Effects of Mixing, Aging and pH on Lipid Nanoparticle RGTM

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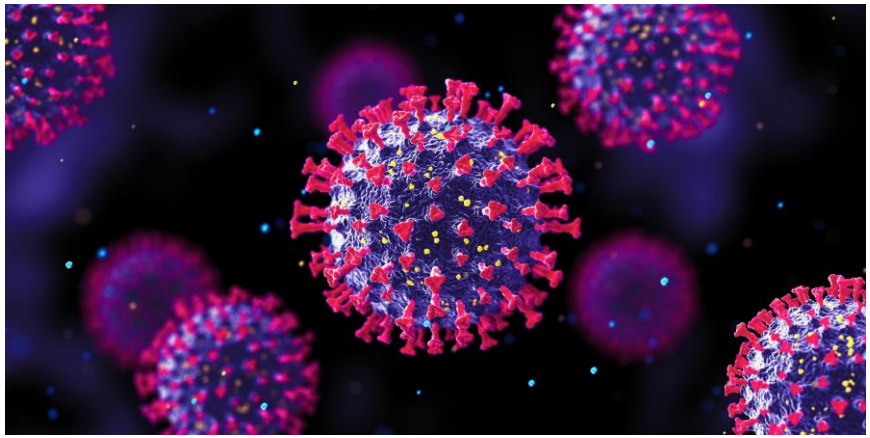
Mentor: Chelsea Edwards

NIST CENTER FOR NEUTRON RESEARCH

CHRNS CENTER FOR HIGH RESOLUTION NEUTRON SCATTERING NIST

Lipid Nanoparticles (LNP)



NIST

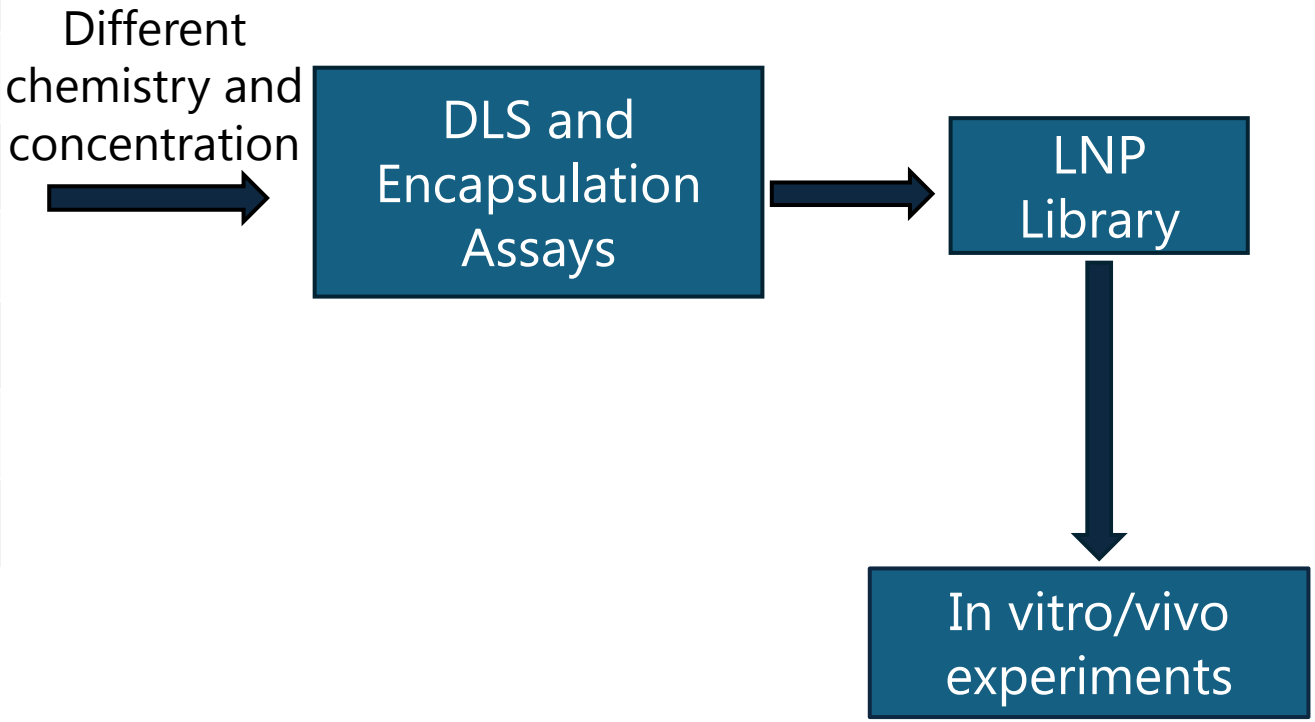
PROJECTS/PROGRAMS

LNP-RNA Research Grade Test Material

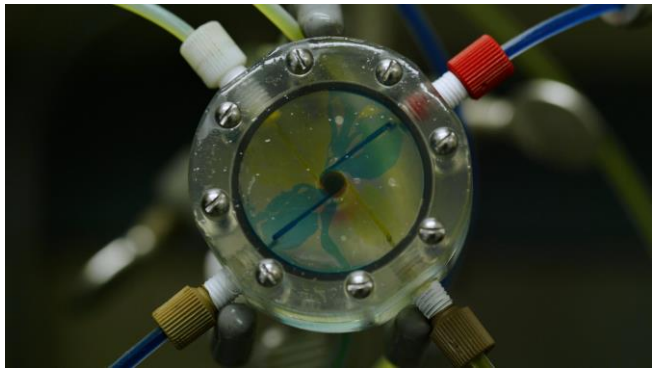
https://www.nist.gov/programs-projects/lnp-rna-research-grade-test-material

Lipid Nanoparticle (LNP) Formulation

Components
Cationic ionizable lipid
Zwitterionic lipid
PEG Lipid
Cholesterol
Nucleic acid cargo
Aqueous Buffer
Ethanol

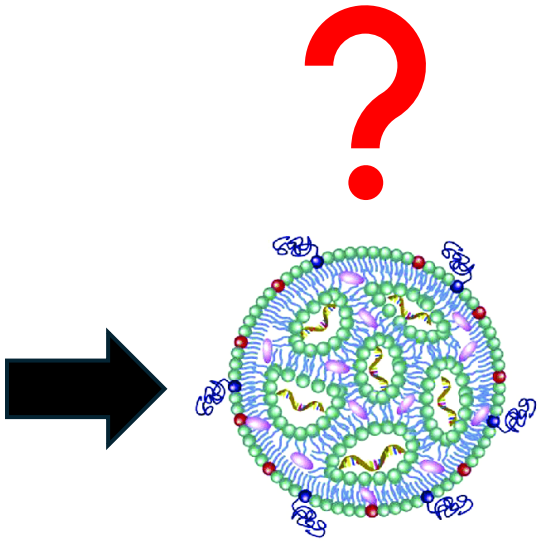


Variables Involved in LNP Production



Variables

- **Mixing modality**
- Flow rate
- **pH**
- Concentration
- **Aging,** etc.



