Constructing Native Extracellular Matrix Scaffolds for Tissue Repair

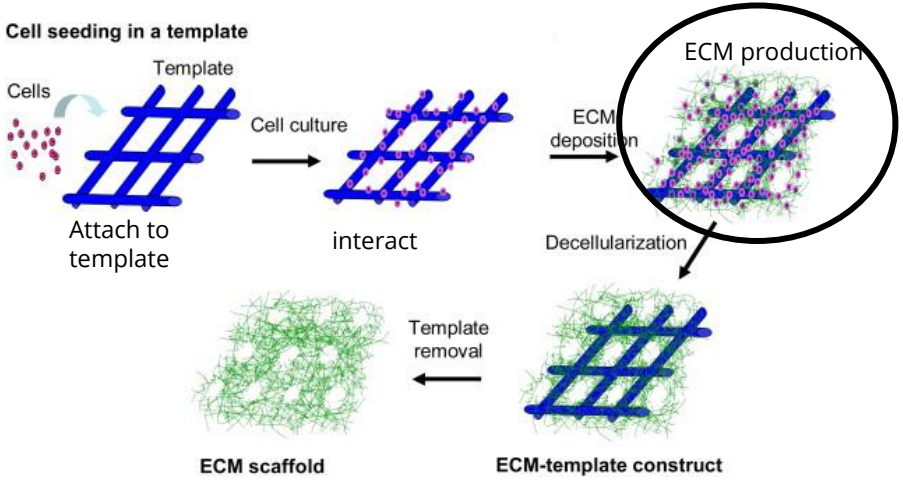
Sanjana Sureshbabu
Mentor: Minh Phan

CHRNS
NIST CENTER FOR NEUTRON RESEARCH

AMERICAN UNIVERSITY
WASHINGTON, DC

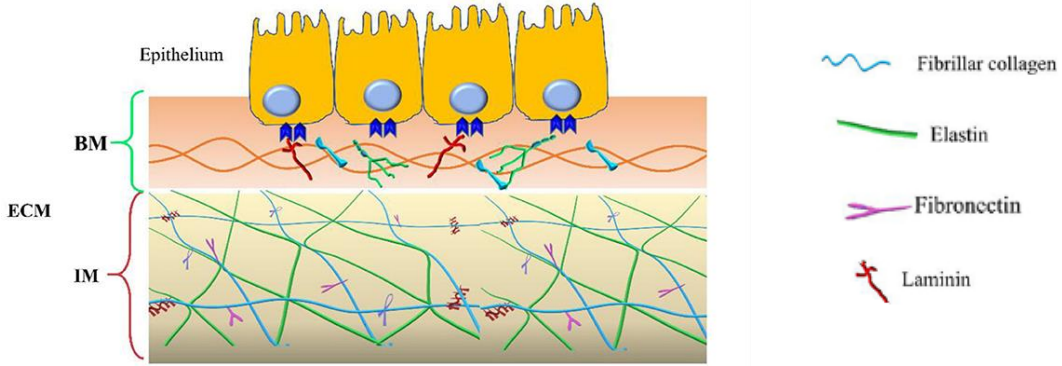
Why Tissue Engineer?

- Biocompatible polymer is used for tissue repair and implantation, but cell communication may be inhibited.
- Native scaffolds from tissues have risks of disease, rejection, and is it hard to isolate.
- Cell derived ECM can be taken from human cell cultures, but ECM production can take months to occur.
- **Goal:** Engineer pre-scaffolds to significantly speed the process up.



Assunção, M., Dehghan-Baniani, D., Yiu, C. H. K., Später, T., Beyer, S., & Blocki, A. (2020, November 10). *Cell-derived extracellular matrix for tissue engineering and Regenerative Medicine*. Frontiers.

