Characterizing Actin-Coated Lipid Membranes with Single-Molecule Imaging and Elasticity Measurements

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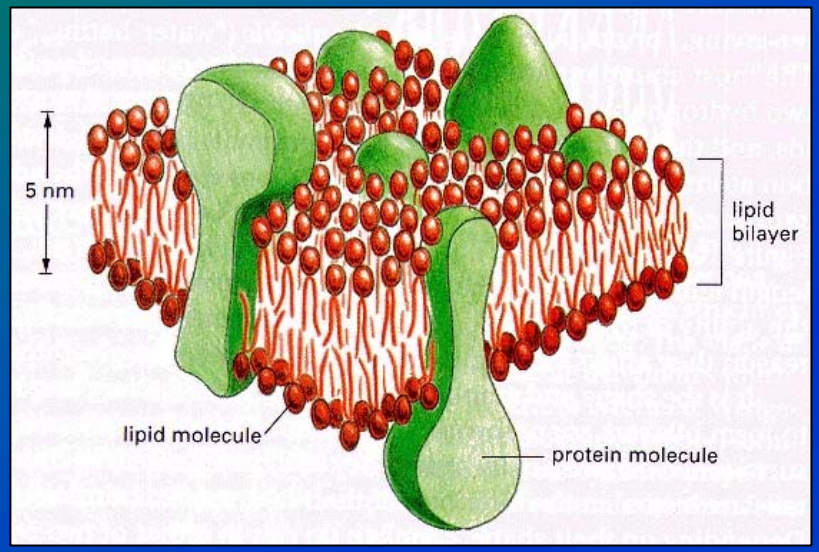
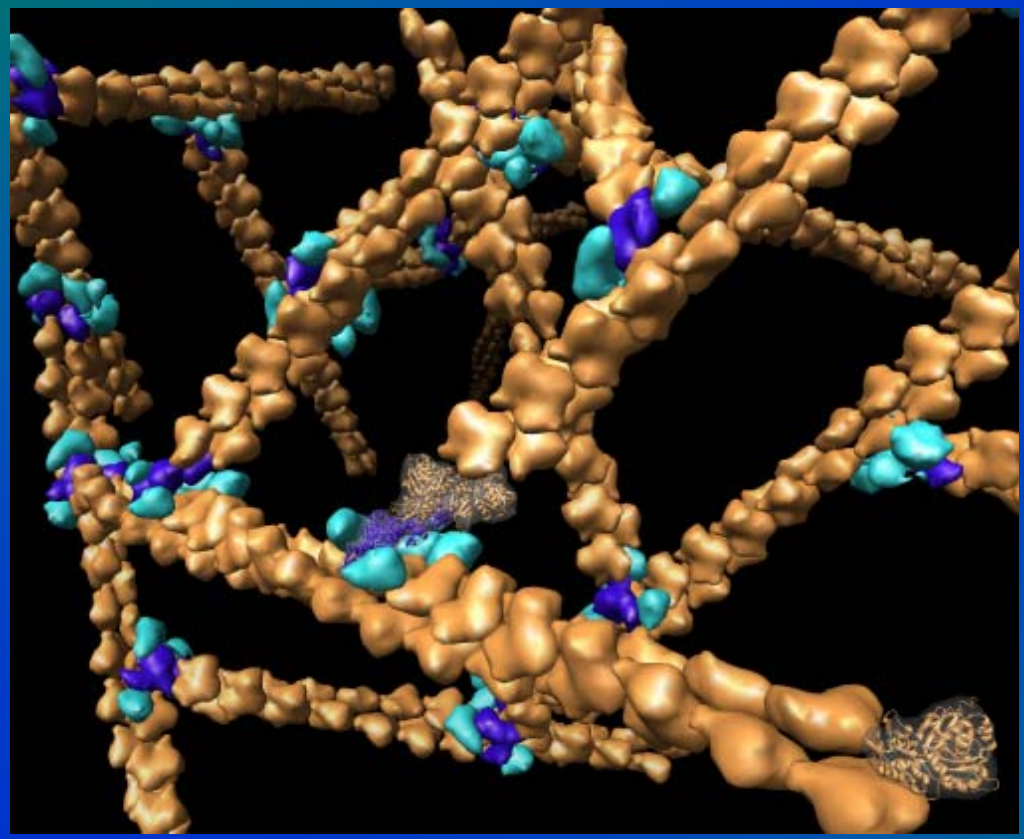
Bilayer Lipid Membranes (BLM)



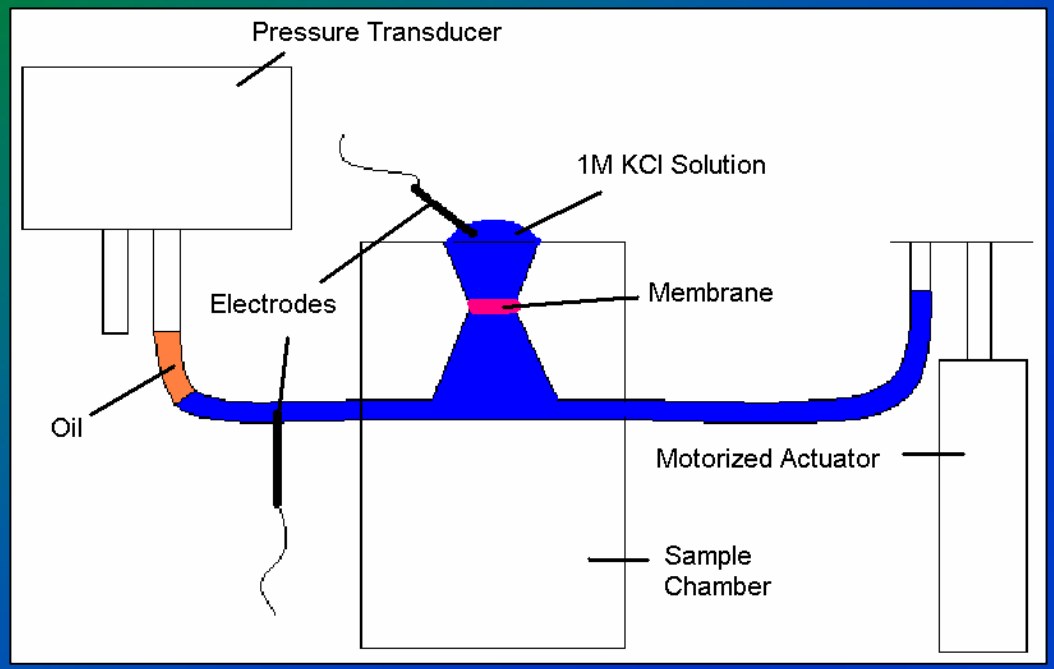
- Important subject of research in drug design, biophysics, biosensors, and nanotechnology
- Highly unstable and extremely fragile
- Natural cells possess a cytoskeletal structure consisting primarily of actin filaments and microtubules to protect the cell and prevent rupture

