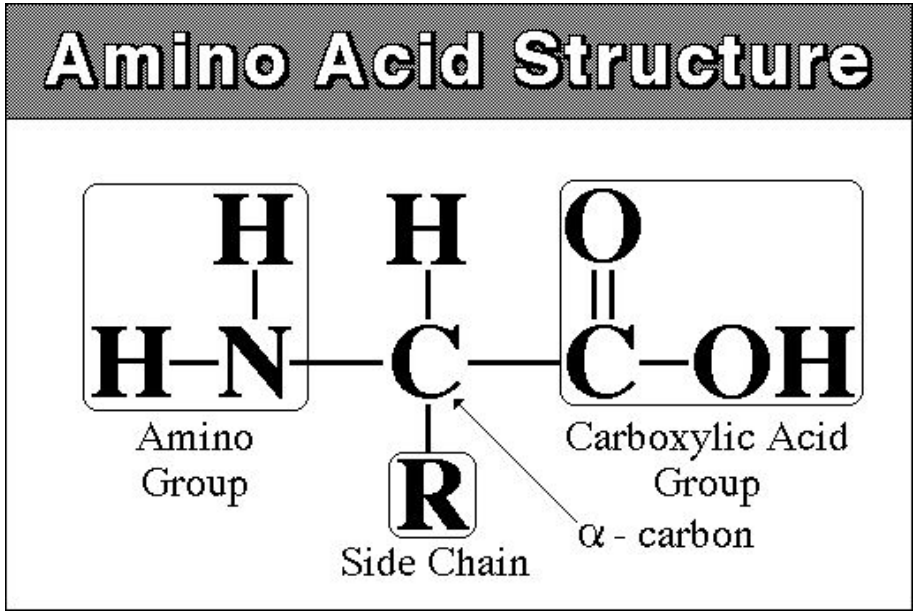
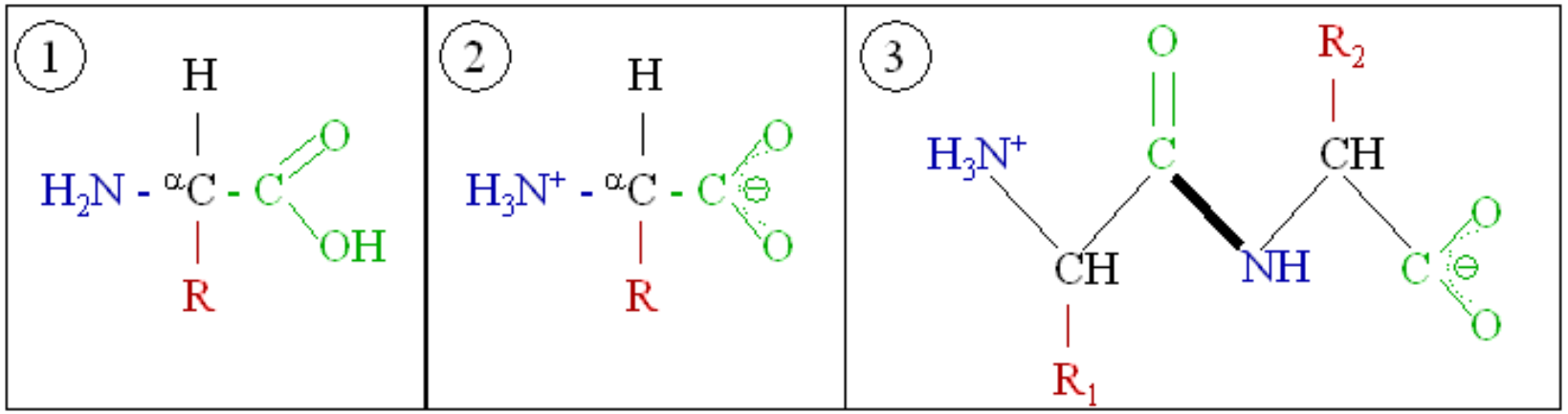
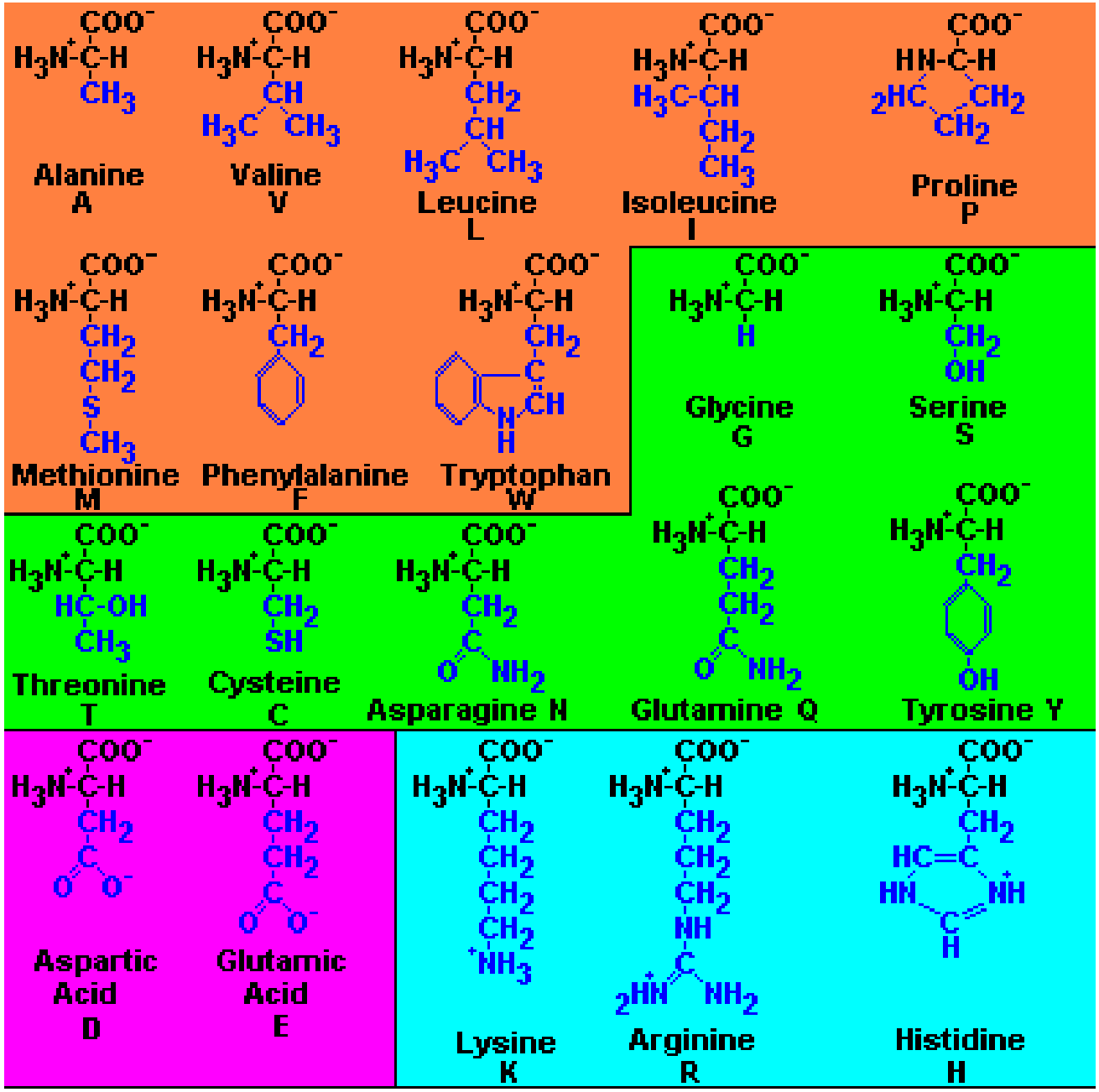
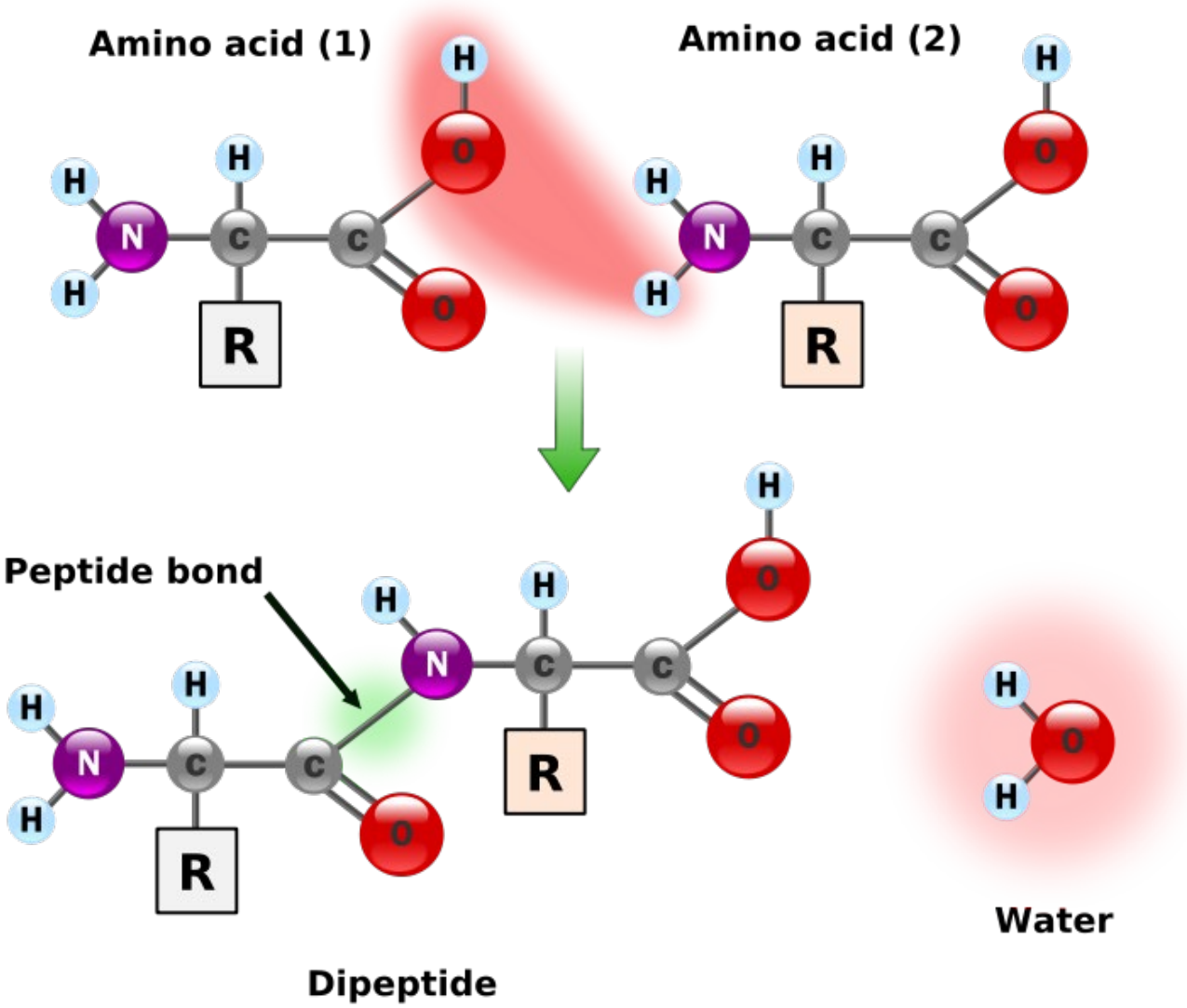
# Amino Acid


Amino Acid Structure
H
H
O
H–N
C
C–OH
Amino Group
Carboxylic Acid Group
R
α - carbon
Side Chain


1
H
$H_2N$ - $^{\alpha}C$ - C
O
OH
R
2
H
$H_3N^+$ - $^{\alpha}C$ - C
O
O
R
3
O
$R_2$
$H_3N^+$
C
CH
CH
NH
C
O
O
$R_1$

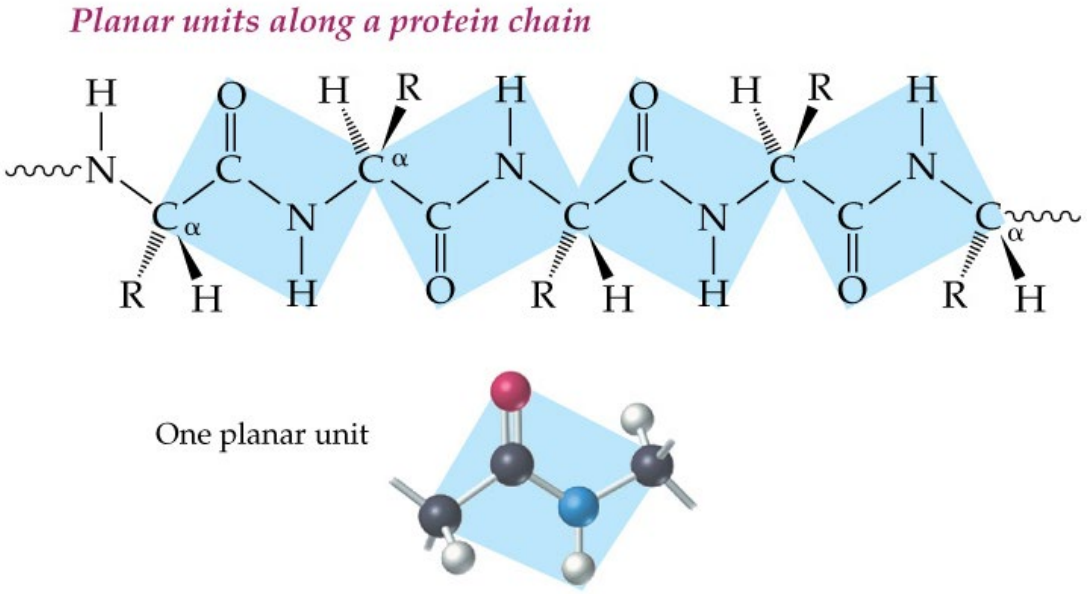

Alanine
A
Valine
V
Leucine
L
Isoleucine
I
Proline
P
Methionine
M
Phenylalanine
F
Tryptophan
W
Glycine
G
Serine
S
Threonine
T
Cysteine
C
Asparagine N
Glutamine Q
Tyrosine Y
Aspartic
Acid
D
Glutamic
Acid
E
Lysine
K
Arginine
R
Histidine
H

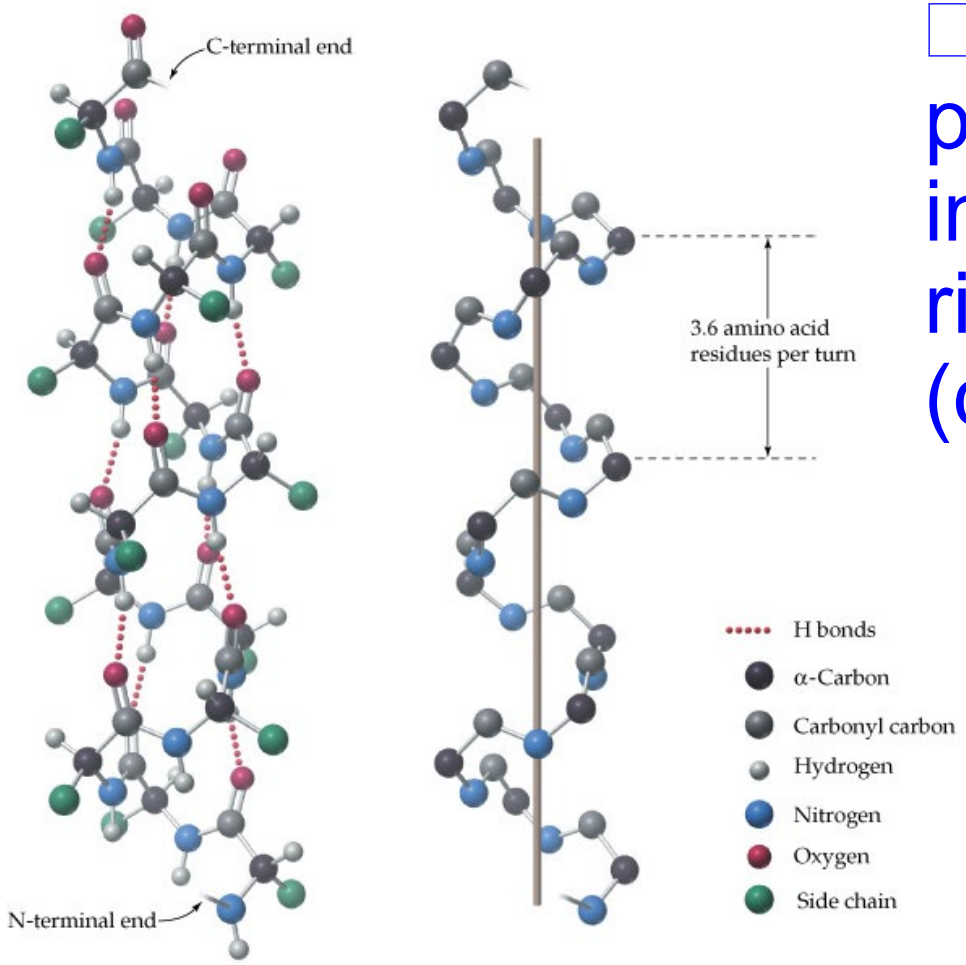
# Peptide bond



# Primary Protein Structure

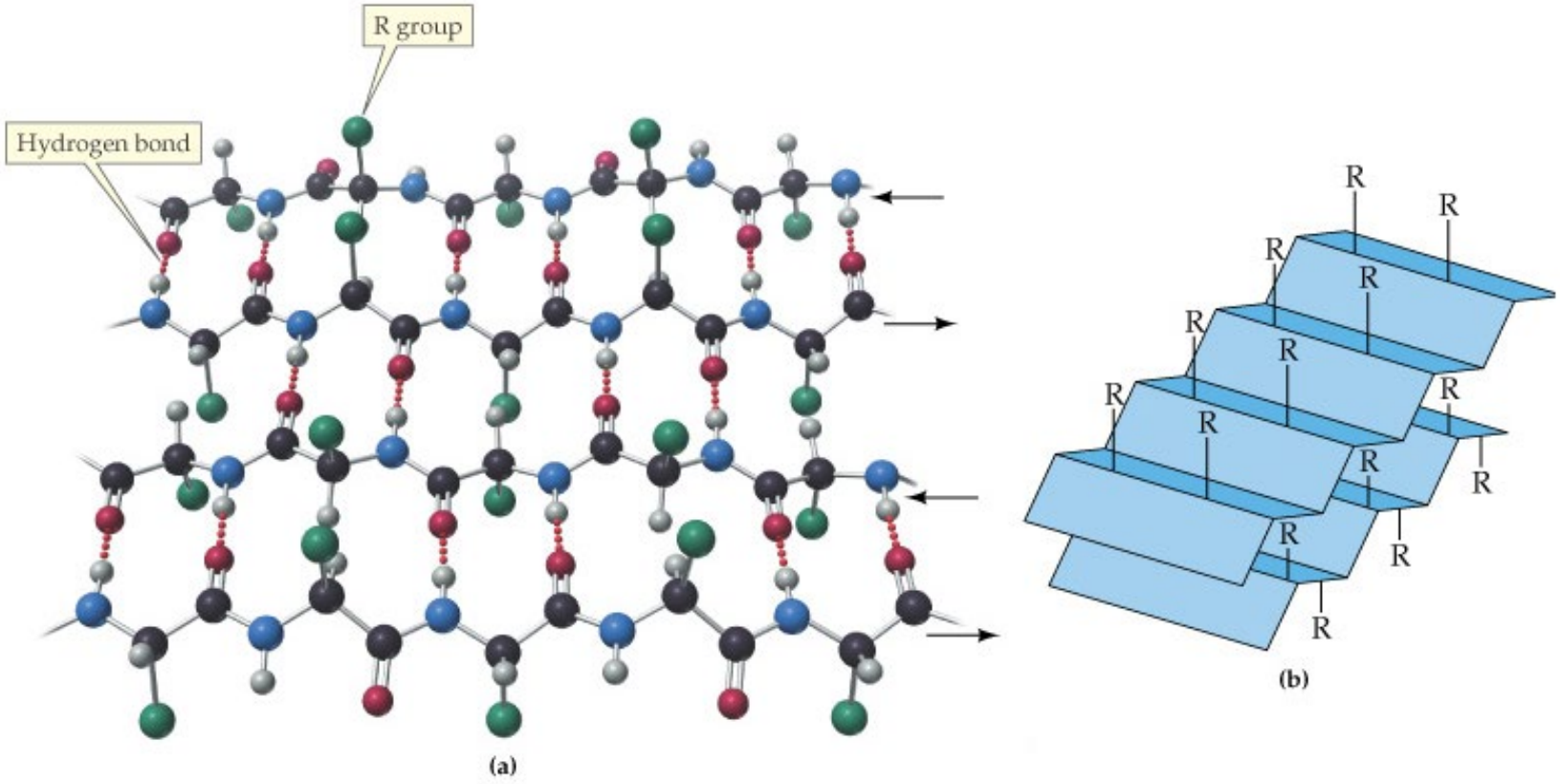
- Primary structure of a proteins is the sequence of amino acids connected by peptide bonds. Along the backbone of the proteins is a chain of alternating peptide bonds and $\alpha$-carbons and the amino acid side chains are connected to these






- ***α-Helix:*** A single protein chain coiled in a spiral with a right-handed (clockwise) twist.

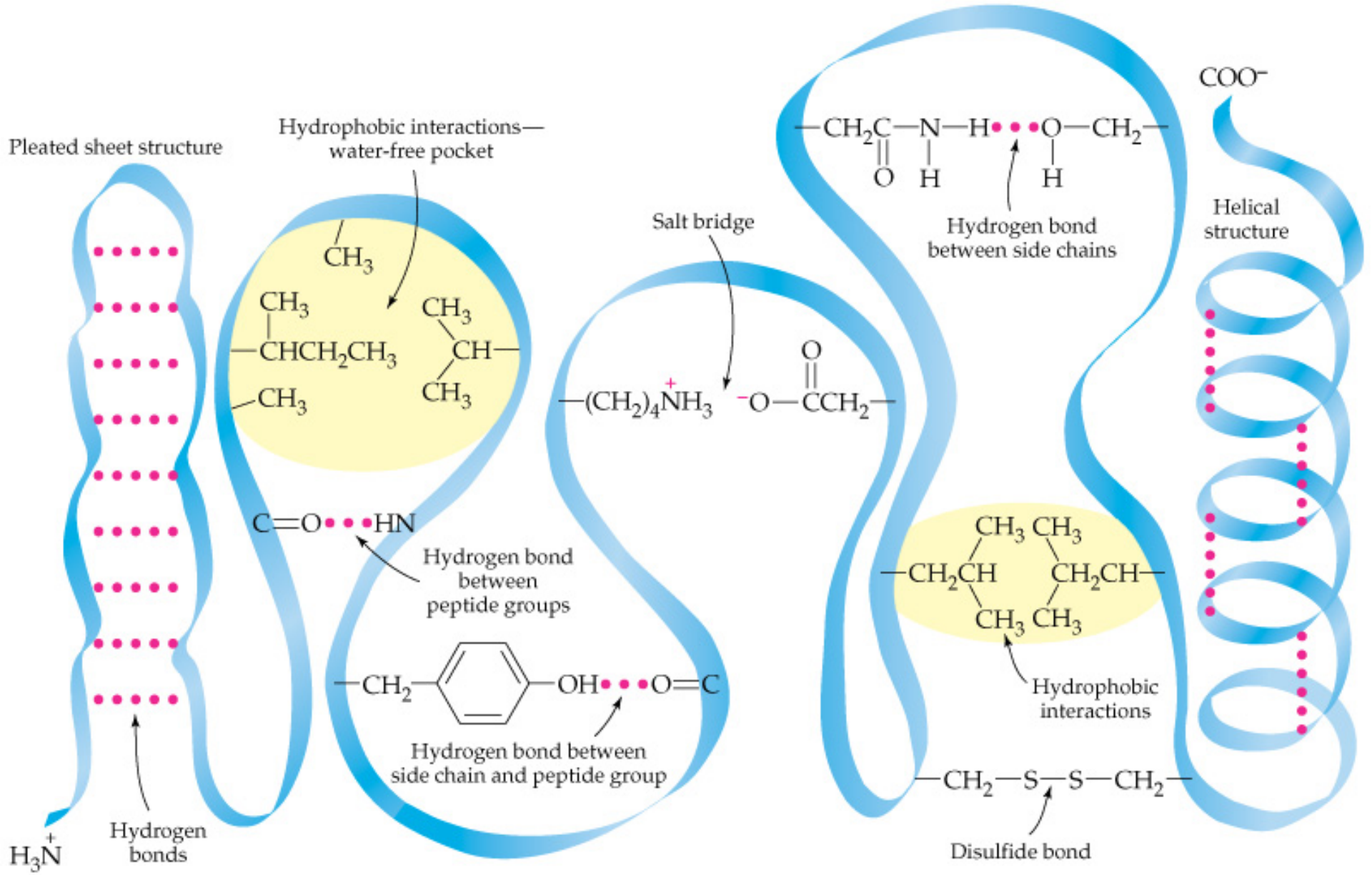
**β-Sheet:** The polypeptide chain is held in place by hydrogen bonds between pairs of peptide units along neighboring backbone segments.

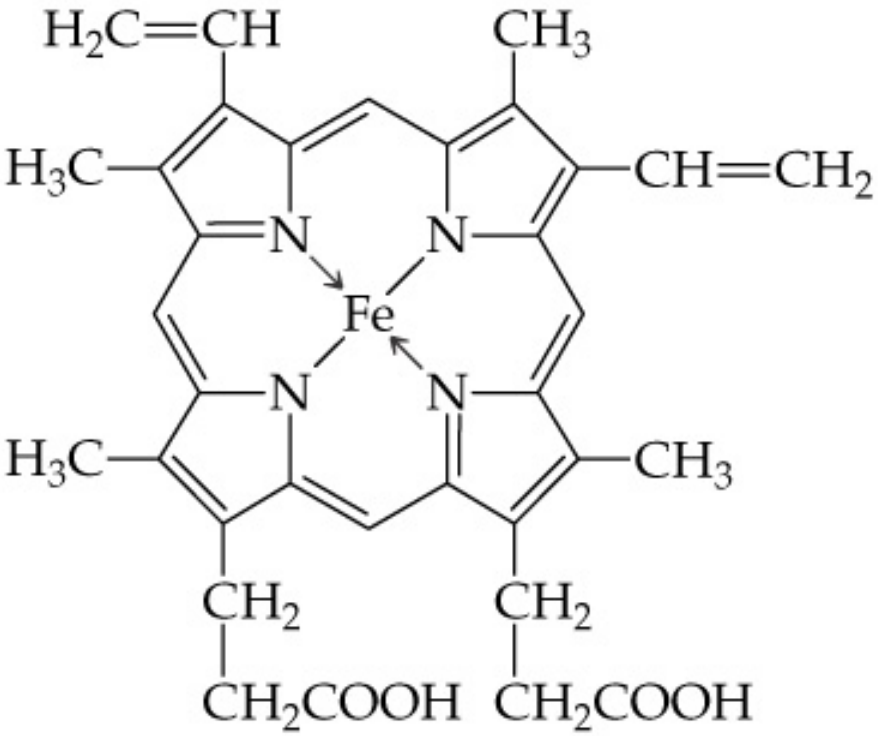


# Tertiary Protein Structure

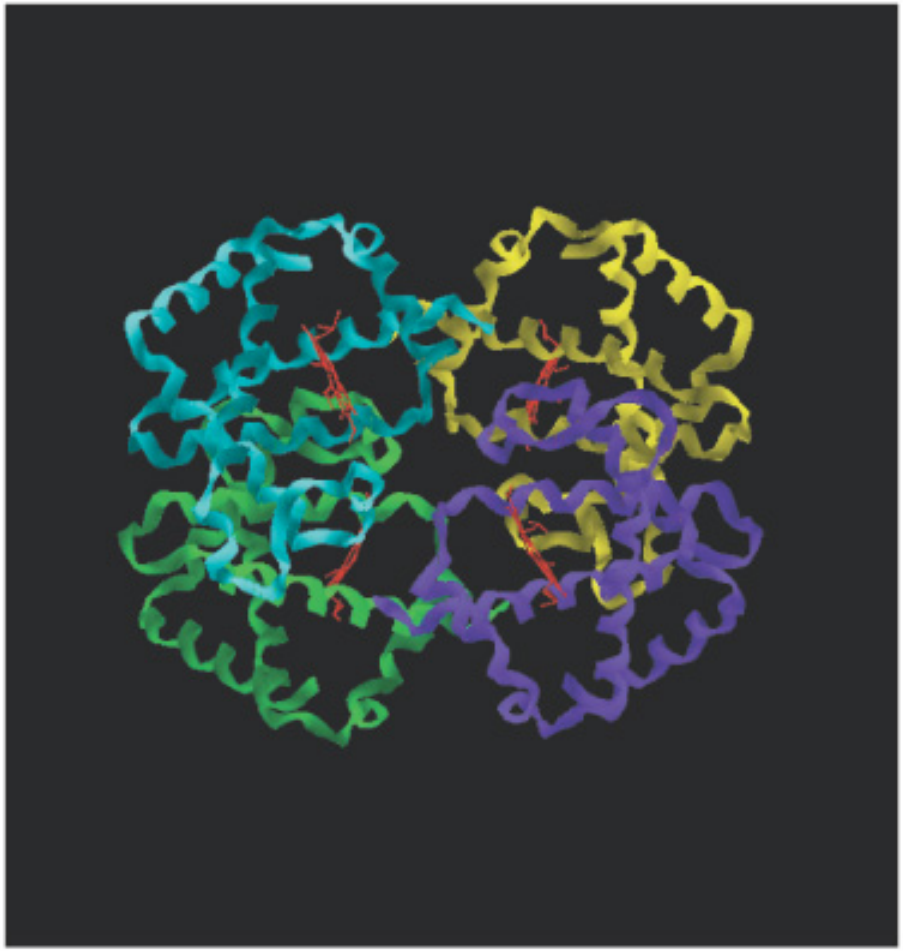
- ***Tertiary Structure of a proteins*** The overall three dimensional shape that results from the folding of a protein chain. Tertiary structure depends mainly on attractions of amino acid side chains that are far apart along the same backbone. Non-covalent interactions and disulfide covalent bonds govern tertiary structure.
- A protein with the shape in which it exist naturally in living organisms is known as a *native protein.*

- Protein shape determining interactions are summarized below:
- Hydrogen bond between neighboring backbone segments.
- Hydrogen bonds of side chains with each other or with backbone atoms.
- Ionic attractions between side chain groups or salt bridge.
- Hydrophobic interactions between side chain groups.
- Covalent sulfur-sulfur bonds.