Pre-Scaffold Construct “The Bricks”



Collagen- main structural element, mechanical strength

Elastin- elasticity of tissues (large arteries, heart valves, etc)

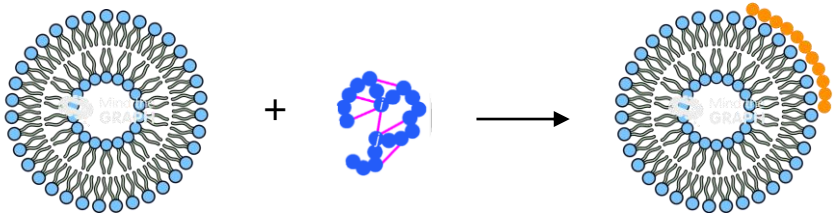
Fibronectin- promotes cell adhesion and migration

Laminin- component of basement membrane, supports growth and differentiation of cells

Pompili, Simona, et al., (22 Mar. 2021) The Charming World of the Extracellular Matrix *Frontiers*

What do we do to make this construction possible?

- Extracellular Matrix proteins tend to fold in native form, this will cause it to be inactive.
- Utilizing lipid membranes can unfold proteins, and they can make it functional



Paten, J. A., Martin, C. L., Wanis, J. T., Siadat, S. M., Figueroa-Navedo, A. M., & Ruberti, J. W. (2019, June 6). *Molecular interactions between collagen and fibronectin: A reciprocal relationship that regulates de novo fibrillogenesis*. Chem.

Arranging Protein on Membrane

1. Individually
2. Combinations
    a. Fibronectin and Collagen
        i. Accelerates the onset of fibril growth
    b. Fibronectin and Laminin
        i. Fibrillogenesis occurs the same way in both proteins due to β-sheet structures

Ahn, Seungkuk et al. (2018) Formation of Multi-Component Extracellular Matrix Protein Fibers

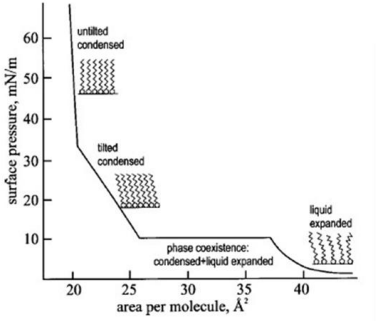
Goals

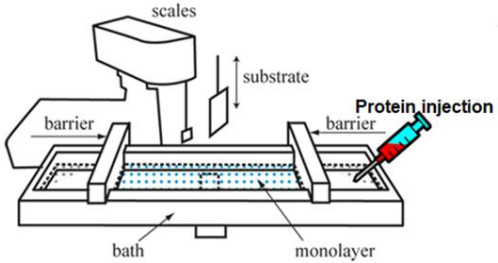
- To determine the membrane compositions that best supports the proteins and construct a scaffold on the lipid particles
- The particles should be able to put on wounds to establish cell connection, tissue reconstruction, and healing

Langmuir Isotherms (X & Y Axes) and Adsorption Assays

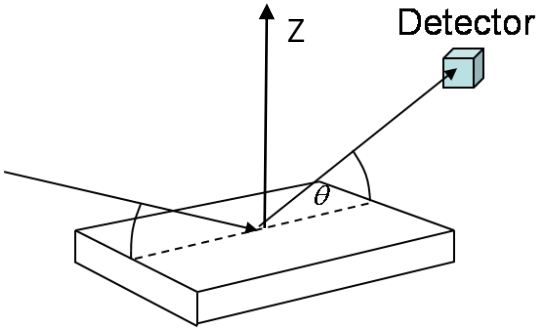
- Phase diagram that gives you insight of the two dimensional phase behavior of sample
  - To find the optimized pressure for the protein to be injected
  - Shows if protein is adsorbed at all; kinetics of the adsorption

ex)





X-ray Reflectivity(Z Axis)



- How protein arranges under the membrane
- Measures thickness, density, roughness, etc.

