Development of Lipid-Based Scaffolds with Extracellular Matrix Proteins for Tissue Repair

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NIST CENTER FOR NEUTRON RESEARCH

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Materials Science and Engineering
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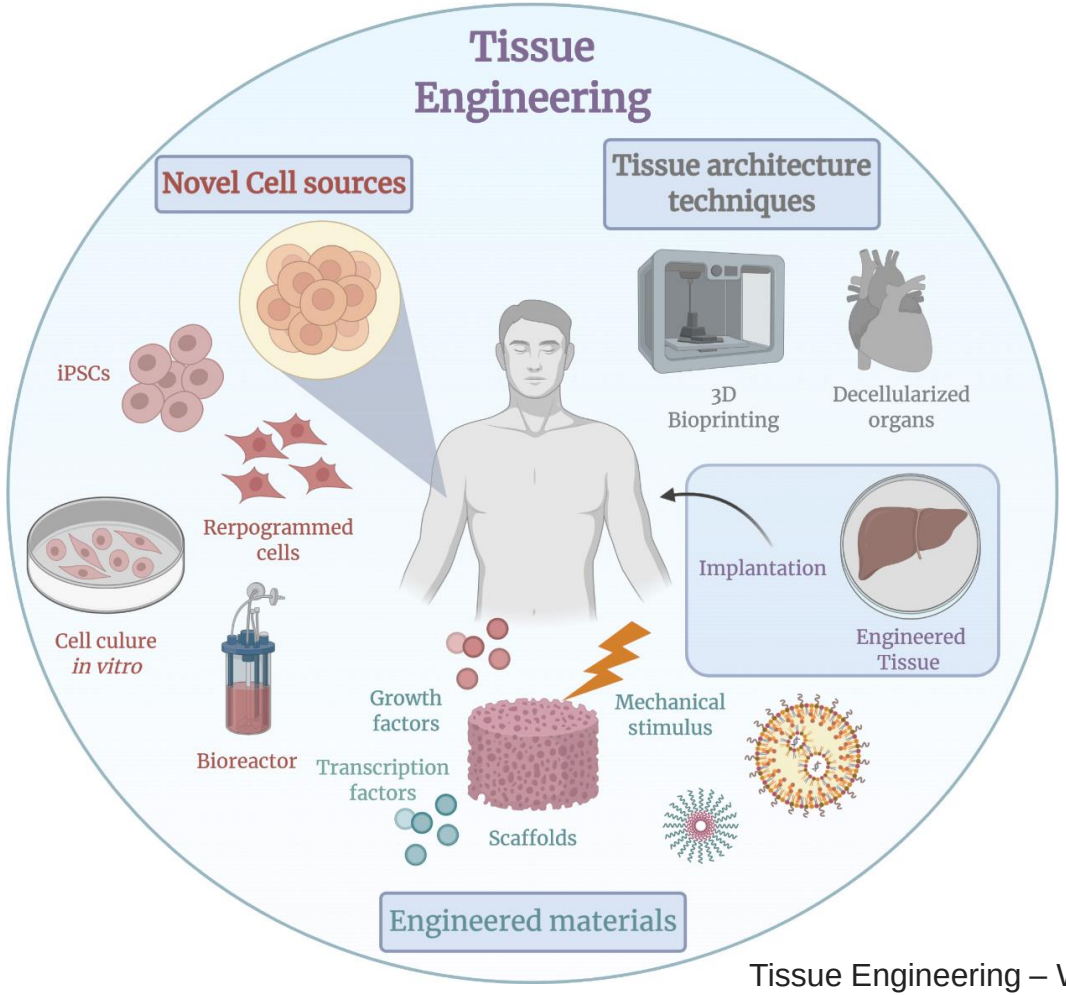
NSF CHRNS CENTER FOR HIGH RESOLUTION NEUTRON SCATTERING NIST

Tissue Engineering

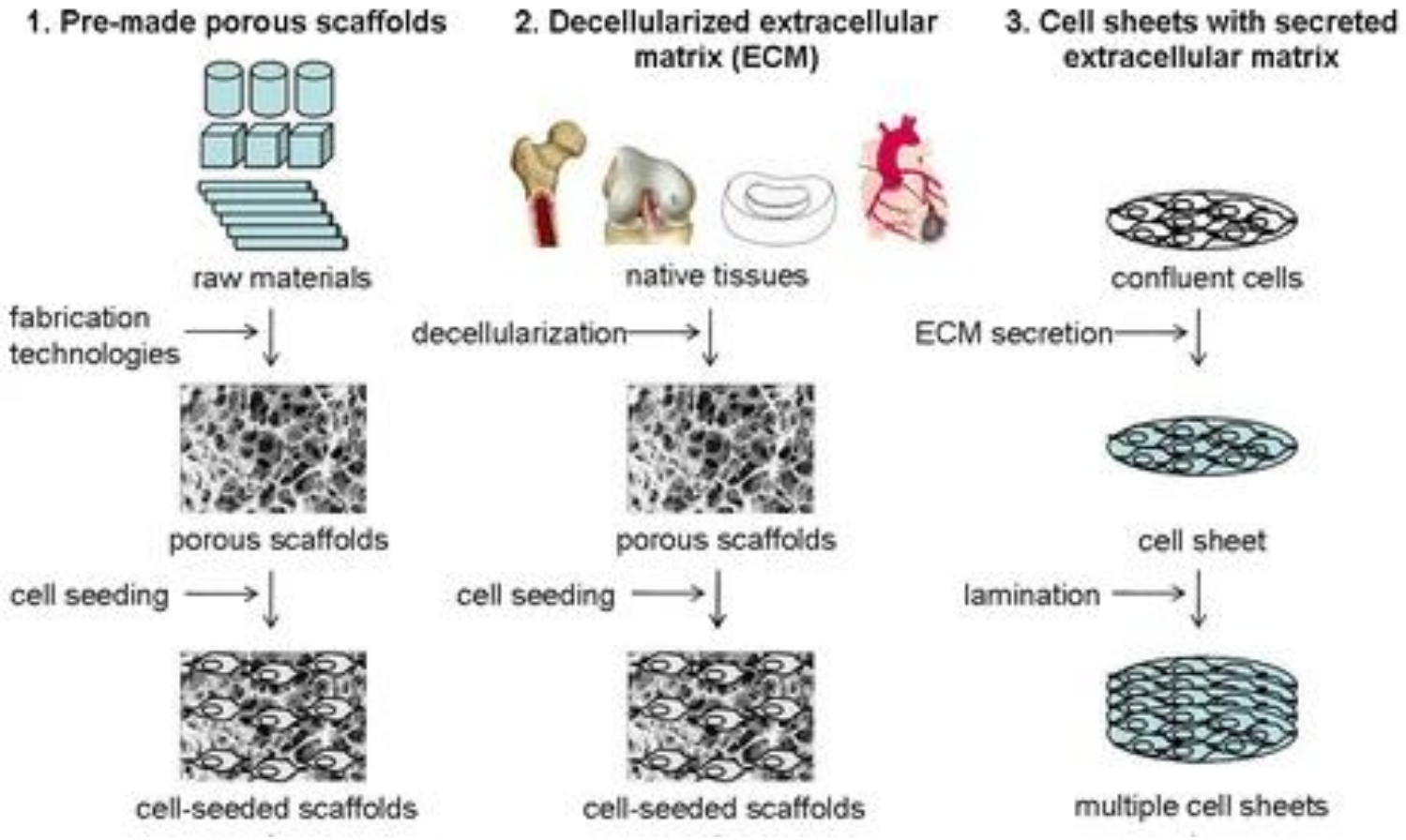
Tissue engineering relies on:

- **Scaffolds**
- **Repopulate cells**
- **Cell-growth stimuli factors**



Tissue Engineering – Wikipedia

Scaffolds



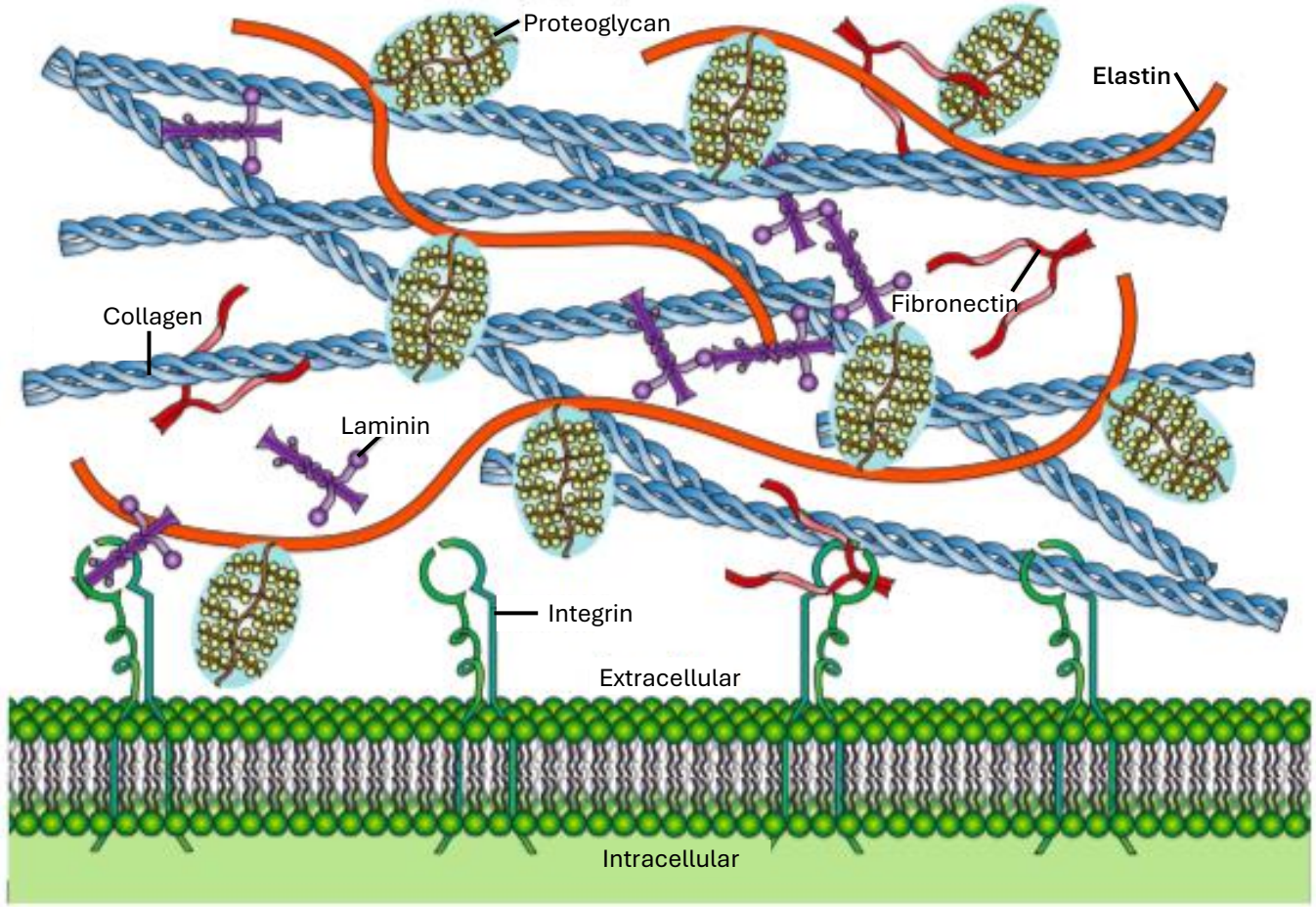
Chan, B.P. and Leong, K.W., 2008. Scaffolding in tissue engineering: general approaches and tissue-specific considerations. *Eur. Spine J. 17*, 467-479

