Supplementary Information

Ganglioside-incorporating Lipid Nanoparticles as Polyethylene Glycol-free mRNA Delivery Platform

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Supplementary Methods

To enhance the stability of ganglioside-LNPs, some parameters on the preparation were first optimized. The stability of LNPs in liquid medium can be explained by DLVO theory, which was postulated by Derjaguin, Landau, Verwey, and Overbeek[1]. The theory demonstrates the stabilization of a colloidal system, which involves a combination of the attractive van der Waals interactions (V_A) and the repulsive electrostatic Coulomb interactions (V_R)[1]. Particles are more stabilized when the total energy of aforementioned interactions is low or negative[2].

$$V_{total} = V_A + V_R$$

The magnitude of van der Waals interactions depends on the size, shape, and chemical composition of colloidal particles, which is characterized by the Hamaker constant (A)[1]. In the case of spherical particles with a radius of a and a separation distance of H, the van der Waals force can be expressed as follow

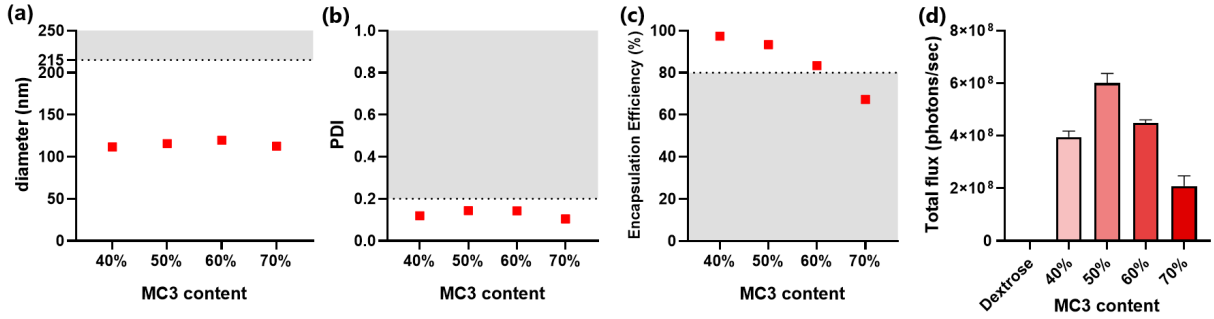
$$V_A = -\frac{aA}{12H}$$

Due to the presence of ionizable cationic lipids, each LNP bears positive charge at low pH, which leads to the formation of electrostatic double layer that repulses the adjacent particles[1]. The electrostatic interaction between two particles is given as

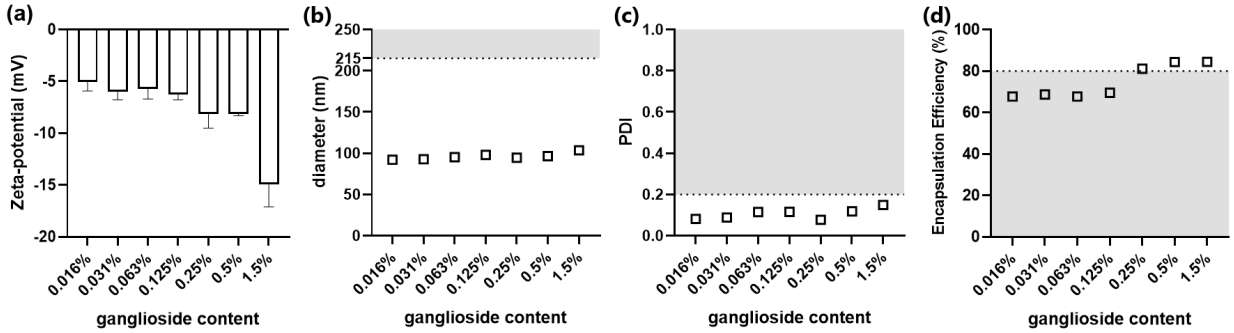
$$V_R = 2\pi a \varepsilon_r \varepsilon_0 {\varphi_0}^2 \exp(-KH)$$

