STUDY OF THE STRUCTURE OF RHODOPSIN DURING PHOTOACTIVATION

BY

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Abstract

G-protein coupled receptors are a superfamily of transmembrane proteins that are heavily involved in signal transduction within cells. They are extremely important to study because they are involved in many physiological processes such as sight, smell, taste, etc. GPCRs are also heavily targeted by drugs and therefore understanding the way they function can help improvements with the development of drugs. This study uses Bovine Rhodopsin as a prototypical model GPCR, as it is very commonly studied. Rhodopsin has an inactive Metarhodopsin I and an active Metarhodopsin II state that allow for the functionality of the protein. The equilibrium that exists between the two states are studied in order to observe the structural features of the activation process. Changing the metal cofactors in solution, and varying the osmotic pressure, can cause a shift in the equilibrium. The effect these factors have on the shift are studied in these experiments. Analyzing the different Meta I and Meta II states can be done by using UV-visible spectroscopy. The different environments created by the factors listed above will be studied and can be applied to other GPCR proteins as well.

Introduction

Rhodopsin

Rhodopsin is a GPCR found in the retina of the eye, more specifically in the rod outer segments. Rhodopsin is a class A GPCR and is one of the most commonly studied. G-protein coupled receptors are the largest family of transmembrane proteins involved in signal transduction (Palczewski, 2006). They represent the proteins that are used for sight, smell, and many other things. Figure 1 illustrates that they are composed of seven alpha helices with short connecting loops that span the entire membrane. Because the motifs in the secondary structure in Rhodopsin (Figure 1) can be observed in most other GPCRs, the studies performed on Rhodopsin are typically considered to apply to the other GPCRs as well (Palczewski, 2006).

GPCRs are traditionally responsible for binding some type of external molecule that acts as a signal. This binding then allows the GPCR to undergo a conformational change which changes the internal shape of the molecule. The conformational change causes binding of guanylate nucleotide-binding protein (G-protein) to the intracellular surface of the receptor allows for the bound guanosine diphosphate (GDP) to be exchanged for guanosine triphosphate (GTP) (Palczewski, 2006). The newly bound GTP causes the beta and gamma subunits to dissociate while the alpha subunit can continue to activate further reactions in the cell via signal transduction (Hurowitz et al., 2000).

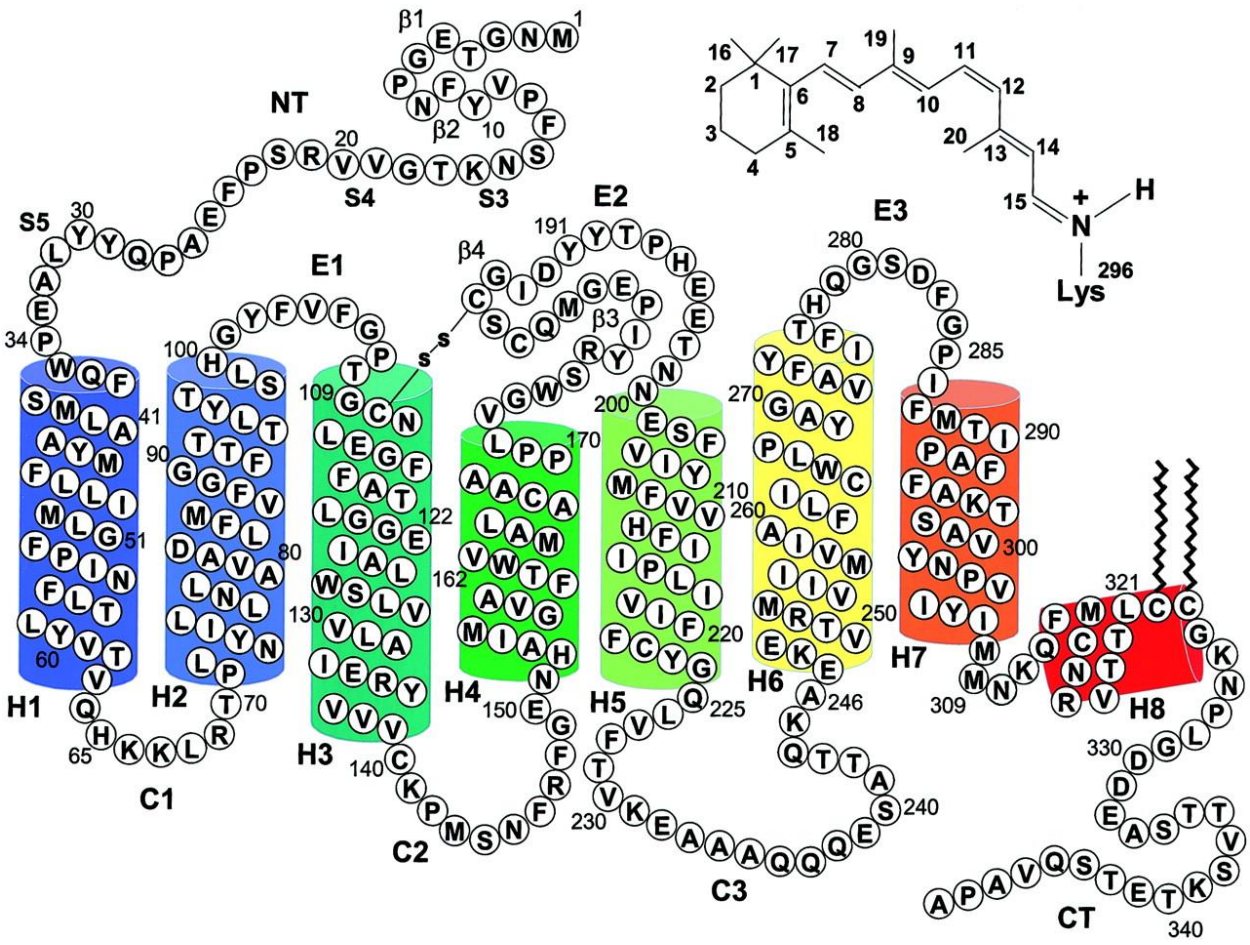


***Figure 1**. Secondary structure of a GPCR molecule. It is composed of seven alpha helices and is a transmembrane protein. Both the extracellular and cytoplasmic sides are shown. The GPCR shown in this figure is Rhodopsin. The ligand 11-cis-retinal is also shown in the top right corner (Menon, 2001).*

Rhodopsin contains a protein called opsin and a photosensitive retinal ligand. Retinal is buried within the opsin protein as seen in Figure 2. Because retinal is extremely light sensitive, it is responsible for absorbing photons of light (Menon et al, 2001). When a photon of light is absorbed it causes the 11-*cis*-retinal ligand to undergo isomerization into the all-*trans* form (Smith, 2010). Retinal is covalently bonded to the opsin molecule,

specifically the lysine at position 296 found in the 7th helix of the protein (Stenkamp et al., 2002).

