

“Leviathan” Polymersomes Formed via Simple Gel-Assisted Rehydration

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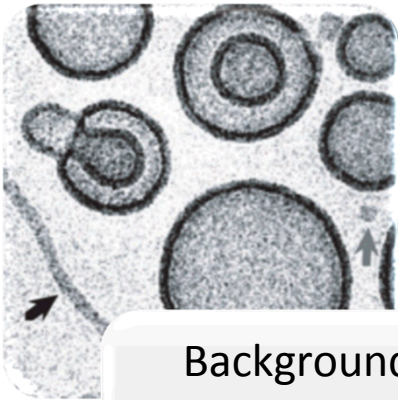
Center for Integrated Nanotechnologies
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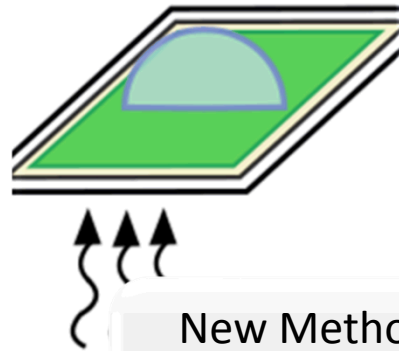


Outline for Today's Talk



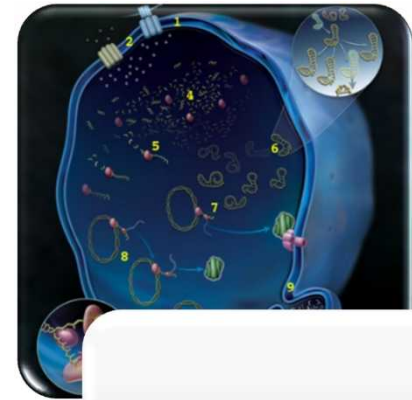
Background

- Forming polymer vesicles (polymersomes)



New Method

- Gel-assisted rehydration
- Size tunability
- Mechanism of formation

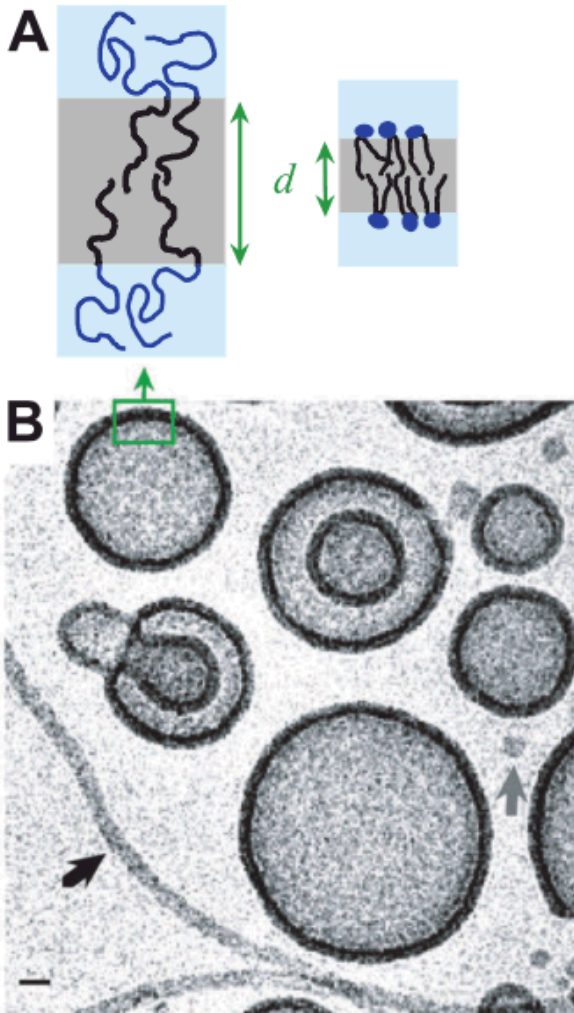


Overall Goal:
Building
polymersome-
based protocells



Polymersomes as Liposome Alternatives

Polymer vesicles, or polymersomes are beginning to supplement and supplant lipid vesicles.

