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Los Alamos National Laboratory · Sandia National Laboratories

CINT

The Center for Integrated Nanotechnologies

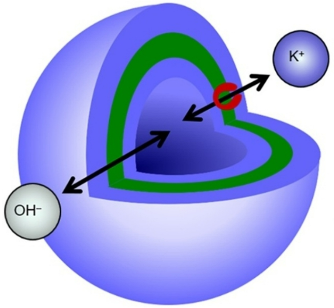
Nanomaterials *Integration*

A U.S. DOE Nanoscale Science Research Center

Monitoring and Modulating Ion Traffic in Hybrid Lipid/Polymer Vesicles

Walter F. Paxton, *Patrick T. McAninch, Komandoor E. Achyuthan, Sun Hae Ra Shin, Haley L. Monteith*

(CINT / SNL)



255th ACS National Meeting
New Orleans, LA / March 19, 2018

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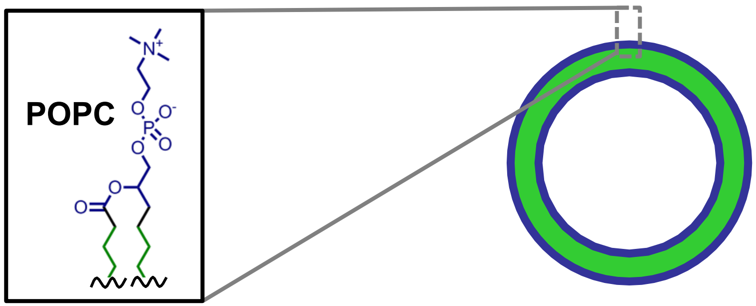
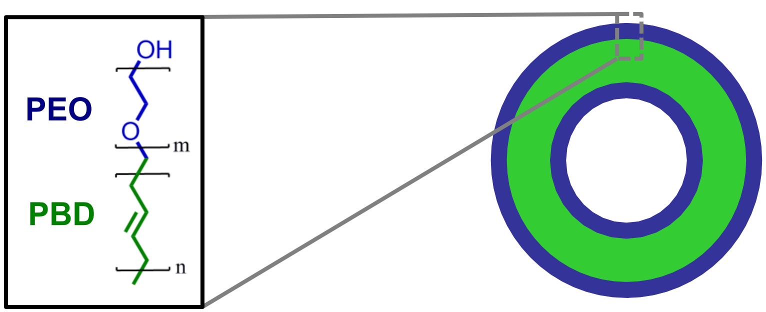
Mimicking Biological Membranes

Liposomes



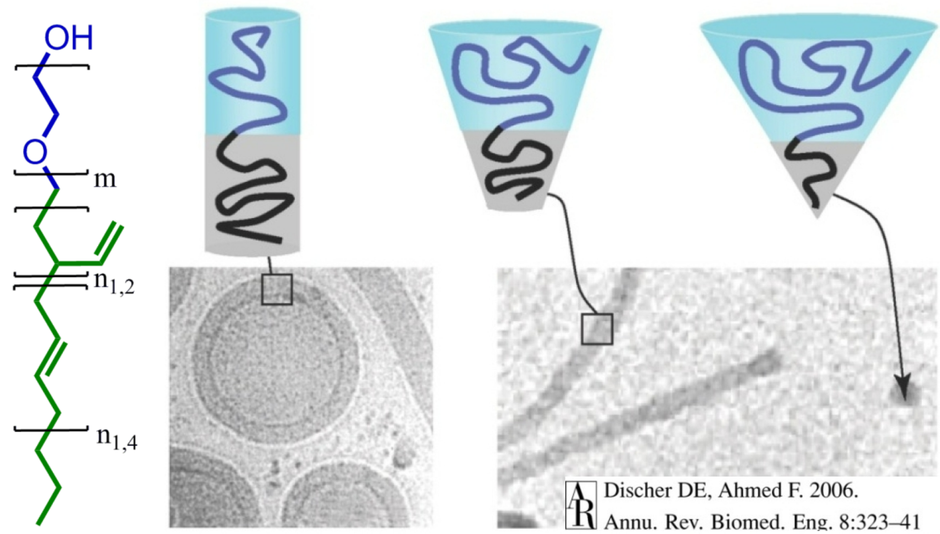
2 Major Challenges:
Limited Chemical and Mechanical Stability
Limited Modification Chemistries

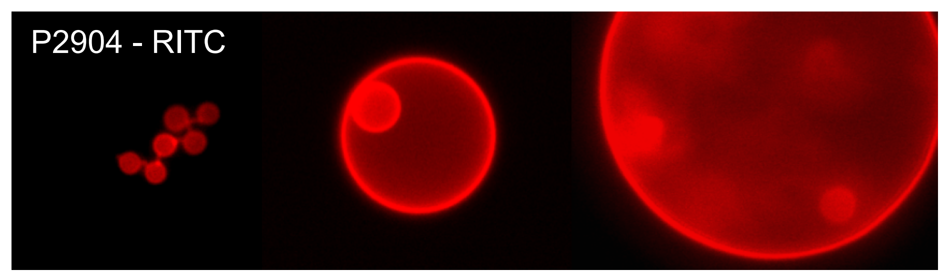
Polymersomes



Polymersomes Can Help
Enhanced Chemical and Mechanical Stability
Unlimited Modification Chemistries

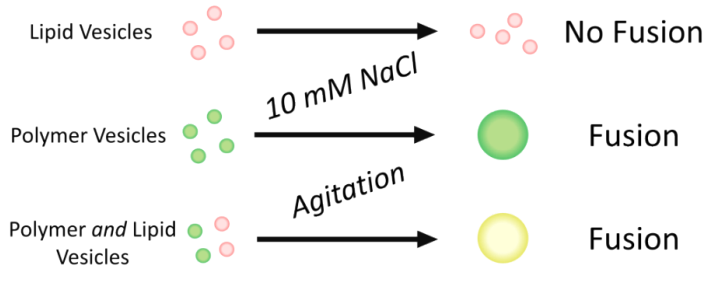
Can we incorporate or mimic **properties** and **functions** of biological cells to create robust **advanced materials**?





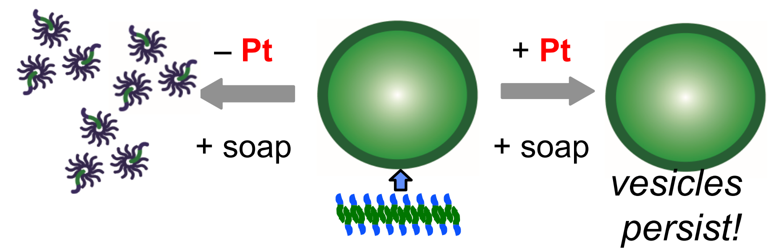
Dynamic Polymer Vesicle Membranes

Mechanically-Activated *Fusion*



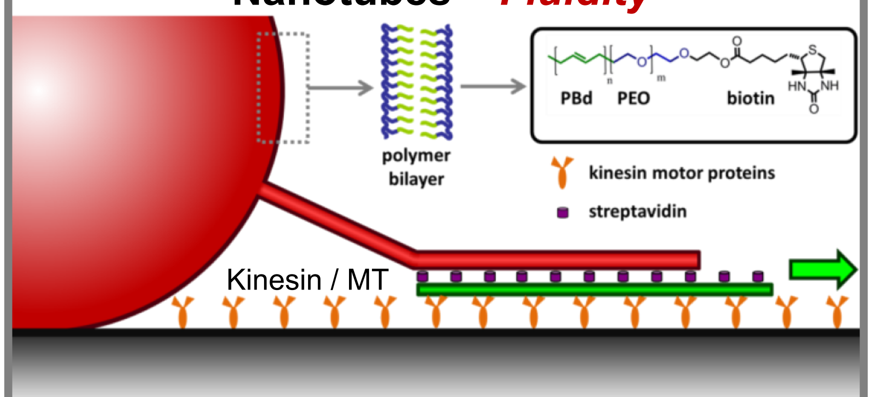
Angew. Chem. – Int. Ed. ***2014,*** *53,* 3372-3376; *J. Poly. Sci. B,* **2014,** *53,* 297–303

Catalytically-Active Cross-Links (*Reactivity and Stability*)