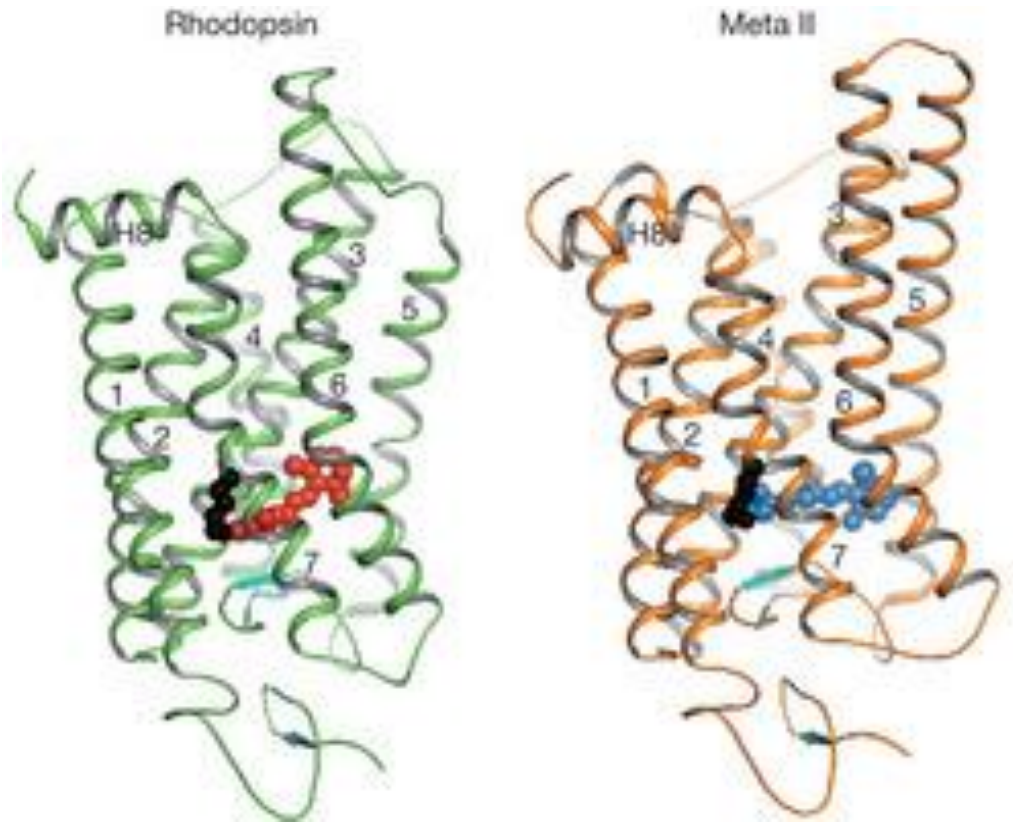


Figure 2. *To the left is the inactive dark state of Rhodopsin. To the right is the activated Metarhodopsin II state. The left has the 11-cis-retinal whereas the structure on the right contains the all-trans retinal after it has undergone photoactivation. This causes a conformational change in the protein structure (Choe, 2011).*

The retinal ligand is the important for converting Rhodopsin between its inactive and active states. Retinal in regards to rhodopsin is found in two different conformations. The first conformation is in the *cis* position of the 11th carbon and is known as 11-*cis*-

retinal. This is originally bound within rhodopsin and is known as the inactive state. As mentioned previously, 11-*cis*-retinal absorbs a photon of light because it is photosensitive. This causes the 11-*cis*-retinal to undergo isomerization into the all-*trans* form. Because the retinal is covalently bound deep within the opsin protein, the isomerization of retinal causes a conformational change within the quaternary structure of Rhodopsin. The all-*trans* form is converted into the inactive Metarhodopsin I (Meta I) state which is the forerunner for the active Meta II state illustrated in figure 3 (Mahalingam et al, 2008).

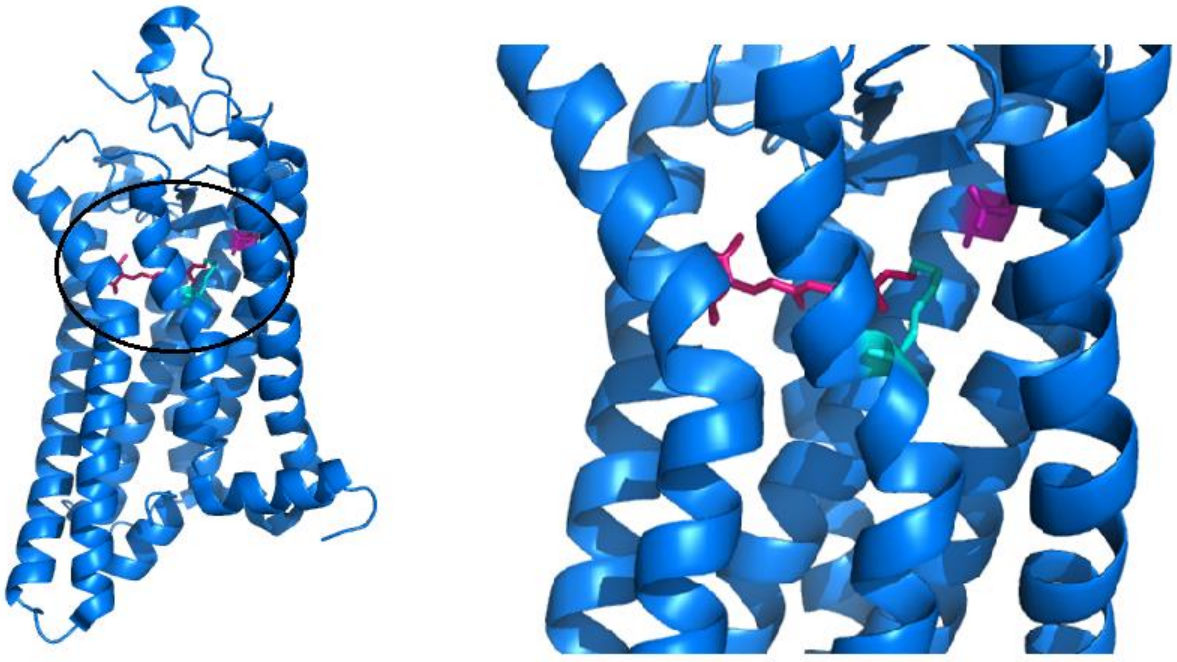


Figure 3. *Expansion of wild-type Metarhodopsin II shows the retinal in pink covalently bound to the lysine found at position 296 shown in aqua. The glutamate at position 113 is also highlighted in purple because it is a counterion that forms a salt bridge with the lysine when the retinal is not present. Crystal structure obtained from PDB ID: 3PQR.*

Metarhodopsin II is now able to start the signal cascade that eventually allows for vision in vertebrates. The active form is able to bind transducin which is a G-protein also

located within the disk membranes of the cells. The transducin is then free to continue signal transduction which sends signals to the brain. After the signal cascade has started Metarhodopsin II is no longer able to accept more photons to continue producing more signals, because only the 11-*cis* retinal form is actually light sensitive. However, the all-*trans* form of rhodopsin is not able to be converted back in to the 11-*cis* form directly. Therefore, retinal leaves the protein and is converted back to the *cis* form by retinoid isomerase. It then enters an empty opsin protein and is ready to start a new cycle. Rhodopsin is found in countless amounts within the photoreceptor cells. Therefore, our vision is not dependent on just one molecule of Rhodopsin. This gives the protein time to be converted back to its inactivated state. (Stenkamp et al., 2002).

