SAND2010-4959C

pH Analysis of Polyacrylamide Membranes in Microfluidics

Hanna Miller
2010 Summer Intern
Department of Biotechnology and Bioengineering
Organization 8621

August 5, 2010

Outline

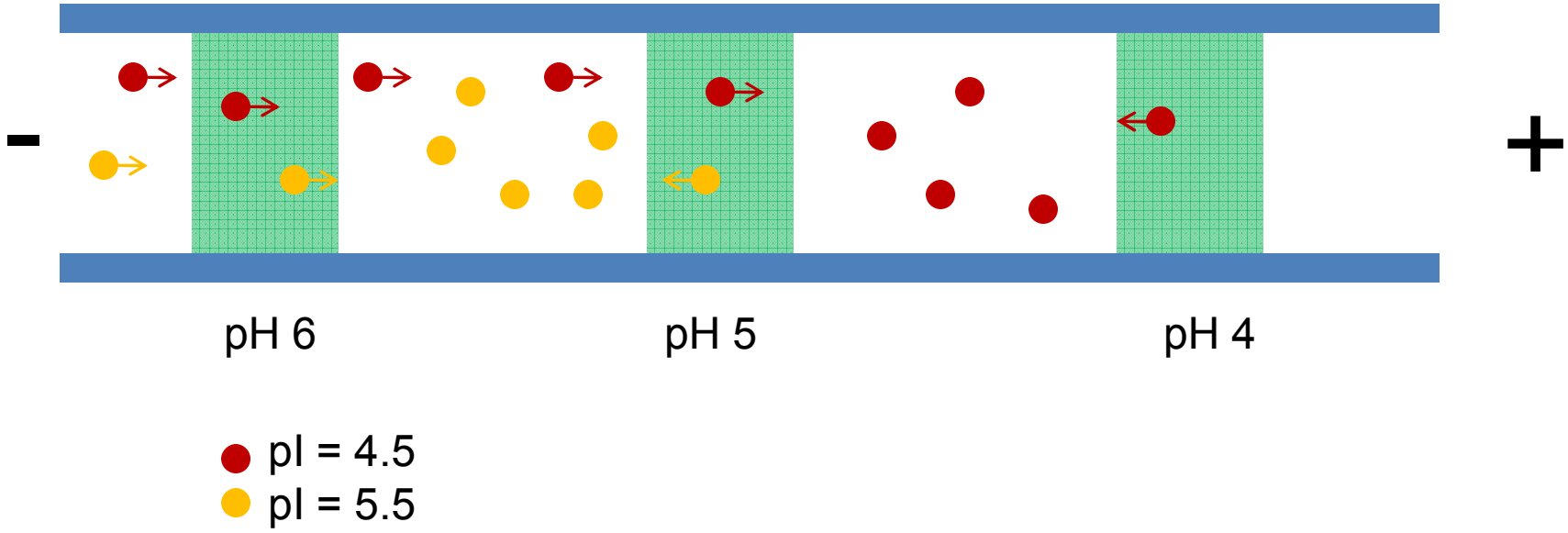
- Background
  - Polyacrylamide Membranes in Microfluidics
  - Concentration Polarization
  - Carboxy SNARF-1 pH Indicator
- Methods and Results
  - Characterization of pH specific polyacylamide membranes
  - pH characterization of preconcentration membranes
- Conclusions
- Future Work
- Acknowledgments
- References

Polyacrylamide Membranes in Microfluidics

- Use of microfluidics in diagnostics allows for faster detection with smaller sample size
- Variety of uses including:
  - Separation of proteins using pH membranes
  - Preconcentration of proteins in pretreatment of samples

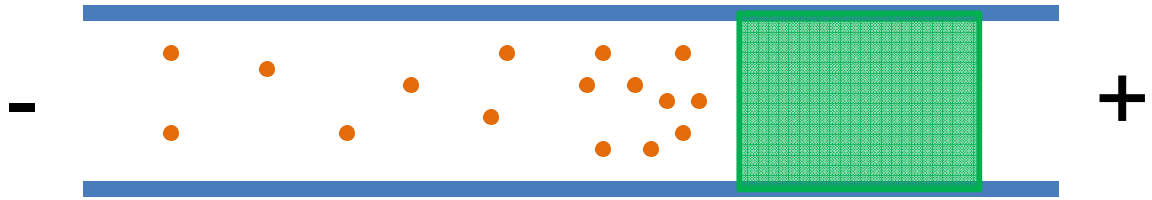
pH Membranes

Proteins are sepperated based on their isoelectric points (pI)



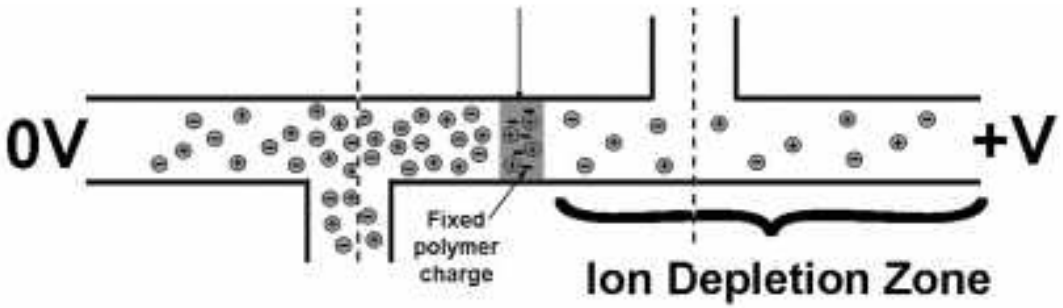
Preconcentration Membranes

- Apply a voltage to bring proteins to a membrane
- Incorporates sample preparation with analysis
- Can concentrate as much as 1000 times