Experimental Design: Lipid Composition

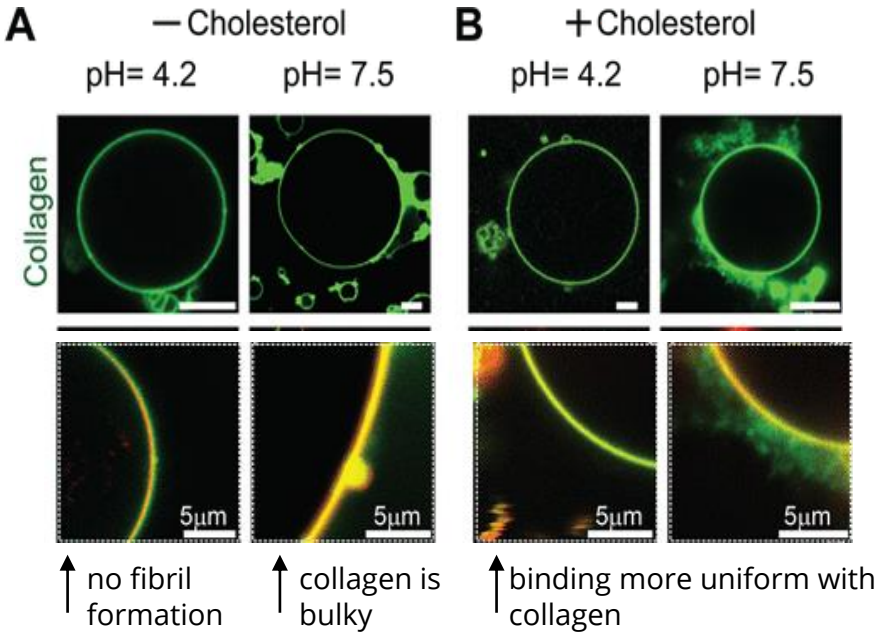
DMPC: the skeleton of the membrane

DMPE: regulates the non-lamellar structure in the membrane

DMPS: negatively charged lipid, helps fibronectin and laminin binding

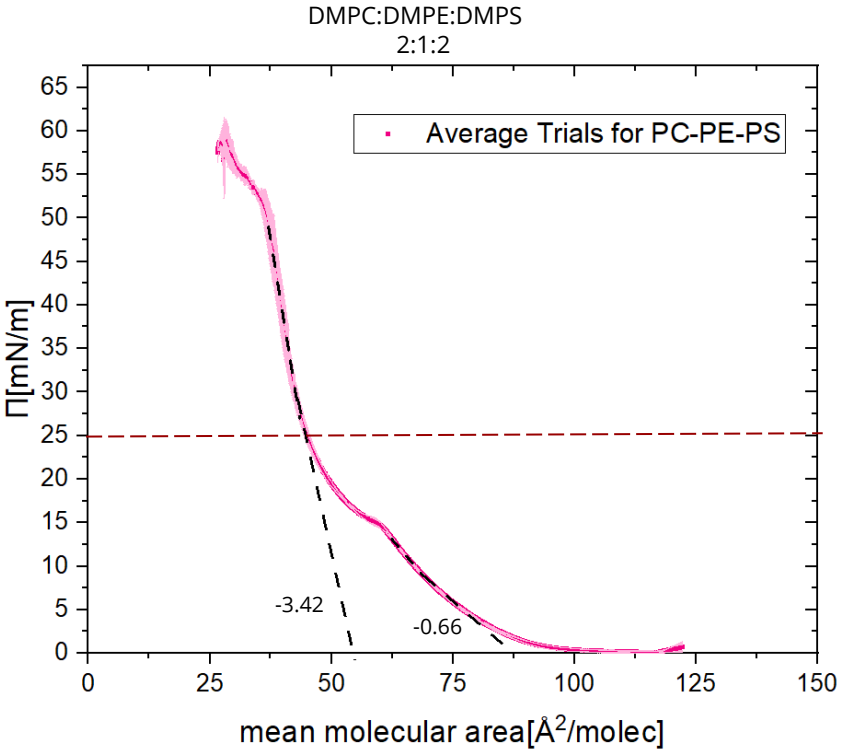
Cholesterol: increases the lateral lipid headgroup separation on membrane surface, this promotes the association degree of collagen monomers