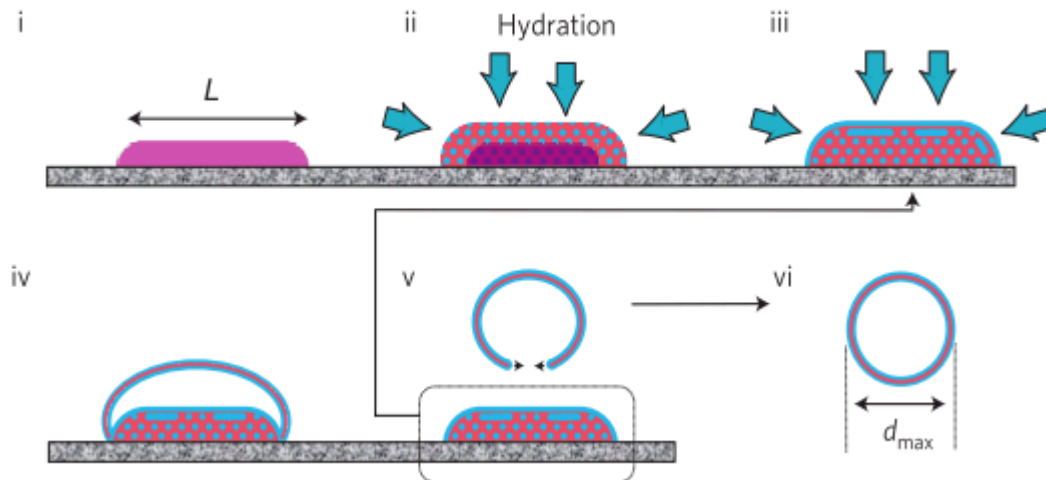
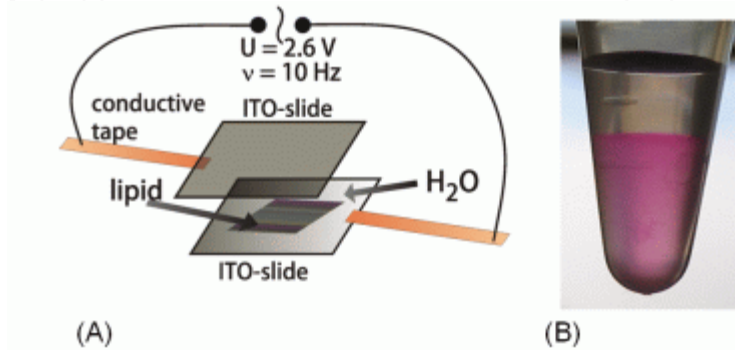


- Polymersomes are created through the self-assembly of amphiphilic block copolymers
- Polymer vesicles are “tough” synthetic vesicles, being explored as analogs of lipid vesicles for drug delivery and synthetic biology applications
- Polymersomes have enhanced properties:
 - Stability
 - Robustness
 - Chemical versatility
 - Barrier properties
 - Tunable physical attributes



Methods to Make Giant Polymersomes (>4 μm)

Typical methods to make giant polymersomes are lengthy, require specialized equipment and produce low yields of intact polymersomes



- Polymersomes can be made by:
 - Electroformation
 - Templated rehydration
- Electroformation is lengthy and limited typically to rehydration in sucrose solutions
- Templated rehydration uses several complex steps including UV photolithography, spin coating

Using Gel-Assisted Rehydration to Form Polymersomes

Gel-assisted rehydration is a simple, robust and rapid method to form hundreds of fully intact, symmetric polymersomes

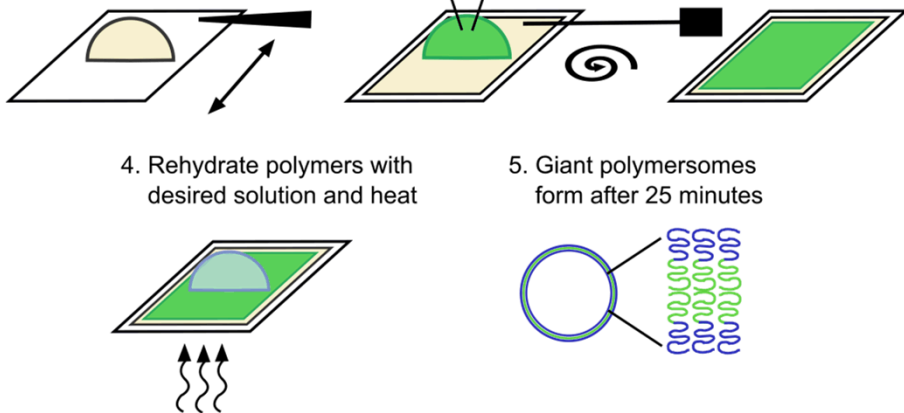
1. Spread dissolved agarose on coverslip with a pipette tip

