Advances in COVID-19 mRNA vaccines

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Advances in COVID-19 mRNA vaccine development

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Jakeline de Oliveira Carvalho

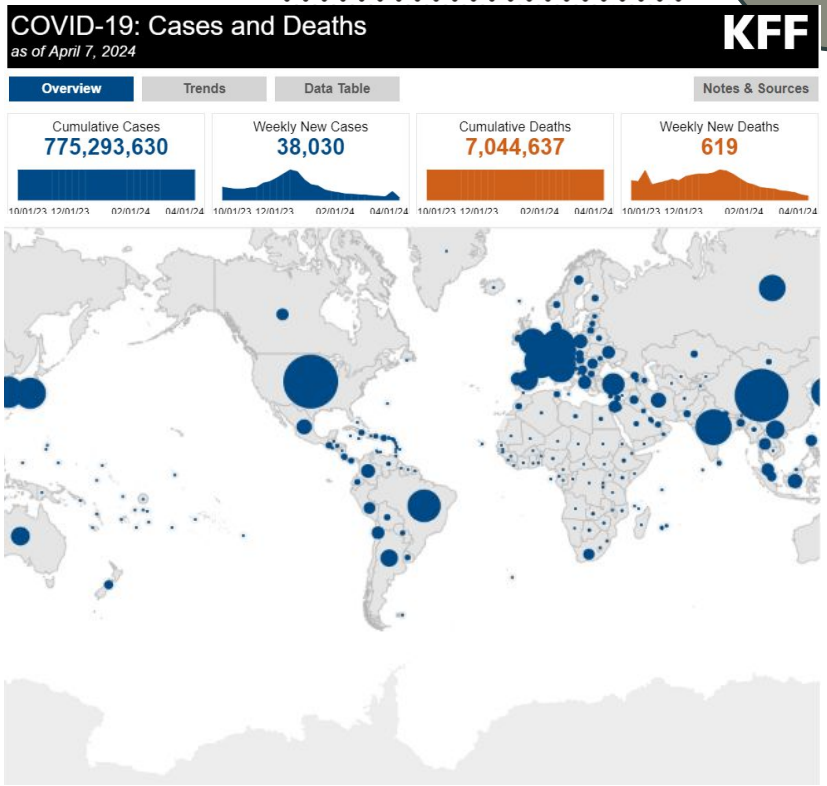
INTRODUCTION

COVID-19

Is an emerging disease caused by severe acute respiratory syndrome coronavirus 2 (SARSCoV-2).

SARSCoV-2

Enveloped positive-sense ssRNA virus of the Betacoronavirus genus included in the Coronaviridae family.



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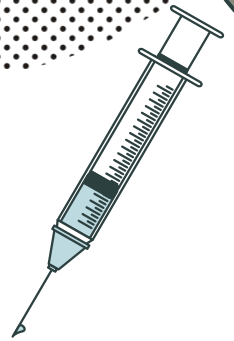
Control the spread of the epidemic

Several approaches have been developed for the COVID-19 vaccine.

Inactivated-virus	Adenovirus vector
Live attenuated	Influenza virus vector
Recombinant protein	mRNA and DNA vaccines



