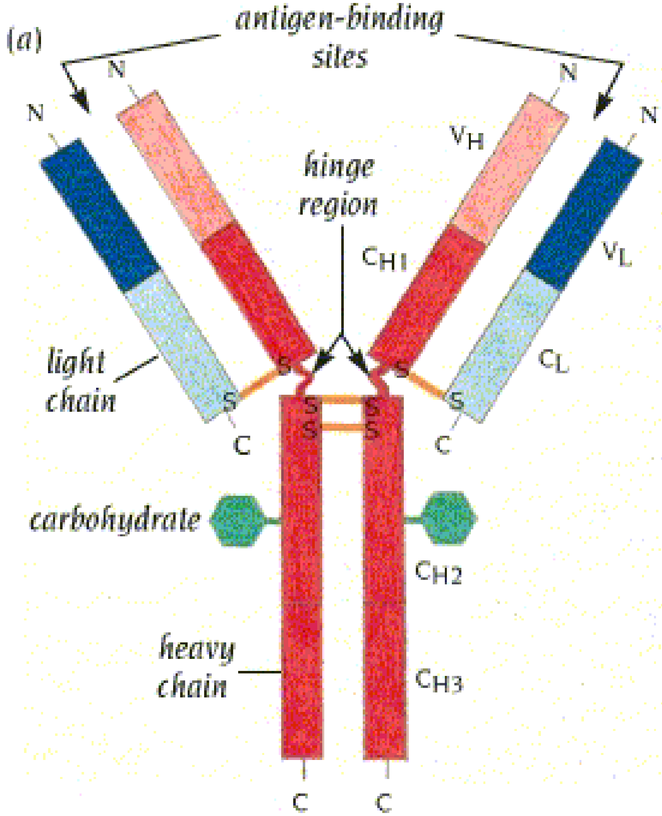
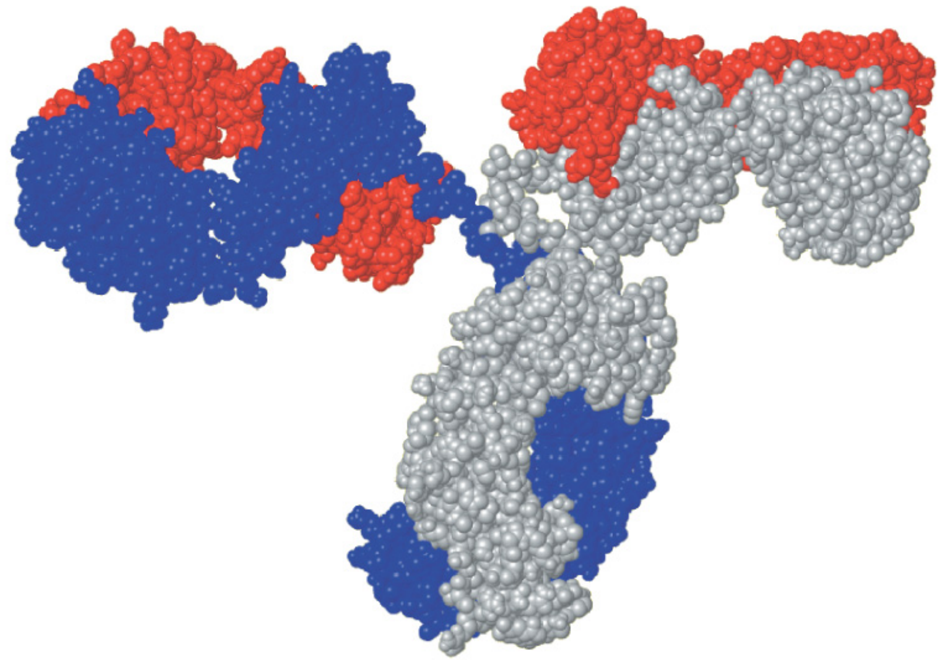
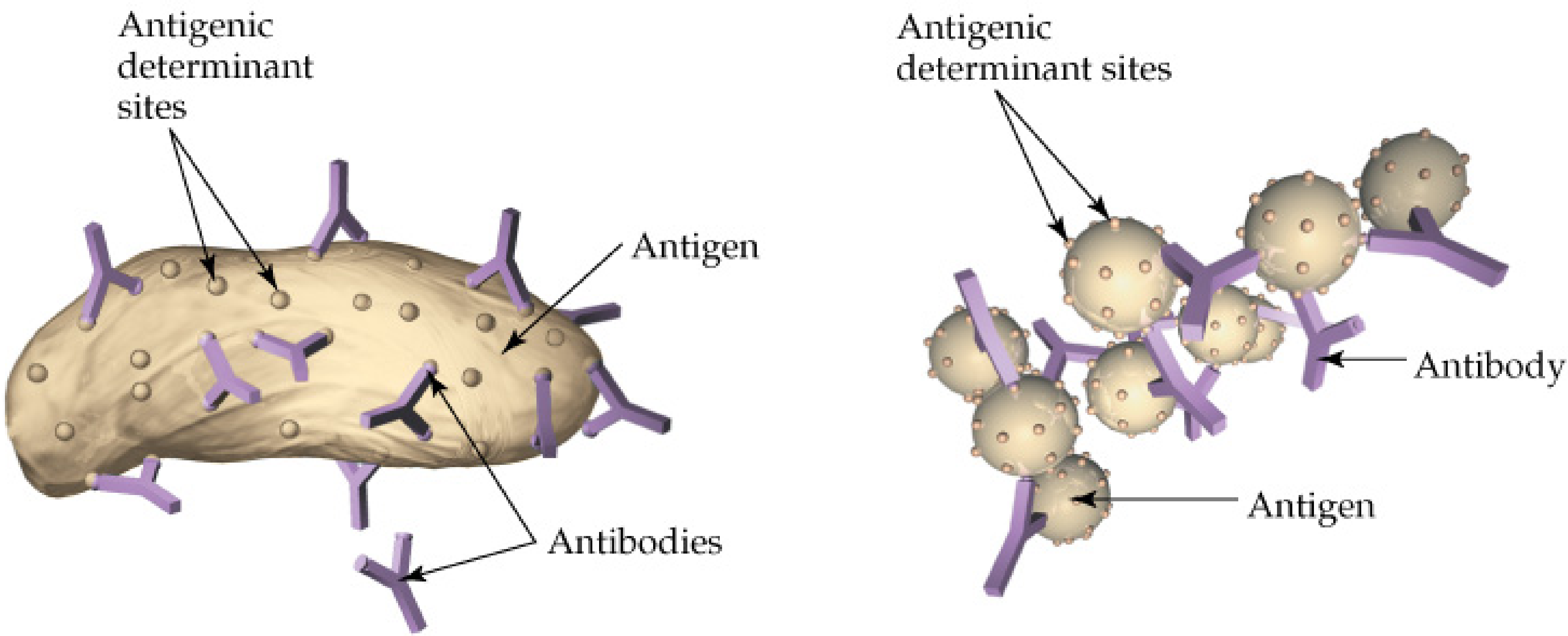

(a)
antigen-binding sites
N
N
N
N
VH
hinge region
CH1
VL
light chain
S
S
S
S
S
S
S
S
C
C
CL
carbohydrate
CH2
heavy chain
CH3
C
C

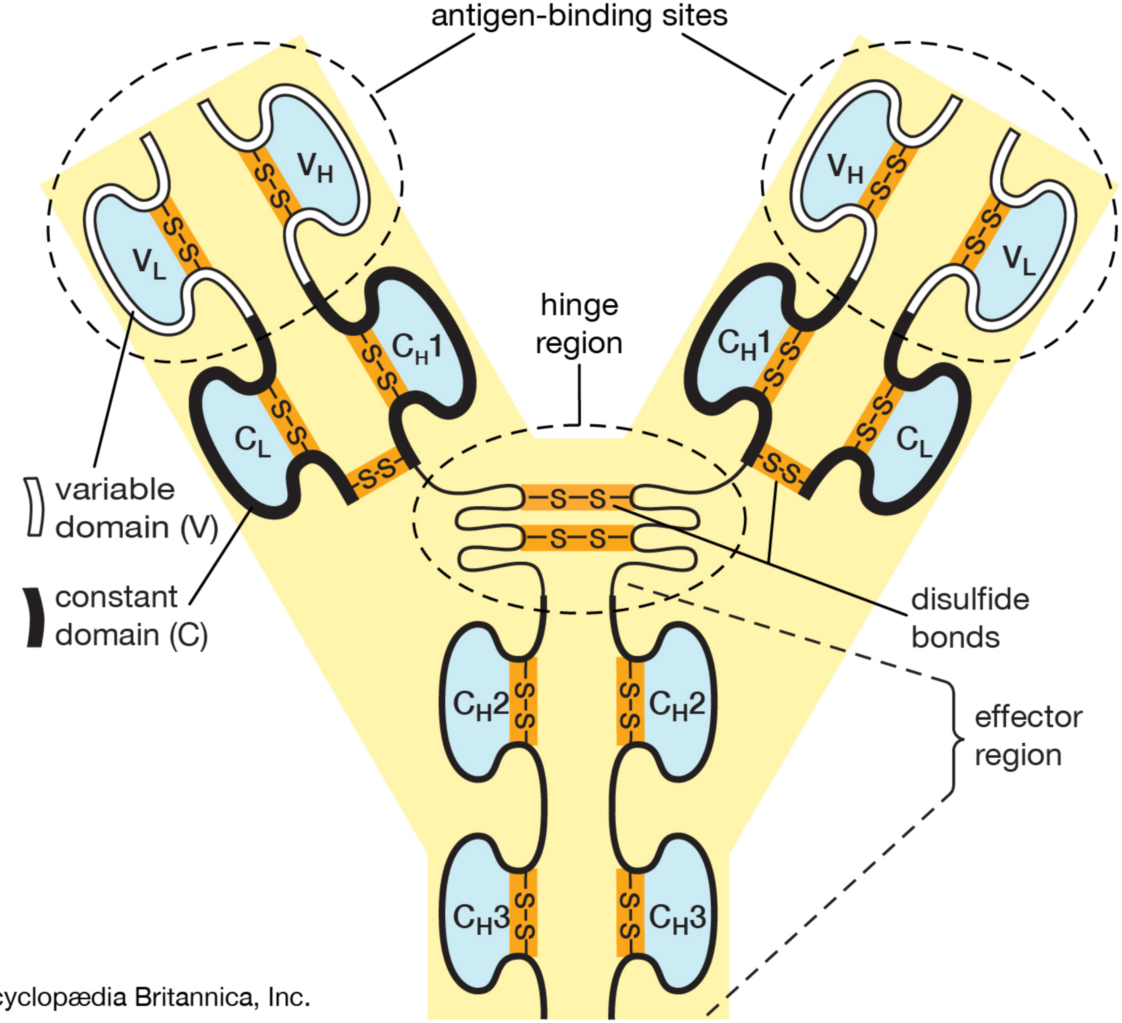


(b)

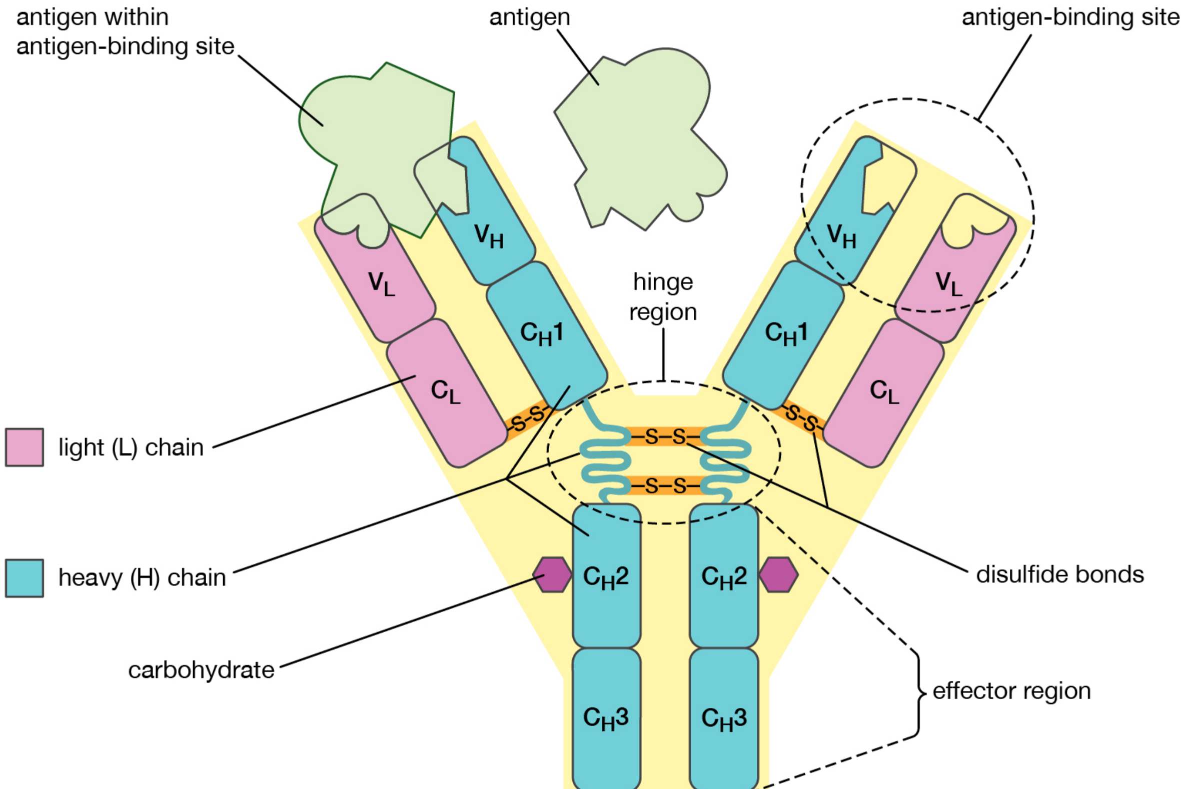

Antigenic
determinant
sites
Antigen
Antibodies
Antigenic
determinant sites
Antibody
Antigen

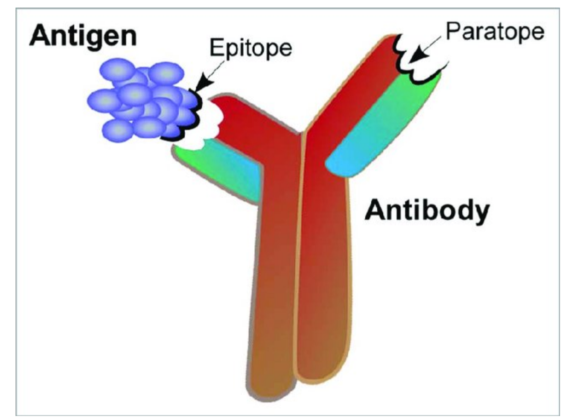
(a) (b)

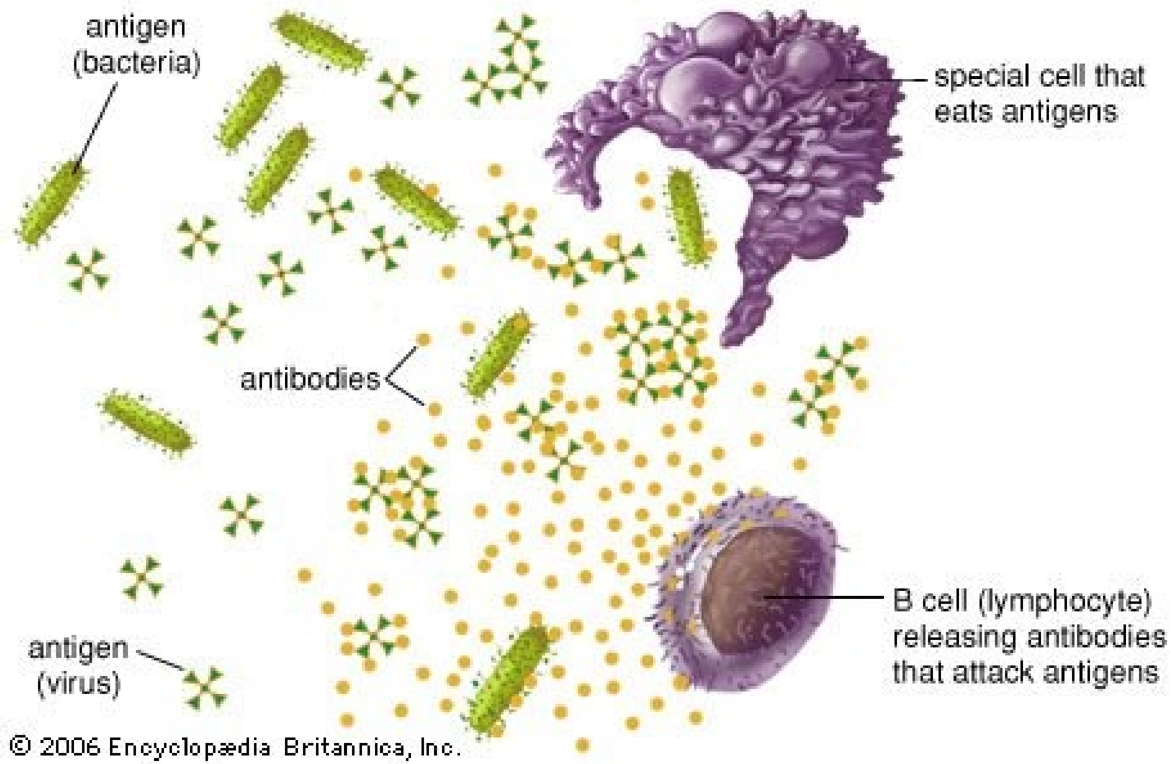
Antibody


antigen-binding sites
hinge region
variable domain (V)
constant domain (C)
disulfide bonds
effector region
V_H
V_L
C_H1
C_L
C_H2
C_H3
-S-S-

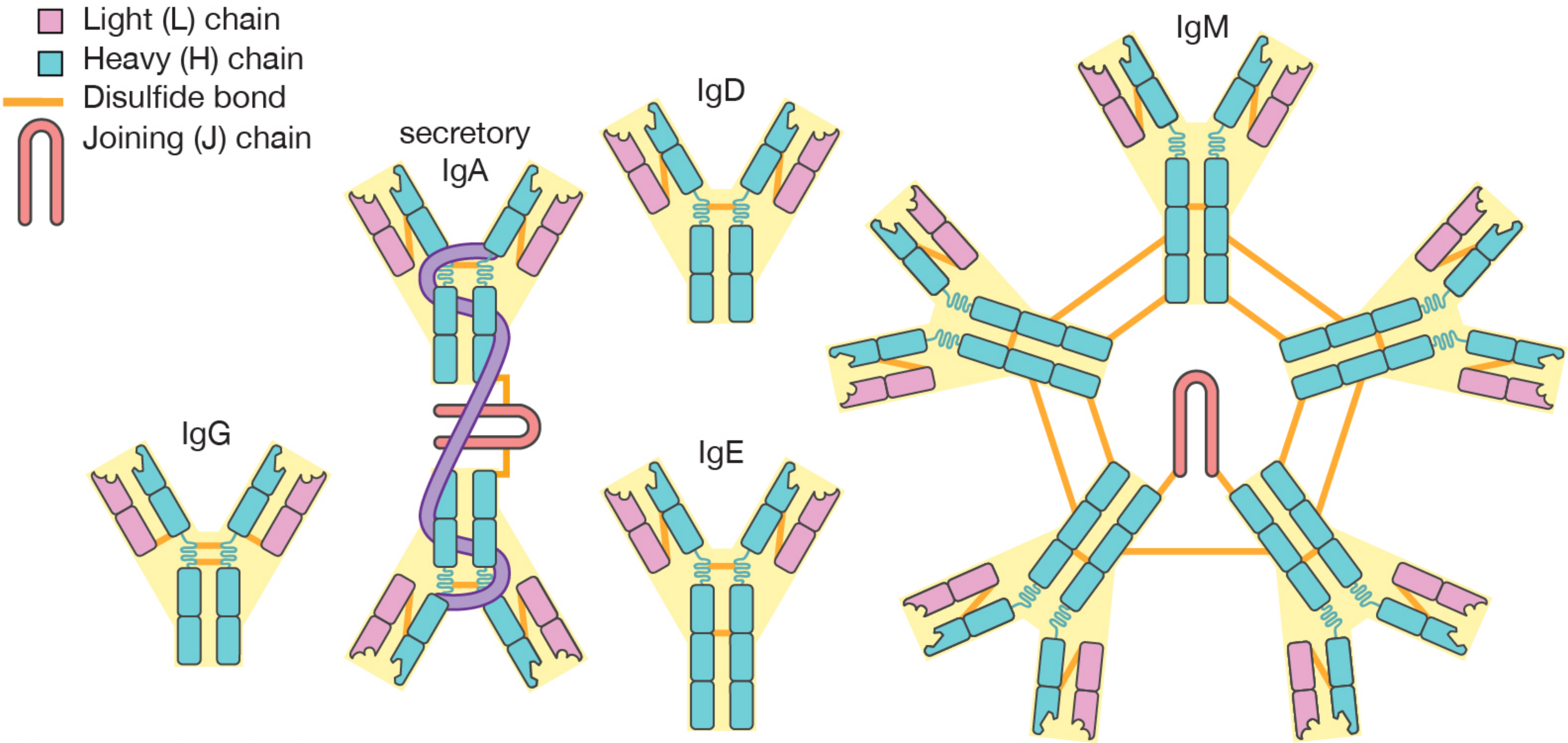
Antibody Binding Sites






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

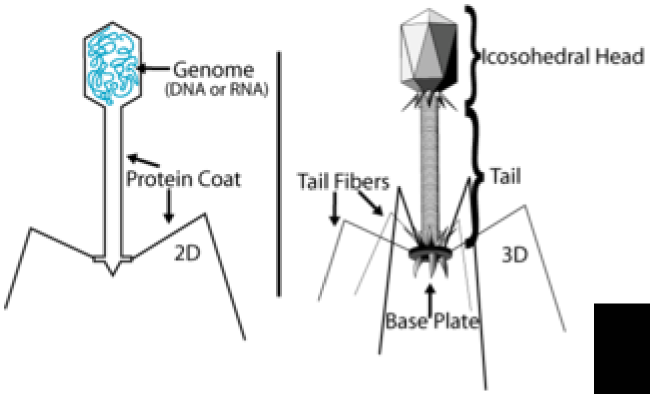
Different Types of Antibodies

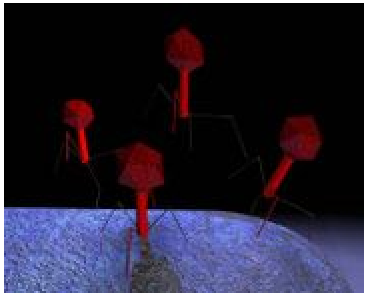


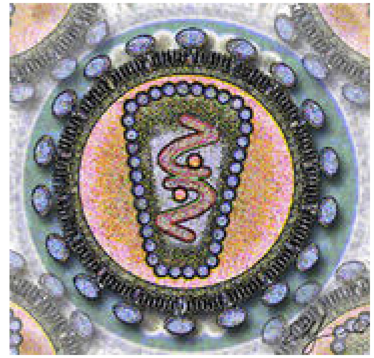
Antibody

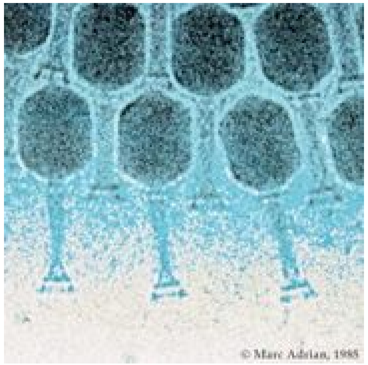
Check
https://www.youtube.com/watch?v=Cvu1ApHkhYM

Virus

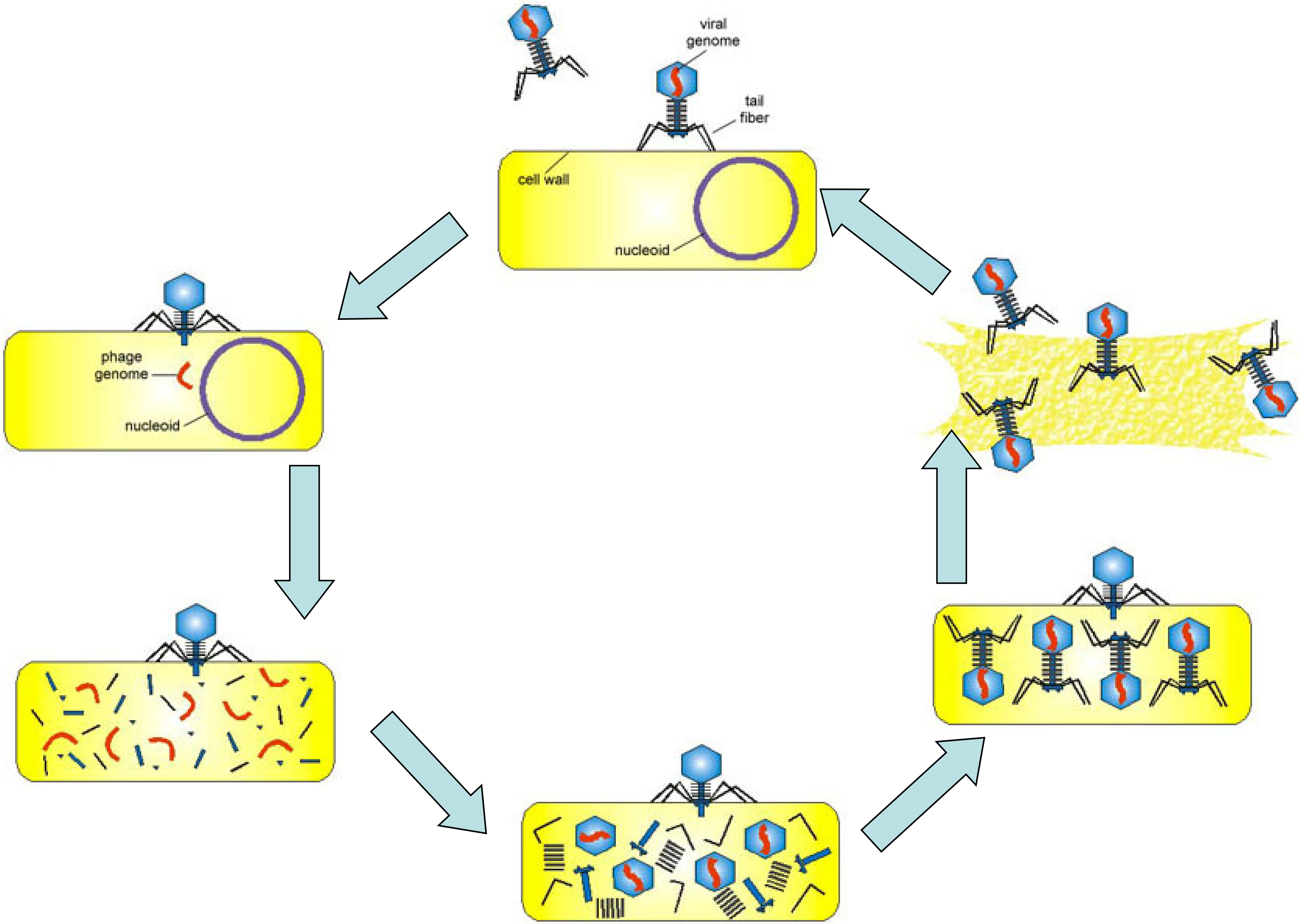

Genome
(DNA or RNA)
Protein Coat
2D
Icosohedral Head
Tail
Tail Fibers
Base Plate
3D



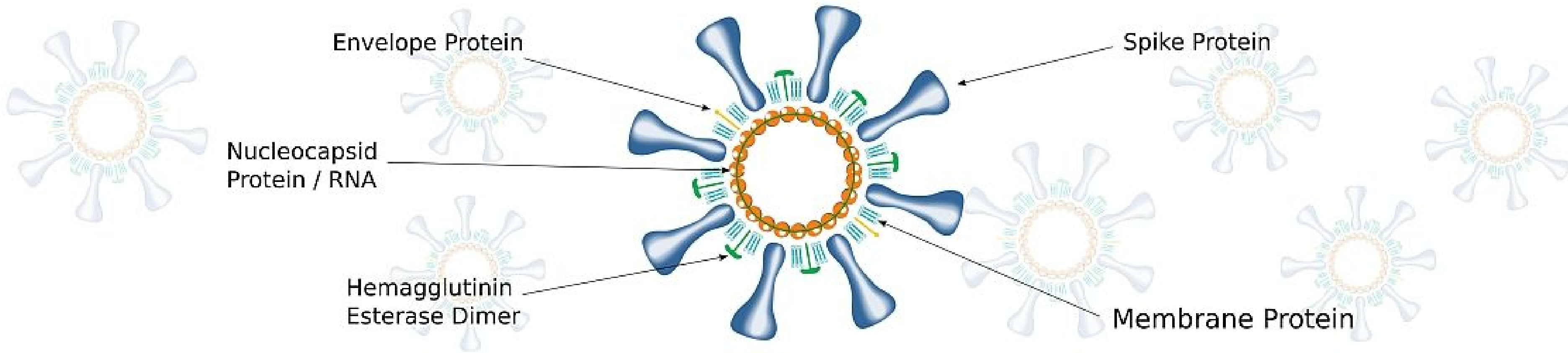


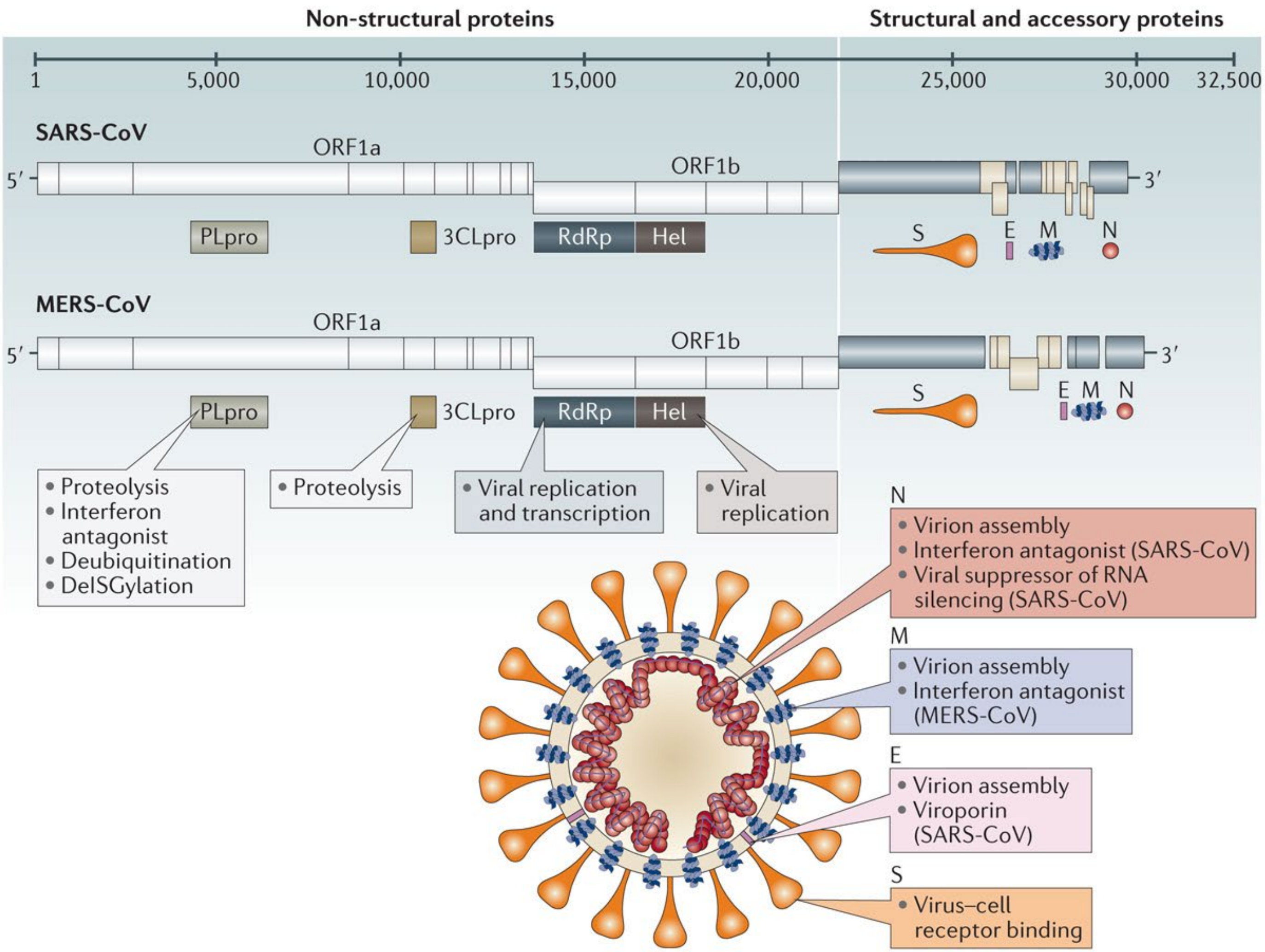

© Marc Adrian, 1985

Virus Reproduction



SARS-CoV-2


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

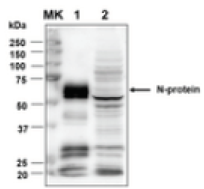


Nature Reviews | Drug Discovery

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

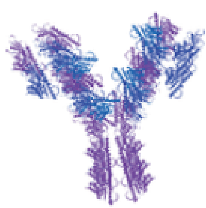
Rabbit Anti-SARS-CoV-2 Coronavirus Nucleocapsid Protein

CODE: 128-10165-1

$1,450.00

SELECT SIZE

ADD TO COMPARISON LIST



Mouse Anti-SARS-CoV-2 Nucleocapsid Protein

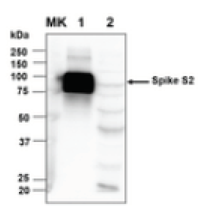
Mouse Anti-SARS-CoV-2 Coronavirus Nucleocapsid protein