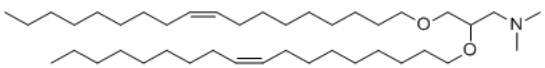
Final size and structure of LNP

Subraveti, S. N., ..., Prud'homme, R. K. *Journal of Visualized Experiments* (2024).

LNP RGTM Production

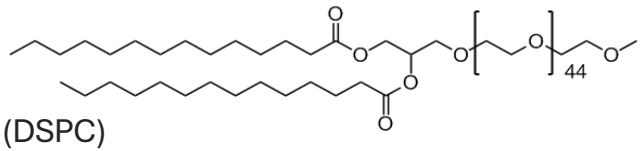
(DODMA)

1,2-dioleyloxy-3-dimethylaminopropane



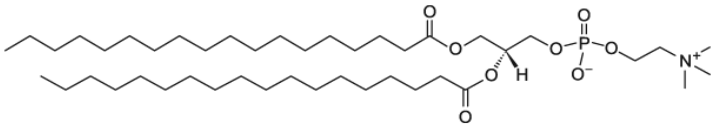
(DMG-PEG 2000)

1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000

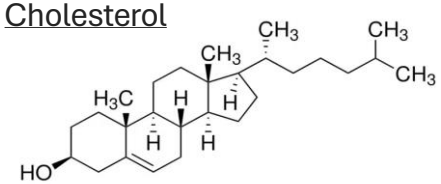


(DSPC)

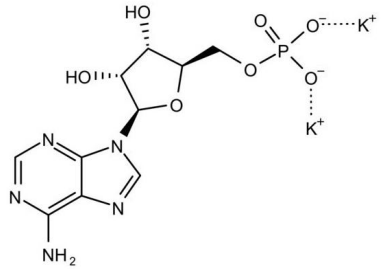
1,2-distearoyl-sn-glycero-3-phosphocholine



Cholesterol

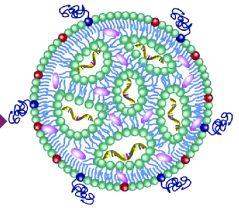


Adenylic Acid (polyadenylic acid, polyA)



Ethanol Stream

Aqueous Stream



Project Objective

- Testing 3 variables on formation and structure of LNP with RGTM composition:
  - Mixing modality
  - pH of aqueous buffer – Ionizable lipid and cargo charge
    - pH 3.5, 4.1, 4.7, 5.3, 5.9
  - Aging

Experimental Approach

Mixing Modalities:

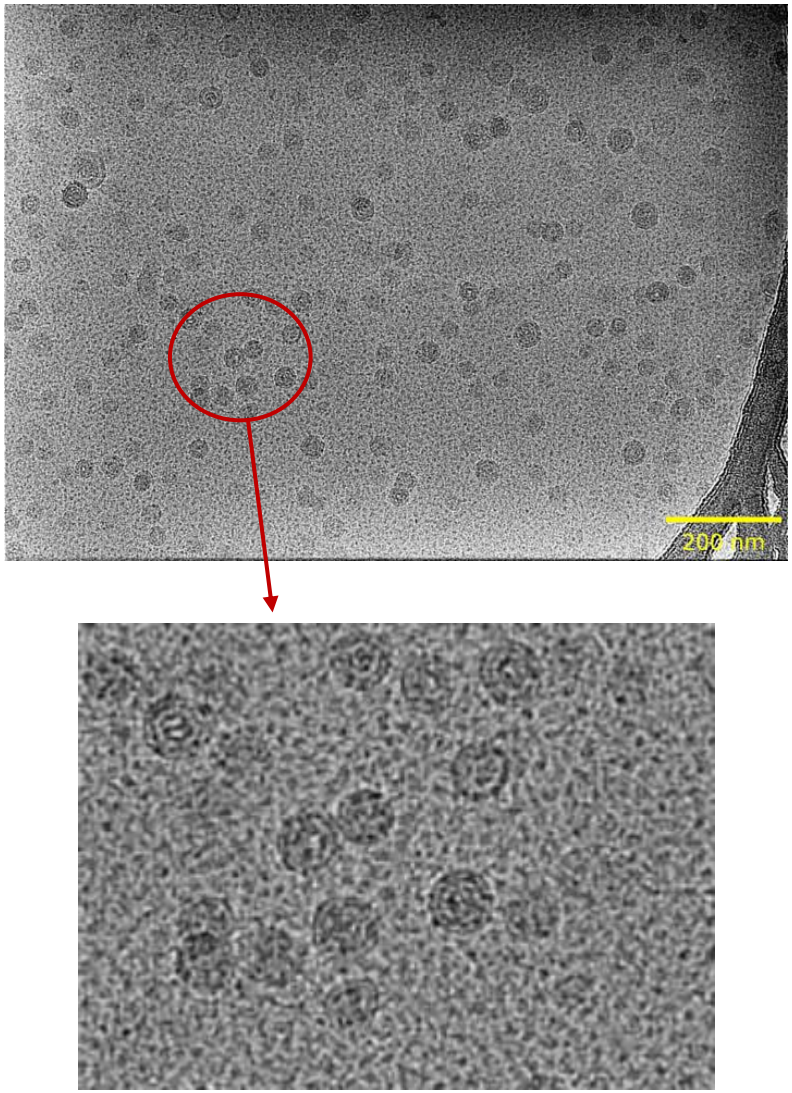
- Multi-Inlet Vortex Mixer (MIVM)
- Stopped Flow Mixer (SFM)

Compare loaded & unloaded LNPs

<u>Analysis:</u>

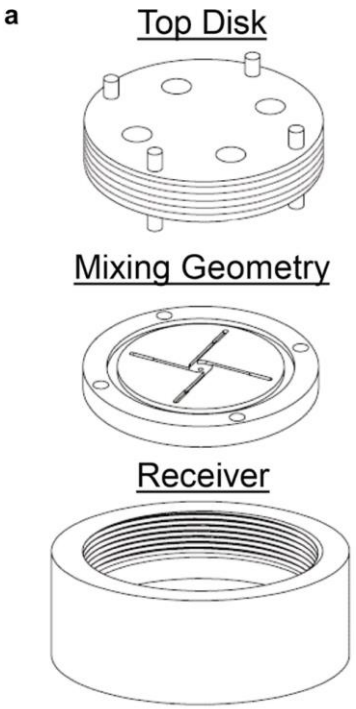
Dynamic Light Scattering (DLS)

- Size distribution



Thomas Cleveland, cryo-EM image of an LNP/RNA, https://www.nist.gov/image/cryo-em-image-lnprna

