Lipid Nanoparticle (LNP) Concerns, Charges, Mutations, Plasmids, and Adverse Events

By Christie Grace
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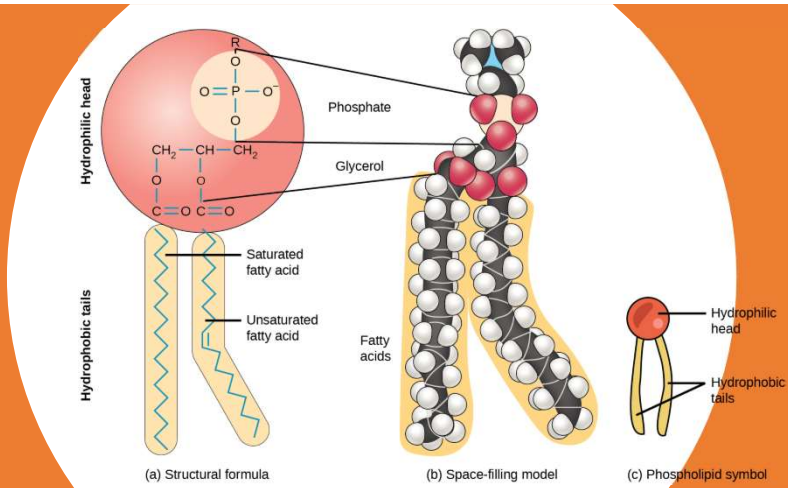
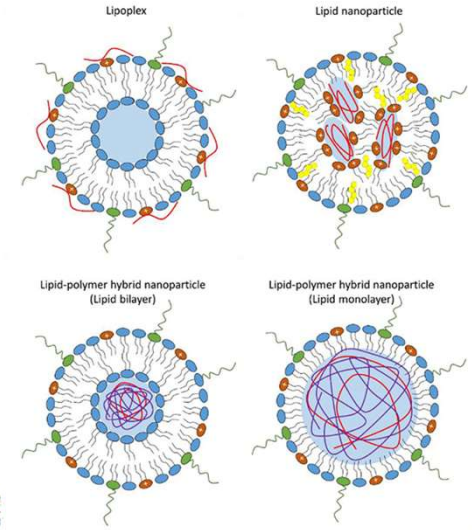
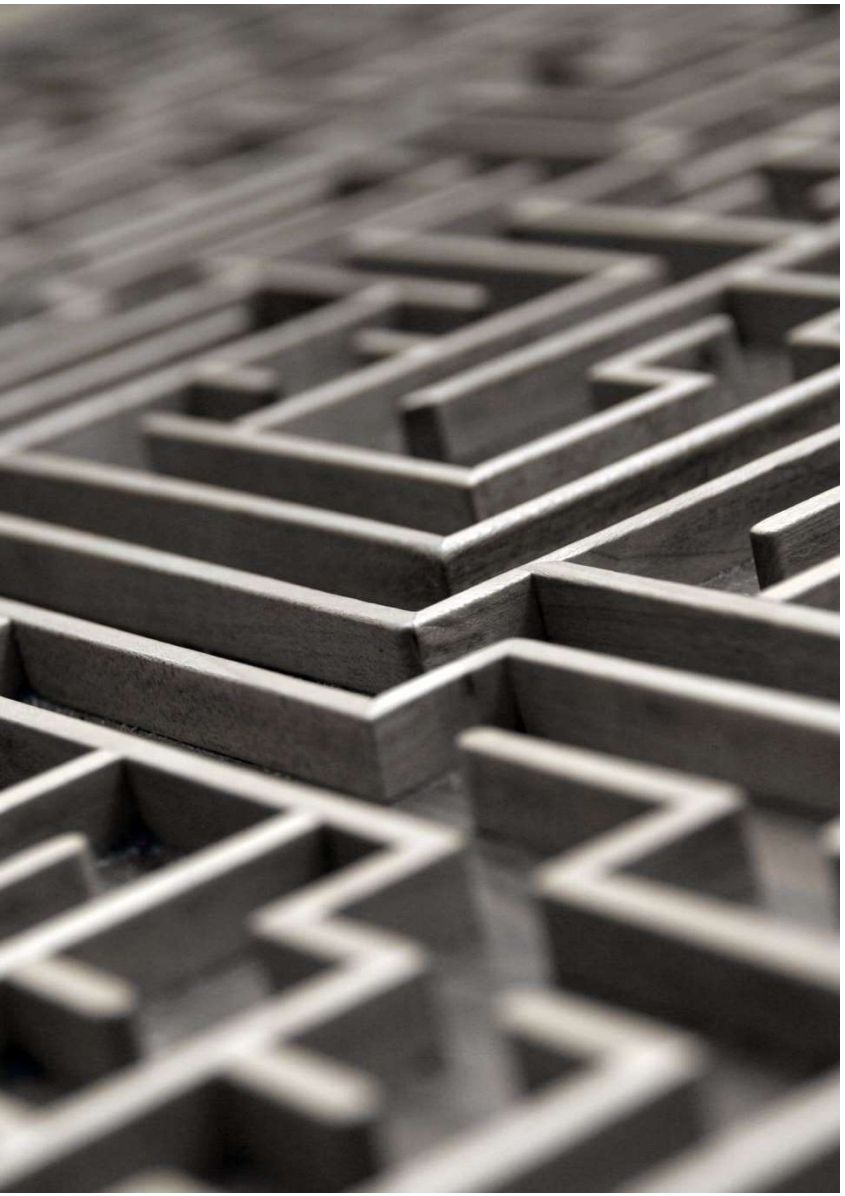






Schedule/Programme:

(macro/birds-eye view highlights due to time)

BRIEF LNP Construction

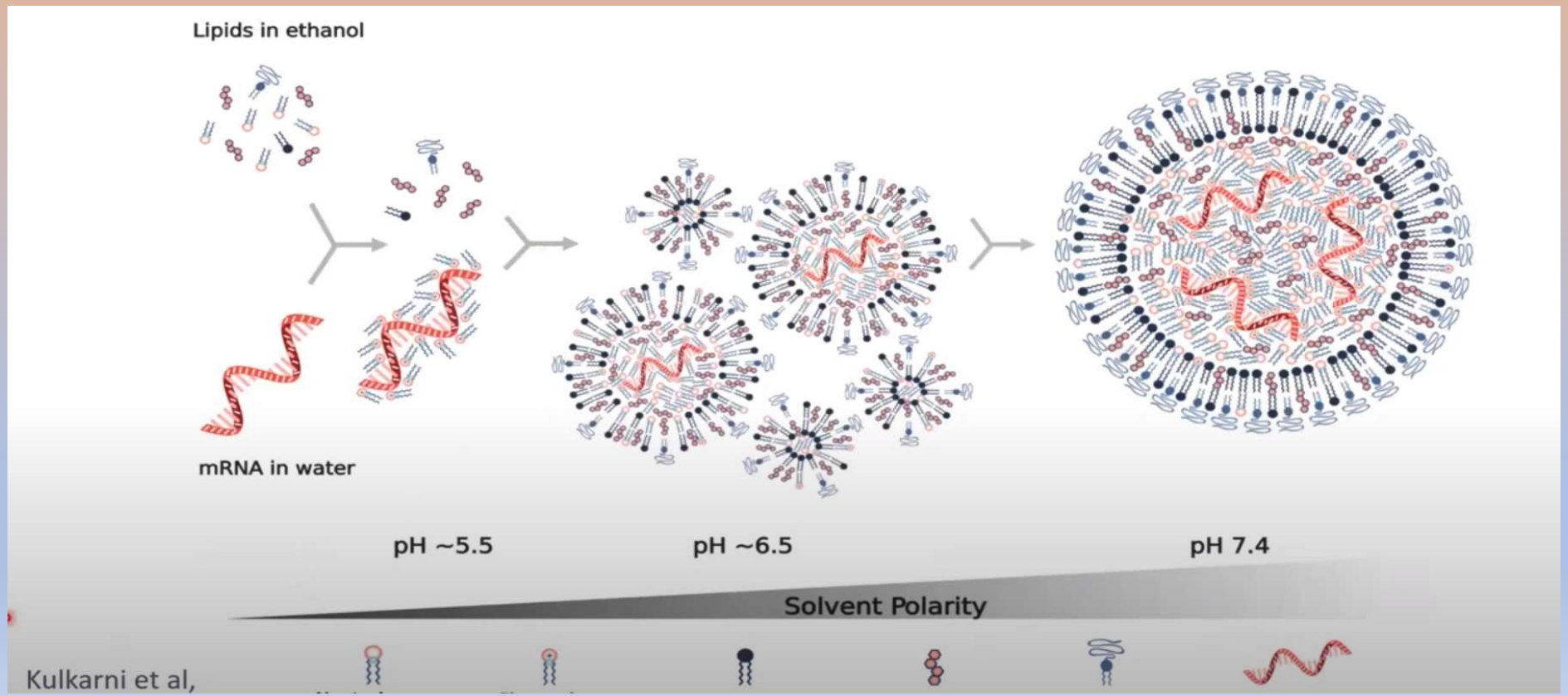
WHAT is happening (definite concerns/potential concerns)

WHY is it happening (definite causes and pathways/potential causes and pathways)

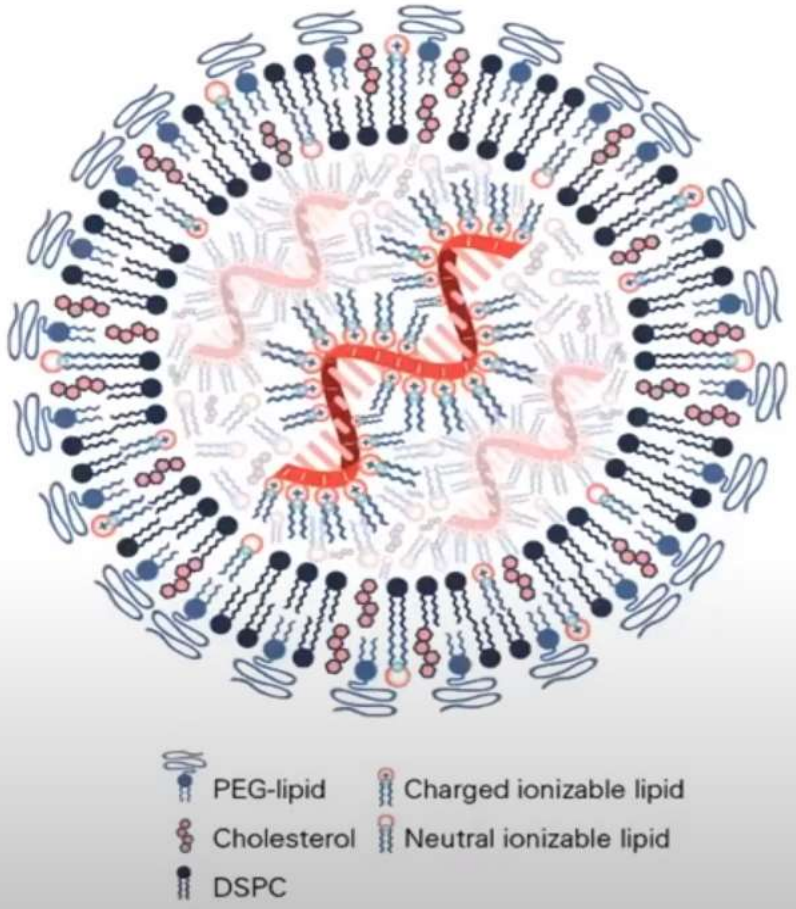
Ancillary/Supplemental Topics of Concern/Active FOIAs (under Christie Grace)

References: Links/Citations

The Construction of a Lipid Nanoparticle with mRNA



Kulkarni et al,



Contents of Lipid Nanoparticles with mRNA

***mRNA: Negatively Charged**
***Cholesterol: no charge**
***Charged Ionizable Lipid: Positively Charged (cation)**
***Neutral Ionizable Lipid (positively charged in lower pH 4.5-5)**

**DSPC (helper lipid—least of your worries)*
**PEG (the very least of your worries)*

WHAT IS (could be) Happening

Positive Charges (+)

Positively Charged Ionizable Lipids in the LNP (positive net charge on LNP/positive Zeta potential on LNP):

Clots

(Sperling et al., 2017)

Inflammation, serum sickness, thrombohemorrhagic phenomena, colloidal instability, and potential SUDDEN DEATH (Davidson and Seneff, 2012)

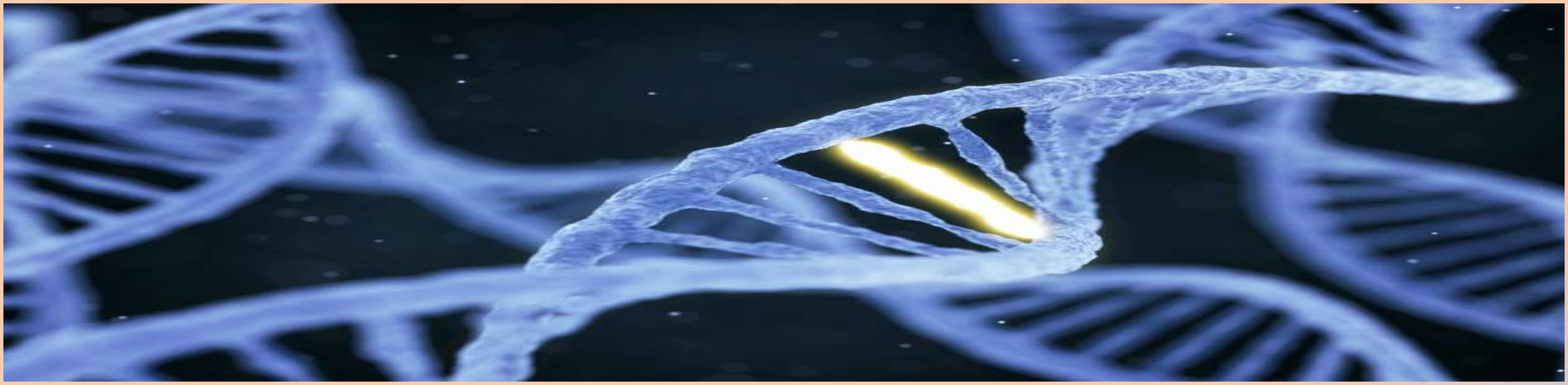
Goes to lung (clots)

Crosses Blood Brain Barrier

WHAT IS (could be) Happening

Positive Charges (+)

Positively Charged Cationic Lipids Appear to be MUTATING the mRNA in the LNP; meaning, they have the potential to MUTATE the RNA and DNA in the HUMAN BODY if they encounter the naturally occurring RNA and DNA!



WHAT IS (could be) Happening

Positive Charges (+)

RNA and DNA MUTATIONS

Not only is the mRNA inside the LNP being mutated by the cationic lipid--this action should have the same impact on human RNA and DNA (which the researchers in the study MISSED!)
(Packer et al., 2021)

* The reactive species that lead to impurity formation are believed to result from oxidation and subsequent hydrolysis of certain chemical groups in the ionizable cationic lipid. These processes can render the mRNA ineffective by altering its structure.

*This should be causing mutations to the base pairs (point mutations).

*The electrophilic impurities from the ionizable cationic lipid component can react with electron-rich functional groups on the mRNA, forming covalent adducts. These adducts can disrupt the mRNA's structure and its interactions with the translation machinery, causing loss of translation and protein expression. The specific functional groups affected could be those critical for ribosome binding, proper folding (misfold), and accurate base pairing during translation (mutation).

This has far-reaching consequences. (non-coding RNA fragments can cause CANCER)

WHAT IS (could be) Happening

Positive Charges (+)

RNA and DNA MUTATIONS

"Reversed-phase ion pair high performance liquid chromatography (RP-IP HPLC) was used to identify a class of impurity formed through lipid: mRNA reactions; such reactions are typically undetectable by traditional mRNA purity analytical techniques." (Packer et al., 2021)

The impurities responsible for the interactions with the mRNA and electrophilic attack are derived from the ionizable cationic lipid component of the lipid nanoparticles. These impurities are electrophilic, meaning they can react with other molecules. **This can cause other structures to form, aggregation, misfolding of proteins, non-coding of the mRNA (oncogenic) and mutations in nucleic acids they would interact with (RNA/DNA) and cause unwanted effects.**

https://www.the-scientist.com/features/long-noncoding-rnas-and-microproteins-can-spark-cancer-or-sometimes-squelch-it-70961

WHAT IS (could be) Happening

Positive Charges (+)

RNA and DNA MUTATIONS—HOW it HAPPENS

1. Electrophilic attack in the study: the cationic lipid is an electrophilic due to its positive charge. It can undergo chemical reactions with the nucleic acid molecules (RNA) and DNA, specifically the bases.

2. The cationic lipid contains positively charged functional groups (electrophilic) which are attracted to electrons (in nucleic acids). Nucleophilic Target (Nucleobase in RNA): The bases in RNA/DNA contain electron-rich atoms that can act as nucleophiles and can "attack" the electrophilic cationic lipid.

3. The nucleophilic electron pair from the nucleobase (RNA/DNA) attacks the electrophilic site on the cationic lipid, to the forming a new covalent bond between the cationic lipid and the nucleobase.

4. The covalent bond formed between the cationic lipid and the nucleobase creates a modified nucleobase, called an "adduct." This modification can alter the structure of the nucleobase and disrupt its normal base-pairing interactions.

5. Depending on the specific nucleobase modified and the extent of the modification, this can lead to altered base pairing, misfolding of the RNA, aggregation, affect the translation of the RNA into protein, mutate the RNA, and mutate DNA if it came in contact with it (downstream and upstream effects), potentially causing catastrophic effects.