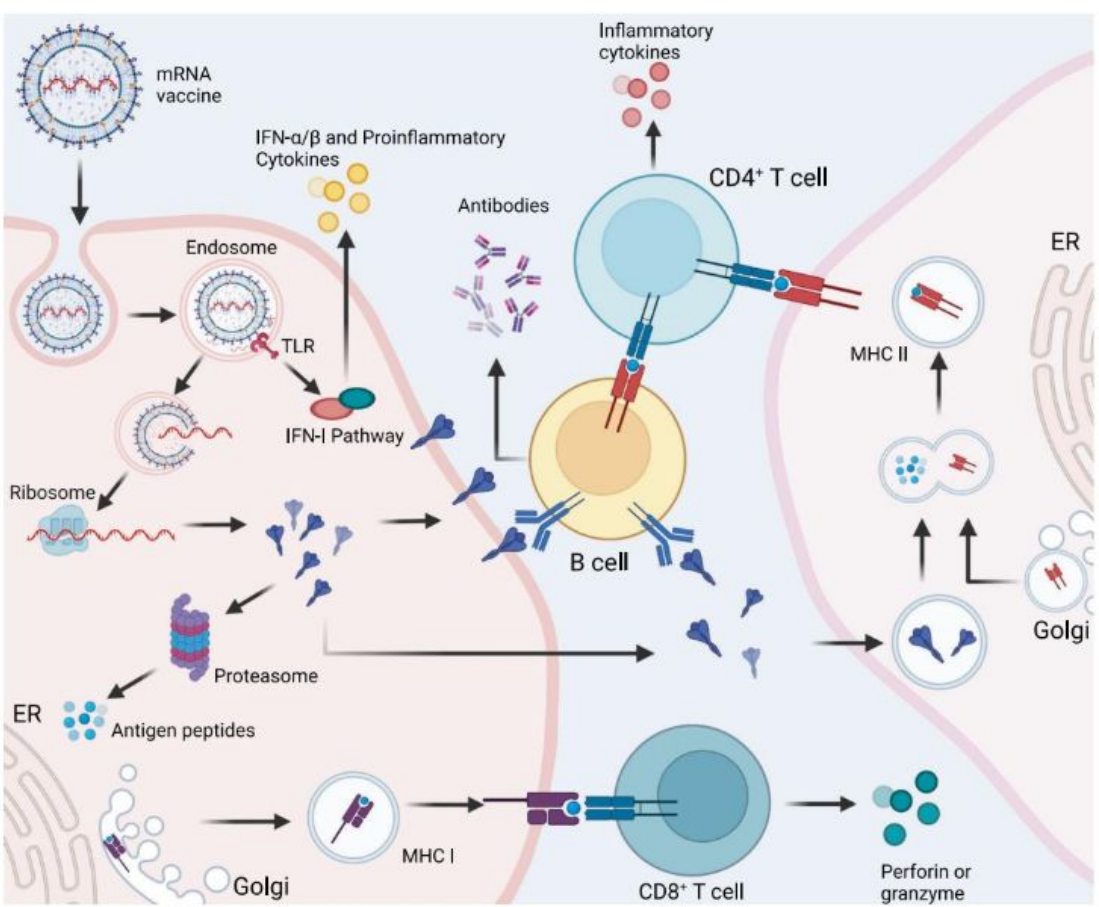
mRNA vaccine

Based on a system that allows the delivery of a nucleic acid molecule that encodes the antigen of interest in the target cell in the host.

Three types of host cells can be transfected

(1) non-immune cells
(2) immune cells found in the tissues at the injection site
(3) immune cells in peripheral lymphoid organs

Cellular and humoral immune responses induced by messenger RNA (mRNA) vaccine.



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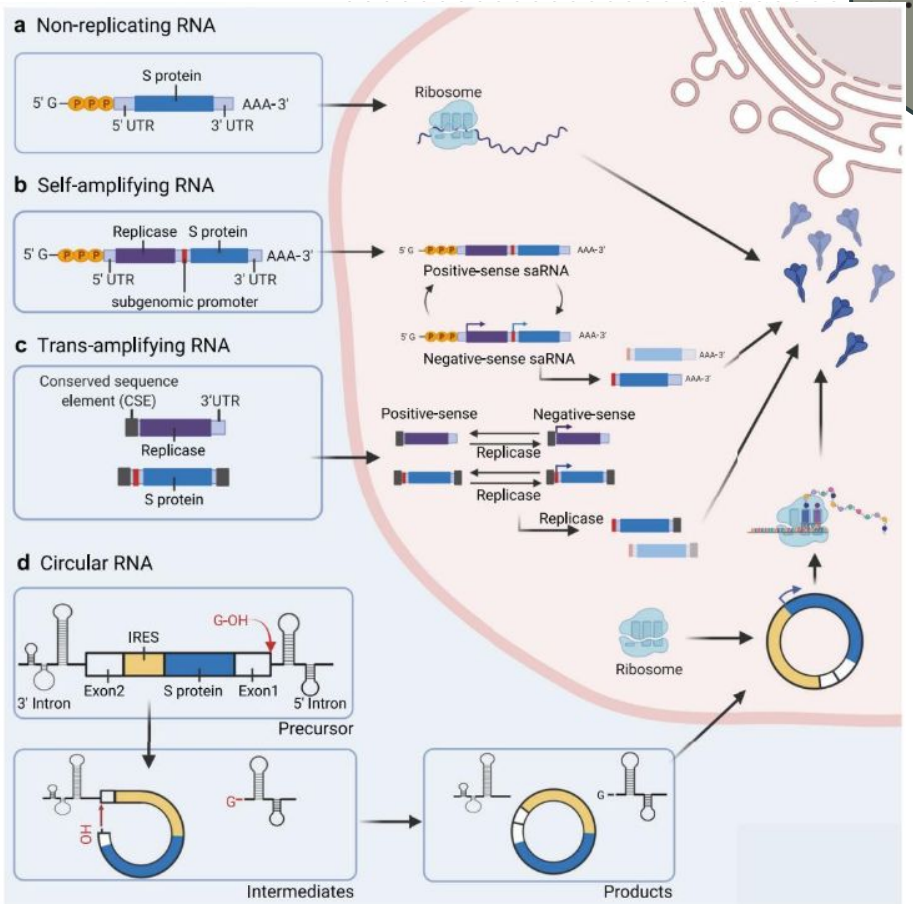
Different types of COVID-19 mRNA vaccines

Types of mRNA vaccine

mRNA vaccines can be categorized as non-replicating mRNA, selfamplifying mRNA (saRNA) and circular RNA (circRNA).



Non-replicating mRNA

Contains an open reading frame (ORF) encoding the gene coding for the target antigen flanked by 5’ and 3’ UTR.

They exclusively offer genetic information that modifies the target antigen.

Important Features

- The 5′-cap structure prevents mRNA from degradation by exonucleases;

- The poly(A) tail plays an important role in maintaining mRNA stability and translation efficiency

- RNA is encapsulated in lipid nanoparticles

Pfizer-BioNTech (BNT162b2) e Moderna (mRNA-1273)

Selfamplifying mRNA (saRNA)

Contain an RNA sequence encoding the target protein and genetic sequences derived from RNA viruses.

