Simple Quantification of mRNA-LNP Encapsulation Efficiency with Scatter-Free Absorption Spectroscopy

Aida López Espinar[1], Eric C. Le Ru[2,3,@], Kirsty Smith[1], Piotr S. Kowalski[4,5], Brendan L. Darby[1]

1. Marama Labs Limited, DCU Alpha, Old Finglas Road, Glasnevin, Dublin, Ireland
2. The MacDiarmid Institute for Advanced Materials and Nanotechnology, School of Chemical and Physical Sciences, Victoria University of Wellington, Wellington, New Zealand
3. Marama Labs Limited, Salamanca Road, Wellington, New Zealand
4. School of Pharmacy, University College Cork, Cork, Ireland
5. APC Microbiome Ireland, University College Cork, Cork, Ireland

@eric.leru@maramalabs.com

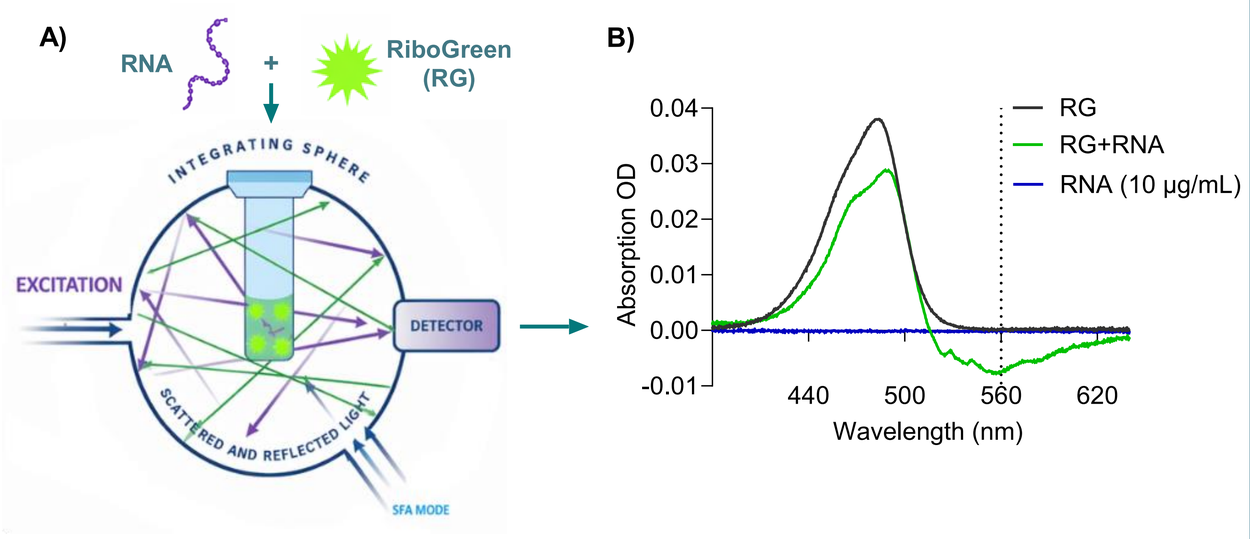
No Lysis Required | **2 Samples VS 10 Samples** | **Total and Free RNA Quantification in a Single Instrument** | **2 min Assay** | **±2% Precision**

Motivation

- Encapsulation Efficiency (EE) is critical to characterize mRNA-LNP formulations [1,2].
- Conventional RiboGreen (RG) EE assay requires detergent-mediated LNP lysis and 10 RG samples.
- Here we report a lysis-free EE method using Scatter-Free Absorption Spectroscopy (SFAS) within an integrating sphere [3]. Only 2 RG samples needed.
- SFAS enables direct quantification of free and total RNA from intact LNP suspensions in a single instrument.

