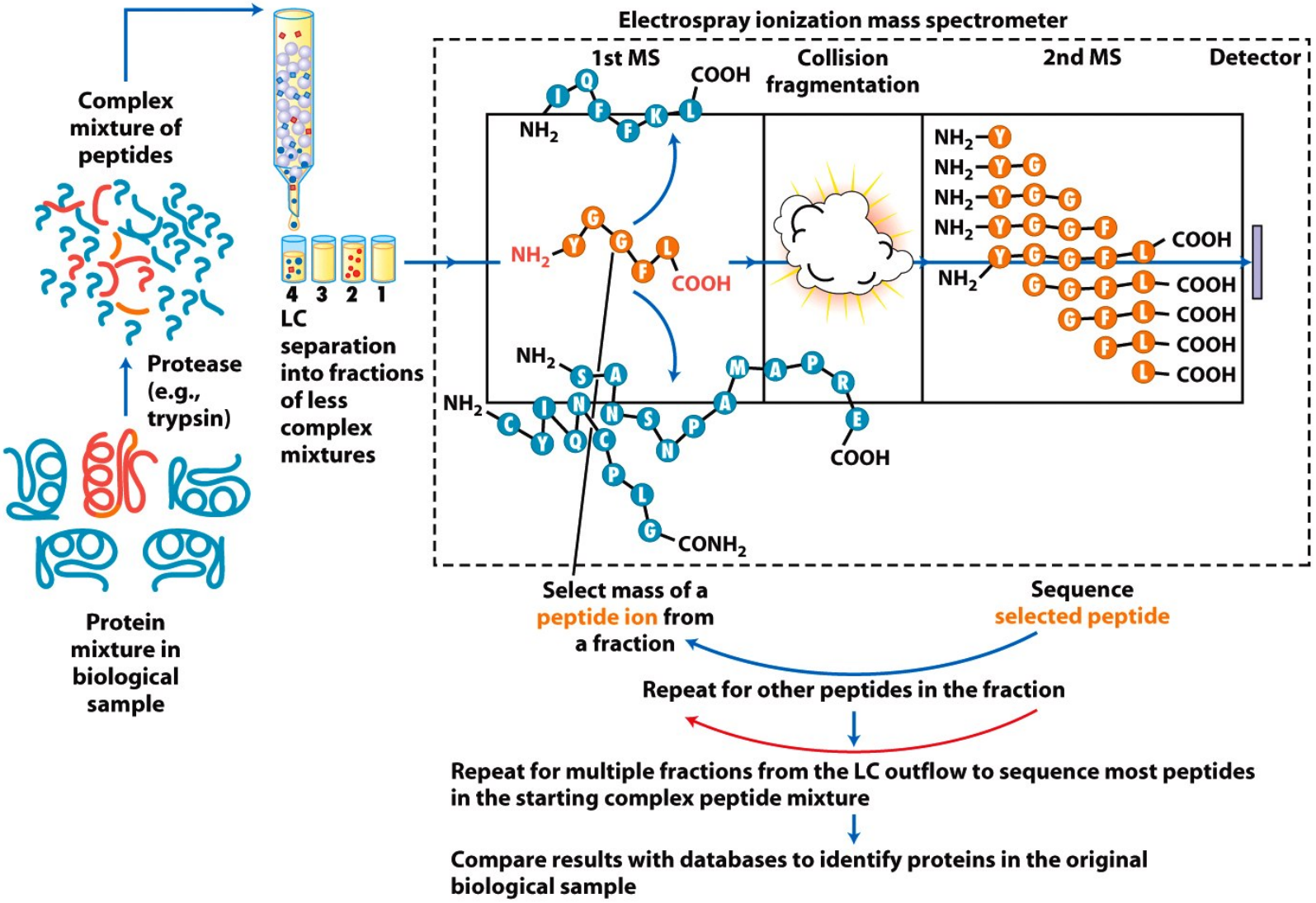


Figure 3-43
Molecular Cell Biology, Sixth Edition

Density-gradinet centrifugation and LC-MS/MS can be used to identify many of the protein in organelle

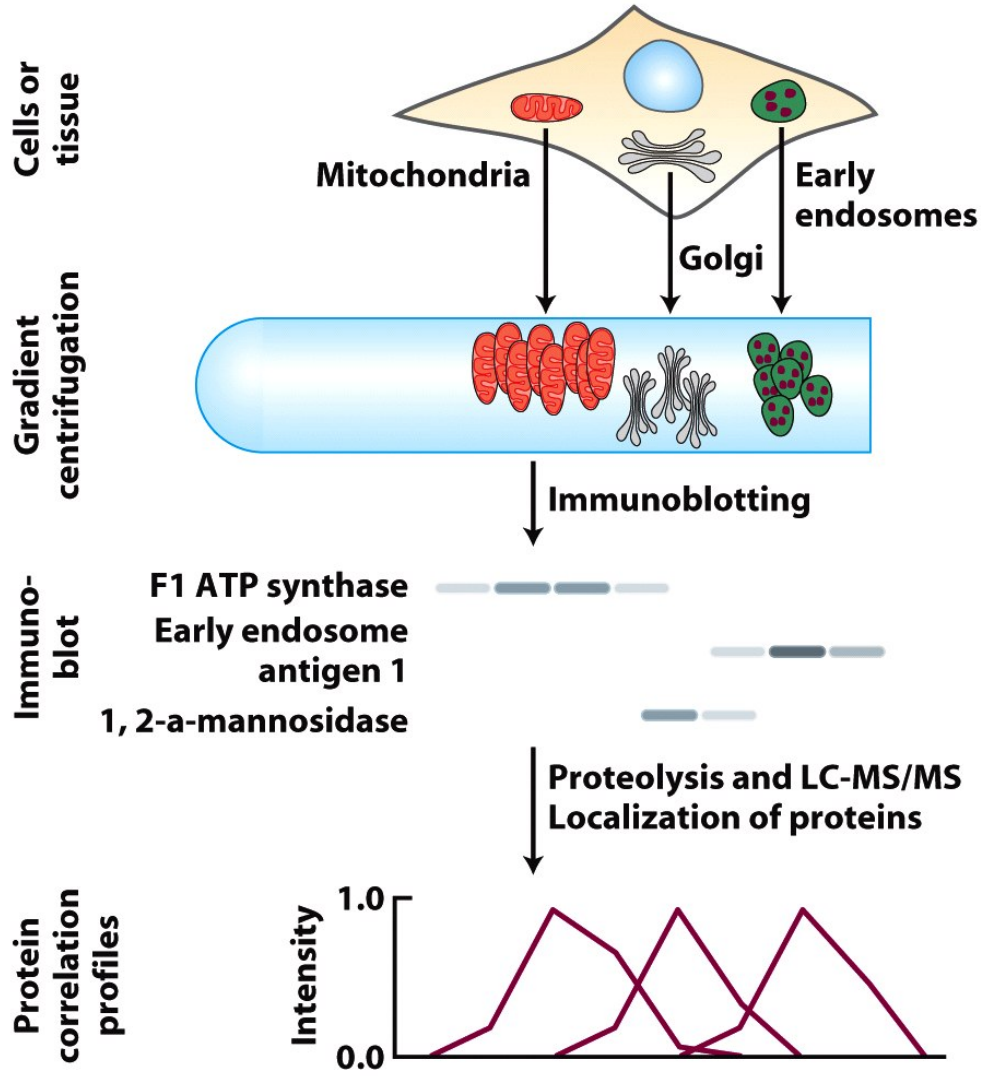


Figure 3-44a
Molecular Cell Biology, Sixth Edition

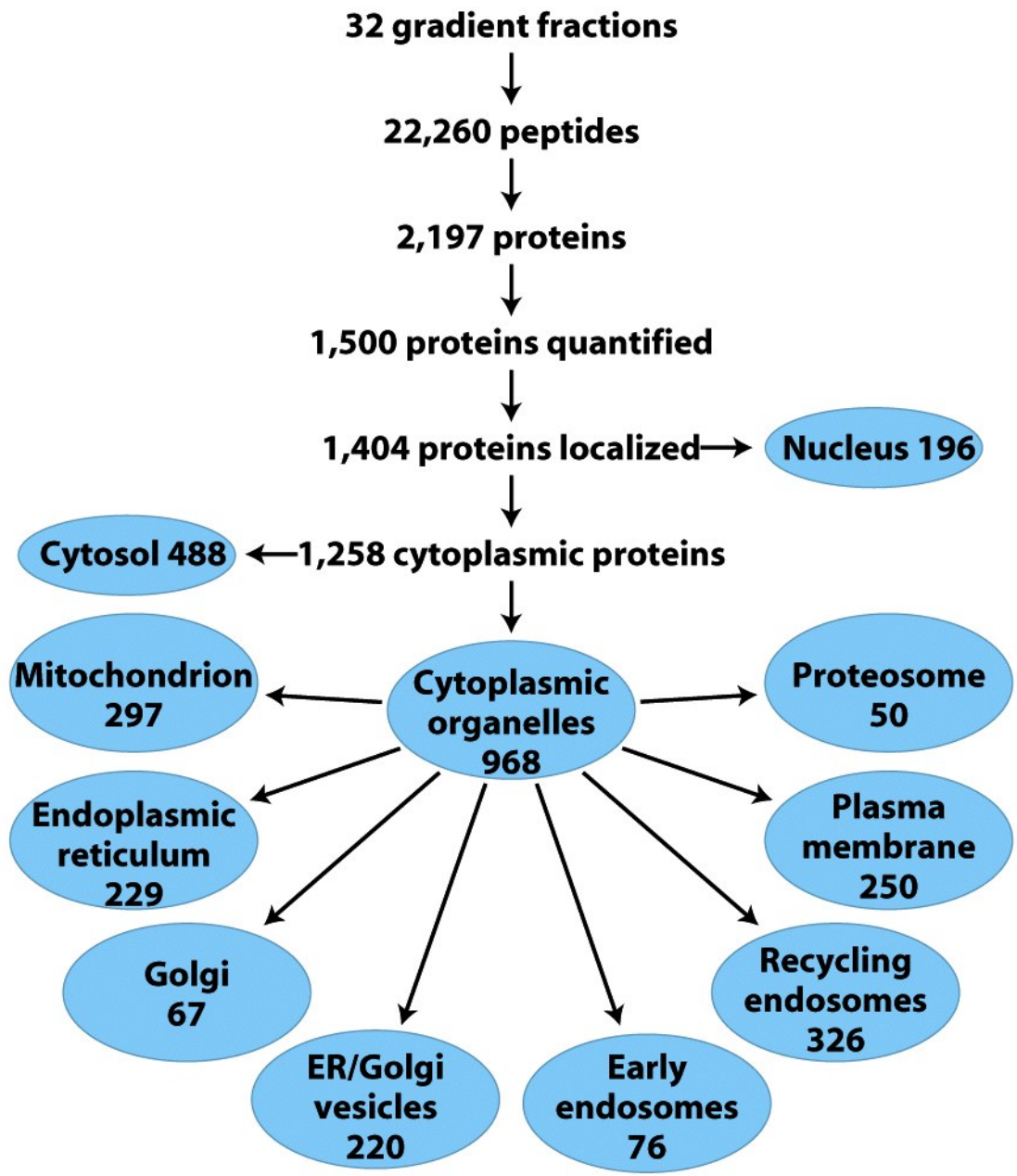
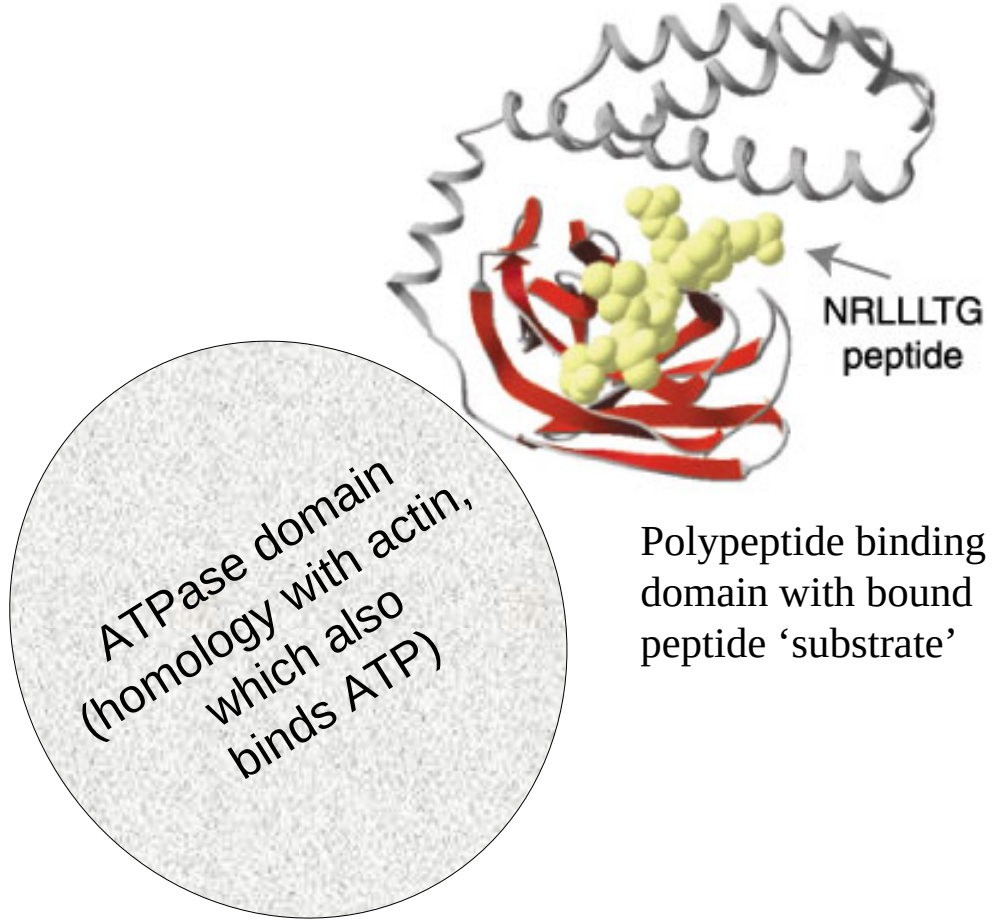


Figure 3-44b
Molecular Cell Biology, Sixth Edition

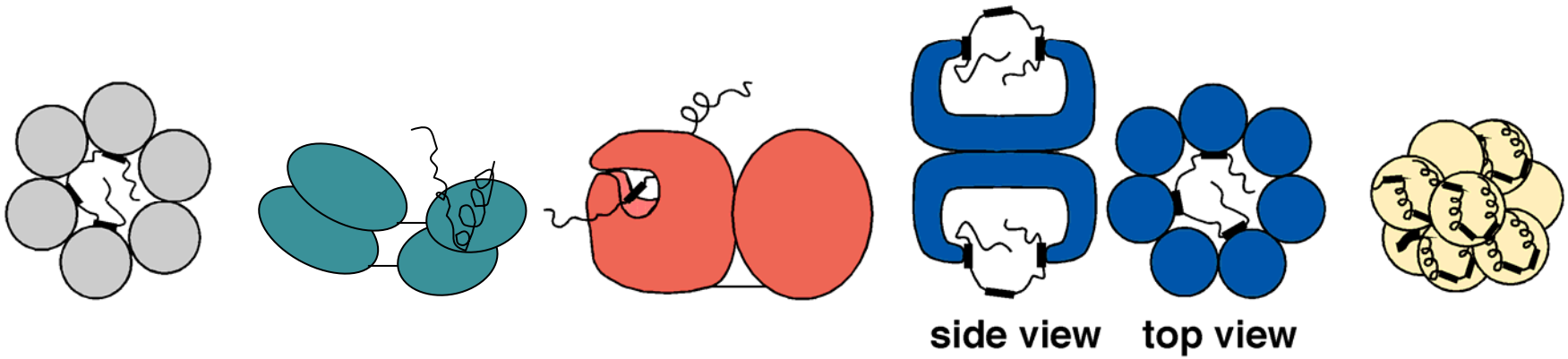
Structure of Hsp70 chaperone

- Structure of entire molecule (~70 kDa) has not been solved
- flexible linkage between ATPase and peptide-binding domains, and different conformations of molecule possible
- polypeptide-binding domain consists of beta-sheet scaffold; loops possess hydrophobic residues that contact peptide
- domain also has an alpha-helical ‘lid’ that is regulated by the ATPase activity