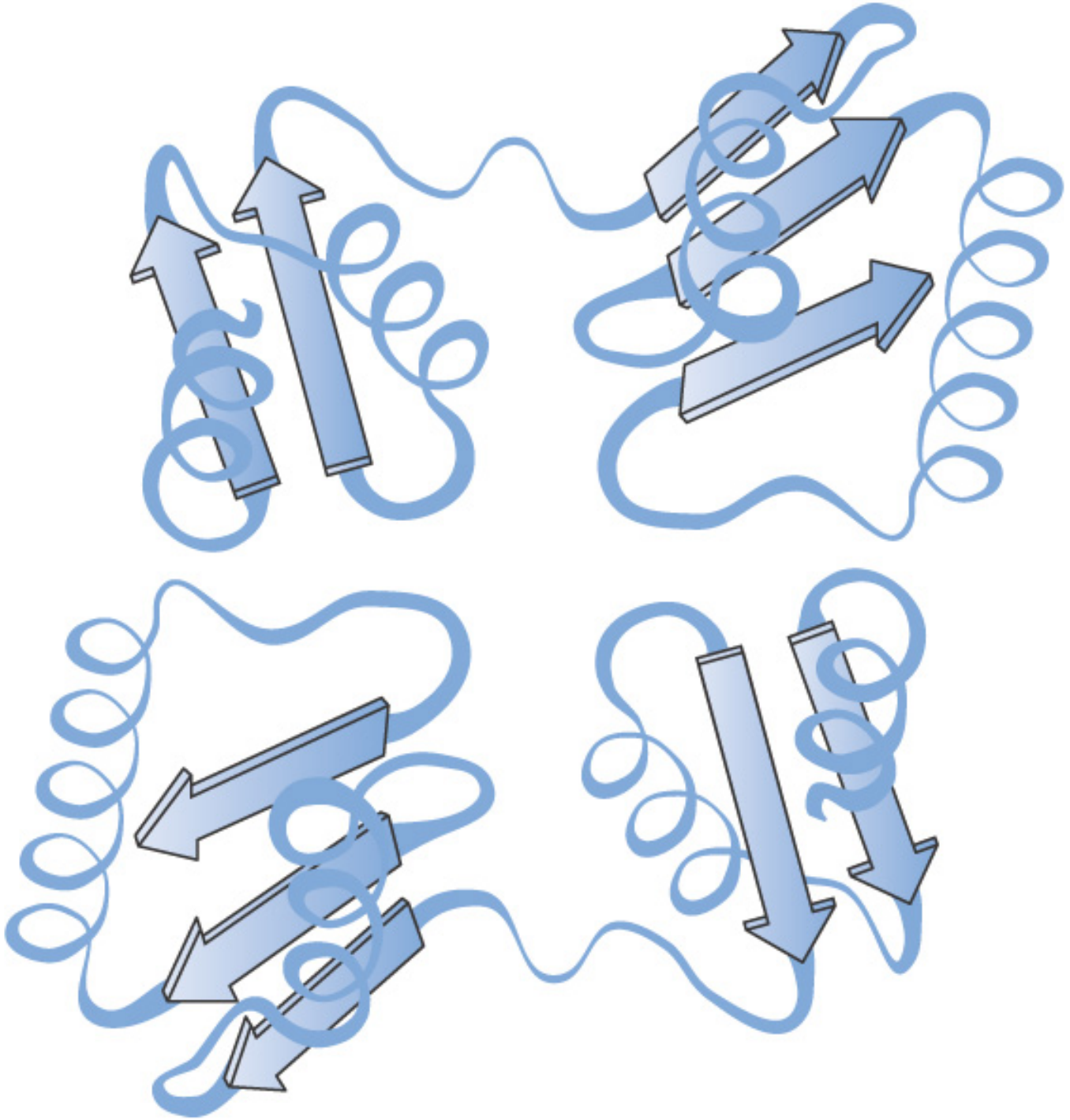

Pleated sheet structure
Hydrophobic interactions—
water-free pocket
Salt bridge
Hydrogen bond
between side chains
Helical
structure
Hydrogen bond
between
peptide groups
Hydrogen bond between
side chain and peptide group
Hydrophobic
interactions
Hydrogen
bonds
Disulfide bond




(a)



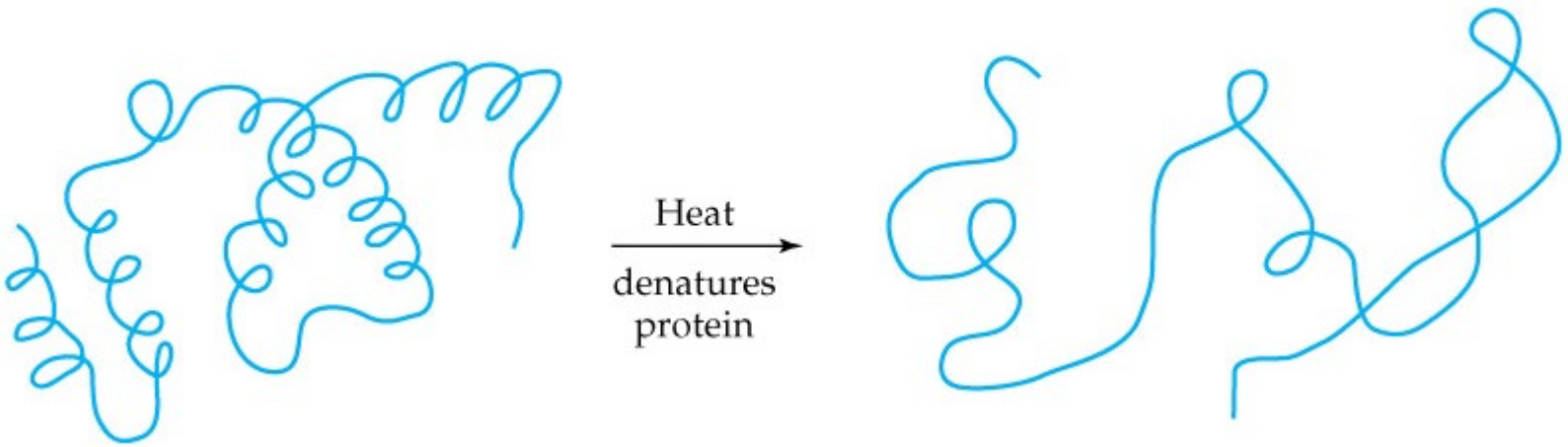
(b)



# Chemical Properties of Proteins

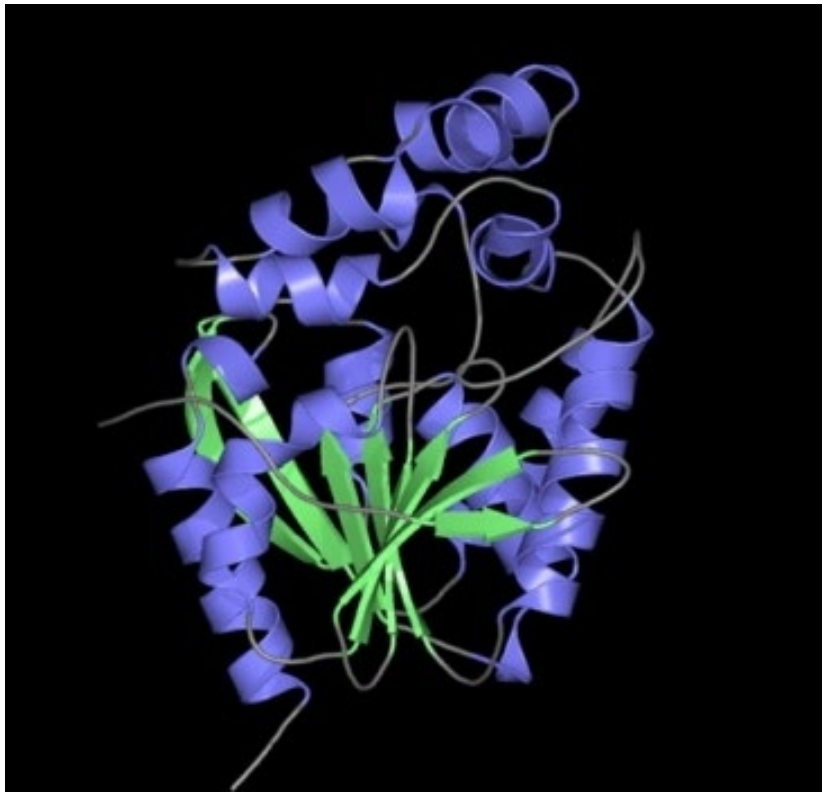
- *Protein hydrolysis:* In protein hydrolysis, peptide bonds are hydrolyzed to yield amino acids. This is reverse of protein formation.

- *Protein denaturation*: The loss of secondary, tertiary, or quaternary protein structure due to disruption of non-covalent interactions and or disulfide bonds that leaves peptide bonds and primary

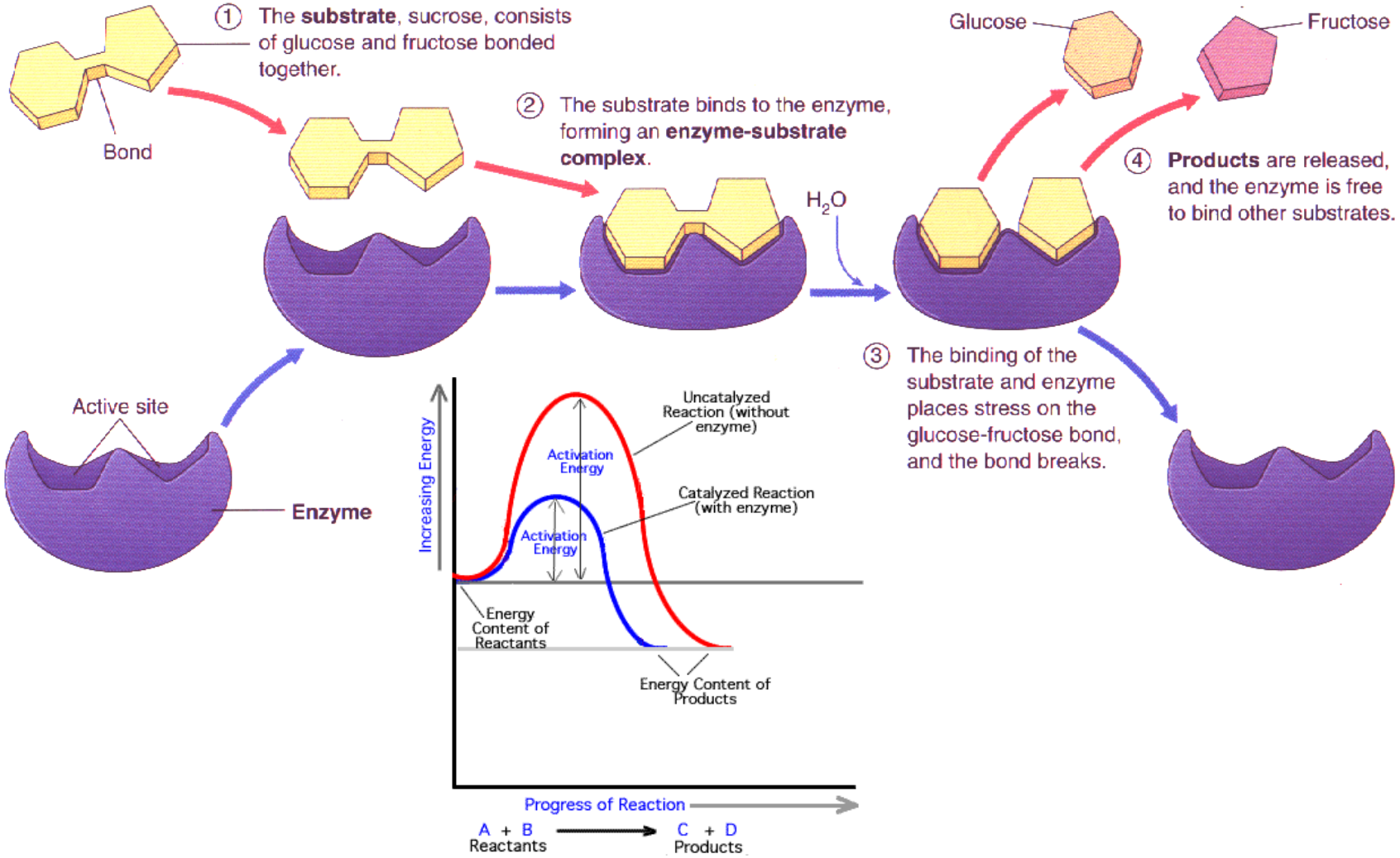


# Catalysis by Enzymes

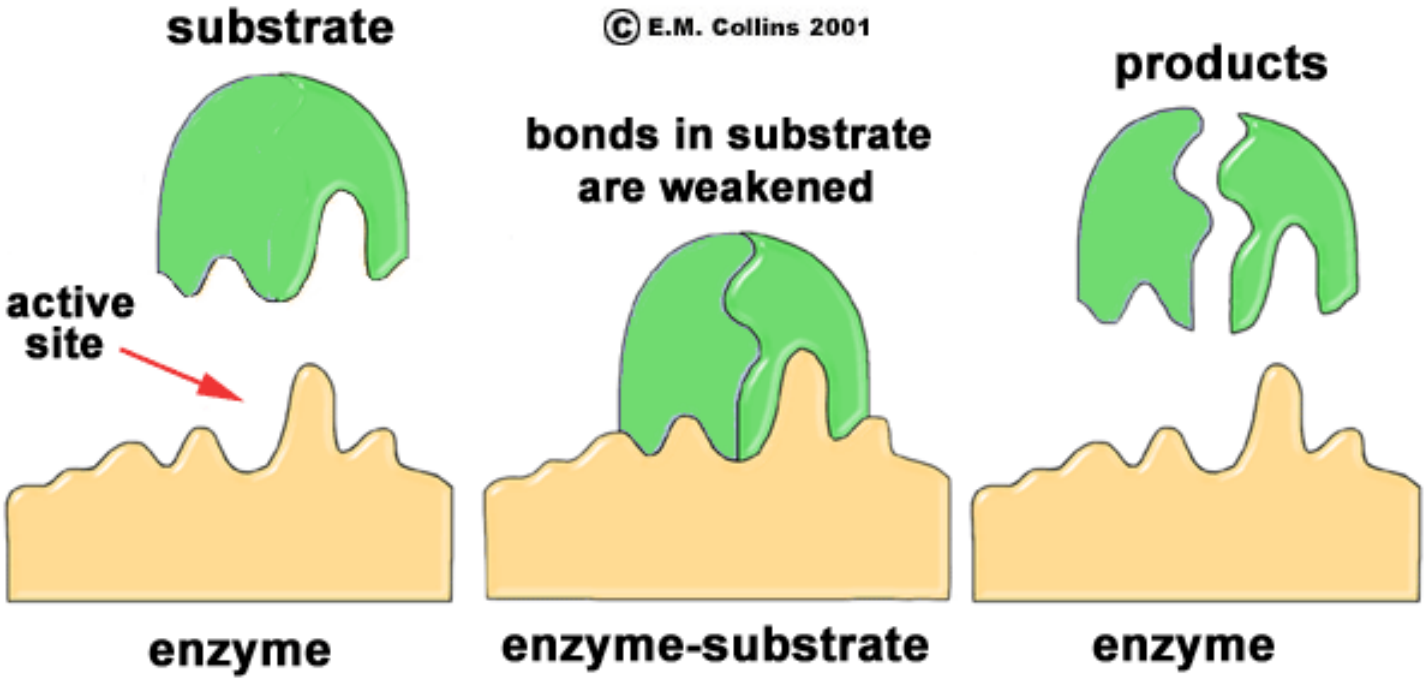
- ***Enzyme*** A protein that acts as a catalyst for a biochemical reaction.



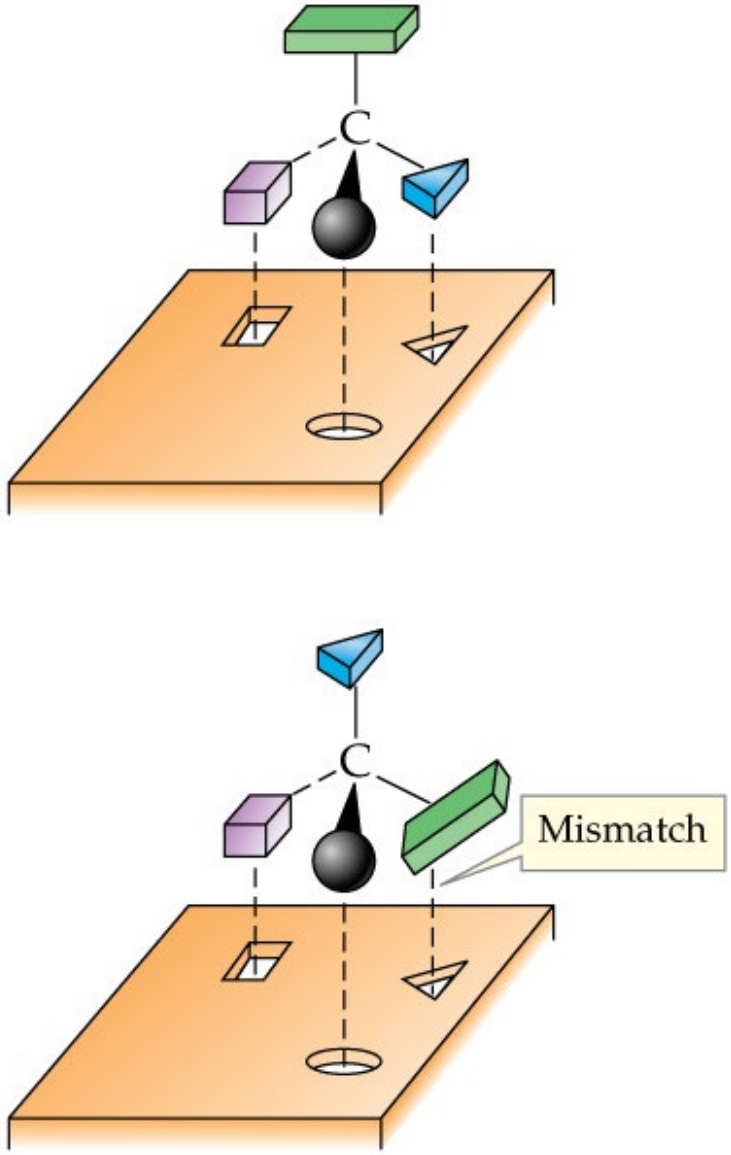
# Enzymatic Reaction



# Specificity



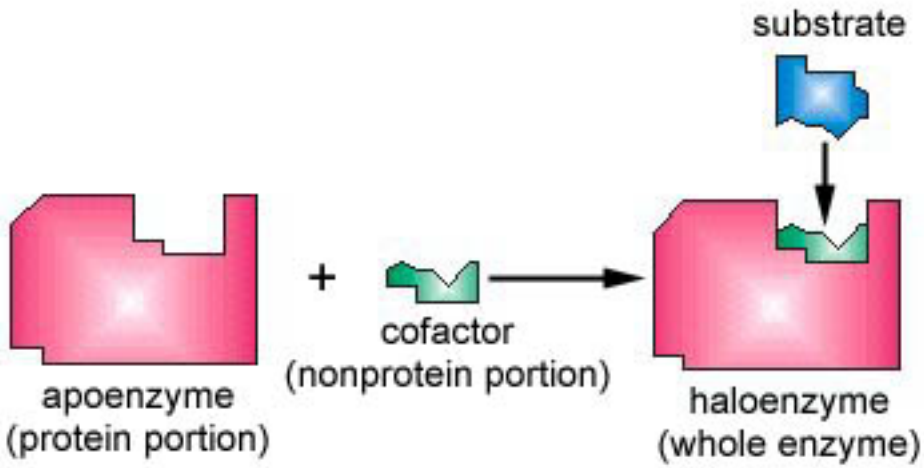
The specificity of an enzyme for one of two enantiomers is a matter of fit. One enantiomer fits better into the active site of the enzyme than the other enantiomer. Enzyme catalyzes reaction of the enantiomer that fits better into the active site of the enzyme.

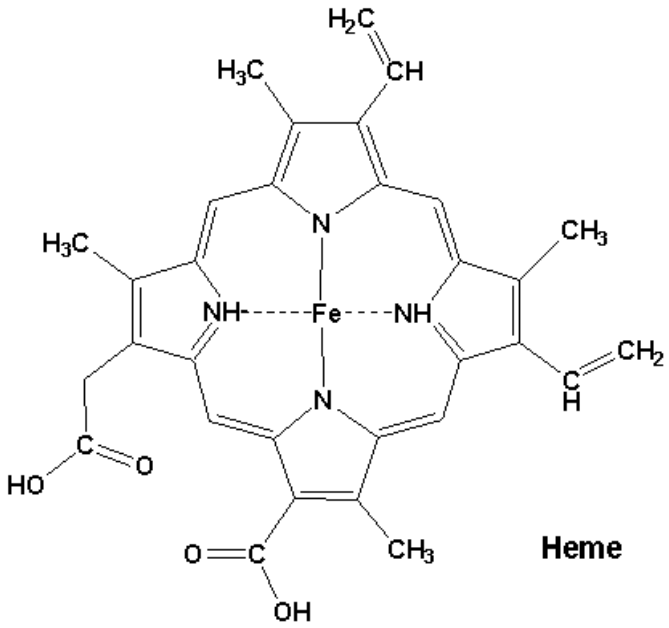


# Enzyme Cofactors

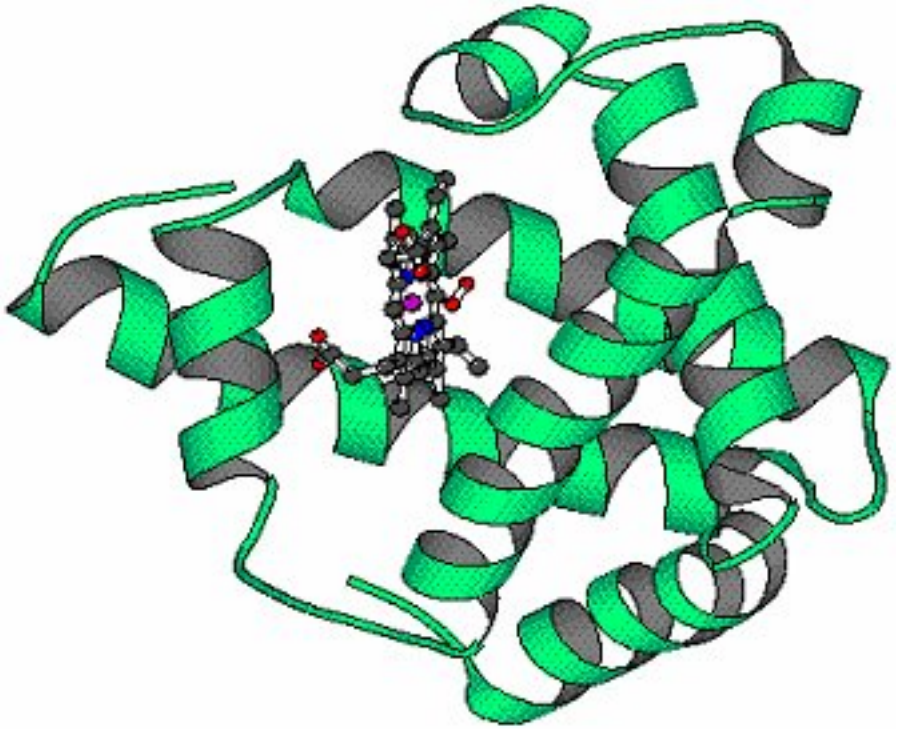
- Many enzymes are conjugated proteins that require nonprotein portions known as ***cofactors***.
- Some cofactors are metal ions, others are nonprotein organic molecules called ***coenzymes***.
- An enzyme may require a metal-ion, a coenzyme, or both to function.

# Cofactor



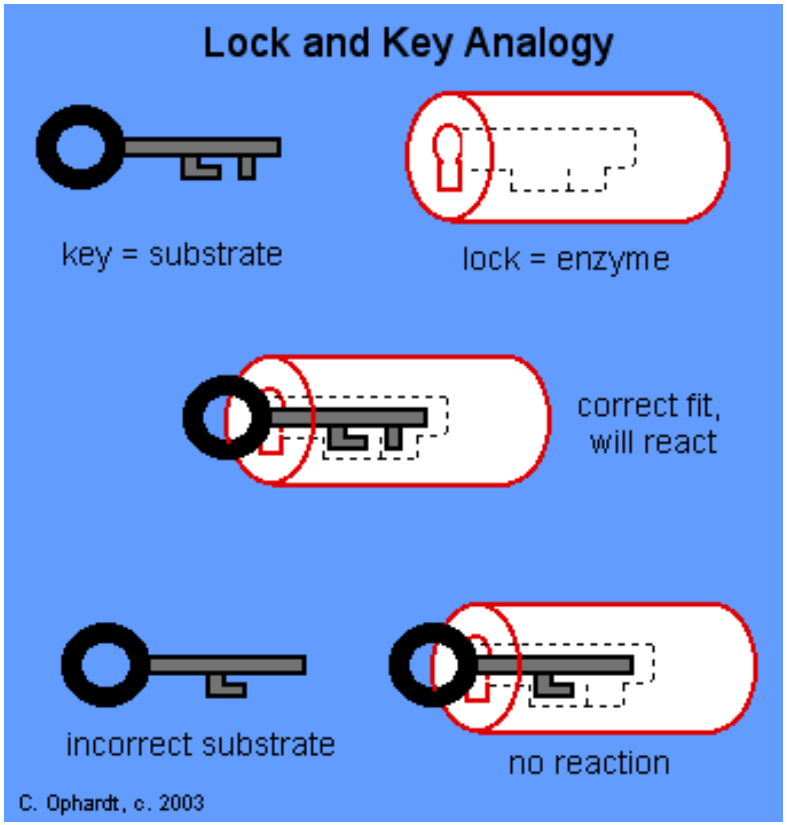

Heme

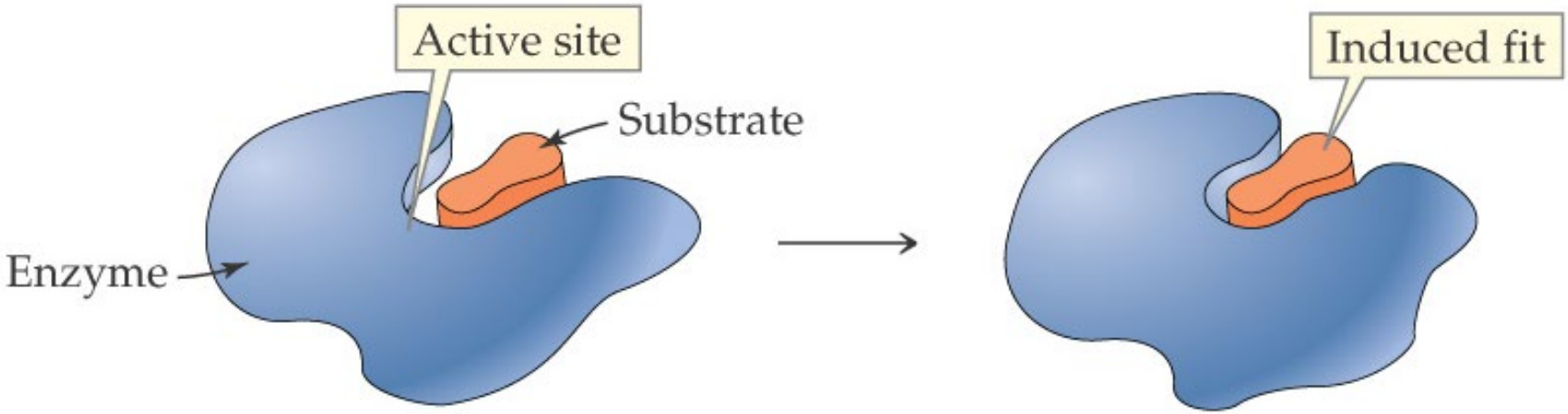


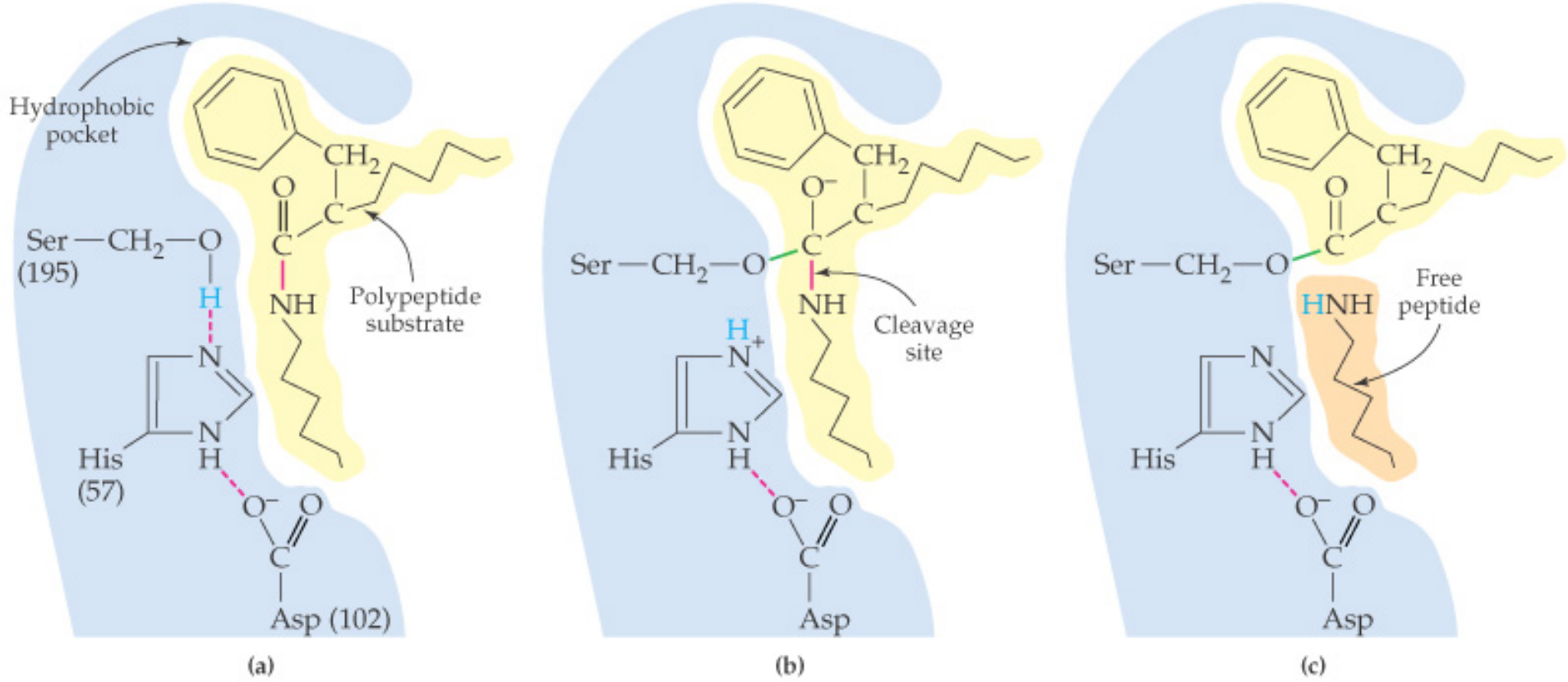
- Cofactors provide additional chemically active functional groups which are not present in the side chains of amino acids that made up the enzyme.
- Metal ions may anchor a substrate in the active site or may participate in the catalyzed reaction.

# How Enzyme Work

- Two modes are invoked to represent the interaction between substrate and enzymes. These are:
- ***Lock-and-key model***: The substrate is described as fitting into the active site as a key fit into a lock.
- ***Induced-fit-model***: The enzyme has a flexible active site that changes shape to accommodate the substrate and facilitate the reaction.


Lock and Key Analogy
key = substrate
lock = enzyme
correct fit,
will react
incorrect substrate
no reaction
C. Ophardt, c. 2003


Active site
Substrate
Enzyme
Induced fit


Hydrophobic pocket
Ser —CH2—O
(195)
H
N
N
H
His
(57)
O⁻ O
C
Asp (102)
CH2
O
C
C
NH
Polypeptide substrate
(a)
O⁻
C
Ser —CH2—O
NH
Cleavage site
H+
N
N
H
His
O⁻ O
C
Asp
(b)
HNH
Free peptide
(c)

# How Does an Enzyme Work?


https://www.youtube.com/watch?v=yk14dOOvwMk

# 19.5 Effect of Concentration on Enzyme Activity

- Variation in concentration of enzyme or substrate alters the rate of enzyme catalyzed reactions.
- *Substrate concentration*: At low substrate concentration, the reaction rate is directly proportional to the substrate concentration. With increasing substrate concentration, the rate drops off as more of the active sites are occupied.

