Understanding the mechanical instability of model lipid-based drug solutions

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Motivation: Why Stability Matters

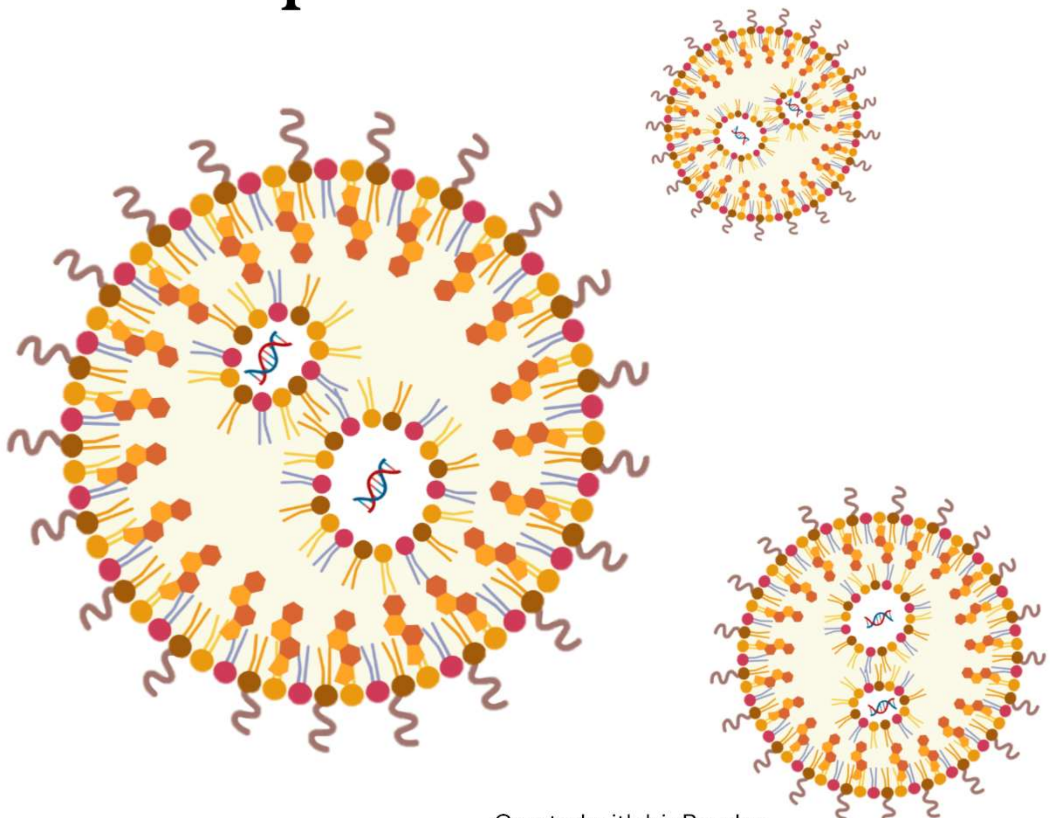
Unicef.org

<u>Goal:</u> Understand mechanical forces that degrade lipid nanoparticles to manufacture more stable drug solutions

- …vaccine reliance on ultra-…orage proved expensive
- Ultra-low freezers, dry ice transport, limited shelf life → distribution challenges

Understanding Lipid Nanoparticles (LNPs)

- Deliver fragile therapeutics
- Physically unstable
  - Aggregate, fuse, and leak
  - Ultra-low temperature
- Mechanical instability persists
  - Alter structure, lose payload, or collapse due to mechanical forces



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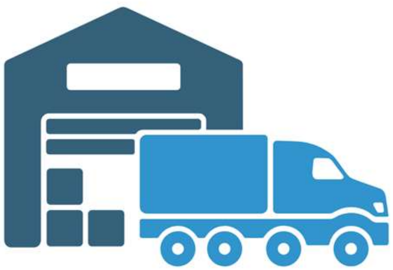
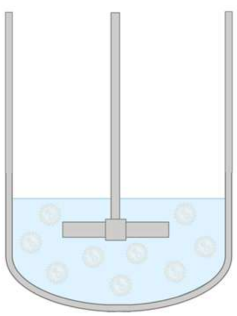
Mechanical Forces

Manufacturing



Shipping

Shaking



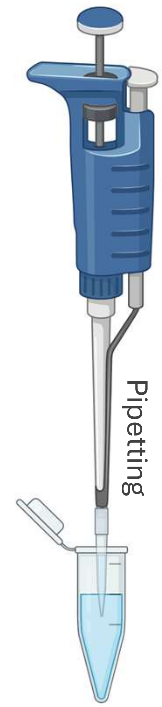
Mixing

Laboratory

Mixing

Injecting

Vortexing



Pipetting

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Literature Review

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Impact of mixing and shaking on mRNA-LNP drug product quality characteristics

Roman Matthessen, Robbe Van Pottelberge, Ben Goffin & Giel De Winter

Stability testing of the Pfizer-BioNTech BNT162b2 COVID-19 vaccine: a translational study in UK vaccination centres

Laila Kudsiova,[1] Alison Lansley,[2] Greg Scutt,[3] Marcus Allen,[4] Lucas Bowler,[4] Sian Williams,[3] Samantha Lippett,[5] Selma Stafford,[6] Michael Tarzi,[5,7] Michael Cross,[5] Michael Okorie[5,7]