Multi-inlet Vortex Mixer (MIVM) Set Up



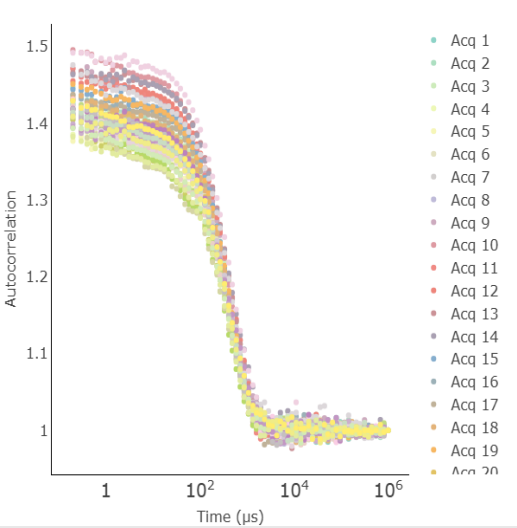
Mixing Ratio 3:1
Total flow rate 40mL/min

Markwalter, Chester E, and Robert K Prud'homme, *Journal of pharmaceutical sciences* (2018), Liu et. al., *Chemical Engineering Science* (2008)

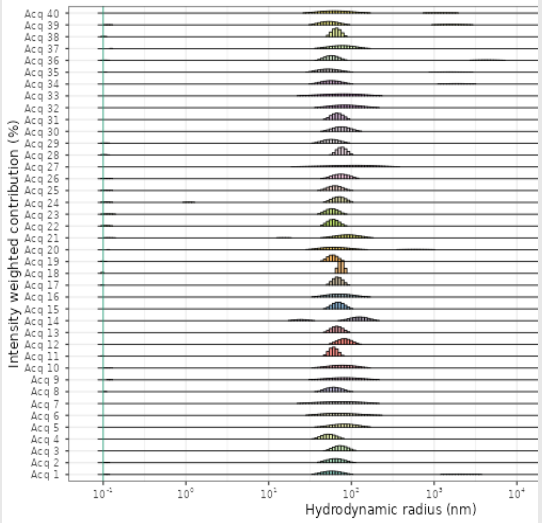
DLS Analysis Protocol

Raw ACF data from DLS instrument → **Raynals DLS Analysis Software** → **Particle Size Distribution CSV File** → **Python code**

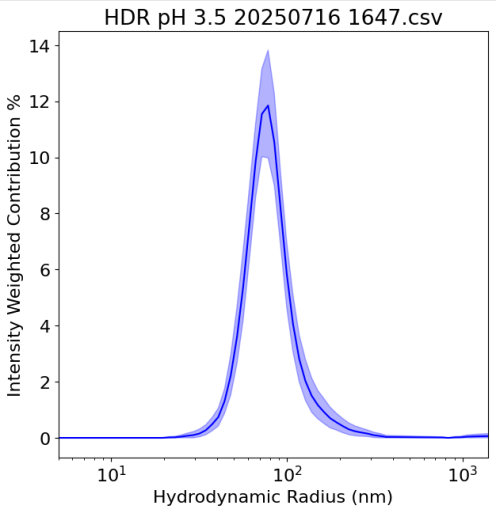
Autocorrelation Functions



Size Distribution Fit

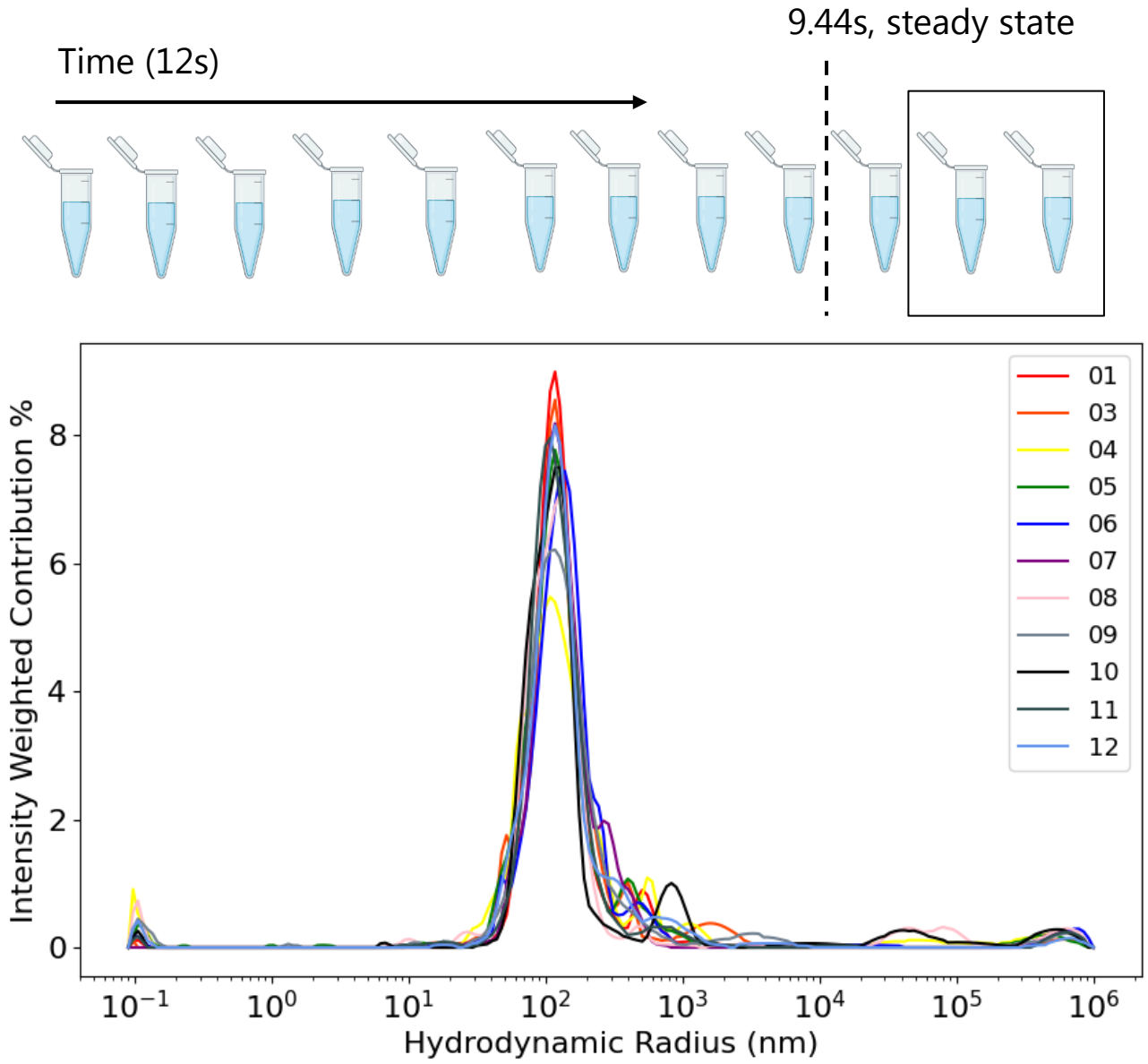


Average



Burastero, O., Draper-Barr, G., Raynal, B., Chevreuil, M., England, P. & Garcia Alai, M. (2023). Raynals, an online tool for the analysis of dynamic light scattering. Acta Crystallographica Section D: Structural Biology, 79(8).