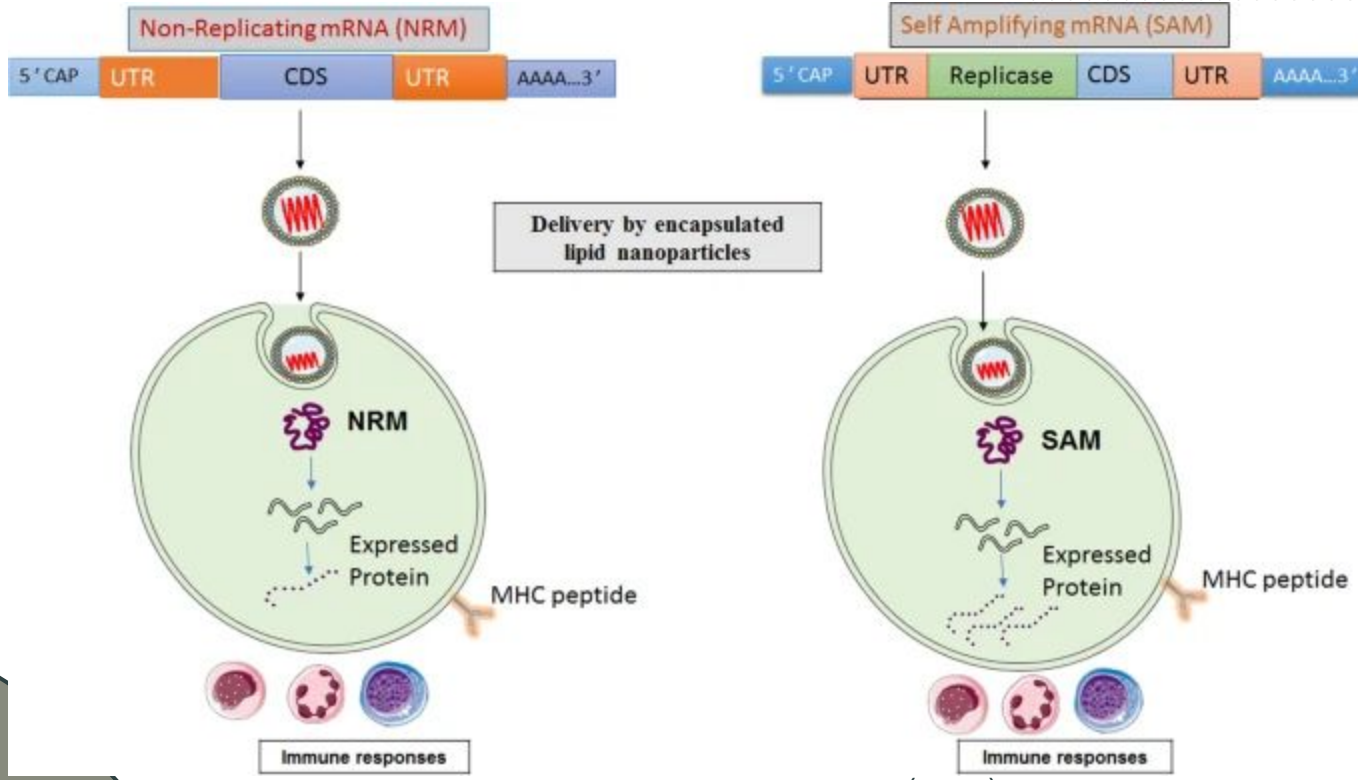
Have the ability to replicate after being introduced into host cells.
Additional sequences used in saRNA vaccines are often derived from the alphavirus.

mRNA in the Mordena vaccine (100ug)
PfizerBioNTech vaccine (30ug)
saRNA (0.1 ~10ug)

Advantages of using the saRNA vaccine

Can be produced with ultra-low doses of saRNA
(1) Greater production potential
(2) Reduced side effects
(3) Allows combination with other vaccines
(4) High levels of antigen expression and long-term duration of immunity

Replicases, transcriptases, or RNA polymerases.



Source: Bhattacharya; et al (2022)

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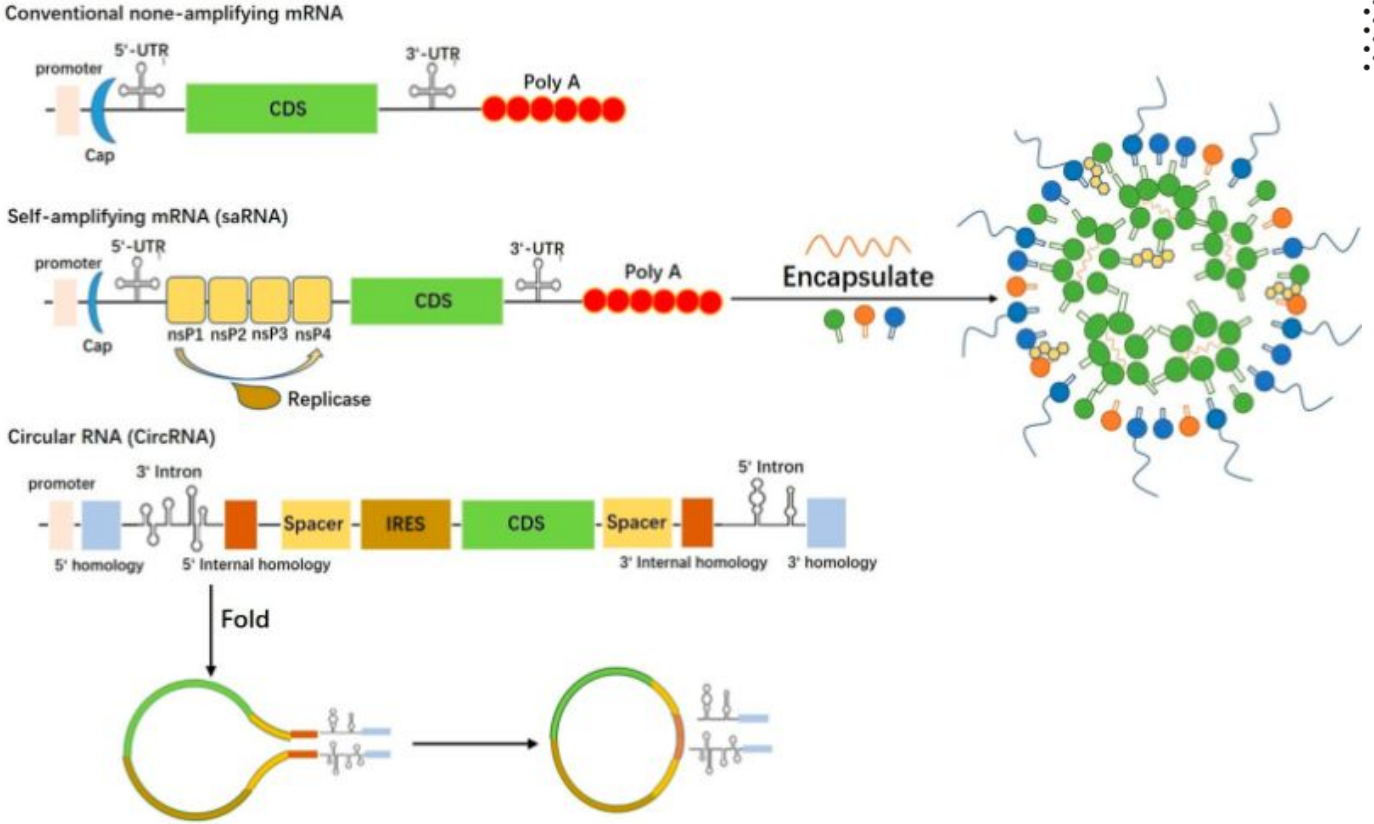
Circular RNA (circRNA)