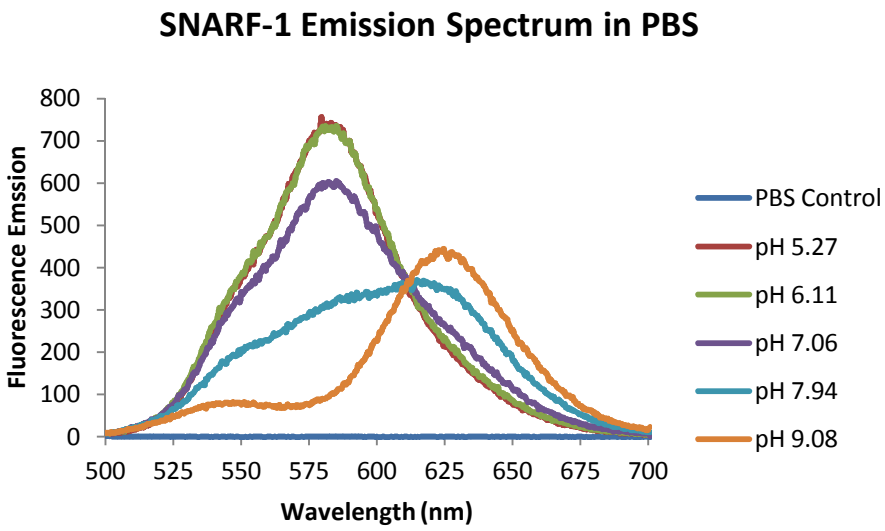
Concentration Polarization

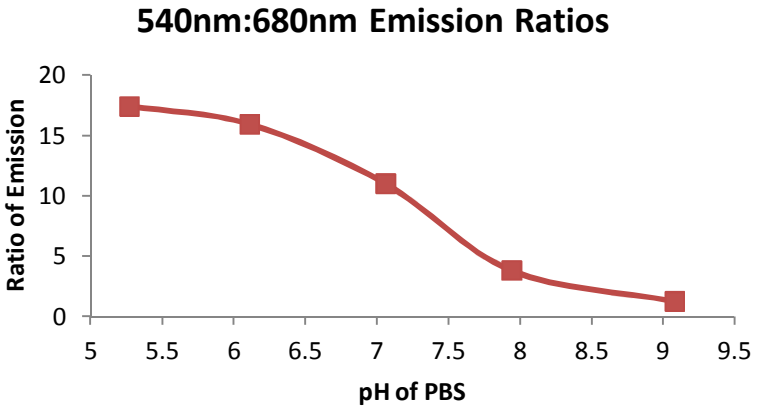
- Voltage causes ions to move across a membrane
- Ions are depleted on the anode side of membrane



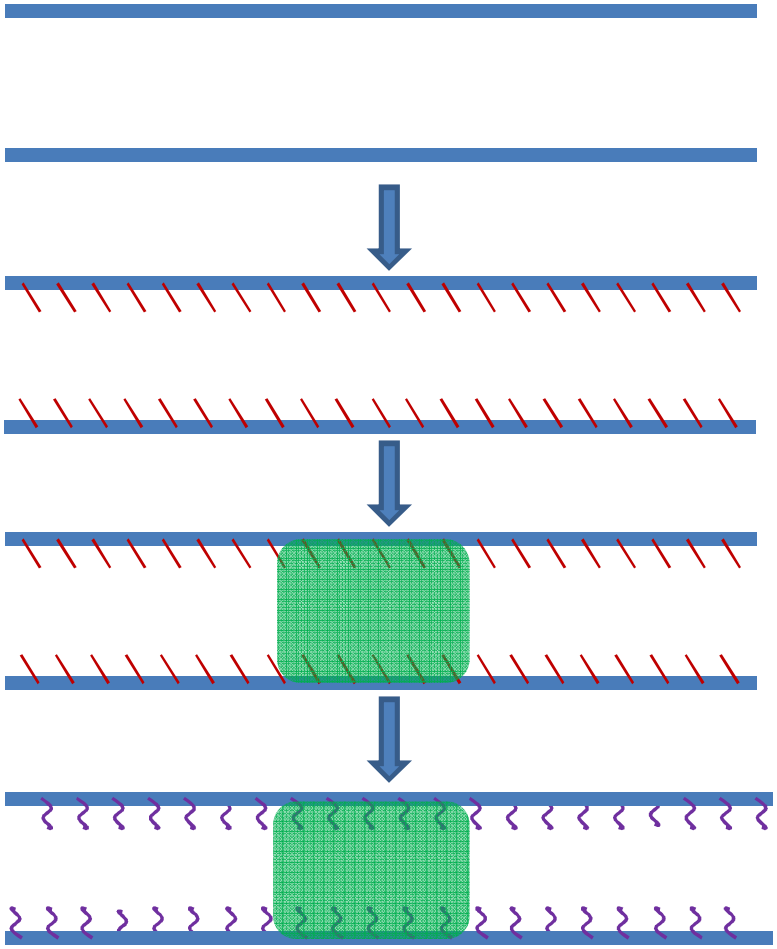
Carboxy SNARF-1 pH Indicator

- Cannot use a pH meter in microfluidics
- Emission spectrum changes based on pH
- pH determined by the ratio of emissions at 540nm and 680nm when excited by 488 nm light