This process of activation is not an on-off switch, however. There is actually an equilibrium that exists between the Meta I and Meta II states. This equilibrium can be perturbed by different elements such as pH, detergents, temperature, and metal cofactors. Like mentioned previously, Rhodopsin is one of the most studied GPCRs because it is believed that the activation of rhodopsin can be easily applied to many other GPCR models.

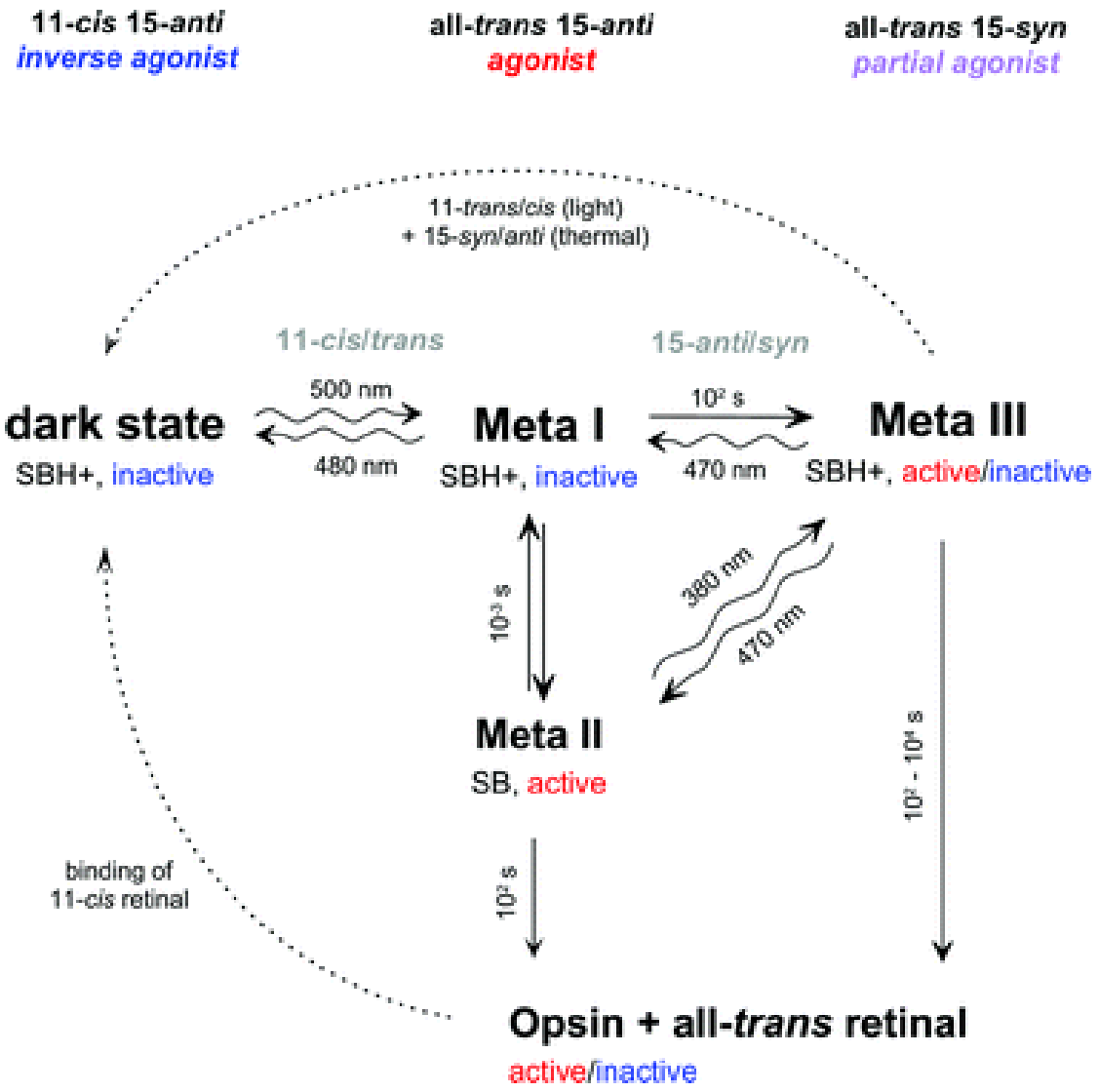


Figure 4. *Photosequence of Rhodopsin. It illustrates Rhodopsin (dark state) at the left absorbing a photon of light which then causes the 11-cis conformation to eventually convert to the all-trans conformation. This is followed by the inactive Meta I state directly followed by the active Meta II state which is then able to activate the "G-Protein Effector" which in this case is transducin. Additionally, it shows the Opsin and a new 11-cis-retinal molecule reforming a Rhodopsin ready to accept a new photon (Bartl., 2007).*

UV-Visible Absorption Spectroscopy

UV-Visible spectroscopy is a form of chemical analysis that can be used to determine both if a certain substance is in the sample, and how much of that substance is present in the sample. UV-Visible spectroscopy uses the wavelength range between 200 nanometers and 760 nanometers, to excite certain electrons in the sample. The absorption is what is collected by the spectrophotometer and a graph of the absorption versus wavelength is created.

The Beer-Lambert Law:

$$A = \epsilon l c \tag{1}$$

is the used to actually quantify the material present. The absorbance *A* in this case that is measured by the spectrophotometer, *l* is the pathlength of the cuvette where the sample is placed, and *c* is the concentration of the sample (Reusch, 2013).

We can use this understanding of UV-Vis spectroscopy to help characterize the Rhodopsin that is purified and used in further experiments. The characterization of the Rhodopsin is important after the purification in order to determine the purity of the final sample, along with the final concentration we were able to obtain. The main technique used in characterizing the protein is UV-Vis spectroscopy between 650 and 250 nm using a 1:9 detergent/buffer solution as the baseline. First the dark rhodopsin is measured, then the rhodopsin is bleached in the light and measured.

When these spectra are obtained there is a lot of scattering due to the particles in the sample. This is a problem because it does not allow us to see the actual structural features associated with the Rhodopsin that can ultimately help us to identify patterns in

structural changes. Figure 5 helps show what a spectra before scattering corrections would look like.