is a highly stable single-stranded RNA with a covalently closed loop structure.

Absence of essential elements for cap-dependent translation
CircRNA can be translated by adding the IRES.

Advantages of using the circ(RNA) vaccine

(1) Structure of circRNA protects from exonuclease degradation
(2) Unmodified circRNA has been shown to induce TLR/RIG-I-mediated innate immune response



ZHOU, Wenshuo et al. Vaccines' New era-RNA vaccine. **Viruses**, v. 15, n. 8, p. 1760, 2023.

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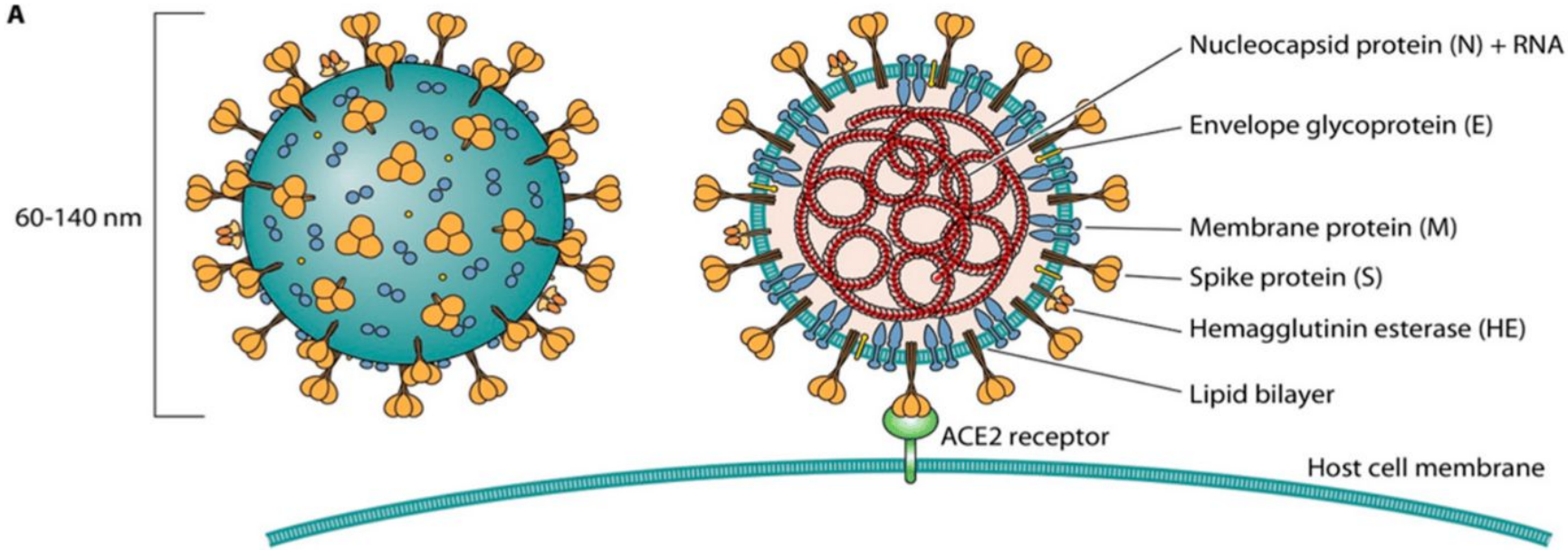
Antigen design for COVID-19 mRNA vaccines



Source: Gitman; et al (2021)

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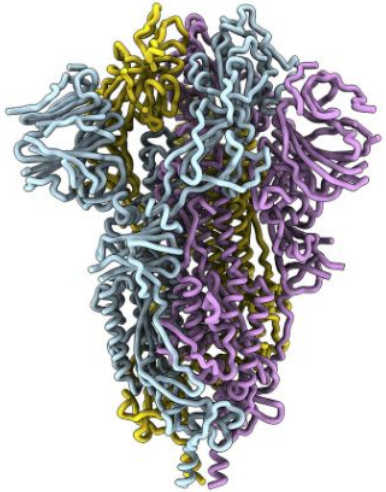

