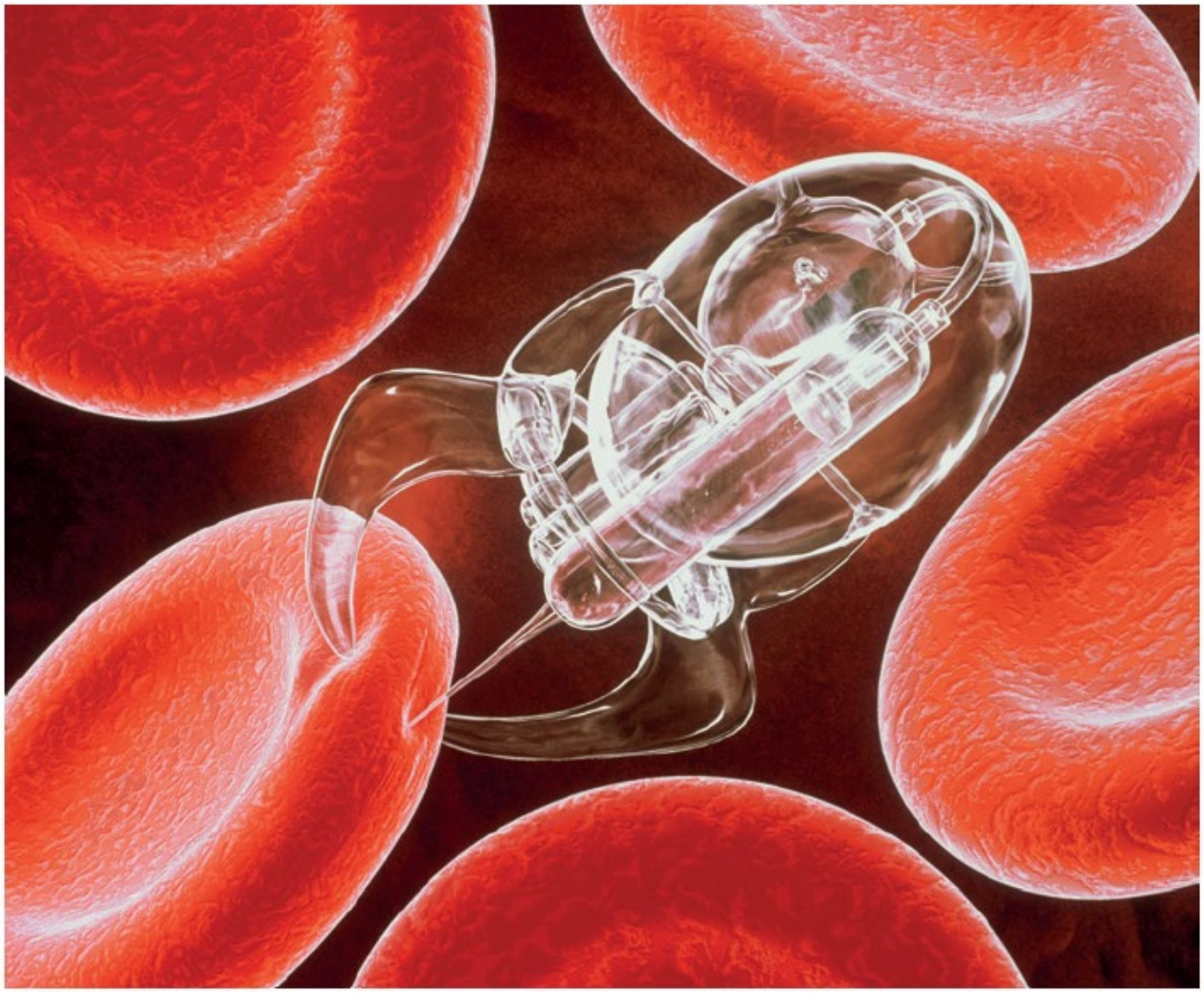
Nanomedicine

Nanobots



Virus Infection

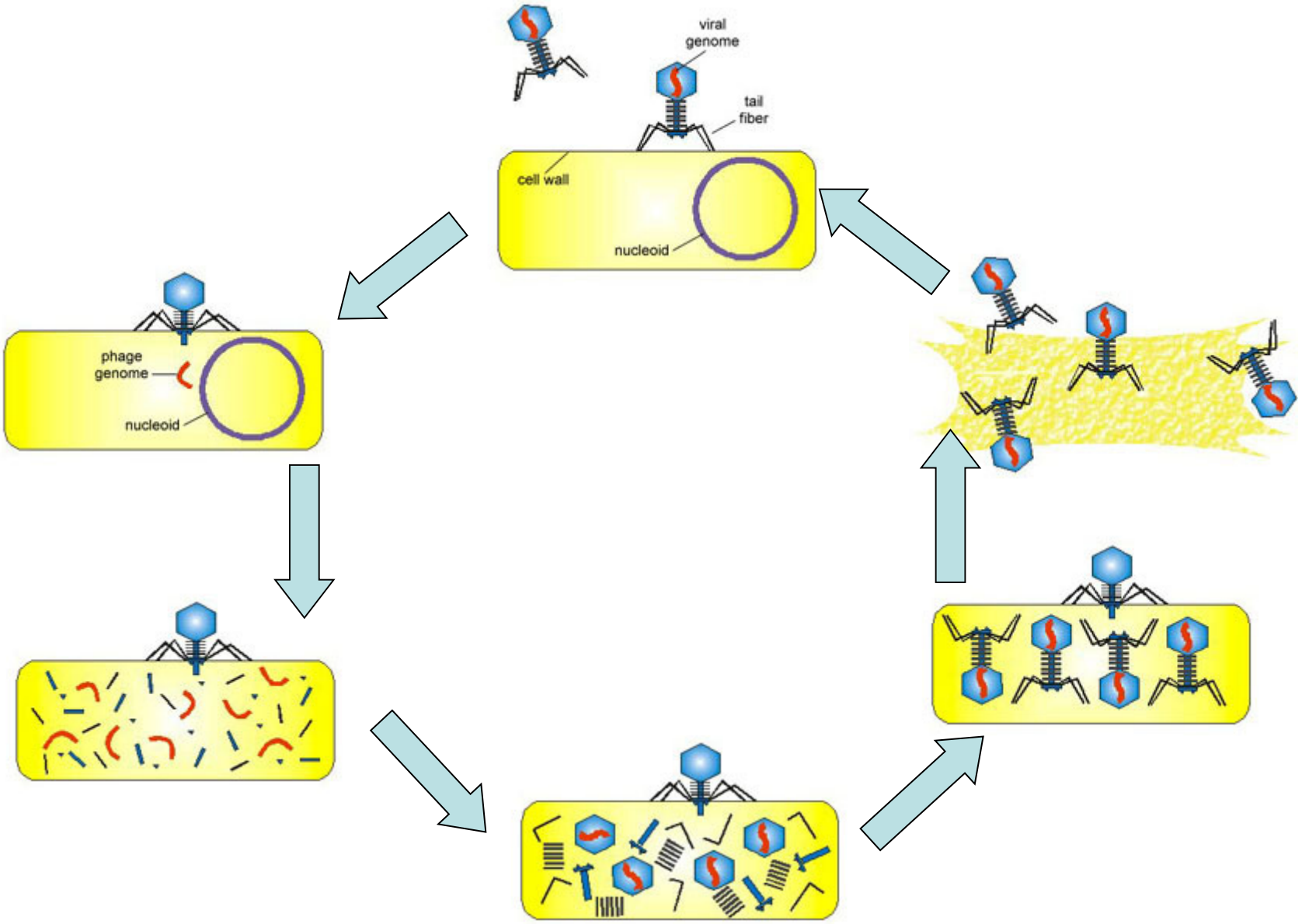
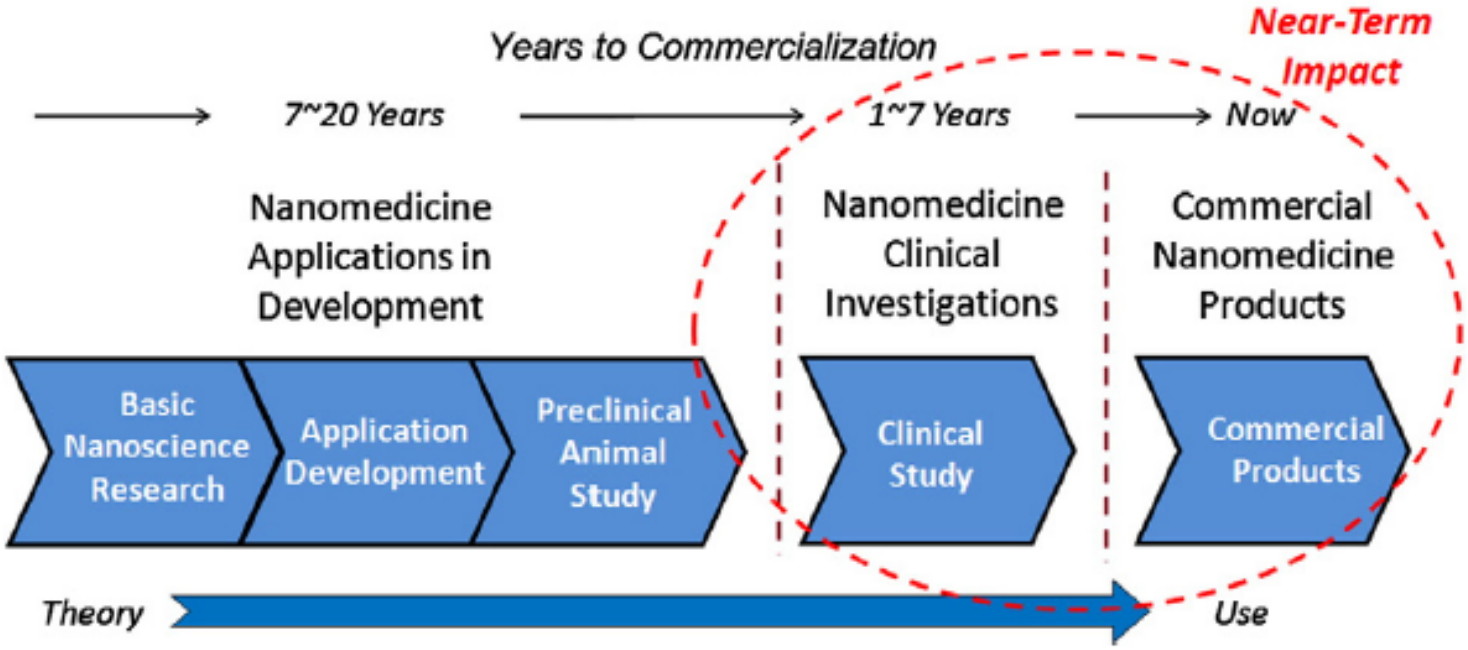


Table 1. Examples of Nanomaterials in Clinical Use.*

Nanomaterial	Trade Name	Application	Target	Adverse Effects	Manufacturer	Current Status
Metallic						
Iron oxide	Feridex	MRI contrast	Liver	Back pain, vasodilatation	Bayer Schering	FDA approved
	Resovist	MRI contrast	Liver	None	Bayer Schering	FDA approved
	Combidex	MRI contrast	Lymph nodes	None	Advanced Magnetics	In phase 3 clinical trials
	NanoTherm	Cancer therapy	Various forms	Acute urinary retention	MagForce	In phase 3 clinical trials
Gold	Verigene	In vitro diagnostics	Genetic	Not applicable	Nanosphere	FDA approved
	Aurimmune	Cancer therapy	Various forms	Fever	CytImmune Sciences	In phase 2 clinical trials
Nanoshells	Auroshell	Cancer therapy	Head and neck	Under investigation	Nanospectra Biosciences	In phase 1 clinical trials
Semiconductor						
Quantum dot	Qdots, EviTags, semiconductor nanocrystals	Fluorescent contrast, in vitro diagnostics	Tumors, cells, tissues, and molecular sensing structures	Not applicable	Life Technologies, eBioscience, Nanoco, CrystalPlex, Cytodiagnostics	Research use only
Organic						
Protein	Abraxane	Cancer therapy	Breast	Cytopenia	Abraxis Bioscience	FDA approved
Liposome	Doxil/Caelyx	Cancer therapy	Various forms	Hand–foot syndrome, stomatitis	Ortho Biotech	FDA approved
Polymer	Oncaspar	Cancer therapy	Acute lymphoblastic leukemia	Urticaria, rash	Rhône-Poulenc Rorer	FDA approved
	CALAA-01	Cancer therapy	Various forms	Mild renal toxicity	Calando	In phase 2 clinical trials
Dendrimer	VivaGel	Microbicide	Cervicovaginal	Abdominal pain, dysuria	Starpharma	In phase 2 clinical trials
Micelle	Genexol-PM	Cancer therapy	Various forms	Peripheral sensory neuropathy, neutropenia	Samyang	For phase 4 clinical trials

* MRI denotes magnetic resonance imaging.

Nanomedicine Technology Development Pipeline



Nanomedicine: Nanotechnology, Biology, and Medicine
9 (2013) 1 – 14

Gene Therapy

- Gene therapy is a technique for correcting defective genes responsible for disease development. Researchers may use one of several approaches for correcting faulty genes:
  - **A normal gene may be inserted into a nonspecific location within the genome to replace a nonfunctional gene. This approach is most common.**
  - **An abnormal gene could be swapped for a normal gene through homologous recombination.**
  - **The abnormal gene could be repaired through selective reverse mutation, which returns the gene to its normal function.**
  - **The regulation (the degree to which a gene is turned on or off) of a particular gene could be altered.**

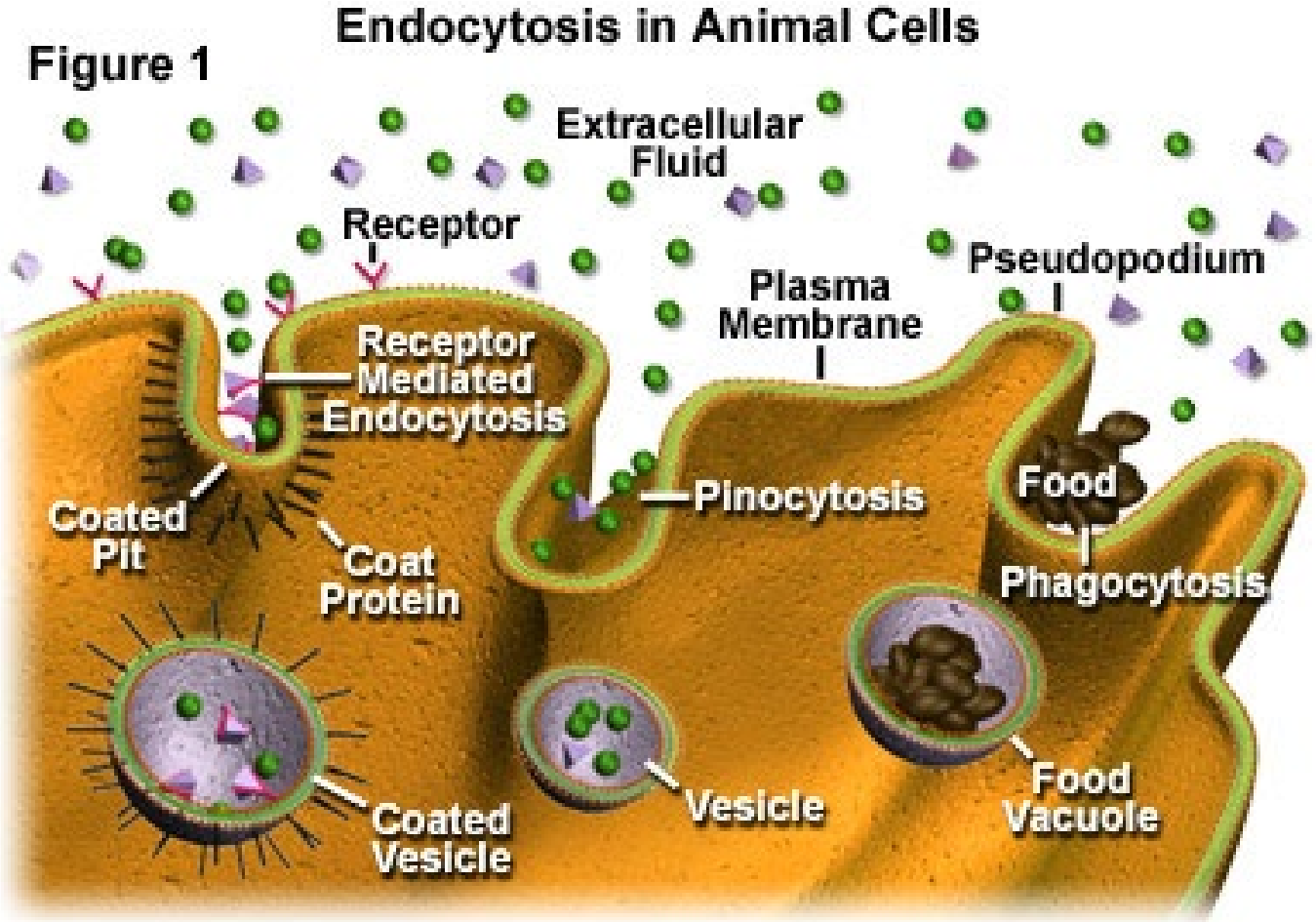
How Gene Therapy Works?

- In most gene therapy studies, a "normal" gene is inserted into the genome to replace an "abnormal," disease-causing gene. A carrier molecule called a vector must be used to deliver the therapeutic gene to the patient's target cells. Currently, the most common vector is a virus that has been genetically altered to carry normal human DNA. Viruses have evolved a way of encapsulating and delivering their genes to human cells in a pathogenic manner. Scientists have tried to take advantage of this capability and manipulate the virus genome to remove disease-causing genes and insert therapeutic genes.
- Target cells such as the patient's liver or lung cells are infected with the viral vector. The vector then unloads its genetic material containing the therapeutic human gene into the target cell. The generation of a functional protein product from the therapeutic gene restores the target cell to a normal state.

Gene Delivery

- Transfection- the delivery of foreign molecules such as DNA and RNA into eukaryotic cells
- Naked DNA is not suitable for in-vivo transport of genetic materials-> degradation by serum nucleases
- Ideal gene delivery system
  - Biocompatible
  - Non-immunogenic
  - Stable in blood stream
  - Protect DNA during transport
  - Small enough to extravagate
  - Cell and tissue specific

Endocytosis



Endocytic pathway in mammalian cells

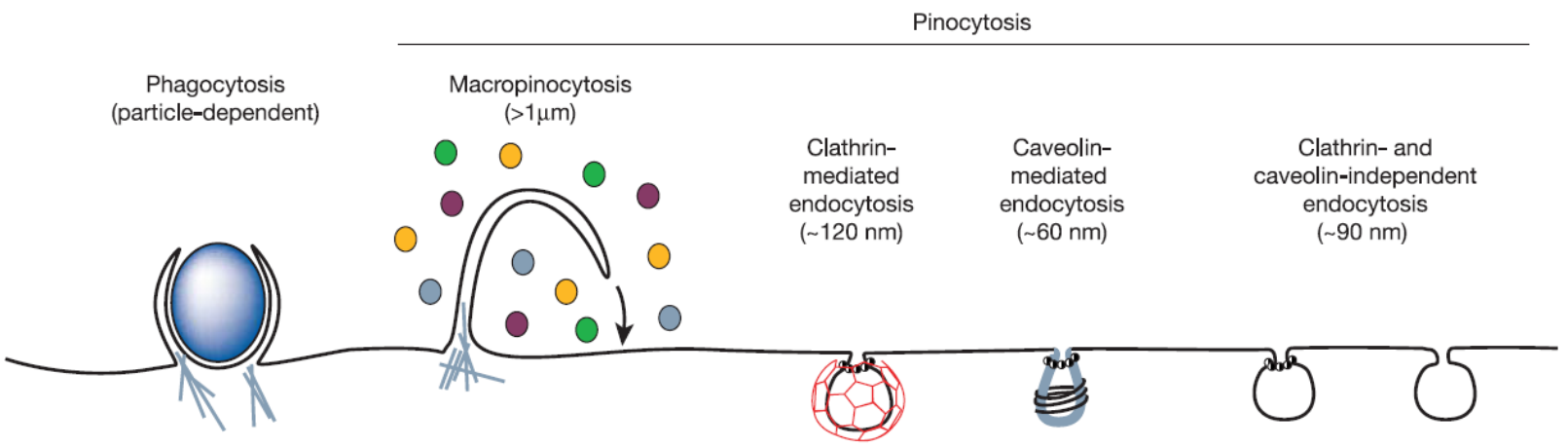


Figure 1 Multiple portals of entry into the mammalian cell. The endocytic pathways differ with regard to the size of the endocytic vesicle, the nature of the cargo (ligands, receptors and lipids) and the mechanism of vesicle formation.

Barrier to non-viral gene delivery

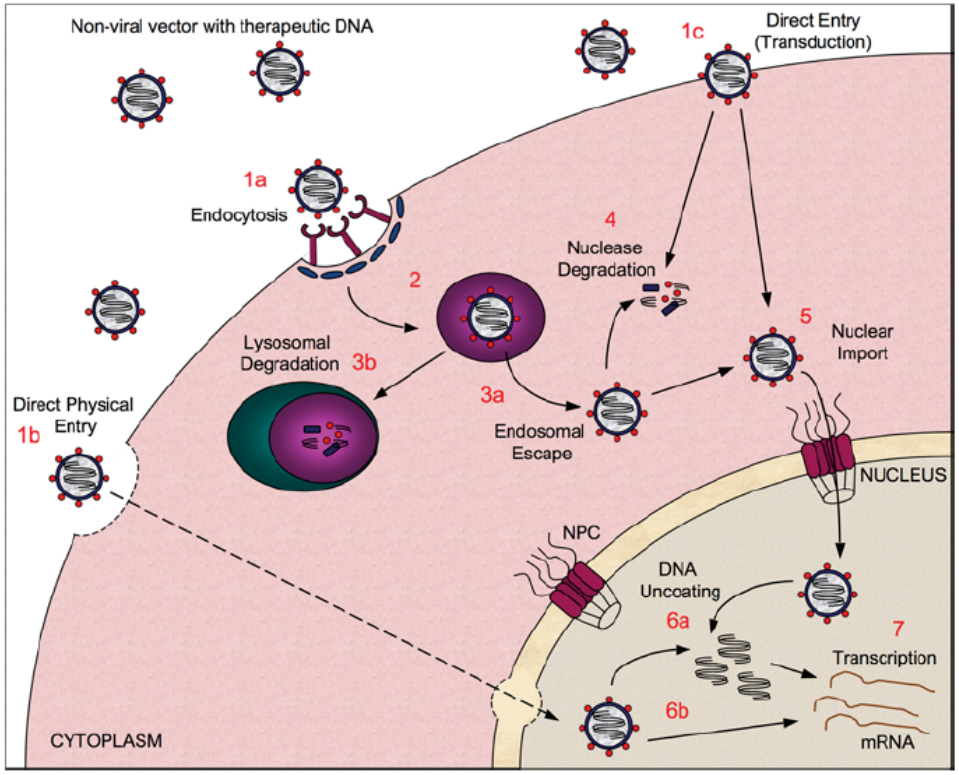


Figure 1 Barriers to non-viral gene delivery

Representation of the route travelled by a non-viral gene-delivery vector carrying therapeutic DNA to the nucleus. A non-viral vector, formed by interaction of the DNA with a carrier compound, must cross the plasma membrane to enter the cell. This can be via several routes, including endocytosis-based entry (1a), direct physical entry routes, such as electroporation or ballistic delivery (1b), or direct entry via protein transduction (1c). Depending on the mode of cellular entry, the vector may become encapsulated in an endosome (2), from which it must escape (3a) or it will become degraded when the endosome fuses with a lysosome (3b). The DNA will at some point be subjected to degradation by cytosolic nucleases (4), as it traverses through the cytoplasm to reach the nucleus. Finally, the vector must undergo nuclear transport (5) through NPCs embedded in the NE in order to gain access to the nucleoplasm. Once in the nucleus, the DNA may (6a) or may not (6b) need to be uncoated, depending upon the vector used, before it can ultimately be transcribed (7).

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NLS-mediated nuclear import

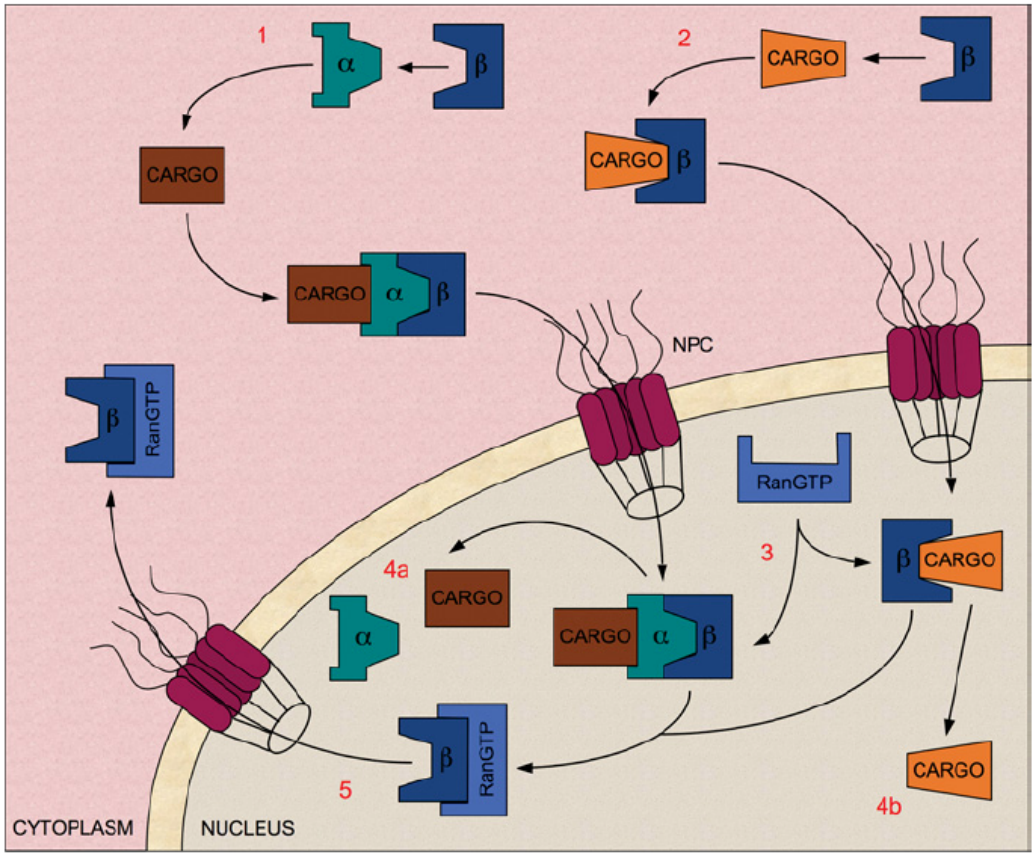


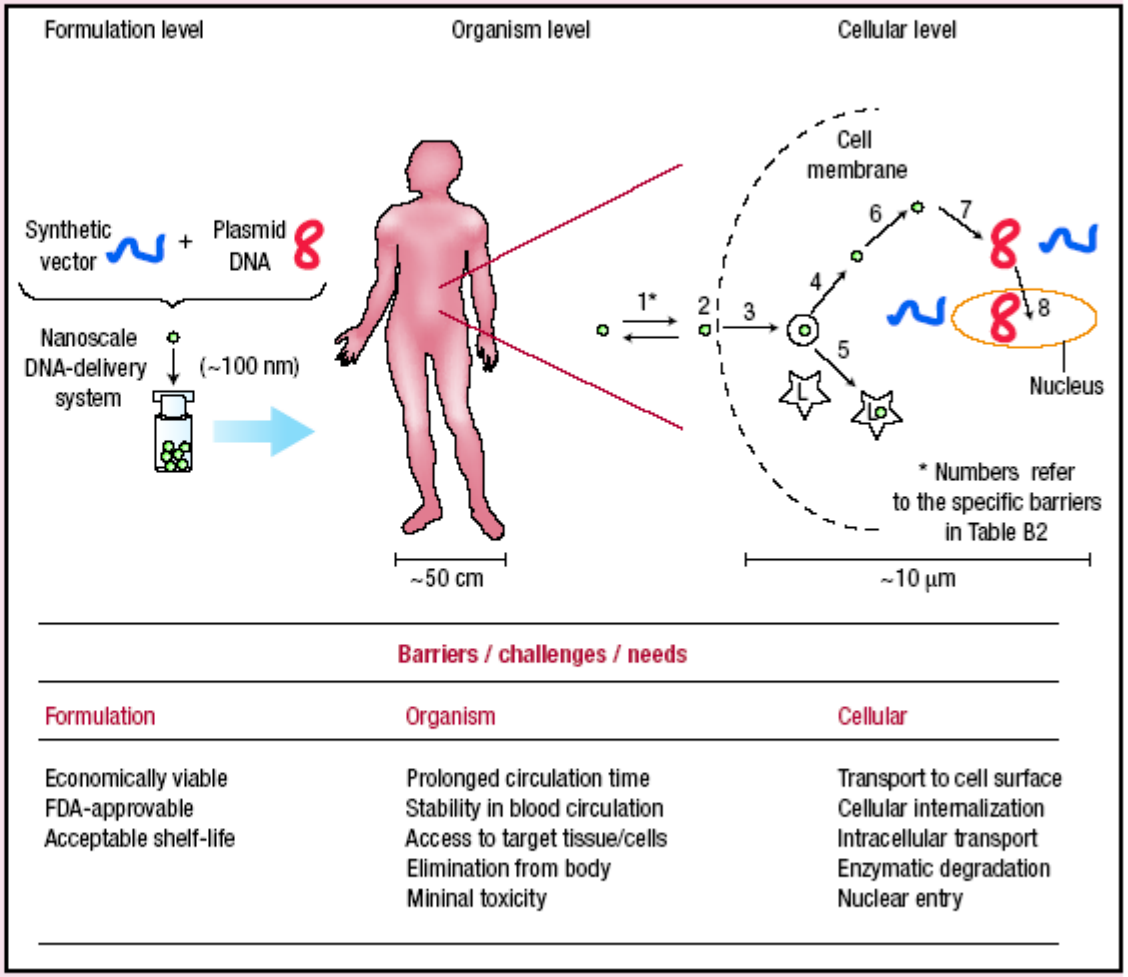
Figure 2 NLS-mediated nuclear import pathways

In classical nuclear import, the NLS found in cargo bound for the nucleus is recognized by the Imp α subunit of the Imp α/β heterodimer (1). However, there are also many examples where Imp β or one of its many homologues can mediate nuclear import or cargo proteins independently of Imp α (2). In both cases, transient interactions between the Imp β and the nucleoporin proteins that line the NE-embedded NPCs mediate translocation into the nucleus. Once inside, RanGTP binds to Imp β (3), releasing Imp α and the cargo into the nucleoplasm (4a and 4b). RanGTP itself is then recycled back to the cytoplasm (5), where it is converted into its RanGDP state (not shown). An animated version of this Figure can be found at http://www.BiochemJ.org/bj/406/0185/bj4060185add.htm

Barriers to DNA Delivery

BOX 1

A number of challenges and barriers face the successful delivery of therapeutic DNA to target cells in the body. Physicochemical, economic and sterilization challenges complicate formulation; the complex environment of the human body hinders its successful transport to the target cell population; and endocytic pathway barriers hinder its successful transport to the nucleus of the cell (the site of action). Each known and major barrier is listed in Fig. B1, using nanoscale DNA-delivery systems as representative examples. Each barrier exists independent of length scale. L = lysosome. A number of clever systems have been devised to overcome these barriers, the general design criteria of which are given in Tables B1 and B2.



Barriers / challenges / needs

Formulation	Organism	Cellular
Economically viable	Prolonged circulation time	Transport to cell surface
FDA-approvable	Stability in blood circulation	Cellular internalization
Acceptable shelf-life	Access to target tissue/cells	Intracellular transport
	Elimination from body	Enzymatic degradation
	Mininal toxicity	Nuclear entry

Figure B1 Barriers to DNA delivery.

Organism Level			
Barrier/challenge/need	**Rationale**	**Example approaches**	**Materials design criteria**
Prolonged circulation time	Maximize total flux past target cell type	PEG conjugates to minimize interaction with serum proteins	Hydrophilic Uncharged
Stability within blood circulation	Maintenance of designed functionality	Crosslinking to maximize overall stability	Stable crosslinks within bloodstream, but reversible upon entry into target cell
Access to target tissue/cells	Transport from capillary lumen to extracellular space to reach target cell surface	Vaso-active protein conjugates (for example, vascular endothelial growth factor)	Retention of protein activity post conjugation
		Targeting restricted to 'leaky' vessel tissues (for example, tumour, liver, spleen).	Small diameter delivery system (for example, <100 nm)
Elimination from body	Minimal build-up of delivery vector over time	Control over molecular weight	Filterable through kidneys
		Engineered biodegradation sites	Biodegradable
Minimal toxicity and immunogenicity	Safety over treatment duration and beyond that required for FDA-approval	Minimize cation density	Non-cytotoxic
		Avoid protein-based materials/conjugates	Non-immunogenic

Cellular Level

Barrier number (from Fig. B1)	Barrier/challenge/need	Example approaches	Materials design criteria
1, 2 and 3	Transport to cell surface, association with cell membrane, internalization	Receptor/ligand interaction (for example, antibody/polymer conjugates, recombinant protein–polymer fusions, carbohydrate conjugates)	Cell-type specificity, low cross reactivity, if desired
			Promiscuous attachment, high cross reactivity, if desired (for example, positive zeta potential, lipid conjugation)
		Non-specific interaction with cell surface (for example, positive zeta potential, lipid conjugates)	
			Endocytic pathway trigger (for example, clathrin-dependent, clathrin-independent, caveolin-dependent)
4 and 5	Escape endosomal vesicle and avoid transport to lysosome	Buffering capacity between pH ~7.2 and ~5.0	Ability to disrupt endosomal membrane and/or fusion of endosome with lysosome
		Fusogenic peptide conjugate	
6	Transport through cytosol to perinuclear space with minimal degradation	'Higher' molecular weight to maintain complex stability within cytosol	Thermodynamic and kinetic stability of complex within cytosol
			Minimize DNA degradation within cytosol
7	Separation of complex to allow nuclear translocation	Hydrolytically or reductively degradable polymers to reduce molecular weight	'Triggered' degradation of polymer to reduce thermodynamic and kinetic stability of complex. Release of intact DNA at or near nuclear envelope
8	Nuclear entry	Nuclear localization sequence conjugates	Facilitate nuclear uptake of DNA using virus-derived signals
		Mitosis	Facilitate nuclear uptake during mitosis when the nuclear envelope is dissolved.

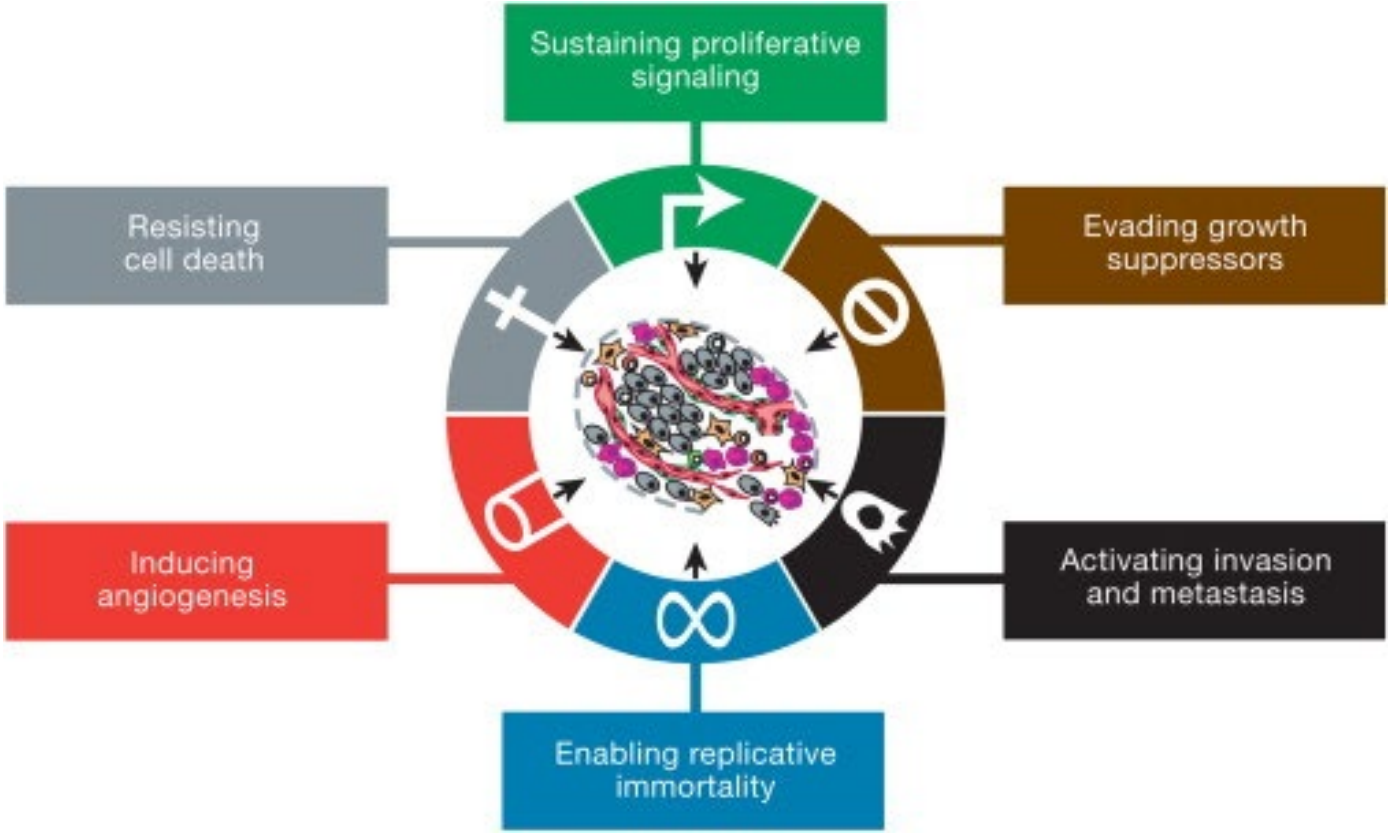
CANCER NANOTECHNOLOGY: OPPORTUNITIES AND CHALLENGES

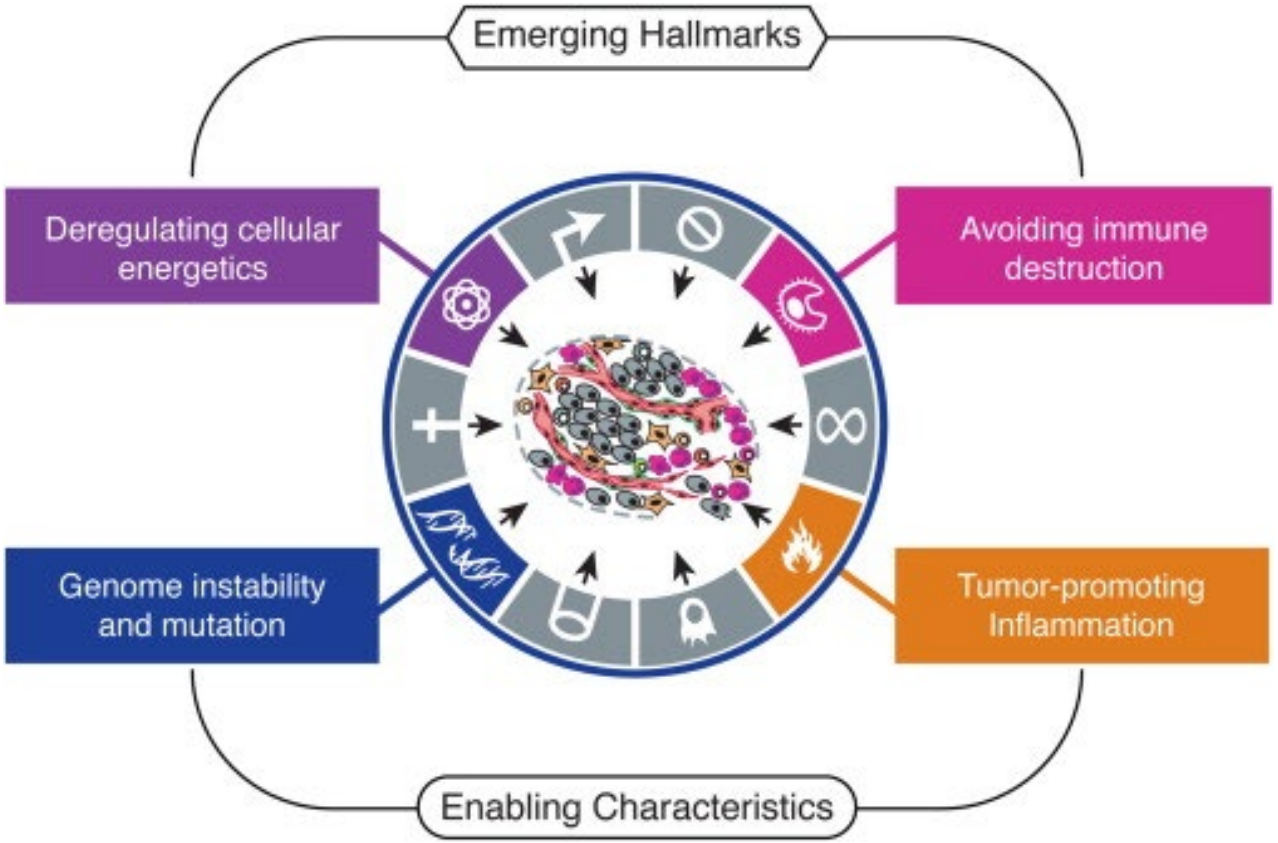
NATURE REVIEWS | CANCER VOLUME 5 | MARCH 2005 | 161

Summary

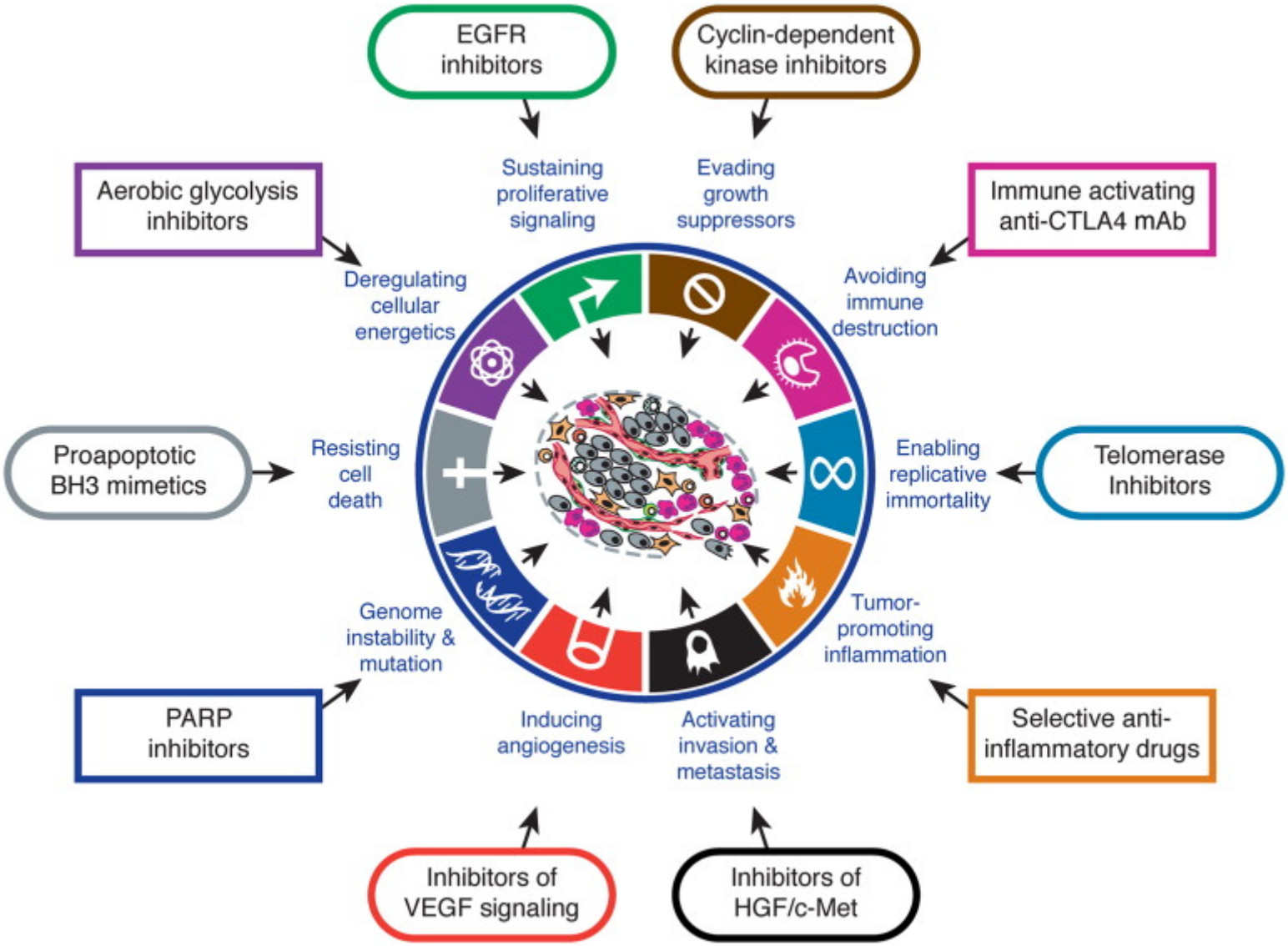
- Nanotechnology concerns the study of devices that are themselves or have essential components in the 1–1,000 nm dimensional range (that is, from a few atoms to subcellular size).
- Two main subfields of nanotechnology are nanovectors — for the administration of targeted therapeutic and imaging moieties — and the precise patterning of surfaces.
- Nanotechnology is no stranger to oncology: liposomes are early examples of cancer nanotherapeutics, and nanoscale-targeted magnetic resonance imaging contrast agents illustrate the application of nanotechnology to diagnostics.
- Photolithography is a light-directed surface-patterning method, which is the technological foundation of microarrays and the surface-enhanced laser desorption/ionization time-of-flight approach to proteomics. Nanoscale resolution is now possible with photolithography, and will give rise to instruments that can pack a much greater density of information than current biochips.
- The ability of nanotechnology to yield advances in early detection, diagnostics, prognostics and the selection of therapeutic strategies is predicated based on its ability to 'multiplex' — that is, to detect a broad multiplicity of molecular signals and biomarkers in real time. Prime examples of multiplexing detection nanotechnologies are arrays of nanocantilevers, nanowires and nanotubes.
- Multifunctionality is the fundamental advantage of nanovectors for the cancer-specific delivery of therapeutic and imaging agents. Primary functionalities include the avoidance of biobarriers and biomarker-based targeting, and the reporting of therapeutic efficacy.
- Thousands of nanovectors are currently under study. By systematically combining them with preferred therapeutic and biological targeting moieties it might be possible to obtain a very large number of novel, personalized therapeutic agents.
- Novel mathematical models are needed, in order to secure the full import of nanotechnology into oncology.

Cancer Hallmark





An increasing body of research suggests that two additional hallmarks of cancer are involved in the pathogenesis of some and perhaps all cancers. One involves the capability to modify, or reprogram, cellular metabolism in order to most effectively support neoplastic proliferation. The second allows cancer cells to evade immunological destruction, in particular by T and B lymphocytes, macrophages, and natural killer cells. Because neither capability is yet generalized and fully validated, they are labeled as emerging hallmarks. Additionally, two consequential characteristics of neoplasia facilitate acquisition of both core and emerging hallmarks. Genomic instability and thus mutability endow cancer cells with genetic alterations that drive tumor progression. Inflammation by innate immune cells designed to fight infections and heal wounds can instead result in their inadvertent support of multiple hallmark capabilities, thereby manifesting the now widely appreciated tumor-promoting consequences of inflammatory responses.


EGFR inhibitors
Cyclin-dependent kinase inhibitors
Sustaining proliferative signaling
Evading growth suppressors
Aerobic glycolysis inhibitors
Immune activating anti-CTLA4 mAb
Deregulating cellular energetics
Avoiding immune destruction
Proapoptotic BH3 mimetics
Resisting cell death
Enabling replicative immortality
Telomerase Inhibitors
Genome instability & mutation
Tumor-promoting inflammation
PARP inhibitors
Inducing angiogenesis
Activating invasion & metastasis
Selective anti-inflammatory drugs
Inhibitors of VEGF signaling
Inhibitors of HGF/c-Met

Protein Corona

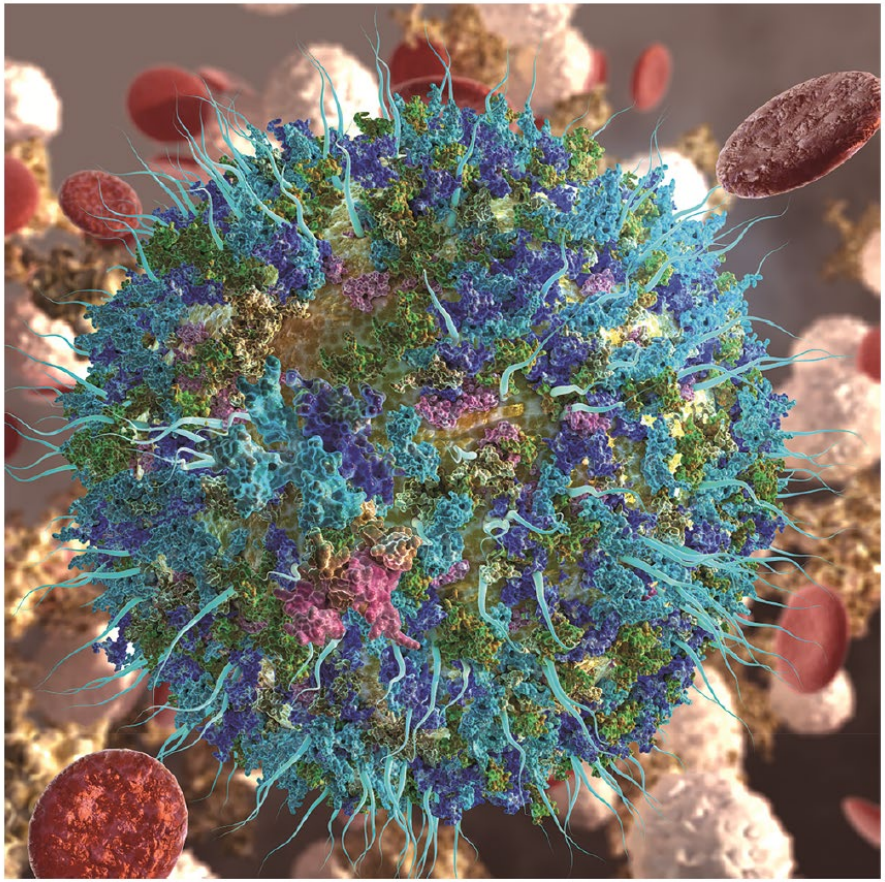


Figure 1. A nanoparticle gains a new biological identity upon its dynamic interactions with biological fluids, giving rise to a protein corona (shown as adsorbed green, blue, and cyan globules), which consequently influences drug delivery and targeting of the functionalized nanoparticle (illustrated as aqua blue fibrils).