$$K^2 = \frac{2nz^2e^2}{\varepsilon_r \varepsilon_0 kT}$$

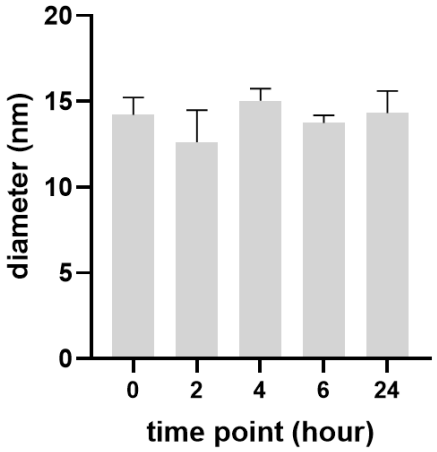
where the constants e is elementary charge and k is the Boltzmann constant[2]. The other terms a, $\varepsilon_r\varepsilon_0$, φ_0, H, n, z, T are particle size, solvent permittivity, the surface potential, distance between particles, ion concentration, ion valence, and temperature, respectively[2]. Two approaches that increase the value of V_{total}, which results in an enhanced particle stabilization, are by increasing the surface potential (φ_0) and reducing ion concentration (n) in the solvent.



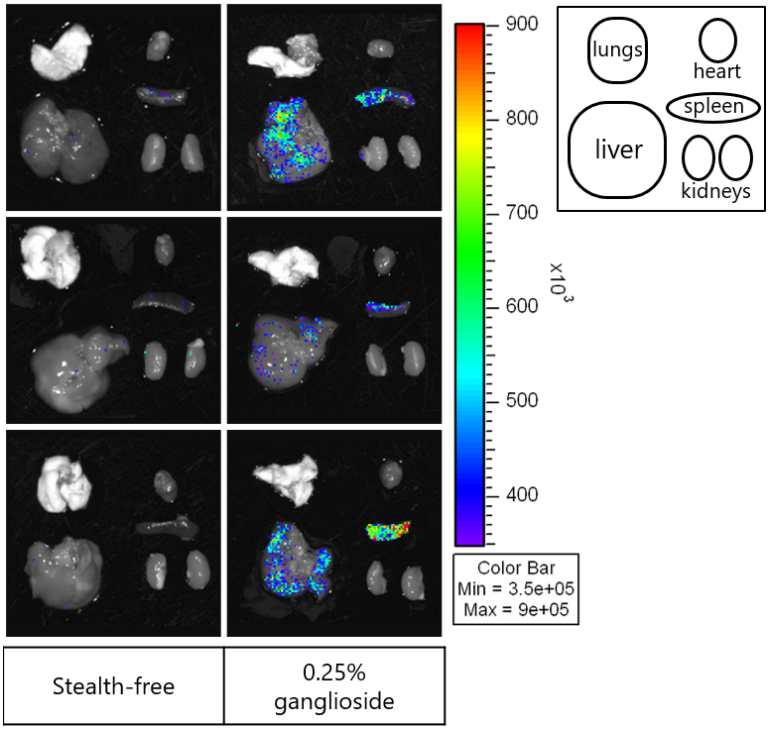
Supplementary Figure 1. Characterization of ganglioside-LNP formulations with varying mol percentages of ionizable lipid MC3. (a) Particle size was measured using dynamic light scattering (DLS). The horizontal line at 215 nm indicates the cutoff for sterile-filtration. (b) PDI was measured using DLS. The horizontal line at 0.2 indicates the cutoff for polydispersity. (c) mRNA encapsulation efficiencies (EE) of candidate LNPs. The horizontal line at 80% EE indicates the cutoff for insufficient mRNA encapsulation. (d) *In vitro* transfection efficiency of candidate LNPs in HEK293T cells. Bioluminescence data was quantified as total flux (photons/second) (N=3). As a control, cells were treated with the 5% dextrose (D5W) solution. According to the transfection efficiency data, 50% was identified as the optimal MC3 concentration, which was adapted for all future formulations.



