Therapeutic Drug Delivery

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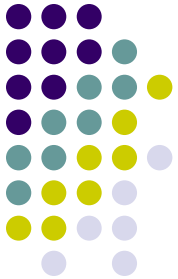
California NanoSystems Institute (CNSI)
University of California Santa Barbara (UCSB)

Current anti-cancer drug delivery methods are not satisfactory

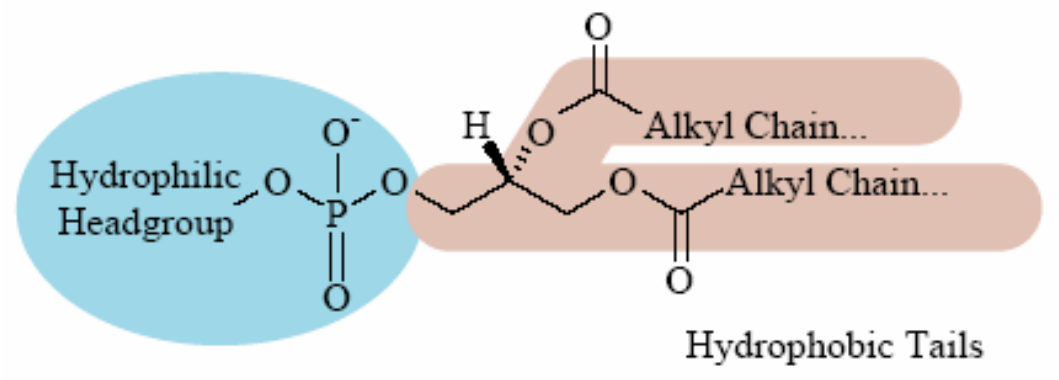


- Typically, a few percent of drug dose reaches intended tissues due to premature release from vesicles
  - Higher dose causes side-effects
- Current delivery technology produces a new generation of vesicles known as vesosomes
  - Their current large size makes them vulnerable to the immune system

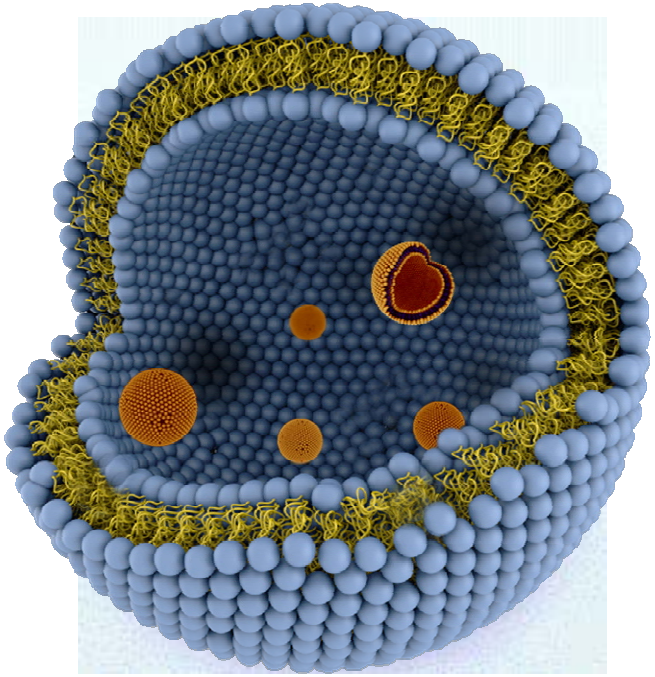
Objective: synthesize smaller vesosomes, that are biocompatible and stable within the human body

Nano-Encapsulation for Targeted Delivery of Drugs

- Liposome-Based Delivery Vehicle
- Cell-Mimic: Vesosomes
- Unilamellar Vesicles



General Structure of double-tailed phospholipids.