Microfluidic Chip Preparation



Glass microfluidic chip

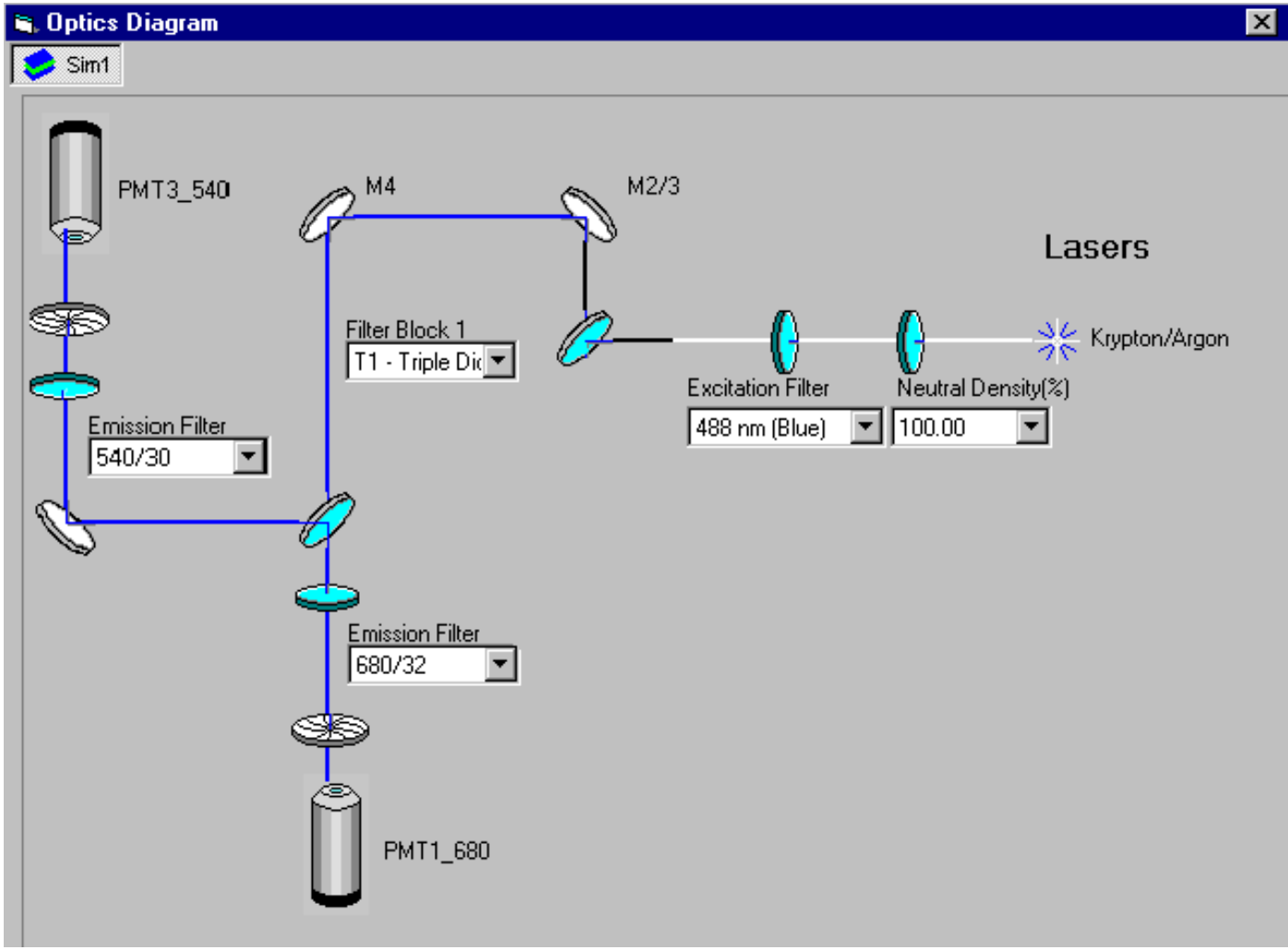
Silane treatment

Membrane fabrication: expose acrylamide-bisacrylamide solution with UV-photoinitator to a UV laser

Acrylamide coating: prevents non-specific binding

Data Collection and Analysis

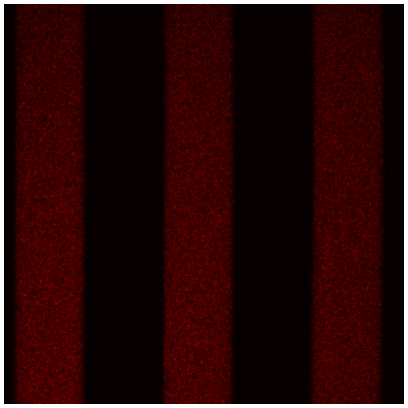
Confocal microscope configuration for fluorescent measurements



