# Biophysical characterization of viral and lipid-based vectors for vaccines and therapeutics with light scattering techniques

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## Introduction

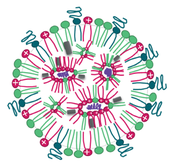
The structural complexity of viral vectors and lipid nanoparticles (LNPs) is integral to their function and successful formulation. Particle characterization is becoming increasingly important when such molecules are used as delivery platforms for nucleic acids (mRNA, DNA). During design, product development and process control, characterization and quality control of physical and chemical attributes require fit-for-purpose and complementary analytical tools.

Selecting the most suitable technique to monitor the sample will depend on: the properties of the vector, the development stage at which the measurement takes place, and the measurement's purpose. In-depth methodological expertise and an holistic approach to data analysis are required for robust measurements and to enable adequate interpretation of data. Here, the combination of complementary, label-free biophysical techniques, including dynamic light scattering (DLS), multiangle-DLS (MADLS), electrophoretic light scattering (ELS), nanoparticle tracking analysis (NTA), multiple detection SEC, and differential canning calorimetry (DSC) have been successfully used to characterize the physical and chemical attributes of viral vectors and LNPs encapsulating mRNA.[1]

## Viral and non-viral vectors used:

**mRNA-LNPs:**

**~50-150nm**

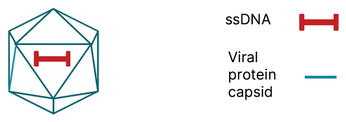



**Liposomes:**

**~30 to ~100s nm**


Multilamellar liposomes can go to µms

**Adeno associated virus (AAV):**




## Instruments and technologies used (with measured parameters):

**Zetasizer Ultra (Red)**

- Non-invasive backscatter (NIBS) DLS (Z-average, PdI)
- Multi-angle DLS (MADLS) **(size distribution, particle concentration)**
- Electrophoretic Light scattering (ELS) **(zeta potential)**

**NS 300**

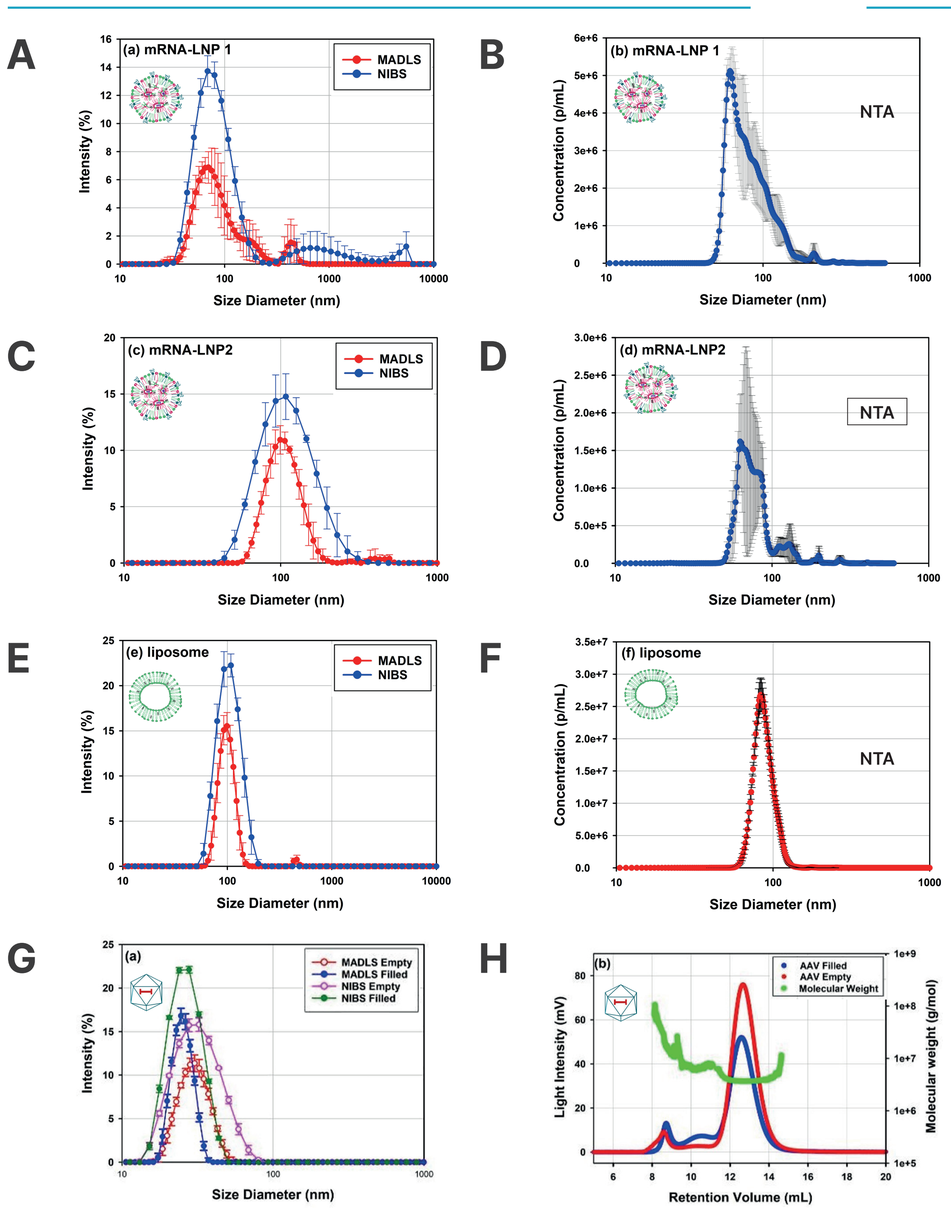
Nanoparticle tracking analysis (NTA), **(size distribution and particle concentration)**

