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Holistic Learning: Seeking a Purposeful Life By Engaging Science and the Humanities

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HOLISTIC LEARNING: SEEKING A PURPOSEFUL LIFE BY ENGAGING SCIENCE AND THE HUMANITIES

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by

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SCIENCE AND THE HUMANITIES

Throughout the research of *Escherichia coli* alkaline phosphatase (AP), I have engaged in personal reflection about the connections between a holistic education combining science and Humanities and the notion of finding purpose in life, which are essential components in developing my thesis. Alkaline phosphatase is a ubiquitous enzyme that serves the advancement of medicinal and technological purposes. By producing a mutant alkaline phosphatase enzyme, more knowledge could be acquired about the kinetic necessity of amino acids located in the active site. Conclusive data was obtained regarding influence of the mutation on the kinetic performance of the enzyme, however our scientific inquiry is only a small contribution to the vast breadth of knowledge about alkaline phosphatase. Mutating the arginine amino acid to a histidine, does not provide concrete knowledge about the whole enzyme, and it does not confirm information about the whole active site because there are many other structural features that have an influence to consider. In essence, research begins as a focused inquiry that leads to answers and then progresses to further questions. Engaging in scientific research that is not cookbook science, or completely planned, provides an opportunity to explore a critical way of thinking and develops an acceptance of uncertainty and an appreciation for the mystery of life. “The scientific mind fundamentally changes our expectations about what constitutes a meaningful life and how it can be obtained” (Ford, 2007, p. 81).

Science has the potential to influence a person to be critical of their sources and have the confidence to challenge everything they learn, for the purpose of being an interactive learner, and bring their sources into conversation with one another. From discussion-based science curriculum, students come to consider information learned as knowledge contributing to an always developing whole picture rather than a concrete final answer.

However, people often fall for the illusion that science has the profound ability to discover concrete final answers about the material world, and this is due in part to our science education that informs students about science by compartmentalizing the material world into distinct parts and encouraging students to memorize information. Prior to graduate-level education, science is often presented as tried and true knowledge, written in a textbook, and to be mastered through memorization. This form of learning leaves little room for students to ask "why" or see the application behind what they are learning. "The problem is to understand the whole; not just the parts but also their interactions need to be understood. And these interactions are not contained in the parts themselves" (Sheldrake, 2012, p146). Throughout our biochemistry research with alkaline phosphastase, we were exploring unknowns. We did not know how the enzyme would respond to our mutation or if the *E. coli* cell would transform with the mutant DNA. After our cells died three times rather than growing, we truly had to keep our sights set on the whole purpose of our research, and also continue to participate in discussion about why our results were not what we expected. We were continually

learning to ask why, learning to engage what we did not know, and trying to contribute possible answers to the frustrating results. It was essential to realize that although our research provides only a small contribution to the plethora of knowledge and research about alkaline phosphatase, every aspect of knowledge is important to understanding the whole enzyme. We supported our hypothesis that mutating Arg166 to His166 will result in similar stabilization of the enzyme, even though the kinetics will likely be altered, since Arg166 is a highly conserved residue in the alkaline phosphatase enzymes of all organisms. We also learned about the activity of alkaline phosphatase, which is valuable information for medical and technological purposes.

Reflecting on my research experience, I acknowledge the contribution of having an autonomous laboratory experience in igniting my scientific imagination and expanding my view of the science. I also recognize the role of the humanities core curricula, which exposed me to philosophy, religion, art, and literature, in developing my ability to think outside the framework of the text to develop my own perspective and ideas about what the authors are saying. These two aspects of my education fostered an appreciation for the dialogue, or "an exchange of ideas or opinions, a joint exploration" in the humanities courses in developing a more critical yet open, scientific mindset (Sheldrake, 2012, p. 330). Regis provides a holistic education dedicated to development of the whole person and integrates science and humanities courses. Through a discussion engaging scientific experience and exposure to the humanities dialogue, I witnessed the interconnectedness of the two

different ways of thinking and considered the contribution of both essential in discerning purpose. Therefore the working thesis for this paper is that blending science and the humanities core curriculum enhances holistic learning because it encourages the metaphysical and material dialogue in seeking a purposeful life.

INTRODUCTION REFERENCES

Ford, D. (2007). *The Search for Meaning: A Short History* (p. 81). Las Angeles, CA: University of California Press.

Sheldrake , R. (2012). *The Science Delusion: Feeling the Spirit of Enquiry.* (p. 26-330). Coronet.

MUTATION OF ACTIVE SITE RESIDUE ARGININE166 TO HISTIDINE166: ANALYSIS OF THE CATALYTIC IMPACT OF MUTATION ON *ESCHERICHIA COLI* ALKLAINE PHOSPHATASE

A single mutation, Arg166 to His166, was introduced into *Escherichia coli* Alkaline Phosphatase (AP) to observe the kinetic implications. AP is a phosphomonoesterase enzyme, and the Arg166 is a highly conserved AP residue essential for placement of phosphate substrate into the active site. The histidine-generated mutation was selected, because histidine also has a delocalized positive charge and similar hydrogen bonding capabilities to arginine. Comparing our data for k_{cat} and K_m values of the wild type enzyme, $0.0509 s^{-1}$ and 82.027μM, to those of the Arg166His mutant enzyme, $5.39 \times 10^{-5}\ s^{-1}$ and 1596.1μM demonstrates the enzyme's lowered affinity for the substrate. Based on the comparison of the catalytic efficiency of the wild type enzyme, $6.2 \times 10^{-4} s^{-1} \mu M^{-1}$, to the mutant enzyme, $3.38 \times 10^{-8} s^{-1} \mu M^{-1}$, the mutation resulted in about a 20,000-fold decrease in catalytic efficiency of the enzyme. Results were expected to show some alteration of the kinetic properties of the enzyme, but not in such significant magnitude. The data illustrates destabilization of the enzyme's hydrogen bonding arrangement and ability to release substrate efficiently, which are changes that have not occurred in previous studies when Arg166 was mutated to neutral residues (Butler-Ransohoff et al, 1988). Further studies, with more qualifying data, regarding the Arg166 residue are needed to explicate its role in Alkaline Phosphatase activity.

