Assessing Efficiency of mRNA Lipid Nanoparticle Formulations Using Fluorescence Microscopy

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Statement of Purpose:

Lipid nanoparticles (LNPs) have emerged as promising vehicles for effective delivery of nucleic acids to cells. Cellular uptake and transfection efficiency of mRNA LNPs can vary based on the lipid composition of the nanoparticles, the cell type being delivered to, and the incorporation of targeting ligands onto the nanoparticle surface. A high-throughput method for screening formulations at different stages in the delivery process (binding to cell surface, internalization, and translation of cargo) would be useful for optimizing final LNP designs. Here, we have developed a high throughput screening method using computational analysis of fluorescence microscopy to rapidly evaluate sub-cellular localization of mRNA LNP delivery and protein translation efficacy.

Methods:

1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) LNPs loaded with mCherry mRNA were synthesized, and transfection efficiency was assessed using MetaExpress image analysis software (Molecular Devices, San Jose, CA). Lipid nanoparticles were synthesized by mixing an aqueous phase consisting of 30 µg mCherry mRNA or 30 uL PBS, nuclease free water, and citrate buffer with an ethanol phase comprised of a ~1:1 DOTAP:DOPE containing 0.035 mol% nitrobenzoxadiazole (NBD)-DOPE at a 3:1 ratio. The mixture was sonicated for 15 minutes and dialyzed against PBS to remove the ethanol. mRNA encapsulation efficiency was then assessed using a Ribogreen assay. Cellular uptake and mCherry expression were then imaged in Hoechst-stained HEK293T cells after being incubated with a 350-ng dose of mRNA for 6 and 18 hours using an ImageXpress Micro XLS Widefield High-Content Analysis system (Molecular Devices, San Jose, CA).

Results:

We developed an algorithm to assess lipid nanoparticle localization and protein expression. Briefly, after defining a multi-wavelength cell scoring module in MetaExpress, the average NBD and mCherry intensity could be quantified in individual cells across different timepoints. For instance, NBD and mCherry localization and expression were quantified at 6-hour and 18-hour timepoints. A greater than 3-fold change in expression intensity was observed between the 6-hour and 18-hour timepoints, allowing evaluation of sub-cellular protein expression kinetics to be readily tracked over time.

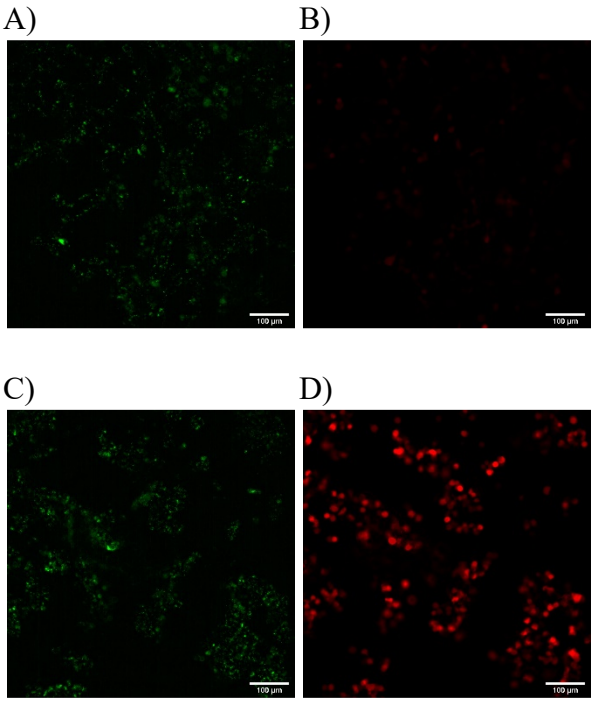

Figure 1. A (6 hr) and C (18 hr) images showing NBD intensity as measured on the GFP channel. B (6 hr) and D (18 hr) images showing mCherry intensity as measured on the Texas Red channel on the microscope. All scale bars are 100 µm and images are taken at 20X magnifcation. Images for LNPs containing no mCherry mRNA (Empty) not shown.

Conclusions:

High throughput screening systems are required to rapidly evaluate whether poor protein expression is due to limited LNP internalization, limited mRNA translation or other factors. Using this system, we can decouple LNP delivery, internalization, and endosomal escape from protein translation. For example, in formulations and different cell lines where mCherry expression is found to be low, we can use the NBD intensity per cell to confirm whether the poor transfection efficiency is due to particle delivery or is cell line specific. By determining the internalization to protein translation ratio, we can quickly screen and optimize LNP formulations and mRNA; mRNA sequences can be optimized for a given formulation to improve protein expression.