DAISY nanoparticle tracking analysis of Lipid nano-particles and liposomes

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Introduction

Lipid-based nanocarriers such as liposomes and lipid nanoparticles (LNP) are clinically proven delivery vehicles for a range of therapeutic agents. Liposomes, consisting of one or more lipid bilayers surrounding an aqueous core, can encapsulate a wide variety of compounds, increasing their bioavailability, stability, and target tissue delivery. As a result, this versatile delivery approach has been used in clinically approved small molecule and protein-based treatments for a range of diseases and illnesses. LNPs are particularly useful for nucleic acid-based drug delivery. Poor cell membrane permeability and susceptibility to degradation means that nucleic acids require a delivery vehicle to be effective. In nucleic acid LNPs, the negatively charged nucleic acids are complexed with positively charged lipids within the core of the particle, offering protection from degradation and improved cellular entry[1,2].

The importance of particle size for lipid-nanocarrier based treatments is well understood. It is also increasingly clear that internal particle structure influences efficacy and safety. Thus, there is a clear and growing need for analytical approaches allowing accurate characterization of lipid nanocarrier size and structure.

In this application note we describe how DAISY nanoparticle tracking analysis (**DAISY-NTA**) provides new insights into lipid nanocarrier preparations.

Contents:

Highlights

- DAISY measures individual particles to provide accurate size, concentration, polydispersity, and refractive index
- DAISY reliably quantifies LNPs, down to 50 nm in diameter
- DAISY can detect small shifts in hydrodynamic size (10 - 20 nm) following surface functionalisation
- DAISY can differentiate large particles from aggregates
- DAISY provides information on internal mass distribution of particles, allowing discrimination between hollow (liposomal) and more homogenous (LNP) particles

Figure 1. Structures of commonly used lipid-based nano-carriers

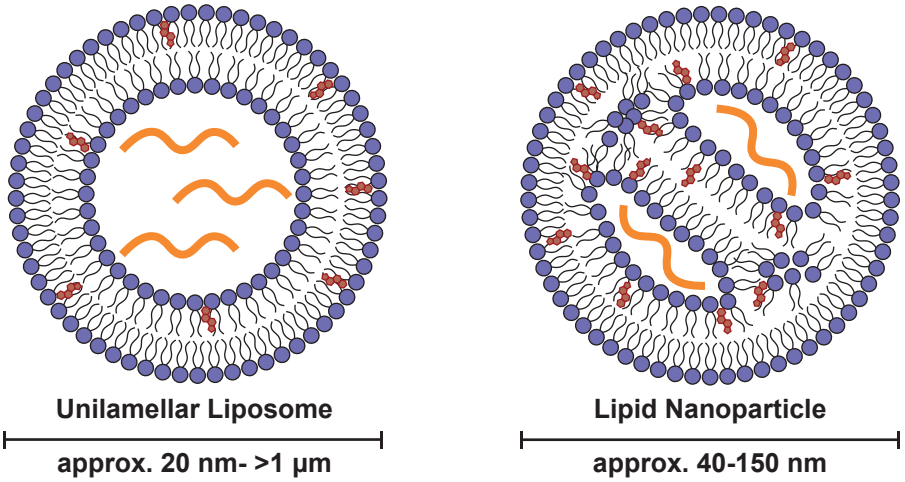
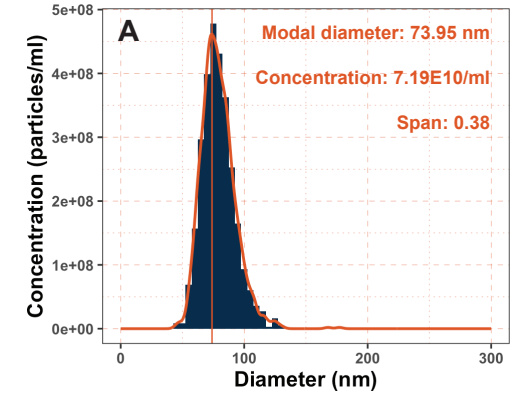
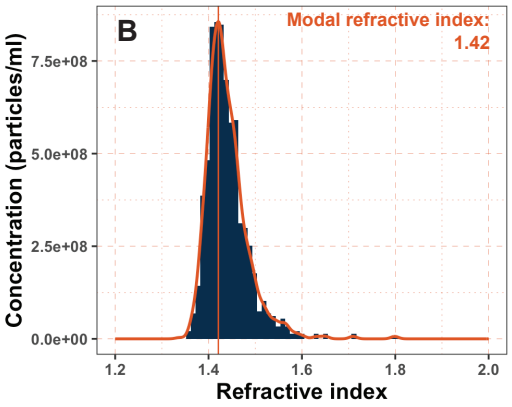