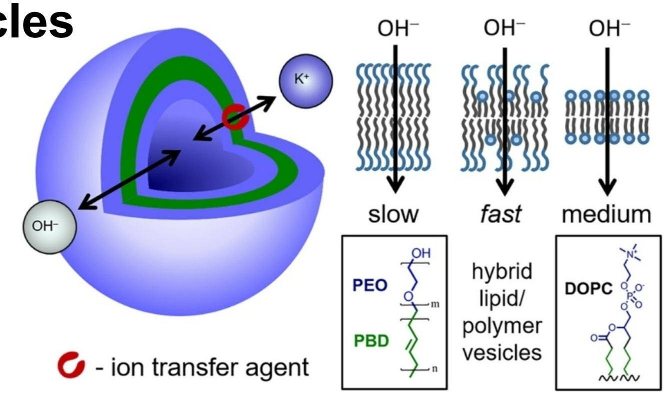
Chem. Mater, **2015,** *27, 4808–4813*

Dynamic Assembly of Polymer Nanotubes – *Fluidity*

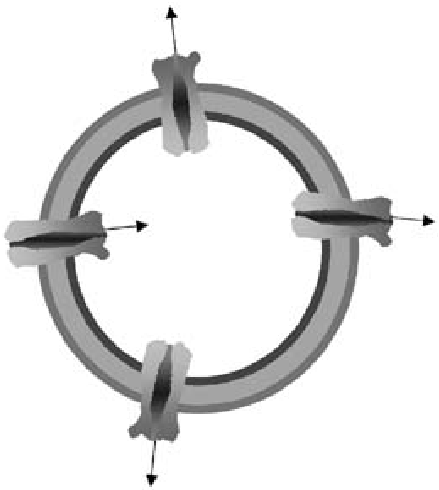


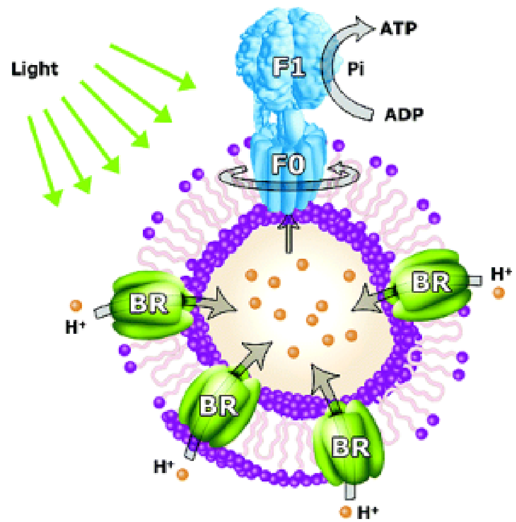
Nanoscale, **2015,** *7,* 10998–11004

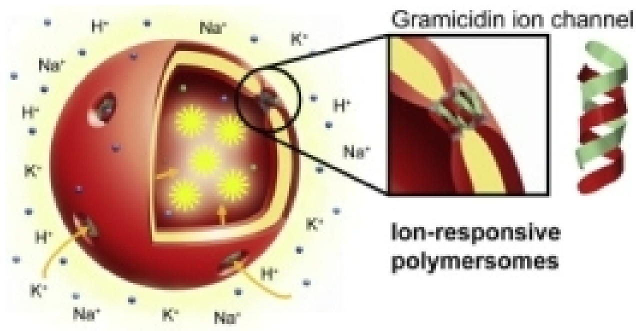
Modulating *Permeability* in Hybrid Vesicles



Colloids and Surfaces B, **2017,** *159,* 268–276





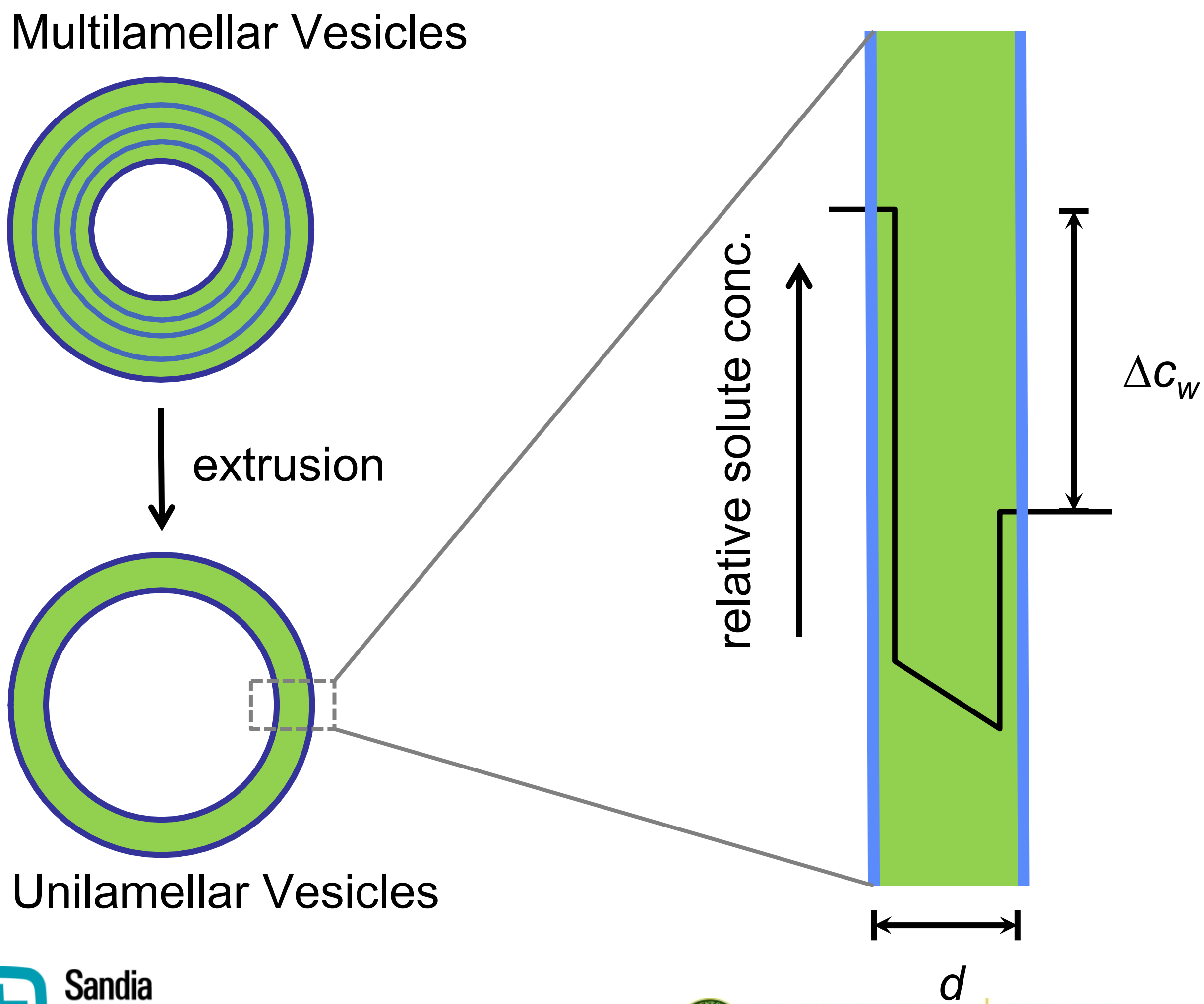


Gramicidin A (Palivan et al, 2015)

Bacteriorhodopsin
F1-ATP-ase (Montemagno et al, 2005)

Aquaporin (Meier et al, 2004)

