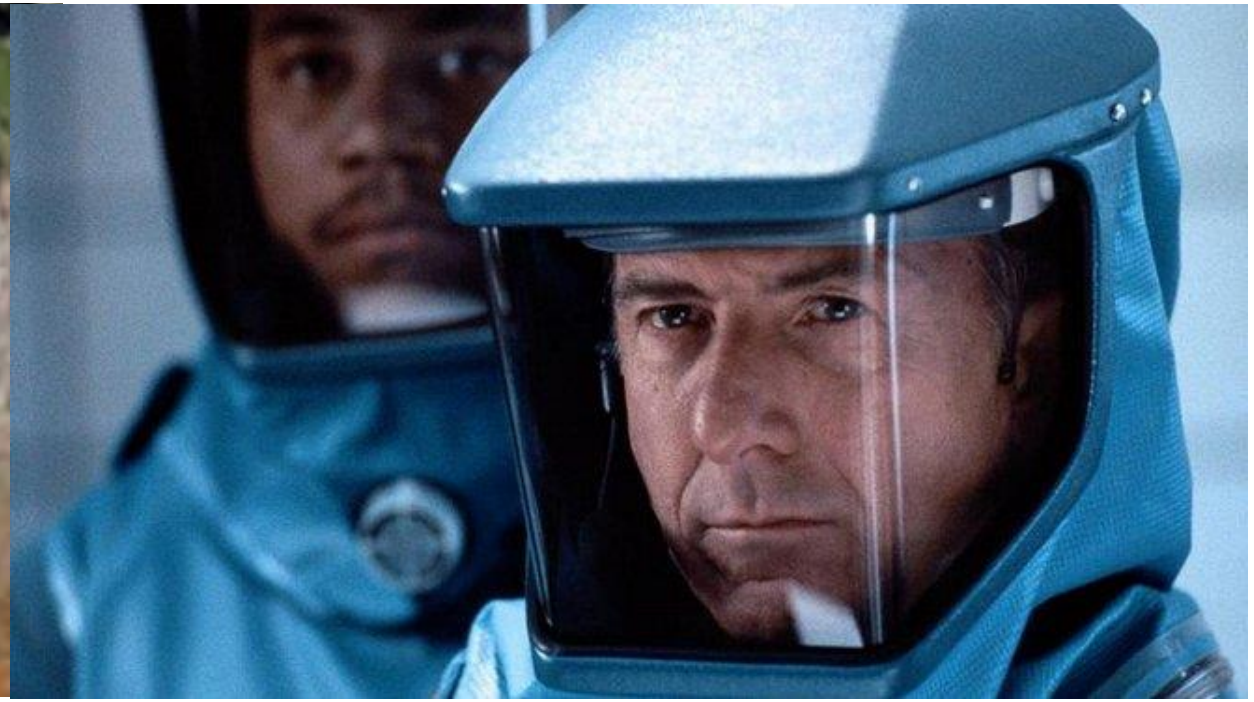
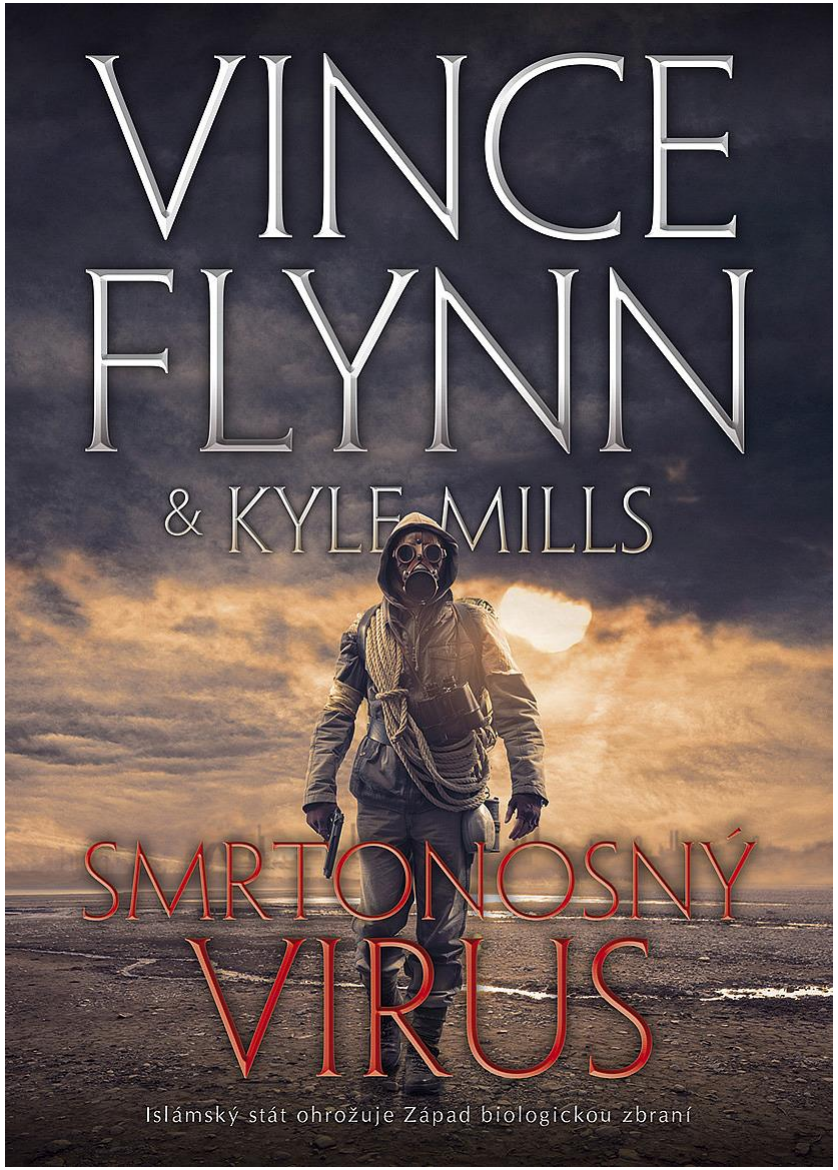
PRIMUM NON NOCERE
Genetické vakcíny proti covid-19: mechanismy potenciálních vedlejších účinků

Prof. RNDr. Jaroslav Turánek, DSc.

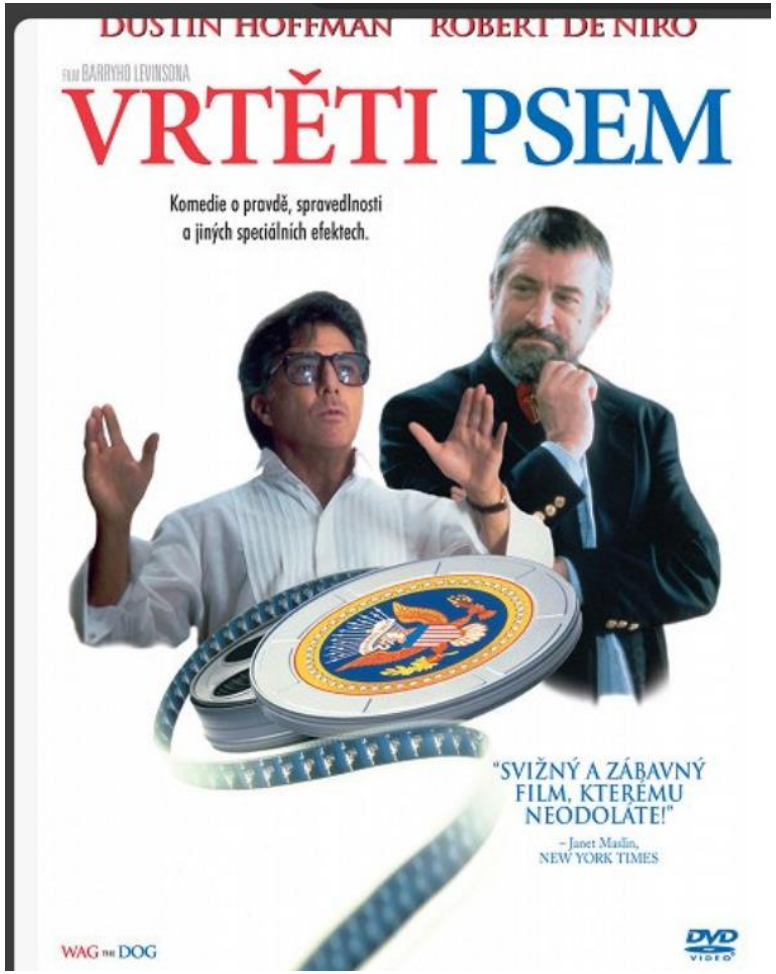
Příprava psychických vzorců




VINCE
FLYNN
& KYLE MILLS
SMRTONOSNÝ
VIRUS
Islámský stát ohrožuje Západ biologickou zbraní

Tento snímek ukazuje, jak s námi média dokážou manipulovat a rozhodně ho doporučuji všem, kteří všemu v televizi slepě důvěřují.

„Je to pravda. Bylo to v televizi."



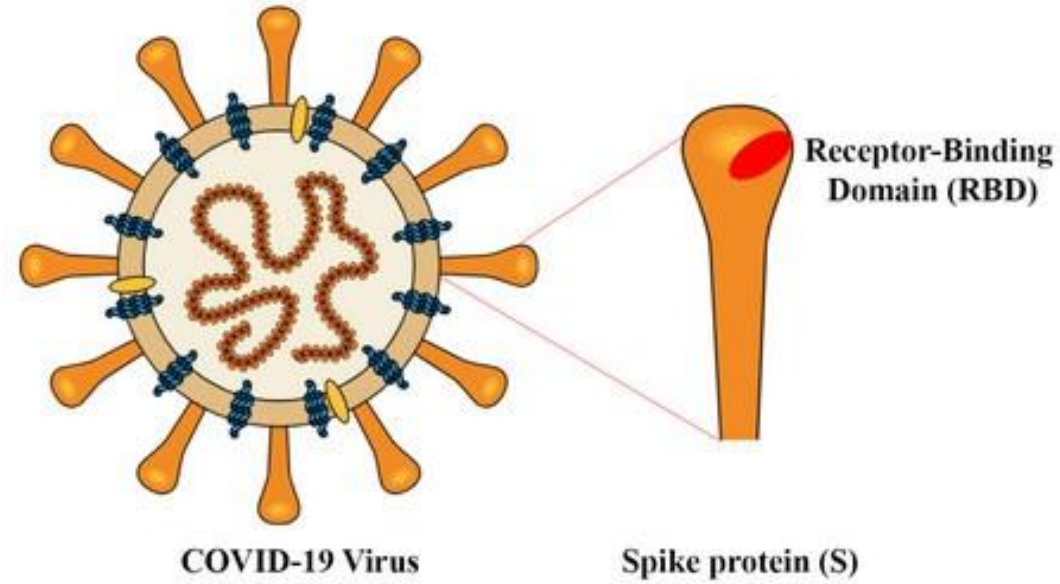
Odkud pocházím a kam kráčím

Týrání začíná u zvířat a končí u lidí

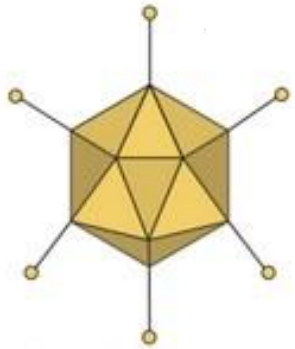
Tržnice ve Wuhanu

WUHAN INSTITUTE OF VIROLOGY

Typy vakcín proti Covid-19

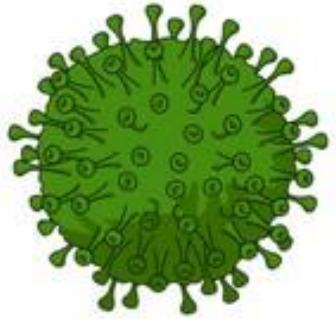


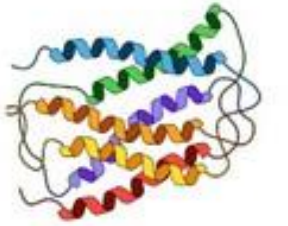
Astra Zeneca,
Jansen
Sputnik



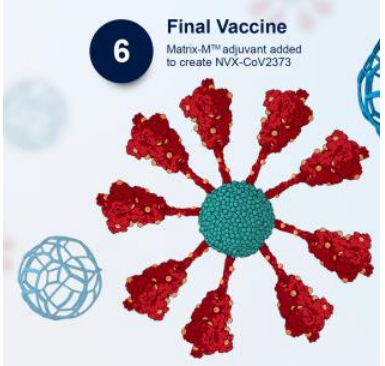
Moderna, Pfizer

Čína, Indie, Valneva





Novavax



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Bimervax – nová rekombinantní vakcína proti Covid-19

- **1. NÁZEV PŘÍPRAVKU**
- BIMERVAX injekční emulze
- Vakcína proti onemocnění covid-19 (rekombinantní, obsahující adjuvans)
- **2. KVALITATIVNÍ A KVANTITATIVNÍ SLOŽENÍ**
- Jedná se o vícedávkovou injekční lahvičku, která obsahuje 10 dávek po 0,5 ml.Jedna dávka (0,5 ml) obsahuje **40 mikrogramů fúzního heterodimeru rekombinantní receptorové**
- **vazebné domény (RBD) spike (S) proteinu viru SARS-CoV-2*** (kmeny B.1.351 a B.1.1.7) navázaného na adjuvans SQBA.
- *Vyrobeno technologií rekombinantní DNA za použití plazmidového expresního vektoru v buněčnélinii ovariálních buněk křečíka čínského (CHO).
- Adjuvans SQBA obsahuje v jedné 0,5ml dávce: skvalen (9,75 mg), polysorbát 80 (1,18 mg), sorbitantrioleát (1,175 mg), dihydrát natrium-citrátu (0,66 mg), monohydrát kyseliny citronové (0,04 mg) a vodu pro injekci.
- **3. LÉKOVÁ FORMA**
- Injekční emulze (injekce)
- Bílá homogenní emulze.
- Dávkování a způsob podání
- Dávkování -Osoby ve věku 16 let a starší
- Má být podána jedna intramuskulární dávka (0,5 ml) přípravku BIMERVAX s odstupem nejméně 6 měsíců mezi podáním předchozí mRNA vakcíny a podáním vakcíny BIMERVAX (viz bod 5.1).
- Starší populace - U starších osob ve věku ≥ 65 let není nutná žádná úprava dávkování.
- Pediatrická populace - Bezpečnost a účinnost přípravku BIMERVAX u dětí a dospívajících mladších 16 let nebyla dosud stanovena. Nejsou dostupné žádné údaje.
- Způsob podání
- Přípravek BIMERVAX je určen pouze k intramuskulárnímu podání, nejlépe do deltového svalu horní části paže.
- Tuto vakcínu nepodávejte **intravaskulárně**, subkutánně ani intradermálně. PROČ???!!! Protože žádná vakcína se nesmí podávat nitrožilně, protože olejová adjuvans po podání do kůže nebo podkožně projevují nekroický účinek zjevně viditelný. V hloubce svalu to neuvidíte.

Genetické mRNA a pDNA vakcíny

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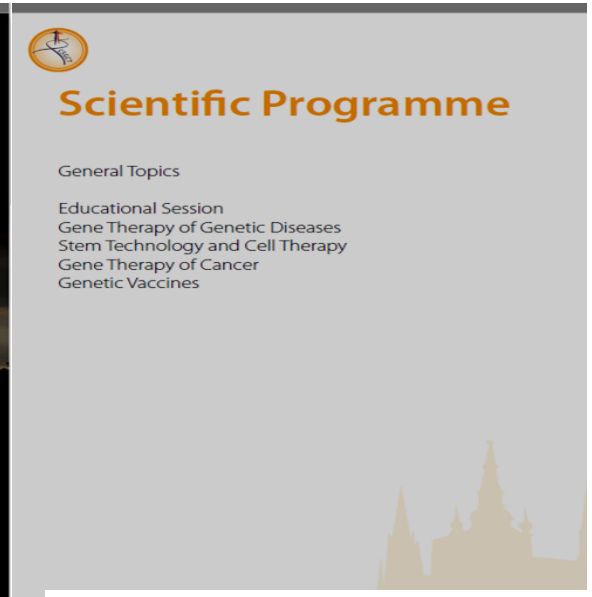
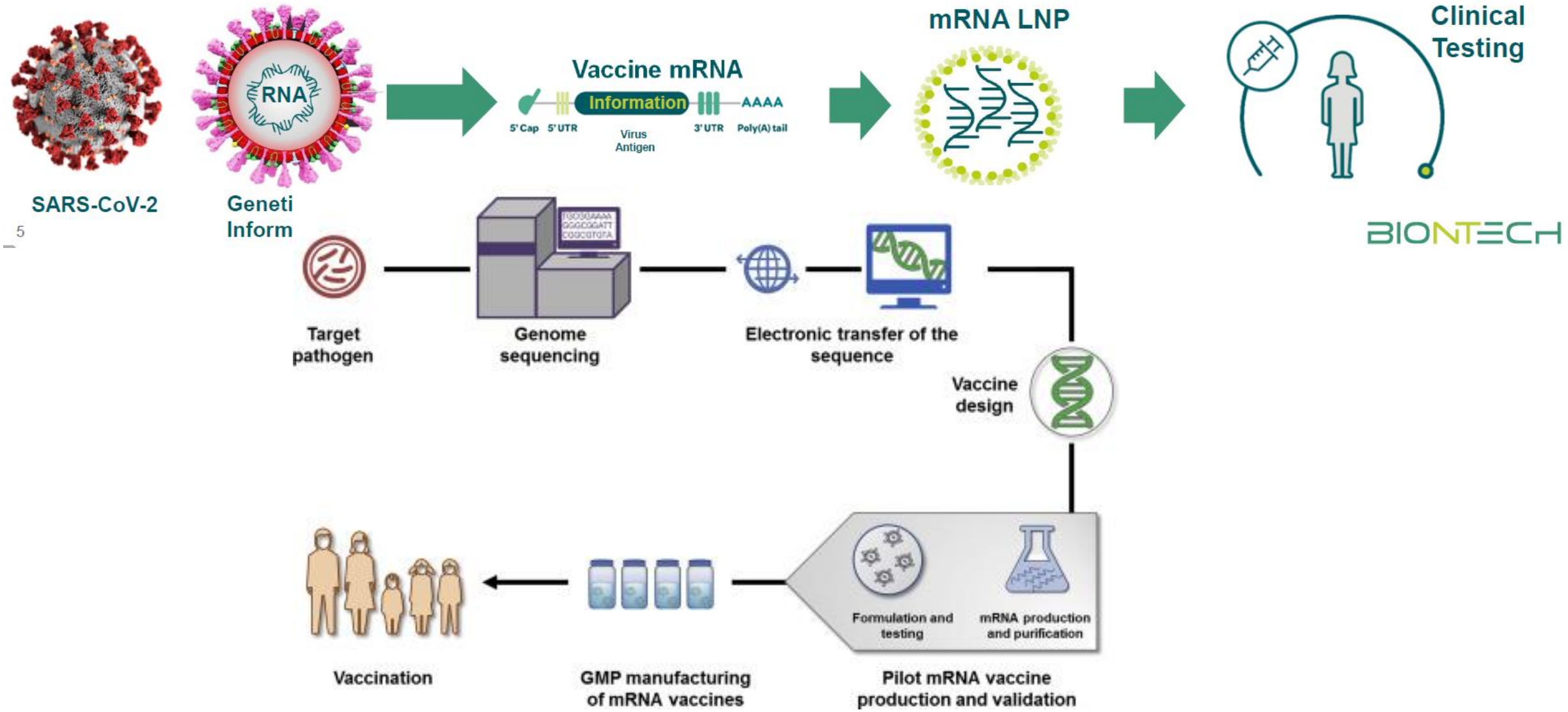
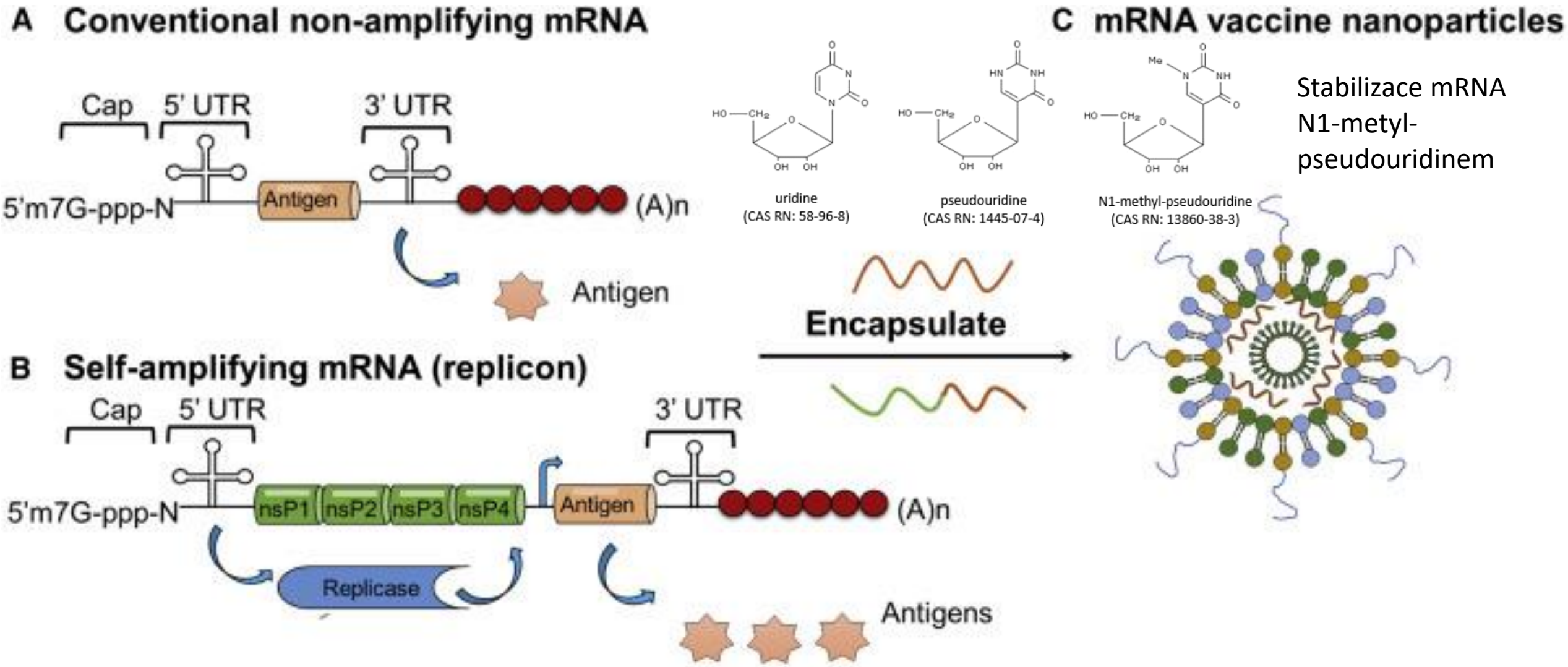
Konference na téma Genetické vakcíny v ČR



