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Initiation of Photochemical Reactions via FRET from Fluorescent Conjugated Polymer Nanoparticles

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Initiation of Photochemical Reactions via FRET from Fluorescent Conjugated Polymer Nanoparticles

A thesis submitted in partial fulfillment of the requirement for the degree of Bachelor of Science in Chemistry from The College of William and Mary

by

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Accepted for Honors

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This Honors thesis is dedicated:

To my parents, who have helped me countless times along the way.

To Dr. Harbron, for guiding me during my time here at W&M.

To Lauren, for always being there to support me.

To God, for creating such an amazing universe to be discovered.
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Chapter 1
Introduction

The inspiration for these experiments came from my research from freshman to junior year. In this chapter I am going to use my research from that time period to introduce the concepts necessary to this field as well as to show the specific observations from which I drew inspiration for the projects represented here, all of which utilize nanoparticles that are composed of conjugated polymers. A conjugated polymer contains a pi system that runs the length of the backbone of the chain (see Figure 1.1).

![Long conjugated backbone](image)

Figure 1.1: MEH-PPV as an example of a conjugated polymer.

The nanoparticles are formed following a literature procedure\(^1\). A precursor solution is prepared consisting of a dilute solution of the desired polymer in THF (~10 -40 ppm). This solution is injected into water while sonicating and then the THF is removed, leaving the polymers in the solution. The polymers are hydrophobic, so they curl onto themselves, forming a sphere, in order to minimize their surface area in contact with the water. The spheres are typically less than 10nm in diameter. The polymer chains curl in on themselves

---

instead of around other polymer chains, as they are very dispersed in the dilute solution. In solutions with higher concentrations of polymer chains, several chains may be incorporated into the same nanoparticle.

Conjugated polymers in solution are often modeled as rigid rods due to the rigidity of the double bonds in the backbone. The hydrophobic effect is strong enough to overcome the rigidity of the double bonds and bend the polymer into the nanoparticle. This was modeled by David Chandler et al.\textsuperscript{3} Oxygen in the water reacts with defects in the polymer to give the nanoparticle a net negative charge\textsuperscript{4}. The negatively charged nanoparticles repel each other, which prevents the hydrophobic effect from aggregating the nanoparticles.

\textsuperscript{2}Figure is from Nanoscale, 2010, 2, 484-494 DOI: 10.1039/B9NR00374F Figure 2
\textsuperscript{3} Drying induced hydrophobic polymer collapse; ten Wolde, Pieter Rein; Chandler, David Los Alamos National Laboratory, Preprint Archive, Condensed Matter (2002), 1-6, arXiv:cond-mat/0207126
\textsuperscript{4} Chemical Defects in the Highly Fluorescent Conjugated Polymer Dots; Clafton, Scott N.; Beattie, David A.; Mierczynska-Vasilev, Agnieszka; Acres, Robert G.; Morgan, Alan C.; Kee, Tak-W. Langmuir (2010), 26(23), 17785-17789.
The nanoparticle is composed of a large number of straight conjugated segments of the polymer backbone bent into a sphere. Long conjugated pi systems (fluorophores) are capable of absorbing and emitting light in a process known as fluorescence. In fluorescence, a fluorophore absorbs incoming light, exciting an electron in the pi system to the pi* orbital. The solvent around the fluorophore adjusts to stabilize the excited state and lowers the excited state’s energy slightly. The fluorophore then returns to the ground state by emitting light of a lower energy wavelength than what was absorbed. The solvent readjusts to stabilize the ground state. This is shown in the Jablonski diagram, Figure 1.3.

Figure 1.3: Jablonski diagram of fluorescence.

Nanoparticles have a large number of fluorophores in a very small area. Not all the segments of polymer backbone in the nanoparticle are of the same length. Longer segments are slightly lower energy fluorophores. When excited, fluorophores in the nanoparticle will transfer their energy to these lower energy fluorophores, which will then fluoresce. Figure
1.4 shows an example of the absorbance and fluorescence of a conjugated polymer nanoparticle. Absorbance and fluorescence are graphed as functions of wavelength.

Figure 1.4: Absorbance and Fluorescence of PFBT nanoparticles. Fluorescence from excitation at 465 nm.

Nanoparticles offer a significant advantage over single particle fluorophores. The multitude of fluorophores in a single nanoparticle makes individual nanoparticles far brighter than single particle fluorophores. Polymer nanoparticles also have a quantum yield that is better than all but the brightest of single particle organic fluorophores\(^5\). Additionally, if a fluorophore in a nanoparticle becomes damaged, the nanoparticle remains fluorescent, whereas a single particle fluorophore stops being fluorescent when damaged.

It should be noted that fluorescence is a slow process when compared to other processes of decaying excited state energy. If there is another process present for the decay of the excited state, the fluorescence of the nanoparticle will be quenched.

---

The project prior to my honors project involved doping these nanoparticles with small molecules (dyes). Doping is a process that relies on the hydrophobicity of the dye molecules. The nanoparticles can be modeled as small droplets of oil suspended in an aqueous solution. If a hydrophobic dye molecule is introduced in this system, it prefers the environment of the nanoparticle over the water. This preference effectively binds the dye to the nanoparticle. Doping is a very efficient method of introducing a dye molecule to a nanoparticle, as it only requires that the dye molecule be added to the nanoparticle precursor solution prior to injection into the water. My previous project studied different ways of attaching dye molecules to nanoparticles. That research determined that doping the nanoparticle with a dye gave similar results to covalently bonding the dye to the polymer backbone.

Photochromic dyes have two forms, a thermally stable form and a photoinduced form (Figure 1.5). These two forms have different absorbance spectra. When the thermally stable form is irradiated, it converts to the photoinduced form. The photoinduced form converts back to the thermally stable form over time. This establishes an equilibrium between the thermally stable form and the photoinduced form known as the photostationary state. Increases in intensity of the irradiating light increase the amount of the photoinduced form of the dye in the photostationary state. The dyes used in this work are all reversible photochromic dyes. Dyes where the thermally stable form of the dye has a higher wavelength that the photoinduced form of the dye are considered reverse.

---

6 Later in Chapters 2 and 3 I use methods of doping nanoparticles with dye after the nanoparticle has been formed. However, the same hydrophobic effect causes the binding to the nanoparticle.
8 For a reversible photochromic dye. Nonreversible photochromic dyes convert entirely to the photoinduced form.
Figure 1.5: Reverse Photochromism

My previous work diverted the energy from the fluorophores to a dye in order to prevent fluorescence. Remember, the nanoparticle funnels the energy from the absorbed light to only a few of the longer chain fluorophores. If the energy could be transferred to a different molecule from the longer chain fluorophores, a very small number of molecules could quench the fluorescence of the entire nanoparticle. In that project, we introduced a photochromic dye molecule to the nanoparticle. The photoinduced form of the dye could accept energy transferred from the fluorophores. The dye molecule then dissipated the excited state energy as heat in order to return to the ground state (see Jablonski diagram Figure 1.6). Since the dye molecule could only accept energy from the nanoparticle if it was in its photoinduced form, the nanoparticles’ fluorescence could be toggled on and off via irradiation of the photochromic dye with ultraviolet light.
Figure 1.6: Jablonski diagram of previous work.

This energy transfer to the dye molecule was the inspiration for my honors thesis project. Instead of the dye molecule dissipating the energy as heat, I proposed to use that energy for a photochemical reaction. I envisioned the nanoparticle as a giant antenna that would absorb incoming light with its hundreds of fluorophores and then funnel all that energy to drive photochemical reactions in a dye molecule. Both of the projects for my honors thesis use the energy of the nanoparticle to drive a photochemical reaction. One project uses nanoparticles to drive a reverse photochromic reaction and the other project uses them to drive a photobase generator.

The energy is transferred to the dye molecule using Förster resonance energy transfer (FRET). FRET transfers energy through a magnetic interaction between the dipole
moments of the donor (nanoparticle fluorophore) and the acceptor (dye). The rate of FRET is given by the following equation:\(^9\):

\[
\kappa_T(r) = \frac{Q_D \kappa^2}{\tau_D} \left( \frac{1}{r^6} \right) \frac{9000 (\ln 10)}{128 \pi^5 N n^4} J
\]

There are two variables of particular importance in the equation\(^10\). The first is the \(\frac{1}{r^6}\) term. \(r\) is the distance between the two molecules. \(\frac{1}{r^6}\) falls off very quickly as the molecules get farther apart. Therefore, it is important that the two molecules be very close together to have efficient FRET. Remember, the dye molecule is held close to the nanoparticle because it is not water soluble. If the dye were water soluble then it would float away from the nanoparticle and very little FRET would be observed.

The second variable of particular importance in the equation is the \(J\) term. This term represents the overlap between the fluorescence of the nanoparticle transferring the energy and the absorbance of the molecule receiving the energy.

\[
J = \int_0^{\infty} F_D(\lambda) \varepsilon_A(\lambda) \lambda^4 d\lambda
\]

Figure 1.7: Illustration of the overlap integral \(J\) (purple). Donor Fluorescence (red) and acceptor absorbance (blue).

