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Photosystem and Philosophy

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PHOTOSYSTEM AND PHILOSOPHY

**A thesis submitted to
Regis College
The Honors Program
in partial fulfillment of the requirements
for Graduation with Honors**

by

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May 2010

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Preface and Acknowledgements

This thesis focuses on the limitation of scientific knowledge due to human perception. The author explores this question by conducting three separate experimental studies which focus on inhibition, purification and immobilization of the enzyme photosystem II, the biological enzyme responsible for producing molecular oxygen. Then, the author examines the limitations of scientific knowledge in the context of method and how this shapes his own understanding of the society of science.

The first chapter briefly introduces some historical basis which is foundational to the philosophy of science while also introducing the function, structure, and catalytic properties of photosystem II. The second chapter examines the inhibitory effects of two herbicides which bind strongly to photosystem II. The third chapter proposes a plan on how to construct a tether molecule which could be used to bind photosystem II to a gold electrode. The fourth chapter focuses on the attempt to purify photosystem II, using a histidine-tagged subunit, from the photosynthetic bacteria *Synechocystis*. In the fifth and final chapter, the author then analyses the experience gained in the laboratory as a working case study to inquire about the boundary of natural science, the limitations of scientific method, and how the society of science functions.

Thank you Dr. James McEvoy for advising this thesis, trusting my intellectual and laboratory skills, and always inspiring me to excellence. Thank you Dr. Karen Adkins for reading this thesis and helping ground the work in the realm of philosophy. Thank you Dr. Tom Bowie for constricting and moderating an Honors program which has taught

personally me to approach this world with hope. Thank you to the biology and chemistry staff and faculty for supporting this work for start to finish. Thank you to my parents Vince and Christine Gallegos, for their love and support who have allowed me to complete a project of this nature. Thanks to my friends and colleagues (especially my laboratory partners, Saul Falcon, Son Nguyen, and John Otten, for their diligent help in the purification of *Synechocystis*). Finally, let thanks be given to God, for He makes all things possible.

Chapter I: Introduction

The preliminary working thesis for this study is grounded in two aspects of thinking. The first and predominate argumentation will center on the importance of laboratory research, conducted by the author, manipulating the biochemical enzyme of photosystem II. The second will be focusing on the importance and the boundary of knowledge that can be concluded by scientific inquiry. Thus, the working thesis for this literary work is: scientific knowledge is limited by the constraints of experimentation which is exemplified in the specific example of the manipulation of photosystem II, in vitro.

Introduction to Philosophy in Science

A fundamental key to understanding the philosophy of science is to indicate what constitutes scientific knowledge. A major authority on this matter is Samir Okasha (2002). Okasha begins to establish that the classification for science is grounded in the commonalties of all the disciplines. Thus this feature is able to concisely elaborate not the question of what is science, but what makes something a science. Clearly, the latter question is much more provocative. To begin this inquiry, the origins of modern science must first be described in the historical background of past discoveries. Okasha (2002) explains that these origins begin with the Aristotelian world view. This world view was challenged by the Copernican revolution which occurred nearly 1,800 years later.

Copernicus paved the way for the first fundamentals of modern physics. Okasha continues to explain that Johannes Kepler (1571-1630) solved many problems of previous astronomers in forming new planetary laws and Galileo Galilei (1564-1642) was instrumental in furthering the use of mathematics in the description of moving objects. The scientific revolution climaxed with the work of Isaac Newton (1643-1727). Another figure who is important in the history of scientific thought was Charles Darwin. Darwin explained that species have evolved from ancestral species by a process named natural selection. Even with intense theological tension, this theoretical mechanism is now the basis for the modern biological world view. The discovery of the structure of DNA by Watson and Crick fundamentally ushered in the era of molecular biology. The current culture of science is based in specialization in the many disciplines that have been so rapidly created in the past century.

Additionally important is the lens which scientists describe the world. Okasha first explains the ideas of the American philosopher Carl Hempel. Hempel concluded that scientific reasoning is based on previously held laws which would facilitate the explanation of new phenomena in a deductive line of reasoning. However, there is a problem with this type of reasoning. A problem arises when an irrelevant set of laws are used to describe an unrelated phenomena. Another approach which differs from Hempel's approach is to examine causality. Okasha outlines that the relationship of causality and explanation of phenomena are intimately linked. However, Okasha explains that causality-based explanations do not completely describe the plethora of scientific knowledge we currently hold. The conclusion which is gathered then explains that the

philosophical perspective which is presently held in science is a mixture of these two perspectives.

Introduction to Photosystem II

Photosystem II (PSII) is a magnificent and awesome example of nature's molecular machines. This protein is an enzyme which intricately catalyses a highly important reaction in nature. This enzyme has been described as, "engine of life" (Baber, 2006), for its importance in the existence of life on earth.

PSII performs a key step in photosynthesis. The enzyme PSII uses light energy from the sun to power the thermodynamically challenging reaction of splitting apart the water molecule. This reaction yields the production of usable electrons harnessed within the NADPH structure which allows the conversion of carbon dioxide to organic molecules used in cellular metabolism. The by-product of the water splitting reaction which PSII catalyses is oxygen gas. With oxygen present, aerobic respiration is able to occur at an efficiency of around 20 times higher than seen in anaerobic respiration (respiration occurring in the absence of oxygen gas). Thus, photosystem II is responsible for almost all of the life-sustaining oxygen in the atmosphere.

PSII is a multisubunit protein in the thylakoid membrane found in all plants, algae, and cyanobacteria. PSII splits apart the water molecule in the reaction center, or RC, by converting light energy into electrochemical potential energy. This RC is made up of two homologous proteins that are labeled D1 and D2 and two chlorophyll containing

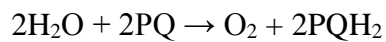
proteins that are labeled CP43 and CP47. Barber also explains that there are many other small subunits present in the PSII. Of these, two bind the redox protein cyt b_{559} .

Studies documented in literature have captured a crystal structure of the PSII reaction center core that was isolated from the cyanobacterium *Thermosynechococcus elongatus*. Each monomer of the dimeric core complex contained 19 different proteins subunits possessing a total mass of around 350 kDa. There are six transmembrane helices of CP43 and CP47 that are related to five transmembrane proteins of D1 and D2 proteins regions. Barber explains that other small subunit proteins most likely establish stability in binding of chlorophylls and carotenoids which are present and located at the peripheral of the CP43/D1/D2/CP47 interface.

Electron transport is extremely important in PSII. Barber continues to explain that the D1 and D2 subunits of the complex bind cofactors which establish the charge separation needed to oxidize water and reduce the terminal electron acceptor associated with PSII, which is plastoquinone. Light energy is passed to plastoquinone through many of redox molecules. First, the light energy is collected in light harvesting chlorophylls and passed to the CP43, and CP47 subunits. Light energy is finally passed to a chlorophyll center near D1 and D2. One of these chlorophyll molecules becomes the P_{680} radical cation denoted, $P_{680}^{+\cdot}$. Transfer of the electrons finally reduces plastoquinone (PQ), to form plastoquinol (PQH_2), which is bound to the PSII complex called the Q_B site. The complete transfer of electrons takes a few milliseconds. Importantly, the $P_{680}^{+\cdot}$ is explained to drive the splitting of water due to its high redox potential of 1.3 V compared to the standard hydrogen electrode, occurring near the D1 protein at a catalytic site

composed of a cluster of Mn ions and a Ca²⁺ ion. The redox protein cyt b₅₅₉ is associated with the protection of the protein from photoelectric damage and is located on the D2 side of PSII.

Interesting, PSII catalyzes the important water splitting reaction. The reaction of water splitting which occurs in PSII is a four electron process which requires the absorption of four photons of light, 2 molecules of water and two molecules of plastoquinone. This reaction can be written in the follow reactions equation.



The four oxidizing equivalents needed to oxidize water are produced by the removal of electrons from Mn ions. The mechanism of the oxidation of water then consists in five intermediate steps termed the S-state cycle. During the S state cycle, two Mn ions lose a total of four electrons as they shift in oxidation state. The shift in oxidation states allows for the first molecule of water to become coordinated to one Mn(V) creating an oxo intermediate. The second water molecule is then coordinated by the Ca²⁺ ion. The oxo intermediate formed is incredibly electron deficient due to the coordination of the highly oxidizing Mn(V) and the oxidizing force created from the other three Mn ions. This relationship makes the oxo vulnerable to nucleophilic attack from the second water molecule (Barber, 2006). This proposed reaction mechanism by Barber and colleagues is an essential description of the reaction which PSII catalyses.

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Chapter II: Inhibition studies on PSII

Introduction

Herbicides have been shown to inhibit the oxygen production in PSII. One such binding site where a herbicide can bind to PSII is the Q_B site. The native substrate of the Q_B site is plastoquinone, so a reasonable inhibitor could be an analogue containing a similar structure. Benzoquinones and naphthoquinone derivatives have been shown to effectively inhibit molecules. 2,3-dichloro-1,4-naphthoquinone was introduced as a herbicide and algicide as early as 1946 by the US Rubber Co. (Oettmeier, 1992).

This inhibitor molecule can be studied in relation to other herbicidal molecules to validate the relative strength of the inhibitor. The molecule 2,3-dichloro-1,4-naphthoquinone was tested and reported to have a pI_{50} of 5.48 (Oettmeier 1986). The effectiveness of quinones acting as inhibitors may be due to their steric and electronic properties.

The pI_{50} is a value which indicates the inhibitor concentration which produces 50% inhibition. This value is found in relation to the substrate concentration, the K_m and K_i (Segel, 1935). The determination of the pI_{50} requires a complex amount of kinetic experimentation to determine the K_m , V_{max} , and K_i of the system. Apparent pI_{50} values can be measured and different inhibitors can be related simultaneous to each other as long as the inhibitor substrate concentration stays constant. Thus holding the substrate concentration constant throughout experimental trials, the relative pI_{50} values can be

related without needing to perform complicated kinetic studies on the enzyme and inhibitor system. This assumption was used for the follow inhibitor study.

Materials and Methods

All chemicals were purchased from Sigma Aldrich unless otherwise noted.

Oxygen Monitoring Chamber

The oxygen evolving assays were conducted using a Clark-type electrode functioning in a 7 mL custom manufactured plastic chamber. The assay chamber was made water tight upon arrival to the lab. The oxygen evolving chamber contained a channel where an optical light guide was placed to supply the needed illumination for the assay chamber. Illumination was supplied by use of a Newport power supply (69931-0250) driving a 250 watt quartz tungsten halogen lamp (Newport 66881), fitted with a cold mirror (Oriel 66245) to reduce heat and infrared radiation. The oxygen probe (YSI 5331A) contained a 0.025” diameter platinum cathode and a silver anode which applied a current of 0.33 microamps in air at 37°C (YSI Incorporated 2007). The probe operates with a polarizing voltage of -0.8 vs. the standard hydrogen electrode, SHE.

Experimental data was acquired in real time using the YSI oxygen Monitor (5300A) system connected to a computer via WinDaq acquisition software. Percentage oxygen change could then be analyzed by graphing the experimental data in Microsoft Excel.

Integrity of the probe membrane was inspected daily by performing a voltage plateau test. The voltage plateau test consisted of dropping the polarizing voltage to 0.7 VDC and measuring the resulting change in oxygen saturation reading. A change of less than 3% is considered acceptable, which translates to less than 0.2% digression linearity (YSI Incorporated 2007). Calibration of the oxygen evolving chamber utilized ultrapure water as a reference for 100% oxygen saturation.

Spinach PSII BBY Membranes

Thylakoid membranes enriched in PSII from spinach leaves were utilized from frozen stocks, kept at -70°C, which were prepared by the method of Berthold et al (1981). Membrane concentration of stock crude samples was calculated using chlorophyll assays (500 fold sample dilution in 80% acetone) measuring absorbance readings at 663, 645, and 652 nanometers using a Beckman DU-640 spectrophotometer. The absorbance readings then were used in the following equation to gain numerical representation of chlorophyll concentration, mg/mL.