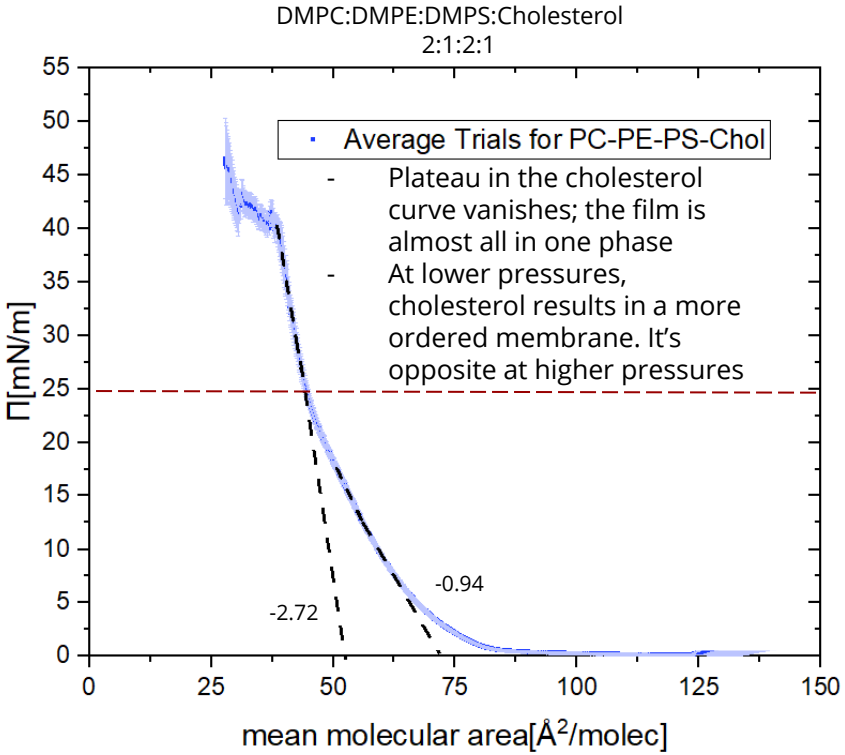
DMPC: DMPE :DMPS: Chol
2: 1: 2-4: 1 ← mole ratio



Phan, Minh D et.al Langmuir (2020) The Effect of Cholesterol on Membrane Binding and Self-Assembly