Cellular Microenvironment and Healing

Extracellular Matrix (ECM)

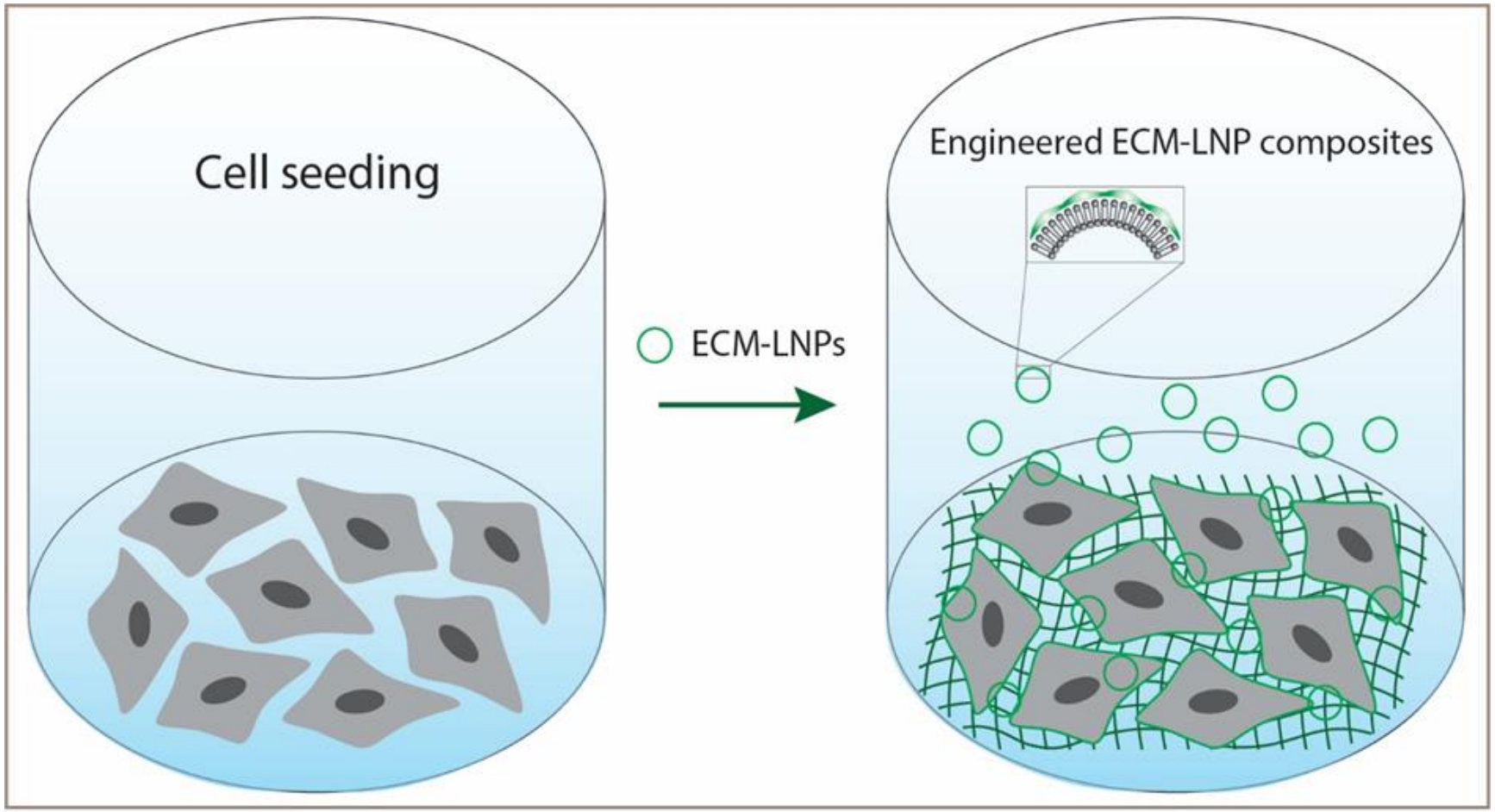
1. Basement Membrane
   a. Adhesive layer
   b. Migration and Adsorption
2. Interstitial Matrix
   a. Structural basis and support
   b. Mechanical Properties

Elastin, Fibronectin, Collagen, Laminin

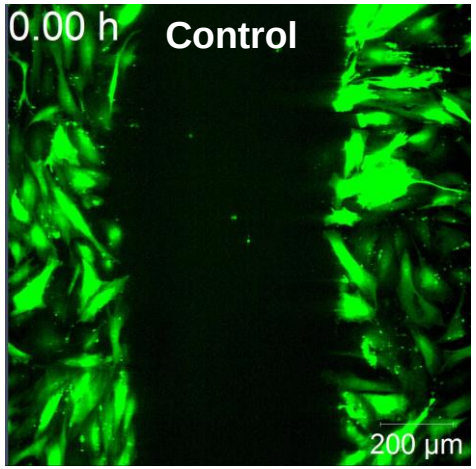


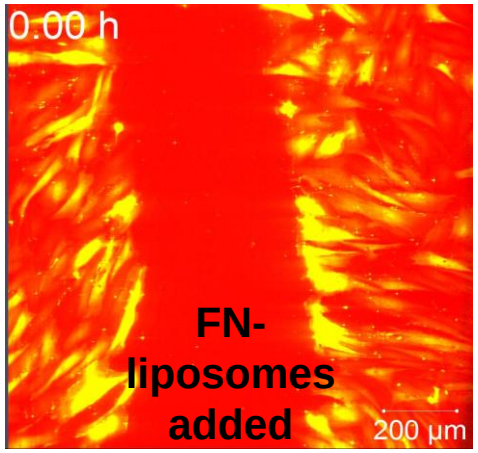
https://www.myopainseminars.com/resources/blog/the-extracellular-matrix-you-dont-say/

Pre-Scaffold and Approach



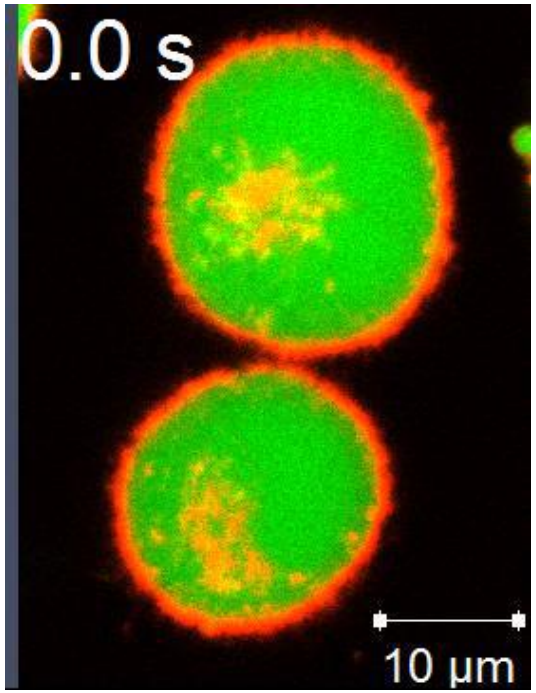
Influence of ECM Proteins on Bio membranes

