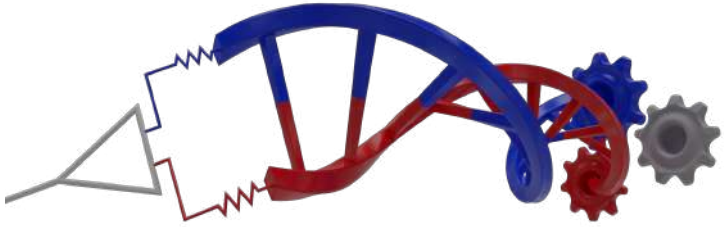
COVID-19 Aşılarının Geleceği ve Yeni Aşı Teknolojileri

Urartu Şeker

Bilkent Üniversitesi UNAM

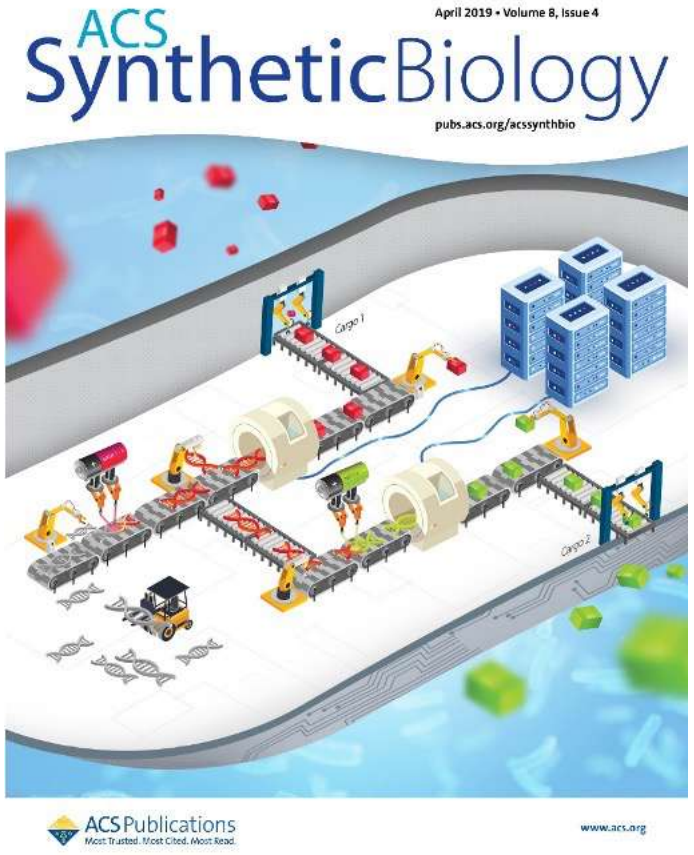
SynBioSys - SekerLab



KLİMİK 2023
Antalya

Synthetic Biosystems Lab @ Bilkent University UNAM

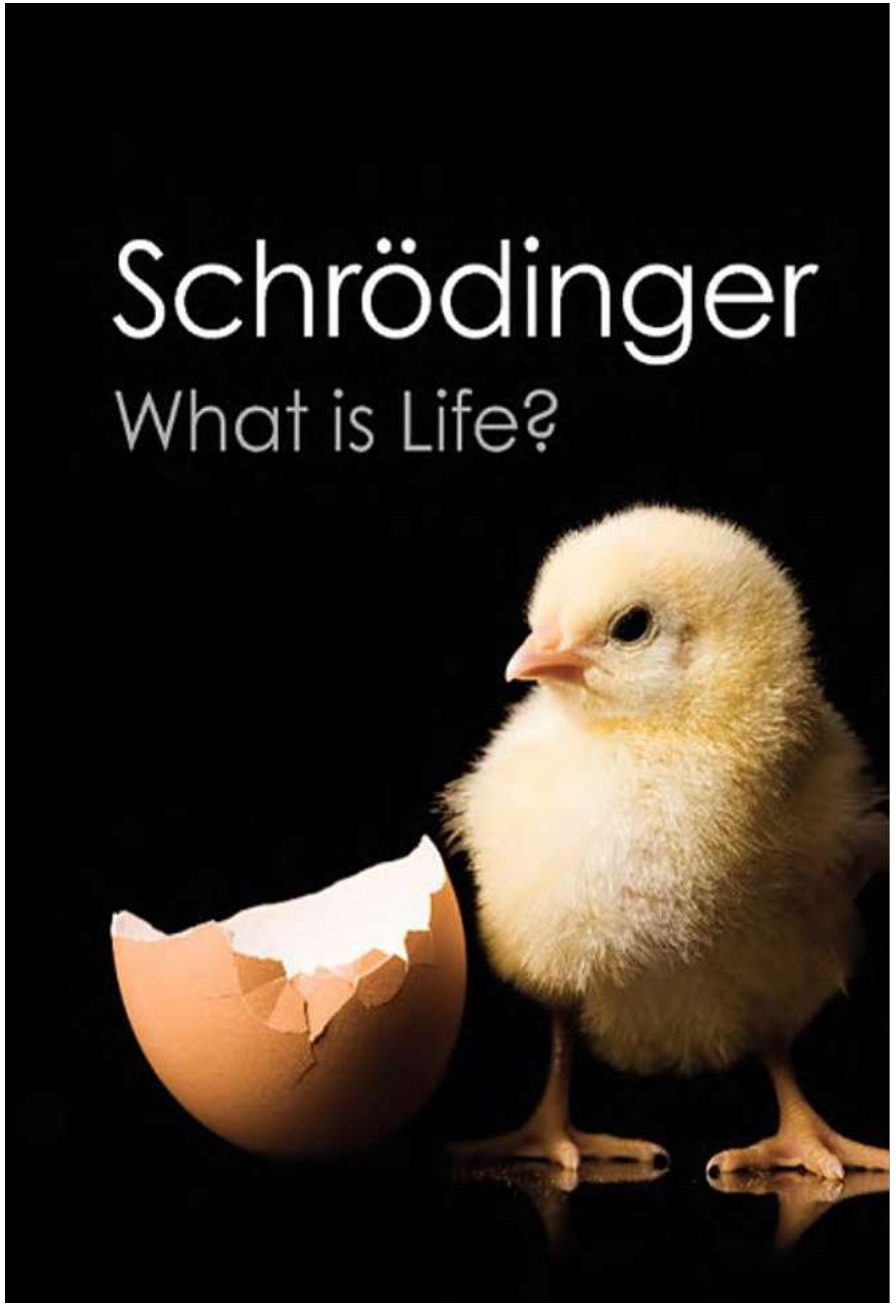
Our interests



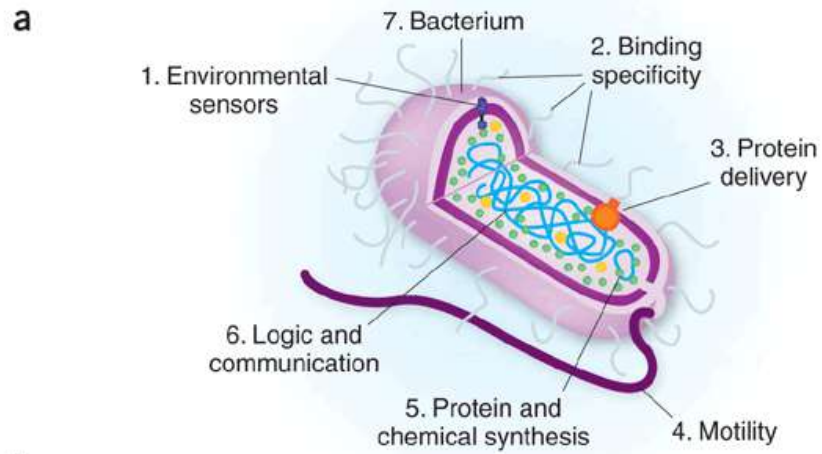
Synthetic biology , *genetic circuit design, living drugs, living materials and living sensors*

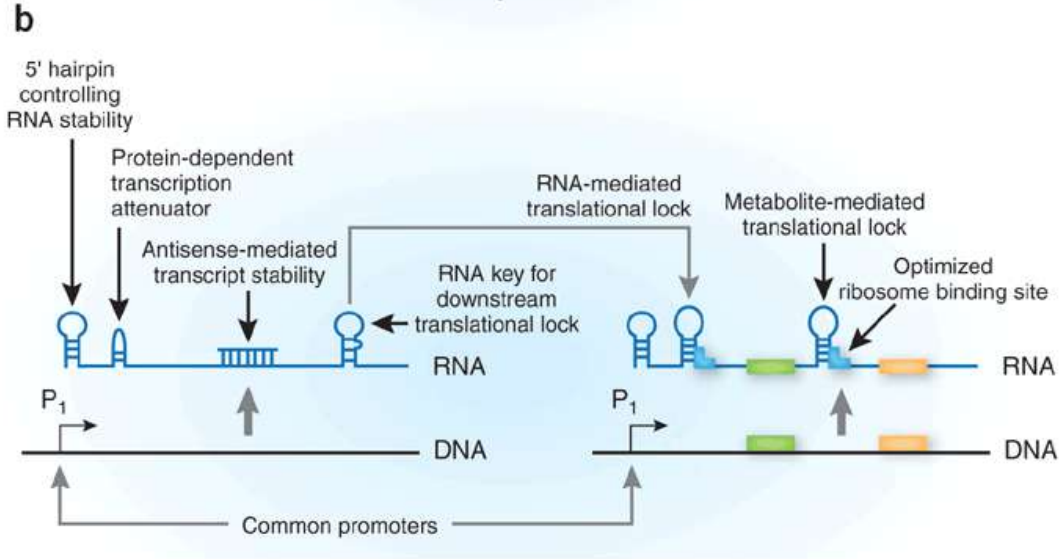
Cell free synthetic biology : *mRNA based Diagnostics, mRNA Therapeutics and mRNA Vaccine development*

Biotechnological Drug Development : *mAbs, host development, cell line development*

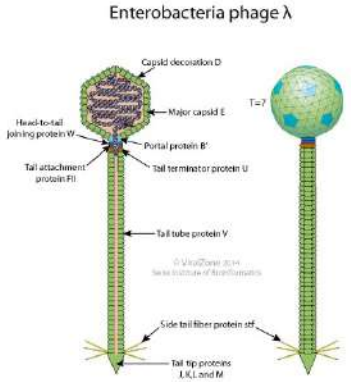

Schrödinger
What is Life?

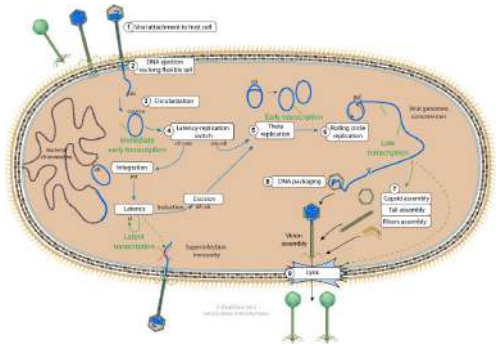
Biological Devices : Biomaterials Through Evolution