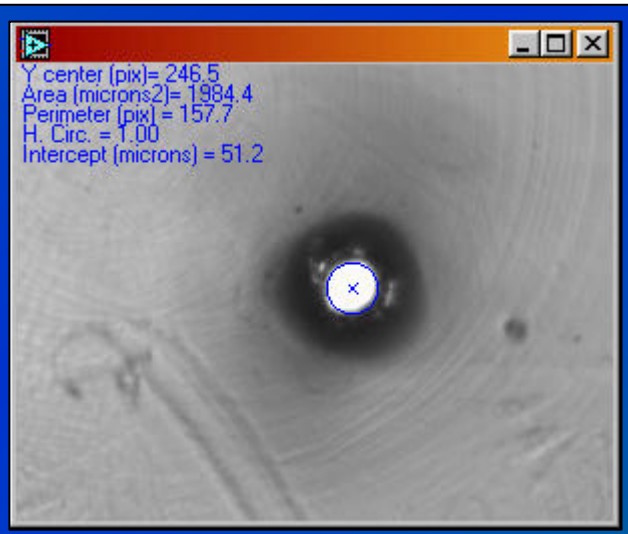
Cytoskeletal Structure



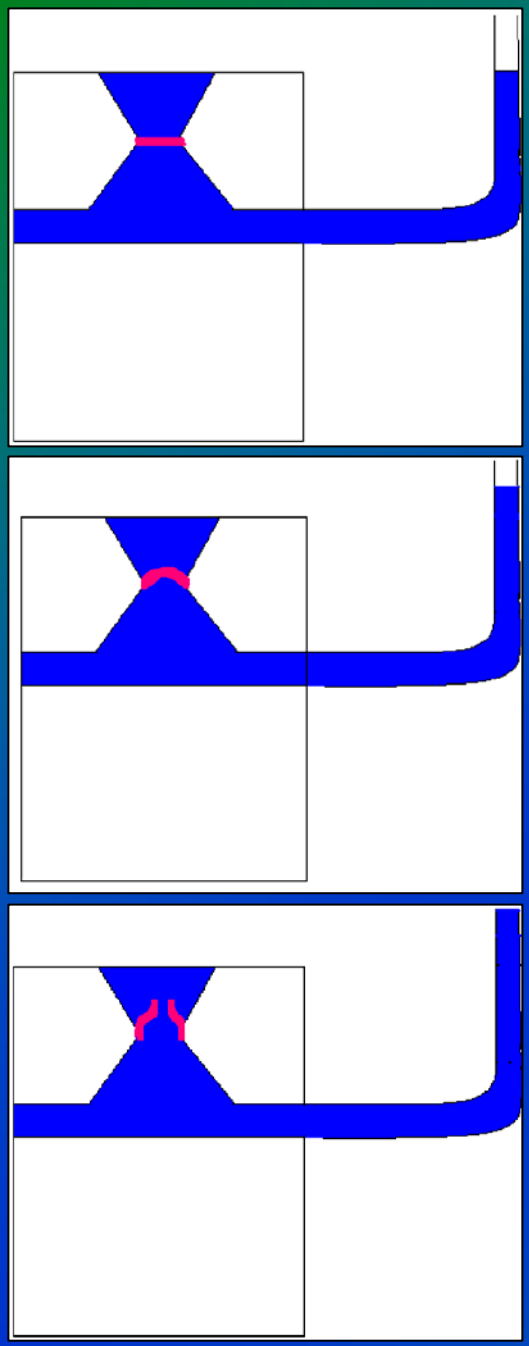
- Actin filaments and microtubules form a network around a cell
- Polymerize from monomer form

Formation of Artificial BLM





- Apply disassembled lipids in two steps onto an approximately 25μm circular aperture
- Use a pipet to blow bubbles across the aperture
- Lipids stick to air bubble and stretch like a soap film, depositing on far side of aperture