WHAT IS (could be) Happening/Positive Charges (+)/RNA MUTATIONS

Electrophilic attack, steric hindrance, and base pair disruption:

RNA MUTATIONS (The RNA content of the cytoplasm is from 70 to 85 per cent—not the nucleus):
Adenine (A) in the mRNA pairs with Uracil (U)
Cytosine (C) in the mRNA pairs with Guanine (G)
Any disruption or modification in these base pair interactions could lead to misreading of the codons, incorrect amino acids being incorporated into the growing protein chain, or even premature termination of translation.

Binding Specificity. Tertiary structures enable RNA to bind to specific proteins and molecules with high specificity. This binding is essential for processes like splicing, translation, and regulation of gene expression.

Catalytic Activity. Some RNA molecules, known as ribozymes, have catalytic activity. Their tertiary structures facilitate the precise positioning of functional groups necessary for catalysis.

Stability. Tertiary structures contribute to the stability of RNA molecules. The interactions between distant parts of the molecule help protect it from degradation by cellular enzymes.

Gene Regulation. Regulatory RNAs, like microRNAs and long non-coding RNAs, rely on their tertiary structures to interact with target RNAs and regulate their expression.

ALL OF THESE INTERACTIONS CAN BE DISRUPTED DUE TO CATIONIC LIPID INTERACTIONS WITH RNA

WHAT IS (could be) Happening/Positive Charges (+)/RNA MUTATIONS

Electrophilic attack, steric hindrance, and base pair disruption:
IMPACTS of cationic ionizable lipids on RNA in the CELL may result in:

Loss of Translation. If modifications by electrophilic interactions of cationic lipids disrupt base pairing and secondary/tertiary structures, it could lead to a loss of translation, meaning ANY HUMAN PROTEIN might not be expressed or at significantly reduced levels. The human proteome core is comprised of spprox. ten thousand to billions of species (more than 150,000 sequence-unique peptides aggregated into 10,000 proteins across total liver, and the major liver cell types alone!)

Aberrant Protein Expression. If the modifications result in incorrect base pairing or frame-shift mutations, it could lead to the translation of an aberrant protein. An aberrant protein could have altered amino acid sequences, folding patterns, and functional properties. This could potentially lead to malfunctioning or non-functional proteins, including misfolding leading **to CANCER, AGGREGATION, NEURODEGENERATIVE DISEASE, ALZHEIMER'S AND ALS!**

Partial Translation or Misfolding. In some cases, the modified mRNA might undergo partial translation, resulting in truncated proteins or proteins with missing functional domains. Alternatively, if the modifications cause the mRNA to fold inappropriately, it might lead to misfolding of the nascent protein during translation, causing **CANCER, OTHER DISEASE, ALS, and ALZHEIMER'S!**

WHAT IS (could be) Happening/Positive Charges (+)/DNA MUTATIONS

Electrophilic attack, steric hindrance, and base pair disruption:

What would happen if DNA PLASMIDS contaminated the LNP and formed DNA LIPOPLEXES or DNA/RNA POLY-LIPOPLEXES and contained an SV40 PROMOTER and a NUCLEAR LOCALIZATION SIGNAL? WHAT IF THESE PLASMIDS (negative charge -256 each) were electrostatically bound to CATIONIC LIPIDS, and they were driven into the NUCLEUS and the CATIONIC LIPIDS INTERACTED WITH THE DNA OF CELLS?

Impaired Gene Regulation. Cationic lipids could interact with DNA in the nucleus. If they bind to specific regulatory regions of genes, such as promoters or enhancers, they COULD influence gene expression, AND lead to upregulation or downregulation of gene expression, depending on the nature of the interaction and the genes involved.

Structural Changes. If cationic lipids disrupt the DNA structure or introduce modifications, they could impact DNA replication, transcription, and repair processes. This could potentially lead to genomic instability, mutations, or DNA damage response activation **(CANCER, and other major diseases)**

Epigenetic Effects. Cationic lipid-DNA interactions might also influence epigenetic modifications, such as DNA methylation or histone modifications. Epigenetic changes can have long-lasting effects on gene expression patterns. **CANCER (in the WOMB? DEFECTS/PREMATURE BIRTH/STILLBIRTH)**

WHAT IS (could be) Happening/Positive Charges (+)/LIPIDS--DNA MUTATIONS—POINT MUTATION CONSEQUENCES

Genetic Disorders: Cystic Fibrosis, Sickle Cell Anemia, Huntington's Disease, and **Familial Hypercholesterolemia**.

CANCER: Examples: mutations in the TP53 tumor suppressor gene commonly found in many types of cancer and mutations in the BRAF oncogene seen in melanoma and other cancers.

Mutations or dysregulation of DEAD box proteins can have significant implications for cell function/disease,

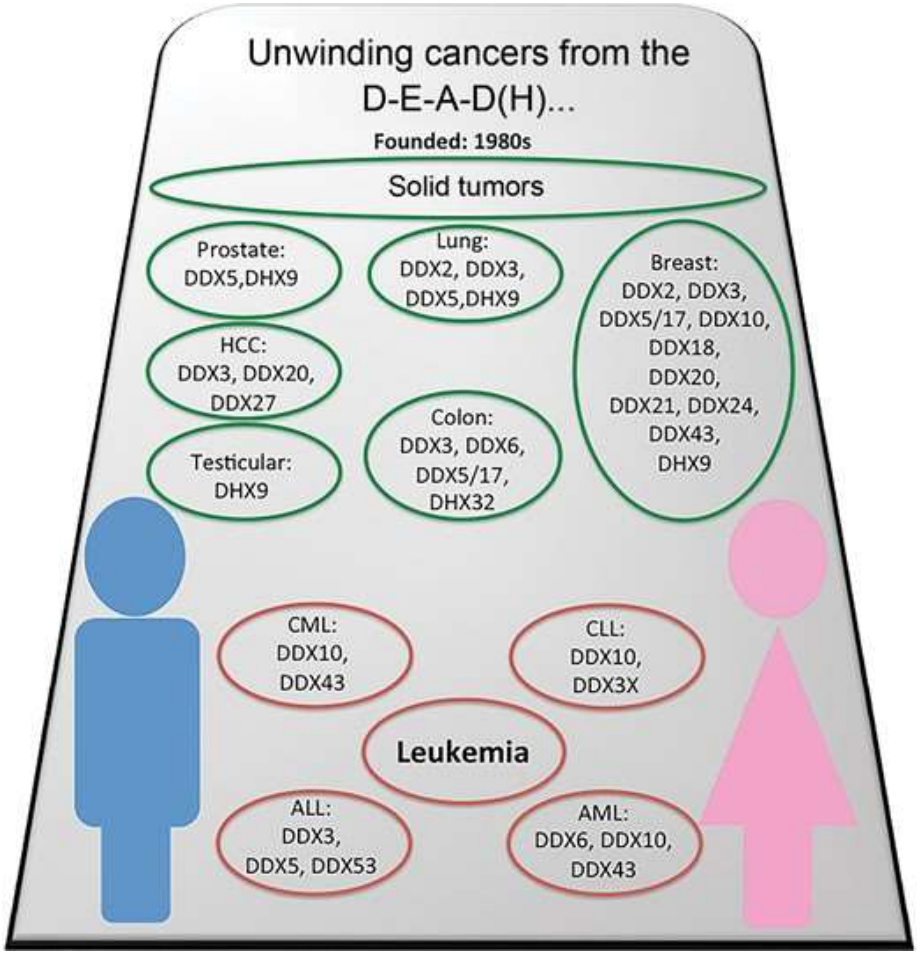
DDX3X (DEAD-box helicase 3, X-linked): Mutations have been associated with developmental delay, intellectual disabilities, and neurological symptoms. Also involved in transcription, translation, and RNA metabolism.

DDX24 (also known as DBP6) and DDX59 (also known as DBP6): :. neuronal development and axon guidance. Mutations in DDX24 have been linked to intellectual disabilities and developmental disorders.

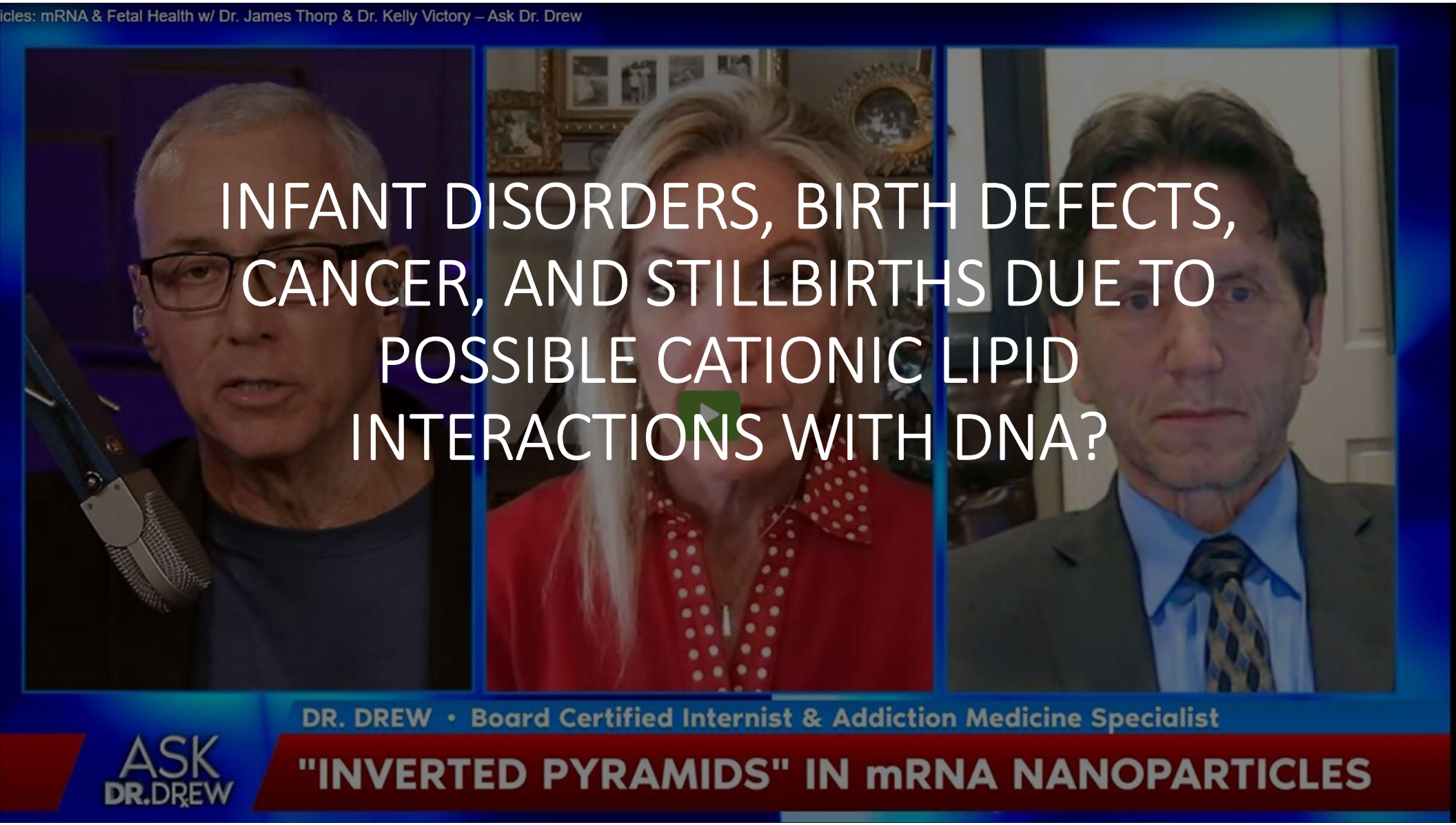
DDX5 (also known as p68) RNA processing and transcriptional regulation. **Dysregulation of DDX5: amyotrophic lateral sclerosis (ALS) and Alzheimer's disease**

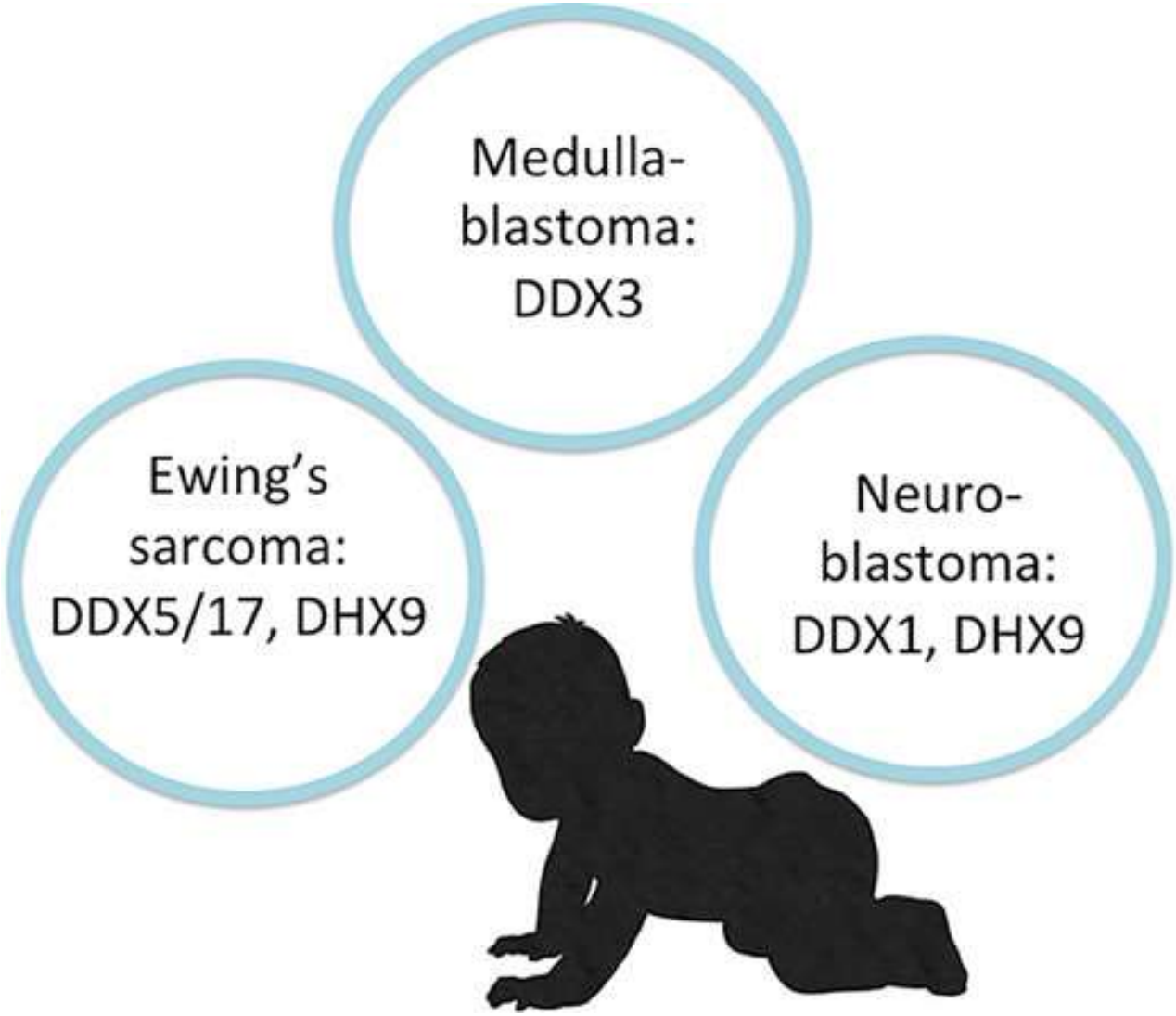
DHX9 (also known as RNA helicase A). Dysregulation of DHX9 : neurodevelopmental disorders and neurodegenerative diseases.

DEAD BOX PROTEIN DYSREGULATION AND CANCERS

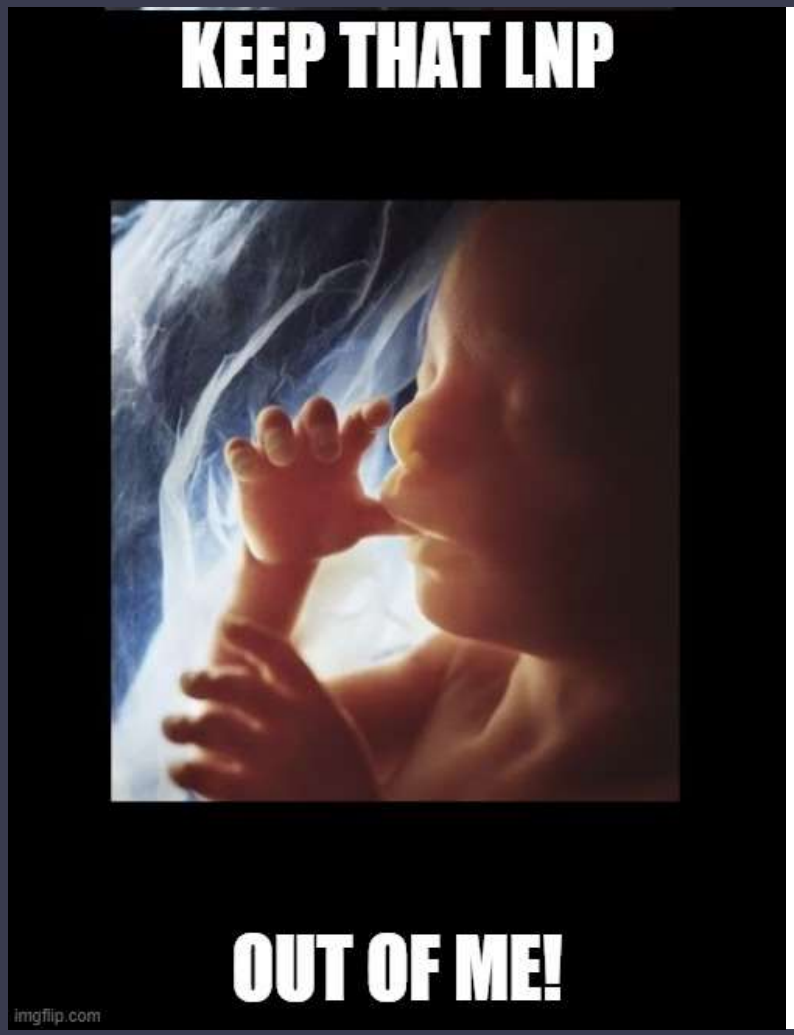


https://academic.oup.com/jnci/article/109/6/djw278/2957323


icles: mRNA & Fetal Health w/ Dr. James Thorp & Dr. Kelly Victory – Ask Dr. Drew
INFANT DISORDERS, BIRTH DEFECTS, CANCER, AND STILLBIRTHS DUE TO POSSIBLE CATIONIC LIPID INTERACTIONS WITH DNA?
DR. DREW • Board Certified Internist & Addiction Medicine Specialist
ASK DR.DREW
"INVERTED PYRAMIDS" IN mRNA NANOPARTICLES



INFANT DISORDERS, BIRTH DEFECTS, CANCER, AND STILLBIRTHS DUE TO POSSIBLE CATIONIC LIPID INTERACTIONS WITH DNA?