2. Spread polymer solution in circular motion on agarose films with needle

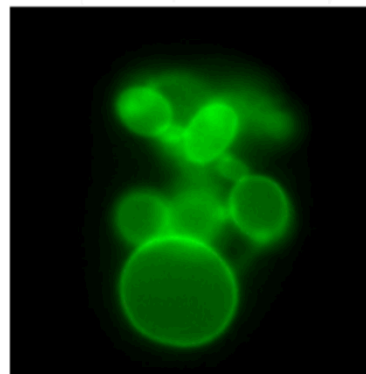
3. Dehydrate polymers on agarose films

4. Rehydrate polymers with desired solution and heat

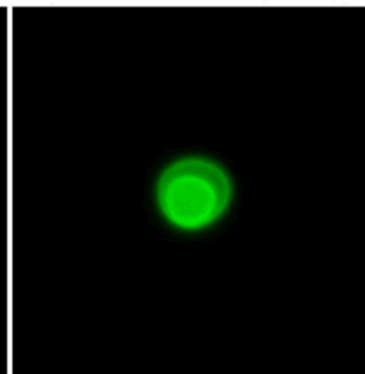
5. Giant polymersomes form after 25 minutes



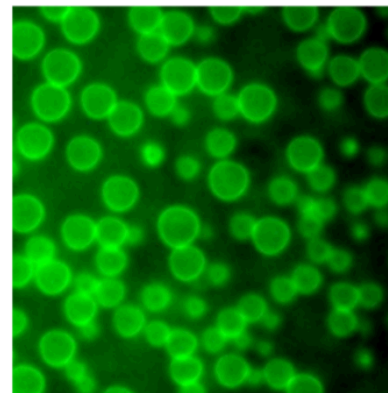
Electroformation



Electroformation



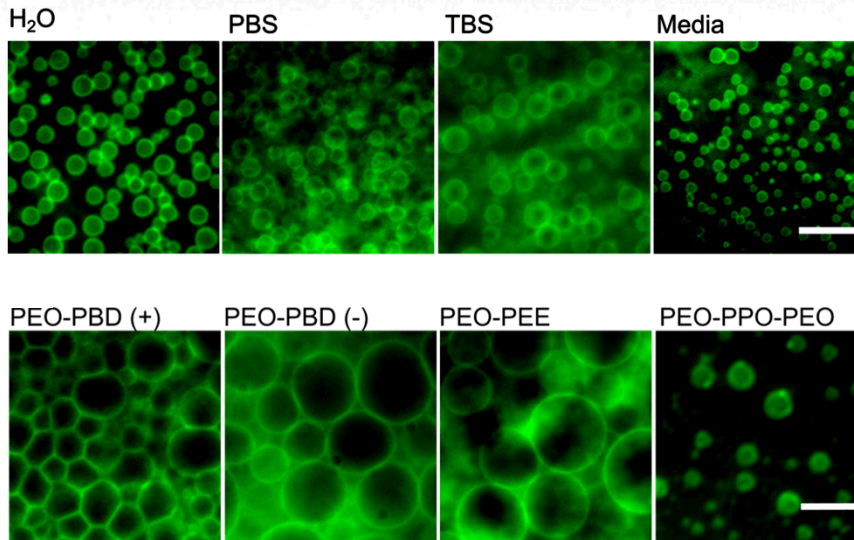
Gel Formation



- Gel-assisted rehydration is versatile, inexpensive and uses standard laboratory equipment
- Polymersomes formed using gel-assisted rehydration exceed the quality of those made using electroformation

Gel-Assisted Rehydration is a Versatile Method

Polymersomes can be formed in a variety of rehydration solutions, including biologically compatible buffers, as well as from a variety of polymer compositions and are fluid



Gel-assisted rehydration is compatible for forming polymersome-based synthetic cells:

- Charged polymers are mimics of naturally charged lipids, typically found in the plasma membrane
- Formation in physiologically-compatible buffers required in biomimetic or synthetic cell studies
- Membrane integrity was confirmed using FRAP
 - Diffusion coefficients calculated and are as expected

