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An Investigation of Protein-DNA Binding Characteristics by Fluorescence Anisotropy

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An Investigation of Protein-DNA Binding Characteristics by Fluorescence Anisotropy

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Abstract

Recent studies have illustrated the presence of several secondary, non-B DNA structures that are associated with the promoter regions of several oncogenes, such as the intensely studied MYC gene. Two such secondary structures, G-quadruplexes and i-Motifs, have become topics of interest due to their prevalence within oncogenic promoters and their potential accessibility for future drug targeting due to their globular natures. Though fairly extensive research has been conducted to understand different factors affecting G-quadruplex formation and stability, similar studies for i-Motifs, particularly related to thermodynamics, are still evolving. One factor known to contribute to the additional energy required for duplex DNA to transition to its single-stranded form capable of forming i-Motifs is protein-DNA binding. This has been observed using EMSA, providing an estimated equilibrium binding constant. Therefore, other methods should be explored to acquire a truer equilibrium binding constant and determination of the energy contributed by protein binding to i-Motif DNA. This thesis explores the method of fluorescence anisotropy as an alternative way of examining the thermodynamic effects protein-DNA binding has on i-Motif stability by using a sequence within the c-MYC promoter and one domain of a Poly Cytosine Binding Protein. Because of the problems incurred throughout various experimental techniques, however, further studies are needed to explore the usefulness of fluorescence anisotropy with this particular protein and DNA sequence.
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1. Introduction

Cancer treatments have been continuously evolving over the course of history with therapies becoming more specific to the disease over the years, such as with DNA targeting agents. Recent research has shown that several forms of non-B DNA structures, such as i-Motifs and G-quadruplexes, are frequently associated with the promoter region of several oncogenes (Brooks and Hurley, 2009; Bhavsar-Jog et al., 2014). Studies have also indicated that the regions capable of forming these secondary structures are prevalent in the transcriptional start sites in regulatory regions of genes (Eddy and Maizels, 2008; Balasubramanian et al., 2011). While fairly extensive research has been conducted regarding G-quadruplexes, far fewer studies have explored all the factors influencing i-Motif structure and stability. An i-Motif is a four-stranded intramolecular folding of single-stranded DNA that has hydrogen bonds formed between hemiprotonated cytosines (Dai et al., 2010) (Figure 1). An i-Motif structure is typically formed at slightly acidic pH (~6.5), and it can form either by internal folding of one single-strand (ss) DNA or from multiple individual single strands (Bhavsar-Jog et al., 2014). Thermodynamically, secondary DNA structures like i-Motifs are less stable than its duplex form, and so additional energy is required for duplex DNA to transition to two single-stranded structures and form G4s and i-Motifs (Bhavsar-Jog, 2013). Recent research has begun to tackle the problem of identifying physiologically relevant i-Motif structures (Sun and Hurley, 2009; Brooks et al., 2010; Cui et al., 2013), but there has been little research addressing the question of how protein binding, as well as other physiological stressors, affects i-Motif structures thermodynamically. To tackle this particular issue, one recent graduate student in the Wadkins lab explored protein-DNA interaction between the
promoter region of an intensely studied oncogene known to form i-Motifs, the c-MYC gene, and a protein known to bind to cytosine-rich DNA, the Poly Cytosine Binding Protein 2 (PCBP2) (Bhavsar-Jog, 2013).

Figure 1. Formation of Secondary Non-B DNA Structures. (A) G/C-rich DNA sequence from the c-MYC promoter capable of forming the secondary DNA structures, G-quadruplexes and i-Motifs. (B) Schematic drawing of a G-quadruplex structure formed in the c-MYC promoter. (C) Schematic drawing of the i-Motif secondary structure formed in the c-MYC promoter. The nucleobases cytosine, adenine, guanine, and thymine are represented by the yellow, green, red, and blue circles, respectively. (Adapted from Kendrick and Hurley, 2010; ©2010 IUPAC)
The product of the c-MYC gene is an oncoprotein transcription factor that has major functions in regulating cell growth and apoptosis (Sun and Hurley, 2009). The MYC oncoprotein family has been widely studied and is known to play a vital role in several human cancers such as lymphoma, breast cancer, prostate cancer, gastrointestinal cancer, and others. Over-expression of the MYC gene can be caused by several different mechanisms, but its irregular expression is typically due to transcriptional regulation (Nesbit et al., 1999; Nilsson and Cleveland, 2003). In addition to MYC’s well-known role in various cancers, previous studies have demonstrated the ability of c-MYC promoter sequences to form stable i-Motifs in acidic solution of pH 4.5-5.5, so understanding the factors affecting these i-Motif structures within the promoter region of c-MYC is vastly significant (Cui et al., 2013). The human c-MYC gene is located on chromosome 8 and consists of four promoter start sites (P0-P3) that regulate transcription, but over 90% of transcription is controlled by the nuclease hypersensitive element (NHE III₁) upstream of P1 and P2 (Chen et al., 2014; Cui et al., 2013) (Figure 2). The NHE III₁ is composed of a G/C-rich DNA sequence and is known to form the i-Motif secondary structures (Cui et al., 2013). Thus, this is one of several promoters of particular interest in studying the regulatory effects of secondary DNA structures.

