SAND2017-12334C

Monitoring and Modulating Ion Traffic in Hybrid Vesicles

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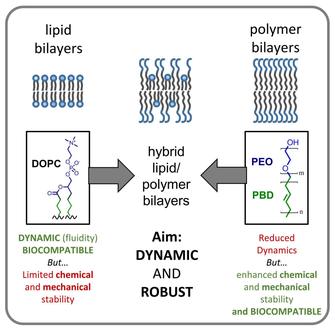
Introduction

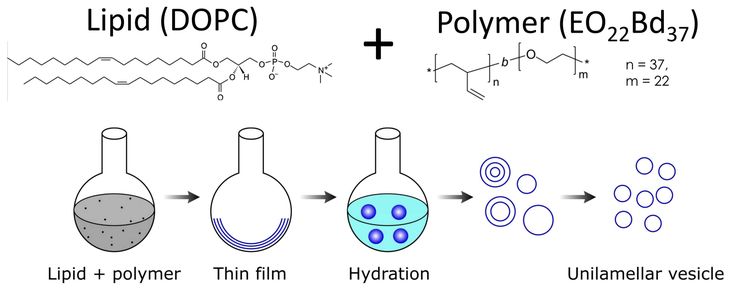
Controlling the traffic of molecules and ions across membranes is a critical feature in a number of biologically relevant processes and highly desirable for the development of technologies based on membrane materials.

Membranes that combine the advantages of lipid and polymer-based vesicles and incorporate membrane transport proteins may also be highly desirable for artificial organelles or other compartmentalized structures capable of controlling molecular traffic.

We looked at transport behavior across hybrid lipid/polymer membranes in the absence and presence of ion transfer agents.



Hybrid Lipid/Polymer Vesicles



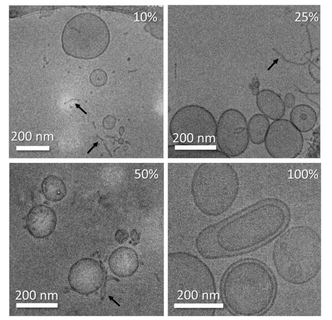


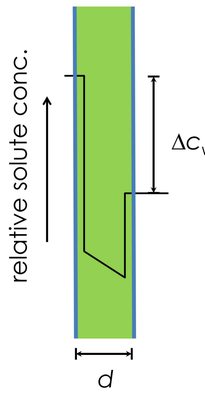
Table 1. Vesicle size distributions by DLS and cryo-EM.

Sample	DOPC (moles)	$EO_{22}Bd_{37}$ (moles)	DLS Size[a] (nm)	Cryo-EM Size[b] (nm)	Bilayer thickness[c] (nm)	worm diameter[c] (nm)	v:w ratio[d]
0%	100%	0%	170±50	180±40	6.7±0.6	n/a	n/a
10%	90%	10%	150±30	140±60	6.5±0.3	10.9±1.1	1.2
25%	75%	25%	150±30	140±60	6.5±0.6	11.4±0.9	0.46
50%	50%	50%	200±30	180±60	8.3±0.6	12.2±0.7	0.50
100%	0%	100%	210±20	250±70	9.4±0.5	n/a	n/a

[a]Z-average diameter from cumulants analysis ± standard error from at least 3 measurements. [b]average diameter ± standard error of >25 vesicles from image analysis of cryo-EM images. [c]average vesicle bilayer thickness or diameter ± standard error of >10 vesicles or worm-like micelles observed in cryo-EM images. No worms were observed in the samples containing pure lipid (0%) or the pure polymer (100%) vesicles. [d]ratio of the number of vesicles to the number of worms observed in cryo-EM images of hybrid vesicles.

