Homology Modeling, Docking Studies and Functional Site Analysis of Various Accessory Interacting Proteins of MnSOD of *Nostoc* PCC7120 and FeSOD of *Thermosynechococcus elongatus*

Bikash Thakuria, Phiralang Diengdoh, *Samrat Adhikari[3]

Bioinformatics Centre, Department of Biotechnology, St. Edmund's College, Shillong, Meghalaya, India

Abstract: *Antioxidant enzymes studies have been evolved to be a potent area of study in context to bioinformatics tools. These antioxidant enzymes are produced by the cell to scavenging the stress effect of and external stimuli. In context to the present study, FeSOD and MnSOD have been investigated using bioinformatics tools. The complex biological systems and their behaviours have now become easy to study via the available bioinformatics tools. Homology modelling has emerged as a powerful tool in predicting an unknown structure of a protein and through phylogenetic analysis we can predict a relationship between the two species of cyanobacteria taken in our current study. Modelling and molecular docking studies is an important step in systems biology study. Despite availability of hundreds of known protein sequences, accurate information about their role in pathways is still largely inaccurate. Here, an attempt is made to explore the structural and the interactions of the accessory proteins with the two enzymes viz. MnSOD (from Nostoc sp. PCC7120) and FeSOD (from Thermosynechococcus elongates) based on the maps available from STRING database with the help of molecular docking studies.*

Keywords: Homology modelling, protein motifs, physiological characterization, pathway analysis, molecular docking

1. Introduction

Cyanobacteria, also referred to as blue–green algae are a large and morphologically diverse group of oxygenic phototrophic prokaryotes, which occur in almost every habitat on earth (Thajuddin, N., Subramanian, G., 2005). These groups of bacteria have both beneficial and detrimental properties when judged from a human perspective. Their extensive growth can create considerable nuisance for management of inland waters and at the same time they might be highly toxic (Gorham, P.R., Carmichael J.I.., 1988). As a consequence, the negative aspects of cyanobacteria have gained more research attention and public concern not only concluding them as a potential candidate for bioremediation activities but also throws a limelight intro their deep assets for scavenging the cell vitality & viability by production of oxidative stress enzymes at intracellular levelfor maintaining cell integrity.This process of induction of oxidative stress is generally linked with the generation of free reactive oxygen radicals causing inhibition of microorganism development. Molecular oxygen is however unreactive, but when activated through reduction, forms reactive oxygen species (ROS) such as superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-). ROS interact rapidly with biological molecules (proteins, lipids, DNA) causing oxidative stress which can result in cell death via apoptosis or necrosis (Kannan, K, Jain, S.K., 2000). One among them is the enzyme named Superoxide dismutase (SOD, EC 1.15.1.1) which belongs to a large and ubiquitous family of metalloenzymes that catalyzes the dismutation of a highly toxic and reactive superoxide radical (O_2^-) to hydrogen peroxide (H_2O_2) and oxygen (O_2) molecule through a cyclic oxidation-reduction mechanism. It is an efficient antioxidant enzyme that is found in virtually all O_2 respiring organisms and acts as the preliminary basis of defense mechanism to surpass the oxidative stress rendered by external stimuli (McCord, J.M., Fridovich, I., 1969). Superoxide anion (O_2^-) and nitric oxide (NO) have been involved as apoptosis inducers (Richter C., 1993, Estevez, A.G., Radi, R., Barbeito, L. *et al.*, 1995,Raiji, L., Baylis, C., 1995,Susin, S.A., Zamazami, N., Kroemer, G., 1998) and as an anti-oxidant protective effect of SOD during oxidative stress have also been also reported (Yen, H.C., Oberley T.D., Vichitbandhan S. *et al.*, 1996, Ho Y. S., Magnenat J. L., Gargano M. *et al.*, 1998). These enzymes particularly catalyzes the disproportionation of superoxide anion radical to hydrogen peroxide and molecular oxygen to protect the cells against oxidative damage and regulate the cellular concentration of O_2^- and its reactive progeny under both physiological and pathological conditions (Balasubramanian, A., Das, S., Bora, A. *et al.*, 2012). Generally, SODs have been classified into four major canonical forms depending on the catalytic metals availability, FeSOD, MnSOD, Cu/ZnSOD and NiSOD. Besides these four, a cambialistic Fe/MnSOD also exists (Meier, B., Barra, D., Bossa, F. *et al.*, 1982). The MnSOD enzyme is involved in maintaining nanomolar, physiological levels of O_2^- and its progeny. In a very elegant and comprehensible analysis a more complex role of MnSOD in establishing cellular redox environment and thus biological state of the cell has been evaluated based on thermodynamic and kinetic grounds (Buettner, G.R., Ng, C.F., Wang, M. *et al.*, 2006, Buettner, G.R., 2011).FeSOD is found in prokaryotes and in eukaryotes. In eukaryotes it has been isolated from *Euglena gracilis*(Kanematsu, S., Asada K., 1990) and higher plants. There are two distinct groups of FeSOD the first group is a homodimer formed from two identical 20kDa subunit proteins, with 1-2 gram atom of iron in the active centre and the second is prevalent in most higher plants, as a tetramer of four equal subunits with a molecular weight of 80-9- kDa. Members of this family contain 2-4 gram atoms of iron in the active centre (Alscher, R.G., Erturk, N., Heath, L.S., 2002). Although SOD enzymes

