Kevin O'Brien

PHY 583

Final Project

May 16, 2019

Diffusion of Proteins with Varying Ionic Concentration

The rate of diffusion of a protein or lipid or other polymer is an important quantity in identifying the kind of polymer, and how they interact. In the experiment that follows, we used the confocal fluorescence recovery after photobleaching, or FRAP method to examine the rates of diffusion of polymers of varying ion concentration in the microscope, and through the data acquired construct recovery plots of their fluorescence intensity, and thus calculate the diffusion coefficient for molarities of 125 mM, 250 mM, 375 mM, and 500 mM NaCl. The FRAP method requires using a laser of a particular frequency to photobleach a sample, and then timing its recovery to its original state. The paper "Simplified Equation to Extract Diffusion Coefficients from Confocal FRAP Data" was used as a reference throughout the process of analyzing the data. From this paper, there were two equations which allowed us to analyze our results. The first,

$$f(x) = 1 - Kexp(-2x^2/r_e^2)$$

allowed us to calculate the effective radius of photobleaching that was used in our experiment. This value is the radius of the polymer that was actually photobleached, as opposed to the nominal radius which is the area we attempted to measure, and marked in the software. These two values, the nominal and effective radius were used in the next equation from the paper that we employed

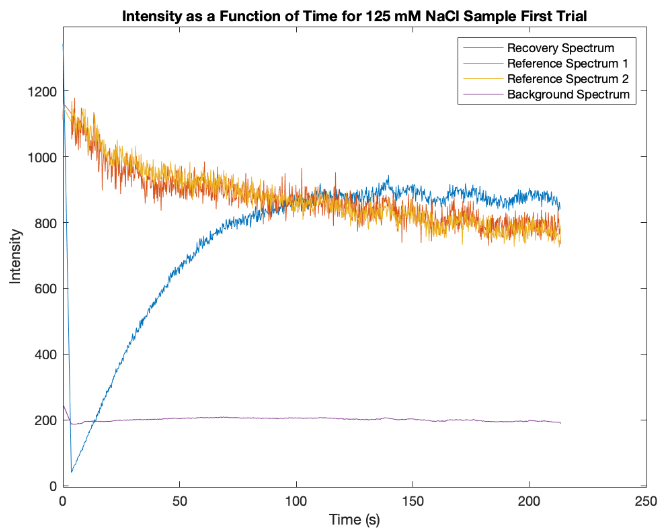
$$D = \frac{r_n^2 + r_e^2}{8\tau_{1/2}}$$

This equation allowed us to calculate the diffusion coefficient using the two radii and $\tau_{1/2}$, or the half time of recovery. To determine the half time of recovery we used the spectral data recorded of the recovery of the polymer's fluorescence as a function of time, and used the method from the paper to determine the half time. Once this was all found, we plotted the diffusion coefficient as a function of concentration.

Due to he small sample size we created a graph of diffusion coefficient vs ion concentration that does not show any sort of strong relationship, however the data analysis performed in the lab effectively employed the methods required.

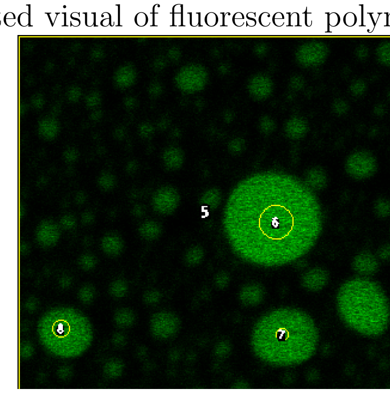
Initially, we gathered data of the polymer droplet that we were bleaching, two reference droplets, and a a background spectrum which was used for reference.

Figure 1: Spectral data gathered of the intensity of polymer droplets with 125 mM Sodium Chloride



From this data, we corrected for the background spectrum by subtracting from our recovered spectrum, and found $\tau_{1/2}$, the half time of recovery. We made similar plots and corrected the samples with the other molarities of NaCl.
Next we used the FIJI software to reconstruct our data, and used it to take measurements of the area of the area we meausred the bleaching of with the microscope. For the first sample, we found an area of 1.712 μm, which allowed us to find a nominal radius r_n of 0.738μm. This same procedure was repeated for the remaining concentrations.

