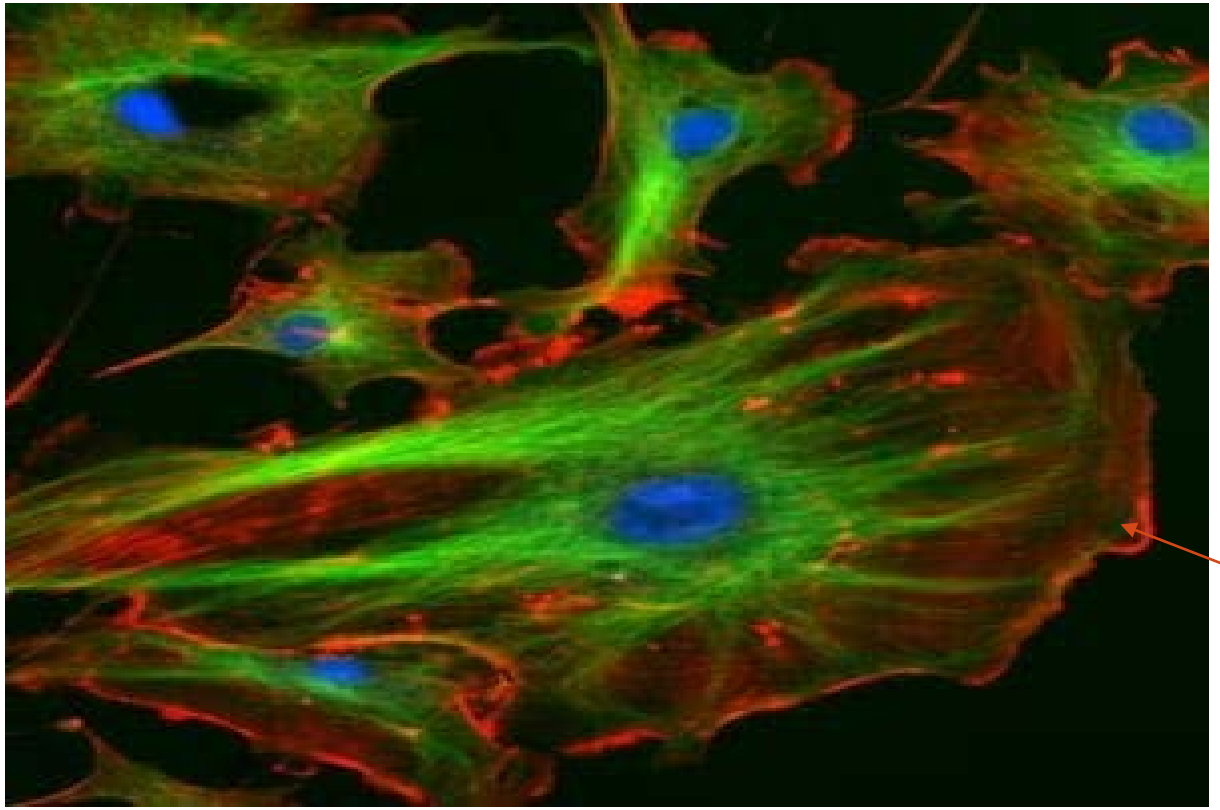
USING VISCOELASTIC PROPERTIES OF POLYMER AND LIPID TO STUDY THE CELL MEMBRANE

Washat Roxanne Ware
NIST Center for Neutron Research
Fayetteville State University

OUTLINE

- Introduction
- Building the Network
  - Characteristics of the Network
    - Data: Dynamic Light Scattering , Rheology, Small Angle Neutron Scattering
- Building the Vesicle
  - Lipid Extrusion
  - Cryogenic Electron Microscopy Images
- Next Steps and Why This Is Important
  - What's left?

THE CELL MEMBRANE

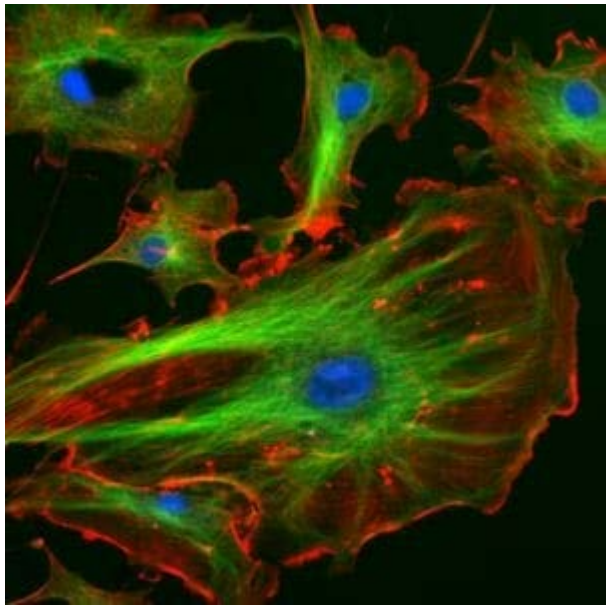


- Cell membrane is made up of a lipid bilayer and is soft and has viscoelastic properties.
- Question: How do the extracellular matrix and cytoskeletal network affect the dynamics of this bilayer?