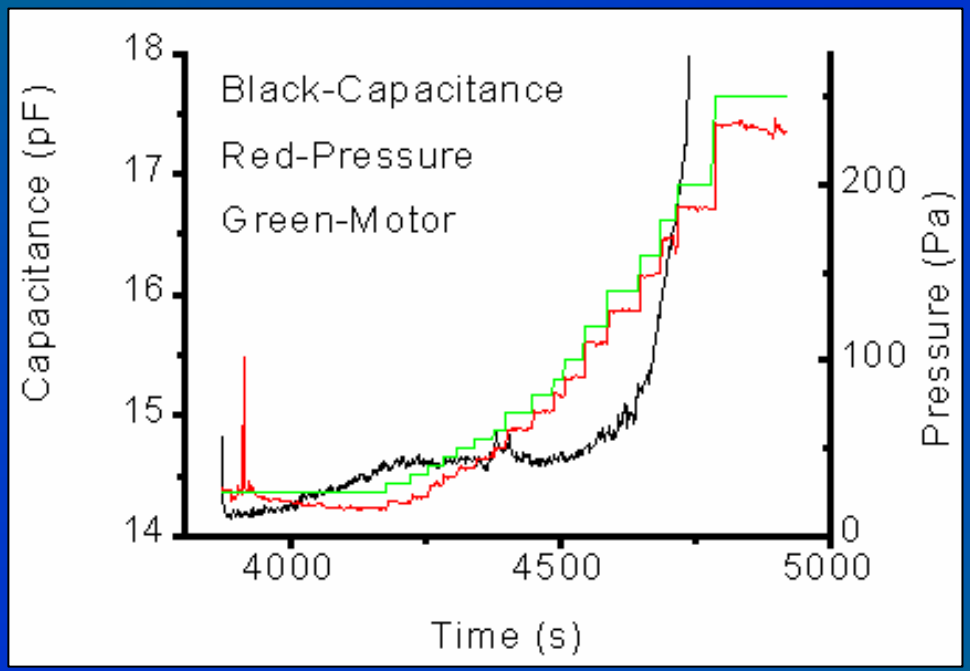


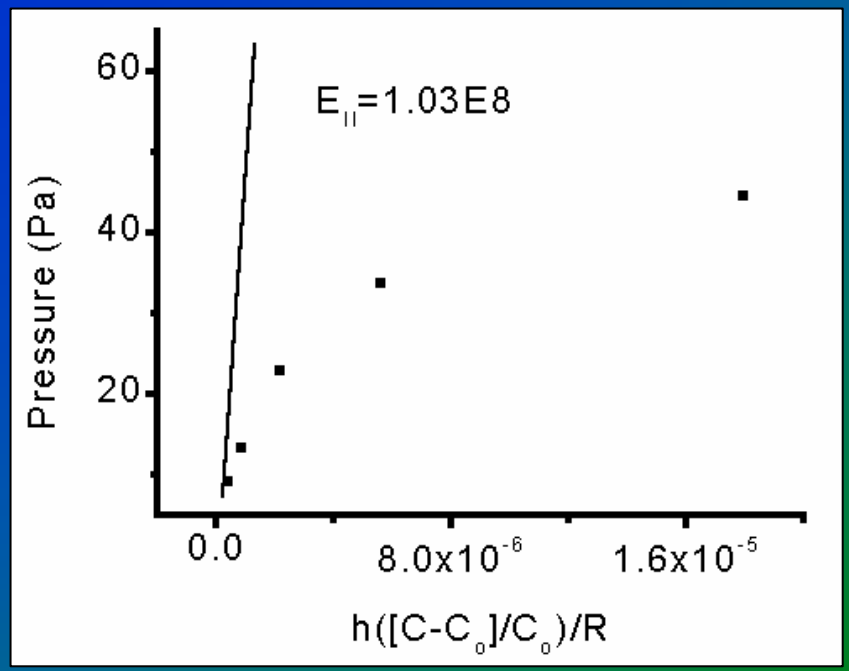
Experiments

- Change in area (bulging) of membrane was measured by calculating the capacitance of the membrane
  - C $\propto$ area of charge collecting surface/distance between surfaces
  - Thickness of the bilayer is constant (5nm), therefore the only quantity changing is the area, if C is changing

Data Analysis: Young's Elasticity Modulus

- $E_{||} = (P*R)/[h*(\Delta C/C)]$
- A measure of stress applied to the system divided by the strain response of the system (how much the membrane stretches when a given pressure is applied)
- Slope of line y=mx+b used to calculated $E_{||}$ where 'y' is pressure, 'm' is $E_{||}$ and 'x' is h[(ΔC/C)/R]; 'b' has no physical significance in calculating $E_{||}$
- A higher elasticity modulus means the membrane stretches less when a given pressure is applied





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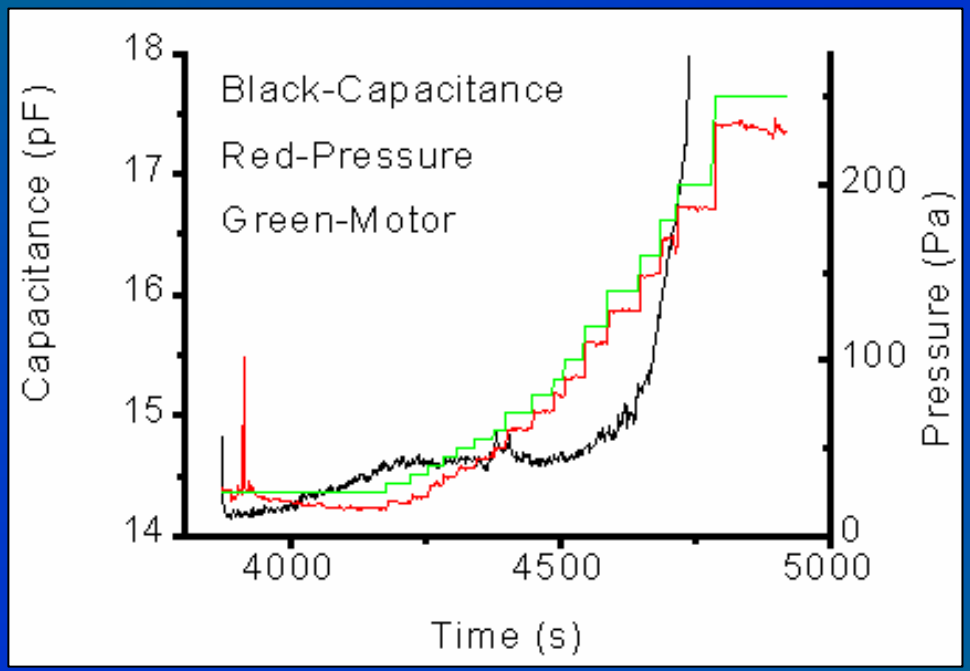

