Project Bubbles:

Quantifying the Effects of Air-Water Interface Renewal on the Stability of Pharmaceutical Products

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Aariana Rao, Richard Montgomery HS

Mentors: Dr. Elizabeth Kelley and Dr. Ryan Murphy

Big Picture

Source: Creative Safety Supply, https://shorturl.at/q2PzJ

NDC 0338-0126-12 Rx only

MYXREDLIN

Insulin Human
in 0.9% Sodium Chloride Injection

100 units per 100 mL
(1 unit / mL)

100 mL Single-Dose Galaxy container
Discard unused portion

(REGULAR) Insulin Human
Sterile Nonpyrogenic

For Intravenous Infusion Only

Cautions: Do not add supplemental medication or additives. Must not be used in series connections. Check for minute leaks and solution clarity.

Each mL contains: 1 unit Insulin Human, USP; 0.412 mg Dibasic Sodium Phosphate Anhydrous, USP; 0.29 mg Monobasic Sodium Phosphate Monohydrate, USP; 9 mg Sodium Chloride, USP; and Water for Injection, USP.

Dosage: See prescribing information.

Store refrigerated at 36°F to 46°F (2°C to 8°C) in the original carton to protect from light. Do Not Shake. Do Not Freeze.

If needed, may store at room temperature up to 77°F (25°C) up to 30 days. Discard after 30 days if stored at room temperature.

Baxter **Code 2G3322**

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07-34-00-2341

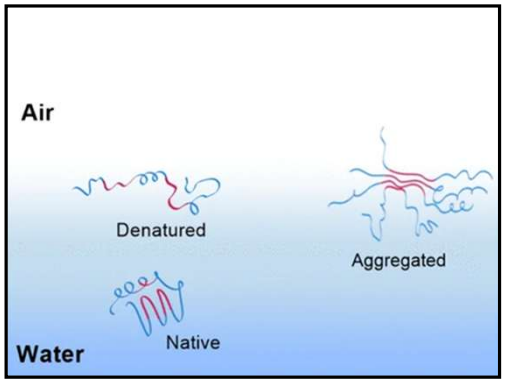
Source: Baxter Healthcare Corporation, 2019, DailyMed

Non-Native Aggregation

Continuous renewal of the air-water interface (bubbles)

Source: 2018-06-water-drop-and-bubbles-01, NJ Productions

Collapse of native structure & aggregation (particles)



Source: Chem. Rev. (2024) 124 (9): 5668–5694

Decrease in drug potency & adverse immune response

Source: iStock, weakened immune system concept, via Getty Images

Project Overview

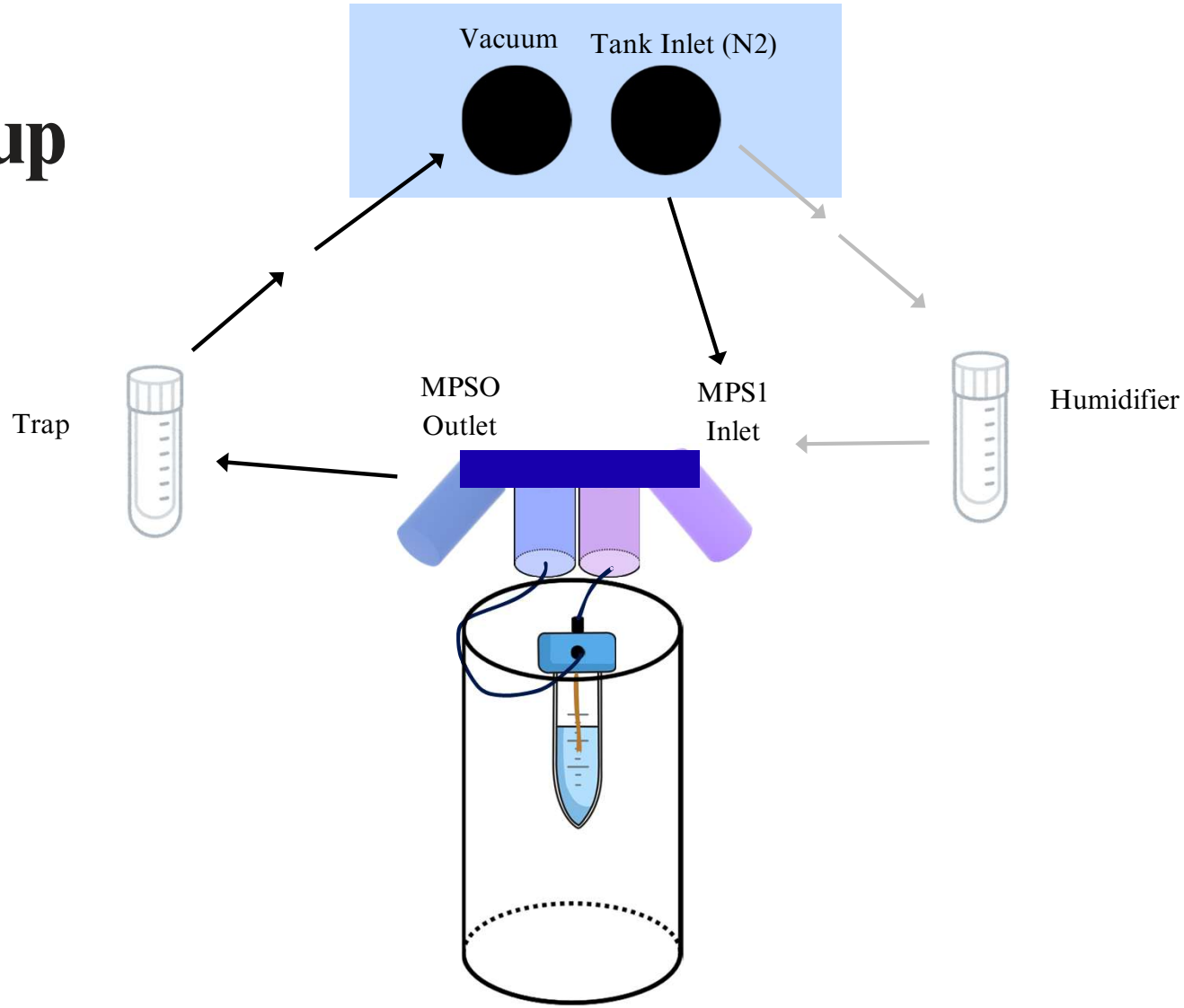
01 Cad Model of the Experimental Setup

02 Produce bubbles in water to test setup and flow rate

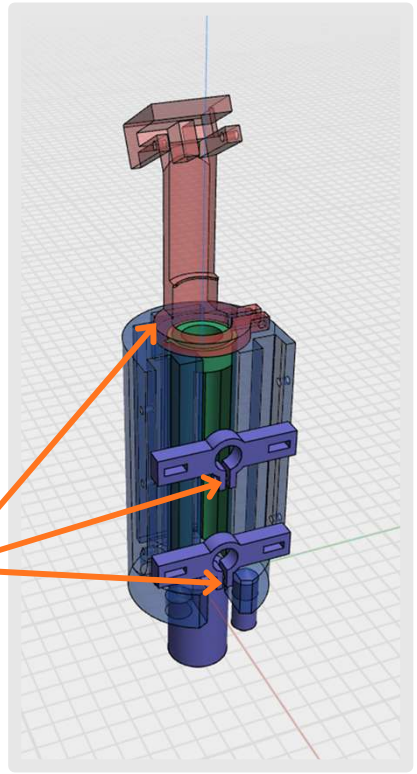
03 Test with proteins: Lysozyme and BSA (Bovine Serum Albumin)