Figure 2. Size, concentration, polydispersity, and refractive index of LNP preparations



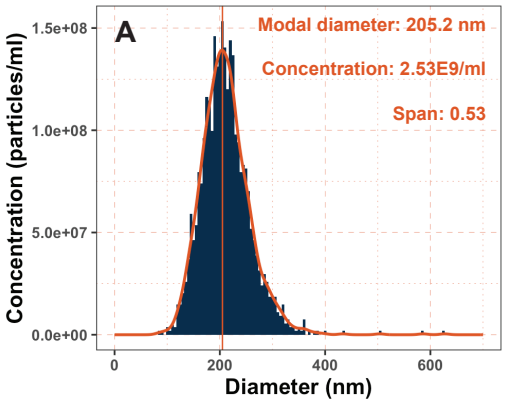


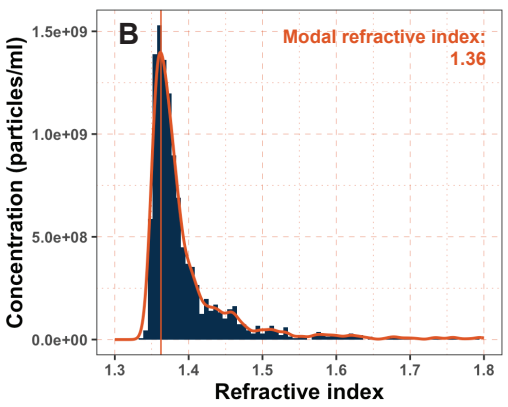
Both size and polydispersity are critical quality attributes for LNP preparations with effects on efficacy and safety. DAISY-NTA accurately quantifies LNP particle size down to 50 nm (**A**). As DAISY tracks and analyses individual particles it is well suited to analyse both mono- and polydisperse samples. The span value reflects the polydispersity of the sample, with monodisperse suspensions giving a span value less than 1. As sample polydispersity increases, so does the span value. With DAISY you you can accurately determine the size, polydispersity, and concentration of your particles with one simple measurement.

Span - Quantifies sample polydispersity	
Span value range	**Sample polydispersity**
0 - 0.5	Highly monodisperse
0.5 - 1	Monodisperse
> 1	Polydisperse

In addition to size distribution and concentration, DAISY-NTA reports the refractive index distribution of your particles (**B**). For LNP samples, the refractive index is directly related to water content, which in turn is related to LNP structure and cargo loading.

Figure 3. Size, concentration, polydispersity, and refractive index of liposome preparations

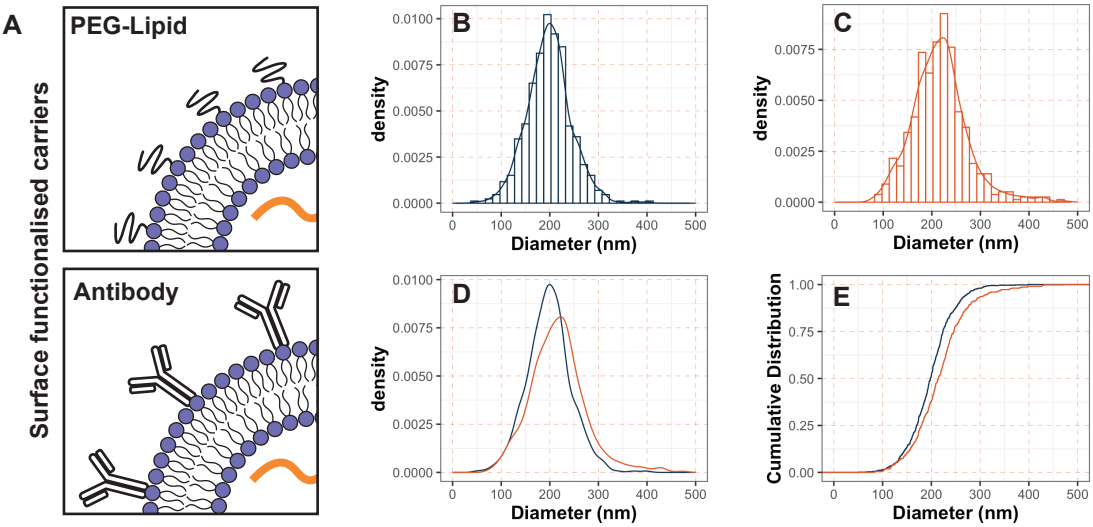




Liposome size and polydispersity have direct consequences for tissue distribution, efficacy, and safety. DAISY-NTA accurately reports size distribution, polydispersity, and concentration derived from a single measurement. Panel **A** shows a DAISY analysis of nominal 200 nm diameter liposomes. The modal particle diameter is assessed to be 205.2 nm. The sample is monodisperse, with a span value of 0.53.

