# Design of Synthetic Protein Membranes Using Droplet Microfluidics

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## Abstract and Introduction:

Protein conjugated beads and particles are often used in biomedical processes [1]. However, these particles suffer a number of limitations. Proteins are chemically attached to fixed locations on the particle surface, and cannot move independently of the particle, even in the presence of external stimulation. This can also lead to partial protein denaturation and loss of activity [2]. The goal of this work was to overcome the limitations of solid particles by studying the behavior of proteins on a liquid-liquid interface. By engineering artificial membrane proteins using the PURExpress *in vitro* protein synthesis platform from New England BioLabs (NEB) and attaching them to droplets using droplet microfluidics, the detrimental effects of denaturation can be greatly mitigated or avoided completely. Preliminary measurements and observations show that protein droplets are uniformly coated with mobile, active proteins. These characteristics distinguish protein droplets from solid particle systems and make them a promising solution to problems in drug delivery, biosensing, as well as the study of protein interactions. Of particular interest is the application of protein droplets to the study of apoptosis.

## Experimental Methods:

Using template DNA provided by NEB, protein-transmembrane helix complexes were synthesized as single proteins using their PURExpress synthesis kit [3]. For the purpose of this work, membrane versions of green fluorescent protein and streptavidin were studied.

Droplets were made using PDMS microfluidic devices, as in Guo, et al. [4], and by simple emulsification. Protein membranes were observed on both water-in-oil and oil-in-water droplets. Droplets were incubated at 37°C after formation to allow proteins to be synthesized and coat the droplet interface.

The structure and properties of these protein droplets were studied using confocal microscopy. Engineered transmembrane green fluorescent protein was used as a model system to study protein droplets and membranes. Fluorescent biotin dyes allowed the use of streptavidin protein drops to demonstrate protein activity.

## Results and Conclusions:

**Protein Density.** While protein-conjugated solid particles vary widely in their degrees of coverage, protein droplets show uniform coatings on the entire surface of the drop. Preliminary measurements also show that droplets are covered with a higher density of proteins than are commercially available particles. Commercially available streptavidin particles are coated with approximately 1.5 ng/cm$^2$, but preliminary data shows that streptavidin drops could have coverage levels above 700 ng/cm$^2$.

**Protein Mobility.** Unlike proteins bound to solid particles, proteins attached to droplets were observed to move in small groups along the plane of the membrane. This could lead to engineered proteins that can respond to stimuli and assemble into complex structures, similar to proteins on the cell membrane.

**Protein Activity.** To determine if attachment to the droplet interface severely damaged the proteins, streptavidin droplets were placed in a solution of biotinylated dye, which only active streptavidin would bind. Fluorescence microscopy demonstrated that the entire surface of the streptavidin drop had the dye molecule bound to it. Further testing is needed to determine the degree of activity and assess the potential of protein droplets as biosensors.

## Future Works:

One of the most promising applications of protein droplets is to the study of apoptosis. Malfunctions of apoptosis, the natural process of programmed cell death, play a major role in the development and progression of cancer [5]. However, the study of apoptosis requires costly and specialized cell culture conditions and soluble synthetic substitutes have proven to be inefficient.

Natural apoptosis occurs when a cell presents a complex of transmembrane proteins called the FAS ligand on its surface, which binds to corresponding receptor on the target cell and induces cell death. Droplets coated with the FAS ligand proteins have the potential to act as synthetic cellular analogs, since they are densely coated with active proteins that can move to assemble into the complete ligand complex.

