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Density Matching Multi-wavelength Analytical Ultracentrifugation to Measure Drug Loading of Lipid Nanoparticle Formulations

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Presented by: Zachary Robinson

Overview

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Goal: Use AUC to Determine the Loading Efficiency of LNPs

Background:

- LNP Practical Applications
- Alternative Methods of Analyzing Loading Efficiency

Methods:

- Preparation of siRNA Loaded LNPS
- MW AUC Analysis
- Density-Matching AUC Analysis

Results:

- AUC Characterization of siRNA Loaded LNPs
- Determination of LNP Loading Efficacy

Pharmaceutical Applications of LNPs

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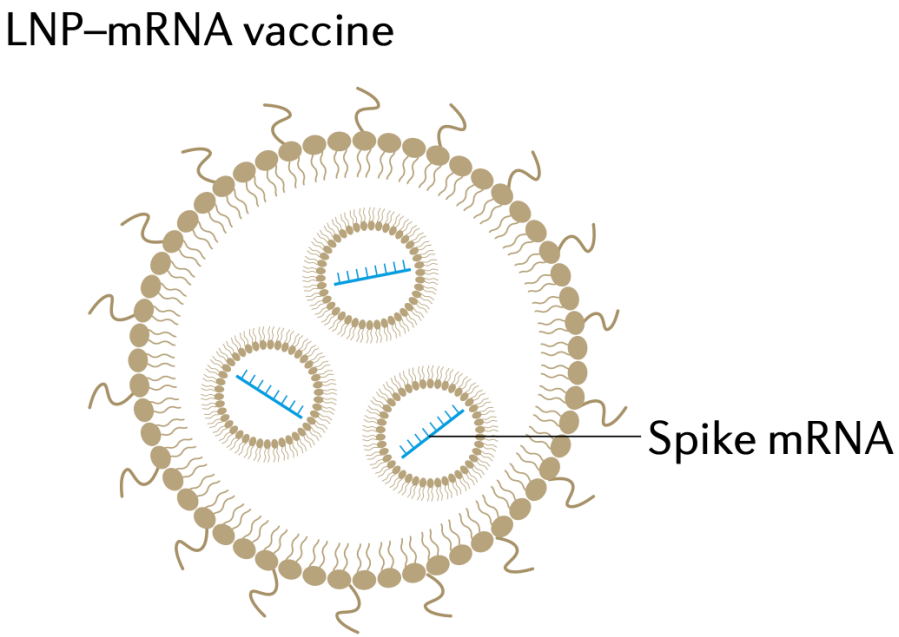
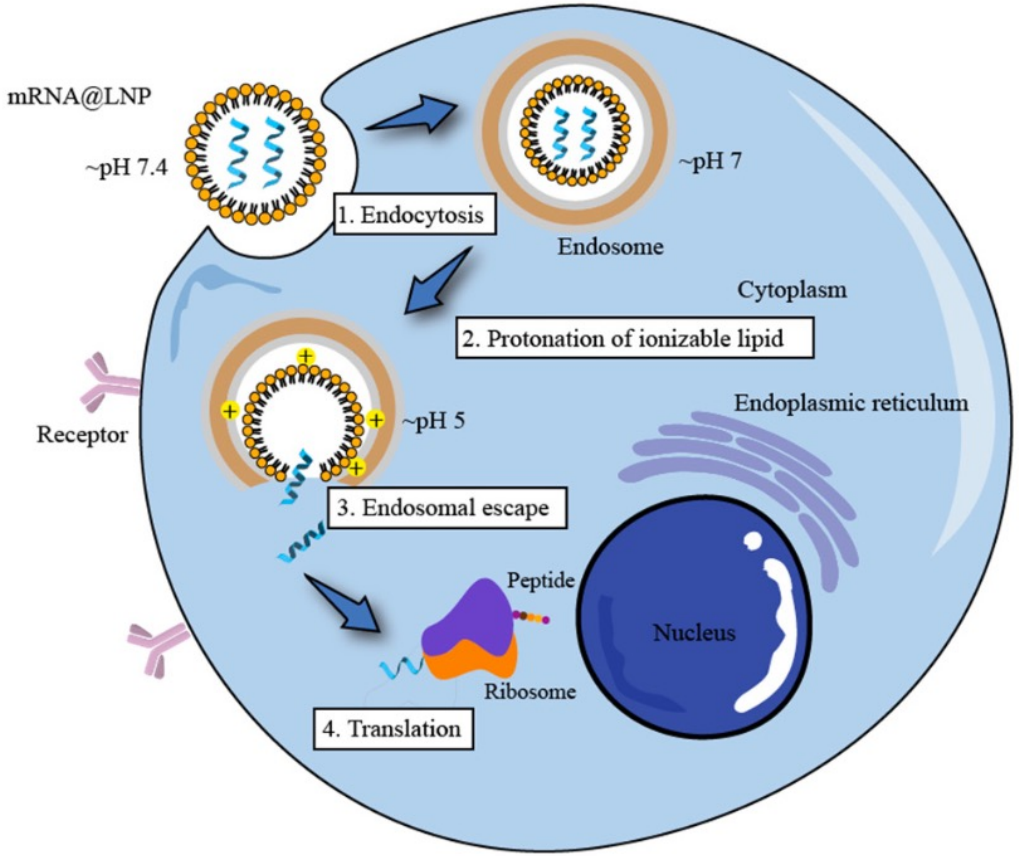
- Method used to facilitate the delivery of nucleic acids through membranes.
- Recent major application in the development of SARS-CoV-2 vaccines.