Stability Study of mRNA-Lipid Nanoparticles Exposed to Various Conditions Based on the Evaluation between Physicochemical Properties and Their Relation with Protein Expression Ability

by Mariko Kamiya [1], Makoto Matsumoto [2], Kazuma Yamashita [1], Tatsunori Izumi [2], Maho Kawaguchi [1], Shusaku Mizukami [3], Masako Tsurumaru [4], Hidefumi Mukai [1,2] and Shigeru Kawakami [1,2,*]

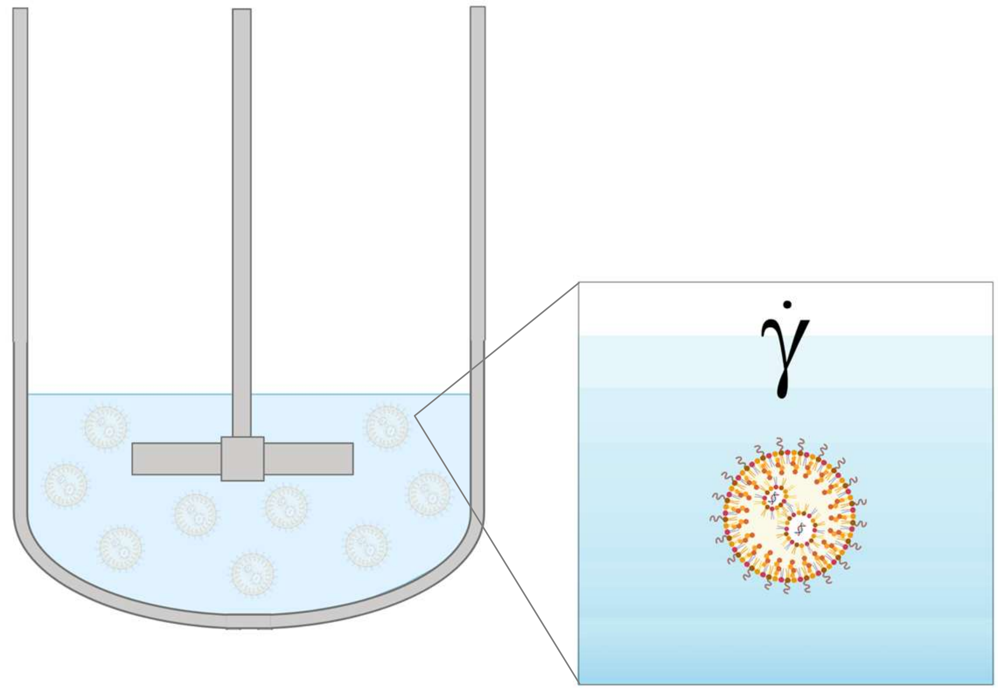
Don't shake it! Mechanical stress testing of mRNA-lipid nanoparticles

Anna Ruppl [a 1], Denis Kiesewetter [a 1], Franziska Strütt [a], Monika Köll-Weber [a], Regine Süss [a], Andrea Allmendinger [a b]

- Literature review of past experiments confirmed that "*vigorous* mixing is bad"
  - How do we define and quantify that?
- When defining vigorous mixing, we investigated two main aspects:
  - *shear rate*
  - *air-water interface*

Shear Rate

- **Shear rate** ($\dot{\gamma}$) – the speed at which different layers of liquid move past each other
  - What most literature attribute LNP mechanical instability to
- Understand whether it's shear causing instability, or other forces during mixing



