Bioengineering

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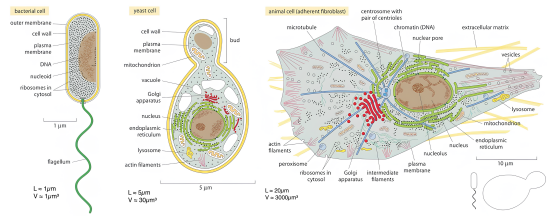
Tera	T	10^{12}	Kilo	k	10^{3}	Nano	n	10^{-9}
Giga	G	10^{9}	Milli	m	10^{-3}	Piko	p	10^{-12}
Mega	M	10^{6}	Mikro	μ	10^{-6}	Femto	f	10^{-15}

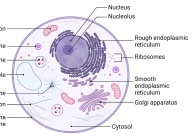
Orientation of the cell

Central Dogma of Molecular Biology

DNA $\xrightarrow{\text{transcription}}$ mRNA $\xrightarrow{\text{translation}}$ proteins

Cells:



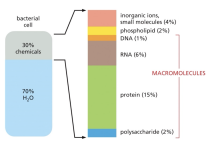


nucleus: houses DNA for EK
nucleolus: produces ribosomes/rRNA
mitochondria: cellular respiration (prod. ATP)
ribosome: produces proteins from mRNA transcripts

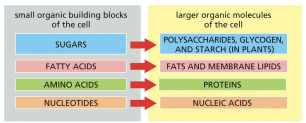
RER, SER and Golgi: involved in protein/lipid synthesis/processing
cytoskeleton: structure to cell, transport mol. in the cell or to enable the cell to move (cell migration)
centrosome: organizes microtubules during cell division allows the mother cell to split into 2 cells

Building Blocks

Cell composition:



Main building blocks:



- **Lipids** (fatty acids): long-term energy storage, cell membrane structure, signaling molecules.
- **Proteins** (amino acids): perform most of the cell's functions, including catalyzing reactions, signaling, and structural support. amino group $\mathbf{NH_2}$, carboxyl group **COOH**
- **Nucleic acids** (nucleotides): store and transmit genetic information (DNA, RNA), carry energy
- **Carbohydrates** (Sugars): short-term energy storages and for structural support.

Bounds

$$K_D = \frac{k_{off}}{k_{on}}$$

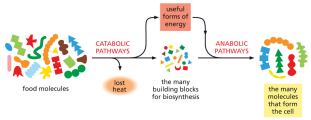
Covalent $\longleftrightarrow 100k_BT$
Ionic $\longleftrightarrow 1 - 10k_BT$
Hydrogen $\longleftrightarrow 1k_BT$
Van der Waals $\longleftrightarrow 0.1k_BT$
Electrostatic $\longleftrightarrow 0.1k_BT$

K_D: Equilibrium const.; indicates the ratio of free & bound units
k_{off}: Dissociation rate constant; inverse of time protein dissociates from the ligand
k_{on}: Association rate constant, speed of the reaction

Enzymes (aka catalysts)

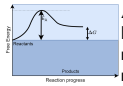
- Accellerate reaction by lowering the activation energy
- Are not consumed in the reaction
- Are specific to the reaction they catalyze
- Do not change the equilibrium point of the reaction.

Energy and Metabolism

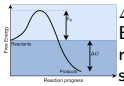


- **Catabolism:** Breakdown (glycolysis, citric acid cycle)
- **Anabolism:** Synthesis (protein synthesis, DNA replication)

Free Energy



$\Delta G > 0$
Endergonic
requires energy
not spontaneously



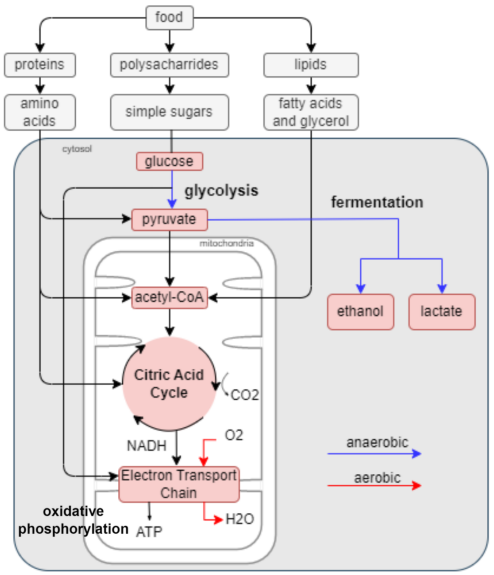
$\Delta G < 0$
Exergonic
releases energy
spontaneously