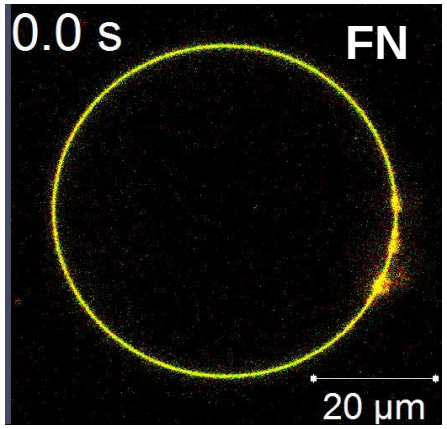


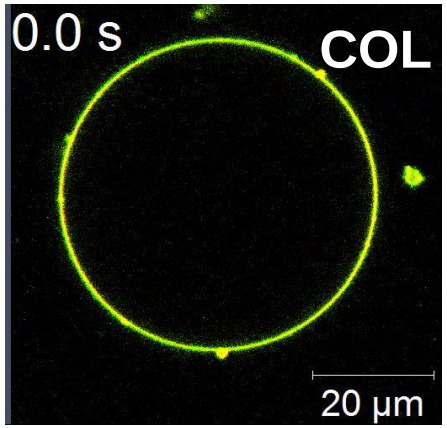


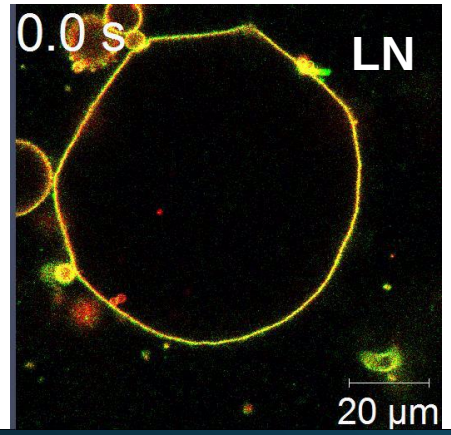
ECM proteins speed up wound healing, migration, and growth

ECM proteins have different roles in mechanical properties and membrane structure



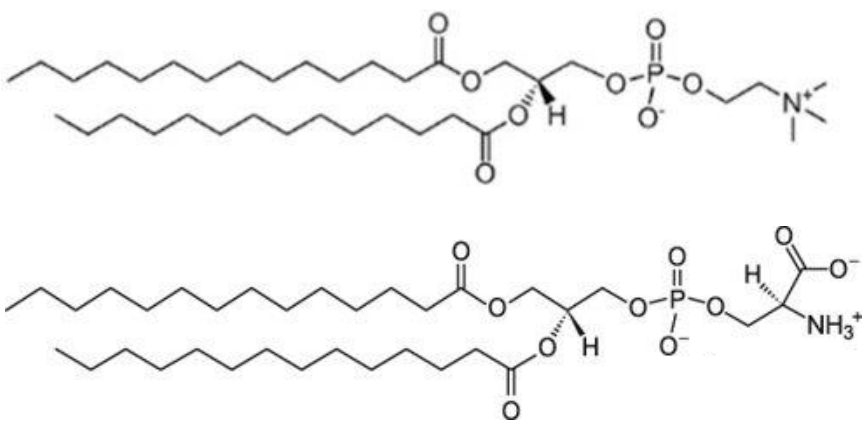


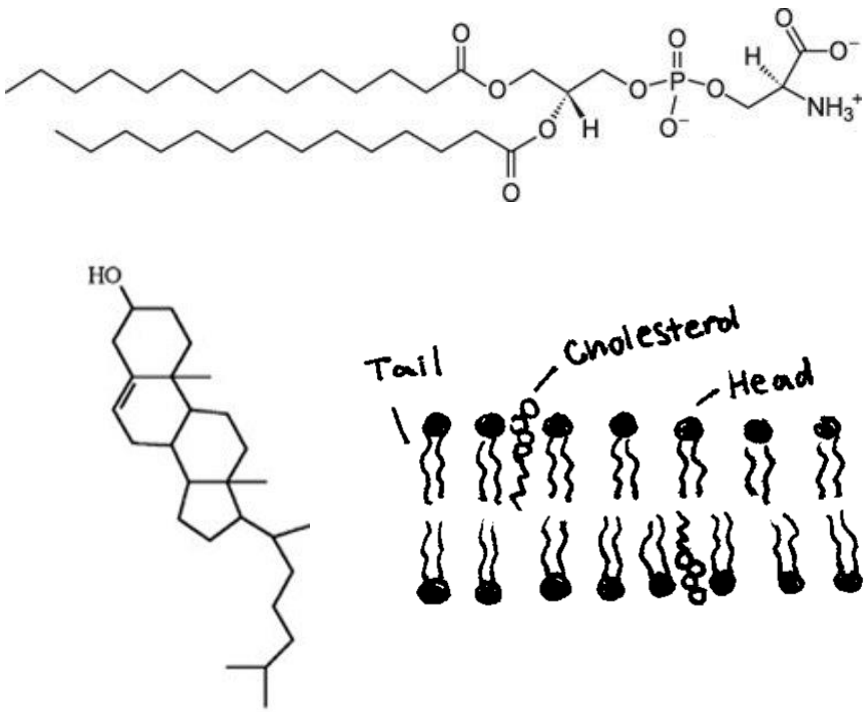




What are the effects of each protein?

Materials and Methods





Buffer: 0.05 M Tris-HCl at 7.2 pH

Lipid Films:

1. (14:0) DMPC + (14:0) DMPS [4:1]
2. (14:0) DMPC + (14:0) DMPS + Cholesterol [4:1, 25 wt.%]

Proteins:

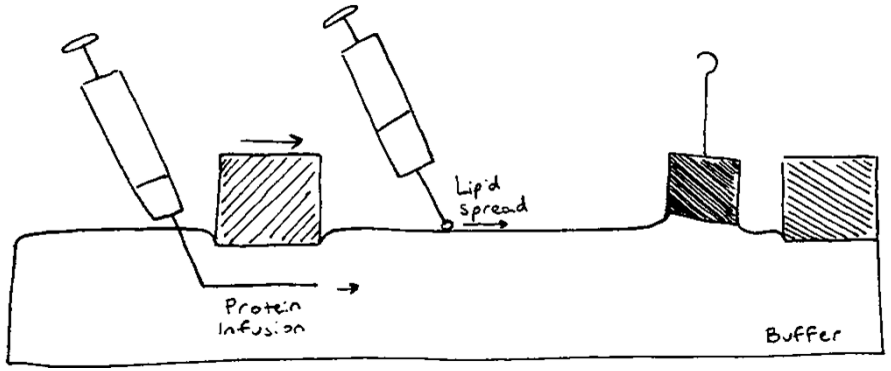
- Elastin
- Fibronectin
- Collagen
- Fibronectin + Elastin
- Fibronectin + Collagen
- Fibronectin + Elastin + Collagen

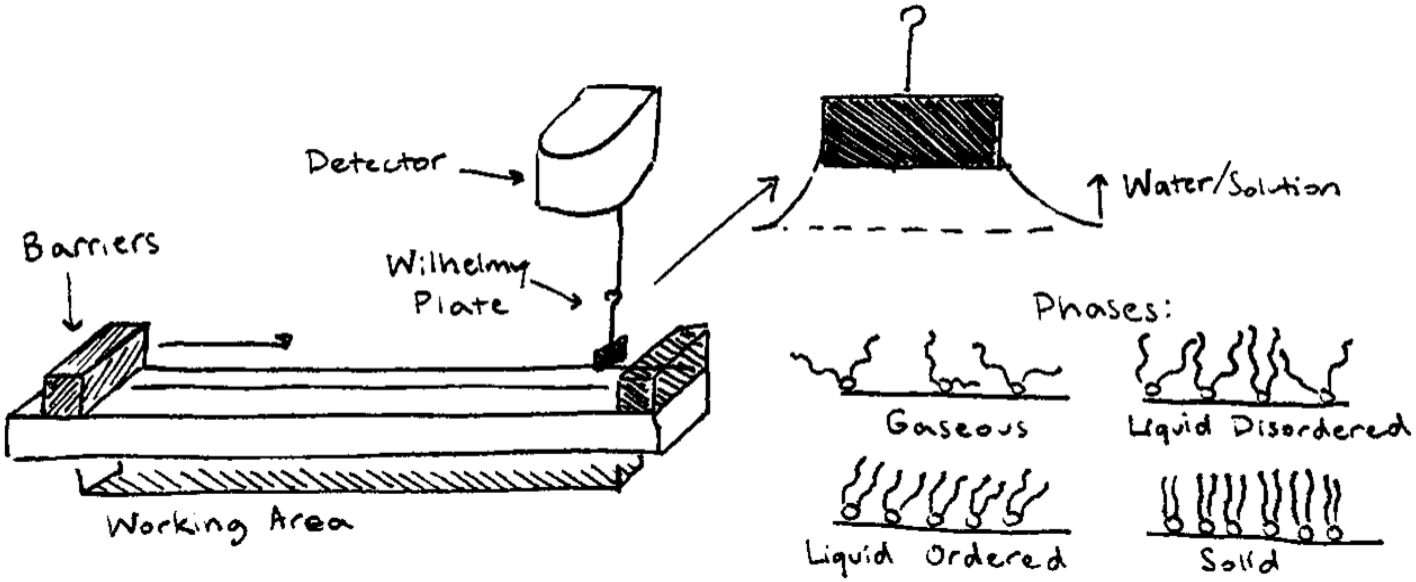
Techniques: Langmuir Isotherm and X-ray Reflectometry

Langmuir Isotherm

$$\pi = \gamma_\circ - \gamma_s$$
$$\gamma_\circ = 0$$
$$\gamma_s < 0$$
$$C_s^{-1} = [(-\frac{1}{A_\pi})*(\frac{dA}{d\pi})]^{-1}$$

