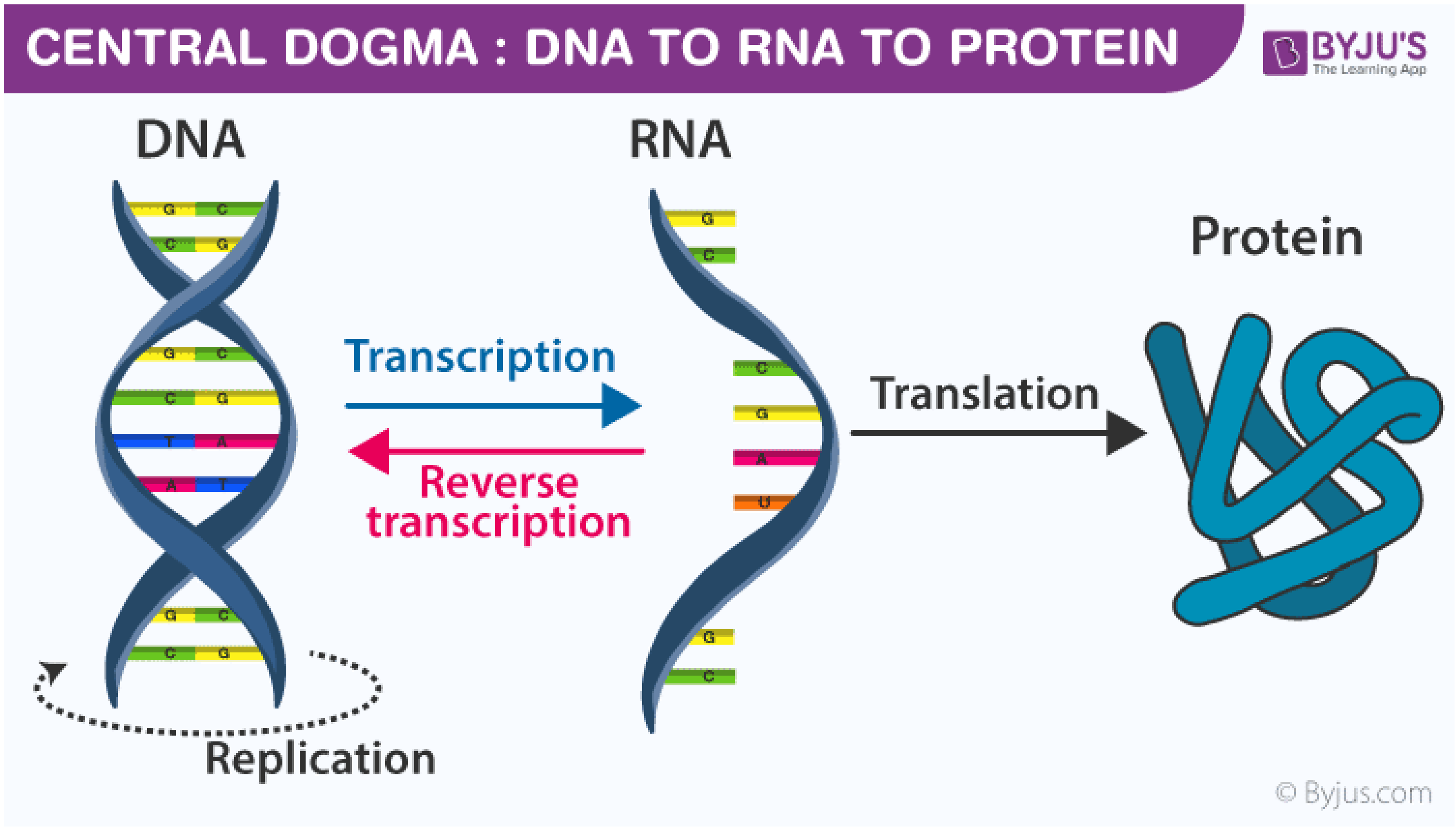
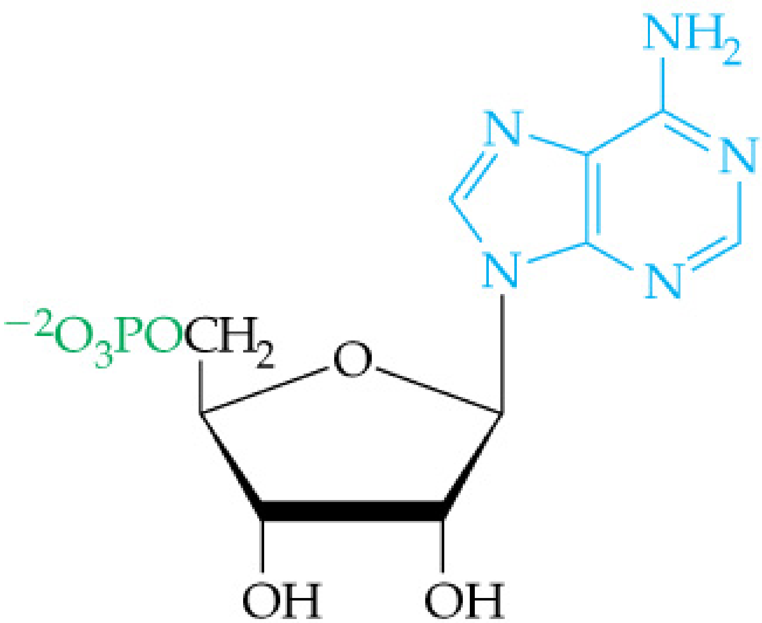
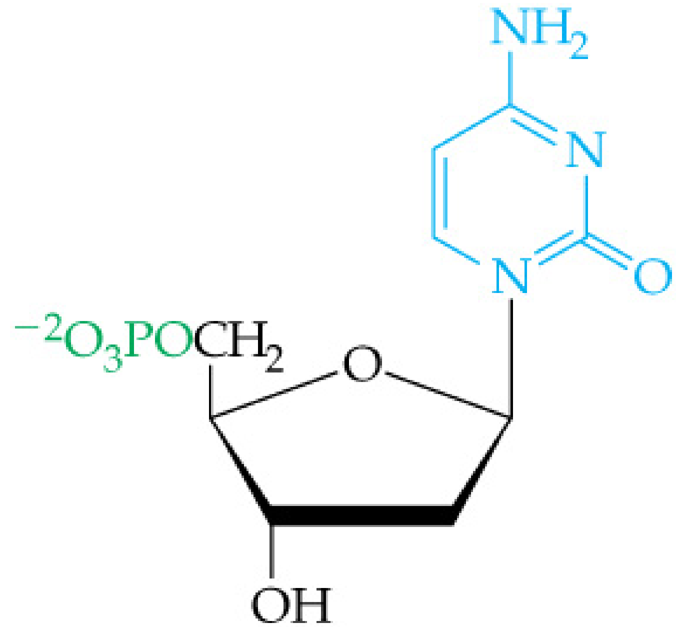
Central Dogma of Molecular Biology



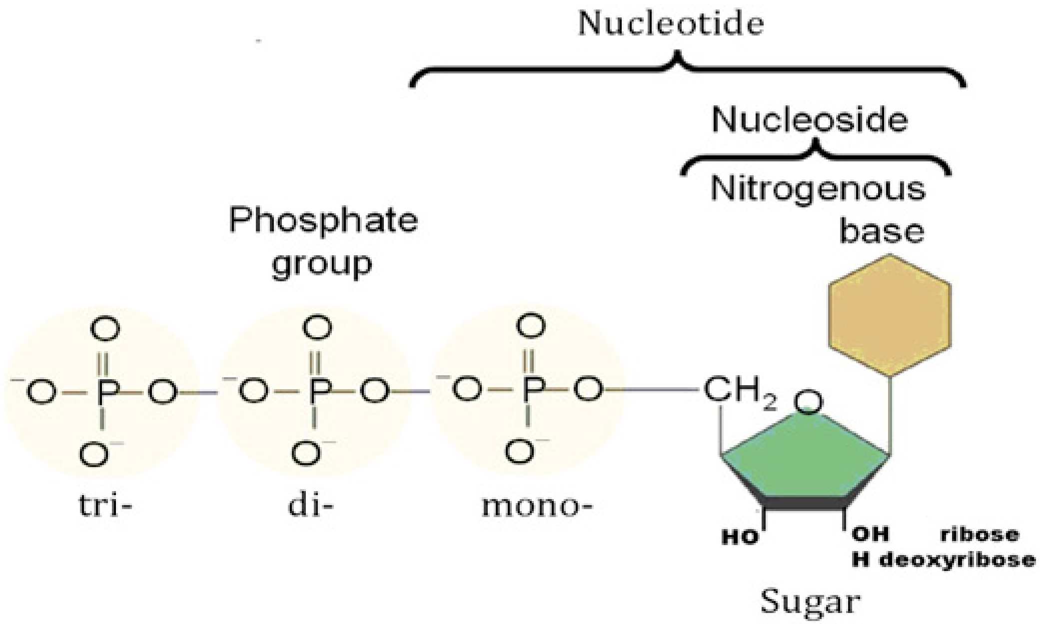
- In RNA, the sugar is ribose.
- In DNA, the sugar is deoxyribose.

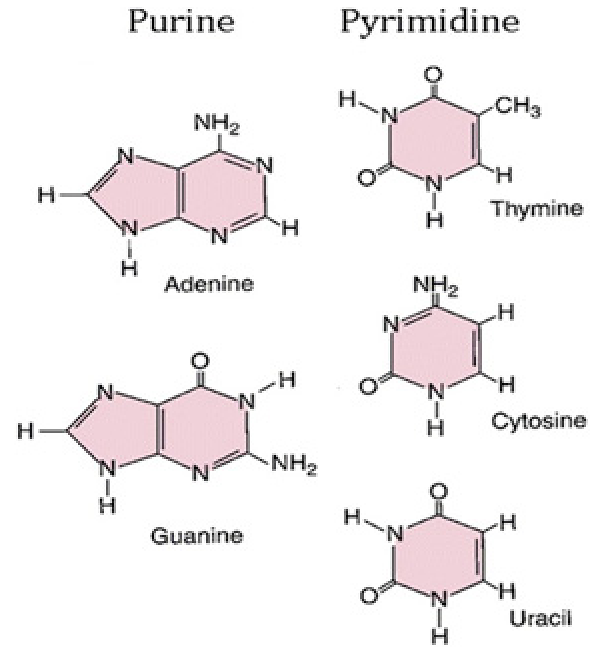


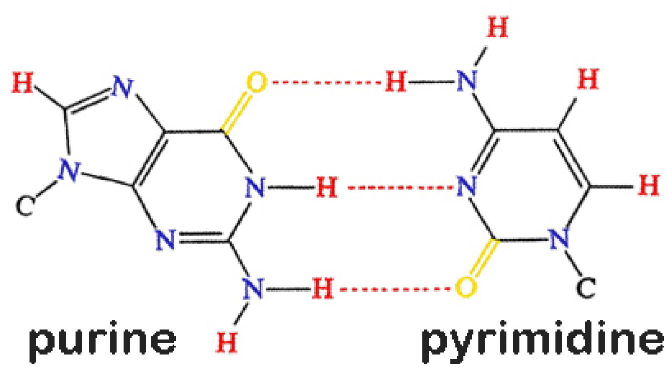
Adenosine 5′-monophosphate (AMP)
(a ribonucleotide)



Deoxycytidine 5′-monophosphate (dCMP)
(a deoxyribonucleotide)


Nucleotide
Nucleoside
Nitrogenous base
Phosphate group
tri-
di-
mono-
CH2
HO
OH ribose
H deoxyribose
Sugar


Purine
Pyrimidine
Adenine
Guanine
Thymine
Cytosine
Uracil


purine
pyrimidine

RNA (ribonucleic acid) and DNA (deoxyribonucleic acid) are both essential types of nucleic acids found in all living organisms, but they differ in structure, function, and role within the cell. Here are some of the key differences between RNA and DNA:

1. Sugar Component
DNA contains deoxyribose sugar, which lacks an oxygen atom at the 2' position of its sugar ring.
RNA contains ribose sugar, which has an oxygen atom at the 2' position of its sugar ring.
2. Nitrogenous Bases
DNA includes the nitrogenous bases adenine (A), guanine (G), cytosine (C), and thymine (T).
RNA includes adenine (A), guanine (G), cytosine (C), and uracil (U) instead of thymine.
3. Structure
DNA is usually double-stranded, forming a double helix structure. This allows it to store genetic information securely.
RNA is typically single-stranded, which enables it to assume various shapes and functions, including coding, decoding, regulation, and expression of genes.
4. Function
DNA serves as the long-term storage of genetic information; it's essentially the blueprint for the organism's entire biological makeup.
RNA plays multiple roles, but its primary function is to convert the genetic instructions found in DNA into the proteins and enzymes that power cellular activities. RNA types include messenger RNA (mRNA), transfer RNA (tRNA), and ribosomal RNA (rRNA), among others, each serving different functions in the process of gene expression.

5. Location within the Cell

DNA is mainly found in the cell nucleus, with a small amount present in mitochondria and, in plants, chloroplasts.

RNA is found throughout the cell, including the nucleus, cytoplasm, and in the organelles where protein synthesis occurs.

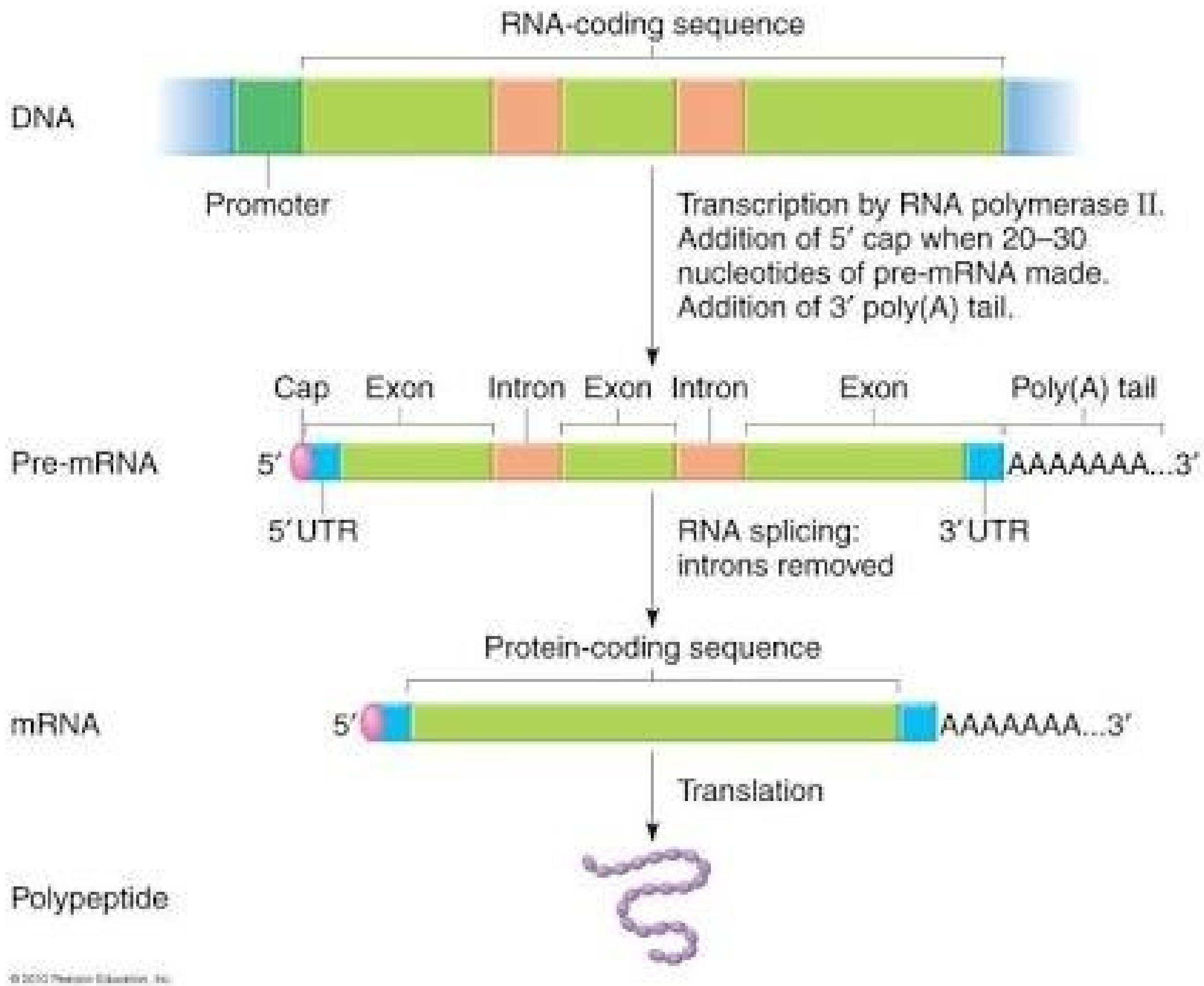
6. Stability

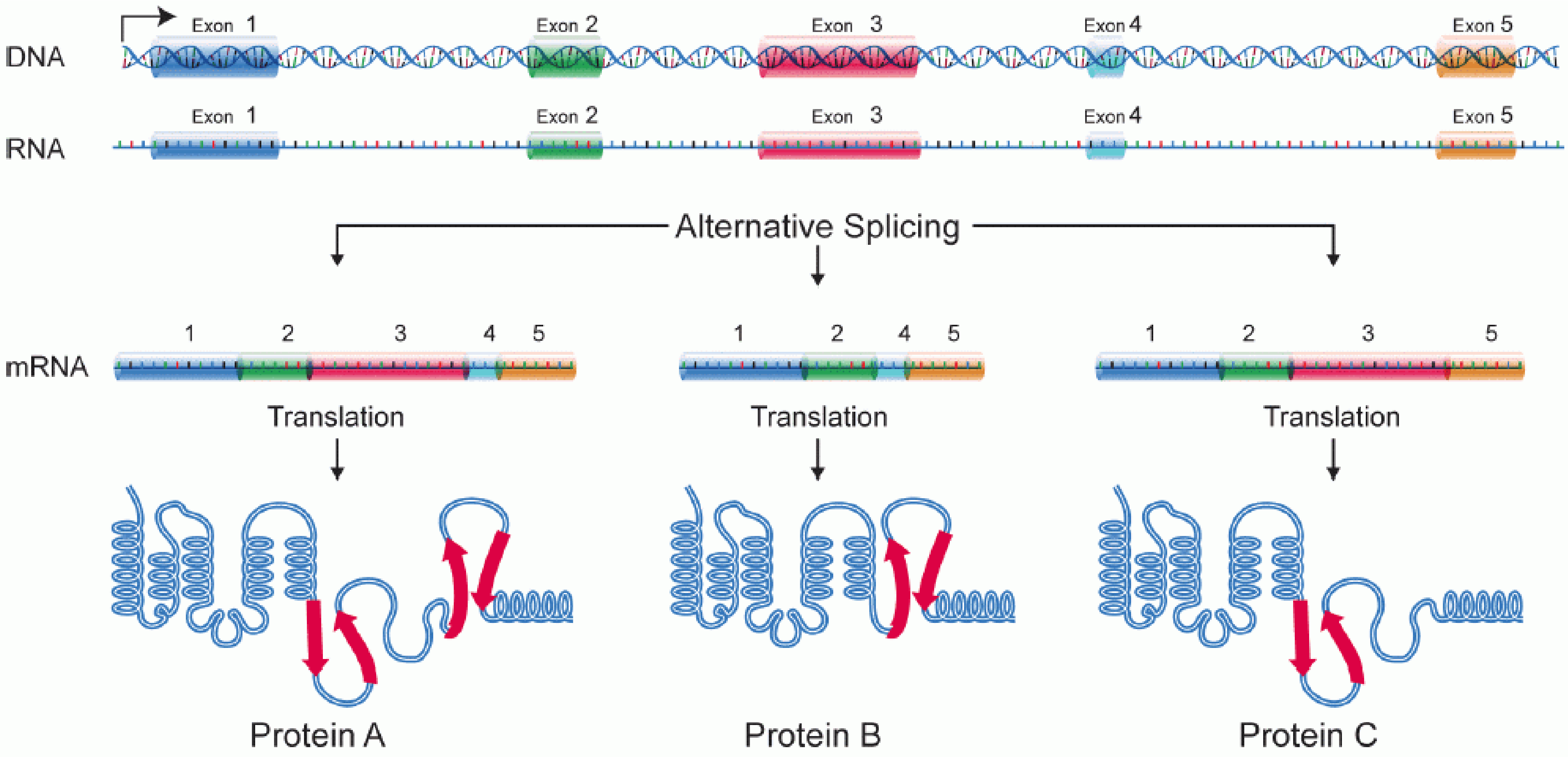
DNA is chemically stable, due in part to its deoxyribose sugar and double-stranded structure, allowing it to function as a long-term storage medium for genetic information.

RNA is relatively unstable and more prone to degradation, which is compatible with its role in transiently guiding protein synthesis and other cellular functions.

Post Transcription Modification of RNA

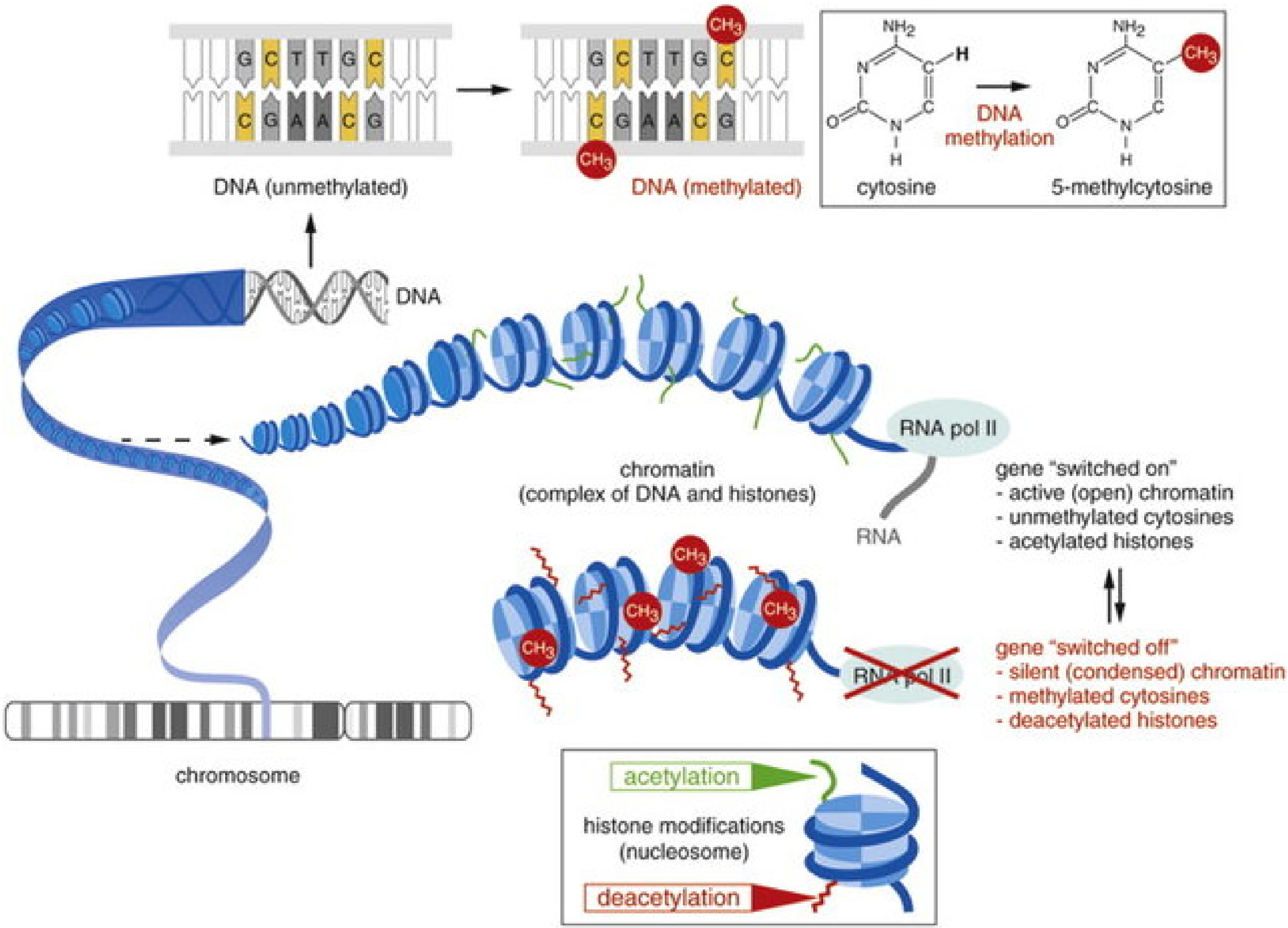
1. RNA capping
2. PolyA tail
3. Splicing

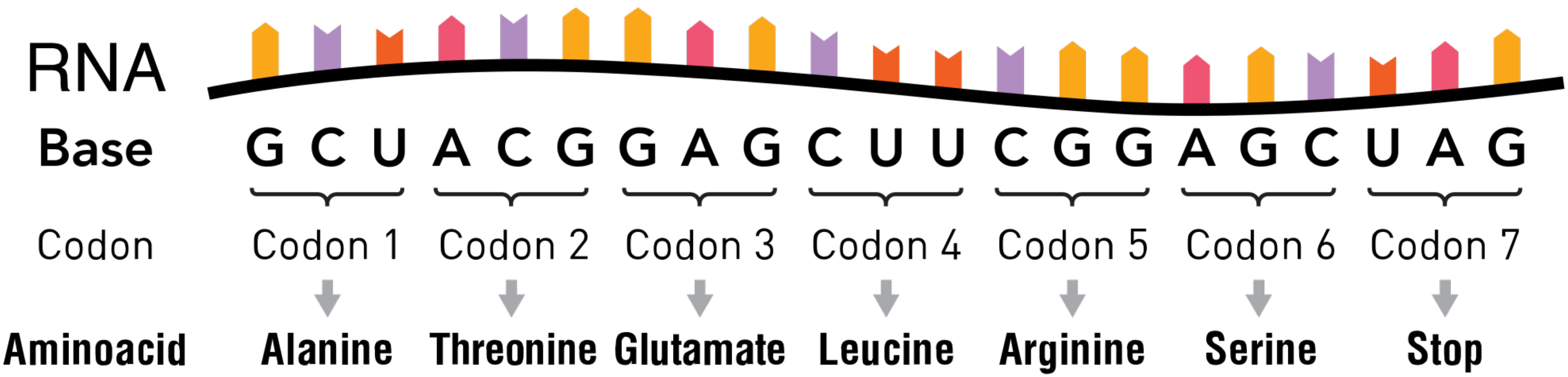




DNA
RNA
Exon 1
Exon 2
Exon 3
Exon 4
Exon 5
Alternative Splicing
mRNA
1
2
3
4
5
Translation
Protein A
Protein B
Protein C

DNA Methylation and Histone Acetylation



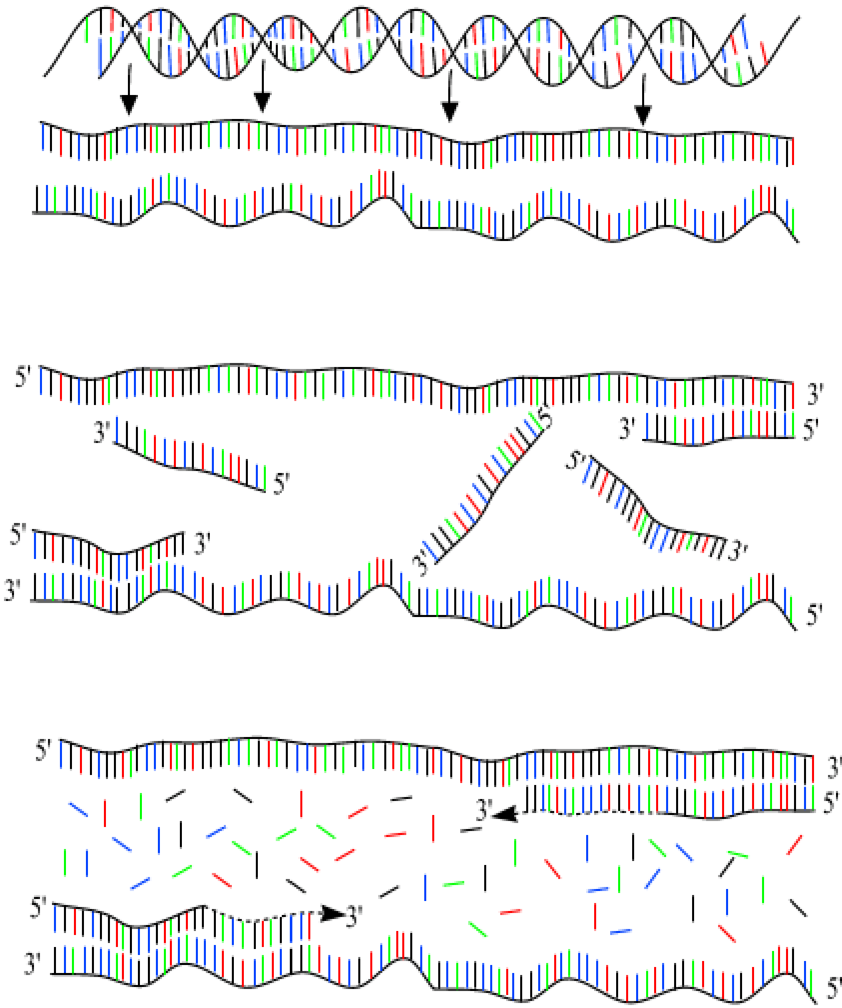


Second letter

First letter	U	C	A	G	Third letter
U	UUU, UUC } Phe UUA, UUG } Leu	UCU, UCC, UCA, UCG } Ser	UAU, UAC } Tyr **UAA Stop** **UAG Stop**	UGU, UGC } Cys **UGA Stop** UGG Trp	U C A G
C	CUU, CUC, CUA, CUG } Leu	CCU, CCC, CCA, CCG } Pro	CAU, CAC } His CAA, CAG } Gln	CGU, CGC, CGA, CGG } Arg	U C A G
A	AUU, AUC, AUA } Ile **AUG Met**	ACU, ACC, ACA, ACG } Thr	AAU, AAC } Asn AAA, AAG } Lys	AGU, AGC } Ser AGA, AGG } Arg	U C A G
G	GUU, GUC, GUA, GUG } Val	GCU, GCC, GCA, GCG } Ala	GAU, GAC } Asp GAA, GAG } Glu	GGU, GGC, GGA, GGG } Gly	U C A G

PCR : Polymerase Chain Reaction

30 - 40 cycles of 3 steps :



Step 1 : denaturation

1 minut 94 °C

Step 2 : annealing

45 seconds 54 °C

forward and reverse primers !!!