Redox

Oxidation loss of e^- $(H^+ + e^-)$ | Oxidation of glucose
Reduction gain of e^- $(H^+ + e^-)$| Reduction of pyruvate

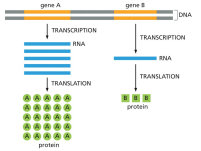
$\text{NADH} \rightleftharpoons \text{NAD}^+ + 2e^- + H^+$ | $\rightharpoonup$ oxidation, $\leftharpoondown$ reduction

Glucose Metabolism



Stage	Molecules	Glycolysis
Invest	2 ATP, 2 NAD^+, 1 glucose	
Payoff	4 ATP (2 net), 2 NADH, 2 H^+, 2 pyruvate	
Net gain	2 ATP, 2 NADH, 2 pyruvate	
Stage	**Molecules**	**Citric Acid Cycle**
Invest	2 Acetyl-CoA, 6 NAD^+, 2 FAD, 2 GDP (ADP)	
Payoff	6 NADH, 2 $FADH_2$, 2 GTP (ATP), 4 CO_2	
Net gain	2 ATP (GTP), 6 NADH, 2 $FADH_2$, 4 CO_2	
Stage	**Molecules**	**Oxidative Phosphorylation**
Invest	10 NADH, 2 $FADH_2$, 6 O_2	
Payoff	34 ATP, 6 H_2O	
Net gain	34 ATP, 6 H_2O	

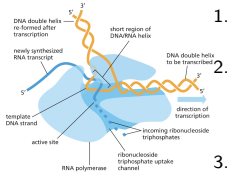
Biosynthesis



Genes are not always on and do not always produce the same number of transcripts or proteins.

Synthesis of proteins is a complicated and tightly regulated process.

Transcription

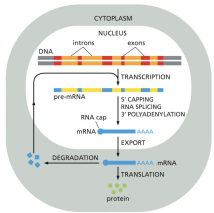


1. Small region of DNA opens and unwinds.
2. RNA polymerase (catalytic enzyme) interacts with one strand in the open region of DNA and adds ribonucleotides to grow an RNA polymer.
3. RNA polymerase further unwinds the DNA in the forward direction.

types of RNA

Type	Function
messenger RNA **mRNA**	code for proteins
ribosomal RNA **rRNA**	form core of ribosom structure and catalize protein synthesis
micro RNA **miRNA**	regulate gene expression
transfer RNA **tRNA**	serve as adaptors between mRNA and amino acids during protein synthesis
other non-coding RNA	RNA splicing, gene regulation, telomere maintenance, etc.

Eukaryotes vs Prokaryotes



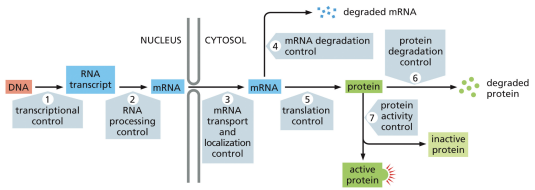
- Multiple types of RNA polymerases transcribe different classes of RNA.
- Transcription initiation is more complex.
- mRNA molecules undergo splicing, where introns (non-coding regions) are removed and exons (coding regions) are joined together to form the final RNA.

- 1 type of RNA polymerase transcribes all types of RNA.
- Transcription initiation is a simpler process.
- mRNA molecules are translated immediately after transcription

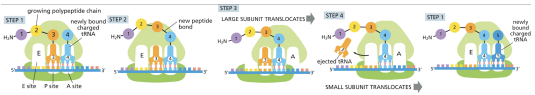
Both use gene regulatory proteins that bind to specific sequences of DNA:

- Repressors: Bind to sequences to turn genes off. Make it more difficult for RNA polymerase to bind to DNA.
- Activators: Bind to sequences to turn genes on. Make it more favorable for RNA polymerase to bind to DNA.

Gene Regulation



Translation by Ribosomes

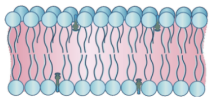


- On the mRNA, every three bases (= nucleotides) form one codon (needs ATP).
- A tRNA brings a matching amino acid. It has an anticodon that is complementary to the mRNA codon.
- The tRNA binds to the mRNA in the ribosome (at the A site).
- The amino acid is added to the growing polypeptide chain.
- The tRNA is ejected (from the E site), and the ribosome shifts forward by one codon.
- The process repeats until a stop codon is reached.