Problem: Are all LNPs loaded with RNA?



Xucheng Hou *et al,* 2021, nature reviews materials



Lei Yang *et al,* 2022, Pharmaceutics

Alternative Approaches

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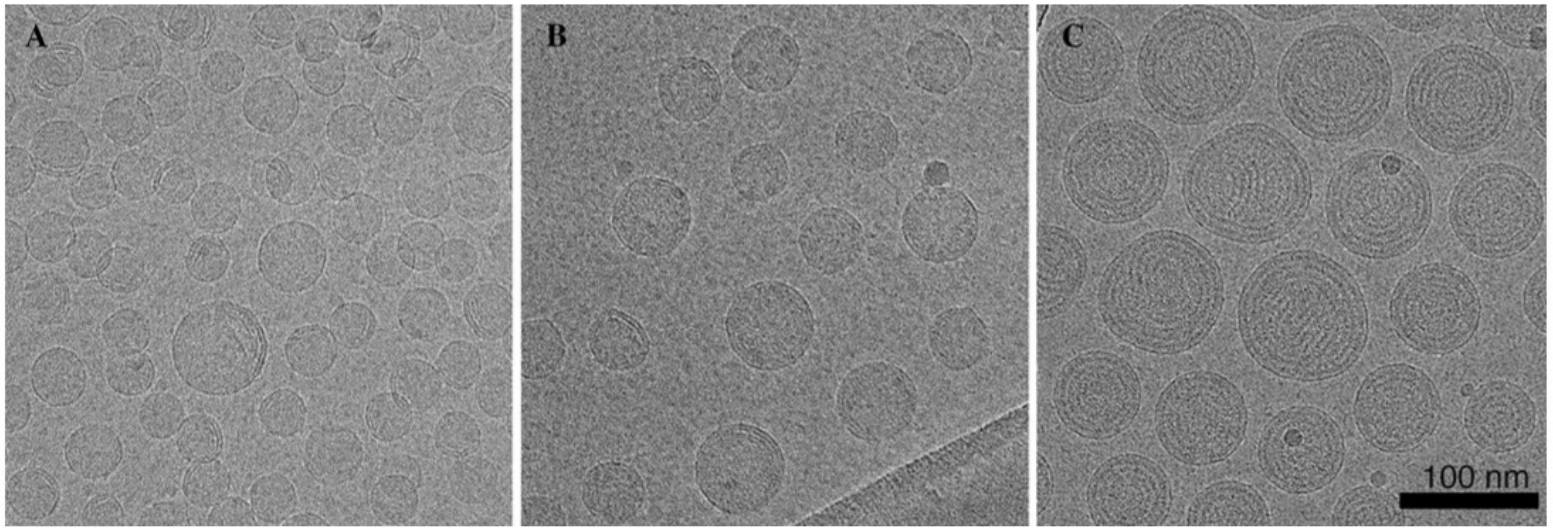
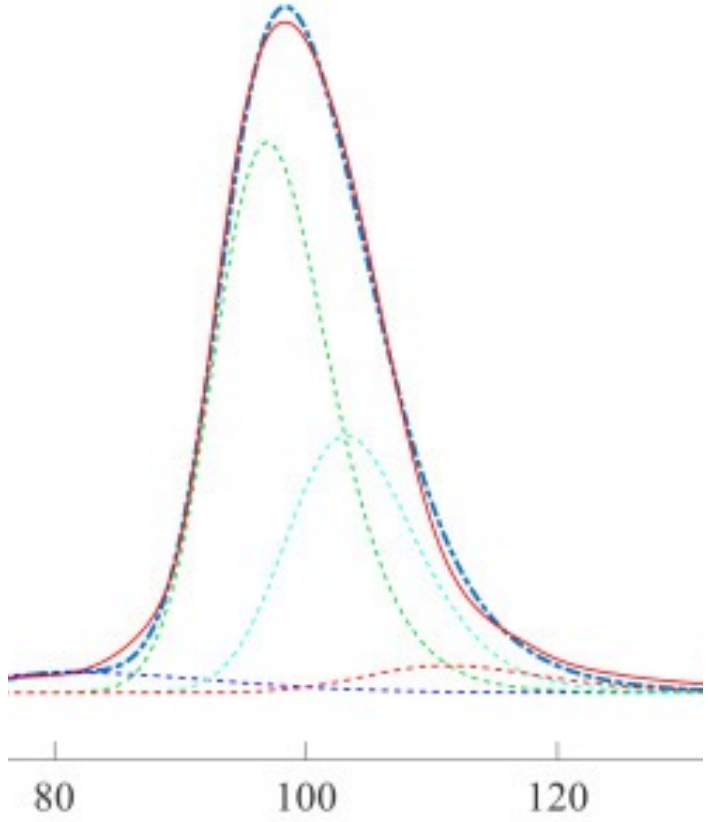
Other methods used to determine loading efficiency:

1. Spectroscopic Methods
2. Dynamic Light Scattering/ Size Exclusion Chromatography
3. Transmission Electron Microscopy



Amy Henrickson *et al,* 2021, ACS Nano



Ryunosuke Kitamura *et al,* 2022, Journal of Chromatography Open

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Preparation of siRNA Loaded LNPs

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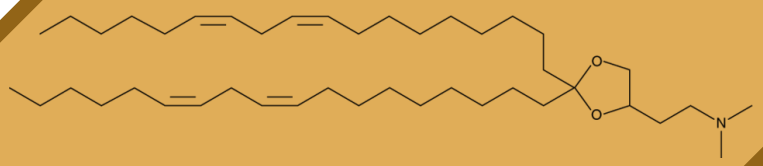
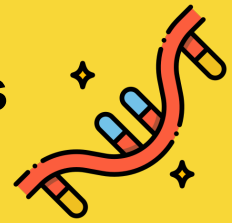
① Dissolve Lipid Components in Ethanol



② Add Aqueous siRNA



NP1: 1 Phospholipid per 1 RNA Phosphate Group

NP6: 6 Phospholipid per 1 RNA Phosphate Group

③ Dialyze into 1x PBS

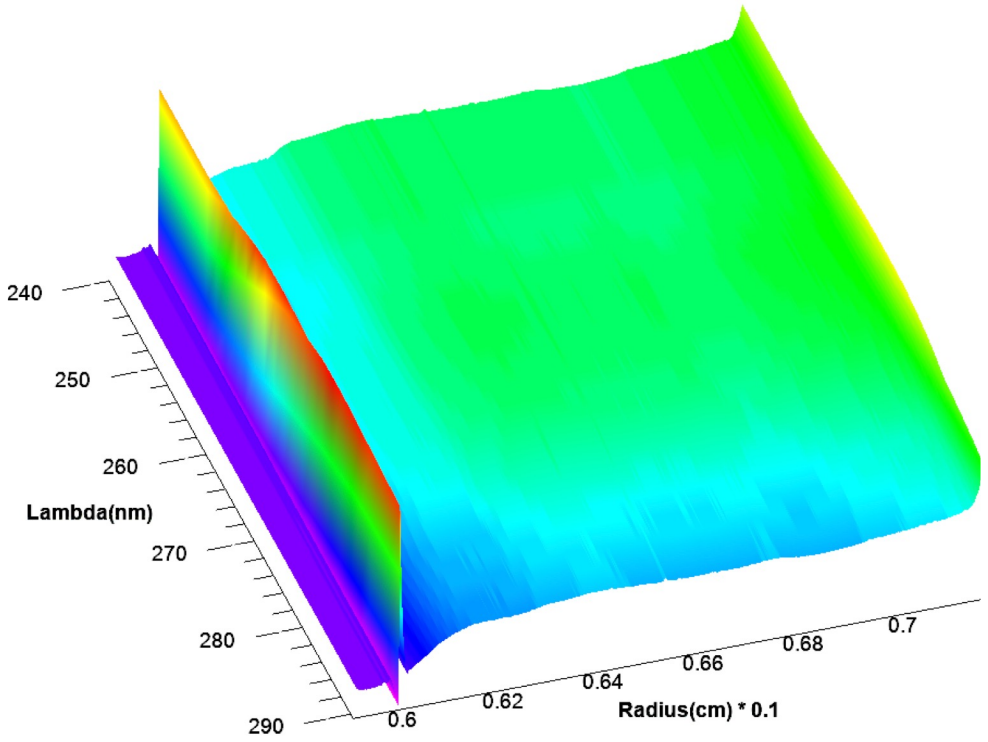
50% Ethanol → 1x PBS

④ Adjust Concentration

Final Concentration: 0.1-1.0 O.D.

Multi-Wavelength Analysis

- Lipid nanoparticle: Mie-scattering
- Nucleic acid: Absorbance at 260nm



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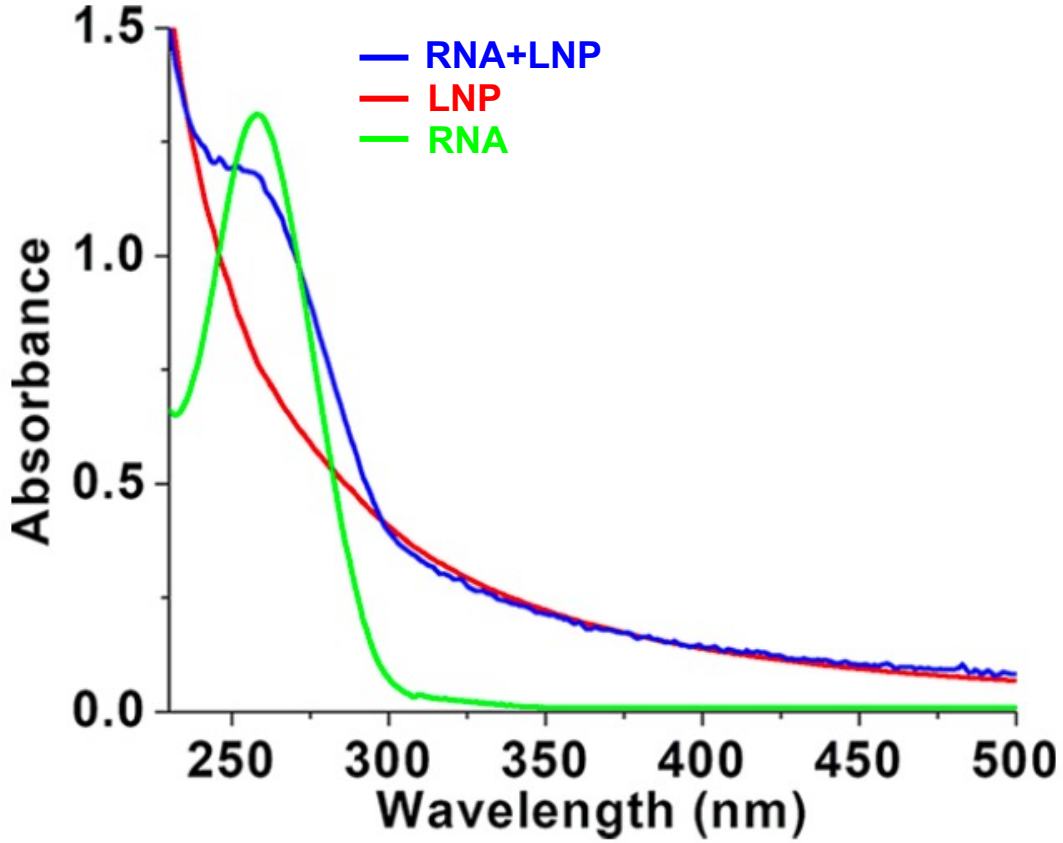