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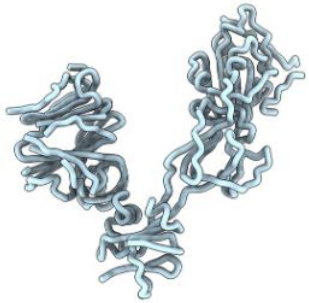
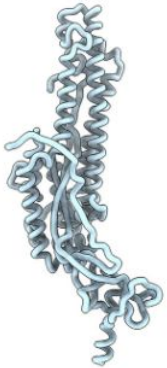


S_1 subunit

S1: Signal peptide (RBD) that interacts with ACE2

S2: Mediates fusion between envelope and cell membrane

C

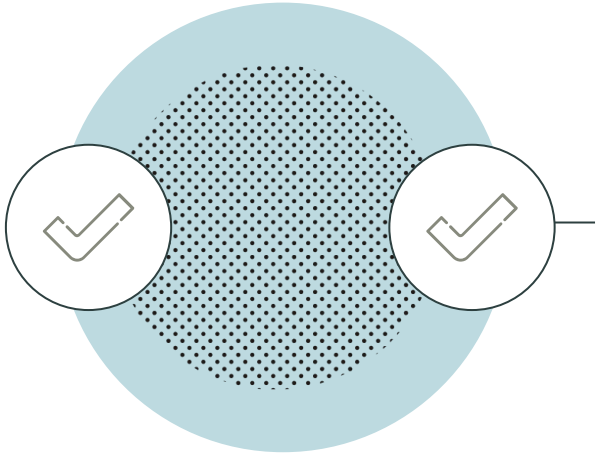


S_2 subunit

Source: Walls; et al (2020)

Insuring stability and safety

Increase in stability of coding sequence and mRNA secondary structure by increasing GC content



Codon optimization- Adjusting the balance between codon usage and tRNA availability

Several optimization tools are available

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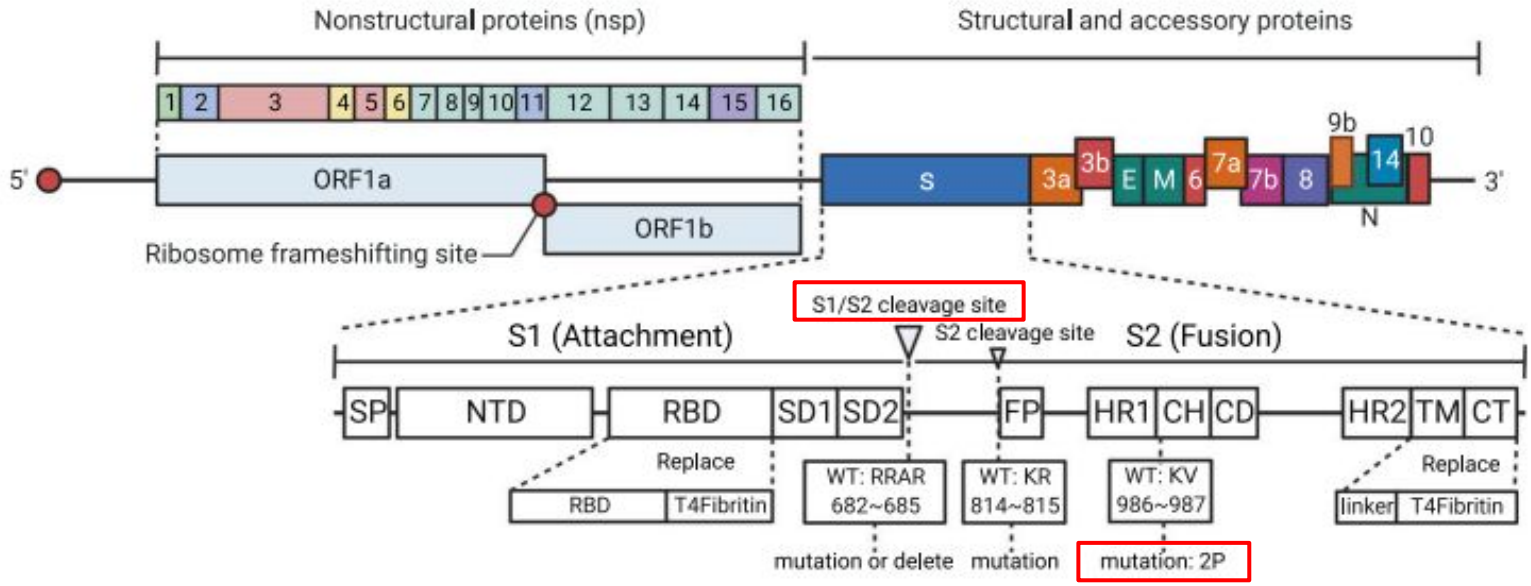
Designing the S protein



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Designing the S protein

2P Mutation strategies

Based on studies with MERS-Cov
Two mutations: K986P and V987P → improve stability of the S protein.
Used by Moderna and BioNtech.