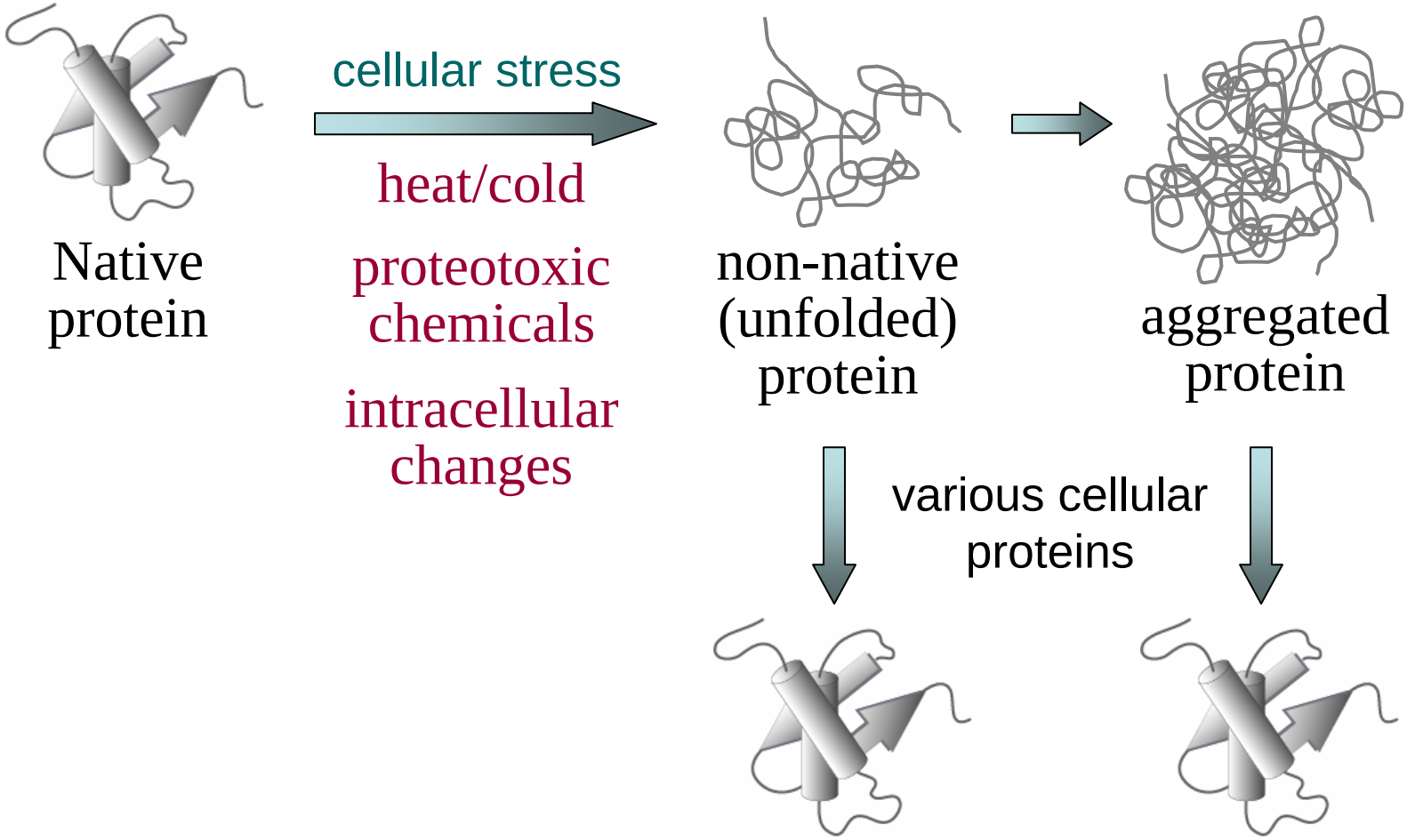
Polypeptide binding domain with bound peptide ‘substrate’

Major chaperones and their interactions with substrates



Hsp100 (ClpB)	Hsp90 (HtpG)	Hsp70 (DnaK)	Hsp60 (GroEL)	sHSP (IbpA/B)
disaggregation	stabilization of folding intermediates	stabilization of folding intermediates → refolding	stabilization of folding intermediates → unfolding ? → refolding	stabilization of folding intermediates

Cellular processes involving non-native proteins: refolding



Summary of chaperon

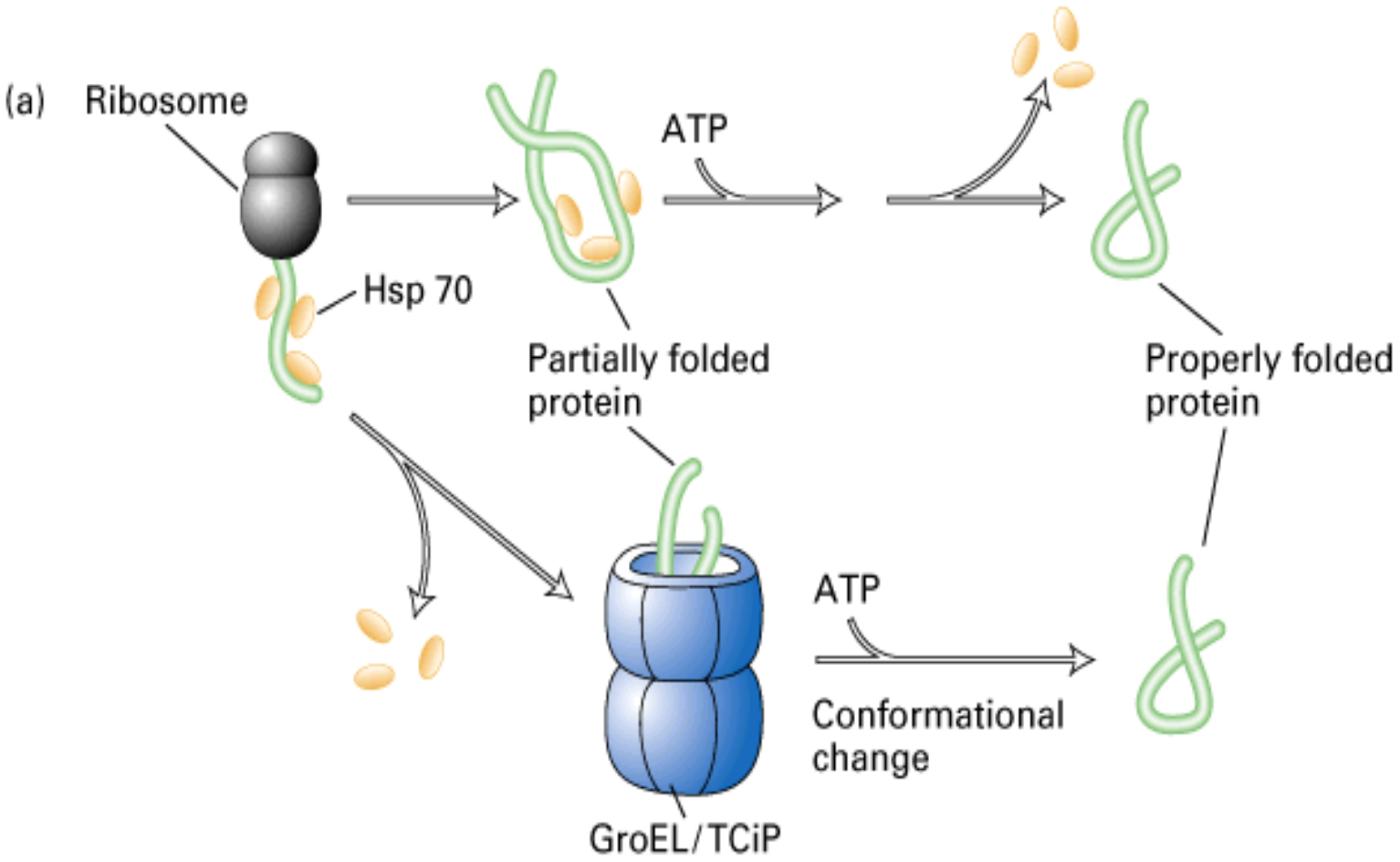


Figure 3-15a: Chaperone-mediated protein folding. Many proteins (step 1) fold into their proper three-dimensional structure with the assistance of Hsp70, a molecular chaperone that transiently binds to a nascent polypeptide as it emerges from a ribosome. Proper folding of some proteins (step 2) also depends on the chaperonin TCiP, a large barrel-shaped complex of Hsp60 units.

Different protein → different function

TABLE 3-1 Selected Molecular Machines

Machine*	Main Components	Cellular Location	Function
Replisome (4)	Helicase, primase, DNA polymerase	Nucleus	DNA replication
Transcription initiation complex (11)	Promoter-binding protein, helicase, general transcription factors (TFs), RNA polymerase, large multisubunit mediator complex	Nucleus	RNA synthesis
Spliceosome (12)	Pre-mRNA, small nuclear RNAs (snRNAs), protein factors	Nucleus	mRNA splicing
Nuclear pore complex (12)	Nucleoporins (50–100)	Nuclear membrane	Nuclear import and export
Ribosome (4)	Ribosomal proteins (>50) and four rRNA molecules (eukaryotes) organized into large and small subunits; associated mRNA and protein factors (IFs, EFs)	Cytoplasm/ER membrane	Protein synthesis
Chaperonin (3)	GroEL, GroES (bacteria)	Cytoplasm, mitochondria, endoplasmic reticulum	Protein folding
Proteasome (3)	Core proteins, regulatory (cap) proteins	Cytoplasm	Protein degradation
Photosystem (8)	Light-harvesting complex (multiple proteins and pigments), reaction center (multisubunit protein with associated pigments and electron carriers)	Thylakoid membrane in plant chloroplasts, plasma membrane of photosynthetic bacteria	Photosynthesis (initial stage)
MAP kinase cascades (14)	Scaffold protein, multiple different protein kinases	Cytoplasm	Signal transduction
Sarcomere (19)	Thick (myosin) filaments, thin (actin) filaments, Z lines, titin/nebulin	Cytoplasm of muscle cells	Contraction

*Numbers in parentheses indicate chapters in which various machines are discussed.

AMINO ACID			SIDE CHAIN
Aspartic acid	Asp	D	negative
Glutamic acid	Glu	E	negative
Arginine	Arg	R	positive
Lysine	Lys	K	positive
Histidine	His	H	positive
Asparagine	Asn	N	uncharged polar
Glutamine	Gln	Q	uncharged polar
Serine	Ser	S	uncharged polar
Threonine	Thr	T	uncharged polar
Tyrosine	Tyr	Y	uncharged polar

POLAR AMINO ACIDS

AMINO ACID			SIDE CHAIN
Alanine	Ala	A	nonpolar
Glycine	Gly	G	nonpolar
Valine	Val	V	nonpolar
Leucine	Leu	L	nonpolar
Isoleucine	Ile	I	nonpolar
Proline	Pro	P	nonpolar
Phenylalanine	Phe	F	nonpolar
Methionine	Met	M	nonpolar
Tryptophan	Trp	W	nonpolar
Cysteine	Cys	C	nonpolar

NONPOLAR AMINO ACIDS

Figure 4-3 Essential Cell Biology, 2/e. (© 2004 Garland Science)

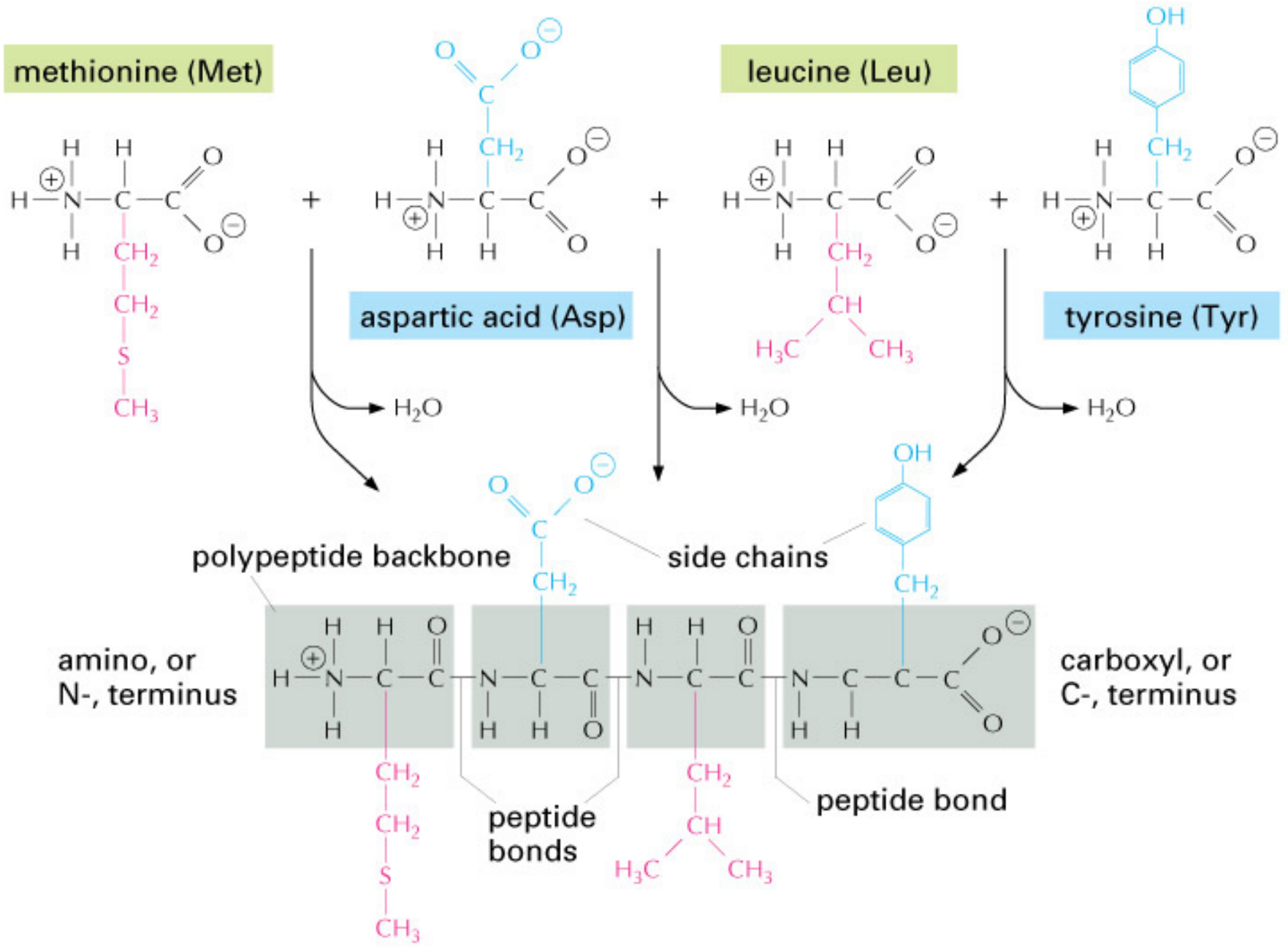


Figure 4-2 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Peptide segments of some protein are removed after synthesis