Average of Trials With and Without Cholesterol





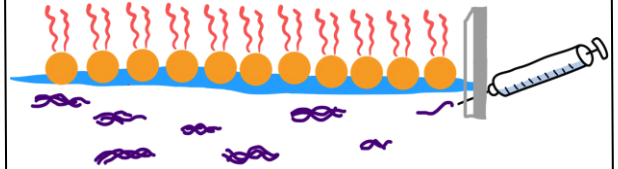
X-ray Reflectivity Experiments

Measurement #1

- Bare membrane
- Only the mixed lipid sample, no protein injected

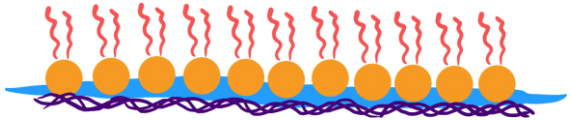
Measurement #2

- Initial injection of protein



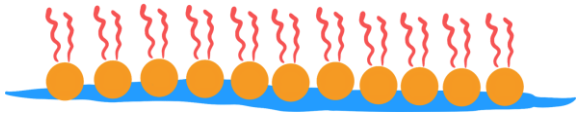
Measurement #3

- Equilibrium measurement, protein is at equilibrium



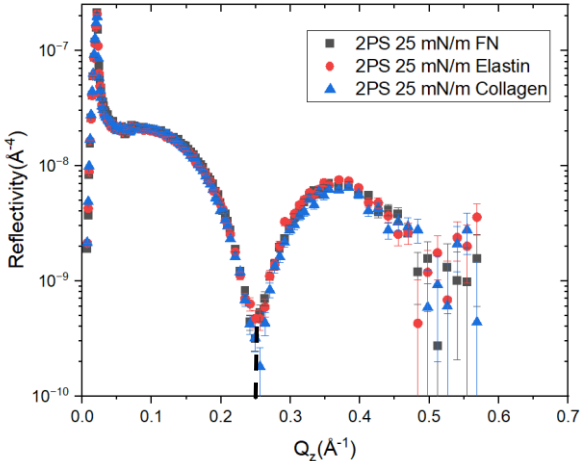
Bare Membranes

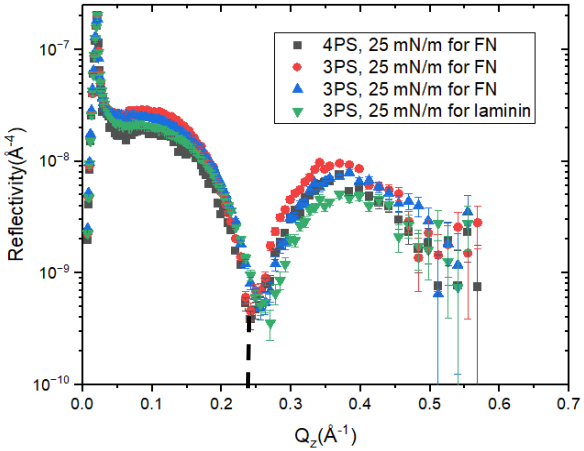
(Before protein injection)



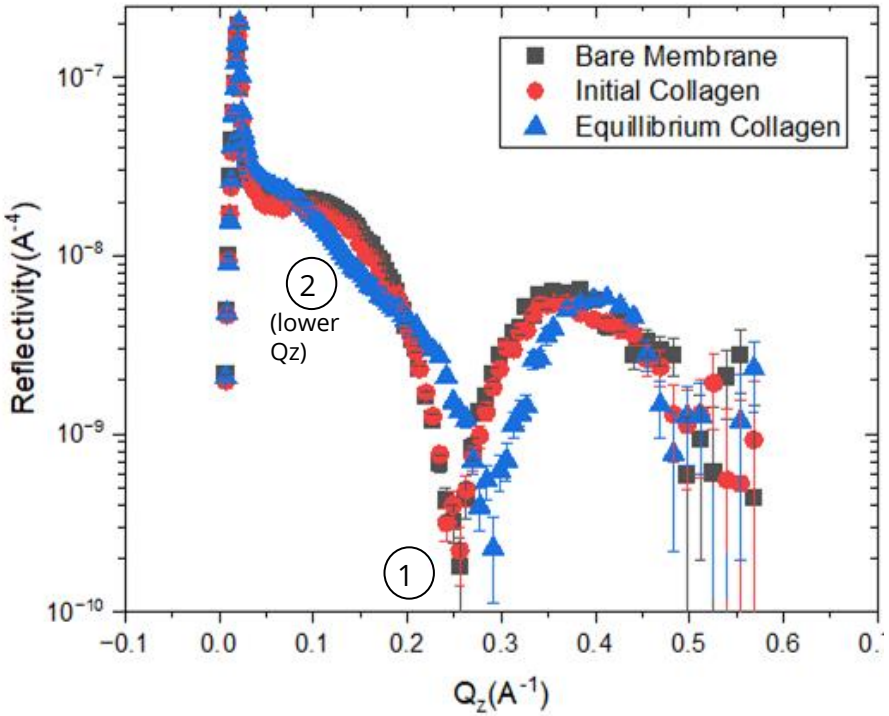
- Collagen and fibronectin graphs are very similar; reproducible
- 3PS & 4PS graphs are shifted inward-- thicker
- Pressure decreases, film is thinner