The equation states that the chlorophyll concentration is equal to the sum of absorbance readings multiplied by their subsequent constants. The constant terms in front of the absorbance readings are determined by specific absorption coefficients as detailed in the

work of Arnon (1949) and Mackinney (1941). The sum is then multiplied by the dilution factor, which in the case of this study is equal to 500.

Photosystem II Assay Protocol

Each oxygen assay consistently contained the final electron acceptor, ferricyanide (1 mM), and primary electron acceptor, DCBQ, 2,5-dichloro-p-benzoquinone, (250 μ M), in 2.5 mL of MES (20mM, pH 6.0) buffer containing NaCl (15 mM) and CaCl₂ (20 mM). Spinach membranes were injected directly into the reaction chamber from thawed frozen stock samples. During inhibitor trials, spinach membranes were incubated in the presence of inhibitor for 25 minutes in the reaction chamber before assaying in the fashion listed below. The two inhibitors used in this study were DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; and DCNQ, 2,3-dichloro-1,4-naphthoquinone.

Oxygen assays were carried by first mixing all needed chemicals in the reaction chamber. Then, spinach PSII (in thylakoid membrane crude) was added to the reaction chamber. The amount of PSII added was depended on chlorophyll concentration being ≤ 10 μ g chl in each assay. Data collection began for one minute under no illumination to insure proper stirring and receive a baseline reading. Then, the chamber was illuminated for a duration of two minutes. After this period of time elapsed, the lamp was turned off and the data analyzed. Percent oxygen change was then achieved by applying plotting percent oxygen versus time. A best fit line was then fitted to the data plot during the time interval of full illumination. The slope of this line was then determined to be in units of change in percent oxygen per second.

Oxygen production of microbiological systems is frequently displayed in micromoles of oxygen per hour per milligram of chlorophyll. Calculation to these units from the experimental data was achieved by consideration of several factors. Factors considered were oxygen solubility of water, atmospheric pressure adjustment, and assay volume. The following equation was used for calculation of all experimental data.

$$\frac{\text{O}_2 \text{ (}\mu\text{Moles)}}{\text{mg Chlorophyll} \times \text{Volume (ml)} \times \text{Time (hr)}} \times \text{Correction Factor}$$

This converts the percent change of oxygen concentration read during the assay to the desired units for reporting oxygen production of biological systems. The correction factor term is used to correct for the drop in atmospheric pressure resulting from altitude. The correction factor for these experiments used was 0.83 as it exists for an elevation of around 5,280 ft.

Results and Discussion

Spinach PSII Assays

Analysis of the PSII activity present in the thylakoid membrane samples was conducted by assaying the membrane with DCBQ and ferricyanide in MES buffer as outlined in the materials and methods section. Five subsequent trials were performed

displaying an average oxygen production of 134 $\mu\text{moles O}_2 \text{ hr}^{-1} \text{ mg chl}^{-1}$ for the thylakoid membrane. Data for the trial is listed in Table 1.

Table 1. Data for Spinach PSII Assays

Assay Number	$\Delta\% [\text{O}_2]$ (sec^{-1})	Oxygen Produced ($\mu\text{moles hr}^{-1} \text{ mg chl}^{-1}$)
1	0.068	136
2	0.060	119
3	0.085	170
4	0.066	126
5	0.063	121
Average O₂ Production		134

The average oxygen production for 1 milligram of spinach PSII membranes in this study is less than half of the activity published by Berthold et al (1981), who achieved activity of around 300 $\mu\text{moles hr}^{-1} \text{ mg chl}^{-1}$ for one milligram of PSII membranes. The low activity of the samples in this study most probably is due to several reasons. The first explanation is rooted in the storage practices utilized for the frozen stock sample. In previous studies, the chloroplasts from spinach were flash frozen and stored in liquid nitrogen until use. The long freezing time and the concurrent thawing and freezing associated with this type of experimentation could have decreased the activity of the PSII membranes. Constant thawing could also decrease the activity of the PSII membranes. An interesting effect of photoinhibition could also be the result of the low activity seen in the PSII assays. More likely the low oxygen production due to photoinhibition could be accounted to the light sources which was used throughout the trial. A white light was

used with any ultraviolet or infrared filter. This could certainly been a sources of photoinhibition of the enzyme.

DCMU Inhibitor Study

PSII assays were conducted with DCMU present to witness the inhibitory effect of the molecule. Concentration of DCMU in the reaction mixture was varied to classify inhibitor activity. DCMU concentration spanned from one-tenth to the 250 micromolar range yielding 27.4% to 75.8% inhibition. This data can be seen in Table 2.

Table 2. Data for DCMU Inhibition Trials

Inhibitor added (μM)	Oxygen Produced ($\mu\text{moles hr}^{-1} \text{ mg chl}^{-1}$)	Percent Activity	Percent Inhibition
0.00	134.35	100	0
0.10	97.56	72.6	27.4
1.00	105.15	78.27	21.73
2.00	91.82	68.34	31.66
100.0	61.95	46.11	53.89
250.0	55.47	41.3	58.7
2.50×10^3	32.52	24.2	75.8

Further analysis of this data was then performed by graphing the values with inhibitor concentration versus percent inhibition. A logarithmic best fit curve was then added to the plot. This data, along with the slope of the best fit curve is seen in Figure 1. The equation expressing the best fit curve is also present in the figure.

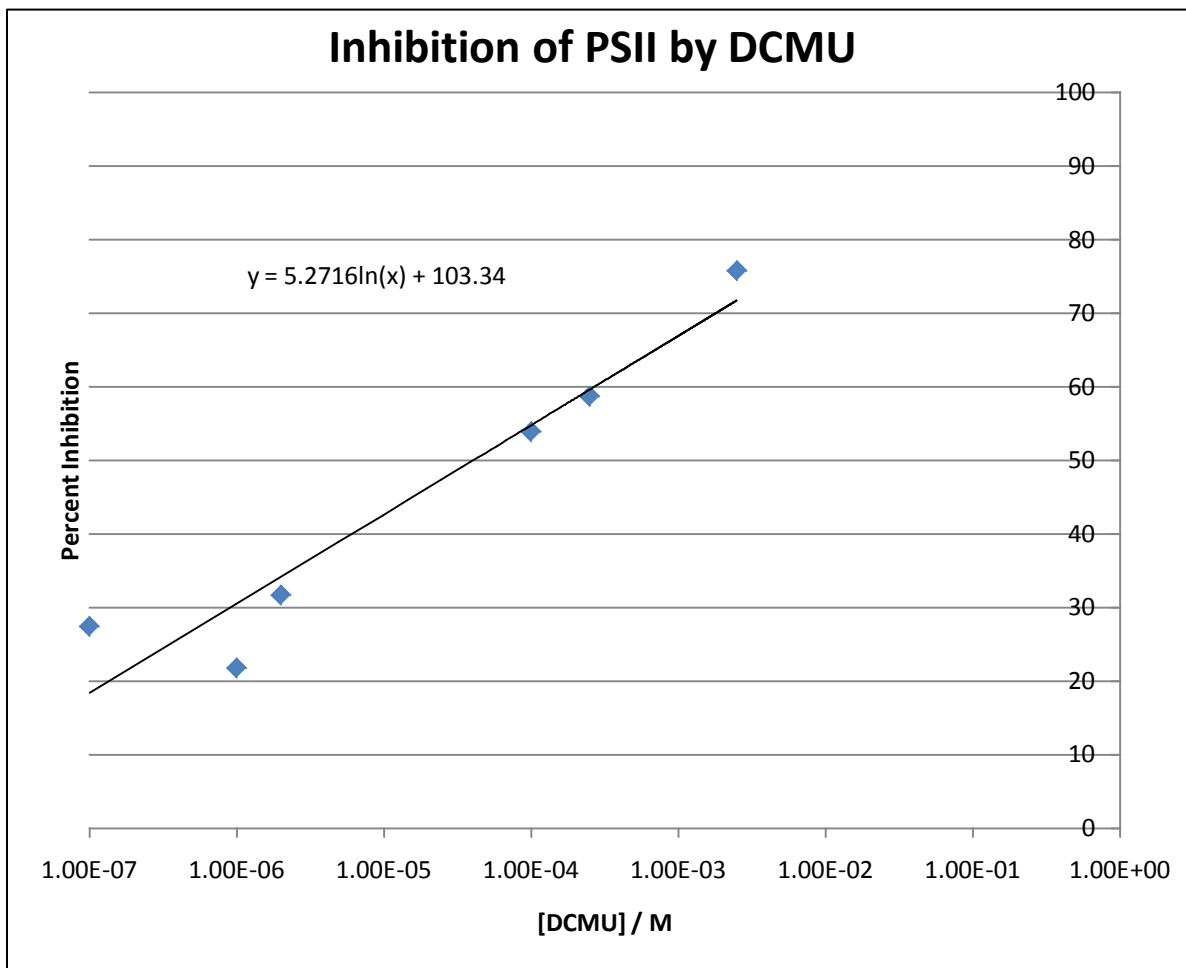


Figure 1. Data of DCMU Inhibition Activity on PSII Membranes.

Using the equation for the best fit curve, the concentration which achieved 50 percent inhibition was calculated to be 4.03×10^{-5} M or 40.3 μ M. This concentration of 50 percent inhibition is much beyond the literature value reported by Gonzales et al (1997), which is given in pI_{50} , which is 6.9 for DCMU. This pI_{50} represents that the value for 50 percent inhibition, for DCMU, is reported as being 0.126 μ M. This is 2.5 orders of magnitude difference in concentration from the experimental value. This means that in

our trials, nearly 320 times more inhibitor must be added to achieve the same inhibition as reported in literature. It may seem surprising at first that the concentration values for 50 percent inhibition are different. However, it must be remembered from above the true meaning of pI_{50} . The literature value reported, pI_{50} , is dependent on K_m , V_{max} and K_i . This fact shows that a true reporting of pI_{50} correctly relates parameters of the reaction, such as V_{max} , K_i and K_m . A consistent reporting of the concentration which achieves 50 percent inhibition must also take into account these three variables to compensate for varying reaction conditions, such as inhibitor, substrate and enzyme concentrations. The reality is that the reported value for this experiment fails to account and incorporate the K_m , V_{max} and K_i into the final reporting of inhibitor concentration needed. The concentration seen in this experimental representation only reports a 50 percent inhibition curve that specific to the reaction circumstances present (inhibitor concentration, enzyme concentration and substrate concentration). The literature value of pI_{50} is divergent from the experimental value because the true pI_{50} is bent to incorporate all reaction circumstances which could possibly occur. However, for this study, allocation of a pI_{50} is not needed. If the reaction conditions are set constant for all assays, then inhibition of PSII can be compared quantitatively across varying inhibitor concentrations and different inhibitor molecules. Thus, this study continued with the testing of the inhibition activity of DCNQ using the same assay conditions.

DCNQ Inhibitor Study

PSII assays were conducted with DCNQ present, to study its inhibitor effect and its relative inhibition strength compared to DCMU. The quantitative analysis contained three separate assay trials at three distinct concentrations of DCNQ. DCNQ concentration spanned from 50 to the 500 micromolar range yielding 26.1 to 58.35 percent inhibition. The data can be seen in Table 3.

Table 3. Data for DCNQ Inhibition Trials

Inhibitor added (μM)	Oxygen Produced ($\mu\text{moles hr}^{-1} \text{mg chl}^{-1}$)	Percent Activity	Percent Inhibition
0.00	134.35	100	0
50.0	99.267	73.9	26.1
250.0	75.804	56.4	43.6
500.0	55.951	41.65	58.35

Further analysis of this data was then performed by graphing the values with inhibitor concentration versus percent inhibition. A logarithmic best fit curve was then added to the plot. This data of DCNQ inhibition activity, along with the slope of the best fit curve is seen in Figure 2. The equation expressing the best fit curve is also present in the figure. DCMU data is also graphed on the same plot for reference.