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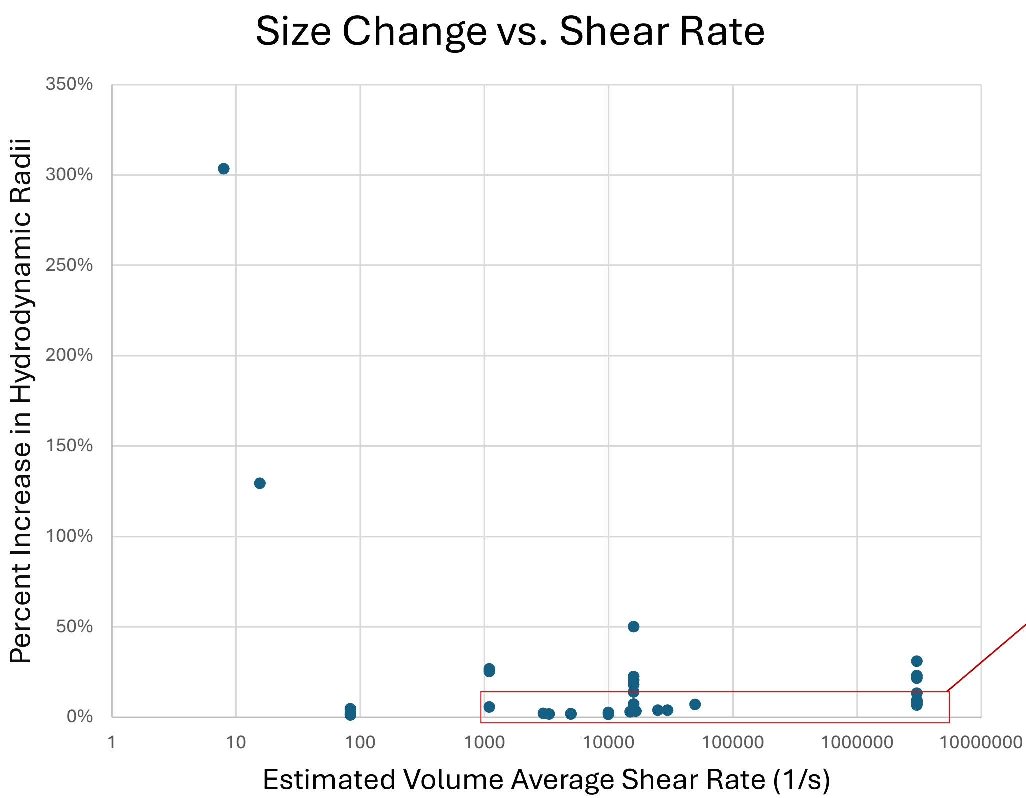
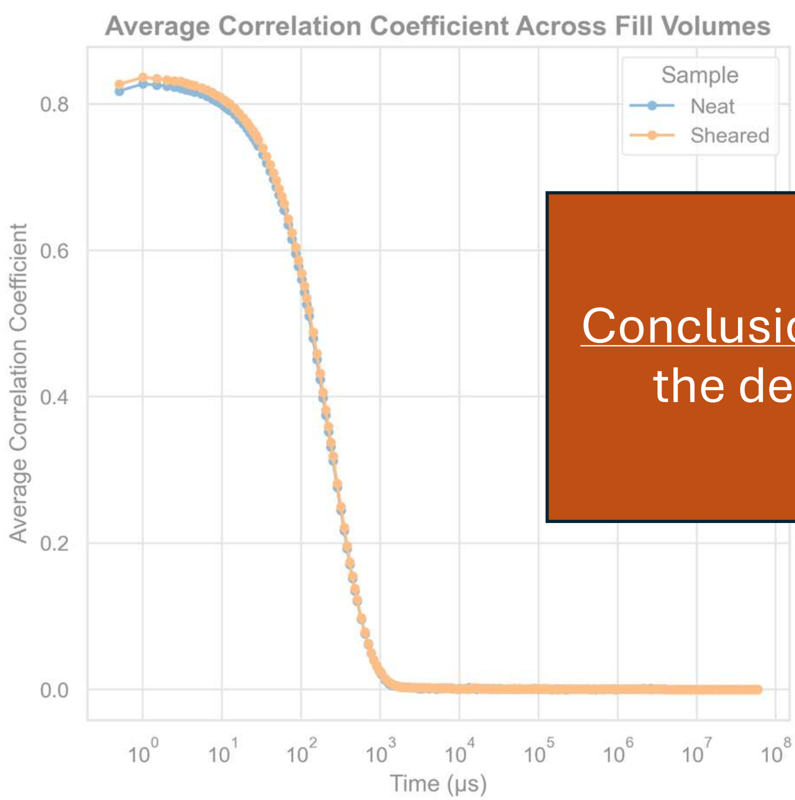
Change in Size vs. Shear Rate



- **Hydrodynamic radius:** effective radius of a particle in fluid

- Low shear rate → large size increase
- High shear rate → no significant size increase

Analyzing Shear Rate

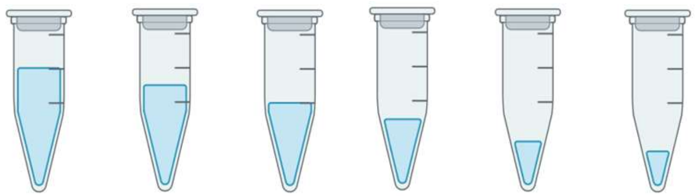


- Mechanical force: shearing
  - air
  - r 30 mins
  - namic light scattering
  - urement of size
- No increase in hydrodynamic radii

<u>Conclusion:</u> Shear rate is not the determinant factor

Air-Water Interface

- **Air-water interface** – boundary where liquid meets air
- Investigate by changing fill volume
  - Greater fill volume → less interface
  - Lower fill volume → greater interface



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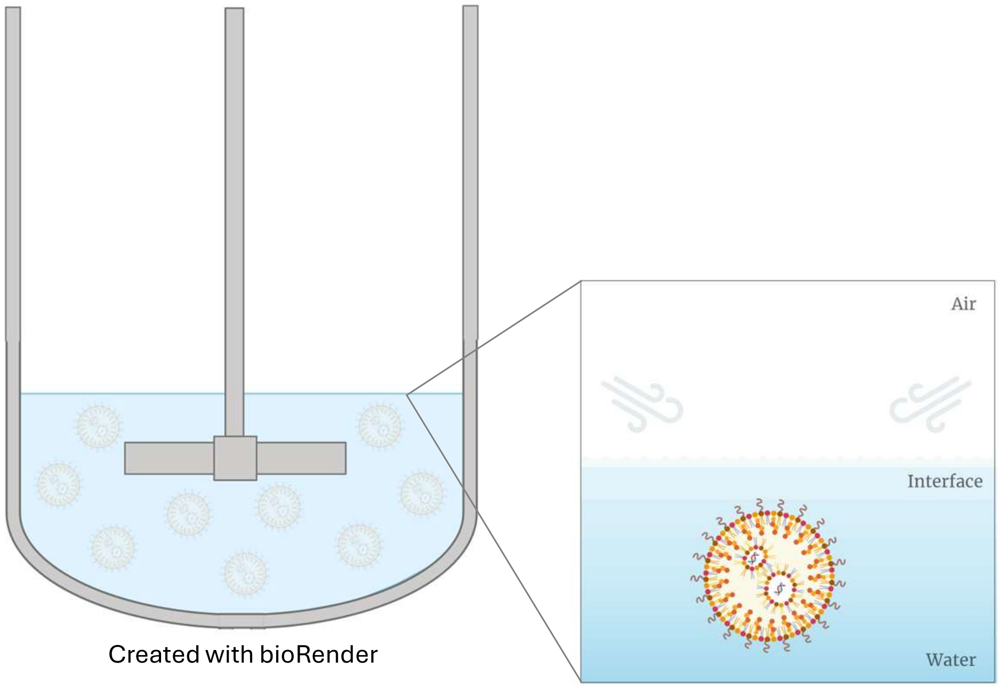

