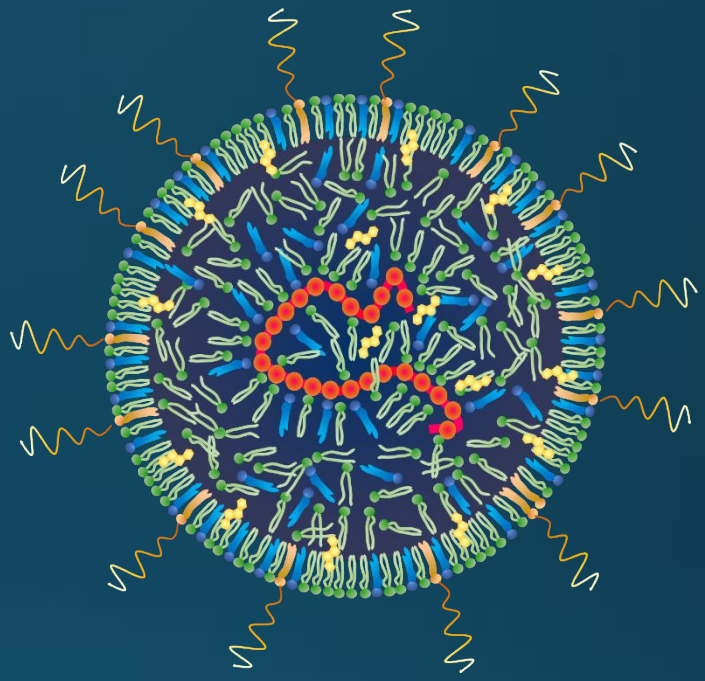
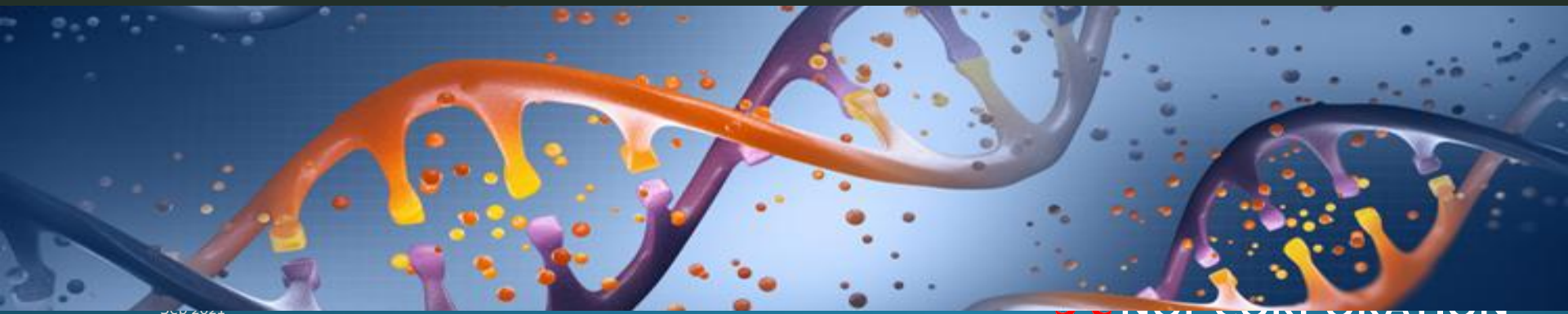


COATSOME® SS-Lipids: Biodegradable Lipid Nanoparticles for Gene Therapy and Vaccines with Room Temperature Stability

Syed Reza, Drug Delivery Consultant, NOF CORPORATION

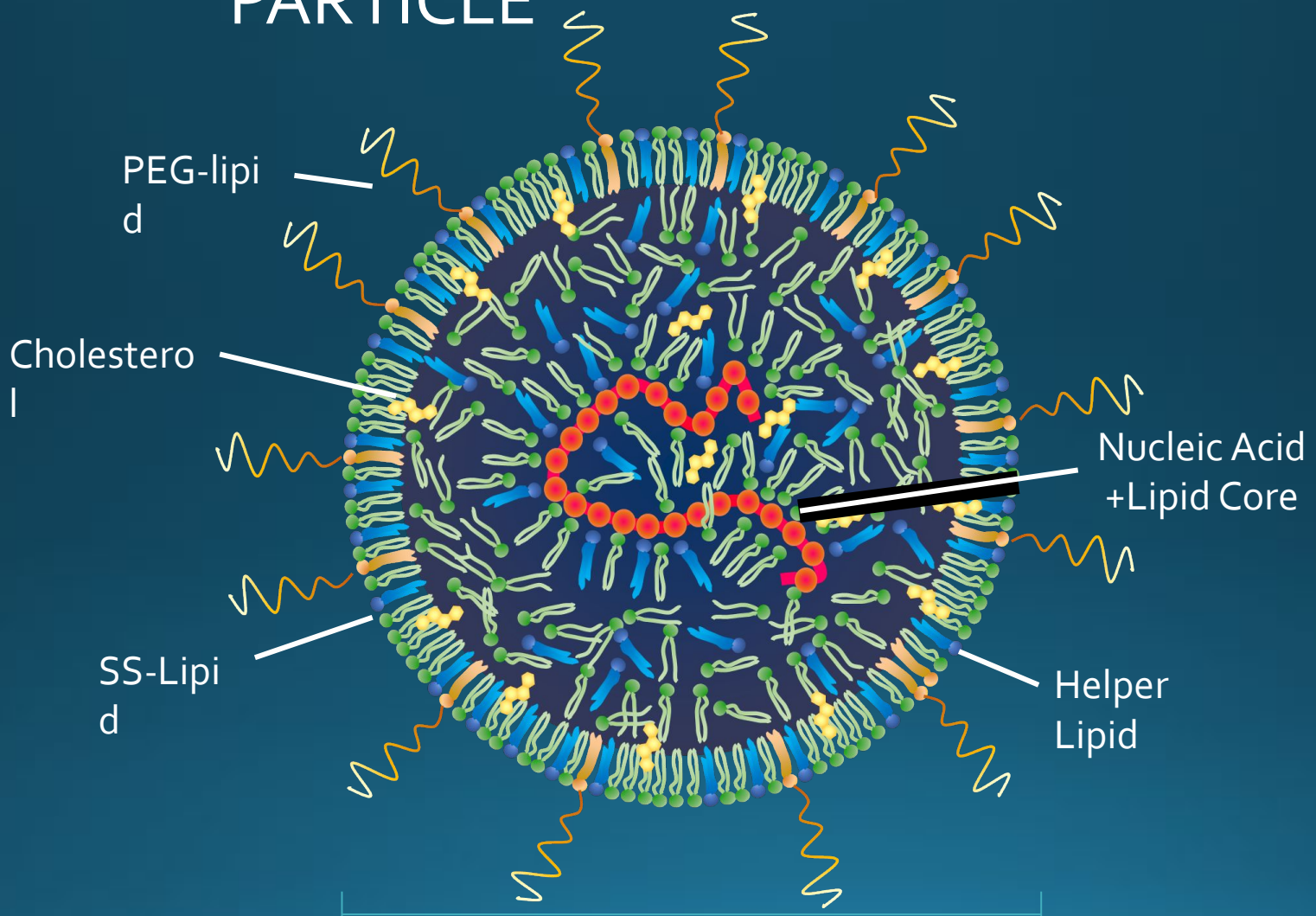


NANO PARTICLES FOR GENE DELIVERY

- Nucleic acid therapeutics encompass a wide range of applications, ranging from vaccines, enzyme replacement, and gene editing
- Lipid Nanoparticles (LNPs) have been developed for delivery of RNA, however the toxicity of the ionizable lipids limits their clinical use.
- A molecular design to minimize the toxicity should be pursued to broaden the pharmaceutical and clinical options.
- A key issue for reducing cytotoxicity and accelerating the intracellular release of cargos is “biodegradability”.

CHEMISTRY OF SS-LIPIDS

SS-LIPID COATSOME PARTICLE



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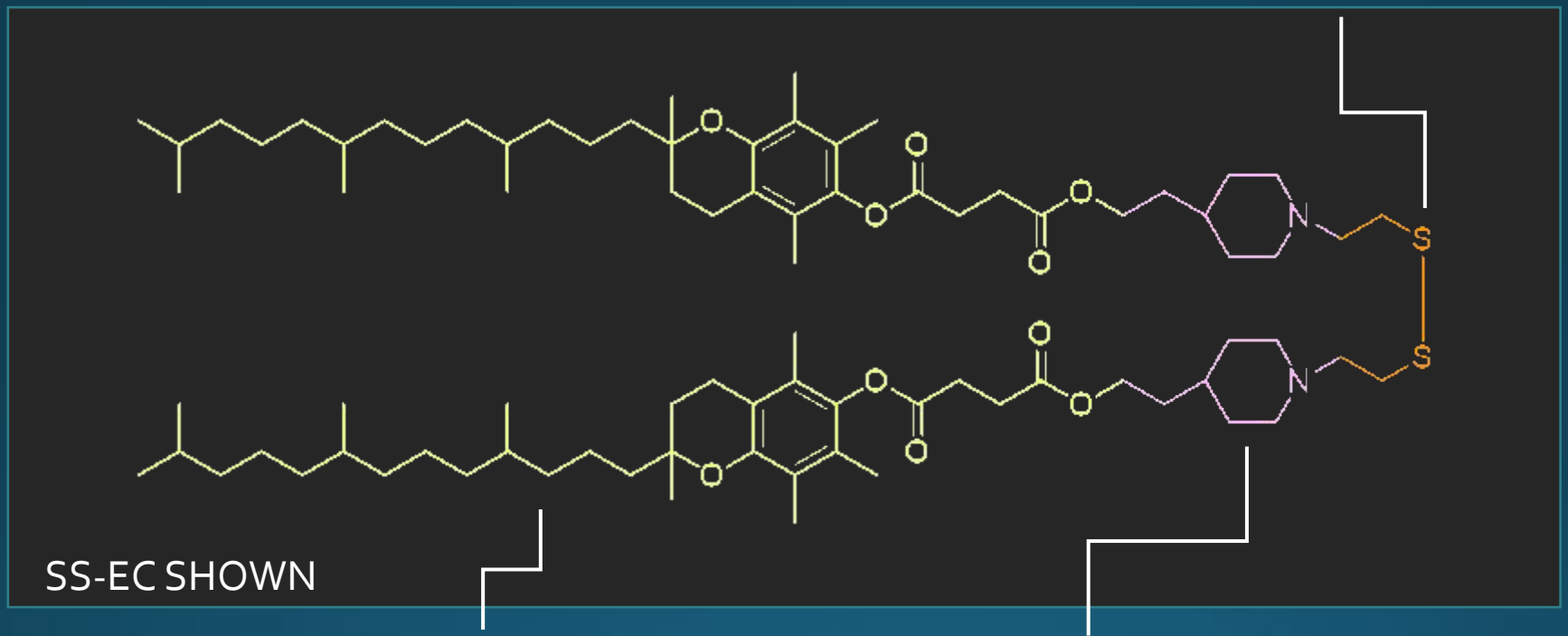
SS-LIPID STRUCTURE