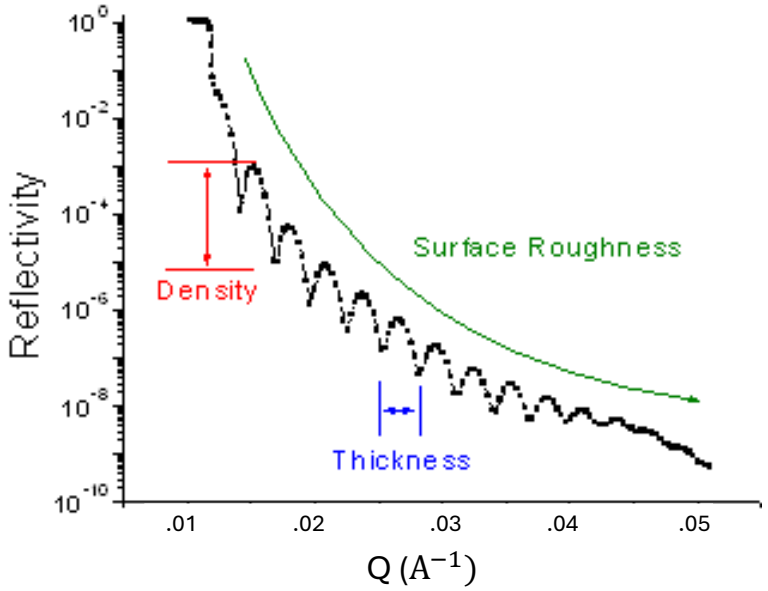
- Investigation of two-dimensional structure and interfacial elasticity of compression
- Shows adsorptions and changes in mechanical properties

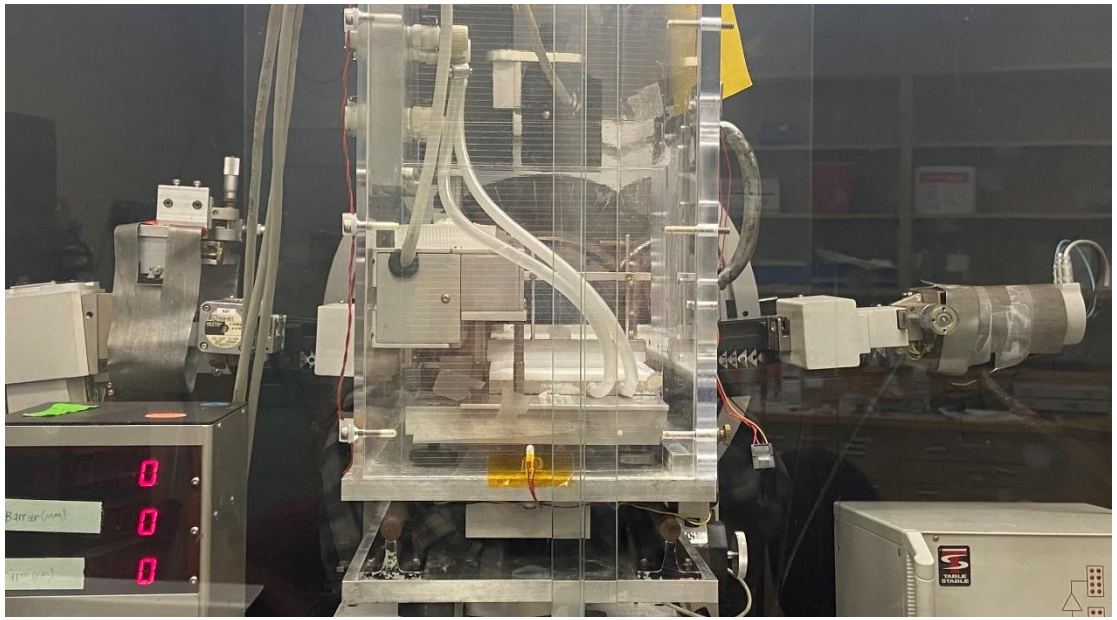


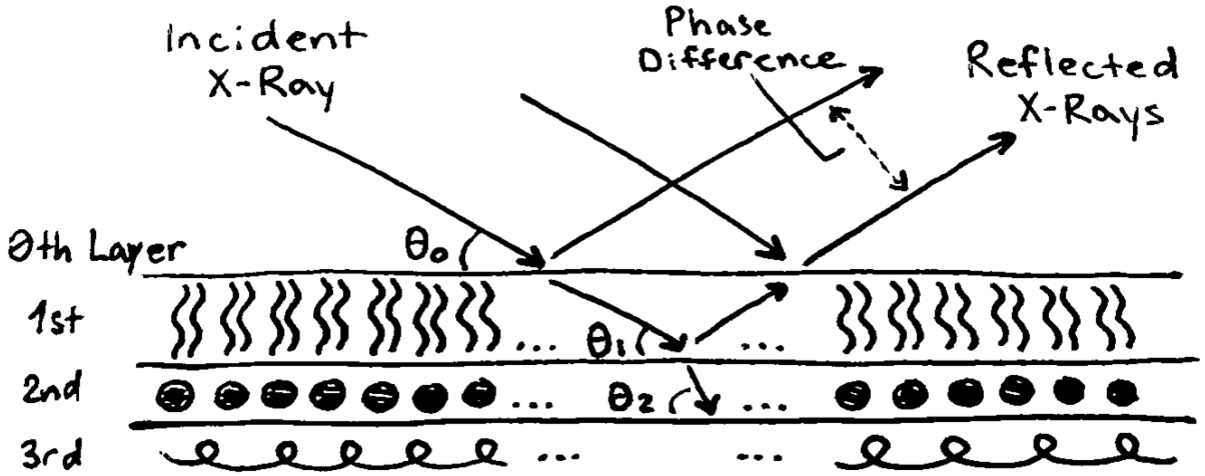


X-Ray Reflectometry

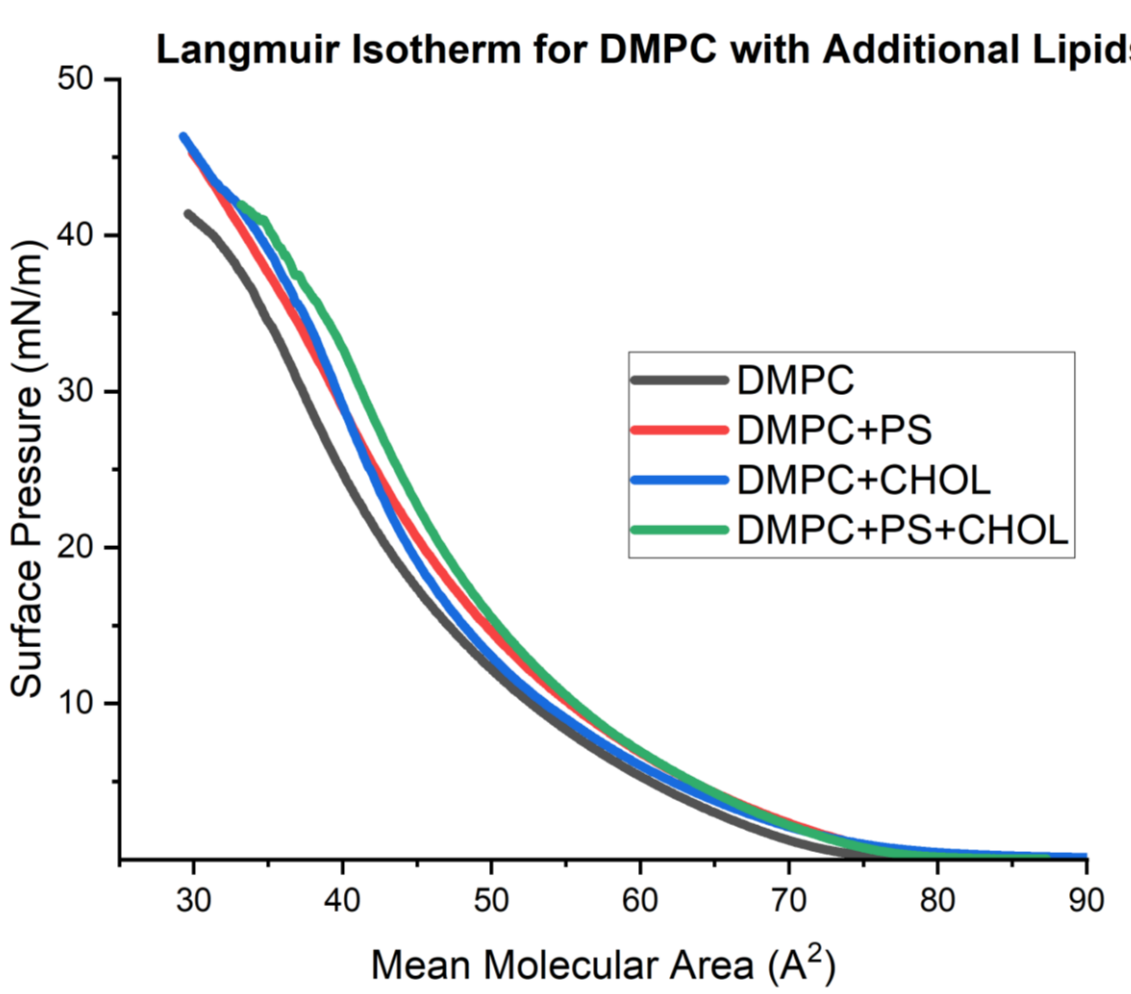
- Investigation of the third dimension through layers
- Shows the incorporation of protein onto film and structure development