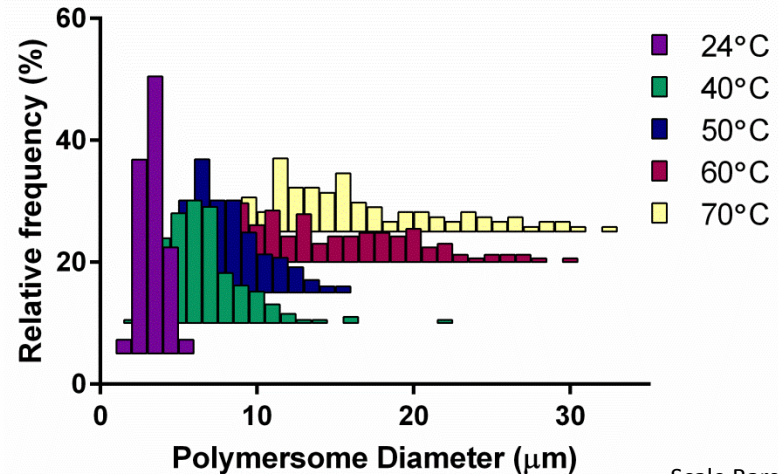
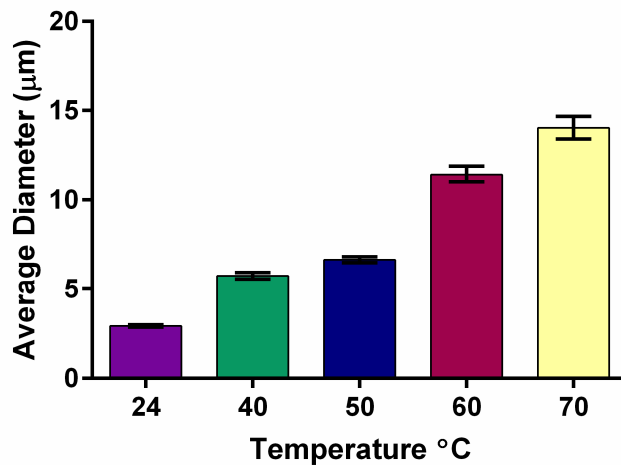
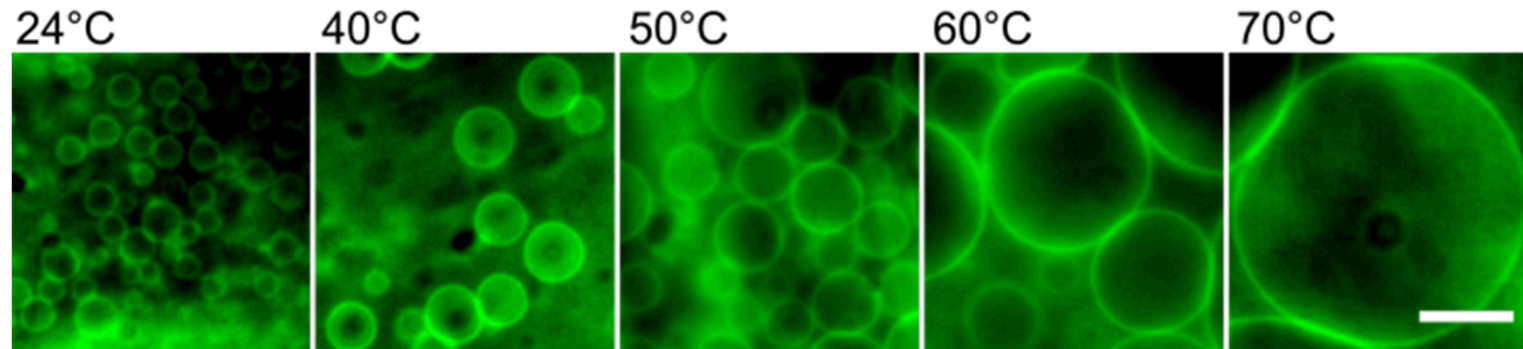
Polymer	Abbreviation
Poly(ethylene glycol)-poly(butadiene)	PEO-PBD
Poly(ethylene glycol)-poly(butadiene)-NH ³⁺	PEO-PBD (+)
Poly(ethylene glycol)-poly(butadiene)-COO ⁻	PEO-PBD (-)
Poly(ethylene glycol)-poly(ethylethylene)	PEO-PEE
Poly(ethylene glycol)-poly(propylene oxide)- poly(ethylene glycol)	PEO-PPO-PEO