Monitoring Vesicle Permeability

Permeability Model



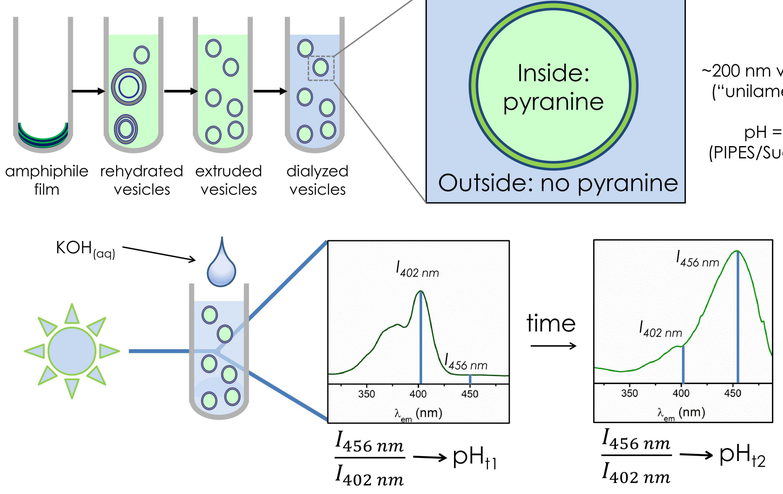
Solubility-Diffusion Mechanism

$$P = \frac{DK}{d} = \frac{J}{\Delta c_w}$$

P = permeability coeff.
D = diffusion coeff.
K = partition coeff.
d = bilayer thickness
J = solute flux
Δc_w = solute gradient

$$J_{OH^-} = \frac{\Delta n_{OH^-}}{\Delta t} \times \left(\frac{1}{S_{ave}}\right)$$ OH⁻ flux across a membrane

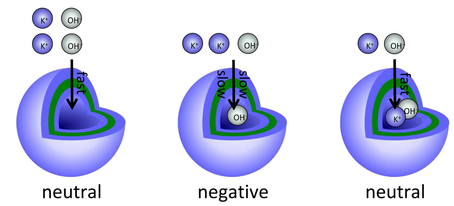
Monitoring H^+/OH^- flux



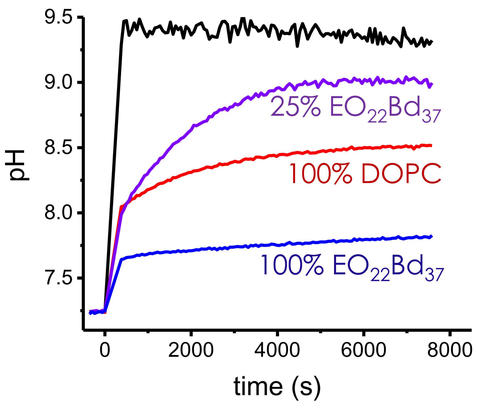
~200 nm vesicles ("unilamellar")

pH = 7 (PIPES/Sucrose)

pH flux reflects K^+ flux

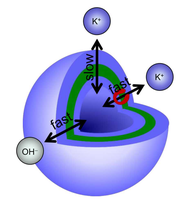


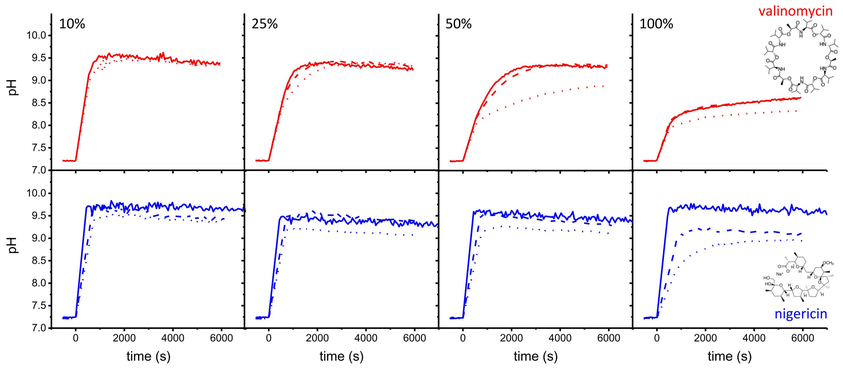
1) Permeability of OH^- is FAST → buildup of negative charge
2) Negative charge compensated by flux of the K^+ counterion…
3) …but permeability of K^+ is SLOW.
4) K^+ flux is the rate limiting step for net flux of OH^-



Internal vesicle pH determined from pyranine fluorescence intensity ratio of emission at 510 nm with 460 and 405 nm (i.e., $I_{\lambda ex=460}/I_{\lambda ex=405}$) excitation for pure DOPC vesicles (red) pure polymer vesicles (blue), and DOPC with 25 mol% $EO_{22}Bd_{37}$ (purple). All samples in 25 mM PIPES / 300 mM sucrose / 0.02% NaN_3. The addition of 5 µL of 22 mM KOH to 50 µL of vesicles suspension at t = 0 s caused an initial jump in pH followed by a more gradual change.

