Development of Functionalized Semipermeable Membranes for Microfluidic Separations

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Biological Separations

- Detection and quantitation of target molecules in a sample is important for research and diagnostics (e.g. viruses or hormones in biological samples
- Size-based separations appropriate for gross and sophisticated separations
  - Gross- "cleaning up" a sample, i.e. blood in order to then perform…
  - Sophisticated- ability to separate smaller molecules in a way that allows for quantitation
- Goal is the ability to accomplish separations on very small samples over a large range of molecule sizes in aqueous medium

Separation Techniques

- Gas Chromatography- inappropriate for charged and/or very high molecular weight molecules
- High Performance Liquid Chromatography (HPLC)- slow, expensive, requiring bulky instrumentation
- Silica-gel column chromatography- standard for macroscale, but ineffective on a microscale
- Membranes with controlled pore sized to affect size-based separations

Microfluidics

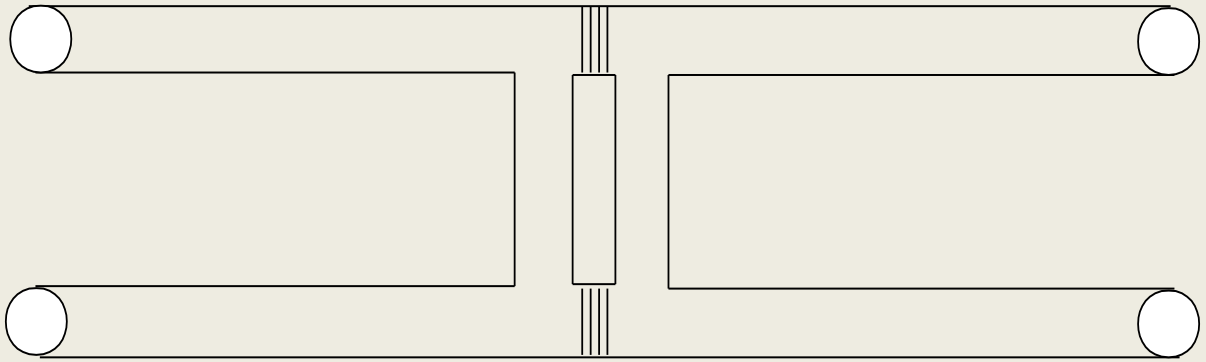
- General method for accomplishing complex separations rapidly, with small sample sizes on a microscale
- Membranes may be synthesized *in situ*
- Enhance sensitivity of common testing methods- performance of separations tends to improve with reduction in size
- Reduced expense and greater mobility of testing

Spring 2010 Research

- Microchip fabrication
- Membrane formation
- Evaluation of membranes

Microchip Fabrication

- Fascinating, but complex, method to create microchips
- Design used for this project specific to function
- 30μm depth channels for introduction of reagents and solutions, membranes formed in shallower channel ~2μm deep.

