

Lipid Nanoparticles Comprising Poly-phosphocholine Conjugates for Nucleic Acid Delivery

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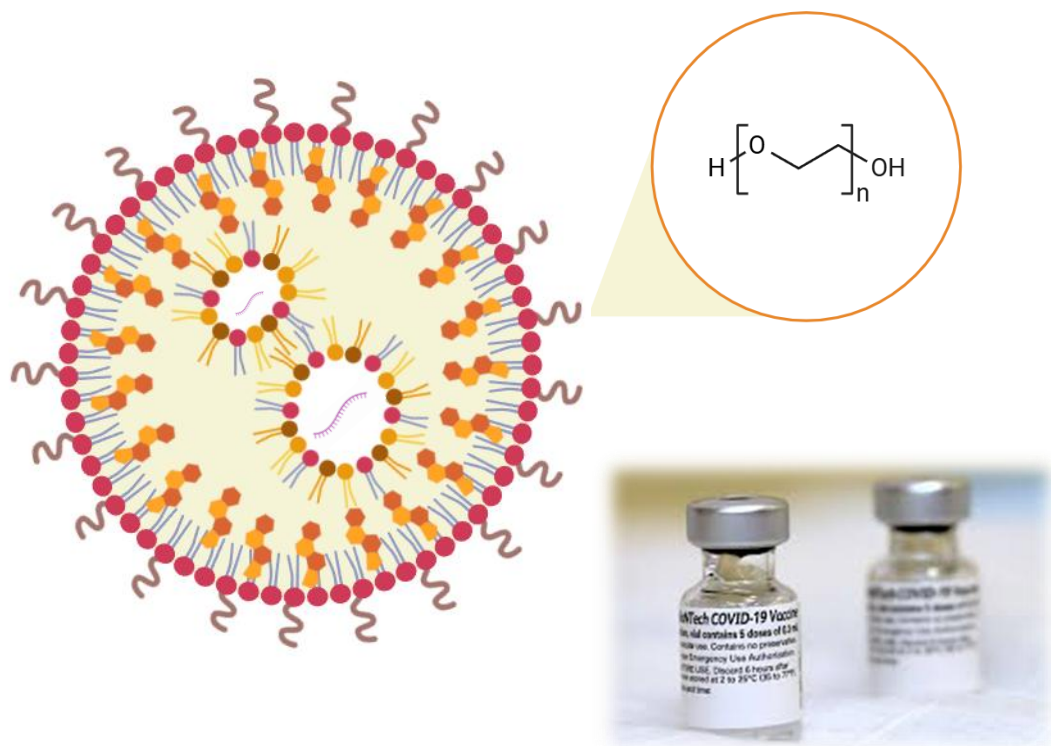


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Lipid Nanoparticles (LNPs)

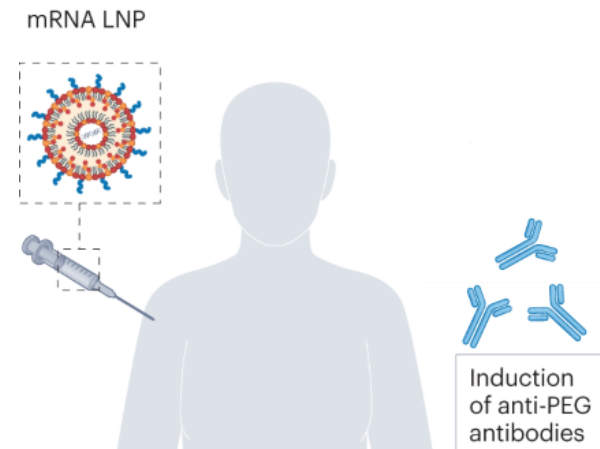
PEGylation in LNPs: Small Components, Big Influence



Preventing aggregation

Increased circulation time

Reduced opsonization



Reduced cellular uptake

Accelerated Blood Clearance (ABC)