The future of the mRNA vaccine technology and production



Non-amplifying and self-amplifying mRNA constructs



Stabilizace mRNA
N1-metyl-
pseudouridinem

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Preparation of nanoliposomes by microfluidic mixing

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SCIENTIFIC REPORTS
natureresearch

Preparation of nanoliposomes by microfluidic mixing in herring-bone channel and the role of membrane fluidity in liposomes formation

Jan Kotouček[1,8], František Hubatka[1,8], Josef Mašek[1], Pavel Kulich[1], Kamila Velínská[1], Jaroslava Bezděková[1,2], Martina Fojtíková[1], Eliška Bartheldyová[1], Andrea Tomečková[1], Jana Stráská[3], Dominik Hrebík[4], Stuart Macaulay[5], Irena Kratochvílová[6*], Milan Raška[1,7] & Jaroslav Turánek[1*]

Introduction of microfluidic mixing technique opens a new door for preparation of the liposomes and lipid-based nanoparticles by on-chip technologies that are applicable in a laboratory and industrial scale. This study demonstrates the role of phospholipid bilayer fragment as the key intermediate in the mechanism of liposome formation by microfluidic mixing in the channel with "herring-bone" geometry used with the instrument NanoAssemblr. The fluidity of the lipid bilayer expressed as fluorescence anisotropy of the probe N,N,N-Trimethyl-4-(6-phenyl-1,3,5-hexatrien-1-yl) was found to be the basic parameter affecting the final size of formed liposomes prepared by microfluidic mixing of an ethanol solution of lipids and water phase. Both saturated and unsaturated lipids together with various content of cholesterol were used for liposome preparation and it was demonstrated, that an increase in fluidity results in a decrease of liposome size as analyzed by DLS. Gadolinium chelating lipids were used to visualize the fine structure of liposomes and bilayer fragments by CryoTEM. Experimental data and theoretical calculations are in good accordance with the theory of lipid disc micelle vesiculation.

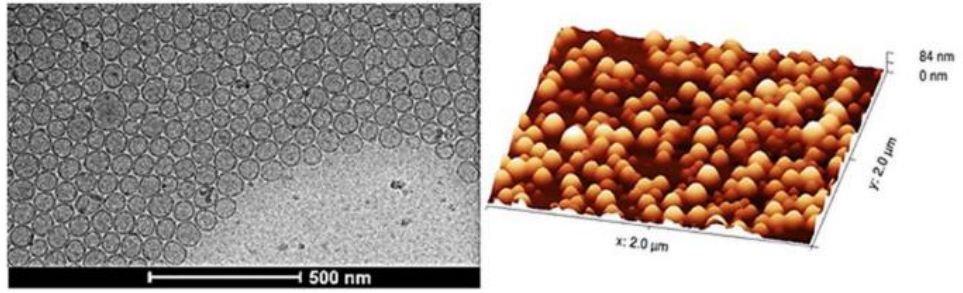
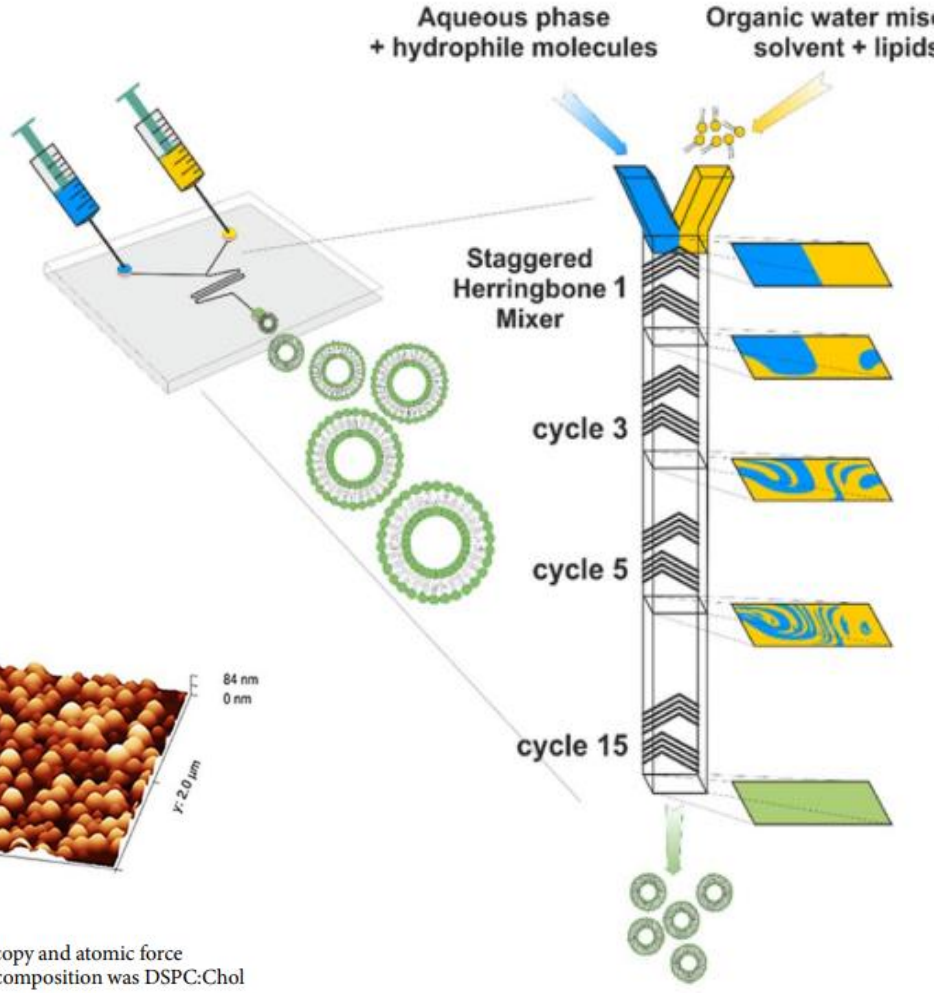


Figure 5. Structure of liposomes revealed by cryoelectron transmission microscopy and atomic force microscopy. Liposomes were prepared by the microfluidic mixing method. The composition was DSPC:Chol 70:30 Cryo-TEM picture (left) and AFM picture (right).



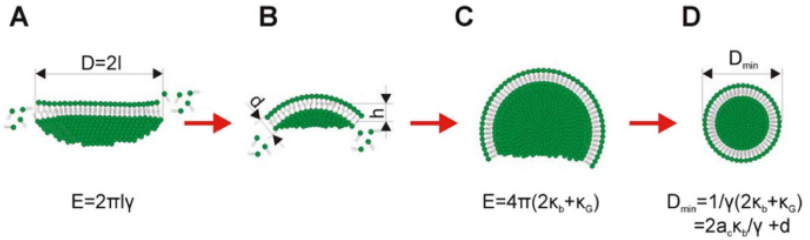


Figure 7. A schematic illustration of vesicle formation in the process of self-assembly. A planar disc-like micelle (**A**) curves into a spherical cap (**B** and **C**) and finally closes to form a vesicle (**D**). Equations are described in the text.

$$E = \frac{1}{2}\pi k_b \cdot \left(\frac{4h}{l} - lc_0\right)^2 + 4\pi k_G \cdot \left(\frac{h}{l}\right)^2 + \frac{16}{l^2}\pi B \cdot \left(\frac{h}{l}\right)^4 2\pi\sqrt{l^2 - h^2}\gamma$$

where $h \in [0, l]$ is the height of the spherical cap that effectively indicates vesicle shape evolution ($h = 0$) corresponds to a planar patch; $h = l$ corresponds to a spherical vesicle, $B = \kappa_1 + \kappa_2 + \kappa_3$, where κ_1, κ_2, and κ_3 are fourth-order moduli; γ is line tension; κ_G is Gaussian bending stiffness, κ_b is the bending stiffness, and c_o is the spontaneous curvature. The last term in Eq. (1) is the line energy along the free edge, where γ is the line tension.

Based on assumption that the number of lipid molecules in the micelle and formed liposome remains constant (it implies the same surface of micelle and vesicle formed from it), during the process of vesiculation, the membrane is assumed to maintain a uniform curvature. For an initially flat disc-liker membrane micelle $h = 0$ and the system energy in Eq. (1) reduces to $E_1 = 2\pi l\gamma$. For a fully vesiculated sphere $h = l$ and Eq. (1) is reduced to $E_2 = 8\pi\kappa_b + 4\pi\kappa_G + 16\pi B/l^2$, which can be further reduced to $E_2 = 4\pi\kappa_b$ for the case of linear elasticity $B = 0$ and $\kappa_G \approx -\kappa_b$[29].

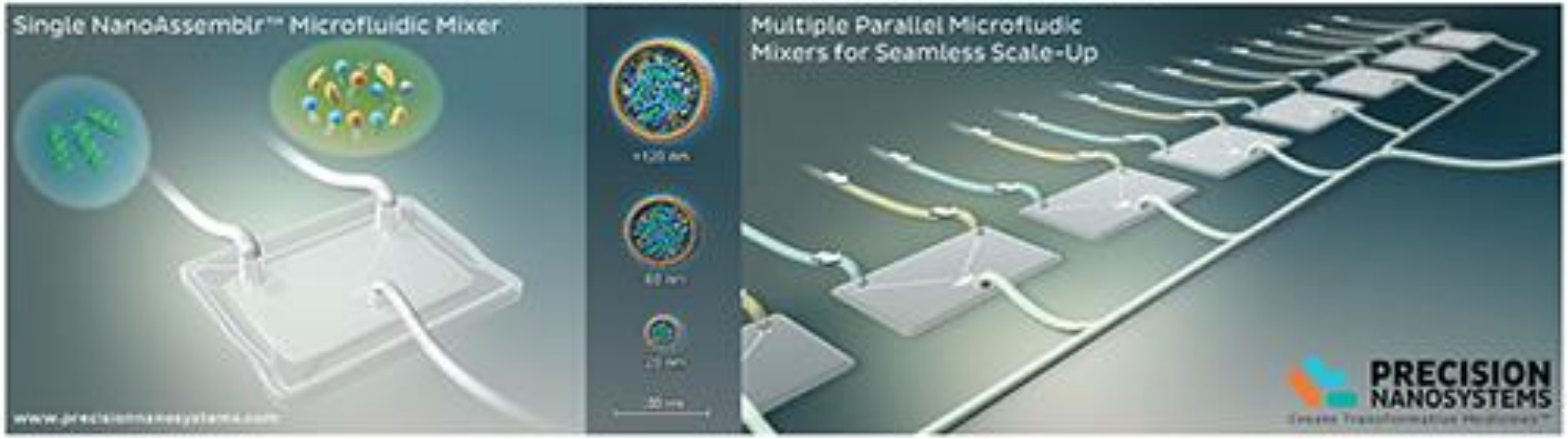
For the transition from the DBM configuration into a spherical vesicle, it must stand that $E_2 \leq E_1$. Based on above assumption the lower diameter of the circular DBM that can possibly form a spherical vesicle is given by $l^* = 2\kappa_b/\gamma$, $l = al^*$, where the scaling parameter $a \geq 1$[15].

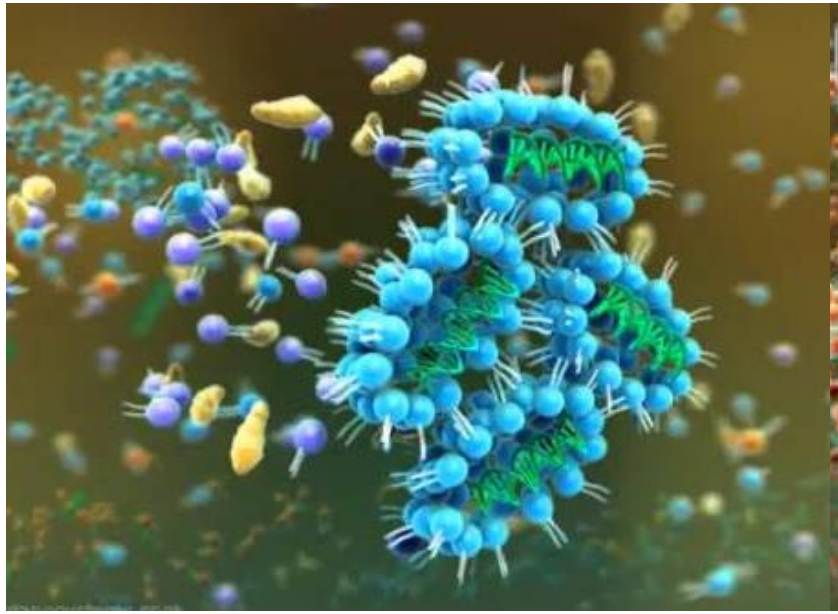
The lower outer diameter of spontaneously self-assembled vesicles can be expressed as:

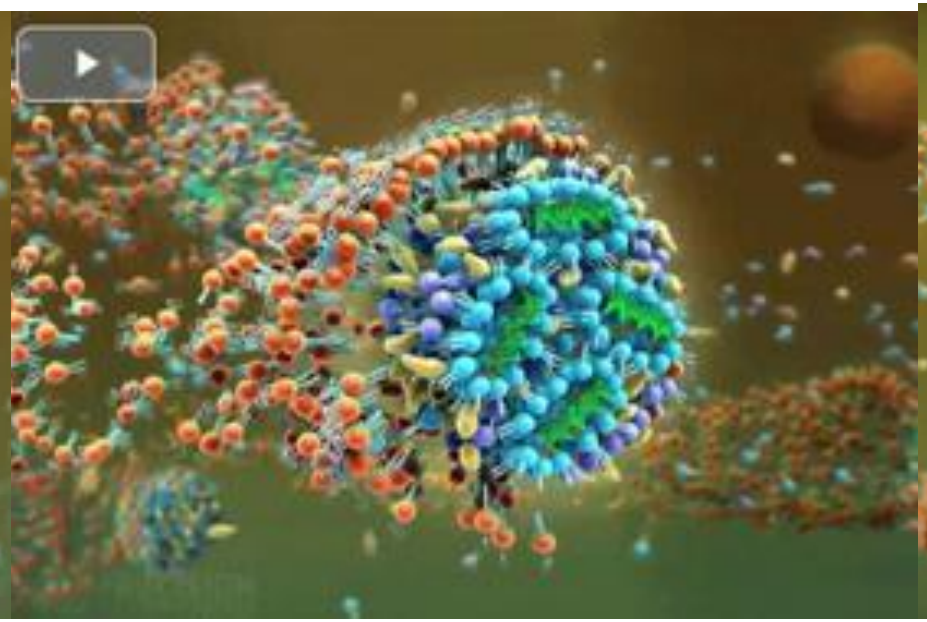
$$D_{\min} = a_c l^* + d = 2a_c \frac{k_b}{\gamma} + d \qquad (2)$$

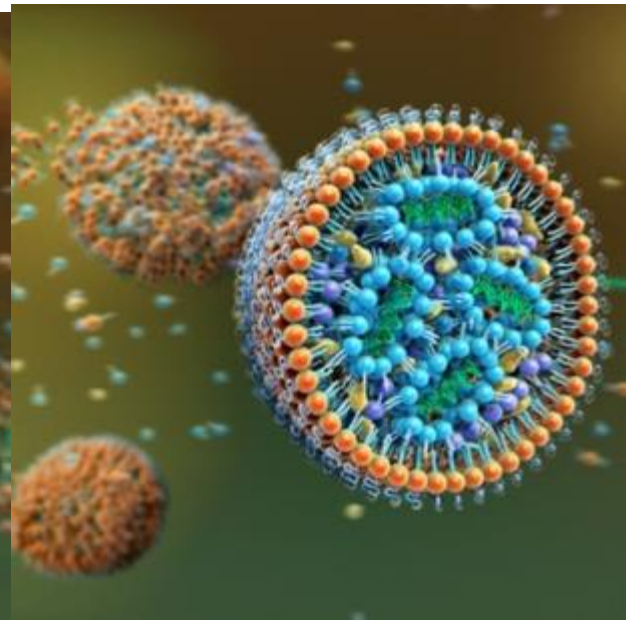
Scientific Reports | (2020) 10:5595 |
https://doi.org/10.1038/s41598-020-62500-2

Microfluidic Nanoparticle Production

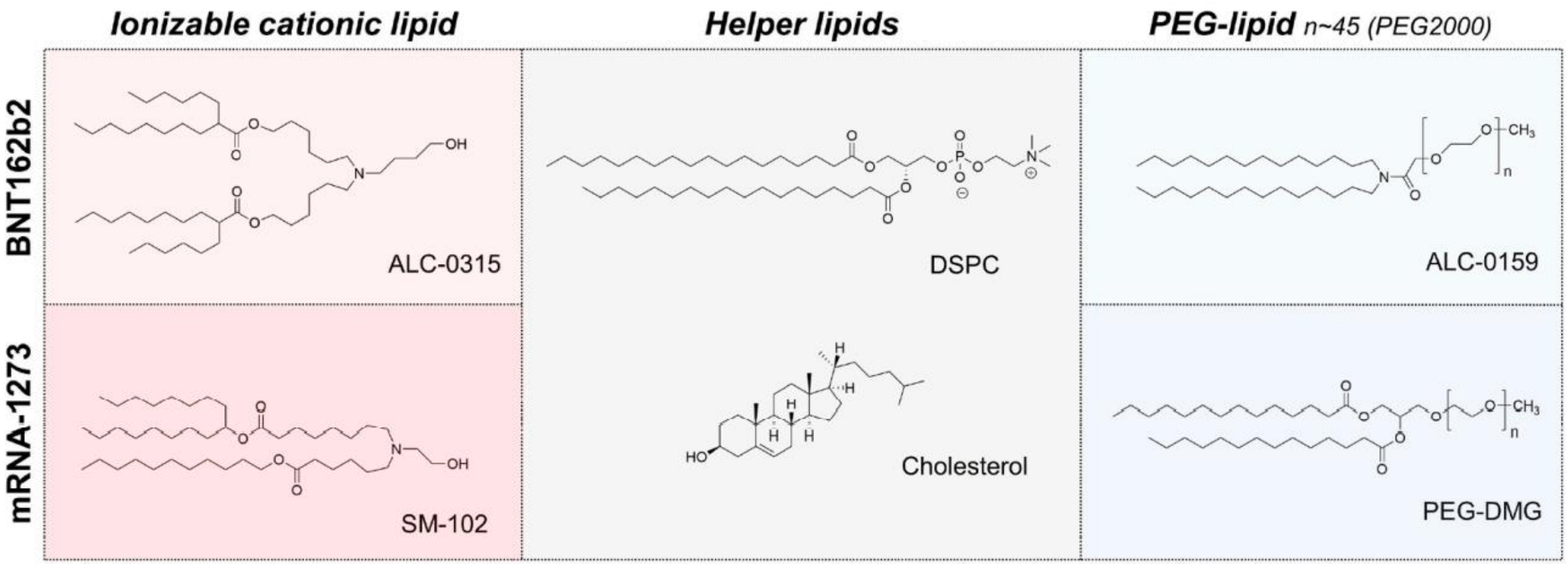








Lipid used in the mRNA-LNP



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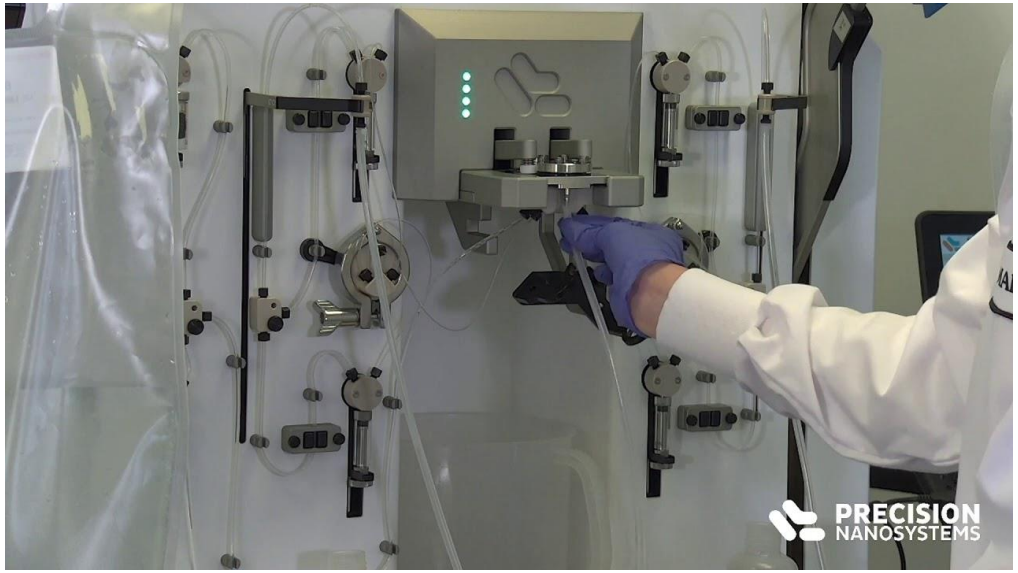
Přístroj Blaze pro průmyslovou výrobu