**OMNISEC Reveal**

Size exclusion chromatography (SEC) with static light scattering (SLS), uv absorbance (UV) and refractive index (RI), SEC-SLS/UV/RI, **(molecular weight distribution, payload fraction)**

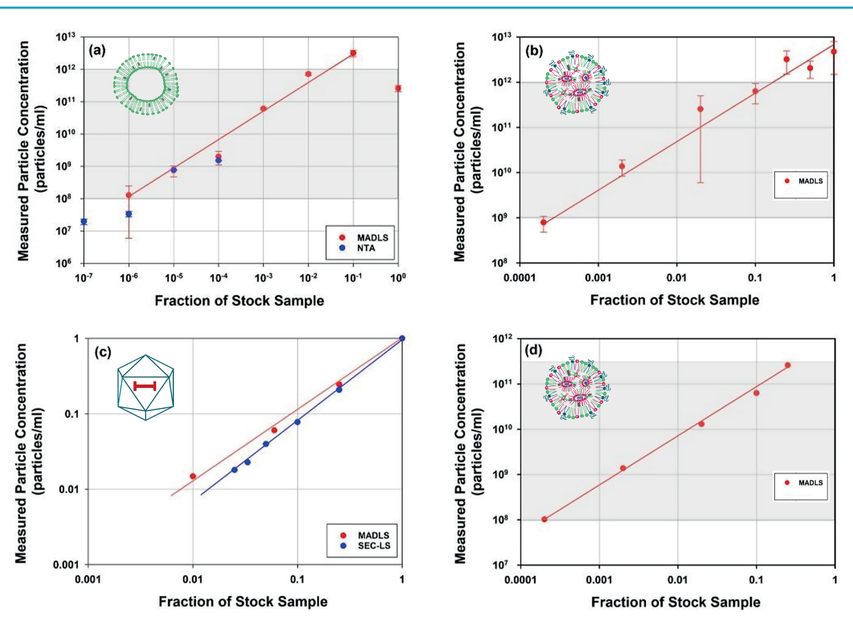
**MicroCal DSC**

Differential Scanning Calorimetry (DSC) **(thermally induced structural transitions)**



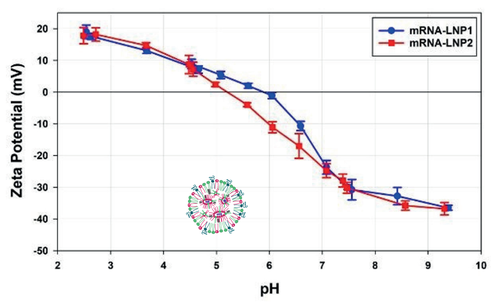

**Size distributions for viral and non-viral vectors with DLS, MADLS, NTA, and SEC-SLS/UV/RI; A/B LNP1, C/D LNP2, E/F liposome, G/H AAV**

