Biodistribution of LNP-mRNA using fluorescence-fLuciferase fusion proteins.

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Background

Lipid nanoparticles (LNPs) are a key tool used to deliver mRNA into cells/ in vivo in novel vaccine technologies. LNPs are less than 100 nm in diameter, consisting of ionisable lipids, helper lipids and mRNA (**Fig 1**).

A key issue of mRNA delivery via LNPs is non-specific protein expression in a wide variety of organs, which has potential to cause off-target effects. Hence, determining the biodistribution of LNPs after administration is critical to improving their safety and efficacy. Key organs for monitoring include the liver, spleen, and lungs.

We are developing tools to track the biodistribution of LNPs post-transfection in cells, skin and mice, both in vivo and ex vivo. We designed mRNA to encode a fluorescent-fLuciferase fusion protein to determine mRNA expression and therefore LNP localisation, via bioluminescent and fluorescent signalling.

Bioluminescence and fluorescence are common pre-clinical, optical imaging modalities. Bioluminescence occurs due to catalytic enzyme activity (e.g. luciferase breakdown of luciferin) emitting light. Fluorescence is a result of absorption of a shorter wavelength photon by a fluorophore protein, which re-emits a quantifiable, longer wavelength, photon in response. Both techniques offer a non-invasive approach to image desired expression in mice, over multiple time points, and multiple animals at once. This reduces the number of mice used. The same techniques can be employed in cellular imaging and skin explants too, replacing the need for animals in early preclinical testing.

Methods

For this project, we generated constructs for in vitro transcription to produce mRNA encoding for a mGreenlantern (mGL) fluorophore and firefly luciferase (fLuc), fusion protein. The generated mRNA was tested using transfection with LNPs and lipofectamine into several cell lines and in human skin explants before testing in animals.

In Balb/c mice, the LNPs were inoculated intramuscularly using C12-200 as an ionisable lipid. Protein expression in vivo was visualised in a non-invasive manner using an Ami HTX (Spectral Imaging). For ex vivo visualisation animals were culled, and organs were exposed to luciferin and imaged in the Ami HTX to visualise the luciferase activity.

Conclusions

- Our mRNA for fluorescent-fLuciferase fusion proteins allow us to detect the biodistribution of LNPs carrying mRNA in cells, skin explants and mice.
- In vivo luminescence signalling showed biodistribution of the LNPs was mainly in muscle, liver and spleen.
- The concentration of mRNA determines the strength of the luminescence signal in vivo, but volume does not.
- Use of optical imaging modalities reduced the number of mice needed for the experiments, as we used the same mice across multiple time points.
- Currently we are quantifying the fluorescence in vivo, via FACS in mouse dissociated tissues.