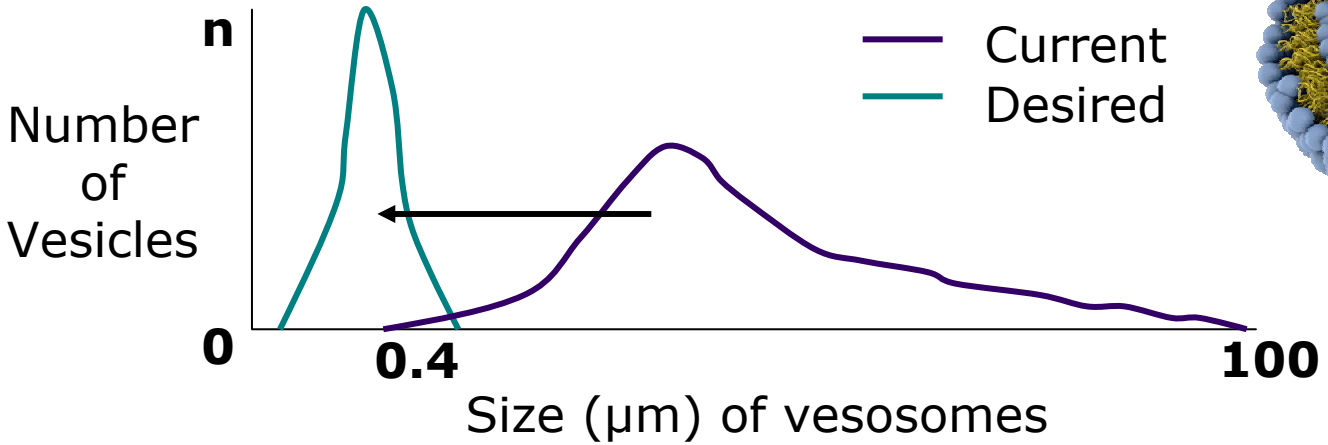


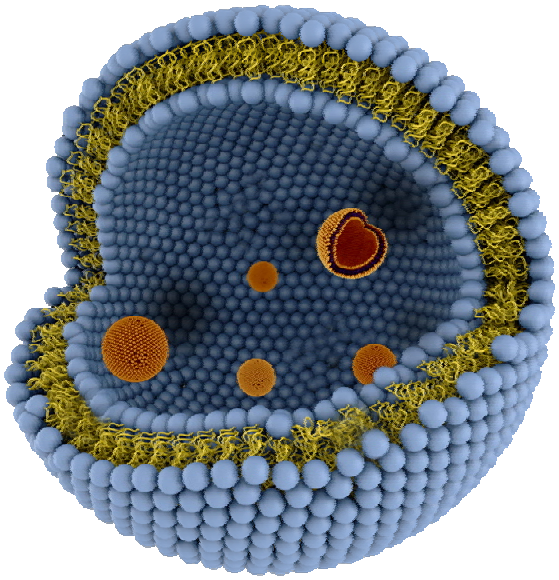
The Liposome

Improving Nanoparticles for Targeted Drug Delivery

Experimental objectives:

- Decrease typical vesosomes from 0.4 – 100 μm to < 0.4 μm
- Narrow size distribution of vesosomes

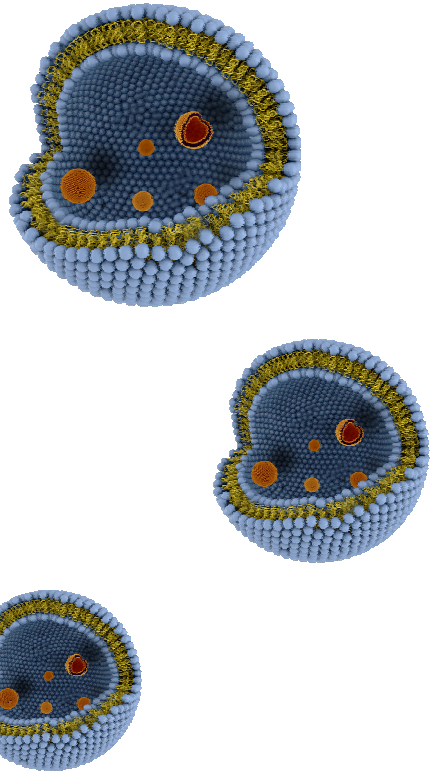




Experimental Design

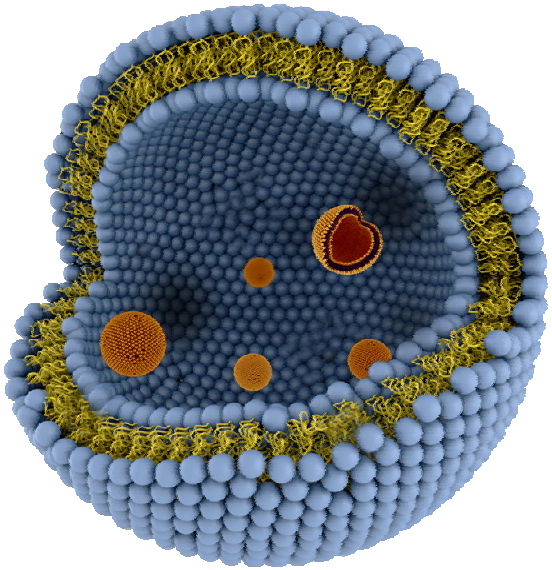
Modifying three critical process variables:

- Polymer selection
  - changes bilayer curvature
    - Poloxamer 188
    - Brij 700
- Concentration optimization
  - Range: 1 – 12 $^{mg}/_{mL}$
- Down scaling synthesis
  - Extrusion
  - Sonication



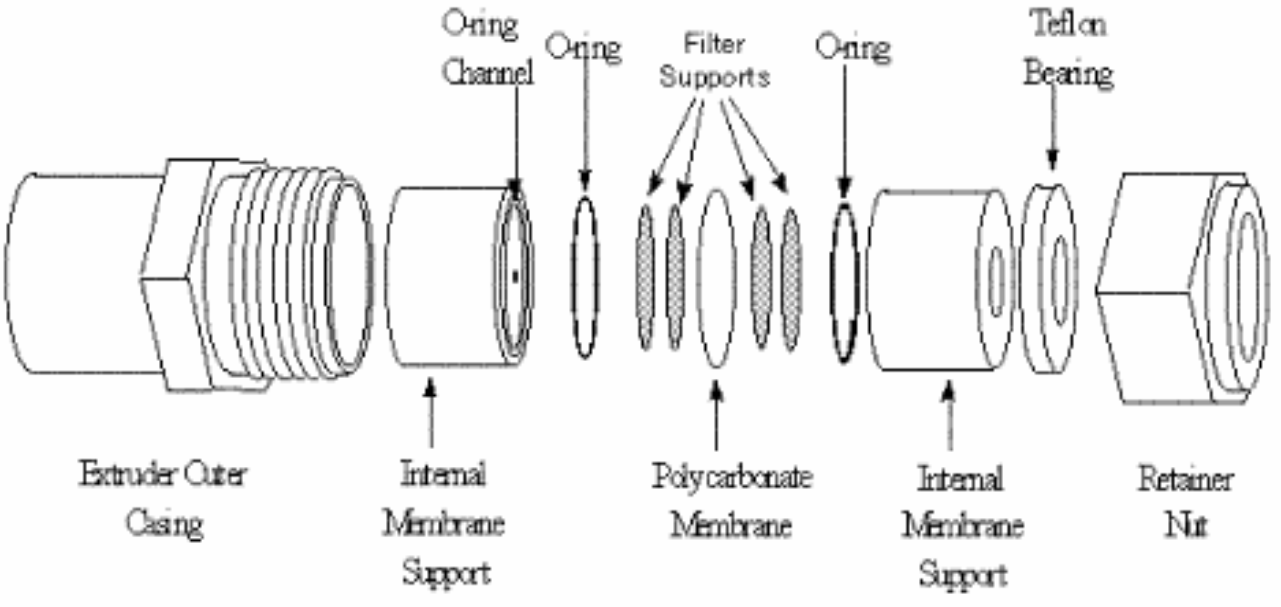
Procedure

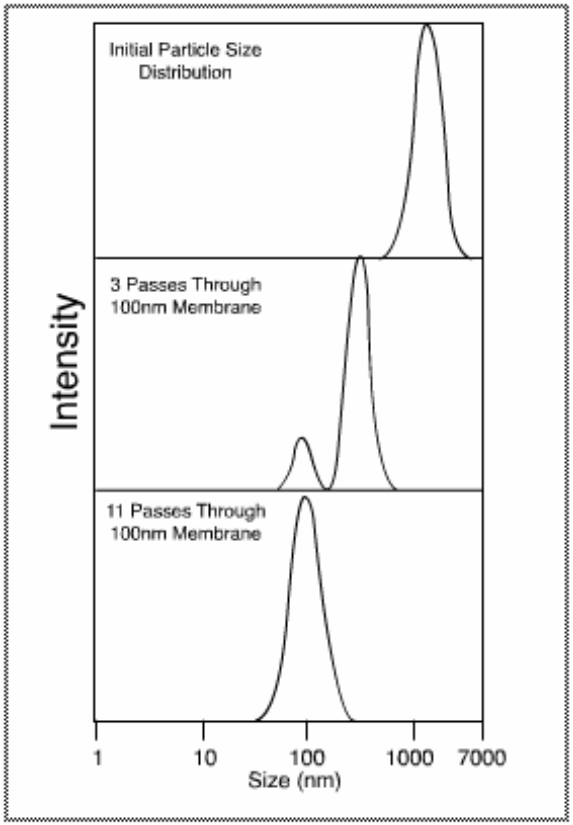
- **Sample:**
  - Dipalmitoylphosphatidychloline lipid (DPPC)
- **Extrusion / Sonication**
- **Interdigitation**
- **Freeze Fracture (FF) / Replication**
- **Transmission Electron Microscopy (TEM)**



