



## DOCKING STUDIES OF STRESS TOLERANT PROTEINS WITH PROTECTIVE MOLECULES

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**Abstract:** The present study was aimed to dock the stress tolerant proteins with the protective molecules. The stress tolerant proteins namely hexokinase, citrate synthase, phosphofructokinase from *Selaginella moellendorffii*, transketolase, transketolase 7 from *Craterostigma plantagineum* which can interact with the protective molecules trehalose sugar and growth regulating compounds IAA and tryptophan. The docking studied was performed by using the docking tool GOLD. The active sites were predicted using Q-site finder. The results were analyzed based on the binding compatibility of H bonds and the interactions energy was mentioned as k.cal/mol.

**Keywords :** The results were analyzed based on the binding compatibility of H bonds and the interactions energy was mentioned as k.cal/mol.

### INTRODUCTION

Carbohydrate plays a very important role to protect the cells due to the accumulation of sugars; their abundance was dramatically changed during dehydration and rehydration [1]. The disaccharide stress response to sugar trehalose plays a key role as osmoprotectant to protect cells, leaves, protein, enzymes from drought [2-3]. Rosa and James [4], Gabriel *et al.*, [5] studied that trehalose as the molecular chaperone protects the citrate synthase from dehydration. During desiccation the trehalose sugar perform stabilization reflects that the substitution for solvent water in H bonding to proteins and prevent lipid transition at membrane, and prevent the aggregation of denatured proteins and form a glass like structure around the proteins [6].

The plant growth regulator IAA present in all plants as free forms or sometimes it can bind with proteins and amino acids, when the auxin bind with the proteins it makes more rigid and stabilized the molecules [7]. IAA concentrations highly occurred during desiccation in *Craterostigma wilmsii* [8]. Auxin protects the plants from various abiotic stresses [9 - 12]. The auxin synthesis and transport offered the plant to adapt various stress [13]. Amino acids deposition has been observed in many plants exposed to abiotic stress [14]. Citrate synthase is an enzyme responsible for the catalyzing the reaction of the citric acid cycle the condensation of acetyl-coA and oxaloacetate to form citrate [15]. John *et al.*, [16] observed the trehalose mediated high level of protection to the phosphofructokinase during dehydration. Phosphofructokinase involved in the regulation of glycolysis in animals [17], plants [18], insects [19], bacteria [20] and yeast [21]. Hexokinase is an enzyme converts glucose into glucose 6- phosphate by phosphorylation. Hexokinase inhibited by its own product of glucose- 6- phosphate glucose [22]. During dehydration the hexokinase activity linked to stress response sugar accumulation lead to decrease the glucose concentration at the desiccated state of *Sporobolus stapfianus* and *X. viscosa* [23]. Transketolase involved two reactions in pentose phosphate pathway, converts ribulose 5-phosphate and xylulose 5- phosphate to

glyceraldehydes' 3 phosphate and sedoheptulose 7- phosphate and also it converts xylulose 5- phosphate and erythrose 4 -phosphate to form glyceraldehyde 3- phosphate and fructose phosphate.

Docking is the interaction process between the orientation of target protein and ligands molecule to produce the stable compound [24]. Orientation offered the strength of association or binding affinity between the target protein and ligands molecules [24]. At present number of docking tools are available in the market. In the present study the GOLD (Genetic Optimization of Ligand Docking) based on genetic algorithm (GA) was employed for the docking of the plant compounds. Deepika *et al.*, [25] confirmed that hydrogen bonding interactions play an important role for stability of the complex. Feruloyl CoA was docked with Cinnamoyl CoA reductase (CCR) of *Leucaena leucocephala* using GOLD [26]. Similarly the *Aeromonas hydrophila* efflux B protein was docked with (EPIs) 1-(1-naphthylmethyl)-piperazine (NMP) and phenyl-arginine-β-naphthylamide (PAβN) [27] and STAT4 protein with Flurbiprofen [25]. Docking study was carried out IAA with lectin protein obtained from *Canavalia maritima* seeds [28]. With this knowledge the present study was aimed dock the following proteins namely hexokinase, citrate synthase, phosphofructokinase from *Selaginella moellendorffii*, transketolase, transketolase 7 from *Craterostigma plantagineum* with trehalose sugar, IAA and tryptophan has been studied using the dock tool GOLD.

### Materials and Methods

**GOLD** (Genetic Optimization of Ligand Docking)

GOLD score can be calculated

Gold score = S (hb\_ext) + S (vdw\_ext) + S (hb\_int) + S (vdw\_int)

Where S (hb - ext) is the protein-ligand hydrogen bond score, S (vdw - ext) is the protein-ligand van der Waals score, S (hb-int) is the score from intermolecular hydrogen bond in the ligand and S (vdw-int) is the score from intermolecular strain in the ligand.