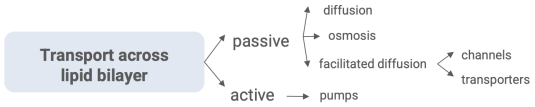
Multiple ribosomes can translate the same mRNA at the same time (making multiple proteins at the time), forming a polyribosome (or polysome).

Transport and Exchange

Cell Membrane



Phospholipid bilayer with hydrophilic head and hydrophobic tail act as barrier between compartements

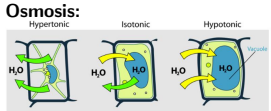


Passive Transport

Diffusion:
Along conzentration gradient, from high to low concentration.

$$\tau_D = \frac{l^2}{D} \quad D = \frac{k_B T}{6\pi\eta a}$$

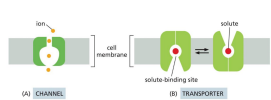
Osmosis:



Hypertronic: high solute concentration
Hypotonic: low solute concentration.

Membrane is **selectively permeable**, allowing some molecules to pass through while blocking others.

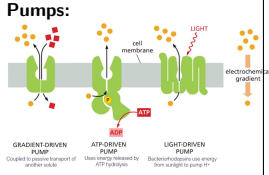
Facilitated Diffusion:



- selective to size and charge of solute
- bidirectional
- gated by external inputs
- Transporter good for large molecules

Active Transport

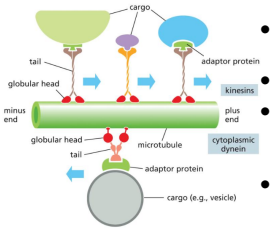
Pumps:



Vesicular Transport

Endocytosis: into cell
Exocytosis: out of cell
Pinocytosis: cell drinking
Phagocytosis: cell eating
Receptor-mediated endocytosis: specific uptake of molecules

Intracellular Transport



- chemical energy (ATP) to mechanical work
- directed motion
- Kinesin: carry out microtubule-based tiretrograde transport (towards cell edge)
- Dynein: carry out retrograde transport (towards cell nucleus)

Cell Sensing and Signaling

Cellular Communication

Long Range:
Endocrine
- into blood stream
- affect whole organism

Neural
- in neurons (electric)
- at synapses (chemical)

Short Range:
Paracrine
- affect local tissue

Contact Dependent
- direct binding

Signaling

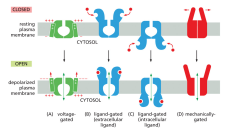
Cell Surface Receptors:
Extracellular signal molecule (hydrophilic or charged) causes receptor to releas intracellular signal molecule.

Intracellular Receptors:
Signal molecule (hydrophobic) cross the membrane and act inside the cell.

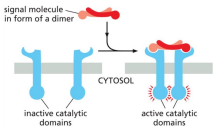
Signal is:
- Amplified
- Integrated
- Distributed
- Modulated (feedback loop)

Receptors

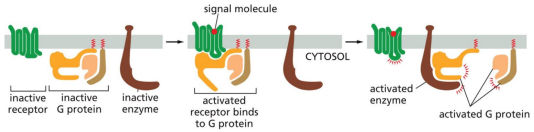
Ion-channel-coupled



Enzyme-coupled



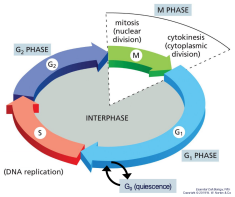
G-protein-coupled receptors



- Ligands (ex.: hormones, neurotransmitters) bind to GPCR (G-protein coupled receptor) which changes conformation
- Activated receptor causes G-protein to exchange its GDP for GTP gets activated
- G-protein modulates the activity of effector molecules generate intracellular second messenger

Growth and Differentiation

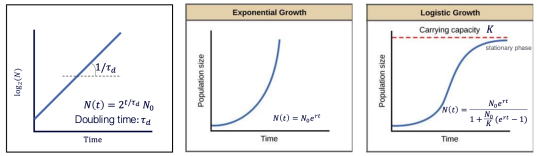
Cell Cycle



G1: Cell growth
S: DNA Synthesis
G2: Growth and preparation for Mitosis
M: Mitosis (cell division)

Quiescent cells: cells pause before replication. Reversible growth arrest (G0 phase)

Growth



Clonal Population

Genotype: ensemble of all the genes of a cell ("all available genes")
Phenotype: output of set of expressed genes ("all visible genes")
Clonal population: same genotype and phenotype, identical cells, can differ due to mutations in genotype

Cell Death

Apoptosis:
- controlled cell death
- directed by extracellular signals
- controlled by intracellular signal cascade
- apoptotic cell gets phagocytosed by macrophages

Necrosis:
- death as result of injury
- cells burst and release their contents

Stem Cells – Cell Differentiation

Differentiated cells in adult organisms contain all the genetic information to form a new organism. But, they express **only a fraction** of genes **specific to their function**.