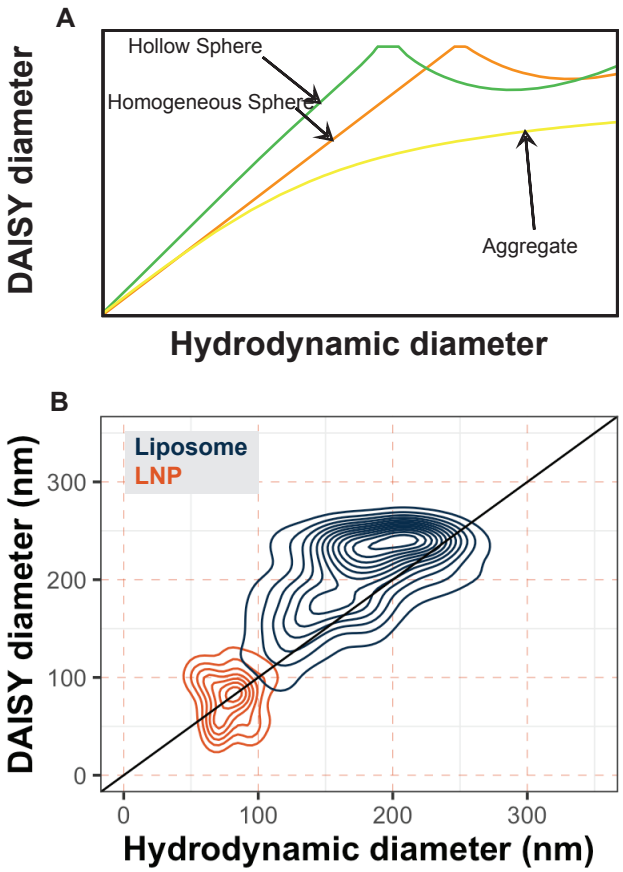
Direct measurement of particle refractive index (**B**) shows that liposomes have a lower refractive index than LNP (above). This reflects their hollow nature, with an aqueous filled core surrounded by lipid membrane. Note that despite their relatively low refractive index, DAISY is able to accurately quantify liposome size below 100 nm (see detection limits, Figure 6, below).

Figure 4. Assessing surface functionalisation



Surface modification of lipid nano-carriers is widely applied to alter their biodistribution and function[2]. Commonly used modifications include the addition of PEG lipids or specific antibodies (**A**). Adding molecules to the surface of the particles in this way increases their hydrodynamic diameter. DAISY-NTA has sufficient sensitivity to detect these small shifts in particle diameter at the population level. Above is an example of 200 nm nominal diameter liposomes, before (**B**) and after (**C**) the addition of antibody to their surface. The modal diameter shifts from 199.5 nm to 224.3 nm, in line with the expected size increase due to antibody association (**D**). A cumulative distribution plot shows the rightward shift in particle diameter following antibody addition (**E**).