Amy Henrickson *et al,* 2021, ACS Nano

Density Matching Analysis

- Determine partial specific volume from Svedberg equation by running the sample in several buffer conditions.

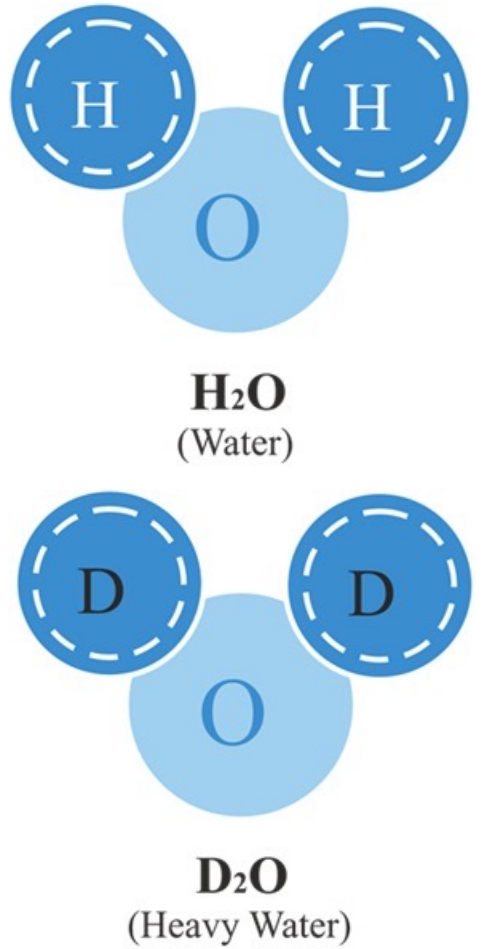
- **Limitation:** Need to be cautious of alterations to partial specific volume from hydrogen deuterium exchange

Svedberg Equation

$$s = \frac{M(1 - \bar{v}\rho)}{Nf}$$

Partial Specific Volume of Analyte

Solvent Density



Reproduced from: https://deuteriumoxide.blog.ir/tag/what%20is%20deuterium%20oxide

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Density Matching Characterization of Loaded LNPs

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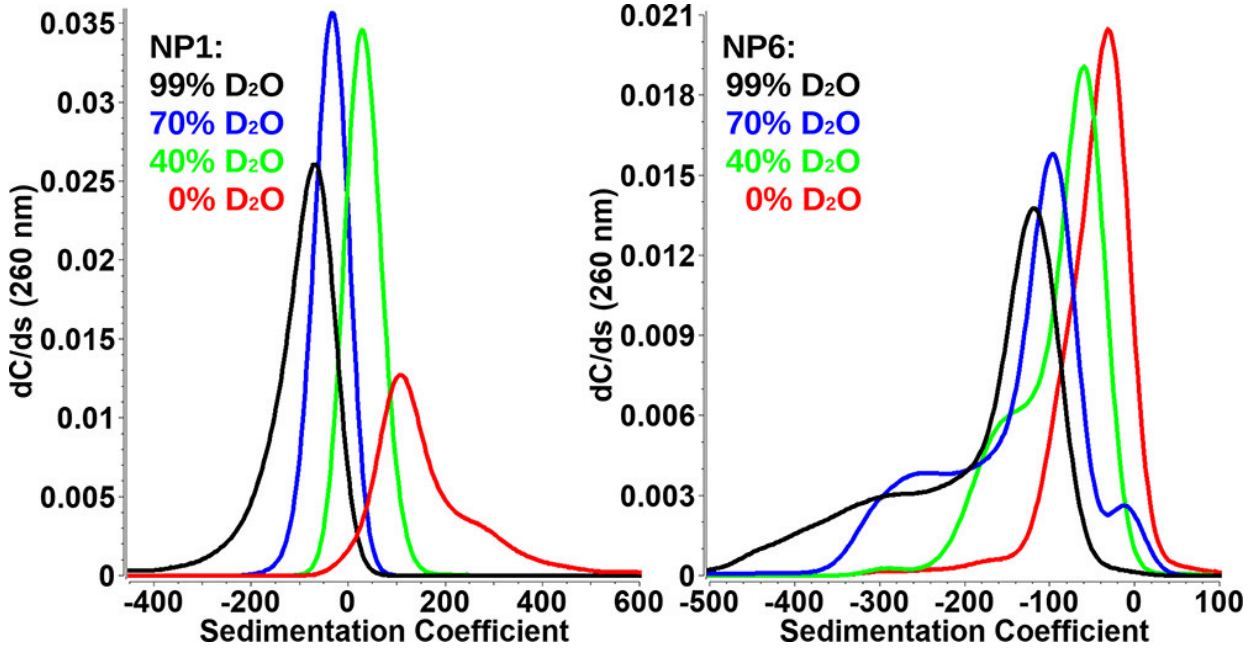
- **Step 1:** Run the loaded LNPs in conditions containing various concentrations of D_2O.