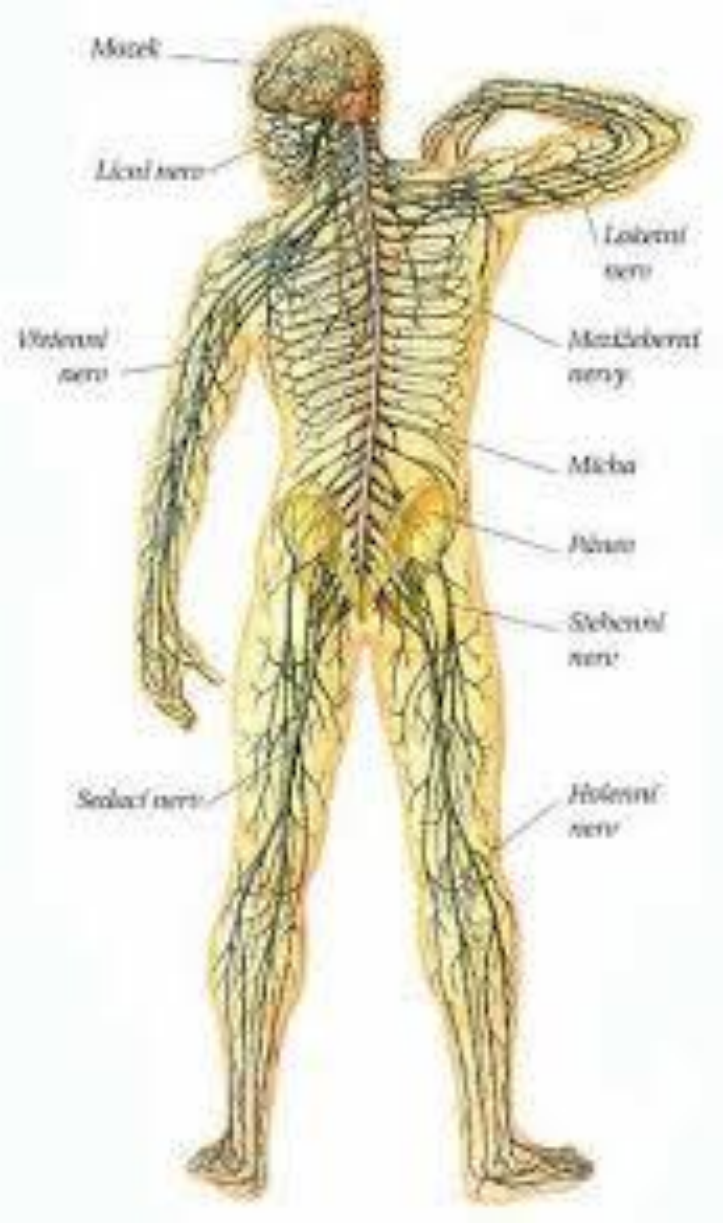
Factors influencing the fate of nanoparticles in the organism

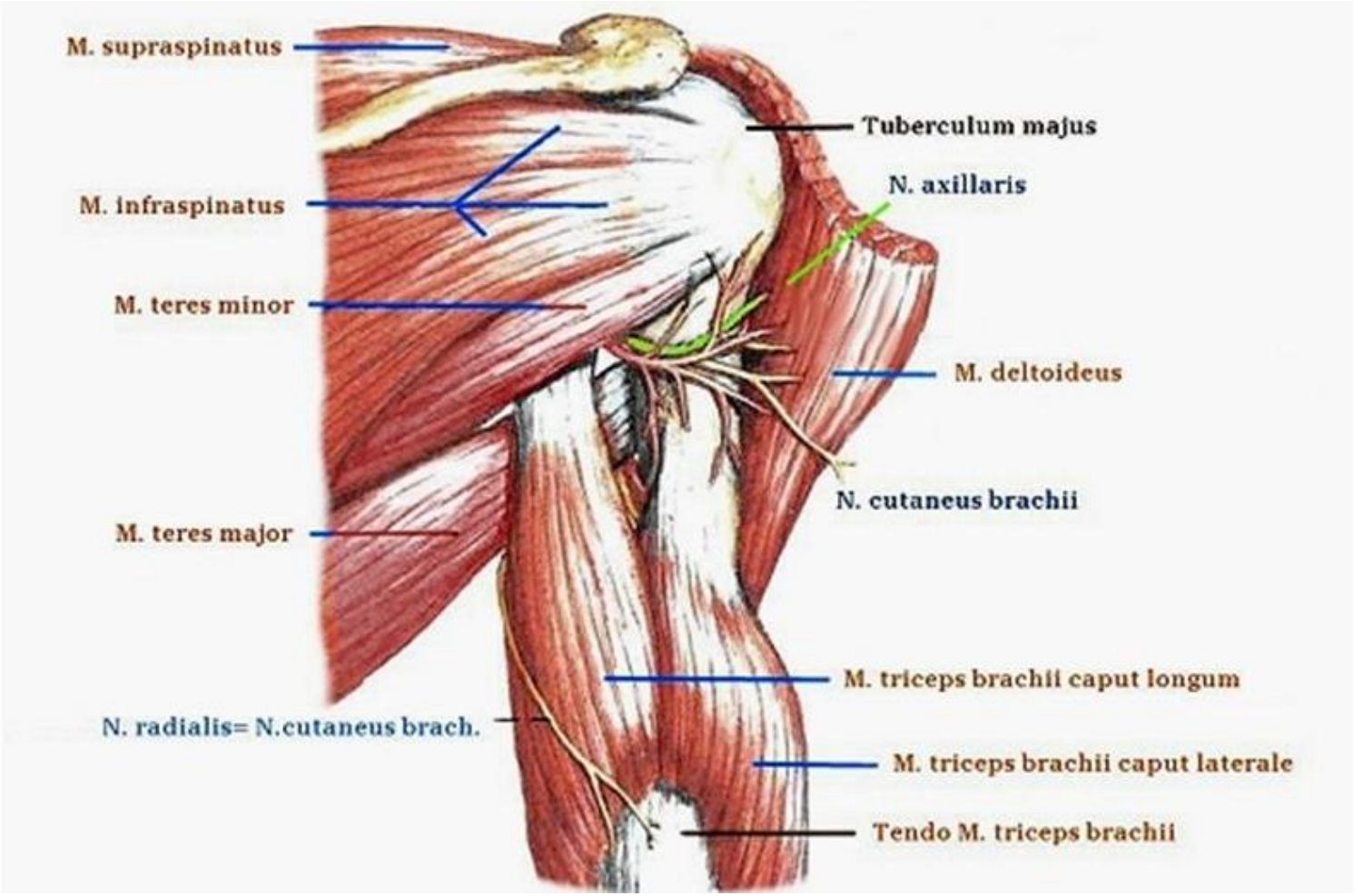
Size of nanoparticle and size distribution

Surface modification – long circulation, specific/ non specific interaction with biomolecules in organism

Stability - biodegradation, bioresistance, morphological stability, stability of components

Zasažení nervů inervujících svaly ramenního pletence po i.m. vakcinaci





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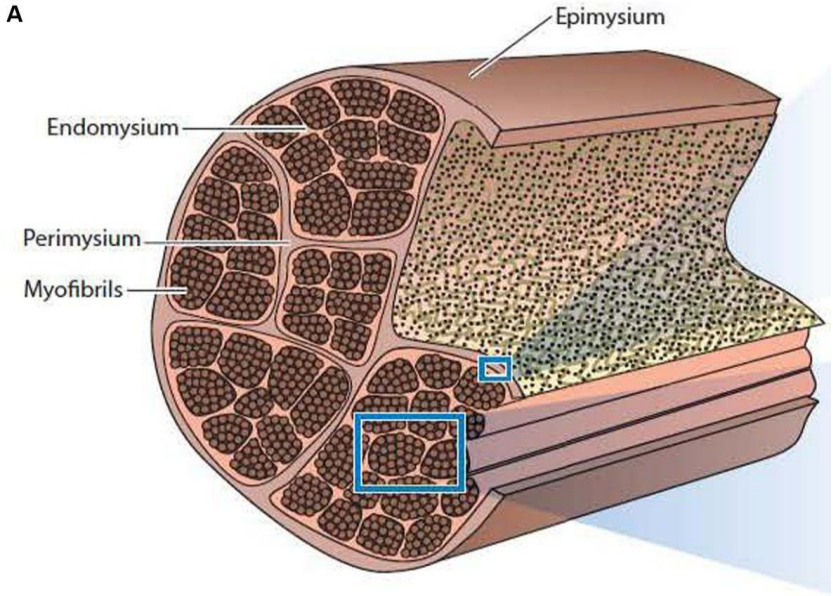
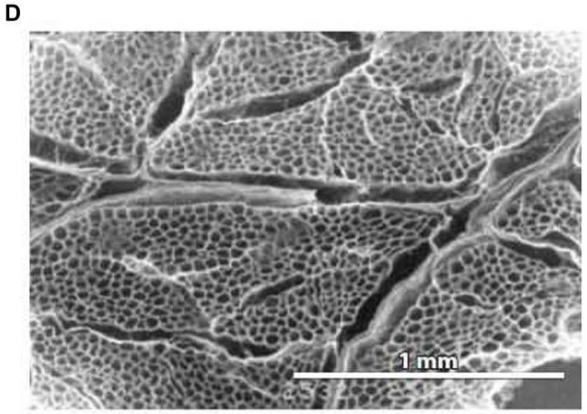
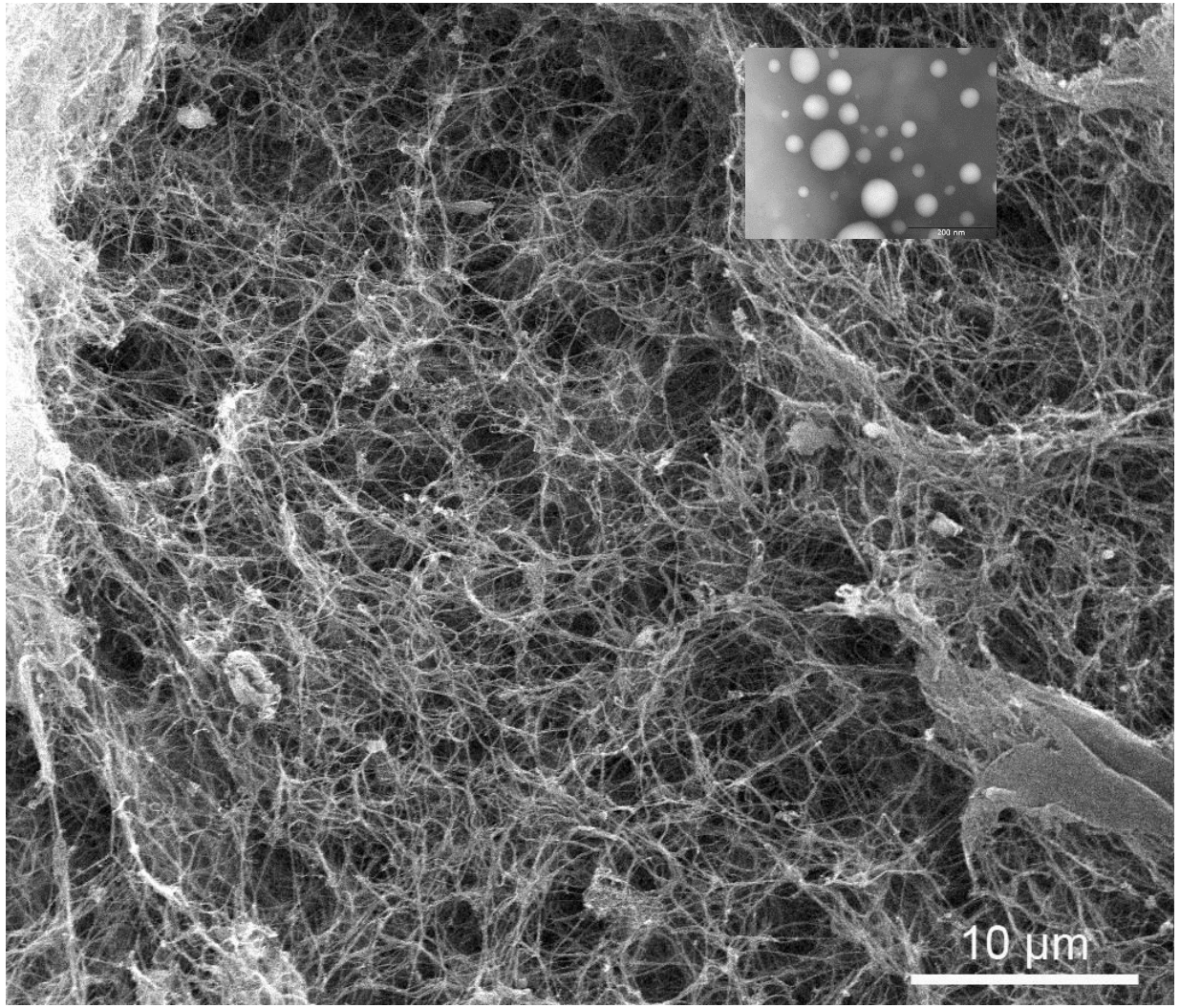
Extracellular matrix







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Lymfatický systém – rozsev mRNA vakcíny po těle po vstupu do krevního oběhu

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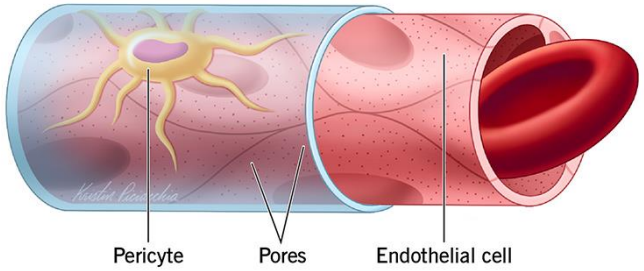
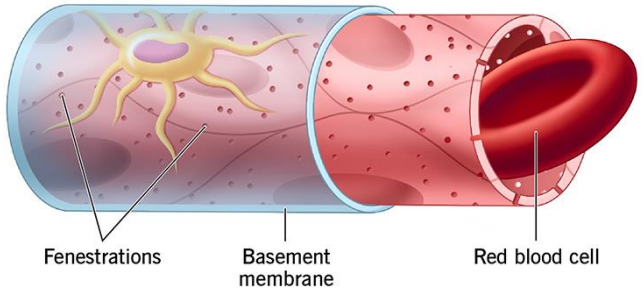
Fenestrations

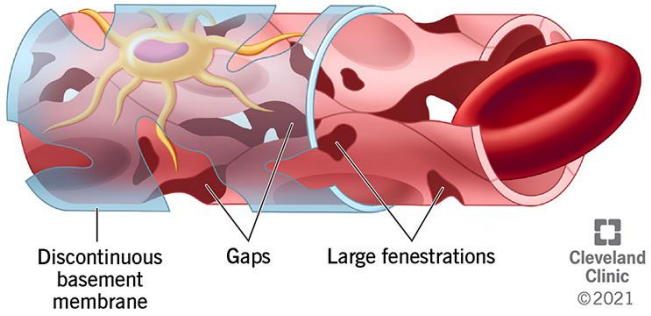
Continuous Capillary



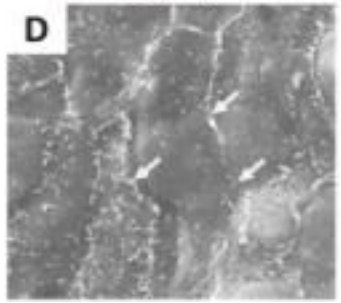
Fenestrated Capillary

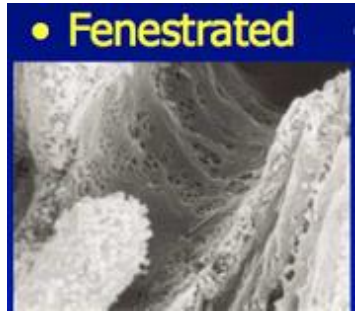


Sinusoidal Capillary

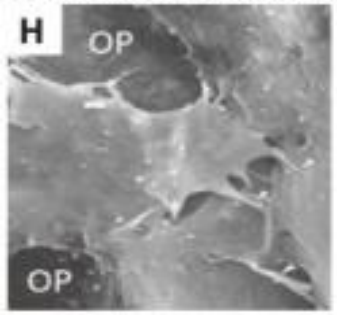


Endothelial surface
Arrows: tight junctions

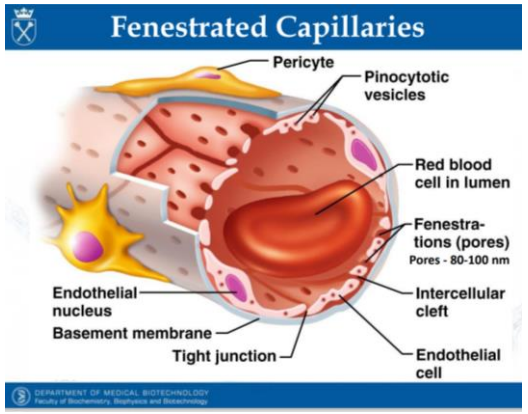




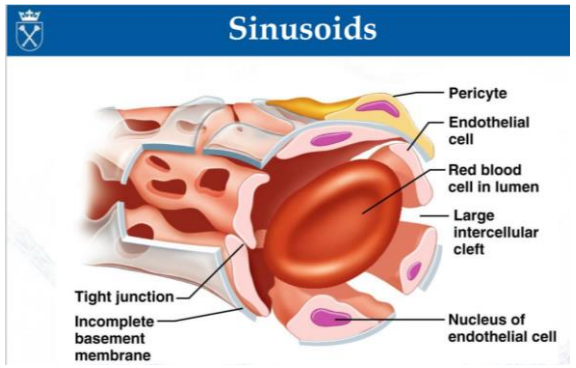
OP: endothelial opening



Found wherever active capillary absorption or filtrate formation occurs (e.g., small intestines, endocrine glands, and kidneys)



Found in the liver, spleen, lymphoid tissue, bone marrow and in some endocrine organs



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mRNA vaccine and Expression of S-protein in the body

1. There is no control over distribution of mRNA vaccine in the body!!!
2. There is no control over intensity and longevity of S-protein expression in tissues and organs!!!
3. There is no control over incorporation into genomic DNA!!! (endogenous reverse transcriptases)
4. Penetration through various barriers (blood- brain batrrier and placental barrier) can hit brain and foetus

Australian Government
Department of Health
Therapeutic Goods Administration

Nonclinical Evaluation Report

BNT162b2 [mRNA] COVID-19 vaccine (COMIRNATY™)

Submission No: PM-2020-05461-1-2
Sponsor: Pfizer Australia Pty Ltd

January 2021

TGA Health Safety Regulation

NONCLINICAL EVALUATION REPORT

Submission type:	New vaccine
Sponsor:	Pfizer Australia Pty Ltd
Generic name:	**BNT162b2 [mRNA] COVID-19 vaccine**
Trade name:	**COMIRNATY**
Dose form and strength:	Solution; 500 µg/mL (diluted to deliver 30 µg/0.3 mL per injection)
Vaccine Type:	mRNA encapsulated in LNP
Submission No:	PM-2020-05461-1-2
Tox file No:	E20-375469
TRIM reference:	D20-3908532
Date:	8 January 2021 (revised 15 January 2021)

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1.3. Product formulation

BNT162b2 vaccine is supplied as a preservative-free concentrated suspension formulation for intramuscular (IM) administration after dilution. The vaccine contains a nucleoside-modified messenger RNA, encoding the SARS-CoV-2 Spike-glycoprotein (S), produced by *in-vitro* transcription process and encapsulated into solid-lipid nanoparticles (LNP). The LNP is composed of four lipids: ALC-0315, ALC-0159, DSPC, and cholesterol. Quantities of mRNA, lipids and excipients are presented below in Table 1-1.