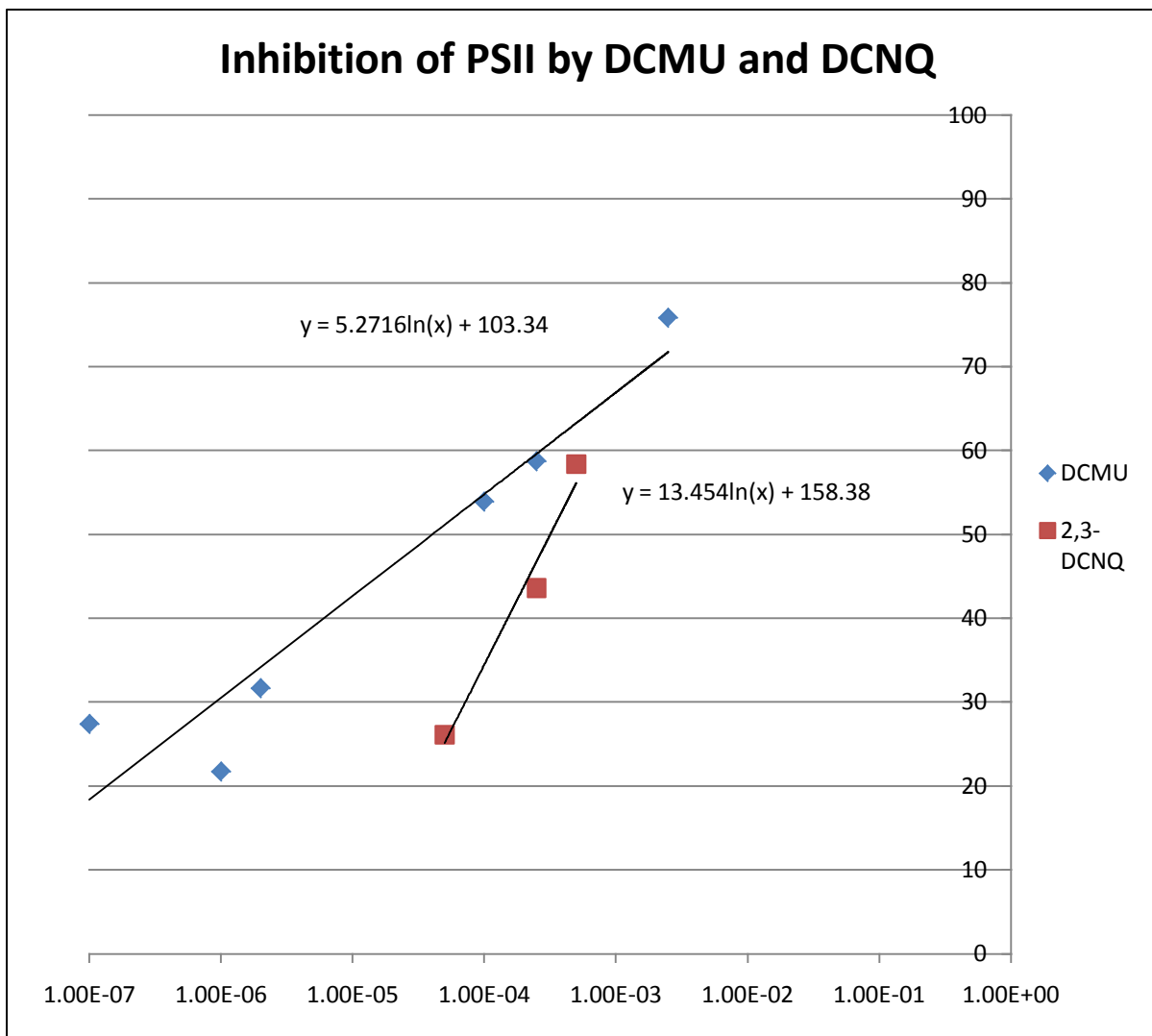


Figure 2. Data of DCMU and DCNQ inhibition activity on PSII

Using the data described above, the inhibition activity of the DCNQ molecule can be compared to the inhibition activity of DCMU. The relative concentration of DCNQ needed to achieve 50 percent inhibition can be calculated from the data plot line. Using the equation for the best fit curve, the concentration of DCNQ which achieved 50 percent inhibition was calculated to be 3.17×10^{-4} M or 317 μ M. In relation to DCMU, which

presented 50 percent inhibition at 40.3 μM , the 50 percent inhibition concentration differed by a factor of nearly 7.81. This means that it took a factor of 7.81 more DCNQ to achieve the same inhibition that DCMU achieved. This shows that DCNQ is a less effective inhibitor than DCMU by about one order of magnitude of concentration. This behavior is expected when indentifying the literature pI_{50} values for both DCMU and DCNQ. The pI_{50} value for DCMU inhibition of PSII, is 6.9, while the pI_{50} value for DCNQ is 5.48. This explains that the concentration to achieve 50% inhibition for both inhibitors is 0.126 μM and 3.31 μM respectively. Thus, according to literate it should take a concentration of 26.3 times more DCNQ than DCMU to achieve 50% inhibition of O_2 production.

Conclusions

In relating the experimental results to the theoretical relationship between enzyme inhibition activity, it has been shown that DCNQ, 2,3-dichloro-1,4-napthoquinone, is a less potent inhibitor of photosystem II in comparison with DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea. The experimental analysis of both these inhibitor's activity confirmed this relationship by relating the relative comparison of concentration needed to achieve 50 percent inhibition.

The results provide an interesting description of the affect of inhibitory molecules on the enzymatic production of oxygen in photosystem II. This study supports a more general purpose of building, a simple, reliable method of PSII assaying which can be performed to compare relative oxygen production of PSII. This method of PSII assaying

can be used in future studies to relate the relative inhibitory effect of various molecules on PSII.

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Chapter III: Proposing a Synthetic Tether Molecule for PSII

Introduction

A current area of study regarding biological enzymes is to use electrochemical experimentations which help to describe the electronic structure within an enzyme. One specific technique which has been used to study PSII is cyclic voltammetry. Natural reduction potentials of several components within PSII have been documented (Stryer et al, 2007).

In order for these types of experiments to proceed, the enzyme PSII must be attached to an electrode surface. One such method of attaching the protein is to utilize a histidine-tagged PSII subunit added in solution. The histidine tag functions as an anchor for a long chain like substituent to attached the protein to one end and to situate the other end on a gold electrode surface. The chain moiety is made up of amino-nitrilotriacetic acid (ANTA) and a disulfide known as DTSP. DTSP spontaneously attached to the gold surface. Nickel binds the ANTA and histidine-tagged PSII (Ataka et al, 2004). The limitation of using this method is that a purified PSII mutant protein must used in order to contain the much needed histidine-tagged PSII subunit.

Another option to attach PSII is to use an organic tether which contains a moiety that binds strongly, as an inhibitor, to one of the binding sites in the molecule. A possible binding site to bind is the Q_B site, the site which normally binds plastoquinone. As seen

in the previous experiment and in literature, herbicides bind strongly to the Q_B active site (Oettmeier 1986). One such inhibitor is DCNQ, 2,3-dichloro-1,4-naphthoquinone. The purpose for using this specific inhibitor is that its chemistry is easily substitutable by a primary amine (Nagata et al, 2008). This reaction undergoes a mechanism of electrophilic addition, where the amine functions as a nucleophile.

The organic tether molecule would need to incorporate a long chain like substituent in order to penetrate into the Q_B site, which lies in a hydrophobic region of the molecule. Also, a moiety that can bind to the electrode surface must also be introduced.

Proposed Synthesis Plan for Organic Tether Molecule

The first function needed step is to substitute one of the chlorides from the DCNQ, identified as compound (1), 2,3-dichloro-1,4-naphthoquinone, molecule with a long hydrophobic chain molecule. The chain molecule must contain both an amine group to substitute into DCNQ, and a carboxylic acid to be used in a later step. The chain like molecule proposed is 12-aminododecanoic acid, identified as compound (2). The substitution process can be accomplished by following a procedure similar to those described in literature (Sarhan, El-Dean, & Abdel-Monem, 1998).

The substituted derivative of DCNQ, identified below as compound (3), can then be reacted with a sulfur containing compound to substitute on the end of the carboxylic acid of (3). The final tether must contain a sulfur group in order to spontaneously attach to a gold electrode surface. The molecule chosen to act as this moiety is cysteamine (4). This molecule is chosen because it contains a thiol group as well as an amine. The amine

is attached to the carboxylic acid of (3) by using the carbodiimide technique. The carbodiimide technique uses a carbodiimide, such as dicyclohexyl carbodiimide (DCC), to activate the carboxylic acid making it susceptible nucleophilic attack by an amine (Montalbetti, & Falque, 2005), (Kurzer and Douraghi-Zadeh, 1966). The reaction mechanism joins the amine and carboxylic acid to produce the tether molecule (5) and simultaneously produces a urea by-product as seen in Figure 1.