Demonstrating a range of size distributions and polydispersities



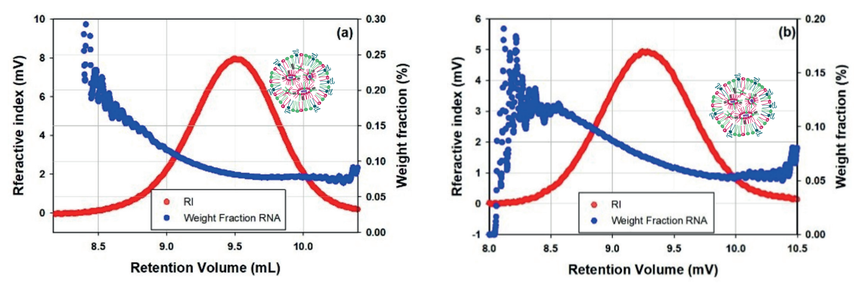

**Particle concentration determination for viral and non-viral vectors with MADLS, NTA and SEC-SLS/RI/UV**

Technology applicability depends on vector size range, which also determines accessible concentration range.




**Zeta potential can be used to infer charge of a particle in a buffer/medium**

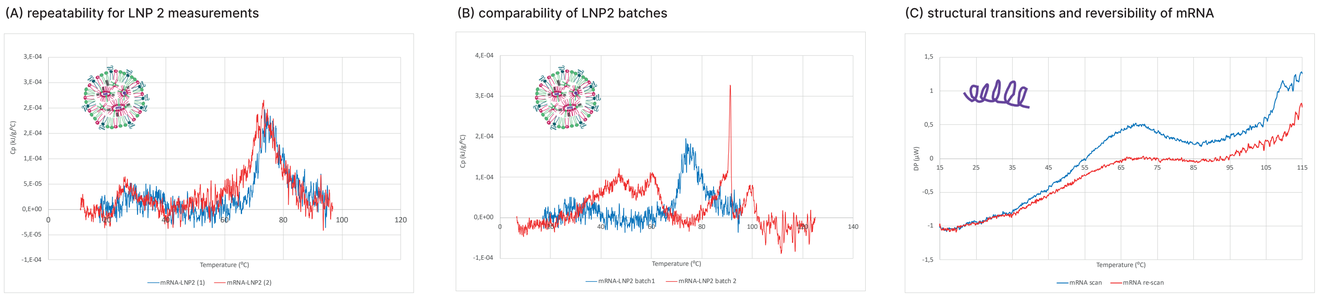
Here the MPT3 titrator was used to assess behaviour across the full pH range (pH2.5-9.5). LNP1 and LNP2 differ only in the ionizable lipid used, which shows in the region pH 4.5 to 7.




**Fraction of mRNA inside the LNPs measured with SEC-SLS/UV/RI, a) LNP1 and b) LNP2**

## Key conclusions

- Complementary and orthogonal techniques are critical to validate and/or add additional insight into sample behaviours
- For size and concentration measurements, the choice of analytical technology depends on vector size and its polydispersity. Also, the question the measurement should answer affects the choice, i.e. has the sample changed since the previous step, or what oligomeric states are present in the sample and how much of each?
- Zeta potential can provide information of charge in a particular buffer, or, as in this case, investigate differences across a pH range due to different lipids being used
- Separation of population and analysis of light scattering and dual concentration detectors allow quantification of lipid and mRNA components separately, as here shown as mass fraction of mRNA in the LNP population
- DSC can be used to map the thermally-induced structural transitions of complex LNP assemblies and their components, such as mRNA. DSC thermograms can serve as qualitative and quantitative fingerprints, enabling analysis of batch comparability




**Overlays of the DSC thermograms showing (A) repeatability for LNP 2 measurements, (B) comparability of LNP2 batches , (C) structural transitions of an mRNA and their reversibility**

Two aliquots of the same LNP2 sample show good reproducibility, whereas in B, the two thermograms show large differences between the batches of LNP2. Partial reversibility of structural transitions of a free mRNA in **solution is** shown in C

### References

[1] Markova N.,Cairns S., Jankevics Jones H., Kaszuba M., Caputo F., Parot J., (2021) Vaccines 10, 49

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