---


\(^{10}\) \(Q_D\) is the donor’s quantum yield, \(\tau_D\) is the donor’s excited state lifetime, \(n\) is the refractive index of the solution, \(k\) is dependent on the orientations of the dipoles, and \(N\) is Avogadro’s number.
Graphically, the overlap is the area below the fluorescence and absorbance graphs (shown in purple in Figure 1.7). $F_D(\lambda)$ is the graph of the fluorescence of the nanoparticle and $\varepsilon_A(\lambda)$ is the graph of the absorbance of the dye. $\lambda$ is the wavelength of the light (x axis of fluorescence and absorbance graphs).

Essentially $J$ is a measure of the extent to which the color of light emitted is the same as the color of light absorbed. The $J$ term leads to the second requirement for a FRET interaction: the donors the fluorescence needs to have good overlap with the absorbance of the acceptor. However, remember that no light is actually emitted in FRET as it is an entirely magnetic interaction with no electric component. The $J$ term leads to another interesting feature of FRET. The interaction only requires sufficient overlap; it does not specify that the acceptor must be “downhill” in energy from the donor. As long as the overlap is the same, the FRET interaction is equally probable.

Figure 1.8: Both have an equal overlap of donor fluorescence (red) and acceptor absorbance (blue) and therefore equal chance of a FRET interaction occurring.
The fact that the FRET interaction can go uphill in energy just as easily as it can go downhill might build a fear that the FRET interaction could simply happen in reverse. However, remember that it is the fluorescence peak of the donor interacting with the absorbance of the acceptor. To go backwards, one would look at the fluorescence of the acceptor and the absorbance of the donor. In all experiments in this paper, the fluorescence of the dye molecule (acceptor) is essentially zero, so there is no FRET from the dye back to the nanoparticle.

Overall, doped conjugated polymer nanoparticles represent a robust system. The absorbance and fluorescence of the nanoparticles can be tailored by the choice of polymer. The function of the nanoparticle can be tailored by the selection of the appropriate dye. Polymer nanoparticles’ fluorescence is extremely bright. Furthermore, the fluorescence can be quenched by a small amount of dye molecules. Lastly, the energy from quenching the nanoparticle can be funneled to the dye. The goal of this work is to accomplish useful work from the energy funneled to the dye molecule.
Chapter 2

“Off to On” Photoswitchable Fluorescent Nanoparticles.

Introduction:

This experiment uses the energy from the nanoparticle to drive a reverse photochromic dye. These dyes switch to a lower wavelength absorbance upon being irradiated. The dyes and polymer are selected such that they have good overlap for FRET between the fluorescence of the nanoparticle and the absorbance of the thermally stable form of the dye. Upon irradiation, the nanoparticle, instead of fluorescing, transfers the energy to the reverse photochromic dye (Figure 2.1). The reverse photochromic dye uses that energy to switch to its form that absorbs at a lower wavelength, with a poor FRET overlap. As it is no longer able to FRET to the thermally stable form of the photochromic dye, the nanoparticle becomes fluorescent.

Figure 2.1: Jablonski Diagram of Off to On Nanoparticles.
When added to the nanoparticle, the dye creates a system where the nanoparticle can be switched from being nonfluorescent to fluorescent. This system has a major advantage over my previous work with photoswitchable fluorescent nanoparticles. Those nanoparticles used an additional wavelength of light (365nm) to irradiate the dye, which can damage samples. In this experiment, the duration and intensity of the light that shines upon the nanoparticles is the controlling factor that switches the fluorescence of the nanoparticle (Figure 2.1).

As a result, this photoswitchable nanoparticle operates entirely within the visible region. At low intensities of light, a large number of the molecules are still in the thermally stable form at the photostationary state. Since the thermally stable form can FRET with the nanoparticle, the fluorescence is blocked. At higher intensities of light, the dyes are mostly in the photoinduced form at the photostationary state. As the photoinduced form cannot FRET with the nanoparticle, the nanoparticle returns to being fluorescent.
Figure 2.2: Scheme of Nanoparticle Quenching

The effect of different intensities allows measurements of the fluorescence of the nanoparticle in the “off” configuration. Short, weak intensity pulses of light from the fluorimeter are used to measure the fluorescence. These short, weak pulses do not have a large effect on the fluorescence, as it takes time for the reverse photochromic dyes to change forms and they lack the intensity to affect a large number of the dyes. To turn “on” the nanoparticles’ fluorescence, a large flood light is shined on the sample. The fluorimeter only reports the change in fluorescence from its short, weak pulses that it sends into the sample. As a result, the reported fluorescence change from the large flood light only represents the increase in fluorescence from the change in the dyes and not an increase in fluorescence from the additional incident light.
Applications:

In 2014, the Nobel Prize in Chemistry was awarded to Eric Betzig, Stefan Hell and William Moerner for their development of super resolved fluorescence microscopy\(^{11}\). Super resolved fluorescence microscopy allows for the resolution of distances below the diffraction limit of the incident light\(^{12}\). The following figure illustrates the dramatic increase in resolution attained by super resolved fluorescence microscopy.

![Figure 2.3: Distribution of GFP-labeled lysosome protein (CD63) without (A) and with (B) super resolved fluorescence microscopy. Image by Betzig et al.\(^{10}\).](image)

A small component of the instrument that they developed is that the proteins they want to image must have fluorescent tags attached to them that can be switched between fluorescent and non-fluorescent states. The application of “Off to On” nanoparticles would be to replace the current small molecules that are being used for the tags. Compared to switchable single molecules, nanoparticles are orders of magnitude more fluorescent and far more robust\(^{13}\). Previous work in Harbron lab has made a variety of polymer nanoparticles by the same method all of which were determined to have less than 10nm in diameter via

\(^{11}\) Betzig et al., Science, 2006, 313, 1642-1645  
\(^{12}\) For a very oversimplified conceptualization, having resolution “below the diffraction limit of light” can be thought of as seeing the distance between molecules that is less than the “size” of the photon of light being used to see them.  
dynamic light scattering. I suspect that the nanoparticles in this work are also less than 10nm. While the nanoparticles are larger than single molecules, they are still far smaller than the cells that are being imaged. This chapter deals solely with the creation of nanoparticles that exhibit the necessary photoswitchable fluorescence and does not attempt to use them for imaging of biological samples. However, Daniel T. Chiu et al. proved that it is possible to attach photoswitchable fluorescent PFBT nanoparticles to cells for cell imaging. The photoswitching wavelength was in the ultraviolet (365nm){14}

**Materials:**

This experiment makes use of one polymer type, PFBT; two different dyes, APESO and APSO; and a coating polymer, PVCoCo. Before discussing the results of the experiment, I will first give the characteristics of these compounds.

**PFBT:**

![Figure 2.4 PFBT Structure](image)

---

A nanoparticle composed of PFBT absorbs green light and emits yellow light as shown in Figure 2.5. This polymer was chosen as it has a very high fluorescent yield and overlaps well with the absorbance of the dyes.

A sample of PFBT without dye was irradiated at 488 nm. It showed minor photobleaching that diminished the fluorescence of the nanoparticle. This blank sample demonstrates that any increases in fluorescence intensity (shown later) are the result of changes in the dye molecule, not a photophysical effect of irradiating the nanoparticle. Additionally, this proves that the fluorimeter is effective at canceling out the additional fluorescence from irradiating the nanoparticle.

Figure 2.5: Absorbance and Fluorescence of PFBT nanoparticles excitation at 456nm
Figure 2.6 APESO structure.

APESO is a reverse photochromic spirooxazine dye. Due to the large group similar to adamantane, it prefers the merocyanine (MC) form. Upon irradiation it switches from its thermally stable MC form to the photoinduced spirooxazine (SO) form. When it converts to the SO form it loses the peak at 550nm in Figure 2.6. With the removal of the irradiation, the SO form returns to the MC form. Figure 2.7 shows the overlap between the fluorescence of PFBT nanoparticles and the absorbance. Note that the two peaks have a massive amount of overlap. This overlap allows for a very efficient FRET interaction between the MC form of

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15 Modulating short wavelength fluorescence with long wavelength light; Copley, Graeme; Gillmore, Jason G.; Crisman, Jeffrey; Kodis, Gerdenis; Gray, Christopher L.; Cherry, Brian R.; Sherman, Benjamin D.; Liddell, Paul A.; Paquette, Michelle M.; Kelbauskas, Laimonas; et al; Journal of the American Chemical Society (2014), 136(34), 11994-12003.
Figure 2.7: Absorbance of APESO in toluene. Also shown is the fluorescence of PFBT. Nanoparticles (ex. 456nm).

When dissolved in toluene, the APESO did not show any switching to the MC form when irradiated for 30 seconds with 488 or 514nm light. The lack of switching shows that the dye is not particularly sensitive to irradiation. I hypothesize that the effective increase in intensity from the energy funneling effect of the nanoparticle will lead to a photoswitching effect.
APSO is a reverse photochromic dye, very similar to APESO. Upon irradiation it switches from its merocyanine (MC) form to the spiroxazine (SO) form. With the removal of the irradiation it returns to the MC form. This dye shows higher photobleaching and worse photomodulation results than APESO dye, as demonstrated in the following experiments.
This polymer is used as an additive to the nanoparticles. We theorized that PVCoCo coats the PFBT nanoparticles, adding a protective layer that can help prevent oxygen from entering the nanoparticle. Use of PVCoCo was inspired by previous work in Harbron lab\textsuperscript{16}. Whether this coating is around the outside of the nanoparticle or incorporated throughout the nanoparticle is unknown. PVCoCo also slightly changes the polarity of the environment of the nanoparticle.

