

## Fluidizing membranes: a simple way to assist in the formation and size-control of giant polymersomes

In the 2005 article “A Giant Step Towards Artificial Life”, David Deamer outlined twelve requirements for creating an artificial cell or “protocell.” The very first of these steps is to form a membrane enclosure, encapsulating all of the materials necessary for sustaining a self-replicating and functional system. To effectively mimic a eukaryotic cell, membrane enclosures with an approximate diameter of 10-100  $\mu\text{m}$  need to be created. Giant unilamellar vesicles (GUVs) are biological membrane model vesicles typically generated through the self-assembly of amphiphilic lipid molecules, creating vesicles in the desired size range for creating protocells. Some of the inherent limitations of lipid GUVs include a fairly short shelf-life due to the inherent instability of the lipid membranes. To overcome these limitations, block copolymers are being explored as synthetic analogs to lipids, specifically in the generation of giant polymer vesicles, or polymersomes.

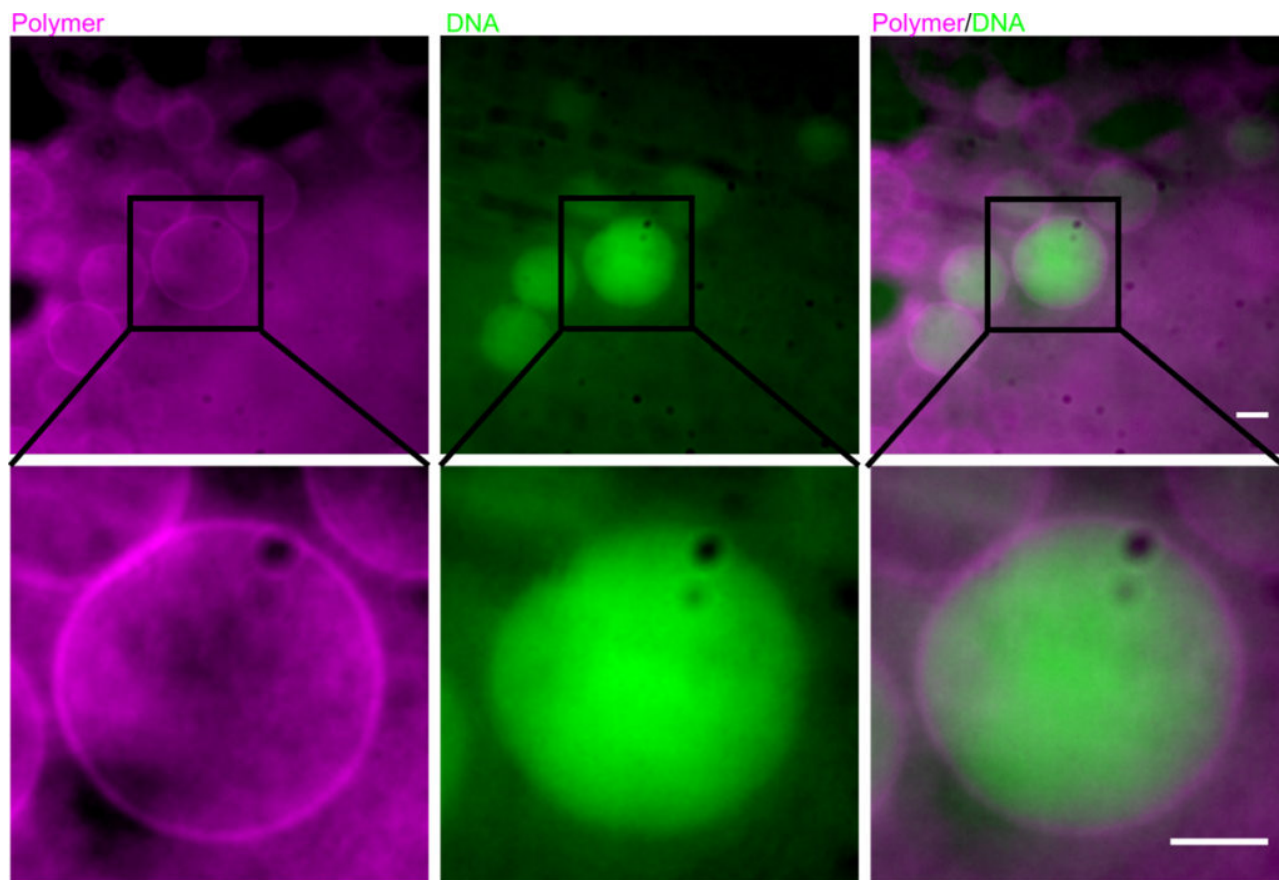


Fig. 1. DNA encapsulated in polymersomes formed using gel-assisted rehydration. Epifluorescence images of Poly(ethylene glycol)-poly(butadiene), or PEO-PBD, polymersomes (shown in magenta) formed at room temperature for one hour. Fluorescently-labeled DNA oligomers (shown in green) were successfully encapsulated in the formed polymersomes. Scale bar = 10  $\mu\text{m}$ .

The formation of polymersomes, however, is typically limited to fairly labor-intensive techniques (i.e. electroformation) and results in rather low-yields. In the present study, a recently reported technique for the formation of lipid GUV vesicles was adapted for the formation of polymersomes (block copolymer vesicles). The gel-assisted rehydration technique involves depositing a layer of agarose on a glass coverslip, and fully dehydrating the agarose film. Polymer solutions are then spread on the dried agarose film, and the solvent is fully evaporated. Rehydration of the polymer layer results in rapid formation of giant polymersomes, with comparable membrane fluidity to polymersomes formed from traditional techniques. In contrast to the traditional techniques, polymersomes formed using gel-assisted rehydration may be generated using a wide variety of buffers (e.g., water, PBS, TBS, etc.) and various polymer compositions (e.g., diblock, triblock, charged, neutral, etc.), demonstrating the versatility of this technique.