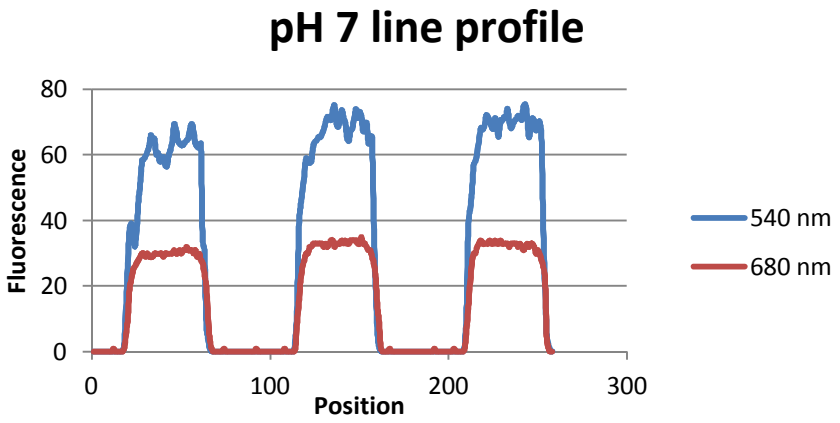
Data processing with excel and Image J

Calibration of SNARF-1

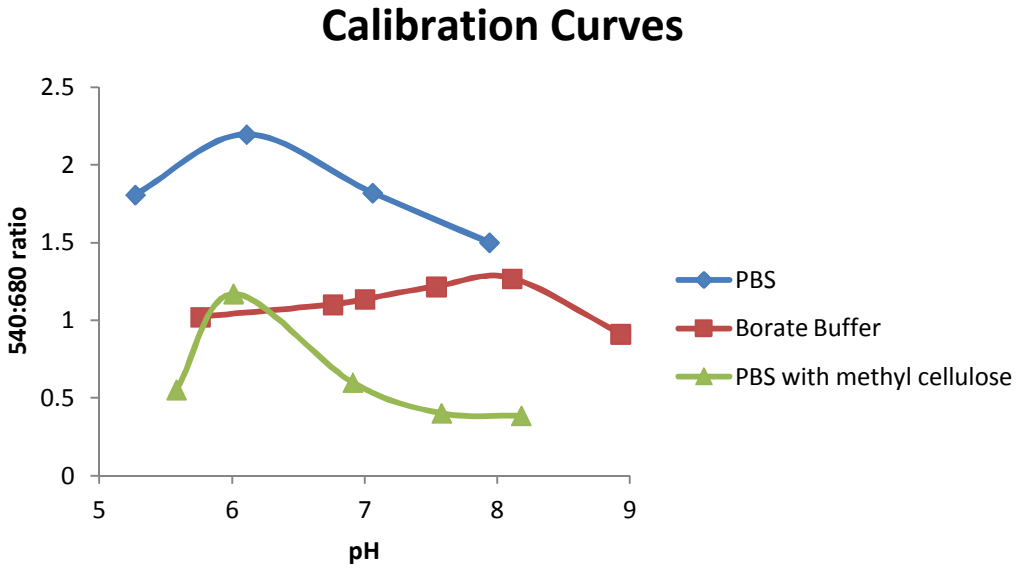
680 nm emissions



540 nm emissions

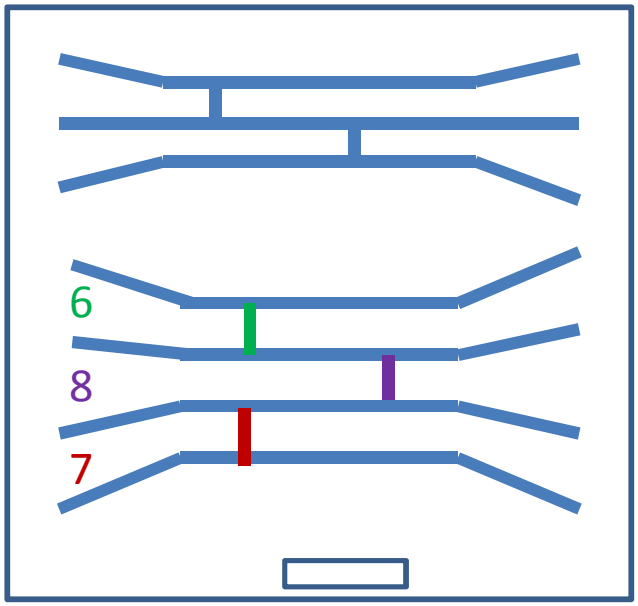


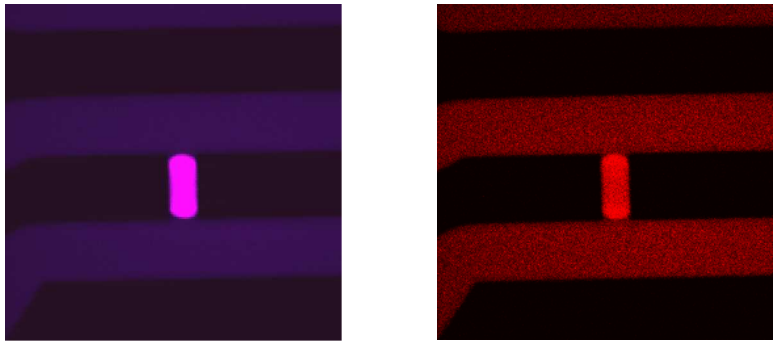
- Line Profiles were taken of the channels at the 540nm and 680 emissions
- Repeated for multiple pH values



pH Membrane Synthesis

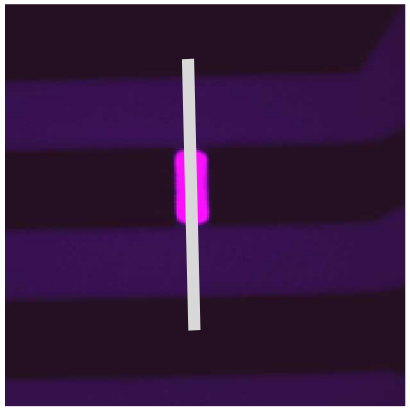
- Made acrylamide-bisacrylamide solutions with various concentrations of immobilines with different pK values
- Membranes made one at a time in the proper channels