Vesicle Permeability Model



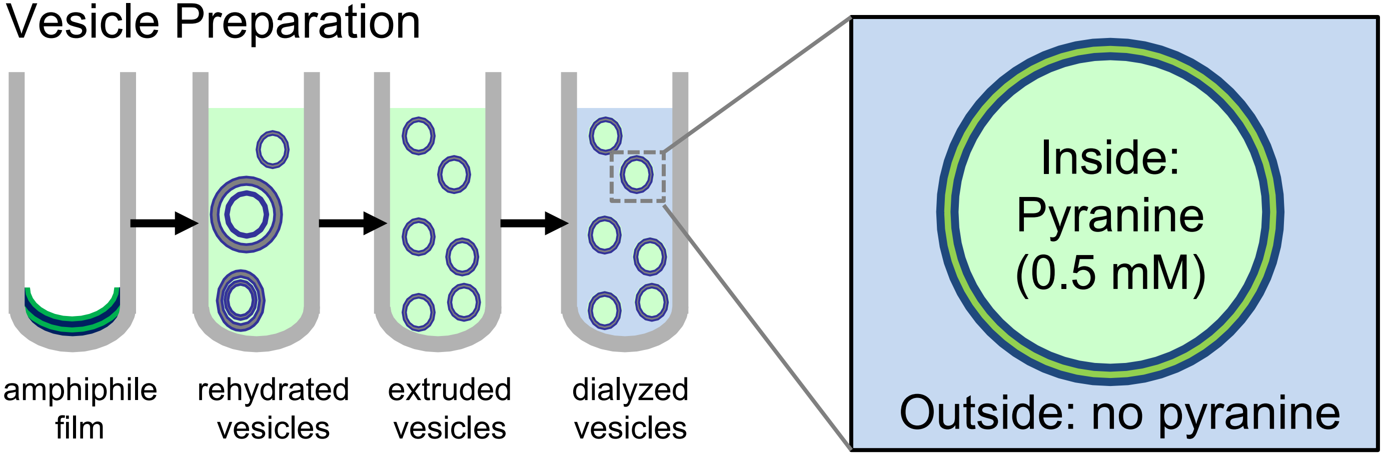
Solubility-Diffusion Mechanism

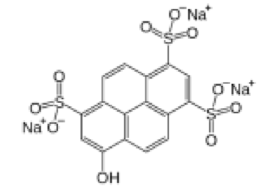
$$\boldsymbol{P} = \frac{DK}{d} = \frac{J}{\Delta c_w}$$

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

Ion Flux Monitored by Fluorescence

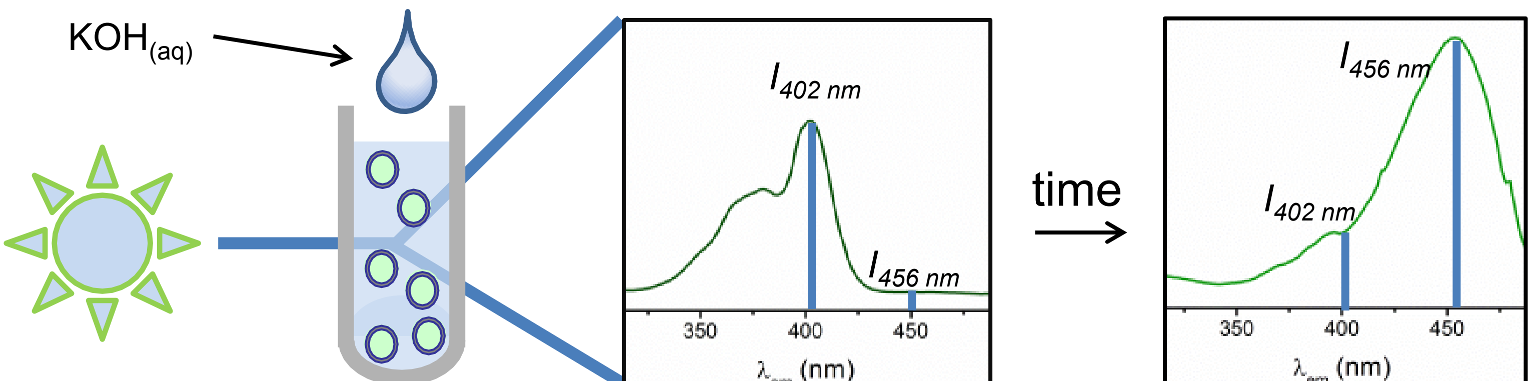
Vesicle Preparation





~200 nm vesicles (“unilamellar”)

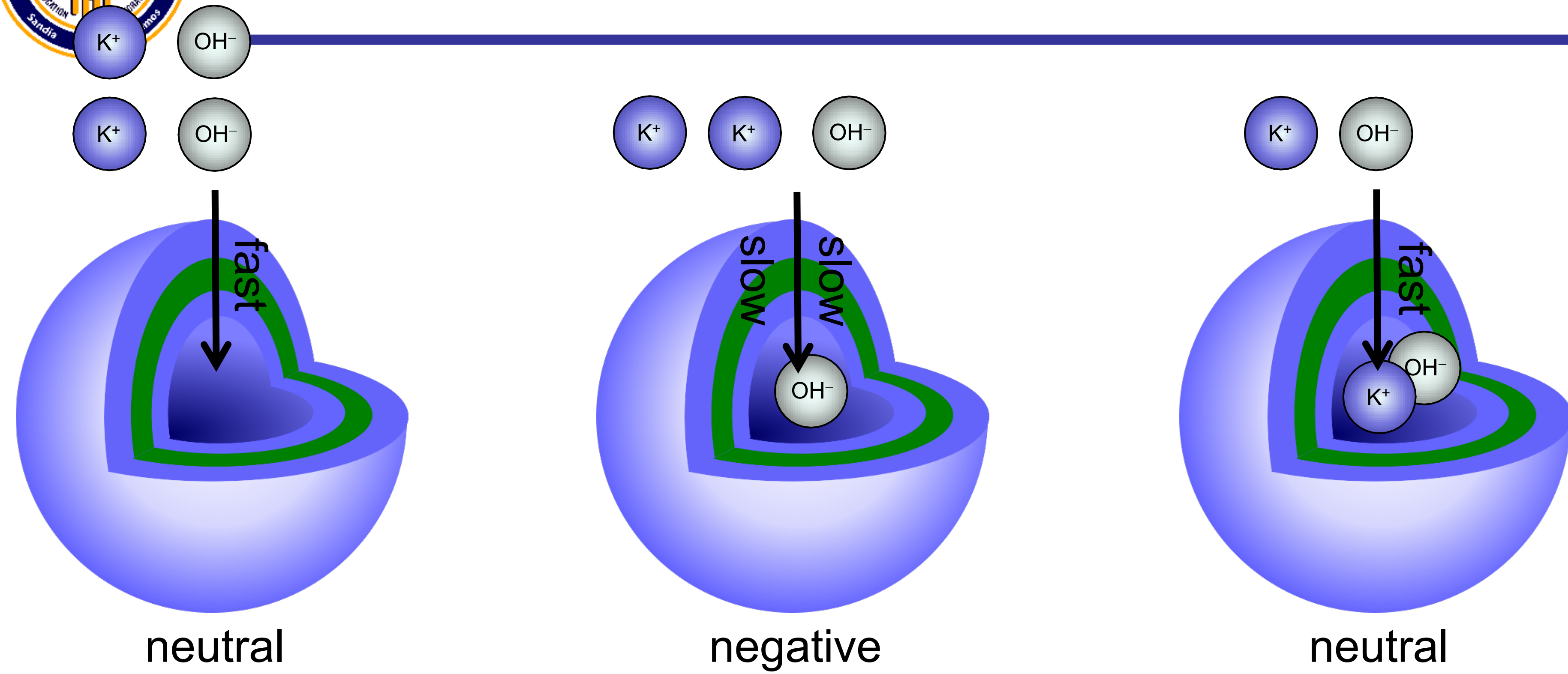
pH = 7 (PIPES/Sucrose)