DISULFIDE BOND
(CLEAVABLE)



SS-EC SHOWN

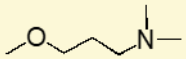
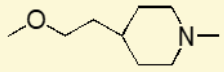
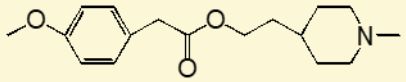
TOCOPHEROL
(ADJUVANT)

TERTIARY AMINE
(IONIZABLE)

NOF CORPORATION

SS-Lipids "COATSOME® SS Series" PRODUCT RANGE

$$RCOO\text{-}X\text{-}CH_2CH_2\text{-}S$$
$$RCOO\text{-}X\text{-}CH_2CH_2\text{-}S$$

Product name	RCO-	-O-X-	MW
COATSOME® SS-E	α-D-Tocopherolsuccinoyl		1322.0
COATSOME® SS-EC			1402.2
COATSOME® SS-OC	Oleoyl		905.5
COATSOME® SS-OP	Oleoyl		1173.8

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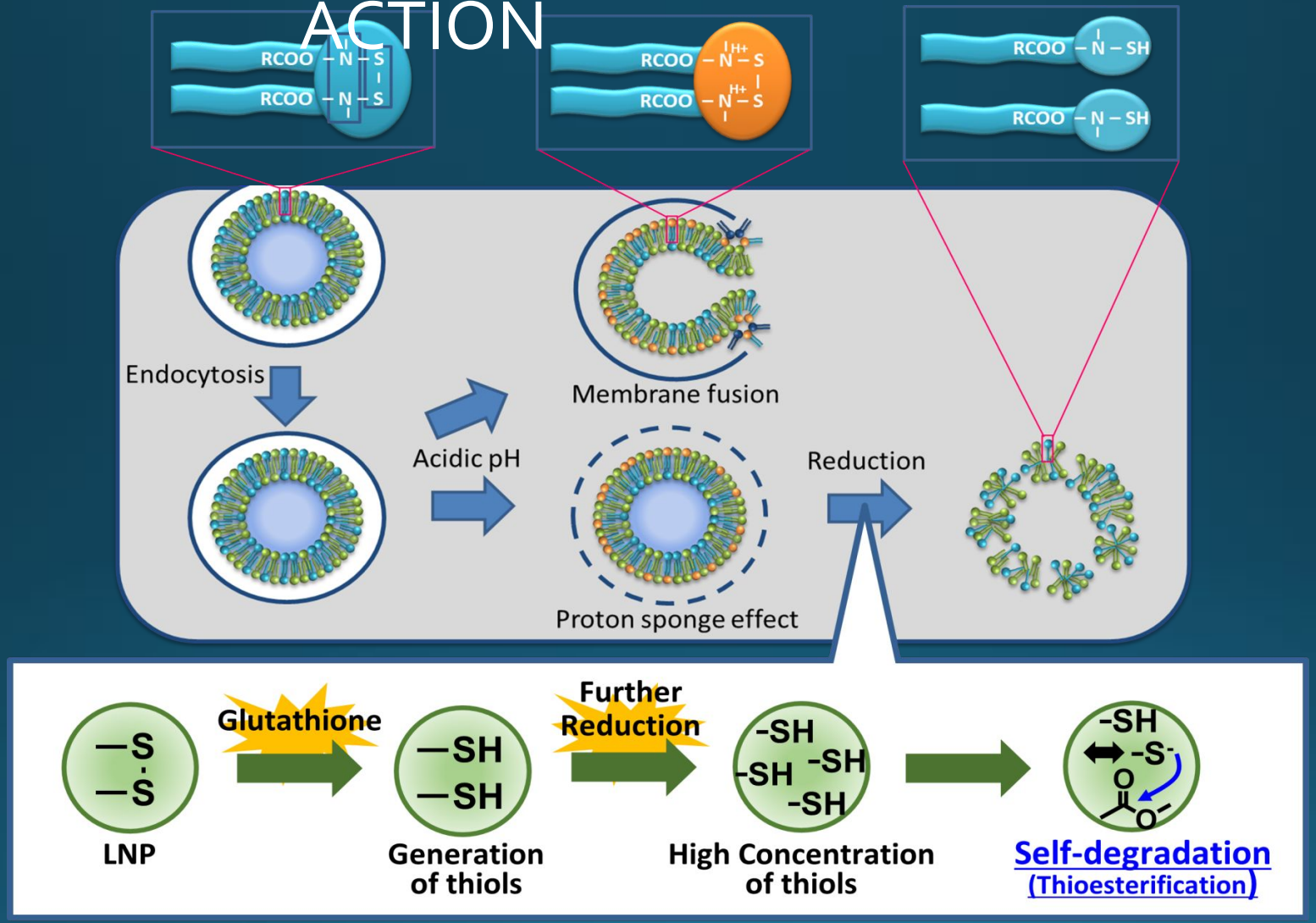
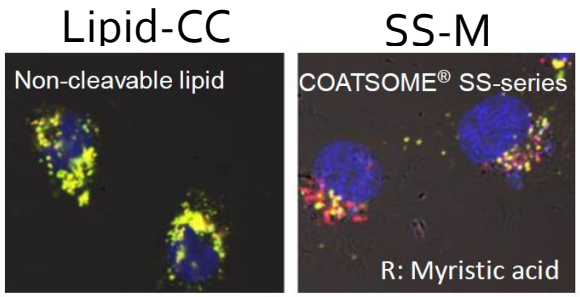
MECHANISM OF ACTION



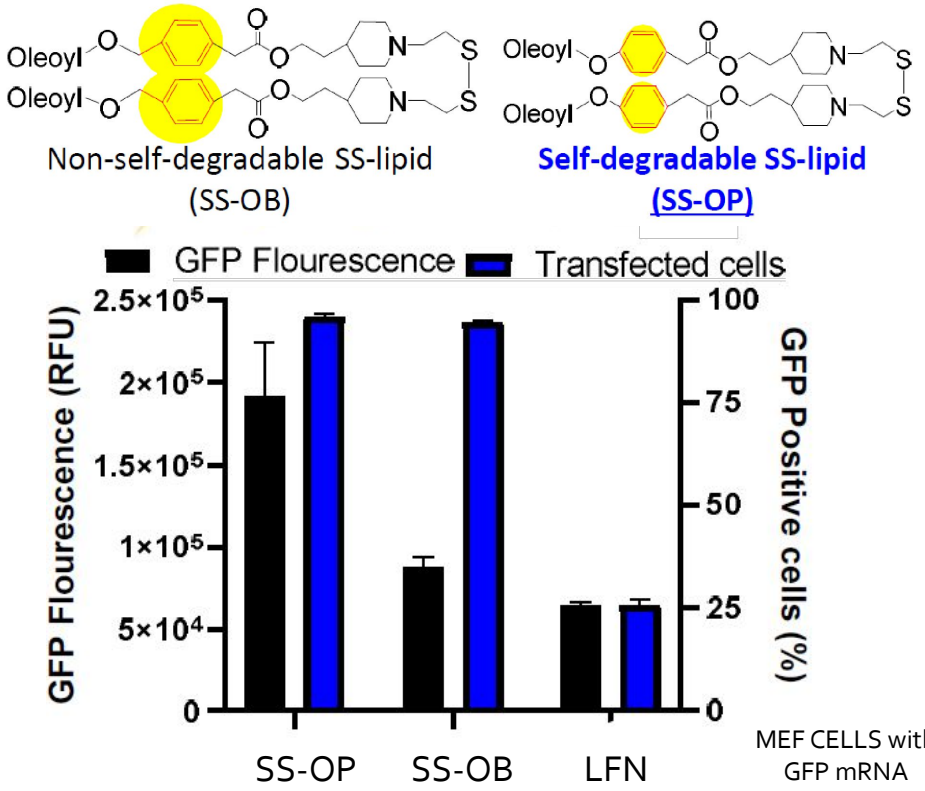
BIODEGRADABILITY = HIGH GENE EXPRESSION



Blue:Nucleus (Hoechst33342)
Green: Lipid membrane (NBD)
Red: pDNA (Rhodamine)

(L): pDNA in control liposomes remain bound to membrane (yellow)

(R): pDNA (red) delivered by COATSOME dissociates from endosome lipid (green).



NON BIODEGRADABLE LIPID TRAPS DNA AFTER ENDOSOMAL RELEASE

GENE EXPRESSION OF BIODEGRADABLE SS-OP IS HIGHER THAN MORE STABLE ANALOGS

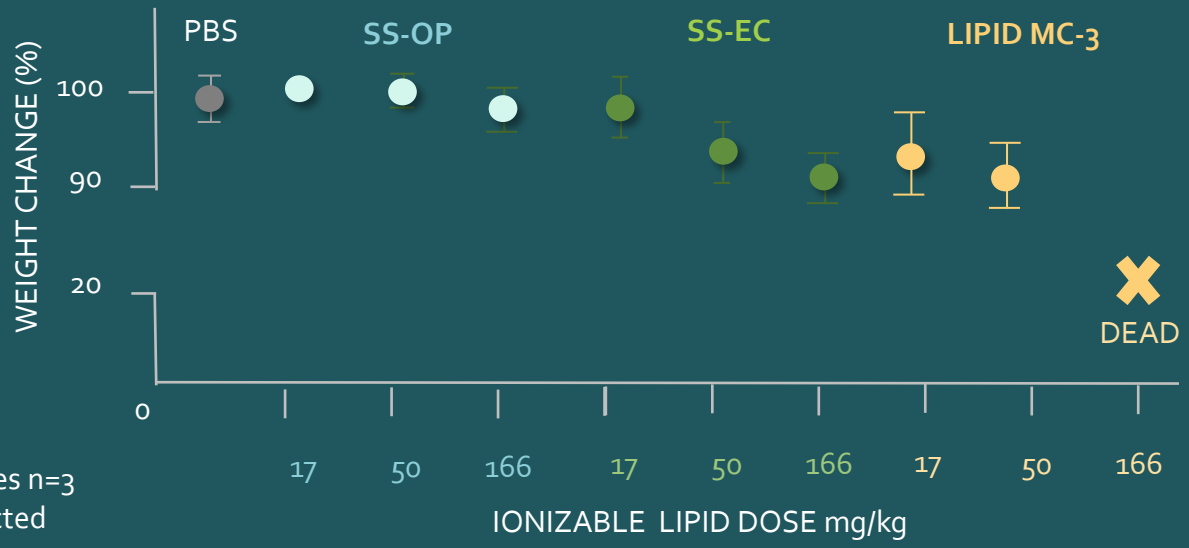
SAFETY AND TOXICITY STUDIES

SS LIPIDS HAVE LOW SYSTEMIC TOXICITY

LIPID COMPOSITION

70% LIPID
30% CHOL
3% DMG-PEG-2000

WEIGHT CHANGE
(24 HOURS AFTER SINGLE DOSE)