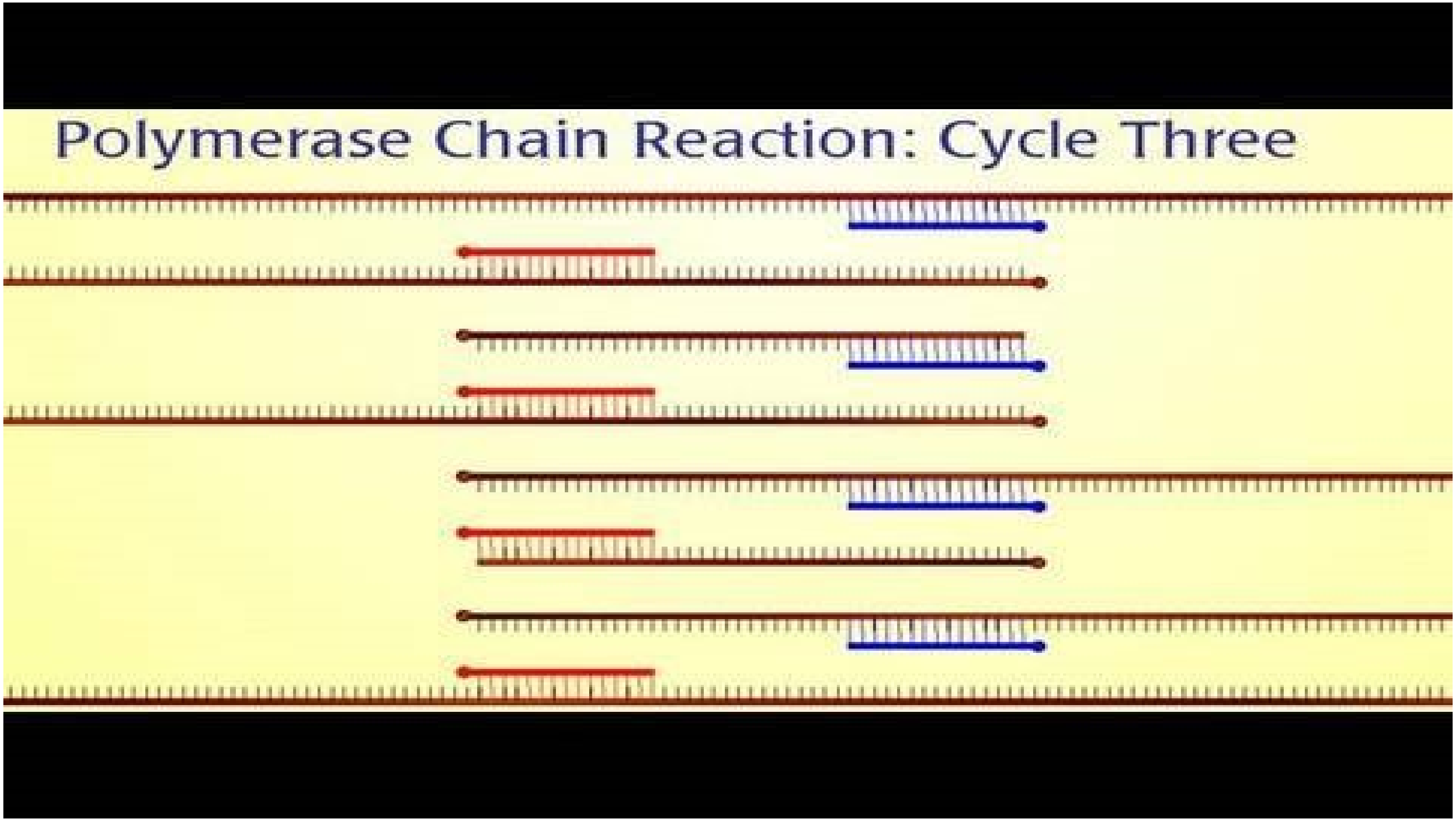
Step 3 : extension

2 minutes 72 °C

only dNTP's

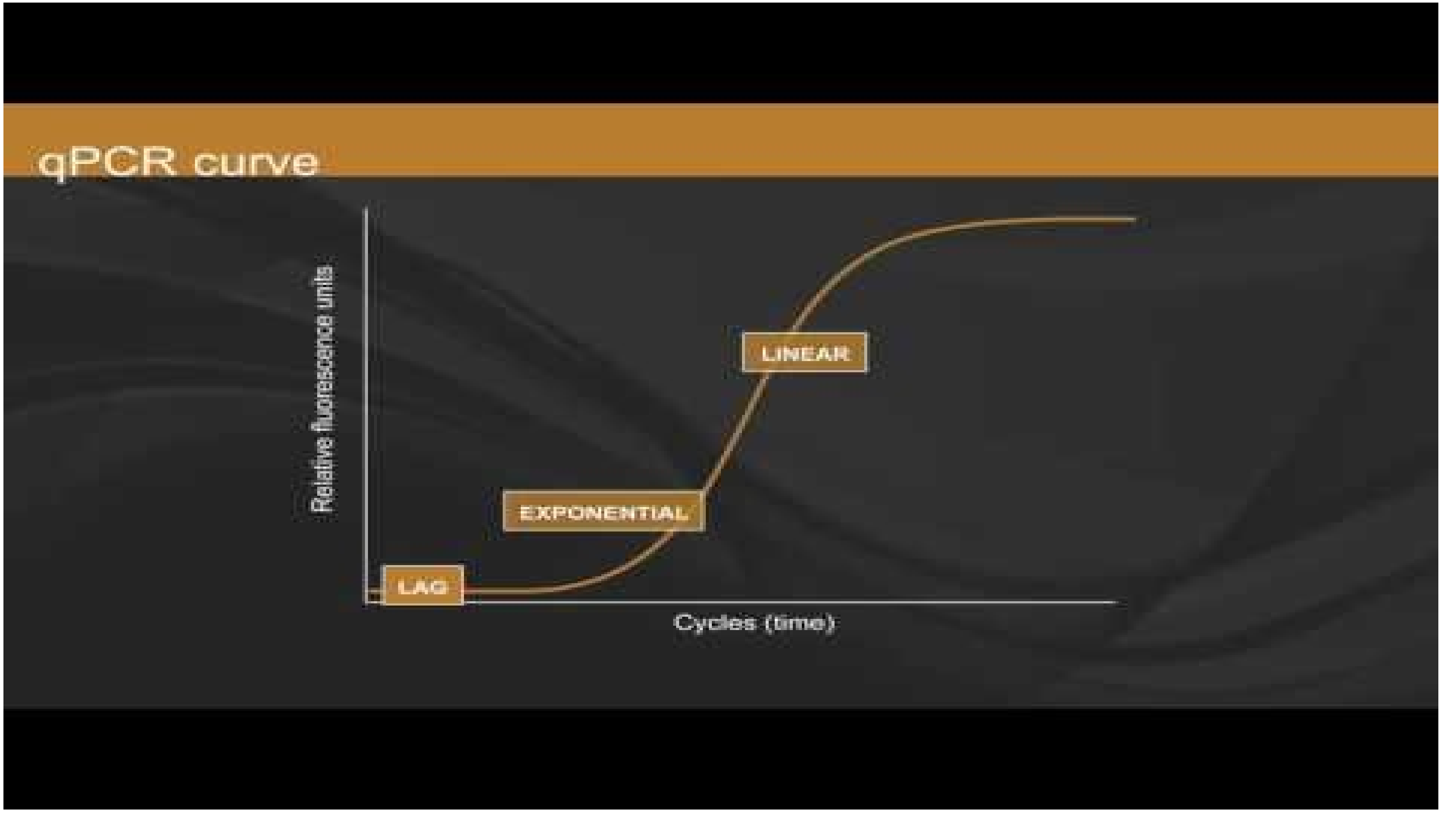
(Andy Vierstraete 1999)

PCR



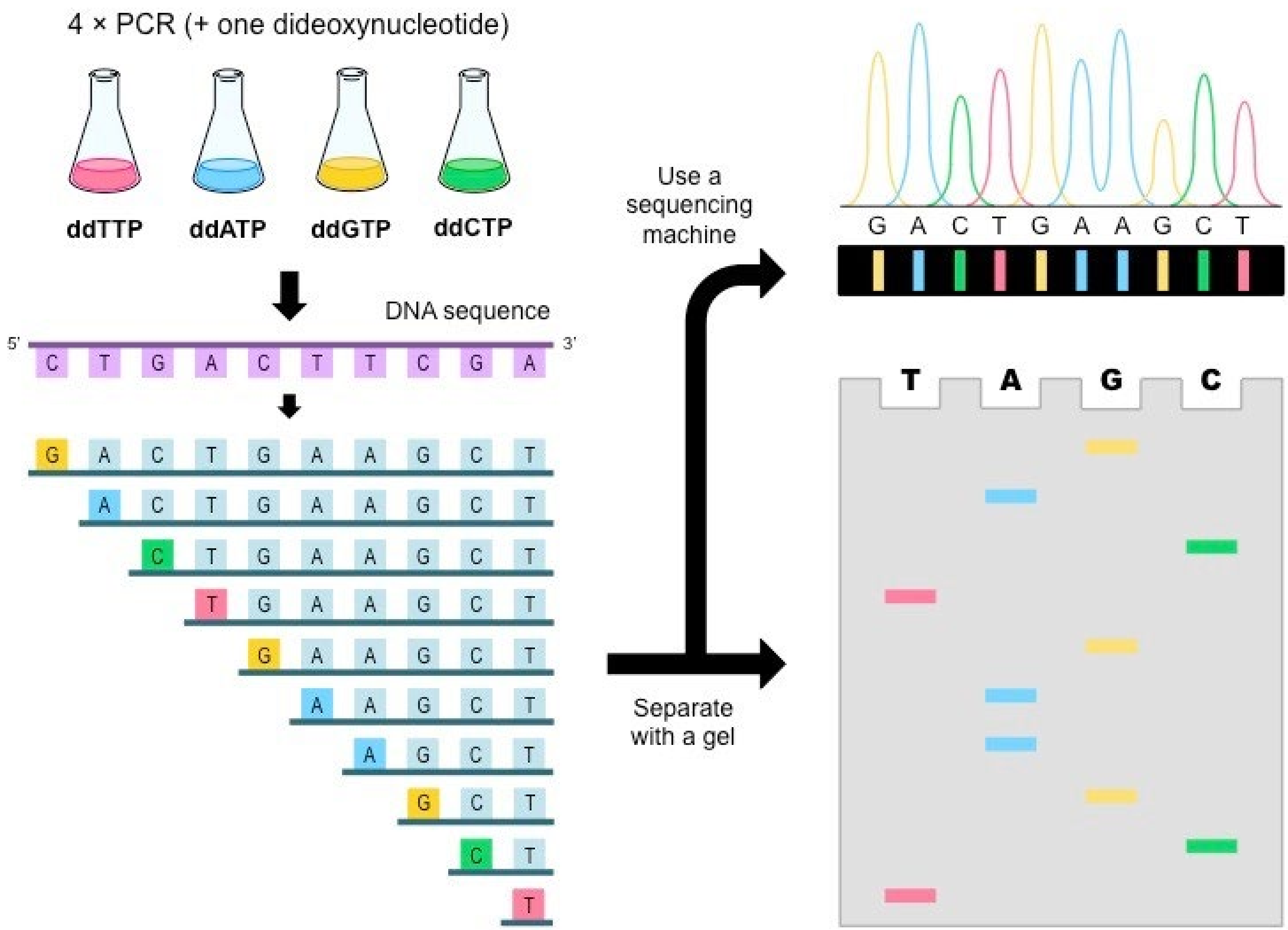
https://www.youtube.com/watch?v=JRAA4C2OPwg

Real-time PCR



https://www.youtube.com/watch?v=1kvy17ugI4w

DNA Sequencing

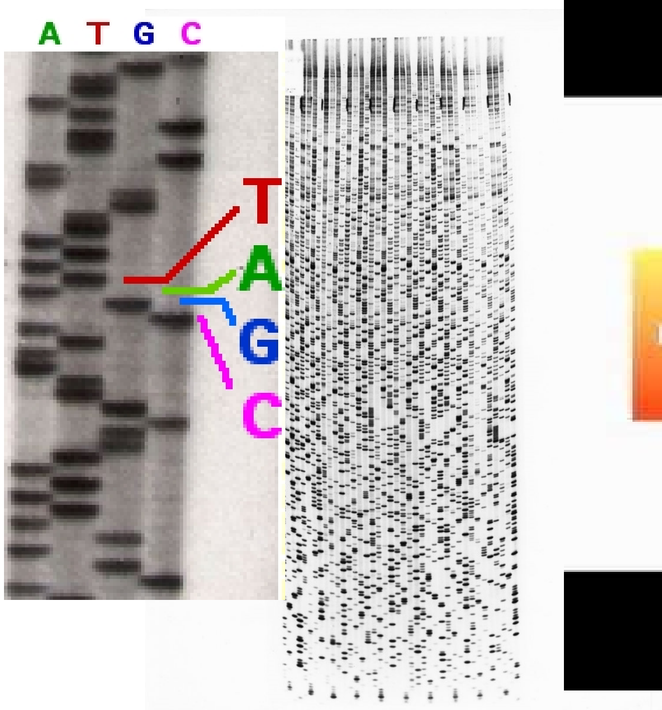


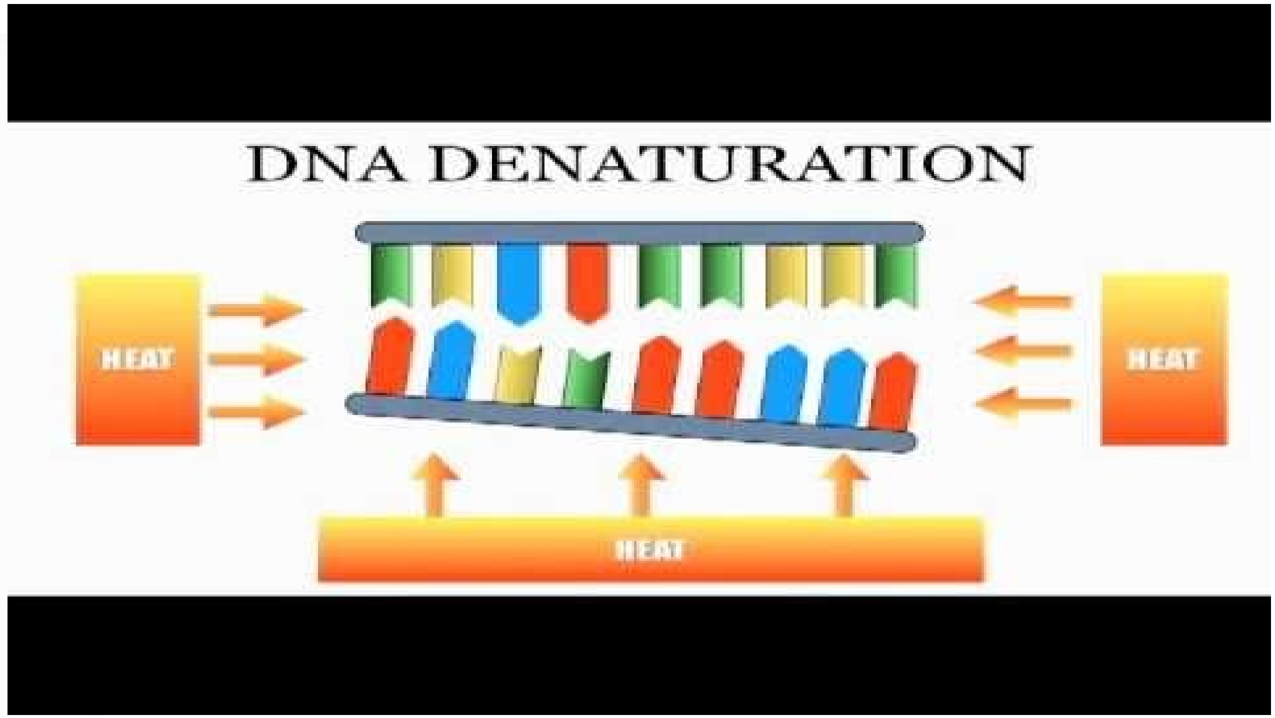
What is Sanger Sequencing

DNA Template Preparation: The DNA sequence to be determined is used as a template. This DNA is denatured to create single-stranded templates.
Primer Annealing: A short, single-stranded piece of DNA, known as a primer, which is complementary to a region of the template, is annealed (bound) to the template to initiate DNA synthesis.
Chain Elongation: A special mixture is prepared that includes the normal deoxynucleotide triphosphates (dNTPs) required for DNA synthesis and a small proportion of chain-terminating dideoxynucleotide triphosphates (ddNTPs). The DNA polymerase enzyme extends the primer by adding nucleotides complementary to the template until a ddNTP is incorporated, causing the extension to terminate.
Fragment Separation: The result is a mixture of new DNA strands of varying lengths, each terminating at a different point. These fragments are then separated by size through capillary electrophoresis.
Result Interpretation: The terminal base of each fragment can be identified based on the label of the ddNTP (each of the four ddNTPs is labeled with a different fluorescent dye). By ordering the fragments by size, the DNA sequence can be read from the shortest to the longest fragment, providing the sequence of the template DNA.
While newer sequencing technologies (next-generation sequencing methods) offer faster and cheaper ways to sequence DNA, Sanger sequencing is still used today for its high accuracy, especially for sequencing small DNA fragments and validating DNA sequences obtained by other methods.

GPT4

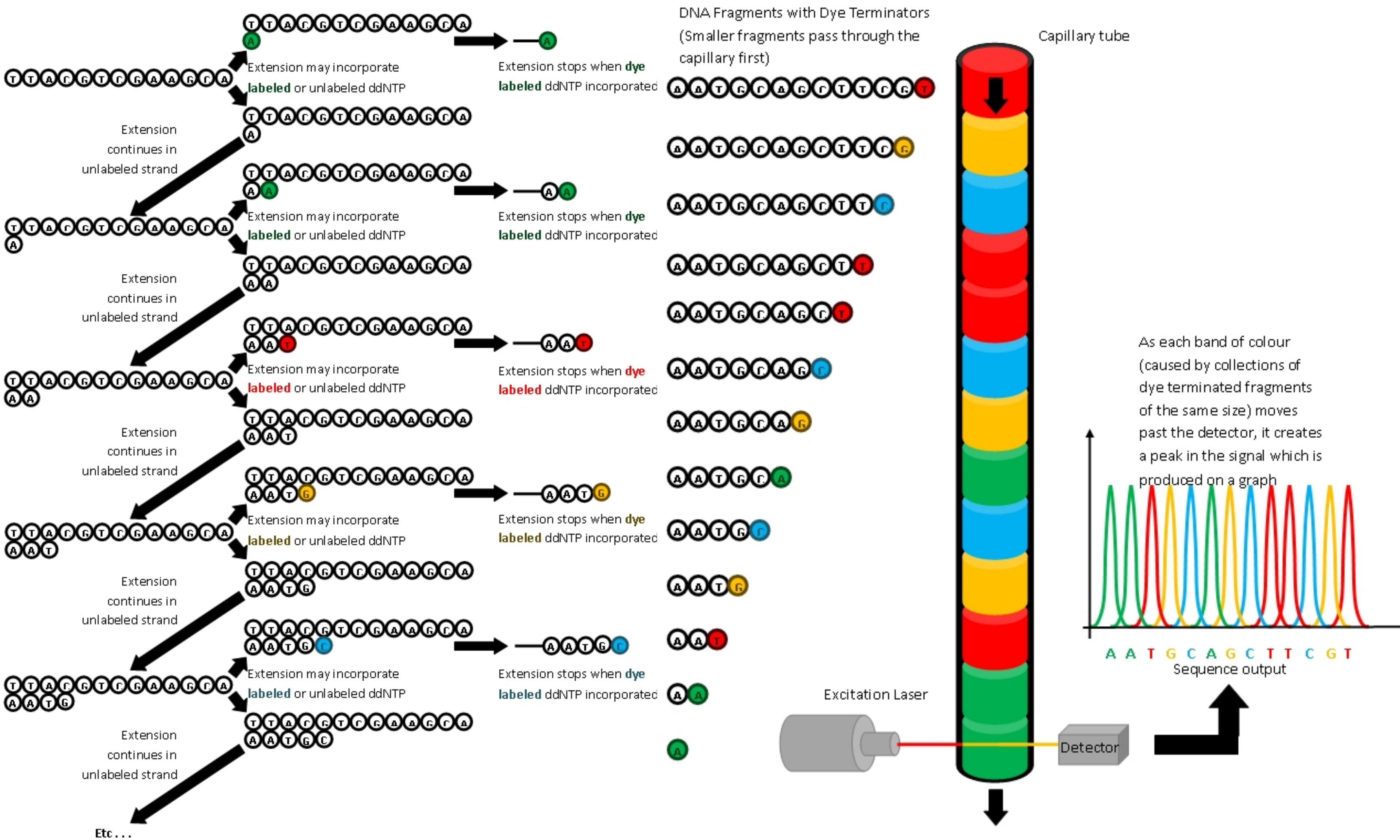
DNA Sequencing



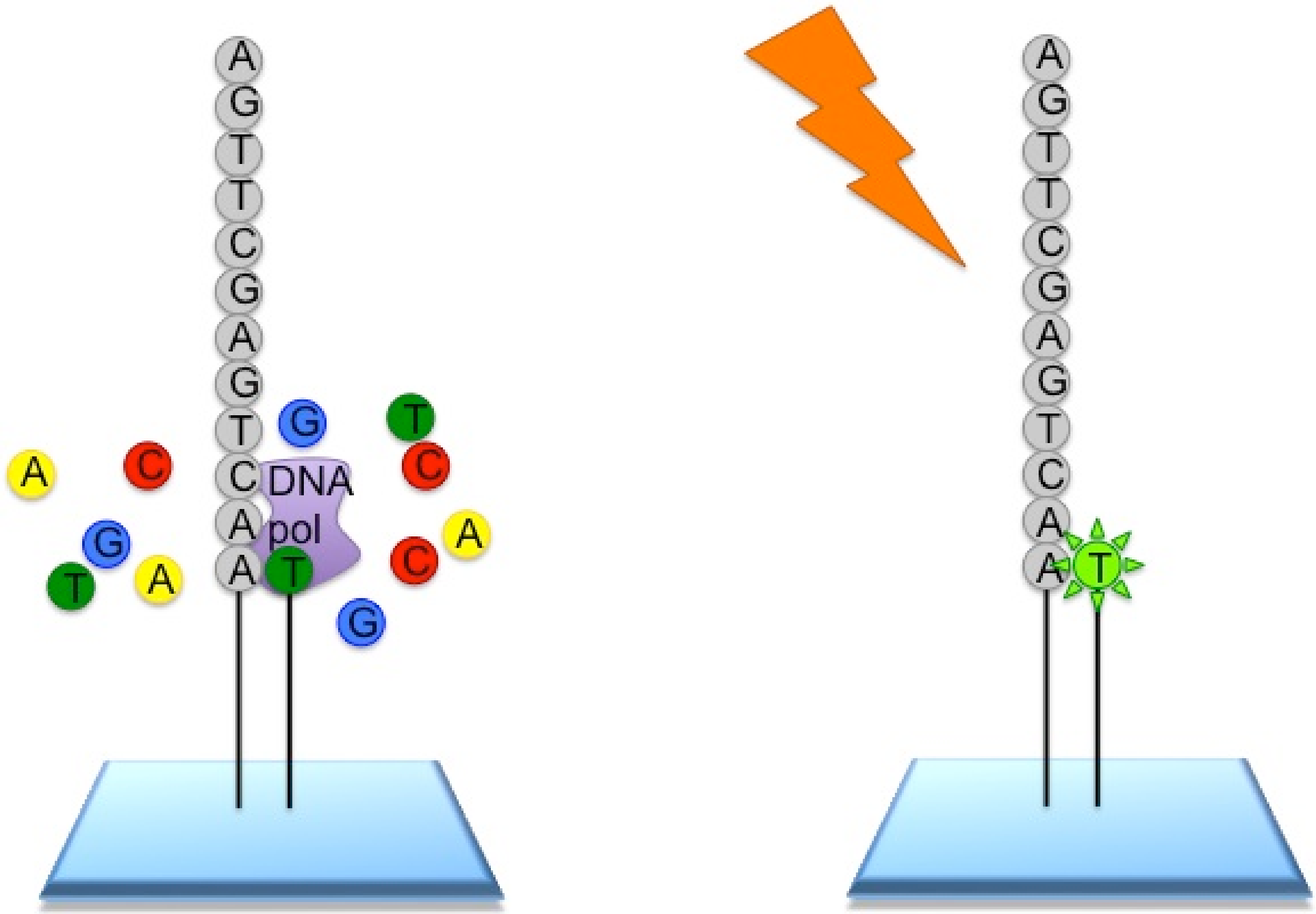


https://www.youtube.com/watch?v=vK-HIMaitnE

Dye Terminations



NGS Illumina



100-150 bp

What is NGS

Here's a basic overview of how NGS works:

Library Preparation:

The first step in NGS is preparing a library of the sample DNA (or RNA, in the case of RNA sequencing). This involves fragmenting the sample DNA into smaller pieces, then attaching adapters to both ends of the fragments. These adapters serve multiple functions: they allow the fragments to bind to the sequencing platform, include sequencing primer binding sites, and may include unique barcodes that enable the multiplexing of different samples in a single sequencing run.

Amplification:

Once the library is prepared, the DNA fragments are amplified, typically using a method like PCR or bridge amplification on a solid surface. This step increases the quantity of DNA, ensuring there is enough signal for detection during sequencing.

Sequencing:

NGS platforms use different methods to sequence the amplified DNA fragments. Common approaches include:
Sequencing by Synthesis (Illumina): This is the most widely used method, where DNA polymerase synthesizes a complementary strand to the template, incorporating fluorescently labeled nucleotides that are detected by the sequencer.

Data Analysis:

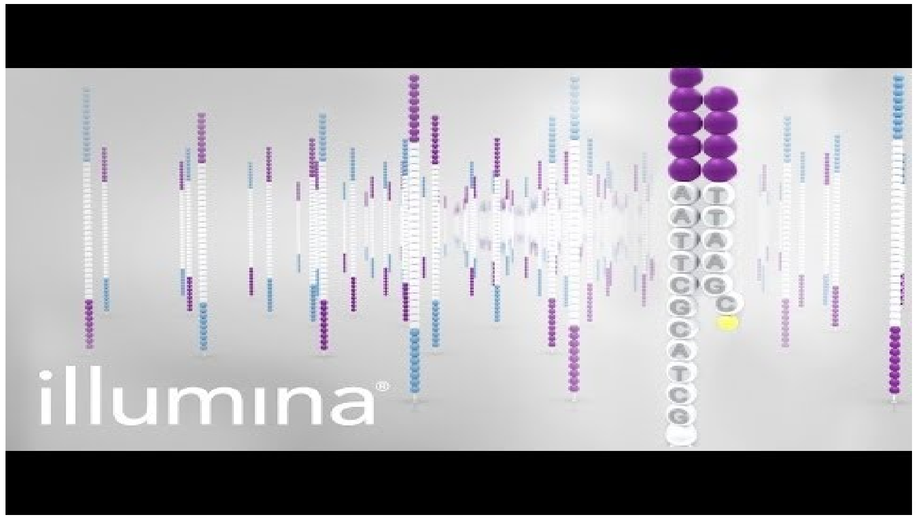
The raw data from an NGS platform consist of millions to billions of short DNA sequences (reads). These reads are then processed and analyzed using bioinformatics tools. Depending on the application, this may involve aligning the reads to a reference genome, assembling them into longer sequences, identifying variants, or quantifying gene expression levels.

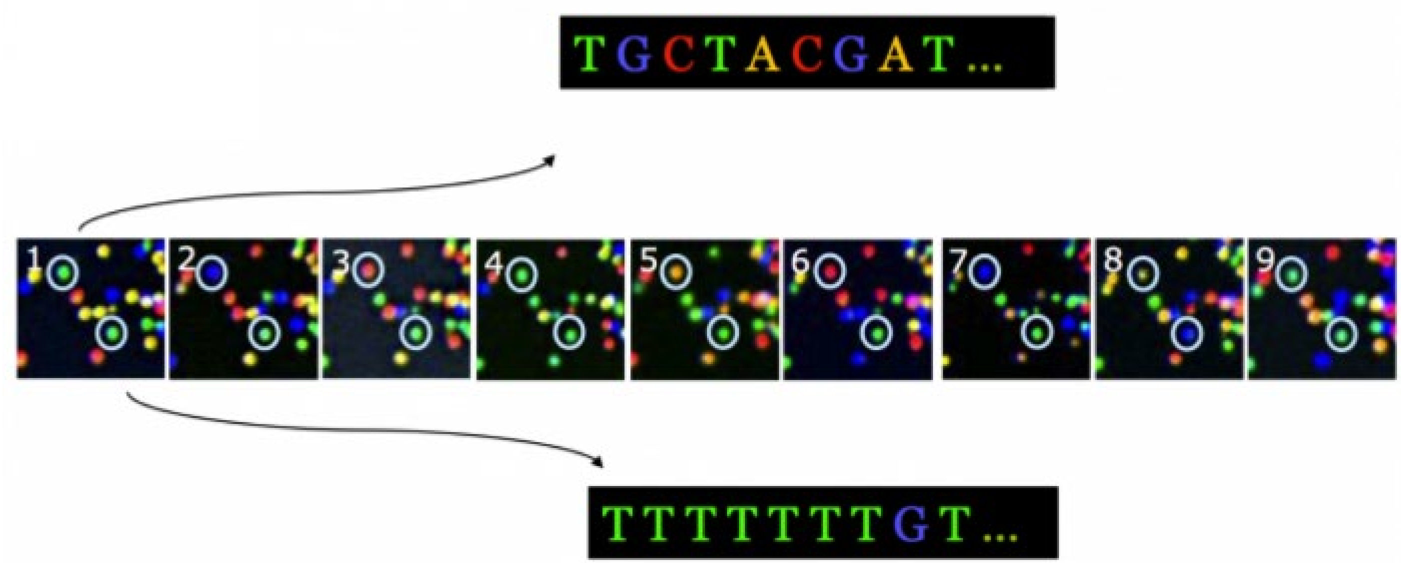
Applications:

NGS can be used for a wide variety of applications, including whole-genome sequencing, targeted sequencing of specific genes or regions, transcriptome analysis (RNA-seq), metagenomics, and epigenomics.
Overall, NGS technologies provide a powerful set of tools for exploring the genetic and molecular basis of life, with applications ranging from basic biological research to clinical diagnostics and personalized medicine.

NGS Illumina

https://www.youtube.com/watch?v=fCd6B5HRaZ8



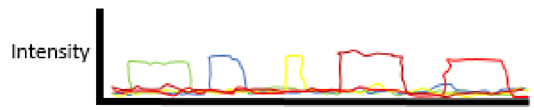


Third Generation Sequencing

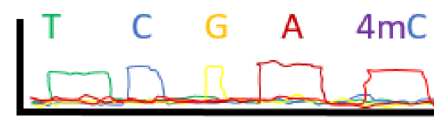
PacBio SMRT seq

DNA passes thru polymerase in an illuminated volume

Raw output is fluorescent signal of the nucleotide incorporation, specific to each nucleotide

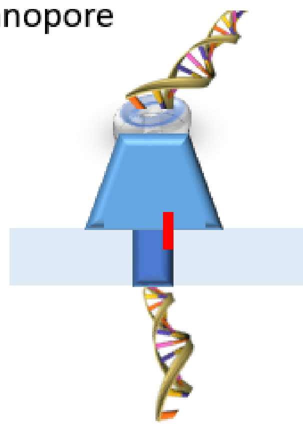


A,C,T,G have known pulse durations, which are used to infer methylated nucleotides

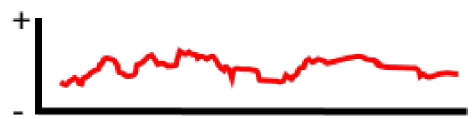


Oxford Nanopore

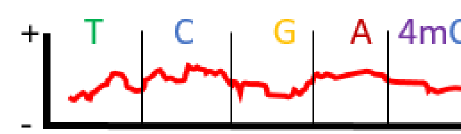
DNA passes thru nanopore



Raw output is electrical signal caused by nucleotide blocking ion flow in nanopore



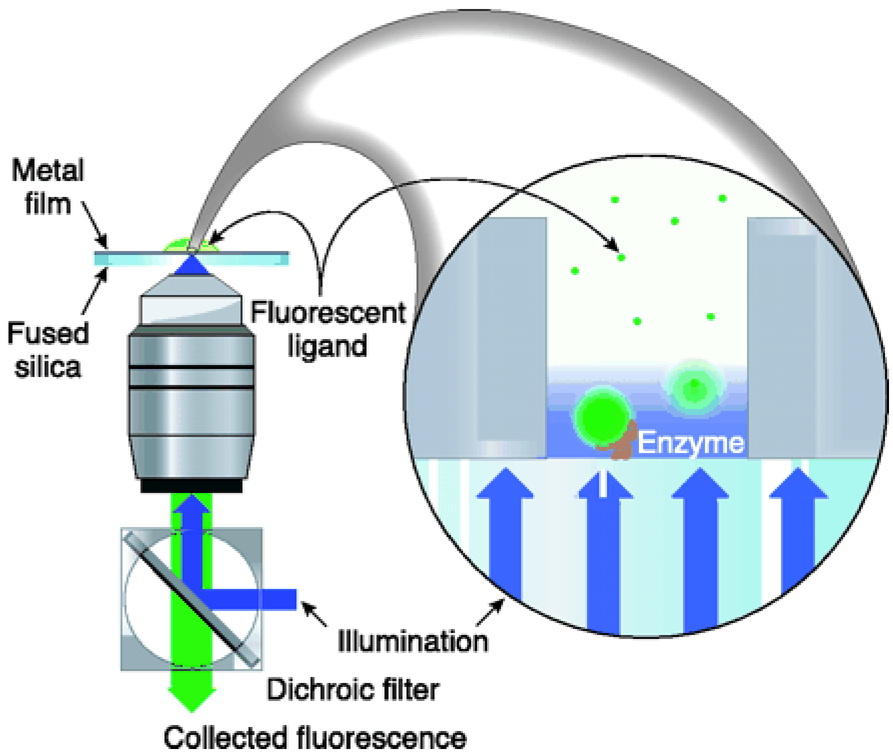
Each nucleotide has a specific electric “signature”



PacBio SMRT Sequencing:
Single Molecule, Real-Time (SMRT) sequencing by Pacific Biosciences uses zero-mode waveguides (ZMWs), tiny wells that allow the observation of a single DNA polymerase molecule as it incorporates nucleotides into a DNA strand.
During sequencing, each of the four DNA bases is attached to a different fluorescent dye. As a DNA polymerase adds a nucleotide to the growing DNA strand inside a ZMW, the incorporated base emits a fluorescent signal, which is detected in real time.
SMRT sequencing enables the reading of very long DNA fragments (**up to 20 kb or more**), which helps in resolving complex genomic regions, identifying large structural variations, and improving genome assembly.
Oxford Nanopore Sequencing:
This technology uses protein nanopores set in an electrically resistant polymer membrane. When a voltage is applied across the membrane, an ionic current flows through the nanopores.
As a DNA or RNA molecule passes through a nanopore, it causes characteristic disruptions in the current. Each type of base (A, C, G, T for DNA; A, C, G, U for RNA) disrupts the current in a unique way, allowing the sequence to be determined.
Nanopore sequencing can process very long strands of DNA or RNA directly, which provides advantages in mapping long repetitive regions and characterizing full-length transcripts in transcriptome studies.
Both technologies have greatly expanded the capabilities of genomic analyses by offering:
Longer Reads: They provide much longer reads than second-generation sequencing, which is crucial for de novo genome assembly, spanning repetitive sequences, and fully characterizing genomic rearrangements.
Single-Molecule Sequencing: Both technologies sequence individual DNA molecules, which helps in detecting base modifications such as methylation directly during the sequencing process, without the need for additional chemical treatments.
Real-Time Data Access: These technologies allow for the monitoring of the sequencing process in real time, enabling rapid access to data and the potential for dynamic experimental adjustments.
Despite these advantages, **third-generation sequencing methods also have limitations, such as higher error rates compared to second-generation sequencin**g. However, these errors are random rather than systematic, which means they can often be overcome with sufficient coverage or by combining with short-read sequencing data for validation and error correction. As the technology continues to evolve, improvements in accuracy, cost, and throughput are expected to further expand the applications of third-generation sequencing.