Principle: RiboGreen Fluorescence in SFAS

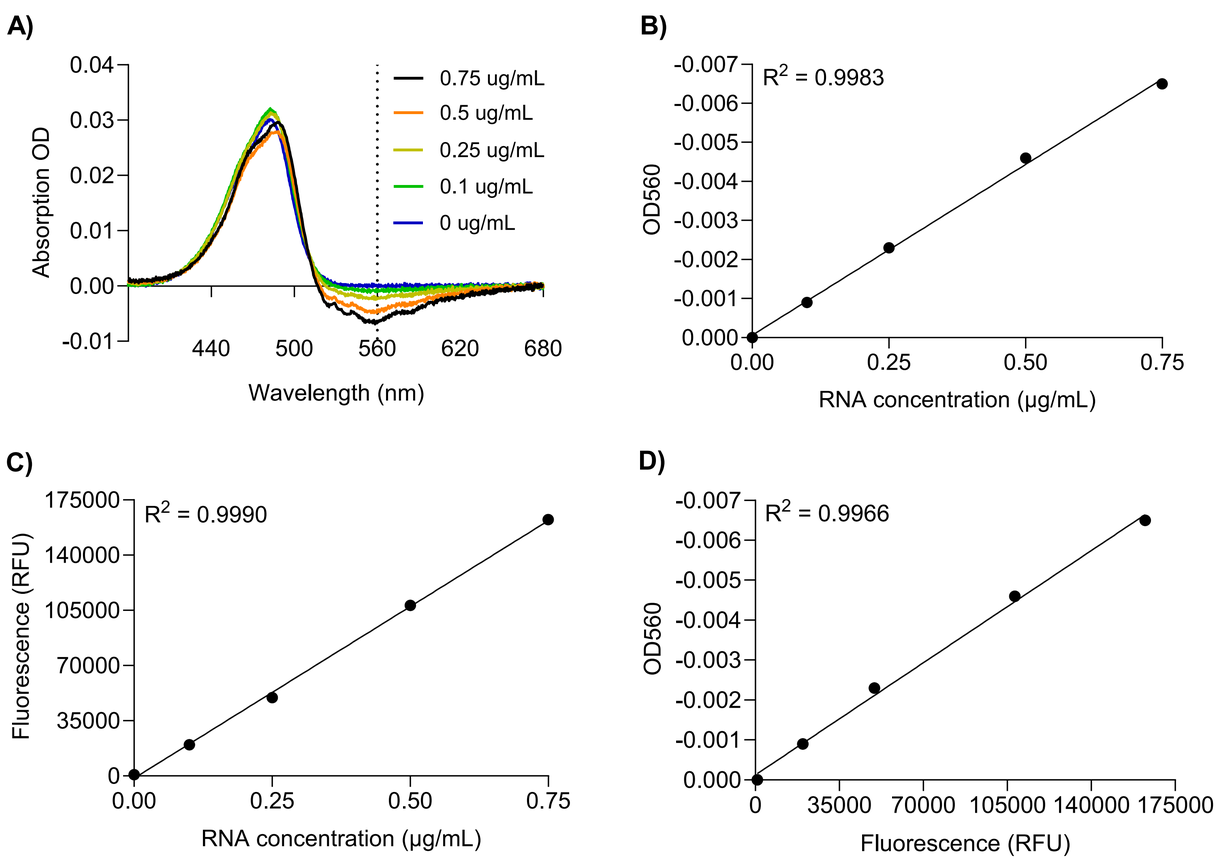
- RG fluoresces when bound to RNA.
- Our measurements used the **CloudSpec** instrument for SFAS.
- The integrating sphere collects all scattered and emitted light, including RG fluorescence (**A**).
- Fluorescence signal appears as negative absorbance feature ~560 nm in the SFAS spectrum (**B**).



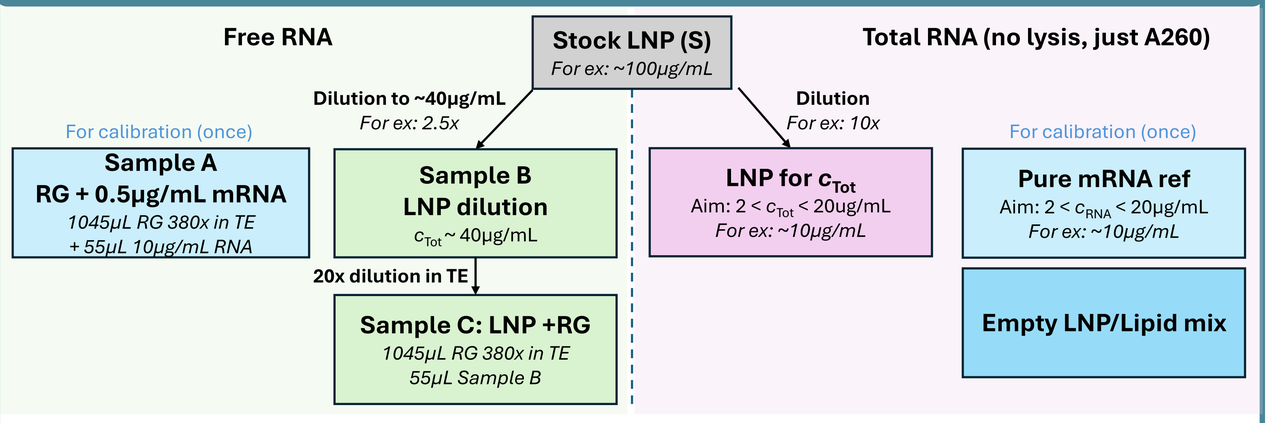
Demonstration: SFAS RNA Quantification Using RG

- Calibration curve for $0 \leq c_{RNA} \leq 1\mu$g/mL in TE+RG.
- |A560| increases linearly with RNA concentration (**A, B**).
- Independent plate reader (PR) check shows strong correlations (**C**,**D**).