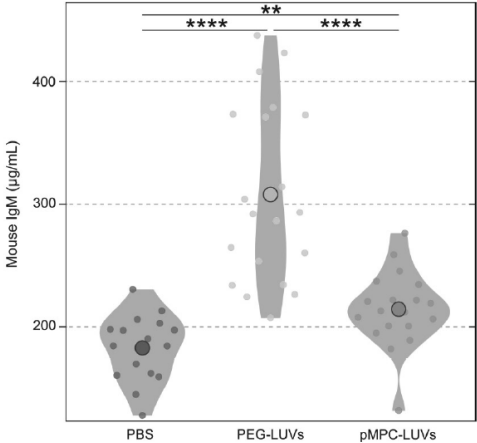
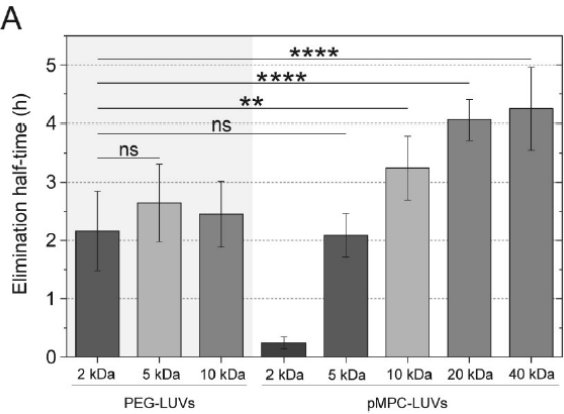
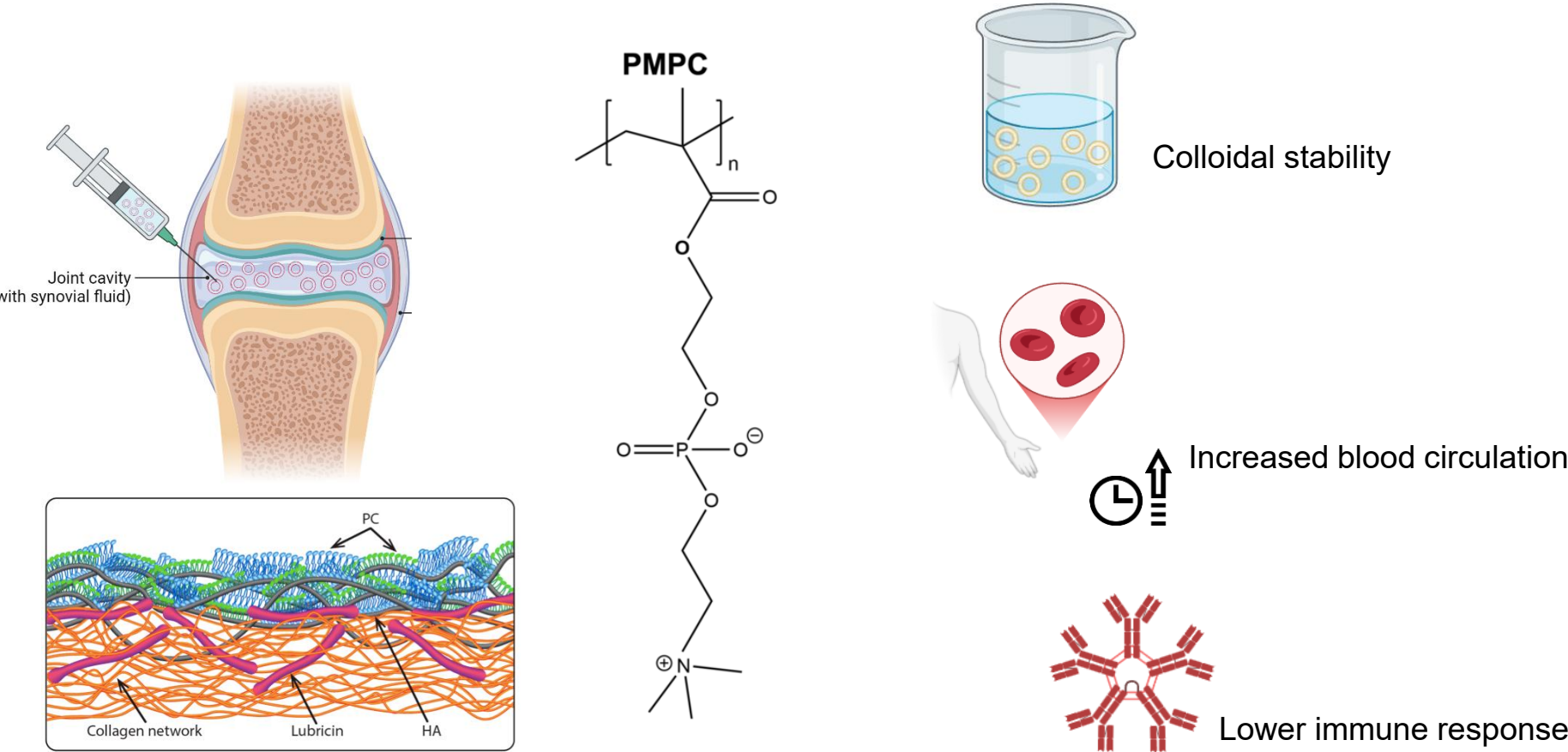
Hypersensitivity reactions