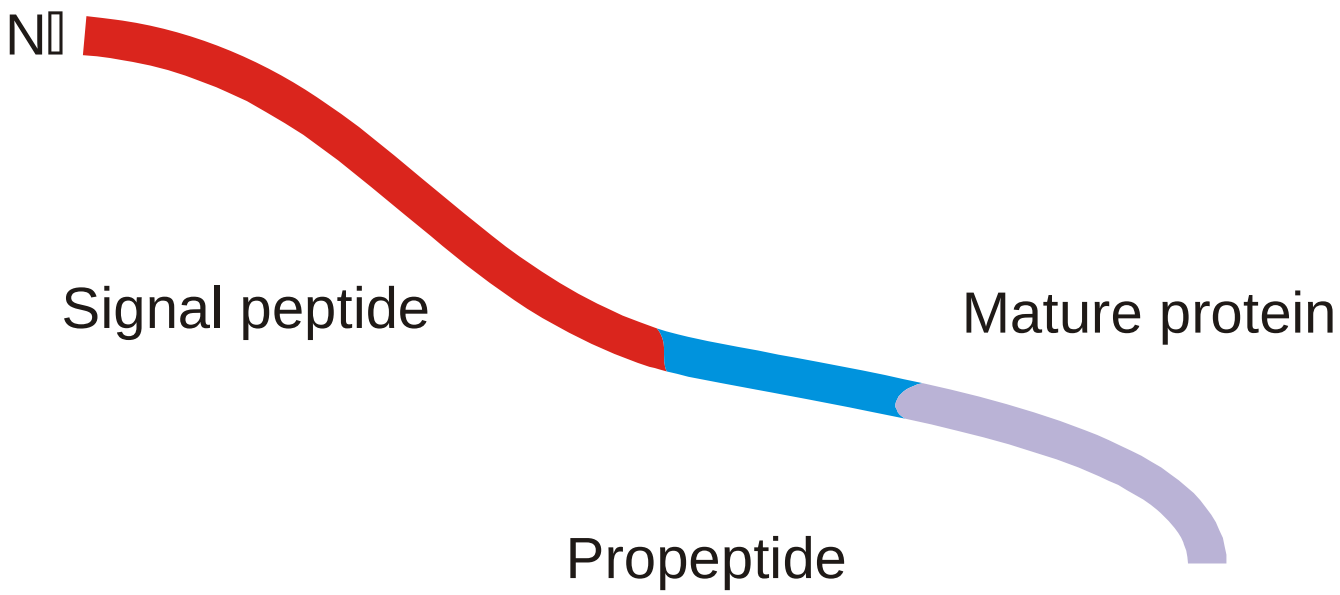
Protein targeting/localization signals

- **Signal peptide**
- **Mitochondrial targeting peptide**
- **Chloroplast targeting peptide**
- **Peroxisomal targeting signal (PTS2)**
- **Signal anchor**
- **Nuclear localization signal**
- **ER/Golgi retention signal**
- **Peroxisomal targeting signal (PTS1)**
- **Transmembrane helices**

Cleaved

Uncleaved

Signal peptide or propeptide



Characteristics of signal peptides

	Length	n-region	h-region	c-region	-3, -1
Euk	**22**	**only slightly Arg-rich**	**short, very hydrophobic**	**short, no pattern**	**small and neutral residues**
Gram-	**25**	**Lys+Arg-rich**	**slightly longer, less hydrophobic**	**short, Ser+Ala-rich**	**almost exclusively Ala**
Gram+	**32**	**Lys+Arg-rich**	**very long, less hydrophobic**	**longer, Thr+Pro-rich**	**almost exclusively Ala**

Ubiquitin–Mediated Proteolysis in Cellular Processes

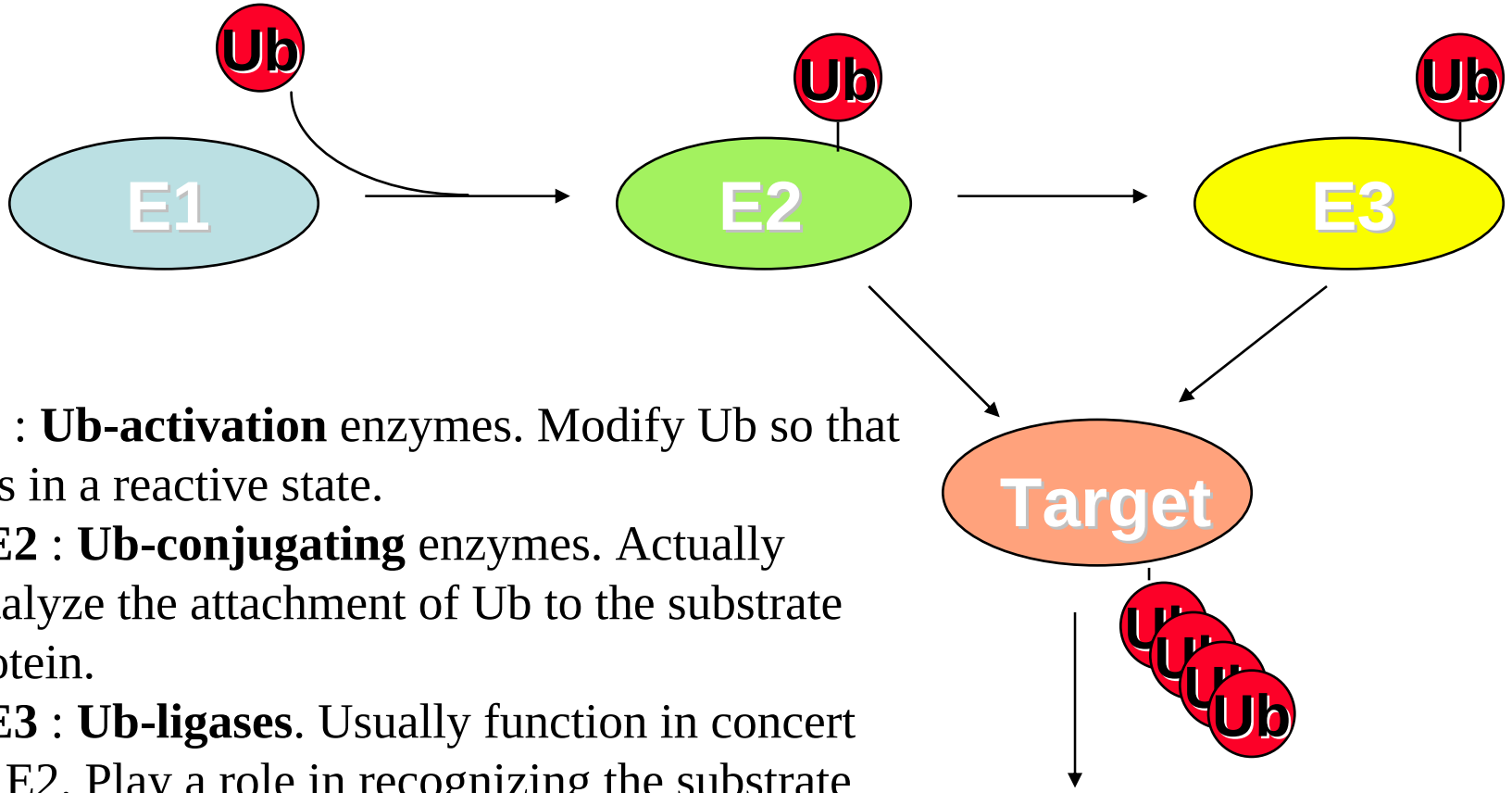
Regulation of:

- Cell cycle
- Differentiation & development
- Extracellular effectors
- Cell surface receptors & ion channels
- DNA repair
- Immune and inflammatory responses
- Biogenesis organelles

Proteins Targeted by Ubiquitin

- Cell cycle regulators
- Tumor suppressors & growth modulators
- Transcriptional activators & inhibitors
- Cell surface receptors
- Mutant and damaged proteins

The Ubiquitin Modification Pathway



E1 : **Ub-activation** enzymes. Modify Ub so that it is in a reactive state.

E2 : **Ub-conjugating** enzymes. Actually catalyze the attachment of Ub to the substrate protein.

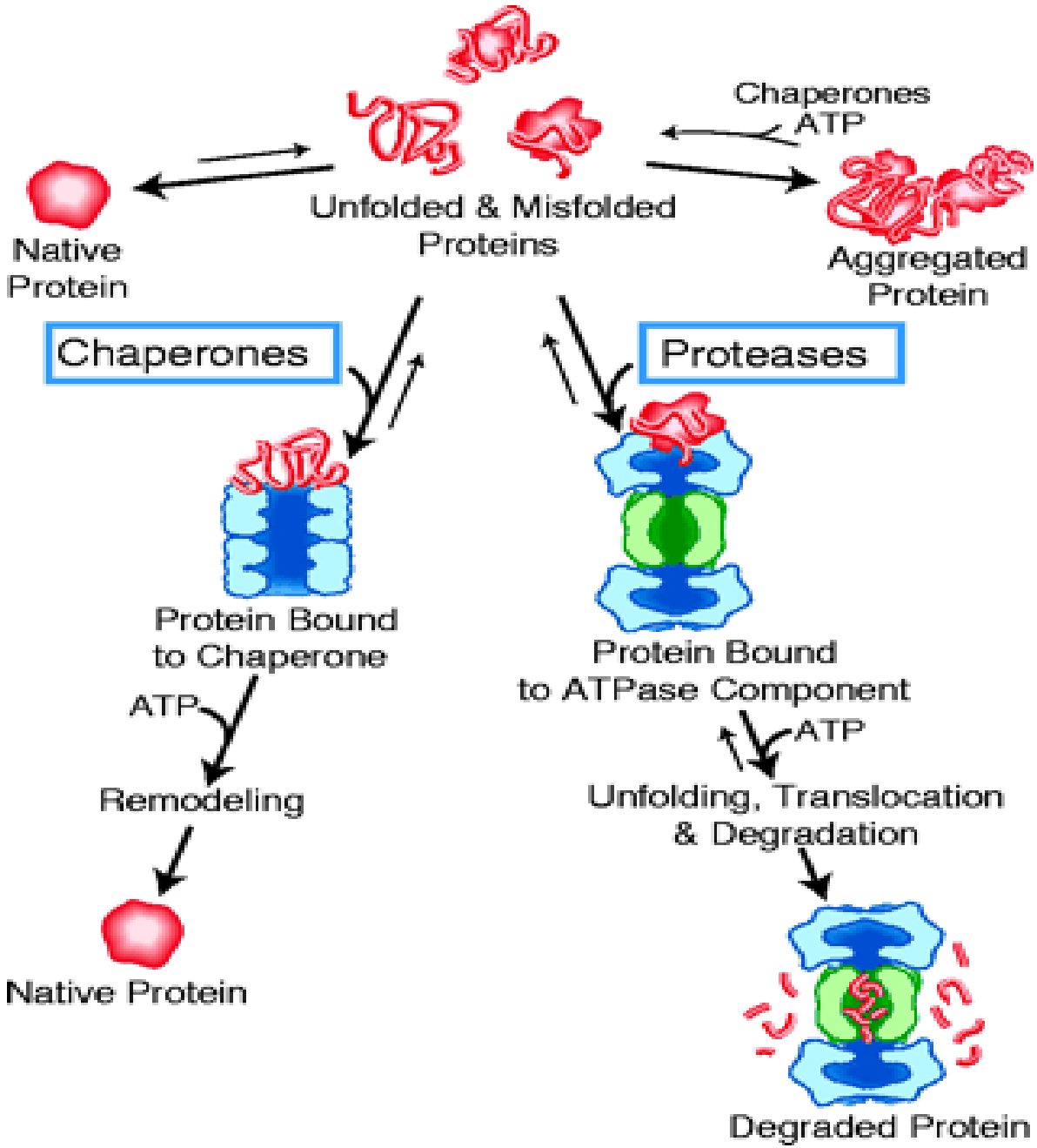
E3 : **Ub-ligases**. Usually function in concert w/ E2. Play a role in recognizing the substrate protein.

26s proteosome degradation

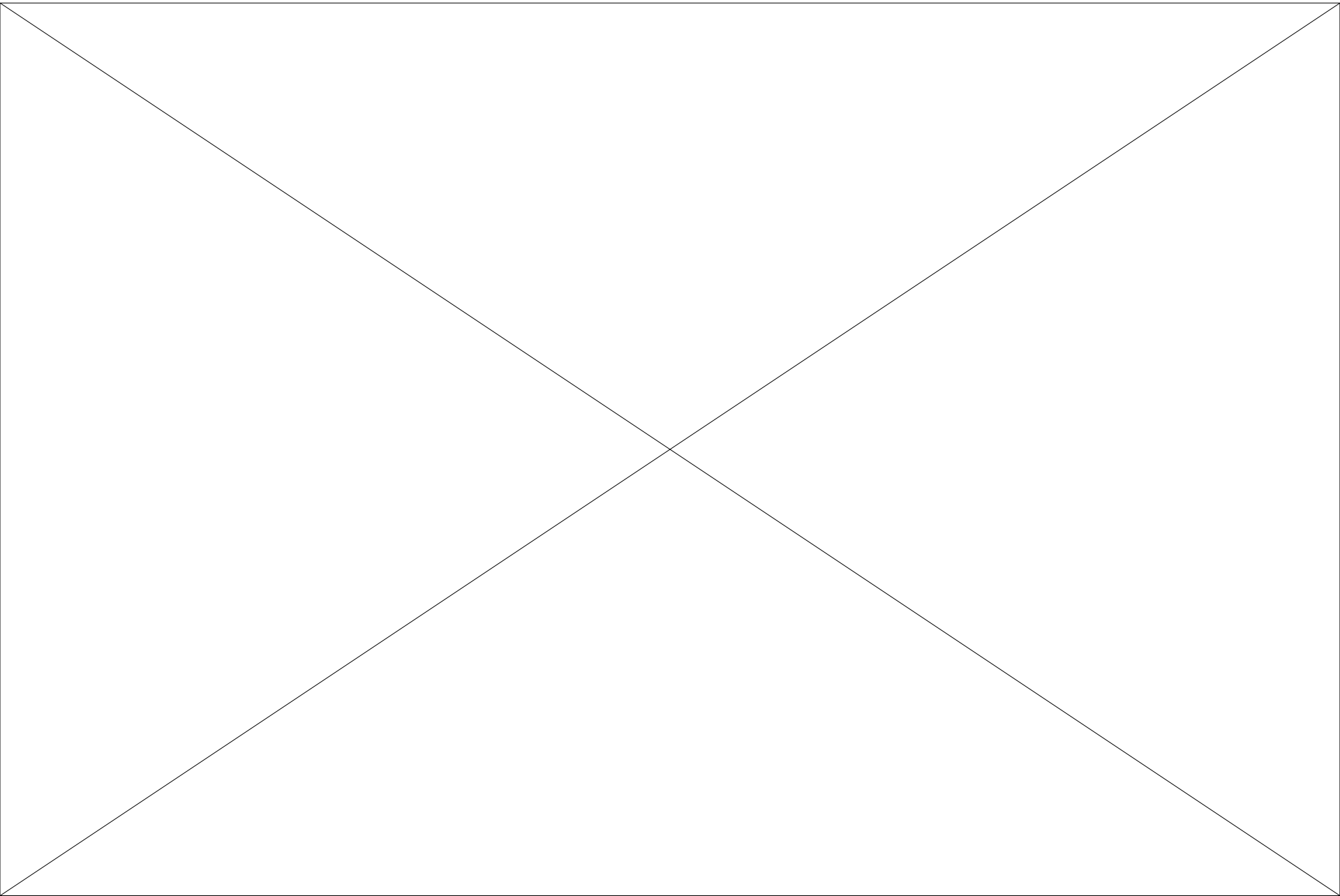
Ubiquitin Conjugation: A 3 Step Mechanism