"Ionizable lipid nanoparticles for in utero mRNA delivery"
Rachel S. Riley et al. ,Ionizable lipid nanoparticles for in utero mRNA delivery.Sci Adv.7,eaba1028(2021).DOI:10.1126/sciadv.aba1028

"target progenitor cells in multiple organs are more prevalent and highly accessible during gestation, and many physical barriers, such as the blood-brain barrier, are not as developed as they are after birth"

WHAT IS (could be) Happening/Positive Charges (+)/CATIONIC LIPIDS/ BRIDGING EFFECT/ION BRIDGING

Cationic lipids can act as bridges that bring nucleic acids (RNA/DNA) together, potentially leading to the formation of higher-order structures or complexes.

Consequences:
Cationic (+) lipids can bind to the negatively charged phosphate groups along the nucleic acid chains (RNA/DNA), causing the formation of **aggregates, complexes, or networks.**

This can also result in the formation of larger structures, such as duplexes or hybrid complexes, where the cationic lipid acts as a bridge between the molecules.

Cationic lipids can bring multiple RNA molecules close enough for their individual secondary structures to interact, potentially leading to the aggregation of RNA molecules. This can affect the RNA's function and behavior.

The bridging effect can also stabilize interactions between RNA molecules or between RNA and other molecules, such as proteins, leading to the formation of stable complexes with altered functions.

Consequences of ION BRIDGING via CATIONIC IONIZABLE LIPID INTERACTIONS WITH NUCLEIC ACIDS

Nucleic Acid Condensation. The cationic lipids neutralize the negatively charged phosphate groups along the nucleic acid backbone, causing the nucleic acids to condense and form particles or complexes.

Biological Interactions. Excessive ion bridging can lead to the formation of aggregates or complexes that interfere with cellular processes, potentially affecting normal cellular functions.

Aggregation and Misfolding. When aggregates form, they interfere with proper folding of nucleic acids or other cellular components. Nucleic acids have specific three-dimensional structures that are crucial for function. Aggregation can cause misfold.

Impaired Cellular Uptake. Ion bridging can lead to the formation of very large complexes that are difficult for cells to internalize. These oversized complexes might get stuck on the cell surface, preventing efficient uptake of other components.

Altered Intracellular Localization. Aggregates could become trapped in cellular compartments such as endosomes, preventing their release into the cytoplasm where cellular processes take place.

Interference with Cellular Machinery. Excessive ion bridging can result in the formation of complexes that are too large or irregular to be properly recognized by these cellular components. As a result, the nucleic acids within the complexes might not be processed as intended, leading to compromised gene expression or interference with other cellular functions.

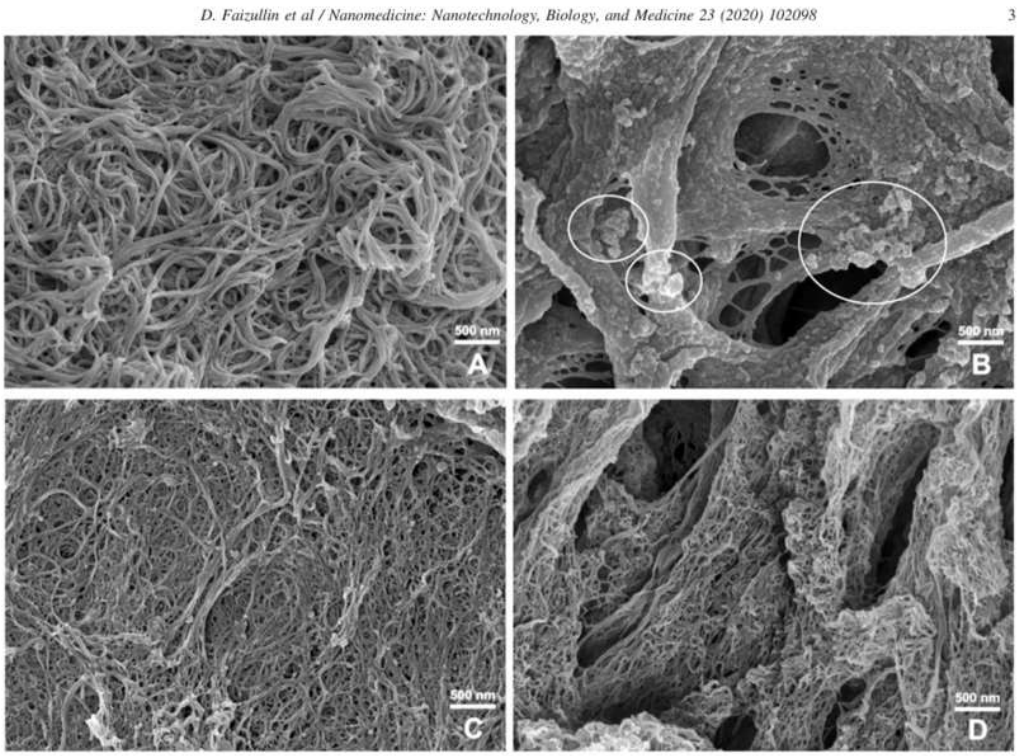
Toxicity and Immune Responses.
Large aggregates can trigger cellular stress responses, activation of cellular defense mechanisms or even cell death. These aggregates can be recognized by the immune system as foreign entities, triggering immune responses, inflammation, and adverse effects on the cells and tissues.

Alteration of Signaling Pathways. Formation of non-native complexes due to ion bridging could disrupt interactions and lead to unintended activation or inhibition of signaling pathways.

WHAT IS (could be) Happening

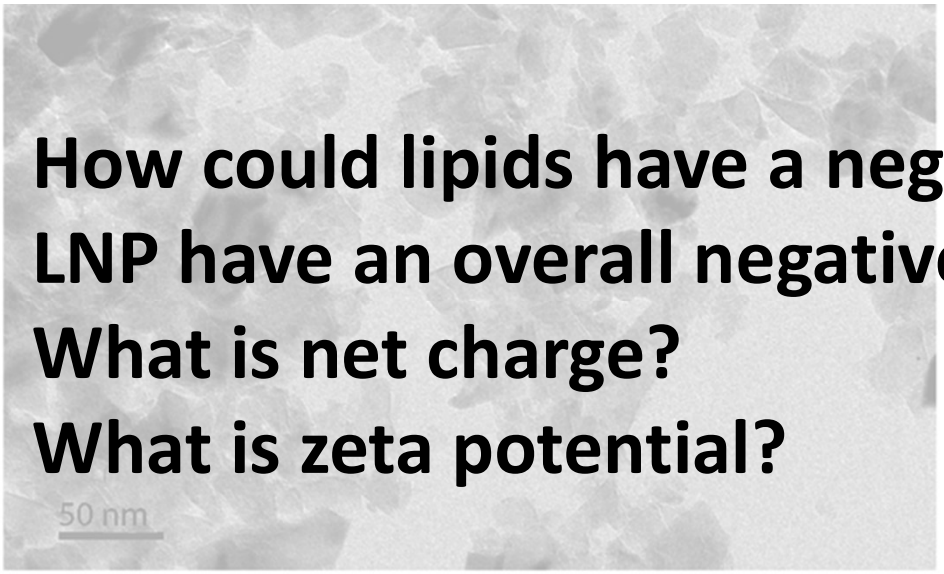
NEGATIVE CHARGES (BOTH ZETA POTENTIAL MORE NEGATIVE AND NEGATIVE NET CHARGE—CHARGED LIPOSOMES

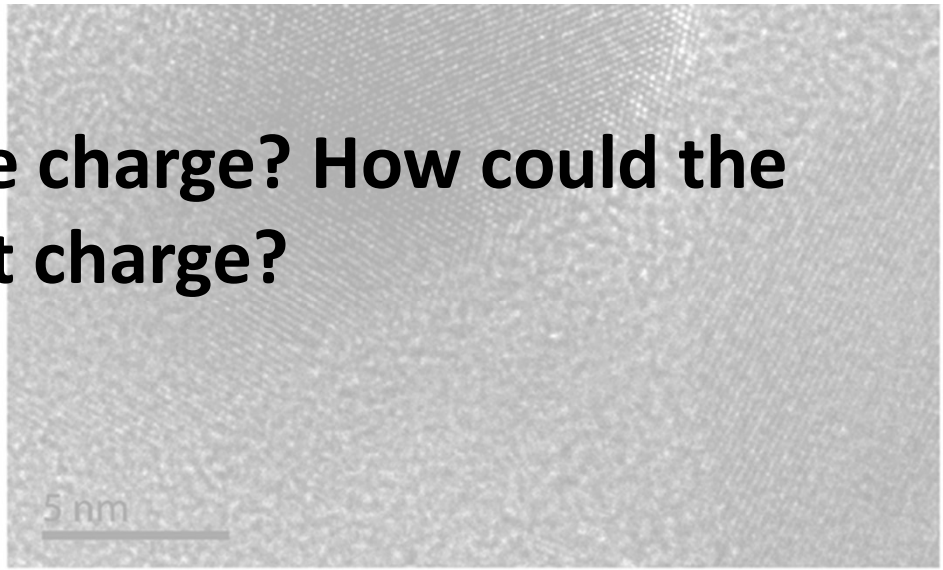
The thick spindly clots that embalmers and others are finding!



"Direct interaction of fibrinogen with lipid microparticles modulates clotting kinetics and clot structure" (Faizullin et al., 2020)

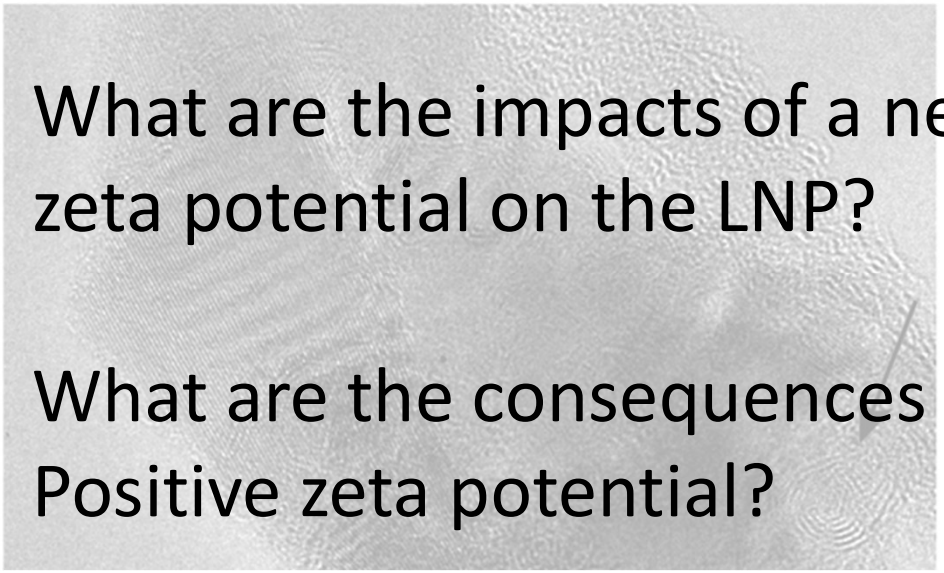
"Coagulation cascade is greatly accelerated upon the binding of coagulation factors to **negatively charged lipids** of membranes. **Being bound to the membrane surface, coagulation proteases become thousands of times more active** due to the formation of spatially arranged multi-enzyme complexes facilitating the surface-directed transfer of the activated coagulation factors between the various complexes."





How could lipids have a negative charge? How could the LNP have an overall negative net charge?
What is net charge?
What is zeta potential?

c



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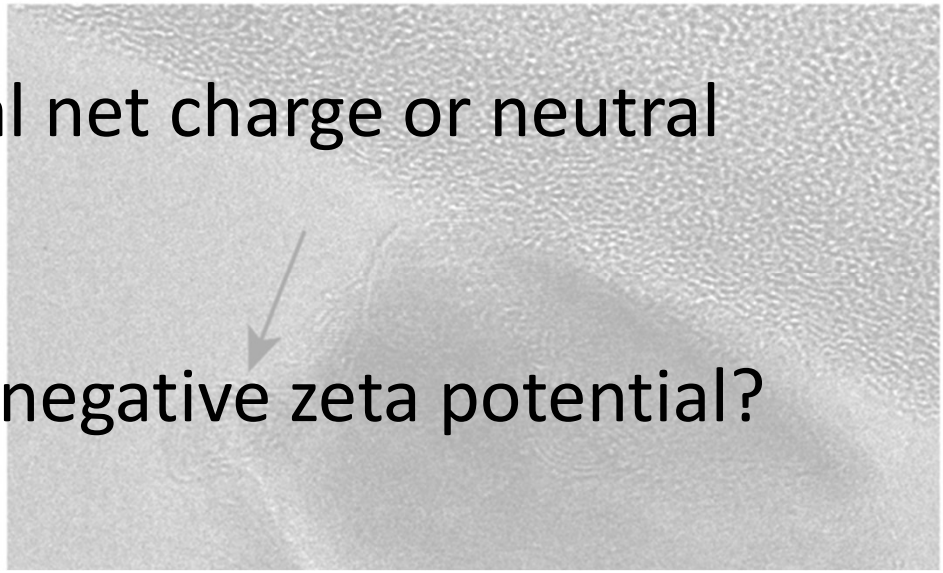
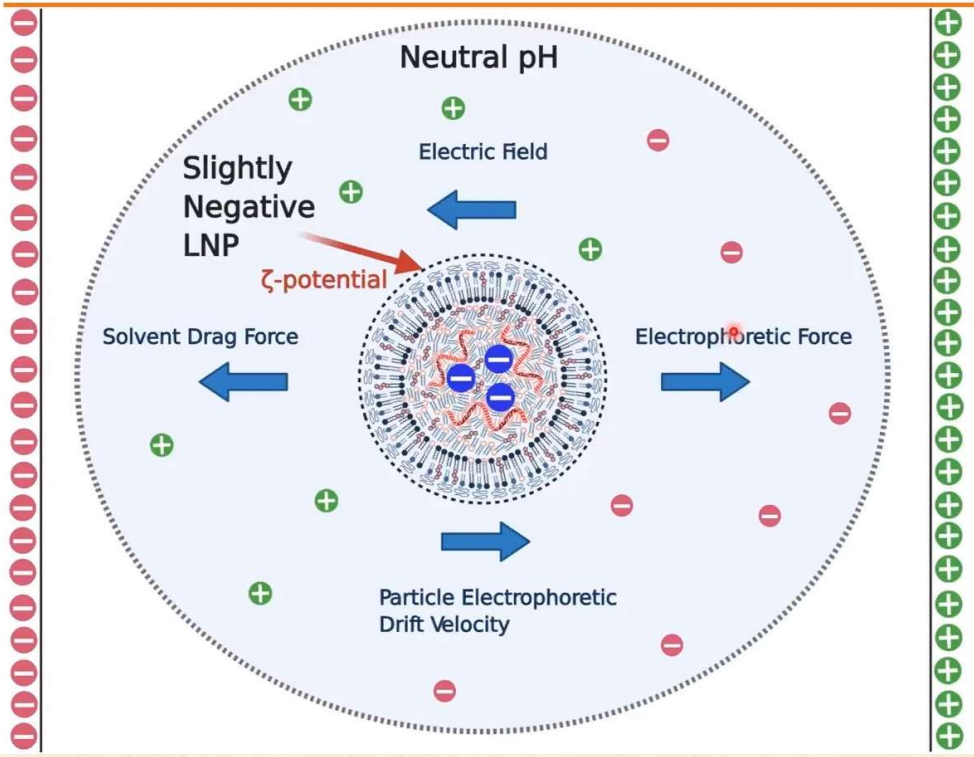


What are the impacts of a neutral net charge or neutral zeta potential on the LNP?

What are the consequences of a negative zeta potential? Positive zeta potential?

