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Structure modeling and inhibitor prediction of NADP oxidoreductase enzyme from *Methanobrevibacter smithii*

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Abstract:
The F420-dependent NADP oxidoreductase enzyme from *Methanobrevibacter smithii* catalyzes the important electron transfer step during methanogenesis. Therefore, it may act as potential target for blocking the process of methane formation. Its protein sequence is available in GenBank (accession number: ABQ86254.1) however no report has been found about its 3D protein structure. In this work, we first time claim 3D model structure of F420-dependent NADP oxidoreductase enzyme from *Methanobrevibacter smithii* by comparative homology modeling method. Swiss model and ESyPred3d (via Modeller 6v2) software's were generated the 3D model by detecting 1JAX (A) as template along with sequence identities of 34.272% and 35.40%. Furthermore, PROCHECK with Ramachandran plot and ProSA analysis revealed that swiss model produced better model than Modeller6v2 with 98.90% of residues in favored and additional allowed regions (RM plot) as well as with ProSA Z score of -7.26. In addition, we investigated that the substrate F420 bound at the cavity of the model. Subsequently, inhibitor prediction study revealed that Lovastatin (-22.07 Kcal/mol) and Compactin (Mevastatin) (-21.91 Kcal/mol) produced more affinity for model structure of NADP oxidoreducatse as compared to F420 (-14.40 Kcal/mol). It indicates that the Lovastatin and Compactin (Mevastatin) compounds (Negative regulator) may act as potential inhibitor of F420 dependent NADP oxidoreducatse protein.

Keywords: Methanogenesis, Homology modeling, Functional site prediction, Substrate finding, Oxidoreductase Inhibitor, Docking, Autodock

Background:
The decomposition of organic compounds in the absence of oxygen produces methane as a main product **[1]**. Likewise, the anaerobic bacteria are mostly performing the function of decomposition in the absence of oxygen known as anaerobic microbial decomposition. Most of the methane producing bacteria belongs to the domain archaea **[1]**. They further extended to the kingdom Euryarchaeota. These methane producing bacteria i.e methanogens are classified in to five orders i.e Methanopyrales, Methanococcales, Methanobacteriales, Methanosarcinales and Methanomicrobiales **[2, 3]**. Apart from these, Methanobrevibacte phlotypes are the most dominant phylotype of methanogens present in Australia, Canada and European countries. These bacteria's used different substrates for completing their energy requirement and produce methane. The well known substrates for methanogenic bacteria are carbon dioxide, hydrogen, acetate and methanol etc **[1]**. But this increasing level of methane in the environment is a matter of global concern. Everyday scientists are making experiments in order to reduce methane by mitigating methanogens. A major portion of the methane in the environment is coming from the livestock sector so it is worthwhile to stress majorly on the strategies or methods that will mitigate the methane emission from rumen **[4, 5]**. Methane producing pathway in methanogens that utilize carbon dioxide and molecular hydrogen involves ten methanogen specific enzymes which catalyze unique reactions using unique coenzymes **[1, 4, 6, 7]**.

One of them is F420 H2: NADP oxidoreductase (Fno) that catalyzes the electron transfer step between NADP+ and F420 **[8]**. During the reaction, NADP isreduced to NADPH by accepting one or more hydrides (H−) from F420 **[9]**. It is an important step for regulation of methane formation in methanogen bacterium such as*Methanobrevibacter smithii* (ATCC 35061). Therefore, the NADP oxidoreductase enzyme may play vital role in the formation of methane in *Methanobrevibacter smithii*. This group of methanogens also finds in human gut and helps in the digestion of polysaccharides which affects harvesting of host calorie **[10]**. It also dominants the 10% of anaerobes in the human gut. *Methanobrevibacter smithii* uses large carbohydrates as sole carbon source and degrades them to methane which is toxic to body. Furthermore, the excreta with high methane content is consumed by bacterium in order to release free methane which is a major issue for