Figure 5. Assessing mass distribution with DAISY

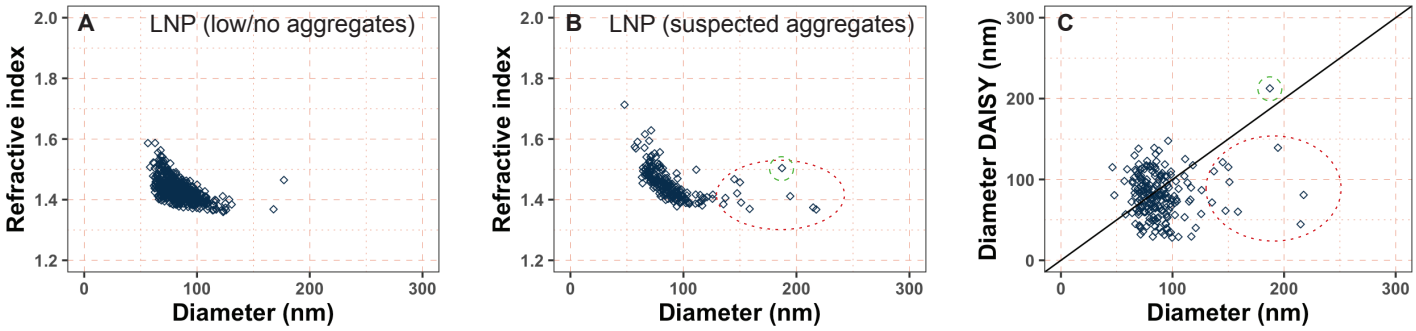


DAISY-NTA is unique in measuring both hydrodynamic size and purely light scattering-derived optical size (the DAISY diameter). The relationship between these two size measurements follows a strict 1 to 1 relationship for particles with homogenous internal mass distributions, i.e. solid spheres. However, when particle mass and geometry differ from this solid sphere model the relationship between DAISY diameter and hydrodynamic diameter deviates in predictable and quantifiable ways (**A**). Particles such as vesicles with a mass distribution biased towards the periphery will scatter light more intensely, giving a measured DAISY diameter higher than the hydrodynamic diameter. Conversely, particles with a relatively lower mass density at the periphery, such as aggregates of proteins or vesicles will scatter light less strongly than a solid sphere of the same hydrodynamic diameter.

This unique feature of DAISY-NTA is clearly demonstrated when comparing hollow liposomes to more homogenous LNPs. Liposomes are distributed above the 1 to 1 line, due to their vesicular structure, whereas LNPs fall exactly on the line (**B**).

It is increasingly clear that differences in LNP internal structure as well as size can have dramatic effects on function[3]. Integrating DAISY-NTA into routine LNP analytics will give vital insights into size, concentration and LNP structure in a single fast and straightforward assay.

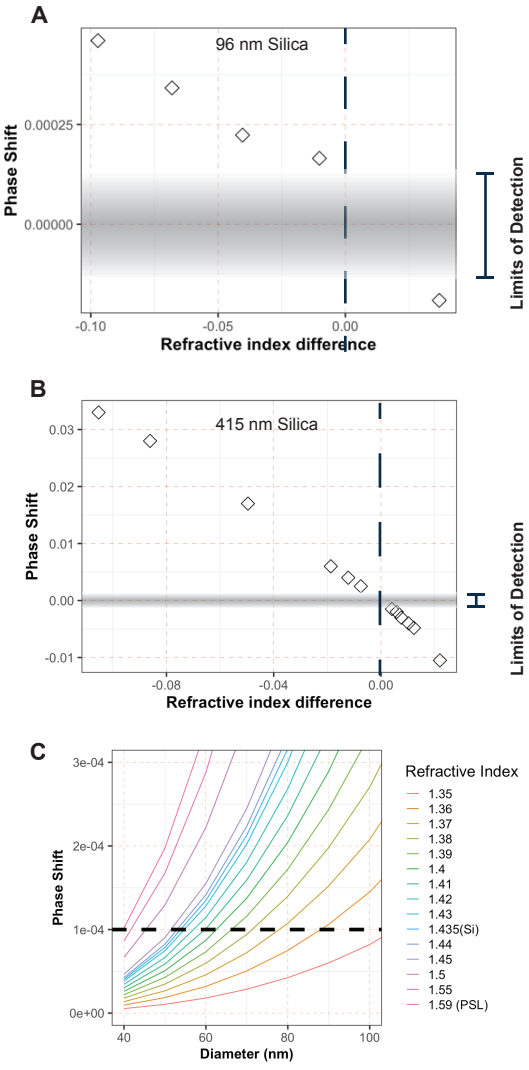
Figure 6. Detecting and identifying aggregates



Aggregation of lipid nanocarriers is detrimental to safety and efficacy. Detection and monitoring of aggregation is therefore vital. DAISY-NTA not only detects larger particles, but can also differentiate between nanocarrier aggregates and other contaminants. Above we show two LNP preparations, one with little to no aggregation present (**A**), and one with suspected LNP aggregates (**B**). As would be expected for aggregates, these larger particles largely show a decreased refractive index when compared to the bulk population (red ellipse in **B**). Furthermore, DAISY analysis shows that most of these larger particles do indeed show aggregate-like geometry (**C**). Note however that one particle, circled in green, appears to have a relatively high refractive index, and shows a homogenous mass distribution, suggesting that this particle is not an LNP aggregate.

Thus, DAISY is able to not only detect aggregates but also examine their physical properties to define their make-up.

Figure 7. Detection limits



As with any light scattering microscopy-based approach, the detection limits of DAISY are related to particle size and refractive index. Altering the refractive index of the surrounding media, and therefore the effective refractive index of the particles, objectively demonstrates the detection limits of our instrument.

Our experimentally determined detection limit, in terms of refractive index difference, for 96 nm particles is ~0.01 refractive index unit (**A**). For larger particles, such as 415 nm silica (**B**) this detectable refractive index difference drops to 0.005 refractive index units or below.

Combining this experimentally derived information with well validated optical simulations (**C**) demonstrates detection limits in terms of size for particles with a wide range of refractive indices.

Rigorous experimental determination of precise detection limits allows researchers to make a fully informed choice about the suitability of DAISY-NTA for their samples.

References

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