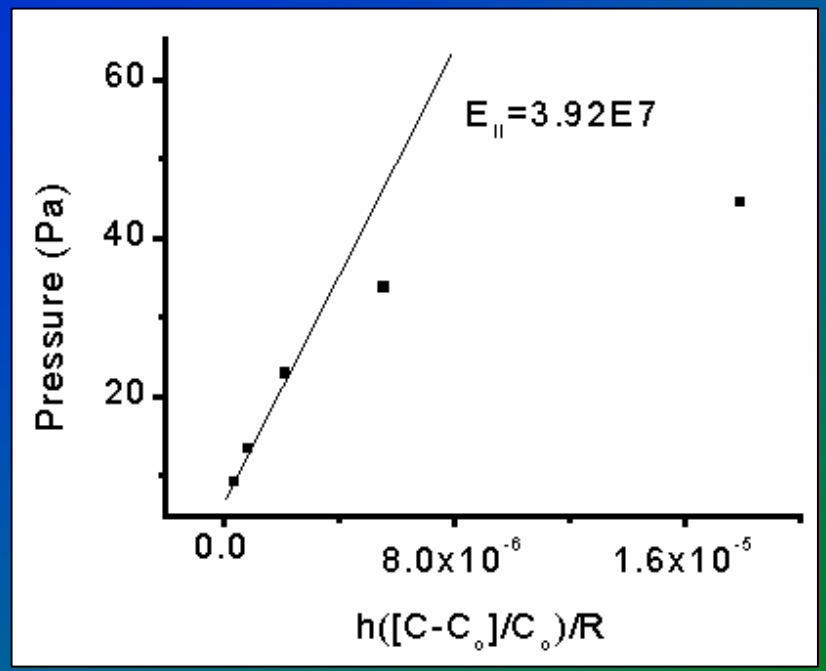

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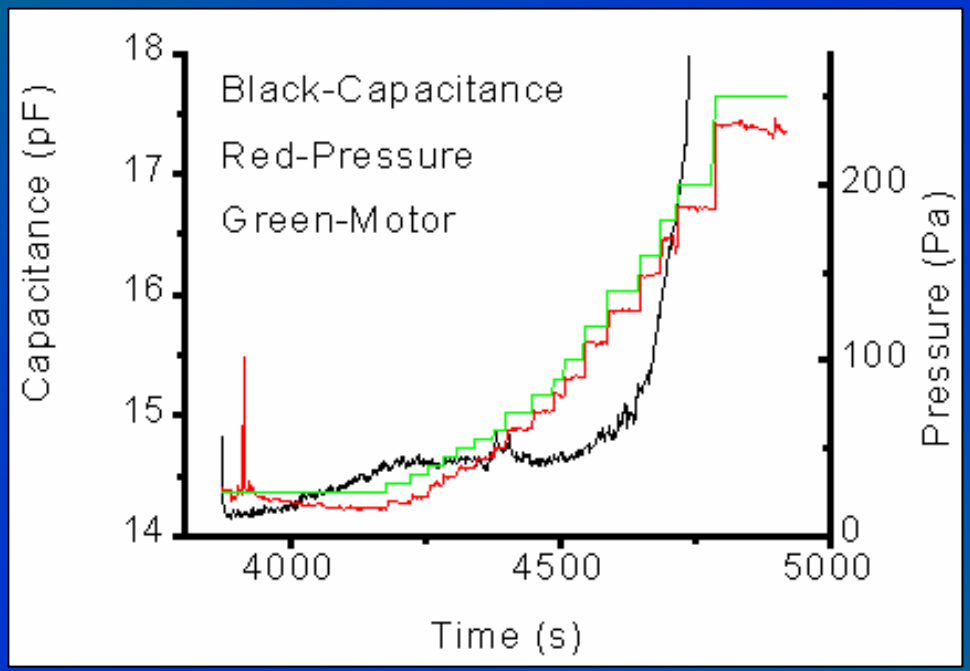