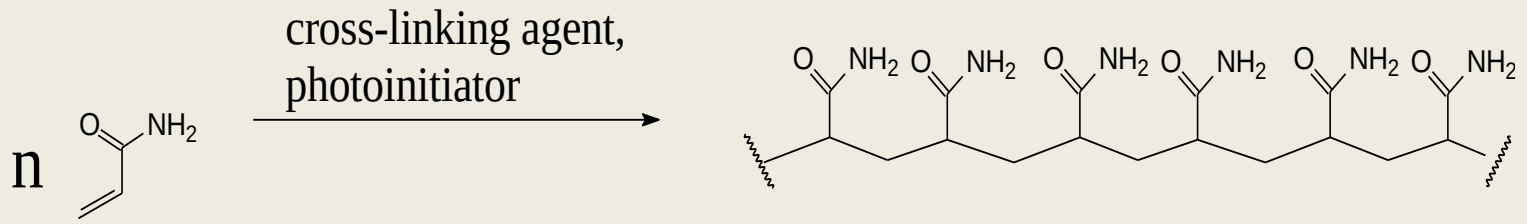


Semipermeable Membranes

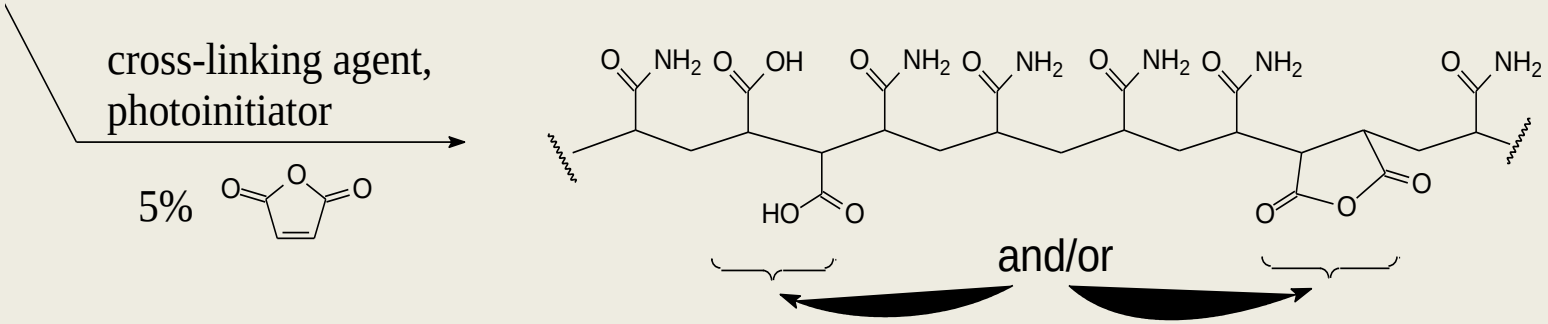
- Polyacrylamide membranes used to trap biological molecules of MW=5,000- 1,000,000
- First goal is to vary permeability of membranes through synthetic means
- Desire ability to trap MW≥ 200- 500 molecules
- Second goal is incorporation of silica and amine derivatives into the membrane, to give a functionalized membrane