Zero Mode Waveguide

https://www.youtube.com/watch?v=NHCJ8PtYCFc



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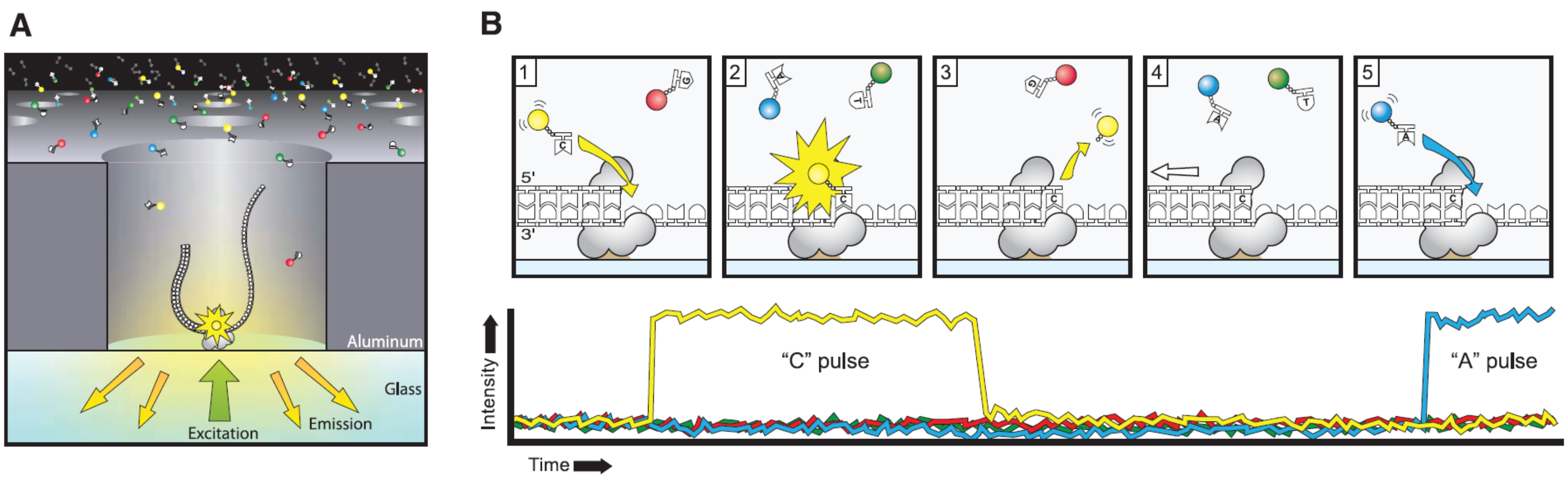
Real-Time DNA Sequencing from Single Polymerase Molecules



Nanopore Sequencing

https://www.youtube.com/watch?v=RcP85JHLmnI

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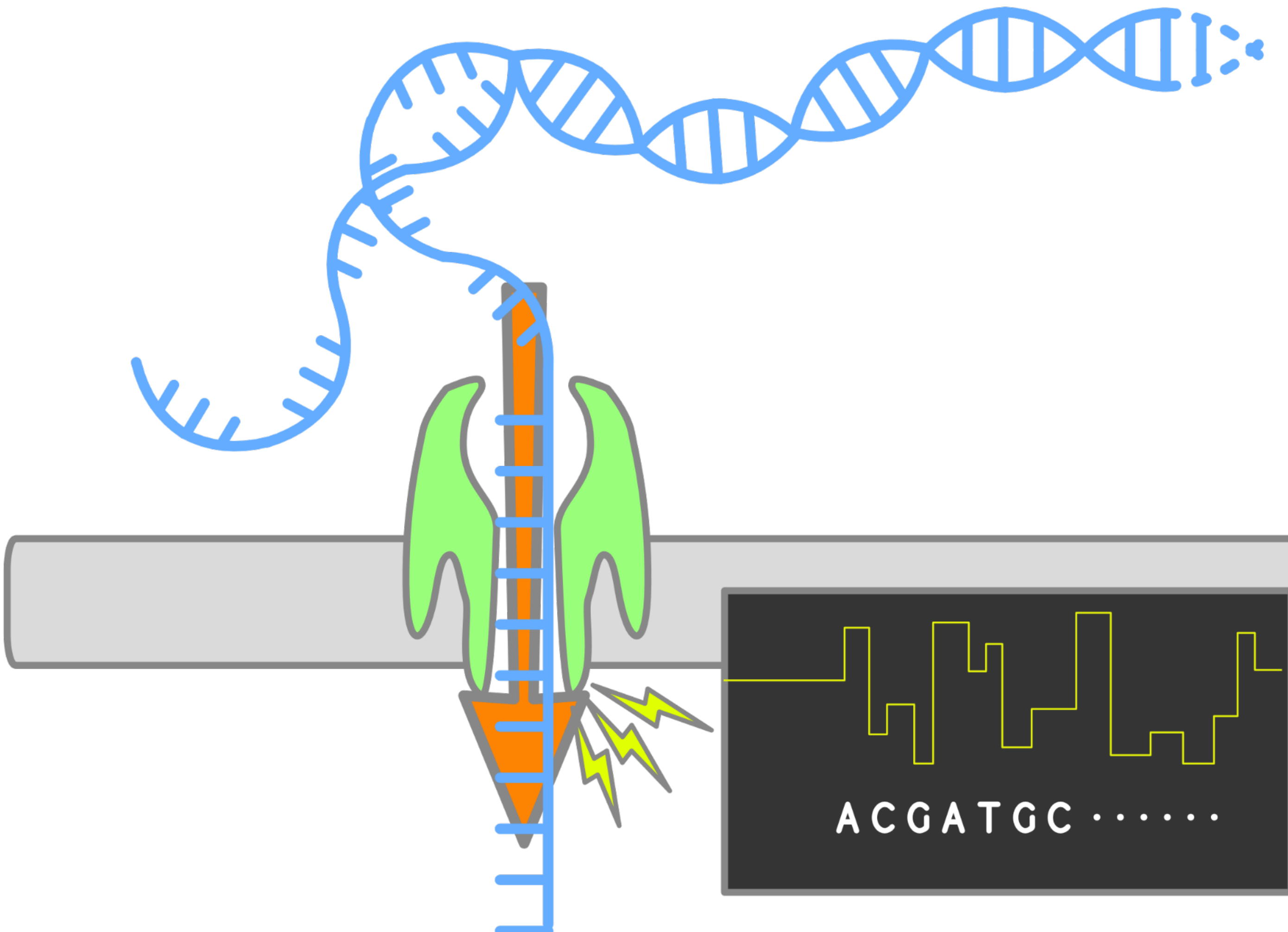
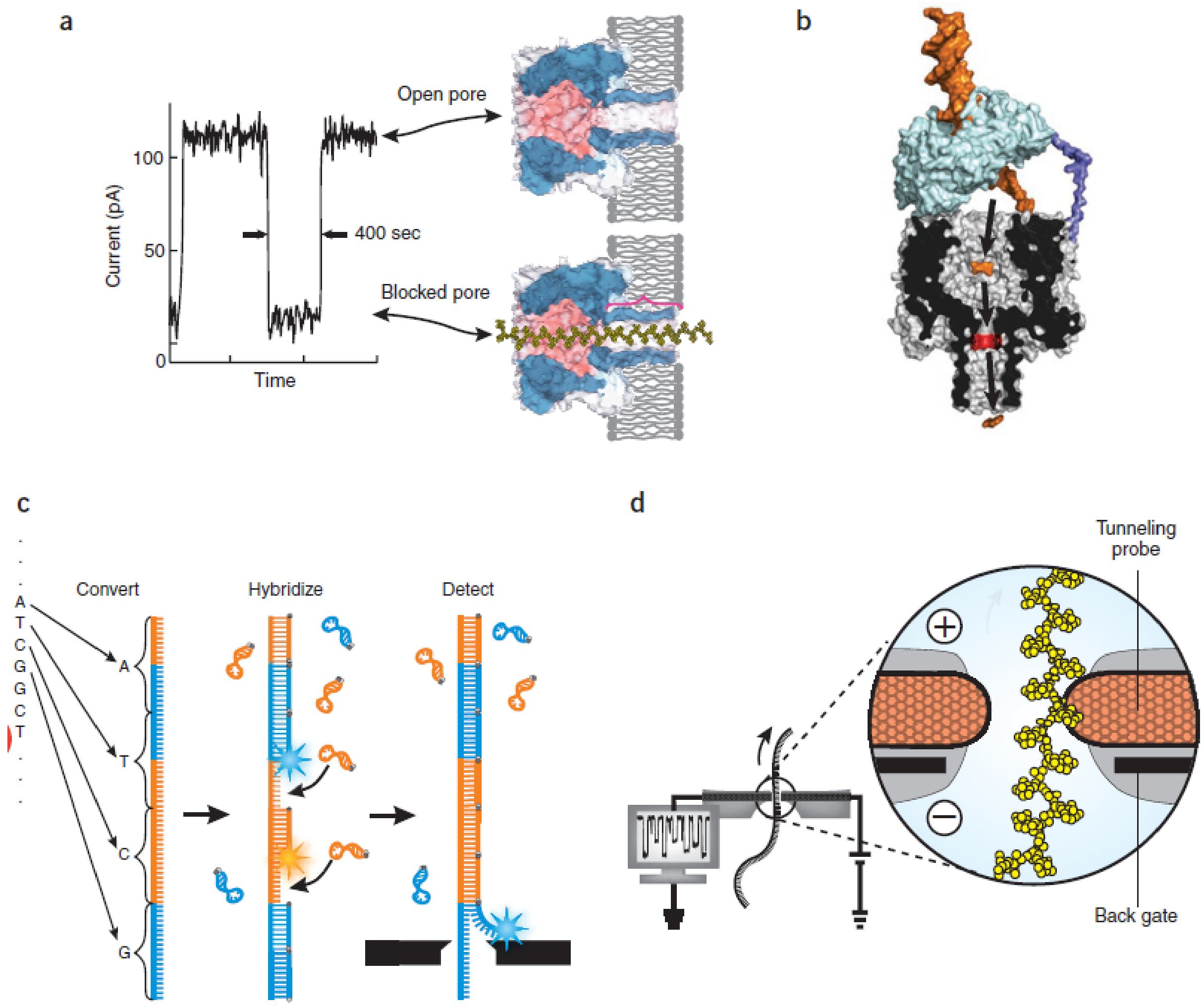



a
Open pore
Blocked pore
400 sec
Current (pA)
100
50
0
Time
b
c
Convert
Hybridize
Detect
A
T
C
G
G
C
T
d
Tunneling
probe
Back gate

Single-cell analysis refers to the study of individual cells isolated from tissues in a mixed population, which provides a detailed examination of the cellular differences and a deeper understanding of the biological function and complexity at the single-cell level. This approach is crucial in various fields of biological sciences and medicine, including developmental biology, neuroscience, immunology, and cancer research. Below are key aspects and methodologies of single-cell analysis:

Cell Isolation: The first step in single-cell analysis is to isolate individual cells from a tissue or cell culture. Various techniques can be used for this purpose, including flow cytometry, microfluidics, laser capture microdissection, and manual cell picking.

Single-Cell Sequencing: Once individual cells are isolated, their genomic, transcriptomic, or epigenomic content can be analyzed. Single-cell RNA sequencing (scRNA-seq) is one of the most common methods, allowing researchers to measure gene expression levels in individual cells. This provides insights into the cellular heterogeneity within a tissue and can identify distinct cell types and states, even within seemingly homogeneous cell populations.

Single-Cell Genomics and Epigenomics: Beyond transcriptomics, single-cell DNA sequencing can reveal genomic variations at the single-cell level, such as mutations or copy number variations. Single-cell epigenomics, including methods like single-cell ATAC-seq, can assess chromatin accessibility and other epigenetic features, offering clues about the regulatory mechanisms driving gene expression in individual cells.

Proteomics and Metabolomics: Advanced techniques also enable the analysis of proteins and metabolites at the single-cell level. These approaches can provide functional data that complements genomic and transcriptomic information, offering a more comprehensive view of the cell's state and activity.

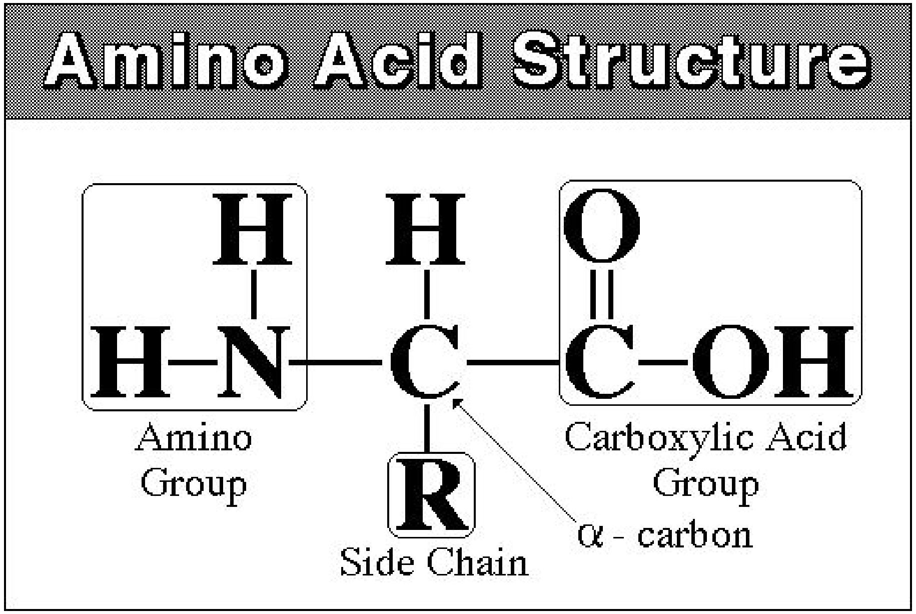
Spatial Analysis: Recent advancements have enabled the spatial characterization of cells within their native tissue contexts. Techniques like spatial transcriptomics and imaging-based methods allow researchers to understand not only the individual cell profiles but also their spatial organization and interactions within the tissue.

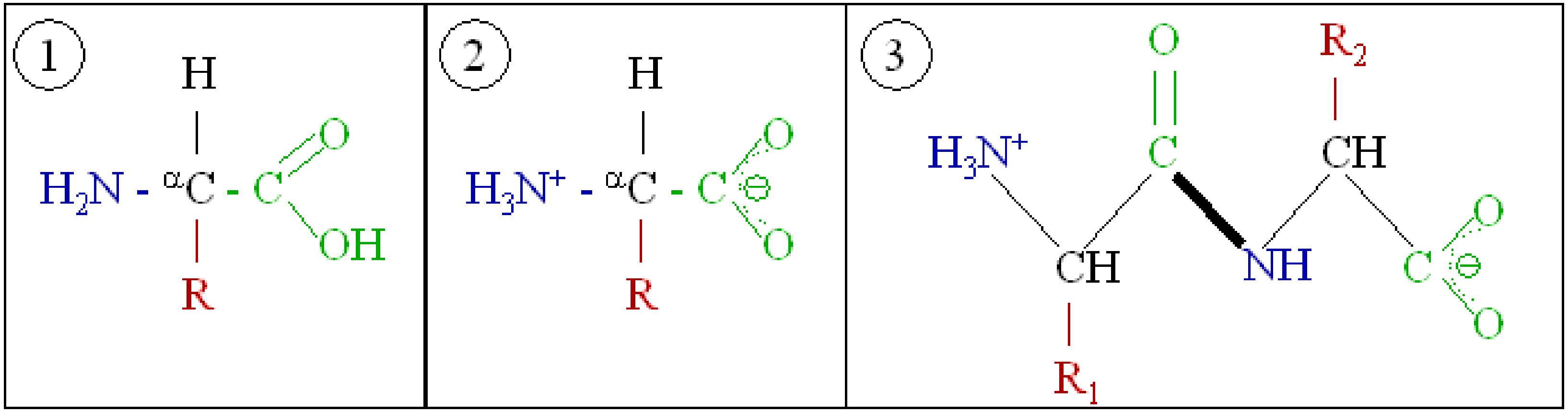
Data Analysis and Integration: Single-cell datasets are typically large and complex, requiring advanced computational tools for analysis and interpretation. Bioinformatics and data analysis methods are employed to cluster cells into distinct groups, identify marker genes, analyze differential expression, and infer developmental trajectories or cell-cell interaction networks.

Applications: Single-cell analysis has numerous applications across various fields of biology and medicine. For example, in cancer research, it can reveal the heterogeneity within tumors, identify rare cancer stem cells, or uncover mechanisms of drug resistance. In developmental biology, it can elucidate cell lineage relationships and developmental pathways. In immunology, it helps in characterizing immune cell diversity and responses.

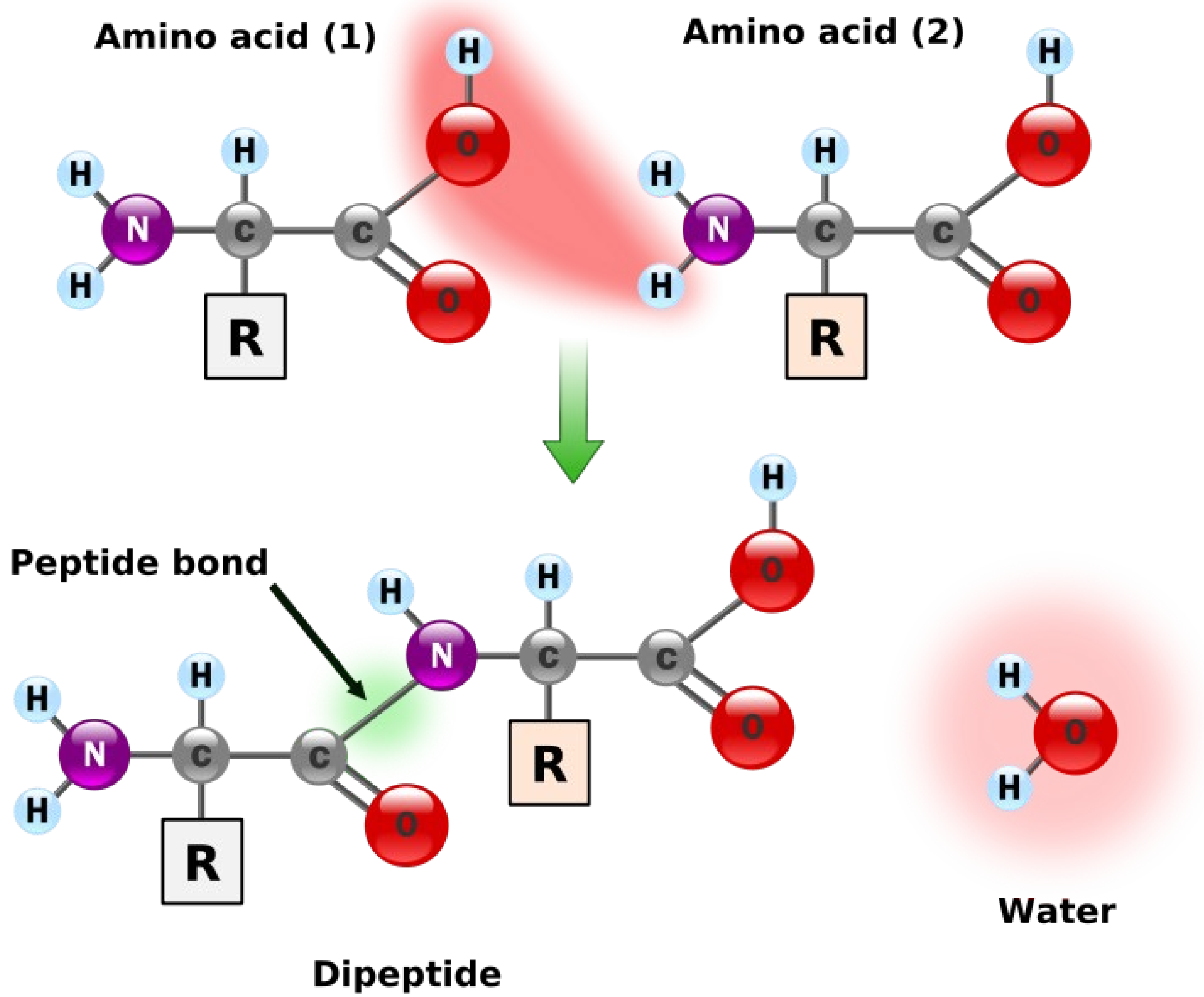
Quality Control and Data Preprocessing:
Quality control (QC) is crucial to remove low-quality cells and genes that could distort the analysis. This step typically involves filtering out cells with extremely high or low total gene counts or high mitochondrial gene expression, which might indicate dead or damaged cells.
Dimensionality Reduction:
High-dimensional single-cell data are often reduced to lower dimensions for visualization and further analysis. Techniques such as PCA (Principal Component Analysis), t-SNE (t-Distributed Stochastic Neighbor Embedding), and UMAP (Uniform Manifold Approximation and Projection) are popular for this purpose.
Dimensionality reduction serves to highlight the inherent structure of the data, revealing clustering of cells that might correspond to different cell types or states.
Clustering:
Once the data are in a reduced dimension space, clustering algorithms can identify groups of cells with similar expression profiles. These clusters often correspond to different cell types or subtypes within the sample.
Various clustering algorithms can be used, such as k-means, hierarchical clustering, or graph-based clustering methods (e.g., Louvain algorithm). The choice of algorithm and parameters (e.g., resolution in Louvain) can significantly affect the results, so it may require optimization based on the data.
Differential Expression Analysis:
To characterize the identified clusters and infer their biological significance, differential expression analysis is performed to identify genes that are significantly up- or down-regulated between clusters.
This step can highlight marker genes that define cell types or states and provide insights into the biological processes and pathways active in different cell populations.
Annotation and Interpretation:
The next step is to annotate the identified cell clusters with known cell types, which can be done by comparing the expression of marker genes in each cluster with known profiles from the literature or reference datasets.
Further biological interpretation can involve pathway analysis, gene set enrichment analysis, or network analysis to understand the functions and interactions of the differentially expressed genes.
Integration and Comparison:
If analyzing data from multiple samples or conditions, integration methods can be used to align datasets, allowing for comparative analysis and identification of condition-specific responses or cell types.
Techniques for data integration include canonical correlation analysis, mutual nearest neighbors, or specialized tools like Seurat or Scanpy's integration methods.
Trajectory Inference:

Amino Acid


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


1
H
H2N - αC - C
O
OH
R
2
H
H3N+ - αC - C
O
O
R
3
O
R2
H3N+
C
CH
CH
NH
C
O
O
R1

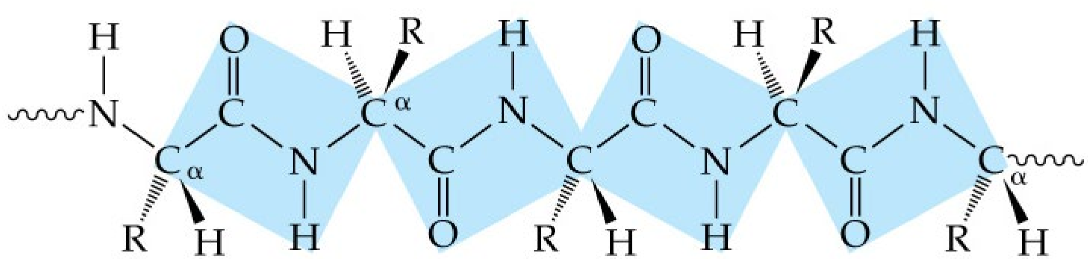
Peptide bond



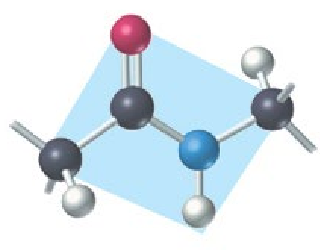
Primary Protein Structure

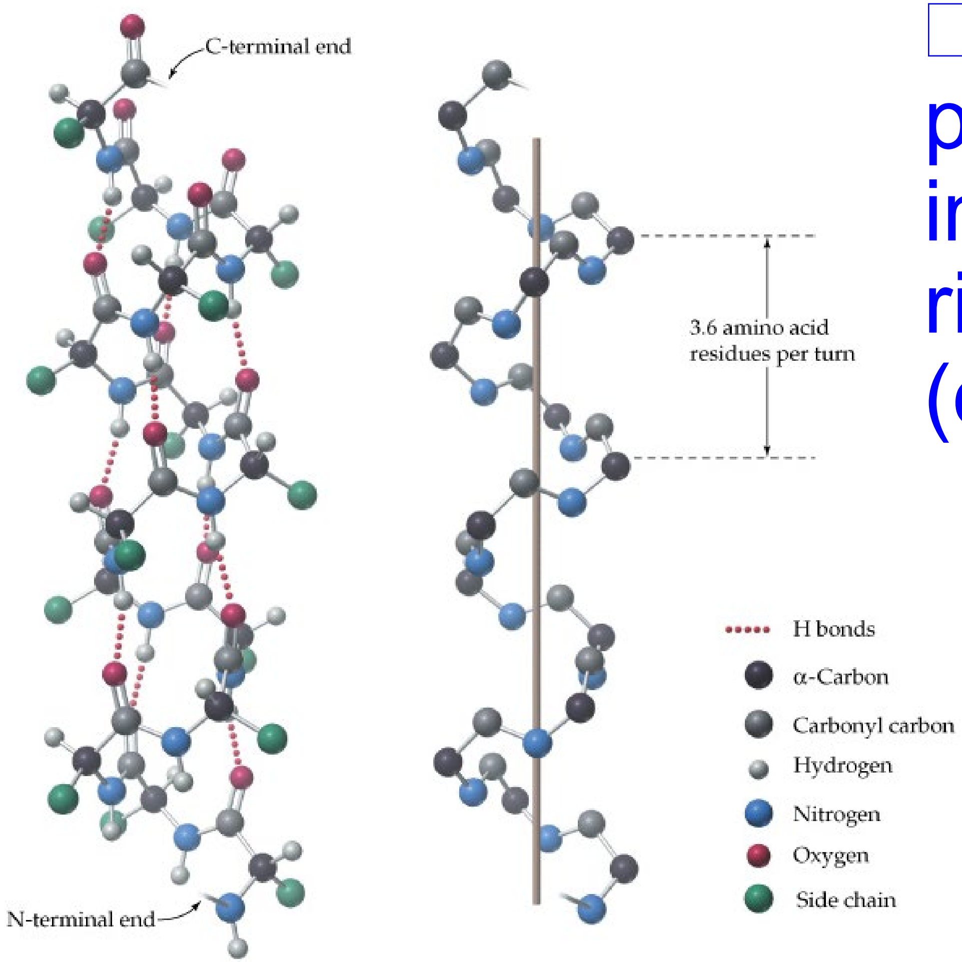
- Primary structure of a proteins is the sequence of amino acids connected by peptide bonds. Along the backbone of the proteins is a chain of alternating peptide bonds and α-carbons and the amino acid side chains are connected to these

Planar units along a protein chain



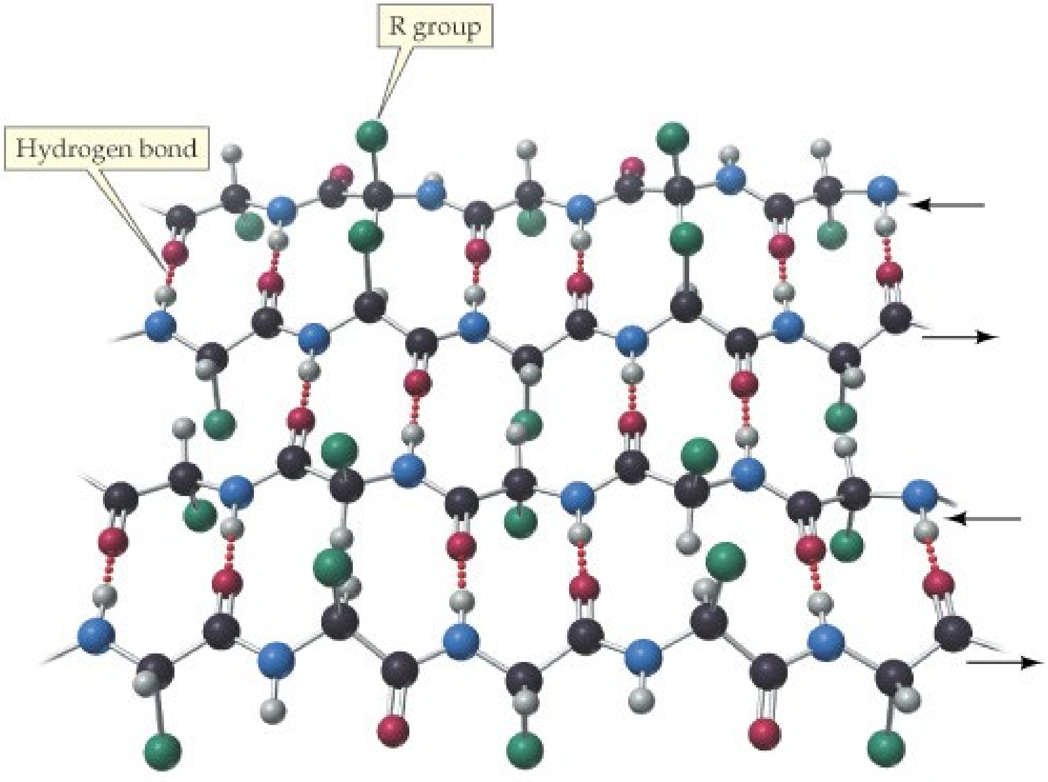
One planar unit



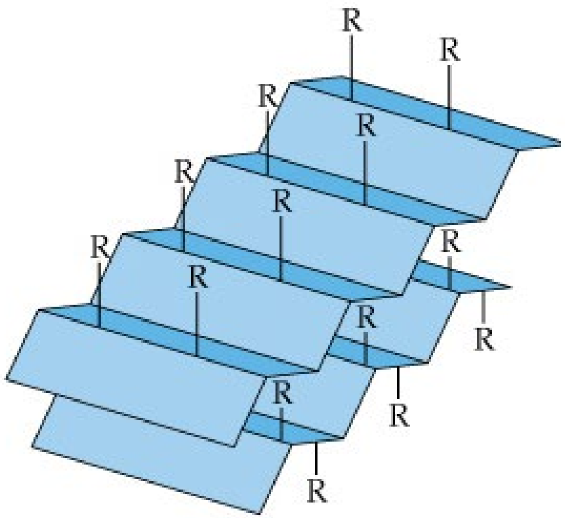


□ ***α-Helix:*** A single protein chain coiled in a spiral with a right-handed (clockwise) twist.

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



(a)

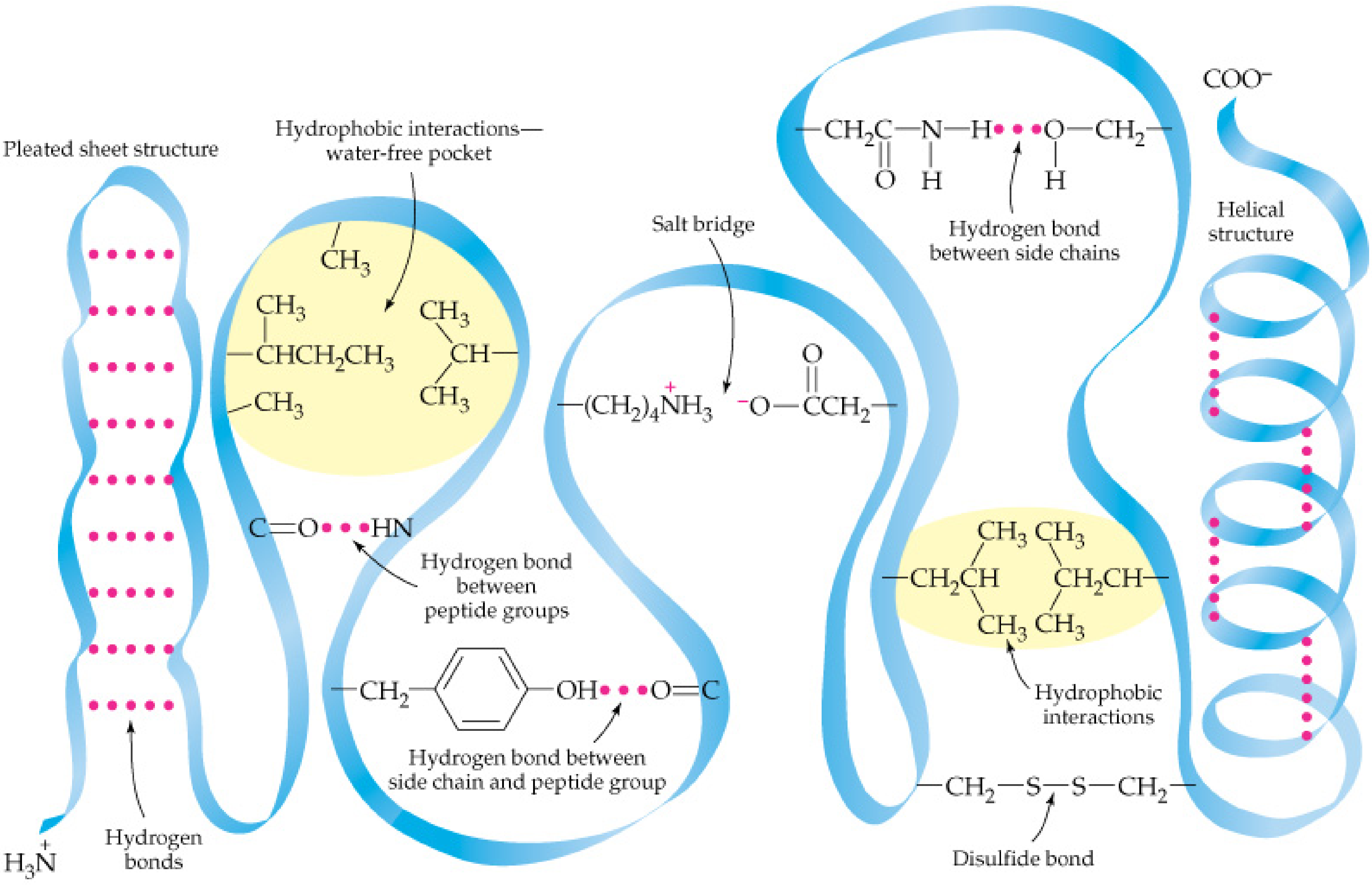


(b)

Tertiary Protein Structure

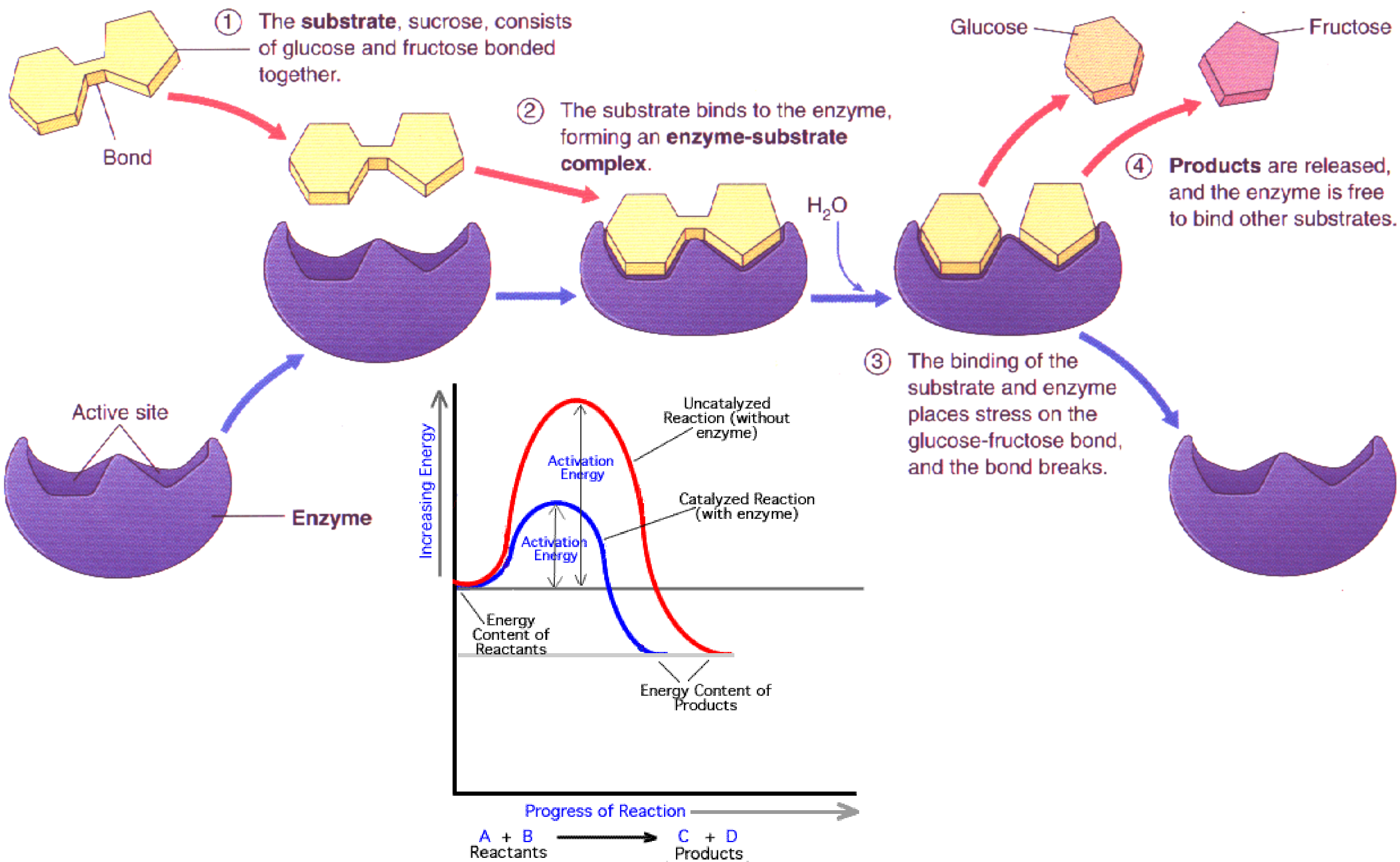
- ***Tertiary Structure of a proteins*** The overall three dimensional shape that results from the folding of a protein chain. Tertiary structure depends mainly on attractions of amino acid side chains that are far apart along the same backbone. Non-covalent interactions and disulfide covalent bonds 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.