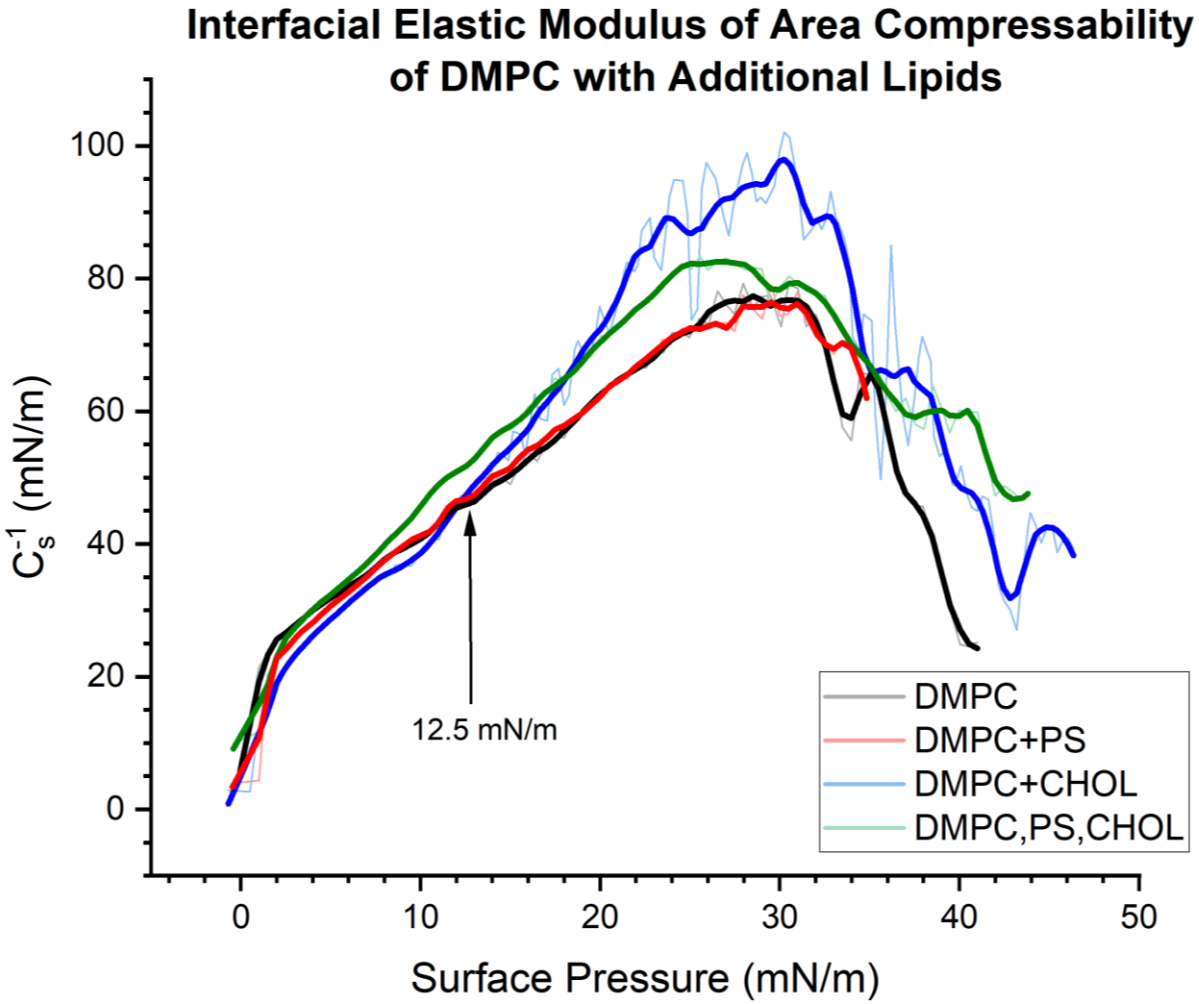






Stage 1: Bare Membrane





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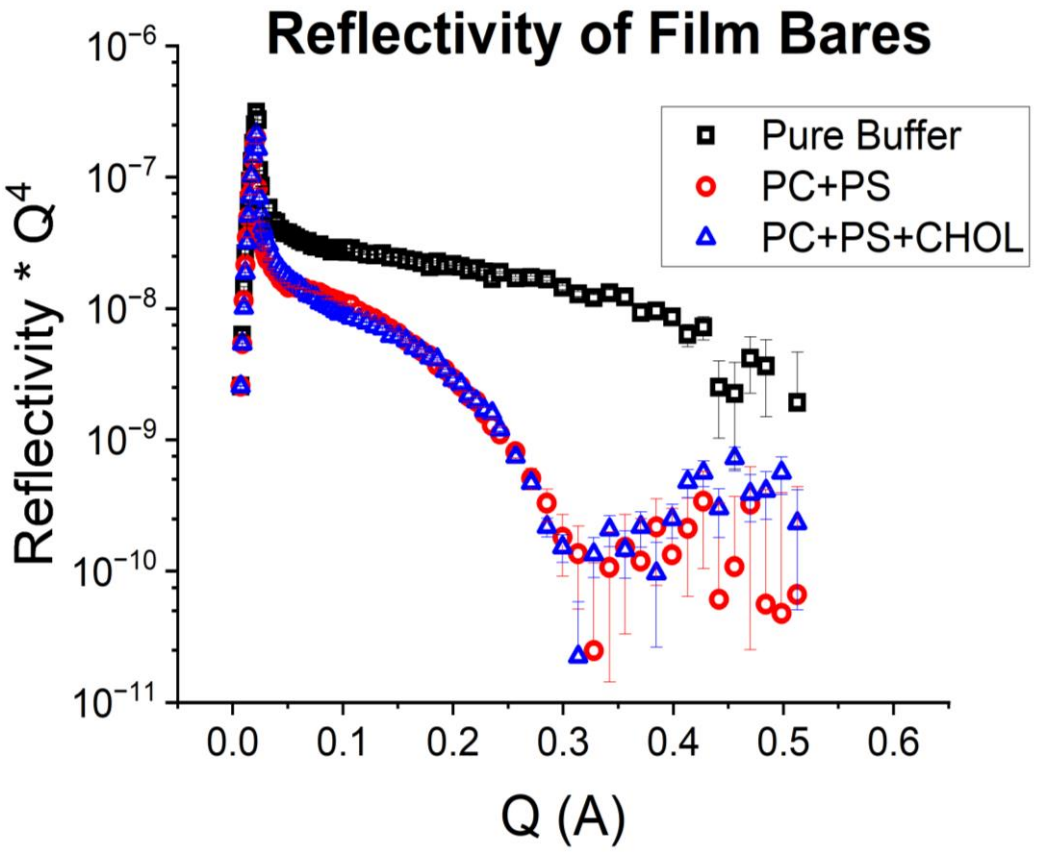
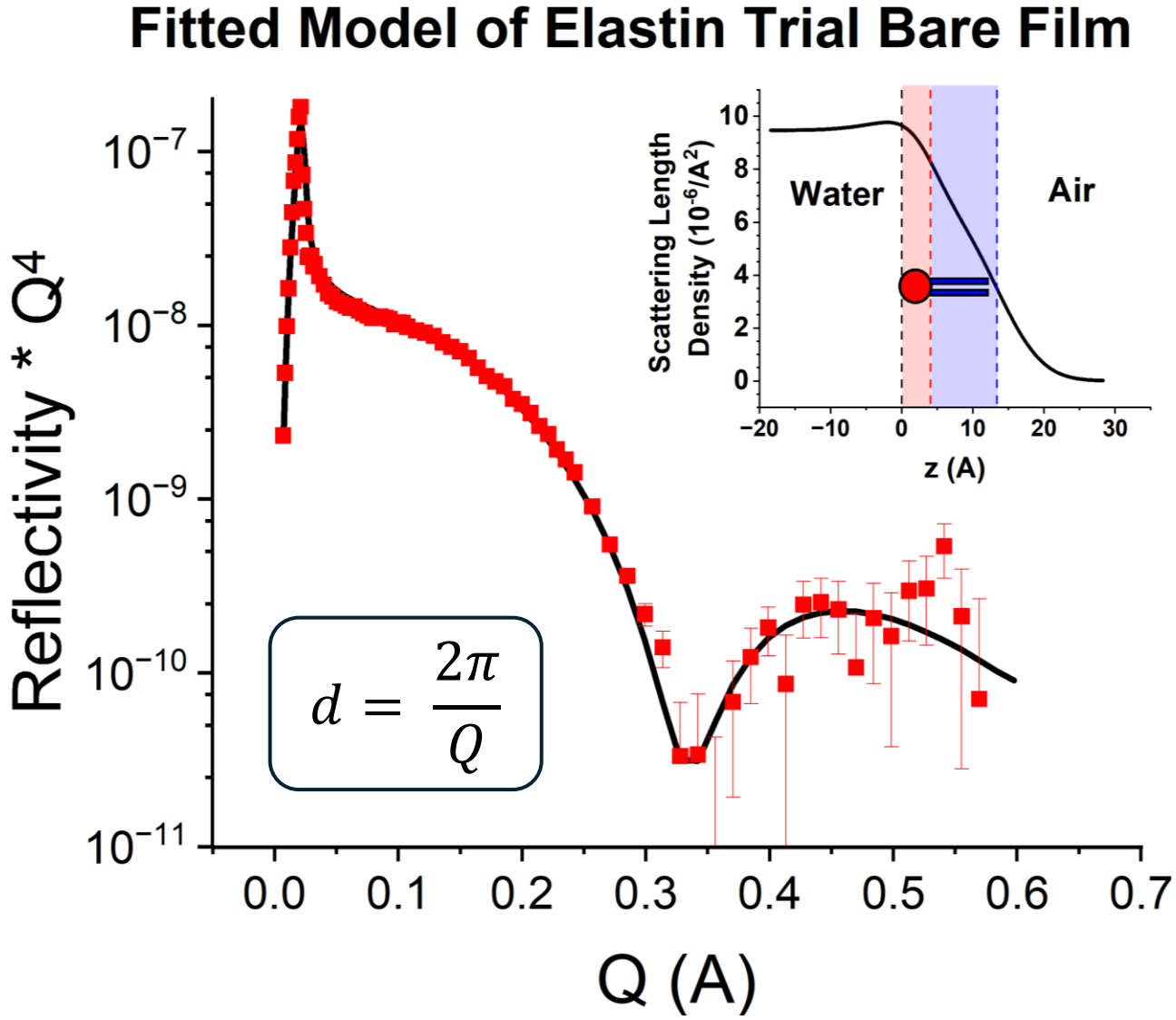
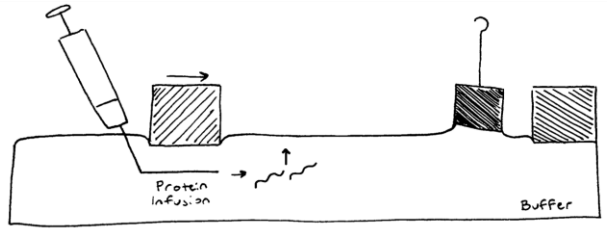
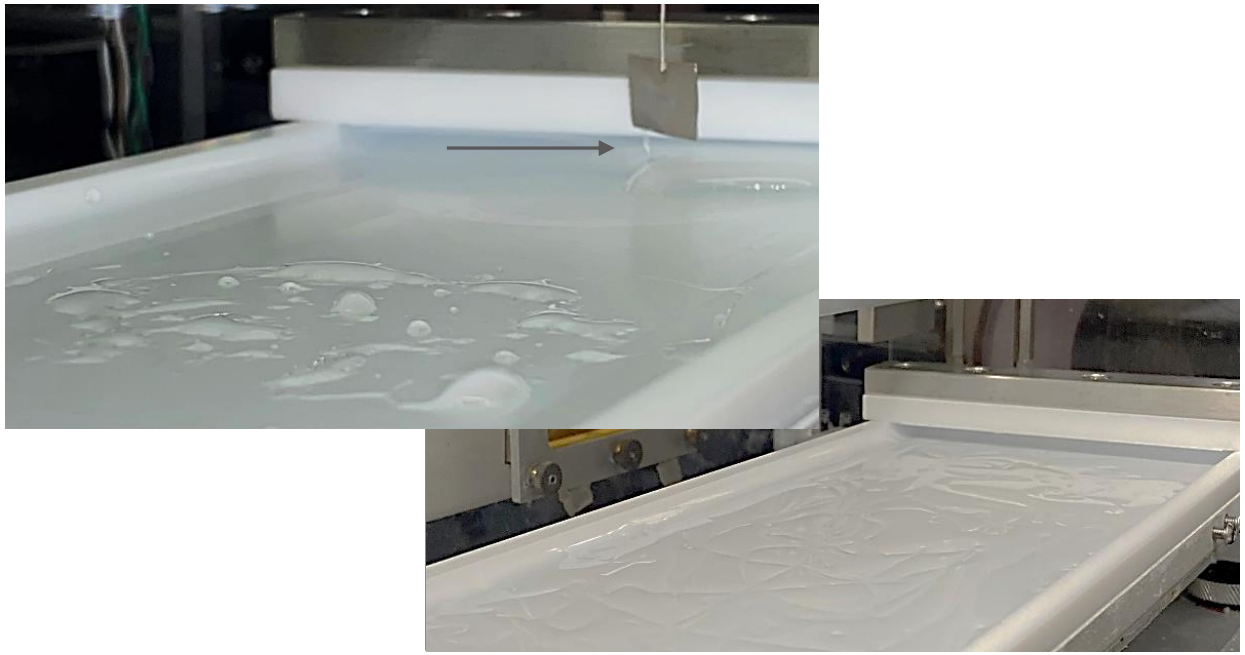