Materials / Methods

The Mini - Extruder





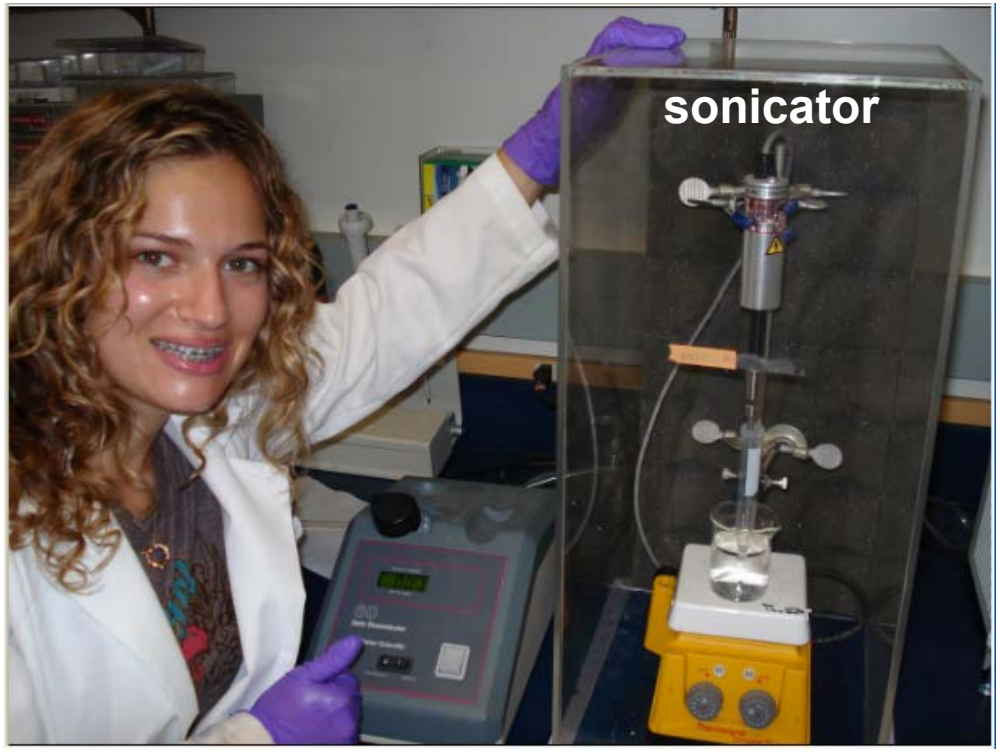
Reduces the size of the vesicles

http://www.avantilipids.com/Extruder.html

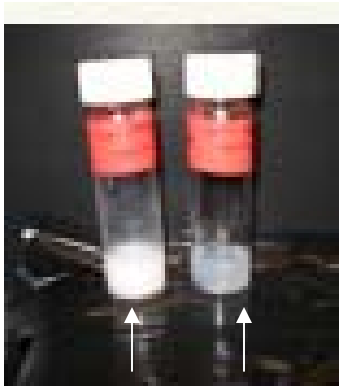
Size reduction from:

- 200 nm
- 100 nm
- 50 nm

Materials / Methods
Sonication



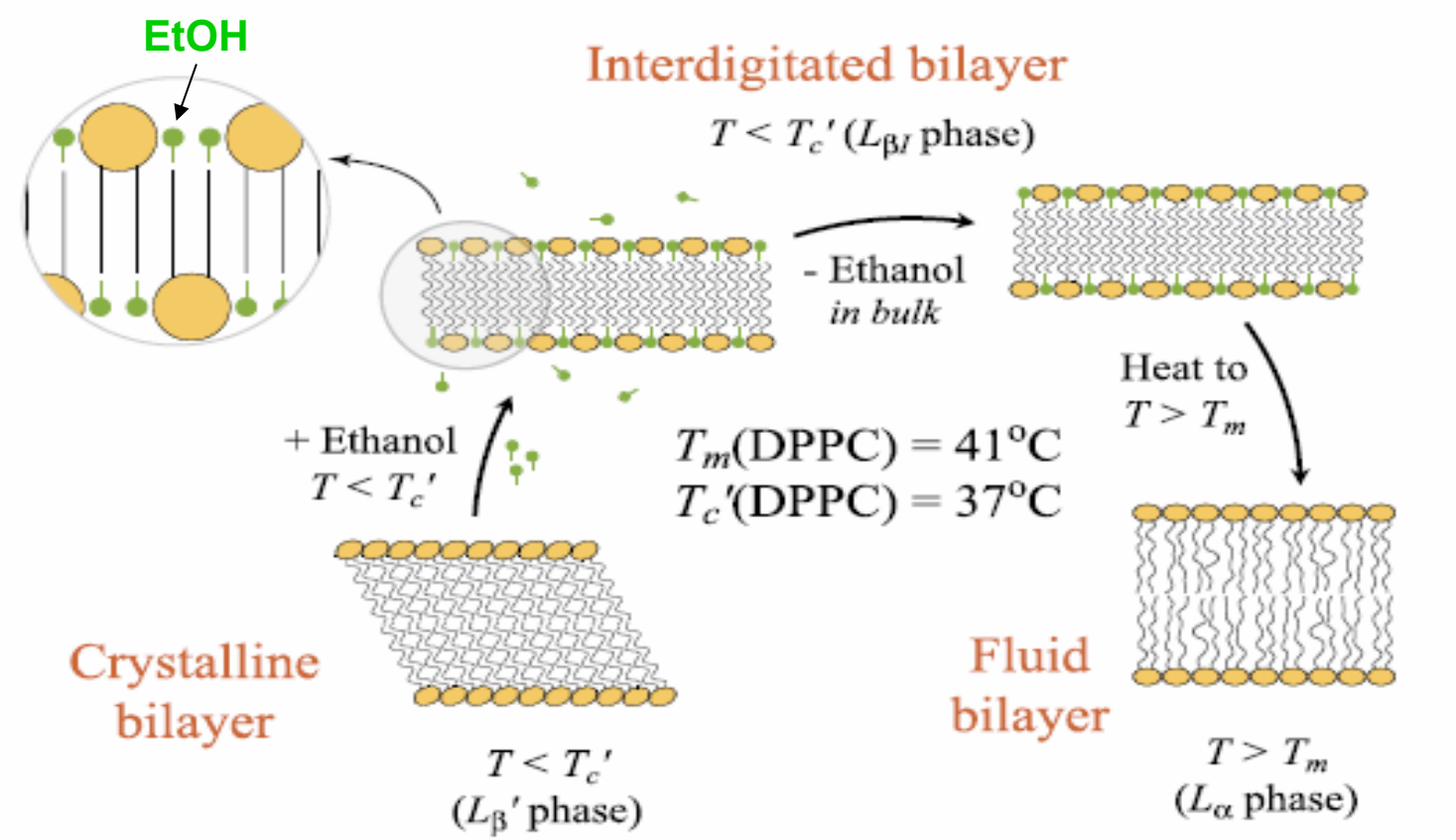
- Provides energy waves to breakdown vesicles



Before / After

Shift in color from opaque/white to translucent
Indicates a decrease in particle size

Materials / Methods
Interdigitation



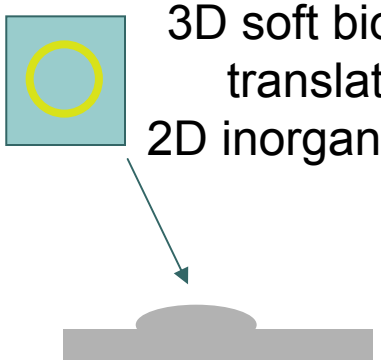
At T < Tm (the main transition temperature) ethanol molecules intercalate between the headgroups.

Upon heating above Tm, the bilayer re-forms and reverts to a fluid L □ phase.

