Measuring Permeability of Lipid Membranes to H^+ and Acids

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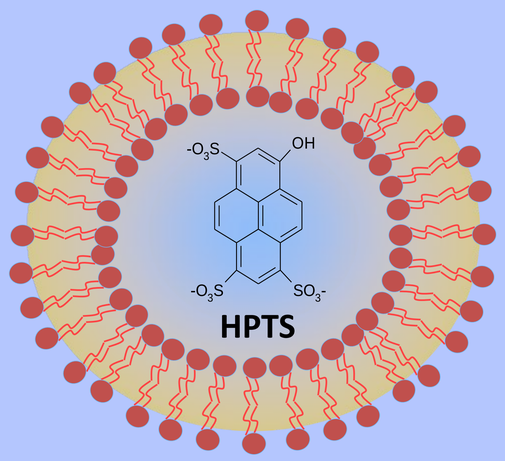
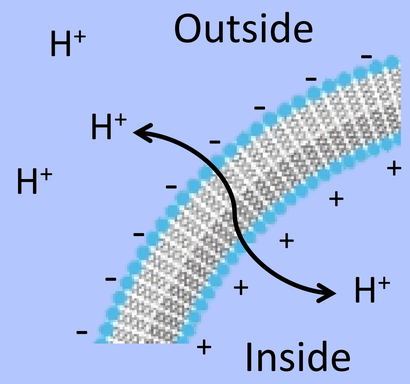
Abstract

Transport through cellular membranes plays a defining role in biological systems. The focus of this research is to study the passive permeability of lipid membranes for small molecules using lipid vesicles (liposomes) as a model. A fluorescent probe that cannot permeate through the membrane was encapsulated inside the liposomes. The spectrum of the probe is pH dependent allowing the H^+ ions concentration to be measured. Various acids and salts were added to the medium outside of the liposomes. Due to the penetration of H^+ or non-dissociated acid molecules through the membrane, the pH inside the liposomes will change. This causes the change in the spectrum of the fluorescent probe inside the liposomes, which was recorded. Phosphatidylcholine, the major component of bacterial and eukaryotic membranes, was used to measure the permeability of lipid membranes in this study.

How we measured the pH inside of the liposomes

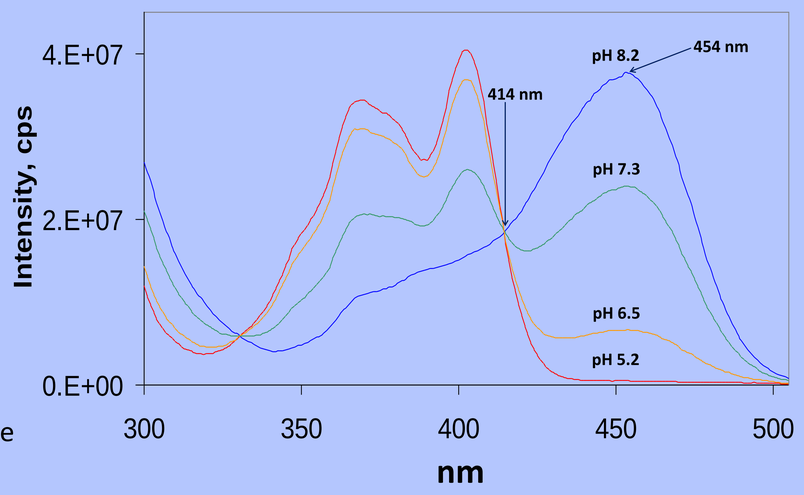


A fluorescent probe was used that can not penetrate through the lipid bilayer. HPTS is a non-penetrating probe with pH-dependent fluorescence. Liposomes were prepared in the presence of HPTS, the probe outside of the liposomes was removed by gel-filtration.



