Protein PKA and Cu(I) Binding

A Major Qualifying Project Report

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Submitted To:

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Acknowledgements:

I would like to thank my advisor and Professor George Kaminski for helping me complete this project and learning objective. His guidance of the Linux software system and using research databanks was vital to the execution and validity of the MQP. Professor Kaminski was always readily available to answer my questions with clear and prompt responses, and without his help, the project would not be as completed to the extent that it is.

Abstract:

Protein PKA and protein-metal binding constraints of Zn(II) ions are scarce in literature and not abundantly kept in one place. It is even harder to find a list of such constants correlated with the corresponding PDB structures. Using the Protein Data Bank website, I compiled Zn(II) protein binding constraints from various articles into an excel spreadsheet where they are sourced. This process was the first part of my MQP, as these values are very valuable to have in one list which will be used in future research

The second part of my MQP was to reproduce the cysteine pKa values previously calculated with the PKA17 software and to fit new parameters for obtaining tyrosine pKa values with pKa. The latter is a new project, this had not been done previously. We used the PKA17 program in Linux. The calculated protein pKa shifts were compared with the experimental values. One can employ PKA 17 with the parameter values obtained in this MQP project to predict tyrosine pKa values in many applications in science, including pharmaceuticals and physical chemistry.

1.0 Introduction:

Binding affinities for various metal protein complexes are useful in science for figuring out which metal ion and protein will bind the best, as well as identifying which metal protein complex being looked at. These interactions are studied and analyzed in the area known as computational chemistry. The binding affinities can be evaluated for many applications including disease treatments and biochemical processes. Finding these values in literature can be a challenge however, as for certain metals, their binding affinities are not readily abundant. Copper (Cu) (I) ions fall under the category of less abundant in literature and are the focus of this project. Copper (I) ions have been correlated to cancer research and more specifically with the cis-platin treatment. This would involve replacing the platinum ions with Cu (I) ions, which are less expensive and more abundant in the world. The issue with this initial research is figuring out if the Cu (I) ions will bind to the proteins the same way the platinum ions do, hence comparing the binding affinity values. By searching various data banks, the project aims to comprise a table of Zn (II) binding affinity values first, to get used to searching in the data banks, then of Cu (I) binding affinity values that can be easily accessed by scientists and chemists that need them for further research. Binding constants depend on protonation states of protein residues. This is why I have also used and developed parameters for protein pKa calculations with the PKA17 software. I have reproduced cysteine pKa shifts and produced new parameters for calculating tyrosine pKa's. The data produced by these calculations will be critical in determining protein-metal binding constraints.

2.0 Background:

Understanding what binding affinities are and how they impact proteins was vital information to learn going into this project. Just knowing the binding affinities of a protein and metal helps to understand its function, efficacy, and position in a molecule. Diving deep into research papers and website data banks was also a large part of this project, as well as learning a completely new software system that I have never used. The research for the project was done on the Protein Data Bank website, where a plethora of scholarly articles on various different proteins are stored. These articles held the information we were trying to compile into one area so other scientists can access it easily. Understanding the PKA 17 technology we used to calculate the final PKA values, as well as Linux were major factors in completing this Major Qualifying Project (MQP). Learning what assumptions PKA 17 takes into consideration when calculating the final PKA values for each residue is important to understanding the project as a whole, while Linux was the software we used to run the PKA 17 calculations through; thus gaining knowledge of the key commands using throughout the process of the calculations proved to be valuable.

2.1 Metal/Protein Binding Affinities:

Binding affinities in biochemistry measures the strength of a single molecule binding to another. The first molecule in this complex is typically a biomolecule, (usually either a protein of DNA), and the second molecule in the complex is the ligand or binding partner. This second molecule is either a drug or an inhibitor in biochemistry. Binding affinities are measured in dissociation constant, which determine the order of strengths for each complex. The higher the dissociation constant, the lower the chance the complex will bind to the target molecule and the lower the dissociation constant, the stronger the chance the complex will bind to the target

molecule. The various strengths of these binding affinity complexes can be affected by the molecules around them. For example, the binding affinity of a ligand can be affected if it is near a valine amino acid molecule versus a serine amino acid molecule. Binding affinities can be applied to many areas of chemistry, biochemistry, and biology. Drug delivery is a huge area of study when it comes to binding affinities. Figuring out the binding affinities of different proteins within the body can allow scientists to develop drugs to better fight disease. By becoming more knowledgeable about these values, scientists can anticipate the efficacy of the drug, as well as the structure and position.

2.1.1 Zinc (II) and Copper (I) Binding Affinities:

The focus of this MQP is protein binding affinities with first zinc (II), then copper (I) ions. These binding affinities are scarce in scientific scholarly articles, making them hard to incorporate in independent research. Zinc (II) ions were the focus of the first part of my project, as I was to compile binding affinity values from literature. This was done as a gateway into learning how to use website databases, as well as illustrate just how hard finding these binding affinity values are. Zinc (II) binding affinity values are hard to find in literature, however not as hard as finding copper (I) binding affinities. Luckily, the copper (I) values that I was working with were provided by my advisor's previous research. Copper (I) binding plays an important role in the science world, especially when it comes to drug development chemistry and drug delivery. Cancer treatments and cancer research are both evolving topics that take a lot of time and effort to further our understanding. Copper (I) binding affinities can help in this regard, and there is early research being done on their importance in the development of cis-platin treatments, (a common cancer treatment designed to kill as many diseased cells in the body as possible). Platinum ions are currently being used, which are harder to find in nature and can

cause a human more harm than good due to platinum's harmful chemical properties. Copper (I) has been studied as a potential replacement for platinum ions because copper (I) is more abundant in nature and its binding affinities are similar to that of platinum. The binding affinities of platinum and copper (I) being similar is vital because binding affinity goes hand in hand with how effective the drug or treatment will be. The fact that the values are close gives scientists hope that with more research, copper (I) ions can replace platinum ions in cis-platin treatments.

2.2 Protein Data Bank Website:

For the majority of research done for this project, the Protein Data Bank website was utilized. The website is composed of hundreds of thousands of scholarly journal articles on various protein characteristics. Among these characteristics, binding affinity is one of the featured ones as well as structure, Protein Data Bank ID (PDB ID), and size. The PDB ID is a distinguishing four character identification system that corresponds to each protein. By typing this code into the search bar of the Protein Data Bank website, one can access all this information on the protein and more. Publications that include research and experiments done on the specific protein will also be included in the search. This way, gathering information and data from these scientific journal articles can be made much easier. The Protein Data Bank website also digitizes its information by putting it into files that can be downloaded into various computer softwares for analysis. These files are simply called Protein Data Bank files or PDB files. Each PDB file contains all the information that can be accessed on the Protein Data Bank website, however, this data can be manipulated since it is now digitized. In other words, one can focus on one trait of a specific protein, (like binding affinity), and cut out the rest of the data making the PDB file much easier to read and easier to perform analytic tests. This website became a central cog for almost all of the research done for the project, including compiling the copper (I) binding affinity values

from literature into tables, and downloading the specific protein PDB files from the website to the computer software we were using to perform the PKA 17 calculations called Linux.

2.3 Linux Software Program:

The computer software that we used to facilitate the PKA 17 calculations is called Linux. This computer software has been around since the early 1990's and is used as the operating system for android phones to this day. Linux is very versatile as it can be run from anywhere, including remote desktops, which certainly came in handy due to the ongoing pandemic. Its ability to be easily downloaded was another reason why Linux was so attractive for this MQP. It took less than thirty minutes for the operating system to be downloaded onto my laptop, which is blindingly fast compared to downloaded other operating systems. Once downloaded, the "putty-extender" allows you to access Linux by setting up an account on one of the available directories. Linux is free for all users, and was created "by the people, for the people." This ultimately means that Linux is open for anyone to use, can be modified for the betterment of the program, and can be redistributed to others if the modified Linux program proves successful. The main reason in choosing Linux for this project, however, was that it can successfully run the PKA 17 calculations with little edits to the already existing files. The PDB files, (mentioned in a previous section), need minimal tidying up for the program to run, which saves an abundance of time compared to running these calculations through another operating system. The edits themselves are also made easy due to the fact that you can remove large chunks of unwanted data by typing in very short commands. These edits may seem insignificant, but they make the PDB files much more manageable and easy to follow. Linux also organizes the data in a way that is easy to understand, which again supports the myriad of reasons why we chose Linux for this project.

2.4 PKA 17 Calculations:

This project is based around calculating binding affinities through a calculation developed and worked on by my advisor George Kaminski called PKA 17. The calculation is able to give a final PKA value for a binding complex taking into account neighboring residues, and most amino acids. PKA 17 data has been published in many scientific journal articles partially authored by my advisor, but it is still being studied and improved upon to this day. This project will help further validate the PKA 17 calculations as well. By comparing experimental or obtained PKA values from other scientific literature, one can gather a large number of values to compare to the final PKA values the calculation gives. If the experimental PKA values from either experiments or scientific literature are similar to the final PKA values given by PKA 17, then we can consider the final PKA to be a good estimation for the binding affinity.

2.4.1 PKA 17 Assumptions:

The main assumption for the PKA 17 calculation is taking the 20 amino acids and combining them to have 17, (hence PKA 17, meaning conducting the calculations with 17 amino acids instead of 20). This helps with cleaning up the PDB files that are used to do the actual calculations. My advisor has combined valine, leucine, and isoleucine as one, as well as grouping glutamine and asparagine together, and glutamic acid and aspartic acid together. By doing so, the amount of amino acids in the calculation goes from 20 to 17, since the three groups of combined amino acids each count as one. These amino acids were chosen to be combined since their properties and chemical structures are very similar. The PKA 17 calculations would be more inaccurate if all 20 amino acids were included. PKA 17 sees the amino acids that were grouped together as redundant, thus grouping them together corrects this problem.

3.0 Methodology:

The process of completing this MQP was split into two parts; one part was analyzing and finding zinc (II) binding affinities in scientific journal articles, and the other part was analyzing cysteine and tyrosine PKA values my advisor had already gathered through Linux and PKA 17. For the zinc (II) binding affinity values, I used the Protein Data Bank website to look these values up. Once I found just over one hundred zinc (II) binding affinity values from various scientific journal articles, I amassed a table in Microsoft Excel to make them more organized, as well as putting the corresponding PDB ID numbers and citations of the articles the values were derived from in the table. The second portion of the project entailed analytical and computational chemistry skills to replicate and formulate parameters for PKA 17 calculations with cysteine PKA values. I also took my professor's research with tyrosine PKA values and developed new parameters by calculating their final PKA values through PKA 17. Tables were constructed through Microsoft Excel of the data collected during these processes, and were provided as deliverables in the next section of this paper.

3.1 Comprising a Table of Zinc (II) Binding Affinities:

For this component of the project, my advisor recommended I search articles with zinc (II) binding affinities on the Protein Data Bank website, (PDB). In the search bar on the website, you can search by PDB ID #, molecule, or by a protein's specific name. I did not have much to go on since my goal was to find proteins with zinc (II) binding affinities from scientific journal articles on the website. Therefore, I did not know the exact proteins I was looking for; I only know what element they would be complexed to. The first search I tried was "zinc (II) binding affinities." This search yielded the most results for my table of comprised zinc (II) binding affinities. The hardest part about this section of the MQP was actually determining whether all of

the over one thousand articles that came up in my search are relevant. Checking to see if complexes can be made with zinc (II) is the first step of making sure the article goes with our research project. Once the article is clicked on, one can scroll down to the bottom of the page to the section called “small molecules.” Under this heading, there should be a section for “ligands” that should include zinc (II). If this “ligands” sub-section does not include zinc (II), then the article is deemed not relevant for our research and we move to another. However, if zinc (II) was included in the “ligands” sub-section, then we investigated the article. Once the article is deemed relevant to this project’s research, it was key to note that binding affinity is measured in Kd, (dissociation constant). This term for binding affinity is used throughout scientific journals since it is a quicker and easy way to illustrate binding affinity in a written paper. I combed the relevant article for these Kd values, which were usually found in various data tables in the article itself. The catch to finding these values was to make sure that they corresponded to zinc (II) and not various amino acids. Although the binding affinities for amino acids are important, they are not what this project was focused on. I did add these values to my zinc (II) binding affinity table, however since they were values complexed with zinc (II). The binding affinity values that are proteins complexed with zinc (II) is more of what we were looking for. Protein zinc (II) binding affinities were added to a table in Microsoft Excel with the article citation, PDB ID number, type of molecule, and if the value in the article was calculated or experimental. After looking through over one thousand articles, I was able to find one hundred nine values corresponding to our project. This is an example of just how hard finding these values are, and shows how scarce zinc (II) binding affinities are in scientific literature. The table of zinc (II) binding affinities is going to be quite useful in the scientific and chemistry fields since all these values are concentrated in one spot, (in my newly constructed table), versus scattered amongst many different scientific

journal articles. Such tables had not been compiled by other researchers. Some binding constants are available, many PDB structures are available, but structures together with energies are not something that you would easily see in one big table

3.2 Setting up Linux and Downloading PDB Files:

My advisor for this project had already found cysteine and tyrosine PKA values in various proteins, however, I would first have to download the corresponding PDB files from the Protein Data Bank website to my laptop. For my experiment with cysteine PKA values, forty-five proteins were analyzed; thus about forty-five PDB files. The experiment involving tyrosine PKA values included forty-four proteins; thus about forty-four PDB files. The number of proteins to PDB files may not be the same number due to multiple residues being analyzed in the same protein. Once all the correct PDB files were downloaded, the next step was to set up an account for myself on Linux through Worcester Polytechnic Institute, (WPI). I used WPI's solar-2.wpi.edu network on Linux to complete the project, and a directory titled "FOR_GREGGORY" was created by my advisor to use within the solar-2.wpi.edu network. Once I had my accounts all squared away, I decided to do some Linux practice on my own, since I had never used the software before in previous research. Tutorial videos were helpful to learn the base commands, liking opening up files, saving them to my directory, and renaming files so that I could organize them better within my directory. After I felt comfortable with Linux, there was another application that my advisor said would be useful to download, which was WinSCP. WinSCP allows the user to take downloaded files that are already on one's laptop or computer safely to another directory. For example, I could have transferred files from my personal desktop to my Linux directory "FOR_GREGGORY" with this WinSCP application. This came in handy once the PDB files for each individual protein was downloaded to the desktop on my personal

laptop. By clicking on each file individually and then dragging them over to the desired folder or directory, ("FOR_GREGGORY"), all of the files could now be seen and worked on in Linux.

3.3 Preparing PDB Files for PKA 17:

As they sat, the PDB files downloaded directly from the Protein Data Bank website were not ready to be run through PKA 17. They needed to be trimmed up so only the data that concerned our research was present in the files. To assist with the preparation of the PDB files, my advisor gave me one of his published papers regarding cysteine and tyrosine PKA values. This information allowed me to identify which residue to analyze and focus on. Logging into Linux, I selected one of the PDB files, for example 2ovo.pdb, and opened it using the "vi" command. Then, I typed the command "more 2ovo.pdb | grep ATOM | grep CA" to single out the data I wanted in the large PDB file. By typing "| grep ATOM | grep CA", this command tells the operating system that I only want lines of code that contain "ATOM" and "CA". Once this was completed, the next step was to look at my advisor's published papers, and determine which cysteine or tyrosine residue was in question. The residue number is typed at the top of the PDB file, and this let PKA 17 know that the calculation was to be done at this specific cysteine or tyrosine residue. To further trim up the PDB file, lines of code that were identical or described a different atom were deleted by either typing "dd" for a singular deletion, or "d1000000" for multiple deletions. After this step was done, the PDB files were saved under new names using the "mv" command. The cysteine residue files were saved as a number between 001 to 045, and the tyrosine residue files were saved as a number between 0001 to 0044. This was done to ensure organization, and minimize confusion between the two sets of files. Once all the files were organized this way, the next step would be to get all the data in the form of a histogram. To do this step, the "testcys" file was used for the cysteine residue files and "testtyr" was used for the

tyrosine residues. Typing these commands left us with PKA values, and numbers ranging 0-10 across. The PKA values were disregarded, since they were to be replaced with the experimental PKA values my advisor previously researched. The numbers ranging from 0-10 correspond to the number of amino acids residues around the complex. My advisor set up a separate file called "res.20" that the histogram data was transposed onto. After this histogram data has been copied into the "res.20" file, the experimental PKA values from my advisor's published paper and tables were added to the left side to indicate which binding affinity was in question. The "res.20" file was then saved as "res.new" for the cysteine residues and "res.newtyr" for the tyrosine residues. The last step before running the actual PKA 17 calculations was to turn the "res.new" and "res.newtyr" files, (that contained twenty amino acids), to two "res.17" files, (that contained seventeen amino acids). To do this, we looked at the assumptions PKA 17 used, which were talked about in a previous section of this paper. Combining the residue numbers for leucine, isoleucine, and valine allowed us to cut down the number of amino acids in the calculation by one. This combining of amino acids was also done for glutamine and asparagine, as well as glutamic acid and aspartic acid. Without doing these specific amino acid combinations, PKA 17 would not do its calculation properly, as it assumes the amino acids that are in each specific combination previously mentioned have the same chemical properties; thus deeming there to be duplicates in the file. Once both the "res.17" files for cysteine and tyrosine were completed, the PKA 17 calculations were ready to be performed.

3.4 Running PKA 17:

Running the PKA 17 calculations occurred once the PDB files for the various proteins with cysteine and tyrosine PKA values were prepared.. Each separate PDB file had to be run one at a time, so this effort was tedious. The command to run PKA 17 was "/pka17f2" followed by

the number of the PDB file. For the cysteine PKA values, it was a number from 001 to 044. The tyrosine PKA values were numbered 0001 to 0045. Once the command in Linux was entered, the PKA 17 calculation could be obtained. The PKA 17 calculation file has an abundance of information in it including what amino acids are around the residue in focus, the final calculated PKA value, and the histogram data that was previously dealt with in the preparation of the PDB files. The most important piece of information from the PKA 17 calculation file was the final calculated PKA values. These values were the crux of the MQP, and the most valuable data collected in the project. The final PKA values calculated from PKA 17 were tabulated into separate tables for cysteine and tyrosine values. Organizing the newly acquired data into tables made it easier to compare the values that were generated by PKA 17. Experimental PKA values for cysteine and tyrosine were compared to the calculated final PKA 17 values. This comparison led to calculating the error between experimental PKA value and the PKA 17 final calculated values. The error calculations were a key result, (which will be talked about me in-depth in the next section), as these values determined the validity of the PKA 17 calculation.

4.0 Results:

After the process of researching binding affinity values and running files through the PKA 17 software in Linux, results were gathered throughout each process. In the first portion of the MQP, the zinc (II) binding affinities were tabulated for the usage of future researchers. The second portion of the MQP was gathering cysteine and tyrosine PKA values from PKA 17 and proving its validity in science and their validation. All results produced in the project could help move science and chemistry forward if they are applied to certain fields of research. Further discussion of the applications of the results from this project will be included in the conclusion section of this paper. This section will be a broad overview of all deliverables amassed while working on this MQP.

4.1 Zinc (II) Binding Affinity Table:

As described in this paper, the first portion of the project was completing research on zinc (II) binding affinities and comprising a table with them. The table is shown below in the following figures, (as it was too big to fit in just one figure).

	Zn(II) Binding Affinities		
Type	*Zn(II) Affinity (KJ/mol-1)*	*Calculated or Experimental*	*Reference*
Amino Acids	-96.8	Experimental	"A Theoretical Study of Zinc (II) Interactions with Amino Acid Models and Peptide Fragments"
Peptide	-66.7	Experimental	"A Theoretical Study of Zinc (II) Interactions with Amino Acid Models and Peptide Fragments"
Amino Acid	-38.7	Experimental	"A Theoretical Study of Zinc (II) Interactions with Amino Acid Models and Peptide Fragments"
H20	0	Experimental	"A Theoretical Study of Zinc (II) Interactions with Amino Acid Models and Peptide Fragments"
Amino Acids	-4.49	Experimental	"A Theoretical Study of Zinc (II) Interactions with Amino Acid Models and Peptide Fragments"
Amino Acid	-13.4	Experimental	"A Theoretical Study of Zinc (II) Interactions with Amino Acid Models and Peptide Fragments"
Amino Acid	-17.9	Experimental	"A Theoretical Study of Zinc (II) Interactions with Amino Acid Models and Peptide Fragments"
Amino Acid	-60.4	Experimental	"A Theoretical Study of Zinc (II) Interactions with Amino Acid Models and Peptide Fragments"
Amino Acid	-34.3	Experimental	"A Theoretical Study of Zinc (II) Interactions with Amino Acid Models and Peptide Fragments"
Amino Acids	9.63	Experimental	"A Theoretical Study of Zinc (II) Interactions with Amino Acid Models and Peptide Fragments"
Amino Acid	19.3	Experimental	"A Theoretical Study of Zinc (II) Interactions with Amino Acid Models and Peptide Fragments"
Amino Acid	21.4	Experimental	"A Theoretical Study of Zinc (II) Interactions with Amino Acid Models and Peptide Fragments"
Amino Acids	7.36	Experimental	"A Theoretical Study of Zinc (II) Interactions with Amino Acid Models and Peptide Fragments"
Amino Acid	64.3	Experimental	"A Theoretical Study of Zinc (II) Interactions with Amino Acid Models and Peptide Fragments"
Amino Acid	33.1	Experimental	"A Theoretical Study of Zinc (II) Interactions with Amino Acid Models and Peptide Fragments"
Buffer Solution	76.4	Calculated	"The Biological Inorganic Chemistry of Zinc Ions"
Buffer Solution	118.7	Calculated	"The Biological Inorganic Chemistry of Zinc Ions"
Amino Acid	139.3	Experimental	"The Biological Inorganic Chemistry of Zinc Ions"
Amino Acid	82.3	Experimental	"The Biological Inorganic Chemistry of Zinc Ions"
Amino Acid	82.5	Experimental	"The Biological Inorganic Chemistry of Zinc Ions"
Amino Acid	96	Experimental	"The Biological Inorganic Chemistry of Zinc Ions"
Amino Acid	197.1	Calculated	"The Biological Inorganic Chemistry of Zinc Ions"
Amino Acid	125.5	Calculated	"The Biological Inorganic Chemistry of Zinc Ions"
Protein/Enzyme	551.1	Calculated	"Inhibitory Zinc Sites in Enzymes"
Protein/Enzyme	588.2	Calculated	"New Perspectives of Zinc Coordination Environments in Proteins"
Protein/Enzyme	24.9	Experimental	"Redox Biochemistry of Mammalian Metallothioeneins"

Protein	-39.8	Experimental	"Mechanistic Explanation of Two Novel Zn (II) Dithiocarbamate Complexes with Beta-lactoglobulin"
Protein	-44	Experimental	"Mechanistic Explanation of Two Novel Zn (II) Dithiocarbamate Complexes with Beta-lactoglobulin"
Protein	130.2	Experimental	"Insight into the Binding Behavior of Ceritinib on Human alpha-1 acid glycoprotein: Multi-Spectroscopic and Molecular Modeling Approaches"
Protein	kd is 4.6 nanoMolar	Experimental	"Metal binding properties, stability and reactivity of zinc fingers"
Protein	kd is 55nM	Experimental	"Parameters Influencing Zinc in Experimental Systems in Vivo and in Vitro"
Protein	kd is -1.6nM	Experimental	"Parameters Influencing Zinc in Experimental Systems in Vivo and in Vitro"
Protein	kd is 25 picoMolar	Experimental	"Parameters Influencing Zinc in Experimental Systems in Vivo and in Vitro"
Protein	kd is 16nM	Experimental	"Parameters Influencing Zinc in Experimental Systems in Vivo and in Vitro"
Protein	kd is 21 nM	Experimental	"Characterization of the Zn(II) Binding Properties of the Human Wilms' Tumor Suppressor Protein C-terminal Zinc Finger Peptide"
Protein	kd is 1.9 nM	Experimental	"Characterization of the Zn(II) Binding Properties of the Human Wilms' Tumor Suppressor Protein C-terminal Zinc Finger Peptide"
Protein	kd is 300nM	Experimental	"Characterization of the Zn(II) Binding Properties of the Human Wilms' Tumor Suppressor Protein C-terminal Zinc Finger Peptide"
Protein	kd is 36 nM	Experimental	"Characterization of the Zn(II) Binding Properties of the Human Wilms' Tumor Suppressor Protein C-terminal Zinc Finger Peptide"
Protein	kd is 8.5 pM	Experimental	"Characterization of the Zn(II) Binding Properties of the Human Wilms' Tumor Suppressor Protein C-terminal Zinc Finger Peptide"
Protein	kd is 20 nM	Experimental	"Characterization of the Zn(II) Binding Properties of the Human Wilms' Tumor Suppressor Protein C-terminal Zinc Finger Peptide"
Protein	kd is 54 nM	Experimental	"Characterization of the Zn(II) Binding Properties of the Human Wilms' Tumor Suppressor Protein C-terminal Zinc Finger Peptide"
Protein	kd is 12 nM	Experimental	"Characterization of the Zn(II) Binding Properties of the Human Wilms' Tumor Suppressor Protein C-terminal Zinc Finger Peptide"
Complexone	kd is 6.4x10^-16M	Experimental	"Metal binding properties, stability and reactivity of zinc fingers"
Complexone	kd is 3.7x10^-15M	Experimental	"Metal binding properties, stability and reactivity of zinc fingers"
Complexone	kd is 5.6x10^-15M	Experimental	"Metal binding properties, stability and reactivity of zinc fingers"
Complexone	kd is 2.3x10^-14M	Experimental	"Metal binding properties, stability and reactivity of zinc fingers"
Complexone	kd is 6.6x10^-13M	Experimental	"Metal binding properties, stability and reactivity of zinc fingers"
Complexone	kd is 2.3x10^-11M	Experimental	"Metal binding properties, stability and reactivity of zinc fingers"
Complexone	kd is 4.9x10^-10M	Experimental	"Metal binding properties, stability and reactivity of zinc fingers"
Complexone	kd is 6.3x10^-10M	Experimental	"Metal binding properties, stability and reactivity of zinc fingers"
Complexone	kd is 1.2x10^-9M	Experimental	"Metal binding properties, stability and reactivity of zinc fingers"
Complexone	kd is 4.4x10^-9M	Experimental	"Metal binding properties, stability and reactivity of zinc fingers"
Complexone	kd is 9.8x10^-9M	Experimental	"Metal binding properties, stability and reactivity of zinc fingers"

Indicator	kd is 0.7 nM	Experimental	"Parameters Influencing Zinc in Experimental Systems in Vivo and in Vitro"
Indicator	kd is 0.5nM	Experimental	"Parameters Influencing Zinc in Experimental Systems in Vivo and in Vitro"
Indicator	kd is 7.8 micro M	Experimental	"Parameters Influencing Zinc in Experimental Systems in Vivo and in Vitro"
Indicator	kd is 2 micro M	Experimental	"Parameters Influencing Zinc in Experimental Systems in Vivo and in Vitro"
Indicator	kd is 8.9 micro M	Experimental	"Parameters Influencing Zinc in Experimental Systems in Vivo and in Vitro"
Indicator	kd is 1 micro M	Experimental	"Parameters Influencing Zinc in Experimental Systems in Vivo and in Vitro"
Indicator	kd is 40 micro M	Experimental	"Parameters Influencing Zinc in Experimental Systems in Vivo and in Vitro"
Indicator	kd is 3.4 micro M	Experimental	"Parameters Influencing Zinc in Experimental Systems in Vivo and in Vitro"
Indicator	kd is 1.5 nM	Experimental	"Parameters Influencing Zinc in Experimental Systems in Vivo and in Vitro"
Indicator	kd is 3 nM	Experimental	"Parameters Influencing Zinc in Experimental Systems in Vivo and in Vitro"
Indicator	kd is 20 nM	Experimental	"Parameters Influencing Zinc in Experimental Systems in Vivo and in Vitro"
Indicator	kd is 27 nM	Experimental	"Parameters Influencing Zinc in Experimental Systems in Vivo and in Vitro"
Indicator	kd is 23 micro M	Experimental	"Parameters Influencing Zinc in Experimental Systems in Vivo and in Vitro"
Indicator	kd is 65 nM	Experimental	"Parameters Influencing Zinc in Experimental Systems in Vivo and in Vitro"
Indicator	kd is 370 nM	Experimental	"Parameters Influencing Zinc in Experimental Systems in Vivo and in Vitro"
Indicator	kd is 15.5 micro M	Experimental	"Parameters Influencing Zinc in Experimental Systems in Vivo and in Vitro"
Protein	kd is 8.1 micro M	Experimental	"Solution Structure of a Zap1 Zinc-responsive Domain Provides Insights into Metalloregulatory Transcriptional Repression in Saccharomyces cerevisiae"
Protein	kd is 8.8 nM	Experimental	"Solution Structure of a Zap1 Zinc-responsive Domain Provides Insights into Metalloregulatory Transcriptional Repression in Saccharomyces cerevisiae"
Protein	kd is 160 nM	Experimental	"Solution Structure of a Zap1 Zinc-responsive Domain Provides Insights into Metalloregulatory Transcriptional Repression in Saccharomyces cerevisiae"
Protein	kd is 150 micro M	Experimental	"Solution Structure of a Zap1 Zinc-responsive Domain Provides Insights into Metalloregulatory Transcriptional Repression in Saccharomyces cerevisiae"
Protein	kd is 250 nM	Experimental	"Solution Structure of a Zap1 Zinc-responsive Domain Provides Insights into Metalloregulatory Transcriptional Repression in Saccharomyces cerevisiae"
Protein	kd is 130 micro M	Experimental	"Solution Structure of a Zap1 Zinc-responsive Domain Provides Insights into Metalloregulatory Transcriptional Repression in Saccharomyces cerevisiae"
Ligand	4.94 micro M	Experimental	"Structural Basis for Bifunctional Zinc(II) Macrocyclic Complex Recognition of Thymine Bulges in DNA"
Ligand	5.88 micro M	Experimental	"Structural Basis for Bifunctional Zinc(II) Macrocyclic Complex Recognition of Thymine Bulges in DNA"
Ligand	5.54 micro M	Experimental	"Structural Basis for Bifunctional Zinc(II) Macrocyclic Complex Recognition of Thymine Bulges in DNA"
Ligand	5.78 micro M	Experimental	"Structural Basis for Bifunctional Zinc(II) Macrocyclic Complex Recognition of Thymine Bulges in DNA"
Protein	kd is 2.2 micro M	Experimental	"Structural Basis for Bifunctional Zinc(II) Macrocyclic Complex Recognition of Thymine Bulges in DNA"

Ugg **(4V5P)**	Protein	kd is 12 micro M	Experimental	"Structural Basis for Bifunctional Zinc(II) Macrocyclic Complex Recognition of Thymine Bulges in DNA"
Cgg **(2ERG)**	Protein	kd is 185 micro M	Experimental	"Structural Basis for Bifunctional Zinc(II) Macrocyclic Complex Recognition of Thymine Bulges in DNA"
Agg **(4AQL)**	Protein	kd is 200 micro M	Experimental	"Structural Basis for Bifunctional Zinc(II) Macrocyclic Complex Recognition of Thymine Bulges in DNA"
Gcc **(5ZMM)**	Protein	kd is 108 micro M	Experimental	"Structural Basis for Bifunctional Zinc(II) Macrocyclic Complex Recognition of Thymine Bulges in DNA"
HP3 **(1UN6)**	Protein	kd is 142 micro M	Experimental	"Structural Basis for Bifunctional Zinc(II) Macrocyclic Complex Recognition of Thymine Bulges in DNA"
HP4 **(1UN6)**	Protein	kd is 104 micro M	Experimental	"Structural Basis for Bifunctional Zinc(II) Macrocyclic Complex Recognition of Thymine Bulges in DNA"
HPTL **(1UN6)**	Protein	kd is 127 micro M	Experimental	"Structural Basis for Bifunctional Zinc(II) Macrocyclic Complex Recognition of Thymine Bulges in DNA"
HP3nT **(1UN6)**	Protein	kd is 435 micro M	Experimental	"Structural Basis for Bifunctional Zinc(II) Macrocyclic Complex Recognition of Thymine Bulges in DNA"
rHP **(1UN6)**	Protein	kd is 20 micro M	Experimental	"Structural Basis for Bifunctional Zinc(II) Macrocyclic Complex Recognition of Thymine Bulges in DNA"
CCTCC **(1UN6)**	Protein	kd is 25 micro M	Experimental	"Structural Basis for Bifunctional Zinc(II) Macrocyclic Complex Recognition of Thymine Bulges in DNA"
MTF1-all **(1P26)**	Zinc Finger	kd is 6.3 micro M	Experimental	"Metal binding properties, stability and reactivity of zinc fingers"
Zn-F10 **(6OV8)**	Zinc Finger	kd is 6.5 micro M	Experimental	"Metal binding properties, stability and reactivity of zinc fingers"
WT1-3 **(5KL3)**	Zinc Finger	kd is 8.7 micro M	Experimental	"Metal binding properties, stability and reactivity of zinc fingers"
TFIIIA-all **(1TF6)**	Zinc Finger	kd is 8 micro M	Experimental	"Metal binding properties, stability and reactivity of zinc fingers"
MBP-Wild Type **(3HPI)**	Protein	kd is 50 micro M	Calculated	"Structural Studies of an Engineered Zinc Biosensor Reveal an Unanticipated Mode of Zinc Binding"
EZ-MBP-HA **(1BBO)**	Protein	kd is 0.29 micro M	Calculated	"Structural Studies of an Engineered Zinc Biosensor Reveal an Unanticipated Mode of Zinc Binding"
EZ-MBP **(1BBO)**	Protein	kd is 2.7 micro M	Calculated	"Structural Studies of an Engineered Zinc Biosensor Reveal an Unanticipated Mode of Zinc Binding"
MBP-lobe1 **(1BBO)**	Protein	kd is 50 micro M	Calculated	"Structural Studies of an Engineered Zinc Biosensor Reveal an Unanticipated Mode of Zinc Binding"
3GCG (zf222 site) **(2RUV)**	Protein	kd is 61.4 nM	Experimental	"Multiconnection of Identical Zinc Finger: Implication of DNA Binding Affinity and Unit Modulation of the Three Zinc Finger Dominant"
3GCG (zf2222 site) **(2RUV)**	Protein	kd is 32.4 nM	Experimental	"Multiconnection of Identical Zinc Finger: Implication of DNA Binding Affinity and Unit Modulation of the Three Zinc Finger Dominant"
4GCG (zf222 site) **(2RUV)**	Protein	kd is 103 nM	Experimental	"Multiconnection of Identical Zinc Finger: Implication of DNA Binding Affinity and Unit Modulation of the Three Zinc Finger Dominant"
4GCG (zf2222 site) **(2RUV)**	Protein	kd is 18.2 nM	Experimental	"Multiconnection of Identical Zinc Finger: Implication of DNA Binding Affinity and Unit Modulation of the Three Zinc Finger Dominant"
ZnF2-TF **(2GQE)**	Zinc Finger	kd is 2000 micro M	Experimental	"Molecular Characterization of the Ran-binding Zinc Finger Domain of Nup153"
ZnF2-TFM **(2GQE)**	Zinc Finger	kd is 4000 micro M	Experimental	"Molecular Characterization of the Ran-binding Zinc Finger Domain of Nup153"
ZnF3 **(2GQE)**	Zinc Finger	kd is 39 micro M	Experimental	"Molecular Characterization of the Ran-binding Zinc Finger Domain of Nup153"
ZnF4 **(2GQE)**	Zinc Finger	kd is 32 micro M	Experimental	"Molecular Characterization of the Ran-binding Zinc Finger Domain of Nup153"
Npl4 **(2GQE)**	Zinc Finger	kd is 2385 micro M	Experimental	"Molecular Characterization of the Ran-binding Zinc Finger Domain of Nup153"
Npl4-LVA **(2GQE)**	Zinc Finger	kd is 350 micro M	Experimental	"Molecular Characterization of the Ran-binding Zinc Finger Domain of Nup153"
C96-RIDC-14 **(3IQ5)**	Protein	kd is 5.2x10^-10 M	Experimental	"Evolution of Metal Selectivity in Templated Protein Interfaces"

Figures 1, 2, 3, and 4 show the entire zinc (II) binding affinity table I found during this project.

As seen in figures 1, 2, 3, and 4, all of the article titles that the values were taken from are listed. The first section of the zinc (II) binding affinity table is with certain isolated amino acids in aqueous solution. They were not from a specific protein, thus they do not have a PDB identification number. The binding affinity values for these amino acids are measured in kj/mol^{-1}, and the values were recorded as being either calculated or experimental. A calculated value was deemed if the number in the journal article was derived by an equation from the researchers. A value was deemed to be experimental when the researchers conducted an experiment and received a number from that. Most of the data I found in the first section of the zinc (II) binding affinity table were experimental values with few exceptions. It is natural to view the experimental numbers as more reliable than the calculated ones due to the fact that they have been vetted through a scientific experiment. The number of zinc (II) binding affinity values I found for the amino acids was around 22. The majority of the rest of the values were for protein binding of zinc (II). For these values, they were measured in Kd, which is dissociation constant,

(this was defined earlier in the paper). The zinc (II) binding affinity values with proteins all have assigned PDB identification numbers based on the entries in the Protein Data Bank website. For all these protein binding affinities with zinc (II), the articles that the values were found in are listed on the sections of tables, as well as if the values were calculated or experimental. Just like the amino acid section of the zinc (II) binding affinity table, most of the zinc (II) binding constraints were experimental. The significance and applications of these results will be discussed in the conclusion section of this paper.

4.2 Cysteine and Tyrosine PKA's:

The second portion of my MQP yielded final PKA values using PKA 17 of cysteine and tyrosine residues. These results were compared to the experimental PKA values my advisor had given me, and a standard unsigned error was calculated, as well as an average unsigned error.The table was organized by PDB identification number, and there were 44 data points for the cysteine residues as well as 45 data points for the tyrosine residues. A standard unsigned error was calculated for each individual data point for both data sets. The average unsigned error was calculated by averaging all of the individual standard unsigned errors and dividing the total by the number of data points in each data set. Both data sets are shown below in figures 5 and 6.

CYS	PDB ID	Residue #	Experimental pKa	Final pKa	Unsigned Error	Average Unsigned Error
	1HIC	C39	3.76	3.79	0.03	1.121
	3TRX	C32	6.3	6.61	0.31	1.121
	1UAE	C115	8.3	8.96	0.66	1.121
	1L1N	C147	8.86	8.21	0.65	1.121
	1I9D	C12	6.4	6.72	0.32	1.121
	1A23	C30	3.5	4.63	1.13	1.121
	1A23	C33	9.5	8.64	0.86	1.121
	1XOB	C32	7.1	7.7	0.6	1.121
	1XOB	C35	9.9	9.9	0	1.121
	1I7K	C102	10.9	9.38	1.52	1.121
	1JAS	C88	10.2	8.69	1.51	1.121
	1JBB	C87	11.1	8.23	2.87	1.121
	1GSS	C45	4.2	6.23	2.03	1.121
	3BPB	C249	8.9	8.43	0.47	1.121
	1MEK	C36	4.5	5.53	1.03	1.121
	1KCT	C232	6.86	6.3	0.56	1.121
	1CTE	C29	3.6	2.73	0.87	1.121
	1CPJ	C29	3.6	4.25	0.65	1.121
	1THE	C29	3.6	3.18	0.42	1.121
	1QLP	C232	6.86	8.35	1.49	1.121
	4MA9	C46	5.94	7.21	1.27	1.121
	4MA9	C165	8.64	10.58	1.94	1.121
	1P5F	C106	5.4	5.73	0.33	1.121
	1I0E	C283	5.6	4.99	0.61	1.121
	2L90	C72	8.2	6.24	1.96	1.121
	1EH6	C145	5.3	7.42	2.12	1.121
	1PPN	C25	3.32	2.25	1.07	1.121
	2HNP	C215	5.57	5.8	0.23	1.121
	1PPO	C25	2.88	2.43	0.45	1.121
	1M8B	C56	2.27	4.74	2.47	1.121
	1M8C	C56	2.27	2.52	0.25	1.121
	1OMU	C56	2.5	4.7	2.2	1.121
	2OVO	C56	2.23	4.28	2.05	1.121
	1B4Q	C22	3.6	5.73	2.13	1.121
	2H19	C74	8.48	7.7	0.78	1.121
	2H1A	C77	8.33	9.56	1.23	1.121
	2H1B	C77	7.4	8.92	1.52	1.121
	3C71	C74	7.2	6.18	1.02	1.121
	4IP1	C461	10.5	7.82	2.68	1.121
	2FWF	C461	9.3	7.89	1.41	1.121
	1O73	C40	7.2	6.03	1.17	1.121
	3CZ9	C106	6.4	4.41	1.99	1.121
	3CY6	C106	5.7	5.78	0.08	1.121
	3CZA	C106	6.1	5.73	0.37	1.121

Figure 5 shows the cysteine final PKA values generated by PKA 17.

TYR	PDB ID	Residue Number	Expt. PKA	Final PKA	Unsigned Error	Average Unsigned Error
	1BEG	12	~11.5	11.27	0.23	0.496
	1BEG	33	>12.0	11.25	0.76	0.496
	1BEG	47	>12.0	11.83	0.18	0.496
	1BEG	85	10.35	10.67	0.32	0.496
	1BEG	87	>12.0	11.28	0.73	0.496
	1BEO	33	>12.0	12.18	0.17	0.496
	1BEO	47	>12.0	12.56	0.55	0.496
	1BEO	87	>12.0	11.41	0.6	0.496
	1BEO	12	~11.5	11.06	0.44	0.496
	1BEO	85	10.35	11.19	0.84	0.496
	1BF4	8	>9.3	11.34	2.03	0.496
	1BF4	34	>10.0	10.21	0.2	0.496
	1C8C	8	>9.3	10.1	0.79	0.496
	1C8C	34	>10.0	10.37	0.36	0.496
	1RGG	30	11.3	10.97	0.33	0.496
	1RGG	49	10.6	11.16	0.56	0.496
	1PGB	33	~11	10.46	0.54	0.496
	2OVO	11	10.16	10.73	0.57	0.496
	2OVO	20	11.07	10.57	0.5	0.496
	2OVO	31	>12.5	11.24	1.27	0.496
	4PTI	10	10.4	10.74	0.34	0.496
	4PTI	21	11.1	11.8	0.7	0.496
	4PTI	23	11.7	11.64	0.06	0.496
	4PTI	35	11.1	10.85	0.25	0.496
	1RGG	86	>11.5	11.85	0.34	0.496
	1RGG	52	>11.5	11.08	0.43	0.496
	1RGG	55	>11.5	11.49	0.02	0.496
	1RGG	80	>11.5	11.41	0.1	0.496
	1RGG	81	>11.5	11.41	0.1	0.496
	2MB5	103	10.3	10.21	0.09	0.496
	2MB5	151	10.5	10.27	0.23	0.496
	1PGA	3	>11	11.73	0.72	0.496
	1PGA	45	>12	11.33	0.68	0.496
	1PGA	33	~11	10.55	0.45	0.496
	1PGB	3	>11	11.7	0.69	0.496
	1PGB	45	>12	10.61	1.4	0.496
	2LZT	20	10.3	10.49	0.19	0.496
	2LZT	23	9.8	10.44	0.6	0.496
	2LZT	53	12.1	11.61	0.49	0.496
	1MBC	103	10.3	10.65	0.35	0.496
	1MBC	151	10.5	10.44	0.06	0.496
	2ZTA	17	9.82	10.24	0.42	0.496
	1ANS	7	9.7	10.23	0.53	0.496
	1ANS	18	10.1	9.6	0.5	0.496
	1HRC	67	11	10.37	0.63	0.496

Figure 6 shows the tyrosine final PKA values generated by PKA 17.

The error calculations were a critical result for this portion of the project because it validates PKA 17's performance, which was one of the goals of the project. As discussed previously, PKA 17 takes into account assumptions in its calculations; the main one being that it combines similarly structured amino acid residues together. These groups of amino acids are isoleucine, leucine, and valine as one group. Another group is asparagine and glutamine, and the final group is aspartic acid and glutamic acid. Since the calculated average unsigned error for each data set was relatively low, (cysteine was 1.121 and tyrosine was 0.496 pH units), the calculations by PKA 17 can be considered sufficiently accurate.

5.0 Conclusion:

Based on the results gathered from this project, conclusions can be drawn and the results can be applied to different areas of science. For the table of zinc (II) binding affinities, it can be applied to any inorganic chemistry research, metalorganic chemistry research, and analytical chemistry research. The biggest reason for doing this portion of the project was to make zinc (II) binding affinities more abundantly available for researchers. Instead of combing through thousands of research articles to find data points, continuing this table to have a multitude of zinc (II) binding affinity values would not only save time, but also potentially allow experiments and research to be conducted more swiftly. For the cysteine and tyrosine residue data sets, these results can be applied to pharmaceutical research and cancer research. More specifically, these data points can be applied to the cis-platin cancer treatment.

5.1 Zinc (II) Binding Affinity Table Applications:

The zinc (II) binding affinity table has many general applications in the world of science. Knowing now how grueling finding these values was for my project, a concentrated table of values would be best. It is inconvenient for scientists and researchers alike to scour hundreds and hundreds of scientific journal articles, just to find minimal results. This table would help people in the science and chemistry fields by cutting down the time it takes to find these values for other research. While simply finding and amassing a table of zinc (II) binding affinities seems arbitrary, it would be very helpful in completing research and helping all scientists find these values if they need them. However, there are some drawbacks to having a table like this one of zinc (II) binding affinities. For example, the authors of the articles the zinc (II) binding affinities have been taken from need to be vetted. An easy way to do this would be to click the links at the bottom of the article to see where the authors are getting their own information. Another would

be to see how many times the article itself has been cited in other research. Doing these two things will help establish if the articles that the zinc (II) binding affinities are being pulled from are legitimate. Determining the methods in which the zinc (II) binding affinities were acquired was key to seeing if the values belonged in the table, (if the values were experimental). By performing these quick checks to make sure the values are reliable, a table like the one I have created with zinc (II) binding affinities would have many general applications and benefits in the field of science and chemistry.

5.2 Cysteine and Tyrosine PKA Applications:

The cysteine and tyrosine PKA's generated by PKA 17 have applications in pharmaceuticals, mainly in cancer research. Hard acid soft base chemical interactions come into play with these types of results. In the widely known cis-platin treatment used for cancer, cysteine is more of the focus rather than tyrosine. The structure of cysteine allows for it to act like an acid because of its -SH side chain, which is odd considering its PKA suggests cysteine should be basic. This -SH side chain, or thiol chain, participates in many different acidic reactions. Platinum acts like a Lewis acid in this case, as it accepts protons from the thiol bond of the cysteine. Our goals with this portion of the project was to prove the validity of PKA 17 and to see if further research could be done to assert our claim that copper (I) could replace platinum in the cis-platin cancer treatment. With the results from this project, both those claims are proven to be true. First, the average standard unsigned errors from the cysteine and tyrosine final PKA's suggest that PKA 17 calculates PKA's accurately. The average standard unsigned error of the cysteine residue data points was 1.121 and for the tyrosine residue data points it was 0.496. With both of these errors being sufficiently low, it can be concluded that PKA 17 accurately calculates protein PKA values within a standard error. The second claim of potentially copper (I) binding

replacing platinum binding in cis-platin has merit based on the results acquired but more research needs to be done. This project only scratches the surface on this claim, but the results were promising.

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