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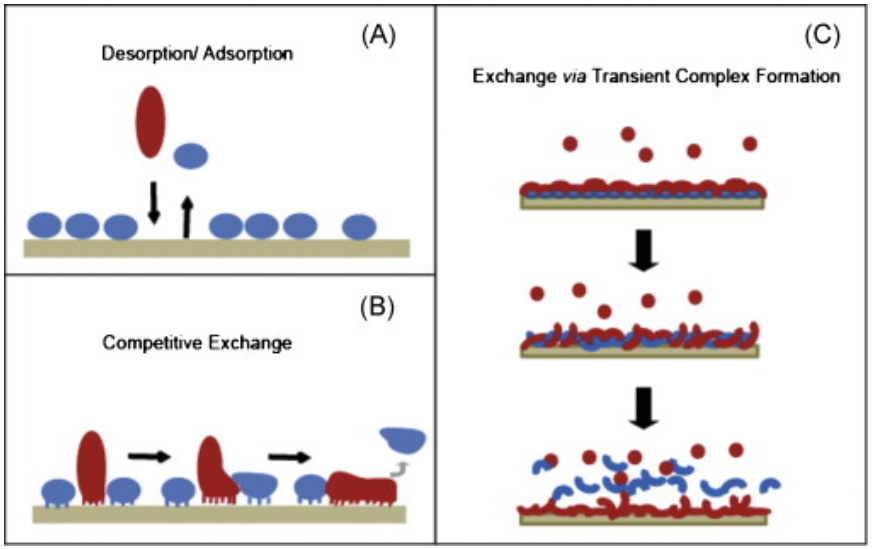
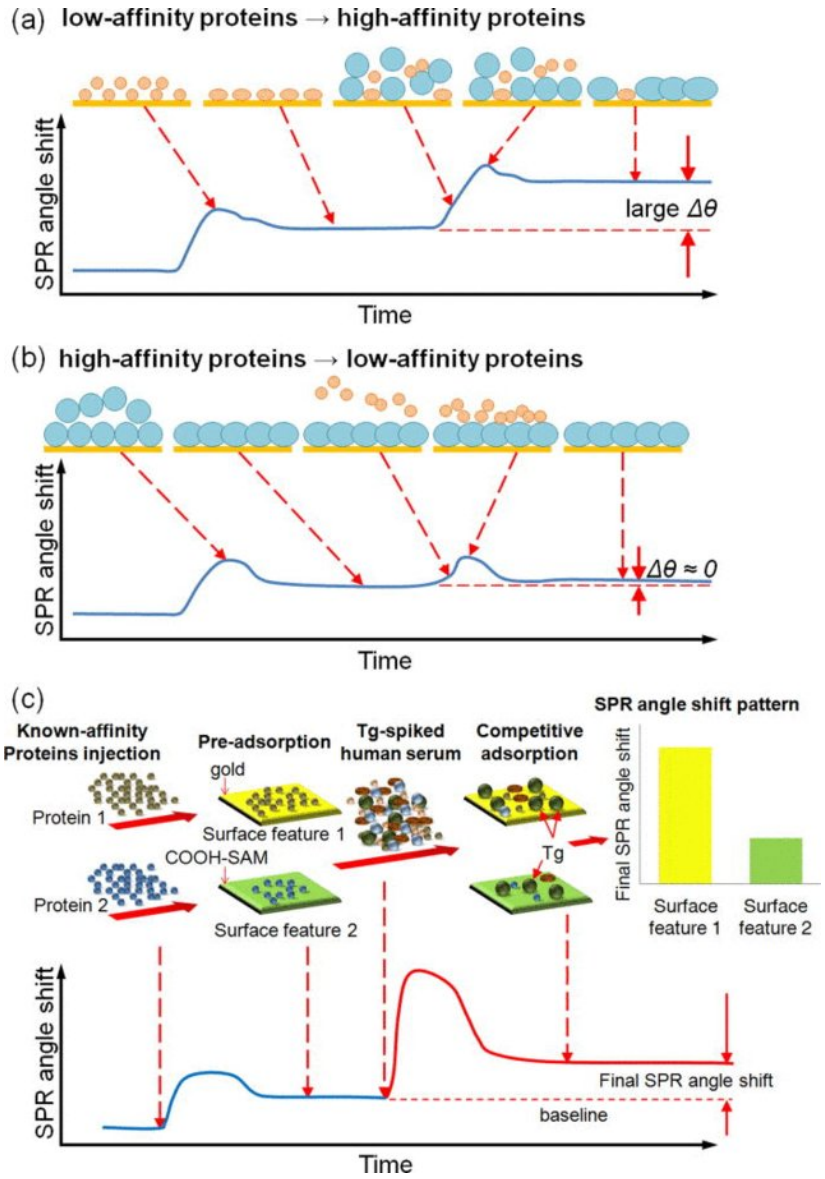
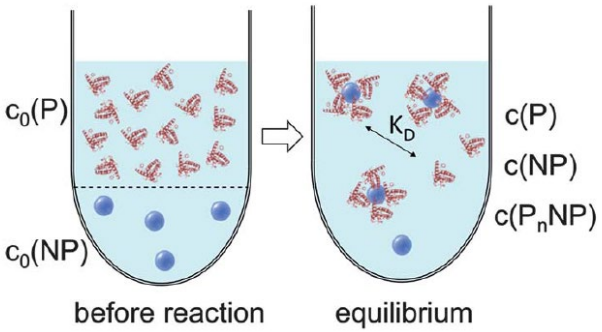
ACS Nano 2017, 11, 11773−11776

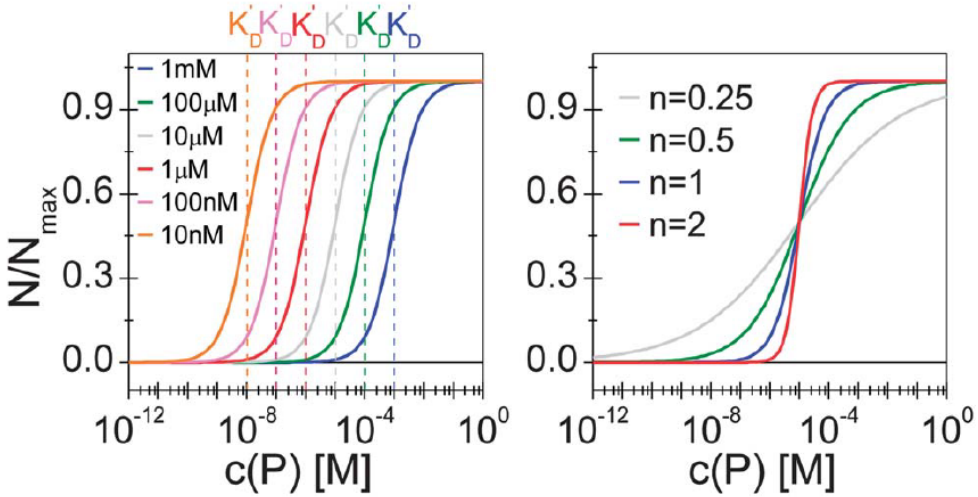
Vroman Effect





Vroman Effect





$$NP + nP \leftrightarrow P_nNP$$

$$K_D = \frac{c(NP)c^n(P)}{c(P_nNP)} = \frac{k_{off}}{k_{on}}$$

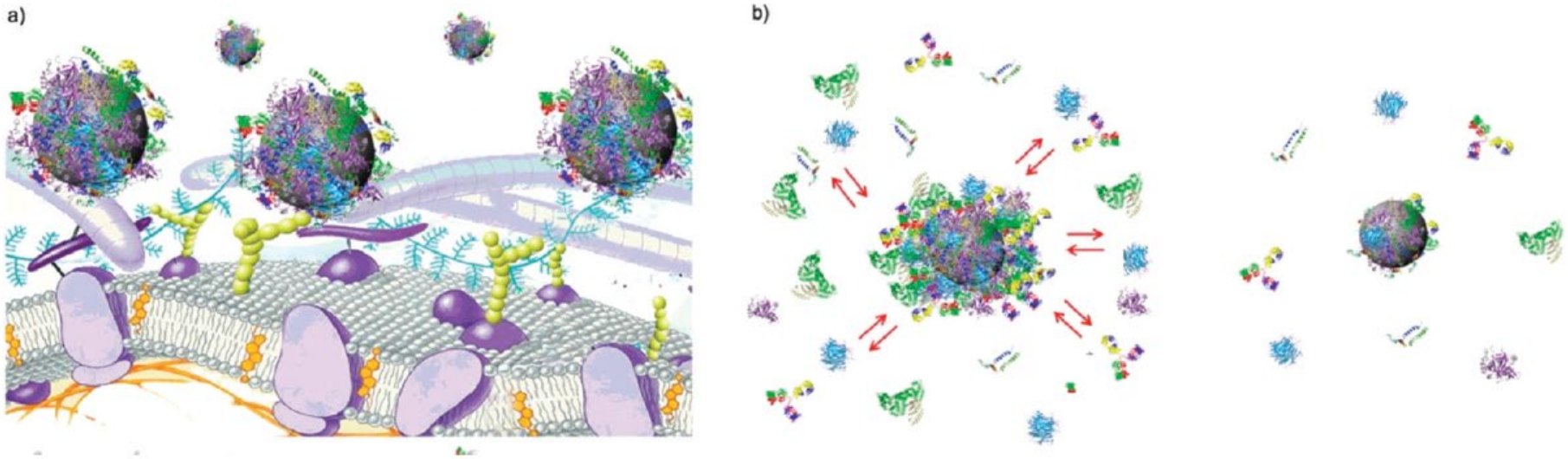


Figure 1. (a) Cartoon representation of the possible exchange/interaction scenarios at the bionanointerface at the cellular level. (b) Schematic drawing of the structure of NP−protein complexes in plasma: the "core" nanoparticle is surrounded by the protein corona composed of an outer weakly interacting layer of protein (left, full red arrows) rapidly exchanging with a collection of free proteins and a "hard" slowly exchanging corona of proteins (right). Diagram is not to scale in representing the proportions of the different objects.

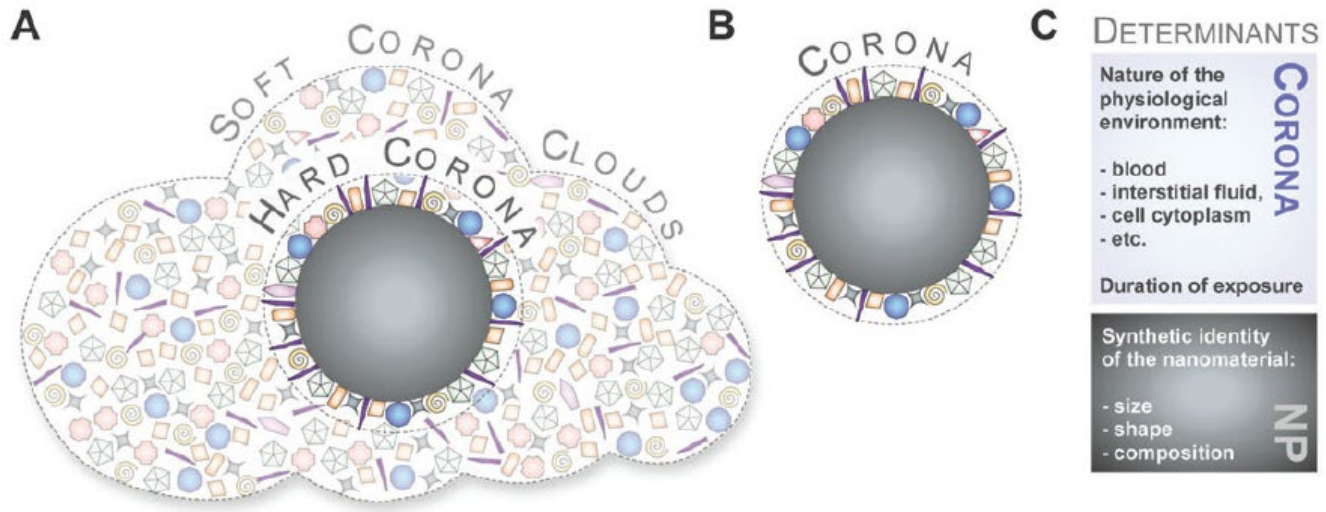


Fig. 3 Illustration of the old and new models referring to the description and determinants of the protein corona. (A) Hard and soft coronas, as well as protein clouds. (B) Coronas as analytically accessible NP–protein complexes. (C) Determinants of corona formation include not only the synthetic identity of the nanomaterial, but also the nature of the physiological environment.

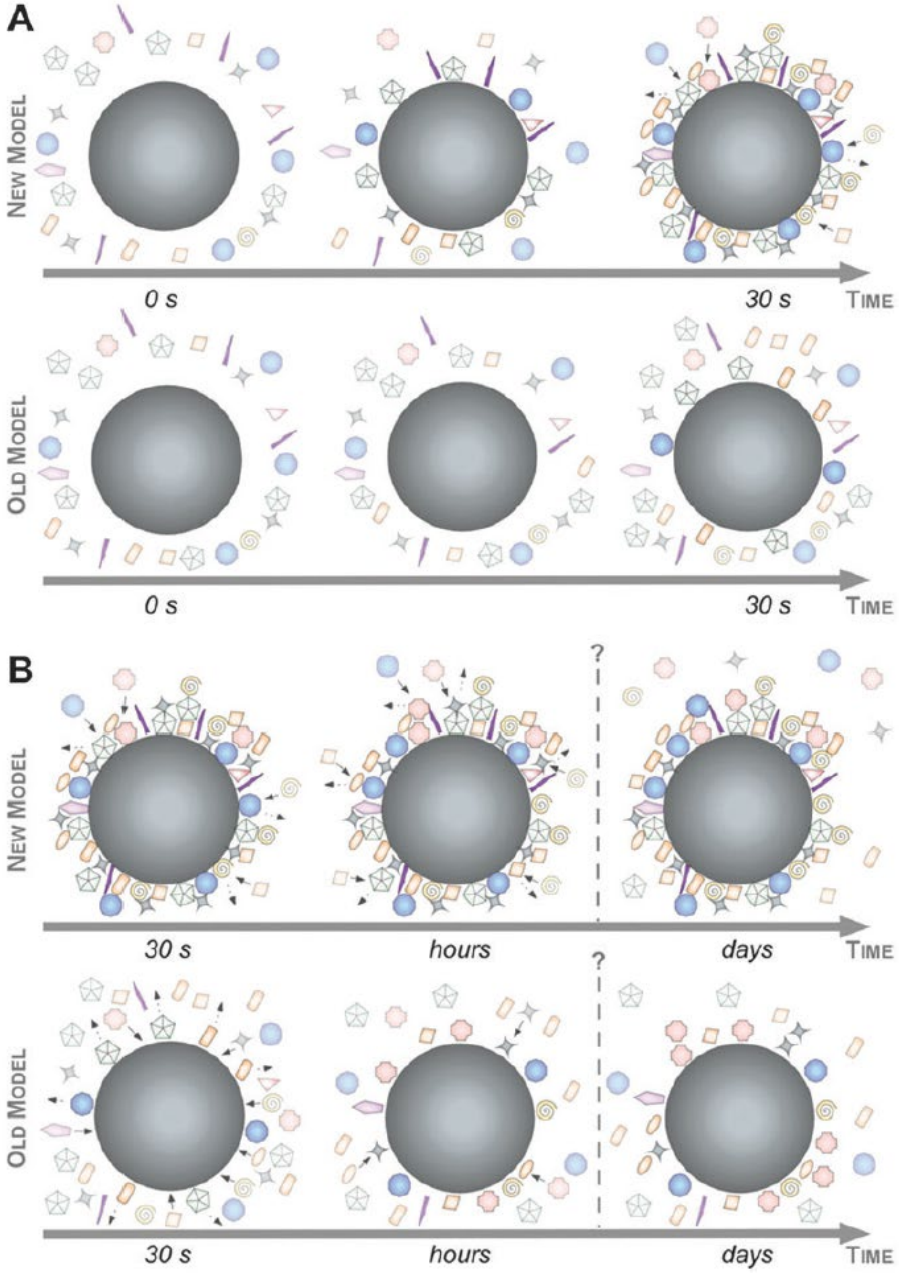


Fig. 6 Complexity and evolution of the biomolecule corona – the old *versus* the new model. (A) The early phase: a highly complex corona is established already in 30 sec, which may be composed of multiple core–shell structures ('new'). A corona of low complexity evolves slowly ('old'). (B) The late phase: corona composition *ex situ* remains stable and changes predominantly quantitatively rather than qualitatively over time with Vroman-effect dependent and independent binding kinetics ('new'). A highly dynamic protein corona, changes significantly over time, controlled by the 'Vroman-effect' ('old'). Note that the objects are not drawn to scale.

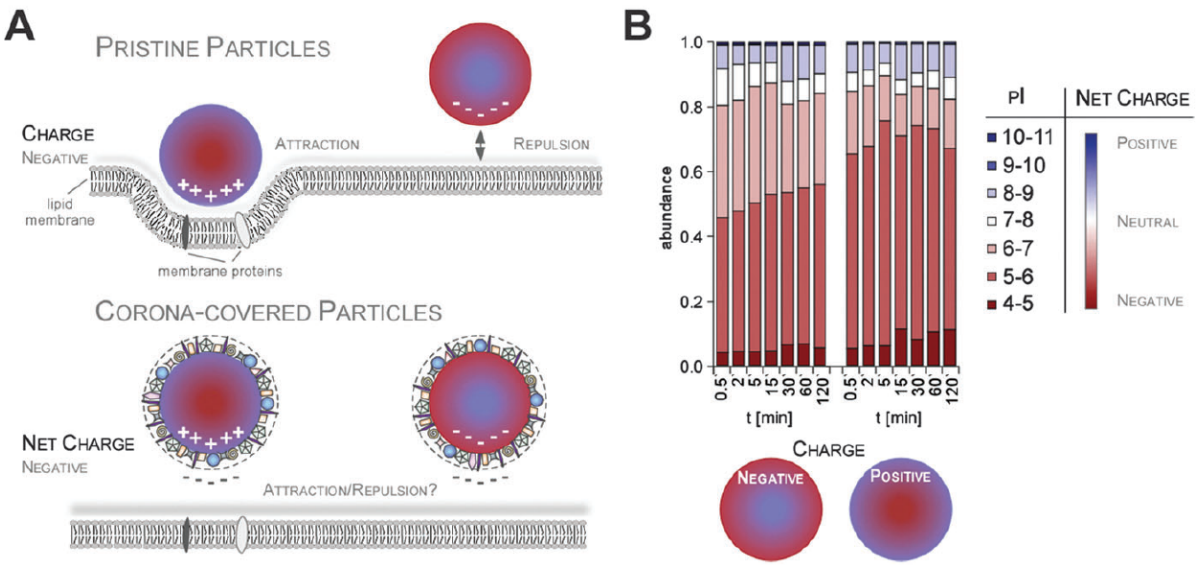


Fig. 7 Impact of NP charge on cellular uptake in the absence or presence of the protein corona. (A) Improved cellular uptake of positively charged NPs may be mediated by enhanced interaction with the negatively charged cell membrane only for pristine NPs (upper panel). In contrast, plasma corona covered NPs are overall negatively charged *in situ*, probably preventing NP-charge driven cell membrane interaction. (B) Plasma corona covered NPs are overall negatively charged, irrespective of the NPs' negative or positive surface functionalization.

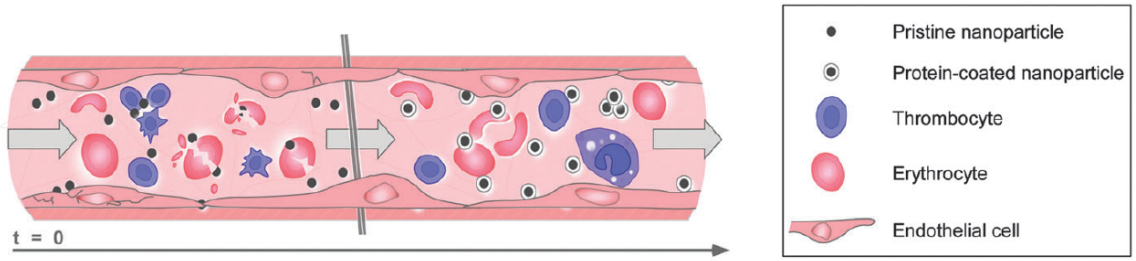
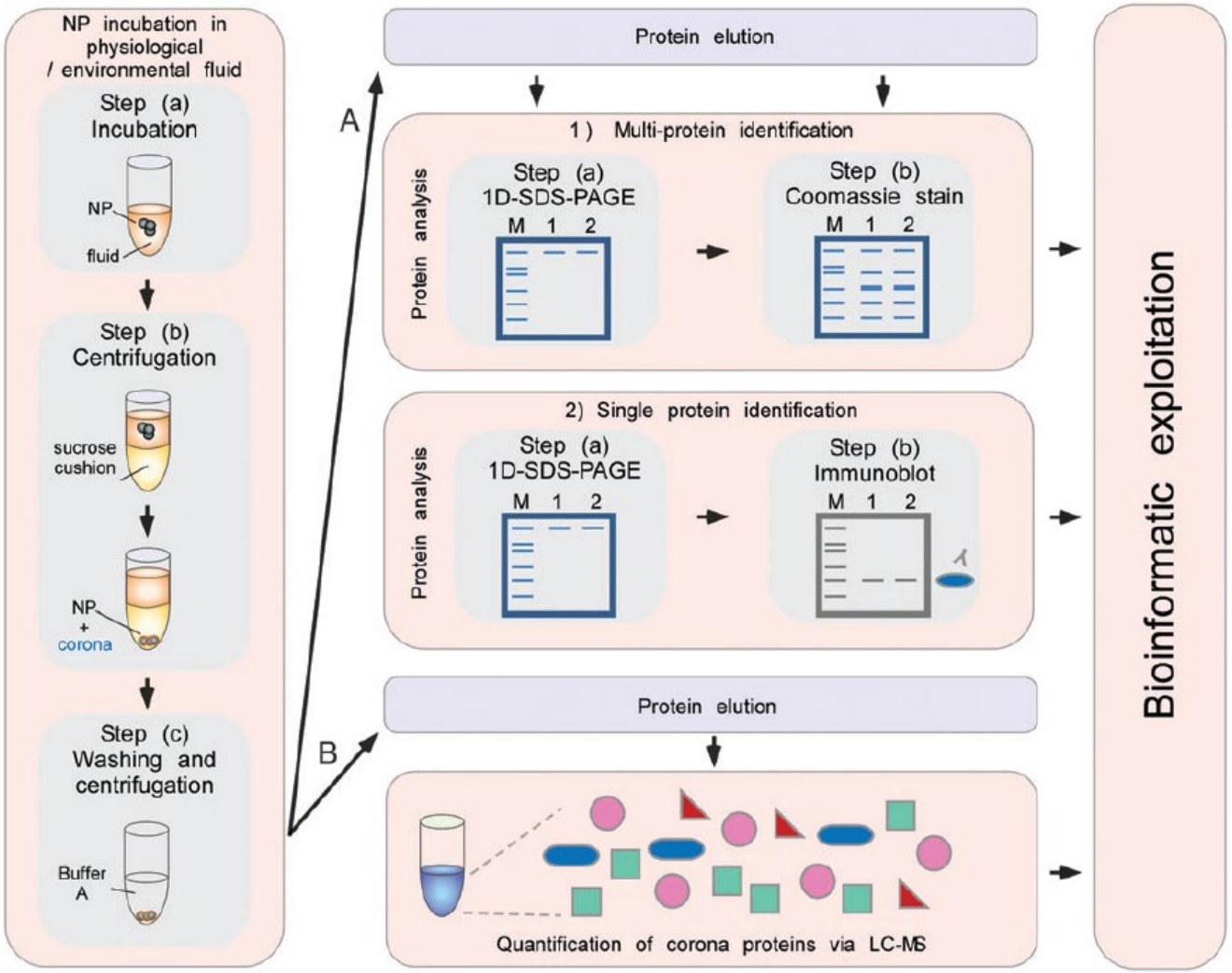
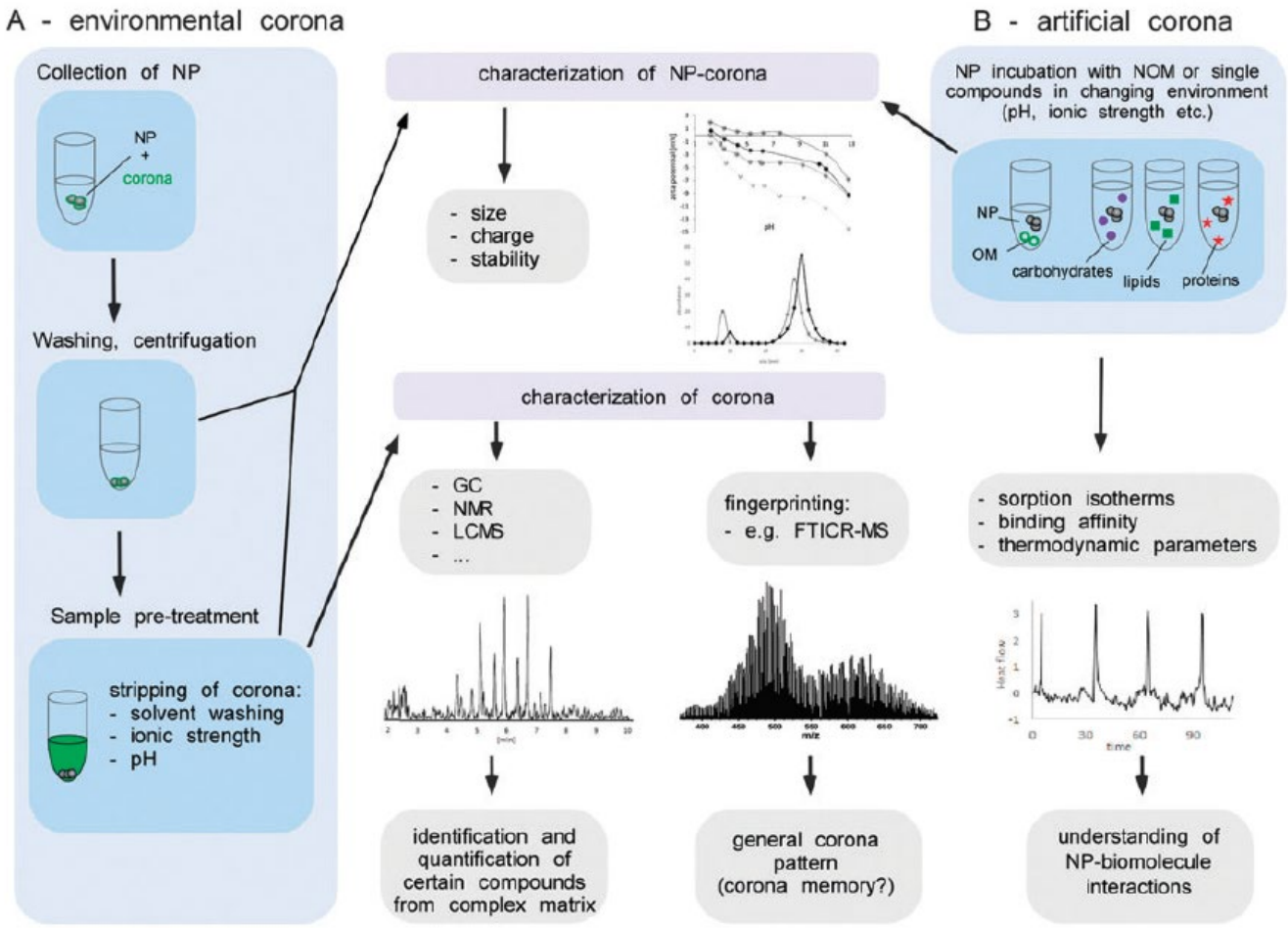


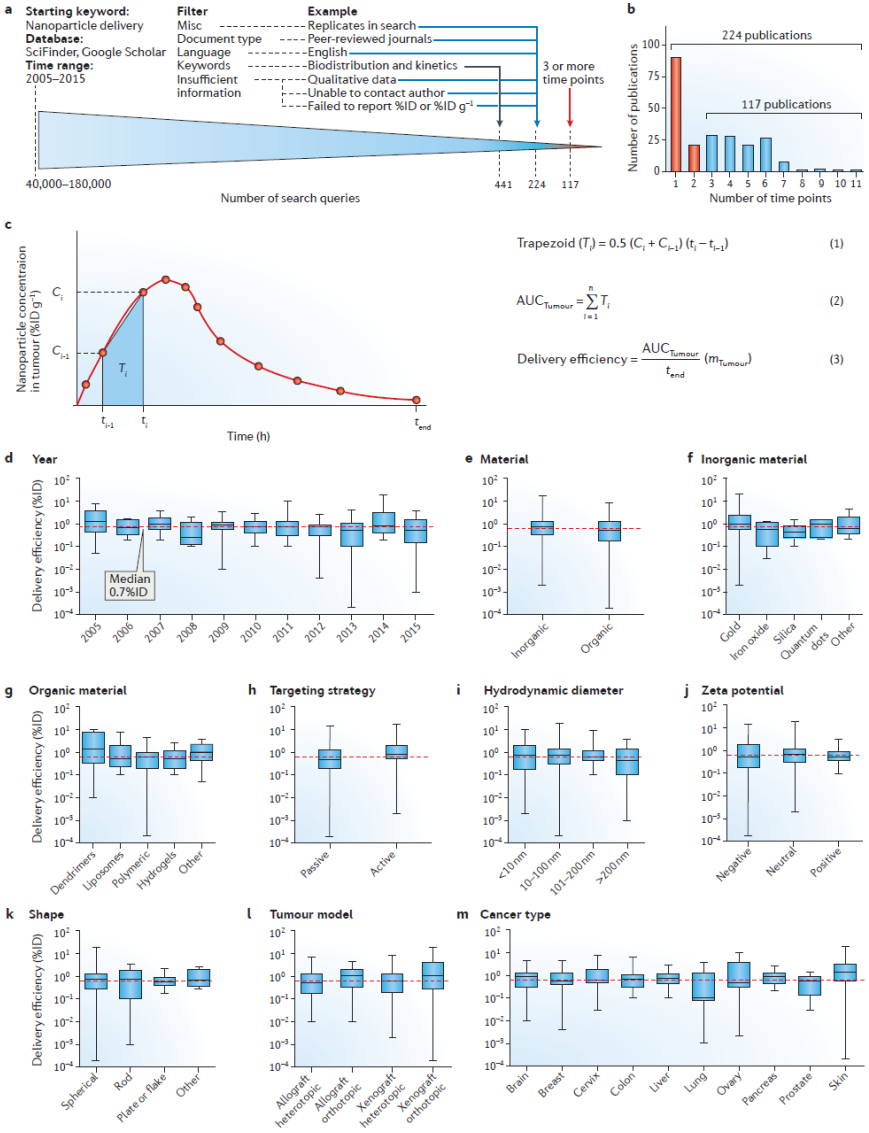
Fig. 8 Illustration of how rapid corona formation kinetically impacts early nanopathology in the human blood system. Upon entry or parenteral application, pristine NPs only exist for a short period of time, but are still capable of immediately affecting the vitality of endothelial cells, triggering thrombocyte activation and aggregation, and may also induce hemolysis. Formation of the biomolecule corona rapidly modulates the NPs' decoration with bioactive proteins protecting the cells of the blood system against nanoparticle-induced (patho)biological processes, and can also promote cellular uptake. Note, the elements are not drawn to scale.

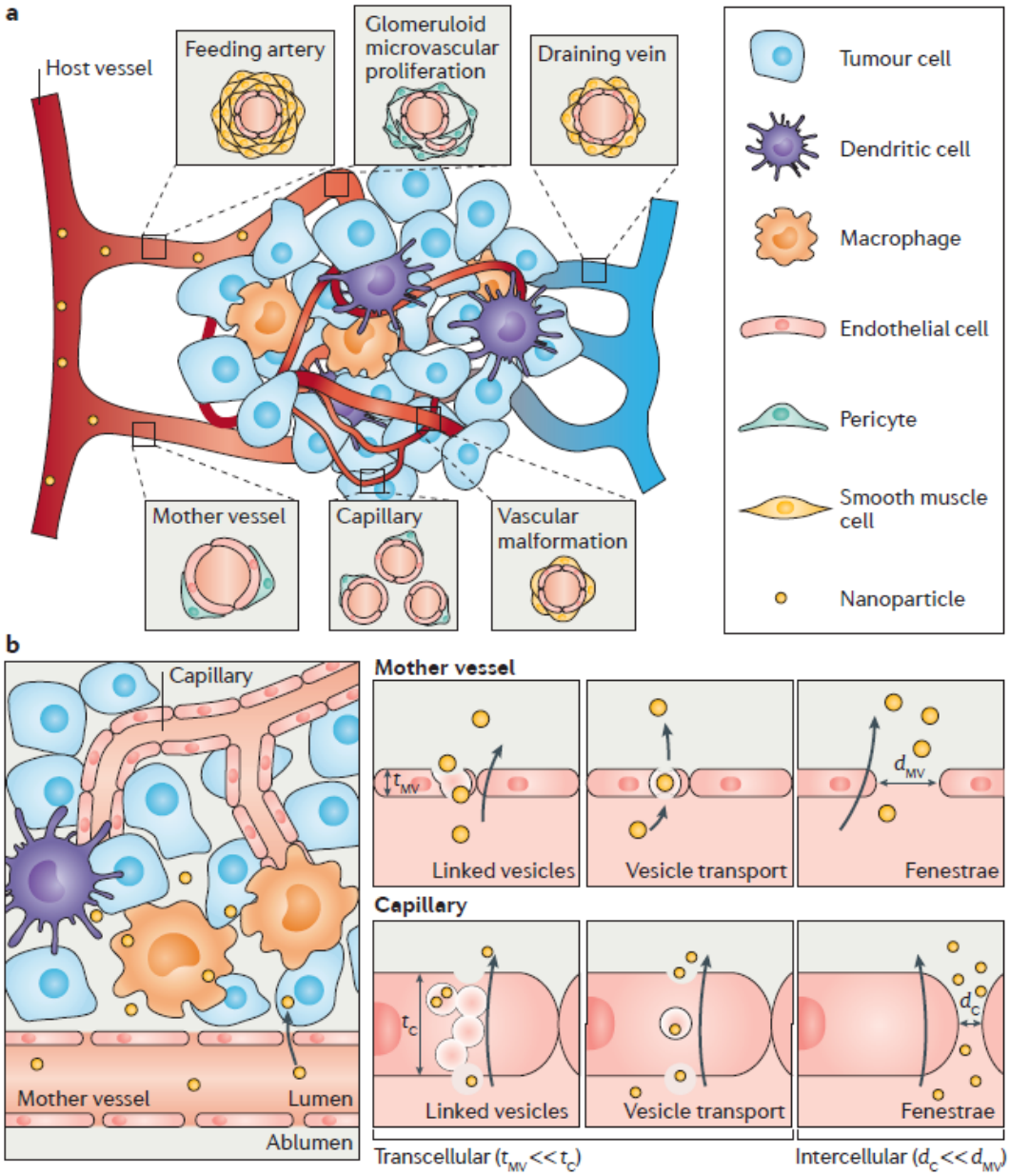

NP incubation in physiological / environmental fluid
Step (a) Incubation
NP
fluid
Step (b) Centrifugation
sucrose cushion
NP + corona
Step (c) Washing and centrifugation
Buffer A
A
B
Protein elution
1) Multi-protein identification
Protein analysis
Step (a) 1D-SDS-PAGE
M 1 2
Step (b) Coomassie stain
M 1 2
2) Single protein identification
Protein analysis
Step (a) 1D-SDS-PAGE
M 1 2
Step (b) Immunoblot
M 1 2
Protein elution
Quantification of corona proteins via LC-MS
Bioinformatic exploitation


A - environmental corona
B - artificial corona
Collection of NP
NP
+
corona
Washing, centrifugation
Sample pre-treatment
stripping of corona:
- solvent washing
- ionic strength
- pH
characterization of NP-corona
- size
- charge
- stability
characterization of corona
- GC
- NMR
- LCMS
- ...
fingerprinting:
- e.g. FTICR-MS
identification and quantification of certain compounds from complex matrix
general corona pattern (corona memory?)
NP incubation with NOM or single compounds in changing environment (pH, ionic strength etc.)
NP
OM
carbohydrates
lipids
proteins
- sorption isotherms
- binding affinity
- thermodynamic parameters
understanding of NP-biomolecule interactions

Delivery Efficiency

< 1%ID




a
Host vessel
Feeding artery
Glomeruloid microvascular proliferation
Draining vein
Mother vessel
Capillary
Vascular malformation
Tumour cell
Dendritic cell
Macrophage
Endothelial cell
Pericyte
Smooth muscle cell
Nanoparticle
b
Capillary
Mother vessel
Lumen
Ablumen
Mother vessel
t_{MV}
Linked vesicles
Vesicle transport
d_{MV}
Fenestrae
Capillary
t_C
Linked vesicles
Vesicle transport
d_C
Fenestrae
Transcellular ($t_{MV} << t_C$)
Intercellular ($d_C << d_{MV}$)

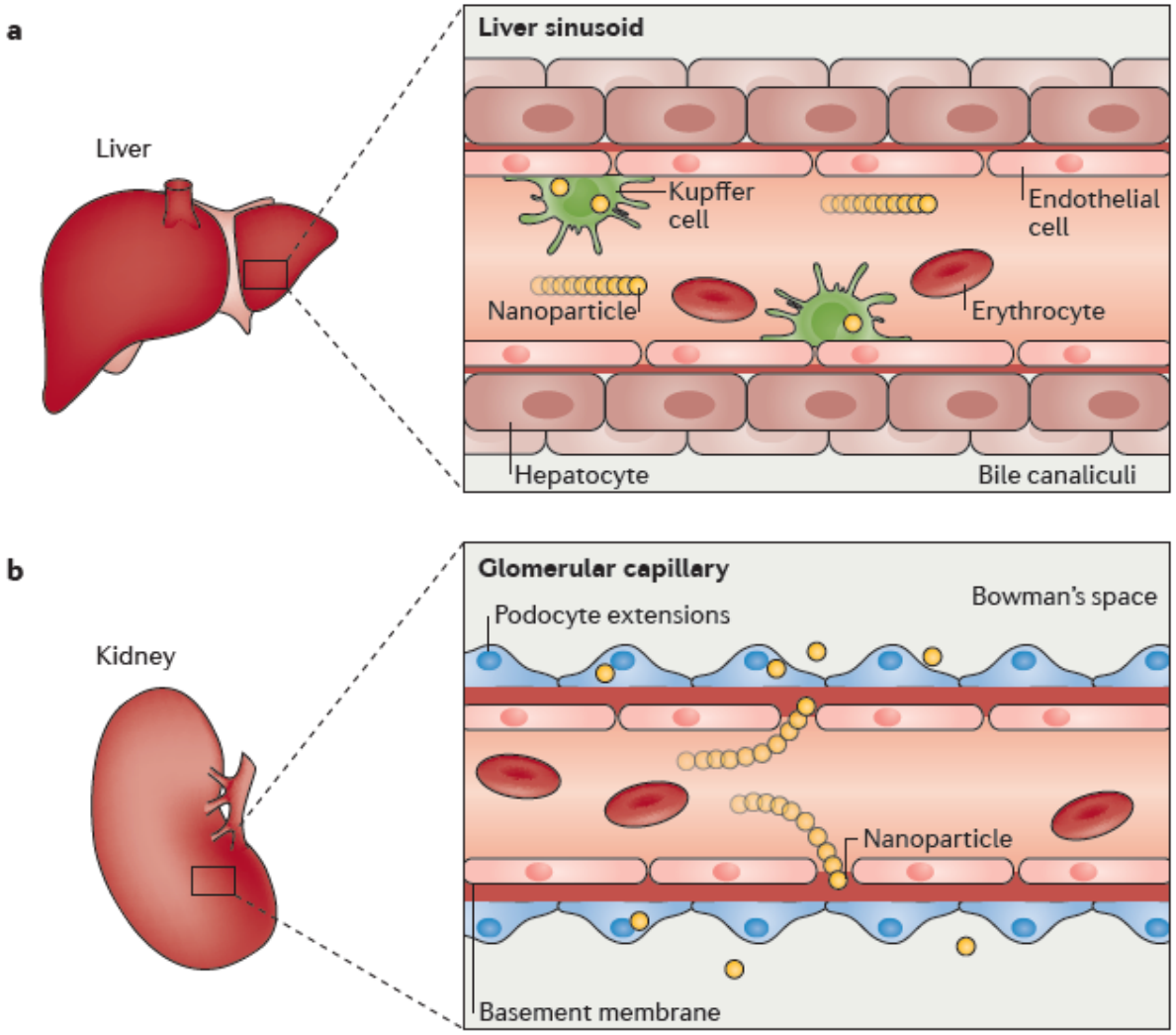
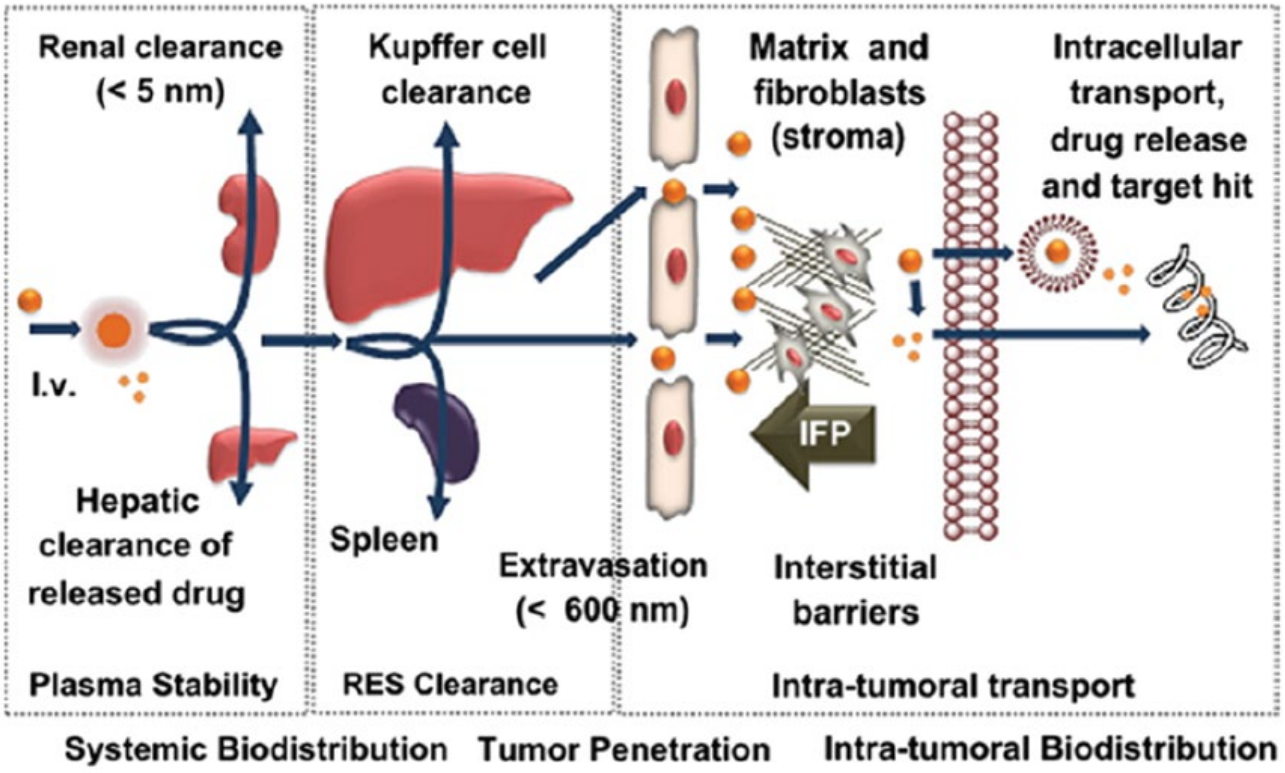


Figure 4 | **Mechanisms for nanoparticle elimination from the bloodstream. a** | The liver is the primary organ of the mononuclear phagocytic system that entraps a vast majority of the administered nanoparticle dose. Phagocytic cells, such as Kupffer cells, line the liver sinusoid. **b** | If the hydrodynamic diameter of a nanoparticle is smaller than 5.5 nm, it may be filtered from the blood via the kidneys and excreted in urine. Other major organs that are involved in the removal of nanoparticles from the bloodstream include the spleen, lymph nodes and the skin.

(A)


Renal clearance
(< 5 nm)
Kupffer cell
clearance
Matrix and
fibroblasts
(stroma)
Intracellular
transport,
drug release
and target hit
I.v.
IFP
Hepatic
clearance of
released drug
Spleen
Extravasation
(< 600 nm)
Interstitial
barriers
Plasma Stability
RES Clearance
Intra-tumoral transport
Systemic Biodistribution
Tumor Penetration
Intra-tumoral Biodistribution

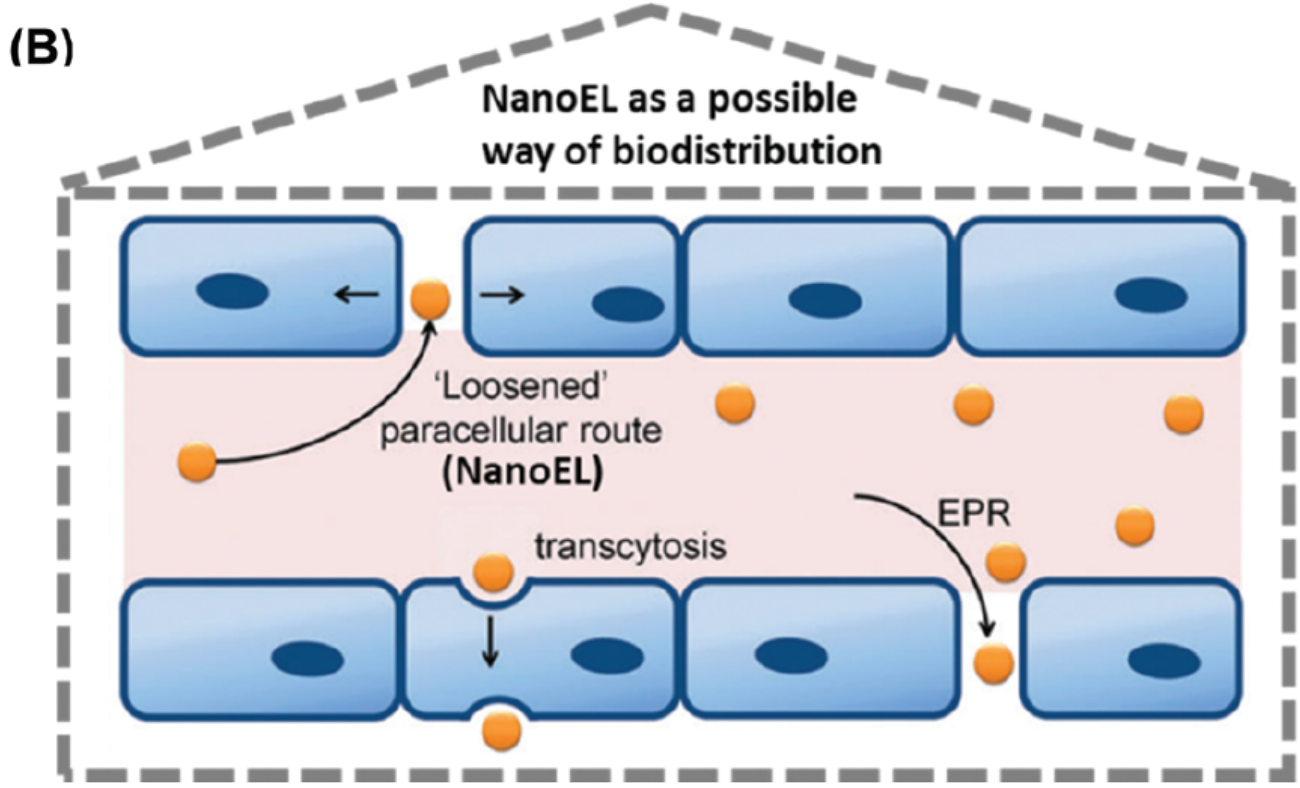
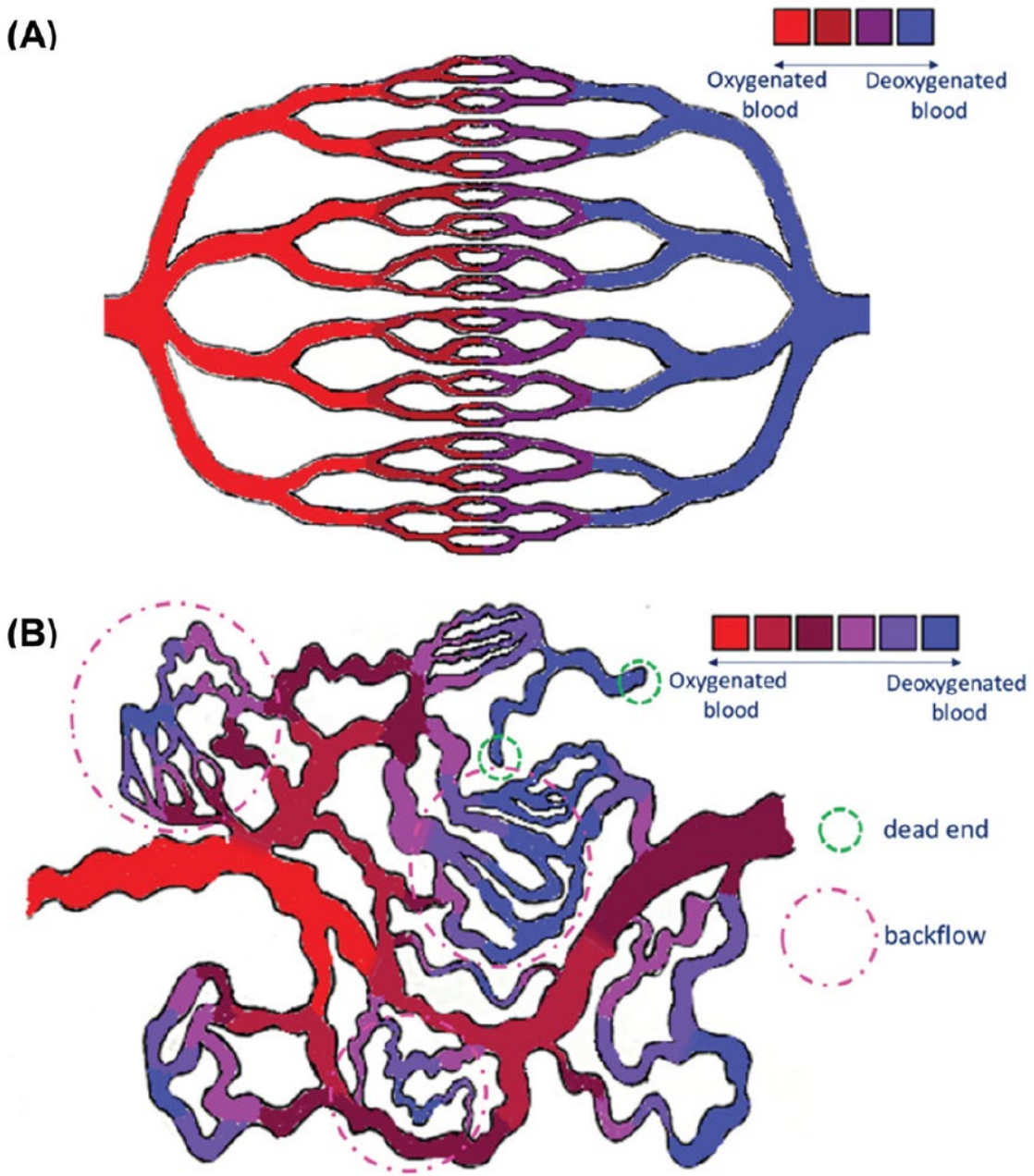


Fig. 1 Drug delivery by NPs are subjected to various factors that limit the overall dose of drugs reaching the target site. (A) These factors can be classified into three separate phases, namely, systemic biodistribution, tumor penetration and intra-tumoral biodistribution. Reproduced with permission from Ernsting *et al.*[24] Copyright 2013 Elsevier. (B) Nanomaterial-induced endothelial leakiness (NanoEL) may be viewed as an emerging strategy to improve the biodistribution of nanomedicine to target sites. Adapted from Setyawati *et al.*[25] with permission from the Royal Society of Chemistry 2015. IFP, interstitial fluid pressure.


