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CHARACTERISING LIPID NANOPARTICLES WITH FIDA: SIZING, PDI, AGGREGATION, INTERACTIONS, VISCOSITY, AND BINDING CAPACITY

Emil G. P. V. Stender, Ph.D.[1], Kritika S. Ray, Dr. rer. nat.[1], Adam Coln Hundahl, Ph.D.[1] and Henrik Jensen, Ph.D.[1]

[1] Fida Biosystems, Copenhagen (Søborg), Denmark, www.fidabio.com, e-mail: techsupport@fidabio.com

Background

Lipid nanoparticles (LNPs) are pivotal in biotechnology and pharmaceuticals, offering promise for drug delivery applications within vaccines and personalised medicine.

To optimize LNPs, comprehensive characterisation is essential, encompassing physical, chemical, and biological properties like particle size, stability, viscosity and interactions. Traditional methods include light scattering for sizing and stability, surface plasmon resonance (SPR) or mass spectrometry for interactions, and viscometry for rheological properties.

In this poster, we present data illustrating the use of Fida Neo for comprehensive characterisation of lipid nanoparticles (LNPs), including size, polydispersity, and aggregation state analysis in simple buffers and serum/plasma environments.

Additionally, we demonstrate FIDA's application in studying LNP interactions by investigating the binding of an anti-PEG antibody to LNPs. This analysis yields affinity constants and elucidates concentration-dependent changes in LNP sample viscosity. Finally, we quantify the average surface binding capacity of LNPs for anti-PEG antibodies.

Method

Flow Induced Dispersion Analysis (FIDA) is a 1st principle technique that provides information of the hydrodynamic radius, polydispersity, and aggregations state of Lipid Nano Particles (LNPs). It works by introducing a 40 nL plug sample (indicator) into an analyte. The indicator and analyte are then mixed by pressure driven flow dispersion. The analyte can be any liquid, be that simple buffers, interaction partners, or serum/plasma.