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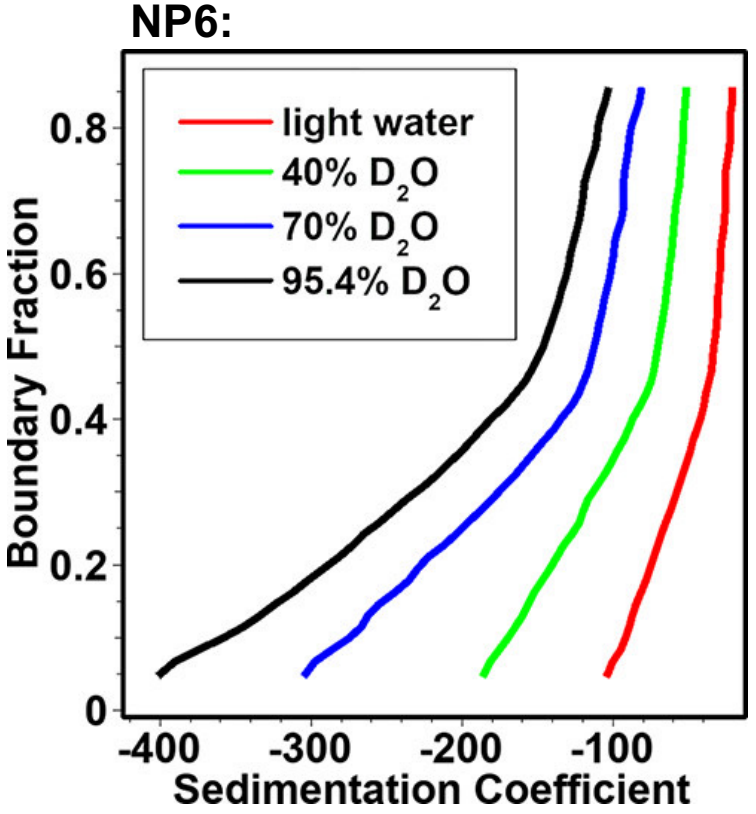

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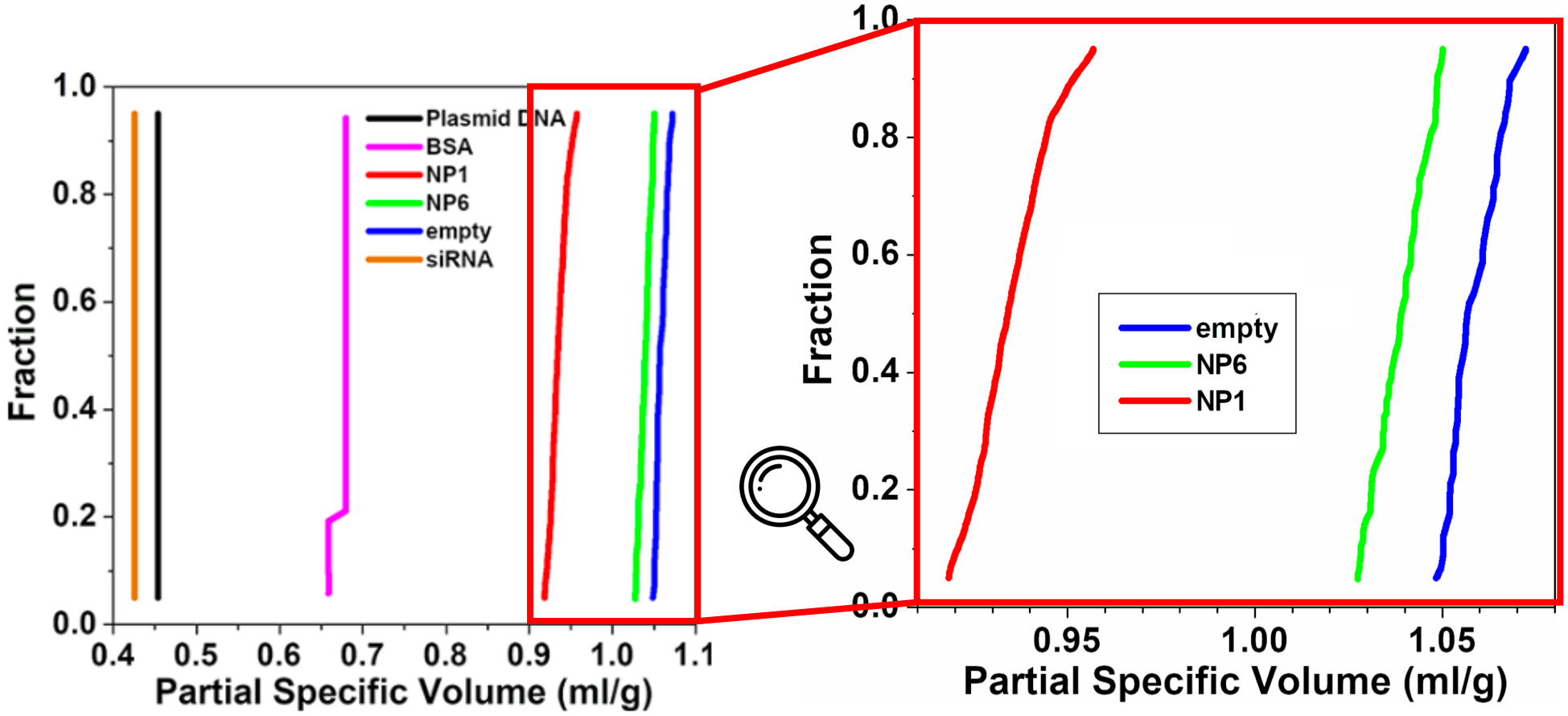
Density Matching Characterization of Loaded LNPs