ALKALINE PHOSPHATASE RESEARCH INTRODUCTION

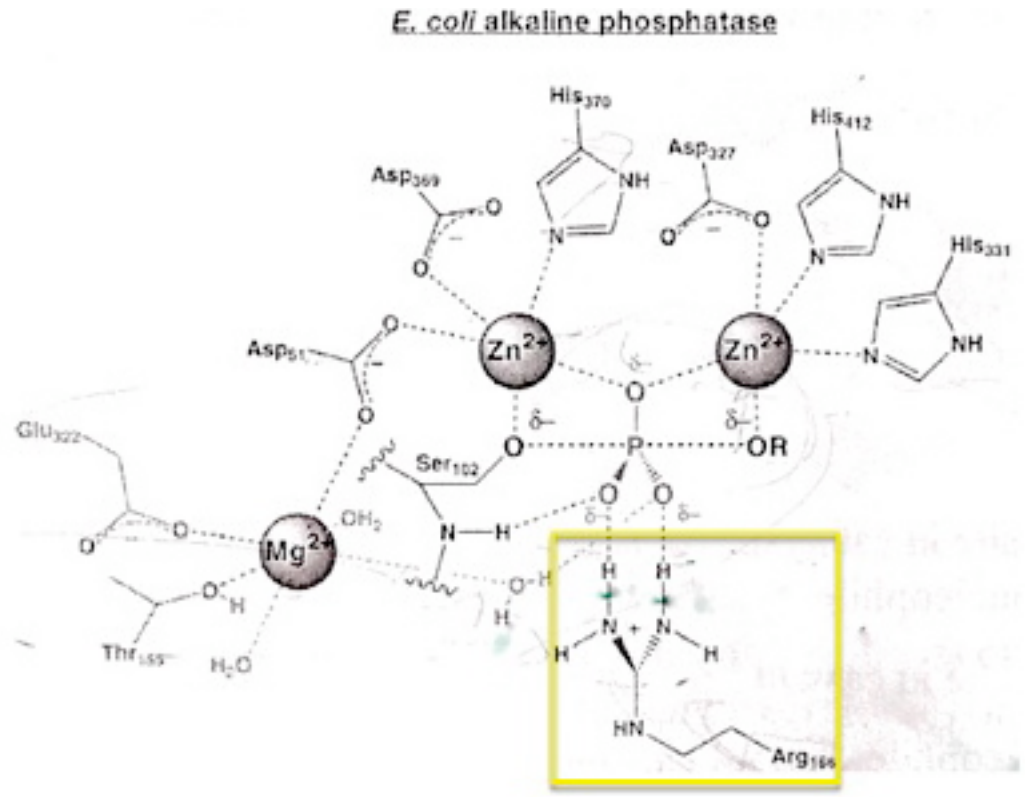
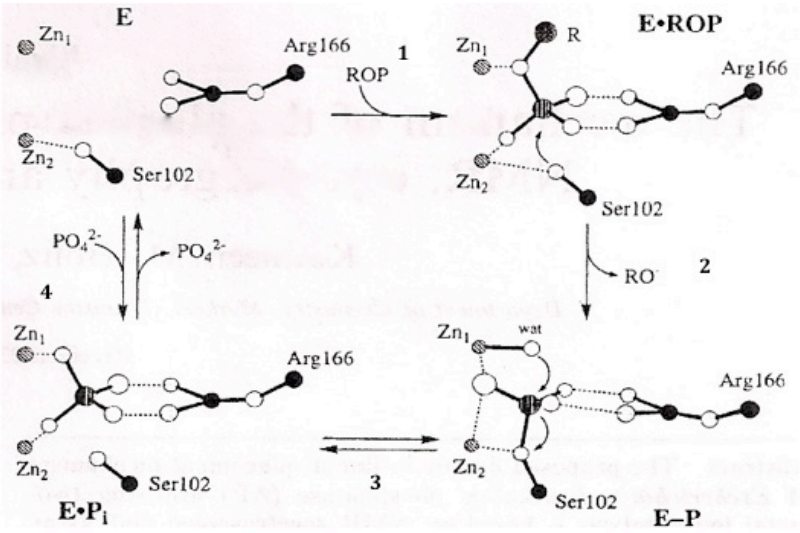


Figure 1: Schematic depiction of *E. coli* Alkaline Phosphatase (AP) Active Site. Figure from Zalatan et al, 2008. Highlighted region is the residue of interest mutated to a histidine residue.

Alkaline phosphatase (AP) is a ubiquitous enzyme that primarily serves to provide free inorganic phosphate via mechanisms of dephosphorylation and transfer of phosphoryl groups (Chaidaroglou et al, 1988). *Escherichia coli* AP is located in the cellular periplasm (Kikuchi et al, 1981) where it dimerizes and each monomer, composed of 449 amino acids, chelates to two zinc atoms and one magnesium atom (Stec et al, 1988). The mammalian and bacterial alkaline phosphatases are approximately 30% similar in sequence (Sun et al, 1999), with

two notable variations in the active site positions of 153 and 328 (aspartate and lysine in *E. coli*;, histidine in mammalian AP) (Murphy & Kantrowitz, 1994). Other conserved residues in the active site include aspartate-91, serine-92 (Holtz et al, 2000), and arginine-166 (Ma & Kantrowitz, 1996). Arg-166 and Zn^{2+} directly interact with the substrate to orientate the phosphate into the active site region (Holtz et al, 2000). Figure 1 shows the design of the *E. coli* AP active site with the location of Arg-166 highlighted. In each active site, two histidines and a serine residue (Figure 1) are also essential for enzyme catalytic activity (Holtz & Kantrowitz, 2000).

The reactions catalyzed by this phophomonoesterase enzyme (Kim & Wyckoff, 1991) include the non-specific hydrolysis of phosphate esters (Sun et al, 1999), transfer of phosphates to water via hydrolysis, and transfer of a phosphate to amino alcohols via transphophorylation (mechanism shown in Figure 2) (Kikuchi et al, 1981). The guanidinium group of Arginine-166 communicates with two hydrogen bonds providing stabilization of incoming phosphate groups (P_i) (Butler-Ransohoff et al, 1988), charged intermediates, and pentacoordinate transition states within the phosphoryl transfer mechanism (O'Brien & Herschlag, 2002). The ability to catalyze the characteristic reactions of the alkaline phosphatase enzyme is altered but not completely diminished upon the loss of the stabilizing Arg166 residue (Figure 1) (O'Brien et al, 2008).



Zn	Zinc atoms
Arg166	Argenine 166 position
Ser102	Serine 102 position
E	Native Enzyme AP (Alkaline Phosphatase)
ROP	Substrate Phosphate Monoester
E-ROP	Enzyme Substrate Complex
RO	Ester Oxygen
E-P	Enzyme Covalent Intermediate with Phosphate
E-P_i	Enzyme Non-Covalent Complex
PO_4^{2-}	Phosphate in Rate Limiting Step

Figure 2. Mechanism illustrating the non-specific hydrolysis of phosphate esters. Step 1: the oxygen of the ROP substrate interacts with Zn_1, and Zn_2 coordinates with an additional oxygen in ROP as well as the hydroxyl part of the Ser102. Concurrently, two other oxygens flanking the phosphorous atom in the substrate interact with the guanidinium group of Arg166, in order to complete the formation of E-ROP. Step 2: The nucleophilic attack by the hydroxyl group in the Ser102 on the phosphoruous atam causes the R group to depart leaving Zn_1 open to bind and activate a water molecule. Note that both Zn_1 and Zn_2 are coordinated to the same oxygen now, and that the phosphate is drawn down further into the active site. Step 3 (Reversible): A nucleophilic attack on the phosphorous atom by the activated water molecule results in the release of the Ser102 and substrate interaction, in addition to causing a transition from the covalent E-P to non-covalent E-P_i form of the complex. Step 4 (Reversible): The dissociation of the phosphate group is rate limiting and results in renewal of the native enzyme structure (Figure and Data adapted from (5).