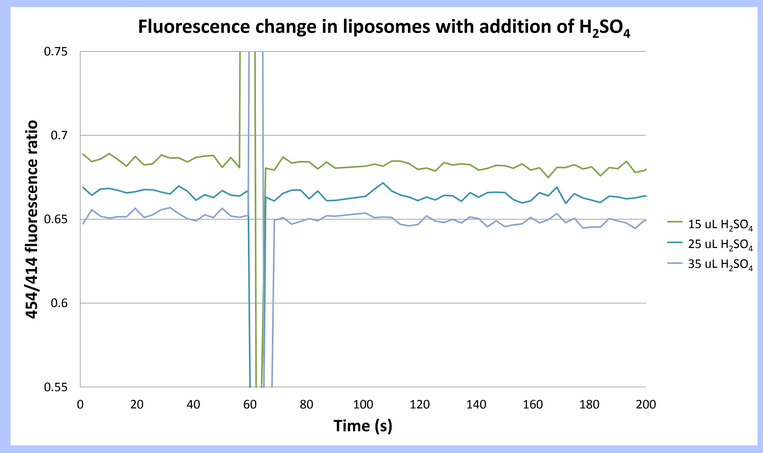
Non-penetrating acid added outside can lower the pH inside of the liposomes. As the pH is lowered outside of the liposome, the concentration of protons increase outside. Protons then begin to enter the liposome, changing the pH and causing the inside to become positively charged. Equilibrium is reached when the developed electric potential counterbalance the protons' concentration difference between inside and outside of the liposome.

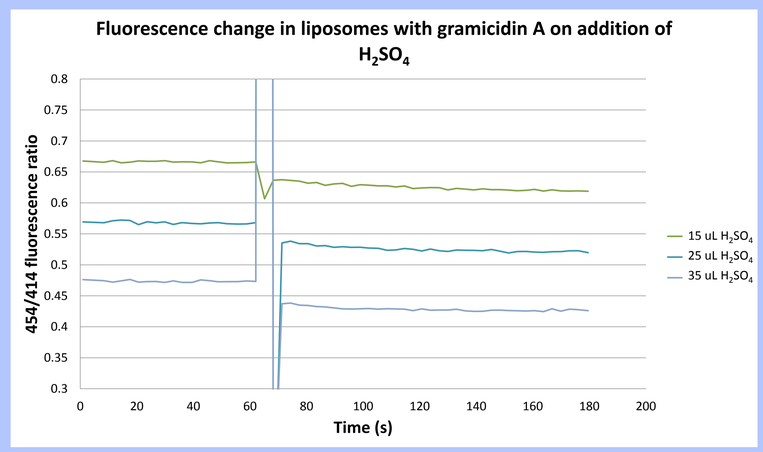
Membrane impermeable fluorescent probe Excitation spectra for HPTS, emission 510 nm



Charge-Uncompensated H^+ Penetration

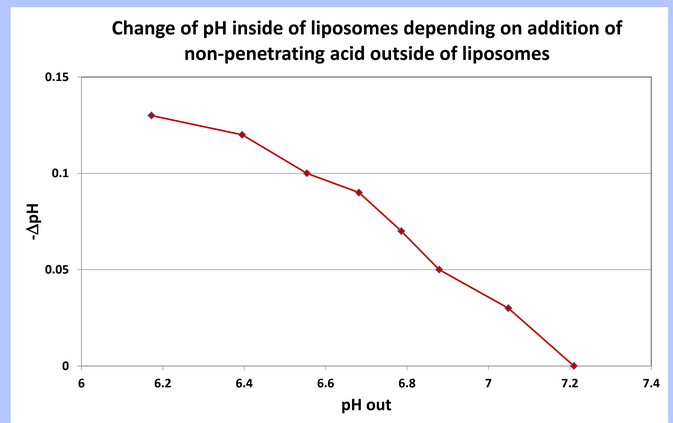
1M Sulfuric acid (H_2SO_4) was added in increments to our liposomes. The fluorescence of our liposomes was measured using a stationary fluorometer. As acid was added, the pH outside the liposomes decreased. Protons crossing the lipid membrane caused the pH inside of the liposome to decrease. Our pH sensitive probe's fluorescence changed with pH change.





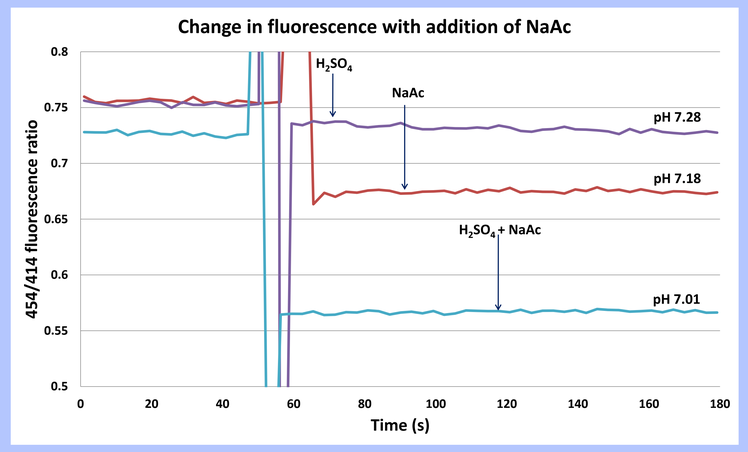
Gramicidin A makes channels in the lipid bilayer, allowing Na^+ ions to move freely out of the liposome, quenching electric potential on the membrane. In this case, pH inside and outside become equal upon acidification.