Results

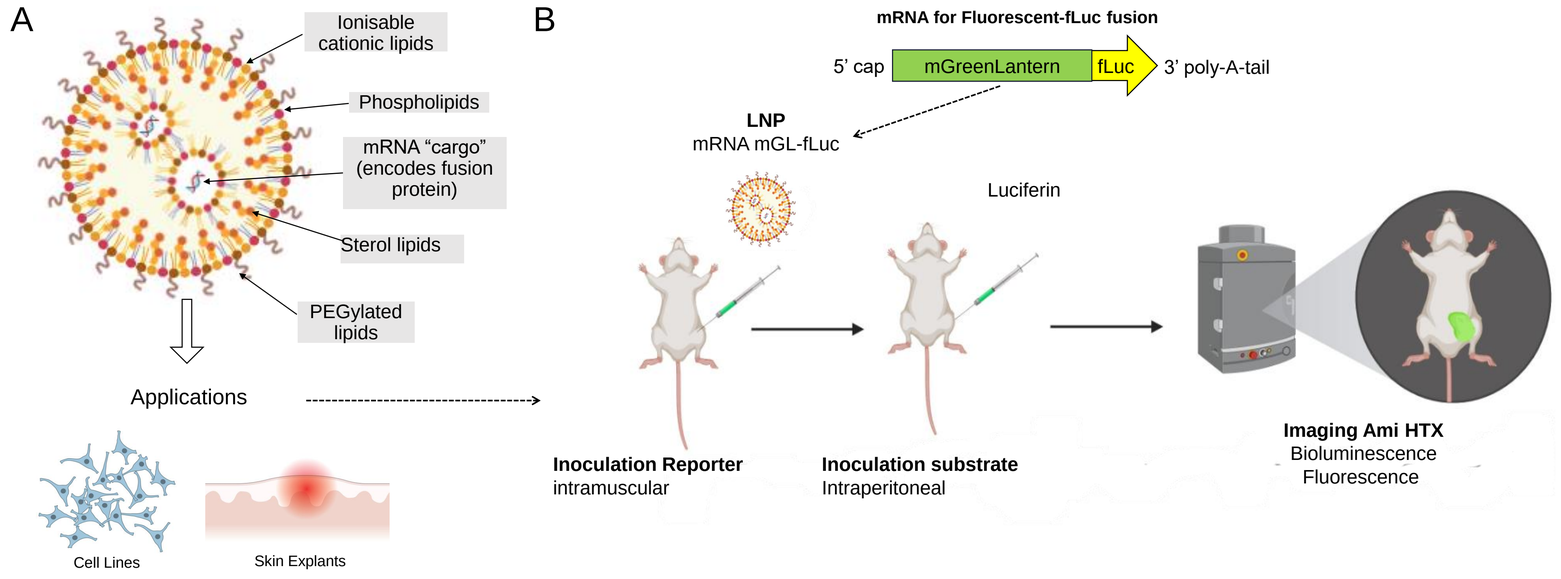

Fig 1. A: Diagram of LNP structure encapsulating the mRNA cargo. The LNP-mRNA product is then tested in cells and skin explants. B: The fusion protein mRNA "cargo" encodes mGreenlantern and firefly luciferase, fused with a short linker molecule in between. It was inoculated in mice intramuscularly. Then the luciferase substrate luciferin was inoculated intraperitoneally. Mice were imaged using the Spectral Imaging Ami HTX system for successful bioluminescent and fluorescent signalling from protein production in vivo.

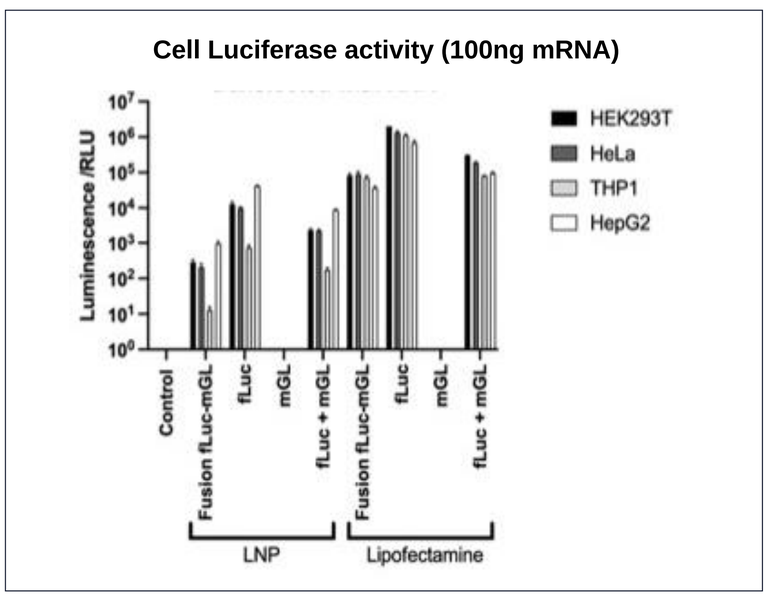

Fig 2. Quantification of the luciferase activity in cells transfected with the mRNA from the fusion protein mGL-fLuciferase in different cells lines: HEK 293T, Hela, THP1, and HepG2. The mRNA derived from these constructs expressed well in a range of cells lines.

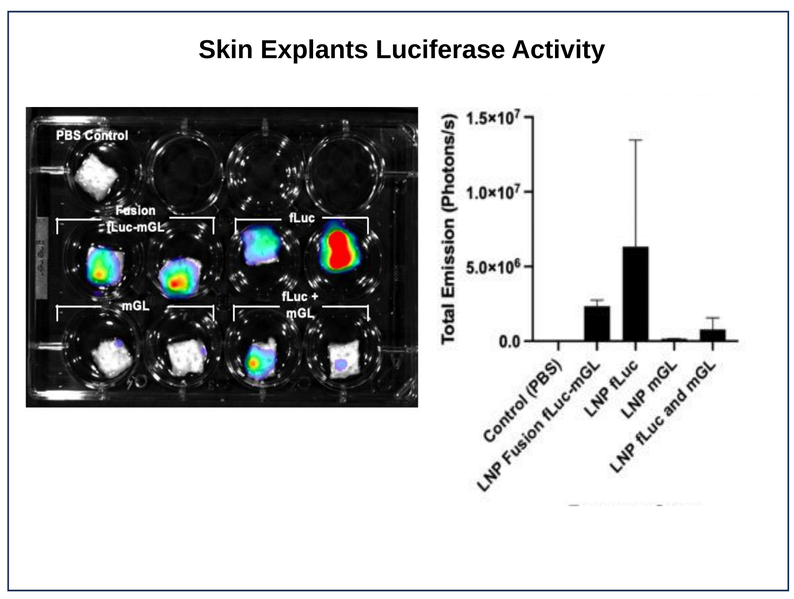

Fig 3. Luminescence detection in human skin explants inoculated with LNPs. Skin explants were prepared by removing the bottom layer of fat and cutting a 1cm^2 slice. The explants were then injected with LNPs containing 2.5 μg of mRNA. We observed a lower luciferase activity in skin explants injected with mRNA for the fusion protein compared with the activity for skin injected with fLuc alone.