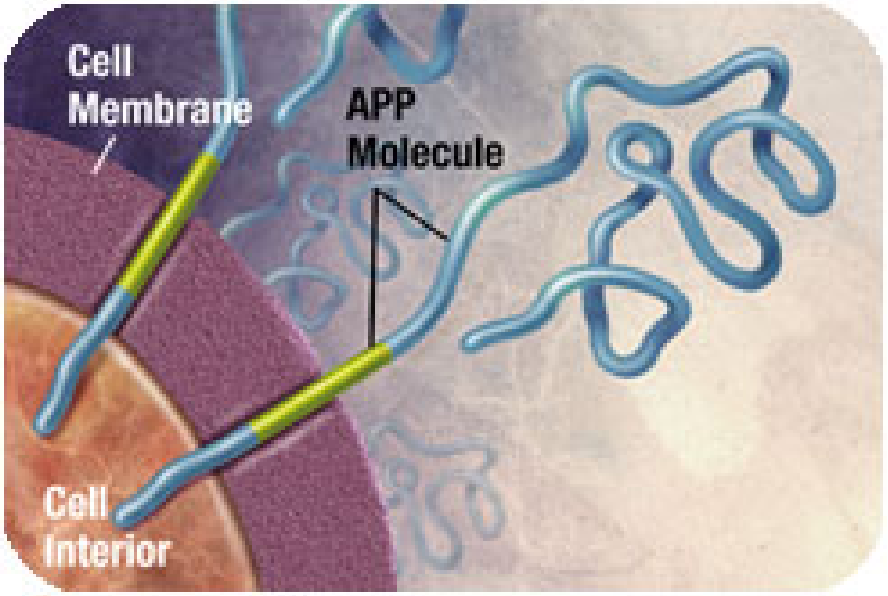
Ubiquitin (Ub) activating enzyme E_1	High energy thiol ester is formed between C-terminal Gly of ubiqutin and a Cys in the E_1 active site (ATP/AMP)
Ubiquitin conjugating enzymes E_2	Ub is transferred to a Cys of E_2 forming a new thiol ester
Ubiquitin ligase E_3	Ub forms isopeptide bond between C-terminal Gly of Ub and ε -amino group of Lys on a target protein

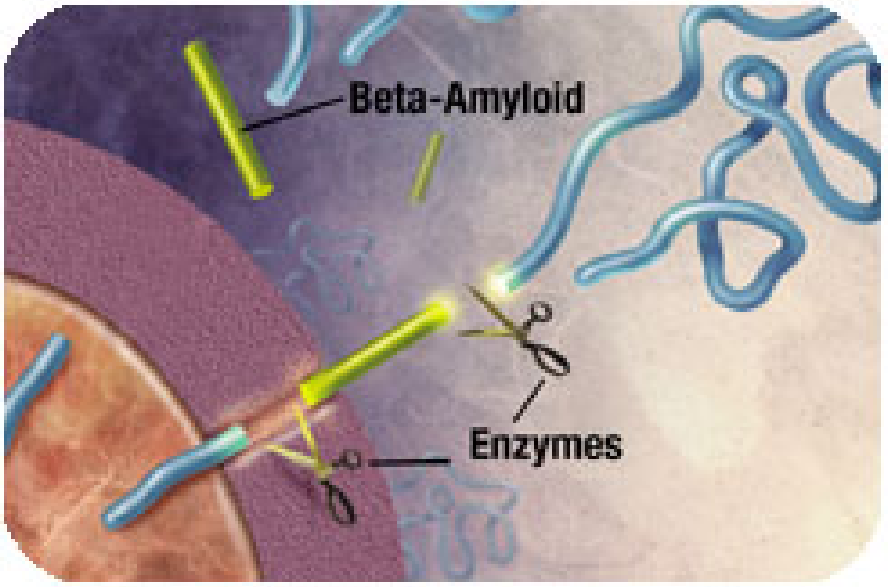
The life of protein

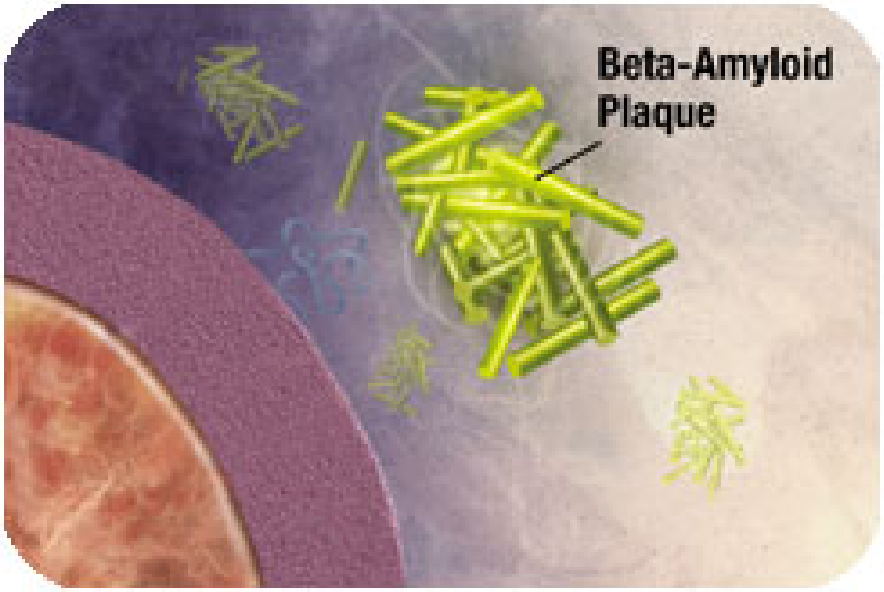


Life cycle of a protein







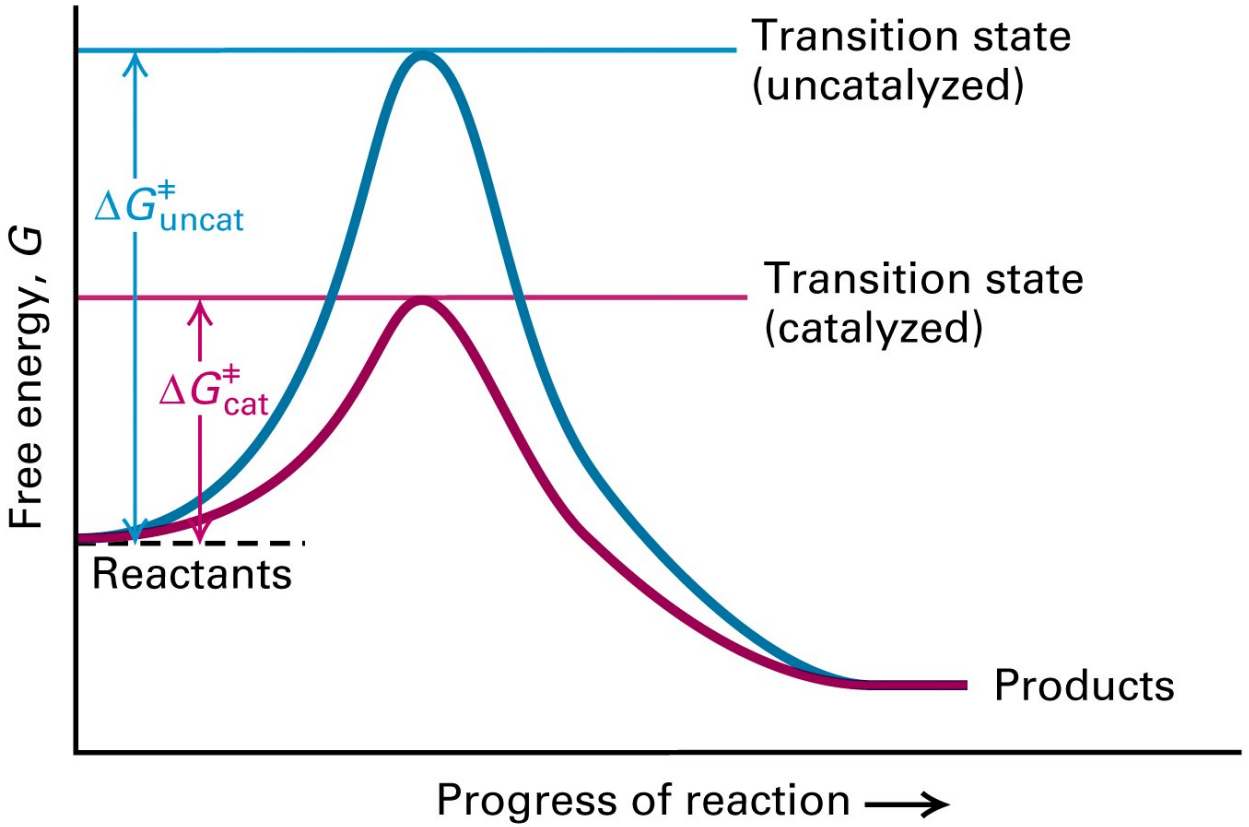


Beta-amyloid Plaques

Amyloid precursor protein (APP) is the precursor to amyloid plaque.

1. APP sticks through the neuron membrane.
2. Enzymes cut the APP into fragments of protein, including beta-amyloid.
3. Beta-amyloid fragments come together in clumps to form plaques.

Enzyme are highly efficient and specific catalysts

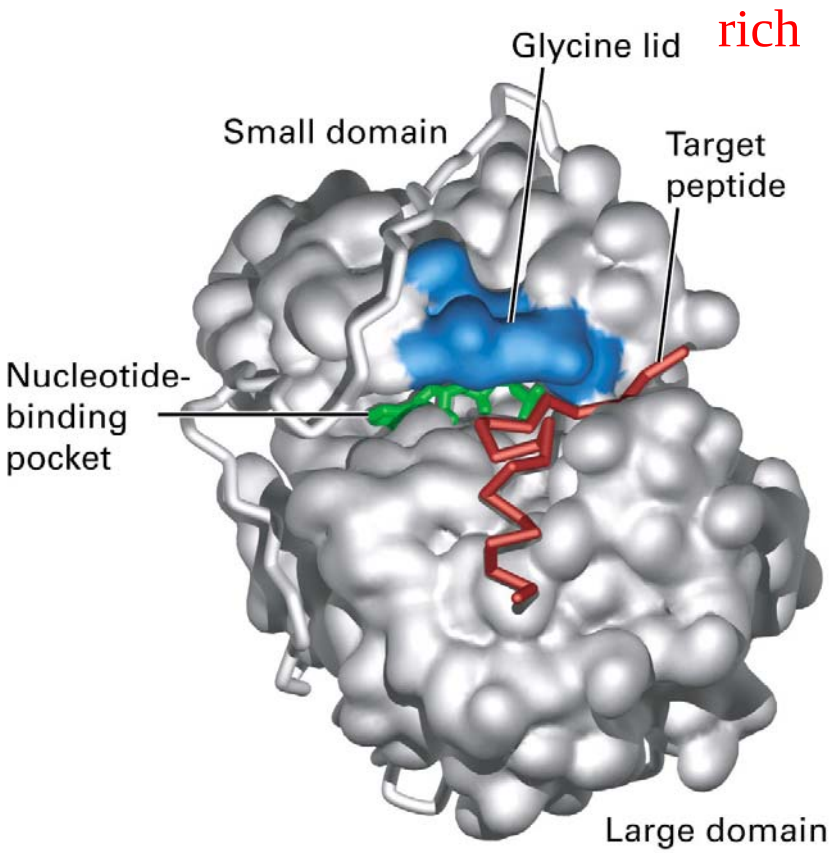


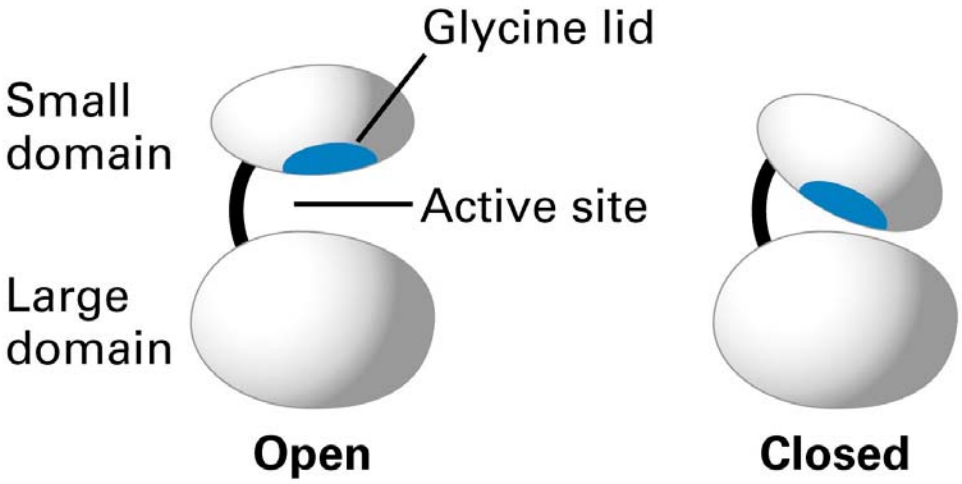
A reaction will take place spontaneously only if the total G of the products is less than that of reactants.