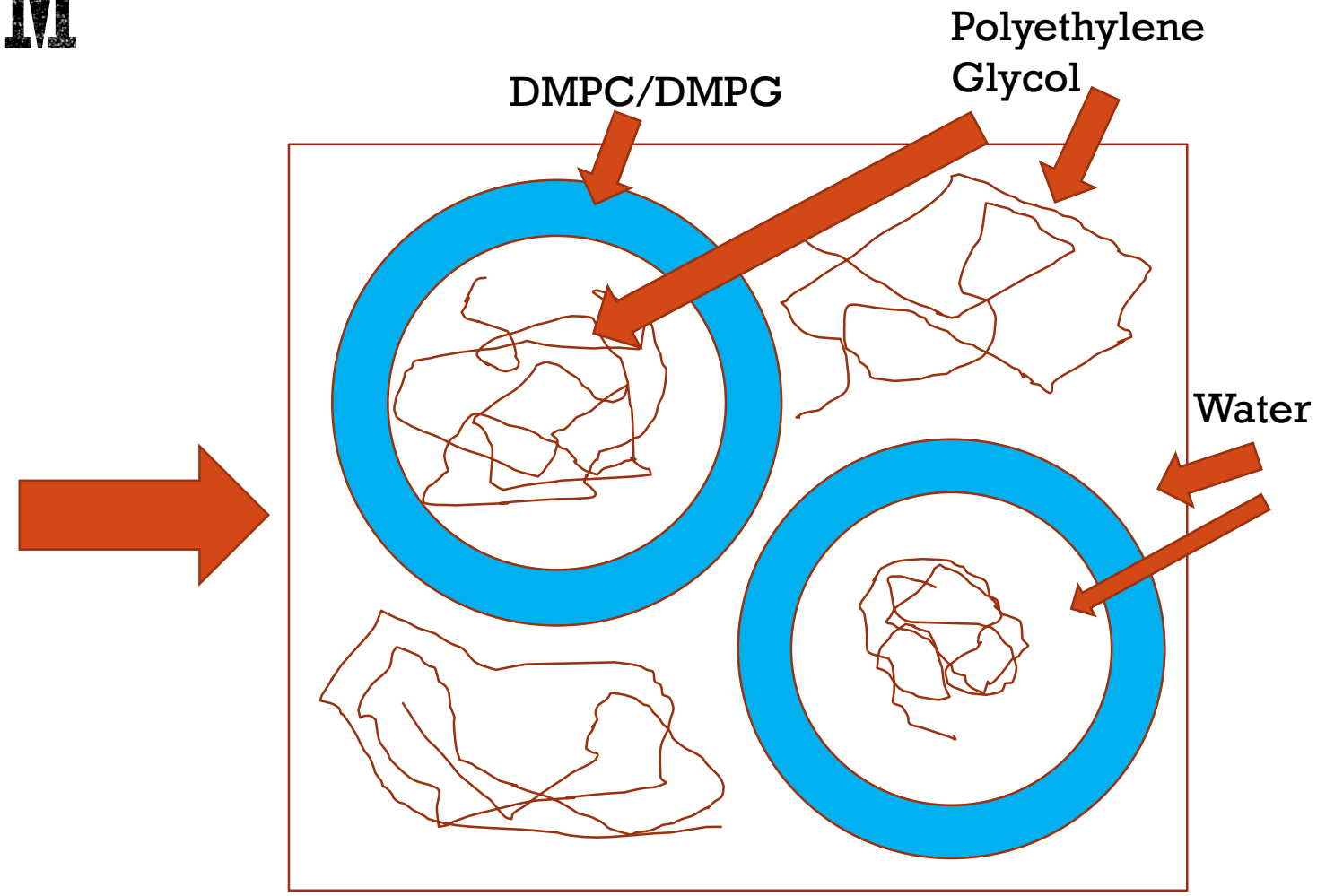
Cell Membrane

*https://biologydictionary.net/cytoskeleton/

A MODEL SYSTEM



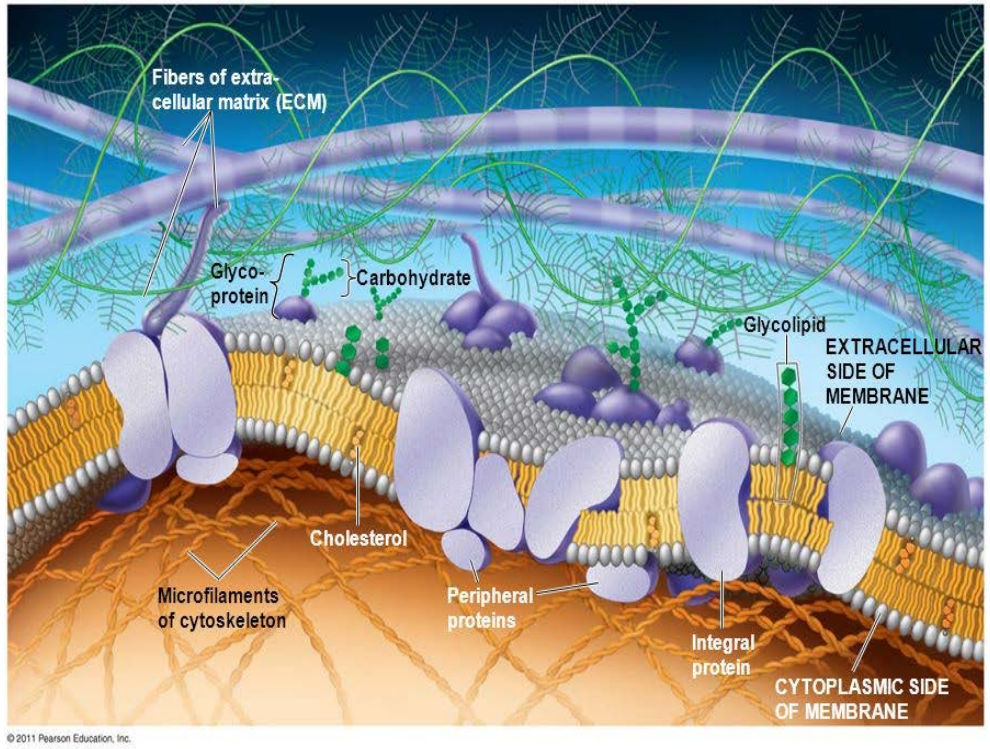
*https://biologydictionary.net/cytoskeleton/



OUTLINE

- Introduction
- Building the Network
  - Characteristics of the Network
    - Data: Dynamic Light Scattering , Rheology, Small Angle Neutron Scattering
- Building the Vesicle
  - Lipid Extrusion
  - Cryogenic Electron Microscopy Images
- Next Steps and Why This Is Important
  - What's left?

PART 1: NETWORK



- Created a network that mimics the cytoskeletal network and extracellular matrix with polyethylene glycol.

- Why polymer?
  - Viscoelastic Properties
  - Cheap and abundant
  - Knowledge about their properties

*https://slideplayer.com/slide/10581332/

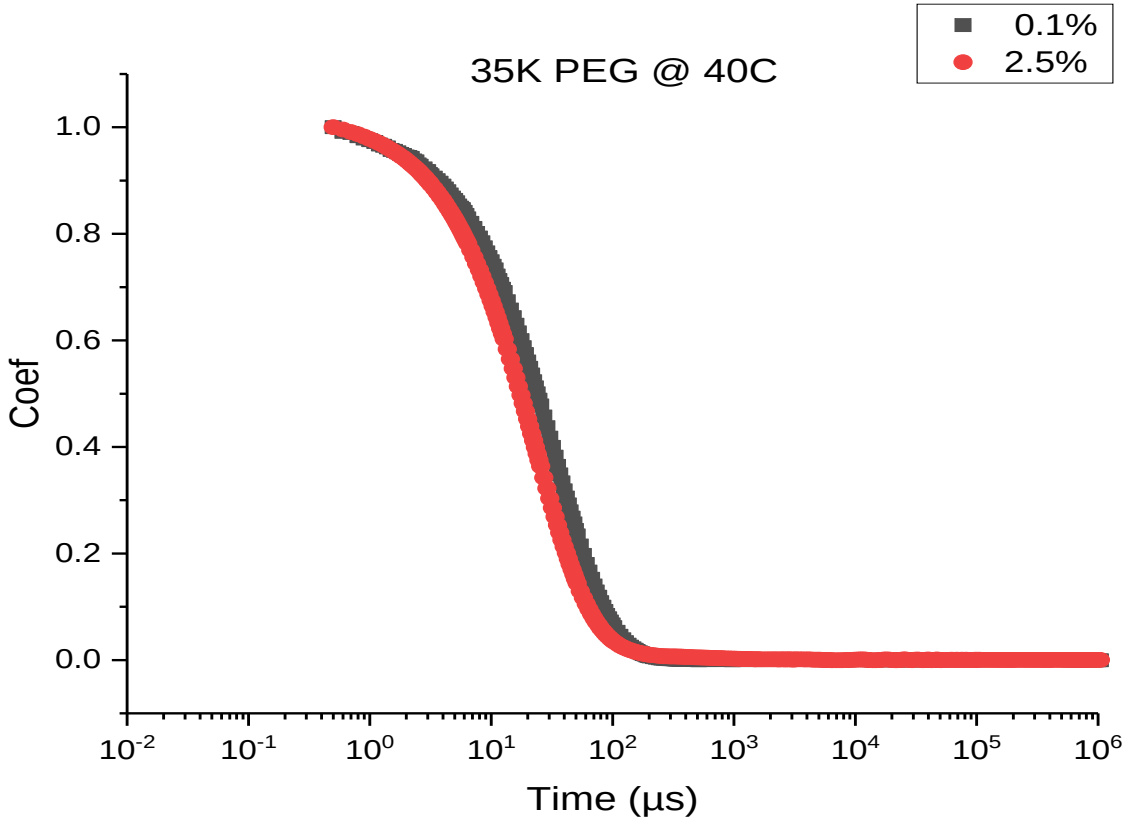
NETWORK: FINDING C*

C<C* C=C* C>C*

*used 100,000 MW and 35,000 MW PEG to create 9 different wt%.