(A)
Oxygenated blood
Deoxygenated blood
(B)
Oxygenated blood
Deoxygenated blood
dead end
backflow

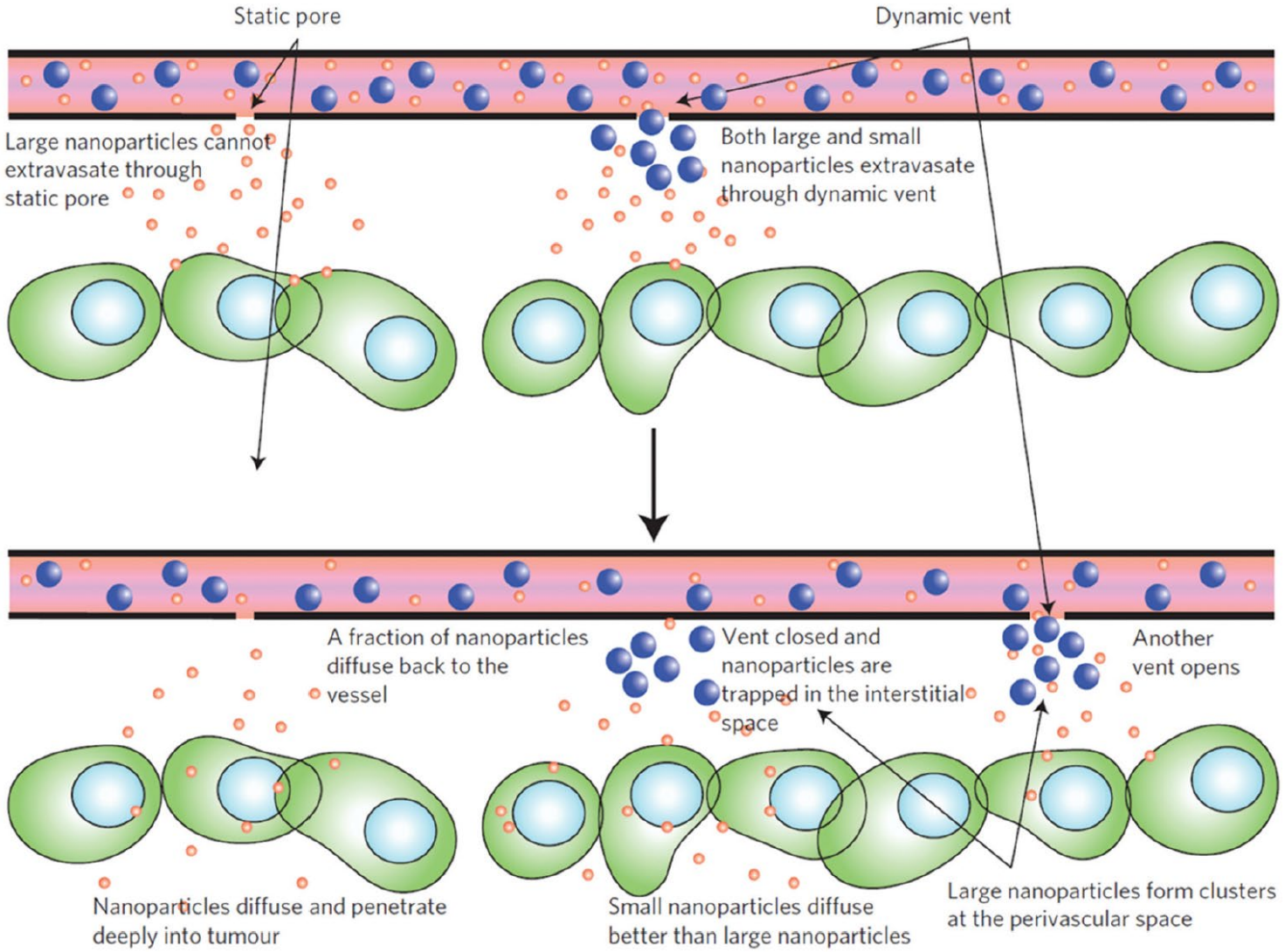
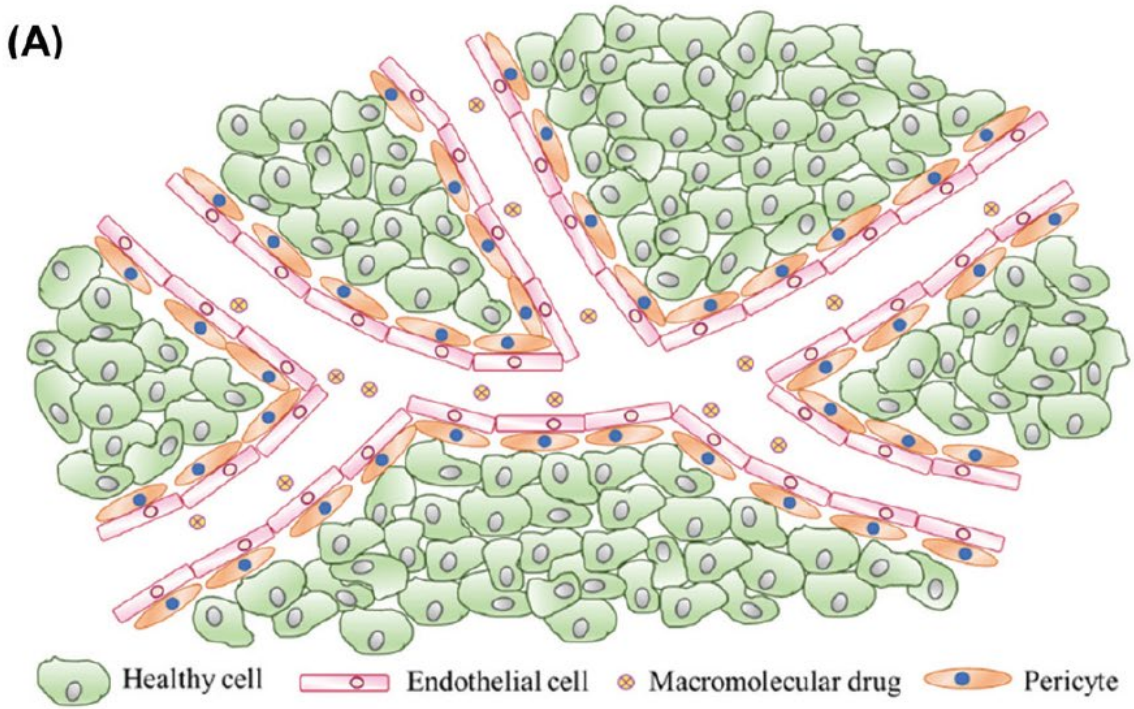
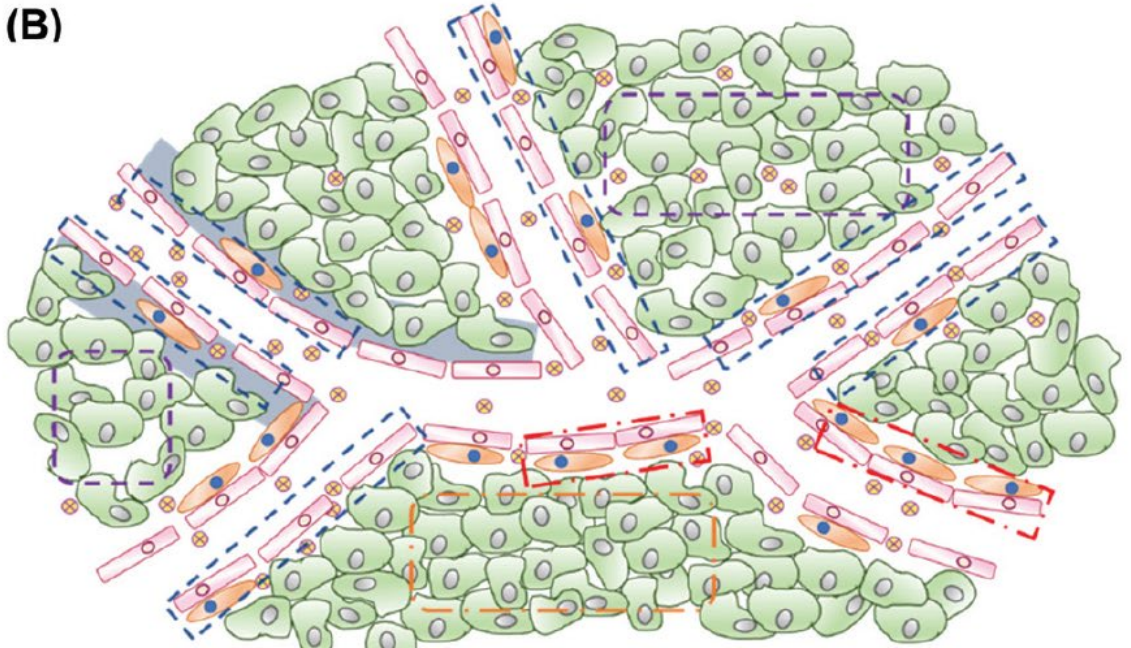
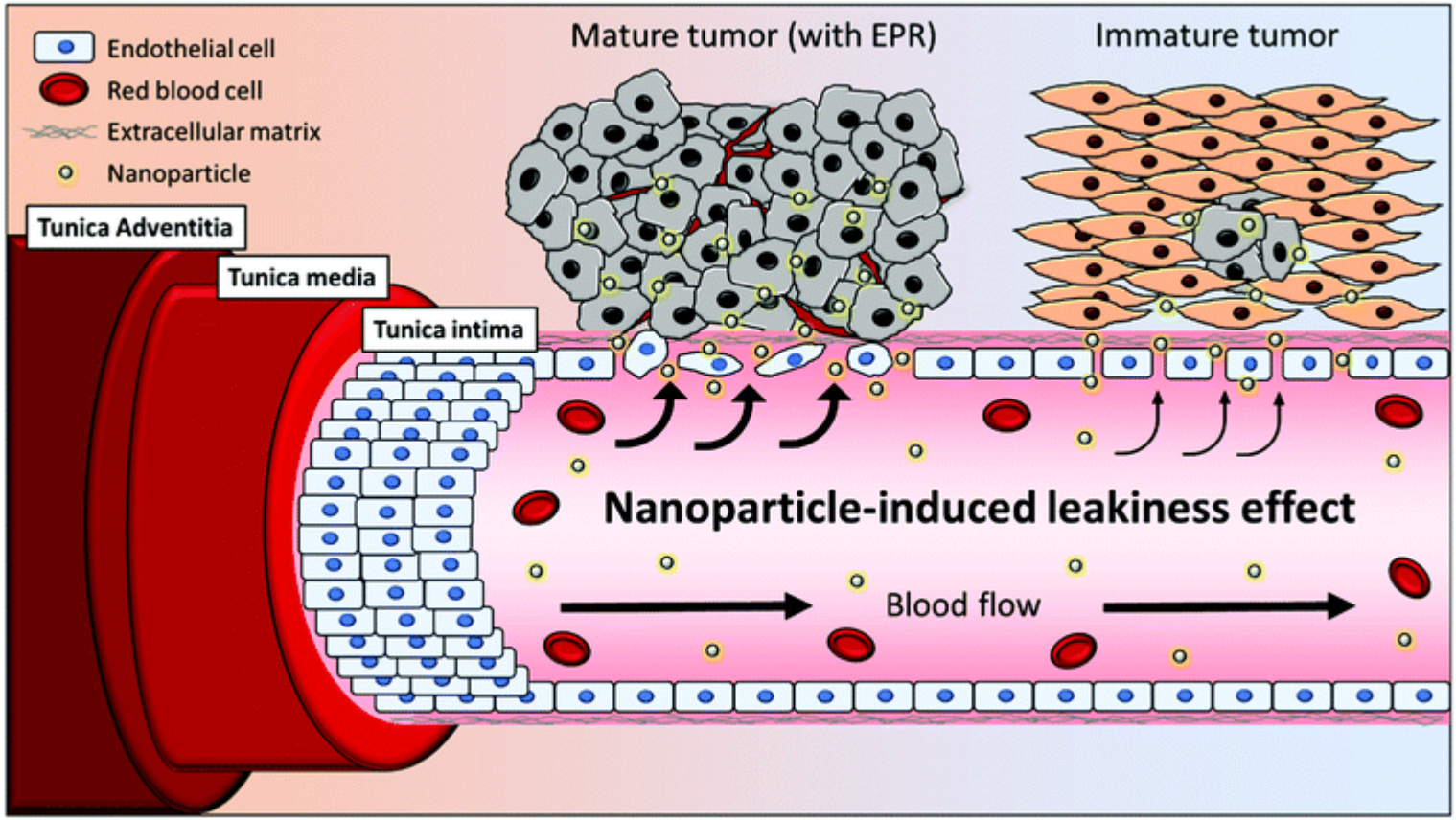


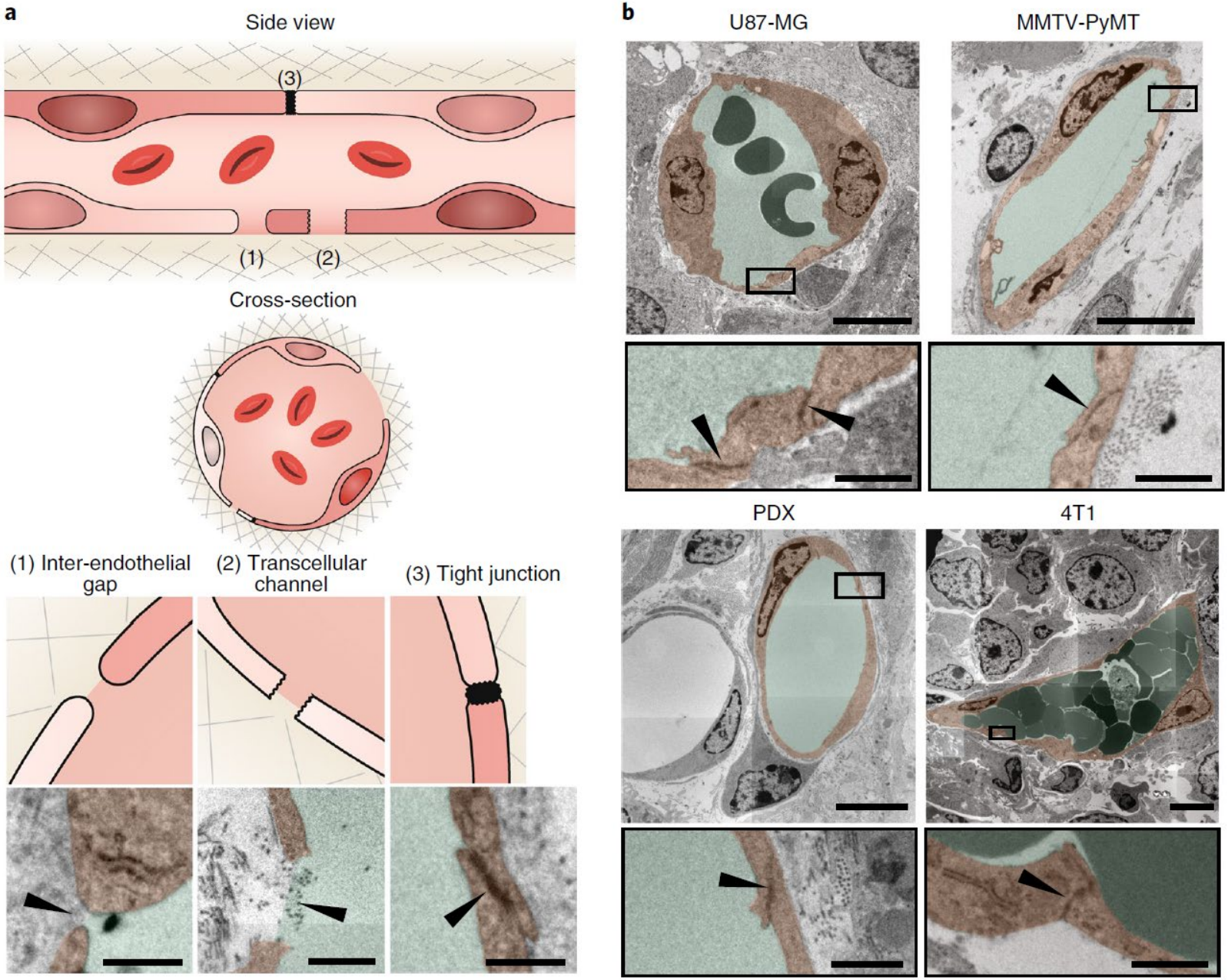
Fig. 3 Presence of static pores and dynamic vents in tumor vessels allows for NPs of differential permeability of NPs to penetrate through the vessel walls to reach the tumor sites. Predominantly for small NPs, static pores promote a deeper penetration of these NPs for a longer period of time. On the other hand, dynamic vents form transient openings which allow both small and large NPs to diffuse across the vessel walls, but over a shorter period of time. Reproduced with permission from Matsumoto *et al.*[107] Copyright 2016 Springer Nature.

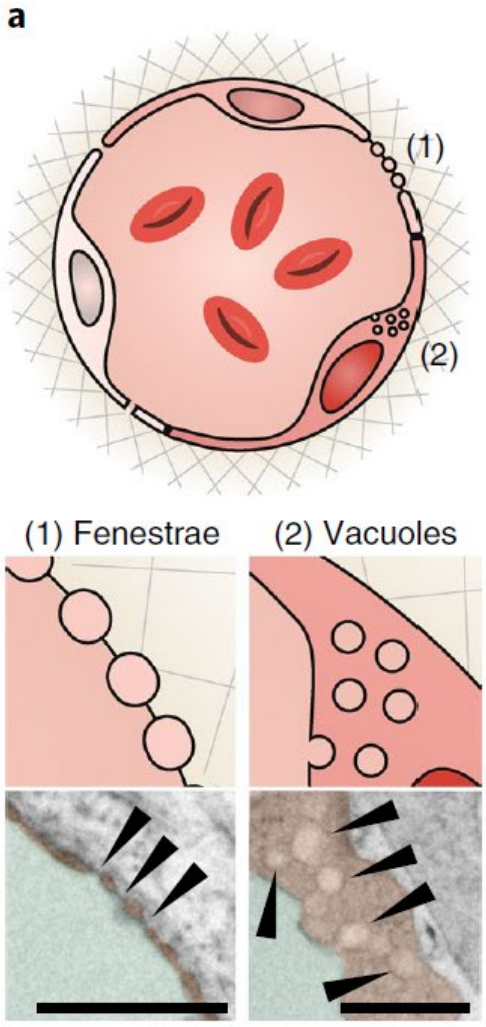

(A)
Healthy cell
Endothelial cell
Macromolecular drug
Pericyte

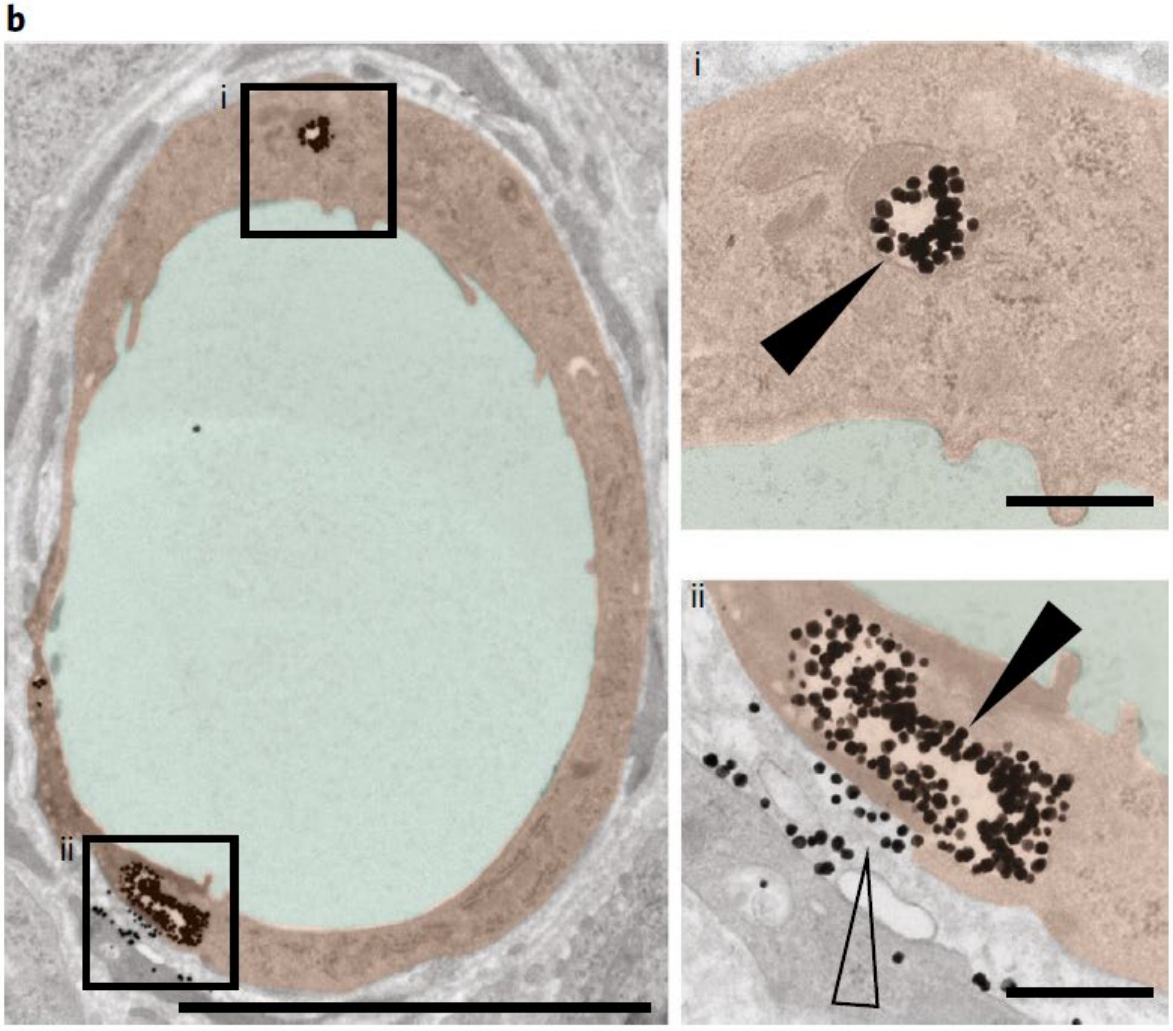

(B)

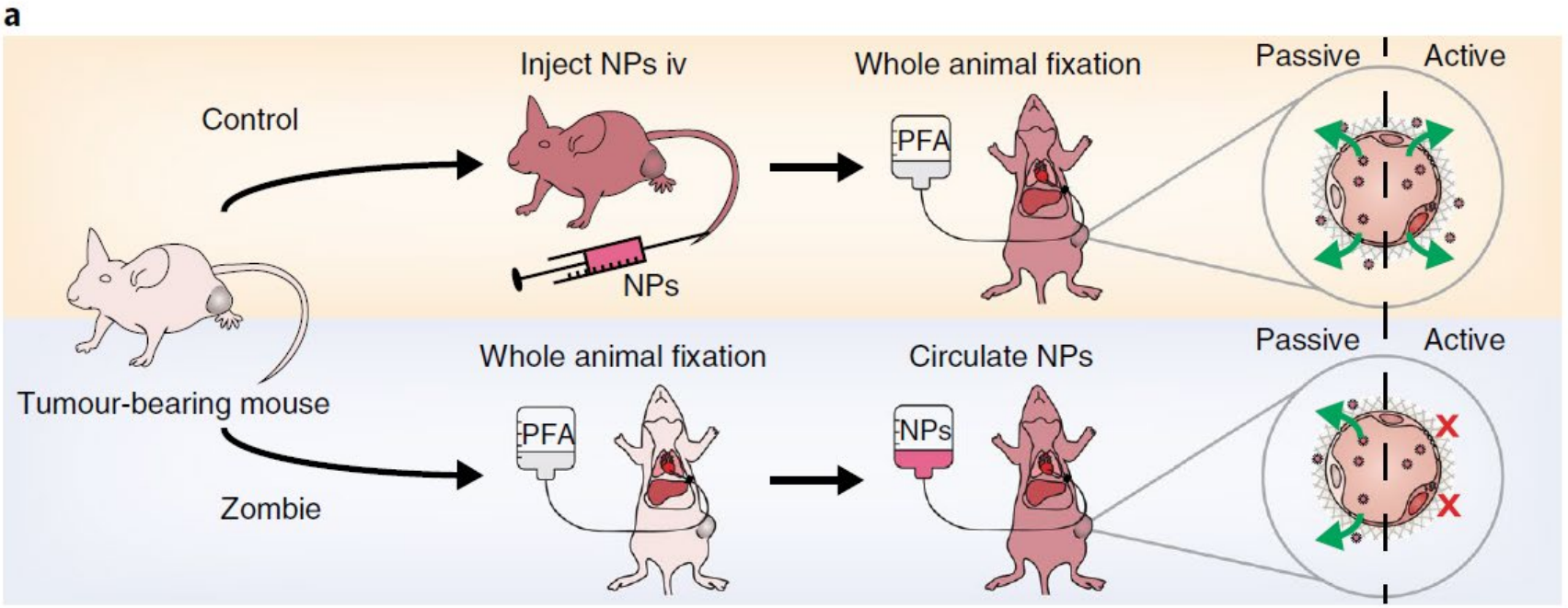
EPR Effect

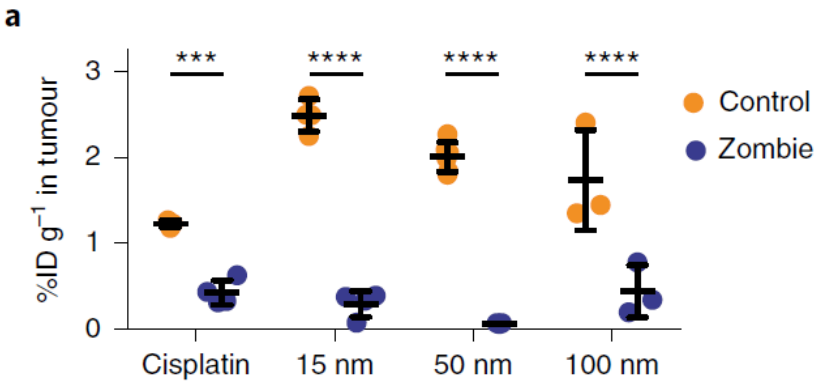

Endothelial cell
Red blood cell
Extracellular matrix
Nanoparticle
Tunica Adventitia
Tunica media
Tunica intima
Mature tumor (with EPR)
Immature tumor
Nanoparticle-induced leakiness effect
Blood flow


a
Side view
(3)
(1)
(2)
Cross-section
(1) Inter-endothelial gap
(2) Transcellular channel
(3) Tight junction
b
U87-MG
MMTV-PyMT
PDX
4T1


a
(1)
(2)
(1) Fenestrae
(2) Vacuoles


b
i
ii
i
ii





b

NP type	% attributed to gaps
Cisplatin	35
15 nm	12
50 nm	3
100 nm	25

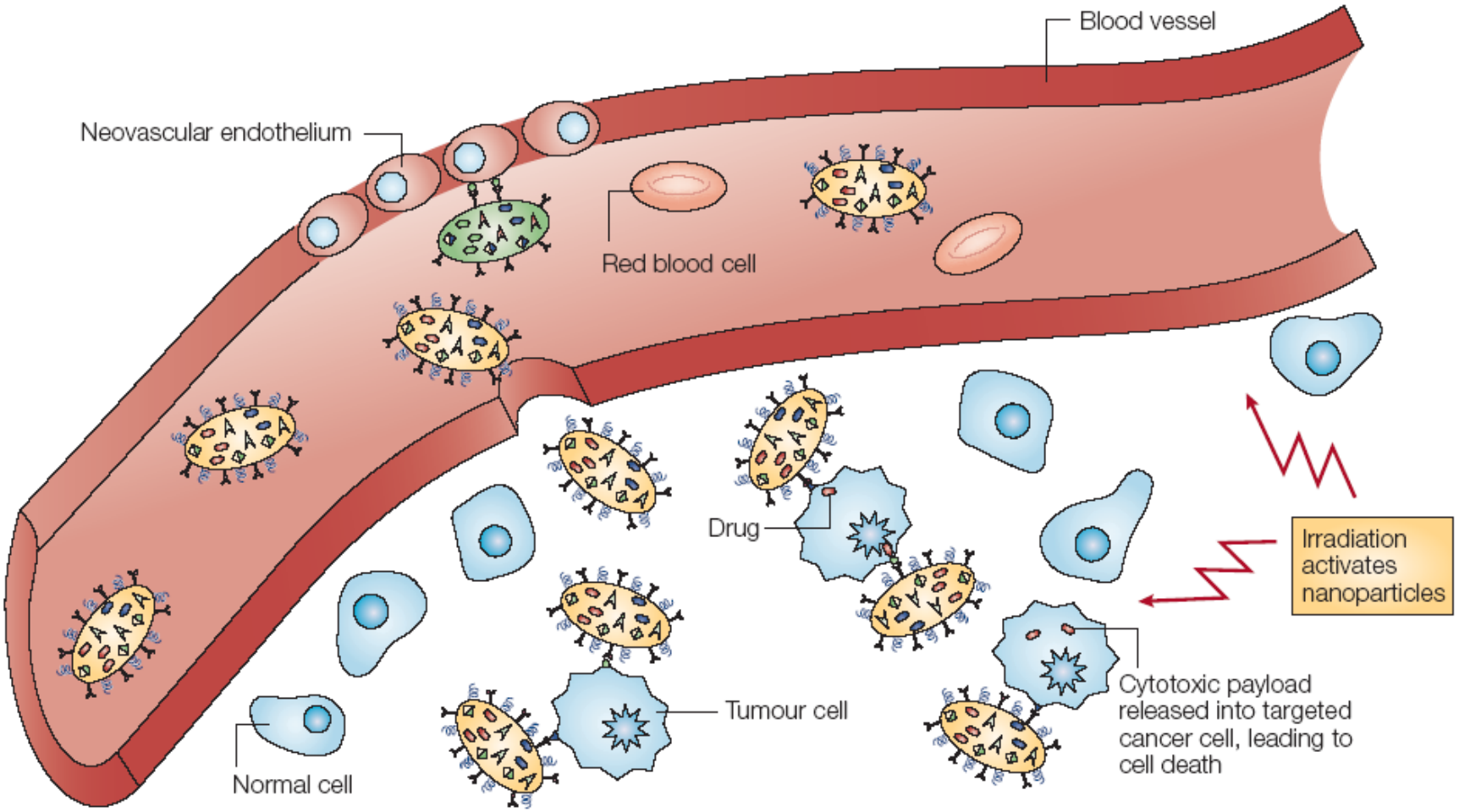


Figure 4 | **Multicomponent targeting strategies.** Nanoparticles extravasate into the tumour stroma through the fenestrations of the angiogenic vasculature, demonstrating targeting by enhanced permeation and retention. The particles carry multiple antibodies, which further target them to epitopes on cancer cells, and direct antitumour action. Nanoparticles are activated and release their cytotoxic action when irradiated by external energy. Not shown: nanoparticles might preferentially adhere to cancer neovasculature and cause it to collapse, providing anti-angiogenic therapy. The red blood cells are not shown to scale; the volume occupied by a red blood cell would suffice to host 1–10 million nanoparticles of 10 nm diameter.

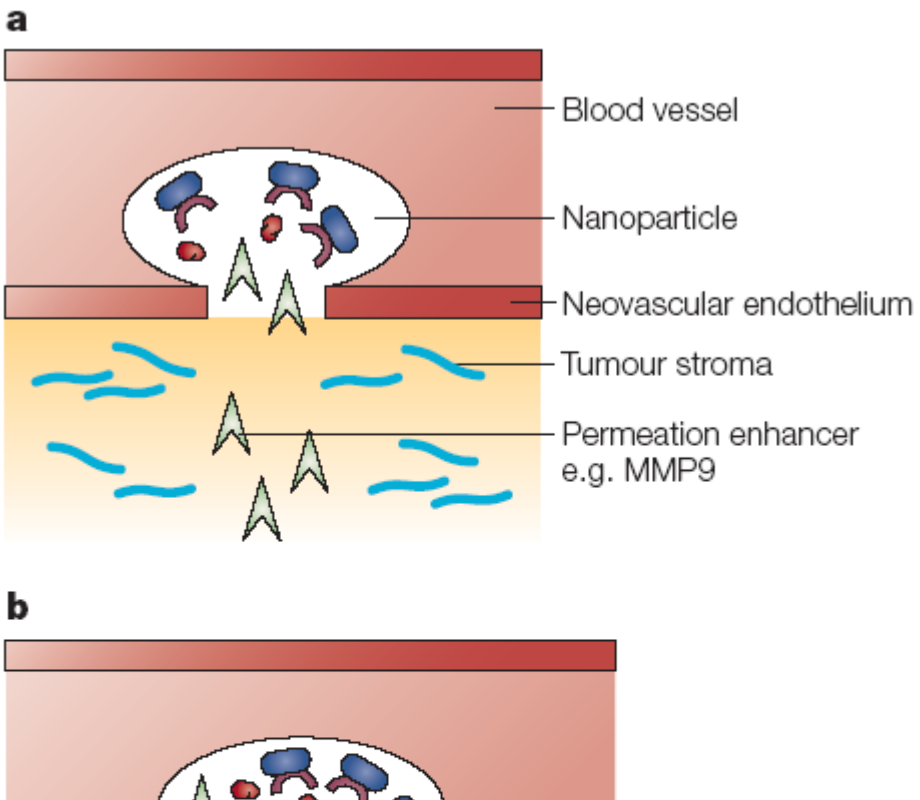
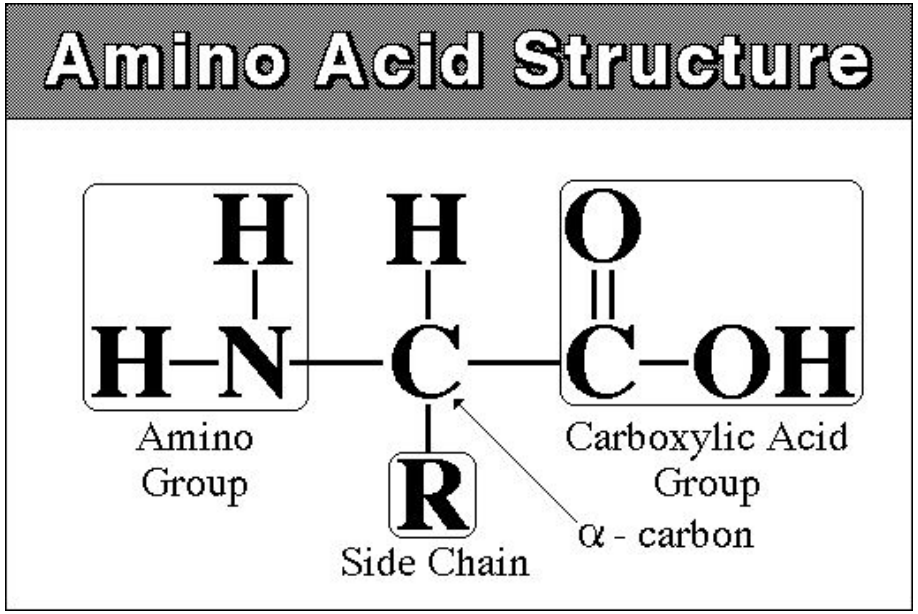
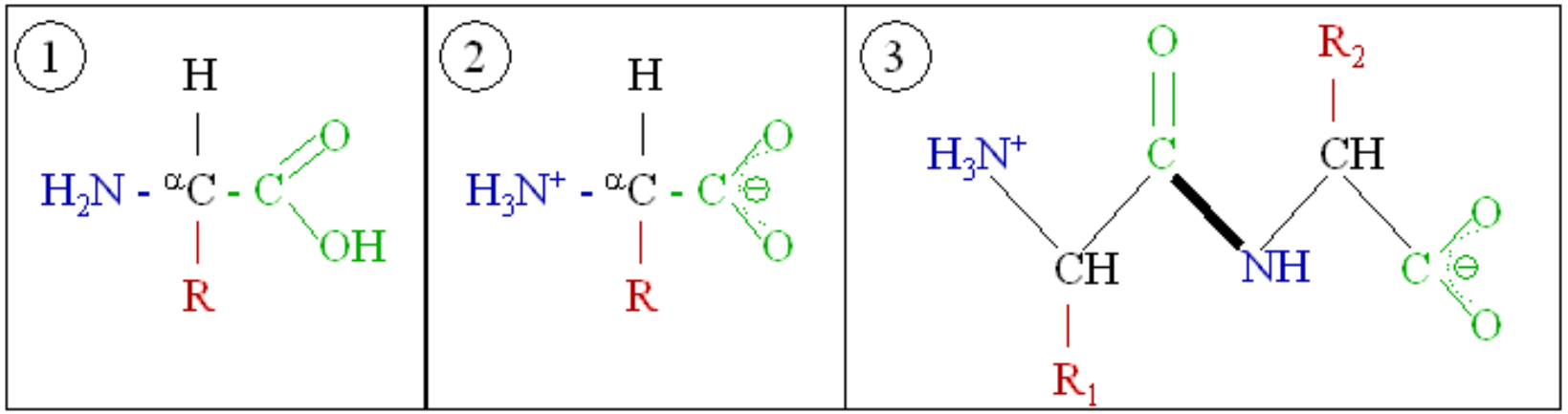


Figure 5 | **A vision for a future multistage nanodevice with multiple-barrier-avoidance capability**. A nanovector selectively binds to the cancer neovascular endothelium, releases a penetration enhancer, generates a fenestration, and deploys through it a track of molecular motor molecules such as actin. Therapeutic agents bound to a conjugate molecule such as myosin are then released by the nanovector, and travel along the 'molecular track' to reach deeply into the cancer lesion, despite the opposing oncotic osmotic pressure.

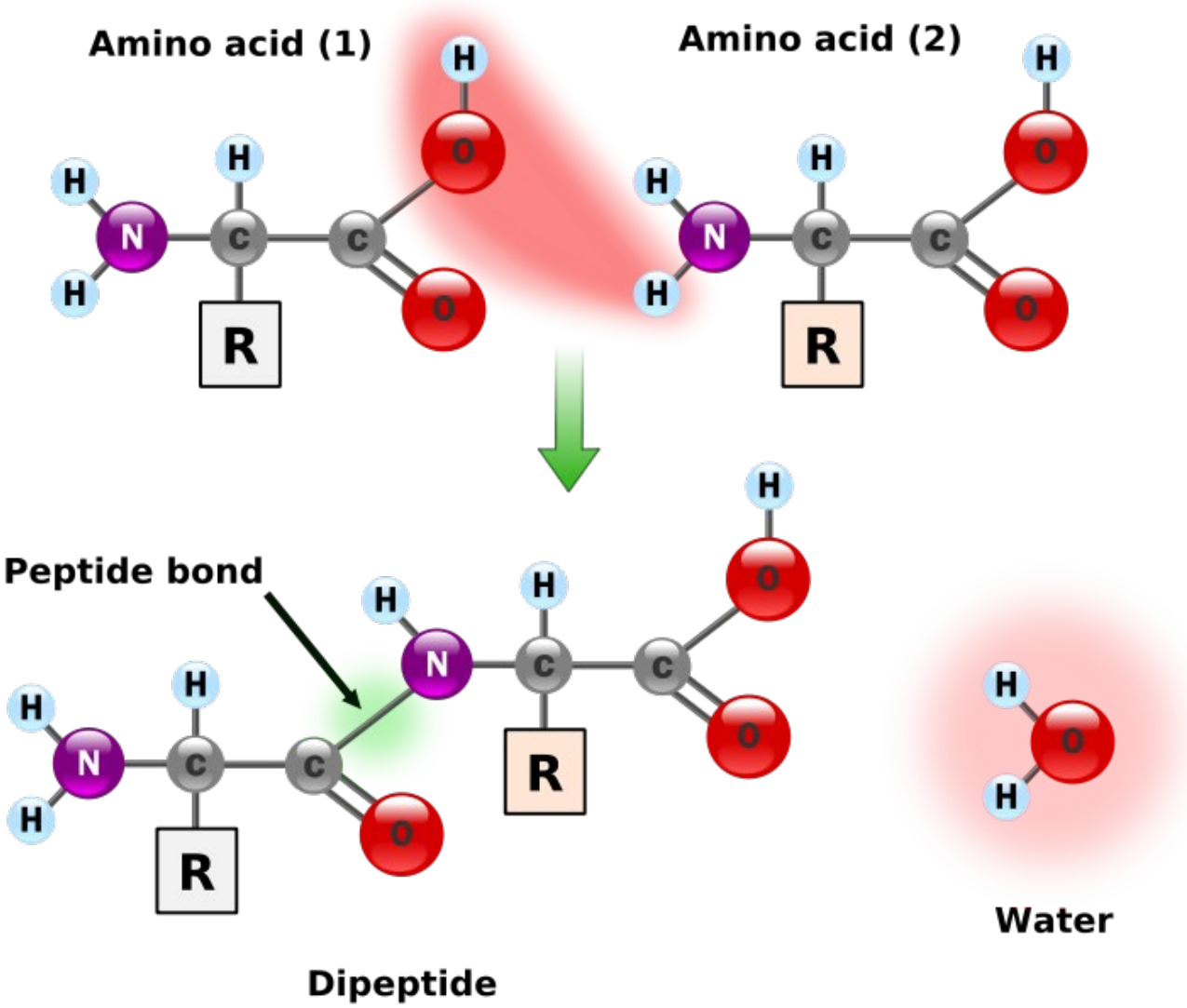
Review

Amino Acid


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

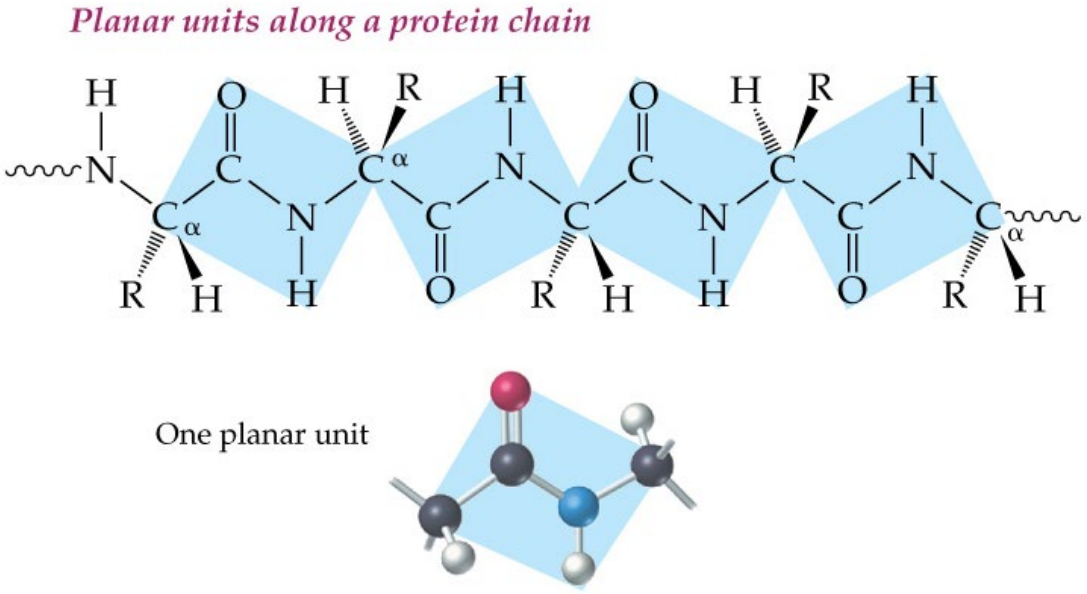

1
H
H_2N - $^{\alpha}C$ - C
O
OH
R
2
H
H_3N^+ - $^{\alpha}C$ - C
O
O
R
3
O
R_2
H_3N^+
C
CH
CH
NH
C
O
O
R_1

Peptide bond



Primary Protein Structure

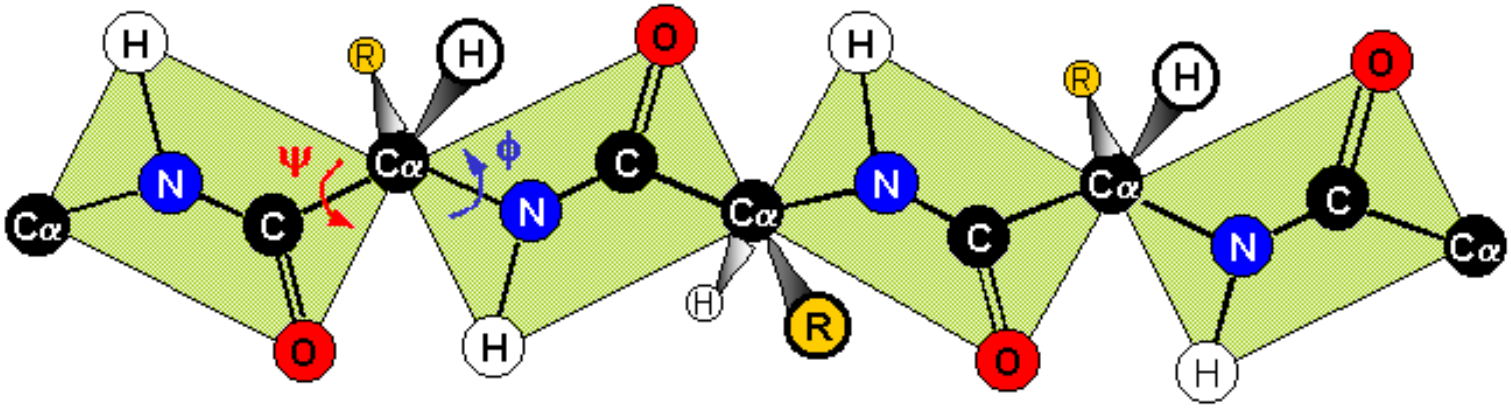
- Primary structure of a proteins is the sequence of amino acids connected by peptide bonds. Along the backbone of the proteins is a chain of alternating peptide bonds and α-carbons and the amino acid side chains are connected to these

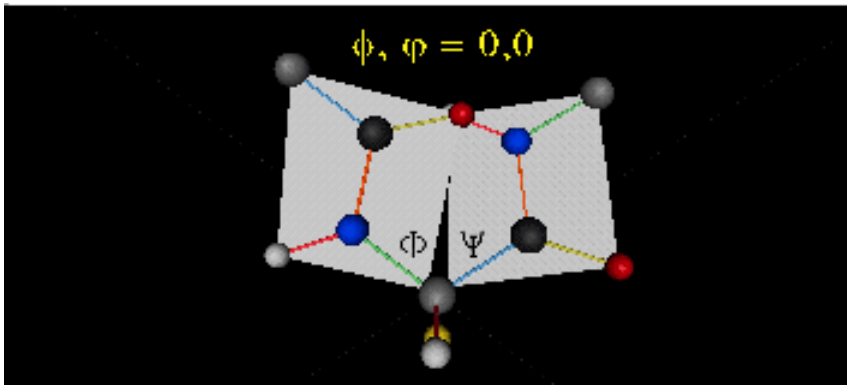


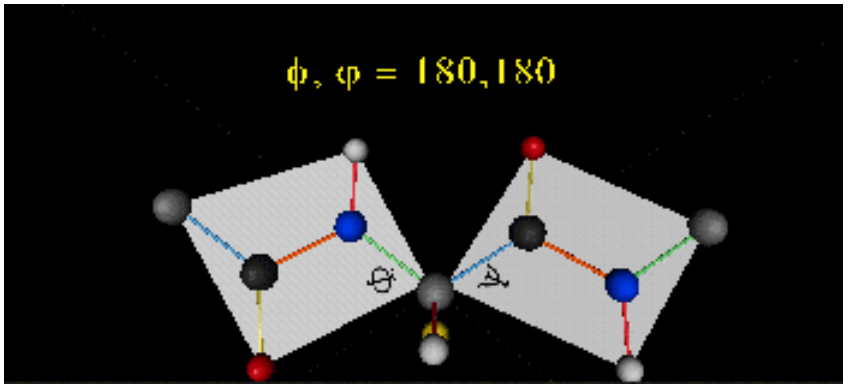
Secondary Protein Structure

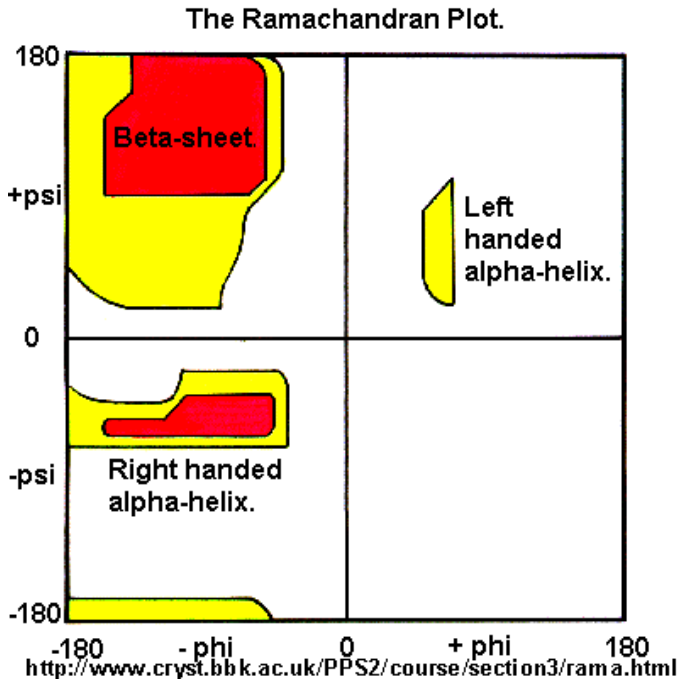
- Secondary structure of a protein is the arrangement of polypeptide backbone of the protein in space. The secondary structure includes two kinds of repeating pattern known as the *α-helix* and *β-sheet*.
- Hydrogen bonding between backbone atoms are responsible for both of these secondary structures.

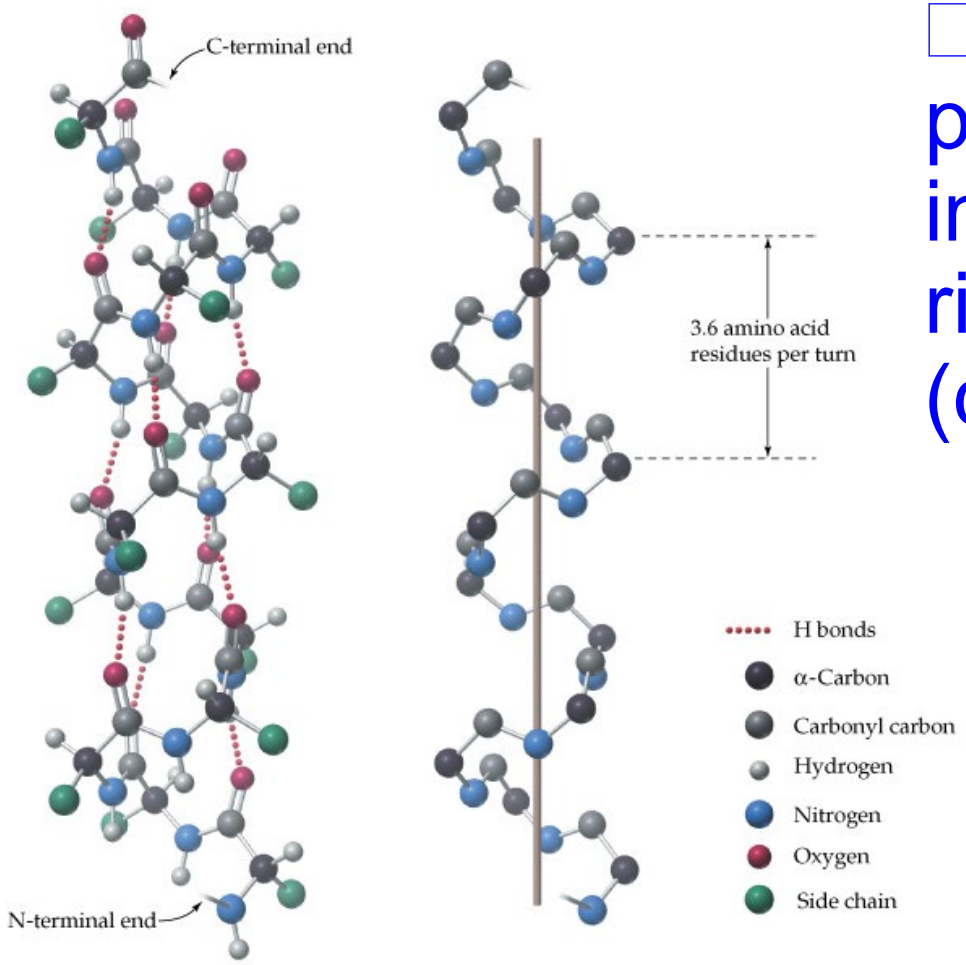
FULLY EXTENDED POLYPEPTIDE CHAIN





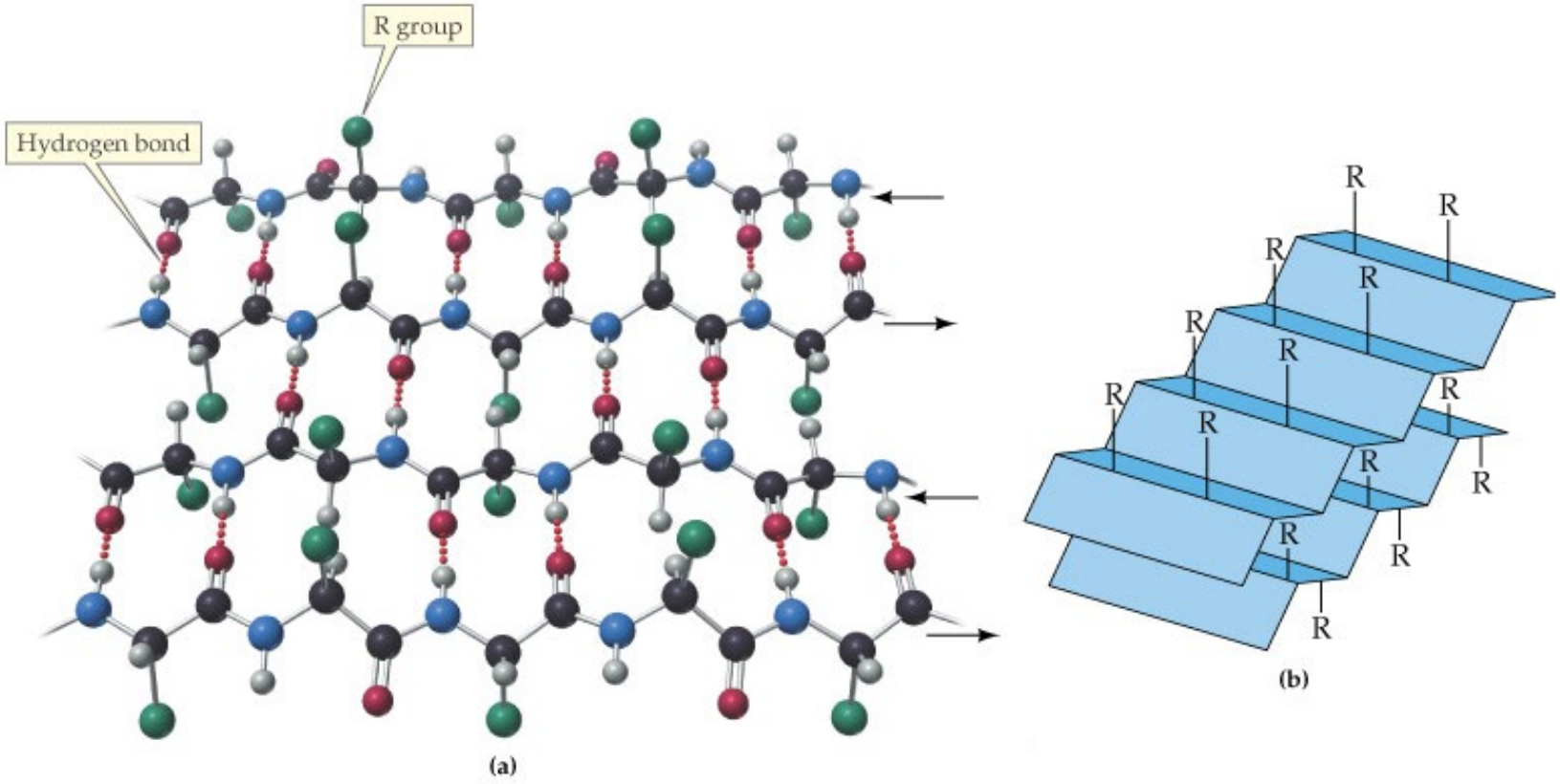






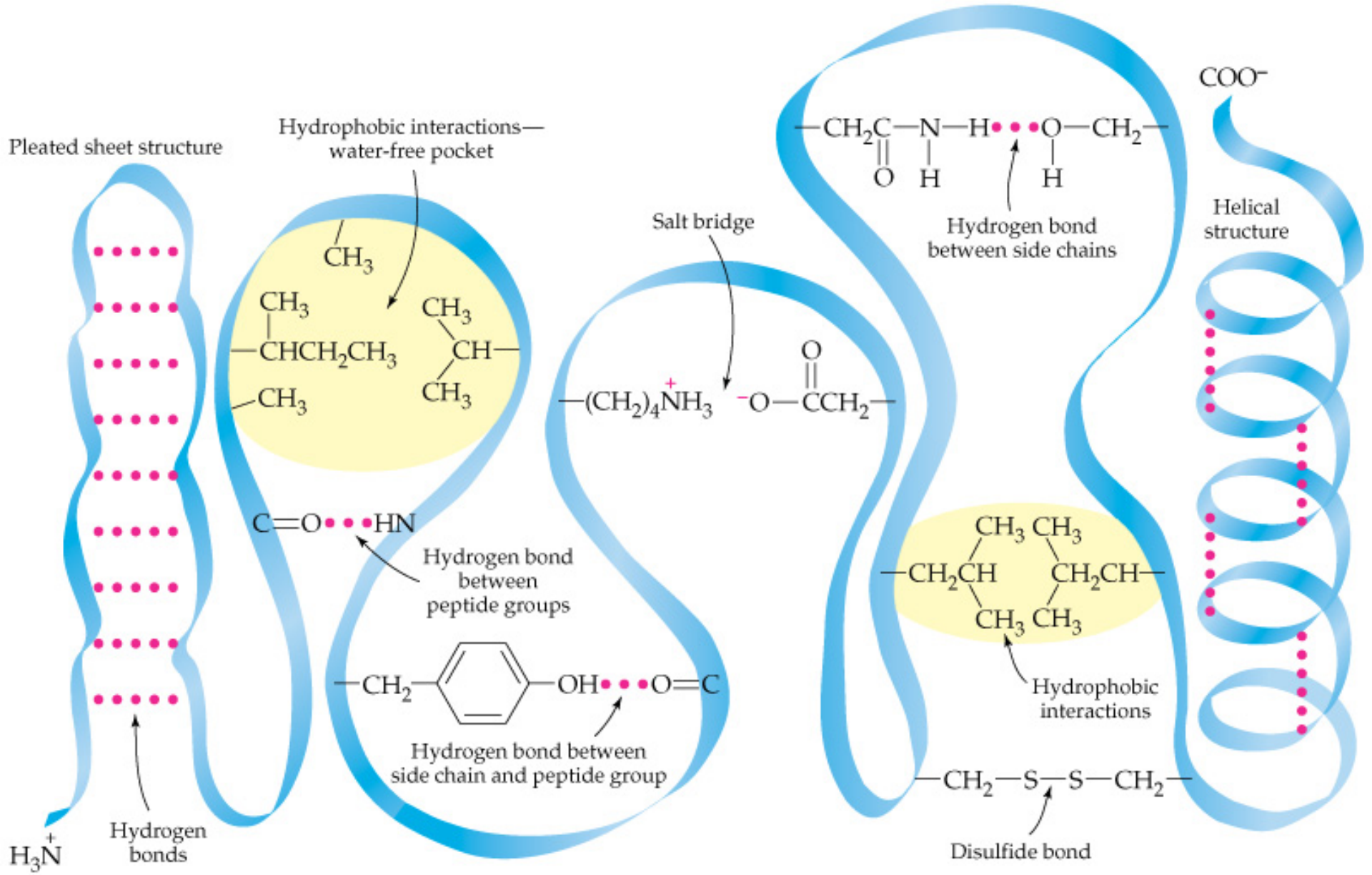
- ***α-Helix:*** A single protein chain coiled in a spiral with a right-handed (clockwise) twist.