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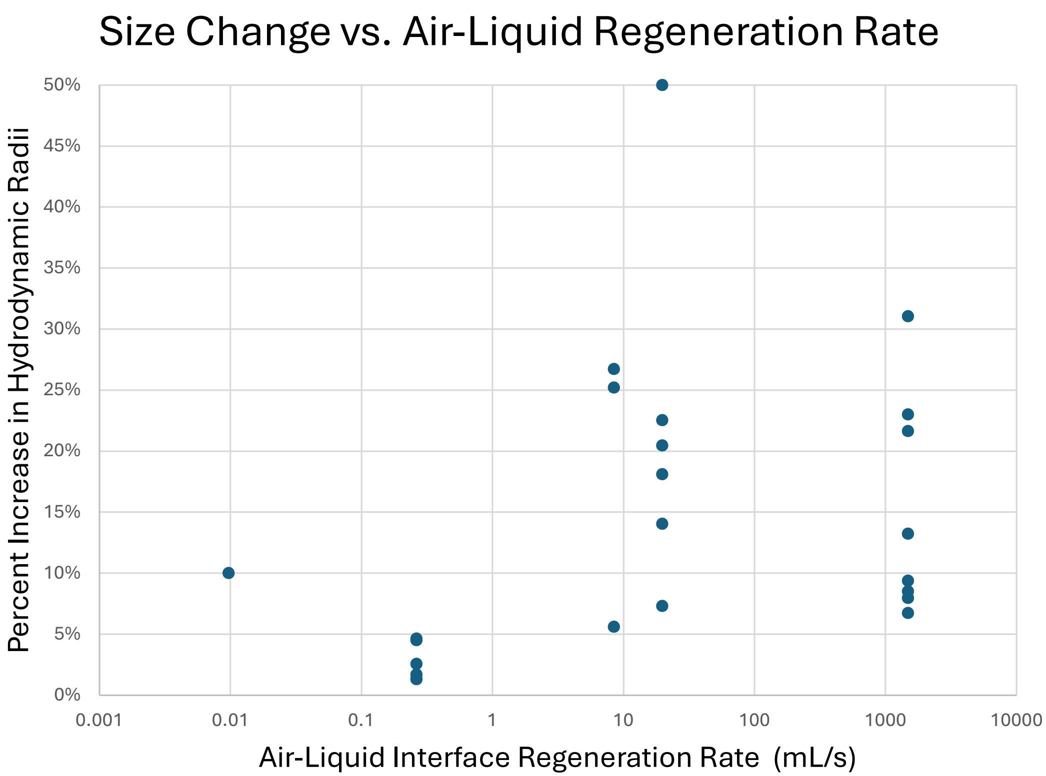
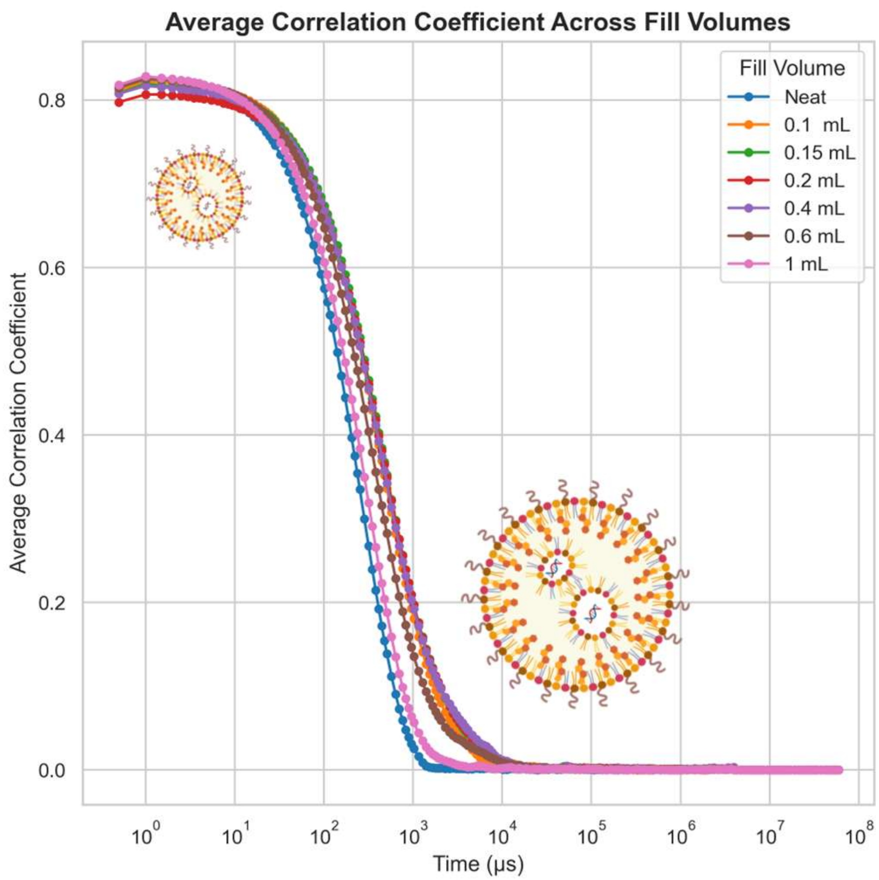
Change in Size vs. Air-Liquid Regeneration



