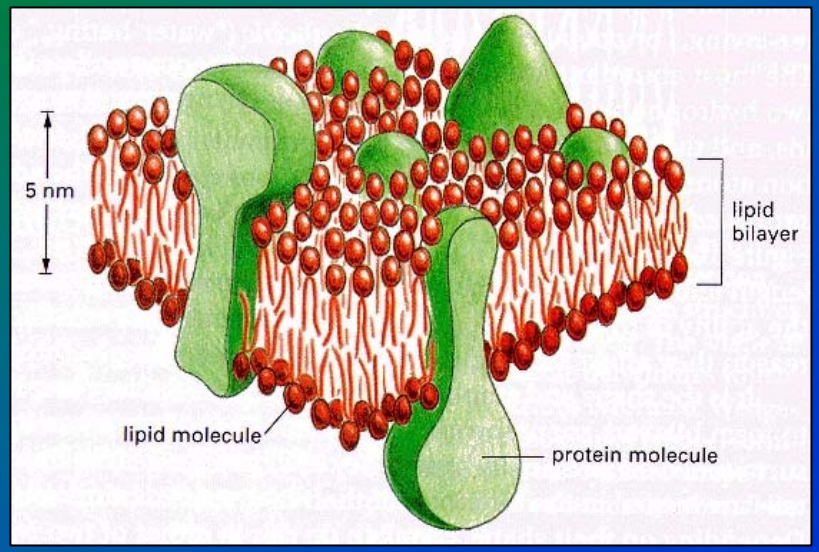
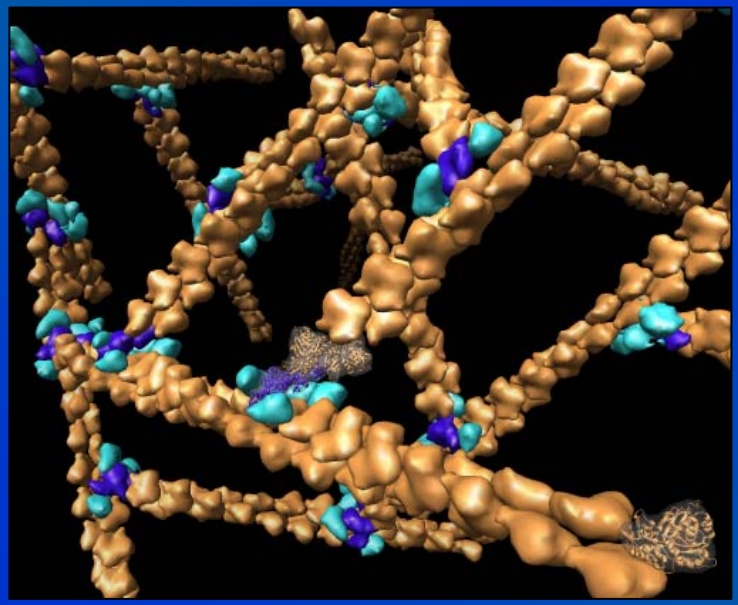
Characterizing Actin-Coated Lipid Membranes with Single-Molecule Imaging and Elasticity Measurements