**Figure 2.** NHE III₁ Region of the MYC Promoter. Brackets illustrate the G/C-rich regions within the promoter capable of forming G-quadruplexes and i-Motifs in single-stranded (ss) DNA and supercoiled (sc) DNA (bolded G’s and C’s) (adapted from Brooks and Wadkins proposal).
It has also previously been shown that hnRNP K, a poly (rC) binding protein that is structurally similar to poly cytosine binding protein (PCBP2), binds to C-rich stranded DNA in the c-MYC promoter and promotes transcription from that promoter (Lee et al., 1996). Unlike hnRNP K, however, PCBP2 is known to be biologically active at acidic pH that stabilizes i-Motif structures. PCBP2 is part of the heterogeneous nuclear ribonuclear protein family, and it consists of three KH domains (Figure 3). Only in a very recent study by a graduate student were the structural consequences and energy contributions of PCBP2 binding to the i-Motif forming sequence of the c-MYC promoter region observed (Bhavsar-Jog, 2013).

**Figure 3.** Sequence of PCBP2 KH1 and KH2. (A) Domain structure of all three KH domains. (B) Schematic depictions of the KH1 and KH2 domains showing the 3 α-helices and 3 β-sheets (Dui et al., 2008).

In the graduate student’s study, it was shown that the protein binding to DNA i-Motifs contributed towards the formation and stabilization of i-Motifs by providing a fraction of the additional energy required for DNA to form i-Motifs. Additionally, the
study showed that both the KH1 and KH3 domains of PCBP2 could bind to the i-Motif forming sequence of the c-MYC promoter region at pH 5.4 and retain the i-Motif conformation after binding. The method used by the graduate student in this study to observe the protein binding and subsequently calculate the binding constant for the KH1- and KH3-DNA interaction was electrophoretic mobility shift assay (EMSA) using non-denaturing PAGE gels (Bhavsar-Jog, 2013). Despite this being an efficient method to verify that protein-DNA binding does contribute to the energy required for i-Motif structures to form, EMSA has limitations in determining the true equilibrium binding constant because of the gel matrix involved. This paper will discuss the use of an arguably more efficient method of measuring protein-DNA binding, which is fluorescence anisotropy changes resulting from protein binding to fluorescently-labeled DNA strands.

Fluorescence anisotropy is a way of describing polarization of fluorescence. The affinity of DNA binding proteins can be assessed with fluorescence anisotropy by using fluorophore-labeled oligonucleotides. After determining a suitable wavelength, a polarized beam of light excites the solution containing a fluorophore, and the subsequent polarized emissions are used to calculate the anisotropy. Changes in anisotropy provide a useful measurement for binding because many fluorophores have a fluorescent lifetime that is on the same nanosecond time scale as the rotation of biological macromolecules in solution. When analyzing a fluorophore’s rotational frequency in respect to its fluorescent lifetime, slowly rotating fluorophores have relatively large anisotropy values, while rapidly rotating fluorophores have nearly no anisotropy. When a protein successfully binds to the fluorescently-labeled oligonucleotide, the fluorophore’s rotation should
decrease due to the additional mass of protein, causing the fluorescence anisotropy measurement to increase. Consequently, the changes in anisotropy measurements due to addition of protein to the solution should adequately measure protein binding. One way in which this method is advantageous to several others is that it allows binding affinities to be determined in solution without requiring the separation of free and bound oligonucleotide, as with EMSA in the previous study. Additionally, protein can be continuously added to the solution in small increments, obtaining a plethora of data points quickly and efficiently (Anderson et al., 2008). This paper will describe using the method of fluorescence anisotropy to determine a true equilibrium binding constant for PCBP2 KH-1 domain and an i-Motif forming 51-mer sequence from the c-MYC promoter.
2. Materials and Methods