Charges Inside the Lipid Nanoparticle (LNP) with mRNA, Net Charge, and Zeta Potential

LNP Net Charge and Endosomal Protonation by Electrophoretic Mobility (Zeta Potential)



$$\zeta = \frac{3\eta\mu}{2\epsilon\epsilon_0 f(d/2\lambda)}$$

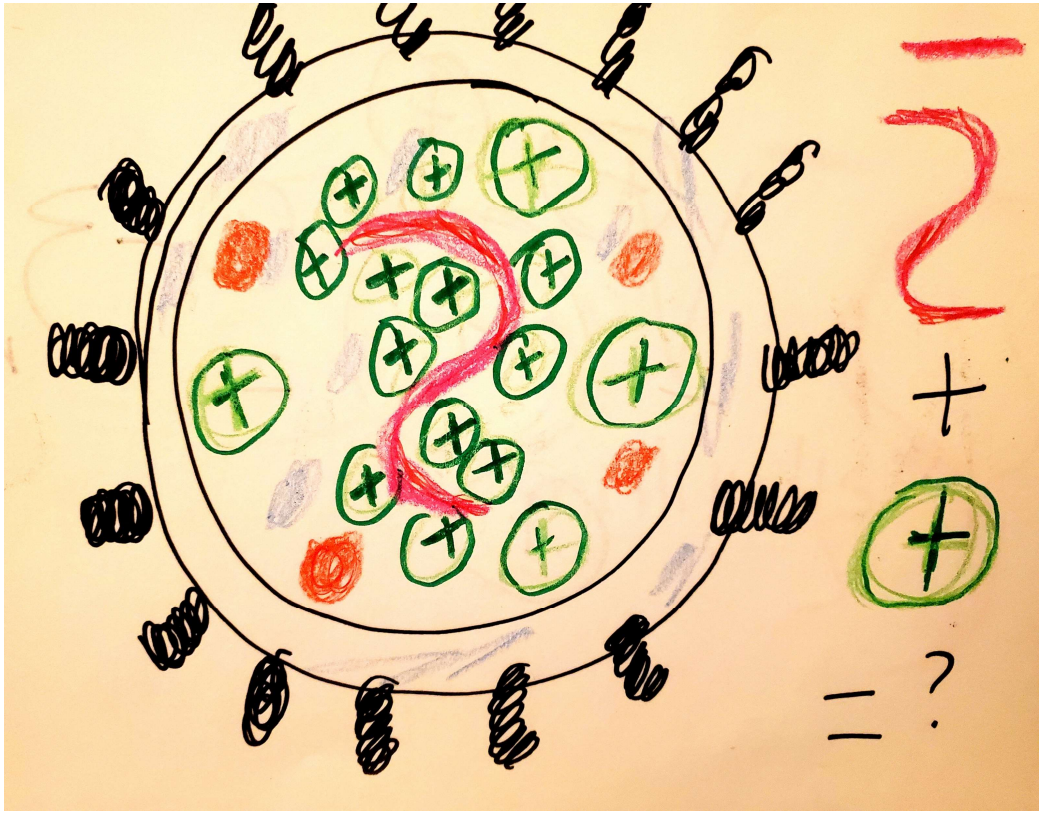
ζ = zeta potential
μ = electrophoretic mobility
η = viscosity
f = Henry's function
d = particle diameter
λ = Debye Length

$$\vec{v} = \mu\vec{E}$$

v = particle electrophoretic drift velocity
E = electric field

Doane et al, Accounts of Chemical Research, 2011

Charges Inside the Lipid Nanoparticle (LNP) with mRNA, Net Charge, and Zeta Potential

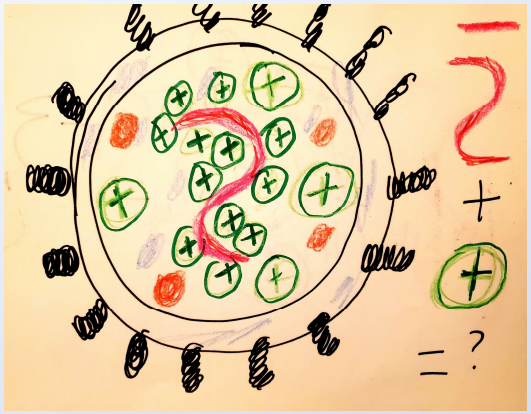


Red Backwards S= negatively charged mRNA

Green Circle with Plus Sign= positively charged ionizable cationic lipid

(not drawn with all contents—we are mainly concerned about the charges here. Cholesterol break down (freeze/thaw) conversion to phosphatidylethanolamine causing clots and oxysterols to neurodegenerative covered later)

Charges Inside the Lipid Nanoparticle (LNP) with mRNA, Net Charge, and Zeta Potential



Charge Refresher: (+) Positively charged items are attracted to negatively (-) charged items.

The mRNA has a negative charge (negatively charged phosphodiester backbone). The positively (+) charged ionizable lipids are electrostatically bound to the (-) mRNA.

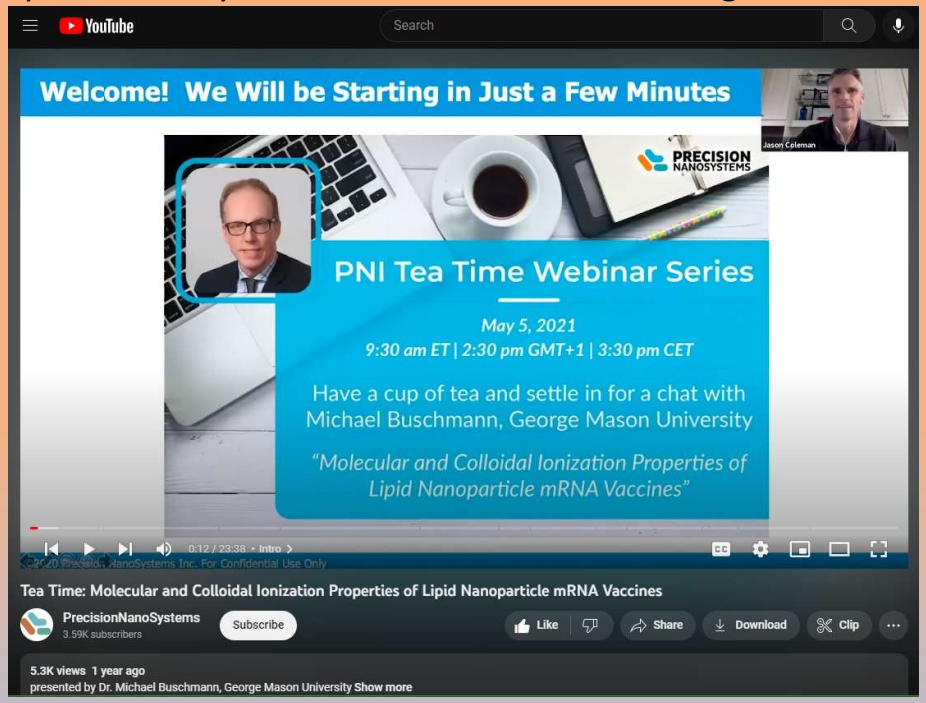
The overall LNP has a net charge (adding the positive and negative charges together). This is “ideally” neutral to slightly negative.

The LNP also has a **ZETA Potential**. The ZETA potential is the charge on the surface of the LNP in aqueous dispersion. You cannot have ZETA potential without pH. ZETA changes with acidity. pH is the most important parameter for zeta potential. For example, if you add acid in nanofluid, pH will decrease, which will increase positive charges on the LNP surface, and make the Zeta potential increase (measured in millivolts here). *The point at which zero electrophoretic mobility occurs is called an isoelectric point.*

Introducing Precision NanoSystems Webinar: “Tea Time: Molecular and Colloidal Ionization Properties of Lipid Nanoparticle mRNA Vaccines”

May 7, 2021

presented by Dr. Michael Buschmann, George Mason University



https://www.youtube.com/watch?v=iK9kFpvxZYA&list=LL&index=161

Pfizer's own documents state their calculations of the ZETA potential on the LNP containing what is "supposed to be" mRNA only, with ONLY the other lipids inside, is -3.13 millivolts, AND that "the nearly neutral LNP surface supports the mechanism that their …drug product avoids non-specific binding events in the blood compartment"

What if the zeta potential changed due to a change in the amount of negatively charged or positively charged particles?

Surface charge

BNT162b2 drug product was subjected to electrophoretic light scattering analysis to determine the zeta potential, which is defined as the electrostatic potential between the particle surface and the bulk solvent. The zeta potential distribution for BNT162b drug product is narrow and monomodal. The average apparent zeta potential is around -3.13 mV, indicating the surface of the LNP is slightly negatively charged. The nearly neutral LNP surface supports the mechanism that BNT162b2 drug product avoids non-specific binding events in the blood compartment.

Precision NanoSystems Study Question/Hypothesis:

Does the pKa of the LNP determine mRNA delivery in vitro for intramuscular (IM) injections?

DOES [CHANGING]THE NET CHARGE OR ZETA POTENTIAL ON THE LNP IMPACT BIODISTRIBUTION AND EXPRESSION FOR INTRAMUSCULAR (IM) DELIVERY OF LNP/mRNA “VACCINES”?

Biodistribution Data from Pfizer: JAPAN

2.6.5.5B. PHARMACOKINETICS: ORGAN DISTRIBUTION CONTINUED

Test Article: [^{3}H]-Labelled LNP-mRNA formulation containing ALC-0315 and ALC-0159
Report Number: 185350

Species (Strain):	Rat (Wistar Han)
Sex/Number of Animals:	Male and female/3 animals/sex/timepoint (21 animals/sex total for the 50 µg dose)
Feeding Condition:	Fed ad libitum
Method of Administration:	Intramuscular injection
Dose:	50 µg [^{3}H]-08-A01-C0 (lot # NC-0552-1)
Number of Doses:	1
Detection:	Radioactivity quantitation using liquid scintillation counting
Sampling Time (hour):	0.25, 1, 2, 4, 8, 24, and 48 hours post-injection

Sample	Mean total lipid concentration (µg lipid equivalent/g (or mL) (males and females combined)							% of administered dose (males and females combined)						
	0.25 h	1 h	2 h	4 h	8 h	24 h	48 h	0.25 h	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	0.271	1.48	2.72	2.89	6.80	13.8	18.2	0.001	0.007	0.010	0.015	0.035	0.066	0.106
Bladder	0.041	0.130	0.146	0.167	0.148	0.247	0.365	0.000	0.001	0.001	0.001	0.001	0.002	0.002
Bone (femur)	0.091	0.195	0.266	0.276	0.340	0.342	0.687	--	--	--	--	--	--	--
Bone marrow (femur)	0.479	0.960	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	0.007	0.013	0.020	0.016	0.011	0.010	0.009
Eyes	0.010	0.035	0.052	0.067	0.059	0.091	0.112	0.000	0.001	0.001	0.002	0.002	0.002	0.003
Heart	0.282	1.03	1.40	0.987	0.790	0.451	0.546	0.018	0.056	0.084	0.060	0.042	0.027	0.030
Injection site	128	394	311	338	213	195	165	19.9	52.6	31.6	28.4	21.9	29.1	24.6
Kidneys	0.391	1.16	2.05	0.924	0.590	0.426	0.425	0.050	0.124	0.211	0.109	0.075	0.054	0.057
Large intestine	0.013	0.048	0.093	0.287	0.649	1.10	1.34	0.008	0.025	0.065	0.192	0.405	0.692	0.762
Liver	0.737	4.63	11.0	16.5	26.5	19.2	24.3	0.602	2.87	7.33	11.9	18.1	15.4	16.2
Lung	0.492	1.21	1.83	1.50	1.15	1.04	1.09	0.052	0.101	0.178	0.169	0.122	0.101	0.101

Biodistribution Data from Pfizer: JAPAN

マスキング箇所：調整中

SARS-CoV-2 mRNA Vaccine (BNT162, PF-07302048)
2.6.5 薬物動態試験の概要表

2.6.5.5B. PHARMACOKINETICS: ORGAN DISTRIBUTION CONTINUED

Test Article: [^{3}H]-Labelled LNP-mRNA formulation containing ALC-0315 and ALC-0159
Report Number: 185350

Sample	Total Lipid concentration (µg lipid equivalent/g [or mL]) (males and females combined)							% of Administered Dose (males and females combined)						
	0.25 h	1 h	2 h	4 h	8 h	24 h	48 h	0.25 h	1 h	2 h	4 h	8 h	24 h	48 h
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	1.37	--	--	--	--	--	--	--
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	12.3	0.001	0.009	0.008	0.016	0.025	0.037	0.095
Pancreas	0.081	0.207	0.414	0.380	0.294	0.358	0.599	0.003	0.007	0.014	0.015	0.015	0.011	0.019
Pituitary gland	0.339	0.645	0.868	0.854	0.405	0.478	0.694	0.000	0.001	0.001	0.001	0.000	0.000	0.001
Prostate (males)	0.061	0.091	0.128	0.157	0.150	0.183	0.170	0.001	0.001	0.002	0.003	0.003	0.004	0.003
Salivary glands	0.084	0.193	0.255	0.220	0.135	0.170	0.264	0.003	0.007	0.008	0.008	0.005	0.006	0.009
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	1.28	1.30	1.47	0.024	0.130	0.319	0.543	0.776	0.906	0.835
Spinal cord	0.043	0.097	0.169	0.250	0.106	0.085	0.112	0.001	0.002	0.002	0.003	0.001	0.001	0.001
Spleen	0.334	2.47	7.73	10.3	22.1	20.1	23.4	0.013	0.093	0.325	0.385	0.982	0.821	1.03
Stomach	0.017	0.065	0.115	0.144	0.268	0.152	0.215	0.006	0.019	0.034	0.030	0.040	0.037	0.039
Testes (males)	0.031	0.042	0.079	0.129	0.146	0.304	0.320	0.007	0.010	0.017	0.030	0.034	0.074	0.074
Thymus	0.088	0.243	0.340	0.335	0.196	0.207	0.331	0.004	0.007	0.010	0.012	0.008	0.007	0.008
Thyroid	0.155	0.536	0.842	0.851	0.544	0.578	1.00	0.000	0.001	0.001	0.001	0.001	0.001	0.001
Uterus (females)	0.043	0.203	0.305	0.140	0.287	0.289	0.456	0.002	0.011	0.015	0.008	0.016	0.018	0.022
Whole blood	1.97	4.37	5.40	3.05	1.31	0.909	0.420	--	--	--	--	--	--	--
Plasma	3.97	8.13	8.90	6.50	2.36	1.78	0.805	--	--	--	--	--	--	--
Blood:Plasma ratio[a]	0.815	0.515	0.550	0.510	0.555	0.530	0.540	--	--	--	--	--	--	--

If the CHARGE on the LNP is MORE POSITIVE (+), it will go to the LUNGS even if given IM (positive charge also linked to CLOTS and other adverse events, more on that in a bit).

If the CHARGE on the LNP is closer to NEUTRAL (o), it will go to the LIVER and distribute from there (still concerns, more on that in a bit).

If the CHARGE on the LNP is a little NEGATIVE (a little bit), it will go the SPLEEN.

If the CHARGE on the LNP is a LOT NEGATIVE, it will LEAK into the VASCULAR SYSTEM EVEN IF ASPIRATION AT SITE AND INTO THE MUSCLE CAUSING A CASCADE OF ADVERSE EVENTS (CLOTS AND MORE).

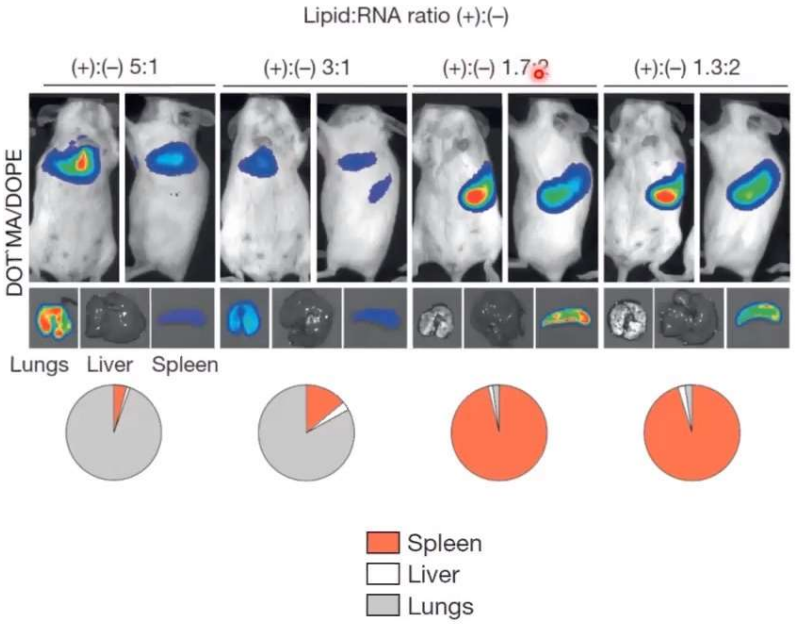
Carrasco et al , Nature 2021 Ionization and structural properties of mRNA lipid nanoparticles influence expression in intramuscular and intravascular administration https://www.nature.com/articles/s42003-021-02441-2
"off target expression of immunogens could however generate systemic cytokines, activate complement, amplify the frequency or severity of adverse events that have been observed in recent clinical trials21,22, and/or impair immune response generation"

LNP DISTRIBUTION BY CHARGE

Precision NanoSystems

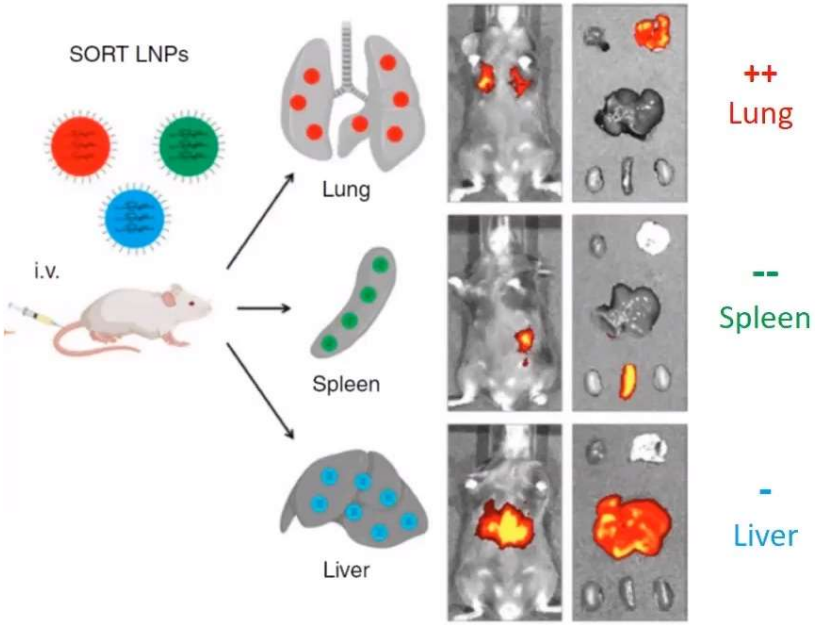
Carrasco, M.J., Alishetty, S., Alameh, MG. et al. Ionization and structural properties of mRNA lipid nanoparticles influence expression in intramuscular and intravascular administration. Commun Biol 4, 956 (2021). https://doi.org/10.1038/s42003-021-02441-2

+LNPs go to lung, -LNPs go to spleen



Kranz et al, *Nature,* 2016

++LNPs go to lung, --LNPs go to spleen
-LNPs go to liver



Cheng et al, *Nature Nanotech,* 2020

Now remember, Pfizer stated the close to NEUTRAL charge on the LNP, in their OWN WORDS, when evaluating the ZETA POTENTIAL of their PRODUCTS, states they are avoiding adverse event potential of the following: **"The nearly neutral charge….AVOIDS NON-SPECIFIC BINDING EVENTS IN THE BLOOD COMPARTMENT."**

THAT MEANS CLOTTING

Surface charge

BNT162b2 drug product was subjected to electrophoretic light scattering analysis to determine the zeta potential, which is defined as the electrostatic potential between the particle surface and the bulk solvent. The zeta potential distribution for BNT162b drug product is narrow and monomodal. The average apparent zeta potential is around -3.13 mV, indicating the surface of the LNP is slightly negatively charged. The nearly neutral LNP surface supports the mechanism that BNT162b2 drug product avoids non-specific binding events in the blood compartment.