- **Step 2:** Extrapolate to find the zero-sedimentation point.



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Density Matching Characterization of Loaded LNPs

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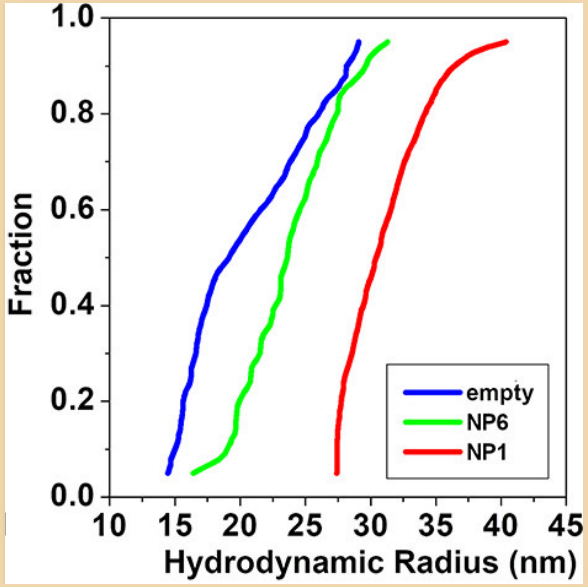
- **Step 3:** Determine anisotropy, frictional ratio, and other parameters.

AUC



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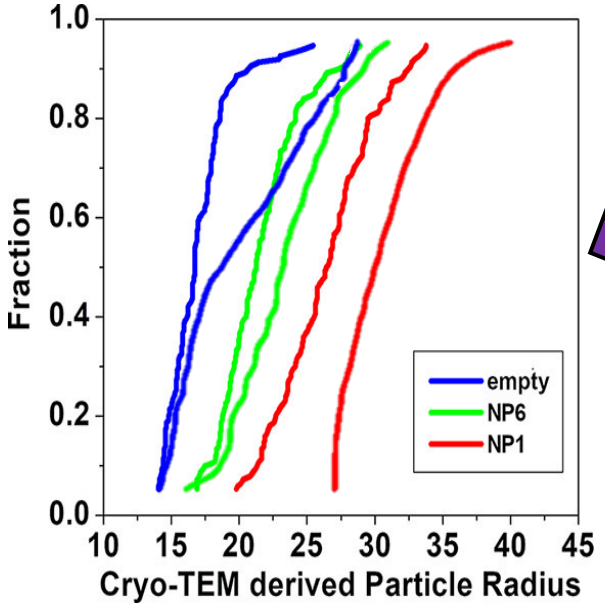
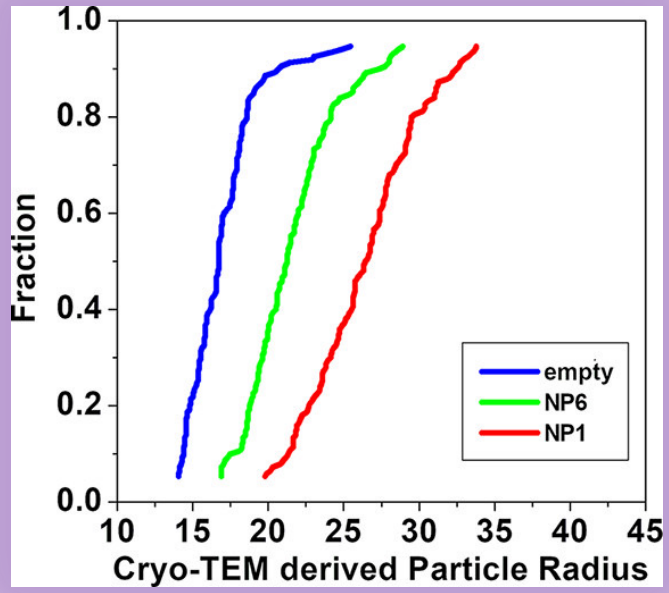