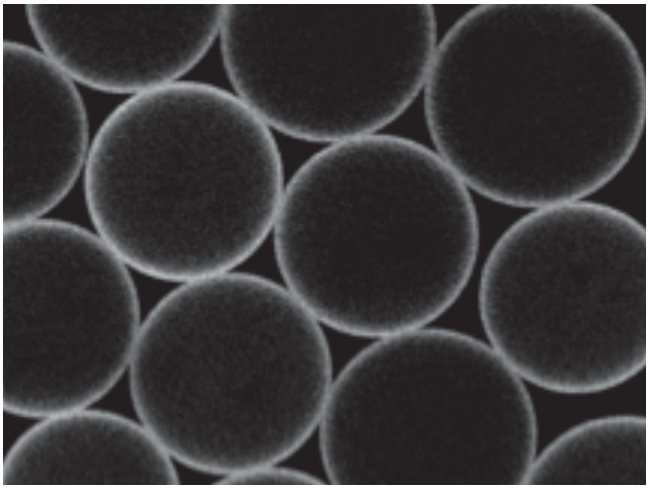

*Figure 1: Droplets covered in engineered transmembrane green fluorescent proteins.*

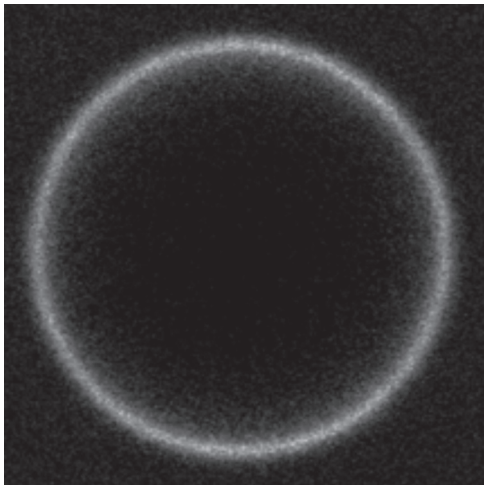

*Figure 3: Active streptavidin-coated droplet binds fluorescent biotin dye.*

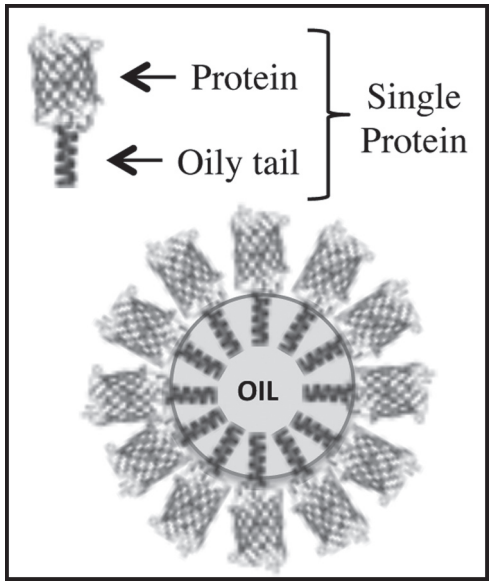


*Figure 2: Schematic of engineered membrane proteins (top) with fully coated oil droplet (bottom).*

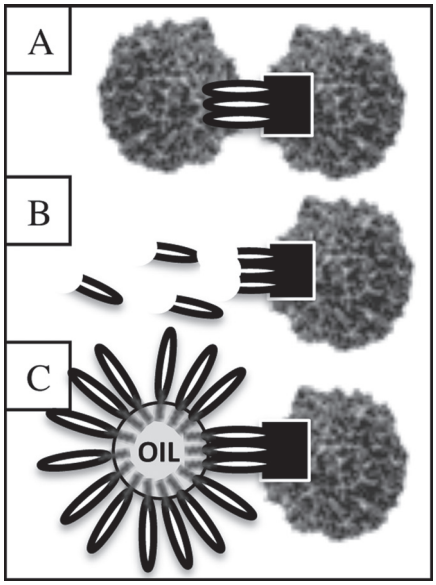


*Figure 4: FAS ligand binding to receptor on target cell bound to (A) another cell, (B) soluble FAS ligand fragments, and (C) FAS ligand protein droplet.*

Droplet protein technology has the potential to greatly reduce the cost barrier of entry for researchers hoping to study apoptosis for the development of cancer therapies.


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