The S1/S2 cleavage sites

Direct deletion of the sequence Q677TNSPRRARYSV687.
Prevents the S protein from cleavage → structural stability and stronger immune response

Table 2. Antigen design strategies adopted for COVID-19 mRNA vaccines

Developers/Vaccine Name	Antigen	Nucleotide modification	2Pmut	S1/S2 Cleavage site	Additional design	Reference (s)
BioNTech/BNT162b2	Spike	+	+	−	NA	27
Moderna/mRNA1273	Spike	+	+	−	NA	272
CureVac/CVnCoV	Spike	−	+	−	RNActive® technology	480
RiboBio	Spike	+	+	+	T4 Fibritin; S2 mut; Delete FP, TMD, CTD	141
Abogen/ARCoV	RBD	+	NI		NA	185
BioNTech/BNT162b1	RBD	+	NI		T4 Fibritin	278
CanSinoBIO	RBD	+	NI		RBD-CTB fusion protein; RBD-CRM197 fusion protein; CPG adjuvant; TLR adjuvant	481
Stemirna	Spike; S1 subunit; RBD; M; N; E	+	−	−	Insert additional sequences before ORF; LPP delivery systems	276,330
LIVERNA	Spike; S1 subunit; RBD	+	NA	NA	NA	479
Institute of Microbiology, Chinese Academy of Sciences	Spike; S1 subunit; RBD	+	NA	NA	NA	482

2P mut: two proline mutations (K986P, V987P) on the S2 subunit of the S protein to maintain its stability; *NA*: not applicable; *NI*: not involved; *CTB*: cholera toxin B subunit; *CPG*: non-methylated short nucleotides cytosine and guanine; *TLR*: toll-like receptor; *FP*: fusion peptide; *TMD*: transmembrane domain; *CTD*: C-terminal domain; *RBD*: receptor binding domain; *LPP*: lipopolyplex.

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Delivery systems

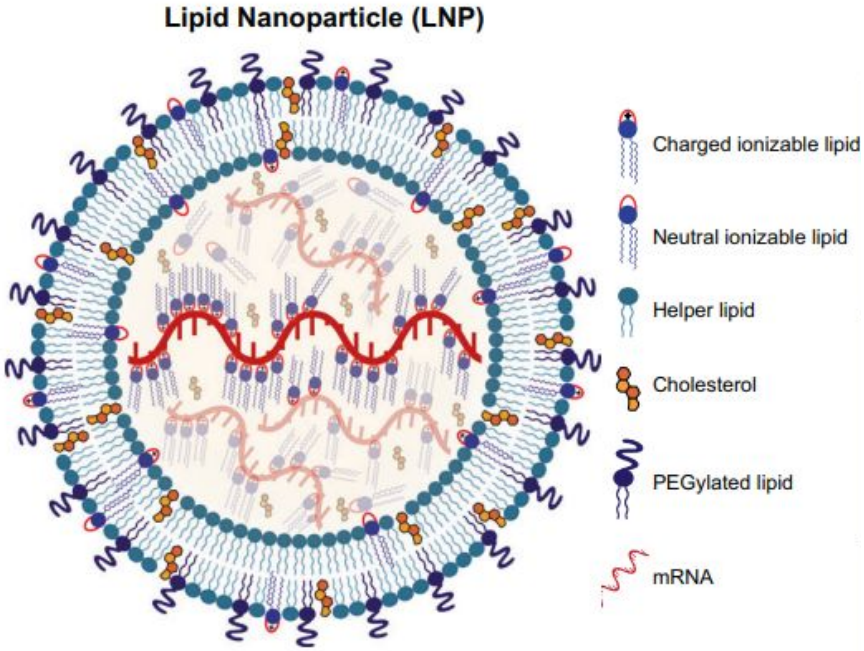
mRNA delivery through the cell membrane can be difficult:

- Large molecular weight (10 kDa - 100 kDa);
- Negative charge;
- Degradation by nucleases.

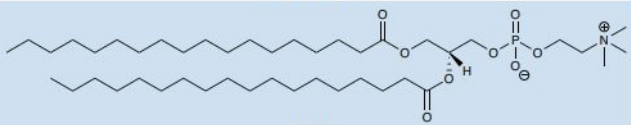
Necessity for new delivery systems

1) LNPs

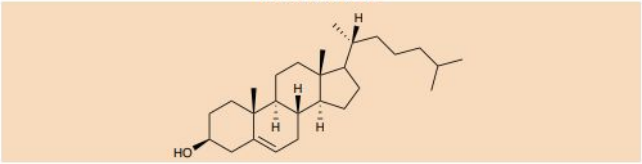
- Lipid Nanoparticle;
- LNP is a nano-scale vesicle which simulates the lipid structure of the cell membrane;
- The most used delivery system;
- Four components: ionizable lipids, helper phospholipids (DSPC), cholesterol, and PEGylated lipids reduce aggregation and non-specific uptake by immune cells (PEG2000-DMG);
- Cons: pro-inflammatory effects, some are prone to degradation and cumulative toxicity;



