Peptide alternative to PEG in Lipid Nanoparticles: Preliminary data

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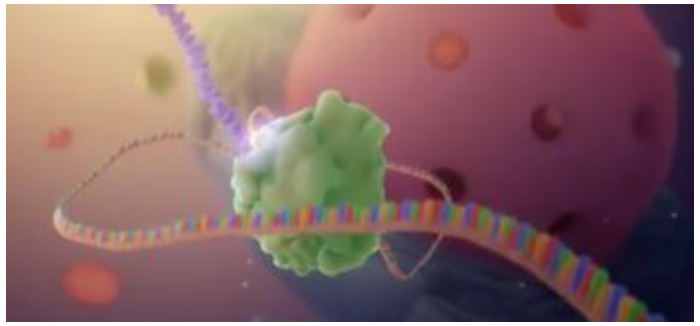
Company highlights



Arcturus is a Commercial-Stage mRNA Vaccines and Medicines Company

Publicly Traded (Nasdaq: ARCT)

Headquarters: San Diego, CA

Number of Employees: 161

Founded: 2013

Promising Therapeutic Candidates

LUNAR-COV19 (COVID-19 Vaccine Approved in Japan)

LUNAR-OTC (Ornithine Transcarbamylase Deficiency- Phase 2)

LUNAR-CF (Cystic Fibrosis – Phase 1b)

LUNAR-FLU – Preclinical

Additional Earlier Stage Programs

Arcturus Technologies Validated by Multiple Strategic Partners

Johnson & Johnson

CYSTIC FIBROSIS FOUNDATION

DukeNUS Medical School

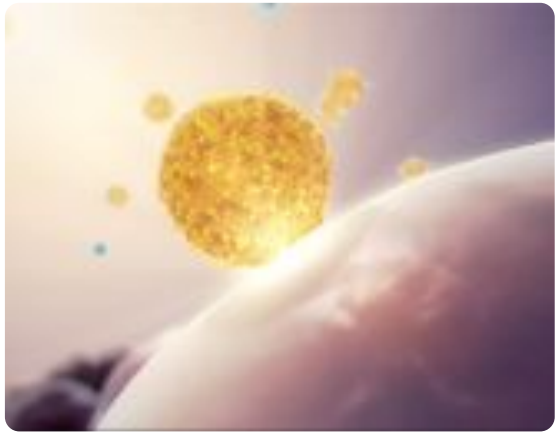
Takeda

ultragenyx pharmaceutical

LUNAR® Delivery Technology

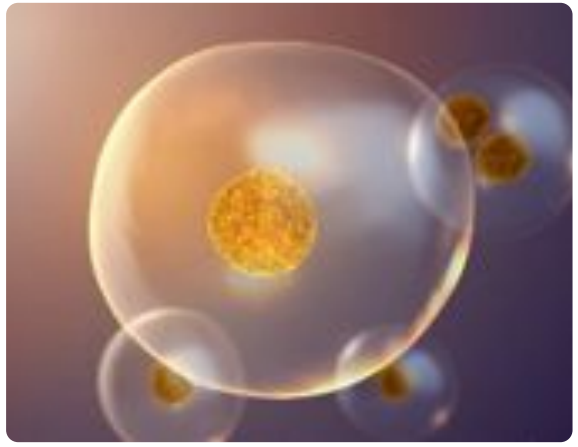
Biodegradable, highly optimized for each cell type

LUNAR® binds to Cell Membrane



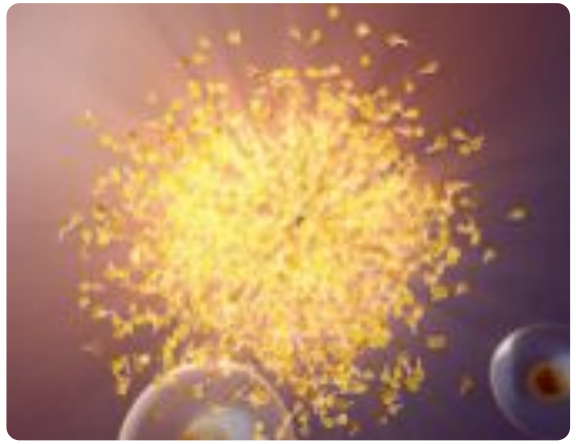
Transfection

LUNAR® packaged inside Cell



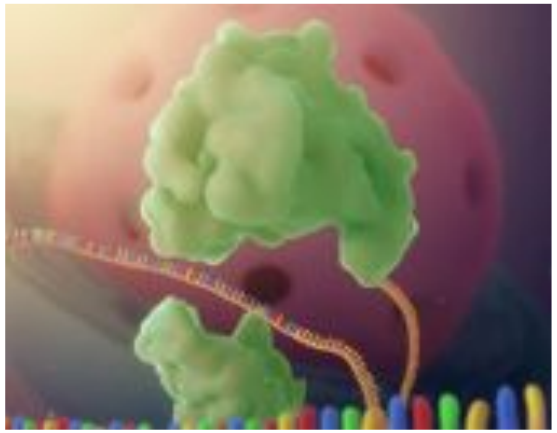
Endosome

mRNA release



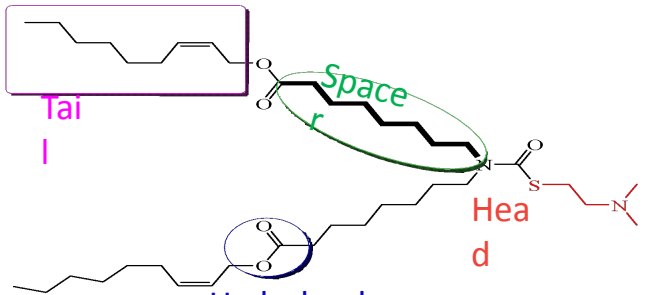
Aged acidic Endosome rupture

mRNA translates into protein

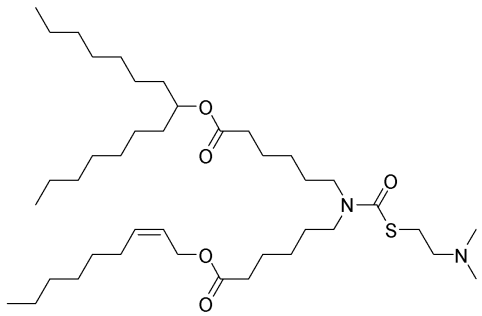


STARR™ / mRNA Technology

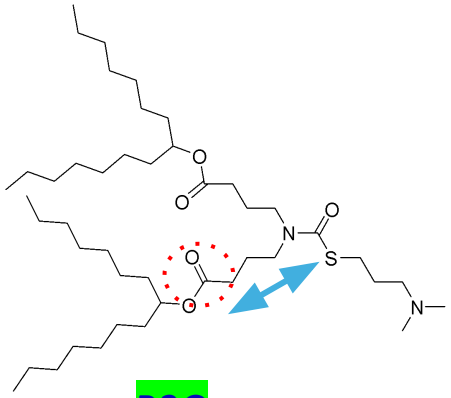
Generations of ATX-lipids to improve potency