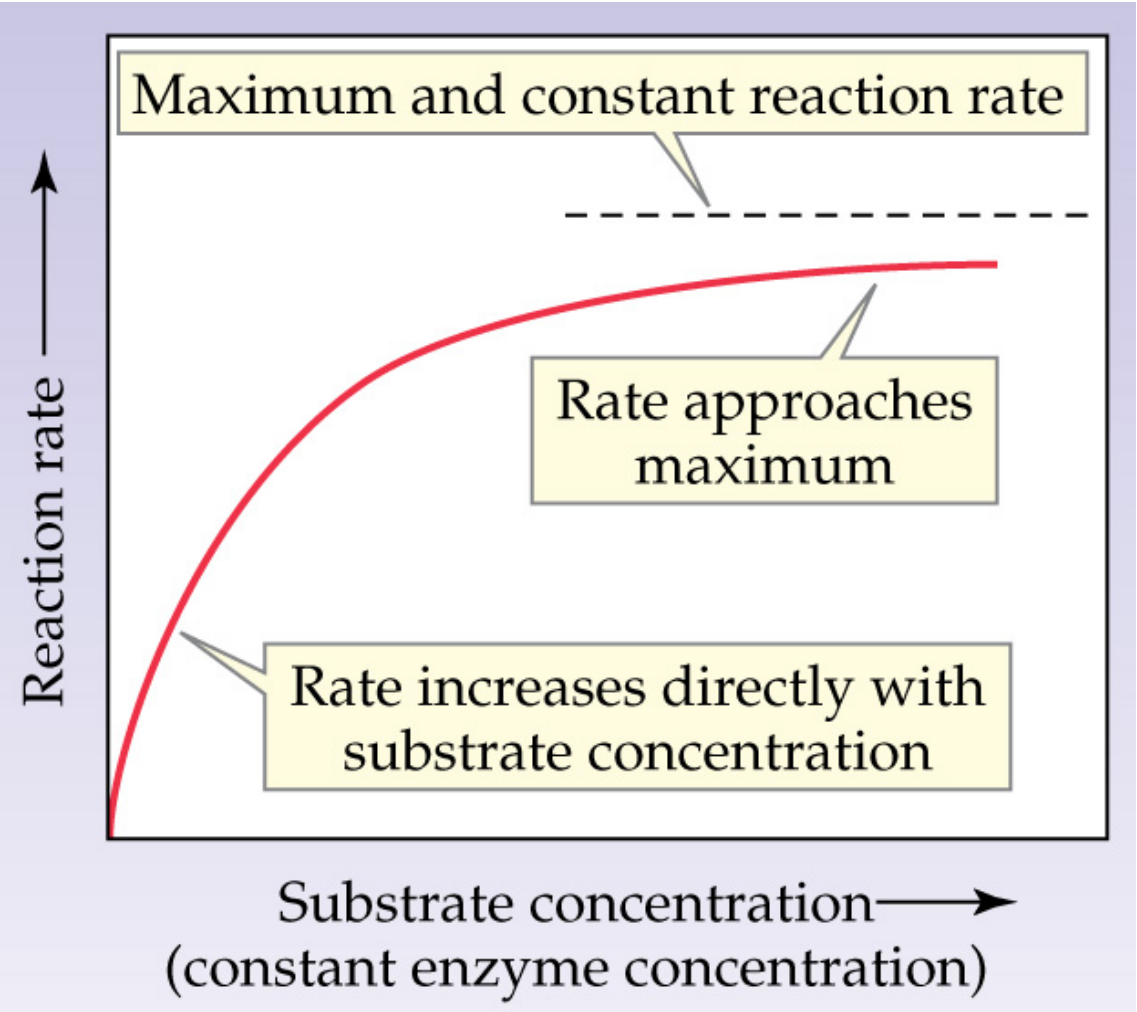
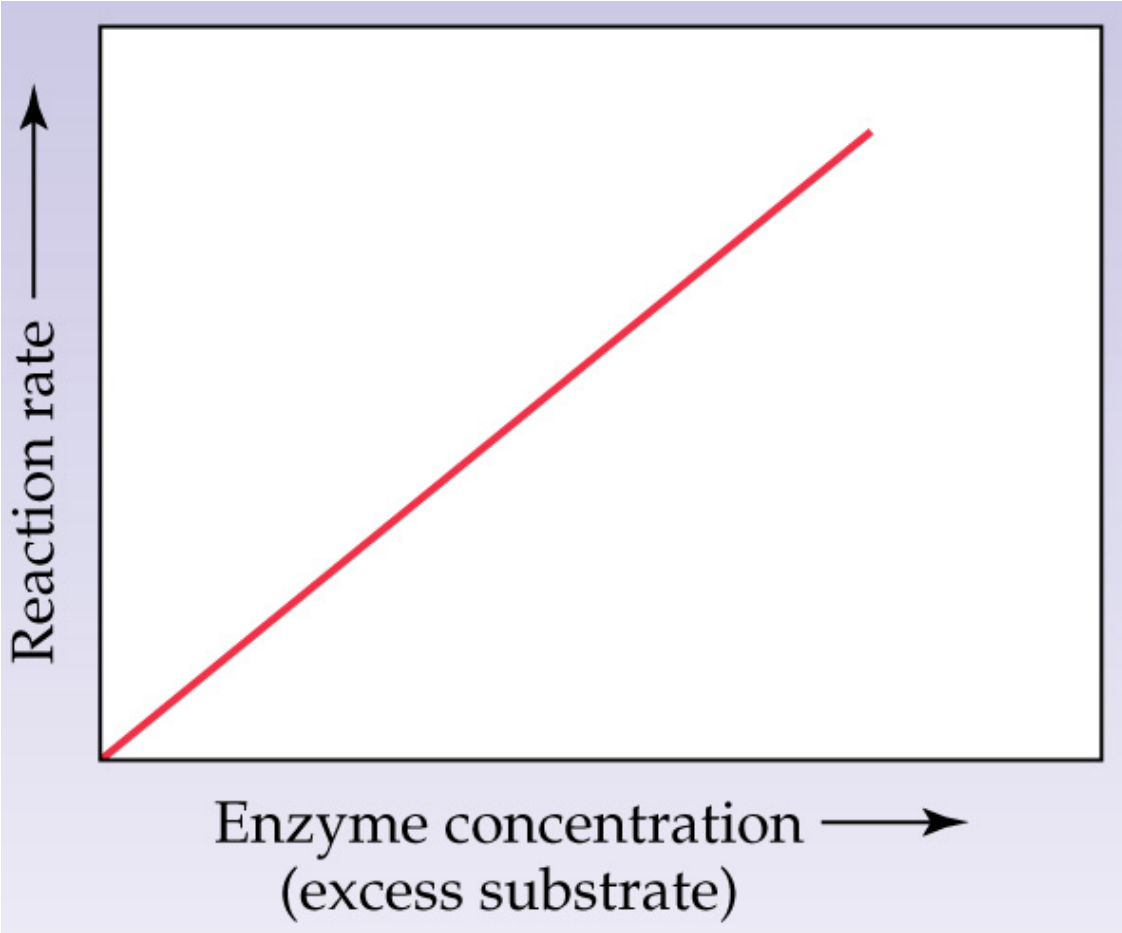



Fig 19.5 Change of reaction rate with substrate concentration when enzyme concentration is constant.

- *Enzyme concentration*: The reaction rate varies directly with the enzyme concentration as long as the substrate concentration does not become a limitation, Fig 19.6 below.



# 19.6 Effect of Temperature and pH on Enzyme Activity

- Enzymes maximum catalytic activity is highly dependent on temperature and pH.
- Increase in temperature increases the rate of enzyme catalyzed reactions. The rates reach a maximum and then begins to decrease. The decrease in rate at higher temperature is due to denaturation of enzymes.

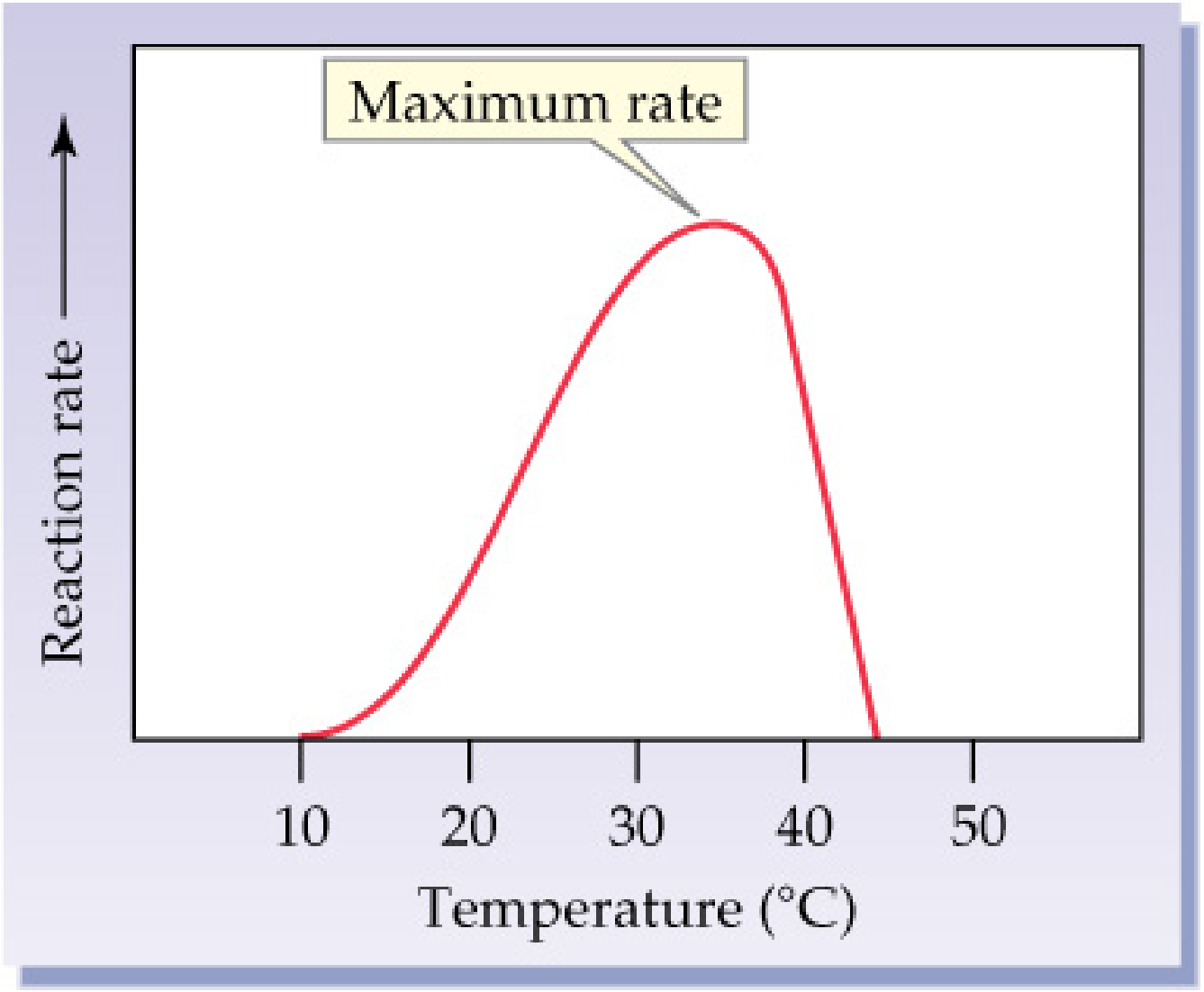
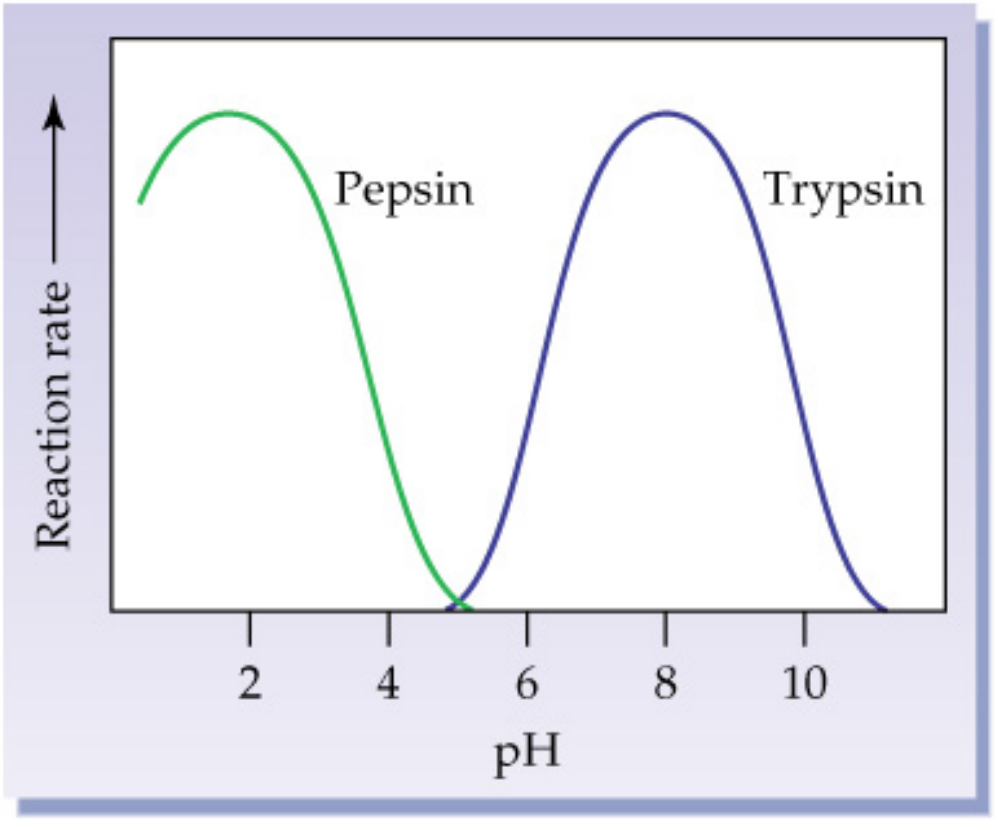



Fig 19.7 (a) Effect of temperature on reaction rate

- Effect of pH on Enzyme activity: The catalytic activity of enzymes depends on pH and usually has a well defined optimum point for maximum catalytic activity Fig 19.7 (b) below.

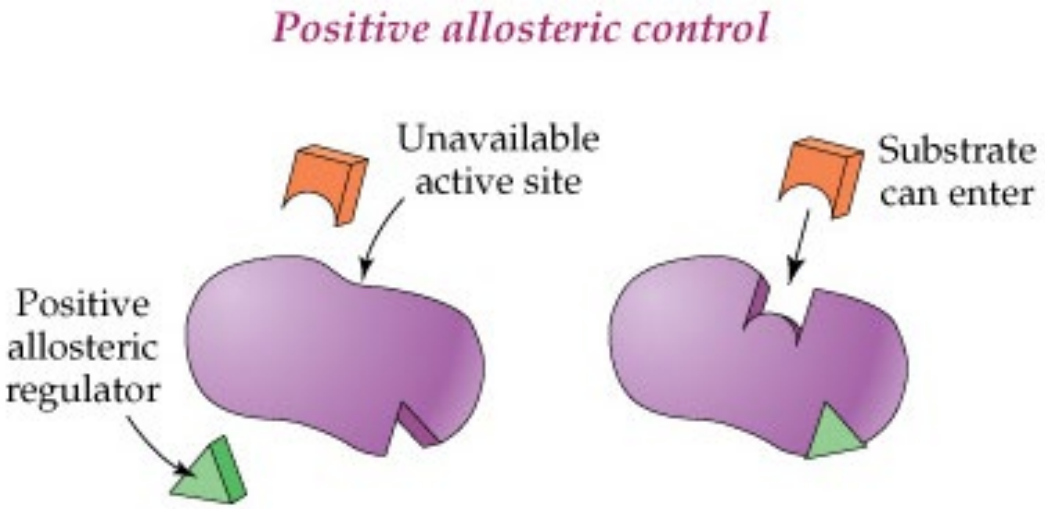


# 19.7 Enzyme Regulation: Feedback and Allosteric Control

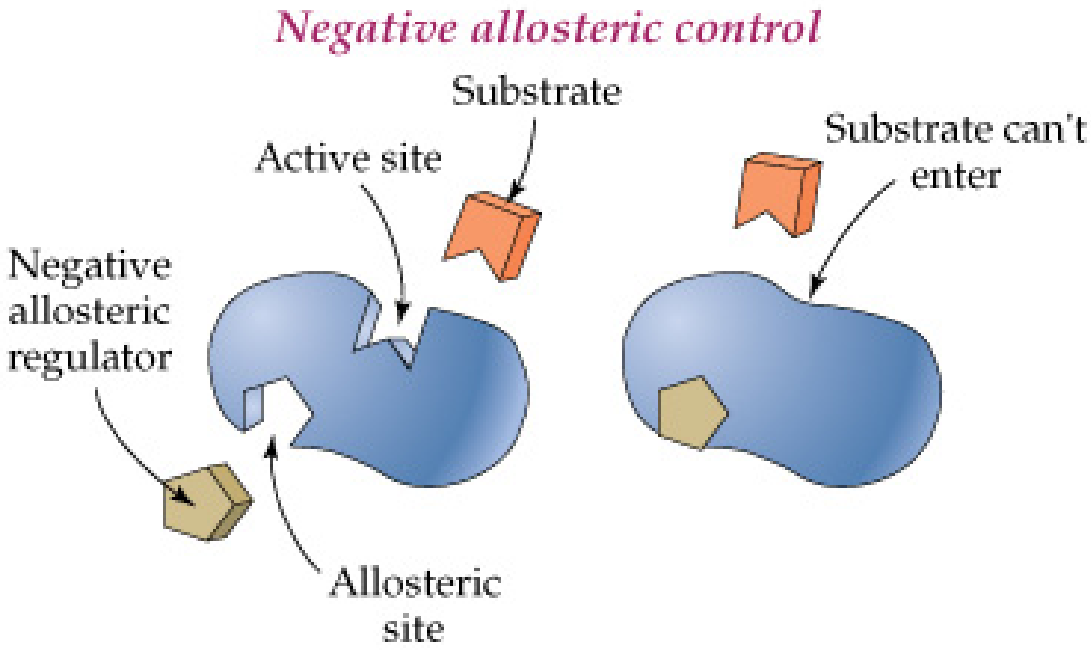
- Concentration of thousands of different chemicals vary continuously in living organisms which requires regulation of enzyme activity.
- Any process that starts or increase the activity of an enzyme is ***activation.***
- Any process that stops or slows the activity of an enzyme is ***inhibition.***

# Two of the mechanism

- ***Feedback control***: Regulation of an enzyme's activity by the product of a reaction later in a pathway.
- ***Allosteric control***: Activity of an enzyme is controlled by the binding of an activator or inhibitor at a location other than the active site. Allosteric controls are further classified as positive or negative.
  - A positive regulator changes the activity site so that the enzyme becomes a better catalyst and rate accelerates.
  - A negative regulator changes the activity site so that the enzyme becomes less effective catalyst and rate slows down.




A positive regulator changes the activity site so that the enzyme becomes a better catalyst and rate accelerates.

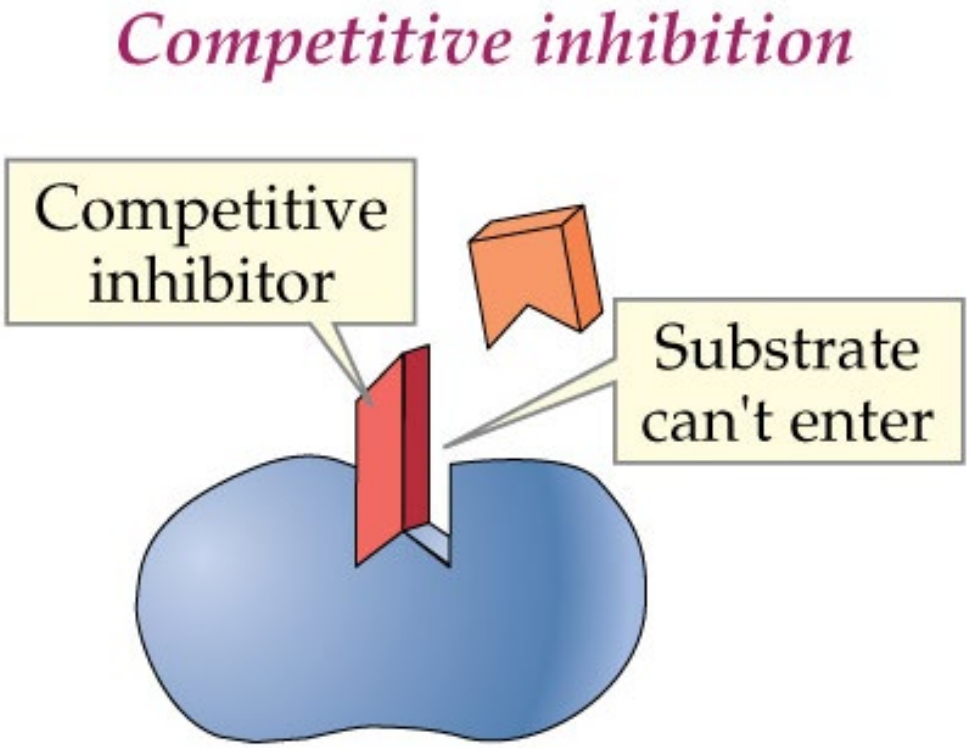



A negative regulator changes the activity site so that the enzyme becomes less effective catalyst and rate slows down.

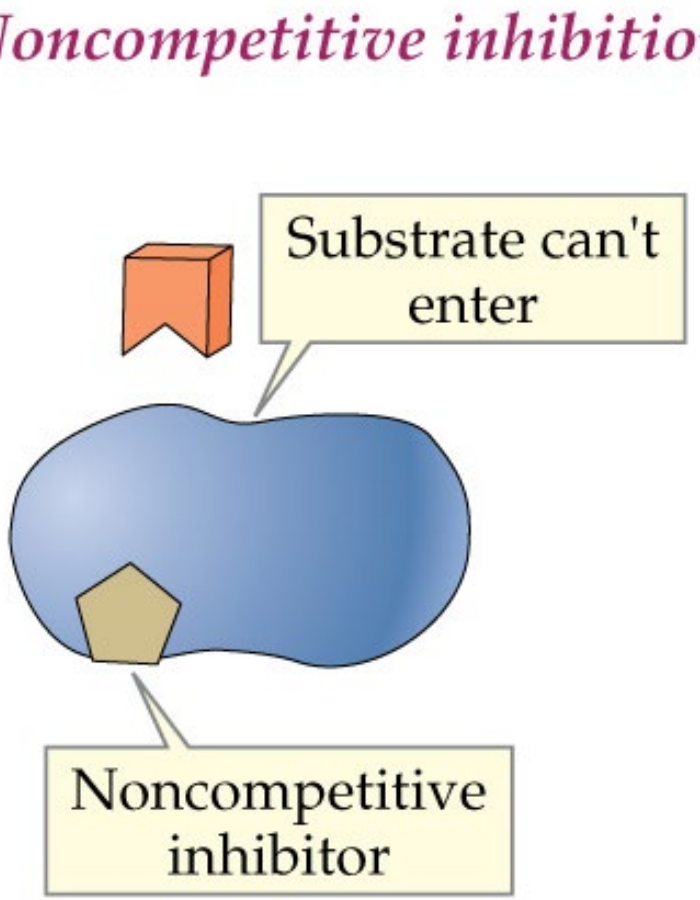
# 19.8 Enzyme Regulation: Inhibition

- The inhibition of an enzyme can be *reversible* or *irreversible.*
- In *reversible inhibition,* the inhibitor can leave, restoring the enzyme to its uninhibited level of activity.
- In *irreversible inhibition,* the inhibitor remains permanently bound to the enzyme and the enzyme is permanently inhibited.

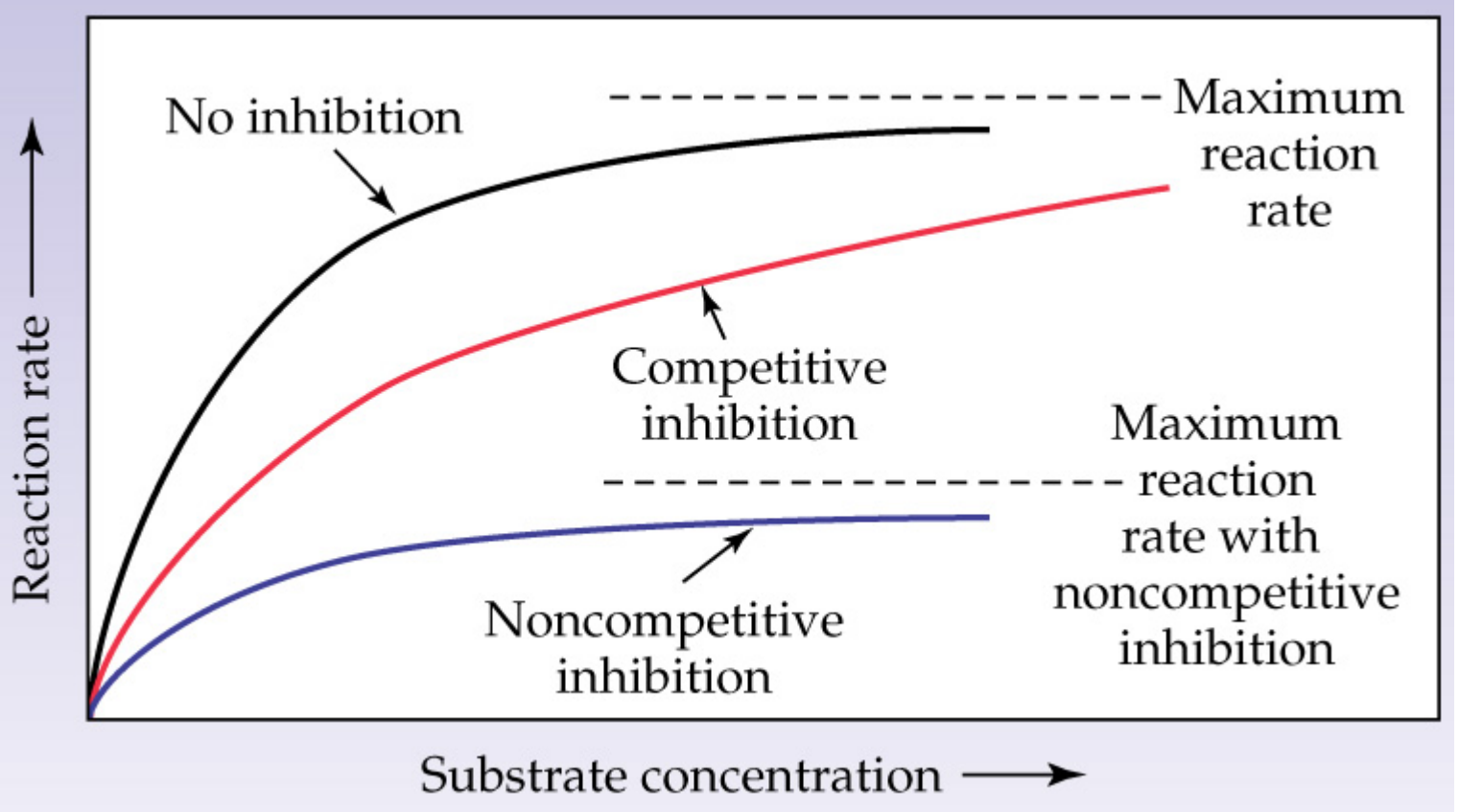
- Inhibitions are further classified as:
- *Competitive inhibition if* the inhibitor binds to the active site.



- *Noncompetitive inhibition,* if the inhibitor binds elsewhere and not to the active site.

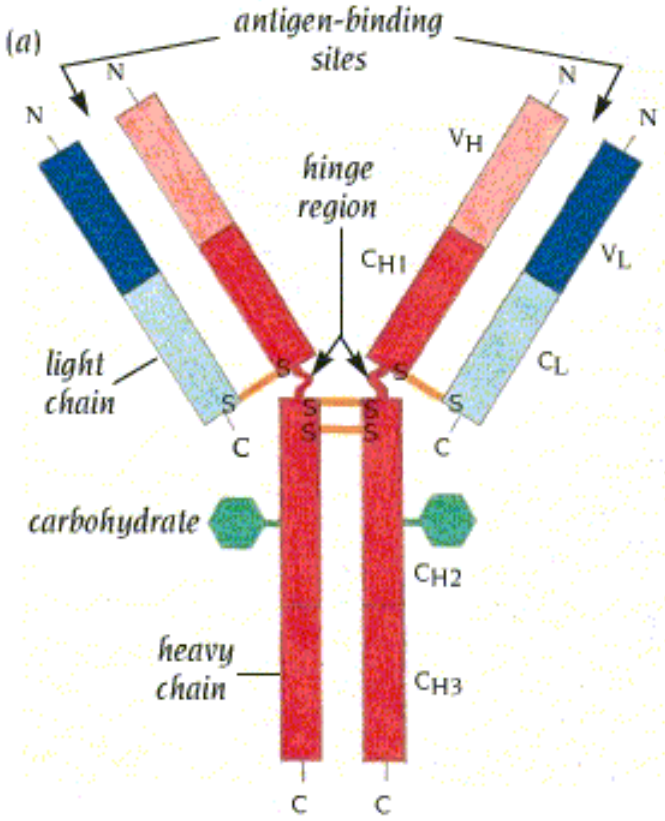


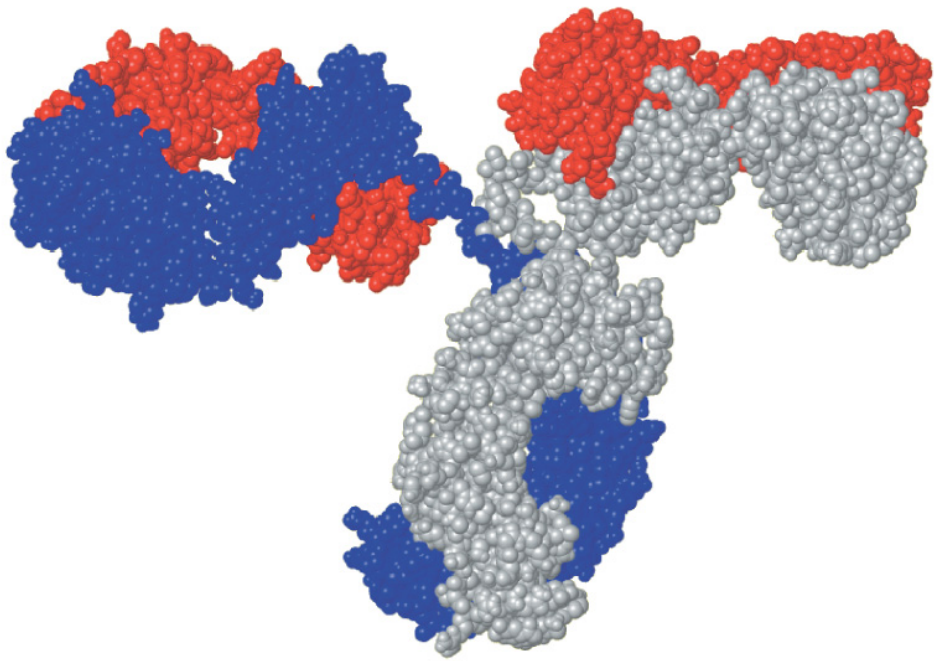
•The rates of enzyme catalyzed reactions with or without a competitive inhibitor are shown in the Fig 19.9 below.



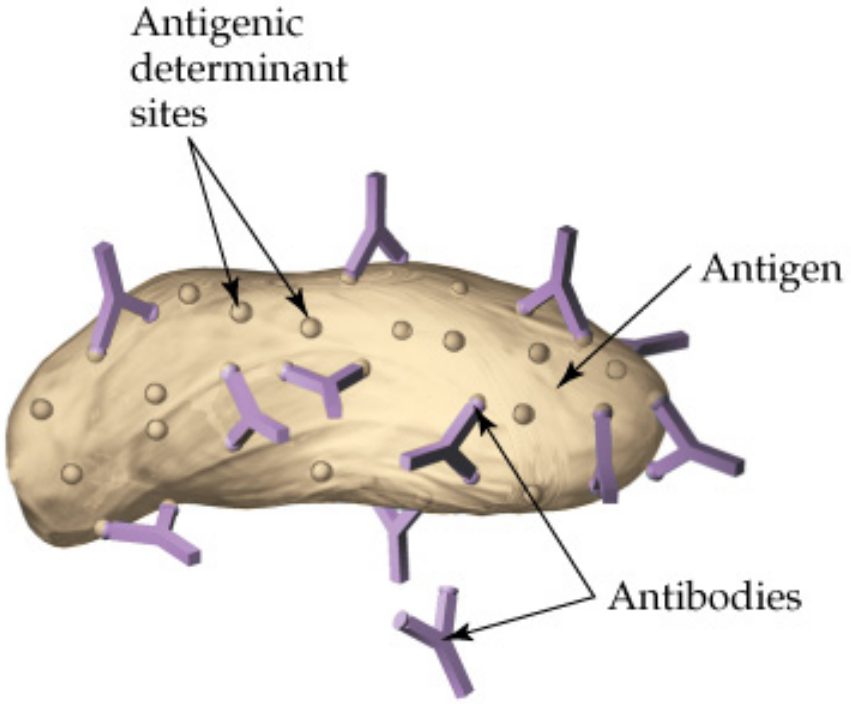
# Shape-Determining Interactions in Proteins

- The essential structure-function relationship for each protein depends on the polypeptide chain being held in its necessary shape by the interactions of atoms in the side chains.