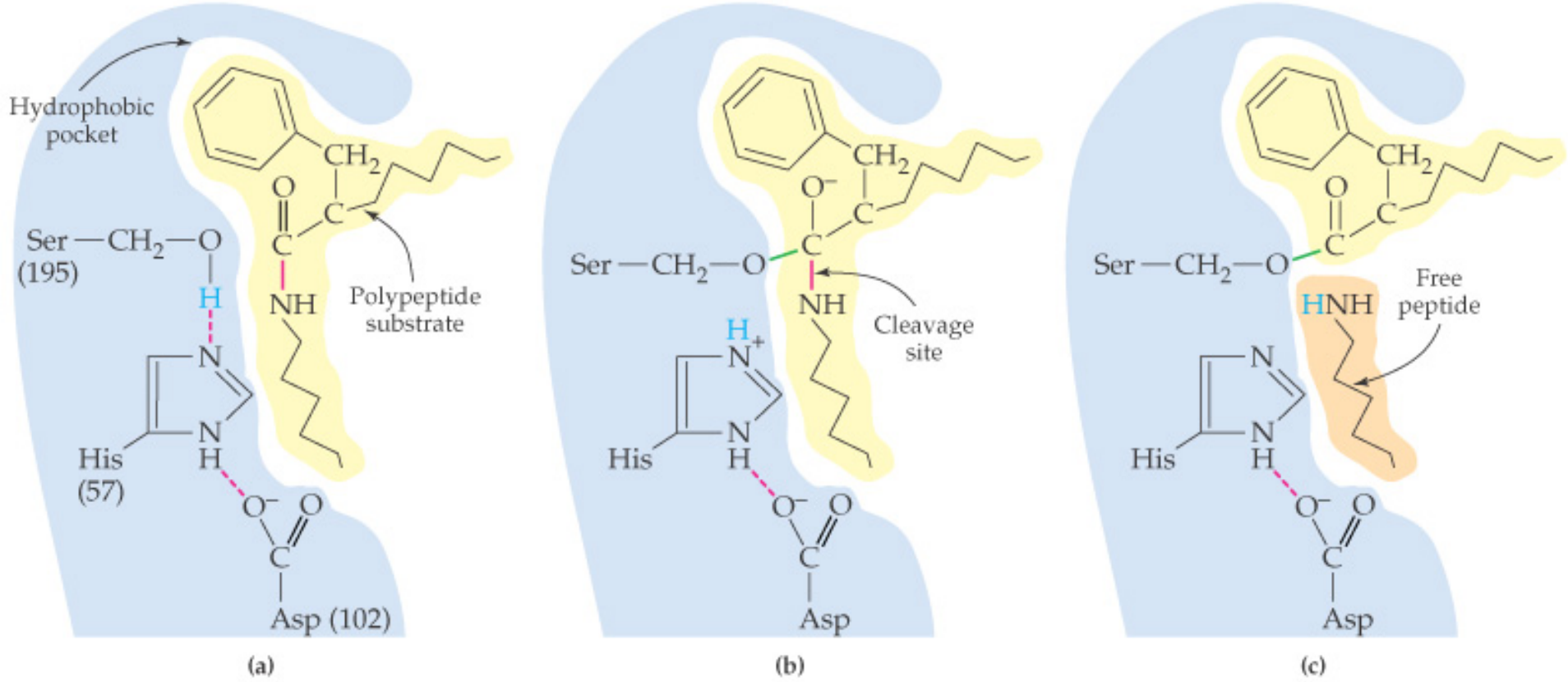
β-Sheet: The polypeptide chain is held in place by hydrogen bonds between pairs of peptide units along neighboring backbone segments.

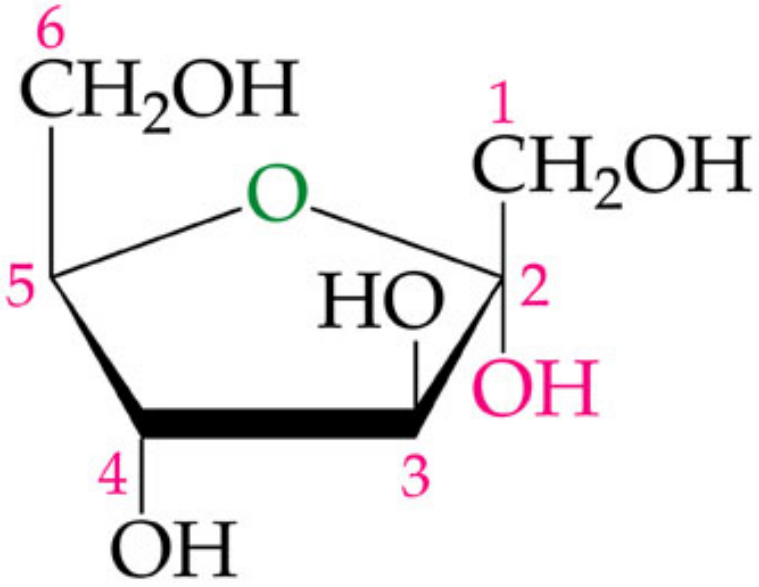


Tertiary Protein Structure

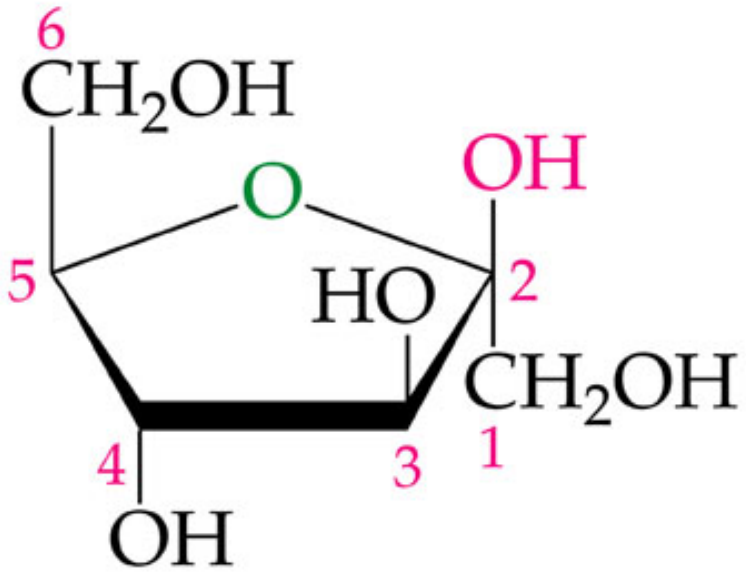
- ***Tertiary Structure of a proteins*** The overall three dimensional shape that results from the folding of a protein chain. Tertiary structure depends mainly on attractions of amino acid side chains that are far apart along the same backbone. Non-covalent interactions and disulfide covalent bonds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


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

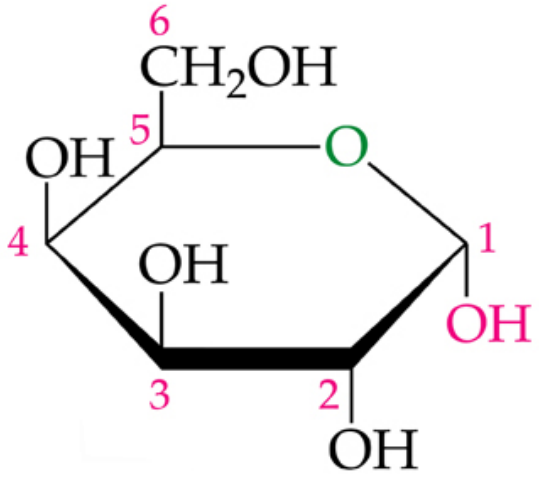

Hydrophobic pocket
Ser —CH2—O
(195)
H
N
N
H
His
(57)
O⁻ O
C
Asp (102)
CH2
O
C
C
NH
Polypeptide substrate
(a)
O⁻
C
Ser —CH2—O
NH
Cleavage site
H+
N
N
H
His
O⁻ O
C
Asp
(b)
HNH
Free peptide
(c)



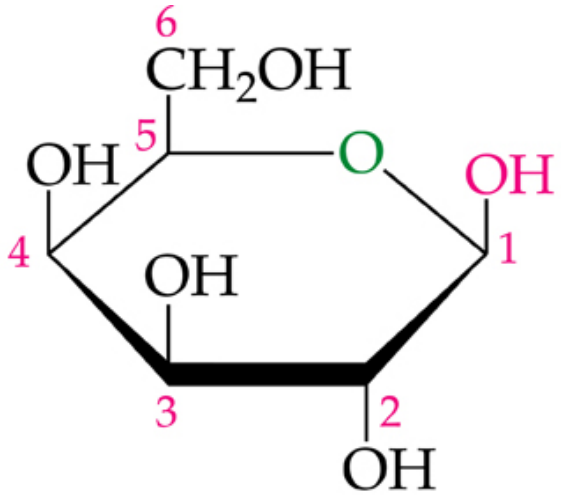
α-D-Fructose



β-D-Fructose

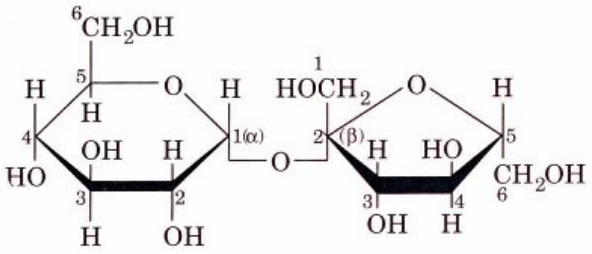


α-D-Galactose

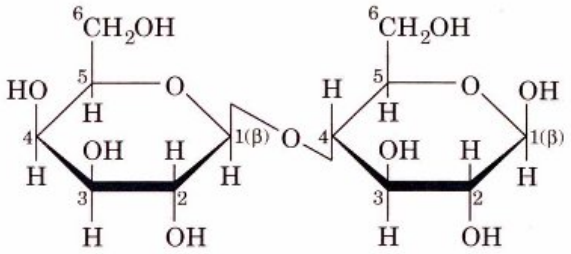


β-D-Galactose

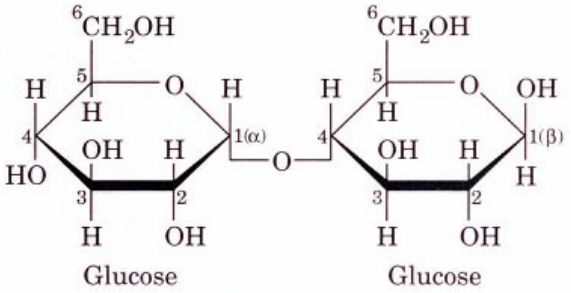
Some Common Disaccharides



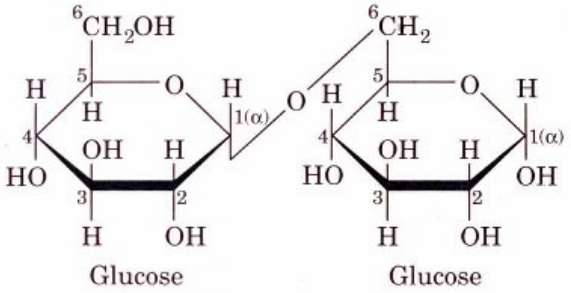
Sucrose



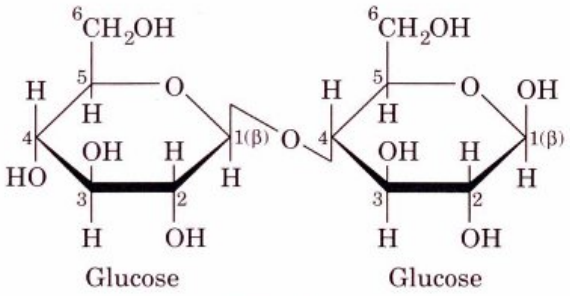
Lactose



Maltose

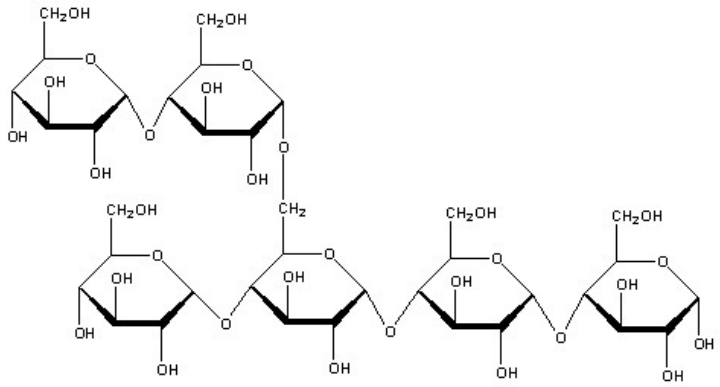


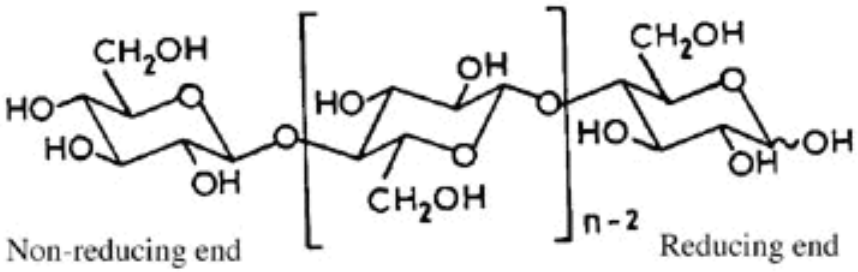
Isomaltose



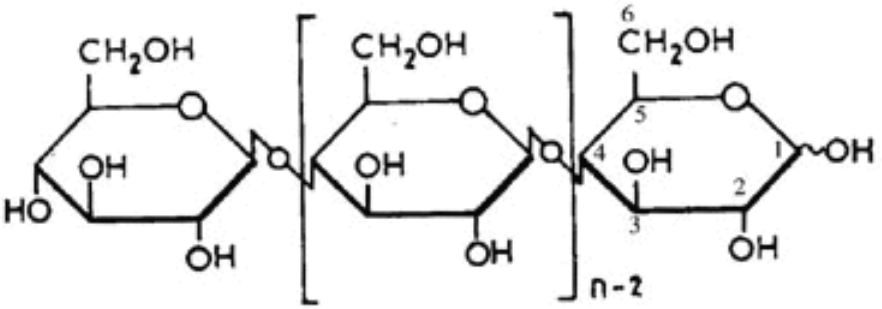
Cellobiose

Polysaccharides

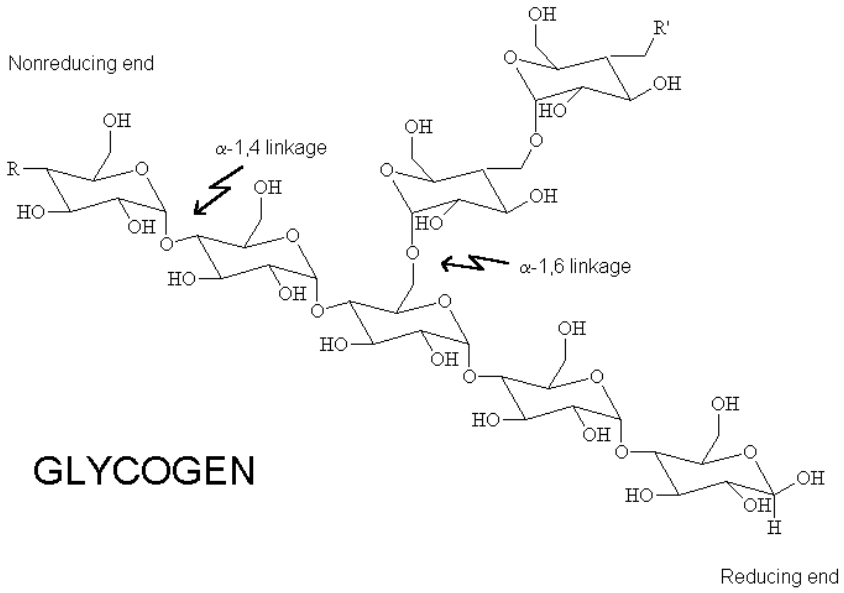




Sometimes shown as



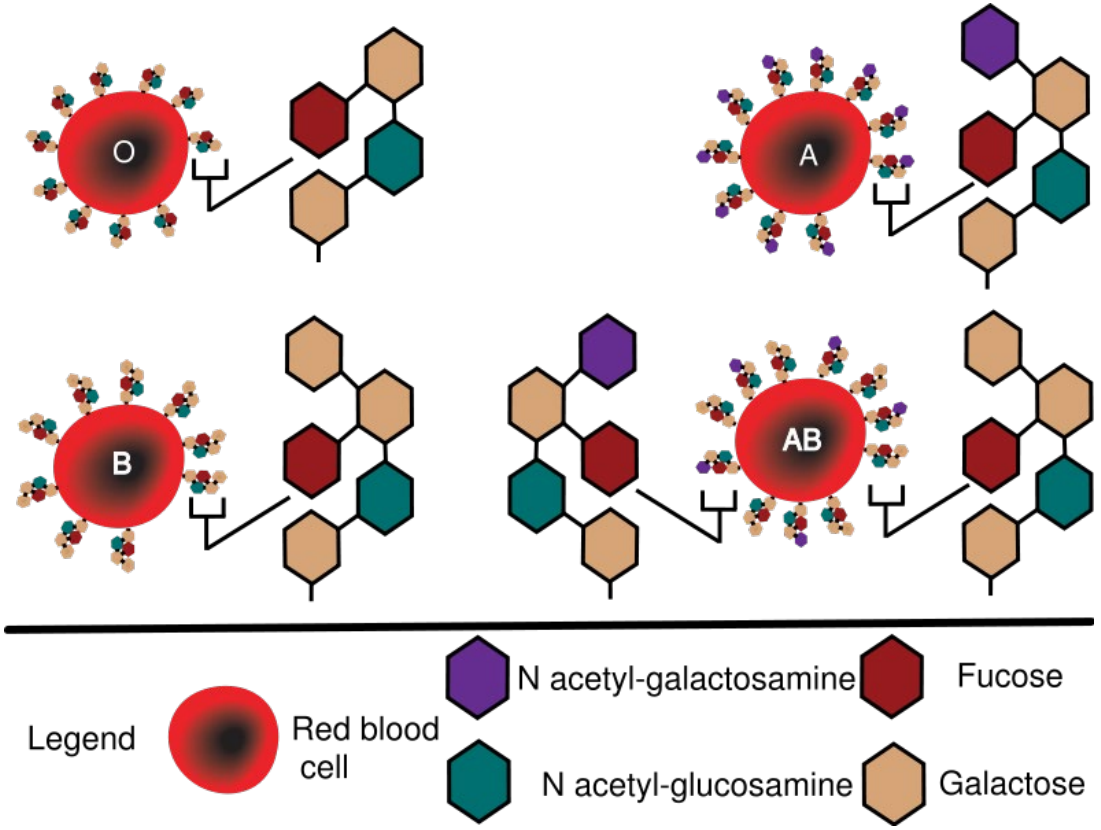
Cellulose



GLYCOGEN

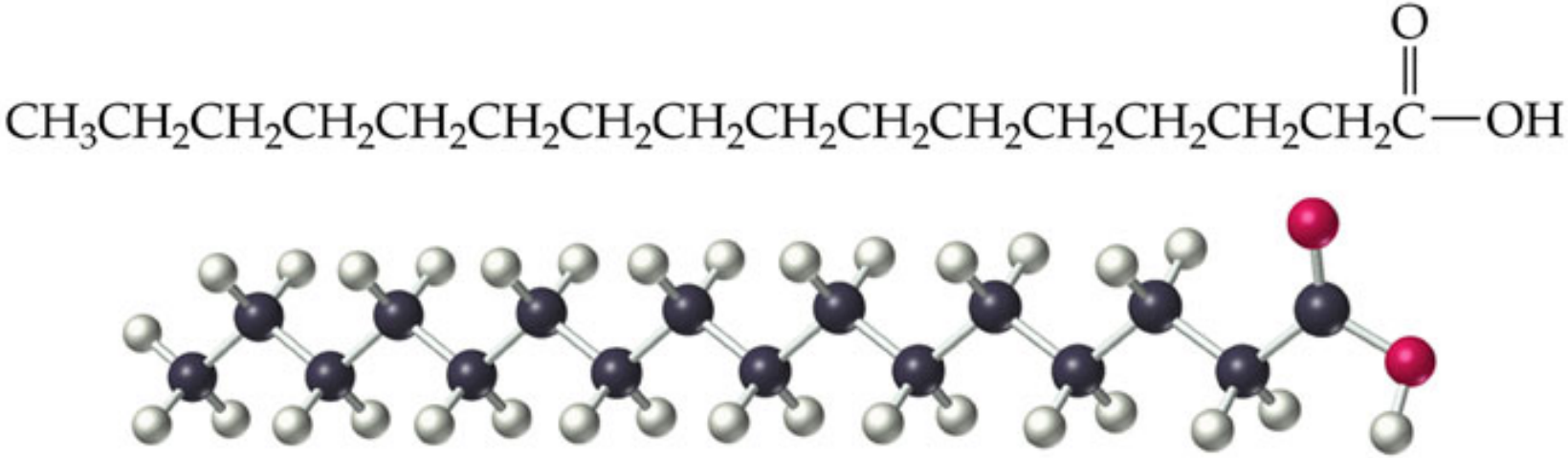
Blood Type

	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

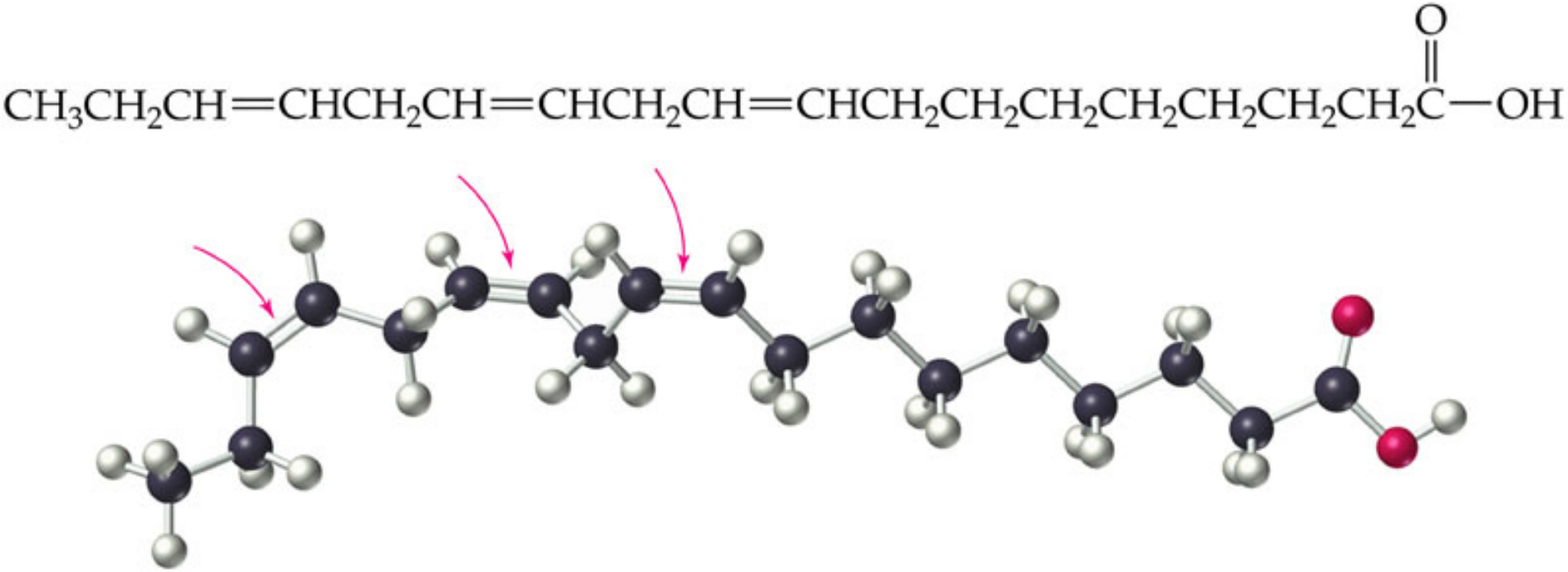


Lipid

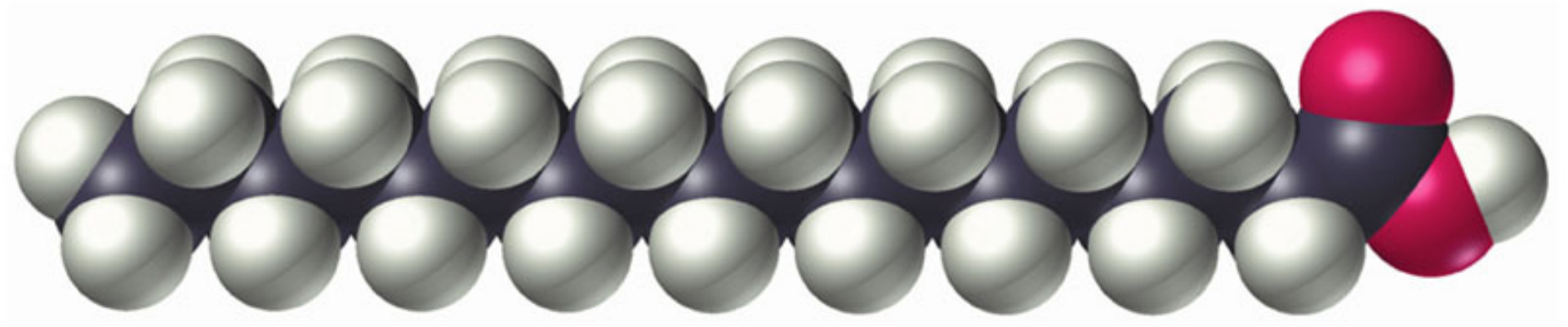
- ***Lipids*** are naturally occurring molecules from plants or animals that are soluble in nonpolar organic solvents.
- Lipid molecules contain large hydrocarbon portion and not many polar functional group, which accounts for their solubility behavior.



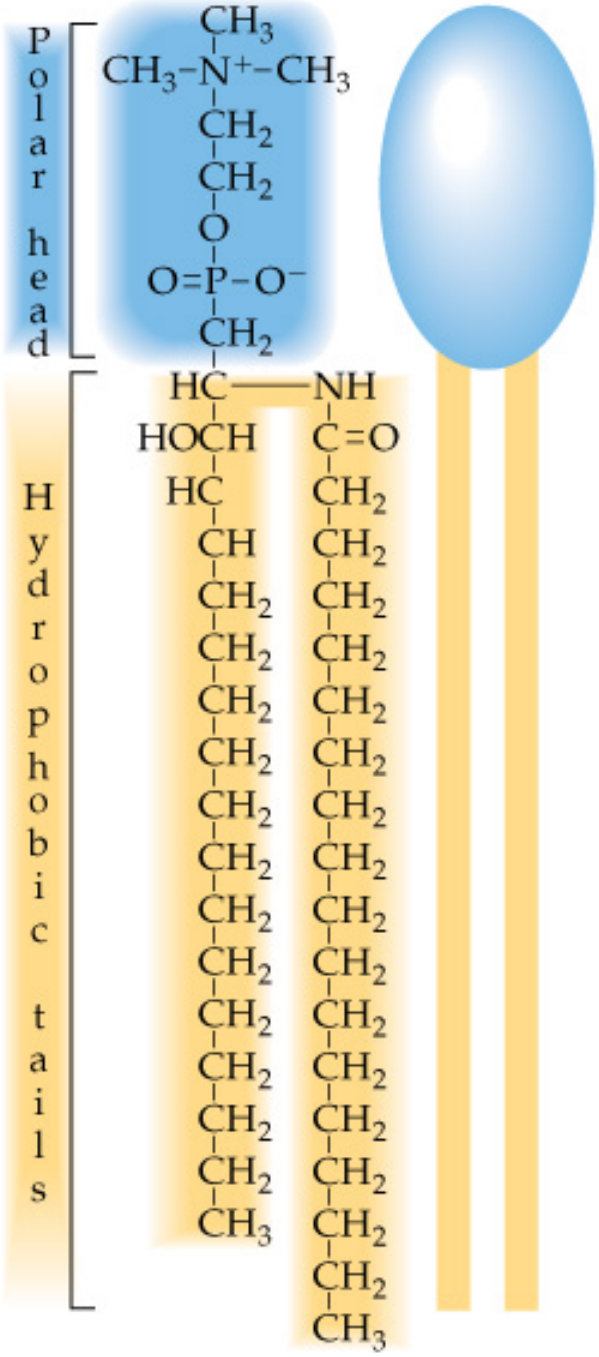
A saturated fatty acid
(palmitic acid)



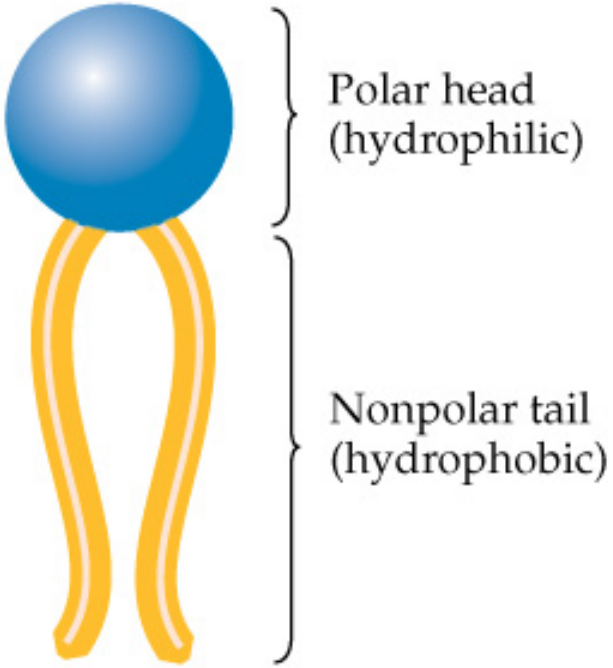
A *cis* unsaturated fatty acid
(linolenic acid)



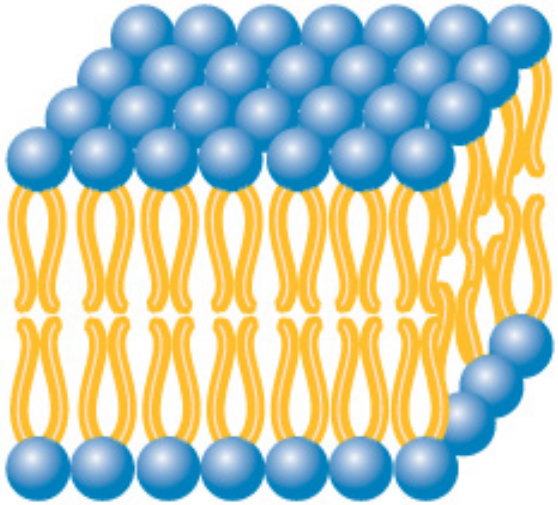
Stearic acid, an 18-carbon saturated fatty acid



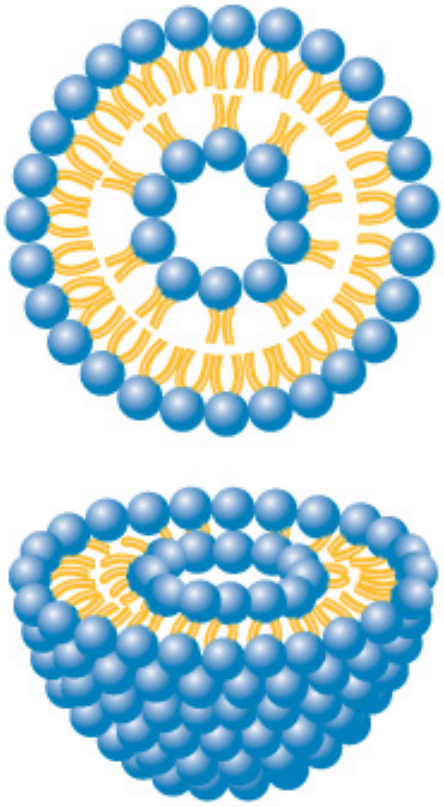
A sphingomyelin



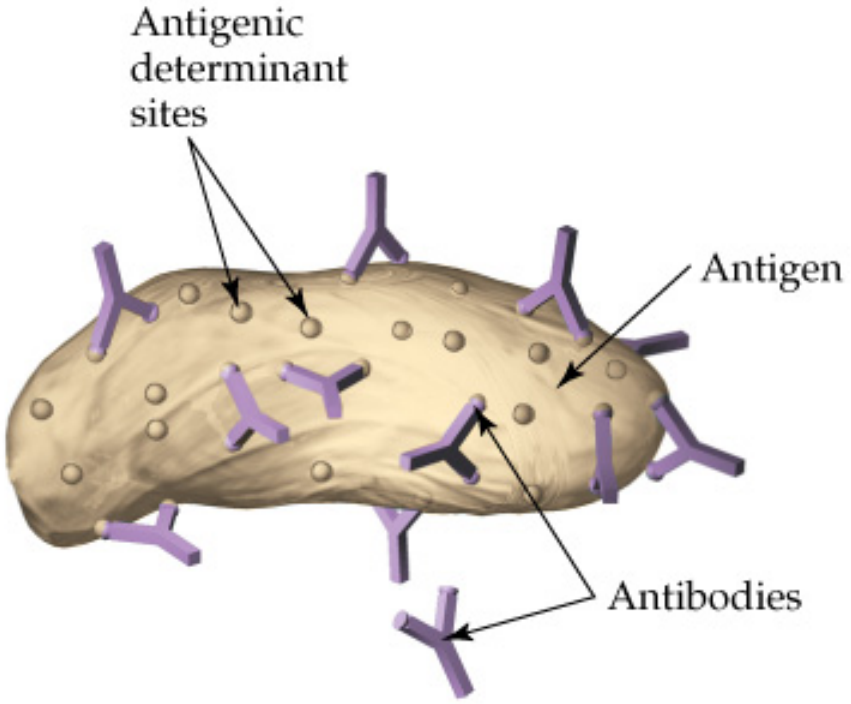
Membrane lipid



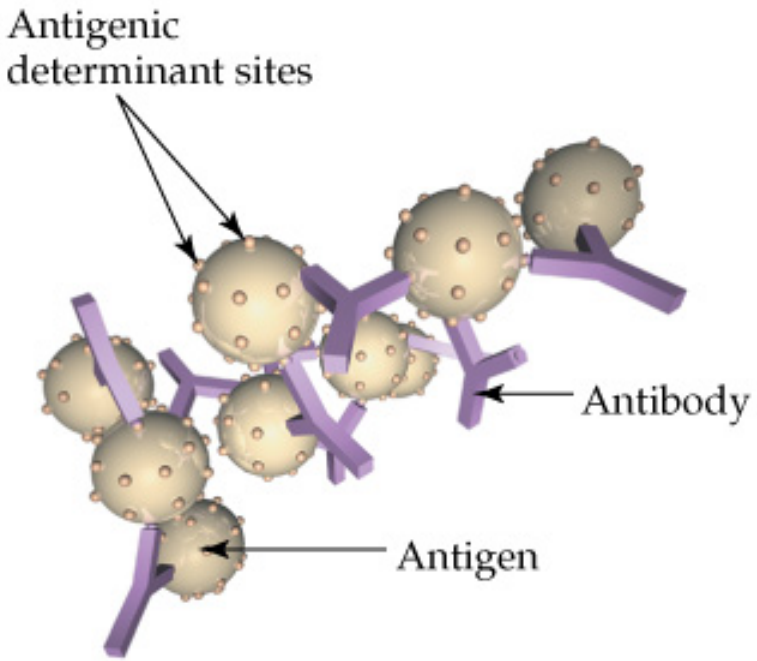
Lipid bilayer



Liposome

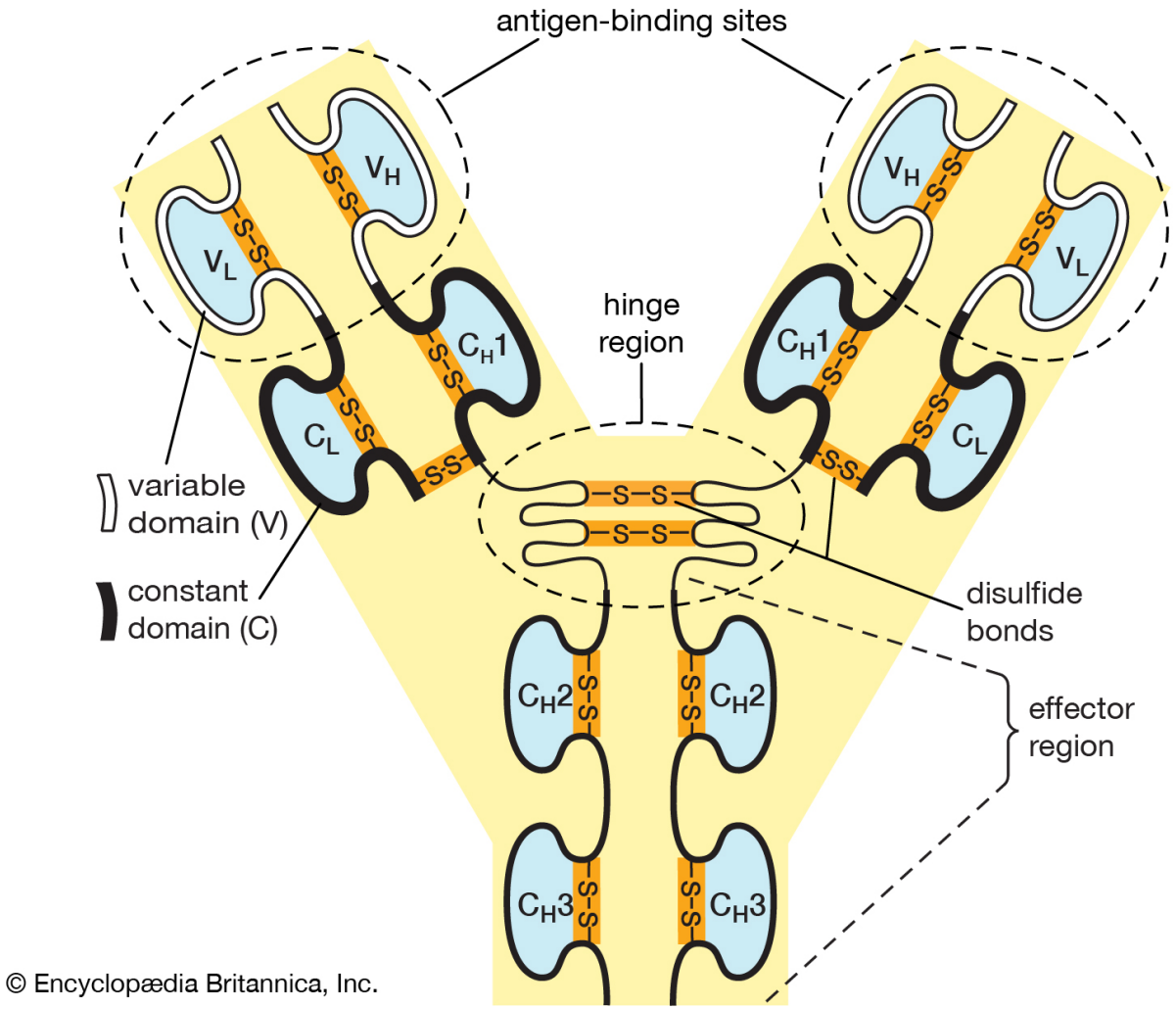

Antigenic determinant sites
Antigen
Antibodies

(a)


Antigenic determinant sites
Antibody
Antigen

(b)

Antibody


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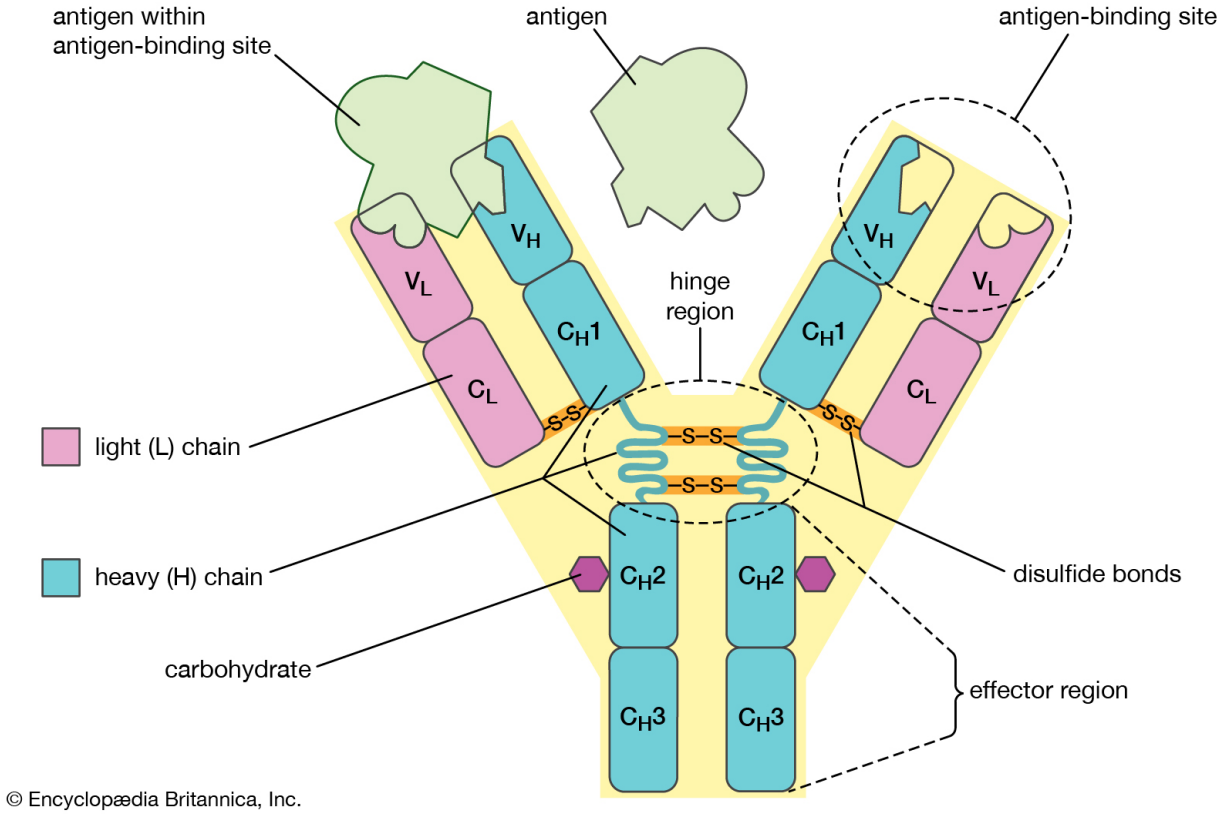
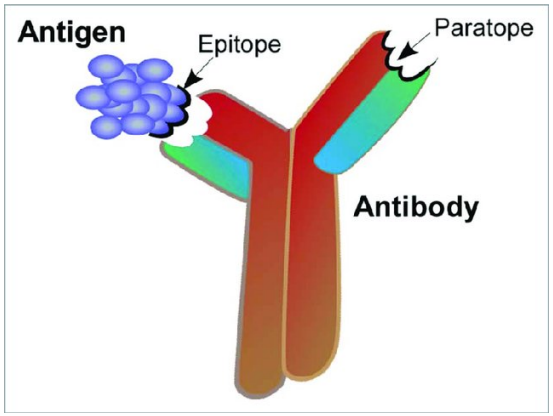
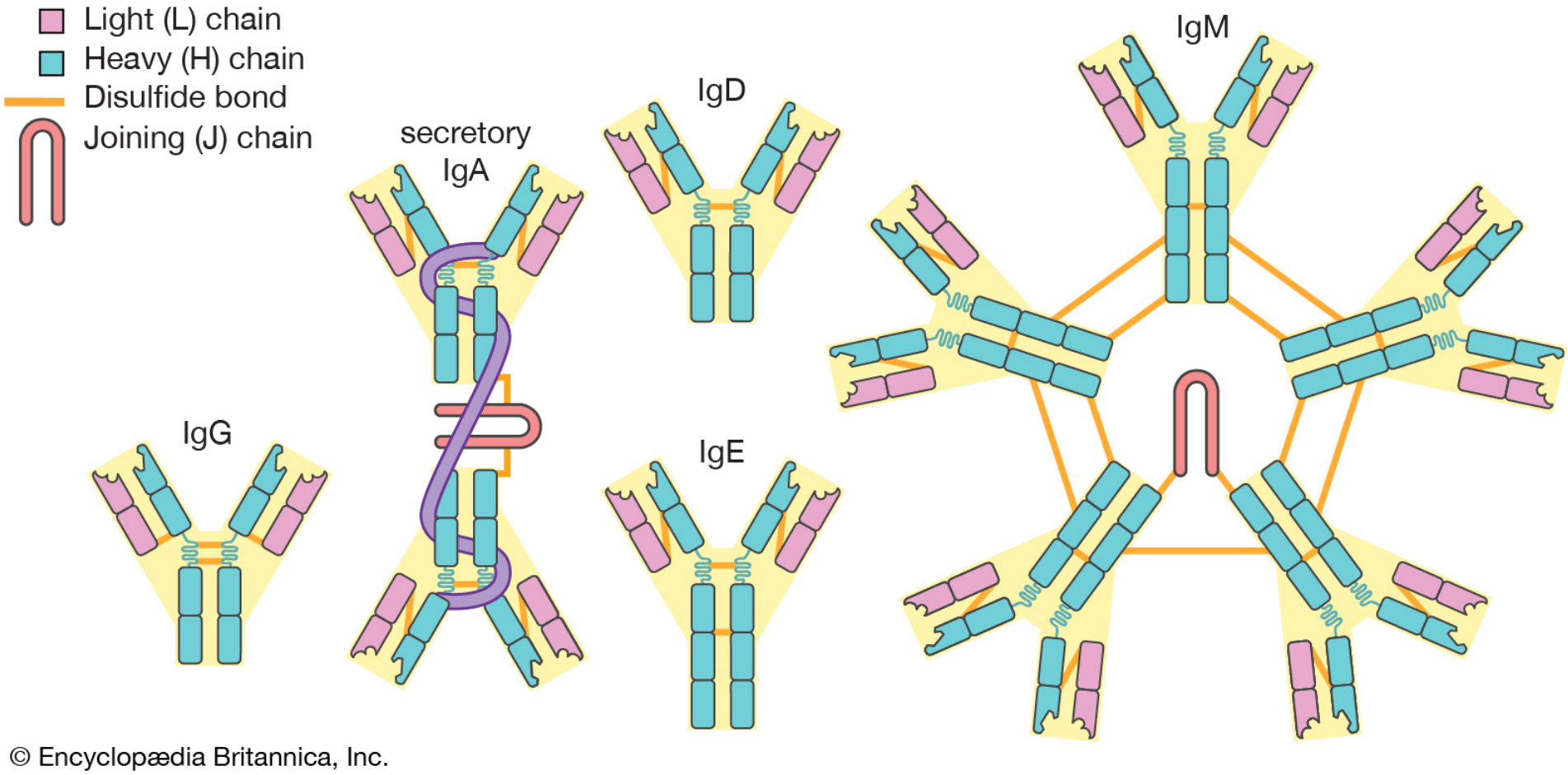
Antibody Binding Sites

