Stability of Liposomal Nanomedicines

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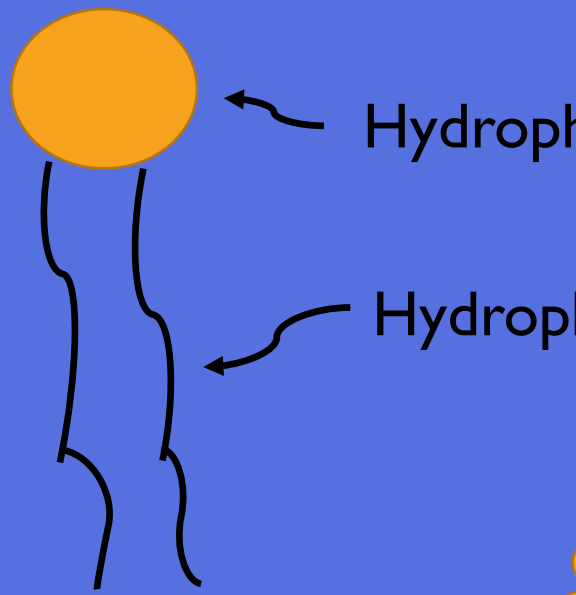
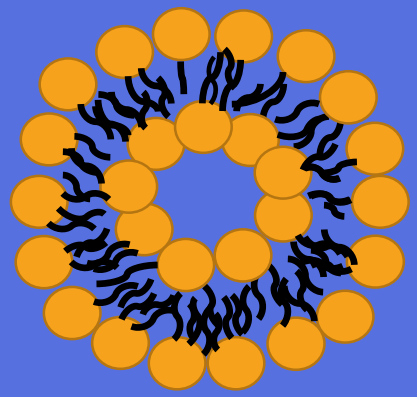
OUTLINE

1. **Introduction**
2. Materials and methods
3. Results and discussions
4. Conclusions

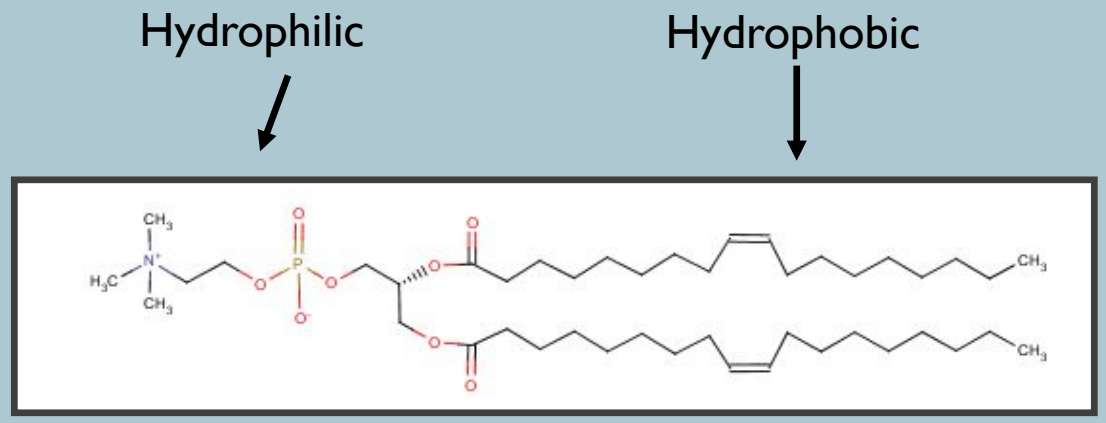
WHAT ARE LIPOSOMES?





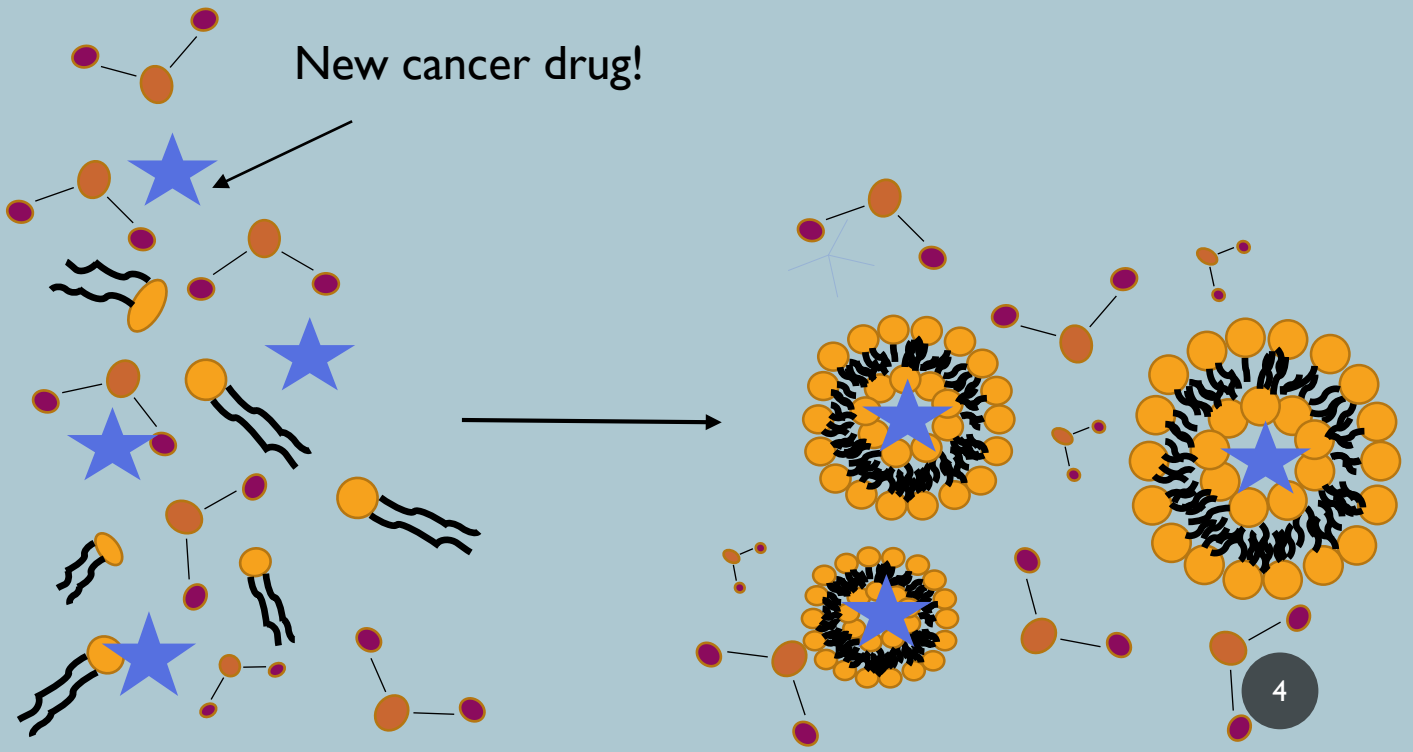
Unilamellar Liposome

- Lipids in solution form **liposomes**
  - Enclose aqueous solutions
- Vesicles can have one or more bilayers
  - Unilamellar vesicles simplest model for drug delivery



WHY LIPOSOMES?

- Liposomes are promising candidates for future drug delivery applications
  - Similar morphology to the cellular membrane
  - Biocompatible, biodegradability, and low toxicity

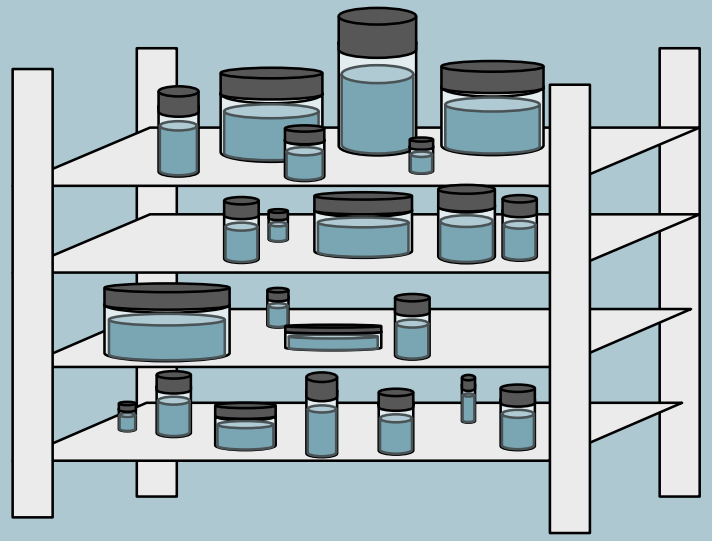


Doxil: Liposomal Injection for Ovarian Cancer

Exparel: Non-opioid pain relief

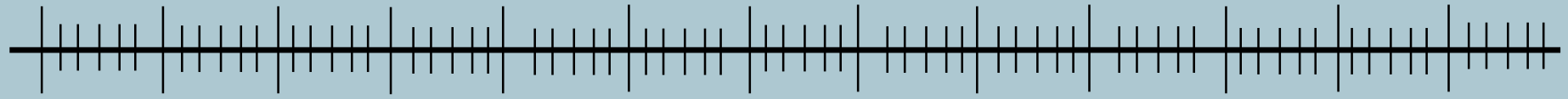
Liposome Real Toning Ampoule: Skin Care

PHARMACEUTICAL USE: WHAT WE STILL DON'T KNOW

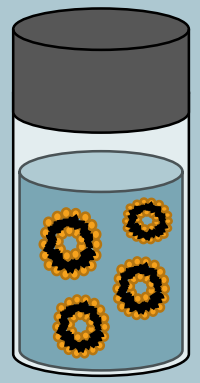


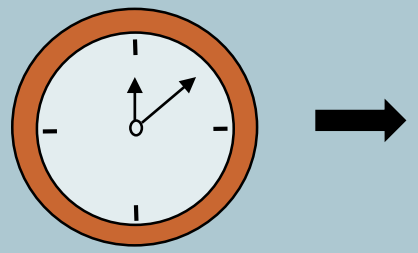
- Shelf life and storage conditions for liposomes are unknown details
  - Crucial for pharmaceutical use
- My experiments look closely at vesicle **stability**
  - **Chemical Instability**
  - **Structural Instability**