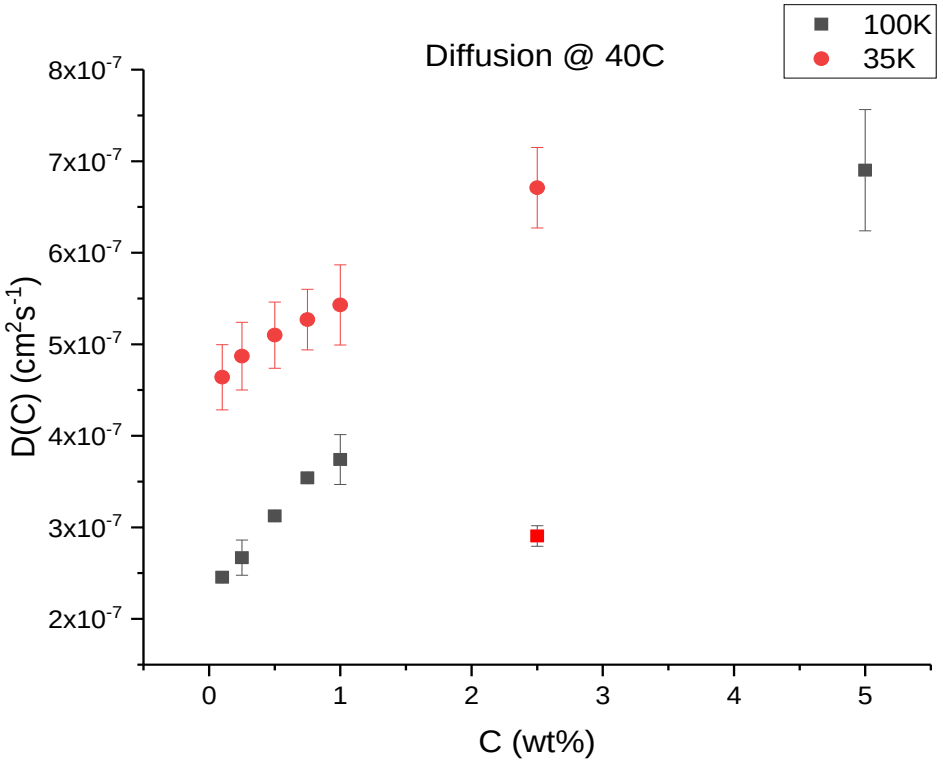
- C*-point at which polymer coils/chains begin to overlap in solution
  - Important because we are trying to recreate a matrix that mimics ECM/cytoskeletal network

DYNAMIC LIGHT SCATTERING: DECAY CURVE



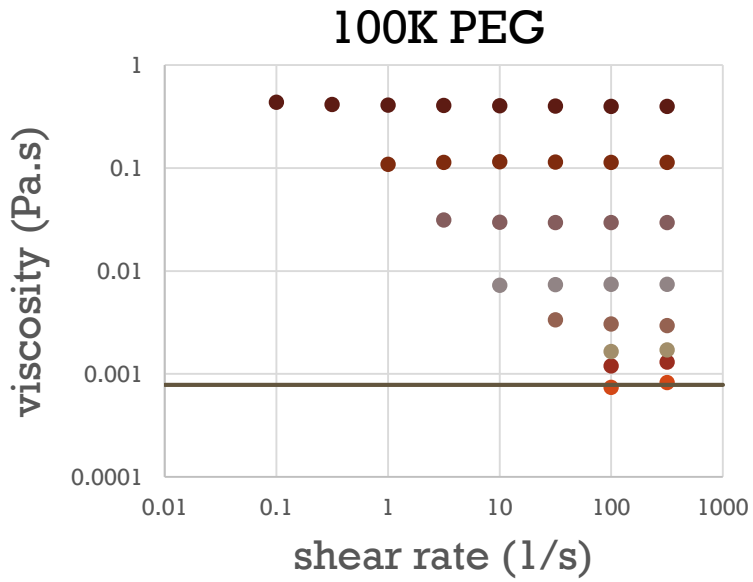
- Key Findings: Higher concentrations have a steeper curve->shifted to the left->meaning smaller particles that are moving rapidly.

DYNAMIC LIGHT SCATTERING: DIFFUSION



- DLS can tell you about the size of particles in solution and their diffusivity in solution
- Key Findings: Positive slope so we know water is a good solvent for PEG; slopes start to converge between 2.5 wt% and 5 wt%.
- Important: DLS couldn't pick up good readings after 5 wt%.

RHEOLOGY

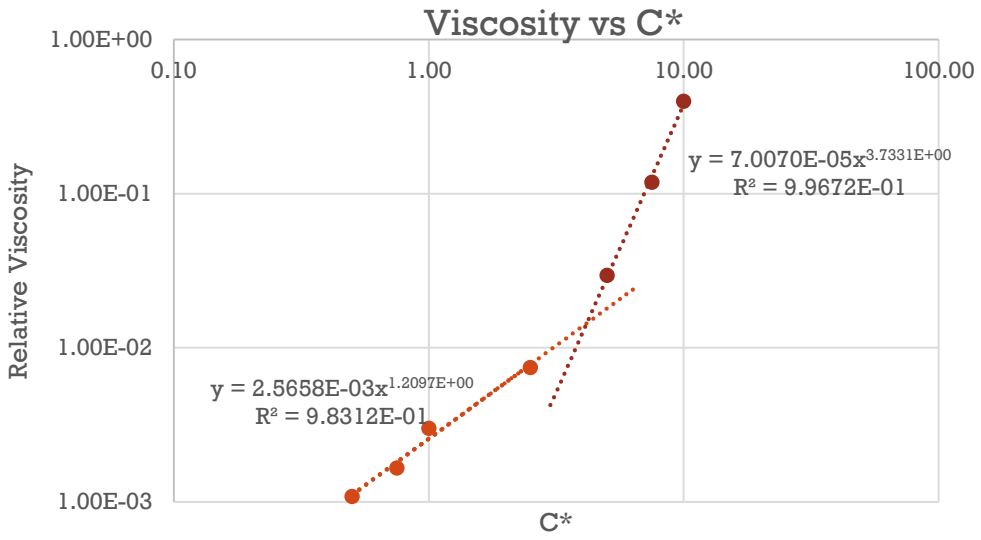


Concentrations

- 10%
- 7.50%
- 5%
- 2.50%
- 1%
- 0.25%
- 0.75%
- 0%
- Literature Value of D2O

- Test: Does increasing concentration increase viscosity?
- Key Findings: Viscosity increases with concentration, with a more dramatic increase between 2.5 and 5 wt%, which could suggest that something is happening to our polymer coils in solution.
- Polymers in solution are Newtonian fluids.