Structure of film changes as PS and pressure are varied



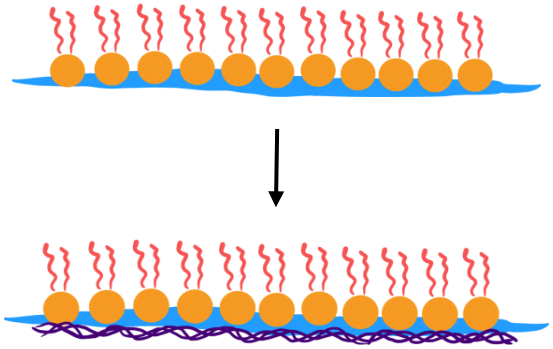


Collagen XRR

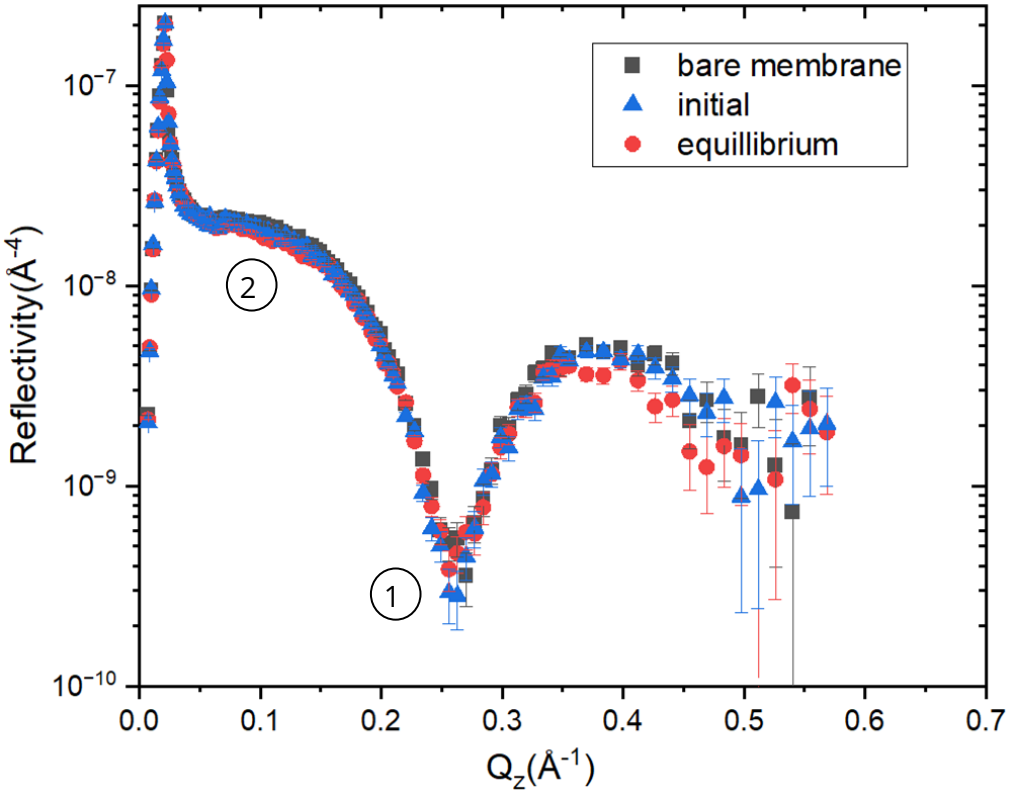


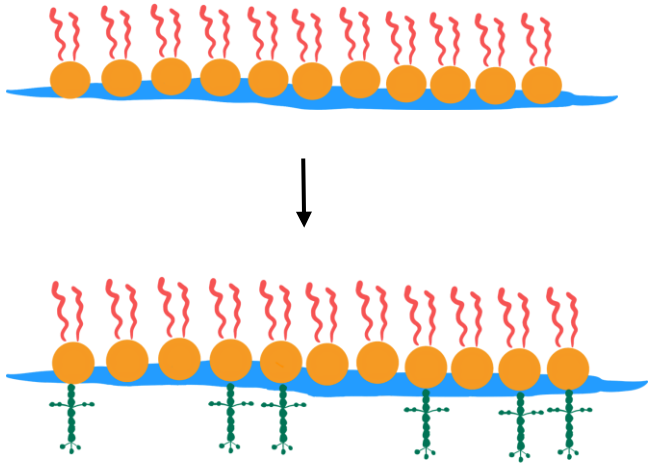
DMPC:DMPE:DMPS:Cholesterol
2:1:2:1

- Uniform collagen assembly on membrane
- Non-specific binding to membrane, not dependent on negative charge