A mutation of the Arg166 residue to a neutral amino acid has been found to cause a hundred fold increase in inhibition of the enzyme (Kikuchi et al, 1981), most likely due to changes in phosphate hydrogen-bonding in the active site (Kim & Wyckoff, 1991). Replacement of the neutral residue at 166 with a histidine may restore enzyme activity, given arginine and histidine's similar hydrogen-bonding capabilities (Cruz-Ramos et al, 2004). Furthermore, arginine residues are found at position 166 in mammalian AP and histidine residues coordinate with zinc metal ions in the active site (Figure 1) (Ma & Kantrowitz, 1996). Histidines are highly pH specific residues (Murphy & Kantrowitz, 1994) and a mutation of 2.4 of the histidine residues in a subunit, as determined stoichiometrically, results in loss of activity in the enzyme (Ding et al, 2002). We hypothesize that mutating Arg166 to His166 will

result in similar stabilization of the enzyme, even though the kinetics will likely be altered, since Arg166 is a highly conserved residue in the alkaline phosphatase enzymes of all organisms.

In addition to providing data for kinetic inquiry, studying *E. coli* alkaline phosphatases may contribute to clinical practice, due to the relationship between increased blood activity of alkaline phosphatases in the presence of skeletal and liver diseases (Koncki et al, 2006). Levels of alkaline phosphatase are also monitored for skeletal related physiological and pathological disturbances that lead to a fatal disease, such as hypophosphatasia (Murphy & Kantrowitz, 1994). Beyond health care implications, alkaline phosphatase is economical and efficient, thus making it a popular immunoreagent and marker for genosensing devices (Koncki et al, 2006). Knowing more about the inhibition and structural mechanism impacting the enzyme's transition state can provide more insight for health care technology and knowledge about the enzyme (Koncki et al, 2006).

MATERIALS & METHODS

Bioinformatics

The DNA sequence of interest (EG10727, *E. coli* AP) was researched through ExPASy (http://www.expasy.ch) and Protein Data Bank (PDB) (http://www.rcsb.org). A mutation site was selected where Arginine-166 residue was mutated to a Histidine.

Primer Design

Mutagenic primers were designed following guidelines from the QuikChange Lighting Site-Directed Mutagenesis (Agilent Technologies). Integrated DNA Technologies provided the following information (mutations are highlighted):

DNA Sequence:

Sense 5' - G GCA CAT GTG ACC TCG C**GC** AAA TGC TAC GGT CCG AG - 3'

Antisense 5' - C CGT GTA CAC TGG AGC G**CG** TTT AATG CCA GGC TC - 3'

Primer Sequence:

Sense 5' - G GCA CAT GTG ACC TCG C**AT** AAA TGC TAC GGT CCG AG - 3'

Tm (50mM NaCl): 68.5°C

GC Content: 56.7%

Molecular Weight: 11,359.4

Antisense 5' - C CGT GTA CAC TGG AGC G**TA** TTT ACG ATG CCA GGC TC - 3'

Tm (50mM NaCl): 68.5°C

GC Content: 56.7%

Molecular Weight: 11,381.4

Mutant DNA Amplification

The mutant AP DNA was amplified in a pET21c(+) plasmid (New England Biolabs) with the phoA insert located between the restriction sites, Nde1 and Xho1. Following QuickChange Lightning Site-Directed Mutagenesis Kit protocol (Agilent Technologies), the plasmid was amplified using the following conditions: 1 µL of oligo primer 10 ng/µL and 3.5 µL of 20 ng/µL dsDNA were the conditions used in the16 cycles of PCR. The product was then treated with Dpn 1 endonuclease to select for the mutant DNA, by consuming the parental strand, as described in the QuikChange protocol.

Cell Transformation and DNA Midi-Prep

Novablue (DE3) (EMD Scientific) cells were transformed using 1 µl and 2 µl of DNA generated via PCR and added to 20 µl of cells. These cells were incubated on ice for 10 minutes, heated at 42°C for 30 seconds, followed by 2 minutes on ice. 80 µl of room temperature SOC media was added and transformations were plated on LB-Ampicilin agar plates (40 µl/mL) and incubated overnight at 37°C. Single colonies were picked and used to inoculate 5 mL of LB containing 80 µg/mL of Ampicilin, which were then incubated for eight hours at 37°C. From these samples, we inoculated a large scale, 30 mL culture (50µg/mL) incubated overnight at 37°C. These cells were used for purification and evaluation of the plasmid DNA. 850 µL of the cells were added to 50 µL of glycerol then frozen at -70°C.

DNA was purified using the Qiagen Mini-prep kit (Alameda, CA), generating 0.265 mg/mL mutant DNA. The midi-prep DNA was then stored at -20°C. Samples of the original transformed dsDNA and the purified DNA were examined using restriction digests Nde1 and Xho1 (New England Biolabs, MA) and in gel electrophoresis (1% Agarose, 0.5M EDTA, acetic acid, 10 mg/mL ethidium bromide, and TAE buffer) for one hour at 150 V. Samples were sent to Functional Biosciences (Madison, Wisconsin) for sequencing.

Mature Protein Expression

Origami-BL21 (DE3) cells were transformed with mutant DNA and plated as described above (Amp 40µg/mL). A small 7mL culture of LB broth, containing 80µg/mL of Ampicillin , was inoculated with a single colony and grown overnight at 37°C followed by inoculation of a large scale culture containing 200 mL LB broth and 80µg/mL Ampicillin. This culture was grown for 5 hours at 37°C until an A600 of 0.572. IPTG (1 mM) was added to induce protein expression. 2.5 hours later, the cells were harvested by centrifuging at 10,000 rpm for 30 minutes then stored at -70°C.

The cells were resuspended in 25 mL resuspension buffer (50mM Tris-Cl pH 8.0, 10mM EDTA, 5mM mercaptoethanol, 20% w/v sucrose) and frozen in a dry ice/ethanol bath followed by a 37 °C water bath to thaw. 1mL lysozyme (4mg/mL in 100mM Tris pH 8.0, 5mM EDTA; Sigma-Aldrich), was then added to the cells for 15 minutes. Following incubation, freeze lysate at -20°C. The ammonium sulfate (AS) precipitation was initiated with addition of 0.5 mL of DNase I (1mg/mL in 0.5 $CaCl_2$), with the first cut

containing 25% (18g/mL AS) and the second cut having 90% (59.5 g/mL). Samples were centrifuged after each cut 15,000 rpm for 30 minutes. The supernatant was collected for the 25% cut and the pellet in the 90% cut. The pellet was resuspended in IEX application buffer (10mM Tris pH 7.4, 5mM $MgCl_2$), and dialyzed against the IEX buffer (6mm diameter, 12-14 kDa cut-off, SpectrumLabs.com) at 4°C.

DEAE Sephacel (GE Healthcare, 1.8 cm x 2.4 cm, volume of 6.1 cm^3) was equilibrated with 60 mL IEX buffer. The sample was loaded and washed with three volumes IEX application buffer, followed by elution over a gradient 0 to 125 mM NaCl (60 mL total volume), and a final high-salt wash (125 mM NaCl in IEX buffer). Protein elution was monitored via absorbance at 280 nm. The protein eluted over a NaCl concentration range of 99.96mM to 110.67 mM.