**Experimental:**

Nanoparticle precursor solutions of PFBT (30ppm) in tetrahydrofuran (THF) were prepared. These solutions were filtered at .7μm with a Fisher micro filtration apparatus. The solutions were sonicated for 30 seconds, and then 1ml of solution was injected into 8ml of ultrapure water. This solution was sonicated for another 2 min. A solution of dye and THF was injected into the nanoparticle solution to bring the nanoparticle to 10% dye and 90% PFBT by wt. The sample was sonicated for another 10 seconds. The solution was placed under high vacuum for 30-45min at room temperature to remove the THF. The solution was filtered at .7 and .22 μm with a Fisher micro filtration apparatus to remove aggregates of the nanoparticles.

For the low dye concentration experiments, the nanoparticles were prepared in the same manner except the dye was included at 0.5% dye to 99.5% PFBT by wt. in the nanoparticle precursor solution, and no dye was added after the 2 min of sonication.

For the coating polymer, PVCoCo was included in the nanoparticle precursor solution. The amounts were adjusted to 10% dye, 10% PVCoCo and 80% PFBT by wt.

\textsuperscript{16} Ratiometric Fluorescence Detection of Mercury Ions in Water by Conjugated Polymer Nanoparticles Childress, Elizabeth S.; Roberts, Courtney A.; Sherwood, Desmarie Y.; LeGuyader, Clare L. M.; Harbron, Elizabeth J.; Analytical Chemistry (Washington, DC, United States) (2012), 84(3), 1235-1239.
Excepting the changes in ratios of dye and the addition of PVCoCo, the nanoparticles were prepared in the normal manner.

Absorbance measurements were made with a Varian Cary 50. Fluorescence measurements were made with a Varian Eclipse at an excitation wavelength of 467 nm. All measurements were made open to the atmosphere except the low dye concentration experiments as noted, which were degassed in argon gas for 10 min and measured in a sealed cuvette. Irradiation of the samples was done with 488nm argon ion laser for the low dye percentage samples. For safety reasons, the light source was switched to a 455 nm LED floodlamp for the other samples.

**Results & Discussion:**

The sample’s fluorescence change upon irradiation was assessed by two measures. The first measure was percent photomodulation, which measures the increase in fluorescence upon irradiation. The second was recovery efficiency, which measures the extent to which the nanoparticle returns to being nonfluorescent after the irradiation is removed.

The percent photomodulation is defined as:

\[
\frac{\text{Maximum intensity upon irradiation}}{\text{starting intensity}} \times 100\%
\]

The recovery efficiency is defined as:

\[
\frac{\text{maximum intensity} - \text{final intensity}}{\text{maximum intensity} - \text{starting intensity}} \times 100\%
\]
Low Dye Percentage

The rationale for using a very low level of dye is as follows. In my previous work with nanoparticles, 3% of doped dyes underwent conversion upon irradiation\textsuperscript{17}. A very small number of dye molecules are needed to block the fluorescence of the nanoparticle. Additional dye molecules have little effect on diminishing the fluorescence. A very low percentage of dye molecules were used to ensure that all dye that photo converted would create noticeable change in the fluorescence.

Ultimately it was discovered that this line of reasoning was flawed as 51.4% of the dye molecules converted to the spirooxazine form upon irradiation. I hypothesize that the change from 3% to 51.4% photoconversion can be attributed to the amplification of the effective “intensity” of the incident light via FRET from the nanoparticle.

Low dye percentage results

Use of low percentages of dyes gives interesting results. The dye percentage is so low that the dye cannot be detected by absorbance spectroscopy. The dye molecules precipitate out of solution prior to the nanoparticles forming. Thus, nanoparticles with extremely small amounts of dye are made. As absorbance spectroscopy cannot detect the dye and a large amount of the dye is filtered out, the amount of dye present in the nanoparticles is unknown. The presence of the dye can only be determined by fluorescence which does not give a quantitative amount of dye. As a result, the dye filtering out was not discovered until late in this experiment.

\textsuperscript{17}Functionalization of Conjugated Polymer Nanoparticles for Fluorescence Photomodulation; Chamberlayne, Christian F.; Lepekhina, Elena A.; Saar, Brooklynd D.; Peth, Kathryn A.; Walk, Jordan T.; Harbron, Elizabeth J.; Langmuir (2014), 30(48), 14658-14669.
Nonetheless, the low dye percentage nanoparticles give useful results. As the nanoparticles have very few dye molecules, they are particularly sensitive to side reactions that degrade the dye in the nanoparticles. This allows for causes of these side reactions to be easily determined. First, the nanoparticles show a large bleaching effect. Alternate side reactions were taking place that destroyed the dye. This permanent loss of dye gave rise to photomodulation that did not return to the same fluorescence intensity after the light was removed as shown in Figure 2.9 below.

![Figure 2.9: Photobleaching of low dye concentration APESO nanoparticles that were not degassed. Yellow shows periods of irradiation at 488nm.](image)

It was hypothesized that oxygen was a key component in these side reactions. To test this hypothesis, APESO containing nanoparticles samples were degassed with argon. The argon displaces the oxygen dissolved in the water. Upon irradiation these degassed samples showed a massive decrease in photobleaching\(^{18}\) as shown in figure 2.10. This confirms that oxygen plays a key role in the side reactions.

\(^{18}\) Photobleaching of the dye is destruction of the dye molecule (via side reactions) caused by irradiation. The loss of the dye increases the fluorescence on the nanoparticle.
Figure 2.10: Fluorescence of low dye concentration APESO nanoparticles after degassing with argon. Yellow shows periods of irradiation at 488nm.

**Low percentage dye with coating polymer:**

PVCoCo as a coating polymer is added to the nanoparticle. The purpose of the coating polymer is to protect the dye from any remaining oxygen in the water after degassing. In addition to reducing the photobleaching, the coating polymer caused an increase in the photomodulation of the nanoparticles to 8% as shown in Figure 2.11 below. An important note about the spectra in both Figures 2.9 and 2.10 is that the y axis starts at 355 and 520 respectively. These low dye concentration nanoparticles are more a case of “bright to a bit brighter” instead “off to on”.

In Figure 2.11, the initial slight rise in fluorescence intensity at 0 seconds is caused by the light from the fluorimter converting some of the dyes to the SO form. This rise shows that the fluorimeter light used to determine the fluorescence of the nanoparticle when it is “off” does have a minor effect on the sample.
Figure 2.11: Fluorescence of low dye concentration APESO nanoparticles with coating polymer. Yellow shows periods of irradiation at 488nm.

Prior to addition of the coating polymer, the photomodulation was about 2%. It is unclear exactly why the coating polymer increased the fluorescence modulation. Three possible explanations are: 1) the coating polymer helped to retain a slightly higher amount of dye in the creation process; 2) the coating polymer changed the polarity of the nanoparticle environment in a way that increased the amount of dye that to the spirooxazine form; and 3) the coating polymer changed the configuration of the nanoparticle, allowing for more room between the rigid segments of the nanoparticle. This extra room allowed a larger percentage of the dyes to be able to convert to the spirooxazine form.

A last discovery in the low percentage experiments was that the APESO dye worked better than the APSO dye. The APSO dye showed more photobleaching. Even when degassed with a coating polymer, the APSO dye showed photobleaching. Overall, APESO is the better dye.
10% APSO dye:

Figure 2.12 shows the change in fluorescence of the 10% APSO dye nanoparticles upon irradiation. The APSO sample increases 83% in fluorescence intensity with an 83% recovery efficiency when irradiated for 20 seconds.

I want to bring attention to a feature of the peaks. They appear to be composed of two distinct processes. At about 4 seconds, the faster process dies out and the slower process begins to dominate the fluorescence change. I believe the fast process represents switching from the MC to the SO form of the dye and the slower process is photobleaching. These two processes are particularly evident in the APSO experiments, as they have more photobleaching. Note in Figure 2.13 how the distance recovered back is roughly approximate to the distance gained by only the fast process. This lends further support to the idea that the fast process is the reversible photoswitch, with the slow process being loss of the dye.
Figure 2.13: Fluorescence 10% APSO nanoparticles. Note the two difference rates at which the fluorescence increases. Yellow is the period of irradiation at 455 nm.

If the sample is irradiated for a long period of time, the dye is destroyed. When the sample is irradiated for 79 seconds instead of 20, the fluorescence continues to increase; however, the fluorescence remains high after the light is removed. This is consistent with the dye being destroyed, resulting in a fluorescence increase. Absorbance scans of the sample also show no APSO present in the sample afterwards.

10% APESO dye:

The 10% APESO dye nanoparticles show excellent photomodulation. As there is a lot of dye present, the nanoparticles initially show almost no fluorescence (4.5 fluorescence units). Upon irradiation (by LED light source) the samples show an increase to 129 fluorescence units. This is a 2766% increase in fluorescence intensity.\(^{19}\) The sample returns to a fluorescence intensity of 5.7 upon removal of the irradiation, a 99% recovery

\(^{19}\) This was from a single trial, More trials are needed to determine the error on this measurement.
percentage. Unfortunately, the amount of bleaching increases with each subsequent irradiation.

