Encapsulation of Cell-Free Synthesis via Emulsion Vesicles

Sampreeti Chowdhuri, Christian M. Cole, Brandon Nichols, Natalya Ballard, Neal Devaraj

Department of Chemistry & Biochemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, California

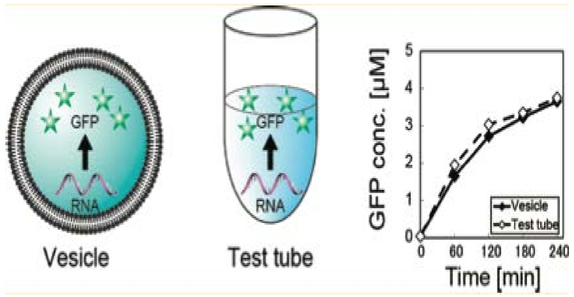
Introduction

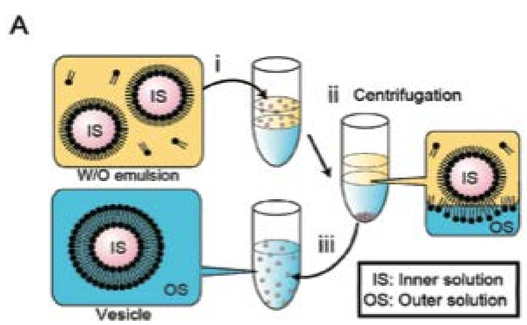
Bacteria such as E. coli are typically used in expressing various proteins. Research has shown that mammalian proteins can have difficulties properly folding when they are expressed in bacterial expression systems. In addition, some proteins are too toxic to express at high levels. By encapsulating the DNA/RNA, and the expression machinery (i.e. polymerases, tRNA synthetases, amino acids, and small molecules), proteins can be expressed without concern of living bacteria which gives us more control of the expression condition.

We first experimented with the emulsion method of synthesizing large vesicles by encapsulating fluorescent green sucrose solution inside the lipid vesicle. Once we were able to encapsulate the dye, we attempted to encapsulate GFP-expressing E. coli cells in order to test the maximum size of the molecules that the vesicles can hold.

After making sure that the vesicles could encapsulate sufficently large molecules, we created vesicles that contained the necessary components to express green fluorescent protein (GFP). These vesicles were created using palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) or dilauroyl phosphatidylcholine (DLPC).We also created vesicles with a 1:1 ratio of POPC to cholesterol in order to test which vesicle membrane would be able to best encapsulate the components of the cell-free synthesis.