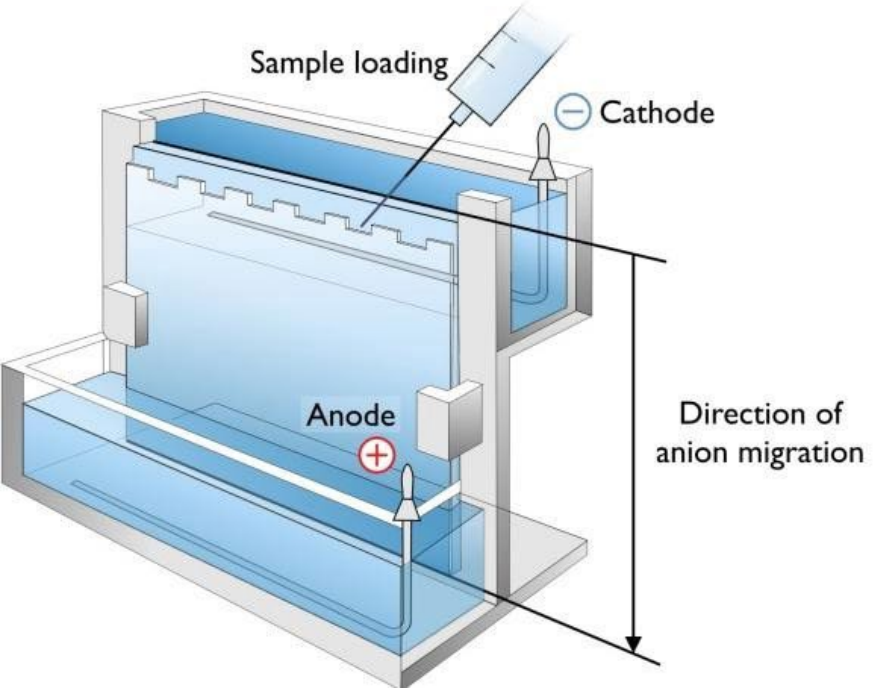


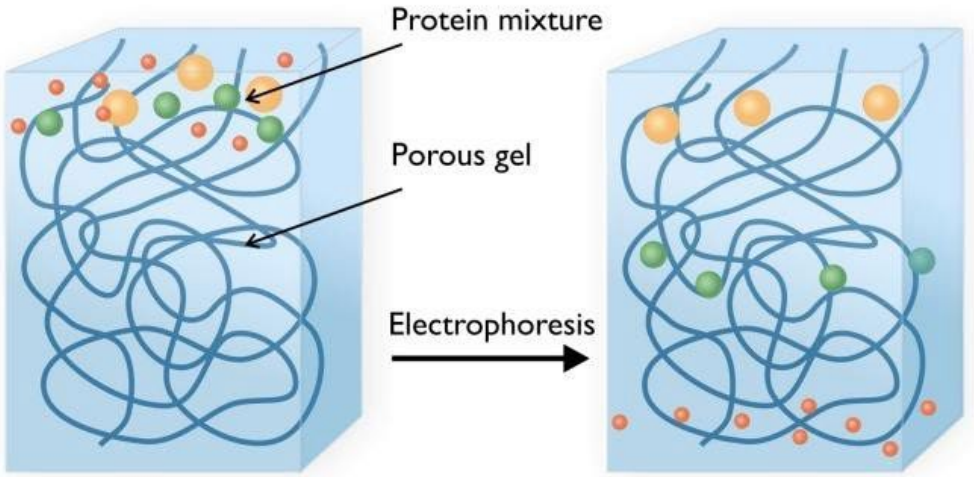


Different Types of Antibodies

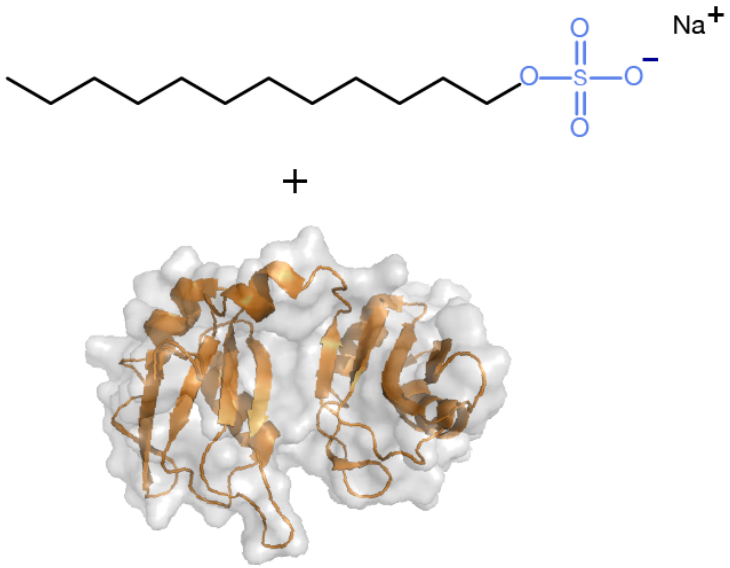


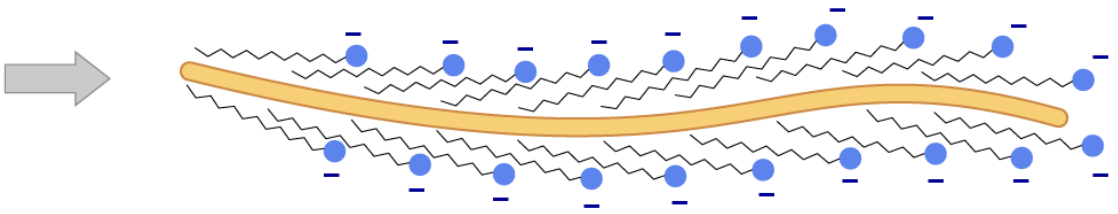
Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)



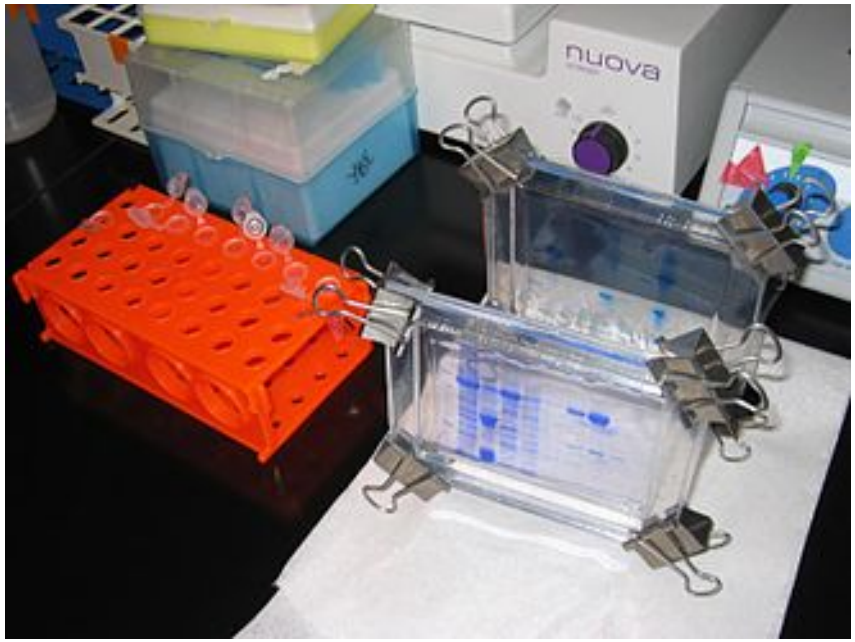


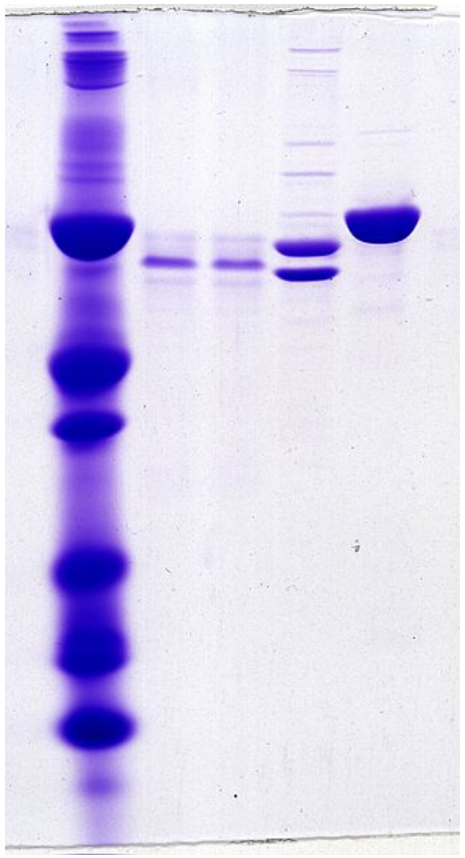
Protein Denature



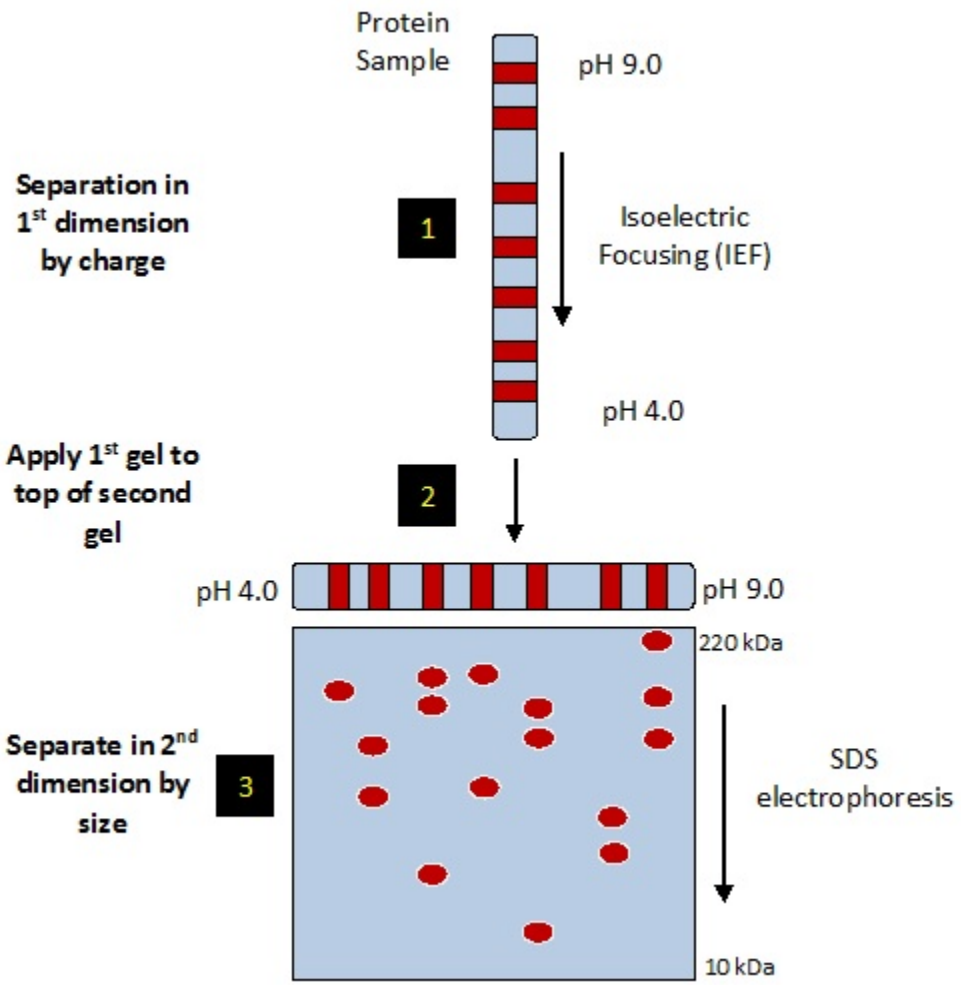


SDS-PAGE

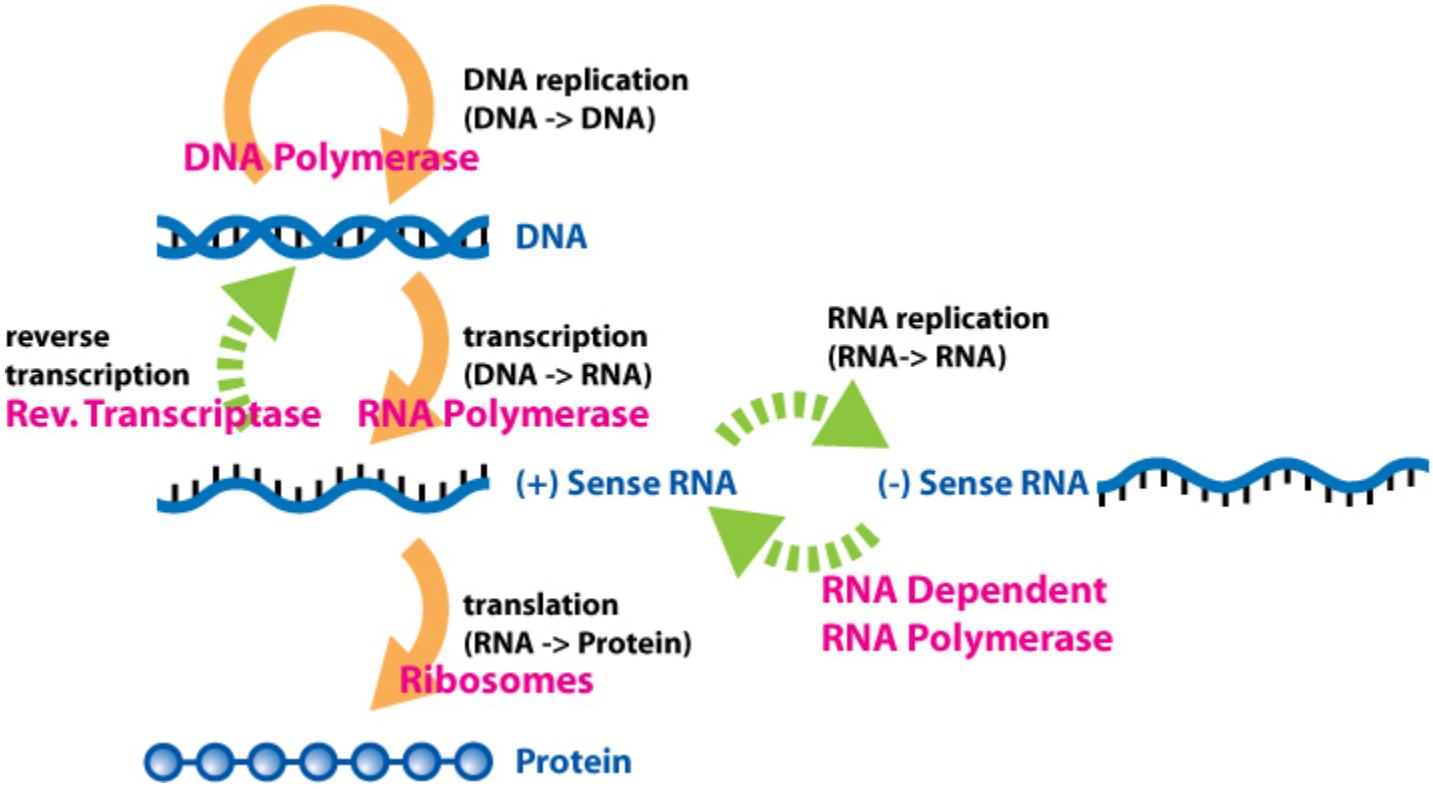




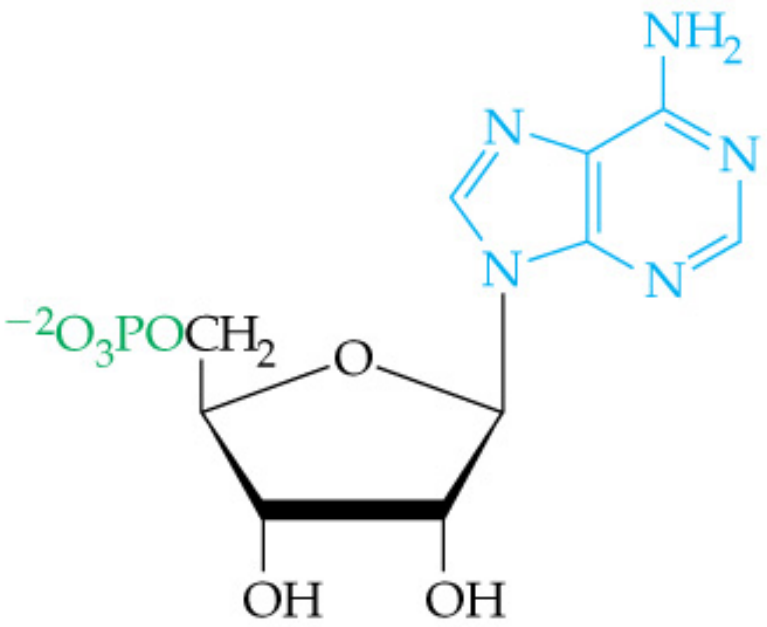
2D PAGE


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

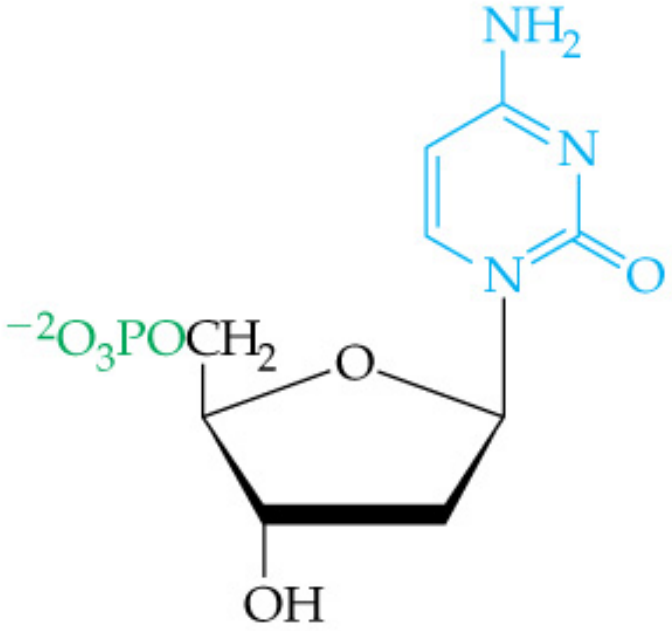
Central Dogma



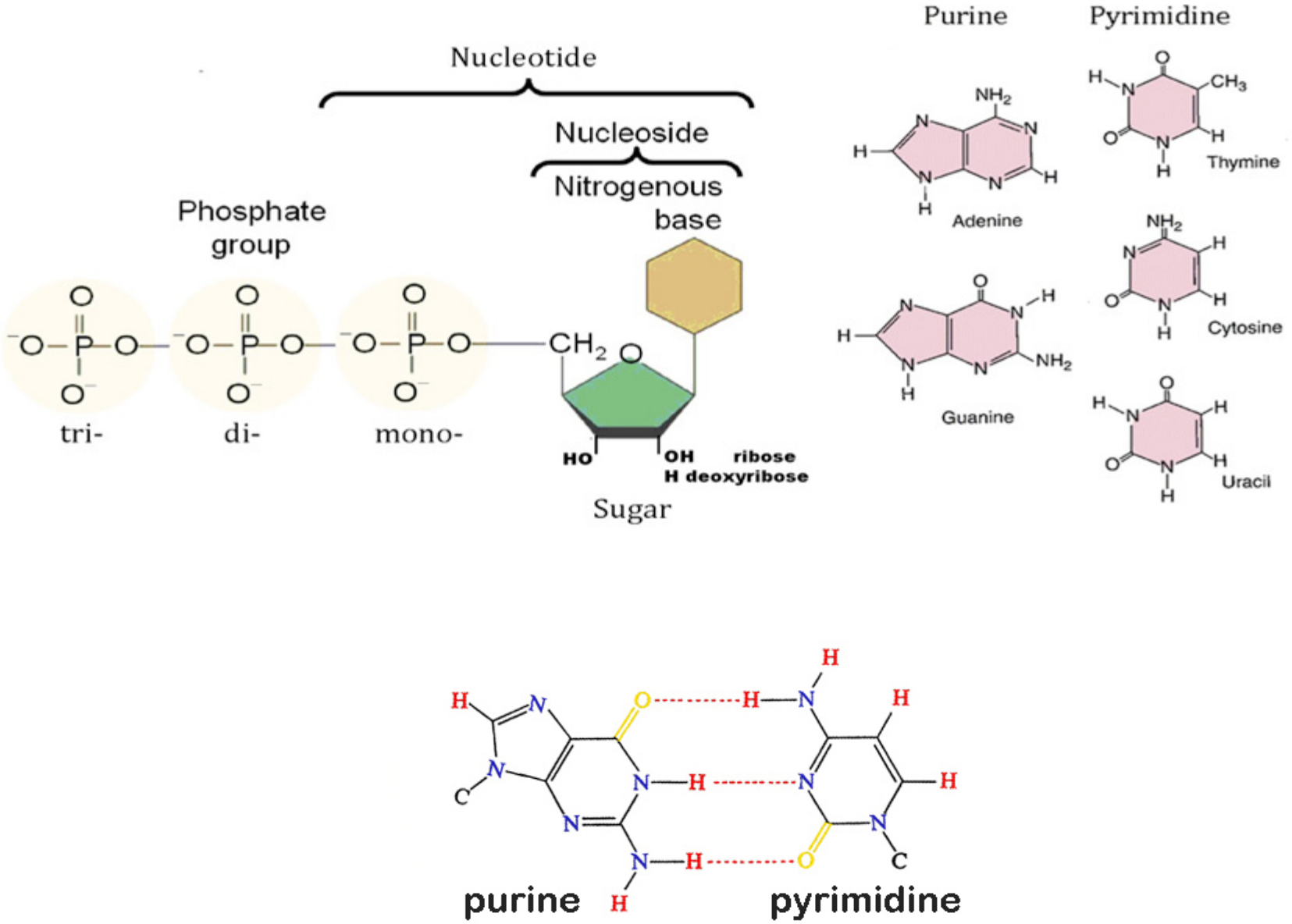
- In RNA, the sugar is ribose.
- In DNA, the sugar is deoxyribose.



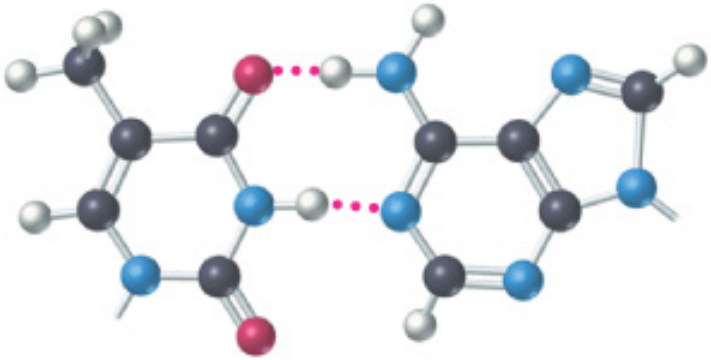
Adenosine 5′-monophosphate (AMP)
(a ribonucleotide)

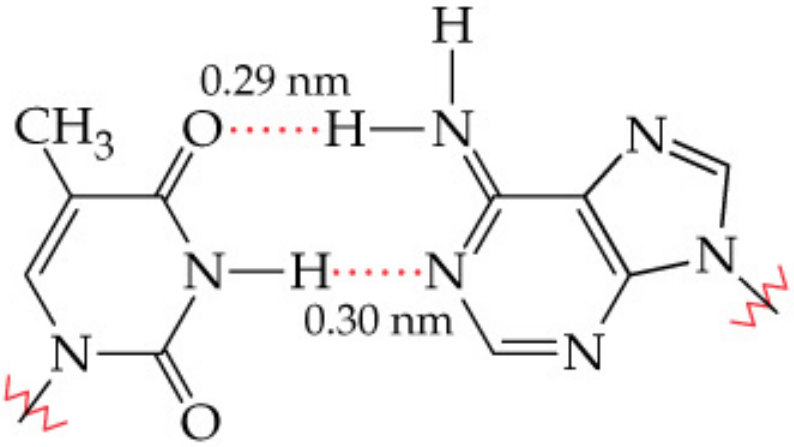


Deoxycytidine 5′-monophosphate (dCMP)
(a deoxyribonucleotide)

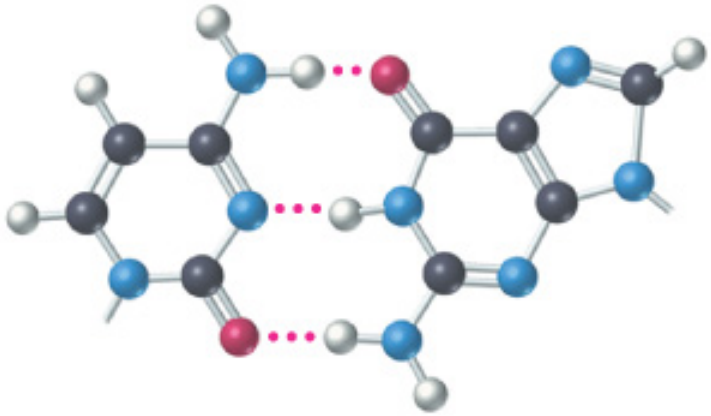

Purine
Pyrimidine
Nucleotide
Nucleoside
Nitrogenous base
Phosphate group
tri-
di-
mono-
HO
OH
H
ribose
deoxyribose
Sugar
Adenine
Thymine
Cytosine
Guanine
Uracil
purine
pyrimidine

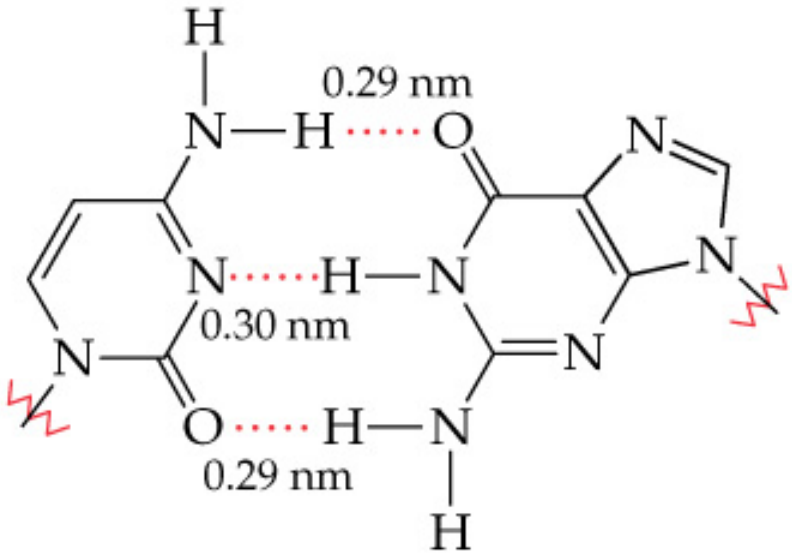
Thymine–Adenine

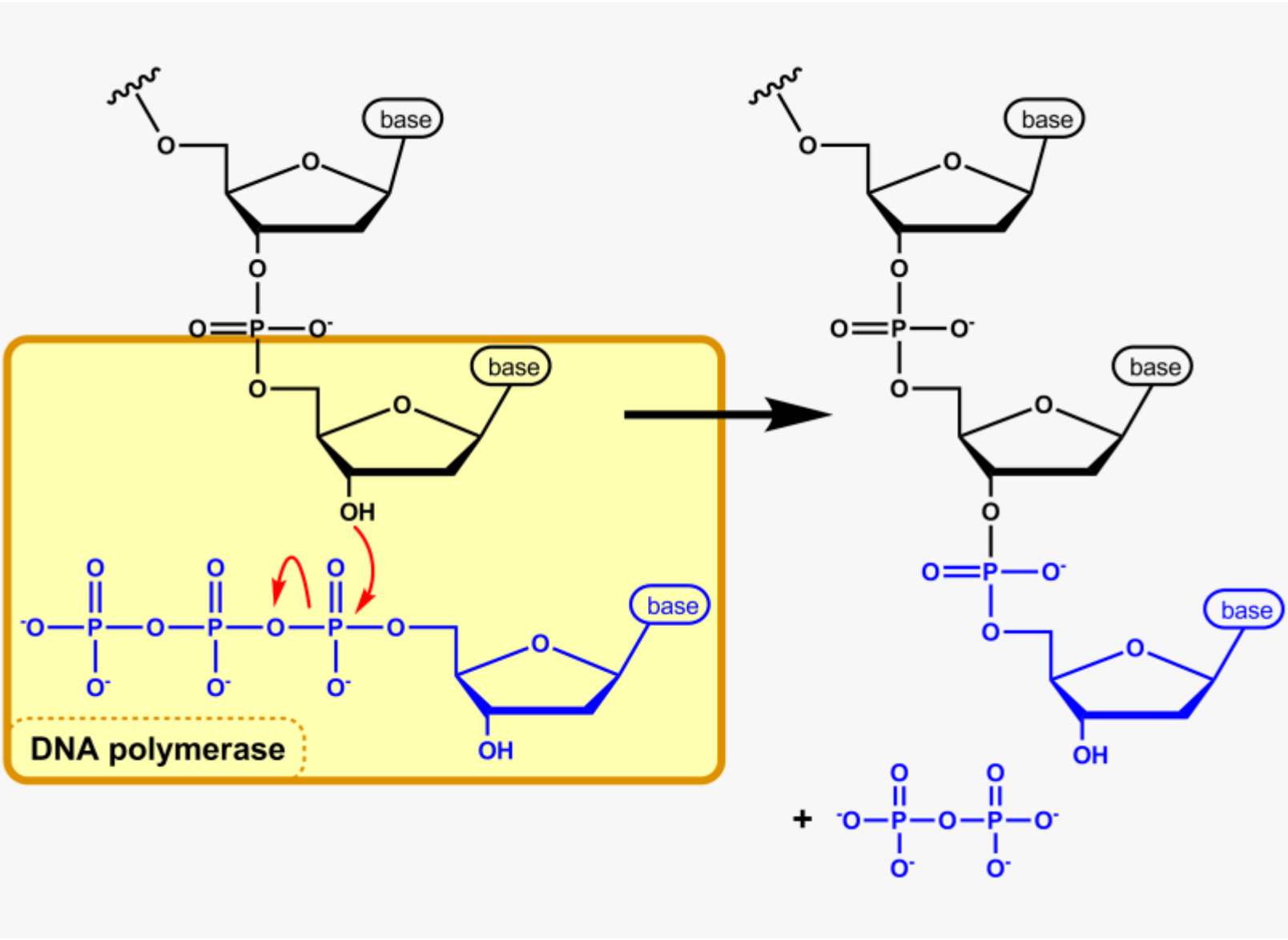


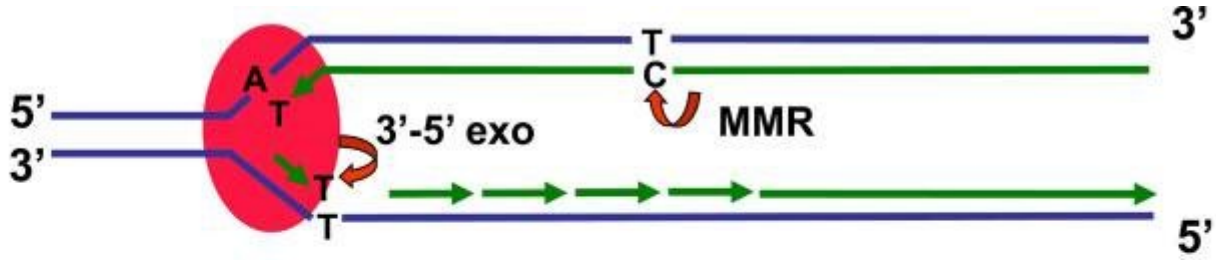


Cytosine–Guanine






base
base
OH
base
DNA polymerase
OH
base
base
OH
+

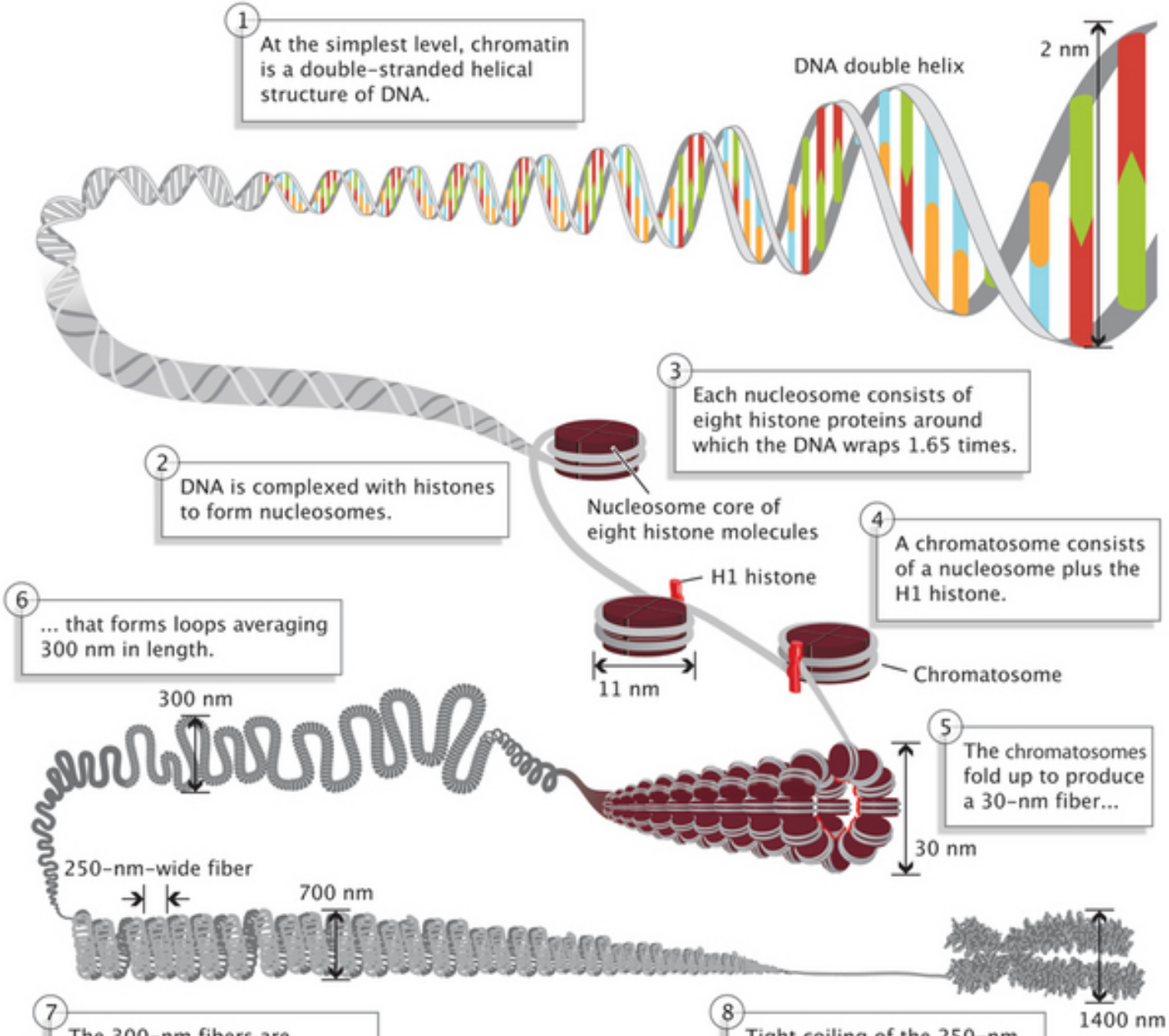


BASE SELECTION $10^{-4} – 10^{-5}$

3'-5' EXONUCLEASE 10^{-2}

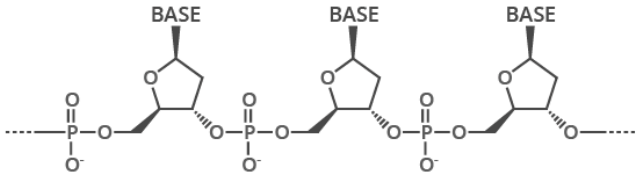
MISMATCH REPAIR 10^{-3}

DNA REPLICATION FIDELITY 10^{-10}


1
At the simplest level, chromatin is a double-stranded helical structure of DNA.
DNA double helix
2 nm
2
DNA is complexed with histones to form nucleosomes.
3
Each nucleosome consists of eight histone proteins around which the DNA wraps 1.65 times.
Nucleosome core of eight histone molecules
4
A chromatosome consists of a nucleosome plus the H1 histone.
H1 histone
11 nm
Chromatosome
6
... that forms loops averaging 300 nm in length.
300 nm
5
The chromatosomes fold up to produce a 30-nm fiber...
30 nm
250-nm-wide fiber
700 nm
1400 nm
7
The 300-nm fibers are compressed and folded to produce a 250-nm-wide fiber.
8
Tight coiling of the 250-nm fiber produces the chromatid of a chromosome.

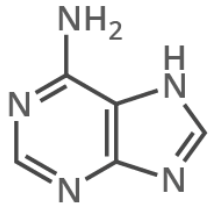
THE CHEMICAL STRUCTURE OF DNA

THE SUGAR PHOSPHATE 'BACKBONE'

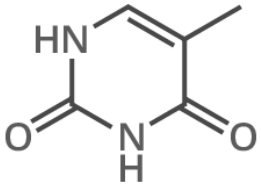


DNA is a polymer made up of units called nucleotides. The nucleotides are made of three different components: a sugar group, a phosphate group, and a base. There are four different bases: adenine, thymine, guanine and cytosine.

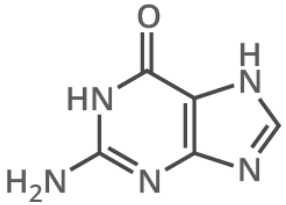
A ADENINE



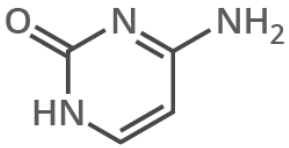
T THYMINE



G GUANINE

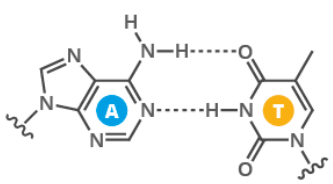


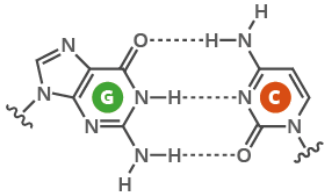
C CYTOSINE



WHAT HOLDS DNA STRANDS TOGETHER?

DNA strands are held together by hydrogen bonds between bases on adjacent strands. Adenine (A) always pairs with thymine (T), while guanine (G) always pairs with cytosine (C). Adenine pairs with uracil (U) in RNA.



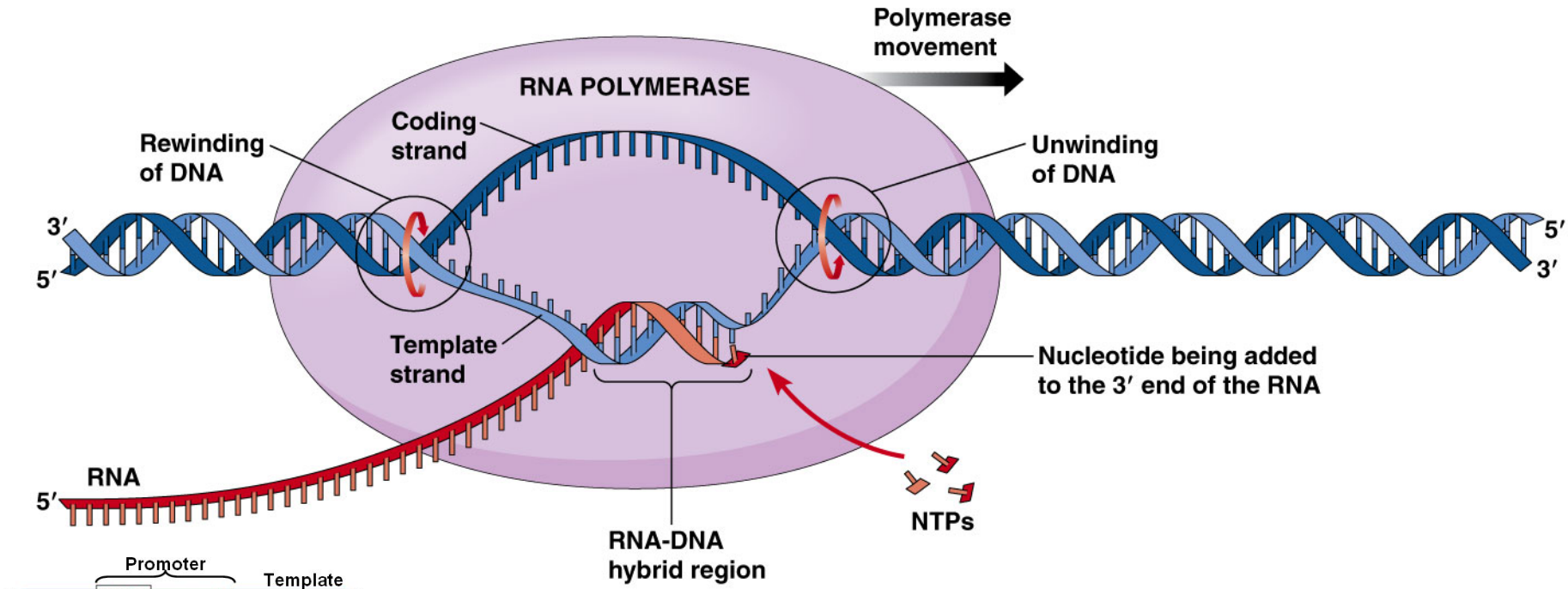


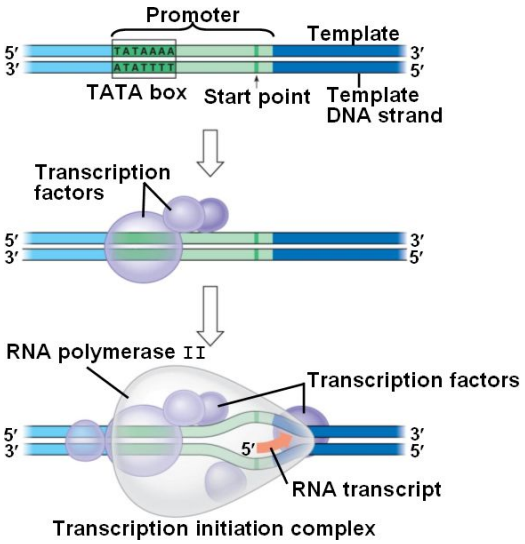
FROM DNA TO PROTEINS

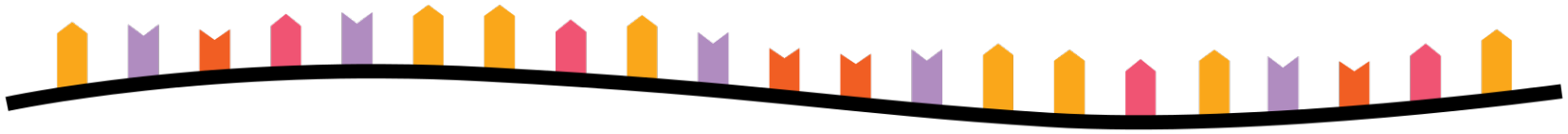
The bases on a single strand of DNA act as a code. The letters form three letter codons, which code for amino acids - the building blocks of proteins.

An enzyme, RNA polymerase, transcribes DNA into mRNA (messenger ribonucleic acid). It splits apart the two strands that form the double helix, then reads a strand and copies the sequence of nucleotides. The only difference between the RNA and the original DNA is that in the place of thymine (T), another base with a similar structure is used: uracil (U).

In multicellular organisms, the mRNA carries genetic code out of the cell nucleus, to the cytoplasm. Here, protein synthesis takes place. 'Translation' is the process of turning the mRNA's 'code' into proteins. Molecules called ribosomes carry out this process, building up proteins from the amino acids coded for.


Polymerase movement
RNA POLYMERASE
Coding strand
Rewinding of DNA
Unwinding of DNA
3′
5′
5′
3′
Template strand
Nucleotide being added to the 3′ end of the RNA
RNA
5′
NTPs
RNA-DNA hybrid region


Promoter
Template
5′
3′
TATAAAA
ATATTTT
3′
5′
TATA box
Start point
Template DNA strand
Transcription factors
RNA polymerase II
Transcription factors
RNA transcript
Transcription initiation complex



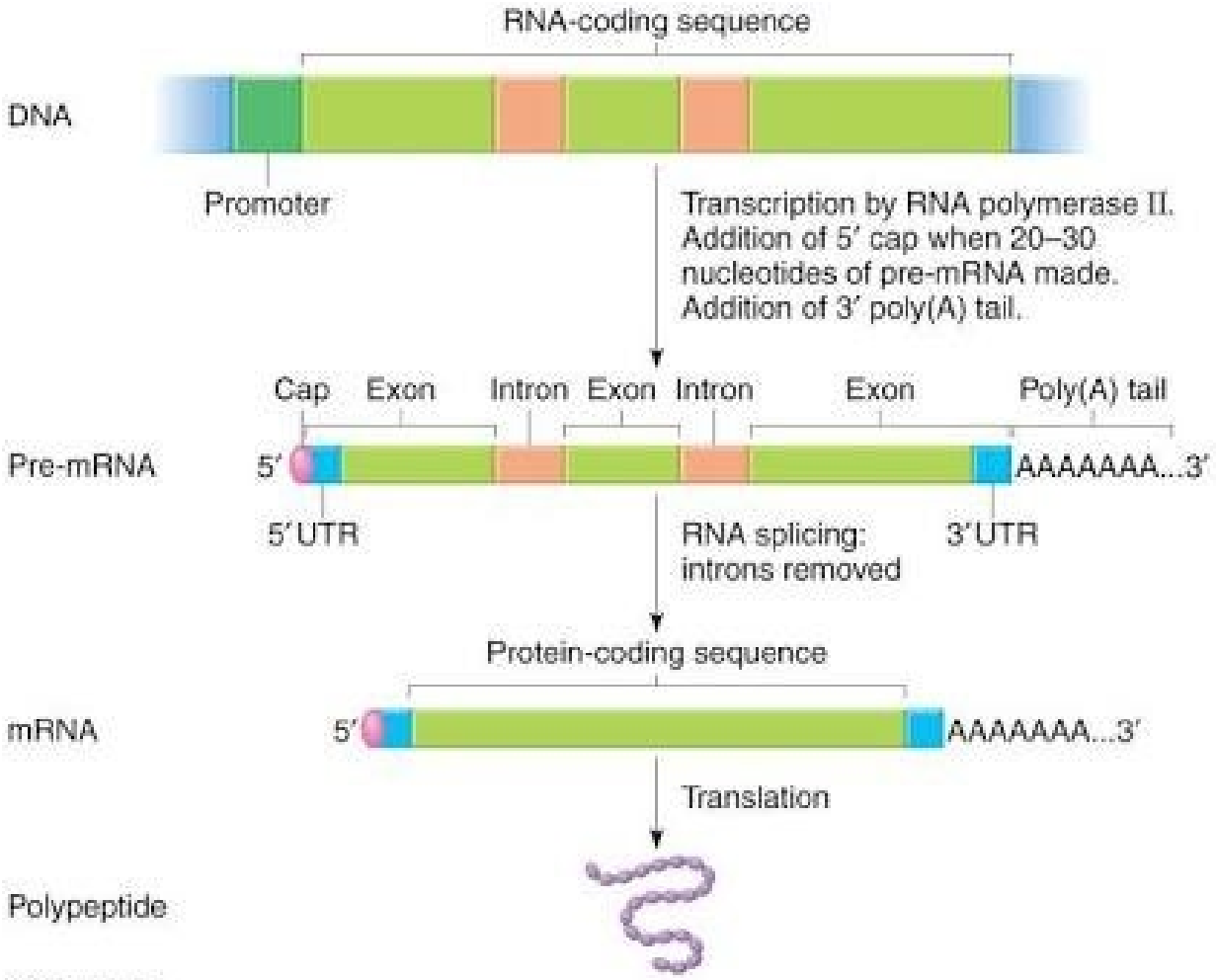
Base	G C U	A C G	G A G	C U U	C G G	A G C	U A G
Codon	Codon 1	Codon 2	Codon 3	Codon 4	Codon 5	Codon 6	Codon 7
Aminoacid	**Alanine**	**Threonine**	**Glutamate**	**Leucine**	**Arginine**	**Serine**	**Stop**

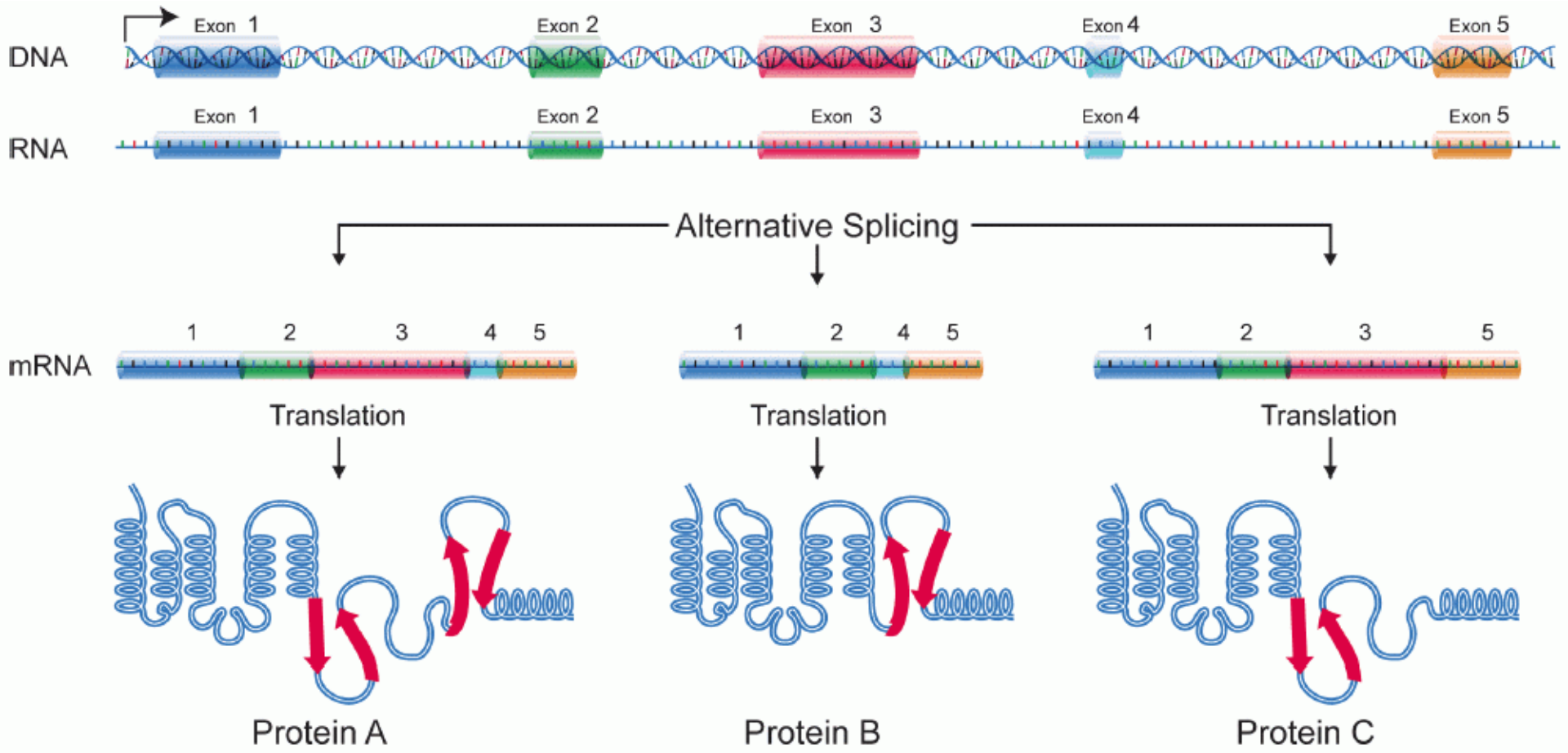
Second letter

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

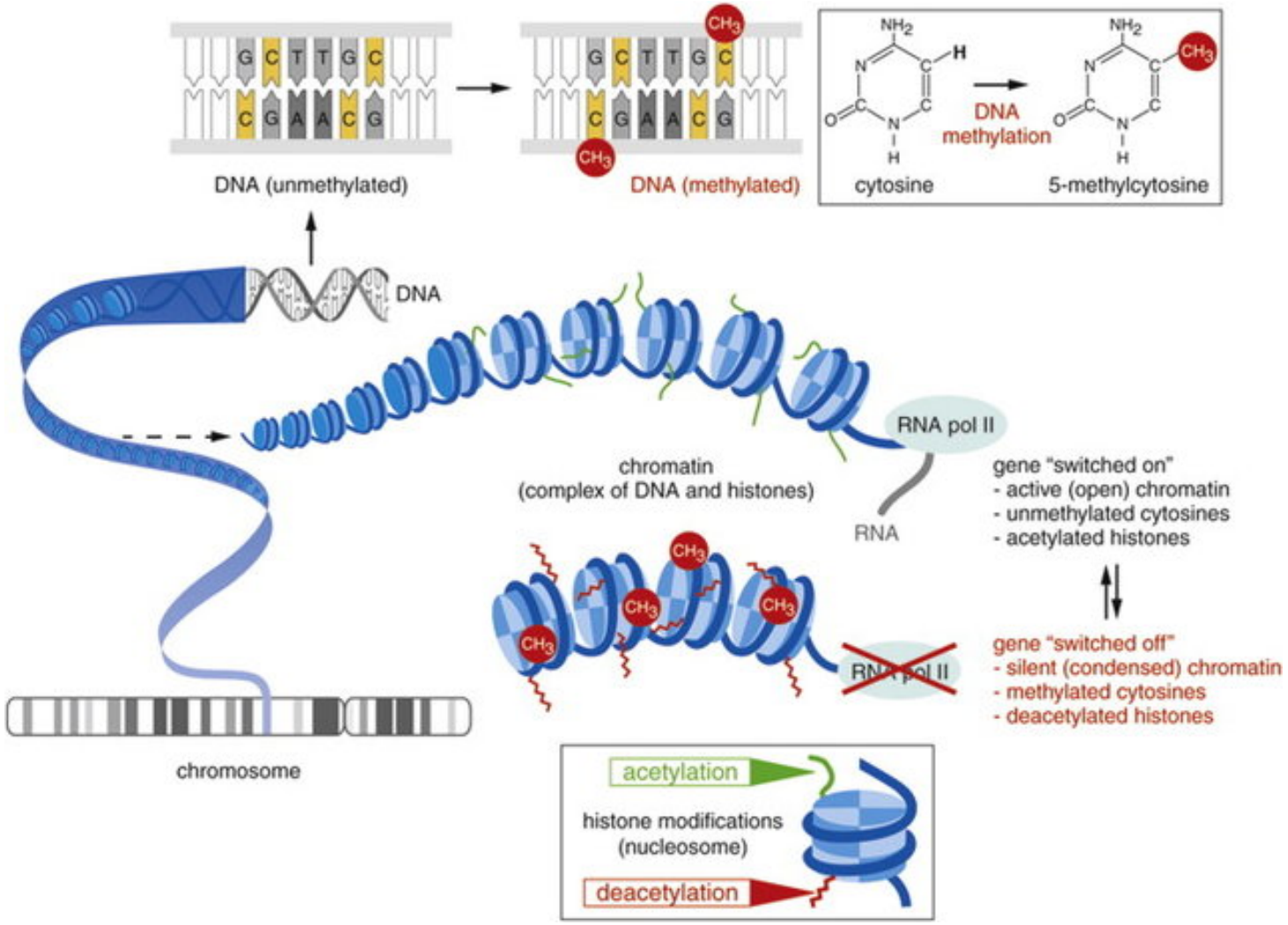
Post Transcription Modification of RNA

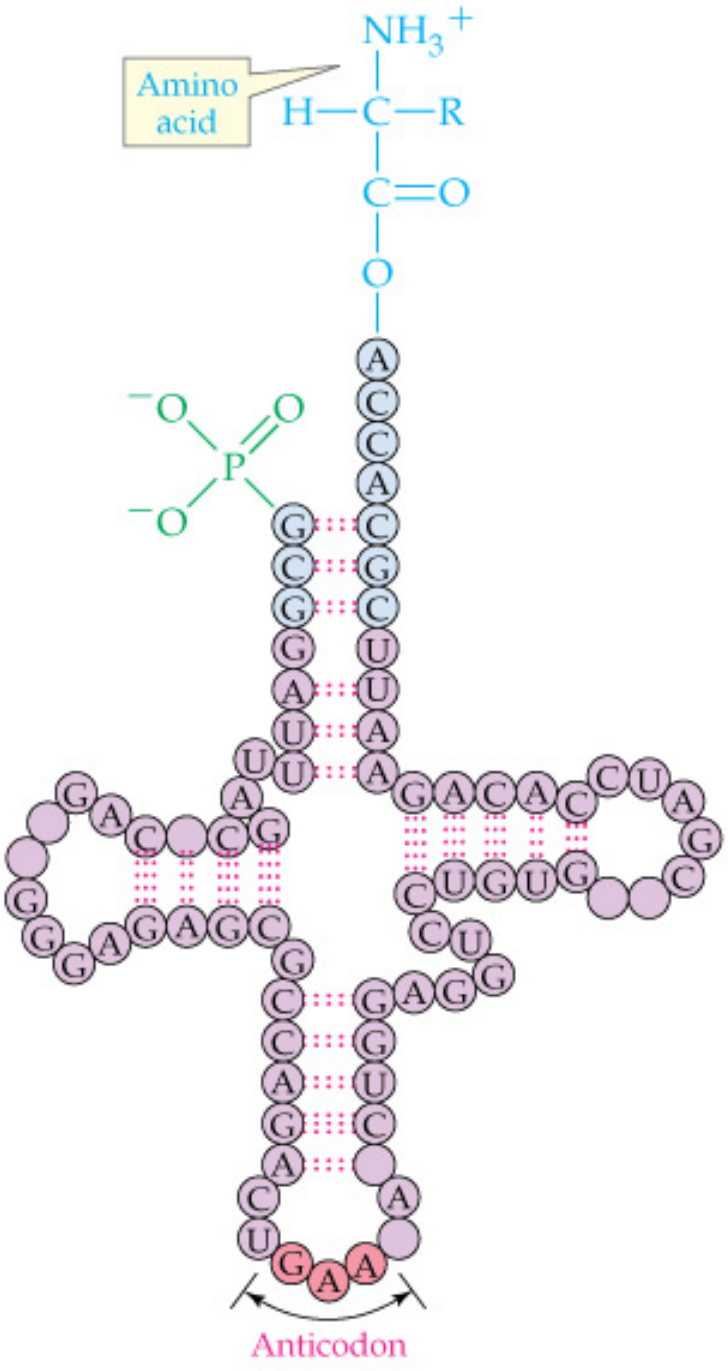
1. RNA capping
2. PolyA tail
3. Splicing