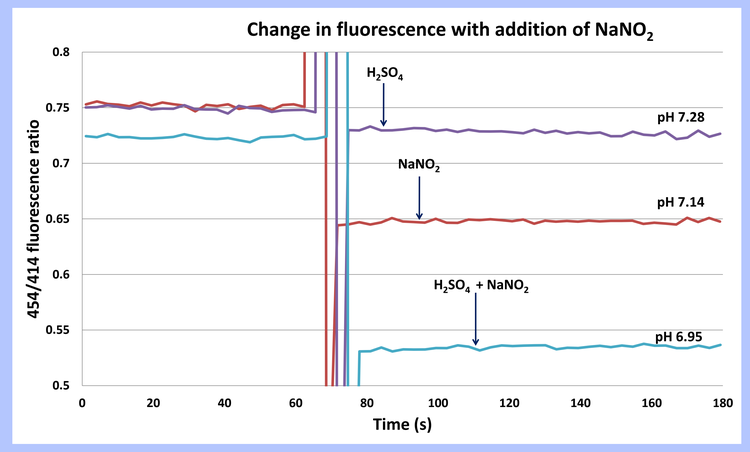
Note the change in scale.

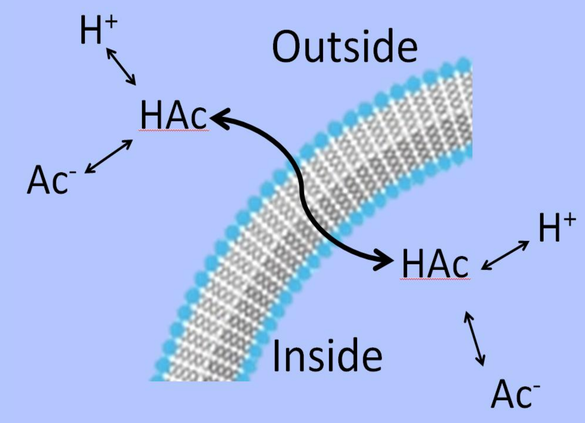


As the pH outside of the liposomes was lowered by H_2SO_4 (non-penetrating acid), pH inside was also lowered, though to a smaller extent. Calculations based on the model of flat capacitor however, predict a 30 times smaller change of inner pH than observed experimentally. Development of an adequate model is currently under way.