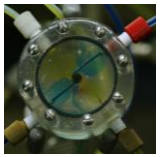
MIVM Steady State Flow Study

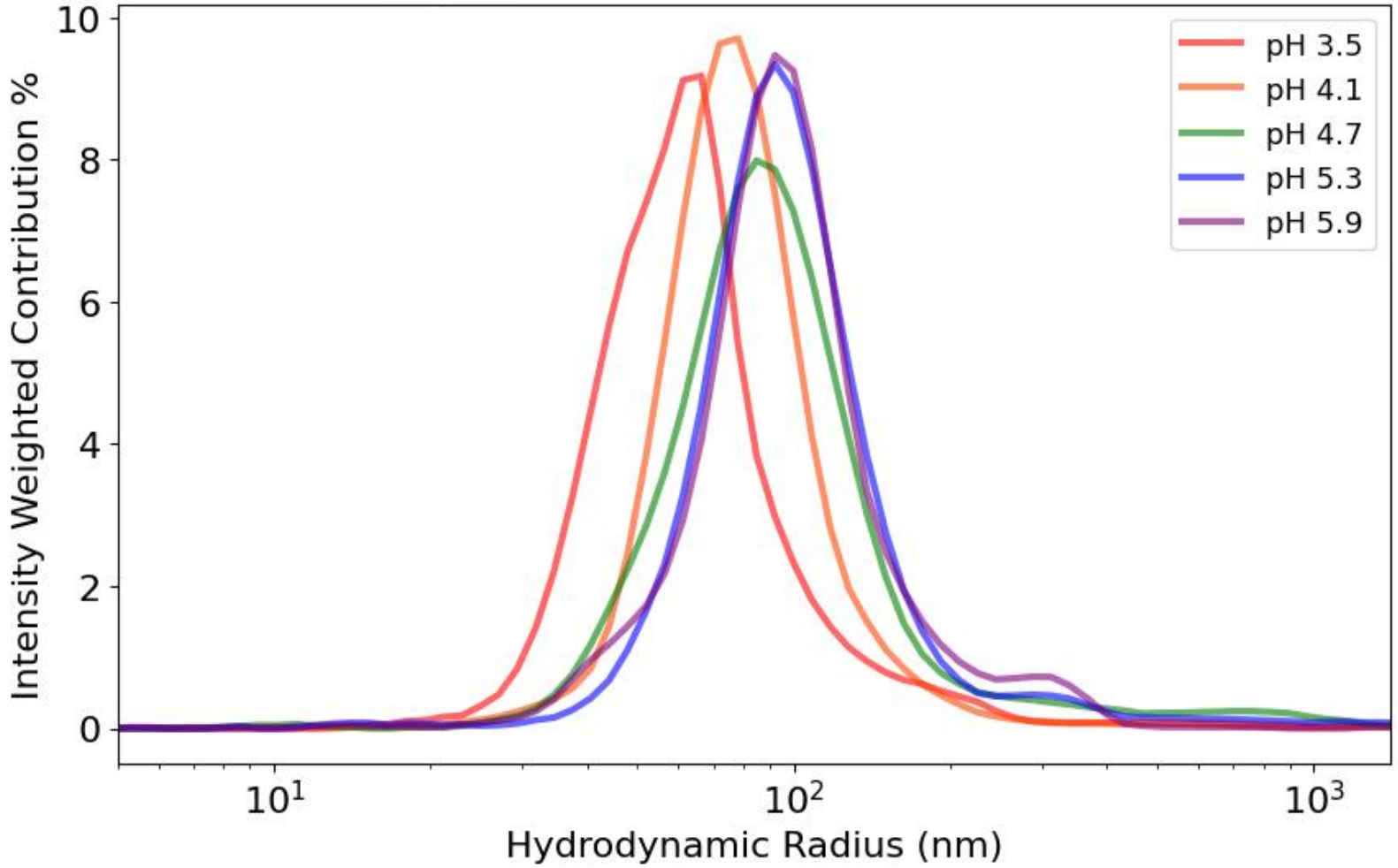


LNP Synthesis and DLS

MIVM: pH-Dependent **Empty** LNP Size

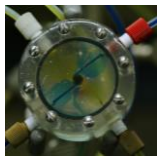
Synthesis 1

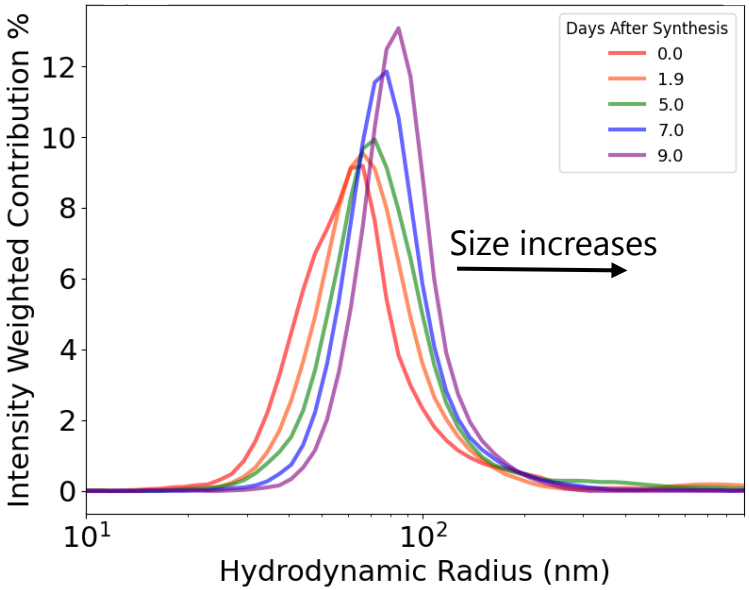




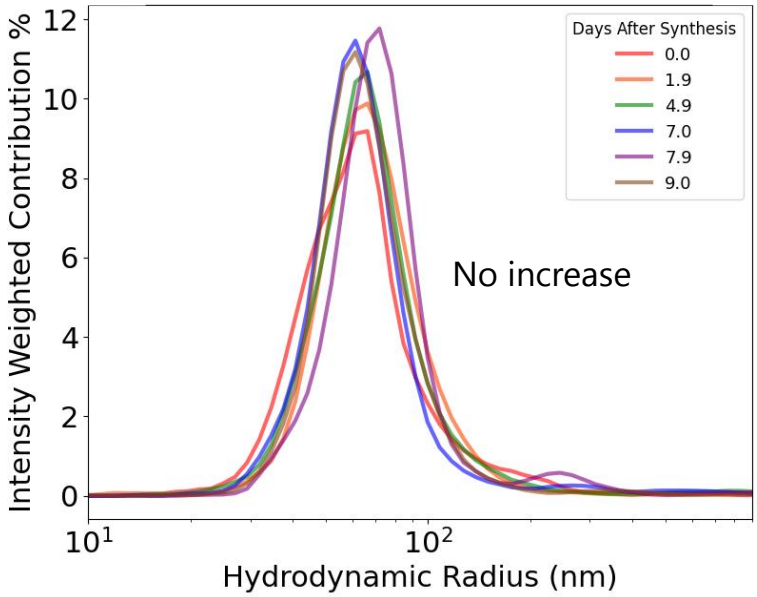