Table 1-1. Product formulation

Ingredient
BNT162b2 mRNA
((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate)*
2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide#
1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC)
Cholesterol
Potassium chloride
Disodium hydrogen phosphate
Potassium dihydrogen phosphate
Sodium chloride
Sucrose
Water for injections

s47

* co

Several manufacturing process changes for BNT162b2 mRNA, including production scale, were introduced during development. The proposed commercial scale manufacturing process includes use of linearised plasmid DNA template for mRNA production, whereas in early development phases PCR-amplification of DNA template was used. The characteristics of the BNT162b2 LNP batches used in the nonclinical studies are given in the following table (*Sponsor's response to TGA nonclinical questions*).

Table 1-3. LNP characterisation in nonclinical studies

Study no.	Batch LNP	Size (nm)	Encapsulation efficacy (%)	RNA (mg/mL)	Lipid (mg/mL)			
					ALC-0315	ALC-0159	DSPC	Cholesterol
R-20-0072	LNP8 FM-1074-D	s47						
072424 (PF-07302048_06Jul2)	FM-1261A	s47						
185350	NC-0552-1	s47						
R-20-0085	BNT162b2 (V9) (RBP020.2 LNP)	s47						
R-20-0112								
VR-VTR-10671								
20GR142								
20256434								
LPT 38166	BNT162b2(V8) (RBP020.1 LNP)	s47						

NA: not available in the certificate of analysis

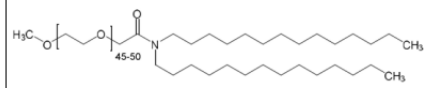
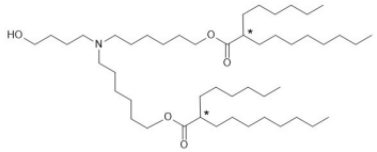
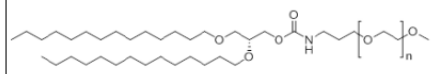
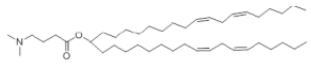
Both RBP020.1 LNP RNA and RBP020.2 LNP RNA were produced using the manufacturing process starting with (linear) PCR product.

1.4. Excipients

2-[(Polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) and ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate)(ALC-0315) are novel excipients and are not listed on the TGA's ingredient database. These excipients will be referred to as ALC-0159 and ALC-0315 in this report, respectively. As per the Sponsor's statement, ALC-0315 has been

optimised for RNA encapsulation and intracellular delivery, and ALC-0159 has been engineered to regulate LNP size (40 – 180 nm) and transfection potency. Batch data for multiple batches of proposed vaccine showed particle size (D50) in the range of 58 – 94 nm (Table 1-3). The lipids protect the mRNA from degradation, and facilitate cell uptake, hence they are a major determinant of potency (Jayaraman et al. 2012). The Sponsor indicated that these novel excipients, ALC-0159 and ALC-0315 are structurally and functionally similar to the lipid excipients PEG2000-C DMG and DLin-MC3-DMA, respectively, used in a siRNA-LNP drug product Onpattro™ (patisiran), which is approved in the US, Europe (by EMA) and Canada.

Details of these lipids are shown below.

Product	Novel lipid excipient (CAS no, Molecular formula and weight in Da)	Structure	Chemical name
BNT162b2 V9	**ALC-0159** (*CAS no.* 1849616-42-7, $(C_2H_4O)_nC_{31}H_{63}NO_2$, n=45-50 and 2400-2600 Da]		2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide
	ALC-0315 (*CAS no* 2036272-55-4, $C_{48}H_{95}NO_5$ and 766 Da]		((4-hydroxybutyl)azanediyl)bis (hex ane-6,1-diyl)bis(2-hexyldecanoate)
Onpattro™ (patisiran)	PEG2000-C-DMG [NA, $C_2H_4O)_nC_{36}H_{73}NO_5$, n=47 and 2650±300 Da]		(α-(3'-{[1,2-di(myristyloxy)proponoxyy] carbonylamino}propyl)-ω-methoxy, polyoxyethylene)
	DLin-MC3-DMA [NA, $C_{43}H_{79}NO_2$ and 642.09 Da]		(6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl-4-(dimethylamino) butanoate

Biodistribuce mRNA vakcíny

Table 4-2. Mean concentration of radioactivity (sexes combined) in tissue and blood following a single IM dose of 50 µg mRNA/rat

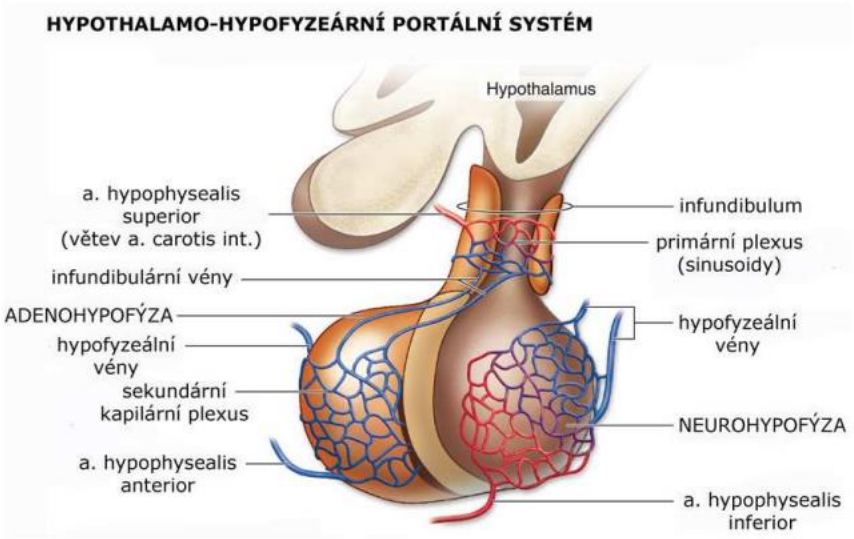
Sample	Total Lipid Concentration (µg lipid equiv/g (or mL))						
	0.25 min	1 h	2 h	4 h	8 h	24 h	48 h
Adipose tissue	0.057	0.100	0.126	0.128	0.093	0.084	0.181
Adrenal glands	0.27	1.48	2.72	2.89	6.80	13.77	18.21
Bladder	0.041	0.130	0.146	0.167	0.148	0.247	0.365
Bone (femur)	0.091	0.195	0.266	0.276	0.340	0.342	0.687
Bone marrow (femur)	0.48	0.96	1.24	1.24	1.84	2.49	3.77
Brain	0.045	0.100	0.138	0.115	0.073	0.069	0.068
Eyes	0.010	0.035	0.052	0.067	0.059	0.091	0.112
Heart	0.28	1.03	1.40	0.99	0.79	0.45	0.55
Injection site	128.3	393.8	311.2	338.0	212.8	194.9	164.9
Kidneys	0.39	1.16	2.05	0.92	0.59	0.43	0.42
Large intestine	0.013	0.048	0.09	0.29	0.65	1.10	1.34
Liver	0.74	4.62	10.97	16.55	26.54	19.24	24.29
Lung	0.49	1.21	1.83	1.50	1.15	1.04	1.09
Lymph node (mandibular)	0.064	0.189	0.290	0.408	0.534	0.554	0.727
Lymph node (mesenteric)	0.050	0.146	0.530	0.489	0.689	0.985	1.366
Muscle	0.021	0.061	0.084	0.103	0.096	0.095	0.192
Ovaries (females)	0.104	1.34	1.64	2.34	3.09	5.24	12.26
Pancreas	0.081	0.207	0.414	0.380	0.294	0.358	0.599
Pituitary gland	0.339	0.645	0.868	0.854	0.405	0.478	0.694
Prostate (males)	0.061	0.091	0.128	0.157	0.150	0.183	0.170
Salivary glands	0.084	0.193	0.255	0.220	0.135	0.170	0.264
Skin	0.013	0.208	0.159	0.145	0.119	0.157	0.253
Small intestine	0.030	0.221	0.476	0.879	1.279	1.302	1.472
Spinal cord	0.043	0.097	0.169	0.250	0.106	0.085	0.112
Spleen	0.33	2.47	7.73	10.30	22.09	20.08	23.35
Stomach	0.017	0.065	0.115	0.144	0.268	0.152	0.215
Testes (males)	0.031	0.042	0.079	0.129	0.146	0.304	0.320
Thymus	0.088	0.243	0.340	0.335	0.196	0.207	0.331
Thyroid	0.155	0.536	0.842	0.851	0.544	0.578	1.000
Uterus (females)	0.043	0.203	0.305	0.140	0.287	0.289	0.456
Whole blood	1.97	4.37	5.40	3.05	1.31	0.91	0.42
Plasma	3.96	8.13	8.90	6.50	2.36	1.78	0.81
Blood:plasma ratio	0.815	0.515	0.550	0.510	0.555	0.530	0.540

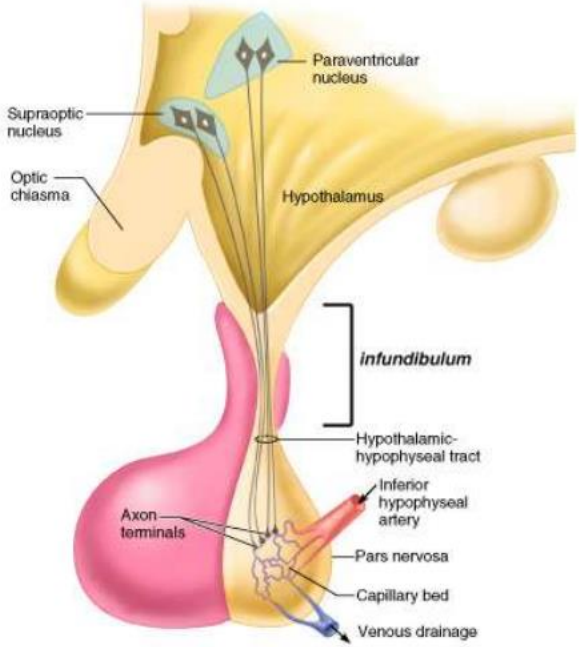
- Mean total radioactivity was greatest at the injection site followed by the liver with much lower total recovery in spleen, adrenal glands and ovaries (Table 4-2). The total radioactivity recovery was less than 100% at all time-points (range = 20 – 60%) probably due to difficulty in collecting entirety of injection site samples and the presence of radioactivity in the carcass, faeces and urine, which were not analysed.
- The tissue distribution pattern was similar in 100 µg mRNA/animal dose group as noted above for 50 µg mRNA/animal dose, with highest distribution into liver, adrenal glands and spleen.
- Draining lymph nodes to the site of injection should have been collected and analysed for radioactivity, given the increased size of draining lymph nodes seen in other nonclinical studies after dosing.

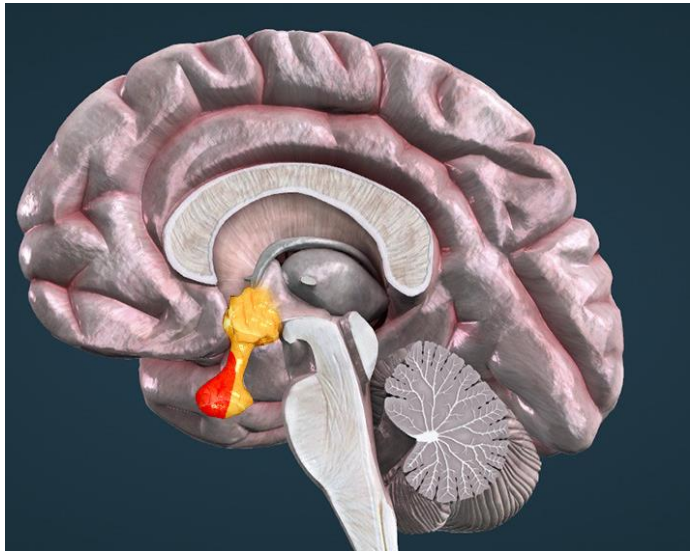
Rozsev mRNA vakcíny byl prokázán do všech orgánů

Účinek mRNA vakcíny na hypotalamus-hypofyzální systém

Endokrinní funkční jednotky: (1) HT – adenohypofýza a (2) HT - neurohypofýza

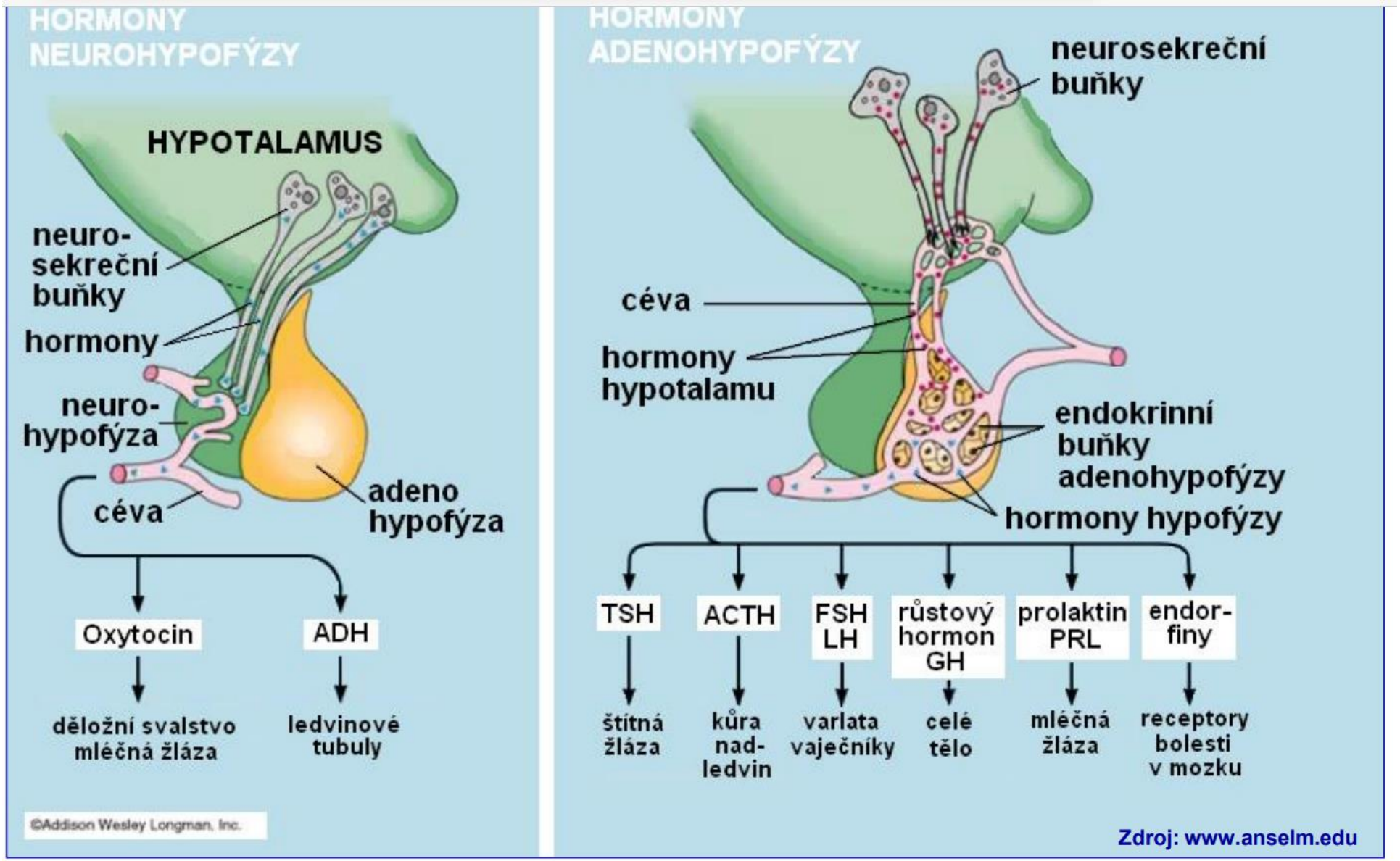






Pozorované vedlejší účinky mRNA vakcíny připomínají patologii poruchy produkce hormonů hypofýzou

Syndrom posturální ortostatické tachykardie



Možné dopady mRNA vakcinace proti covid-19 na imunitní systém

1. Rozsáhle zdokumentovaná **subverze vrozené imunity**, primárně prostřednictvím suprese IFN-α a jeho přidružené signalizační kaskády. Toto potlačení může vést k širokému spektru důsledků, mezi které patří reaktivace latentních virových infekcí a snížená schopnost účinného boje s budoucí infekcí. **Narušení hormonálního řízení organismu**.

2. **Dysregulace systému** jak pro prevenci, tak **pro dohled nad maligní transformací** v buňkách a následná potenciace těchto změn vakcinací.

3. Vakcinace mRNA potenciálně **narušuje intracelulární komunikaci** zajišťovanou exosomy a přiměje buňky k přijímání spikové mRNA, což vede k produkci vysoké hladiny exosomů nesoucích spike. To může vést k vážným důsledkům skrze zánětlivé stavy. Pokud by se některá z těchto potenciálních hrozeb plně realizovala, dopad na miliardy lidí po celém světě by mohla být obrovský a mohl by přispět jak ke krátkodobým, tak dlouhodobým onemocněním, které budou zatěžovat náš zdravotní systém.

Innate Immune Suppression by SARS-CoV-2 mRNA Vaccinations: The role of Gquadruplexes, exosomes and microRNAs Stephanie Seneff, Greg Nigh, Anthony M. Kyriakopoulos, and Peter A McCullough
Food and Chemical Toxicology January 2022 DOI: 10.22541/au.164276411.10570847/v1

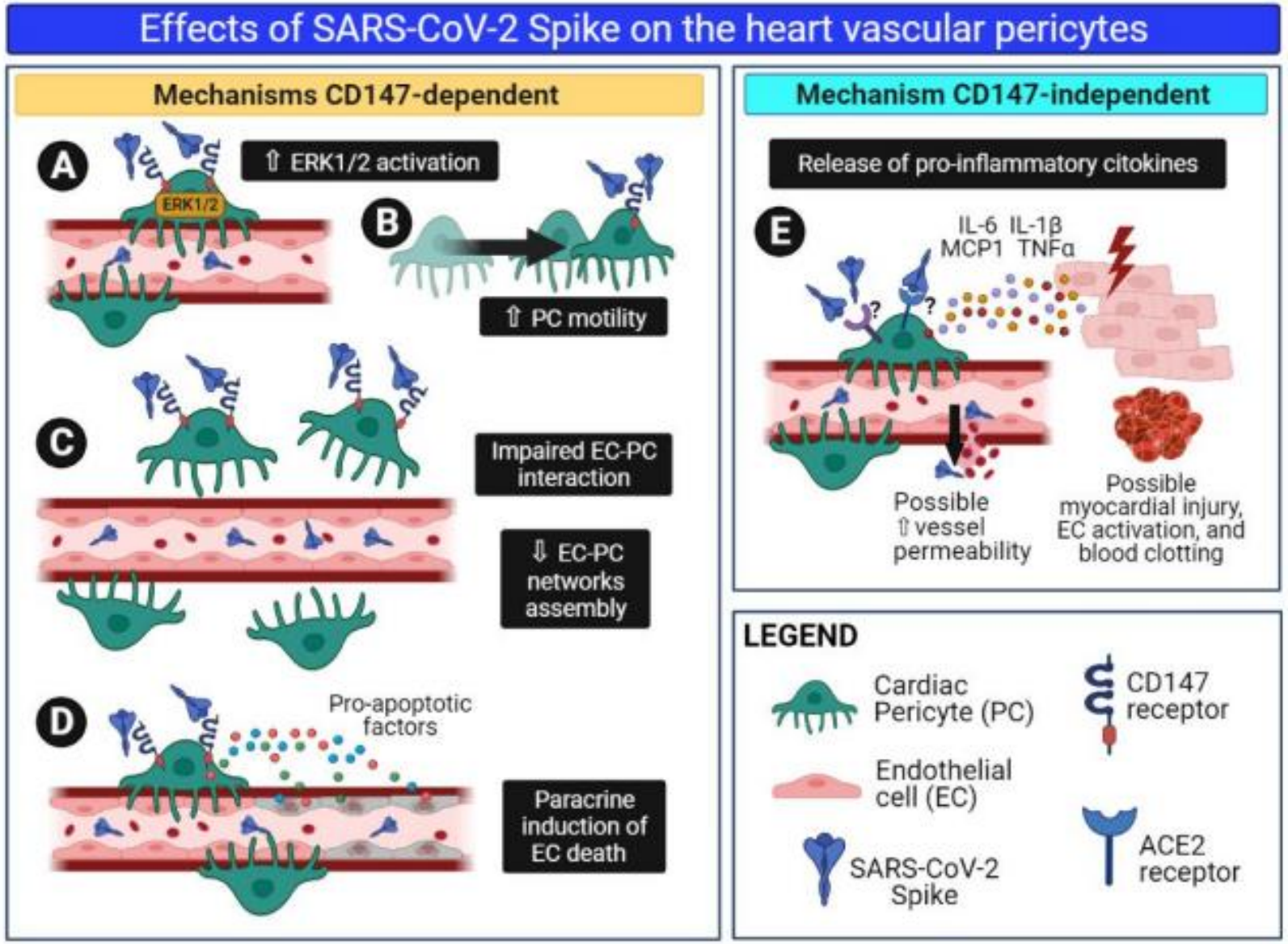
Myokarditida a perikarditida

Značná pozornost médií byla věnována skutečnosti, že vakcíny COVID-19 způsobují myokarditidu a perikarditidu, se zvýšeným rizikem zejména u mužů mladších 30 let. Myokarditida je spojena s aktivací krevních destiček, takže to může být jeden z faktorů reakce na vakcíny. Další faktor by však mohl souviset s exosomy uvolněnými makrofágy infikovanými mRNA vakcínami a specifickými mikroRNA nalezenými v těchto exosomech.