Stem cells can differentiate to tissue-specific cell, but also renew themselves.

Pluripotent stem cells can give rise to all cell types.
Multipotent stem cells can give rise to a limited number of cell types tissue specific.

Bioprocesses

Bioprocesses rely on several key components, including biological components, such as the **target molecule** and the **cells** used to produce it; **culture medium** and one or more **bioreactors**; as well as a **process**.

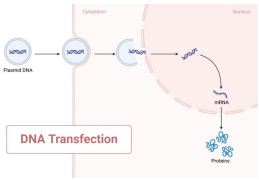
Modern technology used:
- DNA sequencing and synthesis
- Precise gene editing
- Genetic circuit design

Cell source:

Mammalian cells:
- \- slow growth
- \- complex growth media
- \+ proper folding
- \+ glycosylation

E. coli:
- \+ fast
- \+ simple growth media
- \- refolding required
- \- no glycosylation

Transfection



Insert DNA that codes for the wanted biomolecule into cell.
Use:
- viruses
- electroporation
- carriers

store transfected cells in cryogenic conditions

Culture medium

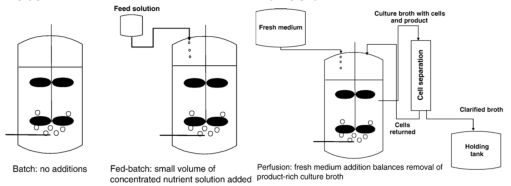
Source of nutrients to support the growth of cells.
Composition:
- building blocks (sugars, aa)
- water
- salts/ions

Bioreactor

Carefully designed **culture medium** that **provides nutrients** (building blocks, water, ions, energy) and a **suitable environmen** for cells to grow and generate biomolecules of interest.

Batch

Perfusion

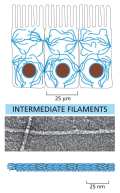


Cell and tissue architecture

Cytoskeleton – "Bones and muscles of the cell"

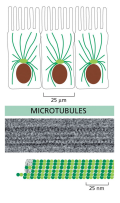
- Resistance to deformation
- Drives movement
- Organizes the cell interior (shape and cargo)
- Physical interactions with the environment
- Present in all eucaryotic cells

Intermediate Filaments



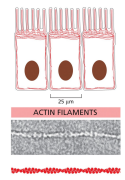
- **Toughest** and most durable filaments
- Primary function: **Withstanding mechanical stress**
- **Assembled** from α-**helical proteins** → Rope-like structures
- Without preferred direction (Diameter 10nm)
- **Major types:** Keratins, Vimentins, Neurofilaments, Lamins

Microutubules



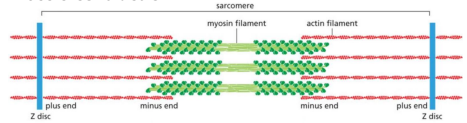
- Essential for **spatial organization**
- **Polar**, have a **distinct orientation**, centrosome → cell membrane
- Are **assembled from** globular proteins: α- **and** β-**tubulins** that assemble in tubulin dimers (25nm diameter)
- **Dynamic** → constantly grow or shrink
- Can form **Cilia**, help in **cell division** or **transport**

Actin Filaments



- Provide **support**, change the **cell shape** (division) and drive **movement**
- Assemble from **globular proteins** ("G-Actin") like microtubules and form **hierarchical structures** by-crosslinking
- **Polar** with **no preferred direction**
- Can form **protrusions** → **exploring** and **sensing environment**
- **"Myosin motors"** → participate in cargo transport and muscle contraction

Muscle contraction:



In a **sarcomere**, **myosin II** binds to actin and **proceeds towards the + side**.
This sliding of myosin on actin filaments **shortens the strands** and leads to muscle contraction

Extracellular Matrix (ECM)

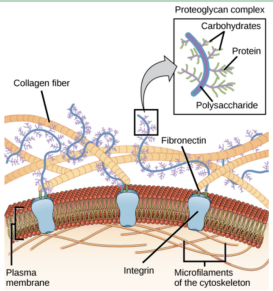
Fibrous elements outside the cell that hold cells and tissues together
Functions:
- Structural support and mechanical scaffold
- Resistance to stretch and compression
- Boundary between tissues
- Water retention
- Reservoir for signaling molecules

Plants have cell walls (cellulose and pectin) instead of ECM.