CODE: 128-10166-1

$1,450.00

SELECT SIZE

ADD TO COMPARISON LIST



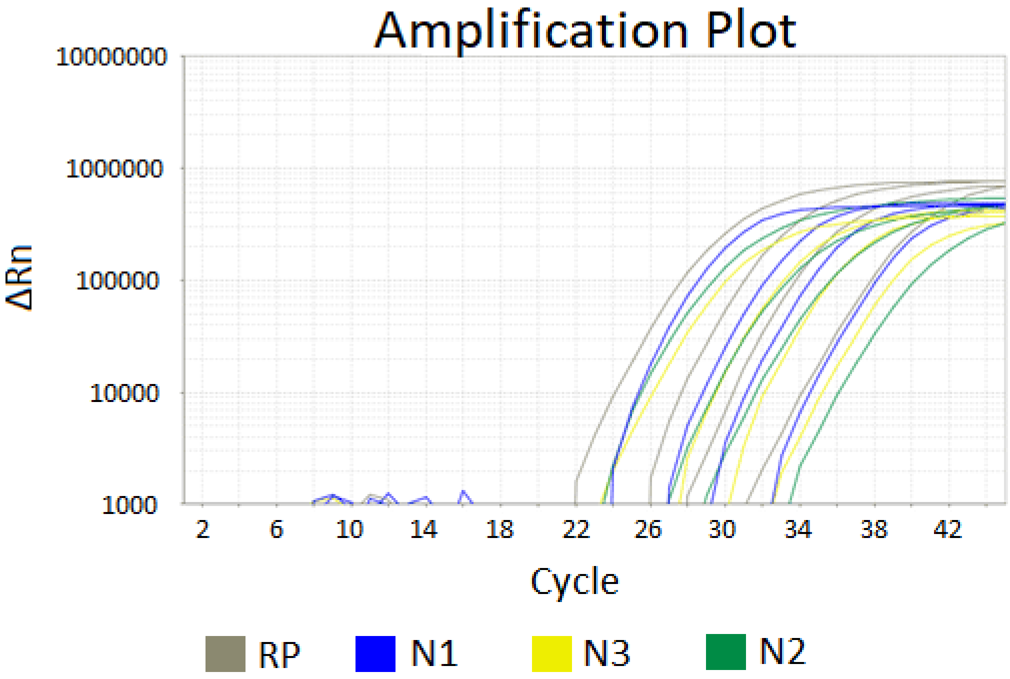
Rabbit Anti-SARS-CoV-2 Spike Protein

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

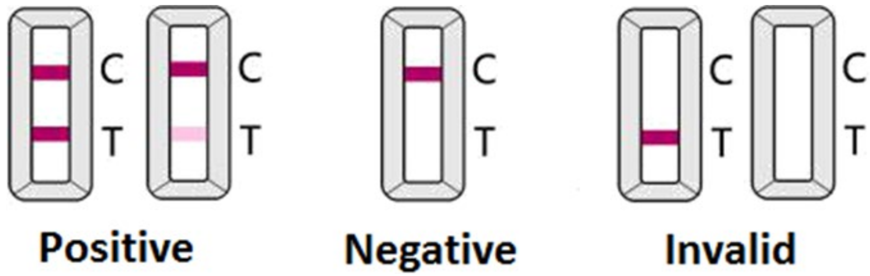
CODE: 128-10168-1

$1,450.00

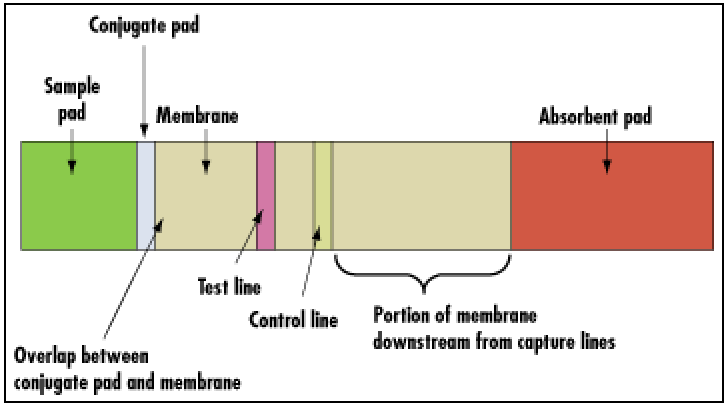
Real-time RT PCR



Fast Screening Kit

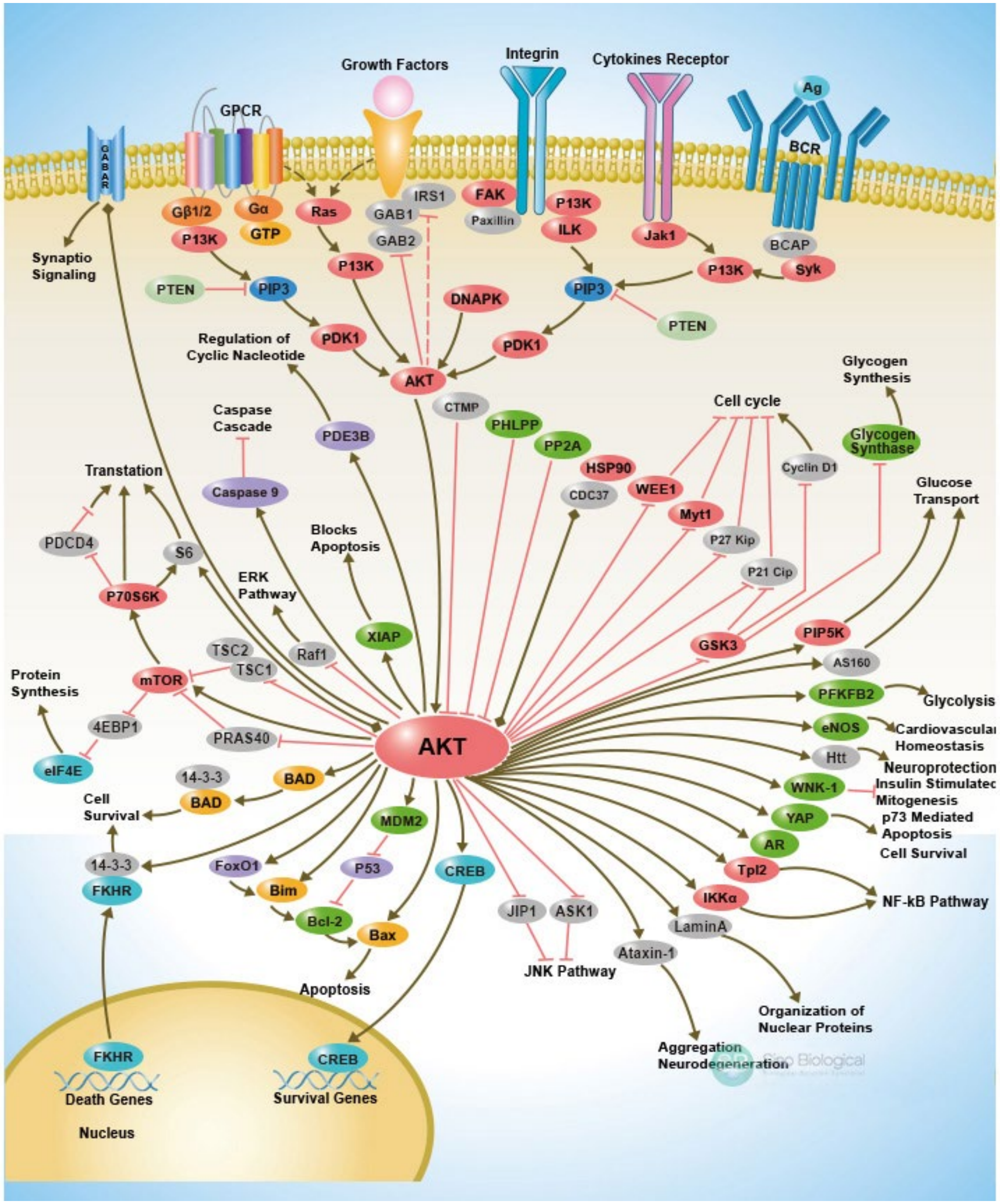


hCG immunoassay

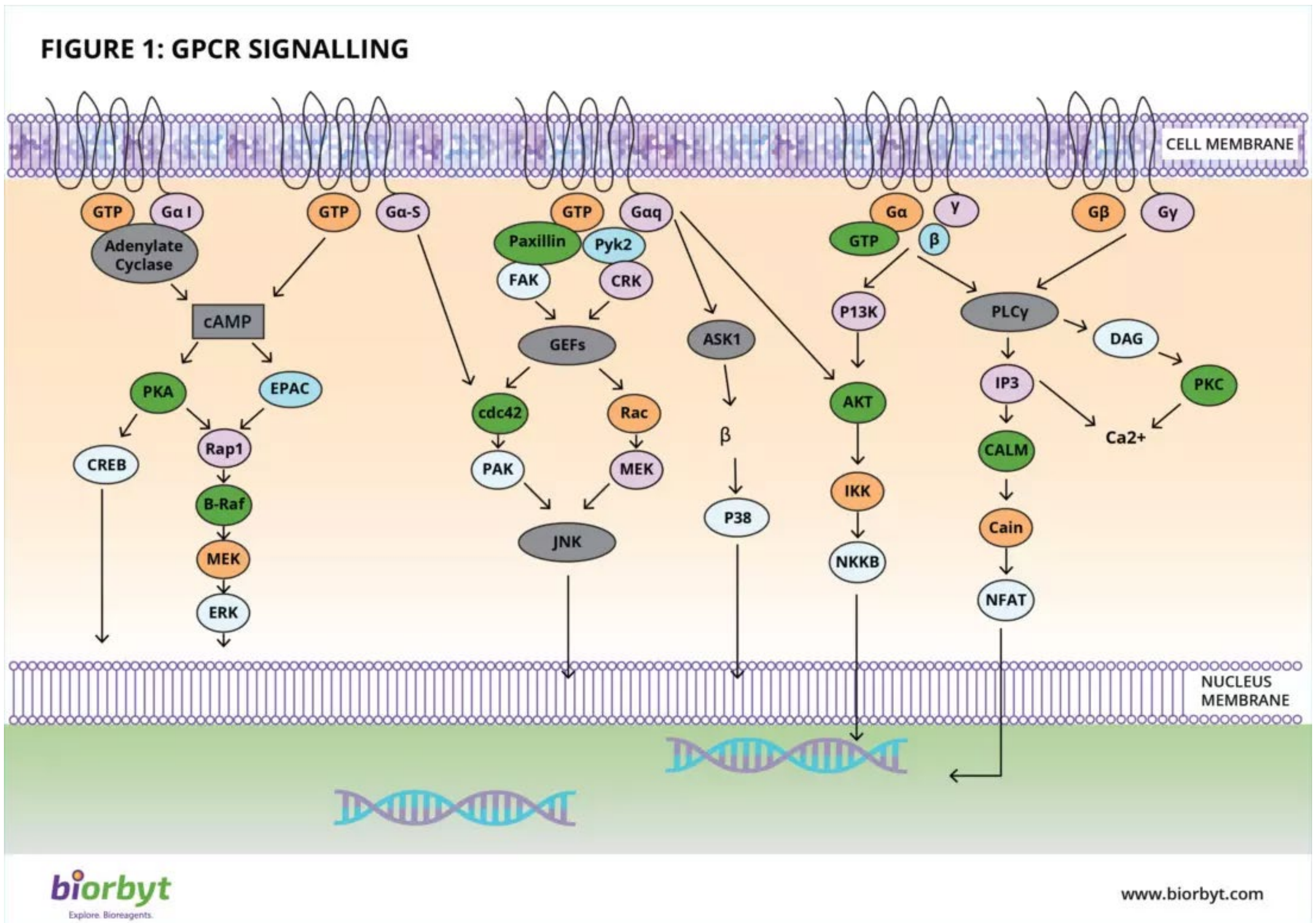


human chorionic gonadotropin (hCG)

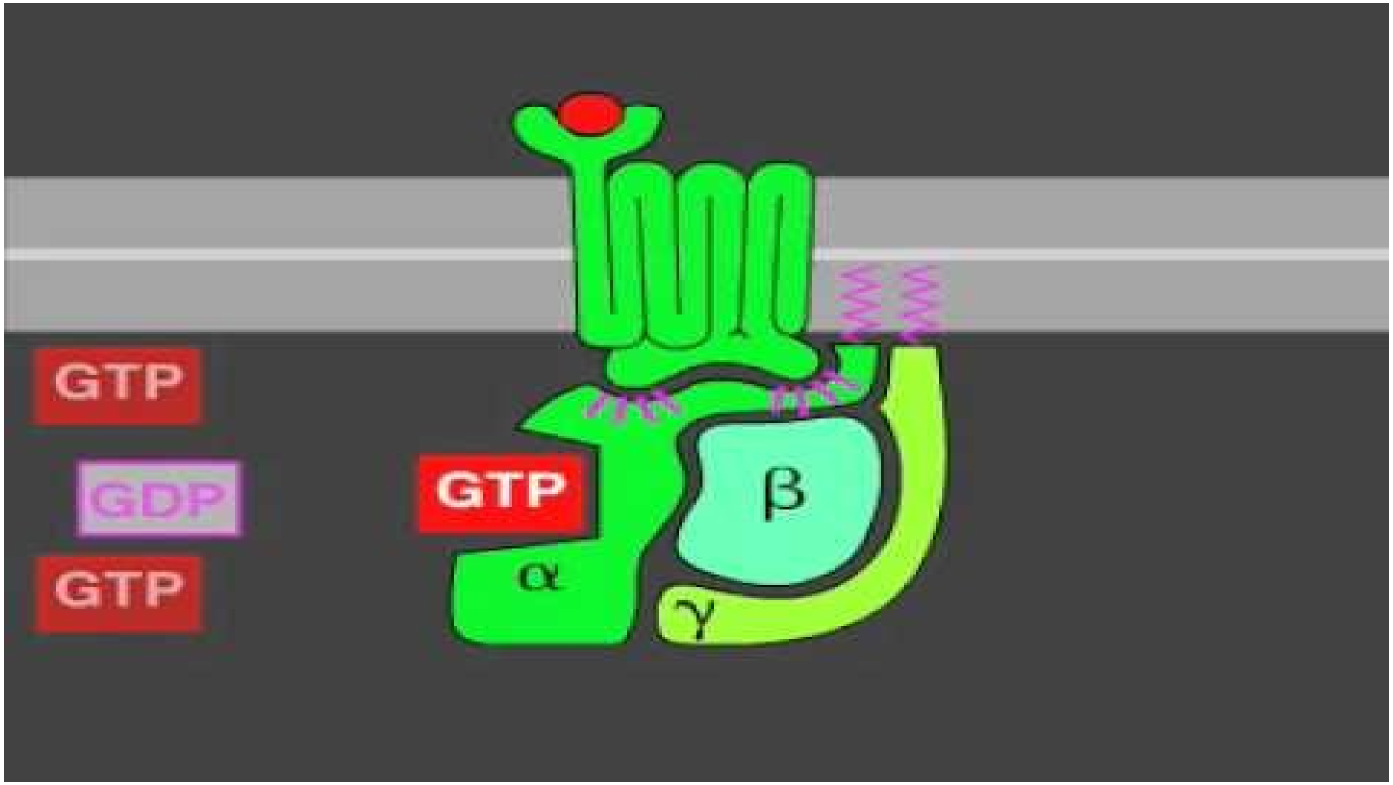
Signal Transduction



G-Protein Signaling Pathway

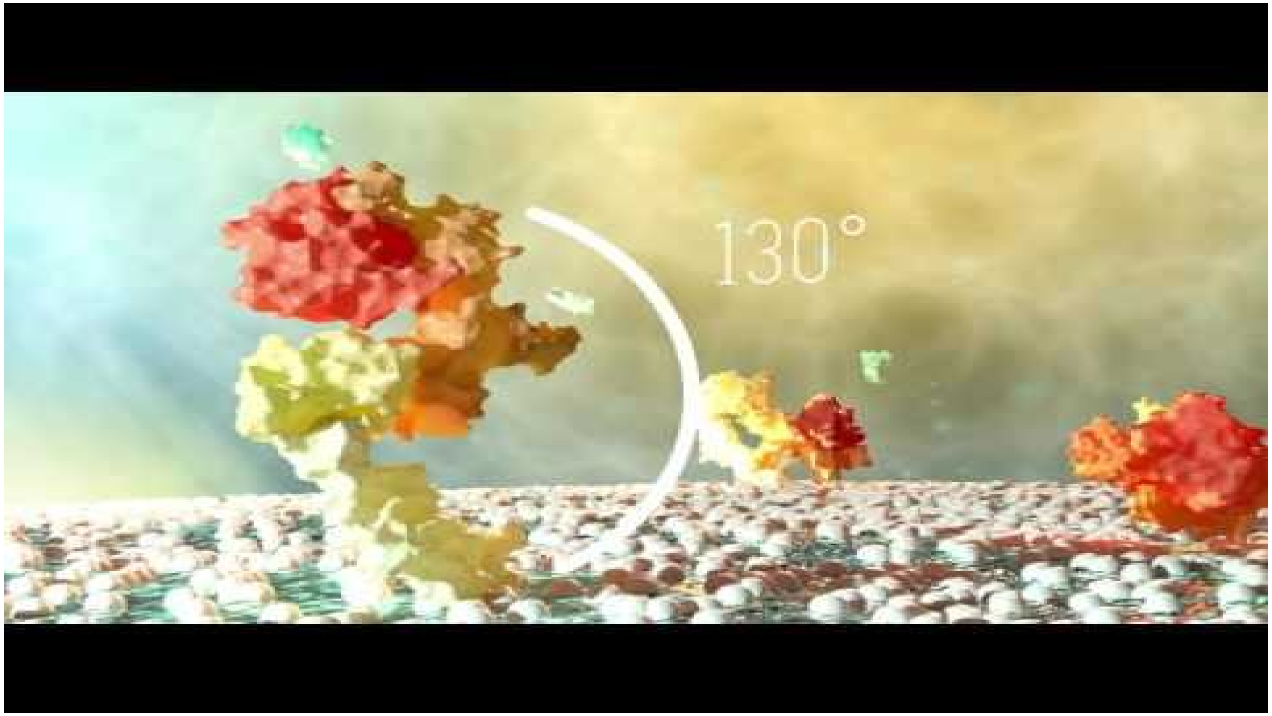


G Protein



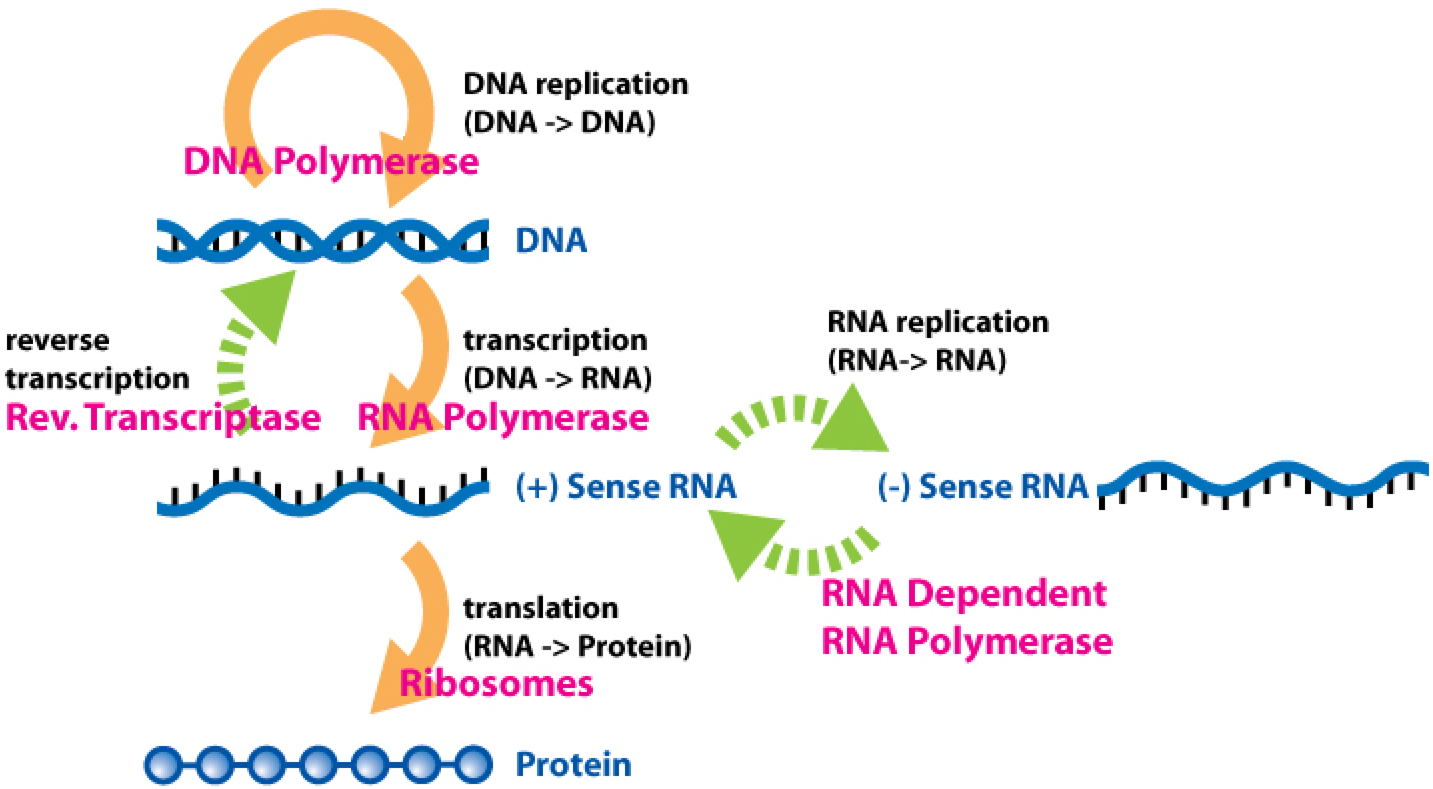
https://www.youtube.com/watch?v=GIu_T6DQuLU

EGFR



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

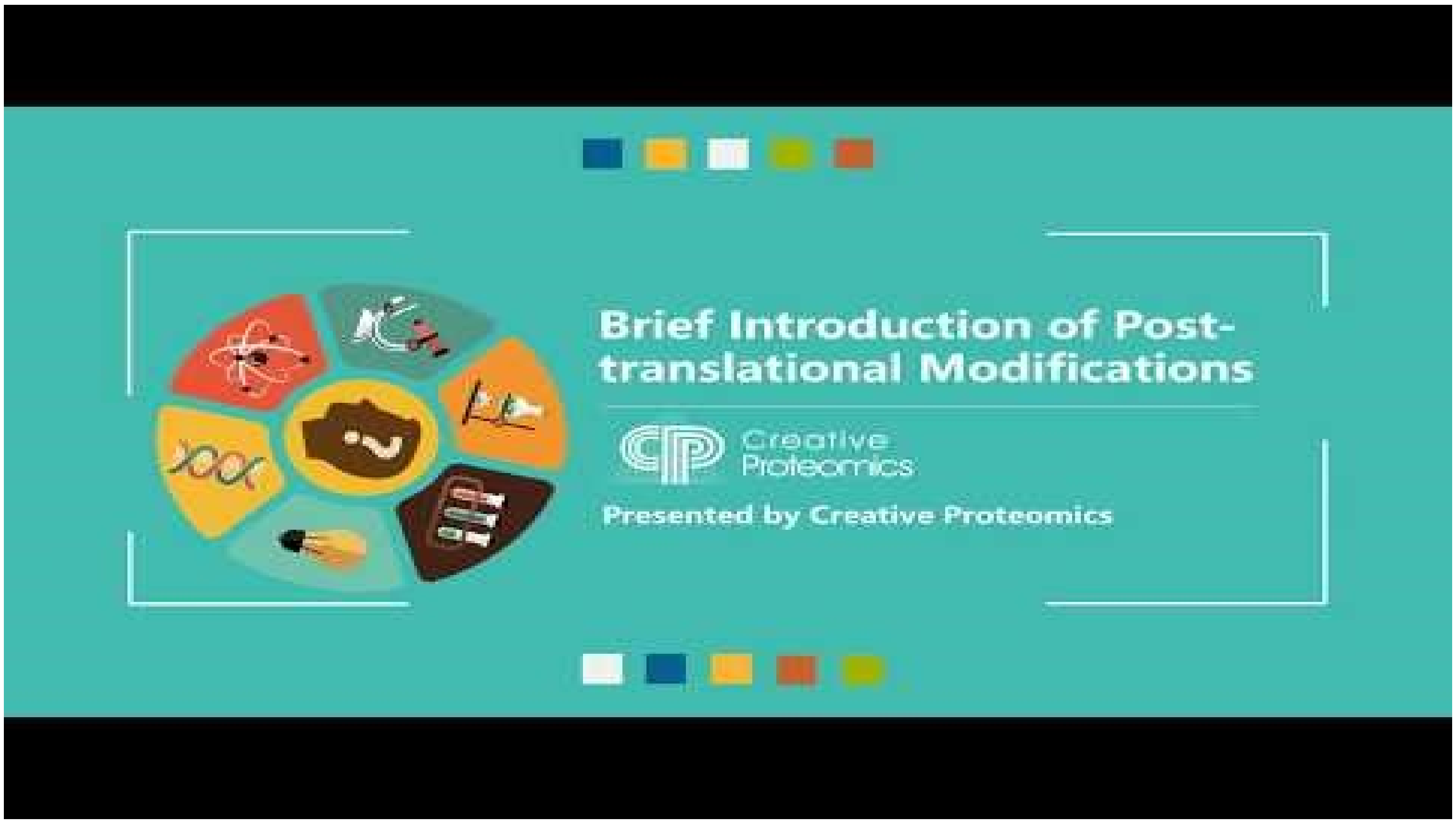
Central Dogma



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



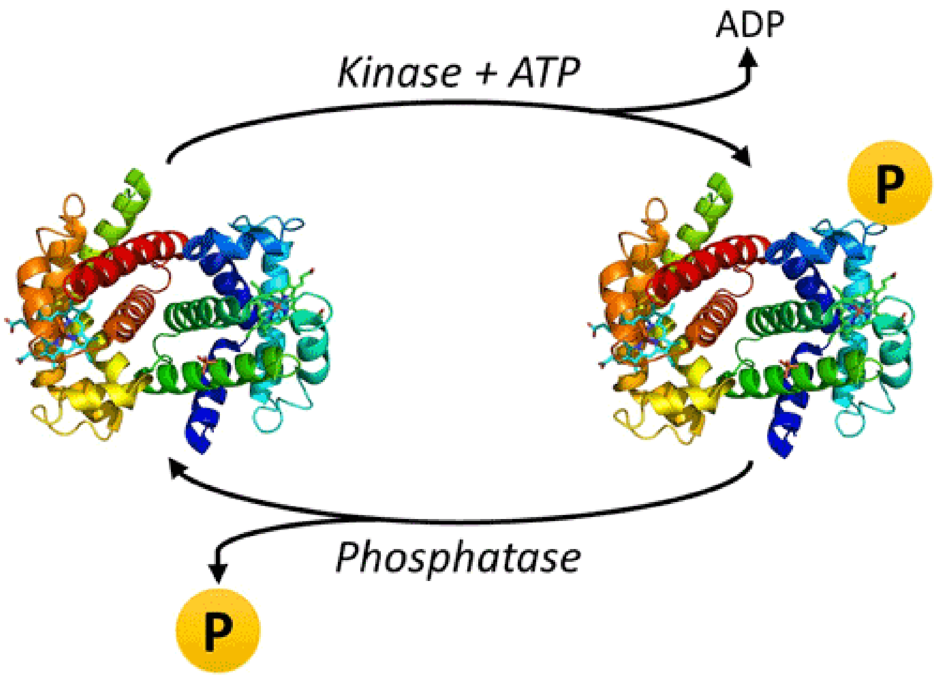
https://www.youtube.com/watch?v=AeVDoDp3lII

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

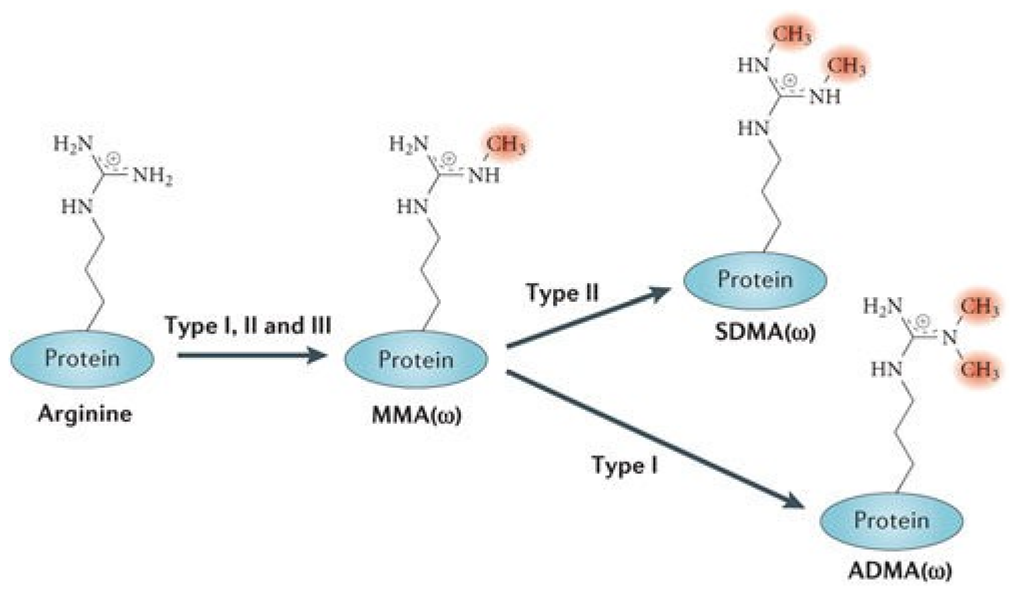
https://www.youtube.com/watch?v=xG2WOd_fWqo

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.

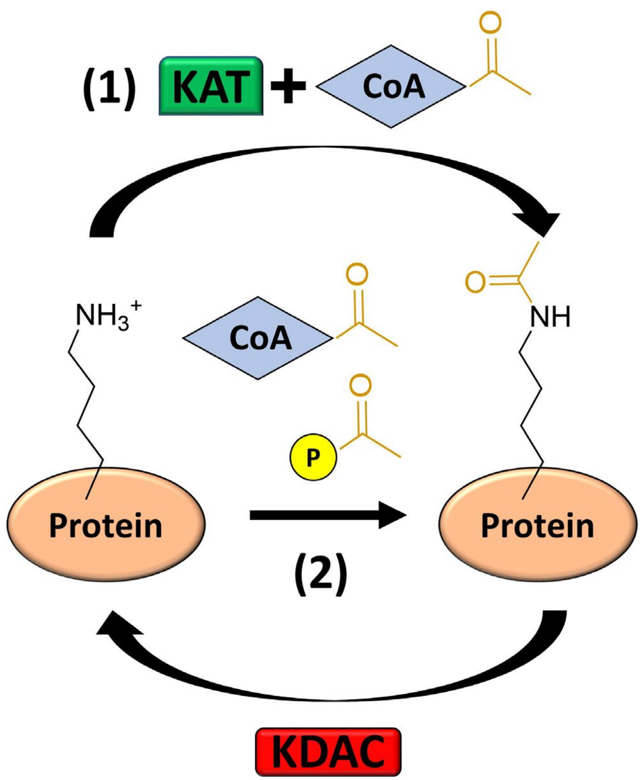
Protein Stability: Methylation can also impact the stability of proteins. Certain methylations can protect proteins from ubiquitin-mediated degradation, thereby prolonging their half-life in the cell.

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:

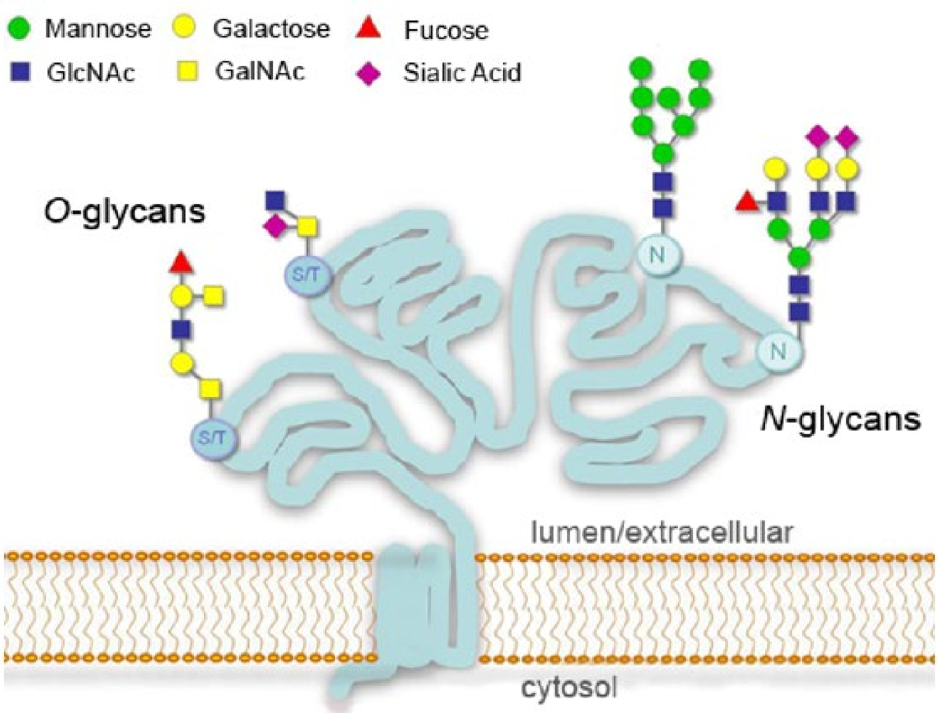
Gene Regulation: Acetylation of histone proteins, which DNA wraps around in chromatin, is crucial for the regulation of gene expression. When histones are acetylated, the chromatin structure becomes more open, allowing gene transcription machinery to access the DNA. Conversely, deacetylation typically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

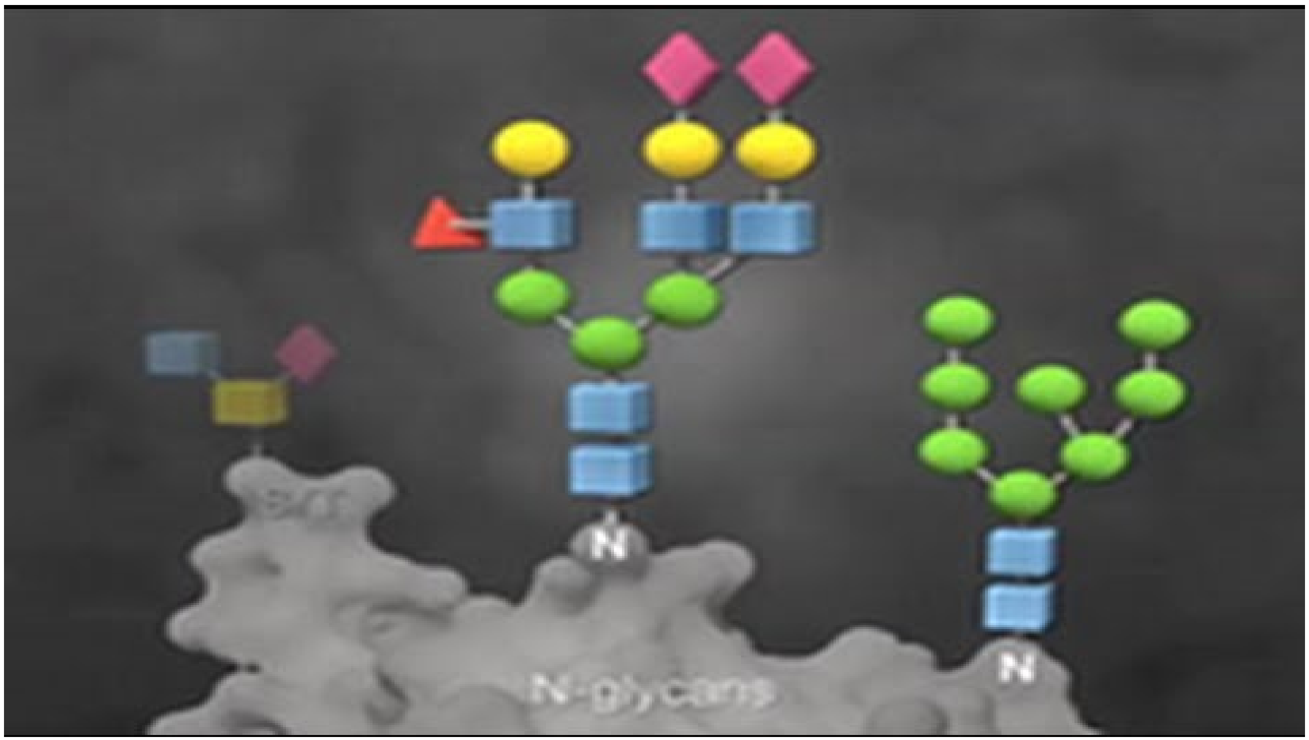
There are two main types of protein glycosylation:

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



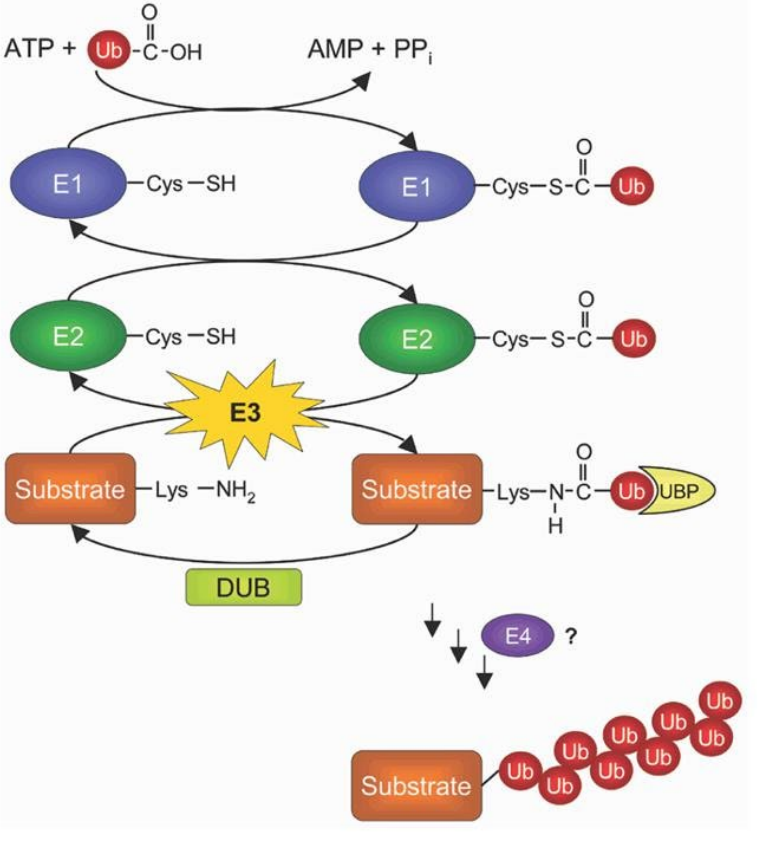
Check
https://www.youtube.com/watch?v=RorGifz6C2Y

The importance of glycosylation includes:

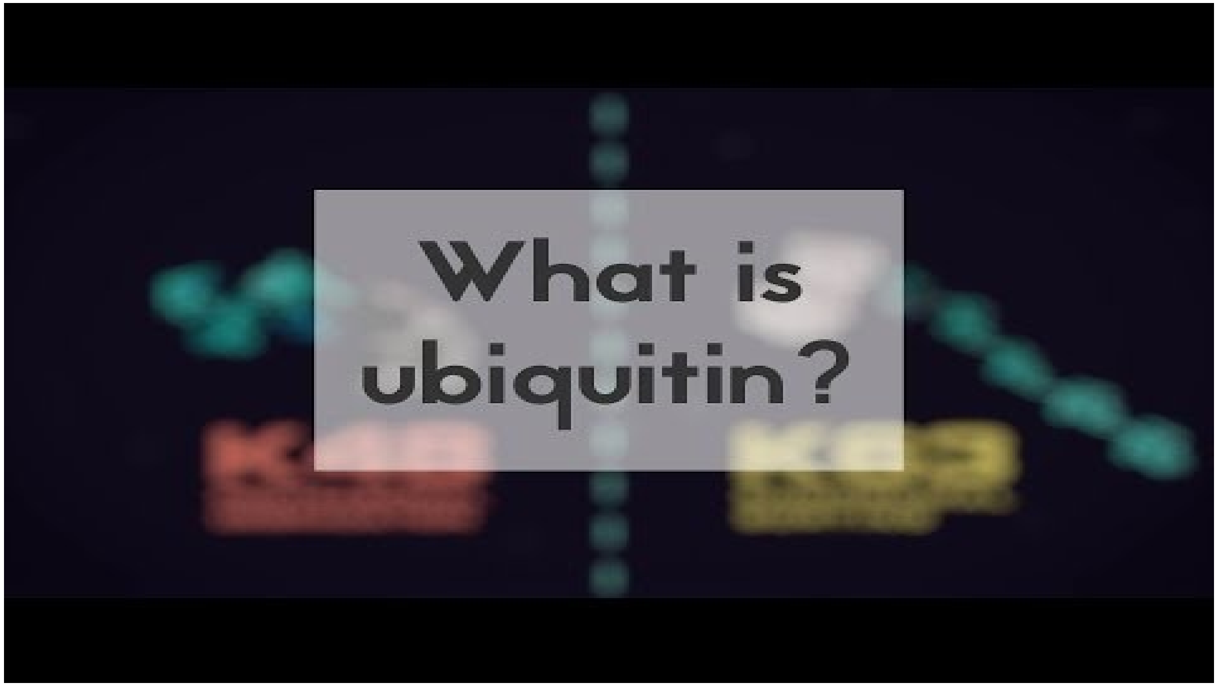
Protein Folding: Glycosylation helps in proper protein folding and stability, aiding in the correct three-dimensional conformation necessary for protein function. It also assists in the quality control mechanisms within the cell, such as in the endoplasmic reticulum, where improperly folded glycoproteins 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



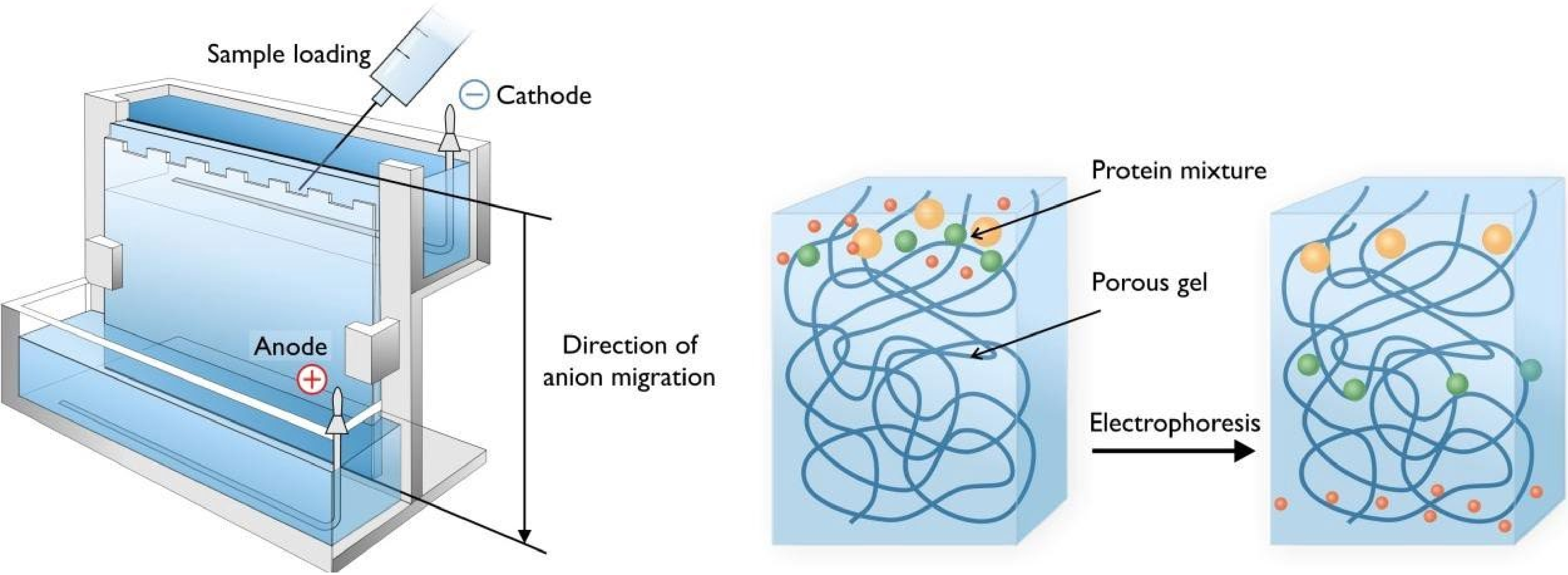
Check
https://www.youtube.com/watch?v=e29F7R3K_5A

Ubiquitination

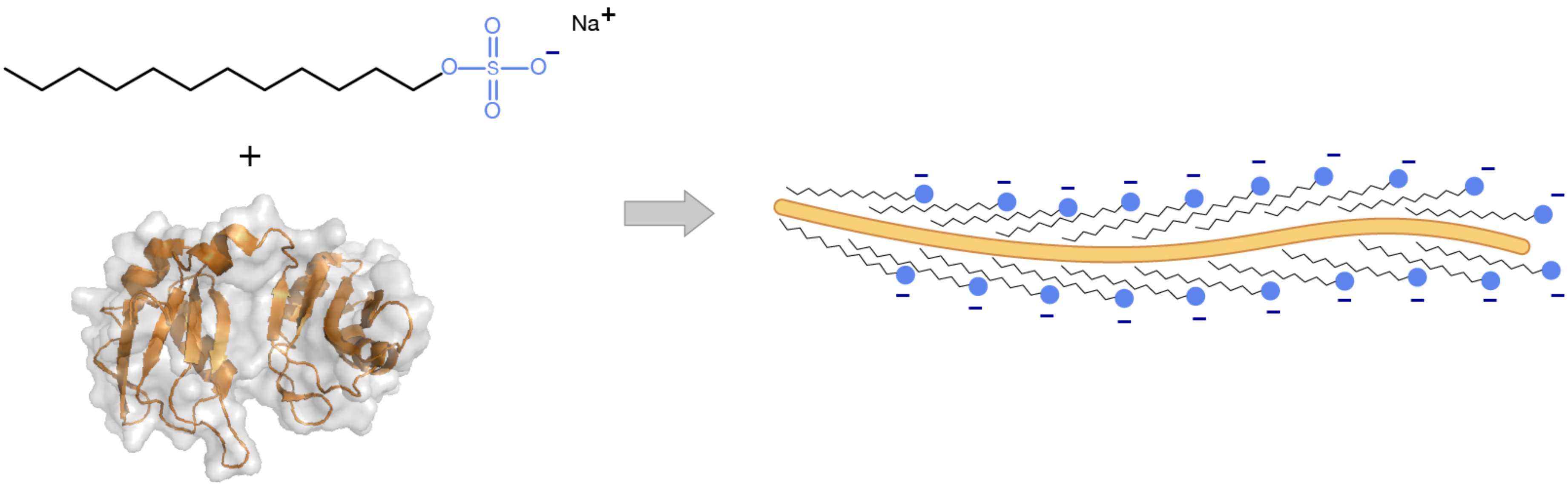
https://www.youtube.com/watch?v=jbc1QCu9hFg

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.