MICE

BALB/c 6wk females n=3
200 ul volume injected

SS-Lipids are well tolerated via systemic route

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COATSOME FORMULATIONS

COATSOME-HEPATIC

COATSOME-IVT

COATSOME-SPLEEN

COATSOME-VAX

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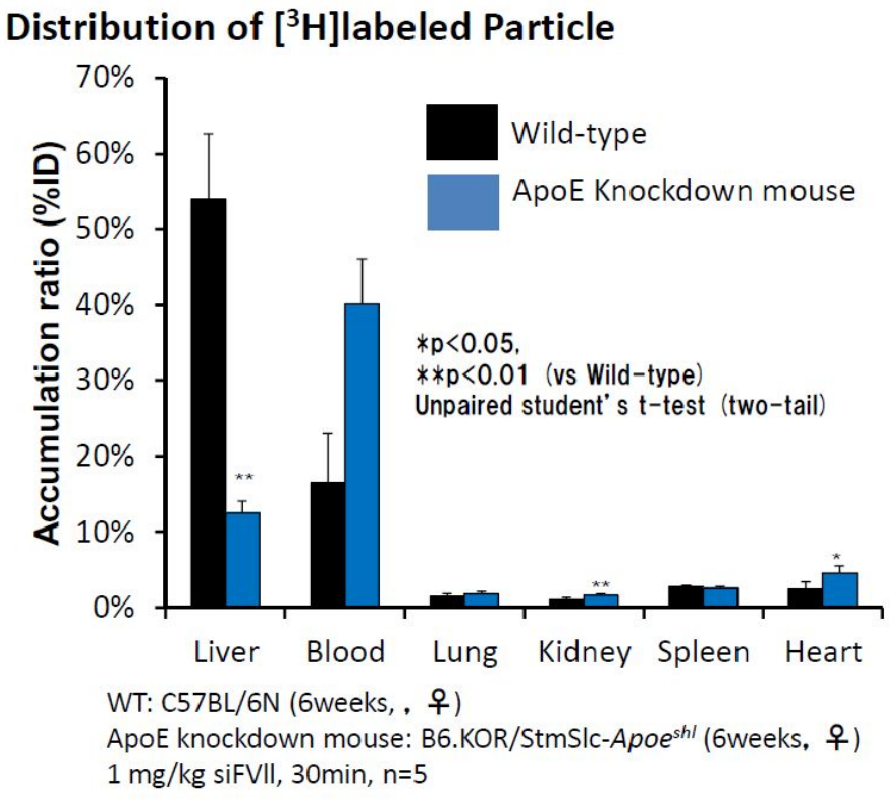
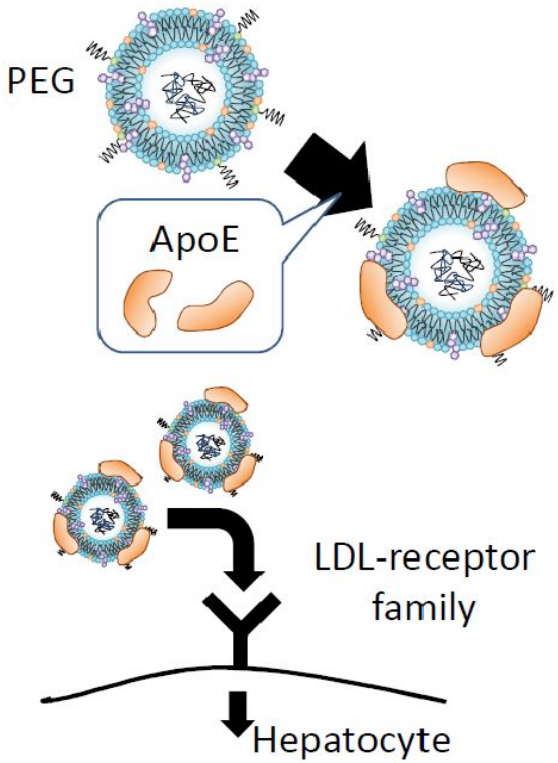
HEPATIC DELIVERY OF mRNA

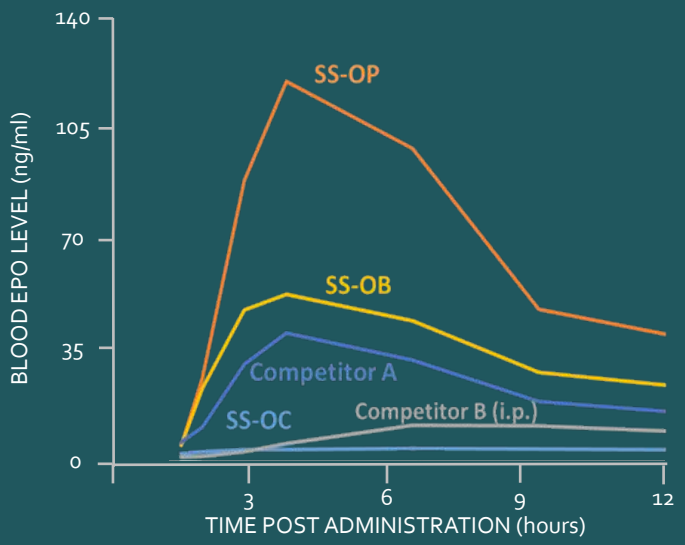
LDL RECEPTOR MEDIATED UPTAKE



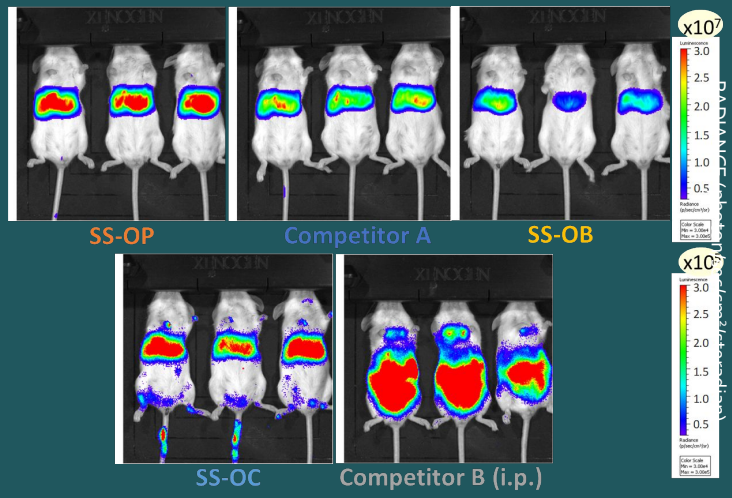


IN VIVO mRNA DELIVERY

ERYTHROPOEITIN mRNA



LUCIFERASE



Intravenously administered LNPs mainly introduced mRNA to liver. LNPs using SS-OP showed higher transgene activity than LNPs using conventional LNP.

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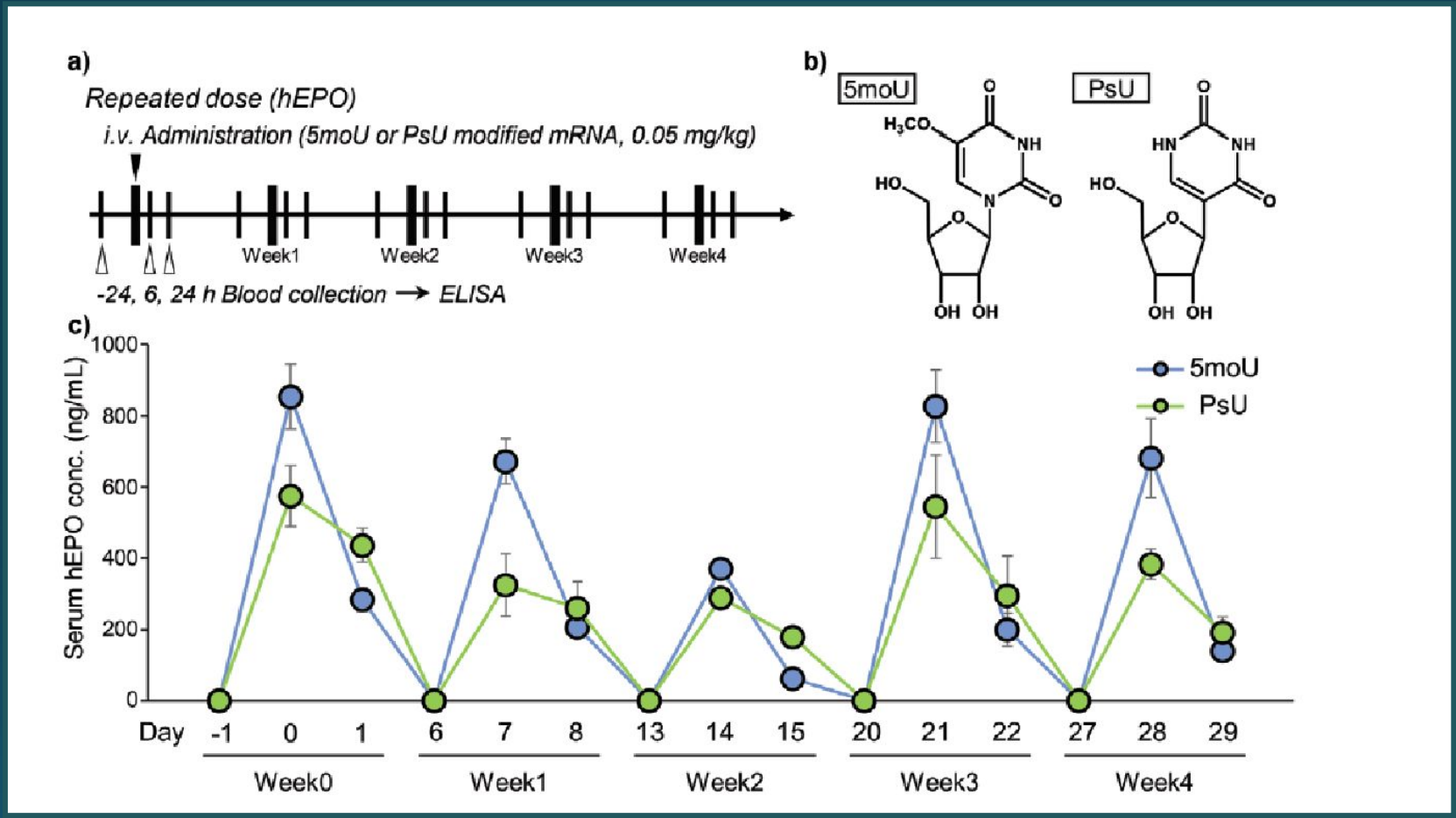
CONSISTENT GENE EXPRESSION WITH REPEAT DOSING