04 Test with lipid nanoparticles (± mRNA)

Setup

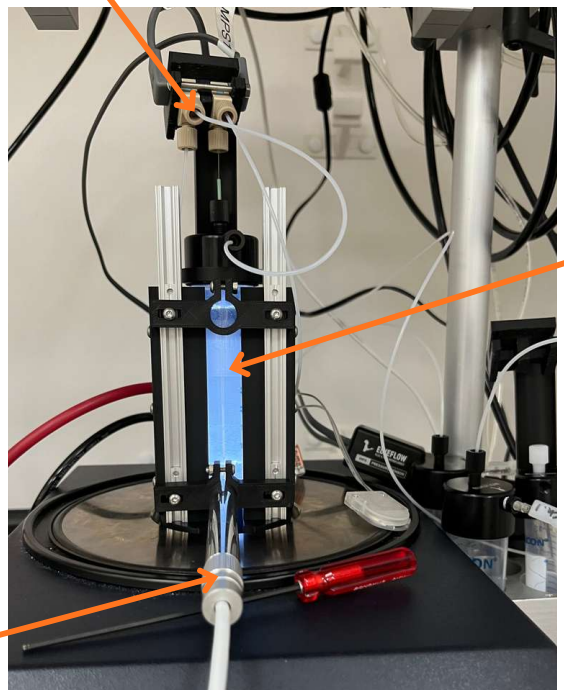

Vacuum
Tank Inlet (N2)
Trap
MPSO
Outlet
MPS1
Inlet
Humidifier

Setup



C-Clips
for vial and
cameras

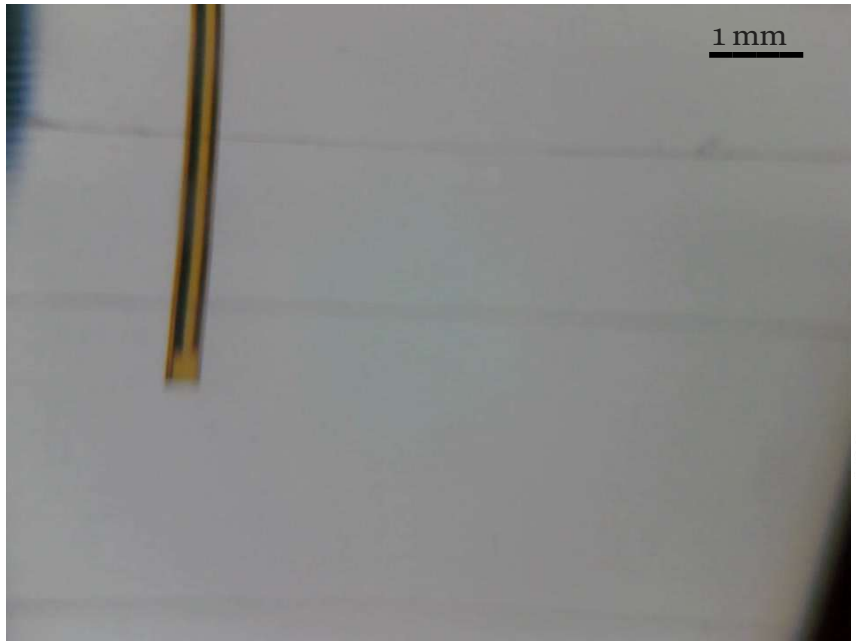
Pressure Sensors (N2)

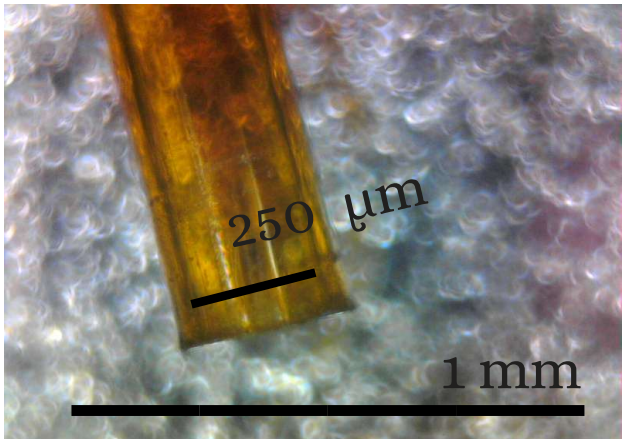


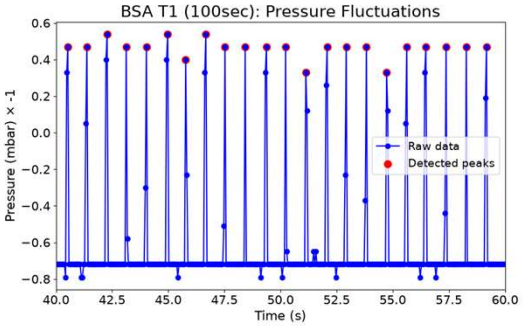
Vial containing
solution and a
capillary tube

Camera

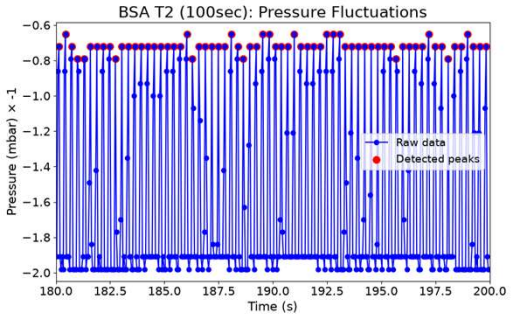
Producing Bubbles







1.1 bubbles/second

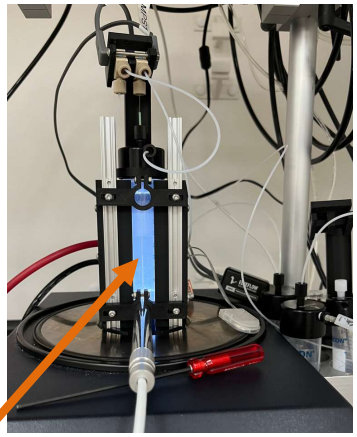


3.0 bubbles/second

Sample Volumes

15 mL vial

50 mL vial



Project Overview

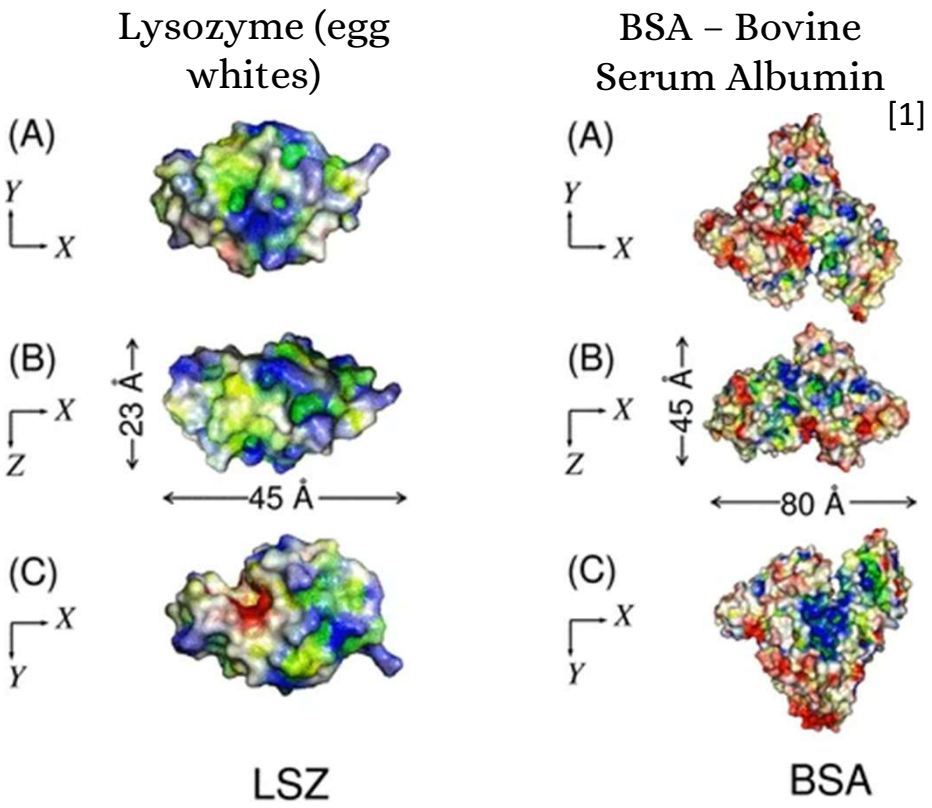
01 Cad Model of the Experimental Setup

02 Produce bubbles in water to test setup and flow rate

03 Test with proteins: Lysozyme and BSA (Bovine Serum Albumin)

04 Test with lipid nanoparticles (± mRNA)

Proteins



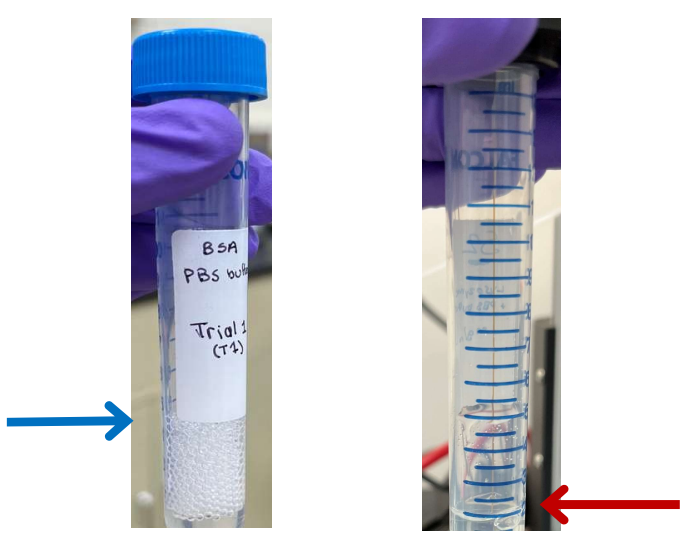
Hydrophobic residues

Positively charged residues

Negatively charged residues

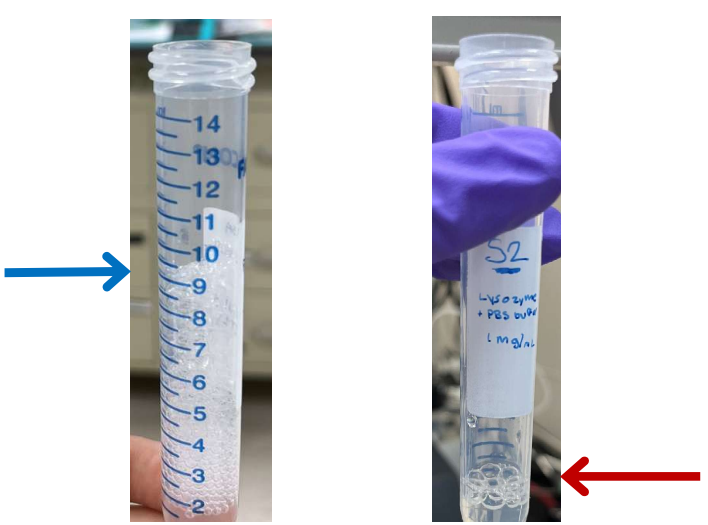
[1] Yano et al. J. Phys. Chem. B (2018) 122 (17): 4662–4666.