$$\frac{I_{456\ nm}}{I_{402\ nm}} \rightarrow pH_{t1}$$

$$\frac{I_{456\ nm}}{I_{402\ nm}} \rightarrow pH_{t2}$$

Measuring Ion Flux in Vesicles

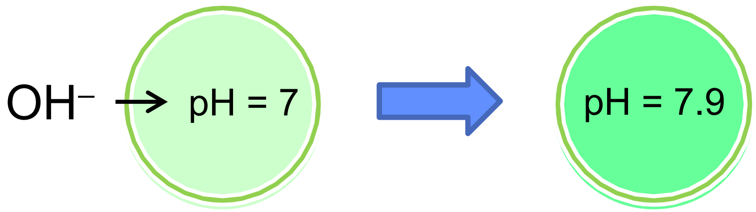


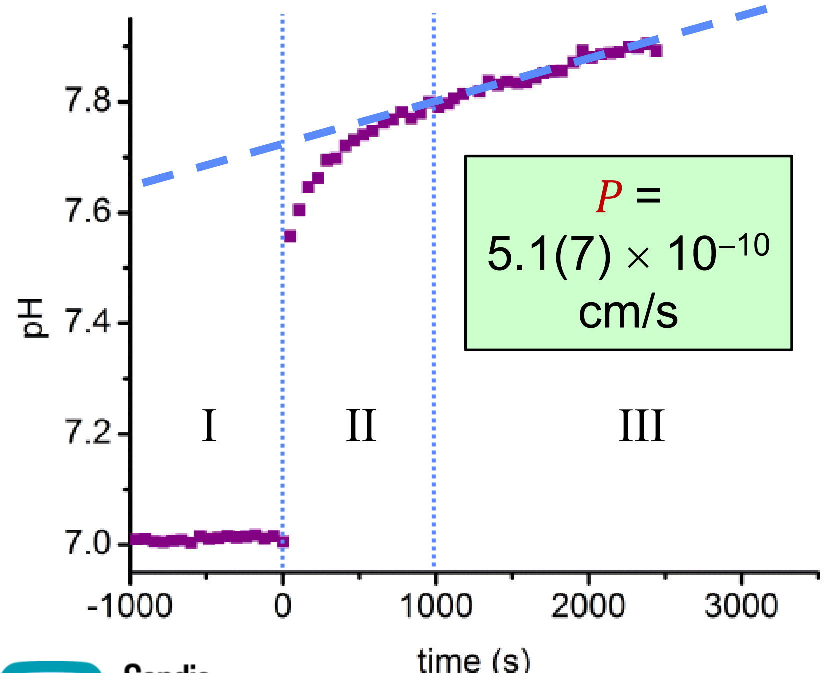
1. Permeability of OH^- is FAST → buildup of negative charge
2. Negative charge compensated by flux of the K^+ counterion…
3. …but permeability of K^+ is SLOW.
4. K^+ flux is the rate limiting step for net flux of OH^-

pH can be used to determine flux of K^+

Calculating Lipid Permeability

DOPC





$$\frac{\Delta n_{OH^-}}{\Delta t} = f([OH^-])$$

via Henderson-Hasselbalch

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

OH^- flux across a membrane

$$\boldsymbol{P} = \frac{J}{\Delta c_w}$$

Effective permeability coeff.

$$\boldsymbol{D^*} = DK = \frac{J_{OH^-}}{\Delta c_w}$$

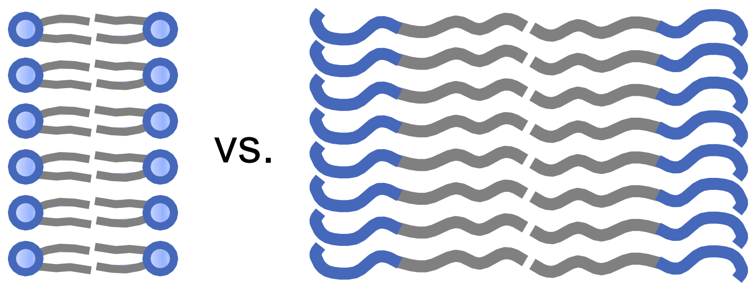
Effective diffusion coeff.

Vesicle Permeability Summary

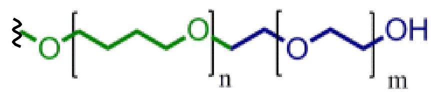
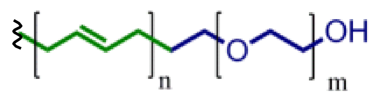
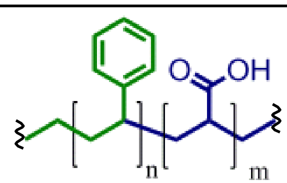
Sample	Vesicle diameter (nm)[a]	Membrane Thickness (nm)	P_{OH^-} ($\times 10^{10}$ cm/s)	D* ($\times 10^{16}$ cm^2/s)
DOPS	170(30)	2.6[b]	5.4(8)	1.4(2)
DOPC	190(30)	2.7[c]	5.1(7)	1.4(2)
$EO_{20}BD_{33}$	200(40)	6.8[d]	1.5(3)	1.0(2)
$EO_{89}BD_{120}$	260(70)	22[d]	0.4(1)	0.9(2)

[a] DLS. [b,c] Literature values. [d] Estimated from published models.

Permeability ↔ Thickness



Permeability
↕
Chemical Composition

Polymer		Thickness (nm)	P_{A^-} (cm/s)	D* (cm^2/s)
PEO-PBO[a]		2.4	10^{-7}	10^{-14}
PEO-PBD		6.8	10^{-9}	10^{-16}
PS-PAA[b]		33	10^{-13}	10^{-18}

[a] Battaglia et al. **2006**; [b] Eisenberg et al. **2006**

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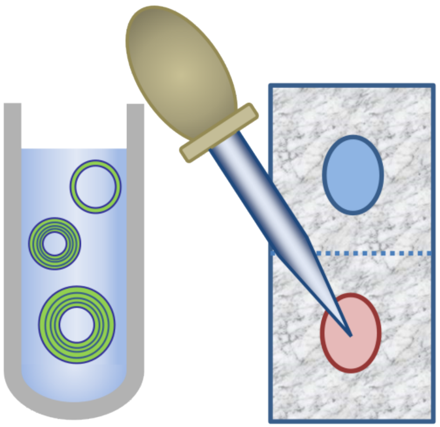
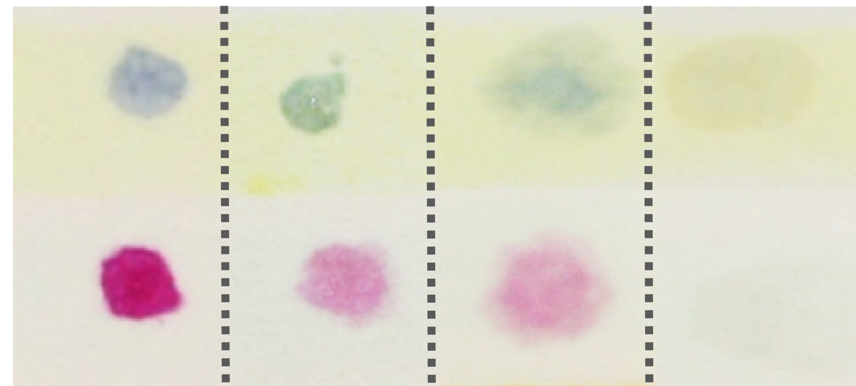
Large Persistent ΔpH in Polymersomes

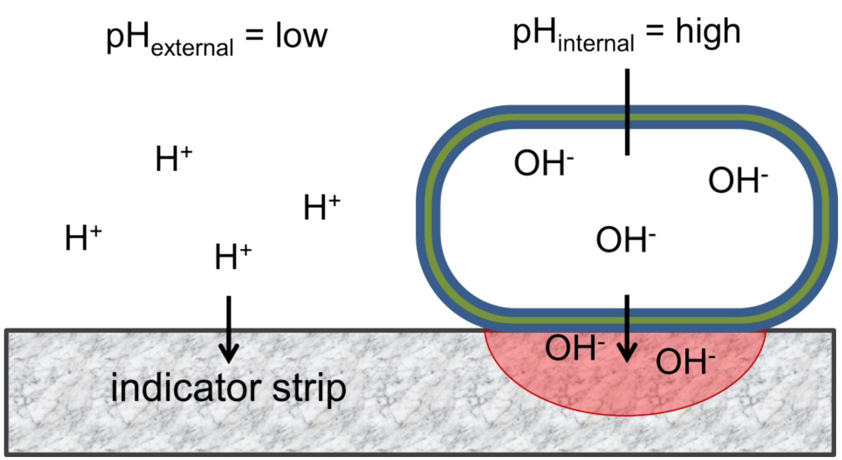


thymolphthalein
(pK_a ~10)

phenolphthalein
(pK_a ~9)



	$EO_{20}BD_{33}$ 1 M KOH	+3 eq H^+ H_3PO_4	After 3 weeks	+0.1% TX100
$pH_{internal}$	14	>10	>10	4.2
$pH_{external}$	14	3.8	3.8	4.2