Myocarditis after immunization with COVID-19 mRNA vaccines in members of the US military. This article reports that in “23 male patients, including 22 previously healthy military members, myocarditis was identified within 4 days after receipt of the vaccine”: https://jamanetwork.com/journals/jamacardiology/fullarticle/2781601

Myocarditis, pericarditis and cardiomyopathy after COVID-19 vaccination: https://www.sciencedirect.com/science/article/pii/S1443950621011562
COVID-19 Vaccination Associated with Myocarditis in Adolescents: https://pediatrics.aappublications.org/content/pediatrics/early/2021/08/12/peds.2021-053427.full.pdf
1.Acute myocarditis after administration of BNT162b2 vaccine against COVID-19: https://pubmed.ncbi.nlm.nih.gov/33994339/

Záněty srdce



The SARS-CoV-2 S protein alters cardiac pericyte function. Schematic summary of the research. We hypothesize that in patients with acute COVID-19, S protein molecules are cleaved from the virus particle and released from the respiratory system into the bloodstream. Through the circulation, isolated S protein fragments reach all organs of the body, including the heart. Here, the interaction of the S protein with the CD147 receptor on cardiac PCs triggers the ERK1/2 signalling (A) and provokes PC dysfunction, including increased cell motility (B) and decreased angiogenic activity in cooperation with coronary ECs (C). In addition, the S protein-CD147 interaction prompts cardiac PCs to release pro-apoptotic factors, which cause EC death (D). Finally, through a mechanism CD147-independent, the S protein induces PCs to release pro-inflammatory cytokines, which include MCP-1, IL-6, IL-1β, and TNF-α (E). These cytokines can damage neighbouring cardiomyocytes and activate ECs, potentially promoting blood to clot and increasing vascular permeability.

The SARS-CoV-2 Spike protein disrupts human cardiac pericytes function through CD147-receptor-mediated signalling: a potential non-infective mechanism of COVID-19 microvascular disease Elisa Avolio, et al.
Clinical Science doi: 10.1042/CS20210735

CD147-spike protein is a novel route for SARS-CoV-2 infection to host cells Signal Transduction and Targeted Therapy (2020) 5:283

Vedlejší účinky vakcinace mRNA vakcínami

Více než 1000 publikací v odborných časopisech dokládá vedlejší účinky vakcinace mRNA vakcínami.
www.saveusnow.org.uk/covid-vaccine-scientific-proof-lethal/

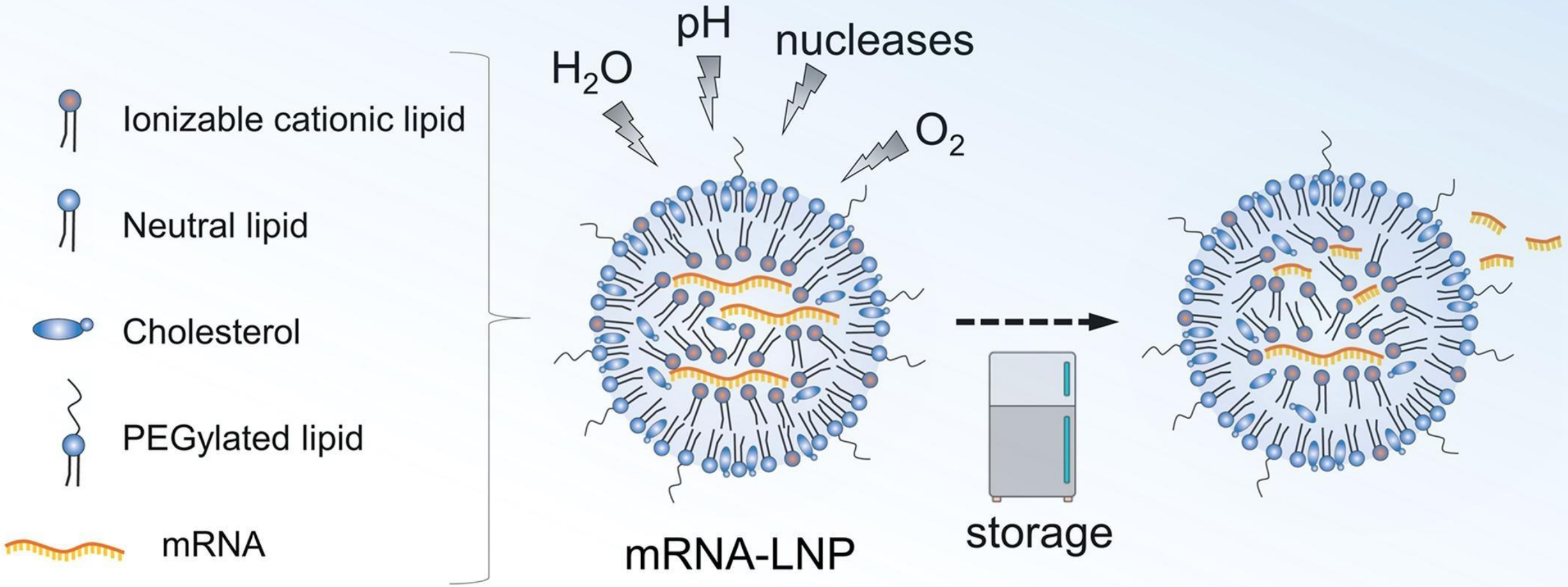
Závažné komplikace po vakcinaci

Zánět srdce a osrdečníku, zánět nadvarlat, cysty na vaječnících, trombózy a trombocytopenie, infarkty a náhlá úmrtí, vyhaslá těhotenství v prvním trimestru, poruchy menstruace a krvácení, nádorová onemocnění, otoky mízních uzlin, autoimunitní onemocnění, pankreatitidy.

Která autorita v České republice kontroluje kvalitu šarží dovážených vakcín proti Covid-19 používaných k vakcinaci obyvatel ?

Kdo nese odpovědnost?!

Stability of mRNA vaccines



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Skutečný postup pro analýzu mRNA a vektorových vakcín

Postup pro analýzu vzorků vakcín zaslán na SÚKL

Zpracoval: Prof. RNDr. Jaroslav Turánek, DSc.

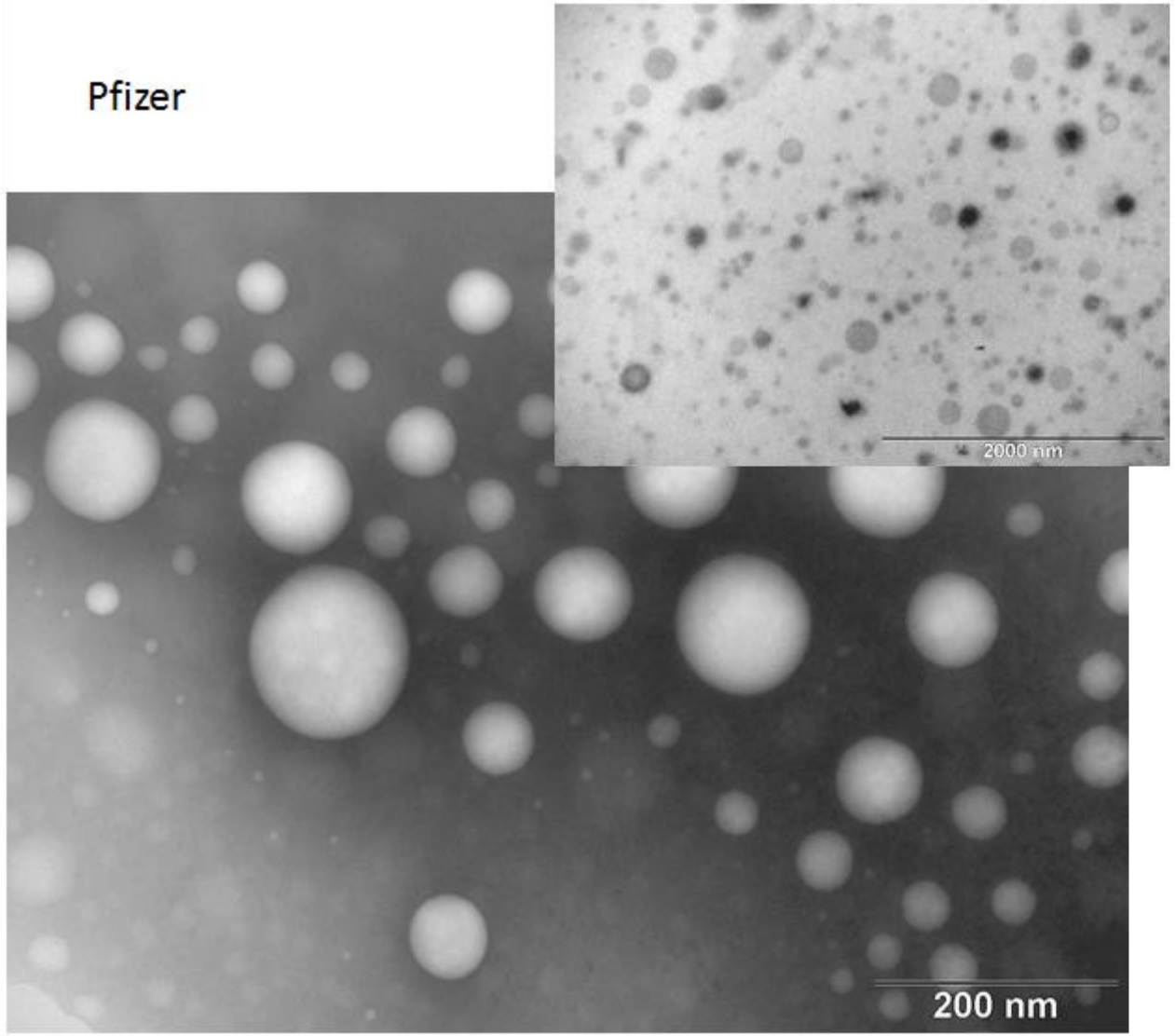
Testy pro analýzu mRNA vakcín od firem Moderna, Pfizer, a virových vektorových DNA vakcín od firme Astra Zeneca a Johnson & Johnson

1. Získání ověřených šarží vakcín s historií skladování, notářské potvrzení pravosti
2. Vizuální posouzení stavu vakcín
3. Změření transmitance na přístroji Spekord 600, pH, refraktometrického indexu a vodivosti (Sytém Mettler Toledo)
4. Změření distribuce velikosti, počtu částic a ζ-potenciálu na přístroji ZetaSizer Ultra+
5. Analýza částic pomocí diferenciální gradientové ultracentrifugace (Ultracentrifuga Optima XPN-90 Beckman) a analýza jednotlivých frakcí pomocí DLS a elektronové mikroskopie
6. Změření distribuce velikosti a počtu částic metodou průtokové nanocytometrie na přístroji nanoAnalyzer
7. Analýza částic ve vakcíně pomocí TEM, SEM a posouzení strukturální integrity nanočástic
8. Analýza mRNA (mRNA vakcíny) pDNA (vektorové virové vakcíny) ve vakcínách; intaktnost mRNA respektive pDNA, kvantifikace a množství nukleové kyseliny pomocí horizontální elektroforézy; konfirmace sekvence; exaktní kvantifikace metodou QRT-PCR, analýza kontaminující DNA pomocí ELFO.
9. Funkčnost – transfekce a exprese S-proteinu *in vitro* a kvantifikace proteinu metodou ELISA, průkaz exprese proteinu v buňkách metodou konfokální mikroskopie a imunochemické vizualizace

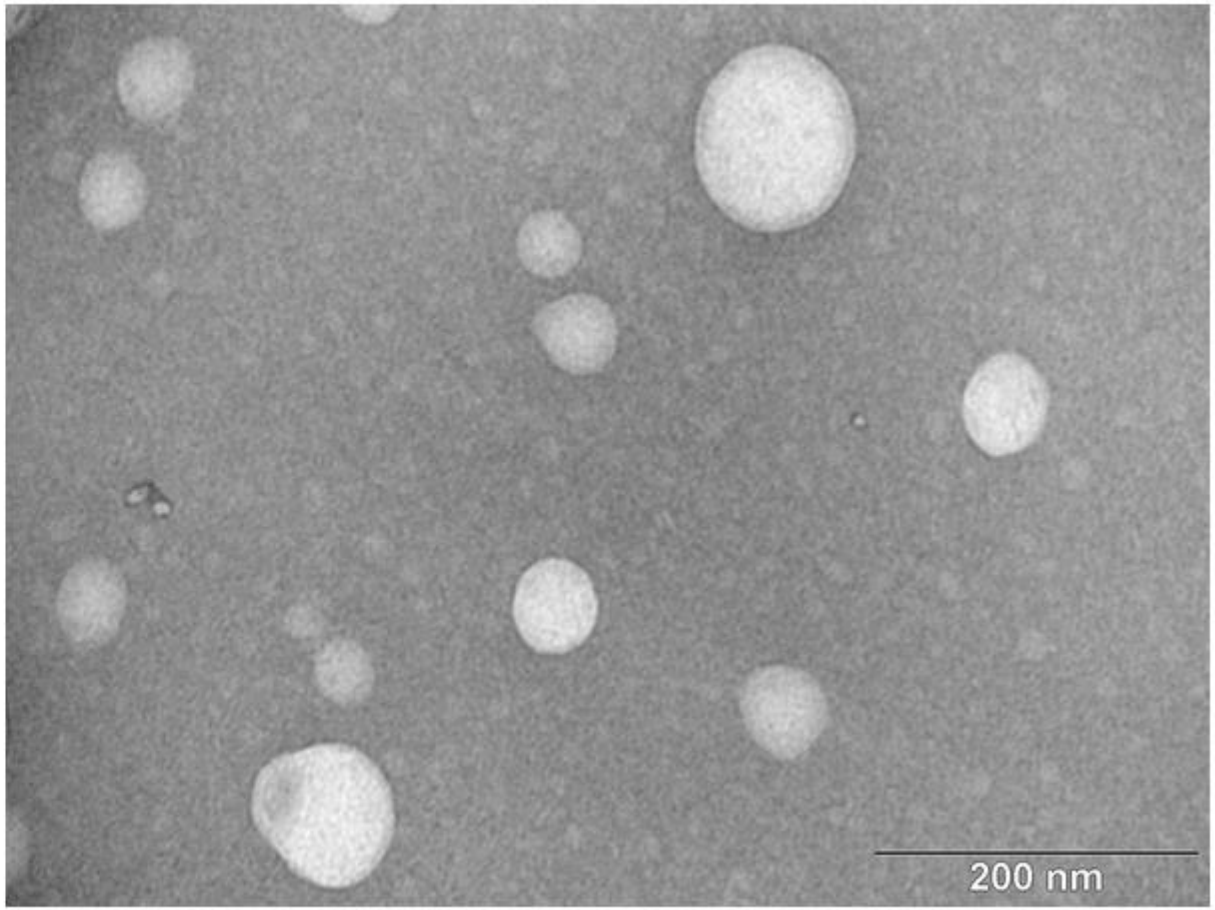
V případe zjištění dalších částic, které nejsou ve složení vakcín deklarovány, budou tyto částice podrobeny další analýze pomocí Ramanovy a infračervené spektroskopie a mikroskopie, elektronové mikroskopie s analýzou prvků (EDX detektor)

Cryo-electron microscopy of mRNA-LNP

Pfizer



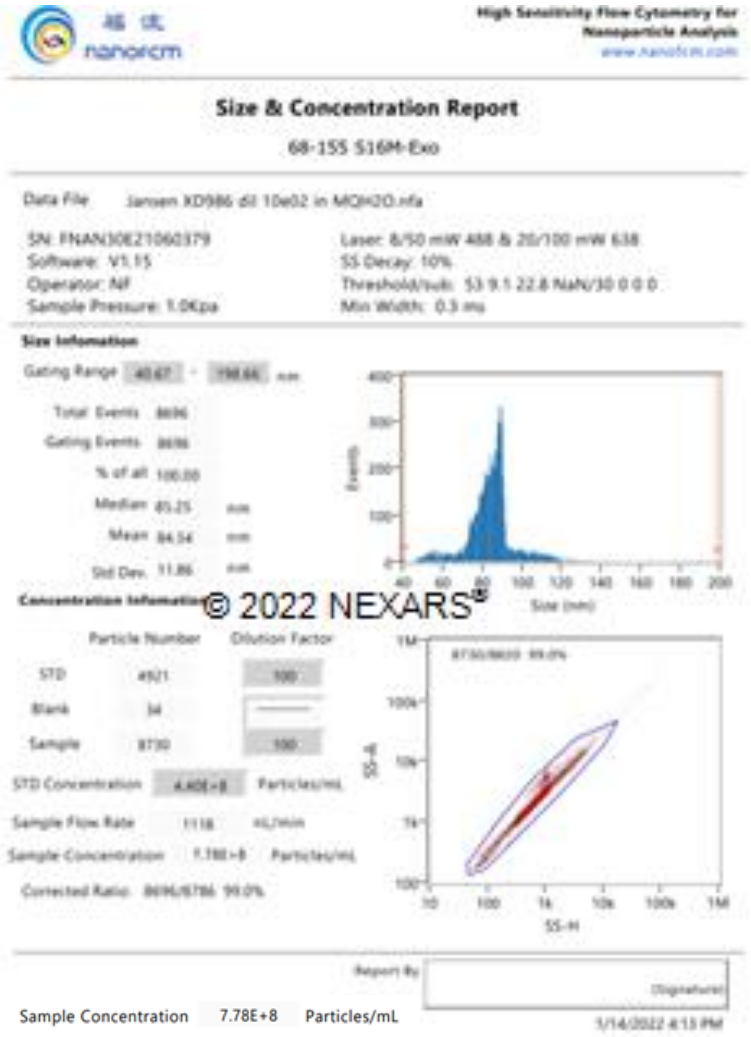
Moderna

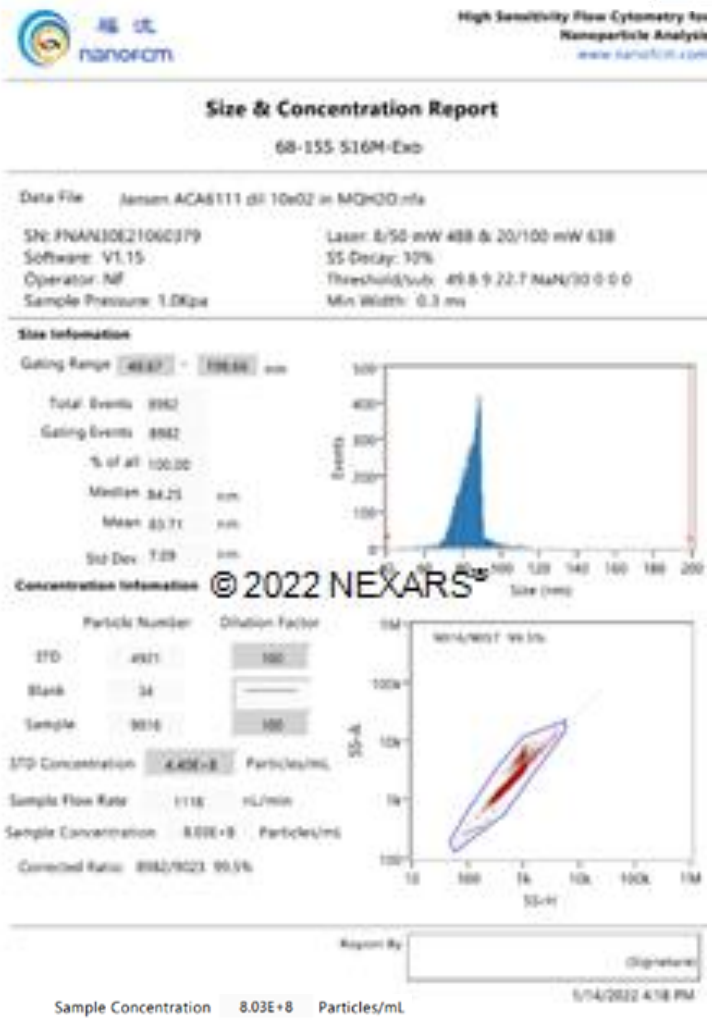


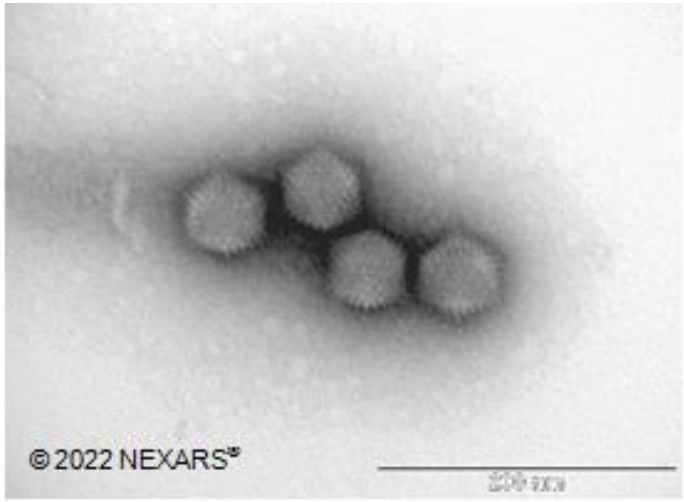
Velikost nanočásti mRNA vakcíny umožňuje diseminaci do všech orgánů