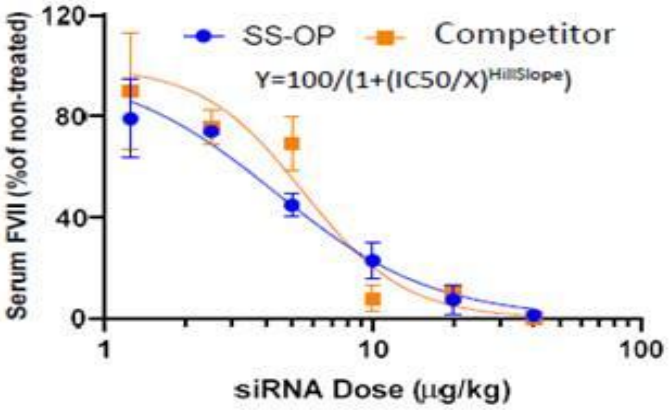


EFFICIENT siRNA DELIVERY TO LIVER

LNPs composition

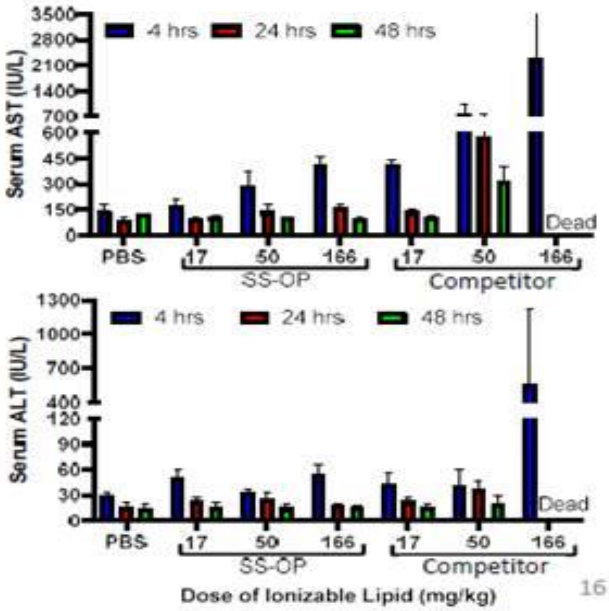
SS-OP/Chol/DMG-PEG2000 = 70/30/1.5 (mol%)
Competitor lipid/DSPC/Chol/DMG-PEG2000 = 50/10/38.5/1.5 (mol%)

Dose-Response curve (Hepatic FVII knockdown by I.V.)



Lipid	Diameter (nm)	PdI	ζ potential (mV)	IC50 (µg/kg)	Hill Slope
SS-OP	96	0,12	-2,4	4,3	-1,5
Competitor	62	0,08	-2,1	5,5	-2,3

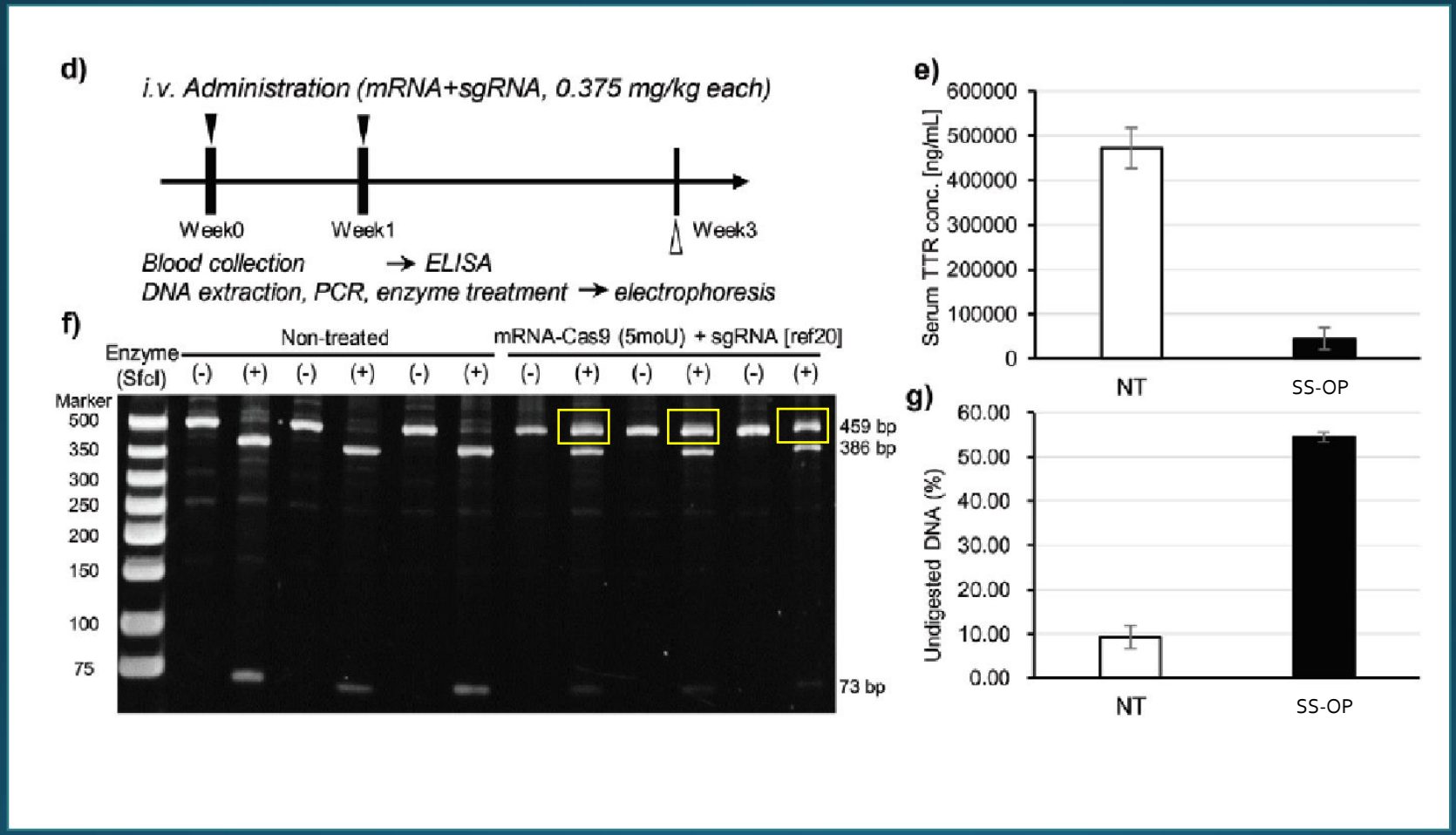
Hepatotoxicity



16

GENE EDITING

TTR GENE EDITING

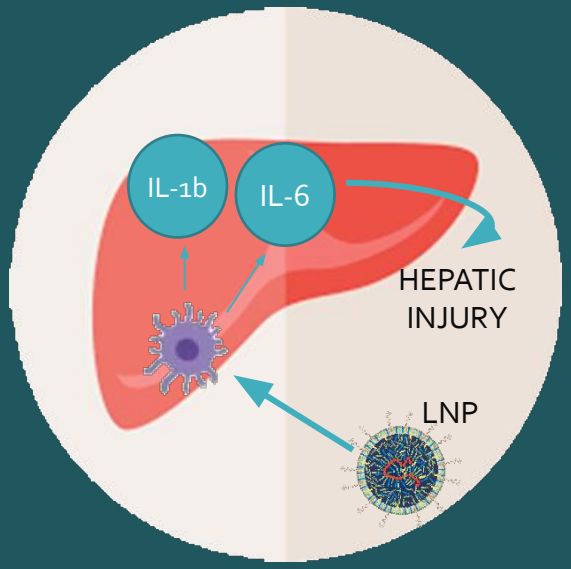


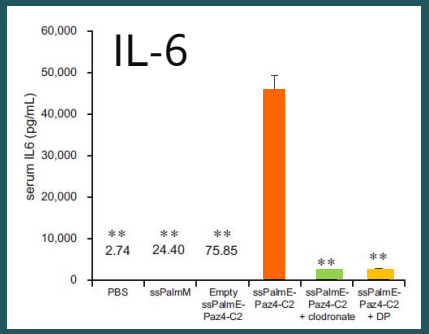
After two weeks 55% hepatocytes edited, >98% decrease in serum TTR

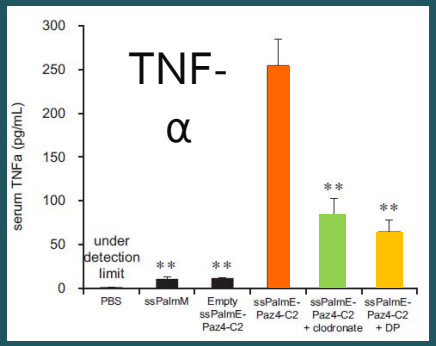
VACCINE

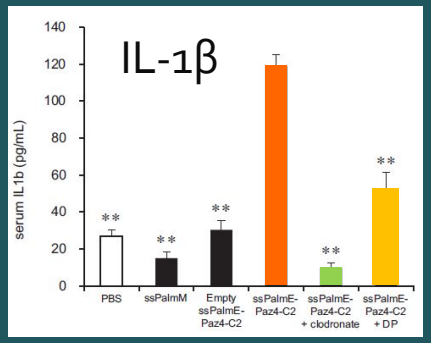
TOXICITY MEDIATED BY MACROPHAGES

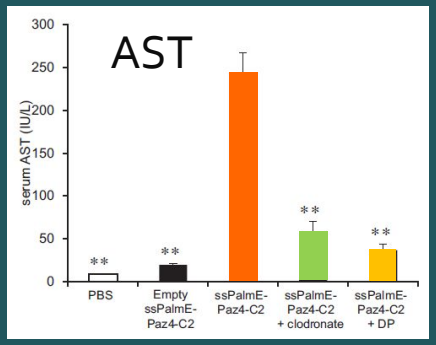
LNP+pDNA
LNP+pDNA+Clodronate
LNP+pDNA+Dexamethasone