### Active Site Prediction

After prediction of proteins, the possible binding sites of citrate synthase phosphofructokinase, hexokinase, transketolase and transketolase 7 proteins were searched using Q-site finder (<http://bmbpcu36.leeds.ac.uk/qsitfinder/>).

#### The binding active sites were obtained for citrate synthase includes.

CYS23, GLY24, GLY25, LEU26, CYS27, VAL87, SER88, ARG89, GLY113, GLY114, ASN115, GLY116, SER117, LYS142, THR143, ILE144, ASP145, ASN146, ASP147, ILE148, LEU149, THR154, PHE157, LYS186, LEU187, MET188, ARG190, GLN191, SER192, GLY193, PHE194, ILE195, GLU244, GLY245, ALA246, GLN248, THR257, ASP258, ALA259, SER260, GLY261, ASN262, PRO263, ILE264, LEU265, GLY266, ASP267, ALA268, TYR291, ASP293, PRO294, THR295, TYR296, MET297, ILE298, ARG299, ALA300.

#### The binding sites were obtained for phosphofructokinase includes.

MET1, ASN2, SER26, ILE27, VAL28, SER29, ALA30, ALA31, SER32, GLY40, GLY41, SER42, GLY43, SER44, LEU45, THR46, VAL47, VAL48, ASP49, PHE58, ALA59, ILE60, SER61, ASP62, GLY63, GLY64, THR65, VAL66, ASN67, ALA68, THR69, ASP70, PHE71, LYS72, GLY89, TYR90, LEU91, ASN92, THR93, ALA94, PRO95, VAL96, ARG97, SER98, SER99, ILE100, SER101, TYR102, ILE103, ASP104, GLY105, LYS107, ILE109, LEU110, ARG111, TYR112, ARG113, GLY114, TYR115, MET134, TYR135, GLY136, GLU279, HIE280, GLU281, MET282, ASN283, CYS284, ALA287, HIE291, HIE354, ARG355, VAL356, LYS358, ARG445, ARG446, SER447, HIE450, SER451, ARG452, HIE453, TRP454, TYR455, PRO465, ARG469, VAL471, ALA475

#### The domain sites were retrieved for hexokinase includes.

ASP103, LEU104, GLY105, GLY106, THR107, ASN108, PHE109, ARG110, VAL111, GLN126, TYR128, LYS129, GLU130, VAL131, SER132, ILE133, PRO135, MET138, THR177, SER179, PHE180, PRO181, THR196, LYS197, GLY198, PHE199, ASN231, ASP232, THR233, THR236, GLY239, GLY240, ILE252, LEU253, GLY254, THR255, GLY256, SER257, ASN258, GLU317, SER321, GLY322, MET323, ARG358, THR359, PRO360, SER363, ASP455, GLY456, GLY457, LEU458, TYR459, GLU460, HIE461, TYR462, ARG488, LEU489, SER490, LYS491, ASP492, GLY493, SER494, GLY495, ILE496.

#### The active sites obtained for transketolase were as follows:

MET59, PHE61, ASN62, LYS64, ASN65, PRO66, TYR67, TRP68, PHE69, ARG71, ASP72, ARG73, PHE74, VAL75, LEU89, TYR94, ASP95, SER96, LYS111, PRO116, GLU117, ASN118, PHE119, GLU120, THR121, PRO122, GLY123, VAL124, GLU125, VAL126, THR127, THR128, GLN133, GLY134, SER137, ALA138, LEU141, ALA144, HIE147, LEU148, THR164, GLU321, LYS343, SER344, ILE345, ILE346, THR347, GLY348, GLU349, LEU350, LEU385, GLY387, ILE389, ALA430, ASN433, GLY434, ALA436, LEU437, HIE438, SER439, PRO440, GLY441, LEU442, VAL443, PRO444, ILE462, ALA464, LEU465, SER466, LYS467, ALA468, ARG469, VAL470, ASN502, VAL522, GLU523, ASN524, ALA525, GLY526, ARG527.

#### The domain active site of transketolase 7 was as follows:

TYR51, MET55, PHE57, ASN58, PRO59, LYS60, ASN61, PRO62, TYR63, TRP64, PHE65, ARG67, ASP68, ARG69, PHE70, VAL71, LEU72, TYR90, ASP91, SER92, ASN114, PHE115, THR117, PRO118, GLY119, VAL120, GLU121, VAL122, SER133, HIE158, GLU317, ILE342, GLY430, ALA432, LEU433, HIE434, SER435, PRO436.