Figure 2.14: Reversible changes in fluorescence of 10% APESO nanoparticles upon irradiation. Yellow represents periods of irradiation at 455 nm.

In the absorbance spectrum of 10% APESO nanoparticle (Figure 2.15), there is a small decrease at 576 nm upon irradiation. This decrease is the loss of the MC form of the APESO dye to its SO form. Note in the inset of figure 2.15 how after 1 min the peak almost returns (green) to the original intensity (red) but not quite. This slight loss in absorbance represents the loss of APESO dye to side reactions.
The change in the absorbance at 566 nm over time was tracked. The purpose of taking a time dependent absorbance scan is to compare it the time dependent change in fluorescence. However, the changes in absorbance and in fluorescence do not have the same rate constants. This difference is to be expected as fluorescence is not linear with regards to dye concentration, whereas absorbance is linear. As shown in Figure 2.16, the absorbance spectrum descent follows a fitted exponential curve upon irradiation but not perfectly. The deviation from an exponential curve comes from contribution of the rate of the photobleaching side reaction. The photobleaching is also evidenced by the absorbance not fully returning to its original value after the light is removed. The return to the MC form of the dye after the light is removed shows an exponential fit. The exponential fit proves that
the return of the dye to MC form is the only reaction taking place after the light is removed. Combined, these two rates prove that the side reactions which degrade the dye use the excited state MC form of the dye as opposed to reacting with the SO form.

![Figure 2.16: Absorbance kinetics of APESO dye. Yellow shows period of irradiation at 455 nm. (cuvette was not zeroed)](image)

The fluorescence does not conform to exponential fits, which further underscores that the amount of quenching does not scale linearly with the amount of dye in the nanoparticle.

**Degassed, 10% APSO dye:**

To help with the photobleaching, a sample was degassed. Initial experiments show that upon degassing, the dye left the nanoparticles and precipitated out of solution. I suspect that the bubbling action encouraged leakage of the dye into the solution and that dye then precipitated. The degassing was done on a different aliquot of the same sample as the results shown above for the 10% APESO dye. The remaining non-degassed sample still had its dye over the same time period.
**10% APESO with 10% Coating Polymer:**

Adding a coating polymer had good effects in the low concentration dye samples giving inspiration for this experiment. The coating polymer may prevent photobleaching by protecting the dye from the oxygen in the sample.

Samples with a coating polymer decreased the photobleaching as expected (see Figure 2.17). However, the coating polymer also increases the intensity of starting fluorescence. The increase is small but noticeable, from 4.5 fluorescence units to a starting point of 17 fluorescence units. Both samples had 10% dye by wt. This increase is unfortunate but expected. The addition of the coating polymer increases the average distance between the dye and the fluorophore. The increased distance leads to a slight decrease in FRET, which is seen as the slight increase in fluorescence intensity.

![Figure 2.17: Fluorescence of Coated 10% APESO dye. Yellow indicates periods of irradiation at 455 nm.](image)

The absorbance spectrum of the nanoparticles shows that the APESO dye is converted to its SO form upon irradiation (inset, Figure 2.18). Also, notice the size of the
absorbance peak of the nanoparticle compared to the size of the peak from the APESO. It is far easier for the nanoparticle to absorb incoming light and transfer that energy to the APESO dye than it would be to get the APESO to absorb the light. The difference in these peaks is the best representation that I have of how the nanoparticle dramatically increases the absorbance (and thus decreases the necessary intensity of light) needed to convert the APESO to its other form.

Figure 2.18: Absorbance spectrum of coated 10% APESO nanoparticles before, immediately and 1 min after 5 sec of irradiation with 455 nm light.

Since the change upon irradiation is very small (figure 2.18), figure 2.19 is a difference spectrum showing the change in the absorbance. The decreases are shown as positive increases to illustrate that the lost absorbance matches the spectrum of the MC form of the APESO dye. Note how the spectrum changes between 500 and 600nm. The
decrease at 500nm - 600nm matches the spectrum of APESO in toluene proving that what is changing in the spectrum is the APESO dye.

Figure 2.19: Inverted and Normalized difference spectra of coated 10% APESO nanoparticles before and after irradiation (red) and normalized absorbance of APESO dye in toluene (black).

Future Direction:

This project has achieved most of its original goal to create an “off to on” fluorescent nanoparticle entirely within the visible region. In the future, I would look to make the nanoparticles more robust by attaining a repeatable recovery of original intensity and make the nanoparticles stabile over longer irradiation times. To achieve these new goals I would suggest the following strategies.

The 10% dye and 10% coating were educated guesses for the best amount. Variation of these amounts to find the optimal amounts of both is the next step in this experiment.
Second, I suggest varying the order of coating polymer addition on creation of the nanoparticles. Instead of creating coated nanoparticles and then adding dye molecules to them, create nanoparticles, add the dye and then add the coating. Changing the order of addition should help trap the dye closer to the center of the nanoparticle where it will have better FRET, it should also protect the dye better from the oxygen in the water. This type of nanoparticle may also be able to be degassed.

Lastly, look into using a normal photochromic dye. These are far more common and can be more robust that reverse photochromic dyes. I did some preliminary studies of a normal photochromic dye system. I took a photochromic spirooxazine dye (SOMe) from my previous work and the PPE polymer from Chapter 3 and combined them. As this is not a reverse photochromic dye, SO is the thermally stable form. The SO overlaps with the fluorescence of the PPE nanoparticle but not particularly well. Upon irradiation of the PPE the nanoparticle should have a small fluorescence, and then the SO converts to the MC form and the fluorescence should increase.

Figure 2.20: MC and SO form of SOMe

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The absorbance showed a new peak at 600 which represents the MC form of the photochromic dye (figure 2.21).

![Absorbance graph](image)

Figure 2.21: SOMe dye in PPE nanoparticle. Absorbance before and after 1 min of irradiation at 365nm.

While the absorbance showed the anticipated change, the fluorescence did the opposite of what was expected; it decreased upon irradiation (figure 2.22).

![Fluorescence graph](image)

Figure 2.22: SOMe dye in PPE nanoparticle. Fluorescence before and after 1 min of irradiation at 365nm (ex 365nm).
Upon further investigation of the system it is observed that the MC form of the dye catches the trailing end of the PPE fluorescence (figure 2.23). This FRET to the photoinduced MC form of the dye quenched the fluorescence of the nanoparticle.

![Absorbance and Fluorescence Spectra](image)

Figure 2.23: PPE fluorescence quenching

This system of SOMe and PPE creates an interesting nanoparticle whose fluorescence decreases when the intensity of the irradiating light increases. This is a quite novel type of nanoparticle and deserves further investigation.

Conclusions:

This experiment successfully created an “off to on” nanoparticle using only visible light. The nanoparticles do suffer from mild photo bleaching on repeated cycling of the “off to on” function. The two best systems are APESO dye, which has the best photomodulation; and APESO with a coating polymer, which has the least photobleaching while still maintaining the off to on switchability.
Chapter 3

pH Changing Nanoparticles

Introduction:

This work proposes to create nanoparticles that alter the pH of a solution upon irradiation. Light responsive changes in pH are commonly accomplished with a series of compounds called photo acid generators (PAG) and photo base generators (PBG). This project intends to demonstrate that when PAGs and PBGs are incorporated into nanoparticles, the energy transferred from the nanoparticle can be used to initiate their photochemical reaction. Additionally, this project intends to demonstrate that the progress of that photochemical reaction can be monitored via the change in the nanoparticle’s fluorescence.

Particles that change the pH of a solution are useful in a variety of applications. Yongfeng Gao and Michael J. Serpe used photo acid generators to make pH responsive, color changing microgel-based etalons into light responsive, color changing microgel-based etalon\(^{21}\). Alternatively, consider the use of pH changing nanoparticle in the following example: studying the mitochondrion of a cell by changing the pH inside the mitochondria without changing the pH of the rest of the cell. The location of the mitochondrion is determined with a microscope. A PAG or PBG is introduced to the cell and laser is shown on the mitochondrion to initiate a photoinduced pH change at that location. A PAG/PBG containing nanoparticle would be better to introduce to the cell than the PAG/PBG alone. When irradiated by the laser, the nanoparticle funnels energy to the PAG or PBG allowing lower intensities of light to be used as opposed to the PAG or PBG by itself. High intensities

\(^{21}\) Light-Induced Color Changes of Microgel-Based Etalons; Yongfeng Gao and Michael J. Serpe, ACS Appl. Mater. Interfaces, 2014, 6 (11), pp 8461–8466 DOI: 10.1021/am501330z
of light are undesirable as they can heat up the cell. Additionally, many PAGs and PBGs are not water soluble, which presents a problem for introducing them into the cell. The nanoparticles host non-water-soluble PAGs and PBGs allowing them to be used in aqueous environments. This example illustrates how a pH changing nanoparticle could be useful.

The system for the pH changing nanoparticle is very similar to the “off to on” nanoparticles from chapter 2. The nanoparticle’s fluorescence matches the absorbance of the PAG or PBG inside the nanoparticle. Upon irradiation of the nanoparticle, the energy is transferred to the PAG or PBG via FRET, and the PAG or PBG then changes the pH. As the PAG or PBG is consumed, FRET to the remaining PAG or PBG is diminished and the fluorescence of the nanoparticle increases.