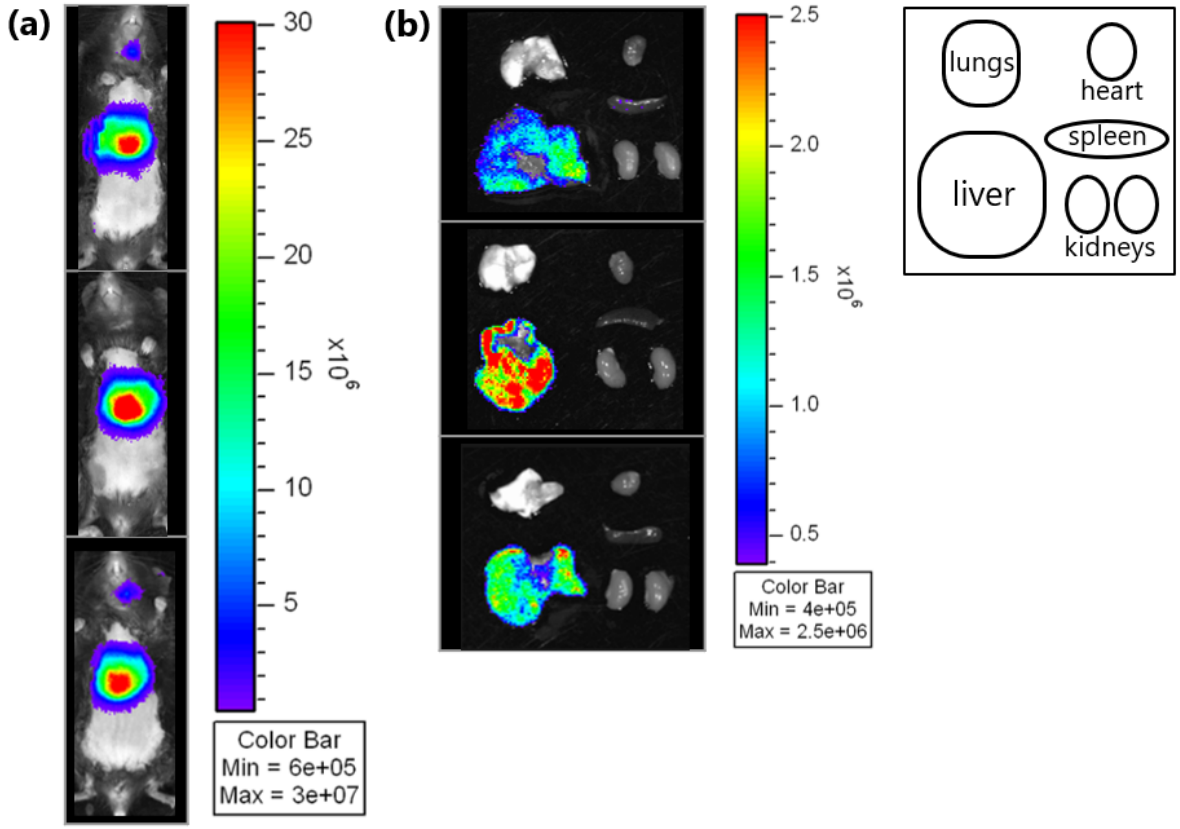
Supplementary Figure 2. Characterization of ganglioside-LNP formulations with varying mol percentages of ganglioside spanning 0.016–1.5%. (a) Zeta-potential was measured using Nano Zetasizer (N=3). (b) Particle size was measured using dynamic light scattering (DLS). The horizontal line at 215 nm indicates the cutoff for sterile-filtration. (c) PDI was measured using DLS. The horizontal line at 0.2 indicates the cutoff for polydispersity. (d) mRNA encapsulation efficiencies (EE) of candidate LNPs. The horizontal line at 80% EE indicates the cutoff for insufficient mRNA encapsulation. Formulations with 0.25% or higher ganglioside concentration which comply the criteria were re-evaluated at smaller intervals, while those with 0.125% or lower ganglioside concentration were excluded from further measurement.



Supplementary Figure 3. Size measurement of fetal bovine serum (FBS) (N=3).



Supplementary Figure 4. *In vivo* transfection of stealth-free LNPs and 0.25% ganglioside-LNPs. Images of bioluminescence from the harvested organs (lungs, liver, heart, spleen, and kidneys) 24 hours after intravenous injection of LNPs loaded with fLuc mRNA.



Supplementary Figure 5. *In vivo* transfection of PEG-LNPs. Images of bioluminescence from the whole body (a) and harvested organs (lungs, liver, heart, spleen, and kidneys) (b), 24 hours after intravenous injection of PEG-LNPs loaded with fLuc mRNA.

Supplementary References

(1) Horinek, D. DLVO Theory. In *Encyclopedia of Applied Electrochemistry*; Kreysa, G., Ota, K., Savinell, R. F., Eds.; Springer: New York, NY, 2014; pp 343–346. https://doi.org/10.1007/978-1-4419-6996-5_7.

(2) Okuda, K.; Sato, Y.; Iwakawa, K.; Sasaki, K.; Okabe, N.; Maeki, M.; Tokeshi, M.; Harashima, H. On the Size-Regulation of RNA-Loaded Lipid Nanoparticles Synthesized by Microfluidic Device. *J. Controlled Release* **2022**, *348*, 648–659. https://doi.org/10.1016/j.jconrel.2022.06.017.