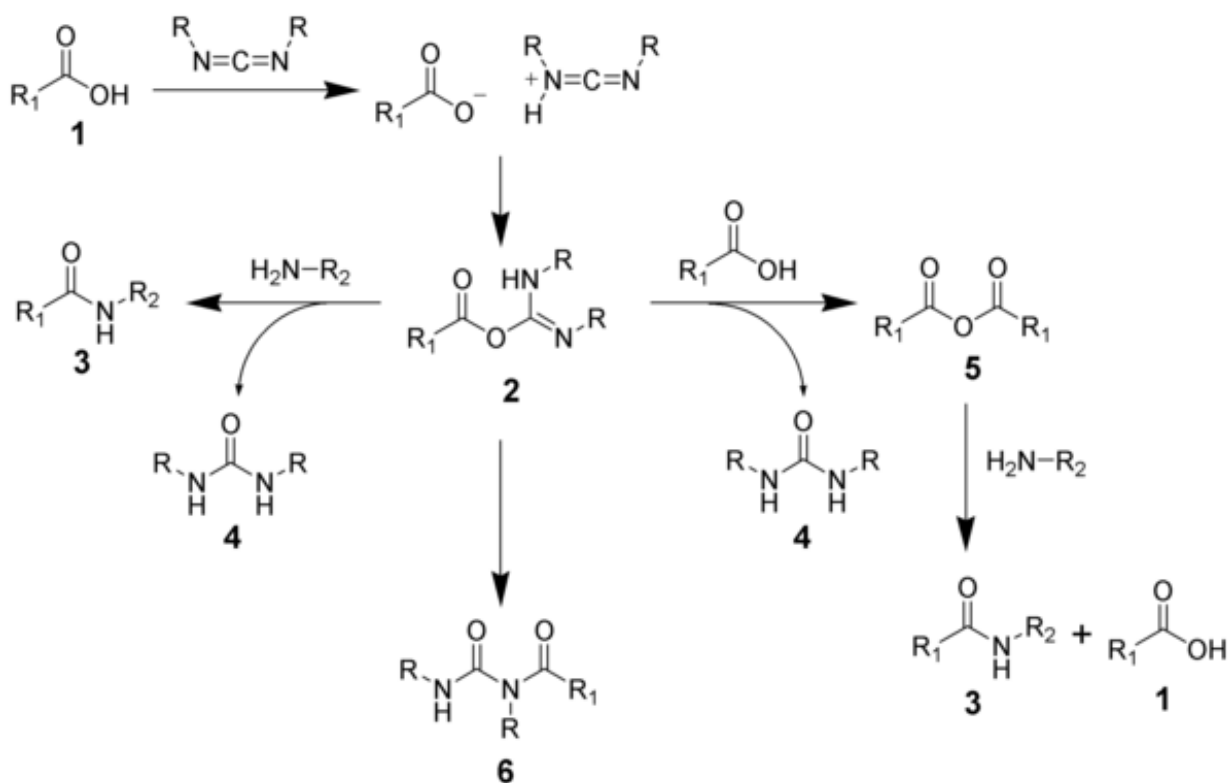
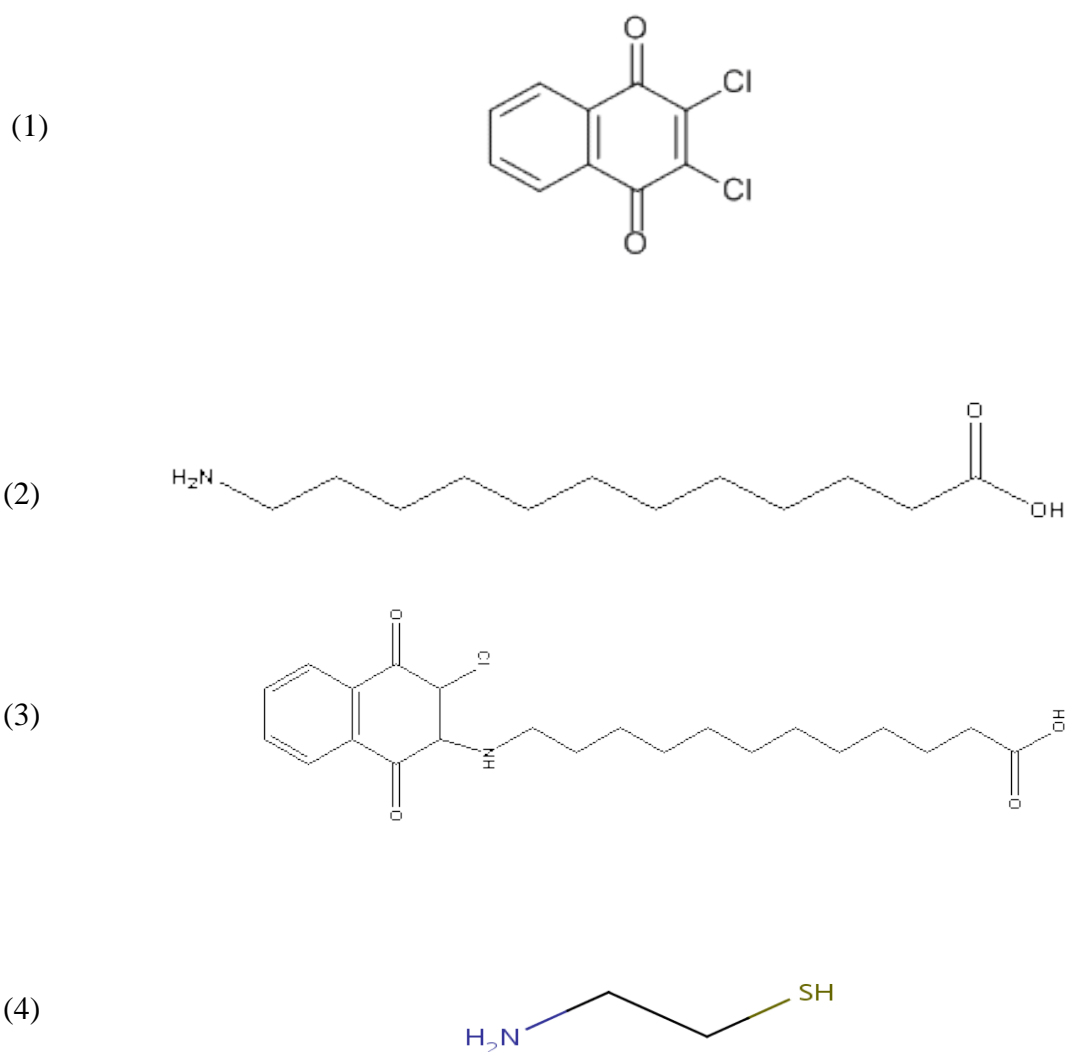
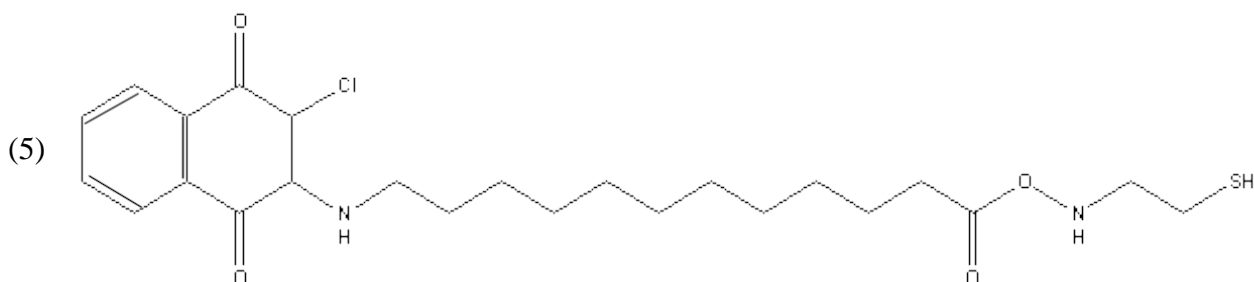


Figure 1. The carbodiimide technique reaction mechanism. The carboxylic acid, 1, will react with the carbodiimide to produce the key intermediate, which is the O-acylisourea, 2. The O-acylisourea will react with amines to give the desired amide, 3, and urea-byproduct, 4 (ChemPep, 2010).

As seen in Figure 1, the important intermediate is the O-acylisourea. This intermediate can be viewed as a carboxylic ester with an activated leaving group. The side reaction of the O-acylisourea can react with additional carboxylic acid to give a carboxylic anhydride or can rearrange to form an N-acylurea. The side reactions can be minimized by using solvents with low dielectric constants (ChemPep, 2010).





To practice the first step in process, the molecule glycine can be used instead of 12-aminododecanoic acid. This first step is able to produce a molecule which is similar to the target molecule while still being cost effective. The reaction would then produce a glycino-naphthoquinone derivate seen in the reaction in Figure 2.

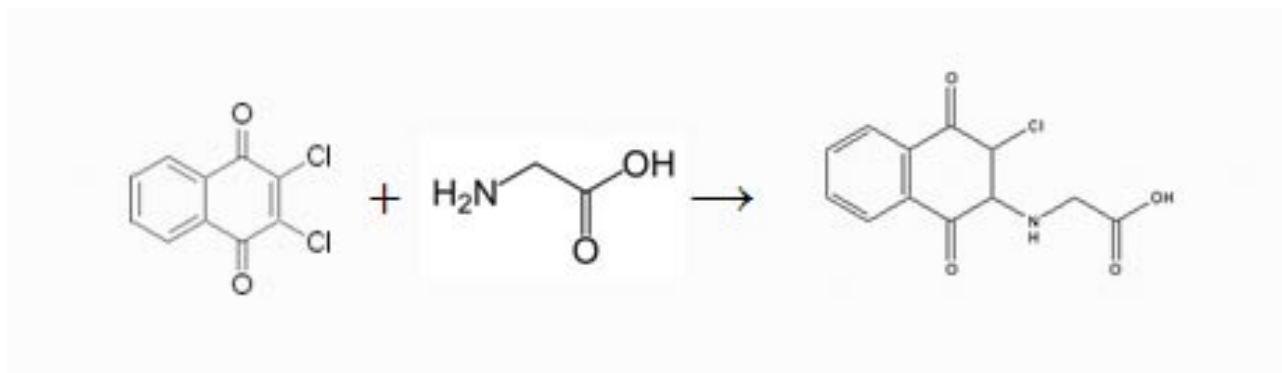


Figure 2. Showing the reaction between DCNQ and glycine to produce the glycino-DCNQ, 2-glycino-3-chloro-1,4-naphthoquinone, derivate.

Experimental

All chemicals were purchased from Sigma Aldrich unless otherwise noted.

A similar reaction method was followed as documented in literature (Sarhan, El-Dean, & AbdelMonem, 1998). Glycine (2.6 mmoles) was reacted with 2,3-DCNQ (1.25 mmoles) in DMF along with triethylamine (2.6 mmoles) at room temperature. The

reaction mixture was stirred for 14 hours and then diluted with an equal amount of water. The resulting red crystals were collected by vacuum filtration then re-crystallized from ethanol to give 31.0% yield, and a melting point of 193.4-195.2°C.

Results and Discussion

The melting point of the product was taken and compared to literature values of known molecules. The known value according to Sigma Aldrich, for 2,3-DCNQ is 192°C, and for glycine is 232-233°C. The melting point for the product is very near to the melting point for the unsubstituted reactant DCNQ.

IR absorption spectroscopy was also conducted on the starting materials, DCNQ and glycine, in addition to the isolated product material. The IR absorption spectra of the product material was identical to the spectra of the starting material DCNQ.

From these two forms of evidence, it is reasonable to conclude that the recovered material was in fact unsubstituted DCNQ. This shows that the substitution reaction did not occur. The failure of the reaction could be accounted for by several circumstances. This first explanation is that glycine has a significantly low solubility in organic solvents such as DMF. The glycine may not have fully dissolved in solution. Also, the triethylamine may have reacted with the DCNQ instead, thus changing the electronic configuration of the conjugated ring of DNCQ, not allowing substitution to occur.

An alternative approach to this method which could increase solubility of the amino acid, would be to add a hydrophobic protecting group onto the carboxylic acid end of the amino acid. This would increase the solubility of the amino acid dramatically and

inherently allow for less solvent to be used in the reaction mixture. This would increase the concentrations of both products in solution and possibly increase product yield.

Conclusions

The practice step performed in the laboratory did not produce a substituted naphthoquinone. The method which was used needs to account for the low solubility of the unprotected amino acid reactant. According to the literature surveyed, the proposed reaction mechanisms for the synthesis of the organic tether molecule seem to still be promising. With a minor change in experimental mechanism, the organic tether molecule is still a possibility. A possible protecting group which could be utilized is methyl ester which could be introduced as methyl chloride.

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Chapter IV: PSII Purification from His tagged *Synechocystis*

Abstract

Q-histidine tagged *Synechocystis* PCC 6803 was grown from frozen stock culture. The cells were broken and purified photosystem II was collected using a nickel-NTA affinity chromatography column. Chlorophyll concentration for the purified PSII aliquot was found to be 4.9 µg/ml. The purified PSII sample was subjected to Western blotting using anti-His Ab to bind the His-tagged Q subunit. The purified PSII sample migrated on the acrylamide gel indicating near 25 kDa in mass, 8.5 kDa larger than the expected value. Examination of the nitrocellulose membrane indicates possible presence of PSII subunits. Future studies should center on achieving greater growth of the *Synechocystis* culture before purification begins.

Introduction

Photosystem II (PSII) is a multisubunit protein found in the thylakoid membrane present in all plants, algae, and cyanobacteria. PSII performs a key step in the photosynthetic pathway, catalyzing the oxidation of water at the reaction center (RC) using light energy. The active site of the RC is thought to contain a cubane-like Mn_3CaO_4 cluster linked to a fourth Mn ion by a mono-oxo bridge (Barber 2006).

The oxidation of water molecules is possible due to electron transport. Light energy is collected in light harvesting chlorophylls and channeled to the CP43 and CP47 subunits. The energy then passes to a chlorophyll molecule named P680. P680⁺ is the final cofactor which drives the water splitting reaction due to its relatively high redox potential of 1.3 V, occurring near the D1 subunit. The electrons finally reduce plastoquinone (PQ), to from plastoquinol (PQH₂) at the Q_B site (Barber 2006). The overall reaction catalyzed by PSII is written below.



The protein core reaction center of PSII contains many subunits of different size. The D1 and D2 subunits are 38 kDa and 39 kDa respectively. The CP47 and the CP43 subunits are 56 kDa and 50 kDa respectively. The psbQ subunit is 16.5 kDa in mass (Barber 2003).

Materials and Method

Q-His synechocystis cells were streaked onto BG-11 solid medium and incubated under full light for 5-7 days at 30° C. A pea-sized culture of cells from this plate was then used to inoculate 100 ml of liquid BG-11 medium and incubated for 3-4 days under full light at 30° C until it became dark green. This liquid culture was then divided and added into seventeen 1L volumes of BG-11 with glucose (5 mM) in 2 L glass flasks. The 2 L

flasks were kept at constant stirring for 5-7 days until darkening occurred. A second batch of culture was also made starting from the solid medium cell culture.