Membrane Synthesis

- Polymerization and cross-linking of acrylamide and bisacrylamide

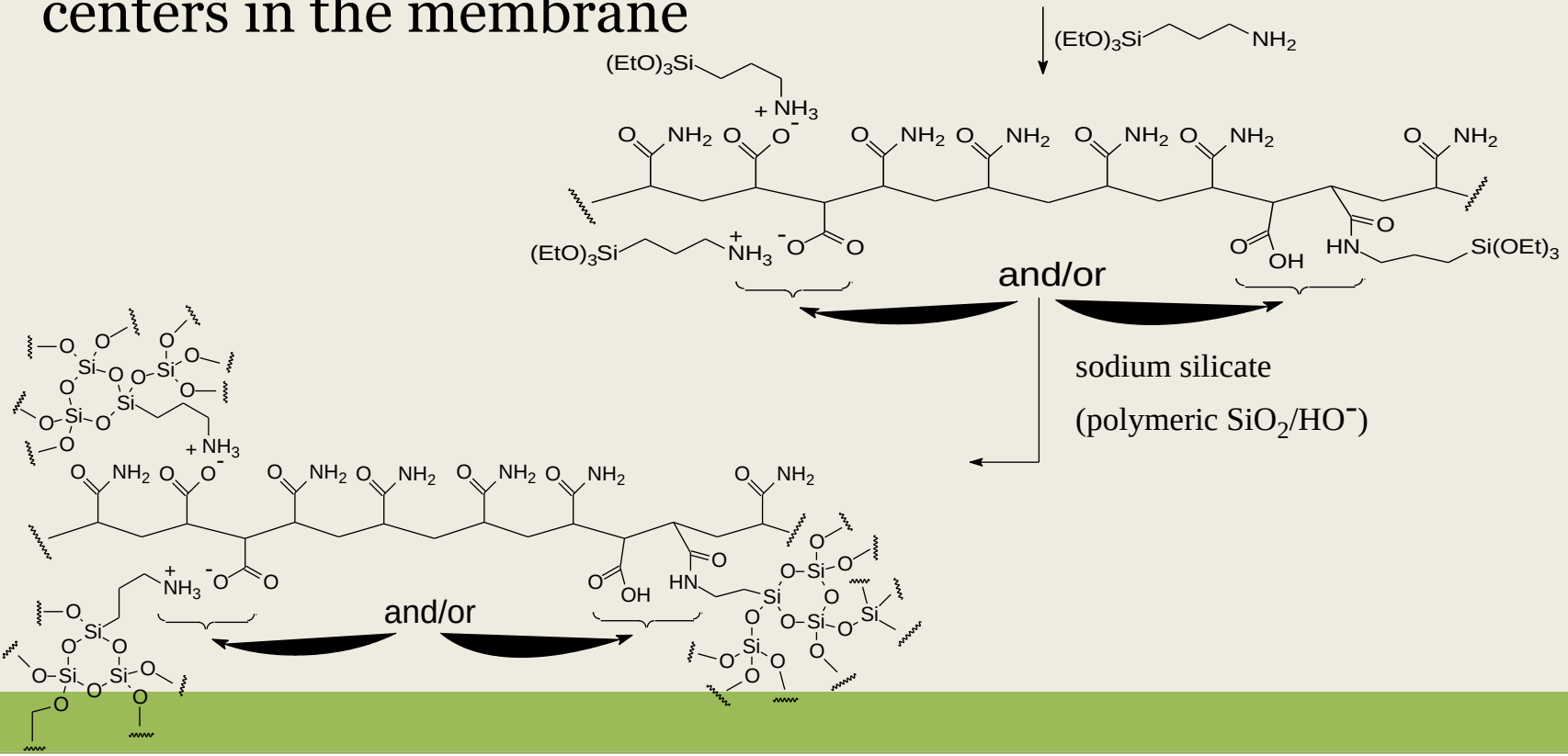


- Maleic anhydride sub-units provide sites for specific molecular associations



Membrane Synthesis

- Modified in a two-step synthetic procedure after polymerization to give a hybrid membrane including organic molecules and silica molecules attached to reactive centers in the membrane

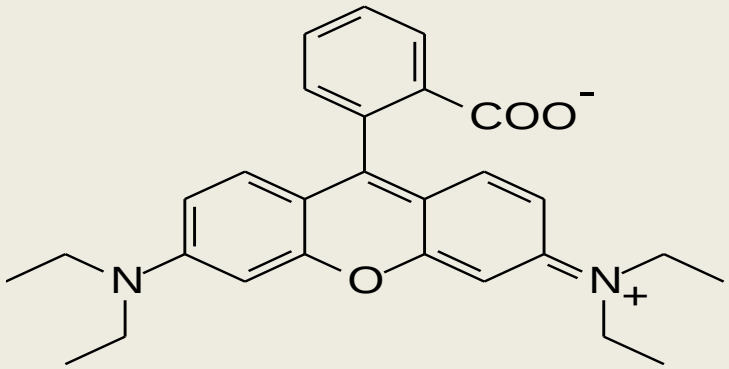


Functionalized Membranes

- Initial membranes contained high concentration of maleic anhydride subunits in order to achieve minimum porosity
- Wanted to test for the passage of buffer molecule while retaining small organic molecules

Evaluation of Membranes

- Electroosmotic flow- neutral molecules carried with bulk solution as it flows from cathode to anode
- Dominant form of flow in microfluidic channels
- Utilized neutral dye molecule, Rhodamine B, MW= 480



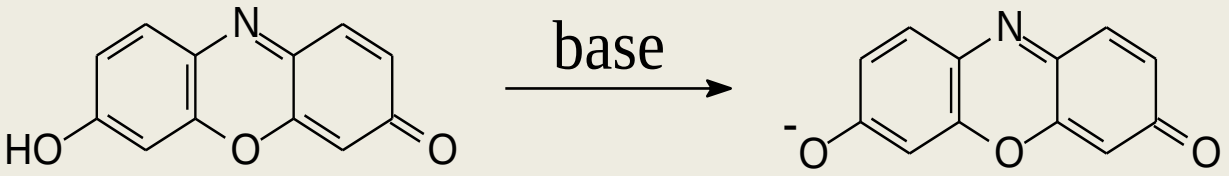
rhodamine B

Evaluation of Membranes

- Electrophoretic flow- ions in solution are attracted to corresponding electrode
- In the absence of electroosmotic flow, may be used to selectively move positive and negative ions
- In this way, molecules can be selectively directed to membranes

Evaluation of Membranes

- Glass has inherent surface charge, must neutralize to reduce interference by electroosmotic flow- formamide coating
- Utilized dye molecule that may be easily ionized, Resorufin, MW= 213.