The Lipid Nanoparticle is CHANGING/BREAKING DOWN when FROZEN (and then THAWED), CAUSING:

The ZETA POTENTIAL TO CHANGE:

-If the mRNA leaves, the LNP, it will have an overall NET POSITIVE CHARGE

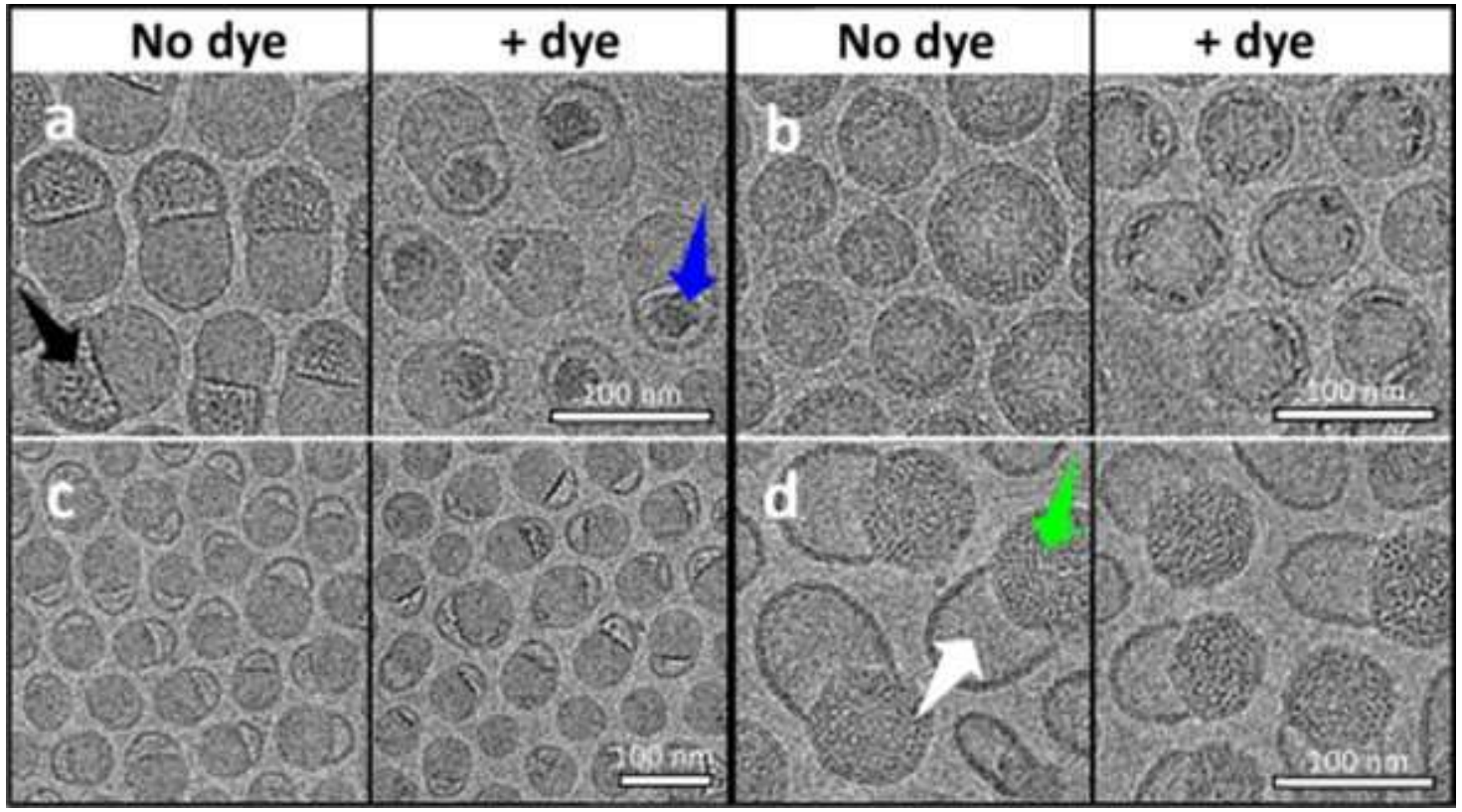
-IF the positively charged lipids leak out, it will have a more NEGATIVE charge, sending it to the SPLEEN or worse, into the VASCULAR, (even if YOU ASPIRATE (does not matter—CLOTS)

-The contents are changing—the cholesterol is becoming ROS, mutated cholesterol, oxysterols, and changing to phosphatidylethanolamine (CLOT POTENTIAL)

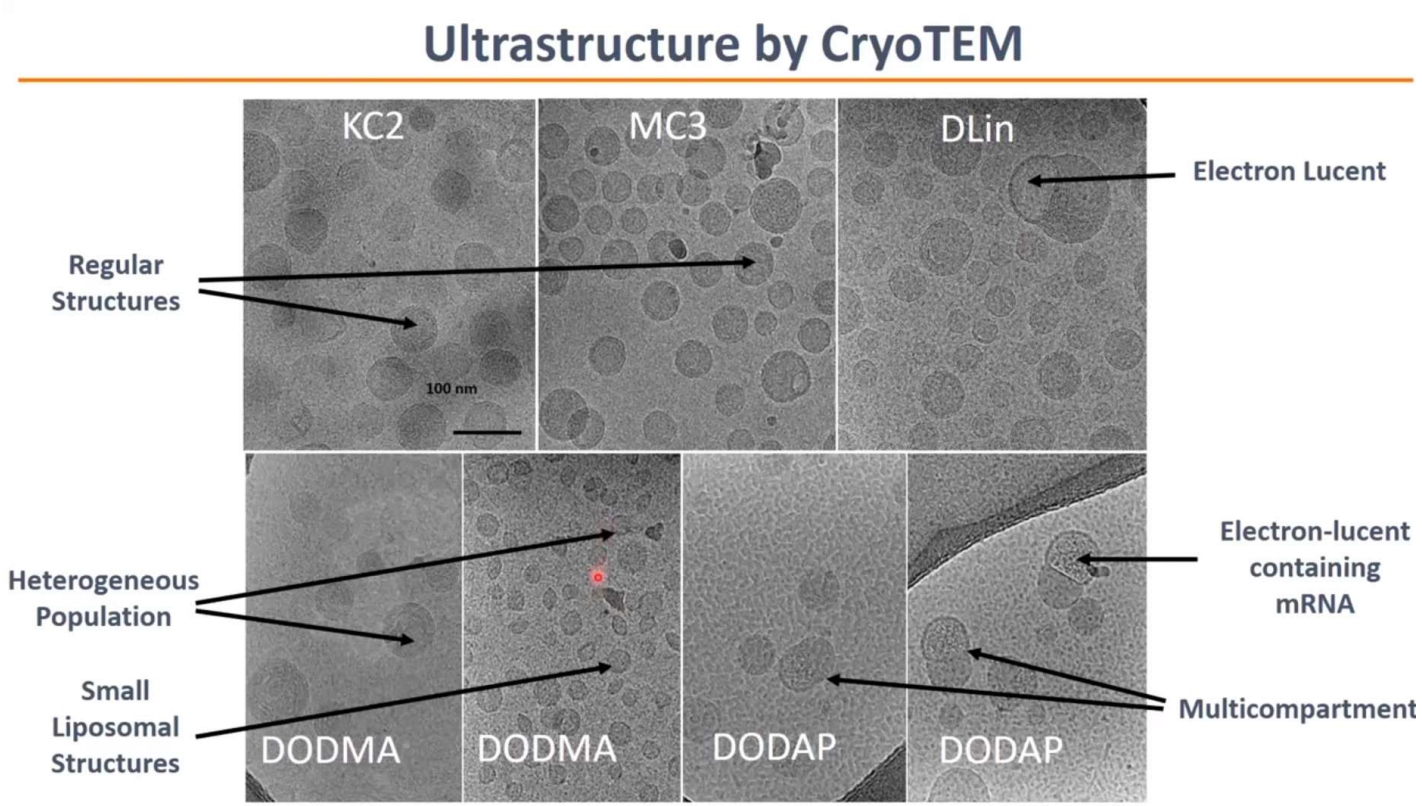
-THE LNP are AGGREGATING/Clumping due to:

- **ION BRIDGING**
- **OSTWALD EFFECT**
- **FLOCCULATION**
- **WHICH CAN CAUSE: Aneurysm, STROKE, etc.**

LNP is BREAKING DOWN



LNP is BREAKING DOWN



LNP BREAKDOWN

De, A., & Ko, Y. T. (2023). Why mRNA-ionizable LNPs formulations are so short-lived: causes and way-out. Expert opinion on drug delivery, 20(2), 175–187. https://doi.org/10.1080/17425247.2023.2162876

EXPERT OPINION ON DRUG DELIVERY
2023, VOL. 20, NO. 2, 175–187
https://doi.org/10.1080/17425247.2023.2162876

REVIEW

Why mRNA-ionizable LNPs formulations are so short-lived: causes a

Anindita De[a] and Young Tag Ko[a]

College of Pharmacy, Gachon Institute of Pharmaceutical Science, Gachon University, Incheon, South Korea

ABSTRACT

Introduction: Messenger ribonucleic acid (mRNA) and small interfering RNA (siRNA) are biological molecules that can be heated, frozen, lyophilized, precipitated, or re-suspended without degradation. Currently, ionizable lipid nanoparticles (LNPs) are a promising approach for mRNA therapy. However, the long-term shelf-life stability of mRNA–ionizable LNPs is one of the open questions about their use and safety. At an acidic pH, ionizable lipids shield anionic mRNA. However, the stability of mRNA under storage conditions remains a mystery. Moreover, ionizable LNPs excipients also cause instability during long-term storage.

Area covered: This paper aims to illustrate why mRNA-ionizable LNPs have such a limited storage half-life. For the first time, we compile the tentative reasons for the short half-life and ultra-cold storage of mRNA-LNPs in the context of formulation excipients. The article also provided possible ways of prolonging the lifespan of mRNA-ionizable LNPs during long storage.

Expert opinion: mRNA-ionizable LNPs are the future of genetic medicine. Current limitations of the formulation can be overcome by an advanced drying process or a whole new hybrid formulation strategy to extend the shelf life of mRNA-ionizable LNPs. A breakthrough technology may open up new research directions for producing thermostable and safe mRNA-ionizable LNPs at room temperature.

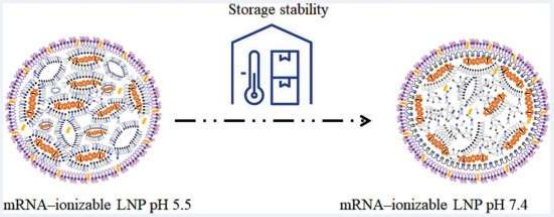
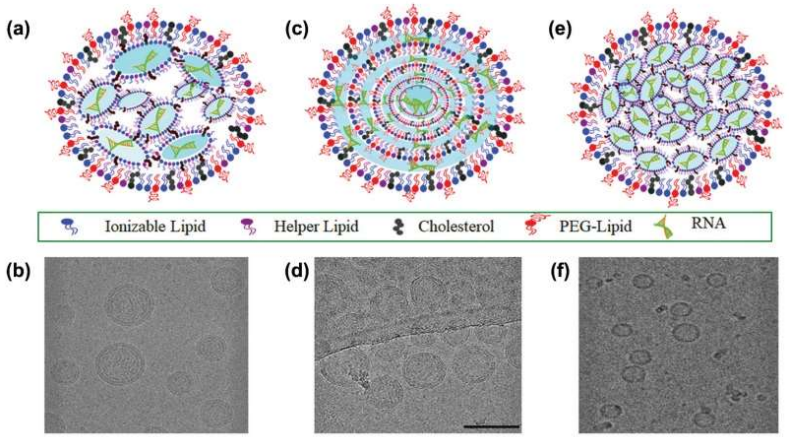
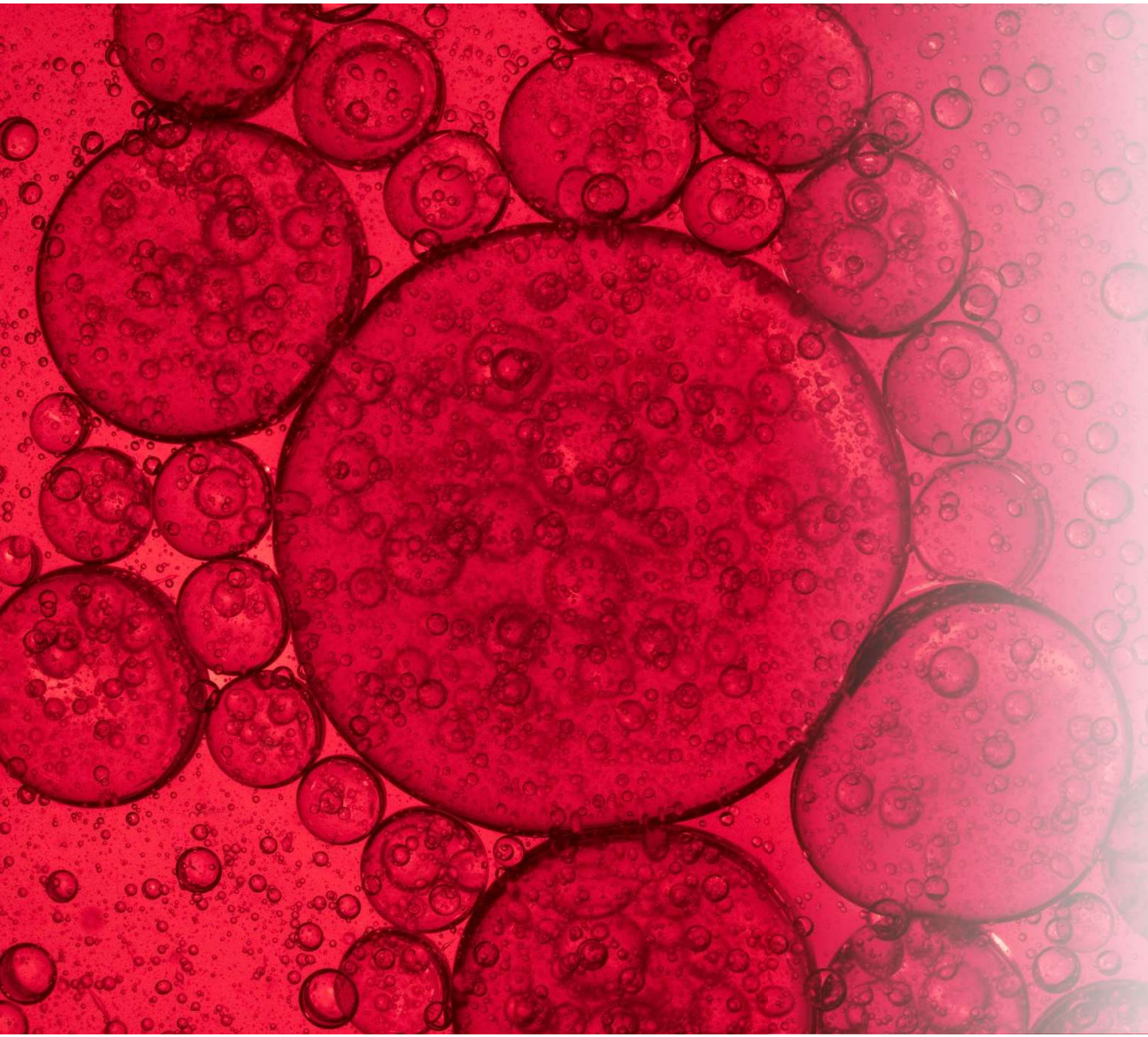


Table 2. Lipid composition of FDA approved commercialized mRNA-ionizable LNPs.