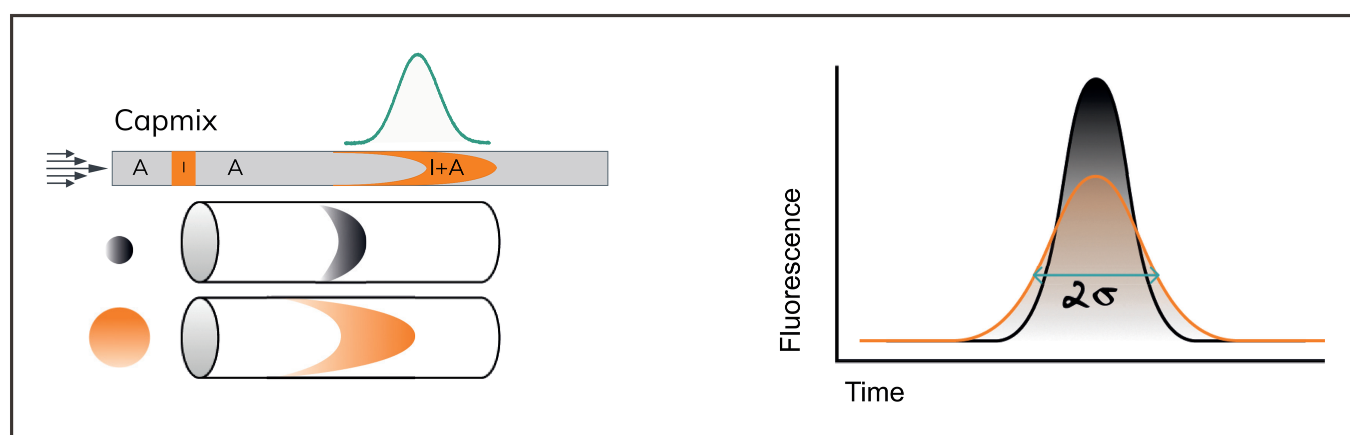
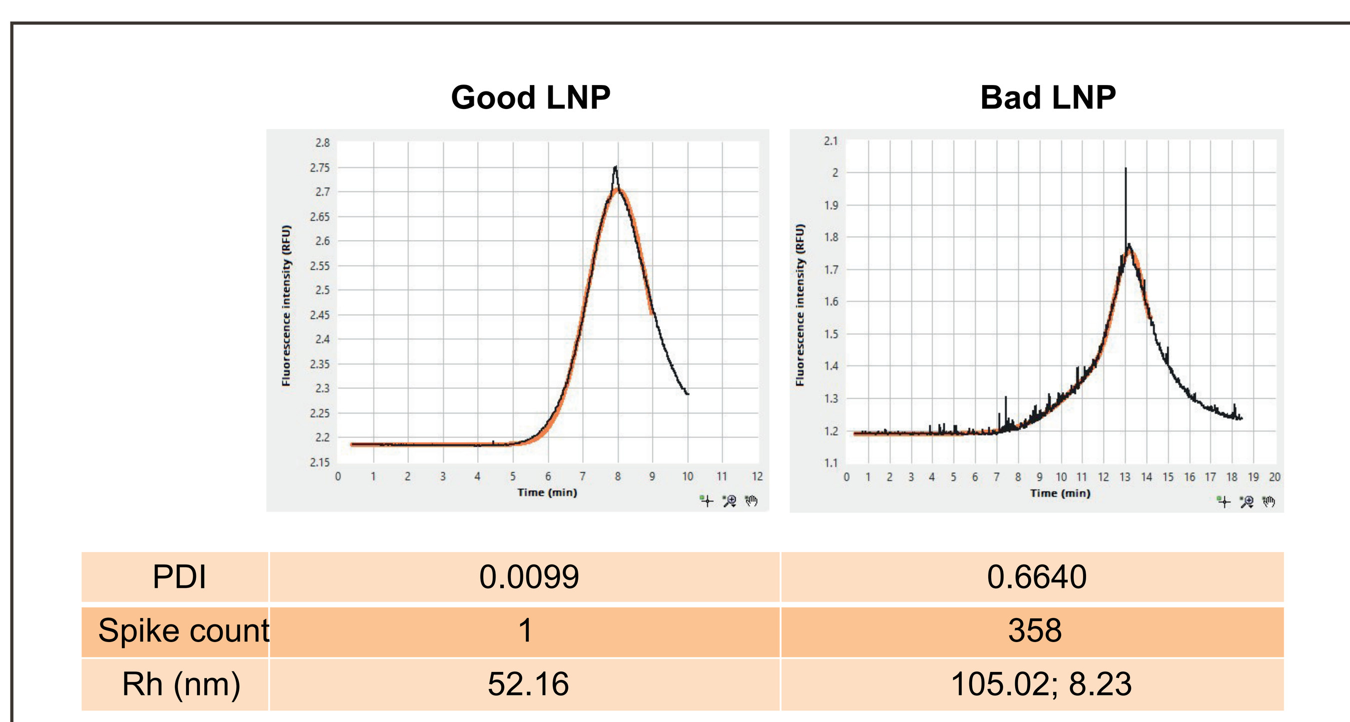


Figure 1: Principle of FIDA, small molecules will have a sharp peak, large molecules will have a broad peak.

Results

In this poster we will demonstrate how to use Fida to characterise and QC LNPs, their interactions, viscosity as well as their binding capacity of biomolecules using a single platform.

Fluorescently labelling an LNP with e.g. C11 TopFluor® Sphingomyelin allows for characterising, sizing, aggregation and polydispersity index in any liquid including serum/plasma and assessing the impact on LNP quality using nL of sample.



	Good LNP	Bad LNP
PDI	0.0099	0.6640
Spike count	1	358
Rh (nm)	52.16	105.02; 8.23

Figure 2: Data example of a good quality monodisperse LNP and a poor quality polydisperse and aggregated LNP.

Binding to LNPs can be characterised in a labelled (serum/plasma) or label free manner (simple buffers) by using a binding partner as the indicator and varying the total concentration of LNP in the analyte. This experiment yields the binding behaviour of the protein, the affinity constant against the LNP and the concentration dependent viscosity in the same experiment.