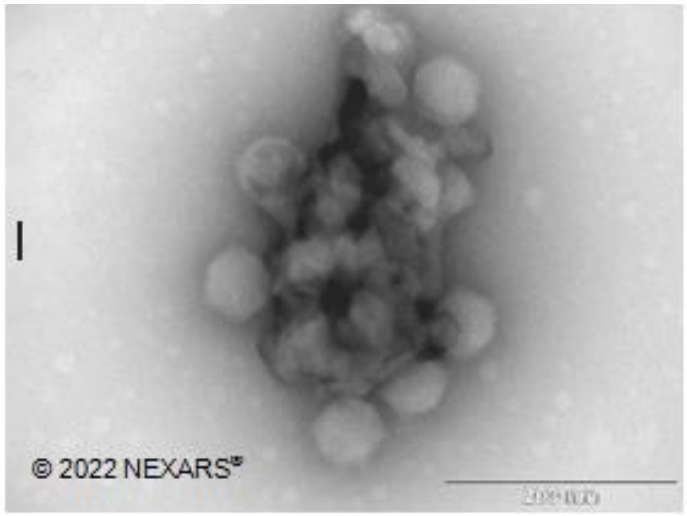
Analysis of viral vector vaccine Jansen

Transmission electron microscopy



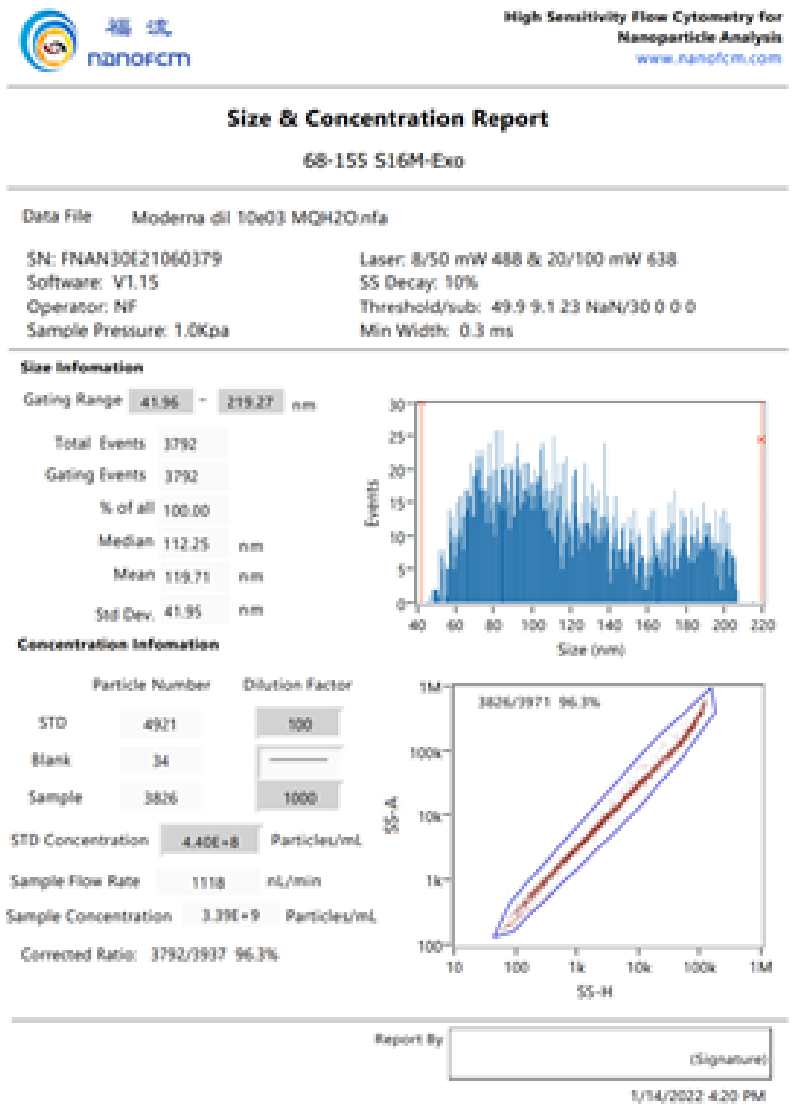






Analýza metodou průtokové nanocytometrie

Ukázka některých výsledků analýzy vakcíny Moderna



nanofcm

High Sensitivity Flow Cytometry for Nanoparticle Analysis
www.nanofcm.com

Size & Concentration Report

68-155 S16M-Exo

Data File: Moderna dil 10e03 MQH2O.nfa

SN: FNAN30E21060379
Software: V1.15
Operator: NF
Sample Pressure: 1.0Kpa

Laser: 8/50 mW 488 & 20/100 mW 638
SS Decay: 10%
Threshold/sub: 49.9 9.1 23 NaN/30 0 0 0
Min Width: 0.3 ms

Size Infomation

Gating Range: 41.96 - 219.27 nm

Total Events	3792	
Gating Events	3792	
% of all	100.00	
Median	112.25	nm
Mean	119.71	nm
Std Dev.	41.95	nm

Concentration Infomation

	Particle Number	Dilution Factor
STD	4921	100
Blank	34	——
Sample	3826	1000

STD Concentration: 4.40E+8 Particles/mL

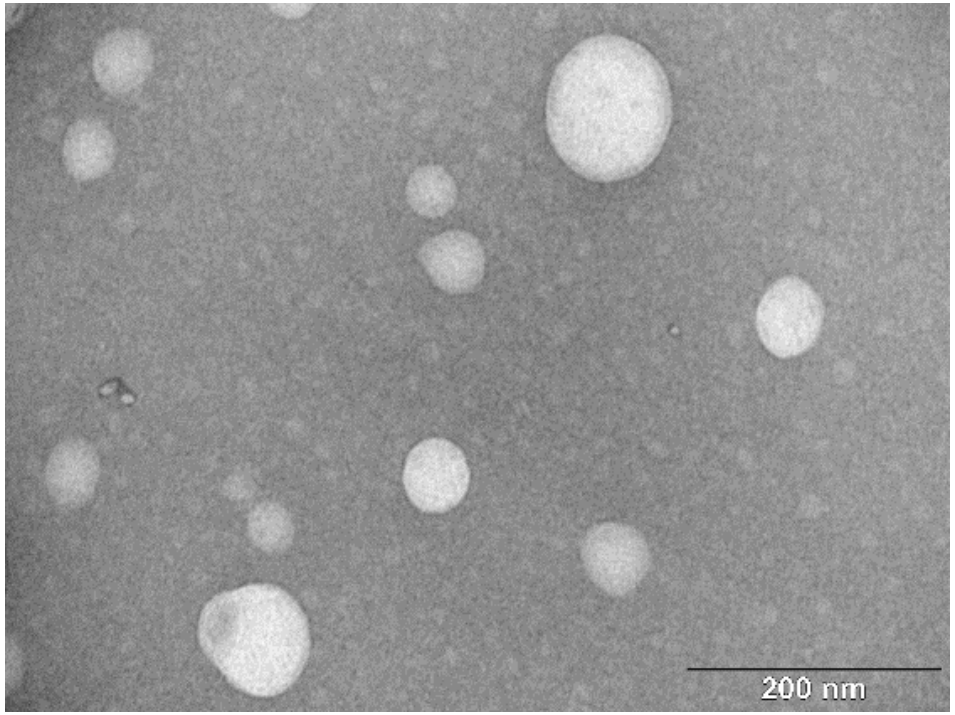
Sample Flow Rate: 1118 nL/min

Sample Concentration: 3.39E+9 Particles/mL

Corrected Ratio: 3792/3937 96.3%

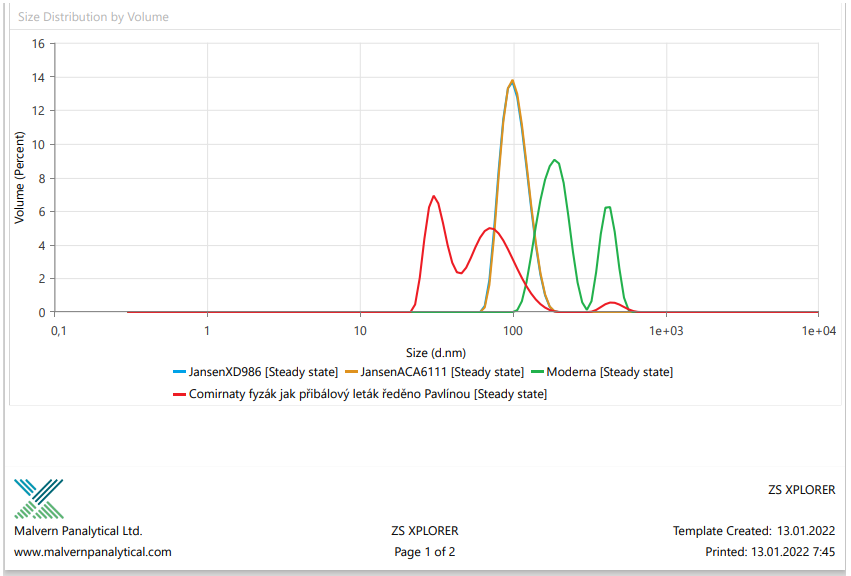
Report By: (Signature)

1/14/2022 4:20 PM



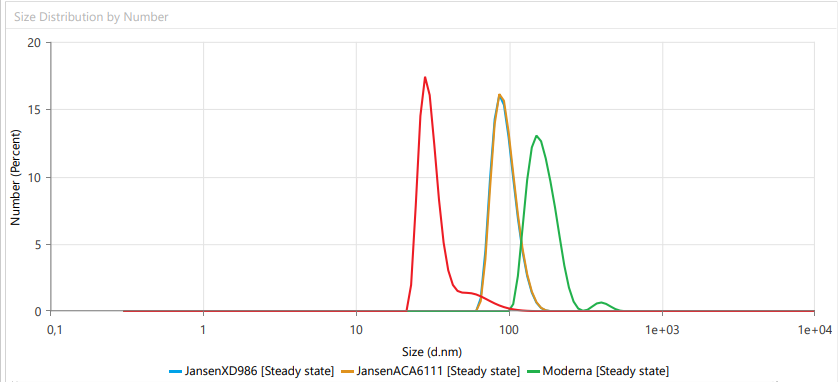
Sample Concentration 3.39E+9 Particles/mL

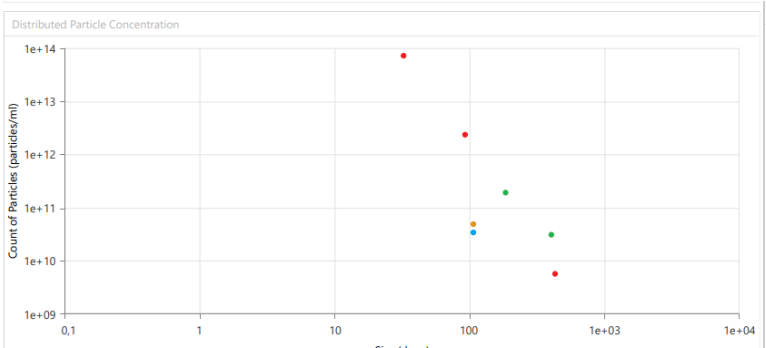
Analyses of mRNA vaccines

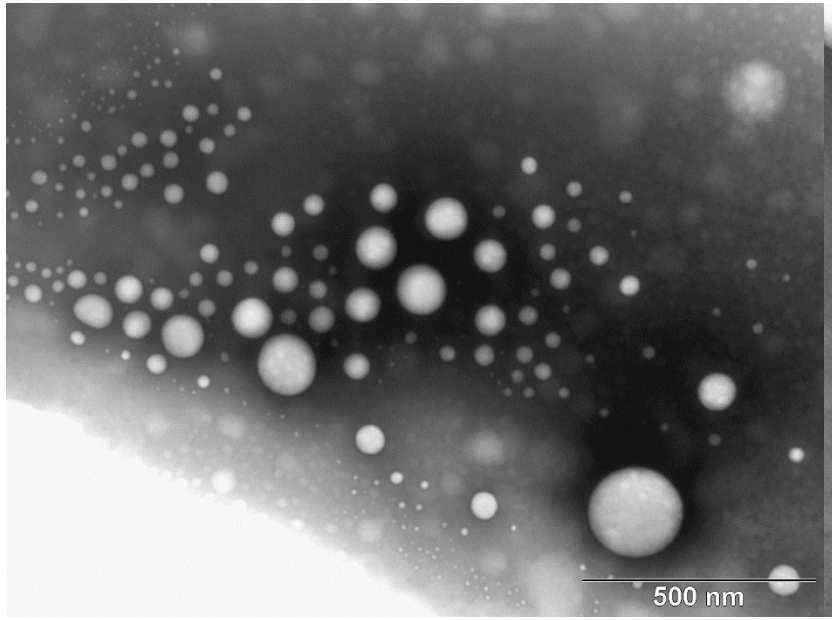


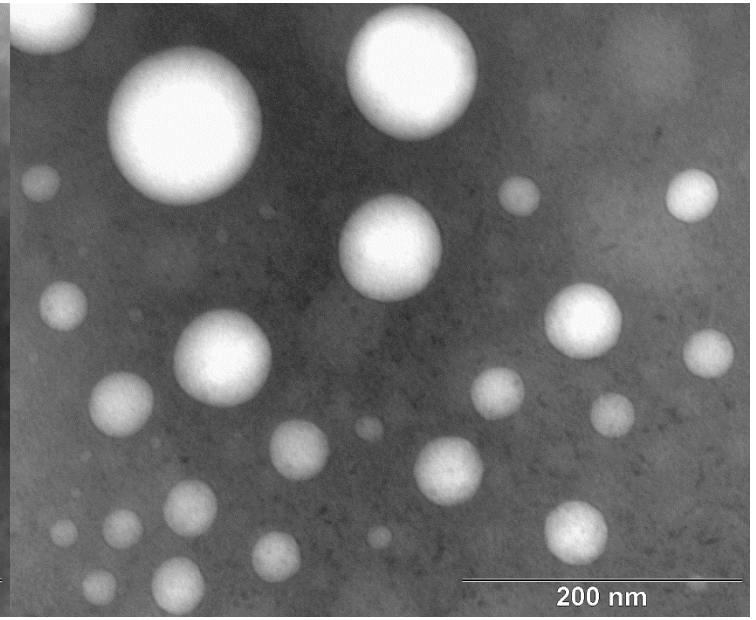
Vakcíny - prosinec 2021

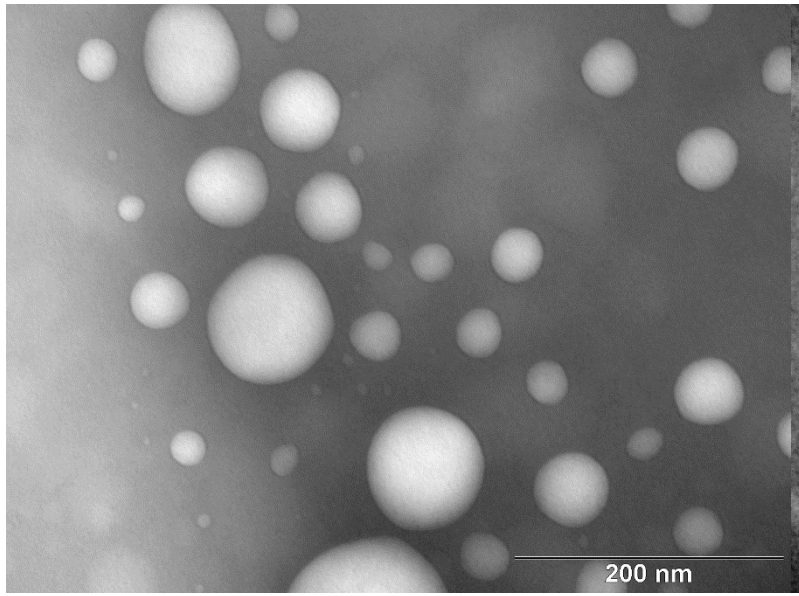
Malvern Panalytical

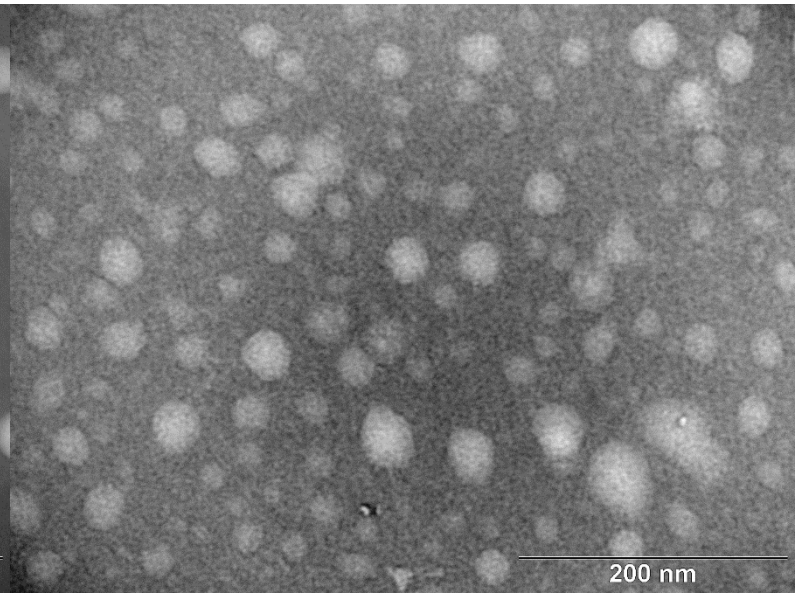








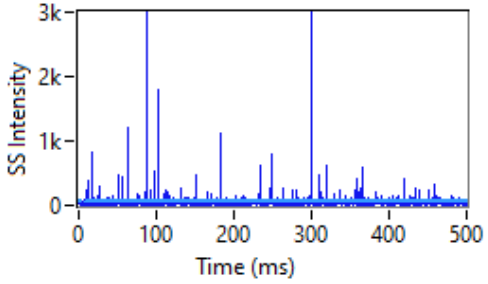


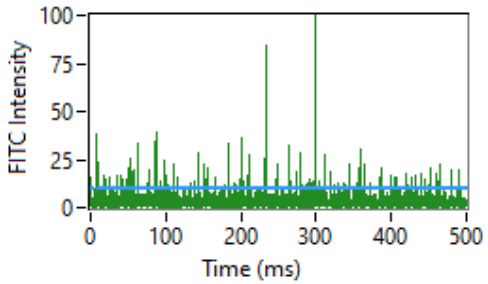


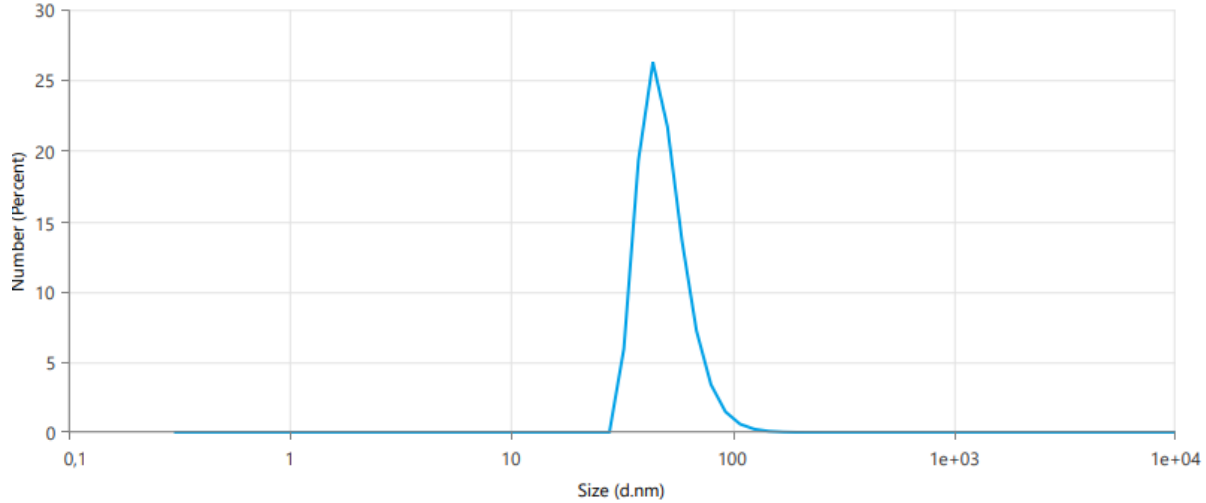
Analýza vakcíny COMIRNATY Omicron BA.4-5 GH9851; Exp 06/23