Plasmid Construction and Cell Lines

The sequence for the PCBP2 KH1 domain was engineered in a pGS-21a plasmid purchased from Genscript (New Jersey, USA). The pGS-21a plasmid contains a selective gene for ampicillin antibody resistance, as well as genes encoding for both GST-affinity and Histidine-affinity tags. The gene encoding for the KH1 domain was inserted directly behind the enterokinase recognition sequence, allowing both tags to be cleaved from the KH1 domain during enterokinase digestion. The plasmid then underwent bacterial transformation into T7 Express Competent *E. coli* cells purchased from Life Technologies (Carlsbad, CA).

Bacterial Transformation and Purification

The T7 Express Competent *E. coli* cells were thawed on ice, and the plasmid DNA was injected into the cell mixture. After remaining on ice for 30 minutes, the mixture was heat shocked at 42 °C and subsequently placed on ice for an additional 5 minutes, allowing the plasmid DNA to enter the bacterial cell. The cells were grown in SOC medium\(^1\) at 37 °C for 60 minutes with shaking at 250 rpm before being plated and incubated overnight at 37 °C.

A single colony of transformed bacteria was used to inoculate a starter culture of LB-ampicillin medium. The cells were harvested by centrifugation at 6000xg for 15 minutes at 4 °C. The pellet was then resuspended in a buffer containing RNaseA.

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\(^1\) SOC medium (20 g/L tryptone, 5 g/L yeast extract, 4.8 g/L MgSO\(_4\), 3.6 g/L dextrose, 0.5 g/L NaCl, 0.186 g/L KCl) is a nutrient-rich broth that helps maximize the transformation efficiency of competent cells.
followed by the addition of a lysis buffer\textsuperscript{2}. Following cell lysis, a neutralization buffer was added, causing the large DNA molecules and proteins to precipitate and the plasmid DNA to remain in solution. The mixture was then centrifuged at 20,000xg for 30 minutes at 4 °C, and the supernatant was subsequently centrifuged at 20,000xg for 15 minutes at 4 °C. A QIAGEN-tip 100 was used to elute the supernatant containing now pure DNA. The eluted DNA was precipitated with room temperature isopropanol\textsuperscript{3} by centrifuging at 15,000xg for 30 minutes at 4 °C. The DNA pellet was washed with 70\% ethanol and then centrifuged again at 15,000xg for 10 minutes. After the pellet was allowed to dry for 5-10 minutes, it was redissolved in TE buffer\textsuperscript{4}.

**UV Spectrometry and Agarose Gel Analysis**

UV spectrometry was used to determine the yield of plasmid DNA at 260 nm. The DNA sample consisted of 960 µl of spectroscopy grade water with 40 µl of DNA.

Gel electrophoresis on an agarose gel was used to confirm the presence of the PCBP2 KH1 domain in plasmid PGS-21a. The restriction endonuclease BamH1\textsuperscript{5} was used to digest the DNA sample. The gel was electrophoresed with Tris-acetate-EDTA (TAE) buffer at 23 volts overnight. Ethidium bromide dye was added to the running buffer to bind to and stain the DNA to allow visualization of the bands.

**Protein Expression**

After the plasmid was transformed into the T7 Express Competent *E. coli* cells, the cells were streaked onto an LB-Ampicillin plate and incubated at 37 °C overnight.

\textsuperscript{2} RNaseA catalyzes the hydrolysis of RNA molecules during the lysis step. Proteins and large chromosomal DNA are denatured, but the plasmid is left stable due to its small, circular size.

\textsuperscript{3} Isopropanol causes the DNA to become insoluble and thus precipitate during centrifugation.

\textsuperscript{4} TE buffer will prevent any contaminative nucleases from degrading the DNA because it contains EDTA that binds with Mg\textsuperscript{2+}, a common cofactor to nucleases.