- **Air-liquid interface regeneration rate** – speed at which a new air-liquid interface is formed as the liquid is mixed

- Regeneration rate increases → percent change in radii increases

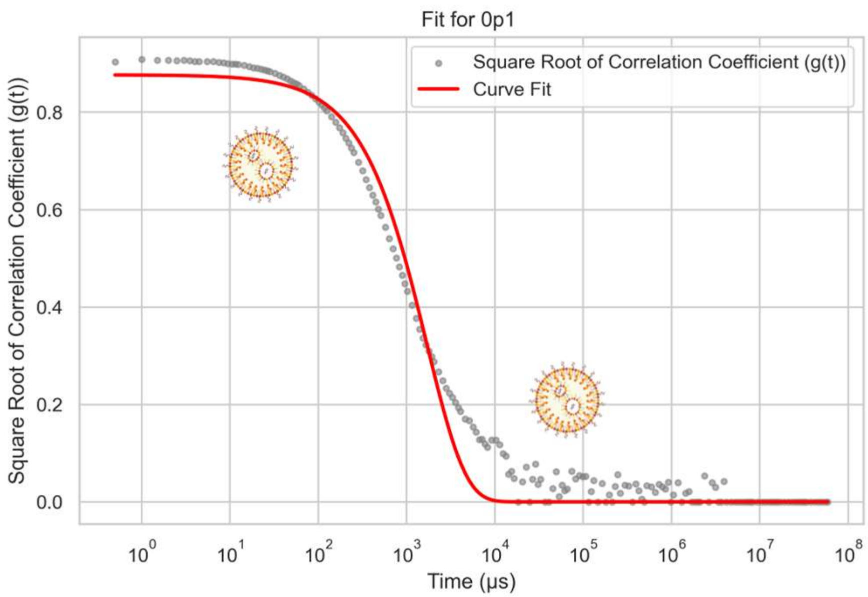
Analyzing Air-Liquid Interface



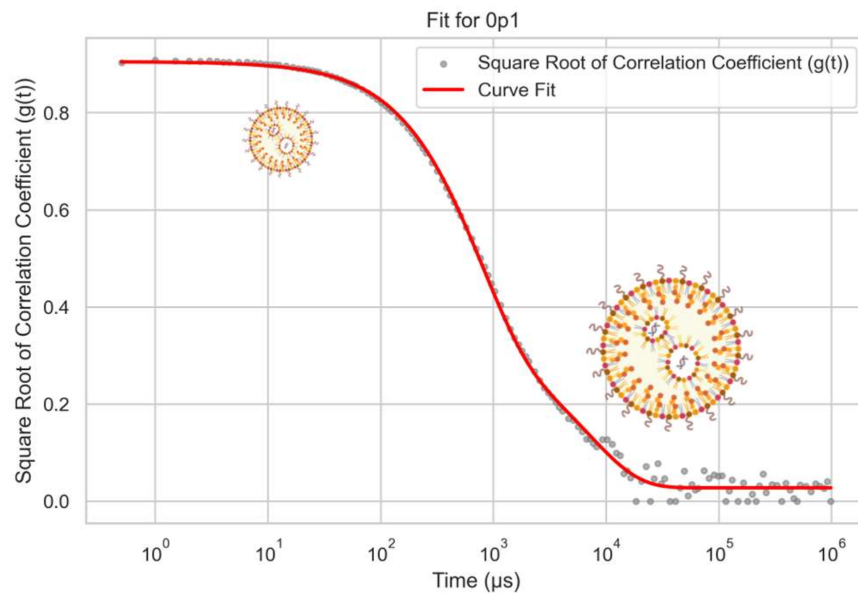
- Mechanical force: vortexing
    - Spins liquid into whirlpool shape
    - Generates most air-water interface
    - Constant time (90s)
- Increasing fill volume (0.1mL - 1.0mL)
- Analyzed samples with DLS

