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Surface-Modified Lipid Nanoparticles for Targeted mRNA Delivery and Immune Modulation in Vaccine Models

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Introduction

Lipid nanoparticles (LNPs) are a clinically established platform for mRNA delivery, with applications ranging from protein replacement therapy to vaccine development. However, conventional LNPs often rely on passive targeting, which can result in suboptimal cellular uptake and off-target biodistribution. To address this, we engineered LNPs functionalized with diverse ligands—including folic acid (FA), D-mannuronic acid (MA), 2-keto-3-deoxy-D-gluconic acid (KDG), Fmoc-glutamine (Fmoc-Gln), DL-α-lipoic acid (ALA), and hyaluronic acid (HA)—each selected for their potential to engage specific cell-surface receptors. LNPs were formulated via microfluidic mixing and characterized for size, polydispersity, zeta potential, and mRNA encapsulation efficiency. In vitro, cytocompatibility and mRNA transfection efficiency were assessed in HEK293, HeLa, and RAW 264.7 cells. In vivo, biodistribution and expression kinetics were evaluated using EZ Cap™ Firefly Luciferase mRNA , while immunogenicity was assessed in mice following intramuscular immunization with OVA mRNA-LNPs. This work aims to identify promising ligand candidates for targeted mRNA delivery and immunomodulation, paving the way for more precise and effective mRNA therapeutics.

Material and method

LNPs were formulated via microfluidic mixing by combining SM-102, cholesterol, DSPC, DMG-PEG2000, and DSPE conjugated with various ligands (folic acid, D-mannuronic acid, 2-keto-3-deoxy-D-gluconic acid, Fmoc-glutamine, DL-α-lipoic acid, and hyaluronic acid). Particle size, polydispersity index, and zeta potential were characterized by dynamic light scattering (DLS), and mRNA encapsulation efficiency was determined using the RiboGreen assay.
In vitro, HEK293, HeLa, and RAW 264.7 cells were treated with LNPs to assess cytotoxicity via the AlamarBlue™ assay and mRNA expression through fluorescence imaging of GFP-mRNA.
Biodistribution and mRNA expression were evaluated at 6, 24, and 48 hours post-injection with Fluc mRNA-LNPs using both ex vivo and in vivo bioluminescence and fluorescence imaging on an IVIS Spectrum system.
For in vivo immunization studies, female BALB/c mice (n=5 per group) were intramuscularly immunized with OVA mRNA-LNPs on days 0 and 28. Serum samples were collected on days 0, 14, 27, and 42 for ELISA quantification of antigen-specific IgG isotypes (total IgG, IgG1, IgG2a).

Results

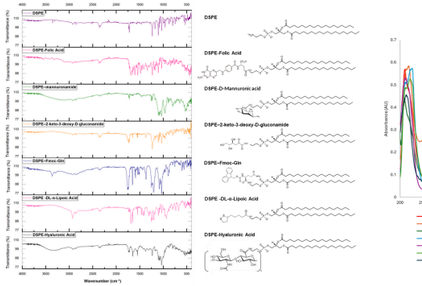

Figure 1: Ligand Conjugation Confirmed. Representative FTIR/UV-Vis spectra.

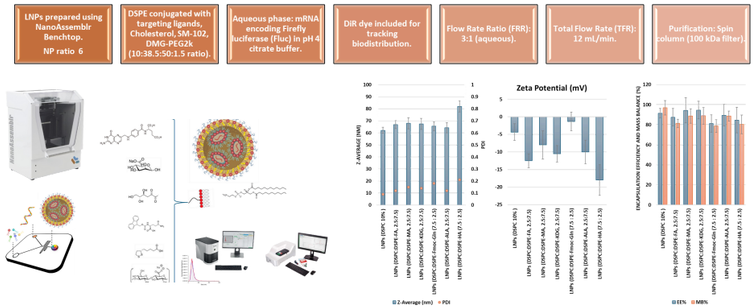

Figure 2. Characterization of ligand-modified lipid nanoparticles (LNPs). (A) Particle size distribution, (B) zeta potential, and (C) mRNA encapsulation efficiency of LNPs formulated with different surface ligands

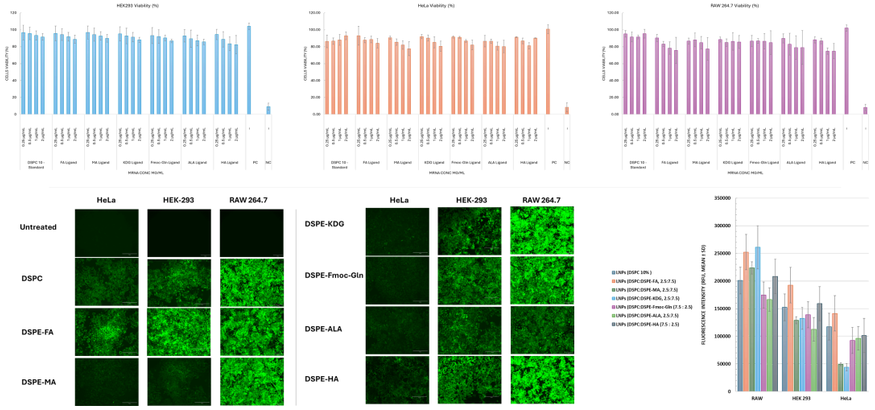

Figure 3: (A) Cell viability following treatment with mRNA-loaded, ligand-functionalised LNPs. (B) In vitro GFP expression in HeLa, HEK293, and RAW264.7 cells after treatment with 3 µg/mL GFP mRNA.