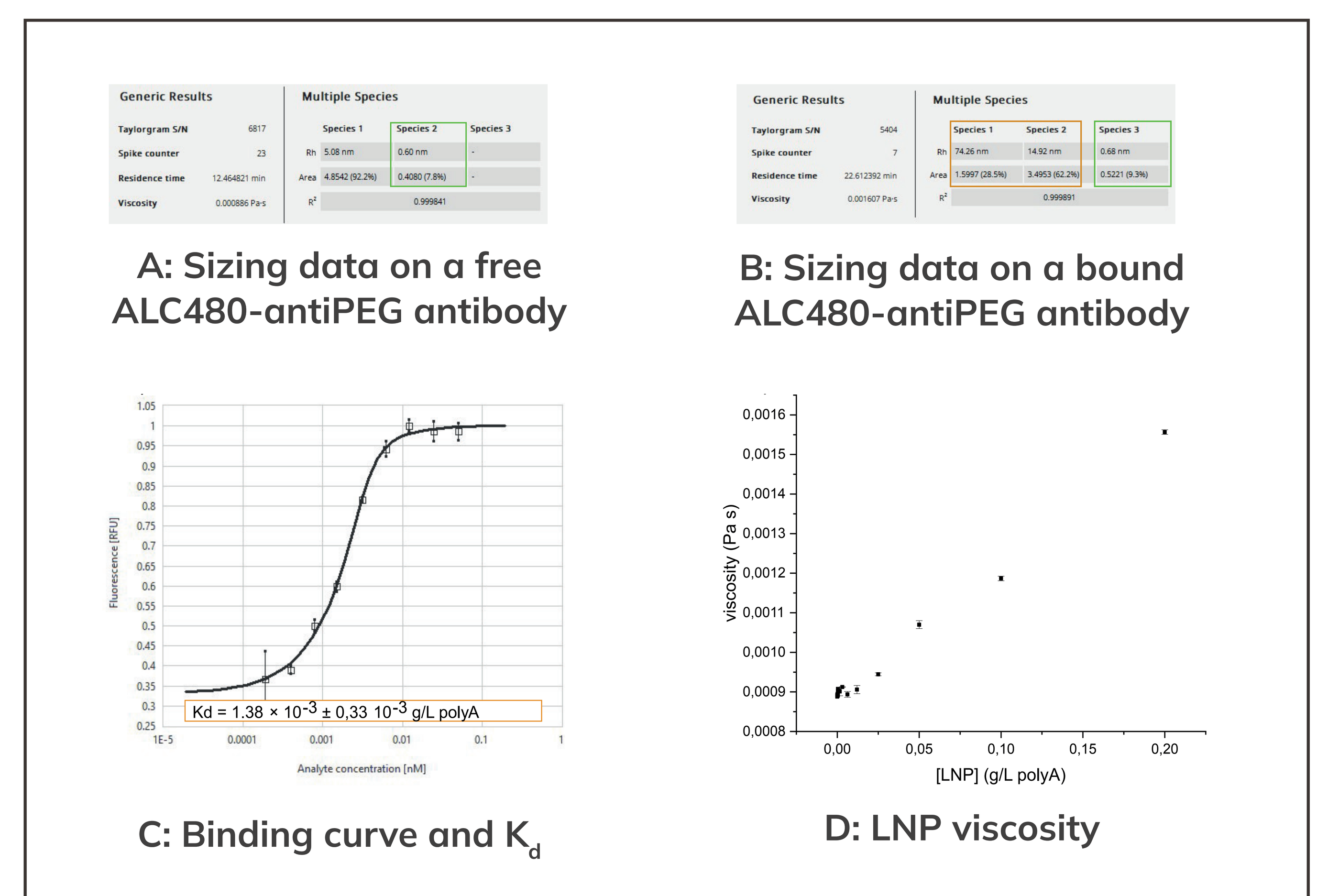


Figure 3: Binding characterisation of LNPs.

The binding capacity for target molecules on the surface of LNPs have long eluted researchers, this parameter can now be characterised using only 20 nL of LNP at a fixed concentration per data point and allowing the Fida Neo to mix it in buffer with a titration of a labelled binding partner. Plotting the fluorescence of the free and bound binding partner can be fitted to two straight lines, their intercept gives the binding capacity of that concentration LNP.