### Ligands (trehalose, IAA and tryptophan) for binding

The ligands selected for this study were trehalose, indole acetic acid and tryptophan "Fig. 1, 2, 3". The interaction of trehalose (sugar joined by 1-1 alpha bond  $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 1)- $\alpha$ -D-glucopyranoside. This bond makes the trehalose stable to high temperature and resist to acid hydrolysis. Trehalose form a clustering tendency due to the hydrogen bond formed between one another. This association in water to form a cluster in various sizes [29]. The ring structure Tryptophan is the precursors of IAA, IAA (indole acetic acid) or auxins are compounds with an aromatic ring and a carboxylic acid group, it generates majority of effects in plants. The 2D structure of Trehalose was drawn using ACD chemsketch ([www.acdlabs.com](http://www.acdlabs.com)).

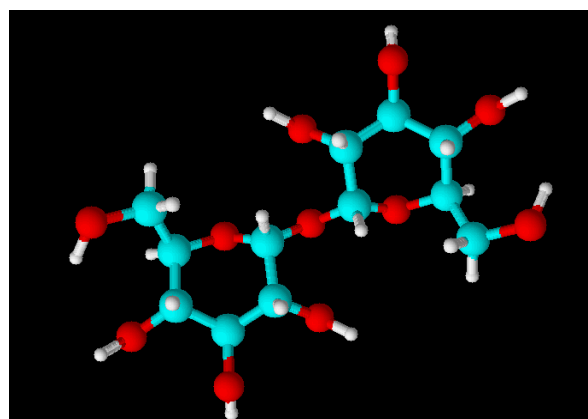


Fig.1: Structure of Trehalose

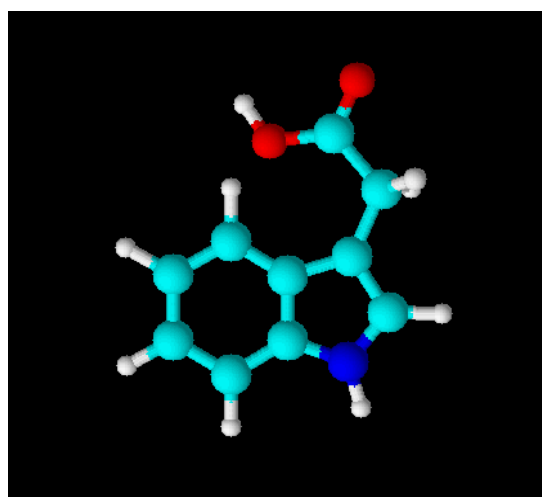
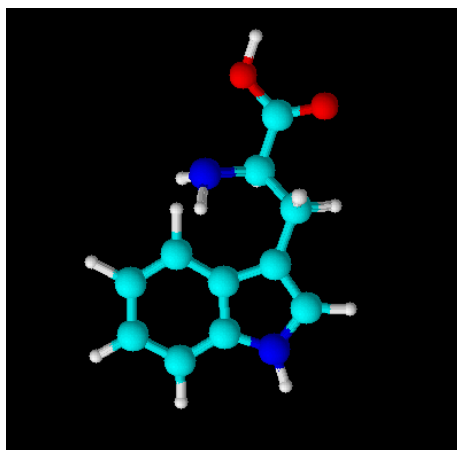
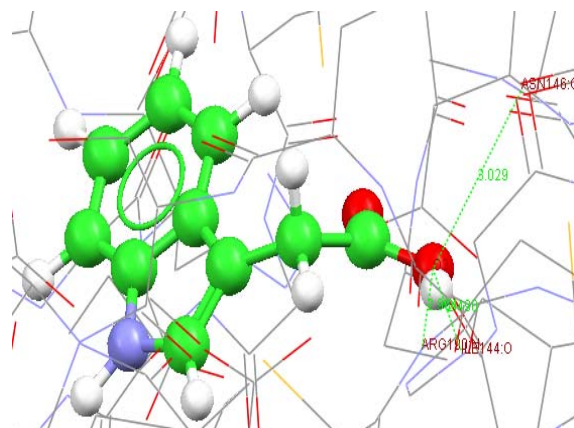


