Spectradyne®
Particle Analysis

Quantifying Lipid Nanoparticle (LNP) Payload with Spectradyne's ARC™ Particle Analyzer

Lipid nanoparticle (LNP) formulations prepared with different mRNA encapsulation efficiency (EE) were used to demonstrate:

1. Spectradyne's ARC particle analyzer can quantify single-particle RNA payload in a LNP formulation.
2. The ARC delivers unique insights for formulation development optimization to maximize per-particle RNA loading.

Experimental Design:

Three LNP formulations were prepared using the Precision Nanosystems Ignite™ platform: Empty LNPs (no RNA payload), high EE and low EE LNPs. High EE and Low EE LNPs were prepared using different exchange buffers, leaving respectively higher and lower concentrations of residual unencapsulated RNA as measured by a RiboGreen assay.

All samples were fluorescently labeled with the intercalating nucleic acid stain SYBR Gold (Thermo Fisher Scientific) according to manufacturer's instructions. Particle size, concentration and fluorescence were measured for all three samples using Spectradyne's ARC particle analyzer.

In a second experiment, High EE LNPs and empty LNPs were mixed together across a range of relative concentrations to demonstrate the expected linear dependence of concentration (of fluorescent particles only) on dilution factor.

Results:

Figure 1 shows the total particle size distribution for the three formulations as measured by Microfluidic Resistive Pulse Sensing (MRPS) on the ARC. The empty LNPs show the lowest overall concentration on the measured size range, and the two loaded LNPs (High EE and Low EE) show very similar overall distributions. The mRNA loading characteristics of each sample are evaluated using fluorescence measurements of each particle, collected simultaneously on the ARC (Figure 2).

Figure 2 shows particle concentration as a function of brightness in each of the three formulations. The LNPs in the high EE formulation are significantly brighter than those in the low EE formulation, implying that the High EE exchange buffer yields particles that contain more RNA on a particle-by-particle basis. This knowledge cannot be obtained using the conventional RiboGreen assay, which measures only residual unencapsulated RNA and cannot evaluate the distribution of RNA payload among particles. The empty LNP formulation contains a negligible concentration of fluorescent particles compared to the loaded formulations, as expected for this control.

Figure 3 shows the expected linear response of fluorescent particle concentration with dilution factor as the High EE LNPs are diluted into the empty LNP control sample.

Discussion:

By accurately measuring the size, concentration, and fluorescence of single particles in nanomedicine formulations such as these lipid nanoparticle based therapeutics, the ARC particle analyzer delivers insights for formulation development and quality control that cannot be obtained in any other way.

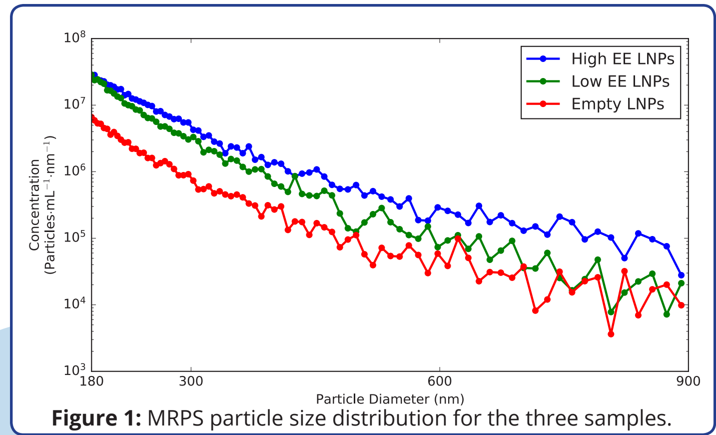

Figure 1: MRPS particle size distribution for the three samples.

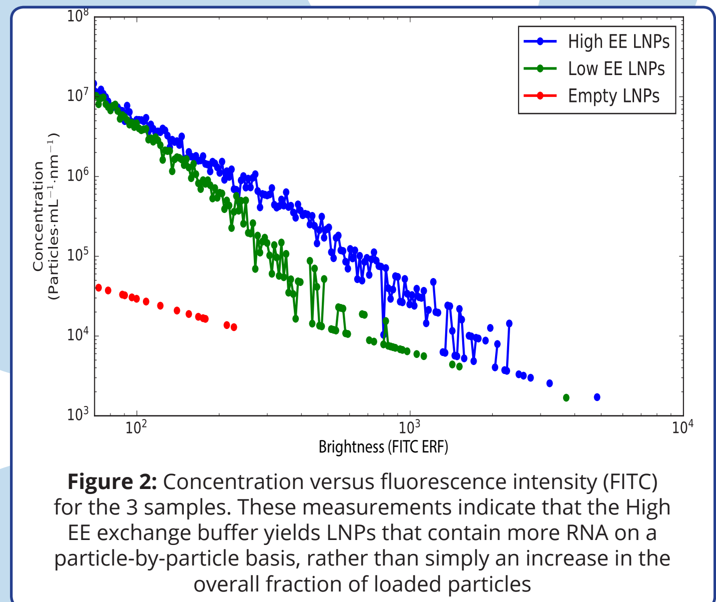

Figure 2: Concentration versus fluorescence intensity (FITC) for the 3 samples. These measurements indicate that the High EE exchange buffer yields LNPs that contain more RNA on a particle-by-particle basis, rather than simply an increase in the overall fraction of loaded particles

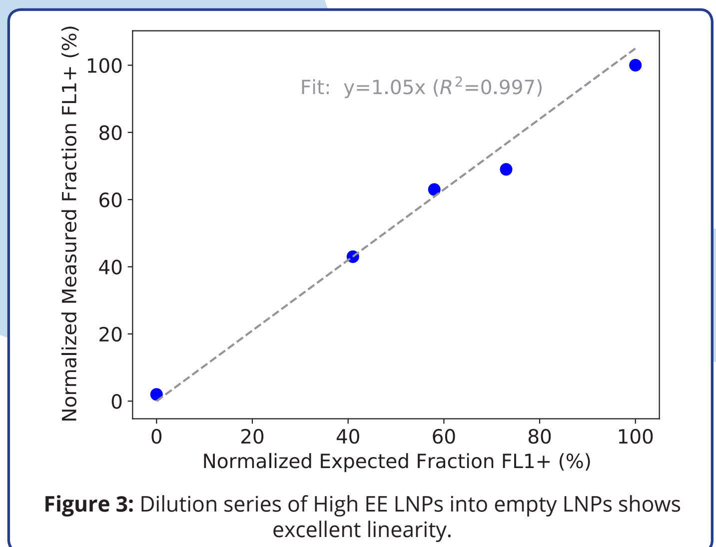

Figure 3: Dilution series of High EE LNPs into empty LNPs shows excellent linearity.

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