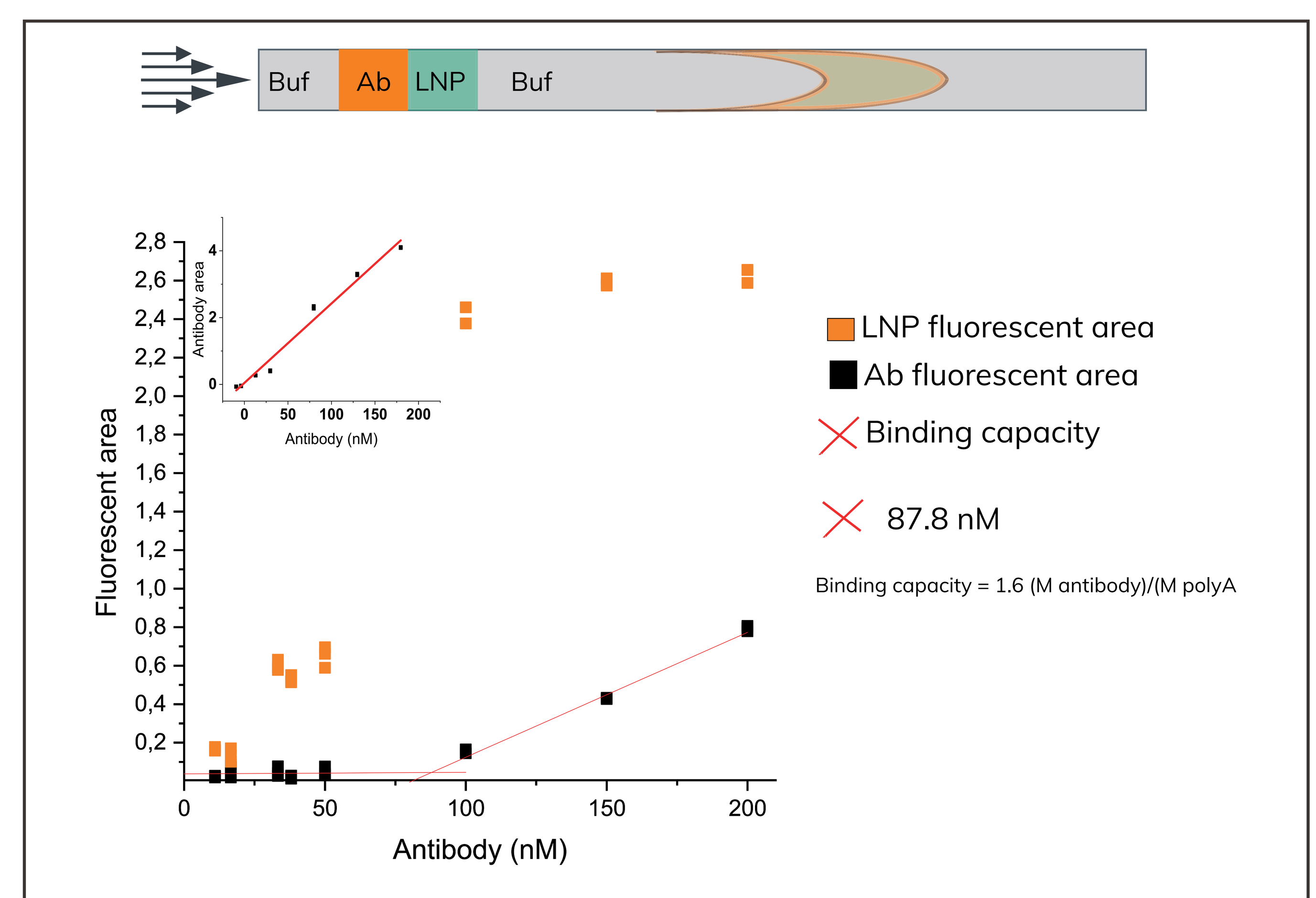


Figure 4: Characterisation of binding capacity. The insert is the antibody titration without LNP present. The binding capacity is calculated assuming an average molecular weight of polyA.

Conclusions

FIDA is a versatile technique for LNP characterisation, providing insights into size, PDI, aggregation, interactions, affinity constants, and binding capacity. Requiring minimal sample volumes, FIDA is effective in both simple and complex biological matrices like serum or plasma. This streamlined method facilitates LNP optimisation facilitating effective drug delivery systems in diverse biological environments.

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