Dobrovolskaia, M.A., 2025. Better the devil you know than the devil you don't—PEG challenges in nanomedicine. *Nature Nanotechnology*, 20(5), pp.580-583.

Poly[2-(methacryloyloxy)ethyl phosphorylcholine] (pMPC)

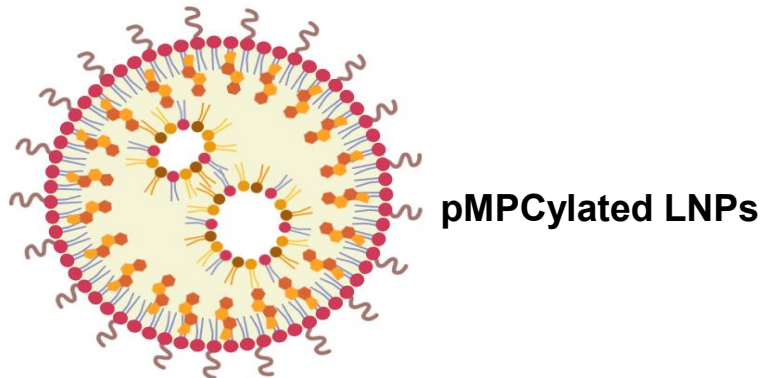
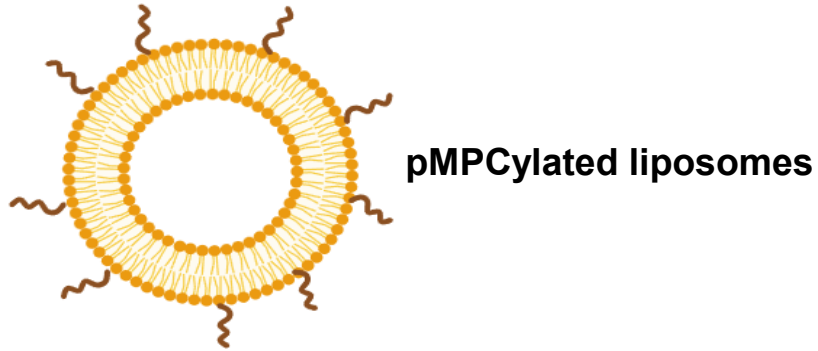
a PEG alternative



Lin, W., Kampf, N., Goldberg, R., Driver, M.J. and Klein, J., 2019. *Langmuir*, 35(18), pp.6048-6054.
Mitsou, E., Kluzek, M., Ptak-Lemos, F., Lan, S., Kamishinsky, R., Kuznetsov, Y., Porat, Z., Klein, J. (in preparation)
Kluzek, M., Hamad, M., Lin, W., Mitsou, E., Porat, Z., Kuznetsov, Y., Oppenheimer-Shaanan, Y., Klein, J. 2025 *Journal of Controlled Release* (under revision)

pMPCylated LNPs

Formulation



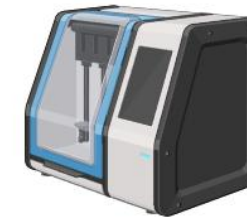
Component	%
Ionizable lipid (ALC-0315)	40.9
Phospholipid (DSPC)	9.4
Cholesterol	42.7
(C18) ₂ - PEG or pMPC	1.6
Stearylamine	5.4