Fig. 2: Indole Acetic Acid



**Fig. 3: Tryptophan**



**Fig .5 : Citrate synthase docking with IAA**

**GOLD docking simulation**

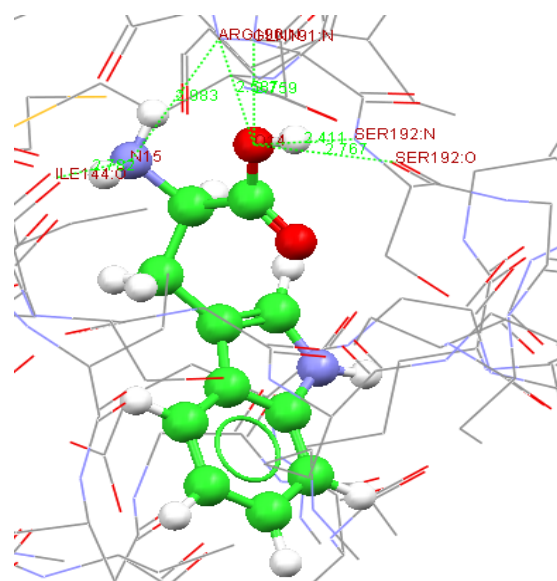
The sugar trehalose was docked with the active site of receptor citrate synthase, phosphofructokinase, hexokinase, transketolase and transketolase7 model using GOLD (Genetic Optimization of Ligand Docking – WWW.CCDC.U.K) [30]. GOLD software based on genetic algorithms (GA) which expose the ligand conformational flexibility and the rational flexibility of selected receptors hydrogen. Grid was drawn for the selected proteins with the center and the size of bounding box 10 Å. The coordination of the closing box (x=121 Å; y = 87 Å z =45 Å) were characterized from the initial set of active site residues.

**Results**

**Molecular docking**

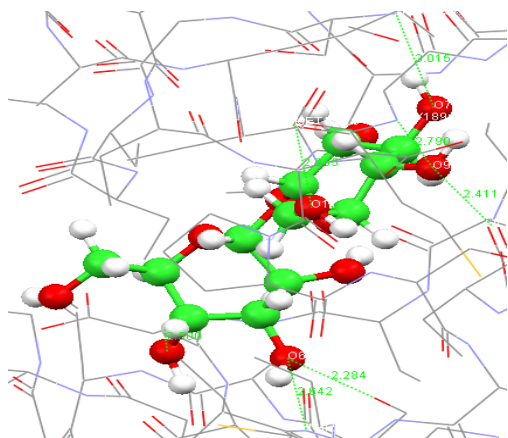
Docking of trehalose sugar with citrate synthase, phosphofructokinase, hexokinase, transketolase and transketolase7 was carried out using GOLD software. The result of docking exposed the best fits of trehalose IAA and tryptophan with the binding receptors of proteins The results were analyzed based on the binding compatibility. Docking energy mentioned as k.cal/mol. 57 amino acids were active site for citrate synthase, 87 amino acids were active site for phosphofructokinase, 64 amino acids were active site for hexokinase, 80 amino acids active site for transketolase and 38 amino acids were active site for transketolase7 were observed.

The docking results of citrate synthase with trehalose six H bonds with the score 54.78 k.cal/mol “Fig. 4”, three H bonds with IAA with the scores of 43.8 k.cal/mol “Fig. 5”, five H bonds with tryptophan with the scores of 49.63 k.cal/mol “Fig.6”.

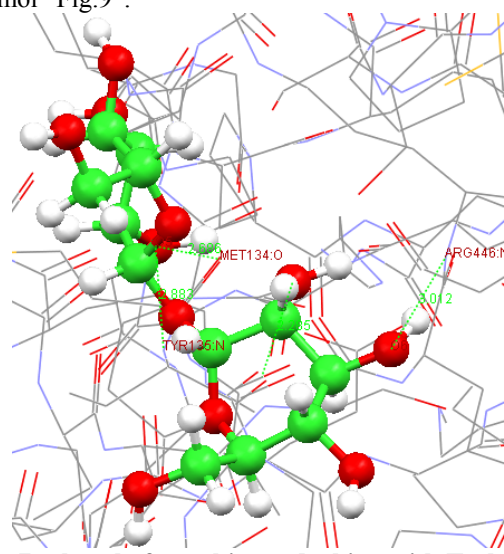


**Fig. 6: Citrate synthase docking with tryptophan**

Trehalose three H bonds with phosphofructokinase with the score of 38.78 k.cal/mol “Fig.7”, IAA two H bonds with PFK with the scores of 45.5 k.cal/mol “Fig.8”, and tryptophan four H bonds with PFK with the scores of 50.55 k.cal/mol “Fig.9”.