Scale Bars = 10 μm

Polysome Size is Easily Tunable

As temperature increases during the rehydration step, polysome size also increases

- Increasing the temperature results in a physical membrane fluidizing effect

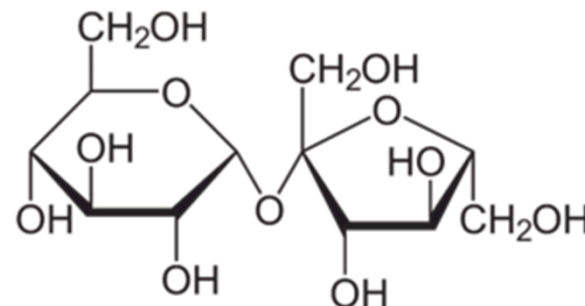
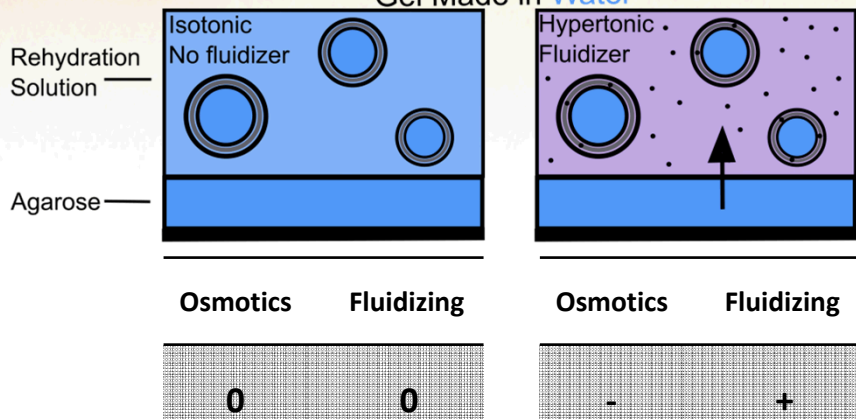


Scale Bars = 10 μm

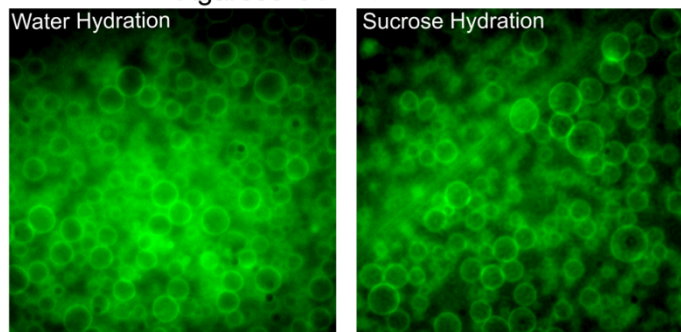


Fluidization and Osmotics Drive Polymersome Formation

Gel Made in Water

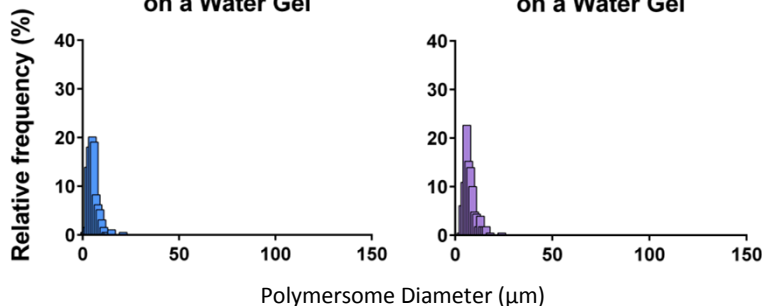


Agarose Gel Made in Water



Water Rehydration on a Water Gel

Sucrose Rehydration on a Water Gel

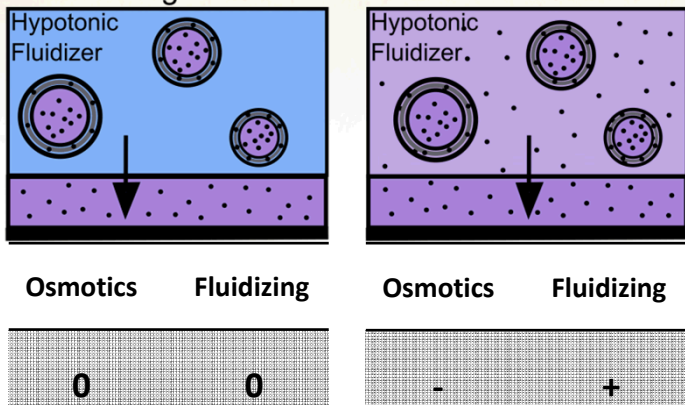


The addition of sucrose has a two fold effect:

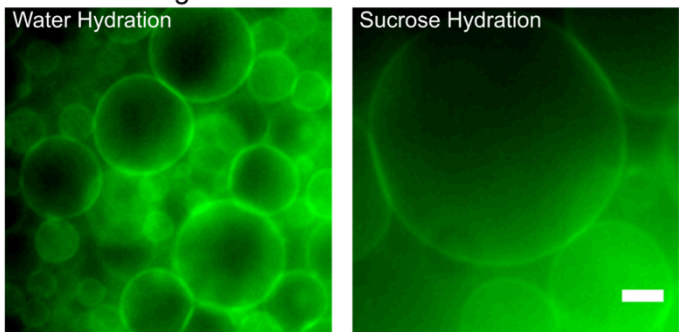
1. It acts a small molecule membrane fluidizer
2. When added asymmetrically, it creates an osmotic gradient

Fluidization and Osmotics Drive Polymersome Formation

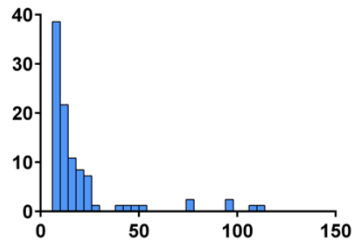
Agarose Gel Made in Sucrose



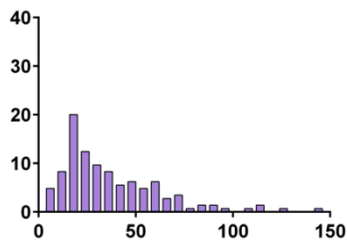
Agarose Gel Made in Sucrose



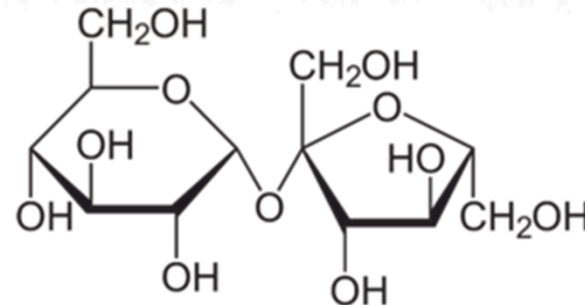
Water Rehydration on a Sucrose Gel



Sucrose Rehydration on a Sucrose Gel



Polymersome Diameter (μm)



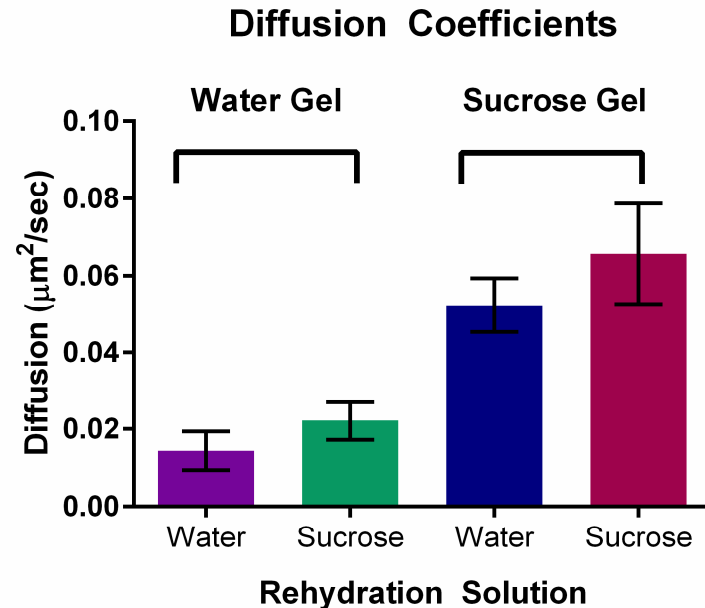
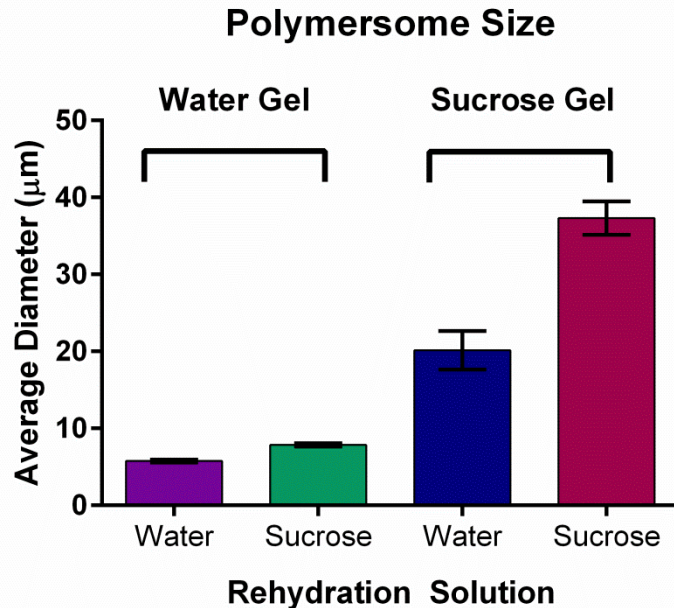
The addition of sucrose has a two fold effect:

1. It acts a small molecule membrane fluidizer
2. When added asymmetrically, it creates an osmotic gradient

Scale Bars = 10 μm

Fluidization and Osmotics Drive Polymersome Formation

The addition of sucrose causes an increase in the average vesicle size as well as an increase in the membrane diffusion coefficient

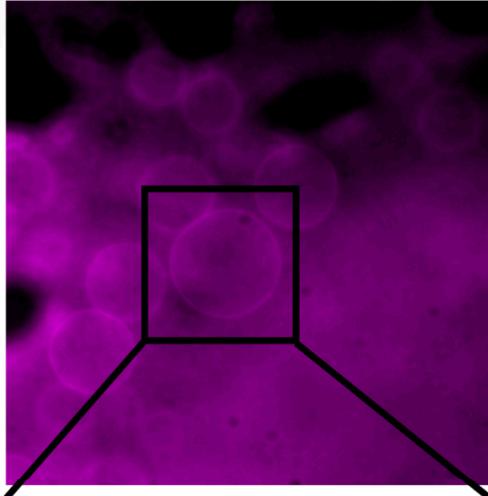


- When sucrose is present in the agarose gel, it creates a positive osmotic gradient, favoring vesicle formation
- The addition of sucrose also acts as a small molecule membrane fluidizer as measured by the increase in the diffusion coefficient

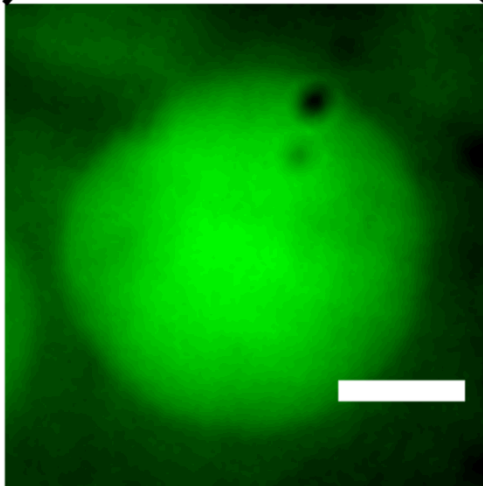
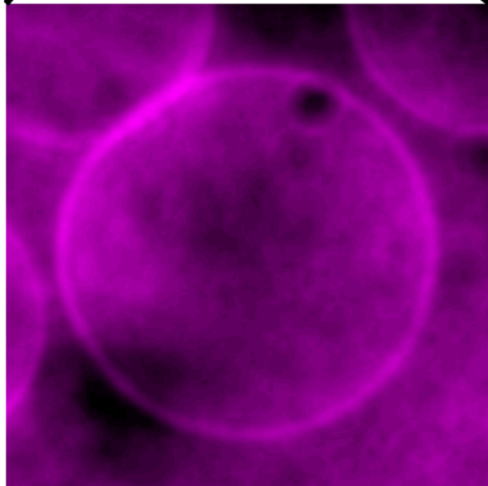
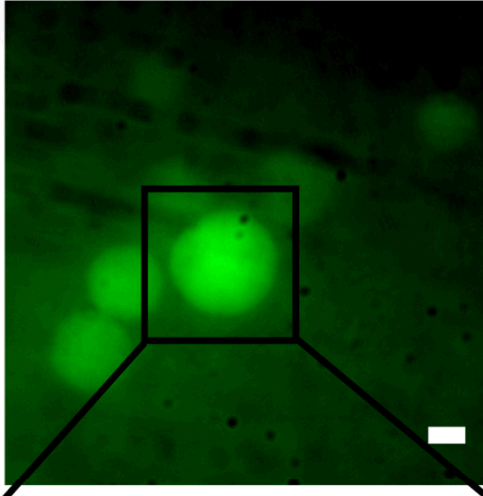
Making Polymersome-Based Protocells