(C18)₂ - PEG 2 kDa

(C18)₂ - pMPC 5 kDa & 20 kDa



Fluc-mRNA
(N/P=6)



Commercial platform

Structural Characterization

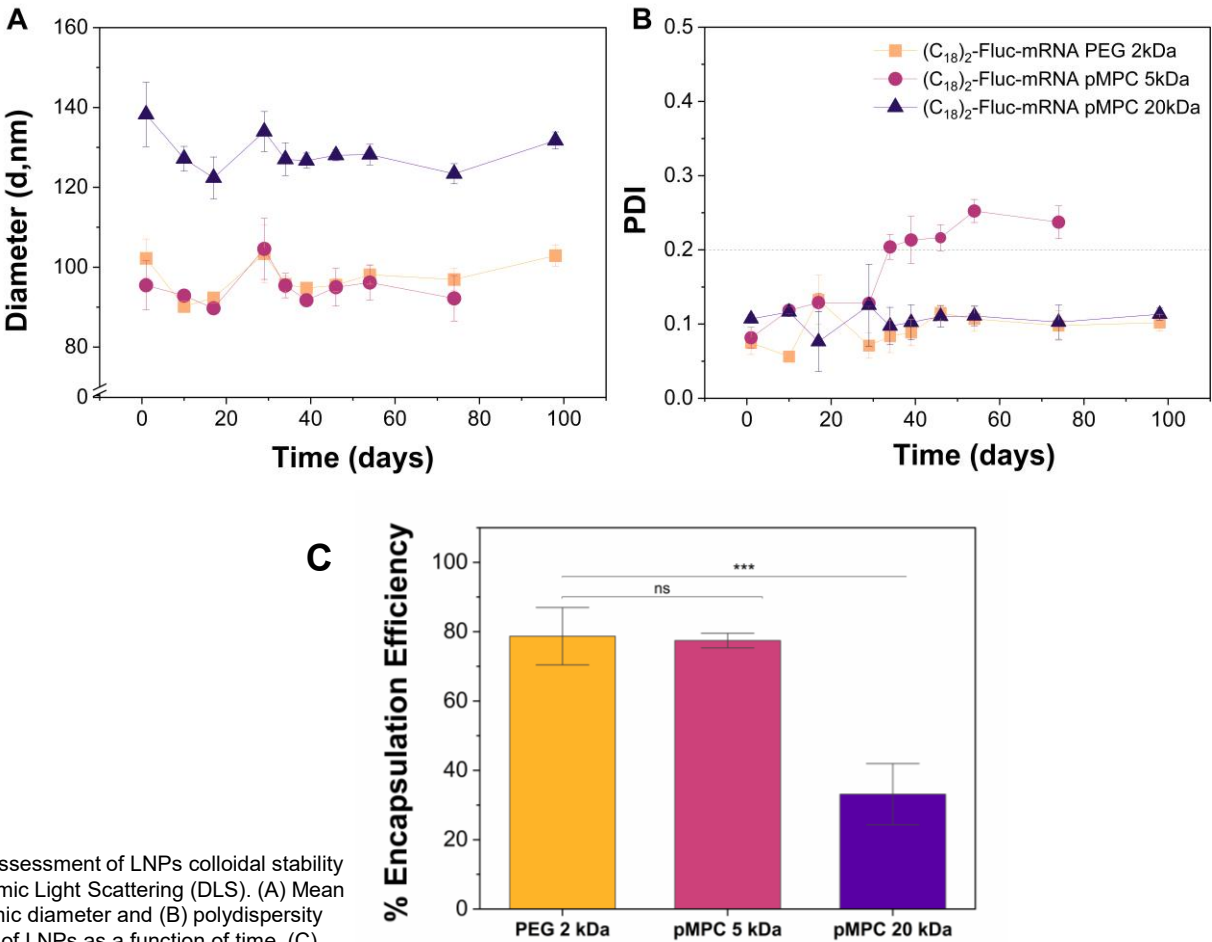


Figure 1. Assessment of LNPs colloidal stability using Dynamic Light Scattering (DLS). (A) Mean hydrodynamic diameter and (B) polydispersity index (PDI) of LNPs as a function of time. (C) Encapsulation efficiency.