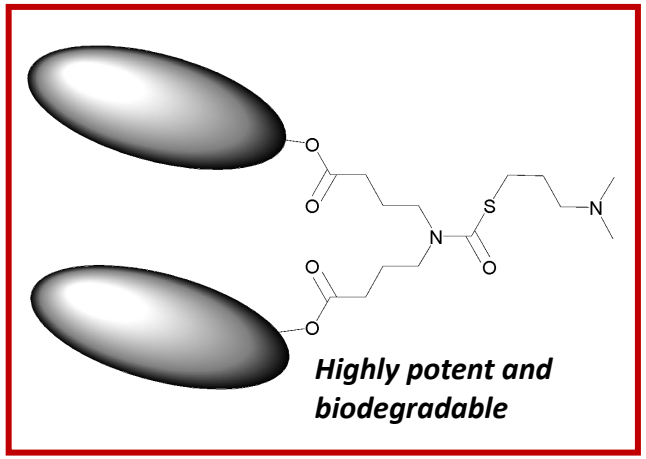
A0C



A9F



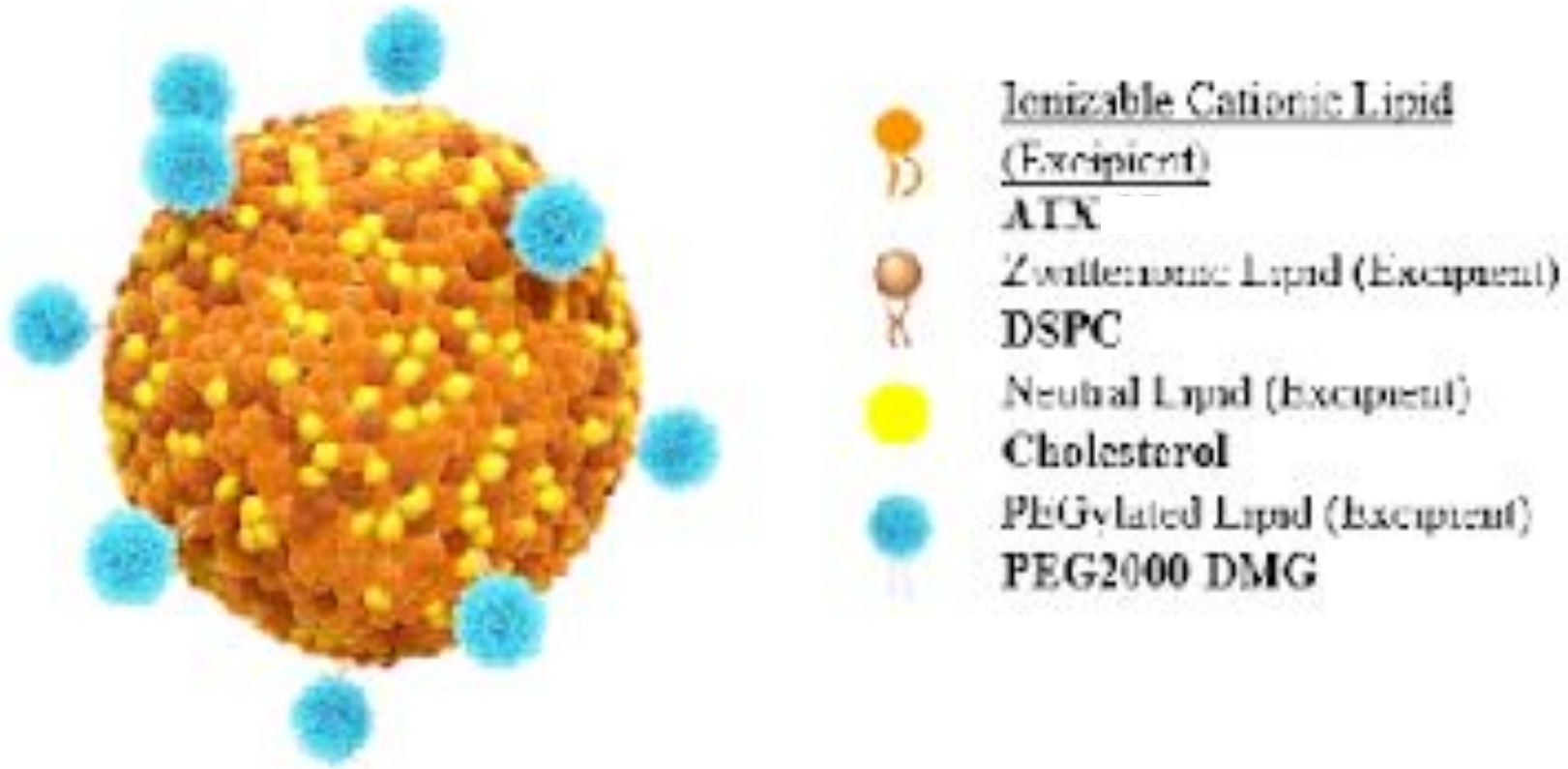
B2G



Note: Not quantitative, only shows trend

- Arcturus has >300 proprietary lipids in the inventory: Some with high potency and bio-degradability
- All LNP formulations (LUNAR) has DMG-PEG as a common component, albeit in low mol%

Role of PEG lipid in lipid nanoparticles (LNPs)



- Provides a hydrophilic steric barrier that prevents aggregation
- Inhibits recognition by the RES system
- Choice of lipid anchor dictates residence time of PEG lipid in the LNP
- PEG may not be the optimal LNP shield : other approaches are needed