Foaming Comparison



BSA Lysozyme

1,000 Seconds



BSA Lysozyme

~ 1.5 hours

UV-Vis

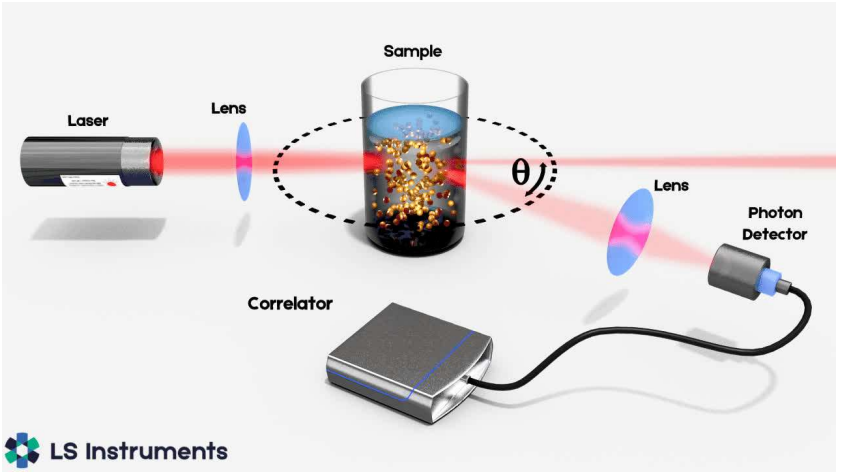
(UltraViolet – Visible Light)

Measures protein concentration

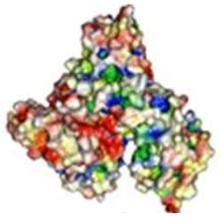
Source: Emily Humphreys, 2016, ThermoFisherScientific

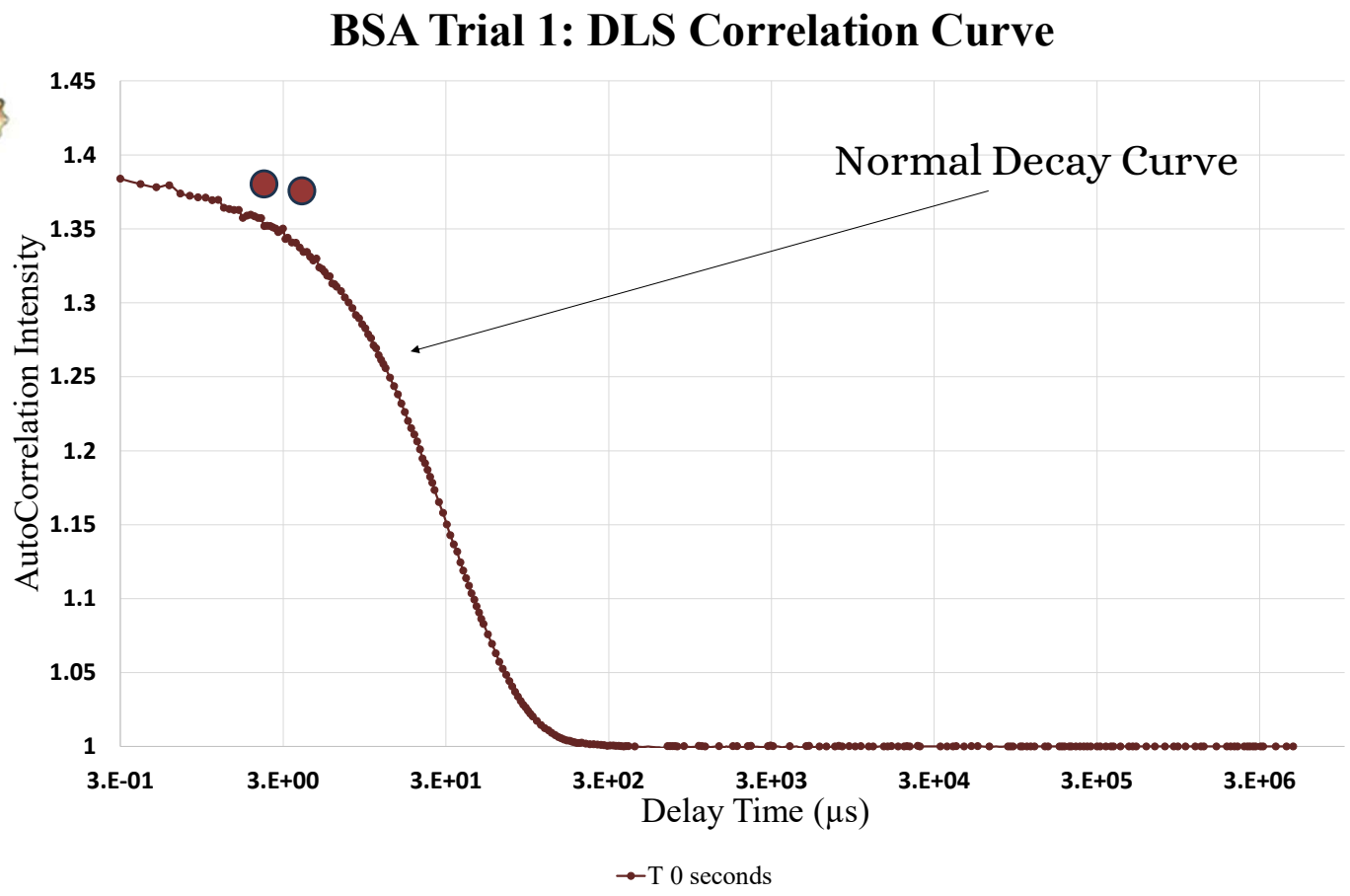
Dynamic Light Scattering (DLS)

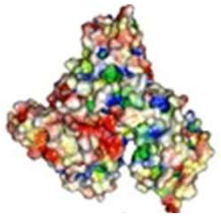
Measures protein / aggregate size



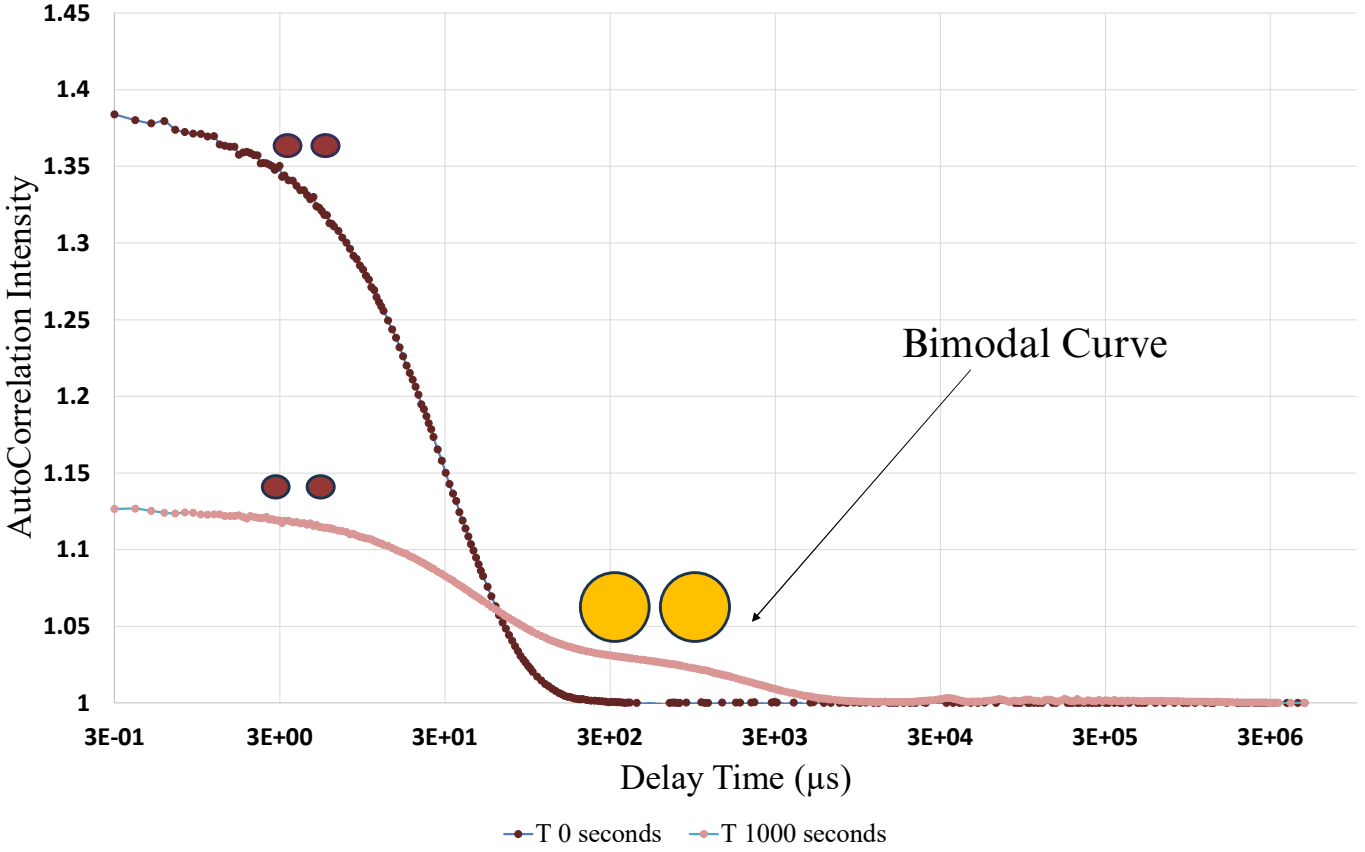
Source: LS Instruments, Dynamic Light Scattering DLS