MIVM Empty LNP **Aging, pH 3.5**

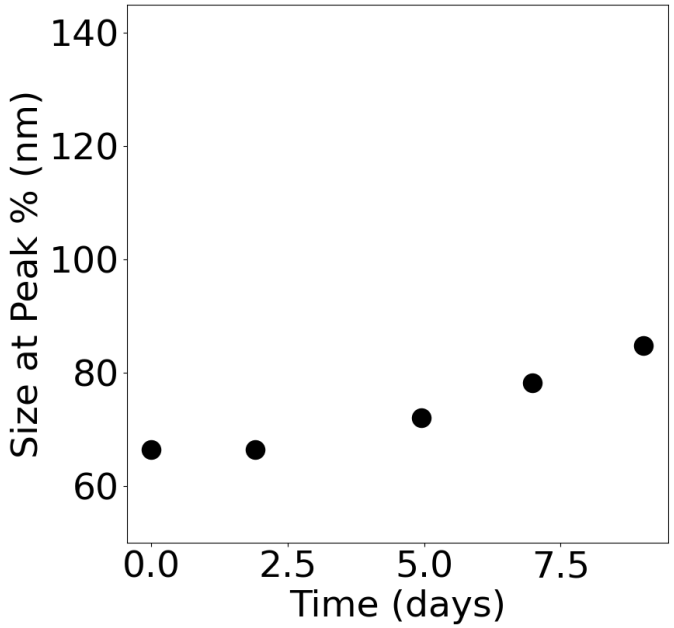
25°C

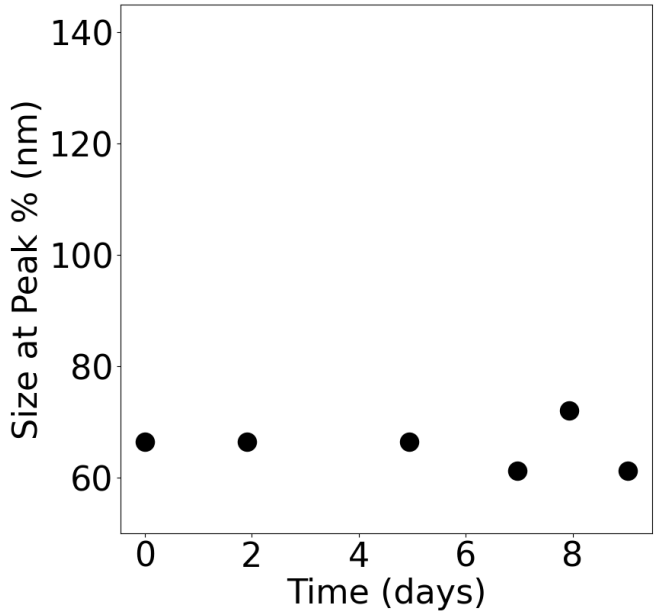




4°C

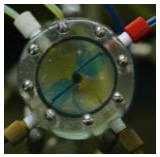


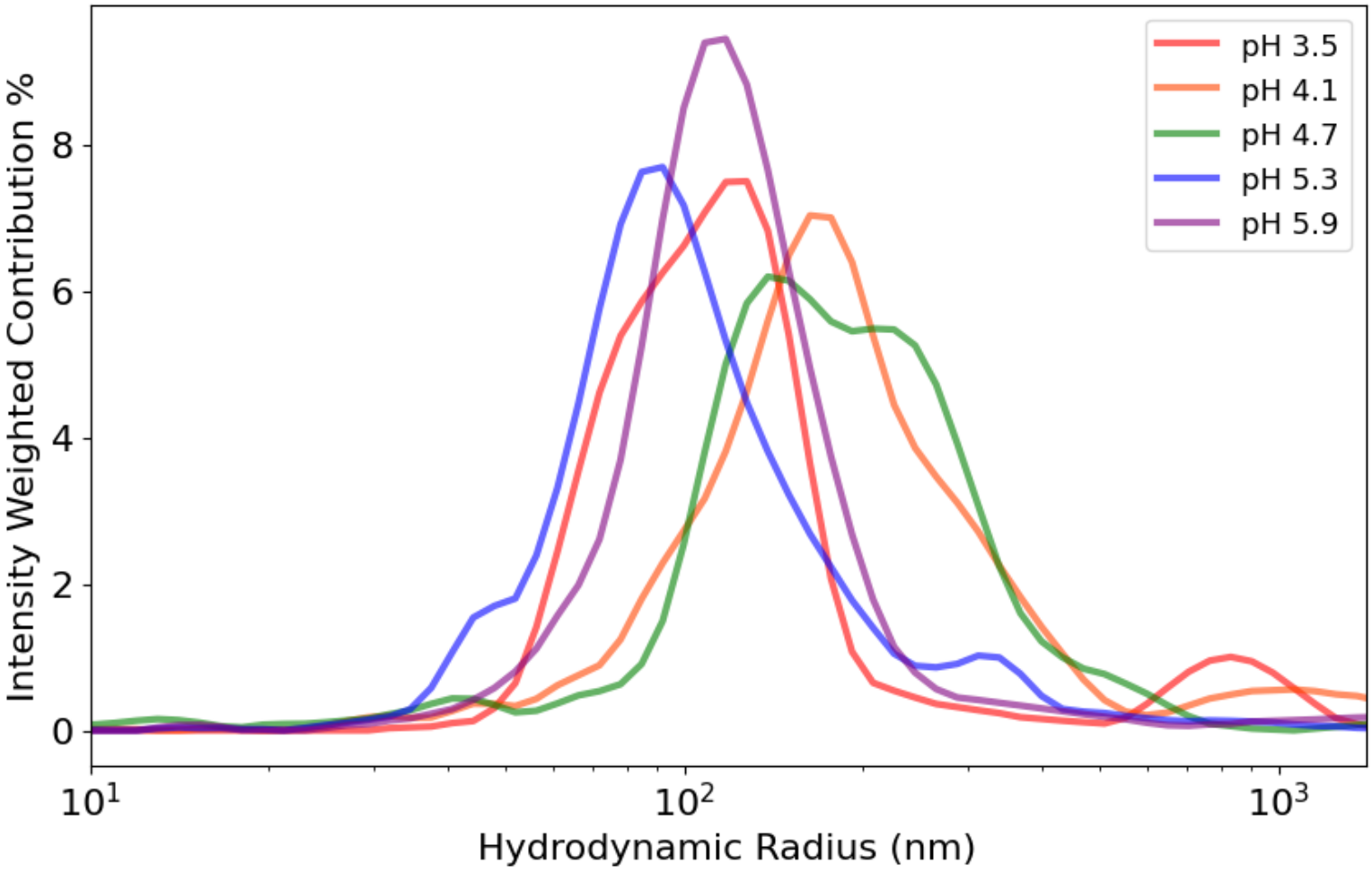




MIVM: pH-Dependent **polyA** LNP Size

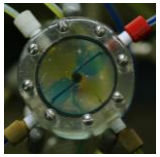
