Electronic Supplementary Material (ESI) for Soft Matter

Hydroxide Ion Flux and pH-Gradient Driven Ester Hydrolysis in Polymer Vesicle Reactors

Walter F. Paxton,[a] Delisia Price,[b] and Nicholas J. Richardson[b]*

[a] Center for Integrated Nanotechnologies, Sandia National Laboratories, P.O. Box 5800, Albuquerque, NM 87185 USA

[b] West Houston Center for Science and Engineering, Houston Community College, 1550 Foxlake Drive, Suite 114 MC 1396 Houston, TX 77084 USA

[*]Correspondence Address
Walter F. Paxton Center for Integrated Nanotechnologies Sandia National Laboratories P.O. Box 5800 Albuquerque, NM 87185 USA Tel: +1 (505) 284 9395 Fax: +1 (505) 284 7778 E-Mail: wfpaxto@sandia.gov

Electronic Supporting Information:

Fluorimetric pH Determination

In order to determine the internal pH of vesicles, a pH vs. fluorescence calibration curve for 8-hydroxypyrene-1,3,5-trisulfonic acid (HPTS) was generated. The pH of a continuously stirred 1 mM HPTS solution in buffer was monitored with an electronic pH meter and adjusted with the addition of 1 M KOH. At regular pH intervals, 300 μL aliquots were analysed via fluorescence spectroscopy, allowing a direct correlation between pH and fluorescence excitation and emission spectra. The calibration curve (**Fig. S1**) was generated by taking a ratio of fluorescence intensity at 509 nm with 456 and 402 nm excitation (i.e., I_{456}/I_{402}) and comparing this ratio with the measured pH.[S1] The data were fit to the following equation with the same functional form as the Henderson-Hasselbalch relation:

$$pH = \frac{A3 - \log\left(\frac{A2 - I_{456}/I_{402}}{I_{456}/I_{402} - A1}\right)}{A4}, \qquad \text{(Eq. S1)}$$

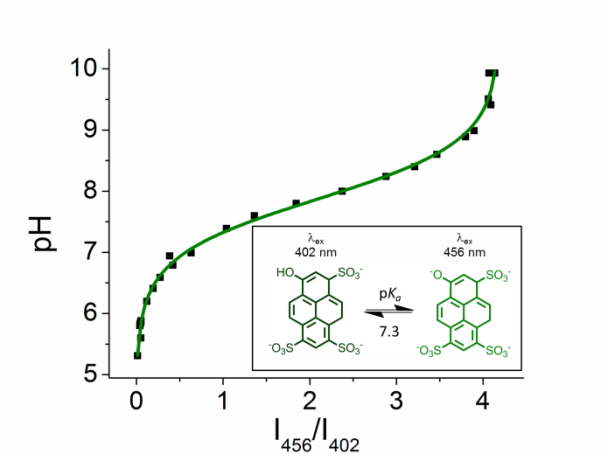

Fig. S1. pH as a function of fluorescence response for HPTS (inset) in aqueous buffer. The ratio of relative fluorescence intensities at 509 nm with an excitation of 456 nm and 402 nm, I_{456}/I_{402}, at 25 °C was used to determine the pH of the bulk solution inside liposomes and polymersomes.

with constants A1, A2, A3, A4 equal to -6×10^{-3}, 4.19, 7.2, 0.92 respectively. The internal vesicle pH was determined by acquiring excitation spectra (λ_{em} = 509 nm) of a vesicle suspension and relating I_{456}/I_{402} to pH through **Eq. S1**. This treatment allowed the determination of pH to within 0.1 units over the range 6 to 9.5. More importantly, the pH measurement was remarkably stable, with relative standard deviations on the order of 0.2% for repeated measurements.

The pH of the external buffer solution was determined from the fluorescence of residual HPTS in the final dialysis reservoir and found to be 7.1, in reasonable agreement with the pH determined with an electronic pH meter (7.0). The pH of the vesicle-entrapped solution prior to the addition of acid was also determined via fluorescence. The internal pH of the different vesicle suspensions varied (DOPC = 7.01; DOPS = 7.14; $EO_{20}BD_{33}$ = 6.80), which may reflect the subtle differences in headgroup/HPTS interactions.

OH^- Permeability Measurements

In order to determine the flux of OH^- across vesicle membranes, we determined the initial pH inside the vesicles suspended in 250 μL of buffer solution, and then added 20 μL of an isotonic aqueous KOH solution (350 mOsm) at t = 0. Excitation spectra for the sample were recorded periodically for at least 30 min. The change in the ratio of fluorescence with an excitation of 456 nm versus 402 nm, i.e. I_{456}/I_{402}, was related to the change in pH using the calibration curve for HPTS fluorescence response versus pH (**Fig. S1**), allowing the pH (and $[OH^-]$) of the internal vesicle solution to be determined over the course of an experiment. We then fit the internal $[OH^-]$ data for each run to a line using regression analysis. To allow a more direct comparison of the OH^- flux across the different membranes, only data after 1000 s from the time KOH was added to the sample were included in the regression analysis.