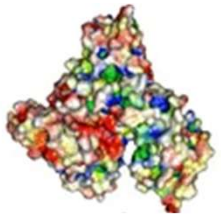



BSA Trial 1: DLS Correlation Curve
Normal Decay Curve
AutoCorrelation Intensity
1.45
1.4
1.35
1.3
1.25
1.2
1.15
1.1
1.05
1
3.E-01
3.E+00
3.E+01
3.E+02
3.E+03
3.E+04
3.E+05
3.E+06
Delay Time (µs)
T 0 seconds

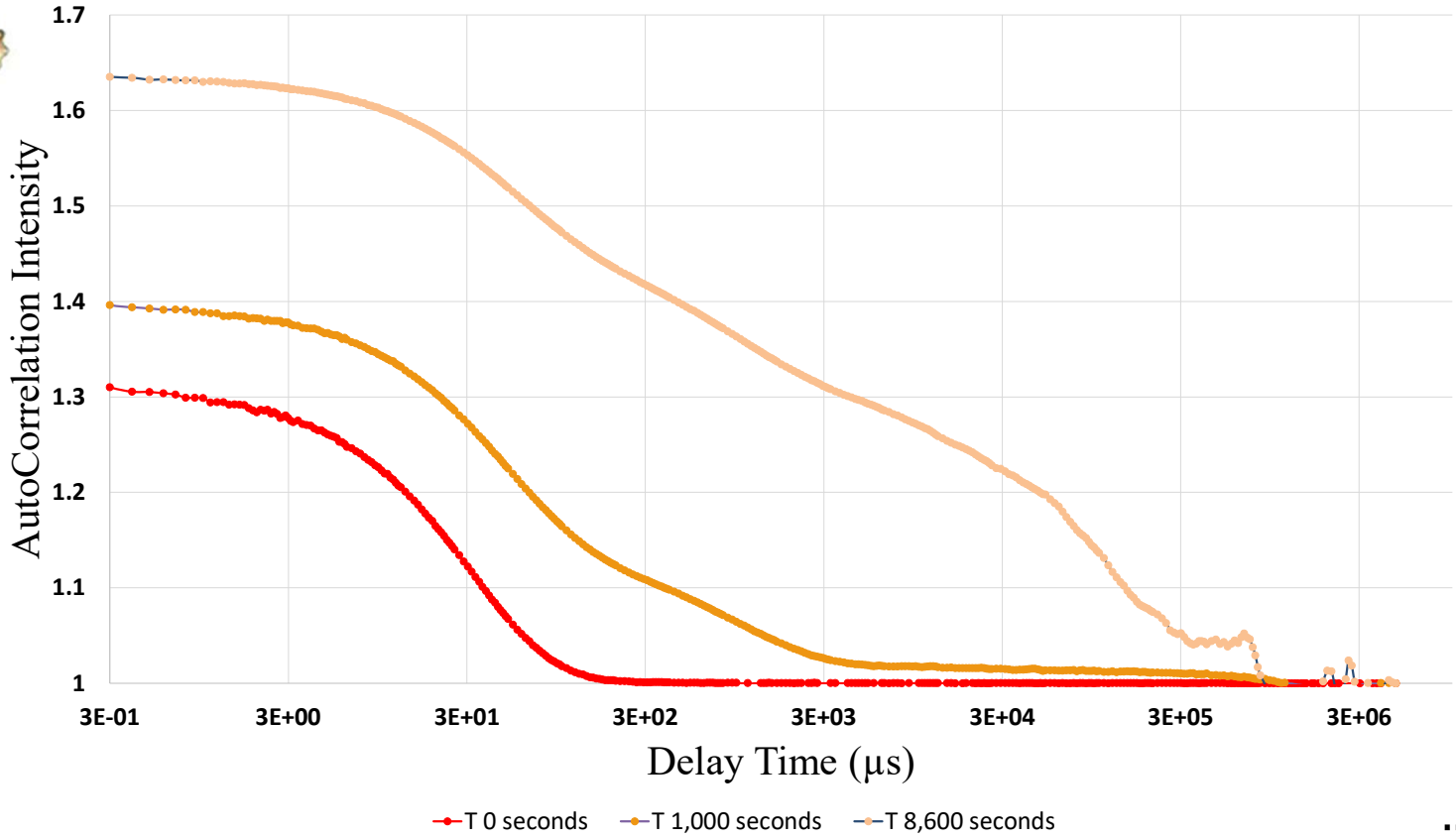


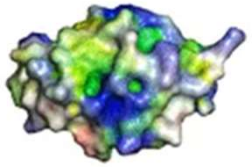
BSA Trial 1: DLS Correlation Curve

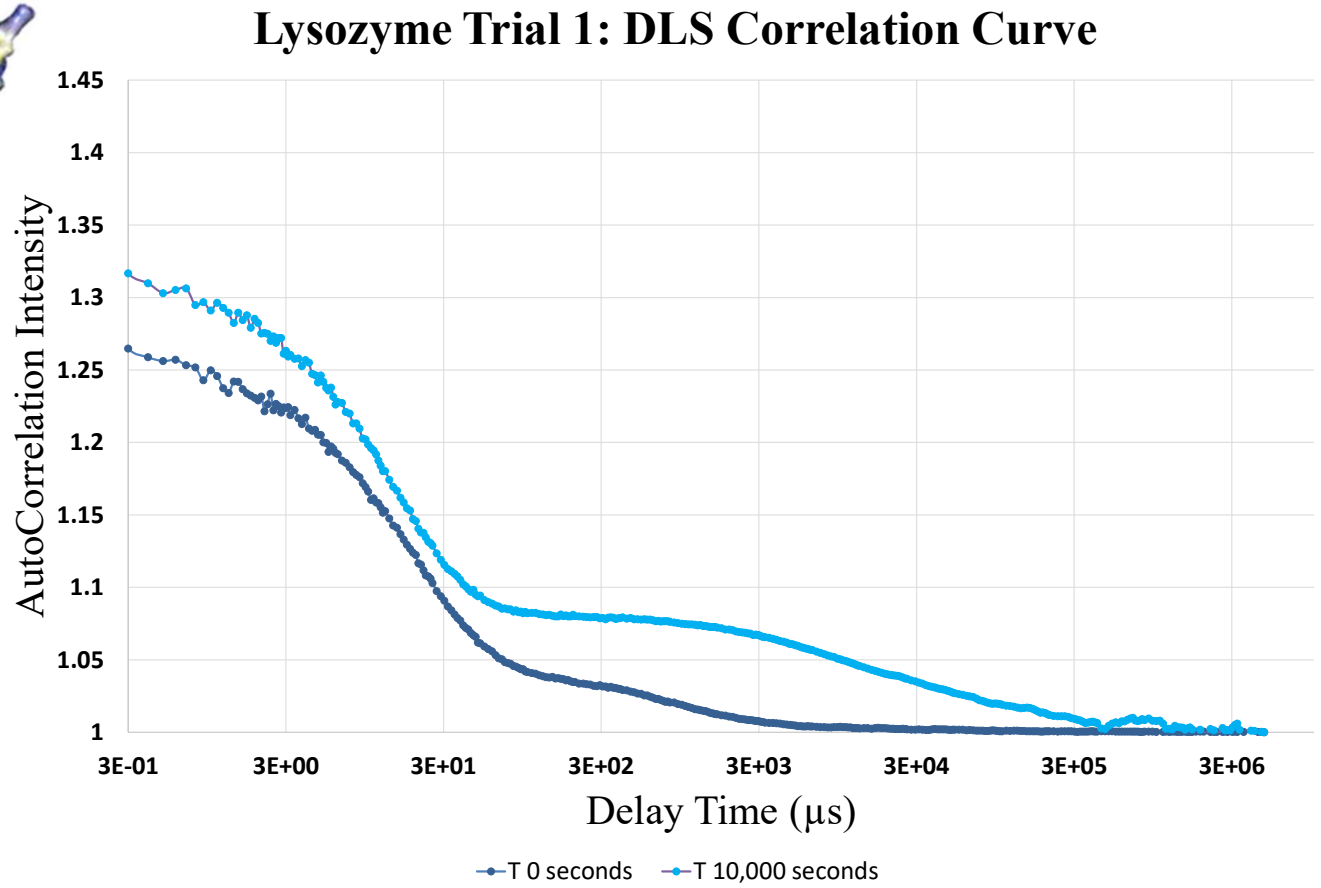


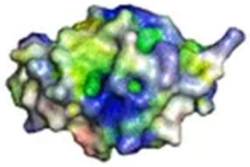


BSA Trial 2: DLS Correlation Curve

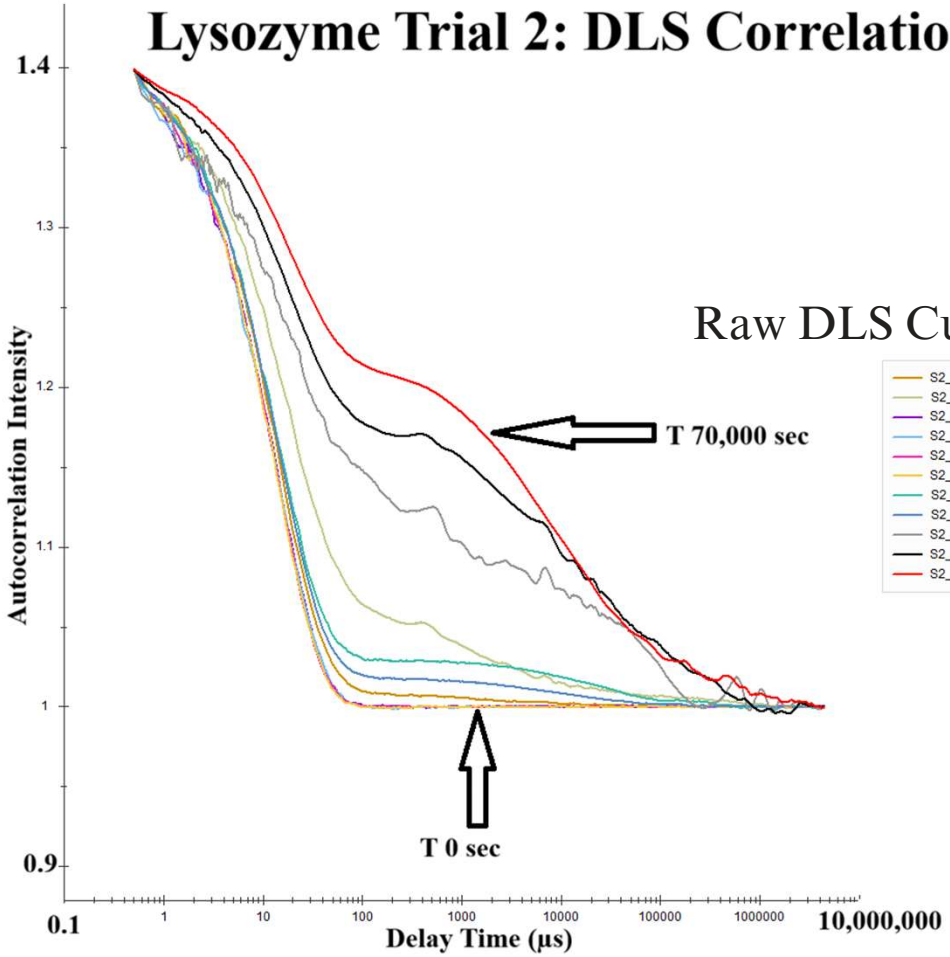




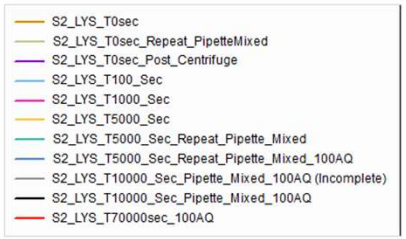

Lysozyme Trial 1: DLS Correlation Curve
AutoCorrelation Intensity
1.45
1.4
1.35
1.3
1.25
1.2
1.15
1.1
1.05
1
3E-01
3E+00
3E+01
3E+02
3E+03
3E+04
3E+05
3E+06
Delay Time (µs)
T 0 seconds
T 10,000 seconds



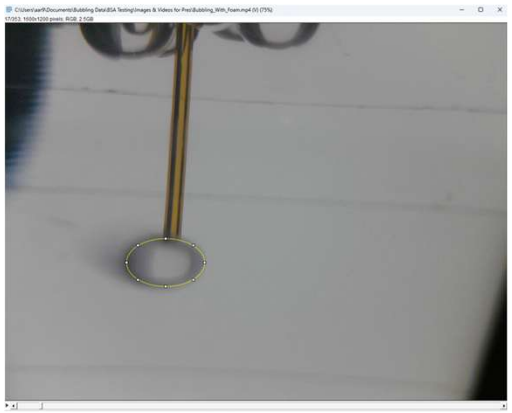