Materials / Methods

Freeze Fracture (FF)

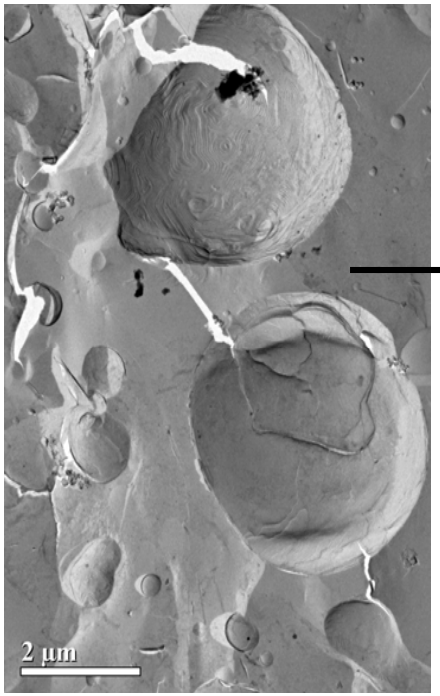
FF in a nutshell:



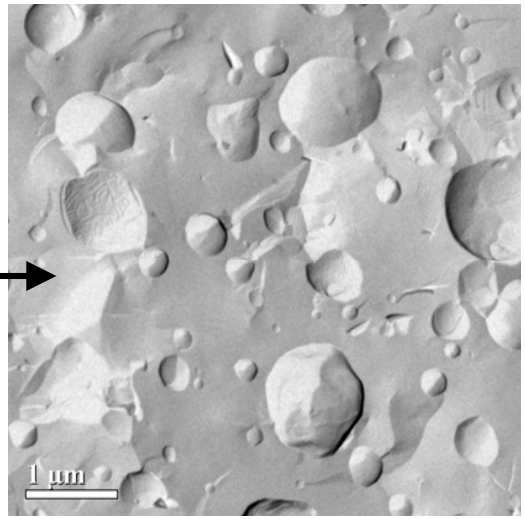
FF is used to image vesicles in their native state

Data Analysis (FF results)
Interdigitation Fusion Vesicles (IFVs)

Unprocessed
Spontaneous vesicles

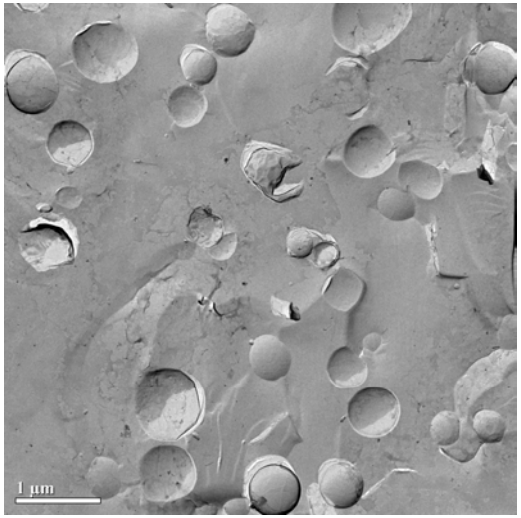


Guohui Wu



Sonicated

- [4 $^{mg}/_{ml}$]
- 0.5 ml DPPC
- 0.125 ml Brij 700



Guohui Wu

Extruded

- [2 $^{mg}/_{ml}$]
- 0.5 ml DPPC
- Poloxamer 188

Processing decreases IFV

Size from 2µm → 500nm -1µm

Conclusion

- Achieved average size reduction by 50 %.
- Combination of the following variables significantly contributed to size reduction:
  - Poloxamer 188
  - [Concentration]: 1-6 $^{mg}/_{ml}$
  - Extruder ≈ Sonicator

Implications

- Experiment with other polymers
- Fluctuate polymer concentrations
- Analyze their natural size contour
- Determine size/number distribution
- Consider file patent claims

Acknowledgements

Funding:

Thanks:

Guohui Wu, Joe Z, James Byrne

Cecile Boyer (Ref)