have ubiquitous occurrence and many researchers have envisaged the biological role of this enzyme in context to various prokaryotic and eukaryotic organism but a detailed analysis on the functional role of various proteins which are involved in the scavenging effects have not been address in details. Therefore the present work signifies an attempt to study the cascade of reaction occurring during disproportionation of superoxide anion radical by the MnSOD enzyme in *Nostoc* PCC7120 and FeSOD of *Thermosynechococcus elongatus*using systems biology approach. Most proteins attain their biological functions through specific interactions with other proteins. Thus, the study of protein-proteininteractions and the interfaces that mediate these interactions is of prime importance for the understanding of biological function (Talavera, D., Robertso, D.L., Lovell, S.C., 2011). It is important to understand the enzyme degradation pathway, its components and the interaction taking place in the pathway if we plan to regulate the pathway for increasing the SOD and better production of free radicals. Systems biology being a holistic and a pivotal approach which involves various molecular modeling, metabolic pathways analysis, and regulatory and signal transduction networks for understanding better cellular behaviour. There are also various levels of abstraction at which variety of techniques that has been employed based on the quality and quantity of data available (Papin, J.A., Hunter, T., Palsson, B.O. *et al.*, 2005). An attempt is also made to predict the protein structure of *Nostoc PCC7120* FeSOD using *Thermosynechococcus elongatus* as template since the same is not available in the protein data bank.

2. Methodology

2.1 Sequence Retrieval

The amino acid sequences of *Nostoc* PCC7120 (formerly *Anabaena* PCC7120) sodA *(Accession No. 1GV3)* [Atzenhofer, W., Regelsberger, G., Jacob, U. *et al.* (2002)] and *Thermosynechococcus elongates* sodB: *Accession No.1MY6* [Kerfeld, C.A., Yoshida, S., Tran, K.T. *et al.* (2003)] were retrieved from the Protein Data Bank. A BLASTp [Altschul, S.F., Gish, W., Miller W. *et al.* (1990)]searchof both sodA and sodB were carried outagainstthenon-redundant protein databasewith the default parameters to find suitable templates for multiple sequence alignment and for selecting the best hits for further analysis. Multiple sequence alignment were carried out using ClustalW2[Larkin, M.A., Blackshields, G., Brown, N.P. *et al.* (2007)] to search for the conserved regions obtained from the BLAST. JALVIEW (JAVA alignmenteditor) program [Waterhouse, A. M. *et al.* (2009)]was used to viewmultiple sequence alignment and to constructa phylogenetic tree. The aligned sequences were then used for evaluating the necessary parameters using Blosum62.

2.2 Domain analysis and Motif searching

Domain analysis was performed using the CDART(Geer, L.Y., Domrachev, M., Lipman, D.J. *et al.,*2002) and Motif search was performed using Motif- Scan server(Naughton B.T., Fratkin E., Batzoglou S. *et al.*, (2006) for the identification of putative domains.

2.3. Physicochemical analysis

The physiochemical characterizations were computed using the ExpasyProtParam server (Wilkins M.R., Gasteiger E., Bairoch A. *et al.*, 1999).

2.4. Homology modelling and evaluation

Homologymodeling of the enzyme wasperformed using SWISS-MODEL (Schwede T., Kopp J., Guex N. *et al.*, 2003). Thegenerated structure was validated using PROCHECK (Laskowski R.A., MasArthur M.W., Moss D.S. *et al.*, 1993) and WHATIF (Vriend G., 1990).

2.5. Pathway analysis

Confidence interval map of sodA and sodB accessory proteins were analyzed from STRING database (Franceschini A**.**, Szklarczyk D**.**, Frankild S**.***et al.*, 2013**)** and availability for authentic structures in Protein data bank was checked comparatively in NCBI Entrez, PDB and SWISSPROT Databases..