Modulating the size of synthetic vesicles is typically quite challenging, resulting in inconsistent size distributions and dispersity. Using gel-assisted rehydration, polymersome size was easily tuned by altering the temperature during rehydration. As the temperature was increased, the size of the polymersomes likewise was increased, with some polymersomes reaching up to ~150  $\mu\text{m}$  in diameter. These results suggest that increased membrane fluidization at higher temperatures assists in increasing the size of polymersomes. To further substantiate this hypothesis, a small molecule membrane fluidizer, sucrose, was added to the agarose layer, the rehydration solution, or a combination of the two; the size of the resulting polymersomes was then measured. As hypothesized, the addition of a small molecule fluidizer resulted in an increased size of polymersomes formed under these conditions. Furthermore, characterization of membrane fluidity confirmed enhanced membrane fluidity with the addition of sucrose, directly supporting the role of membrane fluidization in the formation of giant polymersomes.

Toward the application of polymersomes as artificial cell membranes, gel-assisted rehydration was used to encapsulate genetic material into the giant polymersomes. Here, a fluorescently labeled DNA oligomer was dehydrated directly onto the agarose layer prior to deposition of the polymer solution. Through rehydration, the DNA was successfully encapsulated into the formed polymersome. Overall, gel-assisted rehydration represents a versatile method for forming size-controlled giant polymersomes with the ability to encapsulate cargo, ultimately enabling the formation of synthetic, more-robust membranes enclosures required for creating protocells.

**Adrienne C. Greene<sup>1</sup>, George D. Bachand<sup>2</sup>**

<sup>1</sup>*Nanobiology Department*

<sup>2</sup>*Center for Integrated Nanotechnologies  
Sandia National Laboratories, USA*

## Publication

[The Role of Membrane Fluidization in the Gel-Assisted Formation of Giant Polymersomes.](#)

Greene AC, Henderson IM, Gomez A, Paxton WF, VanDelinder V, Bachand GD

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