Dan Dreyer

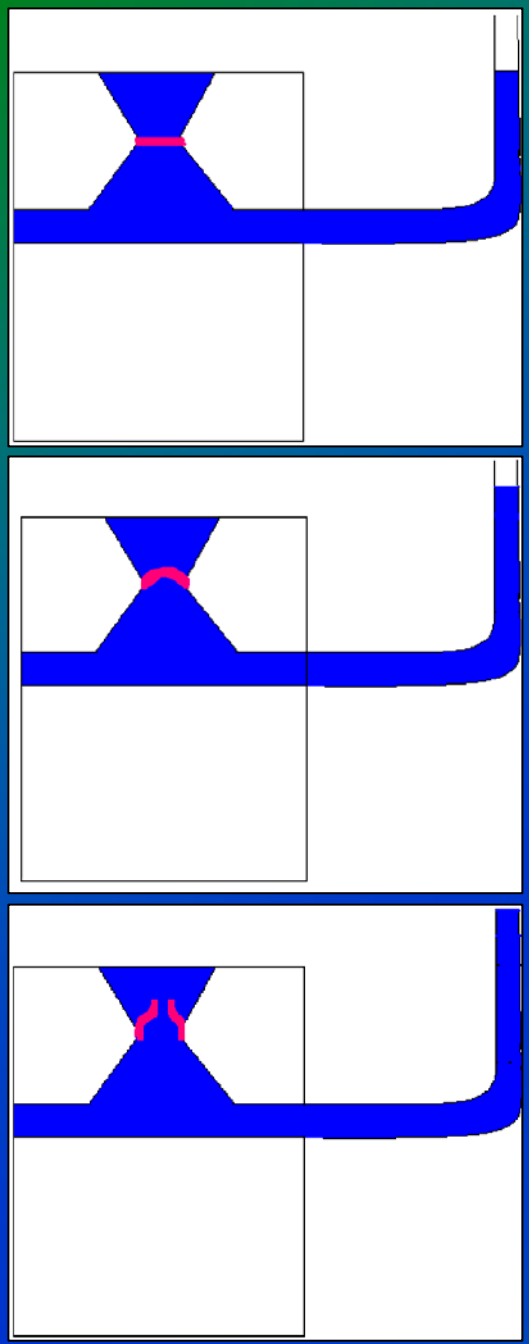
Wheaton College Chemistry Department

Bilayer Lipid Membranes (BLM)





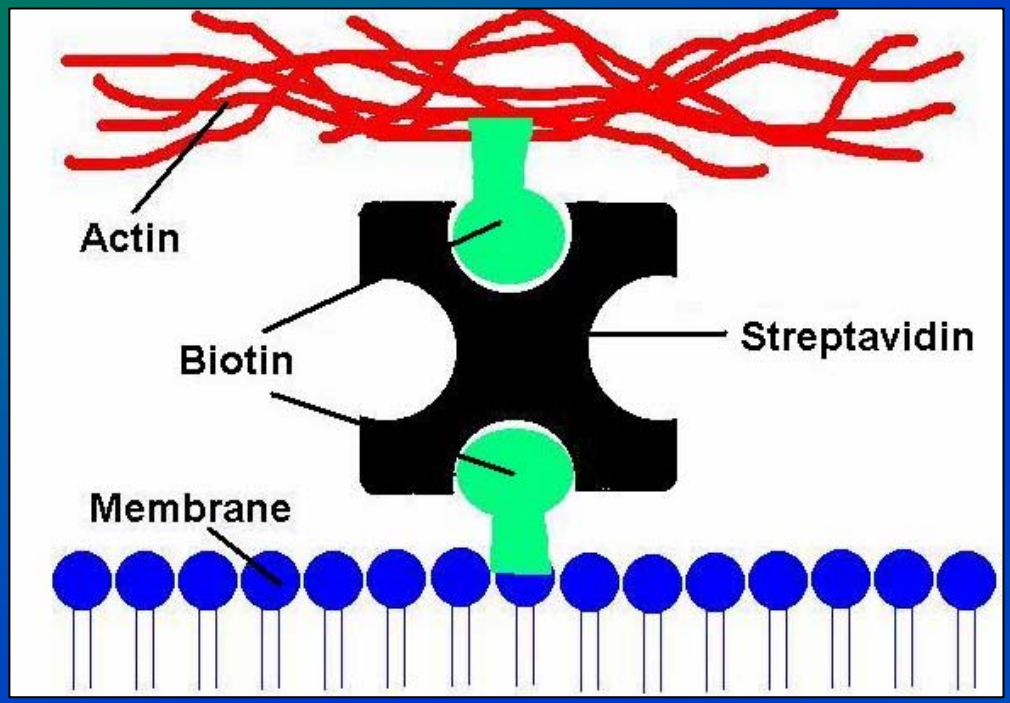
- Important subject of research in drug design, biophysics, biosensors, and nanotechnology
- Highly unstable and extremely fragile
- Natural cells possess a cytoskeletal structure consisting primarily of actin filaments and microtubules to protect the cell and prevent rupture