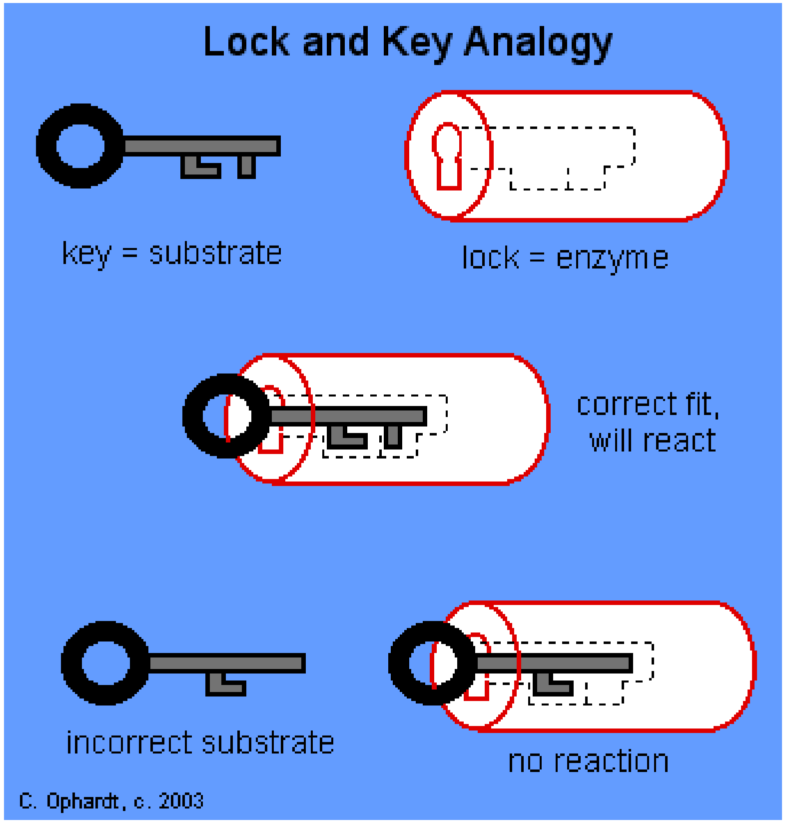
Pleated sheet structure
Hydrophobic interactions—water-free pocket
Salt bridge
Hydrogen bond between side chains
Helical structure
COO⁻
Hydrogen bond between peptide groups
Hydrogen bond between side chain and peptide group
Hydrophobic interactions
Disulfide bond
Hydrogen bonds
H3N+

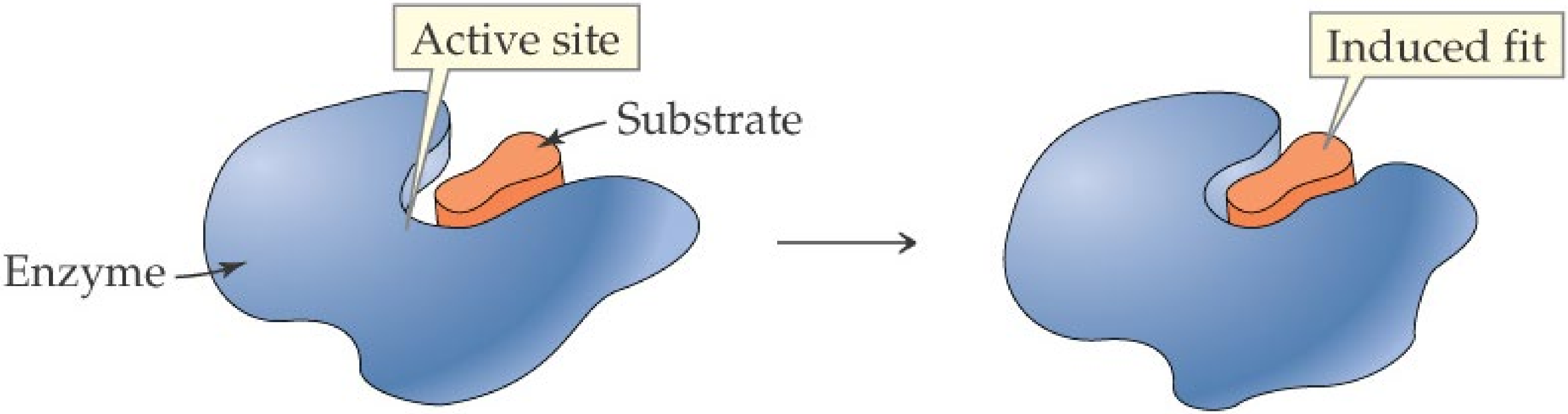
Enzymatic Reaction

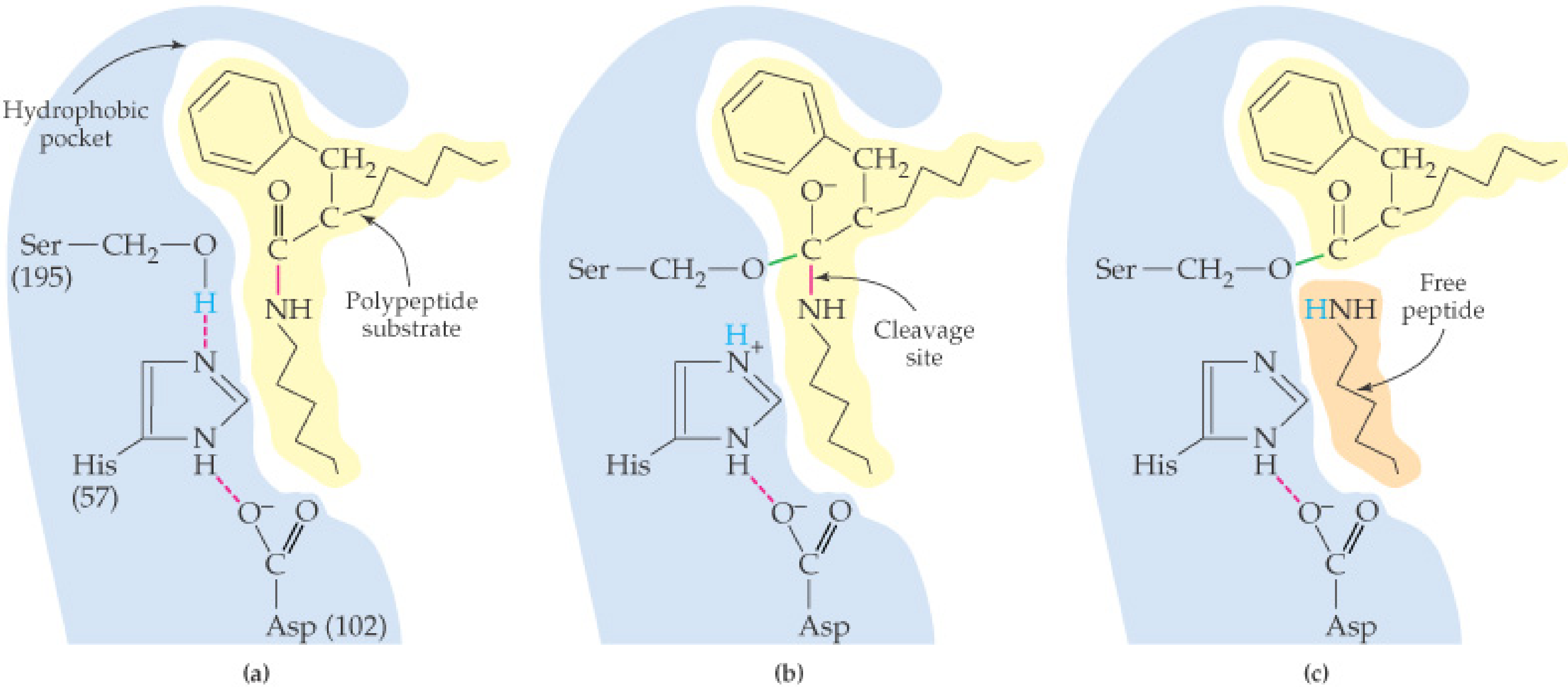


How Enzyme Work

- 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

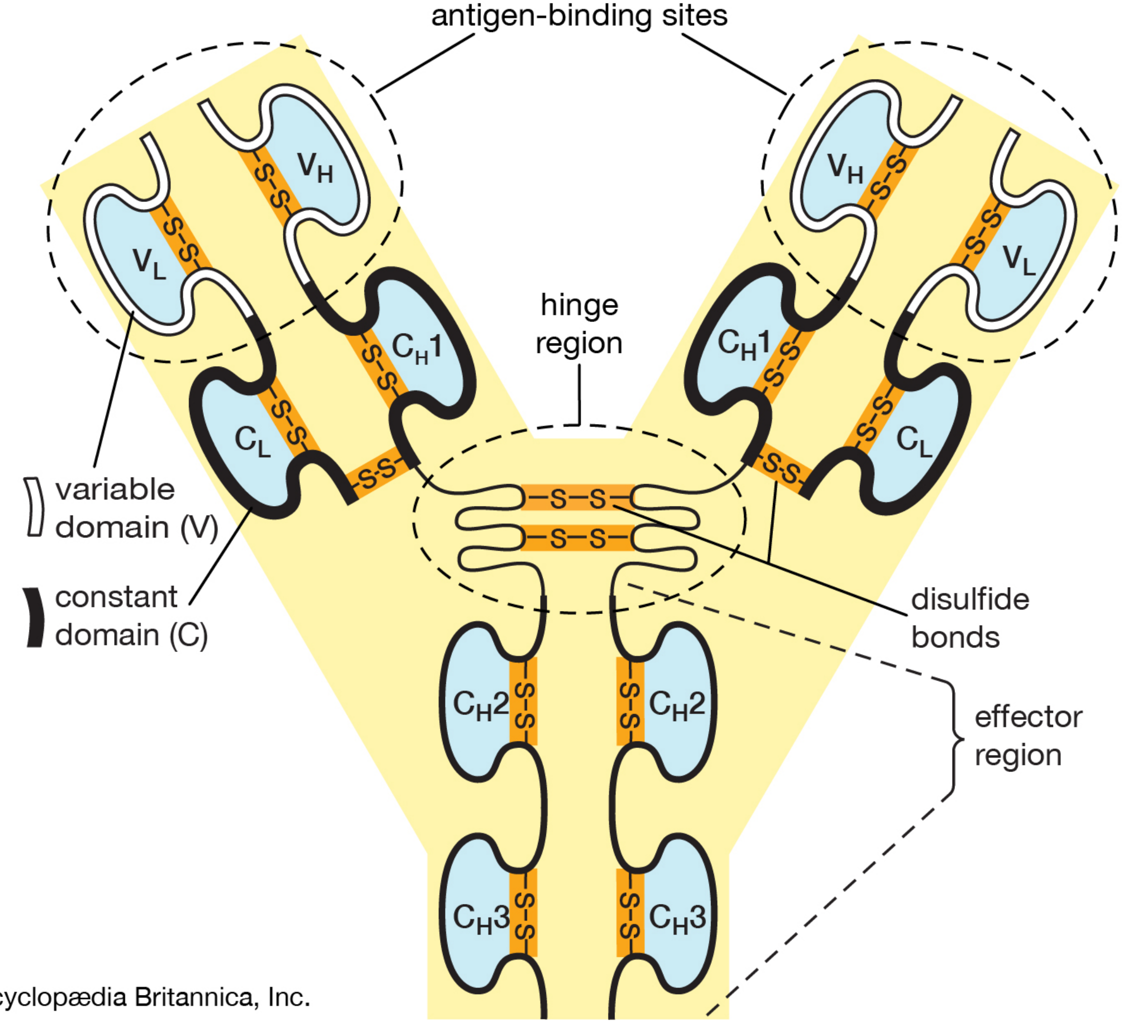

Active site
Substrate
Enzyme
Induced fit


Hydrophobic pocket
Polypeptide substrate
Ser—CH2—O
(195)
His
(57)
Asp (102)
Cleavage site
Free peptide
HNH
(a)
(b)
(c)

Shape-Determining Interactions in Proteins

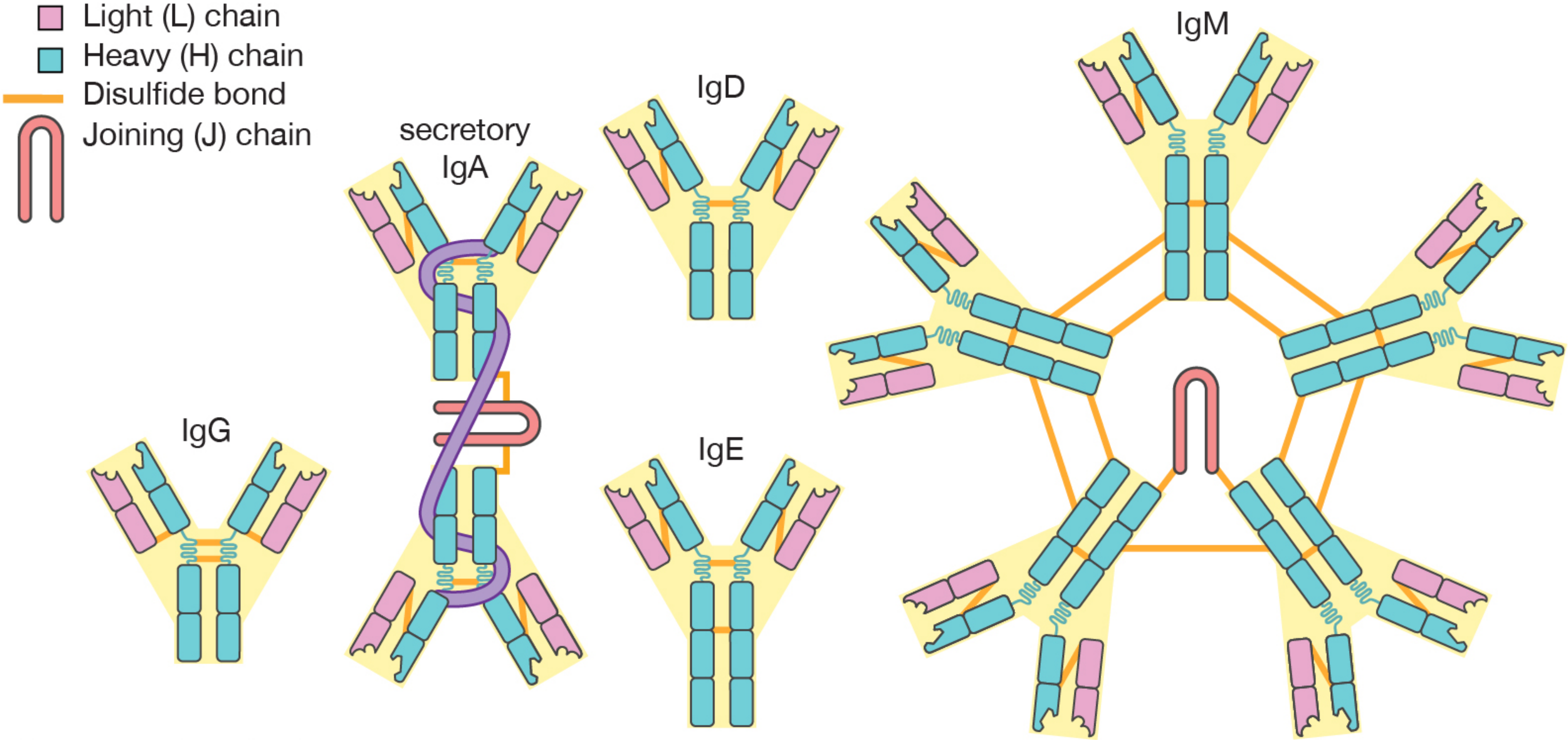
- The essential structure-function relationship for each protein depends on the polypeptide chain being held in its necessary shape by the interactions of atoms in the side chains.

Antibody



antigen-binding sites
hinge region
variable domain (V)
constant domain (C)
disulfide bonds
effector region
V
L
V
H
C
H
1
C
L
C
H
2
C
H
3
S-S

Different Types of Antibodies



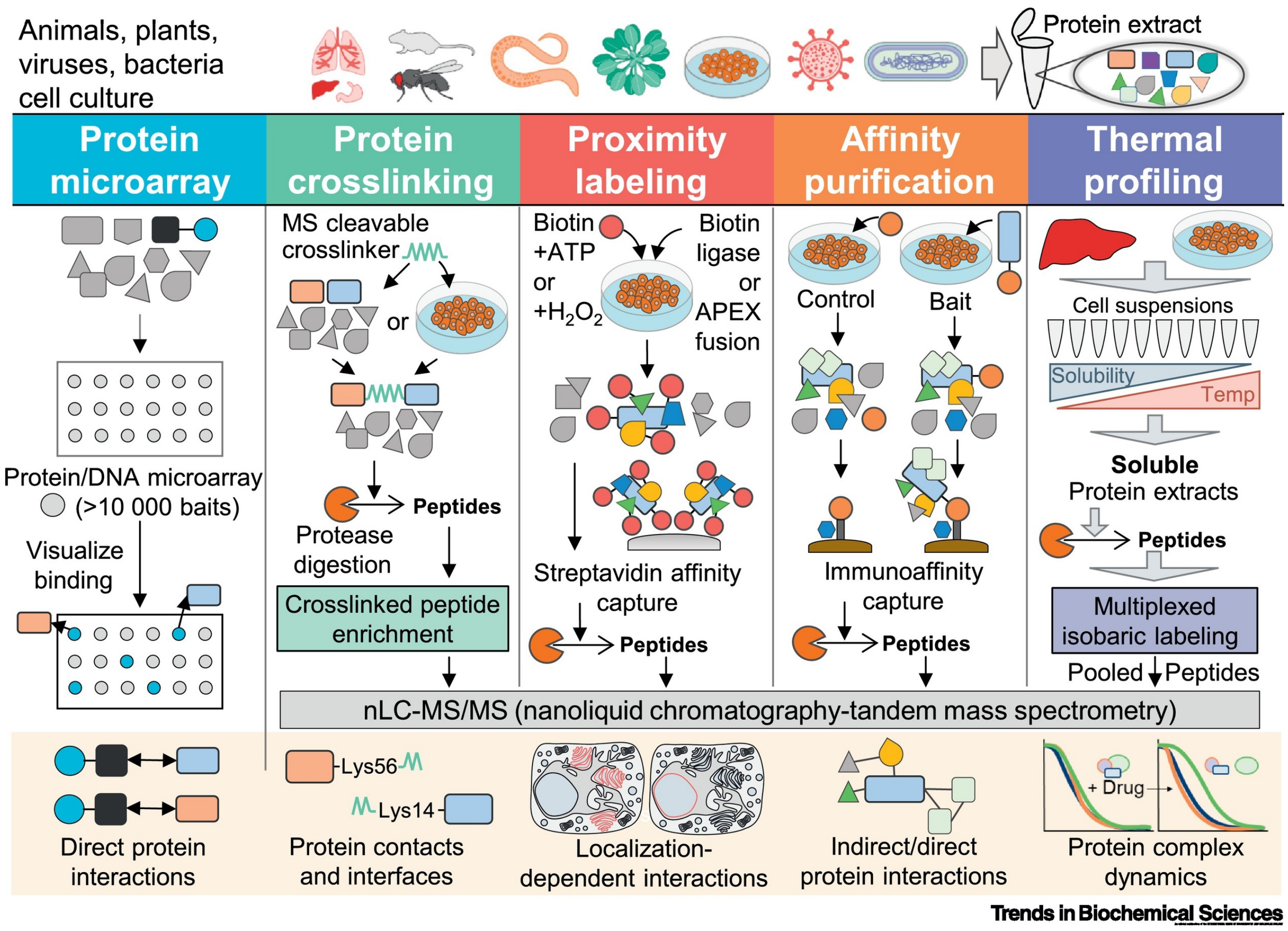
The correlation between RNA expression levels and protein expression is often not perfect due to various factors influencing the process from transcription (RNA synthesis) to translation (protein synthesis) and beyond. Here are several key reasons why RNA expression levels may not always align well with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

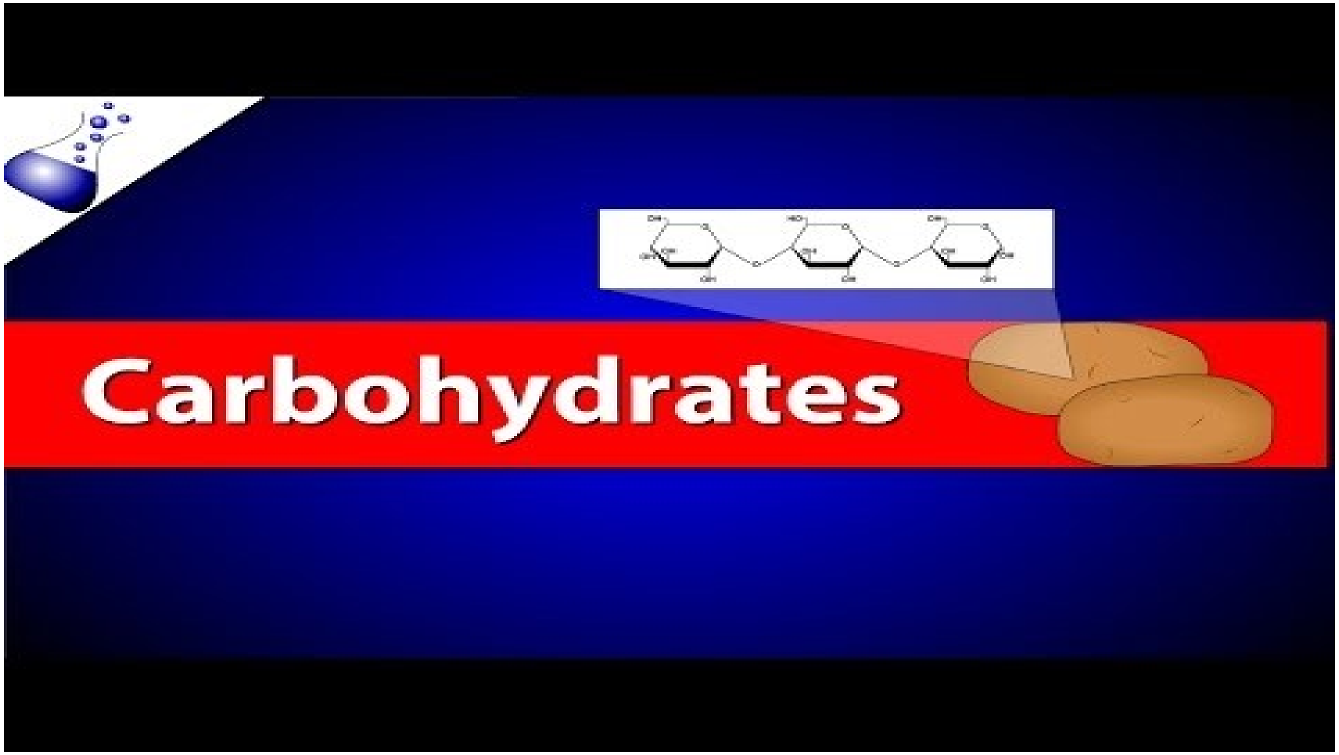
Phosphorylation: The addition of a phosphate group, typically to serine, threonine, or tyrosine residues, affecting the activity, localization, and interaction of proteins.
Ubiquitination: The attachment of ubiquitin, a small regulatory protein, to lysine residues on a target protein, often tagging it 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.

Proteomic

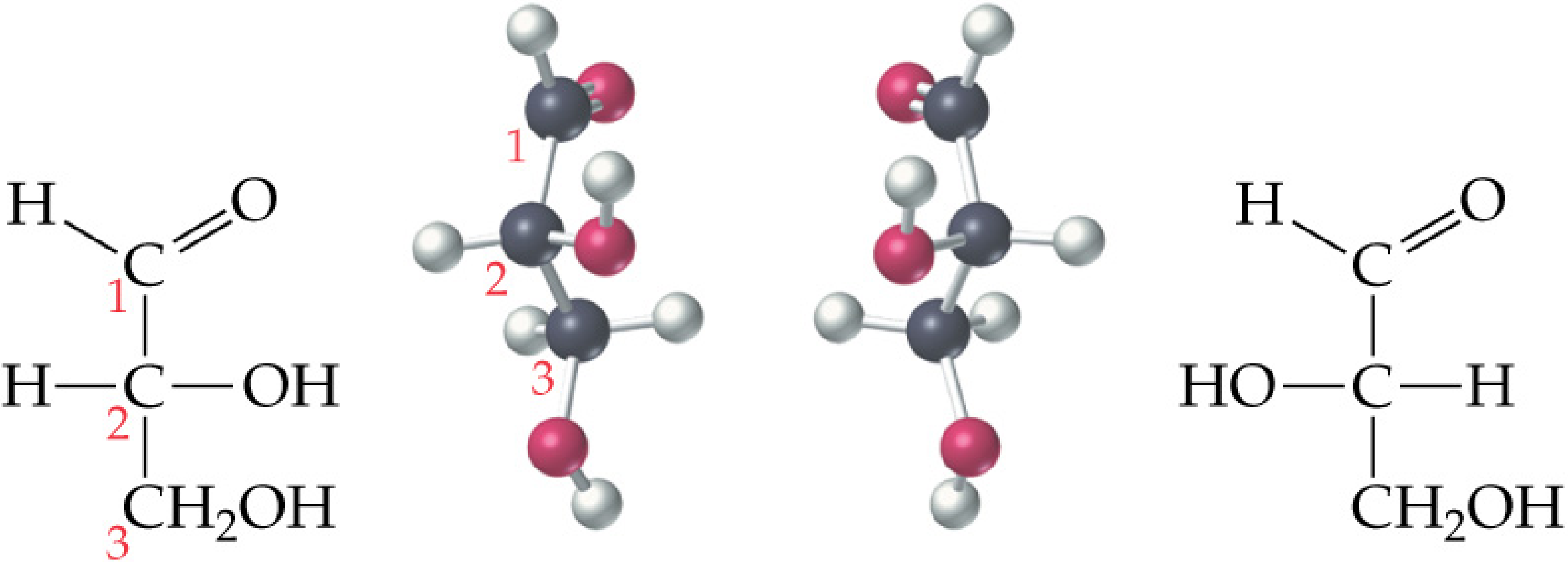


Trends in Biochemical Sciences 2020 45454-455DOI: (10.1016/j.tibs.2020.01.003)