Protein Concentration and Analysis

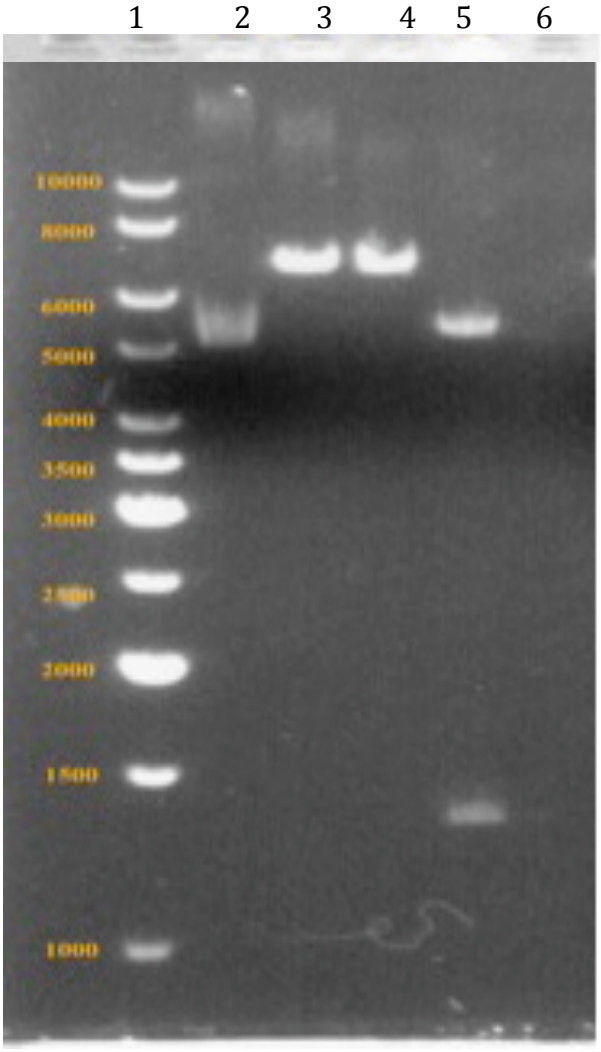
The fractions containing eluted AP were determined using a BSA standard curve and a Bradford Assay (Bradford, 1976). Absorbance readings of serial dilutions of BSA and 1:5 dilutions of Coomassie Brilliant Blue (Bradford Reagent) were taken at A_{595} to create the standard curve. Activity of our protein was examined using p-nitrophenyl phosphate (PNPP), which is hydrolyzed to p-nitrophenol (PNP) and can be observed 410nm. Column elution samples of 100µL of each fraction (in 1.4 mL 0.2M Tris pH 8.0 and 1.5 mL 1mM PNPP, quenched with 0.1 mL 10mM NaOH after three minutes) were analyzed at Abs_{410}. From the plot of the Bradford and activity assay data, fractions containing the most AP were determined. These fractions were pooled together, and then dialyzed in

Tris buffer and 50% glycerol for 48 hours. The dialyzed sample concentration was 0.0054mg/mL.

Kinetics

Michaelis Menten Kinetics plots were derived from PNPP assays with varying conditions including 100µL of protein (0.0054mg/mL) added to concentrations of PNPP and 0.2 M Tris buffer (pH8). Michaelis-Menten and Line-Weaver Burk data were used to determine V_{max} and $K_{M.}$

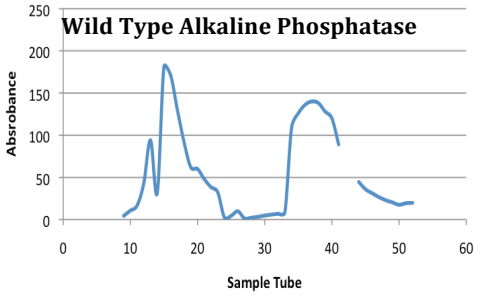
RESULTS



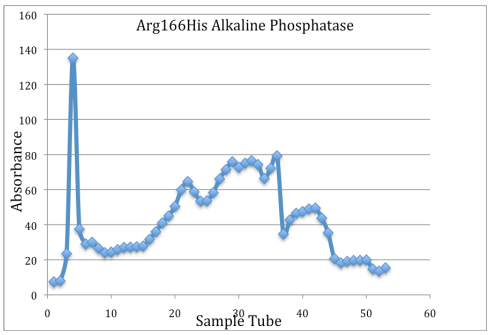
Lane	Content
1	1kb Ladder
2	Uncut
3	Xho1
4	Nde 1
5	Double Cut

Figure 3: Electrophoresis of uncut mutant plasmid and Xho 1 and Nde 1 restriction enzyme mutant plasmid digests.

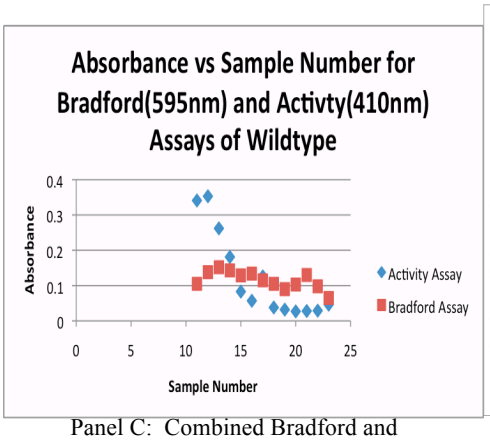
As expected, the size of uncut plasmid was 6000 bp and the size of the cut plasmid was 6500bp. The double cut with Xho1 and Nde1 showed the phoA insert at 1400bp and the remaining plasmid at 6000 bp.



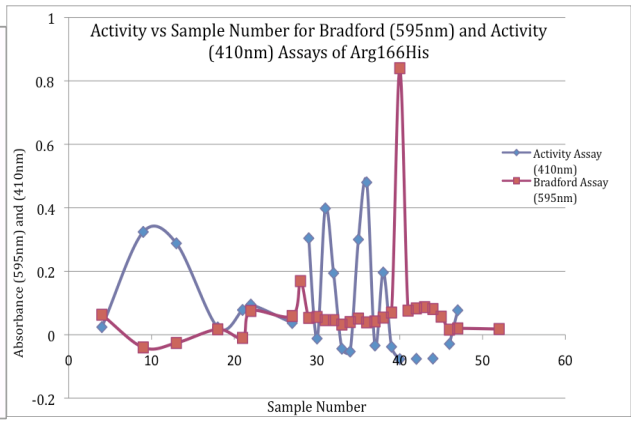
Panel A: A_{280} vs. Fractional Collector Sample Tube Number from anion exchange column procedure for the wild type AP.



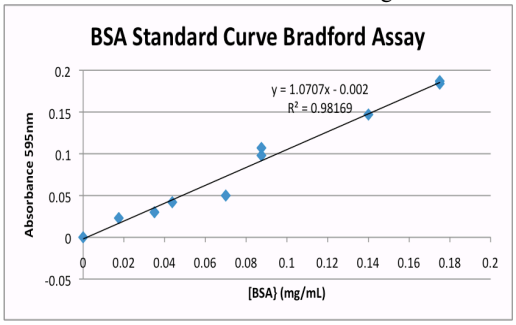
Panel B: A_{280} vs. Fractional Collector Sample Tube Number from anion exchange column procedure for the Arg166His mutant AP.



Panel C: Combined Bradford and Activity Assay plots for the wild type AP Enzyme.



Panel D: Combined Bradford Assay and Activity Assay plots for the mutant Arg166His AP Enzyme.



Panel E. BSA standard curve using Bradford Assay with Absorbance at 595 nm.