LNP UPTAKE BY KUPFFER CELLS CAUSES HEPATIC INJURY

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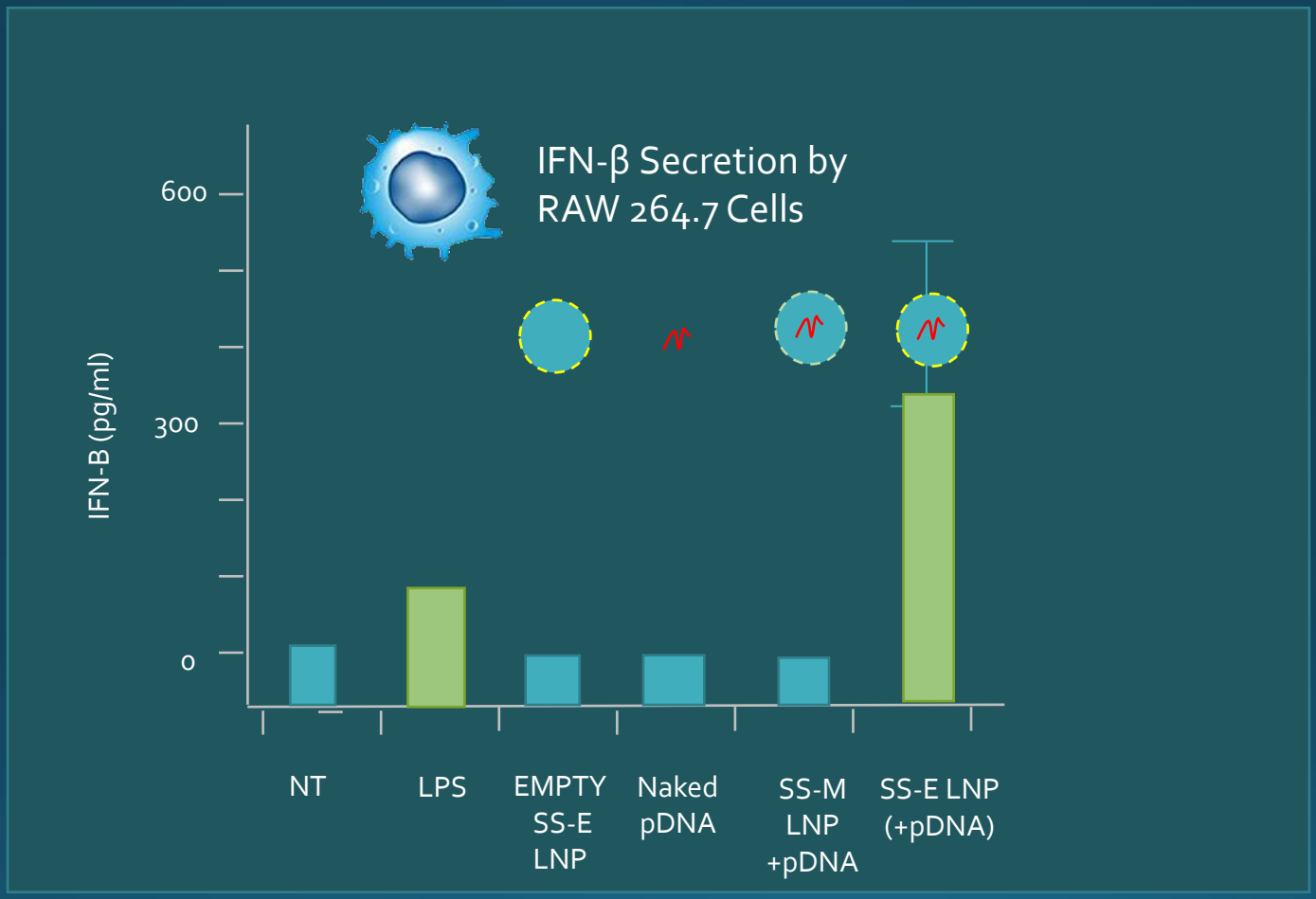
SCREENING OF IMMUNOGENIC LIPIDS



Only combination of SS-E and DNA induces macrophage activation

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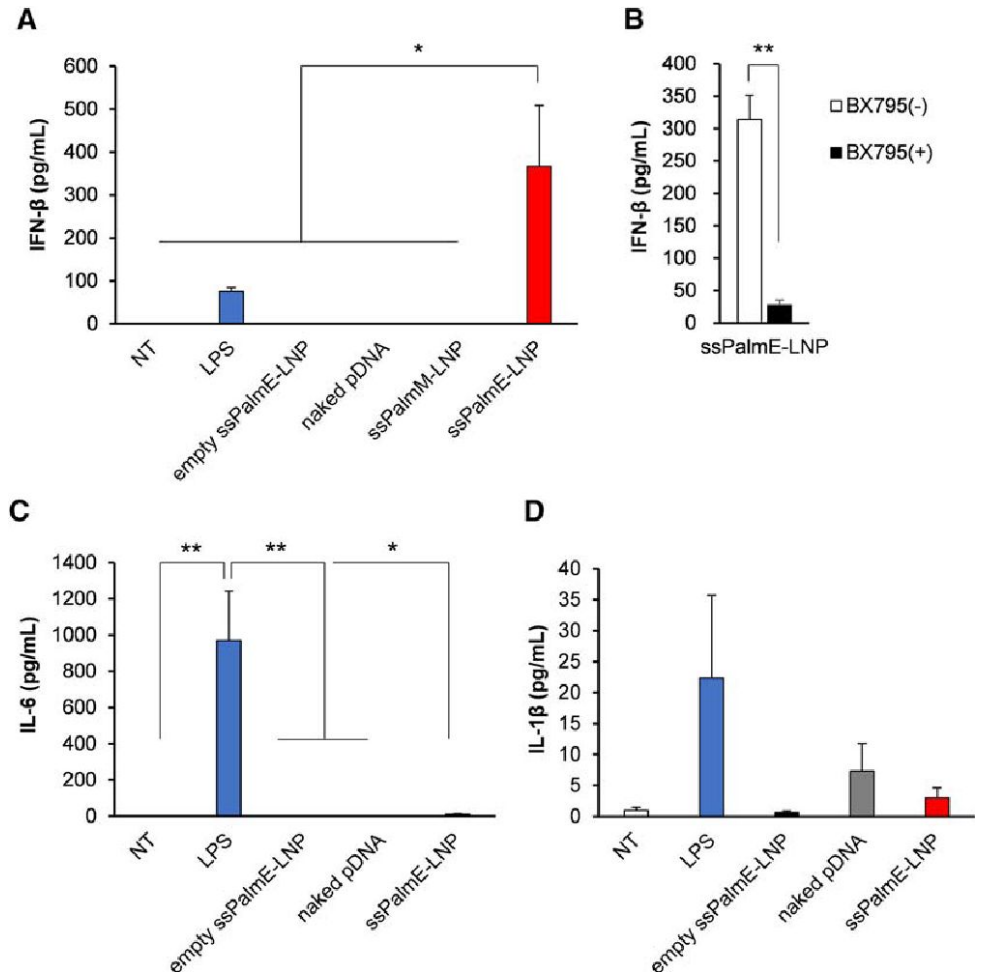
SS-E SPECIFICALLY INDUCES IFN-β



SS-E induces IFN-B but not other broad inflammatory cytokines.

IFN-B stimulation is blocked by TLR/STING inhibitor.

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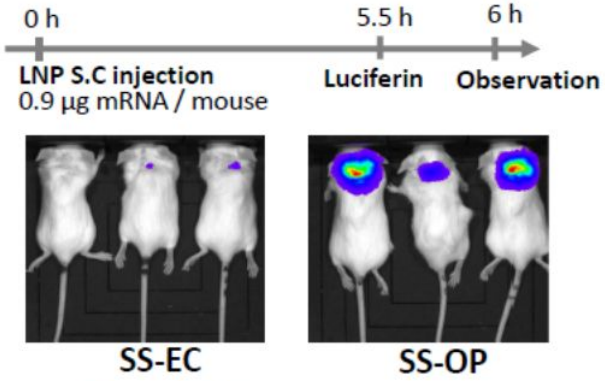
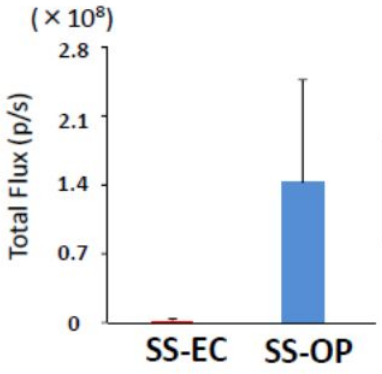
HIGH ANTIGEN EXPRESSION DOES NOT CORRELATE WITH IMMUNE RESPONSE

LNPs composition

SS-lipid / DOPE / Chol / DMG-PEG2000 = 60/30/10/3 (mol%)

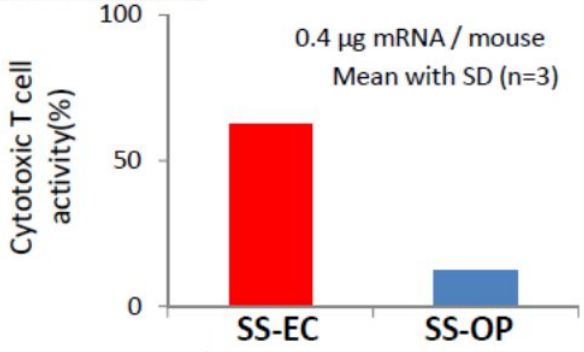
Comparison of Luciferase expression by SC injection