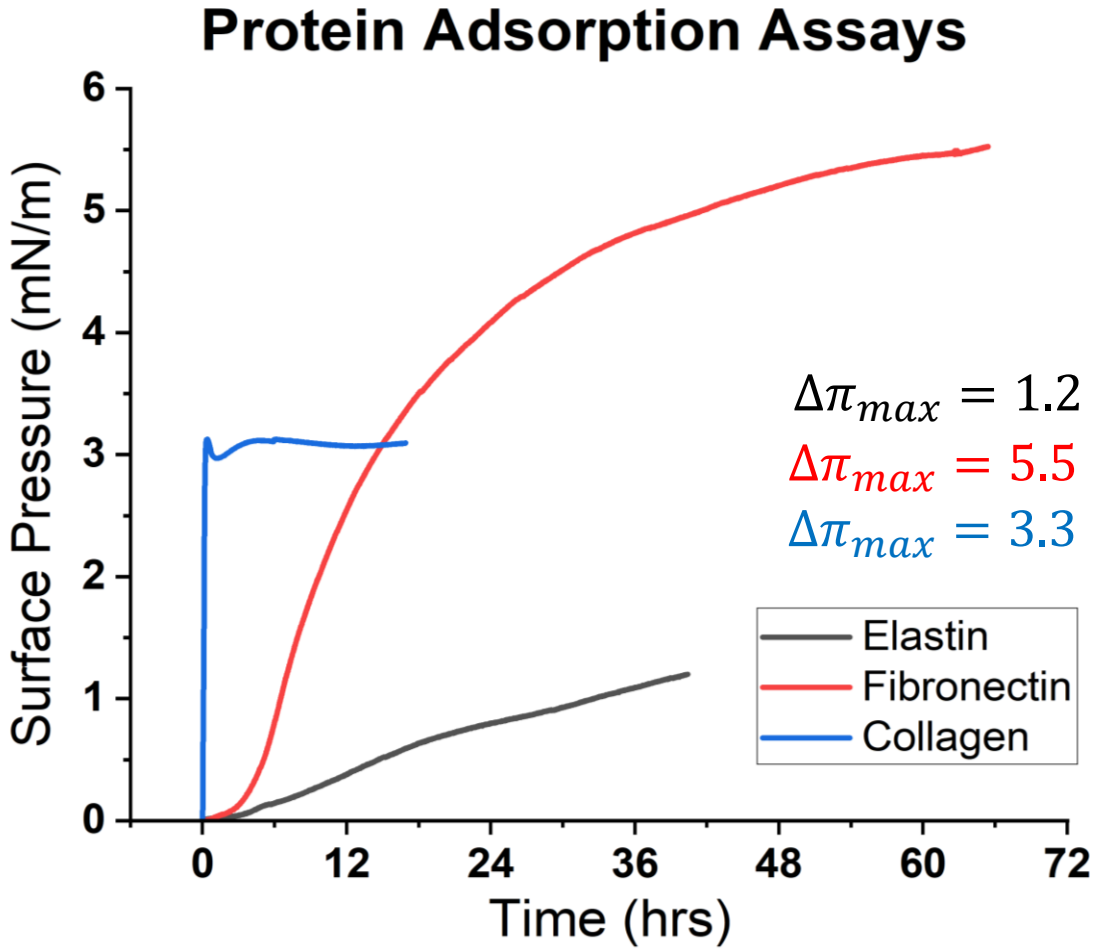


Stage 2: Adsorption



Adsorption Magnitude and Speed varies from protein to protein





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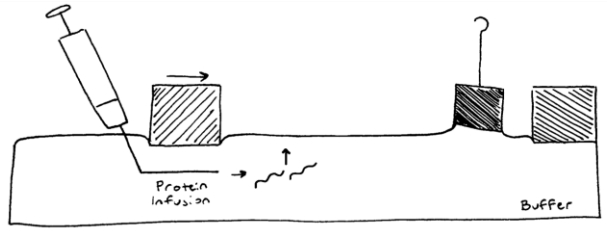
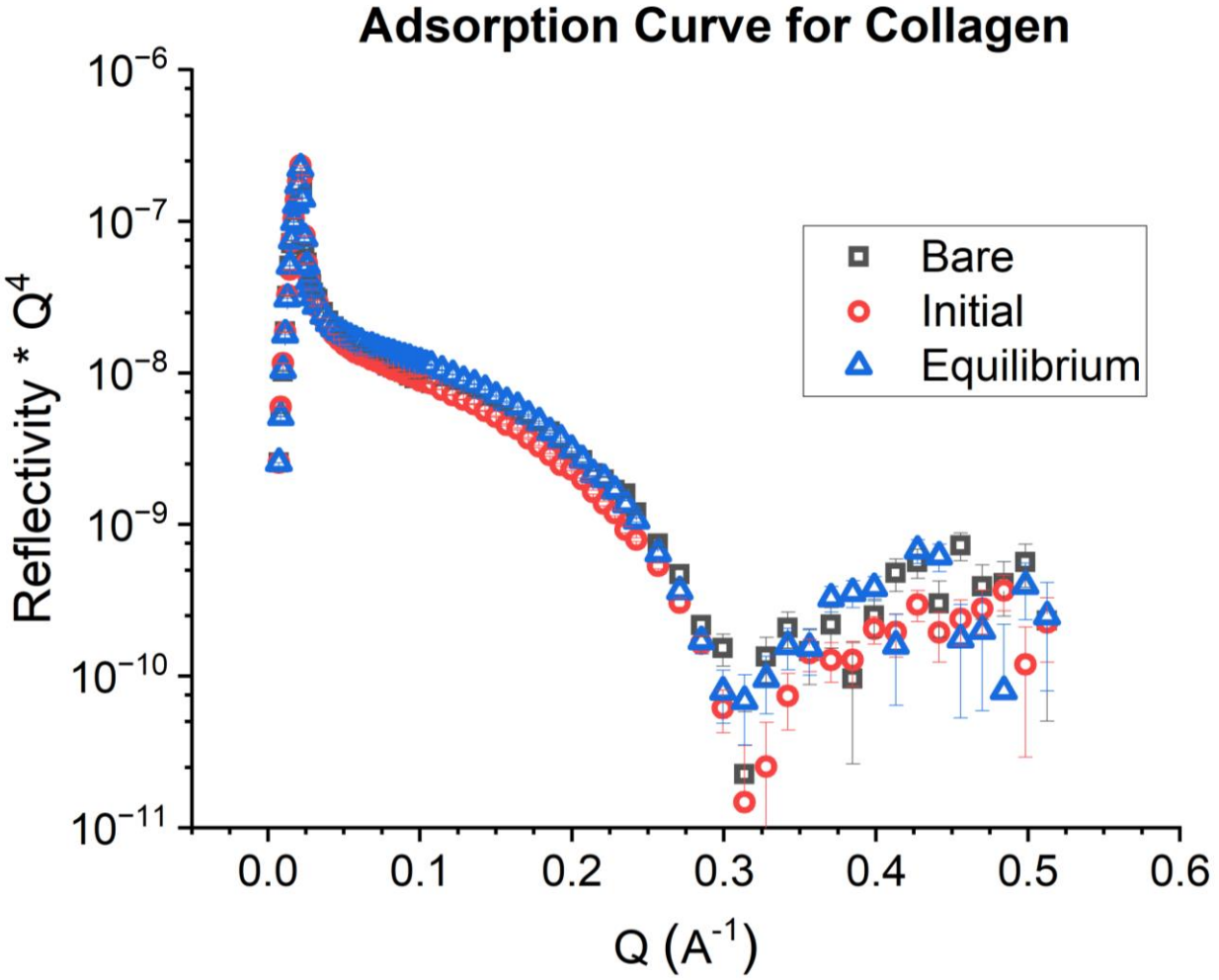
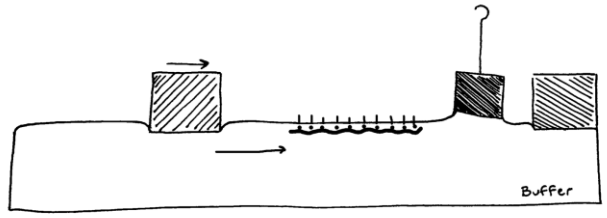