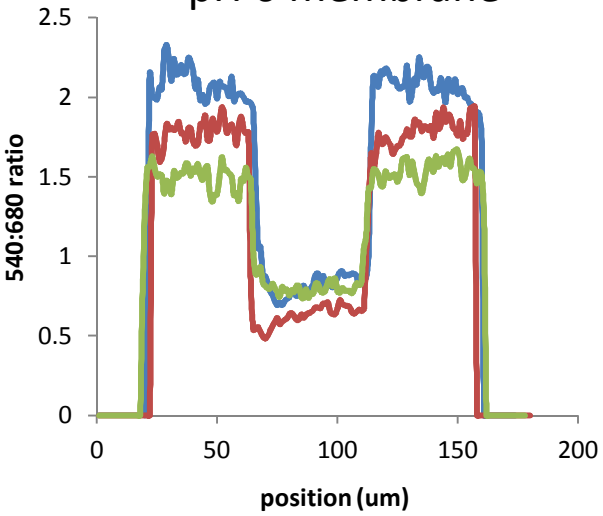
pH 7 membrane in pH 6 PBS

Membranes in Different pH Environments

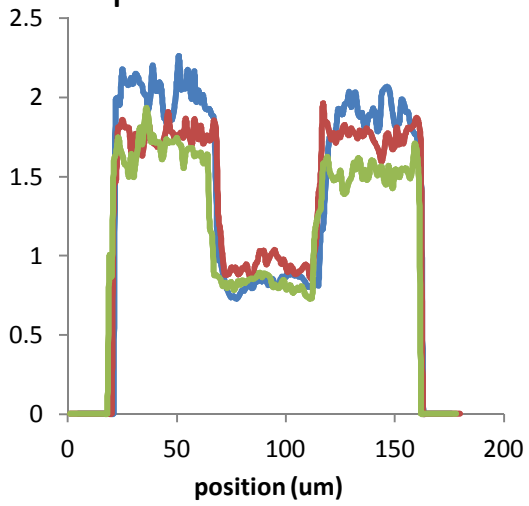


PBS pH6
PBS pH7
PBS pH8

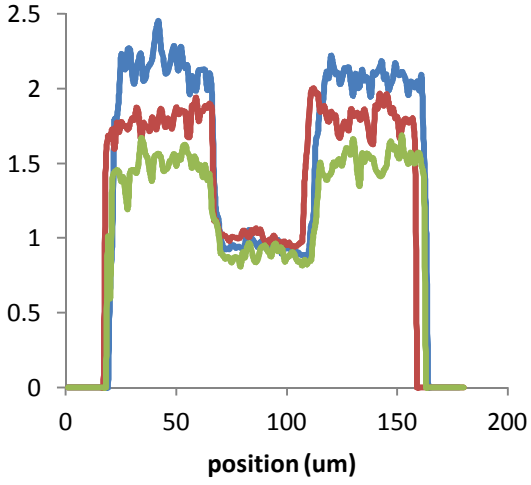
pH 6 membrane



pH 7 membrane

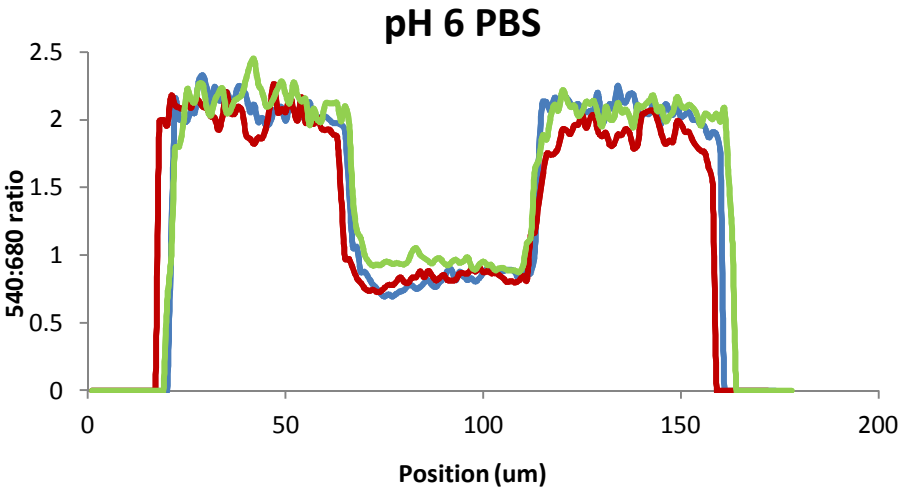


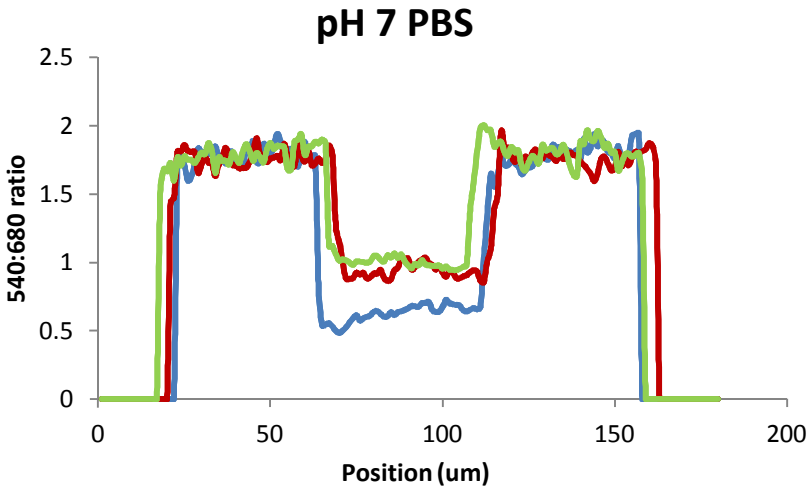
pH 8 membrane

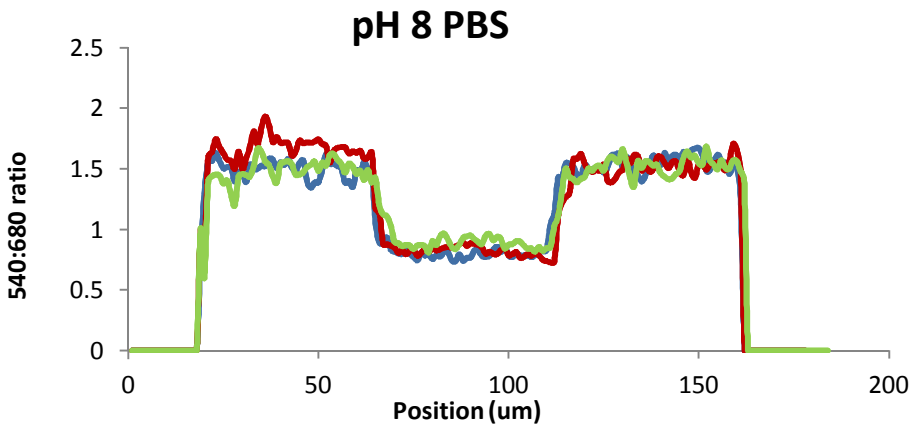


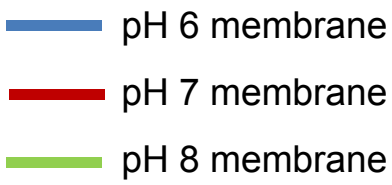
Slight change in membrane pH with changes in environmental pH