Manufacturer and product	Lipid nanoparticle components	Molar ratios (%)Ionizable lipid: helper lipid: cholesterol: PEG-lipid	Molar N/P ratios
Pfizer-BioNTech BNT162b2; **Comirnaty**	ALC-0315[a], ALC-0159[b], DSPC[c] Cholesterol	46.3:9.4:42.7:1.6	6
Moderna mRNA-1273; **Spikevax**	SM-102[d], DSPC[c], CholesterolPEG2000-DMG	50:10:38.5:1.5	6
Curevac CVnCoV	Cationic lipid, Phospholipid, Cholesterol, PEG-lipid	50:10:38.5:1.5	6

[a]**ALC-0315**: ([(4-hydroxybutyl)azanediyl]di(hexane-6,1-diyl) bis(2-hexyldecanoate)), [b]**ALC-0159**: 2-[(polyethylene glycol)-2000]-N,N ditetradecylacetamide, [c]**DSPC**: 1,2-Distearoyl-*sn*-glycero-3-phosphocholine, [d]SM-102: (heptadecan-9-yl 8-((2-hydroxyethyl) (6-oxo-6-(undecyloxy) hexyl) amino) octanoate), **PEG2000-DMG**: 1-monomethoxypolyethyleneglycol-2,3-dimyristylglycerol PEG average Molecular weight 2000





LNP

Ostwald Effect: A smaller lipid is absorbed into a larger lipid—making it larger.

Aggregation by charge.

Can cause strokes/blockages

LNP: Methods of AGGREGATION:

Ann. Univ. Sofia, Fac. Chem. **102/103** (2011) 253-258 [arXiv 1107.2995]

Flocculation of vesicles

Roumen Tsekov
Department of Physical Chemistry, University of Sofia, 1164 Sofia, Bulgaria

The flocculation of liposomes is theoretically studied. An expression for the flocculation activation energy is derived, accounting for the electrostatic and hydrophobic interactions as well as for the correlation area of floc-spots.

In water phospholipid molecules form spontaneously spheroid structures called vesicles or liposomes [1, 2]. Their body is filled by maternal solution and surrounded by a closed bilayer surface. The liposome wall is not penetrable for large molecules and for this reason vesicles are used as original carrier of drags and other substances in medicine and cosmetics [3, 4]. Hence, the stability of liposome suspensions is important for these applications and this is the reason for intensive studies on the flocculation kinetics of vesicles in solutions.

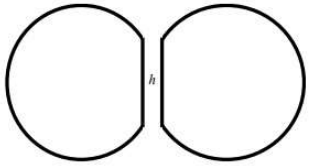
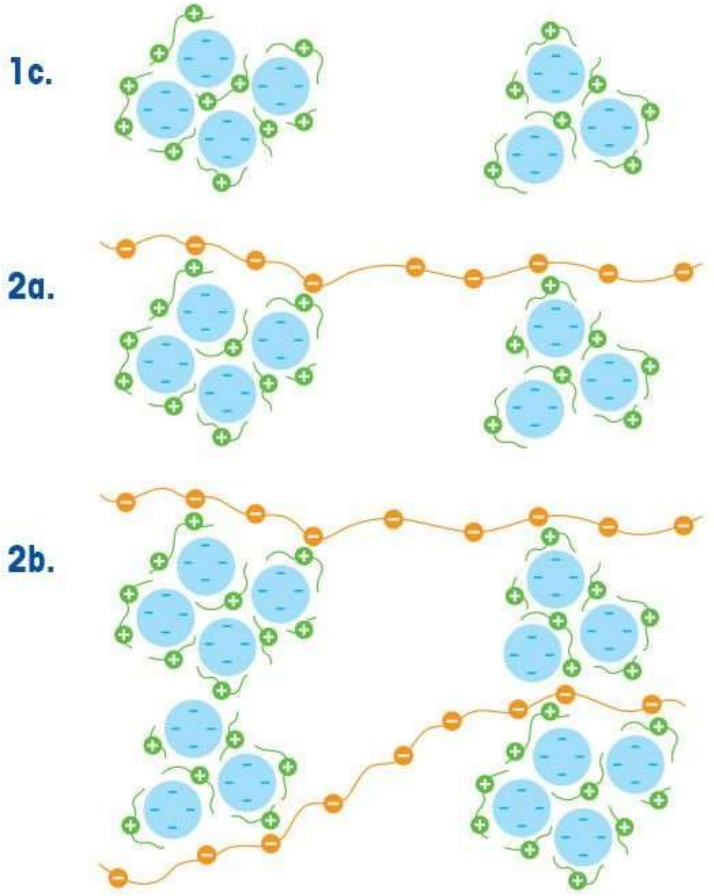


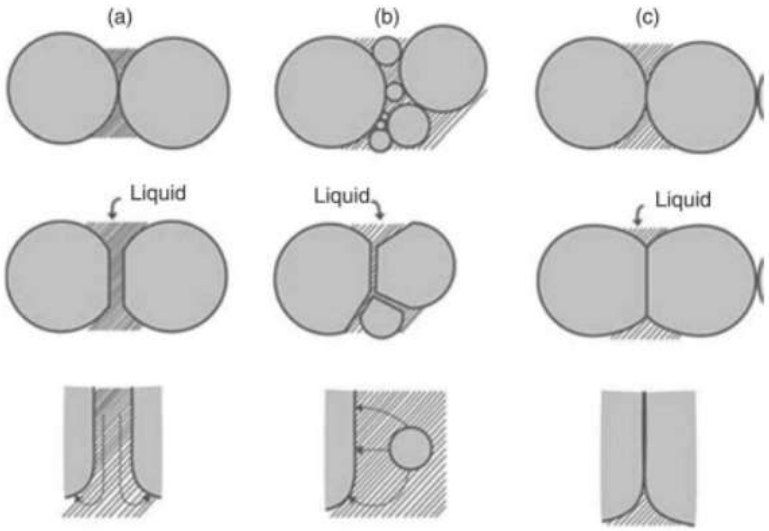
Fig. 1 Schema of a collision of two liposomes or droplets [5]

The problem of coalescence of two liposomes is equivalent to the problem of rupture of the liquid film formed between two vesicles (see Fig. 1). Because vesicles possess almost zero surface tension, they are easily deformable and the hydrodynamic resistance force, due to the vesicle approach, deforms liposomes to form naturally a thin liquid film. Similar picture is observed in deformable droplets [5], which possess, however, large surface tension and, hence, the deformations are smaller. When the film ruptures, the liposomes attach to each other and a primary floc is formed. A very useful idea in colloid science is to consider the film stability as a



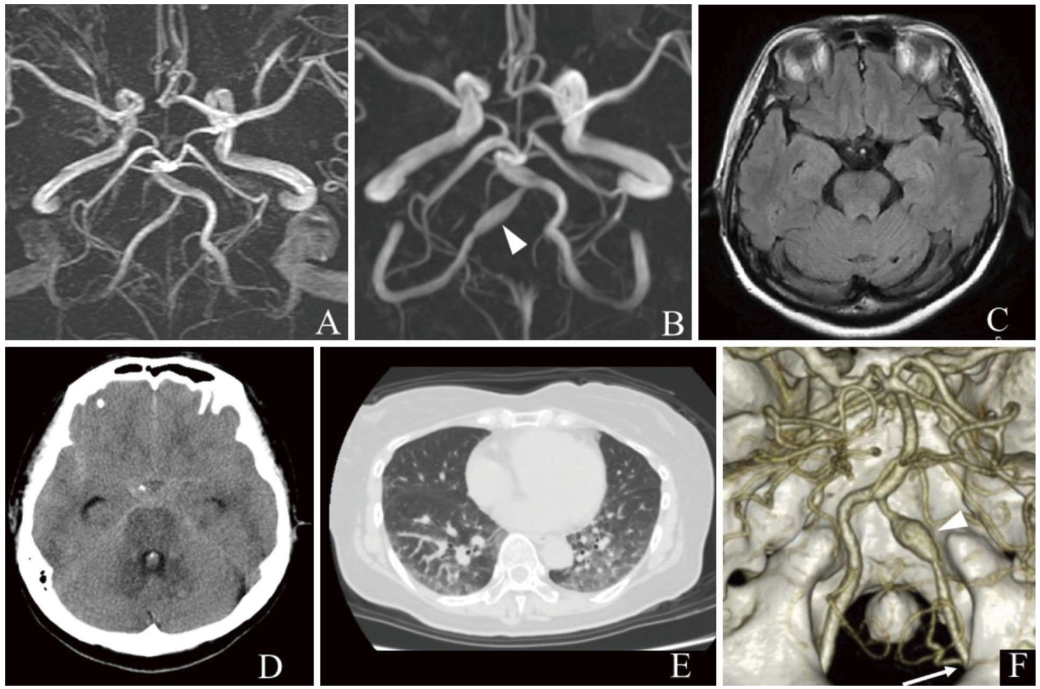
Ostwald ripening

Ostwald ripening occurs via the dissolution of matter at regions with a small radius of curvature and re-precipitation at regions with a large radius of curvature, Fig. 21.13(b). Ostwald ripening leads to the dissolution of smaller solid grains, diffusion of the solute through the liquid and the re-precipitation of the solid onto large grains. The net result is grain growth.



LNP: Methods of AGGREGATION:

Yangi, K., Demir, D. D., & Uzunkol, A. (2023**). Intracranial Hemorrhage After Pfizer-BioNTech (BNT162b2) mRNA COVID-19 Vaccination**: A Case Report. Cureus, 15(4), e37747. https://doi.org/10.7759/cureus.37747



Oshida, S., Akamatsu, Y., Matsumoto, Y., Suzuki, T., Sasaki, T., Kondo, Y., Fujiwara, S., Kashimura, H., Kubo, Y., & Ogasawara, K. (2022**). Intracranial aneurysm rupture within three days after receiving mRNA anti-COVID-19 vaccination: Three case reports**. Surgical neurology international, 13, 117. https://doi.org/10.25259/SNI_1144_2021

IMPLICATIONS OF PLASMID CONTAMINATION of LNP

(Confirmed by Kevin McKernan, Dr. Phillip Buckhaults, (and others) THANK YOU!

Nepetalactone Newsletter

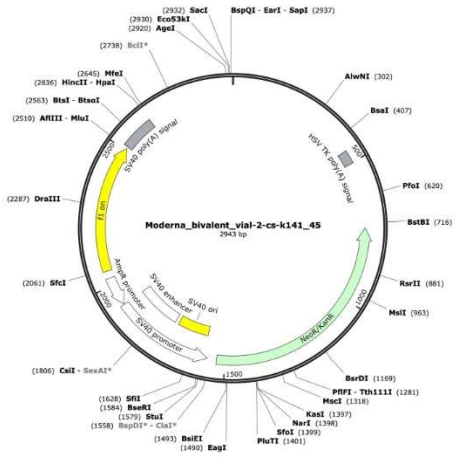


Figure 3. Moderna bivalent vial #2. This may be an empty vector or an assembly artifact that can occur when the insert is 1000X higher in coverage than the vector. Notice the Coverage is only 60X and other vectors are 13,000 X. Likely an index hopping artifact.

>k141_45 flag=1 multi=60.0000 len=2943

CTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTT
TGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAA
ACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAGGATCTTCACCTAGATCCTTTAAATTAAAAATGAAGTTTTAAATCAATCT
AAAGTATATATGAGTAACCTGAGGCTATGGCAGGGCCTGCCGCCCCGACGTTGGCTGCGAGCCCTGGGCCTTCACCCGAACTTGGGGG
GTGGGGTGGGGAAAAGGAAGAAACGCGGGCGTATTGGCCCCAATGGGGTCTCGGTGGGGTATCGACAGAGTGCCAGCCCTGGGACCG
AACCCGCGTTATGAGACAAACGACCCAACACCCGTGCGTTTTATTCTGTCTTTTATTGCCGTCATAGCGCGGGTCCTCCGGTATTGTC
TCCTTCCGTGTTTCAGTTAGCCTCCCCCTAGGGTGGGCGAAGAACTCCAGCATGAGATCCCCGCGCTGGAGGATCATCCAGCCGGCGTCC
CGGAAAACGATTCCGAAGCCCAACCTTTCATAGAAGGCGGCGGTGGAATCGAAATCTCGTGATGGCAGGTTGGGCGTCGCTTGGTCGG
TCATTTCGAACCCCAGAGTCCCGCTCAGAAGAACTCGTCAAGAAGGCGATAGAAGGCGATGCGCTGCGAATCGGGAGCGGCGATACCG
TAAAGCACGAGGAAGCGGTCAGCCCATTCGCCGCCAAGTTCTTCAGCAATATCACGGGTAGCCAACGCTATGTCCTGATAGCGGTCCGC
CACACCCAGCCGGCCACAGTCGATGAATCCAGAAAAGCGGCCATTTTCCACCATGATATTCGGCAAGCAGGCATCGCCATGGGTCACG
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TGATCGACAAGACCGGCTTCCATCCGAGTACGTGCTCGCTCGATGCGATGTTTCGCTTGGTGGTCGAATGGGCAGGTAGCCGGATCAAG
CGTATGCAGCCGCCGCATTGCATCAGCCATGATGGATACTTTCTCGGCAGGAGCAAGGTGAGATGACAGGAGATCCTGCCCCGGCACTT
CGCCCAATAGCAGCCAGTCCCTTCCCGCTTCAGTGACAACGTCGAGCACAGCTGCGCAAGGAACGCCCGTCGTGGCCAGCCACGATAG
CCGCGCTGCCTCGTCTTGCAGTTCATTCAGGGCACCGGACAGGTCGGTCTTGACAAAAAGAACCGGGCGCCCCTGCGCTGACAGCCGG
AACACGGCGGCATCAGAGCAGCCGATTGTCTGTTGTGCCCAGTCATAGCCGAATAGCCTCTCCACCCAAGCGGCCGGAGAACCTGCGTG
CAATCCATCTTGTTCAATCATGCGAAACGATCCTCATCCTGTCTCTTGATCGATCTTTGCAAAAGCCTAGGCCTCCAAAAAAGCCTCCTC
ACTACTTCTGGAATAGCTCAGAGGCCGAGGCGGCCTCGGCCTCTGCATAAATAAAAAAAATTAGTCAGCCATGGGGCGGAGAATGGGC
GGAACTGGGCGGAGTTAGGGGCGGGATGGGCGGAGTTAGGGGCGGGACTATGGTTGCTGACTAATTGAGATGCATGCTTTGCATACT

Nepetalactone Newsletter

Deep sequencing of the Moderna and Pfizer bivalent vaccines identifies contamination of expression vectors designed for plasmid amplification in bacteria

Anandamide
Feb 15

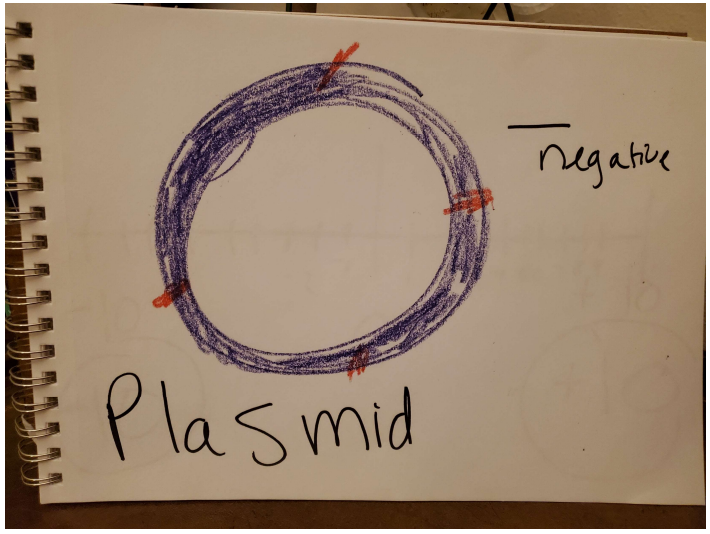
253 138

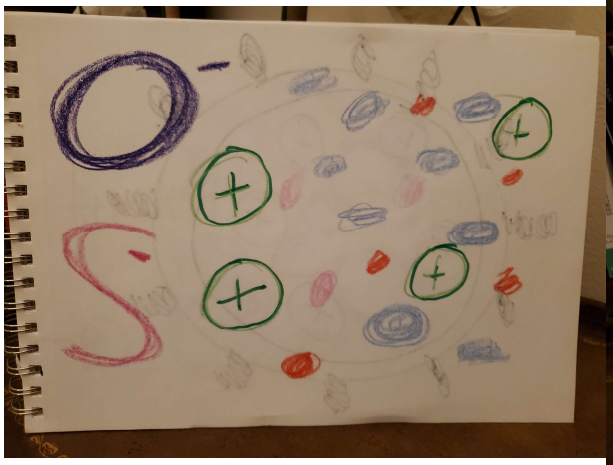
Phillip J. Buckhaults, Ph.D.
@P_J_Buckhaults

An open appeal to health care workers around the world. The Pfizer vaccine is contaminated with plasmid DNA. I want to test tissue and blood samples from people recently vaccinated and see if there is any evidence of this DNA integrating into host genomic DNA. PM me if you would like to help. Thanks. -Dr B.

4:20 PM · Aug 16, 2023 · **22.2K** Views

The Lipid Nanoparticle (LNP) is Breaking Down During the Freeze/Thaw Process, Changing, Leaking, and Mutating the Contents as a Result—DNA PLASMID CONTAMINATION CAUSING A CHANGE IN NET CHARGE/ZETA—ADVERSE EVENTS.





Now remember. Pfizer said their LNP had a zeta potential of APPROX. – 3 millivolts. But what would happen if just ONE DNA PLASMID entered? Taking McKernan's gene sequence of the plasmid, the calculations are:

The electrical charge on a DNA plasmid: charge = (number of base pairs) × (charge per base pair)
The charge on a phosphate group (PO4^-) is -1.
The charge on a sugar molecule is neutral.
The charge on a nitrogenous base depends on the specific base. Adenine and guanine have a charge of +1, while cytosine and thymine have a charge of -1.