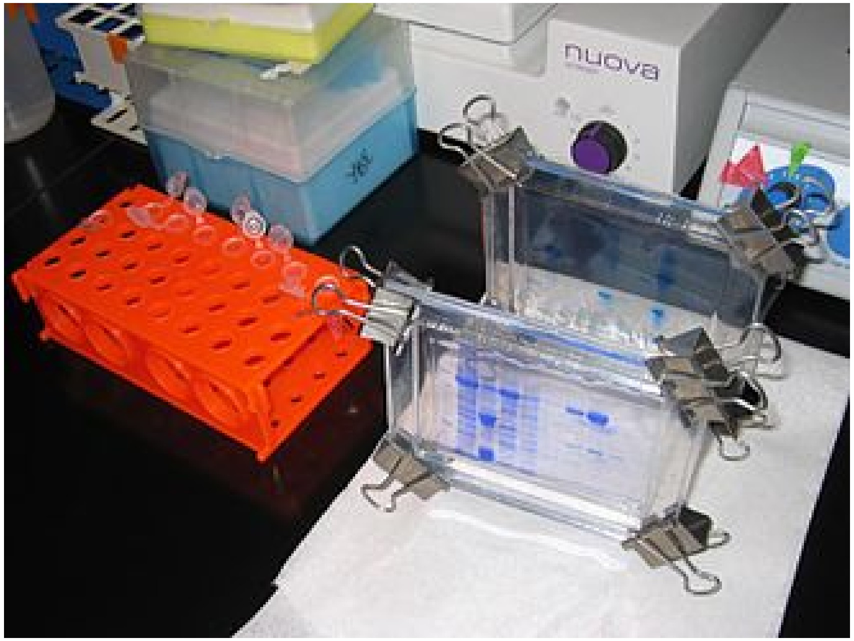
Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

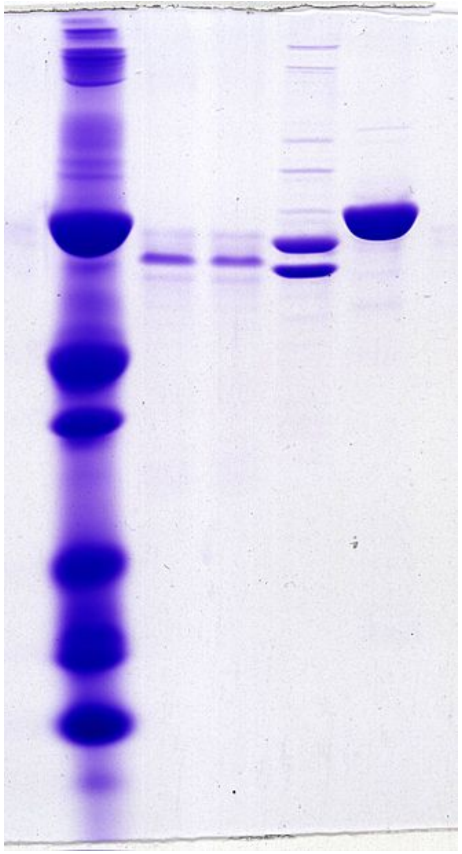


Protein Denature

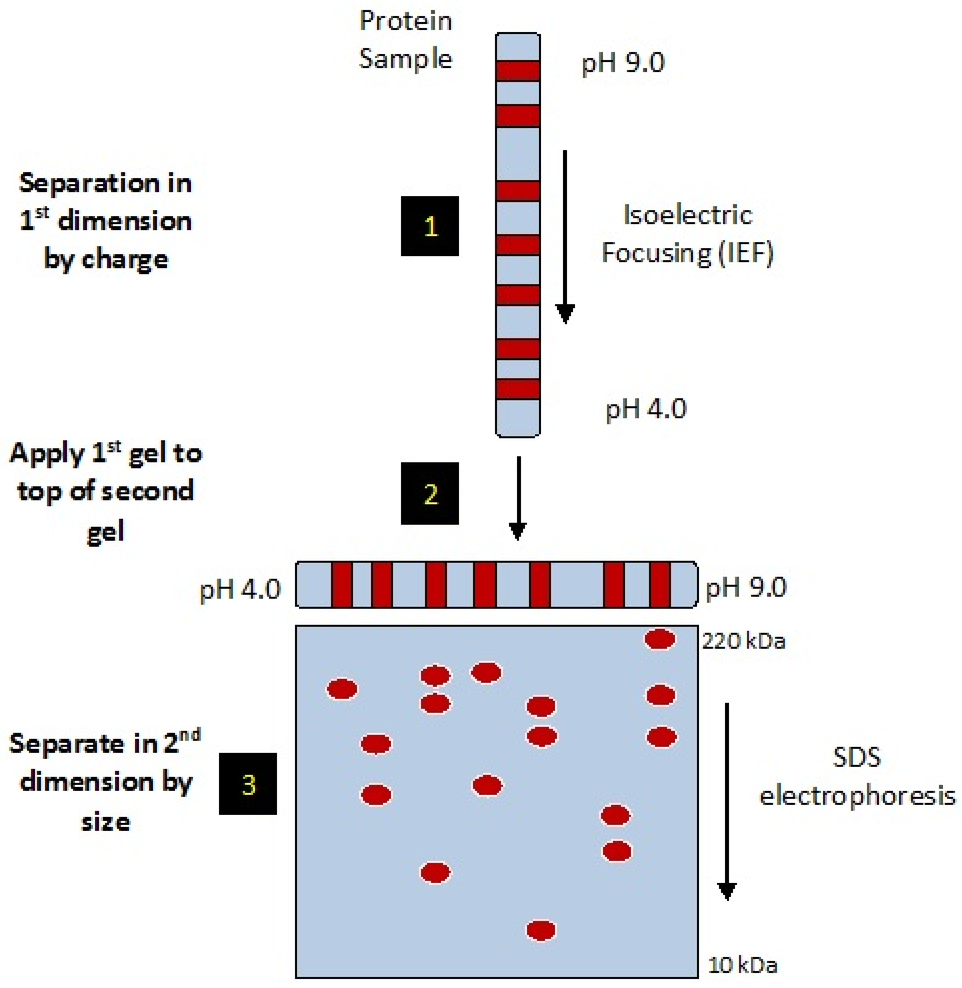


SDS-PAGE

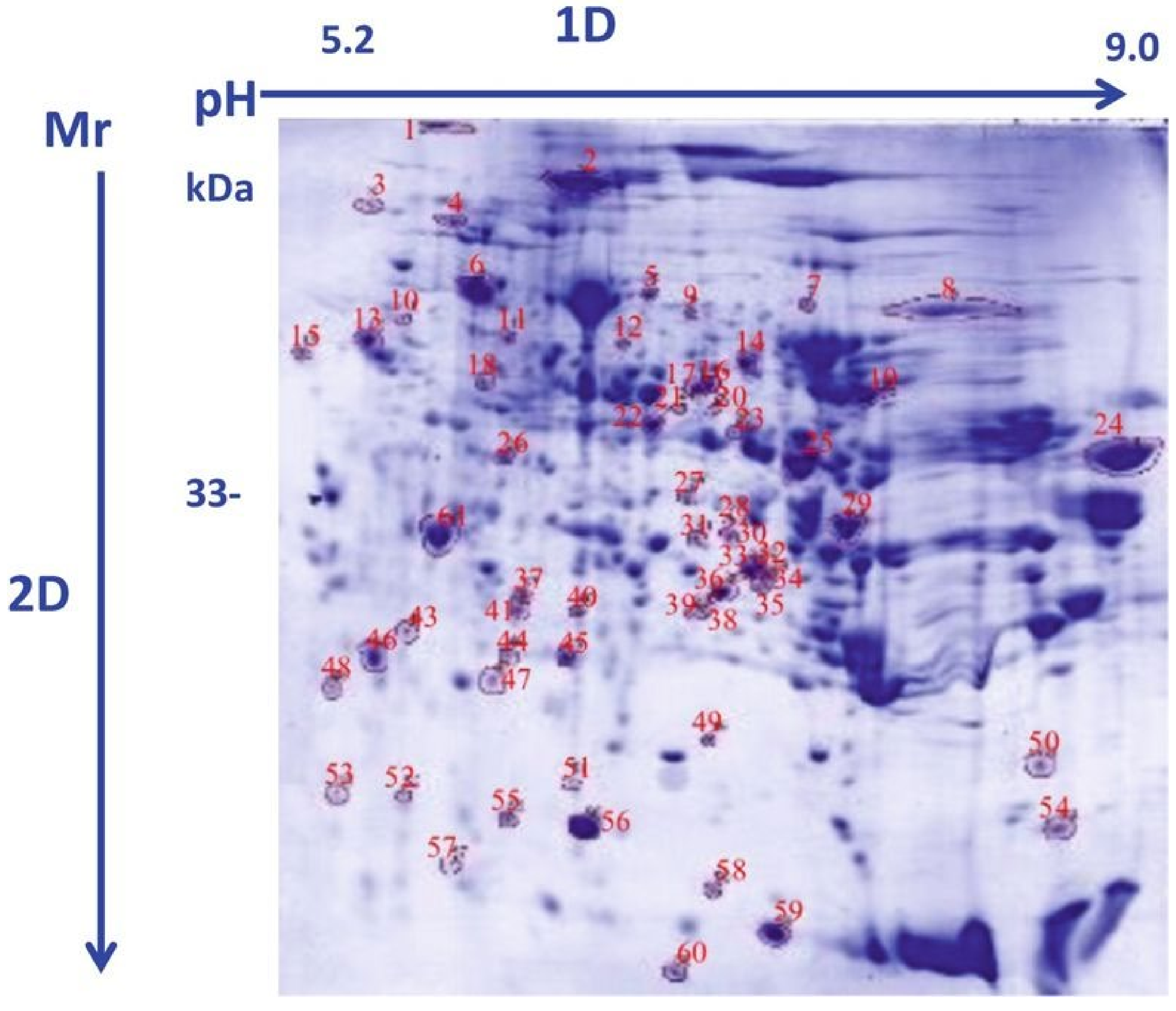


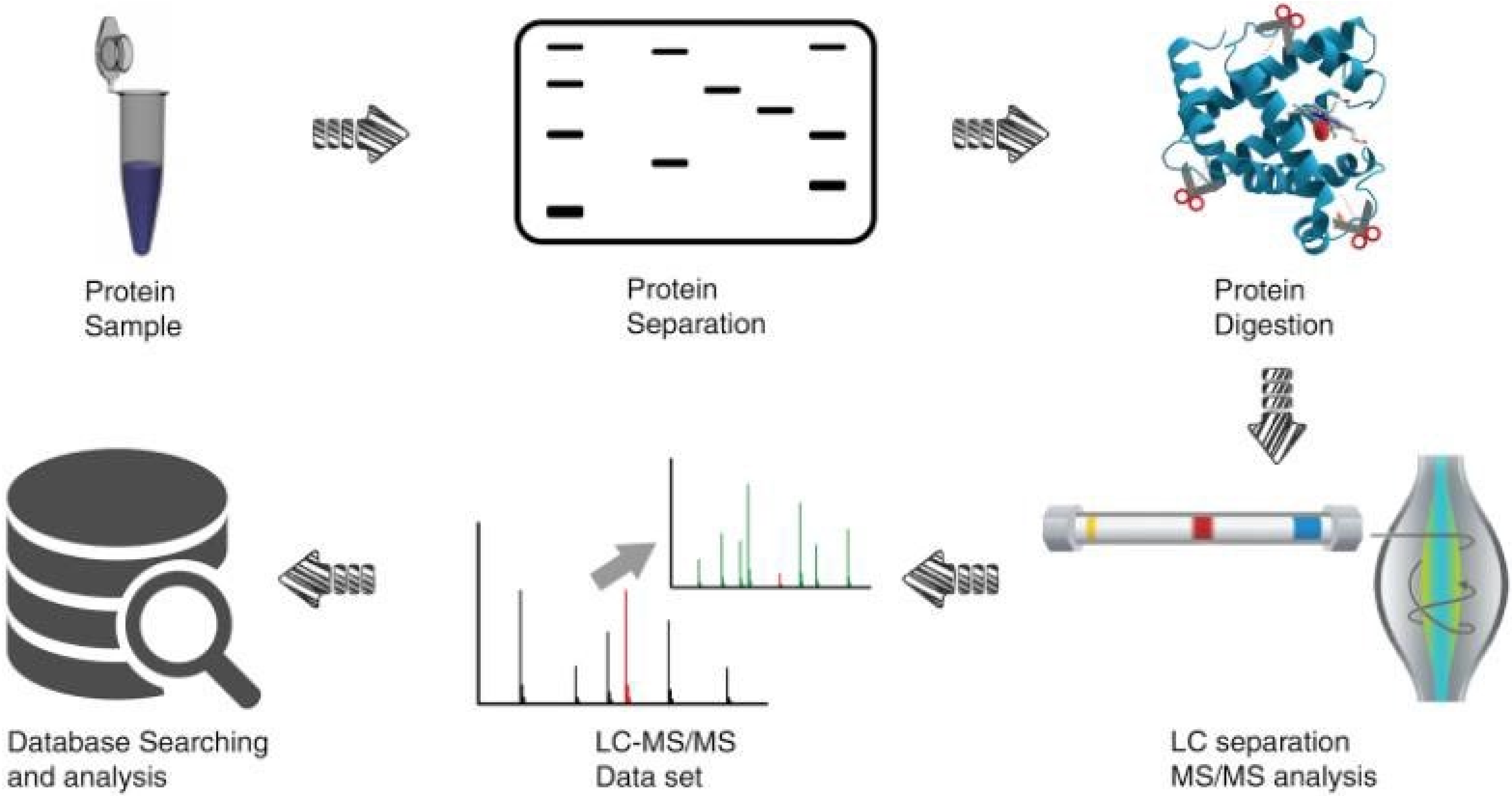


2D PAGE


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

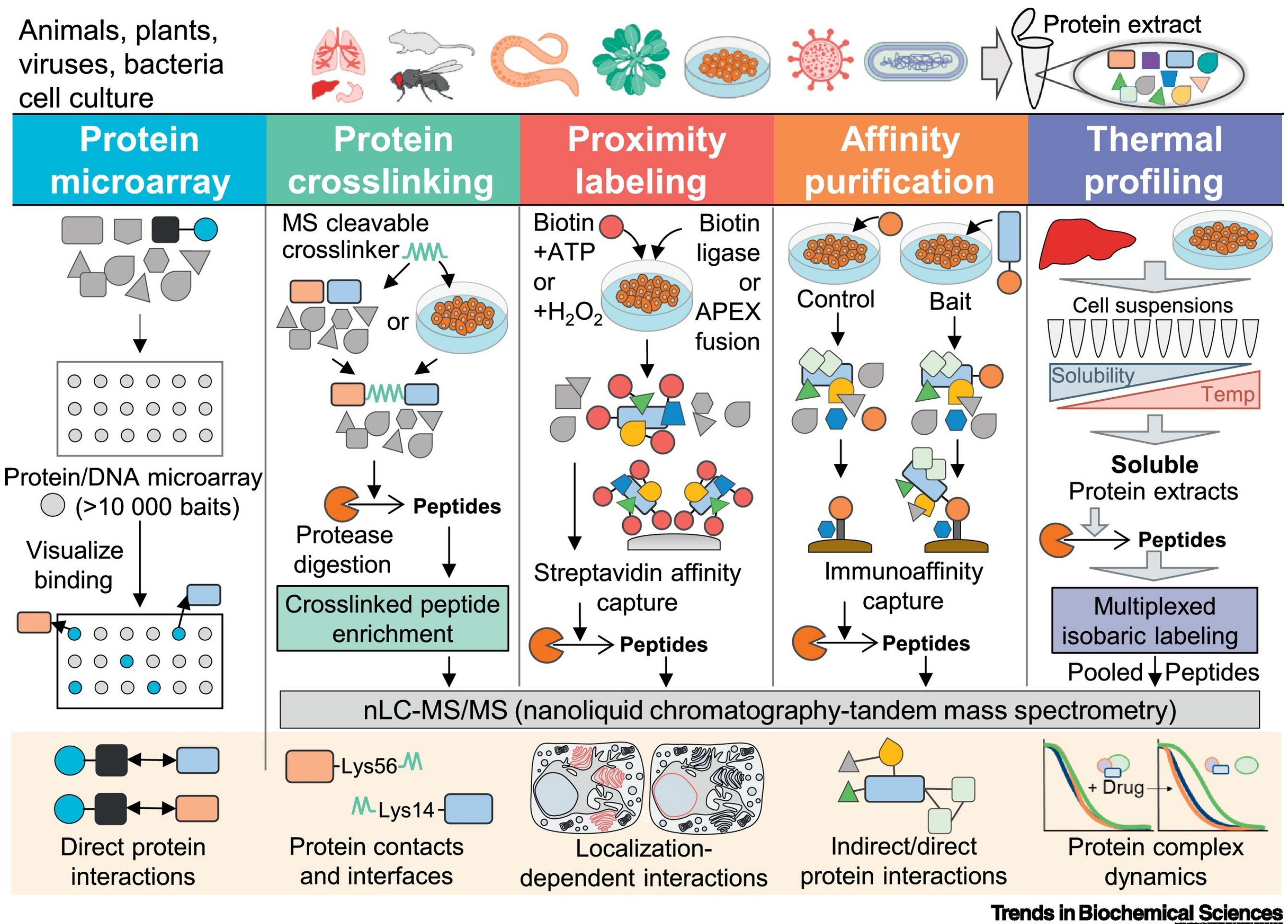
2D PAGE





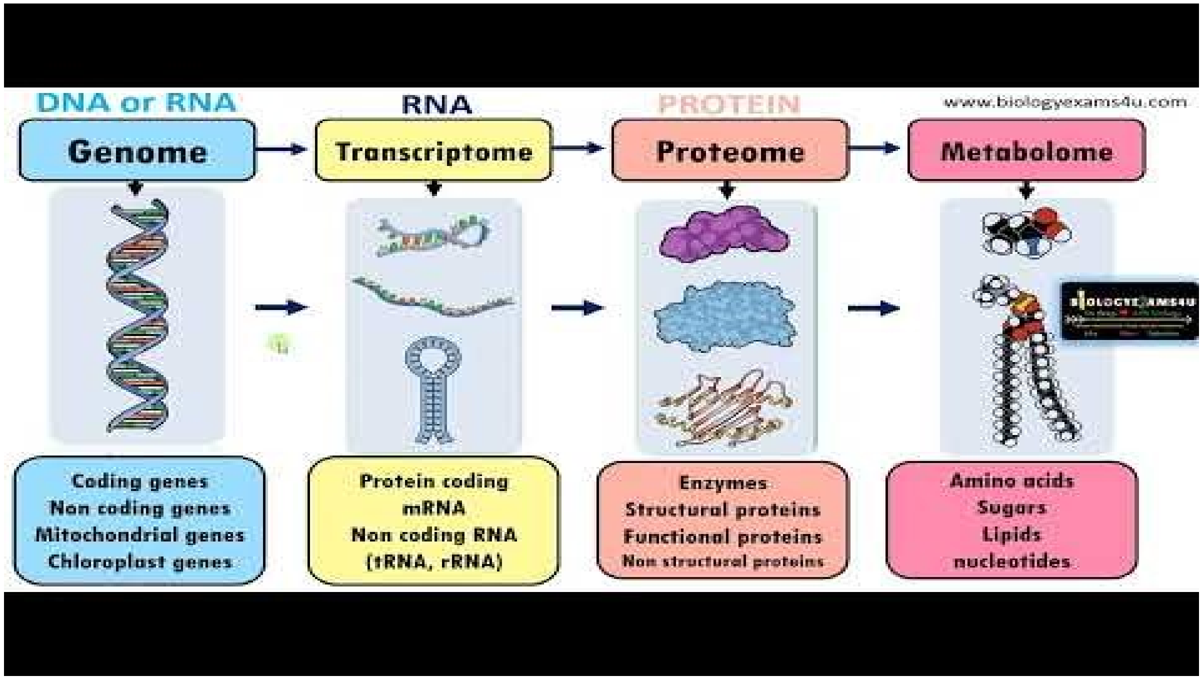
Protein
Sample
Protein
Separation
Protein
Digestion
LC separation
MS/MS analysis
LC-MS/MS
Data set
Database Searching
and analysis

Proteomic



Trends in Biochemical Sciences 2020 45454-455DOI: (10.1016/j.tibs.2020.01.003)

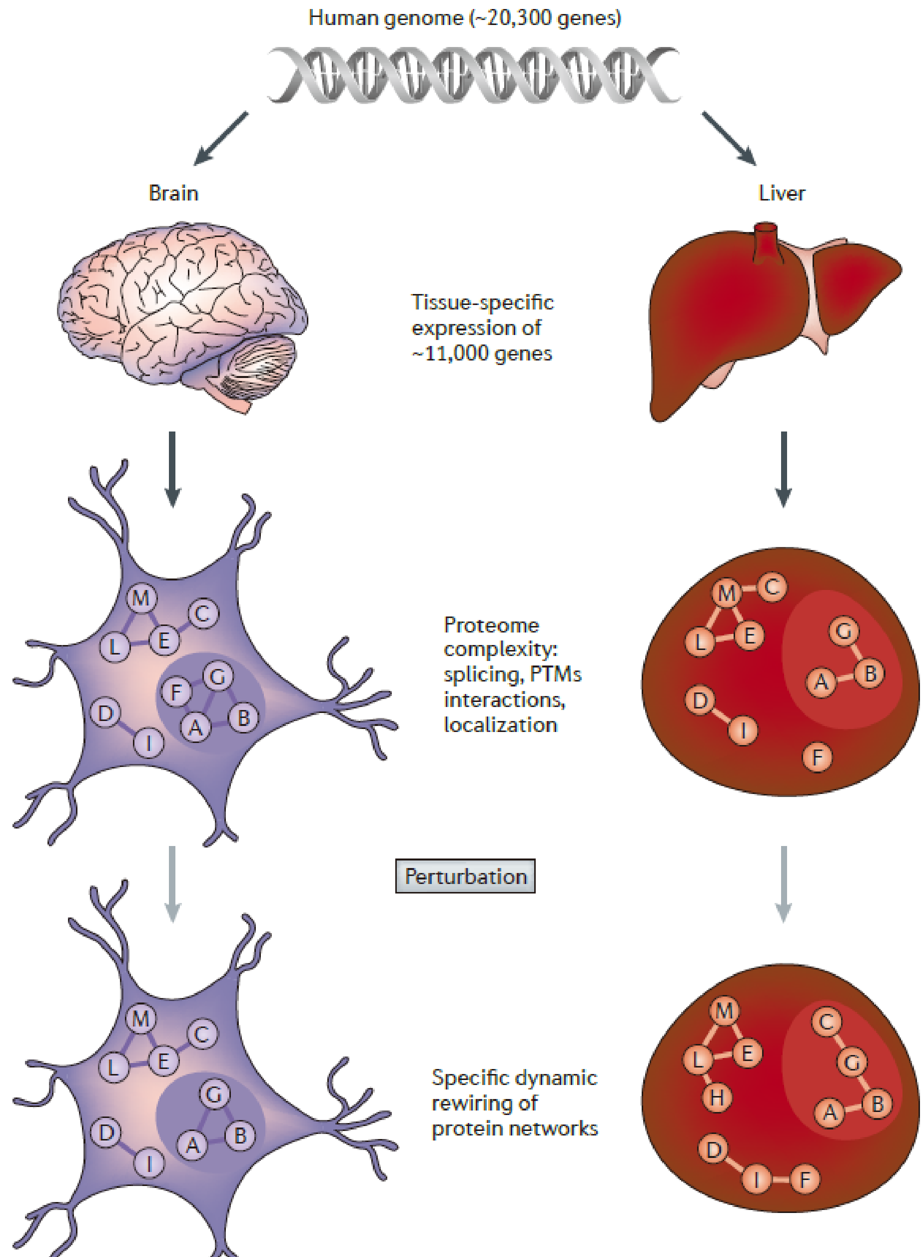
Multiomic



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

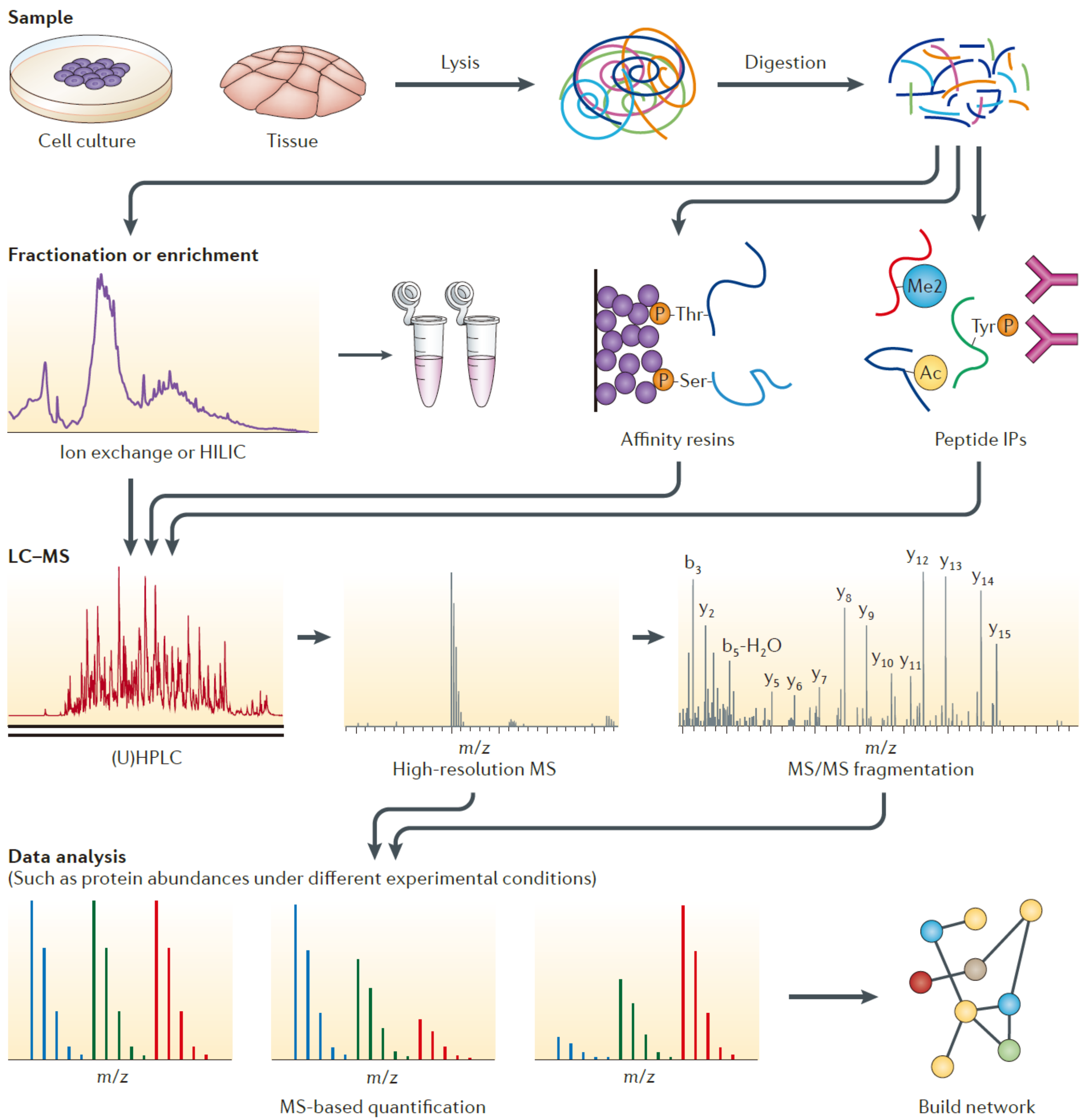
Next-generation proteomics: towards an integrative view of proteome dynamics

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

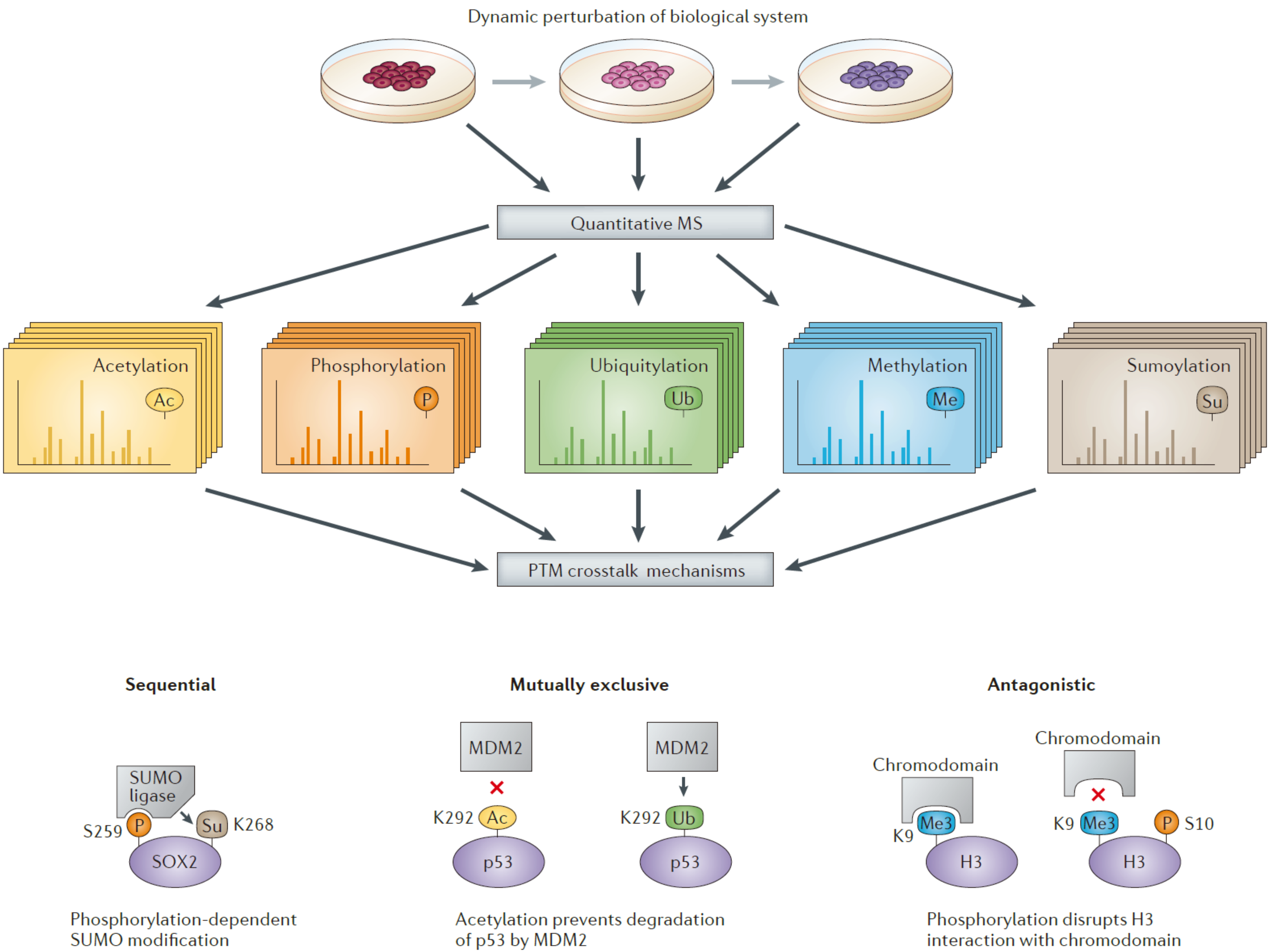


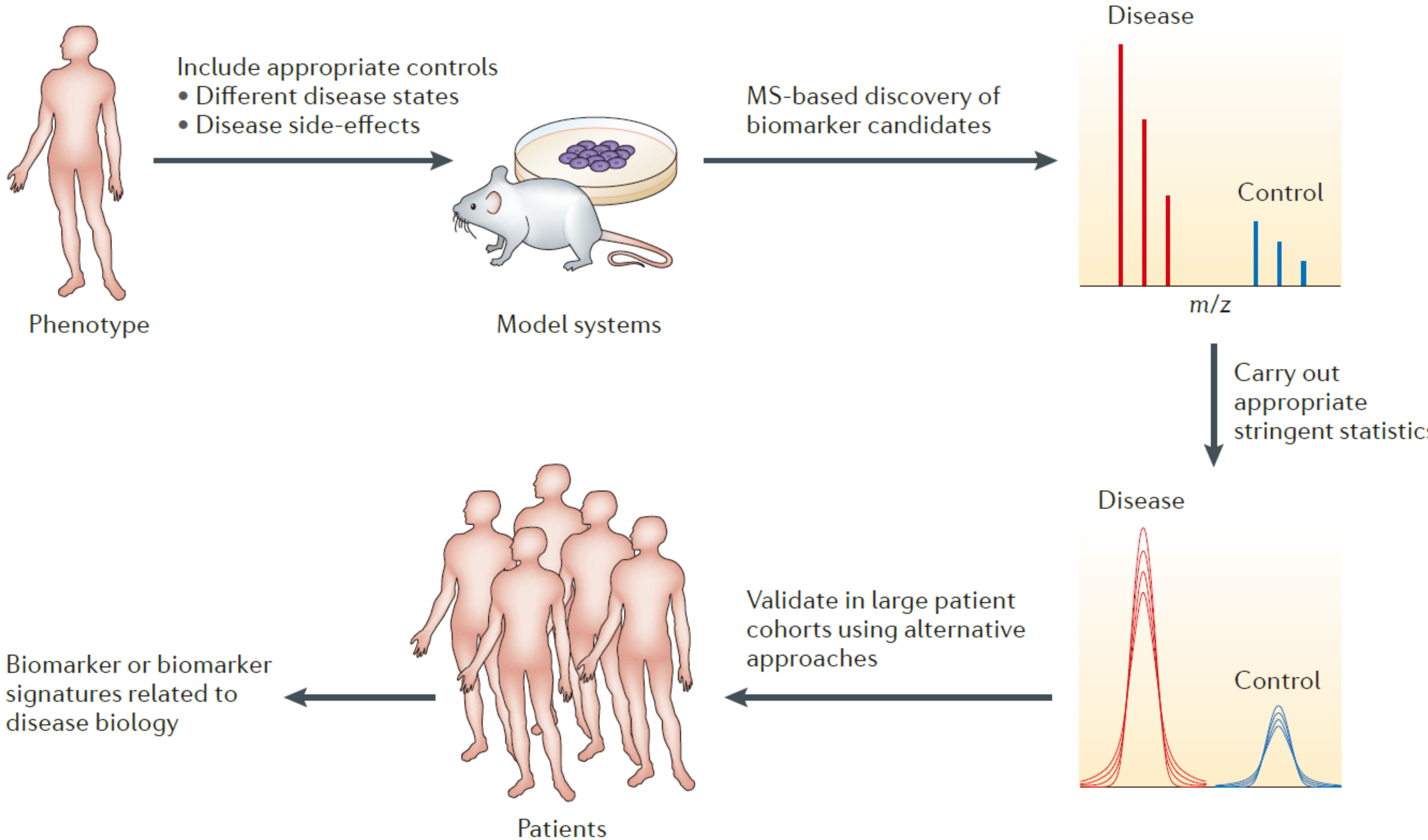
Key Points

- 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
b_3 y_2 b_5-H_2O y_5 y_6 y_7 y_8 y_9 y_{10} y_{11} y_{12} y_{13} y_{14} y_{15}
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


Dynamic perturbation of biological system
Quantitative MS
Acetylation
Ac
Phosphorylation
P
Ubiquitylation
Ub
Methylation
Me
Sumoylation
Su
PTM crosstalk mechanisms
Sequential
Mutually exclusive
Antagonistic
SUMO ligase
S259
P
Su
K268
SOX2
MDM2
K292
Ac
p53
MDM2
K292
Ub
p53
Chromodomain
K9
Me3
H3
Chromodomain
K9
Me3
P
S10
H3
Phosphorylation-dependent SUMO modification
Acetylation prevents degradation of p53 by MDM2
Phosphorylation disrupts H3 interaction with chromodomain