All chemical reactions→high energy transition state→ rate of reaction is inversely to G → So need enzyme for catalysts

Enzyme: formed form protein

Highly efficient and specific catalysts
An enzyme active site binds substrates and carries out catalysis
Active site: specific and chemical reaction site

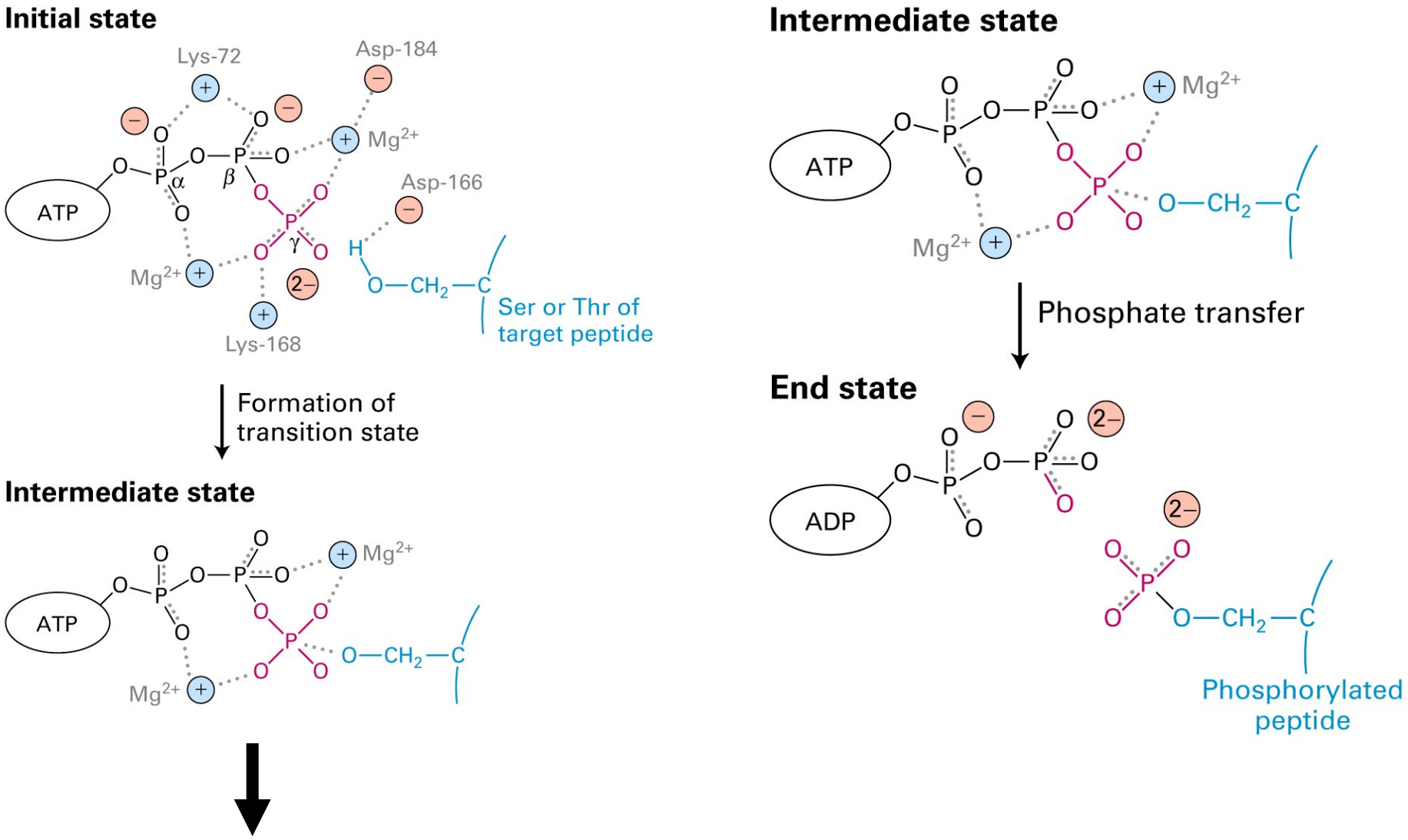


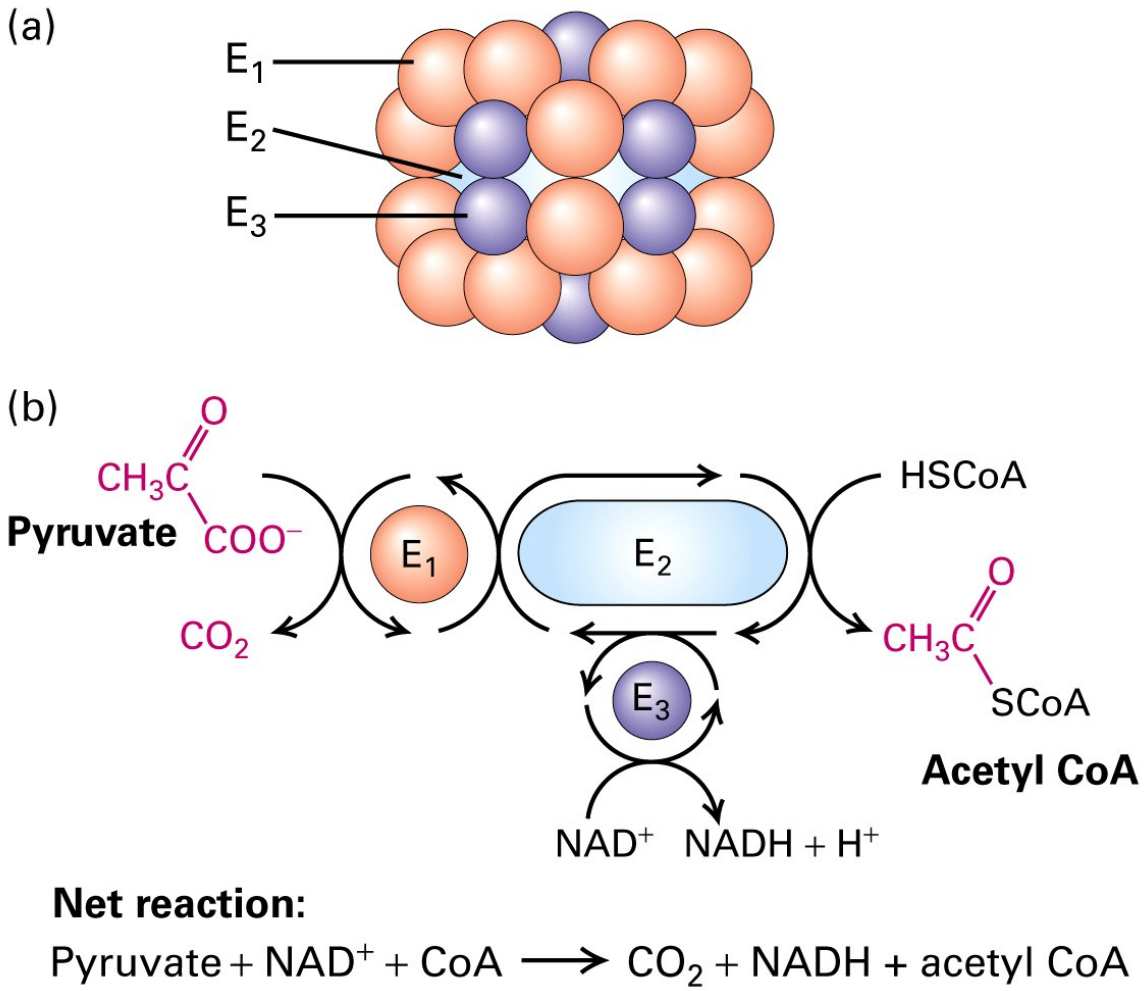


No ATP

Protein kinase A and conformational change induced by substrate binding

Mechanism of phosphorylation by protein kinase A





Structure and function of pyruvate dehydrogenase, a large multimeric enzyme complex that converts pyruvate into acetyl CoA

Molecular motors and mechanical work of cells

Needs energy into motion

Motor proteins (mechanochemical enzymes): generate the forces necessary for many cellular movements, cells depend on specialized enzymes commonly called motor proteins.

Motion types: 1. linear; 2. rotor

Three general properties of the activities of motor proteins:

1. Transduce a source of energy (ATP or ion gradient) for two types movement
2. Bind and translocate along a cytoskeletal filament, nucleic acids strand or protein complex
3. Move direction

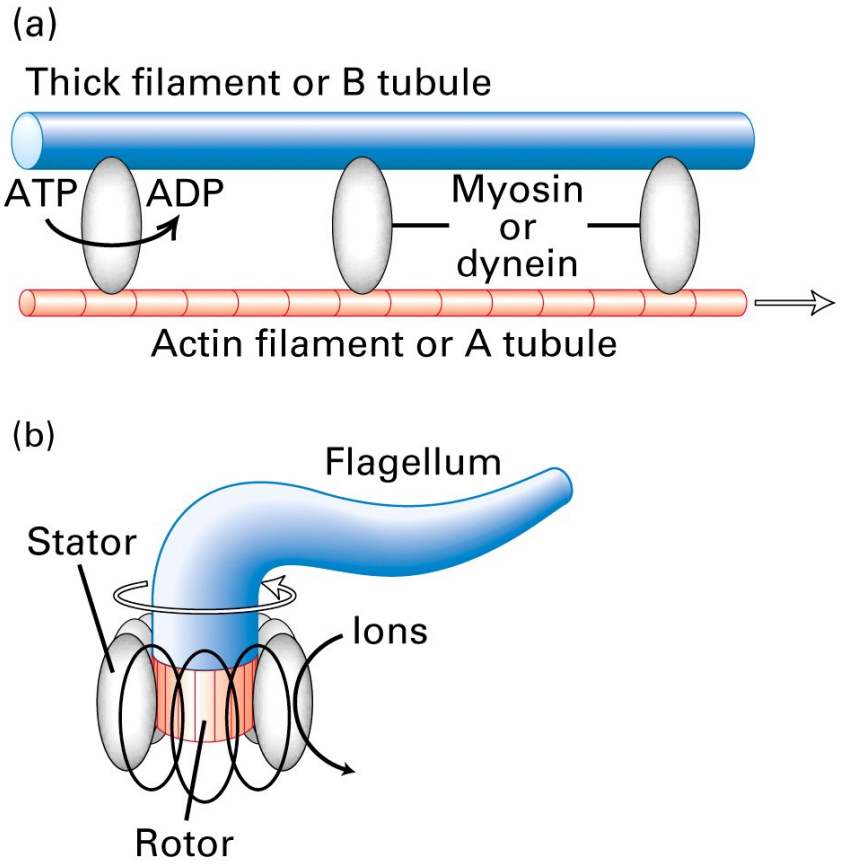


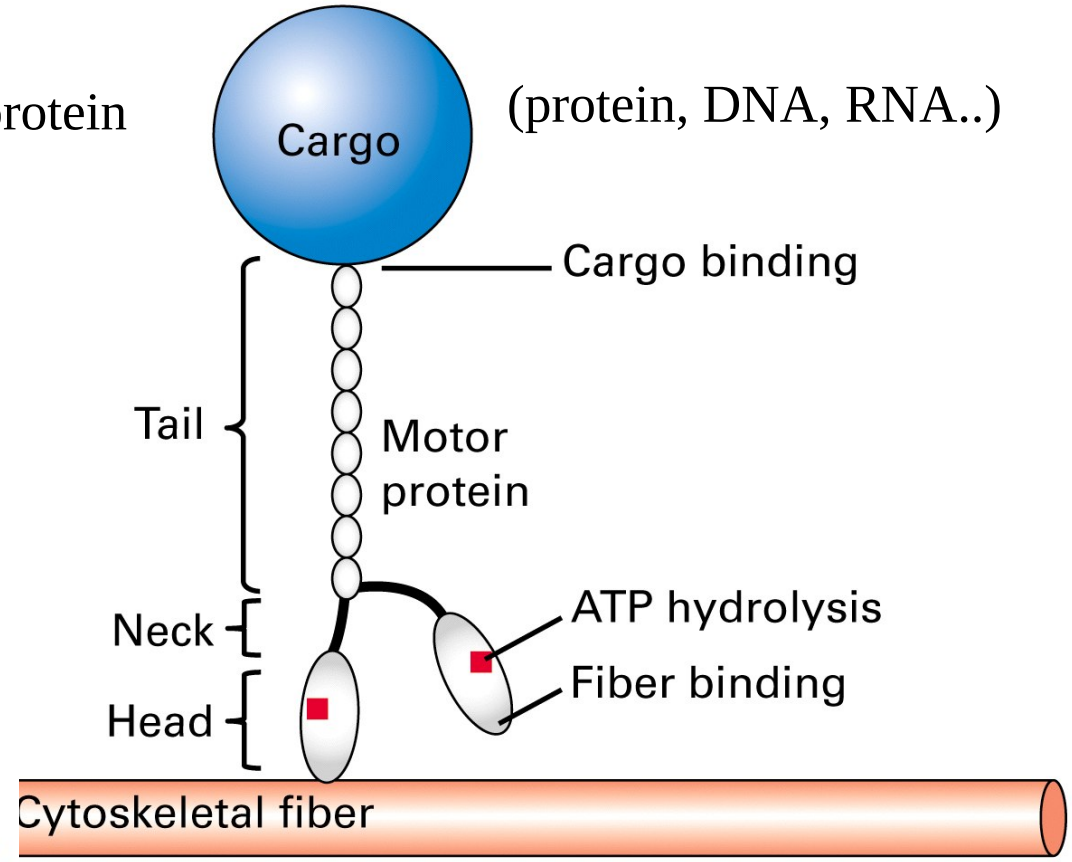
TABLE 3-2 Selected Molecular Motors

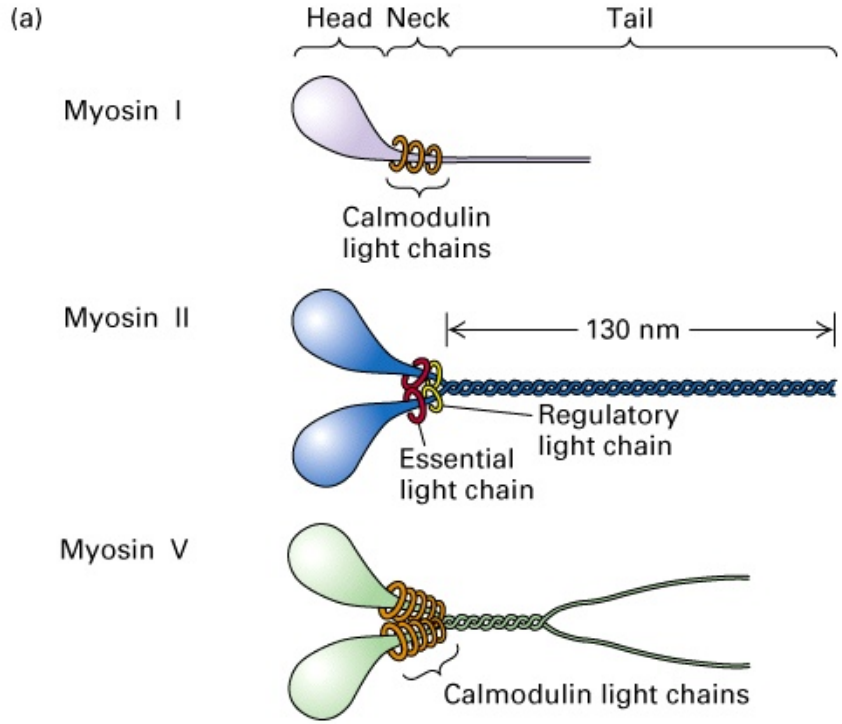
Motor*	Energy Source	Structure/Components	Cellular Location	Movement Generated
LINEAR MOTORS				
DNA polymerase (4)	ATP	Multisubunit polymerase δ within replisome	Nucleus	Translocation along DNA during replication
RNA polymerase (4)	ATP	Multisubunit polymerase within transcription elongation complex	Nucleus	Translocation along DNA during transcription
Ribosome (4)	GTP	Elongation factor 2 (EF2) bound to ribosome	Cytoplasm/ER membrane	Translocation along mRNA during translation
Myosins (3, 19)	ATP	Heavy and light chains; head domains with ATPase activity and microfilament-binding site	Cytoplasm	Transport of cargo vesicles; contraction
Kinesins (20)	ATP	Heavy and light chains; head domains with ATPase activity and microtubule-binding site	Cytoplasm	Transport of cargo vesicles and chromosomes during mitosis
Dyneins (20)	ATP	Multiple heavy, intermediate, and light chains; head domains with ATPase activity and microtubule-binding site	Cytoplasm	Transport of cargo vesicles; beating of cilia and eukaryotic flagella
ROTARY MOTORS				
Bacterial flagellar motor	H^+/Na^+ gradient	Stator and rotor proteins, flagellum	Plasma membrane	Rotation of flagellum attached to rotor
ATP synthase, $F_0F_1(8)$	H^+ gradient	Multiple subunits forming F_0 and F_1 particles	Inner mitochondrial membrane, thylakoid membrane, bacterial plasma membrane	Rotation of γ subunit leading to ATP synthesis
Viral capsid motor	ATP	Connector, prohead RNA, ATPase	Capsid	Rotation of connector leading to DNA packaging

*Numbers in parentheses indicate chapters in which various motors are discussed.