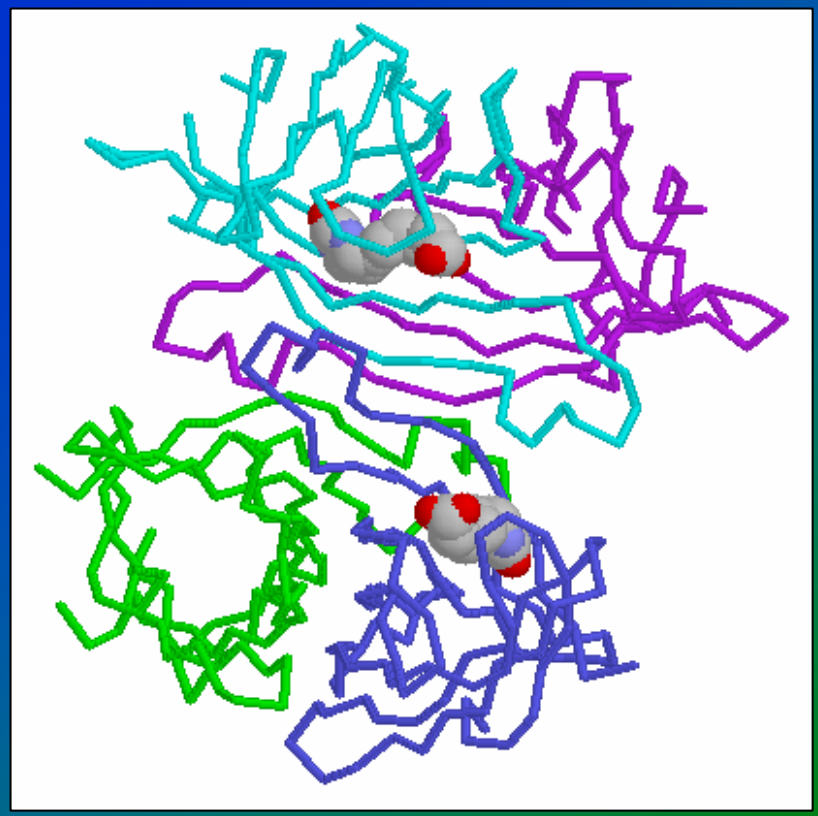
Experiments

- 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

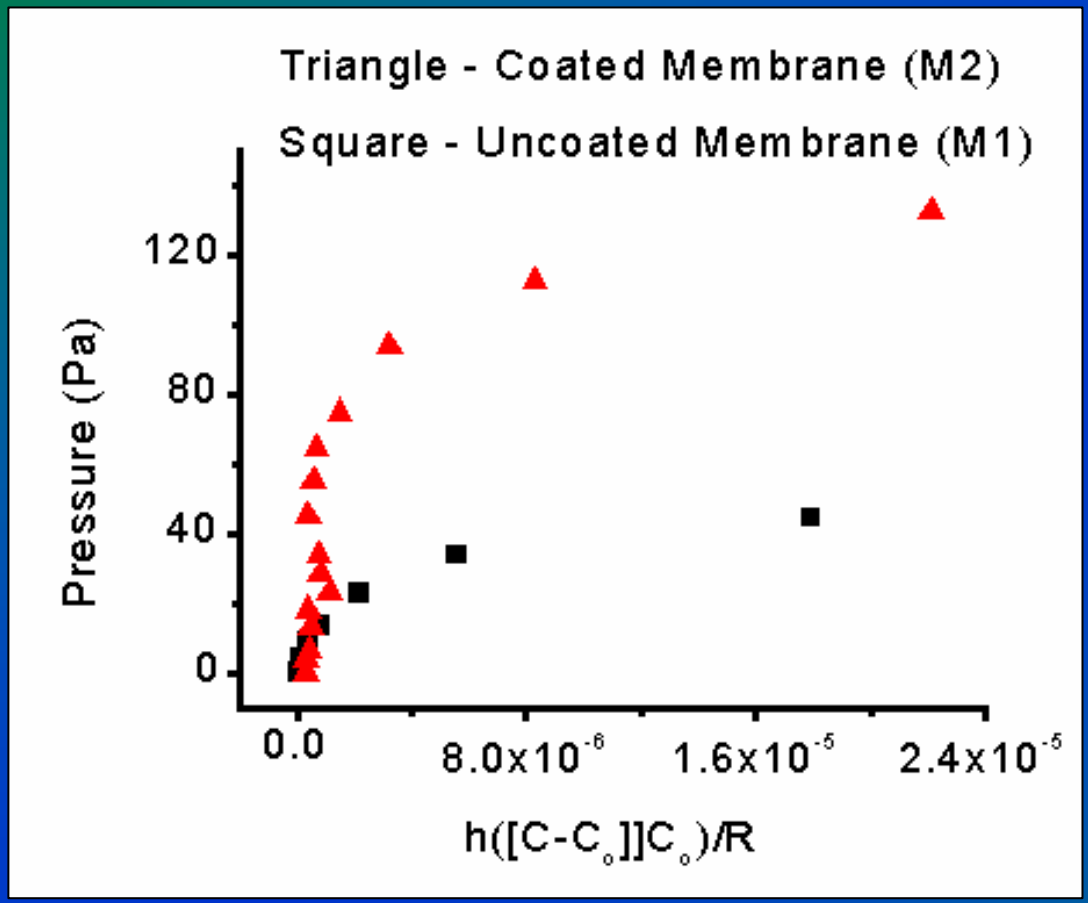
Streptavidin/Biotin Binding



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



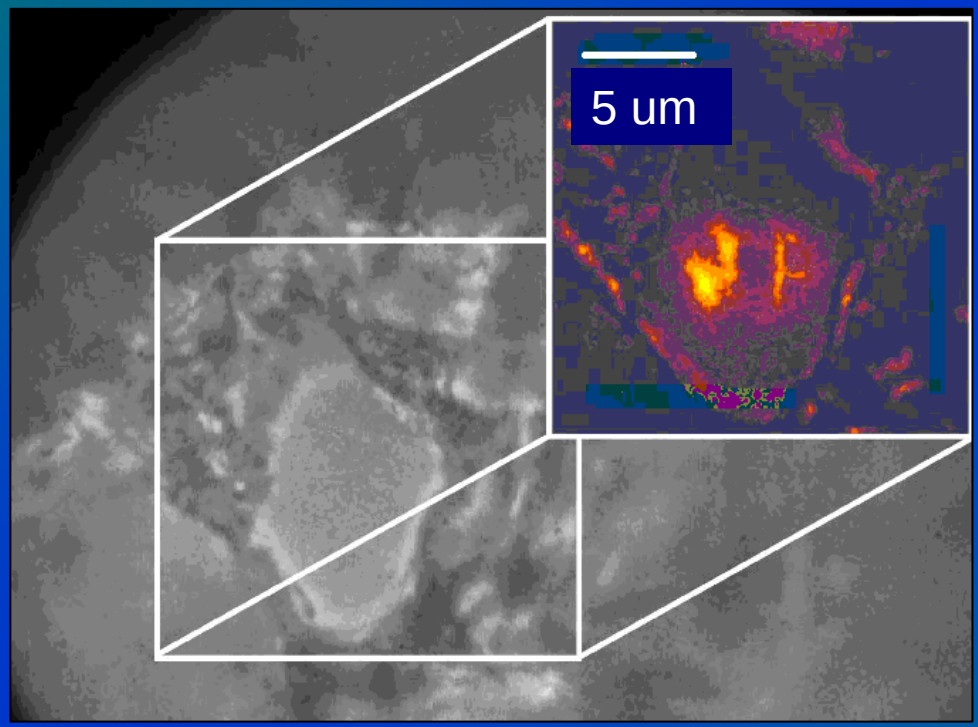
Comparison of Typical Membranes

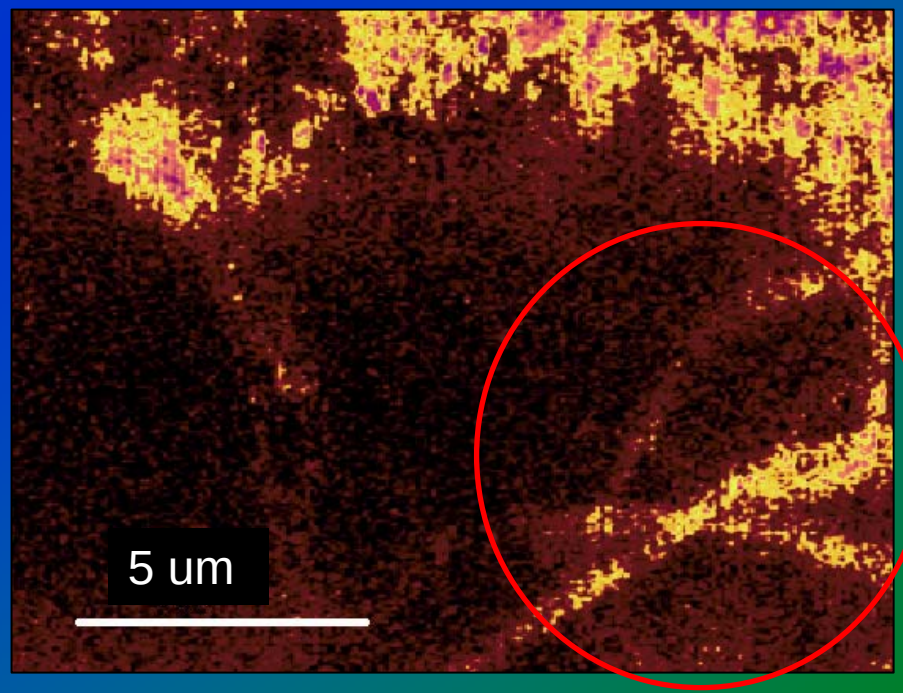


- Actin-coated membranes appear to break at approximately twice the pressure as a similar uncoated membrane

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
- 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

- 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

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