Luciferase expression
SS-EC << SS-OP

Comparison of CTL activity



CTL activity
SS-EC > SS-OP

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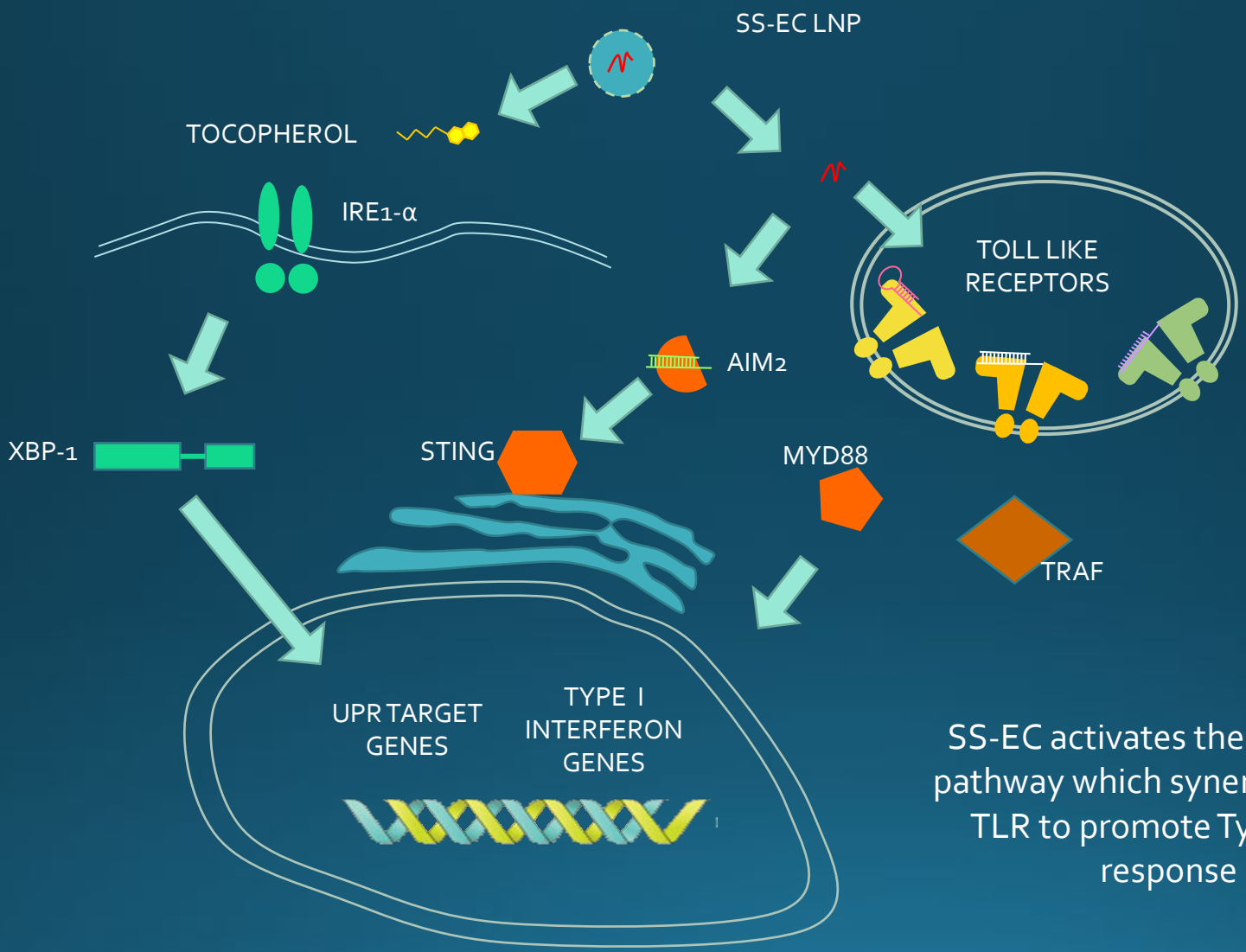
SS-EC LNP MECHANISM OF ACTION



SS-EC activates the ER stress pathway which synergizes with TLR to promote Type I IFN response

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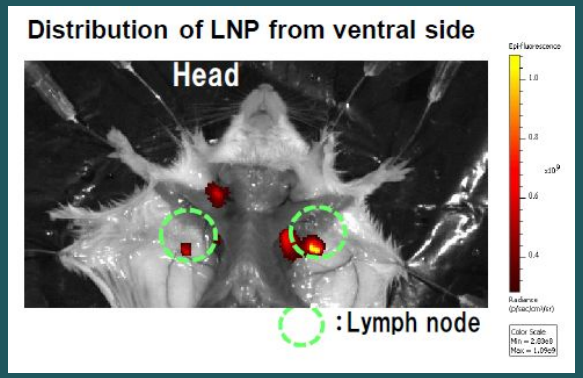
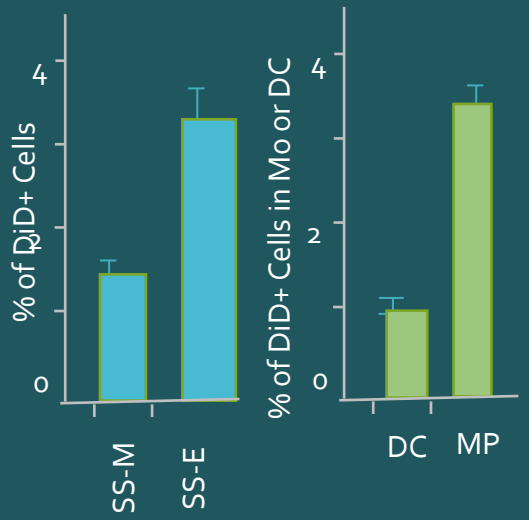
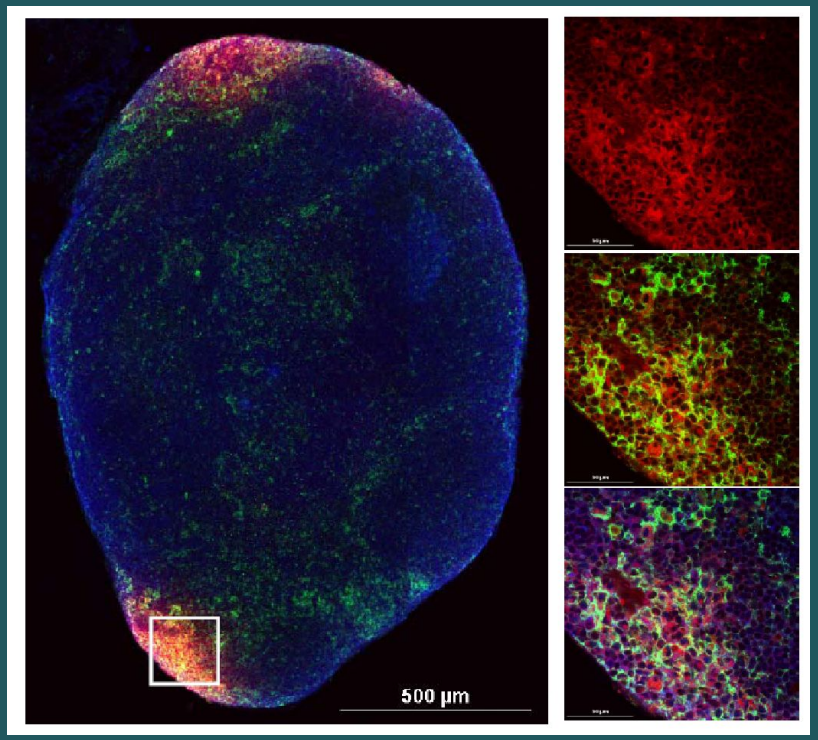
SS-E DELIVERY TO LYMPH NODES



C57Bl/6J mice were injected with DiD labeled LNP in both flanks. Inguinal lymph nodes were harvested at 24 hours and quantified by FACS.
DC= CD11b+ F4/80-, Macrophage= CD11b+ F4/80+





F4/80+ (Green), CD11b (blue), DiD (red)

SC Injected SS-E LNP Migrates To Lymph Node Macrophages

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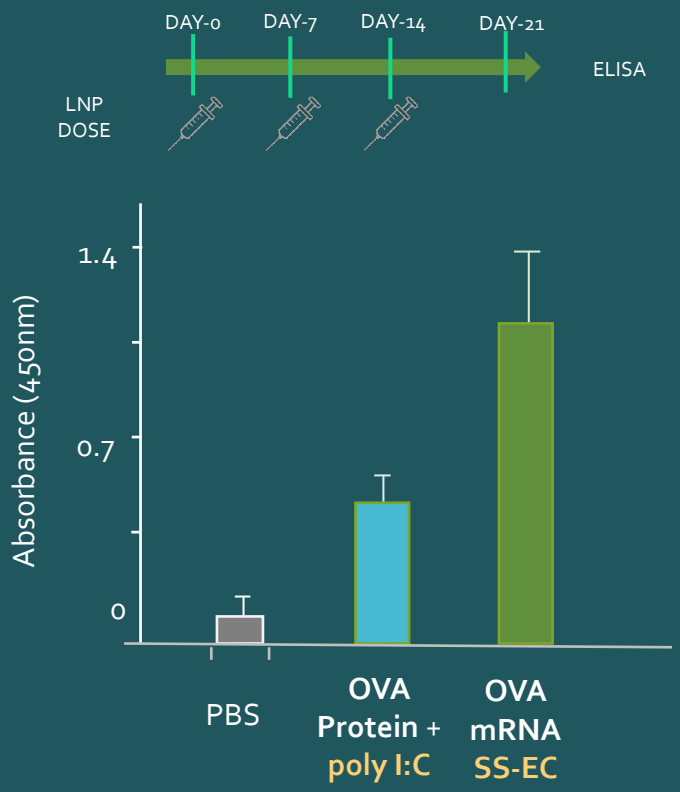
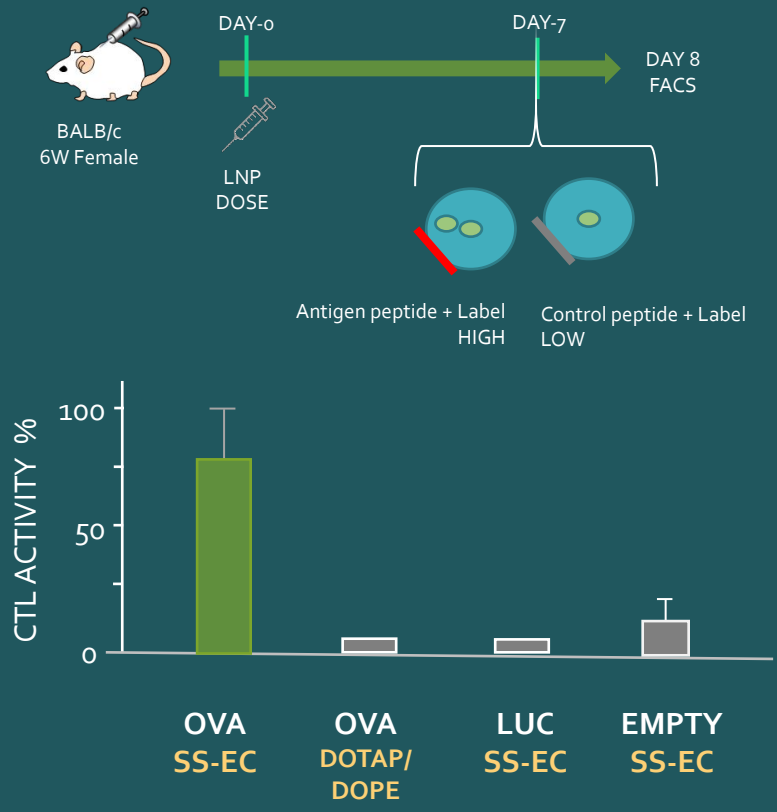
HUMORAL AND CELLULAR IMMUNITY