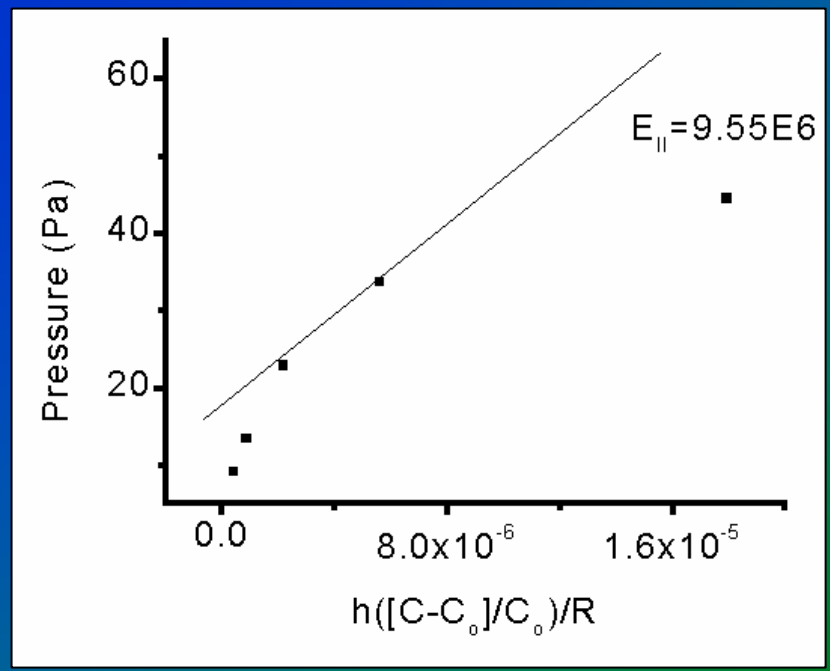

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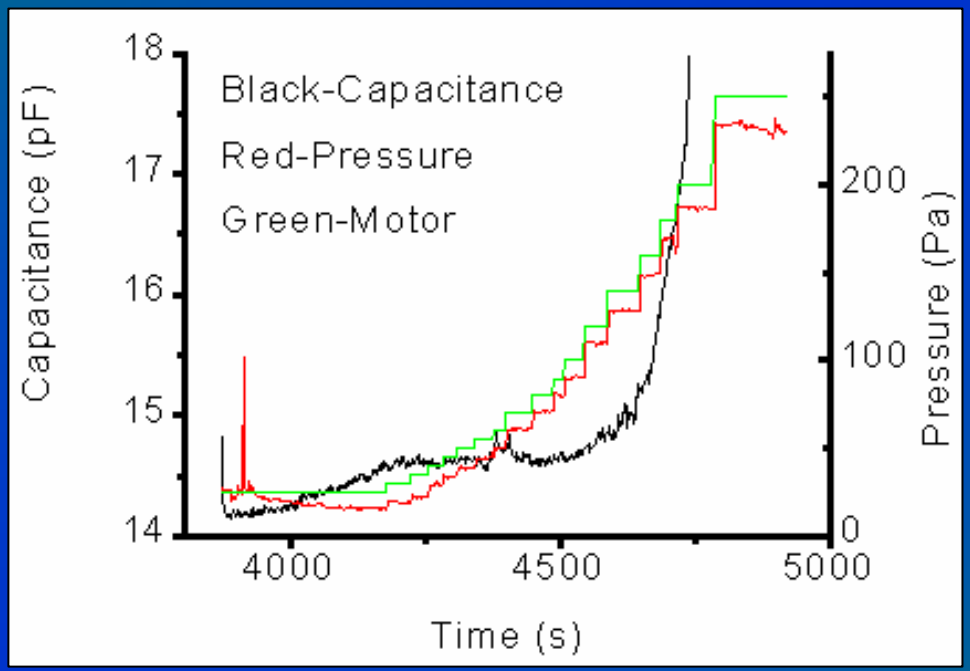
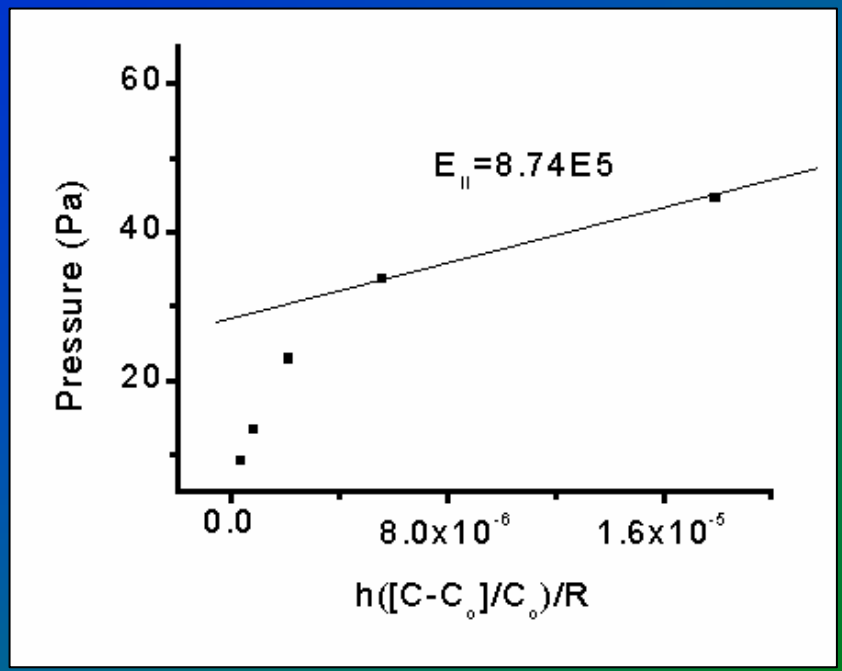
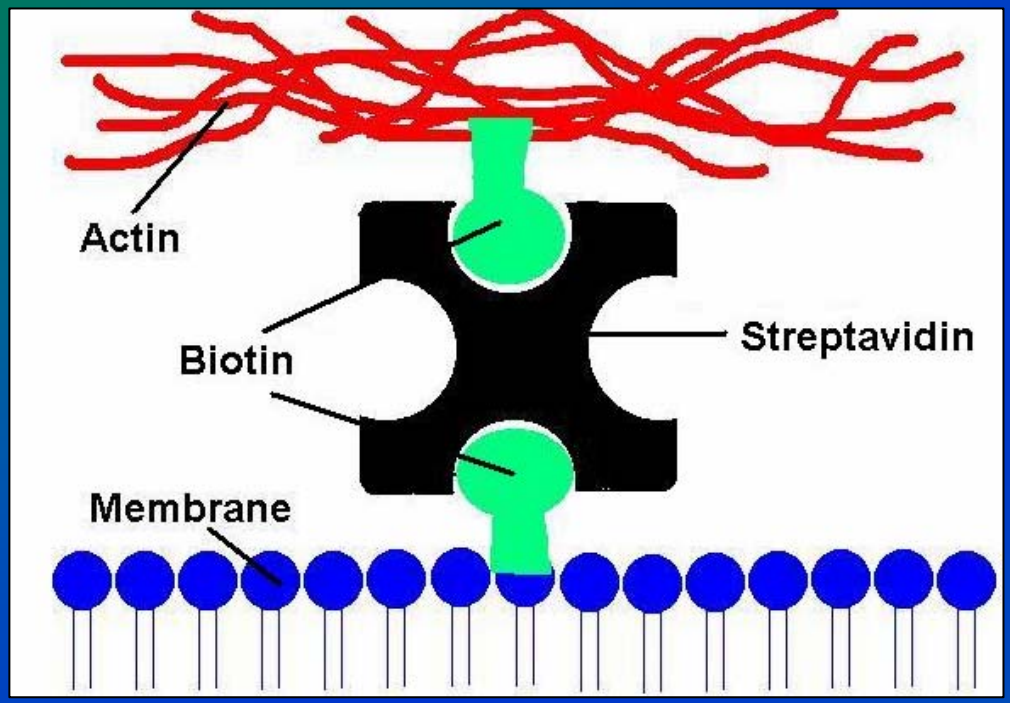
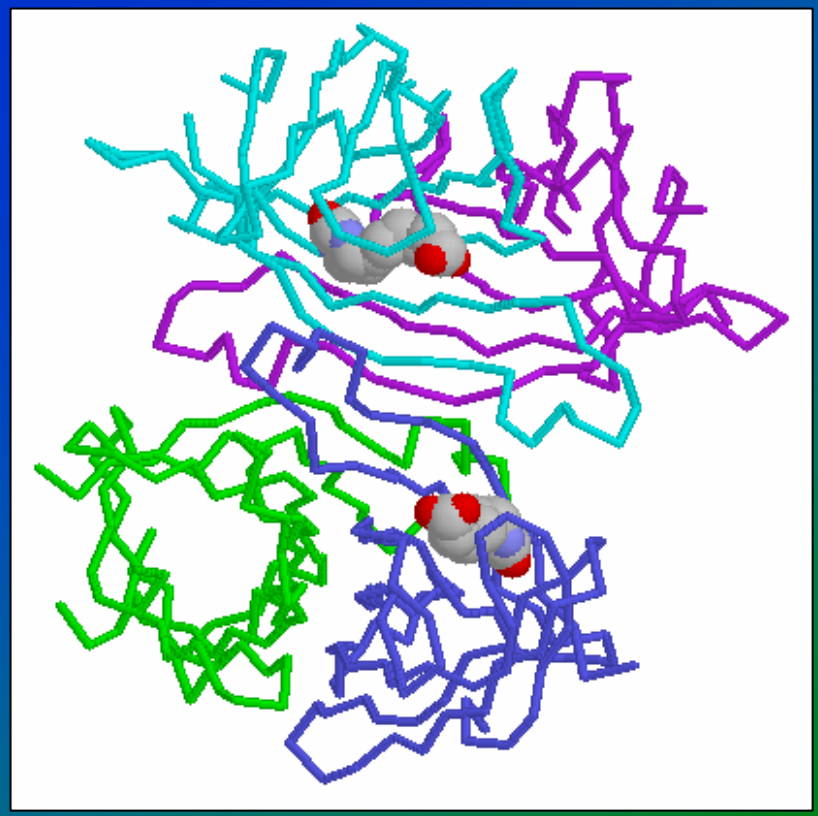