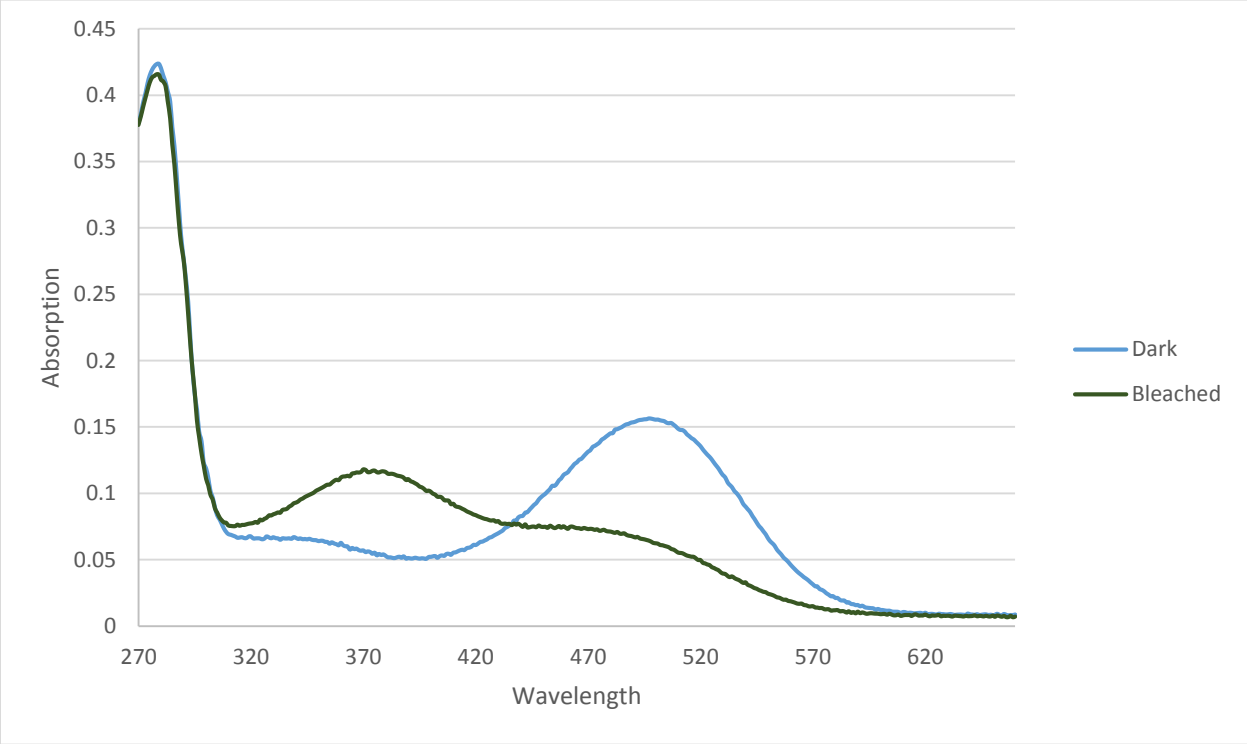


Figure 5. An example of a UV-visible absorption spectra that have not yet accounted for scattering. The blue shows the dark state Rhodopsin while the dark green represents the Rhodopsin after it has been bleached.

Before being able to calculate anything using the spectra obtained, scattering corrections must be performed. This can be done by creating a difference spectrum, where the difference between the light and dark spectra is plotted with respect to the wavelength. This scattering corrections now allow for the structural features of both Metarhodopsin I and Metarhodopsin II to be observed. This is illustrated by figure 6.

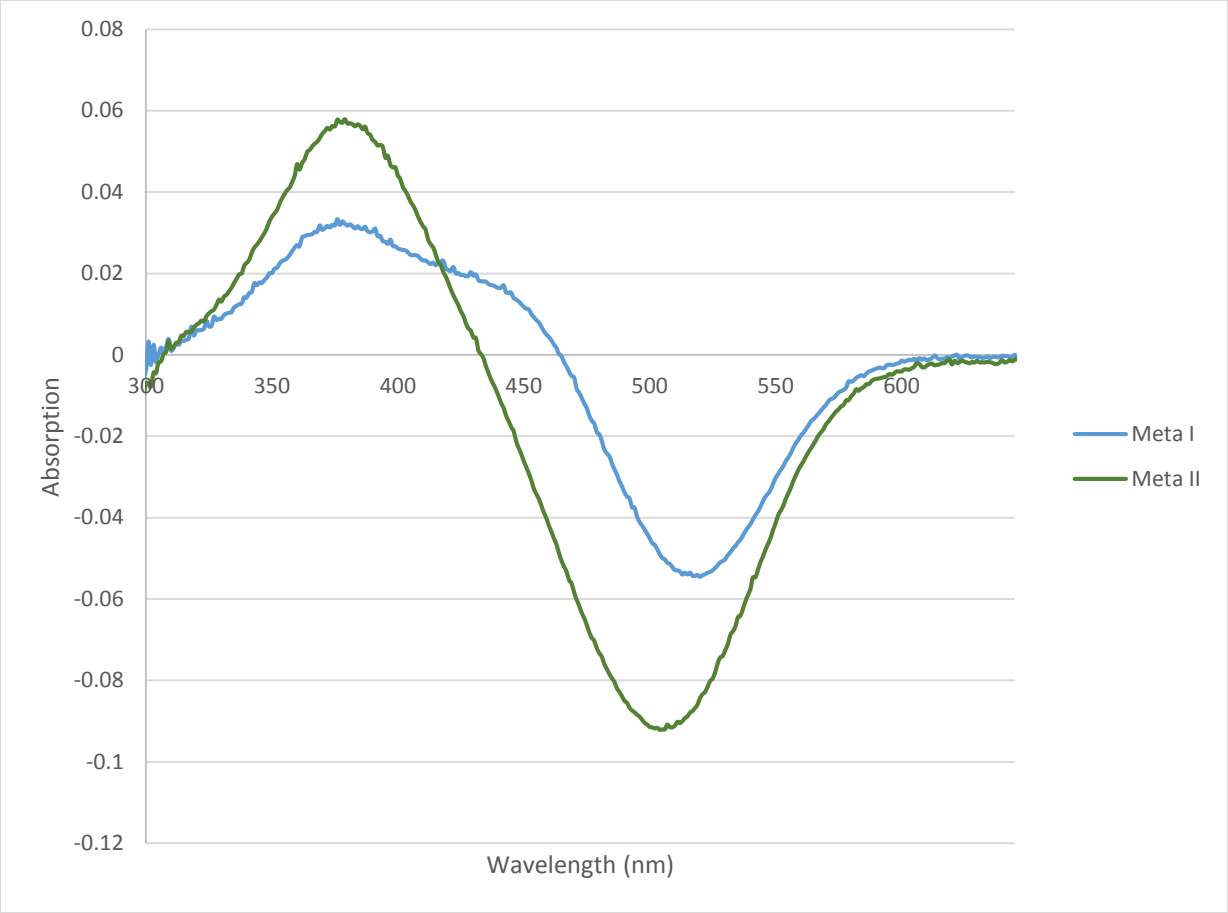


Figure 6*. An example of a difference spectra that allows us to see the maximum wavelengths of both Meta I and Meta II states. This allows us to see the relative amounts with regards to each other. This can also be applied to how this equilibrium shifts over a period of time in response to varying factors.*

Quantity can be determined by:

$$\text{Amount} = (A_{500,dark} - A_{500,light}) \times (\text{dilution factor}) \quad (2)$$

This is using the absorbance measured at 500 nm in the dark, and also the absorbance measured at 500 nm after the sample has been exposed to light.