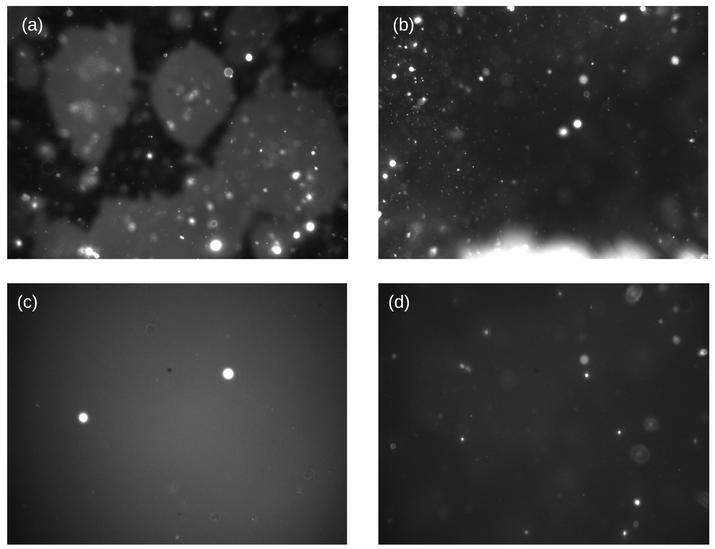
Emulsion Method of Vesicle Synthesis





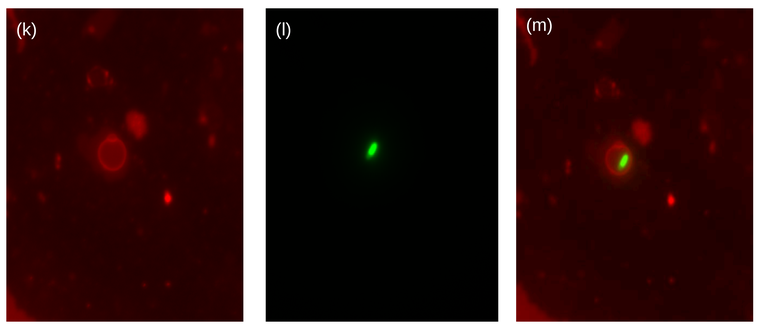
The emulsion method of synthesizing vesicles involves dissolving the inner solution of the vesicles inside 100 microliters of heavy mineral oil, and then placing the solution on top of a lower glucose solution. The lipids in the oil form micells around the inner solution and form a monolayer between the top and lower solutions. After centrifuging, the monolayer wraps around the micells which forms the vesicles.

Sucrose Dye in Vesicles



These pictures show the encapsulation of green fluorescent sucrose dye captured inside POPC vesicles. Using various filters of light we were able to see both the inside and outside parts of the vesicle.

Encapsulation of Bacteria



Texas Red dye was used to stain the outside of the vesicles and the bacteria on the inside expressed green fluorescent protein so it glowed green under the microscope. When the image of the outside of the vesicle and the image of the inside of the vesicle were superimposed, we could see that the bacteria was trapped inside the vesicle and could not escape.

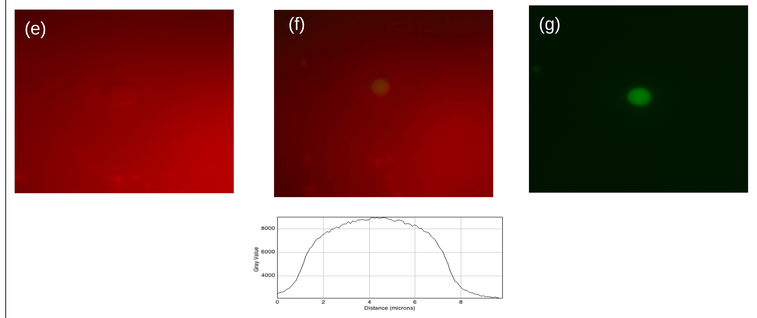
References

Cell-Free Protein Synthesis inside Giant Unilamellar Vesicles Analyzed by Flow Cytometry Koji Nishimura, Tomoaki Matsuura, Kazuya Nishimura, Takeshi Sunami, Hiroaki Suzuki, and Tetsuya Yomo *Langmuir* **2012** *28* (22), 8426-8432

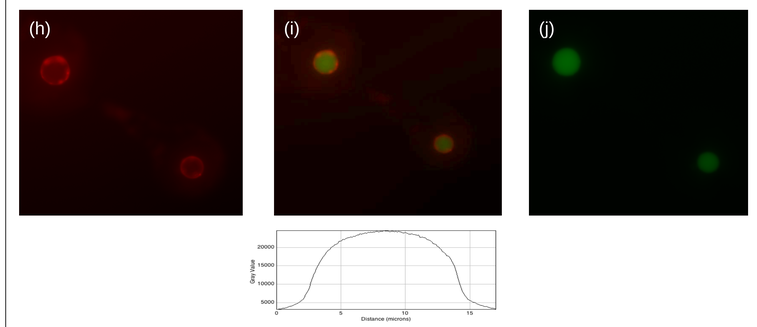
Vincent Noireaux and Albert Libchaber
A vesicle bioreactor as a step toward an artificial cell assembly
PNAS 2004 101 (51) 17669-17674; published ahead of print December 10, 2004, doi:10.1073/pnas.0408236101

GFP Protein Expression inside POPC and DLPC Vesicles

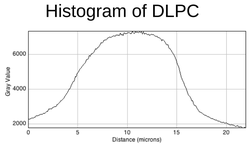
POPC Vesicles



1:1 Cholesterol POPC Vesicles



Histogram of DLPC



After 4 hours of incubation, the vesicles began to express green fluorescent protein. Histograms of the vesicles reveal the intensity of the GFP expression in each type of vesicle that was made. The graphs show that the vesicles made with POPC and cholesterol have the highest intensity of protein expression and the DLPC has the lowest.

Conclusion

We were able to successfully create vesicles using dye, bacteria, and the components of green fluorescent protein. The vesicles that were made using cholesterol tended to express GFP much more intensely than the vesicles made without it. In the future, we will attempt to create vesicles using different lipid composition in order to find the membrane composition that will yield the highest intensity of GFP expression.