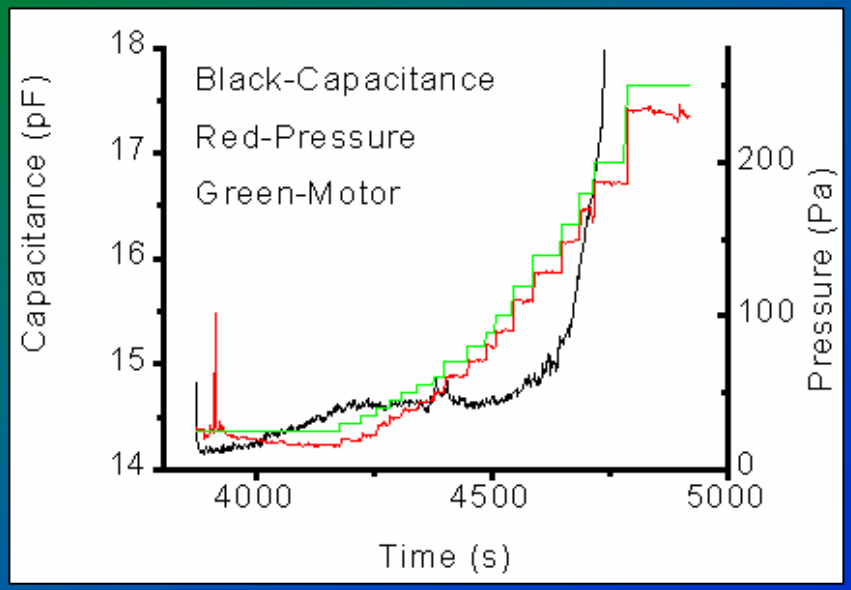
Streptavidin/Biotin Binding

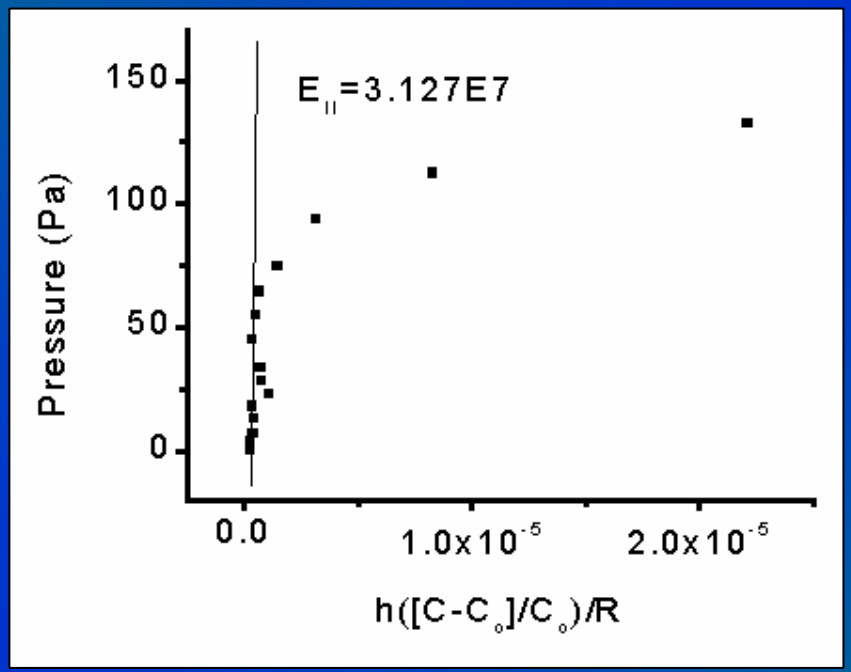


- Streptavidin/biotin system one of the strongest non-covalent binding systems known
- Biotin binds to streptavidin protein