Synthesis 1

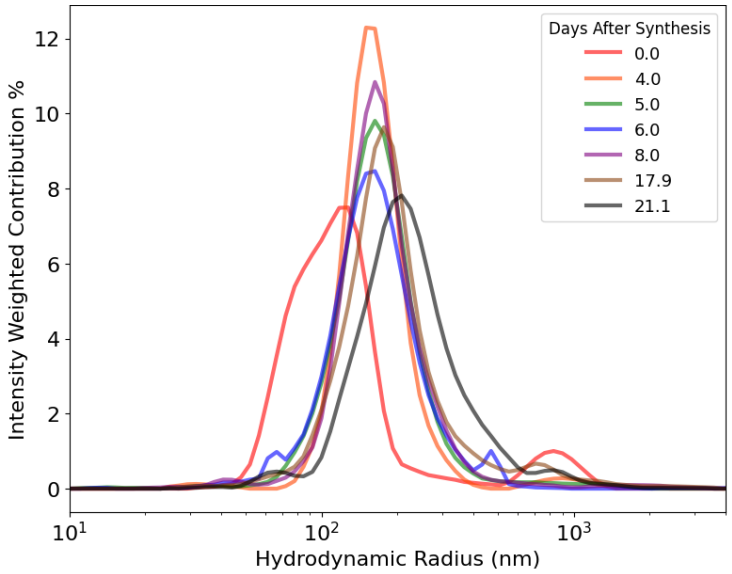


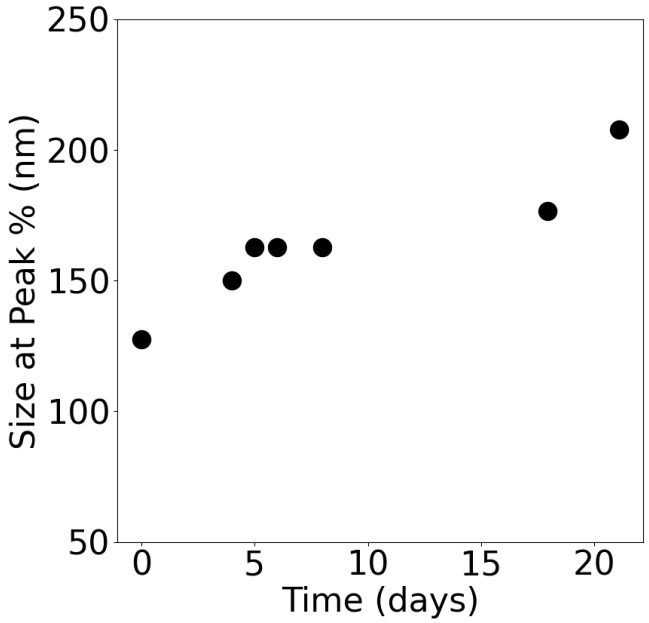


MIVM PolyA LNP **Aging, pH 3.5**

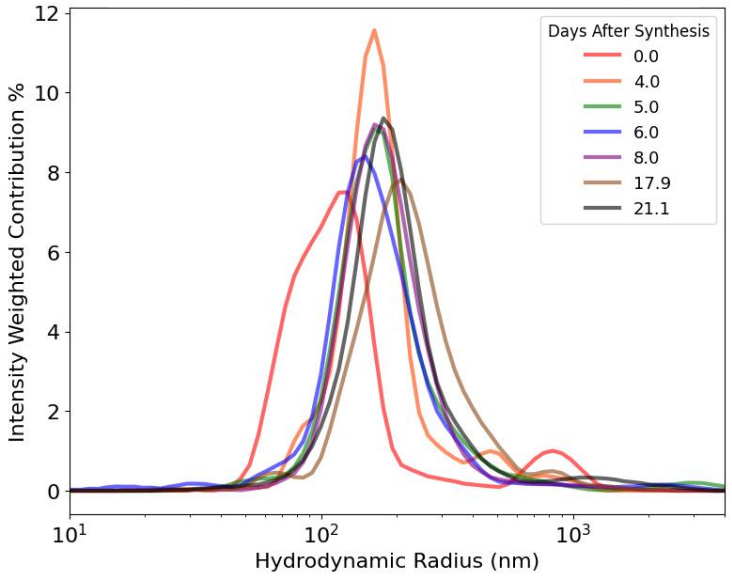
25°C

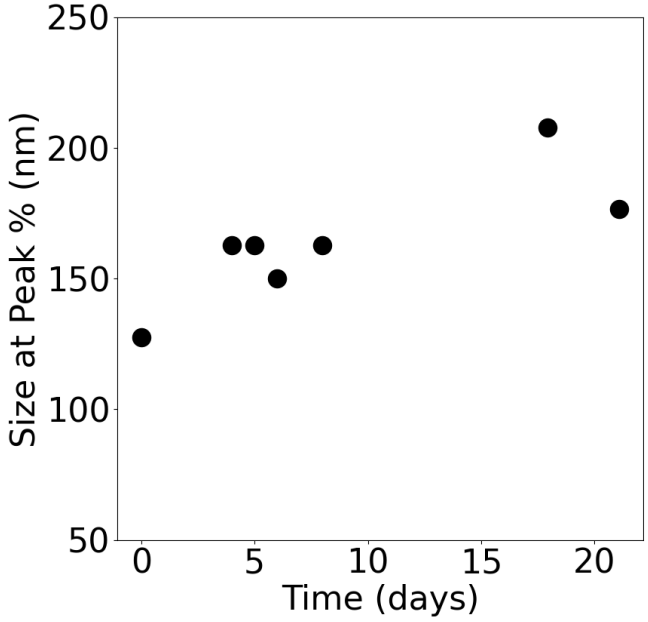




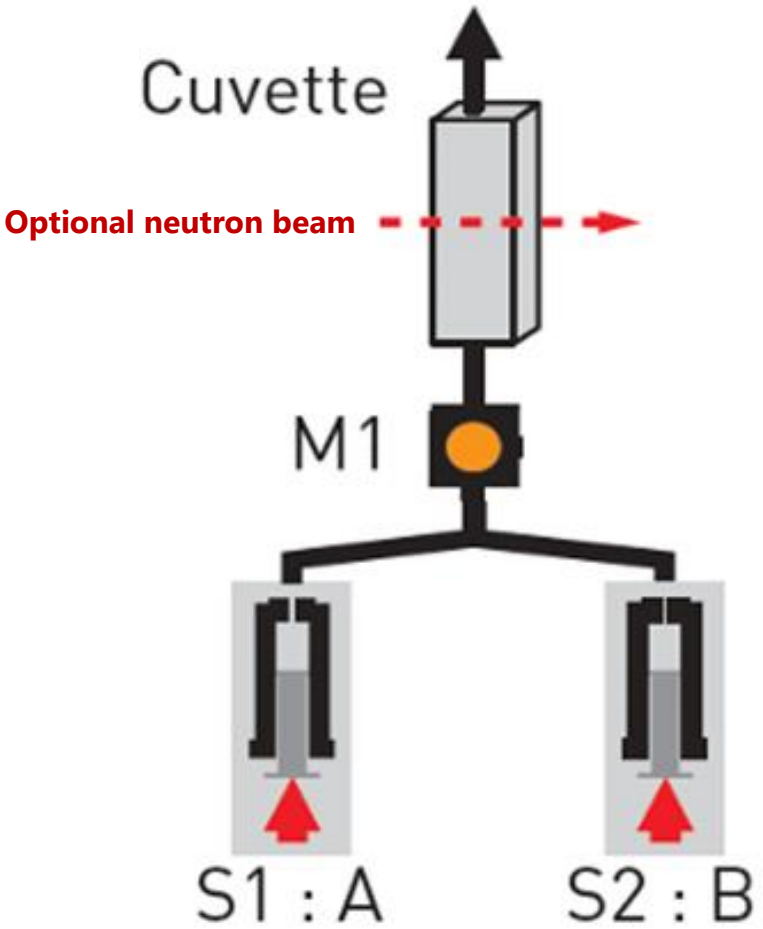


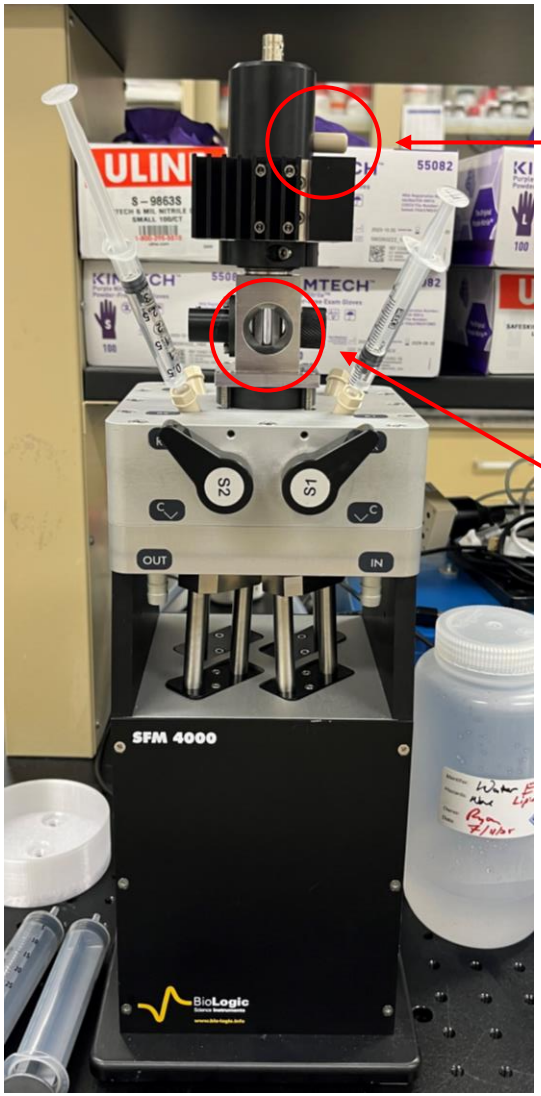