resorufin

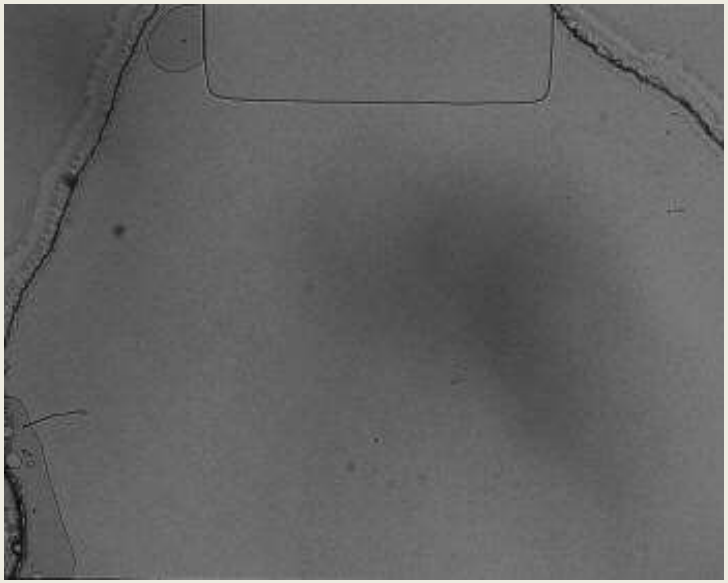
resorufin anion
(highly fluorescent)

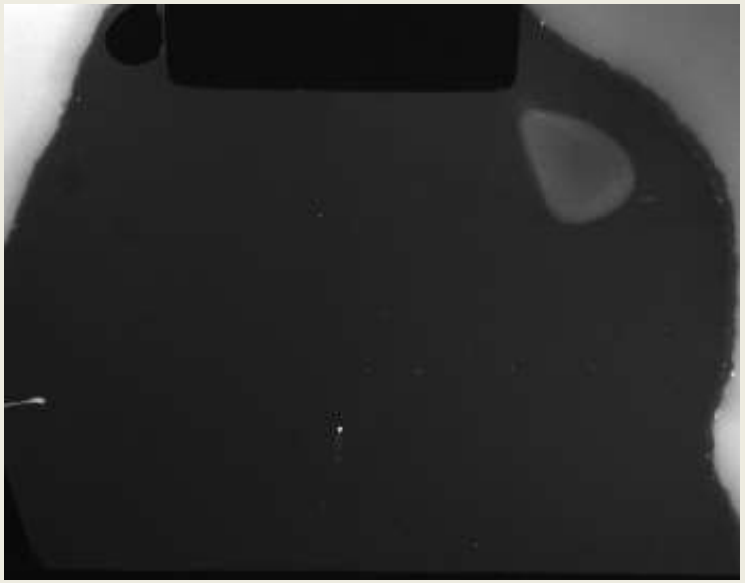
Evaluation Process

- Introduced dye molecules to channels under green light to excite fluorescence
- Applied voltage to ports to enhance flow through microfluidic channels
- Monitored flow of solution to and through membranes in order to evaluate ability of membrane to stop molecules

Results

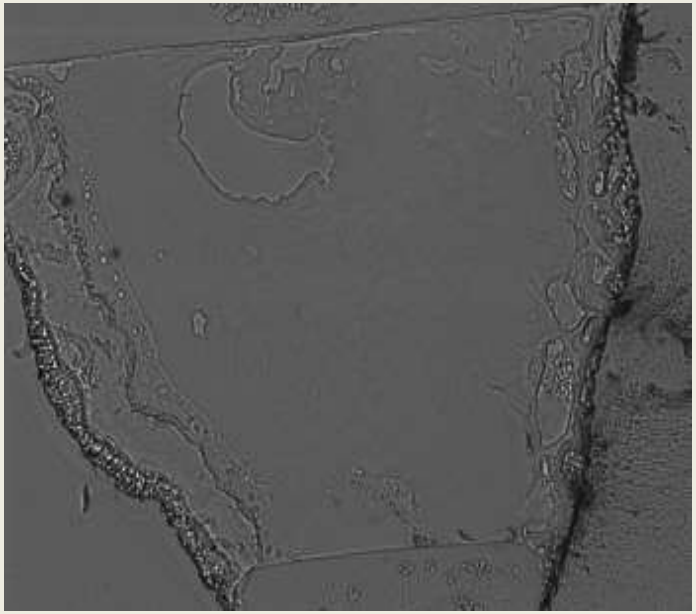
- Electroosmotic flow
- Control membrane, just acrylamide: bisacrylamide polymer