2.6. Molecular Docking studies

Docking study of both MnSOD and FeSOD enzyme and the accessory proteins were carried out using PATCHDOCK server (Duhovny D., Nussinov R., Wolfson HJ., 2002, Schneidman-Duhovny D., Inbar Y., Nussinov R. *et al.*, 2005) and energy minimization was performed before and after docking using QMEAN server(Benkert. P., Künzli M., Schwede T., 2009,Benkert. P., Tosatto S.C.E. and Schomburg D., 2008).

3. Results and Discussion

3.1. Blastp

The retrieved protein sequences from the protein data bank were subjected for BLAST analysis for the effective selection of the suitable templates for both of the sodA and sodB of *Nostoc* PCC7120 and *Thermosynechococcus elongatus. The* Blast result depicted 15 significant hits of the non-redundant proteins for both of the enzymes. The best hits were selected based on the higher identity with maximum score and lower E-value.

3.2. Phylogenetic analysis

The phylogenetic analysis of sodA and sodB were carried out using ClustalW2 with average distances and Neighbour Joining tree were computed using Blosum62 (**Fig 1**). The studies revealed the evolutionary development of both the SODs amongst the various cyanobacteria. In context to the MnSOD *Leptolvngbya* SD KIOST-1(WP_035993274.1) is distantly related to the other proteins and therefore might have evolved from a different ancestor. Except for *Tolypothrix bouteillei* Iicb1 (gb|KIE12033.1) the other 13 proteins appear to be closely related and therefore share a common ancestor. In context to FeSOD, *Nostoc* PCC 7120 (WP_010997089.1) was observed to be distantly related to the other proteins and might have evolved from a different ancestor. The remaining SOD proteins probablymight have

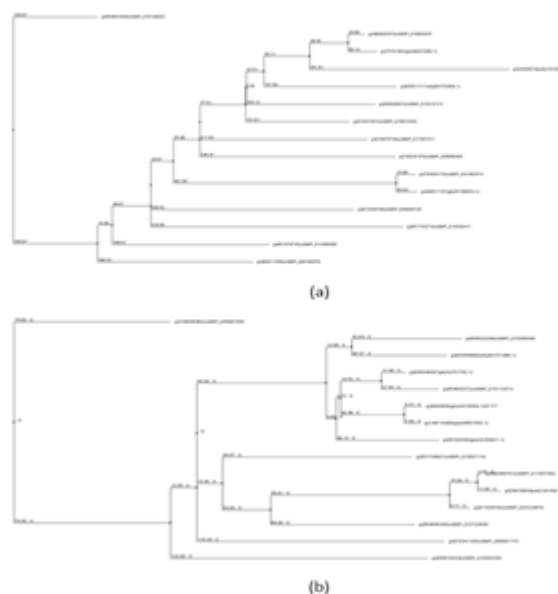evolved from a common ancestor and have further diverged to form closely related clusters of SOD groups.

Figure 1: Phylogenetic tree of of (a)

3.3. Domain analysis and Motif searching

Domain analysis for MnSOD and FeSOD using CDART server and Motifscan revealed the presence of two conserved domains in both the protein sequences (**Fig 2a**). Four hits containing the domains were obtained from MnSOD *viz.* Alpha-hairpin domain (pos: 39-125;raw score: 158.2; N-score: 57.285; E-value: 1.1e-50) belonging to the Sod-Fe-N superfamily and an iron/manganese superoxide dismutases C-terminal domain (pos: 130-234;raw score: 205.7; N-score: 75.358; E-value: 9.3e-69) belonging to the Sod-Fe-C superfamily was observed for the total non-redundant sequences ranging from 284-13453 along with total architectures of 18.
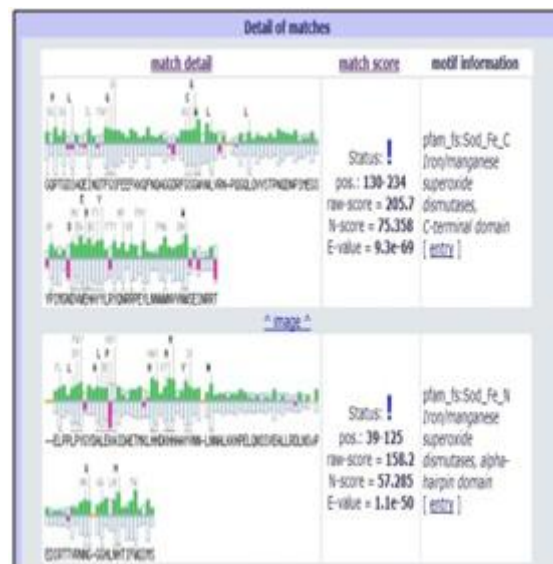