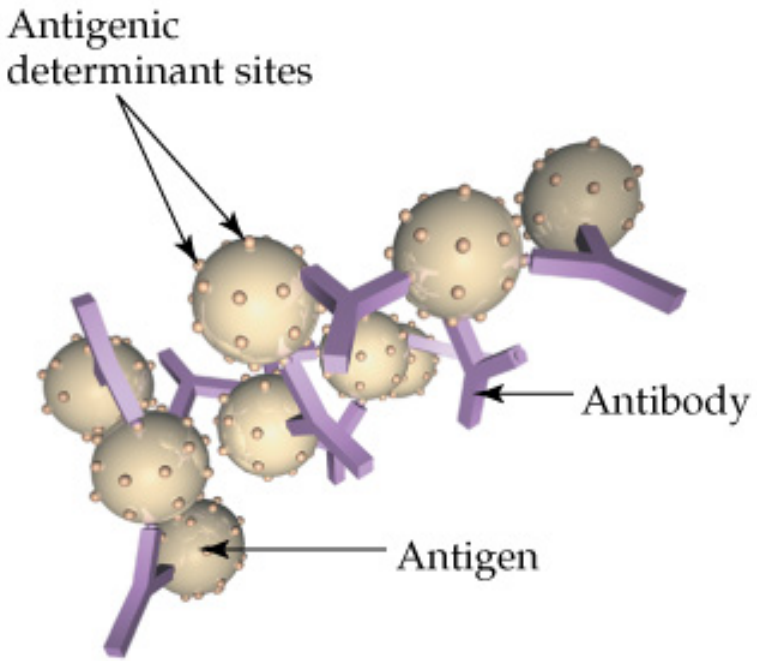

(a)
antigen-binding sites
N
N
N
N
VH
VL
hinge region
CH1
CL
light chain
S
S
S
S
S
S
S
S
C
C
carbohydrate
CH2
heavy chain
CH3
C
C



(b)

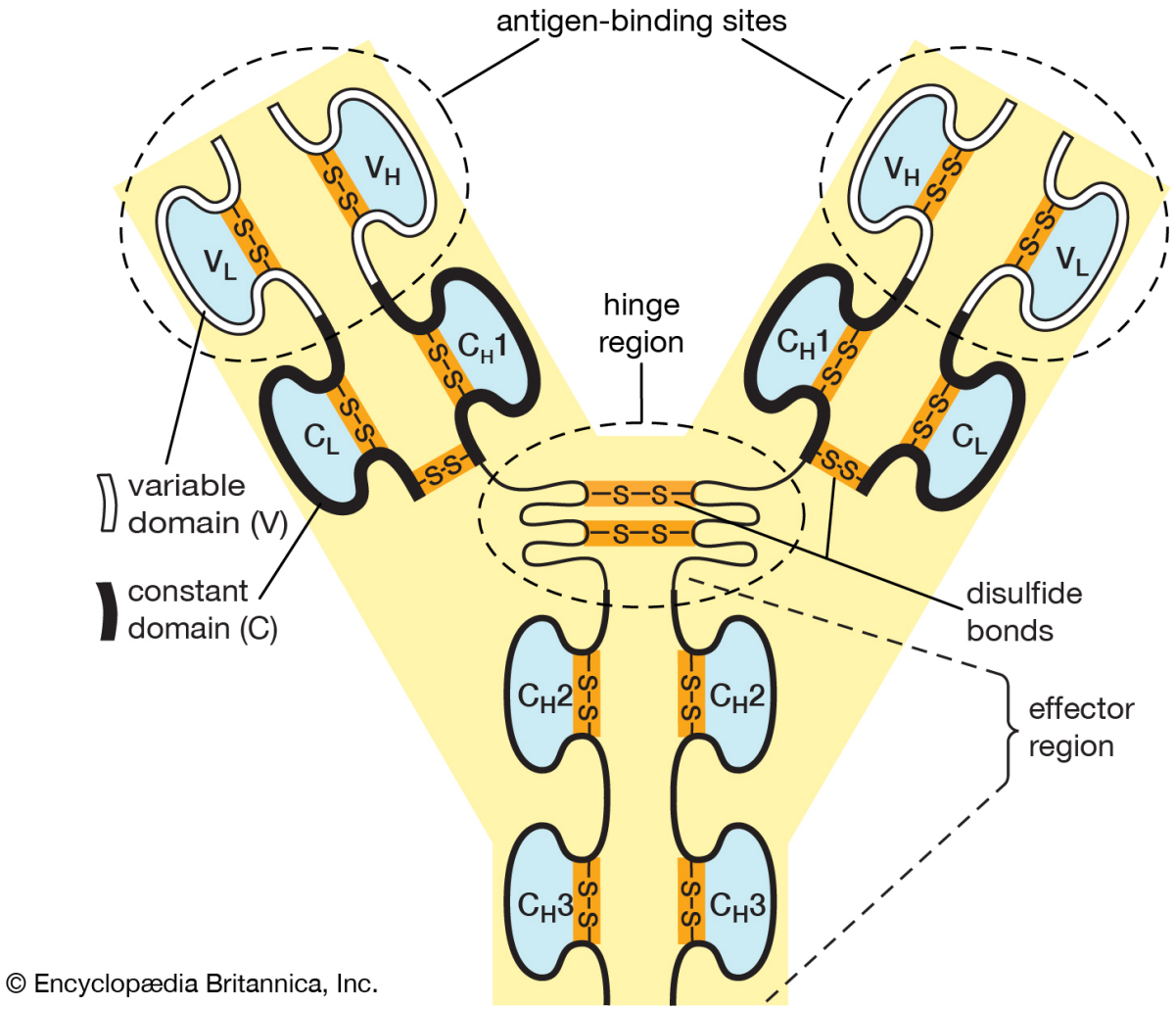

Antigenic determinant sites
Antigen
Antibodies

(a)

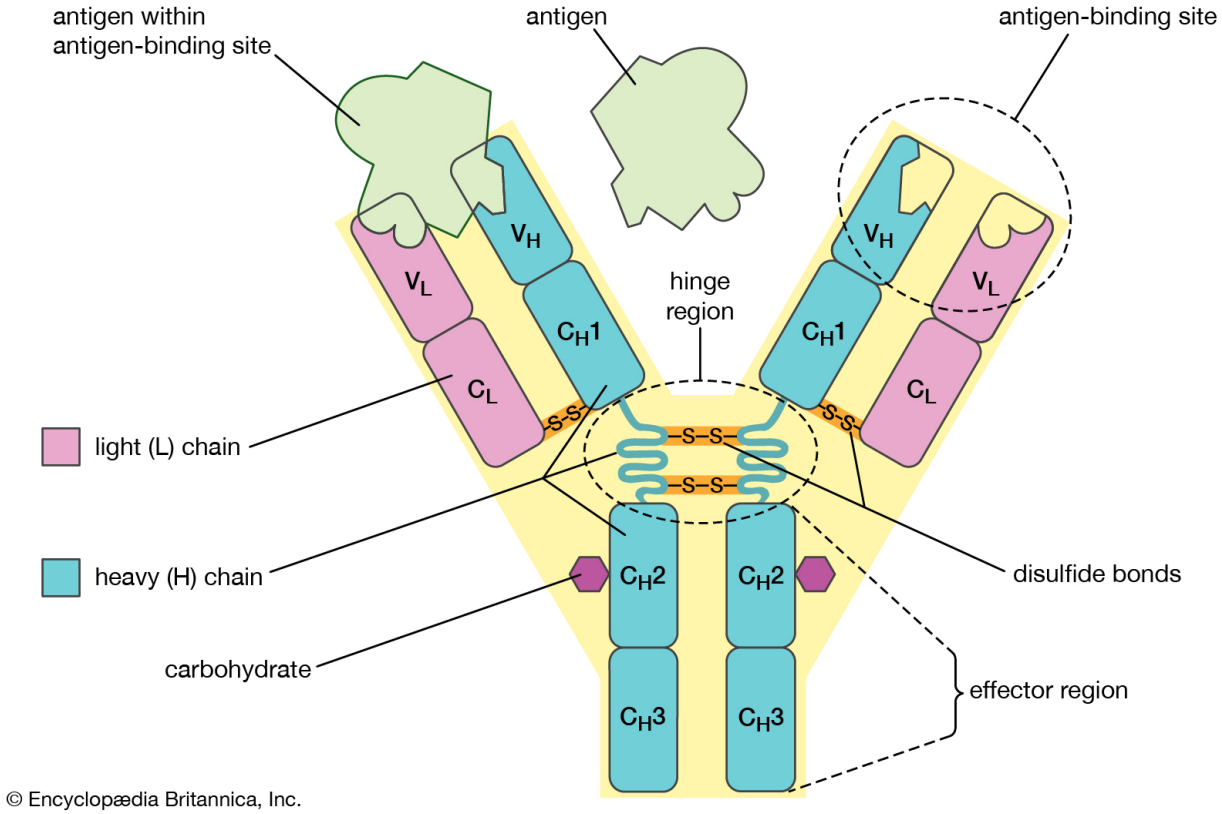

Antigenic determinant sites
Antibody
Antigen

(b)

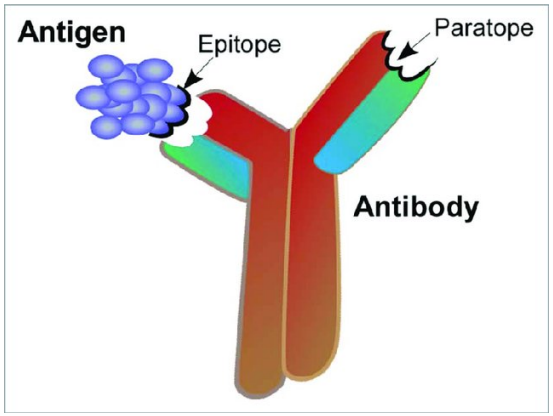
# Antibody

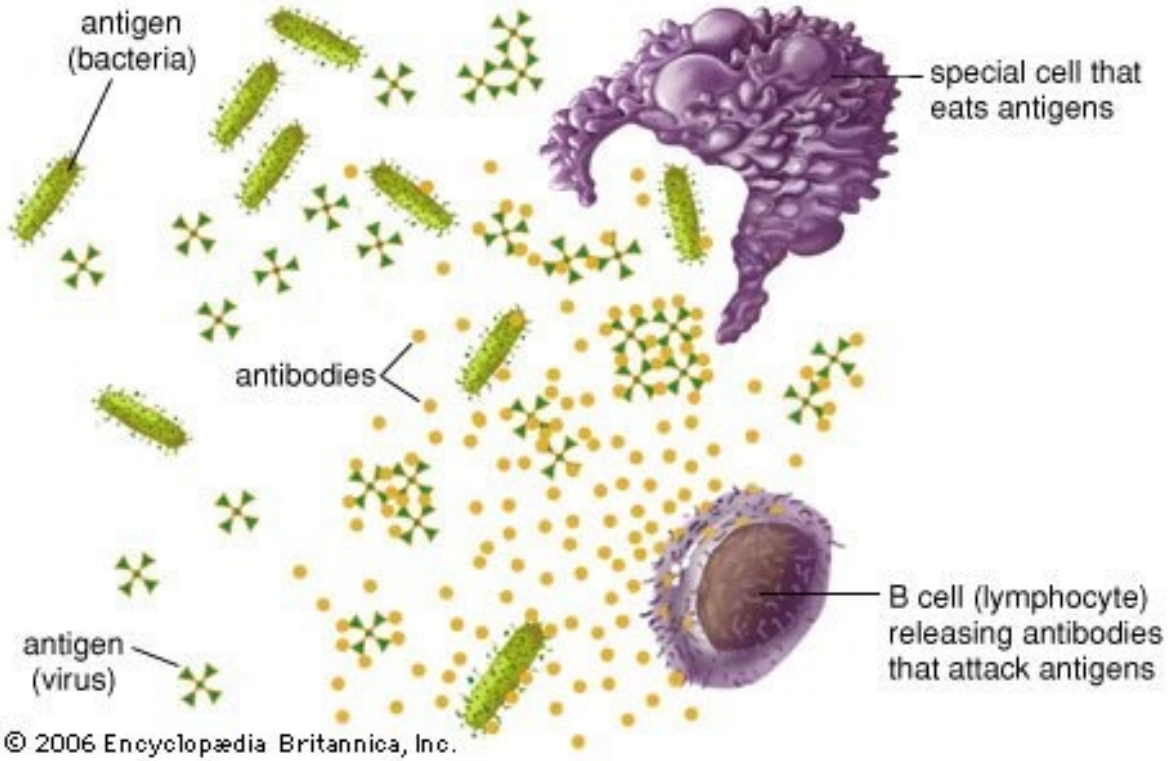

antigen-binding sites
$V_H$
$V_L$
$C_H1$
$C_L$
hinge region
variable domain (V)
constant domain (C)
disulfide bonds
$C_H2$
$C_H3$
effector region
© Encyclopædia Britannica, Inc.

# Antibody Binding Sites

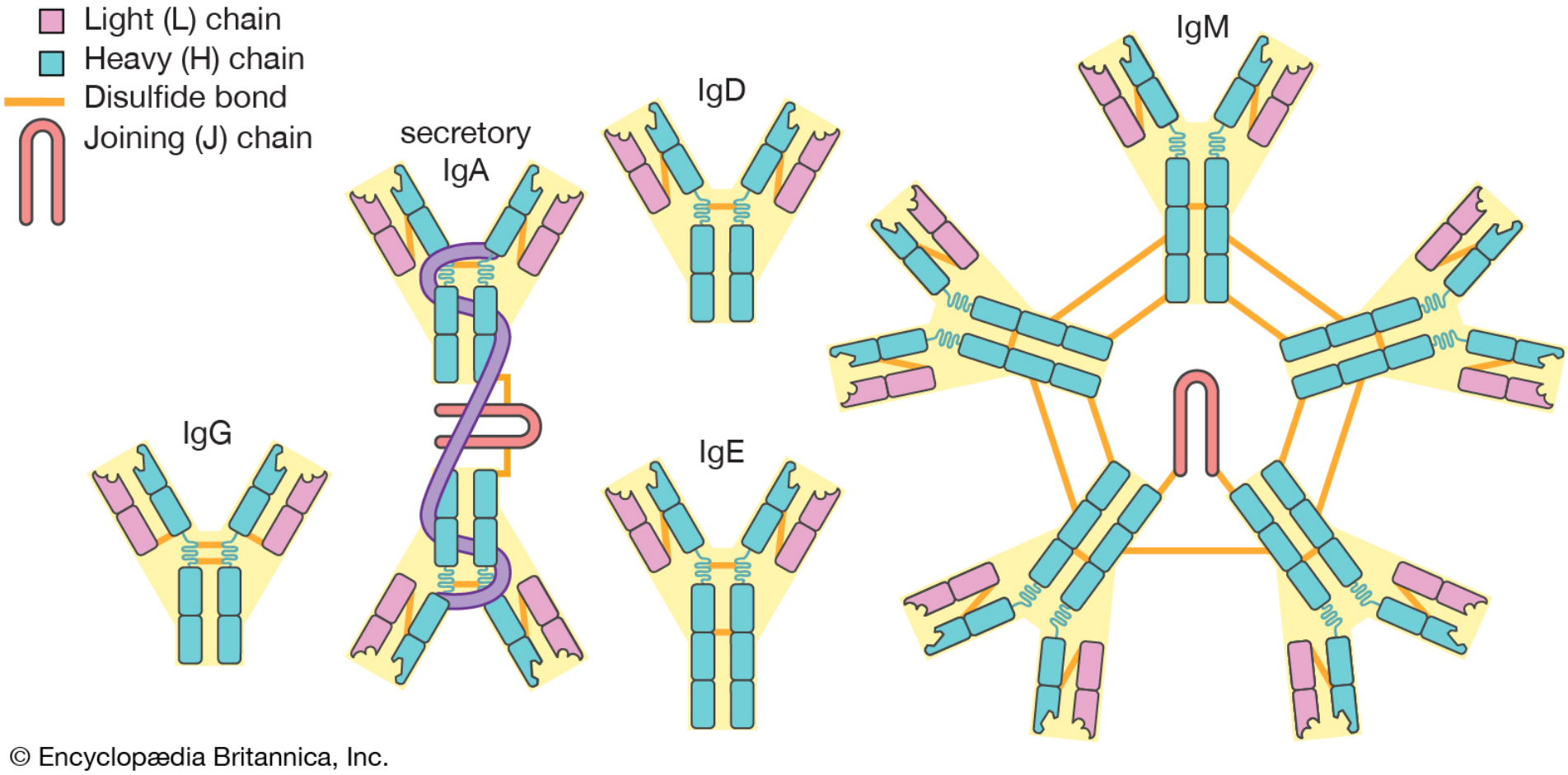





antigen
(bacteria)
special cell that
eats antigens
antibodies
B cell (lymphocyte)
releasing antibodies
that attack antigens
antigen
(virus)
© 2006 Encyclopædia Britannica, Inc.

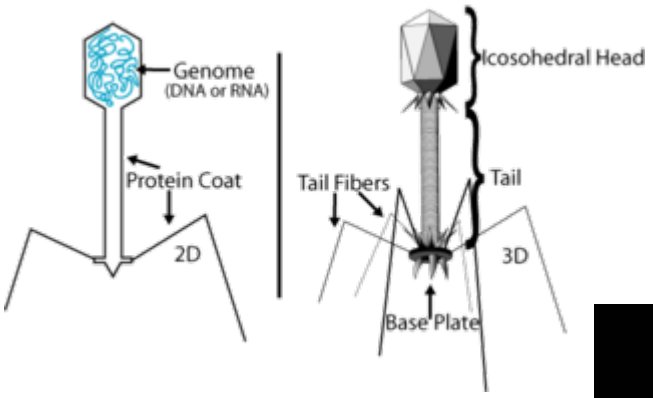
# Different Types of Antibodies

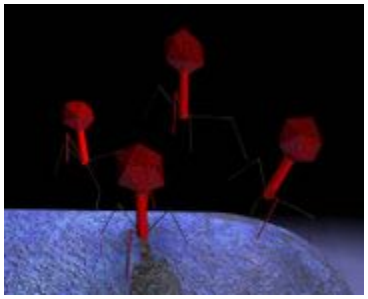


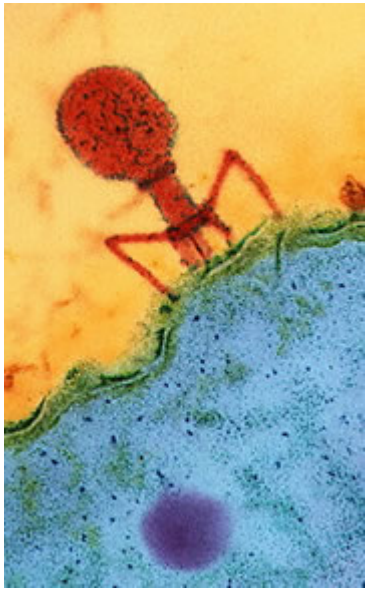
# Antibody

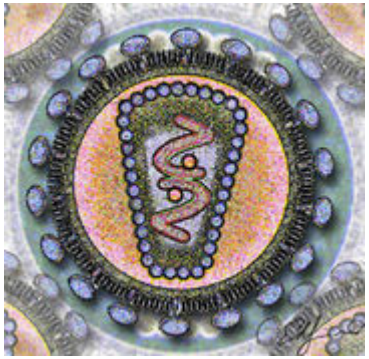

Check
https://www.youtube.com/watch?v=Cvu1ApHkhYM

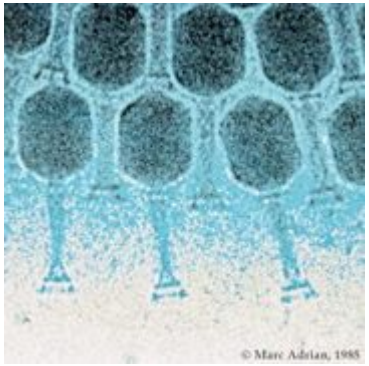
# Virus


Genome
(DNA or RNA)
Protein Coat
2D
Icosohedral Head
Tail Fibers
Tail
3D
Base Plate

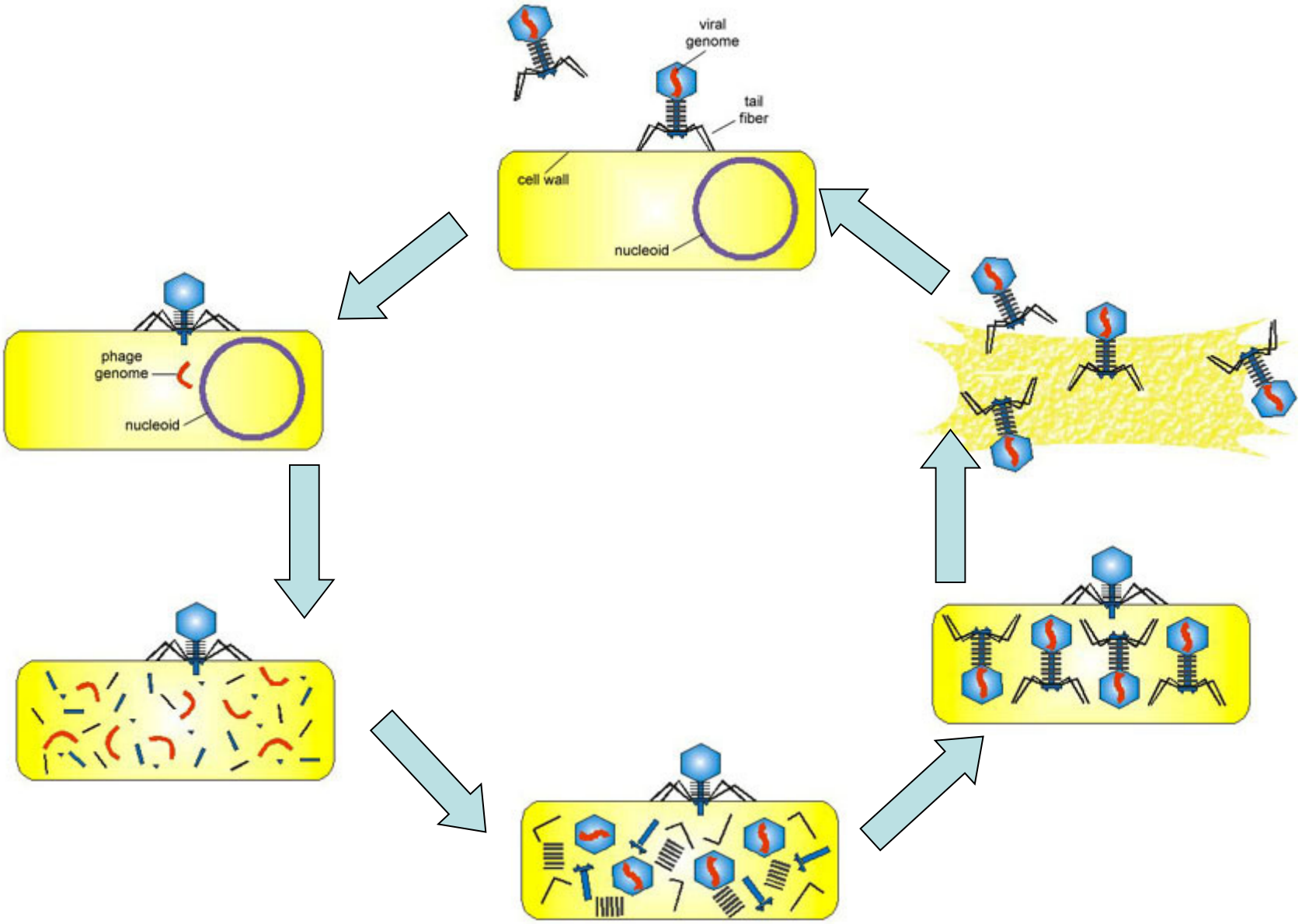




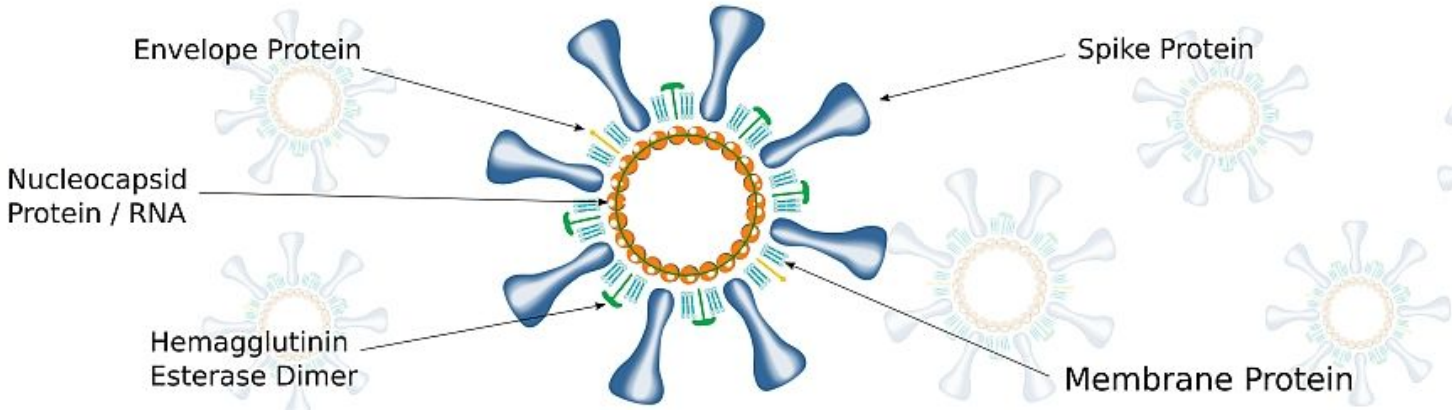


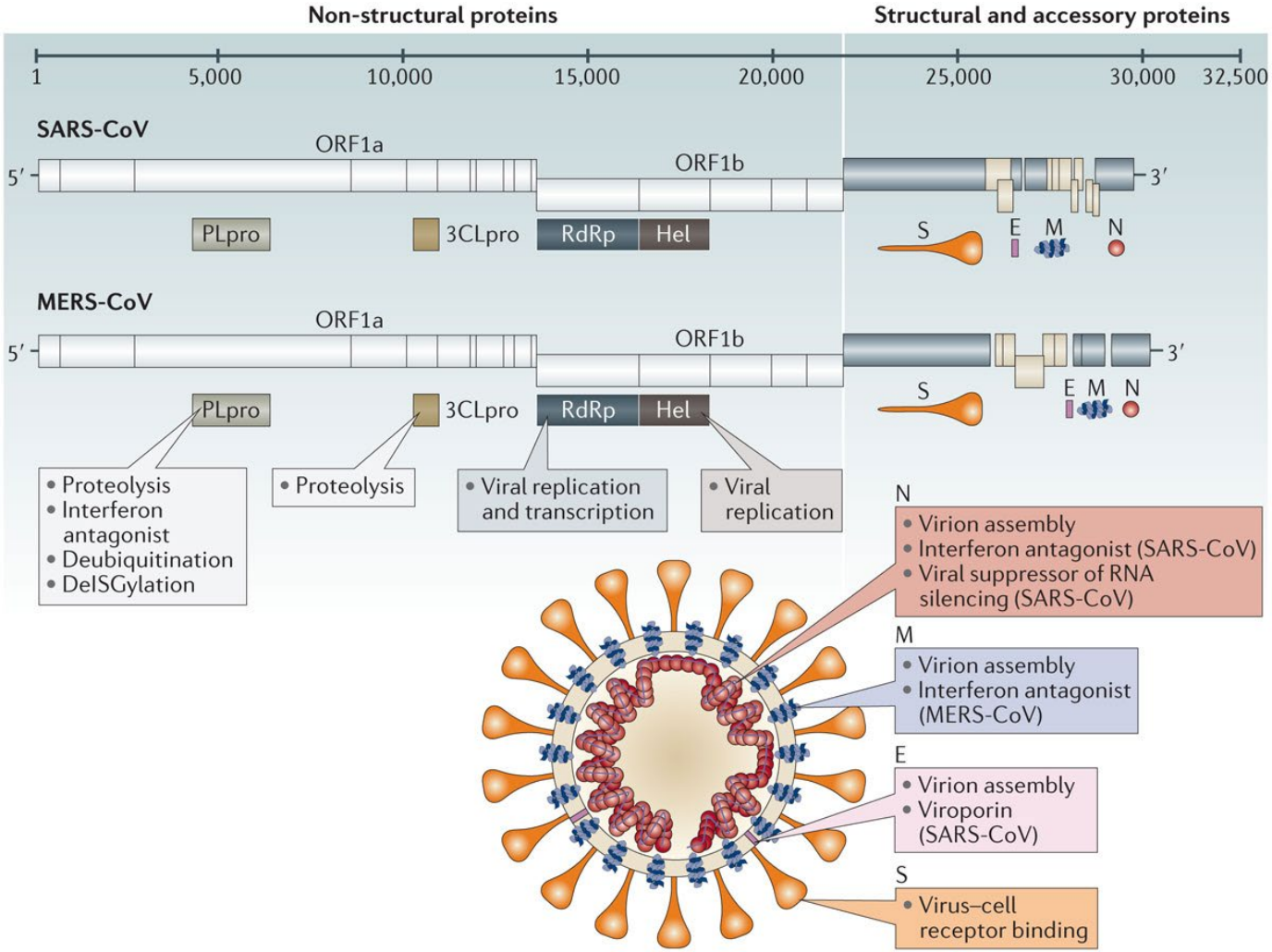

© Marc Adelan, 1985

# Virus Reproduction



# SARS-CoV-2

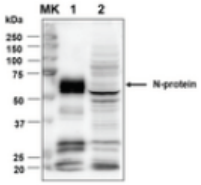

Envelope Protein
Spike Protein
Nucleocapsid
Protein / RNA
Hemagglutinin
Esterase Dimer
Membrane Protein


Non-structural proteins
Structural and accessory proteins
1
5,000
10,000
15,000
20,000
25,000
30,000
32,500
SARS-CoV
ORF1a
ORF1b
5′
3′
PLpro
3CLpro
RdRp
Hel
S
E
M
N
MERS-CoV
ORF1a
ORF1b
5′
3′
PLpro
3CLpro
RdRp
Hel
S
E
M
N
• Proteolysis
• Interferon antagonist
• Deubiquitination
• DelSGylation
• Proteolysis
• Viral replication and transcription
• Viral replication
N
• Virion assembly
• Interferon antagonist (SARS-CoV)
• Viral suppressor of RNA silencing (SARS-CoV)
M
• Virion assembly
• Interferon antagonist (MERS-CoV)
E
• Virion assembly
• Viroporin (SARS-CoV)
S
• Virus–cell receptor binding

# COVID-19 Antibodies

## Monoclonal & Polyclonal Antibodies to SARS-CoV-2

The antibodies available below have been validated to bind to proteins from SARS-CoV-2 (COVID-19), but were developed originally to target proteins from SARS-CoV-1, the virus responsible for the 2003 outbreak. We are currently developing monoclonal mouse and polyclonal rabbit antibodies specific to SARS-CoV-2 spike and nucleocapsid proteins. The polyclonal antibodies will be available in May. The monoclonal antibodies will be available sometime between July - August.