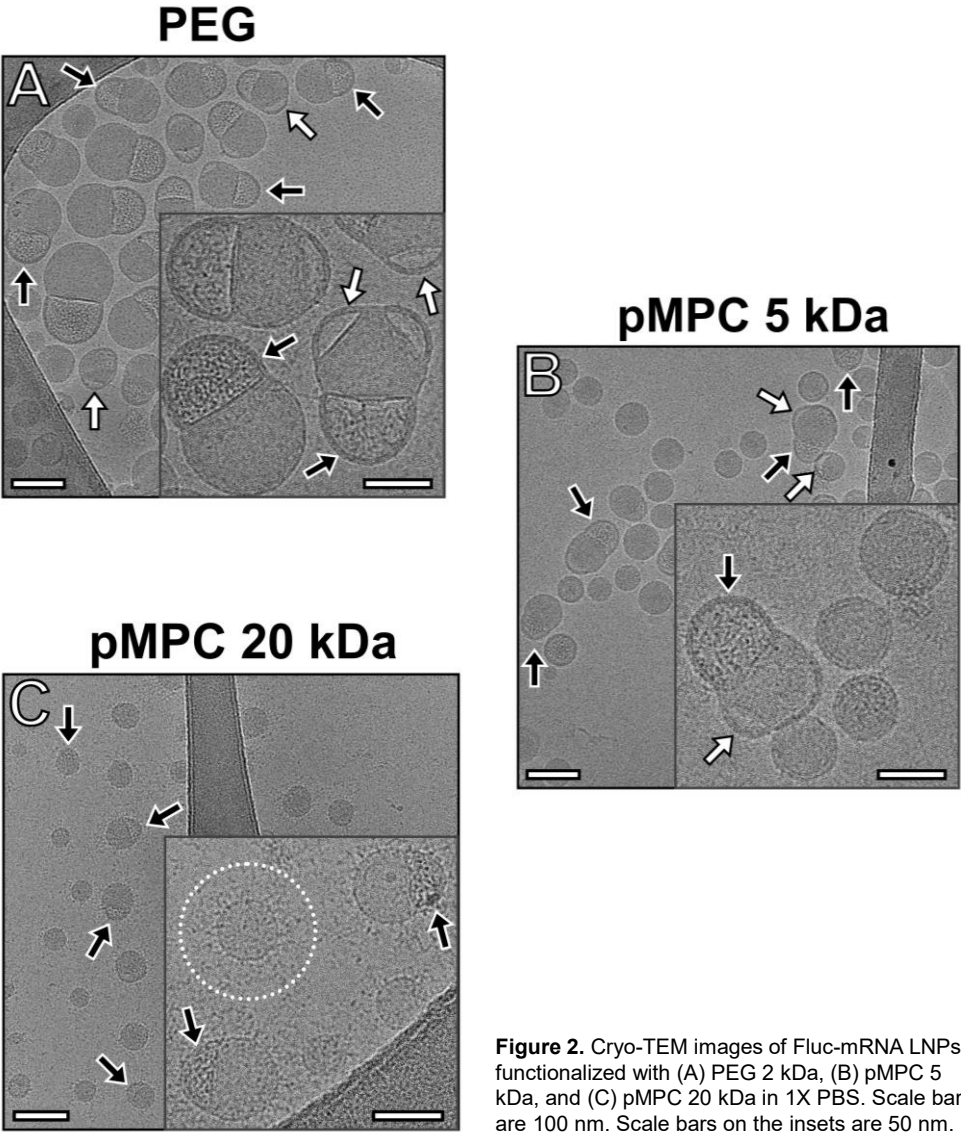


Figure 2. Cryo-TEM images of Fluc-mRNA LNPs functionalized with (A) PEG 2 kDa, (B) pMPC 5 kDa, and (C) pMPC 20 kDa in 1X PBS. Scale bars are 100 nm. Scale bars on the insets are 50 nm.