Composition of ECM

Protein fibres:
Collagen and **Elastin** provide **strength** and **elasticity**

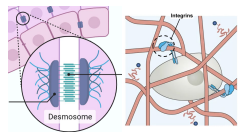
Glycoproteins:
Fibronectin and **Laminin** provide **adhesion** and **signaling**



Glycosaminoglycans (GAGs) and Proteoglycans:
- **Linear**, **rigid** polysaccharide **chains** → form large volumes of porous gels
- They carry **negative charges** → **retention of water**
- Often covalently linked to protein cores called proteoglycans that also provides lubrication
- **Resistance to compression**

All components are **produced** and matured **in the cells** then secreted in extracellular environment
→ Cells engineer their local extracellular matrix

Physical Cell-Cell and Cell-ECM Interactions



Mechanical, electrical, metabolic coupling at cell-cell junctions (desmodrome).
Physical cell-cell and cell-ECM communication (e.g. via integrins)

Woundhealing and Tissue Engineering

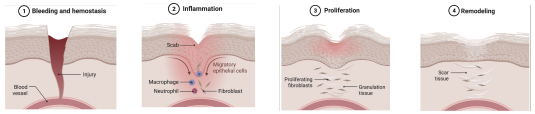
Circulatory system

The circulatory system consists of cardiovascular system and the lymphatic system.

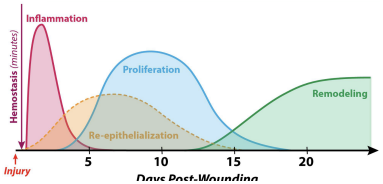
Blood cell types:
- Enucleated: **Erythrocytes** (red blood cells) and platelets
- Nucleated: **Leukocytes** (white blood cells) and other immune cells

Vascular Structure:
Artery, Arteriole, Capillary bed, Venule, Vein

Woundhealing



Blood clot formation | Fight potential pathogens | Rebuilding the tissue | Restoring tissue function



Tissue Engineering

Mimic in vivo conditions so that cells can grow.
- scaffolds
- genetic tools
- biomaterials
- bioreactors

Hydrogel as ECM mimics:
- Hydrophilic networks with tunable mechanical, biochemical and physiochemical properties.
- Matrices can be natural, engineered or hybrid.
- Body tissues have different matrix properties.

Microphysiological Systems and Immune Engineering

Organoids

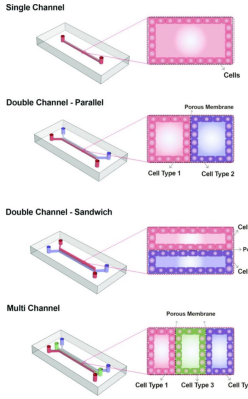
Organoids are "**mini organs**" built in the lab from stem cells.
Organoids **mimic geometric features** and **cell organization** of the original organ (tissue replica). The surrounding **ECM composition** is **custom** designed.

Matrigel: matrix scaffold for organoids, provides **mechanical support** and **adhesion sites** for cells.
Cell source: induced pluripotent SC (iPSC) terminally differentiated cells reprogrammed back to pluripotency.

Possibility to **model genetic diseases** such as Parkinson in cerebral organoids.
Limited to small size, nutrient and oxygen supply is limited by diffusion.

Organs on a Chip

Micro-tissues grown in a controlled microfluidic device where **physical** and **mechanical stimulation** can be applied. OoaC are patient-specific (gender, age, history of disease, etc).



- Controllable flow circuits
- Multiple tissue compartments
- Multiple cell types
- On-demand drug release
- Small scale but high-throughput

Testing:
- interaction between different cell types
- safety in wholeisitc concept
- Measure uptake and clearance rates

Immune Engineering

Consists of using engineering tools and principles to investigate and modulate the **immune system**.