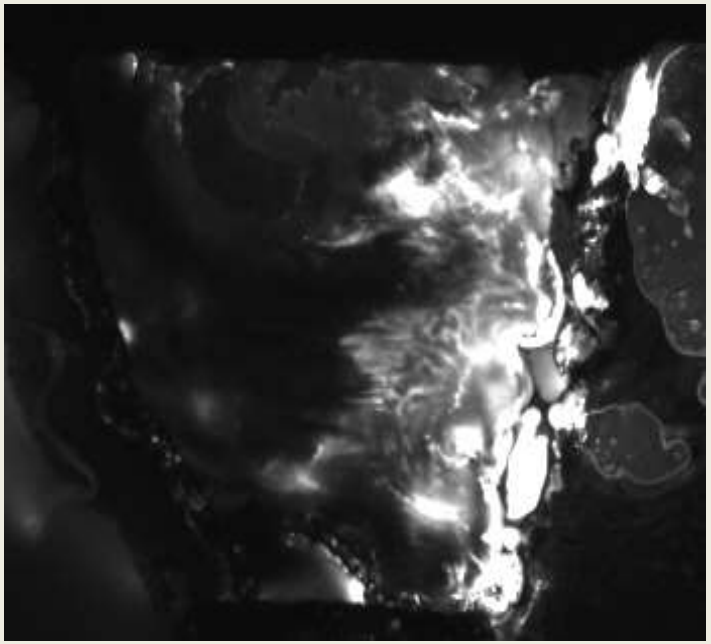




Results

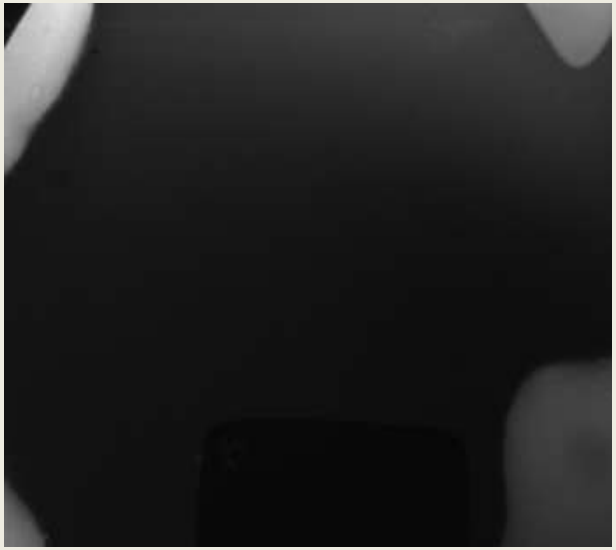
- Electroosmotic flow
- Hybrid membrane with maleic anhydride



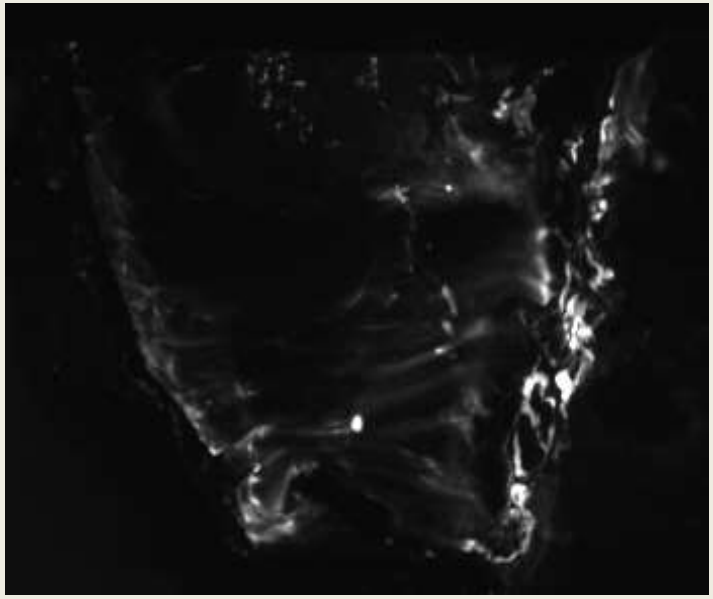


Results

- Electrophoretic flow
- Polymer



Hybrid



Conclusion

- The polyacrylamide membrane was not expected to stop the flow of dye molecules, and it performed as expected
- The hybrid membrane successfully trapped dye molecules slightly larger than MW= 213
- Visible concentration of dye at membrane interface

Future Research

- Synthetically vary concentration of reactive sites (e.g. maleic anhydride) in membrane in order to control permeability of membrane
- Long-term goal is to isolate aliquots containing molecules of certain size, for example MW= 1,000
- Incorporation of functionalized amine groups into membranes in order to perform size- and affinity-based separations

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