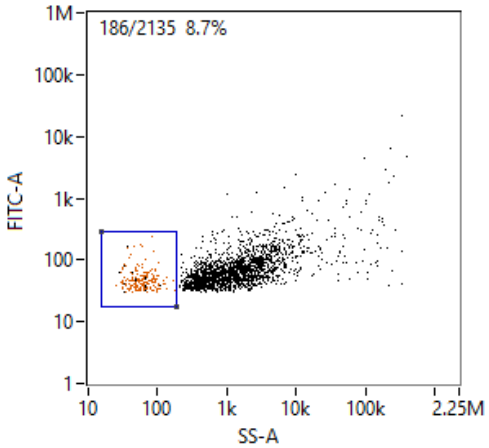
- Vakcína značená **SYTO O9**
- Fluorescence – detekce v SS i FITC kanálu

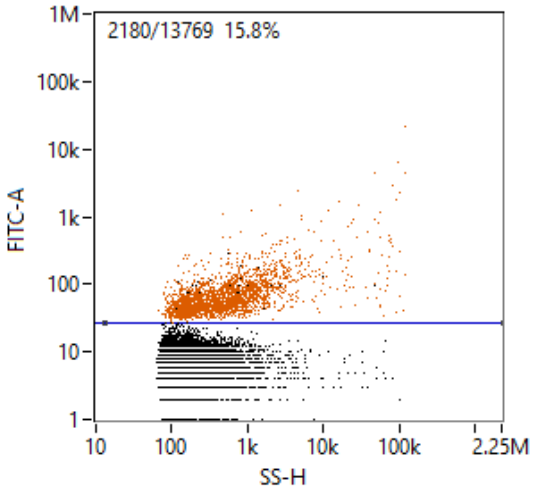
	Velikost [nm]	Conc [par/mL]	PDI [-]
NanoFCM	61,3 ± 12,5	$1,3 .10^{11}$	-
DLS_{number}	50,6 ± 5,8	$8,8 .10^{9}$	0,4 ± 0,1

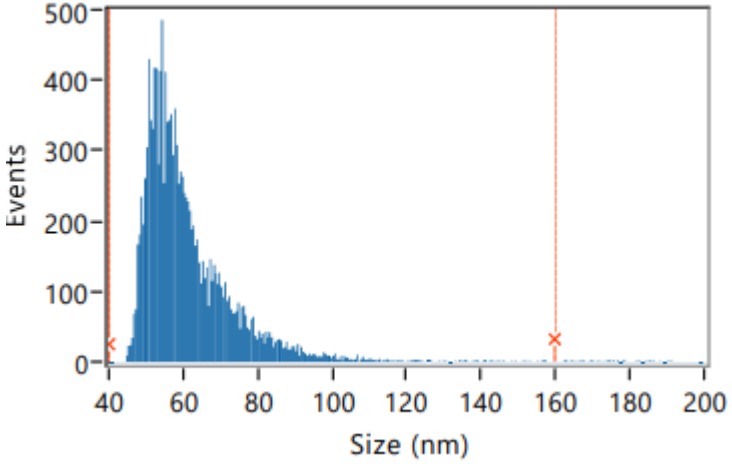


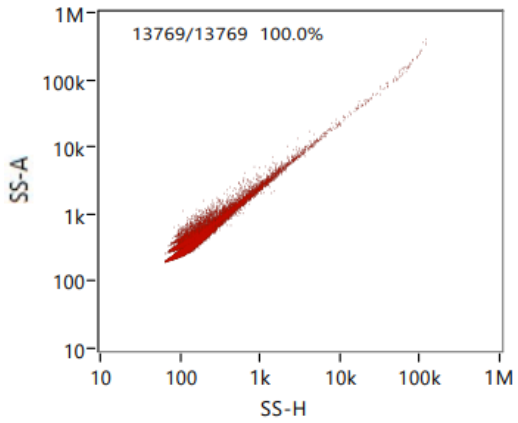


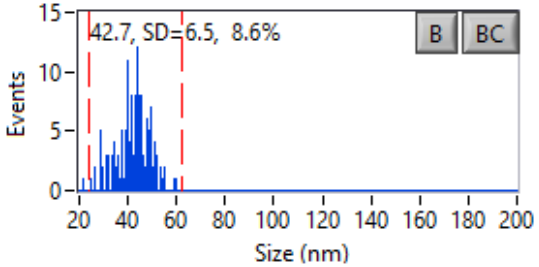


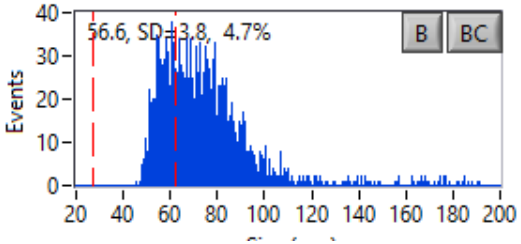












Analýza vakcíny COMIRNATY Omicron BA.4-5 GH9851; Exp 06/23

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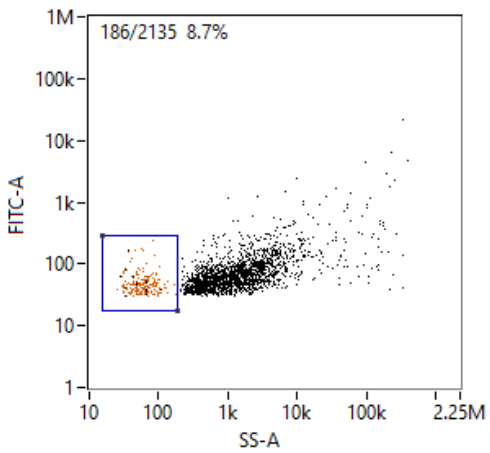
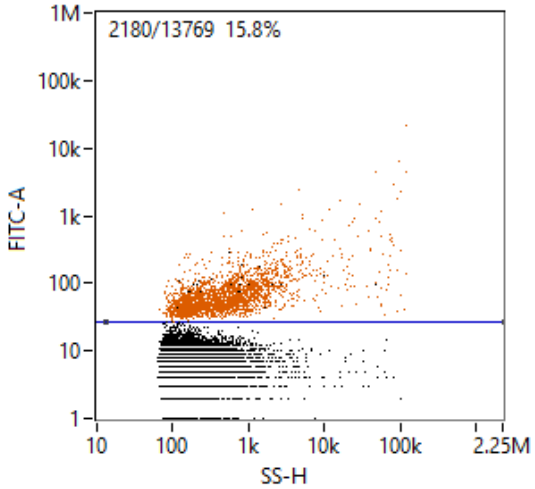
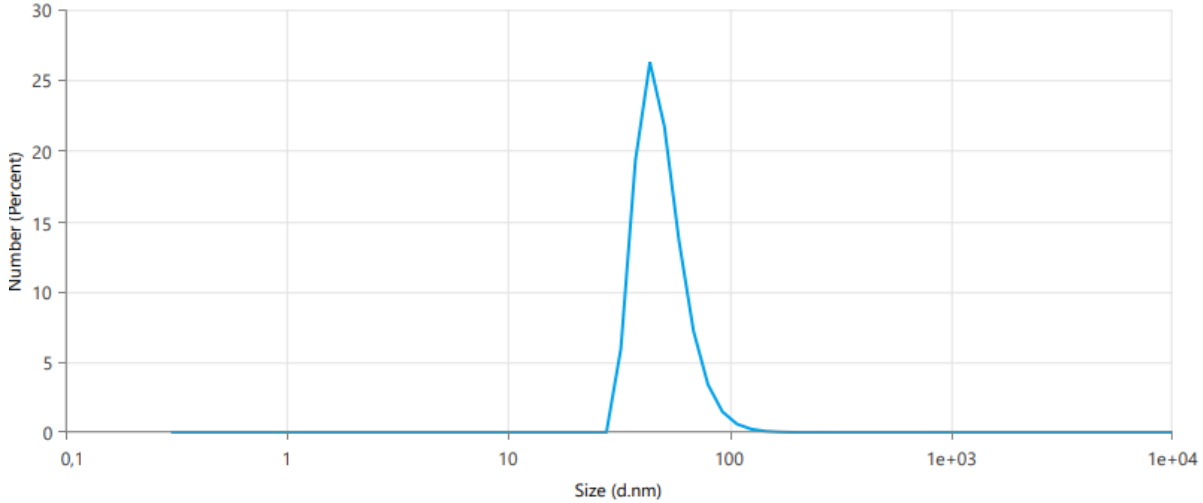
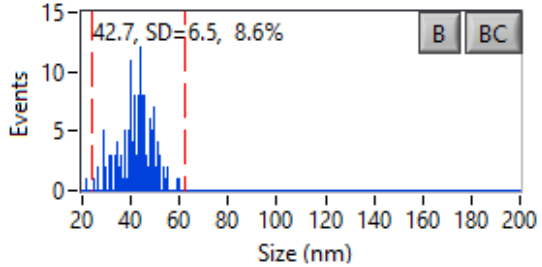
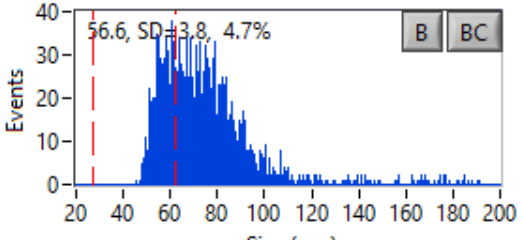
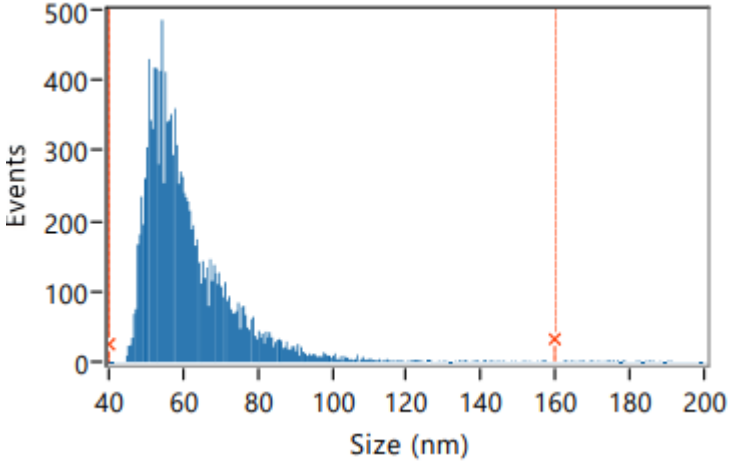


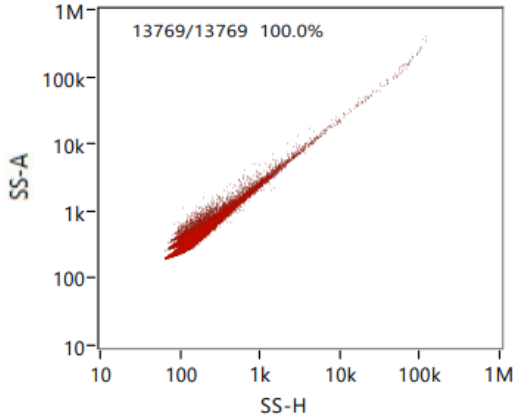




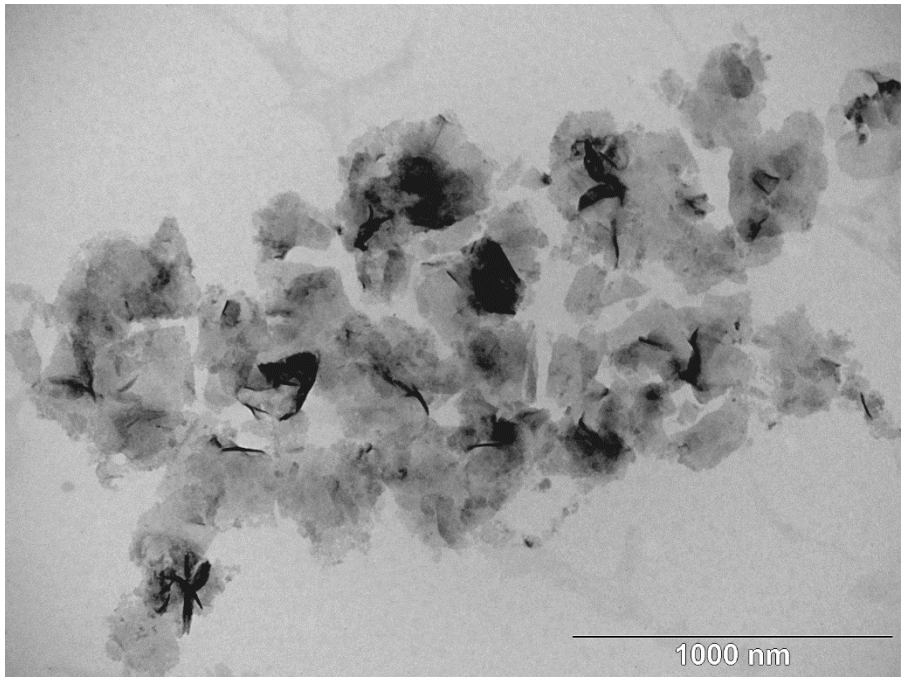




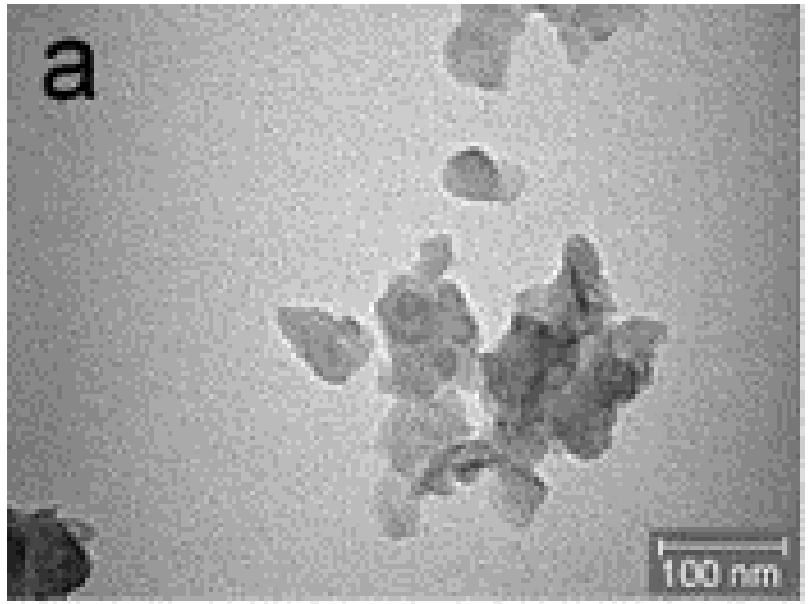


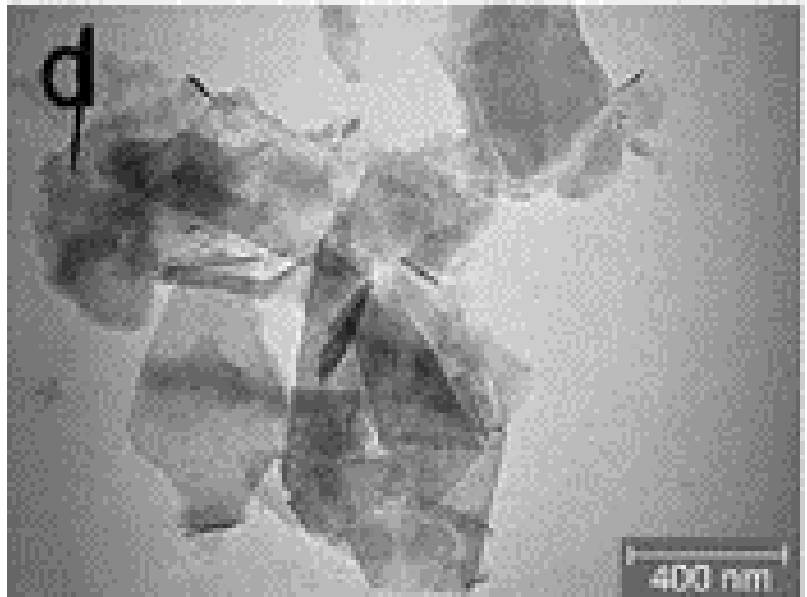


TEM pristine graphene found
in Pfizer mRNA vaccine



Pristine graphene





Proinflammatory Effect of Carbon-Based Nanomaterials: In Vitro Study on Stimulation of Inflammasome NLRP3 via Destabilisation of Lysosomes
Svadlakova, T; Hubatka, F; (...); Turanek, J
Mar 2020 |
NANOMATERIALS 10 (3)

Received: 22 January 2023 | Accepted: 26 March 2023

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RESEARCH LETTER

WILEY

Batch-dependent safety of the BNT162b2 mRNA COVID-19 vaccine

Max Schmeling[1] | Vibeke Manniche[2] | Peter Riis Hansen[3]

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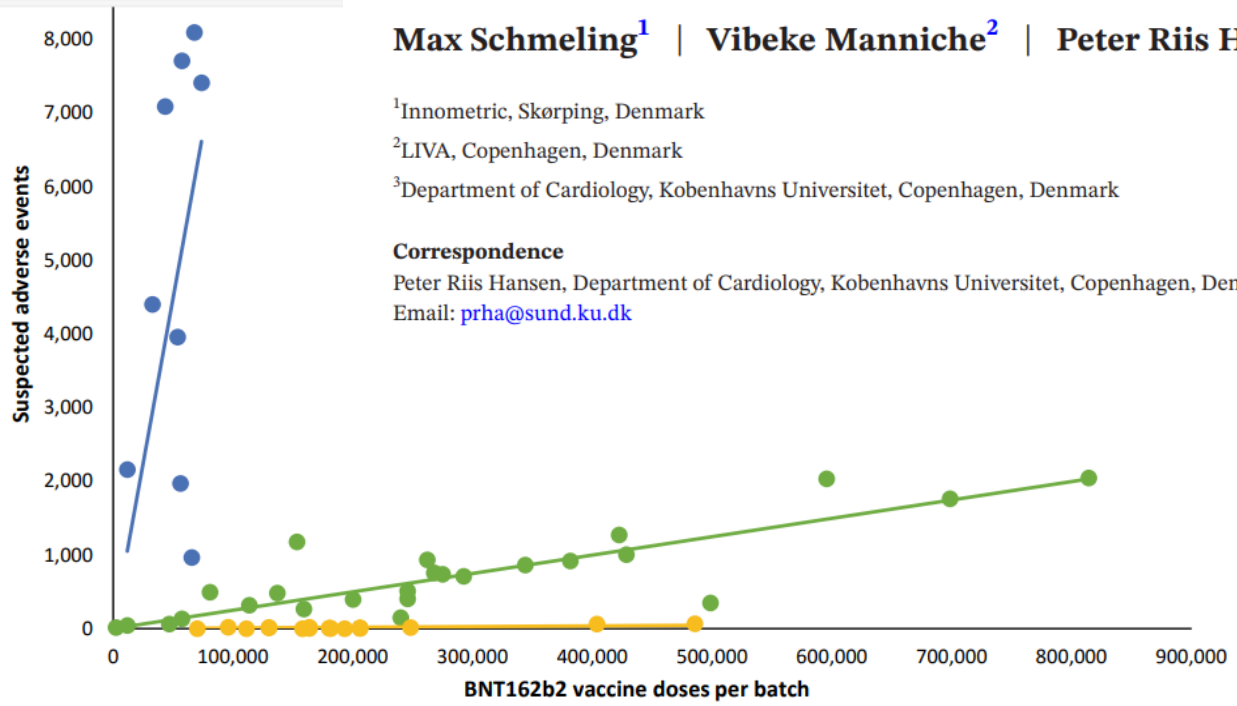

FIGURE 1 Numbers of suspected adverse events (SAEs) after BNT612b2 mRNA vaccination in Denmark (27 December 2020–11 January 2022) according to the number of doses per vaccine batch. Each dot represents a single vaccine batch. Trendlines are linear regression lines. Blue: $R^2 = 0.78$, $\beta = 0.0898$ (95% confidence interval [CI] 0.0514–0.1281), green: $R^2 = 0.89$, $\beta = 0.0025$ (95% CI 0.0021–0.0029), yellow: $R^2 = 0.68$, $\beta = 0.000087$ (95% CI 0.000056–0.000118). Vaccine batches representing the blue, green and yellow trendlines comprised 4.22%, 63.69% and 32.09% of all vaccine doses, respectively, with 70.78%, 27.49% and 47.15% (blue trendline), 28.84%, 71.50% and 51.99% (green trendline), and 0.38%, 1.01%, and 0.86% (yellow trendline) of all SAEs, serious SAEs, and SAE-related deaths, respectively.