![Figure 3.1: Jablonski Diagram of pH Changing Nanoparticles.](image)

An ideal sample would exhibit the following. First, the absorbance spectrum of the sample would show two peaks, one for the nanoparticle and one for the PAG/PBG. Upon irradiation of the nanoparticle peak, the peak representing the PAG/PBG would disappear and a new peak representing the byproduct formed by the PAG/PBG would appear. The
byproduct peak is often blue shifted from the original peak due to a decrease in the size of the chromophore from the loss of the acid/base. These changes to the absorbance spectrum are shown in figure 3.2.

![Absorbance vs Wavelength Diagram](image)

Figure 3.2: Theoretical depiction of changes to UV-vis spectrum upon irradiation.

Changes would also be apparent in the fluorescence spectrum. As the PAG/PBG is consumed, the overlap between the nanoparticle fluorescence and the absorbance of the PAG/PBG would decrease. As the overlap decreases, FRET from the nanoparticle to the PAG/PBG is diminished. With the decrease in FRET, more energy is left for the nanoparticle to use in fluorescence and the fluorescence intensity increases similar to figure 3.3. Coupled with the fluorescence increase would also come a change in pH of the solution generated by the PAG/PBG.
The first task in this experiment is to prove the novel concept that a photochemical reaction can be initiated, not by absorbing light, but rather by energy that is transferred via FRET. The second task is to find a suitable polymer and PAG pair for the nanoparticle.

Overall, this project makes use of two different polymers: PPE and PVK; and three different dye molecules: PAG1, PAG2 and PBG1.

**PVK**: Poly(9-vinylcarbazole):

Unlike the other polymers in this paper, PVK does not have a conjugated backbone. Instead, it has a large pendant carbazole attached to the side of the polymer chain. These pendant carbazoles stack with one another via pi-pi stacking of the aromatic rings. This stacking creates a pi system running the length of the
polymer similar to the pi system that runs the length of the backbone of the other conjugate polymers used in this work. One difference from the polymers with conjugated backbones is the pi-pi stacking is much easier to break than double bonds, so the polymer tends to form fluorophores of shorter lengths when made into a nanoparticle. This gives it the bluest fluorescence and absorbance of all the polymer nanoparticles used in this paper. Its absorbance and fluorescence are shown in figure 3.4. As will be shown later, PVK turned out to be a fairly useless polymer for this experiment.

Figure 3.4: Absorbance and Fluorescence spectrum of PVK nanoparticles
PPE: Poly (2,5-di(2-ethylhexyl)phenylene-1,4- ethynylene)

PPE turned out to be a good polymer for the experiment. Like PVK, it is also a very blue fluorescent polymer. The very blue fluorescence allows it to FRET with compounds that absorb mainly in the near UV. It can also be excited at 405nm which is in the visible range of light. Additionally, PPE has four distinct peaks in the fluorescence (figure 3.5). This opens up the possibility of using a ratio between two peaks instead of simply fluorescence intensity to determine the amount of acid released.
The PPE polymer has a problem. Upon irradiation at 365nm, it photobleaches in a manner that increases its fluorescence intensity. The following graphs (figures 3.6A-C) show the effects of irradiation on PPE nanoparticles that do not have dye molecules in them. Note how the nanoparticles photobleach. The photobleaching breaks the fluorophores into pieces creating two new smaller fluorophores. The increase in number of the fluorophores in the nanoparticle leads to a higher fluorescence intensity. Additionally, bluer fluorophores may have a higher quantum yield\textsuperscript{22}. As the fluorophores are made smaller, there is a slight blueshift in the florescence of the peaks (figure 3.6A). The absorbance also changes slightly a shown by the absorbance difference spectrum (figure 3.6C). Note how there is a decrease in absorbance at 400nm and an increase in absorbance at 250nm. This reflects the

\textsuperscript{22} Quantum yield is the ratio of number of photons absorbed to photons released in fluorescence. Nanoparticles with higher quantum yields are brighter.
shortening of the fluorophores in the nanoparticle.

Figure 3.6: Changes in PPE nanoparticles upon irradiated at 365nm between seconds 60-180 and 400 – 514.

A) Fluorescence before and after irradiation
B) Fluorescence change over time
   Yellow is during irradiation.
C) Difference spectra showing changes in the absorbance spectrum after irradiation.

PAG1\textsuperscript{23}: (E)-3,4,6-trichloro-2-((4-methoxyphenyl)diazenyl)phenol

\begin{align*}
\text{pKa} &= 9.5 \\
\text{trans-form} &\quad \text{proton not released} \\
\text{cis-form} &\quad \text{proton released!}
\end{align*}

\textsuperscript{23}2-Hydroxyazobenzenes to tailor pH pulses and oscillations with light; Emond, Matthieu; Chemistry - A European Journal 2010, V16(29), P8822-8831
PAG1 changes the acidity of its phenolic hydrogen by isomerizing the double bond. In the trans configuration there is additional hydrogen bonding from the nitrogen in the azo group. When irradiated with light it isomerizes to the cis configuration, removing the hydrogen bond and increasing the acidity of that hydrogen. This process is reversible, allowing for the possibility of a reversible pH changing nanoparticle. When the bond isomerizes back to the trans form, it binds the hydrogen and returns the solution back to the original pH.

Figure 3.7: Absorbance spectrum of PAG1 before and after 2 min irradiation at 365nm

Both forms of PAG1 absorb at 400nm as shown in figure 3.7, but the trans form absorbs more strongly as evidenced by the absorption spectrum above. The decrease in absorbance intensity still changes the overlap integral and therefore can affect FRET.
PAG2\textsuperscript{24}: 2-nitrobenzaldehyde

Upon irradiation of PAG2, the aldehyde gains one of the oxygens from the nitro group, turning the aldehyde into a carboxylic acid. This reaction is not reversible. Unlike the case of the PAG1, where the photo-stationary state creates a limit on how much of the acid generating form can be released, the entire amount of the PAG2 can be driven to release acid. This allows for a larger pH change from PAG2 than PAG1. Note that the absorbance of PAG2 increases upon irradiation unlike the other dyes in this work.

Figure 3.8: Absorbance of PAG2 before (red) and after (green) 2min irradiation at 365nm.

**PBG1: (E)-N-cyclohexyl-3-(2-hydroxy-5-nitrophenyl)acrylamide**

![Chemical Structure](image)

(\(E\))-N-cyclohexyl-3-(2-hydroxy-5-nitrophenyl)acrylamide

PBG1 generates base instead of acid when irradiated with light. After irradiation, cyclohexylamine is released as the base. The irradiation isomerizes the trans double bond to a cis double bond. The isomerization allows the alcohol group to displace the amide, generating the amine. PBG1 generates byproducts with an overall similar absorbance to itself. Figure 3.10 shows the slight absorbance changes that PBG1 undergoes when it releases base.

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25365 nm-Light-sensitive photobase generators derived from trans-o-coumaric acid; Koji Arimitsu Ayaka Oguri Masahiro Furutani; Materials Letters. 02/2015; 140. DOI: 10.1016/j.matlet.2014.11.001
Figure 3.10: Absorbance spectrum of PAG1 before and after 1min irradiation at 365nm

Experimental:

Nanoparticles were prepared from solutions of PVK (30ppm) or PPE (30ppm) in tetrahydrofuran (THF) and the intended dye. These solutions were filtered at .7um with a Fisher micro filtration apparatus. The solutions were sonicated for 30 seconds, then 1ml of solution was injected into 8ml of ultrapure water. This was sonicated for another 2min. The solution was placed under high vacuum for 30-45min to remove the THF. The solution was filtered .7 and .22 um with a fisher micro filtration apparatus to remove aggregates of the nanoparticles.

For the PAG1 nanoparticles, the nanoparticle was made without dye. After the THF had been removed via the high vacuum apparatus, the sample was again sonicated and
PAG1 was injected in a 1mg/mL THF solution. The sample was sonicated for 30sec and then returned to the high vacuum apparatus to remove the additional THF. The solution was filtered at .7 and .22 um with a Fisher micro filtration apparatus to remove aggregates of the nanoparticles.

Absorbance measurements were made with a Varian Cary 50. Fluorescence measurements were made with a Varian Eclipse at an excitation wavelength of 365nm for PPE and 344nm for PVK. All measurements were made open to the atmosphere. Irradiation of the samples was done with a 365nm Spectroline pencil lamp for PPE and 254nm Spectroline pencil lamp for PVK.

pH measurements were made with a Oakton Acorn series pH 6 meter.