environmental safety. Therefore, there is need to block the synthesis of methane in *Methanobrevibacter smithii* to reduce its toxicity in the environment. The synthesis of methane can be hindered by decreasing the activity of NADP oxidoreductase enzyme in *Methanobrevibacter smithii*. However, the 3D protein structure of NADP oxidoreductase enzyme from *Methanobrevibacter smithii* is still unknown. Therefore, in the proposed work we developed 3D model structure of F420- dependent NADP oxidoreductase protein from *Methanobrevibacter smithii* whose protein sequence is available in genebank (Accession number: ABQ86254.1). We explored comparative homology modeling method for 3D model development. In addition, we determined the putative functional site for the model protein via various prediction servers which was further validated by molecular docking approach. Moreover, several works have been already reported for direct or indirect inhibition of methane production **[11-15].** Therefore, in our research work we determined putative inhibitor of F420 dependent NADP oxidoreductase protein to inhibit the methane producing pathway in *Methanobrevibacter smithii*.

Methodology:
The protein sequence of F420-dependent NADP oxidoreductase enzyme from *Methanobrevibacter smithii* was obtained from gene bank (Accession number: ABQ86254.1) and furnished as query sequence for homology modeling.

Homology modeling:
The protein sequence was subjected for comparative homology modeling via Swiss model **[16]** and ESyPred3D (via Modeller 6v2) software's **[17]** to generate putative 3D model. The Swiss model performs the sequence alignments and searches the putative template protein for generating the 3D model for query sequence. The ESyPred3D has been incorporated with Modeller (version 6v2) program for generating the putative 3D model. All the modeling parameters were set to be default. The model structure was further verified by PROCHECK and PROSA analysis.

Energy minimization by GROMOS96:
The model structure was further optimized by energy minimization via GROMOS96, implemented in Swiss pdb viewer software. GROMOS96 performs the molecular dynamics of all the bonded and non bonded atoms with in the model structure and obtain the minimum potential energy.

Functional site prediction:
We subjected the model structure to different function and functional site prediction servers e.g. DALI, BLAST, PSI-BLAST, PROFUNC, Q-SITE FINDER and PROSITE. The BLAST and PSI-BLAST were used for function verification. On the other hand, the PROFUNC and Q-SITE FINDER were used for structure based functional site prediction.

Substrate verification:
We determined the putative substrate for the query protein (modeled protein) via Molecular docking. Here, we used the Patch dock **[18]** and Autodock (version 4) **[19]** software's for performing molecular docking. We extracted the substrate molecules files from the template protein pdb file (obtained from homology modeling approach) and converted into 3D structure via CORINA server (http://www.molecular-networks.com/online_demos/corina_demo). The substrates were docked against the model structure via mentioned docking software's. We further divided the docking approach in two steps (1) Blind docking (2) Refined docking. In the blind docking via Patchdock, we considered the whole modeled protein structure as docking target. On the other hand, in refined docking approach via Autodock4, we generated the grid map around the selective active site residues (obtained from fuctional site prediction servers and from blind docking approach) with grid point spacing of 0.375 A^0**(Figure 1).** All docking parameters were set to be default.Subsequently, the substrates were ranked based on docking score obtained after Patchdock. On the other hand, in Autodock4.0, a log file has been generated (dlg) from where we calculated docking energy which is the sum of Intermolecular energy and internal energy .We selected the most stable confirmations of the substrate based on lowest docking energy and their binding mode was analyzed at resulting selected functional site.

Inhibitor prediction:
Finally, the inhibitor compounds were detected for modeled F420-dependent NADP oxidoreductase protein in order to decrease its catalytic activity. We obtained reported inhibitor compounds for methanogenic bacteria from literature. Their SMILES strings were obtained from Pubchem database and 3D structures were generated by CORINA server. The compounds were docked against the modeled structure of F420-dependent NADP oxidoreductase.