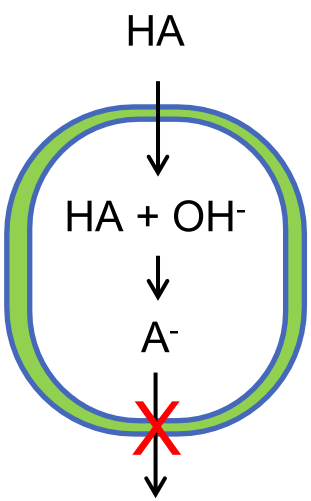


- OH^-/H^+ gradients up to 6 orders…
- …that Persist for several weeks

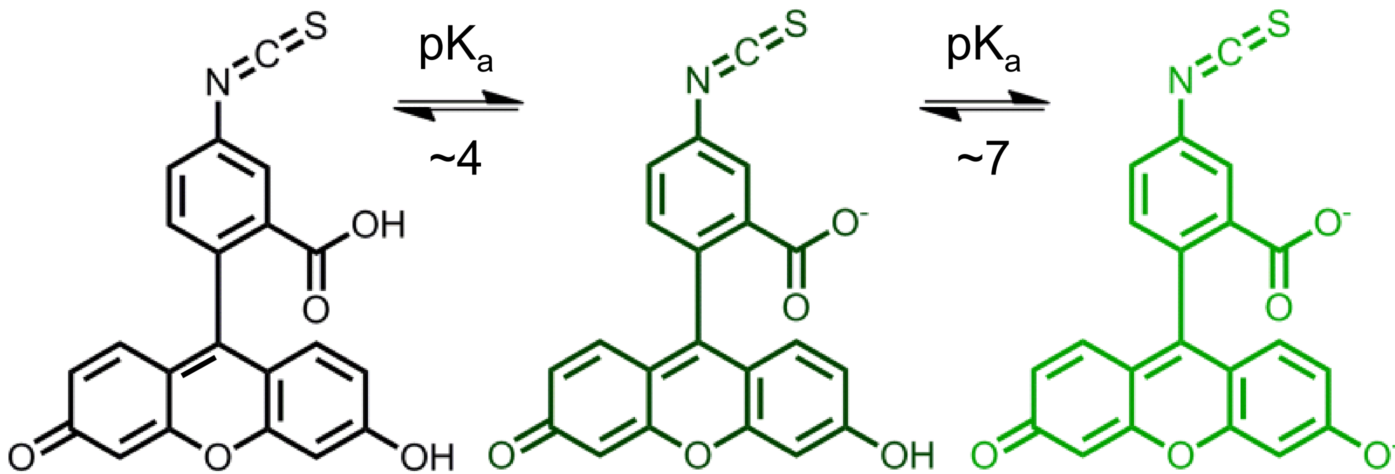
How can we exploit this effect?

Uptake of a Fluorescent Reporter

Neutral species readily penetrates membrane

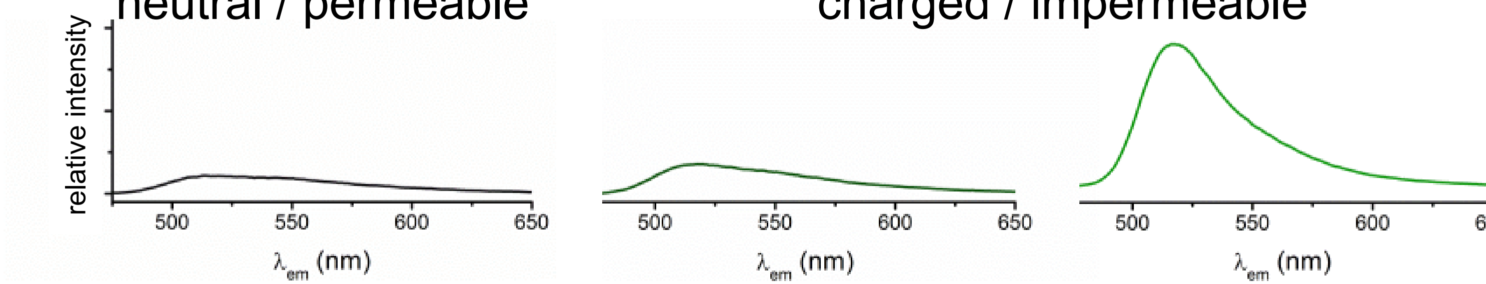


Charged species do not



neutral / permeable

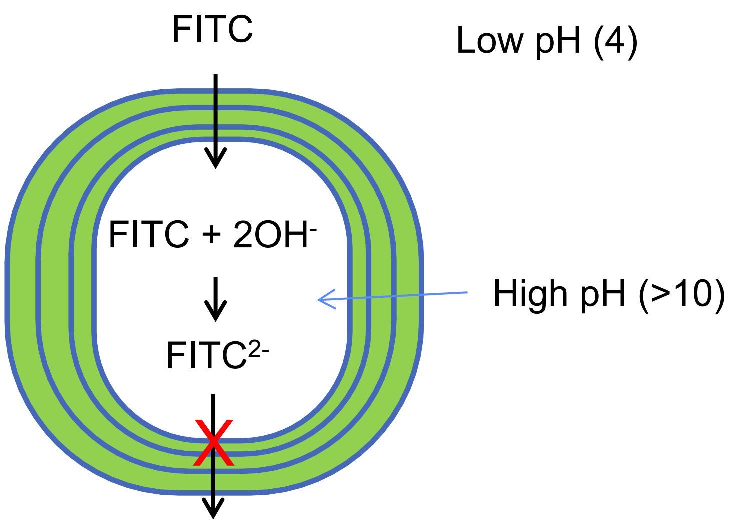
charged / impermeable



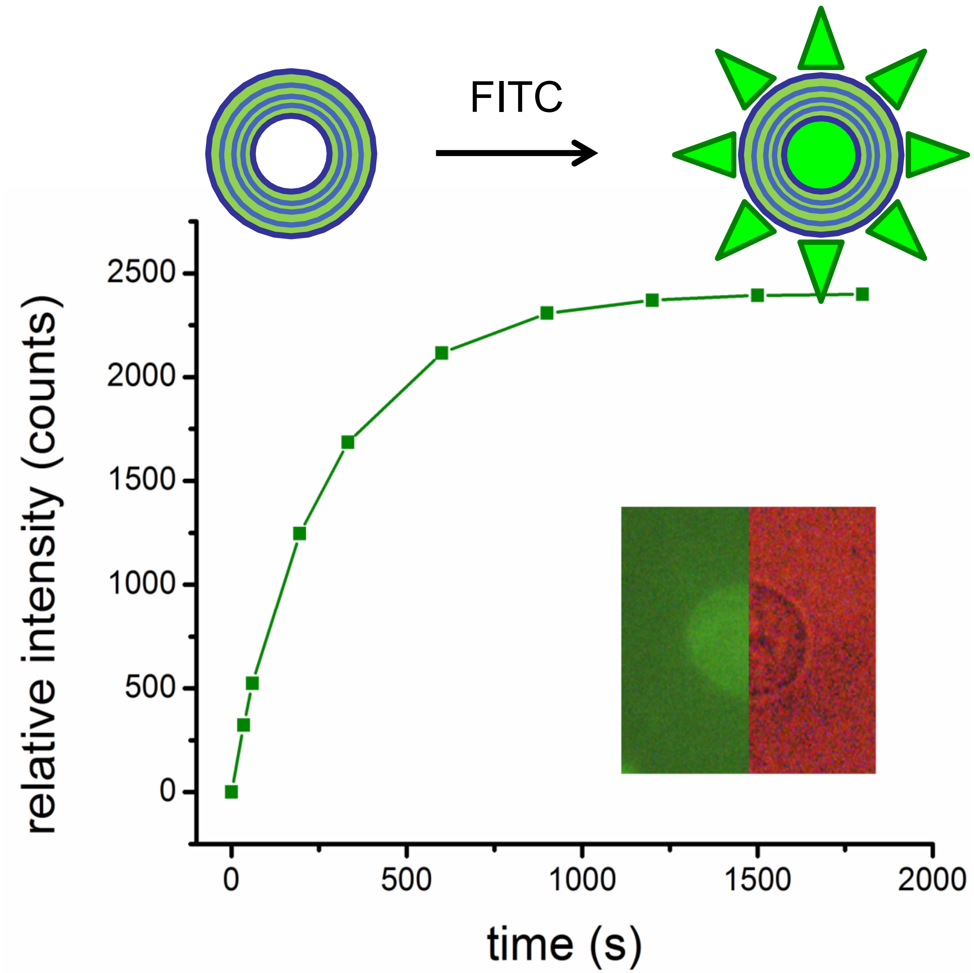
Provides basis for enrichment of a fluorescent reporter.