### Rabbit Anti-SARS-CoV-2 Nucleocapsid Protein

Rabbit Anti-SARS-CoV-2 Coronavirus Nucleocapsid Protein

**CODE:** 128-10165-1

**$1,450.00**

SELECT SIZE ADD TO COMPARISON LIST

---

### Mouse Anti-SARS-CoV-2 Nucleocapsid Protein

Mouse Anti-SARS-CoV-2 Coronavirus Nucleocapsid protein

**CODE:** 128-10166-1

**$1,450.00**

SELECT SIZE ADD TO COMPARISON LIST

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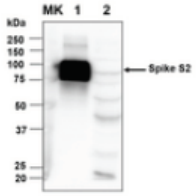


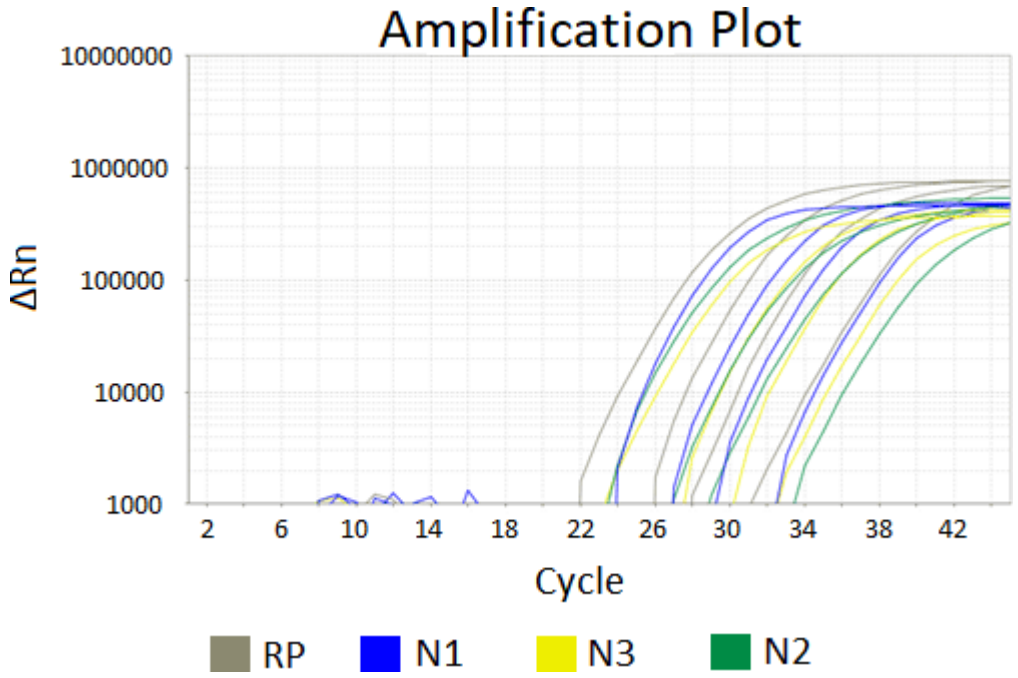

### Rabbit Anti-SARS-CoV-2 Spike Protein

Rabbit Anti-SARS-Associated Coronavirus (COVID-19) Spike Protein

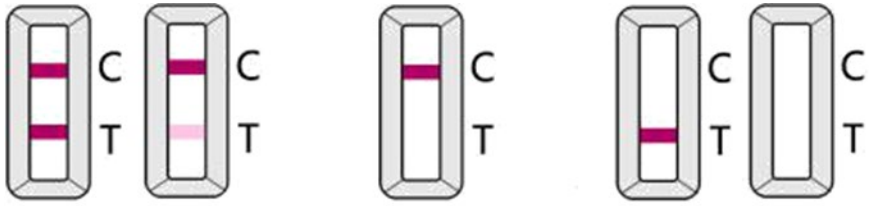
**CODE:** 128-10168-1

**$1,450.00**

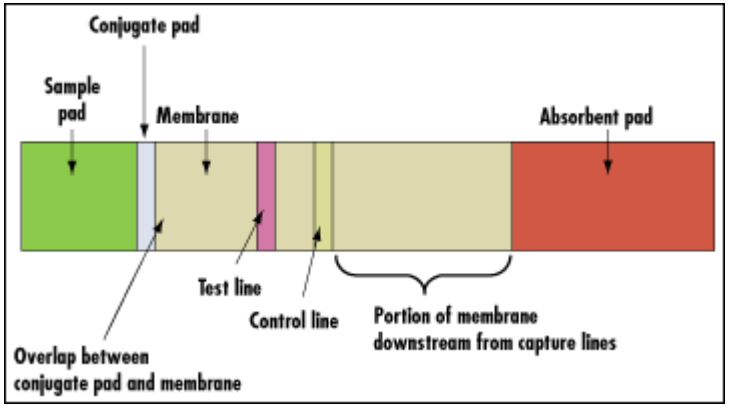
# Real-time RT PCR



# Fast Screening Kit

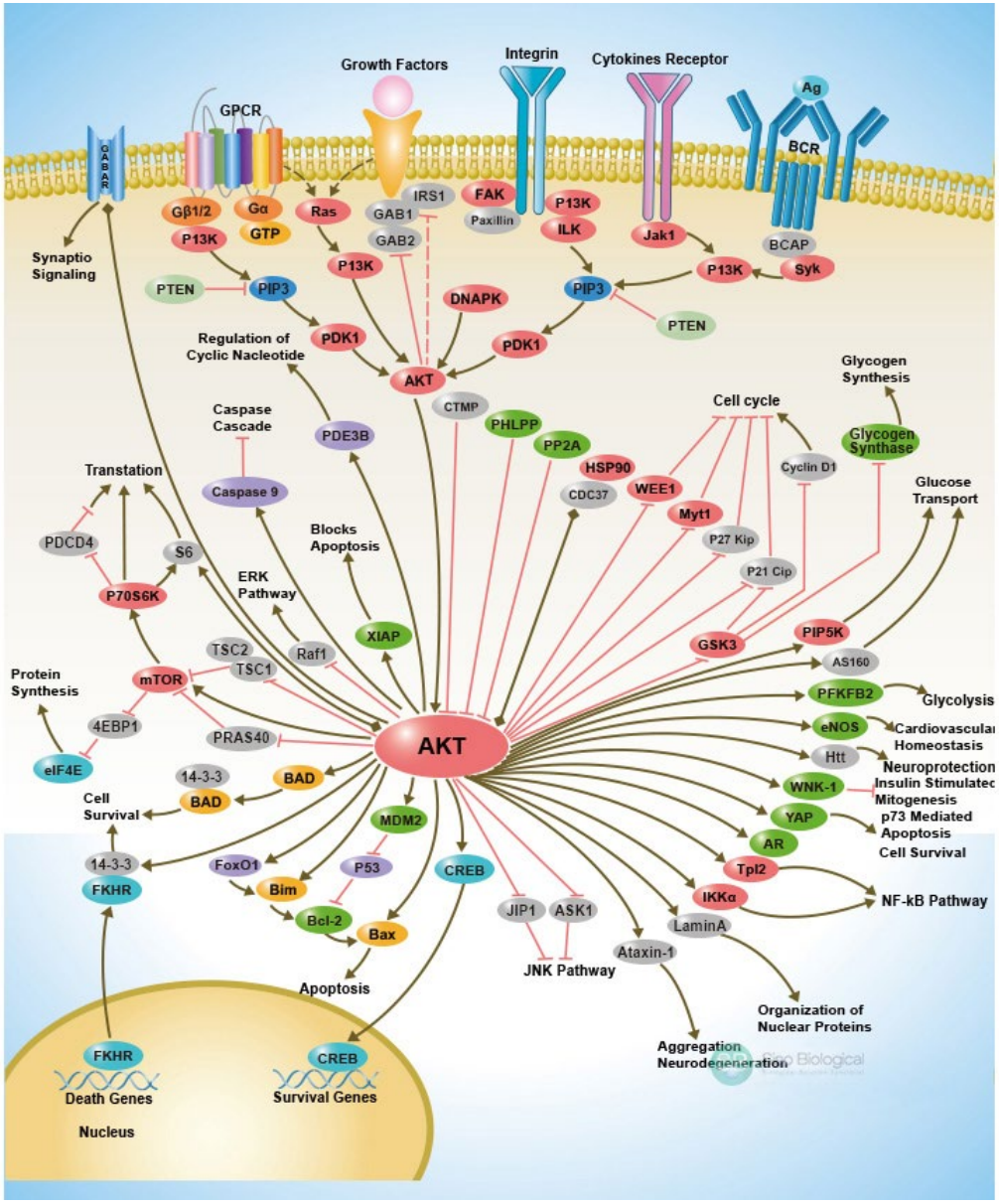



# hCG immunoassay

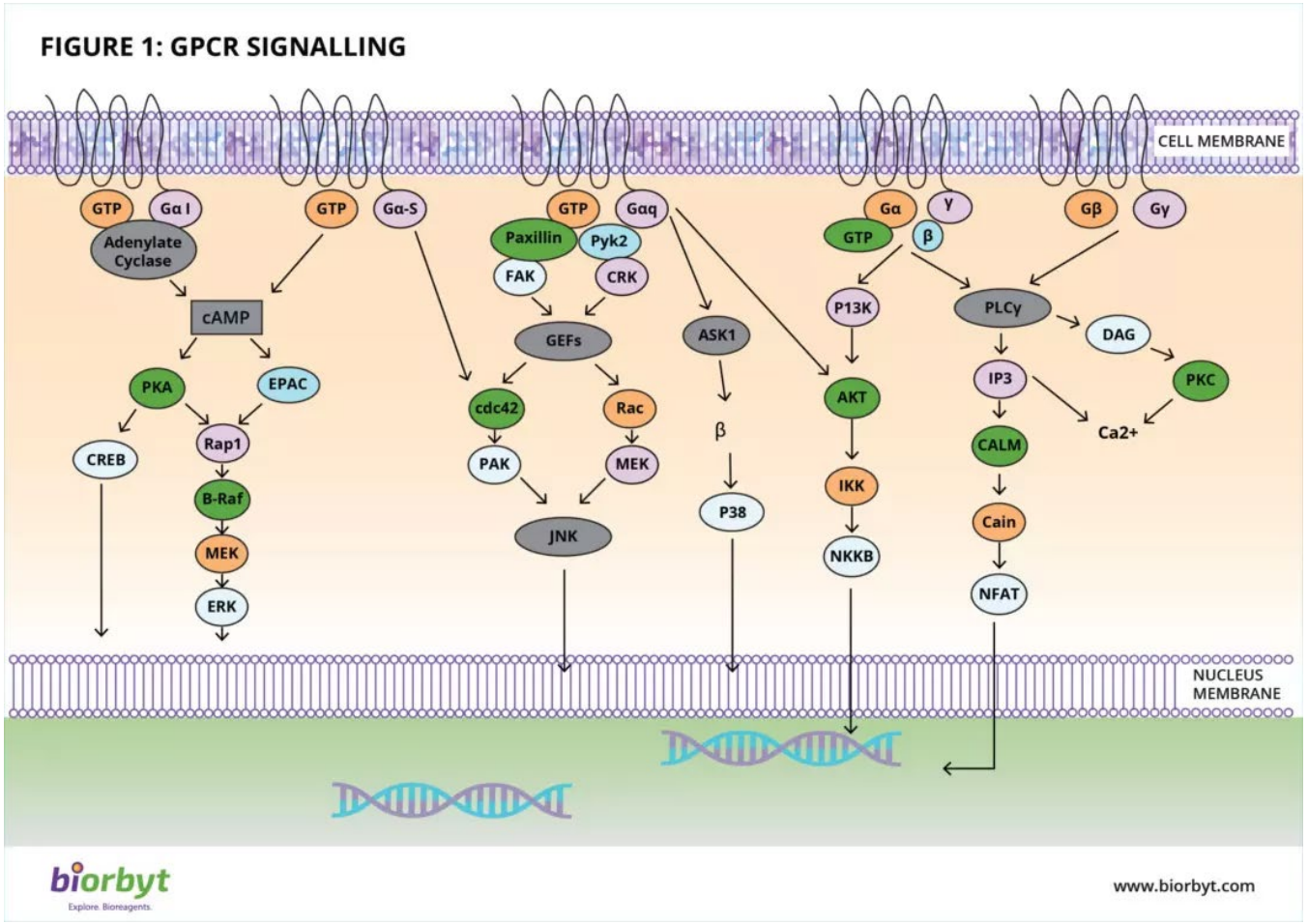




human chorionic gonadotropin (hCG)

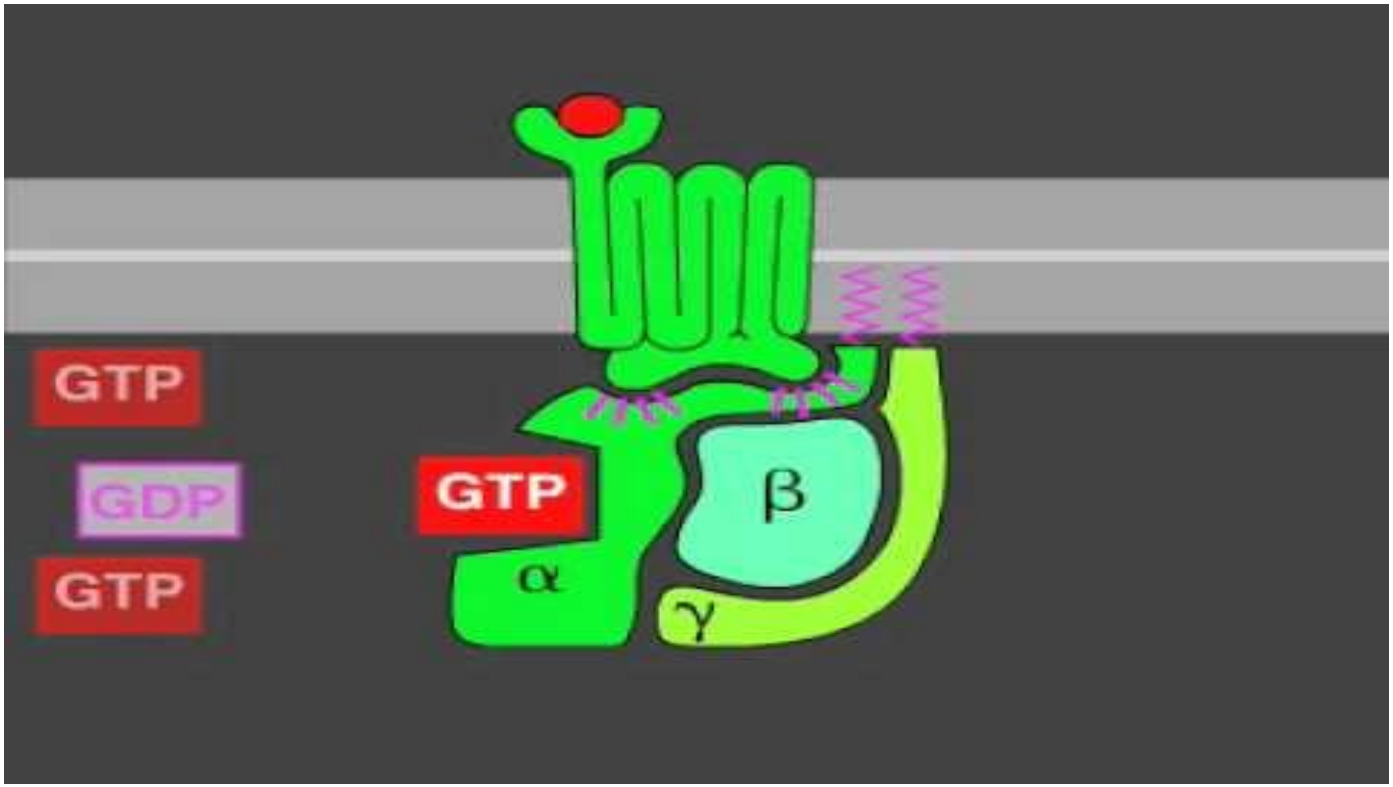
# Signal Transduction



# G-Protein Signaling Pathway

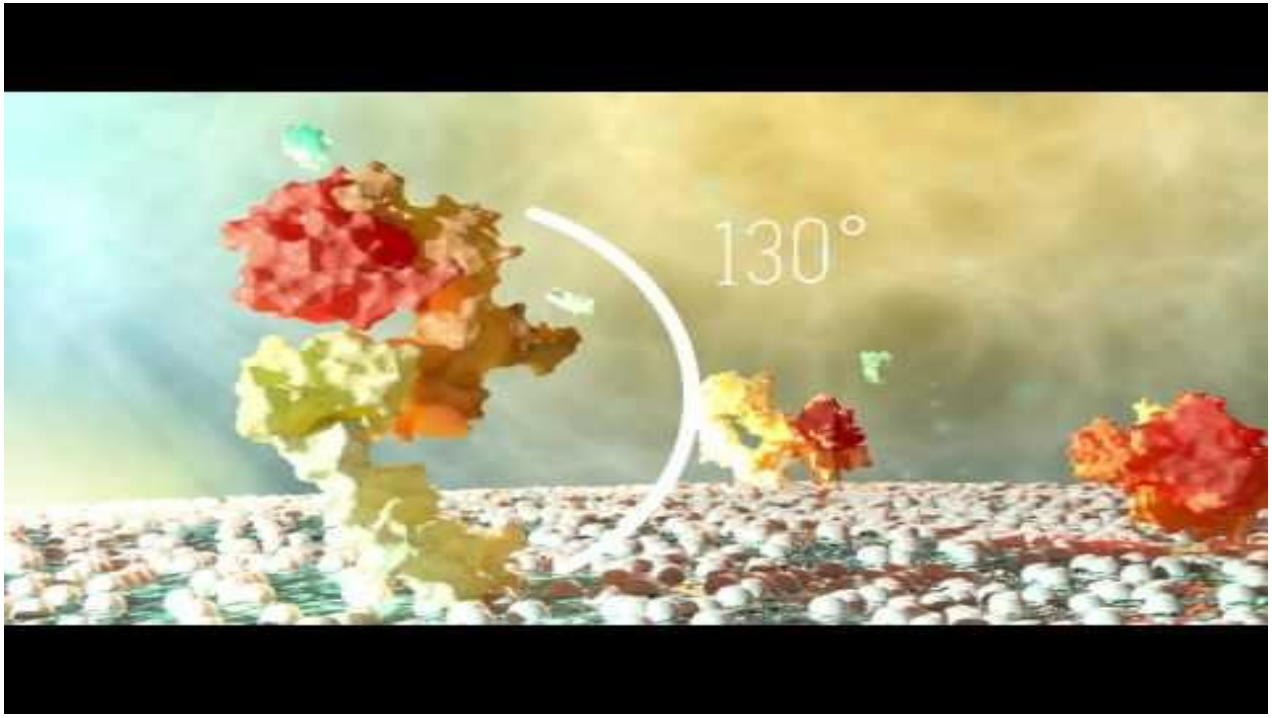


# G Protein



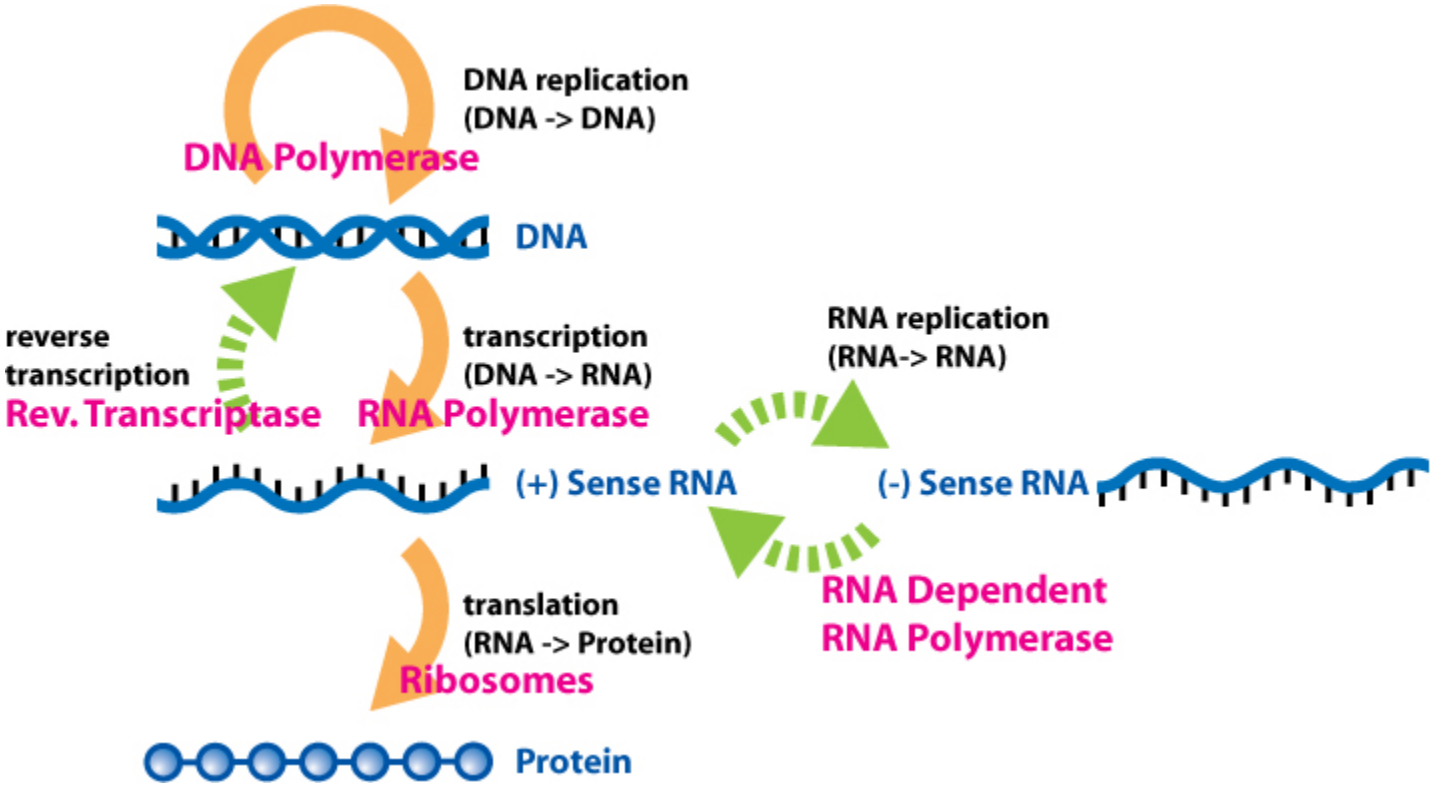

https://www.youtube.com/watch?v=Glu_T6DQuLU

# EGFR




https://www.youtube.com/watch?v=fLGqD5Lm7wY

# Central Dogma



The correlation between RNA expression levels and protein expression is often not perfect due to various factors influencing the process from transcription (RNA synthesis) to translation (protein synthesis) and beyond. Here are several key reasons why RNA expression levels may not always align well with protein expression levels:

**Post-transcriptional Regulation:** After RNA is transcribed, it can undergo various modifications and processing steps that affect its stability, localization, and efficiency of translation. For example, **microRNAs can bind to mRNAs and promote their degradation or inhibit their translation**.

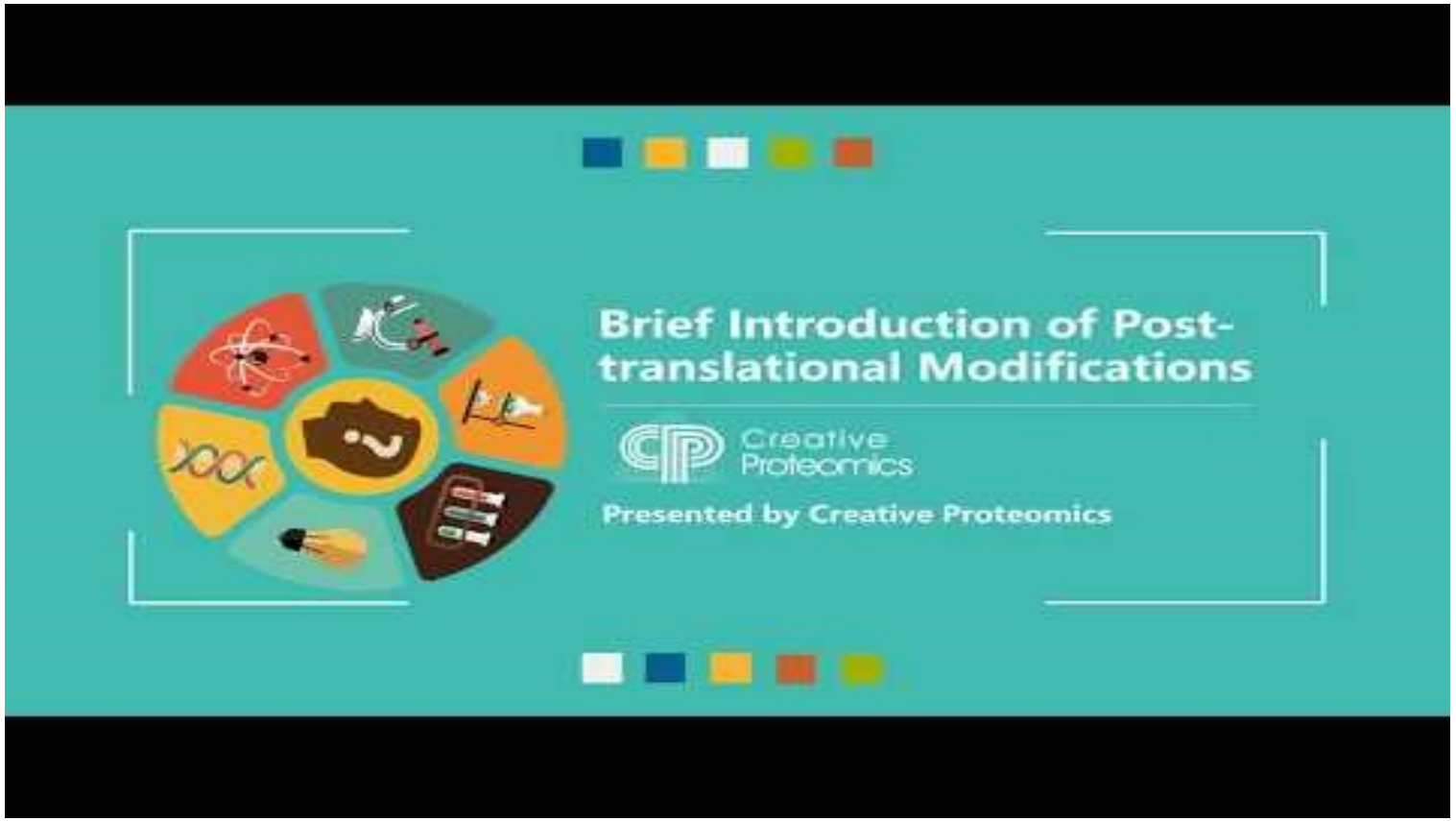
**Translation Efficiency:** The efficiency of translation can vary between different mRNAs depending on factors like the sequence context around the start codon, the length of the 5' untranslated region (UTR), secondary structures, and the availability of tRNAs for rare codons.

**Protein Stability:** Once synthesized, the stability of proteins can vary widely, with some proteins rapidly degraded and others being very stable. This difference can lead to discrepancies between the amount of mRNA present and the level of corresponding protein.

**Post-translational Modifications**: Proteins can undergo various post-translational modifications that can affect their activity, localization, and stability. **These modifications are not predictable from mRNA levels and can significantly influence protein function and abundance.**

**Biological Noise:** Both transcription and translation are subject to stochastic variation, which can lead to cell-to-cell variability in protein levels that is not predicted by mRNA levels alone.

# Post Translational Modification



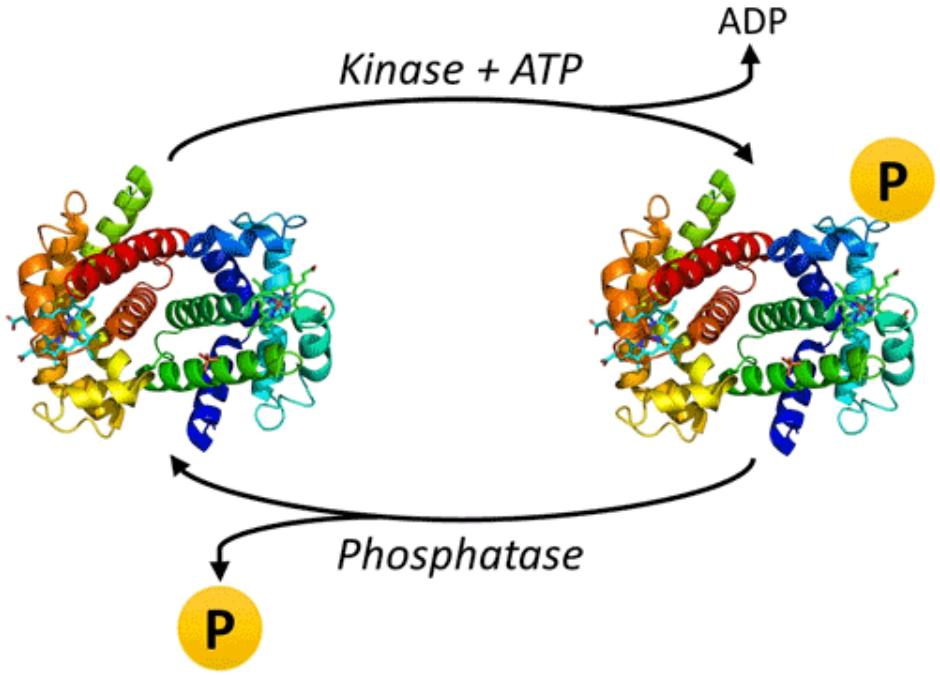

https://www.youtube.com/watch?v=AeVDoDp3lll

Post-translational modification (PTM) refers to the chemical modification of a protein after its synthesis (translation) in the ribosome. PTMs are crucial processes that expand the diversity of the proteome (the entire set of proteins that can be expressed by a cell, tissue, or organism) beyond what is dictated by the genome alone. These modifications can occur at specific amino acid side chains or peptide linkages and significantly influence the protein's function, localization, stability, and interactions with other molecules.

**Phosphorylation:** The addition of a phosphate group, typically to serine, threonine, or tyrosine residues, affecting the activity, localization, and interaction of proteins.
**Ubiquitination:** The attachment of ubiquitin, a small regulatory protein, to lysine residues on a target protein, often tagging it for degradation by the proteasome but also involved in regulating protein activity and location.
**Acetylation:** The addition of an acetyl group, commonly at lysine residues, influencing gene expression and protein stability.
**Glycosylation:** The attachment of sugar moieties to proteins or lipids, impacting their folding, stability, activity, and cellular location.
**Methylation:** The addition of methyl groups, usually on lysine or arginine residues, affecting protein interaction and function.
**Sulfation:** The addition of sulfate groups to tyrosine residues, affecting protein interaction and function.
**Lipidation:** The addition of lipid molecules to proteins, which can affect their membrane localization and function.

# Protein phosphorylation

Phosphorylation in post-translational modification (PTM) refers to the addition of a phosphate group ($PO_4^{3-}$) to a protein, typically to the amino acid residues serine, threonine, or tyrosine in eukaryotic proteins. This modification is catalyzed by enzymes known as kinases, while phosphatases remove phosphate groups. Phosphorylation is a reversible and dynamic modification that plays a crucial role in the regulation of various cellular processes.