Polymersomes efficiently encapsulate fluorescently-labeled single strand DNA

PEO-PBD

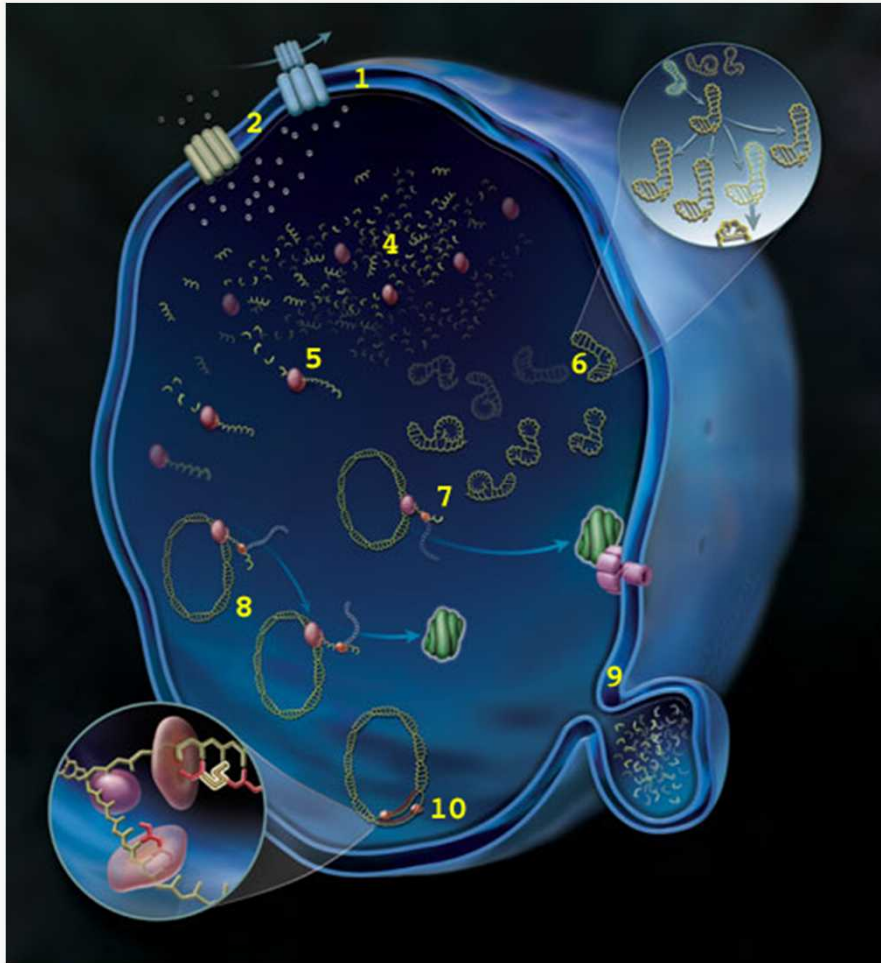


DNA



- Depositing DNA underneath the polymer layer favors DNA encapsulation as the polymers are rehydrated and polymersomes form
- These principles can be expanded to test the encapsulation of proteins, small molecules, larger DNA, etc.

Towards Building a Protocell



- There are 12 requirements to creating a protocell that accurately depicts biology
- The *first step* in building a protocell requires a membrane enclosure
 - Polymersome-based protocells are a robust alternative to liposome-based protocells
- Another key requirement is the ability to encapsulate macromolecules
- *Gel-assisted rehydration is a robust and simple alternative to forming giant polymersomes for studying the properties of polymersomes and also for building protocells*

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