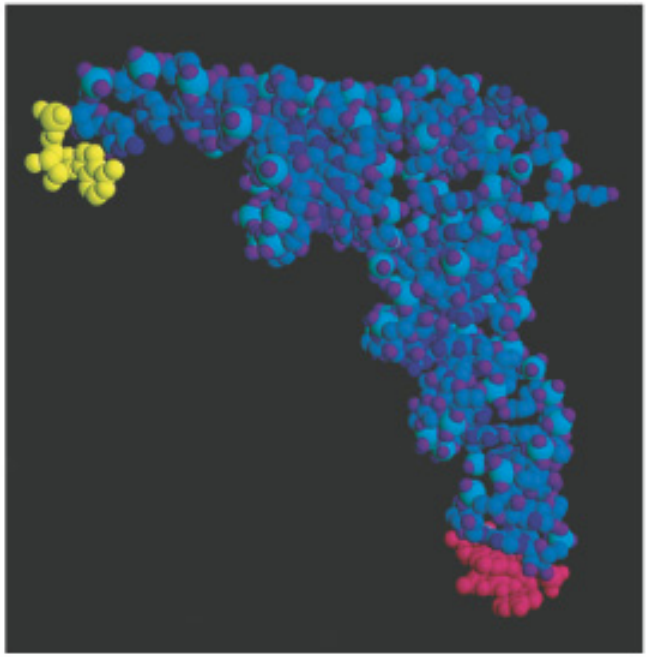

DNA
Exon 1
Exon 2
Exon 3
Exon 4
Exon 5
RNA
Exon 1
Exon 2
Exon 3
Exon 4
Exon 5
Alternative Splicing
mRNA
1 2 3 4 5
1 2 4 5
1 2 3 5
Translation
Translation
Translation
Protein A
Protein B
Protein C

DNA Methylation and Histone Acetylation

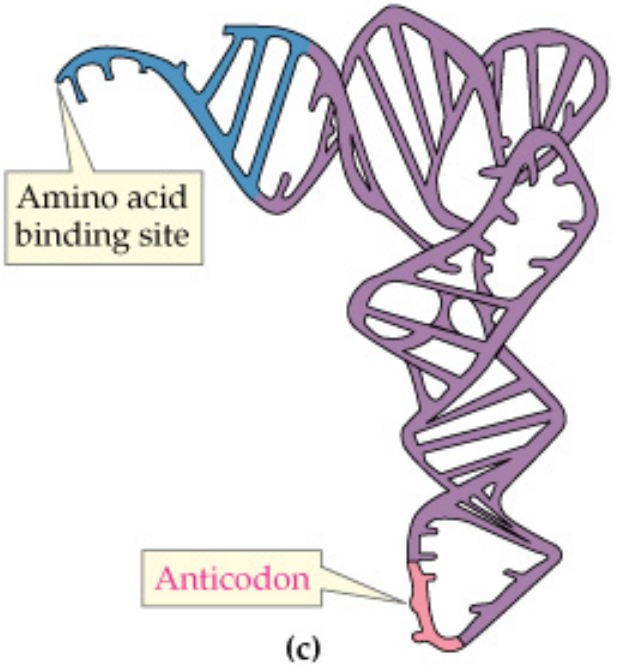



Amino acid
NH_3^+
H—C—R
C=O
O
^-O
O
P
^-O
Anticodon

(a)

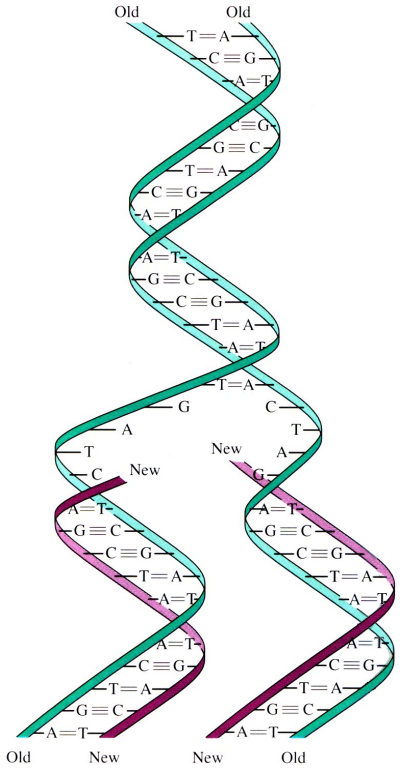


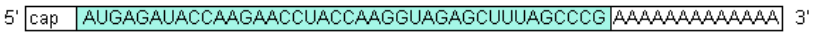
(b)


Amino acid binding site
Anticodon

(c)

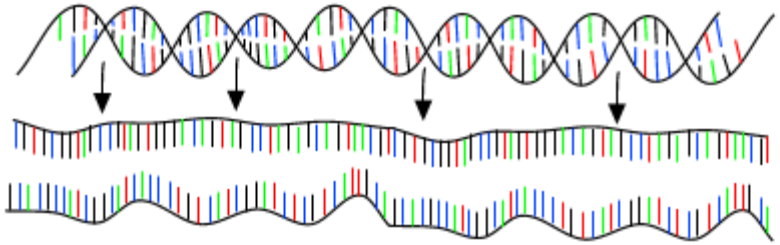
Self-Assembly Process in Nature





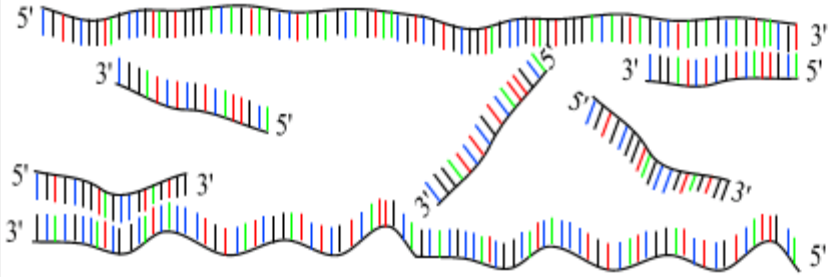
PCR : Polymerase Chain Reaction

30 - 40 cycles of 3 steps :



Step 1 : denaturation

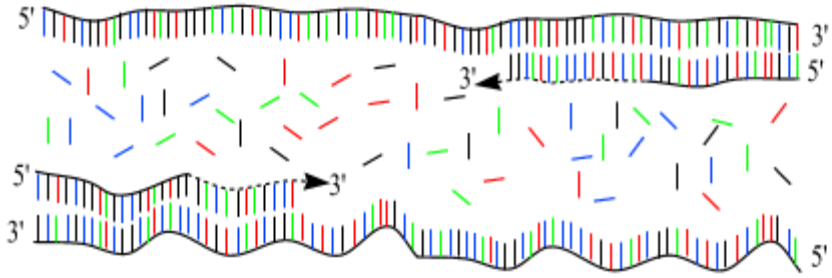
1 minut 94 °C



Step 2 : annealing

45 seconds 54 °C

forward and reverse primers !!!

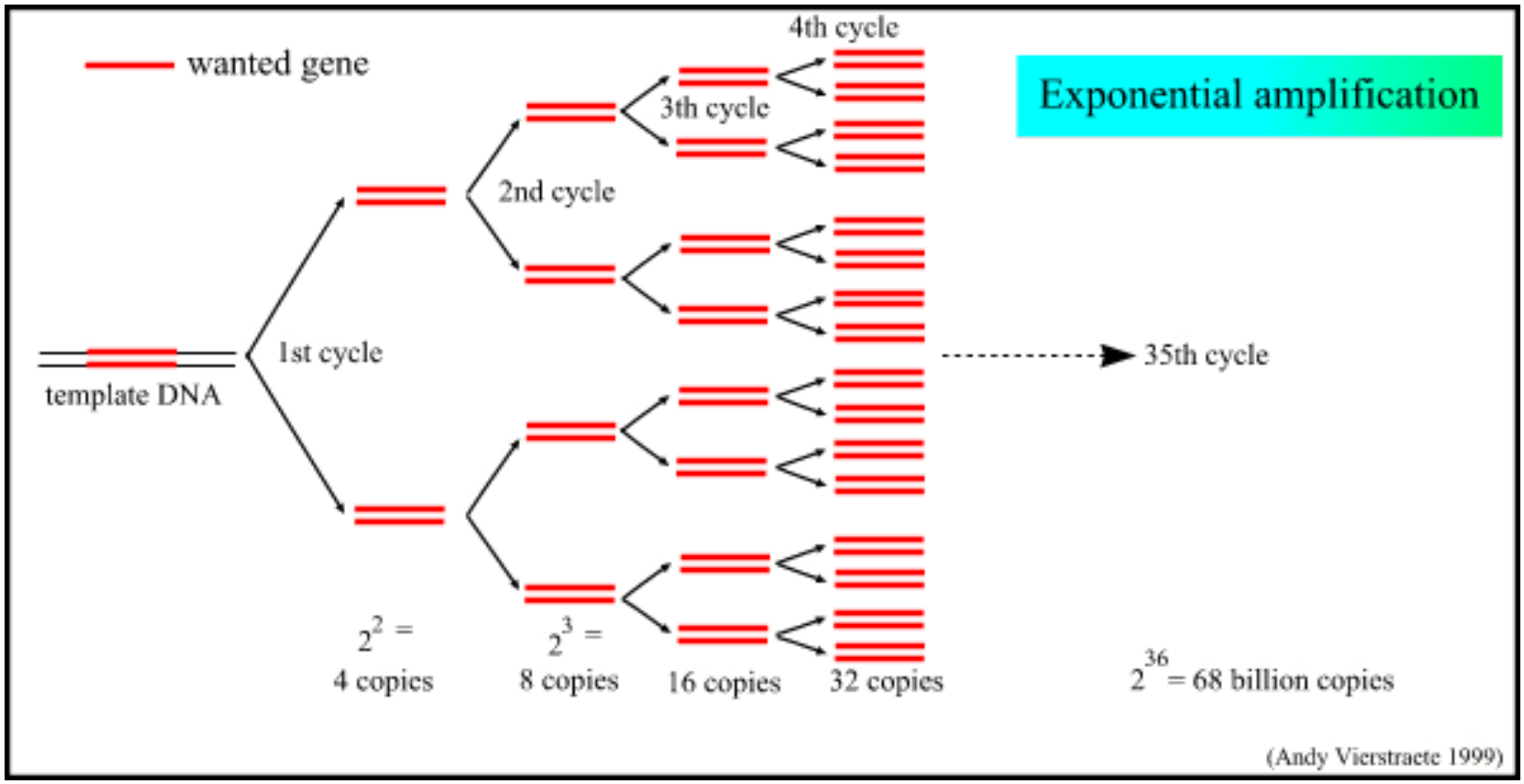


Step 3 : extension

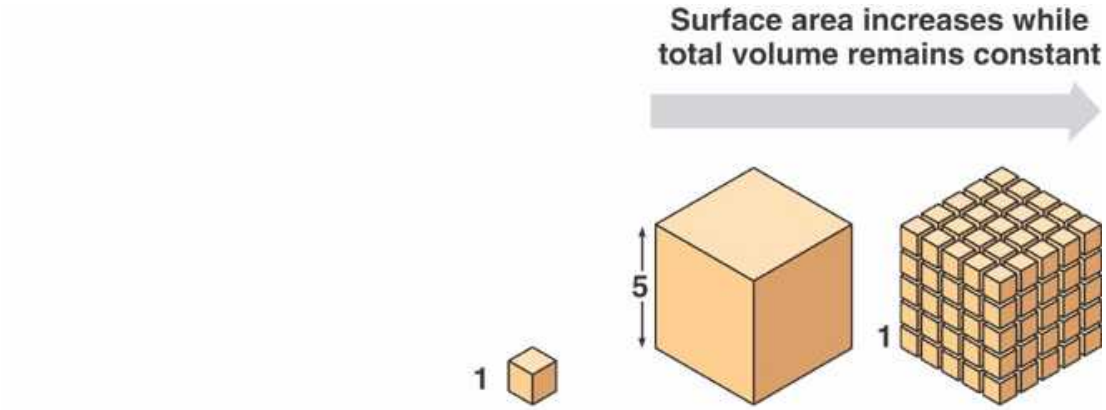
2 minutes 72 °C

only dNTP's

(Andy Vierstraete 1999)


wanted gene
Exponential amplification
4th cycle
3th cycle
2nd cycle
1st cycle
template DNA
35th cycle
2^2 =
4 copies
2^3 =
8 copies
16 copies
32 copies
2^{36} = 68 billion copies
(Andy Vierstraete 1999)

Surface to Volume Ratio



Total surface area (height × width × number of sides × number of boxes)	6	150	750
Total volume (height × width × length × number of boxes)	1	125	125
Surface-to-volume ratio (surface area / volume)	6	1.2	6

Surface Energy

One face surface energy: γ
27 cube: 27 x 6 γ
3 x 9 cube line: 114 γ
3 x (3x3) square: 90 γ
3 x 3 x 3 cube: 54 γ

DLVO Theory

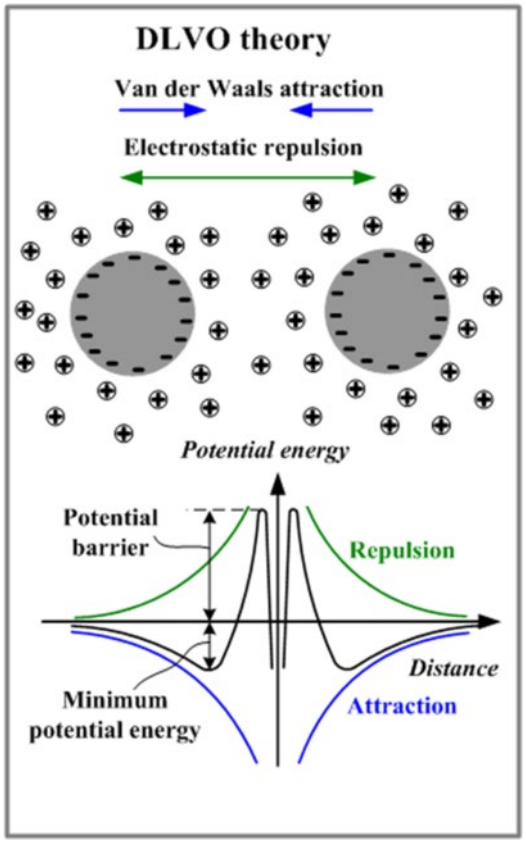
$$V_T = V_A + V_R + V_S$$

$$V_A = -A/(12\ \pi\ D^2)$$

A is the Hamaker constant and D is the particle separation

$$V_R = 2\ \pi\ \varepsilon\ a\ \xi^2\ \exp(-\ \kappa D)$$

a is the particle radius, π is the solvent permeability,
κ is a function of the ionic composition and ξ is the zeta potential

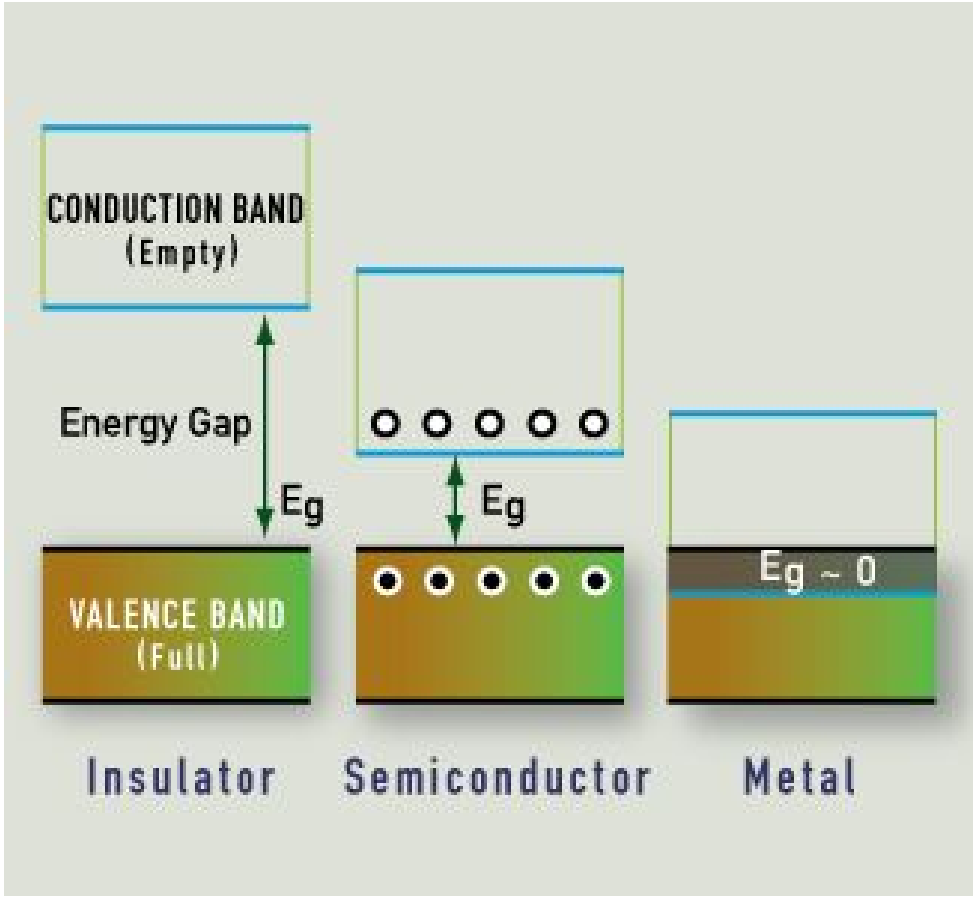


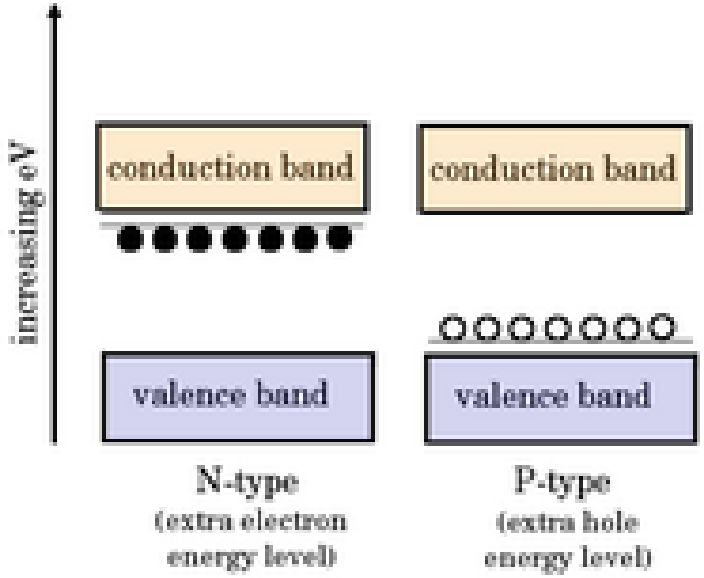
$$\omega = \omega_{el} + \omega_{vdW}$$

$$\omega = 64RTc_{\infty}{\gamma_0}^2 \frac{1}{\kappa} e^{-\kappa d} - \frac{A}{12\pi d^2}$$

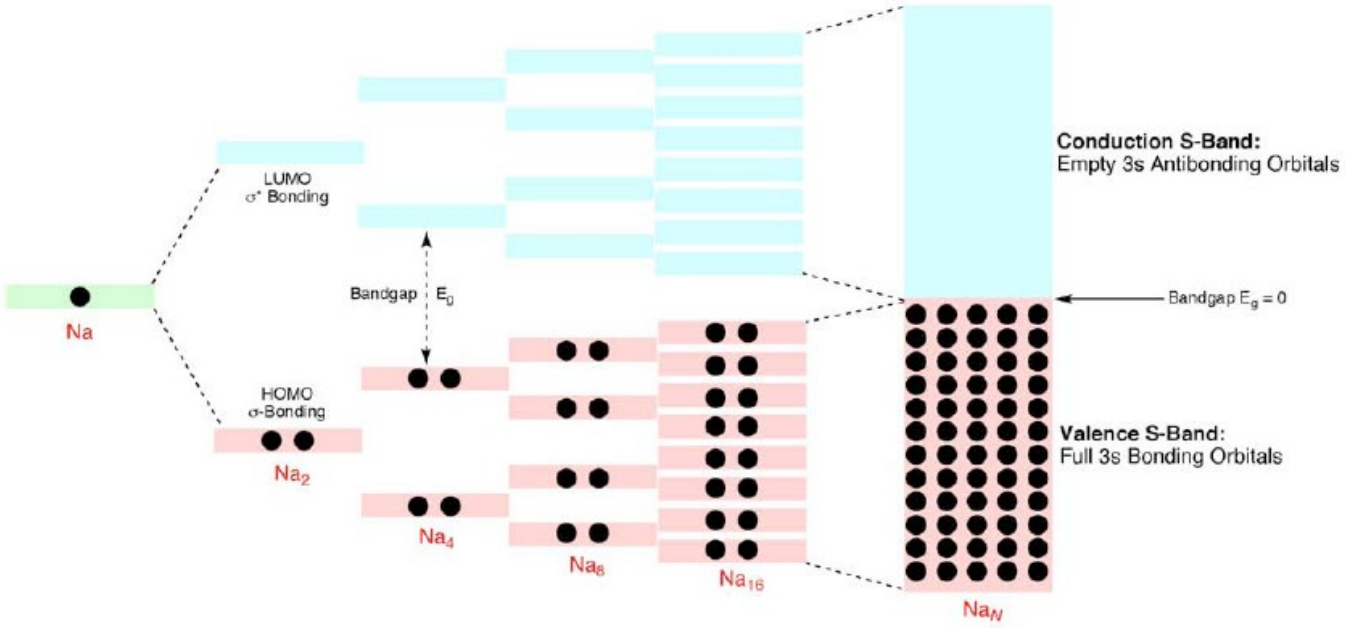
$$\omega = 64RTc_{\infty}{\gamma_0}^2 \sqrt{\frac{RT\varepsilon}{F^2 \sum z^2 c_{\infty}}} e^{-\sqrt{\frac{F^2 \sum z^2 c_{\infty}}{RT\varepsilon}} d} - \frac{A}{12\pi d^2}$$

Bandgap


CONDUCTION BAND
(Empty)
Energy Gap
Eg
Eg
VALENCE BAND
(Full)
Eg ~ 0
Insulator
Semiconductor
Metal


increasing eV
conduction band
conduction band
valence band
valence band
N-type
(extra electron
energy level)
P-type
(extra hole
energy level)

Bandgap


LUMO
σ* Bonding
HOMO
σ-Bonding
Na
Na2
Na4
Na8
Na16
NaN
Bandgap E0
Conduction S-Band:
Empty 3s Antibonding Orbitals
Bandgap Eg = 0
Valence S-Band:
Full 3s Bonding Orbitals

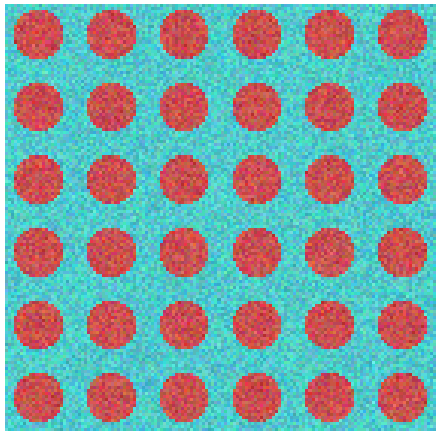
Bloch wave

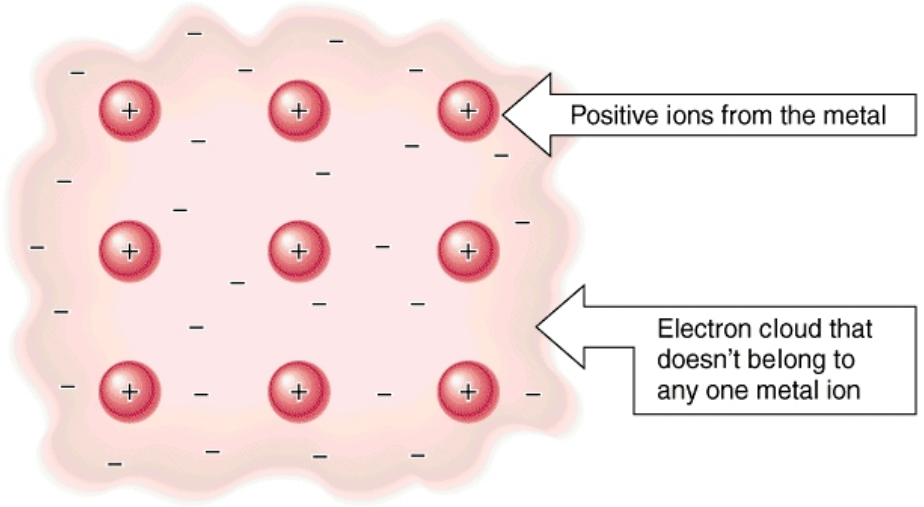
$$\psi_{n\mathbf{k}}(\mathbf{r}) = e^{i\mathbf{k}\cdot\mathbf{r}} u_{n\mathbf{k}}(\mathbf{r})$$

A **Bloch wave** or **Bloch state**, named after Felix Bloch, is the wavefunction of a particle (usually, an electron) placed in a periodic potential.

$$\epsilon n(\mathbf{k}) = \epsilon n(\mathbf{k} + \mathbf{K}),$$

Electron Sea

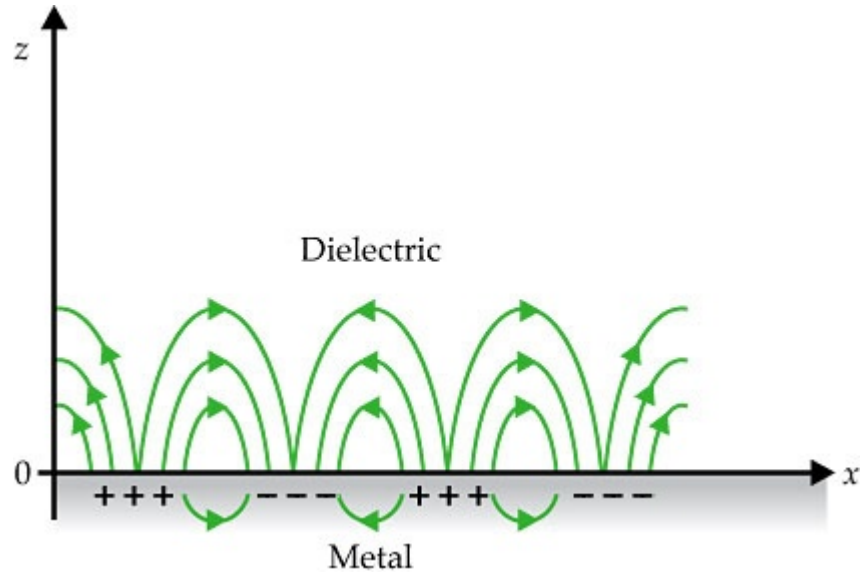




$$m\frac{d^2\delta x}{dt^2} = e\,E_x = -m\omega_p{}^2\,\delta x,$$

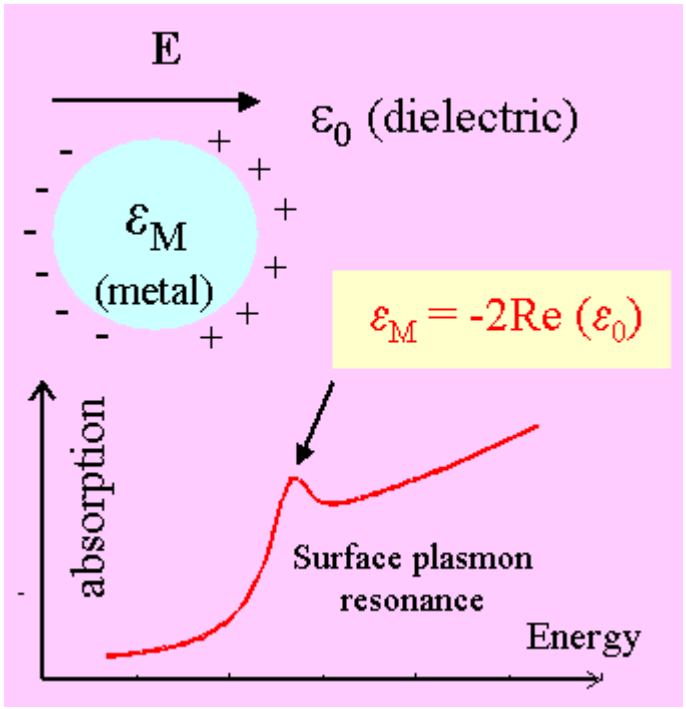
$$\omega_p{}^2 = \frac{n\,e^2}{\epsilon_0\,m},$$

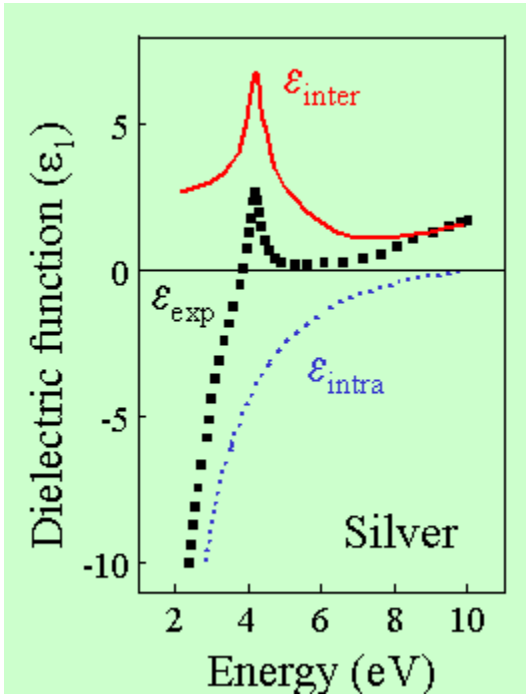
Surface Plasmonon



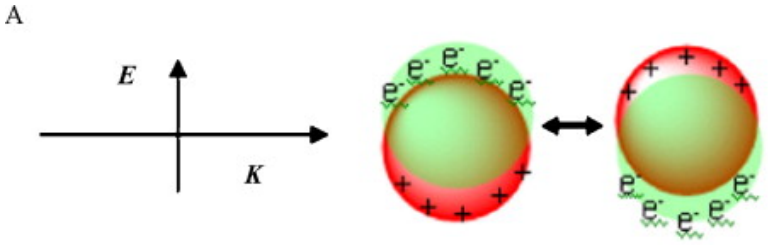
$$\varepsilon_m = 1 - \frac{\omega_p^2}{\omega^2}$$

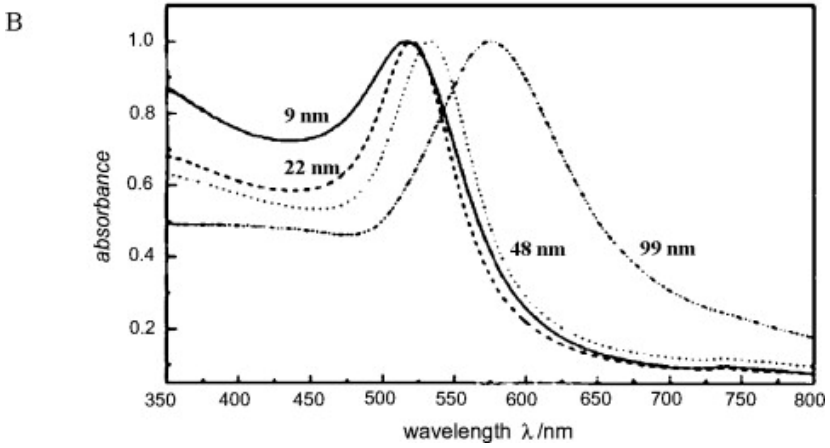
$$\varepsilon_{eff} = \varepsilon_0 + 3N\varepsilon_0 \frac{\varepsilon_M - \varepsilon_0}{\varepsilon_M + 2\varepsilon_0}$$

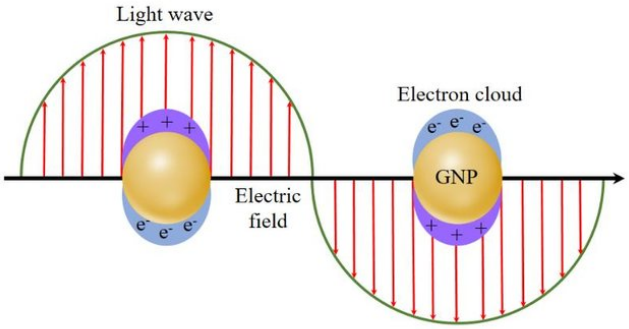




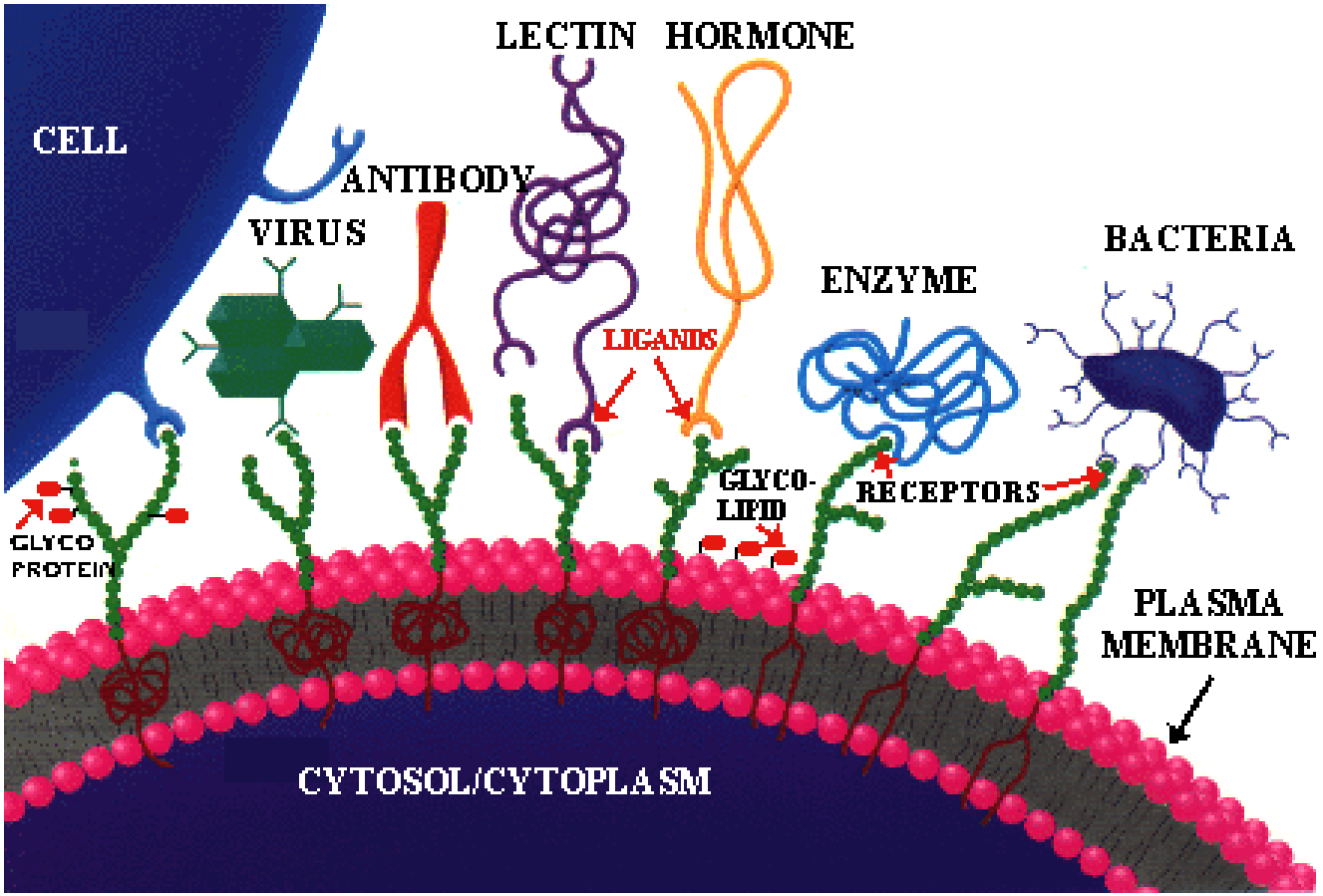
Localized Surface Plasomon



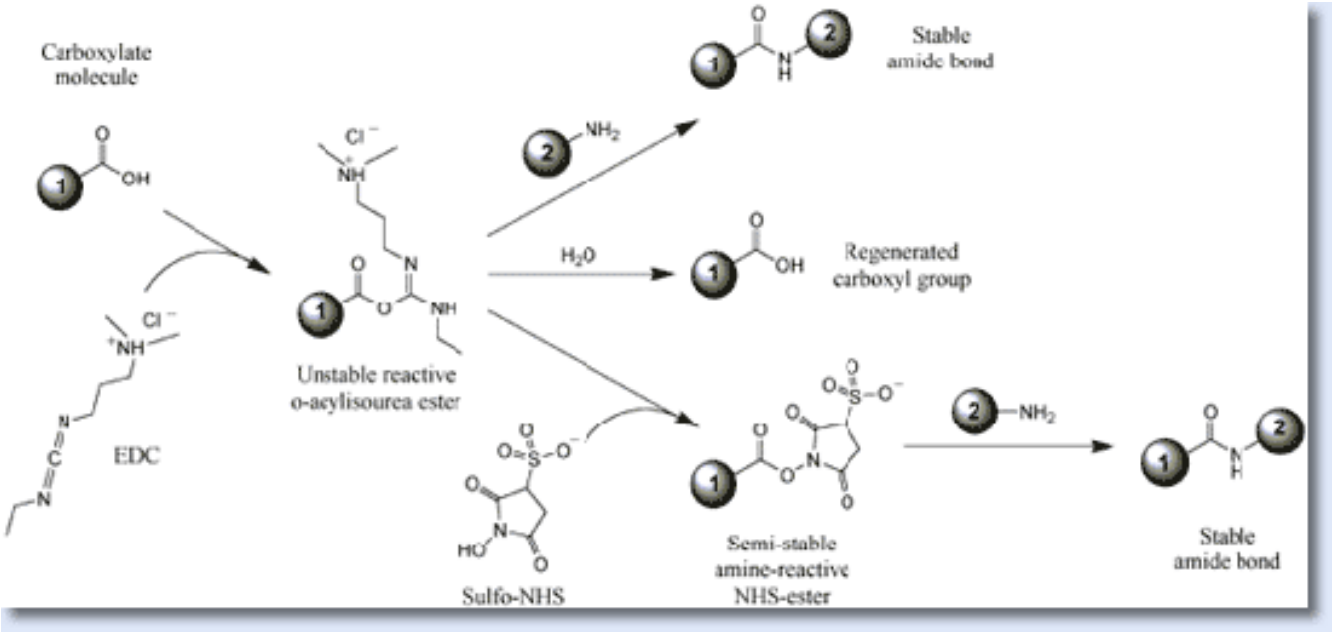


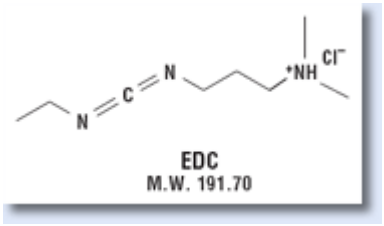


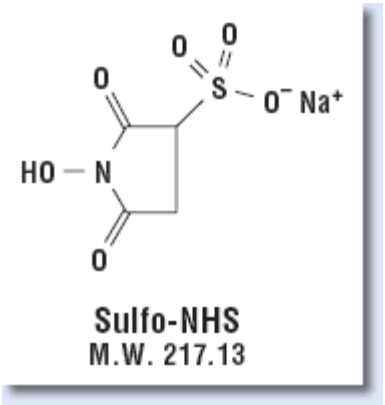
Molecular Recognition



Carboxyl Presenting Surfaces

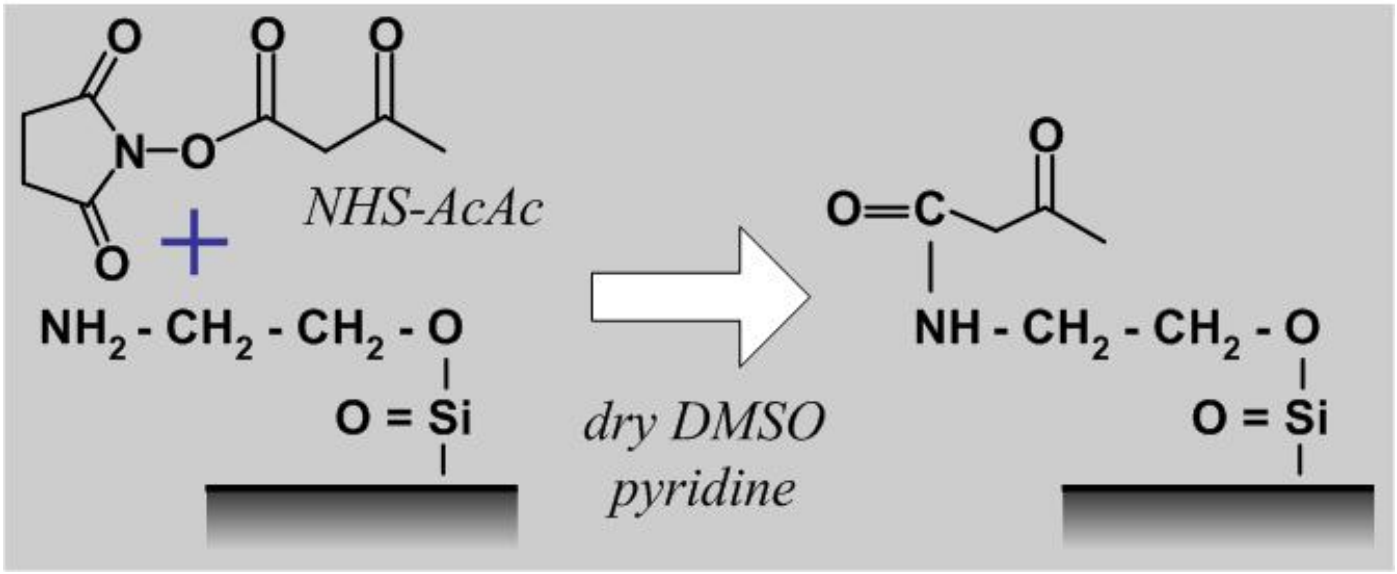




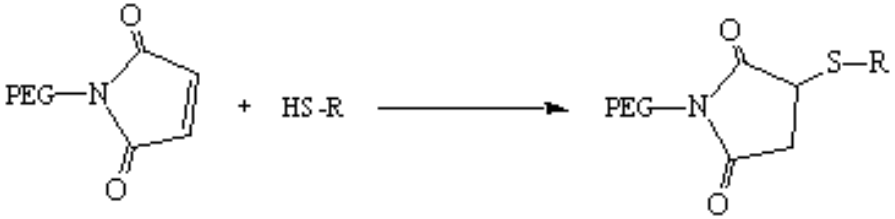


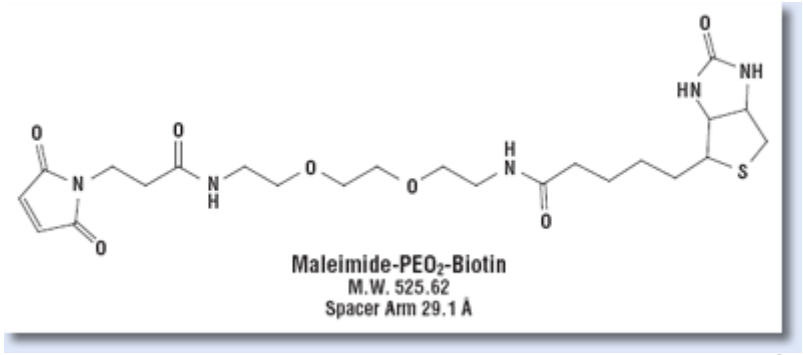
EDC (1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide Hydrochloride)