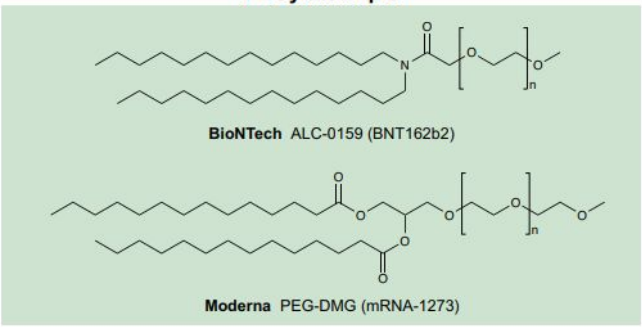
Helper lipid



Cholesterol



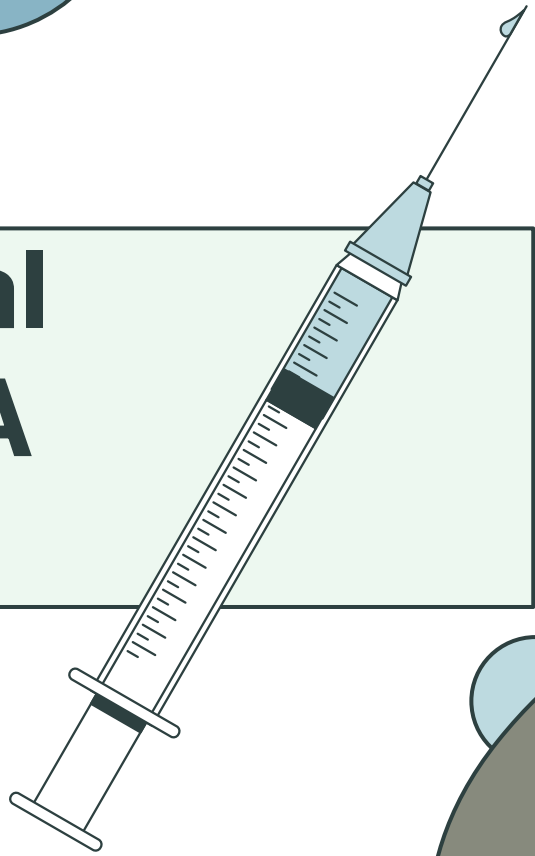
PEGylated lipid



2) Other delivery systems:

- **Polymers** are another widely used mRNA delivery system → better physical stability than lipid carriers;
    - Induces strong cytotoxicity due to the high cationic charge density;
- **Lipid shell-coated LPPs:** condensed mRNA core packaged in a lipid shell;
    - Higher stability, low cytotoxicity, cell delivery and endosomal escape efficiency;
- **Peptide carriers:** com amino acids can electrostatically bind mRNA to form nanocomplexes;
    - Shown to activate TLR7 and TLR8 pathways.

Progress in clinical research on mRNA vaccines



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Progress in clinical research on mRNA vaccines

- The vaccines of mRNA has a delivery systems and nucleic acid modification technology
    - cancer treatment as well as prevention of several infectious diseases
    - myelocytic leukemia (AML), nonsmall cell lung cancer (NSCLC), and melanoma.
- The mRNA vaccine BNT111 developed by BioNTech FixVac platform to treat advanced melanoma.

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Progress in clinical research on mRNA vaccines

- Several mRNA vaccine candidates for viral agents other than SARS-CoV-2 have entered clinical trials
    - influenza virus,rabies virus, Zika virus.
- BioNTech and Pfizer collaborated to develop five COVID-19 mRNA vaccine candidates at the beginning of the pandemic, which were based on nucleoside-modified mRNA (BNT162b2)

Production and quality control of mRNA vaccines

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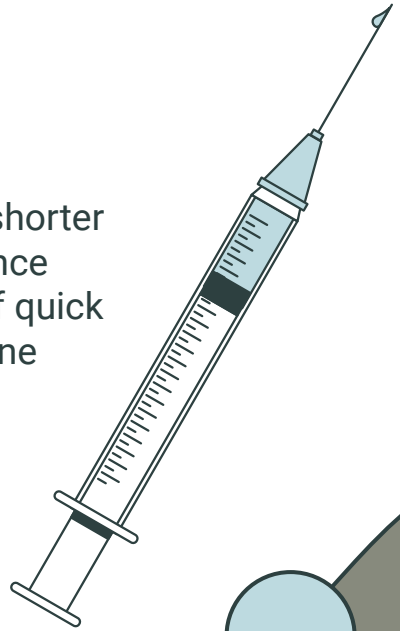
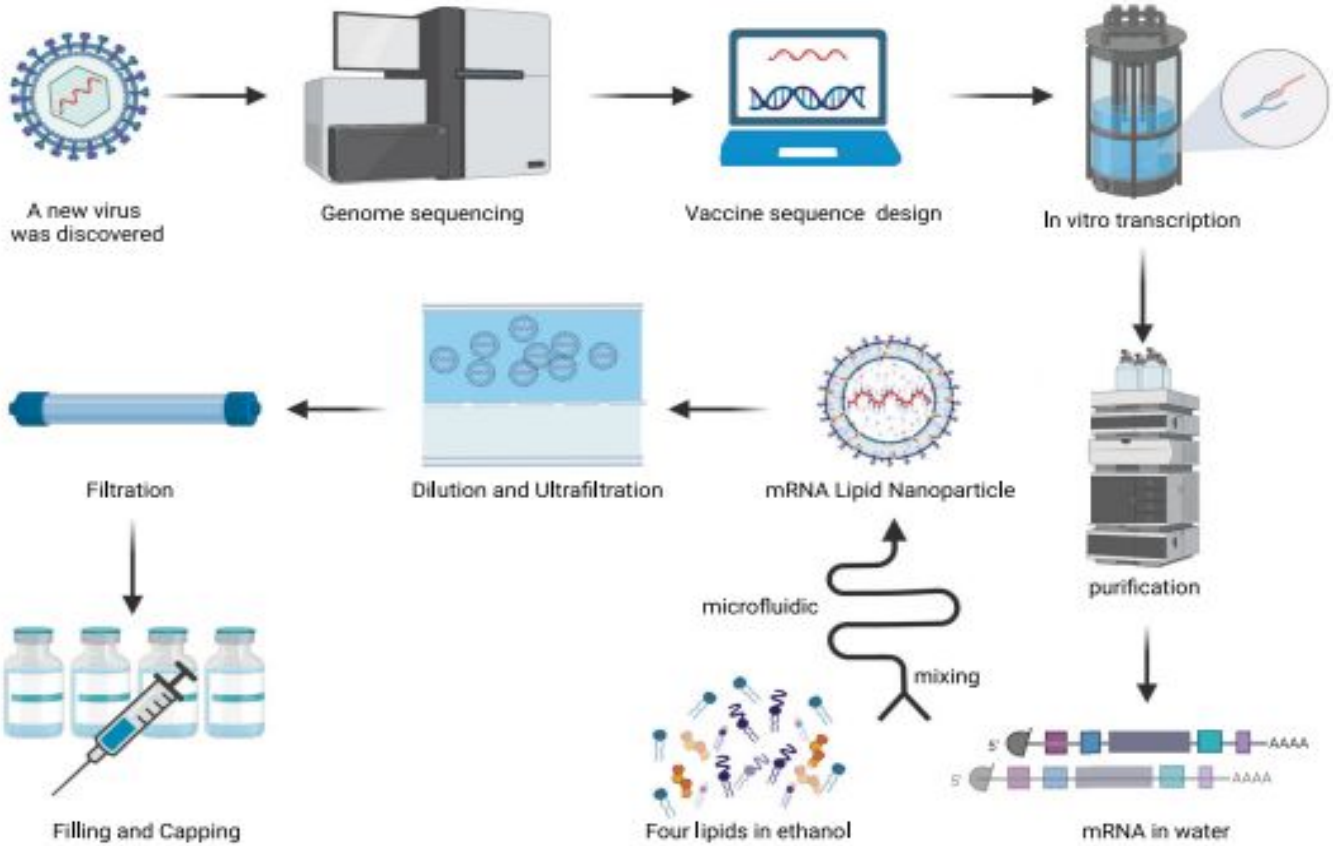
PRODUCTION AND QUALITY CONTROL OF MRNA VACCINES