# Protein phosphorylation

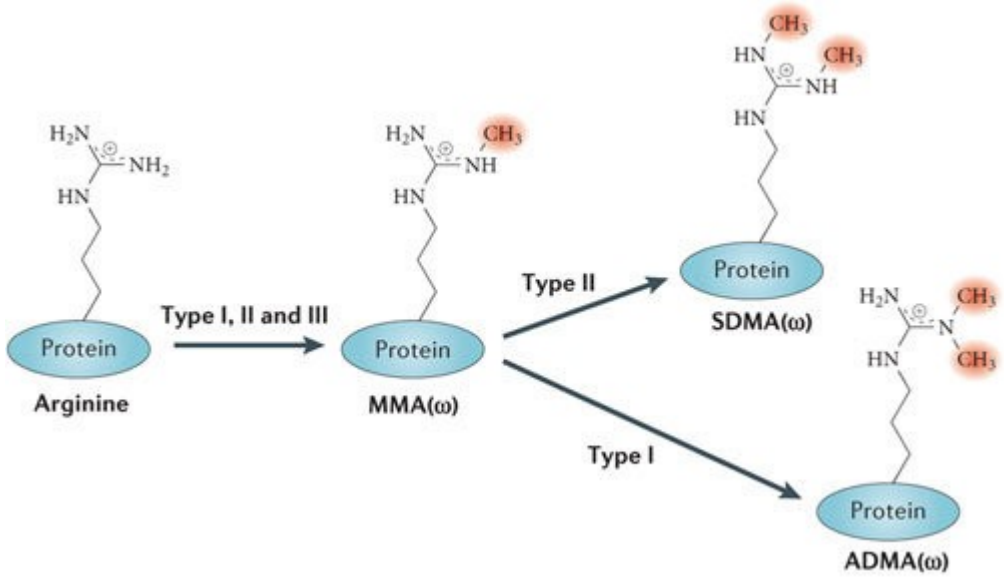

https://www.youtube.com/watch?v=xG2WOd_fWqo

The importance of phosphorylation includes:

**Cell Signaling:** Phosphorylation is pivotal in cell signaling pathways, where it can activate or deactivate enzymes and receptor proteins, thereby transmitting signals inside the cell. This process is fundamental for the cellular responses to hormones, growth factors, and environmental stimuli.
**Protein Function:** Phosphorylation can change a protein's function by altering its conformation, activity, stability, or interaction with other proteins or molecules. This can activate or inhibit the protein's function or redirect its cellular localization.
**Cell Cycle Control**: Phosphorylation regulates the cell cycle, ensuring proper cell division and replication. For example, cyclin-dependent kinases (CDKs) phosphorylate various target proteins to control the progression through different phases of the cell cycle.
**Metabolism:** Phosphorylation plays a key role in metabolic regulation by activating or inhibiting enzymes involved in various metabolic pathways, thus helping to control the energy balance of the cell.
**Transcription and Translation:** Phosphorylation regulates transcription factors and components of the transcription and translation machinery, impacting gene expression and protein synthesis, which are crucial for cell growth, differentiation, and response to external signals.
**Apoptosis:** Phosphorylation is involved in the regulation of apoptosis or programmed cell death, which is vital for removing damaged or unneeded cells and maintaining tissue homeostasis.
**Neuronal Function**: In the nervous system, phosphorylation is essential for neuron function, including neurotransmitter release, receptor activation, and the modulation of ion channel activity, critical for signal transmission and brain function.

# Methylation

Methylation, a specific type of PTM, involves the addition of a methyl group ($CH_3$) to amino acids in a protein, typically to the side chains of arginine or lysine residues in eukaryotic proteins. This modification is carried out by enzymes known as methyltransferases, which use S-adenosylmethionine (SAM) as the methyl donor.



The importance of methylation includes:

**Regulation of Gene Expression:** Methylation of histone proteins, which help package DNA in the nucleus, can influence gene expression. For example, methylation of histone tails can either repress or activate transcription, depending on the specific amino acid methylated and the context within which methylation occurs.

**Protein Function:** Methylation can alter the function of non-histone proteins by affecting their interaction with other proteins, their localization within the cell, and their activity. For instance, methylation can change the conformation of a protein, thereby influencing its function and interactions.

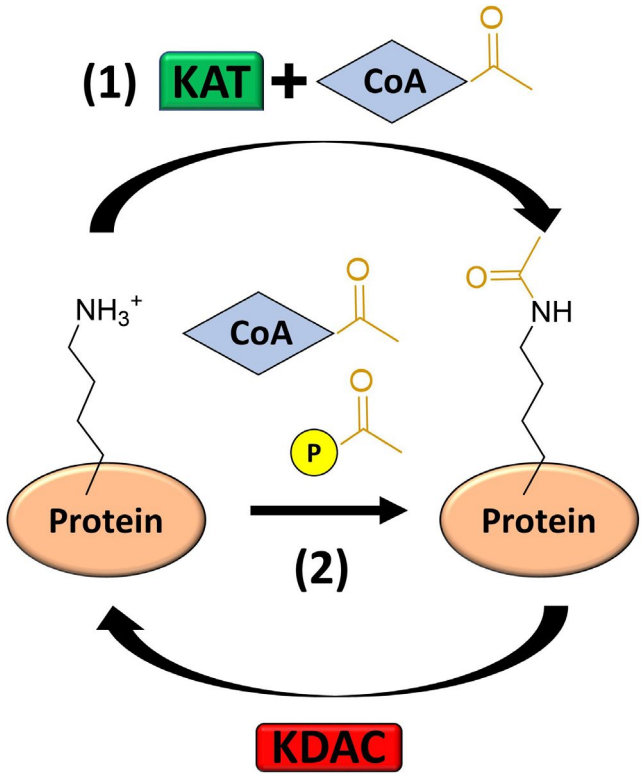
**Protein Stability:** Methylation can also impact the stability of proteins. Certain methylations can protect proteins from ubiquitin-mediated degradation, thereby prolonging their half-life in the cell.

**Signal Transduction:** Methylation plays roles in signal transduction pathways, modifying signaling proteins and modulating their activity to ensure appropriate cellular responses to external stimuli.

**Cellular Differentiation and Development:** Proper methylation is crucial for normal development and cellular differentiation, influencing processes ranging from embryonic development to the maintenance of adult tissue homeostasis.

# Acetylation

Acetylation in post-translational modification (PTM) refers to the addition of an acetyl group ($COCH_3$) to a protein, often at a lysine amino acid residue. This modification is catalyzed by enzymes known as acetyltransferases, which transfer the acetyl group from acetyl-coenzyme A (acetyl-CoA) to the target protein. Conversely, deacetylases remove acetyl groups from proteins.



The importance of acetylation in proteins includes several key aspects:

**Gene Regulation:** Acetylation of histone proteins, which DNA wraps around in chromatin, is crucial for the regulation of gene expression. When histones are acetylated, the chromatin structure becomes more open, allowing gene transcription machinery to access the DNA. Conversely, deacetylation typically tightens chromatin structure and represses gene transcription.

**Protein Function:** Beyond histones, acetylation can regulate the activity, stability, and interaction of non-histone proteins with other molecules. By altering the charge of lysine residues, acetylation can change protein conformations and interactions, influencing various cellular pathways.

**Protein Stability:** Acetylation can also affect the stability and degradation of proteins. For instance, acetylation can prevent ubiquitination at the same lysine residue, thereby inhibiting proteasome-mediated degradation and extending the protein's half-life.

**Cellular Localization:** Acetylation can influence where proteins are located within the cell, affecting their function. For example, certain proteins are shuttled between the nucleus and cytoplasm depending on their acetylation status.

**Signal Transduction:** Acetylation plays a role in signal transduction, impacting how cells respond to external or internal signals. This can have wide-ranging effects on cell growth, division, and response to stress.

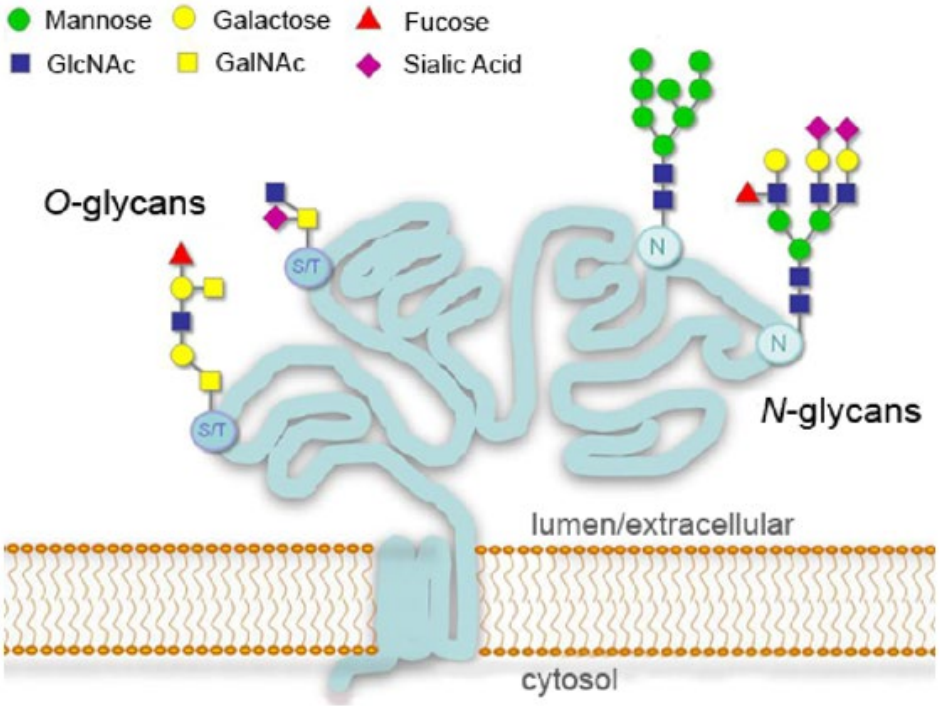
**Interaction with Other Molecules**: Acetylation can regulate the interaction between proteins and DNA, proteins and other proteins, or proteins and small molecules, which is fundamental for numerous cellular processes.

# Glycosylation

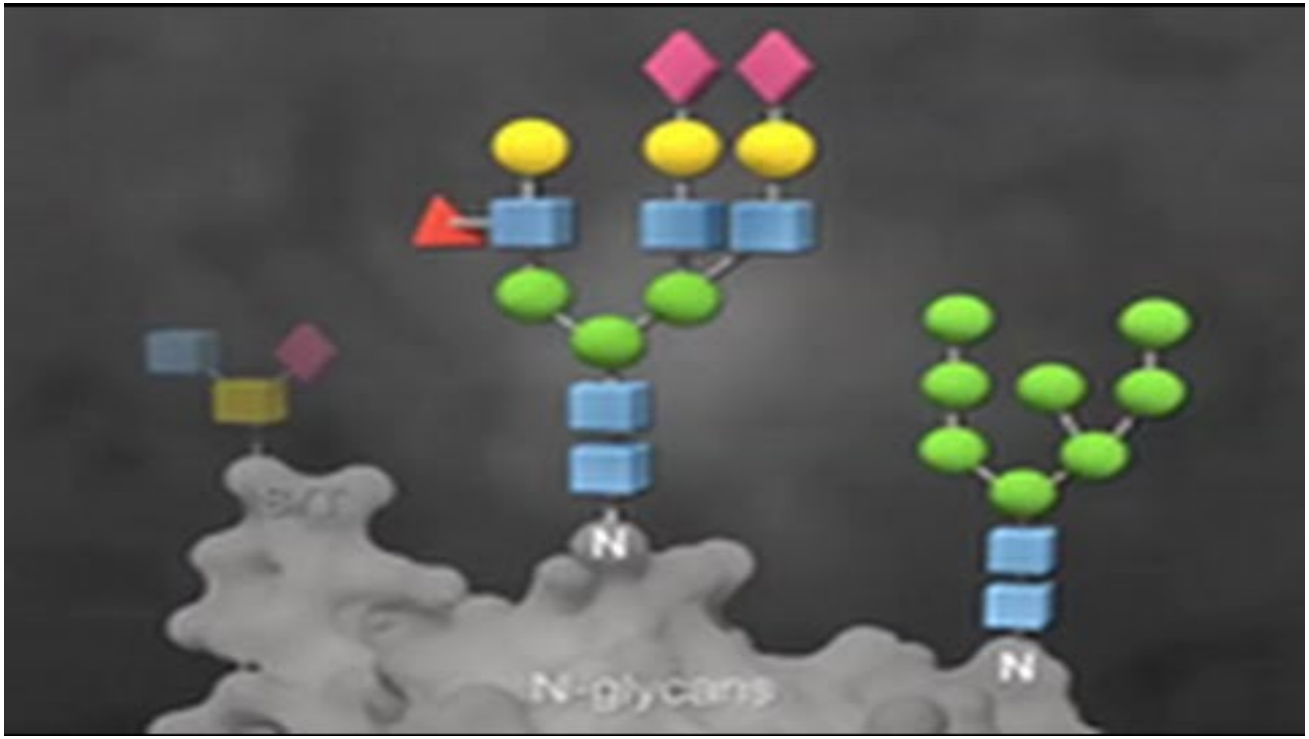
There are two main types of protein glycosylation:

N-linked Glycosylation: This occurs when a sugar molecule is attached to an asparagine (Asn) residue of a protein. It is typically found in the consensus sequence Asn-X-Ser/Thr, where X can be any amino acid except proline.

O-linked Glycosylation: This occurs when a sugar molecule is attached to the oxygen ato of serine (Ser) or threonine (Thr) residues in proteins.



# Glycosylation

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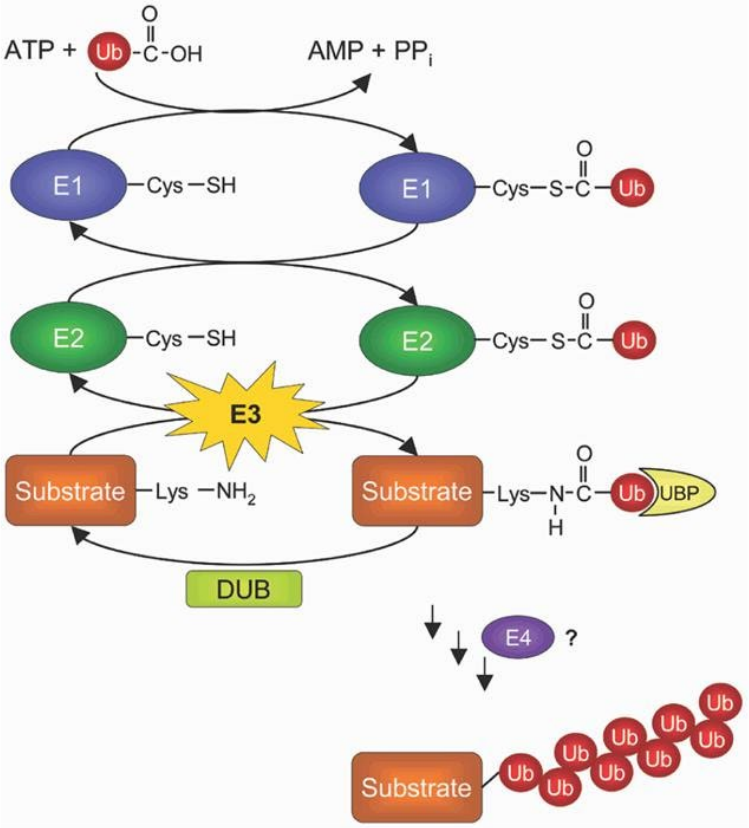
https://www.youtube.com/watch?v=RorGifz6C2Y

The importance of glycosylation includes:

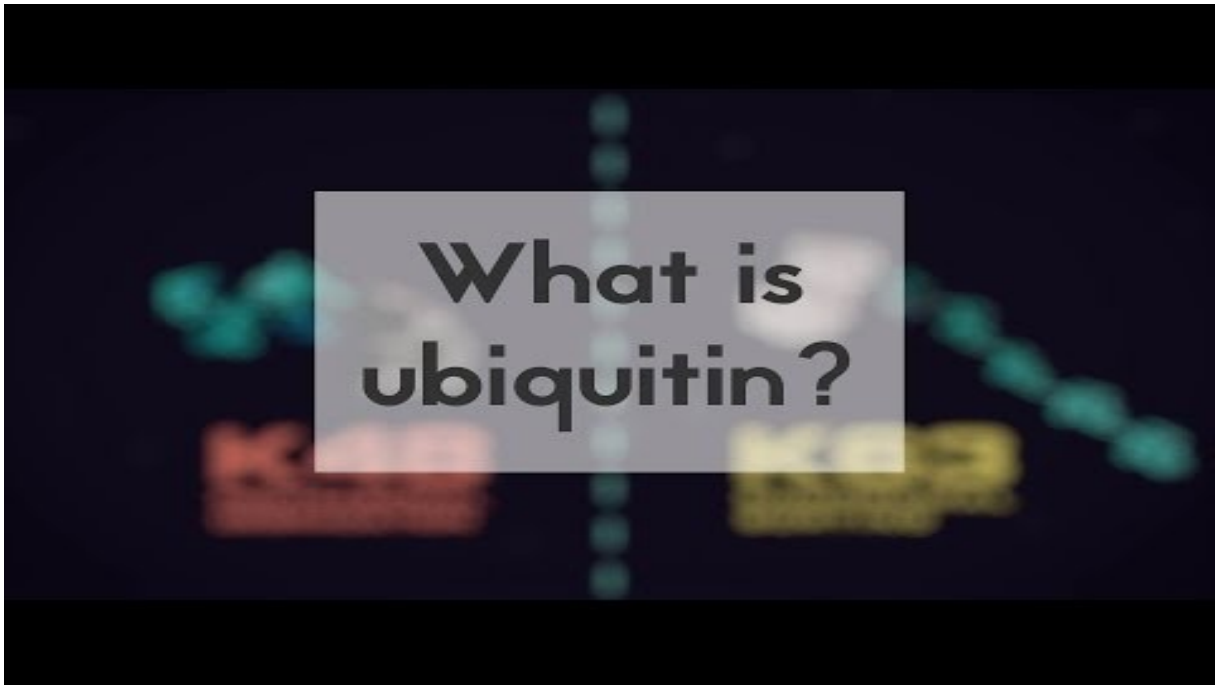
**Protein Folding:** Glycosylation helps in proper protein folding and stability, aiding in the correct three-dimensional conformation necessary for protein function. It also assists in the quality control mechanisms within the cell, such as in the endoplasmic reticulum, where improperly folded glycoproteins are targeted for degradation.
**Cellular Recognition and Signaling:** Glycosylated proteins on the cell surface play critical roles in cell-cell recognition, communication, and signaling. They are involved in various processes such as immune response, where they contribute to the recognition of antigens by immune cells.
**Protein Stability and Half-life:** Glycosylation can increase the stability of proteins and protect them from proteolysis, thus extending their half-life in the circulatory system.
**Cell Adhesion:** Glycosylation contributes to cell adhesion processes, crucial for the development and maintenance of tissues and for the immune system's function, by mediating the interaction between cells and the extracellular matrix.
**Pathogen Recognition:** Many pathogens are recognized by their glycan structures. Host organisms can detect these structures and mount an immune response. Conversely, pathogens can exploit host glycosylation processes for cell entry or immune evasion.
**Therapeutic Proteins:** Many biopharmaceuticals, including antibodies and hormones, are glycosylated. The glycosylation patterns can significantly affect the efficacy and pharmacokinetics of these therapeutic proteins.

# Ubiquitination

Ubiquitination in post-translational modification (PTM) refers to the covalent attachment of a small protein called ubiquitin to a target protein. This process is carried out through a cascade involving three types of enzymes: E1 (ubiquitin-activating enzyme), E2 (ubiquitin-conjugating enzyme), and E3 (ubiquitin ligase), which work together to attach ubiquitin to lysine residues on substrate proteins.



# Ubiquitination

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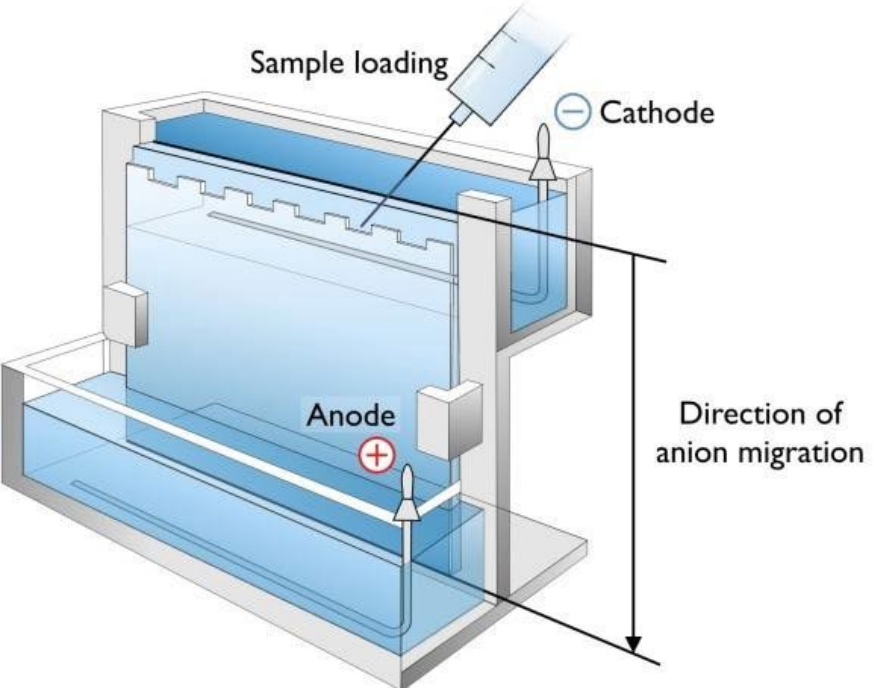
https://www.youtube.com/watch?v=e29F7R3K_5A

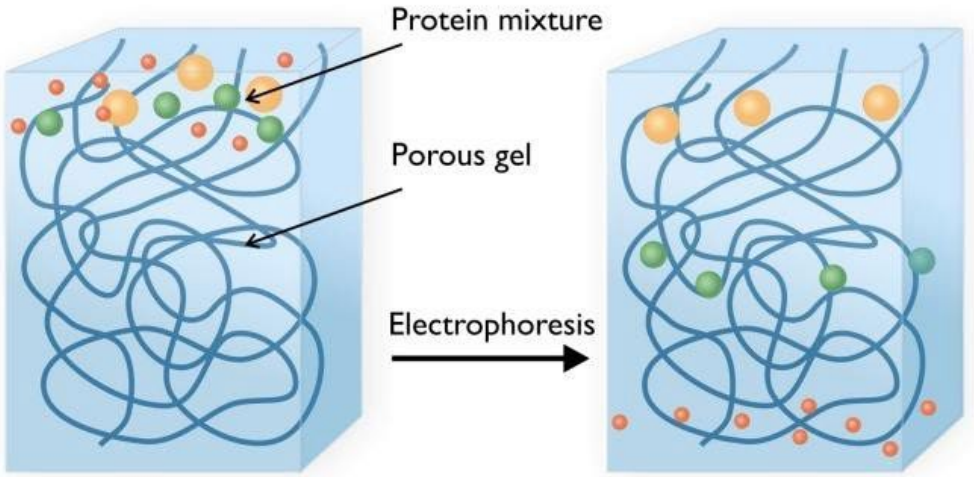
# Ubiquitination

https://www.youtube.com/watch?v=jbc1QCu9hFg

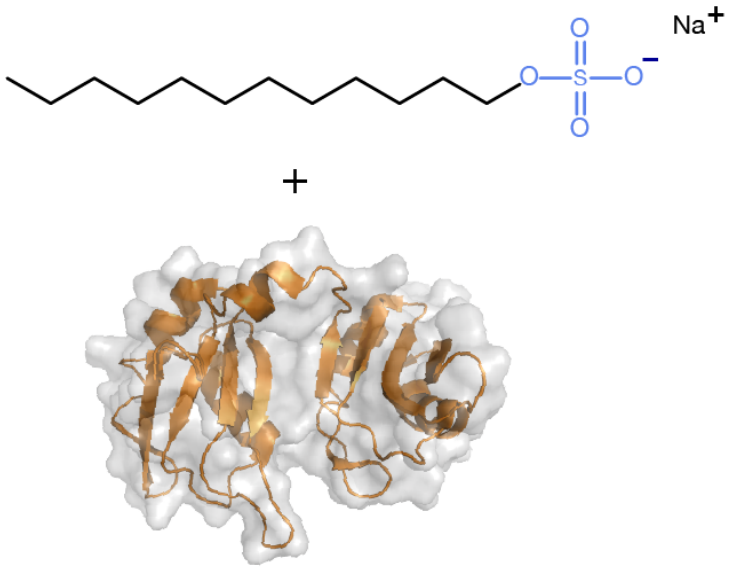
**Proteasomal Degradation:** The most well-known function of ubiquitination is targeting proteins for degradation by the 26S proteasome. Polyubiquitin chains, particularly those linked through lysine 48 of ubiquitin, serve as a signal for proteins to be recognized and degraded by the proteasome, thereby regulating protein levels within the cell.
**Regulation of Protein Function and Activity:** Ubiquitination can also regulate protein activity, function, and localization independently of degradation. For example, monoubiquitination or polyubiquitination with chains linked through other lysine residues (like K63) can influence protein interactions, cellular localization, and activity without targeting the protein for degradation.
**Cell Signaling:** Ubiquitination plays critical roles in various signaling pathways. For instance, in the NF-κB pathway, the ubiquitination of certain components leads to their activation and translocation to the nucleus, where they affect gene expression.
**DNA Repair:** In the cellular response to DNA damage, ubiquitination helps regulate the repair process. Specific patterns of ubiquitination can recruit DNA repair enzymes to damaged sites, facilitating repair mechanisms that maintain genomic integrity.
**Cell Cycle Regulation:** Ubiquitination controls the levels and activity of various cell cycle regulators, ensuring proper cell cycle progression and division. Key regulatory proteins are ubiquitinated and degraded at specific points, allowing the cell cycle to proceed or be halted as necessary.
**Immune Response:** Ubiquitination is involved in the regulation of innate and adaptive immune responses, including the presentation of antigens on major histocompatibility complex (MHC) molecules, the regulation of inflammatory signaling pathways, and the modulation of immune cell function.

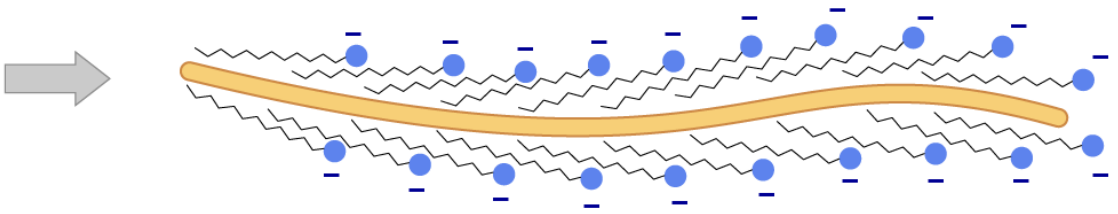
# Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)