T (thymine) - 2929
G (guanine) - 1366
C (cytosine) - 1576
A (adenine) - 1883

charge = (2929 × (-1)) + (1366 × 1) + (1576 × (-1)) + (1883 × 1)
charge = -2929 + 1366 - 1576 + 1883
charge = -256

Therefore, the electrical charge on the DNA plasmid is -256.

Now remember. Pfizer said their LNP had a zeta potential of APPROX. – 3 millivolts. But what would happen if just ONE DNA PLASMID entered (and joined the mRNA and lipids)? Taking McKernan's gene sequence of the plasmid, the calculations are:

Assuming the added DNA plasmid has a charge of -256 at a pH of 7.4, we can estimate the new zeta potential using the following equation:

ζ_new = ζ_old + (k * q)/(ε * η * D)

where:
ζ_old = -3 mV (original zeta potential)
k = Coulomb's constant (1.38 x 10^-23 J/K)
q = charge on the DNA plasmid (-256 e)
ε = dielectric constant of the solvent (water, ~80)
η = viscosity of the solvent (water, ~0.001 Pa*s)
D = electrophoretic mobility of the particle (unknown)

ζ_new = -3 mV + (1.38 x 10^-23 J/K * -256 e)/(80 * 0.001 Pas * -1.0 x 10^-8 m^2/Vs)
ζ_new = -40.6 mV

Therefore, with the addition of the DNA plasmid, the new zeta potential of the lipid nanoparticle is estimated to be approximately -40.6 mV.

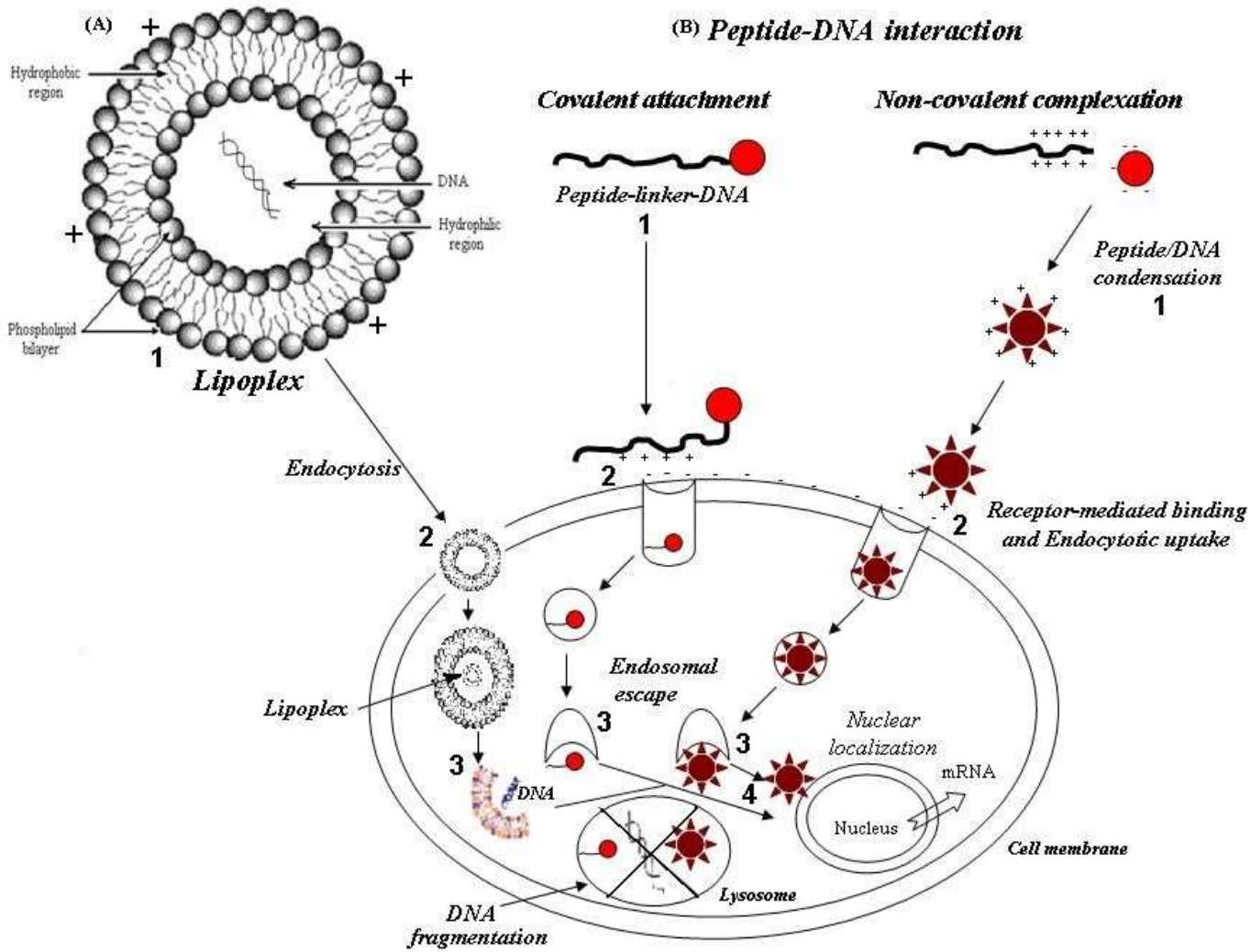
The new zeta potential with the addition of ONE plasmid that may have landed in the LNP, creating a new DNA plasmid poly lipoplex would have a new zeta potential of upwards of minus 40, causing adverse events (leak into the vasculature) and enter heart, brain, blood vessels, cause clots, death, bind to platelets, platelet factor 4 (in those with that genetic predisposition), etc.

But what if the plasmid DNA was only surrounded by positively charged lipids, and created its own lipoplex, and never entered the LNP structure, and was just a stand-alone plasmid surrounded by positively charged lipids?

Assume charge ratio of the lipids to DNA plasmid is 3:1 (common ratio for lipoplex formation):
Number of lipid molecules = 3 x 256 = 768.

Net charge = Total number of positive charges - Total number of negative charges. Net charge = 1536 - 256 = +1280

Wow. Even if that calculation is incorrect, and it was only positive 20, it would be going to: THE LUNGS/causing clots (PE).



https://molecular-cancer.biomedcentral.com/articles/10.1186/1476-4598-10-3/figures/3

DNA Plasmid Contamination Potential Consequences in Human Health

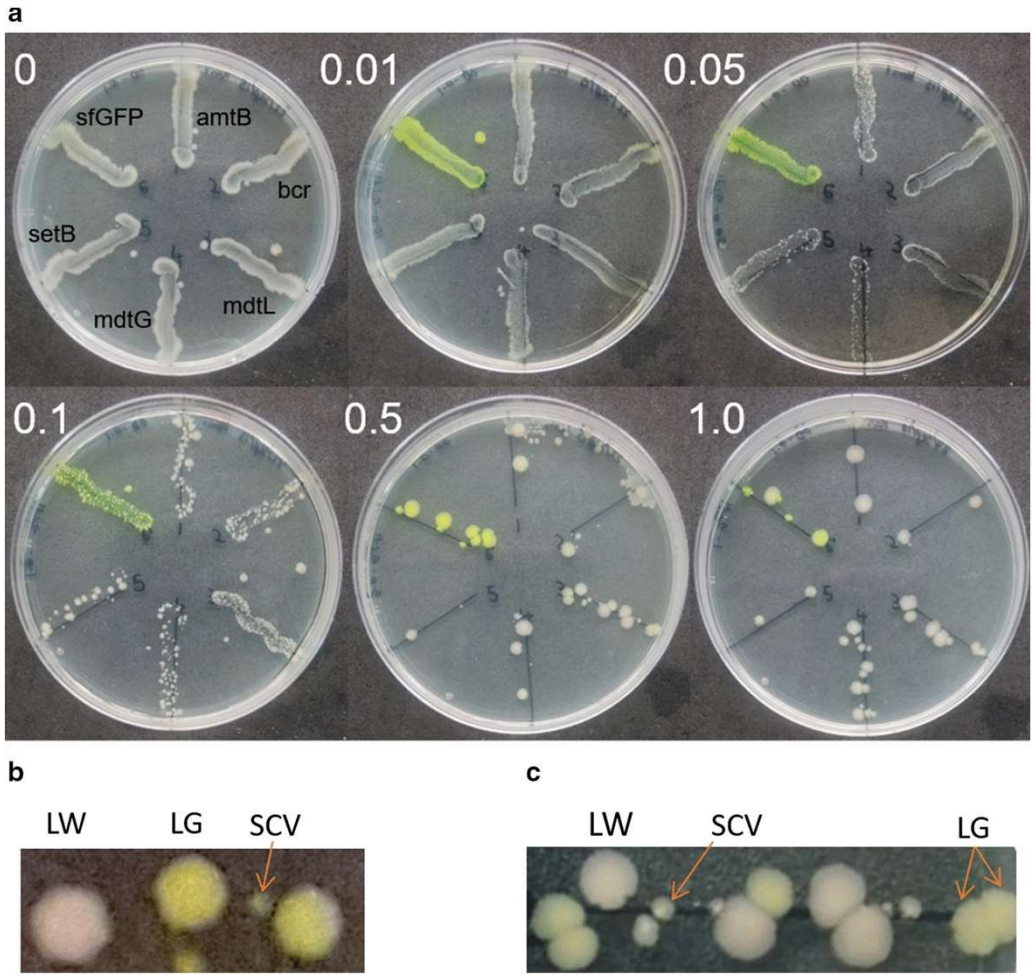
Plasmid DNA/SV40 Promoter (continuous expression)/Antibiotic Resistance Gene Potential Impacts

Destruction of bacteria in the Human Microbiome—Over Expression of the Spike Protein.

Remember, bacteria isn't just in the gut. It exists on the hand/skin, throat and other areas to protect us from pathogens and do other things.

If the plasmids entered our own bacteria, a recent study has shown that overexpression of proteins in bacteria will cause them to "kill themselves" in order to save themselves, in E. coli (James et al., 2021).

https://microbialcellfactories.biomedcentral.com/articles/10.1186/s12934-020-01462-6#citeas



DNA Plasmid Contamination Potential Consequences in Human Health

Plasmid DNA/SV40 Promoter (continuous expression)/Antibiotic Resistance Gene Potential Impacts

Injecting a negatively charged lipid nanoparticle containing RNA intramuscularly with a zeta potential of -40 mV (or even 10 mV), with at least one DNA plasmid, could potentially trigger numerous reactions in the human body, including:

Increased uptake of the nanoparticles by macrophages and monocytes in the liver and spleen, as well as by other immune cells

Activation of the immune system leading to an inflammatory response.

Taken up by cells in the vicinity of the injection site, triggering cellular responses.

Intracellular delivery of RNA: interaction with cellular machinery.

Activation of RNA-sensing pathways, leading to the production of cytokines and chemokines.

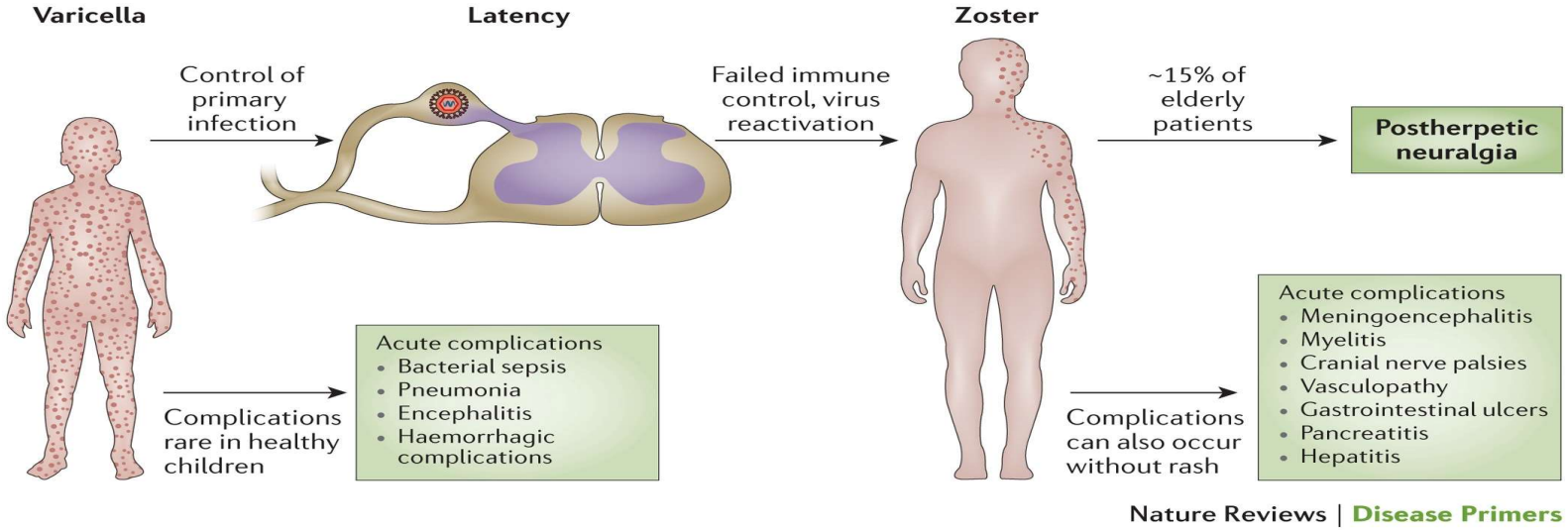
If the nanoparticle is not properly cleared from the body, it could accumulate and cause toxicity.

DNA Plasmid Contamination Potential Consequences in Human Health

Plasmid DNA/SV40 Promoter (continuous expression)/Antibiotic Resistance Gene Potential Impacts

The interaction between the SV40 promoter and intra-condensates may influence viral reactivation. The formation and behavior of intra-condensates can act on the SV40 promoter and the recruitment of factors and co-regulators needed for viral gene expression. .

Varicella zoster virus reactivation following COVID-19 vaccination: a report of 3 cases
https://pubmed.ncbi.nlm.nih.gov/35244157/
Case Report: **Cytomegalovirus Reactivation and Pericarditis** Following ChAdOx1 nCoV-19 Vaccination Against SARS-CoV-2
https://www.frontiersin.org/articles/10.3389/fimmu.2021.784145/full



DNA Plasmid Contamination Potential Consequences in Human Health

Plasmid DNA/SV40 Promoter (continuous expression)/Antibiotic Resistance Gene Potential Impacts

Transfected plasmid DNA is incorporated into the nucleus via nuclear envelope reformation at telophase (if it does not transfect) https://www.nature.com/articles/s42003-022-03021-8

Dysregulation of Gene Expression/Oncogene Activation/Tumor Suppressor Inactivation

Interactions with Pol II:

Picture the DNA as a road with bumps and obstacles, and the histones as roadblocks. If we can smoothen the road, remove the roadblocks, or create alternative routes, the cars (Pol II) can zoom through without any hindrances, leading to faster transcription of genes, like driving on a smooth highway. But that is not necessarily a good thing.

DNA Plasmid Contamination Potential Consequences in Human Health

Plasmid DNA/SV40 Promoter (continuous expression)/Antibiotic Resistance Gene Potential Impacts

Turbo Cancer?

DEAD/H-Box Helicases in Adult Solid Tumors

Breast Cancer
Lung Cancer
Colorectal Cancer
Hepatocellular Carcinoma
Testicular Cancer
Acute Lymphoblastic Leukemia
Prostate Cancer

DEAD/H-Box Helicases in Childhood Solid Tumors
Ewing's Sarcoma
Neuroblastoma
Medulloblastoma

Cytochrome c oxidase deficiency: SURF1 gene mutations.

Binding of DEAD box proteins to SV40 promoter may affect expression of downstream genes. Dysregulated expression of genes involved in cell cycle control, proliferation, or apoptosis can promote uncontrolled cell growth and contribute to cancer development.

Disruption of RNA metabolism/Perturbation of DNA repair/Modulation of signaling pathways/Enhanced expression of oncogenes/Genomic instability

CTNNB1, BRAF, KRAS,

"Persistence of plasmid DNA and expression in rat brain cells in vivo"

Jiao, S., Acsadi, G., Jani, A., Felgner, P. L., & Wolff, J. A. (1992). Persistence of plasmid DNA and expression in rat brain cells in vivo. Experimental neurology, 115(3), 400–413. https://doi.org/10.1016/0014-4886(92)90205-5

DNA Plasmid Contamination Potential Consequences in Human Health

Plasmid DNA/SV40 Promoter (continuous expression)/Antibiotic Resistance Gene Potential Impacts

DEAD BOX Protein LOCATIONS:

Nucleus: Many dead box proteins, including DDX3, DDX1, and DDX5, are predominantly localized in the nucleus of human cells. They participate in various nuclear processes such as RNA processing, transcription regulation, and DNA repair.

Cytoplasm: Dead box proteins are also found in the cytoplasm of cells, where they play crucial roles in RNA metabolism, translation regulation, and other cytoplasmic processes. For example, DDX3 is known to be involved in mRNA transport from the nucleus to the cytoplasm and in modulating translation initiation.

Stress granules and P-bodies: Under certain conditions, dead box proteins can accumulate in specialized cytoplasmic structures called stress granules and processing bodies (P-bodies). These structures are involved in mRNA storage, degradation, and translation repression, particularly during cellular stress responses.

Mitochondria: Some dead box proteins, such as DDX1 and DDX3, have been detected in mitochondria, which are the cellular organelles responsible for energy production. These proteins are implicated in mitochondrial RNA metabolism and may contribute to mitochondrial function.

DNA Plasmid Contamination Potential Consequences in Human Health

Plasmid DNA/SV40 Promoter (continuous expression)/Antibiotic Resistance Gene Potential Impacts

Off-target effects: If the DNA plasmid entered cells and was transcribed into RNA, it could potentially produce off-target effects, such as the production of unintended proteins that could cause harm.

Immunogenicity: DNA plasmid in the vaccine could trigger an immune response, leading to the production of antibodies against the plasmid.