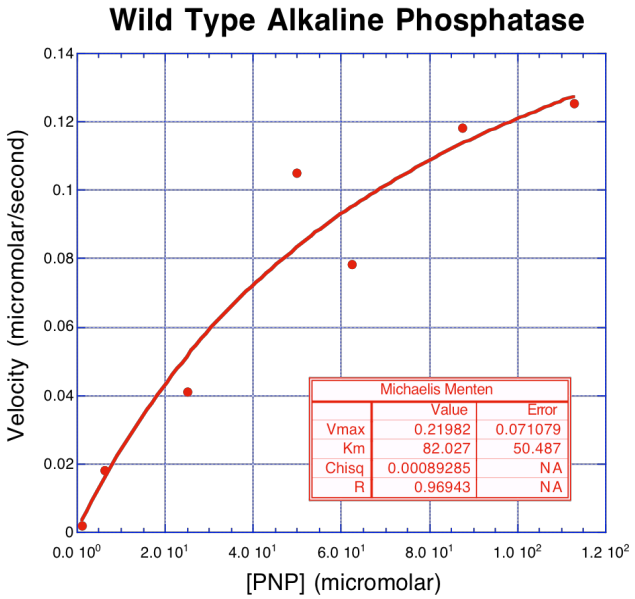
Figure 4: Plotted data from Wild Type and Mutant *E.coli* Alkaline Phosphatase Activity and Bradford Assays and the BSA standard Curve utilized for comparison.

The first two peaks in Figure 4: Panel A are a result of other proteins eluting off the column, and the third peak is the location corresponding to AP elution, which is about sample tube 38. The calculated NaCl elution for the wild type enzyme was in the range of 47 mM – 77 mM. In Figure 4: Panel B Most of the peaks correspond to the proteins, other than AP, dissociating from the anion exchange column. Fraction samples within the range of 35-38 contained the AP enzyme, as determined by PNPP cleavage activity, which eluted over a NaCl concentration range of 99.96mM – 110.67 mM.

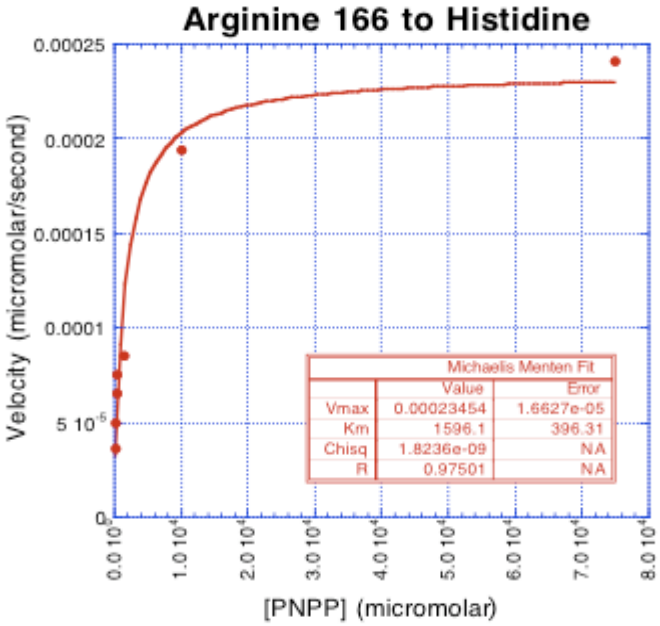
The Bradford Assay provided information about the protein concentration in each sample, and the activity assay produced data regarding the activity of AP in the presence of substrate p-nitrophenyl phosphatate (PNPP). The Activity Assay shows a peak in sample numbers 11-13, but the Bradford Assay did not provide any significant analysis (Figure 4: Panel C).

From the overlapping data in Figure 4: Panel D, it was hard to determine where the mutant protein concentration was the greatest corresponding to the activity of the AP protein in the presence of substrate p-nitrophenyl phosphatate (PNPP). Thus, samples 7-14, 28-31, 19-25, and 35-38 were pooled and dialyzed.

The line equation from the standard curve (Figure 4: Panel E) was used to calculate the concentration of the mutant Alkaline Phosphatase enzyme contained in the pooled elution fractions 4.35μM wild type enzyme and the calculated 1.49μM H166 mutant.



Panel A: Michaelis Menten Plot for wild type Alkaline Phosphatase Enzyme kinetics.



Panel B: Michaelis Menten plot for Arg166His mutant Alkaline Phosphatase Enzyme kinetics.

Figure 5: Michaelis Menten Plots for Wild Type and Mutant *E. coli* Alkaline Phosphatase Enzyme Kinetics.

The Michalelis Menten plot (Figure 10) illustrates the relationship between the substrate (PNPP) and the reaction velocity as it approaches V_{max}. The V_{max}, 0.220 μM/s, from the graph and the $[E]_t$ calculated from the BSA Standard Curve were applied to calculate the k_{cat} for the wild type enzyme $0.509s^{-1}$ (Table 1). The K_m was 82.027μM. The overall catalytic efficiency was $6.21x10^{-4}\mu M^{-1}s^{-1}$. Figure 5: Panel B plot represents the relationship between the substrate (PNPP) and the reaction velocity as it approaches V_{max}, 0.000235 μMs^{-1}, the k_{cat}, $5.39x\ 10^{-5}\ s^{-1}$, using the V_{max} for the Arg166His mutant *E. coli* AP, and the $[E]_t$ calculated from the BSA Standard Curve. The K_m was determined to be 1596.1μM, and the overall catalytic efficiency $3.38x10^{-8}\mu M^{-1}s^{-1}$.

Table 1: Kinetic Data for wild type and Arg166His Alkaline Phosphatase

Sample	**[Enzyme]**	**K_m**	**V_{max}**	**k_{cat}**	**K_{cat}/K_M**
Wild Type	4.32μM	82.027μM	0.220 μM/s	$0.509s^{-1}$	$6.21x10^{-4}\mu M^{-1}s^{-1}$
Arg166His	4.35μM	1596.1μM	0.000235 μMs^{-1}	$5.39x\ 10^{-5}\ s^{-1}$	$3.38x10^{-8}\mu M^{-1}s^{-1}$

Table 1 summarizes the data from Figure 10 and Figure 11, which provided data for the V_{max}, used to calculate k_{cat}, and the K_m needed to calculate the k_{cat} / K_m. The enzyme concentrations, $[E]_t$, calculated with the BSA standard curve line equations are also included.

DISCUSSION

We hypothesized that the Arg166His *E. coli* Alkaline Phosphatase mutant would maintain similar active site stabilization as the wild type enzyme, since replacing a neutral amino acid mutation with a histidine mutation has been shown to restore AP activity (Cruz-Ramos et al, 2004). However, we also purposed that the kinetics would have slightly altered catalytic efficiency and lowered binding affinity for the substrate and less efficient product release after phosphate cleavage, resulting from more complex electrostatic interactions between the phosphate and histidine residue in the active site. Based on the kinetic data from Table 1, the catalytic efficiency, k_{cat}, and $k_{cat}/K_{,m,}$ for the mutant Alkaline Phosphatase, 5.39x 10^{-5} s^{-1} and $3.38x10^{-8}\mu M^{-1}s^{-1}$, is significantly lower in comparison to the wild type Alkiline Phosphatase data for k_{cat}, and $k_{cat}/K_{,m}$, 0.509s^{-1} and $6.21x10^{-4}\mu M^{-1}s^{-1}$, which supports our hypothesis regarding the lowered catalytic efficiency and binding affinity. We did not expect the difference to be of such great magnitude, since we expected the active site with His166 to have relatively similar hydrogen bonding capabilities and product affinity as the Arg166 environment due to similarities in the electrostatics of the amino acids. Our results are most likely due to displacement of the hydrogen-bonding scaffold provided by Arginine's guanidinium group, which uses a positively charged side chain offering two hydrogen bonds to place the phosphate in the active site during hydrolysis of the substrate. Furthermore, Asp101, which positions the guanidinium group of Arg166, side

chains of neighboring residues, and the substrate through a hydrogen bond network around the active site, may not be able to associate with histidine to position the His166 residue in preparation of hydrolyzing the phosphate (Kim & Wyckoff, 1991; Ma & Kantrowitz, Murphy& Kantrowitz, 1994). Thus the ability of the product to be bound and released is not as efficient in the Arg166His mutant. Based on the change in catalytic efficiency and also the behavior of our protein, the decreased affinity for substrate is consistent with our data in Table 1, where the mutant AP had a K_M of 1596.1μM, where as the wild type 82.027μM.