Formulation



Usage

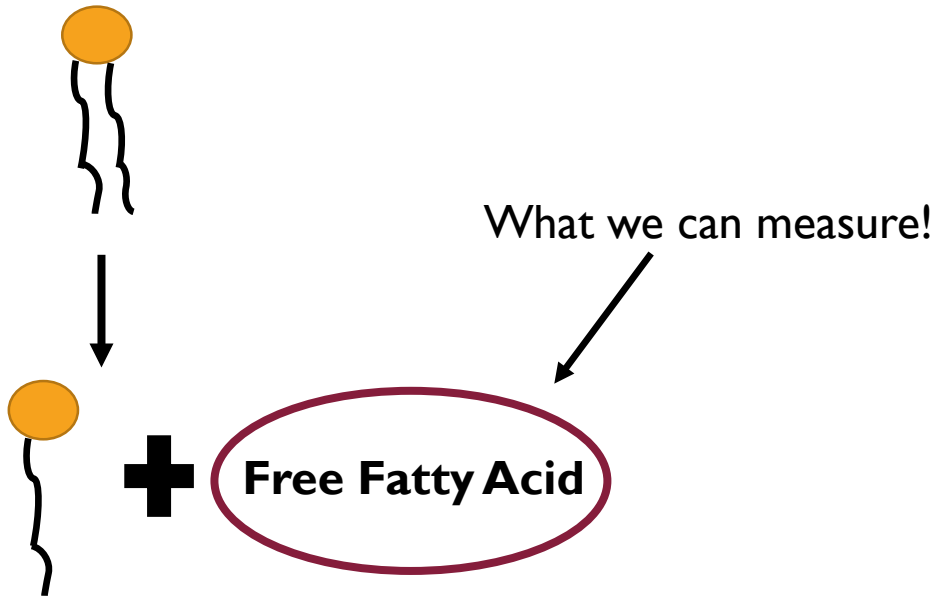




CHEMICAL INSTABILITY

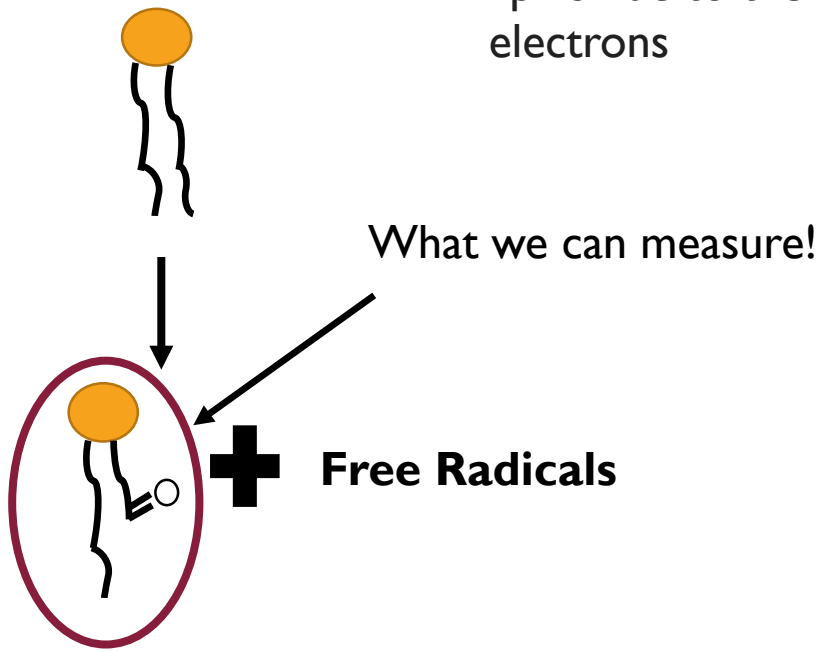
HYDROLYSIS

- Break down of lipids from water moisture

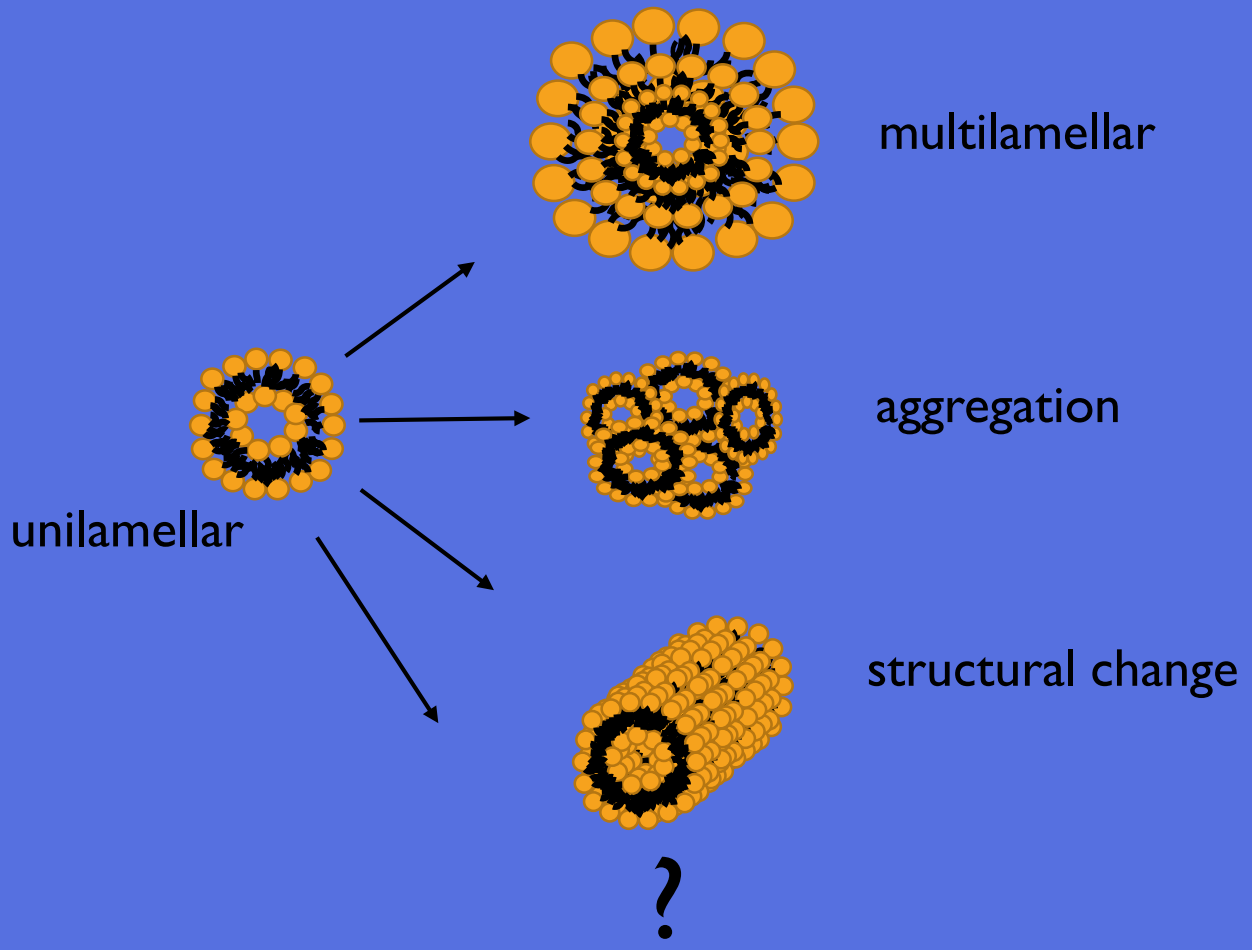


OXIDATION

- Oxidative degradation of lipids due to the loss of electrons



STRUCTURAL INSTABILITY



Structural Instability:

- Result of chemical degradation
- Unstable liposomes can form many different structures
  - Multilamellar?
  - Aggregation?
  - New Structure?
  - ?

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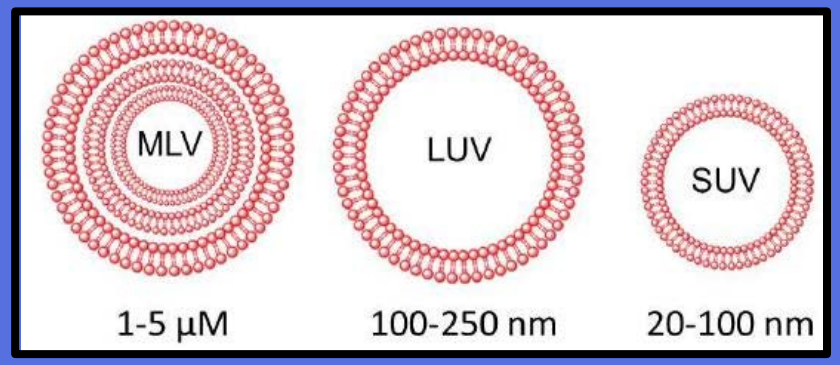
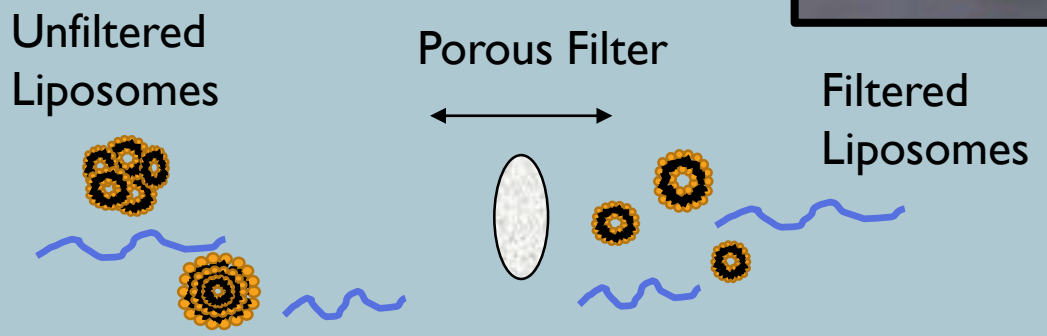
MATERIALS

- Using model system 1,2-Dioleoyl-sn-glycero-3-phosphocholine **(DOPC)**
  - Commonly used lipid in research
- Unilamellar vesicles



Lipid Extrusion:

- Lipids in solution forced through a filter
- Defined pore size
  - Decrease filter size throughout extrusion



METHODS

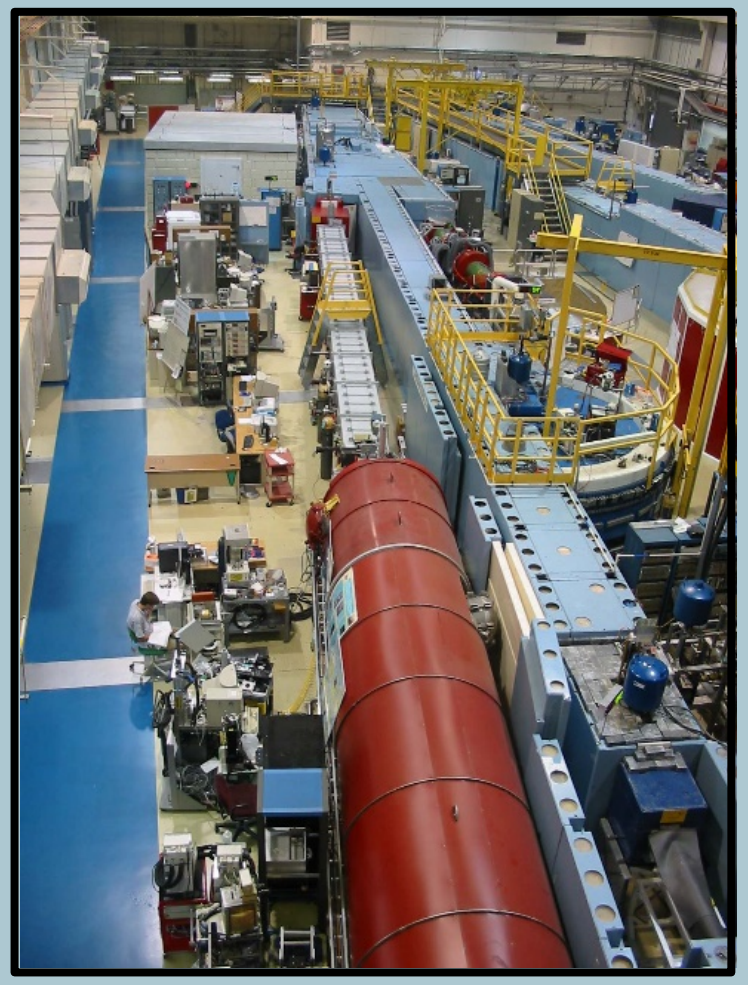
1. UV Vis → Used to measure chemical instability:
   hydrolysis: measure free fatty acid
   oxidation: measures peroxidized lipid

2. Dynamic Light Scattering → Measures the hydrodynamic radius of liposomes

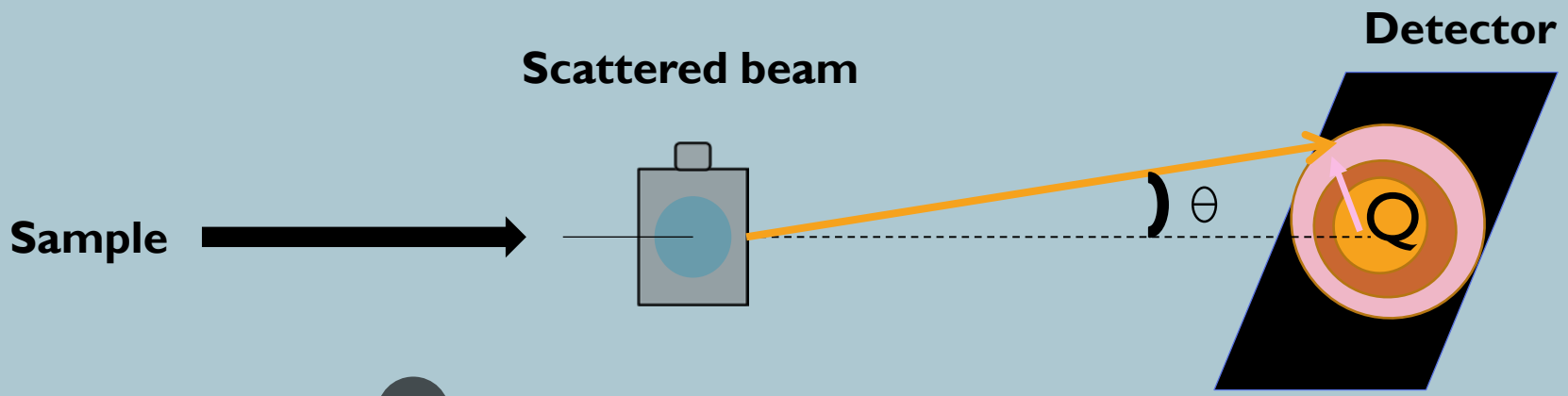
3. Cryogenic Transmission Electron Microscopy (Cryo TEM) → Captured high-resolution structures of liposomes in solution

4. Small-Angle Neutron Scattering → Provide information of vesicle structure and size under different storage conditions

SMALL ANGLE NEUTRON SCATTERING



- Probes material structures by interacting with nucleus in sample
- Neutron scattering can be measured by a detector
- Scattering vector Q can tell us about structures in our sample to nanometer scale



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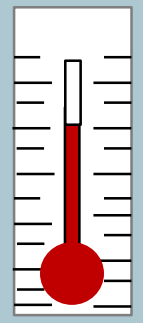
RESULTS

1. **Temperature Effect**: Increasing temperature storage conditions increases degradation kinetics
   A. Hydrolysis and oxidation assays show more chemical degradation with increased storage temperature
   B. DLS shows change in vesicle size occurs at shorter time points at higher temperature
   C. SANS shows more significant change to vesicle structure at higher temperature