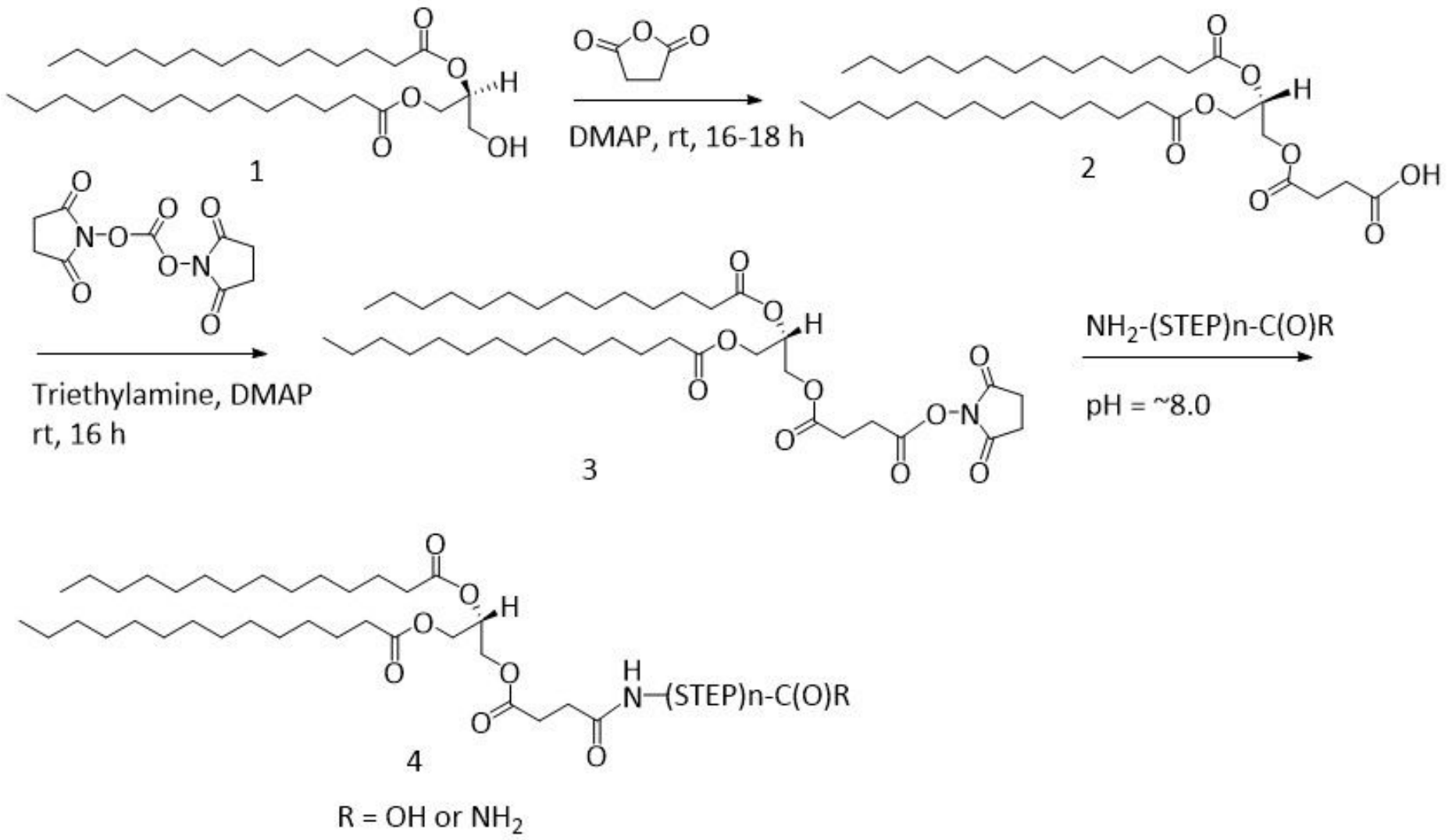
Rationale for alternatives to PEG

- Multiple reports have shown that peptides such as polysarcosine, XTEN, and zwitterionic polypeptides can be used as surrogates for PEG in enzyme replacement therapy
- Incorporation of a zwitterionic motif into the repeat unit of an intrinsically disordered peptide polymer can increase the plasma circulation of the polypeptide
- A functional PEG ‘analogues’ may be tunable in terms of its hydrophilicity, molecular weight, and ability to conjugate with wide varieties of diacylglycerols
- We selected a Serine-Threonine-Glutamic acid-Proline as zwitterionic motif and made 4or 5 units polymers that has approximately the same molecular weight as PEG2K and conjugated these to dimyristoyl glycerol to create DMG-PEG2K analogs
- Such tetrapeptides have a serine, that can be left free or masked, and an amino acid with an additional carboxylic acid group, such as a glutamic acid, that can be kept free or converted to its amide form (glutamine) so that overall hydrophilicity and terminal charges can be modulated as needed

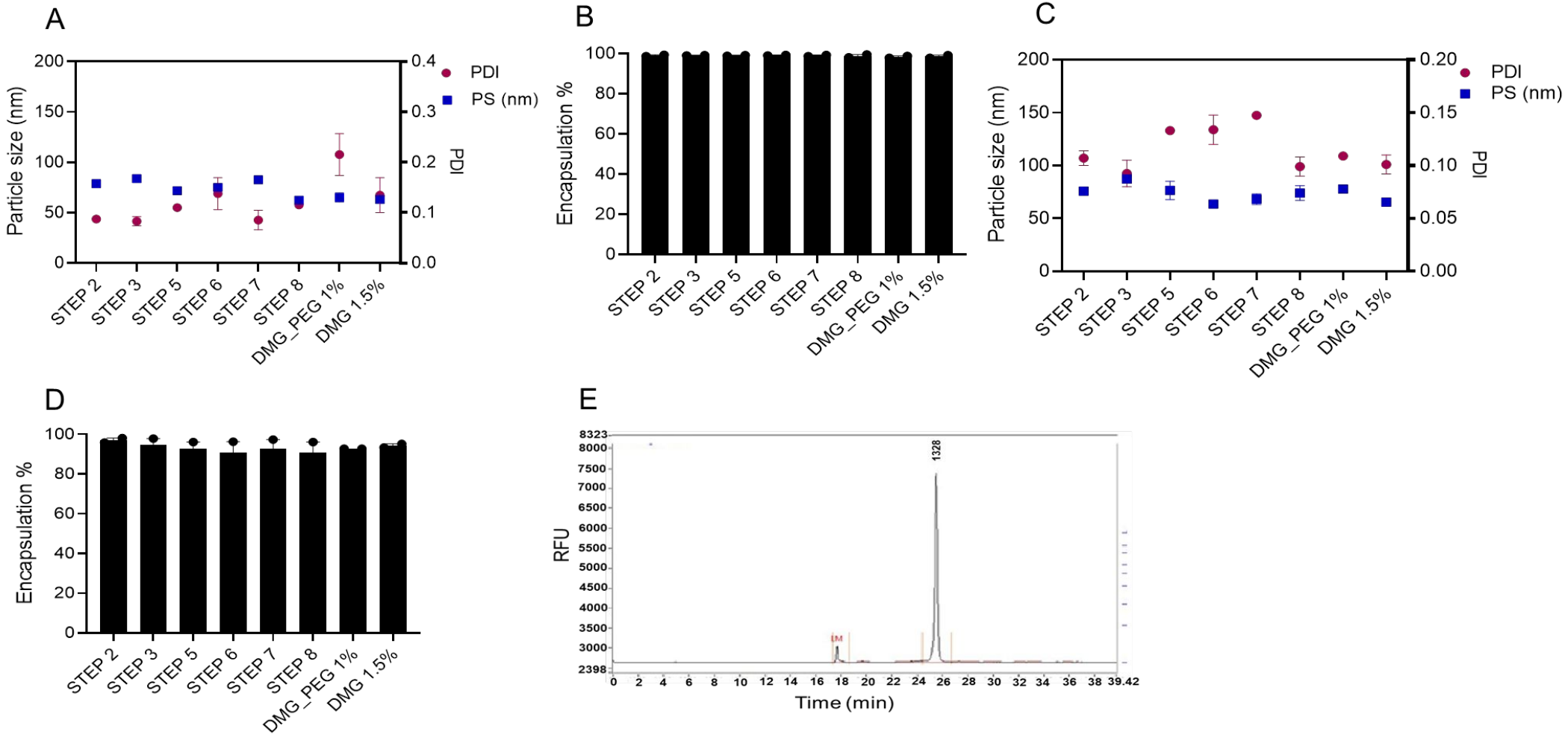
Selected peptides to test the DMG-STEP hypothesis