Membrane Comparison



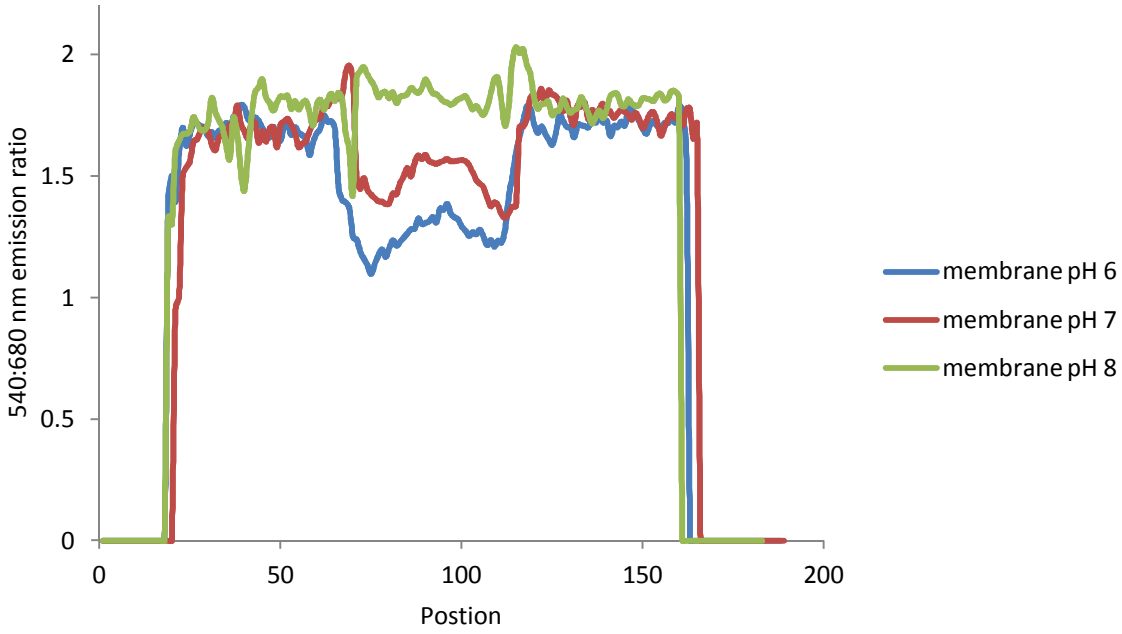






Very small differences between the different pH membranes in the same buffer. Could be due to different conditions inside each membrane, affecting SNARF.