The cells were centrifuged in a GS-3 rotor for 5 minutes at 4,500 rpm (3,422 g) in order to pellet. The pellet of cells were then resuspended to volume of 55 mL in buffer A (50 mM MES-NaOH, pH 6.0, 5 mM CaCl₂, 5 mM MgCl₂, 20% (v/v) glycerol). This volume was then placed in a 88 mL bead-beater chamber with glass beads and 55 μ L of 1M PMSF in DMSO, 1 M benzamidine, 1 M aminocaproic acid, and 3-4 mg of DNase I. The cells were broken by running the bead-beater for nine 15 second intervals with 5 minutes of cooling between.

The broken cells were centrifuged at 3500 rpm (1,992 g) for 5-7 minutes in GSA rotor cooled at 4° C. This step is performed to remove cell debris and unbroken cells. The supernatant was then rotated at 40,000 rpm (100,000 g) for 20 minutes at 4° C to pellet the thylakoid membranes. The supernatant was saved and the membranes were resuspended in buffer B (Buffer A + 15 mM CaCl₂) and centrifuged again at 40,000 rpm for 20 minutes, at 4° C. The cells were resuspended in buffer B.

The cells, or crude extract, was then placed in a Ni-NTA affinity chromatography column at 5mM imidazole. The column was rotated for 45 minutes. The column was then drained with six washes of buffer E (50 mM MES-NaOH, pH 6.0, 20 mM CaCl₂, 5 mM MgCl₂, 25% (v/w) glycerol, 0.20 % (w/v) β -DM, 5 mM imidazole) to remove membrane debris. The column was then washed with three bed volumes of buffer F (50 mM MES-NaOH, pH 6.0, 20 mM CaCl₂, 5 mM MgCl₂, 25% (v/w) glycerol, 0.03 % (w/v) β -DM, 250 mM imidazole). The elute sample, the three bed volumes of buffer F wash, was

noticed to be clear. The wash buffer E contained a green color and was then used to continue the study. The buffer E aliquots were placed in Amicon Ultra-15, 100,000 NMWL filter, tubes and spun at 15,000 rpm (30,100 g) to concentrate. Throughout this entire purification, the cells and all parts were kept at 4° C.

The concentrated extract was frozen in N₂ (l). The extract and the supernatant of the 40,000 rpm spin were subjected to western blotting. The primary antibody used in the western was a Qiagen α -penta His mouse antibody. The secondary antibody was a goat α -mouse Ap antibody.

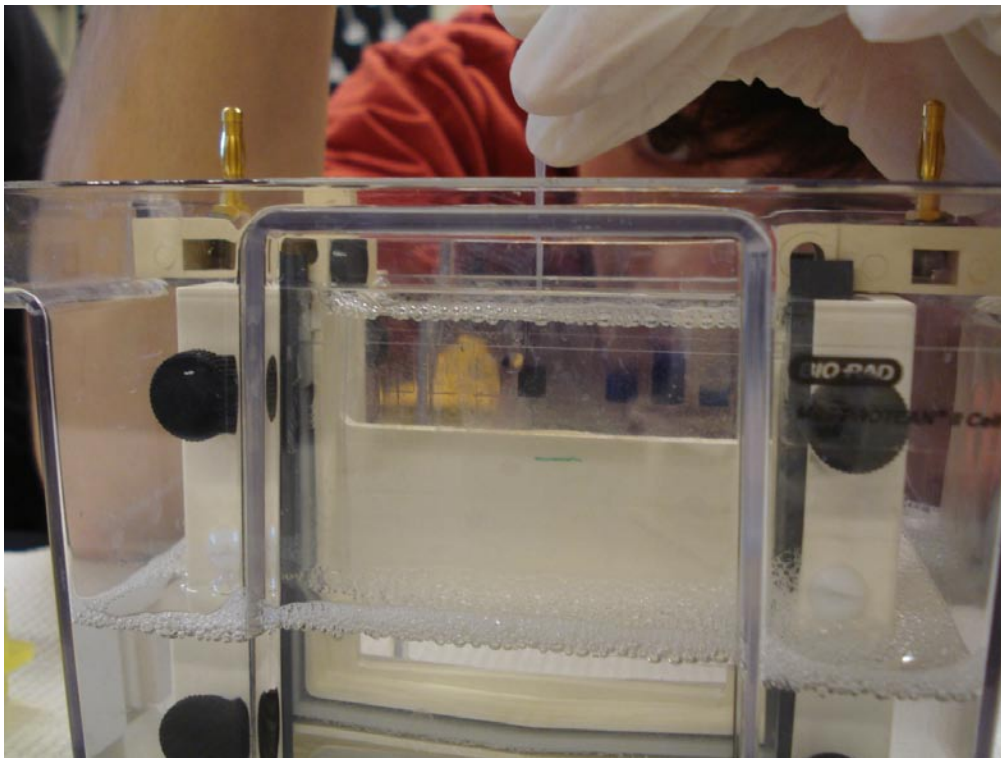


Figure 1. SDS-PAGE chamber used to run the protein against a marker and broken, unpurified cells. From left to right, 1: Marker, 2: Purified PS II, 3: Purified PS II, 4: Empty, 5: *Syn.* Cells



Figure 2. Preparation of the nitrocellulose membrane for antibody staining with marker lane on the right in blue and cell line in center with orange tint.

Results and Discussion

Culture Growth and Harvesting of Cells

Dark green color was seen in the bacterial culture plates that were streaked with Q-His tagged *Synechocystis* cyanobacteria as seen in Figure 3. The dark green color correlates with healthy growth of *Synechocystis* cyanobacteria indicating active photosynthetic metabolism. After inoculation, the 100 mL liquid preculture of Q-His

tagged *Synechocystis* demonstrated a light green color seen in Figure 4. This light coloration of the liquid medium was well under the ideal OD₇₃₀ value of 1.0. The low coloration indicates a diluted preculture with low PSII concentration.

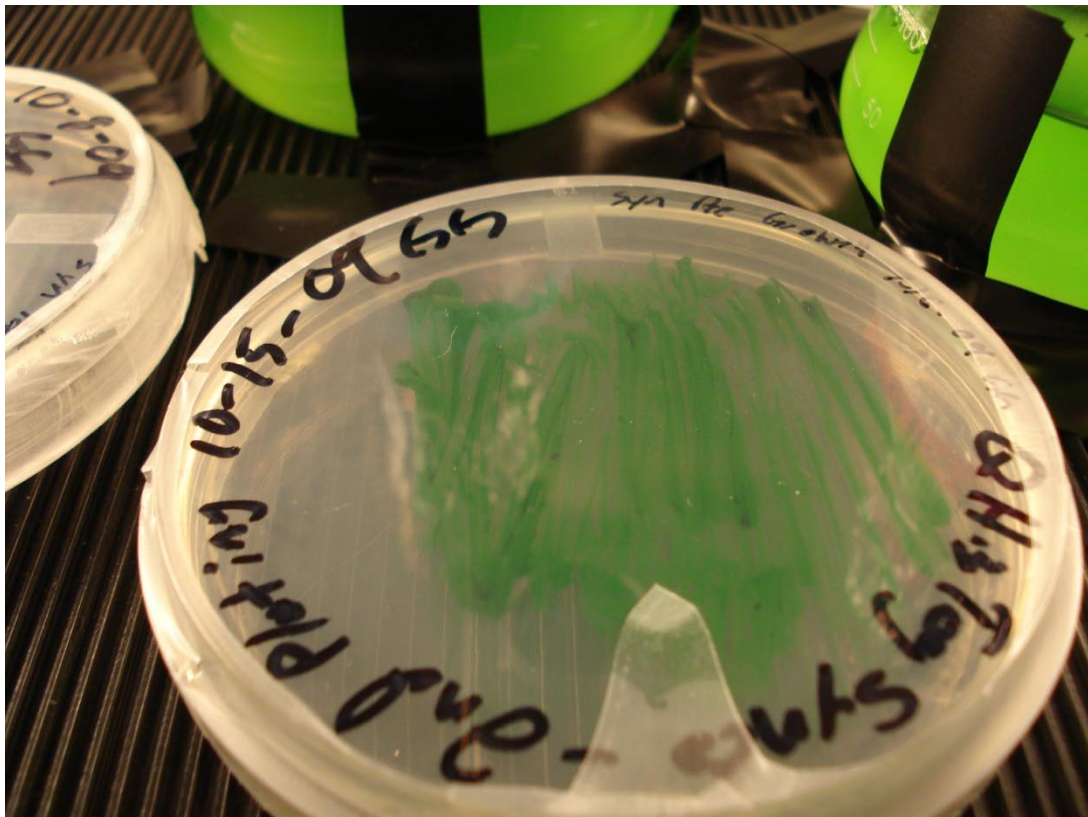


Figure 3. Q-His tagged *Synechocystis* streaked on BG-11 solid medium



Figure 4. 100 mL precultures of Q-His tagged *Synechocystis* after 4 days incubation in BG-11 liquid media

Visual Examination of the Western Membrane and Gels

The two poly-acrylamide gels were stained using Coomassie Blue. Bands were visible as seen in figures 5 and 6. With staining, bands were visible on the non-transfer gel in lanes 1, 2, 5, and 6. When examining figure 5, the bands in lane 1 appear to be bold and distinct, which pertains to the broad range marker. The broad range marker indicates protein mobility on the gel. The bands in lane 2 are faded, but appear close to the 21 kD marker and the 125 kD marker which belong to the purified sample of PSII protein. A large number of distinct bands were seen in the lane containing broken *Syn.* which were

very dark. The large number of bands seen in the lane illustrate the large amount of protein subunits that are present in cytoplasm of the cells. The bands are very dark in this lane due to the high concentration of protein in the sample. In lane 6, the visible bands correlated with the Phycobilisome solution, indicating the possible presence of Phycobilisome protein.

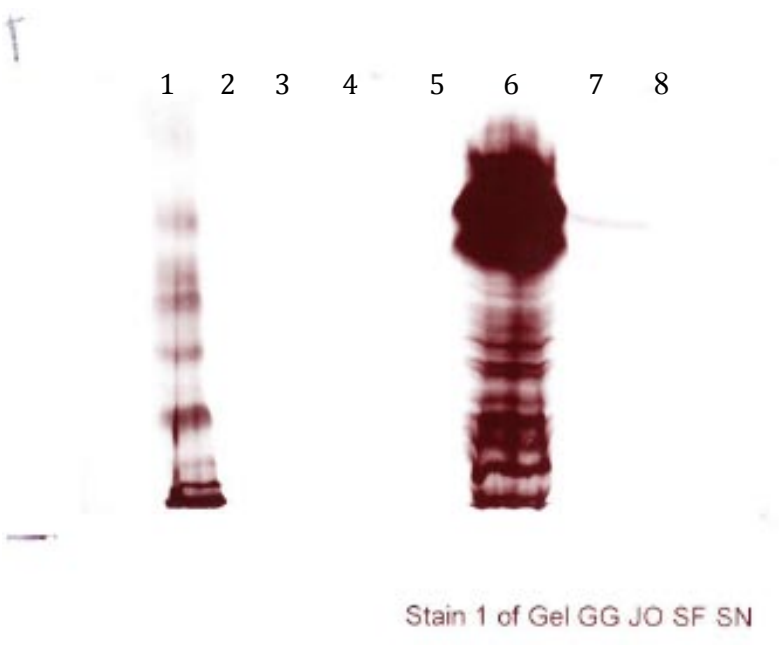
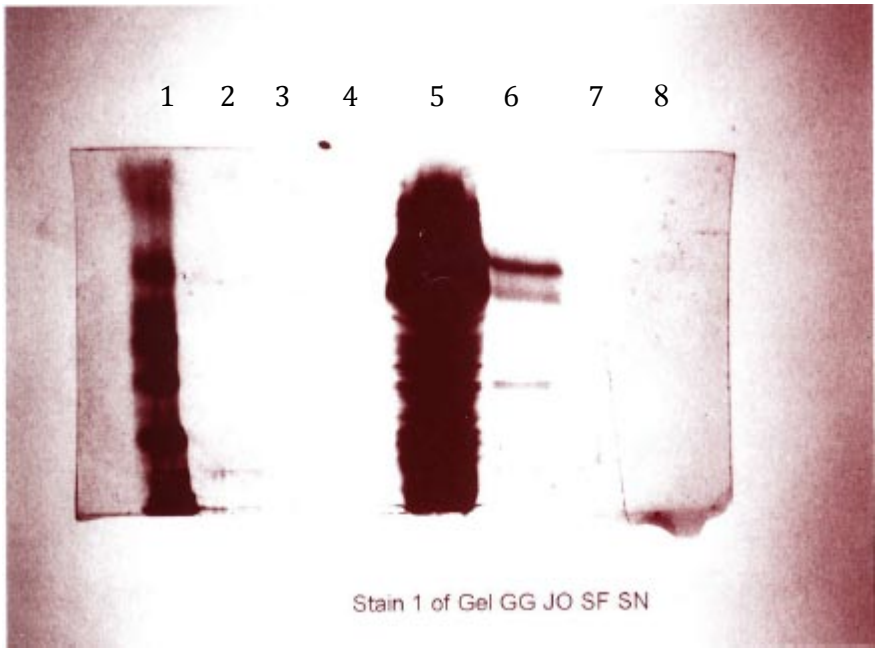