**Results & Discussion:**

Table 3.1: Combinations of polymers and dyes used in this paper.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Dye</th>
<th>Coating</th>
<th>Notebook reference</th>
<th>Problem</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVK</td>
<td>PAG1</td>
<td>none</td>
<td>A9</td>
<td>PVK binds PAG1</td>
</tr>
<tr>
<td>PPE</td>
<td>PAG1</td>
<td>none</td>
<td>A14</td>
<td>nanoparticles absorb all acid or wrong pH</td>
</tr>
<tr>
<td>PPE</td>
<td>PAG1</td>
<td>Poly(ethyleneimine)</td>
<td>none</td>
<td>always filtered out</td>
</tr>
<tr>
<td>PVK</td>
<td>PAG2</td>
<td>none</td>
<td>AR10</td>
<td>Only works with 254nm</td>
</tr>
<tr>
<td>PPE</td>
<td>PAG2</td>
<td>none</td>
<td>AR15 and AR13</td>
<td>Too little FRET overlap</td>
</tr>
<tr>
<td>PPE</td>
<td>PBG1</td>
<td>none</td>
<td>BR1</td>
<td>Works!</td>
</tr>
</tbody>
</table>

49
PAG1 and PVK.

PVK is one of the bluest fluorescent nanoparticles; therefore I paired it with PAG1 as PAG1 absorbs in the blue and near UV. Unfortunately, this combination did not work. The absorbance spectrum did not change even when PAG1 was irradiated directly at 365nm. This result shows that the PAG1 did not isomerize to its acid releasing form. The structure of PVK has pedant groups that I believe pi stacked with the phenol groups of the trans form of PAG1 (see figure 3.11). As converting to the cis form would require breaking the pi stacking, the PAG1 did not convert to the cis configuration and thus no acid was released.

Figure 3.11 hypothetical PAG1 pi stacking to a carbazole

PAG2 and PVK

PVK nanoparticles do not absorb at 365nm; therefore, irradiation at 365 had no effect on the particles. A 254 nm light was used which the nanoparticle can absorb. Irradiation at 254nm was effective at releasing a proton. It had a pH change of .25 from 7.04 to 6.77. Another trial had a pH change of .2 from pH 7.23 to 7.03 upon irradiation.
The absorbance shows the increase after irradiation that is consistent with the increase that PAG2 has upon irradiation (figure 3.12).

![Absorbance and Wavelength Graph]

Figure 3.12: Increase in absorbance of PAG2-PVK nanoparticles upon 2 min of irradiation at 254nm.

Similarly the fluorescence spectra show a decrease upon irradiation (figure 3.13) consistent with the increase in FRET overlap from the increased absorbance. Interestingly, the fluorescence increases at 500nm which opens up the possibility of a ratiometric measurement to correlate to pH change. Unfortunately, 254nm is not a very good wavelength to use with biological samples due to damage from the ultraviolet light.

![Fluorescence and Wavelength Graph]

Figure 3.13: Fluorescence of PAG2-PVK nanoparticles before (red) and after (blue) 2min of irradiation at 254nm.
PAG1 and PPE

PPE does not have as good overlap for FRET as PVK, but the PPE fluorescence still overlaps with the PAG1 absorbance enough for FRET to occur. Upon irradiation both the absorbance and the fluorescence spectra show signs of the acid being released. The fluorescence increases upon irradiation. After the irradiation stops the sample returns to original fluorescence intensity after 10 min (figure 3.14).

![Graph showing fluorescence changes before and after irradiation](image)

**Figure 3.14**: Fluorescence of PAG1-PPE nanoparticles before and after 2 min irradiation at 365nm

The following spectrum of the absorbance has had an absorbance spectrum of PPE nanoparticles subtracted out of it, leaving only the contribution to the absorption from
PAG1. The change in absorbance shows that very little PAG1 converts to the cis form.

Figure 3.15: Absorbance spectra of PAG-PPE nanoparticles minus PPE nanoparticles spectrum.

The following spectra were recorded immediately after the irradiation of the sample was stopped, allowing for the process of PAG1 converting back to the trans form to be studied. On a short time scale (figure 3.16), PAG-PPE nanoparticles’ absorbance rises with a similar trending decrease in fluorescence intensity. Note that the fluorescence does not scale linearly with the amount of PAG1 present.
Figure 3.16: Absorbance kinetics at 365nm (red) and Fluorescence kinetics at 420nm, ex. 365nm (blue) after 2 min of irradiation at 365nm of PAG1-PPE nanoparticles.

On a longer time scale (figure 3.17), the fluorescence rises without a corresponding increase in the amount of trans form of PAG1. Notably it returns to the same intensity as the sample prior to irradiation. I believe that the initial drop in fluorescence is due to the formation of the deprotonated trans form of PAG1 and then the protonation of the trans form of PAG1 gives the slow slight rise in fluorescence by slightly decreasing the FRET efficiency. I do not know the reason for the decrease in fluorescence intensity between the protonated and deprotonated form of PAG1, but I suspect that the deprotonated form has a slightly broader absorption peak.
Figure 3.17: Absorbance kinetics at 365 nm (red) and fluorescence kinetics at 420 nm, ex. 365 nm (blue) after 2 min irradiation at 365 nm of PAG-PPE nanoparticles.

Unfortunately, the pH of the solution did not change even though it appears that the PAG1 had switched to its cis form. It became apparent that the nanoparticle was adsorbing the proton that was released. Remember, the nanoparticles remain separated from each other because they are negatively charged. Protons are positively charged. When the acid was released from the PAG1, it promptly attached to the nanoparticle and thus did not change the pH of the solution. Oddly, this theorized attachment was reversible. After the removal of the irradiation, PAG1 converted to its transform and was reprotonated.

Alternatively, the nanoparticle solution may have been acidic. As the “acid released” form of PAG1 only has a pKa of 7, it is possible that it was not deprotonated, though this would require a different explanation for the fluorescence rise after 100 sec.
PAG1 and PPE with coating polymer:

To fix the problem of the nanoparticles absorbing the proton, I decided to make the nanoparticles positively charged. Polyethyleneimine has a large number of amines. At equilibrium in a water solution, some of these amines will be protonated giving net positive charge to the polymer. Polyethyleneimine was added to the nanoparticles after the nanoparticles had formed. The idea was to wrap the negative charged nanoparticles in the positively charged polymer and then place the photoacid generator on the outside as shown in figure 3.18. The wrapping of the nanoparticles was inspired by a literature procedure. The wrapping failed as all attempts to wrap the nanoparticle in the positively charged polymer aggregated the nanoparticle solution. Additionally, we lack an instrument in the lab that can determine the charge on the nanoparticles. Without that instrument it was too difficult to determine what a good ratio of nanoparticle to positive polymer was to achieve wrapping instead of aggregation.

Figure 3.18: Cartoon of positively coated nanoparticle with dye molecules

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26 Singlet Oxygen Generation from Polymer Nanoparticles-Photosensitizer Conjugates Using FRET Cascade; Bhattacharyya, Santanu; Barman, Monoj Kumar; Baidya, Avijit; Patra, Amitava Journal of Physical Chemistry C (2014), 118(18), 9733-9740.
**PAG2 and PPE:**

Due to PAG2’s nonreversible nature, it can be driven entirely to its proton releasing form and thus release more protons. This version of the experiment intends to use PAG2 to overwhelm the nanoparticles’ ability to absorb protons, and thus still generate a pH change. There is a significant worry that if all protonatable sites on the nanoparticle were protonated, it would remove the negative charge on the nanoparticles and they would aggregate. The polymer PPE is used in this experiment as it can be excited at 365nm. The problem with PPE is that is has very little overlap with the absorbance of PAG2 especially when compared against PVK, as shown in figure 3.19.

![Figure 3.19: PAG2 absorbance in 2:1 THF:water, fluorescence of PPE and PVK nanoparticles](image)

In Figure 3.20, the absorbance increases, indicating that PAG2 released acid. Both PAG2 and PPE nanoparticles absorb 365nm light. In order to determine if the acid release was caused by FRET from the polymer or from direct irradiation, the fluorescence spectrum was examined.
Figure 3.20: PAG2 and PPE absorbance change after 5 min irradiation at 365nm

the fluorescence starts very high and increases steadily until it exceeds the maximum of the detector upon irradiation for 5min (data not shown). This increase in intensity supports that the PPE photobleached as the increase is similar to the increase the blank PPE sample. The release of proton from PAG2 was then caused by the irradiation at 365nm not by FRET from the nanoparticle. A defective pH meter prevented accurate measurements of the pH change. This system does not appear to work due to a lack of enough FRET overlap.

PBG1 and PPE:

PGB1 generates a base, cylohexylamine. This prevents the negatively charged nanoparticle from interfering with the pH change. The photo base generator’s byproduct also absorbs at a similar wavelength to the photo base generator. Note how the absorbance spectrums change between the photobase generator and its products.
Figure 3.21: PBG1 in THF before and after 1min of irradiation at 365nm.

The two peaks at ~ 260nm and 310nm both decrease upon irradiation and the peak at ~340nm increases (figure 3.21). Examine however, the range of 400 to 440nm. This is the only area where the absorbance overlaps the emission from the PPE nanoparticles (figure 3.22). The changes in the peaks at 260nm, 310nm and 340nm do not affect the FRET as there is no overlap of those peaks with the nanoparticle fluorescence spectrum. Within the region that has overlap with the fluorescence - 400 to 440nm - there is a notable decrease in absorbance upon irradiation.
Irradiation of PPE nanoparticles containing this photobase generator worked as expected. The absorbance spectrum showed similar peak changes as the photobase generator did in THF. This confirms that the photobase generator is successfully converted to its products.