CLOTS: highly negative charged LNPs can activate platelets and promote the formation of clots.

Plasmids and the BRAIN?

DNA Plasmid Contamination Potential Consequences in Human Health

Plasmid DNA/SV40 Promoter (continuous expression)/Antibiotic Resistance Gene Potential Impacts

PLASMIDS AND THE BRAIN

Expression of specific genes or proteins within the targeted cells. Pof new proteins, altered protein levels, or modification of existing cellular processes.

Glial cells, including astrocytes, oligodendrocytes, and microglia, play crucial roles in supporting neuronal function, synaptic plasticity, and immune responses in the brain. If the plasmids enter these glial cells and alter their gene expression—alteration of neurotransmitter levels or synaptic activity, while modifications in microglia might impact immune responses or neuroinflammation.

DNA plasmids: trigger inflammation or other immune-related reactions. Off target effects on neighboring cells or unintended alterations in gene expression could occur, which might disrupt normal brain function.

Alterations in : Chondroitinase ABC (ChABC), Matrix metalloproteinases (MMPs), genes encoding various neurotrophic factors, such as brain-derived neurotrophic factor (BDNF) or nerve growth factor (NGF), genes encoding anti-inflammatory molecules, such as interleukin-10 (IL-10) or interleukin-1 receptor antagonist (IL-1ra), TP53 and PTEN, neurofibromatosis type 1 (NF1), Li-Fraumeni syndrome, and tuberous sclerosis complex (TSC), PI3K/AKT or MAPK pathways, and cell migration, such as focal adhesion kinase (FAK) or RhoA.

DNA Plasmid Contamination Potential Consequences in Human Health

Plasmid DNA/SV40 Promoter (continuous expression)/Antibiotic Resistance Gene Potential Impacts

Potential Implications of the SV40 Promoter

The SV40 promoter is recognized and bound by cellular transcription factors such as Sp1, AP-1 (activator protein 1), and ATF (activating transcription factor) proteins. These factors bind to specific DNA sequences within the promoter region and regulate the initiation of gene expression.

Once the SV40 promoter is bound by transcription factors, RNA polymerase II is recruited to the promoter. RNA polymerase II is responsible for transcribing DNA into RNA, which is a crucial step in gene expression.

The SV40 promoter can also interact with enhancer and silencer elements present in the genome. These regulatory elements, when bound by specific transcription factors, can enhance or suppress the activity of the SV40 promoter, modulating gene expression levels.

The activity of the SV40 promoter can be influenced by the local chromatin structure and epigenetic modifications. DNA methylation, histone modifications, and chromatin remodeling factors can affect the accessibility of the SV40 promoter to transcriptional machinery and impact gene expression.

The SV40 promoter can interact with various co-activators and co-repressors that modulate gene expression. These proteins can either enhance or inhibit the activity of the promoter by influencing the assembly and function of the transcriptional complex.

DNA Plasmid Contamination Potential Consequences in Human Health

Plasmid DNA/SV40 Promoter (continuous expression)/Antibiotic Resistance Gene Potential Impacts

Potential Implications of the SV40 Promoter—Layman's Terms

Compare a promoter to a light switch in a room. When you flip the switch on, the light turns on and illuminates the room. Similarly, when a promoter is activated, it "turns on" gene expression, leading to the production of the corresponding protein or RNA molecule. Turning the switch off represents the inactivation of the promoter, resulting in no gene expression.

Compare a promoter to the title and opening instructions of a recipe. The recipe title tells you what dish you're about to make, while the opening instructions guide you on how to start preparing it. Similarly, the promoter provides the necessary information about the gene (the dish), and the opening instructions initiate the transcription process (preparation) to produce the desired protein or RNA molecule.

The activated SV40 promoter "switches on" the expression of the p53 gene (light bulb), leading to the production of the p53 protein. The p53 protein, often referred to as the "guardian of the genome," plays a critical role in regulating cell growth and preventing the formation of cancerous cells.

DNA Plasmid Contamination Potential Consequences in Human Health

Plasmid DNA/SV40 Promoter (continuous expression)/Antibiotic Resistance Gene Potential Impacts

Potential Implications of the SV40 Promoter—Layman's Terms

The promoter can be likened to the starting gate in a dog racing competition. The starting gate holds back the dogs until the race officially begins. Similarly, a promoter acts as a regulatory region that holds back gene expression until it receives the necessary signals to initiate transcription.

Gene expression can be compared to the race itself. Once the starting gate (promoter) opens, the dogs are released, and the race begins. Similarly, when the promoter is activated, it initiates the transcription process, allowing the gene to be expressed and resulting in the production of RNA molecules or proteins.

A strong promoter can be compared to a race where the dogs have a clear path, with minimal obstacles or distractions. It provides a powerful signal for the race to begin, resulting in high levels of gene expression, similar to how a strong promoter leads to robust expression of the target gene.

Transcription factors can be compared to the race trainers or handlers who influence the dogs' performance. They interact with the promoter and can enhance or inhibit gene expression, similar to how trainers can positively or negatively impact the dogs' racing abilities.

SUMMARY

- Cationic lipids within the LNP can cause adverse events, such as clots.
- Cationic lipids can mutate RNA and DNA, through electrophilic attack, potentially leading to nucleic causing a cascade of consequences.
- Negatively charged lipids can cause severe adverse event, including clots.
- Net charge determines biodistribution, and adverse events.
- A negative zeta potential can leak into the vascular department, per Pfizer and Precision Nano promoting adverse events, including clots.
- Lipids can aggregate via Ostwald effect, flocculation, and ion bridging and cause blockages in vessels, immune concerns, and other cellular malfunctions.
- Overexpression of spike protein can destroy bacteria.
- Plasmids can infiltrate the cell, causing multiple adverse events.
- The SV40 promoter can negatively impact multiple cellular process, including gene expression.

Ancillary/Supplemental Topics of Concern/Active FOIAs

Class switch toward noninflammatory, spike-specific IgG4 antibodies after repeated SARS-CoV-2 mRNA vaccination
https://www.science.org/doi/10.1126/sciimmunol.ade2798

Previous studies have already used the SV40 promoter to DRIVE the switching of antibodies, the class switching of antibodies.
If the SV40 promoter was present in the mRNA "vaccine", this would be a concern.

Sen, R., & Kastner, P. (1993). Enhancer interference and immunoglobulin heavy chain class switch recombination. Trends in Biochemical Sciences, 18(5), 176-180. doi: 10.1016/0968-0004(93)90161-t

Chaudhuri, J., et al. (2007). Evolution of the immunoglobulin heavy chain class switch recombination mechanism. Advances in Immunology, 94, 157-214. doi: 10.1016/s0065-2776(06)94006-3 — use of SV40 promoter in antibody class switching

Sale, J. E., et al. (2001). The molecular mechanism of class switch recombination: Balancing long-range synapsis and exonuclease processing. Genes & Development, 15(23), 3266-3277. doi: 10.1101/gad.943001

Immunoglobulin class switch and SV40 promoter

Ancillary/Supplemental Topics of Concern/Active FOIAs

Can positively charged lipids cause cancer?

Dysregulation or mutations in kinases can lead to various diseases, including cancer, autoimmune disorders, and neurodegenerative diseases.

There are hundreds of kinases in the human genome, each with its own specific functions and targets.

Human kinases are proteins that have specific regions called binding sites.
These binding sites can interact with different things in a cell, including lipids.

When a kinase binds to a lipid, it involves a process called electrostatic interaction.

https://pubs.acs.org/doi/10.1021/jacs.1c10154

Electrostatic Interactions as Mediators in the Allosteric Activation of Protein Kinase A RIα
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5495472/

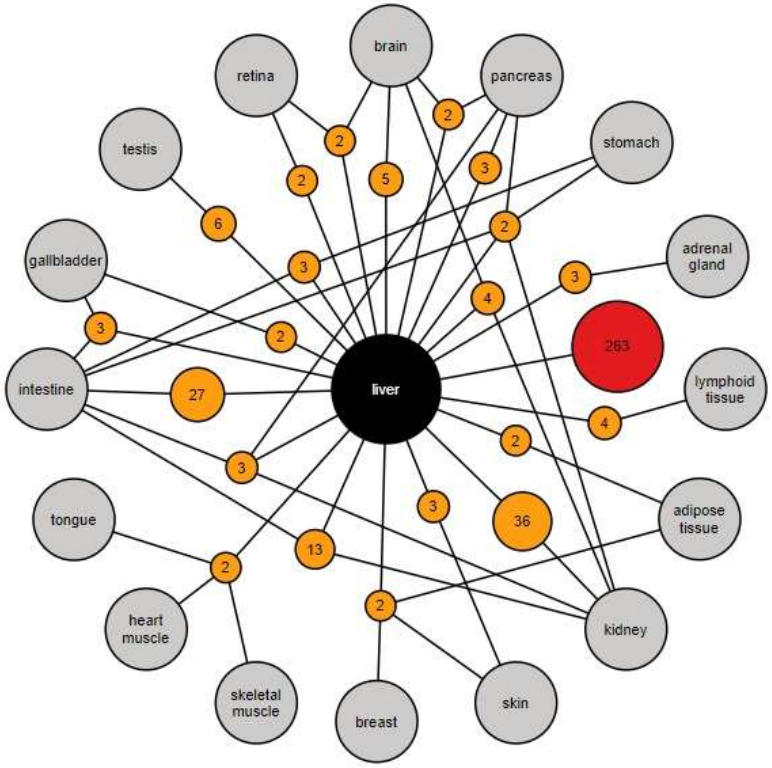
Ancillary/Supplemental Topics of Concern/Active FOIAs

What about liver proteomics? If the primary landing zone for the LNP is the liver…..

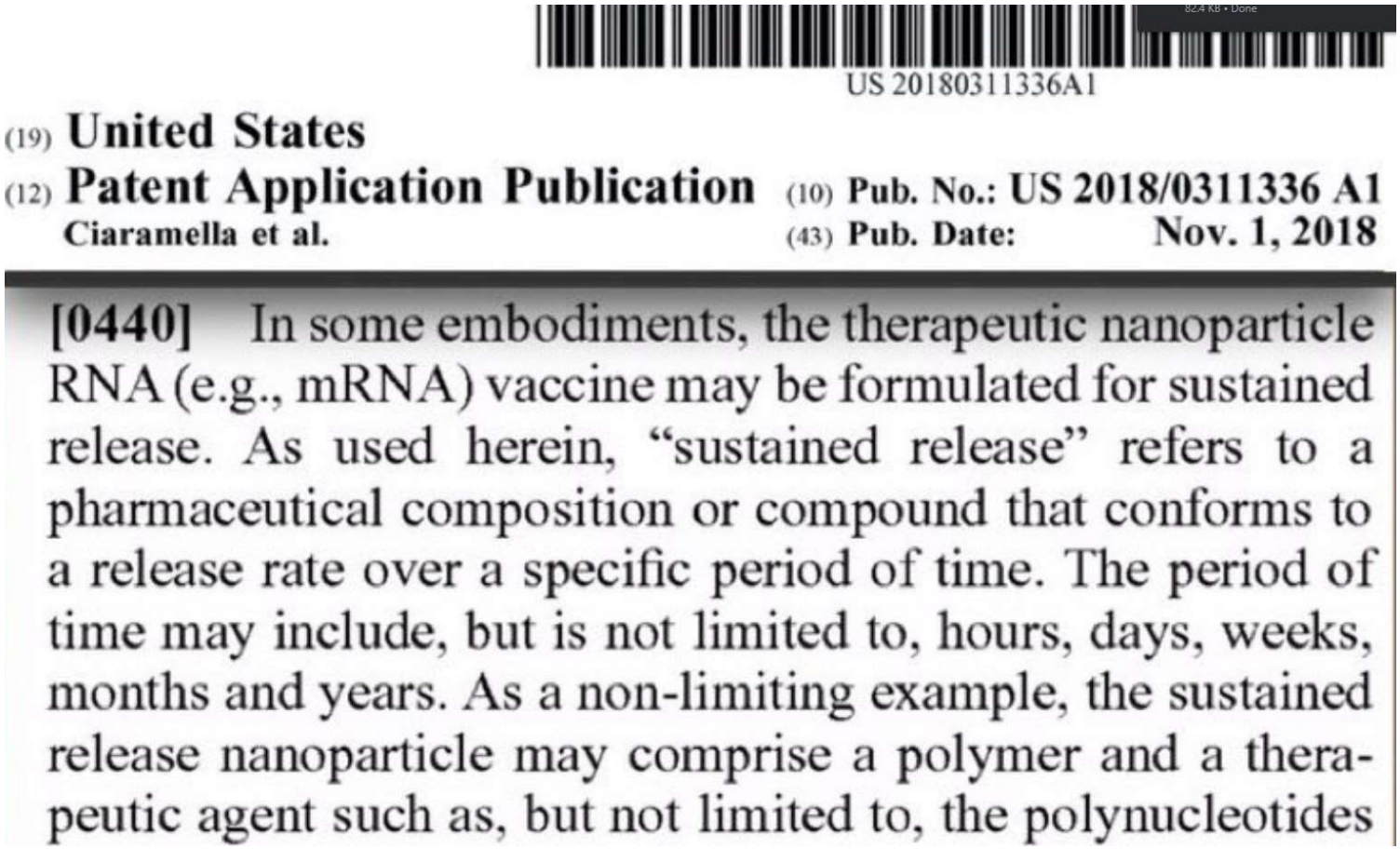
https://www.proteinatlas.org/humanproteome/tissue/liver

https://www.proteinatlas.org/search/tissue_category_rna:liver;group+enriched+AND+show_columns:groupenriched

There are 178 group enriched genes expressed in liver. Group enriched genes are defined as genes showing a 4-fold higher average level of mRNA expression in a group of 2-5 tissues, including liver, compared to all other tissues.



Ancillary/Supplemental Topics of Concern/Active FOIAs



US 20180311336A1

(19) **United States**

(12) **Patent Application Publication**

Ciaramella et al.

(10) **Pub. No.: US 2018/0311336 A1**

(43) **Pub. Date: Nov. 1, 2018**

[0440] In some embodiments, the therapeutic nanoparticle RNA (e.g., mRNA) vaccine may be formulated for sustained release. As used herein, "sustained release" refers to a pharmaceutical composition or compound that conforms to a release rate over a specific period of time. The period of time may include, but is not limited to, hours, days, weeks, months and years. As a non-limiting example, the sustained release nanoparticle may comprise a polymer and a therapeutic agent such as, but not limited to, the polynucleotides

Ancillary/Supplemental Topics of Concern/Active FOIAs

FDA U.S. FOOD & DRUG ADMINISTRATION

July 14, 2023

CHRISTIE GRACE

US

In Reply refer to
FOIA Control #:
2023-6034

Requester reference:

Dear Requester:

The Food and Drug Administration (FDA) has received your Freedom of Information Act (FOIA) request for records regarding:

As per the FDA Freedom of Information Act (FOIA) regarding the 1.Comirnaty vaccine (Pfizer-BioNTech COMIRNATY® COVID 19 Vaccine, mRNA suspension for injection, for intramuscular use), 2. The Pfizer-BioNTech COVID-19 Vaccine, Bivalent 3. the Moderna COVID-19 Vaccine, and etc

In processing your FOIA request, FDA will apply, as appropriate, the FOIA exemptions in 5 USC 552(b) and the foreseeable harm standard in 5 USC 552(a)(8)(i). We will respond as soon as possible and may charge you a fee for processing your request. If your informational needs change, and you no longer need the requested records, please contact us to cancel your request, as charges may be incurred once processing of your request has begun. For more information on processing fees, please see http://www.fda.gov/RegulatoryInformation/FOI/FOIAFees/default.htm. PLEASE NOTE: HOURLY RATES FOR SEARCH AND REVIEW INCREASED FOR ALL REQUESTS RECEIVED ON OR AFTER JUNE 1, 2023.

Due to an increase in the number of incoming requests, we may be unable to comply with the twenty-working-day time limit in this case, as well as the ten additional days provided by the FOIA. The actual processing time will depend on the complexity of your request and whether sensitive records, voluminous records, extensive search, and/or consultation with other HHS components or other executive branch agencies are involved. Please note that requests for medical device approval records (e.g. 510K, PMA, DEN) may take up to 18 to 24 months to process.

If you have any questions about your request, please call Wilson M. Russ, Freedom Of Information Specialist, at (301) 796-8981 or write to us at:
Food and Drug Administration
Division of Freedom of Information
5630 Fishers Lane, Room 1035
Rockville, MD 20857

If you call or write, use the FOIA control number provided above which will help us to answer your questions more quickly.

You also have the right to seek dispute resolution services from:

Office of Government Information Services
National Archives and Administration
8601 Adelphi Road – OGIS
College Park, MD 20740-6001

and/or

FDA FOIA Public Liaison
Office of the Executive Secretariat
US Food and Drug Administration
5630 Fishers Lane, Room 1050

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LINKS

Mechanisms of cellular uptake and intracellular trafficking of nanoparticles": This review article discusses the various mechanisms by which nanoparticles, including cationic lipids, are taken up by cells and transported to the nucleus for transfection.
Link: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5050052/

"Lipoplex size determines lipofection efficiency and intracellular trafficking": This study investigates the relationship between lipoplex size and transfection efficiency, as well as the intracellular trafficking of lipoplexes.
Link: https://pubmed.ncbi.nlm.nih.gov/16380105/

"Nuclear import of nonviral DNA: a case for active nuclear transport": This study explores the mechanisms by which nonviral DNA, including DNA with cationic lipids, is transported across the nuclear envelope for transfection.

"Intracellular Trafficking and Gene Transfection of pH-Responsive and Folated Polyethylenimine-Dioleoylphosphatidylethanolamine (PEI-DOPE) Polyplexes in Cancer Cells," Journal of Controlled Release, 2011.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3059643/

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