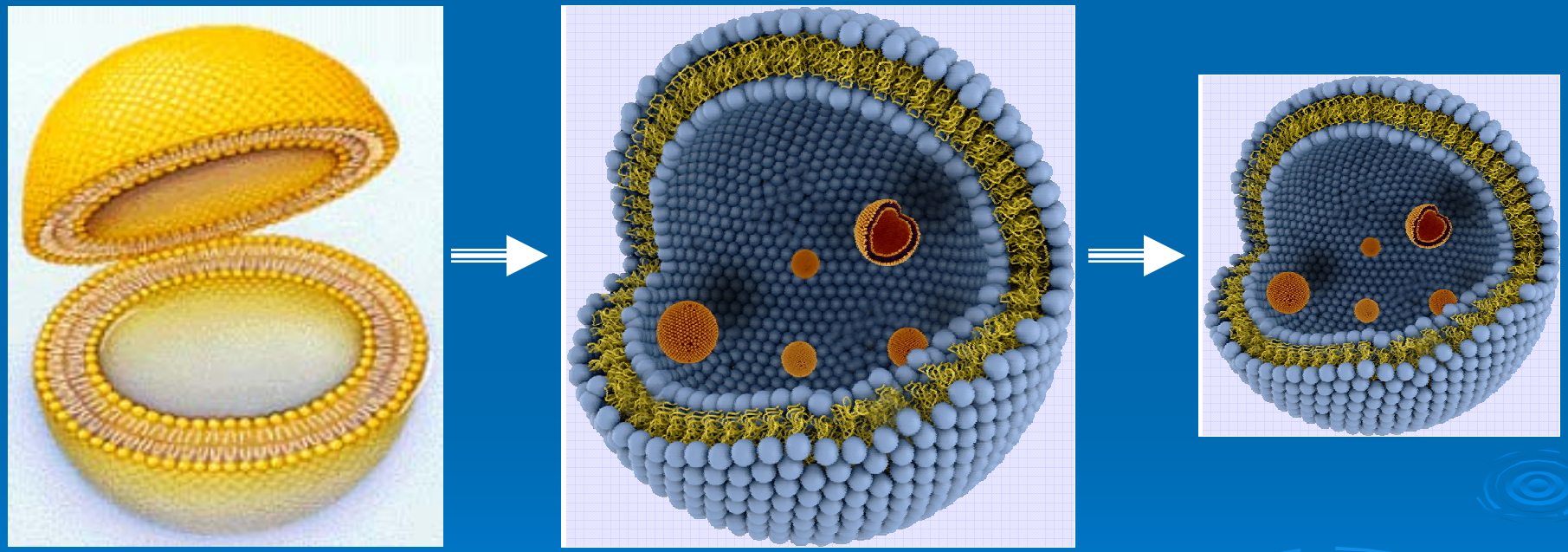
Group members both past and present

Samantha Freeman, faculty, students, and friends

My parents

YOU the audience

QUESTIONS!