4°C





Stopped Flow Mixer (SFM) Set Up





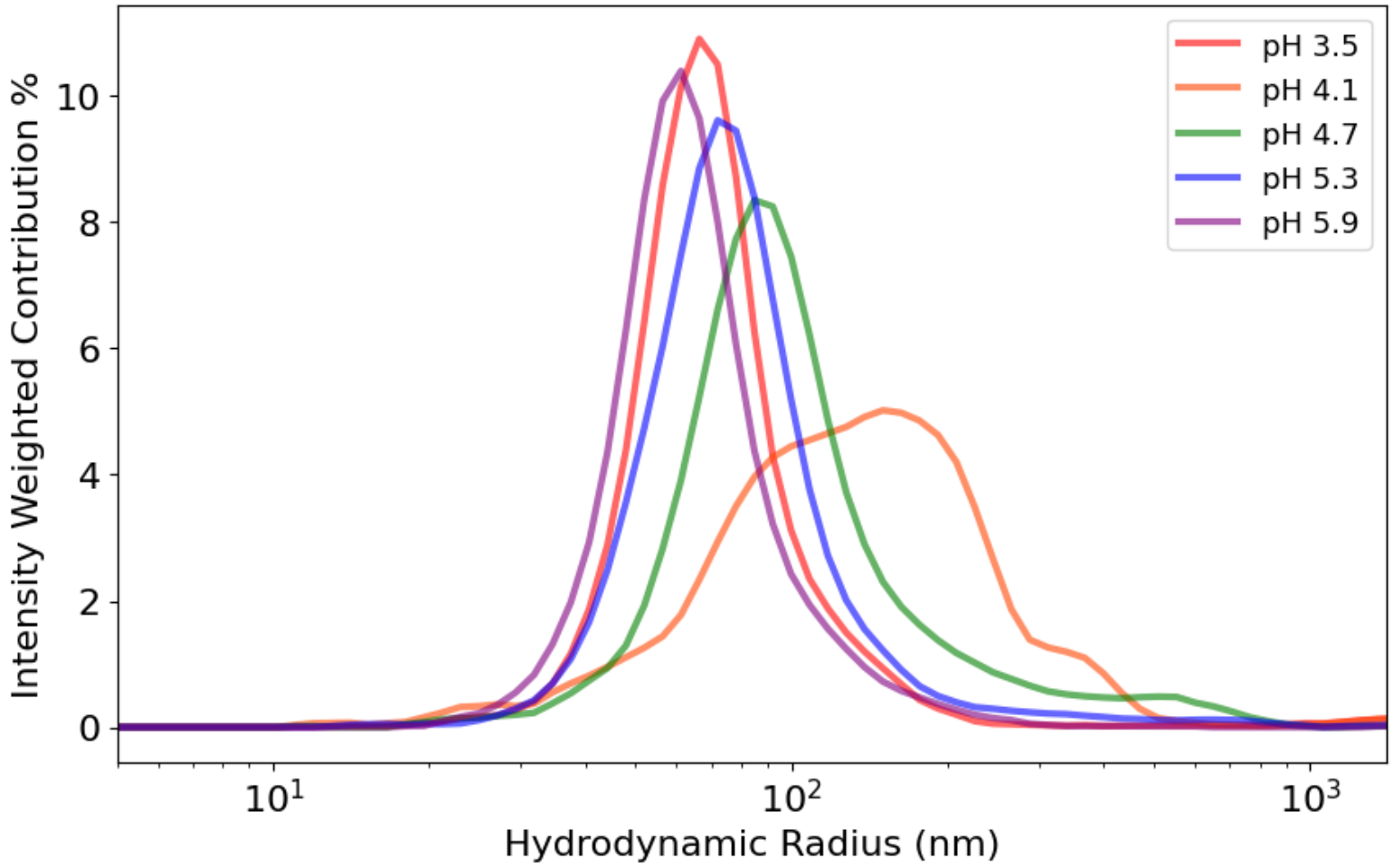
Removed waste outlet tubing to collect samples directly from the cell

Cell where neutron beam would pass through

Matthews, Theyencheri. (2023). Colloid and Polymer Science. 301. 1-8. 10.1007/s00396-023-05093-2.