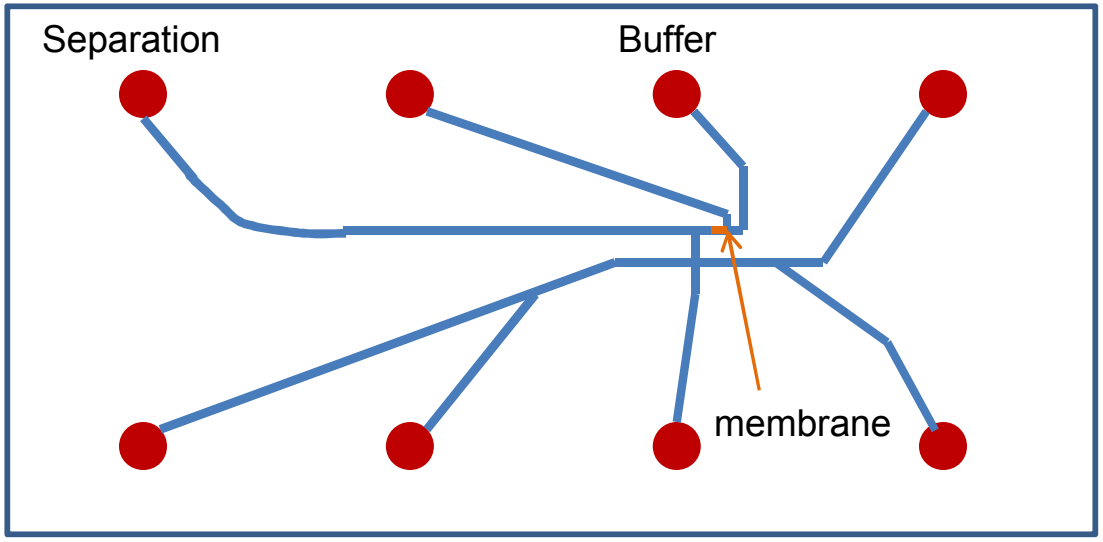
Membrane Analysis with Borate Buffer



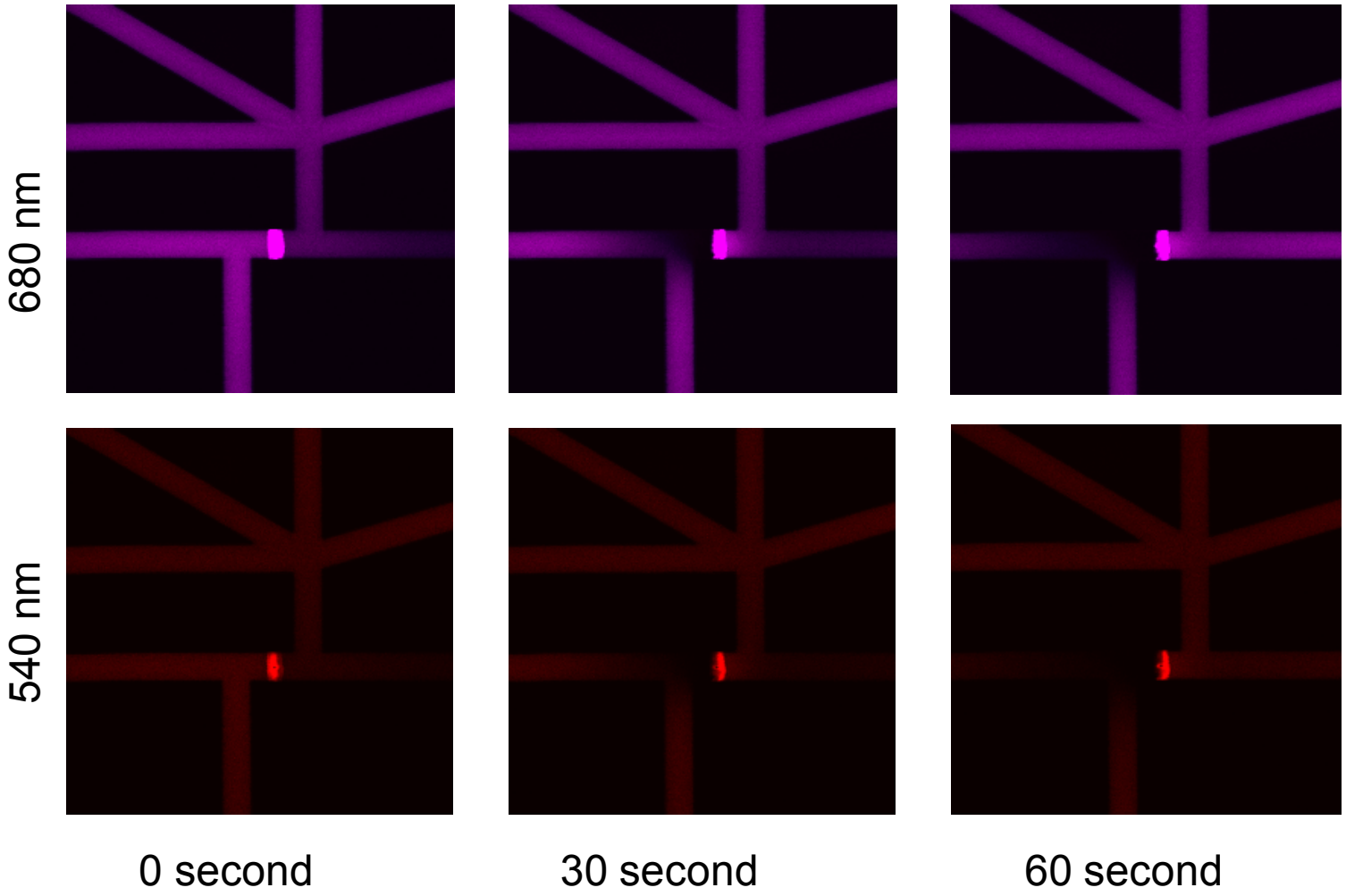
Membranes showed a larger difference in Borate Buffer.

Preconcentration Membranes

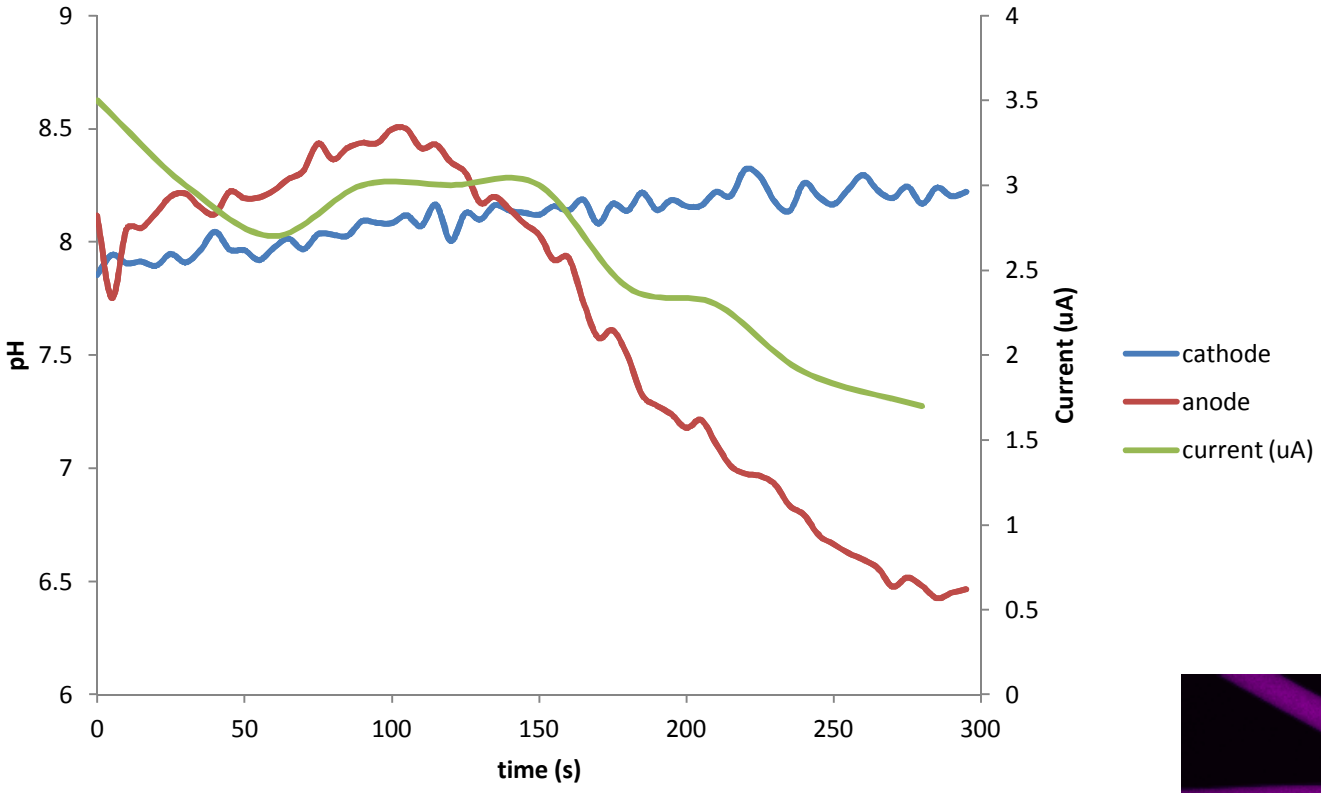
- Synthesize a denser membrane which does not allow large proteins to flow through
- Fill channels with PBS with 1% methyl cellulose