VISCOSITY VS. C*



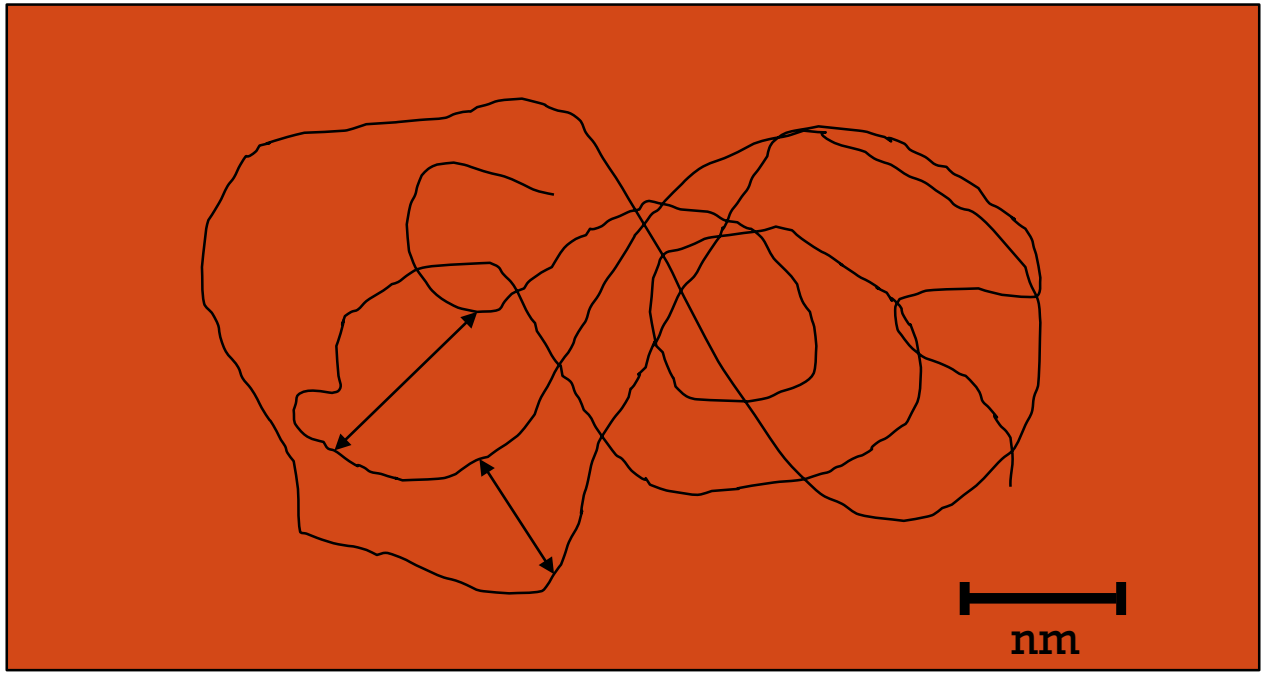
Molecular Weight (kg/mol)	Experimental C* (weight %)
100	3.21

Key Findings: Power law for low concentration and high concentration have different slopes. The point at which they overlap is our c*. Experimental c* is approx. 3.21 wt%.

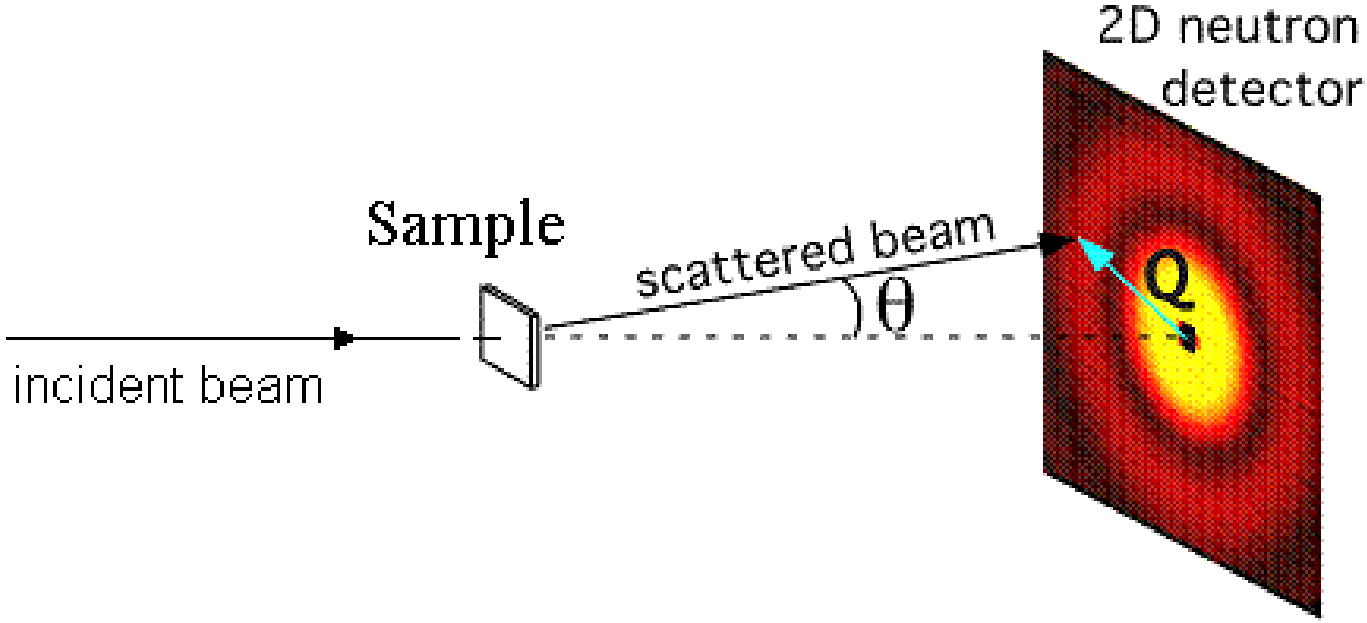
At which concentration do we begin to see overlap in our PEG solutions?

HOW BIG ARE THE "HOLES" IN NETWORK?