Figure 2a: MnSOD motifs from MotifScan

In the case of FeSOD, also four hits containing the conserved domains were obtained from CDART and MotifScan analysis (**Fig 2b**). Alpha-hairpin domain (pos: 1-87; raw score: 132.0; N-score: 49.096; E-value: 1.7e-42) belonging to the Sod-Fe-N superfamily and an iron/manganese superoxide dismutases C-terminal domain (pos: 92-196; raw score: 232.7; N-score: 84.212; E-value: 1.3e-77) belonging to the Sod-Fe-C superfamily was observed.
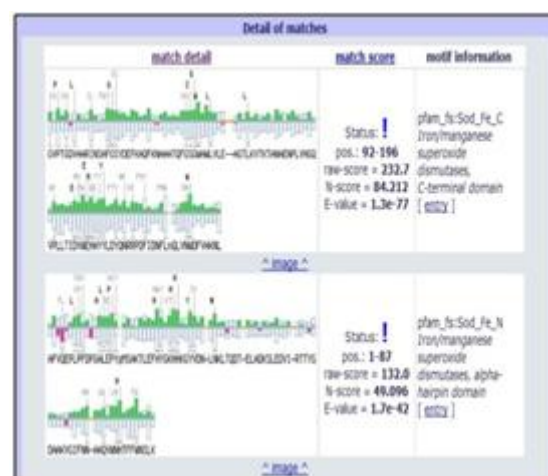

Figure 2b: FeSOD motifs from MotifScan.

3.4. Physicochemical characterization

ProtParam analysis results for *Nostoc* PCC7120 MnSOD and *Thermosynechococcus elongatus* FeSOD revealed the various physical and chemical properties of the two proteins including the molecular weight, theoretical pI, aliphatic index, GRAVY etc. The ProtParam results obtained indicated that MnSOD was an unstable protein with a fairly high instability index (45.96) compared to the stable FeSOD protein with a relatively low instability index (18.91)**Table1**

Table 1: Physiological characterization through ProtParam analysis showing molecular weight, theoretical pI, total number of negatively charged residues, total number of positively charged residues, estimated half life, extinction coefficient, aliphatic index, instability index and Gram Average Hydropathicity (GRAVY).

| Proteins (Accession No.: NCBI/SWISS-PROT)	Molecular weight	Theoretical pI	Total number of negatively charged residues (Asp + Glu)	Total number of positively charged residues (Arg + Lys)	Estimated half-life (w.r.t. E.coli, in vivo)	Extinction coefficients (M^{-1} cm^{-1}, at 280 nm)	Aliphatic index	Instability index (II)	(Gravy)
Manganese superoxide dismutase (1GV3)	28178.0	6.38	25	21	> 10 hours	50420; 1.789	65.28	45.96	-0.858
Iron superoxide dismutase (1MY6)	22084.7	5.44	22	16	> 10 hours	44920;2.034	77.54	18.91	-0.302

3.5. Model evaluation

Protein homology modeling was carried out for both the MnSOD and FeSOD proteins using the Swiss-Model server taking the crystallized PDB structures as queries .i.e. 1GV3 and 1MY6, respectively. For MnSOD, Blastp was carried out against non-redundant database. *Nostoc* PCC7120MnSOD (WP_010994247) with E-value 4e-177 and 99%identity was selected as the target protein. These were then aligned usingClustalW2 and submitted to Swiss-Model server. Altogether two protein structures were modeled, each based on the individual chains of the template dimer. Similarly, *Nostoc* PCC7120 FeSOD (WP_010997089.1) with E-value 1e-102 and 72% identity was selected as the target protein. The ClustalW2 aligned sequences were then submitted to Swiss-Model server. Two predicted protein structures were obtained; each modeled using a separate chain of the template protein. **Fig 3.**

The modeled protein structures obtained from both the MnSOD and FeSOD were subjected to energy minimization and refinement steps using the online QMEAN server followed by Ramachandran plot analysis. The QMEAN Z-score for the two MnSOD models were 2.1.**Fig 3** The Ramachandran plot analysis using Rampage server showed that for the MnSOD model 1, the number of residues in the favored region is 96.7% whereas that in the allowed region is 3.3%.The number of residues in the outlier region is 0%. This means that the modeled structure is a good protein model. MnSOD model2 also showed the same results. For the FeSOD model1, the number of residues in the favored region is 97.2% whereas that in the allowed region is 2.8%.The number of residues in the outlier region is 0%.FeSOD model2 also showed the same results. The modeled protein structures are good stable models.

The ProCheck Ramachandran plot for MnSOD showed that 89.7% of the amino acid residues were found in most favored regions, 9.2% residues in additional allowed regions, 0.6% residues in generously allowed regions and 0.6% residues in disallowed regions. For FeSOD, the ProCheck Ramachandran plot showed that 89.9% residues were found in most favored regions, 8.9% residues in additional allowed regions, 0.6% residues in generously allowed regions and 0.6% residues in disallowed regions. These predicted 3D structures are fairly good protein models. **Fig 3.**

Energy minimization using SwissPdb Viewer and QMEAN server for MnSOD models was found to be e= -31099.742 while for FeSOD it was e= -19807.551
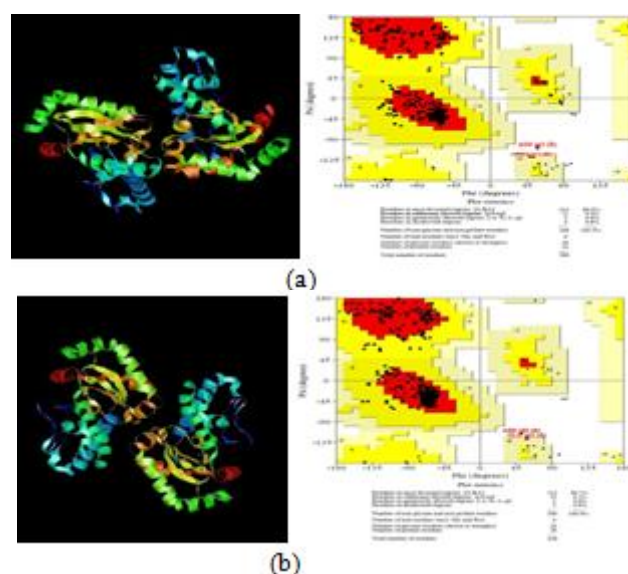
Figure 3: Homology models and Ramachandran Plot analysis of (a) MnSOD and (b) FeSOD

3.5. Pathway analysis

Using STRING 9.1 database, the confidence view showed a number of proteins predicted to interact with the cyanobacterial MnSOD protein with respect to *Nostoc* PCC7120. These proteins were peroxiredoxin (alr4641), preproteintranslocase subunit SecY (secY), AhpC/TSA family protein(alr4404), preproteintranslocase subunit SecG (asl4181), hypothetical protein (alr3090), 30S ribosomal protein S7(rpsG), 30S ribosomal protein S16(rpsP), hypothetical protein (alr0998), preproteintranslocase subunit SecA (secA) and 30S ribosomal protein S15 (rpsO).**Fig 4.**

The confidence view for FeSOD protein with respect to *Nostoc* PCC7120 showed the involvement of the same proteins as in MnSOD but the interactions may differ.

Figure 4: STRING analysis of (a) MnSOD and (b) FeSOD

3.6. Docking studies

In the present study a flexible body docking analysis mode was carried out using the PATCHDOCK server of MnSOD and FeSOD with the available structures of the accessory proteins which were reported to have an interactive part with both the SODs. The molecular docking study has revealed a significant interactions of geometry based algorithm and the computed Global Energy, Docking Score, Attractive VdW, Repulsive VdW, ACE and Hydrogen Bonds parameter are depicted in the **Table. 3** and the docking interactions of the proteins with both of the SODs are shown in **Fig. 5, 6**. The result of docking analysis suggested that the protein 2IPC has shown the highest docking score (Score: 19506) with the projected area covering of the interacting complex (3808.80) followed by the other accessory proteins with the Mn and Fe SOD. The docking results of the protein 3BBN could not be evaluated because of the enormous complexity of the protein structure although the docking score and the area covered were calculated.(Fig and Table in the next page).
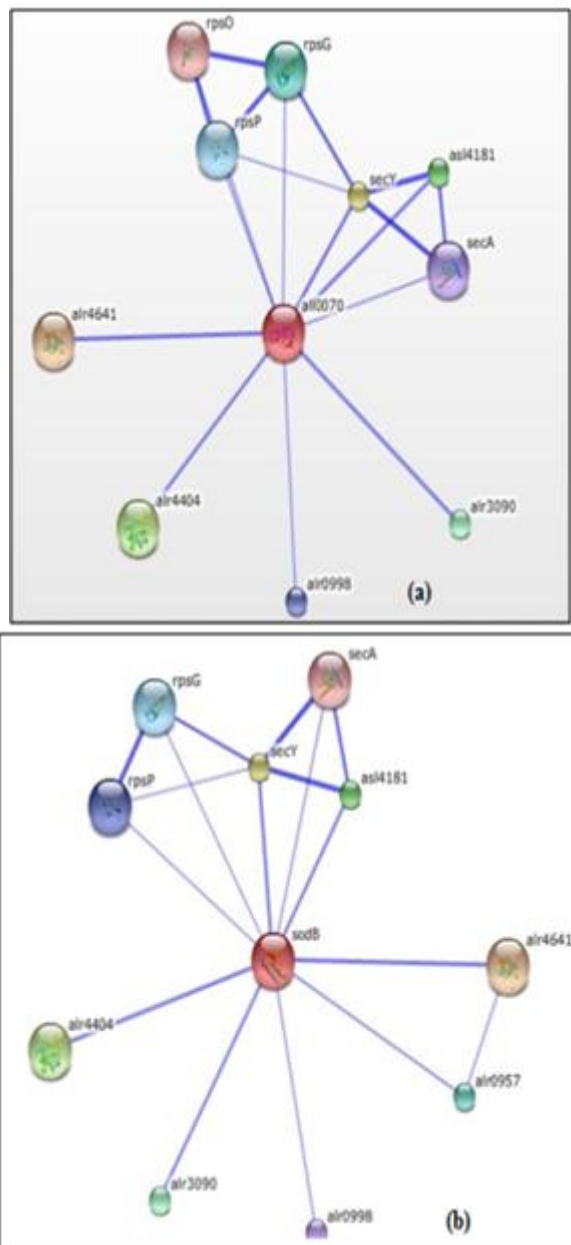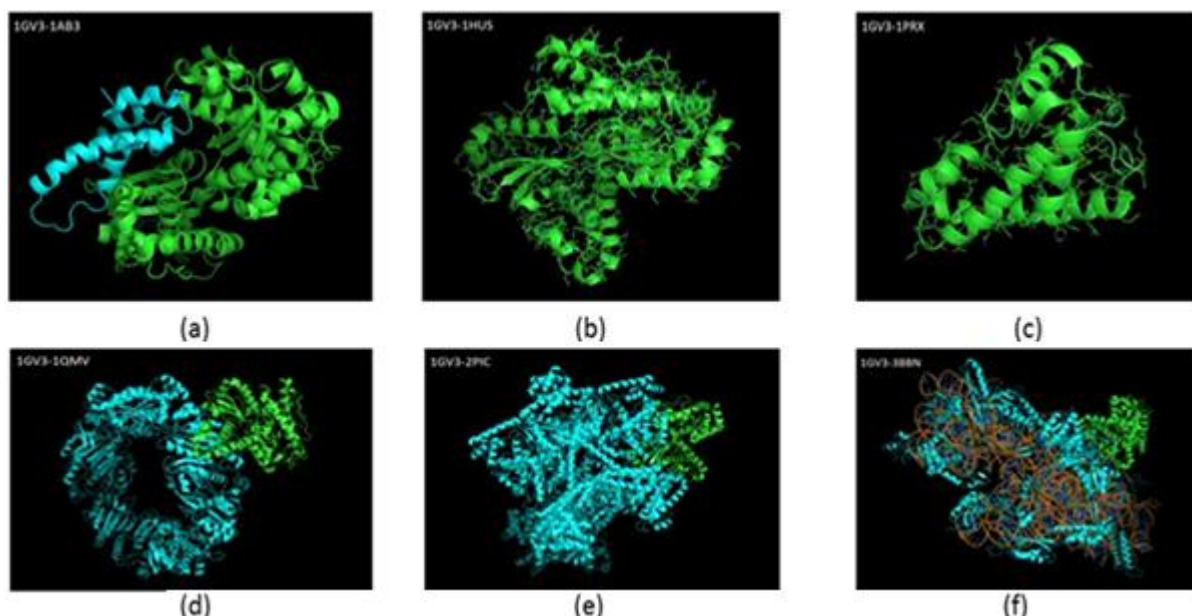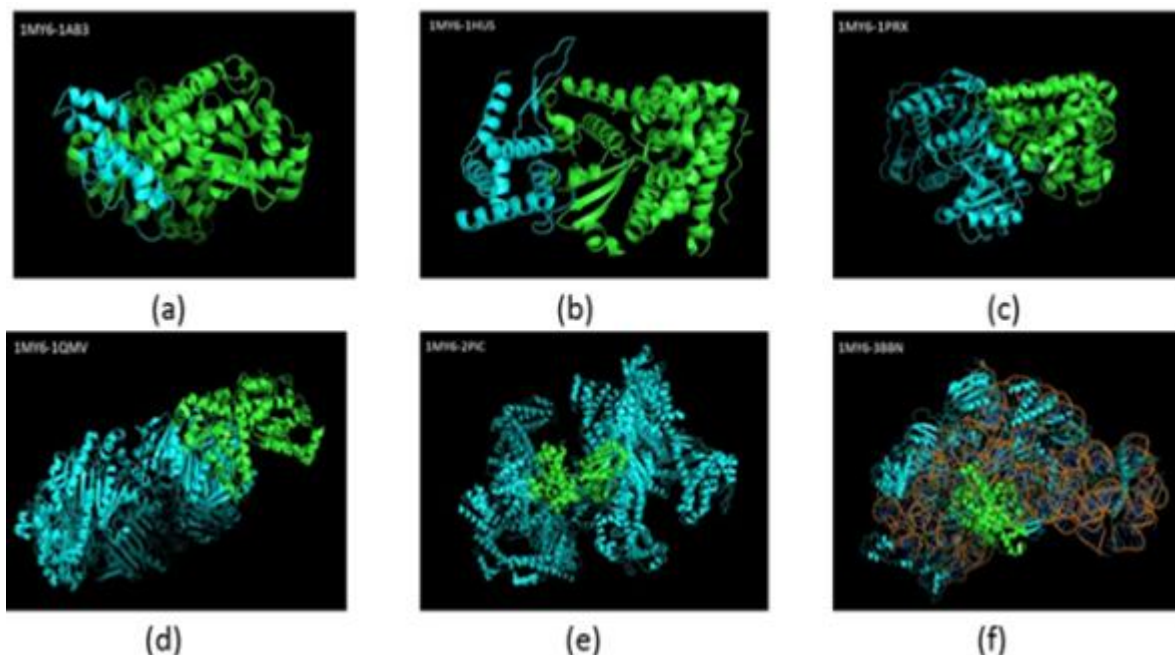

Figure 5: Showing the molecular interactions of MnSOD (1GV3) with its accessory proteins

Figure 6: Showing the molecular interactions of FeSOD (1MY6) with its accessory proteins.

Table 3: (a) and (b) Showing the docking scores of the accessory proteins with both MnSOD (1GV3) and FeSOD (1MY6). The docking refinement for 3BBN was not retrieved as the protein is very big hence only the score and the area covered for docking was retrieved.

Proteins docked with 1GV3	Score	Area	Global energy	Attractive VdW	Repulsive VdW	ACE	HB
1QMV	13414	1578.90	10.82	-2.99	1.13	2.43	-0.27
2IPC	19506	3808.80	11.09	-7.97	8.14	1.99	-0.68
3BBN	16862	2694.70	-	-	-	-	-
1PRX	17022	2518.30	-11.01	-28.85	18.69	6.80	-2.42
1HUS	13984	1833.60	-4.94	-11.11	3.60	4.88	-0.26
1AB3	14360	2010.10	-0.67	-35.47	28.25	12.15	-4.42

(a)

Proteins docked with IMY6	Score	Area	Global energy	Attractive VdW	Repulsive VdW	ACE	HB
1QMV	13414	1578.90	10.82	-2.99	1.13	2.43	-0.27
2IPC	19506	3808.80	11.09	-7.97	8.14	1.99	-0.68
3BBN	16862	2694.70	-	-	-	-	-
1PRX	17022	2518.30	-11.01	-28.85	18.69	6.80	-2.42
1HUS	13984	1833.60	-4.94	-11.11	3.60	4.88	-0.26
1AB3	14360	2010.10	-0.67	-35.47	28.25	12.15	-4.42

(b)

4. Discussion

The integrated behavior of a complex biological process can now be predicted by bioinformatics databases and molecular systems biology tools (Ma H. W., Zeng A. P., 2004, Chellapandi P., Sivaramakrishnan S., Viswanathan M. B., 2010). Homology modeling provided us two fairly good protein models namely 1151 and 1152 for MnSOD and FeSOD respectively and was validated by their corresponding Ramachandran Plots (Laskowski R.A., MasArthur M.W., Moss D.S. *et al*., 1993). The current work shows the presence of the accessory proteins present in both MnSOD and FeSOD and their molecular interactions. All the proteins are similar except for rpsO in MnSOD and alr0957 in FeSOD. On the basis of their behavioral molecular interactions of the accessory proteins with both the superoxide dismutases and the calculated docking analysis, we can predict that both the superoxide dismutases are similar in nature (Schneidman-Duhovny D., Inbar Y., Nussinov R. *et al*., 2005). The docking studies also revealed that protein secA, bearing a PDB code of 2IPC, for both the

superoxide dismutases has the highest docking score, however, alr4641 bearing a PDB code of 1PRX, shows the best confidence scores for the superoxide dismutases from STRING database. Knowledge of important accessory proteins in any pathway enables identification of appropriate targets and co-targets (Priyadarshini P., Adhikari S., 2012). This establishes a relationship between the superoxide dismutases and their accessory proteins [Franceschini, A**.**, Szklarczyk, D**.**, Frankild S**.***et al*., 2013). This has been further verified by the presence of similar domains and motifs (Geer, L.Y., Domrachev, M., Lipman, D.J. *et al.,* 2002, Naughton, BT., Fratkin, E., Batzoglou S. *et al*., 2006). The computed physicochemical properties for MnSOD and FeSOD also shows a fair level of physiological similarity, however, a relatively unstable MnSOD to that of FeSOD was found owing to its structural characterization of the previous. Progress continues apace for all the types of superoxide dismutases. However, the direction this is taking is strikingly different. For Iron and Manganese superoxide dismutases, spectroscopic methods are advancing our ability to understand their electronic bases for relativity and how

these vary from enzyme to enzyme (Han, W.G., Lovell, T., Noodleman, L., 2002, Anne-Frances Miller., 2004).

5. Conclusions

From the present study of the MnSOD (from Nostoc sp. PCC7120) and FeSOD (from *Thermosynechococcus elongates*), we attempted to study the structural analysis and the interactions of various accessory proteins associated with the two antioxidant enzymes via homology modeling and molecular docking based on the maps available from STRING database. It also reveals that both the enzymes are similar in nature.

6. Acknowledgement

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Author Profile

Bikash Thakuria was born in Guwahati, Assam (India) in 1988.He has obtained a master's degree inthe fieldofbiotechnology from Bangalore University (India)in2012. Currently he is working as a Research Associate under Bioinformatics Infrastructure Facility(BIF), funded by the Department of Biotechnology, Govt. of India,inSt. Edmund's College Shillong and holds a research experience of 3 years.He has published 4 papers as the First author in them. His field of research is proteomics and computational biology. He has also participated in different national level work shops in the field of Biotechnology and Bioinformatics. He is also associated with the docking and interaction studies of different azodyes and their environmental impact. Currently he is workingona medicinalplant named*Smilax aspera*and its proteins for cancerstudies. His future plan is to incorporate the Bioinformatics and Remote Sensing/ GIS techniques in the field of Medicinal plants/Agroforestry.

Phiralang Diengdoh did his graduation in Biotechnology from St.Anthony's College, Shillong and then went on to complete his post graduation in Bioinformatics from Kuvempu University, Karnataka. He worked as a teacher in schools for two years before joining the Bioresources Development Centre, Upper Shillong as a Junior Research Fellow and then as a Technical Assistant for a period of four and a half years. He had worked intensively on the conservation and propagation of indigenous as well as hybrid orchids and medicinal plants through various plant tissue culture methods and their subsequent lab to land transfer. He later joined St. Edmund's College, Shillong as a project trainee in the Bioinformatics Facility, Department of Biotechnology and worked on various research topics pertaining to in silico studies of enzymes involved In environmental bioremediation.

Samrat Adhikariwasborn in Shillong, Meghalayain 1979.He has obtained the doctoral degree from North Eastern Hill University, Shillong (2011) in the field of environmental biotechnology and the masters with specialization in molecular microbiology (2002) from Bangalore University, Bangalore. He has received the Biotech Industrial Training Programme fellowship (2003) and also has a vast teaching and research experience for 12 years. Currently he is an assistant professor & the head of the Biotechnology Department, St. Edmund's College, Shillong. He has published 5 papers in peer reviewed journals and 3 in national & international conferences. He has under gone many trainings and also has organized many workshops for young researchers. He is also the reviewer of many academic journals. He has supervised for 3 M.Tech thesis and is currently handling 3 projects sponsored by the different funding agencies of Govt. of India. He is presently a member of many academic bodies in universities. At present, he is working on environmental biotechnology & bioinformatics