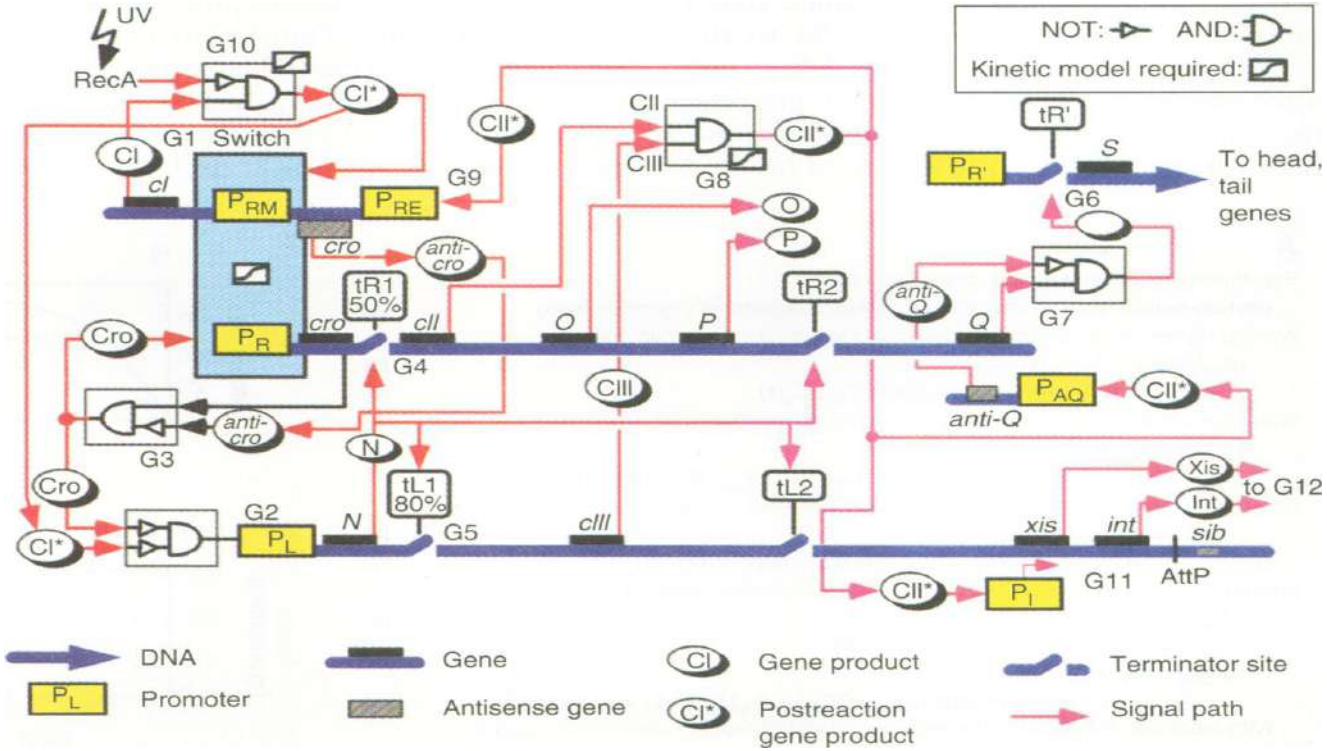


Arkin, A. ***Nature Biotechnology*** *26, 771 - 774 (2008)*



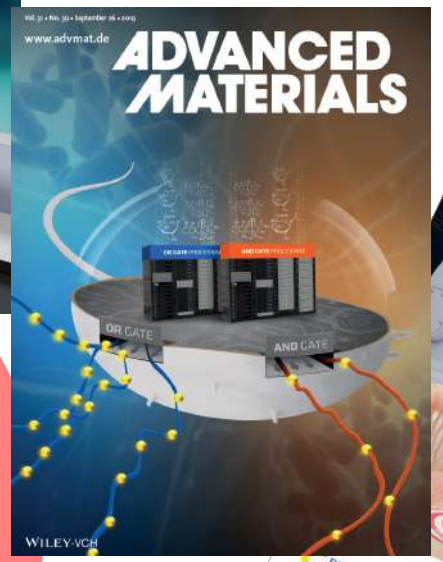


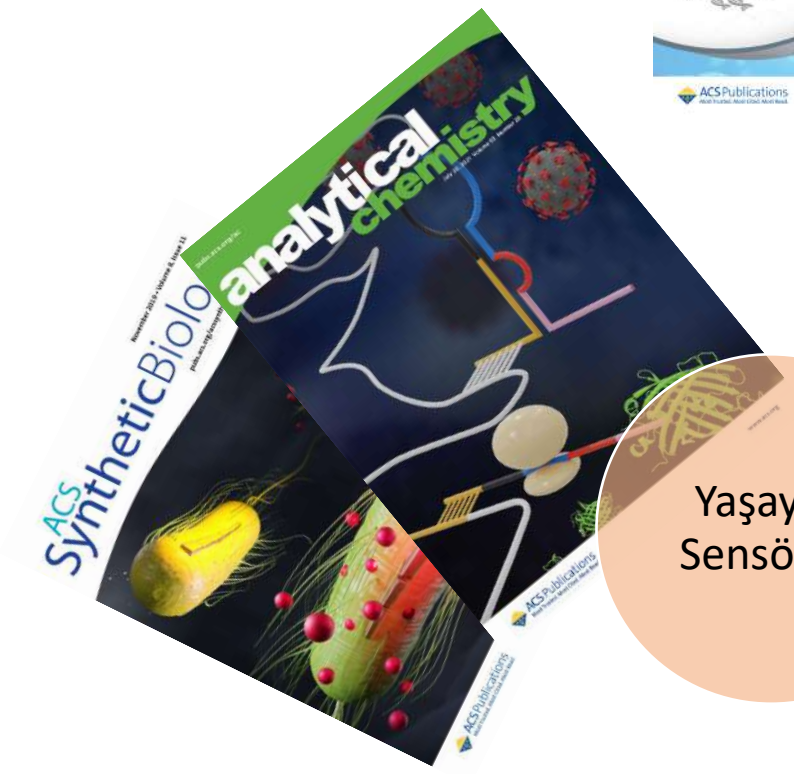
Decision making circuitry used by phage lambda