**Figure 3.22:** Overlap of PGB absorbance (black) with PPE Fluorescence (red)

**Figure 3.23:** PBG1 and PPE nanoparticles absorbance before and after 1min irradiation at 365nm
Figure 3.23 shows very little change as the absorbance of the nanoparticle dwarfs the absorbance of PBG1. To facilitate seeing the change in absorbance, figure 3.24 shows the difference in absorbance between before and after irradiation in figure 3.23. Also plotted in figure 3.24 is the difference in absorbance of PGB1 in THF before and after irradiation. Note how the changes are similar to one another in the number and direction of the new peaks. The differences in amount of change come from the PGB1 in the nanoparticle being in a different environment than in THF.

![Normalized absorbance plot](image)

**Figure 3.24**: Difference spectrum of PBG1 and PPE nanoparticles absorbance before and after 1 min irradiation at 365 nm and difference spectrum of PGB1 in THF absorbance before and after 1 min irradiation at 365 nm. Peaks are normalized to -1 for the peak at ~330 nm.

The change in absorbance correlates with a change in the fluorescence. After irradiation, the fluorescence increased. The fluorescence increase is consistent with a
decrease in FRET. The area of the fluorescence increases by 55% upon irradiation (figure 3.25).

Figure 3.25: Fluorescence spectrum of PBG1 and PPE nanoparticles before and after 1min irradiation at 365nm

Note the shape of the fluorescence spectrum; the fluorescence of each peak is in descending order from left to right. In the undoped PPE nanoparticle, the second peak is the highest (figure 3.22). This shows a change in the ratios of one peak to another. Also, there is a noticeable change in the ratios of peaks two and three between the before and after irradiation spectra. Ratiometric determinations tend to be more accurate for change in fluorescence as the ratios are less sensitive to instrumental parameters. While not in this paper, a future direction of this project could be to correlate the ratios of the peaks to the dye released instead of the fluorescence intensity.

Figure 3.26 shows the overlap between the absorbance and fluorescence, even though the change in the absorbance is very small, there is a large change in the $J$ term of the FRET equation between before and after irradiation.
Figure 3.26: Overlap between PBG and PPE before and after irradiation for 1min at 365nm.

There was a pH change of 0.51 pH units after irradiation from pH 8.19 to pH 8.70. Let us compare this to the theoretical pH change assuming that all PBG is consumed.

First the moles of PBG present.

\[
\frac{0.0222 \text{mg PBG}}{8 \text{ml}} \times \frac{1 \text{mmol}}{291.13 \text{mg}} = 9.5318 \times 10^{-6} \text{M PBG}
\]

Take a starting pH of 8.19.

\[
pH + pOH = 14
\]

\[
14 - 8.19 = pOH 5.81 = -\log([OH])
\]

\[
[OH] = 10^{-5.81} = 1.55 \times 10^{-6} \text{ original } [OH]
\]

Assume all cyclohexylamines are protonated as it is a strong base (pKa = 10.63)

\[
1.55 \times 10^{-6}M + 9.5318 \times 10^{-6}M \text{ PBG} = 1.108 \times 10^{-5}
\]

\[
-\log([OH]) = -\log(1.108 \times 10^{-5}) = 4.955 \text{ pOH } = 9.044 \text{ pH}
\]
Due to the pH of 8.70, it would appear that about a third of the base was released. This suggests that a large amount of the PBG is stuck deep inside the polymer unable to decompose due to steric limitations of the constrained space within the nanoparticle that the dye resides in.

**Future Directions:**

This project has succeeded in changing the pH upon irradiation. There are several more directions this project can investigate. First, the pH change needs to be correlated to the fluorescence intensity in order to be able to use the fluorescence as a measurement of base released. This part of the project has difficulty as we lack a way to take a time dependent pH measurement in lab. I would suggest acquiring such a device for this project. Alternately, irradiating for intervals of time with pH measurements in between may allow correlation of pH to fluorescence intensity to be done. There is also a possibility of correlating the pH change to the ratio of the fluorescence peaks.

Second, the starting pH needs to be moved into the more applicable biological window of pH 5-7. This might be accomplishable by adding a small amount of acid to the solution prior to irradiation or by putting acid into the ultrapure water prior to creating the nanoparticles.

Third, ideally we want to use visible light instead of 365nm light. In principle the PPE polymer should be able to absorb and use 400 - 420nm light. Demonstration of pH change using a 400 - 420nm light source would prove this to be a visible light photobase release. This should be accomplishable with the 405nm laser that we have in lab. There are some
safety issues with the methodology of this irradiation. I would suggest irradiation of the sample in the laser lab and then moving the sample to the instruments as the base release is not reversible. Alternately, to create a system that absorbs further into the visible, one would need a photobase generator that absorbs further into the visible and a corresponding change in the polymer of the nanoparticle.

Fourth, I would suggest revisiting the PAG1- PPE nanoparticles. I believe that the pH of the nanoparticle solution was such that it did not deprotonate the PAG1 in the “acid released” form. Note that the “acid released” form only has a pKa of 7.0. I would suggest making the nanoparticle solution more basic by adding a small amount of NaOH after the nanoparticles have been formed. Then irradiate and see if there is a pH change.

Conclusion:

This project successfully demonstrated that FRET can be used as an alternative to direct excitation to initiate photochemical reactions. This was successfully demonstrated in PAG1-PPE, PAG2-PVK and PBG1-PPE nanoparticles. Additionally, PAG2-PVK had a pH change of 0.25 pH units when irradiated 254nm and the PBG1-PPE nanoparticles where shown to create a pH change of 0.51 pH units upon irradiation at 365nm.
Appendix I: Creation of Nanoparticles.

The purpose of this appendix is to describe the technique used to create nanoparticles as well as the parameters that can be varied to change the properties (size, concentration) of the nanoparticles.

Basic Procedure.

1. Prepare a pre-nano solution\(^\text{27}\)

   a. Stir a 1 mg/ml solution of polymer in anhydrous THF under argon atmosphere in the dark for at least 2 hours. This is to ensure that the polymer is fully dissolved.

   b. Prepare and a solution that is between \(\sim 10 – 40\) ppm conjugated polymer in anhydrious THF. The optimal ppm depends on the particular conjugated polymer used. Remain under argon atmosphere.

   c. Add dye molecule to the solution. Proper amount varies by experiment. This can be as high as 50% in some cases without adversely affecting the nanoparticles.

   d. (Optional) add a coating polymer.

   e. Filter the solution with .7 micrometer filtration paper using the microfiltration set up.

\(^{27}\) Pre-nano solution refers to the nanoparticle precursor solution
i. This removes any chunks of polymer that are not fully dissolved.

ii. Be careful not to leave the filtrate in the microfiltration setup too long as the vacuum can evaporate off your THF changing the concentration of your solution.

2. Place 8mL of ultrapure water in a 50ml round bottom flask.
   a. Rinse the graduated cylinder and round bottom with ultrapure water first.

3. Clamp the 50ml round bottom flask of ultrapure water and a 50ml round bottom containing the prenano solution into the sonicator. To have adequate sonication of the sample during sonication, ensure the following:
   a. Sonicator is at the proper operating level.
   b. The round bottom with the water is centered in the sonicator. The position of the round bottom with the prenano solution is less important.
   c. Most importantly, ensure the round bottom with the water is at least halfway submerged into the sonicator.

4. Turn on the high vacuum now (there will not be enough time later)

5. Sonicate both round bottoms for 30 sec.
a. The purpose of this step is to ensure that the prenano solution is homogeneous and does not have clumps of polymers in it as those clumps will become aggregates.

6. Using a micropipette, transfer 1ml of prenano solution into the round bottom with the sonicator still on. Do this quickly.
   a. Place the tip of the below the water to inject
   b. Swirl the tip slightly as you inject. This helps spread out the THF so there is not a pocket of high THF concentration at the injection point. (pockets of high concentration THF create aggregates in the solution)
   c. Inject in one smooth quick motion. It does not have to be superfast, but injecting too slowly can cause problems.
   d. Alternately, the micropipette can be replaced by a syringe.

7. Sonicate the sample for 2 min.

8. Remove the sample and transfer to the high vacuum to pull off the THF.
   a. This is the most time critical step. Based on my experience, I estimate that you have about 10 sec between the sample leaving the sonicator and
pulling vacuum on the sample before the sample will begin to aggregate due to the THF still in the solution.

b. This 10 seconds is why it is important to already have the high vacuum on. Most of the time, if I had to stop to turn on the high vacuum those samples would aggregate and filter out later on.

9. High vacuum for around 30-40 min to pull off all the THF.

   a. Place a room temperature bath of water on round bottom containing the nanoparticles while they are on the high vacuum. This prevents the solution from freezing from evaporative cooling. Freezing is very bad for the nanoparticles.

   b. There will initially be a large amount of bubbling from the solution. On one hand you want to pull the THF out as fast as possible, on the other hand, if the entire solution is only bubbles it will destroy the nanoparticles. To control the rate at which it bubbles adjust how open the two valves that bring the vacuum to the sample are.

   c. If you take the nanoparticles off the high vacuum and you can still smell THF from the solution, put it back on the high vacuum.

   d. The solution should be colored but clear. Cloudiness is caused by aggregates. Do not lose all hope if it is cloudy, some of it may still be nanoparticles that you can recover after filtering out the aggregates.
e. Final note: Fluorescence is a temperature dependent phenomenon. Bear this in mind if you take the sample really quickly from the high vacuum (where it was evaporatively cooled) to the fluorimeter.