Carbohydrate

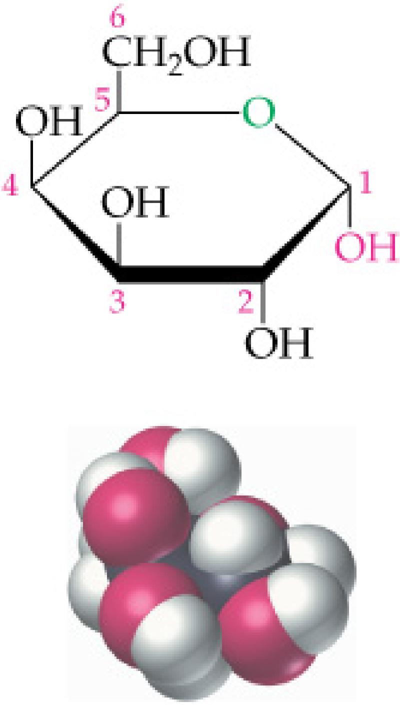


Check
https://www.youtube.com/watch?v=LeOUIXbFyqk

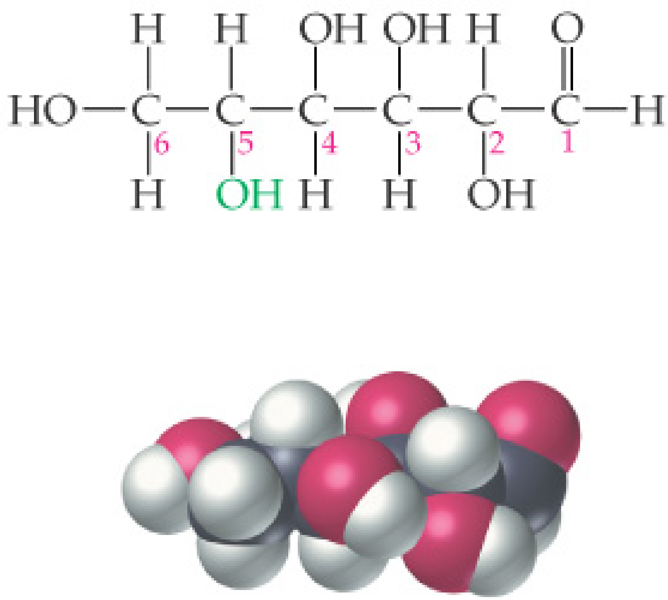


D-Glyceraldehyde
Right-handed

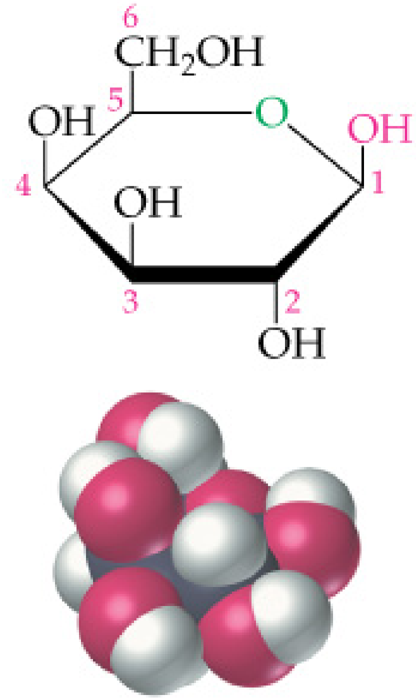
L-Glyceraldehyde
Left-handed



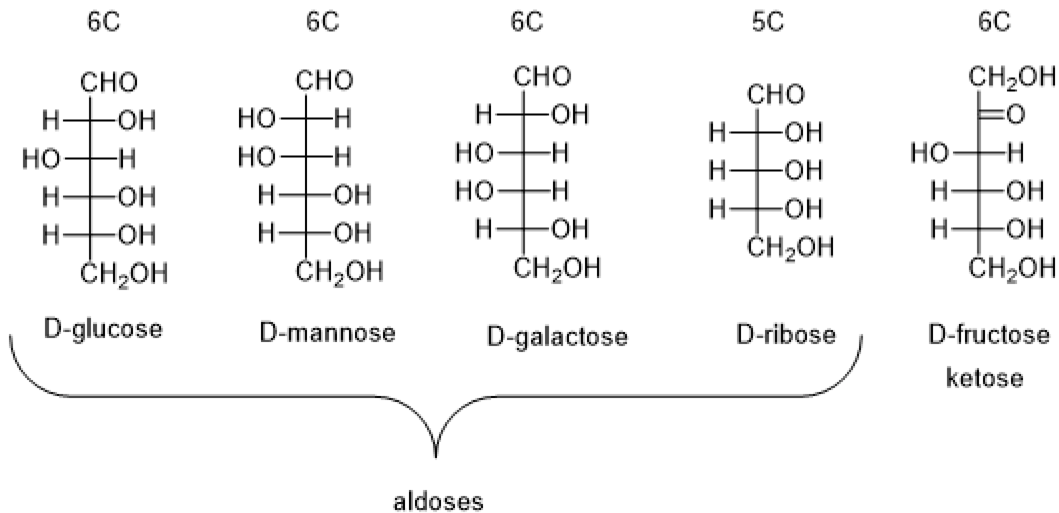
α-D-Galactose

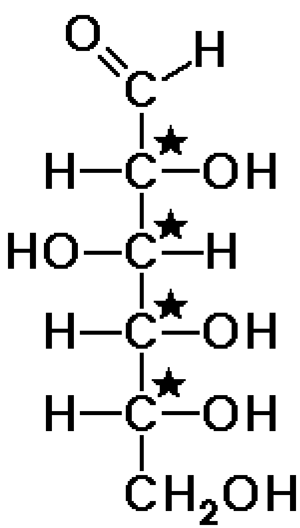


Open-chain galactose



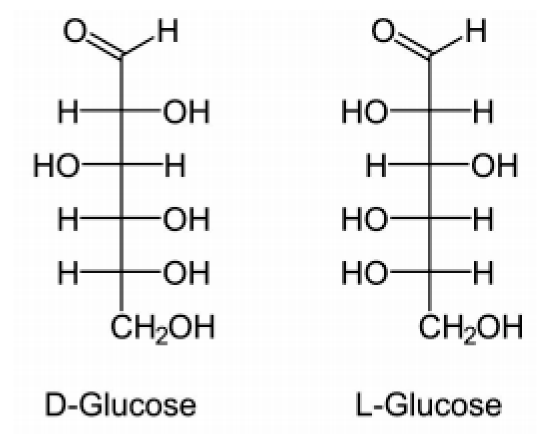
β-D-Galactose



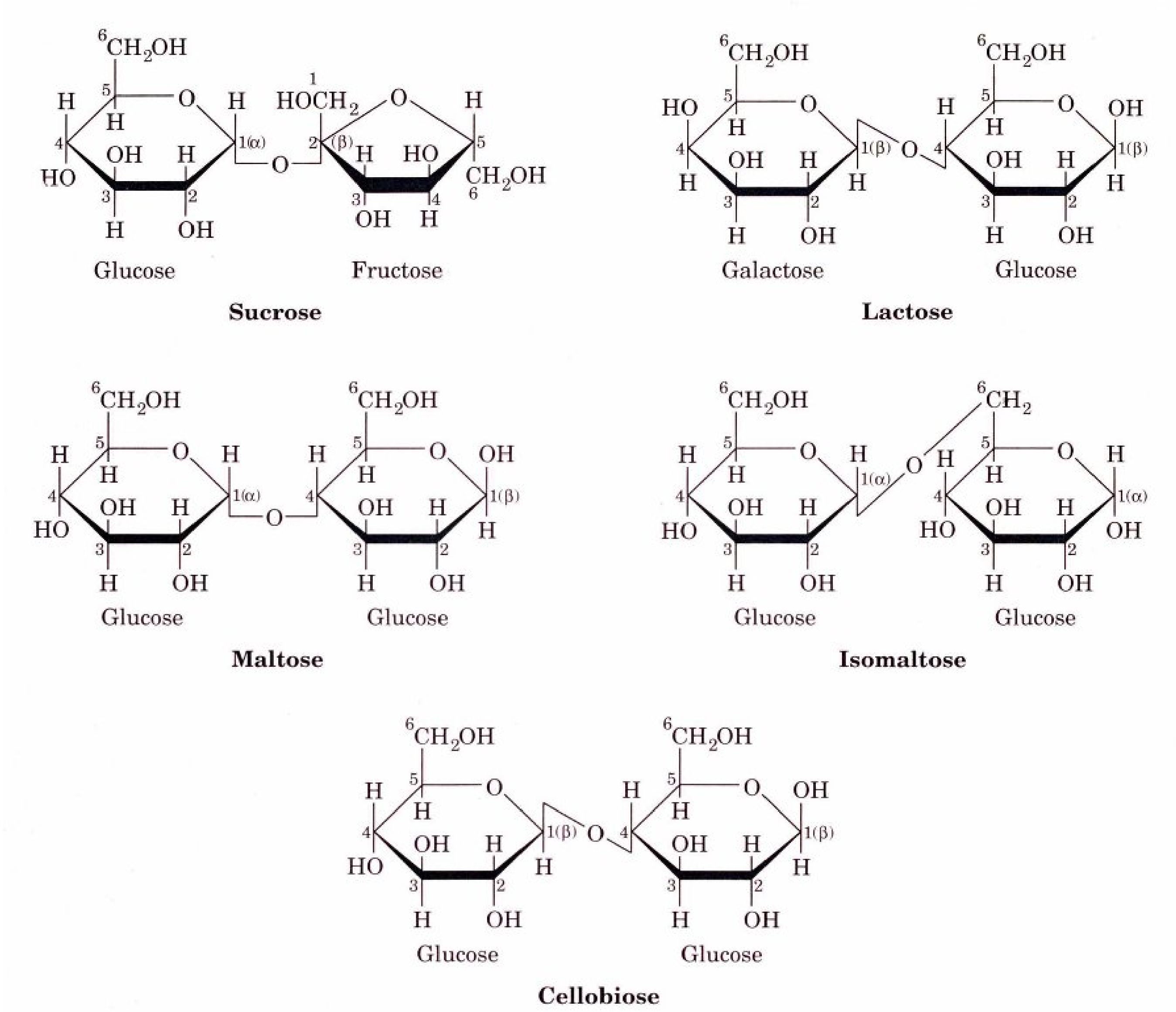


D-glucose

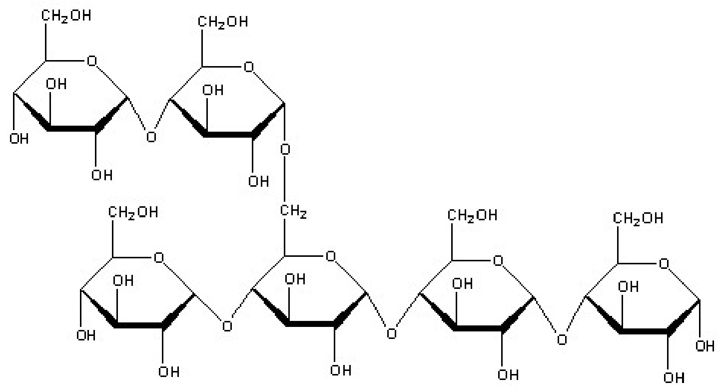
2,3,4,5,6-pentahydroxyhexanal
(with 4 chiral centers)

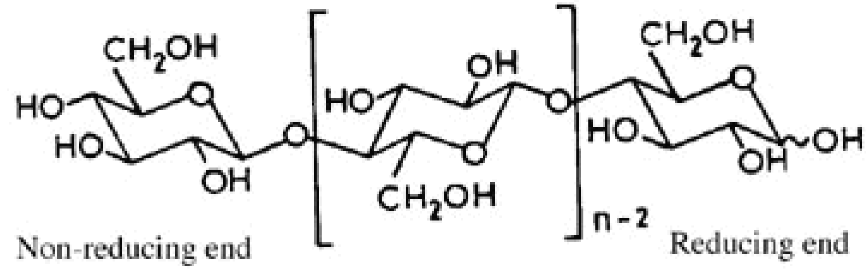


Some Common Disaccharides

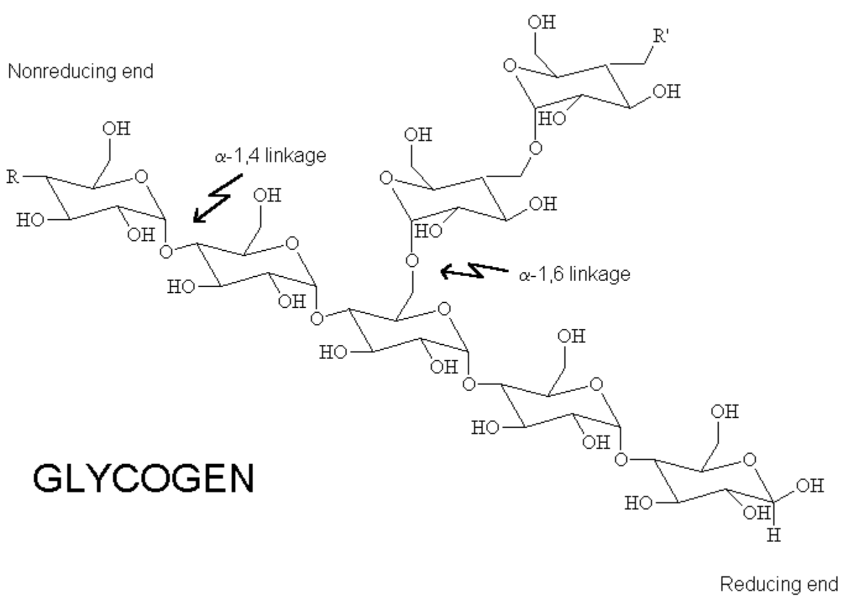


Polysaccharides

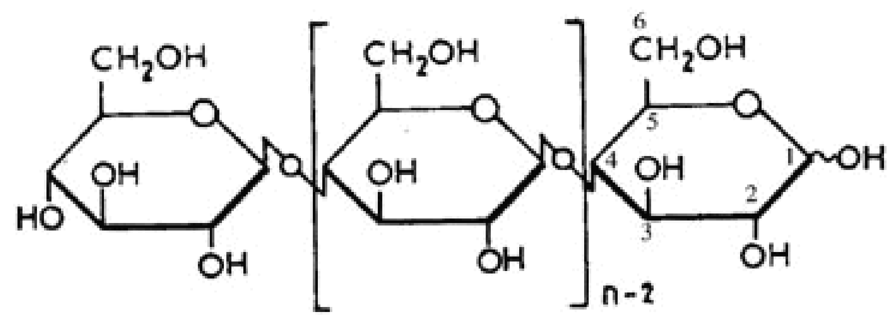




Sometimes shown as

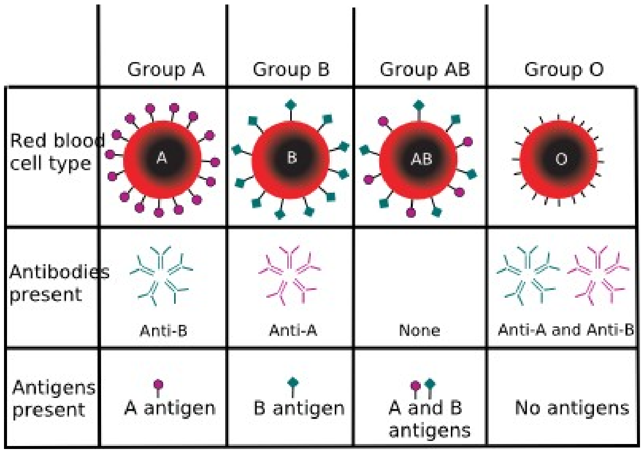


GLYCOGEN

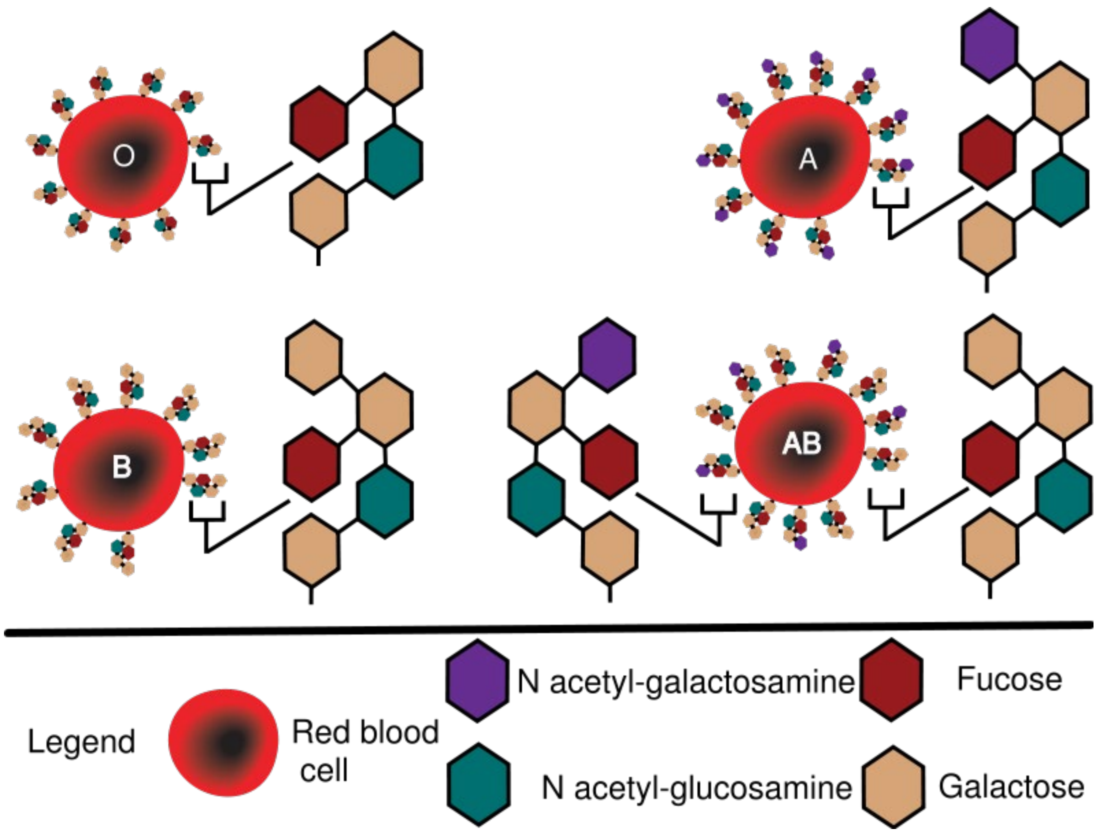


Cellulose

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



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.

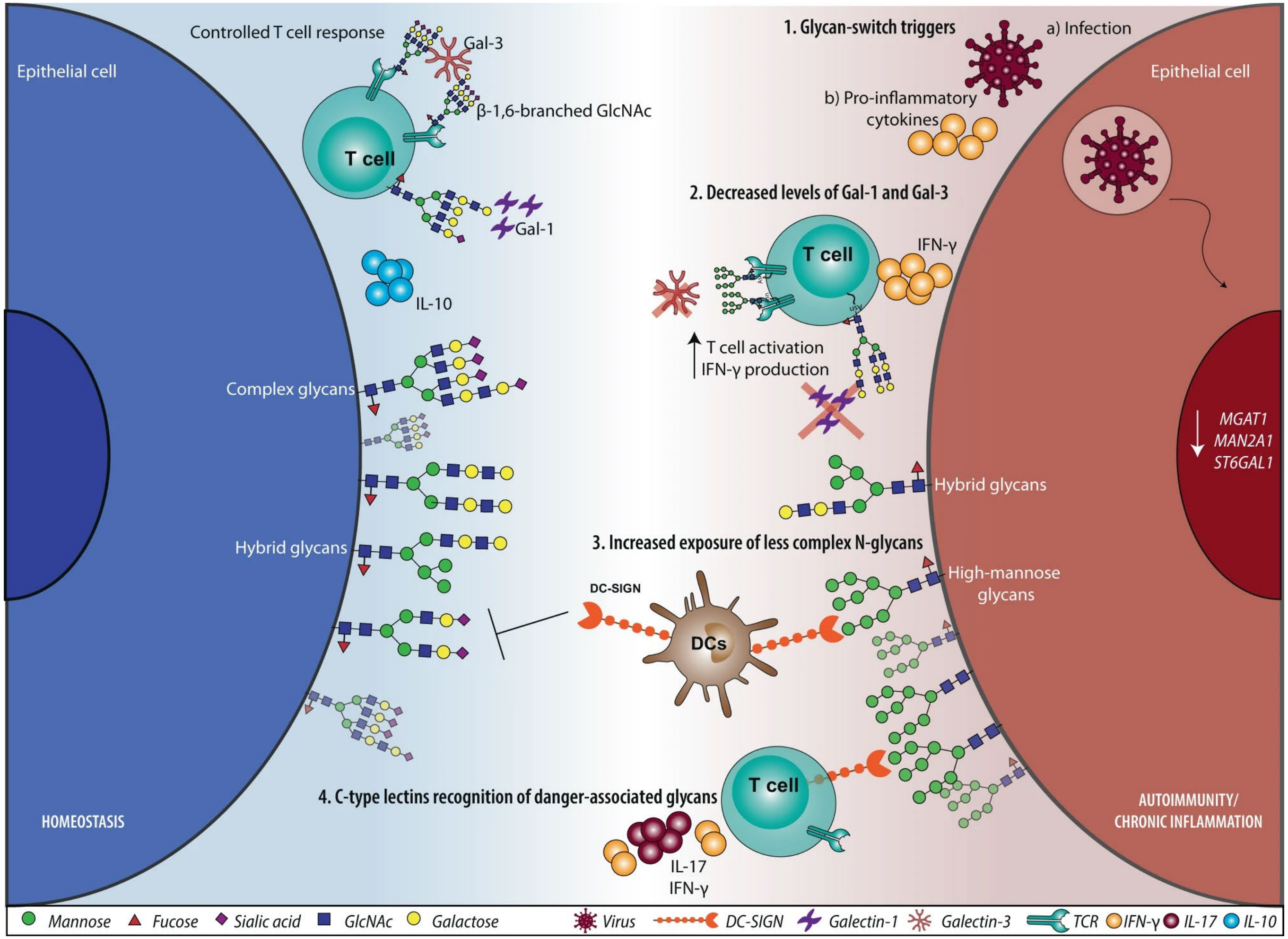
Carbohydrates are essential biomolecules that play numerous vital roles in biological systems, impacting both the structure and function of organisms. Here are some key aspects of their importance:
Energy Source: Carbohydrates are a primary energy source for most organisms. Glucose, a simple sugar, is 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 Binding in IImmune Cells



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.

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.

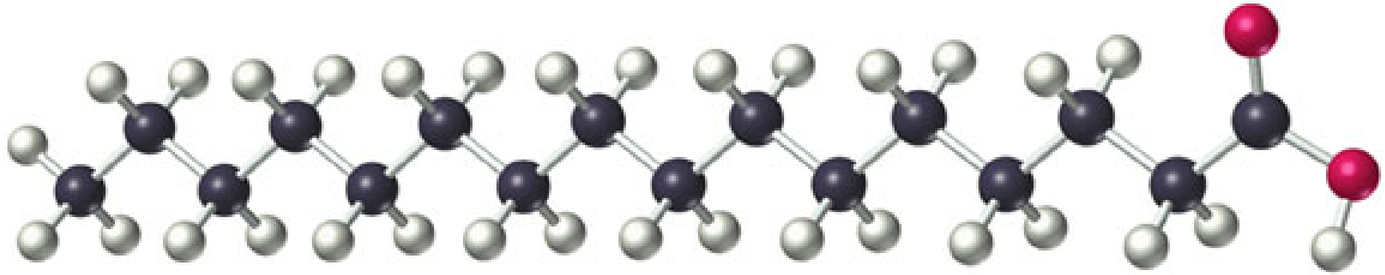
Anchoring Membrane Proteins: Lipids can covalently attach to proteins to anchor them within the cell membrane. This lipid modification is crucial for the localization, function, and signaling of membrane proteins.

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.

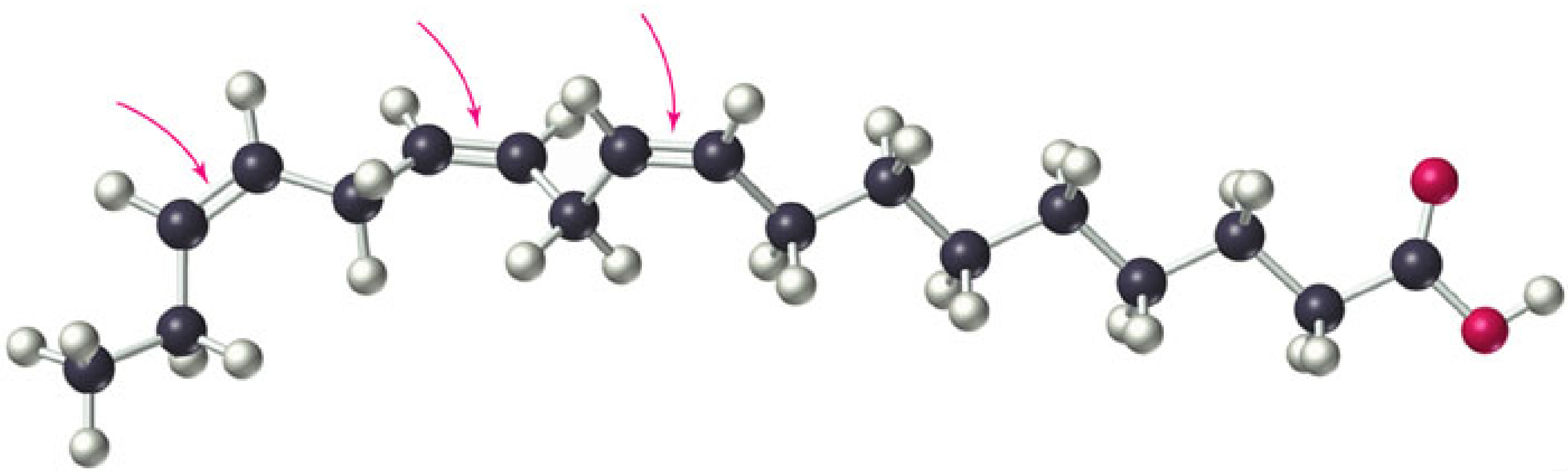
Modulating Membrane Fluidity: The composition of lipids in membranes can influence their fluidity, which in turn affects various membrane-associated functions such as vesicle formation, fusion, and the activity of membrane-bound enzymes and receptors. For instance, cholesterol in animal cell membranes modulates fluidity and permeability.

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



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

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.
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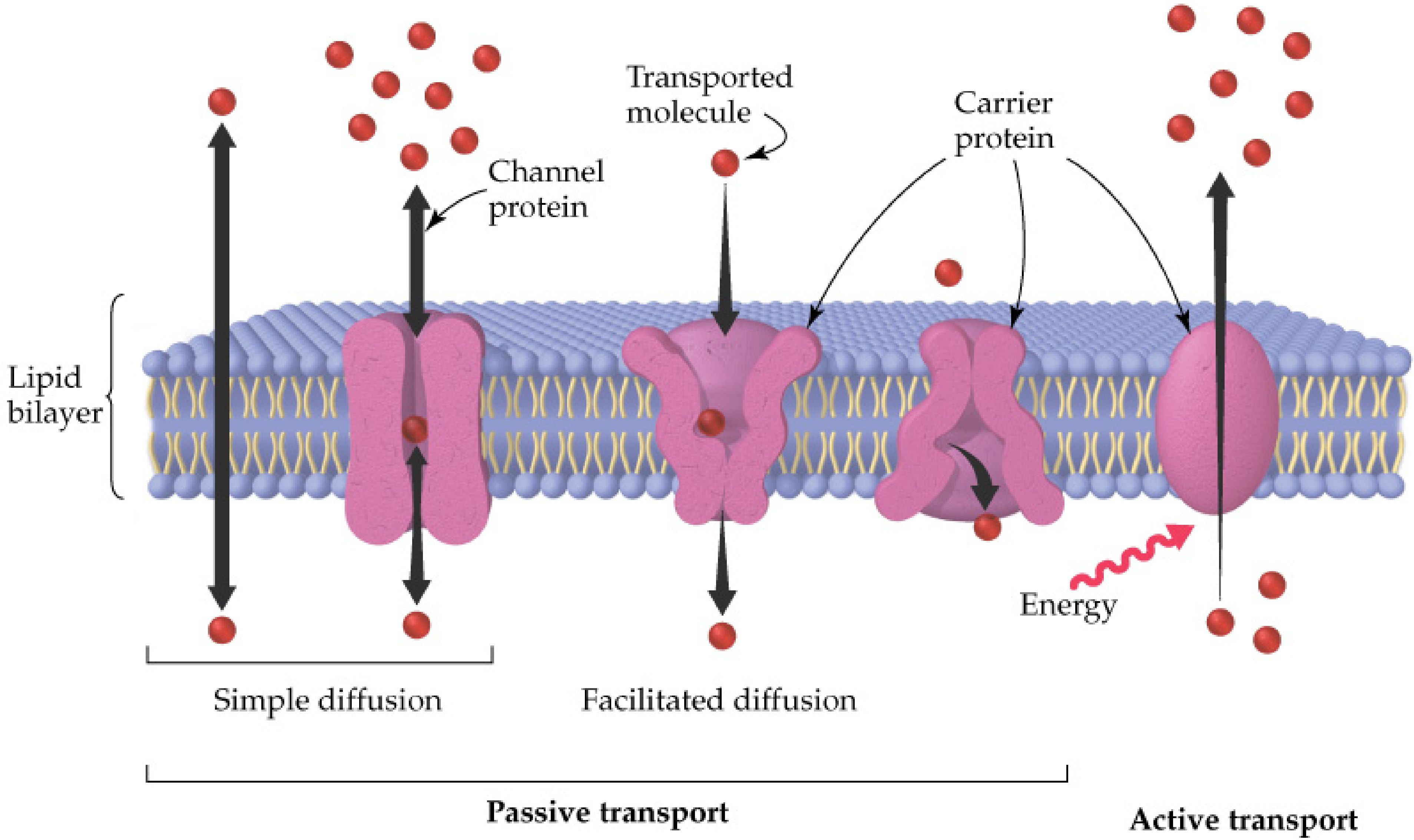
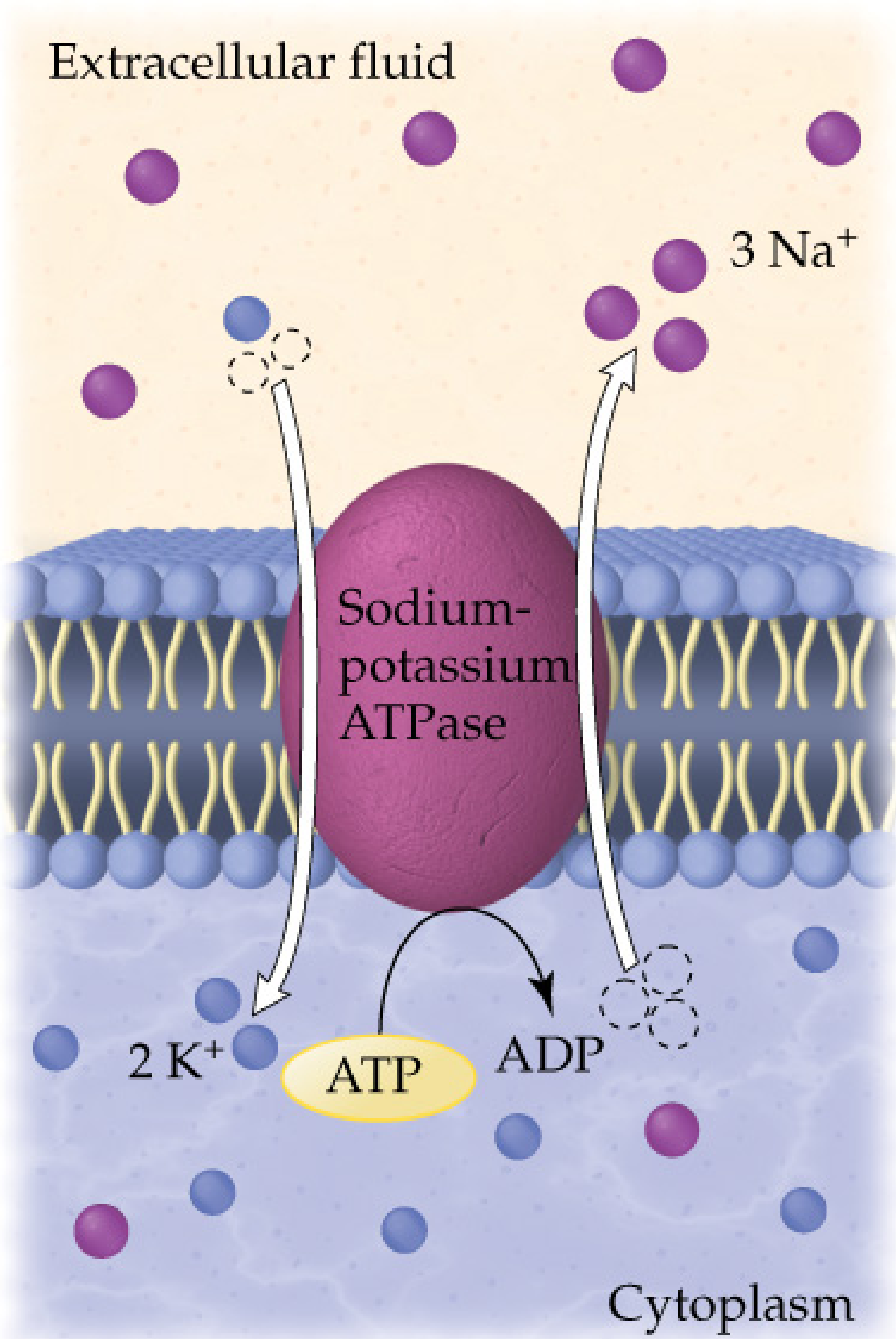
- Na^+, K^+, and other substances that maintain concentration gradients inside and outside the cell cross with expenditure of energy and the aid of proteins.


Transported molecule
Channel protein
Carrier 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:

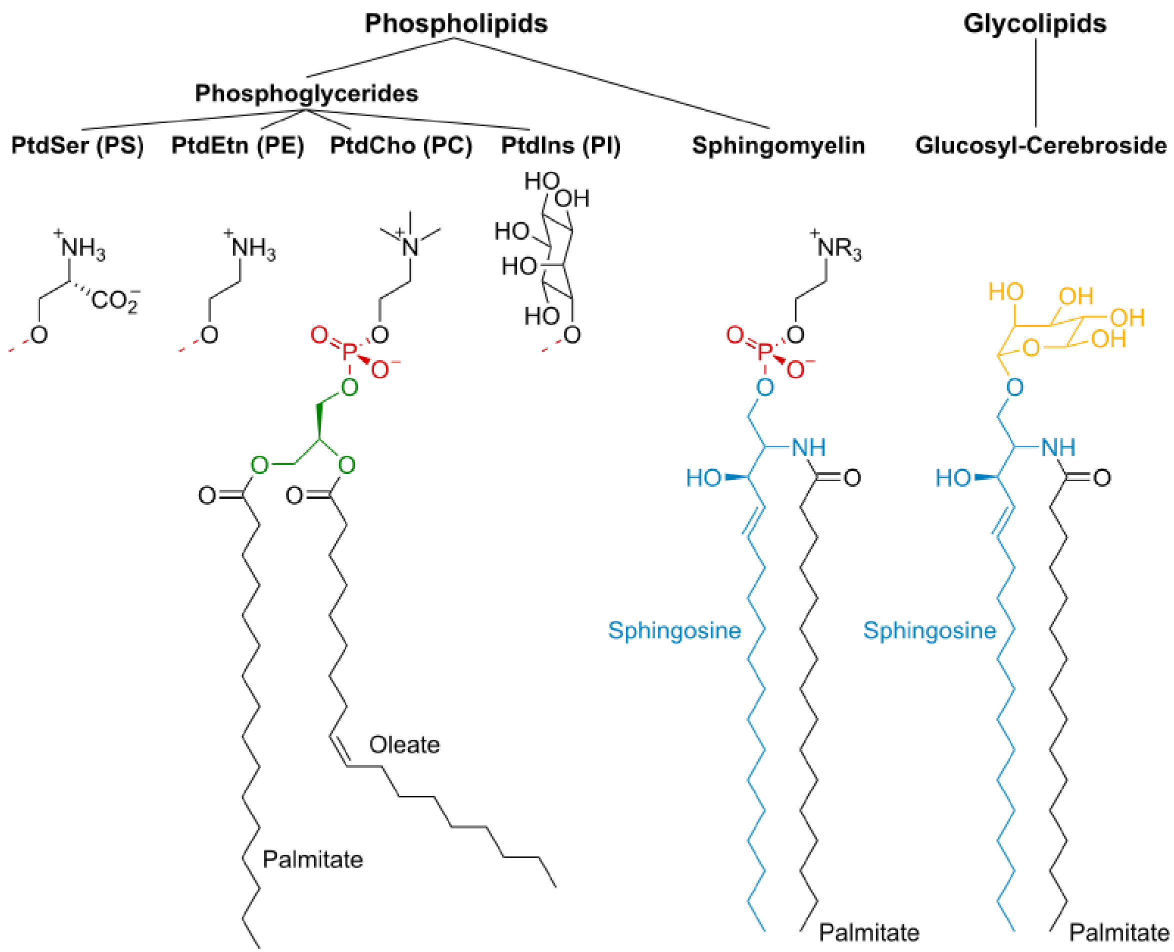
Phospholipids: These are the most abundant lipids in cell membranes. Phospholipids are amphipathic molecules, meaning they have both hydrophilic (water-attracting) and hydrophobic (water-repelling) regions.