FITC Uptake Results

Neutral species readily penetrates membrane

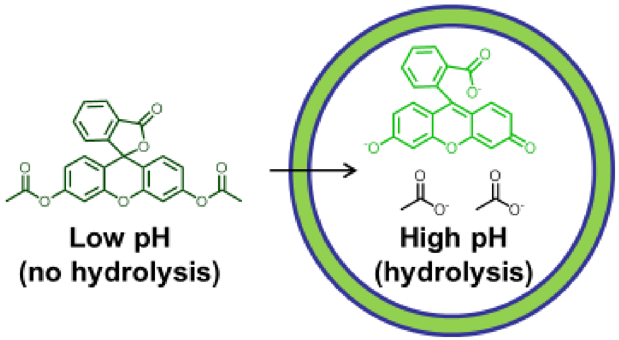


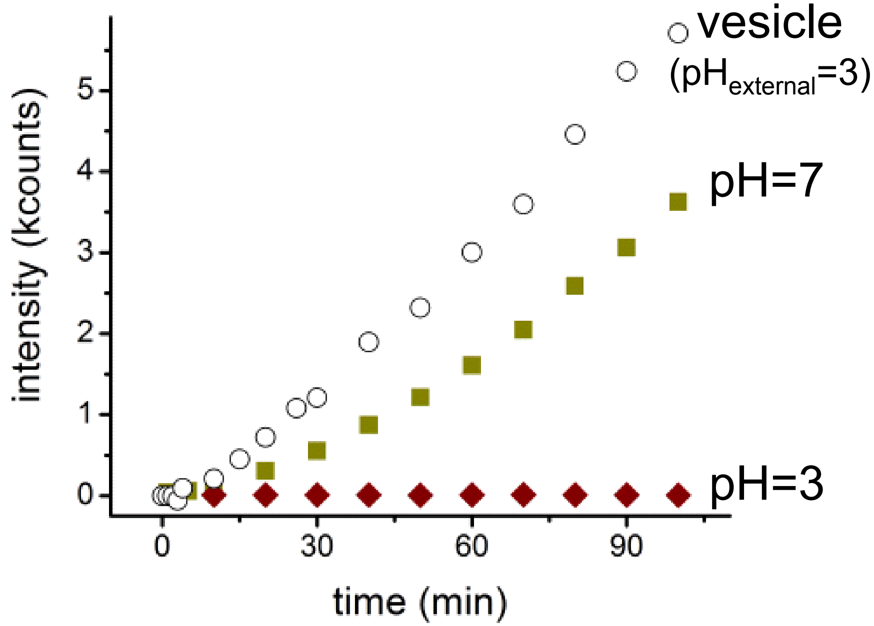
Charged species do not

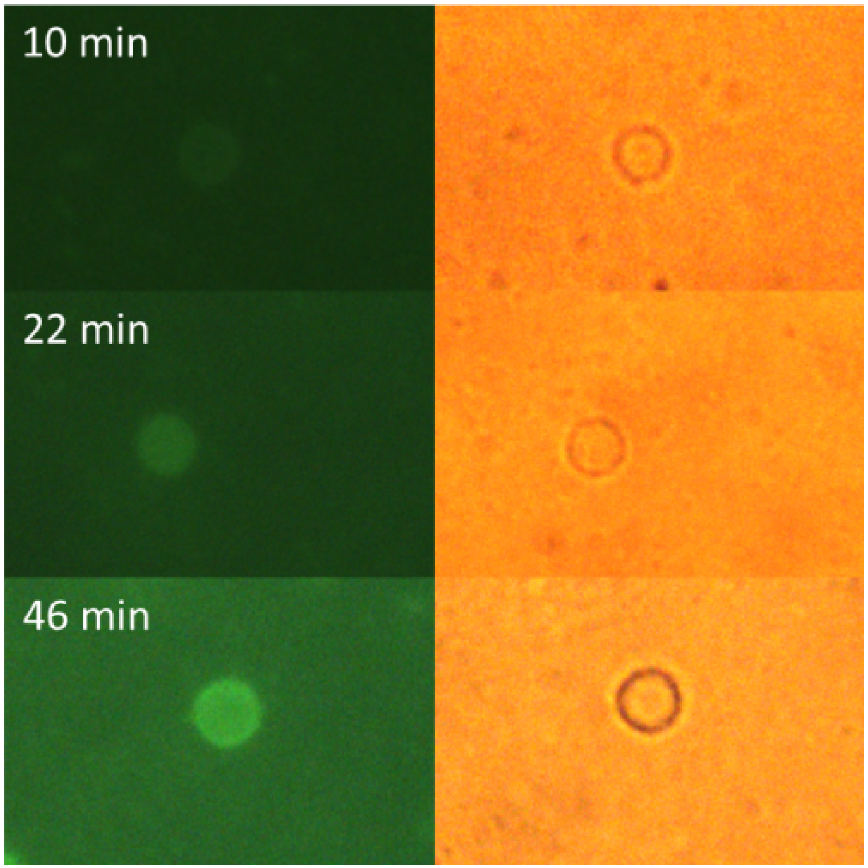


ΔpH in polymersomes to sequester acidic compounds

A Nanoreactor for Ester Hydrolysis



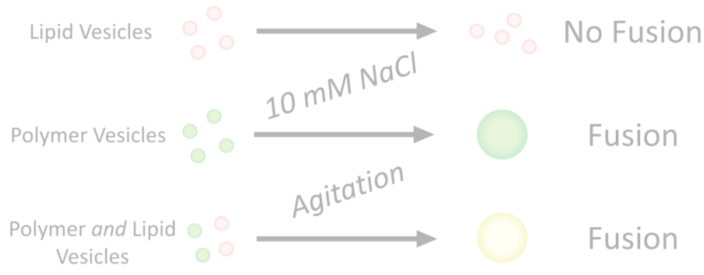




Polymersomes act as an ***artificial lysosome****, collecting and digesting hydrolysable materials*

Dynamic Polymer Vesicle Membranes

Mechanically-Activated *Fusion*



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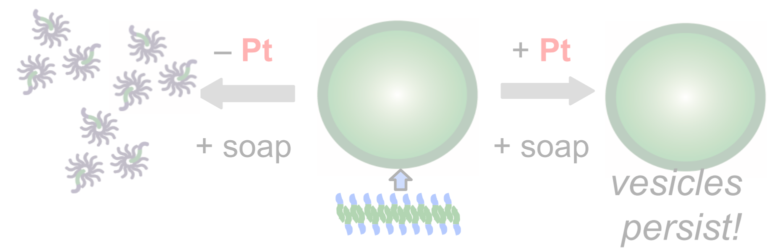
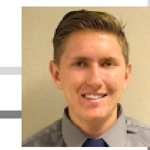
Catalytically-Active Cross-Links (*Reactivity and Stability*)