⇒ **This confirms equivalence of SFAS and PR for RNA quantification.**



Encapsulation Efficiency Using SFAS



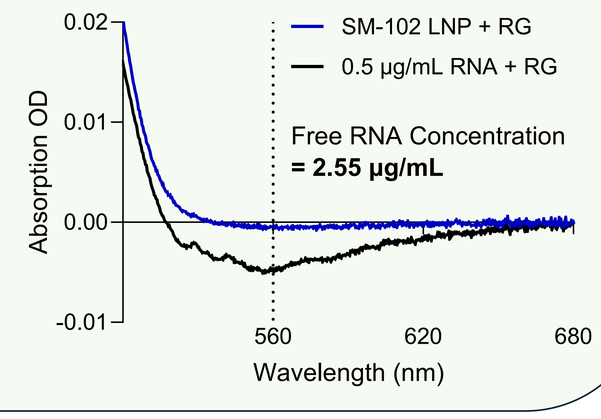
Free RNA Concentration with RG

- RG cannot penetrate intact LNPs — the SFAS signal from RG+LNP reflects free RNA.
- Our protocol (above) ensures same buffer (e.g. 95:5 TE/PBS) in all RG samples.
- Free RNA concentration (c_{Free}) is calculated by comparing LNP+RG fluorescence at 560 nm with a reference RNA of known concentration (c_{Ref}) — see **Equation 1.**

Equation 1:

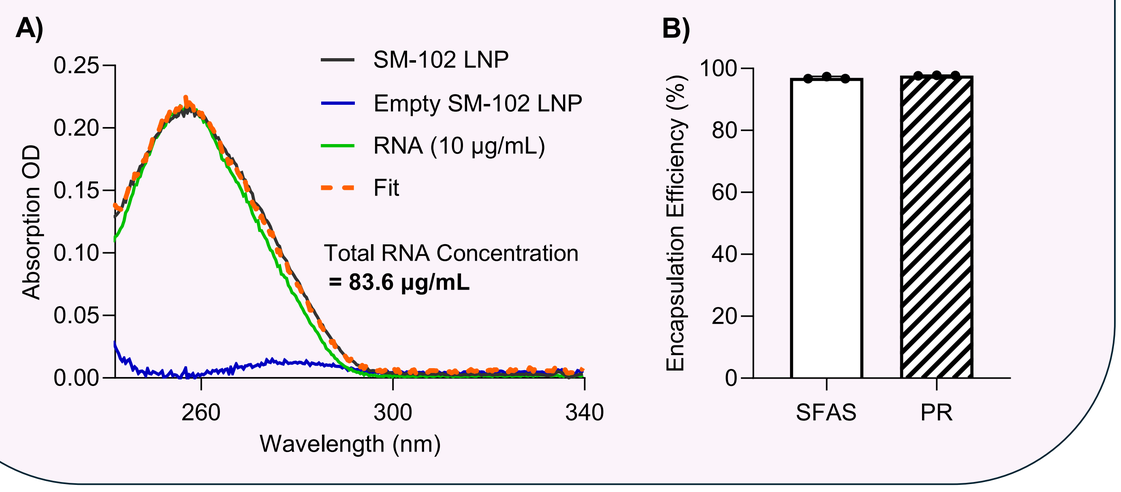
$$c_{Free} = c_{Ref} \frac{|A560\ (LNPs)|}{|A560(Ref)|}$$

Linearity range:
$0.05 \leq c_{Free} \leq 0.5\mu$g/mL
(in sample C)



Total RNA Concentration (A260, no Lysis)

- c_{Tot} is determined from the RNA scatter-free absorbance at 260 nm — no detergent disruption required (**A**) [3,4].
- Deconvolution may be used to account for lipid absorption (**A**).
- EE = ratio of encapsulated RNA (c_{Free}) to total RNA (c_{Tot}):
- $$EE\ (\%) = \left(1 - \frac{c_{Free}}{c_{Tot}}\right) * 100$$
- Excellent agreement with 98% EE from a conventional plate-reader assay (**B**)



Summary

- SFAS enables rapid, reproducible determination of LNP encapsulation efficiency.
- Works on intact suspension. No Triton!
- Only 3 measurements (2 with RG):
  - RNA reference + RiboGreen (measure once and reuse)
  - LNP sample + RiboGreen
  - LNP sample without RiboGreen for total RNA

Pre-print publication here!

References

1. Tsalmpouris et al. Anal. Chem. **2025**, *97*, 19275.
2. Parot et al. J. Control Release **2024**, *367*, 385.
3. Le Ru et al. Nano Lett. **2025**, *25*, 6813.
4. López Espinar A *et al,* Anal Chem. 2025(97):29928.