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

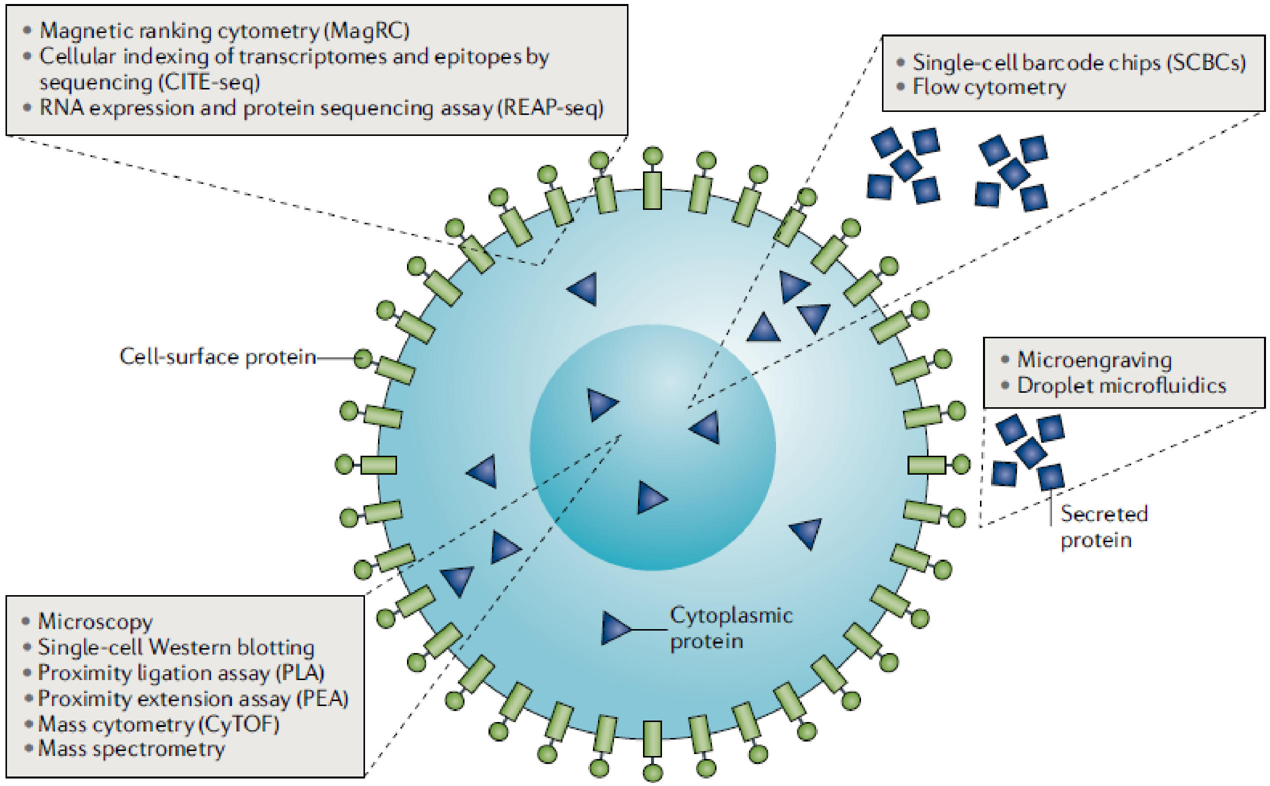
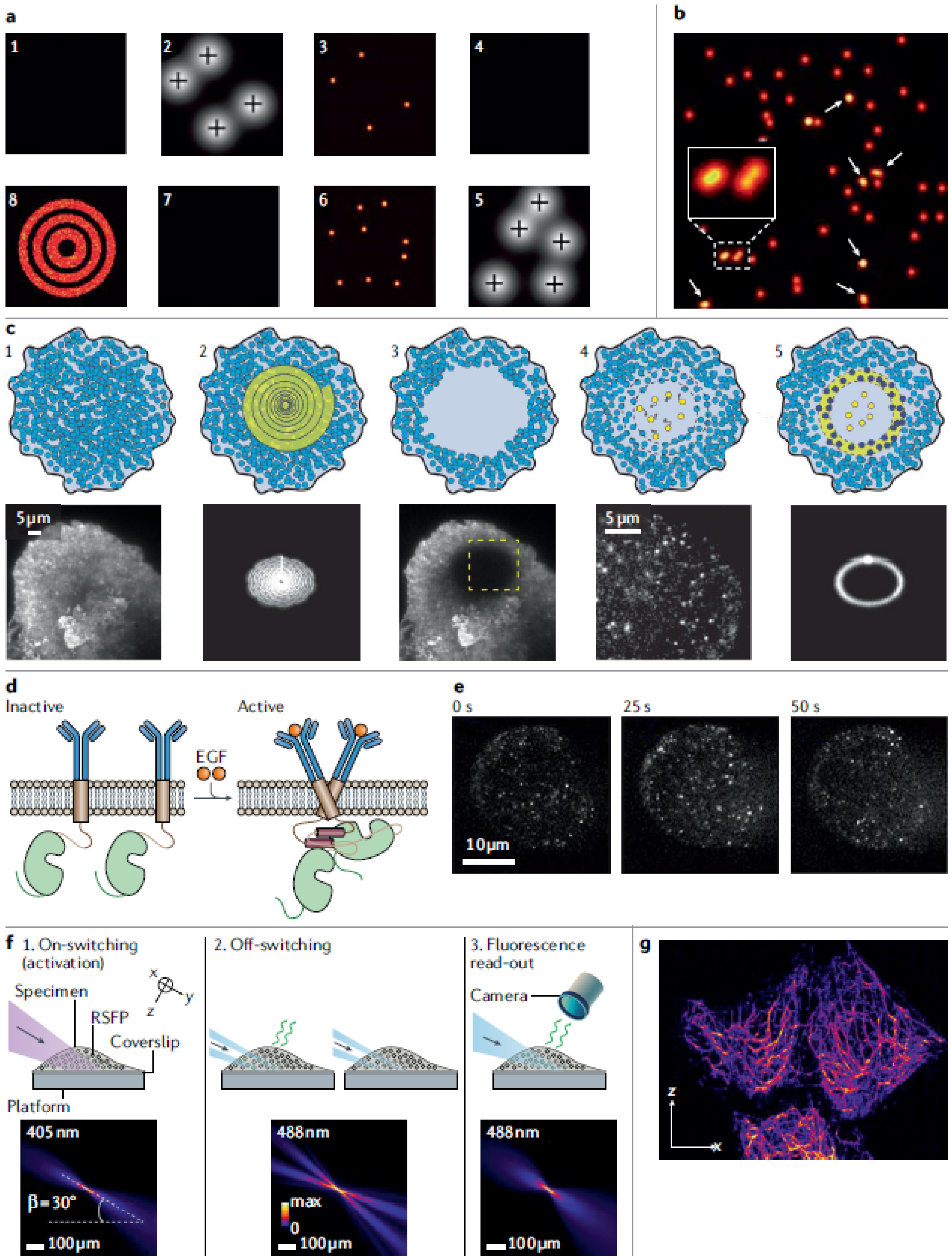
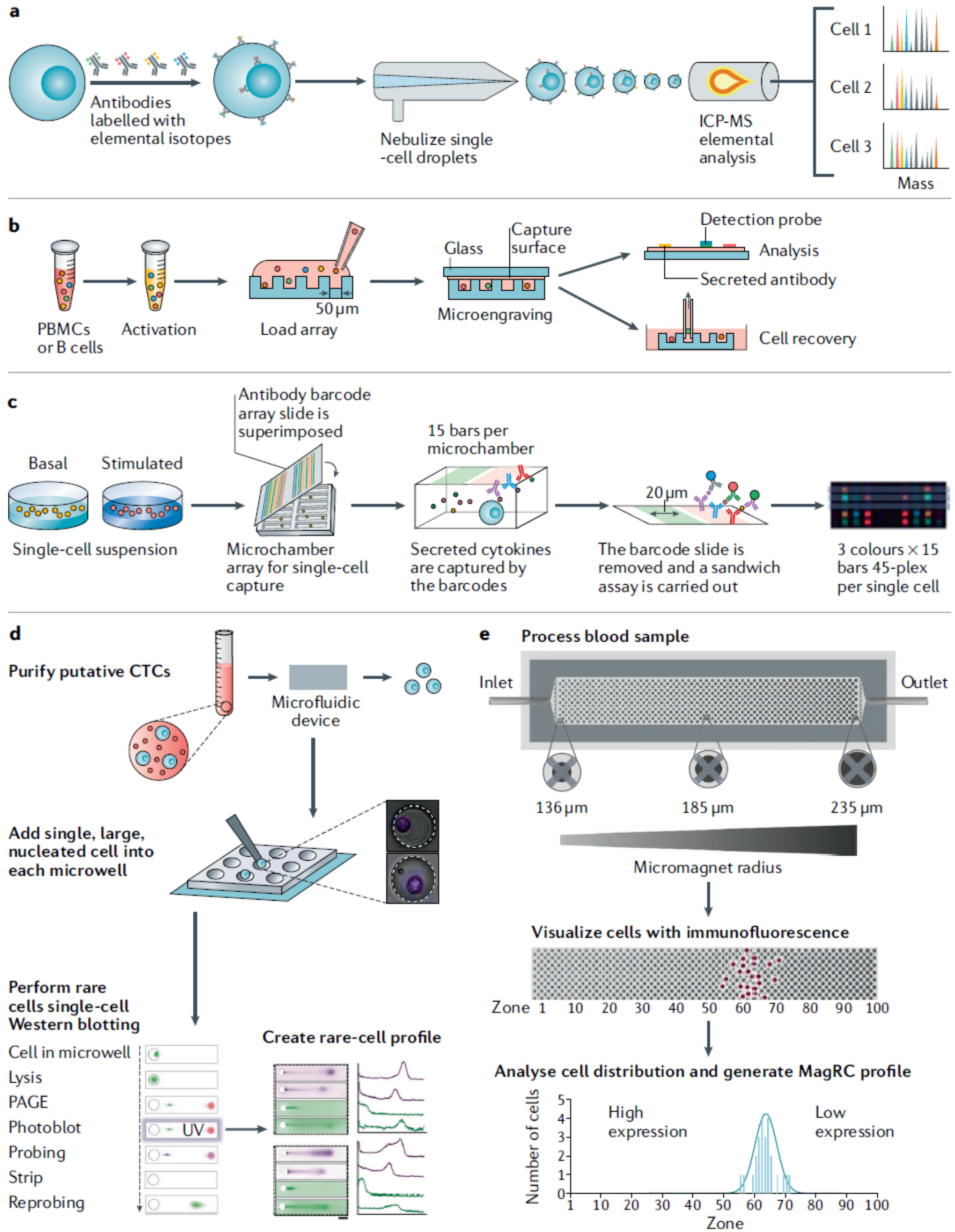
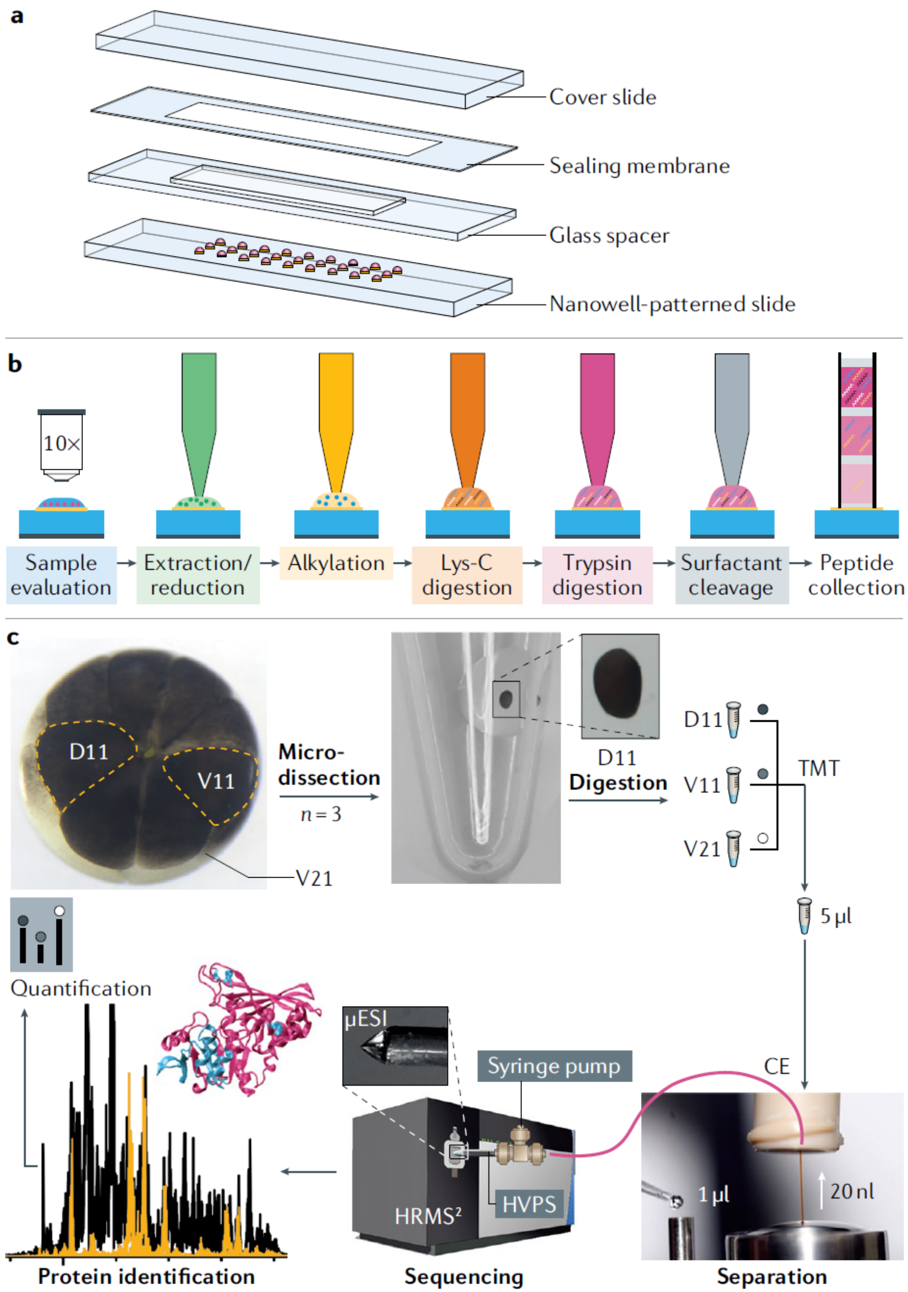


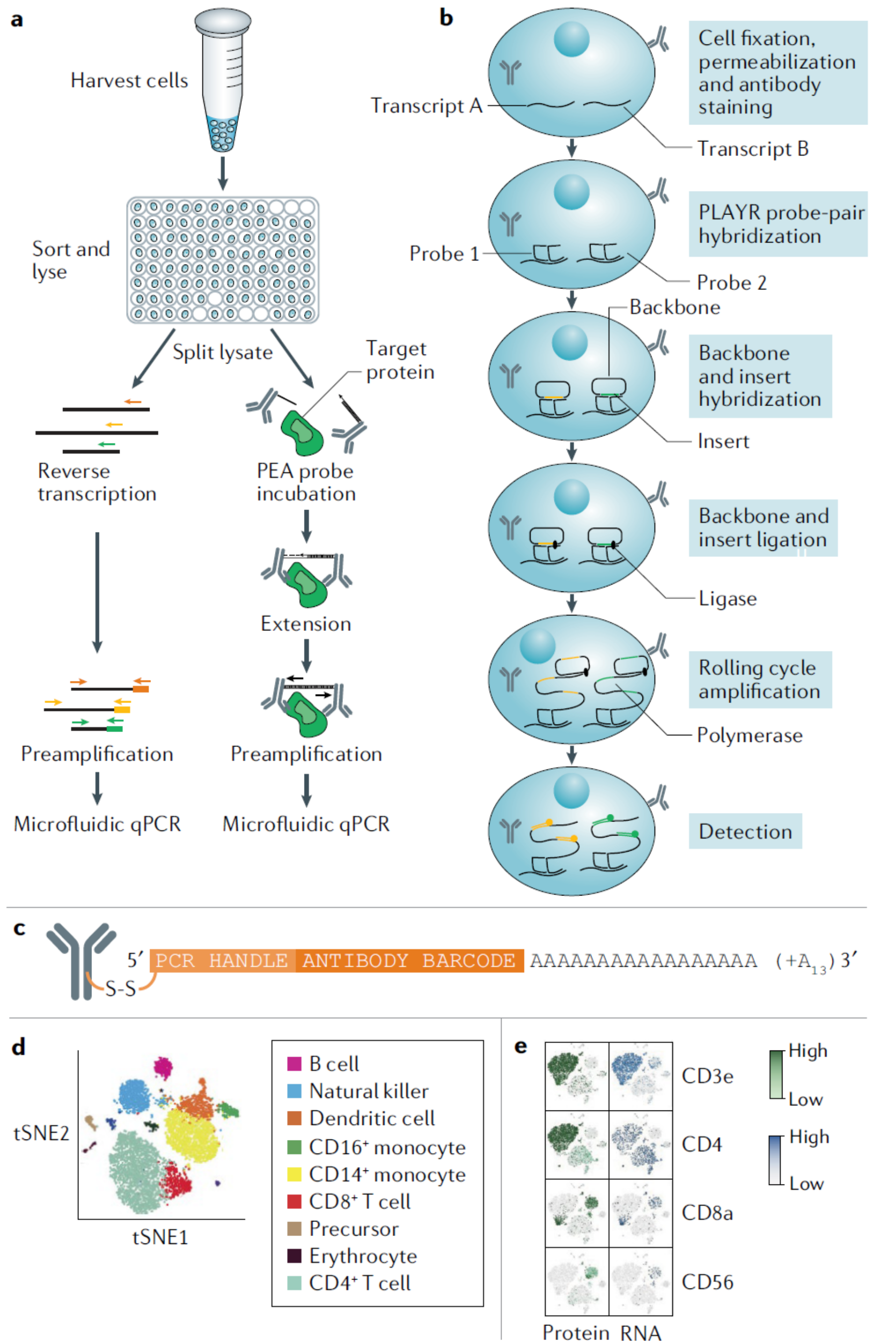
Fig. 1 | **Classification of single-cell protein analysis methods based on the location of target protein.** Cell-surface-protein analysis methods include magnetic ranking cytometry (MagRC), cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq), and RNA expression and protein sequencing 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
Load array
50 μm
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
High expression
Low expression
Number of cells
0 1 2 3 4 5
1 10 20 30 40 50 60 70 80 90 100
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
1 μl
20 nl
HRMS²
HVPS
Protein identification
Sequencing
Separation



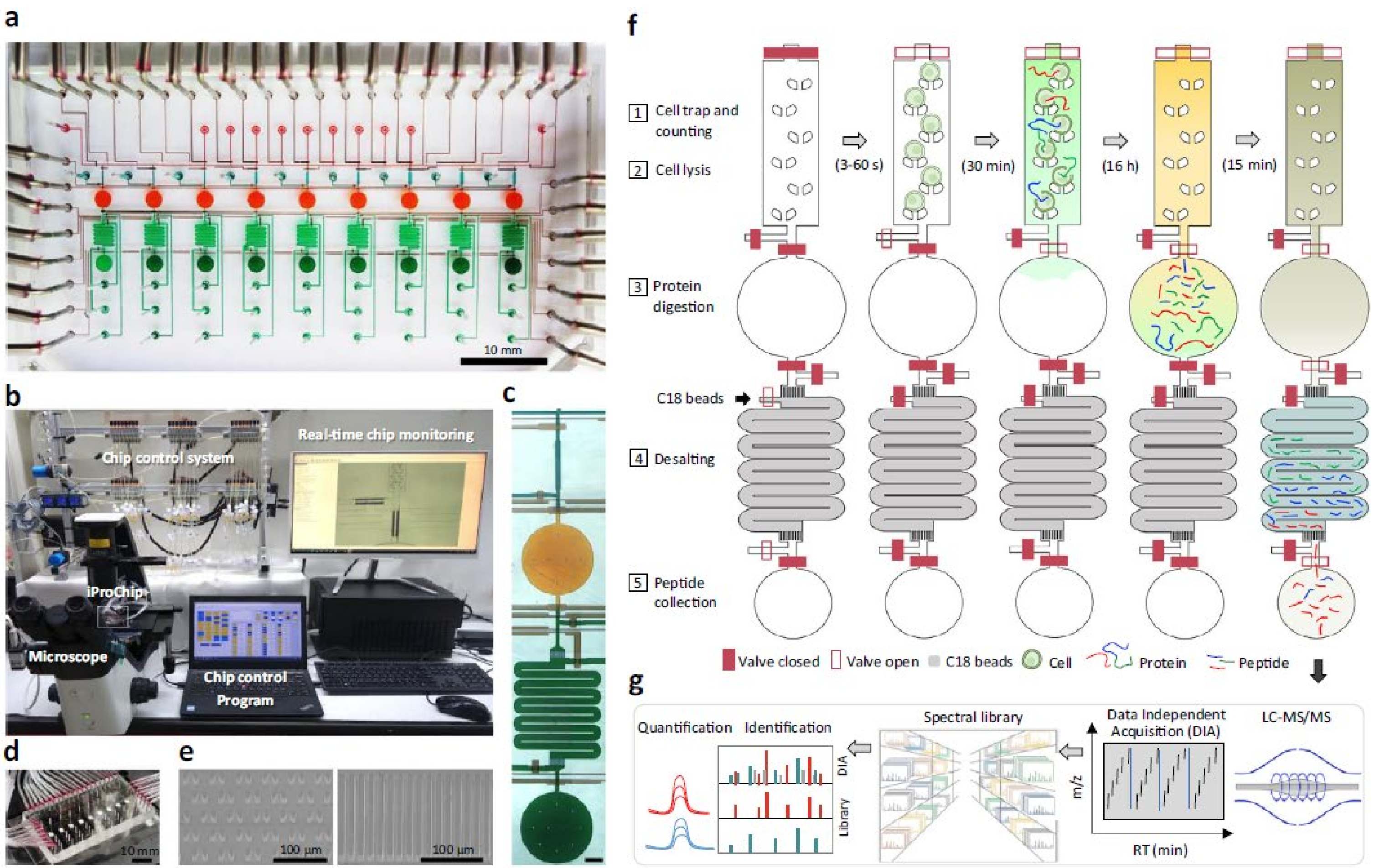
a
Harvest cells
Sort and lyse
Split lysate
Target protein
Reverse transcription
PEA probe incubation
Extension
Preamplification
Preamplification
Microfluidic qPCR
Microfluidic qPCR
b
Cell fixation, permeabilization and antibody staining
Transcript A
Transcript B
PLAYR probe-pair hybridization
Probe 1
Probe 2
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

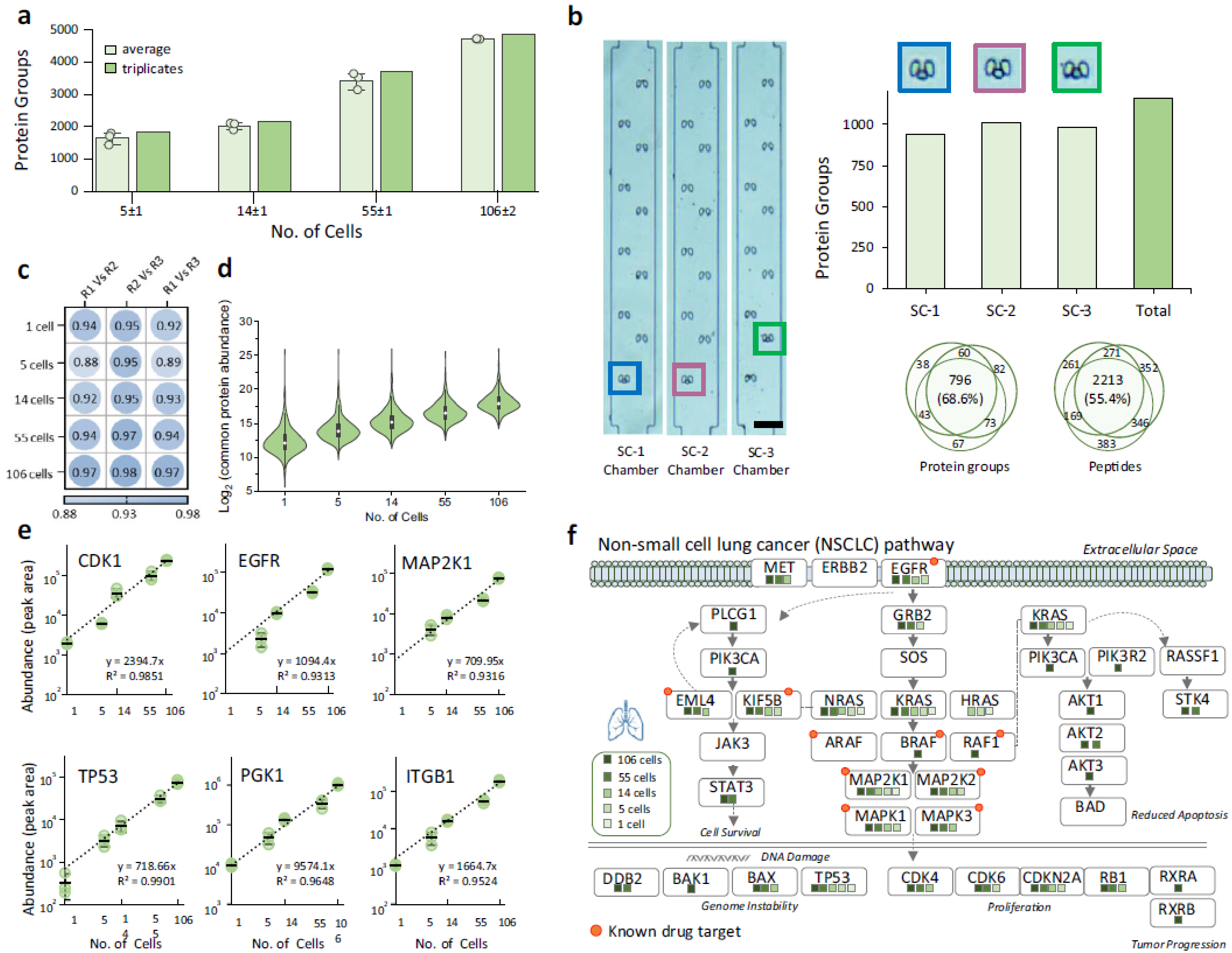
ARTICLE

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

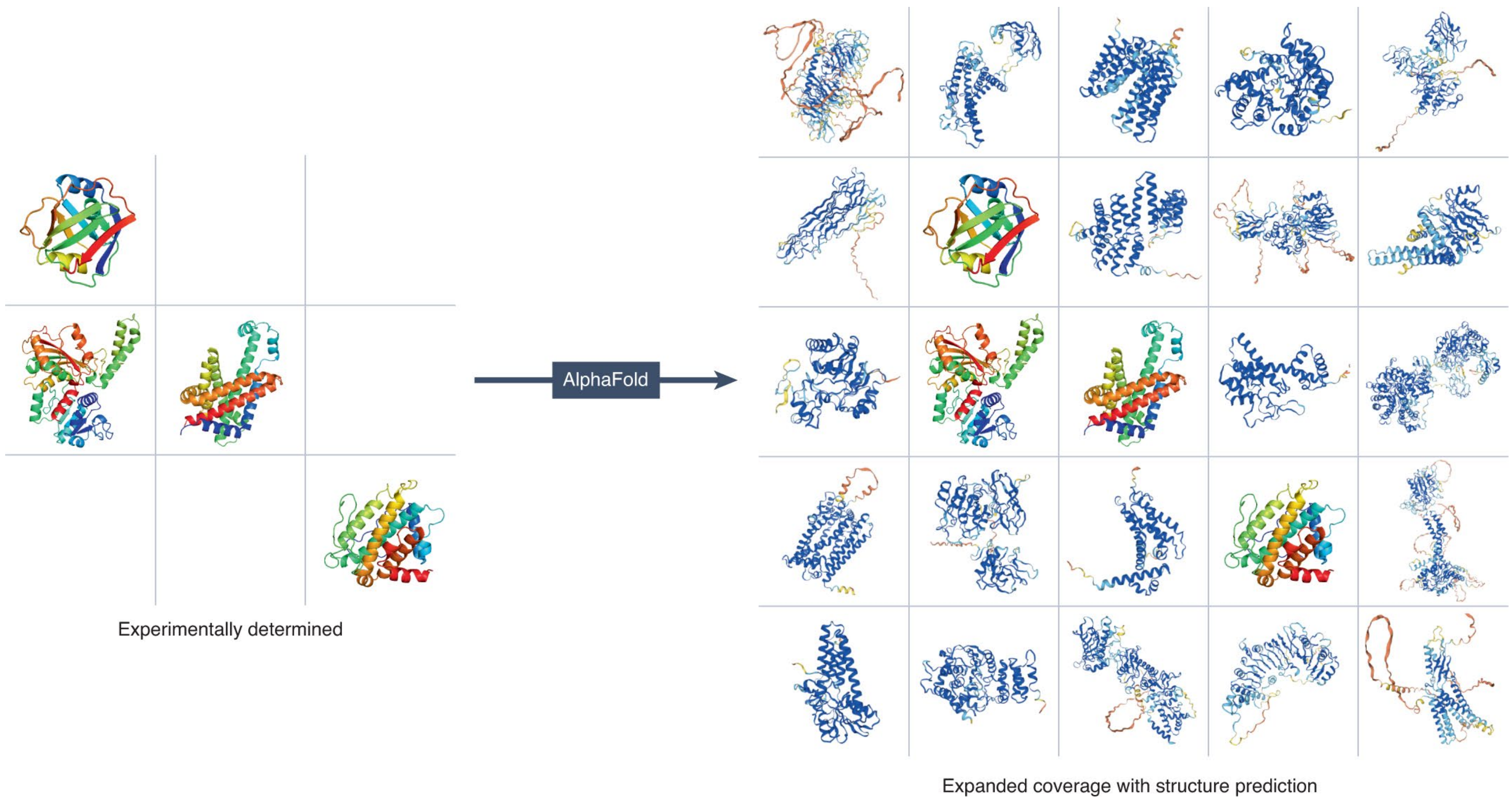
Streamlined single-cell proteomics by an integrated microfluidic chip and data-independent acquisition mass spectrometry

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✉]

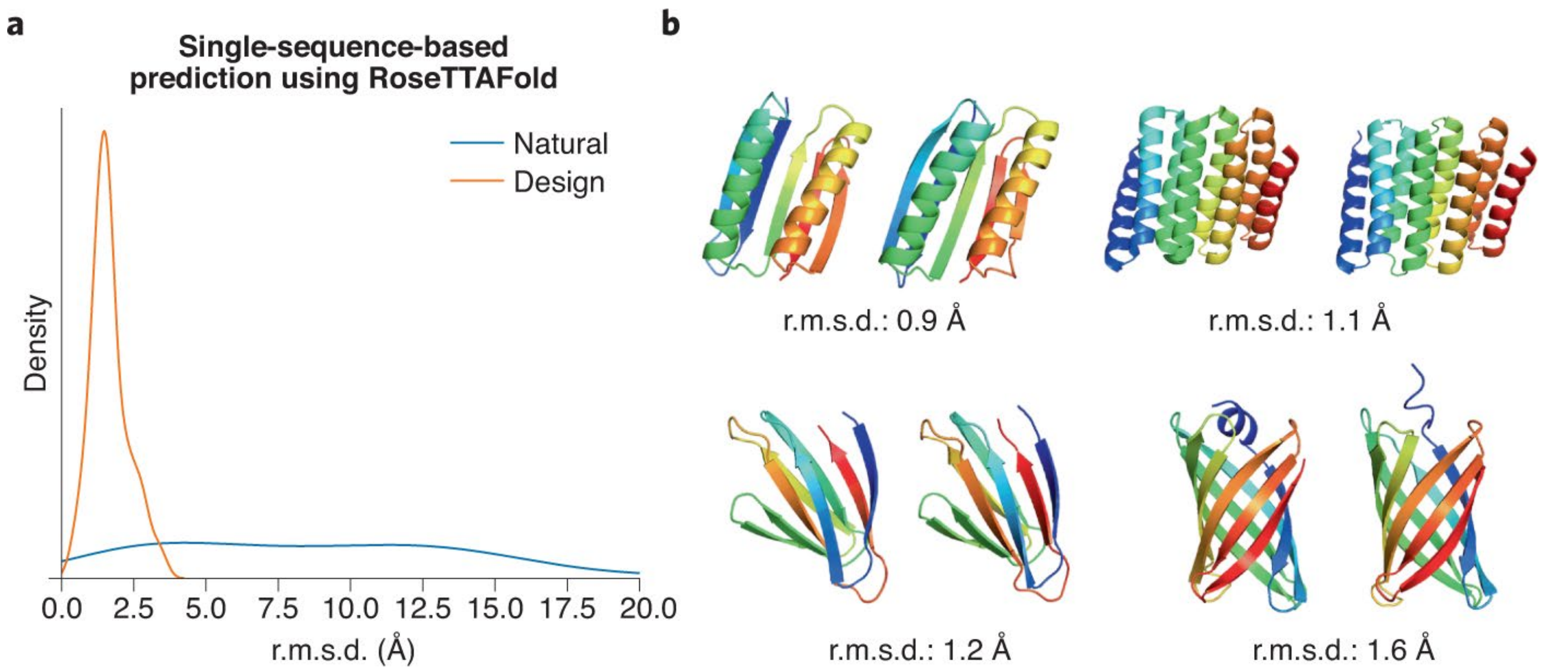



a
Protein Groups
average
triplicates
5±1
14±1
55±1
106±2
No. of Cells
b
SC-1 Chamber
SC-2 Chamber
SC-3 Chamber
Protein Groups
SC-1
SC-2
SC-3
Total
796 (68.6%)
Protein groups
2213 (55.4%)
Peptides
c
R1 Vs R2
R2 Vs R3
R1 Vs R3
1 cell
5 cells
14 cells
55 cells
106 cells
d
Log2 (common protein abundance)
No. of Cells
e
CDK1
EGFR
MAP2K1
TP53
PGK1
ITGB1
Abundance (peak area)
y = 2394.7x
R² = 0.9851
y = 1094.4x
R² = 0.9313
y = 709.95x
R² = 0.9316
y = 718.66x
R² = 0.9901
y = 9574.1x
R² = 0.9648
y = 1664.7x
R² = 0.9524
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
STAT3
MAP2K1
MAP2K2
AKT3
MAPK1
MAPK3
BAD
106 cells
55 cells
14 cells
5 cells
1 cell
Cell Survival
Reduced Apoptosis
DNA Damage
DDB2
BAK1
BAX
TP53
CDK4
CDK6
CDKN2A
RB1
RXRA
RXRB
Genome Instability
Proliferation
Tumor Progression
Known drug target

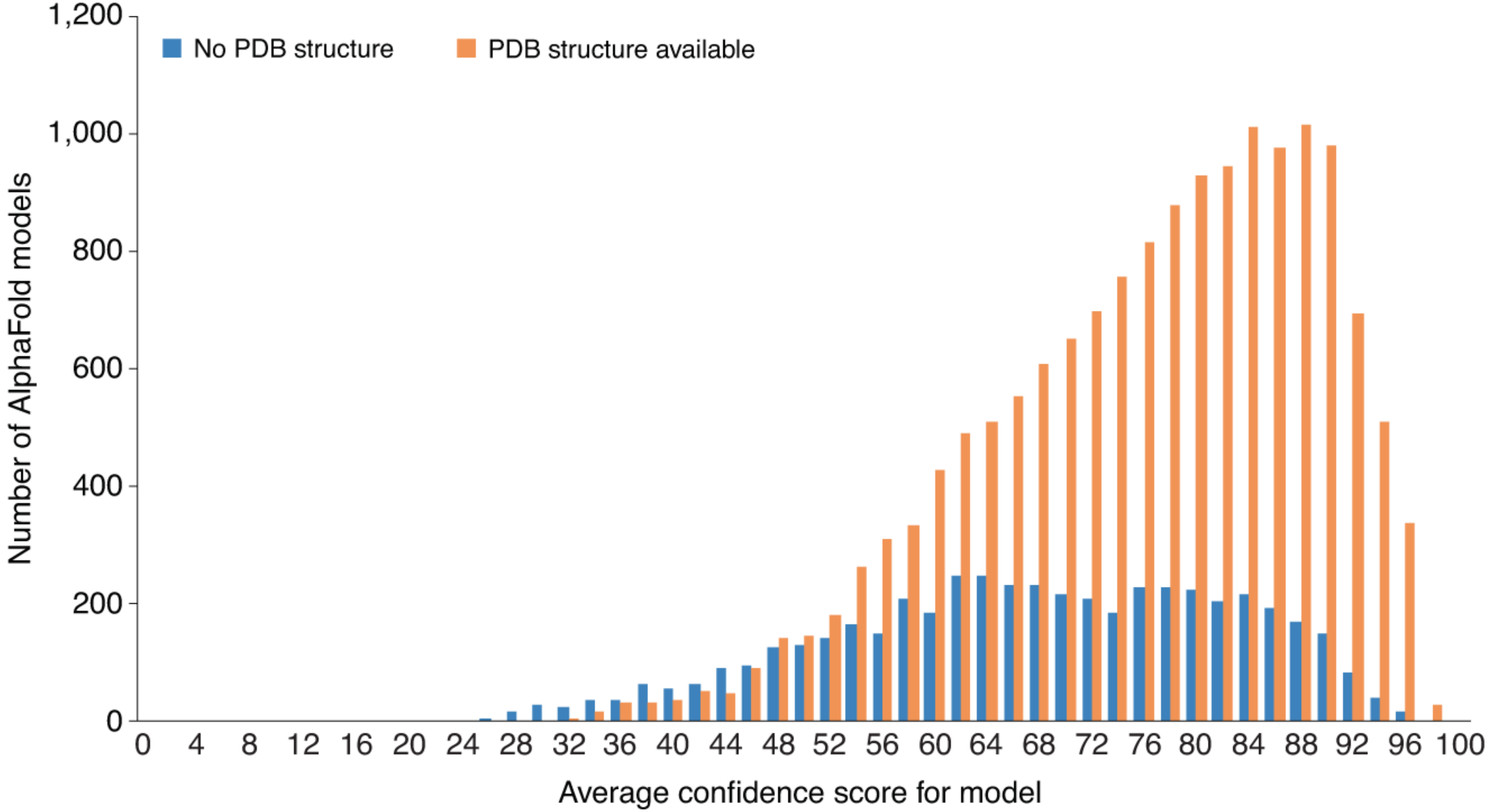
Protein structure predictions to atomic accuracy with AlphaFold



RoseTTAFold accurately predicts structures of de-novo-designed proteins from their amino acid sequences.

