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IN SILICO INTERACTIONS OF LEAD (Pb), CADMIUM (Cd), AND ARSENIC (As) WITH GSTM1: A MOLECULAR MODELLING AND DOCKING STUDY

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ABSTRACT: The GSTM1 molecular model was constructed using sequence collected from Uniprot and with the aid of the usage of homology modeling methods by MODELLER software followed by molecular dynamics with the same software. An equilibration strategy was carried out by NAMD 2.9 and CHARMM27 where as lipids and proteins at the side of the TIP3P model for water and the active site of GSTM1 identified using CASTp server. After modeling of the GSTM1 molecule was docked with lead (Pb), Cadmium (Cd) and Arsenic (As) and the interaction of these changed into very well studied the usage of calculations of molecular mechanics. All evaluation tools were confirmed the reliability of predicted model and the binding pockets were revealed. GSTM1 of Homo sapiens became accrued from NCBI database and drawn the use of READMOL software program which became a molecule generator set of rules. Then the heavy metals were docked with residues of GSTM1 and binding studies were carried out to discover the adjustments within the interactions of GSTM1 with heavy metals.

KEYWORDS: GSTM1, Modelling, Docking studies, Heavy metals.

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1. INTRODUCTION

The glutathione S-transferases (GSTs), enzymes that play an important role within the cleansing of numerous toxicants, and toxic intermediates from the body. It catalyzes the conjugation of electrophile to GSH, consequently alteration in this protein each in expression and activity would

possibly affect the character's response to the oxidative harm or infection resulted by way of lead exposure and mu magnificence of GST enzymes mainly GSTM1 entails in the detoxing of electrophilic compounds consisting of cancer causing agents, therapeutic capsules, environmental pollutants and products of oxidative pressure, by conjugation [3,23]. The mu genes are positioned on chromosome 1p 13.3 and are known tremendously polymorphic and these variations may additionally fluctuate in individual susceptibility to unique cancer agents and pollution; null kind of this gene has connected to lead the various sicknesses consisting of cancer. Heavy metals such as Lead (Pb), Cadmium (Cd) and Arsenic (As) are widely recognized to cause dangerous health consequences and distributed in the mind, liver, kidney and bones [26]. Continuous exposure to heavy metals has the capability to cause many deleterious systematic consequences such as high blood pressure, frank anaemia, cognitive deficits, infertility, immune imbalances, behind schedule skeletal and deciduous dental development, nutrition D deficiency, and gastrointestinal outcomes. The GSTM1gene has 8 exons spanning a place of 21,244 bases with transcript duration of 1,161 bps and translation length of 218 residues (in line with ensemble GRCh37 launch 78) and the scale of the gene is ready 20 kb in length carefully flanked through different mu class gene sequences. The endpoints of the polymorphic GSTM1 deletion incorporates inside the left 5 kb repeated place downstream from the three'-stop of the GSTM2 gene and 5 kb upstream from the beginning of the GSTM1 gene. The right side repeated place 5 kb downstream from the 3'-cease of the GSTM1 and 10 kb upstream from the five'-give up of the GSTM5 gene [22, 25]. The GSTM1 gene has four extraordinary alleles i.e. GSTM1-zero, GSTM1-A, GSTM1-B and GSTM1-1x2 alleles in M1 magnificence [24,4]. GSTM1-zero (GSTM1 null allele) arose from a recombination occasion during evolution between 2 fairly homologous regions flanking this locus, ensuing in deletion of a 20-kb segment [25]. This deletion produces a unique 7.4-kb HindIII fragment with the loss of 10.3- and 11.Four-kb HindIII fragments; as a result homozygotes for GSTM1 null allele produce no GSTM1 protein. The incidence of GSTM1 deletion polymorphisms varies throughout ethnic groups, from 18% to 66% (median, 50%), aside from Asians, for whom it is 38%-58% [24]. GSTM1-A and B are fluctuate by using a unmarried base in exon 7 i.E C-G substitution at base function 534, ensuing in a substitution of Lys-Asn at amino acid 172 [23]. The substitution similarly consequences inside the formation of monomers (GSTM1A-1A, GSTM1B-1B) or heterodimers (GSTM1A-1B), despite the fact that in vitro research advocate that their activities are similar [23, 25]. A particular GSTM1 variant dGSTM1-1x2, containing a duplicated GSTM1 gene has been diagnosed in Saudi Arabian population, [8]. In the present study, we aimed to observe the interplay between GSTM1 protein with the heavy metals like lead (Pb), Cadmium (Cd) and Arsenic (As) using molecular modeling and docking processes.

2. MATERIALS AND METHODS

Sequence retrieval and 3-D version building

The amino acid sequence of GSTM1 of Homo sapiens encoding protein collected from Uniprot database. The preliminary version of GSTM1 constructed by way of the usage of homology modeling methods and with the MODELLER software [5, 9, 10] and submitted to area fishing server [7] for domain prediction observed by related protein shape as a template by way of the BLAST (Basic Local Alignment Search Tool) [1, 2] against PDB (Protein Databank). The collection that showed most identity with the excessive score and e-cost either zero or less terrible values were aligned (Figure. 1) and this was used as a reference shape to build a 3-D version for GSTM1. The coordinates for the structurally conserved areas (SCRs) for GSTM1 have been assigned from the template the usage of multiple sequence alignment, primarily based at the Needleman-Wunsch set of rules [17].

Molecular Dynamics

The structure attained from the MODELLER had been applied to NAMD 2.9 software for equilibration of model for dynamics studies [12] and the lipids and proteins force fields calculated using CHARMM27 [15, 12] alongside the TIP3P model for water [11]. All non-hydrogen atoms with a damping coefficient of one ps-1 were utilized in retaining a regular temperature of 310 K at some stage in the gadget according to Lengevin dynamics. The equilibrium state of the GSTM1 was confirmed using RMSD (Root Mean Square Deviation) of the complete protein's spine upon a successful of three runs for average energy values. Distance distributions are for an unmarried run that was ordinary of the set of runs to ensure the readability of interpretation.

Structure Validation and Active web site Identification

RMSD (Root Mean Square Deviation) were calculated and used for the structure having the least energy with low for further analysis and the initial model turned into improved on this step. The final structure analyzed with the aid of Ramachandran's map using PROCHECK (Programs to test the Stereochemical Quality of Protein Structures) [6]. The environment profile turned into constructed the usage of ERRAT graph (Structure Evaluation server) [13]. This version was used for the identity of the lively website and for docking of the substrate with the enzyme. The energetic website of GSTM1 version was identified with CASTp server [14] and brand new software, CASTp, changed into used to measuring protein pockets and cavities, is based on specific computational geometry methods which incorporates alpha form and discrete drift idea.

Docking Analysis

Docking with GOLD 3.0.1

GOLD (Genetic Optimization of Ligand Docking) software is a genetic algorithm (GA) based software which specially utilizes an evolutionary strategy involving three genetic operators which incorporates crossovers, mutations, and migrations [21]. Generally, it imports the partial flexibility to proteins and complete flexibility to inhibitors. The heavy metals, Pb, Cd and As were subjected for docking with GSTM1 and the interplay of these heavy metals at the side of GSTM1 were thoroughly studied the use of calculations of molecular mechanics. In addition, studied the interaction of GSTM1 and heavy metals separately and in combination. 100 populace size, 1.1 selection strain, 10,000 operations, 1 island and nich size parameters had been used for genetic set of rules. Operator parameters for crossover, mutation, and migration had been set to one hundred, a hundred and ten respectively. Default cut off values are, three.0A° (dH-X) for hydrogen bonds and six. 0 A° for Van der Waals had been employed. The default set of rules pace turned into selected and the protein binding web site in ORIC was defined within a 10A° radius. The number of poses for each inhibitor become set to a hundred and early termination turned into allowed if the pinnacle 3 bound conformations of inhibitors were inside 1.5 A° RMSD. After docking, the character binding poses of every heavy metal had been found and their interactions with the GSTM1 place were studied. The best and most energetically favorable conformation of each heavy metal with GSTM1 were selected.

GOLD Score health feature

The 4 components viz, protein-protein hydrogen bond energy (external H-bond); protein-protein van der Waals power (external vdw); protein internal van der Waals strength (inner vdw); and protein intramolecular hydrogen bond energy (internal- H- bond) were considered for calculating the health feature of GOLD score. The protein-protein hydrophobic contact turned into recommended by way of making an empirical correction through multiplying external vdw score with 1.375. Prediction of ligand binding positions changed into optimized for the health function.

3. RESULTS AND DISCUSSION

Homology modeling of GSTM1 domain

BLAST seek is the supply for the sequence and it ensures extra correct alignment among target collection and template shape. 1YJ6 sequence has a high degree of series identification with GSTM1 domain in PDB. The Structurally conserved areas (SCRs) for the model and the template have been decided by way of superimposition of the two systems and a couple of sequence alignment with forty two percent identities.

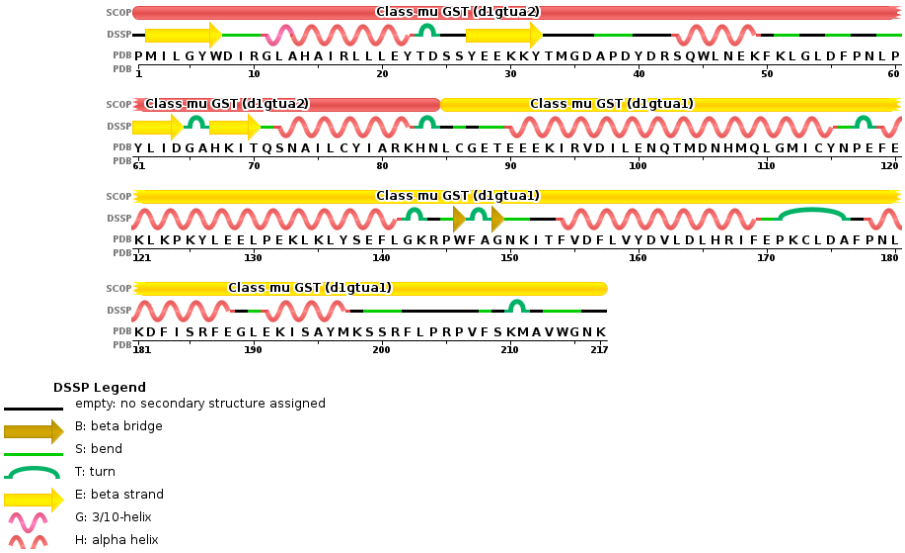

Fig 1: Amino acid Sequence of GSTM1 Domain

In our study, initially 1YJ6 sequence was consideration as a reference shape for modeling GSTM1 domain which coordinates from its protein (1YJ6) to the SCRs, structurally variable areas (SVRs), N-termini and C-termini were assigned to the target collection based at the pride of spatial restraints. GSTM1 became absolutely aligned with the template and this used to broaden three-D structure of the protein. Out of 20 PDB files found in MODELLER, we selected a least power PDB record for in addition studies and obtained final solid structure of the GSTM1 is shown in Figure-2 after loop modeling. 9 helix and 3 sheets have been discovered in GSTM1 area and was confirmed with the aid of SPDBV.

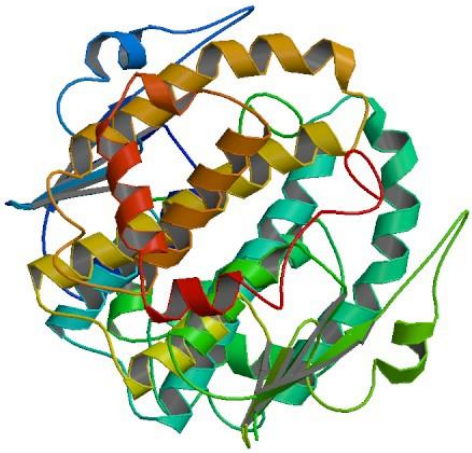

Figure 2: Final refined structure of GSTM1

Low RMSD (Root Mean Square Deviation) and low energy of GSTM1 domain was obtained by NAMD and the same is in Figure 3.

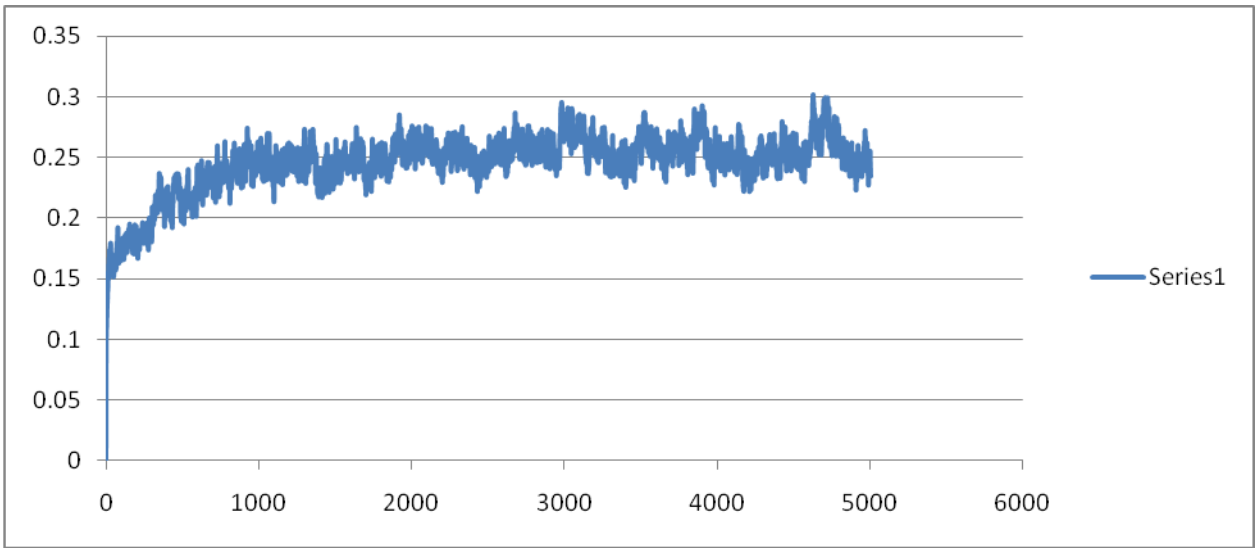

Figure 3: Calculated RMSD graph of molecular dynamics simulations of GSTM1 using NAMD software. Time (Ps) was taken in X-axis and RMSD in Y-axis

The final structure was further checked by VERIFY-3D graph and the results are shown in Figure 4: The overall scores indicate acceptable protein environment.

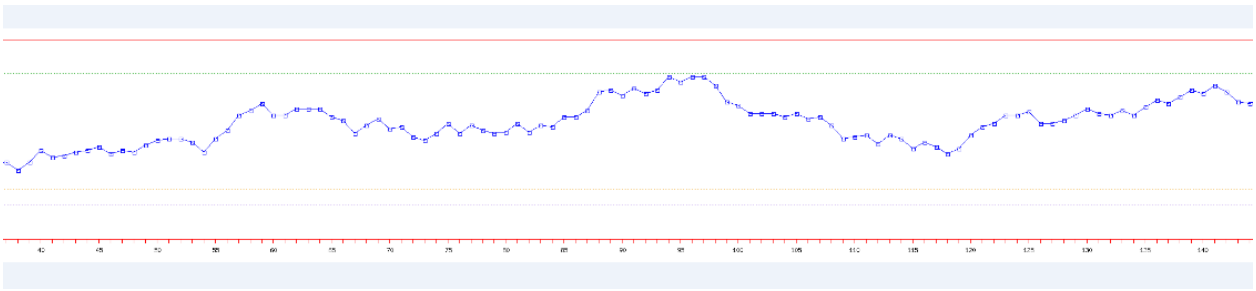

Figure 4: The VERIFY-3D profile results of GSTM1; overall quality score indicates residues are reasonably folded

Validation of GSTM1 Domain

Ramachandran plot calculations were computed with the PROCHECK software after the refinement procedure, validation of the version and Ψ and θ distributions values for non-glycine, non-proline residues are summarized and presented in Table 1. The RMSD (Root Mean Square Deviation) deviation values for covalent bonds and covalent angles relative to the standard dictionary of GSTM1 is -3.27 and -0.Sixty five Aº. Altogether one hundred % of the residues of GSTM1 are in favoured and allowed regions and the overall PROCHECK G-issue of GSTM1 is –1.32 and demonstrated three-D surroundings profile was correct.

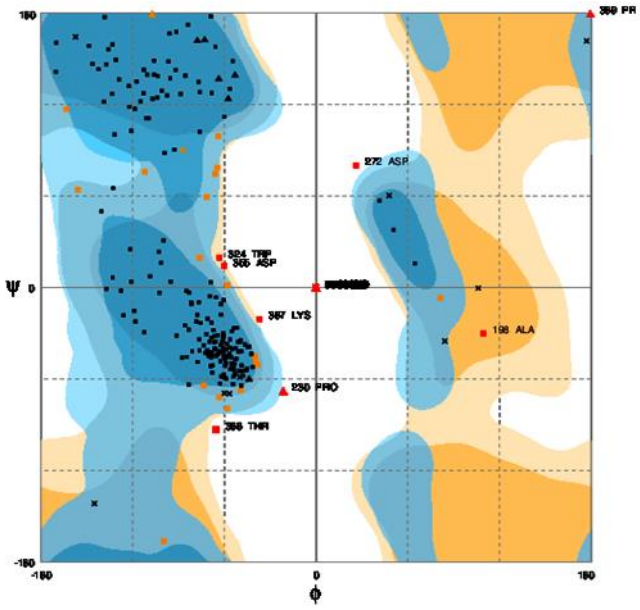

Figure 5: Ramachandran plot

Table 1: % of residue falling in the core region of the Ramachandran's plot

The number of residues in a favoured region (~98.0% expected) :	205 (91.3%)
The number of residues in an allowed region (~2.0% expected) :	10 (4.1%)
The number of residues in outlier region :	7 (4.6%)

Active site Identification of GSTM1

The feasible binding web sites of GSTM1 searched based on the structural assessment of a template, the version construct and additionally with CASTp server after deciding on the receptor from PDB and isolated the A-chain in SPDBV and the equal is proven in Figure 7[14].

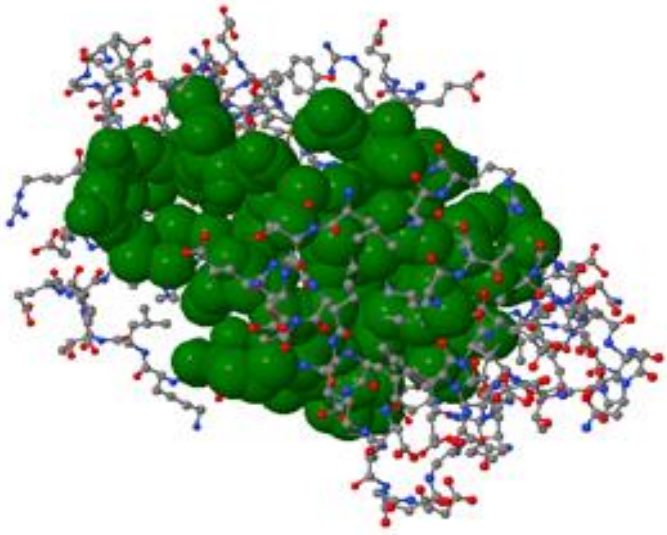

Figure 6: Active site pockets of the GSTM1 showing highest area and volume

After confirmation of GSTM1 structure, Pb, Cd and As were docked using GOLD software. In the binding pocket, common H-bonding interactions have been formed between all heavy metals and gene place. The specific H-bonding interaction changed into best determined inside the binding of GSTM1 [20, 21]. In order to provide an explanation for the binding of those proteins; the H-bonding interactions with the opposite surrounding residues in the hydrophobic binding pocket have been also examined. In the existing examine, CASTp server become used to locate the possible binding web page of GSTM1. From the binding site analysis, it turned into found that binding wallet is recognized and the most important binding pocket became selected for the docking research. The heavy metals had been docked into GSTM1 location using GOLD 3.0.1 and all docking solutions had been ranked in line with the GOLD health function.

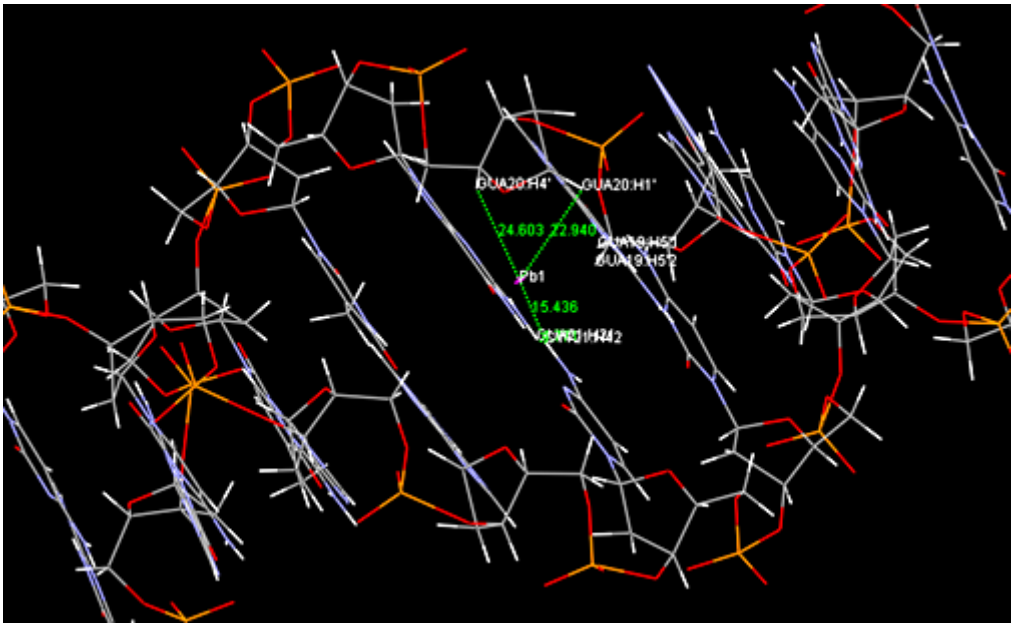

Fig 7: Docking results of Pb with GSTM1

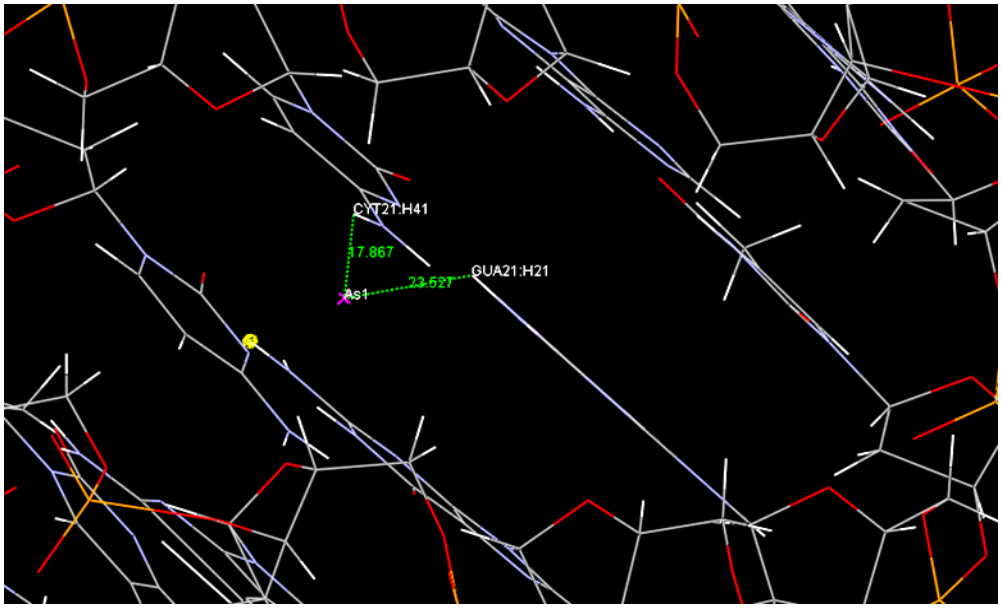

Fig 8: Docking results of Arsenic with GSTM1

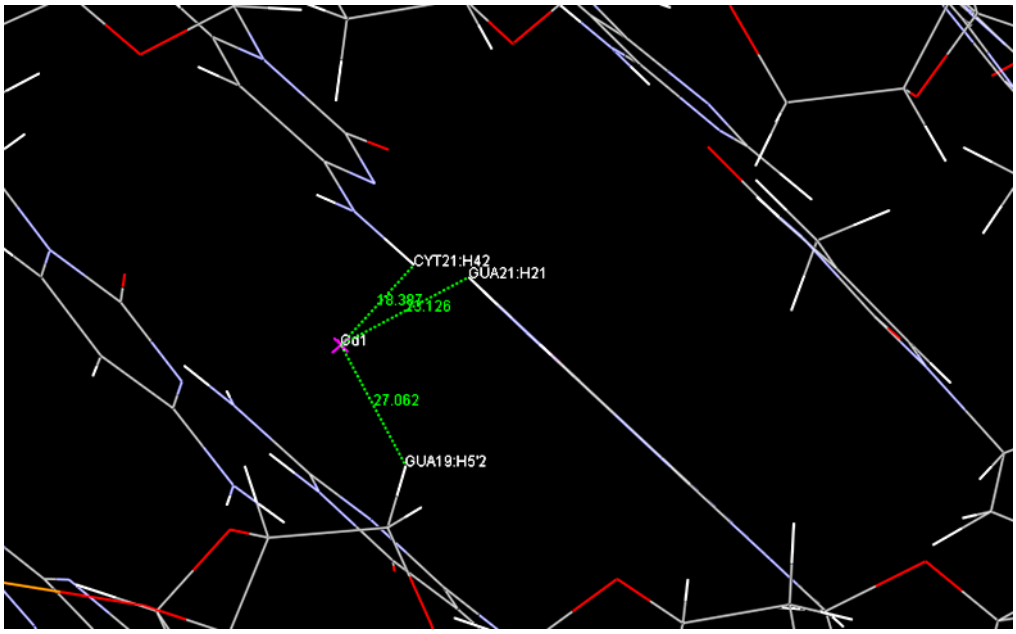

Fig 9: Docking results of Cadmium with GSTM1

4. CONCLUSION

In this study, in silico methodologies such as homology modeling and docking analysis were carried out to predict the effect of heavy metals on GST genes. Three dimension structure of GSTM1 was modeled by employing the crystal structure template. The predicted structure of GSTM1 has a good degree of accuracy. The final refined model was assessed by different evaluation programs. Ramachandran plot values indicated ideal results of predicted model as residues in favoured regions which are 91.3%, while only 7 (4.6%) residue was in the outlier region. The binding studies confirmed the changes in the GSTM1 were due to binding of heavy metals and helpful to synthesis to novel drugs for various diseases.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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