Purity can be calculated by:

$$\text{Purity} = A_{280,dark}/A_{500,dark} \quad (3)$$

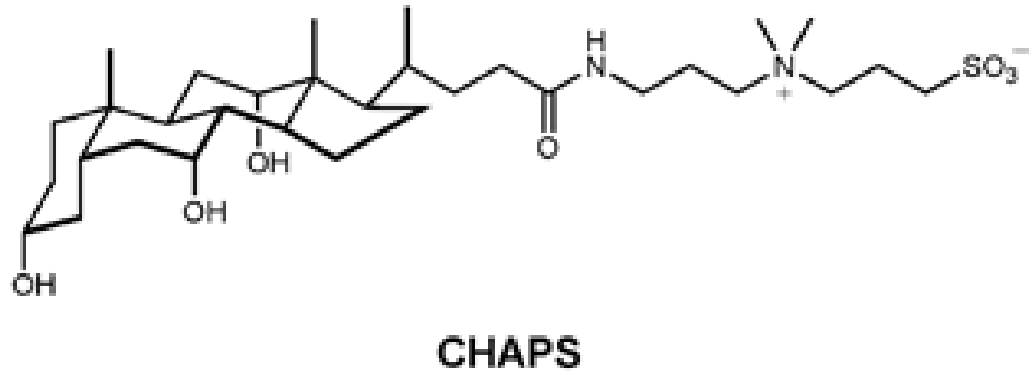
This is using the absorbance measured at 280 nm and 500 nm both in the dark.

In addition, UV-vis spectroscopy can also be used to observe the equilibrium between the Meta I and Meta II states that were associated with the inactive and active states of Rhodopsin respectively. Using difference spectra (photoproduct-dark) produces a graph that allows us to compare the two states. The max wavelength (λ_{max}) of Meta I is around 480 nm, while the λ_{max} of Meta II is found at 380 nm (Ryan, 2006). The concentrations of each state is directly proportional to the absorbance found at their corresponding maximum wavelengths. This allows us to see how the equilibrium between the two states shift in various experiments (Figure 6).

Detergents

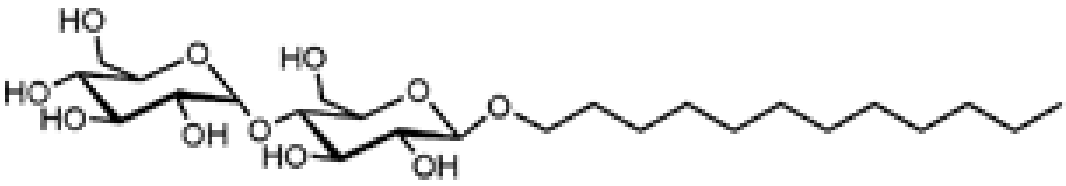
Detergents can be chemically used to solubilize certain proteins. They are non-denaturing and allow for the protein to remain intact. This is important for the case of Rhodopsin because it is a transmembrane protein. The detergent is used to help purify the Rhodopsin, because the amphiphilic reagents are able to completely remove the protein from the membrane. These amphiphilic reagents are able to form micelles around the protein primarily composed of hydrophobic regions. These micelles along with the protein are soluble in aqueous solution and can therefore be centrifuged and separated from the substances that are insoluble.

The two main detergents that are used in the lab are CHAPS and DDM. The structure of the detergent CHAPS is shown in figure 7 and is an abbreviation for (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate. It is a zwitterionic detergent that is known to trap Rhodopsin in the inactive Meta I state. DDM stands for *n*-dodecyl β-D-maltoside and is shown in figure 8. This is a neutral detergent that traps Rhodopsin in its active Meta II state.



CHAPS

Figure 7. The chemical structure of the CHAPS detergent (Chae, 2010)



DDM

Figure 8. The chemical structure of the DDM detergent (Chae, 2010)

Methods

Rod Outer Segment (ROS) Purification

Purification of Bovine Rhodopsin from ROS is important because it allows us to take the retinas and to obtain the actual rhodopsin that can successfully be used in further experimentation. Purifying Rhodopsin is a lengthy process that involves homogenization of the protein, centrifugation, and the use of a sucrose density gradient in order to separate and collect the part of the protein that we are interested in. As mentioned earlier, the Rhodopsin responds to photoactivation and therefore the entire purification process is performed only under dim red light. In addition, the process is also performed in the cold room or over ice around 4°C in order to prevent denaturation of the protein.

Solultions:

Homogenizing medium:

162 g of 42% sucrose

13 mL of 1.0 M NaCl

.4 mL of 0.1 M $MgCl_2$

.4 mL of 0.1 M EDTA

1.0 mL of 1.0 M Tris-acetate

Add H_2O until entire solution reaches 226 g

Density Gradient Solutions:

All solutions:

.1 mL of 1 M Tris-acetate

.1 M $MgCl_2$

1.10 g/mL solution:
Add 62.4 g of 42% sucrose
H_2O until total weight reaches 110 g

1.11 g/mL solution:
Add 68.4 g of 42% sucrose
H_2O until total weight reaches 111 g

1.13 g/mL solution:
Add 81.4 g of 42% sucrose
H_2O until total weight reaches 113 g

1.15 g/mL solution:
Add 68.4 g of 42% sucrose
H_2O until total weight reaches 111 g

The retinas were thawed by putting the bottle in a beaker of water for about an hour. The centrifuge was turned on and a bucket of ice was obtained. Under the red dim light, argon was added to the loose homogenizer was and then the thawed retinas were added. The retina container was rinsed with 5 mL of homogenizing solution to make sure all the retinas were obtained. This step was repeated one additional time. Next, 10 mL of homogenizing solution was added to the homogenizer and argon was added again. Thereafter, 100 strokes were applied using the loose homogenizer, with argon being added at least once every 10 strokes. The retinas were then transferred to GSA bottles, and the contents of the homogenizer were rinsed out with 3 mL of homogenizing solution two separate times. The centrifuge bottles were balanced and argon was added once

more before centrifuging for 20 minutes at 4°C at 2,600 x *g* (Sorvall GSA Rotor). After centrifugation the supernatant was removed and kept on ice wrapped in aluminum foil.

The pellet was returned to the tight Teflon homogenizer and the bottles were rinsed with 3 mL of homogenizing solution three separate times. Then, 12 strokes were applied and argon was added after each stroke. The mixture was then returned into the GSA bottles, and the homogenizer was rinsed with 5 mL of homogenizing solution. Argon was added to the centrifuge bottle and they were balanced by weight. The solution was centrifuged 20 min at 4°C at 2,600 x *g*. After centrifuging, the second supernatant was combined with the supernatant that was stored from the first centrifugation. Twice the volume of 10 mM Tris-acetate were added to the solution while slowly stirring. The swinging bucket rotor was placed in the cold room to allow time for it to cool down. The supernatant mixture was equilibrated and centrifuged for an hour at 4°C at 8,000 x *g* (Sorvall GSA Rotor). The pellet is what was collected after centrifugation.

While waiting for the centrifuge to finish, the sucrose density gradient were prepared. A 10mL graduated cylinder was used to add 10mL of 1.11 g/mL sucrose solution to each swinging bucket tube. An 18 gauge syringe was then used to add 9.5 mL of 1.13 g/mL, and then 7.5 mL of 1.15 g/mL to each tube to form different density layers. It was important not to disrupt the layers because it makes it difficult to extract the Rhodopsin later on. The pellet obtained after centrifuging was resuspended in less than 25mL of 1.10 g/mL sucrose solution using a 20 gauge syringe. The resuspended pellet was then transferred equally to six different tubes, and they were balanced in pairs using 1.10 g/mL to achieve equal weight. Argon was added and the tubes were all then placed in the AH627 swinging rotor so that the two that were balanced were across from each

other. They were centrifuged in the Ultraspeed Centrifuge for 1 hour at 4°C at 113,000 x *g* (Beckman SW 28 Rotor).

