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IN SILICO MOLECULAR DOCKING ANALYSIS TO PREDICT THE ROLE OF METAL IONS IN THE FUNCTION OF DRUG TARGETED PROTEINS

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ABSTRACT

Metal ions are required for many critical functions in living systems. Scarcity of some metal ions can lead to disease. A characteristic of metals is that they easily lose electrons from the familiar elemental or metallic state to form positively charged ions which tend to be soluble in biological fluids. Role of zinc, calcium and copper ions in the catalytic mechanism of drug targeted proteins such as Farnesyltransferase, Neuraminidase and Thioredoxin are analyzed using molecular docking, respectively. The docking results show that inhibitors have low Binding, docking and internal energies with proteins in the presence of Zn, Ca and Cu metal ions. However, in the absence of Zn, Ca and Cu metal ions these energies are increases. Metal plays a critical role in function of these proteins. Our results suggested that metal plays a particular role in the binding of the inhibitor with protein as well as structural stability and catalysis.

KEYWORDS: Farnesyltransferase; Metlloproteins; AutoDock; Neuraminidase;Thioredoxin; Metal ions.

1. INTRODUCTION

The importance of metal ions in biological system is increasingly drawing attention as indicated by the recent emergence of terms such as metallic and metallic. Approximately one third of all of structurally-determined proteins are metal-bound,[1] and large percentages of metals present in the human body are bound to proteins.[2, 3] This emphasizes the crucial role of metal ions in stabilizing protein structure.[4] Most of metlloproteins play a key role in biological process, and therefore have been considered to be a promising target for drug discovery. The metal ion in the active site can participate in the enzymatic reaction as a receptor for the lone pair electrons, which has an effect of weakening the chemical bonds in the substrate. Due to such an essential role in enzymatic reaction, a chemical group that can bind to the central metal ion is necessary in the molecular structure of a small-molecule inhibitor that can regulate the activity of metalloproteins. This has made it much more difficult to design an inhibitor of a metalloprotein than that of a protein without a metal cofactor. Despite such a difficulty, a number of chemical groups that chelate and inactivate a metal ion at the active site of metalloproteins have been reported, including carboxylate, passport, hydroxamate, -keto acid, and diol moieties.

Metal ions are required for many critical functions in living systems. Scarcity of some metal ions can lead to disease. A characteristic of metals is that they easily lose electrons from the familiar elemental or metallic state to form positively charged ions which tend to be soluble in biological fluids. It is in this cationic form that metals play their role in biology whereas metal ions are electron deficient, most biological molecules such as proteins and DNA are electron rich. The attraction of these opposing charges leads to a general tendency for metal ions bind to and interact with biological molecules.[5] Well-known examples include pernicious anemia resulting from iron deficiency, growth retardation arising from insufficient dietary zinc, and heart disease in infants owing to copper deficiency. The ability to recognize, to understand at the molecular level, and to treat diseases caused by inadequate metal-ion function constitutes an important aspect of medicinal bioinorganic chemistry.

Metals perform a wide variety of tasks such as carrying oxygen throughout the body and shuttling electrons. Haemoglobin, an iron-containing protein that binds to oxygen through its iron atom, carries this vital molecule to body tissues. Metal ions such as zinc provide the structural framework for the zinc fingers that regulate the function of genes in the nuclei of cells. Similarly, calcium-containing minerals are the basis of bones, the structural framework

of the human body. Zinc is a natural component of insulin, a substance crucial to the regulation of sugar metabolism. Metals such as copper, zinc, iron and manganese are incorporated into catalytic proteins the metalloenzymes-which facilitate a multitude of chemical reactions needed for life.[5]

In addition to the metalloantibiotics, a number of drugs and potential pharmaceutical agents also contain metal-binding or metal-recognition sites, which can bind or interact with metal ions and potentially influence their bioactivities and might also cause damages on their target biomolecules. For instance: Anti-inflamatory drugs, such as aspirin and its metabolite salicylglycine, ibuprofen, the indole derivative indomethacin, bioflavonoid rutin, diclofenac, suprofen are known to bind metal ions and affect their antioxidant and anti-inflammatory activities.

Farnesyltransferase (FTase) is a zinc metalloenzyme that catalyzes the addition of isoprenoid farnesyl, from farnesyl diphosphate (FPP), to a cysteine residue of a protein substrate containing a C-terminal, CAAX motif, in which C is the cysteine that is farnesylated, A is an aliphatic amino acid, and X is the carboxyl terminal residue.[6] Fates play crucial roles in cell growth regulation, signal transduction and proliferation. It has been recognized as a potential target for anticancer therapeutics.

The Swine flu is an infectious disease of swine and human, caused by influenza A virus subtype H1N1.[7] The World Health Organization figures show that worldwide more than 214 countries have reported laboratory confirmed cases of H1N1, including over 18449 deaths (WHO, 2010). Swine influenza A virus belong to the viral family of Orthomyxoviridae. They are RNA viruses with a segmented genome that is comprised of eight negative-sense, single-stranded RNA segments. These eight segments encode eleven proteins in which two are surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA). Hemagglutinin has 16 subtypes (H1, H2, H3, . . ., H16) and neuraminidase has 9 subtypes (N1, N2, N3, . . ., N9) and this novel virus consists of subtype H1 and N1.[8, 9] NA is responsible for cleaving the terminal sialic acid moieties from the receptors to facilitate the illusion of the progeny virions from the infected cell. The cleavage will facilitate the virus to release and form sites of infection in the respiratory tract. Because of its essential role in influenza virus replication and its highly conserved active sites, NA has become the main target for drug design against influenza virus.

Thioredoxin (TRX), a small redox-active multifunctional protein, acts as a potent antioxidant and a redox regulator in signal transduction. TRX expression is elevated in various types of human cancer.

2. MATERIALS AND METHODS

Role of zinc, calcium and copper ions in the function of drug targeted proteins such as Farnesyltransferase, Neuraminidase and Thioredoxin, respectively are analyzing using molecular docking.

2.1 Receptor x-ray structures

The Protein Data Bank (PDB) is a repository for the 3D structural data of large biological molecules, such as proteins and nucleic acids. The data typically obtained by X-ray crystallography or NMR spectroscopy can be accessed at http://www.rcsb.org. The 3D coordinates of Farnesyletransferase, Neuraminidase and Thioredoxin structure with bound ligands were retrieved from PDB database with codes 1X81, 3B7E and 2TRX, respectively and taken as the receptor model in flexible docking program.

Before docking all heteroatoms and water molecules were removed from .pdb file of protein structure by charge method AMI-BCC using chimera. After removing the water molecule, hydrogen atom was added to protein for correct ionization and tautomeric states of amino acid residues such as Asp, Ser, Glu, Arg and His.

2.2 Active site analysis

The active site residues of Farnesyletransferase, Neuraminidase and Thioredoxin were taken from the PDBSUM entries of 1X81, 3B7E and 2TRX. The binding site residue in Farnesyletransferase is ASP359, LEU96, TYR361, TRP106 and TRP102. Neuraminidase has TRY178,GLU227,ASP151,GLU119,ARG371,ILE222,ARG152,GLU277,TRY406,ARG118, ARG292,ASN294,GLU276,SER276 and ASN294 residues. And Thioredoxin has binding site residues LEU94, ALA93, ILE38 and MET37.

2.3 Inhibitors Dataset

The information regarding the experimentally known inhibitors of Farnesyltransferase protein such as Azacitidine, Gemcitabine, Tipifarnib and Toremifene and for Thioredoxin such as Granisetron and Nilutamide was obtained from the Drugbank database. The anti-flu drugs oseltamivir and zanamivir against Neuraminidase were obtained from the literature.[11] The

3D structures of known inhibitors were downloaded from the PubChem compound database. List of experimentally known inhibitors is shown in Table 1.

2.4 Molecular docking

Docking of known inhibitors screened from literature against Farnesyltransferase, Neuraminidase and Thioredoxin structures were done using molecular docking program AutoDock 3.0.5. Gasteiger charges are added to the ligand and maximum 6 numbers of active torsions are given to the lead compounds using AutoDock tool.[10] Kollman charges and the solvation term were then added to the protein structure using the same. We have made the grid and adjusted the number of points in X, Y, Z-axis so that the entire active site of the proteins is covered. The default value is 0.375 Å between grid points, which is about a quarter of the length of a carbon-carbon single bond. The spacing between grid points can be adjusted with another thumbwheel. Grid spacing values of up to 1.0 Å can be used when a large volume is to be investigated. The Lamarckian genetic algorithm implemented in Autodock was used. Docking parameters were as follows: 30 docking trials, population size of 150, maximum number of energy evaluation ranges of 25,0000, maximum number of generations is 27,000, mutation rate of 0.02, crossover rate of 0.8, Other docking parameters were set to the software's default values. After docking, the ligands were ranked according to their docked energy as implemented in the AutoDock program.

3. RESULTS AND DISCUSSION

The medicinal uses and applications of metals and metal complexes are of increasing clinical and commercial importance. The constituents of proteins and nucleic acids offer excellent ligands for binding to a metal ion. The pharmaceutical use of metal complexes therefore has excellent potential. Designing ligands that will interact with free or protein-bound metal ions are also a recent focus of medicinal inorganic research.

3.1 Molecular docking studies of farnesyltransferase

Docking studies predicted the interaction of ligands with protein and residues involved in this complex. In such interaction studies, the most important requirement was the proper orientation and conformation of ligand which fitted to the enzyme binding site appropriately and formed protein-ligand complex. Therefore, optimal interactions and the best autodock score were used as criteria to interpret the best conformation among the 30 conformations, generated by AutoDock program. The docking results of Azacitidine, Gemcitabine, Tipifarnib and Toremifene inhibitors with farnesyltransferase are shown in table 5.1. The

results show that Azacitidine, Gemcitabine, Tipifarnib and Toremifene inhibitors have low binding, docking and internal energies with farnesyltransferase in presence of Zinc metal ion. However, in the absence of Zn metal ion these energies are increasing. This shows that Zinc plays a biological role in the binding of inhibitor at the catalytic site of farnesyltransferase protein.

A close view of the binding interactions of farnesyltransferase with Azacitidine, Gemcitabine, Tipifarnib and Toremifene inhibitors were analysed through PyMol viewer with and without Zinc metal ion are shown below in Figure 1(a-b) to 4(a-b) respectively. Ligand is coloured in red (in stick drawing) where as amino acids involved in hydrogen bonds color by atom. In docking complex between Farnesyltransferase and inhibitor Azacitidine with Zinc, One H-bond is formed between amino acid TYR361 (O) and Azacitidine (H) with bond length 2.15Å (fig. 1a). In case of without Zinc, One H-bond is formed between amino acid TYR361 (O) and Azacitidine (H) with bond length 1.94Å and 5.90 Å (fig.1b).

In docking complex between Farnesyltransferase and inhibitor Gemcitabine with Zinc, three H-bond is formed between amino acid TYR361 (O) and Gemcitabine (H) with bond length 3.11 Å, 2.71 Å, 6.07 Å, 5.14 Å and 2.19Å (fig. 2a). In case of without Zinc, four H-bond is formed between amino acid TYR361 (O) and Gemcitabine (H) with bond length 5.50 Å, 6.68 Å, 1.99 Å and 6.85Å. (fig.2b).

In docking complex between Farnesyltransferase and inhibitor Tipifarnib with Zinc, One H-bond is formed between amino acid TYR361 (OH) and Tipifarnib (H) with bond length 3.07Å and 1.81 Å (fig. 3a). In case of without Zinc, two H-bond is formed between amino acid TYR361 (OH) and Tipifarnib (H) with bond length 2.09Å and 2.90 Å (fig.3b).

In docking complex between Farnesyltransferase and inhibitor Toremifene with Zinc, No H-bond is formed between amino acid with Tipifarnib (fig. 4a). In case of without Zinc, One H-bond is formed between amino acid TYR361 (OH) and Toremifene (O) with bond length 2.93Å (fig.4b).

Role of zinc in Farnesyltransferase structure and function

Farnesyltransferase has two subunits: a 48kDa alpha subunit and a 46kDa beta subunit. Both subunits are primarily composed of alpha helices. The α subunit is made of a double layer of

paired alpha helices stacked in parallel, which wraps partly around the beta subunit like a blanket. The alpha helices of the β subunit form a barrel. The active site is formed by the center of the β subunit flanked by part of the subunit. Farnesyltransferase coordinates a zinc cation on it β subunit at the lip of the active site.

3.2 Molecular docking studies of neuraminidase

The docking results of Oseltamivir and Zanamivir inhibitors with neuraminidase are shown in table 5.2. The result shows that Oseltamivir and Zanamivir inhibitors have low binding, docking and internal energies with neuraminidase in presence of calcium metal ion. However in the absence of Ca metal ion these energies are increase. This shows that calcium play a biological role in the binding of inhibitor at catalytic site of neuraminidase protein.

A close view of the binding interactions of neuraminidase with Oseltamivir and Zanamivir inhibitors was analyzed through PyMol viewer with and without calcium metal ion are shown below in Figure 5(a-b) to 6(a-b) respectively. Ligand is coloured in red (in stick drawing) where as amino acids involved in hydrogen bonds color by atom.

In docking complex between Neuraminidase and inhibitor Oseltamivir with Calcium, Three H-bond is formed between amino acid ASP151(O), GLU119(O), ARG 371(N) and Oseltamivir (H) with bond length 2.82 Å, 2.09 Å, 1.66 Å, 2.79 Å, and 3.61 Å (fig. 5a). In case of without Calcium, Two H-bond is formed between amino acid GLU119 (O), ARG371 (N) and Oseltamivir (O) with bond length 3.18 Å, 1.61 Å and 2.77Å (fig.5b).

In docking complex between Neuraminidase and inhibitor Zanamavir with Calcium, Four H-bond is formed between amino acid GLU277 (O), SER179 (O), TYR406 (O), ASP151 (O) and Zanamavir (O) with bond length 2.10 Å, 1.81 Å, 2.03 Å , 3.76 Å,4.01 Å, 2.35Å. (fig. 6a). In case of without Calcium, Four H-bond is formed between amino acid GLU277 (O), GLU276(O), TYR406(O), ASP151(O) and Zanamavir (H) with bond length 2.39 Å,6.22 Å, 4.07 Å , 3.73 Å (fig.6b).

Role of calcium in influenza virus Neuraminidase protein

In structures of NA determined previously, a calcium ion is observed to coordinate amino acids near the substrate-binding site. In three of the NA monomers determined here this calcium is absent, resulting in structural alterations near the substrate-binding site. These changes affect the conformation of residues that participate in several key interactions

between the enzyme and substrate and provide at a molecular level the basis of the structural and functional role of calcium in substrate and inhibitor binding.

3.3 Molecular docking studies of Thioredoxin

The docking results of Granisetron and Nilutamide inhibitors with Thioredoxin are shown in table 5.3. The result shows that Granisetron and Nilutamide inhibitors have low binding, docking and internal energies with Thioredoxin in the presence of copper metal ions. However, in the absence of Cu metal ion these energies are increasing. This shows that copper plays a biological role in the binding of inhibitor at the catalytic site of thioredoxin protein.

A close view of the binding interactions of neuraminidase with Granisetron and Nilutamide inhibitors was analyzed through PyMol viewer with and without copper metal ion are shown below in Figure 4.16 to 4.19 respectively. Ligand is coloured in red (in stick drawing) where as amino acids involved in hydrogen bonds color by atom.

In docking complex between Thioredoxin and inhibitor Granisetron with Copper, no H-bond is formed between the amino acid and Granisetron (fig. 7a). In case of without Copper, no H-bond is formed between the amino acid and Granisetron (fig. 7b).

In docking complex between Thioredoxin and inhibitor Granisetron with Copper, no H-bond is formed between the amino acid and Granisetron (fig. 7a). In case of without Copper, no H-bond is formed between the amino acid and Granisetron (fig. 7b).

3.4 Biological relevance of Copper

Copper is an essential micronutrient required by all life forms. Cu is a transition metal and hence involved in a variety of biological processes viz., embryonicdevelopment, mitochondrial respiration, regulation of hemoglobin levels as well as hepatocyte and neuronal functions. Being a transition metal, Cu gets biologically converted between different redox states, namely oxidized Cu (II) and reduced Cu (I). This unique attribute has made Cu metal to get manifested as an important catalytic co-factor for a variety of metabolic reactions in biological systems.

Table 1: List of experimentally known inhibitors.

Sl. No.	Inhibitor Name	CID No.	MWT (g/mol)	Molecular formula	X Log P	HBD	HBA
1	Azacitidine	9444	244.20468	$C_8H_{12}N_4O_5$	-2.2	4	5
2	Gemcitabine	60750	263.198146	$C_9H_{11}F_2N_3O_4$	-1.5	3	6
3	Tipifarnib	159324	489.39578	$C_{27}H_{22}Cl_2N_4O$	4.1	1	3
4	Toremifene	3005573	405.95962	$C_{26}H_{28}ClNO$	7.2	0	2
5	Oseltamivir	65028	312.40452	$C_{16}H_{28}N_2O_4$	1.1	2	5
6	Zanamivir	60855	332.3098	$C_{12}H_{20}N_4O_7$	-3.2	7	8
7	Granisetron	5284566	312.40936	$C_{18}H_{24}N_4O$	2.8	1	3
8	Nilutamide	4493	317.22071	$C_{12}H_{10}F_3N_3O_4$	2	1	7

Table 2: The docking results of known inhibitors with farnesyltransferase.

Sl. No	Inhibitor Name	Metal ion	Binding Energy (Kcal/mo	Docking Energy (Kcal/mol	Intermol Energy (Kcal/mol)	Torsional Energy (Kcal/mo	Internal Energy (Kcal/mol)
1	Azacitidin	Zn	-5.66	-6.2	-6.28	0.62	0.08
2	Azacitidin	absent	-11.37	-12.0	-11.99	0.62	-0.01
3	Gemcitabie	Zn	-5.18	-5.43	-5.8	0.62	0.37
4	Gemcitabie	absent	-5.45	-5.64	-6.07	0.62	0.43
5	Tipifarnib	Zn	-8.06	-5.75	-9.3	1.25	3.56
6	Tipifarnib	absent	-12.84	-10.71	-14.09	1.25	3.38
7	Toremifen	Zn	-6.91	-9.74	-9.71	2.8	-0.03
8	Toremifene	absent	-10.25	-13.23	-13.05	2.8	-0.18

Table 3: The docking results of known inhibitors with Neuraminidase.

Sl. No	Inhibitor Name	Metal ion	Binding Energy (Kcal/mo	Docking Energy (Kcal/mol	Intermol Energy (Kcal/mol)	Torsional Energy (Kcal/mo	Internal Energy (Kcal/mol)
1	Oseltamivir	Ca	-7.16	-10.38	-9.96	2.8	-0.43
2	Oseltamivir	absent	-15.01	-18.19	-17.81	2.8	-0.38
3	Zanamivir	Ca	-6.63	-8.48	-8.81	2.18	0.34
4	Zanamivir	absent	-7.51	-10.0	-9.69	2.18	-0.31

Table 4: The docking results of known inhibitors with Thioredoxin.

Sl. No	Inhibitor Name	Metal ion	Binding Energy (Kcal/mo	Docking Energy (Kcal/mol	Intermol Energy (Kcal/mol)	Torsional Energy (Kcal/mo	Internal Energy (Kcal/mol)
1	Granisetro	Cu	-5.32	-6.55	-6.26	0.93	-0.29
2	Granisetro	absen	-5.27	-6.6	-6.2	0.93	-0.4
3	Nilutamide	Cu	-3.15	-4.01	-4.08	0.93	0.08
4	Nilutamide	absen	-3.8	-4.64	-4.74	0.93	0.1

(a) **(b)**

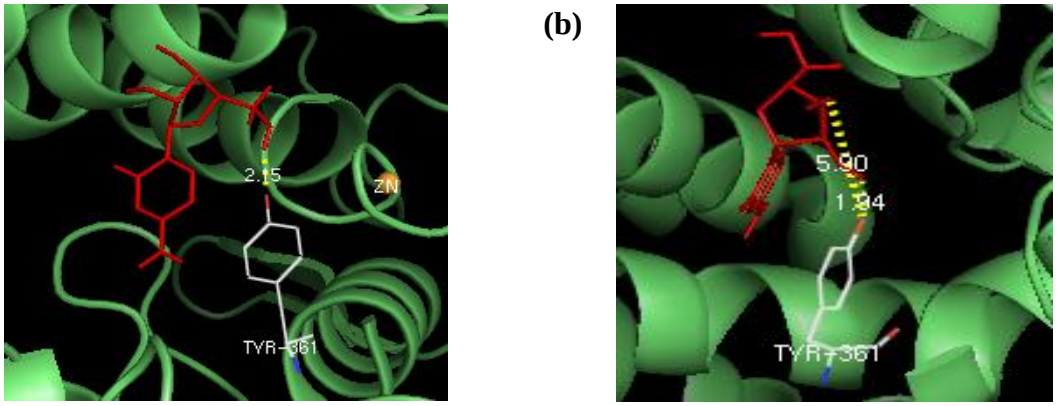

Figure 1(a-b): The docking structure of complex between Farnesyltransferase and inhibitor Azacitidine (a) with Zinc and (b) without Zinc. Inhibitor Azacitidine is show in sticks representation and is colored with red using PyMol viewer. Amino acid residues are represented as lines. Hydrogen bond is represented by yellow dotted line.

(a) **(b)**

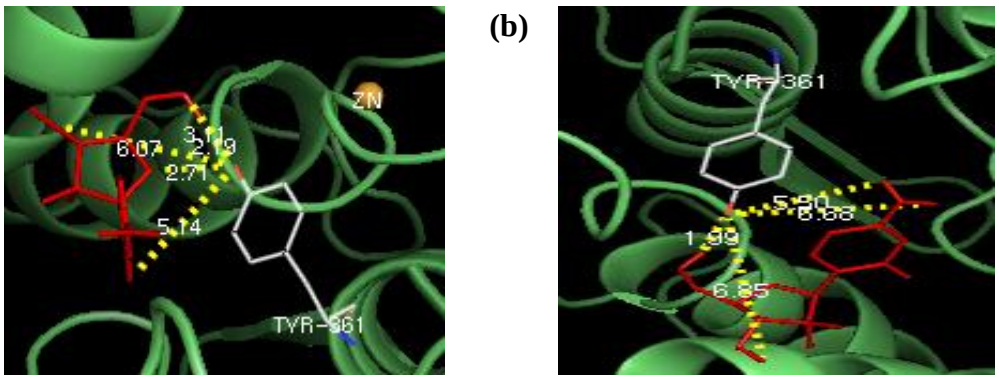

Figure 2(a-b): The docking structure of complex between Farnesyltransferase and inhibitor Gemcitabine (a) with Zinc and (b) without Zinc. Inhibitor Azacitidine is show in sticks representation and is colored with red using PyMol viewer. Amino acid residues are represented as lines. Hydrogen bond is represented by yellow dotted line.

(a) **(b)**

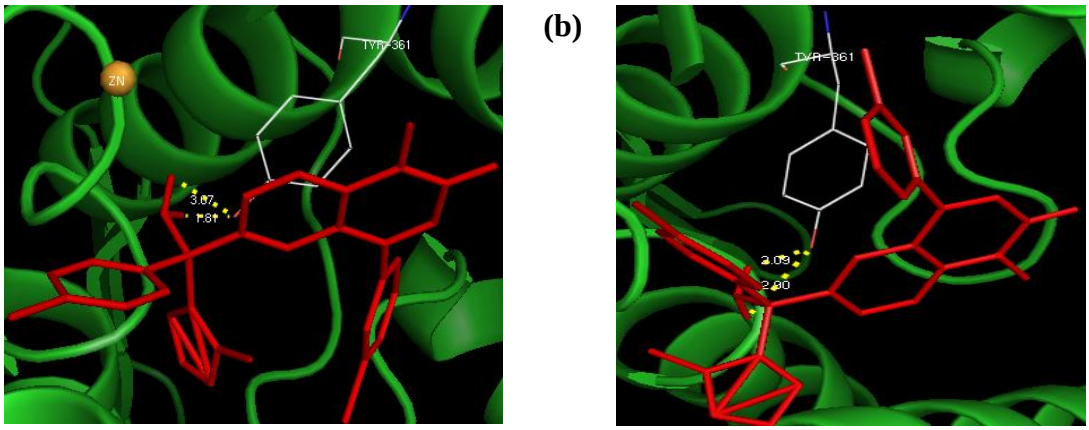

Figure 3(a-b): The docking structure of complex between Farnesyltransferase and inhibitor Tipifarnib (a) with Zinc and (b) without Zinc. Inhibitor Azacitidine is show in sticks representation and is colored with red using PyMol viewer. Amino acid residues are represented as lines. Hydrogen bond is represented by yellow dotted line.

(a) **(b)**

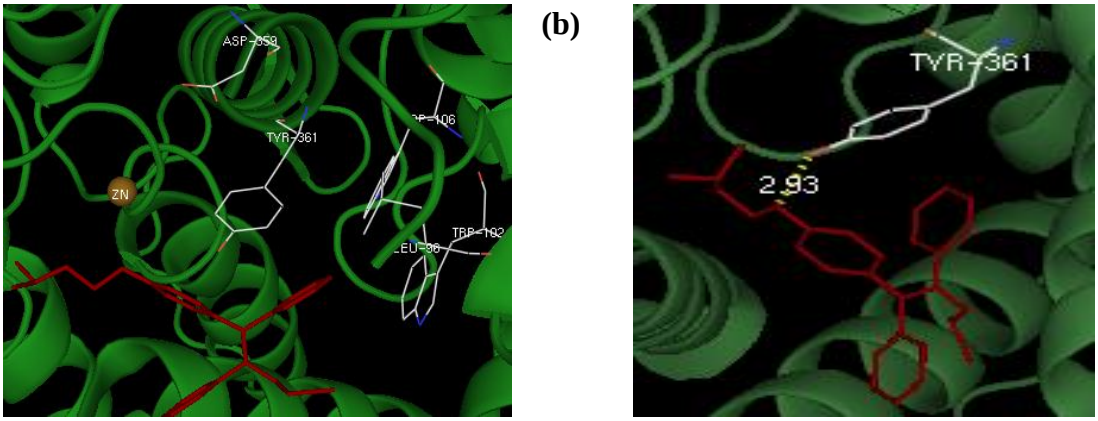

Figure 4(a-b): The docking structure of complex between Farnesyltransferase and inhibitor Toremifene (a) with Zinc and (b) without Zinc. Inhibitor Azacitidine is show in sticks representation and is colored with red using PyMol viewer. Amino acid residues are represented as lines. Hydrogen bond is represented by yellow dotted line.

(a) **(b)**

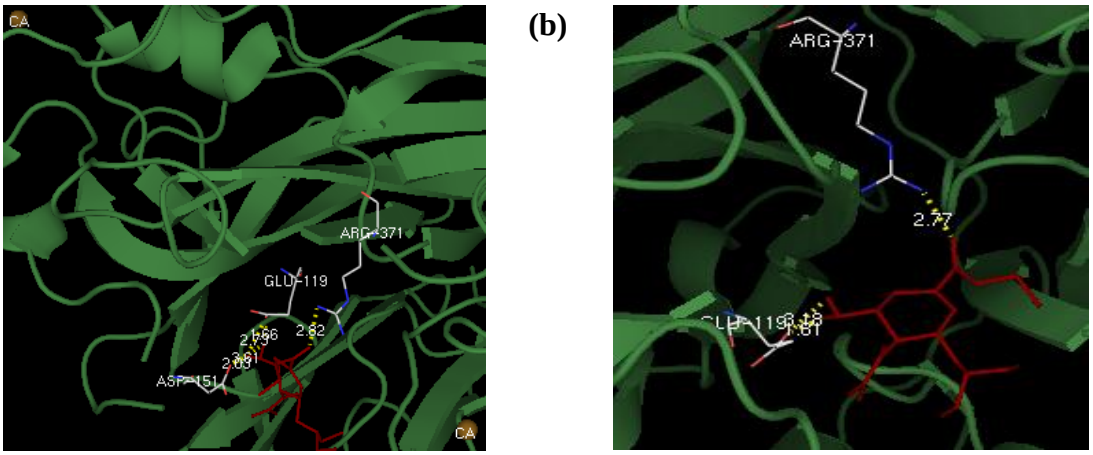

Figure 5(a-b): The docking structure of complex between neuraminidase and inhibitor Oseltamivir (a) with Calcium and (b) without Calcium. Inhibitor Oseltamivir is show in sticks representation and is colored with red using PyMol viewer. Amino acid residues are represented as lines. Hydrogen bond is represented by yellow dotted line.

(a) **(b)**

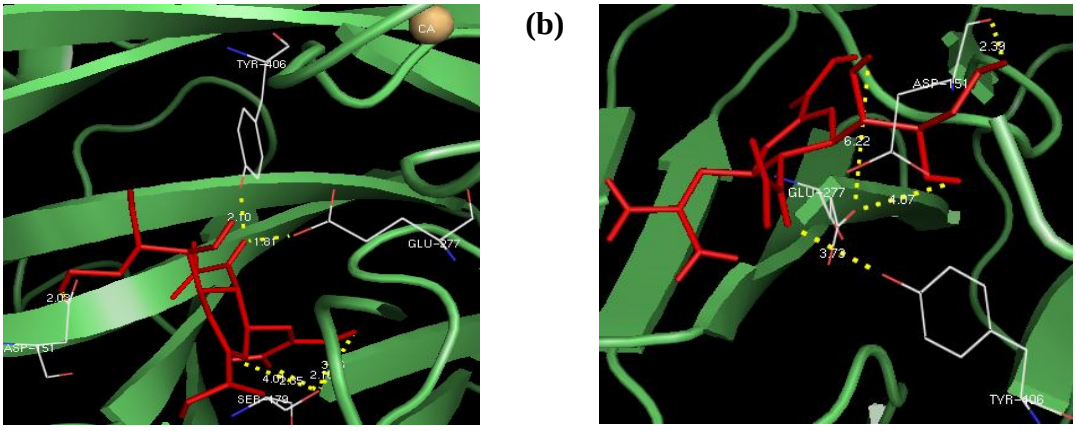

Figure 6(a-b): The docking structure of complex between Neuraminidase and inhibitor Zanamavir (a) with Zinc and (b) without Zinc. Inhibitor Azacitidine is show in sticks representation and is colored with red using PyMol viewer. Amino acid residues are represented as lines. Hydrogen bond is represented by yellow dotted line.

(a) **(b)**

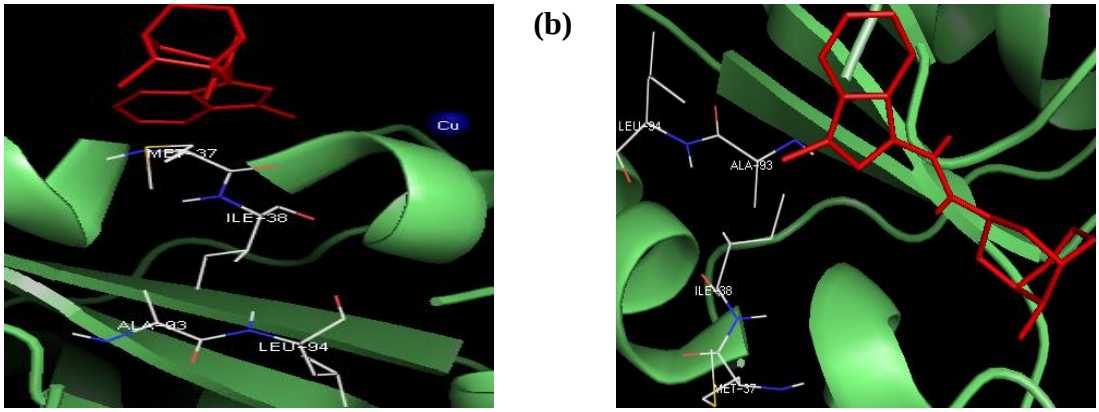

Figure 7(a-b): The docking structure of complex between Thioredoxin and inhibitor Granisetron (a) with Copper and (b) without Copper. Inhibitor Granisetron is show in sticks representation and is colored with red using PyMol viewer. Amino acid residues are represented as lines. Hydrogen bond is represented by yellow dotted line.

(a) **(b)**

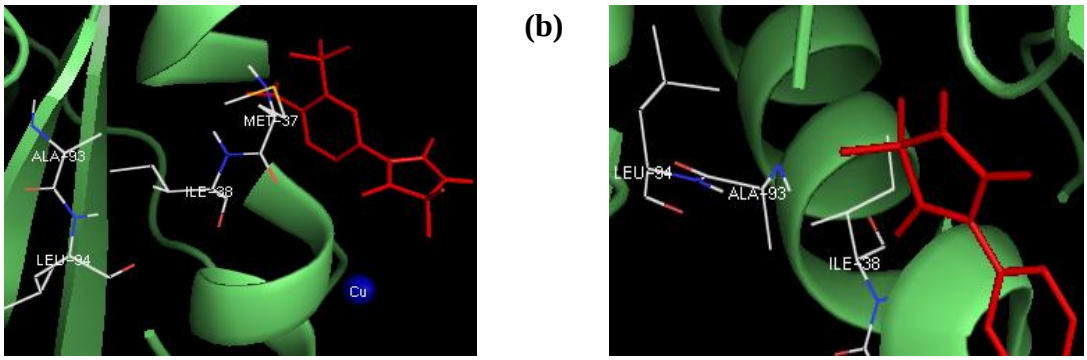

Figure 8(a-b): The docking structure of complex between Thioredoxin and inhibitor Nilutamide (a) with Copper and (b) without Copper. Inhibitor Nilutamide is show in sticks representation and is colored with red using PyMol viewer. Amino acid residues are represented as lines. Hydrogen bond is represented by yellow dotted line.

4. CONCLUSION

Metal play a critical role in the function of Farnesyltransferase, Neuraminidase and Thioredoxin proteins. Metals can have purely stabilized roles, or may be central to protein activity, perhaps involved in enzyme activity. Our results suggested that the environment of metals in proteins is quite different from the aqueous environment and this may confer unusual properties of the metal such that it may be better suited to a particular role in structural stability or catalysis.

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