Cryo-EM



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Multi-Wavelength Analysis of Loaded LNPs

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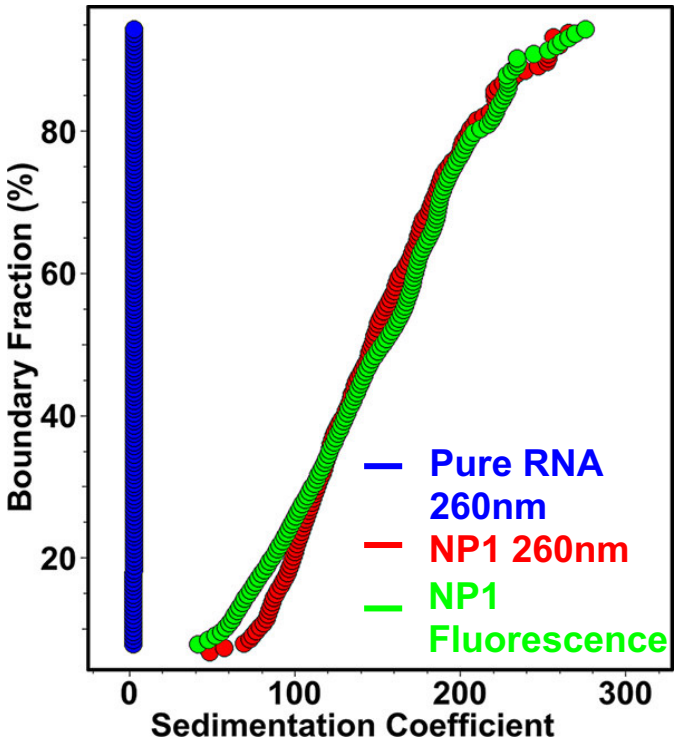
- Validated use of 260nm chromophore on siRNA by labelling fluorescently.
- MWL shows that the siRNA and LNPs sediment together.



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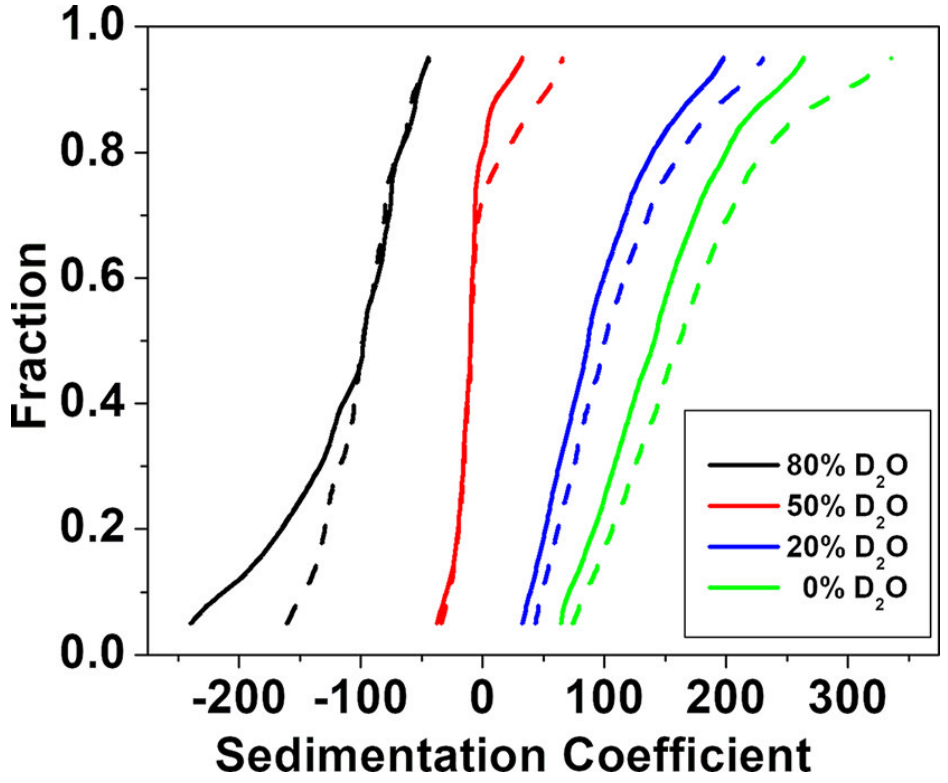

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Key Findings

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1. All siRNA incorporated in the LNPs.