IgG ASSAY



CTL ASSAY



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COMPARISON OF ANTIBODY RESPONSE BY DIFFERENT ADMINISTRATION ROUTE

LNP composition

SS-EC/DOPE/Chol/DMG-PEG2000=60/30/10/3 (mol%)

(Lipid/mRNA=131.5nmol/μg)

Particle property

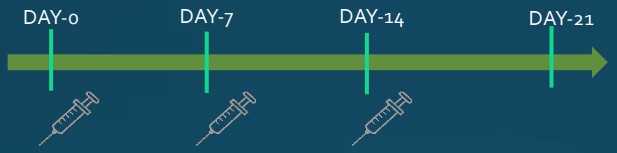
size: 69nm (PdI: 0.17)
Zeta potential: -2mV
Encapsulation ratio: 98%
Recovery ratio: 100%

Route of administration

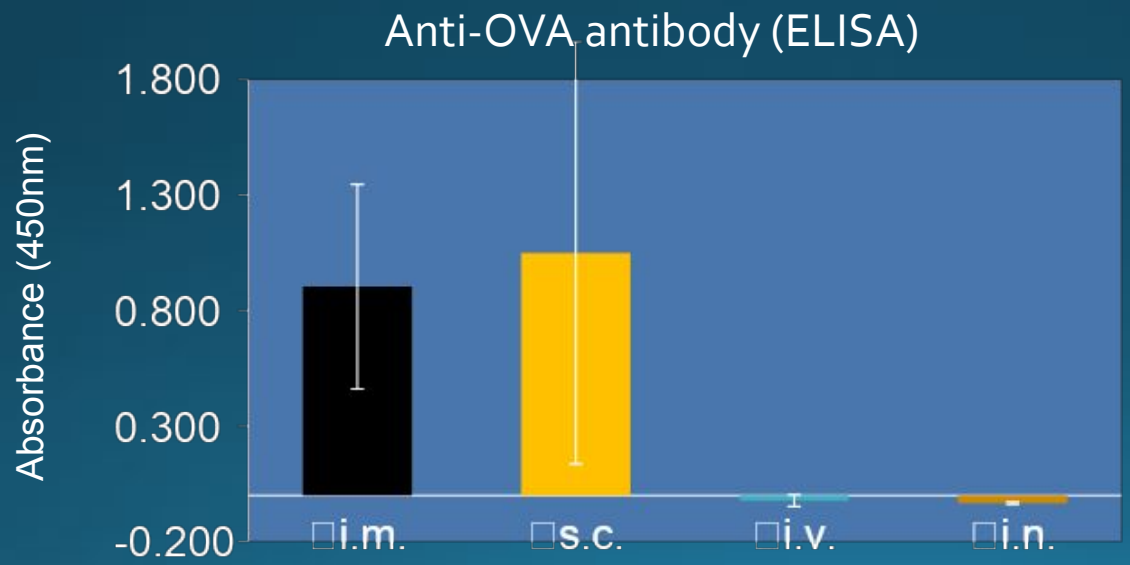
① i.m. (hind thigh muscle)
② s.c. (neck back)
③ i.v. (tail vein)
④ i.n. (nose)

Mice: BALB/c 6w ♀ (n=3)
OVA-mRNA 1.5μg (50μL)

LNP DOSE



ELISA
10,000 fold dilution of serum



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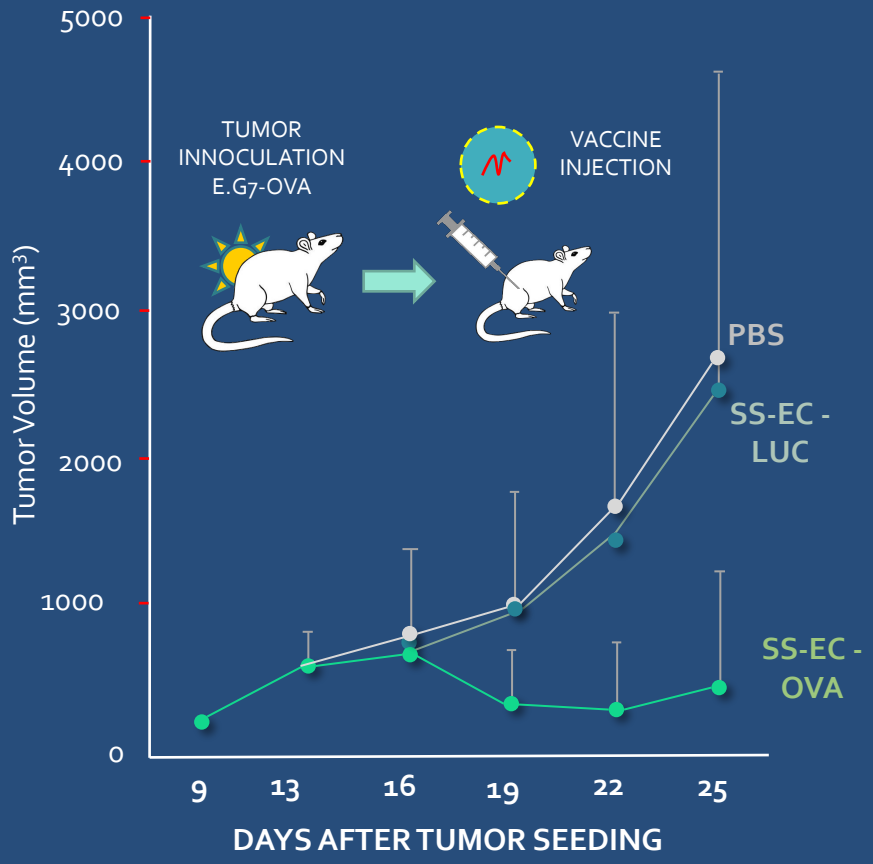
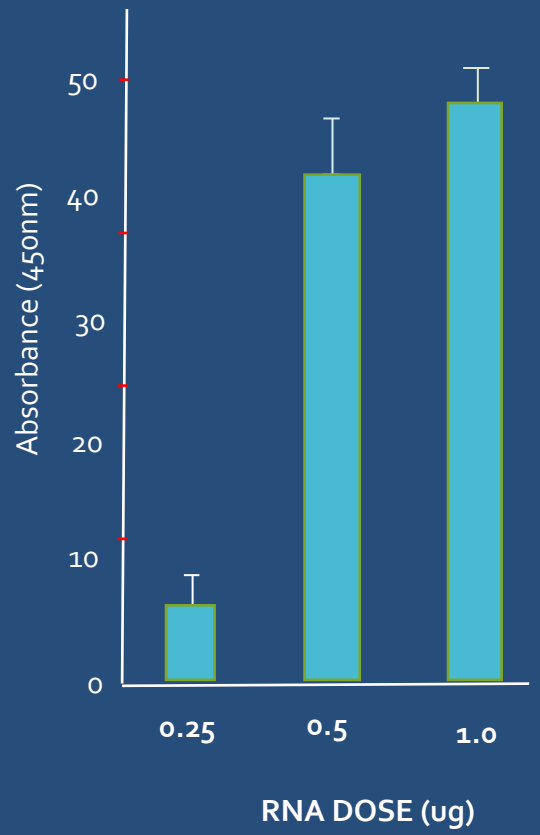
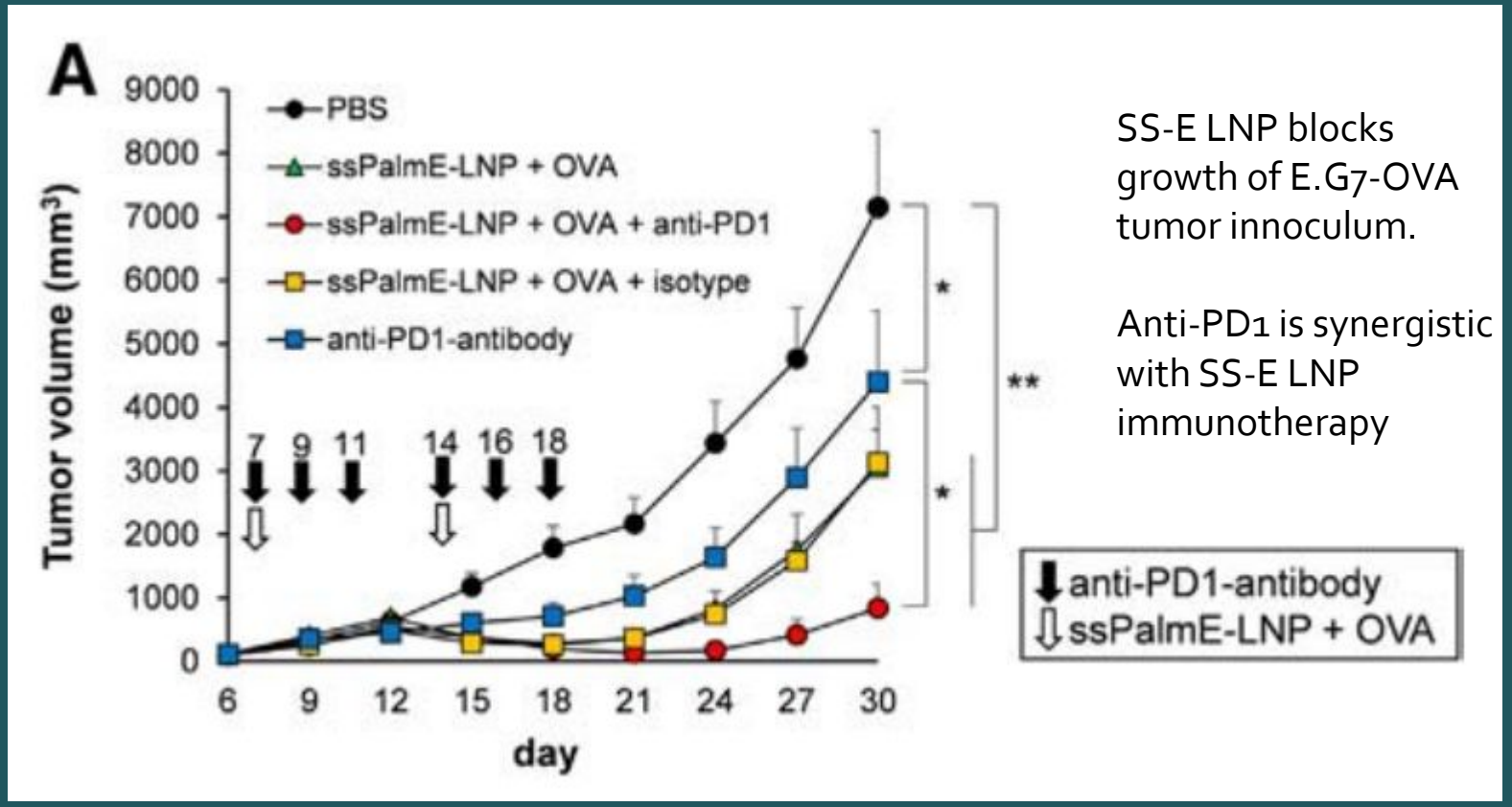
TUMOR VACCINE MODEL