Materials / Methods

Freeze Fracture / Replication

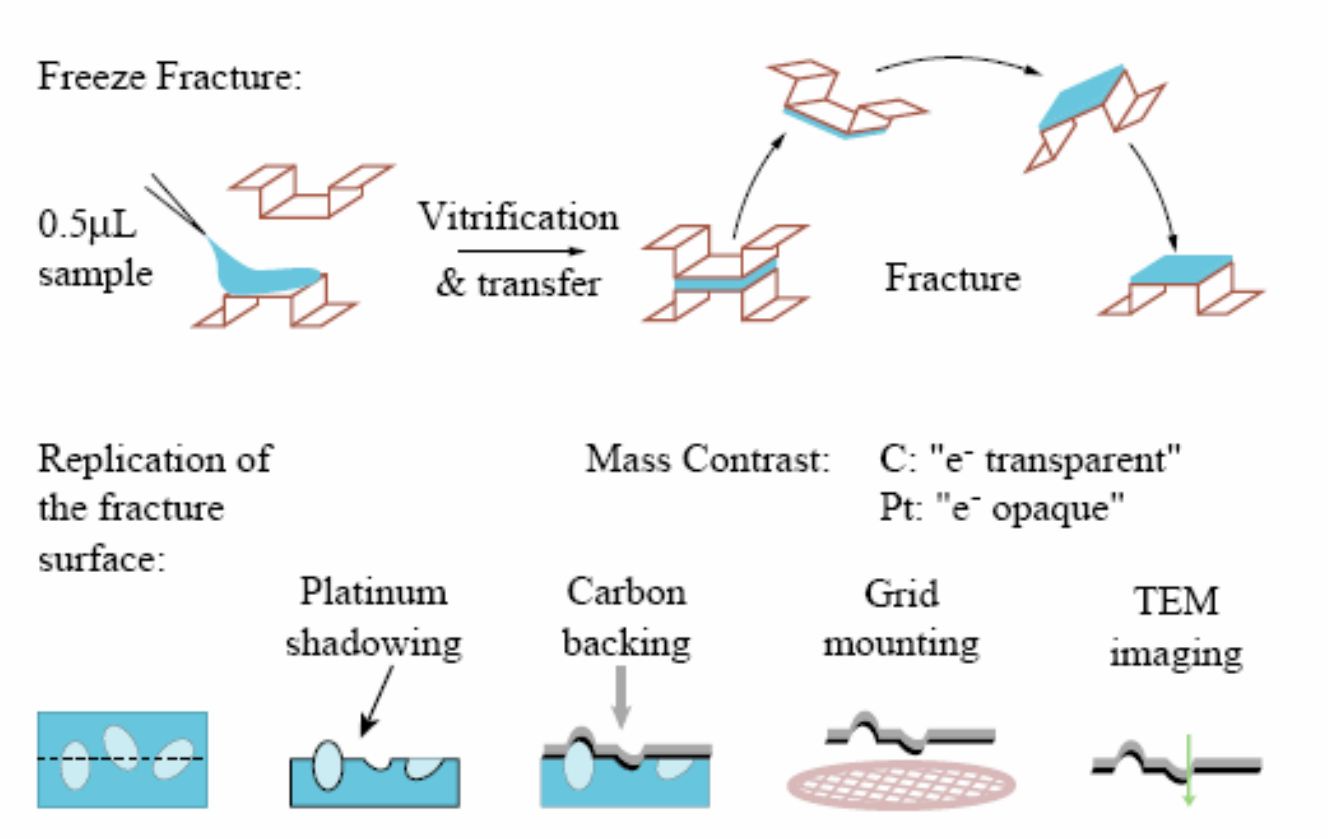
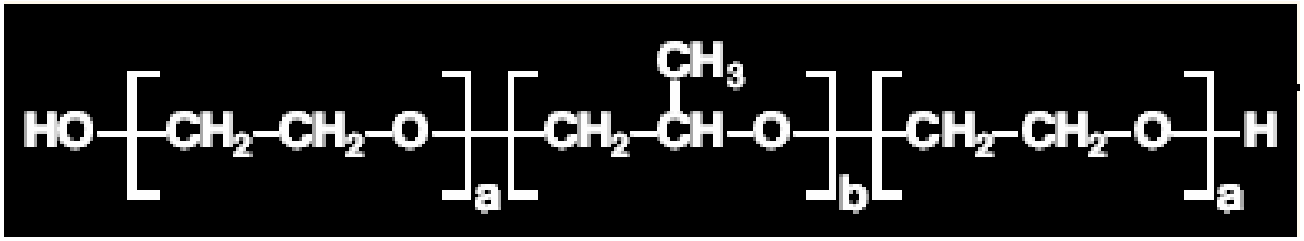


Figure 11: Schematic representation of the Freeze Fracture Replication process: from sample preparation for cryo-fixation, to the mounting of the replica on a TEM grid.

Polymer Fusion

Hydrophobic

Poloxamer 188



Hydrophilic Hydrophilic

Brij 700

$C_{18}H_{37}[OCH_2CH_2]_{100}$- OH

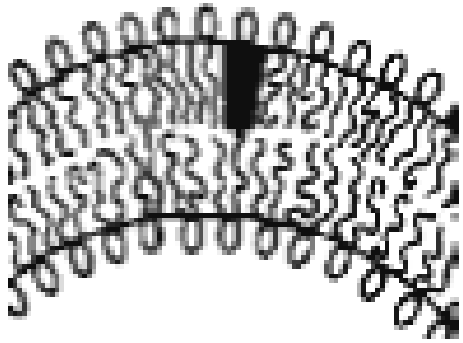
Hydrophobic Hydrophilic

Structures

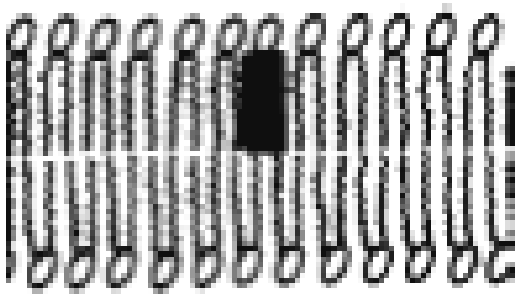
Structures formed

Spherical micelles

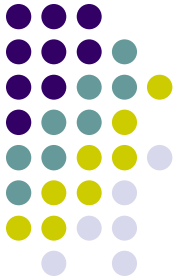
Vesicles



Planar bilayers



Why am I Doing this Research?



- Advance drug delivery efficiency for treatment in diseased tissues
  - Lower dose reduces side effects
- Patient safety
- Lower costs of goods