2. Density matching is a valid approach for determining the degree to which nucleic acid is incorporated into LNPs.

3. Multi-wavelength analytical ultracentrifugation is another feasible approach to quantify nucleic acid incorporation.

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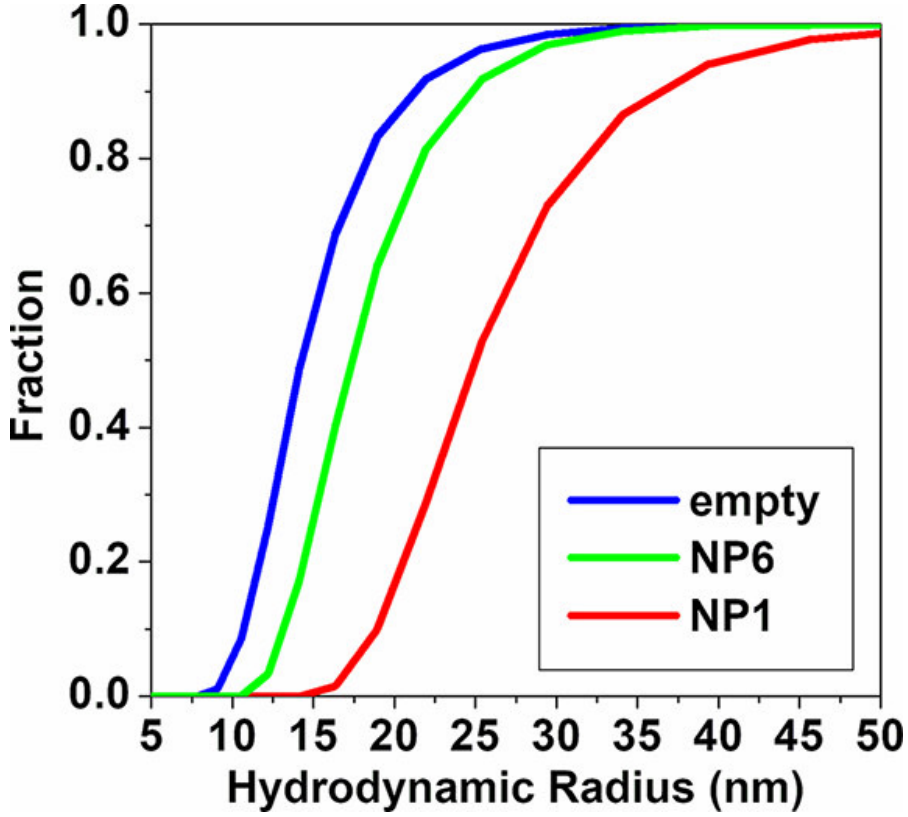
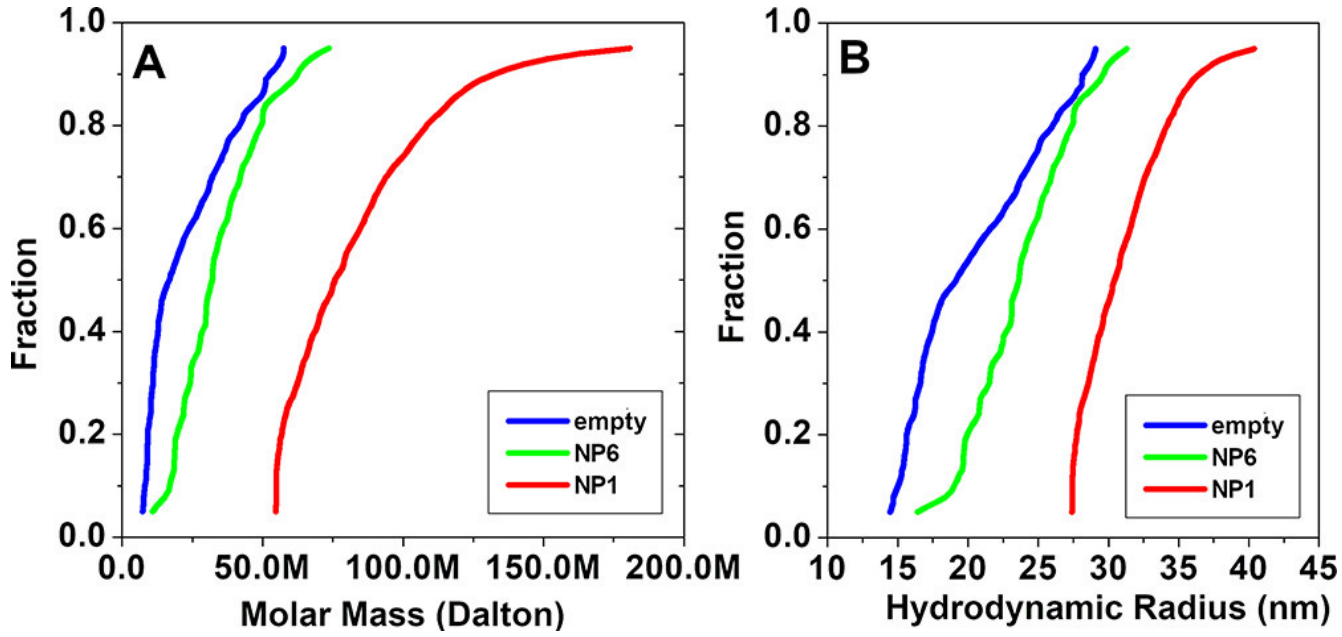
Questions?


1.0
0.8
0.6
0.4
0.2
0.0
Fraction
5
10
15
20
25
30
35
40
45
50
Hydrodynamic Radius (nm)
empty
NP6
NP1


A
B
1.0
0.8
0.6
0.4
0.2
0.0
Fraction
0.0
50.0M
100.0M
150.0M
200.0M
Molar Mass (Dalton)
10
15
20
25
30
35
40
45
Hydrodynamic Radius (nm)
empty
NP6
NP1