ISTANBUL TECHNICAL UNIVERSITY ★ INSTITUTE OF SCIENCE AND TECHNOLOGY

MOLECULAR MODELLING OF PEPTIDE - METAL INTERACTIONS IN A MODEL YEAST *SACCHAROMYCES CEREVISIAE*

M.Sc. Thesis by
Ergi TERZİOĞLU, B.Sc.

Department : Advanced Technologies
Programme: Molecular Biology – Genetics and Biotechnology

Supervisors : Assoc. Prof. Dr. Zeynep Petek ÇAKAR
Assoc. Prof. Dr. Cenk SELÇUKİ

JANUARY 2009

ISTANBUL TECHNICAL UNIVERSITY ★ INSTITUTE OF SCIENCE AND TECHNOLOGY

MOLECULAR MODELLING OF PEPTIDE - METAL INTERACTIONS IN A MODEL YEAST *SACCHAROMYCES CEREVISIAE*

M.Sc. Thesis by
Ergi TERZİOĞLU, B.Sc.
(521051227)

Date of submission : 29 December 2008
Date of defence examination : 22 January 2009

Supervisors (Chairman) : Assoc. Prof. Dr. Zeynep Petek ÇAKAR (ITU)
Assoc. Prof. Dr. Cenk SELÇUKİ (EU)
Members of the Examining Committee: Assoc. Prof. Dr. Ayten KARATAŞ (ITU)
Assis. Prof. Dr. Fethiye Aylin KONUKLAR (ITU)
Dr. Bülent BALTA (BU)

JANUARY 2009

İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

SACCHAROMYCES CEREVISIAE MODEL MAYASINDA PEPTİT - METAL ETKİLEŞİMİNİN MOLEKÜLER MODELLEMESİ

YÜKSEK LİSANS TEZİ

Ergi TERZİOĞLU, B.Sc.
(521051227)

Tezin Enstitüye Verildiği Tarih : 29 Aralık 2008
Tezin Savunulduğu Tarih : 22 Ocak 2009

Tez Danışmanı : Doç. Dr. Zeynep Petek ÇAKAR (ITU)
Doç. Dr. Cenk SELÇUKİ (EU)
Diğer Jüri Üyeleri: Doç. Dr. Ayten KARATAŞ (ITU)
Yrd. Doç. Dr. Fethiye Aylin KONUKLAR (ITU)
Dr. Bülent BALTA (BU)

OCAK 2009

FOREWORD

There are some periods that we can not forget in our lives, the postgraduate program and this study are two of those for me, from the beginnig to the end. I hope it is more than getting a diploma or accomplishing a study because of sacrificing many special things in fact I should have not.

I would like to thank to my supervisors Assoc. Prof. Dr. Zeynep Petek Çakar and Assoc. Prof. Dr. Cenk Selçuki for their support and encourage. My thesis is a product of their guidance.

I would like to also thank to Muhammet Kahveci for his help at the beginning of the study, and other friends for their support.

I send my special thanks to my family for their encourage and patience, and also to my co-workers for their devotion.

December 2008

Ergi TERZİOĞLU

INDUSTRIAL ENGINEER

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

MOLECULAR MODELLING OF PEPTIDE – METAL INTERACTIONS IN A MODEL YEAST *SACCHAROMYCES CEREVISIAE*

SUMMARY

Some metals are essential for living organisms. Copper, as an essential metal, participates in many protein structures for their funtionality and it is transported by copper chaperones inside the cell to the right locations. Atx1, a copper chaperone in *Saccharomyces cerevisiae,* transports copper ions to Ccc2 protein which brings the ion to Golgi. Atx1 has a conserved MXCXXC motif, that is also conserved in Atx1-like proteins in many organisms, to bind copper ions, where M is Methionine, C is Cysteine, and X is any amino acid. It is assumed that this motif binds copper ions regardless of the X residues.

The aim of this study was to investigate the copper binding efficiency of MXCXXC motif according to different amino acids used for X residues by computational analysis. Four amino acids, Alanine, Glycine, Threonine and Valine were used in all possible combinations in the MXCXXC motif instead of X residues. In addition to neutralized peptides, methyl groups were bound at the terminals for comparison of the results. Most stable structures of the peptides were preferred for the interaction with the two types of copper ions, copper(I) and copper(II). Nine fixed locations were identified on peptides for copper ions to be located. First, one copper(I) was located to one of the locations in every interaction, and then two copper(I) were located to two of them; so the same procedure for copper(II). The computational conformational search was applied through CHARMM22 force-field in HyperChem.

The computational results indicated that Threonine is decreasing the energy of peptide – metal complex the most when it is found in the first and the third X positions. For the second X residue, it can be Threonine or any other amino acid for better binding. MTCGTC is the amino acid sequences that bind copper ions the best among the 124 different motifs generated by using four amino acids (A, G, T, and V) including neutralized peptides and peptides with methyl at the terminals.

SACCHAROMYCES CEREVISIAE MODEL MAYASINDA PEPTİT – METAL ETKİLEŞİMİNİN MOLEKÜLER MODELLEMESİ

ÖZET

Yaşayan organizmalar için bazı metaller önemlidir, yaşam için gereklidir. Bu grupta yer alan bir metal olan bakır, birçok proteinin yapısında bulunarak onların fonksiyonlarını gerçekleştirmelerini sağlar ve hücre içinde doğru noktalara taşınması bakır tutan proteinler tarafından gerçekleştirilir. *Saccharomyces cerevisiae*'da bakır tutan bir protein olan Atx1, bakır iyonunu, golgiye bakır taşımayla görevli Ccc2 proteinine aktarır. Atx1, bakır iyonlarını tutabilen ve birçok organizmadaki Atx1 benzeri proteinlerde de bulunan MXCXXC motifine sahiptir; bu motifte M Metiyonin'i, C Sistein'i ve X de herhangi bir amino asidi temsil etmektedir. Bu motifin bakır bağlamasının X yerine hangi amino asit olursa olsun değişmeyeceği düşünülmektedir.

Bu çalışmanın amacı, hesapsal yöntemler kullanarak, X yerine farklı amino asitler deneyerek MXCXXC motifinin bakır bağlama verimliliğini incelemektir. MXCXXC motifindeki X'ler yerine Alanin (A), Glisin (G), Treonin (T) ve Valin (V) olmak üzere dört farklı amino asit, tüm olası dizilimler şeklinde kullanılmıştır. Sonuçların karşılaştırılabilmesi için, her peptidin nötr durumuna ilaveten terminallerine metil eklenmiş halleri de çalışmaya dahil edilmiştir. Bakır(I) ve bakır(II) ile etkileşimleri için her peptidin en karalı hali tercih edilmiştir. Bakır iyonlarının yerleştirilmesinde peptit üzerinde dokuz sabit nokta belirlenmiştir. Her bir etkileşim için önce bir tane bakır(I) bir noktaya, daha sonra iki tane bakır (I) iki ayrı noktaya yerleştirilmiştir. Aynı prosedür bakır(II) için de uygulanmıştır. Konformasyonel analiz için HyperChem moleküler modelleme programındaki CHARMM22 modülü kullanılmıştır.

Hesapsal sonuçlar, Treonin amino asidinin birinci ve üçüncü sıradaki X pozisyonunda yer aldığında peptit – metal kompleksinin enerjisini en fazla düşürdüğünü göstermektedir. İkinci sıradaki X pozisyonu için, Treonin veya diğer amino asitlerin de iyi bağlanma sağladığını söyleyebiliriz. A, G, T ve V amino asitlerini kullanarak oluşturduğumuz 124 farklı motif arasında (nötr durumdaki peptitler ve terminallerine metil bağlı peptitler) bakır iyonlarını en iyi bağlayan olarak MTCGTC öne çıkmaktadır.

1. INTRODUCTION

Metals in cell is a topic containing many aspects such as conditions according to concentrations and metal-protein interactions. Generally yeast is used in determination of pathways including metals. Especially copper systems in *Saccharomyces cerevisiae* are highly investigated.

1.1. The Yeast: *Saccharomyces cerevisiae*

Yeast is an organism placed under the phylum “ascomycetes” and identified as a unicellular fungus. “Ascus” are sac like structures that are formed by yeast for producing and storing spores; and formation of ascus is thought to be the most interesting feature of this class and the most popular member of yeast is *Saccharomyces cerevisiae* are shown in Figure 1.1, which is also called as baker’s yeast (Madigan*etal.*,2003).

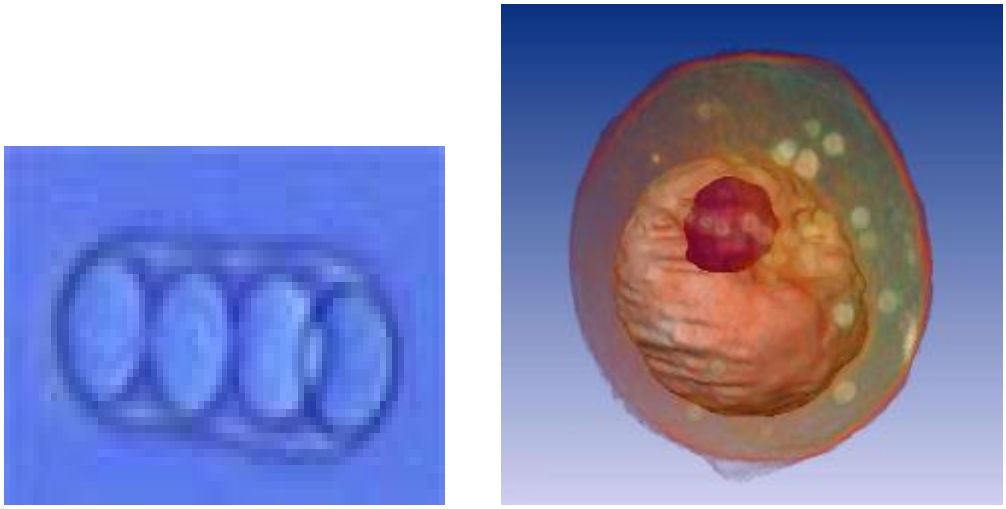

Figure 1.1 Yeast **A.** Yeast ascus, **B.** *Saccharomyces cerevisiae.*
(Url-1 <http://bugs.bio.usyd.edu.au/> and Url-2 <http://media.nih.gov>, respectively)

S. cerevisiae is easily cultivated at large scale because it does not need sophisticated fermentation techniques and expensive growth media to grow (Kapoor and Viraraghavan, 1995). In addition to these characteristics, it is regarded as safe. Thus, *S. cerevisiae* is widely used in industries like food and beverage (Wang and Chen, 2006).

S. cerevisiae is a perfect model organism to identify and investigate the reactions between metal and cell at molecular level because of the genetic manipulation, easiness and the availability of the complete genomic sequence (Peregol and Howell, 1997). Especially, most of the information about copper trafficking pathways in eukaryotic cells were obtained from studies on baker's yeast (Rousselot-Pailley *et al.*, 2006).

1.2. Metals In Biosystems

Metals have vital importance for microorganisms during their life processes. Calcium, cobalt, copper, iron, magnesium are examples of essential metals. Other metals, such as silver, gold, and mercury, are nonessential, so they are not needed biologically. Catalysing biochemical reactions, stabilizing protein structures (as cofactors) and serving in keeping osmotic balance are some functions of essential metals. For instance, while iron, copper, and nickel participate in redox processes, magnesium and zinc stabilize many enzymes and DNA through electrostatic forces. And also, potassium and sodium are necessary for intracellular osmotic pressure.

At high concentrations, metals are toxic to microorganisms even if they are essential. Removal of essential metals from their original binding sites or through ligand interactions causes toxicity. Nonessential metals have greater affinity than essential ones in binding to oxygen sites and groups containing thiol. As a result of toxicity, conformational structures of nucleic acids and proteins change; oxidative phosphorylation and osmotic balance are distrupted (Bruins *et al.*, 2000).

Environmental conditions with metal stress (because of high concentration) resulted with new systems that provided resistance to virtually all toxic metals (Rouch *et al.*, 1995). The genes that are related to metal conditions (essential and nonessential) arose synchronously as mechanisms for carbon and sugar sources. Some metals, such as Fe(II) and Cu(II), are used by changing their oxidation states. This means that oxidation-reduction systems exist in microorganisms for metal use and resistance. Cell surface electron-transport systems and enzyme-reducing systems let the cell detoxify and regulate metal ions' movements.

In situations where metal ions are in excess, microorganisms resist to metals through six mechanisms (Table 1.1). Only one or a combination of these mechanisms can be used by microorganisms according to the extend of resistance (Bruins *et al.*, 2000).

Table 1.1 : Mechanisms of metal resistance in microorganisms (Bruins *et al.*, 2000).

Metal exclusion by permeability barrier
Active transport of the metal away from the cell/organism
Intracellular sequestration of the metal by protein binding
Extracellular sequestration
Enzymatic detoxification of the metal to a less toxic form
Reduction in metal sensitivity of the cellular targets

Under normal conditions, the cell uptakes and transports essential metals by trafficking systems. Cell surface, cytosol, nucleus, golgi, and mitochondria are the main locations of the proteins that are dependent on metal ions for activity. Thus, essential metals, as a cofactor, should be delivered to the right site at the right time through many different locations. Unfortunately, another metal instead of the needed one for the activity can easily bind to the protein. However, the metalloprotein is provided with the correct ion among all the metals present in the cell. Furthermore, during delivery of the right metals to the proteins, the toxic side reactions of the metal ions are avoided. Therefore, it is understood that metal ions do not exist as free agents in the cell. They are detoxified, sequestered, or escorted to the right site in the metalloprotein under surveillance systems. Several pathways for these physiological needs show up after the entrance of metals in a cell (Luk *et al.*, 2003), and copper trafficking system is the most studied and decoded one.

1.3. Copper And Microorganisms

All living organisms need copper, as an essential micronutrient. Organisms perform a conversion between copper's different oxidation states (oxidized Cu(II) and reduced Cu(I)), so copper can participate in different biological processes by many metalloenzymes (Cu/Zn superoxide dismutase – antioxidant defence, cytochrome c oxidase – mitochondrial respiration, lysyl oxidase – development of connective tissue, tyrosinase – melanin biosynthesis, ceruloplasmin – iron homeostasis, hephaestin – intestinal iron efflux, dopamine β-hydroxylase – catecholamine production, peptidylglycine α-amidating mono-oxygenase – peptide hormone processing etc.) as a catalytic cofactor.

Similar to general metal toxicity, copper ions are able to get in and alter proteins, nucleic acids, and lips as a result of reactions through formation of hydroxyl radicals under overload conditions. For instance, copper competes with Zn(II) in zinc-finger transcription which prevents Zn relevant proteins from binding the target sequence. Thus, to keep the balance between nutritional deficiency and toxicity, copper concentration in the cell must be under control. Complicated mechanisms to use copper properly exist from mammalian's cells to unicellular organisms.

The key term for keeping copper concentration in the cell at molecular level in safe is "copper chaperones", a protein family whose duty is to deliver copper to relevant target and to prevent other cellular components from inappropriate copper interactions. The fact that mammals have very similar copper transporters and chaperons like eukaryotes tells us that copper trafficking systems are similar within cells, and gives some clues one to another (Bertinato and L'Abbe, 2004). Studies on *Saccharomyces cerevisiae* lead to most of the information about copper trafficking system.

1.4. Copper Transport Systems

The travel of copper from outside of the cell to the correct site of a protein consists of two main systems: copper uptake system and intracellular copper trafficking. They are mostly enlightened regarding *S. cerevisiae*, that is commonly accepted as a model system for metal studies.

1.4.1. Copper Uptake System

Nonessential and essential metal ions are taken into the cell by nonspecific uptake system under normal conditions. But nonessential metals should be excluded by specific ion efflux systems and essential metals should be limited through transporting in situations with high metal ion concentration (Bruins *et al.*, 2000).

S. cerevisiae has plasma membrane reductases, Fre1p and Fre2p, for process of copper uptake. These reductases lower the valence state of copper ion to Cu(I) through redox reactions, so the reduced copper ions are then able to be carried into the cell (Hassett and Kosman, 1995). After the reduction, surface transporters take place for transferring Cu(I) through cell membrane. There are two types of transporter in baker's yeast, high affinity and low affinity transporters.

1.4.1.1. High Affinity Copper Transporters

High affinity copper transporters, Ctr1p and Ctr3p, are responsible for copper uptake maximization to provide growing under the situations where copper is limited. While Ctr1p is a big multi spanning plasma membrane transporter protein, Ctr3p is a small intracellular cysteine-rich integral one (Hassett *et al.*, 2000). When copper concentration exceeds normal limits, high affinity uptake system is left.

1.4.1.2. Low Affinity Copper Transporters

Low affinity copper transporters, Fet4p and Ctr2p, function when there is no alarming conditions. They deliver the copper metal ion to metallochaperones or vacuole (a store of ions) (Culotta *et al.*, 2001).

1.4.2. Intracellular Copper Traficking

There are two possibilities, being utilized or detoxificated, for a copper ion when entering into a cell.

1.4.2.1. Detoxification Of Metal Ions

Detoxification of metal ions in *S. cerevisiae* is applied with the help of two types of mechanisms: enzymatic and non-enzymatic defence systems.

Enzymatic defence system includes enzymatic activities of catalase and duperoxide dismutase. Under the conditions of high copper concentration, free radicals are formed by the cell. The damages of these free radicals are prevented by these enzymes through redox properties of copper ions. For example, while Sod1 participate in superoxide anion (O^{-2}) decomposition to hydrogen peroxide (H_2O_2) and oxygen, catalase function in formation of O_2 and H_2O from H_2O_2.

Non-enzymatic defence system in baker's yeast includes metallothioneins and glutathione. Metallothioneins are cysteine-rich proteins with low molecular weight. Because of containing sulfhydryl and thiol group, and discluding aromatic amino acids and disulfide bridges, they bind easily a wide number of metal ions. Glutathione is also a cysteine-rich protein like metallothionein. While glutathione functions for detoxification of metal ions, it can also be used as a nitrogen and sulphur source under starvation conditions (Avery, 2001).

1.4.2.2. Utilization Of Copper Ions

Copper utilization means the transportation of the copper ions to the components of the cell that need them for metabolic activities; and there is not one target. This is accomplished with the help of metallochaperones, intracellular metal receptors (Figure 1.2). In *S. cerevisiae*, there are three Cu transport mechanisms consisting of metallochaperone and the target: Atx1 – golgi apparatus, CCS – cytosolic SOD, and Cox17 – mitochondria (O'Halloran and Culotta, 2000).

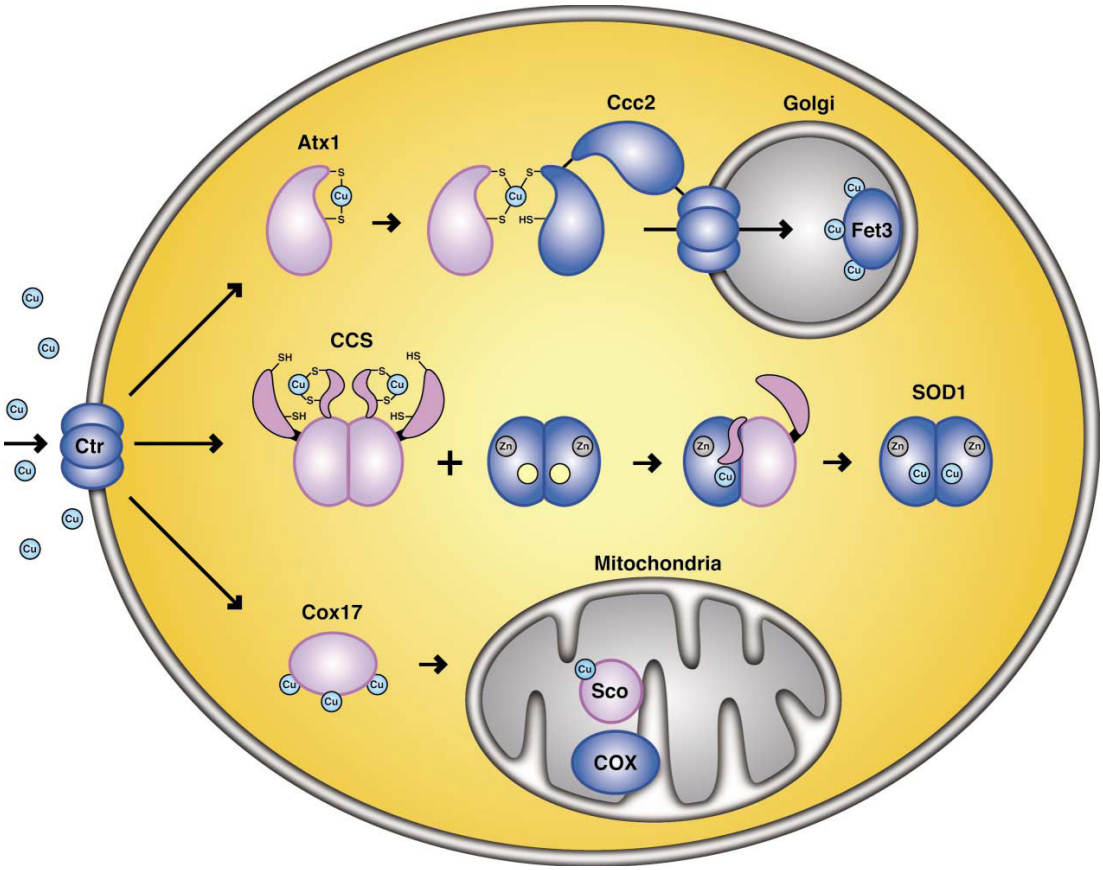

Figure 1.2 : Copper chaperone pathways in yeast. Conservation exists for all three pathways in humans and other eukaryotes. The Ctr membrane transporters transport copper to the cell. Thiol exchange reactions and the formation of a three-coordinate intermediate are proposed to provide copper delivery from Atx1 to the N-terminal domain of Ccc2. Formation of a heterodimeric intermediate is proposed to provide copper delivery from CCS to SOD1 (Rosenzweig, 2002).