Eur J Clin Invest. 2023;53:e13998. | 1 of 3
https://doi.org/10.1111/eci.13998

Food and Chemical Toxicology 164 (2022) 113008

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Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Innate immune suppression by SARS-CoV-2 mRNA vaccinations: The role of G-quadruplexes, exosomes, and MicroRNAs

Stephanie Seneff[a,*], Greg Nigh[b], Anthony M. Kyriakopoulos[c], Peter A. McCullough[d]

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[b] Immersion Health, Portland, OR, 97214, USA
[c] Research and Development, Nasco AD Biotechnology Laboratory, Department of Research and Development, Sachtouri 11, 18536, Piraeus, Greece
[d] Truth for Health Foundation, Tucson, AZ, USA

Thus, the available evidence strongly suggests that **endogenously produced SARS-CoV-2 spike glycoprotein** creates a different microRNA profile than does natural infection with SARS-CoV-2, and those differences entail a potentially **wide range of deleterious effects**.

THE IMPAIRMENT OF INNATE IMMUNITY

A central point of our analysis below is **the important distinction between the impact of vaccination versus natural infection on type I IFN**. While vaccination actively suppresses its production, natural infection promotes type I IFN production very early in the disease cycle. Those with preexisting conditions often exhibit impaired type I IFN signaling, which leads to more severe, critical, and even fatal COVID-19. If the impairment induced by the vaccine is maintained as antibody levels wane over time, this could lead to a situation where **the vaccine causes a more severe disease expression than would have been the case in the absence of the vaccine.**

Another expected consequence of suppressing type I IFN would be reactivation of preexisting chronic viral infections.

DNA repair

Mechanistically, we found that the **spike protein localizes in the nucleus and inhibits DNA damage repair** by impeding key DNA repair protein BRCA1 and 53BP1 recruitment to the damage site."

Reactivation of herpes viruses

There are multiple additional case reports of herpes **zoster reactivation following COVID-19 vaccination** in the literature (Psichogiou et al., 2021b; Iwanaga et al., 2021).
Llado ´ et al. (2021)
n 82-year-old woman who had acquired a hepatitis C viral (HCV) infection in 2007. A strong increase in HCV load occurred a few days after vaccination with an mRNA Pfizer/BioNTech vaccine, along with an appearance of jaundice. **She died three weeks after vaccination from liver failure** (Lensen et al., 2021).

Integration of mRNA/DNA into human genom

Sequencing of bivalent Moderna and Pfizer mRNA vaccines reveals nanogram to microgram quantities of expression vector dsDNA per dose

Kevin McKernan, Yvonne Helbert, Liam T. Kane, Stephen McLaughlin
Medicinal Genomics, 100 Cummings Center, Suite 406-L, Beverly Mass, 01915

Several methods were deployed to assess the nucleic acid composition of four expired vials of the Moderna and Pfizer bivalent mRNA vaccines. Two vials from each vendor were evaluated with Illumina sequencing, qPCR, RT-qPCR, Qubit™ 3 fluorometry and Agilent Tape Station™ electrophoresis. Multiple assays support DNA contamination that exceeds the European Medicines Agency (EMA) 330ng/mg requirement and the FDAs 10ng/dose requirements. These data may impact the surveillance of vaccine mRNA in breast milk or plasma as RT-qPCR assays targeting the vaccine mRNA cannot discern DNA from RNA without RNase or DNase nuclease treatments. Likewise, studies evaluating the reverse transcriptase activity of LINE-1 and vaccine mRNA will need to account for the high levels of DNA contamination in the vaccines. The exact ratio of linear fragmented DNA versus intact circular plasmid DNA is still being investigated. Quantitative PCR assays used to track the DNA contamination are described.

Introduction

Several studies have made note of prolonged presence of vaccine mRNA in breast milk and plasma (Bansal et al. 2021; Hanna et al. 2022; Castruita et al. 2023). This could be the result of the stability of N1-methylpseudouridine (m1Ψ) in the mRNA of the vaccine. Nance *et al*. depict a vaccine mRNA synthesis method that utilizes a dsDNA plasmid that is first amplified in *E.coli* prior to an *in-vitro* T7 polymerase synthesis of vaccine mRNA (Nance and Meier 2021). Failure to remove this DNA could result in the injection of spike encoded nucleic acids more stable than the modified RNA. The EMA has stated limits at 330ng/mg of DNA to RNA (Josephson 2020-11-19). The FDA has issued guidance for under 10ng/dose in vaccines (Sheng-Fowler et al. 2009).

Residual injected DNA can result in type I interferon responses and can increase the potential for DNA integration(Ulrich-Lewis et al. 2022).

current issues in molecular biology

MDPI

Article

Intracellular Reverse Transcription of Pfizer BioNTech COVID-19 mRNA Vaccine BNT162b2 In Vitro in Human Liver Cell Line

Markus Aldén [1], Francisko Olofsson Falla [1], Daowei Yang [1], Mohammad Barghouth [1], Cheng Luan [1], Magnus Rasmussen [2] and Yang De Marinis [1,*]

[1] Department of Clinical Sciences, Lund University, 20502 Malmö, Sweden; ma7440al-s@student.lu.se (M.A.); francisko.olofsson@gmail.com (F.O.F.); daowei.yang@med.lu.se (D.Y.); mohammad.barghouth@med.lu.se (M.B.); cheng.luan@med.lu.se (C.L.)
[2] Infection Medicine, Department of Clinical Sciences, Lund University, 22362 Lund, Sweden; magnus.rasmussen@med.lu.se
* Correspondence: yang.de_marinis@med.lu.se

Abstract: Preclinical studies of COVID-19 mRNA vaccine BNT162b2, developed by Pfizer and BioNTech, showed reversible hepatic effects in animals that received the BNT162b2 injection. Furthermore, a recent study showed that SARS-CoV-2 RNA can be reverse-transcribed and integrated into the genome of human cells. In this study, we investigated the effect of BNT162b2 on the human liver cell line Huh7 in vitro. Huh7 cells were exposed to BNT162b2, and quantitative PCR was performed on RNA extracted from the cells. We detected high levels of BNT162b2 in Huh7 cells and changes in gene expression of long interspersed nuclear element-1 (LINE-1), which is an endogenous reverse transcriptase. Immunohistochemistry using antibody binding to LINE-1 open reading frame-1 RNA-binding protein (ORFp1) on Huh7 cells treated with BNT162b2 indicated increased nucleus distribution of LINE-1. PCR on genomic DNA of Huh7 cells exposed to BNT162b2 amplified the DNA sequence unique to BNT162b2. Our results indicate a fast up-take of BNT162b2 into human liver cell line Huh7, leading to changes in LINE-1 expression and distribution. We also show that BNT162b2 mRNA is reverse transcribed intracellularly into DNA in as fast as 6 h upon BNT162b2 exposure.

Keywords: COVID-19 mRNA vaccine; BNT162b2; liver; reverse transcription; LINE-1; Huh7

check for updates

Citation: Aldén, M.; Olofsson Falla, F.; Yang, D.; Barghouth, M.; Luan, C.; Rasmussen, M.; De Marinis, Y. Intracellular Reverse Transcription of Pfizer BioNTech COVID-19 mRNA Vaccine BNT162b2 In Vitro in Human Liver Cell Line. *Curr. Issues Mol. Biol.* **2022**, *44*, 1115–1126. https://doi.org/10.3390/cimb44030073

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1. Introduction

Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was announced by the World Health Organization (WHO) as a global pandemic on 11 March 2020, and it emerged as a devasting health crisis. As of February 2022, COVID-19 has led to over 430 million reported infection cases and 5.9 million deaths worldwide [1]. Effective and safe vaccines are urgently needed to reduce the morbidity and mortality rates associated with COVID-19.

Several vaccines for COVID-19 have been developed, with particular focus on mRNA vaccines (by Pfizer-BioNTech and Moderna), replication-defective recombinant adenoviral vector vaccines (by Janssen-Johnson and Johnson, Astra-Zeneca, Sputnik-V, and CanSino), and inactivated vaccines (by Sinopharm, Bharat Biotech and Sinovac). The mRNA vaccine has the advantages of being flexible and efficient in immunogen design and manufacturing, and currently, numerous vaccine candidates are in various stages of development and application. Specifically, COVID-19 mRNA vaccine BNT162b2 developed by Pfizer and BioNTech has been evaluated in successful clinical trials [2–4] and administered in national COVID-19 vaccination campaigns in different regions around the world [5–8].

BNT162b2 is a lipid nanoparticle (LNP)–encapsulated, nucleoside-modified RNA vaccine (modRNA) and encodes the full-length of SARS-CoV-2 spike (S) protein, modified

Pět aspektů současné vakcinační kampaně

1. **Vakcinace v epidemii** – tlak na vznik nových mutací a možný vznik ADE efektu
2. **Vakcinace toxinem** – vedlejší závažné reakce S-protein: Inflammagen, penetruje přes BBB, charakter prionu, fůzní protein tvoří syncitia, indukuje vznik krevních sraženin a stabilizuje fibrinová vlákny proti účinku trombolytických enzymů, způsobuje poškození endotelií a jejich disfunkci, indukuje disbalanci receptorů ACE2/ACE, penetruje do jádra a interferuje s reparací DNA, aktivuje retroviry a endogenní reverzní transkriptázy – zabudování mRNA do genomu
3. Fakticky se u mRNA vakcín jedná o **intravenózní vakcinaci** – neexistuje kontrola nad distribucí v organismu a mírou exprese ve tkáních
4. **Masová vakcinační kampaň s využitím genetické vakcíny**, u které nejsou známy dlouhodobé účinky a mechanismy působení na úrovni genomu
5. **Vakcinační kampaň je nátlaková** s využitím nepřípustných donucovacích postupů v rozporu se zákonem a medicínským poznáním.

„Pokud vás někdo musí přesvědčovat, napomínat, tlačit, lhát, lákat na pobídky, nutit, zastrašovat, odhalovat, obviňovat, vyhrožovat, trestat a kriminalizovat… Pokud je toto všechno považováno za nezbytné k získání vašeho souhlasu –

<u>můžete si být naprosto jisti, že to, co se propaguje, není pro vaše dobro</u>.“

Britský autor sci-fi literatury Ian Watson

Pár moudrostí na závěr pro politiky

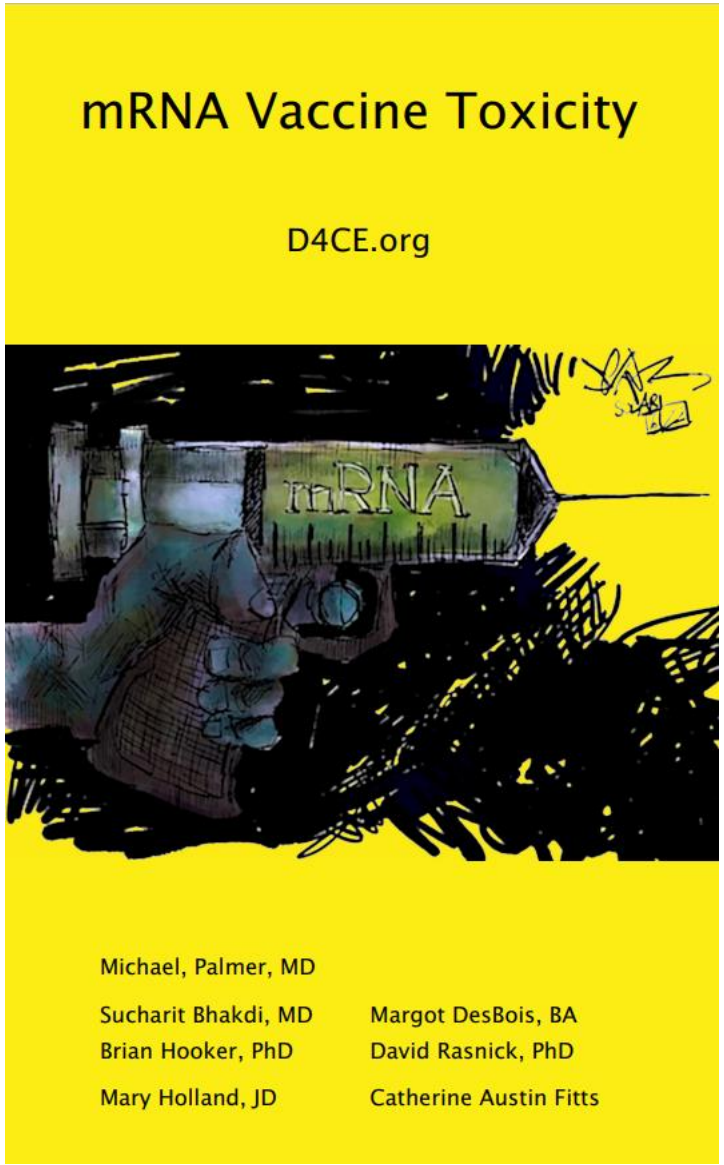
„Věda je národům nezbytná. Stát, který ji nerozvíjí, se nevyhnutelně mění v kolonii.“ – Frédéric Joliot-Curie (Je třeba také rozvíjet odpovídající průmyslovou základnu)

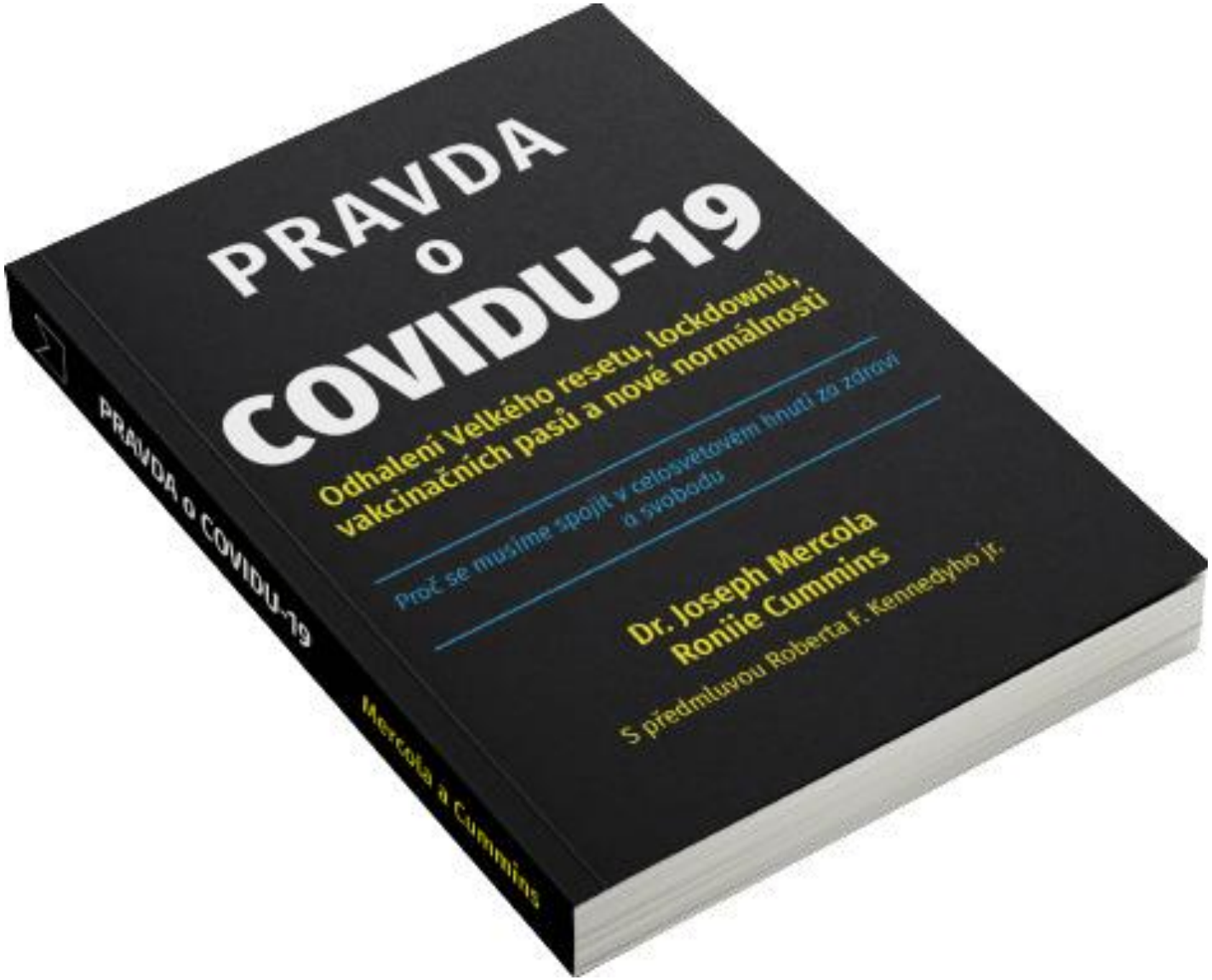
„Lidé, kteří dnes ovládají vědu, skutečně ani sami nepředvídají všechny důsledky toho, co se děje.“ – Norbert Wiener (Jsou schopni je předvídat a nést zodpovědnost za důsledky?)

„Dnešní vědecký výzkum v mnoha oborech už dávno nemá ctižádost ani svobodu naplňovat touhu lidského ducha po poznání pravdy, nýbrž poslušně plní zadání diktovaná ekonomickými, případně politickými zájmy svých mecenášů. Zisk se stal pravdou vědy.“ Tomáš Halík (v době kovidismu mlčel, Amen)

„Žijeme ve společnosti, naprosto závislé na vědě a technologii, a přesto skoro nikdo vědě a technologii nerozumí. To je jasný recept na katastrofu.“ – Carl Sagan (Proto je tak snadné pro politiky poroučet větru, dešti a přírodním zákonům)

„Jakožto humanista miluji vědu. Nesnáším pověry – jejich přičiněním bychom dodneška neměli atomovou bombu.“ Kurt Vonnegut


mRNA Vaccine Toxicity
D4CE.org
mRNA
Michael, Palmer, MD
Sucharit Bhakdi, MD
Brian Hooker, PhD
Mary Holland, JD
Margot DesBois, BA
David Rasnick, PhD
Catherine Austin Fitts


PRAVDA
o
COVIDU-19
Odhalení Velkého resetu, lockdownů, vakcinačních pasů a nové normálnosti
Proč se musíme spojit v celosvětovém hnutí za zdraví a svobodu
Dr. Joseph Mercola
Ronjie Cummins
S předmluvou Roberta F. Kennedyho Jr.
PRAVDA O COVIDU-19
Mercola a Cummins

Kdo se nenechá navakcinovat, zemře!!!

Ministr zdravotnictví Vlastimil Válek říká, že „se musíme psychicky připravit na to, že se budeme každý rok pravděpodobně přeočkovávat“

Jaroslav Flegr - To to letí. Je tam třetí!

Flégr po třetí dávce

Epidemiolog zároveň přiznal, že vakcíny mají i své slabiny. "Dominantně se využil transportní systém na bázi mRNA, který se sice vyvíjel už v podstatě desítky let zpátky, ale nevyvíjel se pro vakcíny. Vyvíjel se pro léčbu onkologických onemocnění. Takže se úplně neřešilo, jestli tento systém má nějaké drobné komplikace, nebo nikoliv, což u vakcín, které se dávají preventivně, samozřejmě řešíme. Takže tady máme určité spektrum komplikací, které se logicky musí připustit, objektivně tady prostě jsou," dodal Prymula.Zdroj: https://tn.nova.cz/zpravodajstvi/clanek/478533-zastance-ockovani-prymula-si-pro-ctvrtou-davku-nepujde-priznal-i-slabiny-vakcin?campaignsrc=tn_clipboard

Odborníci mají jiný názor Podle českých odborníků je podání čtvrté dávky naprosto bezpečné. Většina z nich už dokonce další očkování podstoupila. "Ano, už jsem očkován. Samozřejmě je to bezpečné," řekl TN.cz vakcinolog Roman Chlíbek. "Čtvrtou dávku mám, nechal jsem si ji aplikovat," uvedl epidemiolog Petr Smejkal.Zdroj: https://tn.nova.cz/zpravodajstvi/clanek/478533-zastance-ockovani-prymula-si-pro-ctvrtou-davku-nepujde-priznal-i-slabiny-vakcin?campaignsrc=tn_clipboard

Život je jen sen a jedinou skutečností v něm je LÁSKA
František Bílek