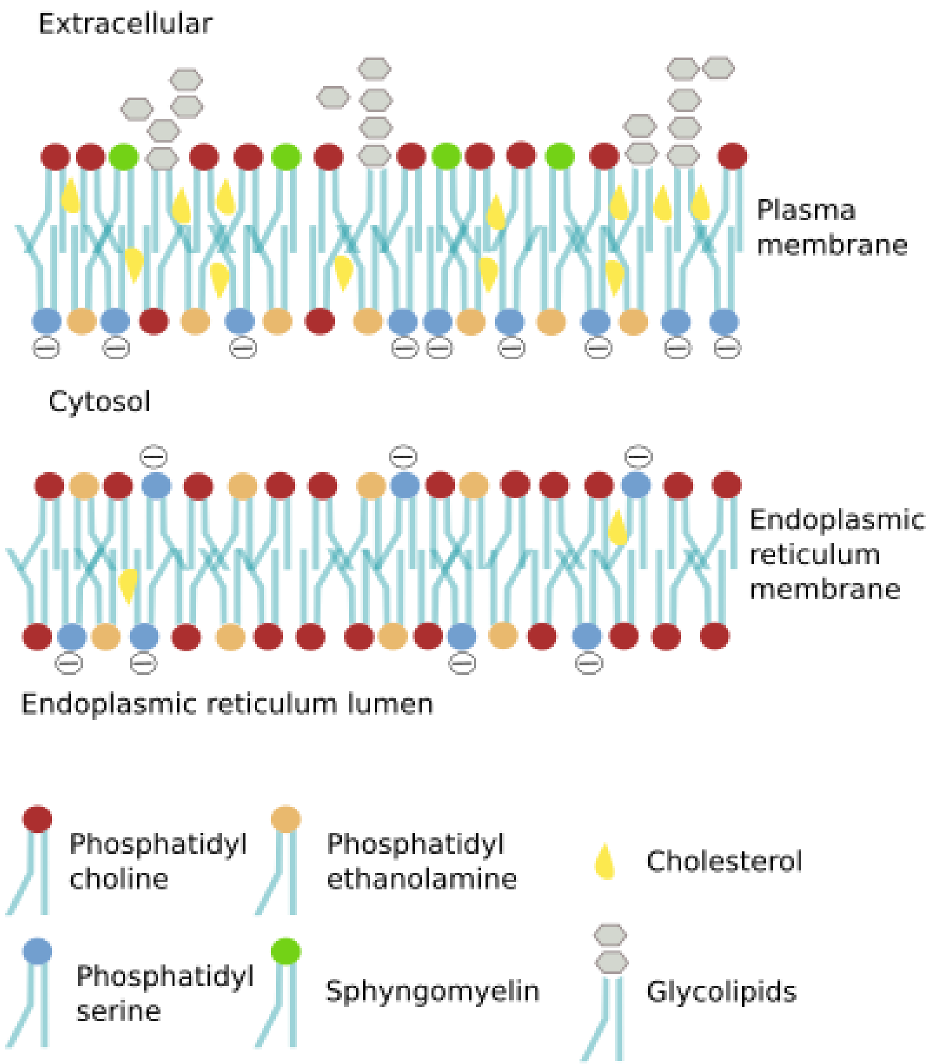
Glycolipids: Comprised of a lipid moiety and one or more sugar residues, glycolipids are primarily found on the extracellular surface of cell membranes. They play crucial roles in cell recognition, communication, and immune responses. The sugar moieties of glycolipids interact with specific molecules 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.

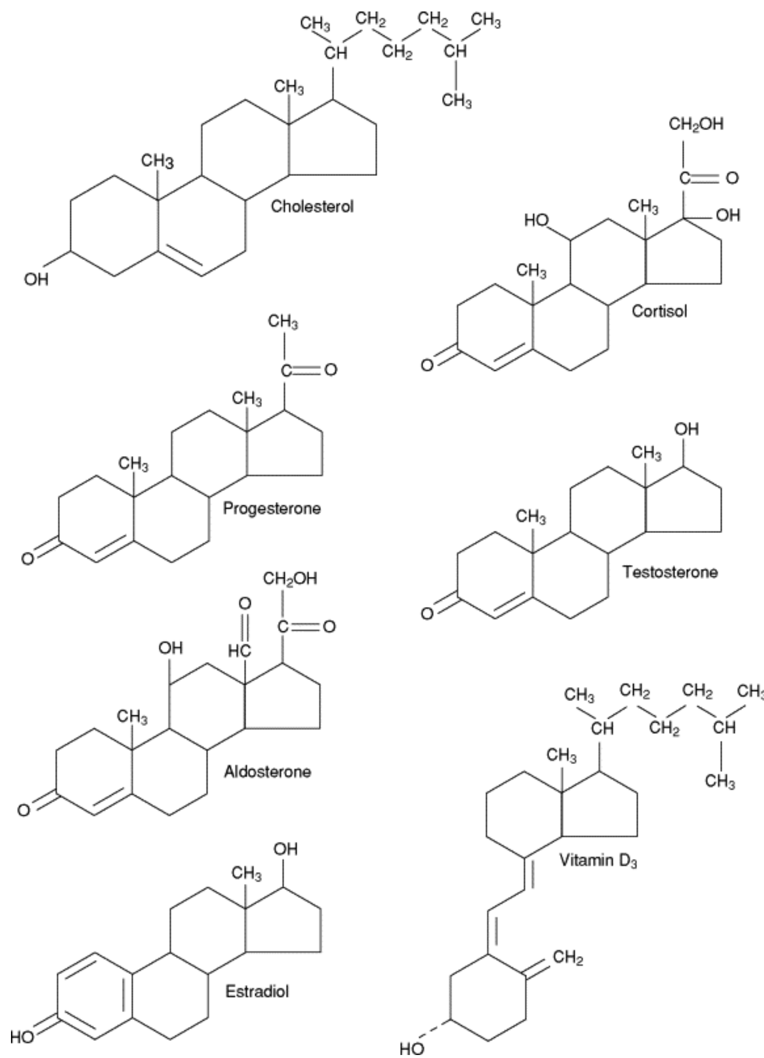
Lipids on Cell Membranes



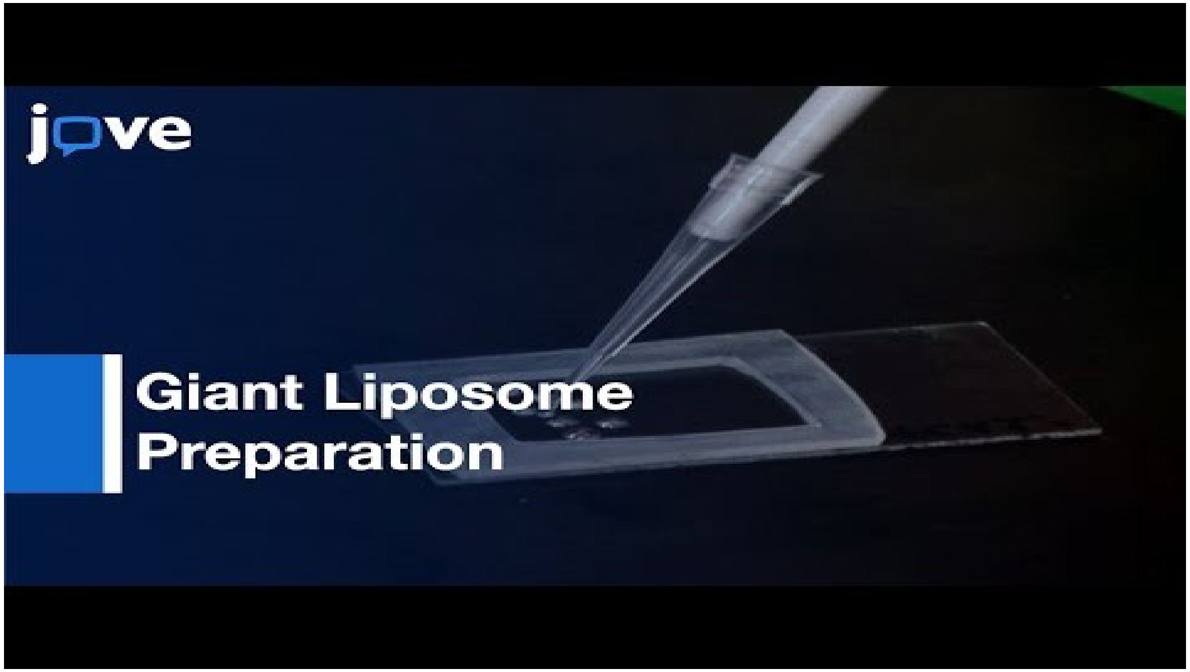


Extracellular
Plasma membrane
Cytosol
Endoplasmic reticulum membrane
Endoplasmic reticulum lumen
Phosphatidyl choline
Phosphatidyl ethanolamine
Cholesterol
Phosphatidyl serine
Sphyngomyelin
Glycolipids

Cholesterol

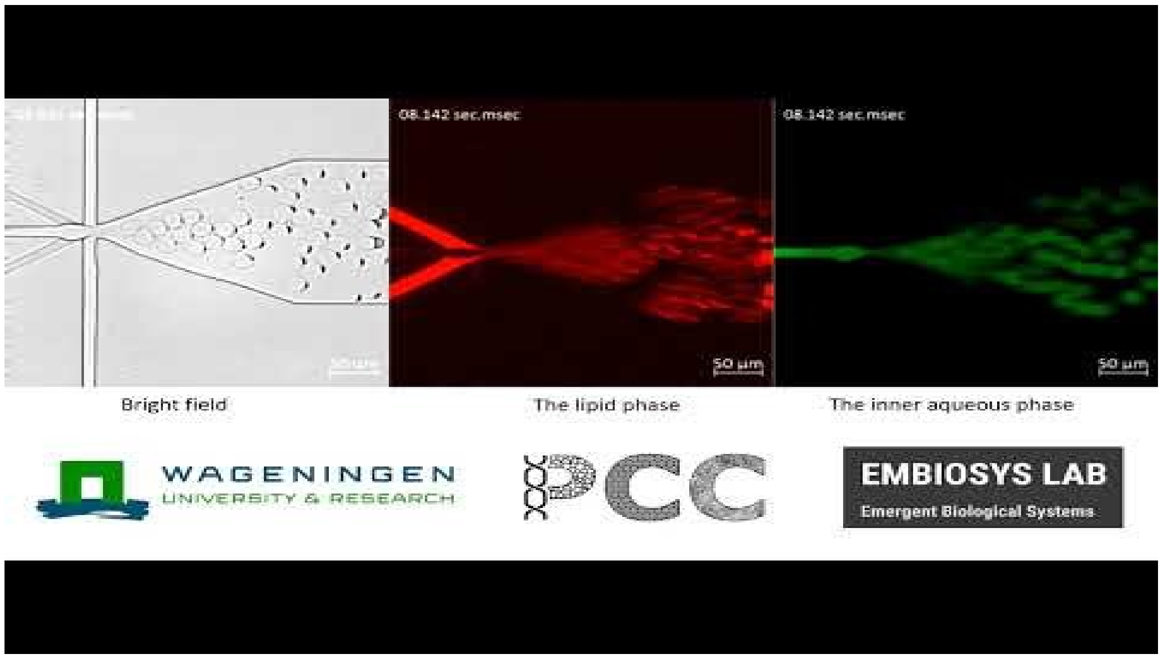


Liposome Preparation



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

Liposome Preparation by Microfluidics



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

Nanoparticles for Biomedical Applications

Nanoparticles have been extensively explored for various biomedical applications, ranging from drug delivery and diagnostic imaging to therapy and regenerative medicine. The diversity in their composition, size, shape, and surface properties allows for their tailored application in different biomedical fields. Here are some of the key types of nanoparticles used in biomedical applications:

Metal Nanoparticles:

- **Silver nanoparticles, Gold nanoparticles, Iron oxide nanoparticles:**.

Quantum Dots:

Lipid-Based Nanoparticles:

- **Liposomes**

Polymeric Nanoparticles:

Dendrimers:

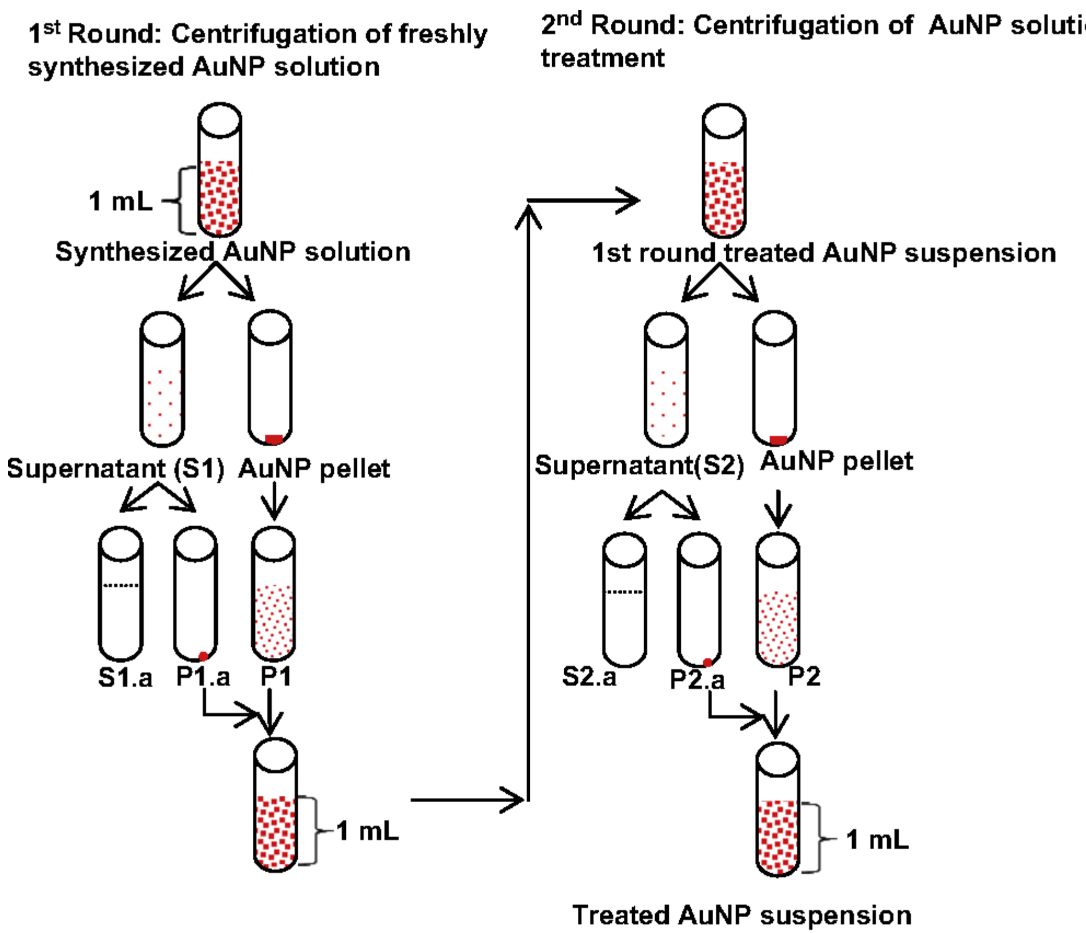
Carbon-Based Nanoparticles:

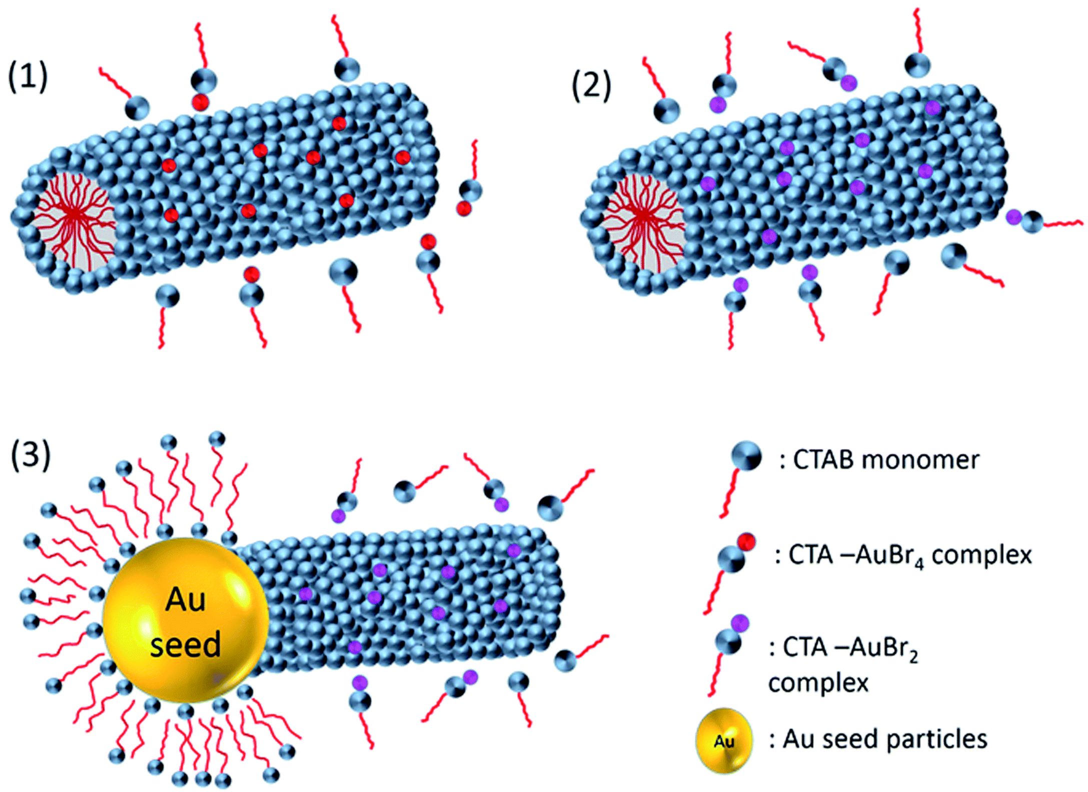
- **Carbon nanotubes and graphene:**
- **Nanodiamond**
- **Carbon dot**

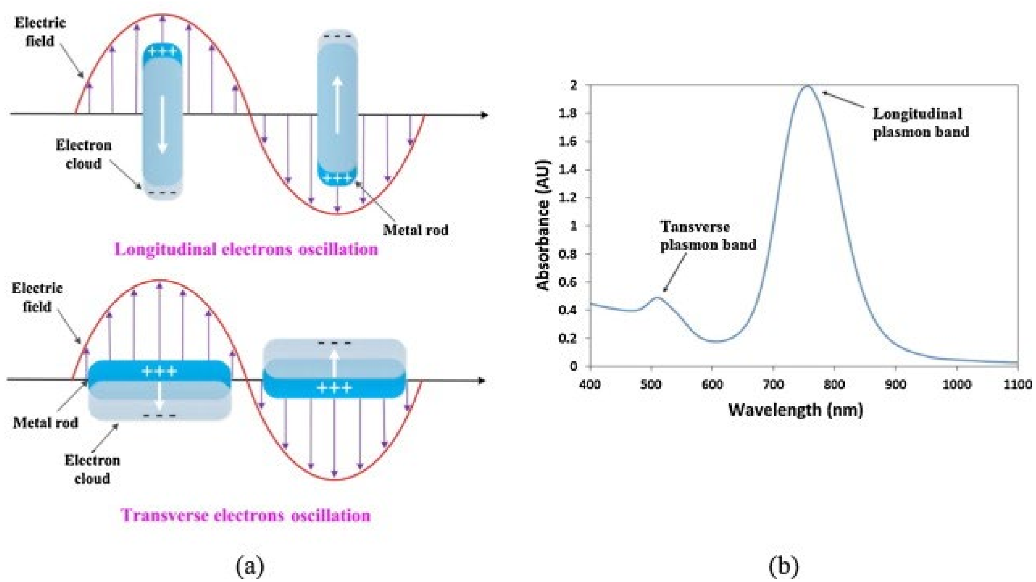
Silica Nanoparticles:

Porous Nanoparticles

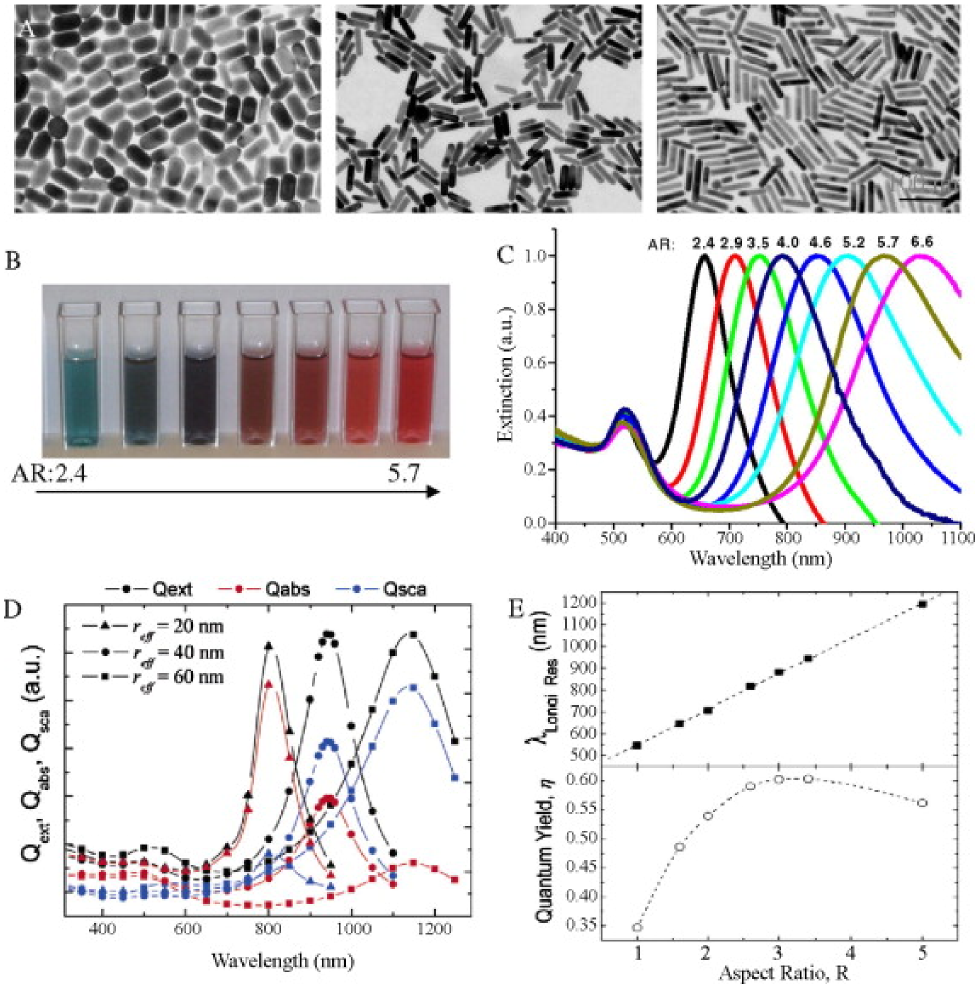
Purification of Au nanoparticles




(1)
(2)
(3)
Au
seed
: CTAB monomer
: CTA –AuBr4 complex
: CTA –AuBr2
complex
Au
: Au seed particles

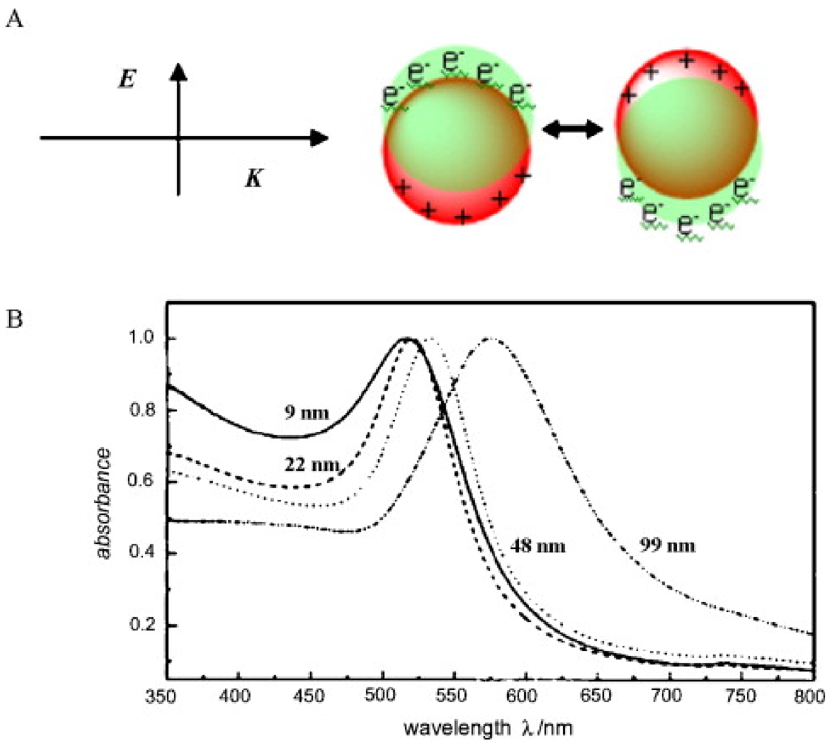

Electric
field
Electron
cloud
Metal rod
Longitudinal electrons oscillation
Electric
field
Metal rod
Electron
cloud
Transverse electrons oscillation
Absorbance (AU)
Wavelength (nm)
Longitudinal
plasmon band
Tansverse
plasmon band
(a)
(b)

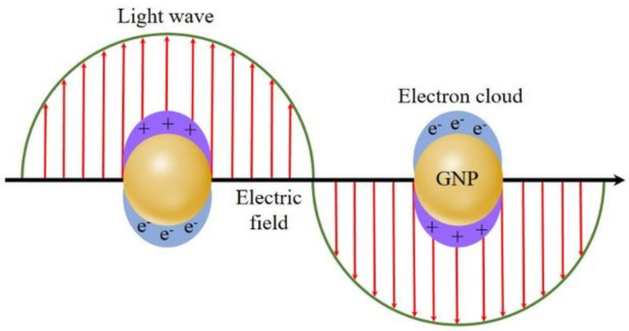
Nanorods



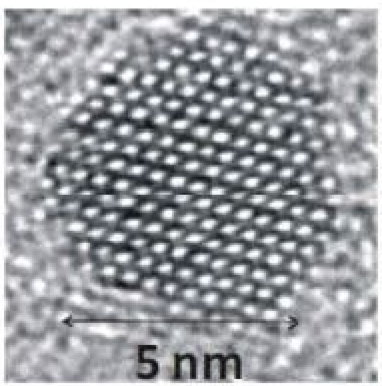
A
B
AR:2.4
5.7
C
AR: 2.4 2.9 3.5 4.0 4.6 5.2 5.7 6.6
Extinction (a.u.)
Wavelength (nm)
D
Qext
Qabs
Qsca
r_{eff} = 20 nm
r_{eff} = 40 nm
r_{eff} = 60 nm
Q_{ext}, Q_{abs}, Q_{sca} (a.u.)
Wavelength (nm)
E
$\lambda_{Longi\ Res}$ (nm)
Quantum Yield, η
Aspect Ratio, R

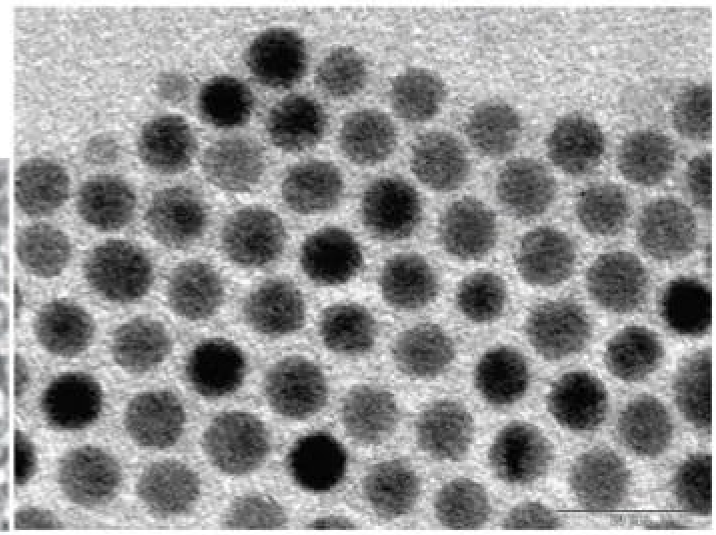
Localized Surface Plasomon





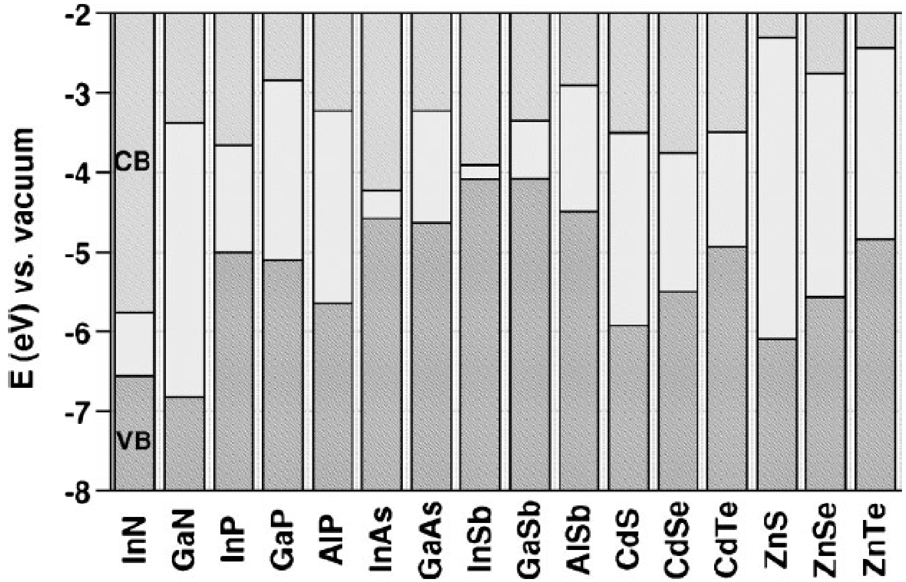
Quantum Dots





Type I Quantum Dots:
Band Alignment: In Type I quantum dots, both the conduction band minimum and the valence band maximum of the shell material lie outside the energy levels of the core material. This means that both electrons and holes are confined within the core material.
Carrier Confinement: Since both charge carriers (electrons and holes) are confined in the same region (the core), they have a higher probability of recombining. This results in strong photoluminescence as the recombination of electrons and holes releases energy in the form of light.
Applications: Due to their efficient confinement of charge carriers and strong luminescence, Type I quantum dots are widely used in applications that require high fluorescence efficiency, such as in bioimaging, light-emitting diodes (LEDs), and quantum dot displays.

Type II Quantum Dots:
Band Alignment: In Type II quantum dots, the conduction band minimum and the valence band maximum are staggered between the core and shell materials. This typically results in the confinement of electrons and holes in different regions; for example, electrons may be confined in the core while holes are localized in the shell, or vice versa.
Carrier Confinement: The spatial separation of charge carriers (electron and hole) reduces their recombination probability, which can lead to longer exciton lifetimes. While this separation can decrease the photoluminescence efficiency, it provides other useful properties, such as tunable emission from the visible to the infrared spectrum.
Applications: The extended carrier lifetimes and tunable emission properties make Type II quantum dots useful for applications in solar energy conversion, where they can help in creating more efficient photovoltaic cells, and in sensors, where their tunable absorption can be exploited for detecting various substances.



Scheme 1. Electronic energy levels of selected III–V and II–VI semiconductors using the valence-band offsets from Reference [12] (VB: valence band, CB: conduction band).

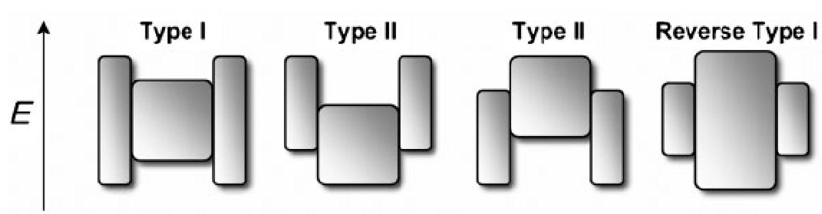
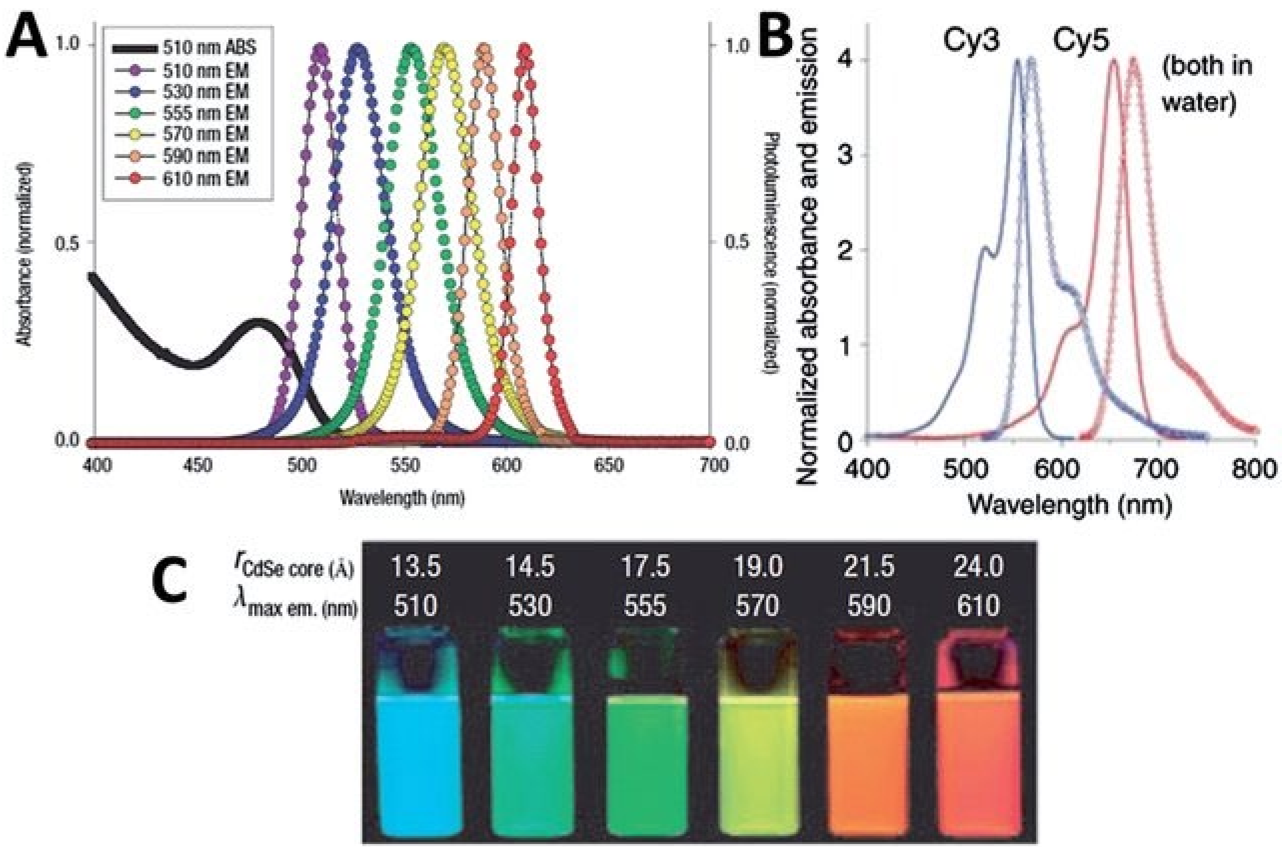


Figure 1. Schematic representation of the energy-level alignment in different core/shell systems realized with semiconductor NCs to date. The upper and lower edges of the rectangles correspond to the positions of the conduction- and valence-band edge of the core (center) and shell materials, respectively.

