**Introduction**

A series of experiments observing Fluorescence Recovery After Photobleaching (FRAP) microscopy were used to find the recovery rates in lipid bilayers formed with dilauroylphosphatidylserine (DLPS), dilauroylglycerophosphocholine (DLPC), dioleoylglycerophosphocholine (DOPC), and a fluorescently labeled dimyristoylglycerophosphoethanolamine formed in a high ionic strength buffer solution containing HEPES, magnesium chloride with varying concentrations of CHAPS detergent. These experiments were run with 0X, 1X, and 5X CHAPS detergent initially. In each of these samples, there was a presence of round domains sporadically located on the bilayer, and FRAP techniques were used on both the bilayer and the round domains in each sample. Research was also done in the absence of the fluorescently labeled lipid, using the same procedures as before, but then laying down a 50μL layer of 100nM Cytochrome P450 Reductase (a membrane protein) labeled with AlexaFluor 555 in HEPES, sodium chloride, and calcium chloride. Again, using 0X, 1X, and 5X concentrations of CHAPS detergent, FRAP techniques were used to note the phase segregation of the protein in the different domains and to measure the varying diffusion coefficients. Further experiments were conducted with labeled 2C9 Cytochrome P450 to determine co-localization with Cytochrome P450 Reductase within the heterogeneous biomimetic lipid assembly.

**Goals**

2. Observe diffusion coefficients of lipid bilayer using FRAP techniques.
3. Observe behavior of Cytochrome 2C9 using FRET microscopy.

**FRAP Microscopy Setup**

- Titanium Sapphire Laser
- Half Waveplate
- Polarizer
- Thermally Controlled Sample Holder
- Staining Stage
- Oil Immersion Objective
- Two Photon Filter
- Diode Laser
- Hammamatsu Detection Camera

**FRAP Results**

- D = 3.3 μm²/sec Recovery = 98%

**Cytochrome 2C9 FRET Sample**

- Cytochrome 2C9 colocalized on the DLPS, DOPC, and DLPC lipid bilayer.
- Cytochrome 2C9 after being rinsed 7 times with 50 μL of 5X HEPES and magnesium chloride and 1X CHAPS detergent on the same sample as above.

**References**


**Acknowledgments**

Special thanks to the National Science Foundation, Adam Barden, Elisa Silva-Lopez, Angela Rudolph, and Adam Golker for their support.