Describing Data Curves

Unimodal fit: Ae^{-Dq^2t}

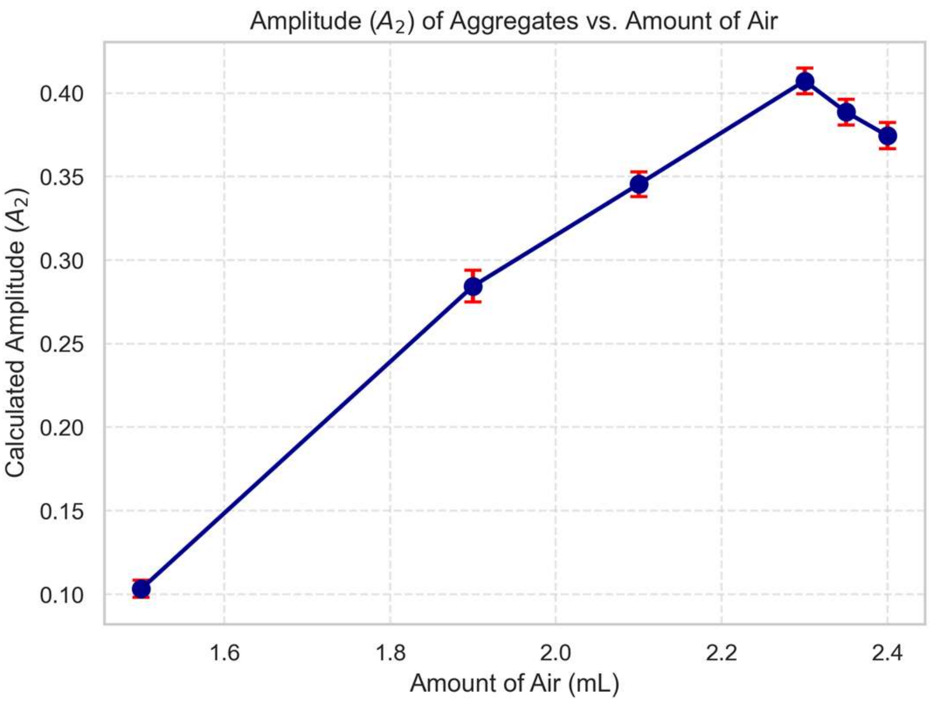


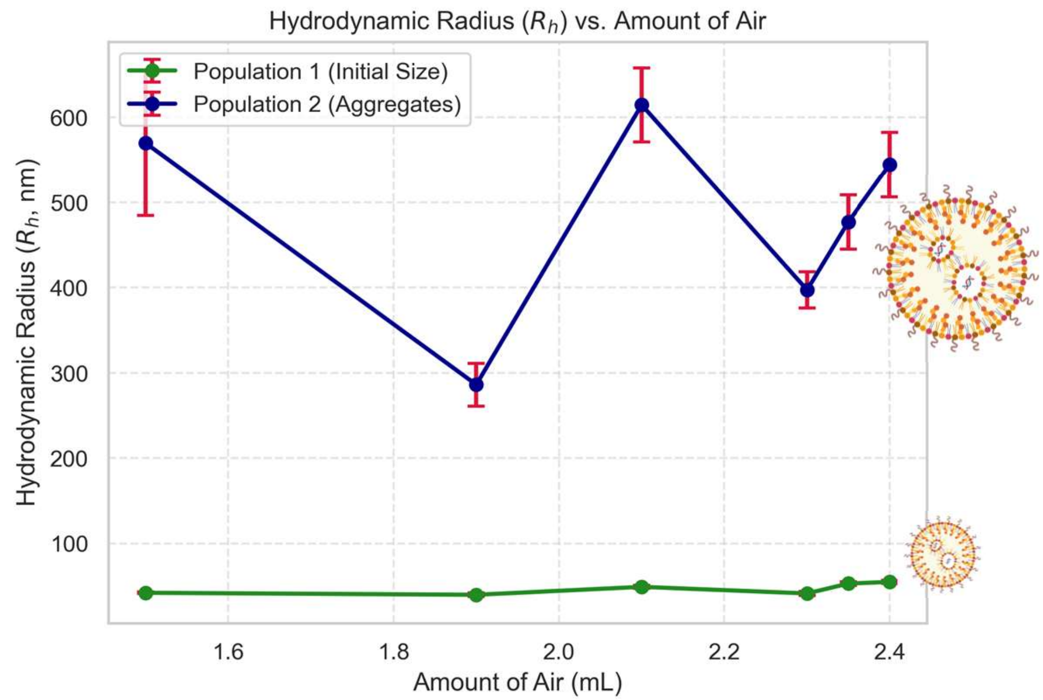
Bimodal fit: $A_1e^{-D_1q^2t} + A_2e^{-D_2q^2t}$



- Fit data to extract quantitative information
- Bimodal model was better fit, confirming second population