Figure 5. Commassie blue stained SDS-PAGE non-transfer gel; 1: Broad range marker, 2: Purified PS II, 3: Purified PS II, 4: Empty, 5: Broken Syn. cells, 6: Phycobilisome solution

The transfer gel was also examined after staining using Coomassie Blue as seen in Figure 6. The larger proteins were visible in lanes 2, 3, 5, 6, 7, 8 corresponding respectively to the samples which were contained. Lanes 2 and 3 contained the purified PSII at the same concentration placed in the non-transfer gel. Lane 5 contained the *Syn.* cell lysate while lane 6 contained phycobilisome solution. Lanes 7 and 8 contained the undiluted purified PSII sample. Part of the gel is broken due to handling. The interesting relevance to staining the transfer gel is to indicate how much of the samples have transferred from the SDS-PAGE gel to the western membrane. Some small bands can be seen in the upper region of the gel in the lanes containing the purified PSII indicating that some larger proteins may have not transferred. This is understandable knowing that larger proteins take longer amounts of time to be removed from the SDS-PAGE gel. Dark bands were seen in the *Syn.* cell lysate in lane 5. It is possible to conclude that these multiple bands were still seen on the transfer gel because not all the protein from this lane could transfer to the membrane due to such a large amount of protein in the sample.

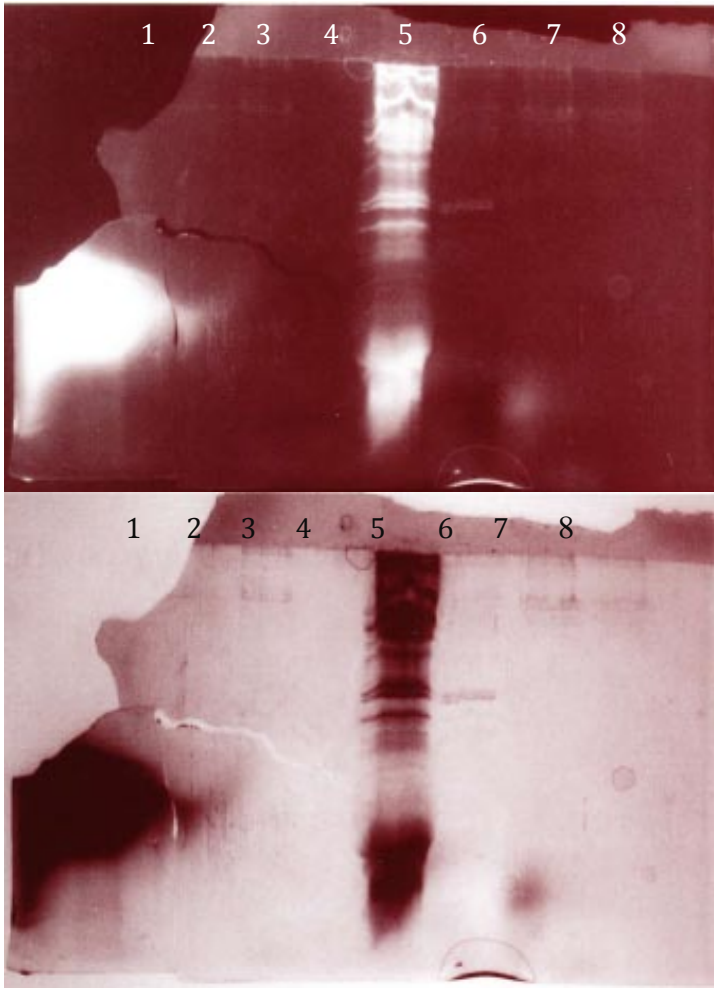


Figure 6. Coomassie blue stained SDS-PAGE transfer gel, top is negative photo, bottom is normal color, Lanes; 1: Broad range marker, 2: Purified PS II, 3: Purified PS II, 4: Empty, 5: Broken Syn. cells, 6: Phycobilisome solution, 7: Purified PSII, 8: Purified PSII

The western membrane was stained with Ponceau S after the transfer. This is can be seen in Figure 7, showing evidence that smaller proteins successfully transfer as there

were bands in lane 5 and lane 6. The most concentrated band appears in the lane 5 around 21kD marker. The marker lane is also defined in the image.

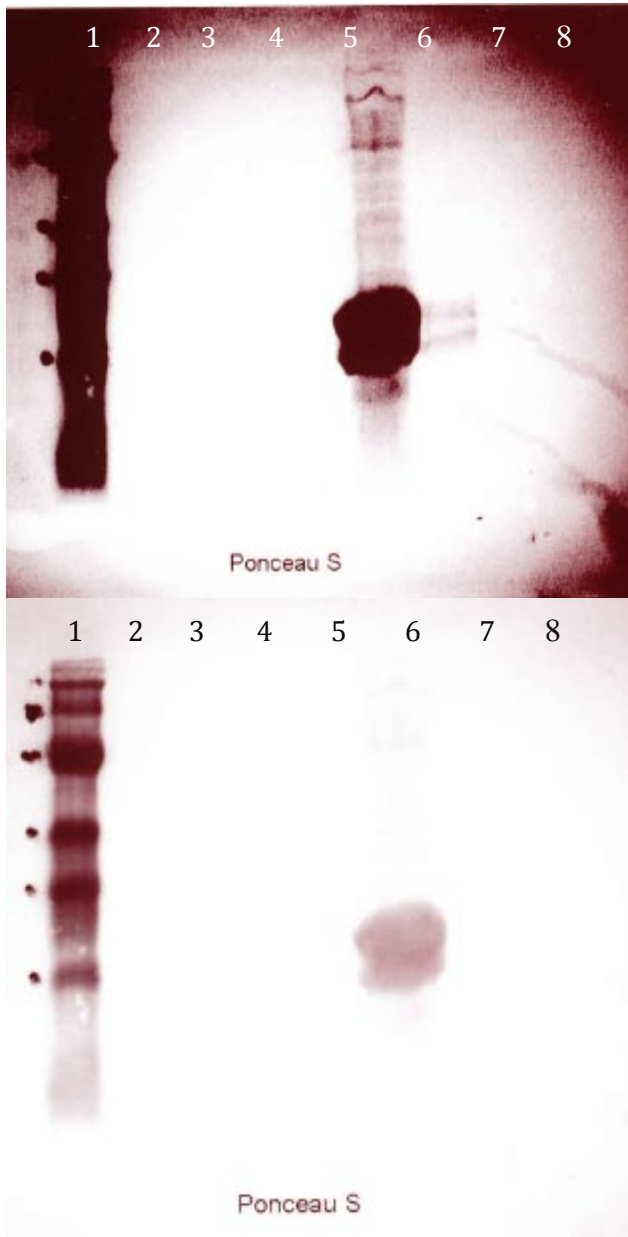


Figure 7. Ponceau S stained transfer membrane. 1: Broad range marker, 2: Purified PS II, 3: Purified PS II, 4: Empty, 5: Broken Syn. cells, 6: Phycobilisome solution, 7: Purified PSII, 8: Purified PSII

In figure 8 the anti-His antibody is seen after development with BCIP (5-bromo-4-chloro-3'-indolyphosphate p-toluidine xalt) and NBT (nitro-blue tetrazolium chloride). Coloration of the membrane highlights several important features. Some coloration is present in the lanes 2 and 3 in the migration length around the first marker. These seem rather inconclusive. Possible bands can be seen in lane 5, 7, and 8 occurring just before the 21 kD marker. The band in lane 5 is subtle and poorly defined while the bands in lane 7 and 8 are cloudy but are darker in color. Lane 8 is completely covered while lane 7 is partially covered. These bands seem to represent the protein witnessed in lane 2 of the non-transfer SDS-PAGE gel, occurring near the 21 kD marker. Lane 5 corresponds to the broken *Syn.*, and lane 8 corresponds to the purified PSII.

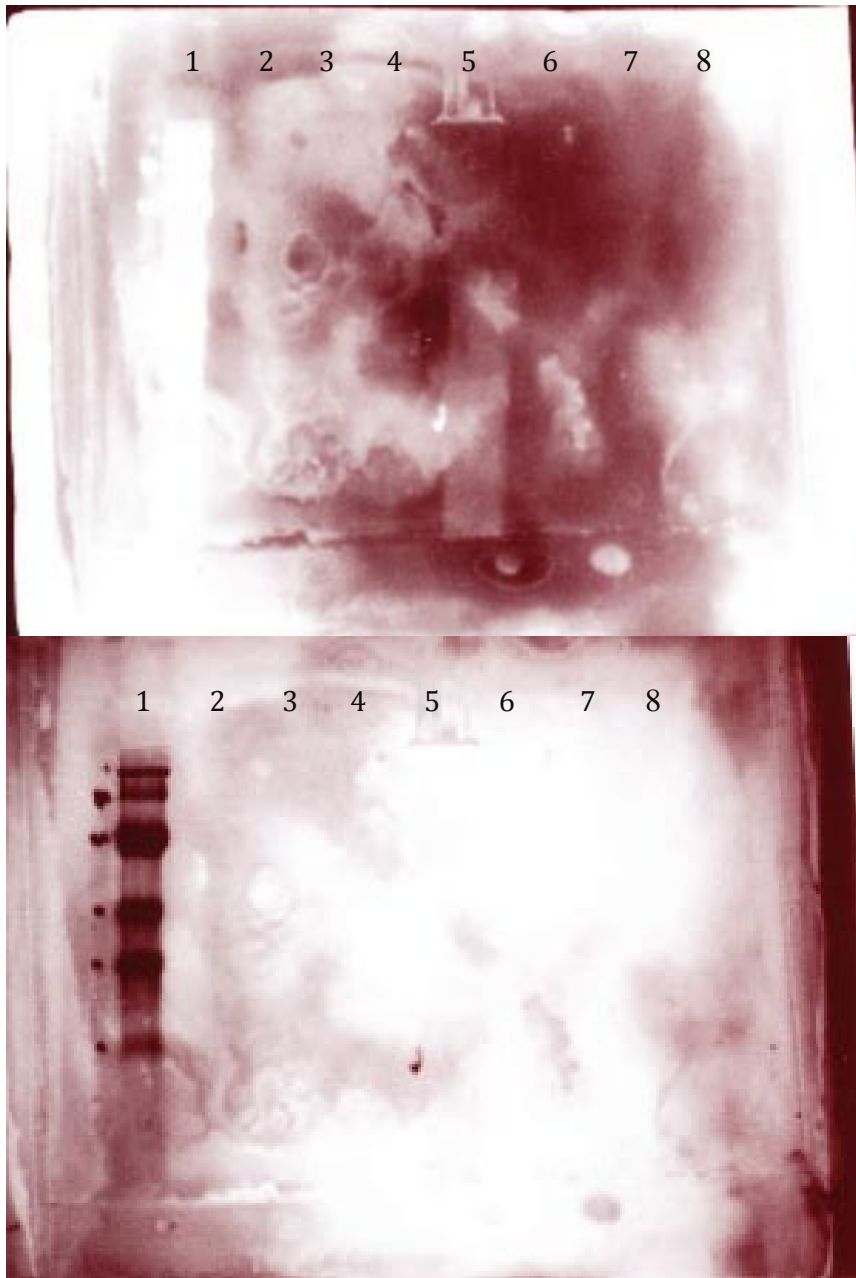


Figure 8. Antibody stained transfer membrane, top is negative photo, bottom is normal color, Lanes; 1: Broad range marker, 2: Purified PS II, 3: Purified PS II, 4: Empty, 5: Broken Syn. cells, 6: Phycobilisome solution, 7: Purified PSII, 8: Purified PSII