Production of mRNA vaccines

- Production of mRNA vaccines does not require culturing cells or viruses as in traditional vaccine production technology.
- The production cycle is shorter and easy to scale up, hence offering the possibility of quick industrialization of vaccine production.




A new virus was discovered
Genome sequencing
Vaccine sequence design
In vitro transcription
purification
mRNA in water
Four lipids in ethanol
microfluidic mixing
mRNA Lipid Nanoparticle
Dilution and Ultrafiltration
Filtration
Filling and Capping
5'
AAAA

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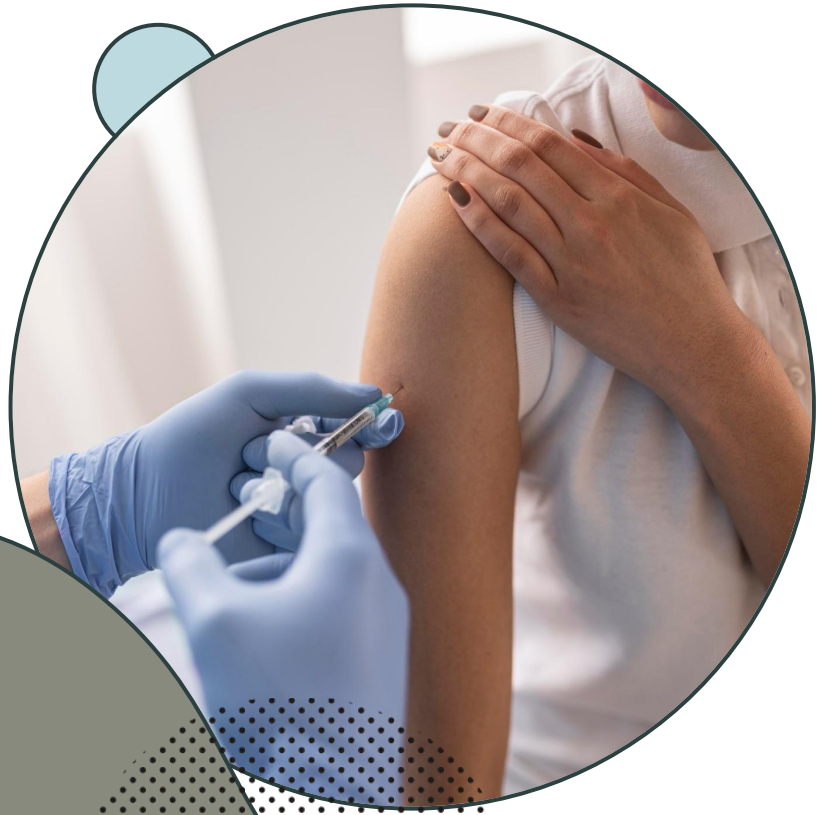
PRODUCTION AND QUALITY CONTROL OF MRNA VACCINES

Quality control of mRNA vaccines

- Safety, efficacy, and quality control of vaccine production are determined by measuring critical process parameters.
- Quality control of mRNA vaccines should adhere to criteria preconized by laws and regulations of the producing countries.
- The mRNA vaccine should be stored and transported under ultra-low temperature conditions, due to the intrinsic instability of mRNA.



CONCLUSION AND FUTURE PERSPECTIVES

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Conclusion

- After over 30 years of research, mRNA vaccines have become a promising technology platform for vaccine development.
- Prior to the emergence of COVID-19, mRNA technology was mostly used for developing novel cancer therapeutic drugs showing promising results.
- In the future, the mRNA technology platform will enable preventing and managing infectious diseases as well as treating other disorders.

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