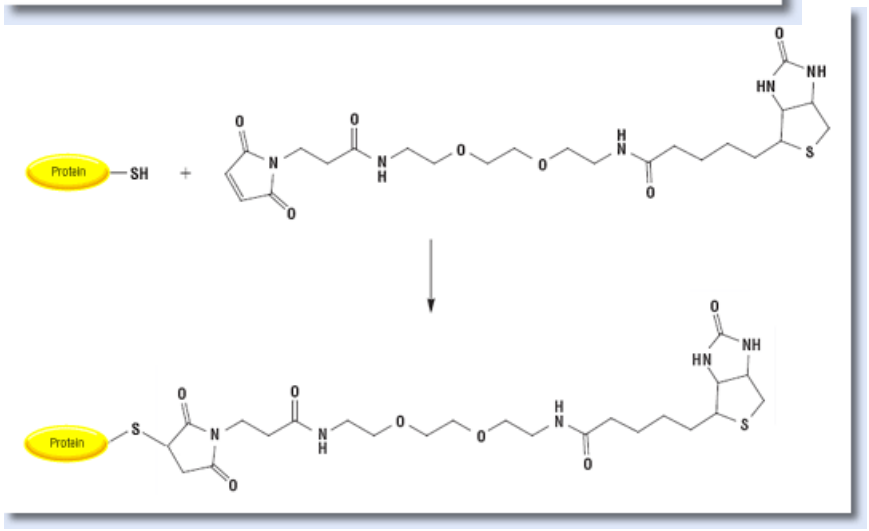
Amine Presenting Surface

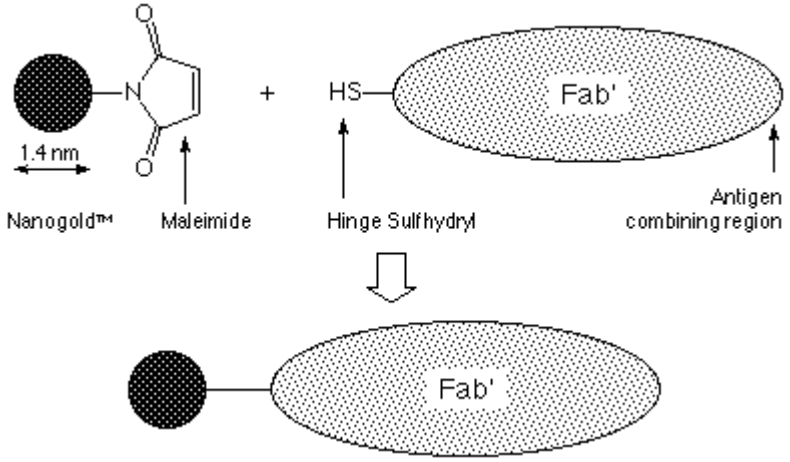


Sulfhydryl Labeling

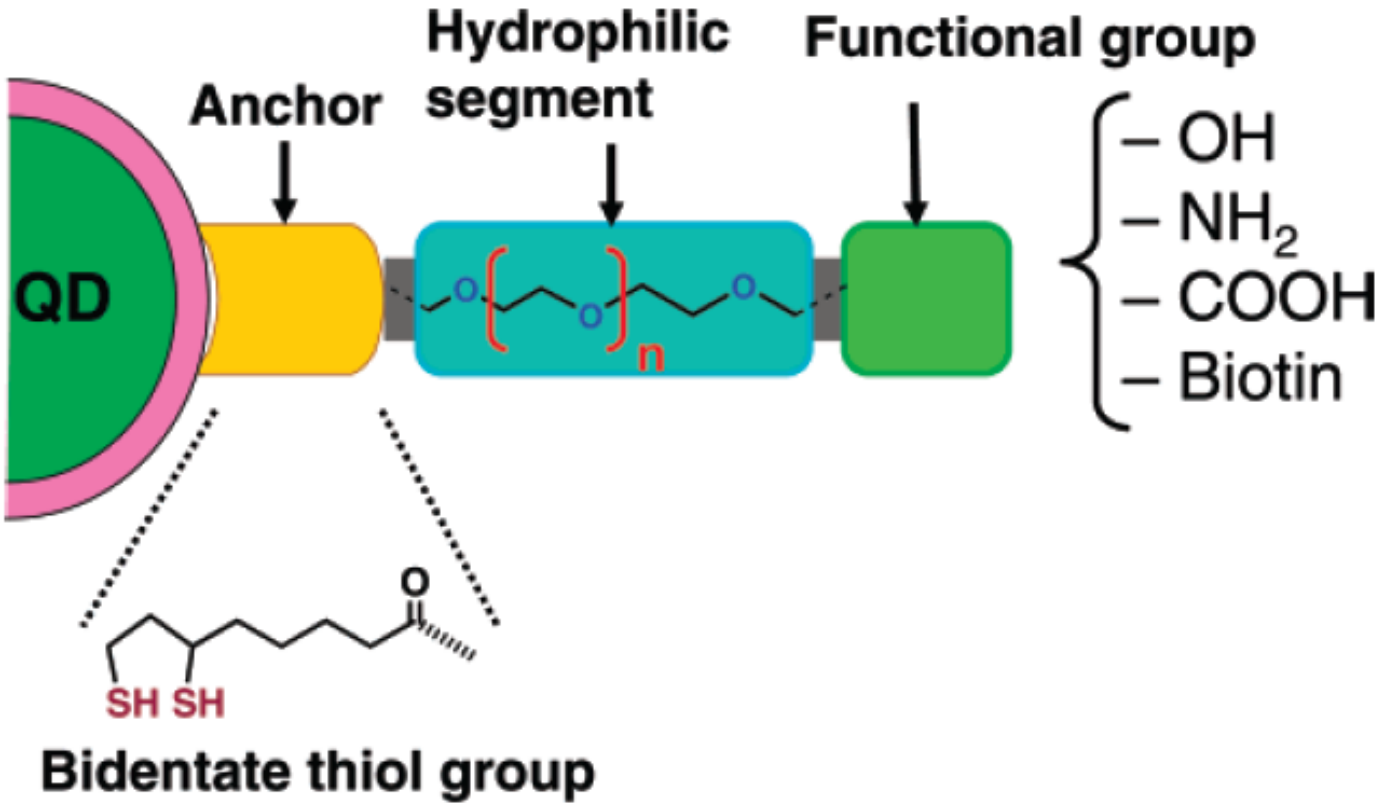




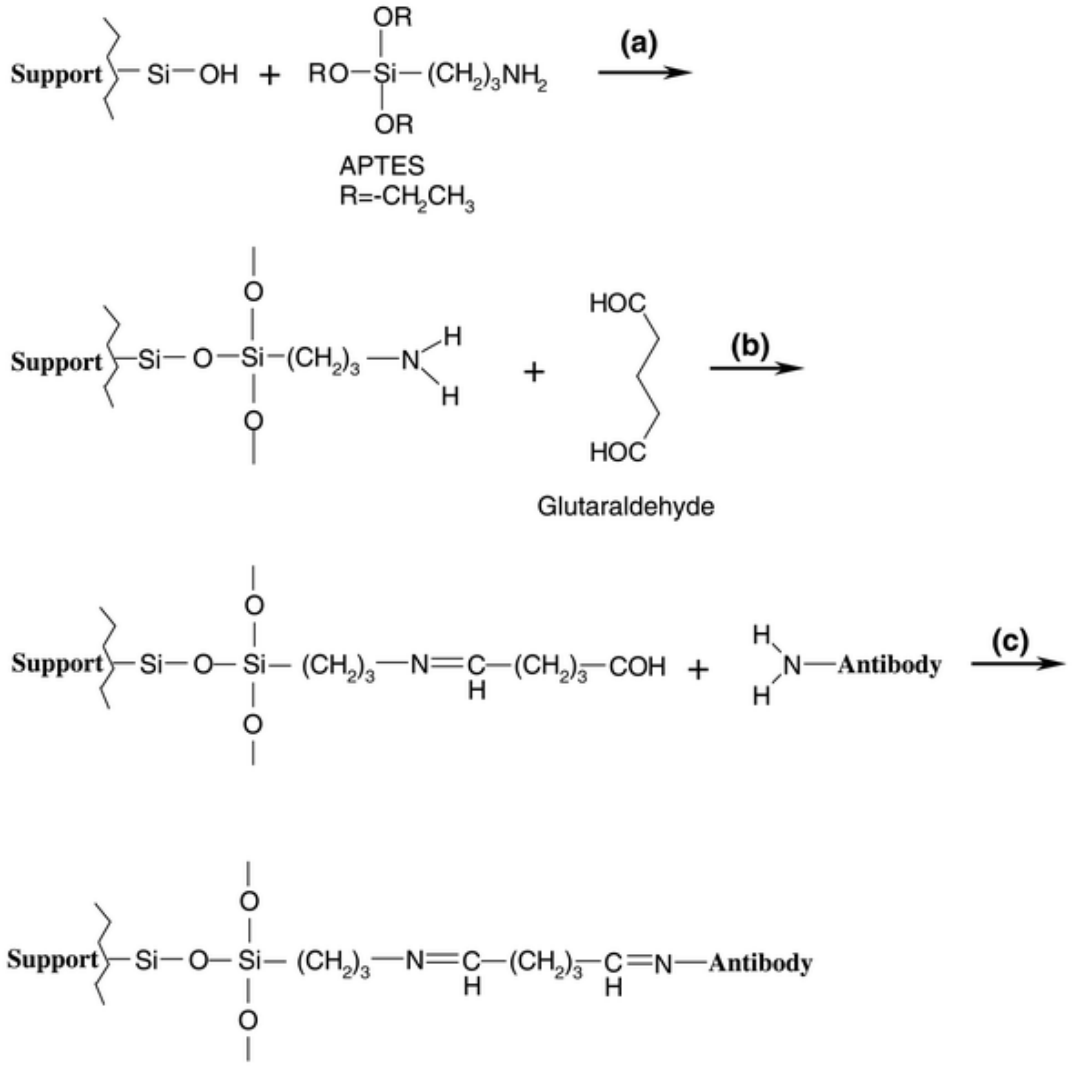




Scheme 1. Modular Design of Hydrophilic Ligands with Terminal Functional Groups Used in This Study



Silica Modification



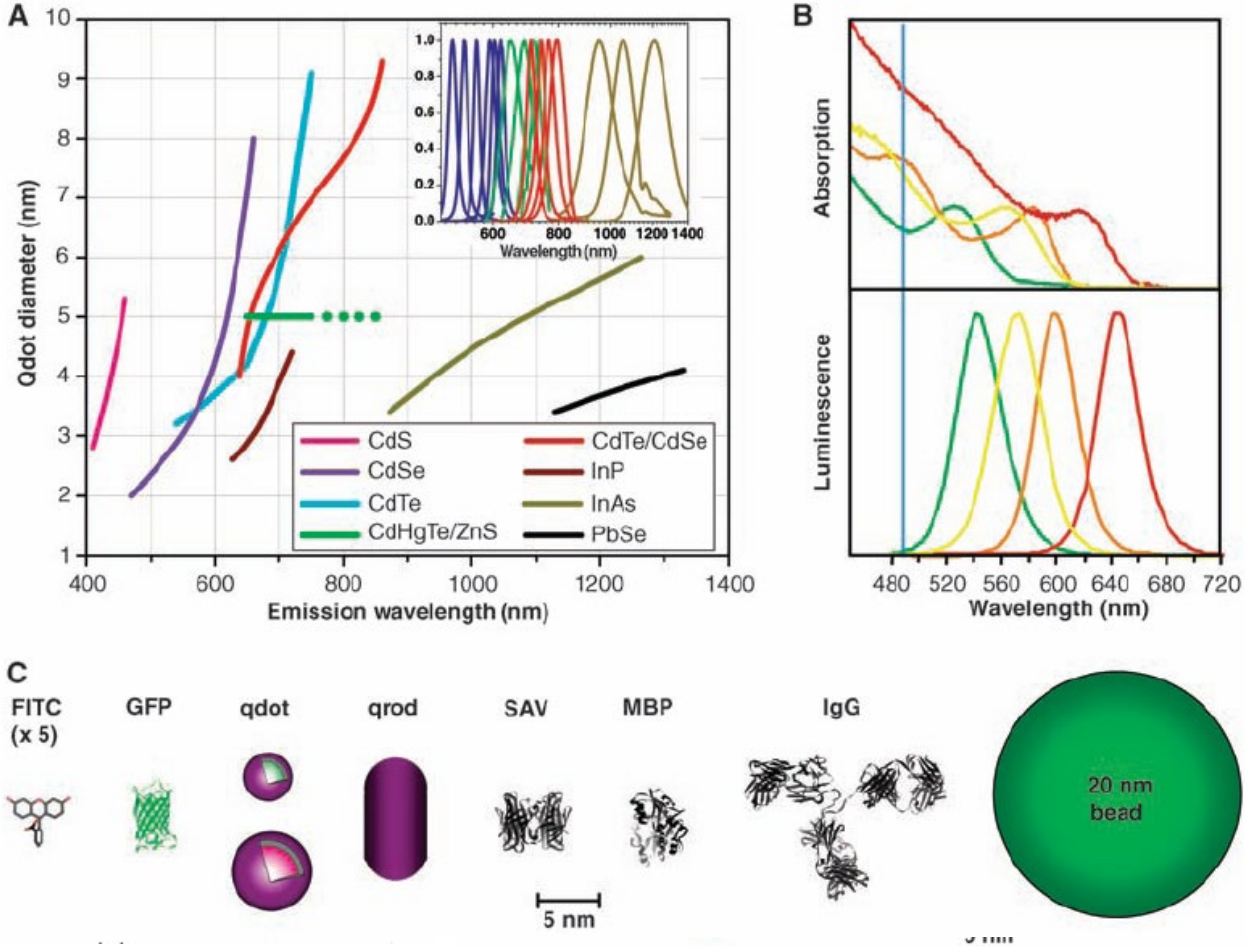


Fig. 1. (**A**) Emission maxima and sizes of quantum dots of different composition. Quantum dots can be synthesized from various types of semiconductor materials (II-VI: CdS, CdSe, CdTe…; III-V: InP, InAs…; IV-VI: PbSe…) characterized by different bulk band gap energies. The curves represent experimental data from the literature on the dependence of peak emission wavelength on qdot diameter. The range of emission wavelength is 400 to 1350 nm, with size varying from 2 to 9.5 nm (organic passivation/solubilization layer not included). All spectra are typically around 30 to 50 nm (full width at half maximum). Inset: Representative emission spectra for some materials. Data are from (*12*, *18*, *27*, *76*–*82*). Data for CdHgTe/ZnS have been extrapolated to the maximum emission wavelength obtained in our group. (**B**) Absorption (upper curves) and emission (lower curves) spectra of four CdSe/ZnS qdot samples. The blue vertical line indicates the 488-nm line of an argon-ion laser, which can be used to efficiently excite all four types of qdots simultaneously. [Adapted from (*28*)] (**C**) Size comparison of qdots and comparable objects. FITC, fluorescein isothiocyanate; GFP, green fluorescent protein; qdot, green (4 nm, top) and red (6.5 nm, bottom) CdSe/ZnS qdot; qrod, rod-shaped qdot (size from Quantum Dot Corp.'s Web site). Three proteins—streptavidin (SAV), maltose binding protein (MBP), and immunoglobulin G (IgG)—have been used for further functionalization of qdots (see text) and add to the final size of the qdot, in conjunction with the solubilization chemistry (Fig. 2).

Colorimetric Detection of DNA

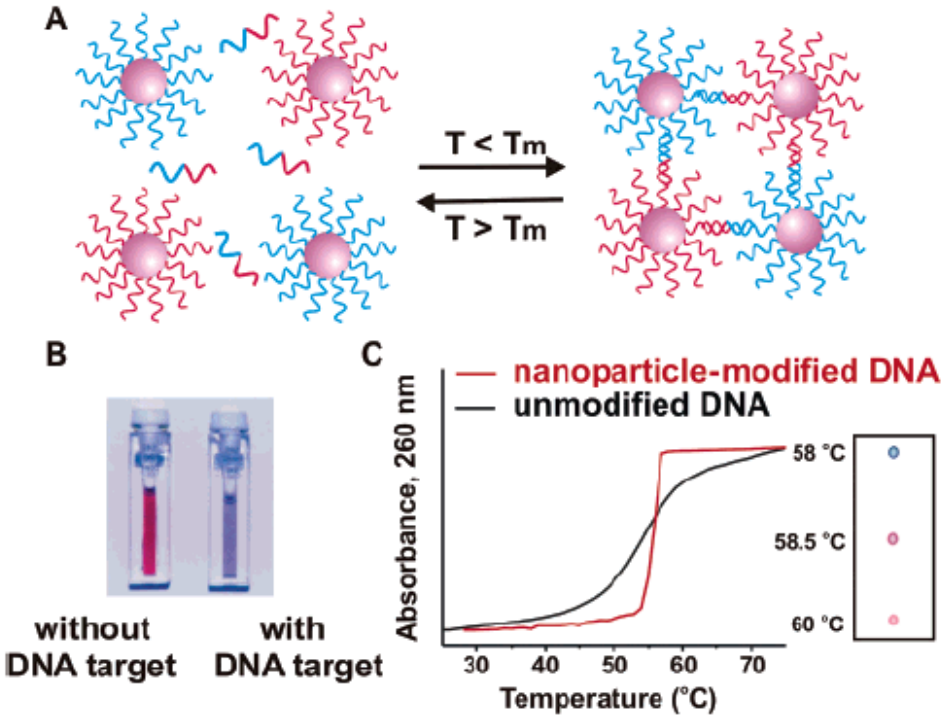
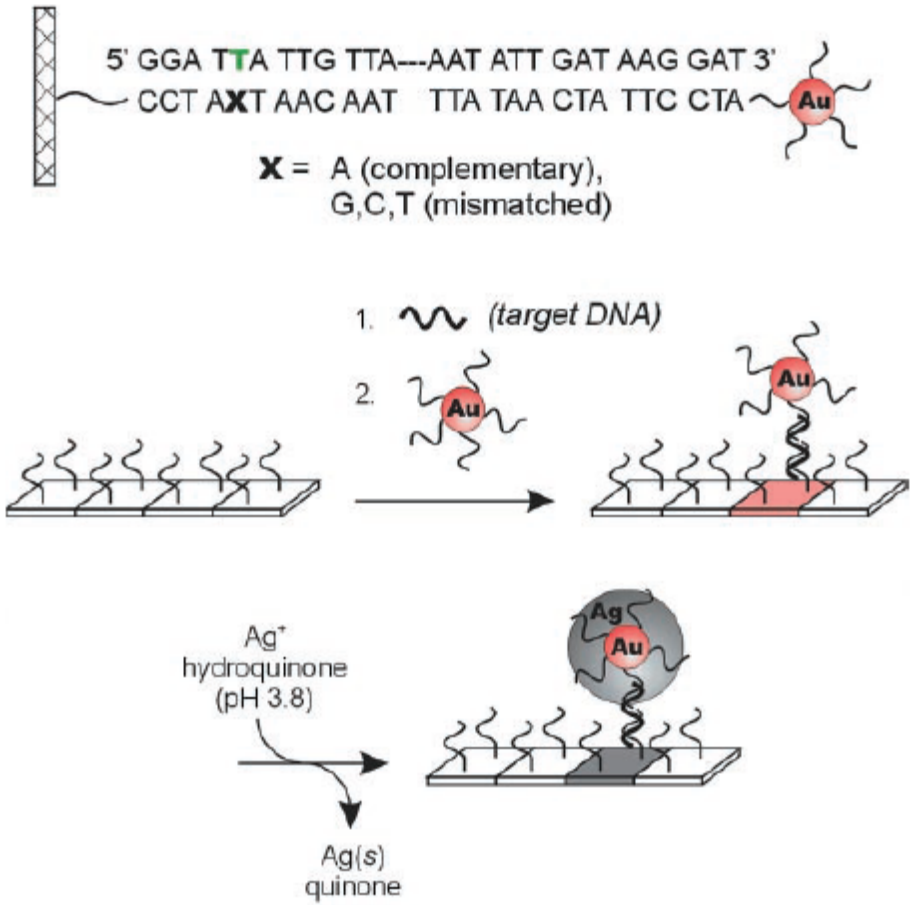


Figure 2. In the presence of complementary target DNA, oligonucleotide-functionalized gold nanoparticles will aggregate (A), resulting in a change of solution color from red to blue (B). The aggregation process can be monitored using UV−vis spectroscopy or simply by spotting the solution on a silica support (C). (Reprinted with permission from *Science* (http://www.aaas.org), ref 29. Copyright 1997 American Association for the Advancement of Science.)

Scanometric DNA Array Detection with Nanoparticle Probes

SCIENCE VOL 289 8 SEPTEMBER 2000

T. Andrew Taton,[1,2] Chad A. Mirkin,[1,2]* Robert L. Letsinger[1]*



50 fM => 0.2 fM

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

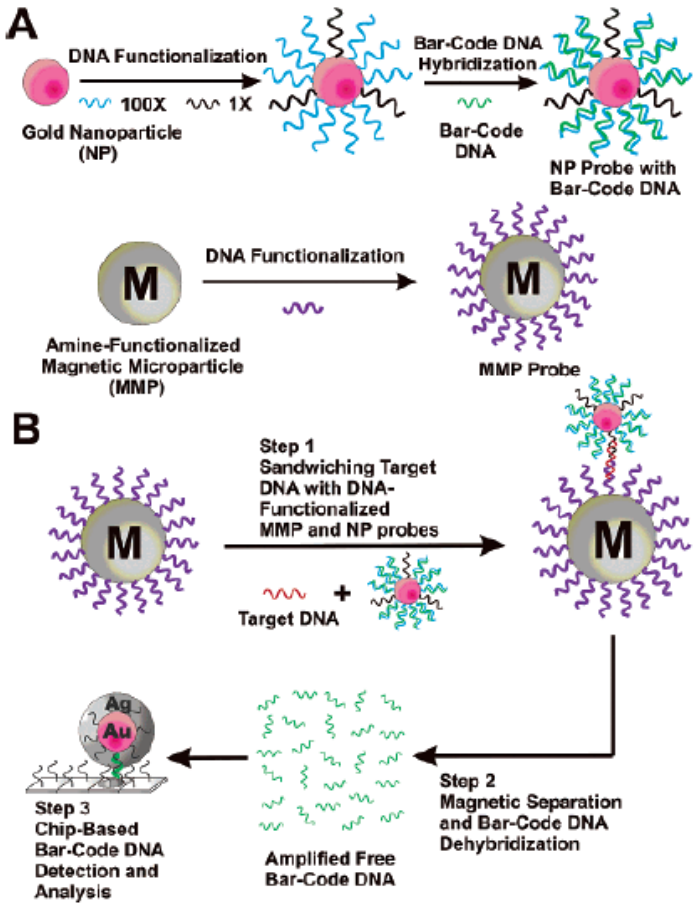


Figure 1. The DNA-BCA assay. (A) Nanoparticle and magnetic microparticle probe preparation. (B) Nanoparticle-based PCR-less DNA amplification scheme.

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†

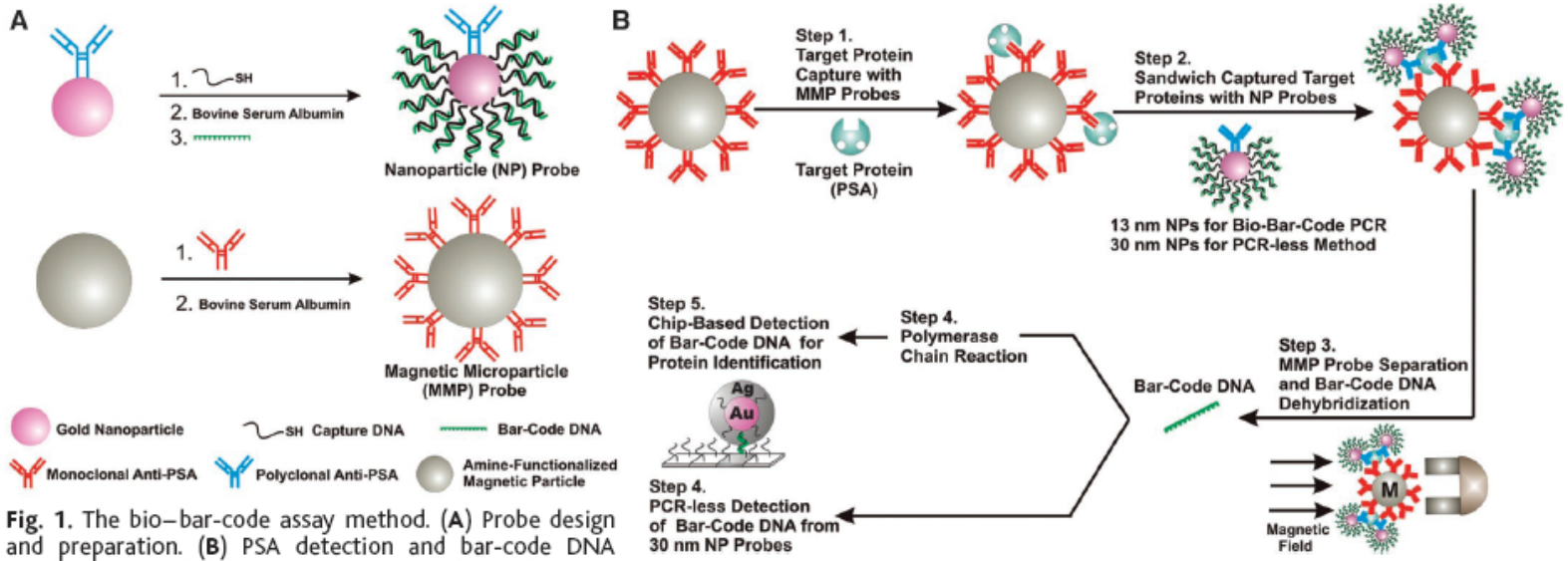
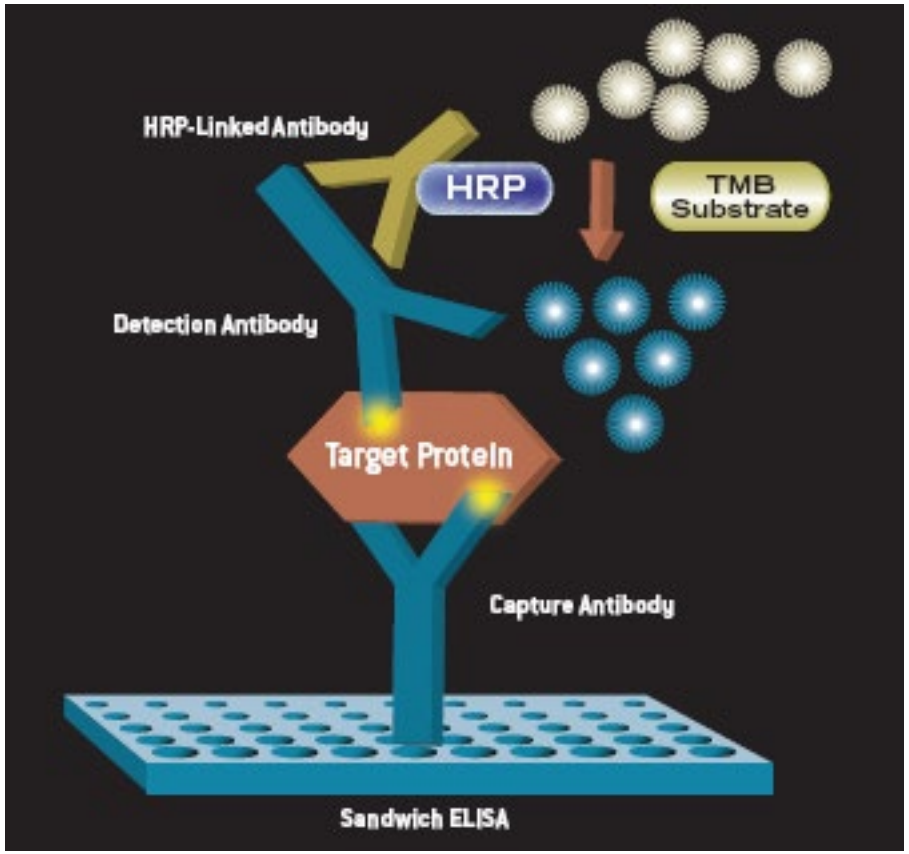


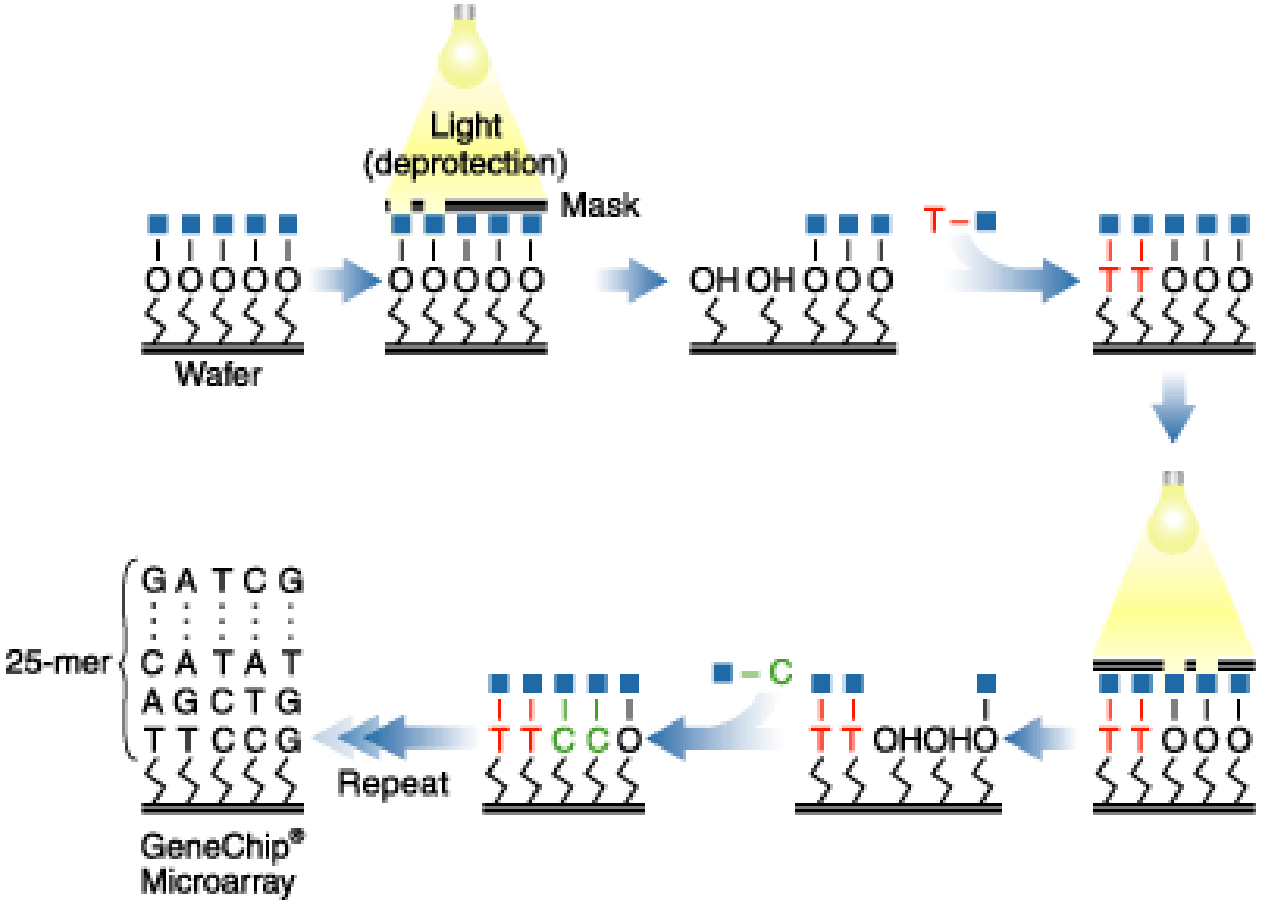
Fig. 1. The bio–bar-code assay method. (**A**) Probe design and preparation. (**B**) PSA detection and bar-code DNA amplification and identification. In a typical PSA-detection experiment, an aqueous dispersion of MMP probes functionalized with mAbs to PSA (50 μl of 3 mg/ml magnetic probe solution) was 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.

Enzyme-Linked ImmunoSorbent Assay (ELISA)

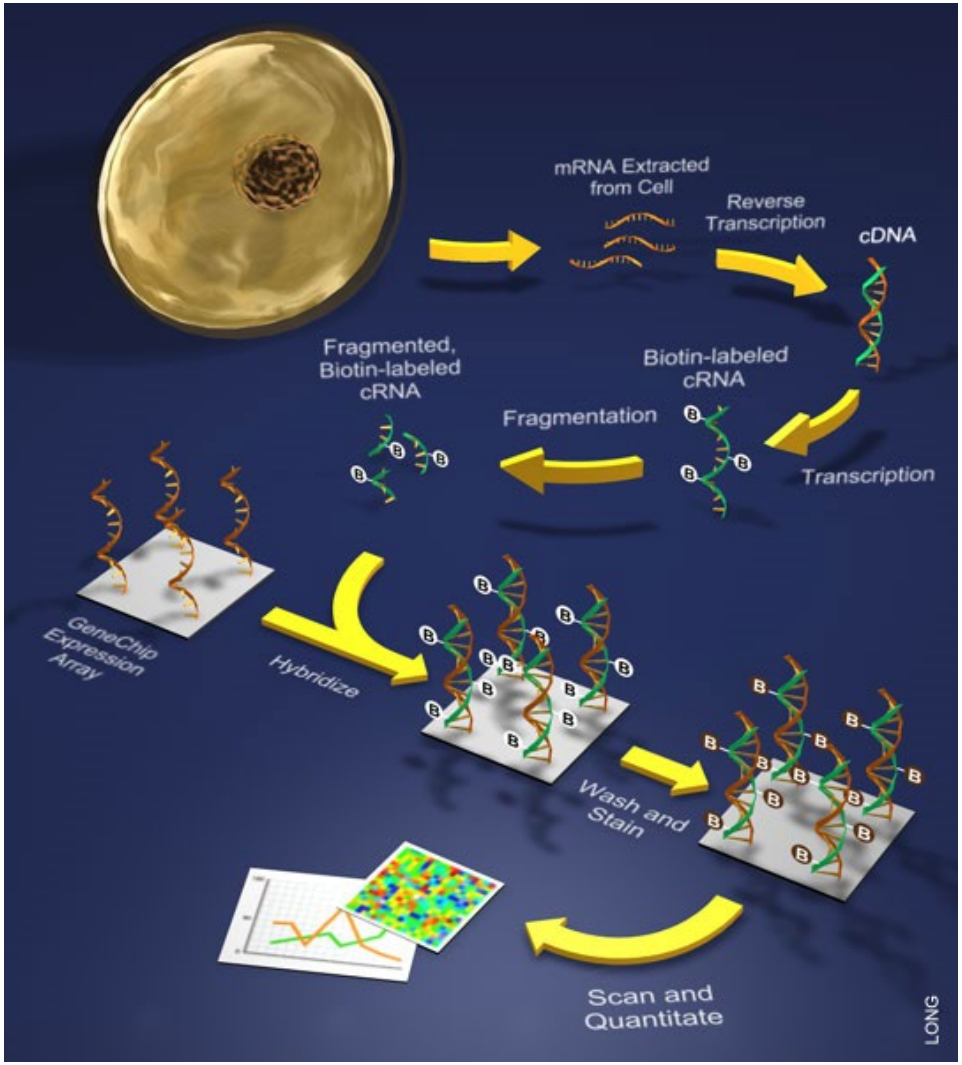


Labeling
BSA/PEG

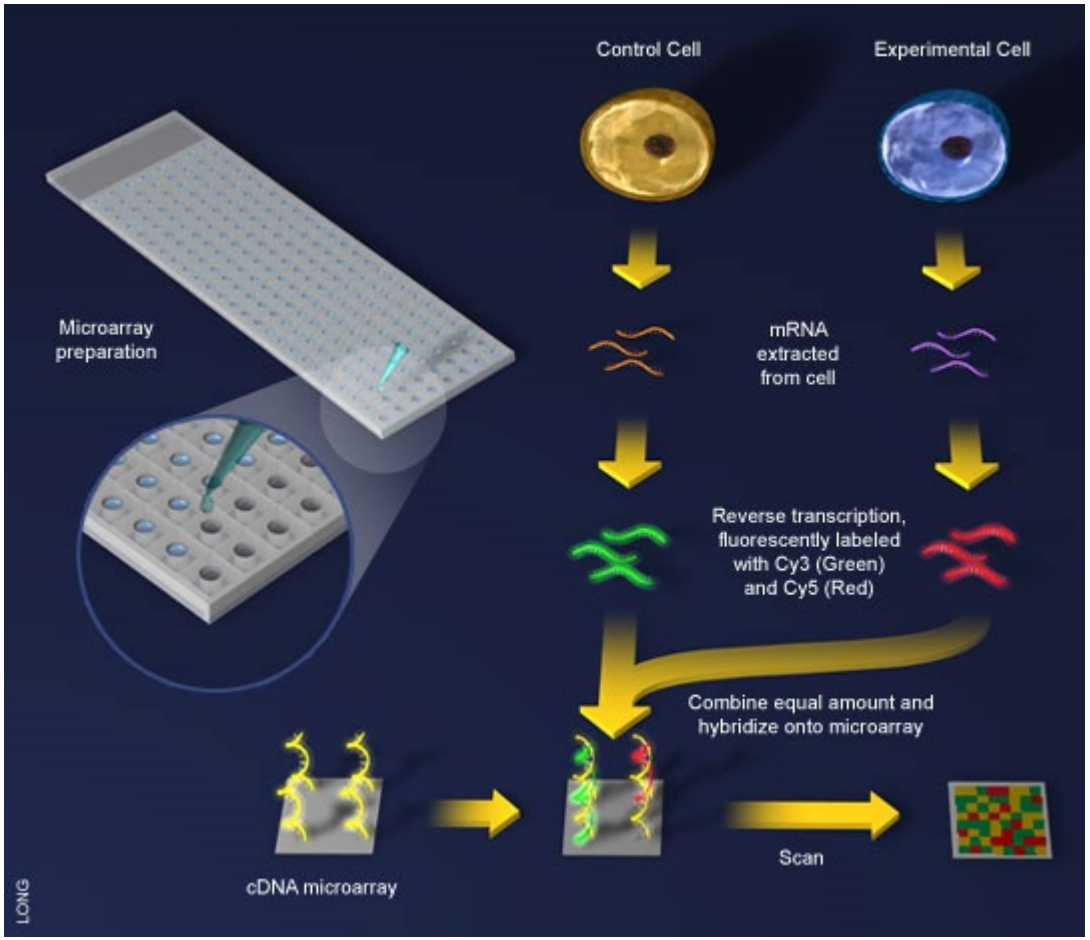
GeneChip


Light
(deprotection)
Mask
Wafer
T
C
Repeat
25-mer
G A T C G
C A T A T
A G C T G
T T C C G
GeneChip®
Microarray

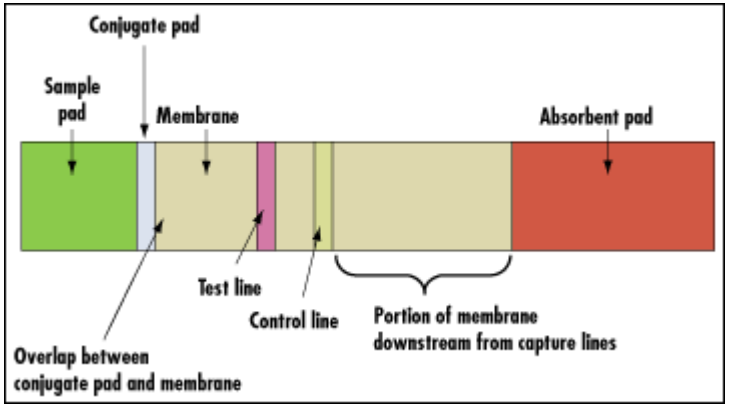
Scheme


mRNA Extracted from Cell
Reverse Transcription
cDNA
Fragmented, Biotin-labeled cRNA
Biotin-labeled cRNA
Fragmentation
Transcription
GeneChip Expression Array
Hybridize
Wash and Stain
Scan and Quantitate
LONG

cDNA Microarray

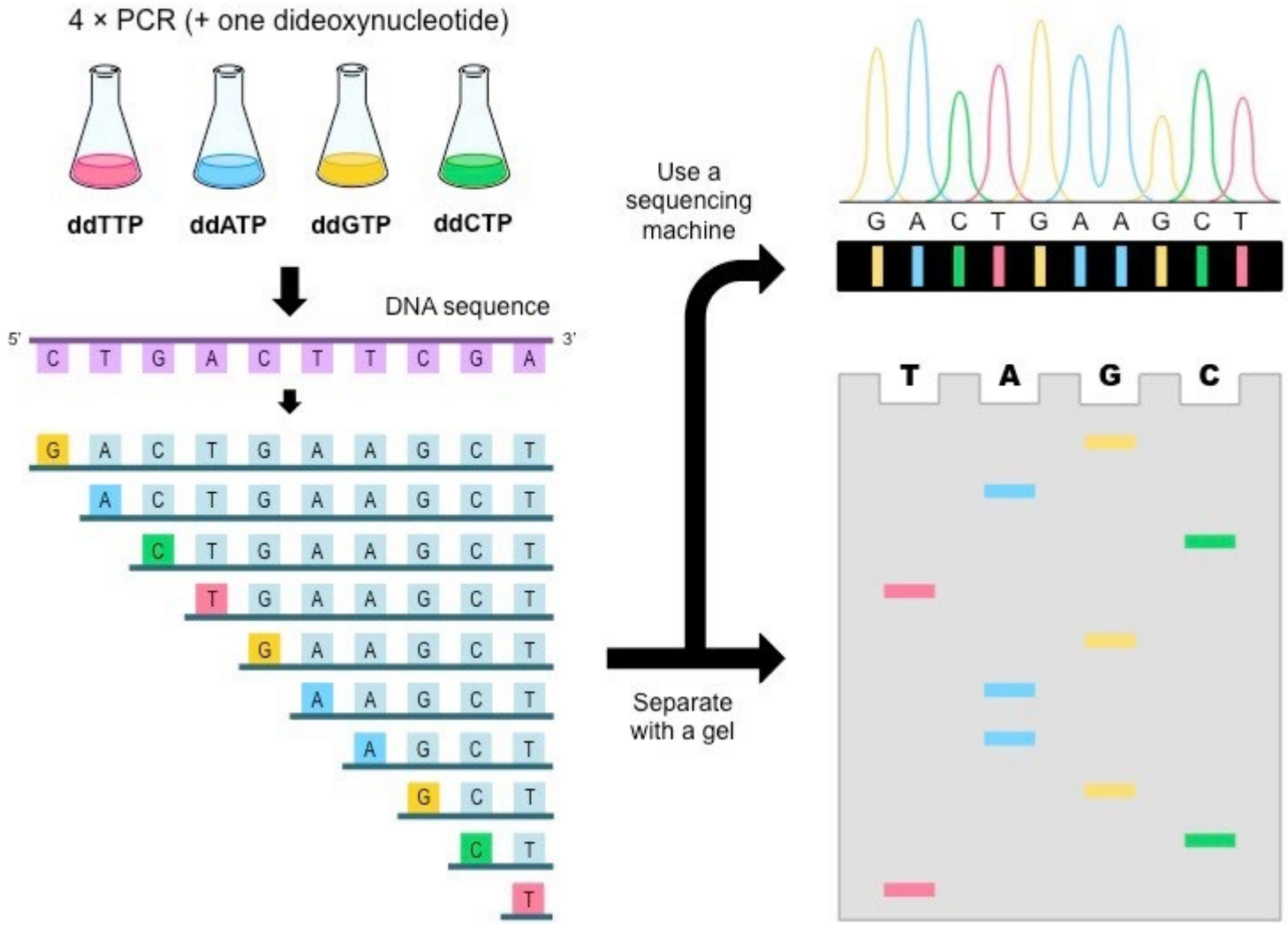


hCG immunoassay

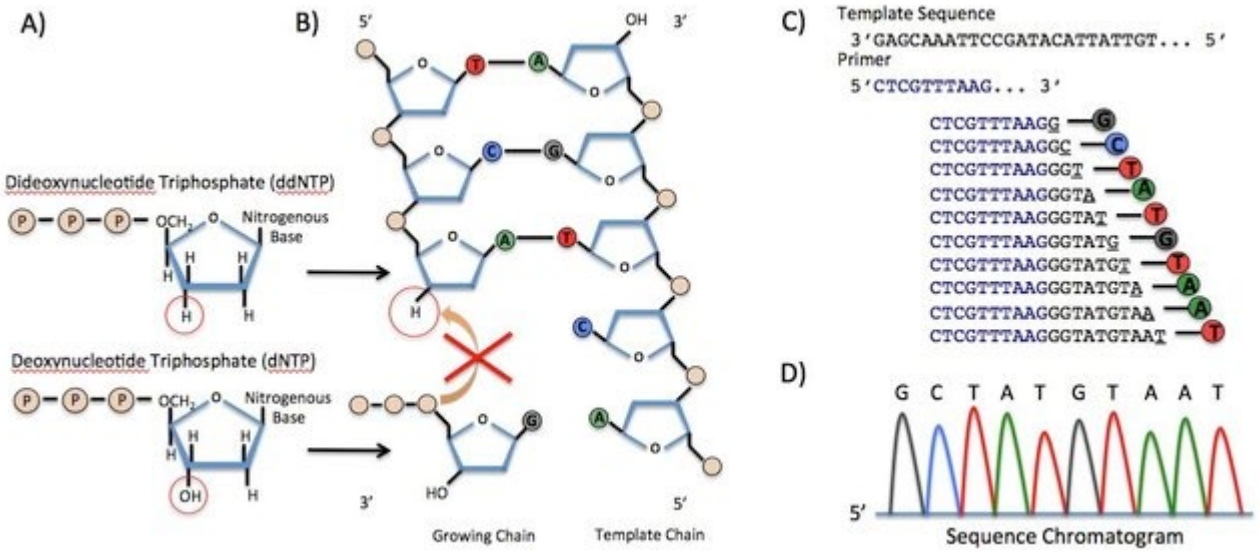


human chorionic gonadotropin (hCG)

DNA Sequencing



Sanger Sequencing



Dye Terminations

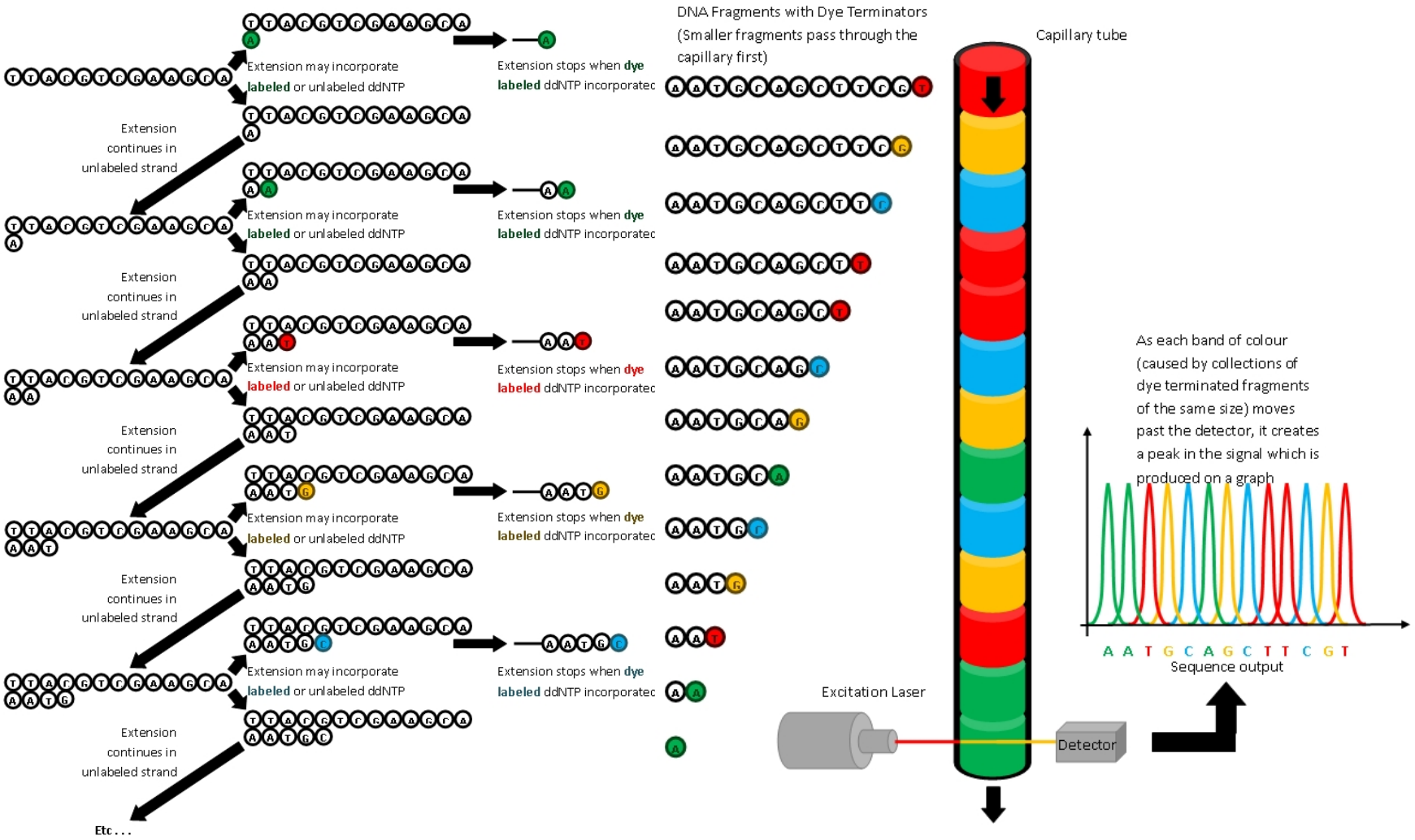
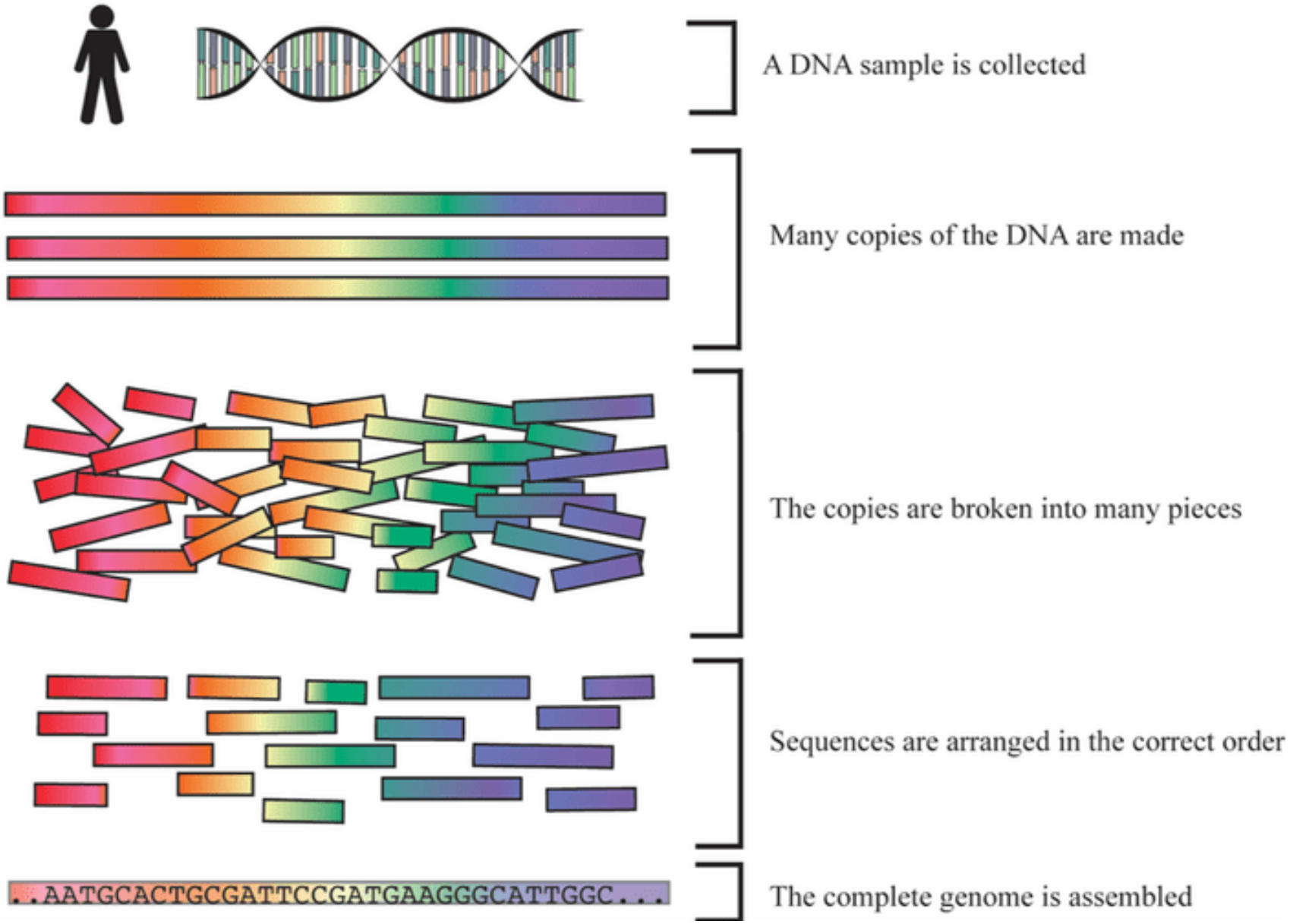
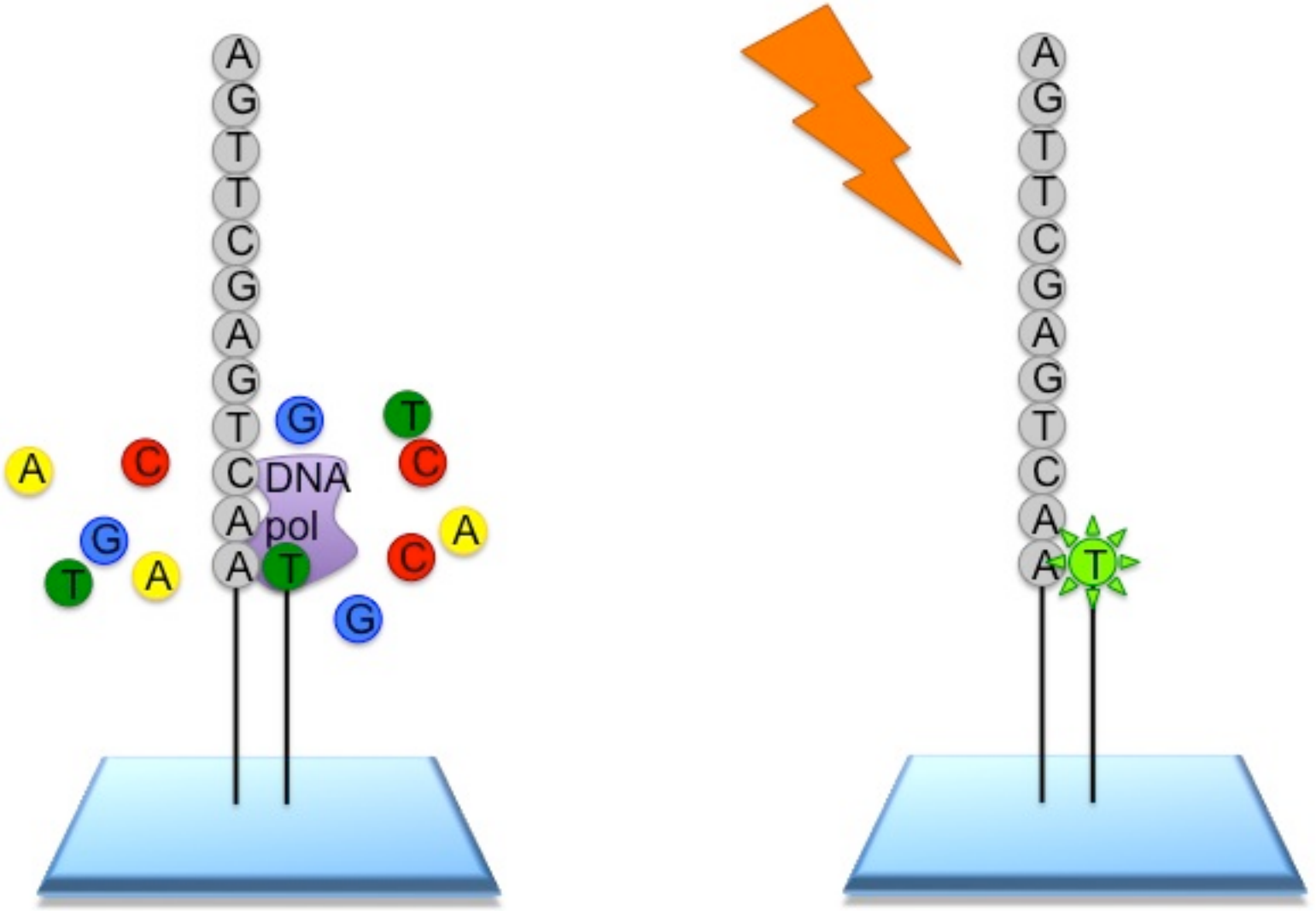
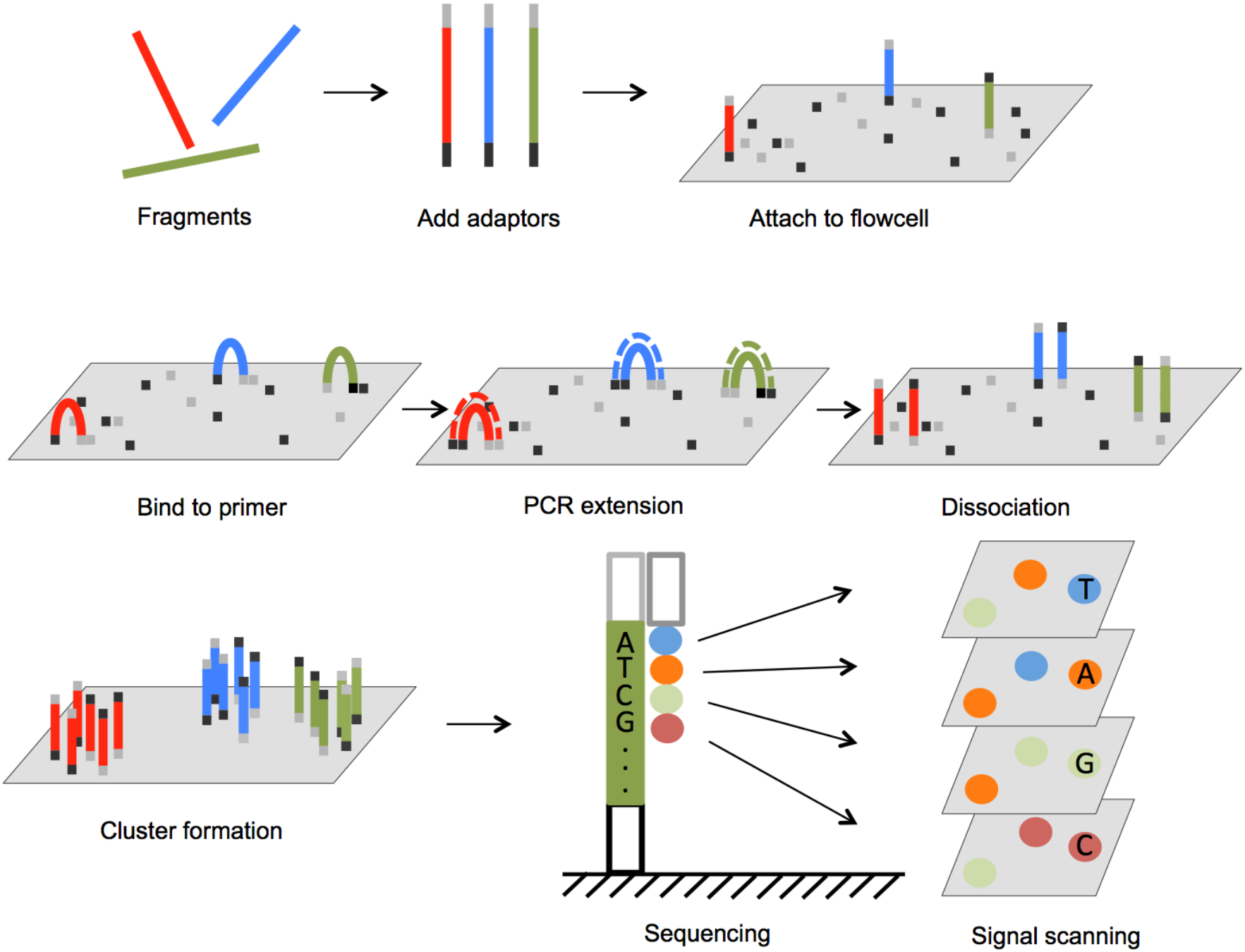