- Correlation Length

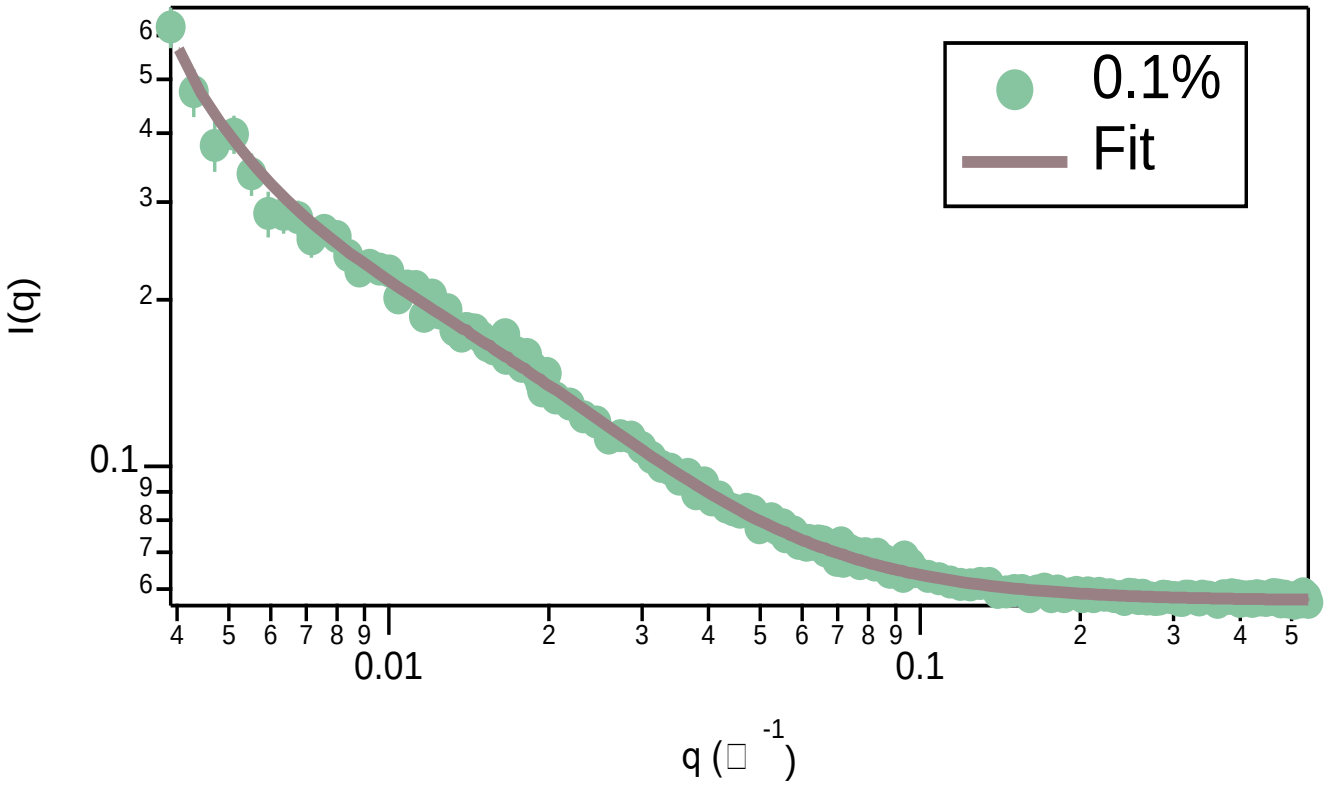


SMALL ANGLE NEUTRON SCATTERING (SANS)



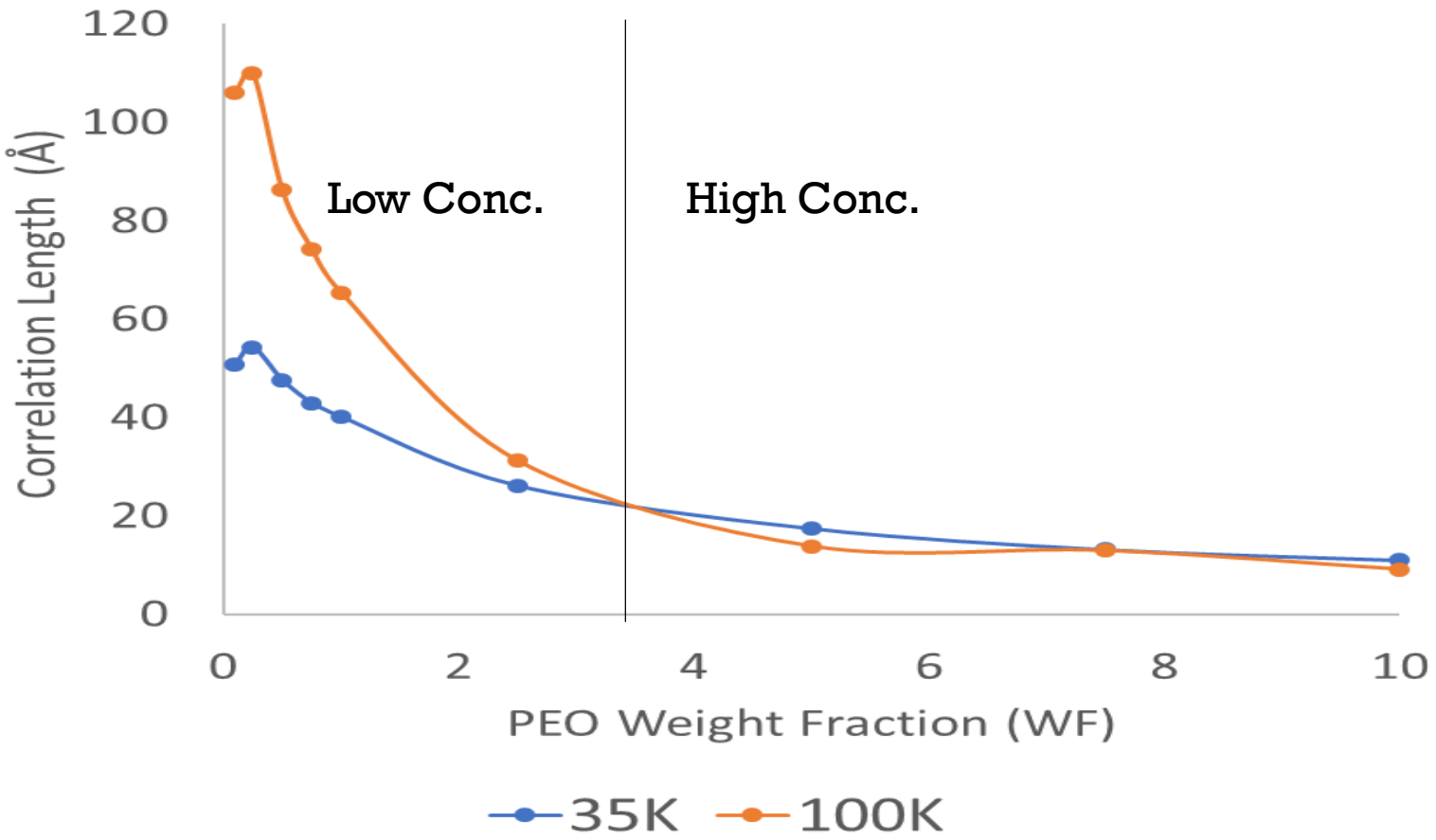
- Nanoscale Structures
- Neutrons are shot through a beam onto sample, where they scatter at different Q's (scattering angles).
- Structure, size, etc.
- Length of instrument is 30m.

SANS DATA



- Data fit to model to determine correlation length.
- Key Findings: Model fit data over entire q range.

CORRELATION LENGTH



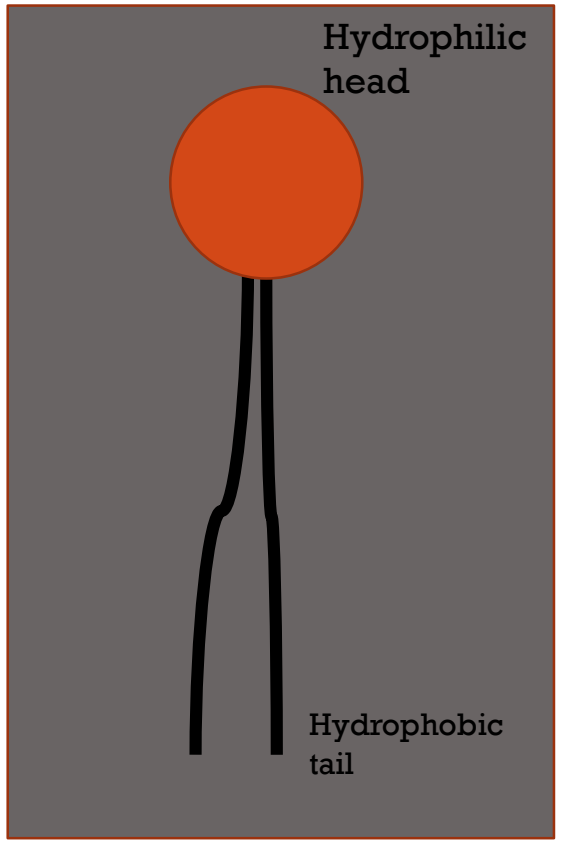
Key Findings:

- We found that correlation length is inversely proportional to concentration.
  - Higher concentrations have smaller correlation lengths
  - Higher molecular weights have smaller correlation lengths.
- Important: SANS measures entire length of coil in wt. % ‘s lower than 2.5.

