Spectradyne®
Particle Analysis

Measurment of mRNA payload in Lipid Nanoparticles (LNPs) with Spectradyne's ARC™ Particle Analyzer

Three LNP formulations were measured after staining RNA payload with a membrane permeable dye, demonstrating:

1. Spectradyne's ARC particle analyzer can differentiate between particles with payload and empty particles within the same sample.
2. Efficacy of payload uptake in LNPs as small as 65nm can be directly measured and compared.

Experimental Design:

Three LNP formulations were prepared using different preparation conditions. All were loaded with an mRNA payload.

The RNA payload was fluorescently labeled with a membrane-permeable intercalating nucleic acid stain according to manufacturer's instructions. Particle size, concentration and fluorescence were measured using Spectradyne's ARC particle analyzer.

Results:

Figure 1: Microfluidic Resistive Pulse Sensing (MRPS) particle size distributions for all particles in each sample, showing median size and concentration for each. The three samples show only slight differences.

Figure 2: Distribution of payload brightness for the three samples. Sample C shows the lowest concentration of payload-containing particles, followed by Sample A and Sample B, in increasing overall concentration. The dotted vertical lines show the median brightness (MFI) for each sample. The inset scatterplot shows payload brightness vs diameter for each particle measured using absolute, translatable units. Each dot on the scatterplot represents a single particle with measurements taken for size, concentration and fluorescence, all in absolute, referencable units.

Figure 3: The scatterplot in Figure 2 is used to gate all particles having payload brightness >30 FITC MESF, and the resultant particle size distributions now represent absolute concentration of only the LNPs containing mRNA above the detection limit. The median size and concentration for each sample is also shown.

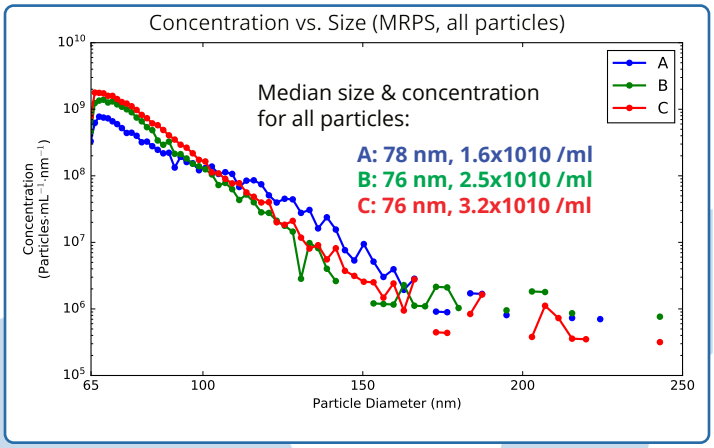

Figure 1

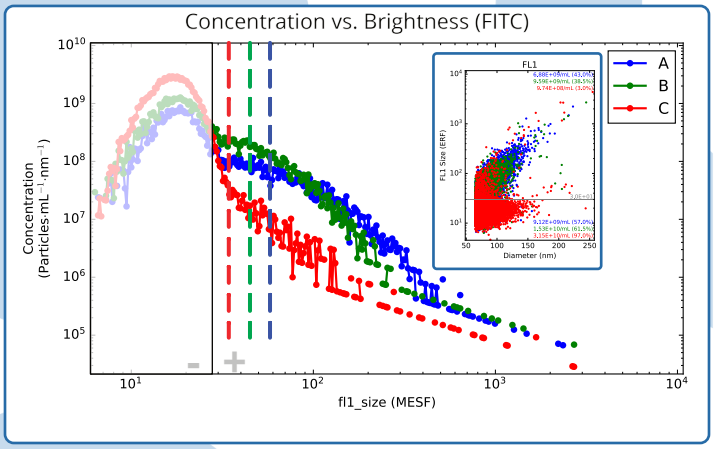

Figure 2

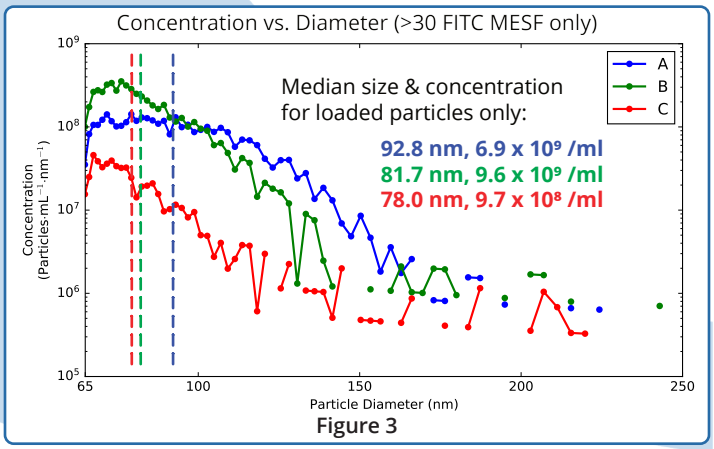

Figure 3

Discussion: The ARC delivers accurate measurements of nanoparticle size, concentration and single-particle payload. This type of quantitative analysis, in which the RNA loading into LNPs is assessed on a particle-by-particle basis, cannot be obtained using any other commercial technology at this size scale, and represents a much more direct measure of therapeutic dose than can be obtained using conventional loading assays such as Ribogreen assessment of bulk RNA. This powerful data can be used to determine the most effective loading strategies for nanoparticle-based RNA therapeutics.

info@spectradynellc.com