Lysozyme Trial 2: DLS Correlation Curve



Raw DLS Curves Normalized



Total Interfacial Turnover



Surface Area Formula for Ellipsoid

$$A = \frac{\pi D_{eq}^2}{2} + \frac{\pi D_{pol}^2}{4e} \ln\left(\frac{1+e}{1-e} \right)$$

Bubble Surface Area * Number of Bubbles Produced = Total Interfacial Turnover

Results

File Edit Font Results

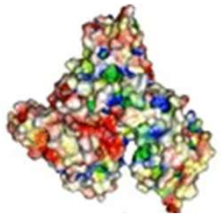
	Area	Mean	Min	Max	Major	Minor	Angle	Circ.	AR	Round	Solidity
1	29812	116.811	63	149	248.061	153.018	0	0.918	1.621	0.617	1.001
2	21228	151.483	121	159	200.043	135.113	0	0.945	1.481	0.675	1.000
3	19096	109.738	53	153	206.021	118.016	0	0.893	1.746	0.573	1.000
4	18449	115.301	44	154	191.005	122.981	0	0.931	1.553	0.644	1.000

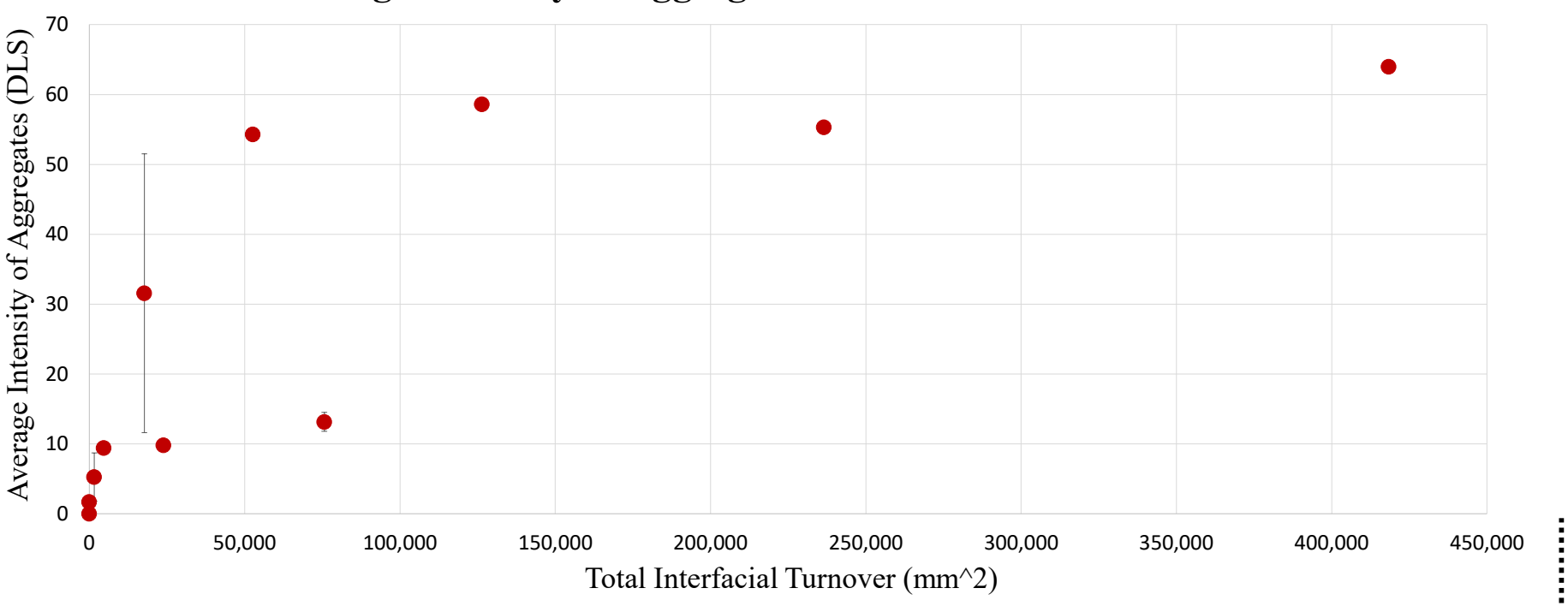
A = Total Surface Area of the oblate spheroid (flattened) bubble

