ZINC-DEPENDENT INTERACTION OF TRANSCRIPTIONAL CO-ACTIVATOR CBP/p300 WITH MTF-1

A THESIS IN
Cellular and Molecular Biology

Presented to the Faculty of University
Of Missouri-Kansas City in partial fulfillment of
the requirements for the degree

MASTER OF SCIENCE

by
VUGAR AZIZOV

B.S., The University of Kansas, 2010

Kansas City, Missouri
2012

ZINC-DEPENDENT INTERACTION OF TRANSCRIPTIONAL CO-ACTIVATOR CBP/p300 WITH MTF-1

Vugar Azizov. Candidate for the Master of Science degree

University of Missouri-Kansas City, 2012

ABSTRACT

One of the major functions of the metal response element-binding transcription factor 1 (MTF-1) is to sense and maintain sub-nanomolar to nanomolar zinc levels in response to influxes of labile zinc within the cell. MTF-1 responses to elevated zinc include up regulation of metallothionein (*MT-I & II*) and efflux transporter (*ZnT1*) genes. MTF-1 also responds to oxidative stress and heavy metal loads. Due to a lack of liver development, MTF-1 is essential for embryogenesis as determined from knockout mice. The zinc dependence of DNA-binding and interactions with other transcription factors has been identified as major determinants in the homeostatic regulation of labile intracellular zinc by MTF-1. p300 along with its paralog, cyclic-AMP response element binding protein (CBP), have histone acetyltransferase protein scaffold functions and interact with other transcription factors. Previous studies have shown that p300, Sp1 and MTF-1 form a complex. It was also found that a zinc dependent interaction between p300 and MTF-1 is essential for activation of *MT-I*

transcription in mice. Herein, we present NMR evidence for a physical interaction between MTF-1 and a zinc binding domain of p300. This new observation suggests a complex molecular basis for the zinc dependence of *MT-I* activation, involving direct zinc coordination to the MTF-1 DNA binding domain and the TAZ2 domain of p300.

APPROVAL PAGE

The faculty listed below, appointed by the Dean of the School of Graduate Studies, have examined a thesis titled "Interaction of transcriptional co-activator CBP/p300 with MTF-1 in zinc-dependent manner." presented by Vugar Azizov, candidate for the Master of Science degree, and certify that in their opinion it is worthy of acceptance.

Supervisory Committee

John H. Laity, Ph.D., Committee Chair
Cell Biology and Biophysics

Brian Geisbrecht, Ph.D.,
Cell Biology and Biophysics

Karen Bame, Ph.D.,
Molecular Biology and Biochemistry

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

List of Tables

Table Page

ABBREVIATIONS

MTF-1	Metal response element (MRE)-binding transcription factor-1
p300	Transcriptional Co-activator
TAZ1	Transcriptional adaptor zinc binding 1 domain of p300
TAZ2	Transcriptional adaptor zinc binding 2 domain of p300
PCR	Polymerase Chain Reaction
NMR	Nuclear Magnetic Resonance
HSQC	Heteronuclear Single Quantum Coherence
AMP	Adenosine Monophosphate
HPLC	High Pressure Liquid Chromatography
ACN	Acetonitrile
TFA	Tri-Fluoro acetic Acid
ITC	Isothermal Titration Calorimetry
znT1	Zinc transporter 1 gene
mtI	Metallothionein I gene
DNA	Deoxyribonucleic Acid
cDNA	Complimentary Deoxyribonucleic Acid
CIP	Calf Intestinal Phosphotase
MRE	Metal Response Element
kDa	Kilo Daltons, 1 Dalton = 1 g/mol,

ACKNOWLEDGEMENTS

I have been very lucky to be working in Dr. John Laity's laboratory. Not only he taught and mentored me, he also gave me the push I needed to do my best. I have learned a great deal from Dr. Laity and I am very thankful for his patience. I deeply benefit from his openness, support, knowledge and faith in me in pursuit of my degree. His teaching method; working at the bench, going through every single detail patiently with me, not only created a perfect environment for learning but his trust, also allowed me to apply my knowledge independently. I would like to acknowledge Dr. Laity's immense influence on my post-baccalaureate career.

I would like to acknowledge my committee members, Dr. Brian Geisbrecht, and Dr. Karen Bame for their countless advices, helpful comments and contributions. I would like to acknowledge Dr. Andrew Keightley for his valuable help with Mass Spectrometry and results analysis.

I would also like to thank our valuable lab members; Linda Feng for her enormous help with day to day questions and techniques; Ryan Huyck for his help with MTF-1 zf6-acidic domain and various techniques. Surely, without their help I would not be able to learn various methods quickly and efficiently. My appreciations extend to other members of School of Biological Sciences as well.

My deepest thanks and gratitude extends to my family - parents, brothers for their moral and material support. I would like to thank my friends for their help in keeping me focused, and having faith in me.

I would like to dedicate this thesis to my mother, Tofiga Askerova; she is, and always will be the beacon of light in my life.

CHAPTER 1
INTRODUCTION

1.1 Metal Response Element Binding Transcription Factor 1

MTF-1 is 650 amino acid protein with functionally distinct DNA binding, acidic, proline-rich, serine-threonine rich, and cysteine rich domains (Figure 1a) (Radtke, et al. 1993) (Heuchel, et al. 1994). The DNA binding domain is comprised of six Cys2-His2 zinc fingers. Each zinc finger can reversibly bind zinc, and recognizes short *cis*-acting metal response elements (MREs) DNA sequences. These MREs are found in multiple places in the promoter region of *MT-I* and *MT-II* (Stuart, Searle and Palmiter 1985) (Lee, et al. 1987) and other genes such as *ZnT1* (Langmade, et al. 2000). Elevated zinc levels in the cell, whether due to exposure or zinc redistribution, are believed to initiate translocation of MTF-1 to the nucleus (G. K. Andrews 2000) (Saydam, Georgiev, et al. 2001) where it binds to MREs in the promoter region of the *MT-I* gene (G. K. Andrews 2001) (Laity and Andrews 2007) and recruits p300/CBP and Sp1 (Li, et al. 2008). There are other factors that bind *MT-I* gene promoter constitutively such as Sp1, c-Jun, and USF (Li, et al. 2008). It has also been shown that the promoter of *MT-I* gene becomes fully bound and transcriptionally activated by the binding of MTF-1 and p300/CBP recruitment, in addition to transcription factors that are bound constitutively (Daniels and Andrews 2003) (Li, et al. 2008).

1.2 p300/CBP

p300 and CBP are transcriptional co-activators (Chrivia, et al. 1993) (Eckner, et al. 1994). CBP and p300 are encoded by different genes and have 58% identical polypeptide sequences. (Chan and La Thangue 2001) (Eckner, et al. 1994). CBP is named for its hallmark activity, which is binding to cyclic-AMP response elements. p300/CBP maintain basal transcription levels of various genes (Eckner, et al. 1994), and have histone acetyltransferase activity and protein scaffold/adapter domains. p300 is 2412 amino acid protein that is comprised of the following substructures: nuclear receptor interaction domain, CREB, MYB interaction domain, cysteine/histidine rich domains CH1/TAZ1 and CH3/TAZ2 (Figure1b) and the interferon response binding domain. Germ-line mutations of p300 or its paralog CBP causes Rubinstein-Taybi syndrome (Iyer, Ozdag and Caldas 2004). Both p300 and CBP have two homologous zinc binding domains, TAZ1 and TAZ2, which function as scaffold/adapter domain (Chan and La Thangue 2001) (Ponting, et al. 1996) (De Guzman, et al. 2000) (De Guzman, et al. 2005). These domains have been shown to bind and recognize more than 30 different transcription factors. Despite the sequence and structural homology between TAZ1 and TAZ2 (NCBI Blast 33% identity) (Altschul, et al. 1997), they have been shown to bind different factors (De Guzman, et al. 2005) (De Guzman, et al. 2000) (Iyer, Ozdag and Caldas 2004) (Figure1B). Solution structural studies of CBP TAZ domains have revealed the 3D structure consisting of four amphipathic α-helices (De Guzman, et al. 2005) (Freedman, Sun and Poy, et al. 2002). Each of these TAZ domains binds 3 zinc atoms, which is crucial to the structure of the TAZ domains (De Guzman, et al. 2000) (De Guzman, et al. 2005). Interaction of TAZ1 with HIF-1α (Dames, et al. 2002) (Freedman, Sun and Poy, et al. 2002) ,

or CITED2 (Freedman, Sun and Kung, et al. 2003) (De Guzman, et al. 2004) reveal that the intrinsically unstructured HIF-1α and CITED2 domains become structured upon TAZ1 binding. The same has been shown for the TAZ2 domain in interaction with other ligands (De Guzman, et al. 2000). Whereas their target proteins differ, the His-Cys-Cys-Cys zinc binding sites within both TAZ domains have equivalent 1:3 zinc binding stoichiometry.

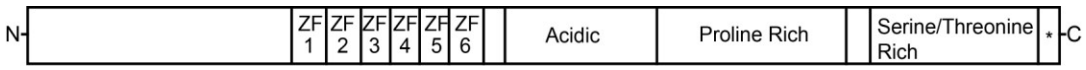
1.3 MTF-1 and p300 Interaction

In this study and in the study by Li et al, MTF-1 construct is from *Mus musculus* (mouse). Mouse MTF-1 is similar to human MTF-1 especially in the acidic and zinc finger zinc sensing domains (Huyck, Keightley and Laity 2012). Studies show that MTF-1 interacts with p300 and both proteins are co-immunoprecipitated by anti-MTF-1 and anti-p300 antibodies. (Li, et al. 2008). Depletion of p300, but not CBP, by siRNA (RNA silencing) knockdown in mice, attenuates MTF-1-dependent transcription of *MT-I* (Li, et al. 2008). Thus, the studies presented in this thesis have focused solely on the MTF-1 interaction with p300 rather than CBP.

Studies by Li and coworkers (Li, et al. 2008) established that p300 binds to the zf6-acidic domain of MTF-1. When they constructed multiple deletion mutations of MTF-1, the potential p300 binding site was identified to be within the zf6-acidic domain. The mutant MTF-1 lacking the acidic domain (MTF-1-ΔAD) was able to bind to the promoter of *MT-I*, but was unable to activate transcription of the gene. Over-localization of MTF-ΔAD in the nucleus was also observed by Li et al. Mutation of seven leucine residues within the MTF-1 acidic domain to alanine resulted in a dramatic (~8 fold) decrease in recruitment of p300 to the *MT-I* promoter region, as demonstrated by co-immunoprecipation experiments. Acidic

domain leucine residue mutations also impaired MTF-1's ability to be excluded from the nucleus. This observation is consistent with the hypothesis that five of the seven acidic domain leucine residues mutated are part of a MTF-1 nuclear exclusion sequence (Li, et al. 2008).

It is well established that exposure to elevated zinc levels stimulates expression of *MT-I* (Davis, McMahon and Cousins 1998). The binding of MTF-1 to MREs in the *MT-I* promoter region is zinc dependent. Significantly, NMR studies of the MTF-1 zf6-acidic domain (residues spanning zf6 to residue number 405) reveal no defined secondary structure of this polypeptide in the absence or stoichiometric excess of zinc (up to 3-fold excess zinc) (Li, et al. 2008). Since recruitment of p300 directed by the MTF-1 zf6-acidic domain is crucial for *MT-I* gene transcription it is important to understand the mechanism by which p300 interacts with MTF-1. Research presented in this thesis investigates p300 TAZ domains as the potential binding partner of the MTF-1 zf6-acidic domain. Given the zinc-dependence of the MTF-1 and p300 functional synergy observed earlier, we searched for zinc binding domains in p300 to probe for the molecular basis of the MTF-1:p300 functional synergy (Huyck, Keightley and Laity 2012). Specifically the goal of this thesis was to determine, and to provide preliminary characterization of a physical interaction between the two proteins. Accordingly, we determined the TAZ1 and TAZ2 domains of p300 were the most likely candidates to pursue, since they are the only known zinc binding domains within this transcriptional coactivator that are heavily involved in interacting with various factors. Research presented herein shows the results of NMR studies of p300 TAZ1 and MTF-1 zf6-acidic domain, p300 TAZ2 and MTF-1 zf6-acidic domain.



* Cysteine rich domain

Figure 1a. MTF-1 represented schematically. Zinc fingers (ZF) 1-6 represented by digits.

p300 TAZ1: N'-MSGAHTADPEKRKLIQQQLVLLLHAHKCQRREQANGEVRQCNLPHCRTMK-
p300 TAZ2: N'-MSPGDSRRLSIQRCIQSLVHACQCRNANCSLPSCQKMKRVVQHTKGCKRK-
p300 TAZ1: -NVLNHMTHCQSGKSCQVAHCASSRQIISHWKNCTRHDCPVCLPLKNAGDKR-C'
p300 TAZ2: -TNGGCPICKQLIALCCYHAKHCQENKCPVPFCLNIKQK-C'

Figure 1b. p300 TAZ1 and TAZ2 amino acid sequences N' terminus to C' terminus and reported zinc binding sites. In green are the zinc binding His-Cys-Cys-Cys residues for TAZ1 and in red for TAZ2.

CHAPTER 2
MATERIALS AND METHODS

2.1 Materials

Mouse p300 cDNA (accession NC_000081.6) was purchased from Thermo Fisher Scientific Open Biosystems (Lafayette, CO) in pCR-XL-TOPO vector. *Escherichia coli (E.coli) BL21(DE3) Codon Plus RIPL* cells were purchased from Agilent Technologies (Santa Clara, CA). DNA expression vector pET21a was purchased from Novagen-EMD (Darmstadt, Germany). Primers were purchased from Integrated DNA Technologies (Coralville, Iowa). Platinum Supermix High Fidelity DNA Polymerase was purchased from Invitrogen (Grand Island, NY). MaxiPrep Plasmid Purification kit , MiniPrep Plasmid Purification kit, Gel Extraction, and Nucleotide Removal kits were purchased from Qiagen (Valencia, CA). Calf intestinal phosphatase, T4 DNA Ligase, and Restriction Endonucleases were purchased from New England Biolabs (Ipswich, MA).

2.2 Cloning of TAZ1/TAZ2 Domains of p300

2.2.1 PCR amplification and purification

The p300 TAZ1 (residues 326-425) and TAZ2 (residues 1725-1811) domains were PCR amplified from p300 cDNA using primers listed in Table 1 and a Techne Touchgene Gradient thermal cycler. An N-terminal Methionine at the 5' end and a stop codon at the 3' end were added by integration of ATG and TTA sequences to sense and anti-sense primers, respectively. *XbaI* and *XhoI* digestion sites (Appendix E) were also added to primers at sense and anti-sense primers, respectively.

Table 1. Sequence of primers used to amplify TAZ1 and TAZ2 domain of p300.

TAZ1 anti-sense primer	5'-GCCTCGAGTTATCGCTTATCCCCAGCATTTT-3'
TAZ1 sense primer	5'-GCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGA TATACATATGATGTCTGGAGCACACACA-3'
TAZ2 anti-sense primer	5'-GCCTCGAGTTATTTTTGCTTGATGTT-3'
TAZ2 sense primer	5'-GCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGA TATACATATGAGCCCAGGAGACTCC-3'

Table 2. PCR conditions (30 cycles; Denaturation through Extension)

STEP	Temperature	Time
Initial Denaturation	95 ^{o}C	2 minutes 30 seconds
Denaturation	95 ^{o}C	0 minutes 45 seconds
Annealing	63 ^{o}C	0 minutes 45 seconds
Extension	72 ^{o}C	1 minute 00 seconds
Final Extension	72 ^{o}C	10 minutes 00 seconds

PCR reactions were prepared using Platinum High Fidelity DNA Polymerase Supermix with addition of appropriate primers to final concentration of 200 nM and template DNA of 9.6 ng. The PCR reactions were performed as shown in Table 2.

5µL of each PCR reaction were analyzed on a 1%(w/v) agarose gel in order to visualize and confirm the PCR product had been generated. PCR products were purified from excess nucleotides and primers using the Nucleotide Removal kit from Qiagen and quantified by absorbance at 220 nm using a Varian Cary Bio, UV visible spectrophotometer (Cary).

2.2.2 Cloning of TAZ1 and TAZ2 genes into pET21a T7 intracellular expression plasmid.

Aliquots of purified TAZ1 and TAZ2 PCR products were made in order to achieve 10:1 (TAZ to pET21a), and 3:1 ratios based on the 500 ng (0.14 pmoles) of pET21a. For TAZ1, a 10:1 ratio was 1.4 pmoles equaling 335 ng, while a 3:1 ratio was 0.42 pmoles that equaled 101ng. For TAZ2, a 10:1 ratio was 1.4 pmoles equaling 304.92 ng, while a 3:1 ratio was 0.42 pmoles that equaled 91.476 ng. TAZ1, TAZ2, and pET21a were digested with *XbaI* and *XhoI* restriction endonucleases simultaneously in NEB Buffer 4 at room temperature for 4 hours. Following digestion, reactions were purified using the Nucleotide Removal kit. The digested pET21a plasmid was incubated with calf intestinal phosphotase to remove the terminal phosphates, and then incubated with TAZ1 or TAZ2 in the ratios previously described. Ligations were done overnight by using T4 Ligase at room temperature. Successful ligation reactions yielded the pET21a-TAZ1 and pET21a-TAZ2 expression vectors. These vectors were transformed into *E. coli DH5α* for plasmid amplification, and also for confirmation by sequencing of insert-positive clones. (Appendix C).

2.2.3 Transformation of pET21a-TAZ1/TAZ2

Expression vectors pET21a-TAZ1/TAZ2 were amplified in *E. coli DH5α* and purified by MaxiPrep. Purified expression vector pET21a-TAZ1 or pET21a-TAZ2 was transfected into *E. coli BL21 DE3 Codon Plus RIPL* cells by the heat shock method (Agilent). 2μL of 10x dilution of XL-10-Gold β-mercaptoethanol (Agilent) were added to 100μL of competent *E. coli* BL21 DE3 Codon Plus RIPL cells. The cells were incubated on ice for 10 minutes. Following this 10 minute incubation on ice, 50 ng of expression vector (pET21a-TAZ1 or pET21a-TAZ2) were added, and cells were incubated on ice for an additional 30 minutes. As a negative control, plasmid pUC18 was transfected into *E. coli BL21 (DE3) Codon Plus RIPL* cells. Cells were incubated in a 42^{o}C water bath for 20 seconds, then transferred to ice for 2 minutes. Following this, 900 μL of freshly made pre-heated 37^{o}C SOC were added to cells, and the samples were incubated in 37^{o}C incubator at 225 rpm for 1 hour. Next, cells were spread onto LB plates supplemented with 200 μg/mL of ampicillin, 34 μg/mL of chloramphenicol and 75 μg/mL of streptomycin antibiotics. Plates were incubated at 37^{o}C overnight. A single colony was picked from plates with successful clone colonies and inoculated into 20 mL of LB broth supplemented with 200 μg/mL ampicillin to serve as a highly selective starter-culture for large-scale overexpression.

2.3 Expression of TAZ1/TAZ2

Cultures were grown in shaking incubator at 225 rpm, at 37^{o}C overnight. The next day, cultures were checked for turbidity. Cells were spun down at 2070 xg for 10 minutes, decanted and drained. The cell pellet was resuspended in 1x M9 Minimal Media (1.28%(w/v) Na_2HPO_4, 0.3%(w/v) KH_2PO_4, 0.05%(w/v) NaCl, 1mM $MgCl_2$, and 1mM $CaCl_2$)

supplemented with 50 μg/mL of ampicillin, trace elements, 2 grams per liter of NH_4Cl and Glucose. For NMR studies, the cells were incubated with $^{15}NH_4Cl$ to label the recombinant protein. The culture was grown at 37°C until the OD_{600} reached 0.8. Protein over-expression was induced by addition of 0.1 M IPTG to final concentration of 0.1mM. Samples for analysis were collected immediately before induction (BI), and then at times corresponding to one, two, and three hours (hrs) after the inducer was added (AI-1hr, AI-2hrs, AI-3hrs). After 3 hours of expression at 37°C for TAZ1 expression and after 6 hours of expression for TAZ2, cultures were harvested by spinning at 6,084x gravity (g) for 20 minutes at 4°C, decanted and drained.