In vitro evaluation

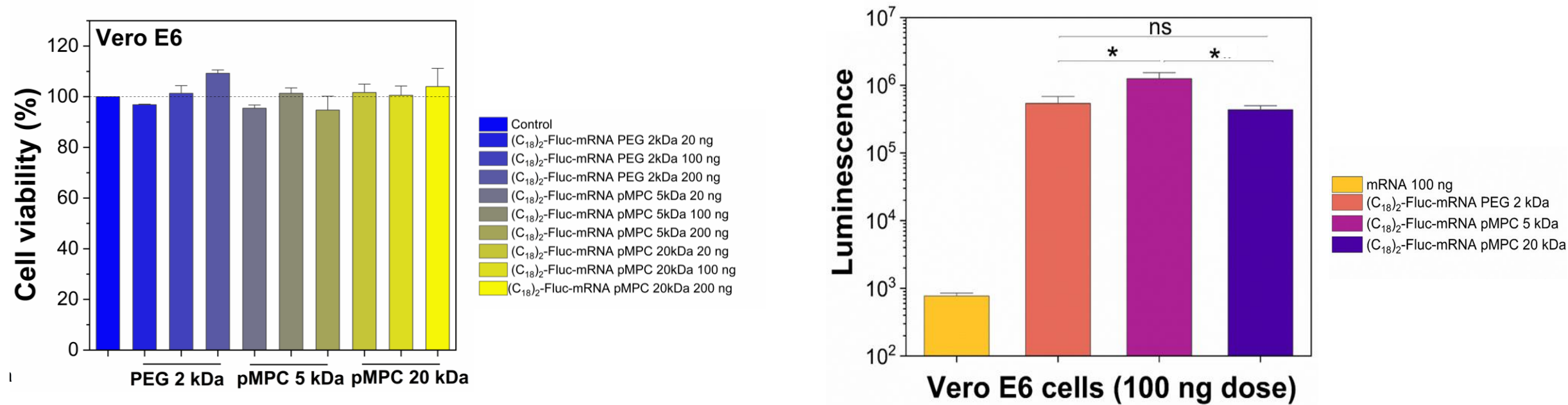


Figure 4. Cell viability (XTT assay) for Vero E6 cells upon administration of three different mRNA doses 20 ng, 100 and 200 ng per well after 72 h. Transfection efficiency of Fluc-mRNA LNPs (100 ng/well) after 48 h. Data are presented as the mean $\pm$ SD of three independent experiments with 6 technical repeats.