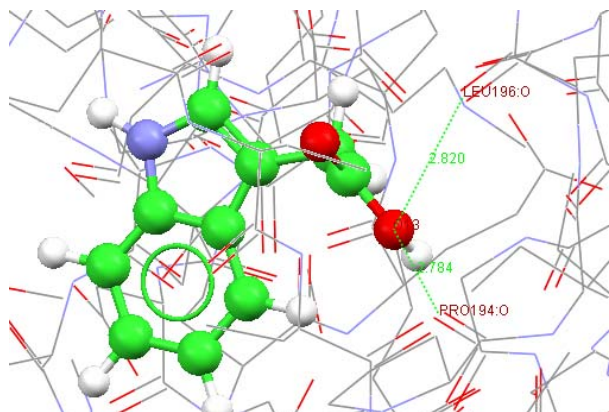


**Fig. 4: Citrate synthase docking with Trehalose**

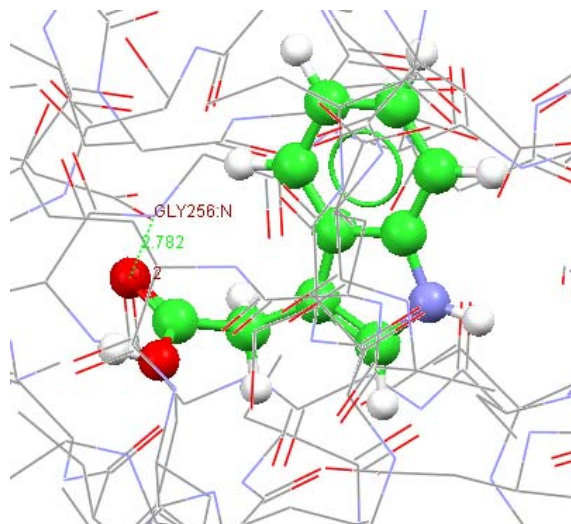


**Fig. 7: phosphofructokinase docking with Trehalose**

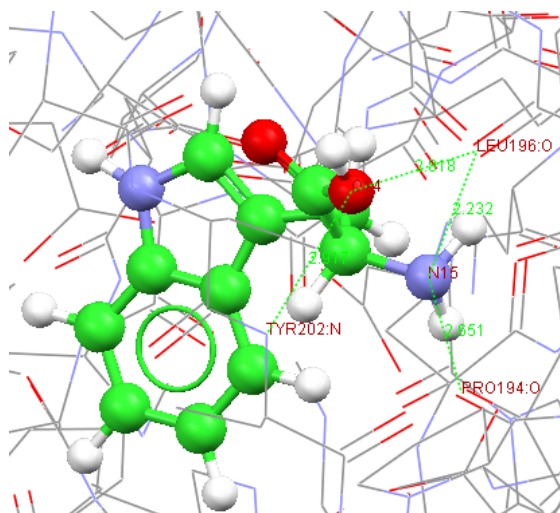




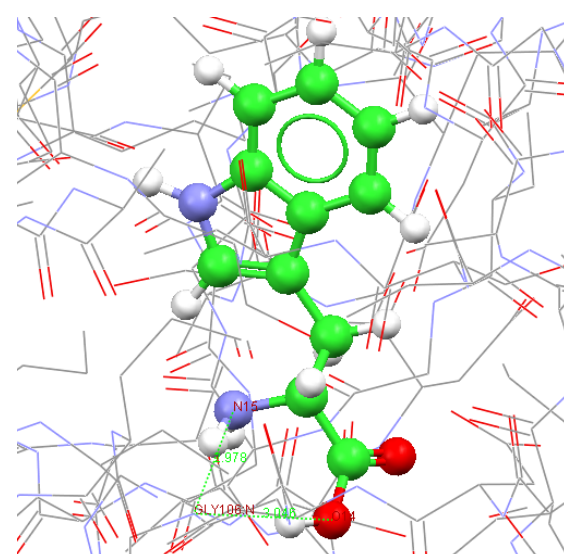
**Fig. 8: Phosphofructokinase docking with IAA**



**Fig. 11: Hexokinase docking with IAA**



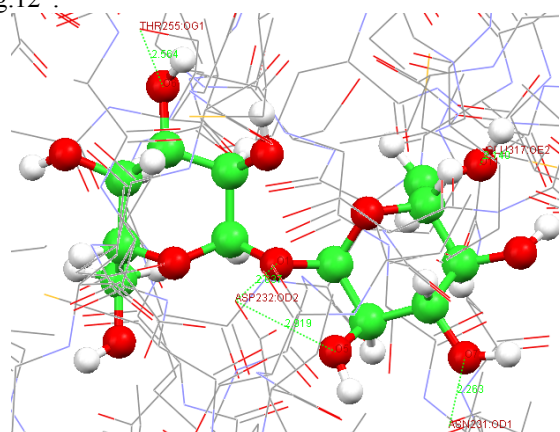
**Fig. 9: Phosphofructokinase docking with tryptophan**