The pH of an aqueous solution reflects the equilibrium concentration of hydroxide at any given time. The buffering capacity of the solution (e.g., containing 25 mM PIPES), however, requires that substantially more OH^- crosses the membrane to elicit the observed change in pH. Because $d[OH^-]/dt$ is approximately

linear beyond 1000 s, we calculated the equilibrium hydroxide concentration after 1000 s and at the end of the experiment (at least 1000 s later) from the linear fit, and then determined the amount of hydroxide ion that must be added to elicit the observed change in equilibrium pH over the course of the experiment according to **Eq. S2**:

$$\frac{\Delta n_{OH^-}}{\Delta t} = \frac{([OH^-]_{final} + [PIPES]_{final} - [PIPES]_{initial})}{\Delta t} \times V_{ave} \qquad \textbf{(Eq. S2)}$$

where Δn_{OH^-} is the amount in moles of hydroxide ion that crosses the vesicle membrane, $[OH^-]_{final}$ and $[PIPES]_{final}$ are the final equilibrium concentrations determined from the Henderson-Hasselbalch equation using pK_{b2} = 7.24 for PIPES, and $[PIPES]_{initial}$ is the equilibrium concentration of PIPES at t = 1000 s, Δt is the elapsed time in seconds, and V_{ave} is the average internal vesicle volume calculated from the average vesicle diameter measured by DLS. The average internal volume consists of the total vesicle volume minus the volume occupied by the hydrophobic core of the vesicle membrane.[S2] We used the thicknesses of lipid membranes determined by Nagle et al. (DOPC = 2.7 nm,[S3]; DOPS = 3.0 nm, ref [S4]), and the polymer bilayer thickness was estimated from models described by Klein et al.[S5] ($EO_{20}BD_{33}$ = 6.8 nm, $EO_{89}BD_{120}$ = 22 nm). This treatment yields the net rate of transfer of OH^- across a vesicle membrane, $\Delta n_{OH^-}/\Delta t$, which may be converted to the flux per unit area of membrane (**Eq. S3**):

$$J_{OH^-} = \frac{\Delta n_{OH^-}}{\Delta t} \times \left(\frac{1}{S_{ave}}\right) \qquad \textbf{(Eq. S3)}$$

where S_{ave} is the average vesicle surface area from the DLS measured vesicle diameter.

For transport across a membrane bilayer according to the solubility-diffusion mechanism, the flux of a given solute is proportional to the permeability coefficient of the rate limiting species diffusing across the membrane, P, and the concentration difference across the barrier, Δc (**Eq. S4**, manuscript **Eq. 1**). The flux can also be expressed in terms of an apparent diffusion coefficient:

$$J = P\Delta c = D^* \frac{\Delta c}{d} \qquad \textbf{(Eq. S4)}$$

where D^* is the product of the solute bulk diffusion coefficient, D, and the partition coefficient, K, of the solute in a water/hydrocarbon (headgroup/tail) system, and d is the average thickness of the bilayer.

In our experiments, the permeability coefficient, P, and the apparent diffusion coefficients, D^*, were determined from the measured hydroxide ion flux and the difference in hydroxide ion concentration across the vesicle membrane. This concentration difference in our experiments was 2.0 mM, and while there was a corresponding gradient of H^+ in the opposite direction, it was more than four orders of magnitude lower than the OH^- gradient over the experimental pH range (~7–10 inside the vesicles), and therefore not expected to make any substantial contribution to the observed changes in pH.

Curve Fitting

Data fits for the HPTS calibration and the OH^- flux data were performed using OriginPro (v8.5.1 SR1).

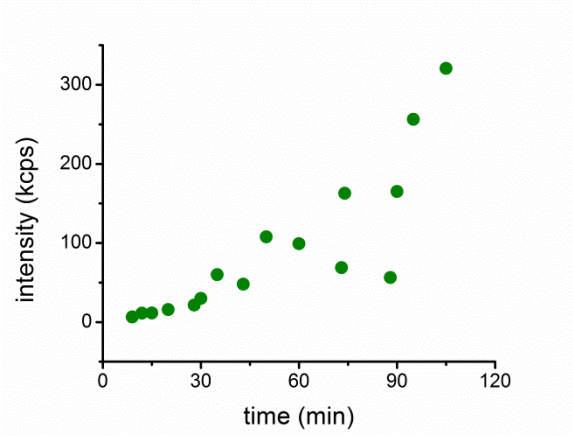

Fig. S2. Background corrected fluorescence intensity of individual PEO-PBD vesicles (~5 μm diameter) with $pH_{internal}$ >10 and $pH_{external}$ = 3 in the presence of 5 uM fluorescein diacetate (FDA) obtained from fluorescence images. Data point values were calculated by subtracting the background fluorescence intensity from the average fluorescence intensity of the vesicles.