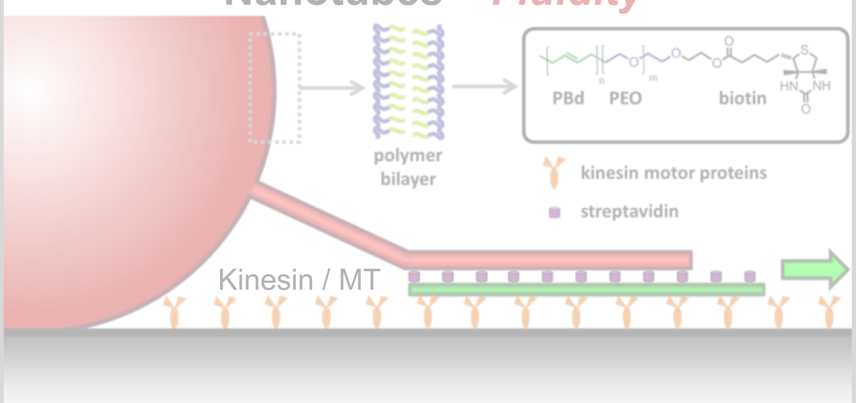


...n. Mater, **2015,** *27,* 4808–4813

w/Hae Ra Shin & Patrick McAninch

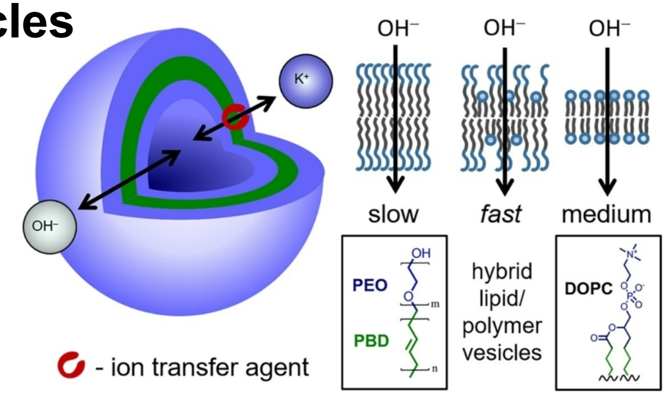


Dynamic Assembly of Polymer Nanotubes – *Fluidity*



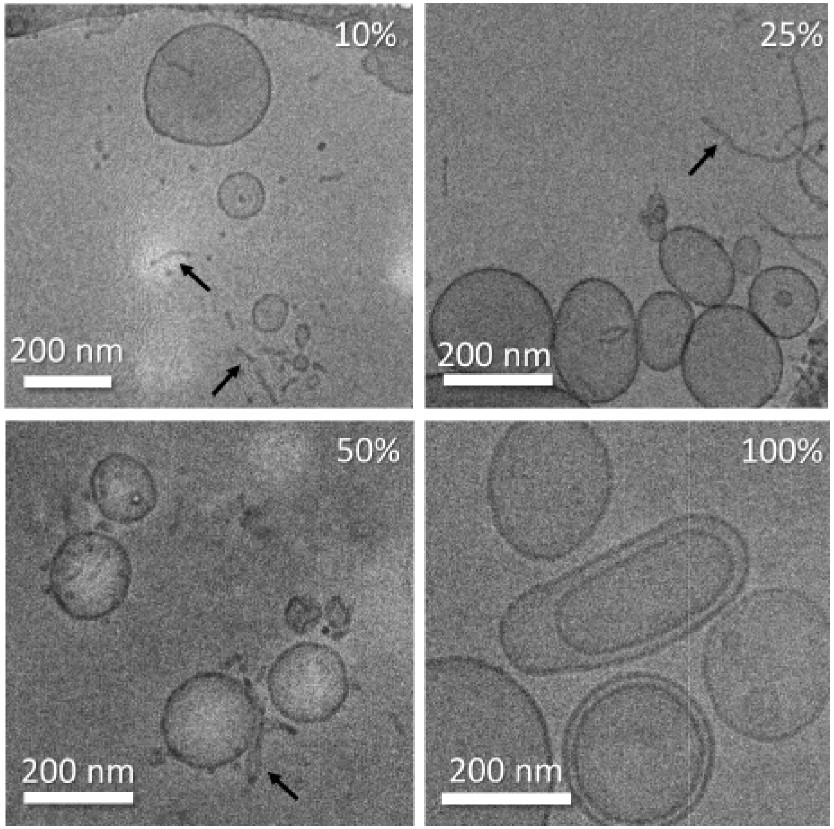
Nanoscale, **2015,** *7,* 10998–11004

Modulating *Permeability* in Hybrid Vesicles



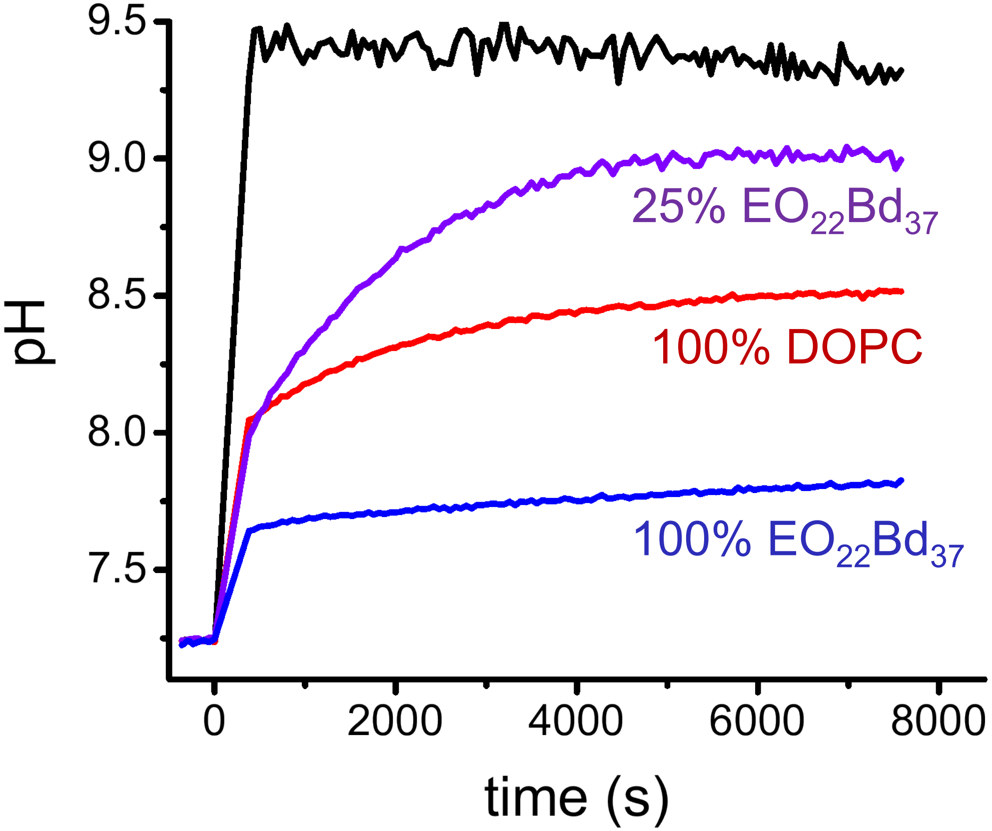
Colloids and Surfaces B, **2017,** *159,* 268–276