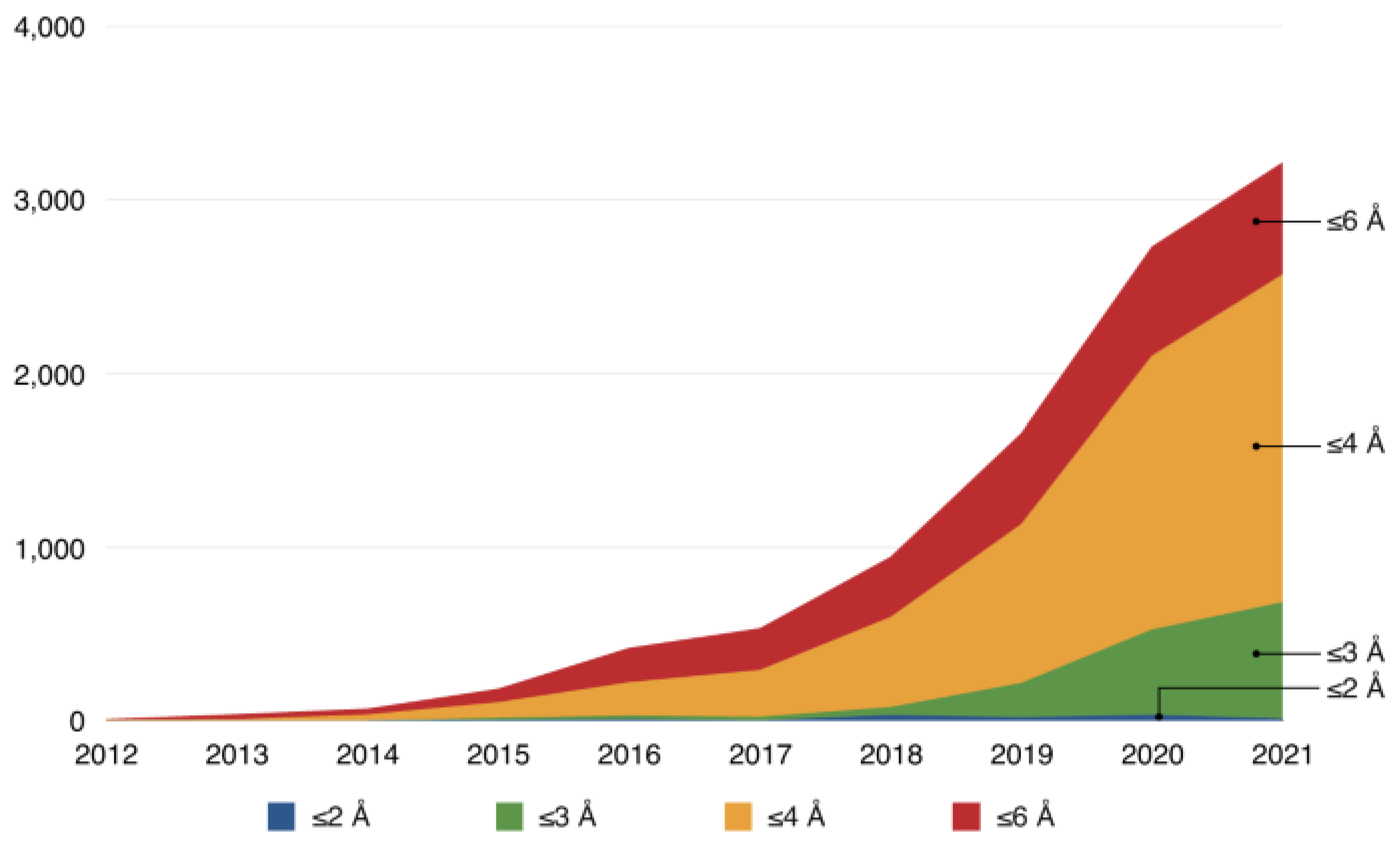


Distribution of average confidence scores for AlphaFold2 models of human proteins with and without homologs available in the PDB.



>200 M protein structure prediction

The number of entries at resolutions better than 6 Å released by the Electron Microscopy Data Bank per year from 2012 to 2021

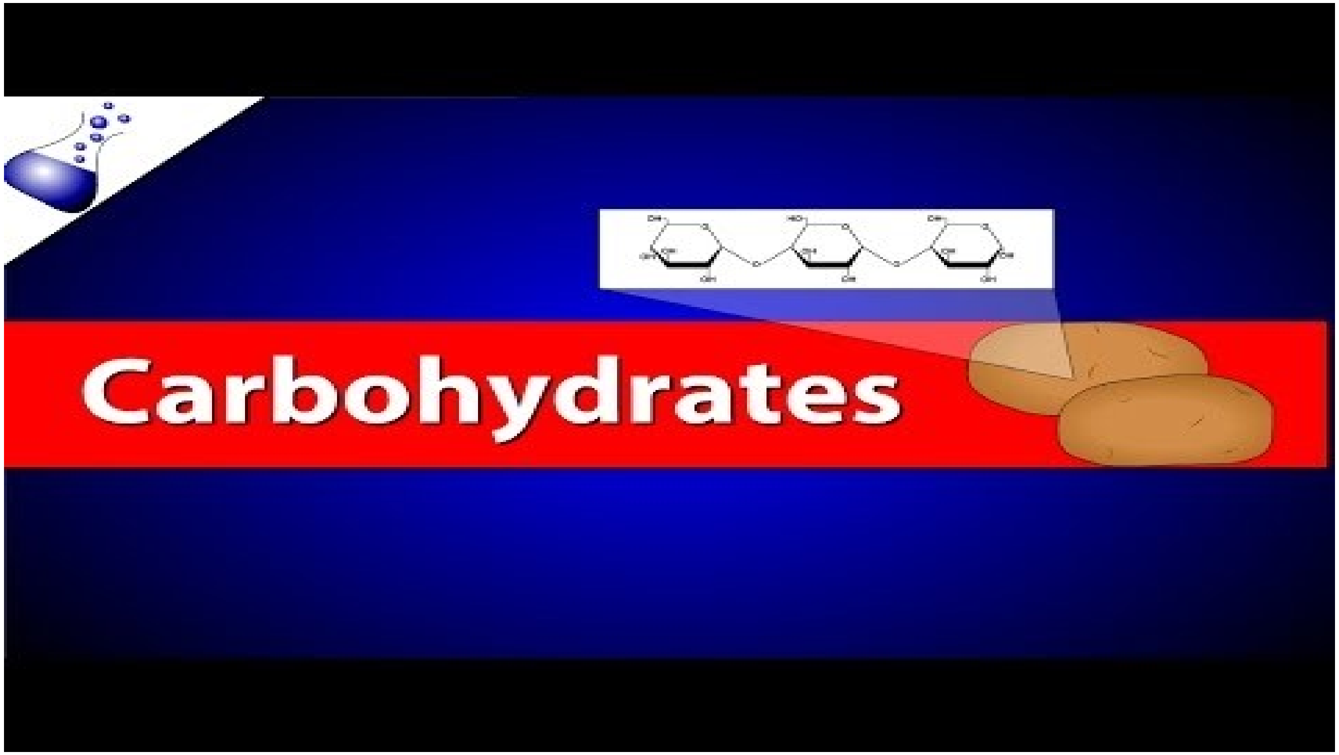


An Introduction to Carbohydrates

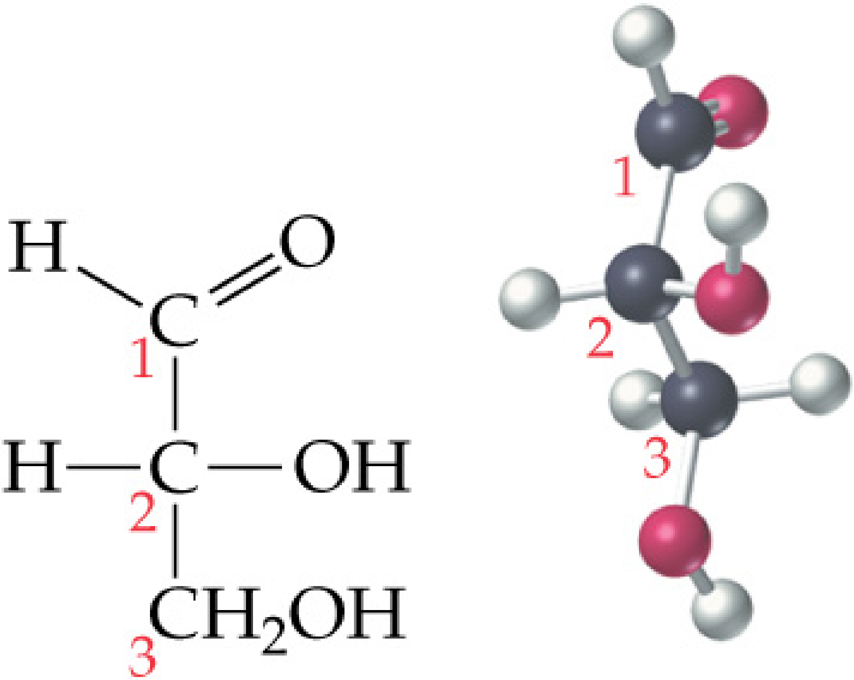
- *Carbohydrates* are a large class of naturally occurring polyhydroxy aldehydes and ketones.
- Monosaccharides also known as simple sugars, are the simplest carbohydrates containing 3-7 carbon atoms.
- sugar containing an aldehydes is known as an aldose.
- sugar containing a ketones is known as a ketose.

- The number of carbon atoms in an aldose or ketose may be specified as by tri, tetr, pent, hex, or hept. For example, glucose is aldohexose and fructose is ketohexose.
- Monosaccharides react with each other to form disaccharides and polysaccharides.
- Monosaccharides are chiral molecules and exist mainly in cyclic forms rather than the straight chain.

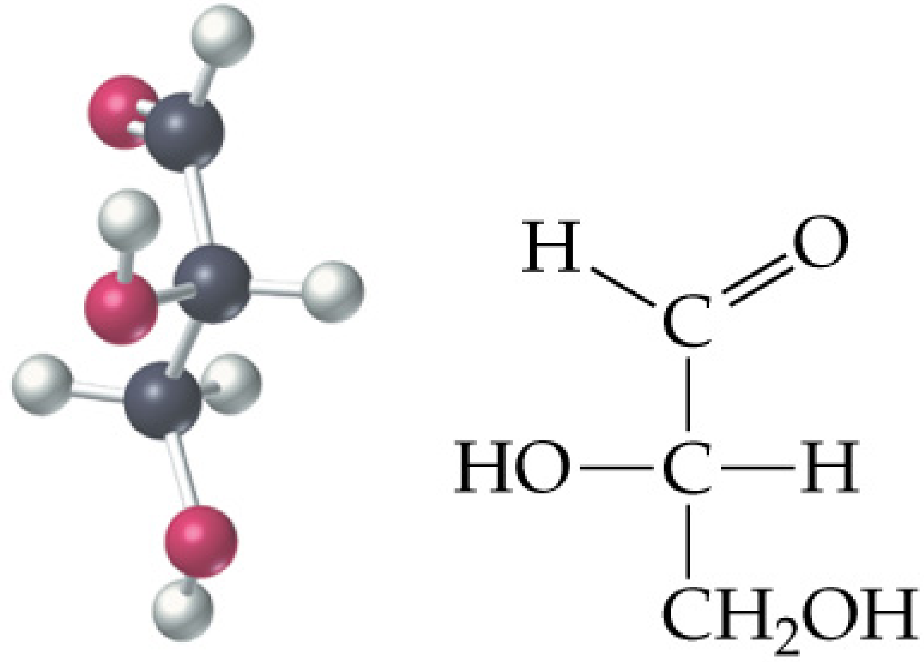
Carbohydrate



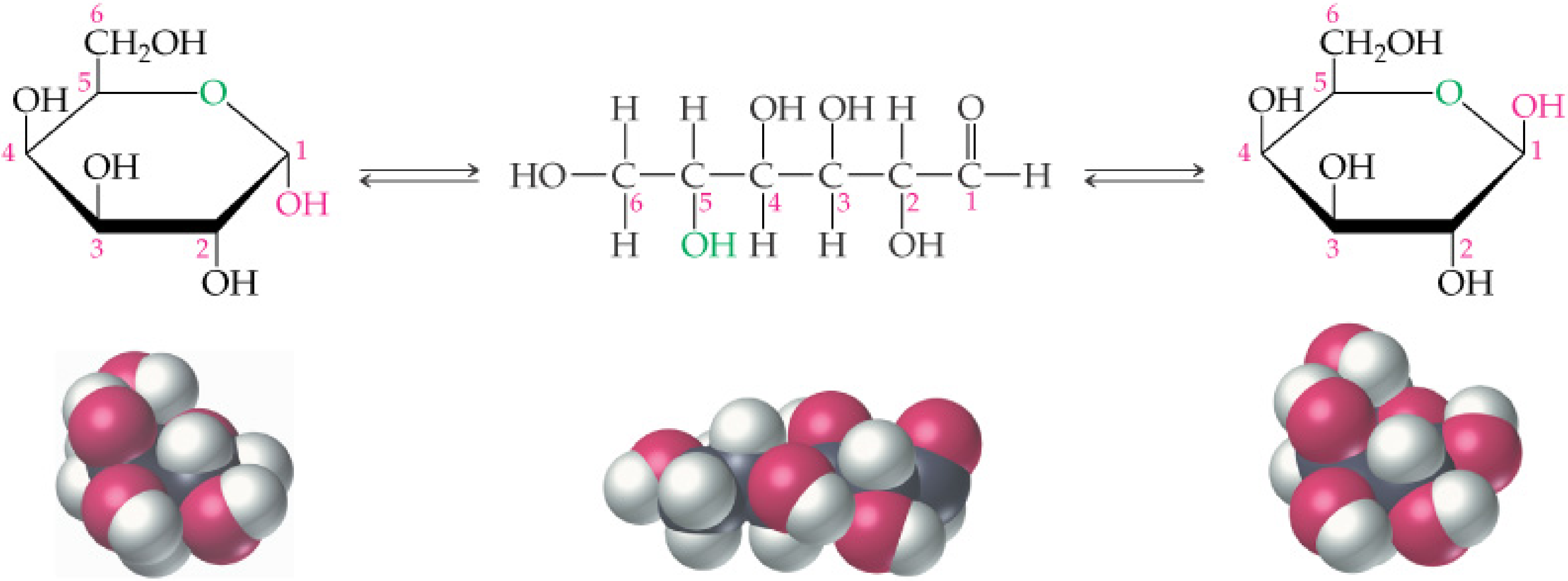
Check
https://www.youtube.com/watch?v=LeOUIXbFyqk



D-Glyceraldehyde
Right-handed



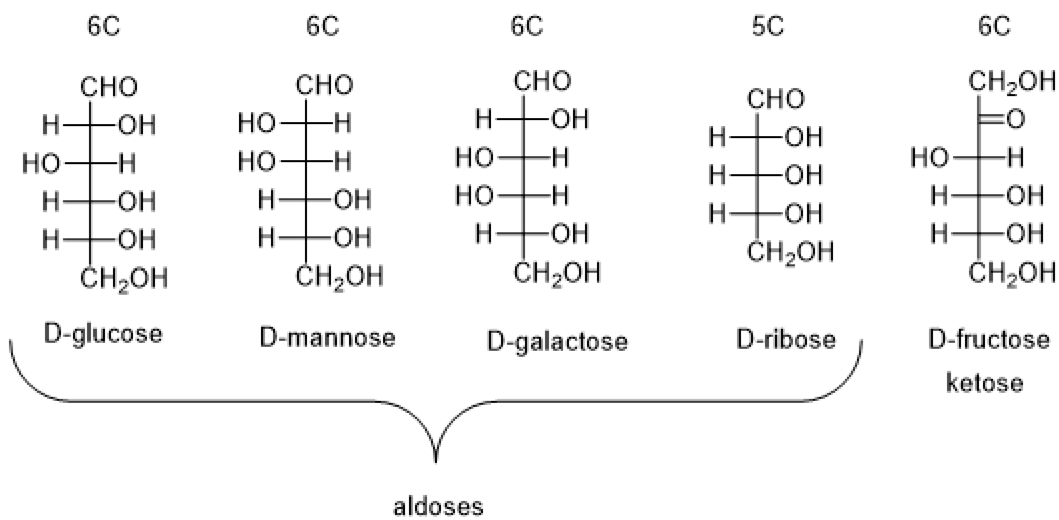
L-Glyceraldehyde
Left-handed

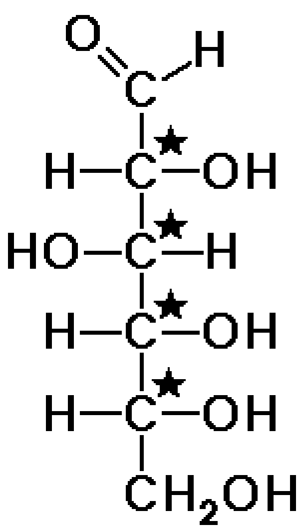


α-D-Galactose

Open-chain galactose

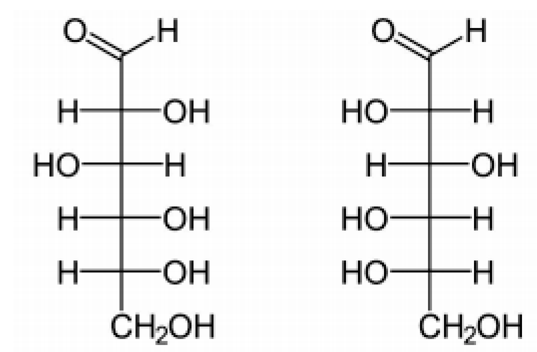
β-D-Galactose





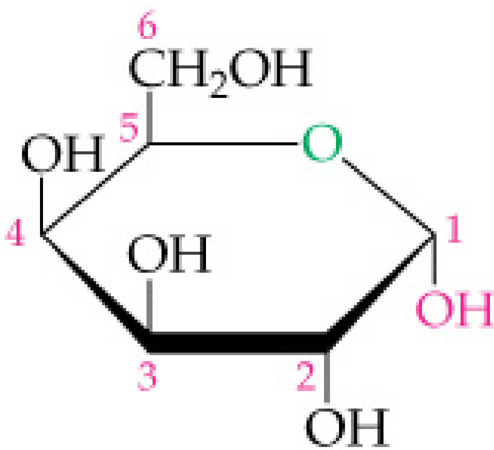
D-glucose

2,3,4,5,6-pentahydroxyhexanal
(with 4 chiral centers)

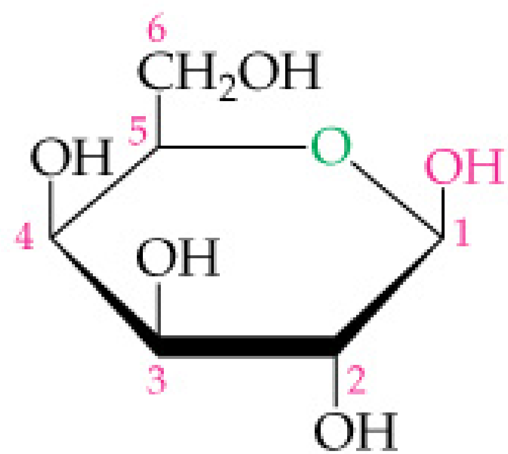


D-Glucose L-Glucose

- *Anomers:* Cyclic sugars that differs only in positions of substituents at the hemiacetal carbon; the α-form has the –OH group on the opposite side from the $–CH_2OH$; the β-form the –OH group on the same side as the $–CH_2OH$ group.



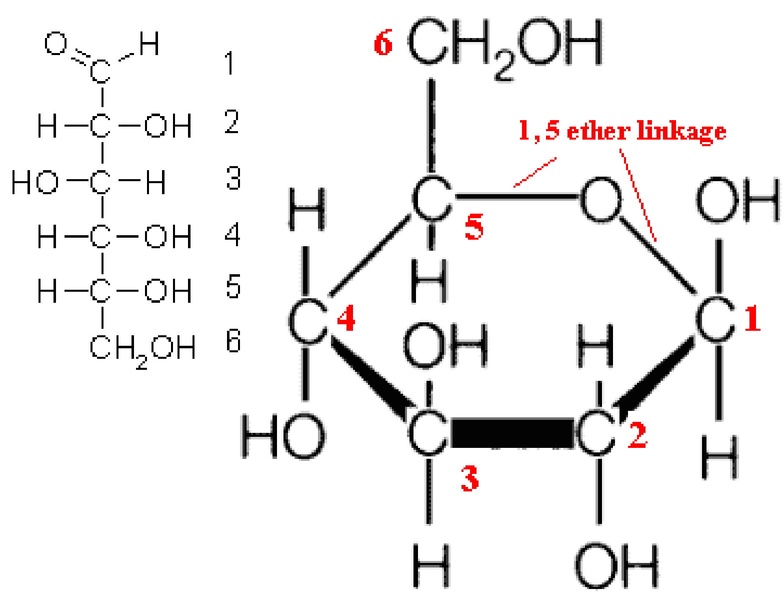
α-D-Galactose



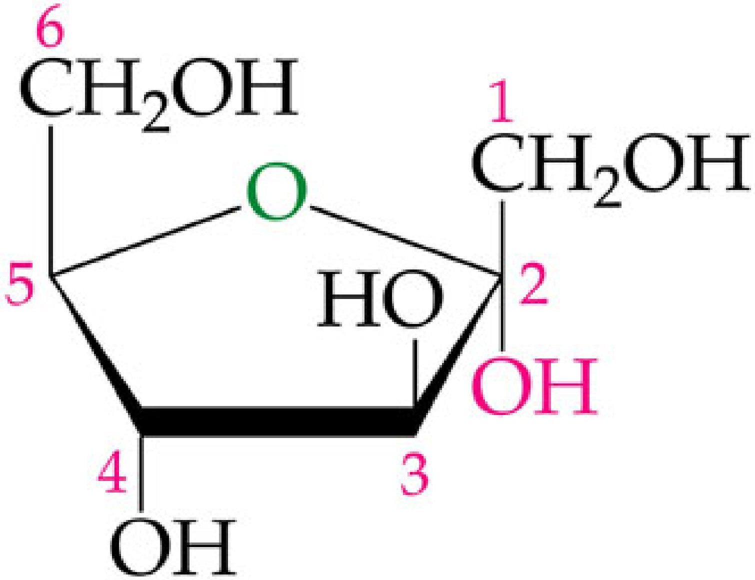
β-D-Galactose

Some Important Monosaccharides

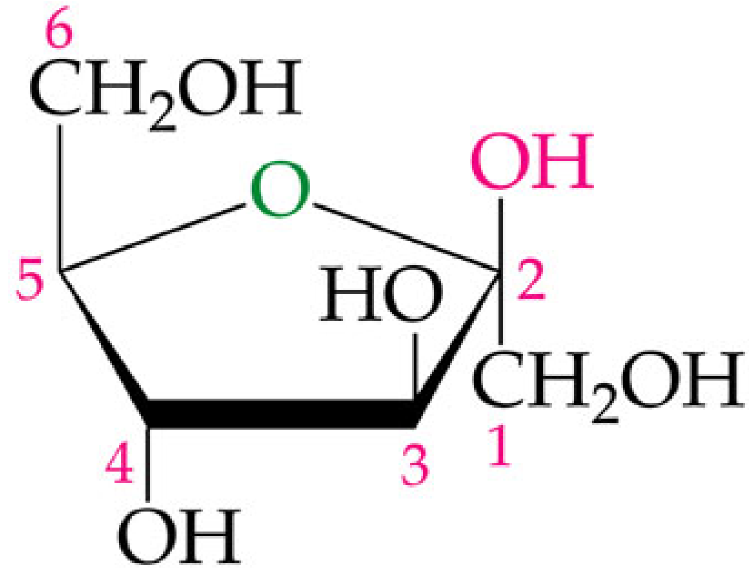
Monosaccharides are generally high-melting, white, crystalline solids that are soluble in water and insoluble in nonpolar solvents. Most monosaccharides are sweet tasting, digestible, and nontoxic.



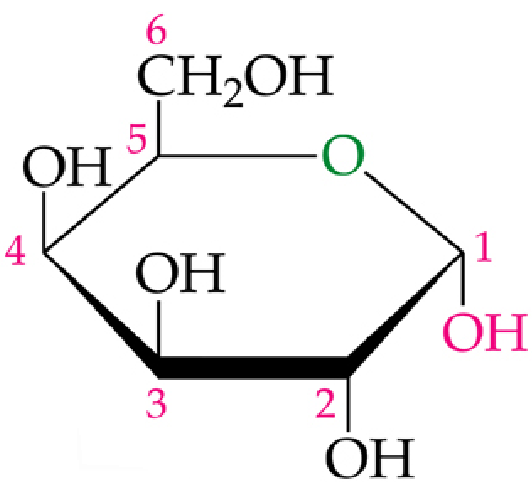
D-glucose



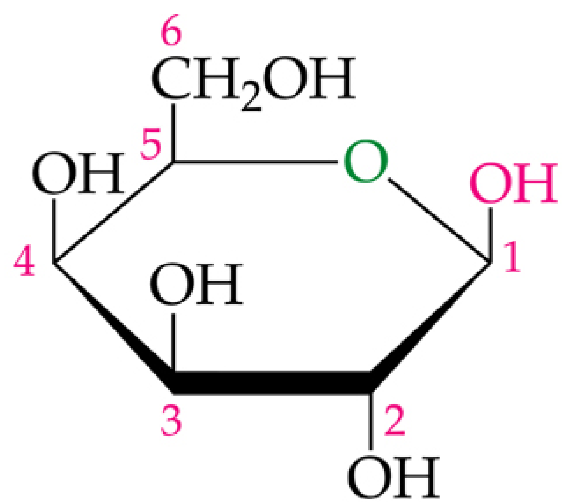
α-D-Fructose



β-D-Fructose

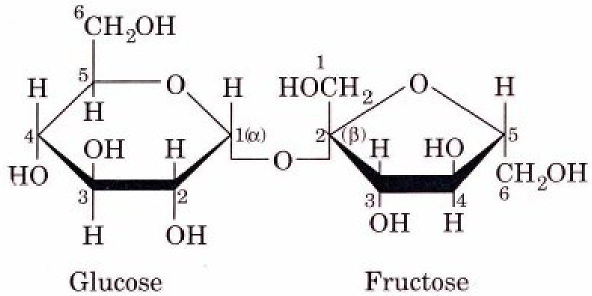


α-D-Galactose

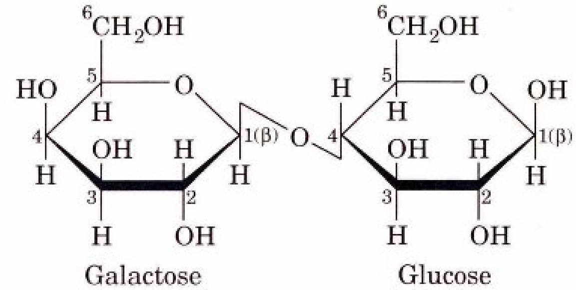


β-D-Galactose

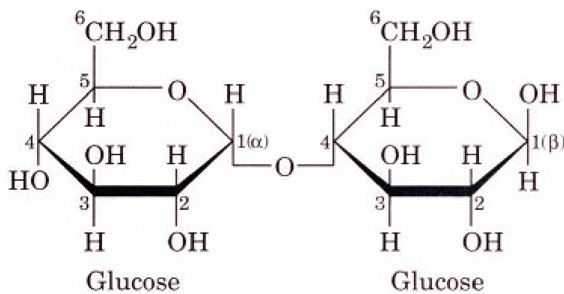
Some Common Disaccharides



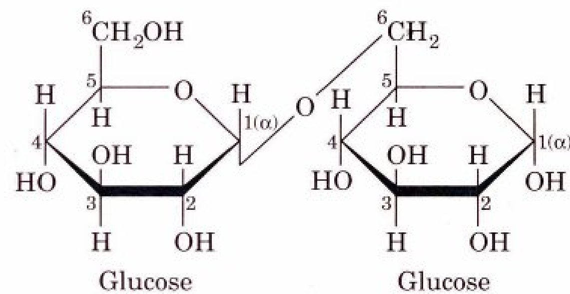
Sucrose



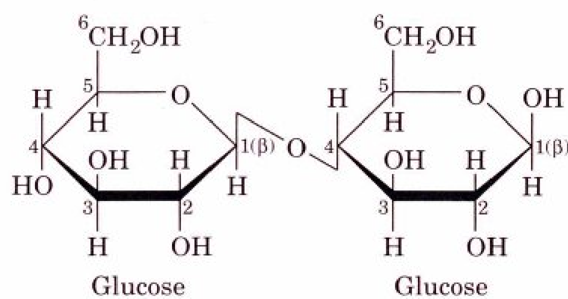
Lactose



Maltose

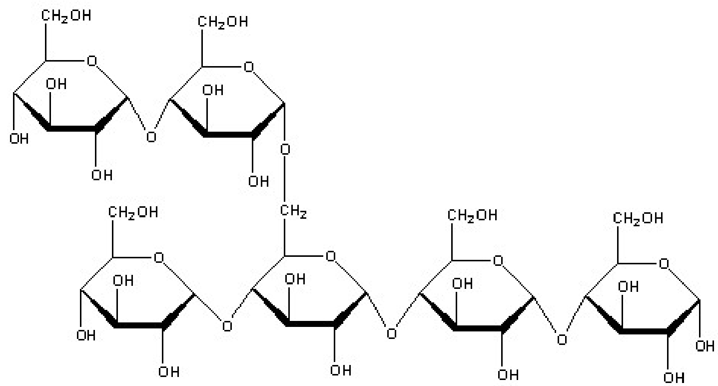


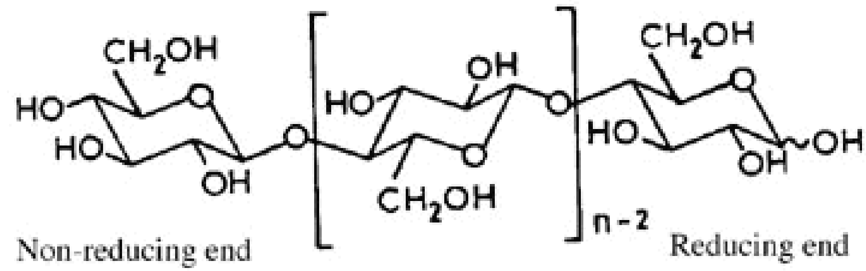
Isomaltose



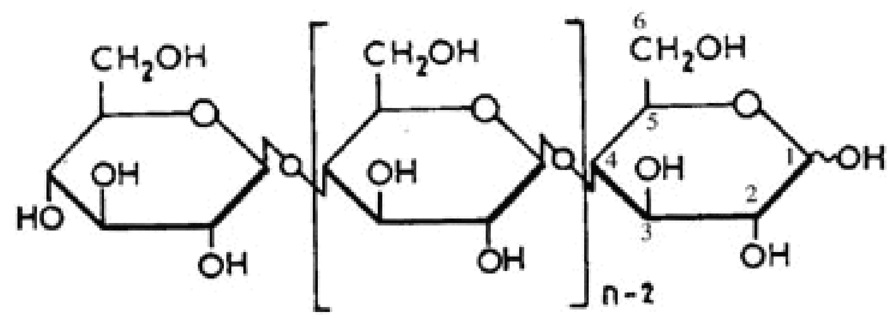
Cellobiose

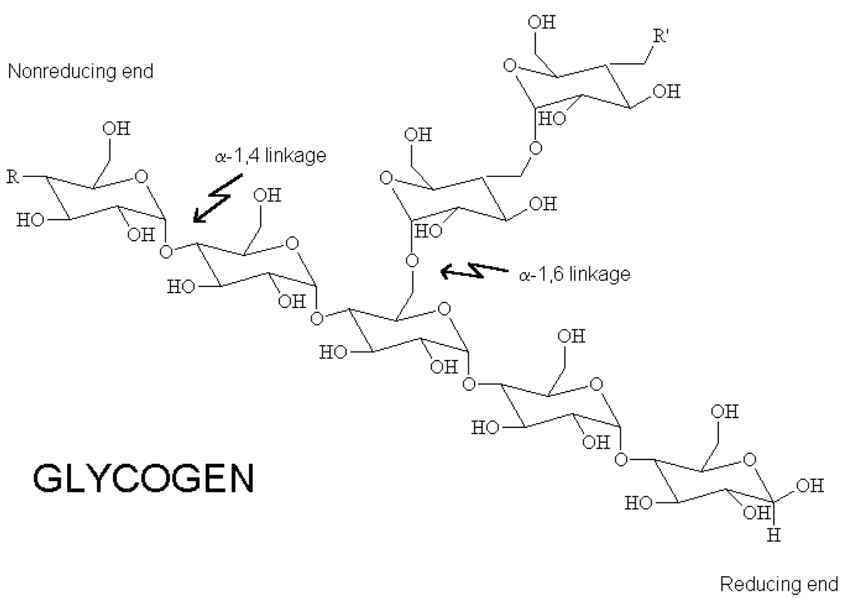
Polysaccharides





Sometimes shown as

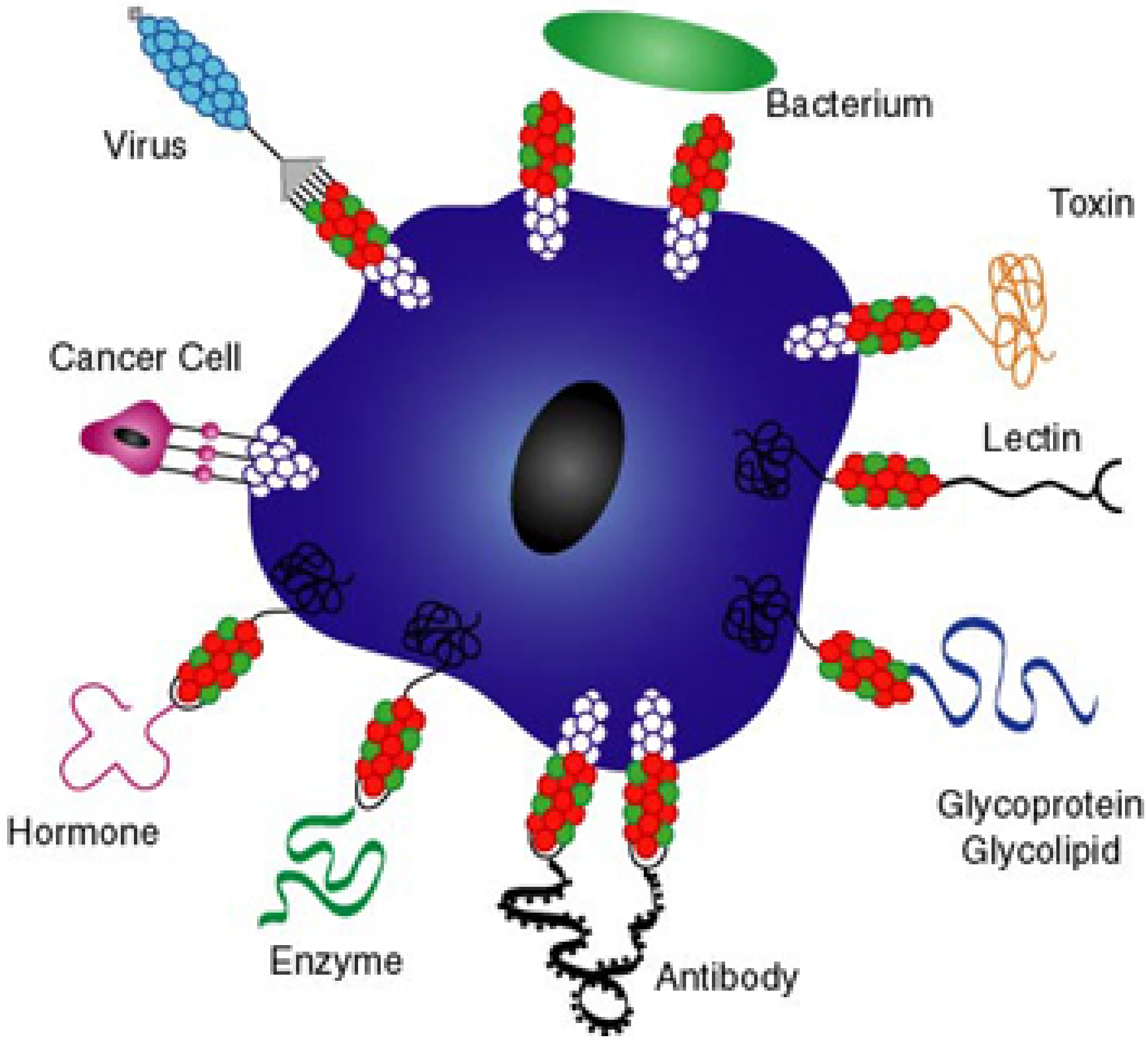




GLYCOGEN

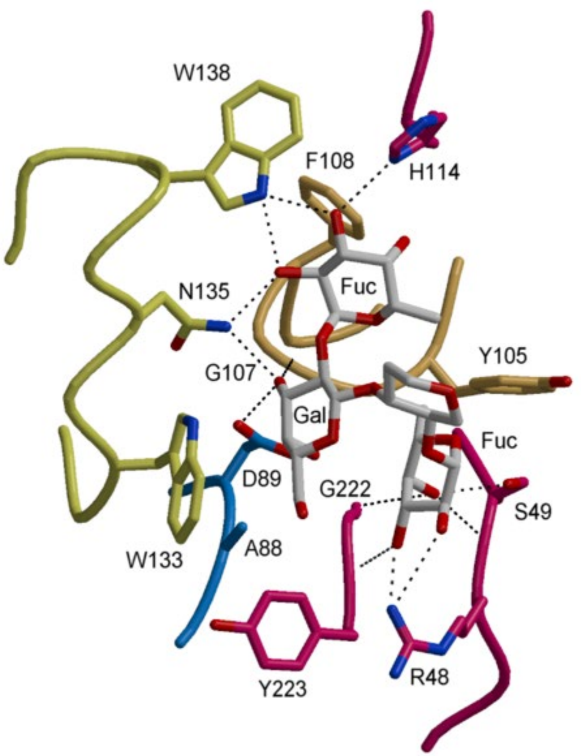
Cellulose

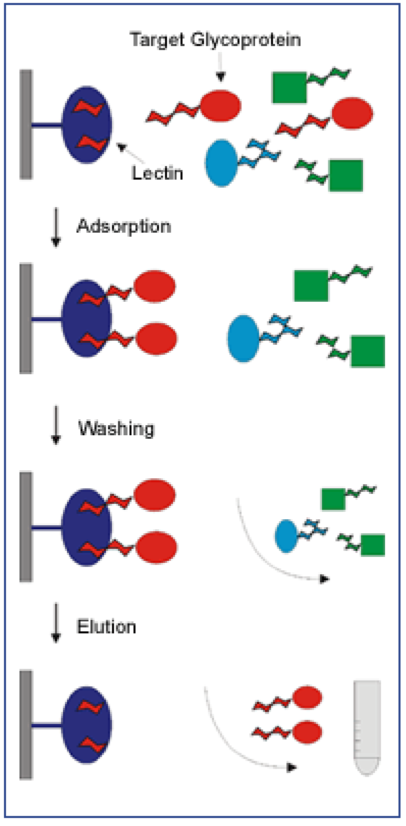
Cell-Surface Carbohydrates Involved in Molecular Recognition



Lectin

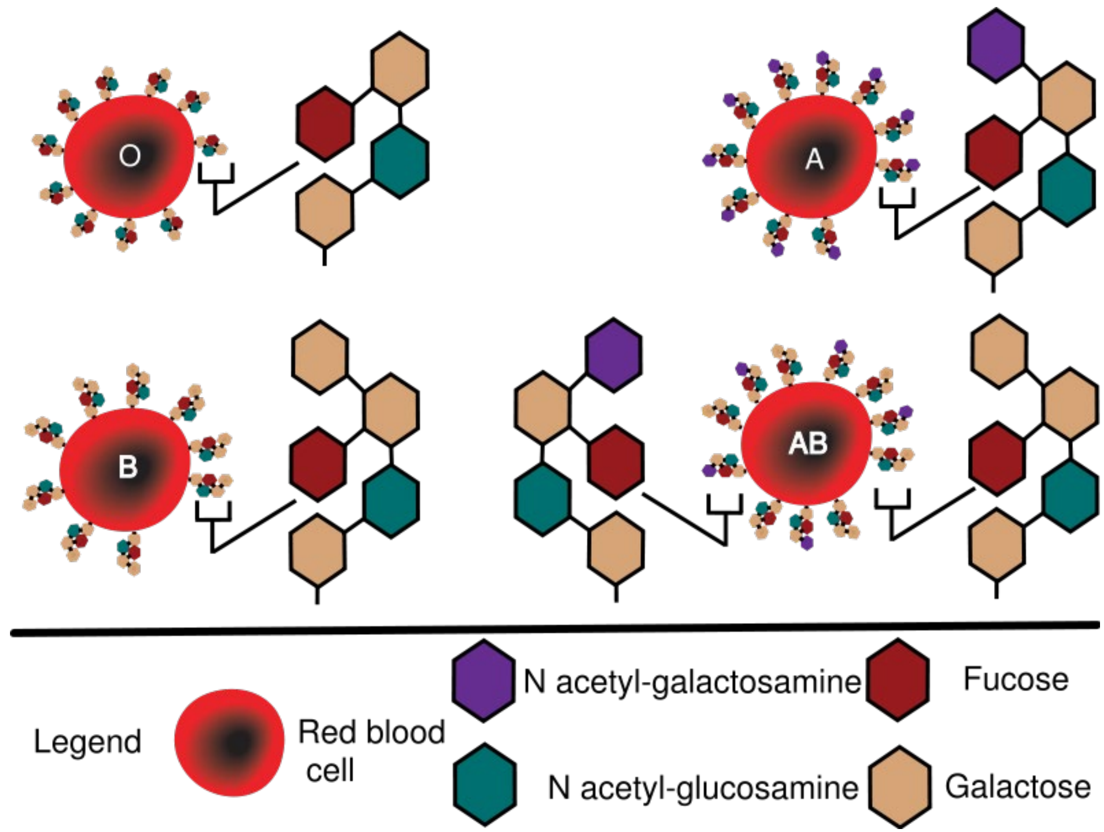
Lectins are sugar-binding proteins which are highly specific for their sugar moieties. They typically play a role in biological recognition phenomena involving cells and proteins. For example, some bacteria use lectins to attach themselves to the cells of the host organism during infection.