Peptide ID	Paptide Motif	Number of STEP units	Molecular Weight	Unique attributes
STEP 2	X-STEP-STEP-STEP-STEP-OH	4	2270.13	Simple 4 STEP unit with MW < 2509, the MW of PEG-DMG
STEP 3	X-STEP-STEP-STEP-STEP-STEP-OH	5	2684.55	Five STEP units with MW>2509
STEP 5	X-S(Me)T(Me)QP-S(Me)T(Me)QP-S(Me)T(Me)QP-S(Me)T(Me)QP-OH	4	2378.21	STEP 2 with S andT hydroxyls masked and E to Q to block H-bonding
STEP 6	(Me)T(Me)QP-S(Me)T(Me)QP-S(Me)T(Me)QP-S(Me)T(Me)QP-S(Me)T(Me)QP-O	5	2819.65	STEP 3 with S and T hydoxyls masked and E to Q to block H-bonding
STEP 7	X-STEP-STEP-STEP-STEP*-NH2	4	2269.14	STEP 2 with terminal proline carboxylic acid converted to an amide
STEP 8	X-S(Me)T(Me)QP-S(Me)T(Me)QP-S(Me)T(Me)QP-S(Me)T(Me)QP-NH2	4	2377.22	STEP 5 with terminal proline carboxylic acid converted to an amide