NOF CORPORATION

TUMOR VACCINE MODEL



SS-E LNP blocks growth of E.G7-OVA tumor innoculum.

Anti-PD1 is synergistic with SS-E LNP immunotherapy

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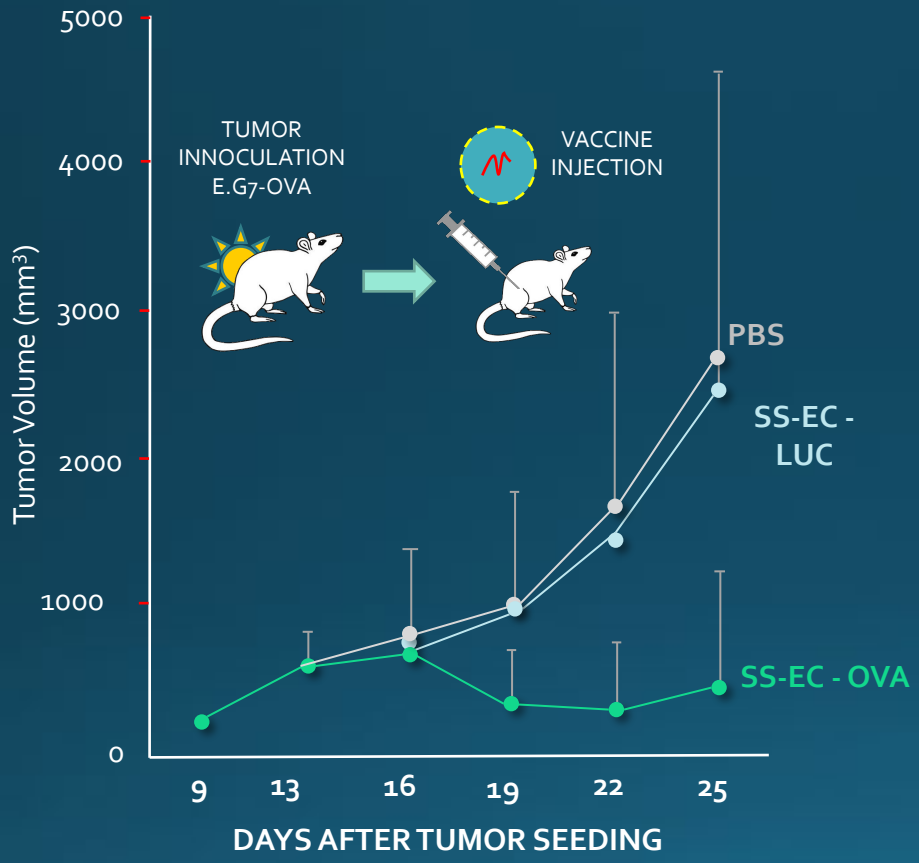
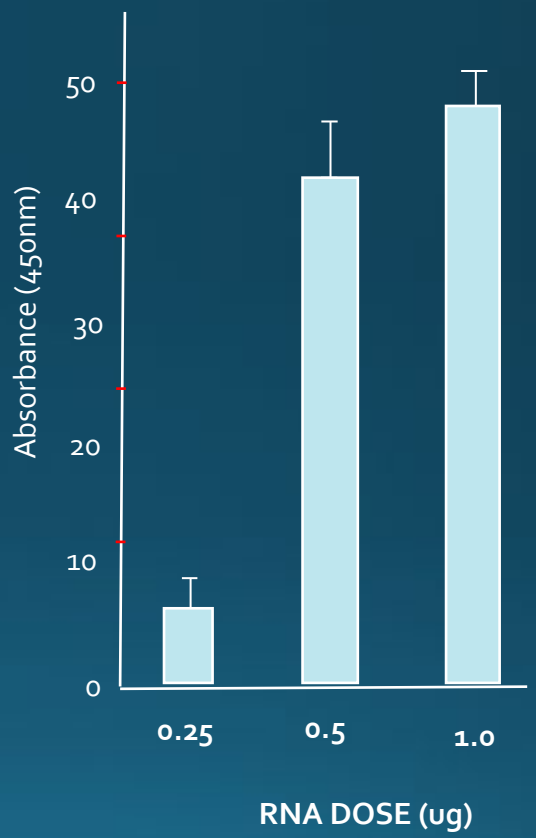
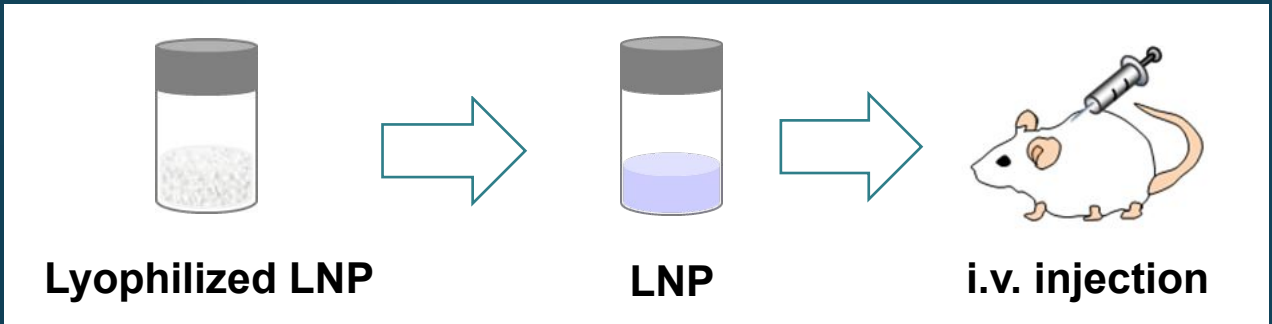
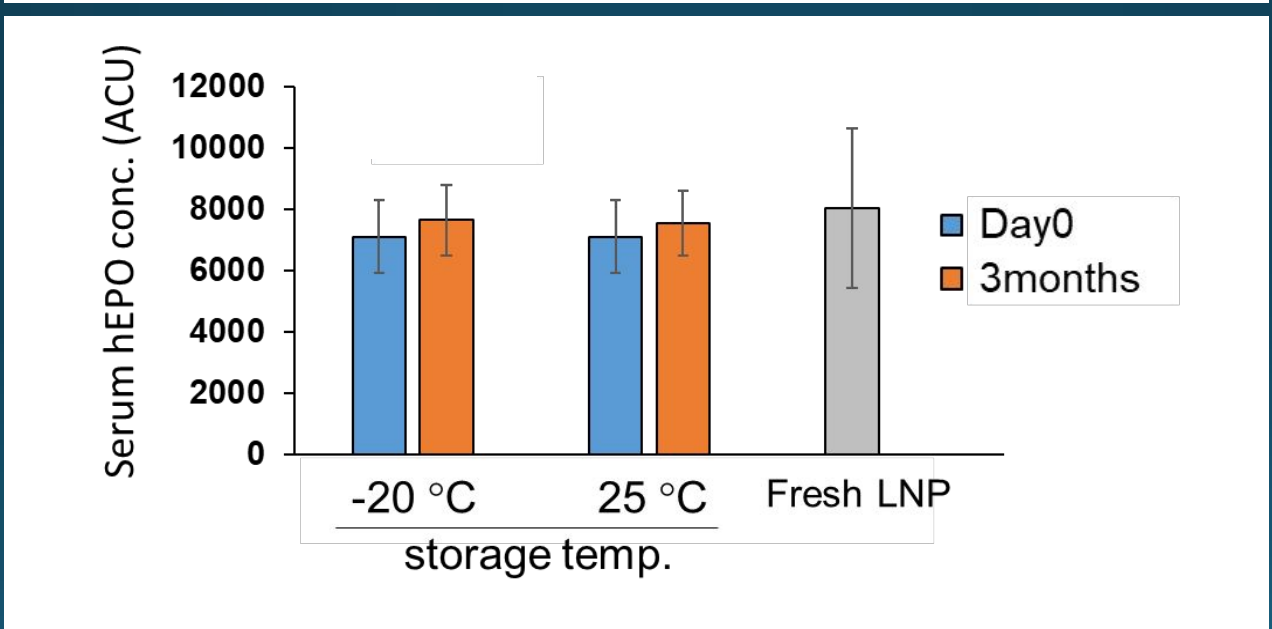
TUMOR VACCINE MODEL





ROOM TEMPERATURE STABLE mRNA LNP





The gene expression activity in vivo of the lyophilized LNP was comparable to that of the fresh LNP across all storage conditions.

BENEFITS OF NOF PARTNERSHIP

- Protocol for Vortex mixing method for initial screening
- Protocol for NanoAssemblr™ for process development stage
- Analytical Methods
- Stability and solubility data on neat lipids
- Method for Lyophilized Drug Product (stability ongoing)
- Scale up and cGMP Manufacturing of Lipids
- Regular updates from our preclinical program (metabolism study, anti-drug antibody assays, rodent and NHP studies)

Thank you!

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www.nofamerica.com