Motor protein-dependent movement of cargo

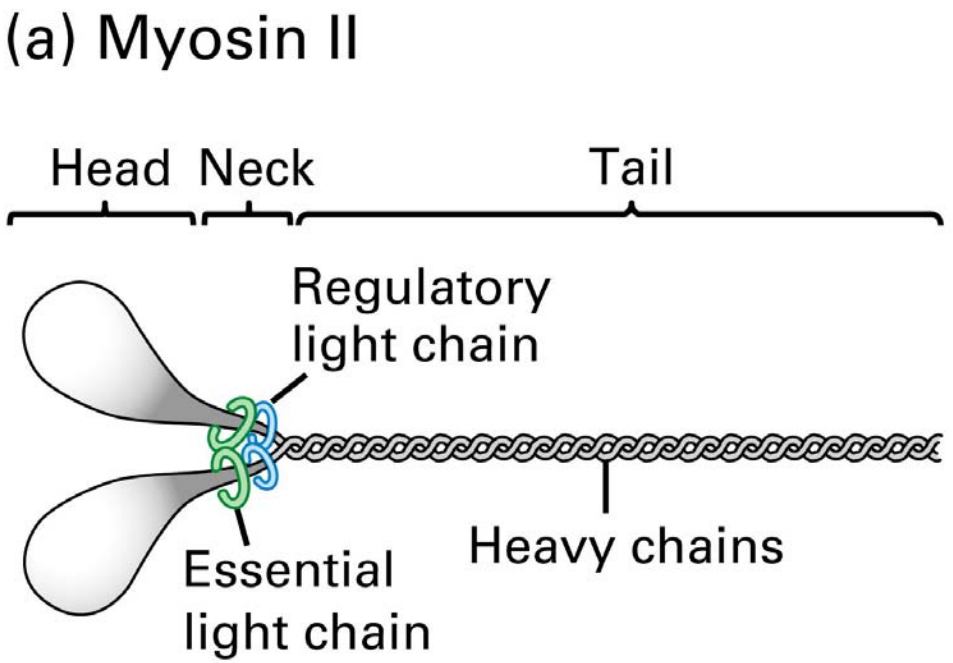
Head: myosin, dynein, kinesin motor protein

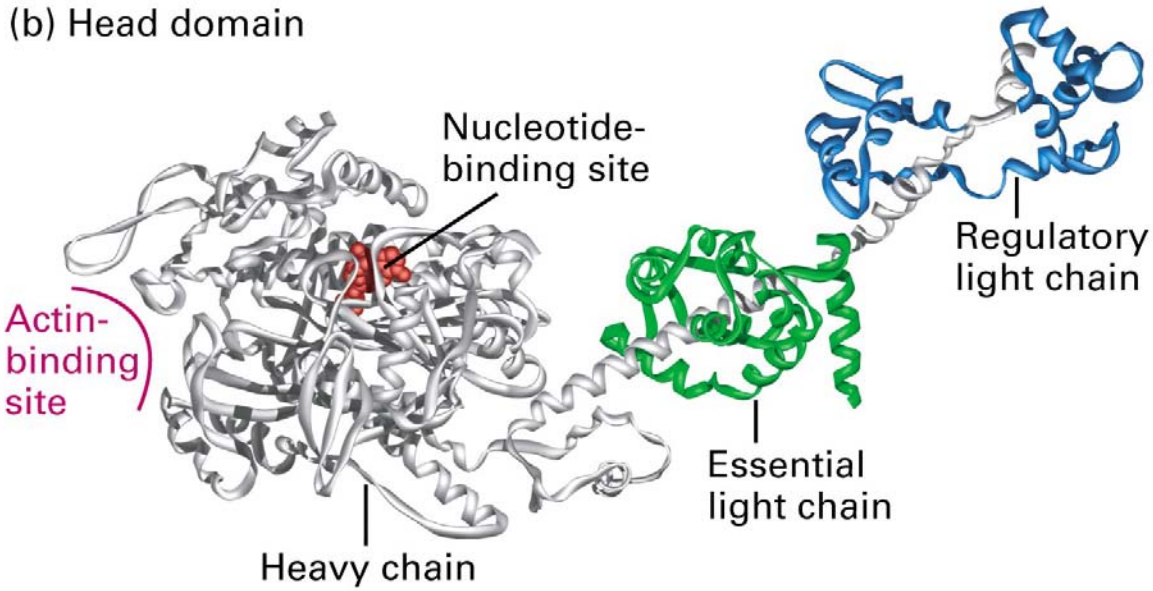




All myosins have head, neck, and tail domains with distinct functions

Conformational changes in the myosin head couple ATP hydrolysis to movement

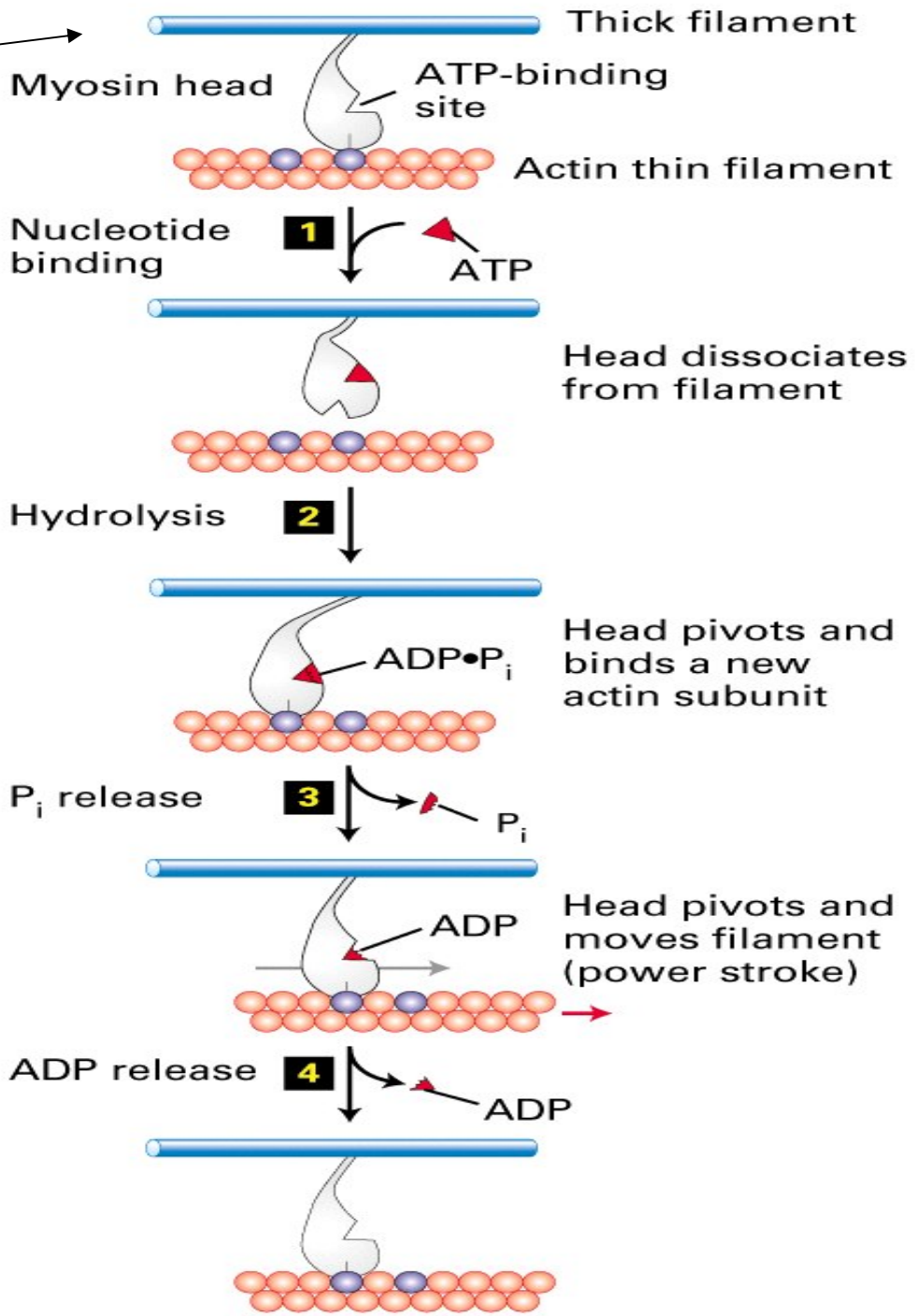




May be other cargo

1. Binding ATP → disrupting actin-binding site
2. Hydrolysis ATP → head, conformational change → move to new position → rebind
3. Pi release → head conformation second change → move the actin
4. Release ATP → new cycle

flash



Functional design of proteins

Protein function generally involves conformational changes

Proteins are designed to bind a range of molecules (ligands)

- Binding is characterized by two properties: affinity and specificity

Antibodies exhibit precise ligand-binding specificity

Enzymes are highly efficient and specific catalysts

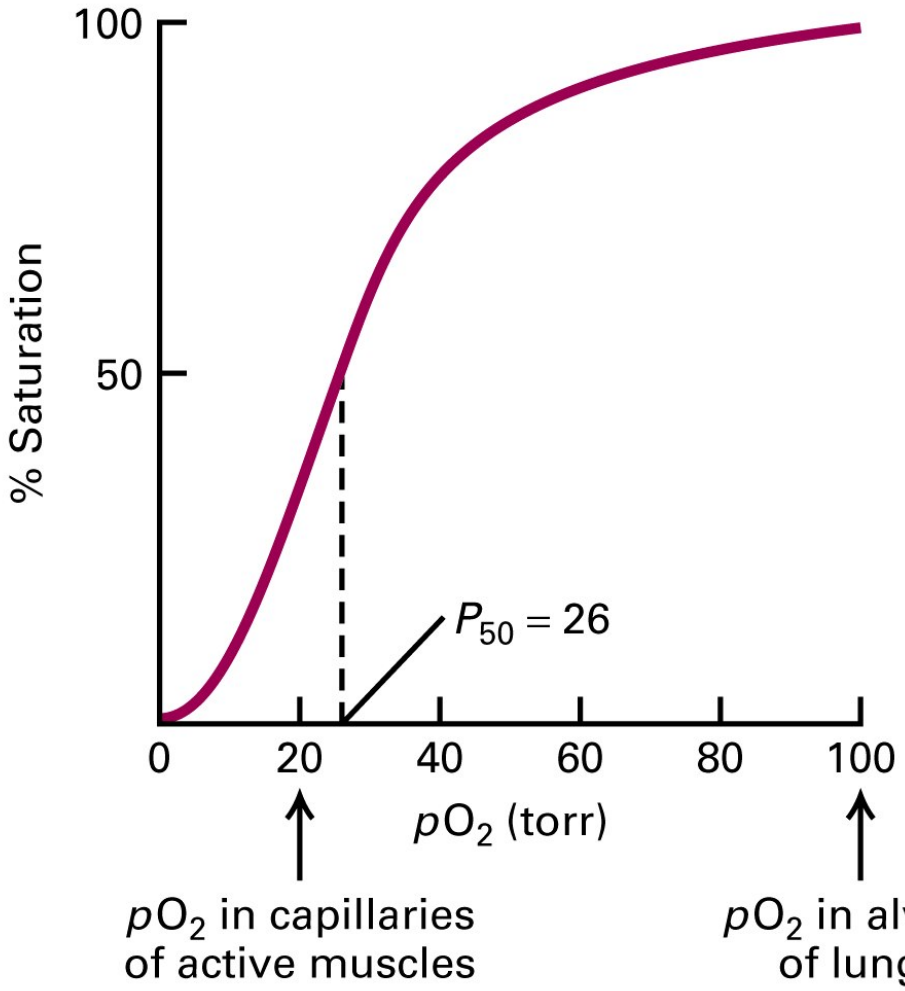
- An enzyme’s active site binds substrates and carries out catalysis

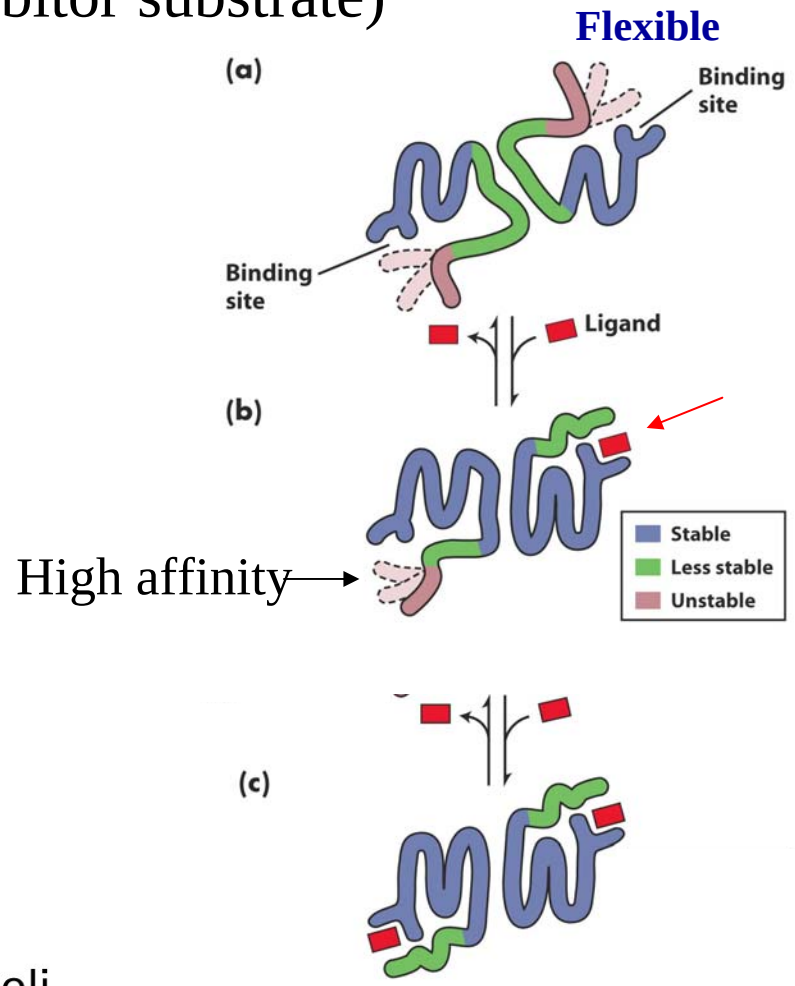
Mechanisms that regulate protein function

- Allosteric transitions
  - Release of catalytic subunits, active ⌦ inactive states, cooperative binding of ligands
- Phosphorylation ⌦ dephosphorylation
- Proteolytic activation
- Compartmentalization

異位性調節

Allostey: any change in a protein's 3 or 4 structure or both induced by the binding of a ligand (activator, inhibitor substrate)





結合一個後→讓另一個更容易結合 **(postive regulation)**

釋放一個後→讓另一個結合力下降→更容易釋放

Allosteric protein – a protein in which the binding of a ligand to one site affects the binding properties of another site on the same protein (also called induced fit model). The conformational changes induced by the **modulator(s)** interconvert more-active and less-active forms of the protein.