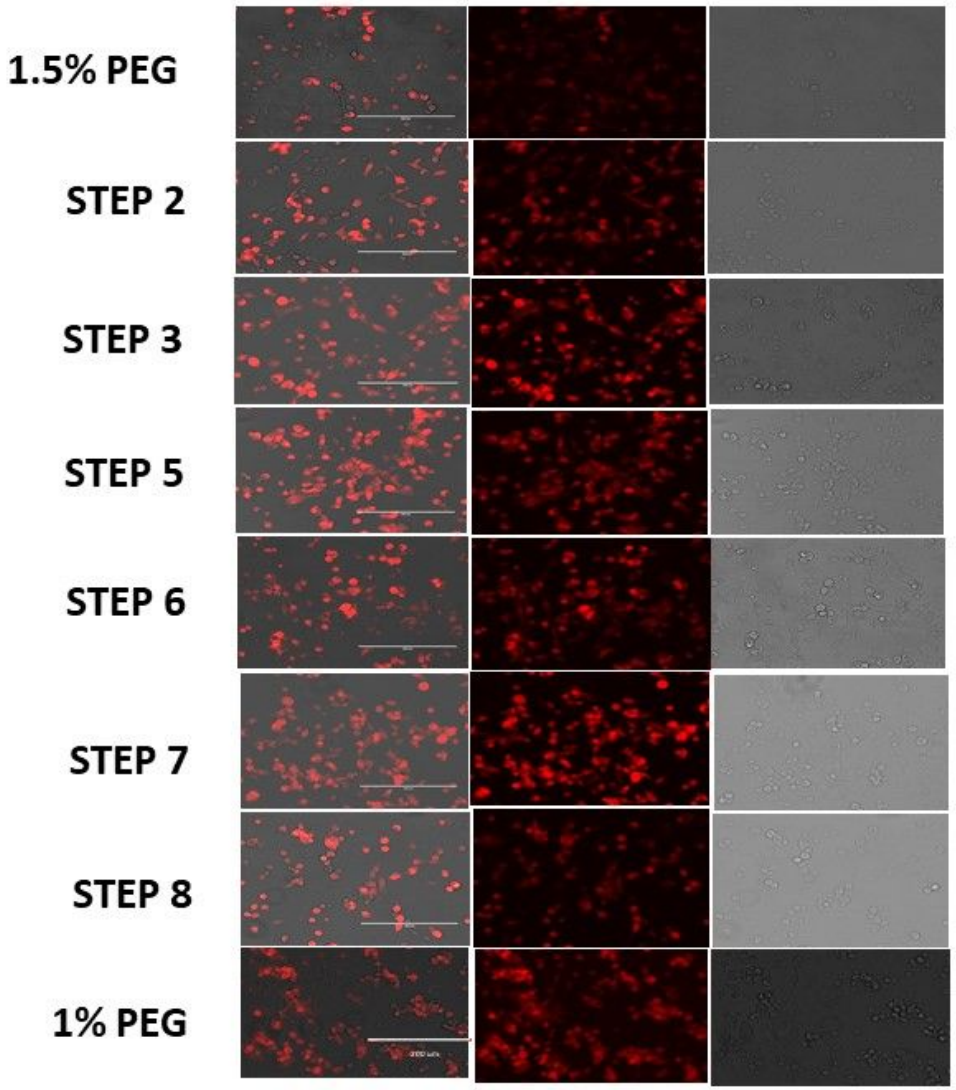
Synthesis of DMG-STEP



STEP-LNP particle characteristics



STEP-LNP mRNA delivery comparison with PEG-LNP

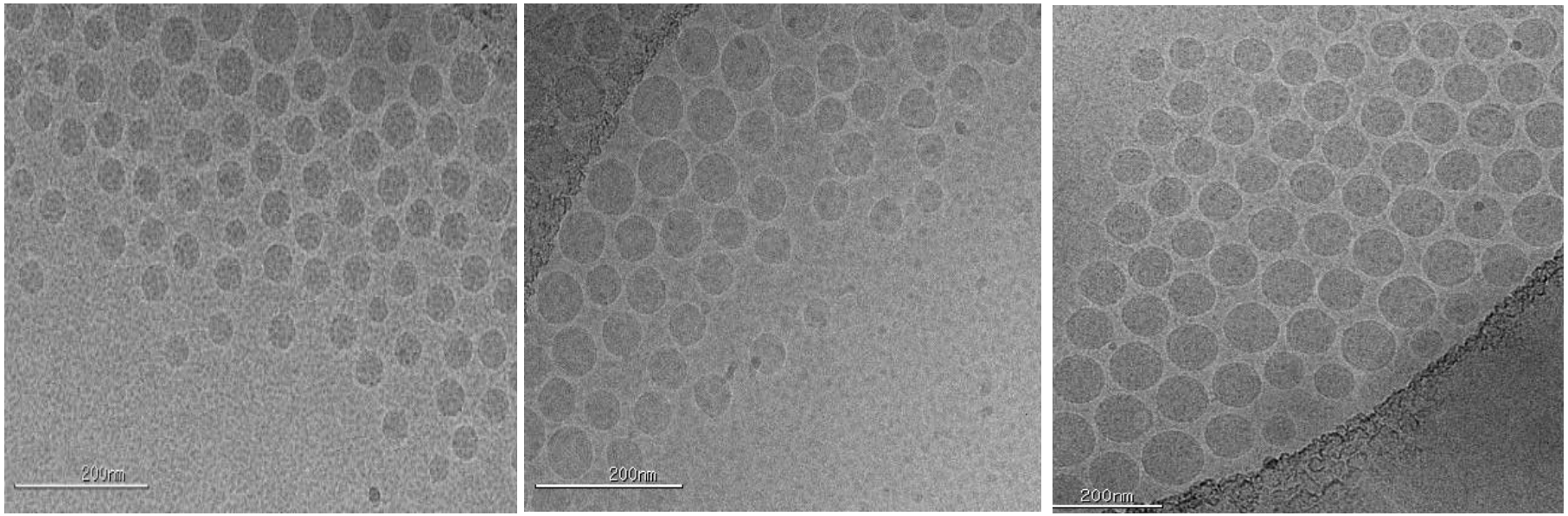


Cryo-TEM of STEP-LNP particles

A PEG-LNP

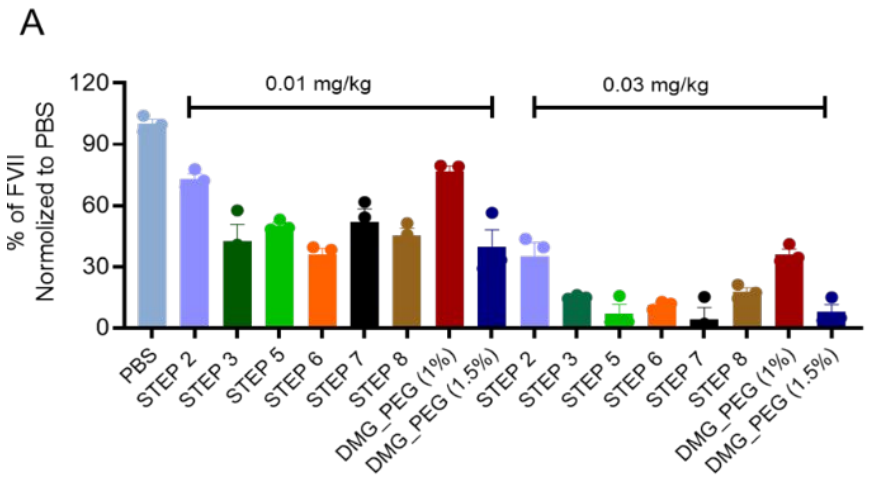