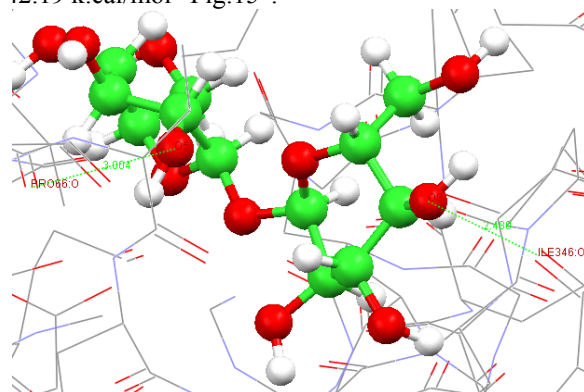
**Fig. 12: Hexokinase docking with tryptophan**

Hexokinase docking with trehalose five H bonds with the score of 41.99 k.cal/mol “Fig.10”, with IAA one H bond with scores of 37.5 k.cal/mol “Fig.11”, with the tryptophan two H bonds with the scores of 42.68 k.cal/mol “Fig.12”.

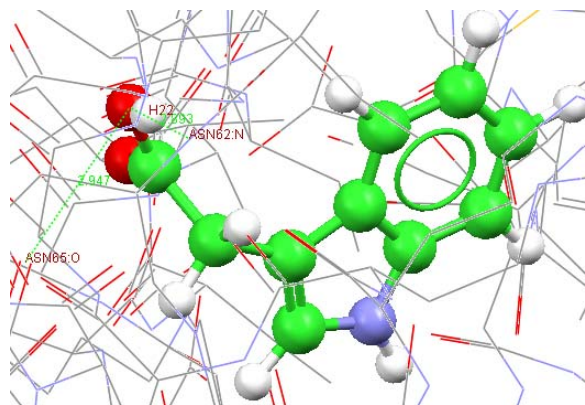
Trehalose two H bonds with transketolase with the score of 35.52 k.cal/mol “Fig.13”, IAA two H bonds with transketolase with the score of 41.29 k.cal/mol “Fig.14”, tryptophan three H bonds with transketolase with the score of 42.19 k.cal/mol “Fig.15”.



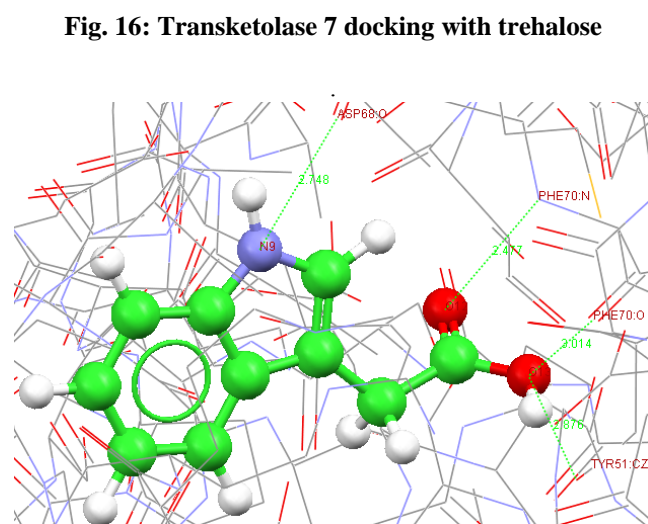
**Fig. 10: Hexokinase docking with trehalose**



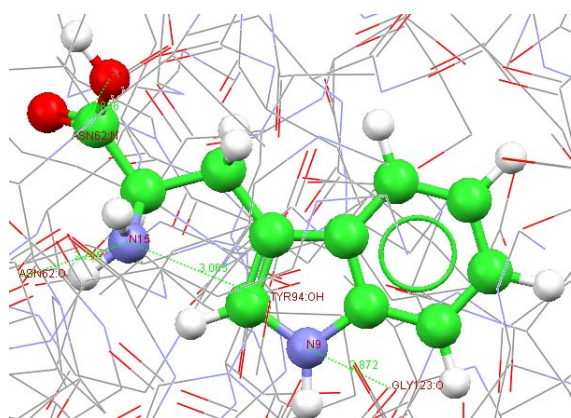
**Fig. 13: Transketolase docking with trehalose**



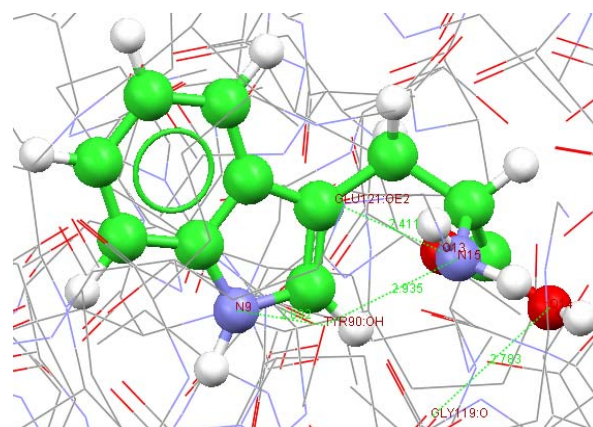
**Fig. 14:** Transketolase docking with IAA



**Fig. 17:** Transketolase 7 docking with IAA

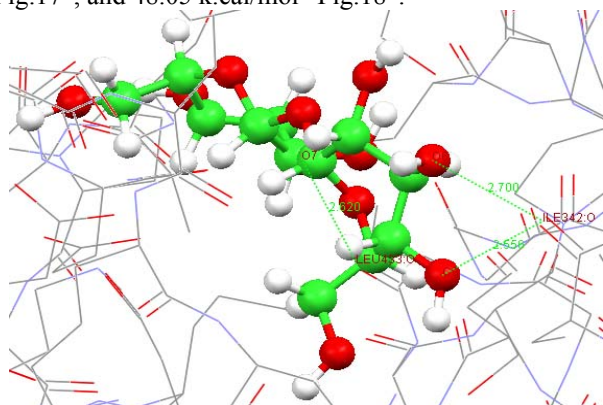


**Fig. 15:** Transketolase docking with Tryptophan



**Fig. 18:** Transketolase 7 docking with tryptophan

Transketolase 7 docking with trehalose three H bonding with the score of 35.34 k.cal/mol “Fig.16”, four H bonds with IAA and tryptophan with the scores of 42.26 k.cal/mol “Fig.17”, and 48.05 k.cal/mol “Fig.18”.



The hydrogen bond interactions and gold scores of docking conformations are depicted in Table - 1. High number of hydrogen bond interactions, it is supposed to be most active residue in interacting complex. Trehalose exhibits the highest Gold fitness with citrate synthase score of 54.78 and interacts with six hydrogen bonds and the best docking conformation showed residues SER, MET, ILE, GLY, THR. This kind of interaction may help to stabilize protein during desiccation.

**Table 1: Docking statistics of substrates and its ligands**

Substrate	Ligand	Atom in protein	Atom in ligand	H-Bond Distance	Gold score
Citrate synthase	Trehalose	SER192:N	O7	3.015	54.78
		MET188:O	O11	2.965	
		ILE144:O	O9	2.411	
		GLY189:N	O9	2.79	
		THR154:N	O6	2.642	
		THR154:OG1	O6	2.284	
	IAA	ASN146:O	O3	3.029	43.8
		ILE144:O	O3	2.490	
		ARG190:N	O3	2.964	
	Tryptophan	ARG190:N	O1	2.983	49.63
		GLN191:N	O1	2.597	
		SER192:N	O1	2.411	
		SER192:O	O1	2.767	
	ILE144:O	N15	2.782		
phosphofruktokinase	Trehalose	ARG446:N	O6	3.012	38.78
		MET134:O	O5	2.686	
		TYR135:N	O5	2.883	
	IAA	LEU196:O	O13	2.820	45.5
		PRO194:O	O13	2.784	
	Tryptophan	LEU196:O	N15	2.232	50.55
		LEU196:O	O14	2.318	
PRO194:O		N15	2.651		
TR202:N		N14	2.917		
Hexokinase	Trehalose	THR255:OG1	O6	2.564	41.99
		ASP232:OD2	O1	2.637	
		ASP232:OD2	O5	2.919	
		ASN231:OD1	O7	2.263	
		GLU317:OE2	O11	2.140	
	IAA	GLY156:N	O12	2.782	37.5
	Tryptophan	GLY106:N	N15	2.978	42.68
		GLY106:N	O1	3.046	
Transketolase	Trehalose	PRO66:O	O5	3.004	35.52
		ILE346:O	O8	2.480	
	IAA	ASN65:O	H22	2.947	41.29
		ASN62:N	H22	2.892	
	Tryptophan	GLY123:O	N9	2.972	42.19
		TYR94:OH	N15	3.065	
ASN62:O		N15	2.235		
Transketolase 7	Trehalose	LEU433:O	O7	2.620	35.34
		ILE342:O	O6	2.700	
		LEU433:O	O6	2.556	
	IAA	ASP68:O	N9	2.748	42.26
		PHE70:N	O12	2.477	
		PHE70:O	O13	3.014	
		TYR51:CZ	O13	2.876	
	Tryptophan	GLY118:O	O14	2.703	48.05
TYR90:OH		N9	2.992		