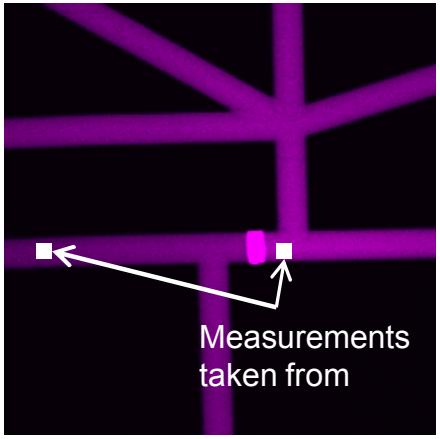
100 Volts Applied Across Membrane



pH Change

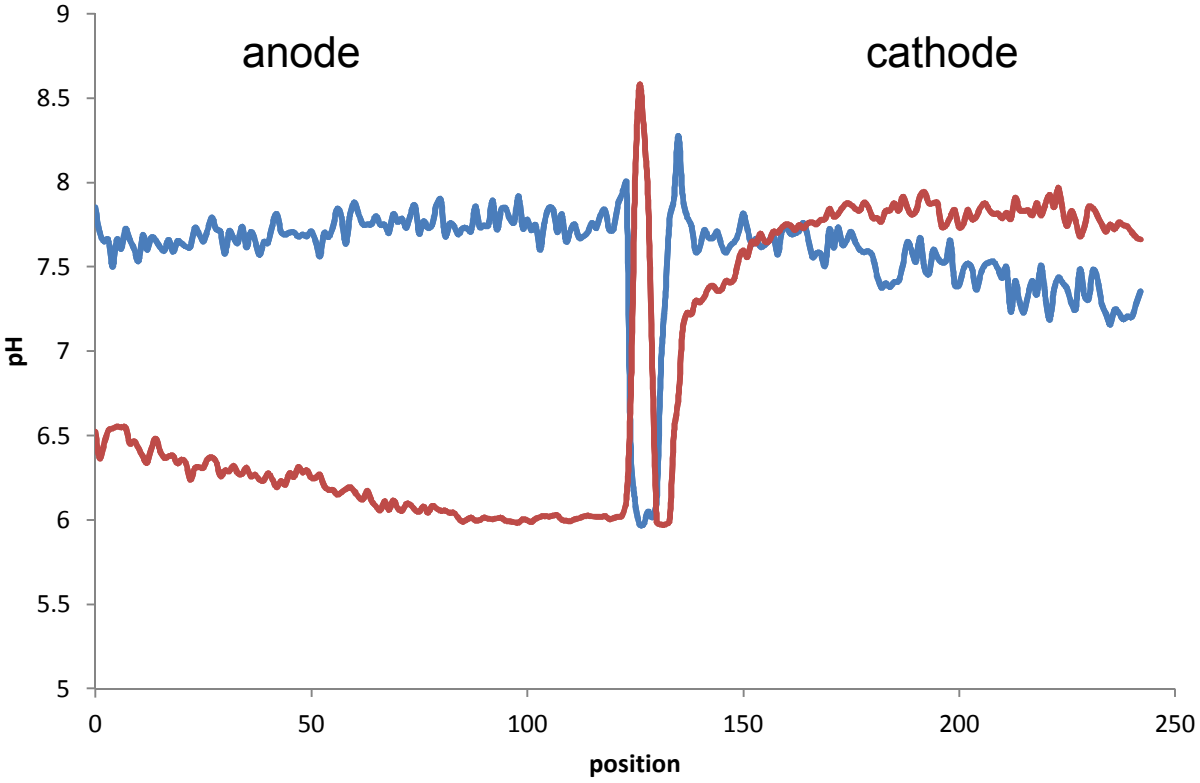


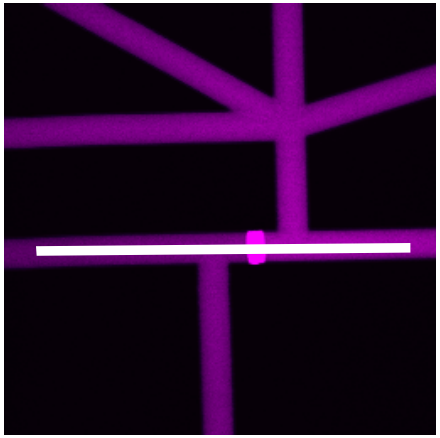
+100 V
anode



0 V
cathode

Line Profile of pH in Channel





Conclusions

The pH membranes are not greatly affected by outside changes in pH but the internal pH of the membranes is inconclusive.

The voltage does affect the pH on either side of a preconcentration membrane. There is a large decrease in pH on the anodic side and a smaller increase on the cathodic side of the membrane

Future Work

Asses the effect of charged membranes on the pH fluctuation in the preconcentration membrane.

Acknowledgements

Junyu Mai

Anson Hatch

Department of Homeland Security

Sandia National Laboratories

"Sandia is a multiprogram laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the United States Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000."

References

- Hatch, A, Herr, A, Throckmorton, D, Brennan, J, Singh, A. *Anal Chem* 2006, 78, 4976-4984.
- Timperman, A, Tracht, S, Sweedler, J. *Anal. Chem* 1996, 68, 2693-2698.
- Sommer, G, Hatch, A. *Electrophoresis* 2009, 30, 742-757.