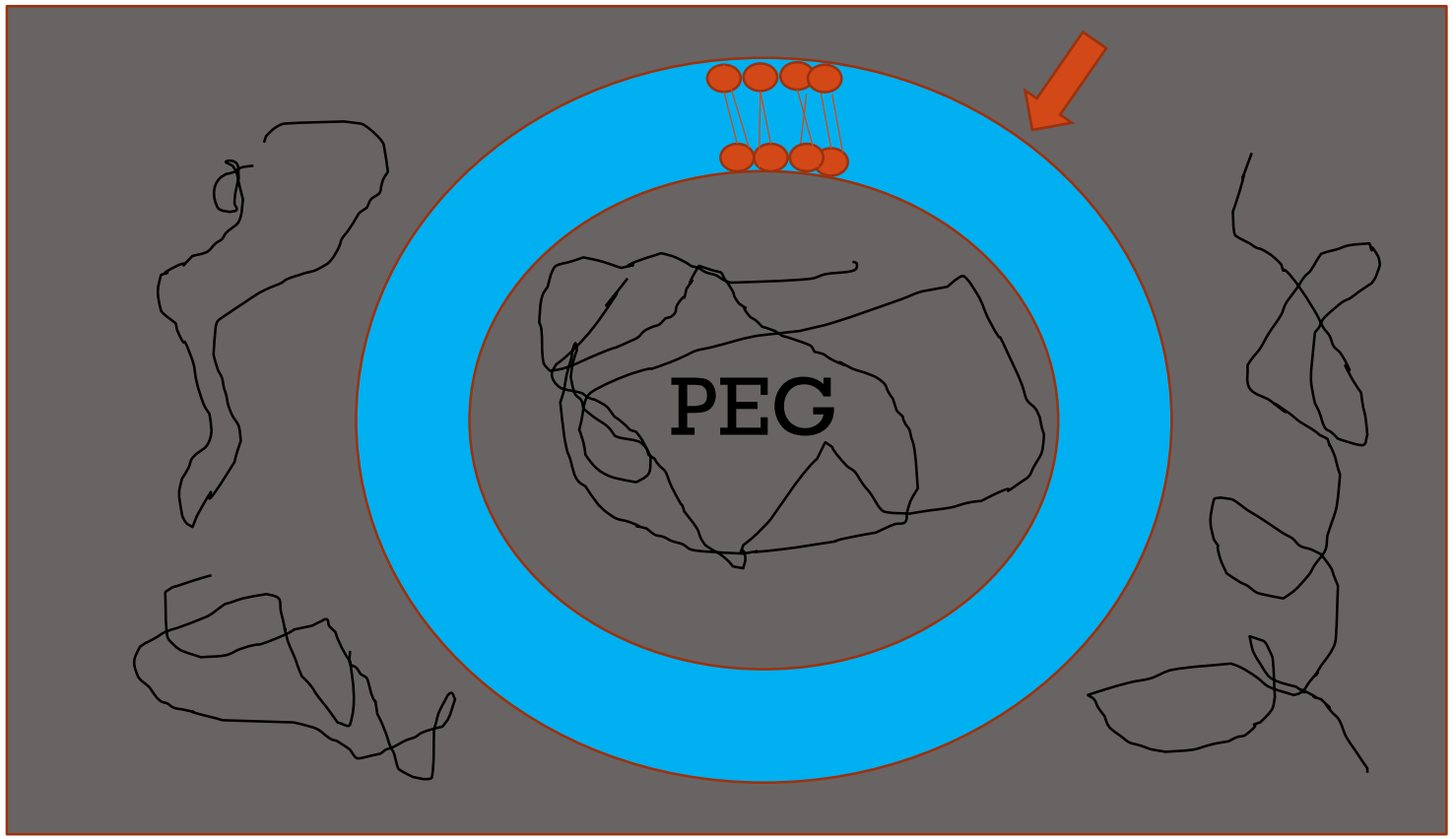
OUTLINE

- Introduction
- Building the Network
  - Characteristics of the Network
    - Data: Dynamic Light Scattering , Rheology, Small Angle Neutron Scattering
- Building the Vesicle
  - Lipid Extrusion
  - Cryogenic Electron Microscopy Images
- Next Steps
  - What's left?
- Why This Is Important