In Vivo Evaluation

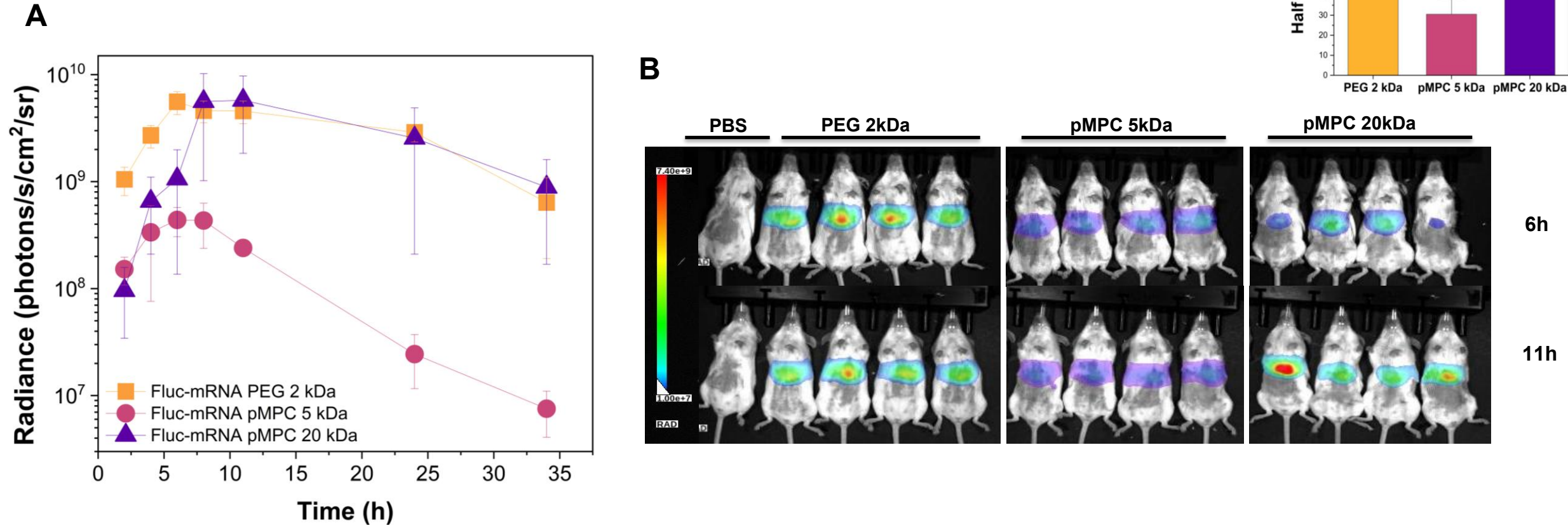


Figure 5. Kinetics of Fluc-mRNA expression following IV administration using various LNPs. (A) Quantification of luminescence (n=4 mice per group, 0.15 mg/kg Fluc-mRNA). Data are shown as mean values $\pm$ S.D (B) Representative bioluminescence images

IgM and Toxicity studies (single dose)