B STEP2-LNP

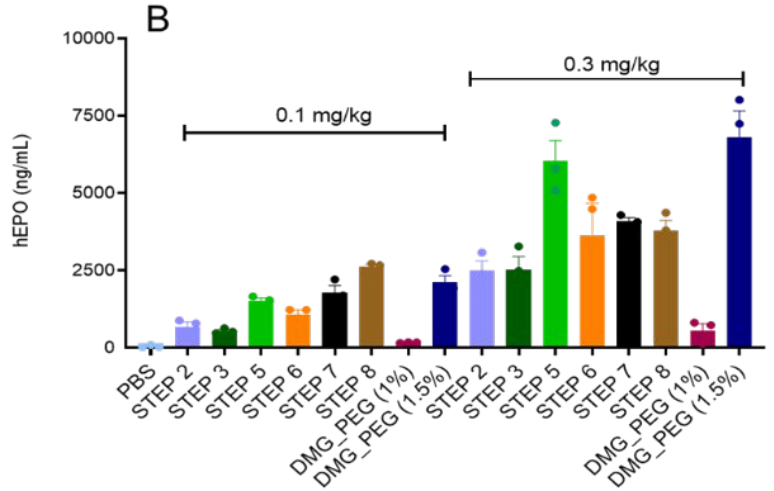
C STEP5-LNP

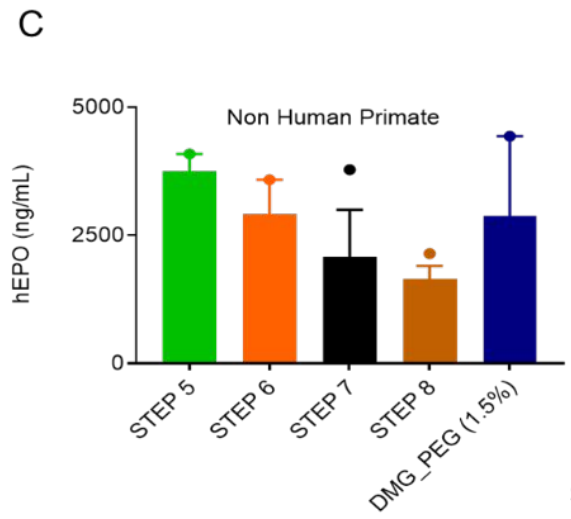


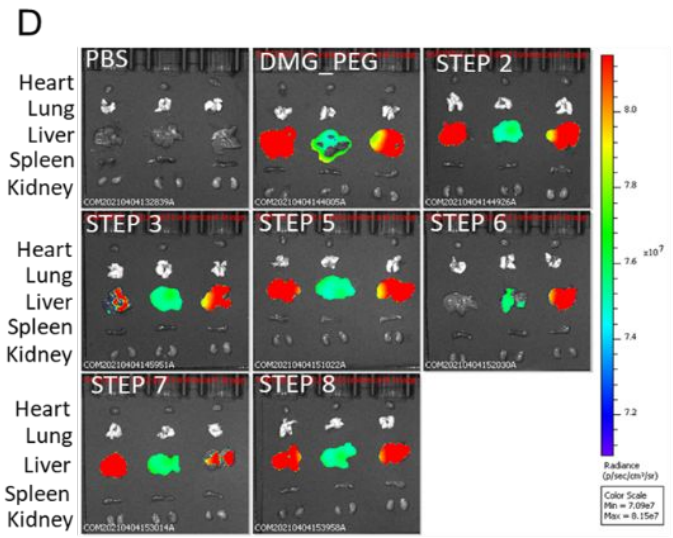
Scale bar 200 nm

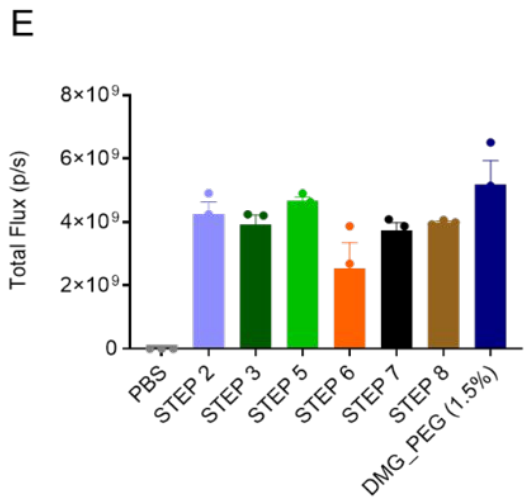
Potencies of STEP-LNPs: FVII KD and hEPO expression