Trends with Fill Volume

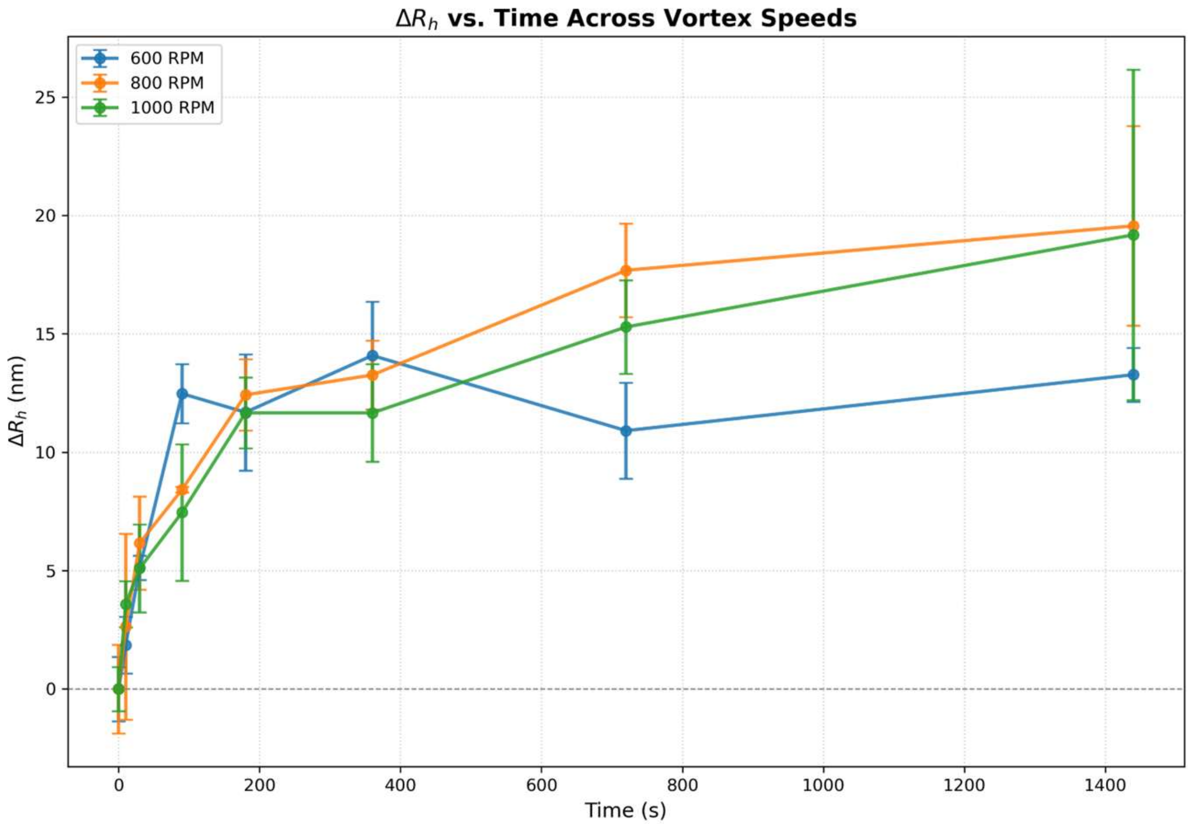




- Slight upward trend for both confirmed air-water interface factors into instability
- **Outliers** → **Follow up:** Only fill volume or an additional variable?

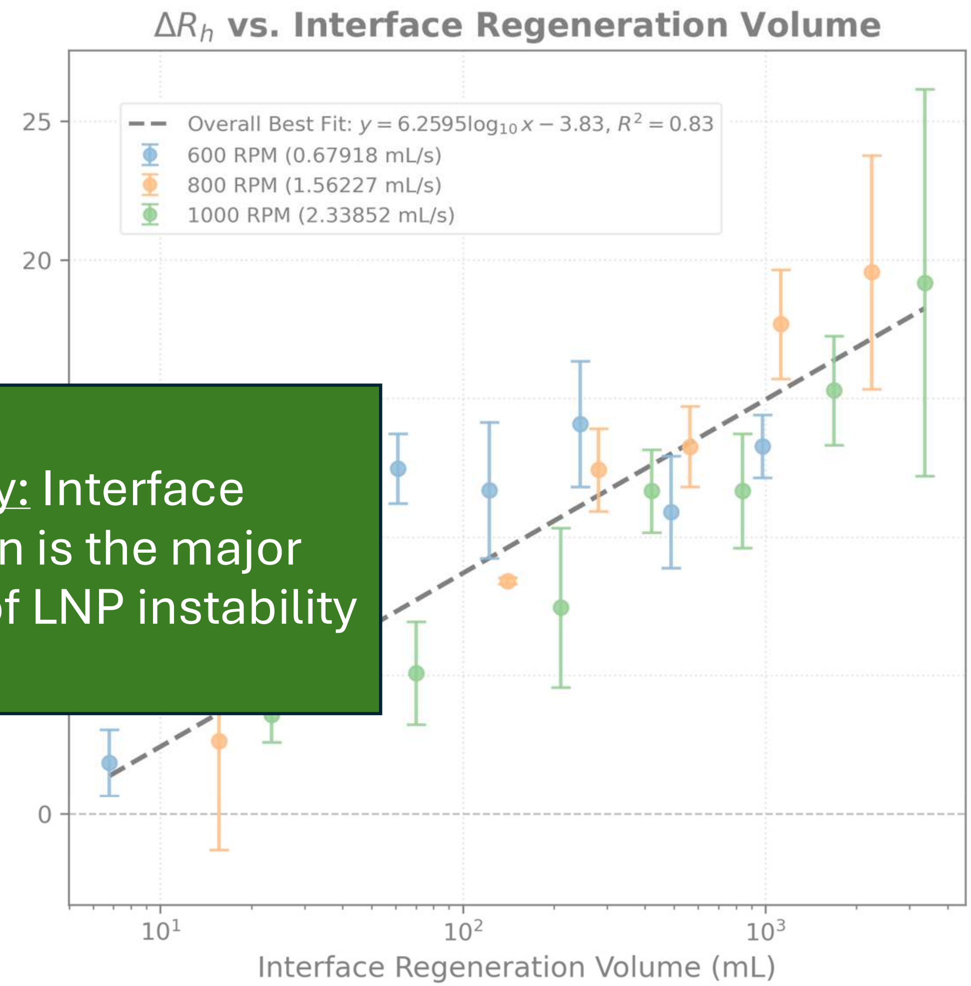
Effects of Interface Regeneration

- Interface regeneration experiment
  - Constant fill volume
  - Vortex time intervals
- Plotted ΔR_h vs. time
  - Fill volume not sole factor
- Different trends across speeds
  - How can we better visualize this data?