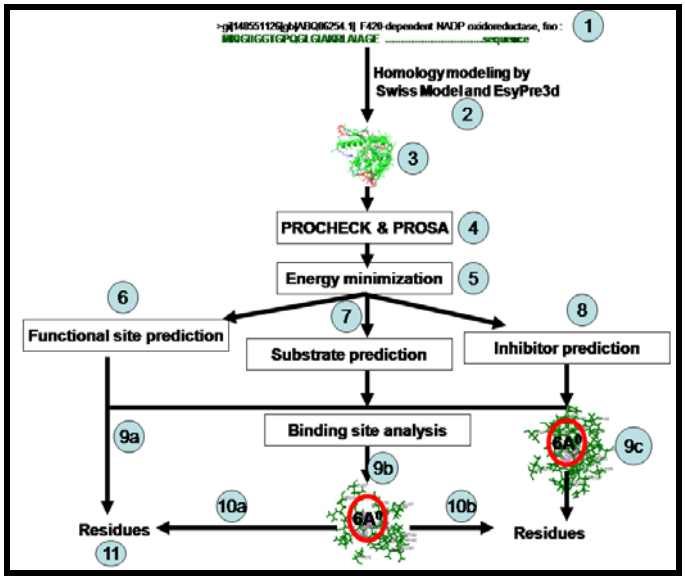

Figure 1: Schematic of the Methodology: (1) The protein sequence of F420-dependent NADP oxidoreductase enzyme from Methanobrevibacter smithii was obtained from gene bank database, (2) The query sequence was subjected to homology modeling via Swiss model and ESyPred3d (Modeller6v2) software's, (3) The 3D model structure was obtained, (4) The quality of 3D model was further verified by PROCHECK and PROSA analysis, (5) The selective 3D model was further furnished for energy minimization by GROMOS96, (6) Next, the optimized model was subjected for functional site prediction by PINTS, PROFUNC and Q-SITE FINDER servers, (7) In addition, the substrate prediction was performed for modeled NADP oxidoreductase enzyme, (8) Subsequently, the potential inhibitor compounds were predicted for the model protein of NADP oxidoreductase enzyme. Furthermore, the binding site residues were extracted from model protein. The analysis was divided in three steps (9a) The putative functional site residues were extracted from servers, (9b) The substrate and (9c) inhibitors binding cavity was determined after docking, (10) The binding site residues were enumerated which surrounded (10a) substrate and (10b) inhibitor (as center) with 6Å of radius and (11) The extracted residues from steps 10a, and 10b were compared with the residues from functional site prediction servers.

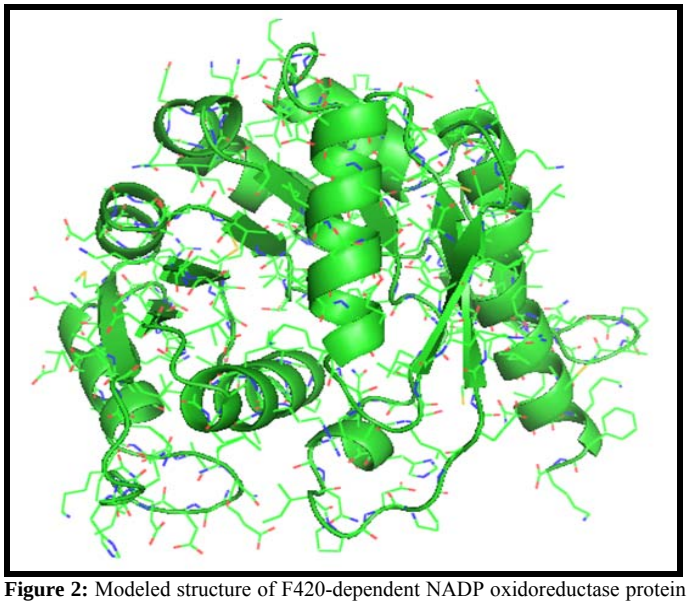

Figure 2: Modeled structure of F420-dependent NADP oxidoreductase protein (Gene bank accession number: ABQ86254.1)

Results:

Molecular Modeling:

We are interested in uncovering structure, substrate and inhibitor of F420dependent NADP oxidoreductase (target) from ***Methanobrevibacter smithii***. The Swiss model software generated the 3D model structure of query protein F420dependent NADP oxidoreductase at resolution of 1.8 Å based on template protein 1JAX (chain A) with sequence identity of 34.272% and e-value of 2.40e-26 **(Figure 2**). On the other hand, the EsyPred3D (via Modeller (version 6v2)) developed model structure based on same template protein 1JAX (chain A) with sequence identity of 35.40% (using ALIGN program). The Ramachandran plot analysis (by PROCHECK) of the model structure from ESyPred3D is showed that 97.30% of the residues are in favored and additional allowed regions. However structure from Swiss model is showed 98.90% residues are in favored and additional allowed regions .Quality assessment of the model via ProSA revealed that the models from both EsyPred3D and Swiss model matched with NMR region of the plot with Z score of -7.25 and -7.26 **(Figure 3)**. ProSA analysis indicated that the model structure of NADP oxidoreductase obtained match with the known proteins whose structures have been already determined by NMR experiments. It signifies good quality of our model. Furthermore, we considered Swiss model structure for our further study **(Figure 2).** The measurement of bad non-bonded interaction for Swiss model structure showed that at 2.0 Å of resolution, the number of bad contacts is of five residues per 100 residues. In addition, Secondary structure prediction of F420dependent NADP oxidoreductase revealed that the modeled structure consist of Helix-Strand-Coil like structure. Energy minimization was performed by GROMOS96 which optimized the model structure from initial energy -4019.835 KJ/mol to final energy of -5671.013 KJ/mol.

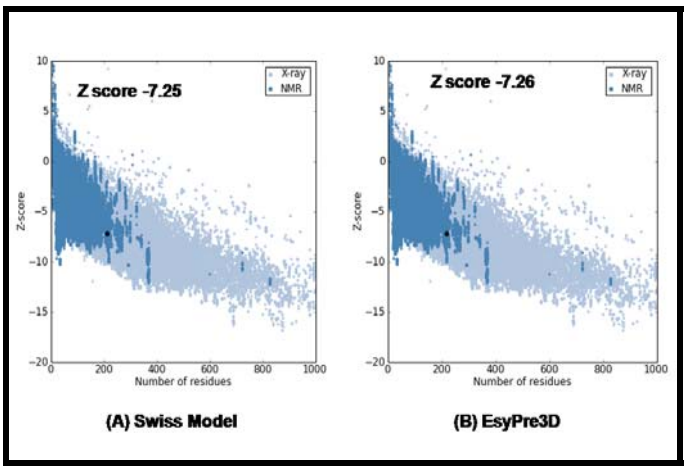

Figure 3: ProSA analysis of Modeled protein structure of F420-dependent NADP oxidoreductase protein via (A) Swiss model (B) ESyPred3d. It represents that the model structures from both the software's are matching well with the known proteins whose structures have been determined by NMR experiments.

Functional site residues:

Functional site prediction by state of art methods such as DALI, PINTS, Profunc and Q Site Finder enumerated the putative active site residues in the model structure **(Figure 1)**. DALI server produced close match with 1JAX (chain A) (Z score 37.4, RMSD 0.7) and 1JAY (chain A) (Z score 34.9, RMSD 0.9). These are known oxidoreductase enzymes with structures in RCSB data bank and were also detected during homology modeling of query protein. These servers detected the following putative functional site residues in the target protein: GLY7, GLY 8, THR 9, GLY 10, PRO 11, GLN 12, GLY 10 13, LEU 14, SER31, ARG32, LYS36, LEU 73, THR 74, VAL75, PRO 76, LEU 77, GLN 80, ALA 99, THR 100, VAL101, ASN 142, ASN 146, THR 201. The ligand molecules F420 and NADP were separated from pdb file of 1JAY. Note that no ligand has been reported for 1JAX.We download their SMILES strings from Pubchem and converted into 3D structures by CORINA server and saved in pdb file format **(Figure 4)**. The docking of the ligands against modeled oxidoreductase protein produced higher docking score for F420 (score 6150) as compared to NADP (score 4195). The ligand F420 binds at the cavity of oxidoreductase protein. We took docked F420 ligand as a centre and at 6 Å of radius to select the surrounding residue environment. The following active site residues surround the ligand F420: GLY7, GLY8, THR9, PRO11, GLN12, GLY13, ILE16, ILE72, LEU73, THR74, VAL75, PRO76, LEU77, GLN78, ALA79, GLN80, LEU97, ASP98, ALA99, THR100, VAL101, PRO102, LEU103 ,GLU104, THR105 ,PRO114, LEU115, ILE117, ALA122, ALA140 ,PHE141, ASN142, ASN143, ILE144 ,SER145, ASN146, SER147, HIS148, LEU149 ,ASP154, ILE156, CYS158, ILE197, GLU198, SER199, ILE200 ,THR201, PRO202, LEU203, LEU204, ILE205, GLY206, MET207, ASN208, ILE209, LYS210, PHE211 and LYS212. These residues are in agreement with the functional site residues predicted by different function site prediction servers. Furthermore, refined docking by Autodock4 for selective functional site (obtained by blind docking approach and predicted by servers) produced lower docking energy of - 14.40 Kcal/mol for F420 **(Figure 5).**