	TYR90:OH	N15	2.935
	GLU121:OE2	O13	2.411

## DISCUSSION

The proteins of citrate synthase, phosphofructokinase, hexokinase, transketolase and transketolase 7 were used for the docking studies with desiccation tolerant sugar trehalose, IAA and tryptophan. Trehalose is the essential components of the stress adaptive mechanisms [4]. Trehalose protects the macro molecules during desiccation [31]. Auxin regulates plant defence response and growth and structures [32-34]. When IAA interact with sugars mono and disaccharides produced low molecular compounds, as the result of IAA binds with proteins or polysaccharides produced high molecular weight compounds [35]. Tryptophan and IAA possess low binding affinity with lectin proteins *Canavalia maritima* seeds [29]. Branimir [7] studied the auxin binding protein and molecular modelling of auxin and antiauxin proteins. Nan [36] studied the interaction of auxin with S-adenosyl- L-homo-Cys (SAH). The interaction of sugars with citrate synthase involved the thermal protection was proved through *in vitro* studies [4]. The docking results explored the H bonding interaction of trehalose with citrate synthase and phosphofructokinase. Some of the proteins are classified as desiccation tolerance they worked both in dehydration and rehydration condition. Some of the proteins play a role of plant stress adaptations [37]. Stress induced proteins play a role to protect cellular damage involved in antioxidant defence, deposition of sugars, cell wall protection and remodelling [1]. Dehydration and heat stable enzymes occurred in plants throughout desiccation and also involved in carbohydrate metabolism, transcription factors and regulatory molecules [38-40]. Hexokinase is also a heat stable enzyme involved in carbohydrate metabolism during dehydration. Hexokinase is the very important enzyme which can be worked both in dehydration and rehydration, in desiccated state which converts all the simple sugars to stress response sugars sucrose during desiccation [23]. IAA one of the hormones which control stresses and lead to fight against defence response and plant growth response [41]. Transketolase are involved in reductive and oxidative pentose phosphate pathways and its isoforms transketolase 7 responses to synthesis octulose [42]. The genes of transketolase were identified in resurrection plant *C. plantagineum* [43]. Wang *et al.*, [44] studied the protein transketolase response in stress tolerance in *S. lepidophylla* and the enzyme concentration was increased during dehydration of *P. patens*. Sugars protect protein through the H bonding to the polar amino acids in the protein [45], [46]. During interaction of sugars with phospholipids in the dried state can replace water molecules around the polar head groups [47]. Trehalose acts as the effective stabilizing molecules to protect the biological molecules against desiccation [48], [49]. Trehalose possesses unique interactions with biological systems in the dried condition which is based on water replacement hypothesis [46]. The low concentration of sugar cause to break water-water hydrogen bond and promote sugar water hydrogen bonds. The increase concentration above 25-30% of trehalose cause to increase water-water interactions lead to cause more stable and widely hydrogen bonded water clusters, these

concentrations of sugars called as structure makers [50]. Trehalose, mannitol and lactose these combined sugars can preserve some enzyme proteins namely lactate dehydrogenase or phosphofructokinase through water replacements [51]. The present study revealed the interactions of stress tolerant proteins with trehalose sugar and these three stress tolerant enzymes possess weak interaction with trehalose sugar which was proven by in-silico method using GOLD software.

## Conclusion

The selected proteins were undergoes docking with bio molecules viz trehalose, IAA, and tryptophan through H bonds by GOLD software. Out of 10 results, the best one was selected based on the high score. In the docking process hydrogen bonds play a major role in structure and function of biomolecules. In this study SER 192, MET 188, ILE 144, GLY 189, THR 154, ASN 154, 146, ILE 144, ARG190, GLN191, SER192 amino acids in Citrate synthase, MET134, TYR135, ARG446, LEU196, PRO194, TR202 in Phosphofructokinase, THR255, ASP232, ASN231, GLU317, GLY156, 106 in hexokinase. POL66, ILE346, ASN65, ASN62, GLY123, TYR94, ASN62 amino acids transketolase. LEU 433, ILE 342, ASP68, PHE 70 in transketolase7 are structure interaction with ligand molecules

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