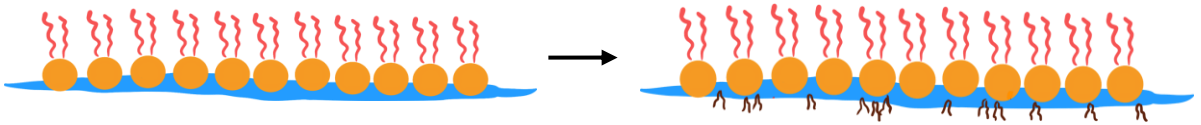


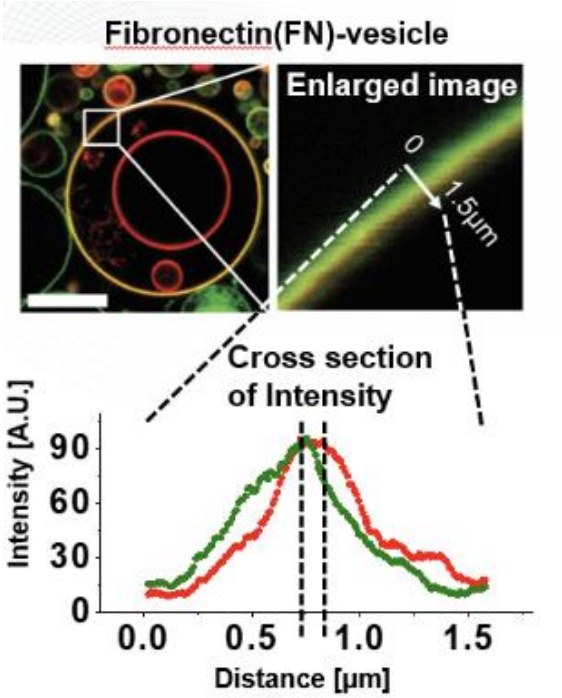
Laminin XRR


bare membrane
initial
equillibrium
Reflectivity(Å^{-4})
10^{-7}
10^{-8}
10^{-9}
10^{-10}
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Q_z(Å^{-1})
1
2