Fluorescence change in liposomes with addition of salts at different pH's

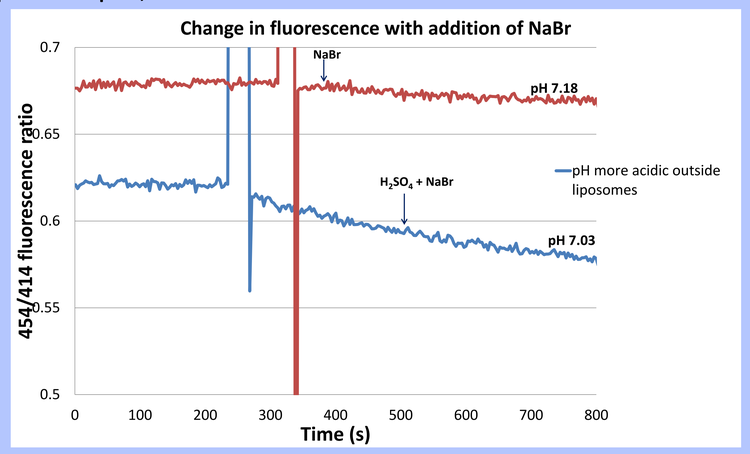


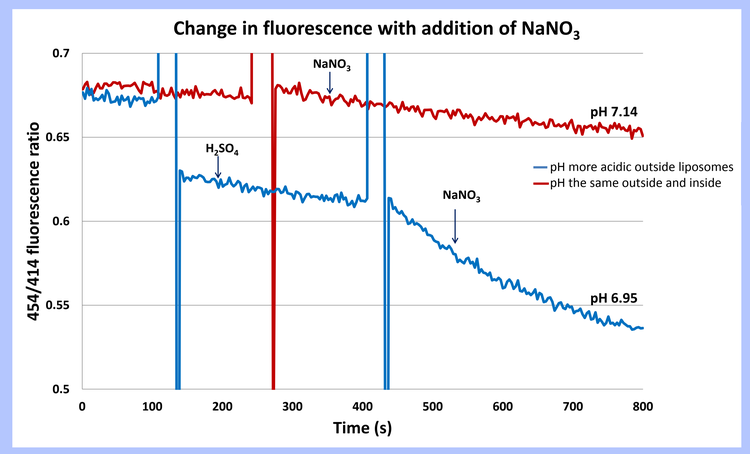




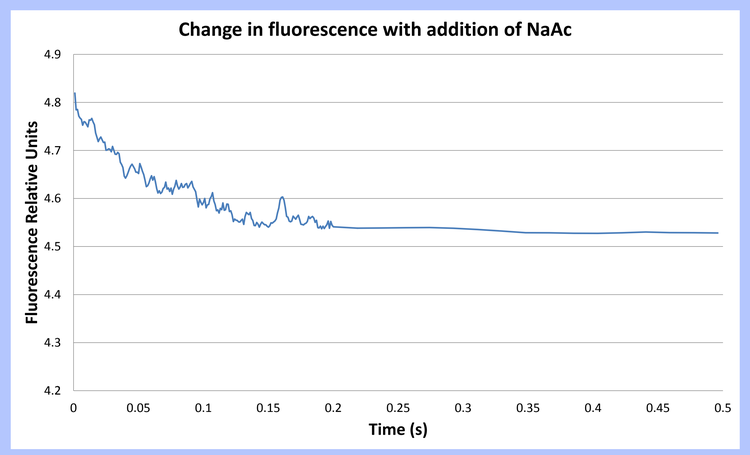
With pH the same outside and inside of the liposomes, there should be no fluorescence change upon addition of salt with the same pH. However, we see the pH inside the liposome drops when we add NaAc or $NaNO_2$ outside. This happens because non-dissociated acid (HAc or HNO_2, pK_a 4.7 and 3.14, respectively) can freely cross the membrane. If we acidify the medium outside, the inside pH drop is deeper, because the concentration of non-dissociated acid was increased.

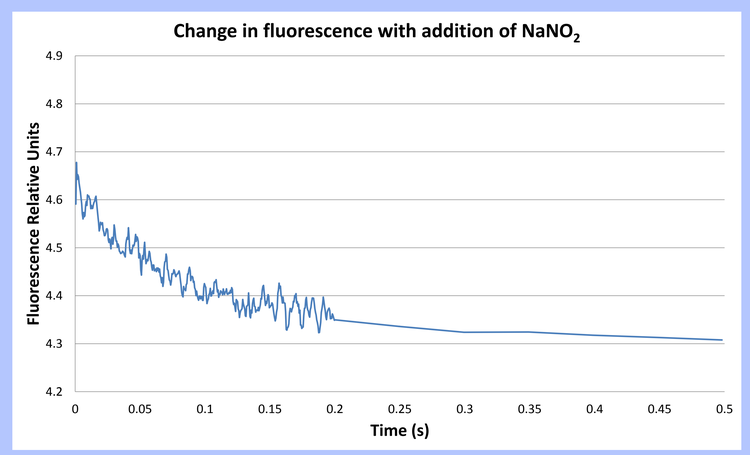
As the uncharged HAc crosses the lipid bilayer, it dissociates. This causes the pH inside the liposome to decrease.





Acidification of internal media takes much longer after addition of NaBr or $NaNO_3$ due to lower concentrations of non-dissociated acid at corresponding pH. The pK_a for HBr is -8 and the pK_a for HNO_3 is -1.44.





The change in fluorescence of liposomes was measured using the KinTek stopped flow fluorometer. This machine allows us to see the first kinetics of fluorescence change. These kinetics are usually so fast that they are not observable on the stationary fluorometer. The rate constant approximately describing the penetration of HAc or HNO_2 through lipid membrane is 14.7 s^{-1} and 8.8 s^{-1} respectively.

Future Experimentation:

Our experimental data were not adequately described by the simple model we used. Further research involving the permeability of liposomes containing HPTS probes needs to be performed and change in fluorescence measured. With this data, we hope to be able to apply similar test to membranes of thermophilic archaea to study permeability of archaeal lipid membranes for small molecules.