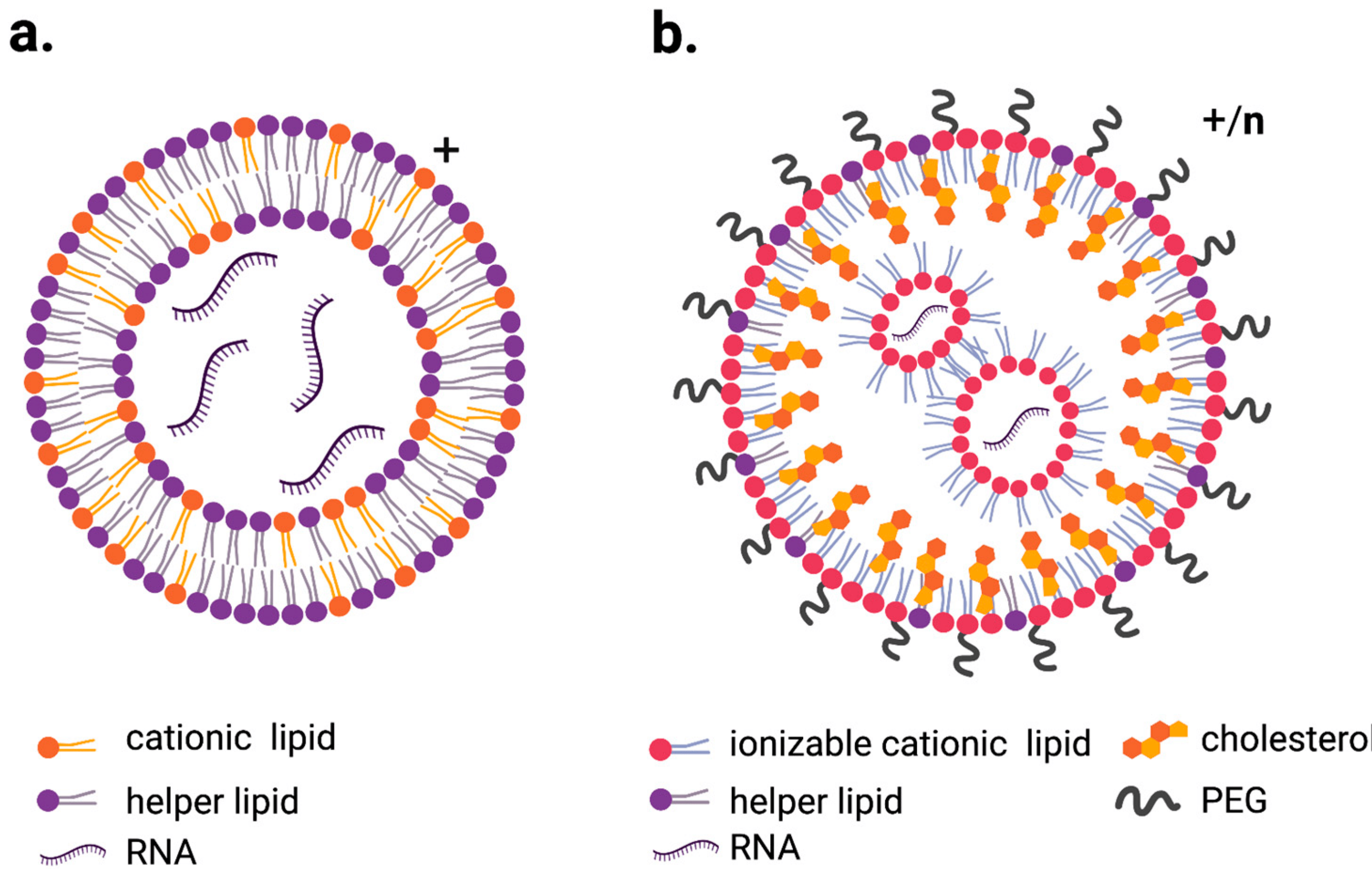
Optical Properties



Advantages of QDs for Imaging

Broad Excitation and Narrow Emission Spectra: Quantum dots can be excited by a wide range of wavelengths but emit light at very specific wavelengths. This narrow emission spectrum allows for the simultaneous use of multiple quantum dot colors for multiplexed imaging, enabling the visualization of several targets within a single sample.
Size- and Composition-Tunable Fluorescence: The emission wavelength of quantum dots can be precisely tuned by changing their size or composition, allowing for the generation of a wide palette of colors from the same material simply by adjusting the quantum dot size. This tunability is advantageous for creating highly multiplexed imaging assays.
High Photostability: Quantum dots are much more resistant to photobleaching than traditional fluorescent dyes and proteins. This property allows for prolonged imaging sessions and repeated exposures, which are essential for long-term studies of dynamic processes in live cells or tissues.
High Quantum Yield: Quantum dots generally have high quantum yields, meaning they are very efficient at converting absorbed light into emitted light. This results in bright signals that can improve the sensitivity and detection limits of bioimaging applications.
Versatility in Functionalization and Bioconjugation: Quantum dots can be functionalized with various biological molecules, such as antibodies, peptides, or nucleic acids, enabling targeted imaging of specific molecules or structures within cells and tissues. This specificity is crucial for studying complex biological processes and for diagnostic purposes.

Lipid Nanoparticles



Selection of Lipid Materials: The first step involves selecting appropriate lipid materials. The typical components of LNPs for vaccine delivery include:

Ionizable cationic lipids: Facilitate endosomal escape of the RNA into the cytoplasm.

Phospholipids: Provide structural integrity.

Cholesterol: Stabilizes the lipid bilayer.

Polyethylene glycol (PEG)-lipids: Confer stealth properties to avoid rapid clearance from the body.

Nucleic Acid Encapsulation: The mRNA or other nucleic acid is mixed with the lipid components. The encapsulation is usually achieved through one of the following processes:

Ethanol Dilution Method: Lipids dissolved in ethanol are rapidly mixed with an aqueous solution containing the nucleic acid. This process results in the spontaneous assembly of LNPs encapsulating the mRNA.

Microfluidics: This method involves the controlled mixing of lipid and nucleic acid streams under laminar flow conditions in a microfluidic device. Microfluidics allows for precise control over the nanoparticle size and encapsulation efficiency.

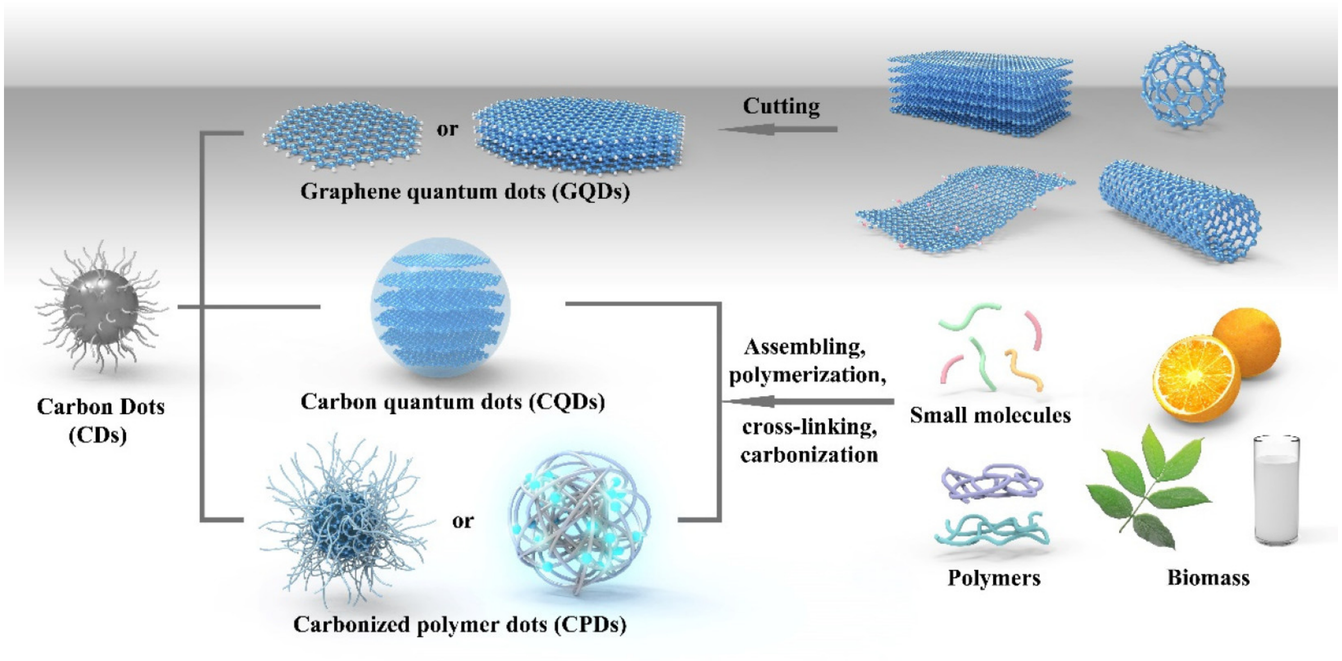
LNP Formation and Size Adjustment: The size of LNPs is crucial for their effectiveness and is typically adjusted to be around 80-100 nanometers.

Sterilization and Quality Control: The LNPs must be sterile for use in vaccines. Sterilization can be achieved by filtration through a sterile filter with a suitable pore size (e.g., 0.22 micrometers).

Formulation: The final LNP formulation is typically prepared in a buffer suitable for injection. The formulation process must ensure that the LNPs are stable and maintain their integrity until they are administered.

Storage: The stability of LNPs is temperature-dependent. They are usually stored at low temperatures (e.g., -80°C) to maintain their structural integrity and functional properties until they are ready to be used.

Carbon Dots



Classification of CDs: including graphene quantum dots (GQDs), carbon quantum dots (CQDs), and carbonized polymer dots (CPDs), and their main preparation approaches.

DOI: (10.1021/acscentsci.0c01306)

Carbon quantum dots (CQDs) are nanometer-sized carbon-based materials that exhibit quantum mechanical properties, including fluorescence.

Characteristics of Carbon Quantum Dots

Size: Carbon quantum dots are typically less than 10 nanometers in diameter.

Composition: They are composed primarily of carbon elements, with some containing heteroatoms like nitrogen, oxygen, or sulfur to modify their electronic and optical properties.

Structure: The structure of CQDs can vary but often includes **graphene-like sp2-hybridized carbon sheets and sp3-hybridized carbon domains**.

Properties

Fluorescence: CQDs exhibit strong fluorescence, which is tunable based on **their size, surface functional groups, and doping elements**. This fluorescence is due to **quantum confinement and edge effects**, where the electronic properties are influenced by the size and structure of the quantum dots.

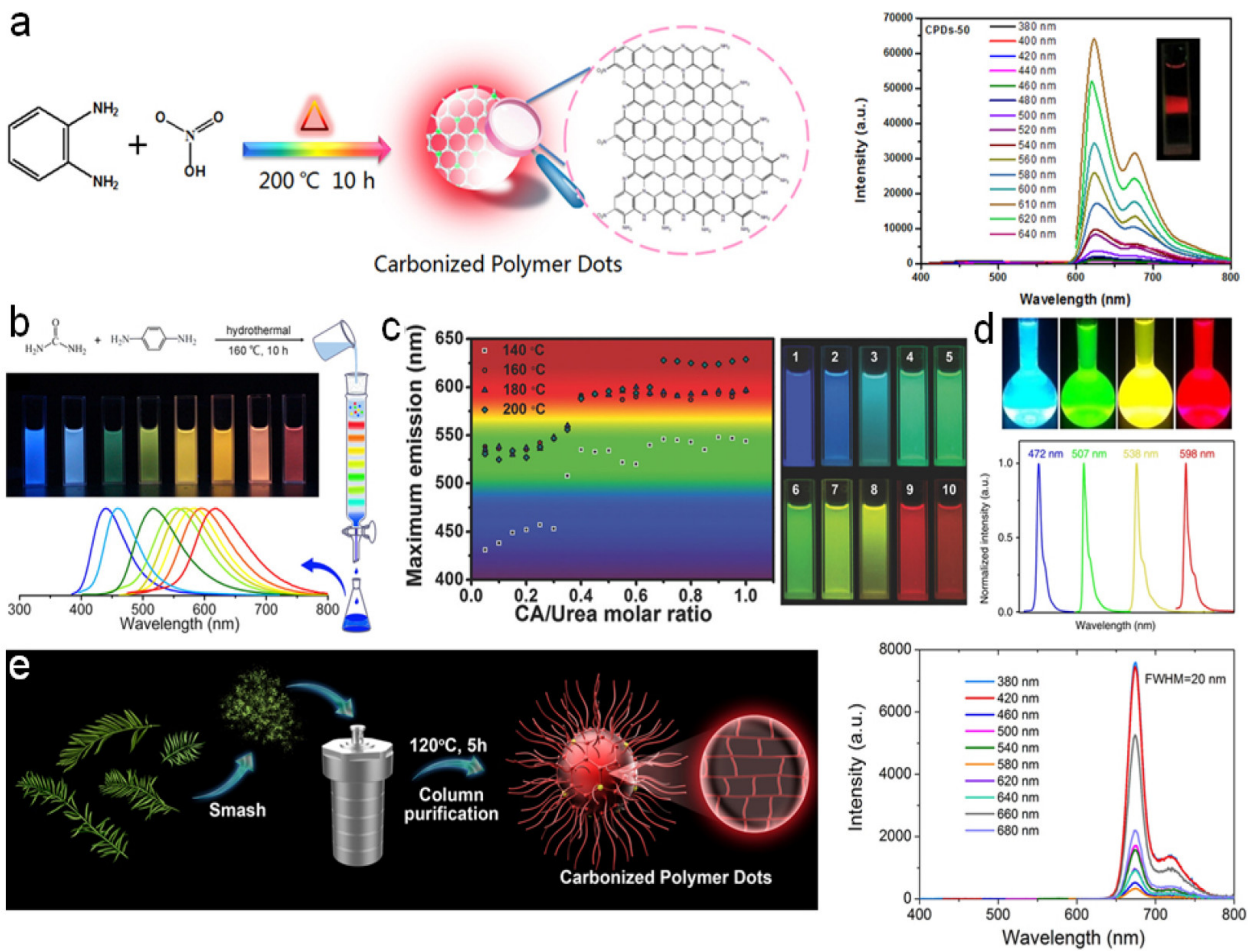
Photostability: They have high photostability, resisting photobleaching under prolonged illumination.

Biocompatibility: Carbon quantum dots are generally considered to be biocompatible and less toxic than traditional semiconductor quantum dots, making them suitable for biological and medical applications.

Water Solubility: Many CQDs are hydrophilic and easily solubilized in water, facilitating their use in biological environments.

Reasons for Fluorescence
The fluorescence of carbon quantum dots originates from several mechanisms, including **quantum confinement effects**, **surface states**, and **molecular fluorescence**. The exact mechanism can depend on the synthesis method, surface functionalization, and the presence of doping elements. Quantum confinement plays a significant role in smaller CQDs, where the movement of electrons is restricted to such a degree that it alters the electronic and optical properties of the material. Surface states, related to the functional groups attached to the surface of CQDs, also significantly influence their optical properties. Additionally, the presence of specific dopants can introduce localized energy states in the band gap, further affecting fluorescence.

Applications
Bioimaging: Their biocompatibility and tunable fluorescence make them excellent candidates for fluorescent labeling and imaging in biological systems.
Sensing: CQDs can be engineered to detect various chemicals and biomolecules, making them useful in environmental monitoring and diagnostics.
Photocatalysis: Their ability to absorb and transfer energy efficiently makes them useful in photocatalytic applications, such as degradation of pollutants and water splitting.
Optoelectronics: CQDs are explored for use in light-emitting diodes (LEDs), solar cells, and other optoelectronic devices due to their electronic and optical properties.
The research and development of carbon quantum dots continue to evolve, revealing new insights into their properties and expanding their range of applications.



Syntheses and optical properties of CDs. Synthesis and PL spectra of (a) red emissive CPDs and (b) multicolor CPDs. Optical properties of (c) multicolor CPDs and (d) CQDs. (e) Synthesis and PL spectra of deep red emissive CPDs.

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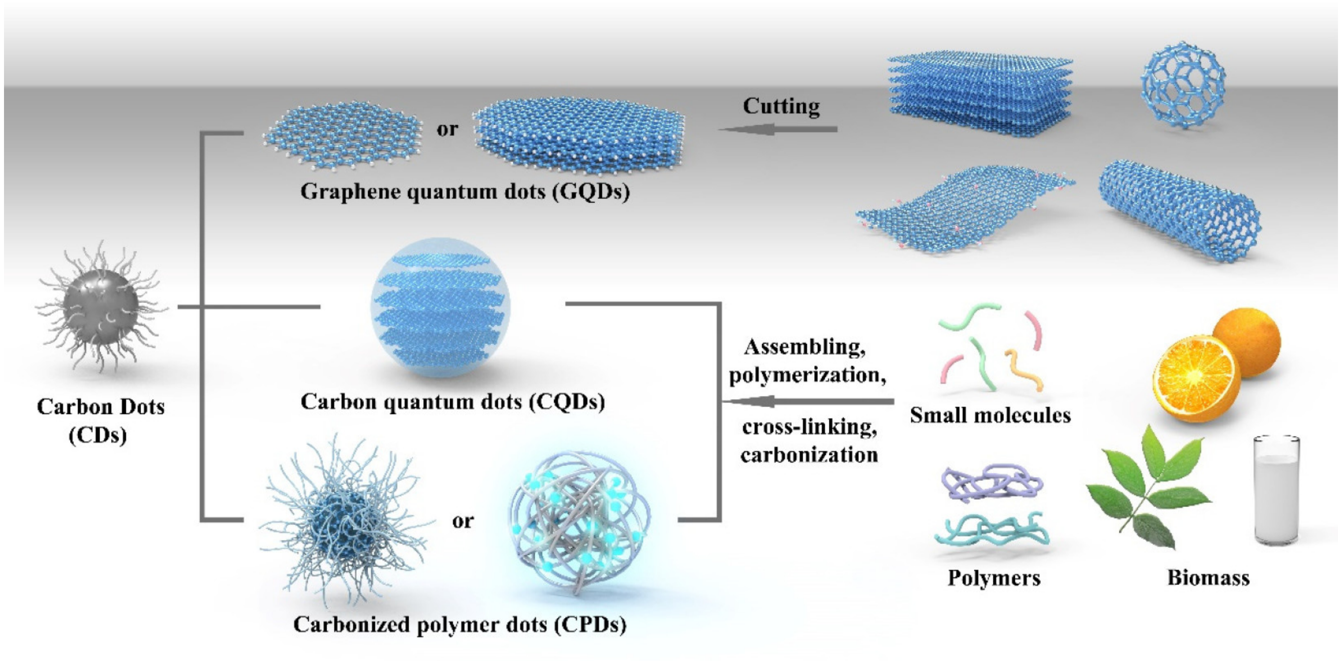
Carbon Dots



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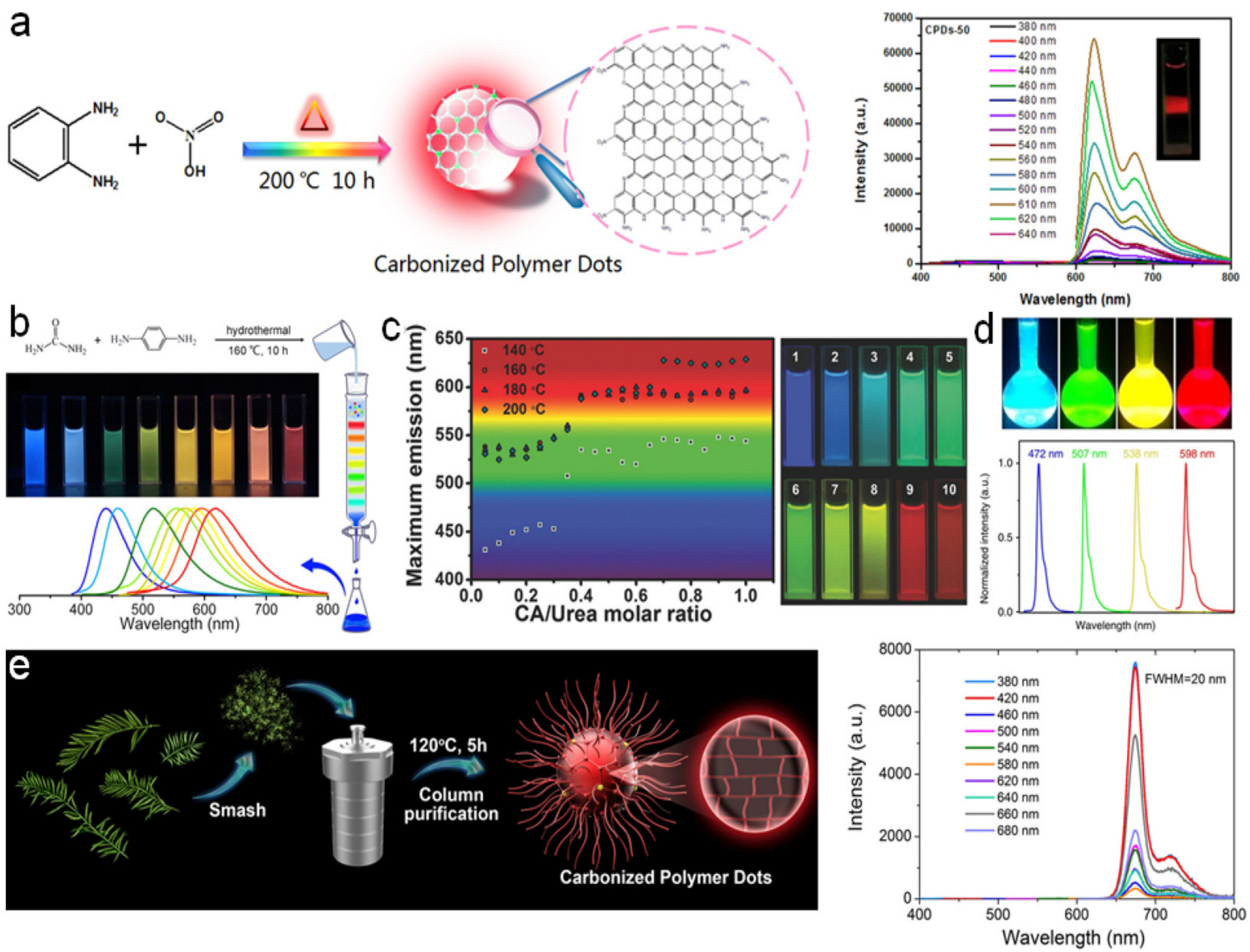

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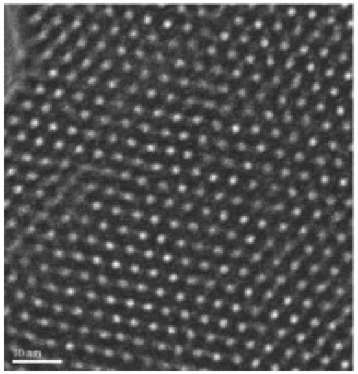
Porous Materials

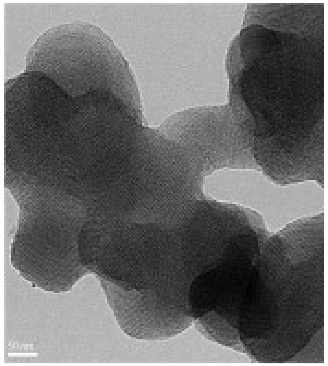
- AAO
- MCM-41

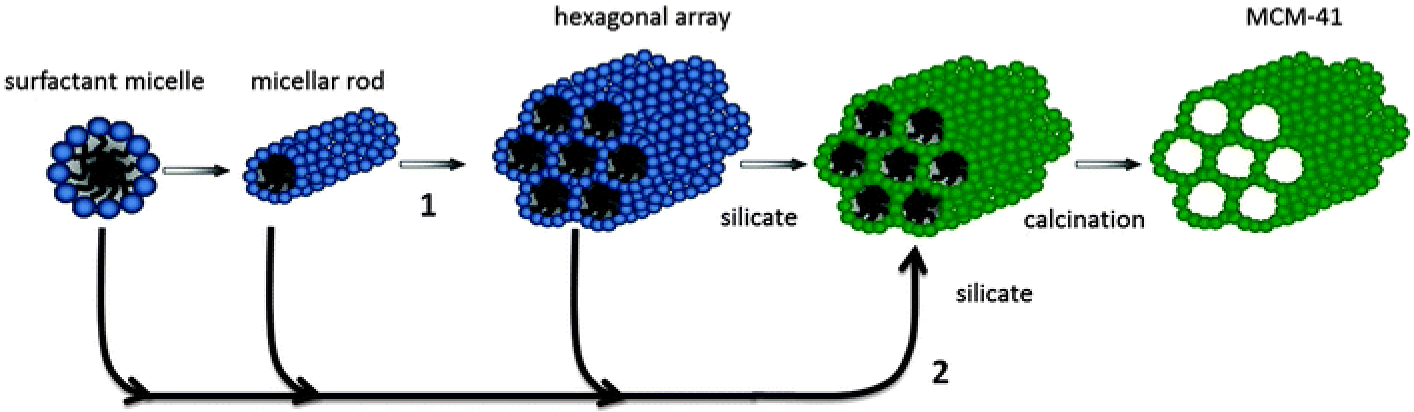
Mobil Crystalline Materials, or MCM-41

Santa Barbara Amorphous type material, or SBA-15

- Micro: < 2nm
- Meso:
- Macro: > 50nm







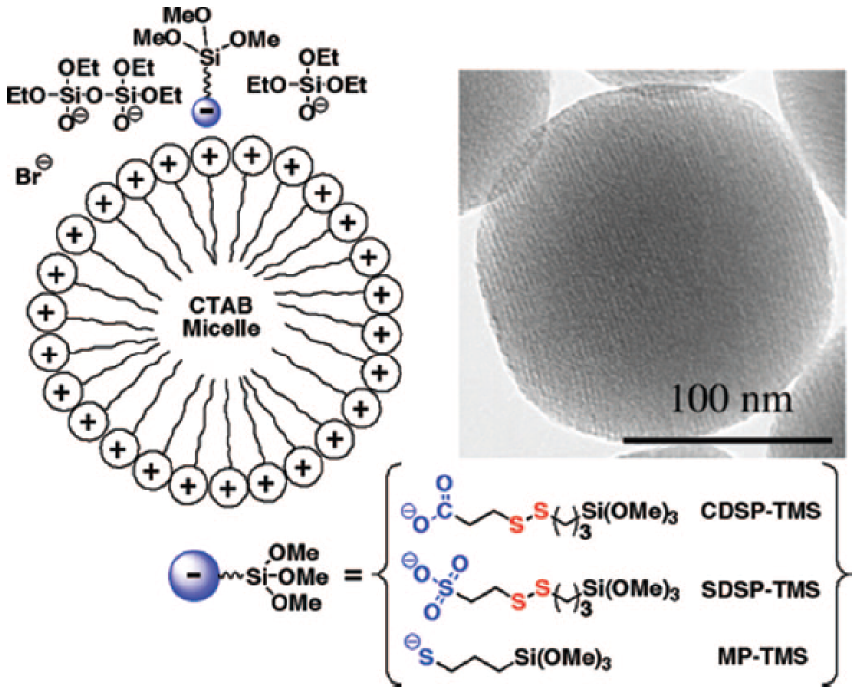


FIGURE 3. Schematic representation of the use of anionic organoalkoxysilanes for controlling the functionalization of the MSN materials. The MCM-41-type mesoporous channels are illustrated by the parallel stripes shown in the transmission electron microscopy (TEM) micrograph of the MSN–SH material. Reproduced with permission from ref 15. Copyright 2005, Royal Society of Chemistry.

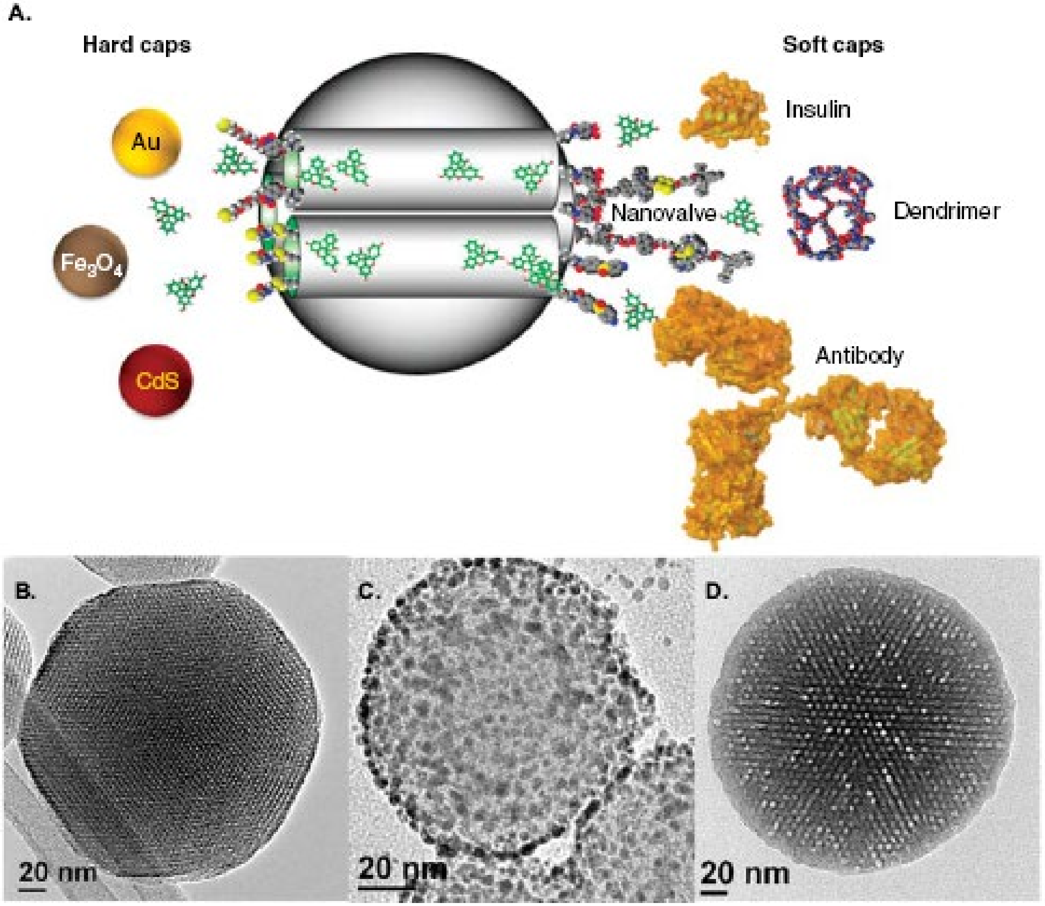


Figure 1. A. Schematic representation of a MSN loaded with drugs and capped with hard caps and soft caps highlighted in this review. Transmission electron microscopy images of (B) a MSN along the axis of the mesopores, (C) capped with hard (Au NP) and (D) with soft (polymer) caps.

MSN: Mesoporous silica nanoparticle.