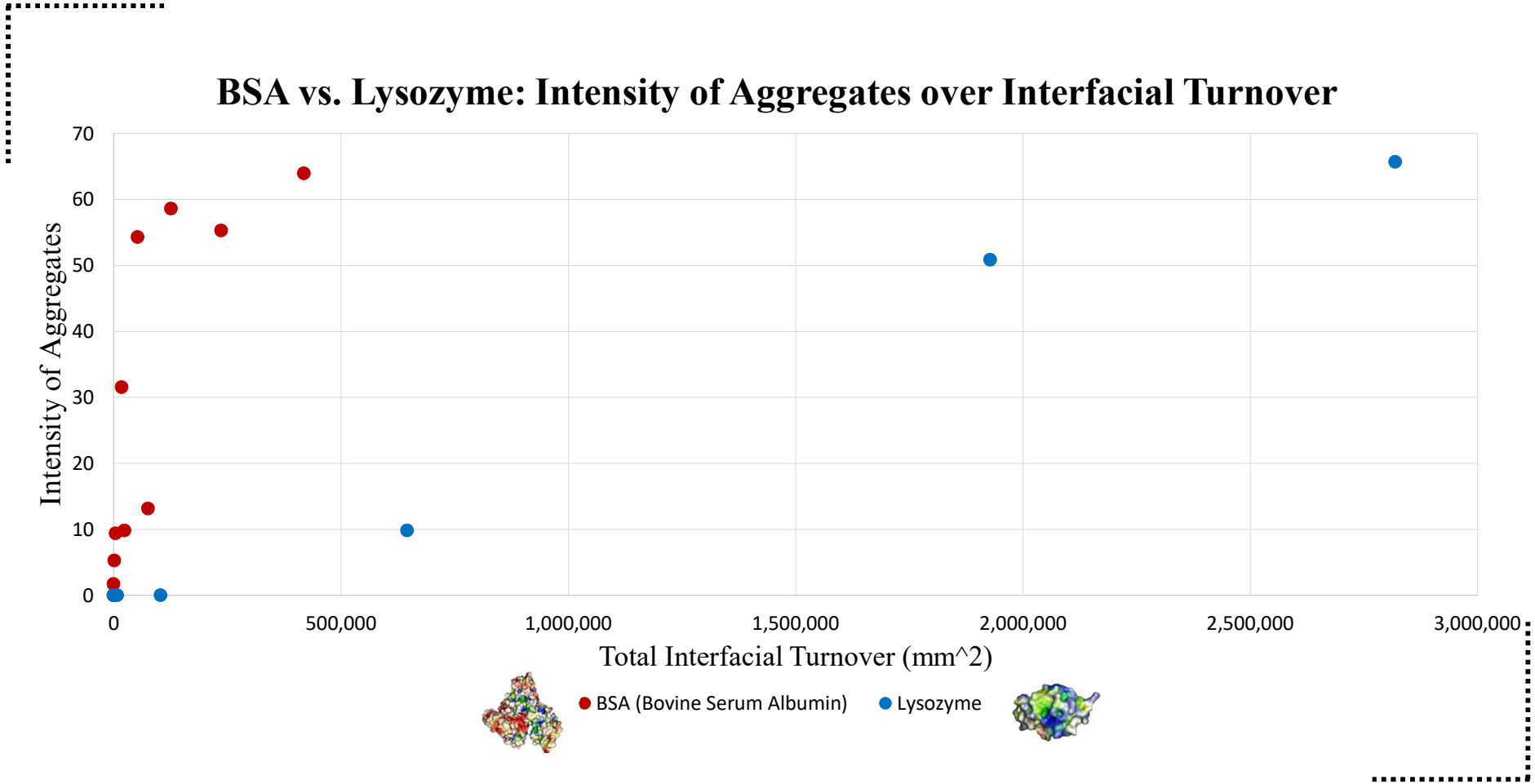
D_{eq} = Equatorial Diameter (Major)

D_{pol} = Polar Diameter (Minor)

e = Eccentricity of the spheroid




BSA: Average Intensity of Aggregates over Total Interfacial Turnover
Average Intensity of Aggregates (DLS)
70
60
50
40
30
20
10
0
0
50,000
100,000
150,000
200,000
250,000
300,000
350,000
400,000
450,000
Total Interfacial Turnover (mm^2)


BSA vs. Lysozyme: Intensity of Aggregates over Interfacial Turnover
Intensity of Aggregates
70
60
50
40
30
20
10
0
0
500,000
1,000,000
1,500,000
2,000,000
2,500,000
3,000,000
Total Interfacial Turnover (mm^2)
BSA (Bovine Serum Albumin)
Lysozyme

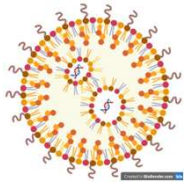
Project Overview

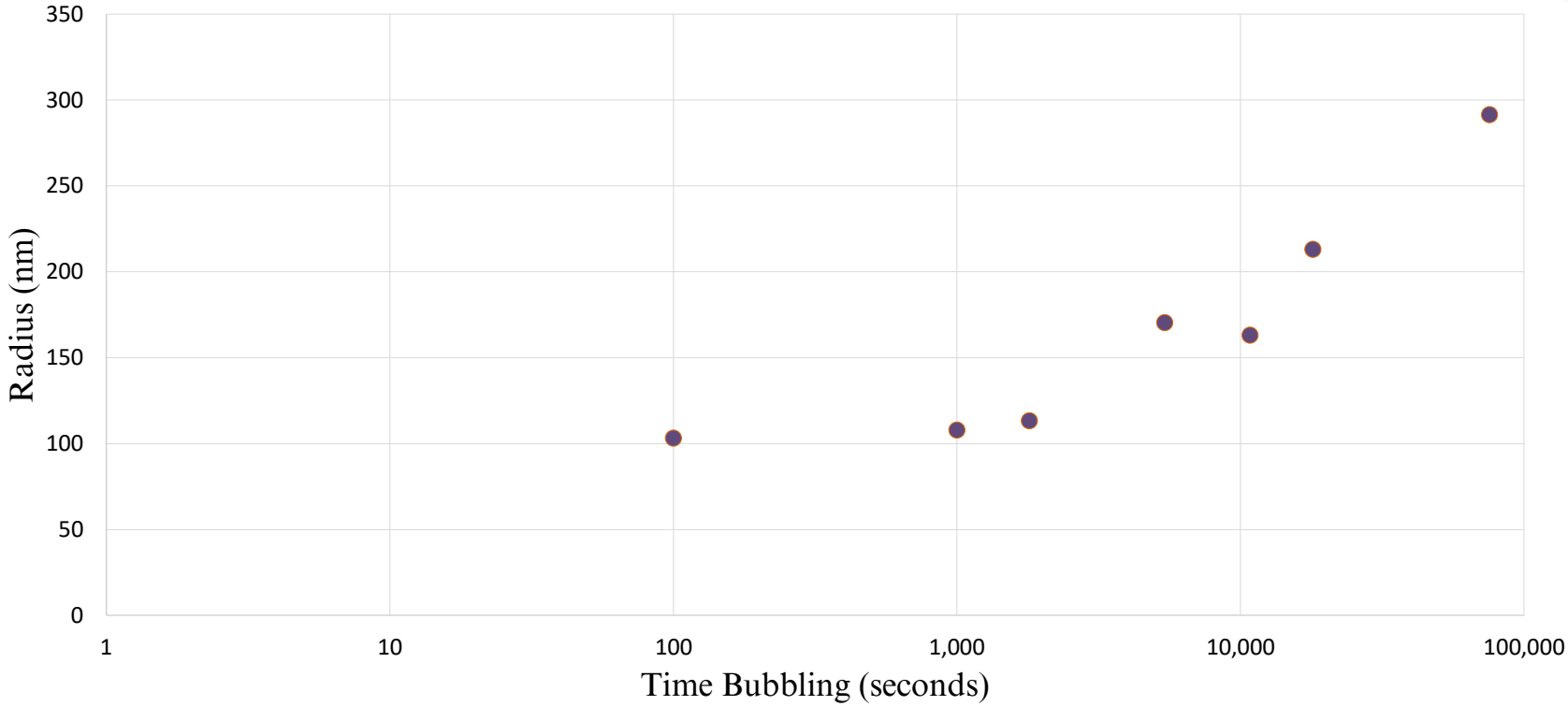
01 Cad Model of the Experimental Setup

02 Produce bubbles in water to test setup and flow rate

03 Test with proteins: Lysozyme and BSA (Bovine Serum Albumin)

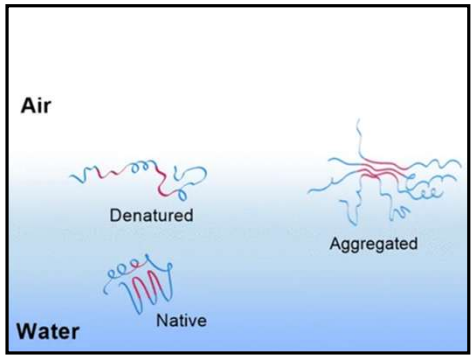
04 Test with lipid nanoparticles (± mRNA)

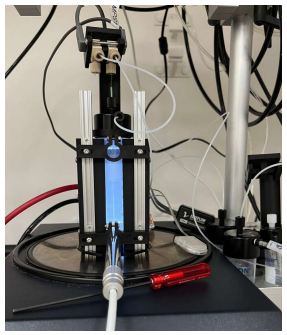



LNP with mRNA Average Radius over Bubbling Time
Radius (nm)
350
300
250
200
150
100
50
0
1
10
100
1,000
10,000
100,000
Time Bubbling (seconds)

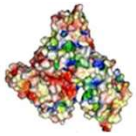
Conclusions

- Exposure to an air-water interface induces aggregation

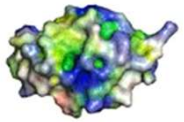




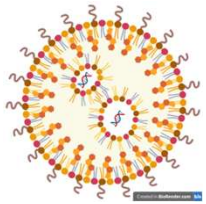
- Bubbling device effectively demonstrates kinetics for aggregation



- Initial finding: changes seem to occur on a logarithmic scale
- Results vary for BSA and Lysozyme over timescale

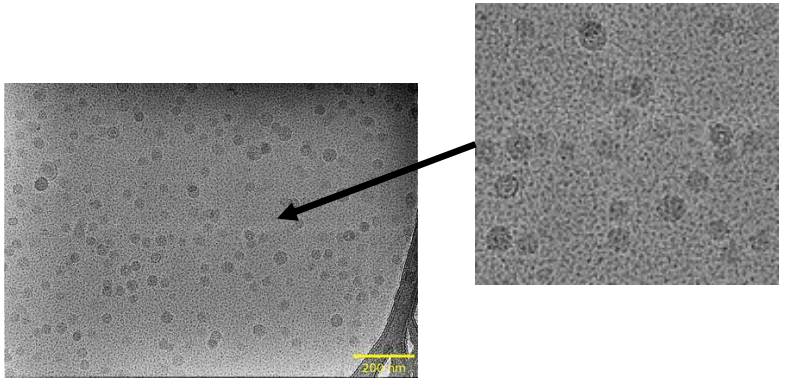


- Lipid Nanoparticles aggregate differently from proteins

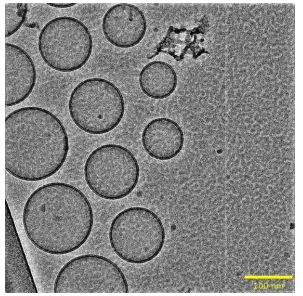


Future Work

- Further testing with BSA and Lysozyme samples to establish a trend
- Testing with Lipid Nanoparticles without mRNA
- Testing with lipid vesicles to see different trends
- Using Cryo-TEM microscopy to analyze the shape of aggregates

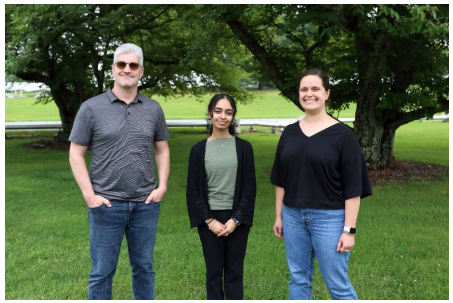


Source: Tom Cleveland, https://www.nist.gov/programs-projects/lnp-rna-research-grade-test-material



Cryo-TEM image of Lipid Vesicles

Acknowledgements



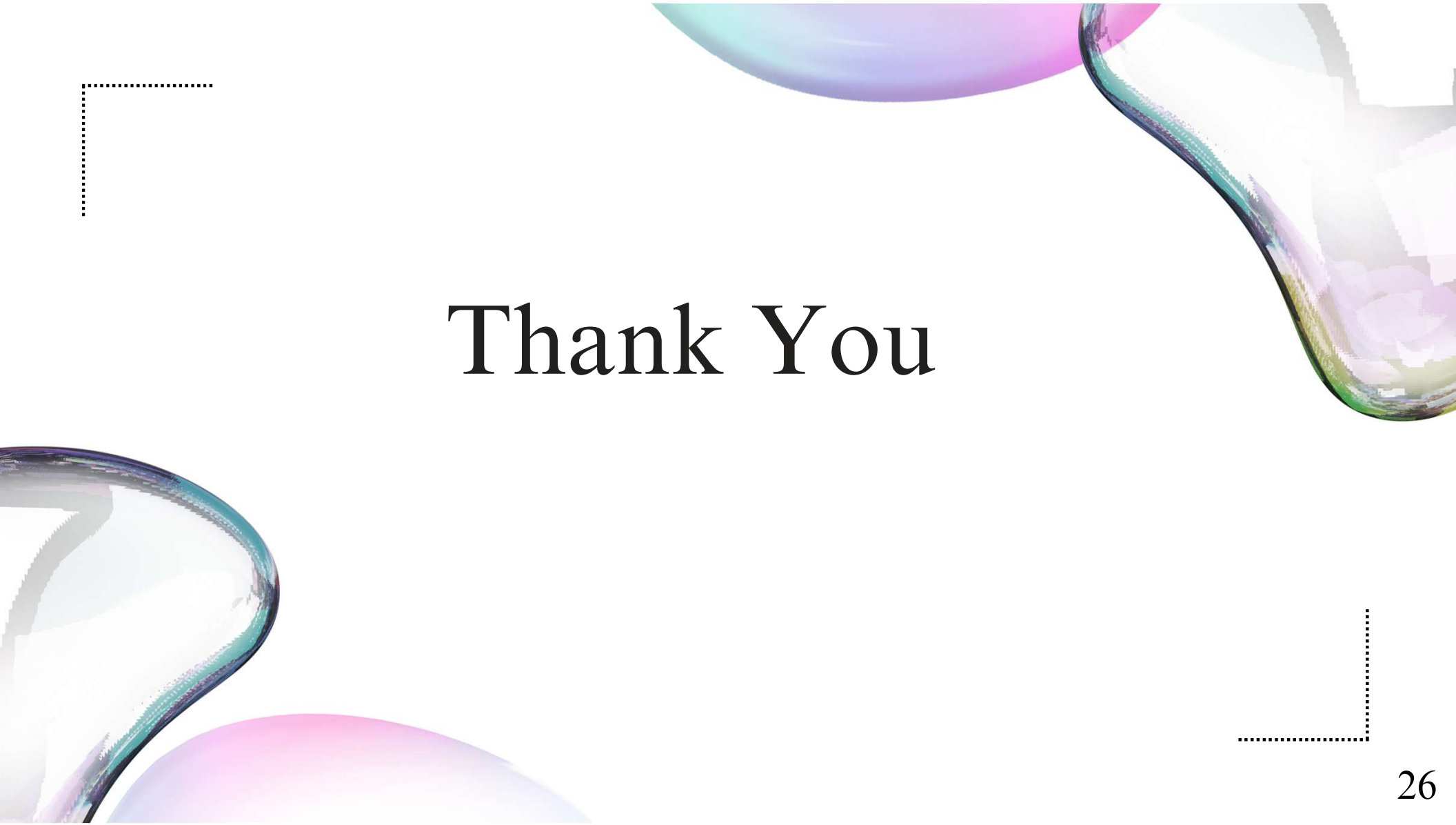
Thank you!

Mentors: Dr. Elizabeth Kelley, Dr. Ryan Murphy for their guidance

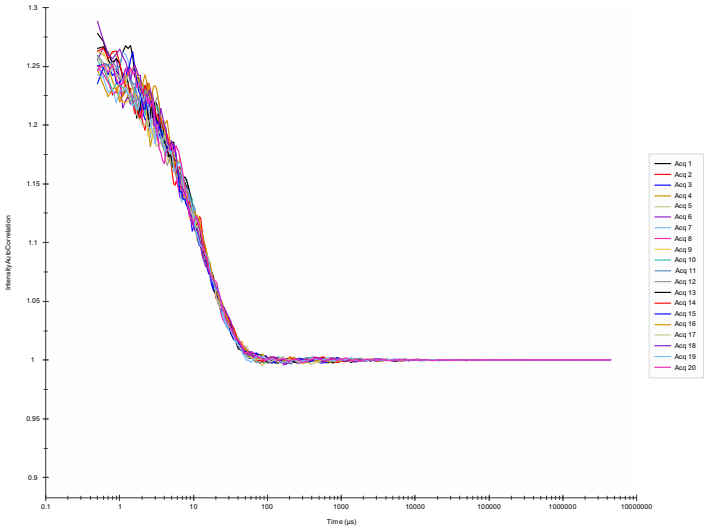
SHIP Directors: Dr. Cara O'Malley, Dr. Leland Harriger

NCNR SHIP Leads: Dr. Julie Borchers, Dr. Kelsi Rehmann, Dr. Susana Marujo Teixeira