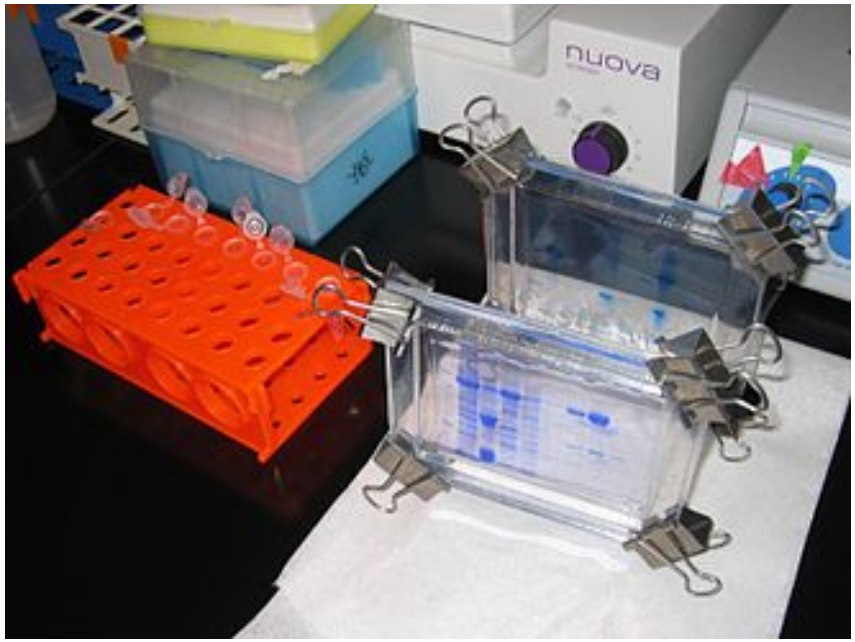



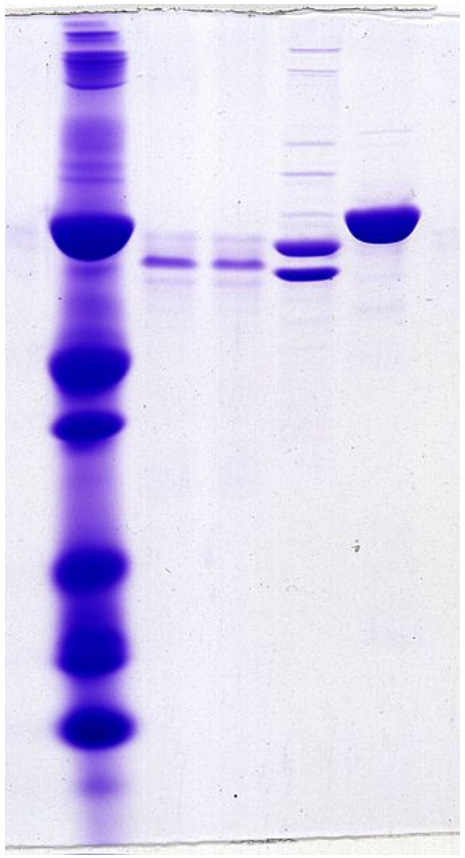
# Protein Denature



# SDS-PAGE



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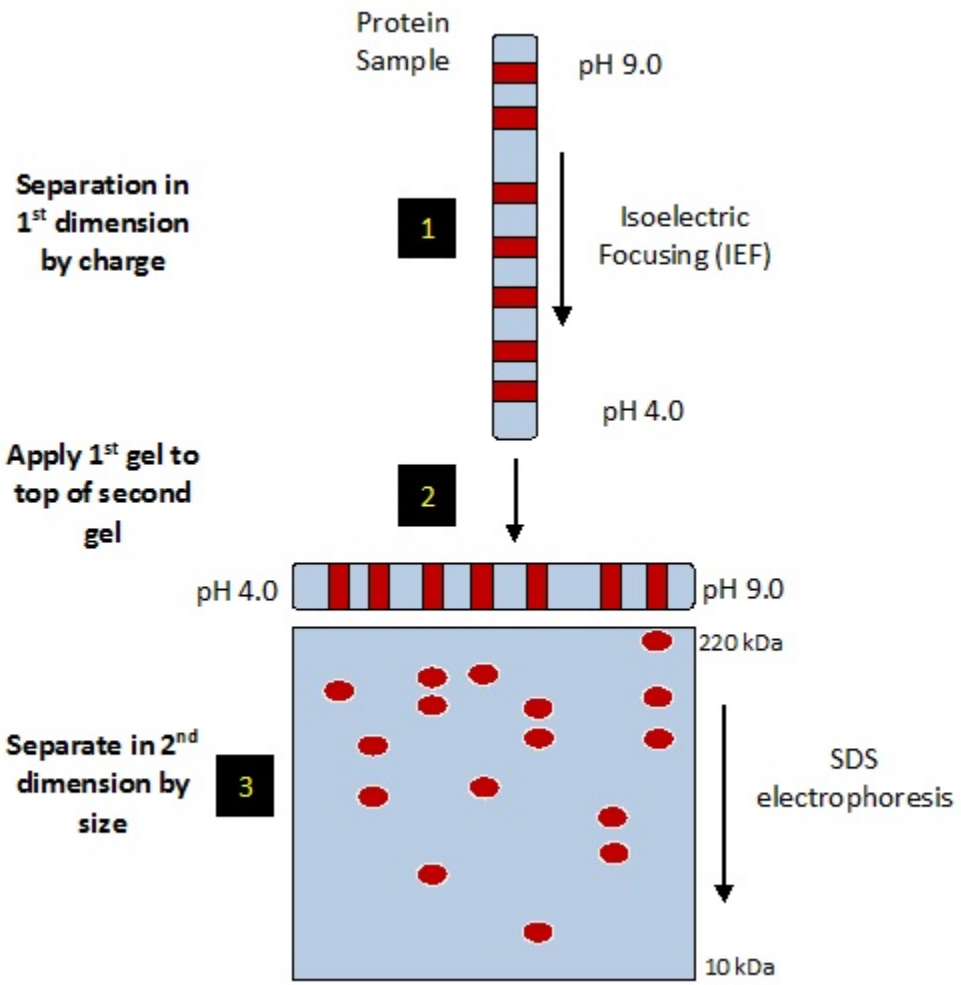
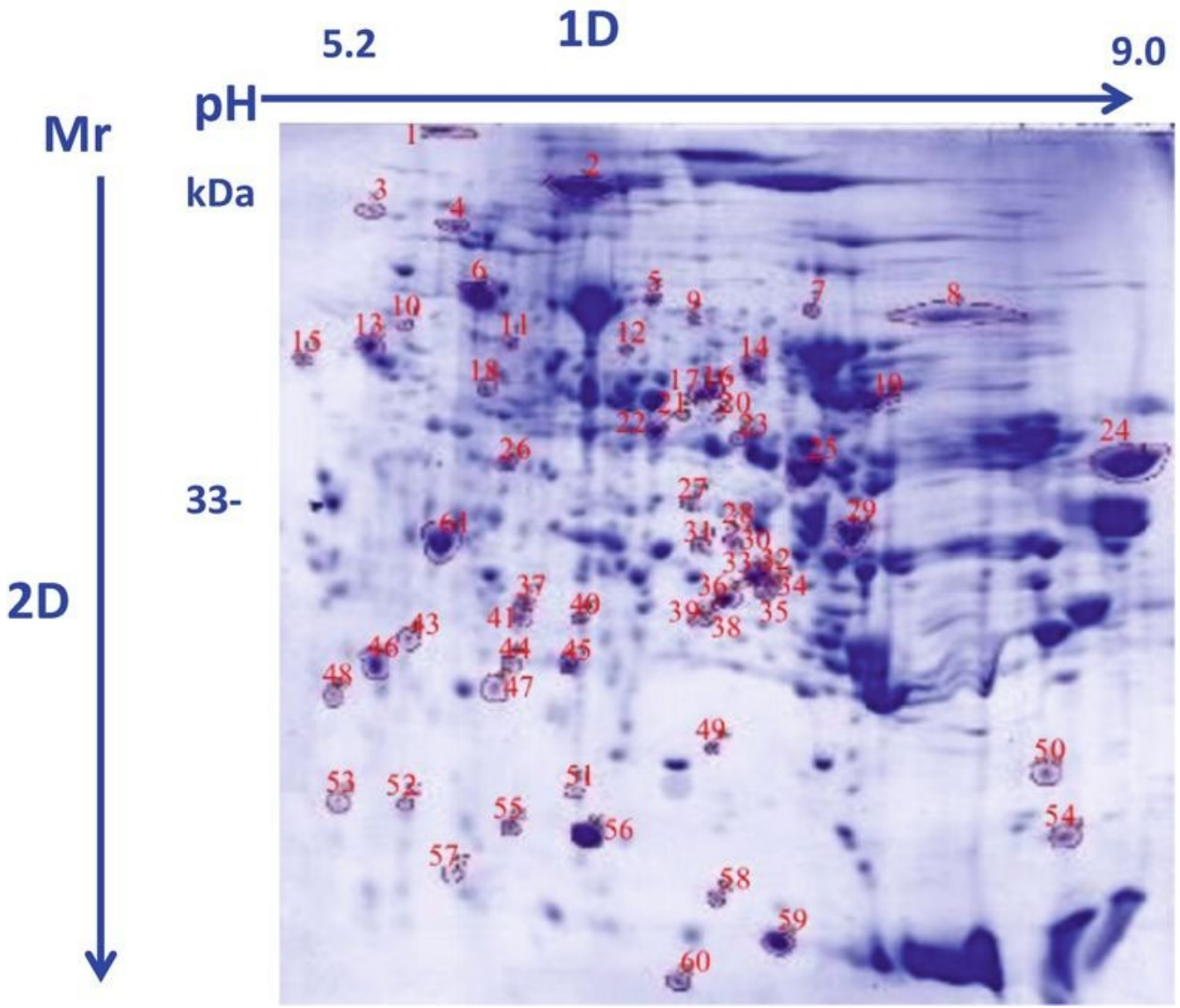
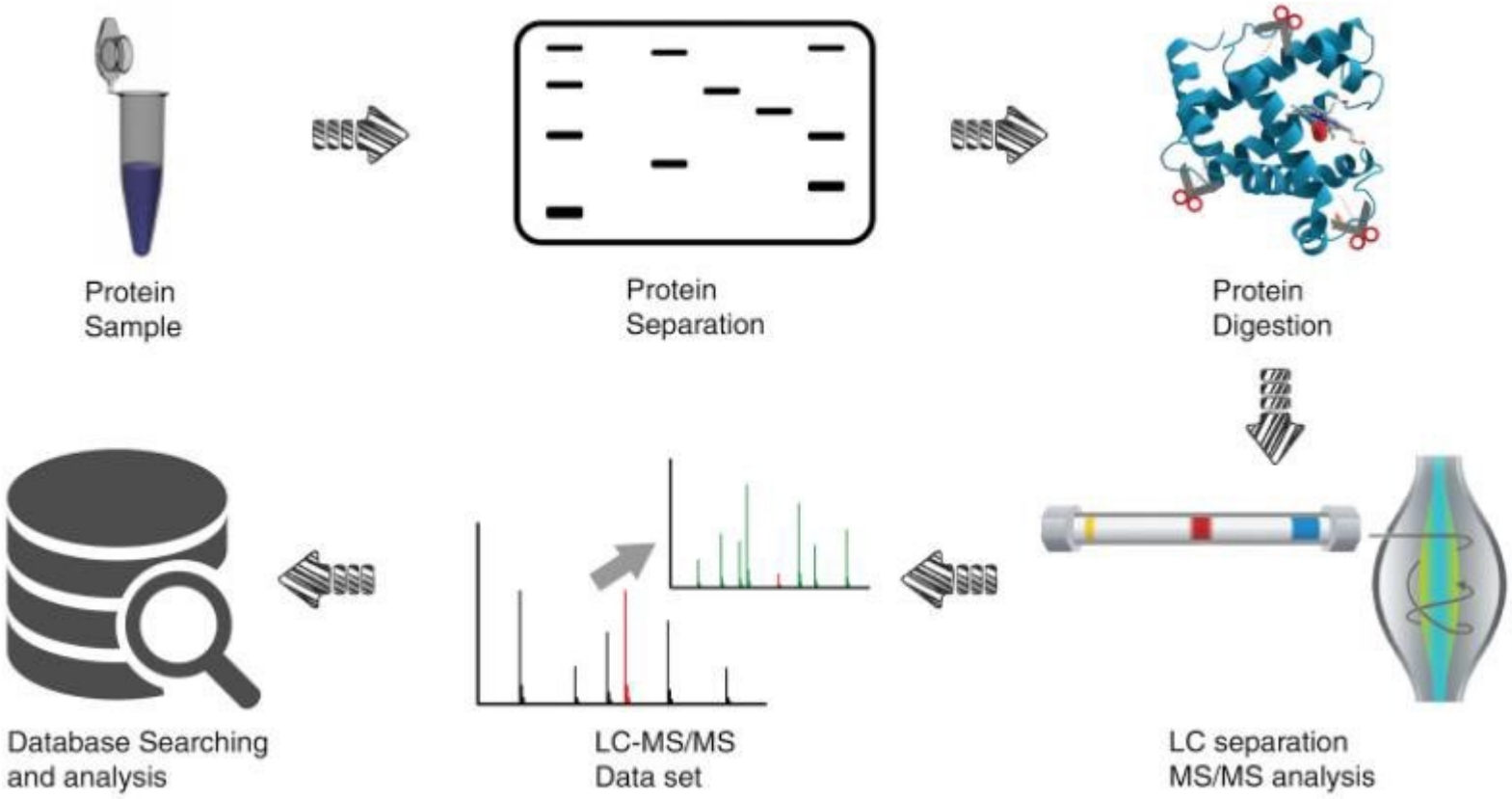
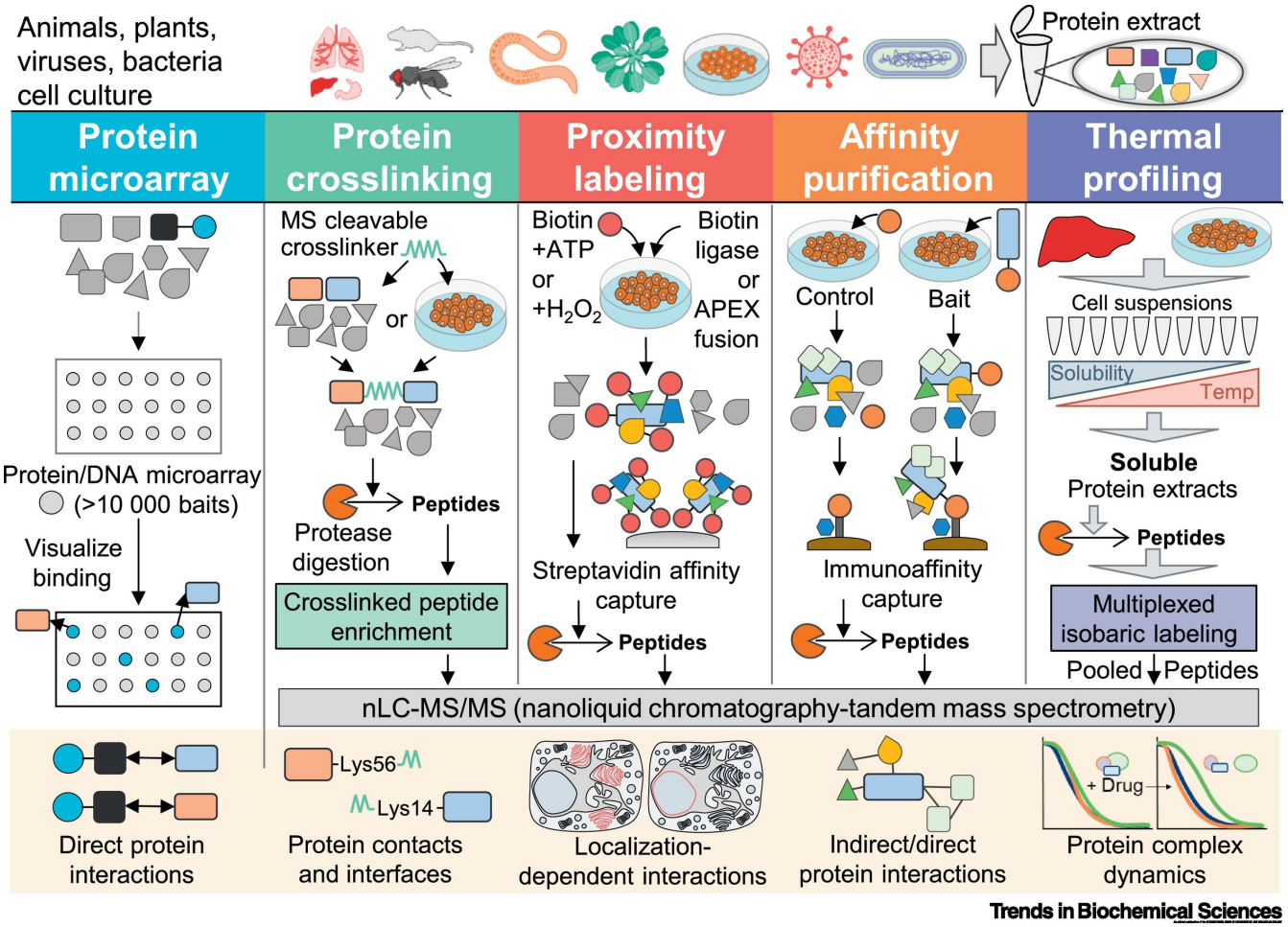

Protein Sample
pH 9.0
Separation in 1st dimension by charge
1
Isoelectric Focusing (IEF)
pH 4.0
Apply 1st gel to top of second gel
2
pH 4.0
pH 9.0
220 kDa
Separate in 2nd dimension by size
3
SDS electrophoresis
10 kDa

# 2D PAGE




Protein
Sample
Protein
Separation
Protein
Digestion
Database Searching
and analysis
LC-MS/MS
Data set
LC separation
MS/MS analysis

# Proteomic

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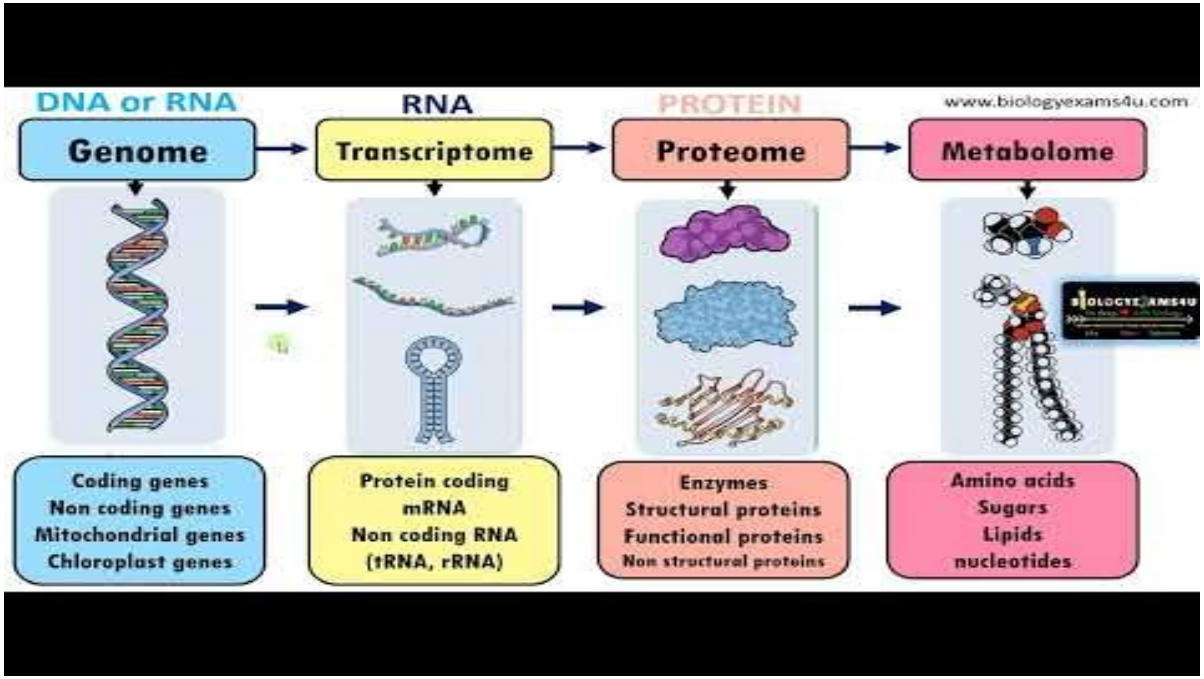
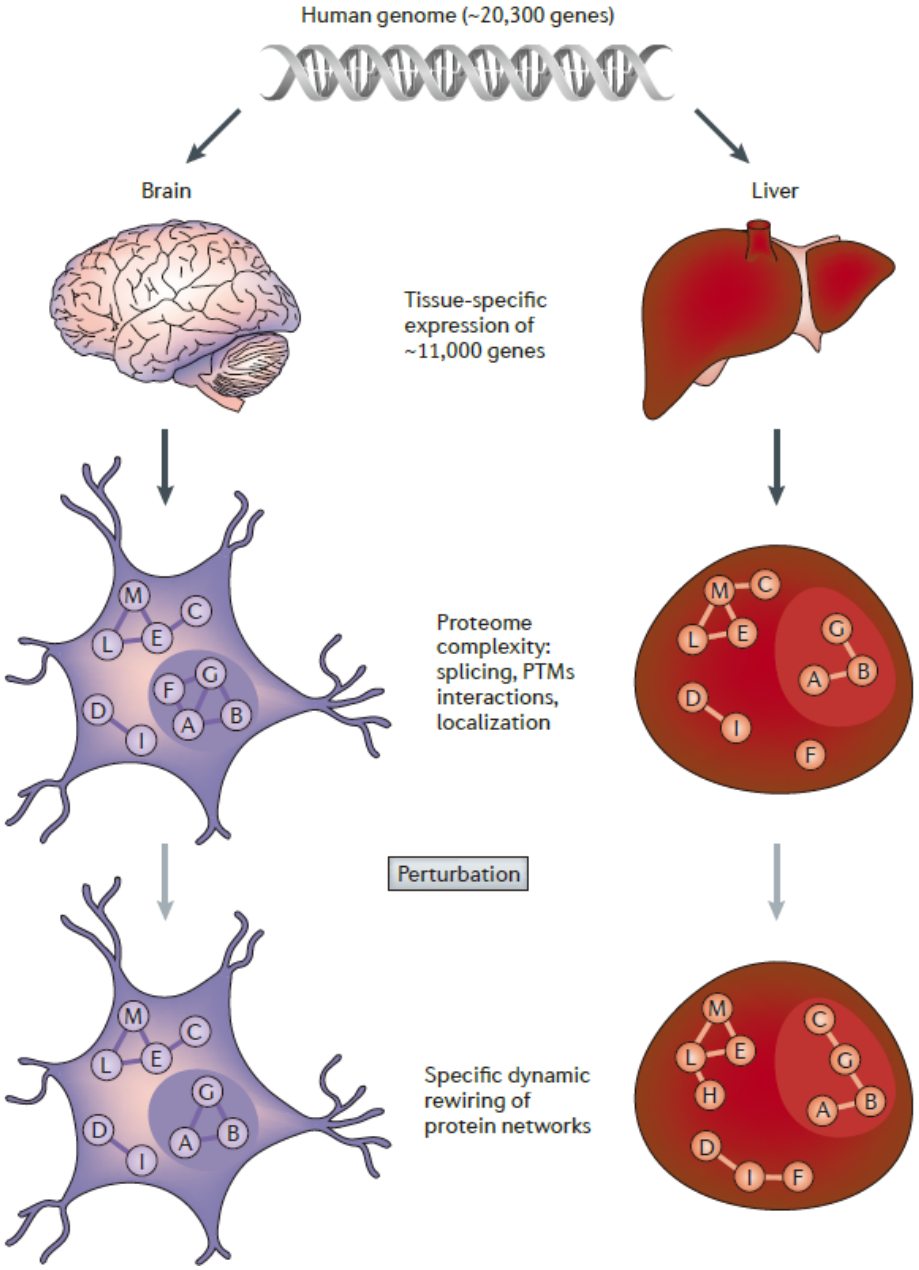
# Multiomic




https://www.youtube.com/watch?v=FJ5iN-v0MFM

# Next-generation proteomics: towards an integrative view of proteome dynamics

*A. F. Maarten Altelaar[1,2]*, Javier Munoz[1,2,3]* and Albert J. R. Heck[1,2]*



**Key Points**

•Our understanding of cellular function depends on exquisite knowledge of all of the molecular components acting in a system. Mass spectrometry (MS)-based proteomics has matured immensely in the last decade, allowing quantitative system-wide analysis of the proteome, including post-translational modifications (PTMs), protein–protein interactions and cellular localization.

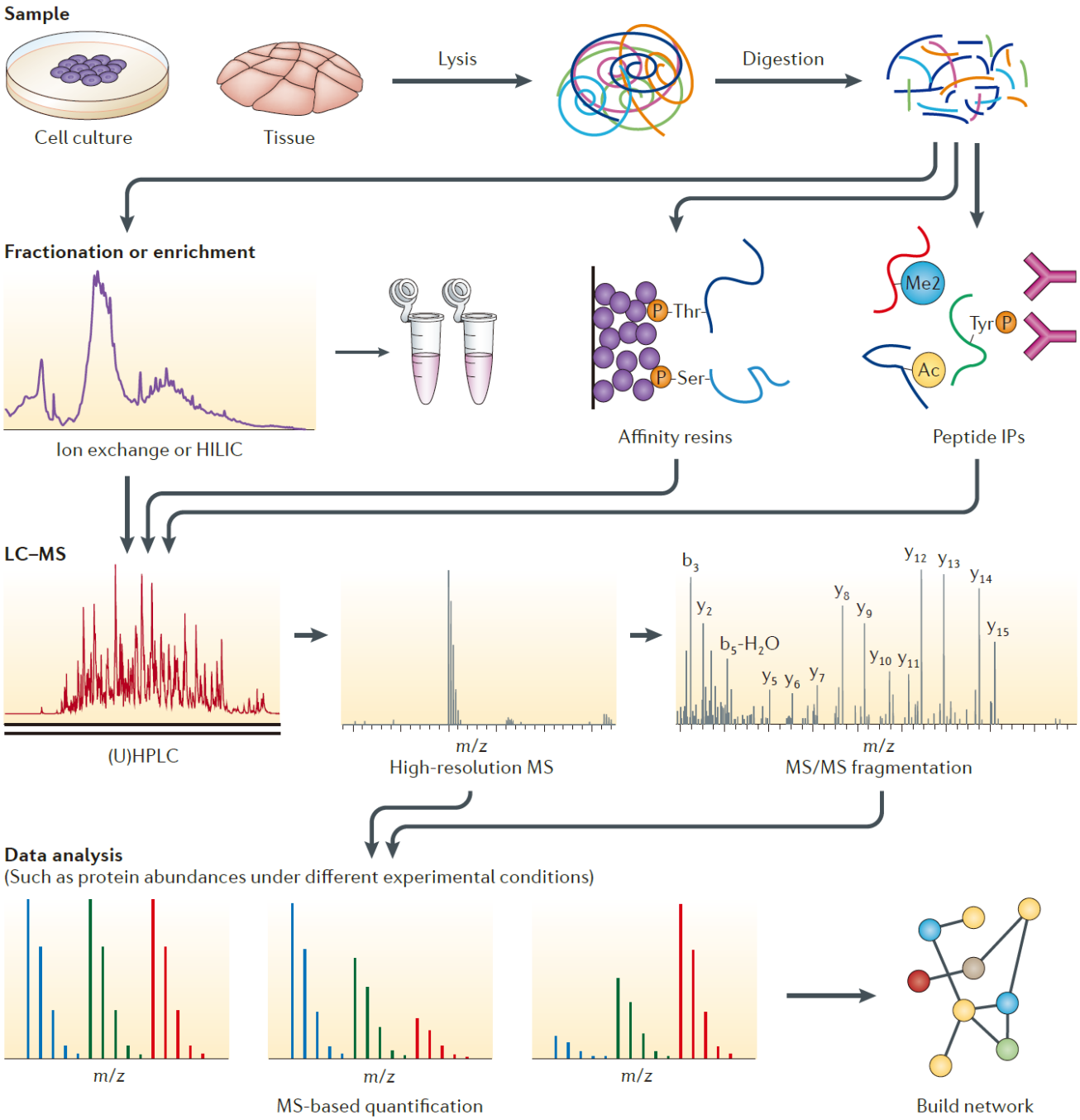
•Quantification of the entire set of proteins expressed in a complex biological system (for example, mammalian cells) is now possible with a high sensitivity and in a reasonable amount of time.

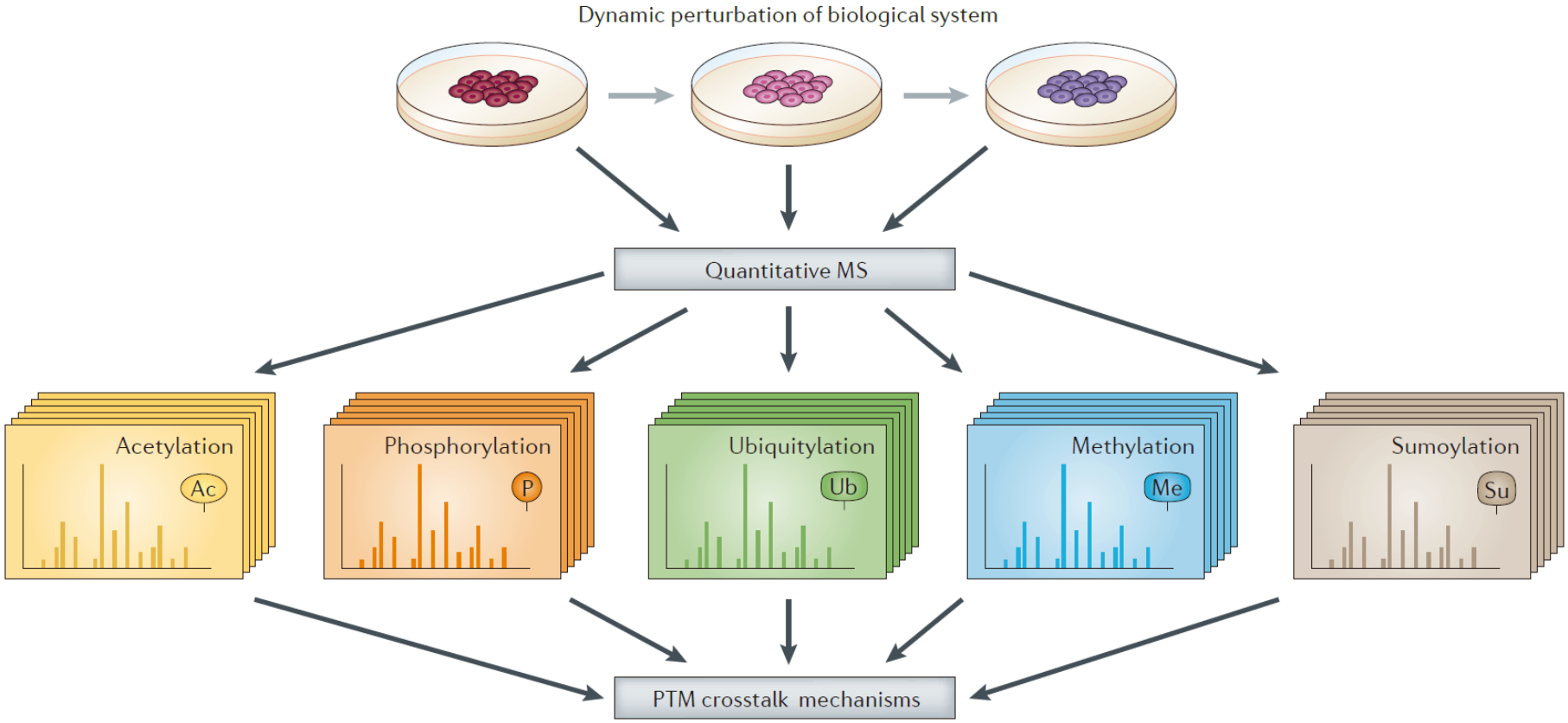
•With the availability of genomic information, the massive capacity for peptide identification by MS is being used to annotate gene sequences and to find new protein-coding genes and splicing variants.

•In combination with new approaches to isolate specific PTMs, MS-based studies are revealing a much higher order of proteome complexity in which most proteins are modified by several PTMs that crosstalk in intricate mechanisms to regulate protein function.

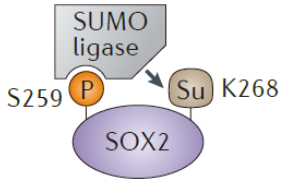
•Protein affinity strategies allow purification of candidate proteins and their interacting partners, which are subsequently identified by MS. These studies describe, with a high degree of detail, dynamic and context-specific protein–protein interaction networks and protein complexes.

•The improvements in sensitivity, robustness and high-throughput of MS-based proteomics now permits applications in the clinical field, including the possibility of discovering disease-related biomarkers and screening molecular targets of candidate drugs.