Incorporating Valinomycin and Nigericin

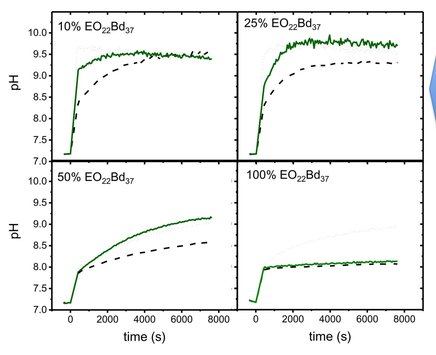




pH response of DOPC vesicles with 10%, 25%, 50% or 100% $EO_{22}Bd_{37}$ w/valinomycin (red traces) or nigericin (blue traces). In these experiments the concentration of either valinomycin or nigericin was varied: 0.027 mM (solid traces); 0.0054 mM (dash traces); and 0.0011 mM (dot traces).

The pH response of hybrid vesicles can be modulated by varying the concentration of iono-phore.

Integrating Gramicidin A

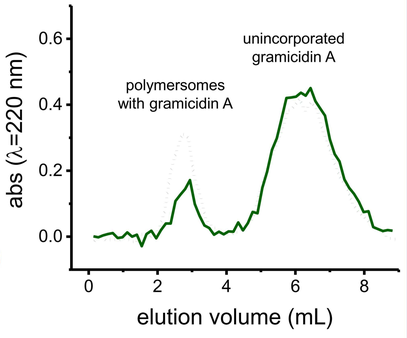


Internal vesicle pH with gramicidin A for hybrid DOPC/polymer vesicles comprised of 10%, 25%, 50%, and 100% $EO_{22}Bd_{37}$ without gramicidin A (dashes) after incubating with gramicidin A (solid green) and after rehydrating with gramicidin A (dotted green).

Gramicidin A in suspensions of polymer vesicles fractionated through a PD-10 buffer exchange column:

- **Incubated** for 20 min in PIPES buffer solution containing 0.0027 mM gramicidin A solution (solid line)
- **Rehydrated** in PIPES buffer solution containing 0.0027 mM gramicidin A and then extruded (dotted line).

Vesicles eluted at ~2 mL while the unincorporated gramicidin A eluted after 5 mL.



Summary

- Hybrid lipid/polymer vesicles MORE permeable than liposomes or polymersomes
- Ion permeability in hybrid vesicles modulated by incorporating ion transfer agents
- Gramicidin A reconstitution enhanced via long rehydration vs. short incubation.

Paxton, W. F.; McAninch, P. T.; Achyuthan, K.; Shin, S. H. A.; Monteith, H. L. "Monitoring and Modulating Ion Traffic in Hybrid Lipid/Polymer Vesicles," Colloids and Surfaces B: Biointerfaces 2017, 159, 268-276.

Why it matters…

The utility of hybrid lipid/polymer bilayers in practical applications can be greatly enhanced through an understanding of the physical properties of these membranes. The work presented here reveals counter-intuitive permeability in hybrid systems. These results highlight some important features of hybrid lipid/polymer vesicles that ought to be taken into consideration when designing dynamic and functional systems. Strategies to incorporate biofunctional molecules and facilitate their activity in synthetic systems will be highly desirable for developing artificial organelles and other biomimetic membrane-based technologies for energy transduction, sensor development, and therapeutics.

This work was performed, in part, at the Center for Integrated Nanotechnologies, an Office of Science User Facility operated for the U.S. Department of Energy (DOE) Office of Science. Sandia National Laboratories is a multi-mission laboratory managed and operated by National Technology and Engineering Solutions of Sandia, LLC., a wholly owned subsidiary of Honeywell International, Inc., for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-NA-0003525.

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