Chlorophyll Concentration and Protein Mobility on SDS-PAGE Gel

Table 1: Concentration of Proteins at Various Steps In the Purification

Samples	Amount of Chl (mg)	[Chl] (mg/ml)
Cells from First Harvest	21.76	0.0474
Cells from Second Harvest	30.47	0.6095
Pooled Sample Prior to Lysing	52.23	
Purified Protein	0.0140	0.0049

Table 2: Determination of Unknown Protein Sizes based on Mobility

Lane 1: Broad Marker		Lane 2: Purified PS II		Lane 6: Phycobilisome	
cm	kDa	cm	kDa	cm	kDa
0.15	209	0.5	124	1.4	56
0.5	124	2.6	25	2.5	26
0.9	80			2.8	22
1.6	49				
2.1	34				
2.4	29				
2.9	21				
3.7	7				

Conclusions

The growth of the preculture was determined to be minimal, indicated by the dark pale green color of the liquid culture. Optical density readings for this color was well

under the desired value of 1.0. This was evident in the low protein concentration when assayed. A healthy culture would have resulted in a dark green color and higher concentration. This phenomenon may affect the detection of protein during gel electrophoresis and Western Blotting. However, light visible bands after Coomassie Blue stain indicate some presence of protein. This indicates that the concentration used for the sample was low.

By comparing the transfer gel and the nitrocellulose membrane, it is evident that the smaller proteins from lanes 2, 3, 5, 6, 7, and 8 were transferred. Some large proteins were left on the transfer gel in lanes 2, 3, 7, and 8. The transfer procedure may have not ran for long enough time to achieve a full transfer of protein on the western membrane, however, transfer did occur.

It can be concluded that PSII was separated based on the separation and collection of phycobilisomes. Visible bands were observed in this lane indicating that the light harvesting protein antenna was concurrently separated from the thylakoid membrane left behind in the supernatant of the 40,000 rpm (100,000 g) spin. Additionally, utilizing the anti-His antibodies, visible bands in lanes 5 and 8 were witnessed indicating possible identity of the target Q-His subunit.

For future experiments, it is imperative to have the cyanobacteria culture at optimal growth. This emphasis may alleviate the problems surrounding the minimal protein concentration. Also, reaction specificity of the anti-His antibody could be called into question. This study utilized an anti-His antibody that binds with a large variety of histidine sequences. In the future, incorporation of a positive control which reacts with

the anti-his antibody would be helpful in establishing if the primary and secondary antibodies are binding properly. Such a positive control could be an enzyme which contains a similar histidine chain as seen in the PSII-Q subunit.

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Chapter V: Philosophical Meditations Regarding Scientific Episteme

The understanding of science is largely based on historical conventions which span from the centuries preceding the present. The present study relies on these conventions. A brief overview of these conventions is applicable in a full synthesis of this thesis. The following is a brief examination of the scientific method in order to describe constraints of the experimentation represented in the above studies.

Science as a Society

Science fundamentally is a perpetuation of ideas created by a dedicated group of individuals who subscribe, consciously or unconsciously, to a fundamental method of knowledge acquisition. The communication of such shared knowledge is fundamental in the continuation of this society. In searching history, the philosopher Steven Shapin explains that science grew out of its infancy in the 1600s. During this time, the scientific community advanced at the method of organizing and sharing thought (Shapin, 1996, p. 12). This advancement allowed science as a whole a greater efficiency at documenting and describing experience. Shapin (1996) explains that the interesting intersection between making observations and constituting experience is the beginning of the scientific method. The communication of such experience is paramount to progression of science as an institution, and it is by communication through this community that value and method are upheld. Science is a progressive entity which is built on the shoulders of

the past. Percy Bridgman explains that when constructing a description of science, scientists are building on the intellect and work of all the ages (Bridgeman 1955). This influence explains that understanding is formulated and compiled in order to build future knowledge. This perpetuation is formally initiated when the members of the society of science express their findings using a standard in method. The standard method of language used in this philosophy are knowledge statements that are based on physical experience. These statements are *matters of fact*. Shapin (1996) explains that fact making can be instituted when the assumption that nature innately possesses characteristics of a machine. Nature progresses according to rules which constitute predictable effects that are replicable. A subsequent method of gathering facts is thus needed for this to occur.

Gathering of facts can be described as actions which are conducted in the physical and mental world of human perception. Bridgman (1955) explains that scientific conventions of measurement are based on a set standard of measurement. For example, the metric system is a set standard of measurement which allows individuals to communicate the volume, length, ect. of an object accurately. Bridgman calls the use of standards of measurement, such as the metric system, “concurrent operations.” These operations are counted as being continuous, as long as the society of scientists keeps them synonymous. In this understanding, the operations are described and supported in their ongoing use. Thus, in beginning to understand scientific method, we first must begin to understand its operational foundations. Boyle was one of the first scientists that described this type of thinking. Shapin (1996) documents that Boyle described this thinking as “systematical.”

Boyle explained that unbiased observation of factual evidence was the only way to gain reliable conclusions. The observation that Boyle explains shows how an ideal form of empiricism should function. Like many ideals, human empiricism has limits and the boundary of natural science must also be investigated in order to fully explain the boundary of scientific method.

It is necessary to explain that first the underlying boundary present to empirical studies is the laws of nature. Boyle, being a pioneer in early empiricism, expressed that matters of fact are gathered to be the foundation of natural philosophy. In their function, matters of fact must be protected from “contamination” from less certain and convincing elements of knowledge (Shapin, 1996, p. 104). The knowledge that Boyle refers to are theology, politics, and moral reflections. By limiting the blooming field of natural philosophy to only a specific type of knowledge, Boyle was able to place emphasis on the need for defining clear boundaries of thought to the field. The boundary which was being described focused on, not what was possible from humans, God, or ethics, but what was possible from processes conducted and governed by the natural world. By placing the focus of scientific knowledge on processes which are naturally governed by the laws of the present universe, Boyle was able to have science coexist with the conception of God while simultaneously strengthening its empirical fact making. Boyle expresses the boundary he believes to be appropriate for natural philosophy,

“None is more willing [than myself] to acknowledge and venerate Divine Omnipotence, [but] our controversy is not what God can do, but about what can be done by natural agents, not elevated above the share of nature,... and in the judgment of true philosophers, I suppose [the mechanical] hypothesis would need no other advantage...than that in ours things are explicated by ordinary course of nature, whereas in other recourse must be had to miracles” (Shapin, 1996, p. 105).

The major boundary of early scientific philosophy is confined to the processes that occur in nature under observable, universal laws. Boyle expresses this in terms of natural “agents”. The agents which he is referring to do not indicate cognitive or conscious beings, but rather refer to the type and possibility of events which can occur under natural, testable law. The hypothesis which stems from this type of reasoning describes the natural world as a mechanical entity. The Aristotelian principle that nature proceeds in accordance to a structured plan gave early philosophers grounds for comparison. Shapin (1996) explains that like human mechanical creations, nature could be investigated. This begins to illuminate the hypothesis which is fundamental to the study of science – nature being described as “the natural machine”. Modern science is also founded on this basic principle; there are natural, observable laws which dictate the course of possible occurring natural events. Thus the natural events which are being referred to are all events in the physical realm of nature.

This synthesis of defining the world as a machine gave Boyle a description of natural philosophy which neither rejected the assertion of God’s presence or God’s

absence. It assumed that the world as we perceive it, functions on observable laws which are held constant throughout creation. By this assumption, facts about the observable world can be ascribed and documented through the process of reproducible operations which illuminate the observable laws by which nature functions.

The universe is ultimately much more complex than any human machine. For this reason, the complexity contained within the interworking of a clock seems simple when compared to the interworking of only one human cell. Furthermore, the causality of the human machine is easily accessible to an observer. Shapin (1996) explains that a simple observer can ask the watchmaker for a schematic of the dials thus illuminating the causal nature of the timepiece. The study of nature, which takes place in science, is somewhat different in kind. Humans do not have resources to find the causal nature of most natural processes. Shapin (1996) describes this as humans do not have direct sensory access to the causal structure of nature. The investigation of the natural world must start not in examining the causal structure of nature, but instead must examine the *effects* of that causal structure. Thus, the boundary of natural science must lie within the observable effects of nature's agents. From the observable events which can be tested through concurrent operations, the scientist is able to learn about the causal structure of nature's laws. In this description of reality, nature proceeds to function, as a machine does, according to set variables. Unlike human technology, the causality of the natural machine is hidden to the human observer.

Trying to understand the boundary of a scientist's endeavors one must look inward. Though it has been stated above implicitly, it must be emphasized further that

any scientific study is also limited by the constraints of human existence. John Stuart Mill highlights this fact by expressing that laws which are deduced by human social groups are constrained by the laws of nature of individual man (Oppenheim and Putnam, 1958). When discussing boundary of scientific thought, the boundary which is inherently present in all observations is the limitations of senses present to the observer. In human observation, the limitation of the senses is the most fundamental obstacle. Human sensation does have limitations. Individuals have thus created technology to widen the range of human observation. Nearly all current science relies on such technology; however, it is the method by which such observers use this technology that is important. Interestingly, human observation will always be limited by each individual's focus and attention.

Understanding the human limitation of science, rules of methodology have been instituted to in order to achieve the empirically unbiased matters of fact which Boyle spoke of centuries before the present. The major underlying philosophy in science is for a theory or matter of fact to be susceptible to revision. The principal resonating behind this statement is the fact that human perception is constantly changing. Whether new technology is made, as mentioned above, or newly acquired data is presented, empirical science is an ever evolving substance. Karl Popper (1959) expresses that empirical statements must be susceptible to revision. In fact empirical statements should be criticized and superseded when better, more plausible empirical theories are presented. It is this philosophy of revision that helps to accommodate the constant evolution of the human society.

Such revision are clearly emphasized in the empirical method of performing science. Understanding the human limitations, and also the limitation of fact making, the testing of scientific theories must undergo systematic testing that is based on method that is appropriate. As Popper (1959) describes, the investigation of the methods employed in the testing of theories is as important as the theory itself. Method which is appropriate and well documented is needed in order to combat the human limitation of human perception. Systematic empirical testing combats the problem of subjectivity. Human limitation dictates that an observer will have biases whenever conducting a study. Popper illuminates the solution to this by expressing the rule, “Only such statements may be introduced into science as are intersubjectively testable” (Popper, 1959, p. 105). Here the role of the society of scientist becomes clear. Intersubjective testing is achieved when the method of the experimentation is documented and presented to the society. Here the role of the society is to both to accept and reject new forms of knowledge within the community. In this regard, the practice of science is a constant, evolving society, which fervently is revising and testing the theories that individual scientists present. Thus, science functions by individuals working to achieve knowledge that is verifiable between individuals based on a given experimental method.