PART 2: BUILD THE VESICLES



DMPC/DMPG

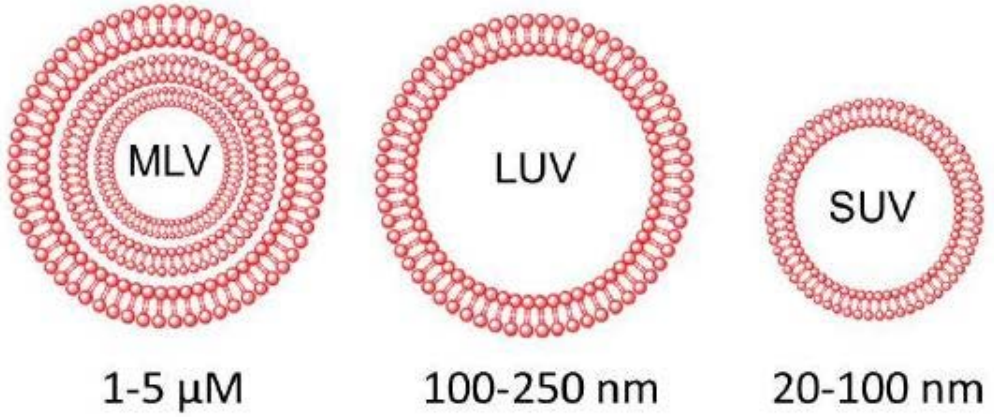


LIPID EXTRUSION

Before Extrusion: Cloudy Solution

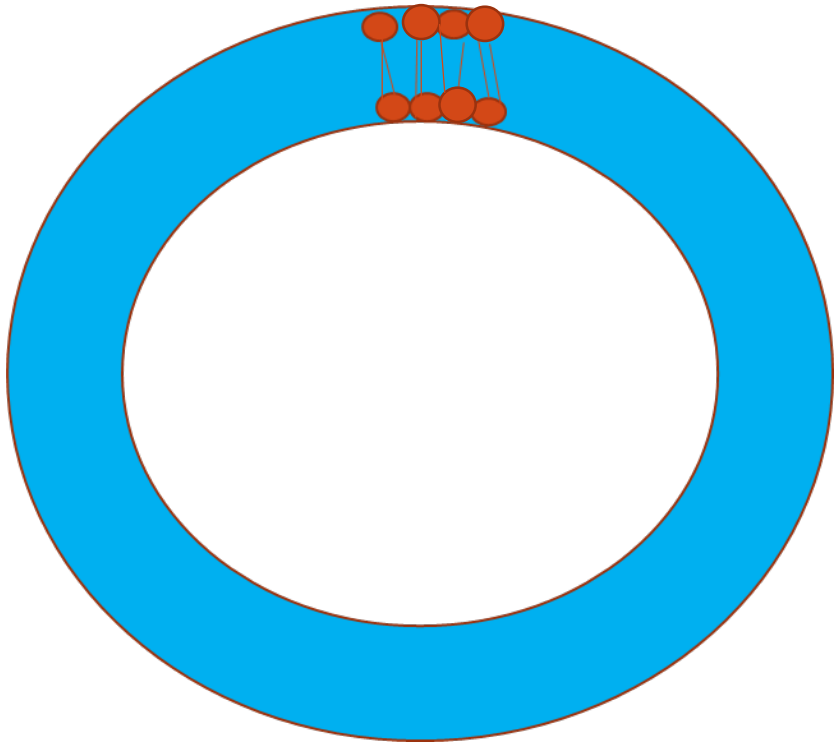
After Extrusion: Clear Solution

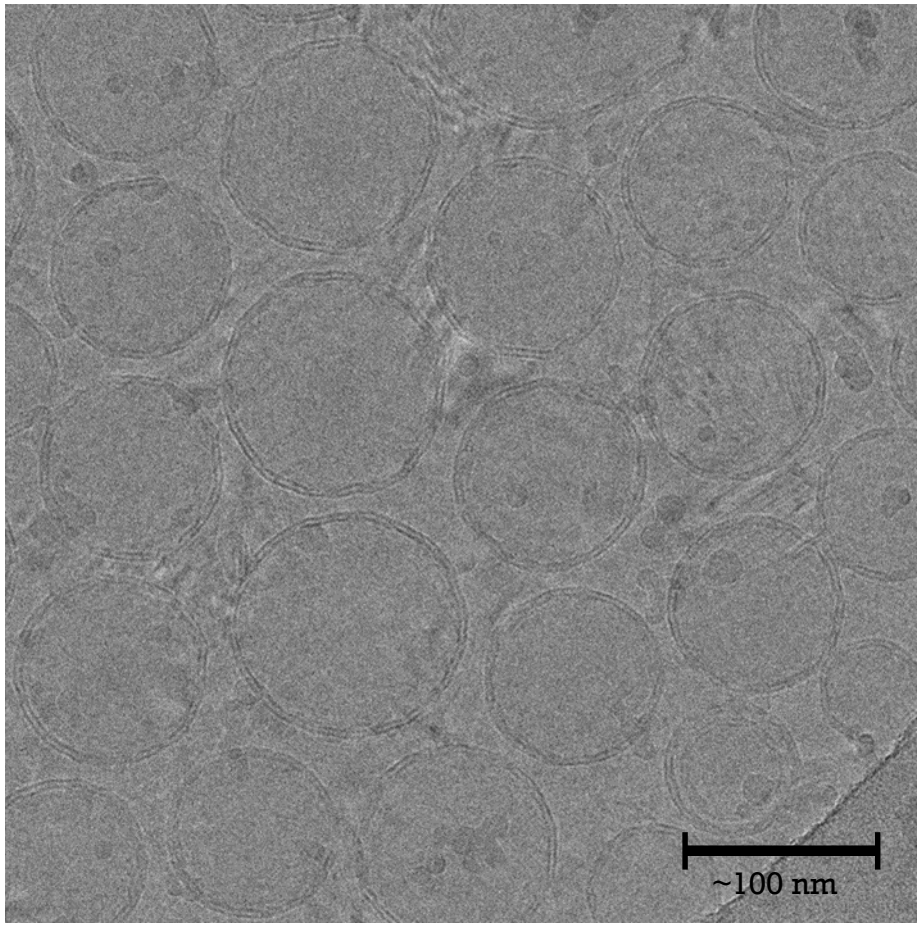
- Mixed DMPC/DMPG charged lipid to form lipid bilayer
- Extruded in solution through 400 nm, 200 nm, and 100 nm filter
- Multilamellar->Unilamellar



https://biopharma.co.uk/blog/2016/11/16/why-you-should-chose-extrusion-for-your-liposomal-enhancement/