McAdams and Shapiro, Science, 1995

Biz neler yapıyoruz





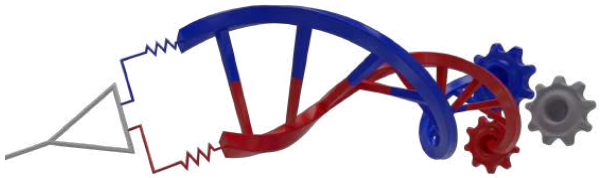
Yaşayan İlaçlar

SENTETİK BİYOLOJİ

Yaşayan Sensörler

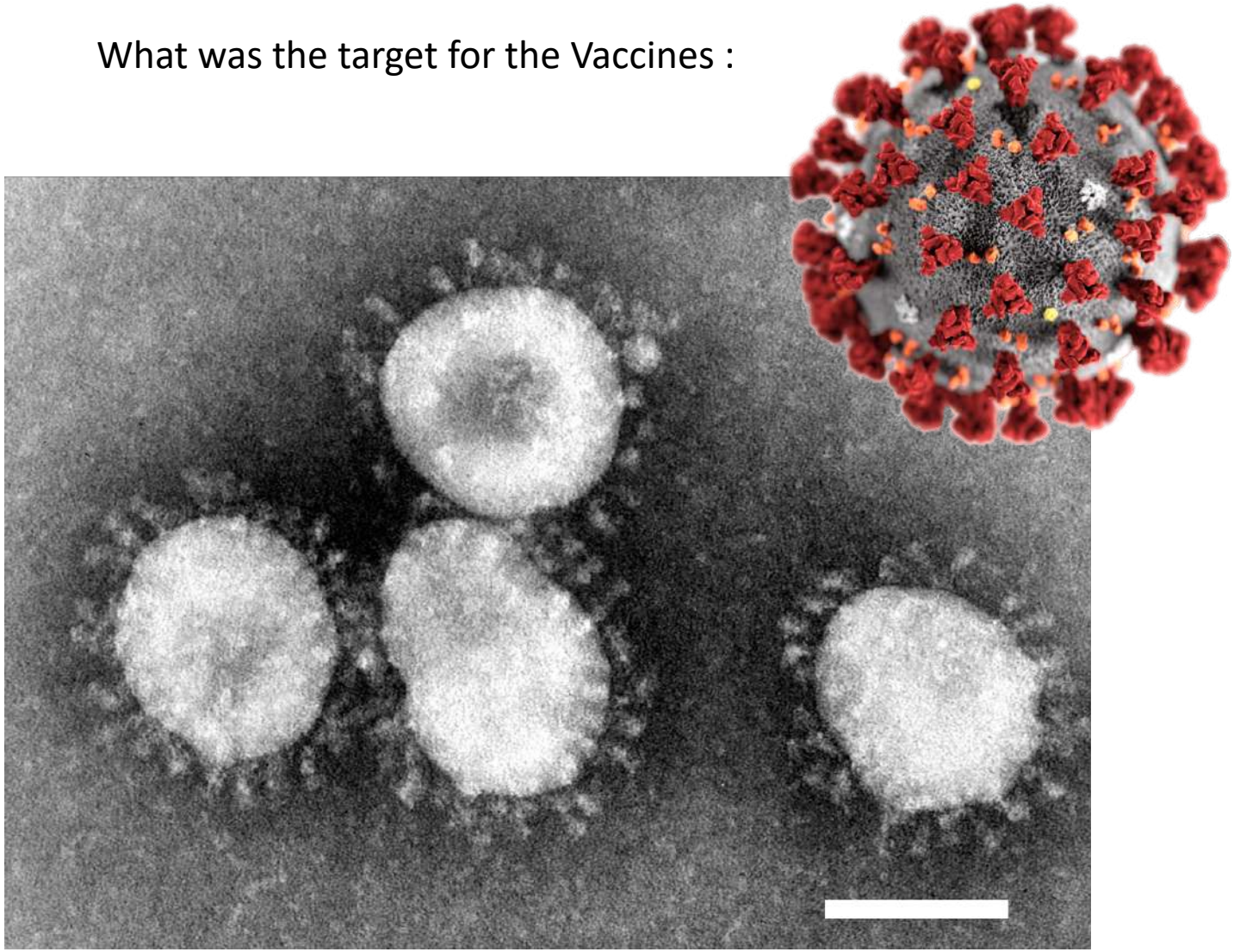
Yaşayan Malzemeler

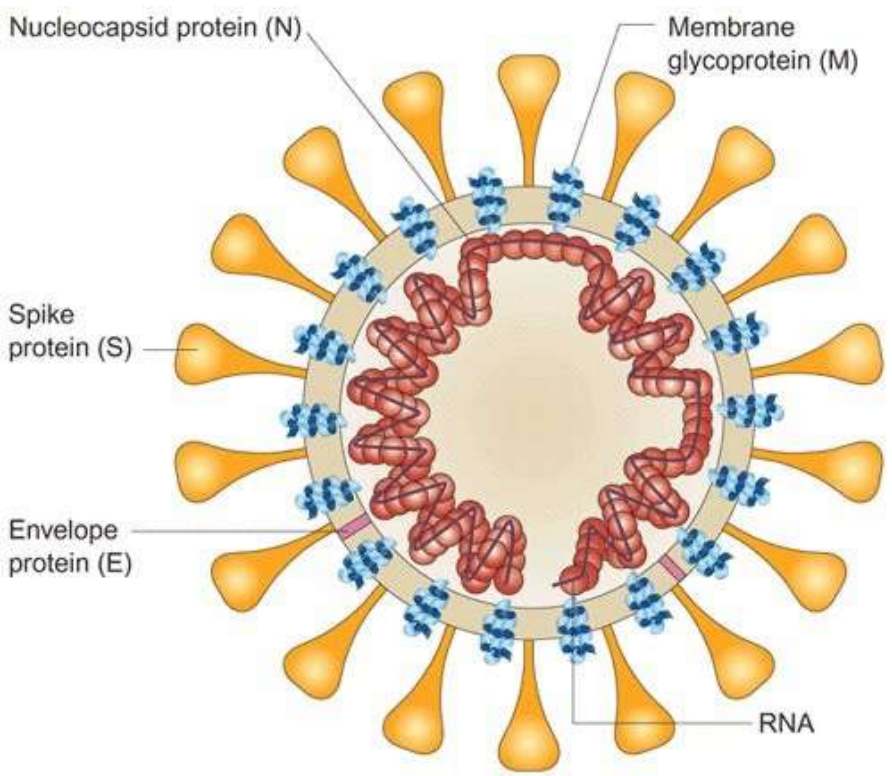
SynBioSys - SekerLab



Vaccines for Emergency Use in COVID-19 Pandemic

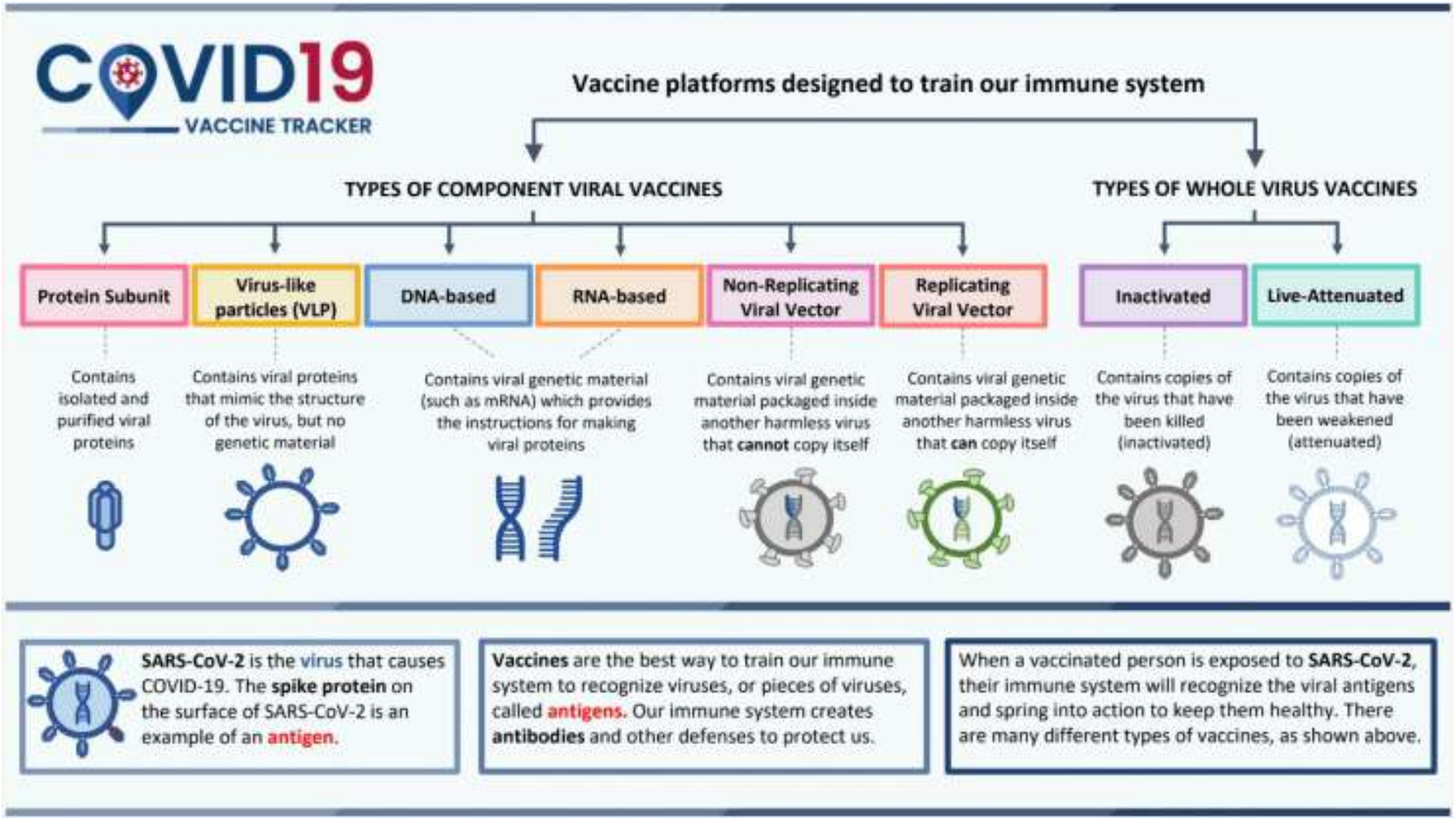
What was the target for the Vaccines :





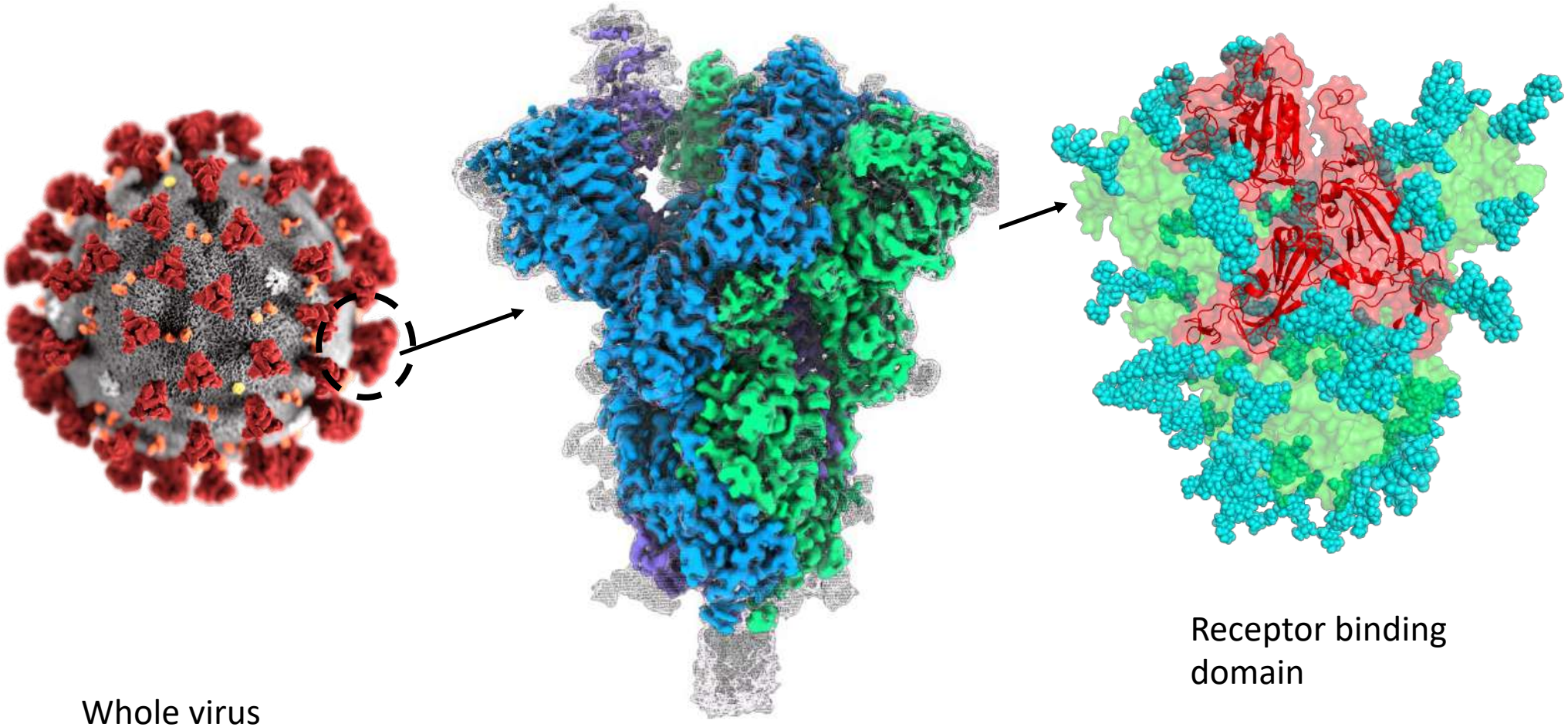
Vaccines for Emergency Use in COVID-19 Pandemic

What was available on the table?



Vaccines for COVID-19 Pandemic

Target antigens



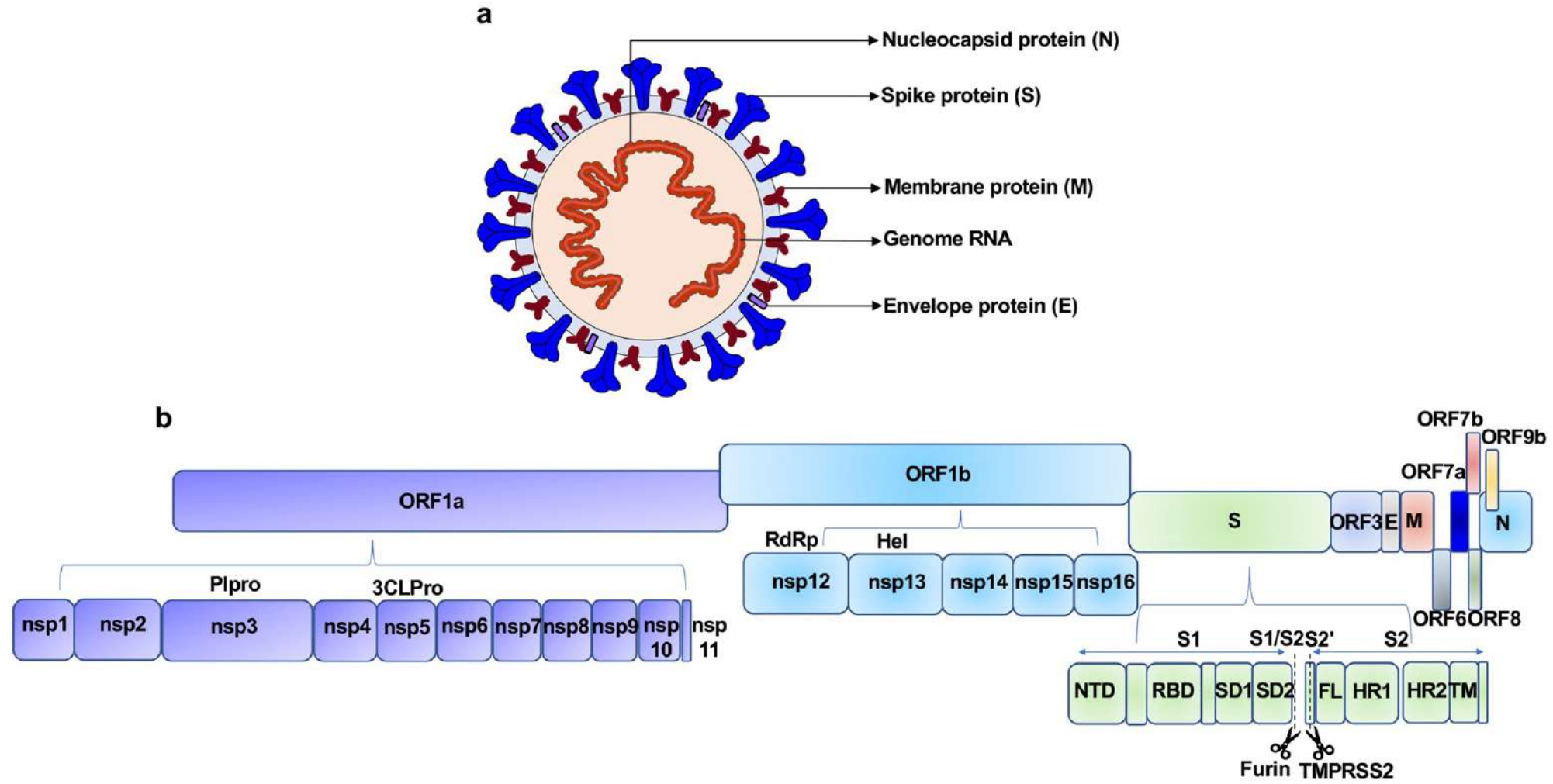
Whole virus

Spike protein

Receptor binding domain

Vaccines for COVID-19 Pandemic

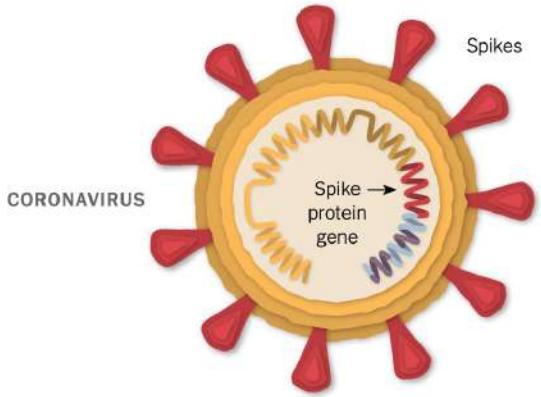
How is the virus encoded?