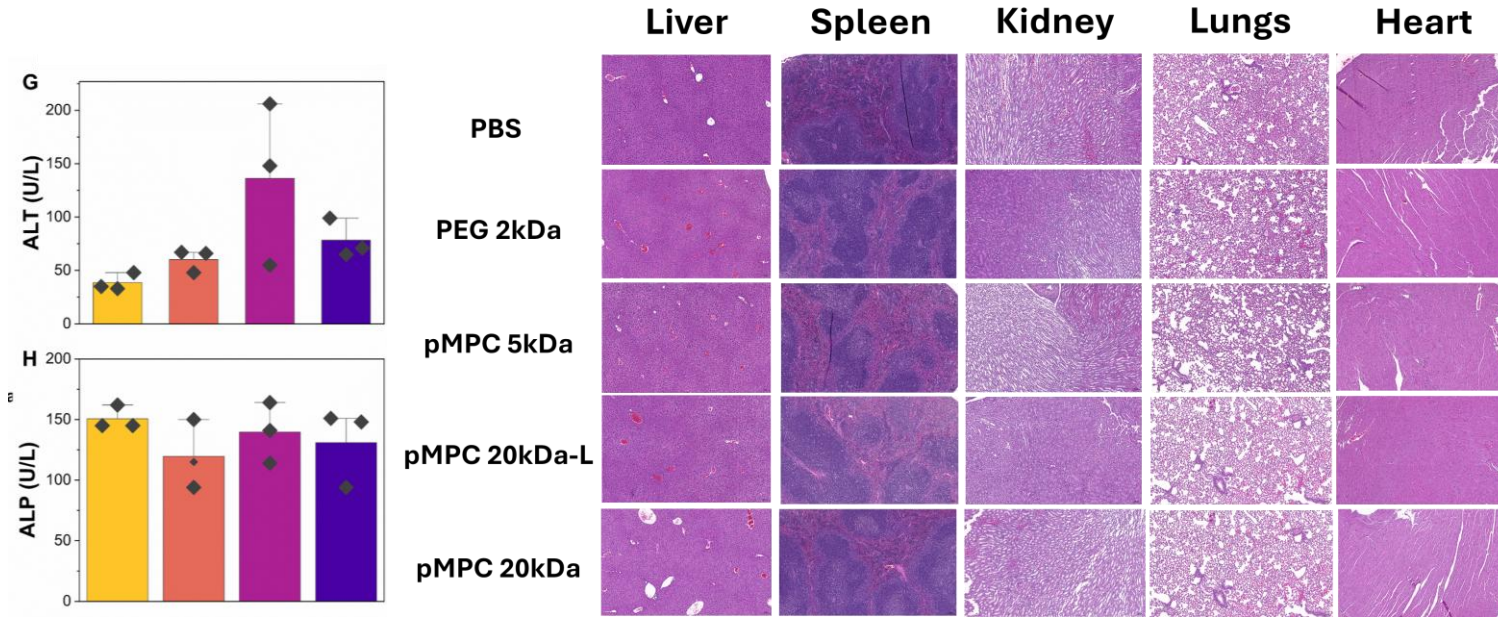


Figure 6. (Left) Serum biochemical parameters of mice as reported by VetScan. (Right) Representative tissue histology images following a single IV injection of Fluc-mRNA encapsulated in different LNPs 24 h upon single IV injection of PBS, and LNPs functionalized with PEG or pMPC (0.15 mg/kg mRNA dose).

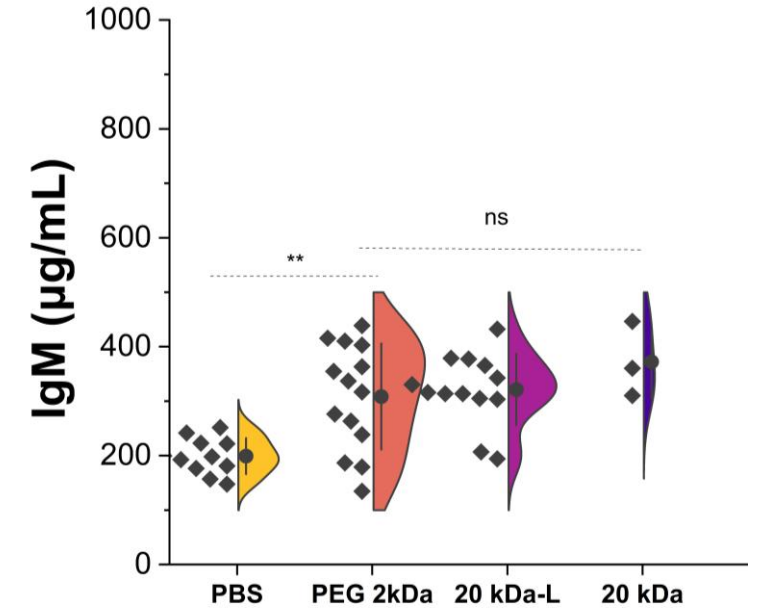
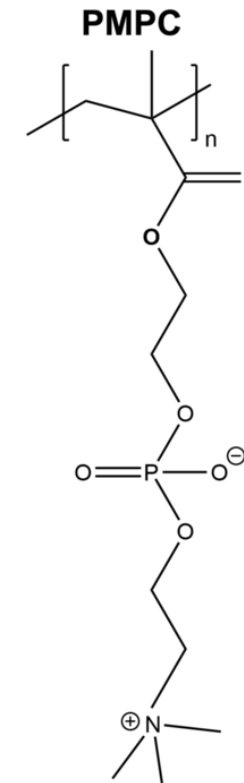


Figure 7. IgM Levels in mice serum following IV injection. Mice received a tail vein injection of 100 μ L containing 0.15 mg/kg of mRNA LNPs or PBS (control). Blood samples were collected 5 days post-injection, and serum IgM levels were quantified using an ELISA kit.

Summary of Findings

- pMPCylation is a viable surface modification strategy for LNPs
- Maintains colloidal stability
- Encapsulation is affected by the MW
- Circulation time is comparable to PEGylated LNPs
- No observable toxicity following a single administration
- Further optimization of formulation parameters (MW, chain length, density) is needed
- Other immune indicators are under investigation



Thank you



ΕΘΝΙΚΟ ΙΔΡΥΜΑ ΕΡΕΥΝΩΝ
National Hellenic Research Foundation

Soft Matter and Surface Forces Lab

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Dr. Di Jin
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Dr. Roman Kamishinsky



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