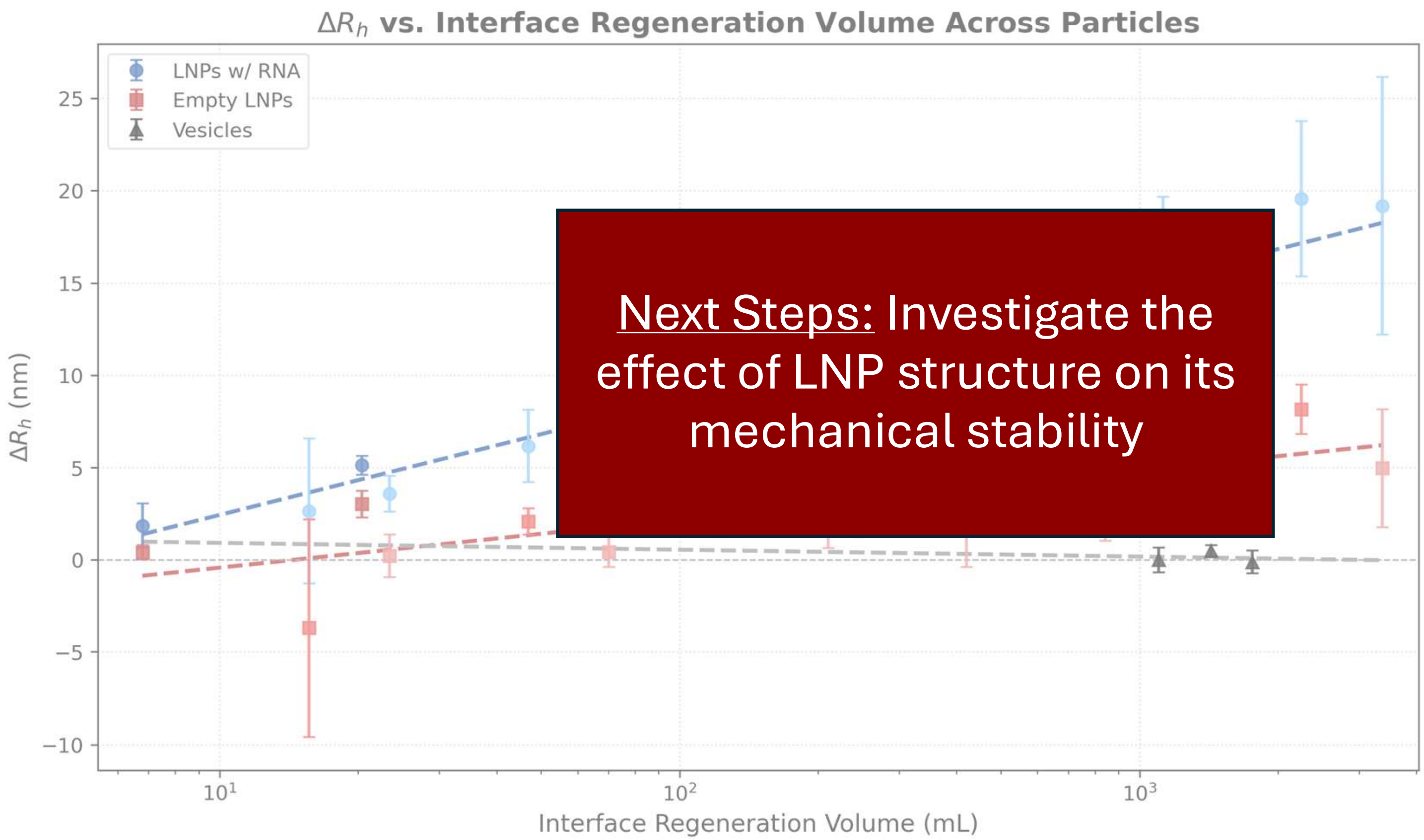
Collapsing Data

- Graphed ΔR_h vs. interface regeneration volume (regeneration rate × time).
  - Data collapses into upw
    with log scale
- Interface regeneration facto
  mechanical instability
  - Explains why vigorous mixing is detrimental

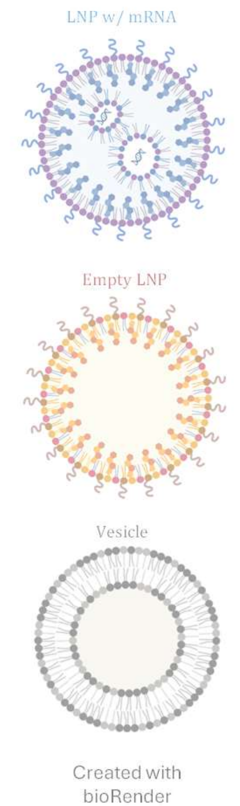


<u>Summary:</u> Interface regeneration is the major determinant of LNP instability

Future Works & Understanding Stability



<u>Next Steps:</u> Investigate the effect of LNP structure on its mechanical stability



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Calculating Hydrodynamic Radii

Bimodal fit: $A_1 e^{-D_1 q^2 t} + A_2 e^{-D_2 q^2 t}$

- Calculated hydrodynamic radii using the Stokes-Einstein equation:

$$D = \frac{k_B T}{6\pi\eta r}$$

Fill Volume	Population 1 (nm)	Population 2 (nm)
Neat	40.81	40.81
0.1 mL	54.79	544.38
0.15 mL	52.81	477.20
0.2 mL	41.24	397.34
0.4 mL	48.94	614.39
0.6 mL	39.66	286.20
1.0 mL	42.00	569.71