\textsuperscript{5} BamH1 recognizes the palindromic sequence GGATCC.
The following day, a single colony was picked from the plate and inoculated into a 5 mL LB-ampicillin starter culture and grown at 37 ºC overnight, with shaking at 250 rpm. A control containing 5 mL of LB-ampicillin solution was also incubated overnight to monitor the growth of the starter culture. Once successful growth of bacteria was confirmed, the starter culture was added to 500 mL of LB-ampicillin media and grown at 37 ºC with shaking at 250 rpm. Checking the absorbance at 600 nm monitored the bacterial cell growth. Once the concentration reached an absorbance of 0.6-0.8, 0.4 mM of IPTG\(^6\) was added to the flask to trigger transcription and induce protein expression. The cells were then allowed to grow for 3 more hours, taking a sample after every hour to monitor expression with a 12% SDS-PAGE gel. The cells were then pelleted by centrifugation at 2054xg for 10 minutes. The cells were then lysed using bacterial protein extraction reagent (BPER) from Pierce Biotechnology (Rockford, IL), followed by centrifugation at 39,000xg for 40 minutes to separate the soluble protein fraction of the lysate.

**Protein Purification**

Glutathione affinity chromatography was used to purify the protein sample. Glutathione S-transferase (GST) protein has a high affinity for glutathione, and thus proteins containing a GST tag can be purified by being washed and eluted through a GST column. The soluble fraction of the lysed bacterial cells was equilibrated with an equal amount of phosphate buffered saline (PBS, 1X: 10 mM Na\(_2\)HPO\(_4\), 1.8 mM KH\(_2\)PO\(_4\), 140 mM NaCl, 2.7 mM KCl, pH 7.3), the GST binding and wash buffer. The protein solution was then poured over the column and subsequently washed with 1X PBS. A freshly made

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\(^6\) IPTG triggers the transcription of the lac operon and is thus used to induce protein expression of genes under control of the lac operator.
10 mM reduced glutathione buffer (0.154 g of reduced glutathione dissolved in 50 mL of 50 mM Tris-HCl, pH 8.0) was used to elute the protein off of the column. The elution samples were concentrated using 30K MWCO concentrator units and then analyzed using a 12% SDS-PAGE gel to confirm the presence of the target protein.

Enterokinase digestion was used to remove the Histidine and GST tags from the KH1 domain. In order for successful digestion, however, glutathione was first removed by overnight dialysis in 50 mM Tris-HCl, as it somehow inactivates enterokinase. The progress of cleavage was observed by analyzing different digestion fractions with a 12% SDS PAGE gel. Once successful cleavage of the tags from the protein is observed, the digested solution is poured back over a GST column. This time, pure KH1 should be eluted.

**Protein Concentration by Bradford Assay**

The protein concentration was determined using the Bradford Assay, which is based on the binding of protein with Coomassie Brilliant Blue G in solution. When protein binds to the dye, the newly formed complex causes an increase in absorption from 495 to 595 nm, with the absorption value being proportional to protein concentration. The standard protein sample used was 1 mg/mL Bovine Serum Albumin (BSA) with concentrations ranging from 62.5 µg/mL to 1000 µg/mL. The unknown KH1 concentration was determined from the linear fit produced by graphing standard sample absorbances versus their concentrations.

**Fluorescence Anisotropy**

Fluorescence anisotropy was used to attempt to determine the binding characteristics between the i-Motif forming region of the c-MYC gene and the PCBP2
KH1 domain. The 51-mer oligodeoxynucleotide containing the region of the c-MYC gene capable of forming i-Motifs was purchased from the Midland Certified Reagent Company (Midland, TX), and it has the sequence 5’-(Cy3)TCC TCC CCA CCT TCC CCA CCC TCC CCA CCC TCC CCA TAA GCG CCC TCC CGG-3’. Cyanine 3 (Cy3) was the fluorophore used, with a peak absorbance value around 550 nm and peak emission value around 570 nm. Approximately 50 nM of DNA in sodium acetate buffer (pH 5.4) was placed in a cuvette and analyzed using an Internal Time-Based Polarization Experiment with Felix software controlling a PTI fluorescence spectrometer. The excitation wavelength was set to 500 nm, and the emission wavelength was set to the DNA’s peak of 570 nm. Both emission spectra and anisotropy measurements were taken with additive fractions of KH1 to the 51-mer oligonucleotide. A line of best fit was then determined from a graph of anisotropy measurements versus the amount of protein added. Assuming accurate measurements are procured, an equilibrium binding constant should then be obtainable.
3. Results

UV Spectrometry and Agarose Gel Analysis

The absorbance of the plasmid DNA stock sample at 260 nm was 0.043, and the concentration of the stock DNA was determined to be 53.75 µg/ml.