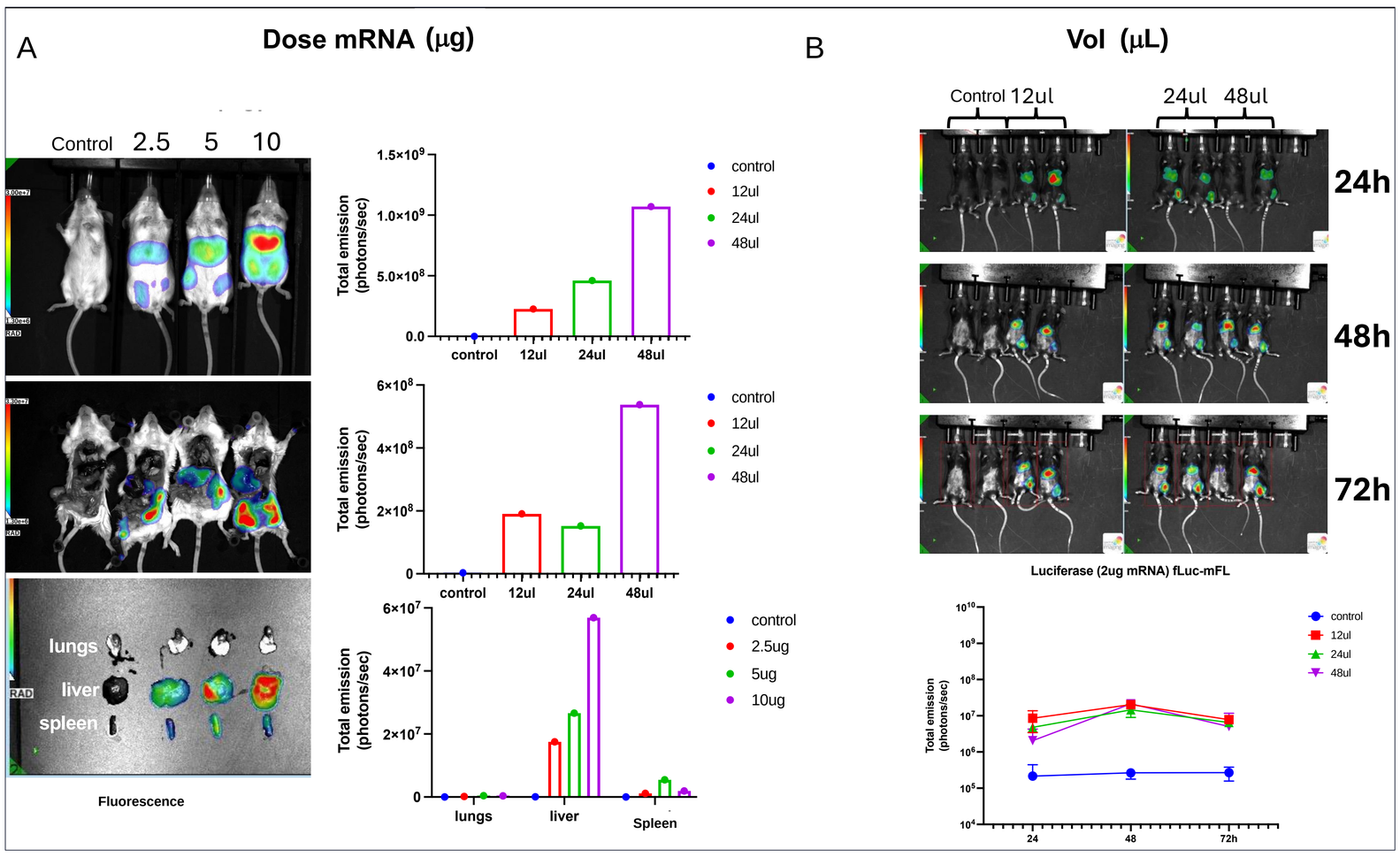

Fig 4. A: Biodistribution of LNPs (C12-200 + mRNA) in mice. The intensity of the luminescence signal correlated with the amount of mRNA delivered by LNPs in vivo. Ex vivo the main organs that showed a signal were muscle (site of injection), liver, and spleen. B: Effect of volume variation for inoculation of LNPs. Volume variation during inoculation of LNPs does not affect the amount of luminescence signal derived from the same amount of mRNA. The dose was 2.5 μg of mRNA and was inoculated in 12 μL, 24 μL, and 48 μL. The group control was inoculated with 12 μL of PBS. The same mice were used for each time point.

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