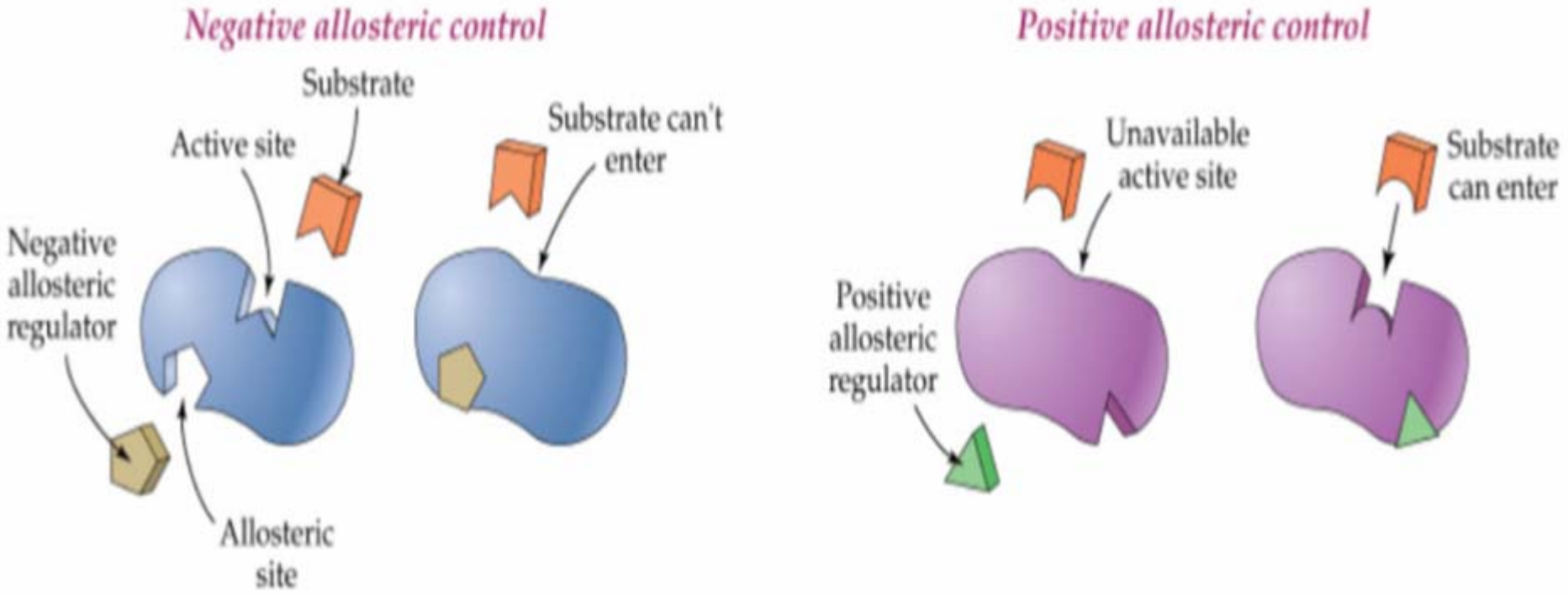
The modulators for allosteric proteins may be either **inhibitors** or **activators**

allos --- other
stereos --- solid or shape
Homotropic interaction --- liagnd = modulator
Heterotropic interaction --- ligand = modulator

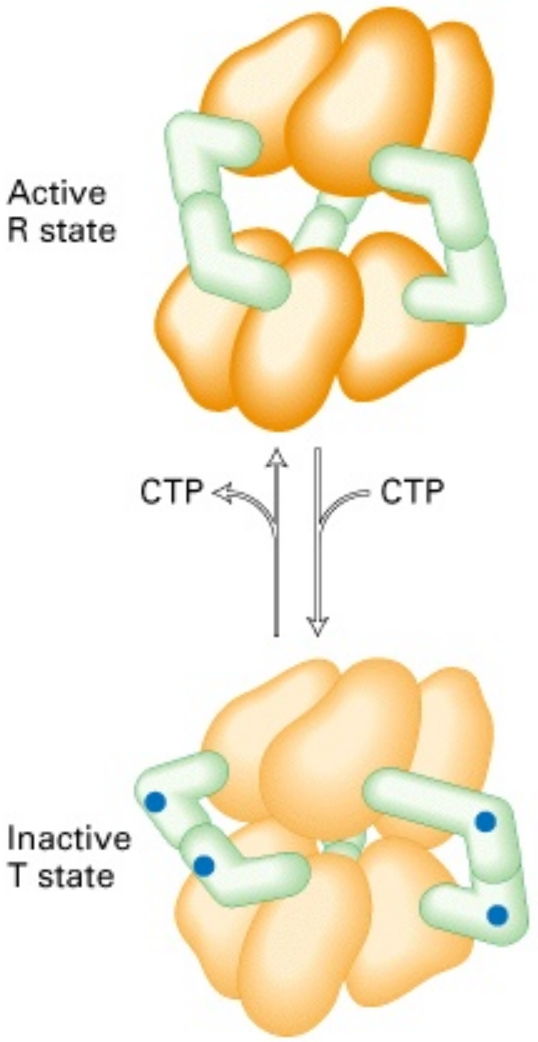
O_2 --- as both a normal ligand and an activating homotropic modulator for Hb

\

<u>Allosteric control:</u> either an activator or inhibitor acts on a portion of the enzyme <u>other than the active site</u> to regulate enzyme function.



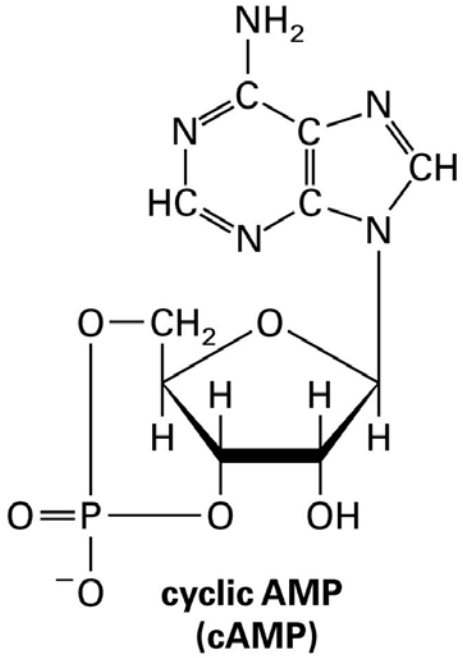
Allosteric transition between active and inactive states

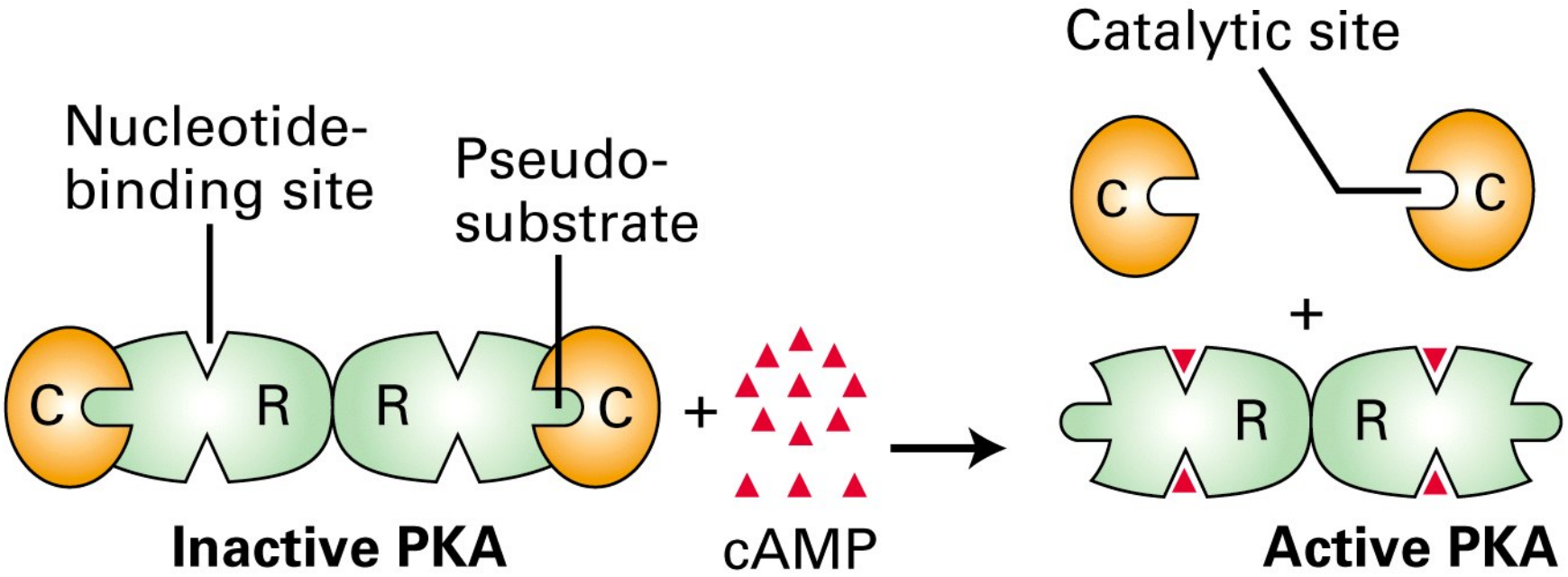


Allosteric release of catalytic subunits

Ligand-indced activation of protein kinase

Ligand binding can induce allosteric release of catalytic subunits or transition to a state with different activity

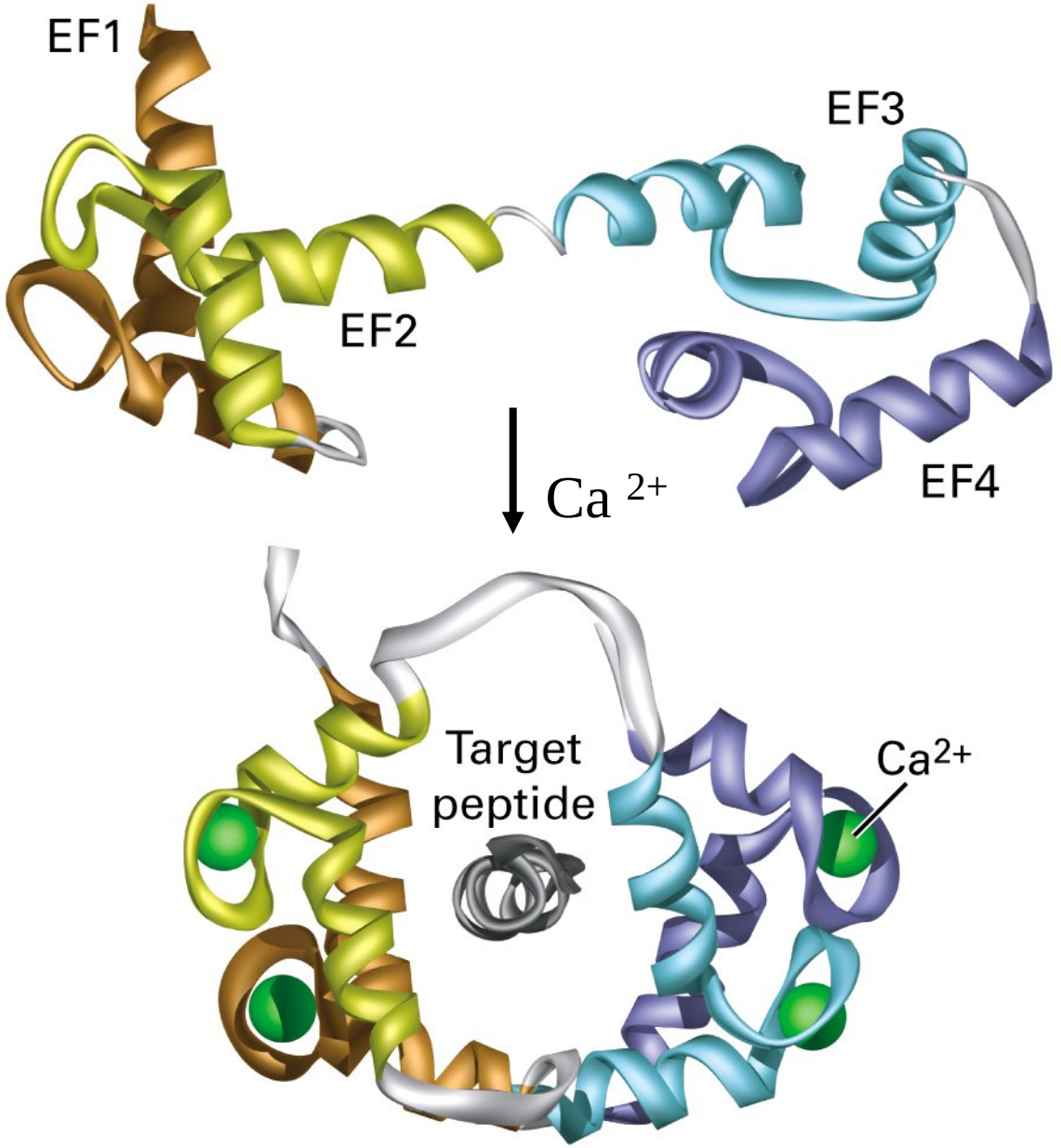




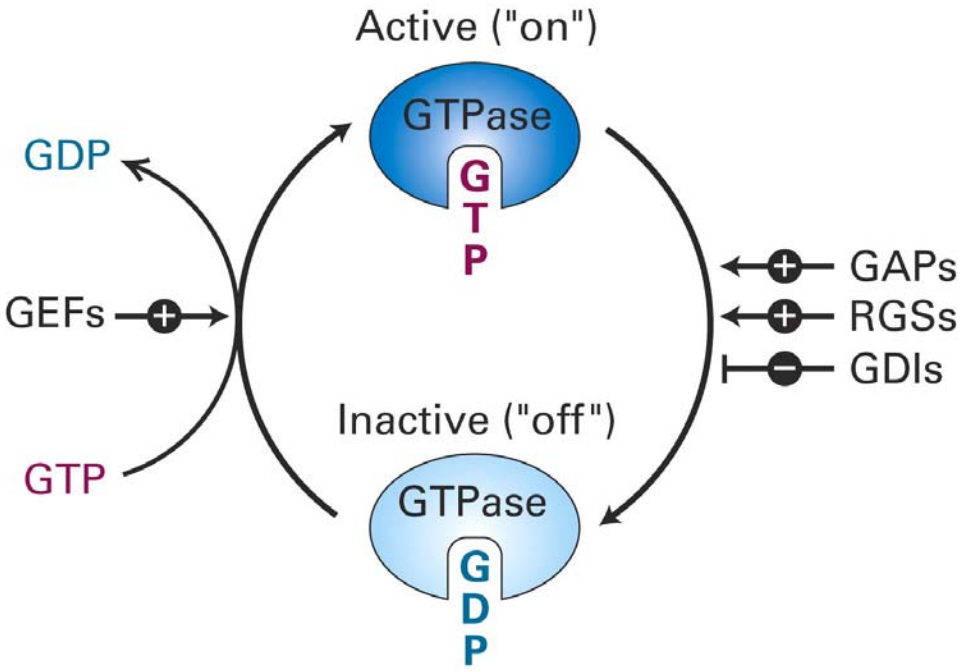
Switch mediated by Ca^{2+}/calmodulin

In normal condition:
- cytosolic calcium is low 10^{-7} M by ER or pump.