After centrifuging, the thick band located between the 1.11 and 1.13 layers was where most of the ROS was located. The carpet was collected using a syringe, being very careful not to collect the carpets from different layers. The carpets were diluted with water (2 volumes). Argon was added and the tubes were balanced before being centrifuged for 25 minutes at 4°C at 20K. The water was removed and 2 more volumes of water were added so the sample could be centrifuged again using the same settings. This step was done as many times as necessary. After the final wash, the water was removed, and all the pellets were resuspended together in a buffer that depended on what experiments would be run with that sample. The purified sample was then placed in Eppendorf tubes, and wrapped in aluminum foil.

Characterization of Rhodopsin using UV-visible Spectroscopy

Baseline:

960 μL of 9:1 buffer to detergent solution

20 μL of NH_2OH

Sample:

Add 20 μL of Rhodopsin

The contents of the baseline listed above were placed into a cuvette. In Scan.link the following settings were used: 700 nm to 270 nm, fast, baseline correction, and plot on

log. The machine was zeroed and a baseline was run with the cuvette. The cuvette was removed and the contents were transferred back into the Eppendorf tube. The sample was added to the same Eppendorf tube and was then mixed. The new sample was returned back into the cuvette. A dark spectrum was obtained. The sample was then bleached for one minute. A light spectrum was the collected. The absorbance values and the equations discussed in the introduction were used to calculate both the purity and the concentration.

Size Exclusion Column Chromatography

The purpose of size exclusion column chromatography is to separate the concentrate the sample into different fractions based off size. Larger molecules of the protein are able to pass through the column at a faster rate than smaller molecules. In the end each fraction can be characterized. The first step was to measure out 6 g of Sephadex G-50 into a clean, dry beaker. The Sephadex was then suspended in 50 mL of Ammonium Acetate that had a pH of 6.5.The solution was then stirred and poured into a column. The Sephadex was given time to settle and excess water was drained. The column had a diameter of 17.18 mm, a height of 26 mm, and a volume of 5,898 mm^3. It was rinsed with 59 mL of ammonium acetate buffer (twice the volume of the column). The protein was spun down using centrifugal filters to obtain a concentration of 10 mg/mL. While this was happening, it was important to make sure the column did not dry. A 300 mL 18% DDM in ammonium acetate solution was created. This was added to the column in order to equilibrate it. After the protein was concentrated they were transferred to Eppendorf tubes and centrifuged once more in the desktop centrifuge for 15 minutes. This

newly concentrated sample was characterized. It was then run through the column and fractions were collected. Each fraction was then characterized.

Zn^{2+} Effect on Photoactivation of Rhodopsin in CHAPS

We mentioned above that there are certain factors that can affect the equilibrium between the inactive Meta I state and the active Meta II state. In this experiment the purpose was to vary the concentration of Zn^{2+} and see how this affected the equilibrium.

The first step was to prepare 1 mL of a 30 µM ROS solution in CHAPS. This was done by adding 200 µL of purified ROS, 614.5 µL of 30% CHAPS and 185.5 µL of $NaPO_4$ buffer. This was spun on the desktop centrifuge at 10,000 RPM for 20 minutes to separate the soluble from the insoluble. Different Zn^{2+} concentrations were created using 1 mM and 1 M stock solutions. Various samples were created as seen in Table 1.

Sample	30 uM ROS in CHAPS	Zn^{2+} (uL)	$NaPO_4$ buffer (uL)
A	100	0	900
B	100	10	890
C	100	100	800
D	100	200	700
E	100	5 (1M solution)	895
F	100	100 (1M solution)	800

Table 1. The different Zn^{2+} concentrations

UV-vis absorption spectroscopy was used to acquire spectra at 20°C of the different samples. Eight spectra were taken in the dark and averaged. The sample was then exposed to light and spectra were taken for another 30 minutes of the bleached sample. Graphs were created plotting the wavelength by the difference between the light spectra and the dark average.

Osmotic Stress

Osmotic Stress occurs when the solute concentration around a cell changes suddenly, and causes osmosis across the membrane to occur very quickly so that water moves outside of the cell (Ho, 2006). The osmolyte that is used to create this stressing environment is PEG also known as polyethylene glycol (Figure 9). The PEG concentrations are directly proportional to the osmotic pressure. The purpose of this experiment was to add a peptide sequence and change the concentration to see how it affects the Metarhodopsin I and Metarhodopsin II states in an osmotic stress environment.

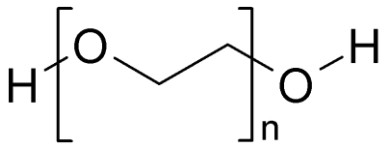


Figure 9. Chemical structure of polyethylene glycol (PEG).

The first step was to create a 500 mL GABI buffer was created using 67 mM BTP (Bis-tris propane), 2 mM $MgCl_2$, and 130 mM NaCl. A 30% PEG 3400 was created by measuring out 3 g of PEG and adding the GABI buffer until the total reached 10 g.

The peptide sequence that was added was ILEWLKDVGLF and was 1260.51 g/mol. A 1 mL 200 µM peptide solution was created. The differing concentrations of peptide were 0 µM, 1.5 µM, 3.0 µM, 6.0 µM, 9.0 µM, 15 µM, 25 µM, and 30 µM. Lastly, a 3 µM sample of ROS was used. The following samples were created as seen in Table 2.

Peptide Concentration (µM)	Volume of Peptide (µL)	Volume of ROS (µL)	Volume of 30% PEG 3400 (µL)	Total Volume (µL)
0	0	40	960	1000
1.5	7.5	40	952.5	1000
3.0	15	40	945	1000
6.0	30	40	930	1000
9.0	45	40	915	1000
15.0	75	40	885	1000
24.0	120	40	840	1000
30	150	40	810	1000

Table 2. Sample preparations for the osmotic stress experiment.

The spectrophotometer was equilibrated to 15° C. The program scanning kinetics was used. The settings were as followed: wavelength was measured from 660 nm to 270 nm, the *y*-max was 0.6, the average time was 0.0125 seconds, and the interval was 4800 nm per minute. Two stages were collected: in stage 1 the cycle was for 0.10 minutes and it stopped after 9 minutes, in stage 2 the cycle was 1 min and stopped after 12 minutes.

A 1 mm pathlength cuvette was used to minimize the light scattering. Each sample was zeroed and a baseline was taken before adding any Rhodopsin. Rhodopsin was then added to the sample in the cuvette. Nine spectra were collected and on the 10^{th} spectra the sample was bleached with an actinic light flash. Spectra 11-19 continued to be collected. The spectra was then analyzed by averaging the dark spectra, averaging the light spectra and then subtracting the dark average from the light average.

Results and Discussion

ROS Purification and Characterization

In order to run the necessary experiments the Rhodopsin has to be purified. In addition, characterization is necessary to see how well the purification went, and to see how much protein was obtained. Figure 10 shows an example of a UV-visible spectra when characterizing the purified Rhodopsin sample.