Further support for the lowered catalytic efficiency of our mutant in comparison to the wild type enzyme was provided by a study by Chaidaroglou et al, which produced an Arg166Ala mutant. The wild type k_{cat} / K_m was $1.8x10^6 M^{-1}s^{-1}$ and the mutant was only decreased to $5.8x10^4 M^{-1}s^{-1}$ (Chaidaroglou et al, 1988). Thus, changing Arg166 to a neutral residue had only a small impact on the overall kinetics of the enzyme, and the positive charge at the 166 location is not essential for the catalytic activity (Chaidaroglou et al 1988). This gave us grounds to purpose that our Arg166His mutant, involving an already positively charged Arg166 residue was mutated to another positively charged residue, would result in only a small decrease of catalytic efficiency. However, the k_{cat}/K_m four our mutant, $3.38x10^{-8}\mu M^{-1}s^{-1}$ was much less than the k_{cat}/K_m for the wild type $6.21x10^{-4}\mu M^{-1}s^{-1}$ (Table 1). Histidine's imidazole ring has a delocalized positive charge similar to the arginine guandinium group, but the hydrogen bonding is different due to presence of the ring structure, and the availability of the dynamics of the

nitrogens. Thus, the process of binding to and positioning the substrate may be more strained. It is also possible that the His166 residue may interact more strongly with other histidines surrounding the active site. The slower turnover process results in a lowered infinity for the substrate, because the active site is occupied longer. It is evident that the positive charge on the Arg166 residue is not the most critical factor, because otherwise the k_{cat} for the Arg166Ala would have been wiped out (Chaidaroglou et al, 1988). In order to determine why there was such a significant change in the catalytic efficiency of our mutant versus the Arg166Ala mutant, I would need to do further analysis of the Chaidaroglou et al study and reproduce their results

Throughout the transformation procedures, we noticed that our cells were very sick, and we were only able to get a few colonies to grow. Even then, they were rather small. Another indication that our protein was going to have altered efficiency in comparison to the wild type was during the anion exchange column procedure, our protein eluted off at a much higher NaCl concentration (99.96mM – 110.67 mM) than the wild type, (45mM -60mM). Thus our protein must have been able to remain on the column in the bound state longer, so future kinetics were suspected to show lower catalytic efficiency. Since both arginine and histidine have a delocalized positive charge, the difference in their structure that influences the catalytic efficiency must be a result of added steric hindrance of the imidizole ring as opposed to the guanidinium side chain (Butler-Ranshoff et al, 1988). Substitutions of the Arg166 residue tend to result in a drastic increase of the enzyme's K_m, which is likely do to the loss of the Arg166 side

chain that is imperative for positioning of the phosphate in preparation of serine's nucleophilic attack during phosphorylation (Hoylaerts et al, 1992).

Our results may have been impacted by the span of several weeks our study proceeded over, because normally protein studies are done within as few of days as possible. A shorter time of study limits the time that the kinetic activity of the purified protein can become altered. The protein may denature over time, which would impact enzyme concentration, thus influencing kinetic calculations. In addition to shorter time, the kinetic results would have benefited by the analysis of a more concentrations of [PNPP], as well as the addition of stopped flow analysis to allow for more high velocity studies. Adding zinc to the activity assays may have allowed for the activity assays to be more conclusive, and efficient to read, since zinc is an important phosphate coordinating metal in the AP active sight. In the case of our protein, zinc coordinates the phosphate with Arginine-166, so this would be interesting addition to our study. Future studies could also include x-ray crystallography to gain more insight into mutant protein structural information. Circular dichroism (CD) could also be used to assess the mutant structure.

CONCLUSIONS

Further studies and data are needed to apply the knowledge gained from this mutagenesis procedure, as well as to derive conclusive data regarding the role of the arginine residue in AP, and other purposes of Alkaline Phosphatase both medically and in industry. An interesting study would be to produce a double mutant Ser102Arg and Arg166Ser and then perform kinetic studies. An addition to the current study would be to create a Arg166Lys to analyze kinetic data for another basic amino acid mutant.

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CURA PERSONALIS & *MAGIS*: THE ROLE OF A HOLISTIC EDUCATION

From my experience with a Jesuit liberal arts education, I have developed an appreciation for the role of philosophical dialogue in gaining a more critical understanding of a scientific education. “It is safe to say that Jesuit pedagogy is not as concerned with a school to work model (get an education and get a job) as it is with impacting the whole person. *Cura Personalis* (care of the whole person) is a theme that runs through every part of Jesuit life. It is educating from the inside out, teaching what it really means to be human“ (Kappus and Jenks, 2010, p. 738). Emphasis on development of the whole person is incorporated into the liberal arts education by requiring students to take courses related to their major, as well as courses directly unrelated to their major. For example, science majors take humanities courses that appeal to new ways of thinking and provide an introduction to alternative opinions to enhance their science education. Furthermore, incorporating a service-learning component of education provides experience with real-life application and critical thinking about the interconnectedness of education and the environment, and more importantly each human being to each other. In addition to *Cura Personalis,* the Jesuit liberal arts education incorporates the ideal of seeking *Magis*. *Magis* pertains to “that which makes us desire and choose only those things that will lead us more toward the end for which we were created” (Kappus and Jenks, 2010, p. 732). Incorporating this ideology into an education is meant to influence a person to consider how to obtain more from life by

having the greatest possible influence working with others to have a resourceful community and by finding purpose in discerning "the end for which human beings are created: the full realization of justice and human dignity" (Father Bart Gregor, Personal Communication, September 14, 2011). In essence, rather than focusing on knowledge learned alone, a liberal arts education is a holistic education concerned with expanding a student's worldview and helping discern to become more fully human through their efforts. "Holistic pedagogy concerns the development of the whole student and acknowledges the cognitive, social, moral, emotional and spiritual dimensions of education" (Tirri, 2009, p. 159). A holistic education influences how a person will approach life by emphasizing the necessity of integrating a balance of community, life learning, and reflection in discerning a purposeful life, and "tying the content of instruction to the student's emotions and personal experience" (Kappus and Jenks, 2010, p. 738). Not only is a student encouraged to reach their potential by learning a wealth of diverse information, but also to emphasize development of an individual, who is actively learning and critically challenging sources of knowledge. By challenging the knowledge learned, students must bring sources into conversation in order to develop thoughts and opinions with the support of key voices of research, professors, colleagues, and personal reflection.