Figure 2: Reconstructed visual of fluorescent polymer using FIJI software



After we found the nominal radius, we found the effective radius r_e by replaying the bleaching of the polymer in FIJI. We used this bleaching to plot the profile of the polymer immediately after bleaching. From this reconstructed profile, we plotted the points and use the equation given by the article "Simplified Equation to Extract Diffusion Coefficients from Confocal FRAP Data",

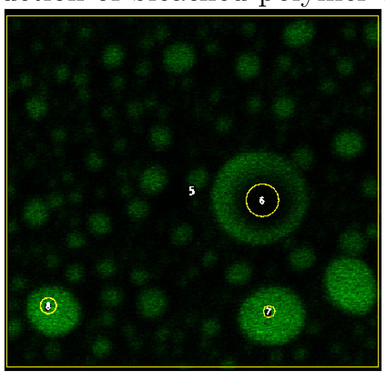
$$f(x) = 1 - Kexp(-2x^2/r_e^2)$$

to determine the effective radius. This radius is determined by using the point at which K has reached 86% of its maximum value, and rearranging the equation such that it reads

$$r_e = \sqrt{\frac{2Kx^2}{ln(y-1)}}$$

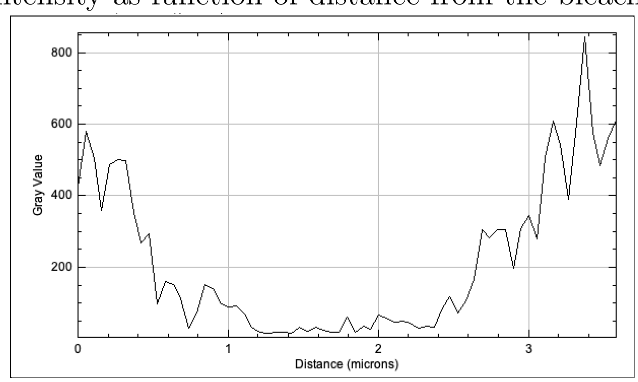
Plugging in our recorded values, we receive a value of r_e for our first sample to be 0.756μm, and repeat the process for the remaining samples.

Figure 3: Reconstruction of bleached polymer using FIJI software



The polymer profile was plotted after bleaching and showed the following.

Figure 4: Intensity as function of distance from the bleached polymer



We then created the following table of values with the addition of a column for D, the diffusion coefficient which was calculated by using the equation

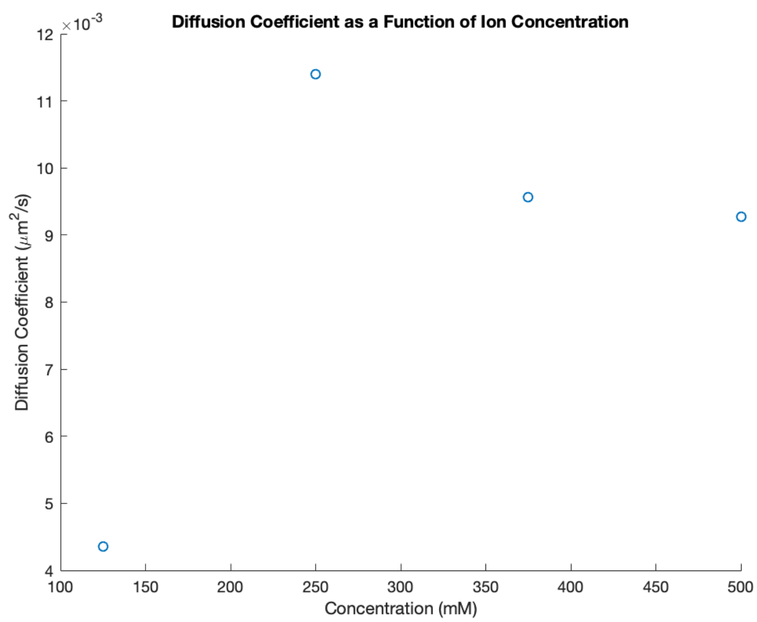
$$D = \frac{r_n^2 + r_e^2}{8\tau_{1/2}}$$

Concentration (mM)	Area (μm^2)	$r_n(\mu$m$)$	$r_e(\mu$m$)$	$\tau_{1/2}(s)$	D (μm$^2/sec$)
125	1.712	0.738	0.756	32	0.00436
250	1.712	0.738	1.17	21	0.0114
375	2.673	0.922	1.68	48	0.00956
500	1.501	0.691	1.53	38	0.00927

Table 1: Table of values of nominal radius, effective radius, and half time of recovery determined from area and fit equations for varying concentrations

These values of D were then plotted as a function of concentration to determine their relationship.

Figure 5: Diffusion coefficient as a function of ion concentration



References

[1] Kang, Minchul. Simplified Equation to Extract Diffusion Coefficients from Confocal FRAP Data. Traffic, 2012, doi:10.1111/tra.12008.