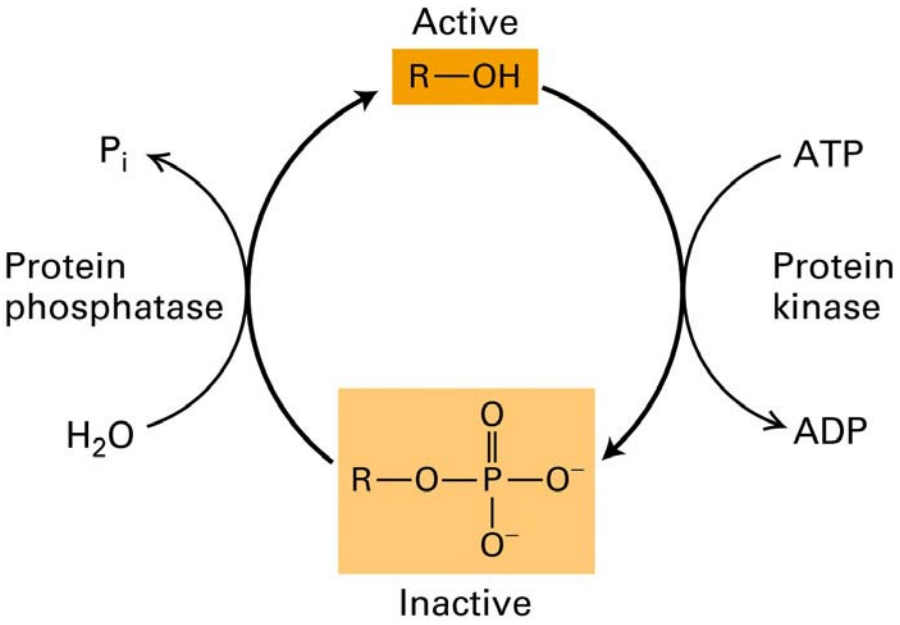
ER release calcium to
- 10-100 fold → sense
- calmodulin →
- conformal change →
- regulated other protein or molecule



Cycling of GTPase switch proteins between the active and inactive forms



Regulation of protein activity by kinase/phosphatase switch



Purifying, detecting, and characterizing proteins

A protein must be purified to determine its structure and mechanism of action

Molecules, including proteins, can be separated from other molecules based on differences in physical and chemical properties

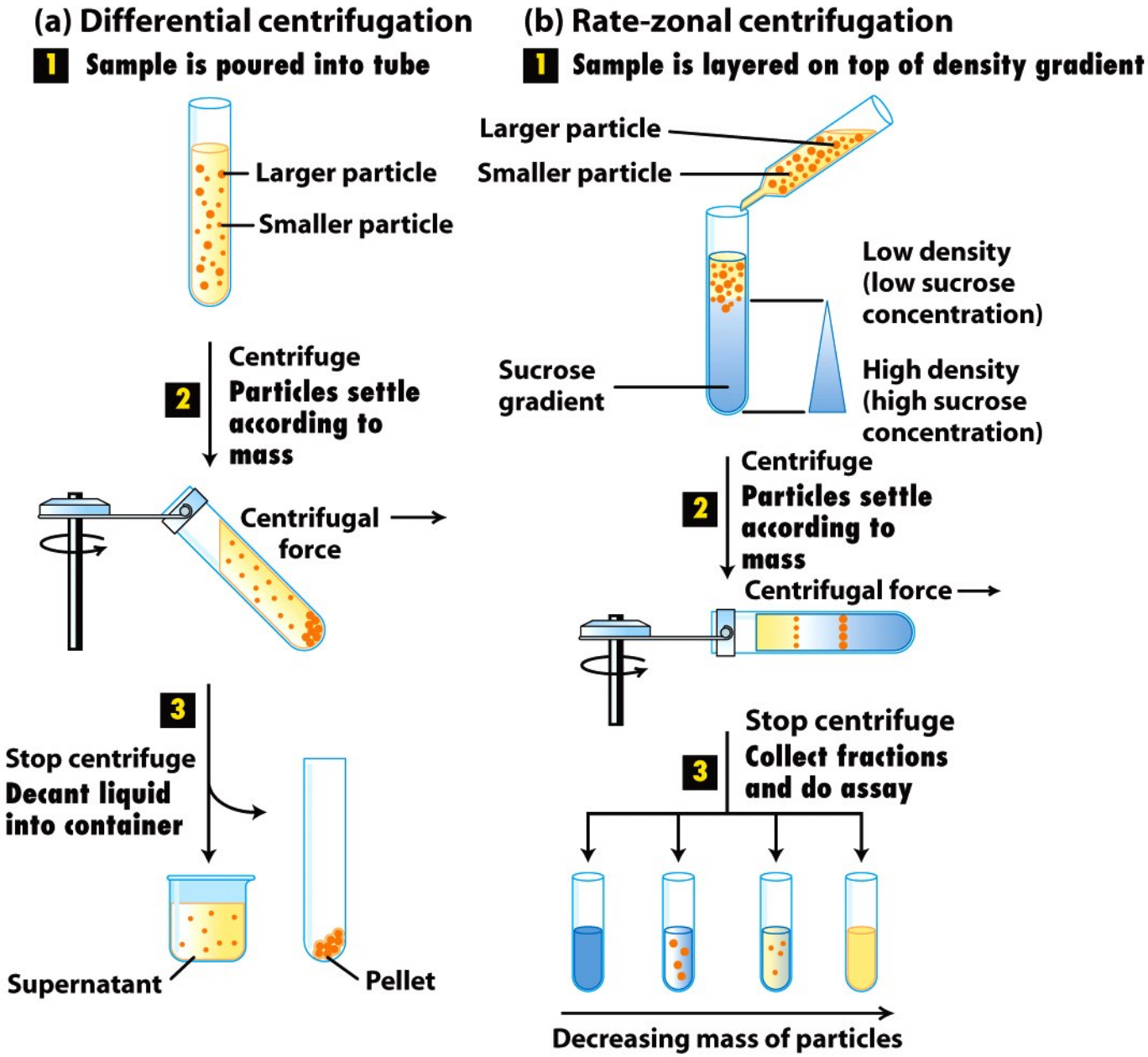


Figure 3-34
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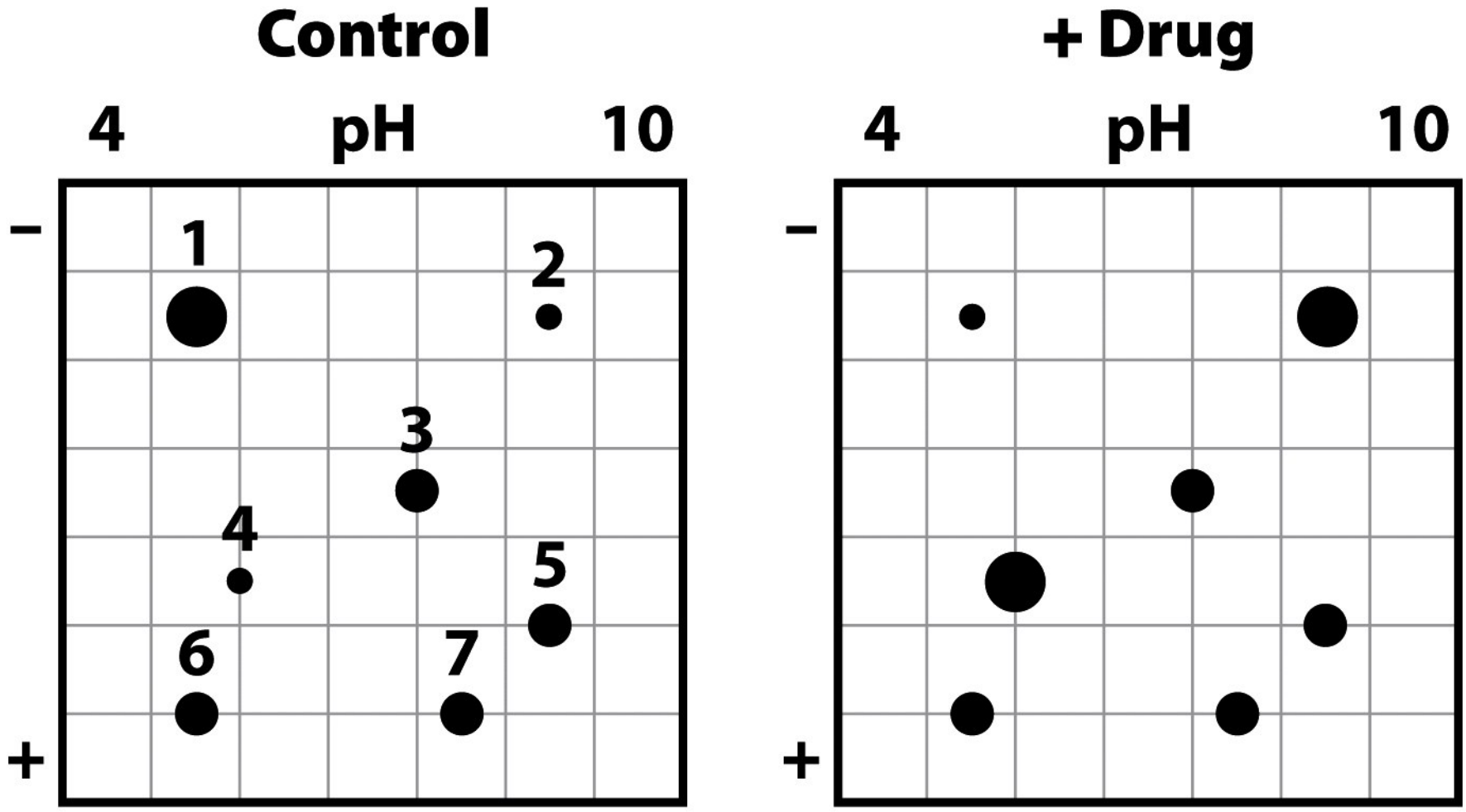

Analyze the Data 3-1
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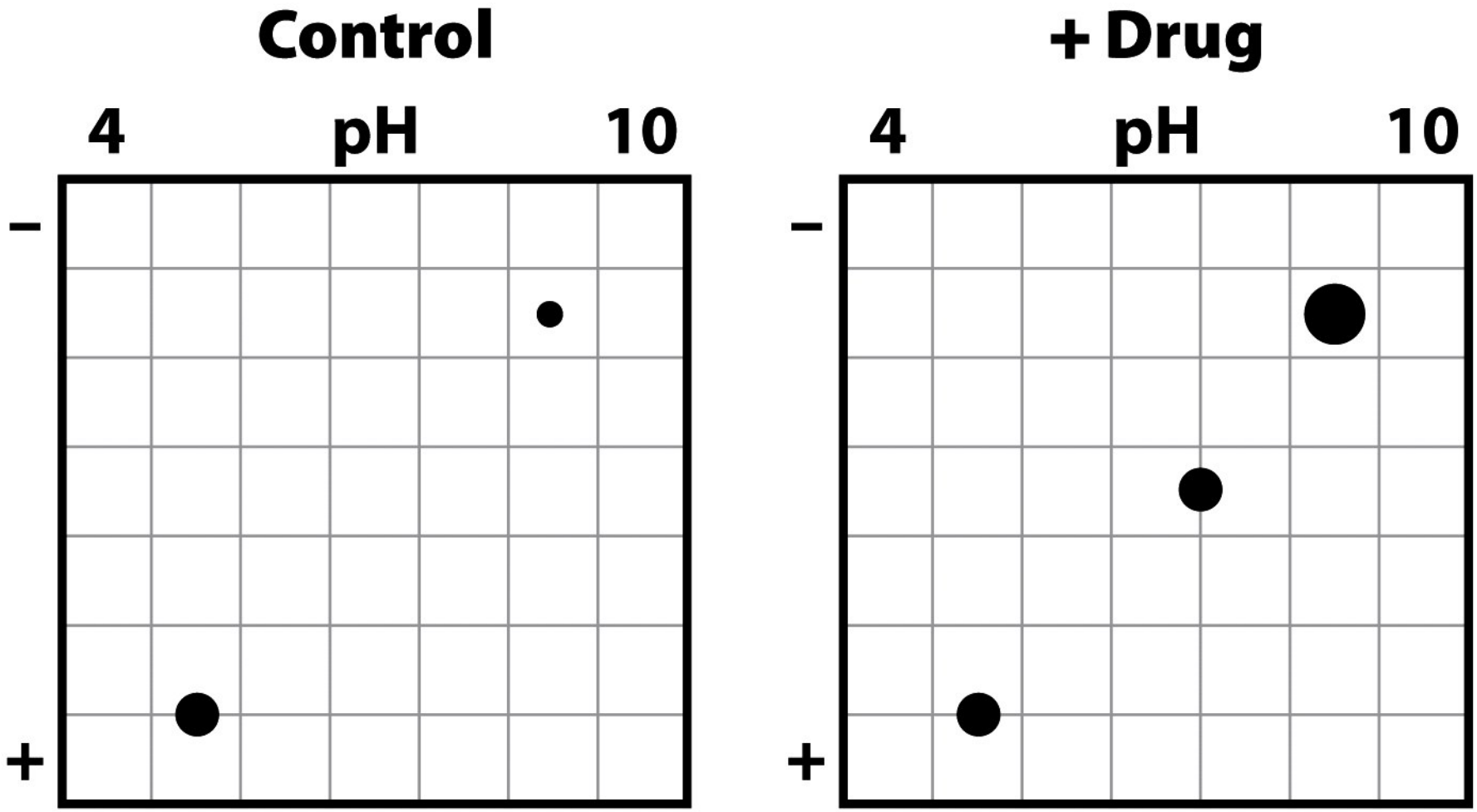

Analyze the Data 3-2
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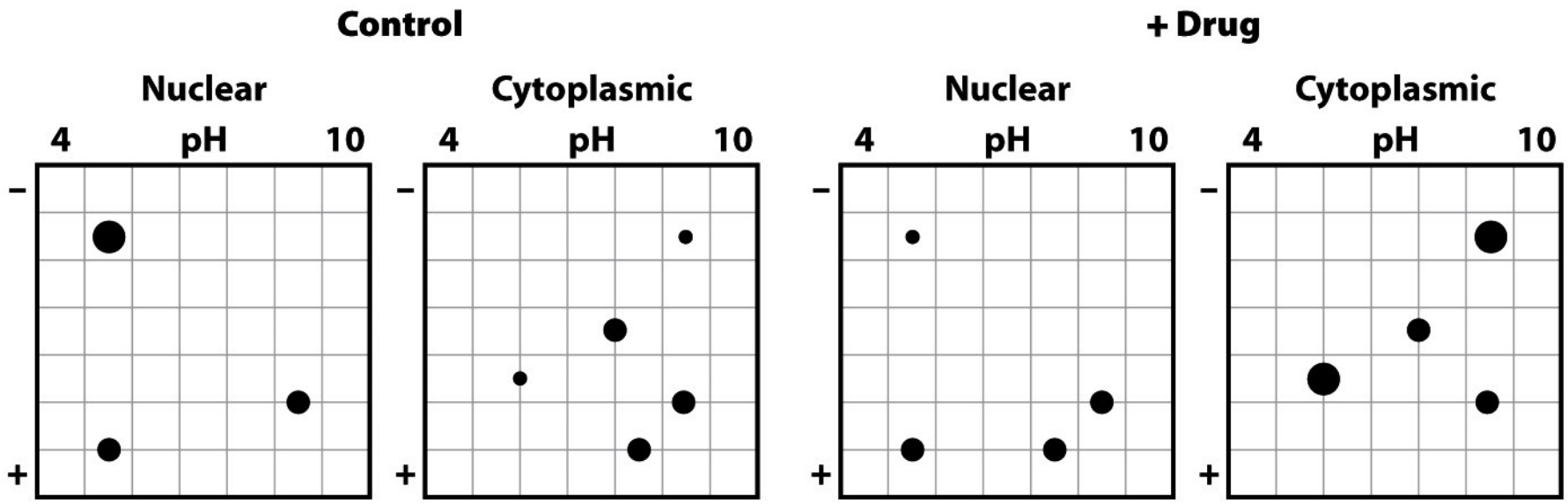

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