Collagen

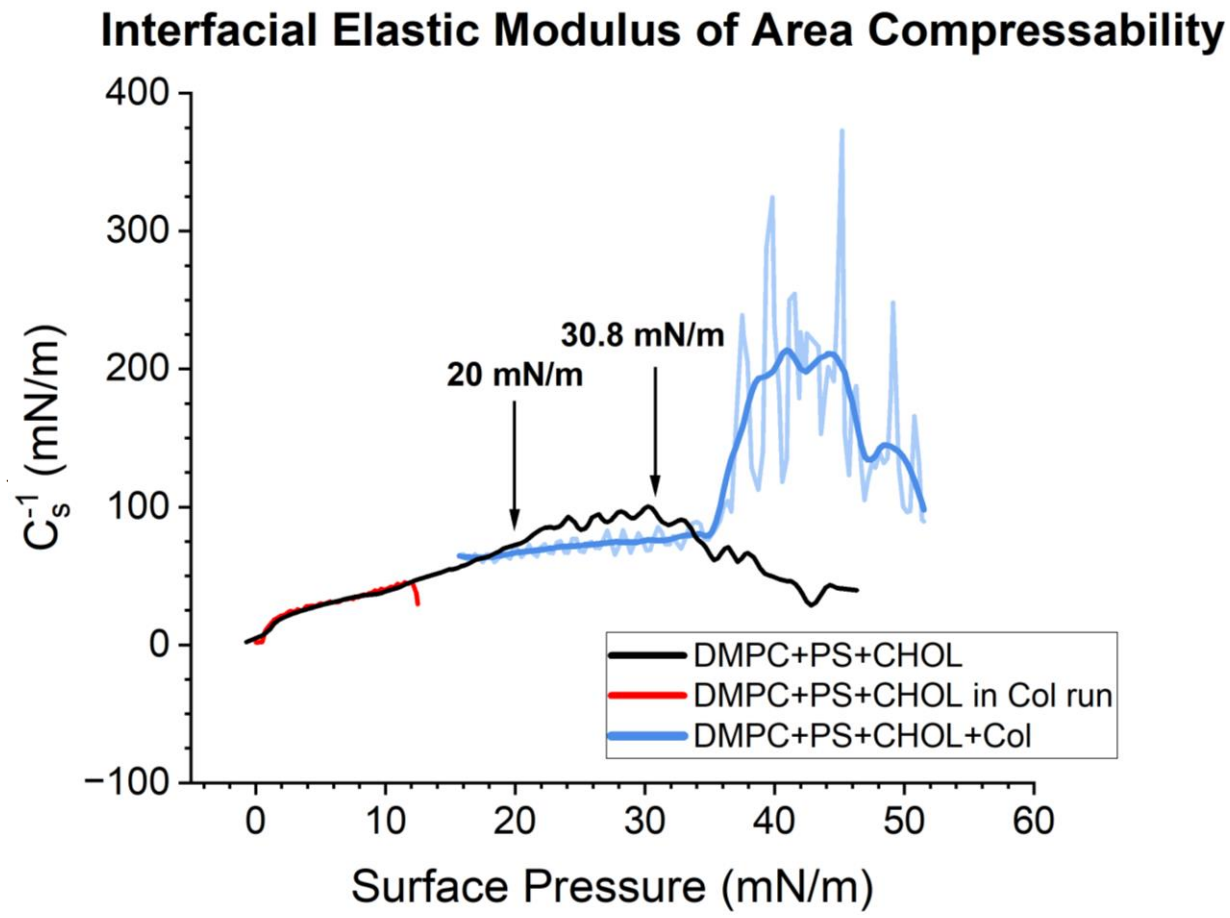


$$d = \frac{2\pi}{Q}$$

Stage 3: Compression

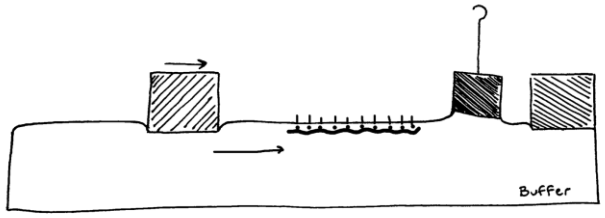


Collagen

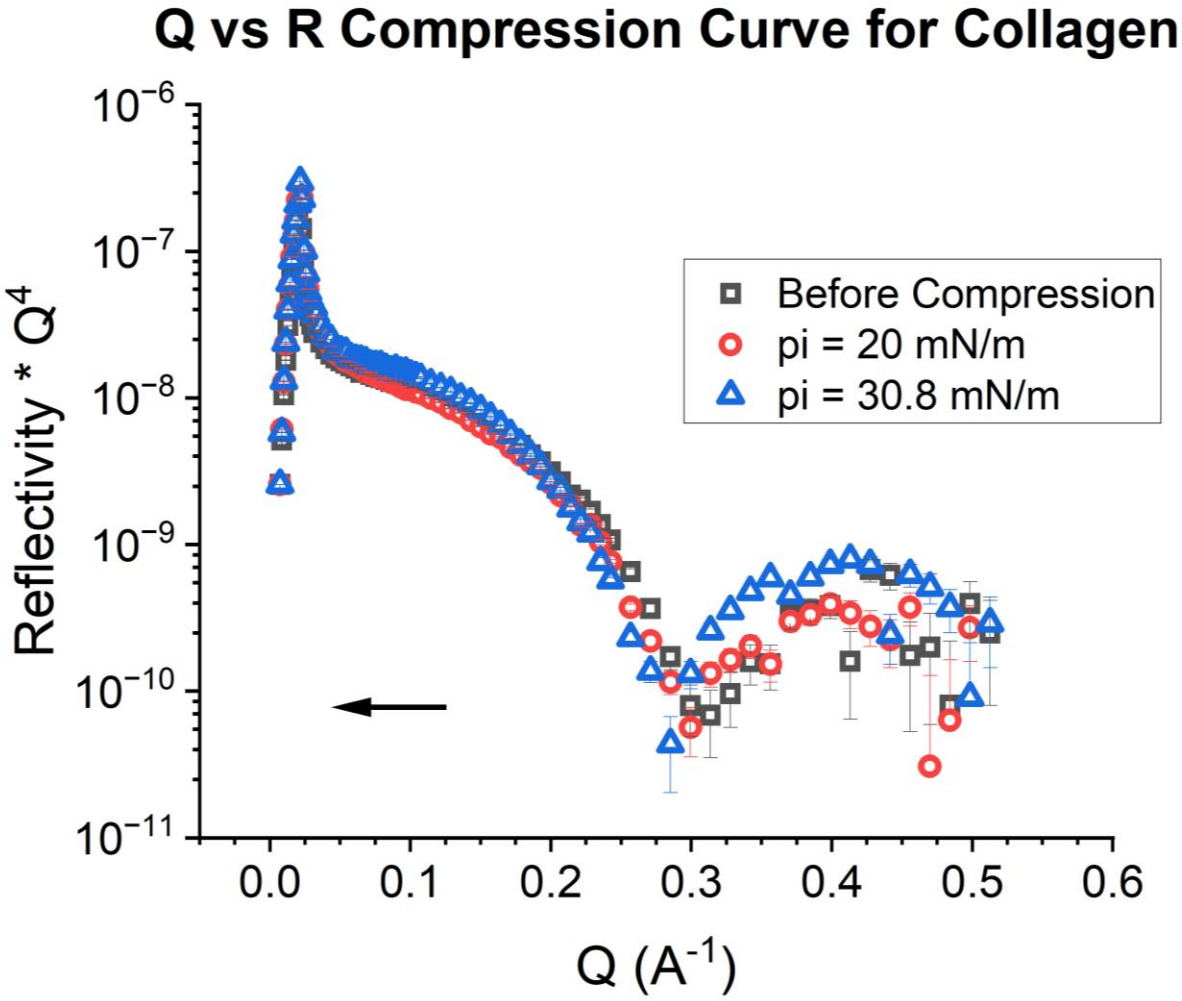


- Most significant change in Modulus
- Very strong after 30.8

Stage 3: Compression

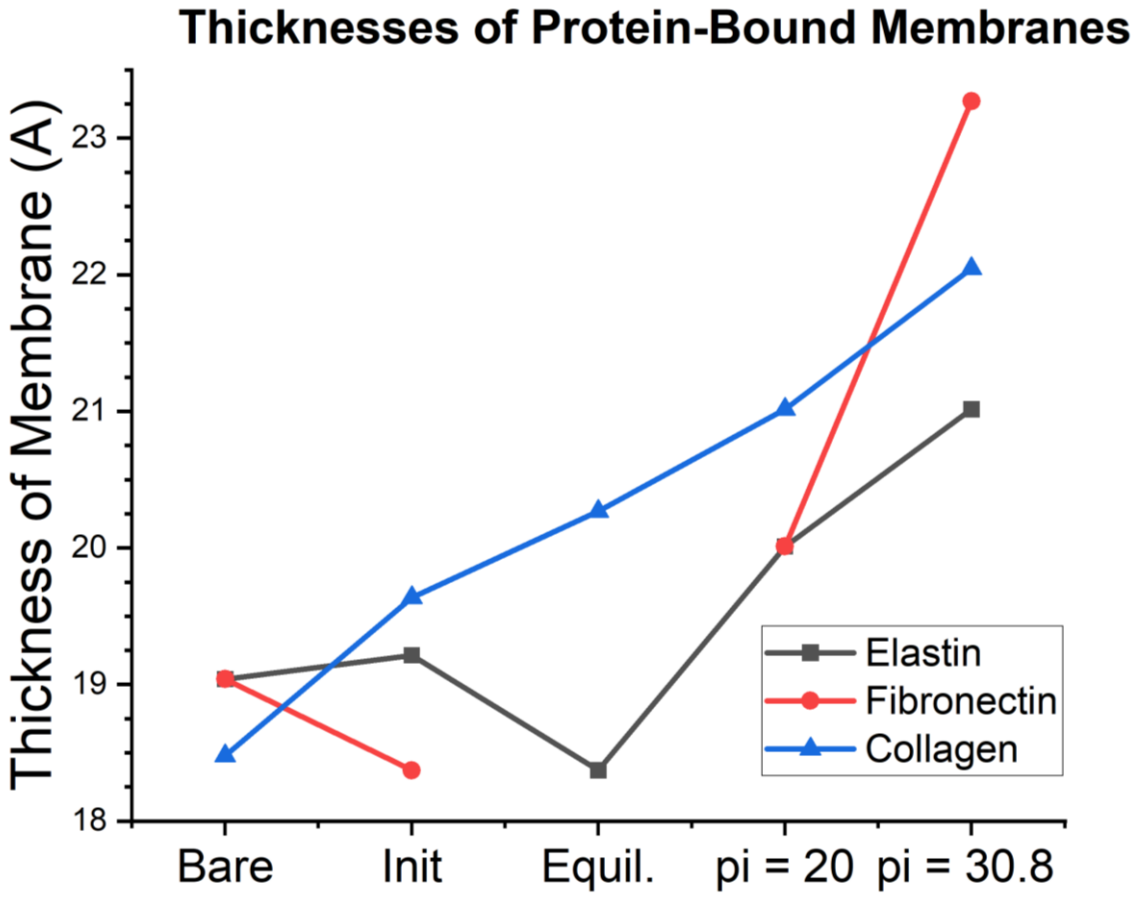


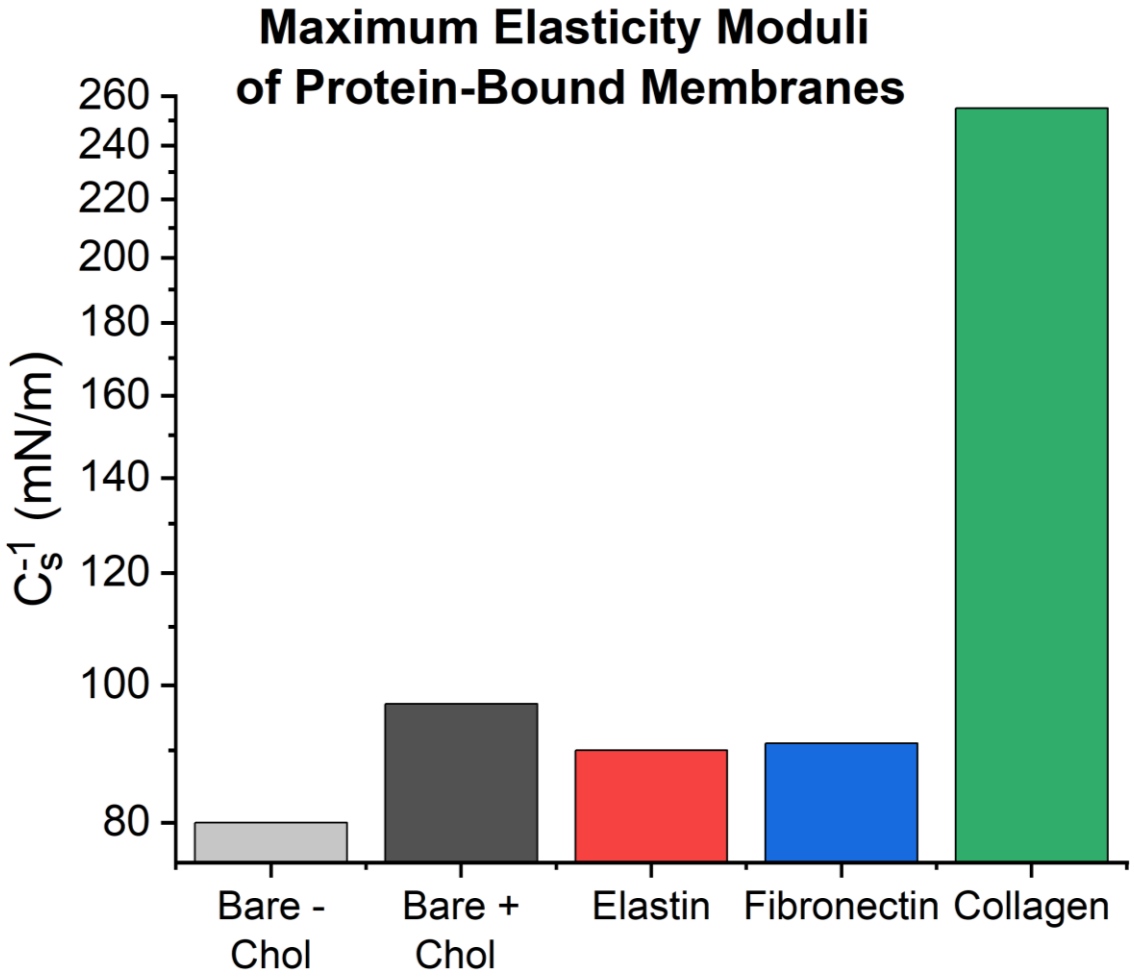
Collagen



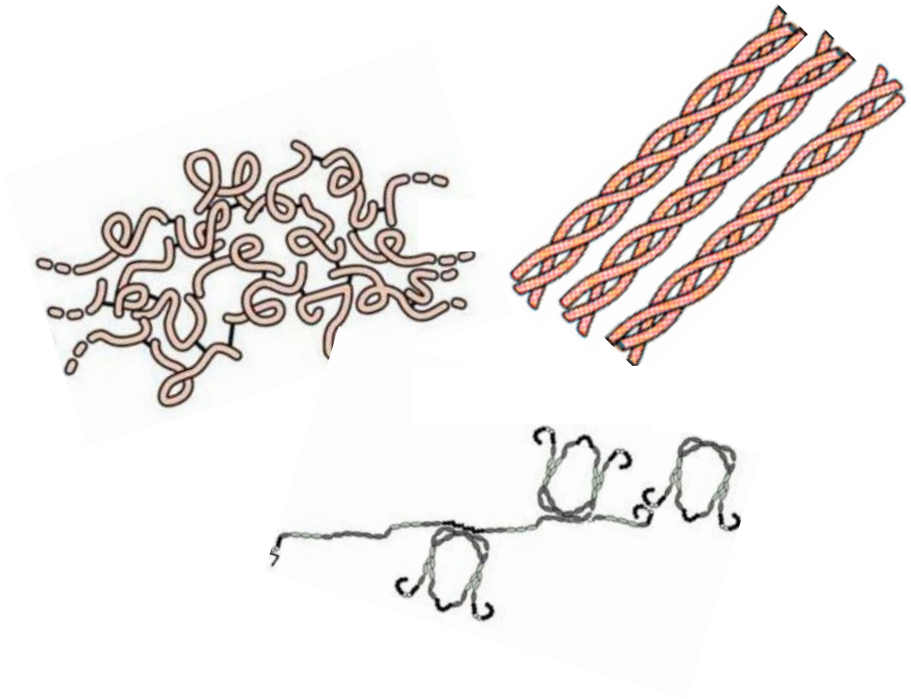
$$d = \frac{2\pi}{Q}$$

Conclusions





Conclusions



Elastin:

1. Poor interaction, minor adsorption
2. Softens membrane (elastic property in ECM)

Fibronectin:

1. Strong interaction, uniform distribution, forms greater structure
2. At physiological pressure, strengthens membrane

Collagen:

1. Moderate adsorption, distributes uniformly
2. Strengthens the membrane significantly (strength in ECM)

Future Work

What is left to do?

Binary and Tertiary Systems

Future plans and applications?

- Find best composition of lipids and proteins
- Replace lipid monolayers -> Liposomes
- What cell interactions are there, can we manipulate cells?

- Biophysical Society Conference 2025

Acknowledgements

NIST CENTER FOR NEUTRON RESEARCH

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Questions?

Veronica Ivanovskaya

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Supplementary Information

- Time constraints – XRR and Langmuir machines being broken
- Loss of data from interference with machines
- Difficulties with exact measurements and repeatability
  - Milli water change mid way
  - Scales being inaccurate
  - Lipid sensitivity to humidity and age of samples
  - Difficulty solubilizing protein in working solution