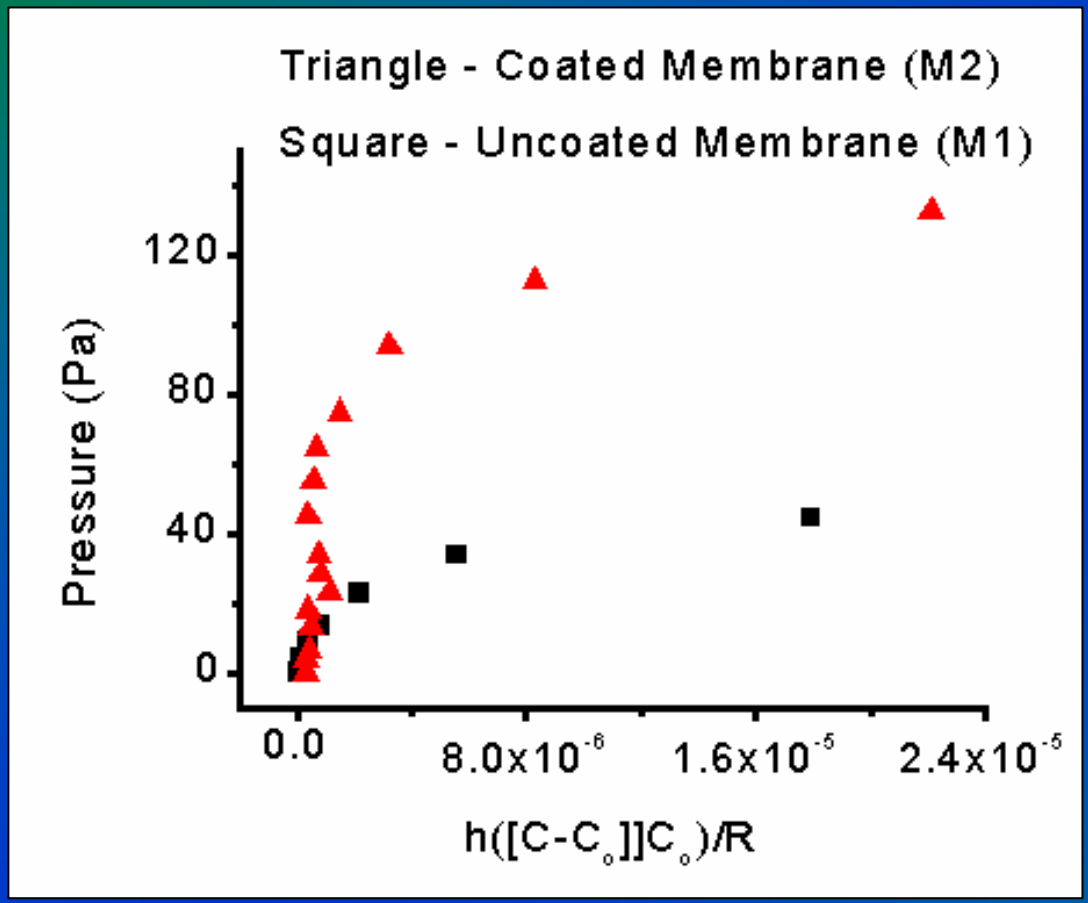
Actin-Coated Membranes





- Actin-coated membranes show very little movement at first, then begin to grow dramatically
- High $E_{||}$ for beginning data points
- Initial $E_{||}$ for coated membranes may be the same as that of uncoated
- At high pressures, $E_{||}$ of coated membranes are also similar to uncoated

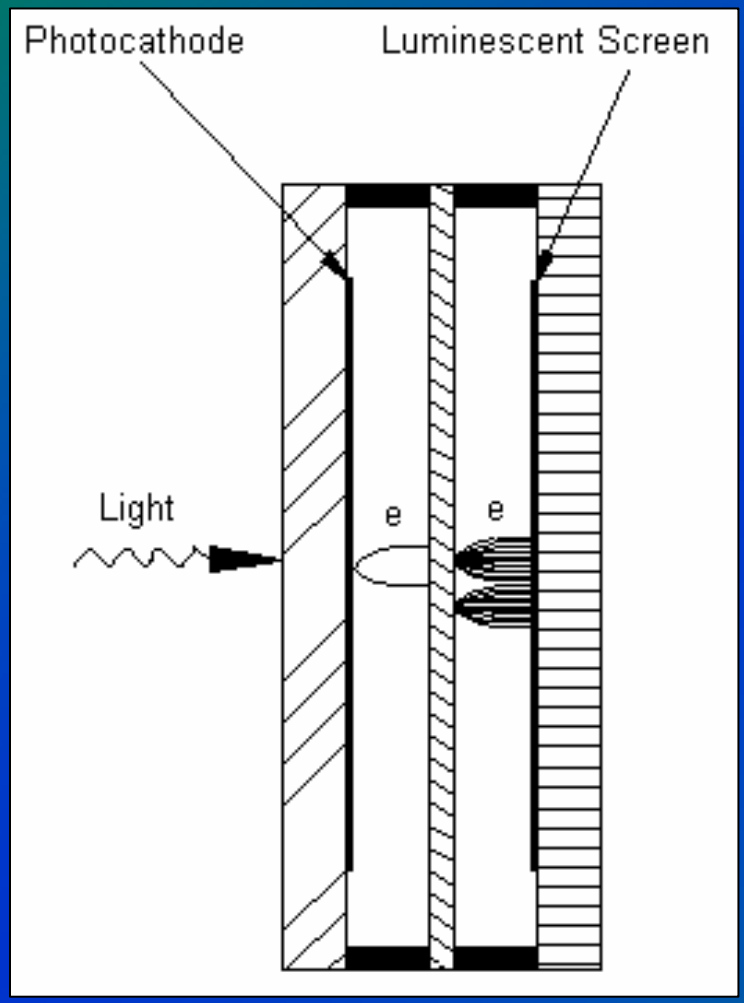
Comparison of Typical Membranes



- Actin-coated membranes appear to break at approximately twice the pressure as a similar uncoated membrane
- The real difference in $E_{||}$ appears to be at intermediate pressures