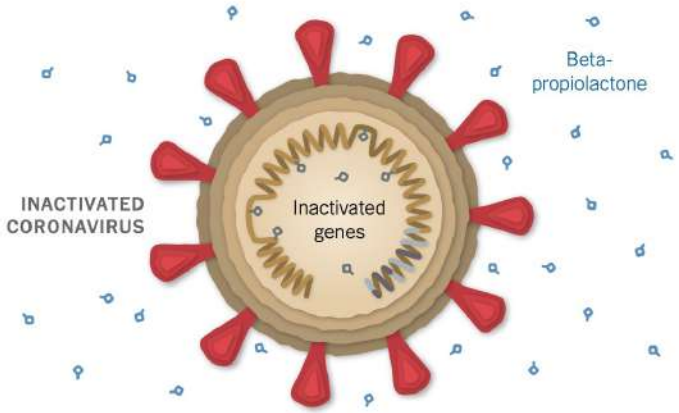


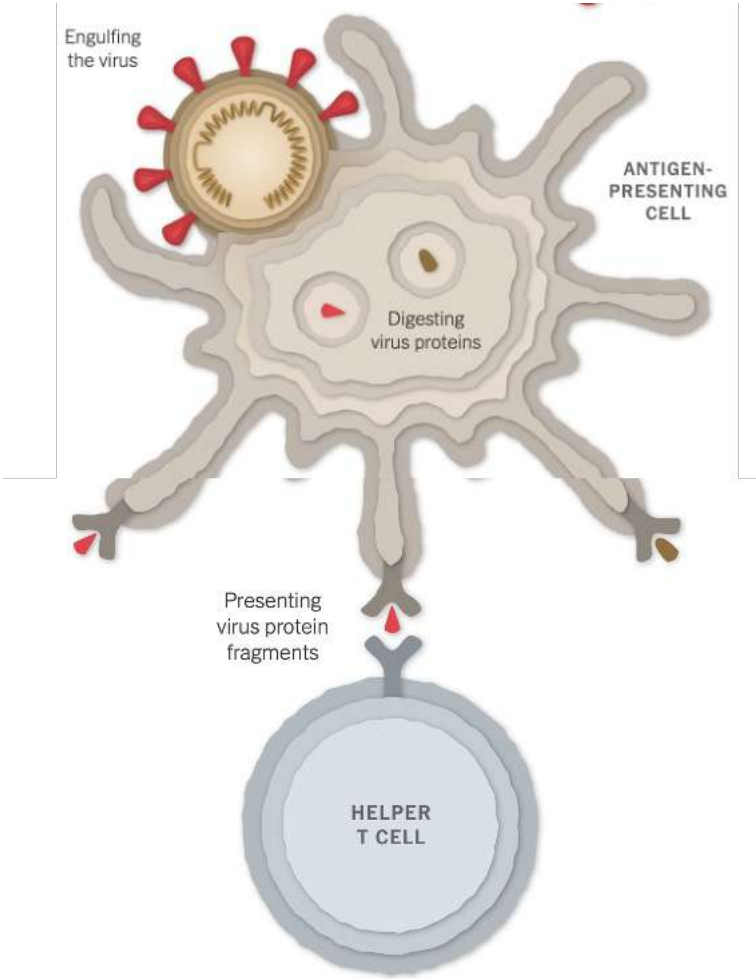
Zhang et al, Sign. Trans. 2021

Vaccines for COVID-19 Pandemic

Use the whole virus as the target





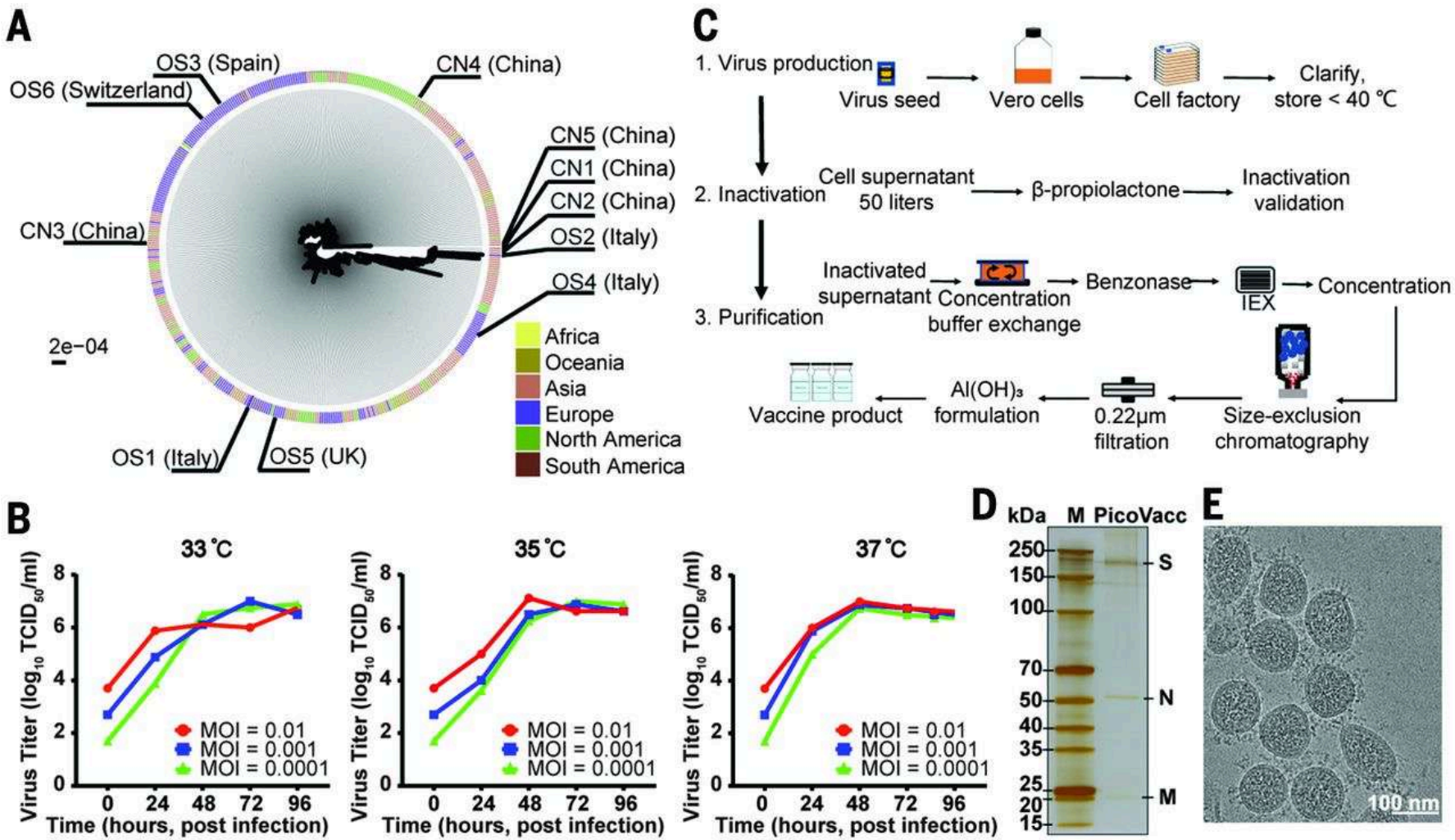


Wikipedia, 2021

Vaccines for COVID-19 Pandemic

Use the whole virus as the target

Coronovac



Gao et al., Science, 2020

Vaccines for COVID-19 Pandemic

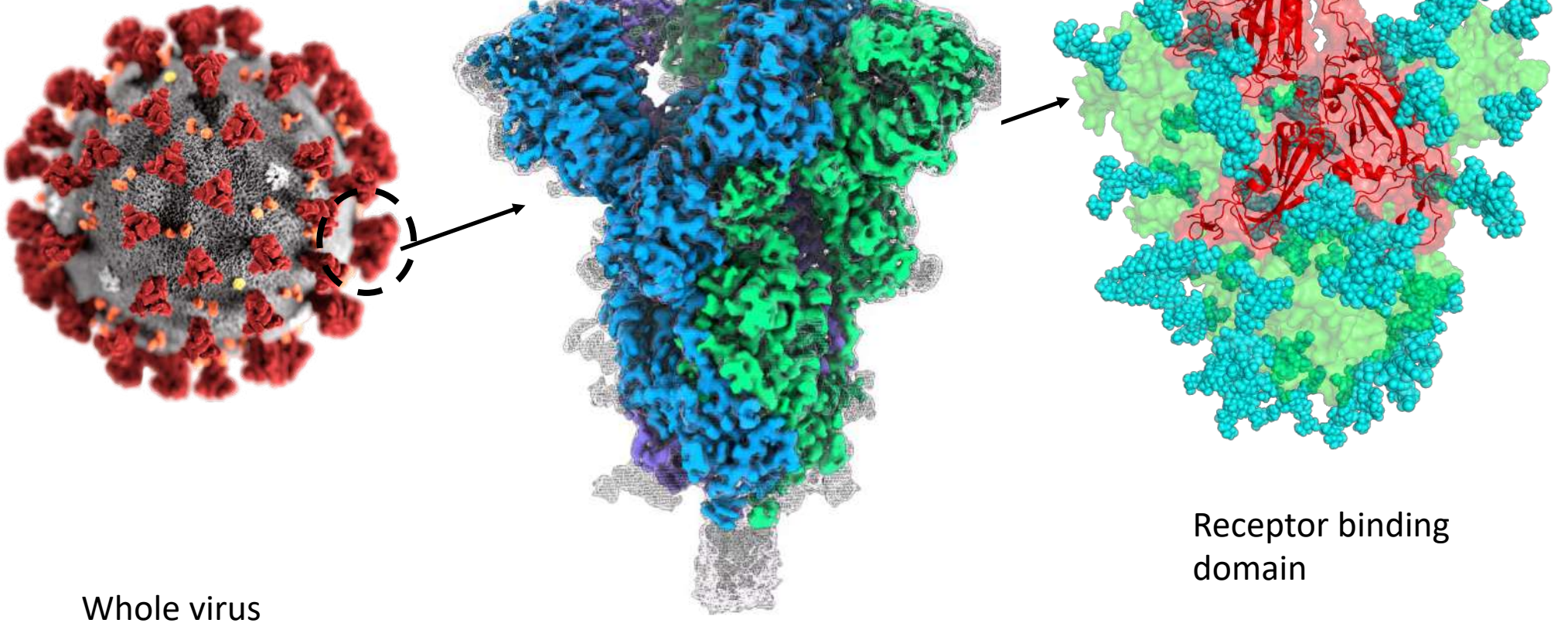
Using the whole virus has some drawbacks (in terms design and production)

- specialized cell lines with high production efficiency
- High level biosafety needs
- Hard to re-engineer for variants

Vaccines for COVID-19 Pandemic

Instead of the whole antigen , code for the core structural domains : Spike protein, N-protein, M protein?

Target antigens



Whole virus

Spike protein

Receptor binding domain

Vaccines for COVID-19 Pandemic

Triggering the immune system with, core domains!
How to deliver ?

- Recombinant proteins, express in the cell line , use your direct injection with adjuvants

Transfection-transcription- translation - purification

- Deliver with a shuttle use cellular machineries , non-replicating viral vectors, adv

Transcription - translation

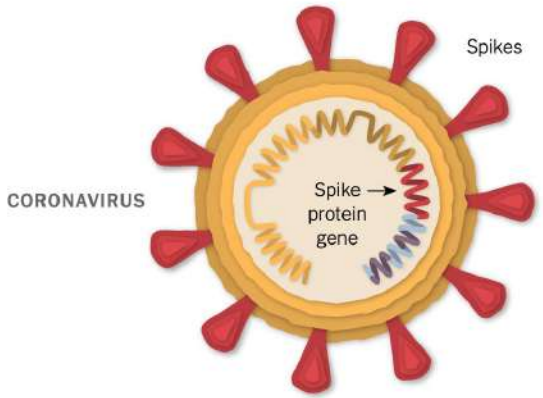
- Deliver the genetic material , let the cell do the job, mRNA