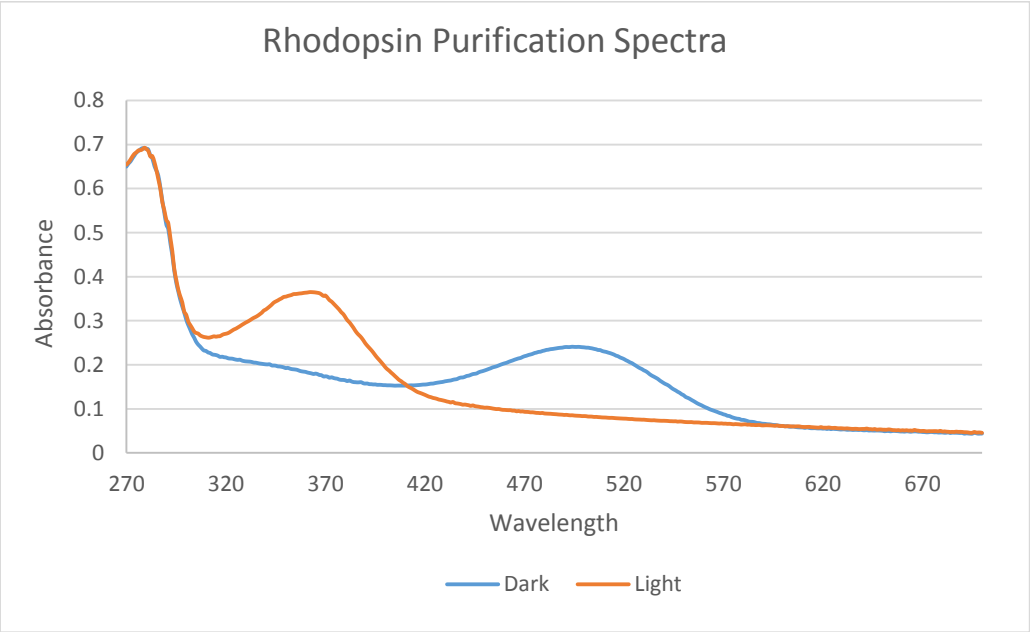


Figure 10. *The purity of the sample is 2.98 in this specific sample. The concentration is 7.635.*

The purity and concentration are found using equations (2) and (3) listed in the methods. Ideally the purity should be between 2.2-2.7 where the lower value suggests it is more pure.

Zn^{2+} Effect on Photoactivation of Rhodopsin in CHAPS

As mentioned earlier, the difference spectra for each of the various samples were taken. Because the sample was bleached, the equilibrium between the Meta I inactive state and the Meta II active state shifted over time. The point of this was to then compare how the different Zn^{2+} concentrations affected the equilibrium in relation to each other. Our hypothesis was that the concentration of Zn^{2+} would affect the rate of decay of Metarhodopsin I. Figure 11 displays the spectra we obtained side by side to one another. The isosbestic point for each graph is around 426 nm. This point at this wavelength suggests that there is indeed a transition between the Meta I and Meta II states.

Samples E and F (Table 1) provided us with spectra that did not resemble any of the other spectra. Sample F showed a lot of scattering and the spectral peaks were not as pronounced. We decided to centrifuge for 15 minutes noting that this might lead to a decrease in the concentration of rhodopsin as well. After centrifuging using the F blank gave an unusual spectrum. When we used baseline obtained from a blank that contained no Zinc, sample F gave the correct spectral features but a negative absorbance.

The difference between samples E and F with the other samples is that the Zn^{2+} concentrations were made with a 1 M Zinc stock solution, which was then diluted to create the desired concentration. The rest of the solutions were made with a 1 mM Zinc stock solution. We are not exactly sure what caused the problem with the spectra. We do not believe it should have caused a difference, because the concentration was not that much higher than the other samples. However, sometimes with higher concentrations

byproducts form. For example the zinc can form complexes with lipids or can form Zinc Hydroxide ($Zn(OH)_2$), which could be a cause for plots that did not follow the trend.

As of right now these results are still inconclusive. We can see that the rate is being affected, but we cannot determine how the rate is being affected without further data points and trials. Future experiments could include repeating the trials that were already conducted, especially samples F and H whose trends did not fit the other samples. This could help illuminate if there was experimental error on our end, or by making them from a different stock solution could help create a more similar trend. In addition, running experiments with higher concentrations of Zinc could help to explain the data further.