The gel was visualized under auto-exposure via a transilluminator and UV preparative (Figure 4). The results from the agarose gel analysis were graphed with a logarithmic fit and showed that the DNA size was approximately 6300 nucleotide base pairs, which is consistent with the known size of the plasmid containing the KH1 insert of approximately 6370 base pairs (Figure 5). Therefore, the bacterial DNA sample used in protein expression was confirmed to be accurate and free of contaminants.

Figure 4. Agarose Gel Electrophoresis. The lane order reading from left to right is as follows: DNA ladder, supercoiled DNA (no BamH1), linear DNA (after BamH1 digestion).
The size of KH1 DNA was determined to be ~6.3 kB using the equation derived from the standard curve.

**Protein Expression and Purification**

The protein expression and purification methods were repeated several times throughout the course of this research project, as some elution fractions proved more difficult to purify than others and caused potential problems with the anisotropy measurements in the next step. Each step of the purification process was analyzed using a 12% SDS PAGE gel and a pre-stained protein ladder (Figure 6). Figure 7a illustrates an example of the steps involved in protein expression, and these results were consistent with each round of protein expression. Figure 7b illustrates the steps involved in the GST column purification and the eluted protein fractions (denoted 1a and 1b) of a single
bacterial colony. These results indicate a relatively pure protein prior to enterokinase digestion with a visible band corresponding to ~38 kDa, the expected molecular weight for the KH1 domain containing the GST and Histidine tags. However, neither fraction provided clear results from fluorescence anisotropy despite their purity.

Figure 6. Protein Ladders for 4-20% SDS PAGE Gels.

Figure 7. SDS-PAGE Analysis of PCBP2 KH1 (1). (A) Stages of protein expression before affinity purification. Lane order from left to right is as follows: protein ladder, pre-induction, post-induction (1 hour), post-induction (2 hours), post-induction (3 hours). (B) Steps involved in GST affinity chromatography. Lane order from left to right is as follows: protein ladder, flow-through, wash 1, wash 2, wash 3, elution 1a, elution 1b. The KH1 band is approximately 38 kDa.

After complications in steps proceeding purification with elution fractions 1a and 1b, two more bacterial samples taken from a glycerol stock (denoted 2a and 2b) were
expressed and subsequently purified via GST column chromatography. However, both elution fractions following GST purification were significantly contaminated, probably causing the unsuccessful enterokinase digestion and lack of pure KH1 bands on the SDS PAGE gel (Figure 8). The 2b elution fraction was put back through a GST column with fresh glutathione resin twice in an attempt to elute pure KH1, but neither gel analysis showed any improvement (Figure 9). A 0.2-micron filter was also used in an attempt to rid the sample of contaminants, but to no avail. Thinking that the contaminant perhaps had a weak affinity for the GST column and was therefore eluting in the first few milliliters while leaving pure KH1 to elute afterwards, the 2b elute was put back over the GST column a third time and eluted in 10, 1.5 mL fractions. The analysis of these results was inconsistent with previous data based on the observable bands.

Figure 8. SDS PAGE Analysis of KH1 Elution Fractions 2a and 2b. Heavy contamination is evident in both fractions as well as unsuccessful enterokinase digestion. Lane order from left to right is as follows: protein ladder, elution 2b pre-digestion, digestion of 2a after 13.5 hours, digestion of 2a after 14.5 hours, digestion of 2a after 16 hours.
Figure 9. SDS PAGE Analysis of KH1 Elution Fractions (2). (A) Prominent band still observed between size 26 and 34 bands after second GST column; elution fractions collected as one. Lane order from left to right is as follows: protein ladder, flowthrough of 2b, wash 1, wash 2, elution 2b. (B) Heavy contamination and unsuccessful enterokinase digestion still evident in both fractions. Bands are inconsistent with previous gels. Lane order from left to right is as follows: protein ladder, elution 2a, elution 2b, digestion of 2b after 15 hours, digestion of 2b after 16 hours.

Protein Concentration

The protein concentration of elution fraction 2a was determined to be 559 µg/mL by using the Bradford Assay 96-well microplate method, and the concentration of 2b was determined to be approximately 742 µg/mL. Figure 10 illustrates the line of best fit determined from graphing the results of 2b. However, due to the contamination within both protein samples, these calculations cannot be considered as completely accurate representations of the concentrations of KH1.
Figure 9. Bradford Assay to Determine Protein Concentration (2b). The linear equation was determined by the standard samples with concentrations ranging from 62.5 µg/mL – 1000 µg/mL. The star denotes the absorbance of KH1, which corresponds to a concentration of ~742 µg/mL.