Applications:
- Evade or delay immune response
- Shut off auto-immunity in auto-immune diseases
- Stimulate immune response (e.g. vaccines)
- Multiply native immune response (T cell activation)

Immune Response

Self:(part of organism)
- **Own** organs, cells and proteins
- Commensal bacteria

Non-self (not part of organism):
- Non-harmful particles of food or pollen
- Pathogens (bacteria, viruses. . .)

Innate:
Unspecific and **immediate** (hours)

Adaptive:
Specific and **slow** (days)
Lymphocytes (B and T cells)

Evading (block reaction):
- Avoid cell attachments or phagocytosis (for example through "self"-markers) – **physical**
- Avoid protein adsorption with hydrophilic, non-fouling coatings –**biochemical**

Activate:
- Deliery of **cytokines** → stimulate immune cell proliferation and recruitment
- **Vaccines** that expose the immune system to specific antigens
- **Hydrogels** loaded with immune-stimulatory substances

Immunotherapies

- use engineered **antibodies** to **boost** the **immune response**
- antibodies can be **modified** and mass-produced to **trigger** an amplified and **targeted** therapeutic **response**
- CAR-T cells: New form of cancer therapy where T cells from patient's blood are **genetically modified** to **attack specific** proteins on **cancer cells**

Foodprocessing

- Yougurt
- Cheese
- Beer
- Milk

Meat alternatives

Plant based meat alternatives, from **pea protein**.
Lab grown meat alternatives, cell cultures grow into synthetic muscle tissue.

Microbiome

All the bacteria and microbes within the GI tract.
Functions:
- Barrier integrity
- Mucus production
- Food metabolism
- Transform food products into chemicals that act as **signaling molecules**.

Dysregulation:
- Leads to diseases (ex: anxiety, depression, insulin resistance, etc)

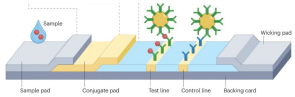
Solutions:
- **Probiotics**: bacteria that metabolize food sources into signals that regulate **homeostasis** in body.
- Design **microphysiological models** (eg. OoaC) that capture the interactions between the gut microbiome intestinal cells.

Drug Delivery

Diagnostics

RT-PCR tests: real time polymerase chain reaction
- Amplification of nucleic acids is done through cycles of **DNA elongation** using **DNA polymerase** (temperature controlled)
- Steps: DNA denaturation, primer annealing, elongation of new DNA strand
- At each cycle, the number of DNA fragments doubles (measured **optically with fluorescent dye**)
- Higher **sensitivity** than lateral flow tests

Lateral flow tests: rapid antigen tests
- **Detection of proteins** (antigens) through immobilization of a **receptor-nanoparticle complex** on a substrate.
- **Antibodies bind to antigens** in the sample and **antigens** simultaneously **bind** to capture **antibodies** immobilized on **test line**.
- The **antigen** is sandwiched between the **capture antibody** and the **detection complex**.
- Signal depends on: the amount of virus, flow, diffusion of antigen proteins and kinetics of reception-antigen binding.



Prophylactics

Vaccine breaktrough (COV 19):
Requirements:

mRNA sequences:
- increased stability
- longer half-life
- higher translation efficiency

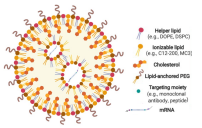
mRNA sequence carrier:
- Protect cargo
- Carry it across the tissue barrier
- Target specific cells
- Allow the mRNA to escape the endosome

Which **barriers** need to be **crossed**?
- Extracellular barriers (blood vessels)
- Intracellular barriers (LNPs endocytosis)
- Endosome and LNP degradation
- mRNA free in cytoplasm

Solution → LNPs

Lipid Nanoparticles (LNPs)

Needed to **avoid digestion of mRNA** by nucleases (foreign body response) and to facilitate endocytosis.



LNP include 4 **main components**:
- **phospholipids:** stabilize shape
- **ionizable lipids:** help RNA to escape endosome
- **cholesterol:** reduce permeability
- **PEGylated lipids:** help avoid immune response

How are **LNPs assembled**?
- **Lipids and cholesterol** are dissolved in an **organic phase** (e.g. ethanol)
- **mRNA** is dissolved in an **aqueous buffer** (low pH)
- **Rapid mixing** of both phases leads to LNPs assembly

Bioengineering FS2025 (Tibitt, Labousse)
Basierend auf TA summary slides und slides der Vorlesung

Keine Gewähr für Richtigkeit und Vollständigkeit
Viel Spass beim Lernen :))

Neuste Version:
`https://github.com/Skinny-King/Bioengineering/tree/main`