SFM: pH-Dependent **Empty** LNP Size

Synthesis 1



SFM: Empty LNP Synthesis **Aging, pH 3.5**

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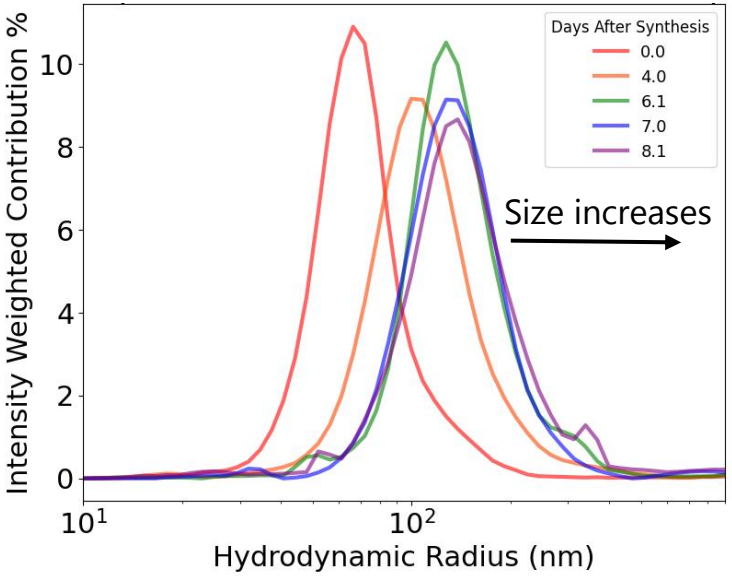
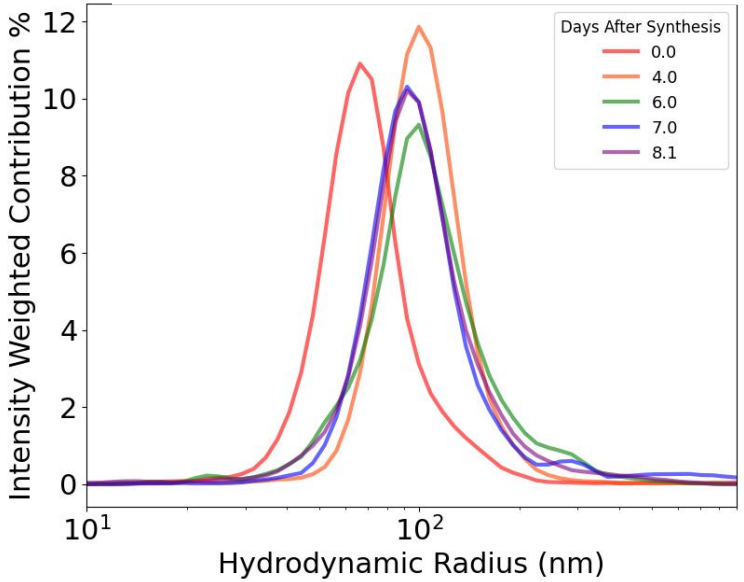
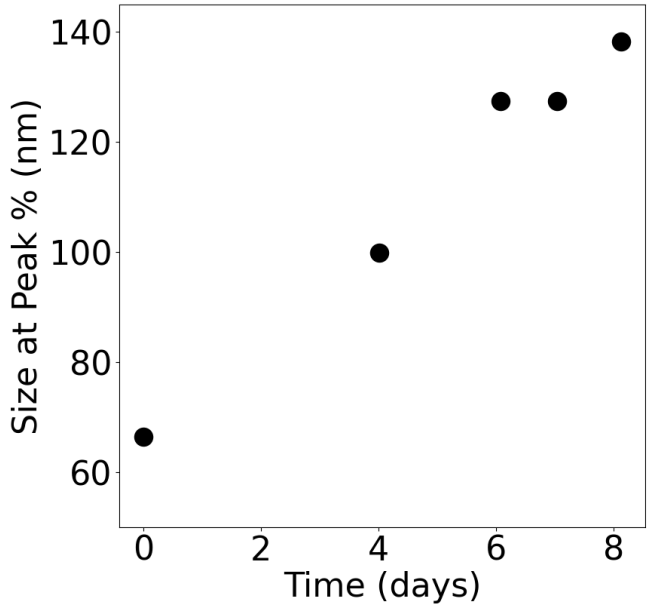
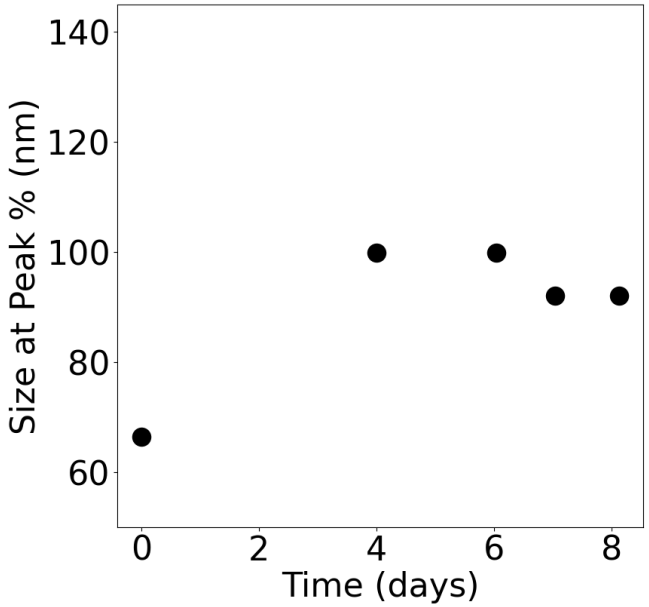
25°C



4°C







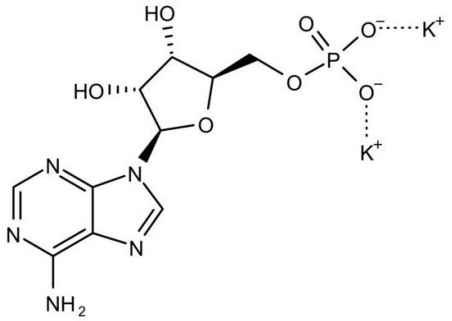
Conclusion

- Similar initial size between MIVM and stopped-flow
- Empty:
  - Aging slower/less significant at 4C
  - Significant aging at 25C
- polyA:
  - Generally, insignificant aging compared to empty LNPs
- Differences between pH, but nonmonotonic

Future Directions

Microfluidic Mixing

PolyA-loaded LNPs



Adenylic Acid (polyadenylic acid, polyA)

Stopped Flow Neutron Scattering

Thank you!

Special thanks to Dr. Yun Liu, Dr. Tom Cleveland, Dr. Ryan Murphy, Dr. Kelsi Rehmann, Dr. Katie Weigandt, Dr. David Hoogerheide, and all others for their contributions and support!

Additional thanks to NCNR SURF Directors, Dr. Susana Marujo Teixeira, Dr. Julie Borchers, and Dr. Leland Harriger