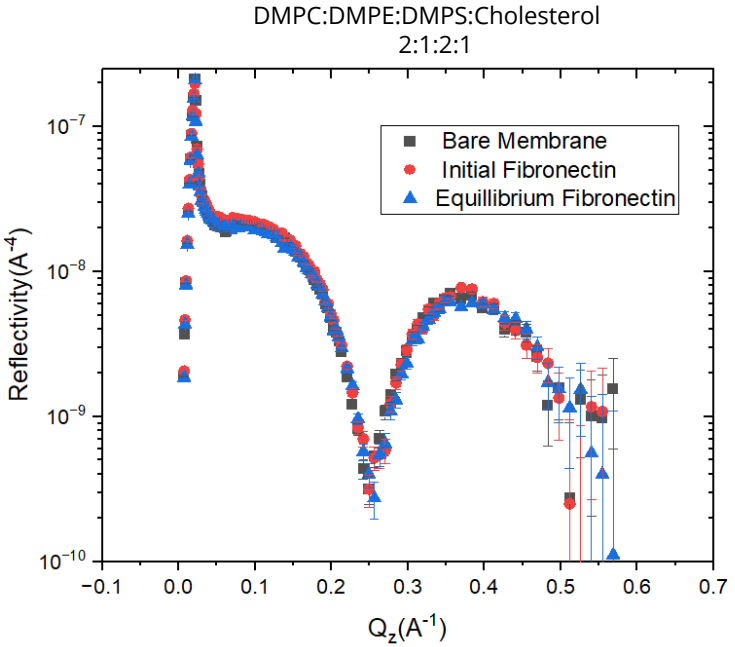
Fibronectin XRR



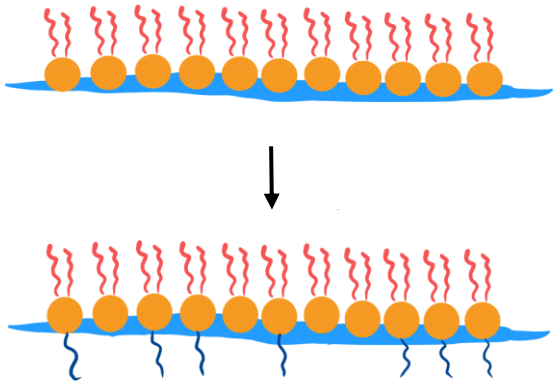


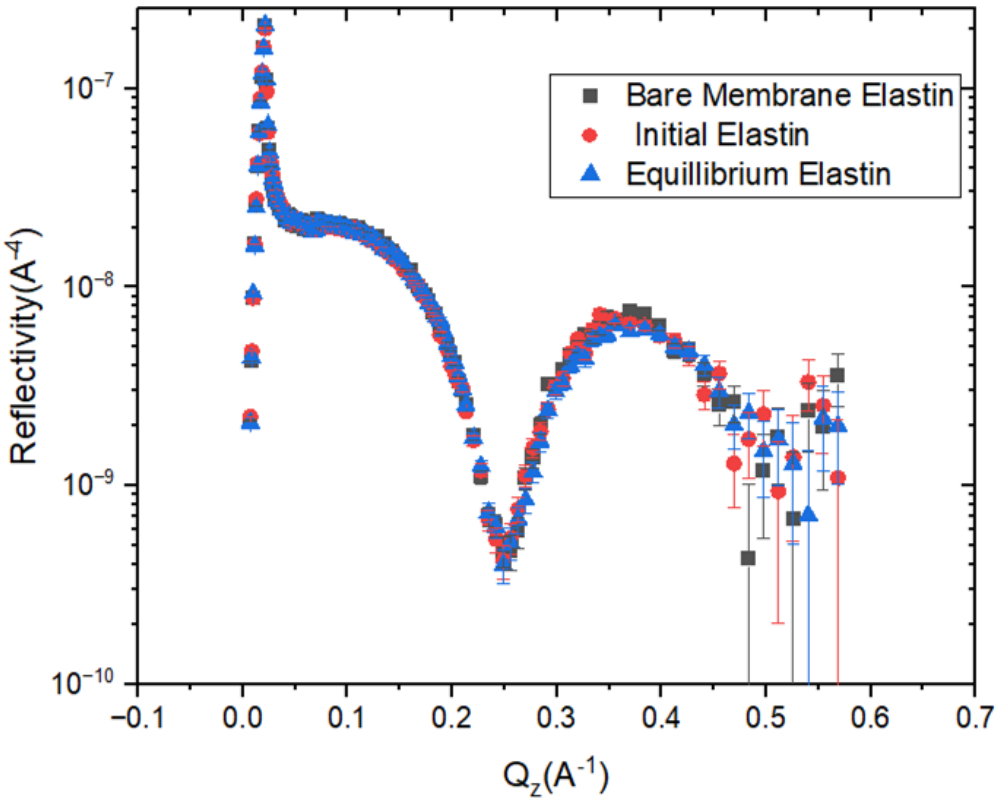
Shown that FN binding is dependent on PS, a negative charged lipid

Increase in PS and loosening packing density → no change



Elastin XRR




Bare Membrane Elastin
Initial Elastin
Equillibrium Elastin
Reflectivity(A^{-4})
10^{-7}
10^{-8}
10^{-9}
10^{-10}
−0.1
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Q_z(A^{-1})

Conclusions

Lipid Sample Conclusion

- The presence of cholesterol results in the film being in mostly one phase
- Raising the PS ratio in the lipid solution makes the bare membrane thicker.

Protein Conclusions

- Successfully fabricated uniform collagen assembly on membrane
- Laminin shows signs of adsorption
- Fibronectin and elastin should bind better in neutral pH where the membrane has more effective negative charges for the proteins to interact with

Acknowledgments

•Minh Phan -- Mentor, XRR procedure, etc.

•Sushil Satija-- Co mentor

•NCNR SURF Directors--
- Julie Borchers
- Susana Marujo Teixeira
- Leland Harriger

•Surabhi Singh -- Lab Partner

•NIST SURF Program

CHRNS NSF NIST
CENTER FOR HIGH RESOLUTION NEUTRON SCATTERING

NIST CENTER FOR NEUTRON RESEARCH

AMERICAN UNIVERSITY
WASHINGTON, DC