Blood Type

	Group A	Group B	Group AB	Group O
Red blood cell type	A	B	AB	O
Antibodies present	Anti-B	Anti-A	None	Anti-A and Anti-B
Antigens present	A antigen	B antigen	A and B antigens	No antigens



The Nobel Prize in Chemistry 2022

The Royal Swedish Academy of Sciences has decided to award the Nobel Prize in Chemistry 2022 to

Carolyn R. Bertozzi
Stanford University, CA, USA
Howard Hughes Medical Institute, USA

Morten Meldal
University of Copenhagen, Denmark

K. Barry Sharpless
Scripps Research, La Jolla, CA, USA

"for the development of click chemistry and bioorthogonal chemistry"

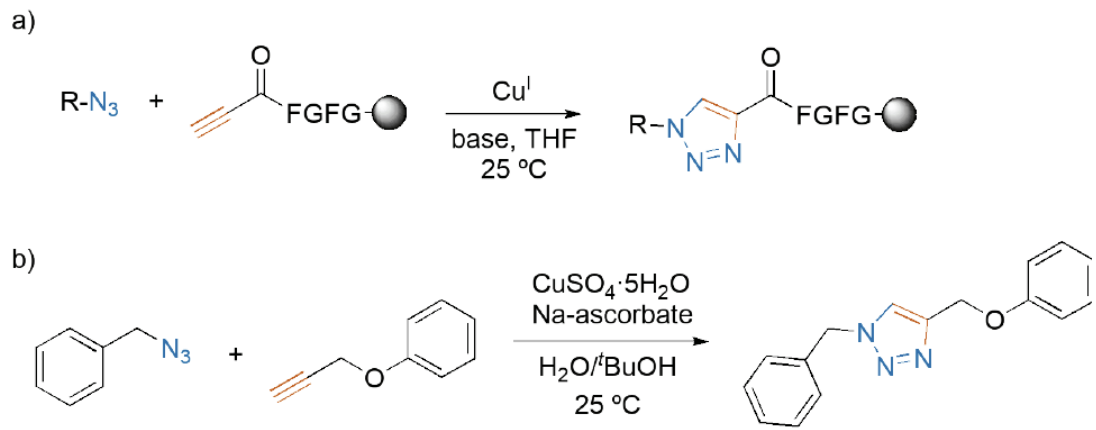


Figure 6: Copper-catalysed reactions from a) **Meldal** and coworkers,[15,16] and b) **Sharpless** and coworkers;[17] F: phenylalanine, G: glycine, filled circle: solid support, THF: tetrahydrofuran, ^{t}BuOH: *tert*-butanol.

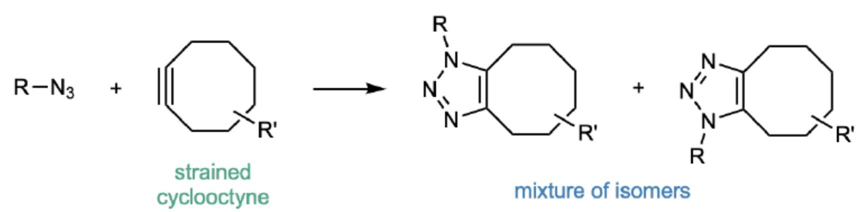
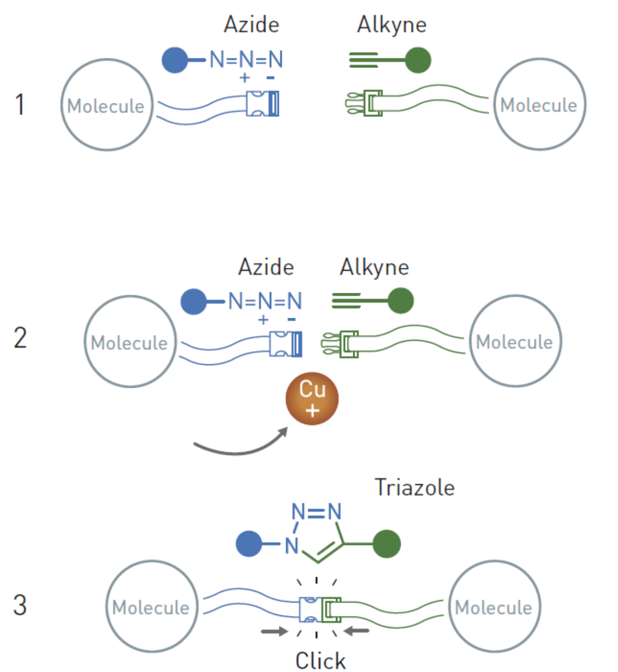


Figure 7: Strain-promoted azide-alkyne cycloaddition (SPAAC).

Click Chemistry

The click reaction that changed chemistry

Azides and alkynes react very efficiently when copper ions are added. This reaction is now used globally to link molecules together in a simple manner.



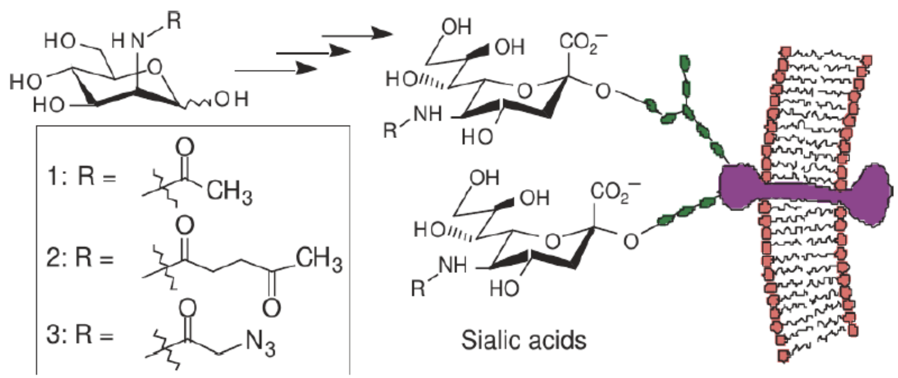
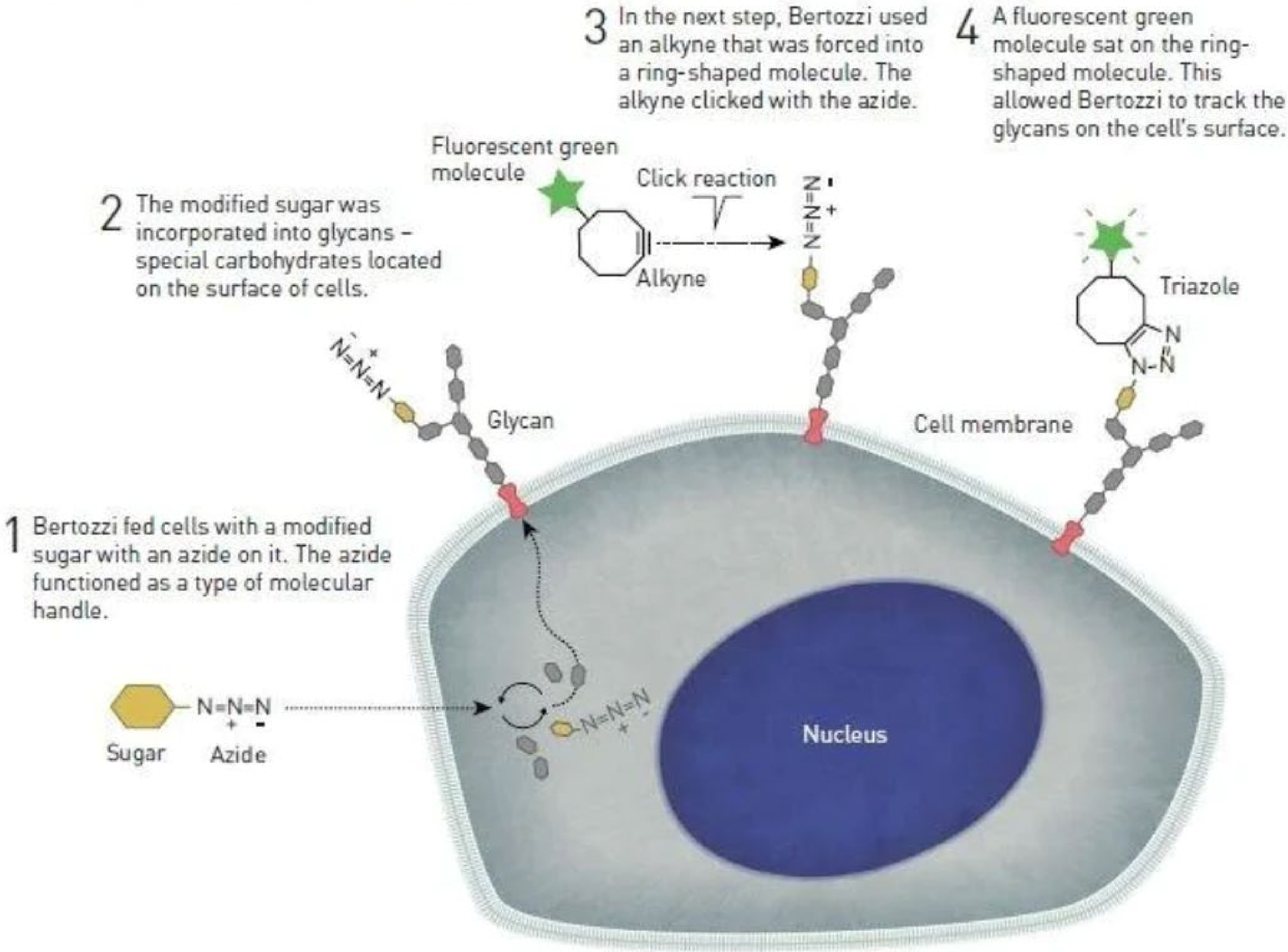


Figure 8: Cell labelling using metabolic engineering. *N*-levulinoyl mannosamine or *N*-azidoacetylmannosamine is fed to cells, converted into functionalized sialic acids, and expressed in glycans at the cell surface.[24]

Bioorthogonal chemistry illuminates the cell



Glycobiology

Glycobiology is the scientific study of glycomes, which are the entire complement of sugars, whether free or present in more complex molecules, of an organism. This field encompasses the structure, biochemistry, and biology of carbohydrates (sugars and their derivatives) and glycoconjugates (molecules that contain sugar residues attached to another structure, such as proteins or lipids).

Carbohydrates are crucial components of all living organisms and are involved in a variety of biological processes, including cell-cell recognition, cell adhesion, immune response, and inflammation. They play key roles in the structure and function of many proteins and lipids, which are modified by the addition of sugar molecules in a process known as glycosylation.

Glycobiology integrates various disciplines, including biochemistry, molecular biology, cell biology, and biotechnology, to understand the roles of carbohydrates in biology and to utilize this knowledge in applications ranging from biomedicine to bioenergy. This research has significant implications for understanding diseases, developing new vaccines and therapeutics, and advancing biotechnological applications.

Carbohydrates are essential biomolecules that play numerous vital roles in biological systems, impacting both the structure and function of organisms. Here are some key aspects of their importance:
Energy Source: Carbohydrates are a primary energy source for most organisms. Glucose, a simple sugar, is a crucial energy substrate in cells and is central to cellular respiration and ATP production, which fuels various biological processes.
Energy Storage: Carbohydrates also serve as energy storage molecules. Plants store energy in the form of starch, while animals store energy as glycogen in the liver and muscles, which can be rapidly mobilized to meet energy demands.
Structural Components: Certain carbohydrates are integral to the structural integrity of cells and organisms. For example, cellulose, a polysaccharide found in plant cell walls, provides structural support to plants. In animals, chitin, a component of the exoskeletons of insects and other arthropods, serves a similar structural role.
Cell Recognition and Signaling: Carbohydrates on the surfaces of cells play key roles in cell-cell recognition and signaling. They are involved in various biological processes, including immune responses, where they help in the identification of foreign substances and pathogens.
Biological Lubrication: Mucins, which are glycoproteins, rely on their carbohydrate components to maintain viscosity and lubrication in biological tissues, crucial for the proper functioning of respiratory, digestive, and reproductive systems.
Glycosylation of Proteins and Lipids: Many proteins and lipids undergo glycosylation, where carbohydrates are covalently attached. This modification can affect the molecules' stability, activity, and localization, impacting various physiological processes.
Dietary Fiber: Some carbohydrates, particularly those that are indigestible by humans like dietary fiber, play important roles in maintaining gut health. They support bowel regularity and can influence the composition of gut microbiota, which is crucial for overall health.
Understanding these roles of carbohydrates is fundamental not only in biochemistry and cell biology but also in fields like nutrition, medicine, and biotechnology, showcasing their broad impact on life and health.

Bioorthogonal chemistry refers to chemical reactions that can occur inside living systems without interfering with native biochemical processes. This concept is crucial in the fields of chemical biology and drug development, as it allows scientists to introduce and track synthetic molecules within biological systems without affecting their normal functions.

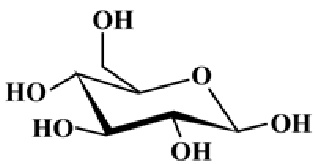
In practical terms, bioorthogonal reactions are highly selective and fast under physiological conditions. They do not cross-react with biological molecules, thereby enabling researchers to label, visualize, or manipulate biomolecules in real-time, in living organisms. Some common bioorthogonal reactions include the copper-catalyzed azide-alkyne cycloaddition (though copper-free versions are preferred in biological contexts to avoid toxicity) and the strain-promoted alkyne-azide cycloaddition.

Overall, bioorthogonal chemistry offers a powerful set of tools for studying biological processes at the molecular level, with applications ranging from imaging specific proteins in cells to targeted drug delivery.

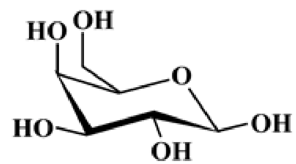
Glycan and Glycomic

https://www.youtube.com/watch?v=NqEgrAYN2Bc

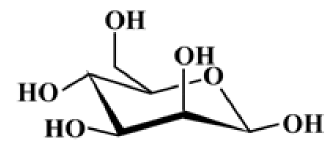
Glycan



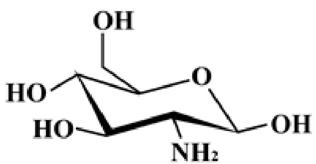
Glucose
(dextrose)



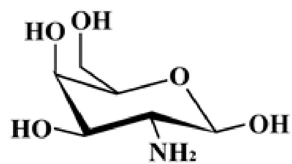
Galactose



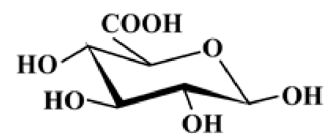
Mannose



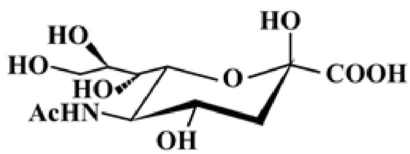
Glucosamine
(to N-acetylglucosamine when acetyl group is attached)



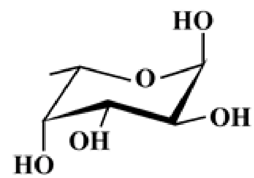
Galactosamine
(to N-acetylgalactosamine when acetyl group is attached)



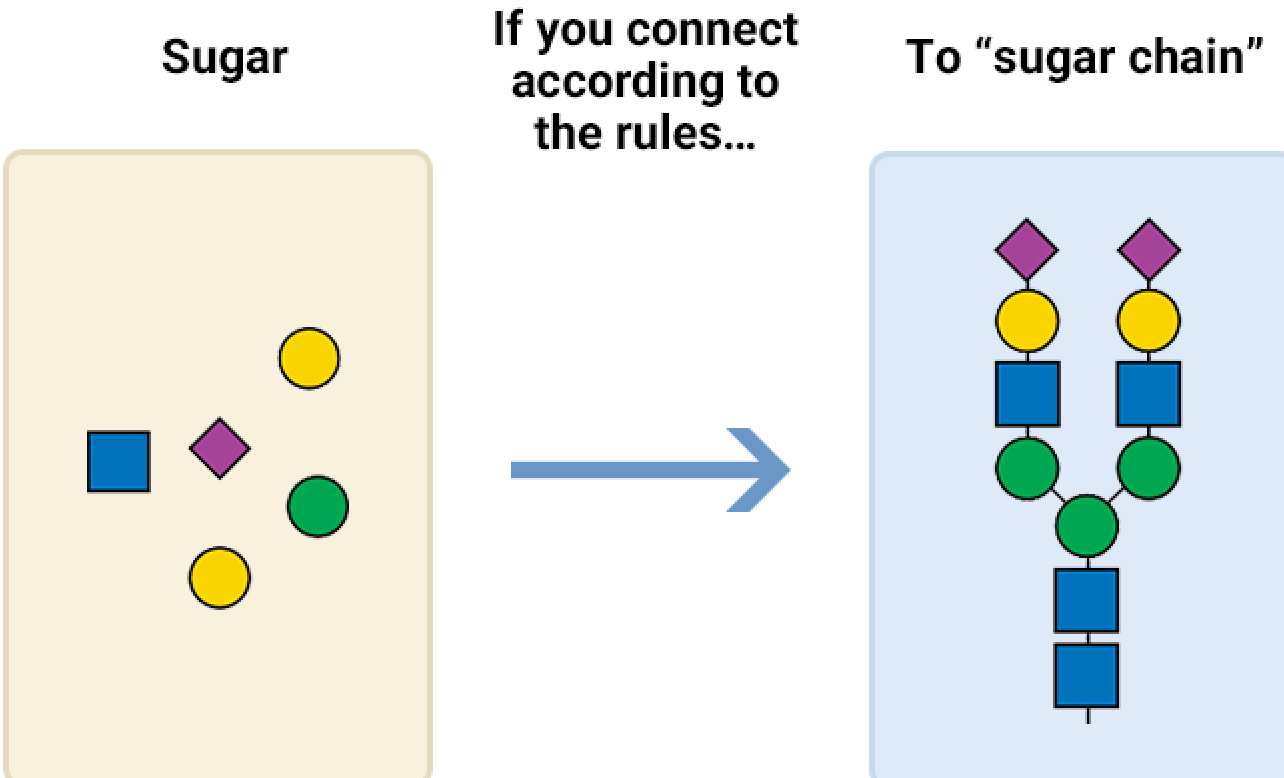
Glucuronic acid

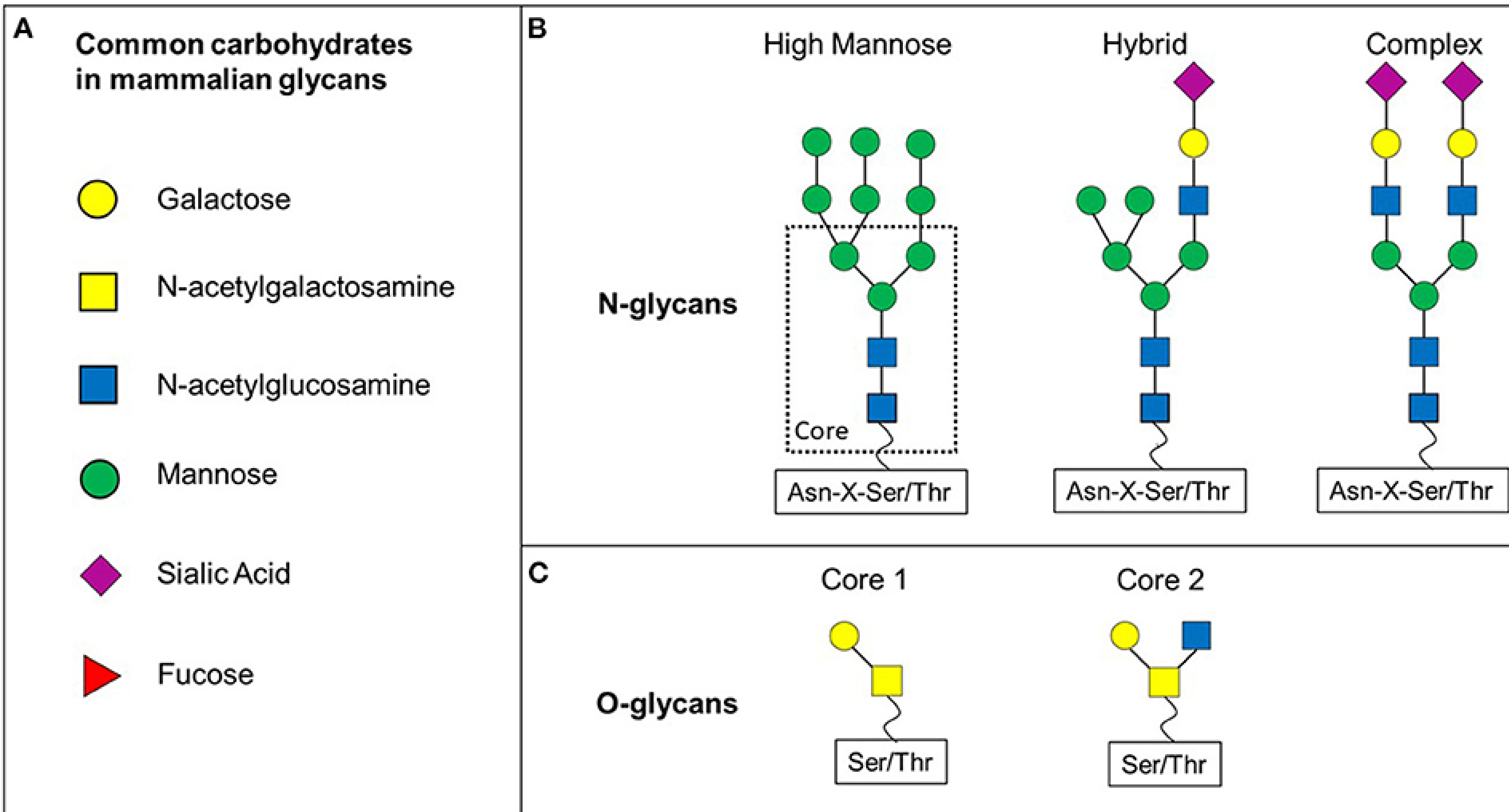


Sialic acid

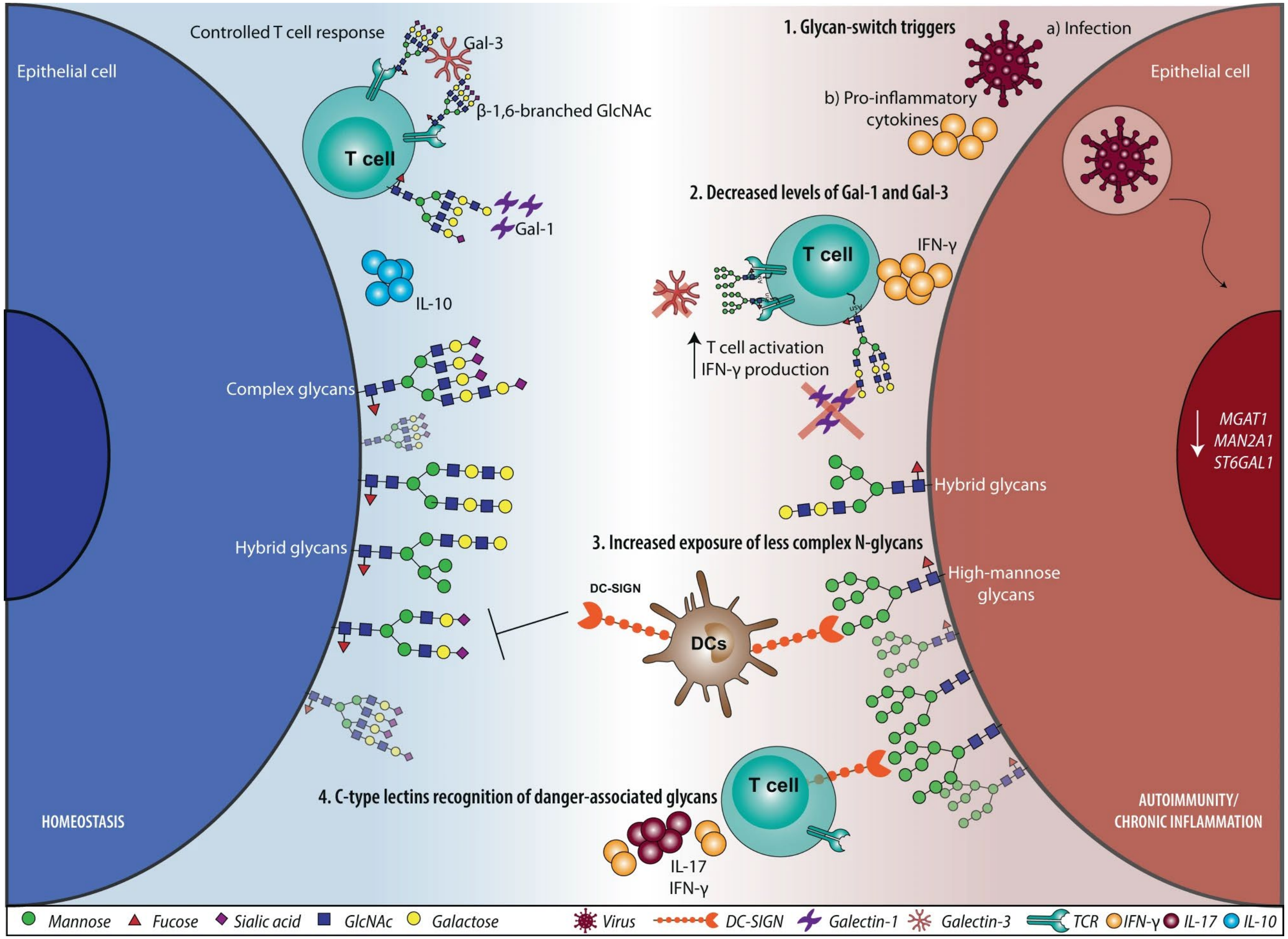


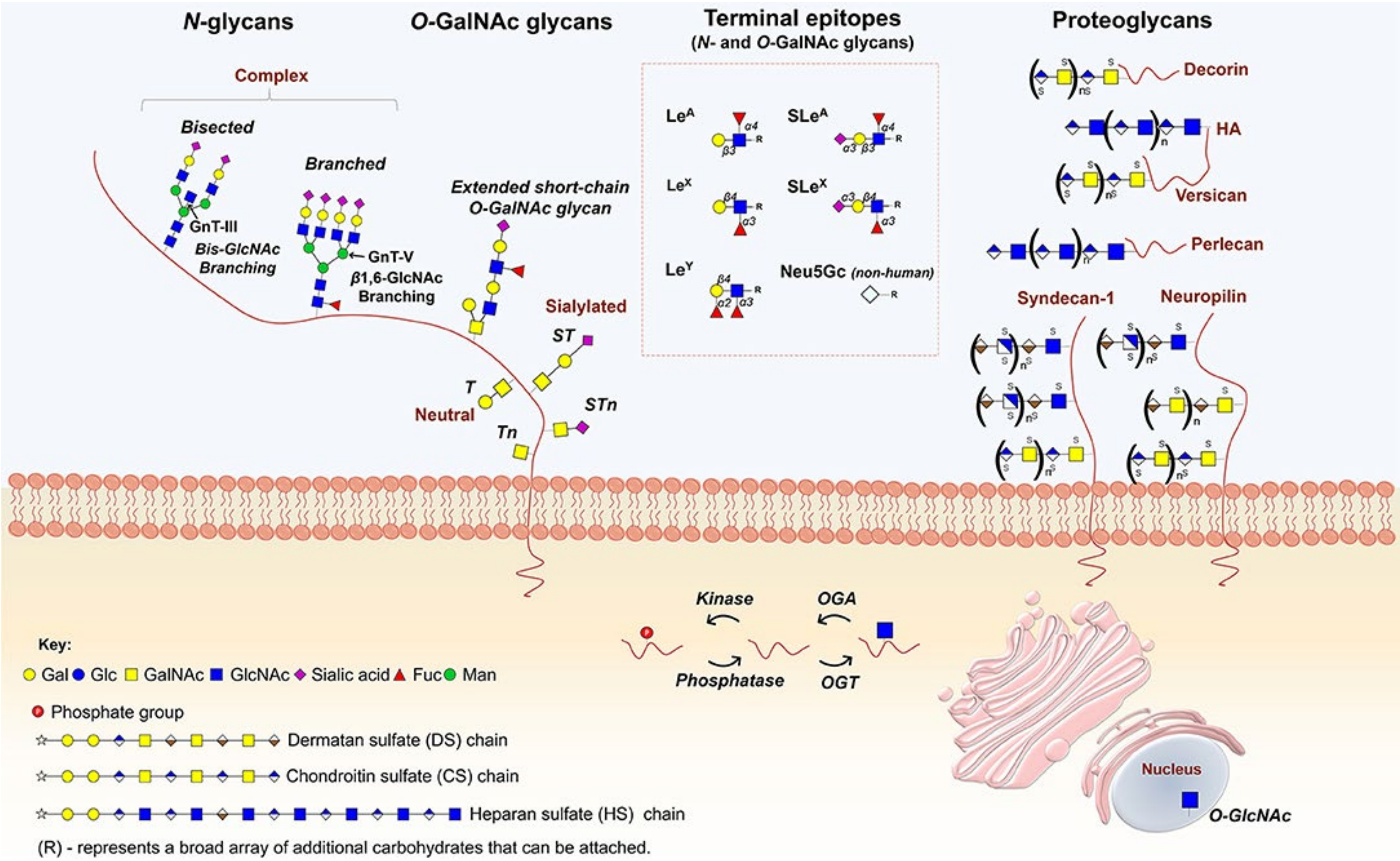
Fucose


Sugar
If you connect according to the rules…
To “sugar chain”

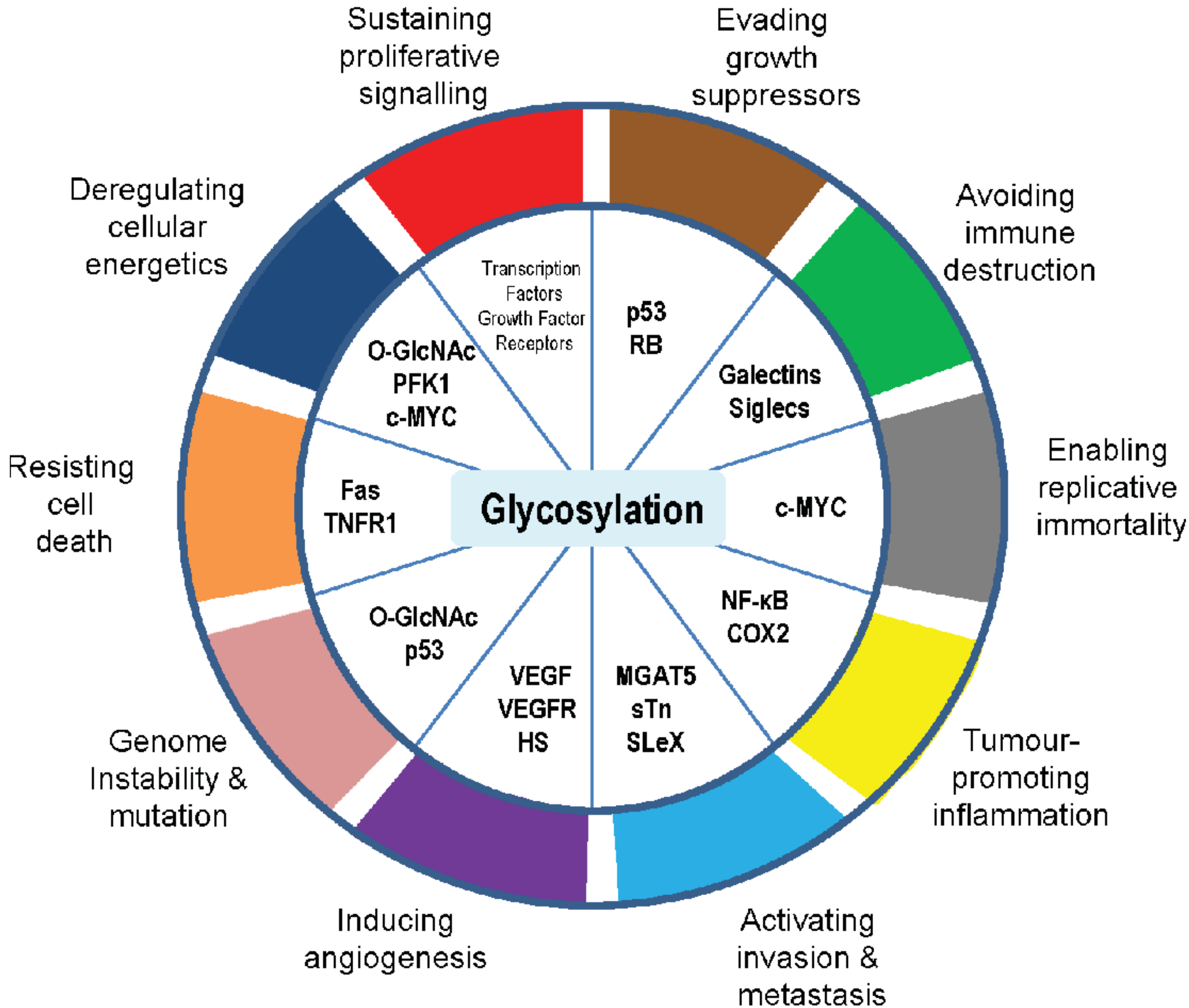

A
Common carbohydrates in mammalian glycans
Galactose
N-acetylgalactosamine
N-acetylglucosamine
Mannose
Sialic Acid
Fucose
B
High Mannose
Hybrid
Complex
N-glycans
Core
Asn-X-Ser/Thr
Asn-X-Ser/Thr
Asn-X-Ser/Thr
C
Core 1
Core 2
O-glycans
Ser/Thr
Ser/Thr

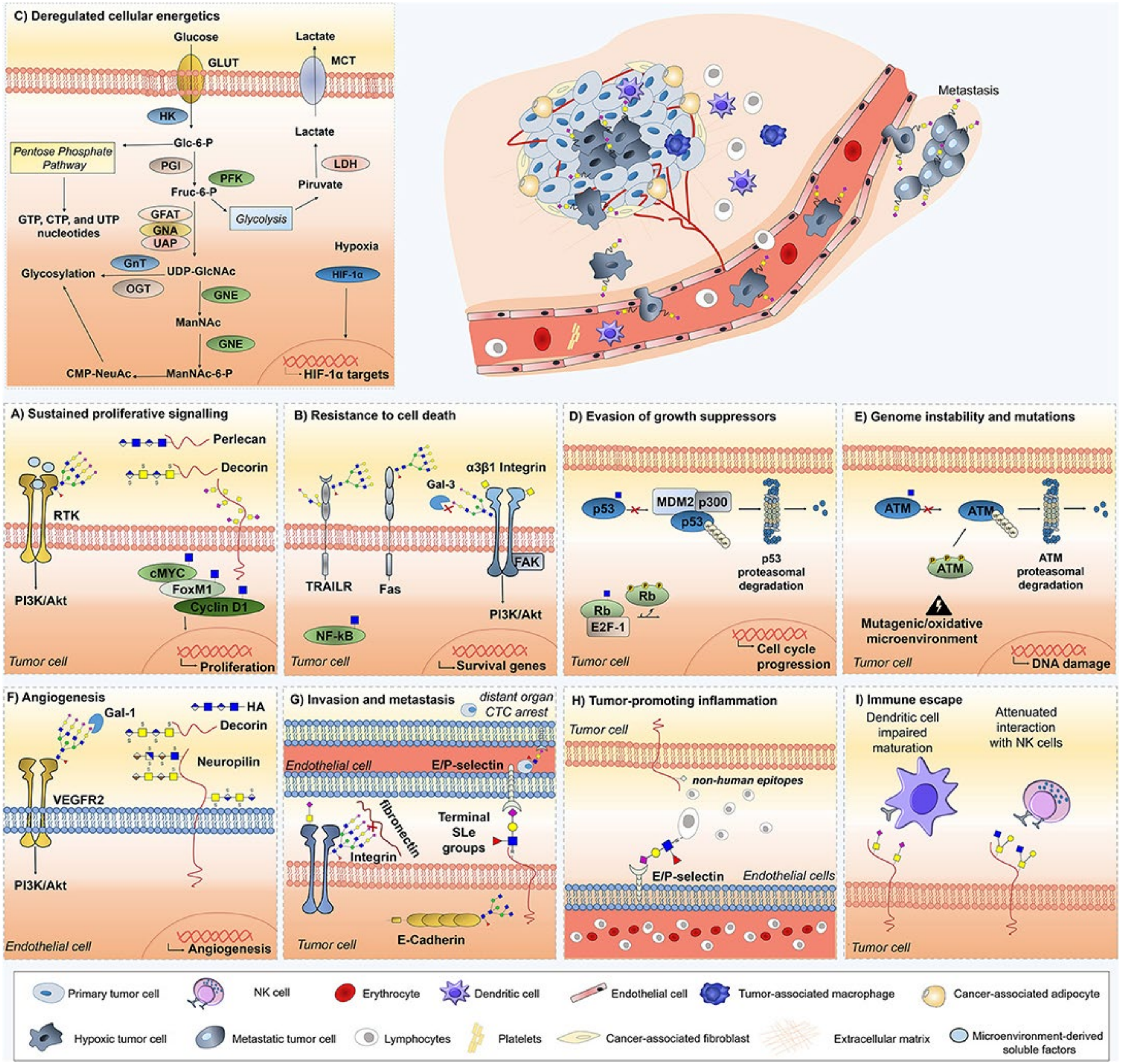
Glycan Binding in IImmune Cells





Main classes of glycans modulating cancer hallmarks**. N-glycans**, whose biosynthesis starts in the endoplasmic reticulum (ER) with the addition of an oligosaccharide chain to an asparagine (Asn) residue, experience further structural maturation in the golgi apparatus (GA) to yield complex bisected and branched structures. **O-GalNAc** glycans, initiated in the GA by the attachment of a GalNAc to the hydroxyl groups of serine (Ser) or threonine (Thr) residues, forming the simplest **O-glycan** Tn antigen (GalNAcα-Ser/Thr), may be further elongated into different core structures that serve as scaffolds for more complex O-GalNAc glycans. Both O- and N-glycan chains are generally branched and/or elongated and may present sialic acids, Lewis blood group related antigens and/or their sialylated counterparts as terminal structures. The figure highlights the structures of some of the most relevant glycans and glycoconjugates driving cancer hallmarks.