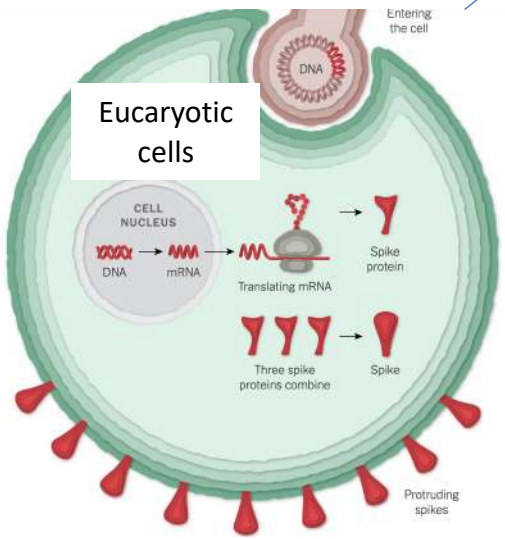
Translation

Vaccines for COVID-19 Pandemic

Novavax-US
Abdala -Cuba

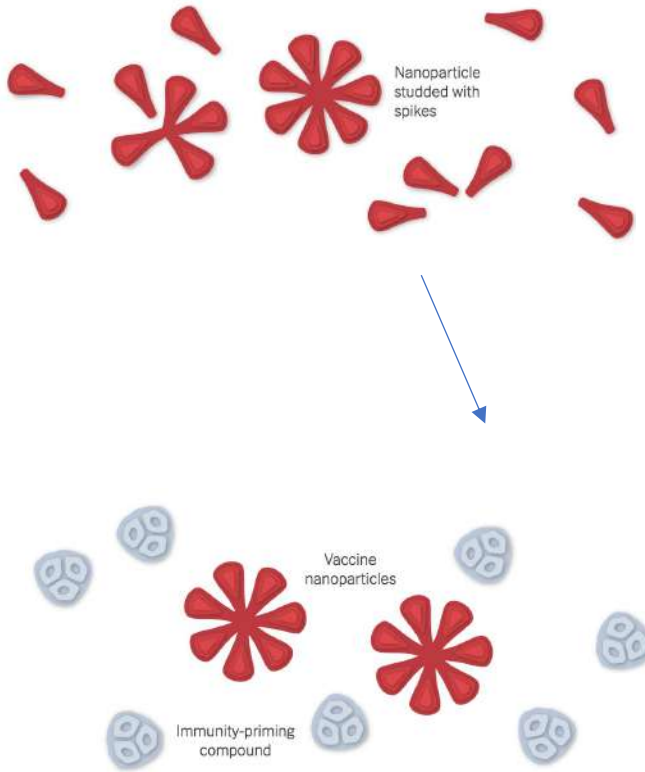
- Recombinant proteins, express in the cell line , use your direct injection with adjuvants