2.4 Purification of TAZ1/TAZ2

Cell pellets were resuspended in 1/20 volume sonication buffer (25mM MOPS, pH 7, 50 μM $ZnCl_2$, 300 mM NaCl, 50mM DTT, and 0.5%(v/v) Triton X-100). 1mM PMSF (phenylmethylsulfonyl fluoride in 100%(v/v) ethanol) was added to the sample just before lysing cells by microfluidizer. After lysis by microfluidizer the cell lysates were spun down at 38,724 x(g) using Sorvall RC5B centrifuge SS-34 rotor for 30 minutes at 4°C to separate the soluble fraction from insoluble debris. The soluble fraction was decanted and the pellet (insoluble fraction) was washed twice with 50mL sonication buffer (with no Triton X-100). Each wash was centrifuged at 38,724 xg (SS-34 rotor) for 30 minutes, decanted and saved. The recombinant proteins were resuspended in solubilization buffer (25mM MOPS pH 7.0, 7M Guanidine HCl, and 200mM DTT), and allowed to sit at 4°C for 24-36 hours. The pellet was broken into smaller pieces and vortexed for higher efficiency of solubilization. After incubation, any solubilized protein was spun down at 38,724 xg (Sorvall SS-34 rotor) for 60

minutes, and the liquid portion was carefully transferred into a different tube. Denatured, solubilized protein was added drop by drop into HPLC loading buffer (10%(v/v) Buffer B: 95%(v/v) Acetonitrile, 5%(v/v) Water, 0.5%(v/v) TFA, and 90%(v/v) Buffer A: 99.9%(v/v) Water, 0.1%(v/v) TFA) while mixing on a stir plate with a stir bar. The volume of the refolding buffer was 150x the volume of the solubilized protein. The refolded protein was centrifuged by spinning at 16,900 xg at 4^{o}C for 60 minutes, and decanted carefully into a clean beaker. The white precipitate of unwanted protein and cell debris was discarded. The clarified solution containing the protein was passed through bottle top 0.2 μm filter, and loaded onto a 25mm diameter x 100mm long C4 cartridge prep column attached to a Waters™ Delta Prep4000 HPLC (High Pressure Liquid Chromatography) system with a 2996 series photo diode array detector for reverse phase purification. A linear gradient of 10%(v/v) Buffer B to 50%(v/v) Buffer B was run at a flow rate of 30mL/min. p300 TAZ1 fractions of 6mL were collected at the rise of the peak on 10th minute to the end of the peak at the 14th minute characterized by the emergence of peak shoulder indicating major impurities. In the process of p300 TAZ2 purification, fractions of 6mL were collected at the rise of the peak on 13th minute to the end of the peak on 17th minute characterized by the peak shoulder. Fractions were analyzed by Waters™ Analytical-Scale HPLC system (Waters 600S Controller, Waters 2996 Photodiode Array Detector, and Waters 626 pump), the purity of fractions were calculated by the Waters: Empower 3 chromatography analysis package by comparing the area under the peak of TAZ1 or TAZ2 to the total area under the peaks in the chromatogram. Suitable pure fractions that are greater than 98% purity were pooled and then quantified based on protein extinction coefficient (ε) generated by Expasy online ProtParam

tool (Appendix G) and calculated by Beer's Law ($A=\varepsilon lc$) where A is absorbance at 280nm measured by spectrophotometer (Cary) and l is the path length. Aliquots of 0.25 - 0.35 μmoles of ~98% pure protein were prepared and lyophilized.

2.5 Expression and Purification of MTF-1 zf6-acidic domain

Production of MTF-1 zf6-acidic domain protein was carried out as described previously from our laboratory (Li, et al. 2008).

2.6 DNA Sequencing

The nucleotide primary sequence of all TAZ1, TAZ2, and the cDNA of p300 purchased were confirmed by DNA sequencing at the University of Missouri DNA Core Facility, Columbia, Missouri (Appendix A). Primers for sequencing of TAZ domains were complimentary to the T7 promoter and T7 terminator regions of the pET21a vector (Appendix C).

2.7 Mass Spectrometry

MALDI-TOF analysis was carried out on purified samples of TAZ1 and TAZ2 protein domains to confirm their respective identities (Appendix H). Mass Spectrometry and MALDI analysis were done at the School of Biological Sciences, The University of Missouri – Kansas City Proteomics Facility, Kansas City, Missouri.

2.8 NMR Spectroscopy

All NMR spectroscopy was conducted using a 14.1T (600MHz at the proton carrier frequency) Varian Inova NMR instrument equipped with a cryogenically cooled ^{1}H, ^{13}C, ^{15}N triple resonance probe at the School of Biological Sciences high field NMR core Facility housed within the Laity Lab. All NMR spectra were recorded at 25^{o}C using an FTS (FTS Systems TC-84) temperature control system integrated with the NMR probe. NMR samples were prepared from lyophilized protein (TAZ1, TAZ2, and/or zf6-405) solubilized in 10mM Tris, 50mM NaCl, 1mM DTT pH 6.9, 0.2mM DSS, 5%(v/v) D_2O to final concentration of

protein 0.5mM for TAZ1, 0.7mM for TAZ2, 0.7mM for zf6-405 (zf6-acidic domain, MTF-1) separately. Complex (TAZ1: MTF-1 zf6-acidic domain, TAZ2:MTF-1 zf6-acidic domain) molar concentration ranged from 0.25mM, to 0.35mM, with significant precipitation formation. ^{1}H-^{15}N-HSQC spectra were collected for TAZ1 titrated in quarter equivalents of MTF-1 zf6-acidic domain, and for TAZ2 in one third equivalents. ^{15}N labeled MTF-1 zf6-acidic domain was resuspended in 20mM MES, 5mM β-mercaptoethanol, 1mM NaN_3, 5%(v/v) D_2O in the presence of 1 equivalent zinc. ^{1}H-^{15}N-HSQC spectra were collected for ^{15}N labeled MTF-1 zf6-acidic domain titrated in one third molar equivalents. NMR data were processed using NMR Pipe Software (NMR Science, Campbell, CA). Data were analyzed using NMR View software (One Moon Scientific, Inc.)

CHAPTER 3

RESULTS

3.1 p300 TAZ1 and TAZ2 Protein Production

The TAZ1 and TAZ2 domains of mouse p300 were amplified, cloned and over-expressed at 37^{o}C (De Guzman, et al. 2005). Both TAZ1 and TAZ2 domains of p300 were found to be expressed and packaged into intracellular inclusion bodies in the *E. coli* host (Figure 2a). The advantage to purifying protein from insoluble fraction is the lower amount of contaminating proteins if the unfolded protein can be purified and subsequently refolded.

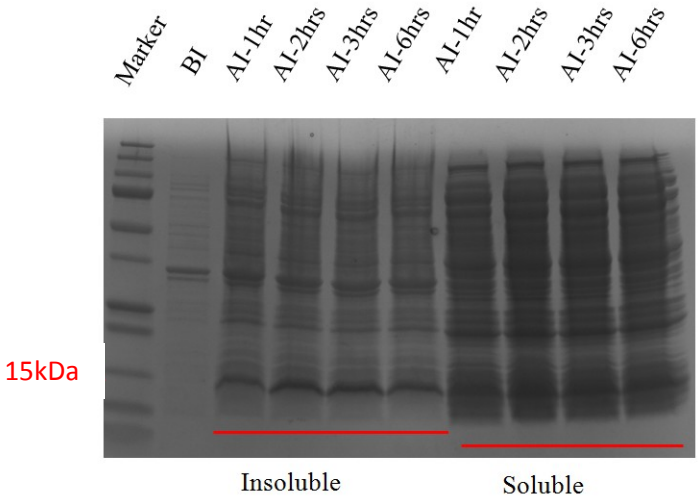


Figure 2a. p300 TAZ1 (band at 15 kDa) overexpression in *E. coli* BL21 Codon Plus RIPL cells. Cell lysates were analyzed by SDS-PAGE, and visualized by Coomassie blue staining. The expressed protein is running at 15kDa molecular weight marker in insoluble fraction. Times points: BI, AI 1hr, AI 2hrs, AI 3hrs, and AI 6hrs.

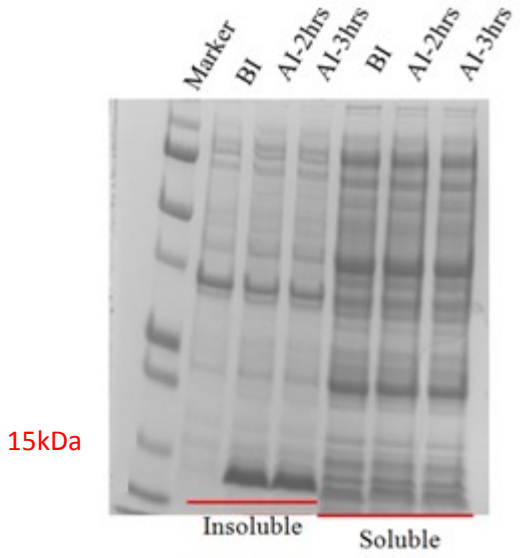


Figure 2b. p300 TAZ2 (band at 15kDa) overexpression in *E. coli* BL21 Codon Plus RIPL cells. Cell lysates were analyzed by SDS-PAGE, and visualized by Coomassie blue staining. The expressed protein is running at 15kDa molecular weight marker in insoluble fraction. Time points: BI, AI 1hr, AI 2hrs, and AI 3hrs.

Separation of the insoluble fraction and soluble fraction (as described in methods) decreased the contaminating proteins (Figure 2). The number of bands is significantly lower in the insoluble fraction where TAZ1 and TAZ2 (Figures 2a and 2b, respectively) can be observed co-migrating with the 15kDa molecular weight marker.

3.2 p300 TAZ1 and TAZ2 purification

p300 TAZ domains were purified to homogeneity by HPLC as described in the methods section. First, an analytical analysis was performed to identify the percentage of solution B that elutes p300 TAZ1 and TAZ2 domains (Figure 3a and 3b, respectively). Significant peaks (AU > 0.05) were collected and analyzed by SDS-PAGE (Figure 3c and 3d). Subsequently, bands corresponding to initial SDS-PAGE after-induction sample showing TAZ1 or TAZ2 expression (Figure 2) at 15 kDa molecular weight marker were identified via mass spectrometry (Figure 4). Mass spectrometry confirmed the identity of an analytical HPLC fraction collected between the 21st minute (34%(v/v) solution B) and 23rd minute to be p300 TAZ1 (Figure 4a). In the same manner, p300 TAZ2 domain was analyzed by analytical HPLC (Figure 3b) (39%(v/v) solution B), SDS-PAGE (Figure 3d) and confirmed by mass spectrometry (Figure 4b).

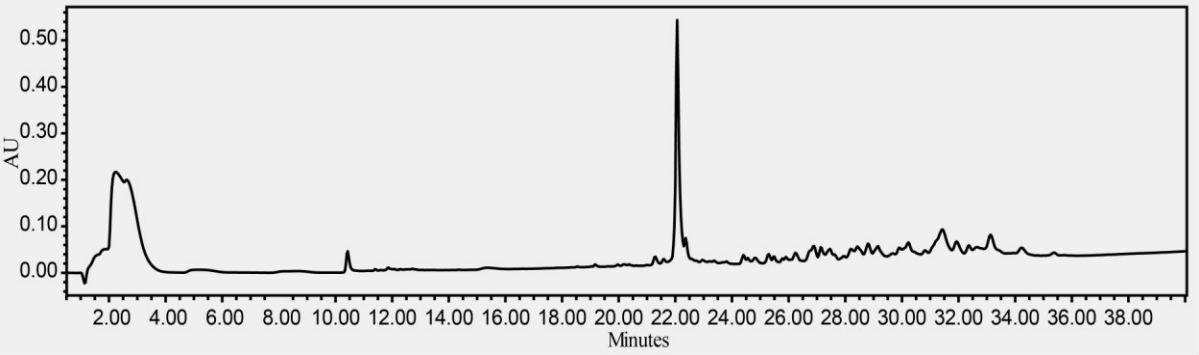


Figure 3a. Analytical HPLC of p300 TAZ1 domain using a C4 column, at a rate of 2mL/min, and gradient of 0% - 100%B. p300 TAZ1 domain observed as a peak between 21 and 23 minutes post injection. Absorbance at 220nm (A_{220}).

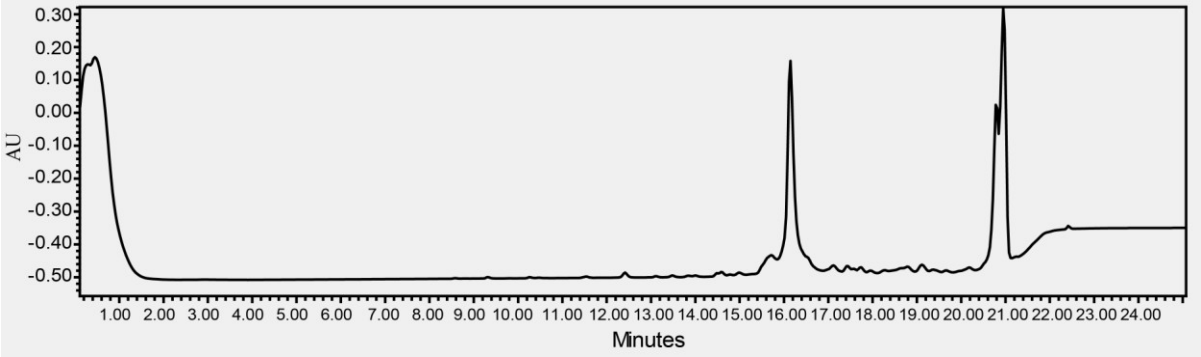


Figure 3b. Analytical HPLC of p300 TAZ2 domain using a C4 column, at a rate of 2mL/min, and gradient of 0% - 100%B. p300 TAZ2 domain observed as a peak between 21 and 23 minutes post injection. (A_{220}).

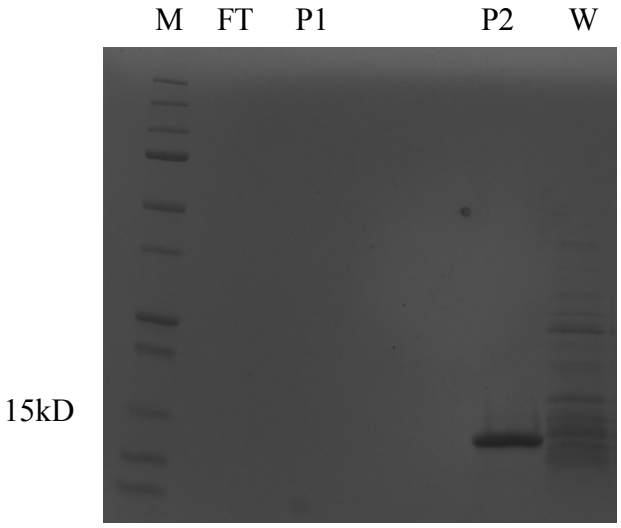


Figure 3c. p300 TAZ1 SDS PAGE analysis of analytical HPLC (Figure 3a). M: Marker; FT: Flow Through - Peak at 2nd minute; P1: small peak at 10th minute; P2: Large peak at 21st minute; W: Wash - 100 % solution B elutes other proteins bound to C-4 column. The band observed at the P2 15 kDa molecular weight marker was analyzed by mass spectrometry.

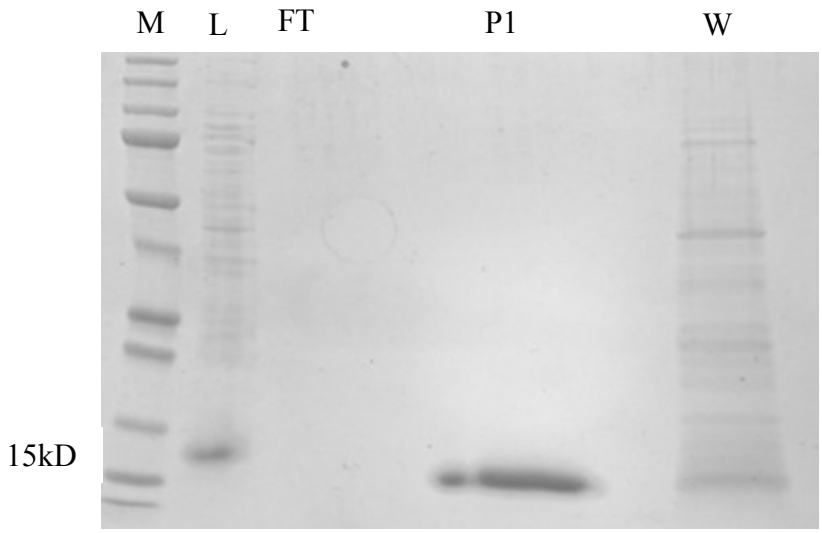


Figure 3d. p300 TAZ2 SDS PAGE analysis of analytical HPLC (Figure 3a). M: Marker; L: Load; FT: Flow Through - Peak at 2nd minute; P1: Large peak at 21st minute; W: Wash - 100 % solution B elutes other proteins bound to C-4 column. The band observed at the P2 15 kDa molecular weight marker was analyzed by mass spectrometry.

20. EP300_HUMAN **Mass:** 266898 **Score:** 115 **Matches:** 23(5) **Sequences:** 8(3) **emPAI:** 0.04

Histone acetyltransferase p300 OS=Homo sapiens GN=EP300 PE=1 SV=2

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Unique	Peptide
623	902.4550	901.4477	901.4253	0.0224	0	27	7.6	4	U	R.EQANGEVR.Q 622 624 625
635	456.3679	910.7212	910.5025	0.2188	0	20	33	7	U	R.QIISHWK.N 634 636
898	542.7999	1083.5852	1083.4702	0.1150	0	40	0.28	1	U	R.QCNLPHCR.T 899 900 904 907
995	561.4064	1120.7982	1119.5746	1.2236	1	9	4.4e+002	2	U	K.LYATMEKHK.E
1138	413.5812	1237.7218	1237.5948	0.1270	0	(20)	26	2	U	R.HDCPVCLPLK.N
1139	619.8869	1237.7592	1237.5948	0.1645	0	51	0.019	1	U	R.HDCPVCLPLK.N 1132 1133 1137
1161	632.0764	1262.1382	1261.5292	0.6091	0	67	0.00069	1	U	K.SCQVAHCASSR.Q 1159 1160
1582	591.0505	1770.1297	1768.8171	1.3125	1	32	1.5	1	U	K.NCTRHDCPVCLPLK.N
1586	595.2595	1782.7567	1781.1039	1.6527	1	49	0.023	1		R.KLIQQQLVLLLHAHK.C

Figure 4a. Mass spectrometry of p300 TAZ1 SDS-PAGE gel band (Figure 3c).

5. gi|109481045 **Mass:** 266301 **Score:** 386 **Matches:** 42(6) **Sequences:** 7(3) **emPAI:** 0.04

PREDICTED: similar to E1A binding protein p300 [Rattus norvegicus]

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Unique	Peptide
287	356.3439	710.6732	710.4075	0.2657	0	44	2.3	2		R.VVQHTK.G 279 281 282 284 285 288 290 291 292
976	504.1439	1006.2732	1005.4372	0.8361	0	41	6.2	2		K.TNGGCPICK.Q 977 981 983
980	1007.6220	1006.6147	1005.4372	1.1775	0	(25)	2.3e+002	7		K.TNGGCPICK.Q 974
1258	568.3504	1134.6862	1133.5322	1.1541	1	50	0.71	1		R.KTNGGCPICK.Q 1255 1257
1451	624.7689	1247.5232	1246.6202	0.9030	0	53	0.32	2		K.CPVPFCLNIK.Q 1449 1450 1457
1516	1278.6620	1277.6547	1277.5492	0.1055	0	(51)	0.57	1	U	R.NANCSLPSCQK.M
1517	640.1459	1278.2772	1277.5492	0.7280	0	64	0.028	1	U	R.NANCSLPSCQK.M 1513 1514 1518
1627	688.9544	1375.8942	1375.6740	0.2202	0	68	0.0087	1		K.QLIALCCYHAK.H 1628 1629
1782	766.7749	1531.5352	1530.6854	0.8499	0	76	0.0015	1		R.CIQSLVHACQCR.N 1776 1777 1778 1779 1780 1781 1783 1784
1787	511.8762	1532.6068	1530.6854	1.9214	0	(32)	38	7		R.CIQSLVHACQCR.N 1785

Figure 4b. Mass spectrometry of p300 TAZ2 SDS-PAGE gel band (Figure 3d).

After the identities of the expressed proteins (p300 TAZ1 and TAZ2) were confirmed and the purification protocol was adapted, TAZ1 and TAZ2 domains of p300 were expressed in large quantities. After expression in large quantities, p300 TAZ1 domain (Figure 5a) and TAZ2 domain (Figure 6a) were purified as described in the methods section by reverse-phase HPLC. p300 TAZ1 domain eluted between the 11th and 13th minute (Figure 5a), while the TAZ2 domain eluted between the 13th and 16th minute. Fractions of TAZ1 and TAZ2 were collected in these time frames and were then analyzed in analytical-scale HPLC. As indicated in the methods pure fractions were quantified, pooled and lyophilized. Subsequently, lyophilized protein was used in further NMR studies.

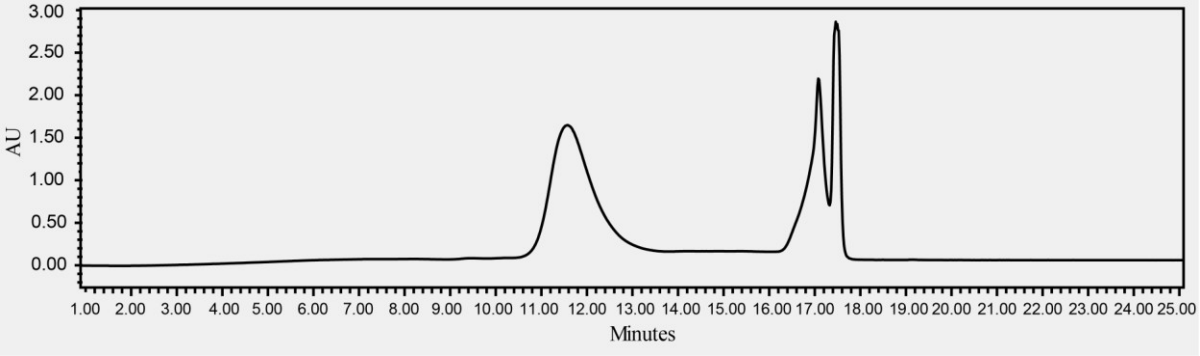


Figure 5a. Reversed-phase HPLC trace of p300 TAZ1 domain. Fractions were collected between 10 and 13 minutes post injection. Gradient: 10%B to 50%B at 2%/min. Flow: 30mL/min, fractions of 6mL analyzed by Waters Analytical-Scale HPLC. (A_{220})

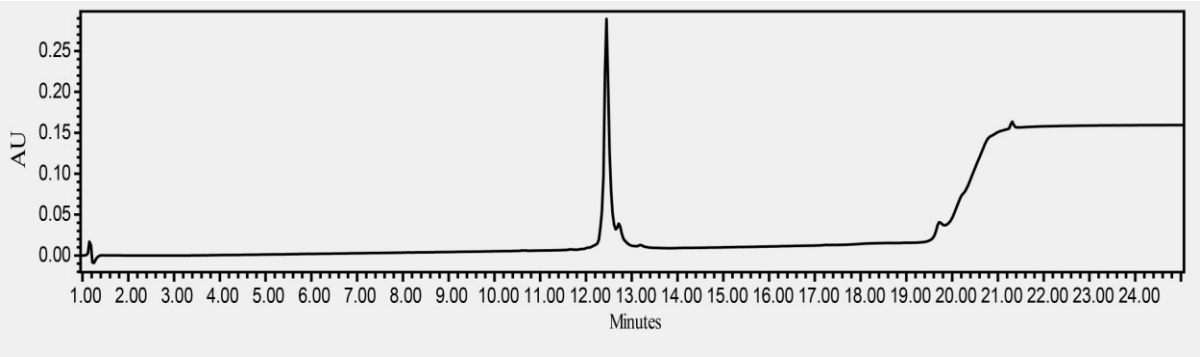


Figure 5b. Analytical HPLC of p300 TAZ1 domain fraction number 29, using a C4 column, at a rate of 2mL/min, and gradient of 10% - 50%B. The p300 TAZ1 domain was observed as a peak between 12 and 13 minutes post injection with minor shoulder indicating impurity. (A_{220}).

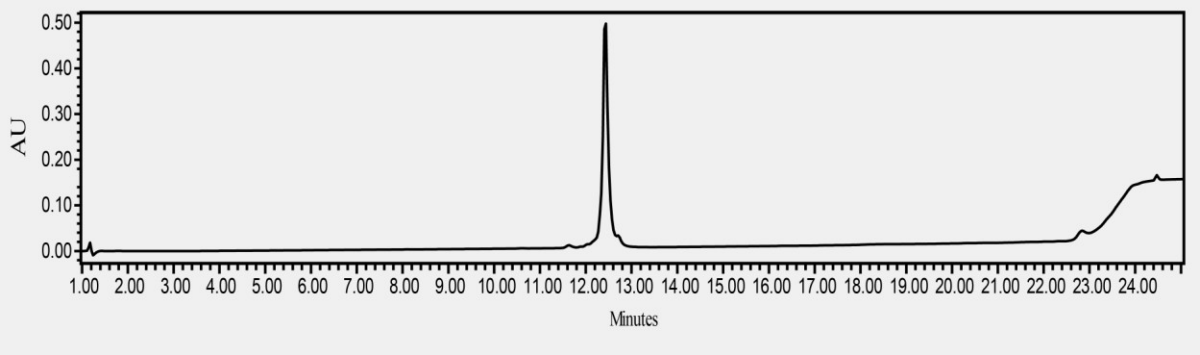


Figure 5c. Analytical HPLC of p300 TAZ1 domain fraction number 23 using a C4 column, at a rate of 2mL/min, and gradient of 10% - 50%B. The p300 TAZ1 domain was observed as a peak between 12 and 13 minutes post injection. (A_{220}).

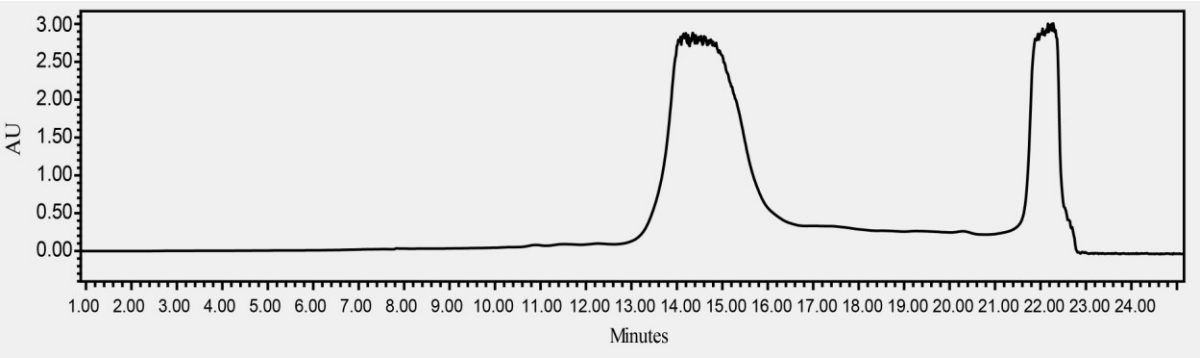


Figure 6a. Reversed-phase HPLC trace of p300 TAZ2 domain. Fractions were collected between 13 and 17 minutes post injection. Flow: 30mL/min, fractions of 6mL analyzed by Waters Analytical-Scale HPLC. (A_{220}).

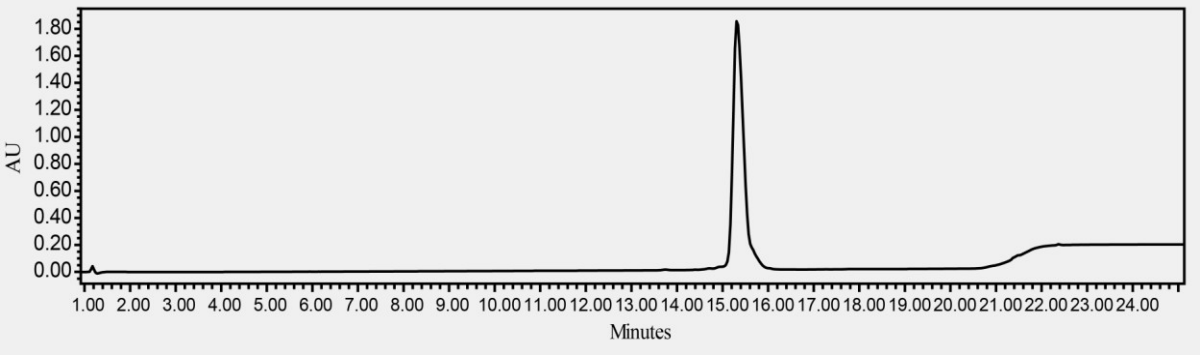


Figure 6b. Analytical-scale HPLC of p300 TAZ2 domain fraction number 23 using a C4 column, at a rate of 2mL/min, and gradient of 10% - 50%B. The p300 TAZ2 domain was observed as a peak between 15 and 16 minutes post injection. (A_{220}).

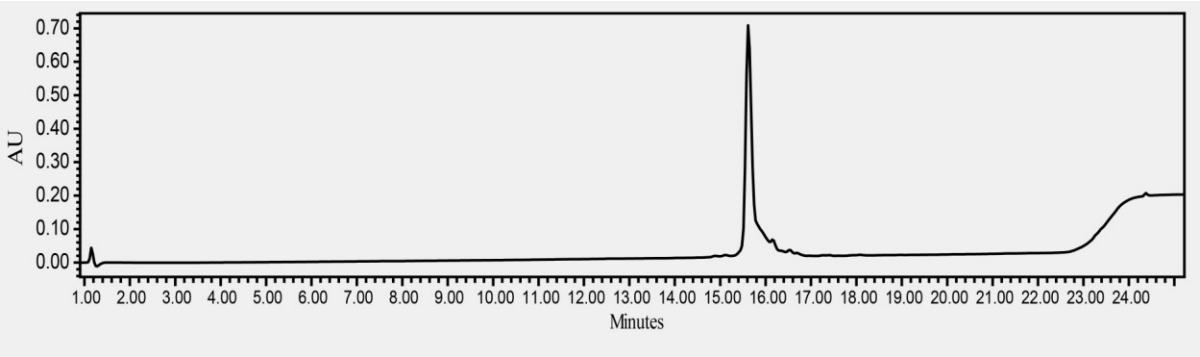


Figure 6c. Analytical-scale HPLC of p300 TAZ2 domain fraction number 33 using a C4 column, at a rate of 2mL/min, and gradient of 10% - 50%B. The p300 TAZ2 domain was observed as a peak between 15 and 16 minutes post injection. (A_{220}).

3.3 NMR Spectra of Uniformly-^{15}N Labeled p300 TAZ Domains, p300 TAZ Domains in Mixture with Unlabeled MTF-1 zf6-acidic domain.

The natural and most abundant form of nitrogen is ^{14}N, but due to even number of protons and neutrons in the nuclei of ^{14}N, NMR cannot sense the atoms, as there is no overall spin of the nucleus. The ^{15}N nucleus has 8 neutrons and 7 protons which yields a nuclear spin quantum number of (-$\frac{1}{2}$). All atoms that have nuclear spin quantum number of $\frac{1}{2}$ are visible to NMR spectroscopy. All atoms present in proteins, aside from oxygen, can be visible to NMR. The HSQC, heteronuclear quantum coherence, experiment collects data on each ^{15}N-^{1}H amide pair of each amino acid. The data collected is in terms of frequency (f) which is then, by Fast Fourier Transform, converted to parts per million (ppm) and visualized as a peak corresponding to x-axis (^{1}H) and y-axis (^{15}N) ppm.

p300 TAZ1 uniformly labeled with ^{15}N was studied by NMR approaches. Initially, an ^{1}H-^{15}N HSQC spectrum was recorded on the p300 TAZ1 domain without zinc; this showed very poor spectral peak dispersion (data not shown), which is indicative of an unstructured protein. However, when three molar equivalents of zinc were added to the p300 TAZ1 domain, the resulting ^{1}H-^{15}N HSQC spectral peaks were much more dispersed, which is indicative of a structured protein (Figure 7). Because the MTF-1 zf6-acidic domain recombinant protein includes zinc finger 6 (zf6) as well, one molar equivalent of zinc was added to sample to ensure that this zf6 site was saturated with metal. Then the unlabeled MTF-1 zf6-acidic domain was added in quarter molar equivalence until a one to one molar ratio was achieved. This allowed four ^{1}H-^{15}N HSQC data sets to be collected (Figure 8).

Similarly, the p300 TAZ2 domain was uniformly labeled with ^{15}N for NMR studies. ^{1}H-^{15}N HSQC data was collected from p300 TAZ2 with 3 equivalents of zinc (Figure 9), and unlabeled MTF-1 zf6-acidic domain was likewise titrated to TAZ2 at third molar equivalence until a one to one molar ratio was reached (Figure 10). The ^{1}H-^{15}N HSQC dispersion of TAZ2 and MTF-1 zf6-acidic domain complex showed subtle differences in peak dispersion. Peak shifts were calculated by a weighted chemical shift formula $\Delta av(NH) = \sqrt{(\Delta H)^2 + (\Delta N / 3.63)^2)}$ (Grzesiek, et al. 1996). The differences in peak positions in ^{1}H-^{15}N HSQC dispersion are indicative of interaction between these two proteins. Figures 11 and 12 showed weighted chemical shift changes for TAZ2 and TAZ1 respectively.

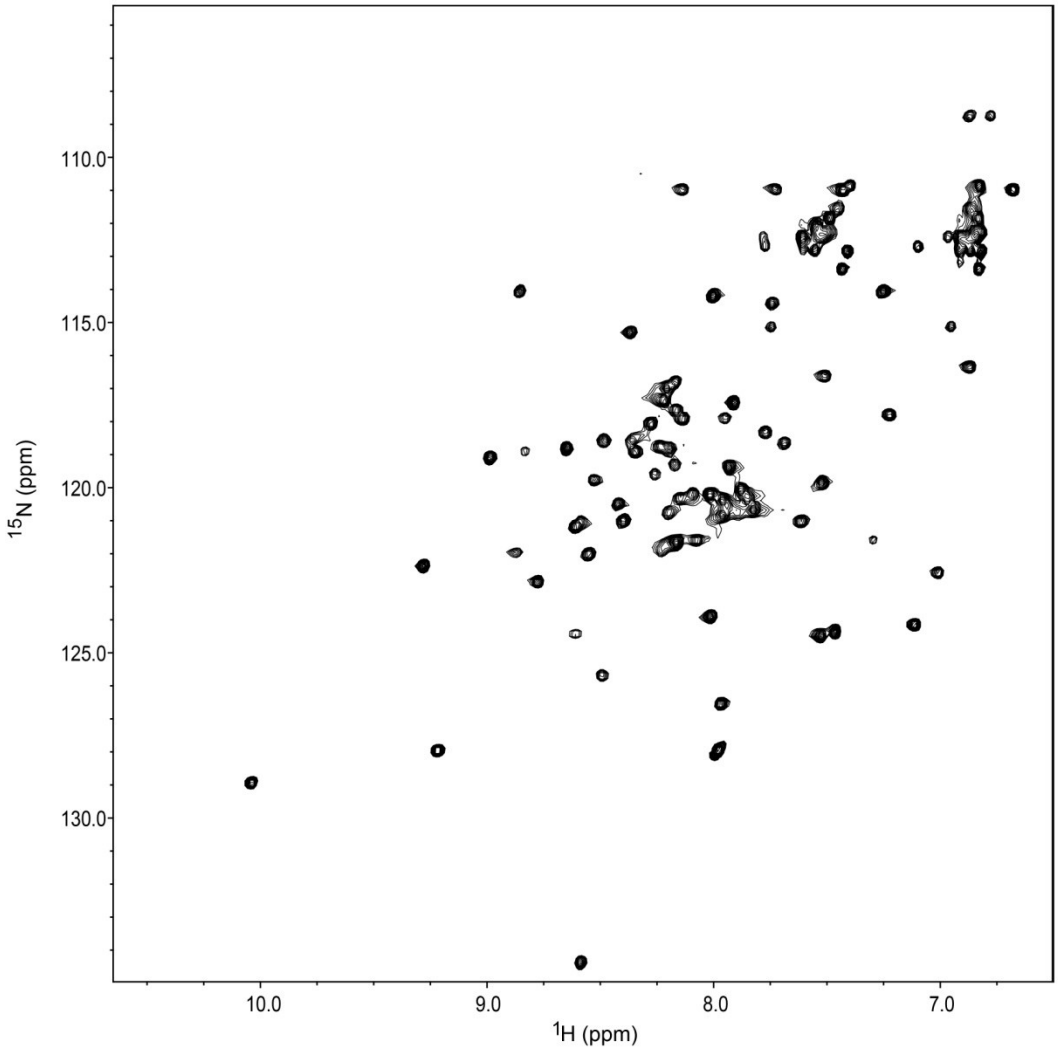


Figure 7. ^{1}H-^{15}N HSQC of p300 TAZ1 domain in the presence of 3 equivalents of zinc.

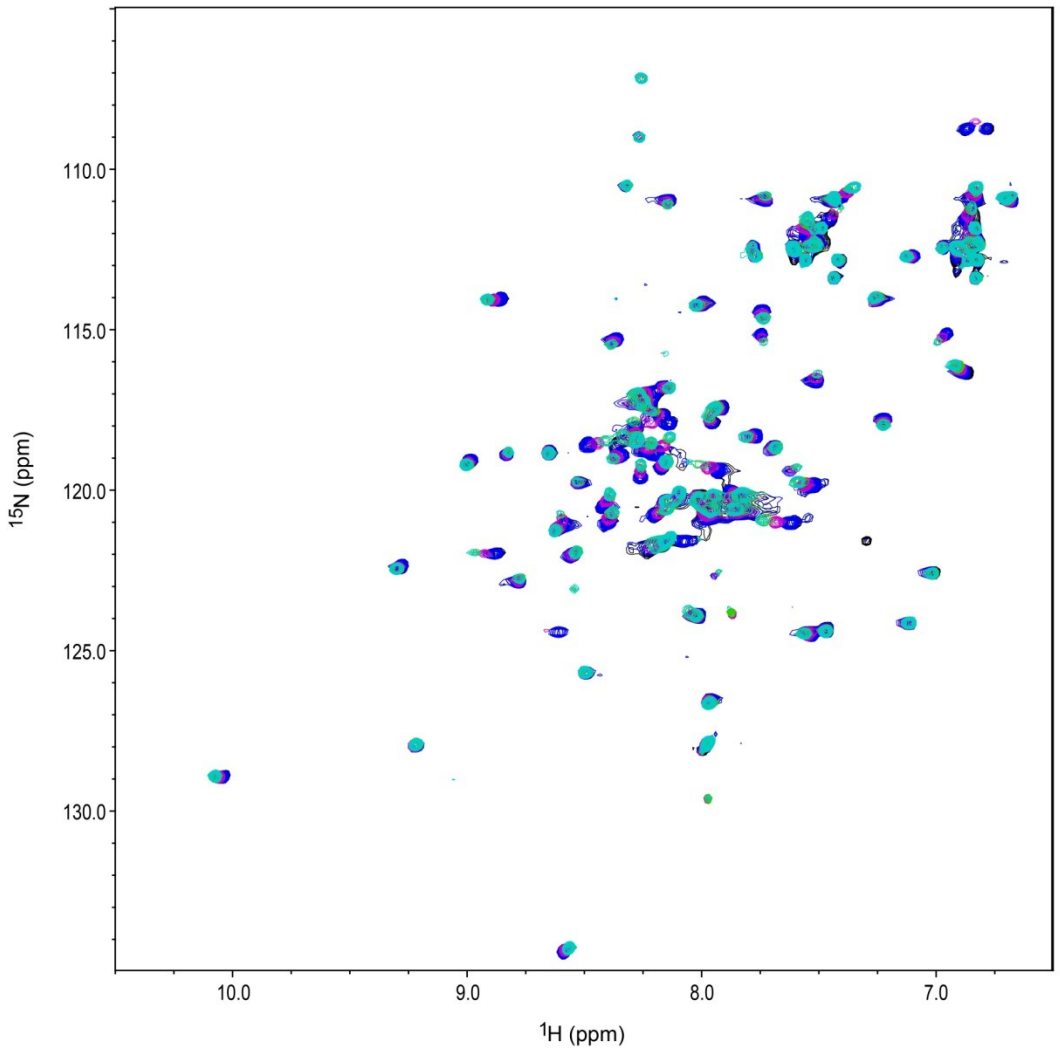


Figure 8. ^{1}H-^{15}N HSQC overlay of p300 TAZ1 domain, in the presence of 3 equivalents of zinc, and a p300 TAZ1 concentration to MTF-1 zf6-acidic domain concentration ratios of 1 to 0 - black, 1 to 0.25 - blue, 1 to 0.5 - purple, 1 to 0.75 - light green, 1 to 1 - cyan.

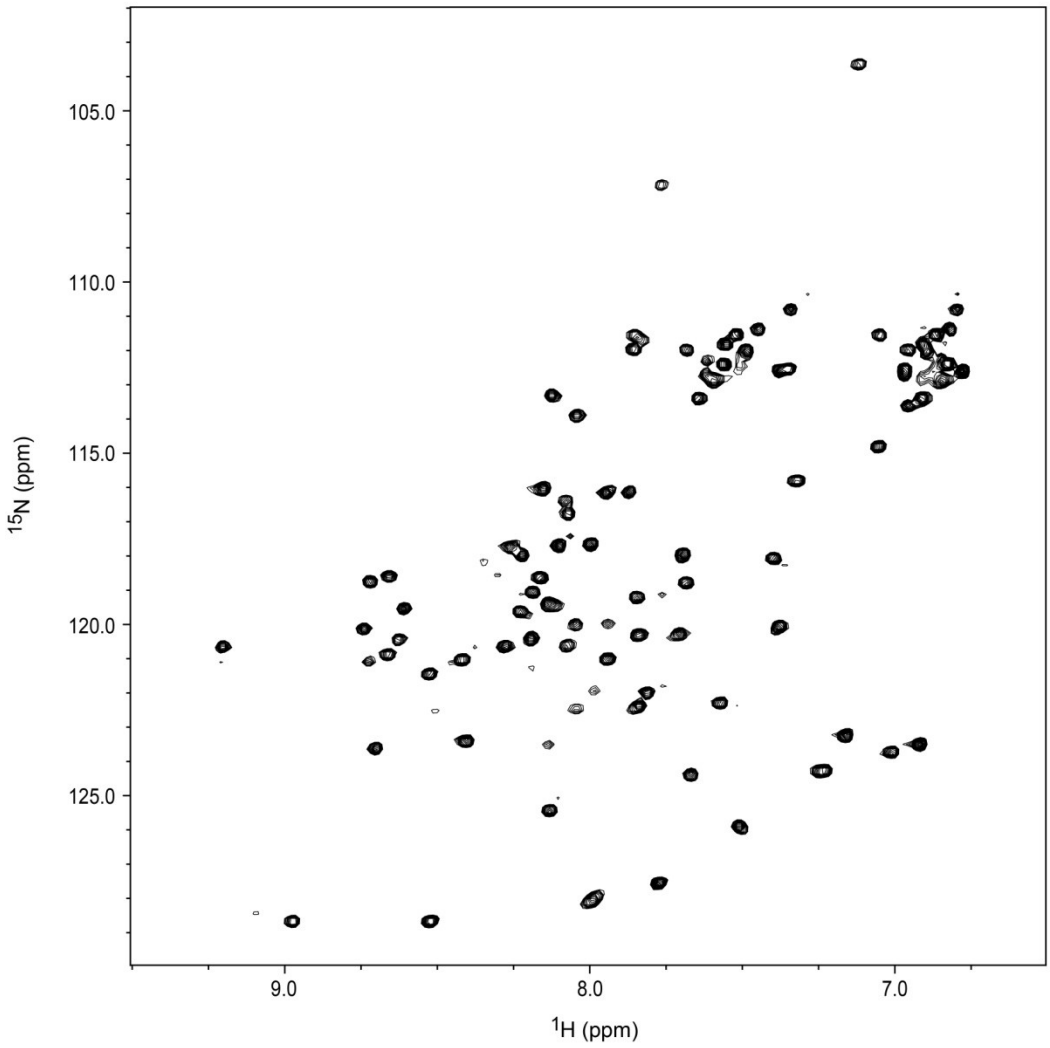


Figure 9. ^{1}H-^{15}N HSQC of p300 TAZ2 domain in the presence of 3 equivalents of zinc.

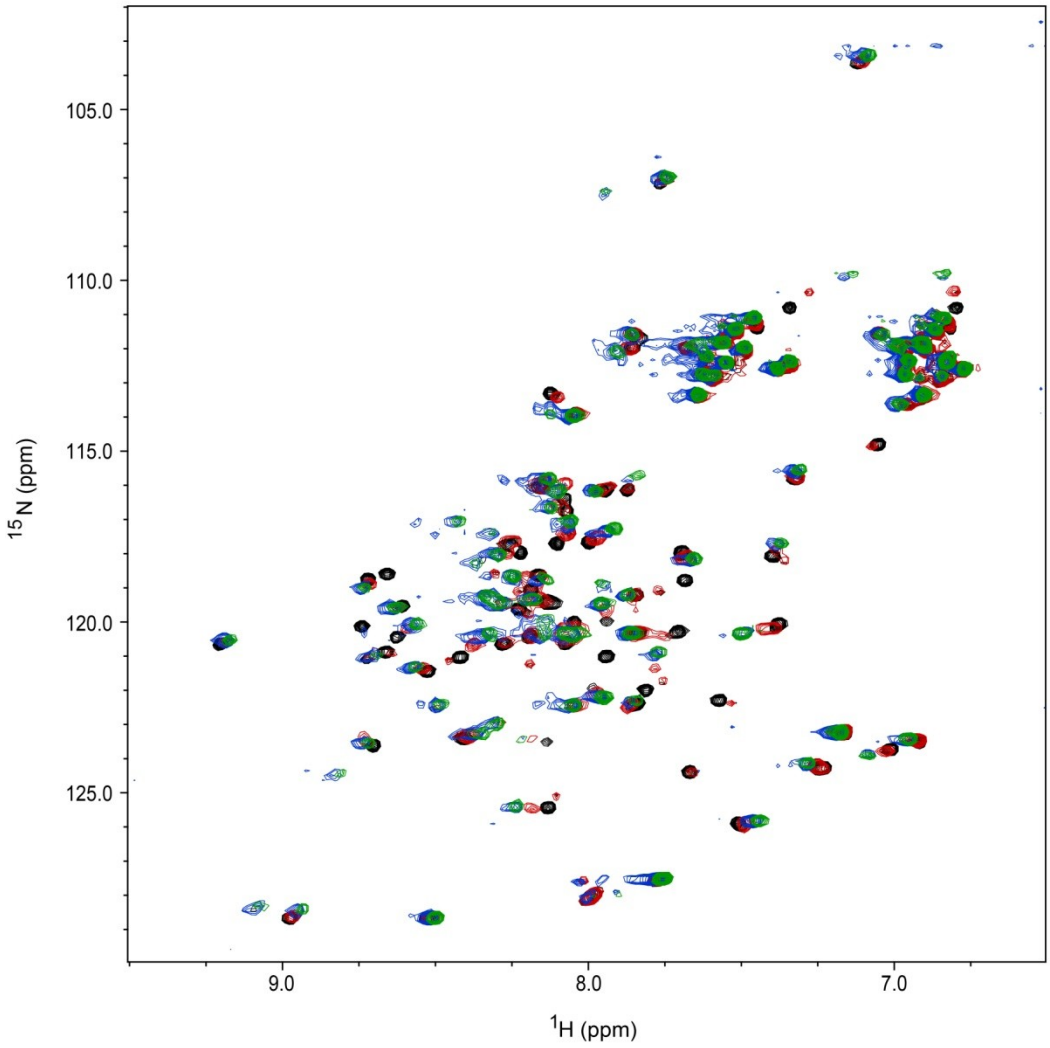


Figure 10. ^{1}H-^{15}N HSQC overlay of p300 TAZ2 domain, in the presence of 3 equivalents of zinc, and a p300 TAZ2 concentration to MTF-1 zf6-acidic domain concentration ratios of 1 to 0 - black, 1 to 0.3 - red, 1 to 0.6 - blue, 1 to 1 - blue.

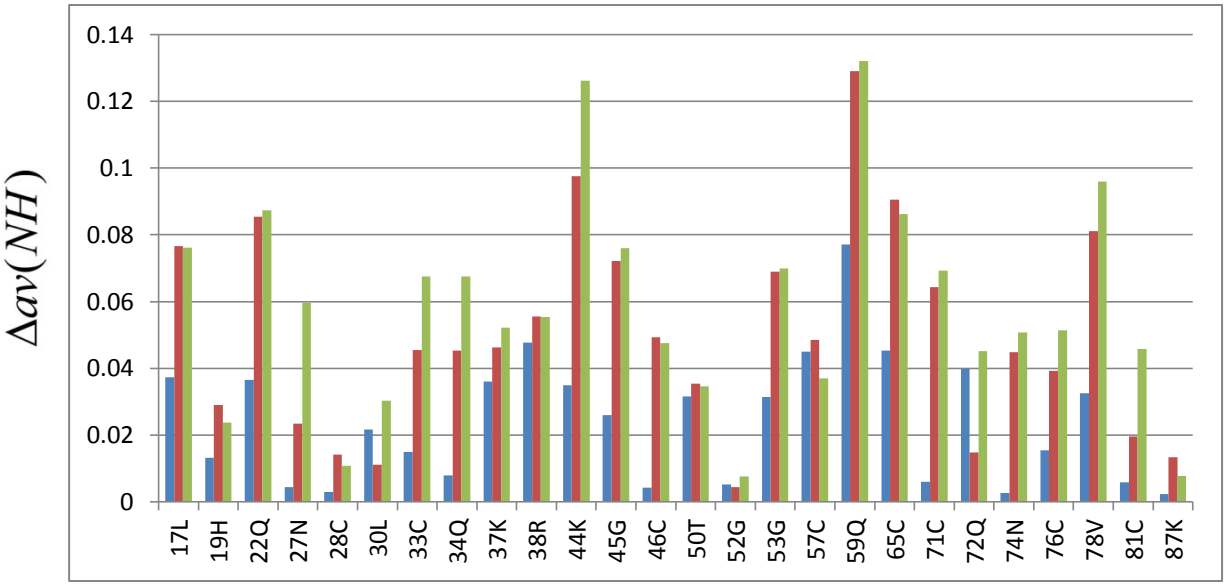


Figure 11. Weighted amide HN chemical shifts differences calculated by the following formula $\Delta av(NH) = \sqrt{(\Delta H)^2 + (\Delta N / 3.63)^2)}$ (Grzesiek, et al. 1996). ^{15}N labeled p300 TAZ2 domain was in the presence of 3 equivalents of zinc. The p300 TAZ2 concentration to unlabeled MTF-1 zf6-acidic domain concentration ratios are 1 to 0.3 - colored blue, 1 to 0.6 - colored red, 1 to 1 - colored green. (Standard deviation of 0.0361, and the mean of 0.0512)

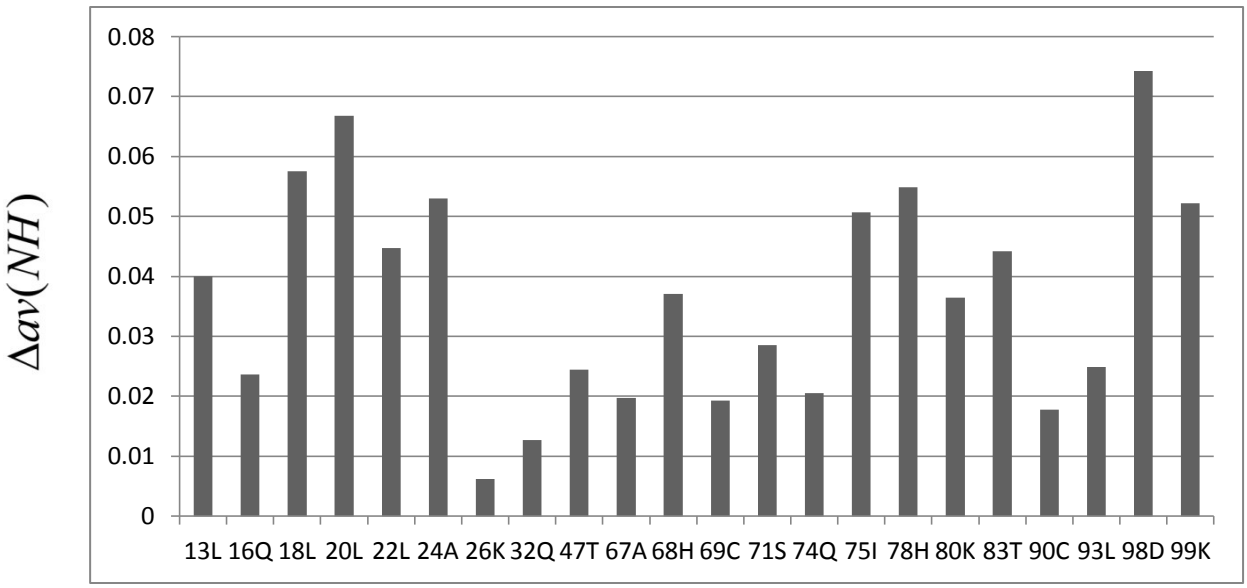


Figure 12. Weighted amide HN chemical shifts differences calculated by the following formula $\Delta av(NH) = \sqrt{(\Delta H)^2 + (\Delta N / 3.63)^2)}$ (Grzesiek, et al. 1996). ^{15}N labeled p300 TAZ1 domain was in the presence of 3 molar equivalents of zinc. Weighted chemical shift differences for molar concentration ratio of 1.0 to 1.0, ^{15}N Labeled p300 TAZ1 to unlabeled MTF-1 zf6-acidic domain are illustrated. (Standard deviation of 0.0185; and the mean of 0.0368)

3.4 Complementary NMR Experiments Involving ^{15}N Labeled MTF-1 zf6-acidic domain and Unlabeled p300 TAZ2 Domain.

MTF-1 zf6-acidic domain was uniformly labeled with ^{15}N. ^{1}H-^{15}N HSQC spectrum was collected for MTF-1 zf6-acidic domain with 1 molar equivalent of zinc in order to occupy zf6. ^{1}H-^{15}N HSQC dispersion showed structured zf6, but very poor dispersion that was localized to the center of spectrum (Figure 13). Unlabeled TAZ2 in the presence of 3 molar equivalents of zinc was titrated to the zf6-acidic domain in third equivalences until a one to one ratio was reached (Figure 14). Then excess TAZ2 was added to the MTF-1 zf6-acidic domain increasing the ratio of p300 TAZ2 concentration to MTF-1 zf6-acidic domain concentration to 1.5:1.0 (Figure 14). Weighted amide HN chemical shifts of assigned residues were plotted and illustrated in figure 15.

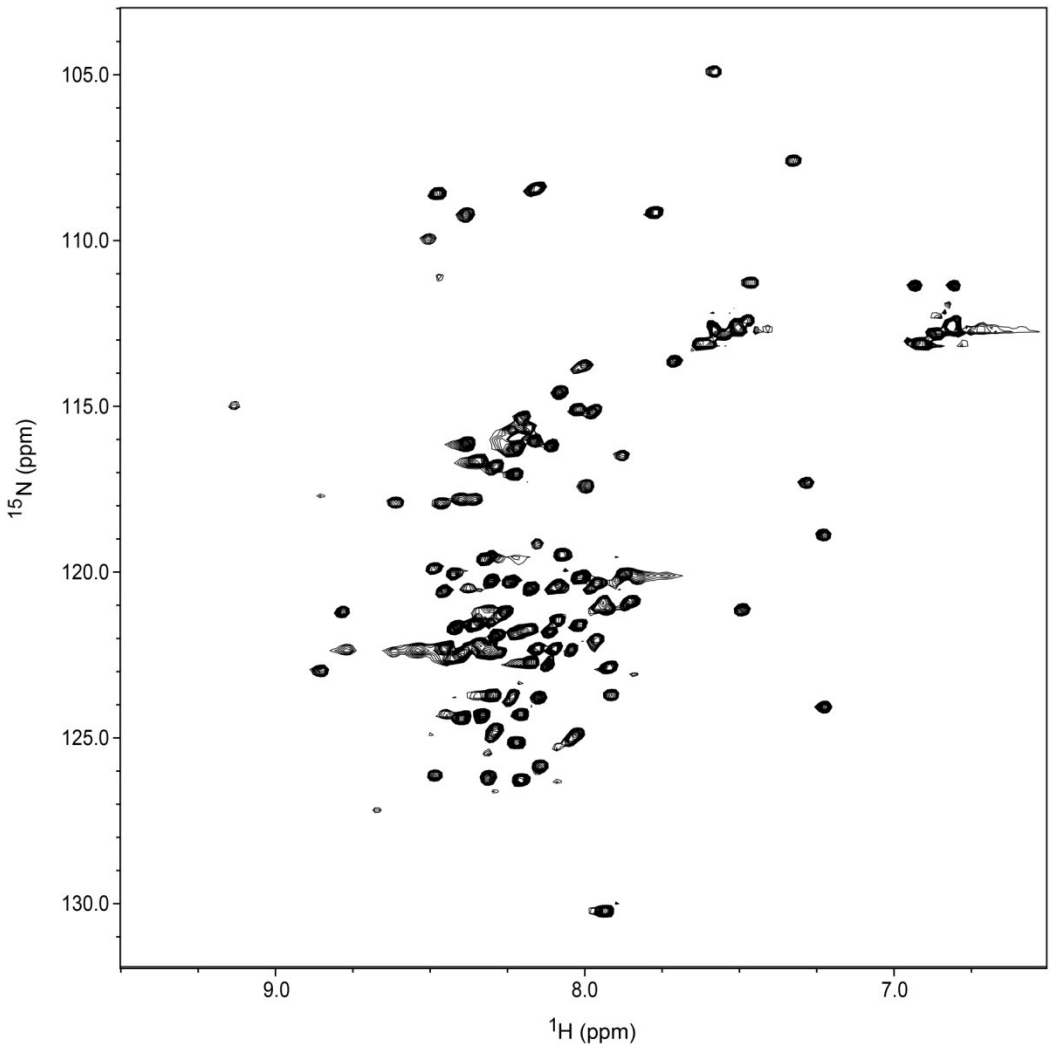


Figure 13. ^{1}H-^{15}N HSQC spectrum of MTF-1 zf6-acidic domain in the presence of 1 molar equivalent of zinc.

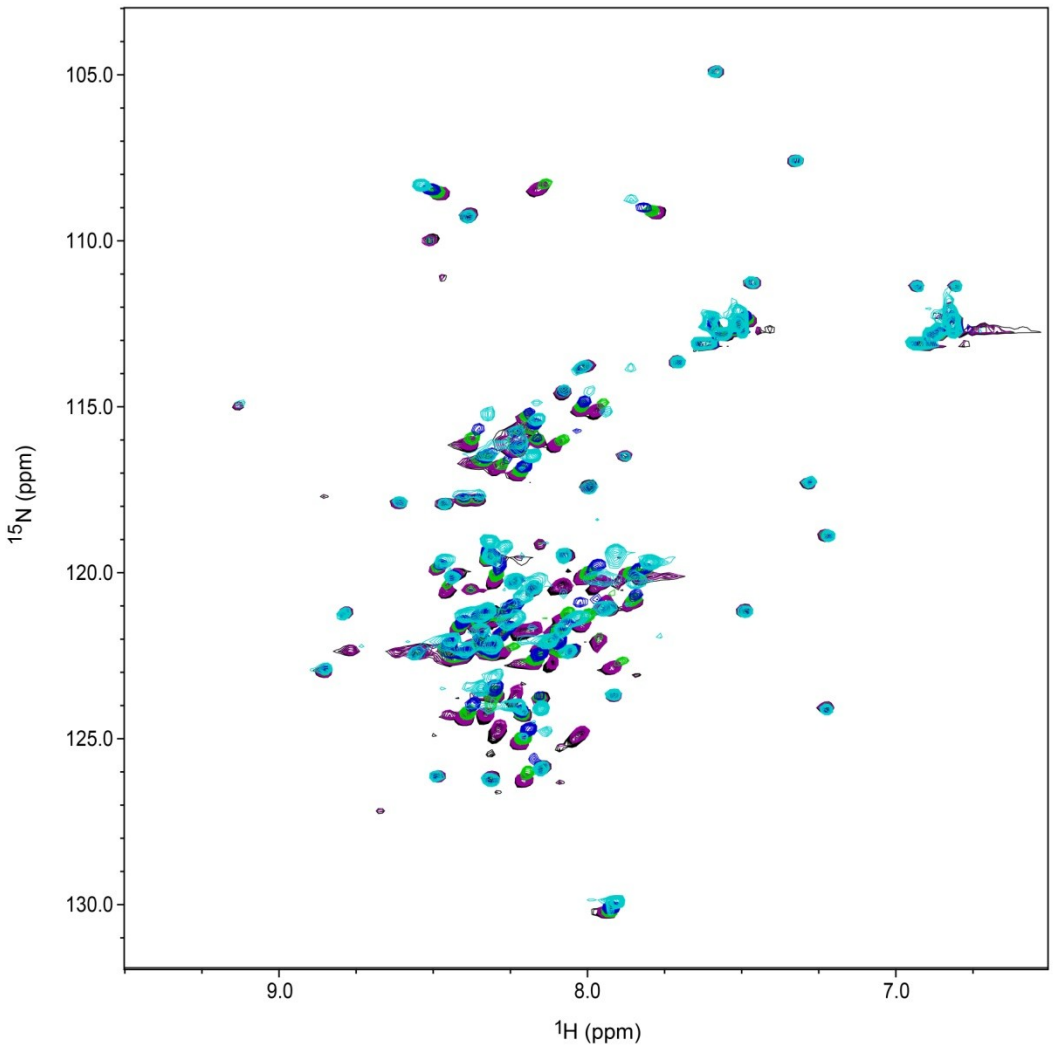


Figure 14. ^{1}H-^{15}N HSQC spectrum of MTF-1 zf6-acidic domain in the presence of 1 molar equivalent of zinc. Unlabeled p300 TAZ2 domain was titrated in 1/3rd molar concentration equivalents and represented in; Black peaks - 0.0 eq. of TAZ2; purple peaks - 0.33 eq. of TAZ2; Green - 0.66 eq. of TAZ2; Blue - 1.0 eq. of TAZ2; Cyan - 1.5 eq. of TAZ2.

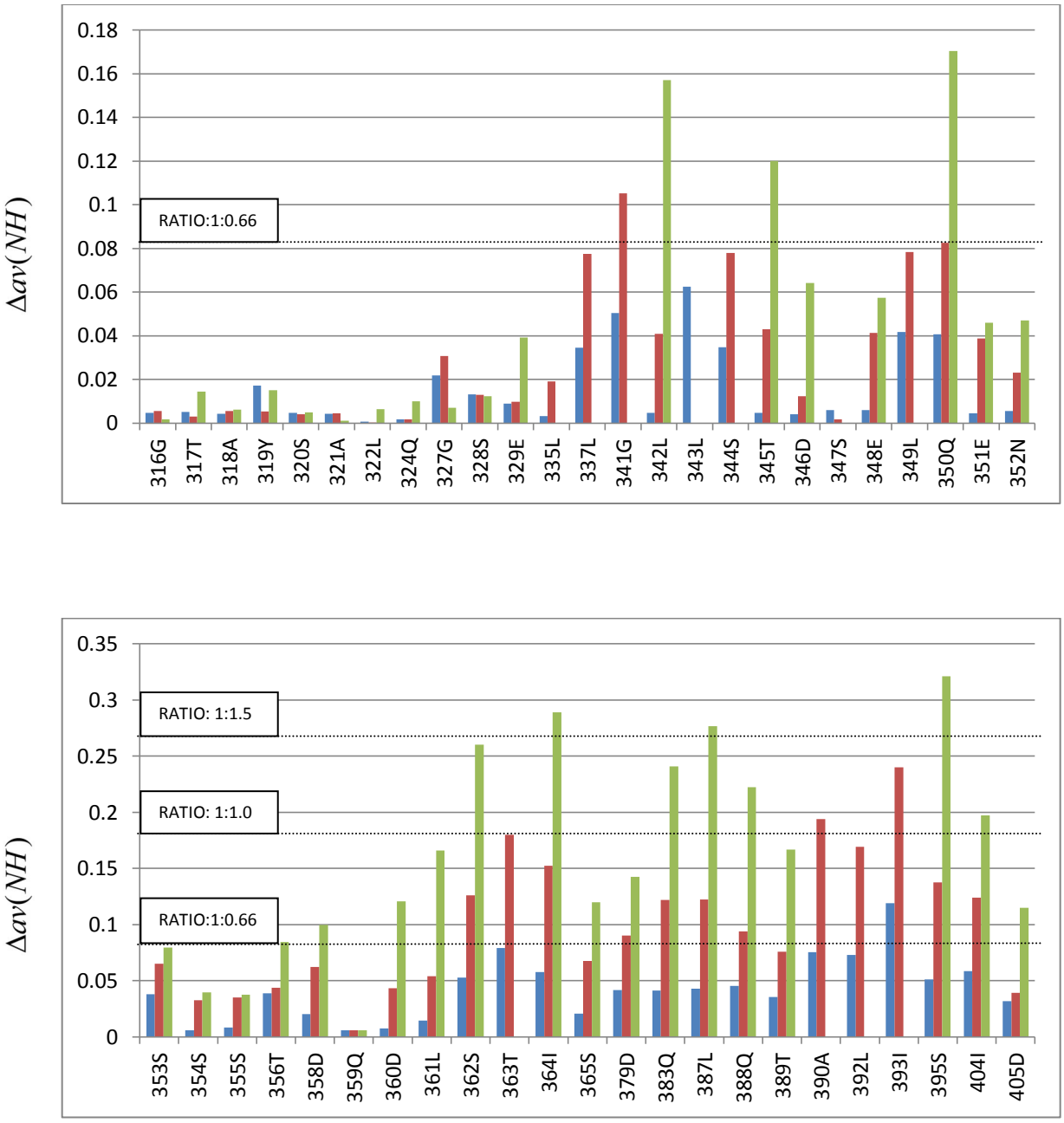


Figure 15. Weighted amide HN chemical shifts differences calculated by the following formula $\Delta av(NH) = \sqrt{(\Delta H)^2 + (\Delta N / 3.63)^2)}$ (Grzesiek, et al. 1996) for MTF-1 zf6-acidic domain and p300 TAZ2 domain titrations in presence of stoichiometric zinc concentrations at the ratios of MTF-1 zf6-acidic domain to p300 TAZ2 domain of 1:0.66 (blue bars), 1:1 (red bars), and 1:1.5 (green bars). Average weighted chemical shift difference per concentration ratio of MTF-1 zf6-acidic domain to p300 TAZ2 plus 2 times the standard deviation is shown.

3.5 NMR Experiments of MTF-1 zf6-acidic domain Titrated with Apo-TAZ2.

^{1}H-^{15}N HSQC spectral analysis of ^{15}N-labeled MTF-1 zf6-acidic domain titrated with unstructured TAZ2 shows no significant weighted amide HN chemical shift difference. Lack of peak shifts indicates that there is no interaction between the MTF-1 zf6-acidic domain and p300 TAZ2 in the absence of zinc. Figure 17 shows an ^{1}H-^{15}N HSQC overlay of different molar ratios of apo-TAZ2 titrations. An ^{1}H-^{15}N HSQC spectrum of MTF-1 zf6-acidic domain in the presence of one molar equivalent of zinc is shown in Figure 16.

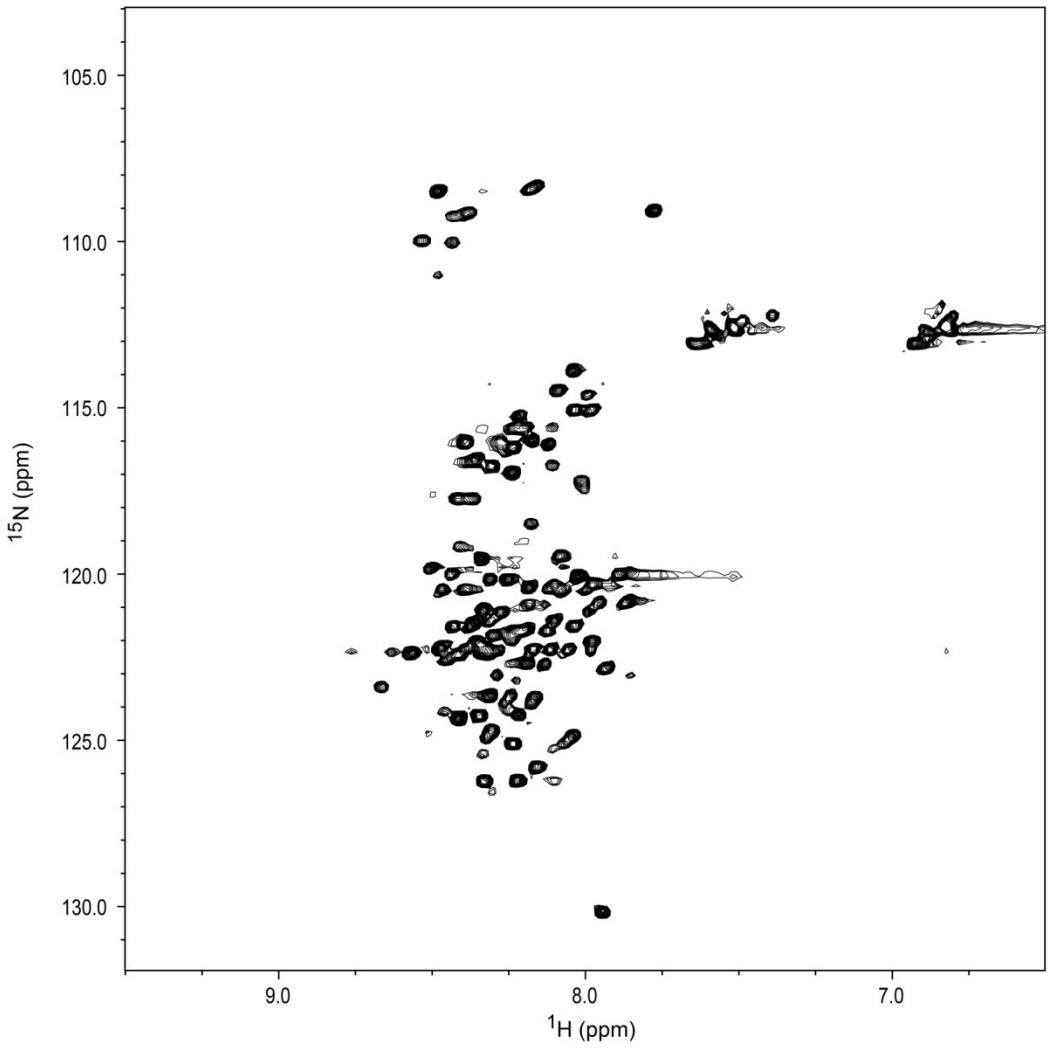


Figure 16. ^{1}H-^{15}N HSQC spectrum of MTF-1 zf6-acidic domain in the presence of 1 molar equivalent of zinc in order to fold zf6 of the MTF-1 zf6-acidic domain construct.

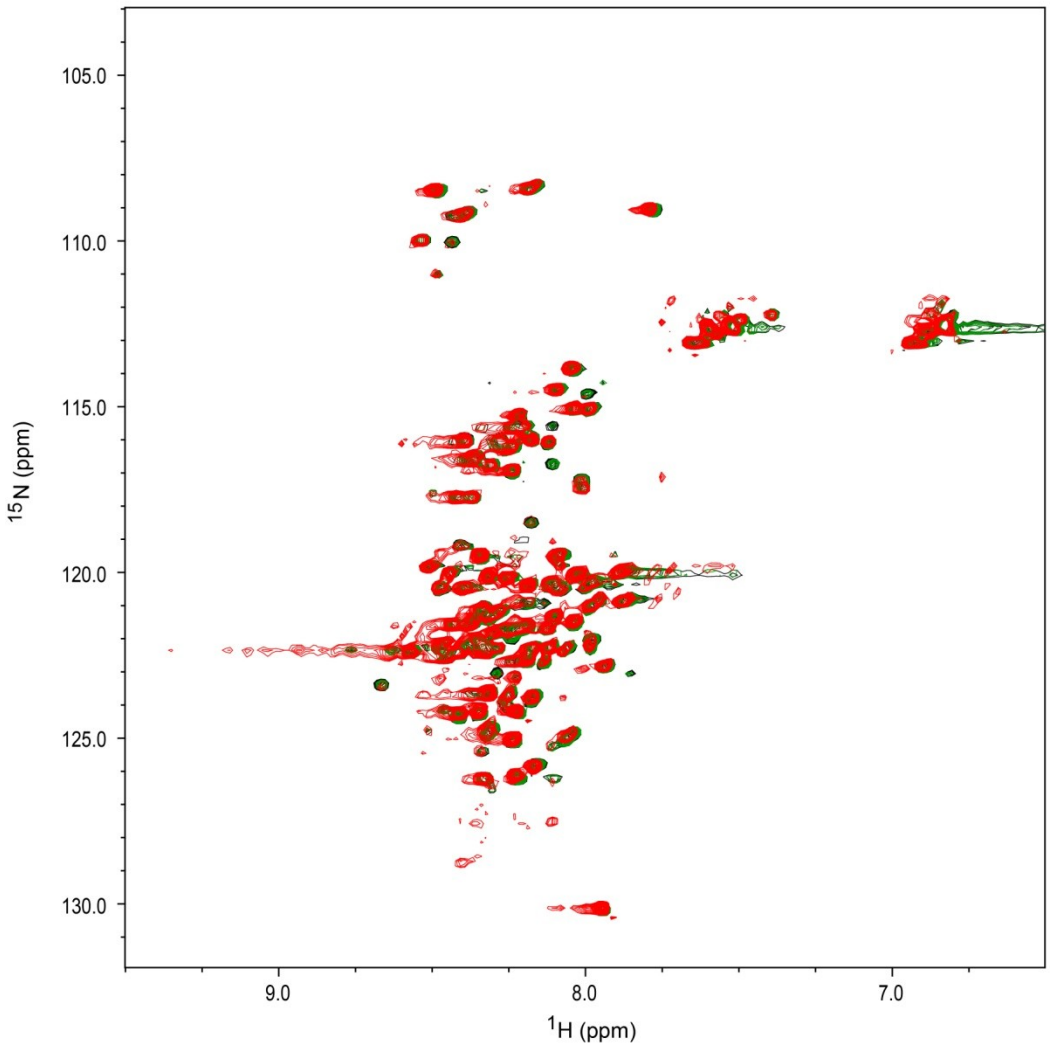


Figure 17. ^{1}H-^{15}N HSQC spectrum of MTF-1 zf6-acidic domain in the presence of 1 molar equivalent of zinc in order to fold zf6 of the MTF-1 zf6-acidic domain construct. Unlabeled p300 TAZ2 domain was titrated in three final molar concentration ratios to ^{15}N labeled MTF-1 zf6-acidic domain of 0 (black), 0.3 (Green), and 0.6 (Red).

CHAPTER 4

DISCUSSION

4.1 NMR Spectral Analysis of p300 TAZ1 Domain Titrations are Inconsistent With Physical MTF-1 zf6-acidic domain interaction.

^{1}H-^{15}N HSQC spectral analysis of ^{15}N-labeled TAZ1 titrated with the unlabeled MTF-1 zf6-acidic domain showed mean weight chemical shifts differences of assigned peaks to be 0.0368. In order to be confident that there is a significant interaction between TAZ1 and the MTF-1 zf6-acidic domain, and to isolate the residues most active in the interaction, only the residues with weighted chemical shifts of more than two standard deviations above the mean were considered (De Guzman, et al. 2004) (De Guzman, et al. 2000). From the residues assigned, only one residue, aspartate-98, met this requirement. These observed differences could be attributed but not limited to non-specific interaction between proteins due to aggregation and minor pH differences. Although the NMR spectrum presented (Figure 8) are inconsistent with a biologically relevant p300 TAZ1 domain interaction with the MTF-1 zf6-acidic domain, more sensitive methods such as isothermal calorimetry, could be used to probe these domains for a low affinity interaction.

4.2 NMR Spectral Analysis of p300 TAZ2 Domain Titrations Consistent with a Physical Interaction with MTF-1 zf6-acidic domain.

^{1}H-^{15}N HSQC spectral analysis of ^{15}N-labeled p300 TAZ2 domain titrated with the unlabeled MTF-1 zf6-acidic domain revealed mean weighted chemical shift differences of 0.052. One residue that had a weighted chemical shift difference greater than two standard deviations above the mean (of total weighted chemical shifts observed), is glutamine-59. Residues leucine-17, lysine-44 are both above weighted chemical shift difference of 0.1. In contrast to TAZ1 weighted chemical shifts (Figure 12), TAZ2 weighted chemical shifts (Figure 11) are higher overall, resulting in a higher mean as well. This observation provides compelling experimental evidence that the TAZ2 domain of p300 is more likely to be interacting with MTF-1 zf6-acidic domain (Appendix G). Additional NMR studies of labeled MTF-1 zf6-acidic domain titrated with unlabeled TAZ2 were done to confirm an interaction. It is expected that if intrinsically unstructured MTF-1 zf6-acidic domain was interacting with p300 TAZ2 domain, it would exhibit a greater chemical shift difference due to a formation of a structure.

4.3 NMR Results of MTF-1 zf6-acidic domain Reveal Subtle Changes Suggesting Binding.

As expected, intrinsically unstructured MTF-1 zf6-acidic domain is characterized in ^{1}H-^{15}N HSQC by low spectral dispersion of amide ^{1}H-^{15}N peaks. Because the MTF-1 zf6-acidic domain construct we used in this study includes zf6 on the N-terminal side of the acidic domain, the addition of one molar equivalent zinc is required in order to fold zf6. Experiments conducted by Li et al demonstrated that the addition of up to three equivalents

of excess zinc to MTF-1 zf6-acidic domain does not affect the unstructured acidic domain. Folded zf6 and unstructured acidic domain HSQC yields more dispersion in contrast to zf6-acidic domain in the absence of zinc. The unstructured acidic domain demonstrates a ^{1}H-^{15}N HSQC spectrum with the majority of the peaks localized to the center of ^{1}H-^{15}N HSQC plot (Figure 13).

At first, after titration of p300 TAZ2 pre-saturated with 3 molar equivalents of zinc, to a molar concentration ratio of 0.33 to 1 p300 TAZ2 to 1 zf6-acidic domain respectively, the zf6-acidic domain ^{1}H-^{15}N HSQC spectrum showed slight to no difference in chemical shifts. As the ratio of TAZ2 to MTF-1 zf6-acidic domain was increased to 0.66 to 1, 1 to 1, and 1.5 to 1, more the peaks shifted (Figure 14). This is indicative of fast-exchange and weak binding between TAZ2 and MTF-1 zf6-acidic domain with K_d (dissociation rate) range of millimolar to sub-millimolar range. As p300 is a transcriptional co-activator, it binds many other proteins/factors (Chan and La Thangue 2001). Strong binding would inhibit p300 from interacting with that diverse range of factors (Eckner, et al. 1994), due to increased competition. Therefore, tighter binding of p300 TAZ2 and MTF-1 zf6-acidic domain in physiological conditions might render it impossible for p300 to dissociate from MTF-1 and interact with other proteins/factors making the observed weaker binding functionally relevent.

Previous work by Li et al demonstrated that seven leucine residues of the MTF-1 zf6-acidic domain -- 335, 337, 340, 342, 343, 349, and 361-- when mutated to alanine (L7A7 mutant) decreased the recruitment of p300 by MTF-1 ~8 fold. Table 3 demonstrates MTF-1 zf6-acidic domain leucine residues mutated by Li et al and the effect of p300 TAZ2 titration

in terms of weighted chemical shift differences. Weighted amide HN chemical shifts of leucine residues 387 and 392 of MTF-1 zf6-acidic domain are significantly higher than that of leucine residues 335, 337, 340, 342, 343, 349, and 361. It is evident from Li et al that recruitment of p300 by MTF-1 was not completely abolished by the L7A7 mutant. It is possible that other leucine residues that demonstrated higher weighted chemical shifts such as 387-Leucine, and 392-Leucine of the MTF-1 zf6-acidic domain are playing a strong role in interaction between the MTF-1 zf6-acidic domain and p300.

Table 3. Weighted amide HN chemical shifts of leucine residues mutated by Li et al, and new leucine residues found.

Leucine Residue Number. * Mutated by Li et al.	Maximum weighted chemical shift upon p300 TAZ2 titration.
335*	0.019
337*	0.077
340*	No Data
342*	0.157
343*	0.062
349*	0.078
361*	0.166
387	0.276
392	0.169

4.4 ^{1}H-^{15}N HSQC Spectra of ^{15}N labeled MTF-1 zf6-acidic domain Titrated with p300 apo-TAZ2.

A peak shift was considered significant if the magnitude of change was calculated to be over two standard deviations above average. The p300 TAZ2 domain showed the most significant change and it was considered to be the possible binding partner. In order to show the zinc dependence of the interaction and also to use the p300 apo-TAZ2 domain as control, a solution of p300 TAZ2 was prepared in the absence of zinc. In this experiment it is assumed that p300 TAZ2 domain is unstructured due to the lack of zinc. If p300 TAZ2 domain would bind zinc that is added to fold zf6 of MTF-1 zf6-acidic domain, p300 TAZ2 would remain unstructured, as p300 TAZ2 domain requires 3 molar equivalents of zinc for proper folding and function (De Guzman, et al. 2000). Unstructured p300 TAZ2 domain was titrated as in earlier experiments. In the absence of added zinc above the 1 molar equivalent for zf6, the MTF-1 zf6-acidic domain does not show any change in peak positions in ^{1}H-^{15}N HSQC spectrum. The above observations are consistent with the hypothesis that the interaction between MTF-1 zf6-acidic domain and p300 TAZ2 domain only occurs in the presence of zinc.

4.5 The Residues of MTF-1 zf6-acidic domain Involved in the Interaction with p300 TAZ2 domain.

Close inspection of the chemical shift difference mapping within the zf6-acidic domain of MTF-1 suggest that the leucine residues discussed in part 4.3 (page 43) are not the sole determinants for the MTF-1 and p300 interaction. Table 4, indicates other residues of the

MTF-1 zf6-acidic domain had weighted chemical shift differences one or more standard deviations above the average. Residues 362S, 364I, 393I, 395S exhibit more than 0.24 weighted chemical shift difference. Polar uncharged residues are capable of interacting with other polar groups via hydrogen bonds and hydrophobic residues via hydrophobic interactions. Future studies involving additional mutations in zf6-acidic domain are needed to resolve these questions.

In conclusion, the study presented herein has shown through NMR analysis that the MTF-1 zf6-acidic domain region shows potential interaction with the p300 TAZ2 domain. This interaction is also zinc dependent, in agreement with the in vivo studies done by Li and coworkers. In vitro co-immunoprecipitation of MTF-1 suggests direct binding of MTF-1 to p300 and that this interaction is not bridged by other transcription factors that are constitutively bound to promoter region of *MT-I* and *MT-II* genes (Huyck, Keightley and Laity 2012). This finding gives an insight into metallothionein gene regulation and activation in response to elevated labile zinc levels.

Table 4. Weighted amide HN chemical shifts difference of MTF-1 zf6-acidic domain residues that have one or more standard deviations above average weighted chemical shift difference.

Residue number, name	Weighted Chemical Shift Difference	Physical nature of residue side chain
345T	0.12	Polar Uncharged
350Q	0.17	Polar Uncharged
360D	0.12	Negatively Charged
362S	0.26	Polar Uncharged
364I	0.29	Hydrophobic
365S	0.12	Polar Uncharged
379D	0.14	Negatively Charged
383Q	0.24	Polar Uncharged
388Q	0.22	Polar Uncharged
389T	0.166	Polar Uncharged
390A	0.194	Hydrophobic
393I	0.24	Hydrophobic
395S	0.32	Polar Uncharged
404I	0.2	Hydrophobic
405D	0.11	Negatively Charged

APPENDIX A

P300 sequencing results:

CGCACTTGCCCCTACCATTTCTGTTGAGTCCGCATCCCTCTCCGGCCACCGCAAC
CAGGCGAANAGAAAAAGGAACTTCCCCCCACCCCTGTTGGGTGGCGGCGGAGCT
CCGGAGCCCACCCTTAGGAGCGGGGCGGGGACCCCGTTTCGGAGAAGAGATTTC
CTCAGGATTCTCGTTTTTTTCCTCGGTTGTATCTCCGAAAGAATTAAAAATGGCC
GAGAATGTGGTGGAACCCGGGCCGCCTTCAGCCAAGCGGCCTAAACTCTCATCT
CCGGCCCTCTCGGCGTCCGCCAGCGATGGCACAGATTTTGGTTCACTGTTTGACC
TGNAACATGACTTACCAGATGAATTAATCAACTCTACAGAATTGGGACTAACCA
ATGGTGGCGATATCAGTCAGCTTCAGACAAGTCTT**GG**CATAGTACAAGANGCAG
CCTCGAAACATAAACAGCTGTCAGAACTGCTGAGGTCTGGTAGCTCCCCAAACC
TCAACATGGGAGTCGGTGGCCCAGGCCAAGCGATGGCCAGCCAGGCCCAACAG
AACAGCCCTGGATTAAGTTTGATAAATAGCATGGTCAAAAGCCCAATGGCACAG
ACAGGCTTGACTTCTCCAAACATGGGGATTGGCAGTAGTGGACCAAATCAGGGT
CCTACTCAGTCCCCAGCAGGTATGATGAACAGTCCAGTGAACCAGCCTGCCATG
GGAATGAACACAGGGATGAATGCTGGCATGAATCCTGGAATGTTGGCTGCAGGC
AATGGACAAGGGATAATGCCCAATCAAGTCATGAACGGTTCCATTGGAGCAGGC
CGGGGACGGCCAAACATGCAGTACCCAAATGCAGGCATGGGCAATGCTGGCAGT
TTATTGACTGAGCCACTACAGCAGGGCTCTCCTCAGATGGGAGGACAGCCAGGA
TTGAGAGGCCCCCAACCACTTAAGATGGGAATGATGAACAATCCCAGTCCTTAT
GGTTCACCATACACTCAGAATTCTGGACAGCAGATTGGAGCAAGTGGCCTTGGT
CTCCAAATTCAGACAAAGACTGTTCTACCAAATAACTTATCTCCATTTGCAATGG
ACAAAAAGGCAGTTCCTGGTGGGGGAATGCCCAGTATGGGCCAGCAGCCTACCC
CATCGGTCCAGCAGCCAGGCCTGGTGACTCCAGTTGCCGCANGAATGGGTTCTG
GAGCACACACAGCTGATCCAGANAAGCGCAAGCTCATCCAGCAGCAGCTTGTTC
TCCTTTTACATGCTCACAAGTGCCAGCGCCGGGAGCAAGCTAATGGGGAAGTGA
GGCAGTGCAACCTTCCTCACTGTCGTACCATGAAAAATGTCCTAAACCATATGAC
ACATTGCCAGTCAGNCAAATCCTGCCAAGTGGCACATTGNGCATCTNCTCGANA
AATCATTTCACACTGGAAAAATTGCACAAGCAGGCCGGGGANGGCCAAACATGC
AGTACCCAAATGCAGGCATGGGCAATGCTGGCAGTTTATTGACTGAGCCACTAC
AGCAGGGCTCTCCTCAGATGGGAGGACAGCCAGGATTGAGAGGCCCCCAACCAC
TTAAGATGGGAATGATGAACAATCCCAGTCCTTATGGTTCACCATACACTCAGAA
TTCTGGACAGCAGATTGGAGCAAGTGGCCTTGGTCTCCAAATTCAGACAAAGAC
TGTTCTACCAAATAACTTATCTCCATTTGCAATGGACAAAAAGGCAGTTCCTGGT
GGGGGAATGCCCAGTATGGGCCAGCAGCCTACCCCATCGGTCCAGCAGCCAGGC
CTGGTGACTCCAGTTGCCGCAGGAATGGGTTCTGGAGCACACACAGCTGATCCA
GAGAAGCGCAGCTCATCCAGCAGCAGCTTGTTCTCCTTTTACATGCTCACAAGTG
CCAGCGCCGGGAGCAAGCTAATGGGGAAGTGAGGCAGTGCAACCTTCCTCACTG
TCGTACCATGAAAAATGTCCTAAACCATATGACACATTGCCAGTCAGGCAAATC

CTGCCAAGTGGCACATTGTGCATCTTCTCGACAAATCATTTCACACTGGAAAAAT
TGCACAAGGCATGATTGTCCTGTGTGTCTTCCTCTCAAAAATGCTGGGGATAAGC
GAAATCAACAGTCAATTTTGACTGGAGCACCAGTTGGGCTTGGAAACCCTAGCT
CTCTAGGAGTGGGGCAGCAGTCCACTCCTAGCCTAAGCACTGTTAGTCAGATTGA
CCCCAGCTCTATAGAGCGAGCTTACGCTGCTCTTGGACTACCCTATCAAGTAAAC
CAGATTCCACCACAACCCCAGGTACAAGCAAAGAATCAACAAAGCCAGCCATCT
GGACAGTCTCCCCAGGGCATGCGGTCTGTGAACAACATGAGTGCTAGTCCTATG
GGTGTAAATGGAGGTGTTGGGGTTCAGACGCCAAATCTTCTTTCTGACTCCATGT
TGCATTCAACTATAAATTCTCAAAACCCAATGATGAGTGAAAATGCTGGTGTGGC
CTCCCTGGGTCCTTTGCCAACAGCAGCTCAGCCATCTAGTACTGGAATTCGAAAA
CAGTGGCATGAAGATATTACTCAGGATCTTCGAAACCACCTTGTTCATAAACTTG
TTCAAGCCATATTTCCCACTCCGGATCCTGCTGCTTTAAAAGACCGACGGATGGA
AAATCTTGTTGCATATGCTCGTAAAGTGGAAGGGGACATGTATGAATCTGCAAA
CAATAGAGCGGAATACTATCACCTCCTAGCCGAGAAGATCTATAAGATCCAGAA
GGAACTAGAAGAGAAACGAAGGACTAGACTACAGAAACAGAACATGCTACCGA
ATGCTCCTGGCATGGGTCCAGTTCCTATGAATACTGGGTCGAACATGGAGCAGC
AACCAACAGGAATGACTACCAATGGTCCTGTACCTGACCCGTCTATGATCCGTG
GCAGTGTGCCGAACCACATGATGCCTCGGATGACTCCACAGCCTGGTTTGAATC
AATTTGGGCAGATGAATATGCCACAGCCCCCTATTGGACCCCGGCAACCCTCTCC
TCTTCAGCACCATGGACAATTGGCTCAGTCTGGGTCACTCAATCCGCCTATGGGC
TATGGACCTCGCATGCAGCAGGCTTCCGGCCAGAACCAGTTCCTCTCCCAGACTC
AGTTCACATCCCAAGGAATGAATGTAACAAACATGCCTTTGGCTCCATCCAGCG
GTCAAGCACCAGTGTCTCAAGCACAAATGTCCAGTTCTTCTTGCCCAGTGAACTC
TCCTATAATGCCTCCAGGGTCTCAAGGGAGCCACATTCACTGTCCTACTCTGCCT
CAACAAGCTCATCAGAATTCACCTTCTCCTGTTCCTAGCCGCACCCCTACTCCTC
ATCATACCCCCCCAAGCATAGGGAATCAACCACCACCAGCAACAGCAATTCCAA
CACCTGTTCCTACACCTCCTGCCATACCACCTGGACCACAGCCCCCATCTCTGCA
TCCCTCATCTAGACAAACACCTACACCACCAACACATCTGCCTCCCCAAGTGCAG
CCTTCACTTCCTGCTGCTCCTTCTGCTGACCAGTCCCAGCAACAGCCTCGGTCAC
AGCAGAGCACAGCAGTTTCTGTTCCTACCCCAACAGCACCATTGCTGCCTCCTCA
GCCTTCCACTCCGCTTTCTCAGCCAGCTGTGAGCATTGAAGGCCAGGTCTCAAAC
CCTCCATCTACTAGTAGCACCGAAGTGAATTCTCAGACCATTCCTGAGAAGCAGC
CTTCACAGGAAGTGAAAATGGAGTCTAAAATGGAGGTGGATAAGCCAGAACCA
GCAGATGCTCAACCTGAGGATACAAAGGAGGCTAAAGGTGAGGATGTTAAAGTA
GAACCTACAGAAATGGAGGAGAGAGGCCCTGAGTTAAAAACTGATGGGAAAGA
GGAGGAAGAACAGCCAAGCACCTCTGCTACCCAGTCCTCCCCAGCTCCTGGACA
GTCAAAGAAGAAGATTTTCAAACCAGAGGAATTACGACAGGCTCTGATGCCAAC
ATTGGAGGCACTTTACCGGCAGGACCCAGAATCTCTTCCCTTTCGTCAGCCTGTG
GACCCGCAGCTTCTAGGAATCCCTGATTACTTTGATATTGTGAAGAGCCCCATGG

ATCTTTCTACAATCAAGAGGAAGTTAGATACTGGACAATACCAAGAGCCCTGGC
AATATATAGATGATATTTGGCTTATGTTCAACAATGCCTGGTTATATAACCGTAA
AACTTCAAGGGTATACAAATATTGCTCTAAACTTTCTGAAGTATTTGAACAAGAA
ATTGACCCTGTCATGCAAAGCCTTGGATACTGTTGTGGCAGAAAGTTGGAGTTTT
CTCCACANACTCTTTGTTGCTATGGGAAACAGTTATGCACAATCCCCCGTGATGC
TACTTACTACAGTTACCANAACAGGTATCATTTCTGTGANAAGTGTTTCAATGAA
ATCCAAGGGGANAGCGTTTCTTTGGGTGATGACCCTTCCCAACCTCAAACTACNN
TANATAAAGAACAGTTTTCCAAGAGAAAAAATGACACGTTGGATCCTGAACTGT
TTGTTGAGTGCACAGAGTGTGGAANAAAGATGCACCAGATCTGTGTCCTTCACC
ACGANATCATCTGGCCATCTGGGTTTGTCTGTGATGGCTGTTTAAAGAAAACTGC
ACGAACTANGAAAGAAAATAANCTTTCTGCTAAAAGATTGCCATCTACCAGACT
TGGGACCTTTCTGGAGAATCGAGTGAATGACTTTCTGAGGCGACAAAATCACCCT
GAATCAGGAGAGGTCACTGTTCGGGTTGTTCATGCTTCTGACAAAACTGTGGAA
NTGAAACCAGGCATGANANCNAGGTTTGTAGATAGTGGAGAGATGGCANAATCT
TTTCCATACCGAACAAANGCCCTGTTTGCCTTTGAAGAAATTGATGGTGTTGACT
TGTGTTTCTTCGGCATGCATGTTCNAGAATATGGCTCTGACTGCCCCCCTCCCAA
CCAGAGGAGGNTATACATATCTTACCTCGATAGTGTTCATTTCTTCCGTCCTAAA
TGCTTGCGGACTGCAGTCTATCATGAAATTCTAATTGGATATTTGGAATATGTCA
AGAAATTAGGATACACAACAGGGCACATCTGGGCCTGTCCACCCAGTGAAGGGG
ATGACTATATCTTCCATTGCCATCCACCTGATCAGAAGATACCAAAGCCCAAGCG
CCTGCAAGAATGGTACAAAAAGATGCTTGACAAGGCTGTATCAGAACGTATTGT
CCATGACTACAAGGATATTTTAAAACAAGCTACTGAAGATCGATTAACAAGTGC
AAAGGAACTACCCTACTTTGAAGGTGATTTCTGGCCCAATGTTCTAGAAGAAAG
CATCAAGGAGCTTGAACAGGAAGAGGAAGAGAGGAAACGGGAAGAGAACACCA
GCAACGAGAGTACTGATGTAACAAAAGGAGACAGCAAAAATGCTAAGAAGAAG
AATAACAAGAAAACCAGCAAAAACAAGAGCAGCCTGAGTAGGGGCAATAAGAA
GAAGCCTGGTATGCCCAATGTGTCTAATGACCTTTCTCAGAAACTGTATGCCACC
ATGGAAAAGCATAAAGAGGTTTTCTTTGTGATCCGCCTCATCGCTTGTCCTGCTC
CCAATTCCCTGCCTCCCATTGTTGATCCTGACCCTCTCATCCCCTGTGACCTGATG
GACGGTAGAGATGCATTTCTCACCCTTGCAAGGGACAAGCACCTGGAGTTCTCTT
CACTTCGAAGAGCCCAGTGGTCTACCATGTGCATGCTGGTGGAGCTCCATACAC
AGAGTCAGGATCGCTTTGTCTACACCTGTAACGAGTGCAAGCACCACGTGGAGA
CACGCTGGCATTGCACTGTGTGTGAGGATTATGACCTGTGCATCACTTGTTATAA
CACTAAAAATCATGACCACAAAATGGAGAAACTAGGCCTTGGCTTGGATGATGA
GAGCAATAACCAGCAAGCTGCAGCTACACAGAGCCCAGGAGACTCCCGCCGCCT
GAGCATCCAACGCTGCATCCAGTCTCTGGTGCATGCCTGTCAGTGCCGCAACGCC
AACTGCTCCCTGCCTTCCTGCCAGAAGATGAAGAGGGTTGTGCAGCACACCAAA
GGCTGCAAACGGAAAACCAATGGTGGGTGCCCCATTTGCAAACAGCTTATTGCC
CTCTGCTGCTATCATGCCAAGCACTGCCAGGAGAACAAGTGCCCGGTGCCATTTT

GCCTCAACATCAAGCAAAAGCTCCGGCAGCAACAGCTGCAGCACCGGCTCCAAC
AGGCTCAGATGCTCCGTAGGAGGATGGCCAGCATGCAACGAACTGGTGTGGCGG
GGCAGCAGCAGGGCCTGCCGTCCCCAACTCCTGCTACACCAACTACCCCTACTG
GCCAACAGCCAGCCACCCCACAGACACCCCAGCCCCAACCCACCTCTCAACCAC
AGCCCACTCCTCCCAACAACATGACACCCTACTTACCCAGGACTCAGACTACTGG
CCCTGTGTCCCAGGGTAAGGCACCAGGCCAGGTGACCCCACCTACCCCACCTCA
GACTGCTCANGCCCCCCCTTCCAGGGCCTCCACCTGCAGCAGTAGAAATGGCAA
TGCAAATTCAGANGGCAGCAGANACACAGCGCCNGATGGCCCATGTACAGATTT
TCCAAAGGCCAATCCAGCACCAGATGCCCCAGATGTCACCCATGGCCCCTATGG
GCATGAACCCACCTCCTATGGCCAGAGGTCCTGGTGGGCATTTGGATCCAGGAA
TAGGTCCAGCAGGAATGCAGCAACAGCCACCTTGGGCCCAAGGAGGAATGCCTC
AGCCCCAGCAGATGCAGTCAGGGATGCCAAGGCCAGCCATGATGTCAGTGGCCC
AGCATGGTCAGCCTTTAAACATGGCTCCACAACCAGGATTGGGCCAAGTGGGTG
TGAGCCCTCTCAAGCCAGGCACTGTGTCTCAACAAGCCTTACAAAACCTTTTGCG
GACTCTCAGGTCTCCCAGTTCTCCCTTACAGCAGCAACAGGTGCTTAGTATCCTT
CATGCCAACCCCCAACTGTTGGCTGCATTCATCAAGCAGCGGGCTGCCAAGTAT
GCCAACCCTAATCCACAGCCTCTCCCTGGACAGCCTGGCATGACCCAGGGACAG
CCAGGGCTGCAGCCACCTACCATGCCTGGCCAGCAAGGTGTCCACTCCAACCCA
GCCTTGCAGAACATGAATCCTCTGCAGGCAGGTGTCCAGAGAGCTGGCCTGCCC
CAGCAGCAGCCGCAGCAGCAGCTCCAGCCACCCATGGGAGCAATGAGTCCCCAA
GCTCAGCAAATGAACATGAATCACAATACCATGCCTTCACAGTTCAGAGACATC
TTAAGACGGCAGATGATGCAACAGCAGGGAGCAGGGCCAGGAATCGGCCCTGG
AATGGCCAACCAGTTCCAGCAGCCCCNANGGAATTGGCTATCCACCGCAGCAGC
AGCAGCAGCAGCAGCAGCAGCGAATGCAGCATCACATGCAGCAAATGCAGCAA
GGAAATATGGGACAAATGGGTCAGCTTCCCCAGGCTCTGGGGGCTGAGGCAGGA
GCCAGTCTACAGGCCTATCAGCAGCGACTTCTTCAACAACAGATGGGGTCTCCTG
CTCAGCCCAACCCTATGAGCCCACAGCAGCACATGCTCCCAAATCAGGCACAGT
CCCCACACCTACAAGGTCAACAAATCCCTAATTCTCTCTCCAATCAAGTGCGCTC
TCCCC
AGCCTGTCCCTTCTCCTCGACCACAGTCTCAGCCCCCCCATTCCAGCCCATCTCC
GAGGATGCAGCCTCAGCCTTCTCCACACCATGTTTCTCCACAGACCAGTTCCCCA
CATCCTGGACTGGTAGCTGCCCAGGCTGCCAACCCTATGGAACAAGGGCATTTT
GCCAGCCCAGACCAGAATTCAATGCTTTCACAGCTCGCTAGCAATCCTGGCATG
GCAAACCTCCACGGGGCAAGTGCCACGGACCTGGGACTGAGCAGCGATAATGCA
GACTTGAATTCAAACCTCTCACAGAGTACACTAGACATACACTAGAGACACCTT
GTAGTATTTTGGAAGCAAAAGATTATTTTCTCTTAACAAGACTTTTTGTACTGAA
AACAATTTTTTTGAATCTTTCTTANCCTAAAAGACCCATTTTCCTTGGAACATGTA
AGAANTGTGCAGTANCCATTTATGGTTTAAAGTAAACNGGCCAGATGAANCNTG

ANGNATAATAGATTACAAAGAATATATTTTTGNTATGGCTNGGTTACCCCAGCTC
TTTTTCCCC

APPENDIX B

Amino Acid sequences of p300, Highlighted TAZ domains.

MAENVVEPGPPSAKRPKLSSPALSASASDGTDFGSLFDLEHDLPDELINSTELGLTNG
GDISQLQTSLGIVQDAASKHKQLSELLRSGSSPNLNMGVGGPGQAMASQAQQNSPG
LSLINSMVKSPMAQTGLTSPNMGIGSSGPNQGPTQSPAGMMNSPVNQPAMGMNTG
MNAGMNPGMLAAGNGQGIMPNQVMNGSIGAGRGRPNMQYPNAGMGNAGSLLTE
PLQQGSPQMGGQPGLRGPQPLKMGMMNNPSPYGSPYTQNSGQQIGASGLGLQIQTK
TVLPNNLSPFAMDKKAVPGGGMPSMGQQPTPSVQQPGLVTPVAAGMG**SGAHTAD**
PEKRKLIQQQLVLLLHAHKCQRREQANGEVRQCNLPHCRTMKNVLNHMTHC
QSGKSCQVAHCASSRQIISHWKNCTRHDCPVCLPLKNAGDKRNQQSILTGAPVG
LGNPSSLGVGQQSTPSLSTVSQIDPSSIERAYAALGLPYQVNQIPPQPQVQAKNQQSQ
PSGQSPQGMRSVNNMSASPMGVNGGVGVQTPNLLSDSMLHSTINSQNPMMSENAG
VASLGPLPTAAQPSSTGIRKQWHEDITQDLRNHLVHKLVQAIFPTPDPAALKDRRME
NLVAYARKVEGDMYESANNRAEYYHLLAEKIYKIQKELEEKRRTRLQKQNMLPNA
PGMGPVPMNTGSNMEQQPTGMTTNGPVPDPSMIRGSVPNHMMPRMTPQPGLNQFG
QMNMPQPPIGPRQPSPLQHHGQLAQSGSLNPPMGYGPRMQQASGQNQFLSQTQFTS
QGMNVTNMPLAPSSGQAPVSQAQMSSSSCPVNSPIMPPGSQGSHIHCPTLPQQAHQN
SPSPVPSRTPTPHHTPPSIGNQPPPATAIPTPVPTPPAIPPGPQPPSLHPSSRQTPTPPTHL
PPQVQPSLPAAPSADQSQQQPRSQQSTAVSVPTPTAPLLPPQPSTPLSQPAVSIEGQVS
NPPSTSSTEVNSQTIPEKQPSQEVKMESKMEVDKPEPADAQPEDTKEAKGEDVKVEP
TEMEERGPELKTDGKEEEEQPSTSATQSSPAPGQSKKKIFKPEELRQALMPTLEALYR
QDPESLPFRQPVDPQLLGIPDYFDIVKSPMDLSTIKRKLDTGQYQEPWQYIDDIWLMF
NNAWLYNRKTSRVYKYCSKLSEVFEQEIDPVMQSLGYCCGRKLEFSPQTLCCYGKQ
LCTIPRDATYYSYQNRYHFCEKCFNEIQGESVSLGDDPSQPQTTINKEQFSKRKNDTL
DPELFVECTECGRKMHQICVLHHEIIWPSGFVCDGCLKKTARTRKENKLSAKRLPST
RLGTFLENRVNDFLRRQNHPESGEVTVRVVHASDKTVEVKPGMKARFVDSGEMAE
SFPYRTKALFAFEEIDGVDLCFFGMHVQEYGSDCPPPNQRRVYISYLDSVHFFRPKCL
RTAVYHEILIGYLEYVKKLGYTTGHIWACPPSEGDDYIFHCHPPDQKIPKPKRLQEW
YKKMLDKAVSERIVHDYKDILKQATEDRLTSAKELPYFEGDFWPNVLEESIKELEQE
EEERKREENTSNESTDVTKGDSKNAKKKNNKKTSKNKSSLSRGNKKKPGMPNVSN
DLSQKLYATMEKHKEVFFVIRLIACPAPNSLPPIVDPDPLIPCDLMDGRDAFLTLARD
KHLEFSSLRRAQWSTMCMLVELHTQSQDRFVYTCNECKHHVETRWHCTVCEDYDL
CITCYNTKNHDHKMEKLGLGLDDESNNQQAAATQ**SPGDSRRLSIQRCIQSLVHAC**
QCRNANCSLPSCQKMKRVVQHTKGCKRKTNGGCPICKQLIALCCYHAKHCQE
NKCPVPFCLNIKQKLRQQQLQHRLQQAQMLRRRMASMQRTGVAGQQQGLPSPTP
ATPTTPTGQQPATPQTPQPQPTSQPQPTPPNNMTPYLPRTQTTGPVSQGKAPGQVTPP
TPPQTAQAPLPGPPPAAVEMAMQIQRAAETQRQMAHVQIFQRPIQHQMPQMSPMAP
MGMNPPPMARGPGGHLDPGIGPAGMQQQPPWAQGGMPQPQQMQSGMPRPAMMS

VAQHGQPLNMAPQPGLGQVGVSPLKPGTVSQQALQNLLRTLRSPSSPLQQQQVLSIL
HANPQLLAAFIKQRAAKYANPNPQPLPGQPGMTQGQPGLQPPTMPGQQGVHSNPAL
QNMNPLQAGVQRAGLPQQQPQQQLQPPMGAMSPQAQQMNMNHNTMPSQFRDILR
RQMMQQQGAGPGIGPGMANQFQQPQGIGYPPQQQQQQQQQRMQHHMQQMQQGN
MGQMGQLPQALGAEAGASLQAYQQRLLQQQMGSPAQPNPMSPQQHMLPNQAQSP
HLQGQQIPNSLSNQVRSPQPVPSPRPQSQPPHSSPSPRMQPQPSPHHVSPQTSSPHPGL
VAAQAANPMEQGHFASPDQNSMLSQLASNPGMANLHGASATDLGLSSDNADLNSN
LSQSTLDIH

Highlighted yellow - TAZ1 domain
Highlighted turquoise - TAZ2 domain

APPENDIX C

Clone: TAZ2-6-1-pET21a

Primer: T7

TTTTGTTTAACTTTAANAANGNNNNATACATATGAGCCCAGGANNNNNNNNNNN
NGAGCATCCAACGCTGCATCCAGTCTCTGGTGCATGCCTGTCAGTGCCGCAACGC
CAACTGCTCCCTGCCTTCCTGCCAGAAGATGAAGAGGGTTGTGCAGCACACCAA
AGGCTGCAAACGGAAAACCAATGGTGGGTGCCCCATTTGCAAACAGCTTATTGC
CCTCTGCTGCTATCATGCCAAGCACTGCCAGGAGAACAAGTGCCCGGTGCCATTT
TGCCTCAACATCAAGCAAAAATAACTCGAGCACCACCACCACCACCACTGAGAT
CCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAG
CAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTGC
TGAAAGGAGGAACTATATCCGGATTGGCGAATGGGACGCGCCCTGTAGCGGCGC
ATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAG
CGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCG
GCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGC
TTTACGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGG
CCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAA
TAGTGNACTCTTGTTCCAAACTGGANNAACACTCAACCCTATCTCGGTCTATTCT
TTTGATTTATAAGGGATTTTGCCNATTTCGGCCTATTGGTTAAAAANNGAGCTGA
TTTAACAAAAATTTAACGCGAATTTTAACAAAATATTAACGCTTACAATTTNNNG
GCACTTTTCNGGGAAATGTGCGCGGAANCCCCNATTTGTTTATTTTTCTAANACA
TTCAANNATGTATNNNCNNCATGANANANNNCCCNGANNAANGNNTNNAATA

TAZ2-6-1

Primer – T7 terminator

GCNNTGTTAGCAGCCGGATCTCNGTGNNGNNGGTGGTGGTGCTCGAGTTATNNN
NNNNNNNNNNNGAGGCAAAATGGCACCGGGCACTTGTTCTCCTGGCAGTGCTTG
GCATGATAGCAGCAGAGGGCAATAAGCTGTTTGCAAATGGGGCACCCACCATTG
GTTTTCCGTTTGCAGCCTTTGGTGTGCTGCACAACCCTCTTCATCTTCTGGCAGGA
AGGCAGGGAGCAGTTGGCGTTGCGGCACTGACAGGCATGCACCAGAGACTGGAT
GCAGCGTTGGATGCTCAGGCGGCGGGAGTCTCCTGGGCTCATATGTATATCTCCT
TCTTAAAGTTAAACAAAATTATTTCTAGAGGGGAATTGTTATCCGCTCACAATTC
CCCTATAGTGAGTCGTATTAATTTCGCGGGATCGAGATCTCGATCCTCTACGCCG
GACGCATCGTGGCCGGCATCACCGGCGCCACAGGTGCGGTTGCTGGCGCCTATA
TCGCCGACATCACCGATGGGGAAGATCGGGCTCGCCACTTCGGGCTCATGAGCG
CTTGTTTCGGCGTGGGTATGGTGGCAGGCCCCGTGGCCGGGGGACTGTTGGGCG
CCATCTCCTTGCATGCACCATTCCTTGCGGCGGCGGTGCTCAACGGCCTCAACCT
ACTACTGGGCTGCTTCCTAATGCAGGAGTCGCATAAGGGAGAGCGTCGAGATCC
CGGACACCATCGAATGGCGCANAACCTTTCGCGGTATGGCATGATAGCGCCCGG
AAGAGAGTCAATTCAGGGTGGTGAATGTGAAACCAGTAACGTTATACGATGTCG

CANANTATGCCGGTGTCTCTTATCANANCGTTTCCCGCGTGGTGAACCAGGCCAG
CCACGTTTCTGCGAAAACGCGGGAAAAANTGGAANCGGCGATGGCGGAGCTGA
ATTACATTCCCAACCGCGTGGNACNACAACTGGCGNGNNNNCAGTCGTTGCTGA
TTGGCGTTGCCNCCTCCNGTCTGGNCCNNGCACGCGCCGNNNCAAATNGTCGCG
GCGATNAAATCNCNCGCCNATNAACTGGNNNNCNNNNN

TAZ1-pET21a nt sequence

Primer – T7

CATATGATGTCTGGNNNNCACACAGCTGATCCAGAGAAGCGCANGCTCATCCAN
CANCANCTTGTTCNCNNTTTACATGCTCACAAGTGCCAGCGCCGGGAGCAAGCT
ATGGGGAAGTGAGGCAGTGCAACCTTCCTCACTGTCGTACCATGAAAAATGTCC
TAAACCATATGACACATTGCCANTCANGCAAATCCTGCCAAGTGGCACATTGTG
CATCTTCTCGACAAATCATTTCACACTGGAAAAATTGCNCNNNGCATGATTGTCC
TGTGTGTCTTCCTCTCAAAAATGCTGGGGATAAGCGATAACTCGAGCACCACCAC
CACCACCANTGANATCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCT
GCTGCCACCGCTGANCAATAACTANNATAACCCCTTGGGGCCTCTAAACGGGTC
TTGAGGGGTTTTTTGCTGAAAGGAGGAACTATATCCGGATTGGCNAATGGGACG
CGCCCNGTANCGGCGCATTAANCGCGGCGGGTGTGGTGGTTACGCNNANCNNGA
CCGCTACACTTGCCAGCGCCCTANCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTT
CNNGCCACGTTCNNNNGCTTTCCCCGTCNAGCTCTANANCNGGGGCTCCCTTTAN
GGTTCCNANTTANNTGCNTNACAGCACCNCNANNNCNANNANANTTGATTANGG
TGANGGTTCAC

Primer – T7 terminator

GGCTTTGTTAGCAGCCGGATCTCAGTGGTGGTGGTGGTGGTGCTCGAGTTATCGC
TTATCCCCAGCATTTTTGAGAGGAAGACACACAGGACAATCATGCCTTGTGCAAT
TTTTCCAGTGTGAAATGATTTGTCGAGAAGATGCACAATGTGCCACTTGGCAGGA
TTTGCCTGACTGGCAATGTGTCATATGGTTTAGGACATTTTTCATGGTACGACAG
TGAGGAAGGTTGCACTGCCTCACTTCCCCATTAGCTTGCTCCCGGCGCTGGCACT
TGTGAGCATGTAAAAGGAGAACAAGCTGCTGCTGGATGAGCTTGCGCTTCTCTG
GATCAGCTGTGTGTGCTCCAGACATCATATGTATATCTCCTTCTTAAAGTTAAAC
AAAATTATTTCTAGAGGGGAATTGTTATCCGCTCACAATTCCCCTATAGTGAGTC
GTATTAATTTCGCGGGATCGAGATCTCGATCCTCTACGCCGGACGCATCGTGGCC
GGCATCACCGGCGCCACAGGTGCGGTTGCTGGCGCCTATATCGCCGACATCACC
GATGGGGAAGATCGGGCTCGCCACTTCGGGCTCATGAGCGCTTGTTTCGGCGTG
GGTATGGTGGCAGGCCCCGTGGCCGGGGGACTGTTGGGCGCCATCTCCTTGCAT
GCACCATTCCTTGCGGCGGCGGTGCTCAACGGCCTCAACCTACTACTGGGCTGCT
TCCTAATGCAGGAGTCGCATAAGGGAGAGCGTCGAGATCCCGGACACCATCGAA
TGGCGCAAAACCTTTCGCGGTATGGCATGATAGCGCCCGGAAGAGAGTCAATTC
AGGGTGGTGAATGTGAAACCAGTAACGTTATACGATGTCGCAGAGTATGCCGGT
GTCTCTTATCAGACCGTTNCCCGCGTGGTGAACCAGGCCAGCCACGTTTCTGCGA
AAACGCGGGAAAAAGTGGAAGCGGCGATGGCGGANCTGAATTACATTCCCAAC
CGCGTGGNNNNACANCTGGCGGGCAAACNNTCGTTGCTGATTGGNGTTGCCACC
TCCAGTCNGGNCCNGCACGCGNCGNCNCAANTNNTCGCGNCNANTAAANCNCN
CNCNATNAACTGGGNGCC

APPENDIX D

P300 TAZ domains DNA sequence.

TAZ1 DNA sequence:
TCTGGAGCACACACAGCTGATCCAGAGAAGCGCAAGCTCATCCAGCAGCAGCTT
GTTCTCCTTTTACATGCTCACAAGTGCCAGCGCCGGGAGCAAGCTAATGGGGAA
GTGAGGCAGTGCAACCTTCCTCACTGTCGTACCATGAAAAATGTCCTAAACCATA
TGACACATTGCCAGTCAGGCAAATCCTGCCAAGTGGCACATTGTGCATCTTCTCG
ACAAATCATTTCACACTGGAAAAATTGCACAAGGCATGATTGTCCTGTGTGTCTT
CCTCTCAAAAATGCTGGGGATAAGCG

TAZ2 DNA sequence:
AGCCCAGGAGACTCCCGCCGCCTGAGCATCCAACGCTGCATCCAGTCTCTGGTG
CATGCCTGTCAGTGCCGCAACGCCAACTGCTCCCTGCCTTCCTGCCAGAAGATGA
AGAGGGTTGTGCAGCACACCAAAGGCTGCAAACGGAAAACCAATGGTGGGTGC
CCCATTTGCAAACAGCTTATTGCCCTCTGCTGCTATCATGCCAAGCACTGCCAGG
AGAACAAGTGCCCGGTGCCATTTTGCCTCAACATCAAGCAAAAG

APPENDIX E

The restriction endonucleases XbaI and XhoI.

XbaI
Recognition and digestion site:

XhoI
Recognition and digestion site:

APPENDIX F

TAZ1 Protein Parameter - Expasy

ProtParam

User-provided sequence:

```
      10        20        30        40        50        60
MMSGAHTADP EKRKLIQQQL VLLLHAHKCQ RREQANGEVR QCNLPHCRTM
KNVLNHMTHC

      70        80        90       100
QSGKSCQVAH CASSRQIISH WKNCTRHDCP VCLPLKNAGD KR
```

Number of amino acids: 102

Molecular weight: 11631.5

Theoretical pI: 9.53

Amino acid composition:

Ala (A)	7	6.9%
Arg (R)	8	7.8%
Asn (N)	6	5.9%
Asp (D)	3	2.9%
Cys (C)	9	8.8%
Gln (Q)	9	8.8%
Glu (E)	3	2.9%
Gly (G)	4	3.9%
His (H)	9	8.8%
Ile (I)	3	2.9%
Leu (L)	9	8.8%
Lys (K)	8	7.8%
Met (M)	4	3.9%
Phe (F)	0	0.0%
Pro (P)	4	3.9%
Ser (S)	6	5.9%
Thr (T)	4	3.9%
Trp (W)	1	1.0%
Tyr (Y)	0	0.0%
Val (V)	5	4.9%

Pyl (O) 0 0.0%
Sec (U) 0 0.0%

(B) 0 0.0%
(Z) 0 0.0%
(X) 0 0.0%

Total number of negatively charged residues (Asp + Glu): 6
Total number of positively charged residues (Arg + Lys): 16

Atomic composition:

Carbon	C	484
Hydrogen	H	802
Nitrogen	N	168
Oxygen	O	140
Sulfur	S	13

Formula: $C_{484}H_{802}N_{168}O_{140}S_{13}$
Total number of atoms: 1607

Extinction coefficients:

Extinction coefficients are in units of M^{-1} cm^{-1}, at 280 nm measured in water.

Ext. coefficient 6000
Abs 0.1% (=1 g/l) 0.516, assuming all pairs of Cys residues form cystines

Ext. coefficient 5500
Abs 0.1% (=1 g/l) 0.473, assuming all Cys residues are reduced

Estimated half-life:

The N-terminal of the sequence considered is M (Met).

The estimated half-life is: 30 hours (mammalian reticulocytes, in vitro).
>20 hours (yeast, in vivo).
>10 hours (Escherichia coli, in vivo).

Instability index:

The instability index (II) is computed to be 50.22
This classifies the protein as unstable.

Aliphatic index: 66.96

Grand average of hydropathicity (GRAVY): -0.731

TAZ2 Protein Parameters - Expasy

ProtParam

User-provided sequence:

```
      10        20        30        40        50        60
MSPGDSRRLS IQRCIQSLVH ACQCRNANCS LPSCQKMKRV VQHTKGCKRK
TNGGCPICKQ

      70        80
LIALCCYHAK HCQENKCPVP FCLNIKQK
```

Number of amino acids: 88

Molecular weight: 9902.8

Theoretical pI: 9.55

Amino acid composition:

Ala (A)	4	4.5%
Arg (R)	6	6.8%
Asn (N)	5	5.7%
Asp (D)	1	1.1%
Cys (C)	13	14.8%
Gln (Q)	8	9.1%
Glu (E)	1	1.1%
Gly (G)	4	4.5%
His (H)	4	4.5%
Ile (I)	5	5.7%
Leu (L)	6	6.8%
Lys (K)	10	11.4%
Met (M)	2	2.3%
Phe (F)	1	1.1%
Pro (P)	5	5.7%
Ser (S)	6	6.8%
Thr (T)	2	2.3%
Trp (W)	0	0.0%
Tyr (Y)	1	1.1%
Val (V)	4	4.5%
Pyl (O)	0	0.0%
Sec (U)	0	0.0%

(B)	0	0.0%
(Z)	0	0.0%

(X) 0 0.0%

Total number of negatively charged residues (Asp + Glu): 2
Total number of positively charged residues (Arg + Lys): 16
Atomic composition:

Carbon	C	413
Hydrogen	H	697
Nitrogen	N	137
Oxygen	O	115
Sulfur	S	15

Formula: $C_{413}H_{697}N_{137}O_{115}S_{15}$
Total number of atoms: 1377

Extinction coefficients:

This protein does not contain any Trp residues. Experience shows that
this could result in more than 10% error in the computed extinction coefficient.

Extinction coefficients are in units of M^{-1} cm^{-1}, at 280 nm measured in water.

Ext. coefficient 2240
Abs 0.1% (=1 g/l) 0.226, assuming all pairs of Cys residues form cystines

Ext. coefficient 1490
Abs 0.1% (=1 g/l) 0.150, assuming all Cys residues are reduced

Estimated half-life:

The N-terminal of the sequence considered is M (Met).

The estimated half-life is: 30 hours (mammalian reticulocytes, in vitro).
>20 hours (yeast, in vivo).
>10 hours (Escherichia coli, in vivo).

Instability index:

The instability index (II) is computed to be 82.58
This classifies the protein as unstable.

Aliphatic index: 66.48

Grand average of hydropathicity (GRAVY): -0.455

APPENDIX G

MTF-1 zf6-acidic domain Weighted Chemical Shift differences

316G	0.00460824	0.0054309	0.00161919
317T	0.00511671	0.00300114	0.01433666
318A	0.00430925	0.00551232	0.00607698
319Y	0.01719633	0.00533774	0.01511907
320S	0.00466422	0.00402129	0.00494629
321A	0.00425609	0.00445357	0.0010516
322L	0.00063709	0.00010373	0.00631948
324Q	0.00166904	0.0017623	0.01000584
327G	0.02172156	0.0307439	0.00701386
328S	0.01320046	0.01302126	0.01222265
329E	0.00892834	0.00967903	0.03914897
335L	0.00327865	0.01903129	0
337L	0.03452638	0.07738081	0
341G	0.05041004	0.10518707	0
342L	0.00461292	0.04094008	0.15694351
343L	0.06236678	0	0
344S	0.03479483	0.07779437	0
345T	0.0047238	0.04294563	0.11996664
346D	0.004	0.012184	0.06423523
347S	0.00592511	0.00176534	0
348E	0.00591644	0.04132978	0.057338
349L	0.04163426	0.07838422	0
350Q	0.04069599	0.08249198	0.17038539
351E	0.00453234	0.03872878	0.04593017
352N	0.00542531	0.02304587	0.04701208
353S	0.03783331	0.06490913	0.07937916
354S	0.0058042	0.03240896	0.03957778
355S	0.00824369	0.03494271	0.0374286
356T	0.0387993	0.04382927	0.08433487
358D	0.02007862	0.06236023	0.09950043
359Q	0.00560677	0.00564918	0.00566986
360D	0.00733386	0.04341997	0.12060425
361L	0.01456872	0.05378088	0.16578656
362S	0.05279905	0.1258675	0.26000898
363T	0.07886087	0.180047	0
364I	0.05749613	0.15236762	0.28888573
365S	0.02042576	0.0674067	0.11978151

379D	0.04163822	0.09020176	0.14251536
383Q	0.04126961	0.12178285	0.24059655
387L	0.04278902	0.12230643	0.27645331
388Q	0.04509811	0.09385993	0.22218367
389T	0.03523001	0.07553801	0.16660756
390A	0.07534416	0.19393676	0
392L	0.07298011	0.1690661	0
393I	0.11896356	0.24009668	0
395S	0.05086558	0.13755597	0.32108491
404I	0.05860384	0.12374974	0.19712626
405D	0.03172832	0.03898302	0.11464598
StDev	0.02637112	0.05889767	0.09316341
Mean	0.02815648	0.06246547	0.07837173
2xStDev+mean	0.08089873	0.18026082	0.26469855

p300 TAZ2 Weighted Chemical Shifts

17	LEU	0.104194
19	HIS	0.033513
22	GLN	0.076433
26	ALA	0
27	ASN	0.084363
28	CYS	0.014967
29	SER	0.057109
30	LEU	0.031703
33	CYS	0.082192
34	GLN	0.0544
37	LYS	0.053916
38	ARG	0.058055
43	THR	0
44	LYS	0.139976
45	GLY	0.071886
46	CYS	0.049589
50	THR	0.030302
52	GLY	0.010603
53	GLY	0.063743
57	CYS	0.033935
59	GLN	0.117068
62	ALA	0
63	LEU	0
65	CYS	0.075769
69	LYS	0
71	CYS	0.081824
72	GLN	0.041311
74	ASN	0.048712
76	CYS	0.059975
78	VAL	0.089225
81	CYS	0.045047
	stdev	0.036101
	average	0.051929

p300 TAZ1 Weighted Chemical Shifts

13L	0.04001518
16Q	0.02362321
18L	0.0575538
20L	0.06680478
22L	0.04476129
24A	0.05300286
26K	0.00618696
32Q	0.01269638
47T	0.02442958
67A	0.0197126
68H	0.03709456
69C	0.01930033
71S	0.02850225
74Q	0.02055362
75I	0.05067197
78H	0.05484305
80K	0.03648703
83T	0.04417846
90C	0.01776891
93L	0.02492122
98D	0.07423873
99K	0.05222449
STDEV	0.01846302
MEAN	0.03679869

Appendix H

p300 TAZ1 domain mass spectrometry

20. EP300_HUMAN **Mass:** 266898 **Score:** 115 **Matches:** 23(5) **Sequences:** 8(3) **emPAI:** 0.04

Histone acetyltransferase p300 OS=Homo sapiens GN=EP300 PE=1 SV=2

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Unique	Peptide
623	902.4550	901.4477	901.4253	0.0224	0	27	7.6	4	U	R.EQANGEVR.Q 622 624 625
635	456.3679	910.7212	910.5025	0.2188	0	20	33	7	U	R.QIISHWK.N 634 636
898	542.7999	1083.5852	1083.4702	0.1150	0	40	0.28	1	U	R.QCNLPHCR.T 899 900 904 907
995	561.4064	1120.7982	1119.5746	1.2236	1	9	4.4e+002	2	U	K.LYATMEKHK.E
1138	413.5812	1237.7218	1237.5948	0.1270	0	(20)	26	2	U	R.HDCPVCLPLK.N
1139	619.8869	1237.7592	1237.5948	0.1645	0	51	0.019	1	U	R.HDCPVCLPLK.N 1132 1133 1137
1161	632.0764	1262.1382	1261.5292	0.6091	0	67	0.00069	1	U	K.SCQVAHCASSR.Q 1159 1160
1582	591.0505	1770.1297	1768.8171	1.3125	1	32	1.5	1	U	K.NCTRHDCPVCLPLK.N
1586	595.2595	1782.7567	1781.1039	1.6527	1	49	0.023	1		R.KLIQQQLVLLLHAHK.C

p300 TAZ2 domain mass spectrometry

5. gi|109481045 **Mass:** 266301 **Score:** 386 **Matches:** 42(6) **Sequences:** 7(3) **emPAI:** 0.04

PREDICTED: similar to E1A binding protein p300 [Rattus norvegicus]

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Unique	Peptide
287	356.3439	710.6732	710.4075	0.2657	0	44	2.3	2		R.VVQHTK.G 279 281 282 284 285 288 290 291 292
976	504.1439	1006.2732	1005.4372	0.8361	0	41	6.2	2		K.TNGGCPICK.Q 977 981 983
980	1007.6220	1006.6147	1005.4372	1.1775	0	(25)	2.3e+002	7		K.TNGGCPICK.Q 974
1258	568.3504	1134.6862	1133.5322	1.1541	1	50	0.71	1		R.KTNGGCPICK.Q 1255 1257
1451	624.7689	1247.5232	1246.6202	0.9030	0	53	0.32	2		K.CPVPFCLNIK.Q 1449 1450 1457
1516	1278.6620	1277.6547	1277.5492	0.1055	0	(51)	0.57	1	U	R.NANCSLPSCQK.M
1517	640.1459	1278.2772	1277.5492	0.7280	0	64	0.028	1	U	R.NANCSLPSCQK.M 1513 1514 1518
1627	688.9544	1375.8942	1375.6740	0.2202	0	68	0.0087	1		K.QLIALCCYHAK.H 1628 1629
1782	766.7749	1531.5352	1530.6854	0.8499	0	76	0.0015	1		R.CIQSLVHACQCR.N 1776 1777 1778 1779 1780 1781 1783 1784
1787	511.8762	1532.6068	1530.6854	1.9214	0	(32)	38	7		R.CIQSLVHACQCR.N 1785

Bibliography

Adilakshmi, T, and R O Laine. "Ribosomal protein S25 mRNA partners with MTF-1 and La to provide a p53-mediated mechanism for survival or death." *The Journal of Biological Chemistry*, 2002: 4147-4151.

Altschul, Stephen F, et al. "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs ." *Nucleic Acids*, 1997: 25:3389-3402.

Andrews, G K, et al. "The transcription factors MTF-1 and USF1 cooperate to regulate mouse metallothionein-I expression in response to the essential metal zinc in visceral endoderm cells during early development." *The UMBO Journal*, 2001: 1114-1122.

Andrews, Glen K. "Cellular zinc sensors: MTF-1 regulation of gene expression." *Biometals*, 2001: 223-237.

Andrews, Glen K. "Regulation of metallothionein gene expression by oxidative stress and metal ions." *Biochemical Pharmacology*, 2000: 95-104.

Chan, H M, and N B La Thangue. "p300/CBP proteins: HATs for transcriptional bridges and scaffolds." *Journal of Cell Sciences*, 2001: 2363-2373.

Chrivia, J C, R P Kwok, N Lamb, M Hagiwara, M Montminy, and R H Goodman. "Phosphorylated CREB binds specifically to nuclear protein CBP." *Nature*, 1993: 855-859.

Cramer, M, et al. "NF-kappaB contributes to transcription of placenta growth factor and interacts with metal responsive transcription factor-1 in hypoxic human cells." *Biological Chemistry*, 2005: 865-872.

Dames, S A, M Martinez-Yamout, R N De Guzman, H J Dyson, and P E Wright. "Structural basis for Hif-1 alpha /CBP recognition in the cellular hypoxic response." *Proc. Natl. Acad. Sci. U.S.A.*, 2002: 5271-5276.

Daniels, P J, and Glen K Andrews. "Dynamics of the metal-dependent transcription factor complex in vivo at the mouse metallothionein-I promoter." *Nucleic Acids Research*, 2003: 6710-6721.

Davis, S R, R J McMahon, and R J Cousins. “Metallothionein knockout and transgenic mice exhibit altered intestinal processing of zinc with uniform zinc-dependent zinc transporter-1 expression.” *Journal of Nutrition*, 1998.

De Guzman, R N, M A Martinez-Yamout, H J Dyson, and P E Wright. "Interaction of the TAZ1 domain of the CREB-binding protein with the activation domain of CITED2: regulation by competition between intrinsically unstructured ligands for non-identical binding sites." *The Journal of Biological Chemistry*, 2004: 3042-3049.

De Guzman, Robert N, Yu Liu Hong, Maria Martinez-Yamout, Jane H Dyson, and Peter E Wright. "Solution structure of the TAZ2 (CH3) Domain of the Transcriptional Adapter Protein CBP." *Journal of Molecular Biology*, 2000: 243-253.

De Guzman, Roberto N, Jonathan M Wojciak, Maria A. Martinez-Yamout, Jane H Dyson, and Peter E Wright. "CPB/p300 TAZ1 Domain Forms a Structured Scaffold for Ligand Binding." *Biochemistry*, 2005: 490-497.

Eckner, R, et al. "Molecular cloning and functional analysis of the adenovirus E1A-associated 300-kD protein (p300) reveals a protein with properties of a transcriptional adaptor." *Genes Development*, 1994: 869-884.

Freedman, S J, et al. "Structural basis for recruitment of CBP/p300 by hypoxia-inducible factor-1 alpha." *Proc. Natl. Acad. Sci. U.S.A.*, 2002: 5367-5372.

Freedman, S J, Z Y Sun, A L Kung, D S France, G Wagner, and M J Eck. "Structural basis for negative regulation of hypoxia-inducible factor-1alpha by CITED2." 2003: 504-512.

Grzesiek, S, et al. “The solution structure of HIV-1 Nef reveals an unexpected fold and permits delineation of the binding surface for the SH3 domain of Hck tyrosine protein kinase.” *Nature Structural Biology*, 1996: 340-345.

Heuchel, R, F Radtke, O Georgiev, G Stark, M Aguet, and W Schaffner. "The transcription factor MTF-1 is essential for basal and heavy metal-induced metallothionein gene expression." *EMBO J*, 1994: 13, 2870-2875.

Iyer, N G, H Ozdag, and C Caldas. “p300/CBP and Cancer.” *Nature Publishing Group*, 2004: 4225-4231.

Laity, John H, and Glen K Andrews. "Understanding the mechanisms of zinc-sensing by metal-response element binding transcription factor-1 (MTF-1)." *Elsevier ABB*, 2007: 201-210.

Langmade, S J, R Ravindra, P J Daniels, and Glen K Andrews. "The transcription factor MTF-1 mediates metal regulation of the mouse ZnT1 gene."

Lee, W, A Haslinger, M Karin, and R Tijan. "Activation of transcription by two factors that bind promoter and enhancer sequences of the human metallothionein gene and SV40." *Nature*, 1987: 325, 368-372.

Li, Yong, Tomoki Kimura, Ryan W Huyck, John H Laity, and Glen K Andrews. "Zinc Induced Formation of a Coactivator Complex Containing the Zinc-sensing Transcription Factor MTF-1, p300/CBP, and Sp1." *Molecular and Cellular Biology*, 2008: 4275-4284.

Lichtlen, P, and W Schaffner. "Putting its fingers on stressful situations: the heavy metal-regulatory transcription factor MTF-1." *Bioessays*, 2001: 1010-1017.

Lichtlen, P, et al. "Target gene search for the metal-responsive transcription factor MTF-1." *Nucleic Acids Res*, 2001: 1514-1523.

Murphy, B J, B G Sato, T P Dalton, and K R Laderoute. "The metal-responsive transcription factor-1 contributes to HIF-1 activation during hypoxic stress." *Biochemical and Biophysical Research Communications*, 2005: 860-867.

Murphy, B J, T Kimura, B G Sato, Y Shi, and Glen K Andrews. "Metallothionein induction by hypoxia involves cooperative interactions between metal-responsive transcription factor-1 and hypoxia-inducible transcription factor-1alpha." *Molecular Cancer Research*, 2008: 483-490.

Ogra, Y, K Suzuki, P Gong, F Otsuka, and S Koizuma. "Negative regulatory role of Sp1 in metal responsive element-mediated transcriptional activation." *Journal of Biological Chemistry*, 2001: 16534-16539.

Ponting, C P, D J Blake, K E Davies, J Kendrick-Jones, and S J Winder. "ZZ and TAZ: new putative zinc fingers in dystrophin and other proteins." *Trends in Biochemical Sciences*, 1996: 11-13.

Potter, Belinda M, et al. "The Six Zinc Fingers of Metal-responsive Element Binding Transcription Factor-1 Form Stable and Quasi-ordered Structures with Relatively Small Differences in Zinc Affinities." *The Journal of Biological Chemistry*, 2005: 28529-28540.

Radtke, F, et al. "Cloned transcription factor MTF-1 activates the mouse metallothionein I promoter." *EMBO J*, 1993: 12, 1355-1362.

Saydam, N, F Steiner, O Georgiev, and W Schaffner. "Heat and heavy metal stress synergize to mediate transcriptional hyperactivation by metal-responsive transcription factor MTF-1." *The Journal of Biological Chemistry*, 2003: 31879-31883.

Saydam, N, O Georgiev, M Y Nakano, U F Greber, and W Schaffner. "Nucleo-cytoplasmic trafficking of metal-regulatory transcription factor 1 is regulated by diverse stress signals." *The Journal of Biological Chemistry*, 2001: 25487-25495.

Smirnova, I V, D C Bittel, R Ravindra, H Jiang, and Glen K Andrews. "Zinc and cadmium can promote rapid nuclear translocation of metal response element-binding transcription factor-1." *The Journal of Biological Chemistry*, 2000: 9377-9384.

Stuart, G W, P F Searle, and R D Palmiter. "Identification of multiple metal regulatory elements in mouse metallothionein-I promoter by assaying synthetic sequences." *Nature*, 1985: 317, 828-831.

VITA

Vugar Azizov was born on February 19th, 1987 in Baku, Azerbaijan. He graduated from Azerbaijani-Turkish Private High School in 2004. Following his graduation, he started his undergraduate studies at the Independence Community College in Independence, KS in 2004. He completed his undergraduate studies at The University of Kansas with a degree of Bachelor of Science in Microbiology in 2010. Following graduation, he started volunteering at one of the labs in The University of Missouri- Kansas City School of Biological Sciences. In September of 2010, Vugar joined the laboratory of Dr. John Laity, studying the interaction of transcriptional co-activator p300 with MTF-1 zf6-acidic domain.