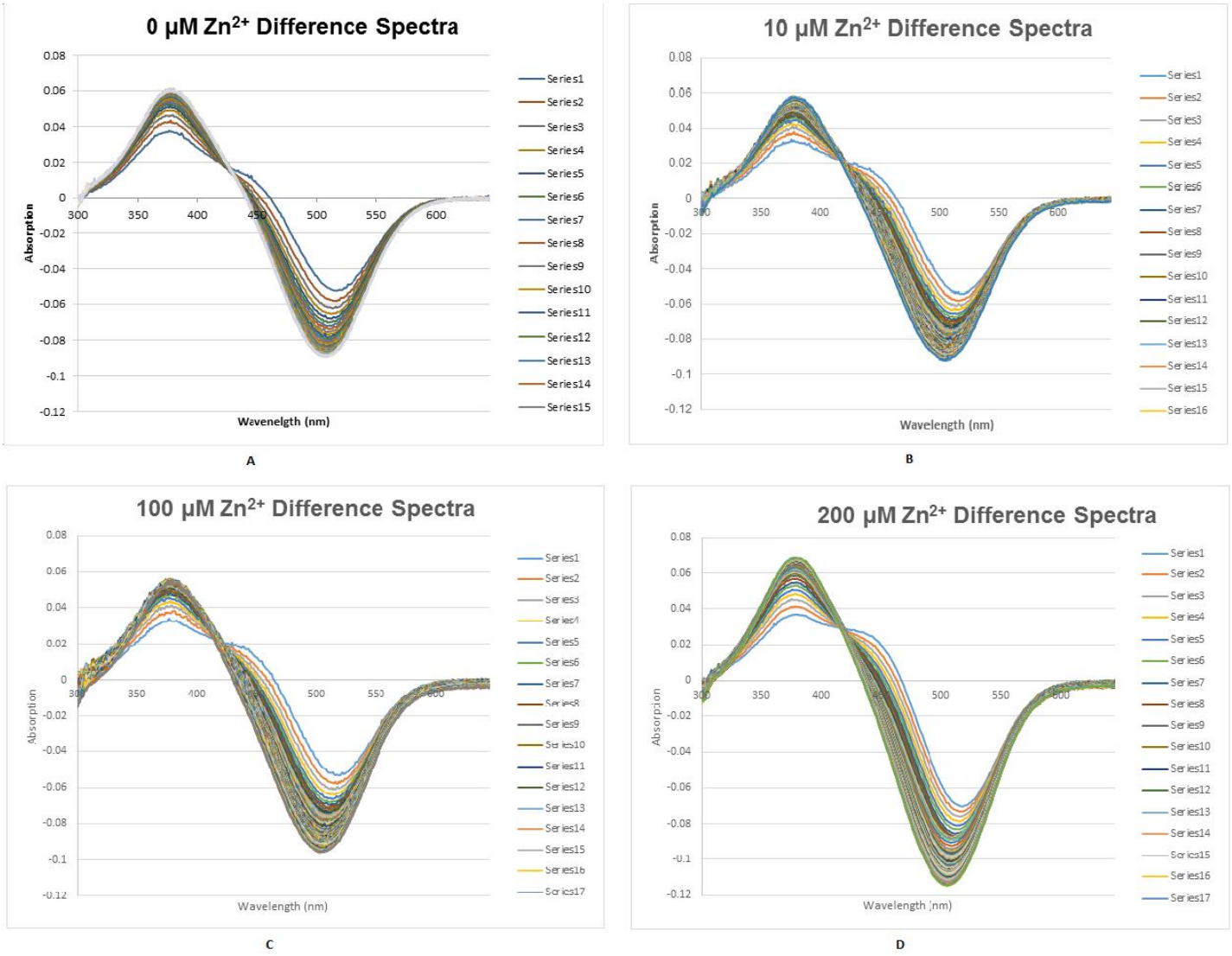


Figure 11*. The presence of zinc affected the rate of decay of Metarhodopsin I. A contains 0 μM Zinc and is represented by Sample A in Table 1. B contains 10 μM Zinc and is represented by Sample B in Table 1. C contains 100 μM Zinc and is represented by Sample C in Table 1. D contains 200 μM Zinc and is represented by Sample B in Table 1. All spectra were obtained at 20°C*

Osmotic Stress

PEG 3400 was the osmolyte in this reaction. It is responsible for removing water out of system. Without this water the inactive Meta I state is favored. In this specific experiment the PEG concentration was held constant but the concentration of peptide was varied. Figure 12 shows the results.

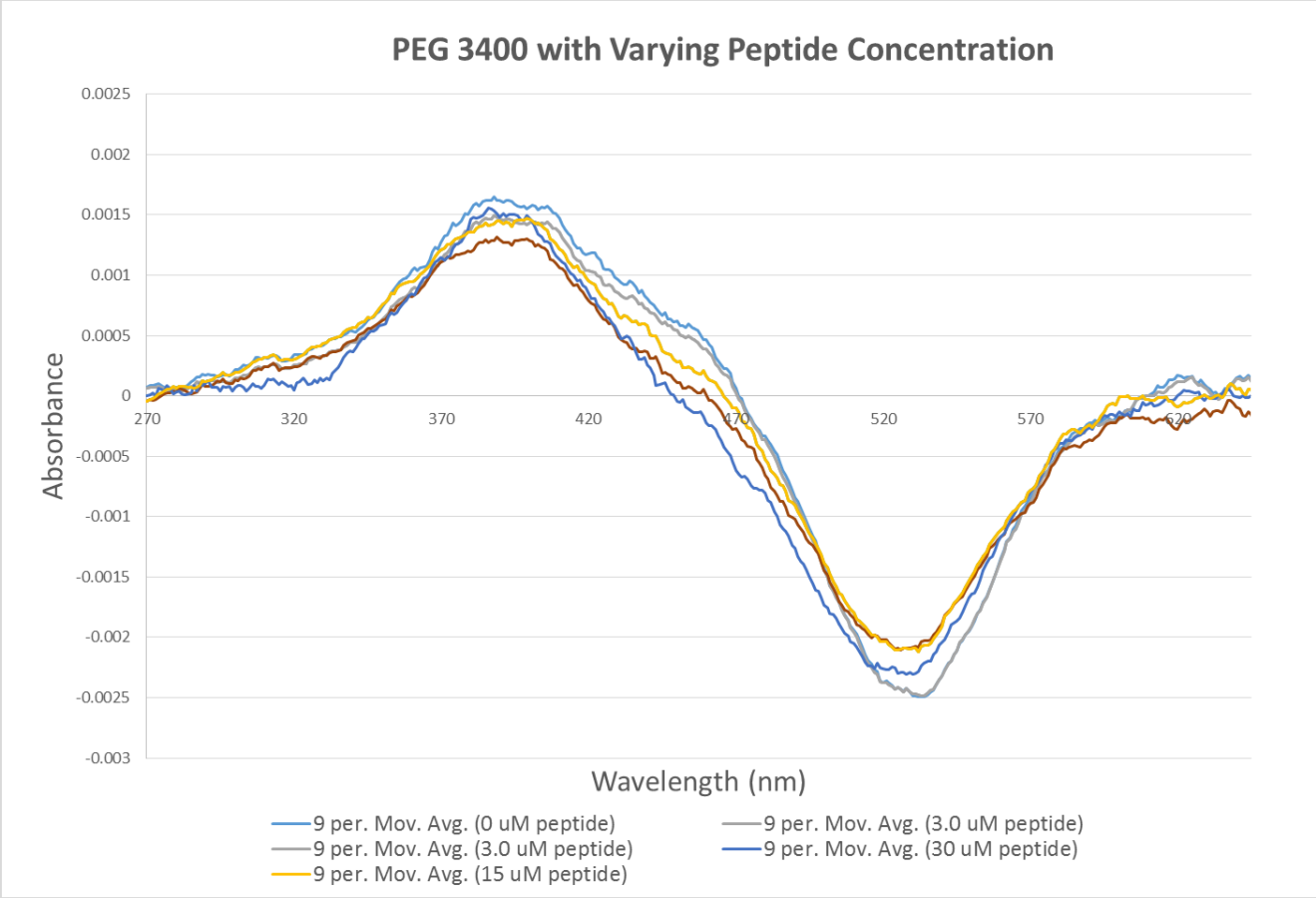


Figure 12. As the peptide concentration increased the shift towards the active Metarhodopsin II state was favored. Starting with a 0 uM peptide concentration in the light blue to a 30 uM peptide concentration in dark blue.

The idea behind this experiment is that the peptide has more affinity to the binding cavity where transducin would normally bind. This would favor the active Metarhodopsin II state. The higher the concentration of peptide the greater the shift towards Meta II. Although this is the suspected reasoning for the results we obtained, more trials need to be conducted in order to justify and form an actual conclusion.

Conclusion

This experiment has combined many aspects of laboratory techniques. From centrifugation, creating density gradients, characterizing samples through UV-visible spectroscopy, to column chromatography, it has largely helped to open my eyes to many techniques that can be applied to further research.

There were two major projects that were conducted in these experiments: both observing the equilibrium of the protein Rhodopsin, specifically between the inactive Metarhodopsin I and active Metarhodopsin II states. The first had to do with changing the concentration of Zn^{2+}. The second had to do with osmotic stress while adding a peptide sequence, and varying the concentration of that sequence. The first project helped show that Zn^{2+} did affect the rate of decay of the inactive Metarhodopsin I state. The sample was in CHAPS which is known to favor Meta I, but with differing concentrations of Zinc there were different rates of decay of Meta I. In the second project, the osmolyte PEG 3400 favored the Meta I state. However, by adding the peptide and increasing its concentration there was a shift towards a favoring of the active Meta II state.

Both projects helped to shine some light on some new factors that could affect the equilibrium. However, due to a lack of time only the foundation of the experiments could be completed, and future work is desirable. Additional trials must be run for each of the experiments with other controls to our understanding of what is going on. Being able to continue with these projects could allow for a better understanding of the structure of Rhodopsin, and could be related to other G-protein coupled receptors as well.

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