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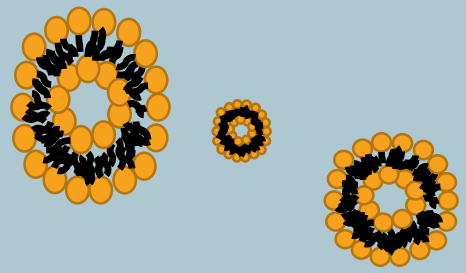
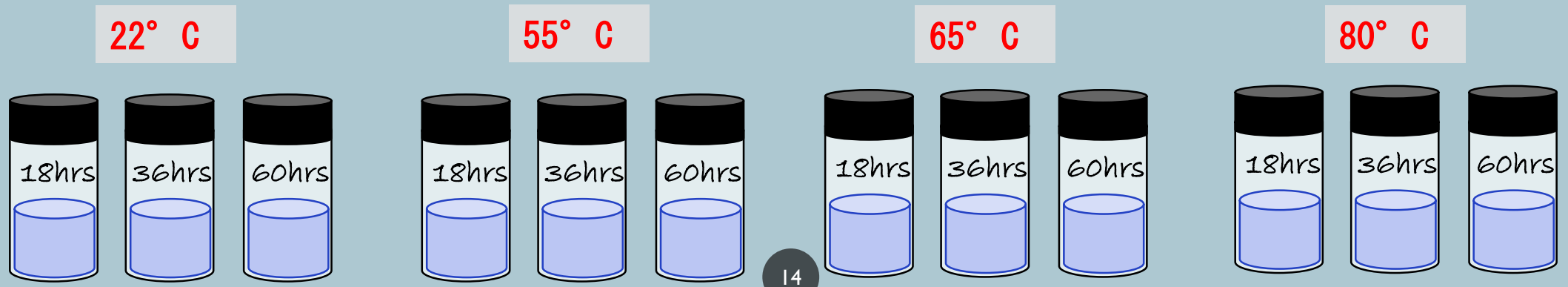


3. **Size Effect**: Smaller vesicles will degrade faster than larger vesicles
   A. Smaller vesicles show more hydrolysis than larger vesicles
   B. DLS shows greater change in vesicle size for small vesicles
   C. SANS shows more change to vesicle structure for smaller vesicles
4. **Structural evolution:** Morphology differs at various stages during vesicle degradation

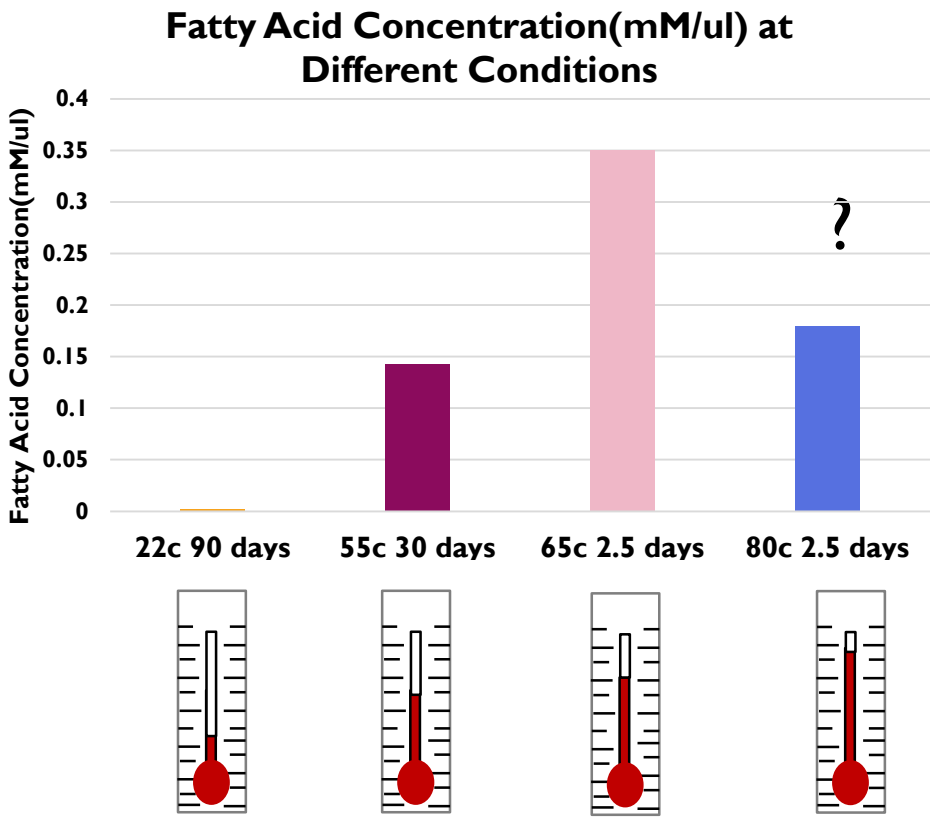
THE TEMPERATURE EFFECT: THE EXPERIMENT

- Put 3 vials of vesicle solutions at 4 temperatures
- Remove vials at varying time points
- Measure vesicle degradation with DLS and chemical assays
- Use SANS to study structure change over time



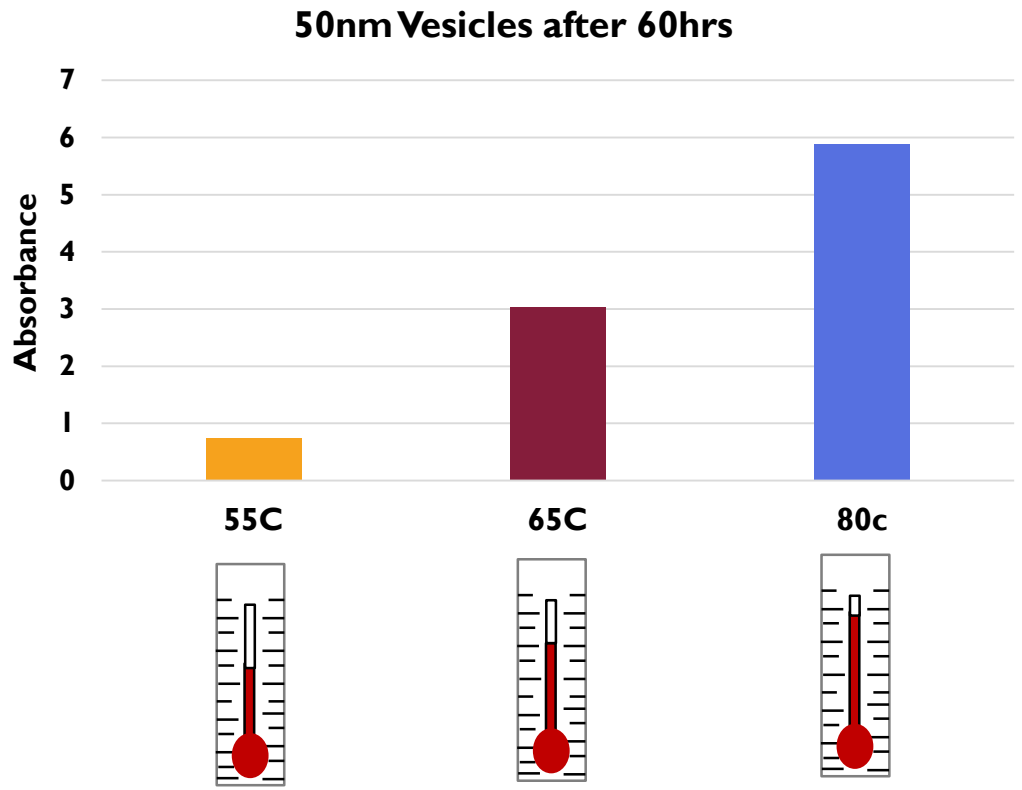
TEMPERATURE EFFECT: CHEMICAL ASSAYS

HYDROLYSIS

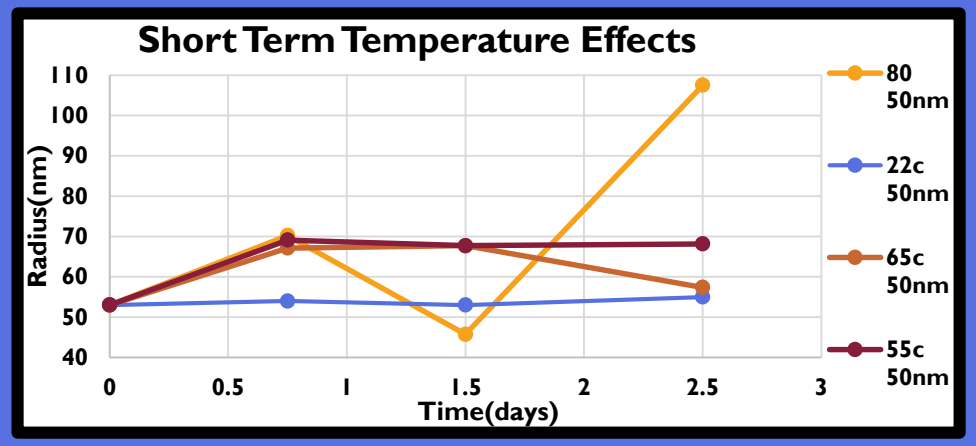


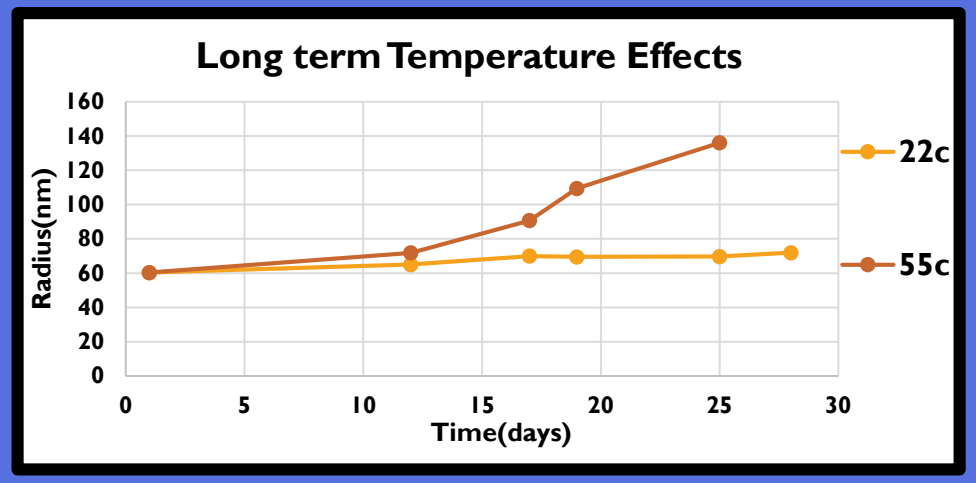
OXIDATION

- More oxidation at higher temperatures!

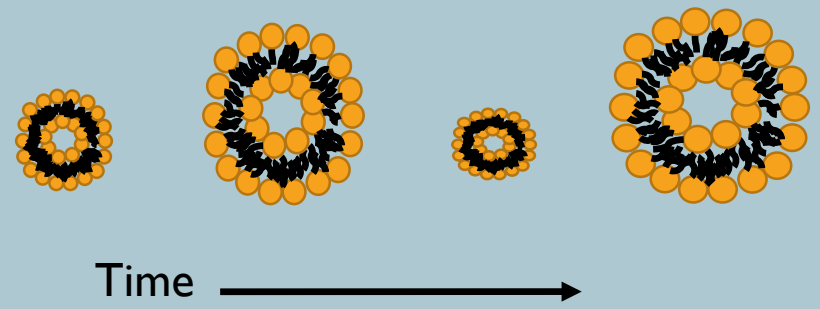


TEMPERATURE EFFECT: DLS



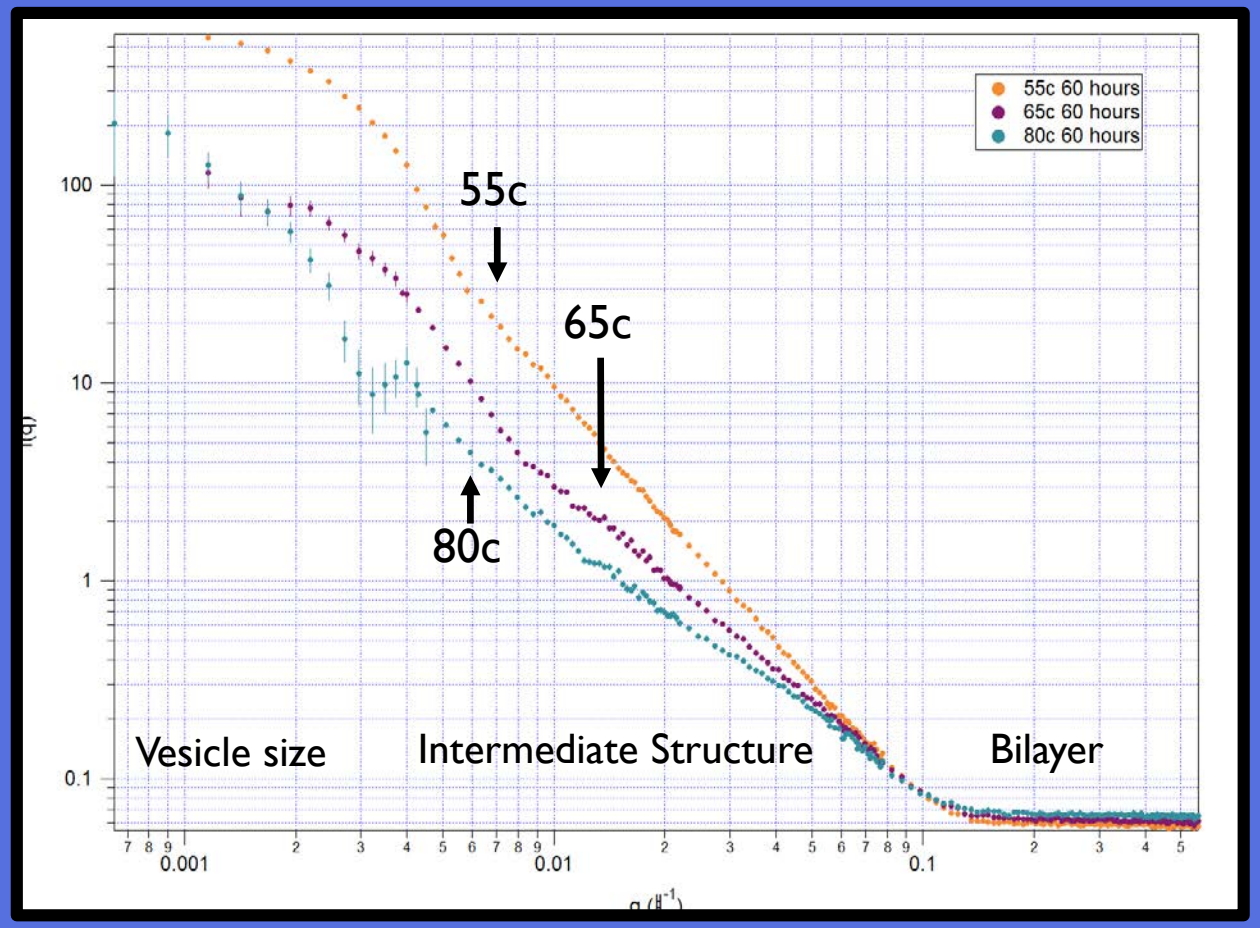


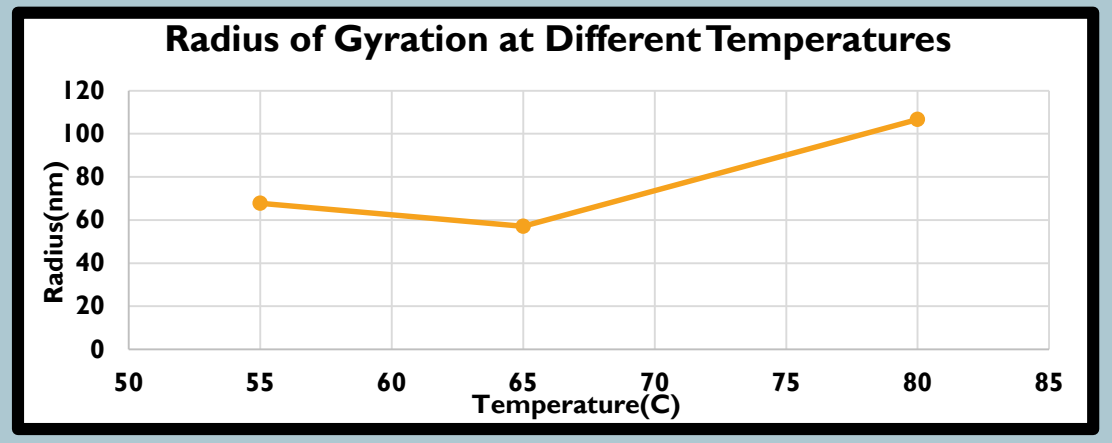
- Vesicles at higher temperatures change size quicker
  - **Degradation kinetics increase with temperature**
- Initially see an increase in vesicle size, followed by a decrease and then eventually an increase again



- At 22c vesicles will remain unchanged even after 30 days!
- Note: DLS only tells us about vesicle size not structure

TEMPERATURE EFFECT: SANS





- Significant change at low q and Rg showing change in vesicle size
- Intermediate structure change
- At high q samples look similar
  - Similar bilayer length scale
- Intermediate structure change

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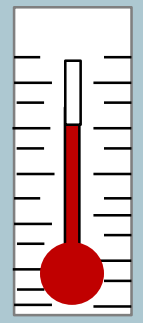

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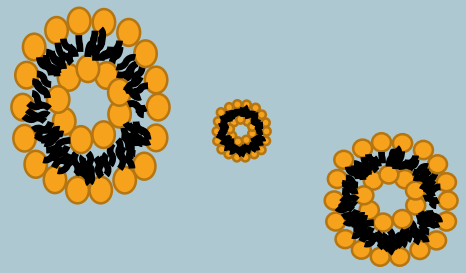
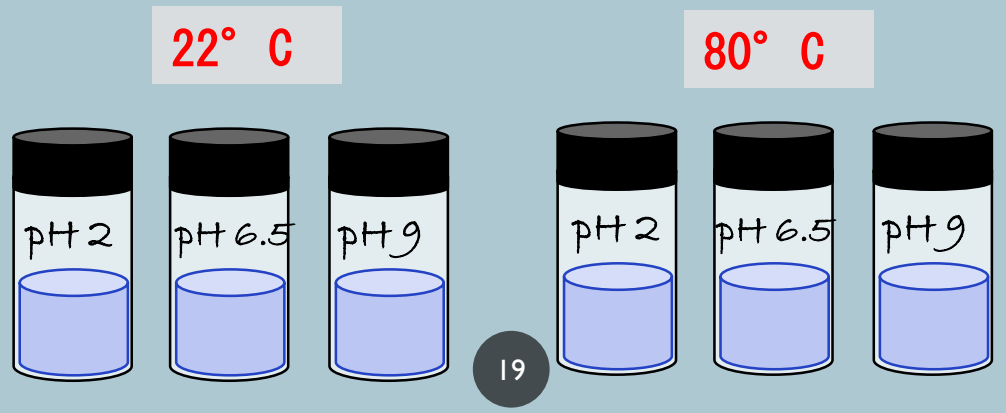


4. **Structural evolution:** Morphology differs at various stages during vesicle degradation

THE PH EFFECT: THE EXPERIMENT

- Put 3 vials at different pH values at 22C and 80C for 24 hours
- Measure vesicle degradation with DLS and chemical assays
- Use SANS to study structure change over time



PH EFFECT: CHEMICAL ASSAYS

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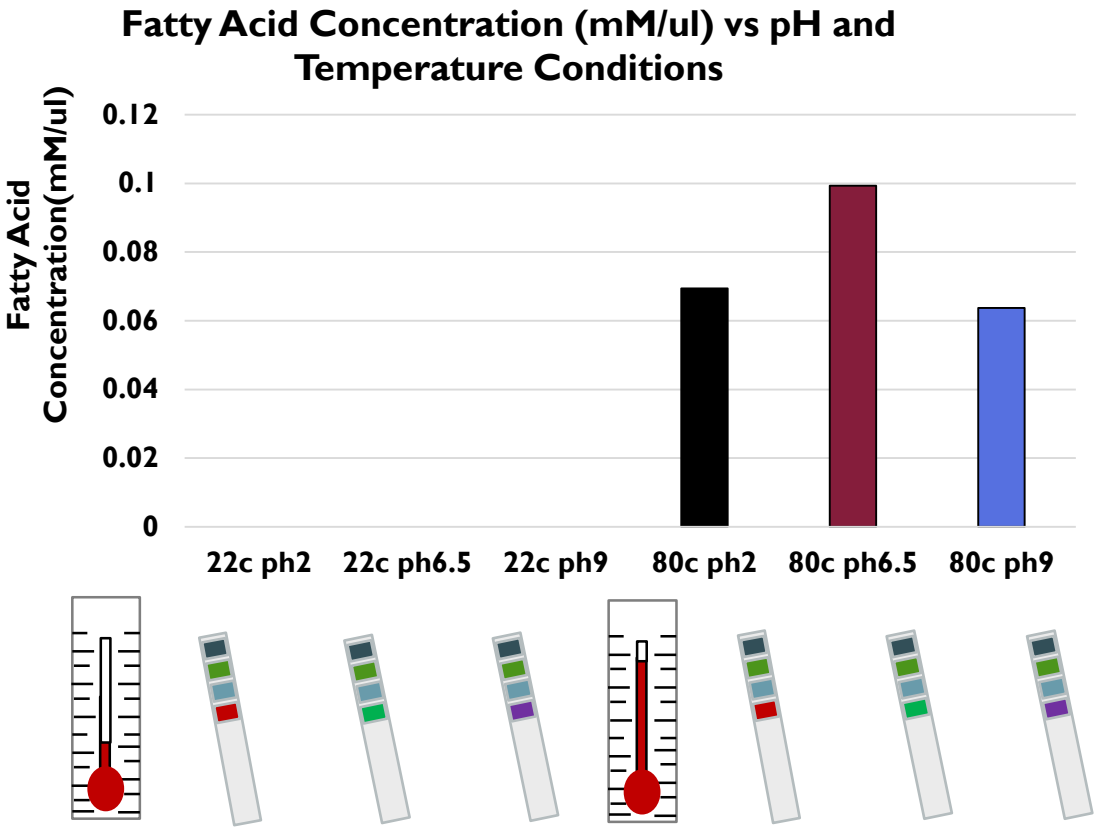
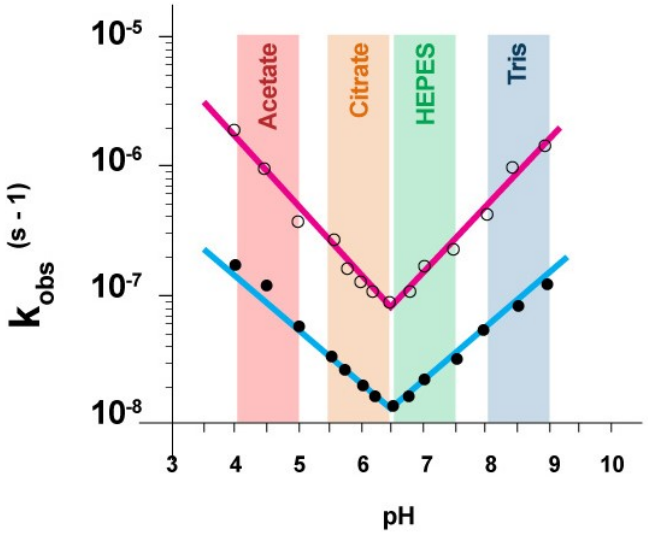


HYDROLYSIS RATE AND PH IN LITERATURE

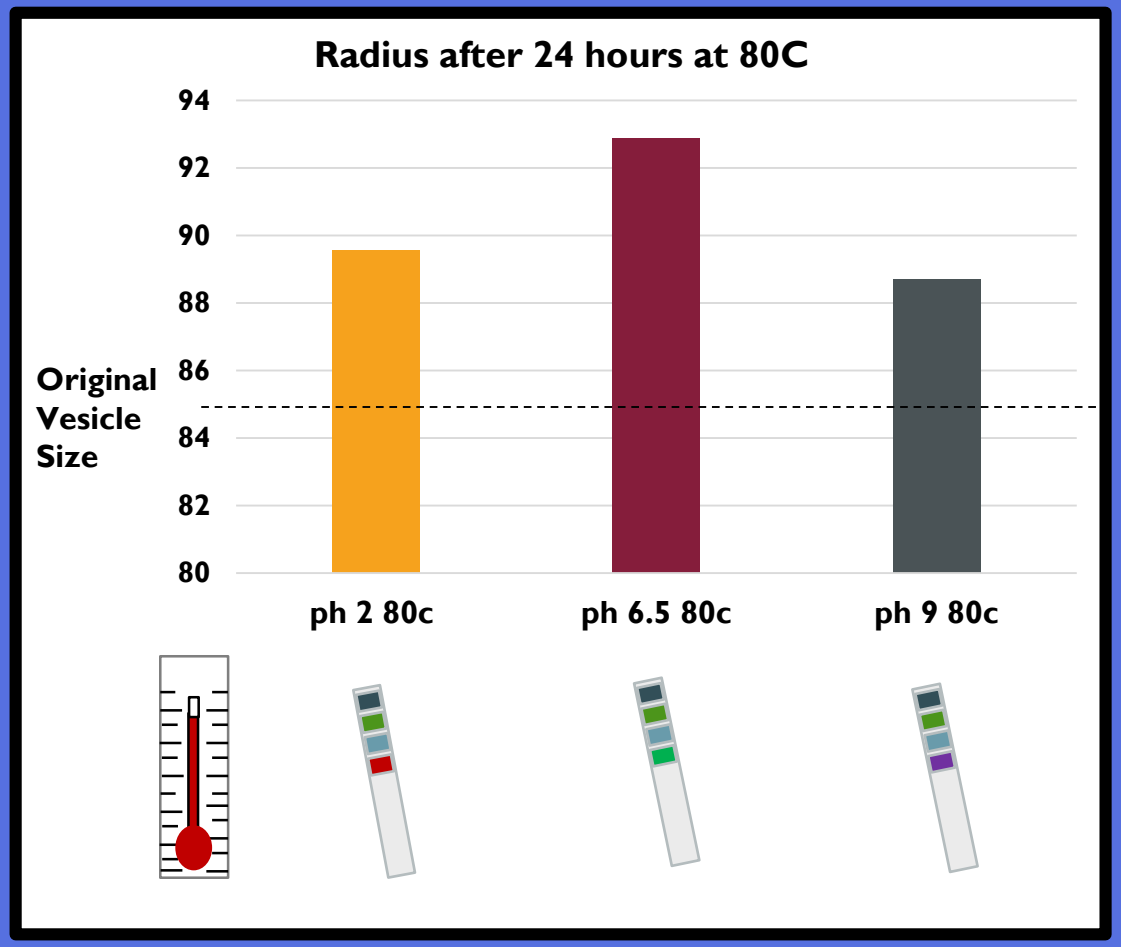


Rate of hydrolysis of HSPC in 0.05 M buffer.

"Liposome Stability" https://encapsula.com/hydrolysis-and-oxidation-of-liposomes/

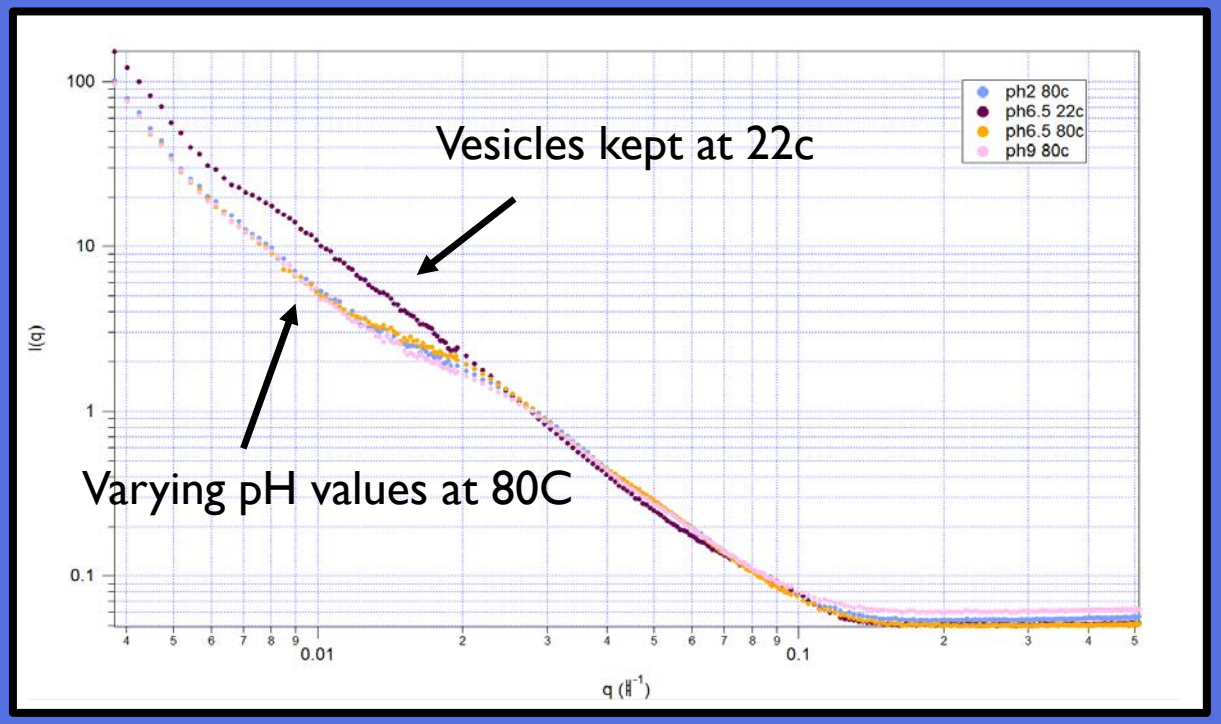
- Slowest hydrolysis rates at neutral pH's
- Faster hydrolysis rate at 3 and 10

PH EFFECT: DLS



- Vesicles at neutral pH grew larger than vesicles at pH of 2 and 9
- Opposing what is suggested in literature
- Different membranes may have changing stability properties

PH: SANS



- Temperature has a much larger impact on vesicle structure than pH
- See slight change in structure at different pH values at 80C

RESULTS

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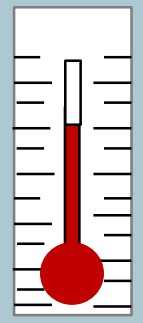

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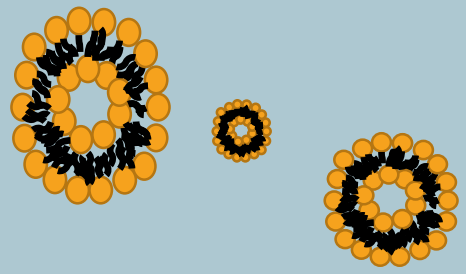
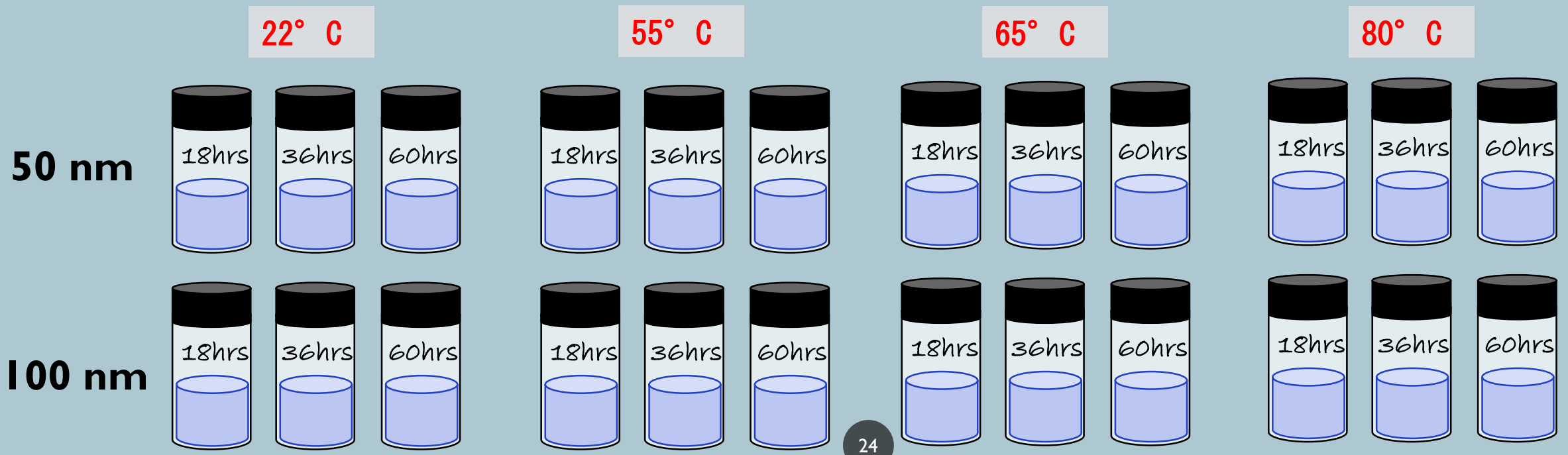


4. **Structural evolution:** Morphology differs at various stages during vesicle degradation

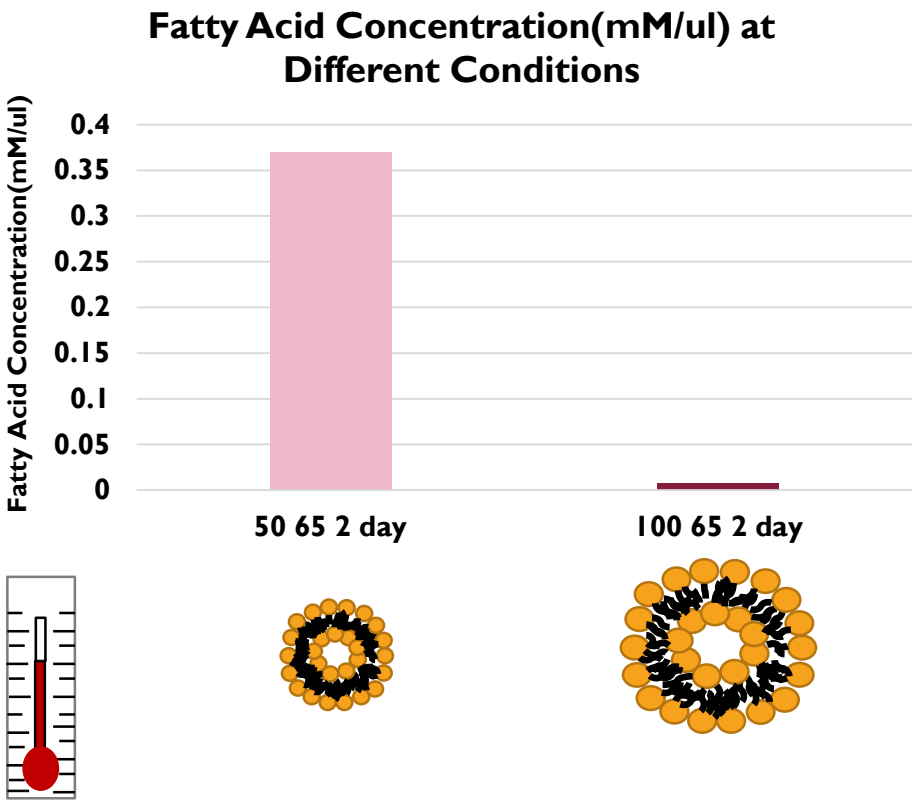
THE SIZE EFFECT: THE EXPERIMENT

- Repeated temperature effect experiments with duplicates at 50nm and 100nm

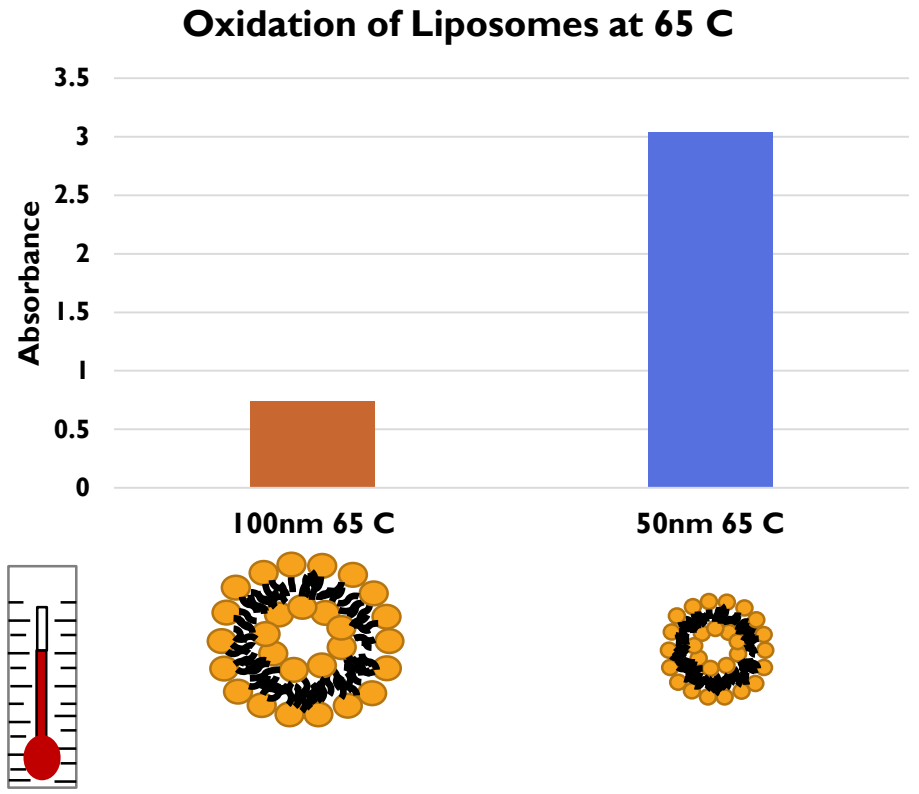


SIZE EFFECT: CHEMICAL ASSAYS

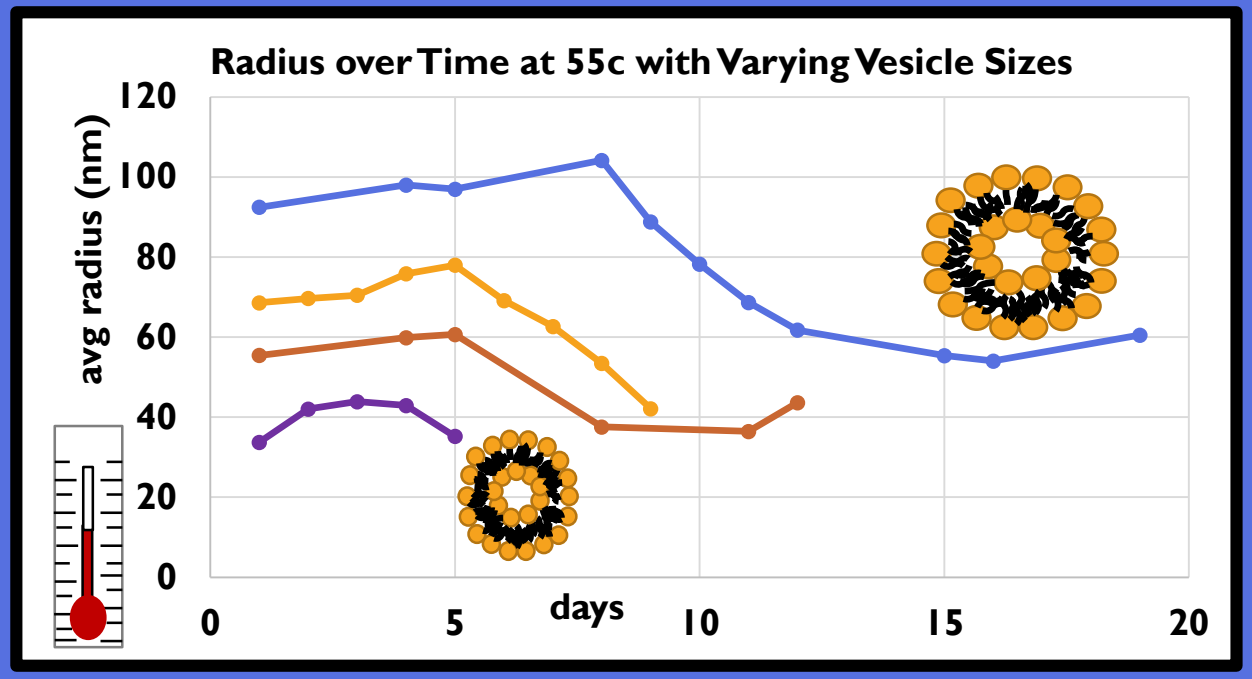
HYDROLYSIS



OXIDATION



SIZE EFFECT: DLS

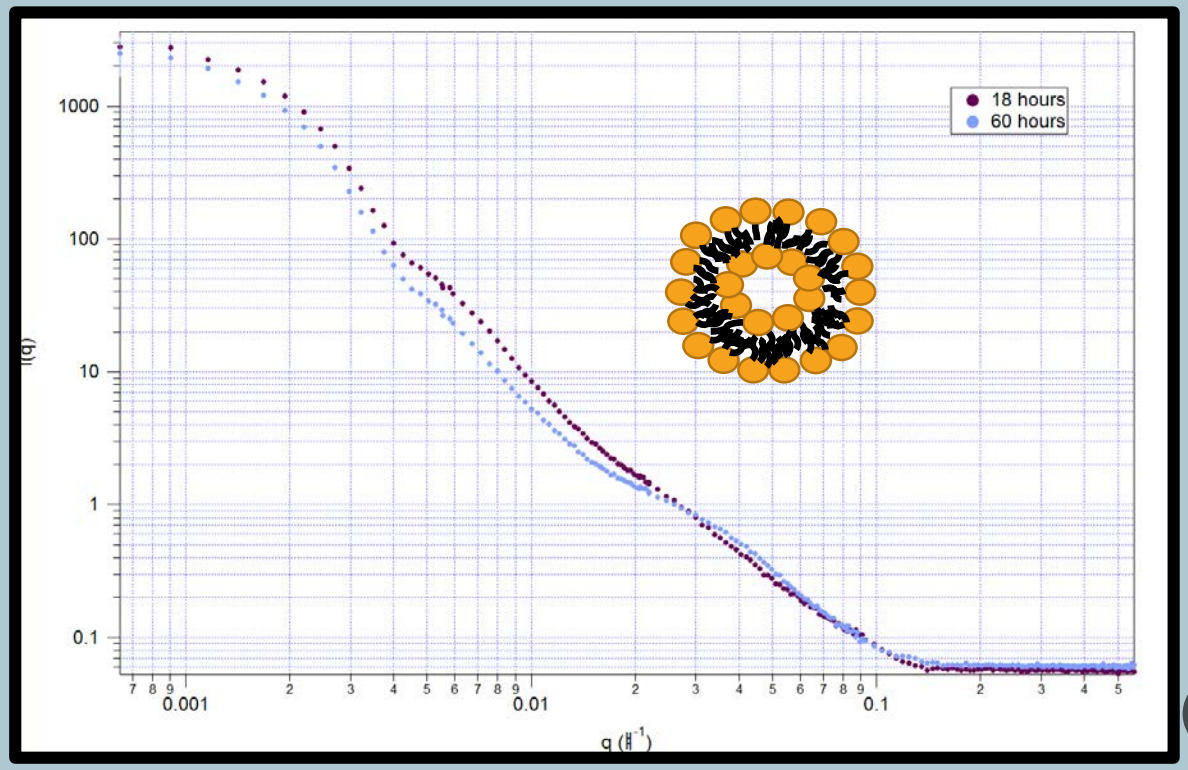


- Smaller vesicles change size more quickly at the same temperature!
- Data shown here is 55c but reproducible at other temperatures

SIZE EFFECT: SANS

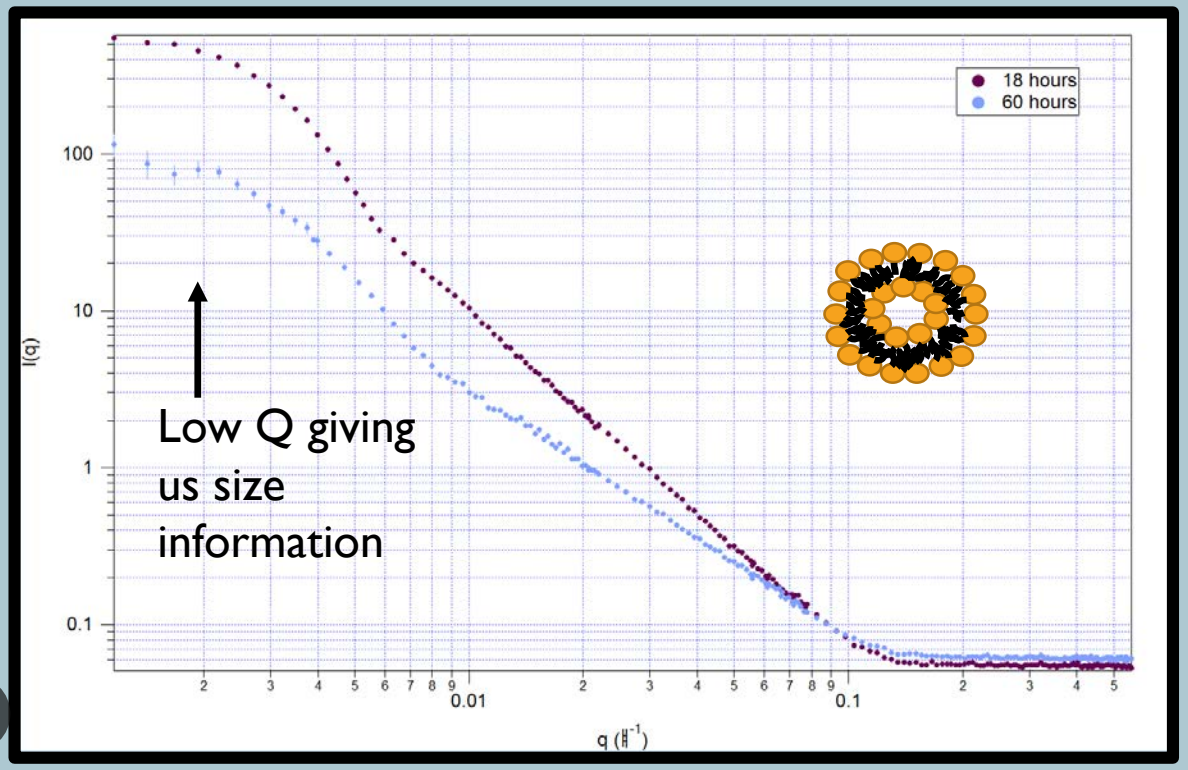
100NM VESICLES AT 65C

- Minimal change in vesicle scattering between 18 hours and 60 hours



50NM VESICLES AT 65 C

- Greater change in vesicle size and structure between 18 hours and 60 hours



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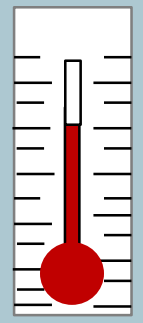
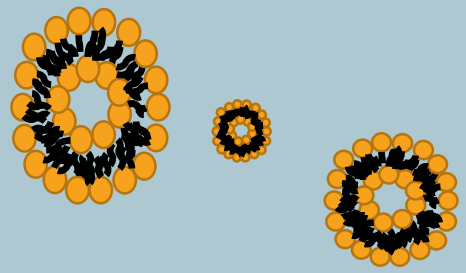
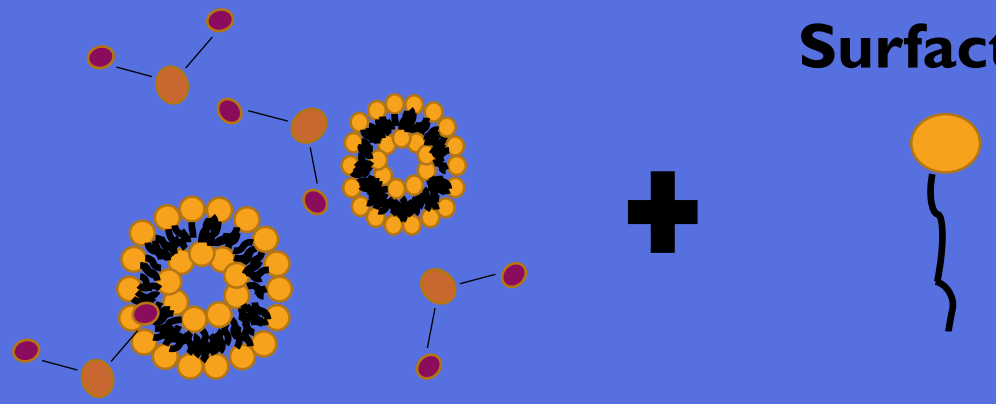
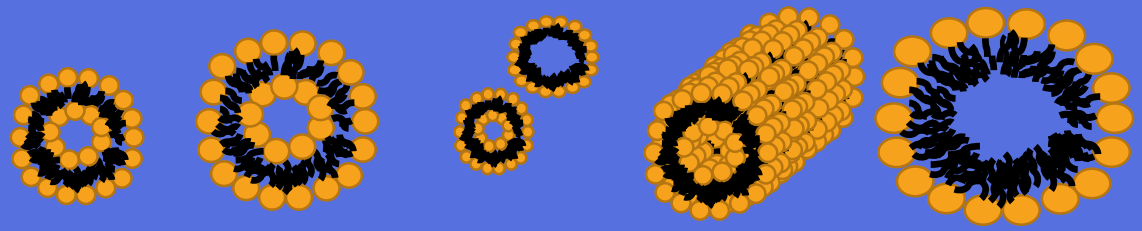




MORPHOLOGY: LITERATURE RESULTS

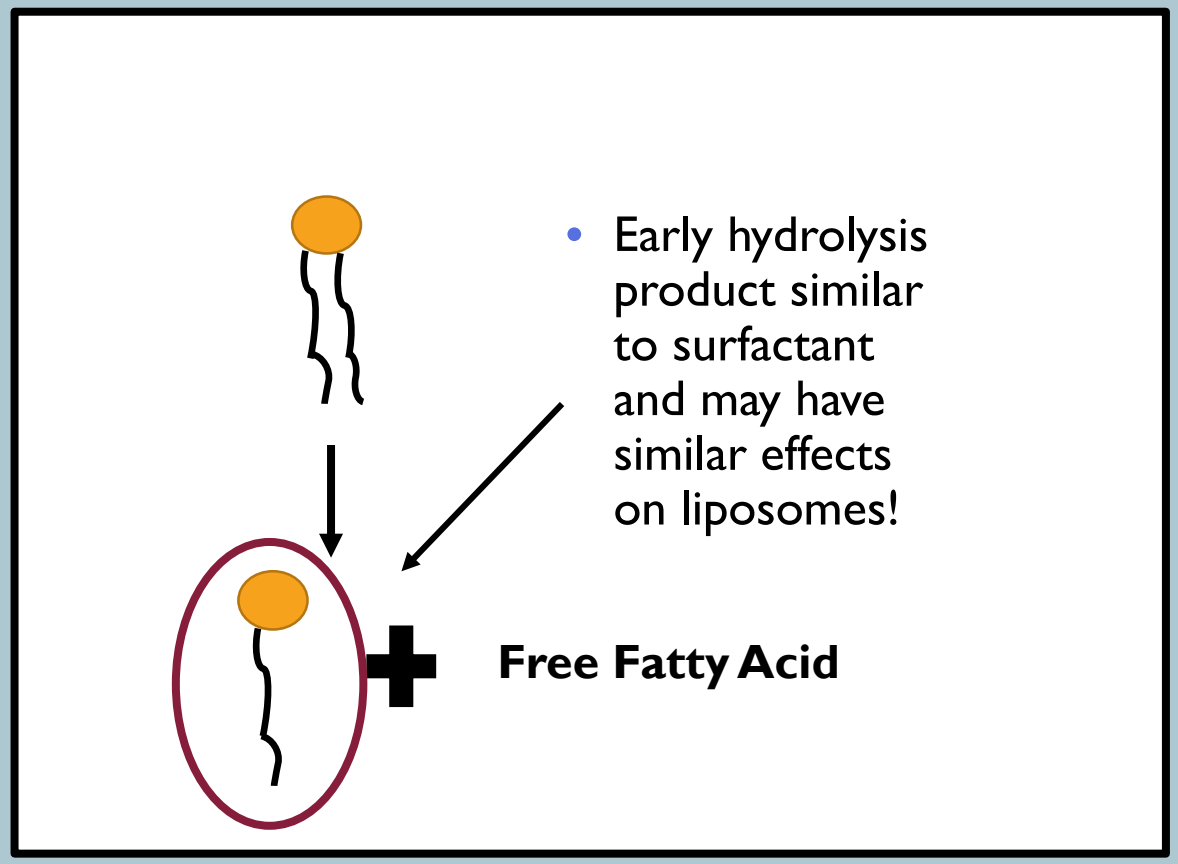


Literature has studied the effects of adding surfactants to liposomal solutions



Increasing Surfactant Concentration →

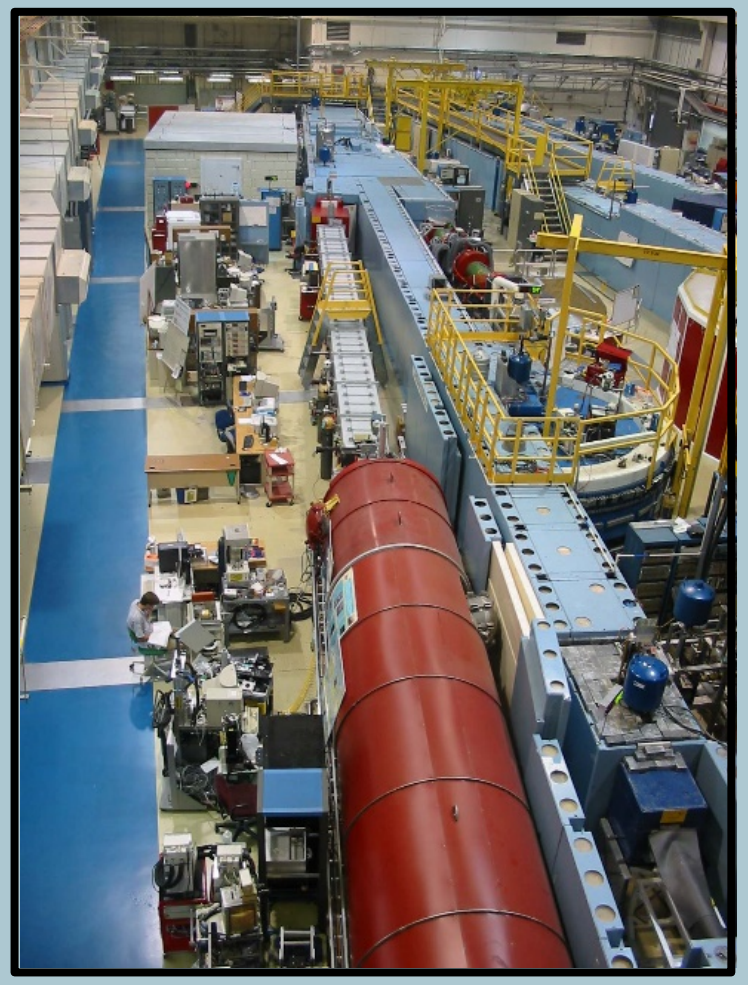
Why is this similar to my work?



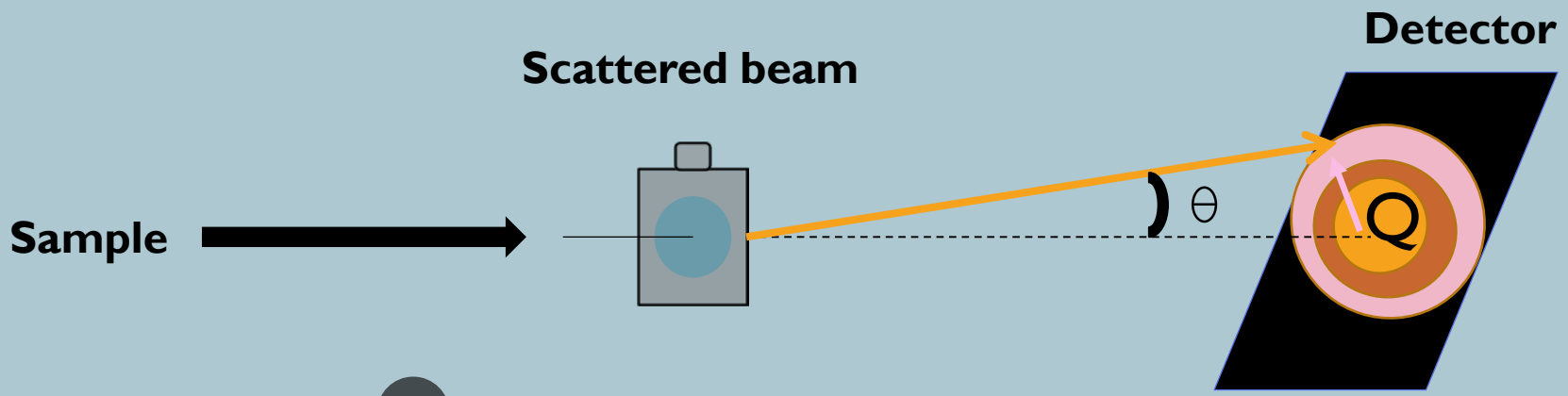
No known studies showing this pattern without causing liposomal structure change with addition of surfactant

Almgren, Mats, "Mixed micelles and other structures in the solubilization of the bilayer lipid membranes by surfactants"

SMALL ANGLE NEUTRON SCATTERING

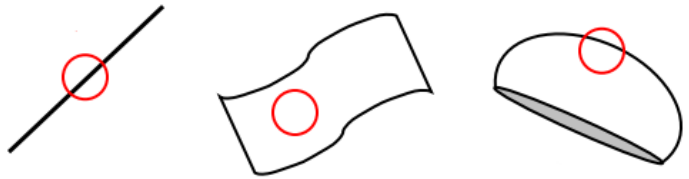


- Able to generate a 2D scattering pattern on detector
- Generate 1D plots that contain structural information



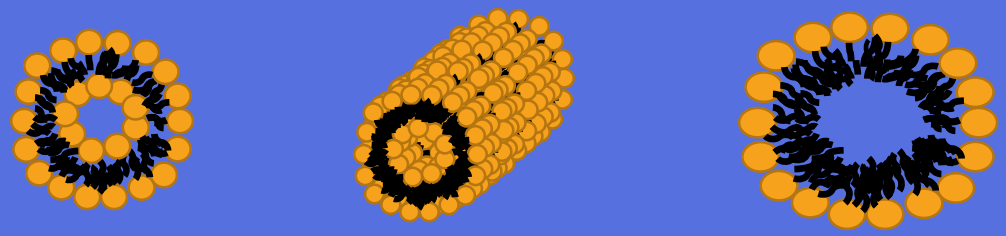
MORPHOLOGY: SANS DATA

Scattering curve can tell us about vesicle structure!

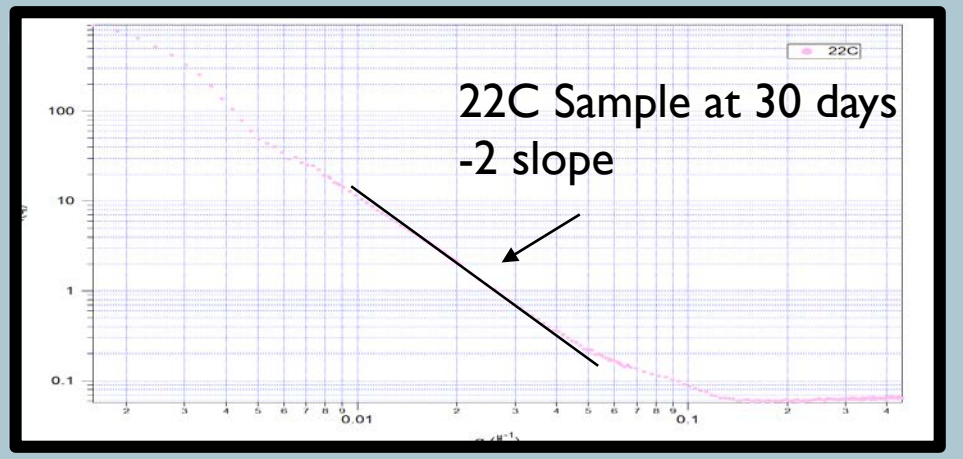


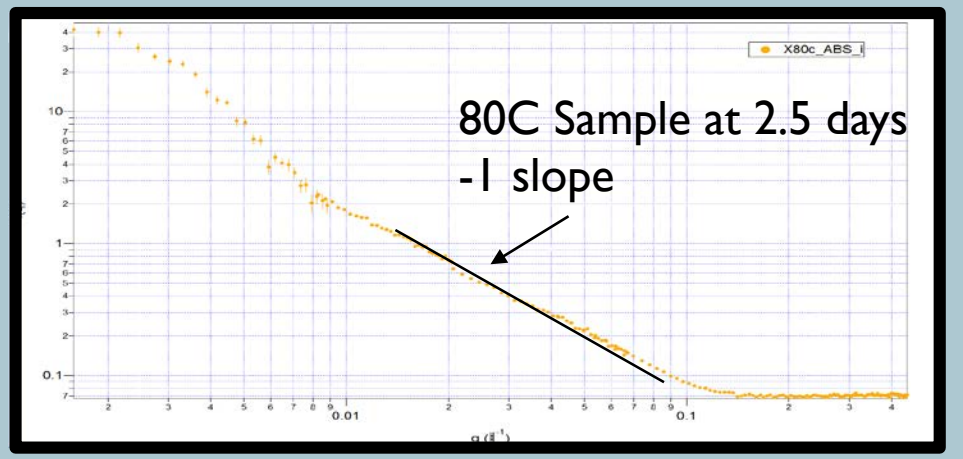
1D object	2D object	3D object
$I(Q) \sim Q^{-1}$	Q^{-2}	Q^{-4}

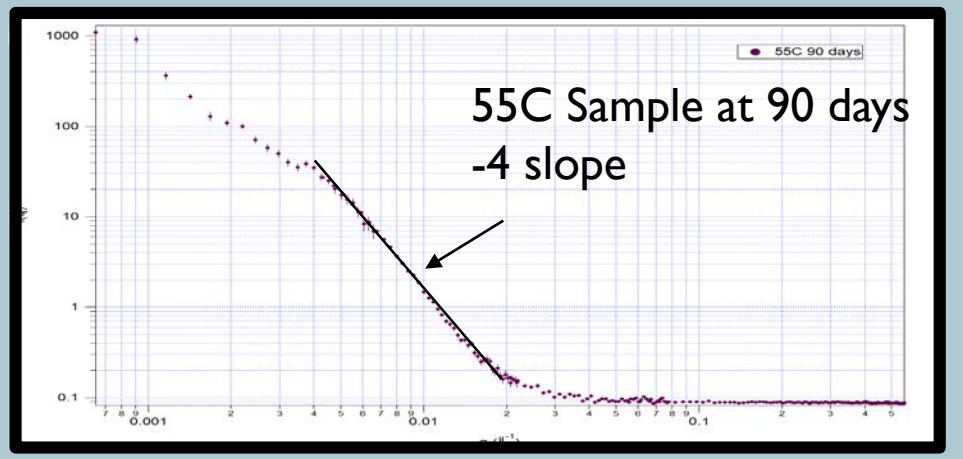
Increasing degradation →



22c 30 Days | 80c 2.5 Days | 55c 90 Days





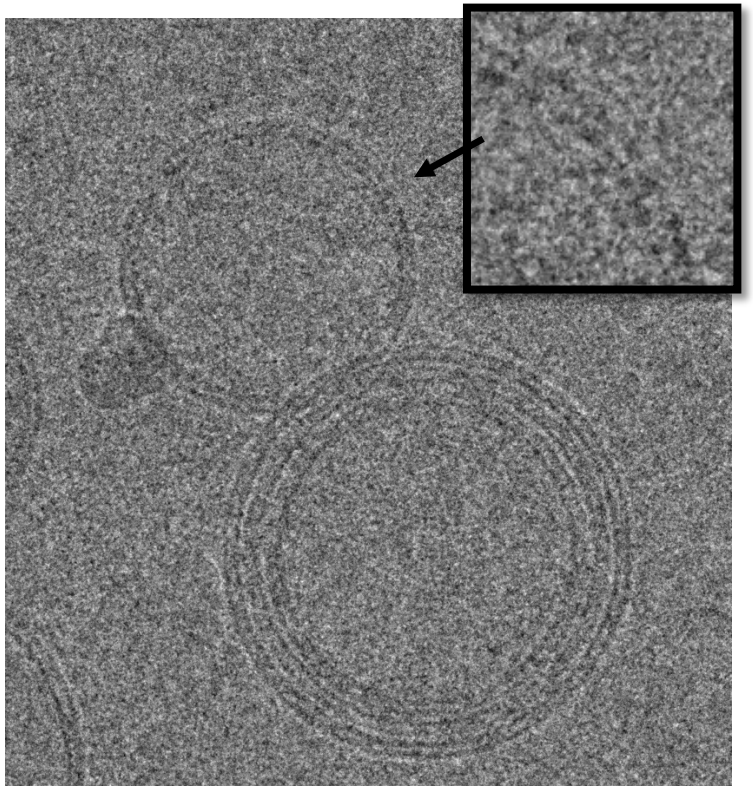


MORPHOLOGY: CRYO TEM

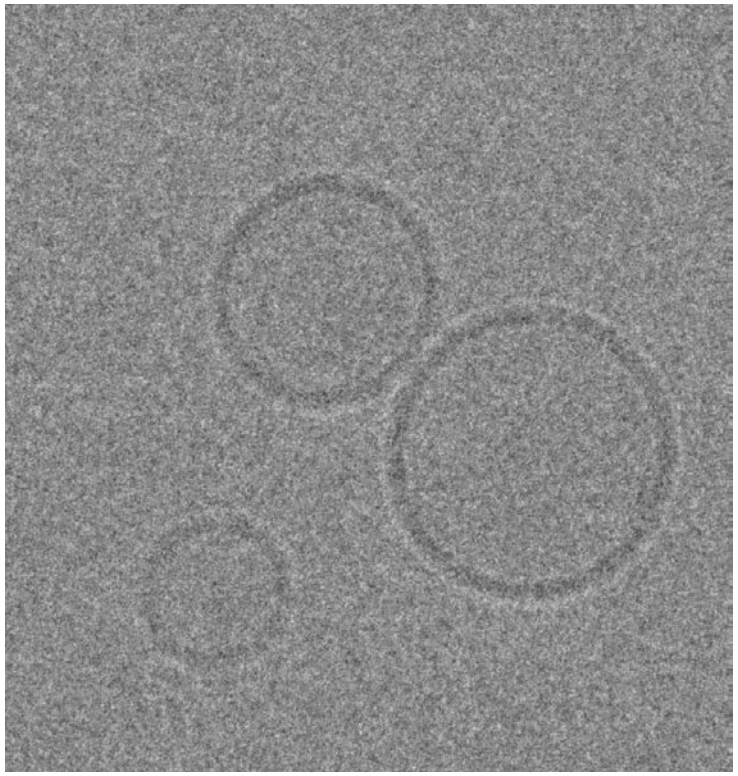
22C SAMPLE 30 DAYS

- Overall 22C samples remained unchanged and unilamellar after 30 days!

Multilamellar



Unilamellar



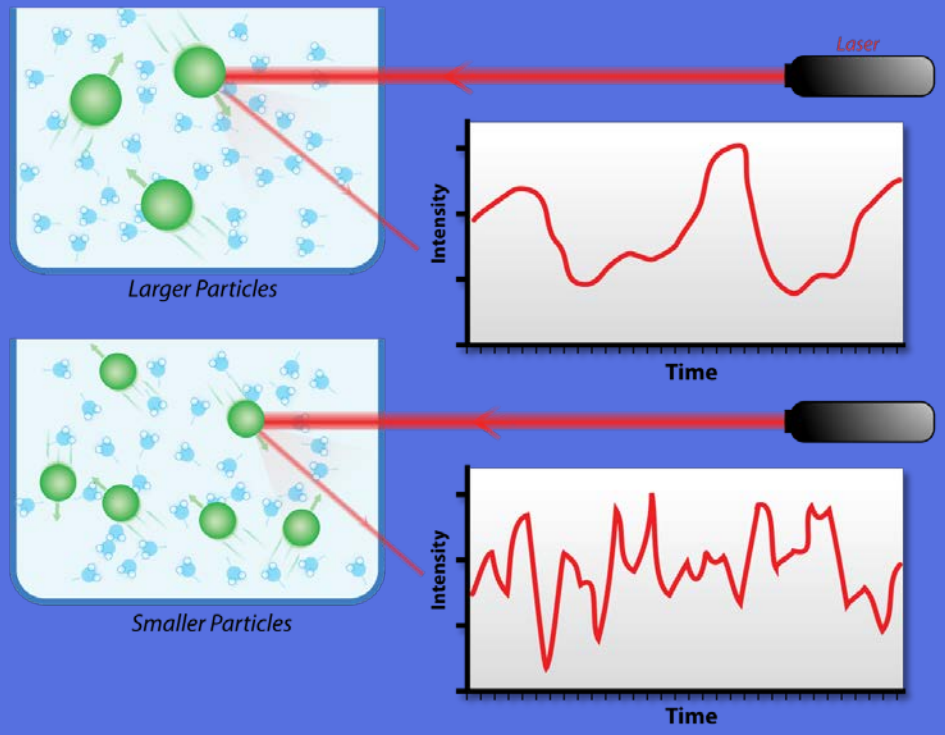
CONCLUSIONS

- **Temperature Effect**: Increasing temperature storage conditions increases degradation kinetics
  - Temperature increases the hydrolysis and oxidation rate
- **pH Effect:** Vesicles in solution with pH 6.5 degrade faster than pH's of 9 and 2
  - Opposing what is reported in literature
  - Showing various properties will differ between membranes
- **Size Effect:** Smaller vesicles will degrade faster than larger vesicles
  - More hydrolysis and oxidation products present in smaller liposome solutions
- **Structural evolution:** Morphology differs at various stages during vesicle degradation
  - Vesicles undergo transitions into 1D structure and eventually large 3D structure

ACKNOWLEDGMENTS

- Center for High Resolution Neutron Scattering
- NIST Center for Neutron Research
- Yun Liu
- Elizabeth Kelley
- Joe Dura
- Julie Borchers

DYNAMTIC LIGHT SCATERING

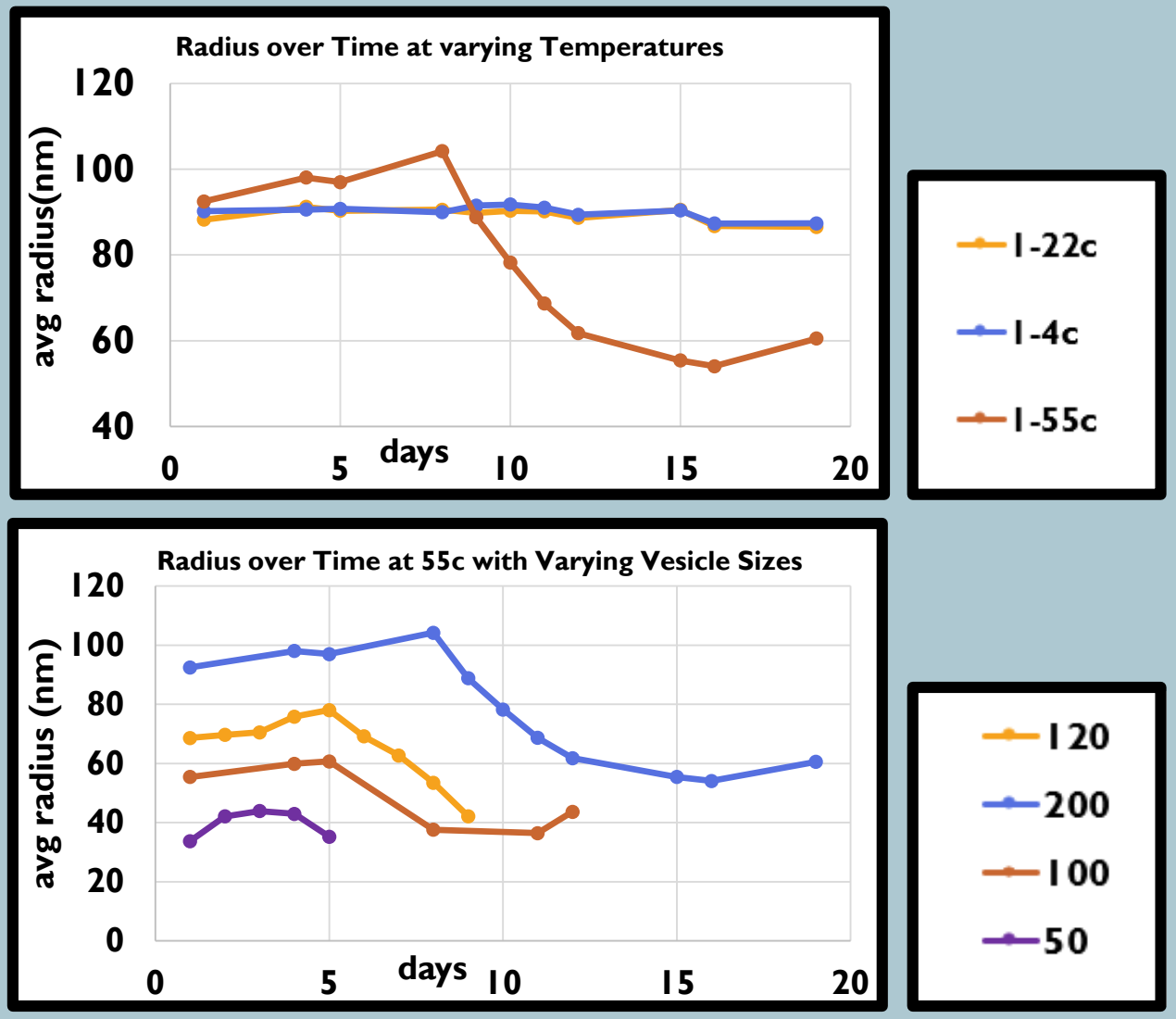


- Popular light scattering technique
- Laser beam shot at sample and detector senses changes in scattering light
  - Particles scattered at known angle
- Can provide diffusion coefficient
  - Can be related to particle size
- Only valid at low concentrations
- All samples at 1mg/ml

$$D = \frac{kT}{6\pi\eta R}$$

Radius!

MY PREVIOUS CONCLUSIONS

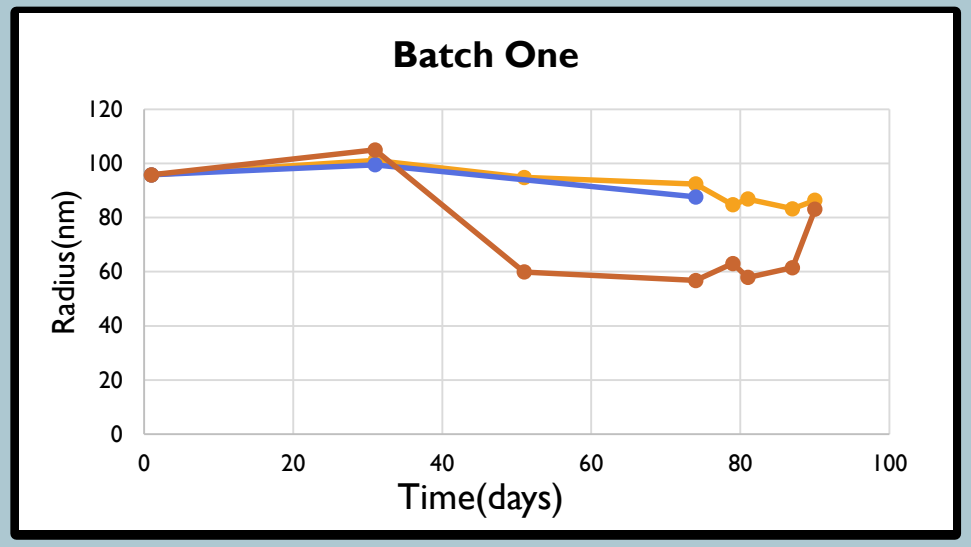


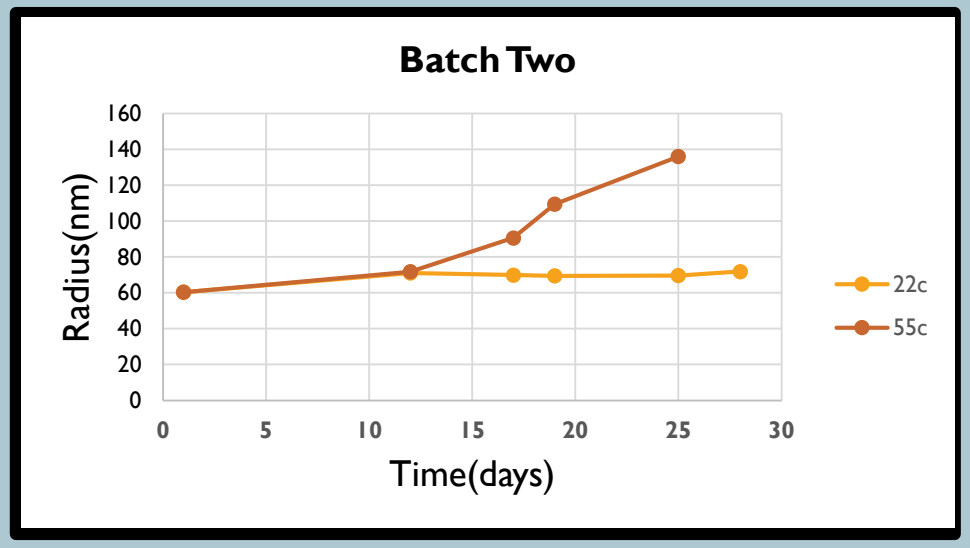
- Last summer used DLS to study vesicle size change at different temperatures over time
- Saw significant change in vesicle size at 55c
  - Initial expansion and then following contraction of vesicle
- Surprising results not well understood
- Saw dependency on vesicle size with the rate of degradation

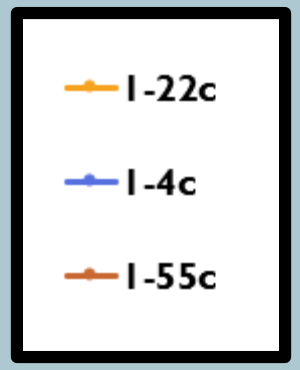
Future Work

- Run longer-term stability experiments
- Study the pathways of chemical decomposition
- Use small angle neutron scattering to understand liposome structure at different degradation time points at different temperatures

LONG-TERM STABILITY EXPERIMENTS







- Ran the same experiments as last summer twice over longer time periods
- Changes:
  - Controlled for light by completely wrapping everything in foil
  - Ran chemical assays to check for degradation
  - Checked pH measurements
  - Decided to not continue with 4c because so similar to 22c
- Again saw significant change in vesicle size at 55c
  - Initial expansion and then following contraction of vesicle
  - Slower than last summer
- Conclusions:
  - Vesicles are degrading at 55c and forming another structure
  - Exposure to light accelerates this process
  - Vesicle size contributes to degradation kinetics