Preparing and Characterizing *Hybrid* Vesicles



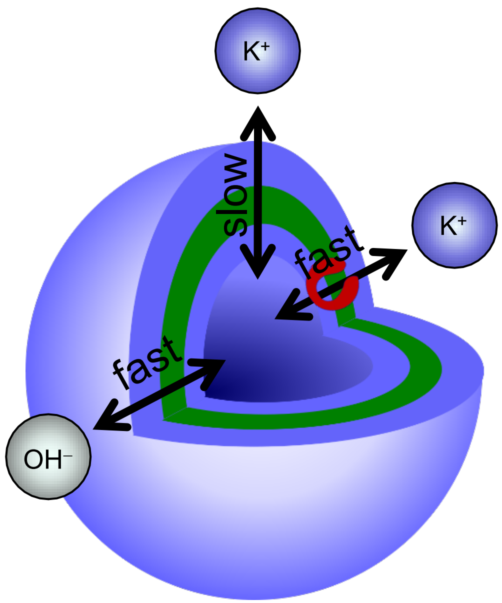
Good vesicles w/worm-like micelles

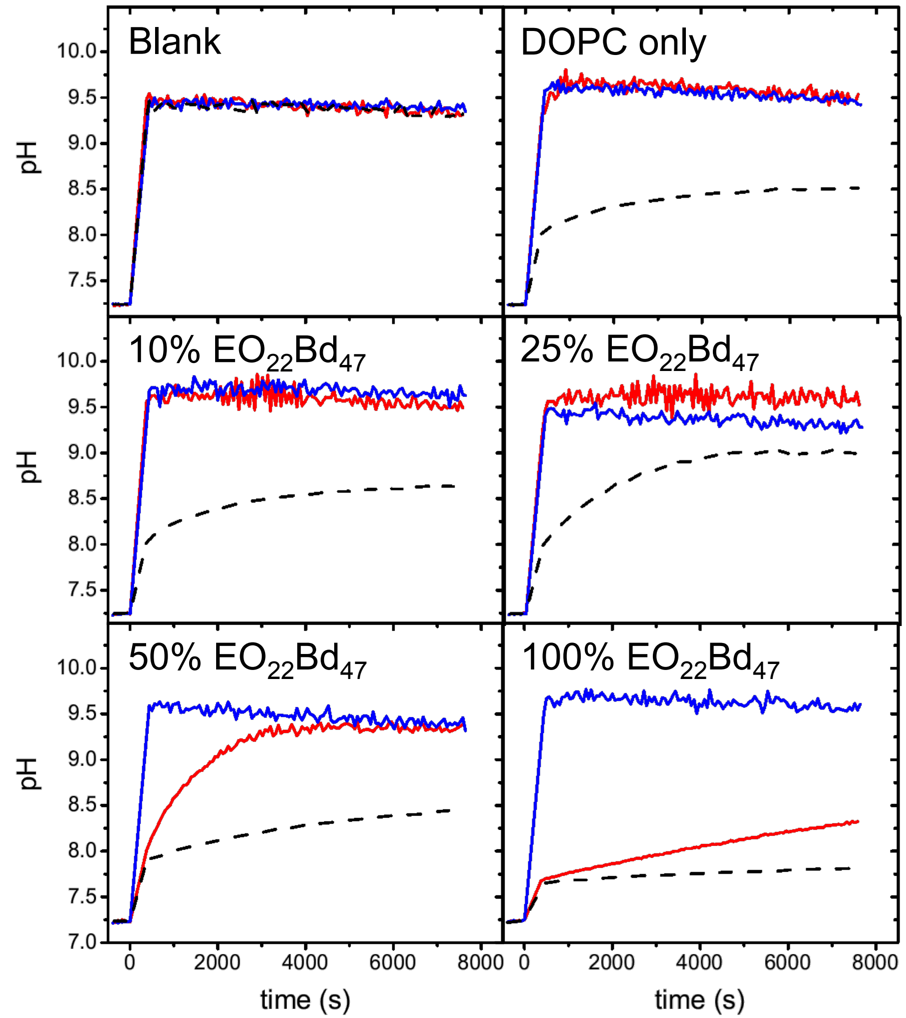
Hybrid Vesicle Permeability



$$J = P\Delta C \rightarrow P \sim 10^{-9}\ \text{cm/s}$$

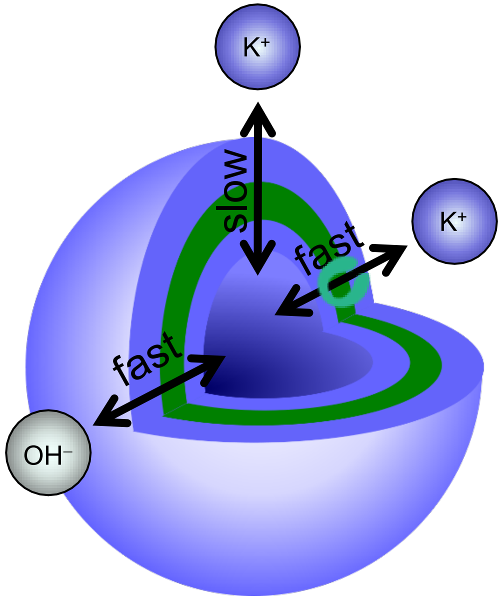
Modulating Permeability with Ionophores

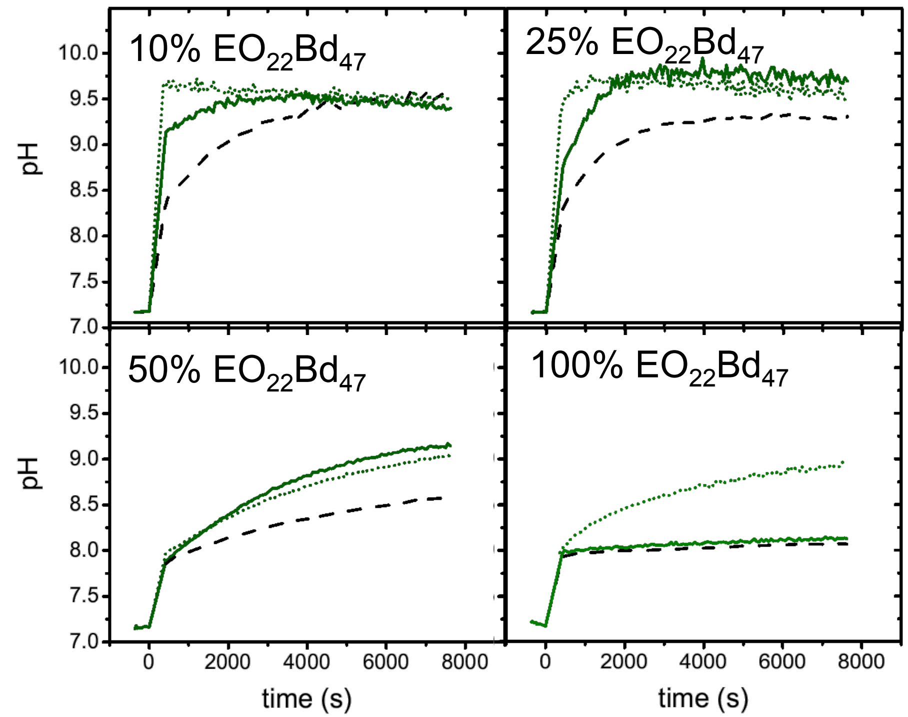




- - - - Control (+KOH)
—— Nigericin
—— Valinomycin

Modulating Permeability with Ion Channels



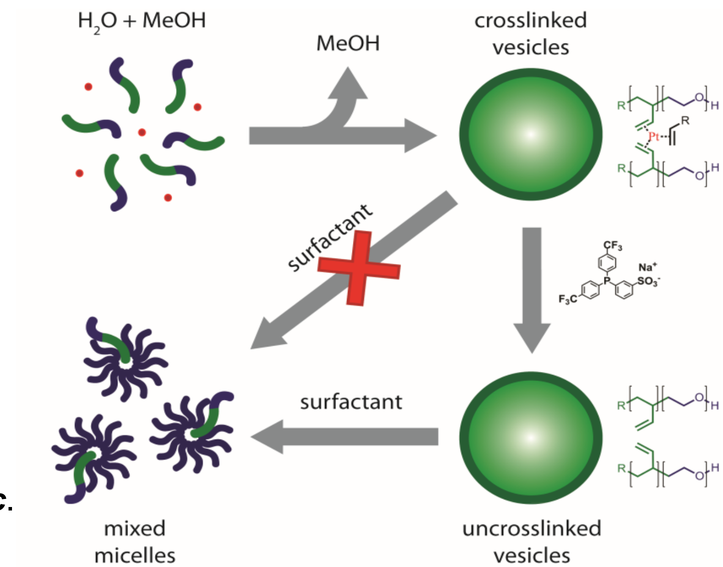


---- Control (+KOH)

—— Gramicidin A (incubated <5 min)

······ Gramicidin A (rehydrated >5 days)

Summary and Conclusions



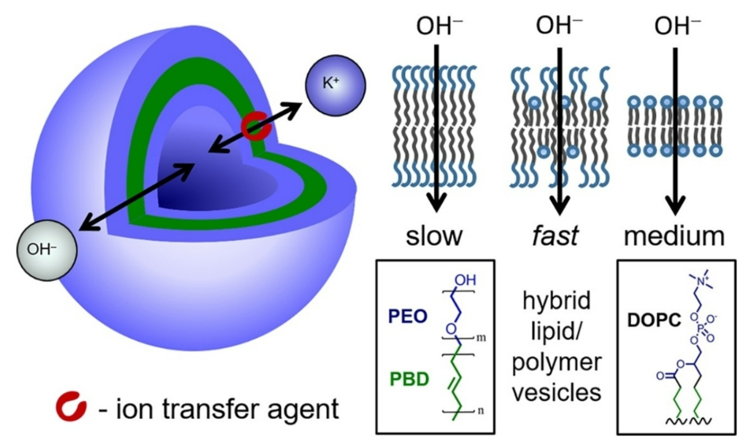
Catalytically Active Cross-Links

Used organometallic interactions to modulate the properties of “normal” polymersomes:

1. Enhanced Stability:
    - Pt--ǁ → Organometallic cross-links
    - Resistant to destabilization
    - Crosslinking can be selectively reversed w/ phosphine ligands
2. Catalytic Activity:
    - Pt centers still active
    - Enable hydrosilation reactions

To produce soft self-assembling material that is both ***robust*** and ***dynamic***.

Monitoring and Modulating Ion Flux in Hybrid Bilayers



Hybrids *MORE* permeable than 100% lipid or 100% polymer vesicles

Modulated ion flux in hybrid vesicles via reconstituted membrane proteins (Nigericin / Valinomycin / Gramicidin)

The permeability of hybrid bilayers is critical property for drug delivery, nanoreactor, and sensing applications.

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Dr. George Bachand (CINT Staff)
Dr. Adrienne Greene (Sandia)
Dr. Nathan Bouxsein (Perspectives)
Dr. Sergei Ivanov (CINT Staff)
Dr. Gabriel Montano (NAU)

CINT / DOE-BES / SNL-LDRD

Thank You!

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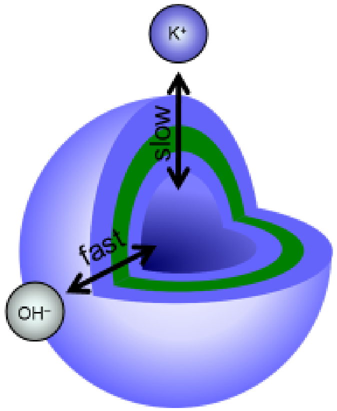
K+
slow
fast
OH−