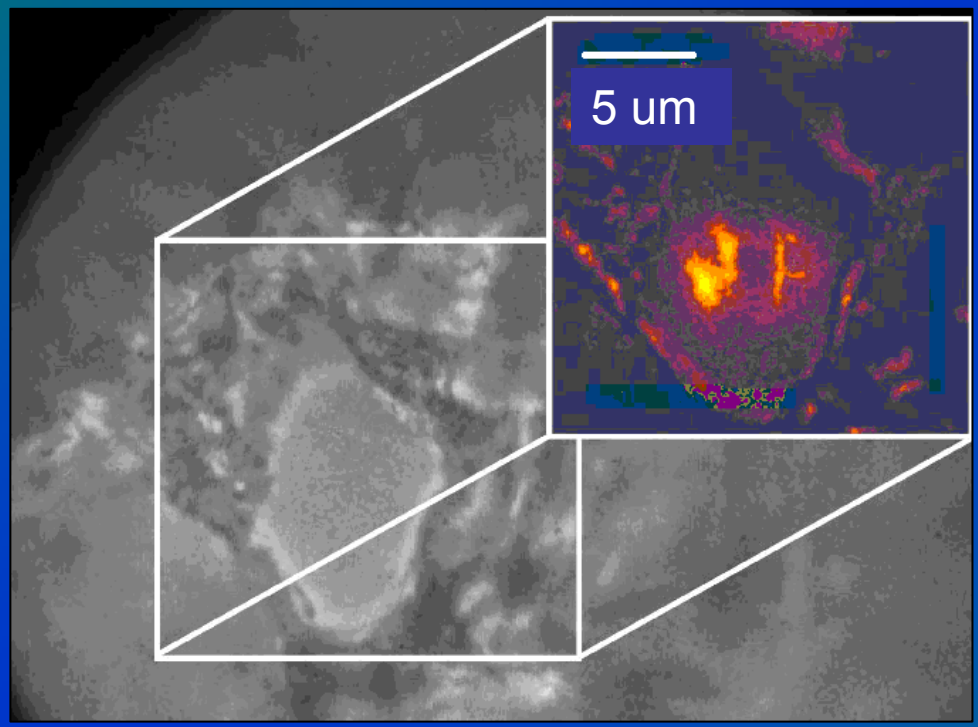
ICCD Imaging

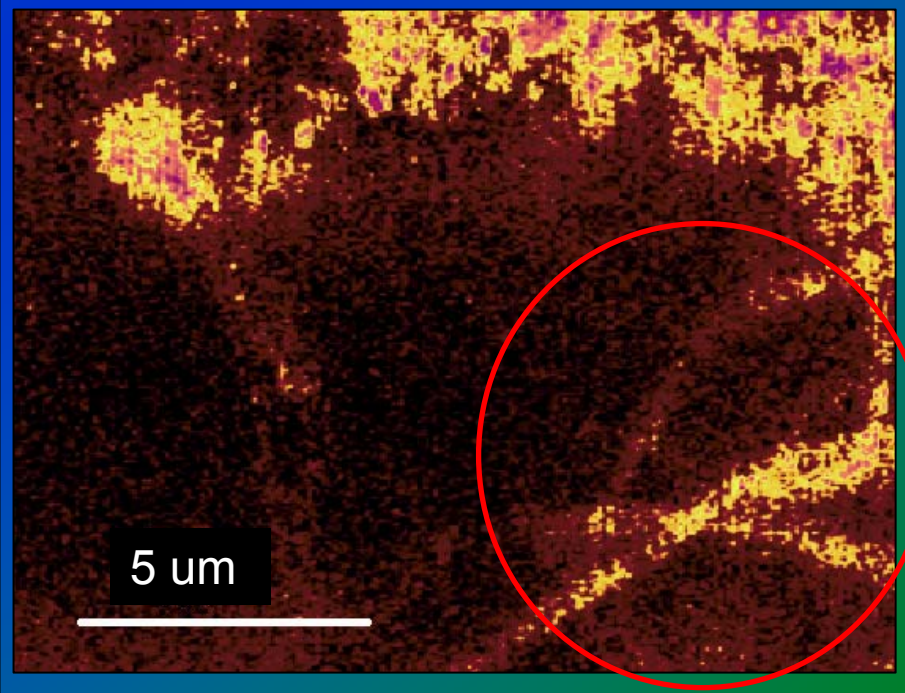
ICCD – Intensified Charge Coupled Device



Imaging Results – ICCD and Confocal

- ICCD – Sensitive, fast 2-D images
- Confocal – Sensitive 3-D images, but takes a long time to obtain





Conclusions

- Actin-coating is an effective method of stabilizing BLM for laboratory purposes
- Although actin loses its effectiveness at elevated pressures and membrane sizes, it has high utility for approximately flat membranes
- Both imaging techniques suggest that actin is indeed binding to the surface of the membrane, but with current procedures appears to only cover the membrane sparsely

Future Work

- Publication
- Other forms of stabilization (e.g microtubules, polymerized membranes, oligosaccharides) will be tested for use as alternate methods of BLM stabilization
- Diffusion measurements of actin-coated lipid bilayers

Acknowledgments

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