A holistic education presents students with the need not only to ask "how", but also "why" life proceeds as it does and emphasizes the necessity of being present in all things, by centralizing on the ideals of *Magis* and *Cura Personalis.* Being present not only

refers to your location, but also concentrating on having your thoughts and actions be connected with the moment. In order to seek more from life and care for the whole person, a student learns to be cognizant and engaged in his/her surroundings and discern purpose by integrating different perspectives, sources of knowledge, and curiosity.

It is imperative to recognize that people will each discern purpose in different ways. Often purpose can be fully recognized when we experience a "pattern interrupt" in our present journey. When, we are forced to step back and question "why" and begin appreciating life rather than going about in automatic mode following a cultural pattern from day to day, we may develop awareness of the people, places, and educational influences that impact our perceptiveness. I experienced the so-called "pattern interrupt" in the biochemistry lab, when I was required to engage the material rather than merely memorizing what I was learning. I had to become comfortable with the unexpected outcomes in the lab, such as the death of our *E. coli* cells, and view the uncertainty as an opportunity to analyze, ask questions, and make suggestions. It was at these points in my education, that I began to ask "why" was I in the lab, and how does this contribute to finding purpose? I also began to recognize how imperative dialogue similar to my humanities classes could benefit the scientific learning approach. I began to see that similar to my discussion based humanities courses, science could also be approached as an opportunity to exchange thoughts and ideas. All contributions to this conversation contribute to enhancing exploration through science and stimulating the

imagination. Each person is an integral component to the learning process and uncertainty is an opportunity rather than a hindrance. This momentarily improved consciousness allowed me to become aware of where I was on my personal journey, and how interactions with people, places, and the books I was reading were intricately interconnected and impacting my perceptions. The holistic education contributes to this enhanced presence, by unveiling the interconnectedness of science, religion, art, and other disciplines.

REFLECTION: INTEGRATING SCIENCE AND THE HUMANITIES

We live in the age of technology; therefore science is very much a part of our everyday reality and influences our worldview. “The materials of science are the materials of life itself. Science is the reality of living, it is the what, the how, and the why in everything in our experience” (Pollack, 2003, p. 5). For example, in healthcare, people depend on knowledge to be able to explicate how the body functions and have answers for how to treat unexpected problems that arise in the human body. From a more literal sense, science investigation is a way of seeking to inquire about the world by asking questions and being observant of all that is happening around us. Science is important because it gives us the confidence to be critical of our sources and drives us to make use of our resources. “Science offers powerful tools for deepening human understanding of the interconnectedness of all life” (Flanagan, 2007, p. 66). There is something inside each of us that strives to obtain more pure understanding and come to terms with uncertainties left to unfold, and this can be satisfied through scientific research. However, to appreciate science, people benefit from viewing science as an open dialogue similar to the interaction of ideas encouraged in humanities classes, such as philosophy. Dialogue incorporates open-minded thought contributions by each person involved in the conversation. It is different from a debate, because the purpose is to enlighten and to enhance the knowledge about the subject, but not to argue or have a winning side. Dialogue welcomes everyone to interact and engage their imagination to

think about things that there may never be final answers to, but each person's thoughts can contribute to the advancement of the ideas and perspectives other people have. I recognized the interconnectedness of all aspects of my education through the discussion format of my biochemistry lab and lecture. The ideal scientific investigation requires you to expand your knowledge by presenting your ideas, whether they are questions or suggestions, where other people can respond to your thoughts. This form of a dialogue is essential to the learning process, as well as demonstrating the relationship between science and the other disciplines. Becoming conscious of this relationship, my appreciation for the holistic education I had embarked upon took a whole new meaning. I recognized the offer to engage the material, people, and experiences through dialogue as the key to discerning my purpose more clearly by requiring me to be present in all things.

Reflecting on my own educational experiences, I recognize a transformation of my personal views and interests based on the presentation of science and my exposure to the liberal arts education. I was privileged to be homeschooled during my elementary and middle school years, which introduced me to a more integrative approach to science, through real life exploration and an interactive curriculum. However, once I entered high school, I quickly became accustomed to the more mechanistic approach to science, and the process of memorizing to regurgitate information later. My interest in science lost fervor as the connection between learning and application diminished. Upon attending Regis, I found the liberal arts education challenging for me because I

was asked to think and engage information I was learning rather than focus on memorizing. The process of holistic learning requires students "to use personal experience and a subjective reflection that acknowledges human excitement, wonder, confusion, and even fear within the strength of a community of experienced leaders and fellow learners (Kappus and Jenks, 2010, p. 742), which can be both enlightening and frustrating. However, a person needs to preparation to be consciously aware of this opportunity and view it as an invitation rather than a burden.

During my freshmen year at Regis, I was introduced to philosophy which presents metaphysical questions, such as considering the role of evil, justice, or the common good, by reading texts that did not have defined answers in the book, rather they were written to stimulate thought and development of opinions to share. Similarly, my honors biology seminar assigned scientific articles to read, as well as excerpts from books by authors who had diverse opinions about the role of scientific dogmas and metaphysical aspects of the scientific world. However, I did not appreciate these experiences, and I considered them unnecessary torture. I was not grasping the concept of the holistic education that exposes students to both the interactive humanities and science courses in order to stimulate questions and dialogue rather than memorization and recall of concrete information. This liberal arts education may be appreciated as an opportunity to have the initiative to think in terms of philosophical and theorizing dialogue to deepen our sense of purpose through clarification of our world picture. Although I had the opportunity to develop my own unique desire for scientific inquiry

during my homeschooling experience, I quickly succumbed to the necessity of the materialistic presentation of science in high school. The inability to recognize this opportunity early on in my college education inhibited me from experiencing the paradigm shift that the holistic education can foster in students. It was not until I took the biochemistry course and was required to engage my science education by coming to class prepared with my own individualized interests, inquiry and discussion contributions, as well as experience a more autonomous project in the lab that I began to awaken the ideals of my education.

The transition was challenging and at first it seemed more stressful than necessary, but as the course continued, I began to appreciate the "more holistic and all encompassing humanities", which actually provided more clarity to the application and presence of science in daily life (Hollenbeck & Reiter, 2004, p. 2). A biochemist, Rupert Sheldrake, who wrote the book "The Science Delusions: Feeling the Spirit of Enquiry", explains his view of science saying,

> "I strongly believe in the importance of the scientific approach. Yet I have become increasingly convinced that the sciences have lost much of their vigor, vitality, and curiosity. Dogmatic ideology, fear-based conformity, and institutional inertia are inhibiting scientific creativity….I believe that the sciences will be more exciting and engaging when they move beyond the dogmas that restrict free enquiry and imprison imaginations" (Sheldrake, 2012, p. 4).

Through the innovative format of my biochemistry course, I was encouraged to come prepared and contribute to discussion rather than come to lecture solely to consume information. I began to correlate the benefit of the integrative

liberal arts education, which exposed me to courses, such as philosophy, that encourage continual dialogue and formulation of questions. The holistic emphasis of my liberal arts education challenged me to be an engaged-learner and devise questions about the authors and textbooks I read by recognizing the imperative concept of perpetual dialogue. In addition, I was asked to analyze the sources and the status quo, which transformed how I look at learning and perceive the role of science and the liberal arts in discerning purpose. Having the opportunity to experience the scientific mindset through research and learning there is more about science than absorbing the facts has allowed science to became more alive to me. I now have the freedom to think beyond the textbook, while also realizing the interdependence of science on other forms of study.