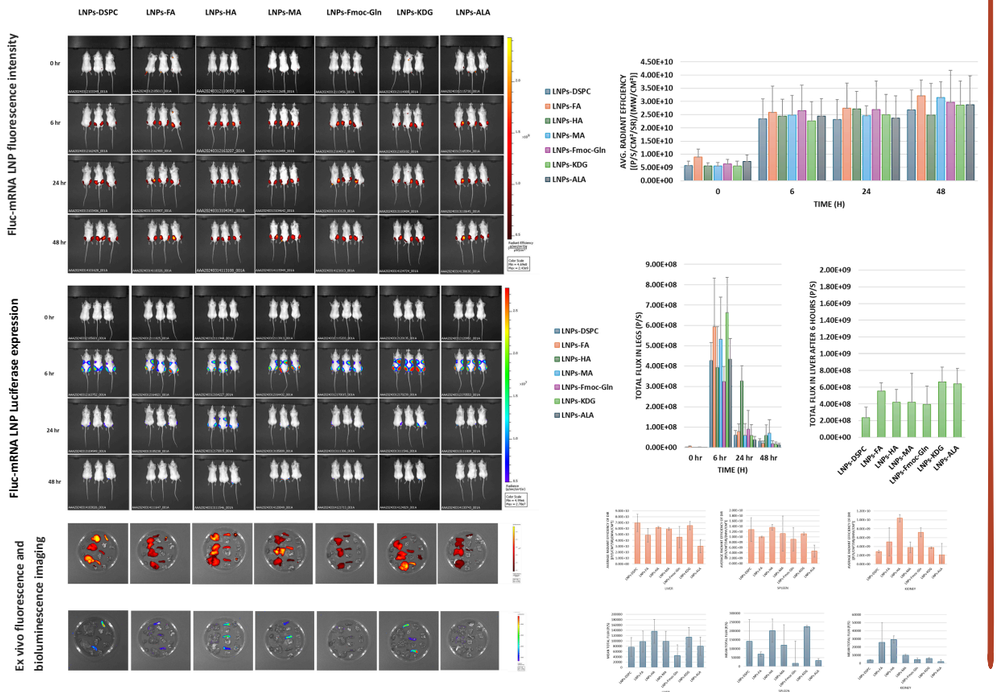

Figure 4: In Vivo Tracking and Luciferase Expression of Fluc-mRNA LNPs via IVIS Bioluminescence Imaging

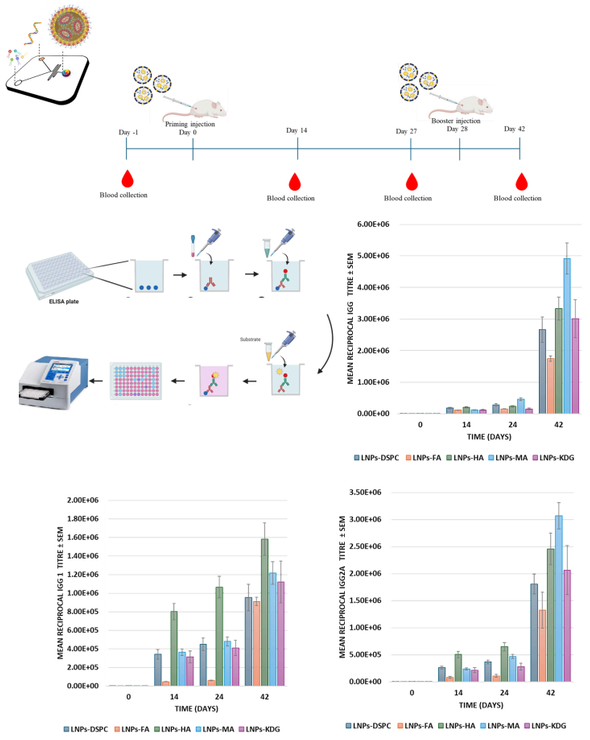

Figure 5. Immune response modulation by ligand-functionalised OVA mRNA-LNPs. BALB/c mice were immunised with 5 µg OVA mRNA-LNPs on days 0 and 28. Serum was collected on days 0, 14, and 42. (A) Total IgG, (B) IgG1, and (C) IgG2a titres. Data are mean ± SEM (n = 5).

Conclusion

Our study demonstrates that surface ligand modification plays a pivotal role in shaping LNP biodistribution and immunogenicity. Specific ligands notably redirected LNP accumulation, highlighting the potential of surface engineering to fine-tune LNP behavior for mRNA delivery.
Among the tested formulations, HA-LNPs and D-mannuronic acid-LNPs showed enhanced immunogenicity, suggesting improved interactions with antigen-presenting cells and potential for precision vaccine development.
These findings suggest a strong link between surface characteristics and immune activation. Future work will focus on elucidating the mechanisms behind ligand-dependent distribution and immune engagement, guiding the rational design of next-generation LNPs for vaccines and RNA therapeutics.

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