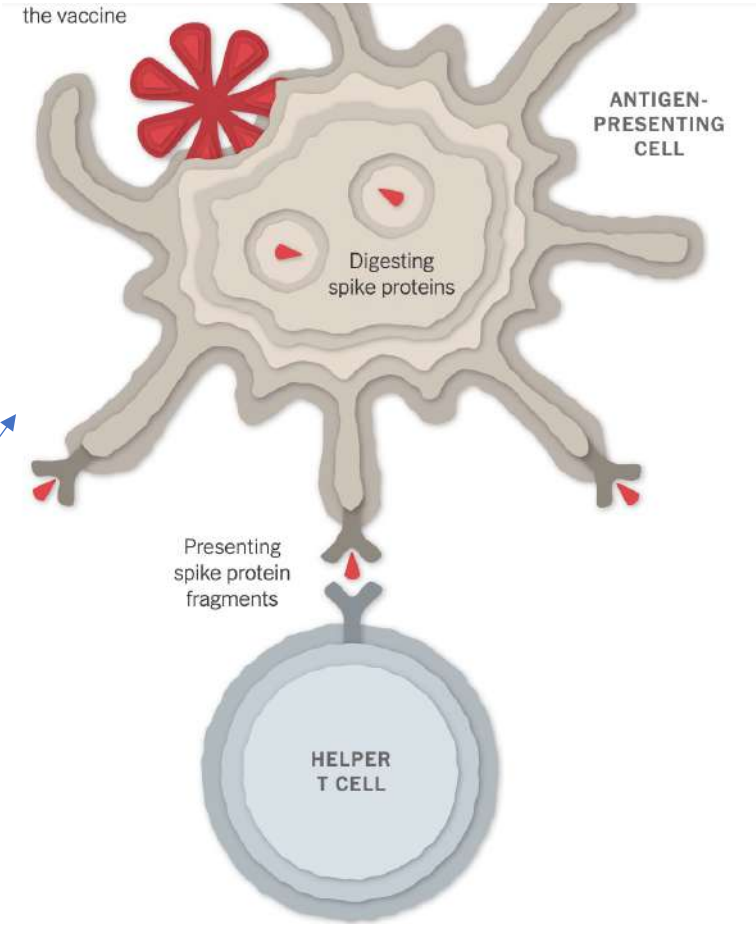




Expression -purification

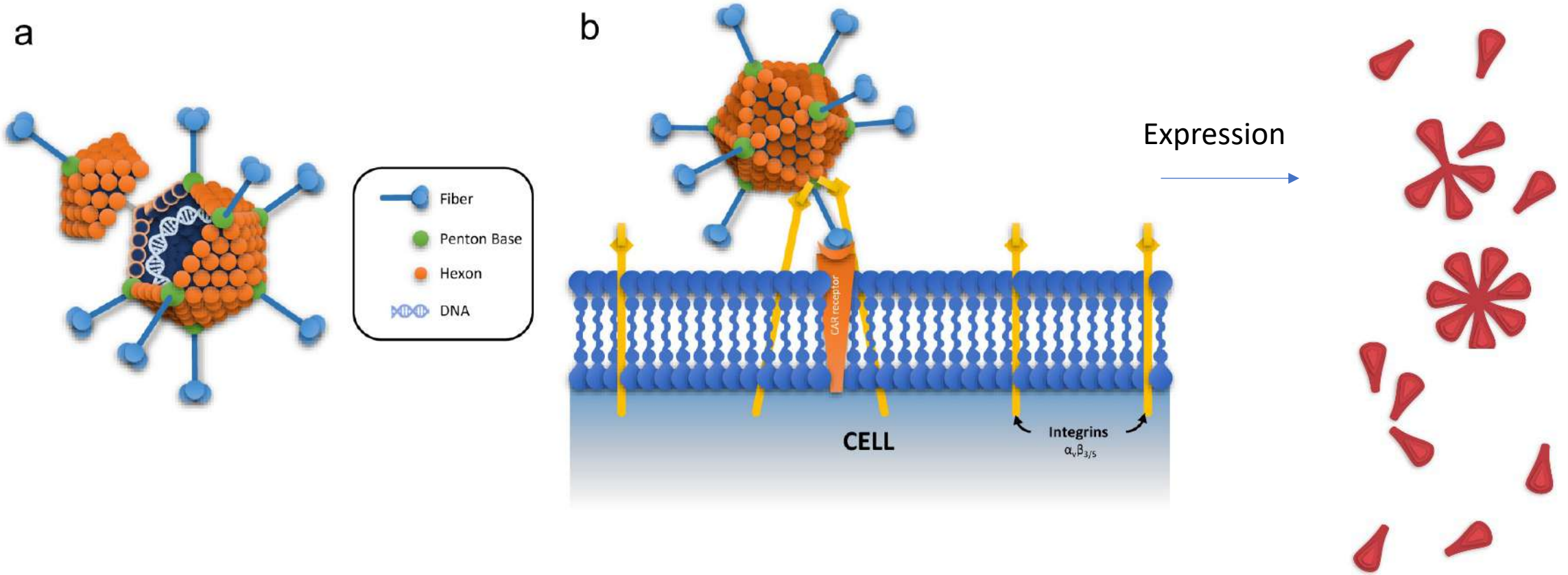
assembly



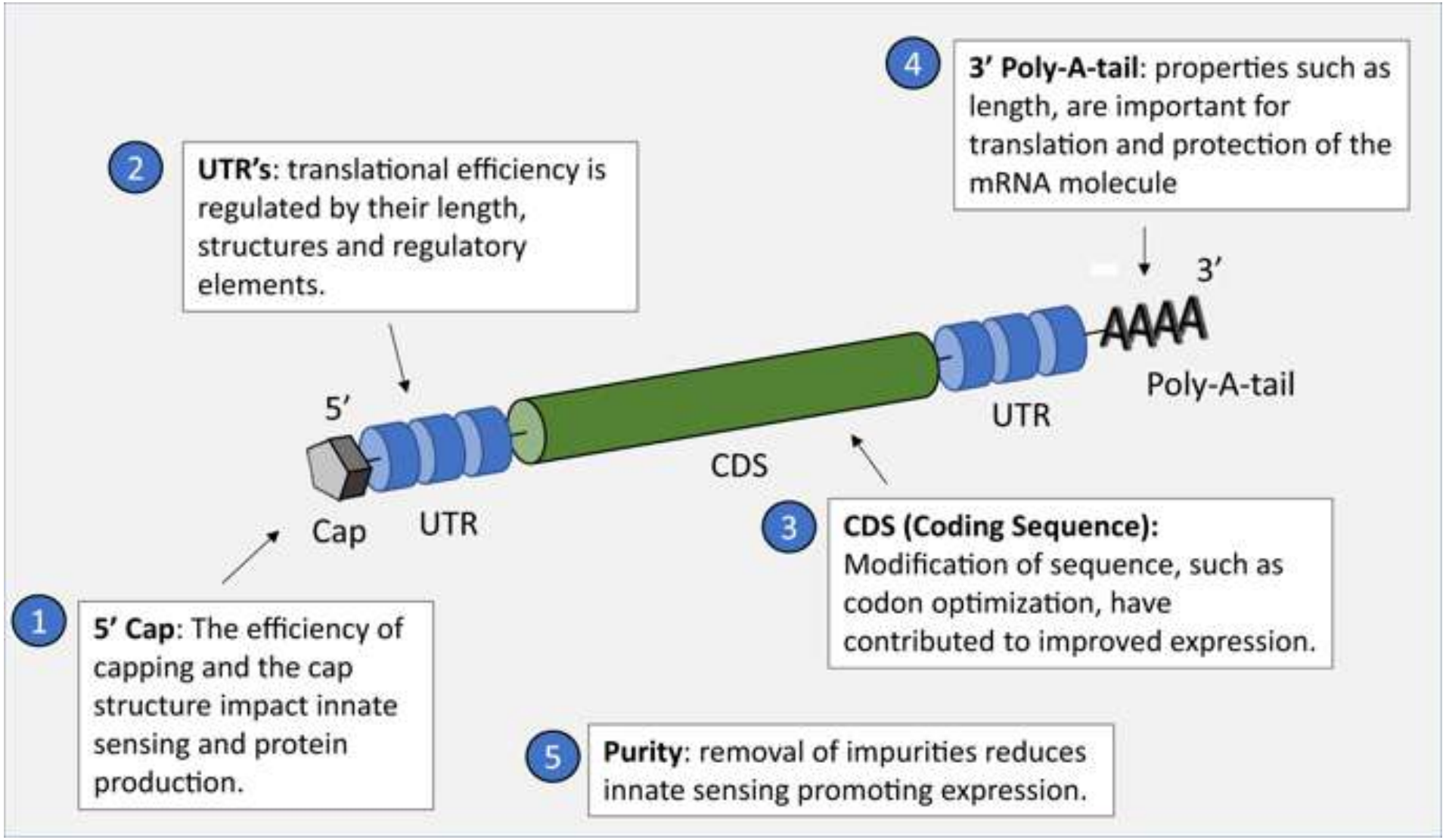


Vaccines for COVID-19 Pandemic

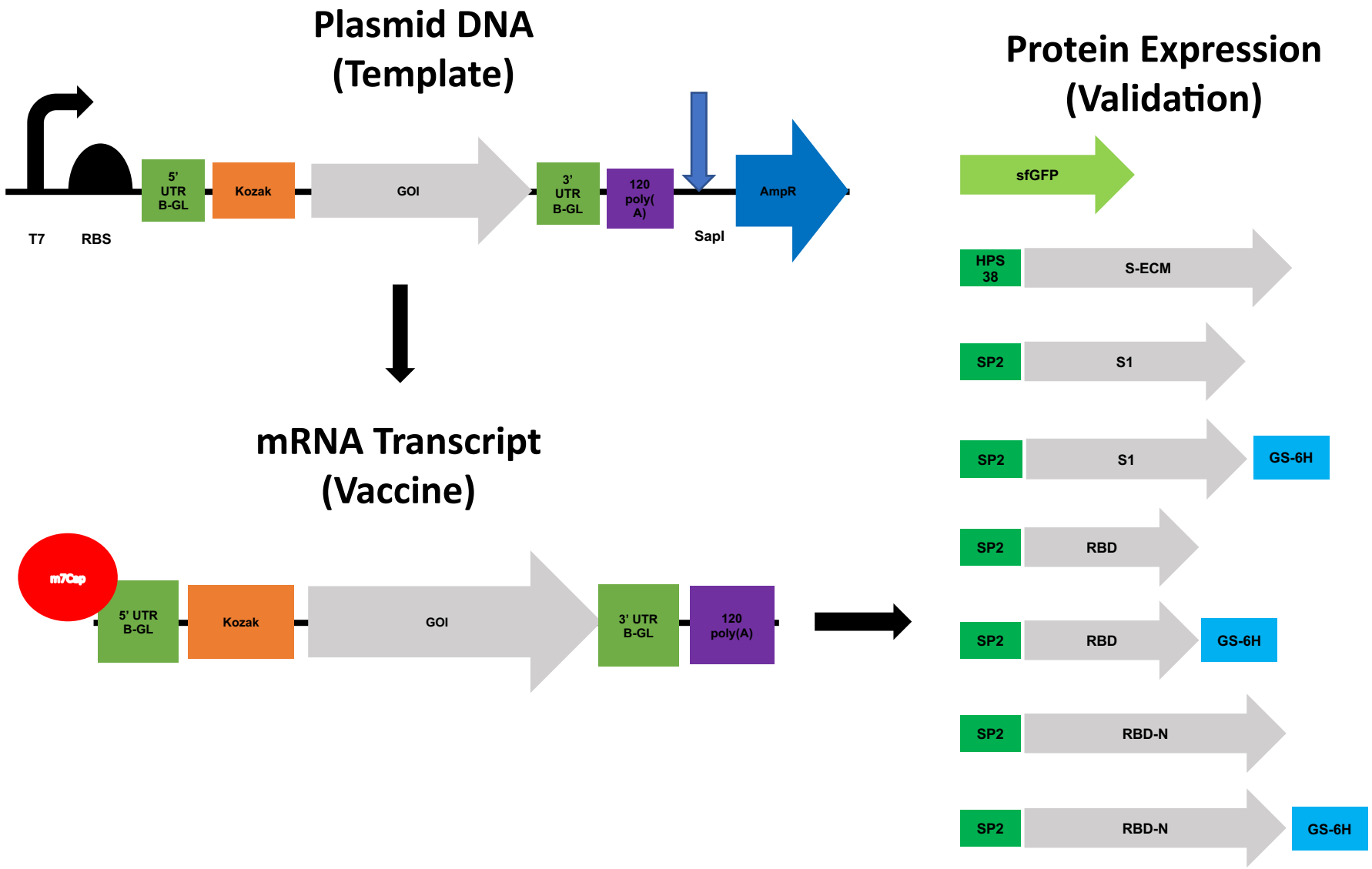
- Deliver with a shuttle use cellular machineries , non-replicating viral vectors,



mRNA Platforms: Let the cell just do the translation

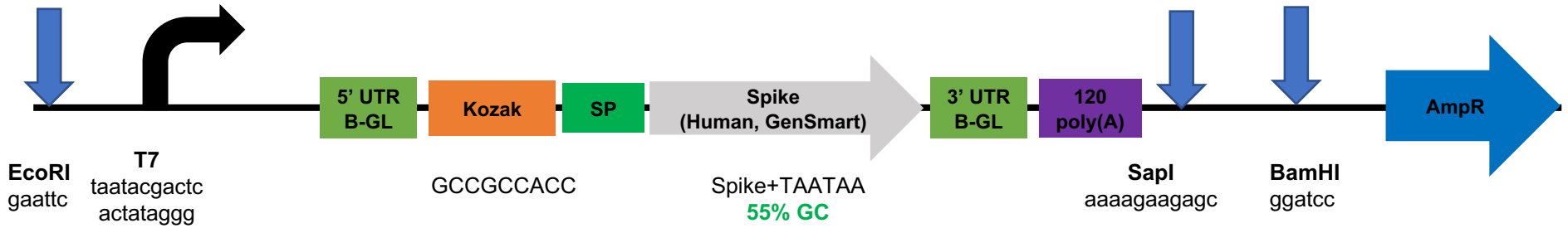


Flow Chart

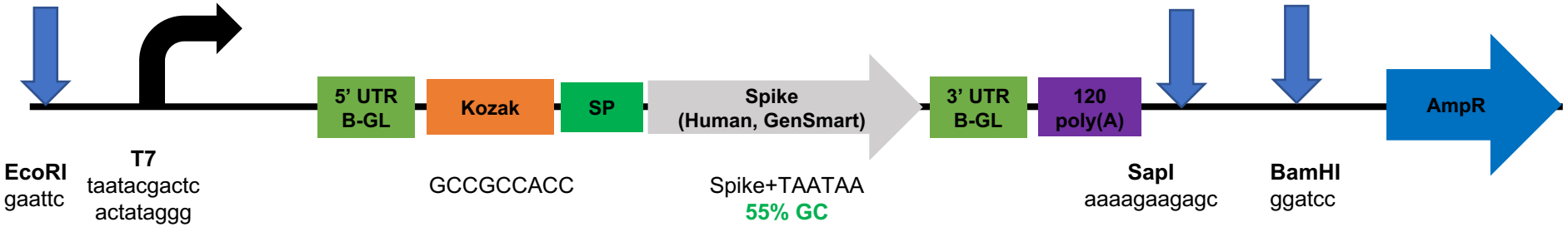


Seker et al ,2021

Genetic design-Spike



Genetic design-Spike ECM



Beta Globin 5' UTR

ACATTTGCTTCTGACACAACTG
TGTTCACTAGCAACCTCAAACA
GACACC

Alternative Start Codons: alternative transcripts

sfGFP: 3,105,102 ,13 aa
Spike: 3, 55, 52 ,37 aa

Reoptimized:
sfGFP: 3,38,35 ,13 aa
Spike: 3, 63, 60 ,37 aa

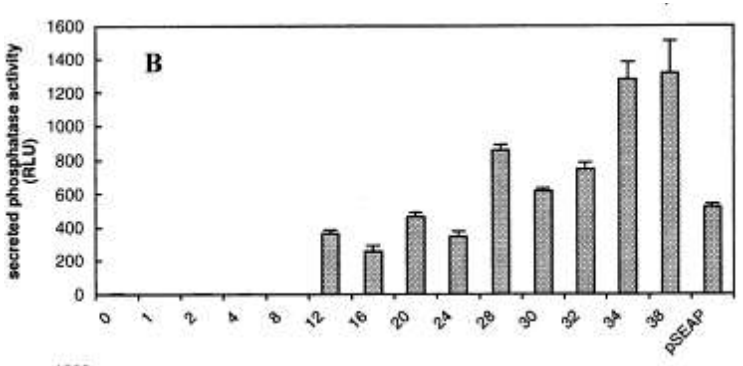
Homo sapiens hemoglobin subunit beta (HBB), mRNA
NM_000518.5

Signal Peptide HSP38

ATGTGGTGGCGGCTGTGGTGG
CTGCTGTTACTGCTGCTGCTG
CTGTGGCCCATGGTGTGGGCC

MWWRLWWLLLLLLLLWPMVWA

Steve Barash, Wei Wang, Yanggu Shi, **Human secretory signal peptide description by hidden Markov model and generation of a strong artificial signal peptide for secreted protein expression**, Biochemical and Biophysical Research Communications, 2002,



Beta Globin 3' UTR

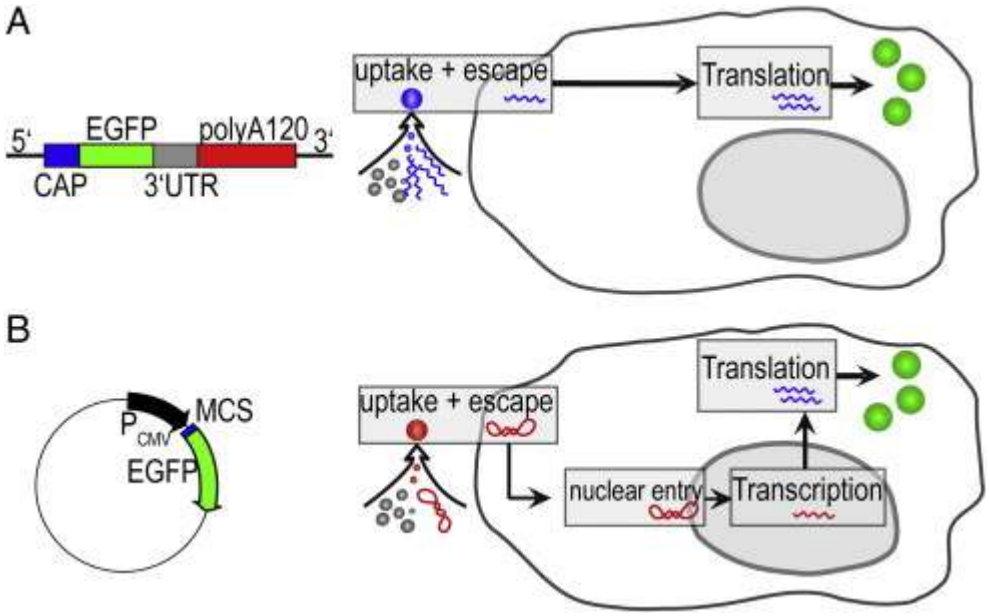
GCTCGCTTTCTTGCTGTCCAAT
TTCTATTAAAGGTTCCTTTGTTC
CCTAAGTCCAACTACTAAACTG
GGGGATATTATGAAGGGCCTTG
AGCATCTGGATTCTGCCTAATA
AAAAACATTTATTTTCATTGCAA

Nucleolin binding site: stability

AU-Rich Element (ARE): instability

Homo sapiens hemoglobin subunit beta (HBB), mRNA
NM_000518.5

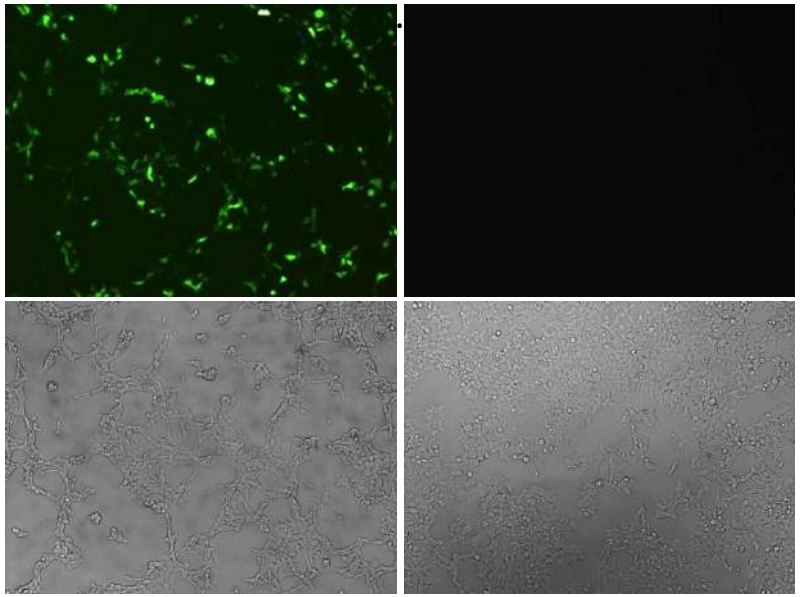
Control Group sfGFP Expression



Leonhardt *et al*. Nanomedicine: Nanotechnology, Biology and Medicine, 2014
https://doi.org/10.1016/j.nano.2013.11.008

HEK293 cell line GFP expression (502-VC-01-0820-RNA)

2.5E05/ml cell concentration, 100 ul/well, 96 well plate
Images taken 16 h after transfection

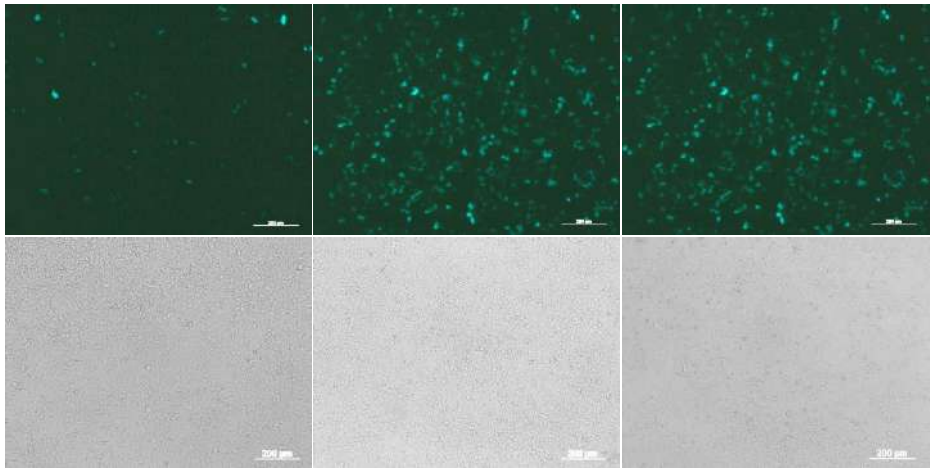


RNA 400 ng
Plus reagent 0.8 ul
Lipofectamine 0.5 ul

HEK293
Control

HEK293 cell line GFP expression (501-RNA)