HOLISTIC DIALOGUE: AWAKEN IMAGINATION & INQUIRY

It is possible that most students never fully experience the exploration of their curiosity and/or the ability to think outside the box in their science education, unless they pursue a science program at a liberal arts institution focused on holistic learning or until they reach the graduate level. Science is often introduced to children in elementary school through small projects and amalgamated stories about the history and foundation of scientific knowledge through the lens of the chosen standardized curricula.

> "Rarely, if ever, do students have opportunities to do science in school science. In other words, texts or teachers control decisions about areas of exploration, questions, or problems to solve, methods of data collection, analysis as well as critique and decisions about conclusions from investigations. Even with inquiry-based learning activities, through which students might believe they are freely constructing knowledge, teachers often guide topic choice, methods, conclusions, and dissemination" (Bencze, & Carter, 2011, p. 655)

Teachers are not intentionally trying to limit students from having the opportunity to experience true science rather it is the basis of our pedagogy in the current paradigm that causes science to be presented in a materialistic manner. Through the emphasis on the particular science compendiums, students are introduced to scientific knowledge perceived necessary for them to memorize. Henry Pollack expounds upon the weakness of the typical science education, in his book "Uncertain Science … Uncertain World". He explains that students are only presented with what scientists do know rather than

illuminating the “the unanswered questions that might excite imaginative students and rekindle the natural curiosity they had as young children”(Pollack, 2003, p. 19). The opportunity to develop my own research project with my partner incorporated with the graduate level lecture format, introduced me to the process of asking why and the desire to explore the world of uncertainty with my own imagination. It is only upon reflection of my biochemistry course, that I have truly realized the value of my experience, because at the time it seemed a brutal adjustment from the memorization format I was accustomed and felt secure about in my prior years. Our biochemistry professor reminded us frequently that although her methods seemed extreme her intention was to prepare us for the real world of integrating science into our lives and thinking critically. Pollack mentioned, “by the time students reach graduate school, they have focused far too long on giving answers instead of asking questions. They have a hard time formulating a research project that poses an interesting non-trivial question, and that lays out a path that may shed some light on it” (Pollack, 2003, p. 20). The approach of our biochemistry lab and lecture was an example of a graduate level experience, because we were forced to ask questions, because our research had not been done before. Thus, the process of predicting the impact of mutating an amino acid, arg166 to his166, in the active site of alkaline phosphatase, exemplifies the opportunity to formulate questions uncovering the imagination of the unknown that Pollack was referring too. We had expectations, but we had to embrace the uncertainty and invest in our own questions, while also applying the knowledge of what research and

technique had already been done in the past in order to pioneer our scientific research.

Dawkins (2000) says, “science progresses by correcting its mistakes, and makes no secret of what it still does not understand” (p. 31). He also mentions that you must have knowledge of what you don’t know in order to learn about it, and that this is a liberating freedom. Being conscience of what you do not know is important because this realization can help you take initiative to embrace the uncertainty and recognize there is always more to learn. My biochemistry professor last year explained to me that I must stop trying to wish to know all the answers and the most in-depth information about everything. Science is not about knowing all the answers. Rather it encourages you to explore what you do not know and have confidence in what you do, so that you can connect with others to build your foundation of knowledge. “The truth is that even the smallest action of the scientist, tucked away in an obscure laboratory, is meaningful, because it contributes to and participates in the story of humankind’s progress” (Ford, 2007, p. 98). This is why minor research can play an integral role in gaining a wealth of knowledge, because it is another piece of the puzzle. I began to acknowledge the opportunity to explore scientific experimentation in a new and more innovative way, by designing my own research project with my lab partner in the biochemistry lab. This was the first time in my education that I was given ownership of my own project, and the lab manual did not provide cookbook style instructions on what to do or expect. The materialistic presentation of science prior to my biochemistry course deterred me from developing the level of scientific inquiry needed in the real laboratory. I believe that the

approach of learning information from the teacher, memorizing, and regurgitating suffocates the imagination and interest in science that everyone could benefit from. I was not particularly engaged in the laboratory, because I did not see the point of doing, what I considered to be monotonous labs. I enjoyed reading about scientists, such as Marie Curie and her research about radium, yet her level of scientific inquiry overwhelmed me. I felt it was beyond my ability to grasp or ever hope to develop such an esteemed level of scientific thought. Science at the university level opened my eyes to critical analysis of research and the glorified dogmas forming our current scientific paradigm, but I still felt restricted by the need to memorize and find absolute word for word answers before I could trust myself to answer a question. Through the process of researching, encountering unknowns, and brainstorming possible solutions and questions about our research with my classmates, an essential realization began to unfold. I had the opportunity "to see how science as a field of inquiry has evolved, and how probing questions and critical thinking contributed to better understanding", as opposed to merely learning for the sake of memorizing information to regurgitate word for word (Pollack, 2003, p. 20). I now acknowledge the importance of embracing what I do not know, and I have developed an appreciation for continuous dialogue and critical analysis of what I know and learn.

FINAL REFLECTIONS: DISCERNING PURPOSE

I feel it is crucial for an individual to be introduced to a holistic presentation of science and liberal arts core for ethical, professional, and personal development, as well as to gain an appreciation for open-minded discussion and seeking purpose in all things. By moving away from the perspective that science will someday obtain final answers and all things must be understood in a material sense, people can instead engage their imagination on new opportunities for innovative research and learning can come to fruition. "The best research is often deeply surprising, because it dispels common myths that we believe about ourselves and the world around us. Research cannot tell us how to live. But interpreted through our own values, it can help free us up to live in ways that more closely align with your own view of the world" (Ecklund, 2010, p. 149). My research of *E. coli* alkaline phosphatase provided only a small addition of knowledge to the world of enzyme research, but the fundamental aspects of my personal engagement in the scientific research process expanded my view of purpose immensely. I developed an appreciation for the value of critically thinking about knowledge, the necessity of exploring uncertainty, and the realization that science encompasses much more than the material aspects of life. I believe that in order to advance the expansion of science literacy and engage people in having an interest to learn about science, as it pertains to them, we need to help them view science as an opportunity to ask questions rather than memorize constipated ideology. People must feel the ability to pursue their curiosity in

order for their inquiry and ownership of scientific engagement to be ignited. “In every one of us there is a scientist who is asleep, and who will not wake up until social and cultural conditions are pushed aside” (Sheldrake, 2012, p. 26). Mechanistic science education is not enough to awaken the scientist within, because it does not compel people to seek knowledge. For example in my healthcare internships, I find that healthcare education is not enough to commit people to make lifestyle changes. People are not prepared to take ownership of their health rather they rely solely on the doctor to manage it for them. This is similar to my behavior as student attending lecture to memorize scientific information, rather than to stimulate my critical thinking by coming prepared for discussion. The belief that science already knows all of answers discourages people from participating in scientific dialogue, thus inhibiting the development of a holistic mindset. By integrating a presentation of science that is more focused on stimulating the imagination in the earlier years of education, students will be more prepared to appreciate the mission of a holistic education. This primary preparation can reveal the interconnectedness of taking ownership of dialogue participation in science and humanities courses to the process of discerning purpose in life.

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