Figure 2: Shotgun Whole-Genome Sequencing



NGS Illumina

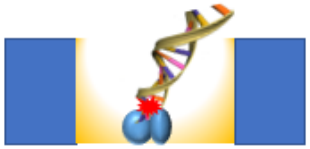



Fragments
Add adaptors
Attach to flowcell
Bind to primer
PCR extension
Dissociation
Cluster formation
ATCG...
Sequencing
T
A
G
C
Signal scanning

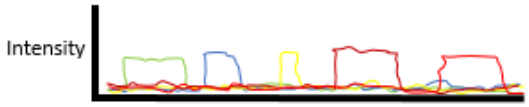
Third Generation Sequencing

PacBio SMRT seq

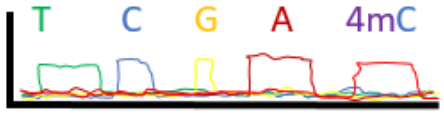
DNA passes thru polymerase in an illuminated volume



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

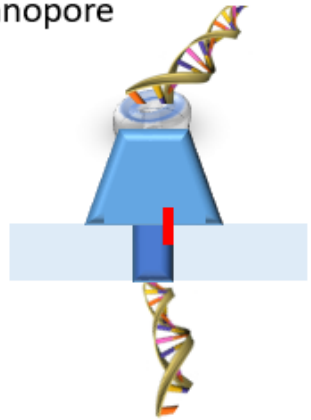


A,C,T,G have known pulse durations, which are used to infer methylated nucleotides

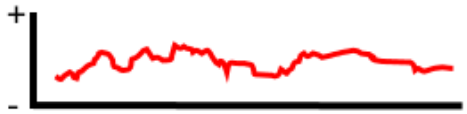


Oxford Nanopore

DNA passes thru nanopore



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



Each nucleotide has a specific electric “signature”

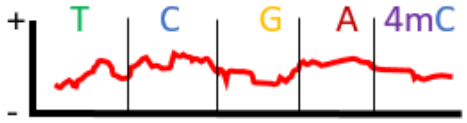
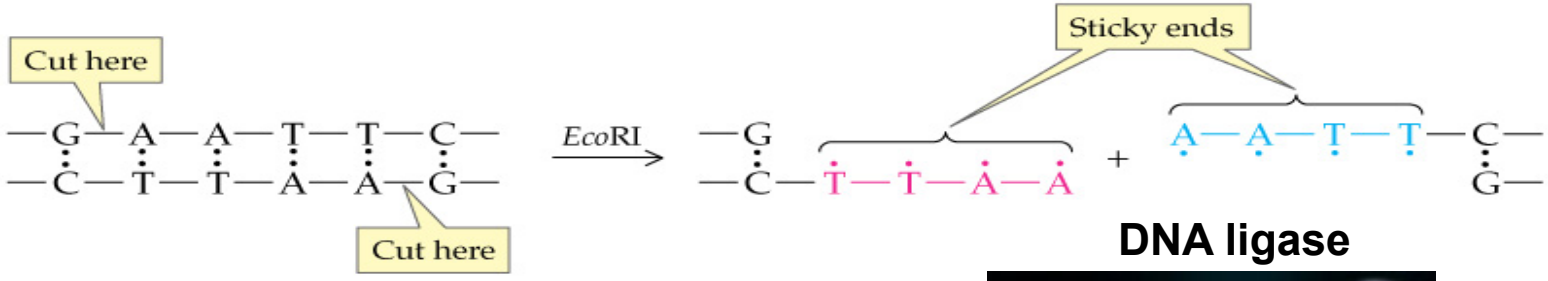


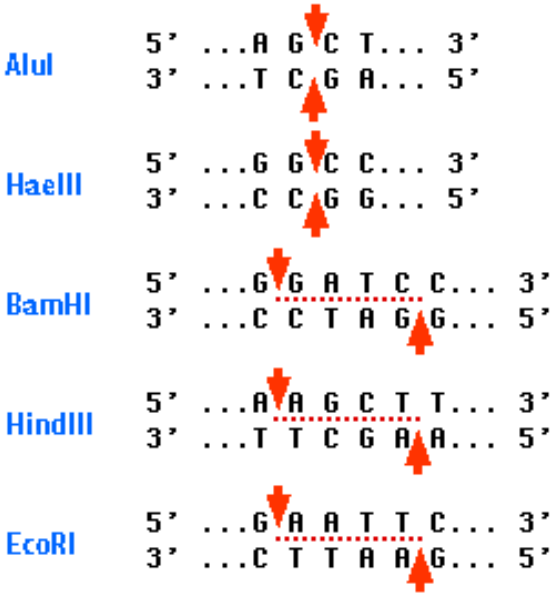
Table 2 Comparison of first-, second-, and third-generation genomic sequencing

	First generation	Second generation	Third generation
Fundamental technology	Size-separation of specifically end-labeled DNA fragments	Wash-and-scan SBS	Single molecule real time sequencing
Resolution	Averaged across many copies of the DNA molecule	Averaged across many copies of the DNA molecule	Single DNA molecule
Current raw read accuracy	High	High	Lower
Current read length	Moderate (800-1000 bp)	Short (generally much shorter than Sanger sequencing)	> 1000 bp
Current throughput	Low	High	High
Current cost	High cost per base, Low cost per run	Low cost per base, High cost per run	Low cost per base, High cost per run
RNA-sequencing method	cDNA sequencing	cDNA sequencing	Direct RNA sequencing
Time to result	Hours	Days	< 1 day
Sample preparation	Moderately complex, PCR amplification is not required	Complex, PCR amplification is required	Various
Data analysis	Routine	Complex (due to large data volumes & short reads)	Complex
Primary results	Base calls with quality values	Base calls with quality values	Base calls with quality values

Adapted from Schadt, et al. Hum Mol Genet 2010[13]



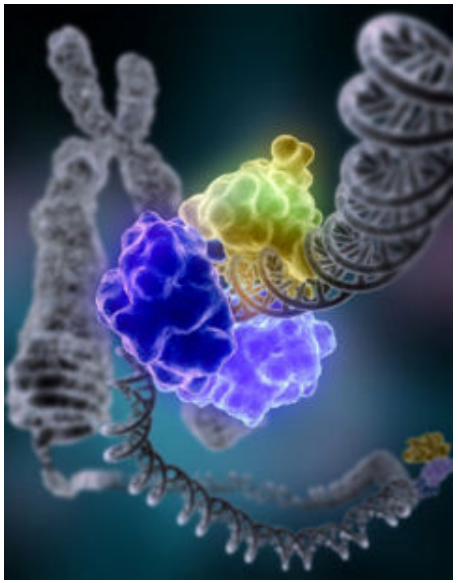
Restriction Enzyme

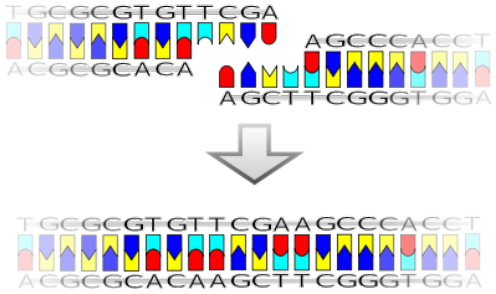


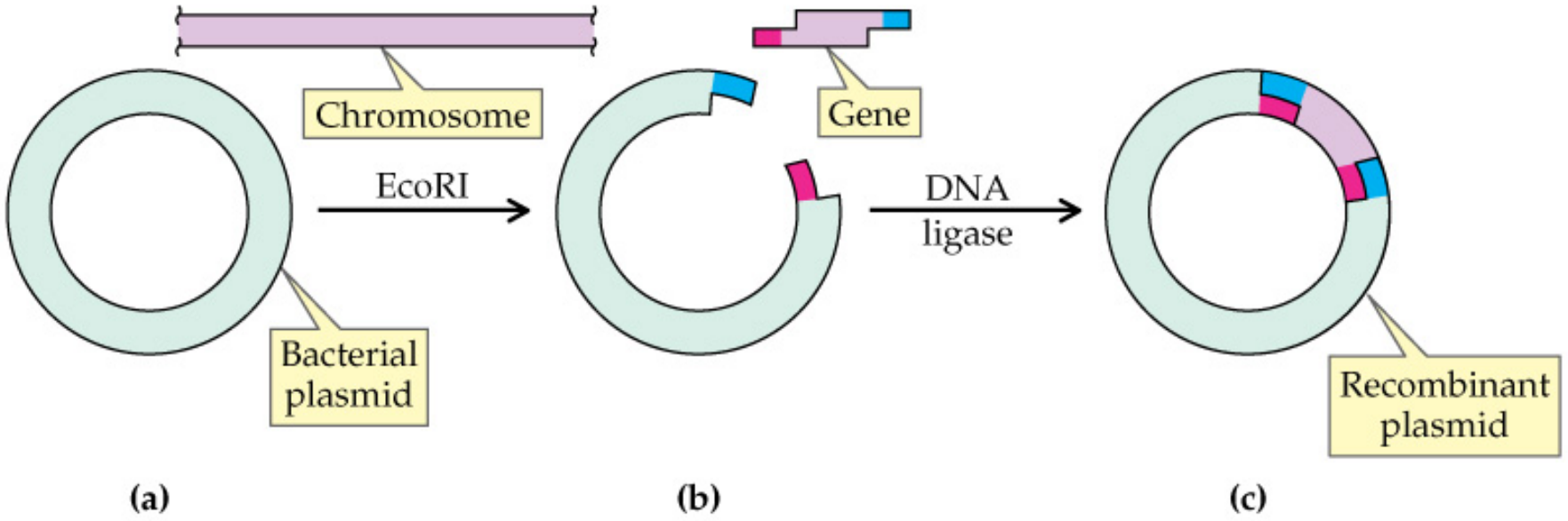
AluI and **HaeIII** produce blunt ends

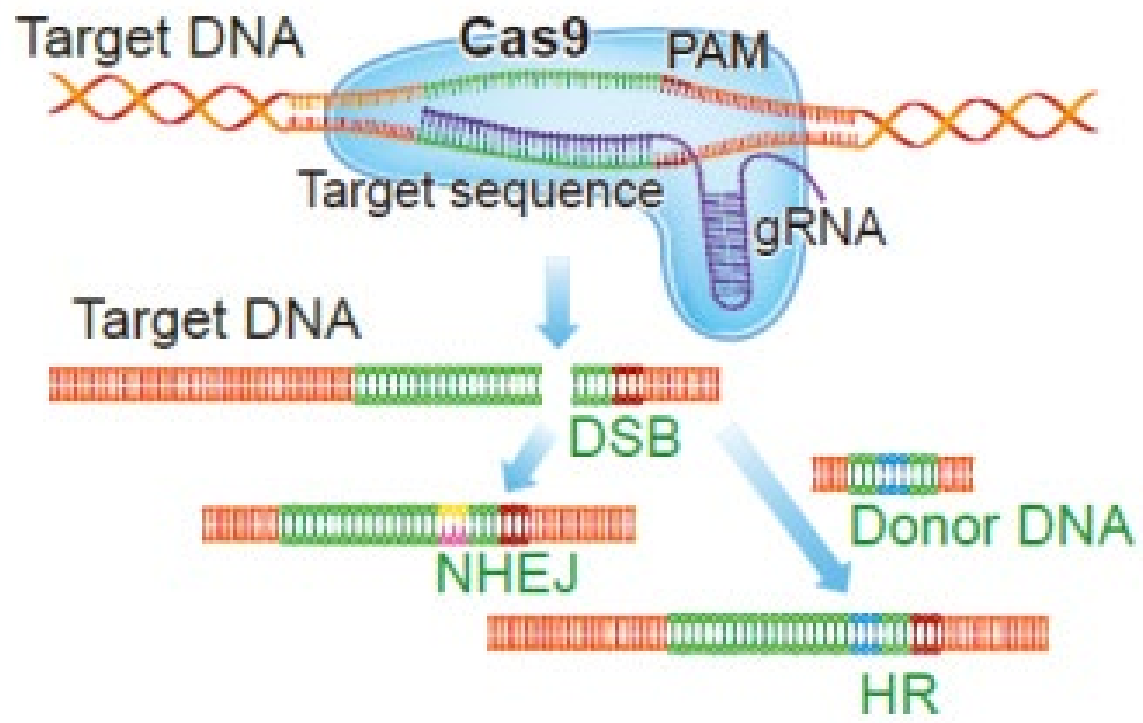
BamHI **HindIII** and **EcoRI** produce "sticky" ends

DNA ligase



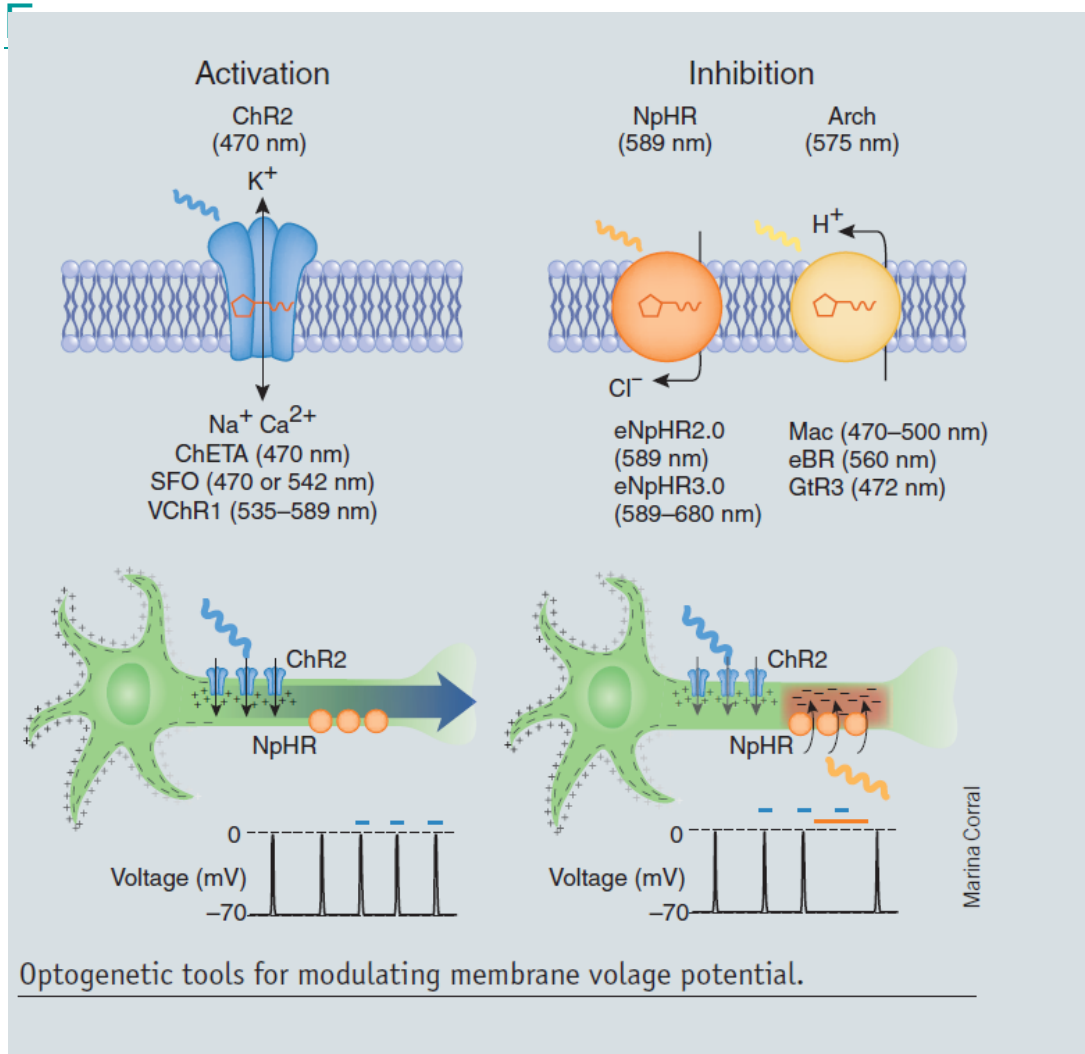



Chromosome
Gene
EcoRI
DNA
ligase
Bacterial
plasmid
Recombinant
plasmid
(a)
(b)
(c)

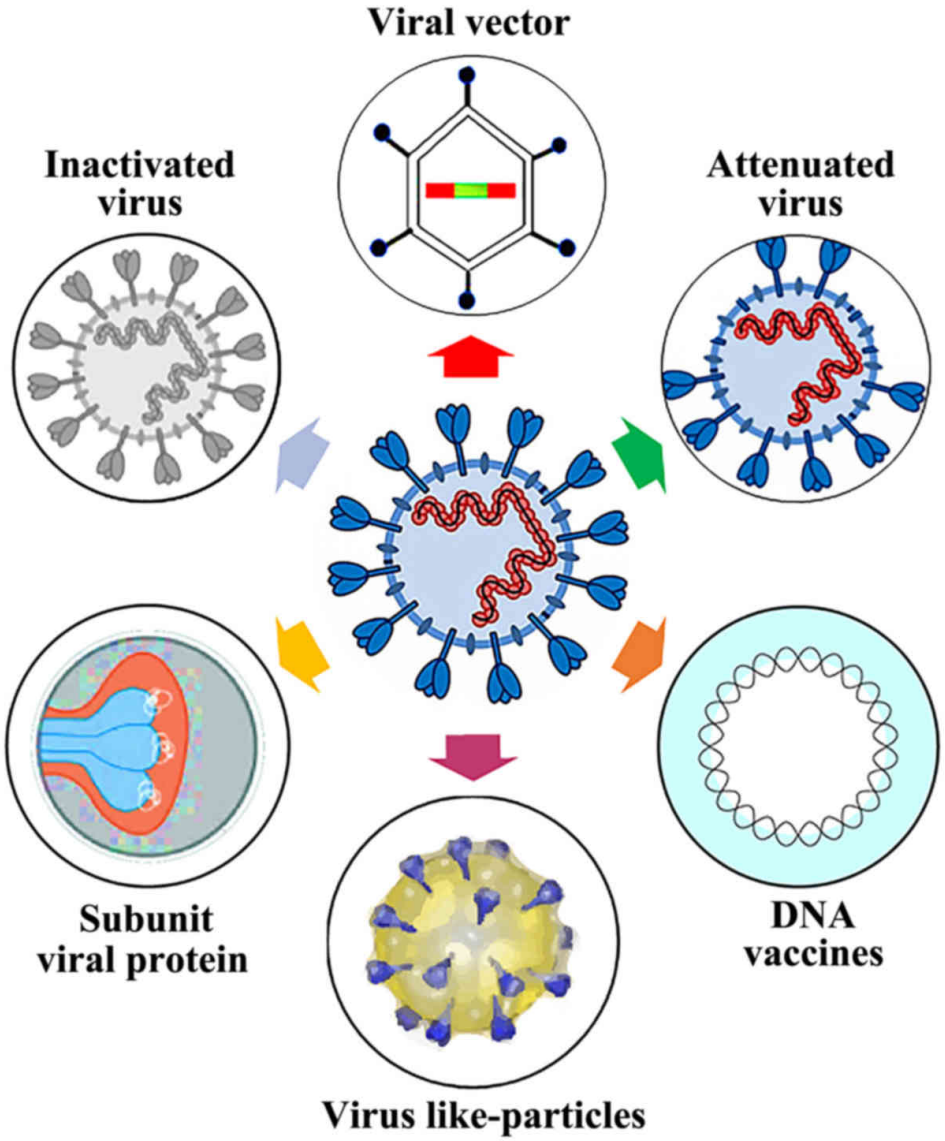

Target DNA
Cas9
PAM
Target sequence
gRNA
Target DNA
DSB
NHEJ
Donor DNA
HR

Optogenetics

https://www.youtube.com/watch?v=I64X7vHSHOE



Optogenetic tools for modulating membrane volage potential.

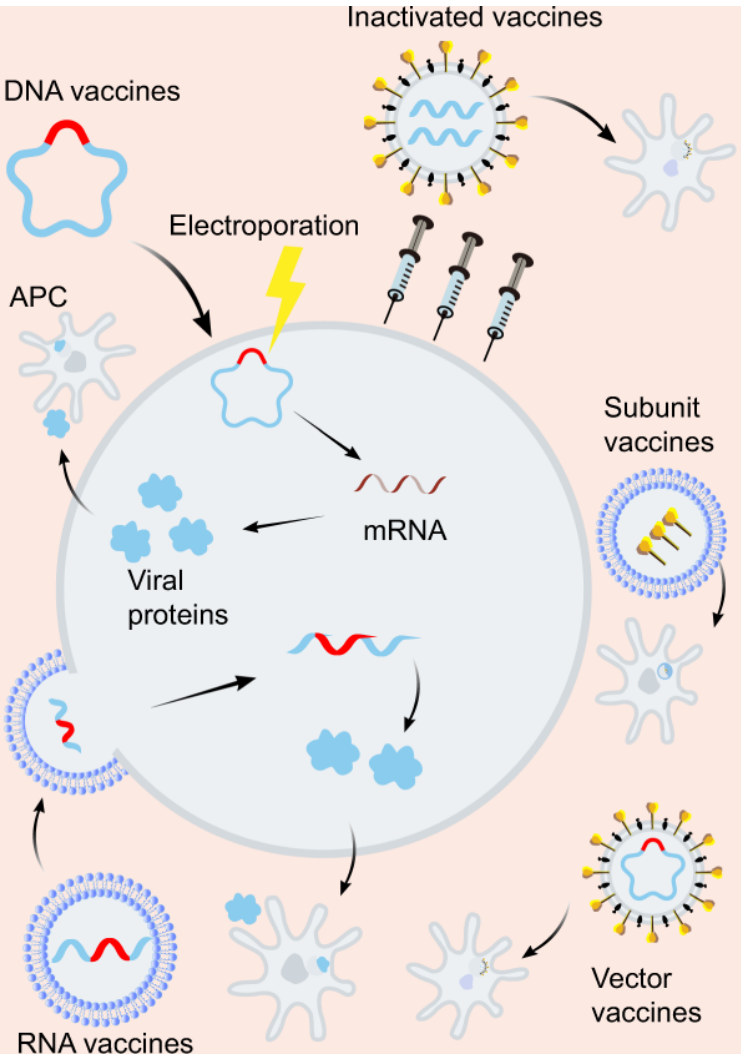

Viral vector
Inactivated virus
Attenuated virus
Subunit viral protein
DNA vaccines
Virus like-particles

Advantages:
- Enhances humoral and cellular immune responses
- Is stable, and can be easily prepared and harvested in large quantities.

Disadvatages:
- The safety and efficacy of vaccines for use in humans remain unknown.

Advantages:
- Can be rapidly developed and have potential for low-cost manufacture.

Disadvatages:
- The properties of mRNA may influence its cellular delivery and organ distribution
- Whether it is safe or not in humans, this remains unknown.



Advantages:
- Can be easily produced and stably express conformation-dependent antigenic epitopes.

Disadvatages:
- The unimportant antigen may skew the immune response
- Needs the biosafety level 3 growth of pathogen.

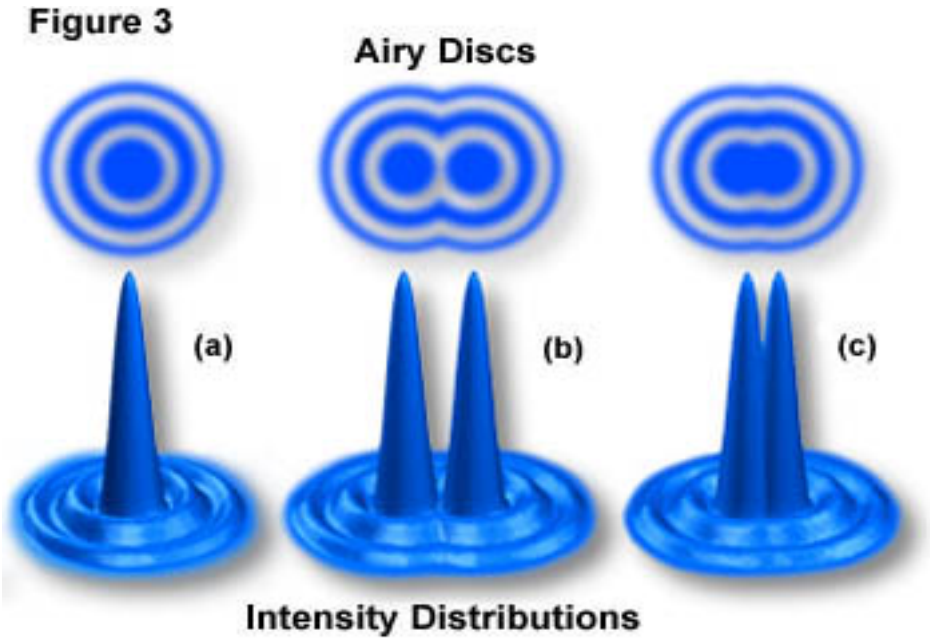
Advantages:
- May protect immunized animals from viral infection.

Disadvantages:
- May have limited efficacy and make immune responses unbalanced.

Advantages:
- Can infect APCs directly, and is physically and genetically stable.

Disadvatages:
- May induce prior immunity to the vector.

Resolution



Resolution (r) = λ/(2NA) (1)

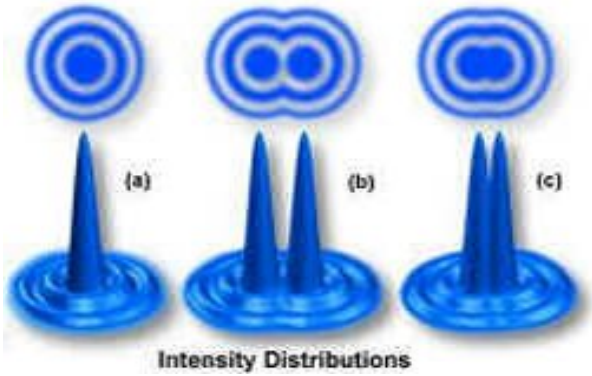
Resolution (r) = 0.61λ/NA (2)

Resolution (r) = 1.22λ/(NA(obj) + NA(cond)) (3)

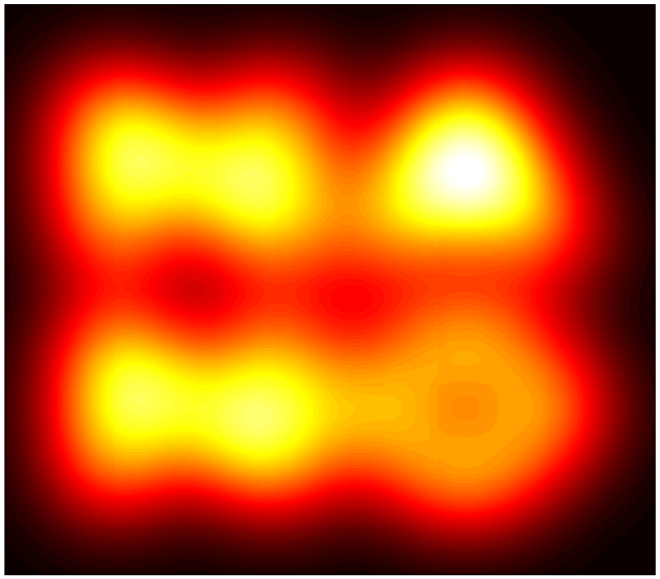
Diffraction Limit

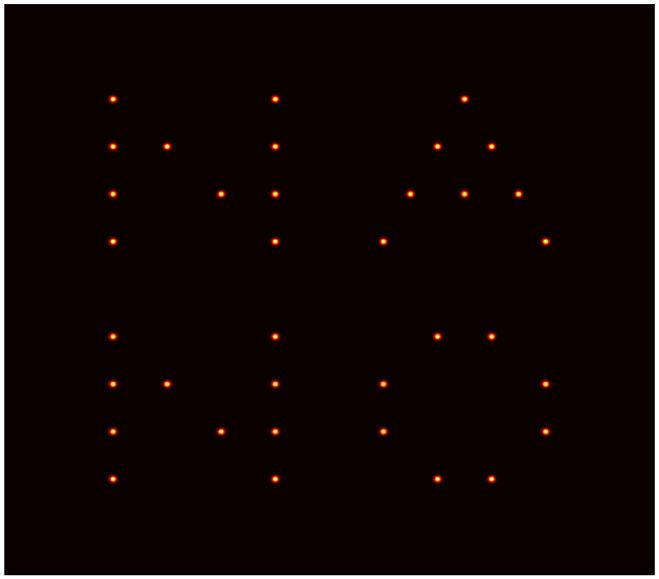
$$d = \lambda/(2n \sin \alpha)$$

$$, k_0 = 2NA/\lambda_{em}$$



Photoactivation localization microscopy (PALM)





Diffraction-limited system:

Lateral resolution $\Delta xy \approx 0.61\,\lambda$ / N.A.
$\approx$ 200 nm

Axis resolution $\Delta z \approx 2\lambda$ / N.A.^{2}
$\approx$ 450 nm

Mean-squared position error:

$$\left(\sigma_{x,y}^2\right)_m \approx \frac{s^2 + a^2/12}{N_m} + \frac{4\sqrt{\pi}s^3 b_m^2}{aN_m^2}$$

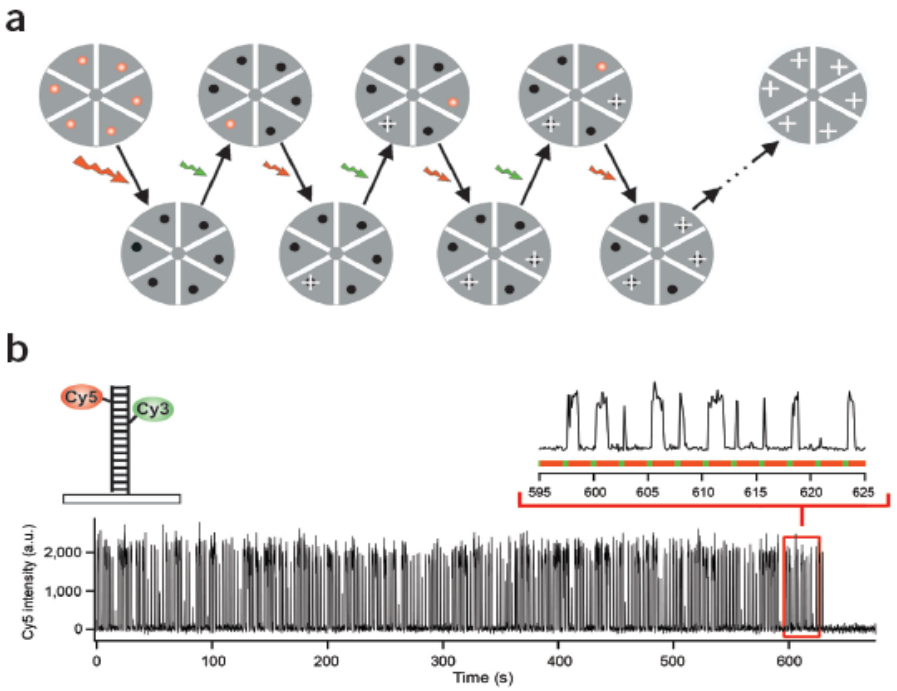
s is the standard deviation of the PSF.
a is the pixel size in the image
N_m is the total number of photons measured from molecule *m*
b_m is the number of background photons collected in the fitting window

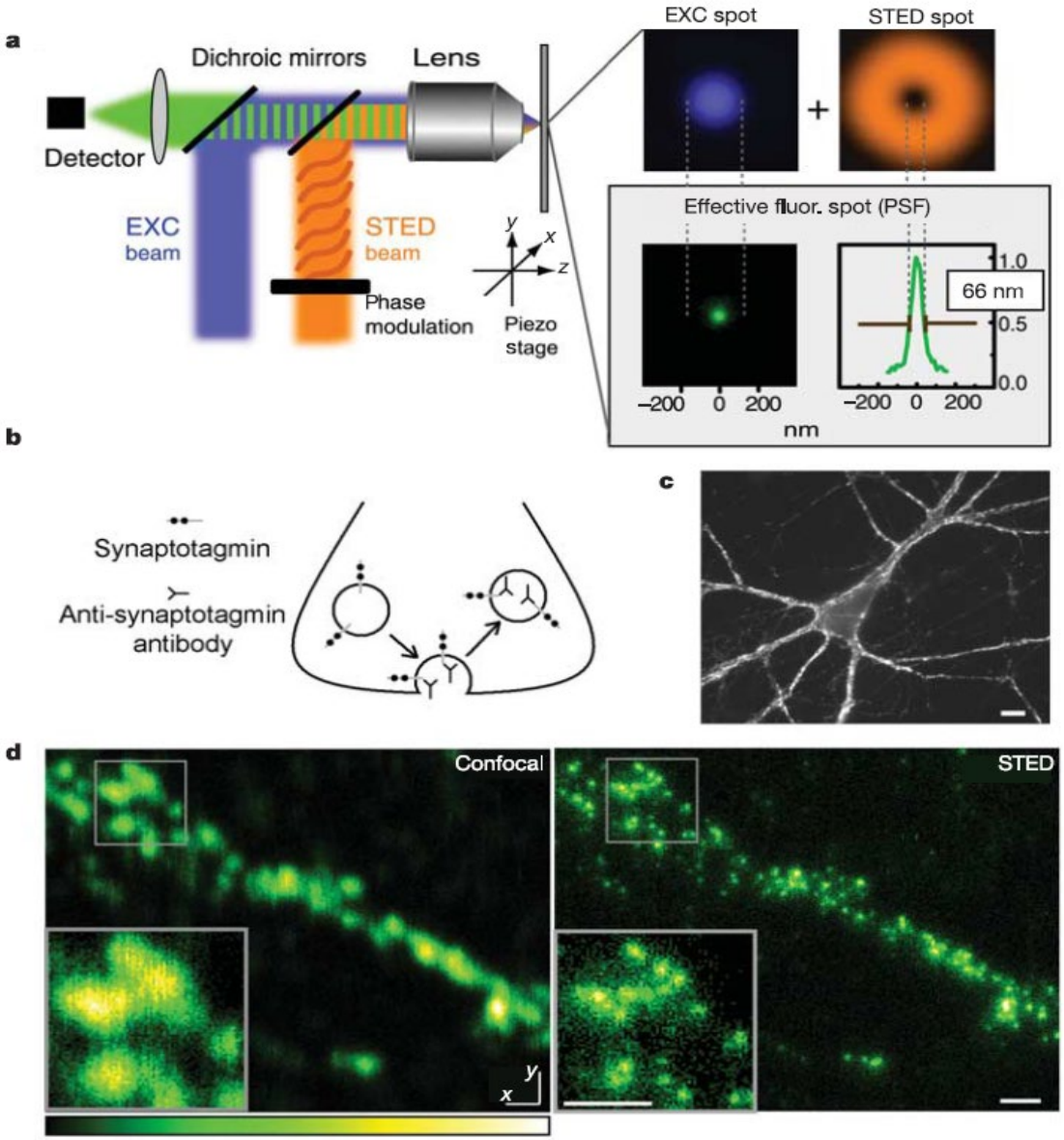
Russell E. Thompson, Daniel R. Larson, and Watt W. Webb, **Biophysical Journal** 82, 2775 (2002).
Eric Betzig, et al., **Science** 313, 1642 (2006).

Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM)

Michael J Rust[1,5], Mark Bates[2,5] & Xiaowei Zhuang[1,3,4]

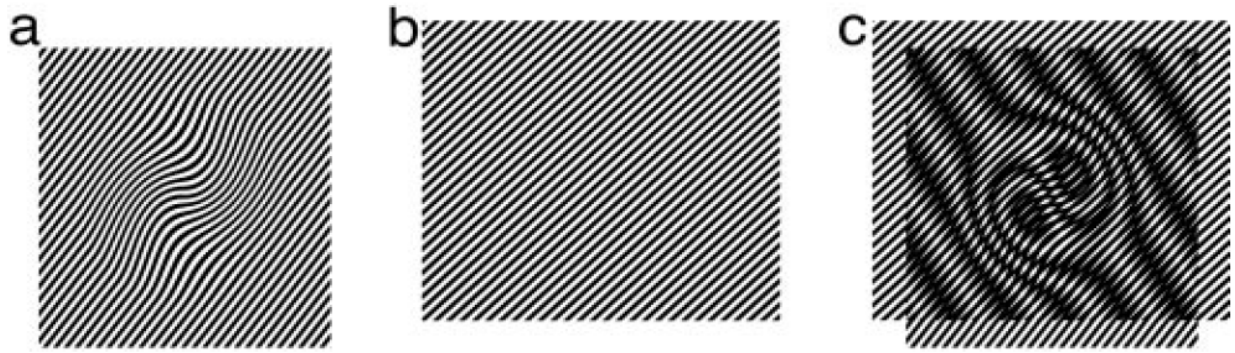
NATURE METHODS




a
Dichroic mirrors
Lens
Detector
EXC beam
STED beam
Phase modulation
Piezo stage
y
x
z
EXC spot
STED spot
+
Effective fluor. spot (PSF)
66 nm
1.0
0.5
0.0
–200 0 200
nm
b
Synaptotagmin
Anti-synaptotagmin antibody
c
d
Confocal
STED

Nonlinear structured-illumination microscopy: Wide-field fluorescence imaging with theoretically unlimited resolution

PNAS | September 13, 2005 | vol. 102 | no. 37 | 13081–13086



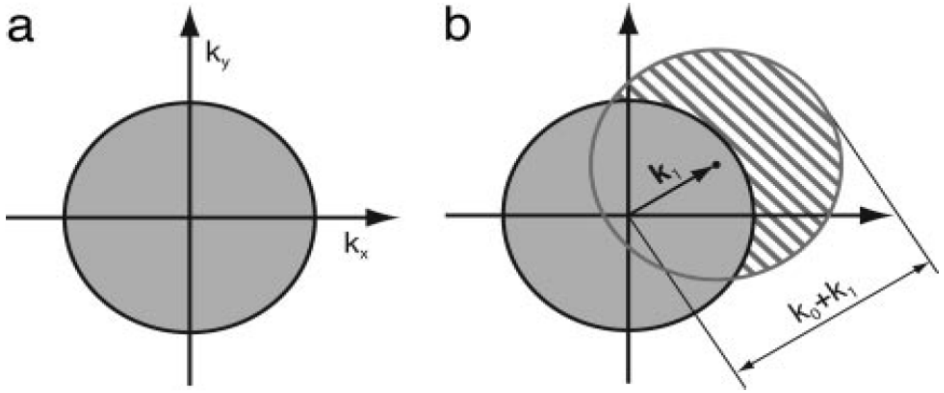
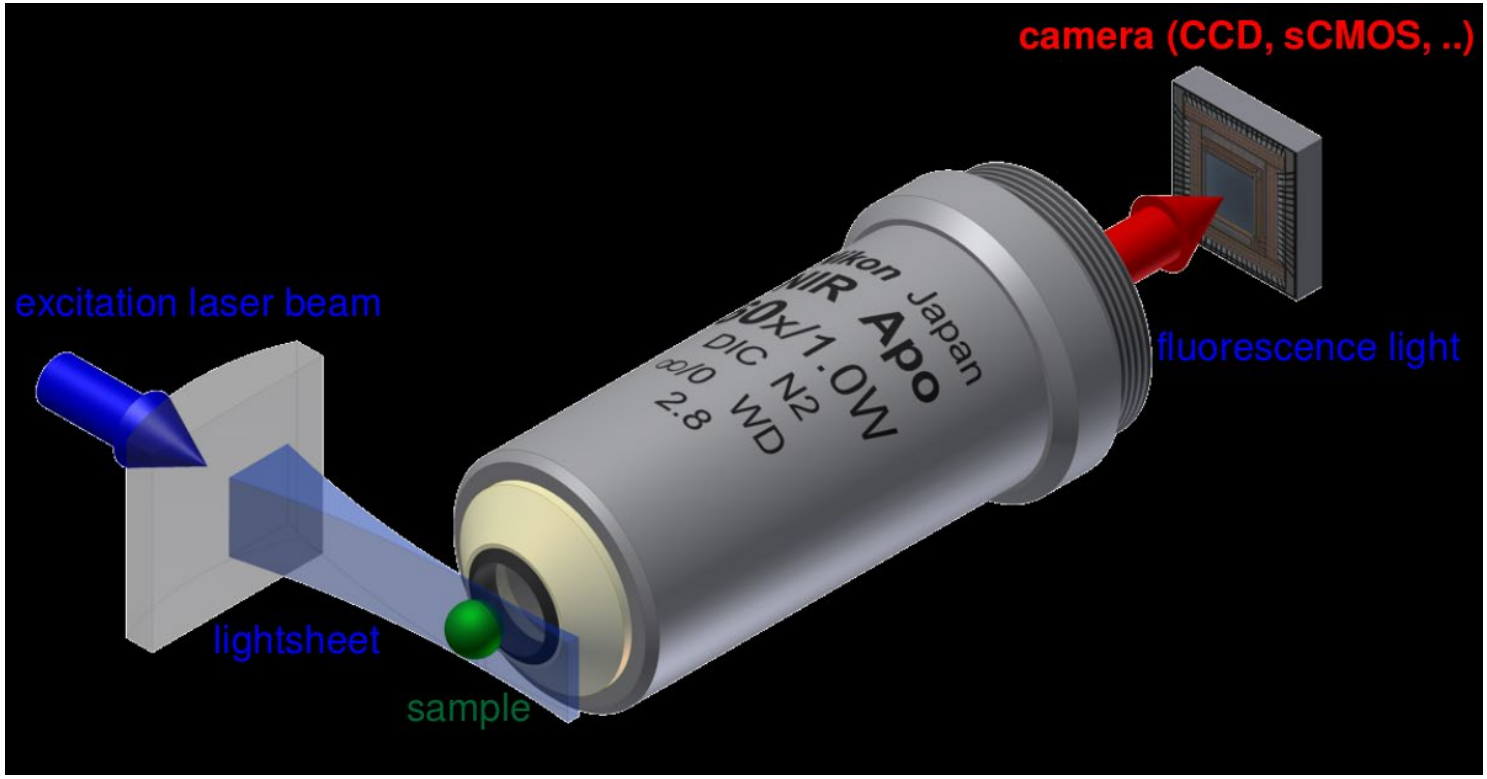
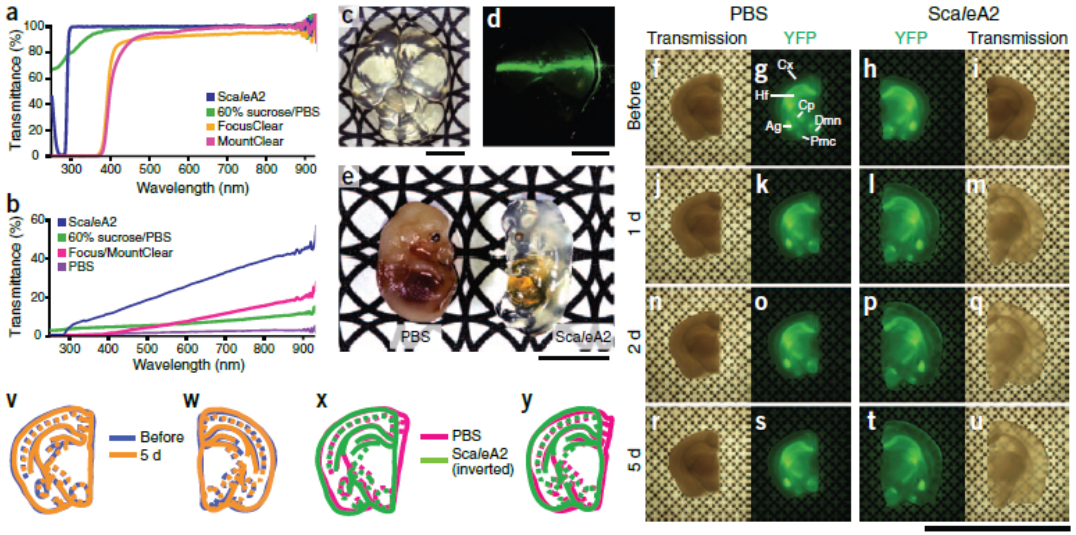


Fig. 2. Structured-illumination concept. (*a*) The set of sample spatial frequencies that can be observed by the conventional microscope defines a circular observable region of radius k_0 in frequency space. (*b*) If the excitation light contains a spatial frequency k_1, a new set of information becomes visible in the form of moiré fringes (hatched circle). This region has the same shape as the normal observable region but is centered at k_1. The maximum spatial frequency that can be detected (in this direction) is $k_0 + k_1$.

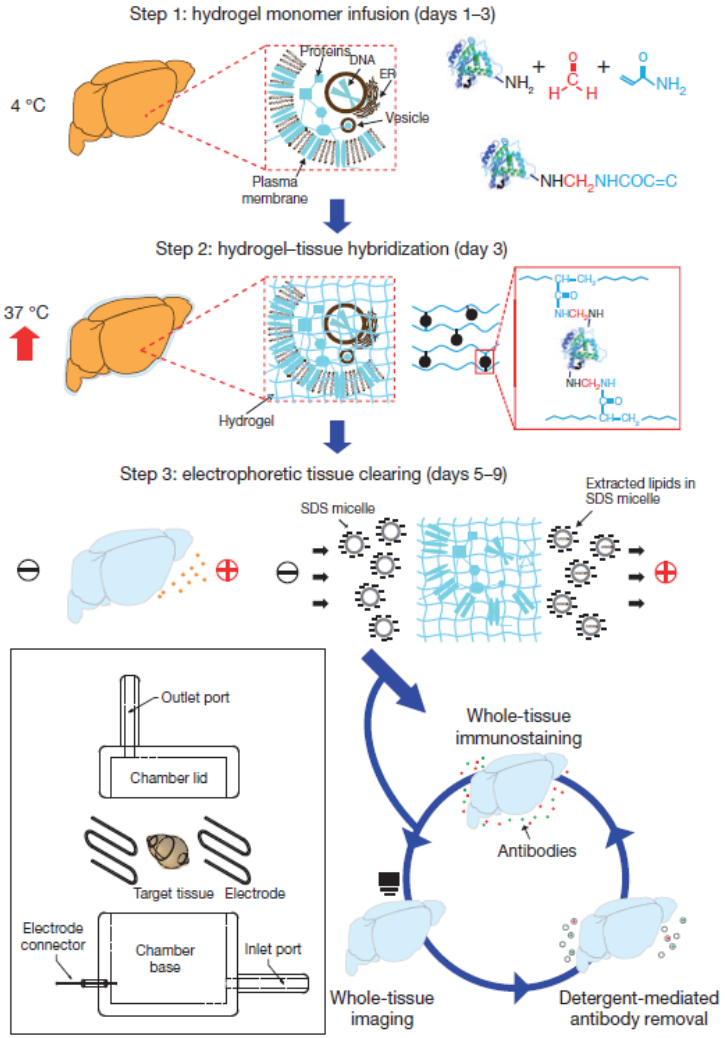
Light sheet fluorescence microscopy

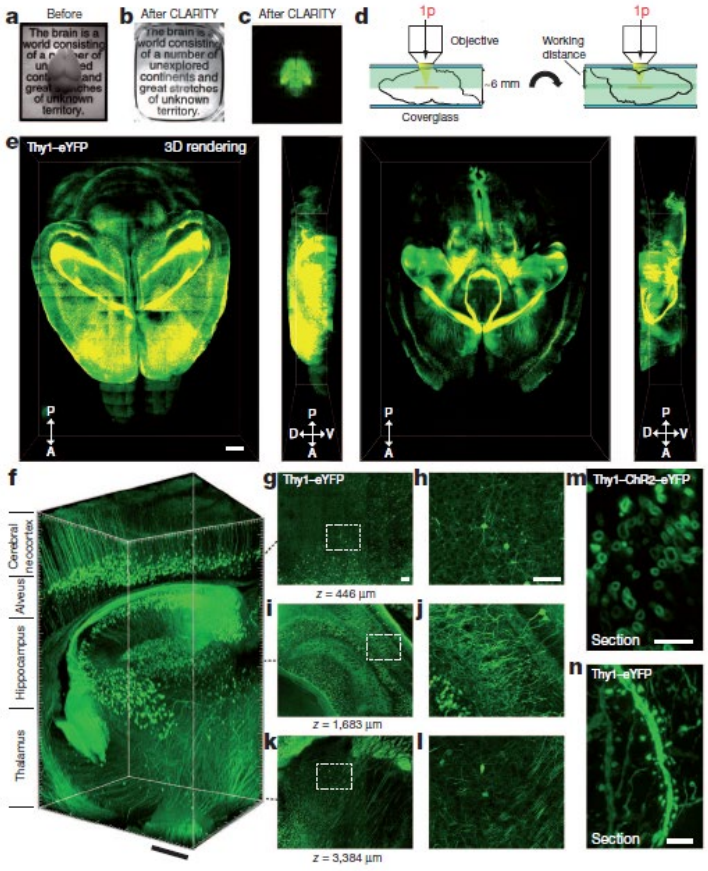


Optical Clearing



Structural and molecular interrogation of intact biological systems





OPTICAL IMAGING

Expansion microscopy

Fei Chen,[1*] Paul W. Tillberg,[2*] Edward S. Boyden[1,3,4,5,6†]