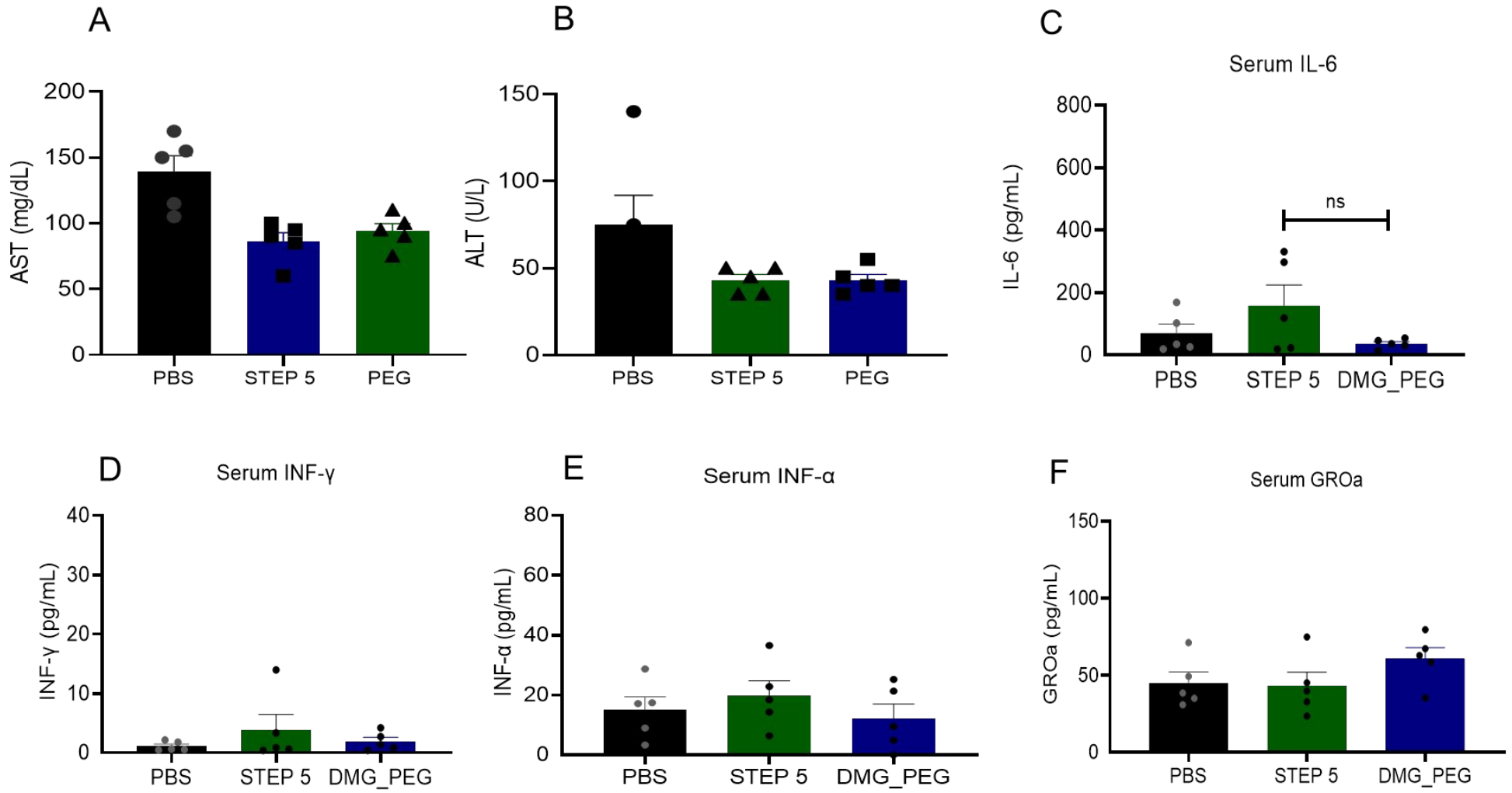




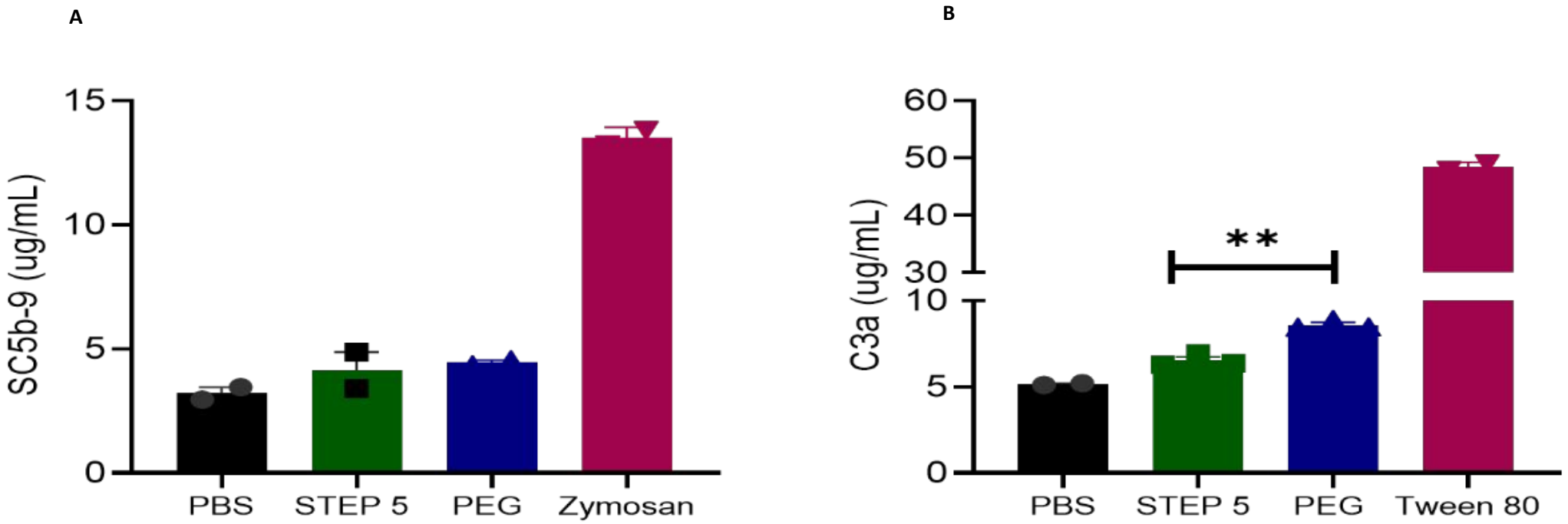




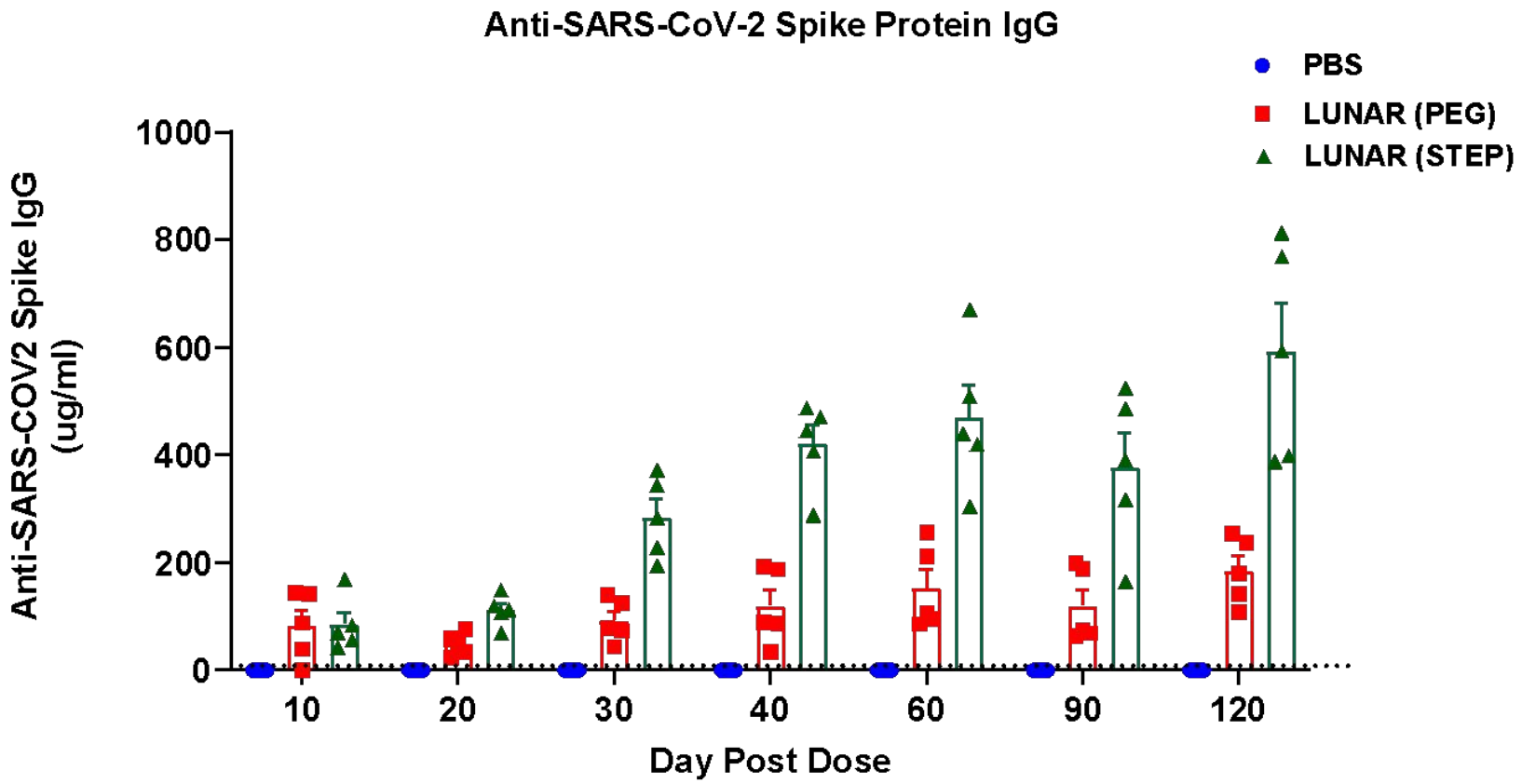
Liver enzyme and cytokine expression of ‘STEP5-LNP’



Complements activation of ‘STEP5-LNP’