Sample
Cell culture
Tissue
Lysis
Digestion
Fractionation or enrichment
Ion exchange or HILIC
P-Thr-
P-Ser-
Affinity resins
Me2
Tyr P
Ac
Peptide IPs
LC–MS
(U)HPLC
m/z
High-resolution MS
b3
y2
b5-H2O
y5 y6
y7
y8
y9
y10 y11
y12 y13
y14
y15
m/z
MS/MS fragmentation
Data analysis
(Such as protein abundances under different experimental conditions)
m/z
m/z
m/z
MS-based quantification
Build network

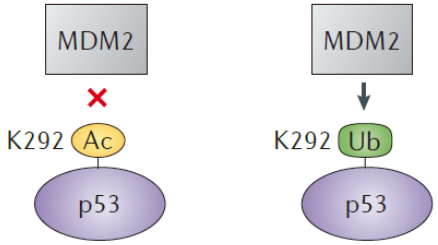



**Sequential**



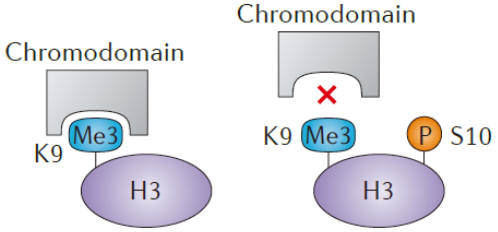

Phosphorylation-dependent SUMO modification

**Mutually exclusive**

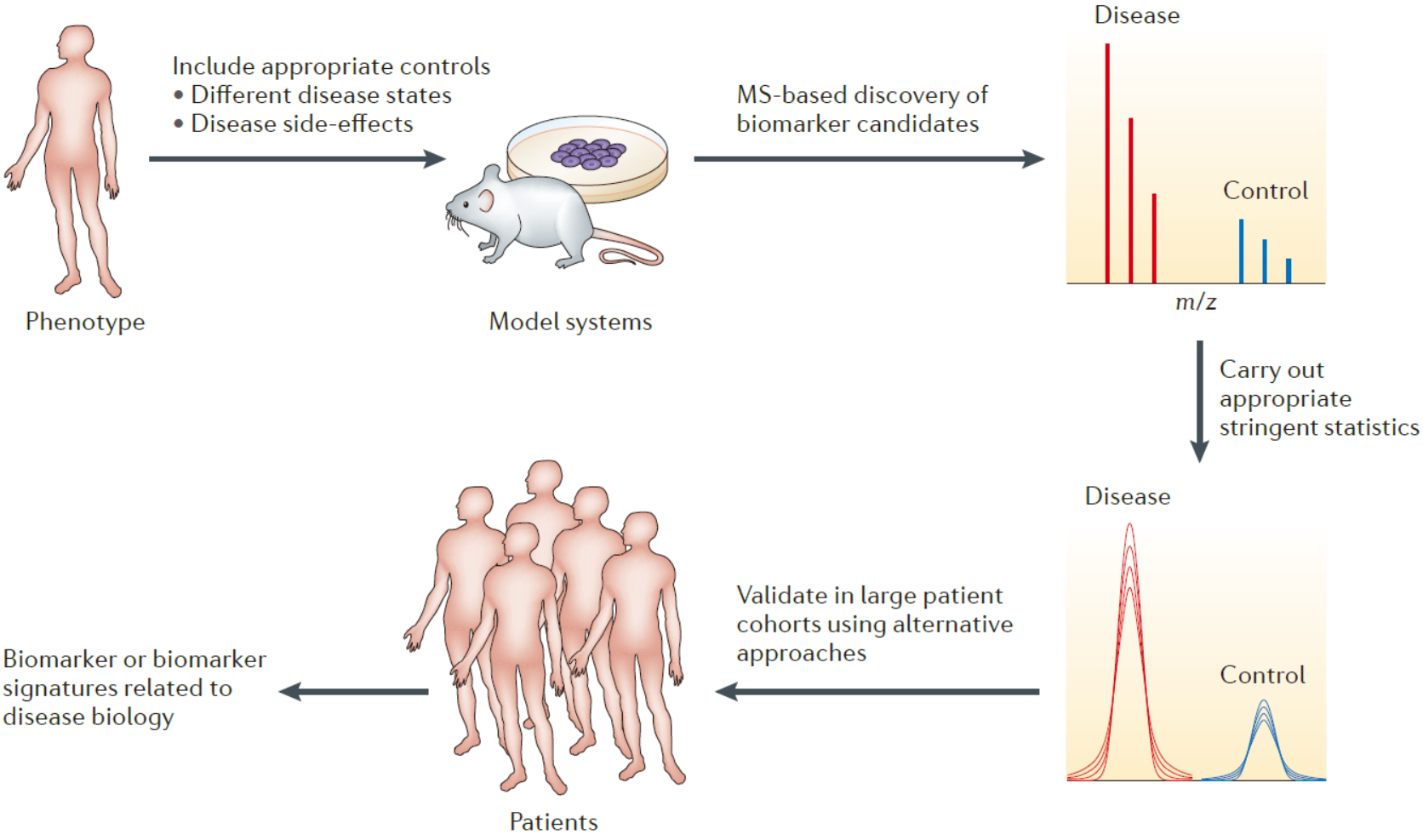



Acetylation prevents degradation of p53 by MDM2

**Antagonistic**




Phosphorylation disrupts H3 interaction with chromodomain


Phenotype
Include appropriate controls
• Different disease states
• Disease side-effects
Model systems
MS-based discovery of biomarker candidates
Disease
Control
m/z
Carry out appropriate stringent statistics
Disease
Control
Validate in large patient cohorts using alternative approaches
Patients
Biomarker or biomarker signatures related to disease biology

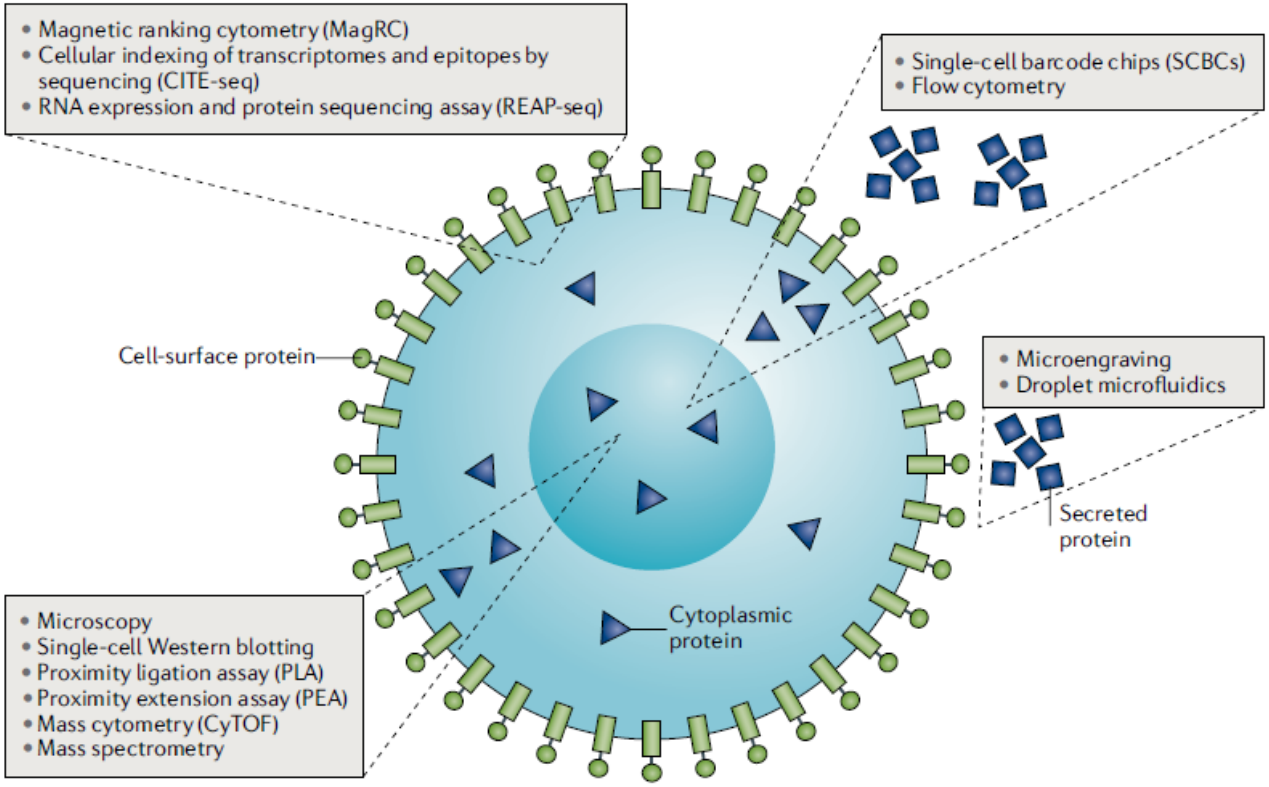
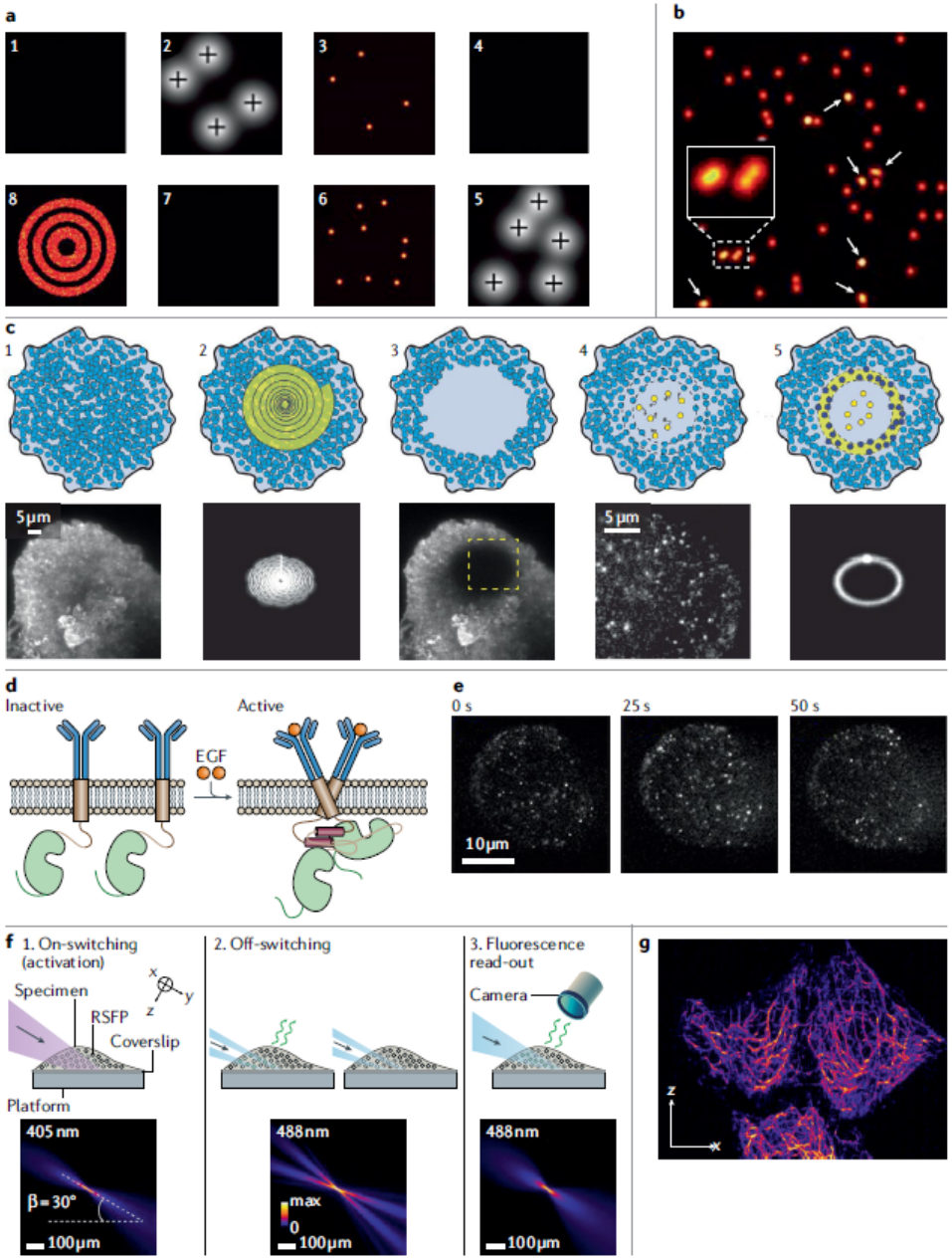
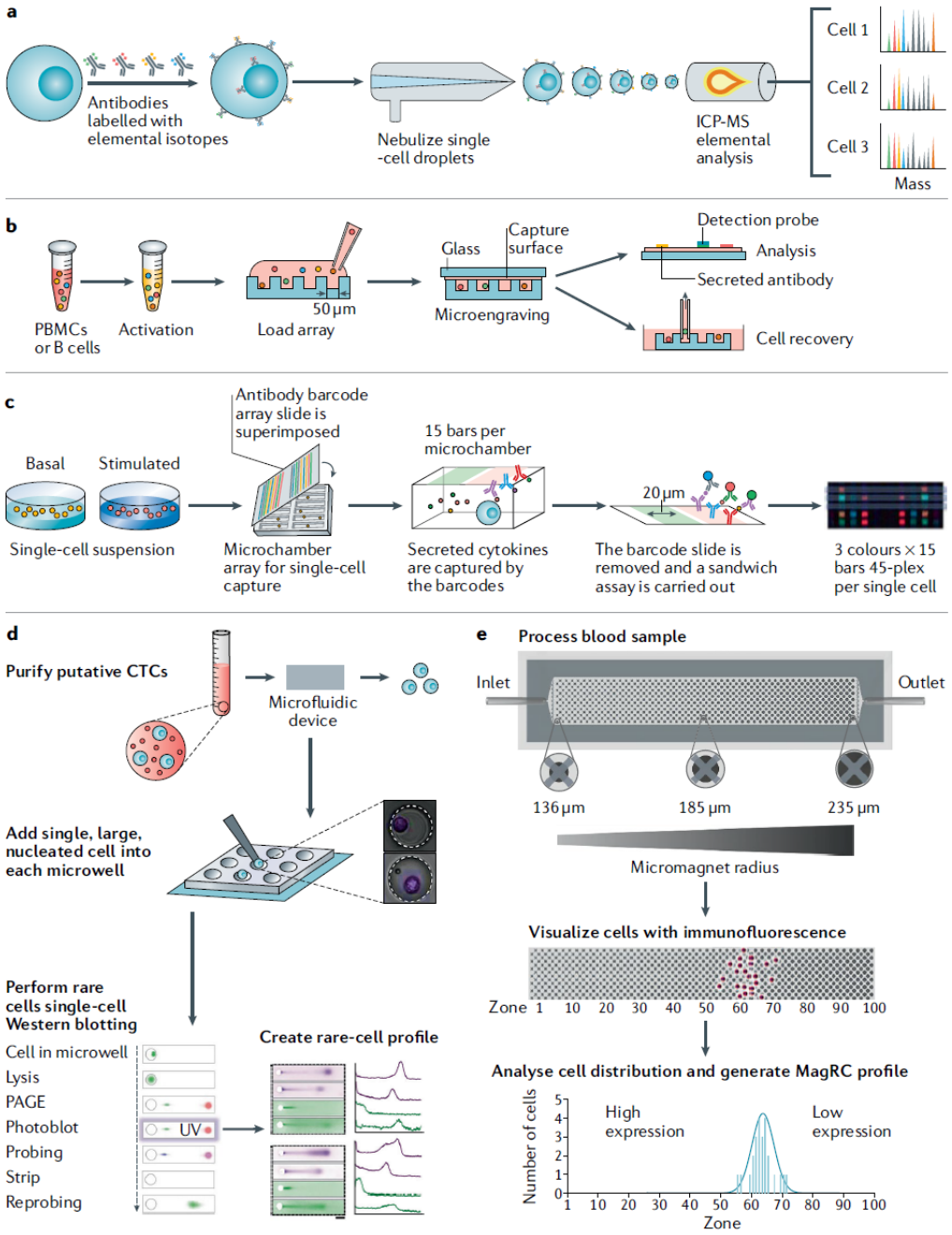
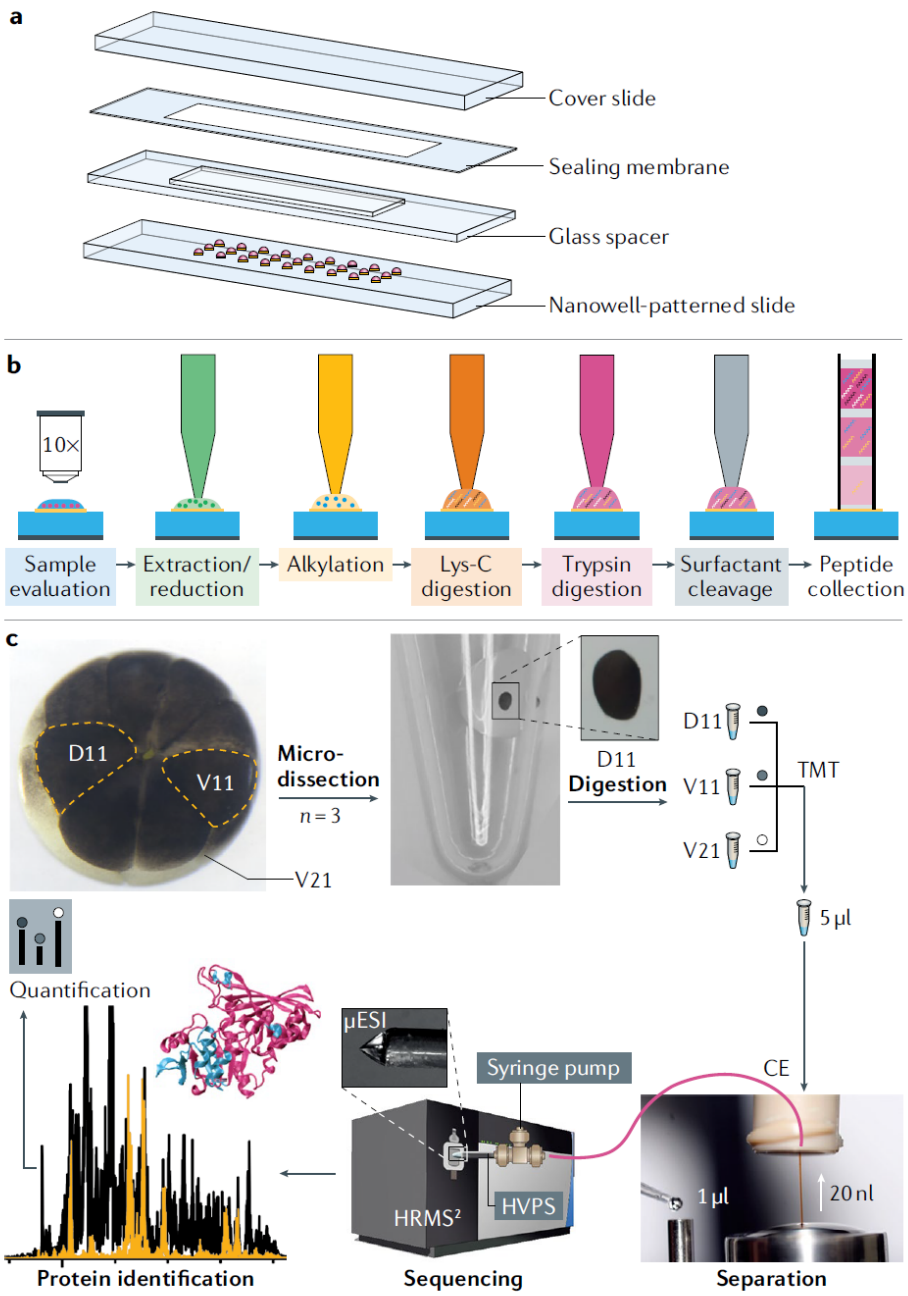
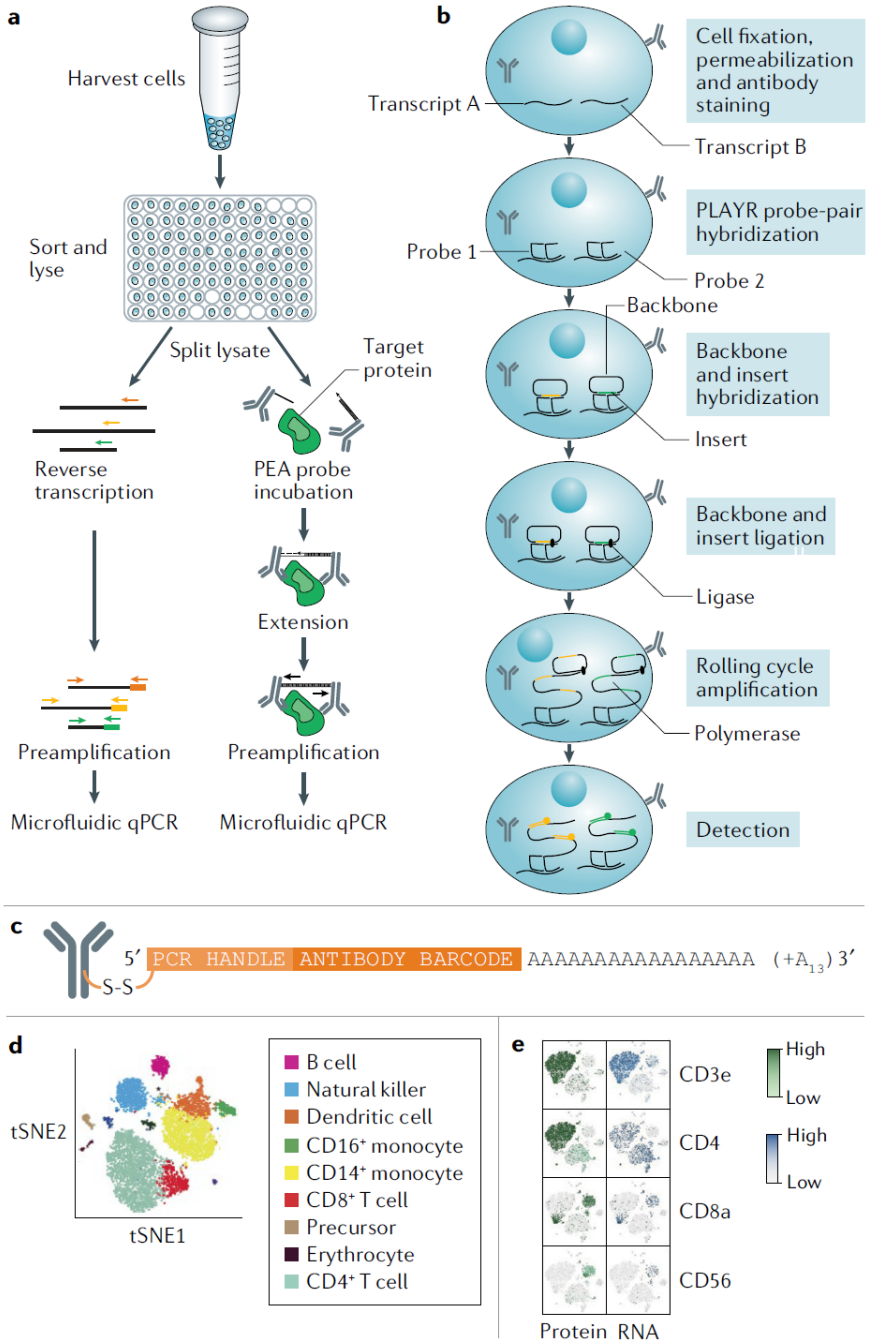



Fig. 1 | **Classification of single-cell protein analysis methods based on the location of target protein.** Cell-surface-protein analysis methods include magnetic ranking cytometry (MagRC), cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq), and RNA expression and protein sequencing assay (REAP-seq). Methods that can be used for the analysis of cell-surface and cytoplasmic proteins include microscopy, single-cell Western blotting, proximity ligation assay (PLA), proximity extension assay (PEA), mass cytometry (cytometry by time of flight; CyTOF) and mass spectrometry. Methods utilized for secreted-protein analysis include droplet microfluidics and microengraving techniques. Methods used for comprehensive analysis of the three proteins include flow cytometry and single-cell barcode chips (SCBCs).


a
1
2
3
4
8
7
6
5
b
c
1
2
3
4
5
5μm
5 μm
d
Inactive
Active
EGF
e
0 s
25 s
50 s
10μm
f
1. On-switching (activation)
Specimen
RSFP
Coverslip
Platform
x
y
z
2. Off-switching
3. Fluorescence read-out
Camera
405 nm
β=30°
100μm
488nm
max
0
100μm
488nm
100μm
g
z
x


a
Antibodies labelled with elemental isotopes
Nebulize single -cell droplets
ICP-MS elemental analysis
Cell 1
Cell 2
Cell 3
Mass
b
PBMCs or B cells
Activation
50 µm
Load array
Glass
Capture surface
Microengraving
Detection probe
Analysis
Secreted antibody
Cell recovery
c
Basal
Stimulated
Single-cell suspension
Antibody barcode array slide is superimposed
Microchamber array for single-cell capture
15 bars per microchamber
Secreted cytokines are captured by the barcodes
20 µm
The barcode slide is removed and a sandwich assay is carried out
3 colours × 15 bars 45-plex per single cell
d
Purify putative CTCs
Microfluidic device
Add single, large, nucleated cell into each microwell
Perform rare cells single-cell Western blotting
Cell in microwell
Lysis
PAGE
Photoblot
UV
Probing
Strip
Reprobing
Create rare-cell profile
e
Process blood sample
Inlet
Outlet
136 µm
185 µm
235 µm
Micromagnet radius
Visualize cells with immunofluorescence
Zone 1 10 20 30 40 50 60 70 80 90 100
Analyse cell distribution and generate MagRC profile
Number of cells
High expression
Low expression
Zone


a
Cover slide
Sealing membrane
Glass spacer
Nanowell-patterned slide
b
10×
Sample evaluation
Extraction/ reduction
Alkylation
Lys-C digestion
Trypsin digestion
Surfactant cleavage
Peptide collection
c
D11
V11
V21
Micro-dissection
n = 3
D11 Digestion
D11
V11
V21
TMT
5 µl
Quantification
µESI
Syringe pump
CE
HRMS²
HVPS
1 µl
20 nl
Protein identification
Sequencing
Separation

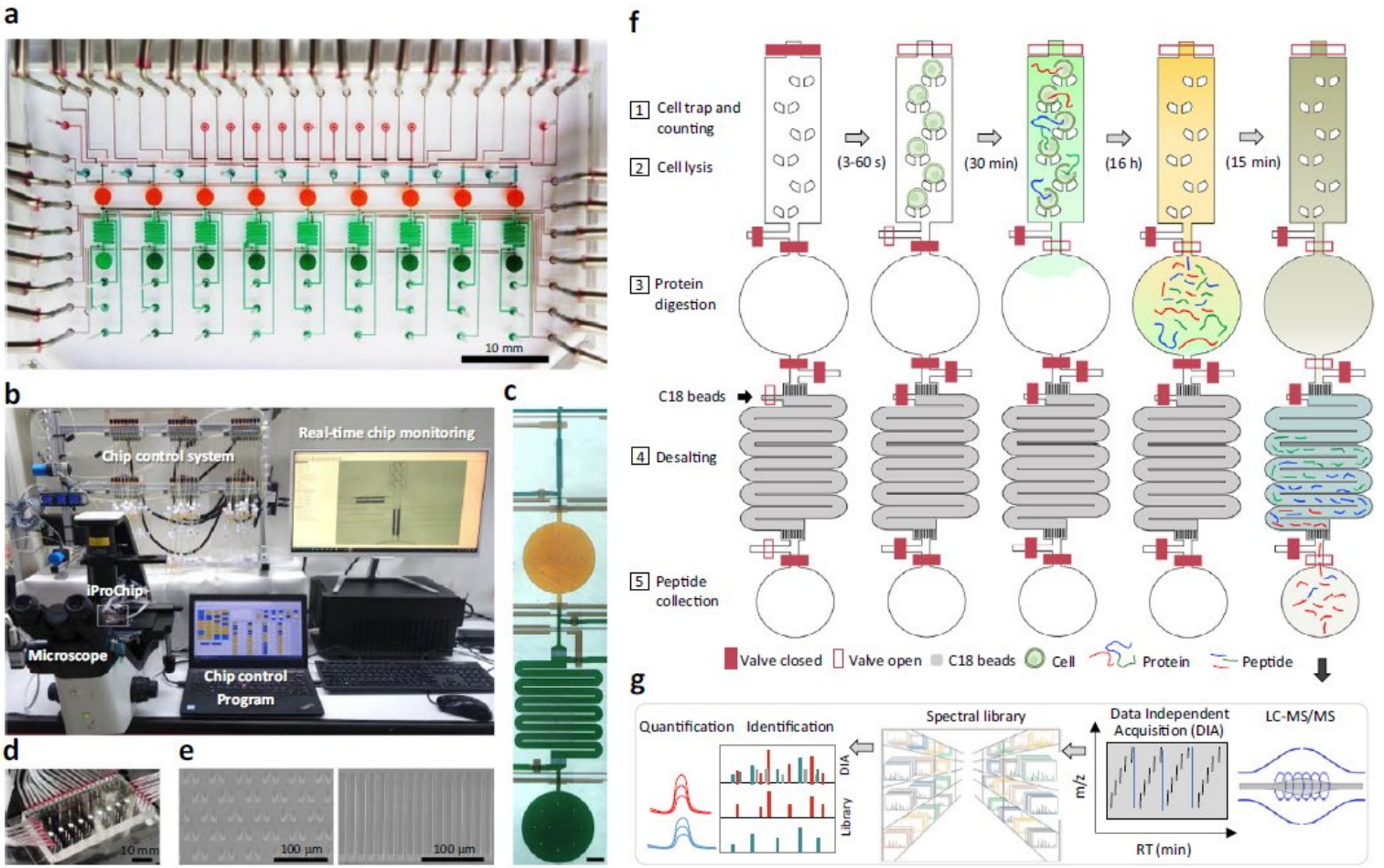

a
Harvest cells
Sort and lyse
Split lysate
Target protein
Reverse transcription
PEA probe incubation
Extension
Preamplification
Preamplification
Microfluidic qPCR
Microfluidic qPCR
b
Transcript A
Transcript B
Cell fixation, permeabilization and antibody staining
Probe 1
Probe 2
PLAYR probe-pair hybridization
Backbone
Backbone and insert hybridization
Insert
Backbone and insert ligation
Ligase
Rolling cycle amplification
Polymerase
Detection
c
5′
PCR HANDLE
ANTIBODY BARCODE
AAAAAAAAAAAAAAAAA (+A13) 3′
S-S
d
tSNE2
tSNE1
B cell
Natural killer
Dendritic cell
CD16+ monocyte
CD14+ monocyte
CD8+ T cell
Precursor
Erythrocyte
CD4+ T cell
e
CD3e
CD4
CD8a
CD56
High
Low
High
Low
Protein
RNA

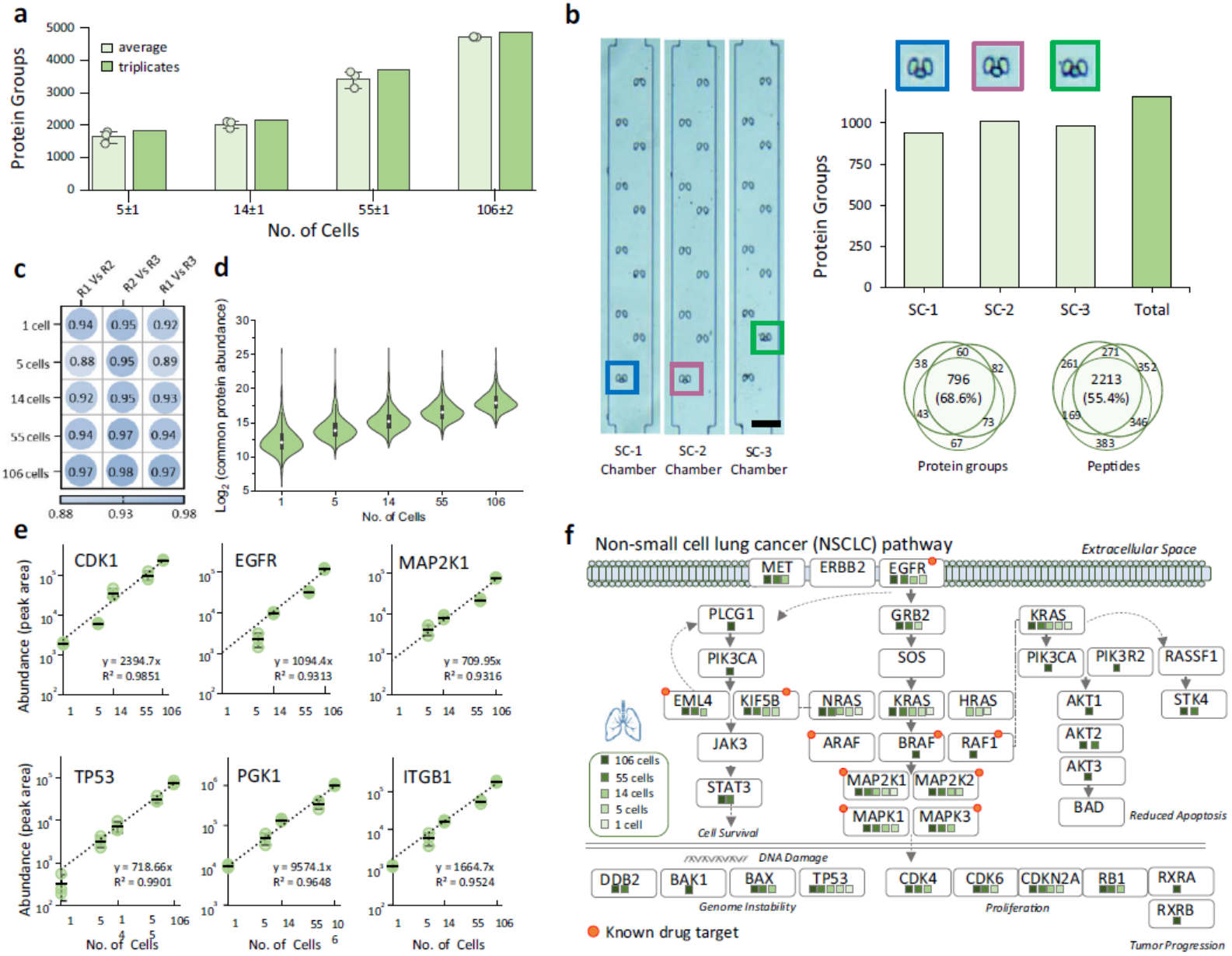
ARTICLE

https://doi.org/10.1038/s41467-021-27778-4 OPEN

# Streamlined single-cell proteomics by an integrated microfluidic chip and data-independent acquisition mass spectrometry

Sofani Tafesse Gebreyesus[1,2,3,9], Asad Ali Siyal[1,4,5,9], Reta Birhanu Kitata[1], Eric Sheng-Wen Chen[1], Bayarmaa Enkhbayar[4,6], Takashi Angata[6], Kuo-I Lin[7], Yu-Ju Chen[1,3,4,8✉] & Hsiung-Lin Tu[1,2,4,8✉]




a
Protein Groups
average
triplicates
No. of Cells
5±1
14±1
55±1
106±2
b
SC-1 Chamber
SC-2 Chamber
SC-3 Chamber
Protein Groups
SC-1
SC-2
SC-3
Total
796 (68.6%)
2213 (55.4%)
Protein groups
Peptides
c
R1 Vs R2
R2 Vs R3
R1 Vs R3
1 cell
5 cells
14 cells
55 cells
106 cells
d
Log2 (common protein abundance)
No. of Cells
e
CDK1
EGFR
MAP2K1
TP53
PGK1
ITGB1
Abundance (peak area)
No. of Cells
f
Non-small cell lung cancer (NSCLC) pathway
Extracellular Space
MET
ERBB2
EGFR
PLCG1
GRB2
KRAS
PIK3CA
SOS
PIK3CA
PIK3R2
RASSF1
EML4
KIF5B
NRAS
KRAS
HRAS
AKT1
STK4
JAK3
ARAF
BRAF
RAF1
AKT2
AKT3
STAT3
MAP2K1
MAP2K2
BAD
MAPK1
MAPK3
Reduced Apoptosis
Cell Survival
DNA Damage
DDB2
BAK1
BAX
TP53
CDK4
CDK6
CDKN2A
RB1
RXRA
RXRB
Genome Instability
Proliferation
Tumor Progression
Known drug target