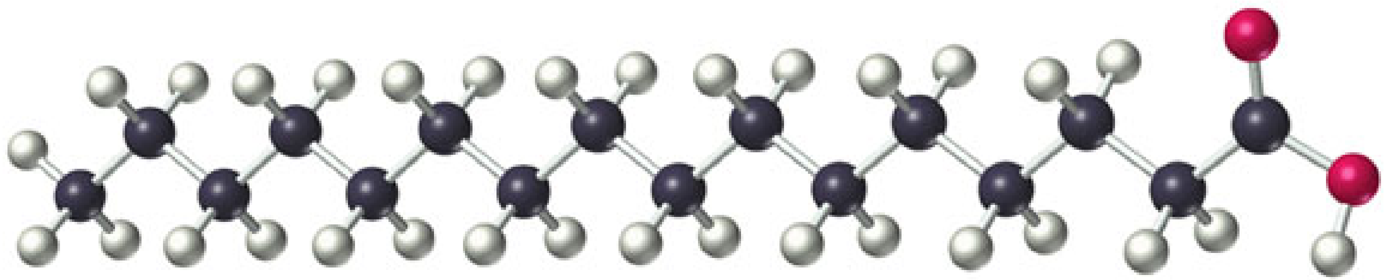

Sustaining proliferative signalling
Evading growth suppressors
Avoiding immune destruction
Enabling replicative immortality
Tumour-promoting inflammation
Activating invasion & metastasis
Inducing angiogenesis
Genome Instability & mutation
Resisting cell death
Deregulating cellular energetics
Transcription Factors Growth Factor Receptors
p53 RB
Galectins Siglecs
c-MYC
NF-κB COX2
MGAT5 sTn SLeX
VEGF VEGFR HS
O-GlcNAc p53
Fas TNFR1
O-GlcNAc PFK1 c-MYC
Glycosylation


C) Deregulated cellular energetics
Glucose
GLUT
Lactate
MCT
HK
Glc-6-P
Lactate
Pentose Phosphate Pathway
PGI
LDH
PFK
Piruvate
Fruc-6-P
GFAT
GNA
UAP
Glycolysis
GTP, CTP, and UTP nucleotides
Hypoxia
GnT
UDP-GlcNAc
Glycosylation
OGT
HIF-1α
GNE
ManNAc
GNE
CMP-NeuAc
ManNAc-6-P
HIF-1α targets
Metastasis
A) Sustained proliferative signalling
Perlecan
Decorin
RTK
PI3K/Akt
cMYC
FoxM1
Cyclin D1
Tumor cell
Proliferation
B) Resistance to cell death
α3β1 Integrin
Gal-3
TRAILR
Fas
FAK
PI3K/Akt
NF-kB
Tumor cell
Survival genes
D) Evasion of growth suppressors
p53
MDM2
p300
p53
p53 proteasomal degradation
Rb
Rb
E2F-1
Tumor cell
Cell cycle progression
E) Genome instability and mutations
ATM
ATM
ATM
ATM proteasomal degradation
Mutagenic/oxidative microenvironment
Tumor cell
DNA damage
F) Angiogenesis
Gal-1
HA
Decorin
Neuropilin
VEGFR2
PI3K/Akt
Endothelial cell
Angiogenesis
G) Invasion and metastasis
distant organ CTC arrest
Endothelial cell
E/P-selectin
fibronectin
Terminal SLe groups
Integrin
Tumor cell
E-Cadherin
H) Tumor-promoting inflammation
Tumor cell
non-human epitopes
E/P-selectin
Endothelial cells
I) Immune escape
Dendritic cell impaired maturation
Attenuated interaction with NK cells
Tumor cell
Primary tumor cell
NK cell
Erythrocyte
Dendritic cell
Endothelial cell
Tumor-associated macrophage
Cancer-associated adipocyte
Hypoxic tumor cell
Metastatic tumor cell
Lymphocytes
Platelets
Cancer-associated fibroblast
Extracellular matrix
Microenvironment-derived soluble factors

Lipid

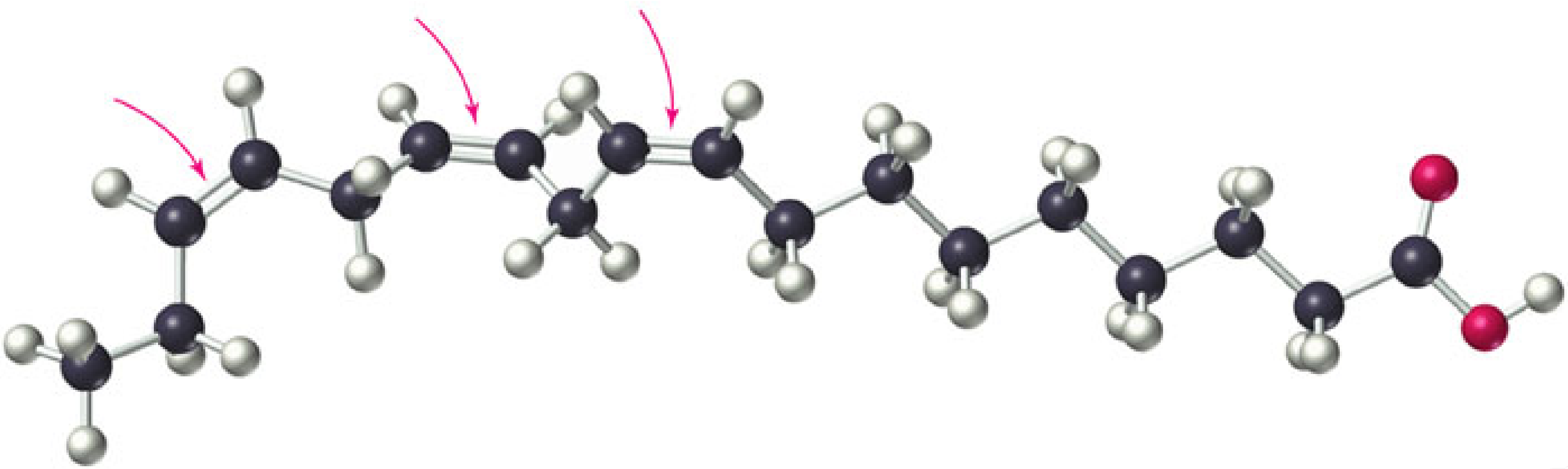
- ***Lipids*** are naturally occurring molecules from plants or animals that are soluble in nonpolar organic solvents.
- Lipid molecules contain large hydrocarbon portion and not many polar functional group, which accounts for their solubility behavior.

$$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2\overset{\overset{\displaystyle O}{\|}}{C}{-}OH$$



A saturated fatty acid
(palmitic acid)

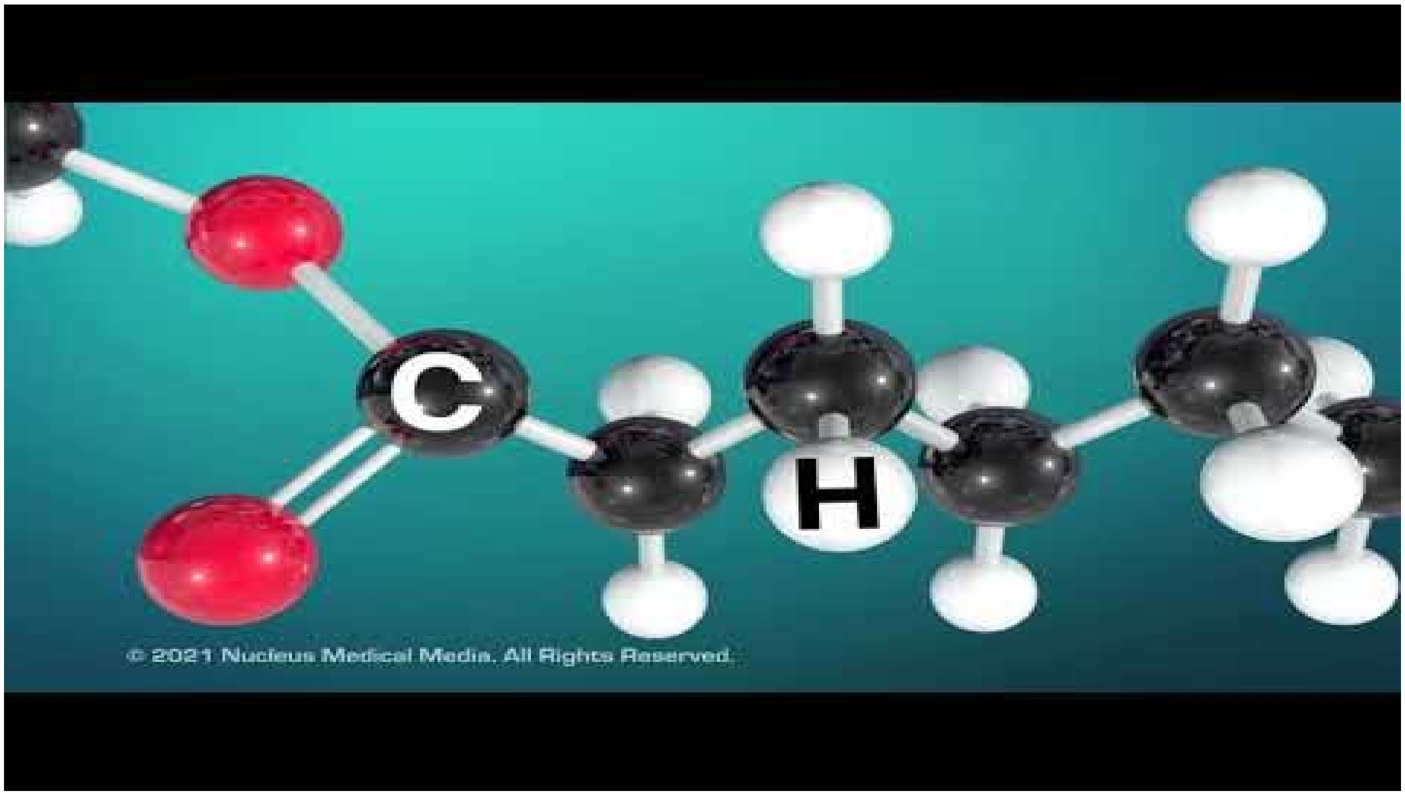
$$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH_2CH_2CH_2CH_2CH_2CH_2\overset{\overset{\displaystyle O}{\|}}{C}{-}OH$$



A *cis* unsaturated fatty acid
(linolenic acid)

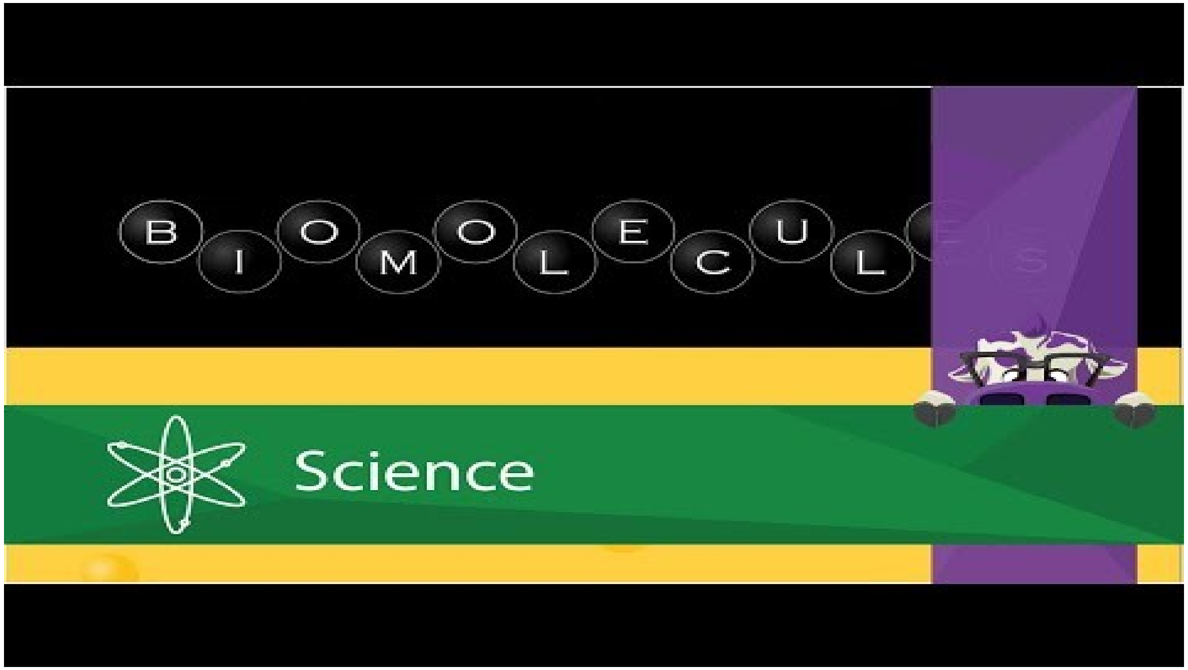
Stearic acid, an 18-carbon saturated fatty acid

Lipid

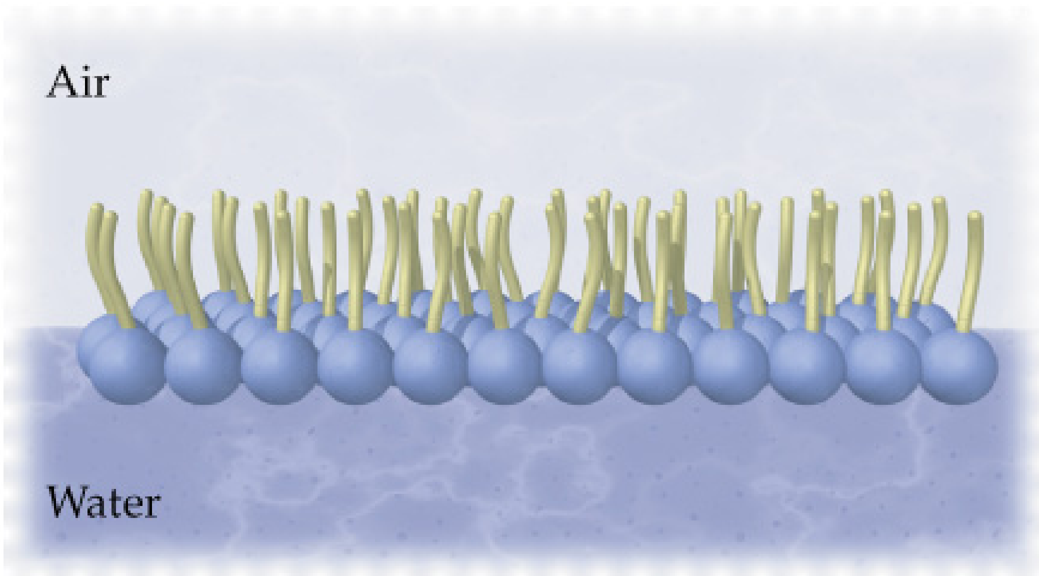


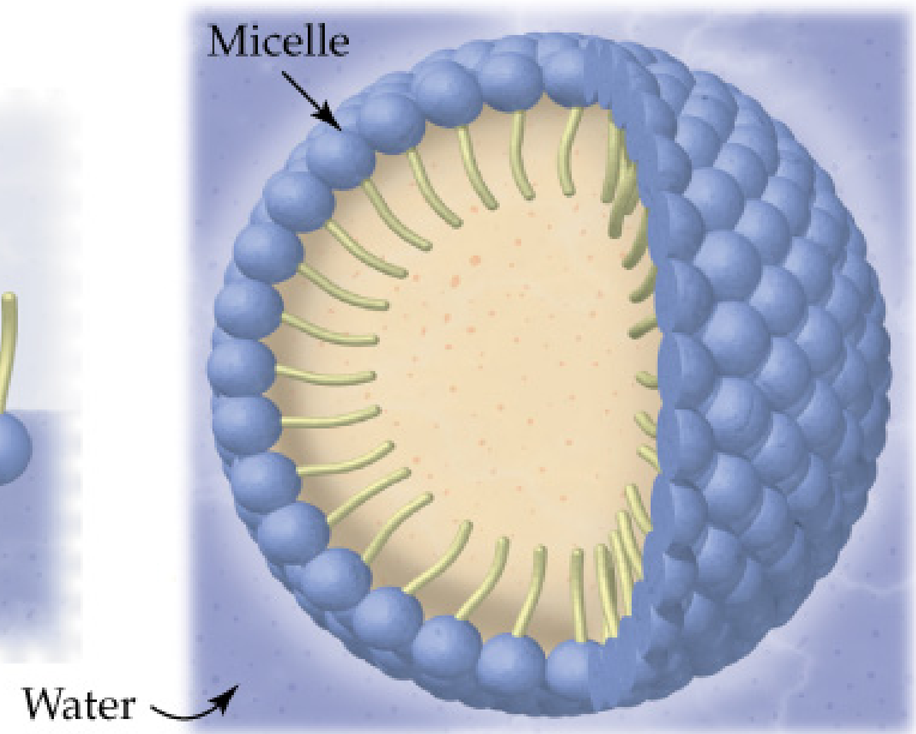
https://www.youtube.com/watch?v=ebScOnAJdu0

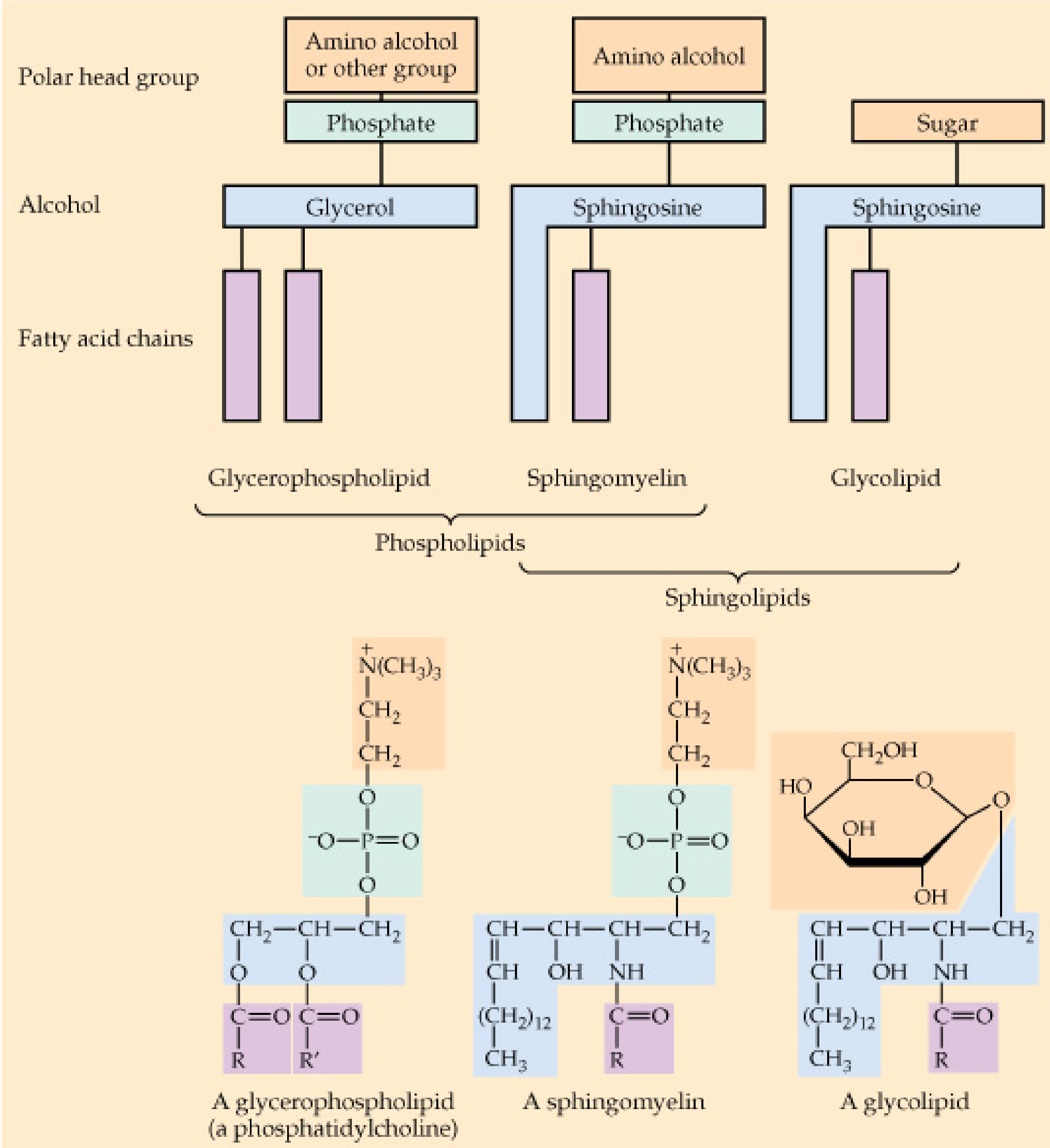
Lipid



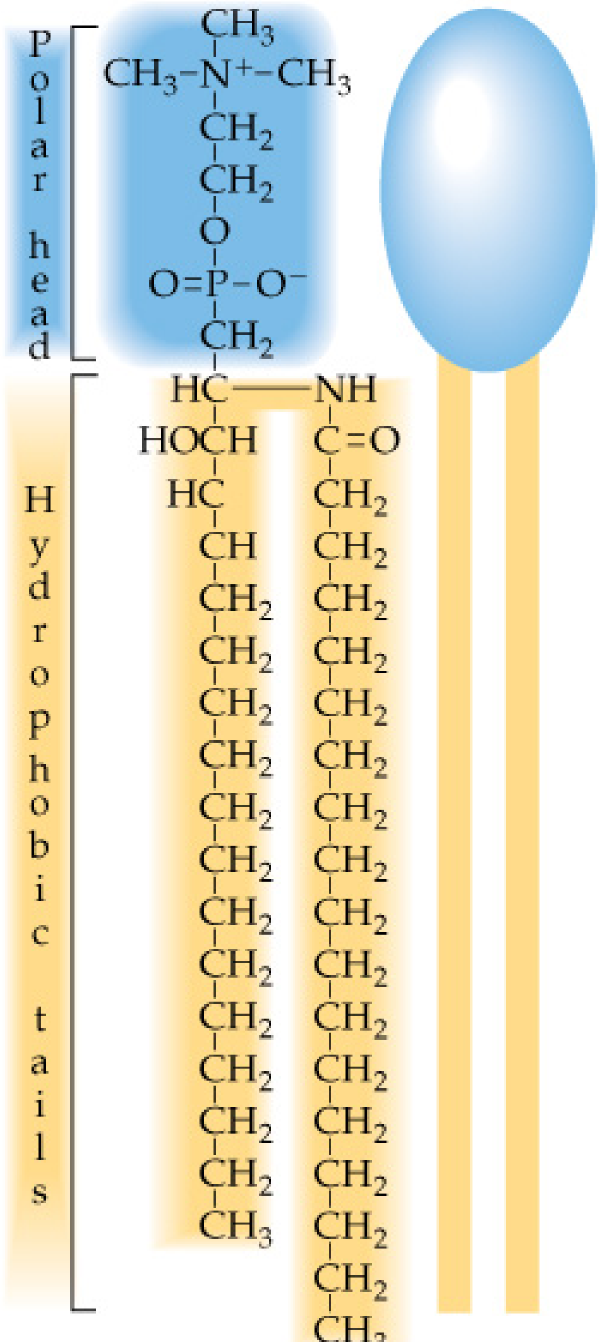
https://www.youtube.com/watch?v=bUaJFg10nkc


Air
Water

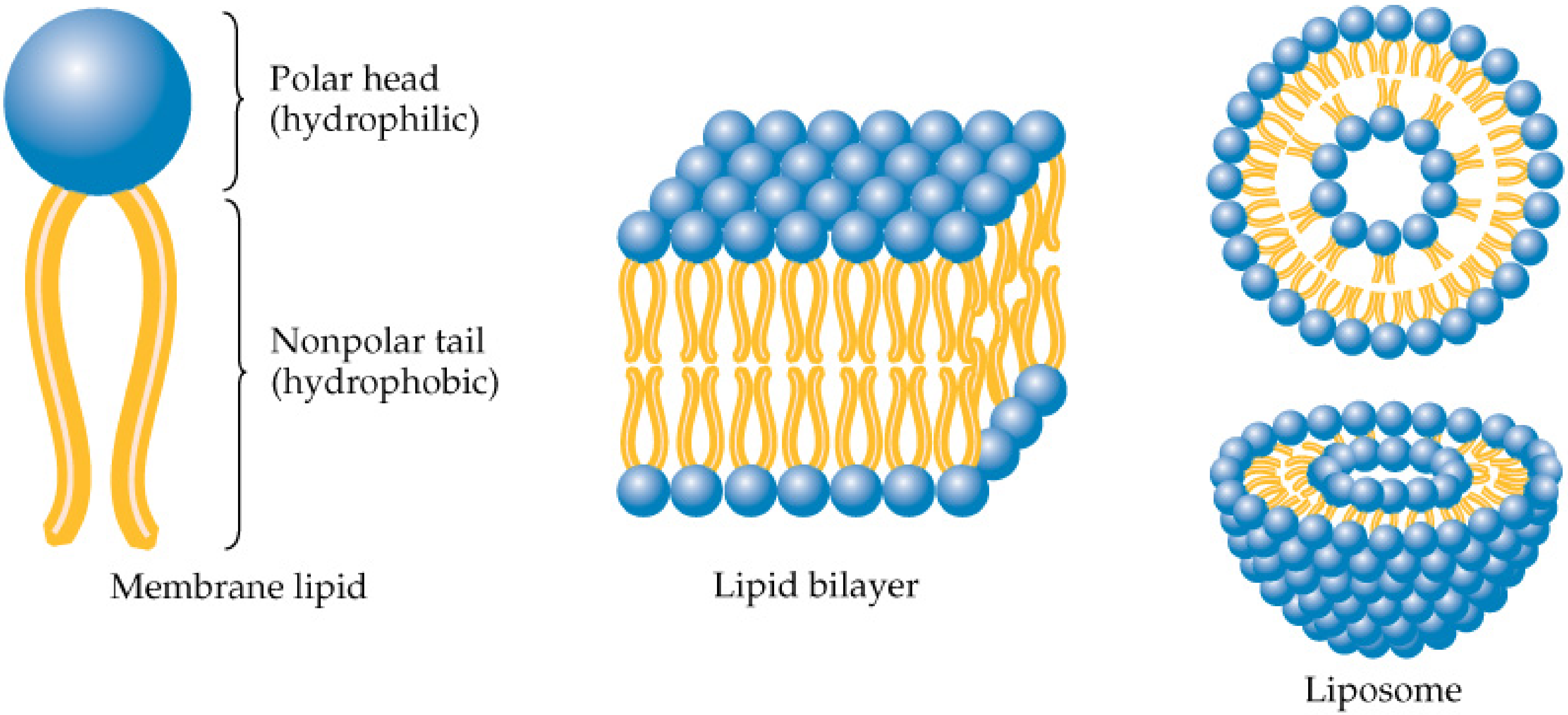

Micelle
Water



Polar head group
Alcohol
Fatty acid chains
Amino alcohol or other group
Phosphate
Glycerol
Amino alcohol
Phosphate
Sphingosine
Sugar
Sphingosine
Glycerophospholipid
Sphingomyelin
Glycolipid
Phospholipids
Sphingolipids
A glycerophospholipid (a phosphatidylcholine)
A sphingomyelin
A glycolipid



A sphingomyelin


Polar head
(hydrophilic)
Nonpolar tail
(hydrophobic)
Membrane lipid
Lipid bilayer
Liposome

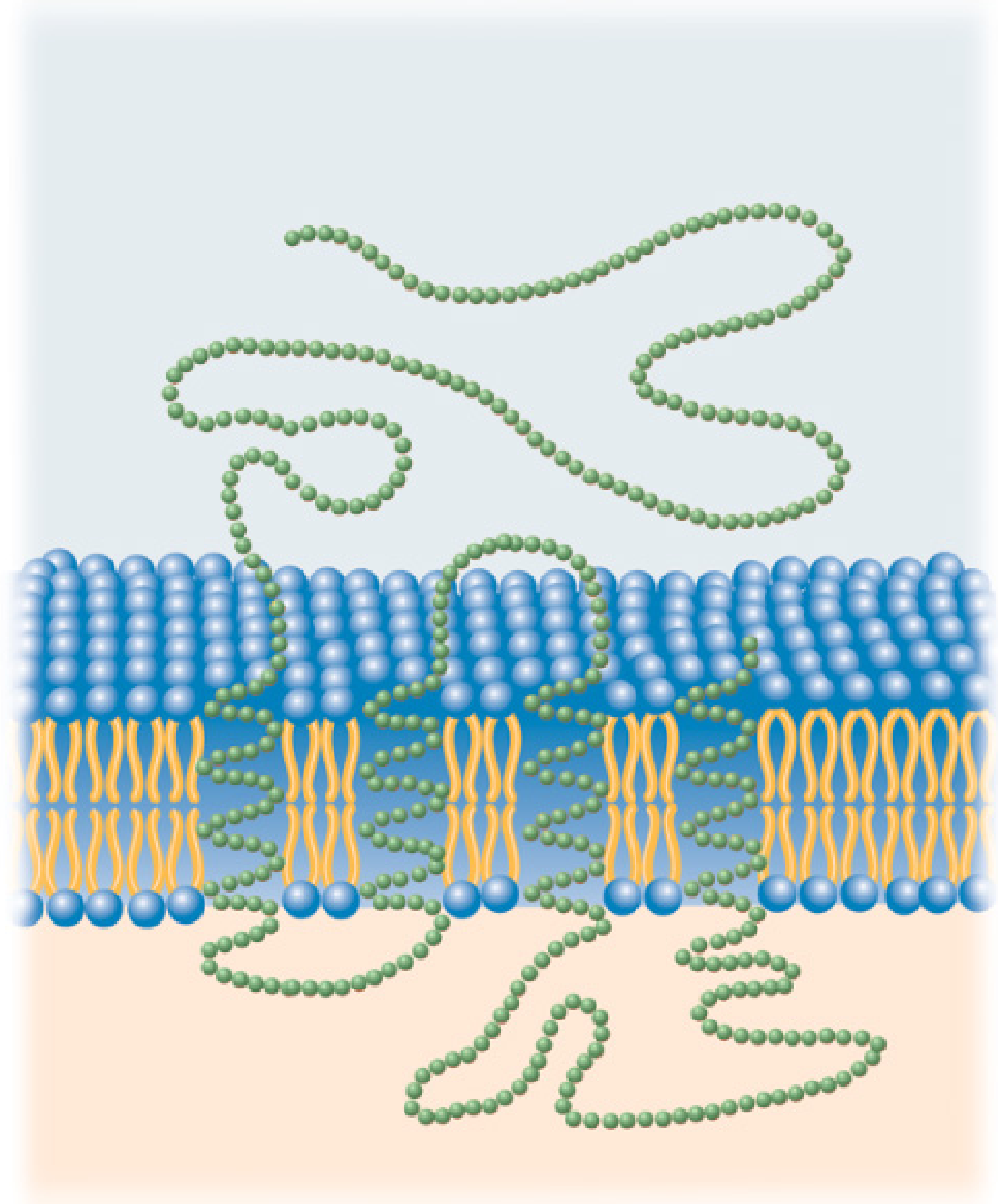
Properties of cell membranes:

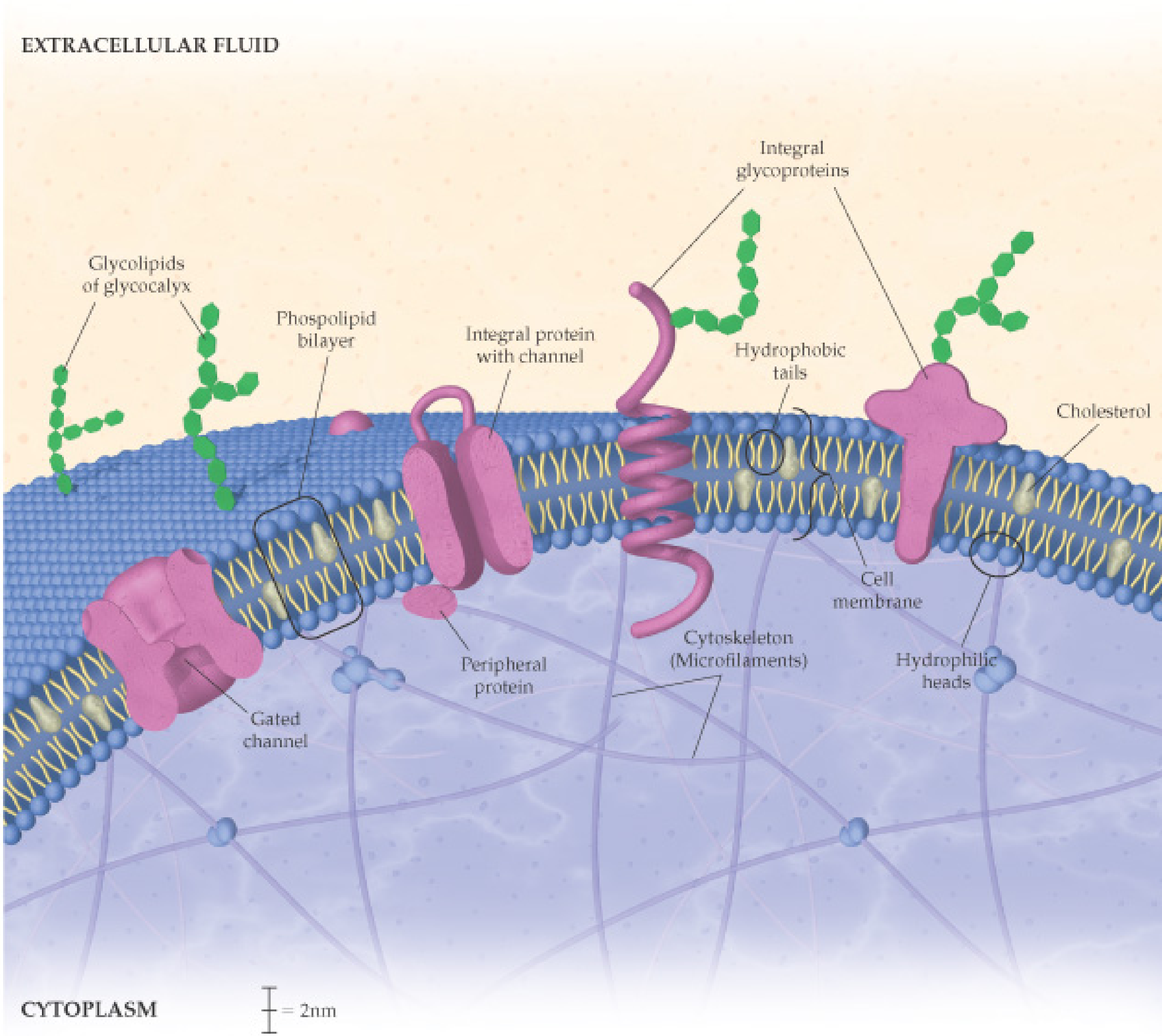
- Cell membranes are composed of a fluid like phospholipid bilayer.
- The bilayer incorporates cholesterol, proteins, and glycolipids.
- Small nonpolar molecules cross by diffusion through the lipid bilayer.
- Small ions and polar molecules diffuse through the aqueous media in protein pores.
- Glucose and certain other substances cross with the aid of proteins without energy input.
- Na^+, K^+, and other substances that maintain concentration gradients inside and outside the cell cross with expenditure of energy and the aid of proteins.

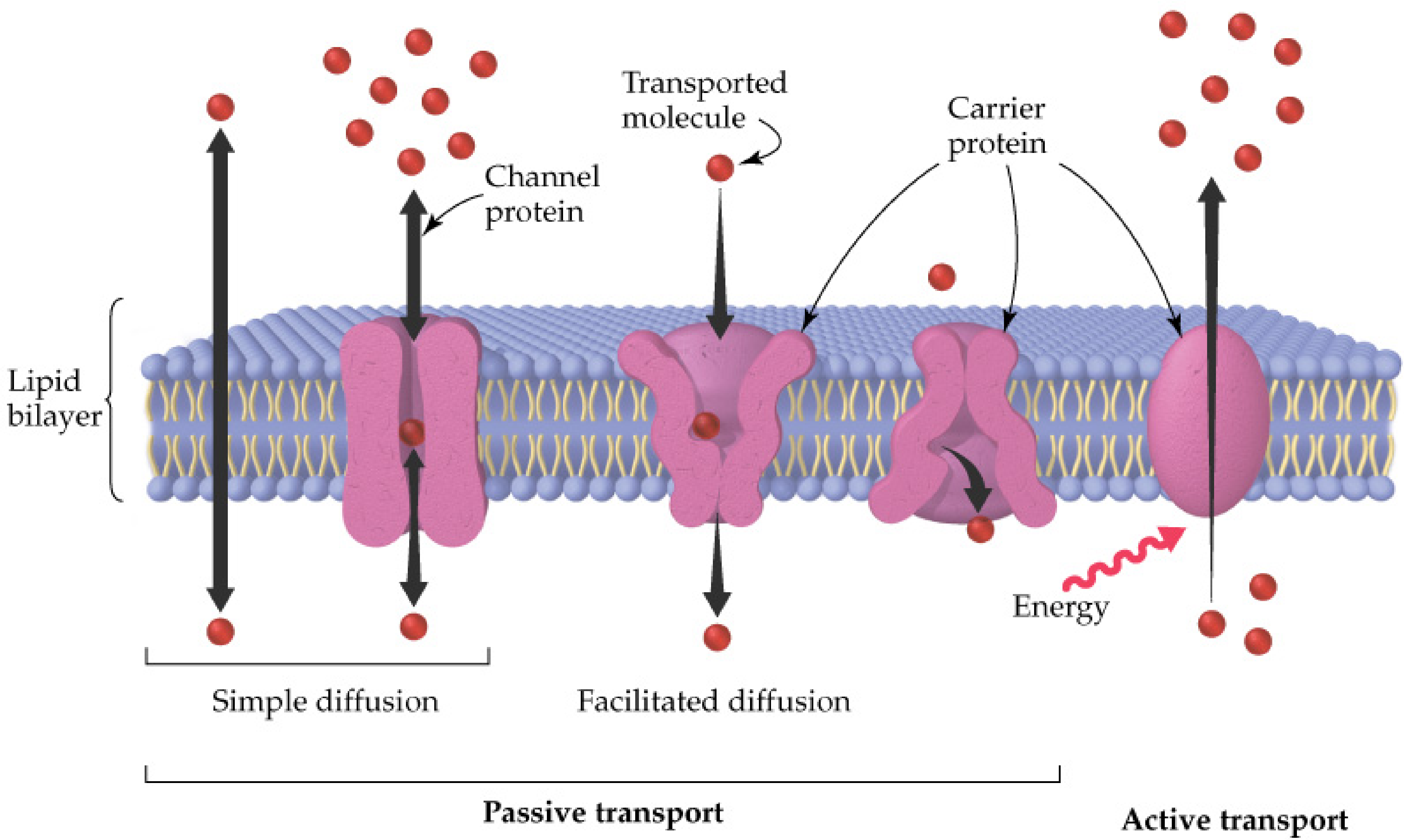
- Small ions and polar molecules diffuse through the aqueous media in protein pores.
- Glucose and certain other substances cross with the aid of proteins without energy input.
- Na^+, K^+, and other substances that maintain concentration gradients inside and outside the cell cross with expenditure of energy and the aid of proteins.