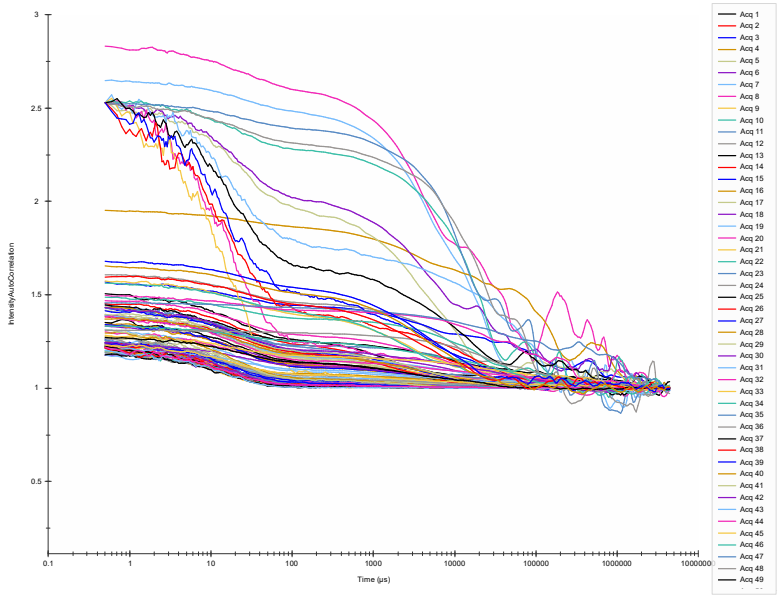
And everyone else who supported me this summer :)


Thank You

DLS Acquisitions - Lysozyme

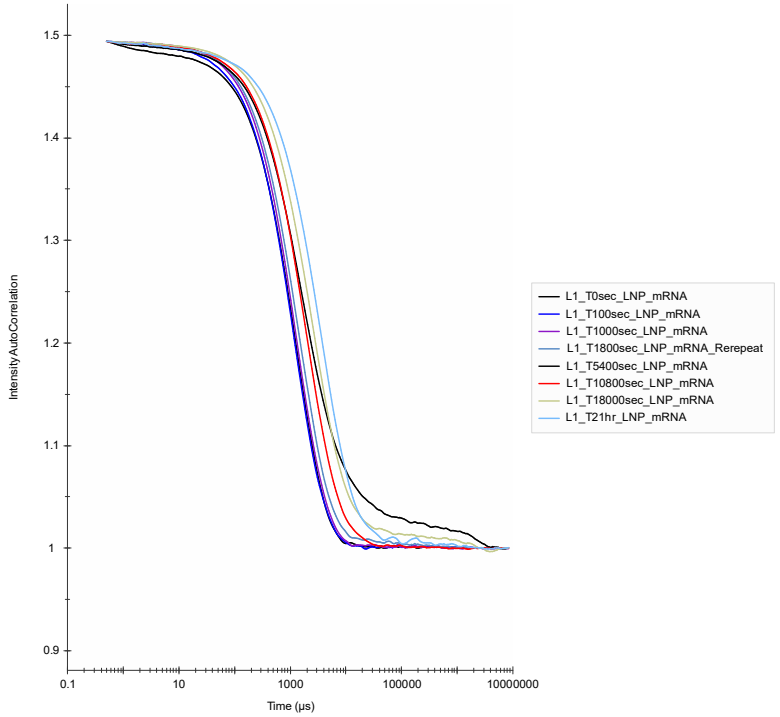


500 seconds

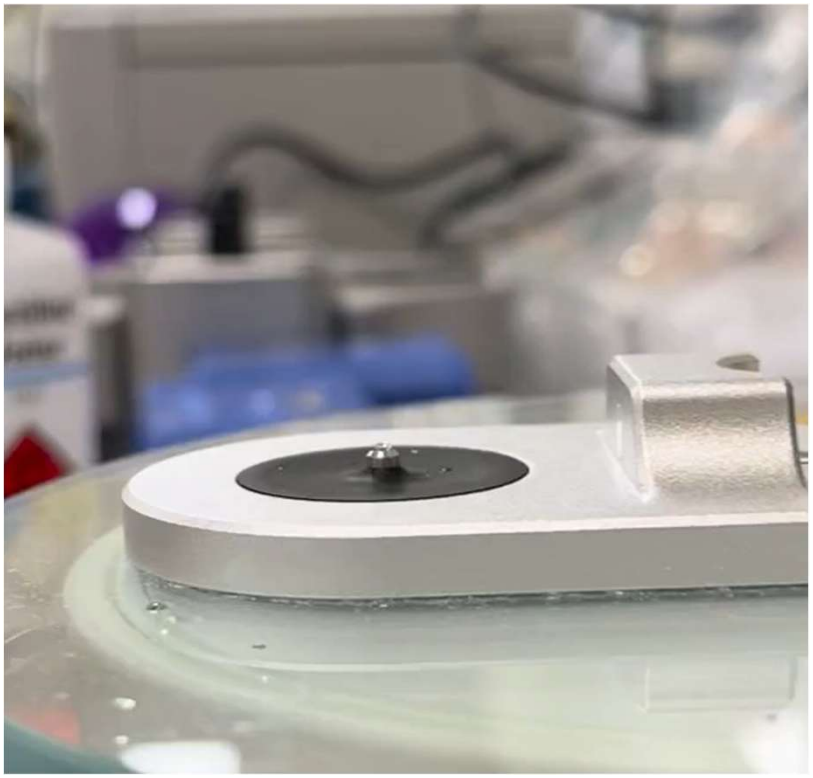


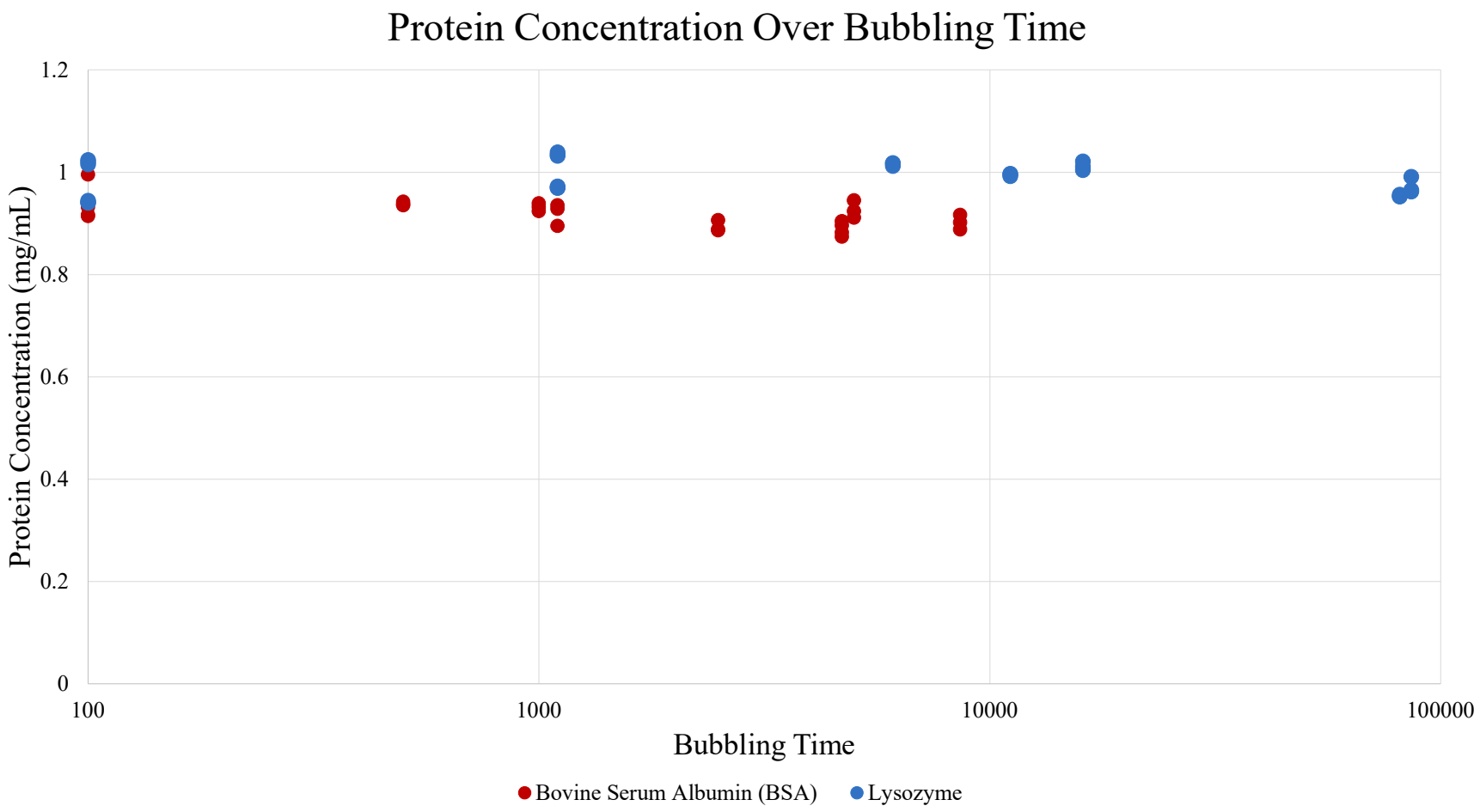
70000 seconds

DLS Acquisitions - LNPS



NanoDrop




Protein Concentration Over Bubbling Time
Protein Concentration (mg/mL)
1.2
1
0.8
0.6
0.4
0.2
0
100
1000
10000
100000
Bubbling Time
Bovine Serum Albumin (BSA)
Lysozyme