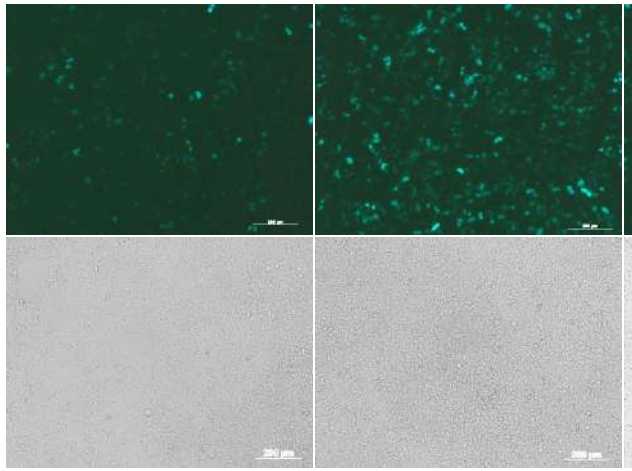
2.5E05/ml cell concentration, 250 ul/well, 48 well plate
Images taken 17 h after transfection
12.08.2020



200 ng RNA+Lipofectamine IVT-ARCA

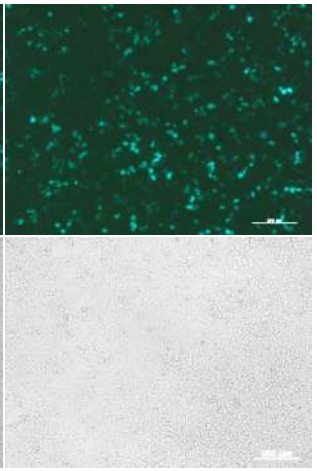
400 ng RNA+Lipofectamine IVT-ARCA

800 ng RNA+Lipofectamine IVT-ARCA

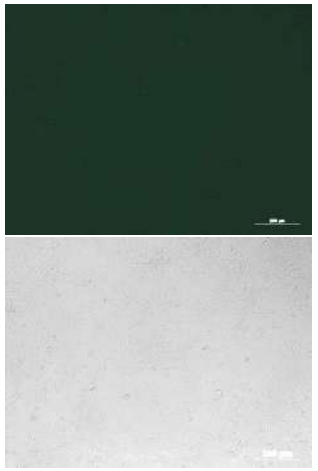


200 ng RNA+Lipofectamine IVT-Vcapping

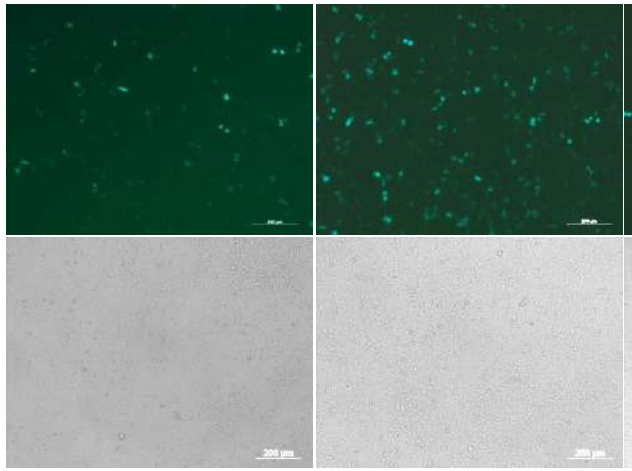
400 ng RNA+Lipofectamine IVT-Vcapping



800 ng RNA+Lipofectamine IVT-Vcapping

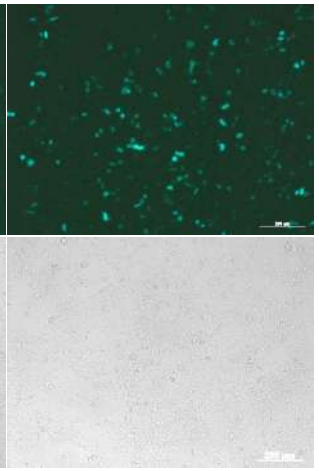


HEK293 Control



200 ng RNA+Lipofectamine IVT-Vcapping-Met

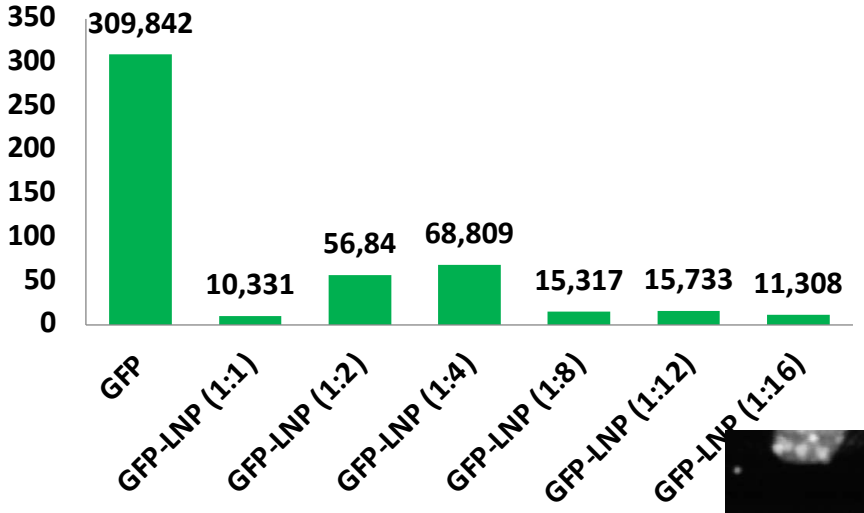
400 ng RNA+Lipofectamine IVT-Vcapping-Met



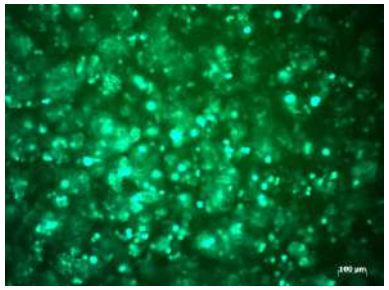
800 ng RNA+Lipofectamine IVT-Vcapping-Met

LNP Transfection-GFP Expression in Expi293 cells

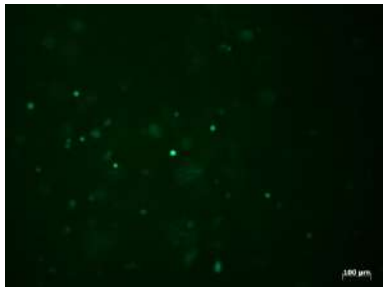
20 mM LNP Mix	Ratio	Final Concentration (mM)
DOTAP	50,00	10,00
HSPC	10,00	2,00
CHOLESTEROL	38,50	7,70
PEG 2000 PE	1,50	0,30
SUM	100,00	20,00