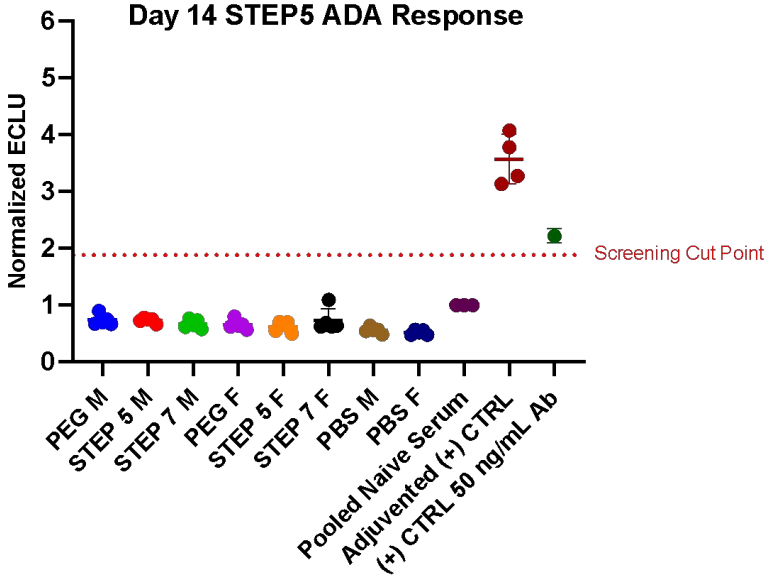


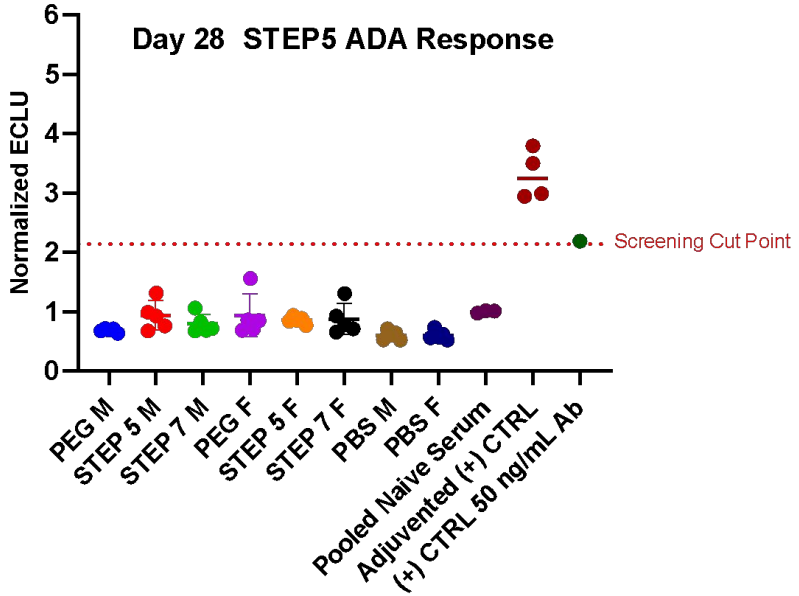
LUNAR-mRNA vaccines: advancing STEP-LNP

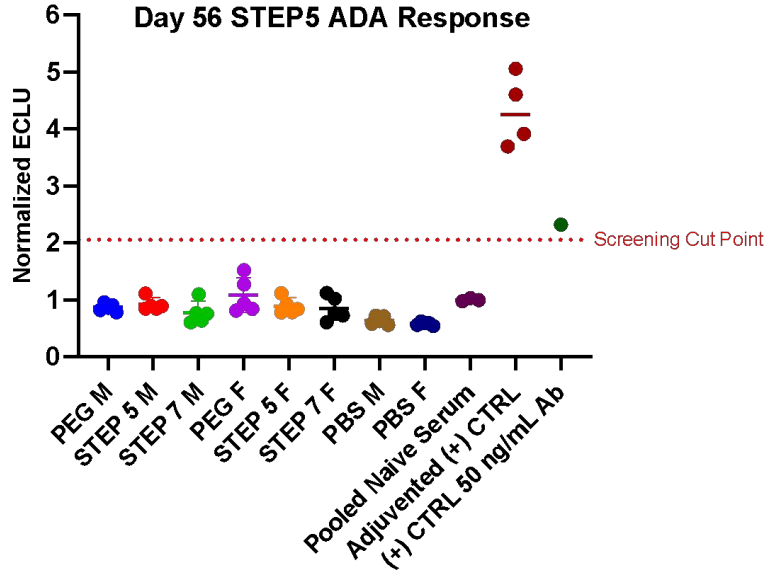


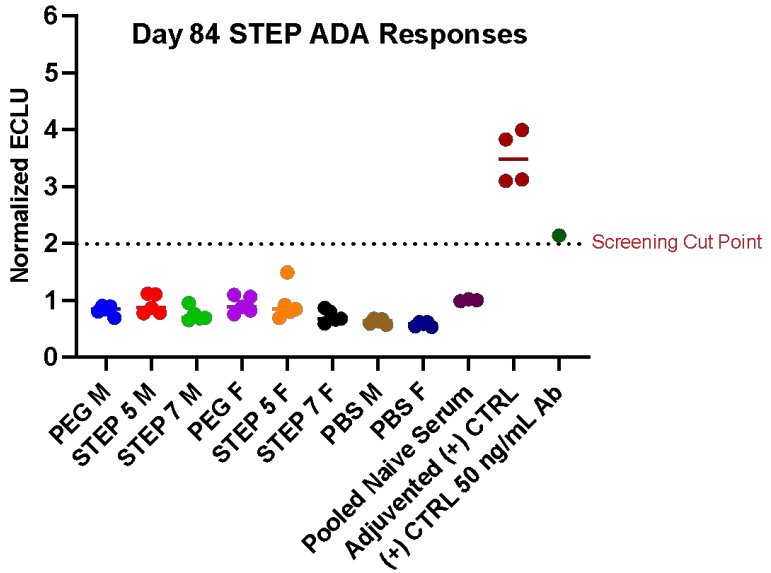
- Replacement of PEGylated lipid in LUNAR® with a non-Pegylated biodegradable alternative imparts superior immunogenicity properties to LUNAR encapsulated mRNA encoding for SARS-COV2 Spike protein

Anti-drug antibody (ADA) response to STEP-LNPs









Summary and conclusions

- DMG-STEPs are readily prepared and incorporated into formulation to produce STEP-LNPs
- STEP-LNPs have similar size, PDI and morphology as PEG-LNPs
- STEP-LNPs are comparable in FVII silencing, and hEPO expression, in both rodents and NHPs, to PEG LNPs
- Tolerability of selected STEP-LNP (STEP5) are comparable to PEG-LNP in ALT and AST elevation, cytokine induction and complement activation
- Antidrug antibody (ADA) assay with STEP-LNP did not show presence of antibody up to day 84. In this assay PEG-LNP also did not show anti-PEG antibody