Fluorescence Anisotropy

The anisotropy results obtained from each KH1 elution fraction were meant to present an analysis of the binding properties between the KH1 domain of PCBP2 and the selected sequence from the c-MYC promoter. However, the data did not reflect a typical protein-DNA binding curve and thus did not generate the desired results. Although the emission spectra of the 51-mer c-MYC sequence in sodium acetate buffer (pH 5.4) showed accurate fluorescent peaks with high intensity, the initial anisotropy value was approximately 0.3, a significantly higher value than expected. The addition of protein elution to the DNA sample created huge fluorescent peaks that were unaccounted for and most likely due to scattering light (Figure 11). To eliminate the possible sources for this light scattering, emission spectra were observed of both 50 mM Tris-HCl buffer (pH 7.5) and reduced glutathione buffer (pH 8). While the fluorescent peak decreased slightly in
both cases, no significant peak was observed such as the one presenting the flawed data (Figure 12).

**Figure 11.** Emission Spectra of c-MYC DNA with Undigested PCBP2 KH1. The blue line represents the 51-mer c-MYC sequence in sodium acetate buffer (pH 5.4) only; the purple and green lines illustrate the additions of 2 µl and 4µl KH1 sample, respectively. Large, unaccounted for peaks along with a decrease in fluorescence was observed upon each addition of protein.
Figure 12. Emission Spectra of c-MYC DNA with Buffers Only. The blue line represents the 51-mer c-MYC sequence in sodium acetate buffer (pH 5.4) only. The lighter lines represent successive additions of (A) reduced glutathione buffer (pH 8) and (B) tris-HCl buffer (pH 7.5), with no significant change in fluorescence, thus ruling them out as sources of discrepancy.

On the other hand, when the protein elution alone was added to the sodium acetate buffer (no c-MYC DNA present), the initial peaks were present, indicating that the protein must be the source (Figure 13). To ensure the unusual results were not due to errors originating within the spectrometer itself, a control analysis of Rhodamine B was performed. The data obtained was consistent with the known properties of Rhodamine B, leading to the conclusion that either contamination within the protein sample, the tags...
attached to the protein, or intrinsic properties of the protein itself must be the cause of the
unusable fluorescence anisotropy results.

Figure 13. Emission Spectra of PCBP2 KH1 Additions Only. The blue and purple lines
represent scans of sodium acetate buffer (pH 5.4) only; the green and orange lines represent
successive additions of 2 µl and 4 µl undigested KH1 sample, respectively.

Due to the continued contamination problems encountered with the 2a and 2b
elution fractions, no emission spectra or anisotropy results were obtained, as the hope
was to see the effect on the emission spectra with pure KH1 elute.
4. Discussion

Though the results obtained from fluorescence anisotropy were not what we hoped they would be, there is still great opportunity for future direction with this explorative project. First and foremost, the problem of obtaining completely pure protein from the column and enterokinase digestion must be solved, as this could be a simple solution to the skewed results that were continuously obtained on the spectrometer. Because relatively pure samples were initially obtained in the 1a and 1b elution fractions, one possibility is that the large GST tag caused the abnormal anisotropy results. With the continuation of this research, a reasonable next step to provide more troubleshooting would be to perform another round of protein expression, this time adding protease inhibitors to the lyse buffer with hopes it would limit possible degradation of the target protein, allowing pure elution from the column followed by successful digestion and anisotropy measurements.

Additionally, because of the high initial anisotropy value of ~0.3 obtained from the DNA sample solution containing a Cy3 fluorophore, future directions with these experiments should incorporate DNA sequences with different fluorophores with the hope that a lower base value anisotropy measurement might be obtained. Once the process of troubleshooting this project is complete and anisotropy measurements begin to normalize, then the equilibrium binding constants between the PCBP2 KH1 domain and the 51-mer c-MYC sequence can be obtained and compared to the previously mentioned graduate student’s results using the EMSA. Upon confirmation that fluorescence anisotropy is, in fact, a more efficient method of determining these binding constants than
the EMSA, further future directions for this project will be to express the entire PCBP2 protein and observe its binding properties within the c-MYC promoter.
List of References