Fluorescein Diacetate (FDA) Kinetics Experiments

A $EO_{20}BD_{33}$ suspension in 1.0 M KOH was prepared and the pH of the external solution was adjusted to pH = 3 with sulphuric acid (final concentration: 1 mM $EO_{20}BD_{33}$). To ~1 mL of this suspension, 2 μL of FDA solution (1 mg/mL in DMSO) was added and the fluorescence of the sample was monitored by fluorescence microscopy for at least 1.5 h (final concentration: 4 μM FDA). We measured the intensity of individual vesicles in each frame and the background fluorescence intensity, which included a contribution of fluorescence from out-of-focus vesicles as well. To minimize effects of photobleaching, each data point represents the measurement of a different unique vesicle. Because fluorescence intensity varied with vesicle size, only vesicles between 4 and 5 μm in diameter were included in the data set. The fluorescence intensity of each vesicle was calculated by subtracting the fluorescence intensity of the background (i.e. CorrrectedVesicleIntensity = RawVesicleIntensity – RawBackgroundIntensity). We plotted the background corrected polymer vesicle intensity in the presence of FDA over the course of ~100 minutes (**Fig. S2**). The observed trend indicates a significant increase in fluorescence intensity over the background fluorescence intensity, consistent with the enhanced hydrolysis occurring inside the polymer vesicles.

For comparison, we fluorometrically measured the relative rates of hydrolysis of FDA at pH = 7 and pH = 3, as well as the rate of hydrolysis at pH = 3 in the presence of polymer vesicles ($EO_{20}BD_{33}$) with an initial internal pH of 14 (**Fig. S3**). For pH control samples, the pH of a sample of deionised water was adjusted to the desired pH with a minimal amount of NaOH or H_2SO_4, and 10 μL of FDA solution (1 mg/mL in DMSO) was added to 990 μL of the pH-adjusted solution. For experiments with polymer vesicles, 100 μL of $EO_{20}BD_{33}$ in 1 M KOH (20 mg/mL polymer) was combined with 500 μL of deionised water and 400 μL of 0.1 M H_2SO_4. To this sample, 2 μL of FDA solution (1 mg/mL in DMSO) was added. The fluorescence of each sample (λ_{ex} = 468 nm; λ_{em} = 509 nm) was monitored at regular intervals for at least 100 minutes, beginning immediately after the addition of the FDA. The data were corrected for differences in FDA concentration.

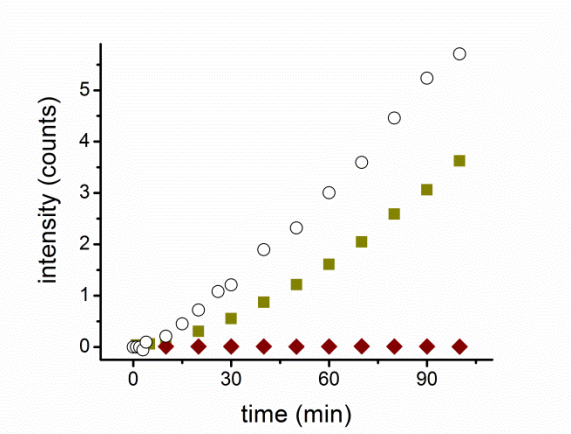

Fig. S3. The hydrolysis of fluorescein diacetate at pH = 7 (squares) and pH = 3 (diamonds) compared to the hydrolysis at pH = 3 in the presence of $EO_{20}BD_{33}$ vesicles with an initial internal pH of 14 (open circles).

In pH = 3 samples, FDA hydrolysis at ANY rate was not observed. Nevertheless, when polymer vesicles containing high pH aqueous solutions were present in pH = 3 samples, the hydrolysis of FDA is significantly faster than even FDA hydrolysis in pH = 7 solution.

References

S1. K. Kano, J. H. Fendler, *Biochim. Biophys. Acta*, **1978,** *509*, 289.
S2. J. Wu, A. Eisenberg, *J. Am. Chem. Soc.*, **2006,** *128*, 2880.
S3. Y. F. Liu, J. F. Nagle, *Phys. Rev. E*, **2004,** *69*.
S4. H. I. Petrache, S. Tristram-Nagle, K. Gawrisch, D. Harries, V. A. Parsegian, J. F. Nagle, *Biophys. J.*, **2004,** *86*, 1574.
S5. G. Srinivas, D. E. Discher, M. L. Klein, *Nat. Mater.*, **2004,** *3*, 638.