CRYOGENIC ELECTRON MICROSCOPY



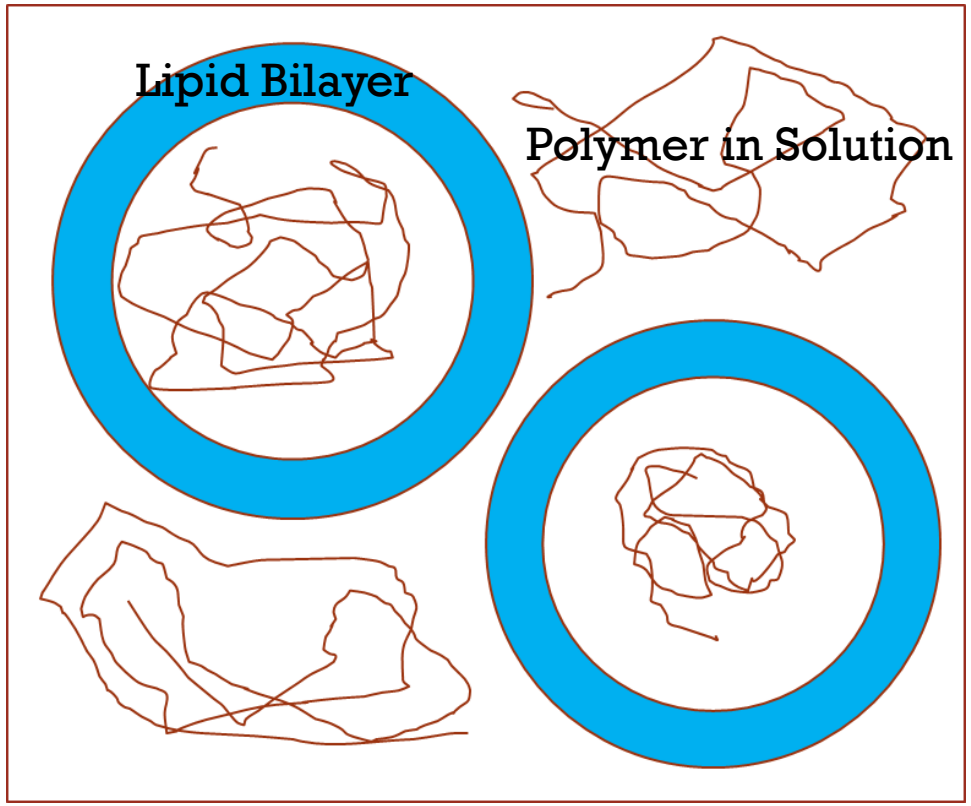


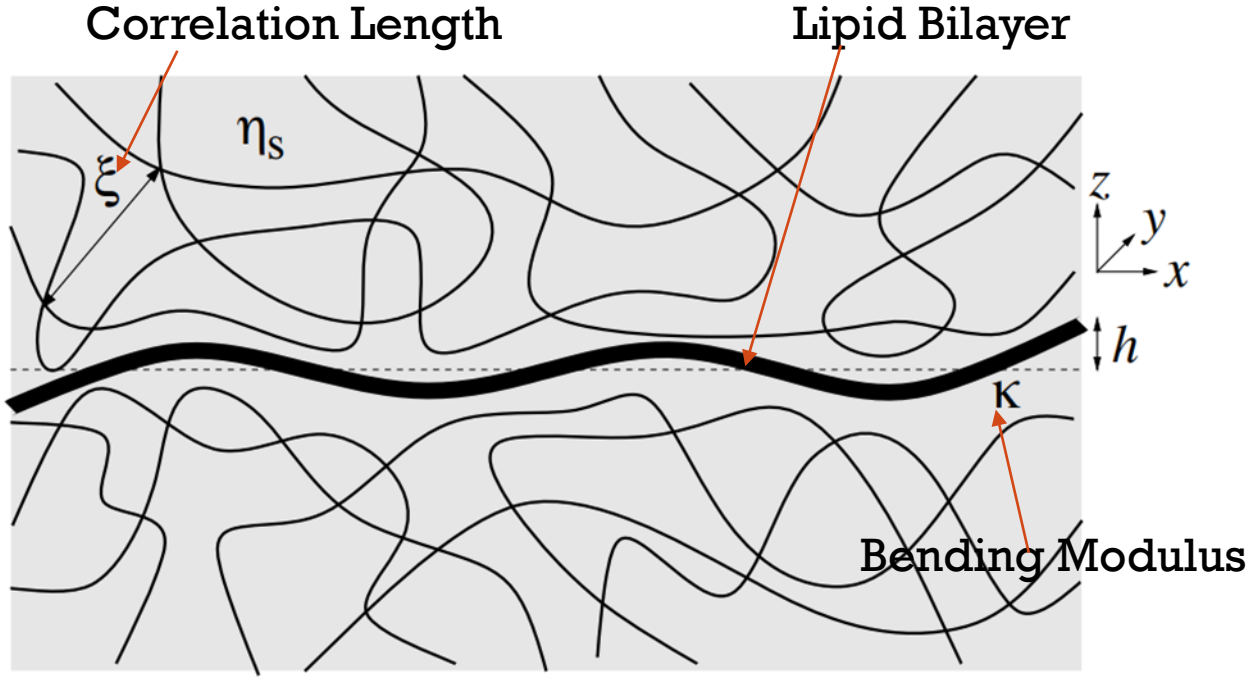
OUTLINE

- Introduction
- Building the Network
  - Characteristics of the Network
    - Data: Dynamic Light Scattering , Rheology, Small Angle Neutron Scattering
- Building the Vesicle
  - Lipid Extrusion
  - Cryogenic Electron Microscopy Images
- Next Steps and Why This Is Important
  - What's left?

NEXT STEPS:

- Next steps include using Neutron Spin Echo Spectroscopy to study the structure dynamics of the liposome vesicles in polymer solution.





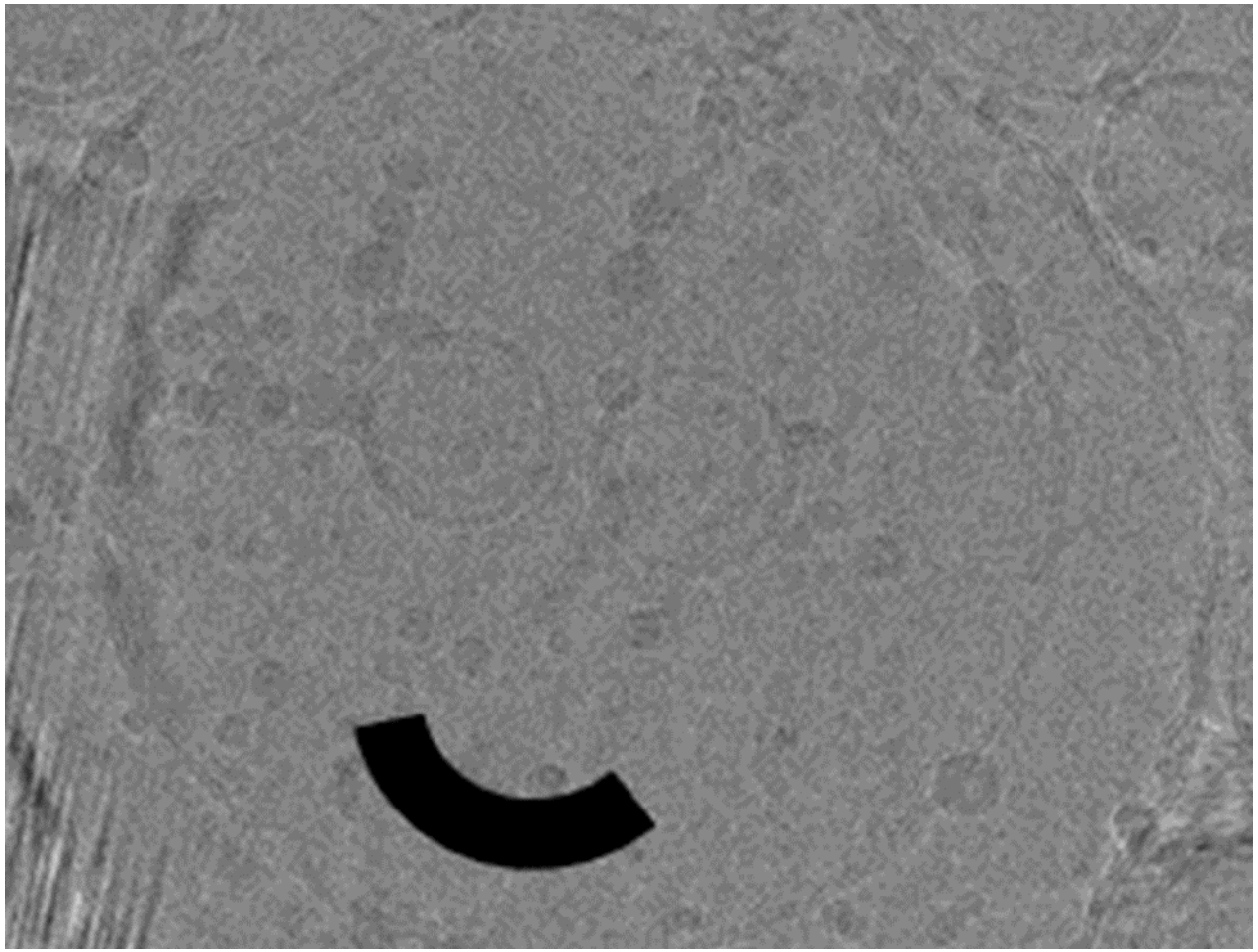
https://en.wikipedia.org/wiki/Liposome

WHY IS THIS IMPORTANT?

- Drug delivery technology
  - Stem Cell Research
  - Gene Therapy
  - Cancer Therapy
- Increasing knowledge about the membrane

Acknowledgements

Dr. Antonio Faraone, NIST
Dr. Elizabeth Kelley, NIST
Dr. Michihiro Nagao, NIST
Prof. Erkan Senses, Koc University
Dr. Tom Cleveland, NIST and IBBR
Dr. Joseph Dura and Dr. Jullie Bourchers, NCNR SURF Coordinators



Questions and Answers?