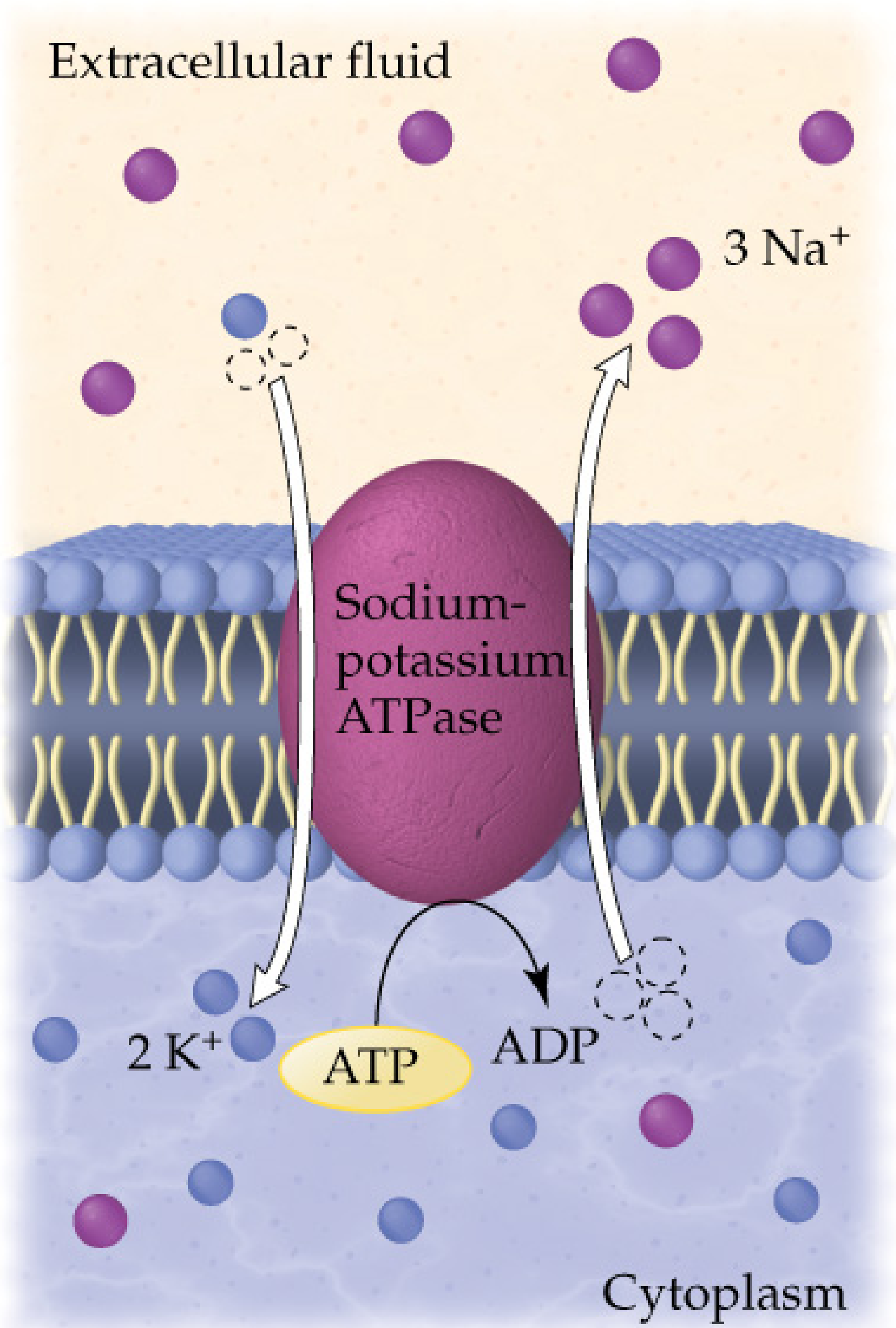
Lipids play several crucial and diverse roles in cells, influencing both their structure and function.

Structural Components of Membranes: Lipids, particularly phospholipids, are fundamental constituents of cellular membranes. They form bilayers that provide the basic structure of the plasma membrane and the membranes of various organelles within the cell. These lipid bilayers are fluid and dynamic, allowing for membrane fluidity and flexibility, which are essential for various cellular processes.
Energy Storage: Lipids serve as an important source of energy.
Signaling Molecules: Various lipids act as signaling molecules or precursors to signaling molecules. For instance, steroid hormones, which are derived from cholesterol, are crucial signaling molecules that regulate a wide range of physiological processes
Coenzymes and Vitamins: Certain lipids act as coenzymes or essential components of coenzymes. For example, the lipid-soluble vitamins A, D, E, and K are critical for various biological functions, including vision, bone metabolism, antioxidant protection, and blood coagulation.
Anchoring Membrane Proteins: Lipids can covalently attach to proteins to anchor them within the cell membrane. This lipid modification is crucial for the localization, function, and signaling of membrane proteins.
Insulation and Protection: In multicellular organisms, lipids provide insulation and protection. Subcutaneous fat serves as an insulator, reducing heat loss, and provides mechanical cushioning to protect internal organs.
Cell Recognition and Communication: Lipids are involved in cell recognition and communication processes. For example, glycolipids, which are lipids with carbohydrate chains, are present on the cell surface and play roles in cell-cell interactions, recognition, and immune responses.
Modulating Membrane Fluidity: The composition of lipids in membranes can influence their fluidity, which in turn affects various membrane-associated functions such as vesicle formation, fusion, and the activity of membrane-bound enzymes and receptors. For instance, cholesterol in animal cell membranes modulates fluidity and permeability.




EXTRACELLULAR FLUID
Integral glycoproteins
Glycolipids of glycocalyx
Phospolipid bilayer
Integral protein with channel
Hydrophobic tails
Cholesterol
Cell membrane
Cytoskeleton (Microfilaments)
Peripheral protein
Hydrophilic heads
Gated channel
CYTOPLASM
= 2nm

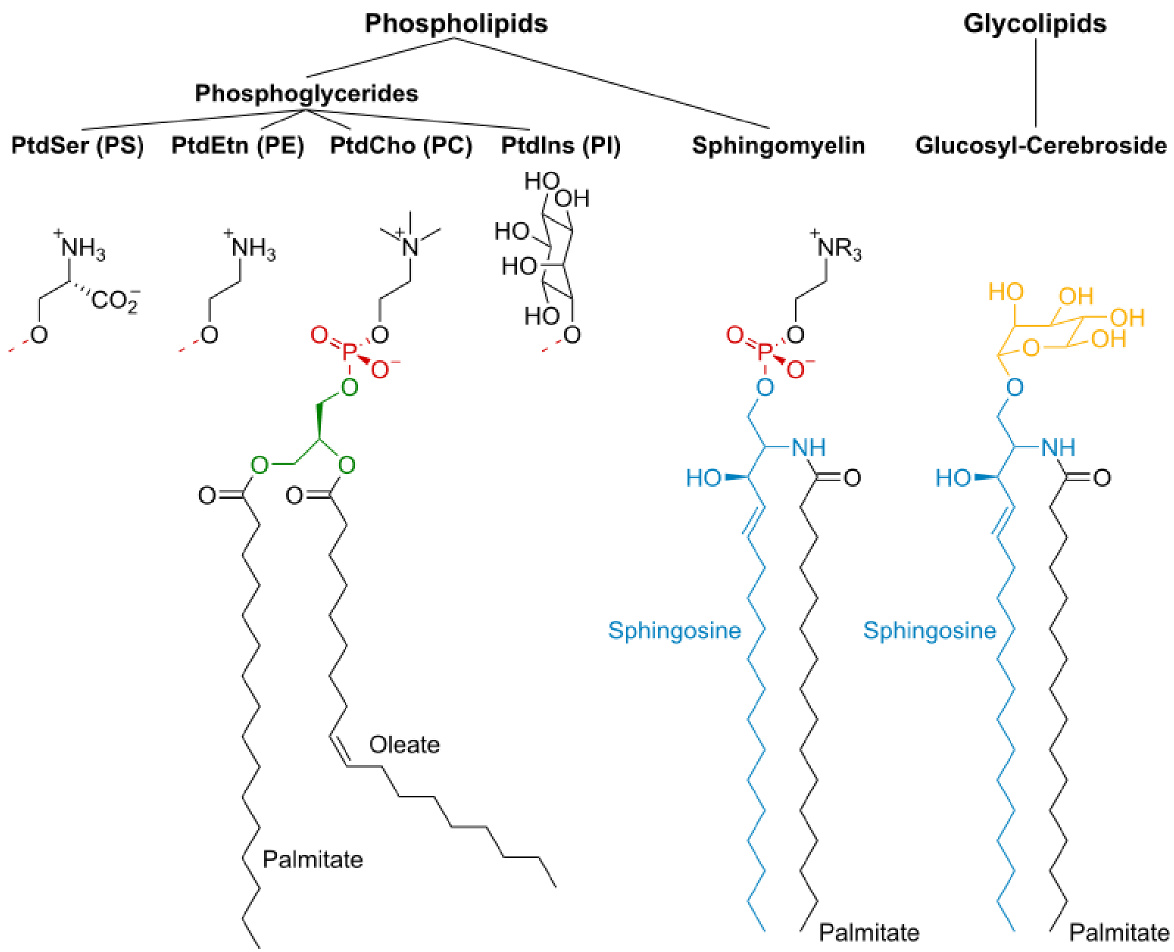

Transported molecule
Carrier protein
Channel protein
Lipid bilayer
Energy
Simple diffusion
Facilitated diffusion
Passive transport
Active transport

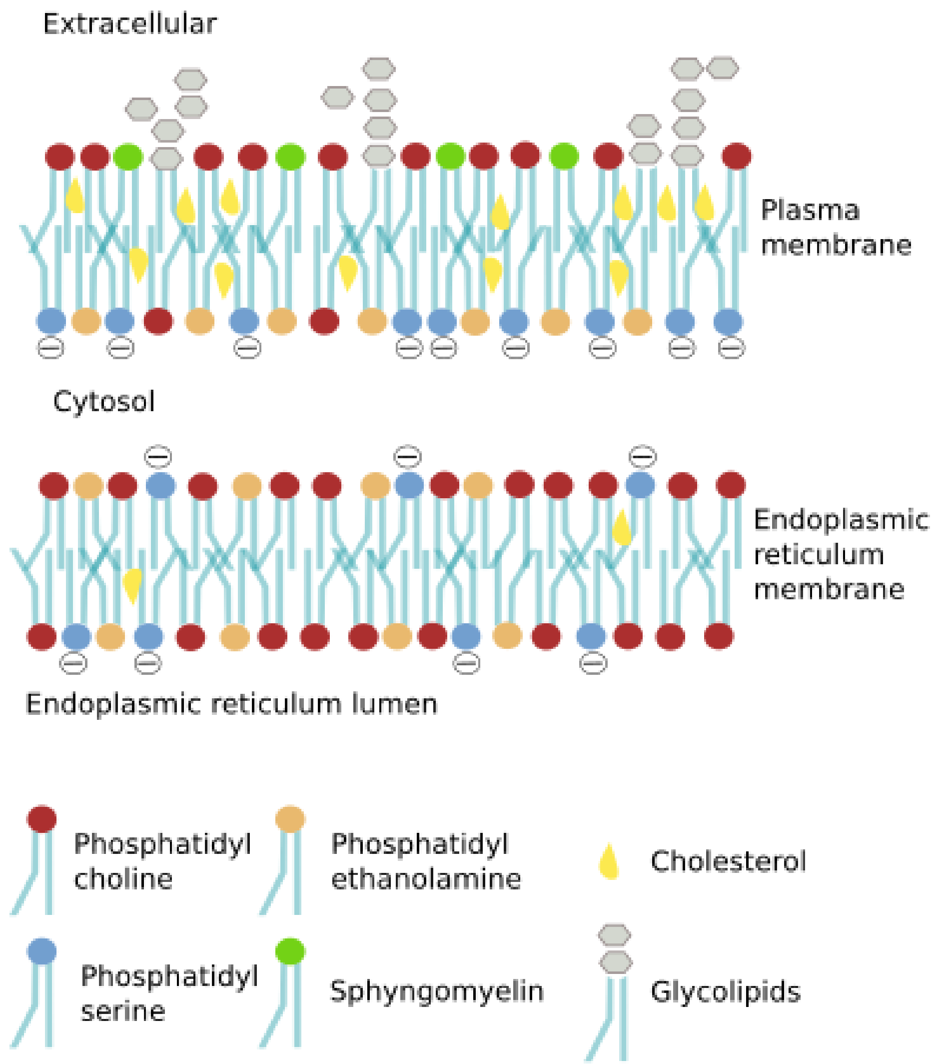

Extracellular fluid
3 Na+
Sodium-
potassium
ATPase
2 K+
ATP
ADP
Cytoplasm

The major classes of lipids found in cells are phospholipids, glycolipids, cholesterol, and triglycerides. Each of these classes plays essential roles in cellular structure and function:

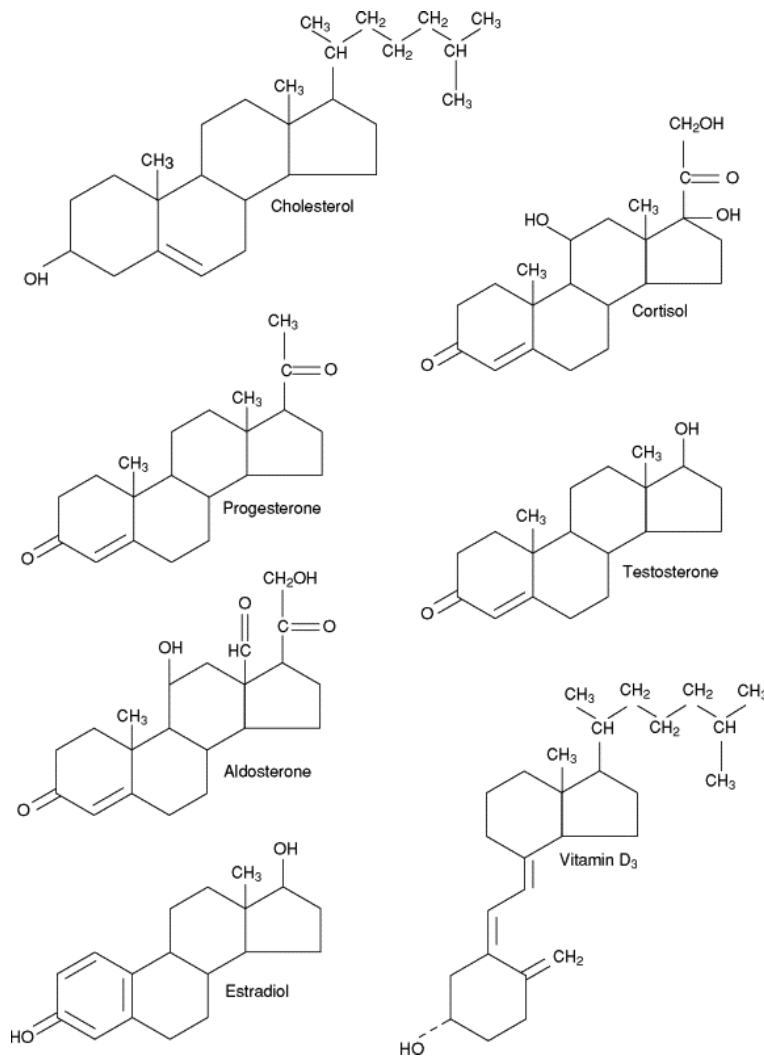
Phospholipids: These are the most abundant lipids in cell membranes. Phospholipids are amphipathic molecules, meaning they have both hydrophilic (water-attracting) and hydrophobic (water-repelling) regions.
Glycolipids: Comprised of a lipid moiety and one or more sugar residues, glycolipids are primarily found on the extracellular surface of cell membranes. They play crucial roles in cell recognition, communication, and immune responses. The sugar moieties of glycolipids interact with specific molecules and cells in the organism's environment, facilitating cellular interactions and signaling.
Cholesterol: Though often associated with health risks when present in excess in the bloodstream, cholesterol is a vital component of animal cell membranes. It modulates the fluidity and permeability of the membrane and is involved in the formation of lipid rafts—specialized membrane domains that serve as organizing centers for the assembly of signaling molecules. Cholesterol is also a precursor for the synthesis of steroid hormones, bile acids, and vitamin D.
Triglycerides (Triacylglycerols): These are the main form of stored energy in many types of cells, particularly adipocytes (fat cells). Triglycerides consist of three fatty acids linked to a glycerol backbone. They are stored in lipid droplets within cells and are metabolized to provide energy when needed. Although not components of cell membranes, triglycerides play a critical role in energy metabolism and homeostasis.

Lipids on Cell Membranes

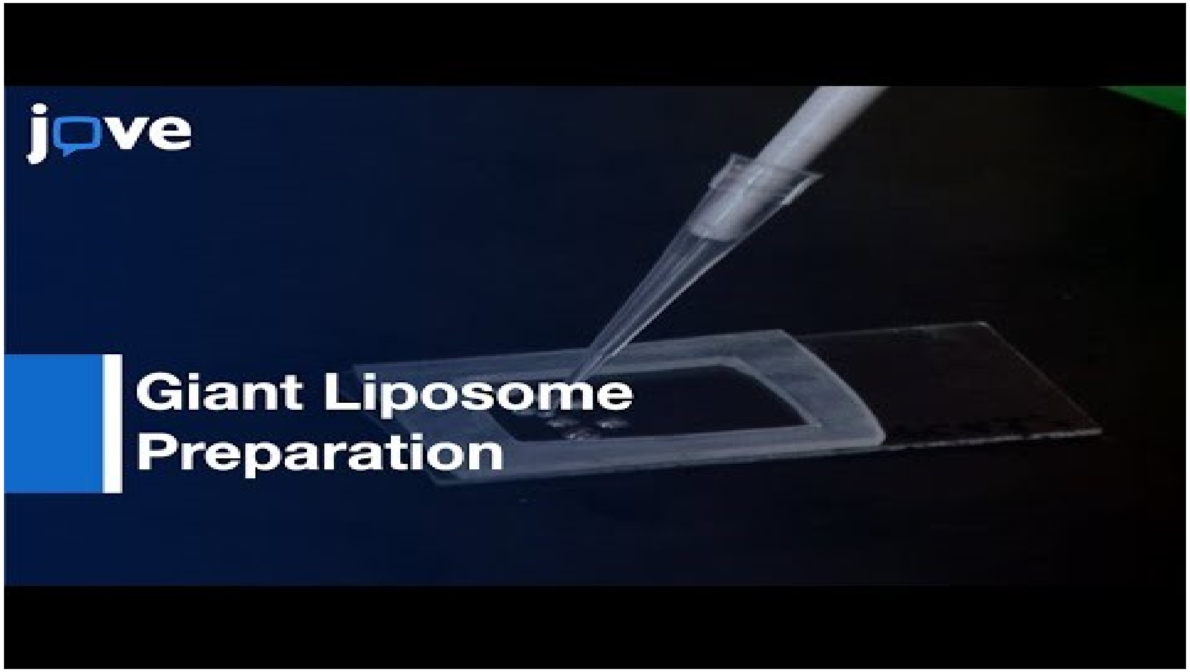



Extracellular
Plasma membrane
Cytosol
Endoplasmic reticulum membrane
Endoplasmic reticulum lumen
Phosphatidyl choline
Phosphatidyl ethanolamine
Cholesterol
Phosphatidyl serine
Sphyngomyelin
Glycolipids

Cholesterol

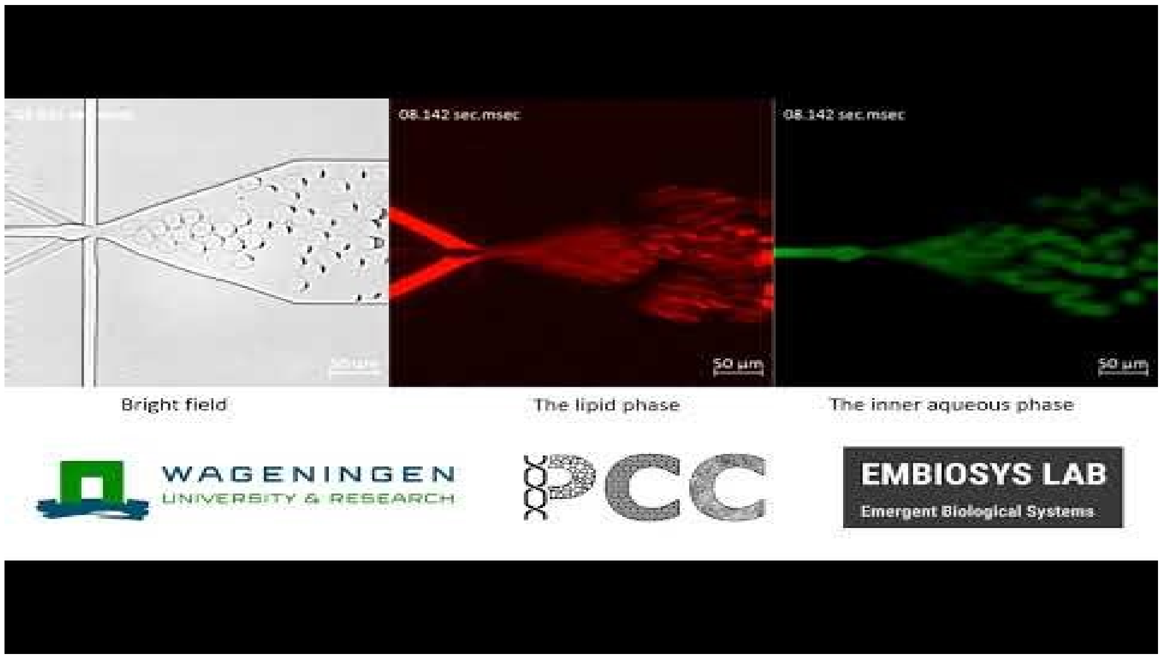


Liposome Preparation



https://www.youtube.com/watch?v=7UvUm2IrZk4

Liposome Preparation by Microfluidics



https://www.youtube.com/watch?v=DmJrsvCLR5w

Liquid-Liquid Phase Separation

Liquid-liquid phase separations in cells refer to the process where biomolecules undergo demixing from a homogenous solution into two distinct liquid phases, resulting in the formation of membrane-less organelles or biomolecular condensates. These phase transitions play crucial roles in cellular organization, function, and regulation:

Compartmentalization: Liquid-liquid phase separation (LLPS) allows cells to organize their intracellular environment without the need for membrane-bound organelles. This compartmentalization facilitates the concentration and sequestration of specific proteins and nucleic acids, enhancing biochemical reactions and processes.

Regulation of Biochemical Reactions: By concentrating specific enzymes and substrates within phase-separated droplets, cells can enhance or regulate the rates of biochemical reactions. This mechanism allows for the spatial and temporal control of metabolic pathways and signaling cascades.

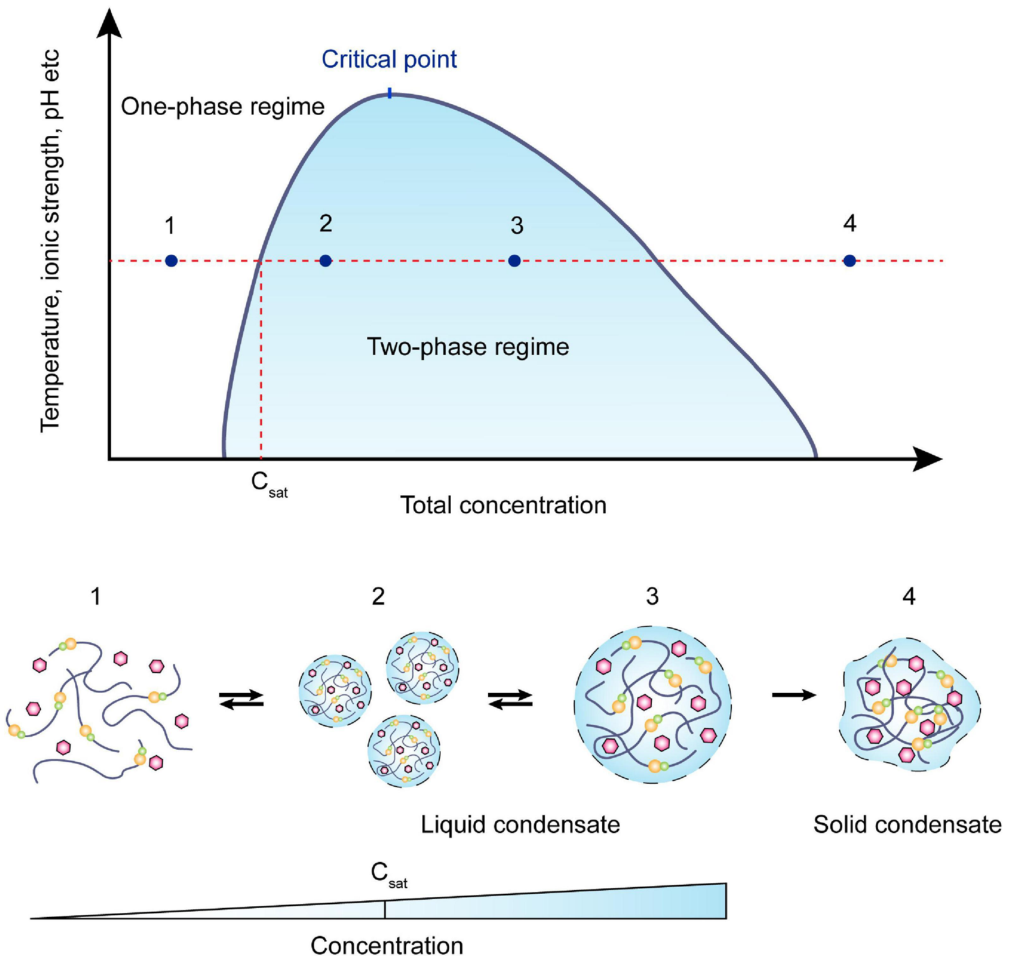
Response to Environmental Stimuli: The dynamics of phase-separated droplets can change rapidly in response to environmental cues, such as changes in temperature, pH, or ion concentration. This responsiveness enables cells to adapt quickly to environmental changes, modulating cellular processes accordingly.

Stress Response: Under stress conditions, cells can form stress granules through LLPS. These granules sequester and protect mRNA and proteins, preventing their aggregation and facilitating their rapid reactivation when stress conditions abate. This process is crucial for cell survival under adverse conditions.

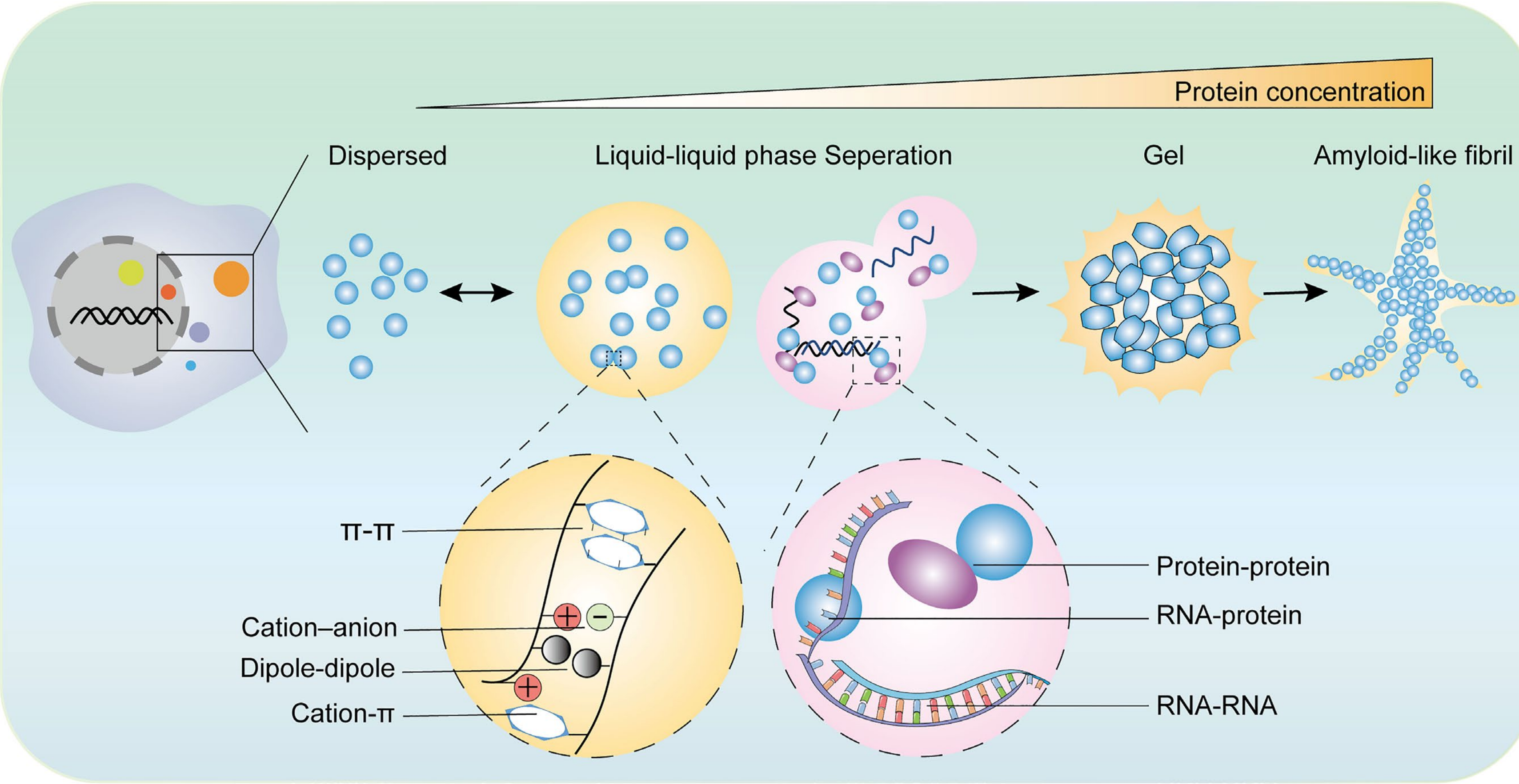
Signal Transduction: Phase-separated compartments can concentrate signaling molecules, enhancing signal transduction pathways. By bringing together key components of a signaling pathway, LLPS can increase the efficiency and specificity of signal transmission.
RNA Processing and Transport: LLPS plays a role in the formation of nuclear speckles and other nuclear bodies that are involved in RNA splicing, processing, and transport. These condensates can regulate gene expression by influencing RNA metabolism and dynamics.
Protein Folding and Stability: By providing a unique microenvironment, phase-separated droplets can influence protein folding and stability. This environment can prevent protein aggregation and assist in the proper folding of proteins, which is essential for their function and longevity.
Membrane Dynamics: LLPS can also influence the organization and dynamics of cellular membranes. For example, the clustering of signaling receptors and other membrane proteins into lipid rafts can be driven by phase separation processes, affecting membrane fluidity and signaling.

In summary, liquid-liquid phase transitions are fundamental for cellular organization, enabling cells to create dynamic, membrane-less compartments that regulate and facilitate a myriad of biochemical processes essential for life.

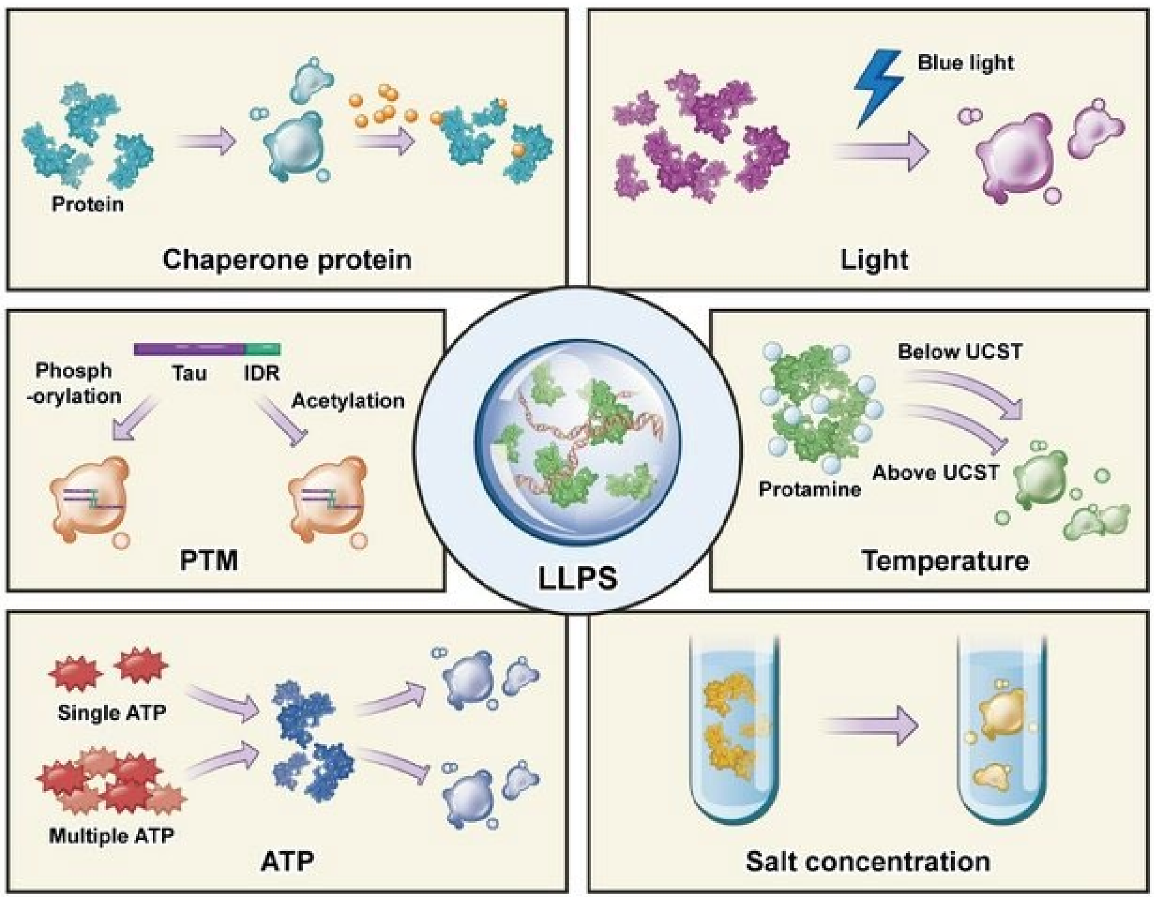
Liquid-Liquid Phase Separation



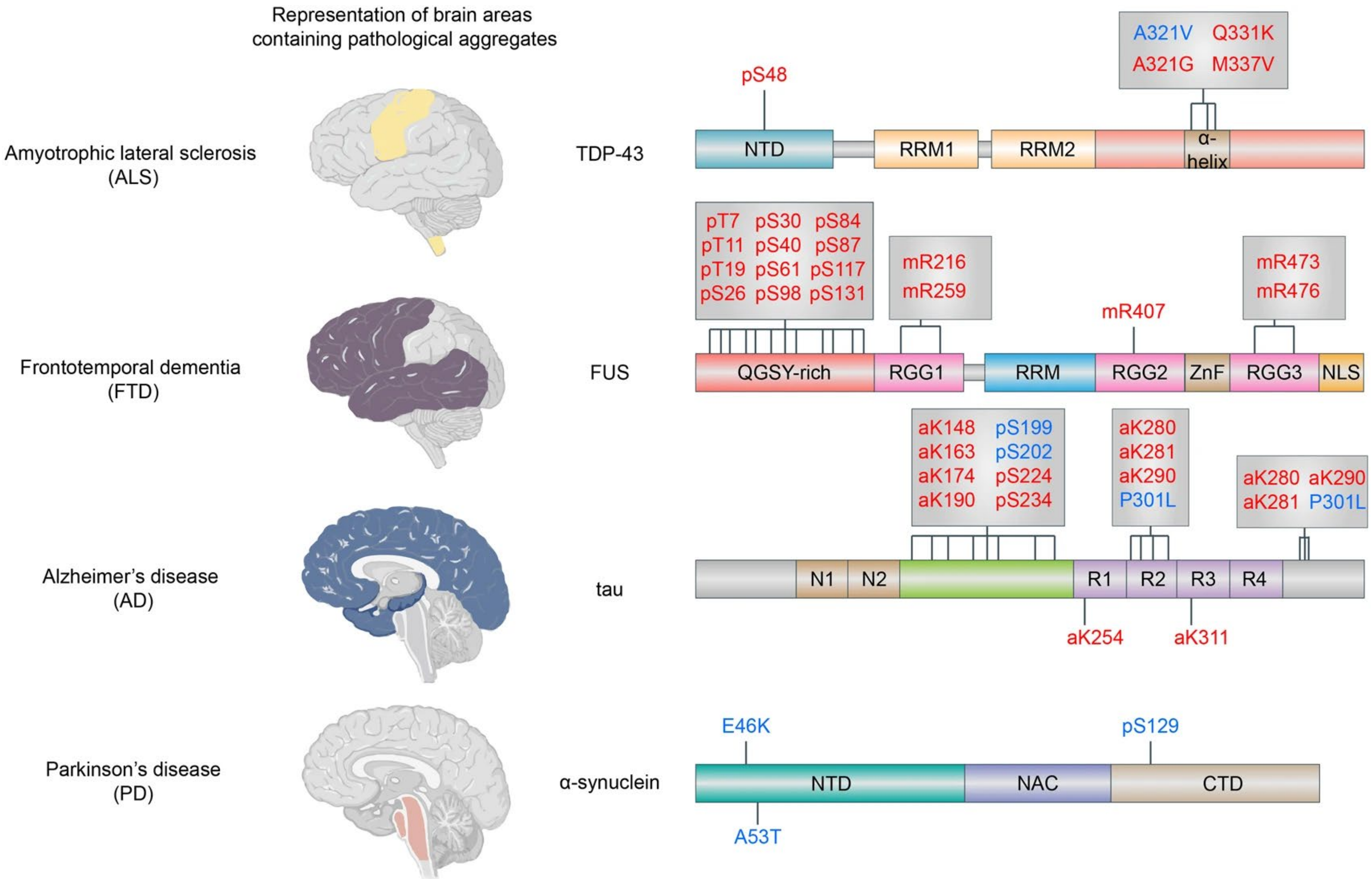
Liquid-Liquid Phase Separation



Factors Regulate LLPS



LLPS in Diseases



LLPS in Cancer