The ideal result from empirical experimentation is to obtain objective results. This however, is understood as an ideal and like most ideal aspirations, is not fully recognized in reality. The boundary of humanity dictates that this ideal is not fully possible. However, science can come close. The primary tool which scientists use to tackle this obstacle is to be cautious in the matters of facts they describe. Also the scientist relies on

a set language of describing his concurrent operations – his experimental method. Methods are buried in the experience of previous individuals. In this complex cycle of thought, the society of science can be understood. Sharon Traweek insightfully summarizes that the goal of achieving objective results relies on the institution of this society. Traweek (1988) adds, “Pure objectivity is tacitly recognized as impossible; but error can be estimated and minimized. The means is peer review, or collective surveillance; the final degree of order comes from human institutions” (p. 152). The human institution of science is broad and large. However, it stands interestingly apart from others, as it is perpetuated by the driving empirical method by which its theories are constantly in motion. The society of science is both self-directing and self-limiting. It is a society which is rigorously validated by its peer-review of methods used.

Method of Discovery Regarding Photosystem II

Speaking directly about the method or experimentation used in this study to test different aspects of PSII, the majority of knowledge dictating the methods used were primarily buried in past journals and published scientific articles. The methods and procedures used in each study are quite specific in terms of what must be done to achieve a measurable result. This is highly recognizable in laboratory science, as much of what is known about the procedure that is trying to be accomplished is knowledge which has been passed from one journal to the next. This documentation of various types of methods dominates the field of thought. In fact, much of what is known about a particular subject is solely found in past empirical documentation. Bruno Latour and Steve Woolgar

notice this structure and describe it in their book, *Laboratory Life: The Construction of Scientific Facts*. Latour and Woolgar express a central importance of the collection of documentation on laboratory methods. Upon visiting a laboratory they were studying, Latour and Woolgar were struck with this realization, that laboratory work can be understood in a continual generation of published and unpublished works which describe the standard methods or concurrent operation used in the experiments. Also, this literature serves as a tool to validate or invalidate the results and work of the scientist (Latour and Woolgar, 1986). These documents even serve to direct the laboratory work as a whole, as seen by the author of this report. Much of the ideas that used in the laboratory are based on the works of the past, as Percy Bridgman indicated above.

In the case of growing the photosynthetic bacteria *Synechocystis*, methods were used from a group at Yale University. The leading Professor of the Yale group offered the procedures to be used by faculty of Regis University, specifically Dr. James P. McEvoy. This documentation highlighted on the appropriate mixture of ingredients that the cells needed to grow. Interestingly, even though this method was appropriately followed the studies showed that the cell growth was not what was expected, being less than half of what was described in the literature. Method in this sense is just a guide. It is also measuring stick, which illuminates if the scientist is on track to arrive with the intended destination.

Journal literature was also instrumental in the case of constructing a plan for the molecular tether molecule. Even though the first step of the synthesis was not successful, all the information which guided the progress of constructing the plan for the synthesis

was acquired from journal articles. Interestingly in this case, there are gaps in knowledge. In the specific case of substituting with an amino acid, no literature could be found to match the exact variable that this study tried to accomplish. Thus, the literature was the foundation of the plan, however, literature did not give the exact method to follow in the specific case found here. After failure of attempting to attach glycine to the naphthoquinone, the author of this report sought other resources. Insight was given by Dr. Kateri Ahrendt, leading to an alternative method of attaching the glycine, or any other amino acid, to the naphthoquinone. Thus the failure of to produce the desired results stemmed from a failure in method.

Likewise, in regard to the inhibition studies, literature provided an almost absolute guide to conducting the experiments. Documented work of past studies showed the appropriate conditions in which PSII should be tested. Literature also concluded the expected trends which should be seen. In the study, the trends did seem to attenuate to the predictions contrived from the literature. In this, literature adds validity to the matters of facts which are collected empirically. The matter of fact that was highlighted in the inhibition studies is that DCNQ, 2,3-dichloro-1,4-naphthoquinone, inhibits photosystem II less in comparison with DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

Thus, method is an extremely powerful tool which all experimentation is built upon. Without the publications of many studies, none of the experiments listed above would be performed. It is this interesting facet that makes the publication of experimental methods so important to the perpetuation of science. Latour and Woolgar (1986) cite that scientist rely on each other's achievements to increase his own production of reliable

results. This concept can be seen in any laboratory across the globe, one felt daily by percipients in the operations of this study presented.

Error, Luck, and Time

One major concern in laboratory life is the effects of error, luck and time. These may seem to correlate more towards a moral or metaphysical ponderings; however, these are facts which weigh heavily on any experimental inquiry. The effect of error may the easiest seen in the laboratory. It is a constant obstacle which infests every single operation which is performed in the laboratory. From the 16th century, researchers have been tackling the problem of error with advancements in method. Bernard Dixon comments on these unusually interesting topics. Dixon (1973) explains that the advancement of method comes with practical innovation in techniques and equipment – advancement in technology. Thus, innovation in method also needed in a working lab, in order to construct, better, more reliable results.

Some would describe this as tinkering, but the important distinction is that lab work demands a highly practical, physical method of discovering the world. Error stems when experimentation is not practical. The presence of error can easily be ascribed to the boundary of the human observer. This boundary is two-fold. There is user error, and error which is produced from mechanical error.

Both these kinds of error can be clearly found in the studies presented above. First, in order for the inhibitor studies to precede a methodological consistency needed to be introduced. Whenever a blank sample was placed in the oxygen evolving chamber,

slight variations in oxygen production were witnessed. These variations were pointed to be produced from factors such as: room temperature, solution stirring speeds, or thermal radiation from the halogen light used to illuminate the chamber. The error which could result in actual experimental trials be less accurate than desired because of these mechanical factors of experimentation. Thus, a technique of blanking a daily sample before starting the experiments was instituted to combat the possible error which arose due to the mechanical nature of the experiments.

Likewise, error due to improper following of method also can be found with the studies. When trying to grow the bacteria, improper plating techniques could have resulted in low cell growth. Also, it was found that some of the early plates being used grow the cells were not properly stored. This error in user attention circumvented the healthy growing of the cells, thus forcing the remaking of petri plates and restart of the whole process. These examples are just two of the daily logistical battle which rages in the laboratory.

Time also greatly affects laboratory work. Not previously discussed, the fourth dimension of time is a boundary which limits every scientific inquiry. This is due to the fact that humans are cemented in the space time continuum. Thus, under working laboratory pressure, experiments are limited by the amount of time which is available for each process. In the case of *Synechocystis* growing, one major limitation in the amount of cultures that could be grown was the amount of time it takes for *Synechocystis* to grow. On average, a batch of mature cells could take up to three weeks to harvest from start to

finish. This includes plating on solid medium, transferring into liquid pre-culture, and then inoculating the large prep for full harvesting of the cells.

Understanding only these two challenges of laboratory life, it is easy to see that gaining new, theory changing results is a complex, arduous process. In all cases, the attention to detail which the lab worker displays dictates the amount of validity that the end result contains. The culmination of the bench work performed in the laboratory is a product of the ingenuity of the scientist and the random ordering of the various factors involved. Thus, the scientist is at constant battle with both the randomness presented in mechanical error and the error which is present in human perception.

Funding Restraints

The final topic which relates directly to the discussion of scientific method is the need for funding and the allocation of such funds throughout the society of scientists. Traweek (1988) indicates that laboratory funding at education institutions is a paramount concern because it dictates the allocation of research funds, thus dictating the amount of resources present to the laboratory. Interestingly, these monetary restraints create a system which conflicts or complicates the perpetuation of scientific thought. As mentioned above, the foundation of scientific research is based on the inter-reliance on each other's discoveries. This notion is complicated by understanding the effects of free trade and the capitalistic economy which science operates.

Scientific society exists in a world where monetary financing is needed. Latour and Woolgar (1986) explain that whether if a lab is funded by private, state, or national

measures much of the direction of modern research is guided by the notion of investment and return (p. 190). This creates an effective tendency for science to be looked at through the lens of laissez-faire economics. In this description, lab research must present monetary profit or the possible gain of knowledge leading to monetary profit in order to be allocated sufficient funds for research. In this system, knowledge is selected not on the potential of discovery, but on the potential for return.

This effect was also felt in the direction and allocation of resources in the studies presented in previous chapters. Much of the instruments, tools, and materials used in the experimentation were dictated by the allowable funds present. These funds were allocated by the supervisor of the studies which were dictated by the amount of grant money given. Thus, the purchasing of chemicals for the synthesis studies were dictated by the amount funds dictated. Likewise, monetary constraints were also seen at the level of the private institution where the laboratory resided.

One example of this limitation was the available spaces and instruments present within the laboratory facility. During the purification of PSII from the broken *Synechocystis* cells, the samples were kept in the dark and refrigerated. The method which kept the cells refrigerated was cumbersome because the affinity chromatography column needed to be set up inside a refrigerated cabinet. This cramped working environments. In a large laboratory facility, with a immense program budget, such as Yale university, the facility would contain a “cold room” (this is a whole room that is refrigerated, allowing for the full working environment to be cooled to a desired

temperature). This is just one distinction of how accessible finances affect the working laboratory.

Conclusion

Much of what science is today relies on conventions and practices that have been evolving for decades and centuries before the present. The limitations of such a complex society of individuals are heavily based on the conventions and ascriptions by which empirical method is carried out. Some of these limitations are monetary, some are from the very nature of human perception. Centered is this society in relation to the discovery of the natural world. A scientist thus ascribes to a culture, a way of life, which is highly dependent on physical operations aimed at testing the observable laws of the universe. This inquiry, though capable of fallacy, attempts most logically to discover the causality buried within the natural machine of nature.

However, I have learned personally that science does not function so easily as a well oiled machine. The description explained above is an ideal of what science could strive for. Indeed the limitation of human perception is increasingly evident the farther specialized the inquiry becomes. In the physical practice of science cements itself in reality as a complex logistical battle. Even simple experiments require the operator to command a significant accuracy, a proficiency in the instruments being used, and an understanding of the variables present.

What has been learned? Specifically from the studies above, some important practical conclusions can be expressed. It was identified from the inhibition studies that

DCNQ relatively acts as a good inhibitor of PSII. This data indicates it also would be a good moiety to attach to a tether molecule in order to bind PSII on an electrode surface. Additionally, a promising method for synthesizing a tether molecule, that could bind PSII to an electrode, was proposed. This method could not be tested significantly due to time limitations. Finally, the limitation or complexity in growing the bacterial cultures of *synechocystis* was indentified. After many trials of unhealthy cultures, the research team will accept that it is more practical to obtain purified PSII from collaborators. The limitation of a small scale bacterial prep program indicates is only found in growth trials. This is an example of limitation which is found only through experimentation. Thus, this final example dictates that laboratory science is largely directed by practicality and time. Overall, the studies listed above were limited by the practical reality of time, space, and technology.

The view of science in relation to the discussion presented in this work exemplifies a more synthesized view of science. The synthesis is based on the understanding that science is a mixture of textbook knowledge and laboratory experience. To explain science without either of these two forms of reality would describe an incomplete picture. Laboratory science is instrumentally founded on the literature documentation of past scientific experiments and the description of the methods used. Anecdotal evidence also expresses that a scientist can learn much through an experiment that does not give an expected result or one which inherently fails. Any scientist who spends time in a lab soon realizes that methods are often inadequate and experiments do not work due to this lack in method. Lab science is grueling and arduous and most

experiments either fail or produce erroneous data. This is strikingly different from the picture of science a layperson may create after reading out of a scientific textbook or completing in an introductory science class. It is important to distinguish this complication in the understanding of science. Scientific textbooks could then be explained as being a photograph, which documents the current knowledge present. The knowledge collected in scientific textbooks is simply a collection of probable scientific conclusions which are existent at the given time the book is written.

Thus, in conclusion, science is a society that is dedicated to the continuation of experimentation investigating the physical universe. This production of knowledge is slow and requires the constant physical battle between natural processes and the methods utilized by scientists. Science is limited by human perception. Due to human limitation, experiments often fail. Scientific method is documented to both validate and progress the endless line of experimentation which will flow from one generation to the next. This form of inquiry aims at suppressing random chaos from an endless supply of variables in order to indentify some reproducible matter of fact.

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