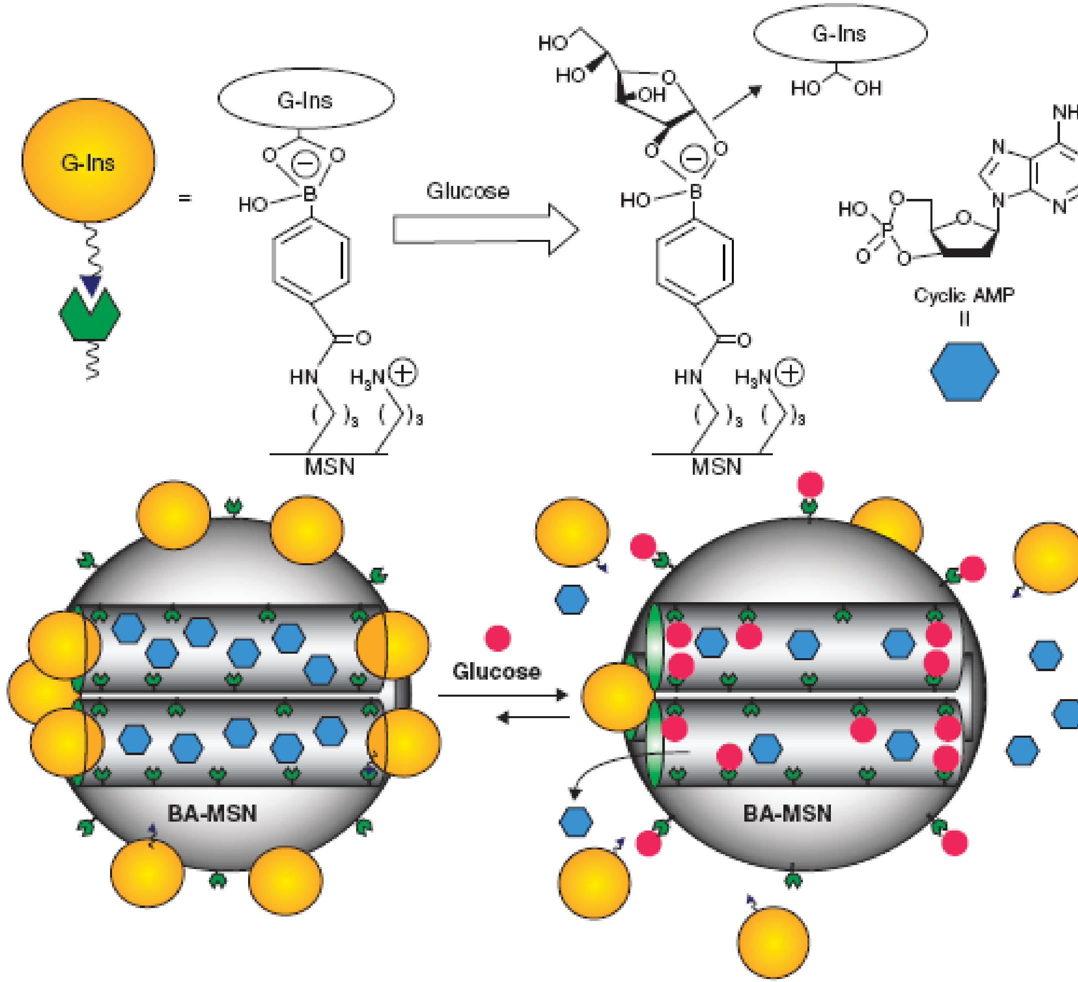
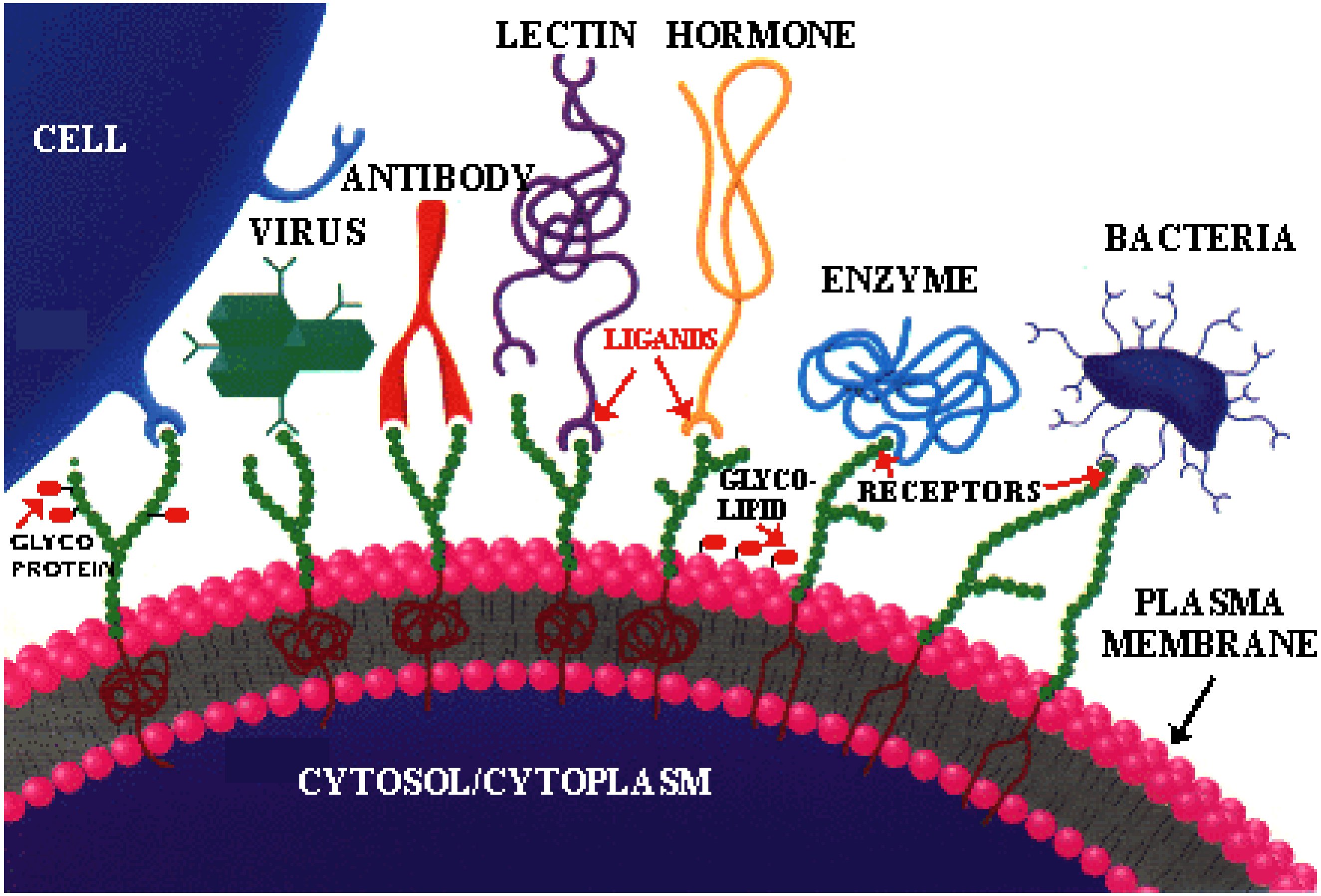


Figure 5. Schematic representation of the glucose-responsive MSN-based double delivery system for controlled release of bioactive G-Ins and cyclic AMP. The controlled release mechanism was achieved by means of the displacement reaction between blood glucose and G-Ins based on reversible boronic acid-diol complexation. High glucose concentration triggers the G-Ins uncapping and the release of cyclic AMP sequentially to diminish the higher than normal level of blood glucose.

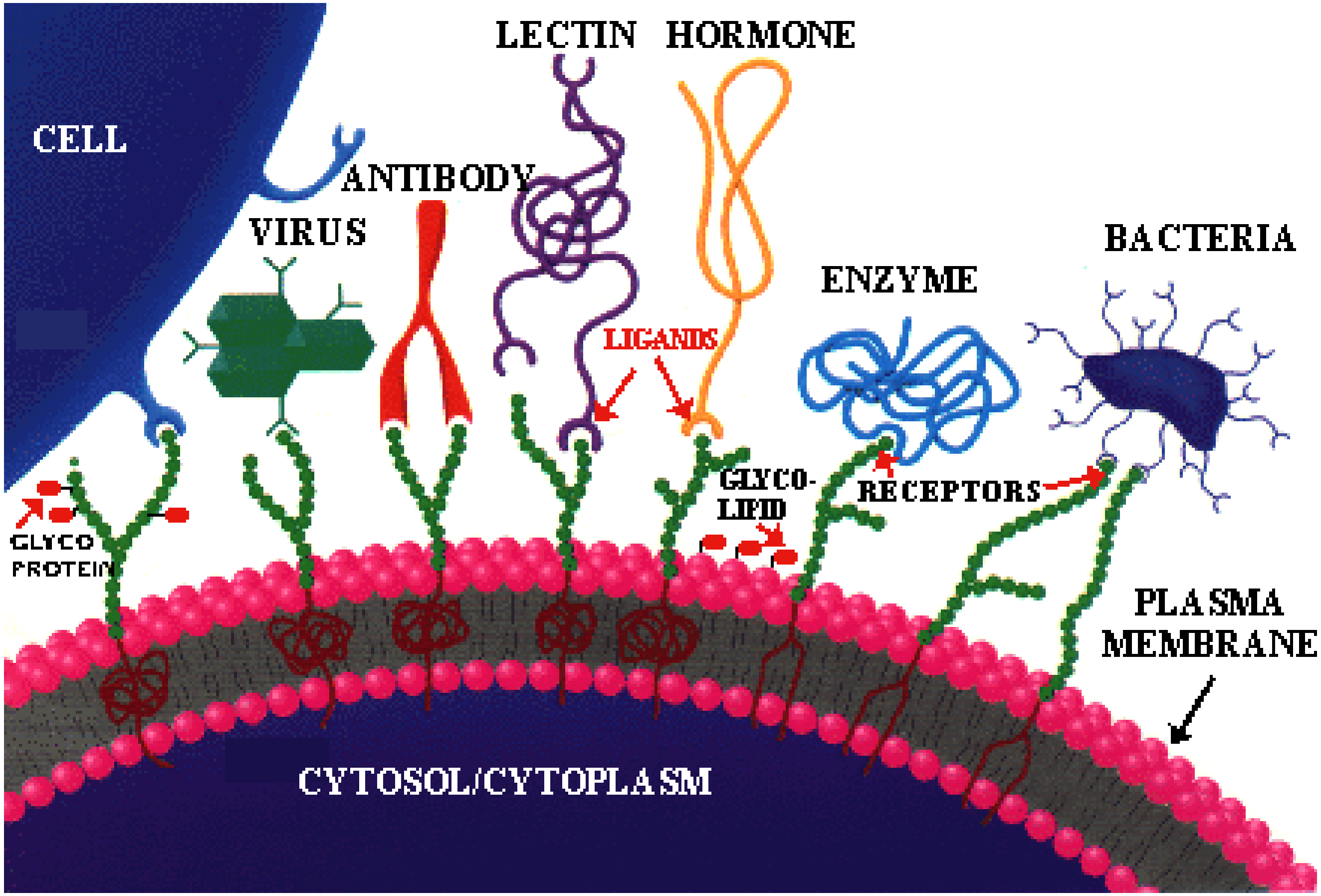
Reproduced with permission from [19].

G-Ins: G-insulin; MSN: Mesoporous silica nanoparticle.

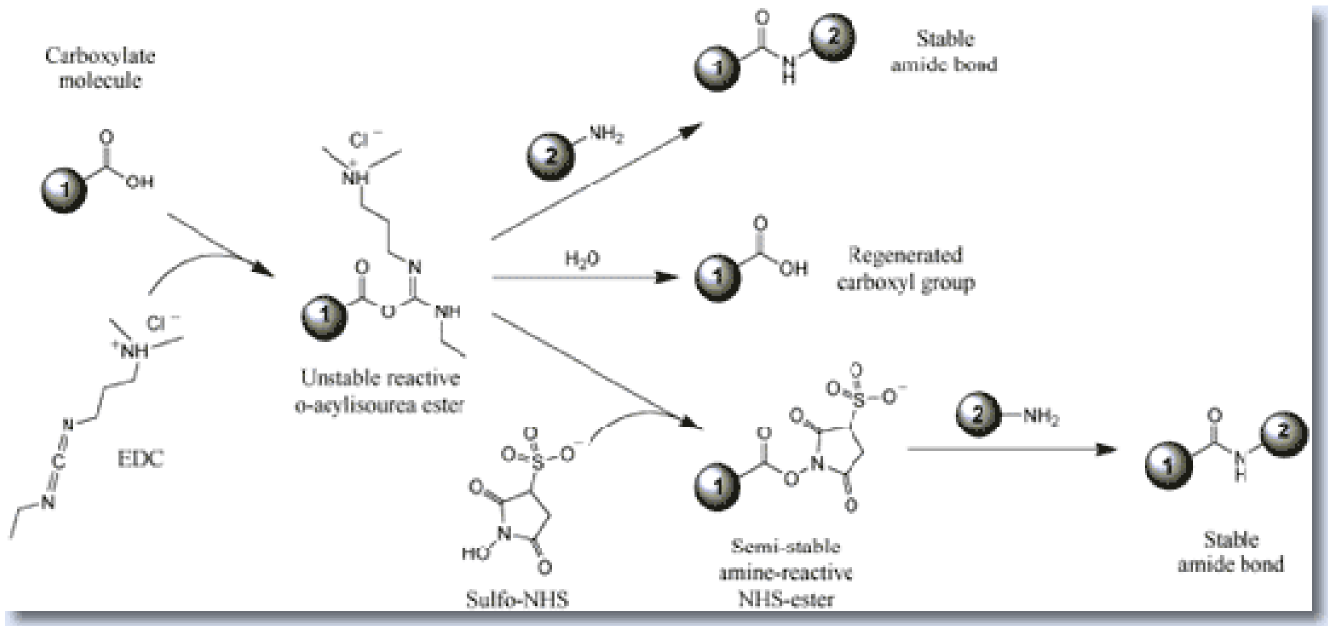
Molecular Recognition

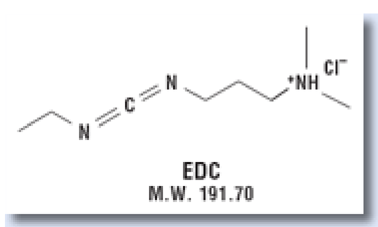


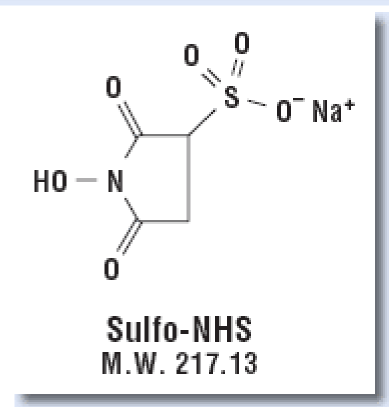
Molecular Recognition



Carboxyl Presenting Surfaces

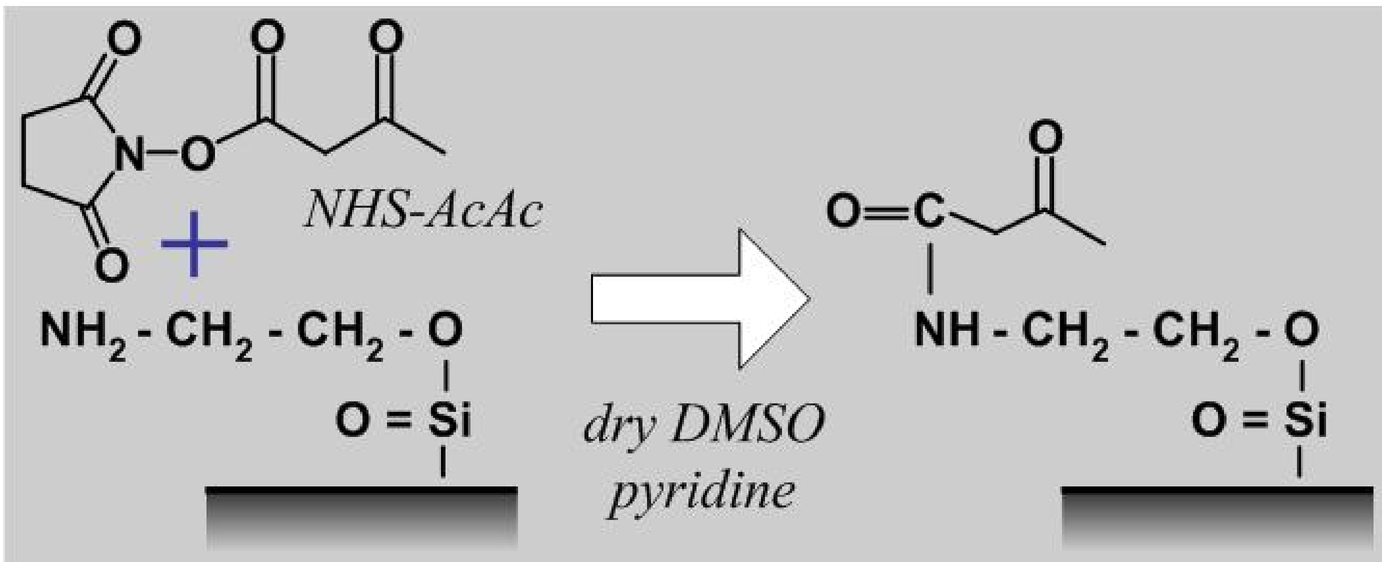




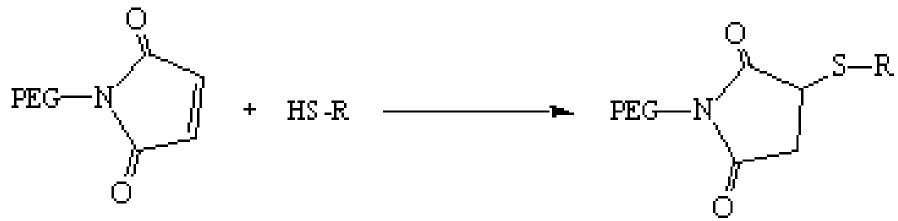


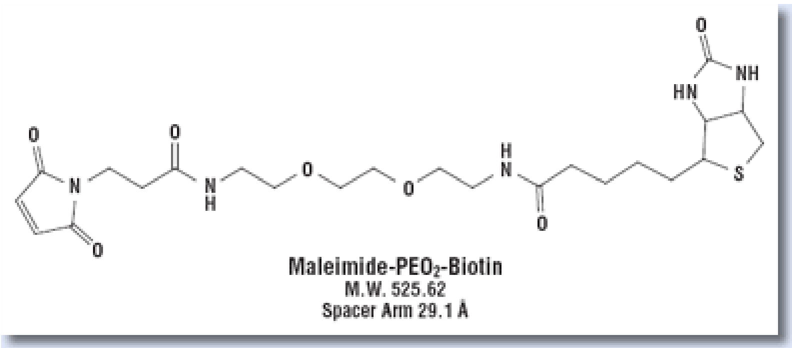
EDC (1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide Hydrochloride)

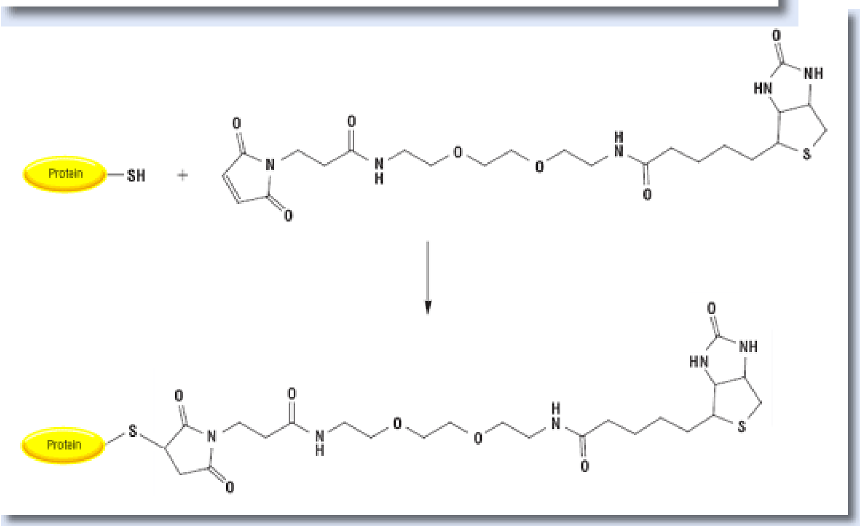
Amine Presenting Surface

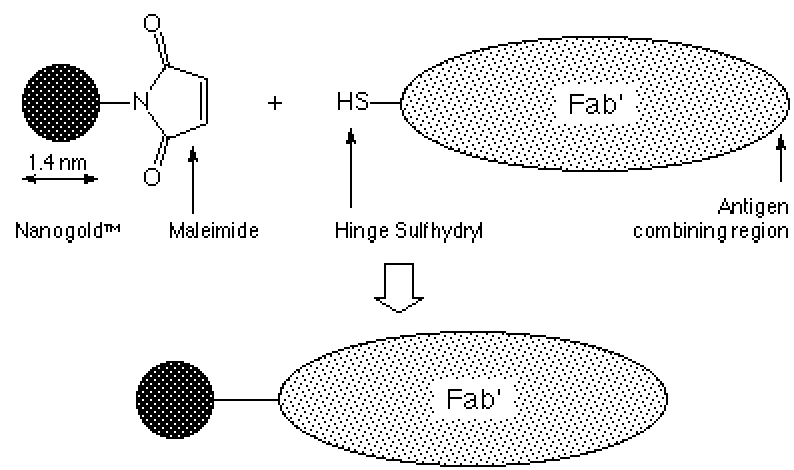


Sulfhydryl Labeling

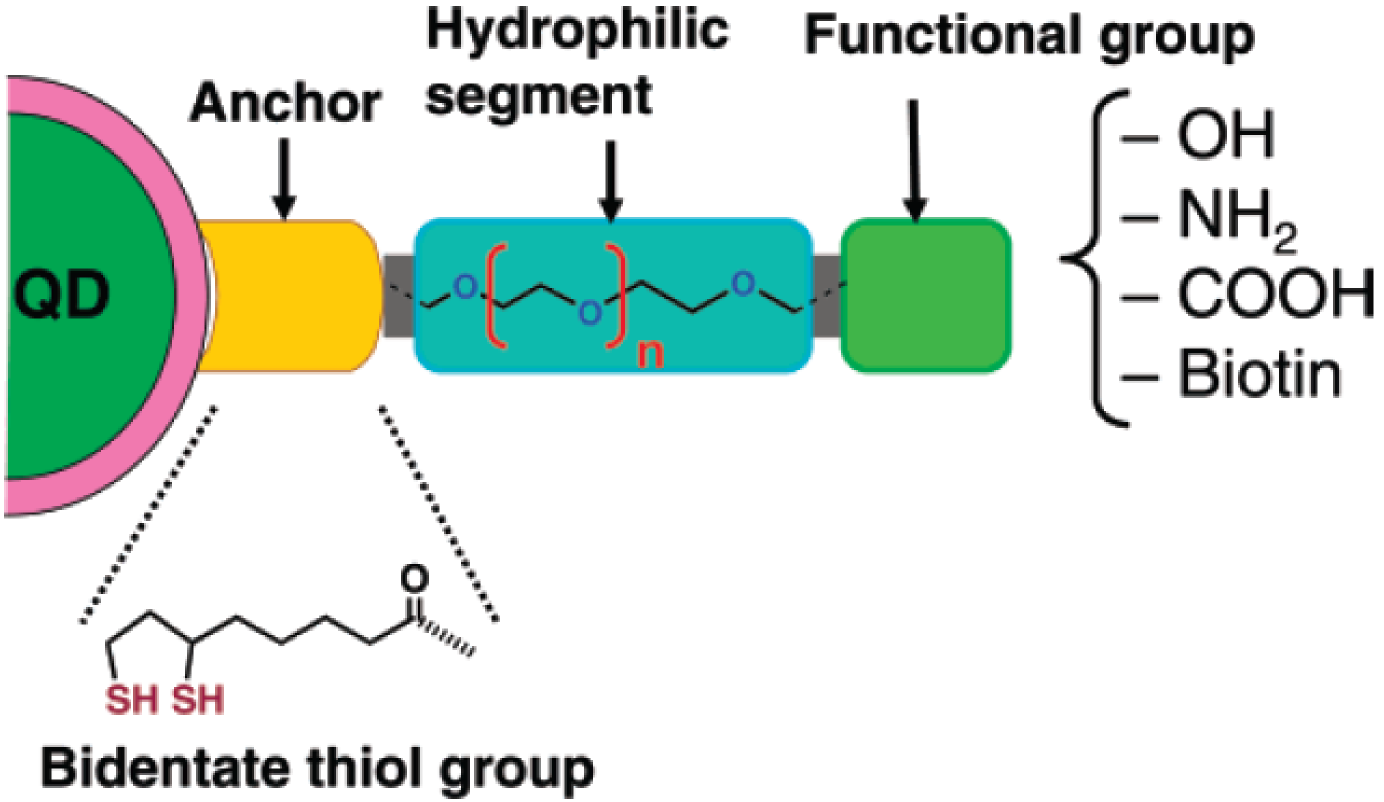








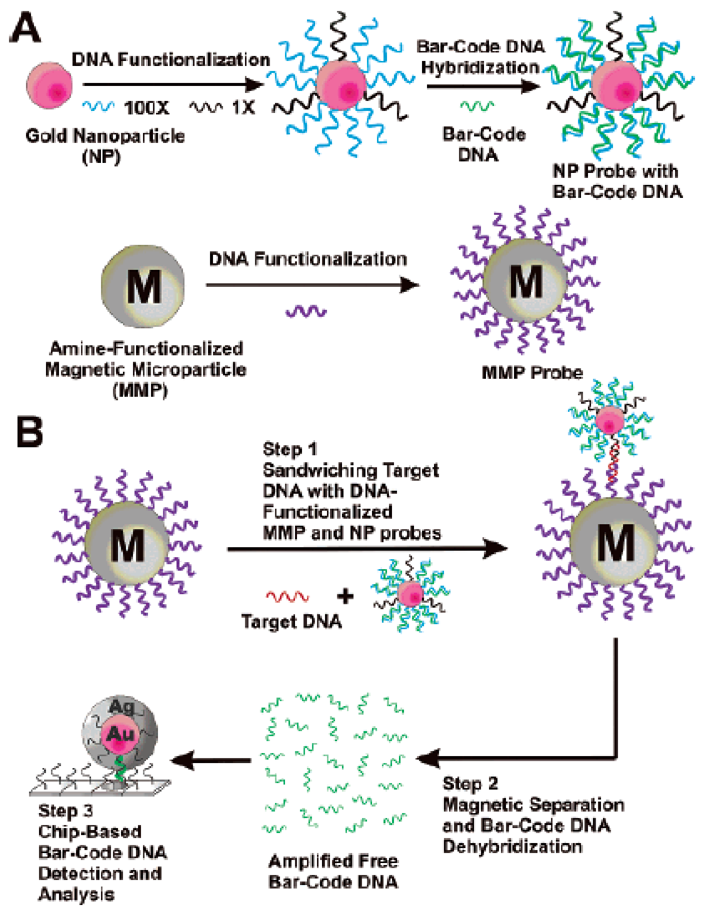
Scheme 1. Modular Design of Hydrophilic Ligands with Terminal Functional Groups Used in This Study



Bio-Bar-Code-Based DNA Detection with PCR-like Sensitivity

Jwa-Min Nam, Savka I. Stoeva, and Chad A. Mirkin*

J. AM. CHEM. SOC. 2004, *126*, 5932−5933



Target DNA (anthrax): 5′ GGATTATTGTTAAATATTGATAAGGAT 3′
Bar-Code DNA: 5′ AGCTACGAGTTGAGAATCCTGAATGCGACG 3′

Figure 1. The DNA-BCA assay. (A) Nanoparticle and magnetic microparticle probe preparation. (B) Nanoparticle-based PCR-less DNA amplification scheme.

Nanoparticle-Based Bio–Bar Codes for the Ultrasensitive Detection of Proteins

26 SEPTEMBER 2003 VOL 301 SCIENCE

Jwa-Min Nam,* C. Shad Thaxton,* Chad A. Mirkin†

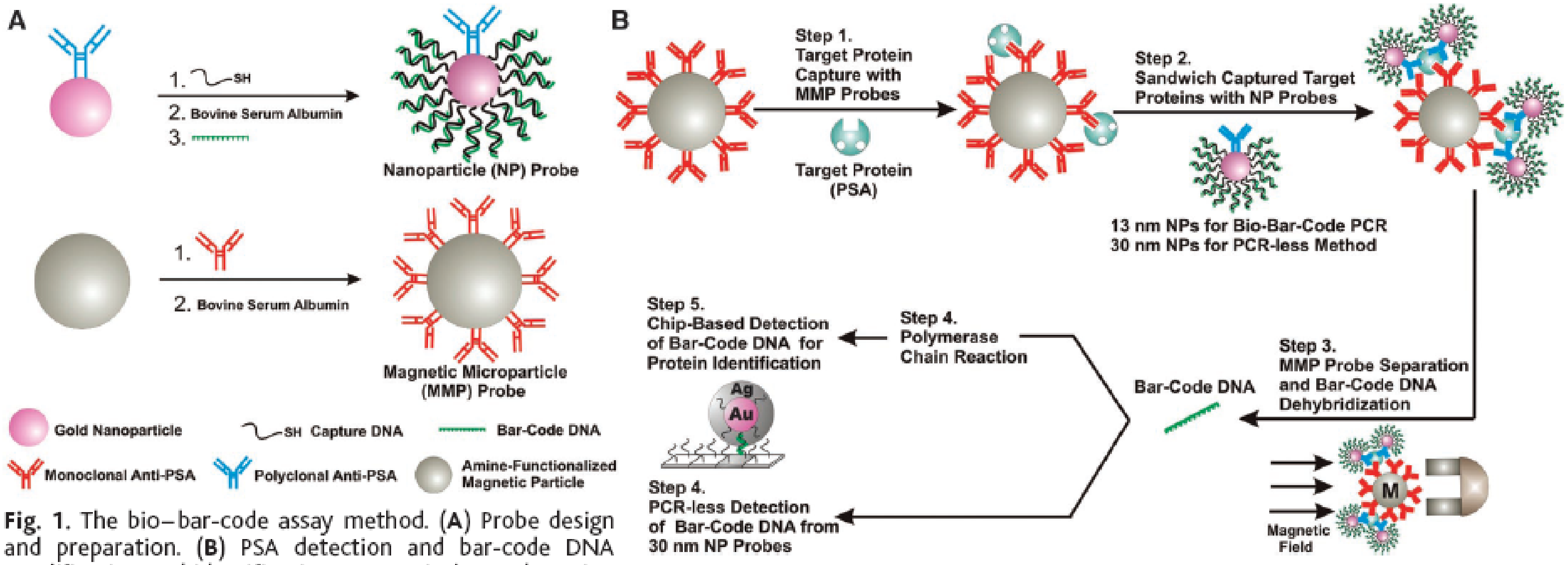
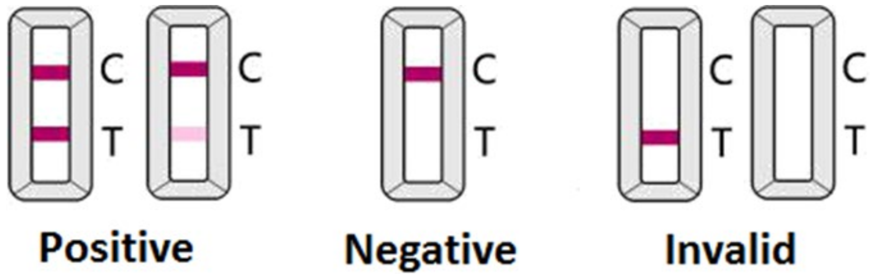
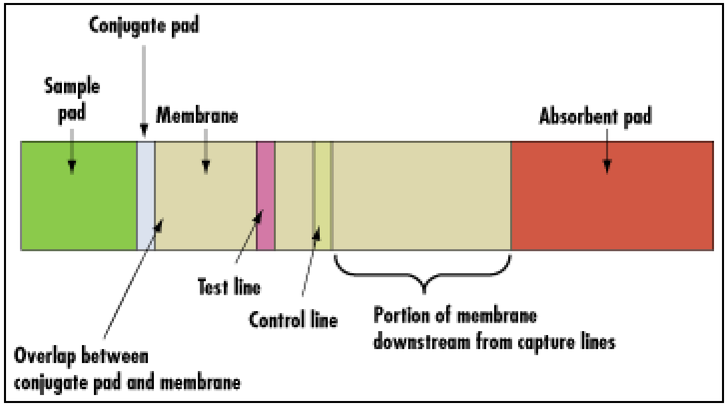


Fig. 1. The bio–bar-code assay method. (**A**) Probe design and preparation. (**B**) PSA detection and bar-code DNA amplification and identification. In a typical PSA-detection experiment, an aqueous dispersion of MMP probes functionalized with mAbs to PSA (50 μl of 3 mg/ml magnetic probe solution) was mixed with an aqueous solution of free PSA (10 μl of PSA) and stirred at 37°C for 30 min (Step 1). A 1.5-ml tube containing the assay solution was placed in a BioMag microcentrifuge tube separator (Polysciences, Incorporated, Warrington, PA) at room temperature. After 15 s, the MMP-PSA hybrids were concentrated on the wall of the tube. The supernatant (solution of unbound PSA molecules) was removed, and the MMPs were resuspended in 50 μl of 0.1 M phosphate-buffered saline (PBS) (repeated twice). The NP probes (for 13-nm NP probes, 50 μl at 1 nM; for 30-nm NP probes, 50 μl at 200 pM), functionalized with polyclonal Abs to PSA and hybridized bar-code DNA strands, were then added to the assay solution. The NPs reacted with the PSA immobilized on the MMPs and provided DNA strands for signal amplification and protein identification (Step 2). This solution was vigorously stirred at 37°C for 30 min. The MMPs were then washed with 0.1 M PBS with the magnetic separator to isolate the magnetic particles. This step was repeated four times, each time for 1 min, to remove everything but the MMPs (along with the PSA-bound NP probes). After the final wash step, the MMP probes were resuspended in NANOpure water (50 μl) for 2 min to dehybridize bar-code DNA strands from the nanoparticle probe surface. Dehybridized bar-code DNA was then easily separated and collected from the probes with the use of the magnetic separator (Step 3). For bar-code DNA amplification (Step 4), isolated bar-code DNA was added to a PCR reaction mixture (20-μl final volume) containing the appropriate primers, and the solution was then thermally cycled (*20*). The bar-code DNA amplicon was stained with ethidium bromide and mixed with gel-loading dye (*20*). Gel electrophoresis or scanometric DNA detection (*24*) was then performed to determine whether amplification had taken place. Primer amplification was ruled out with appropriate control experiments (*20*). Notice that the number of bound NP probes for each PSA is unknown and will depend upon target protein concentration.

Fast Screening Kit



hCG immunoassay



human chorionic gonadotropin (hCG)