Cytochrome oxidase is a mitochondrial enzyme which needs three copper ions for activation. These copper ions are delivered with the cooperation of Cox17 and Sco1, which accomodate in cytosol and mitochondrial inner membrane space respectively. As a metalloprotein, Cox17 transports copper to Sco1 which is the key protein for binding of copper ions to cytochrome oxidase (Thiele and Puig, 2002).

Superoxide dismutase (SOD1) is a cytosolic homodimeric enzyme, contains copper and zinc, and prevents oxidative damage in the cell by avoiding the toxic superoxide anion radicals. SOD1 needs CCS, a copper chaperone, for copper delivery unless there is high concentration of copper in the cell (Luk *et al.*, 2003).

Ccc2 is a protein that transports copper to Golgi (the copper ions in Golgi are then used for Fet3 activation which participates in iron uptake). It consumes ATP during pumping copper through the membrane of Golgi, so it is a ATPase transporter. An intracellular copper receptor, Atx1, leads the copper to Ccc2. Atx1 has many homologs in many organisms (O'Halloran and Culotta, 2000).

1.5. Atx1 Protein

Atx1 was identified in 1995 whose name is originated from the letters in anti-oxidant. It functions on copper delivery in yeast, transporting copper to an intracellular copper transporter located in golgi (O'Halloran and Culotta, 2000). Consisting of approximately 70 amino acids, Atx1 is a small polypeptide with a structure of βαββαβ fold which is common in inorganic ion-binding proteins (Figure 1.3). In this fold, two α-helices are superimposed on a 4-stranded β-sheet, which enables many advantages to Atx1 for its function.

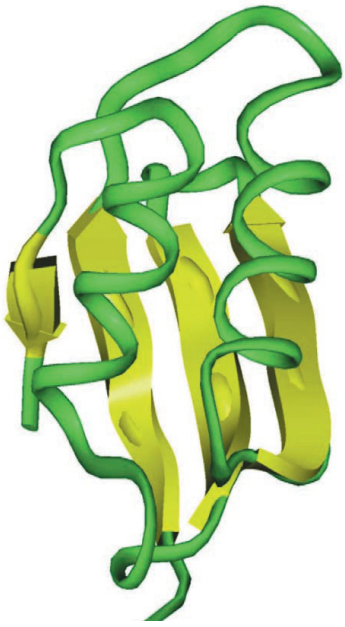

Figure 1.3 : βαββαβ fold of Atx1 (Opella *et al.*, 2002).

The metal-binding site of Atx1 is on the surface, as a result it can interact easily with other proteins like Ccc2. Other copper proteins generally have buried metal-binding sites. Atx1 stabilizes metal ions and performs reactions with fewer donor ligands and less coordination number in metal-binding loop, which is flexible and enables coordination number alterations. Additionally, the surface of Atx1 consists of positively charged residues that let the protein recognise its partner for interaction. For example, mutations on Lys24, Lys28, and Lys65 decrease interactions with Ccc2 (Rosenzweig and O'Halloran 2000).

In *S. cerevisiae*, Atx1 protein has a conserved MXCXXC metal binding motif (where M is methionine, C is cysteine, and X is any amino acid) as well as its target ATPases. While Atx1 possesses only one, target ATPases may contain more than one (for example, Ccc2 has two) repeating motif on N-terminal domains. There should be three sulfur ligands on Atx1 for Cu(I) binding, two of them are from cysteine residues on MXCXXC and the other one could either be another cysteine from another Atx1 or an exogenous thiol. MXCXXC motifs on both Atx1 and Ccc2 perform ligand exchange reactions (Rosenzweig, 2002). Electrostatic interactions (major driving forces of protein-protein recognition) between copper transporter's negatively charged residues and Atx1's positively charged face enable docking of Atx1 with its target, and easiness to metal transfer. Then, Cu(I) center in Atx1 is attacked by a cysteine from the acceptor domain. Through the formation of two- and three-coordinate Cu(I) centers, Cu(I) changes domain (Figure 1.4) (O'Halloran and Culotta, 2000). Chaperones and the soluble domains of ATPases have been observed with S-Cu-S angle of $122^{O}\pm30^{O}$, suggesting that copper is three-coordinated (Banci and Rosetta, 2003).

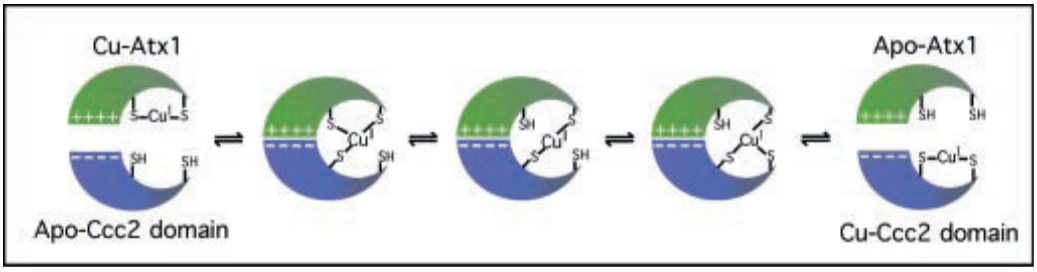

Figure 1.4 : Proposed pathway for copper transfer from Atx1 to Ccc2 (O'Halloran and Culotta, 2000).

1.6. Homologs Of Atx1

There are homologs of Atx1 in many organisms. For example, Atox1, the human homolog of Atx1, transports copper to Menkes and Wilson disease ATPases (homolog of Ccc2); CopZ is the bacterial homolog of Atx1 (Rosenzweig, 2002). They all have βαββαβ fold, which is so-called ferrodoxin-like and whose secondary structure elements are connected by loops. Similar metal-binding sequences of these proteins enable them transport copper to taget proteins. Their length is generally between 70 and 80 residues (Opella *et al*., 2002).

1.7. MXCXXC

The hydrophobic core of these proteins are similar; their secondary structure elements have conserved residues (30-50% sequence identity). Sequence variability increases through the residues far from the metal-binding site. The metal-binding site, CXXC, which contains the two copper-binding cysteines on both sides of any other two amino acids, is completely conserved. The residues located in the loops around CXXC are highly conserved. Especially, there is a methionine just one residue before the first metal-binding cysteine is almost conserved, forming the MXCXXC motif (Banci and Rosetta, 2003). Sometimes MT/HCXXC sequence is regarded as conserved as repeating motif on copper-binding proteins. So while Atx1 and Atox1 should have one repeat of this motif each, the Menkes/Wilson disease proteins should contain six repeats, and Ccc2 should contain two (Rosenzweig *et al*., 1999; Wernimont *et al*., 2000). It seems to be a mechanistic importance for the other sequence variations because of being related to specific subgroups. Metal-binding-site-containing loop is stabilized by other loops. There are highly conserved amino acids participating in protein-protein interaction on these loops. For example, in eukaryotic chaperones Lys on one of these loops is completely conserved (Banci and Rosetta, 2003).

1.8. MXCXXC Selectivity For Other Metals

Regarding merP, a soluble Hg-transport protein, and ZntA, P-type ATPase for binding Zn in Zn-trafficking system and also exploring Pb(II)/Cd(II), MXCXXC is supposed to be also conserved for many soft-metal binding, such as Hg(II), Zn(II), Pb(II) and Cd(II). On the other hand, it is unclear what affects the selectivity of these

metal transporters in vivo. Recent studies demonstrated that MXCXXC motif binds Hg(II) more selectively than Cu ions. The others, after Cu ions are listed Cd(II), Pb(II), and Zn(II) in a row (Rousselot-Pailley *et al*., 2006).

1.9. Aim Of The Sudy

MXCXXC, the conserved motif on metal-binding sequences on the proteins for copper-binding, consists of a Methionine, two Cysteines, and three any amino acids presented as Xs. In this study, we try to detect efficiency differences of MXCXXC motifs on binding Cu(I) and Cu(II) by changing amino acids chosen for X. All the studied structures have been examined by computational tools.

2. METHODOLOGY

In this study, the binding characteristics of copper ions to the metal binding motif MXCXXC was investigated by computational methods. Four amino acids were chosen to represent X residues in every possible combination. Two different sequences were generated from each motif by forming neutral peptides and adding methyl groups to peptide terminals. After the run for the nearest local minimum search, all possible torsions were selected and minimum energy structures were obtained at the end of conformational search. Copper ions (Cu^{+} and Cu^{2+}) were generated and located into the nine fixed locations around the motif. The interaction of motifs with copper ion(s) were observed by geometry optimization. The energy values obtained from the calculations were used for the comparison of copper binding efficiency of the peptides.

HyperChem, the molecular modelling program, is used for all the calculations. HyperChem provided molecular mechanics during energy calculations, energy minimization algorithm for geometry optimization and systematic search method for conformational search. CHARMM22 module was preferred for all steps because this force field is parametrized for amino acids and peptides. The only exception for using another module instead of CHARMM22 was ZINDO/1, for building copper ions. Then the copper ions were transferred into the CHARMM22 module.

Building amino acid sequences, neutralization of peptides and adding methyl groups to the terminals, geometry optimization and conformational search processes were performed in vacuum. The aqueous media will be used according to the results of this study. Other molecular simulation programs with other environments and also experiments with the yeast containing particular metal binding motif in their copper chaperones will be performed and their results will be compared with this study. Taking the environment as vacuum for the computational investigation is the first and the simplest step of a comprehensive study.

2.1. Molecular Mechanics

Molecular mechanics (MM) force fields participate in calculations for conformational analysis. An atom's hybridization and bonding behavior affect the values of bond length, bond angle, and torsion. Molecular geometries, energies, and other features are reproduced by using the equilibrium values of the atoms through mathematical models in MM. The laws of classical Newtonian physics and the parameters obtained from experiments are the basis of these geometry calculations as a function of steric energy. The force field equation is:

$$E_{pot} = \Sigma E_{bnd} + \Sigma E_{ang} + \Sigma E_{tor} + \Sigma E_{oop} + \Sigma E_{nb} + \Sigma E_{el} \qquad \textbf{(1.1)}$$

where E_{bnd}, E_{ang}, E_{tor}, E_{oop}, E_{nb}, and E_{el} are energy of bond streching, angle bending, torsional terms, out-of-plane bending, non-bonded interaction and electrostatic interaction, respectively. E_{pot} is the difference between the ideal molecule's energy and the real molecule's energy. The energies summed together in the formula involves atomic parameters as force constants K_b, K_Q, K_Φ, and K_X, and equilibrium values b_0 and Q_0 which were obtained from X-ray, NMR, IR, microwave, Raman spectroscopy and *ab initio* calculations on some sort of molecules such as alkanes and alcohols. The detailed formula of the force field is given in Equation (1.2).

$$\begin{aligned} E_{pot} = {} & \Sigma 1/2 K_b (b - b_0)^2 + \Sigma 1/2 K_\theta (\theta - \theta_0)^2 + \\ & \Sigma 1/2 K_\phi (1 + \mathrm{Cos} N\phi)^2 + \Sigma 1/2 K_\chi (\chi - \chi_0)^2 \\ & \Sigma ((B/r)^{12} - (A/r)^6) + \Sigma (qq/r) \end{aligned} \qquad \textbf{(1.2)}$$

Some kind of directional derivative techniques are applied for the calculation and minimization of the energy of the atoms in a molecule. This energy consists of bond stretching, angle bending, torsional term, non-bonded interaction and out-of-plane bending values (Url-3 <www.netsci.org>).

2.1.1. Bond Stretching

Bond stretching energy is the square of the difference between stretched and natural bond length (b_0) multiplied by half of the force constant K_b which depends on the force between bonded atoms.

$$E_{bnd}(b)=K_b(b-b_o)^2 / 2 \quad \textbf{(1.3)}$$

This stretched spring energy (E_{bnd}) is described by Hooke's law.

2.1.2. Angle Bending

Angle bending energy, E_{ang}, is obtained when a bond angle is deformed from its natural value. Hooke's law leads to this formula, too:

$$E_{ang}(\theta) = K_\theta(\theta-\theta_o)^2 / 2 \quad \textbf{(1.4)}$$

How much each angle affects the energy is found by using force constant and natural value (K_Q and Q_0 respectively). Bond stretching's force constant is larger than the angle bending's; thus, the contribution to energy function of bond stretching is more significant (Lewars and Errol, 2003).

2.1.3. Torsional Terms

Deforming the torsion or dihedral angle is calculated with the term, E_{tor}. The energy differences as a sequence of single bond rotation should be represented by force fields. So, with the contribution of each bonded quartet of atoms, torsional potentials are used for force field of organic molecules. Torsional potentials are shown as:

$$E_{tor}(\Phi) = K_\Phi [1+\cos(N\Phi)]^2 / 2 \quad \textbf{(1.5)}$$

Φ is the torsional angle and N represent the minimum points in the function as the rotation of the bond through 360^0 (Url-3 <www.netsci.org>).

2.1.4. Non-bonded Interaction

Some atoms are separated by at least two atoms. These atoms have interactions through non-bonded forces that result in having vital importance for modeling the structure of the molecule. Electrostatic and van der Waals interactions are categorized as non-bonded interactions in a force field (Lewars and Errol, 2003).

While non-bonded interaction lead to E_{nb}, Coulombic forces lead to E_{el}. Electrostatic property is the first part of the non-bonded interactions of a molecule. Formula originates from Coulomb's law.

$$E_{el} = qq/r = q_iq_j / 4\pi\varepsilon_0 r_{ij} \quad \textbf{(1.6)}$$

ε_0, r, q_i, and q_j represent local dielectric constant, the distance between charged atoms, and partial charges on the atoms respectively.

The calculation of van der Waals interaction is taken into account the second part of the non-bound interaction.

$$E_{nb}(r) = (B/r)^{12} - (A/r)^{6} \quad \textbf{(1.7)}$$

ε, r, A and B represent well depth, the distance between the center of the non-bonded atoms and constants, respectively (Leach, 2001).

2.1.5. Out-of-plane Bending

E_{oop}, steric energy, is a result of out-of-plane bending.

$$E_{oop} = K_{\chi}(\chi - \chi_o)^2 / 2 \quad \textbf{(1.8)}$$

The molecular properties that electronic forces do not affect such as geometry, rotational barriers, vibrational spectra, relative stability of conformers and heat of formation values are computed by molecular mechanics. The systems involving thousands of atoms and many different combinations can be tested by molecular mechanics because the calculations are efficient and fast. But molecular mechanics depend on parameters derived experimentally, which is considered as a disadvantage when compared with *ab initio* method. Errors can be observed in the calculations on new molecular structures (Url-3 <www.netsci.org>).

2.2. Conformational Analysis

The structure of a molecule in low-energy state in space is defined by conformational analysis by comparing conformational flexibility, characteristics and the function (Leach, 2001). Ground state, global minimum are the terms for the lowest energy conformation. Some conformations which have relatively low energy also indicate the properties of the molecule. Generally, it is not clear which conformation is at the global minimum, so lowest-energy conformation cannot be detected easily. To get a higher probability of determining the scope of the conformational space for a molecule, technical methods should be applied carefully (Schlecht, 1998).

Example of global minimum conformation of butane is shown in Figure 2.1. Two-dimensional diagram involves potential energy curves with global minimum, global

maximum, local minima, and local maxima. For example, when the torsional angle between two end methyl groups of butane is 180^0, the energy is at the global minimum, but if the angle is 60^0, the energy is at the local minima.

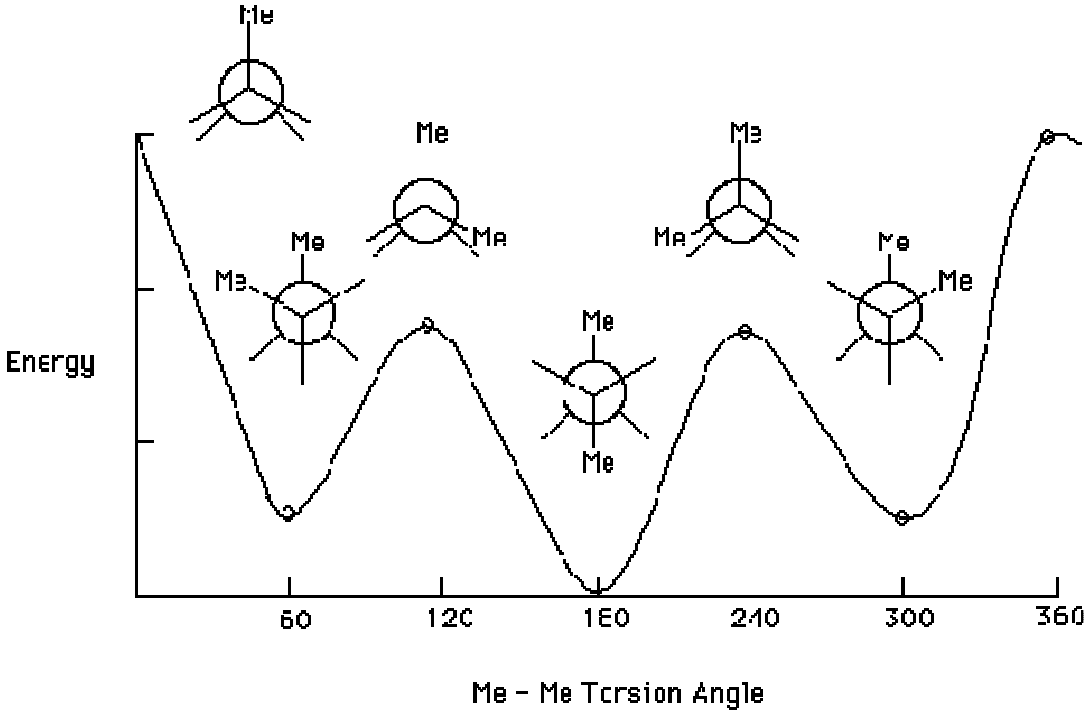

Figure 2.1 : Possible conformations-potential energy curve of butane (Leach 2001).

Rotation of single bonds results in change in the arrangements of atoms in the three dimensional structure which are the conformations of a molecule. Small changes of bond length and angle cause transformation of one conformation to another one, and as a result the energy of the new conformation gets higher or lower (Leach, 2001). The changes in the energy according to different molecular conformations are shown on a multi-dimensional surface based on potential energy and geometry. This multi-dimensional surface is also called saddle points and Figure 2.2 is an example of a saddle point. Every single conformation has a related potential energy point on the surface (Höltje and Folkers, 2003). At the local minimum of the potential energy surface, there is a stable conformer of a molecule, and there may be many local minima but the one that has the lowest energy among these local minima is the global minimum (Becker, 2001).

Conformational search which determines the preferred conformations of a molecule that has a particular behavior is a key part of conformational analysis. These particular conformations generally exist on local minima points on the energy

surface (Leach, 2001). To move to the minimum point which is the closest to the starting structure is a special process of methods for energy minimization. Identifying all minimum energy conformations on the energy surface is a target. On the other hand, to find all of them is sometimes useless because of existence of so many minima points on the energy surface for some molecules, especially for proteins. Although it is not always true, in such conditions, the conformation that has the lowest energy among all the conformations is accepted as the native one (Höltje and Folkers, 2003).

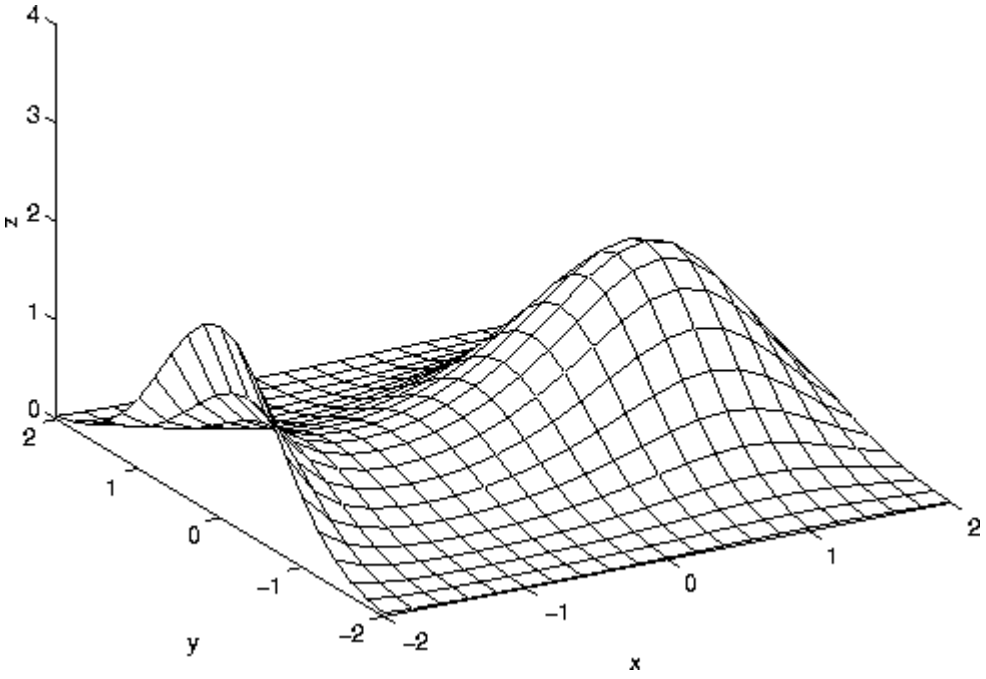

Figure 2.2 : Saddle point (Becker 2001).

2.2.1. Conformational Search

Conformational search is detecting and evaluating of the potential energy surface. Systematic search, stochastic search, molecular dynamics, genetic algorithm, and distance geometry are some examples of methods for conformational search (Schlecht, 1998).

2.2.1.1. Systematic Search

The bond lengths and angles are fixed in the systematic search, and also named as torsional tree method. All bonds in the molecule participate in the calculation system. And by changing each rotatable torsional angle by a set amount, multiple

conformations are produced. Each bond is rotated to an extent of 360^0 by a fixed increment. Local minimum energy conformations are obtained by minimising produced conformations. The search ends when all possible conformations of torsion angles are included in energy calculation and minimization (Leach, 2001).

2.2.1.2. Stochastic Search

The stochastic search is commonly known as Monte Carlo. The method has two different algorithms, one of which depends on torsional space, while the other one depends on coordinates space. The Monte Carlo torsional search is close to the systematic search except the choice of angle variations. Angles are chosen randomly or semirandomly in Monte Carlo. A random increment is applied after the randomly chosen rotatable bond group. The resulting conformation is tested to detect severe steric strain or violation. Energy minimization is applied to the conformations that are successful according to the tests. Whether the structure falls within the accepted energy level or it is a copy of another conformation is controlled.

The algorithm of coordinates space, Metropolis Monte Carlo, provide a high degree of conformational flexibility by placing the molecule in a hot bath. As the bath's temperature decreases, sampling and analysing are applied to the conformations which are produced by random displacements of torsions coordinates. The molecule results into a local-minimum-energy conformation at the end of the cooling cycle. The conformation is evaluated and discarded or kept, then placed in a very hot bath to repeat the process (Schlecht, 1998).

2.2.1.3. Molecular Dynamics Simulation

Adding kinetic energy provides advantage for detecting ability by overcoming energy barriers between local minima in molecular dynamics simulation method. So the molecule is prevented from being stuck in a localized region of conformational space. The process in this method is that first, the sample conformations are added kinetic energy periodically, subjected to energy minimization and then evaluated (Schelcht, 1998). While the aim of conformational search in simulation methods is to find local minimum structure of the molecule, the aim is to join the structures that are not at energy minima region in molecular dynamics or Monte Carlo (Höltje and Folkers, 2003).

2.2.1.4. Distance Geometry

Distance geometry is a method based on the distance between all pairs of atoms which is used for complex molecular systems while building conformational models. A set of constraints is required for generating structures without the need of a starting conformation or an energy function. Conformation space is searched randomly by many distance matrices and then these matrices are changed into conformations in cartesian space. When many distance constraints are known, distance geometry method is useful. Because very sized systems require computational matrix manipulation, which is expensive, distance geometry is restricted to those kind of systems (Becker, 2001).

2.2.2. Energy Minimization

Search methods produce many structures. These conformations should be optimized for further analysis. To obtain local or global minimum structures, energy minimization algorithm is required. The coordinates are gradually changed by numerical methods to produce conformers that have lower energies. When the minimum is reached, it stops. But, the initial conformation is probably close to the minimized structure. So the minimization process is not a search method, just an optimization technique. This method cannot exceed the energy barrier and it finishes with the local minimum energy because of being a downhill procedure. It is almost impossible to get the global minimum of the function with a direct minimization method (Leach, 2001).

Finding a local minimum of a given function is the goal of all minimization algorithms. The first derivative of function is zero at the minimum point (Lewars and Errol, 2003).

The classification of minimization methods is done according to the level of highest derivative in the algorithm. Zeroth-order method is the simplest one that there is no derivative. While first order method require first derivatives, second order methods need both first and second. Although higher order methods are more accurate for computational techniques, they are expensive and time consuming (Becker, 2001).

2.3. CHARMM22

Chemistry at Harvard Macromolecular Mechanics (CHARMM) is a simulation and modeling computer program for macromolecules and has parameter sets of

empirical values. Static energy, molecular dynamics, electrostatics, combined quantum mechanics and molecular mechanics, stochastic dynamics are some applications implemented in the program.

The calculations on proteins, nucleic acids, polypeptides and other related macromolecules are done by CHARMM22 (Schlecht, 1998). CHARMM19, CHARMM22, CHARMM27 are some versions of CHARMM force fields. The parameters of CHARMM22 are defined for protein molecules. The ability of calculating non-polar hydrogens provides the model covering all atoms including hydrogen (HyperChem 7.5, 2003).

2.4. Specific Amino Acids Instead of X Residues

The number of amino acids that would represent X residues was restricted to four regarding the amount of possible motifs. When the three X amino acids were replaced with the four specific ones, 64 different sequences were obtained which is large for computational studies as they require considerable amount of time for each motif.

Small and uncharged amino acids were chosen for this study. Small amino acids prevented time consuming during conformational search and geometry optimization. Atx1 protein has positively charged surface but uncharged copper binding site. Therefore, the amino acids that are uncharged were preferred for the observation of interactions between copper ions and the MXCXXC motifs: Alanine (A), Glycine (G), Threonine (T), and Valine (V) (Figure 2.3).

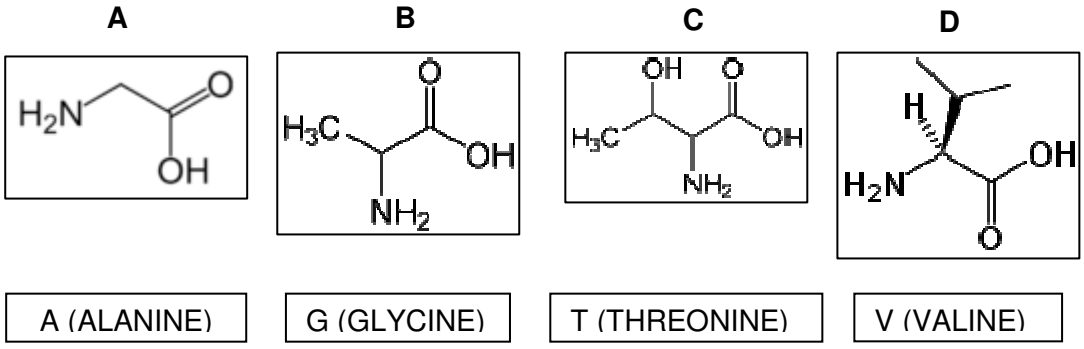

Figure 2.3 : Amino acids for MXCXXC **A.** Alanine **B.** Glycine **C.** Threonine **D.** Valine

During the study, every sequence was investigated with the same procedure. The statements in methodology covers every motif meaning that every process were repeated 64 times. The list of all possible MXCXXC sequences is in Table 2.1.

Table 2.1 : List of all possible MXCXXC.

MACAAC	MGCAAC	MTCAAC	MVCAAC
MACAGC	MGCAGC	MTCAGC	MVCAGC
MACATC	MGCATC	MTCATC	MVCATC
MACAVC	MGCAVC	MTCAVC	MVCAVC
MACGAC	MGCGAC	MTCGAC	MVCGAC
MACGGC	MGCGGC	MTCGGC	MVCGGC
MACGTC	MGCGTC	MTCGTC	MVCGTC
MACGVC	MGCGVC	MTCGVC	MVCGVC
MACTAC	MGCTAC	MTCTAC	MVCTAC
MACTGC	MGCTGC	MTCTGC	MVCTGC
MACTTC	MGCTTC	MTCTTC	MVCTTC
MACTVC	MGCTVC	MTCTVC	MVCTVC
MACVAC	MGCVAC	MTCVAC	MVCVAC
MACVGC	MGCVGC	MTCVGC	MVCVGC
MACVTC	MGCVTC	MTCVTC	MVCVTC
MACVVC	MGCVVC	MTCVVC	MVCVVC

2.5. Formation Of MXCXXC Structures

Atx1 protein has ferrodoxin-like fold and the metal binding site is located between the first loop and the first helix instead of on α-helices or β-sheets. The processes were performed in vacuum.

Two types of sequences were generated from each motif by using the neutral form of the peptide and binding methyl groups to the terminals. One sequence had one neutral type and another type with methyl at both terminals. There had been 64 different sequences initially, at the end of the structure formation process, the number increased to 128. But the evaluation was done according to these two types of peptides; the peptides in the same groups were compared with each other.

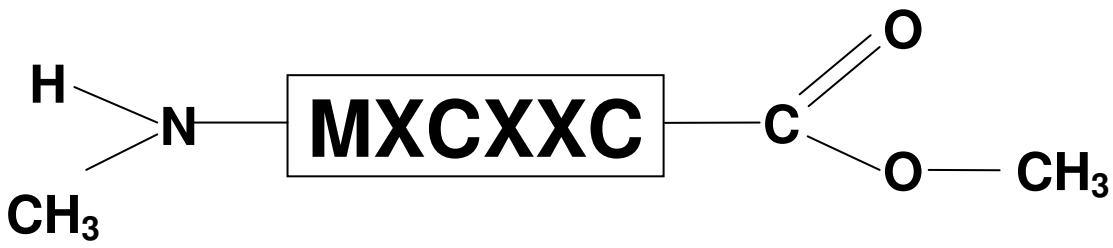

Figure 2.4 : MXCXXC motif with methyl at the terminals.

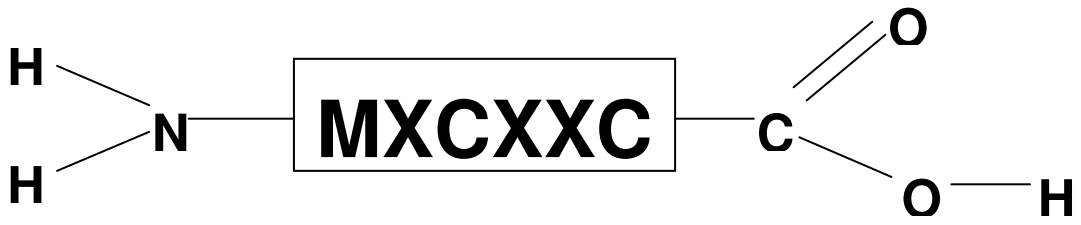

Figure 2.5 : Neutral MXCXXC motif.

2.6. Local Minimums Of Initial Structures

After building the structures of all 128 different sequences, geometry optimization was performed for each sequence. The geometry optimization calculated the nearest local minimum point of the starting structures. This local minimum energy structure was a good starting structure for comformational search. The energy of the initial structure and local minimum were not included in the data that were used for

the evaluation because the aim of the this process was to reach the global minimum structure, that is accepted as the natural structure of metal binding sequence.

2.7. Torsion Determination

Local minimum structures obtained by geometry optimization on initial structures became the starting structures of conformational search. Before conformational search, every possible torsion was chosen in the molecule. During this selection, the torsions that were completely the same were eliminated to decrease the work on the simulation. For example, if a carbon atom is bound with three hydrogen atoms and with another atom that combines it to the rest of the molecule, only one torsion was selected starting with one of those three hydrogen atoms instead of three torsions. This elimination avoided time consuming.

Alanine, Glycine, Threonine, and Valine have different number of bonds, so the number of torsions selected on different amino acid sequences were different. Generally the number of torsions for each sequence was between 27 and 35, covering all the atoms on the molecule. Every torsion selection on sequences were performed with the same starting point, same style, and same end point. It was quite secure for comparing the conformational search results.

2.8. Conformational Search Of Structures

Monte Carlo simulation was used in CHARMM22, involving all the torsions selected. At the end of the conformational search, the program provided a list of energies very close to each other that were the lowest ones. Some sequences had a very long energy list of more than 70, some had as short as 5. Only the lowest energy structure was considered in our study, all the sequences had been investigated with the same approach. Rest of the lists were eliminated. Besides the energy and their structure, many other information were obtained but neglected for the rest of the analysis.

After the conformational search, non-bonded interactions were observed on the minimum-energy structures which were accepted to interact with copper ions. Below, the three different structure of the same sequence (MACAAC) are shown. Figure 2.6, Figure 2.7, and Figure 2.8 are the initial structure that was formed by joining amino acids together, local minimum structure after geometry optimization

and global minimum structure after conformational search respectively. The differences and the bending of the molecule are clear.

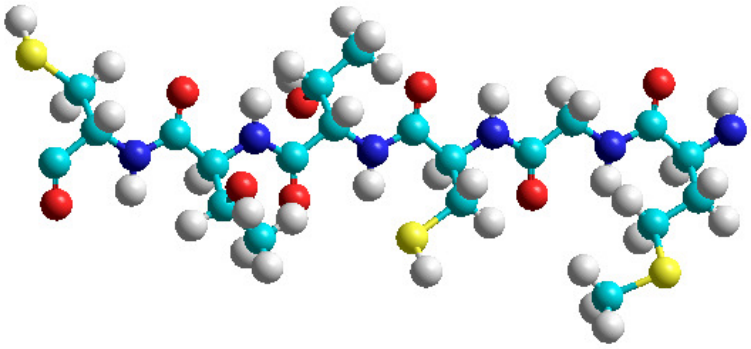

Figure 2.6 : Initial structure of MACAAC.

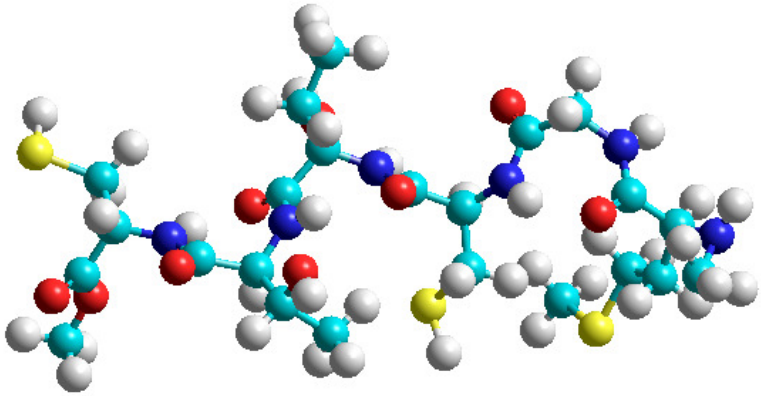

Figure 2.7 : Local minimum structure of MACAAC after geometry optimization.

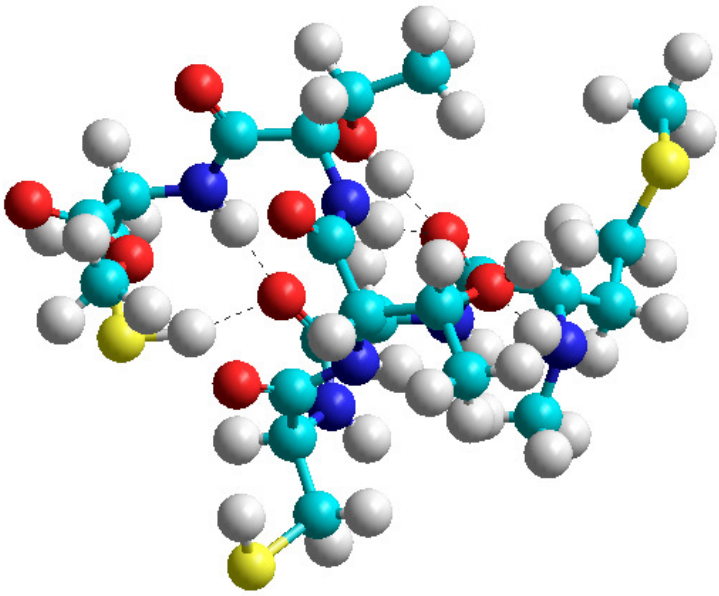

Figure 2.8 : Global minimum structure of MACAAC after conformational search.

2.9. Formation Of Cu^{+} And Cu^{2+}

Copper is a metal and its structure is different from amino acids. In order to form copper ions in HyperChem, ZINDO/1 module was used instead of CHARMM22, which is a module with appropriate parameters for proteins and nucleic acids. And then the copper ions were transferred into the CHARMM22 module for observation of the interaction with metal binding motifs.

In HyperChem images of the structures in this study, copper ions are the green atoms that are not bound to the molecule. In addition to green copper ions, white, light blue, dark blue, yellow, and red atoms are hydrogen, carbon, nitrogen, sulphur, and oxygen, respectively.

2.10. Determination Of Locations For Copper Ions

The locations that the copper ions would be located on global minimum structures of sequences should be the same despite of the change in X residues. Thus, whatever amino acid was used for any X in the motif, the location should be fixed. The backbone of the amino acid sequence and the sulphur atoms in Methionine and the two Cysteines were preferred for locations. There are three sulphur atoms, and six double-bound oxygens independent of the amino acids on the sequence. As a

result, nine locations were determined for copper ions. The locations and their numbers are displayed in Figure 2.9. The location numbers are used in the energy results table.

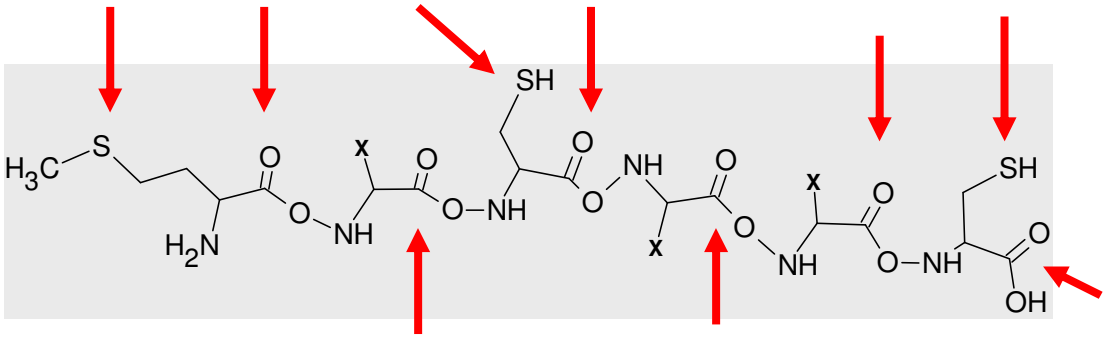

Figure 2.9 : Nine location on the motif MXCXXC for copper ions.

2.11. Copper Organization In Locations

There are two types of copper ions, Cu^{+} and Cu^{2+}, and they should interact with copper binding sequence. It was accepted that there was only one type of copper ion in the environment, which meant that Cu^{+} and Cu^{2+} had never been in the same geometry optimization process for the interaction. Copper organization is shown in Table 2.2. It was a four step organization for each of the 128 sequence.

Table 2.2 Copper organization in locations

Number of copper?	Which copper?	
1	Cu^{+}	Cu^{2+}
2	Cu^{+}	Cu^{2+}

First, only one Cu^{+} was located to a location, so every sequence interacted 9 times with one Cu^{+}. Second, one Cu^{2+} had the same interactions 9 times with each sequence. And then, two Cu^{+} was located into the two locations at the same time, but never in the same number of location. Nine locations with the two Cu^{+} resulted in 36 different structures for each sequence. Last, the process was repeated for two Cu^{2+} and resulted in 36 structures again. The total number of possible structures are shown in Table 2.3.

Table 2.3 : Total number of interaction between copper ions and the sequences.

Number of copper ions	Cu^{+} for one sequence	Cu^{2+} for one sequence	Total 128 sequence
1	9	9	18*128
2	36	36	72*128
Total	45	45	**11520**

All interactions were performed as geometry optimization, which means that the nearest local minimum points were found. Because the structure of the molecule during the optimization was the global minimum one, its structure should not change completely because of the existence of copper ions, the molecule should try to capture the copper ion.

3. RESULTS

11520 structures were obtained after geometry optimizations for the sequences with different copper ion combinations in nine locations. For a detailed investigation, the data was categorized into many groups. There were four kinds of results being investigated so the figures in every group led to these four groups. Additionally, to obtain reliable solutions, two types of approaches were used for the evaluation of the results.

Only figure of the peptides with terminal methyl groups will be evaluated in detail. Results of the neutral peptides will be given in tables without the processes. Finally, all the results will be evaluated by being compared with each other and discussed through compatibility with the recent studies in the literature.

3.1. Categorization According To Terminal Groups

There were two groups of peptides, neutral amino acid sequences and the ones with methyl terminals. So there existed 64 motifs for each group. Their figures were evaluated completely independent from each other. At the end of the evaluation, their results were compared just for comparison. The sign M- was used for the motifs with methyl terminals and H- for neutral motifs. The category can be determined from the letter in front of the motif.

3.2. Categorization According To Copper Ions

The two copper ions, Cu^{+} and Cu^{2+}, were participated in the interactions in different numbers. The results were evaluated according to the copper ion type and the number of copper ions used, which indicates that there would be four type of copper ion categories.

Table 3.1 : Categorization according to terminal groups and copper ions.

Methyl terminals				Neutral			
1 Cu^{+}	2 Cu^{+}	1 Cu^{2+}	2 Cu^{2+}	1 Cu^{+}	2 Cu^{+}	1 Cu^{2+}	2 Cu^{2+}

3.3. Evaluation Methods

Two types of evaluation were used for the figures because of different interpretations on interactions.

3.3.1. The Most Stable Peptide-Copper Complex

Every motif interacted with four types of copper groups. For example MACAAC motif had nine interactions with one Cu^{+}. In this method, the lowest value was taken into account among these nine figures. The reason is that the lowest value showed the best direction of copper for interaction and the most stable peptide-copper complex. Maybe the other 8 interaction energies were significantly high but there was a point that metal binding site captures the copper ion perfectly compared to other motifs.

3.3.2. Stabilization Energy

Four types of amino acids (A, G, T, V) were used for this study. Each of them had different number of atoms which affected the energy values, more atoms more energy, in general. For that reason the global minimum energy of the motif, the energy just before the interaction, was taken into account. Generally, if the starting energy was high, the final energy after interaction was high, too. But it may capture the copper ion better. The stabilization energy, the difference between initial and final energies, was used in this method. This value gave the efficiency of the molecule in binding copper ion.

3.4. Types Of Results

There were four categories of the results providing better binding:

- the best motif for copper ion binding
- the best amino acid for the first X residue
- the best amino acid for the second X residue
- the best amino acid for the third X residue

3.5. Evaluation Of Methyl Group At Peptide Terminal Category As Example

Methyl category will be evaluated as an example in this section. After that, the neutral peptide group evaluation results will be given directly.

3.5.1. The Energy Of Starting Structures Without Copper Ions

Global minimum energy values were used for the calculation of stabilization energy. The global minimum energy values of all motifs with methyl terminals are displayed in the Figure 3.1.

3.5.2. Calculated Energy Of The Peptides With 1 Cu^+

There were nine locations and 1 Cu^+ was placed in one location for every possible interaction, so every motif had 9 figures for evaluation (Table 3.2).

Table 3.2 : Energy values of the sequences with one Cu(I) ion, the titles of columns show the location of the copper ion before interaction (B-111 means Cu (I) (the first number, which copper), only one (in amount, how many, the second number), and where (which location, the third number).

methyl	**Peptide - 1 Cu(I) complex energy (kcal/mol)**								
sequence	B-111	B-112	B-113	B-114	B-115	B-116	B-117	B-118	B-119
MACAAC	54.48	50.95	52.59	51.19	54.35	54.32	49.37	54.41	54.34
MACAGC	50.75	43.89	43.66	55.90	47.50	47.26	43.68	51.67	47.61
MACATC	46.89	46.80	46.81	46.83	57.52	50.48	57.52	55.13	50.83
MACAVC	63.17	65.22	61.01	51.11	51.11	41.85	41.78	41.80	52.51
MACGAC	56.77	48.75	48.79	49.16	49.18	41.55	41.51	38.10	41.55
MACGGC	42.85	42.84	51.28	44.81	42.84	50.86	51.72	42.15	48.16
MACGTC	43.31	45.86	45.89	44.07	45.89	36.46	36.48	36.59	43.9
MACGVC	53.08	47.63	59.06	61.59	57.47	51.96	59.05	56.02	47.63
MACTAC	55.57	50.34	47.90	55.95	39.25	45.09	33.46	33.50	33.48
MACTGC	46.98	36.97	39.20	54.20	43.13	43.12	55.45	46.63	43.15
MACTTC	53.84	51.65	48.79	32.81	43.30	28.78	33.00	32.89	42.57
MACTVC	65.89	65.44	71.24	57.80	58.15	46.78	46.34	46.27	46.18
MACVAC	66.57	61.36	67.44	62.14	46.12	46.11	46.13	63.14	46.17
MACVGC	62.27	50.72	45.73	57.71	50.82	50.55	50.87	55.99	62.44
MACVTC	46.86	46.85	47.92	60.82	47.85	62.78	47.79	63.24	61.18
MACVVC	69.35	68.96	57.87	69.21	58.07	49.03	48.01	48.03	65.86
MGCAAC	58.59	52.94	40.06	40.08	40.09	40.07	40.23	46.89	50.44
MGCAGC	52.20	45.27	46.65	52.01	43.79	46.61	37.68	37.68	43.84
MGCATC	40.78	41.70	50.76	50.90	38.33	28.80	28.81	28.79	50.14
MGCAVC	54.07	52.51	47.83	60.77	47.86	51.71	47.79	54.09	51.96
MGCGAC	37.88	37.93	38.11	47.48	42.14	40.17	39.95	40.68	41.28
MGCGGC	40.23	33.23	39.67	38.04	36.24	40.33	36.28	37.14	40.89
MGCGTC	41.15	41.14	47.50	44.30	45.11	36.62	45.81	40.55	45.99
MGCGVC	39.02	39.03	41.80	39.02	40.13	44.02	48.95	39.01	47.84
MGCTAC	45.36	45.46	35.96	51.16	35.92	35.94	50.19	42.42	35.95
MGCTGC	40.46	40.42	44.37	46.85	43.82	42.94	44.29	39.08	42.87
MGCTTC	50.56	39.10	49.73	40.02	37.50	27.42	27.50	27.57	42.64
MGCTVC	57.66	53.61	44.46	44.39	43.52	43.52	43.81	49.80	43.49
MGCVAC	50.34	50.33	53.06	50.35	50.33	53.08	53.45	54.81	61.18
MGCVGC	52.02	51.76	45.22	55.96	46.93	46.90	45.23	46.55	46.99
MGCVTC	55.24	51.31	44.98	57.19	44.95	44.97	43.82	50.44	44.96
MGCVVC	64.99	56.28	55.80	63.55	64.33	58.02	62.36	58.67	60.00

Table 3.2 Energy values of the sequences with one Cu(I) ion (Cont'd).

MTCAAC	59.58	57.86	49.24	34.66	34.71	34.96	35.10	34.87	36.44
MTCAGC	49.09	45.77	42.96	54.99	41.34	41.32	45.02	46.14	41.42
MTCATC	51.42	51.09	48.18	54.33	26.44	25.66	25.66	25.66	42.77
MTCAVC	60.59	60.38	60.05	60.52	37.94	39.07	38.98	38.92	38.97
MTCGAC	42.46	42.47	48.21	51.30	50.38	46.91	49.13	46.04	39.00
MTCGGC	46.11	39.09	38.54	30.72	38.25	41.98	30.16	30.15	41.31
MTCGTC	49.00	42.61	47.76	35.05	22.72	23.47	23.47	23.49	40.18
MTCGVC	50.38	46.89	54.05	55.91	46.85	51.06	53.42	46.90	43.90
MTCTAC	47.70	45.01	41.13	55.37	42.83	42.16	44.55	47.51	42.78
MTCTGC	33.85	50.98	43.37	50.01	43.28	46.27	33.84	33.83	31.58
MTCTTC	58.00	54.56	54.57	55.58	53.84	55.02	42.60	42.53	43.49
MTCTVC	55.23	52.14	50.06	65.54	51.68	53.9	49.02	55.18	51.63
MTCVAC	64.53	64.16	65.34	40.24	46.36	51.66	40.29	40.27	39.57
MTCVGC	57.63	52.40	49.12	48.20	49.62	51.75	41.53	41.53	49.72
MTCVTC	56.92	57.61	53.70	33.72	34.41	33.97	33.76	34.08	44.91
MTCVVC	66.48	68.75	69.22	56.49	56.52	45.28	45.31	45.44	45.35
MVCAAC	61.10	68.25	52.06	39.45	49.69	38.64	39.45	39.48	49.77
MVCAGC	62.06	45.94	49.81	61.98	46.65	46.61	47.47	47.37	46.62
MVCATC	64.08	63.90	50.40	63.89	47.52	47.68	50.36	50.96	46.58
MVCAVC	71.72	46.62	65.22	48.27	52.12	57.75	53.10	46.69	51.46
MVCGAC	62.93	48.87	48.88	64.66	44.77	45.00	44.77	63.01	61.46
MVCGGC	55.09	47.57	44.36	48.37	44.37	48.38	47.31	47.15	44.51
MVCGTC	55.17	48.15	54.56	55.07	48.18	39.86	39.77	39.31	46.91
MVCGVC	59.84	59.83	58.62	66.88	61.80	52.33	52.33	62.38	52.34
MVCTAC	60.22	53.00	49.42	60.01	50.20	39.42	39.37	39.40	52.05
MVCTGC	58.59	60.22	45.51	45.51	47.14	45.48	47.26	53.98	56.56
MVCTTC	56.15	52.60	54.03	56.05	32.19	33.04	32.31	32.29	53.03
MVCTVC	69.59	61.04	60.52	48.63	56.93	47.64	48.53	48.56	65.54
MVCVAC	67.73	70.18	62.15	56.47	56.34	46.09	46.56	46.58	46.60
MVCVGC	64.14	65.15	57.87	61.00	52.25	57.92	53.49	62.15	62.11
MVCVTC	67.01	55.14	55.13	68.20	68.50	55.16	65.07	69.99	72.71
MVCVVC	51.41	73.07	49.45	73.17	49.34	52.26	51.41	51.39	71.69

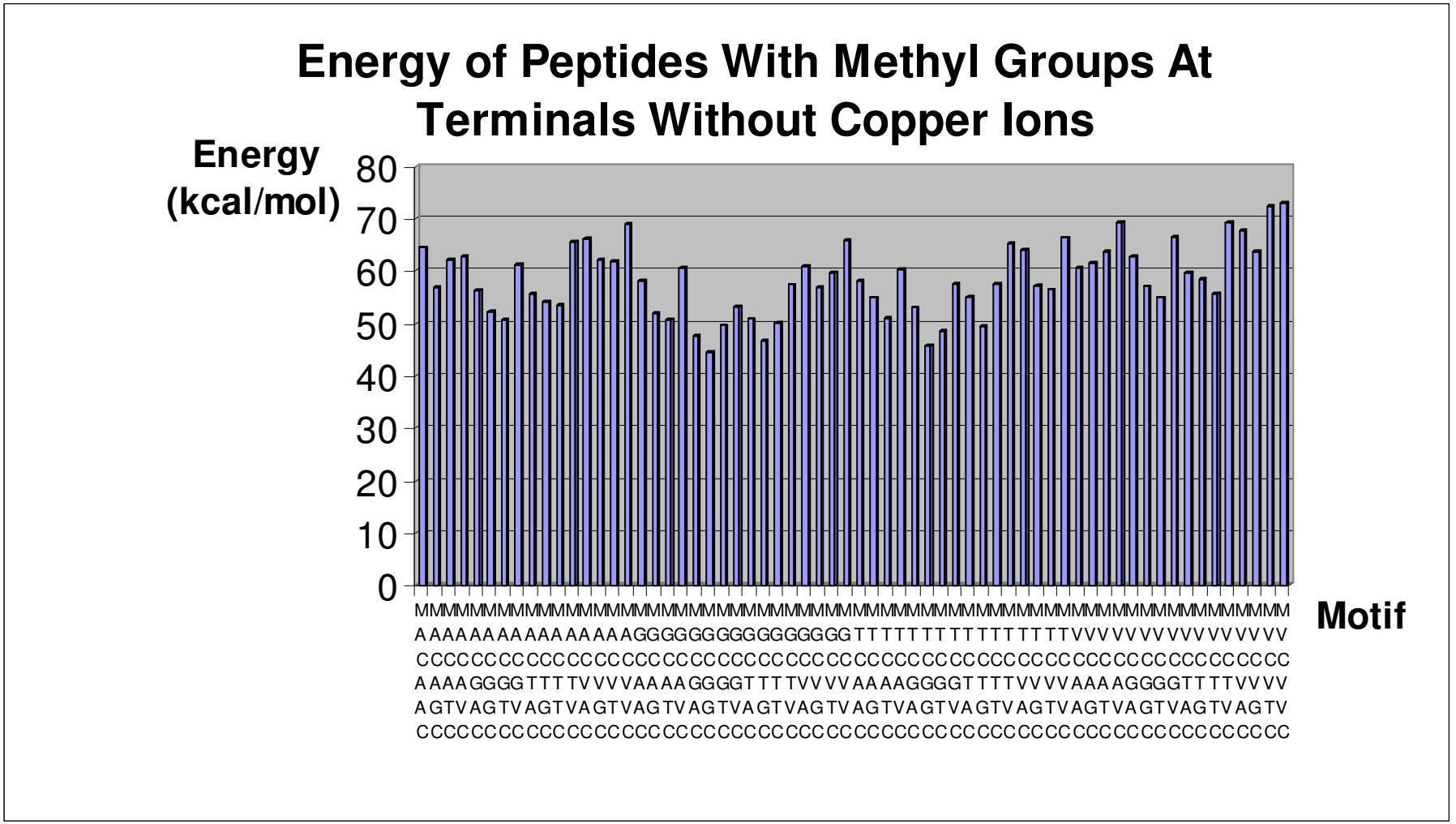

Figure 3.1 : Global minimum energy of peptides with methyl groups at terminals without copper ions

3.5.3. Calculated Energy Of The Peptides With 1 Cu^{+2}

There were nine locations and 1 Cu^{+2} was located in one location for every possible interaction, resulting in 9 structures (Table 3.3).

Table 3.3 : Energy values of the sequences with one Cu(II) ion, the titles of columns show the location of the copper ion before interaction (B-211 means Cu(II) (the first number, which copper), only one (in amount, how many, the second number), and where (which location, the third number).

+ methyl	peptide - 1 Cu(II) complex energy (kcal/mol)								
sequence	B-211	B-212	B-213	B-214	B-215	B-216	B-217	B-218	B-219
MACAAC	36.45	25.56	12.25	31.27	9.22	9.26	0.83	38.04	11.61
MACAGC	41.94	-1.50	-2.74	42.74	-2.66	-2.29	-2.71	34.10	-1.48
MACATC	27.48	26.60	26.61	26.75	24.03	26.37	16.28	61.95	26.99
MACAVC	63.23	59.82	26.54	5.82	6.15	6.14	6.16	6.17	6.16
MACGAC	56.77	29.73	-4.08	39.00	29.69	10.52	10.56	3.16	10.54
MACGGC	29.25	26.12	10.82	35.69	10.78	22.36	5.54	26.53	35.77
MACGTC	30.21	16.58	17.36	35.66	25.37	10.31	10.12	9.12	25.59
MACGVC	38.21	24.56	13.12	61.65	26.04	14.51	18.83	49.01	24.55
MACTAC	55.50	56.26	13.00	55.69	4.72	12.49	0.09	-1.95	32.15
MACTGC	54.11	14.32	3.14	54.13	3.11	4.30	3.19	5.25	4.31
MACTTC	53.61	34.86	11.40	-6.23	12.78	-6.33	-6.24	-6.51	7.94
MACTVC	66.04	40.03	43.82	43.41	40.09	20.40	19.95	19.95	19.51
MACVAC	66.62	18.70	53.07	51.78	19.29	19.30	19.32	14.33	18.63
MACVGC	45.69	5.22	5.17	47.91	5.22	5.20	5.18	47.51	62.57
MACVTC	19.00	15.88	19.76	15.87	13.08	49.10	25.56	59.80	60.25
MACVVC	69.30	60.01	30.31	15.06	13.50	14.26	13.72	14.17	20.76
MGCAAC	58.62	36.86	5.97	47.89	3.90	5.96	4.01	30.82	23.24
MGCAGC	52.29	36.47	16.10	52.29	6.30	16.00	18.74	18.76	6.31
MGCATC	-4.85	15.24	16.08	50.51	-0.55	10.50	-3.04	-5.58	9.98
MGCAVC	44.11	41.77	15.89	60.82	15.95	29.64	15.91	42.65	29.63
MGCGAC	25.84	25.85	4.54	25.76	1.29	10.59	1.97	15.89	30.88
MGCGGC	24.57	9.67	13.44	24.43	2.35	6.83	4.72	20.90	2.31
MGCGTC	30.69	30.71	-2.50	33.09	-2.65	9.90	11.17	27.64	33.95
MGCGVC	15.50	15.40	16.12	36.79	3.93	18.21	14.18	12.33	11.71
MGCTAC	27.17	9.18	6.05	19.15	6.10	6.08	-4.38	19.14	6.11
MGCTGC	29.63	29.65	13.10	29.68	8.99	28.68	-0.36	22.35	28.59
MGCTTC	50.61	12.31	15.38	-2.94	5.36	-8.47	-9.29	-9.27	10.29
MGCTVC	57.67	20.61	13.54	12.65	13.66	13.62	10.59	39.79	13.62
MGCVAC	35.52	35.54	17.55	35.53	11.25	22.11	21.86	47.18	62.23
MGCVGC	40.30	33.22	11.90	54.88	0.75	12.69	0.75	95.21	26.39
MGCVTC	45.88	36.80	20.71	46.95	19.79	19.80	-0.32	1.19	15.61
MGCVVC	65.71	44.25	32.80	51.63	34.16	32.68	30.86	39.70	17.41
MTCAAC	42.62	45.25	16.91	-3.25	14.27	-3.66	-3.87	-0.42	-1.40
MTCAGC	5.26	3.45	2.59	35.00	11.94	11.91	1.24	23.91	11.90
MTCATC	51.63	43.32	0.84	-10.05	-10.07	-10.50	-10.47	-10.49	3.28
MTCAVC	60.55	53.25	21.60	60.39	2.83	2.81	2.82	2.83	2.28
MTCGAC	21.88	21.93	12.04	39.56	11.98	12.07	11.97	33.35	16.45
MTCGGC	46.31	12.40	2.41	-4.02	5.98	9.35	9.44	9.45	-4.81
MTCGTC	48.99	16.26	8.57	-0.64	-13.28	-12.15	-12.94	-13.29	20.22

Table 3.3 Energy values of the sequences with one Cu(II) ion (Cont'd).

MTCGVC	14.08	26.34	17.08	43.75	22.89	24.64	22.99	29.32	13.95
MTCTAC	25.75	20.52	4.23	25.85	4.01	4.02	5.67	25.75	4.39
MTCTGC	13.03	10.85	-0.48	50.14	-0.48	4.30	12.96	13.02	2.55
MTCTTC	58.11	21.77	32.81	46.13	41.77	21.98	22.06	22.05	2.61
MTCTVC	39.07	29.40	13.15	29.45	15.58	13.18	13.10	39.07	15.62
MTCVAC	64.50	35.56	40.41	3.54	10.41	4.47	5.10	3.08	10.42
MTCVGC	57.79	5.81	8.76	-7.40	9.38	19.29	20.77	20.81	2.74
MTCVTC	57.07	40.32	14.05	-1.19	-0.63	-1.23	-1.29	-1.21	30.74
MTCVVC	66.21	53.67	28.17	9.98	9.96	13.89	10.01	9.97	59.70
MVCAAC	61.15	60.07	23.92	60.21	23.66	3.88	3.38	3.24	14.65
MVCAGC	62.22	47.05	13.83	59.63	25.28	25.17	12.35	27.69	25.16
MVCATC	64.07	24.73	12.65	15.92	16.82	22.55	11.46	33.73	15.83
MVCAVC	64.95	63.91	36.89	11.47	17.07	11.41	12.42	10.33	46.14
MVCGAC	62.92	15.42	15.40	49.39	2.87	3.01	2.97	63.06	59.93
MVCGGC	52.29	8.70	25.51	33.07	25.56	33.07	-3.13	31.12	39.66
MVCGTC	55.25	21.67	9.24	43.44	32.78	12.27	12.26	11.97	23.33
MVCGVC	51.03	50.81	55.84	66.98	55.85	33.79	33.80	55.12	33.83
MVCTAC	60.14	50.91	16.11	9.04	21.87	9.68	9.05	9.00	33.49
MVCTGC	58.65	14.01	20.19	20.20	19.19	16.94	24.50	46.95	45.19
MVCTTC	56.89	27.11	17.78	-2.66	12.24	-2.56	-3.23	-3.21	17.75
MVCTVC	69.67	46.02	35.59	28.37	20.62	12.10	10.69	10.71	54.04
MVCVAC	67.56	61.52	14.54	11.24	9.75	10.83	10.95	10.93	58.54
MVCVGC	64.07	23.70	40.51	49.86	27.58	34.15	28.08	32.39	44.23
MVCVTC	23.76	32.27	32.25	55.69	7.13	8.88	25.03	54.93	72.77
MVCVVC	14.95	72.81	42.86	73.89	33.86	15.52	14.99	14.99	24.53

3.5.4. Calculated Energy Of The Peptides With 2 Cu^{+}

There were nine locations and 2 Cu^{+} were located in two different locations for every interaction. Every motif had 36 structures (Table A.1).

3.5.5. Calculated Energy Of The Peptides With 2 Cu^{+2}

There were nine locations and 2 Cu^{+2} were located in two separate locations for every interaction, giving 36 structures (Table A.2).

3.5.6. Calculated Values With Two Methods

All the calculated values of the peptides with methyl terminals through the two methods are displayed in the Table 3.4.

Table 3.4 : Calculated values of the peptides with methyl terminals through the two methods.

+ methyl at the terminals		1 copper(I)		1 copper(II)		2 copper(I)		2 copper(II)	
sequence	**energy without copper (kcal/mol)**	**most stable peptide-copper complex (kcal/mol)**	**stabilization energy (kcal/mol)**	**most stable peptide-copper complex (kcal/mol)**	**stabilization energy (kcal/mol)**	**most stable peptide-copper complex (kcal/mol)**	**stabilization energy (kcal/mol)**	**most stable peptide-copper complex (kcal/mol)**	**stabilization energy (kcal/mol)**
MACAAC	64.43	49.37	15.06	0.83	63.60	41.05	23.38	1.73	62.70
MACAGC	56.86	43.66	13.20	-2.74	59.60	39.26	17.60	-4.76	61.62
MACATC	62.15	46.80	15.35	16.28	45.87	39.35	22.80	1.95	60.20
MACAVC	62.60	41.78	20.82	5.82	56.78	39.16	23.44	7.34	55.26
MACGAC	56.33	38.10	18.23	-4.08	60.41	37.33	19.00	-3.33	59.66
MACGGC	52.15	42.15	10.00	5.54	46.61	37.10	15.05	0.80	51.35
MACGTC	50.74	36.46	14.28	9.12	41.62	33.03	17.71	-11.16	61.90
MACGVC	61.18	47.63	13.55	13.12	48.06	46.41	14.77	3.17	58.01
MACTAC	55.51	33.46	22.05	-1.95	57.46	31.83	23.68	-7.03	62.54
MACTGC	54.04	36.97	17.07	3.11	50.93	33.31	20.73	-8.77	62.81
MACTTC	53.37	28.78	24.59	-6.51	59.88	26.94	26.43	-11.18	64.55
MACTVC	65.43	46.18	19.25	19.51	45.92	41.49	23.94	7.99	57.44
MACVAC	66.13	46.11	20.02	14.33	51.80	44.34	21.79	12.06	54.07
MACVGC	62.09	45.73	16.36	5.17	56.92	43.96	18.13	1.81	60.28
MACVTC	61.72	46.85	14.87	13.08	48.64	43.64	18.08	1.33	60.39
MACVVC	69.00	48.01	20.99	13.50	55.50	46.22	22.78	6.61	62.39
MGCAAC	58.19	40.06	18.13	3.90	54.29	33.88	24.31	-6.60	64.79
MGCAGC	51.87	37.68	14.19	6.30	45.57	32.67	19.20	-2.72	54.59
MGCATC	50.69	28.79	21.90	-5.58	56.27	24.60	26.09	-12.97	63.66
MGCAVC	60.47	47.79	12.68	15.89	44.58	44.42	16.05	10.71	49.76
MGCGAC	47.46	37.88	9.58	1.29	46.17	31.23	16.23	-7.88	55.34
MGCGGC	44.52	33.23	11.29	2.31	42.21	26.62	17.90	-12.81	57.33
MGCGTC	49.59	36.62	12.97	-2.65	52.24	31.19	18.40	-5.90	55.49
MGCGVC	53.09	39.01	14.08	3.93	49.16	34.62	18.47	-2.15	55.24

Table 3.4 Calculated values of the peptides with methyl terminals through the two methods (Cont'd).

MGCTAC	50.83	35.92	14.91	-4.38	55.21	27.83	23.00	-11.88	62.71
MGCTGC	46.76	39.08	7.68	-0.36	47.12	34.07	12.69	-11.91	58.67
MGCTTC	50.10	27.42	22.68	-9.29	59.39	23.22	26.88	-16.79	66.89
MGCTVC	57.32	43.49	13.83	10.59	46.73	39.30	18.02	5.47	51.85
MGCVAC	60.76	50.33	10.43	11.25	49.51	46.90	13.86	10.47	50.29
MGCVGC	56.92	45.22	11.70	0.75	56.17	40.55	16.37	0.92	56.00
MGCVTC	59.76	43.82	15.94	-0.32	60.08	38.56	21.20	-4.47	64.23
MGCVVC	65.88	55.80	10.08	17.41	48.47	49.11	16.77	14.87	51.01
MTCAAC	58.02	34.66	23.36	-3.87	61.89	31.04	26.98	-6.09	64.11
MTCAGC	54.84	41.32	13.52	1.24	53.60	31.63	23.21	-7.73	62.57
MTCATC	50.85	25.66	25.19	-10.50	61.35	22.04	28.81	-13.40	64.25
MTCAVC	60.17	37.94	22.23	2.28	57.89	36.09	24.08	3.03	57.14
MTCGAC	53.00	39.00	14.00	11.97	41.03	34.56	18.44	2.86	50.14
MTCGGC	45.76	30.15	15.61	-4.81	50.57	28.08	17.68	-9.87	55.63
MTCGTC	48.42	22.72	25.70	-13.29	61.71	19.67	28.75	-17.05	65.47
MTCGVC	57.56	43.90	13.66	13.95	43.61	39.13	18.43	6.63	50.93
MTCTAC	55.08	41.13	13.95	4.01	51.07	33.33	21.75	-10.39	65.47
MTCTGC	49.49	31.58	17.91	-0.48	49.97	28.57	20.92	-17.49	66.98
MTCTTC	57.57	42.53	15.04	2.61	54.96	38.64	18.93	-2.41	59.98
MTCTVC	65.18	49.02	16.16	13.10	52.08	43.62	21.56	7.84	57.34
MTCVAC	63.88	39.57	24.31	3.08	60.80	38.53	25.35	3.76	60.12
MTCVGC	57.22	41.53	15.69	-7.40	64.62	37.68	19.54	-7.07	64.29
MTCVTC	56.38	33.72	22.66	-1.29	57.67	30.43	25.95	-4.61	60.99
MTCVVC	66.28	45.28	21.00	9.96	56.32	43.64	22.64	4.85	61.43

Table 3.4 Calculated values of the peptides with methyl terminals through the two methods (Cont'd).

MVCAAC	60.60	38.64	21.96	3.24	57.36	35.67	24.93	2.70	57.90
MVCAGC	61.55	45.94	15.61	12.35	49.20	36.39	25.16	4.65	56.90
MVCATC	63.53	46.58	16.95	11.46	52.07	39.93	23.60	-4.46	67.99
MVCAVC	69.30	46.62	22.68	10.33	58.97	43.61	25.69	11.94	57.36
MVCGAC	62.68	44.77	17.91	2.87	59.81	43.38	19.30	3.06	59.62
MVCGGC	56.98	44.36	12.62	-3.13	60.11	36.51	20.47	-2.54	59.52
MVCGTC	54.85	39.31	15.54	9.24	45.61	33.40	21.45	-5.36	60.21
MVCGVC	66.47	52.33	14.14	33.79	32.68	47.69	18.78	13.99	52.48
MVCTAC	59.55	39.37	20.18	9.00	50.55	35.01	24.54	-1.00	60.55
MVCTGC	58.27	45.48	12.79	14.01	44.26	38.61	19.66	4.52	53.75
MVCTTC	55.61	32.19	23.42	-3.23	58.84	29.68	25.93	-9.01	64.62
MVCTVC	69.15	47.64	21.51	10.69	58.46	40.83	28.32	1.23	67.92
MVCVAC	67.63	46.09	21.54	9.75	57.88	44.45	23.18	10.49	57.14
MVCVGC	63.65	52.25	11.40	23.70	39.95	51.01	12.64	14.56	49.09
MVCVTC	72.18	55.13	17.05	7.13	65.05	51.88	20.30	6.53	65.65
MVCVVC	72.91	49.34	23.57	14.95	57.96	48.37	24.54	14.24	58.67

3.6. All Results

All the results are shown in Table 3.5 and Table 3.6. The figures for these values are not covered in detail.

Table 3.5 : Results of all copper groups for peptides with methyl terminals.

Group	Method	Four Types of Results			
		Best Motif	Best AA for 1st X	Best AA for 2nd X	Best AA for 3rd X
M-1Cu(I)	Most stable	MTCGTC	T	T	T
	stabilization	MTCGTC	T	A-T-V	T
M-1Cu(II)	Most stable	MTCGTC	T	A-G-T	T
	stabilization	MVCVTC	T	V	A-T
M-2Cu(I)	Most stable	MTCGTC	T	T	T
	stabilization	MTCATC	T	A-T	T
M-2Cu(II)	Most stable	MTCGTC	G	T	T
	stabilization	MVCATC	T	T	T

Table 3.6 : Results of all copper groups for peptides with hydroxyl at terminals.

Group	Method	Four Types of Results			
		Best Motif	Best AA for 1st X	Best AA for 2nd X	Best AA for 3rd X
H-1Cu(I)	Most stable	MTCGGC	T	G-T	G
	stabilization	MTCVTC	T-V	A-V	V
H-1Cu(II)	Most stable	MTCGGC	T	A	G
	stabilization	MACAAC	A	V	A-G-T
H-2Cu(I)	Most stable	MTCATC	T	T	G
	stabilization	MACTTC	A	T	T
H-2Cu(II)	Most stable	MGCTTC	T	T	T
	stabilization	MTCTAC	A	T	T

Table 3.5 indicates that MTCGTC is the motif that binds copper ions the best. MTCGTC is the best for the amino acid sequences with methyls bonded at the both peptide terminals. On the other hand, the best motif among the neutral peptides is

unclear (Table 3.6). The tables also indicate that while the best amino acid for the first and the last X residues is Threonine for peptides with methyl terminals, the same conclusion can not be derived for neutral peptides.

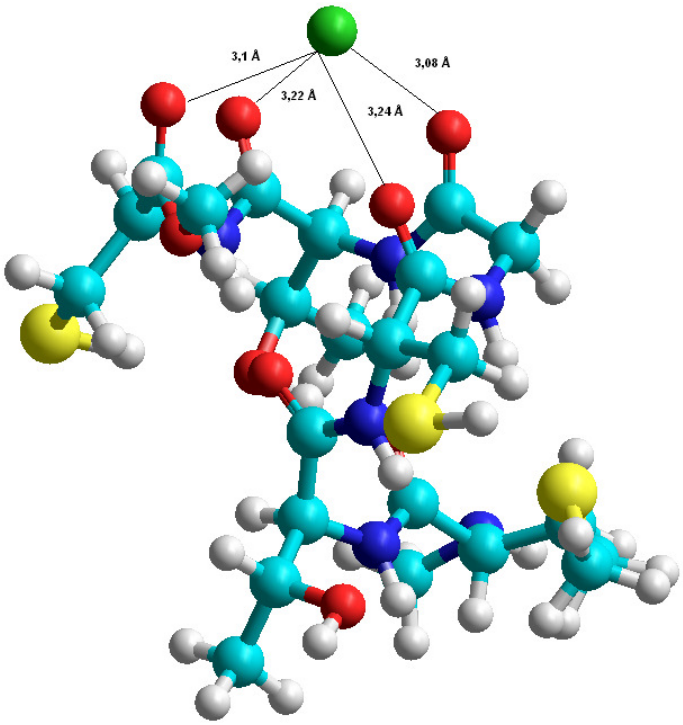

Figure 3.2 : MTCGTC with 1 Cu^{+} (B-115 with methyl terminals).

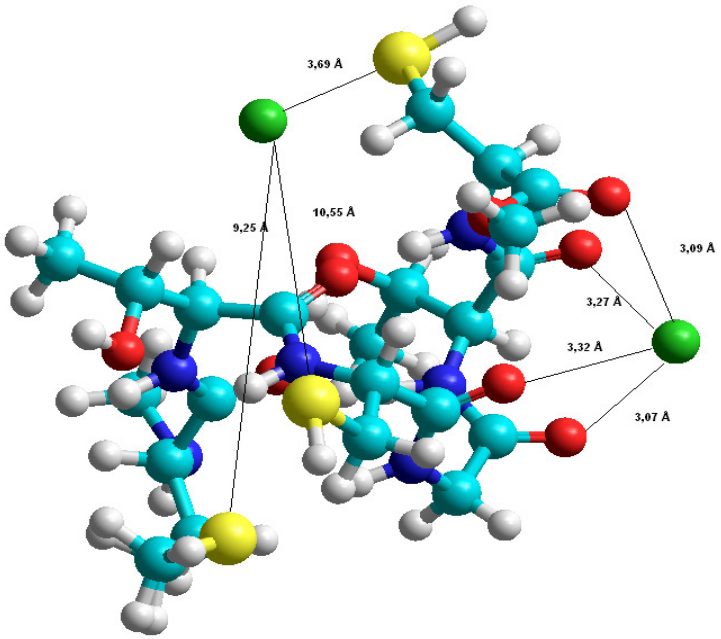

Figure 3.3: MTCGTC with 2 Cu^{+} (B-1279 with methyl terminals).

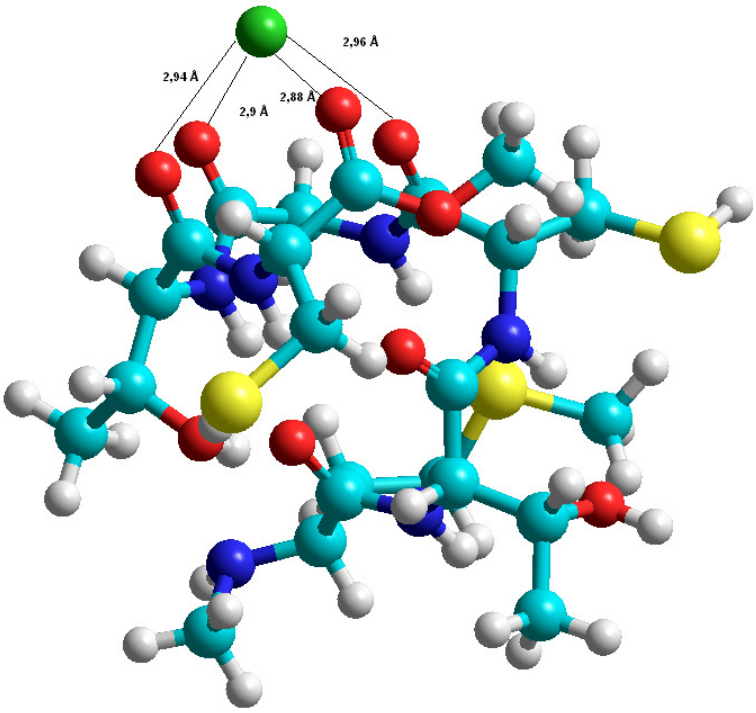

Figure 3.4: MTCGTC with 1 Cu^{2+} (B-218 with methyl terminals).

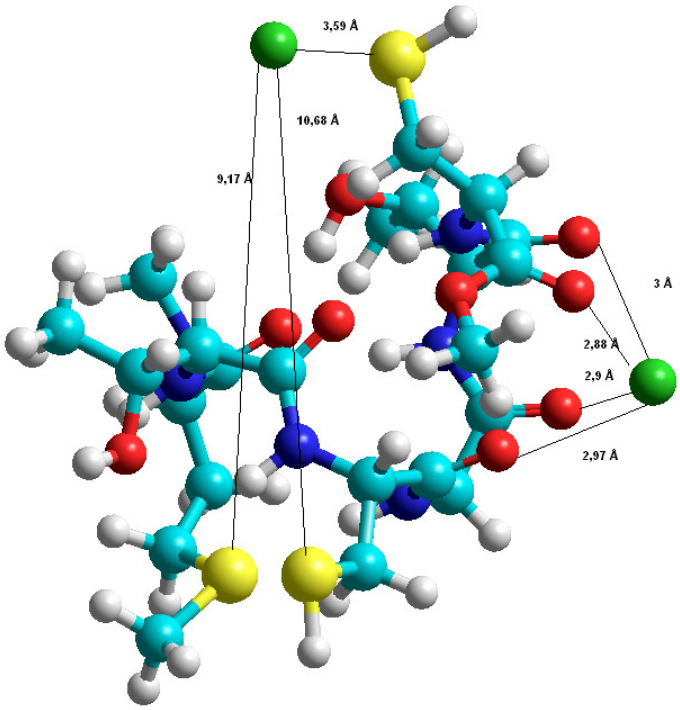

Figure 3.5 : MTCGTC with 2 Cu^{2+} (B-2259 with methyl terminals).

3.7. The Structural Comparison Of Some Peptides

There was one motif considered as the best, MTCGTC. Its energy values with copper ions were better than other motifs' in its categories in general. Structural comparison by taking the distance between the copper ions and some specific atoms on the molecule was a way of comparing the selectivity of the molecule. This structural comparison was just used to see whether there was a noticable difference between the two molecules in binding copper. A good peptide at binding copper MTCATC with methyl groups at terminals in positions B-116, B-1269, B-217, and B-2259 shown in figures 3.6 – 3.9, respectively.

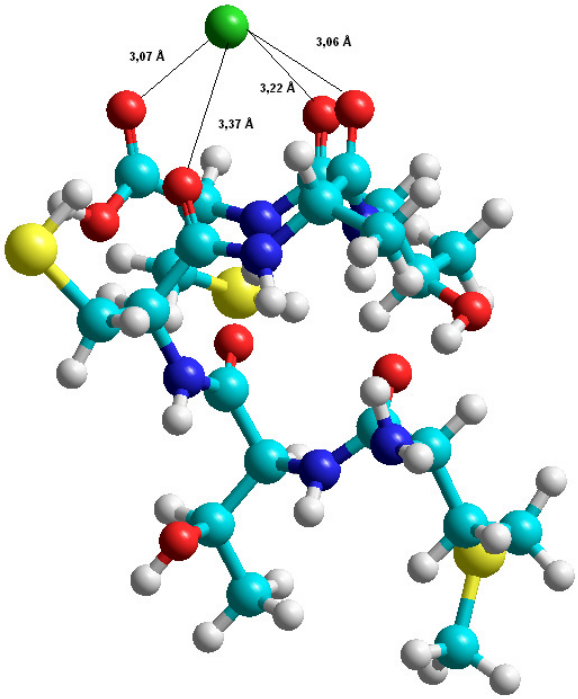

Figure 3.6 : MTCATC with 1 Cu^{+} (B-116 with methyl terminals).

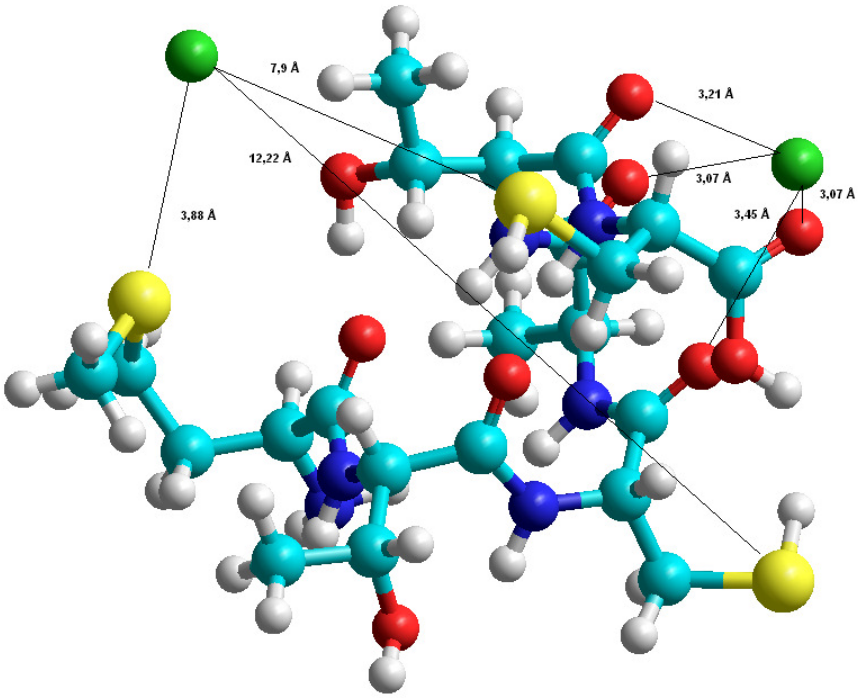

Figure 3.7 : MTCATC with 2 Cu^{+} (B-1269 with methyl terminals).

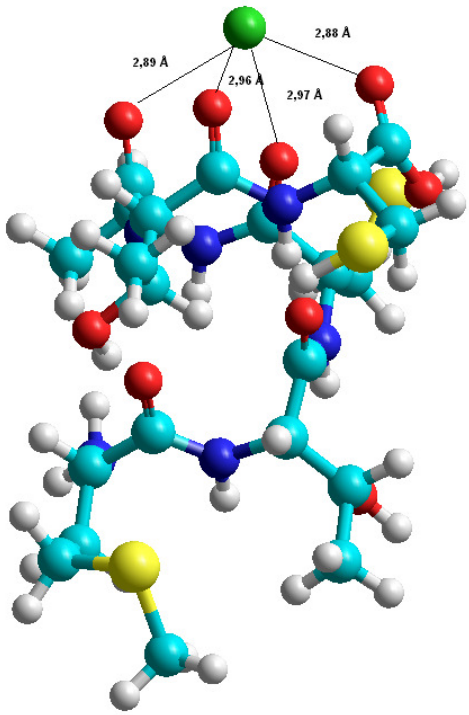

Figure 3.8 : MTCATC with 1 Cu^{2+} (B-217 with methyl terminals).

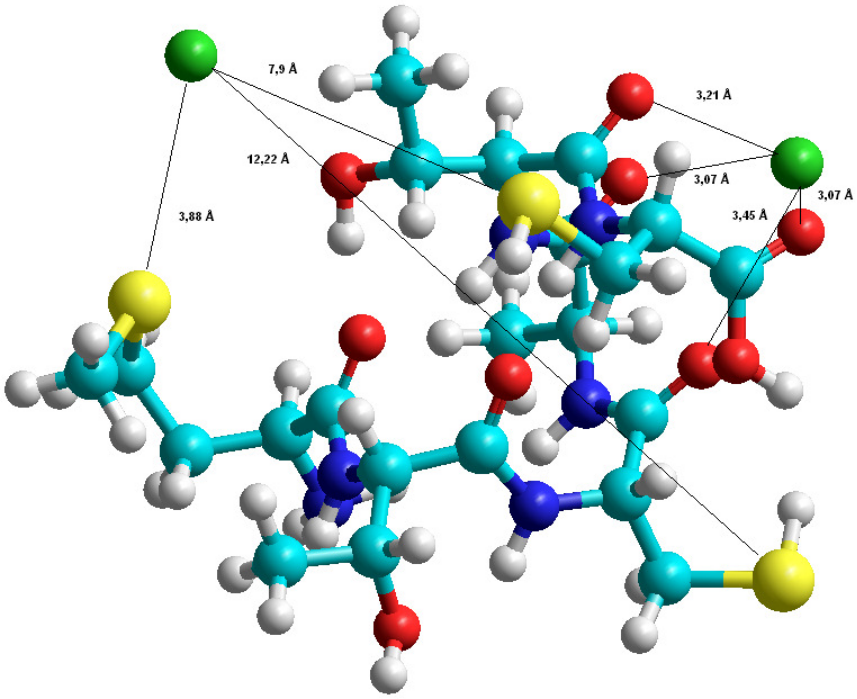

Figure 3.9 : MTCATC with 2 Cu^{2+} (B-2259 with methyl terminals).

A bad peptide at binding copper MVCVVC with methyl groups at terminals in positions B-114, B-1224, B-214, and B-2224 structures after the interactions with four copper groups are shown in figures 3.9 - 3.13, respecively. The efficiencies of copper binding of MTCATC and MVCVVC are quite different and it can be seen clearly by the distance values between copper and the nearest atoms on the peptides.

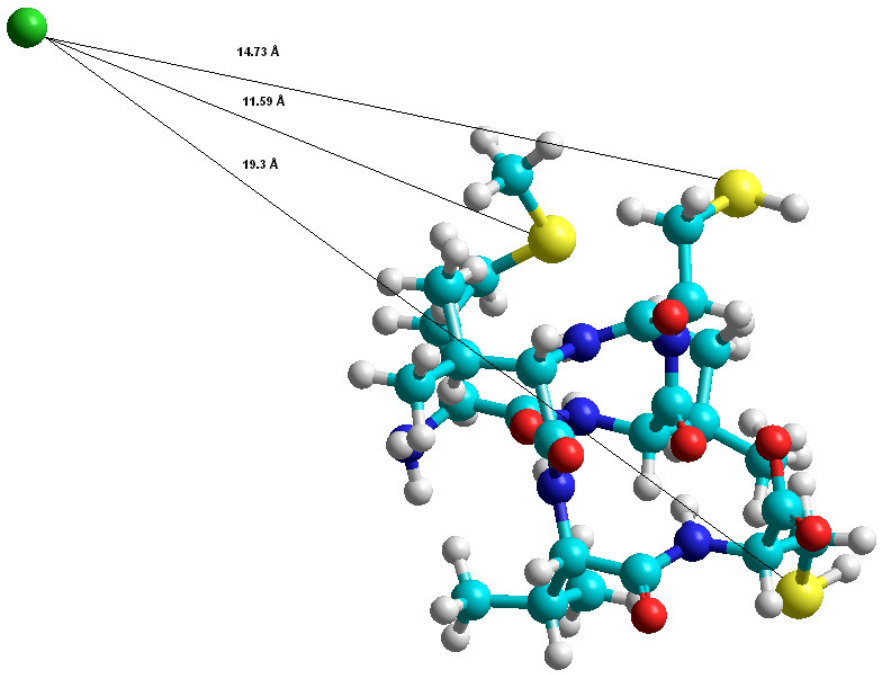

Figure 3.10 : MVCVVC with 1 Cu^{+} (B-114 with methyl terminals).

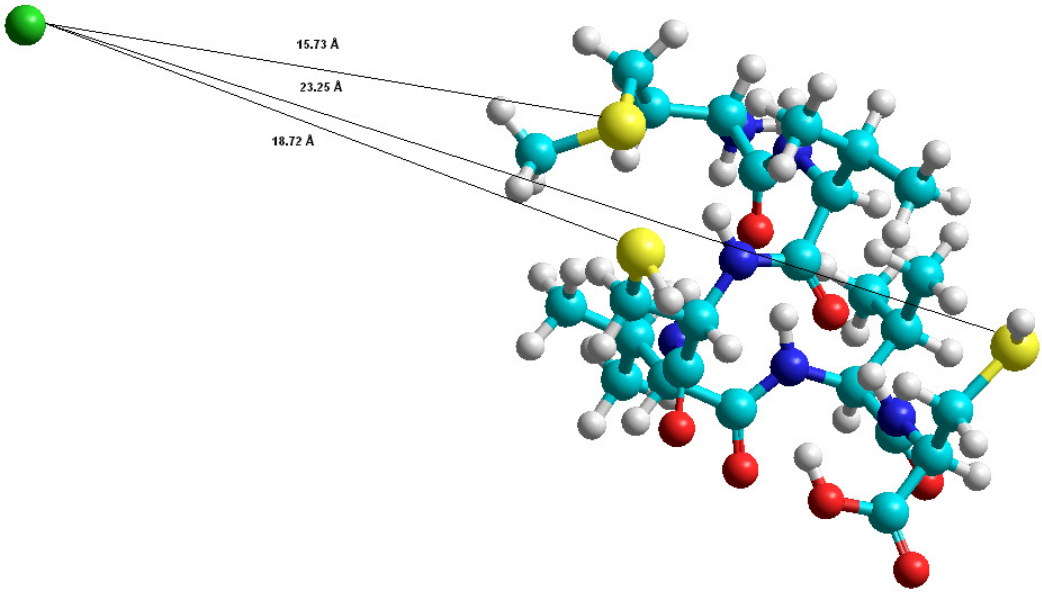

Figure 3.11 : MVCVVC with 1 Cu^{+2} (B-1224 with methyl terminals).

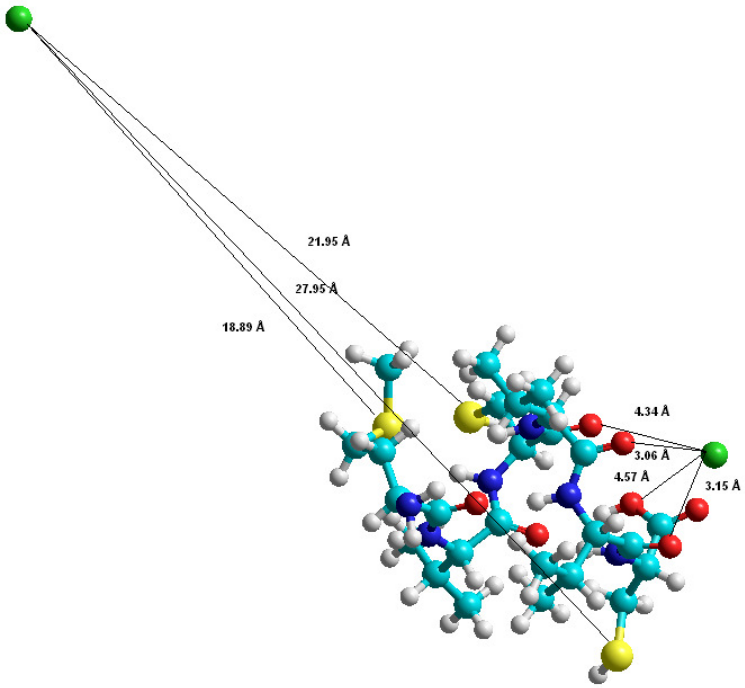

Figure 3.12 : MVCVVC with 2 Cu^{+} (B-214 with methyl terminals).

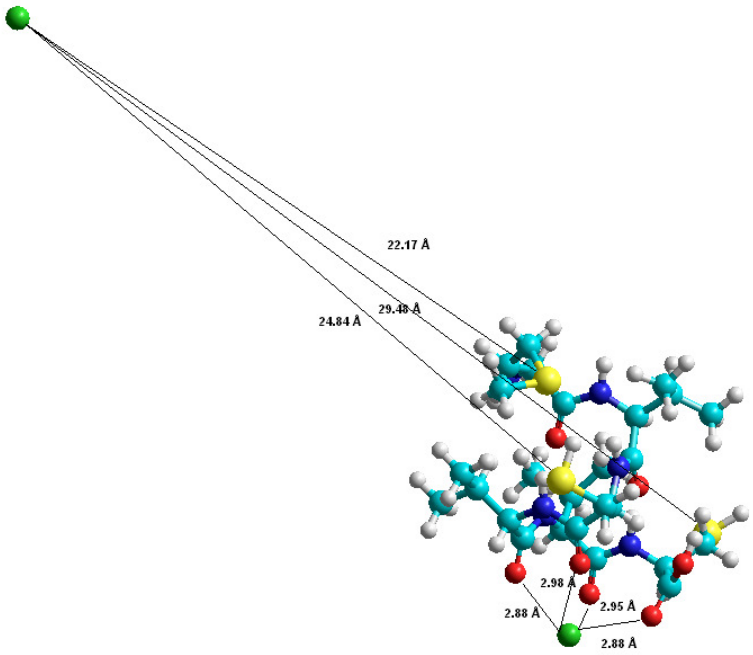

Figure 3.12 : MVCVVC with 2 Cu^{+}

4. DISCUSSION AND CONCLUSIONS

The computational results on copper binding of MXCXXC motif are in agreement with the available literature data. Despite considering results for every X residue independently, a general conclusion is not possible regarding copper binding motif. If this study is followed by experimental ones and confirmed, the data will help clarify protein-metal binding process and its applications.

The results indicate that the first X in the MXCXXC amino acid sequence is Threonine. All the solutions at every level indicate that the motif MTCXXC binds both of the coppers strongly. In general, the results of peptides with terminal methyl groups are supporting Threonine more than the results of neutral ones. Neutral peptides also prefers Threonine for the first X but not as much as peptides with terminal methyl groups. As well as the result for the first X, the best motifs for all categories also have generally Threonine in the position of the first X. The other special aspect of the result for the first X is that the studies on copper binding motif accept the residue between Methionine and the first Cysteine as Threonine. In this study, Threonine at that position was not fixed, and different amino acids were tested along with Threonine to verify the importance of Threonine. Thus, the results are compatible with previous studies reported in the literature.

The results of the first and the third Xs are also similar. At all categories of analysis, Threonine was found to be very dominant over other amino acids, especially for the peptides with terminal methyls. But different from the first X, there are, to our knowledge, no previous reports on the conservation of the third X residue in the literature. This is a new result obtained from our study, which may indicate the importance of Threonine in the third position.

The results for the second X is slightly different from the others. Checking all the results at every category, Threonine is also an alternative for this X residue, but not the favorite one. Alanine, Glycine and Valine are also becoming favorite, not like other X positions where Threonine is dominating. Thus, it can be concluded that the second X residue is any amino acid, preferentially Threonine.

REFERENCES

Avery S.A., 2001. Metal toxicity in yeast and role of oxidative stress, *Advances in Applied Microbiology,* **49,** 111-141.

Banci L. and Rosato A., 2003. Structural genomics of proteins involved in copper homeostasis, *Accounts of Chemical Research,* **36,** 215-221.

Becker O.M., 2001. Computational biochemistry and biophysics, Marcel Dekker Incorporated, New York.

Bertinato J. and L'Abbe M.R., 2004. Maintaining copper homeostasis: regulation of copper-trafficking proteins in response to copper deficiency or overload, *Journal of Nutritional Biochemistry,* **15,** 316-322.

Bruins M.R., Kopil S., Oehme F.W., 2000. Microbial resistance to metals in the environment, *Ecotoxicology and Environmental Safety,* **45,** 198-207.

Culotta V.C., Portnot M.E., Schmidt P.J., Rogers R.C., 2001. Metal transporters that contribute copper to metallochaperones in *Saccharomyces cerevisiae, Molecular Genetic and Genomics,* **265,** 873-882.

Hassett R. and Kosman D.J., 1995. Evidence for Cu(II) reduction as a component of copper uptake by *Saccharomyces cerevisiae*, *The Journal of Biological Chemistry,* **270,** 128-134.

Hassett R., Dix D.R., Eide D.J., Kosman D.J., 2000, The Fe(II) permease Fet4p functions as a low affinity copper transporter and supports normal copper trafficking in *Saccharomyces cerevisiae, Biochemical Journal,* **351,** 477-484.

Höltje H.D. and Folkers G., 2003. Molecular modeling: basic principles and applications, Weinheim, New York.

HyperChem 7.5, 2003. Hypercube Inc., Gainesville, FL.

Kapoor A. and Viraraghavan T., 1995. Fungi biosorption – an alternative treatment option for heavy metal bearing wastewaters: a review, *Bioresour Techno,* **53,** 195-206.

Leach A.R., 2001. Molecular Modelling Principles and Application, Prentice Hall Harlow, New York.

Lewars B. and Errol G., 2003. Computational Chemistry: introduction to the theory and applications of molecular and quantum mechanics, Kluwer Academic Publishers, Secaucus, HJ, USA.

Luk E., Jensen L.T., Culotta V.C., 2003. The many highways for intracellular trafficking of metals, *J. Biol. Inorg. Chem.,* **8,** 803-809.

Madigan M.T., Martinko J.M., Parker J., 2003. Brock Biology of Microorganisms, 10th Edition, Prentice Hall, USA.

O'Halloran T.V. and Culotta V.C., 2000. Metallochaperones, on intracellular shuttle service for metal ions, *The Journal of Biological Chemistry,* **275,** 25057-25060.

Opella S.J., DeSilva T.M., Veglia G., 2002. Structural biology of metal-binding sequences, *Current Opinion in Chemical Biology,* **6,** 217-223.

Peregol P. and Howel S.B., 1997. Molecular mechanism controlling sensitivity to toxic metal ions in yeast, *Toxicol Appl Pharmacol,* **147,** 312-318.

Rosenzweig A.C. and O'Halloran T.V., 2000. Structure and chemistry of the copper chaperone proteins, *Current Opinion in Chemical Biology,* **4,** 140-147.

Rosenzweig A.C., 2002. Metallochaperones: bind and deliver, *Chemistry & Biology,* **9,** 673-677.

Rosenzweig A.C., Huffman D.L., Hou M.Y., Wernimont A.K., Pufahl R.A., O'Halloran T.V., 1999. Crystal structure of the Atx1 metalachaperone protein at 1.02 Å resolution, *Structure,* **7,** 605-617.

Rouch D.A., Lee B.T.D., Morby A.P., 1995. Understanding cellular responses to toxic agents: a model for mechanism choice in bacterial metal resistance, *J. Ind. Microbiol.,* **14,** 132-141.

Rousselot-Pailley P., Seneque O., Lebrun C., Crouzy S., Boturyn D., Dumy P., Ferrand M., Delangle P., 2006. Model peptides based on the binding loop of the copper metallochaperone Atx1: selectivity of the consensus sequence MxCxxC for metal ions Hg(II), Cu(I), Cd(II), Pb(II), and Zn(II), *Inorganic Chemistry,* **45,** 5510-5520.

Schlecht M.F., 1998. Molecular modeling on the PC, John Wiley & Sons, Inc., New York.

Schlick T., 2002. Molecular Modeling and Simulation, Springer-Verlag, New York, Inc.

Thiele D.J. and Puig S., 2002. Molecular mechanisms of copper uptake and distribution, *Current Opinion in Chemical Biology,* **6,** 171-180.

Url-1 <http://bugs.bio.usyd.edu.au/>, accessed at 23.11.2008.

Url-2 <http://media.nih.gov>, accessed at 23.11.2008.

Url-3 <http://www.netsci.org>, accessed at 15.12.2006.

Wang J. and Chen C., 2006. Biosorption of heavy metals by *Saccharomyces cerevisiae*: a review, *Biotechnology Advances,* **24,** 427-451.

Wernimont A.K., Huffman D.L., Lamb A.L., O'Halloran T.V., Rosenzweig A.C., 2000. Structural basis for copper transfer by the metallochaperone for the Menkes/Wilson disease proteins, *Nature Structural Biology,* **7,** 766-771.

APPENDICES

Table A.1 : Energy values of the sequences with two Cu(I) ions, the titles of columns show the initial locations of the copper ions before optimization (B-1212 means Cu (I) (the first number, which copper), two ions (in amount, how many, the second number), and where (which locations, the third and the fourth number), part-I and part-II.

+ methyl	**peptide - 2 Cu(I) complex energy (kcal/mol) part-I**																	
sequence	B1212	B1213	B1214	B1215	B1216	B1217	B1218	B1219	B1223	B1224	B1225	B1226	B1227	B1228	B1229	B1234	B1235	B1236
MACAAC	45.22	49.13	48.64	50.31	50.03	47.41	55.11	49.99	46.66	43.92	46.70	46.71	43.08	45.21	46.60	54.98	50.78	56.65
MACAGC	44.03	46.08	51.11	46.25	43.35	46.09	49.33	44.10	43.98	44.65	48.34	45.70	44.04	39.26	44.06	44.35	42.82	44.28
MACATC	43.43	43.39	44.91	41.24	42.09	45.69	43.06	39.50	48.25	51.08	41.23	39.38	45.80	43.05	39.43	51.03	45.77	39.39
MACAVC	67.20	63.52	52.05	51.88	42.06	42.72	42.54	60.25	63.49	42.66	51.99	42.25	41.42	42.82	42.61	63.45	41.33	49.53
MACGAC	49.31	53.25	41.70	49.98	42.35	42.30	38.59	42.49	42.95	49.94	49.89	37.37	37.36	47.26	37.33	53.13	42.67	42.52
MACGGC	43.49	42.78	43.87	43.63	37.95	46.26	37.14	43.59	44.96	43.62	45.12	38.18	43.85	40.86	42.14	44.80	44.91	44.85
MACGTC	44.75	44.80	39.68	40.62	33.91	33.74	102.68	43.94	50.72	46.47	33.03	33.05	33.06	34.03	40.03	43.47	41.64	33.16
MACGVC	46.41	58.59	53.66	59.57	52.51	59.23	53.05	48.48	54.73	48.22	47.64	49.35	54.77	50.81	48.66	59.26	53.06	61.71
MACTAC	51.10	50.21	55.89	40.35	45.87	34.21	34.12	34.05	50.90	51.06	37.31	43.75	31.83	31.84	31.84	48.43	40.68	41.70
MACTGC	38.16	43.90	46.96	44.12	43.96	43.19	47.55	44.87	34.41	37.61	36.55	36.54	34.43	35.95	36.62	47.85	39.43	33.31
MACTTC	52.03	49.62	33.67	44.02	33.63	33.53	33.77	53.26	49.05	42.02	30.14	33.52	33.51	33.57	43.54	28.51	35.44	37.04
MACTVC	63.64	60.88	60.33	58.64	47.18	47.09	47.11	47.26	58.29	63.60	63.64	42.22	46.52	46.50	46.56	58.27	58.18	41.51
MACVAC	46.55	62.06	62.64	46.91	46.93	46.94	46.51	47.35	62.02	62.90	47.38	47.32	46.82	63.82	46.72	62.04	44.53	44.55
MACVGC	52.17	51.17	60.51	46.05	44.16	48.24	58.54	62.66	49.38	57.11	50.99	49.37	50.96	48.22	51.92	50.64	45.00	60.82
MACVTC	47.47	44.13	48.38	44.11	48.96	47.05	49.52	46.52	44.10	48.37	44.09	49.98	50.86	49.54	47.48	48.46	48.09	49.17
MACVVC	69.62	68.43	69.60	59.02	49.51	49.04	48.79	48.08	67.13	69.80	61.70	51.60	50.86	50.83	61.69	66.55	51.68	51.67

Table A.1 Energy values of the sequences with two Cu(I) ions (Cont'd).

MGCAAC	52.54	40.92	40.74	39.47	40.61	40.66	47.37	47.31	37.65	52.71	35.20	34.38	37.70	47.00	42.49	40.26	40.97	40.56
MGCAGC	45.96	47.24	53.77	44.61	47.30	38.16	38.48	44.59	47.31	52.48	39.10	47.33	32.71	32.67	39.11	47.23	37.26	47.59
MGCATC	43.48	43.96	41.15	34.08	24.62	24.60	29.09	28.84	51.20	46.02	44.07	26.33	26.60	26.32	26.30	41.35	33.65	28.18
MGCAVC	49.88	48.51	54.75	48.51	49.75	48.53	55.03	49.76	48.79	53.02	44.60	45.09	44.68	50.01	45.09	48.65	50.57	47.09
MGCGAC	38.74	38.90	38.17	42.84	40.65	45.80	36.00	37.24	31.83	38.82	37.89	33.85	37.90	34.54	33.76	38.74	31.23	38.48
MGCGGC	27.65	32.04	30.35	42.25	37.84	37.12	37.83	41.41	32.96	33.74	26.62	27.36	29.46	30.89	30.85	40.40	34.43	31.50
MGCGTC	41.94	31.98	31.23	45.80	31.19	38.06	41.28	41.01	47.13	37.60	37.64	31.20	41.21	41.53	41.01	51.55	31.85	42.90
MGCGVC	39.75	34.62	38.52	39.77	35.56	36.87	39.07	43.45	34.63	38.54	37.97	35.56	43.46	39.06	39.04	40.39	42.00	40.36
MGCTAC	46.19	31.40	40.17	32.76	32.79	50.55	40.00	32.81	35.28	46.25	29.59	35.25	27.83	42.73	30.47	36.85	35.62	36.31
MGCTGC	40.42	45.05	47.43	44.75	43.60	44.97	35.64	43.53	42.91	40.40	48.70	39.72	42.87	35.61	39.73	44.93	42.64	45.58
MGCTTC	39.78	48.39	41.06	36.80	28.11	28.04	27.94	42.00	43.98	39.63	24.71	28.10	36.68	28.84	44.61	43.50	40.45	27.54
MGCTVC	54.35	44.32	44.17	44.41	44.35	44.56	117.64	44.33	39.30	54.32	44.29	42.14	39.51	48.03	44.29	44.63	44.47	44.51
MGCVAC	53.14	48.03	51.03	47.68	46.93	46.92	48.43	51.11	48.24	51.12	48.16	47.63	46.90	48.65	51.05	53.79	47.96	53.88
MGCVGC	42.62	44.97	52.86	45.39	44.73	42.29	43.46	44.77	40.55	52.67	47.67	44.92	44.62	42.68	46.40	44.81	47.48	44.58
MGCVTC	51.64	42.93	56.25	42.94	42.93	42.94	50.66	42.93	39.54	47.75	43.73	40.60	41.74	45.49	43.79	45.28	43.53	38.56
MGCVVC	57.14	56.11	64.28	63.03	58.73	56.39	53.92	63.48	49.11	56.54	52.13	51.45	57.32	55.03	56.82	49.19	54.56	58.56
MTCAAC	35.56	37.98	35.75	35.54	34.37	37.00	37.02	56.99	46.67	35.50	32.74	40.72	35.34	35.48	35.10	45.44	37.62	42.86
MTCAGC	40.49	39.45	41.94	38.59	38.59	57.59	44.52	41.99	35.52	43.83	31.63	31.63	40.59	40.44	37.19	41.88	42.42	42.51
MTCATC	25.88	51.22	25.85	26.50	26.49	26.46	26.67	43.83	52.10	51.20	27.07	26.28	26.22	26.30	43.08	48.95	29.49	37.92
MTCAVC	60.83	58.53	60.85	39.25	38.50	39.43	38.68	38.65	61.33	60.71	38.41	39.30	38.65	38.73	38.75	60.74	45.79	46.20
MTCGAC	43.37	45.09	35.83	42.17	41.81	42.19	41.06	39.75	38.84	35.82	35.82	41.84	35.34	35.82	41.32	42.39	47.75	45.19
MTCGGC	39.97	43.71	31.36	39.19	42.22	29.15	30.91	29.09	39.32	44.21	30.10	42.83	32.04	31.06	43.95	39.33	30.86	29.98
MTCGTC	43.19	43.26	35.22	23.40	24.32	24.44	24.75	40.94	42.97	43.19	23.52	24.22	23.38	29.19	39.04	43.30	26.29	31.59
MTCGVC	47.76	48.40	52.79	51.67	50.20	49.92	48.02	44.43	40.82	47.67	40.80	45.96	43.97	40.82	45.56	54.53	51.75	49.08
MTCTAC	41.66	33.58	44.73	35.24	35.26	43.05	49.34	35.19	35.97	44.10	37.79	37.82	59.86	41.55	37.79	37.72	51.38	47.89
MTCTGC	45.08	44.15	34.68	43.68	46.45	40.47	35.23	41.10	38.78	45.97	34.42	40.81	33.85	33.84	37.77	39.41	43.72	36.66
MTCTTC	55.48	55.40	48.33	53.54	54.42	44.40	43.68	44.24	47.16	44.27	41.81	47.28	43.56	43.54	54.84	44.49	41.81	49.57
MTCTVC	55.70	47.24	56.03	49.85	49.98	47.22	56.04	49.75	43.62	52.97	45.76	45.84	43.89	50.18	45.79	51.07	48.47	48.48

Table A.1 Energy values of the sequences with two Cu(I) ions (Cont'd).

MTCVAC	64.43	66.33	40.70	47.22	52.50	41.61	40.74	45.82	70.51	63.98	46.78	52.11	40.87	41.14	40.95	68.41	51.41	46.93
MTCVGC	53.39	50.22	48.73	50.51	54.16	40.67	42.68	50.47	47.80	53.15	40.37	52.78	44.29	39.67	52.60	53.18	49.65	47.17
MTCVTC	56.84	50.68	34.68	35.41	34.55	34.61	34.39	45.34	53.06	45.39	35.60	34.62	34.60	34.82	34.96	52.69	36.28	41.60
MTCVVC	66.12	66.80	57.11	57.21	47.03	46.09	46.06	46.06	72.40	46.20	58.86	48.55	46.40	46.27	46.20	50.66	62.39	51.28
MVCAAC	44.73	52.46	50.88	38.80	40.25	40.36	40.27	58.91	67.88	65.97	56.30	40.85	44.86	45.22	43.98	56.00	35.67	59.23
MVCAGC	46.34	50.50	62.13	47.38	47.29	48.01	48.29	47.31	49.66	46.35	46.52	46.54	36.39	37.27	46.53	58.36	43.98	42.32
MVCATC	64.12	51.20	64.17	47.88	47.89	51.17	51.79	47.8	51.09	64.25	47.02	46.98	51.02	51.63	46.75	51.11	47.09	47.10
MVCAVC	73.77	48.37	72.13	56.85	52.13	52.71	52.10	56.87	71.14	72.57	52.64	52.71	52.64	52.61	52.63	64.47	47.17	47.37
MVCGAC	49.93	48.71	63.23	44.93	45.20	45.31	63.29	62.23	48.06	53.03	51.93	51.82	51.84	49.89	51.60	48.93	46.69	46.70
MVCGGC	48.90	45.15	49.15	49.42	49.55	48.68	47.90	50.56	44.05	49.21	49.17	115.03	45.71	46.93	48.38	45.16	45.17	45.14
MVCGTC	50.50	55.46	55.54	47.99	40.11	40.52	39.79	47.66	52.38	49.10	53.44	42.61	37.68	33.40	46.44	55.64	54.31	44.74
MVCGVC	59.16	58.54	60.72	58.53	47.73	47.71	59.06	47.75	58.56	60.66	58.53	47.69	47.70	59.01	47.72	62.64	51.23	51.23
MVCTAC	53.59	55.59	60.29	50.90	40.19	40.17	40.18	51.31	50.48	53.64	46.83	36.74	36.05	35.97	35.97	55.24	35.01	59.62
MVCTGC	57.80	46.51	48.70	56.23	55.15	47.86	54.45	58.10	53.33	54.12	53.70	49.69	52.70	60.83	60.84	46.30	43.74	60.95
MVCTTC	53.09	50.04	56.44	32.58	33.88	32.91	32.94	53.26	48.41	57.68	35.65	34.87	34.09	34.10	34.11	54.70	37.18	29.68
MVCTVC	61.35	60.82	48.77	57.79	46.68	49.13	49.14	66.26	62.38	65.12	53.11	51.33	53.26	40.83	61.78	60.84	51.14	49.05
MVCVAC	70.60	57.99	57.42	46.32	46.39	47.23	47.41	47.11	72.11	51.22	59.26	51.21	51.34	48.49	51.08	44.83	44.89	53.60
MVCVGC	65.98	58.65	58.99	54.14	60.02	54.25	63.03	62.14	57.79	66.70	58.54	65.91	58.57	65.88	68.78	59.95	51.02	69.06
MVCVTC	54.87	55.06	66.71	60.96	55.59	61.34	67.05	67.79	53.62	55.13	61.68	53.03	60.11	55.78	55.67	55.14	60.63	54.83
MVCVVC	52.00	60.16	51.99	51.86	52.57	51.12	48.39	51.01	49.56	73.27	51.78	51.71	52.66	51.12	51.07	50.54	58.43	58.37

Table A.1 Energy values of the sequences with two Cu(I) ions (Cont'd).

+ methyl	peptide - 2 Cu(I) complex energy (kcal/mol) part-II																	
sequence	B1237	B1238	B1239	B1245	B1246	B1247	B1248	B1249	B1256	B1257	B1258	B1259	B1267	B1268	B1269	B1278	B1279	B1289
MACAAC	43.29	116.22	50.77	49.51	50.93	57.51	47.55	50.58	55.19	41.05	49.71	52.79	47.29	47.64	54.86	46.64	50.02	47.01
MACAGC	46.83	41.11	44.40	48.30	46.27	48.14	51.88	44.64	44.26	41.17	41.10	44.62	44.26	39.51	44.85	41.07	44.15	40.49
MACATC	45.80	43.04	39.45	45.78	39.35	45.77	43.06	39.40	49.90	42.27	58.04	49.91	49.93	51.37	43.02	49.45	49.90	50.00
MACAVC	45.67	47.77	49.57	41.34	41.30	41.11	41.20	39.50	42.73	40.07	39.19	41.47	39.16	42.75	42.73	42.81	41.29	42.78
MACGAC	42.51	54.01	53.21	49.46	42.39	42.27	38.48	37.34	37.39	37.41	48.45	37.47	41.98	42.21	42.58	40.53	44.77	40.81
MACGGC	47.80	40.08	44.90	43.65	37.90	52.15	37.10	37.88	40.01	45.97	37.13	42.15	52.65	39.44	45.40	47.06	46.05	41.48
MACGTC	33.10	33.55	39.99	46.44	33.07	33.09	33.52	40.00	33.09	33.08	34.00	40.02	38.80	36.88	35.21	39.22	35.27	35.30
MACGVC	48.53	60.42	50.73	59.41	57.29	59.34	56.89	48.34	56.35	48.13	59.61	47.71	61.55	54.88	47.13	60.44	54.35	48.52
MACTAC	38.02	43.12	39.18	39.93	45.72	34.22	34.90	34.02	38.67	38.62	33.50	39.82	31.87	31.89	34.94	36.58	36.38	31.88
MACTGC	39.86	41.33	44.00	43.96	43.86	56.01	47.31	44.01	44.84	49.85	39.88	33.31	39.69	40.03	33.31	49.66	49.09	39.89
MACTTC	27.85	27.46	37.07	35.42	30.86	34.17	29.66	32.88	42.71	26.94	26.94	32.91	29.65	29.64	32.79	33.73	28.60	28.49
MACTVC	41.56	41.49	41.52	63.60	41.99	41.56	41.52	41.58	43.67	46.49	46.58	46.52	49.44	49.14	47.01	49.16	47.02	47.35
MACVAC	44.57	44.52	44.54	45.42	45.58	45.53	44.34	45.47	48.15	46.84	47.32	46.97	48.19	48.51	48.86	46.69	48.85	46.61
MACVGC	52.33	61.87	59.44	52.92	45.90	52.81	55.59	58.23	45.44	51.01	45.40	44.18	45.58	58.29	51.07	59.57	43.96	56.76
MACVTC	49.65	48.98	43.64	48.74	63.18	62.66	47.64	47.45	46.63	50.86	49.65	49.30	50.04	63.51	49.53	63.28	63.12	47.49
MACVVC	50.58	51.27	56.27	59.84	49.54	49.05	115.91	55.01	48.23	47.28	46.22	46.23	46.99	49.63	48.98	49.16	49.72	49.35
MGCAAC	38.61	33.88	40.57	39.22	38.99	38.98	48.76	35.90	40.59	43.05	35.69	40.56	47.22	35.28	40.92	33.97	41.06	35.59
MGCAGC	36.54	36.71	49.31	44.46	47.59	38.25	38.73	44.49	42.14	37.27	40.27	44.64	36.53	36.70	37.53	39.34	44.87	40.24
MGCATC	28.02	34.39	38.73	39.59	40.39	28.80	28.82	38.21	28.59	28.57	27.92	28.47	28.67	27.69	28.74	29.36	29.39	29.28
MGCAVC	50.45	44.46	47.12	48.65	48.57	48.64	54.80	52.19	47.12	48.32	49.30	47.11	47.09	49.04	47.10	44.42	47.11	49.07
MGCGAC	35.01	35.80	38.98	42.75	33.47	38.72	35.76	44.61	36.75	31.29	34.99	42.96	36.72	33.18	33.77	37.81	44.65	35.64
MGCGGC	30.90	35.76	40.43	36.98	40.97	37.77	38.70	38.05	32.06	28.56	31.30	32.00	31.98	41.17	40.77	30.86	31.97	38.66
MGCGTC	45.06	51.93	49.82	45.36	34.91	41.99	37.58	42.37	33.52	41.75	43.58	42.39	36.16	31.33	36.80	43.59	45.97	41.59
MGCGVC	42.92	101.22	42.57	44.96	44.48	49.60	38.53	38.53	49.02	48.64	39.65	42.52	46.54	35.69	36.86	39.64	36.17	40.08
MGCTAC	35.59	32.72	36.52	36.72	36.85	51.07	42.50	36.65	35.67	35.60	36.68	36.33	35.61	32.67	35.70	36.24	35.64	32.69

Table A.1 Energy values of the sequences with two Cu(I) ions (Cont'd).

MGCTGC	45.52	44.90	45.54	44.76	43.46	44.79	37.72	43.43	45.53	34.07	39.14	44.37	45.53	38.10	34.54	39.14	45.54	39.16
MGCTTC	27.10	23.22	49.84	37.01	31.78	27.53	28.50	31.71	30.71	28.76	27.94	31.73	28.56	30.31	24.79	30.05	24.83	25.15
MGCTVC	52.79	41.40	44.45	47.77	44.22	43.48	42.24	45.01	44.20	52.70	40.57	47.38	52.70	40.57	44.46	40.04	44.66	41.32
MGCVAC	51.23	52.95	52.71	52.84	53.89	54.75	55.96	52.43	46.91	46.91	48.46	52.80	52.84	52.65	52.88	51.91	54.29	55.55
MGCVGC	47.28	41.18	44.87	42.67	47.44	44.78	45.85	44.89	44.91	47.76	42.09	45.49	44.75	41.34	47.41	41.10	44.62	41.11
MGCVTC	40.94	40.14	43.54	45.29	45.29	43.61	51.99	45.29	45.64	38.57	40.16	46.01	42.24	40.14	45.78	44.55	42.50	40.12
MGCVVC	58.46	53.07	56.47	58.61	50.51	64.29	55.05	63.97	59.59	56.53	58.19	63.05	56.11	51.35	50.86	58.31	56.54	58.45
MTCAAC	41.09	41.21	35.22	35.10	32.53	36.87	32.83	35.08	31.37	31.14	31.04	35.54	32.96	98.06	35.01	37.94	35.47	35.11
MTCAGC	44.79	37.88	38.16	42.03	42.03	45.13	47.14	42.01	42.07	46.85	38.51	42.39	49.12	39.42	42.42	41.78	37.40	38.68
MTCATC	36.91	35.55	48.87	26.36	32.19	26.43	26.27	43.65	24.50	24.52	27.58	22.06	23.71	26.90	22.04	26.88	22.64	26.47
MTCAVC	42.57	46.12	42.69	38.47	38.46	38.47	38.70	48.03	38.53	36.09	37.03	38.46	37.00	40.09	38.90	40.12	44.04	43.94
MTCGAC	47.09	44.98	39.77	42.94	47.38	42.87	42.50	40.63	45.17	48.91	43.66	39.84	45.19	44.02	37.31	46.80	34.56	41.42
MTCGGC	30.64	31.49	38.85	29.18	45.26	31.03	31.38	28.08	30.80	31.72	28.87	29.47	29.35	32.11	46.13	31.17	28.27	38.71
MTCGTC	25.21	31.29	30.86	24.18	24.35	24.20	24.55	40.99	23.77	20.81	23.54	20.05	21.76	24.50	20.05	24.52	19.67	19.72
MTCGVC	55.99	51.61	41.35	55.91	49.15	50.77	50.38	45.53	49.56	53.84	49.09	49.08	49.08	47.63	45.49	49.96	44.54	39.13
MTCTAC	33.57	42.90	33.33	43.59	43.54	44.82	111.81	44.71	41.18	33.35	35.26	35.94	33.34	35.24	43.46	52.26	33.36	35.27
MTCTGC	36.06	29.43	39.32	32.20	47.14	34.56	35.02	44.60	28.57	29.96	29.60	44.45	32.87	31.55	32.91	34.77	34.68	32.16
MTCTTC	45.67	43.55	53.08	39.58	55.67	38.64	42.76	42.68	54.57	39.65	40.32	42.09	42.64	42.66	54.85	40.30	43.66	40.33
MTCTVC	62.43	47.20	50.35	52.18	50.48	50.89	55.84	52.54	44.74	61.92	49.14	50.25	53.82	49.09	54.45	50.70	54.63	47.26
MTCVAC	45.77	53.22	53.23	46.86	46.93	40.84	40.86	40.88	39.83	38.94	38.89	47.14	38.90	39.32	40.60	44.07	39.87	38.53
MTCVGC	45.84	44.84	49.79	50.16	53.90	41.35	42.18	49.86	46.66	44.95	44.76	50.02	38.70	39.02	37.68	44.09	38.48	38.47
MTCVTC	42.29	41.24	54.28	33.55	33.60	35.29	33.04	30.91	32.43	33.22	35.81	31.65	32.48	35.12	30.96	35.78	30.46	30.43
MTCVVC	51.50	53.65	59.41	57.20	45.74	44.83	46.77	46.78	45.73	43.64	44.54	57.33	44.49	46.60	46.96	45.89	47.13	44.54

Table A.1 Energy values of the sequences with two Cu(I) ions (Cont'd).

MVCAAC	43.37	43.36	49.05	38.80	40.22	40.26	40.27	39.61	39.48	38.91	37.59	51.90	40.02	39.97	39.61	39.92	41.78	39.03
MVCAGC	43.05	45.07	42.99	47.36	47.61	48.20	48.08	47.18	47.44	37.41	37.32	50.87	37.41	37.91	37.37	43.62	37.41	37.37
MVCATC	51.23	45.36	48.93	48.35	47.84	51.13	51.72	46.94	54.69	47.17	46.68	47.09	47.31	39.93	46.66	45.93	41.17	40.06
MVCAVC	52.25	51.67	55.55	52.79	47.24	47.36	47.36	47.27	48.02	45.41	53.13	43.61	45.24	45.71	47.76	50.57	50.15	45.43
MVCGAC	46.69	48.92	45.41	45.00	46.56	43.38	60.81	63.78	45.17	54.95	44.82	45.01	44.46	47.96	45.77	44.84	45.49	124.22
MVCGGC	51.90	36.53	36.51	45.01	44.14	52.88	42.22	36.52	44.17	52.86	42.27	36.52	47.67	42.24	42.25	49.01	49.15	47.68
MVCGTC	44.41	44.52	58.79	50.56	40.51	40.70	40.21	48.18	38.35	38.34	38.44	46.07	41.84	39.84	37.20	43.59	37.23	37.24
MVCGVC	51.22	60.99	51.22	62.64	53.08	53.02	63.14	53.26	51.18	51.20	62.15	51.20	56.78	51.98	56.82	52.00	56.79	50.97
MVCTAC	44.56	44.57	59.63	50.49	44.34	40.21	40.29	40.06	40.04	40.01	39.57	50.98	39.69	40.77	40.52	40.16	40.29	41.25
MVCTGC	43.68	45.11	45.34	45.85	46.51	45.36	48.45	49.07	38.61	54.22	47.85	50.36	38.61	45.09	45.35	48.02	46.86	55.67
MVCTTC	37.17	41.65	38.61	32.50	38.88	32.97	33.00	32.72	34.37	30.08	30.07	39.94	31.79	36.41	37.67	36.02	34.36	37.14
MVCTVC	50.03	50.22	62.74	56.08	46.72	49.19	48.42	66.41	45.61	45.59	45.46	56.18	47.72	48.48	46.85	48.77	49.83	49.46
MVCVAC	50.13	57.77	47.18	57.30	46.87	46.07	44.51	46.33	46.02	45.89	44.48	47.04	44.56	44.45	45.62	47.54	47.80	47.69
MVCVGC	51.01	58.60	59.66	57.15	59.82	53.80	61.81	60.61	58.80	56.82	57.05	53.82	58.87	65.89	59.92	54.19	53.82	62.25
MVCVTC	54.66	56.16	55.89	65.55	55.18	51.88	71.35	68.80	55.28	54.72	68.86	60.19	58.37	56.31	55.72	65.57	61.34	70.81
MVCVVC	58.43	61.20	69.42	49.80	51.87	50.96	52.21	51.90	49.25	48.38	48.41	51.03	48.37	49.25	52.45	52.75	51.34	48.40

Table A.2 : Energy values of the sequences with two Cu(II) ions, the titles of columns show the initial locations of the copper ions before optimization (B-2212 means Cu (II) (the first number, which copper), two ions (in amount, how many, the second number), and where (which locations, the third and the fourth number), part-I and part-II

+ methyl	**peptide - 2 Cu(II) complex energy (kcal/mol) part-I**																	
sequence	B2212	B2213	B2214	B2215	B2216	B2217	B2218	B2219	B2223	B2224	B2225	B2226	B2227	B2228	B2229	B2234	B2235	B2236
MACAAC	26.79	4.52	19.99	14.61	18.32	1.80	39.11	9.99	7.56	24.38	5.13	9.08	5.16	17.97	7.62	13.08	1.79	1.73
MACAGC	21.33	-2.13	55.71	-2.57	-1.27	-2.18	-0.87	7.40	3.32	20.61	-1.91	18.14	-2.10	11.39	15.90	-2.23	-4.71	9.42
MACATC	27.60	27.71	36.18	16.60	17.09	29.06	27.90	12.91	8.19	8.00	16.70	12.97	16.93	20.23	13.09	26.98	11.50	13.01
MACAVC	49.15	12.67	7.52	7.34	7.69	7.51	7.94	16.45	44.89	60.91	18.78	16.19	15.46	15.49	20.42	25.99	13.17	12.60
MACGAC	17.73	27.87	40.18	16.76	12.06	11.81	-2.93	11.86	6.34	29.76	3.09	1.12	1.02	1.08	1.08	-2.54	4.68	-2.99
MACGGC	11.90	10.33	30.26	11.55	34.88	6.12	13.40	25.64	11.50	10.33	23.60	22.28	0.80	15.10	24.80	10.47	10.38	10.51
MACGTC	30.16	5.07	24.84	20.56	10.00	-9.90	30.96	31.34	5.77	20.58	20.55	-8.99	-8.16	4.53	30.74	20.33	5.37	-8.08
MACGVC	25.30	41.86	21.93	41.92	41.06	18.02	28.76	17.87	3.48	26.22	25.03	36.99	3.17	29.53	25.70	10.11	15.01	26.64
MACTAC	30.92	12.61	56.39	11.82	-2.44	1.11	0.70	-0.35	21.81	30.50	-0.20	1.18	1.03	-0.05	0.87	27.66	1.80	11.97
MACTGC	30.82	4.27	54.76	4.29	4.18	4.11	6.21	4.05	-3.32	15.84	-3.32	-0.47	-3.32	24.99	9.51	4.44	-5.91	-5.91
MACTTC	35.72	12.52	-5.17	-5.65	-5.34	-4.68	-5.24	24.83	8.99	35.80	-11.18	-7.65	-7.75	-4.11	4.78	12.42	6.16	-7.06
MACTVC	40.97	39.38	44.30	42.58	21.68	21.20	21.06	21.04	39.72	39.54	40.03	10.78	8.98	9.05	10.32	44.82	37.25	16.70
MACVAC	54.04	41.66	52.90	20.54	20.43	20.65	16.51	20.65	25.60	41.73	19.86	19.85	19.86	54.02	19.88	54.15	19.90	19.89
MACVGC	5.52	6.58	49.10	6.56	6.63	6.38	24.89	45.47	6.57	3.24	4.11	6.57	4.15	19.97	6.32	6.70	1.81	6.71
MACVTC	16.88	29.04	16.82	29.04	1.33	11.29	26.44	15.02	29.05	26.50	24.71	21.81	8.93	26.42	20.87	17.53	19.13	8.28
MACVVC	62.21	31.61	69.87	14.96	15.45	14.53	14.93	15.34	31.69	60.60	14.66	15.50	14.77	14.70	14.26	31.67	31.80	15.41
MGCAAC	15.57	7.22	58.85	4.90	4.94	4.87	25.84	24.12	2.45	15.86	2.41	2.48	-0.37	21.33	5.37	7.18	6.66	6.73

Table A.2 Energy values of the sequences with two Cu(II) ions (Cont'd).

MGCAGC	37.80	17.10	52.76	7.06	17.22	7.46	19.89	6.93	7.74	37.44	18.40	26.46	6.14	10.16	18.40	7.62	-2.60	16.15
MGCATC	54.71	17.11	19.43	-0.11	-4.02	-8.22	-0.52	-3.47	17.59	22.41	-12.97	-2.00	-12.27	-2.44	22.32	17.14	2.55	-10.07
MGCAVC	42.75	14.39	42.61	16.88	23.71	16.81	43.22	26.08	12.28	42.69	11.00	25.17	14.35	37.46	19.60	14.42	10.71	17.21
MGCGAC	17.53	11.40	16.30	9.52	11.56	11.59	17.82	12.51	5.49	13.43	14.77	5.50	14.73	22.94	32.29	13.22	6.43	9.52
MGCGGC	8.37	11.84	28.48	-0.35	7.18	5.92	15.56	22.30	-8.22	8.23	-12.81	-12.76	-7.74	4.03	2.21	6.48	-2.59	-2.18
MGCGTC	25.84	7.66	31.78	-5.90	5.94	21.87	25.93	15.79	5.24	23.64	3.57	5.63	16.14	17.59	15.67	7.68	-2.54	0.92
MGCGVC	28.54	9.55	15.79	9.42	8.86	4.67	31.85	24.48	9.47	15.72	7.00	8.87	7.81	31.59	31.49	16.61	3.55	4.53
MGCTAC	3.91	6.06	27.78	6.08	6.23	6.53	20.36	-7.38	-3.83	29.82	-3.82	-3.80	-11.37	17.11	-3.79	7.29	-3.45	-2.44
MGCTGC	30.99	6.85	47.76	9.65	9.65	14.29	24.33	9.19	5.05	32.73	9.68	5.06	4.08	24.29	5.36	5.52	-3.90	10.00
MGCTTC	13.89	13.55	-1.87	6.13	7.71	-7.20	-7.94	11.54	23.31	13.19	-13.86	-13.36	-14.52	-9.55	15.24	16.62	-14.73	-13.65
MGCTVC	21.37	14.88	14.52	14.77	14.77	17.60	39.57	14.89	5.47	23.81	10.54	19.89	18.81	32.21	7.15	14.68	13.61	17.62
MGCVAC	36.78	10.68	36.61	36.68	22.70	34.59	36.91	36.91	18.16	36.73	17.33	34.12	34.12	33.89	36.71	12.17	18.27	10.56
MGCVGC	24.54	1.10	56.19	20.52	21.91	1.29	33.70	20.34	1.52	22.47	0.92	20.64	0.96	24.01	40.88	1.47	4.43	1.08
MGCVTC	41.73	21.29	48.39	16.80	20.64	0.60	0.34	16.70	6.61	41.60	8.98	8.96	-4.47	21.43	8.70	9.75	0.71	0.63
MGCVVC	35.08	28.07	52.74	37.57	28.32	32.03	35.41	23.92	24.46	36.11	28.17	24.38	25.43	38.06	14.87	30.51	22.13	27.70
MTCAAC	-2.25	17.97	4.06	-3.28	-0.18	-2.69	0.20	0.14	26.99	-3.67	-1.29	-1.51	-1.47	2.64	38.54	16.65	4.85	2.58
MTCAGC	36.99	13.48	32.55	13.23	12.87	4.26	10.74	13.14	5.49	9.82	5.45	5.38	-3.66	24.11	5.10	13.39	1.84	2.94
MTCATC	9.55	9.87	-9.65	-9.43	-9.43	-9.17	-9.11	2.50	8.71	13.63	-5.03	2.07	1.87	-6.10	19.90	8.80	-10.46	-11.52
MTCAVC	54.85	22.81	61.07	17.54	5.06	4.26	4.65	14.49	21.74	37.79	26.31	5.41	8.88	8.85	11.54	22.51	20.21	3.42
MTCGAC	15.59	8.49	36.89	11.19	16.08	8.49	9.88	9.37	9.80	22.67	22.61	29.25	9.78	7.41	17.67	8.83	7.25	17.16
MTCGGC	11.49	2.48	-2.57	6.56	12.05	10.63	10.52	6.63	-1.08	7.04	-2.99	-1.82	-9.80	-9.76	10.03	2.86	0.27	8.20
MTCGTC	7.89	12.80	-12.01	-12.14	-11.27	-11.24	-11.51	21.26	6.09	7.73	-10.66	-15.10	-15.15	-13.68	-14.05	-1.70	-11.74	-15.53
MTCGVC	27.28	17.87	26.29	23.92	19.92	14.77	26.54	23.33	15.05	27.32	8.94	32.95	13.01	12.10	21.71	15.11	12.73	22.76
MTCTAC	29.52	-4.23	27.49	-4.24	-4.25	7.65	26.67	1.41	-10.29	21.75	-10.39	-0.34	-5.84	29.36	0.22	4.81	15.40	5.70
MTCTGC	11.92	3.44	13.82	4.18	6.64	-12.74	-12.46	-1.57	-2.29	3.43	-11.32	2.21	-15.65	-17.49	2.71	1.68	2.75	-0.61
MTCTTC	22.05	24.60	47.34	43.20	22.36	14.43	23.10	4.83	23.81	22.09	24.22	2.88	-2.24	-2.41	22.63	22.04	22.33	7.76
MTCTVC	30.73	13.92	40.15	18.34	18.29	13.90	19.72	19.13	9.72	31.28	9.80	14.82	7.84	36.83	9.74	14.18	14.11	14.00
MTCVAC	58.26	41.28	5.03	5.15	5.22	6.53	6.14	4.74	23.78	41.33	3.76	3.82	6.37	11.46	9.79	41.21	11.98	5.36

Table A.2 Energy values of the sequences with two Cu(II) ions (Cont'd).

MTCVGC	6.58	10.02	0.61	11.92	11.57	21.94	21.96	21.16	2.44	16.32	11.09	18.12	-0.42	1.28	11.55	7.05	6.40	14.31
MTCVTC	20.56	15.38	-1.73	0.29	-1.37	-1.47	-1.70	32.25	15.59	24.17	5.07	4.47	4.42	3.84	30.31	15.41	-1.67	-2.47
MTCVVC	54.94	45.12	11.26	11.36	11.42	10.92	11.13	54.28	33.35	11.15	9.66	11.76	11.20	11.19	60.89	46.29	13.43	18.15
MVCAAC	61.43	25.33	61.54	9.11	5.17	4.35	4.88	4.56	35.45	20.41	33.00	16.97	15.27	17.82	2.70	40.44	26.79	9.16
MVCAGC	47.69	14.73	22.08	22.12	26.50	7.57	29.09	14.56	13.68	47.97	21.14	23.64	8.46	21.71	23.67	14.76	13.12	13.09
MVCATC	14.13	13.45	23.59	18.43	15.72	13.13	-0.73	16.31	12.87	25.52	16.86	23.76	13.07	34.90	16.27	13.31	-2.50	-2.52
MVCAVC	53.60	36.42	66.38	18.48	18.52	20.02	21.00	18.46	38.14	66.42	18.42	21.87	20.85	20.78	20.87	16.20	20.90	15.87
MVCGAC	27.06	16.33	50.57	8.32	8.30	8.37	63.55	61.10	33.24	32.60	16.94	16.96	16.90	26.73	27.34	18.53	16.33	14.24
MVCGGC	26.13	26.68	34.24	30.88	34.30	-2.54	40.37	39.07	3.60	7.61	7.46	6.88	16.76	8.81	8.74	26.75	29.28	30.66
MVCGTC	24.34	7.37	55.81	24.33	13.14	13.20	12.44	24.42	14.85	24.17	32.19	5.35	-4.70	24.09	28.56	26.34	14.80	-1.77
MVCGVC	44.07	40.09	52.63	40.03	26.11	26.15	84.12	26.14	40.04	52.00	40.05	26.00	26.00	17.25	26.03	49.29	34.57	34.49
MVCTAC	45.43	18.27	60.18	23.26	7.48	6.48	7.97	6.33	18.66	42.09	20.61	14.34	13.75	13.82	1.00	17.43	20.74	-1.00
MVCTGC	18.01	15.17	36.18	22.21	17.98	20.77	26.67	39.56	17.74	15.25	4.55	21.16	17.09	15.27	15.23	15.28	4.56	17.46
MVCTTC	-0.19	14.79	56.58	13.08	-0.76	-1.53	0.90	5.56	-8.21	17.67	18.97	0.93	-5.07	13.78	27.39	18.85	-9.01	-6.00
MVCTVC	46.53	36.62	69.97	21.25	13.43	11.84	12.00	55.26	34.22	46.71	22.73	4.42	4.98	4.89	52.08	36.70	19.56	1.23
MVCVAC	62.70	30.15	11.82	10.49	12.50	11.96	12.43	21.39	16.87	22.08	11.81	18.32	17.66	17.67	21.77	34.08	15.75	13.24
MVCVGC	24.47	24.36	29.37	29.56	35.00	29.49	64.65	29.48	41.73	24.35	20.65	14.56	27.41	24.40	35.03	35.05	20.65	49.64
MVCVTC	36.66	36.73	19.52	7.31	33.48	26.16	60.91	12.05	9.60	35.30	6.54	14.18	6.53	37.44	33.67	19.29	19.62	14.51
MVCVVC	15.69	59.02	16.77	16.01	15.06	16.67	16.76	34.62	43.22	73.84	15.80	16.49	15.38	15.41	26.32	44.46	36.36	24.47

Table A.2 Energy values of the sequences with two Cu(II) ions (Cont'd).

+ methyl	peptide - 2 Cu(II) complex energy (kcal/mol) part-II																	
sequence	B2237	B2238	B2239	B2245	B2246	B2247	B2248	B2249	B2256	B2257	B2258	B2259	B2267	B2268	B2269	B2278	B2279	B2289
MACAAC	13.76	16.71	16.30	12.51	12.66	2.08	21.56	12.61	28.43	4.19	15.08	16.56	18.68	21.00	9.73	37.61	9.97	15.90
MACAGC	-4.76	7.15	4.34	-1.84	-1.87	-2.22	38.67	44.09	12.39	-1.16	8.79	-0.79	12.38	8.77	-1.87	-1.85	-0.77	21.87
MACATC	16.84	27.57	12.98	12.55	13.04	16.68	20.21	12.97	33.55	6.45	25.49	1.95	6.30	2.00	27.35	34.64	27.31	27.32
MACAVC	18.74	16.22	12.56	25.57	7.78	7.42	7.55	19.25	15.37	18.38	17.72	8.08	17.74	7.51	17.31	28.06	17.50	17.15
MACGAC	-2.99	-3.33	18.61	30.81	11.71	12.05	11.69	12.00	5.51	5.43	1.79	1.02	10.28	17.23	11.69	7.89	8.50	7.94
MACGGC	14.68	17.40	10.41	27.18	18.03	30.26	30.31	23.60	22.29	1.15	13.94	24.84	24.34	27.42	22.50	17.39	8.19	25.49
MACGTC	-8.11	-11.16	19.11	26.27	2.34	9.84	2.96	17.52	8.18	8.14	27.03	17.78	9.91	10.66	13.62	10.74	14.38	14.39
MACGVC	14.57	44.59	15.03	14.84	12.67	26.52	13.71	26.20	45.32	6.78	44.61	24.50	26.47	13.39	23.91	44.62	15.05	17.79
MACTAC	-6.28	-7.03	-4.65	-0.23	-0.24	1.17	1.22	0.75	1.98	12.67	0.32	0.38	13.23	0.63	1.25	15.55	16.04	12.93
MACTGC	5.28	-8.77	29.91	4.38	5.93	0.14	22.79	4.34	4.30	-4.33	-2.85	-6.05	-0.78	-4.02	25.80	4.72	0.19	19.38
MACTTC	-7.09	-7.65	13.12	-7.26	12.52	16.20	9.18	-6.84	4.56	12.48	-5.79	-4.33	9.09	7.64	-9.88	17.77	-10.44	-7.22
MACTVC	10.15	9.95	7.99	39.44	13.27	13.27	13.23	10.62	10.79	10.24	13.87	10.34	8.58	21.23	21.12	36.67	21.31	21.25
MACVAC	19.89	54.08	19.88	18.91	18.93	18.92	53.09	18.94	12.06	12.44	20.51	20.40	32.61	20.57	20.42	20.56	20.44	25.84
MACVGC	6.90	6.76	7.92	6.55	6.68	6.51	47.87	49.04	3.15	14.32	6.57	6.36	3.27	6.71	6.53	6.56	6.44	117.89
MACVTC	2.62	16.50	21.16	13.75	32.94	20.83	24.78	13.91	16.71	2.27	20.75	14.73	13.66	47.77	50.07	6.57	28.74	46.51
MACVVC	14.66	12.28	50.59	14.64	15.21	14.49	14.94	21.39	24.71	30.74	19.70	15.36	24.82	15.15	24.15	33.39	23.29	6.61
MGCAAC	3.89	3.80	7.12	8.73	6.11	4.98	-6.60	32.33	7.00	4.45	3.87	4.85	1.81	3.80	7.07	22.45	5.39	24.00
MGCAGC	-2.70	-0.70	9.91	7.53	17.55	19.87	19.94	6.75	6.83	-2.71	-2.72	18.43	7.24	7.07	12.40	6.12	7.38	6.15
MGCATC	-7.41	-9.96	8.18	0.45	-4.07	-3.96	-4.60	18.62	4.59	5.35	0.26	11.21	5.64	-4.49	-4.35	-4.67	-3.79	-4.39
MGCAVC	12.04	18.15	17.15	14.37	30.94	16.83	34.48	26.31	17.19	10.73	17.14	17.14	17.15	16.33	17.14	17.16	17.14	31.56
MGCGAC	11.38	3.63	-7.88	9.49	11.63	11.54	11.80	11.11	-5.60	18.60	17.48	11.45	5.87	8.11	11.17	3.64	11.48	15.17
MGCGGC	-2.50	9.25	-5.09	-0.62	-2.64	9.48	18.83	16.85	0.57	1.40	3.24	2.21	-3.49	0.06	6.77	4.18	1.97	28.51
MGCGTC	3.42	-2.03	27.13	-2.09	9.41	11.55	23.65	27.34	-2.49	5.95	25.69	6.48	6.91	4.93	13.95	12.06	14.18	31.75
MGCGVC	3.84	9.23	10.15	13.20	27.27	10.20	12.95	37.01	-2.15	20.06	16.57	11.26	-1.58	9.61	25.01	17.46	10.66	18.56

Table A.2 Energy values of the sequences with two Cu(II) ions (Cont'd).

MGCTAC	-11.84	-2.63	4.39	7.17	7.34	6.08	20.41	7.34	4.27	-1.13	6.15	2.97	5.08	6.10	4.38	8.56	-11.88	3.00
MGCTGC	21.68	11.72	10.10	9.60	6.48	1.08	23.07	7.99	9.67	-11.91	12.42	9.72	14.41	11.68	10.19	4.98	13.72	9.53
MGCTTC	-16.79	-14.17	13.93	0.44	-6.48	-8.67	6.47	-0.21	-1.01	-12.55	-13.15	6.22	4.49	10.96	-8.82	-7.40	-8.15	-8.85
MGCTVC	13.06	14.07	13.58	14.46	14.36	9.62	28.59	14.22	13.92	14.20	14.12	34.63	14.96	14.08	34.55	13.58	12.79	15.75
MGCVAC	10.93	10.47	14.05	12.47	10.59	34.73	36.77	36.69	34.12	34.59	36.87	19.66	13.30	14.05	10.66	46.37	45.66	36.40
MGCVGC	1.39	11.67	1.08	1.33	21.29	1.31	33.09	20.37	0.96	23.14	11.56	0.92	1.05	18.15	17.08	11.63	1.05	27.58
MGCVTC	4.37	11.54	0.66	16.69	20.71	-0.51	-2.52	6.62	6.74	14.52	17.02	0.74	6.21	17.14	0.51	15.24	2.66	11.89
MGCVVC	22.17	30.44	27.89	33.01	28.22	25.31	40.00	50.38	27.78	22.19	33.93	30.54	27.71	32.44	28.21	39.05	30.70	30.25
MTCAAC	2.09	-0.03	17.61	15.31	-2.37	15.09	7.96	-2.29	7.90	9.02	-6.09	13.26	9.09	10.44	-2.88	20.13	0.04	64.24
MTCAGC	-5.40	11.14	-7.73	13.15	13.45	21.06	25.60	13.17	29.84	-5.45	12.41	2.68	-5.51	8.35	21.32	7.74	4.95	8.46
MTCATC	-11.52	-11.40	13.92	-8.90	-9.28	0.51	-1.26	24.19	-9.09	-8.96	-9.00	-13.40	-9.16	-9.26	-13.38	-9.30	-13.37	-12.85
MTCAVC	14.35	6.22	3.03	15.45	4.35	4.32	3.65	4.48	16.46	14.98	14.38	4.88	14.38	4.01	15.28	4.22	15.31	15.21
MTCGAC	14.74	16.33	8.50	8.78	14.66	8.81	27.77	18.32	21.41	8.34	27.82	18.31	14.99	2.86	8.42	16.25	9.77	7.44
MTCGGC	-2.15	-2.16	-2.03	4.38	11.70	10.53	10.67	-2.94	-3.03	-0.22	-0.18	-1.95	-9.87	-9.70	9.26	3.71	9.64	2.74
MTCGTC	-15.55	-14.81	9.39	-12.32	-11.27	-11.32	-11.32	21.16	-12.28	-12.32	-12.36	-17.05	-12.52	-11.14	-13.53	-11.91	-17.04	-16.38
MTCGVC	13.78	18.53	6.63	17.89	42.77	15.07	29.72	23.26	19.93	18.08	29.71	23.29	22.14	26.97	23.33	18.54	15.03	31.16
MTCTAC	7.77	-1.48	-3.51	4.81	5.03	6.93	26.96	-1.05	15.98	6.95	-4.26	0.09	22.30	-4.20	16.38	7.56	27.18	-4.24
MTCTGC	-13.01	-1.42	-7.11	0.86	2.96	14.18	14.27	-2.60	-8.25	-13.06	-1.35	-5.17	-11.32	-14.37	13.41	-14.13	16.05	9.95
MTCTTC	7.75	-2.41	23.80	33.21	22.10	15.18	19.36	15.30	22.86	16.99	21.26	2.43	5.11	2.94	22.65	20.02	8.23	20.15
MTCTVC	13.05	14.44	9.00	15.48	15.15	14.94	40.16	15.24	14.28	11.66	17.65	14.30	14.50	20.62	15.32	15.45	14.67	15.07
MTCVAC	6.29	6.24	4.68	5.45	5.76	6.54	5.15	6.15	14.19	17.73	5.12	5.20	17.08	5.82	6.69	22.53	5.55	42.11
MTCVGC	4.32	3.58	8.69	12.53	20.05	26.03	-3.56	3.62	6.08	4.26	6.24	-7.07	-0.46	-0.49	16.55	14.41	15.45	14.88
MTCVTC	-1.78	-4.61	21.20	-0.67	12.24	12.22	5.76	0.97	-0.35	0.23	0.12	-2.11	7.63	-1.91	-2.85	19.04	-2.85	1.81

Table A.2 Energy values of the sequences with two Cu(II) ions (Cont'd).

MTCVVC	14.04	18.43	45.03	20.77	11.33	11.12	24.56	4.85	24.12	25.22	24.54	14.74	24.56	11.35	15.01	10.84	16.23	16.26
MVCAAC	15.88	15.84	23.25	15.61	5.18	4.44	4.76	15.71	16.63	16.67	16.07	4.07	16.01	5.29	3.74	23.33	14.02	14.04
MVCAGC	10.69	10.56	13.17	21.93	21.88	13.04	29.04	26.58	21.99	9.49	9.51	7.85	9.48	9.57	9.49	4.65	9.48	9.46
MVCATC	13.79	7.83	-4.46	16.73	13.71	13.26	34.98	18.49	30.56	6.01	16.29	13.63	6.43	-4.17	-2.47	7.38	6.49	17.88
MVCAVC	16.22	15.57	15.69	11.94	12.48	12.60	12.54	12.49	23.74	24.46	13.24	11.99	23.88	12.72	12.54	28.90	12.61	47.42
MVCGAC	14.55	16.36	16.27	8.76	8.57	8.31	50.54	3.06	13.01	3.17	3.21	27.61	19.12	8.27	8.24	8.32	11.22	41.44
MVCGGC	-0.56	13.31	13.27	26.57	39.17	2.33	18.95	13.33	30.35	13.12	24.43	13.27	-2.51	4.07	3.99	-1.86	-2.01	33.04
MVCGTC	0.11	-1.52	26.19	24.20	12.86	12.24	13.56	24.37	5.33	5.43	-5.36	28.56	7.09	12.28	15.24	14.02	15.16	6.14
MVCGVC	34.54	55.71	34.55	46.89	35.07	35.10	47.51	35.09	20.21	34.52	55.77	31.02	24.95	37.19	13.99	37.16	33.16	37.34
MVCTAC	7.50	5.85	31.19	21.86	7.61	6.50	6.52	5.84	22.24	19.83	86.23	6.16	19.25	6.53	17.48	25.25	18.16	18.15
MVCTGC	4.53	7.84	20.59	22.34	21.23	20.95	34.67	30.38	4.53	19.77	22.21	31.22	4.52	28.17	21.40	26.09	25.61	47.69
MVCTTC	-6.87	-3.25	-6.89	13.06	-0.75	-1.52	-1.25	19.35	2.89	-7.00	10.22	-3.21	12.81	-3.80	-2.14	17.42	8.63	8.75
MVCTVC	8.93	16.40	14.94	22.08	13.56	11.92	12.22	54.97	19.80	20.13	19.88	27.83	21.60	11.11	13.40	11.92	20.18	20.70
MVCVAC	13.30	13.09	15.24	29.84	12.73	24.33	23.34	23.25	13.50	23.93	23.26	11.45	23.29	23.29	12.02	31.56	21.28	21.19
MVCVGC	20.65	34.09	34.60	28.67	35.08	19.82	35.76	53.19	20.66	27.55	29.53	29.10	20.65	34.81	34.61	29.55	29.37	59.71
MVCVTC	7.38	39.23	33.71	12.53	35.13	12.30	40.43	73.24	24.38	29.68	25.83	9.58	26.89	32.68	9.76	8.99	12.31	56.21
MVCVVC	18.08	18.05	51.10	33.87	17.10	16.54	14.24	20.34	27.39	26.76	26.78	16.93	27.40	16.08	16.99	37.17	28.99	21.30

CURRICULUM VITAE

Candidate's full name: Ergi TERZİOĞLU

Place and date of birth: Marktredwitz (Germany), 15 September 1980

Permanent Address: THY Genel Yönetim Binası, Ekip Planlama Müdürlüğü
Yeşilköy, İSTANBUL

University and Colleges attended: Istanbul Technical University, B.S., Industrial Engineering, 2003.