10. Filter the nanoparticles with a .7 and .22 micrometer filtration paper using the microfiltration set up.

a. The solution should be clear and colored after filtration.

b. You can use the black light flashlight to check for fluorescence of the nanoparticles. (this is particularly useful if working with PPE polymer as its nanoparticles are colorless and it is hard to tell if they filtered out of not)

c. If all your nanoparticles filter out, check both filters to see where the nanoparticles are filtering out. If you have lots of color on the .22 but not the .7 micrometer filter than your nanoparticles are forming small aggregates. This means you are close to having the right conditions. If everything is filtering out at the .7 micrometer filter you may need to make more drastic changes to your parameters.

Different Parameters to change and how they affect the resulting nanoparticles.

Changes to prenano solution:
1. The concentration of polymer in the prenano solution.
   a. Increasing the ppm of the polymer in the THF prenano solution increases both the concentration and the size of the nanoparticles. The higher starting concentration increases the likelihood that multiple polymer chains will combine into the same nanoparticle. This makes the average size of the nanoparticles larger. Not all the additional polymer chains add to nanoparticles so more nanoparticle overall also form.

2. Using spec grade instead of anhydrous THF.
   a. Spec grade THF has water in it. This may not seem to be a problem as you are going to inject the solution into water, however it is. Spec grade gives different results than anhydrous THF for the nanoparticles. Normally anhydrous works better. I believe this is caused by the difference in polarity from the extra water in the spec grade THF. This higher polarity causes the polymer chains to curl up more and clump in the prenano solution. The difference in starting configuration of the polymers effects the formation and end result of the nanoparticles. Occasionally, using spec grade THF can promote nanoparticle formation; however, it normally promotes aggregation of the nanoparticles

3. The filtering of the prenano solution removes any polymers that do not completely dissolve. That having been said, I forgot this step once while using one of the more easily soluble polymers and the nanoparticles came out fine.
Changes to sonication procedure:

1. Use of a syringe and needle to inject the prenano solution

   a. I did most of my injections this way. It works perfectly fine. Just be sure to inject the prenano solution in a quick smooth action with the tip of the needle **under the water**. The micropipette is just easier, more convenient and less wasteful of disposable needles.

2. Injecting more prenano solution

   a. This is the easiest way to increase the concentration of the nanoparticles. The additional polymer chains become additional nanoparticles. Since the ppm of the prenano solution has not changed, the polymer chains are still the same distance apart in the prenano solution. As a result they do not form the large nanoparticles like increasing the ppm does. There is an upper limit to increasing the amount of prenano solution. Since there is more THF injected, there are increased issues with aggregates forming and it takes longer for the high vacuum to remove the THF.

   b. If you are injecting more than 1 mL of prenano solution see injecting with a needle above. However, you can also just inject twice with the micropipette in rapid succession.

3. Sonicating longer.

   a. I did not try this. But I do not think that this will have a large effect on the nanoparticles.
4. Removing the THF via rotovap instead of high vacuum.
   a. This also works to form the nanoparticles. It takes longer to remove the THF and there are occasionally issues with the heating decomposing the dye or (less often) the polymer.

5. Scaling up the reaction (example, 40ml water, 5ml prenanosolution)
   a. This also works but tends to yield lower concentrations of nanoparticles. I am not sure why.

After creation changes:

1. Make the nanoparticles less concentrated:
   a. Just add more ultrapure water. This will not harm the nanoparticles. Not sure why you would want to do this other than to increase the volume of the sample (if the nanoparticles are too bright, change the fluorimeter slits and/or detector settings instead of diluting them).

2. Make the nanoparticles more concentrated:
   a. You can high vacuum off some of the water from the nanoparticles after they are created. This takes a long time and be very wary of the nanoparticles freezing. A warm water bath help speed this up. As it is not particularly great for the high vacuum to pull off that much water and very time consuming, I would recommend first trying to increase the amount of prenano solution injected if the sample is not concentrated enough.

3. Adding additional reactants.
a. As long as the reaction will work in water, you can run reactions with the nanoparticles; particularly a click reaction. If the reactants are not water soluble, you can add them to the solution in a minute amount of acetone (or THF but acetone is better) and then high vacuum to remove it. Filter after doing this.

b. $\text{Cu}^{2+}$ causes to the nanoparticles to aggregate. The nanoparticles remain apart from each other as the nanoparticles are negatively charged. I believe the Cu cation acts as a catalyst to draw nanoparticle near each other and aggregate them. You need $\text{Cu}^{2+}$ for a click reaction so use a minimal amount of $\text{Cu}^{1+}$ and add the reducing agent for the Cu last.

4. Adding additional organic solvents:
   
   a. Be very careful doing this. It often causes the nanoparticles to aggregate. Acetone in small amounts is acceptable. You can even add small amounts of THF. This might be a way to polarity of the local environment inside the nanoparticles however I have not explored that option.

5. Changing the pH of the solution.
   
   a. The nanoparticles can handle changes in the pH. pH changes may change the fluorescence spectrum of the nanoparticles. Don’t go to the extremes of pH but the nanoparticles are robust enough to take medium sized changes as showing the pH changing nanoparticles chapter.
Appendix 2 Synthesis:

All chemicals were obtained from Acros or Sigma Aldrich and used as received unless otherwise specified.

Polymers:

**PFBT:** Poly[(9,9-dioctylfluorenyl-2,7-diyl)-alt-co-{1,4-benzo-{2,1’,3}-thiadiazole}]

Obtained from American Dye Source and was used as received. $M_w = 15,000-200,000$

**PPE:** Poly (2,5-di(2’-ethylhexyl)phenylene-1,4- ethynylene)

Obtained from Sigma Aldrich and was used as received.

**PVK:**

Obtained from Sigma Aldrich and was used as received. Avg. $M_n = 25,000-50,000$

**Poly (ethyleneimine) solution**

Obtained from Sigma Aldrich and was used as received $M_n = \sim1800$ by GPC, $M_w = \sim2,000$ by LS. 50% by wt. in water.

**PVCOCO:** Poly(vinyl butyral-co-vinyl alcohol-co-vinyl acetate)

Obtained from Sigma Aldrich and used as received. 80% wt. vinyl butyral

Dyes:
APESO and APSO:

Synthesized by Dr. Natia Frank’s lab\textsuperscript{28}. (University of Victoria)

PAG1

PAG1 was prepared following a literature procedure\textsuperscript{29}. 0.6654g p-anisidine + 2ml concentrated HCL in 2ml of DI water cool in ice bath. Solution turned purple upon addition of the acid. Separate solution of .4376g of Sodium Nitrate +5ml DI water cool in ice bath. Added the sodium nitrate solution to the p-anisidine solution. Solution is now a brown color with a yellow tinge. Separate solution of 1.0774g of 2,4,5 trichlorophenol + .9946g NaOH + 10ml DI water in ice bath. Sonicated to dissolve everything. Solution is a cloudy white. The p-anisidine solution was added dropwise to the 2,4,5 trichlorophenol solution over 45min. Solution temperature was kept below 5\textdegree C. Solution is a cloudy orange. Stir 1 hour in ice bath. Filtered to get a brown paste. High vacuum to remove any water in the sample. Recrystallized in EtOH to get a gray/green powder. Product 89.7mg 6.11\% yield. NMR: 3.922 (3H, s), 7.045(2H, d), 7.519 (1H, s), 7.917(2H, d), 14.600 (1H, s).

PAG2:

Used as received.

PBG1:

\textsuperscript{28} Modulating short wavelength fluorescence with long wavelength light; Copley, Graeme; Gillmore, Jason G.; Crisman, Jeffrey; Kodis, Gerdenis; Gray, Christopher L.; Cherry, Brian R.; Sherman, Benjamin D.; Liddell, Paul A.; Paquette, Michelle M.; Kelbauskas, Laimonas; et al; Journal of the American Chemical Society (2014), 136(34), 11994-12003. Compound 20 (APESO), 21 (APSO).

\textsuperscript{29} Photoinduced pH drops in Water; Mattieu Emond et al. Phys. Chem. Chem. Phys., 2011, 13 6493-6499
PBG1 was prepared following a literature procedure\textsuperscript{30}. 1.5005g 6-nitrocoumarin + 7.5ml cyclohexylamine. Reflux overnight under argon atmosphere. Poured the solution into a beaker containing 20ml of 3.5M HCl and 20ml of CHCl\textsubscript{3}. The water turned cloudy white and the solution started smoking. Stirred the solution, the water turned clear and the CHCl\textsubscript{3} turned a cloudy gray color. Filtered the solution to get a solid white/yellow impure product. CHCl\textsubscript{3} filtrate is a clear amber color (this is the unreacted cyclohexylamine). The product was washed with THF followed by a recrystallization in acetone (10ml). Used high vacuum to remove any remaining acetone. 33.0mg of product. 1.4\%yield.

\textsuperscript{30} 365 nm-Light-sensitive photobase generators derived from trans-o-coumaric acid; Koji Arimitsu Ayaka Oguri Masahiro Furutani; Materials Letters. 02/2015; 140. DOI: 10.1016/j.matlet.2014.11.001
Figure 4.1: H-NMR of PBG1
Figure 4.2: PBG1 H-NMR of aromatic region
Figure 4.3: PBG1 H-NMR of aliphatic region: