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Possible Metsulfuron Herbicide Detoxification by a *Oryza sativa* L. Glutathione S-transferase Enzyme.

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HIGHLIGHTS

- Molecular docking to study molecular anchorage of a GST's rice and herbicides.
- Tau class GST's in plants may be able to detoxify herbicides.
- Tau-class GST in plants may be able to detoxify pesticides.

Abstract: Rice (*Oryza sativa* L.) is one of the most important crops in the world, and it is considered the primary source of nutritional layout in developing countries in Asia. The glutathione S-transferases (GSTs) superfamily confers to rice protection against biotic and abiotic stress, and herbicide resistance. However, the three-dimensional structure of a GST Tau class, is unsolved. The objectives of this work were to develop a reliable comparative model for the s-transferase glutathione class Tau 4 from rice, and simulate docking interactions, against herbicides bentazon and metsulfuron. Results showed that the predicted model is reliable and has structural quality. Ramachandran plot set 91.9% of the residues in the most favored regions. All complexes showed negative binding energies values; and metsulfuron docked to the glutathione tripeptide, and it represents a possible *insilico* evidence of glutathione conjugation with this herbicide.

Keywords: Molecular Modeling, OsGST, Herbicide, Docking, Bioinformatics, Computational Biology.

INTRODUCTION

Rice (*Oryza sativa* L.) is a crop of global significance, due its impact in agribusiness and nutritional value. It is the primary source of Asian developing countries nutritional diet. Despite this remarkable importance, there is a lack of studies to develop techniques that minimizes the potential problems inherent of this grain cultivation, such as competition with invasive plants [1].

In rice crop, the weed control has not been made for a long time due it is an open-area crop, free of invasive plants, in which situation no control measures are necessary. However, there is a lack of technologies and products aimed at the control of weeds in rice [2], in rotation with commercial crops such as soybean and corn. This problem, associated with the low capacity of competition against weeds, is one of the main obstacles of the rice introduction in agricultural systems in corrected soils [1].

Regarding the rice cultivation practices there are no consensus about technical features that are correlated with the competitive ability among weeds [3]. Systems that suffers with intrageneric and/or intraspecific competition explores the same niche that gives competitive advantage for the weed [4] is the actual problem found in the rice and red rice crop in Brazil [5]. Weed management can be done directly or indirectly. In the direct management, the activities are directed to the elimination of weeds by chemical, mechanical, manual and biological methods, while in the rice monoculture the chemical control the most important form of weed management, by the application of multiple herbicides in the cultivated area [1, 6].

Due to the phytotoxicity of these compounds, the herbicides also damages the rice cultivation. The herbicide use can reach 85% of grain decrease [7]. An experiment about evaluative effects of the glyphosate, a broad-spectrum herbicide commonly used in rice monocultures, showed several counterproductive effects such as decreased height, number of juveniles, and grain losses up to 63% in *O. sativa* [8]. In addition to the damage caused by glyphosate, there are associated stress factors (biotic or abiotic), that induce free radicals production, such as oxidative reactions, resulting oxidative stress and irreversible cellular damage [8]. The glutathione S-transferase enzyme superfamily confers to rice catalytic action, protection of biotic and abiotic stress, and herbicide resistance [10-11] by the conjugation reaction of the tripeptide glutathione (GSH) to a hydrophobic compound, and convert it in a more soluble and less toxic conjugated compound [12].

Glutathione S-transferases (GSTs) denotes a superfamily of catalytic proteins that are present in almost all living beings, being distributed in a great diversity of aerobic organisms, ranging from bacteria to human [10-11]. These enzymes are involved in several biological processes [13].

GSTs are usually found in the biological environment as homo or heterodimers (there may be the possibility of complexes), generally presenting two active sites by dimer that express activities independent of each other [14]. Each active site consists of: one for the tripeptide GSH (G-site) which is very specific for this tripeptide and is situated on N-terminal domain, and another binding site which has a lower specificity for the electrophiles (H-site) on C-terminal domain [14].

GSTs have a large spectrum of specificity, which confers them effective metabolic properties several toxic components, characterizing them as the main proteins of phase II of the detoxification process [15].

Others studies demonstrated that the GST enzymes are associated with the tolerance of several crops to the harmful effects of herbicides, and grass resistance to these chemicals. The metabolic process of plant detoxification in some crops is activated by the enzyme as a direct response to the action of herbicides [16]. The aim of this study was to construct a theoretical model for a tau 4 *Oryza sativa* Indica glutathione S-transferase (*OsJGSTU4*) and perform docking simulations against bentazon-sodium and metsulfuron-methyl herbicides.

MATERIAL AND METHODS

Homology modeling and model validation

The *OsJGSTU4* primary sequence was obtained from the NCBI database (<https://www.ncbi.nlm.nih.gov/> - accession number: AAQ02686.1).

A local alignment was made against the PDB database (Protein Data Bank, <http://www.rcsb.org/>) through the local alignment algorithm "BLASTp" (Basic Local Alignment Tool for proteins, <http://blast.ncbi>) identity (46,73%) and good resolution (1.25 Å) were obtained using a tau 4 family *Populus trichocarpa* glutathione S-transferase *PtGSTU30* (PDB ID: 5j4u.1).

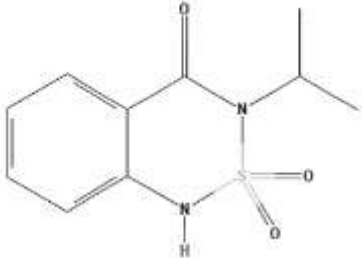
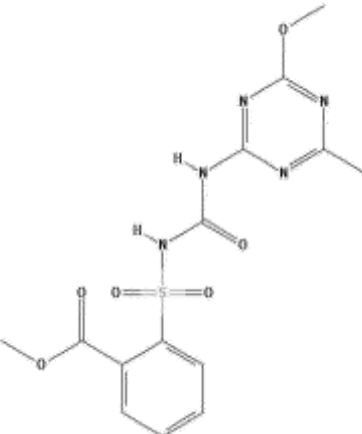
The template were saved in a PDB file, and the SWISS-MODEL server User Template tool (<http://swissmodel.expasy.org/>) was used to generate the model [17-18]. For the model validation, through Ramachandran plot analysis using the PROCHECK program [19] to verify the stereochemical quality of the structure.

Local quality was accessed by ANOLEA [20] and GROMOS force fields [21]. All the generated docked complexes were visualized with Visual Molecular Dynamics software (VMD v. 1.9.3) [22].

Molecular Docking

The structure of the herbicides used as ligands (Table 1.) were obtained from the ZINC database (<http://zinc.docking.org/>) in .mol2 files. These files were converted to .pdbqt in Autodock 4.2.623 (<https://www.chpc.utah.edu/documentation/software/autodock.php>), polar hydrogens were removed and their molecules were assigned with the Gasteiger parameters [24].

Table 1. Herbicides used in docking simulations

Herbicide	Molecular Formula	Metabolic Rout	Structure	Access code
Bentazon e-Sodium	C ₁₀ H ₁₂ N ₂ O ₃ S	Benzothiadiazinone		ZINC 05442053
Metsulfuron n-Methyl	C ₁₄ H ₁₅ N ₅ O ₆ S	Sulfonylurea		ZINC 01532069

The OsJGSTU4 theoretical model, was converted to .pdbqt file in Autodock, hydrogens and Kollman parameters were added [25]. The GSH was treated as a cofactor. Docking simulations were run on the Autodock 4.2.6 program (<https://www.chpc.utah.edu/documentation/software/autodock.php>) and the Lamarckian genetic algorithm (LGA) was chosen. The simulations had the following parameters: 10,000 replicates, energy analyzes per 1,500,000 and 27,000 generations, population size of 150 and mutation rates and crossing-over of 0.02 and 0.08 respectively. The 10 conformations were generated that were ranked based on the lowest energy and analyzed in the VMD [21] (<http://www.ks.uiuc.edu/Research/vmd/>).

Electrostatic Potential Map

The molecular structure of the metsulfuron methyl molecule was obtained through the GAUSSVIEW 5.0 program. Then, this structure was submitted to quantum calculations of molecular geometry and harmonic frequency using the HF / 6-31G method in the GAUSSIAN 09 program package [27]. By the results of these calculations, the metsulfuron methyl electrostatic potential map was analyzed.

RESULTS

Model Quality Evaluation and Validation

The x-ray diffraction template *Pt*GSTU30 (PDB ID: 5j4u.1) presented 2.25 Å resolution [28], and acceptable identity value (46,73%), revealing a homology between the *Os*GSTU4 and *Pt*GST30 proteins appropriate for the modeling what was perceived by the results with other Tau class used by Kilili [29]. The alignment presented a score of 210 bits and an e-value of 6e-67. The Figure 1 shows conserved regions

between *OsJGSTU4* (*Oryza sativa* L. Japonica glutathione S-transferase tau 4) and *AtGSTU* (template) sequences, revealing important anchor residues like GLN 75 (black arrow on position 84) and LYS 111 (black arrow on position 120) conserved, while HIS 54 (black arrow on position 63) and LYS 56 (black arrow on position 65) are not conserved, as shown on Figure 1.

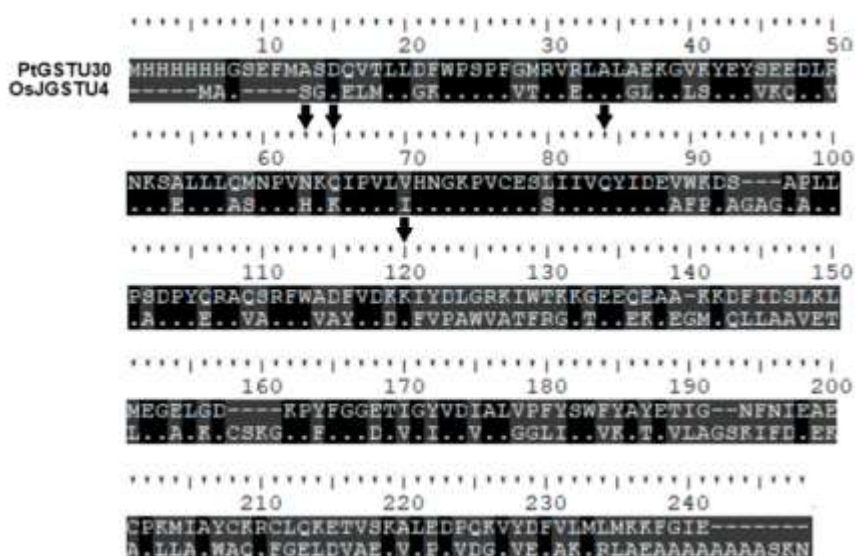


Figure 1. Alignment showing conserved sequences between *OsJGSTU4* (*Oryza sativa* Japonica glutathione S-transferase tau 4) and *PtGSTU30* (template) sequences (“.” Identify matches).

According to Laskowski [19,30], a reliable predicted model are supposed to display over than 90% of residues situated in core regions of Ramachandran plot (A, B and L), for Ho [31] some residues as glycine and proline has predictable and distinct distribution on the Ramachandran plot, as they present different stereochemical patterns. The stereochemical quality was accessed considering the Laskowski [28] critters, the Ramachandran graph showed 94.57% (418/442) of the residues in favorable regions, 100% (442/442) of all residues were in allowed regions as shown in Figure 2a., a model validation results by Maia and Nadvorny [32] had 93.9% of the residues in allowed regions and generated a satisfactory and validated model. Figure 2b. shows the local evaluation of the energy values along the polypeptide chain, and was shown predominantly in the negative region of the graph, revealing good stability of the theoretical model. The Z-score calculated by the server was -7.87 for the theoretical model, and approached it to models elucidated by NMR (Nuclear Magnetic Resonance) technique (Figure 2c.).

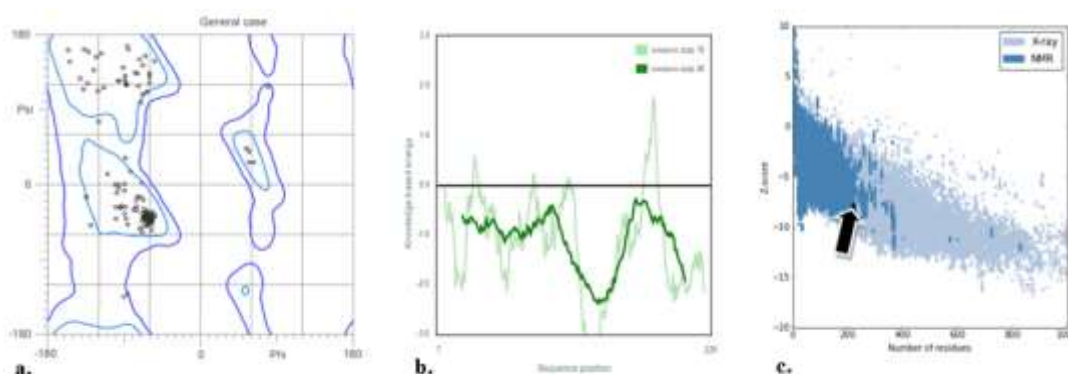


Figure 2. a. Ramachandran plot for *OsJGSTU4* residues. b. Energy local model quality c. Z-score overall quality (black arrow).

According to Melo [20], van Gunsteren and Berendsen [33], ANOLEA and GROMOS the results were generally negative values, revealing a model with stable energy values, such as the results showed by Hamid [34].

Molecular Docking

The docking results for bentazon herbicide and the OsJGSTU4 ranked ten possible complexes based on intermolecular energy and binding energy scores (which were more stable than metsulfuron) the H-bonds formed by the protein and the herbicide molecule, and their respective distances have been identified, as shown in the Table 2 [22].

Table 2 Docking energies for the ten best ranked OsJGSTU4-bentazon complexes (bentazon molecule is identified as "<O>").

Complex	Binding energy (Kcal/mol)	Intermolecular energy (Kcal/mol)	H-bonds (Reside atom – Ligand atom)	Distance Å
1	-3.25	-3.55	LYS 111 :HZ1 - <O> 0:O2	1.68
			LYS 55 :HZ3 - <O> 0:HZ3	2.52
			VAL 53 :O - <O>0:O1	3.0
2	-3.23	-3.53	LYS 111 :HZ1 - <O> 0:O2	1.67
			LYS 55 :HZ3 - <O> 0:HZ3	2.53
			VAL 53 :O - <O>0:O1	3.02
3	-3.24	-3.53	LYS 111 :HZ1 - <O> 0:O2	1.65
			LYS 55 :HZ3 - <O> 0:HZ3	2.54
			VAL 53 :O - <O>0:O1	3.13
4	-3.26	-3.55	LYS 111 :HZ1 - <O> 0:O2	1.67
			LYS 55 :HZ3 - <O> 0:HZ3	2.53
			VAL 53 :O - <O>0:O1	3.07
5	-3.24	-3.54	LYS 111 :HZ1 - <O> 0:O2	1.65
			LYS 55 :HZ3 - <O> 0:HZ3	2.54
			VAL 53 :O - <O>0:O1	3.07
6	3.24	-3.53	LYS 111 :HZ1 - <O> 0:O2	1.66
			LYS 55 :HZ3 - <O> 0:HZ3	2.53
			VAL 53 :O - <O>0:O1	2.96
7	-3.24	-3.54	LYS 111 :HZ1 - <O> 0:O2	1.65
			LYS 55 :HZ3 - <O> 0:HZ3	2.54
			VAL 53 :O - <O>0:O1	3.06
8	-3.25	-3.55	LYS 111 :HZ1 - <O> 0:O2	1.67
			LYS 55 :HZ3 - <O> 0:HZ3	2.52
			VAL 53 :O - <O>0:O1	3.08
9	-3.24	-3.53	LYS 111 :HZ1 - <O> 0:O2	1.65
			LYS 55 :HZ3 - <O> 0:HZ3	2.55
			VAL 53 :O - <O>0:O1	3.19
10	-3.25	-3.55	LYS 111 :HZ1 - <O> 0:O2	1.67
			LYS 55 :HZ3 - <O> 0:HZ3	2.52
			VAL 53 :O - <O>0:O1	3.08

These results shows that herbicide-binding to catalytic site is possible to be performed by OsJGSTU4. All the complexes generated for OsJGSTU4-bentazon showed hydrogens bonds between betazon and the surrounding residues lysines 55, 111 and valine 53 formed H-bonds among molecules what suggest that this are anchor residues (showed in Figure 3C) as shown in Figure 3.

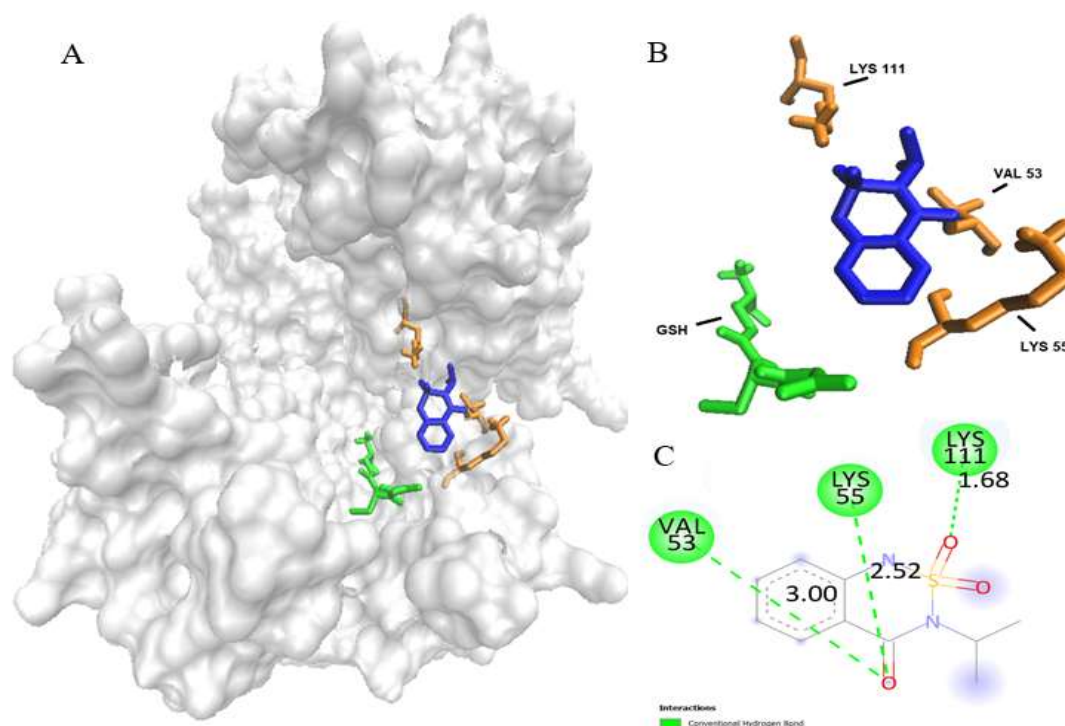


Figure 3. A Docked atoms and residues identification (4 Å cutoff) around bentazon sodium herbicide (<0>) visualization by Visual Molecular Dynamics, B Representation of the approximate residues of the binder, with respective binding distances, C Identification of residues at a distance of 4 angstroms with Discovery Studio Visualizer.

The residues founded showed 10/10 results suggest that the residues are anchor residues for the metabolization process. A work done by Schröder and Collins[37] shows that herbicides like bentazon only can be conjugated among GST after cytP450 enzyme activation, Shimono et al. [38], corroborates that GST's and CYP where expressed during bentazon treatment. Is possible that *OsJGSTU4* present a detoxifying potential for bentazon herbicide.

The, metsulfuron docking complexes showed 2/10 conformations that displays hydrogen bonds with the detoxifying subunit glutathione [12], indicating conjugation glutathione conjugation (Table 3).

Table 3 Docking energies for the ten best ranked OsJGSTU4-metsulfuron complexes (metsulfuron molecule is identified as "<0>").

Complex	Binding Energy (Kcal/mol)	Intermolecular Energy (Kcal/mol)	H-bonds (Reside atom – Ligand atom)	Distance Å
1	-2.34	-4.13	GLN 134: O – <0> 0:N3	3.15
			LYS 111: HZ1 – <0> 0:O5	1.72
			LYS 111: HZ2 – <0> 0:N1	1.79
			LYS 56: HZ1 - <0> 0:O6	1.95
2	-2.31	-4.1	LYS 111: HZ1 – <0> 0:O2	1.76
			LYS 111: HZ3 – <0> 0:O6	2.07
			LYS 56: HZ1 - <0> 0:O3	1.85
3	-2.67	-4.46	ARG 121:HH22 - <0>0:O3	2.02
			ARG 121:HH12 - <0>0:O2	2.03
			LYS 55:HZ2 - <0>0:O6	2.14
			LYS 56 HZ1 – <0> O:O3	2.37
4	-2.39	-4.18	ARG 225 HH11 – <0> 0:N2	1.99
			ARG 121:HH22 - <0>0:O3	1.87
			ARG 121:HH12 - <0>0:O5	2.44
5	-2.4	-4.19	GSH 1:H12 – <0>0:N3	2.05
			LYS 56:HZ1 - <0>0:O2	2.05
			LYS 111: HZ1 – <0> 0:N5	3.04
			HIS 54: HD1 - <0>0:O5	2.72
6	-2.41	-4.2	LYS 55:HZ2 - <0>0:O2	1.81
7	-2.38	-4.17	LYS 111: HZ1 – <0> 0:O3	2.01
			LYS 111: HZ3 – <0> 0:O5	1.72
			LYS 56:HZ1 - <0>0:O2	1.70
8	-3.18	-4.97	LYS 56: HZ1 - <0> 0:O2	1.56
			LYS 111: HZ1 – <0> 0:O3	1.88
			GSH 1:H12 – <0>0:N3	2.13
9	-2.65	-4.44	ARG 121:HH22 - <0>0:O3	1.74
10	-2.56	-4.35	ASP 110:O - <0>0:N3	2.91
			LYS 111:HZ1 - <0> 0:O2	1.63
			LYS 56:HZ1 – <0> 0:O4	1.77
			LYS 56:HZ2 – <0> 0:O5	2.33

The anchors residues seems to be two lysines at 56 and 111 positions, and arginines at 121 and 225 positions interact through hydrogen bonding, all residues located on the catalytic G-site of the enzyme (Figure 3) and a GSH through covalent bound listed in Table 3.

It is probably that OsJGSTU4 metabolizes metsulfuron herbicide through GSH-conjugation[12] and the best distance result (eighth place) is showed in Figure 4.

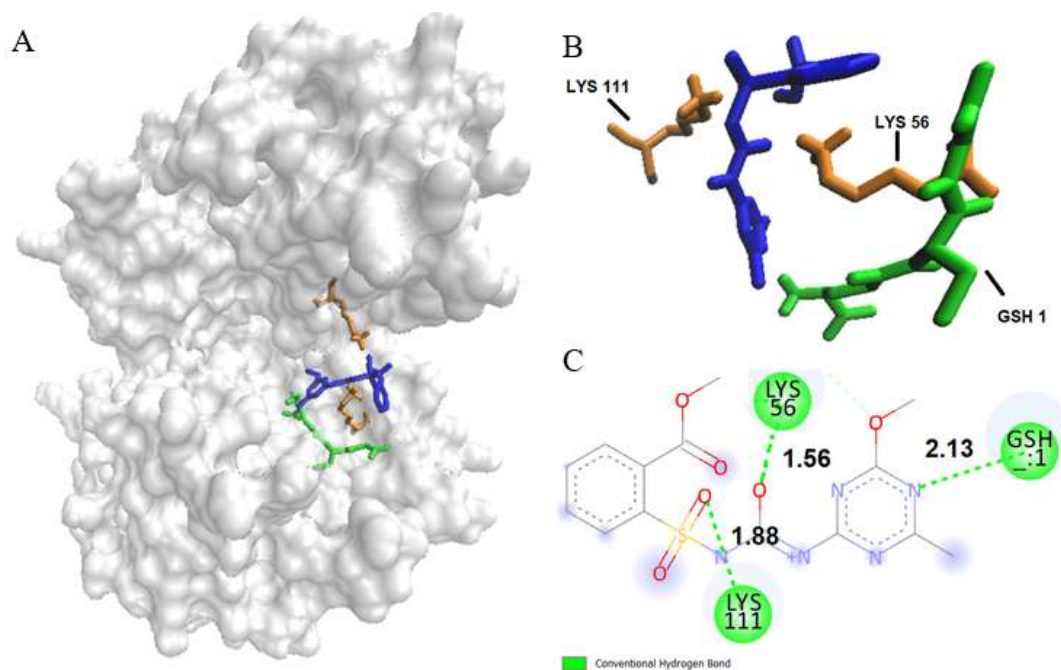


Figure 4. A Docked atoms and residues identification (4 Å cutoff) around metsulfuron-methyl herbicide (<0>) visualization by Visual Molecular Dynamics, B Representation of the approximate residues of the binder, with respective binding distances, C Identification of important residues at a distance of 4 angstroms (with respective bonding distances) visualization by Discovery Studio Visualizer

A work done by Lajmanovich and Junges [39] showed a correlation between metsulfuron methyl and the superfamily of GST expression, another work using *Triticum aestivum* organism done by Lu et al. [40] corroborates with the results above mentioned, its possible that *Osgstu4* is one of the superfamily members that suffer the same expression effect indicating the correlation between the herbicide metsulfuron and *Osgstu4*.

Although all residues displayed in docking tables for bentazon and metsulfuron results are located near to the G binding site, according to GHECOM (http://strcomp.protein.osaka-u.ac.jp/ghecom/cgi-bin/submit_ghecom.cgi) server prediction [35,36] as shown by Figure 5.

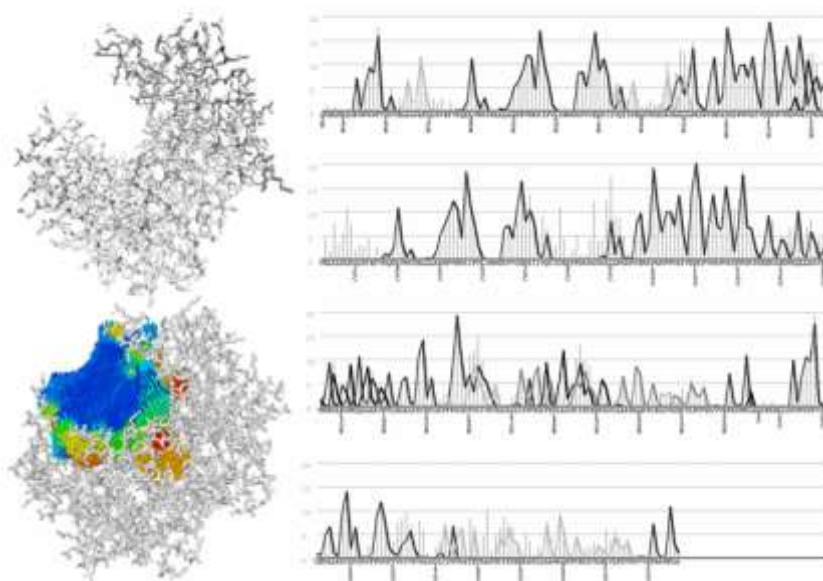


Figure 5. Best theoretical protein (*Osgstu4*) pockets representation (dark spheres region) and the representation of the best result pocket along the polypeptide chain (must high and dark curves) using mathematical morphology.

Electrostatic potential map

Quantum calculations are able to provide detailed information of a particular molecule. This is due to electronic parameters are one of the main factors that rules the interaction between the components of a molecular system. In this context, the molecular electrostatic potential map (MEP) appears as an alternative in order to understand the electrostatic contribution of the environment. The interaction of metsulfuron methyl with the protein can occur by different means, such as hydrogen bonds or electrostatic interactions. One way to assess this issue is by mapping the electrostatic potential. This type of analysis is able to demonstrate the most positive (red), negative (blue) and neutral (green) molecular regions. In this way, we can verify the molecular sites with greater probability of interaction with the more positive or negative regions of the external environment, including the protein.

The Figure 6 clearly shows the presence of electrostatic interactions. Regions of high negative density are observed around sulfur-bound oxygen, while positive sites are more strongly present in the nuclei located at the molecular center.

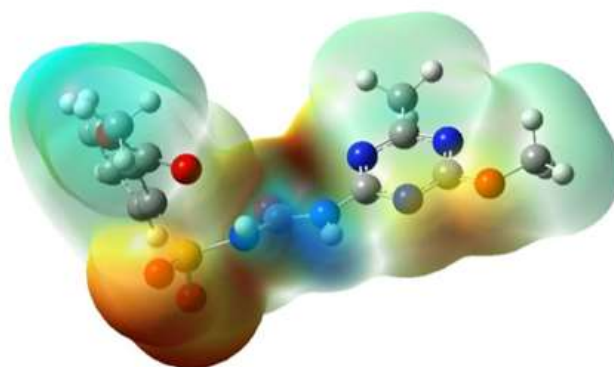


Figure 6. Electrostatic potential of metsulfuron methyl herbicide.

This charge distribution expresses the high probability of molecular interactions occurring between the herbicide and the protein. The negative charges of the chemical structure induces interactions with the negative charged amino acids Lys, Arg and His, from the catalytic site. Thus, in addition to the presence of hydrogen bonds previously discussed, we can suggest that electrostatic interactions also contribute to the complexation of metsulfuron methyl and system stability.

CONCLUSION

Here we present a *in silico* evidence that the inhibition enzyme OsJGSTU4 could display an important role in herbicide tolerance/resistance. Once rice is one of the most important crops around the world, bentazon and metsulfuron is largely used in its culture, the OsJGSTU4 can be used directed to metabolize and confers resistance/tolerance to this herbicides. This lead us to conclude that is highly probable that OsJGSTU4 aims to inhibit and/or metabolize bentazon and metsulfuron and confers to rice a herbicide resistance/tolerance to this compound. The residues situated on positions 43, 53, 54, 55, 56, 111, 121 and 225 are important anchor residues, the majority of interactions with lysines near the G site suggest that lysins play an important role during the anchoring of these xenobiotics. The results presented on this research can be extremely useful for bioengineering by the structural and functional information. The OsJGSTU4 can be a biotechnological target for improve herbicide resistance in *Oryza sativa* L.

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