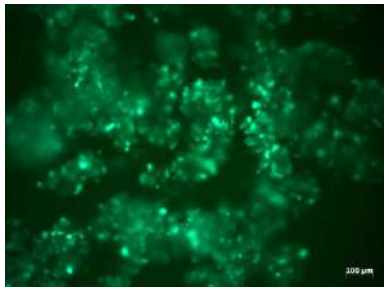
GFP-Expifectamine



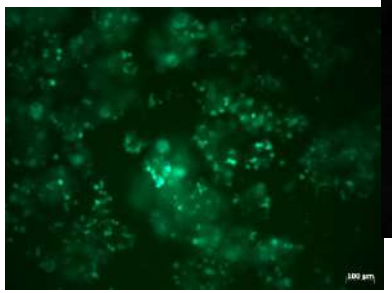
GFP-LNP (1:1, v/v)
N:P = 0.4

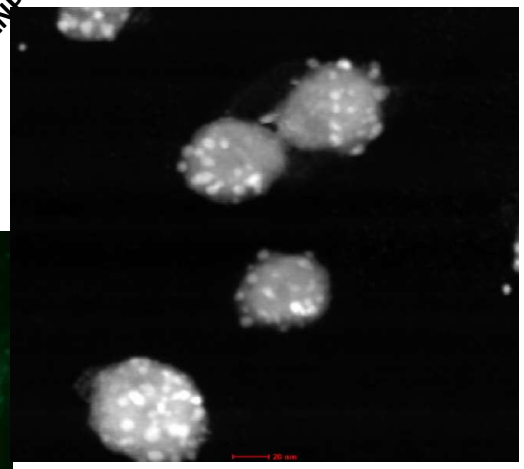


GFP-LNP (1:2, v/v)
N:P = 0.8



GFP-LNP (1:4, v/v)
N:P = 1.6

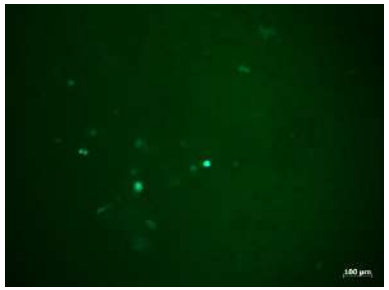




TEM Imaging

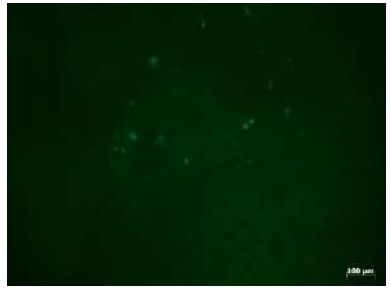
NO GFP

GFP-LNP (1:8, v/v)
N:P = 3.2

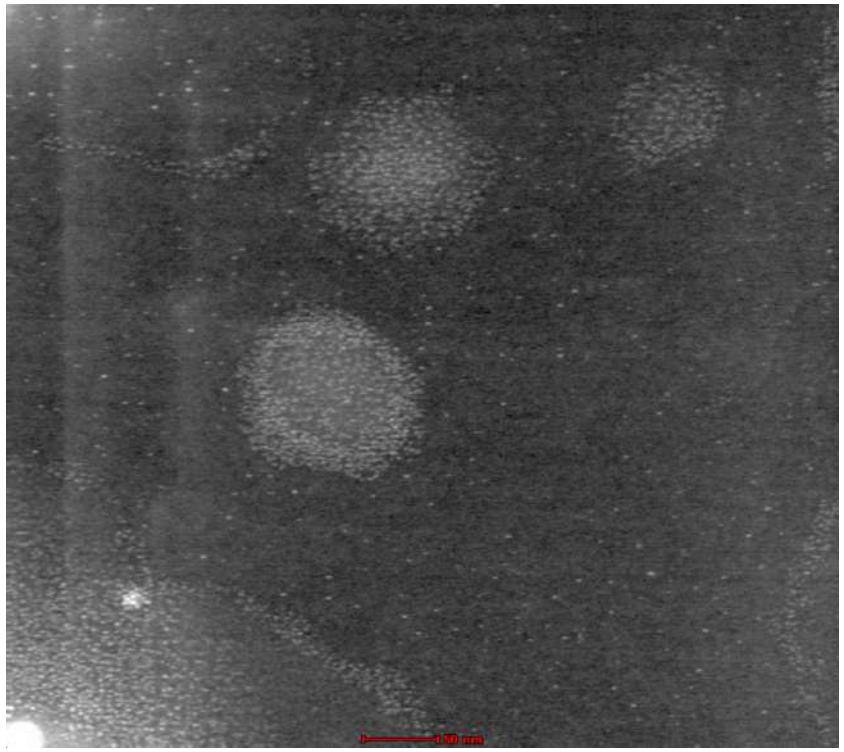


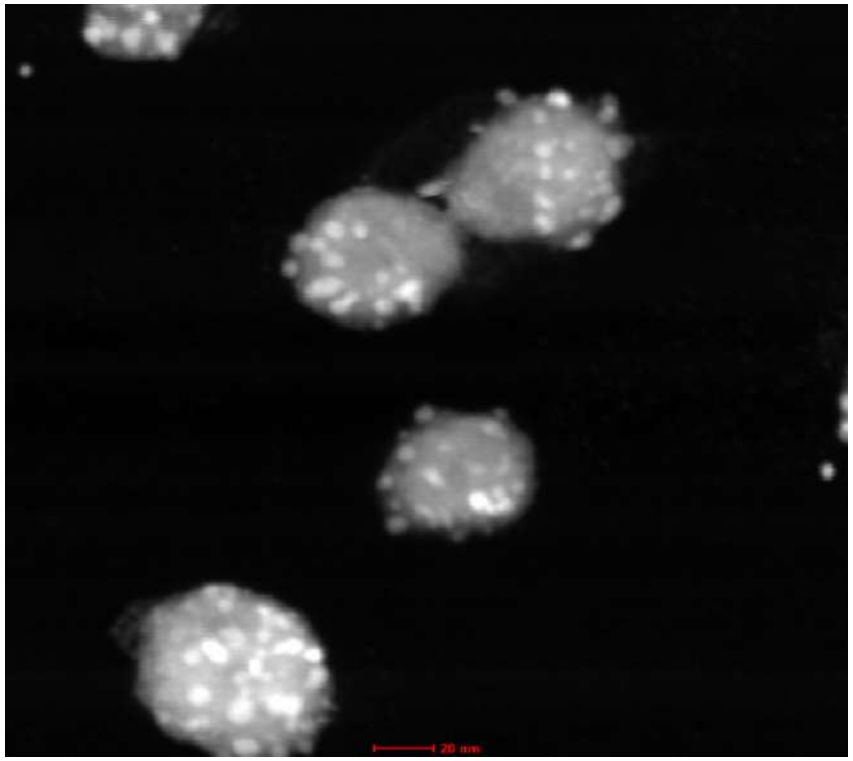
GFP-LNP (1:12, v/v)
N:P = 4.8

GFP-LNP (1:16, v/v)
N:P = 6.4



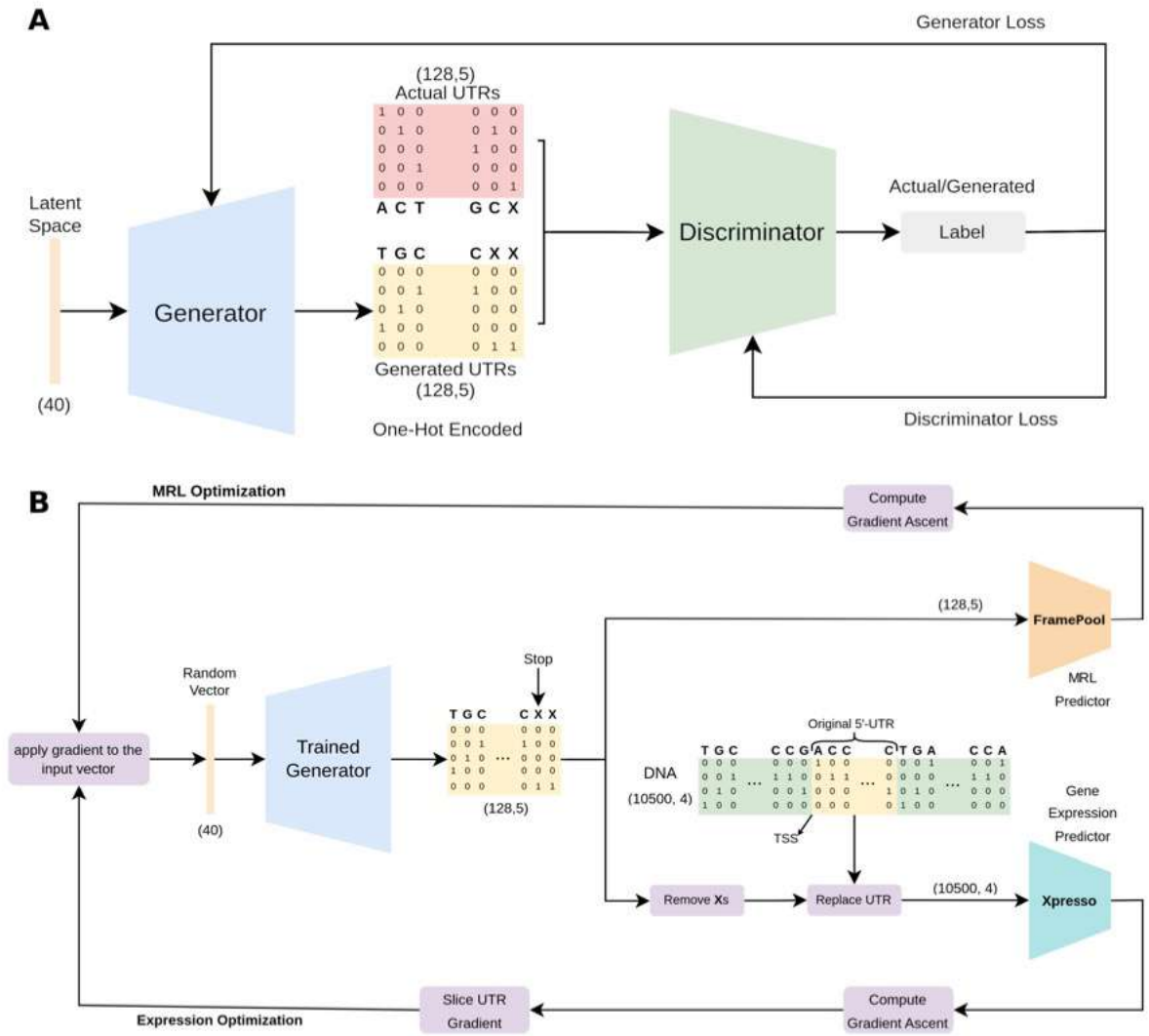
Final Assembly of the mRNA





DOTAP:HSPC:Cholesterol:PE
G200PE
+
GFP
100 nm pore size
Hard to extrude due to precipitation of HSPC after freeze-thawing

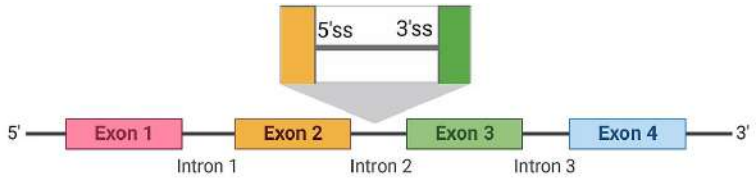
Artificial intelligence for UTR Design

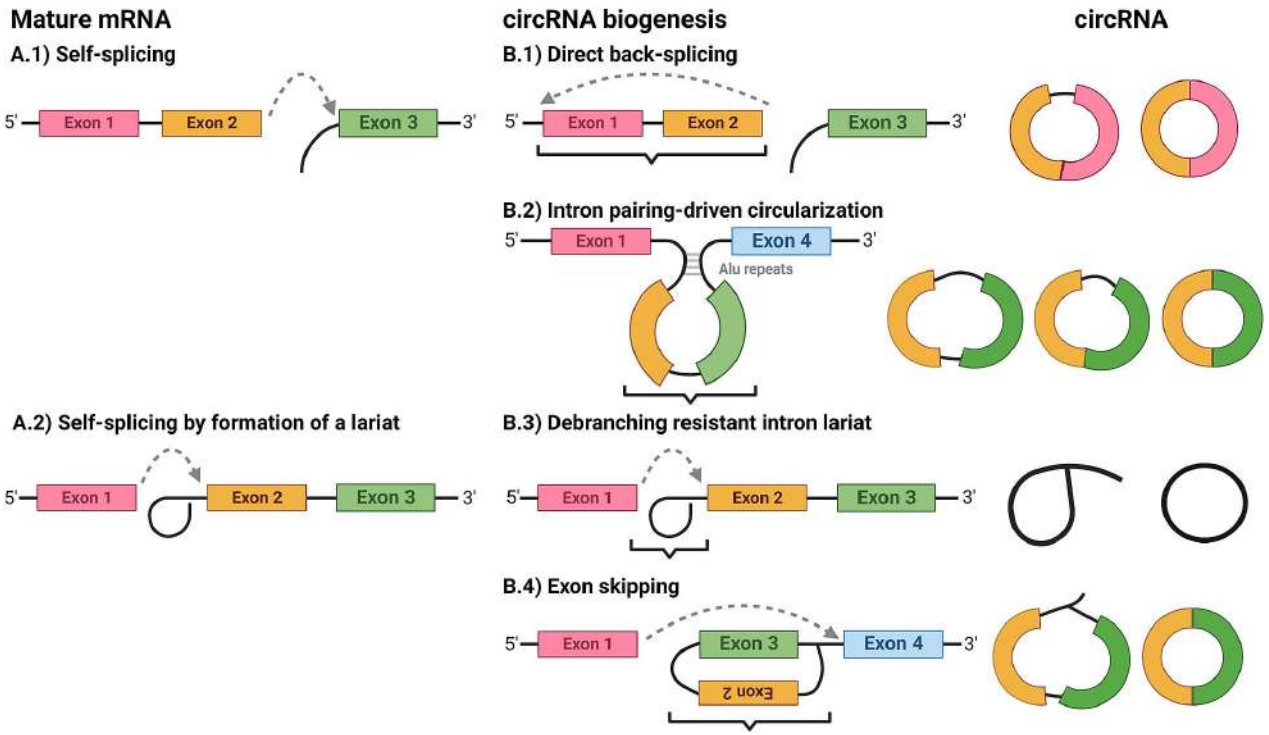


Seker et al ,2023

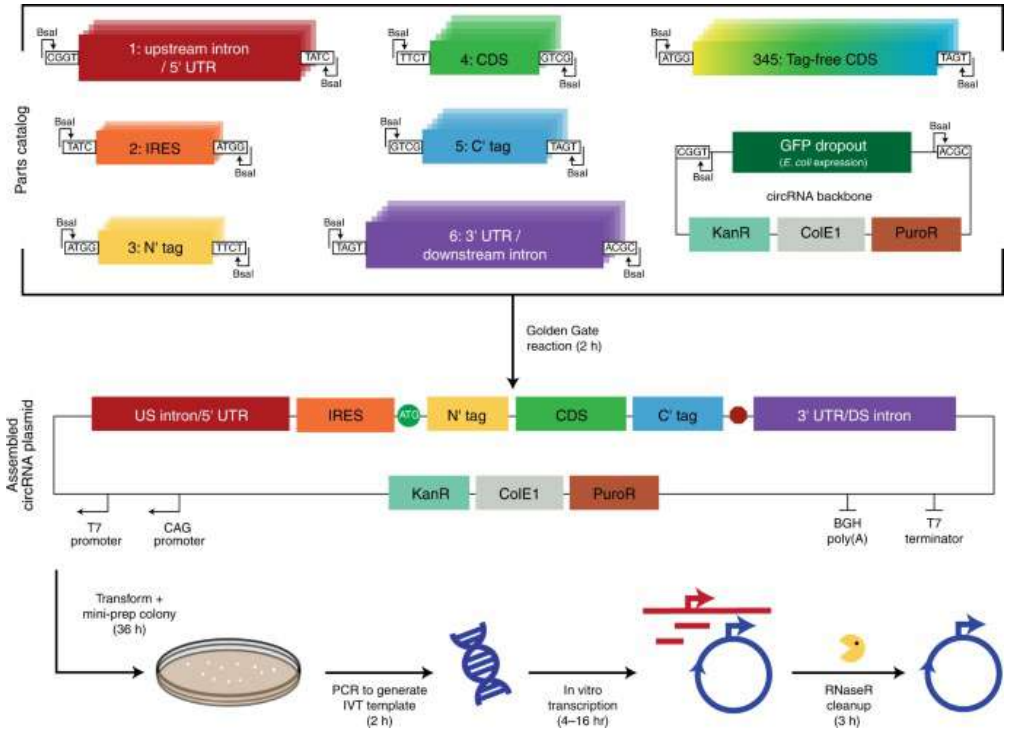
Circular RNA Platforms

In nature





Man made



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