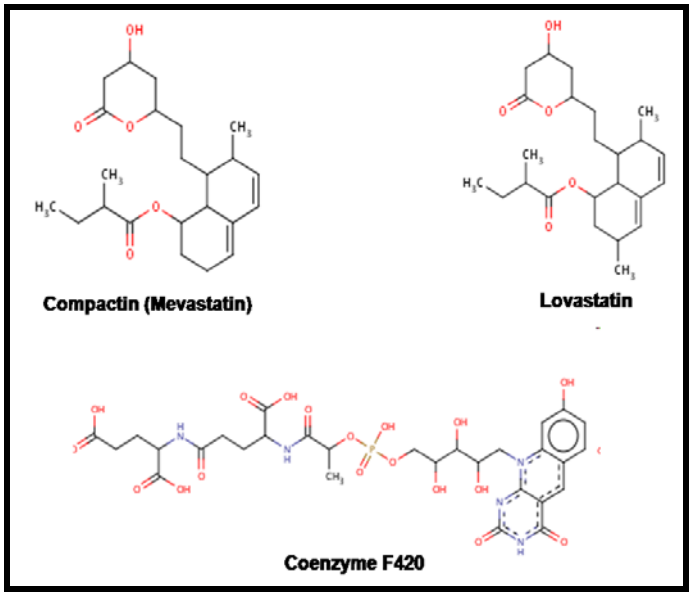

Figure 4: Structure of Compactin (Mevastatin), Lovastatin and F420.

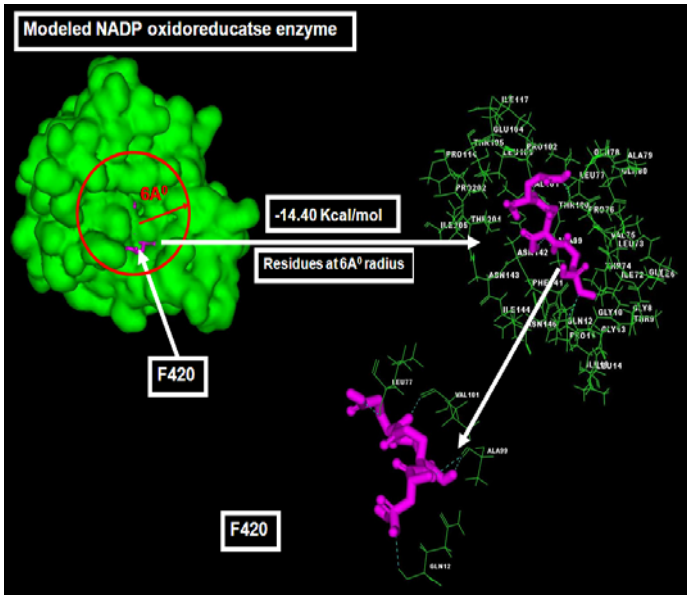

Figure 5: The F420 (pink) binds at the cavity of modeled structure (green) of F420- dependent NADP oxidoreductase protein and residues surrounding the F420 ligand at 6A0 radius. The residues GLN12, LEU77, ALA99 and VAL101 make hydrogen bonds with F420.

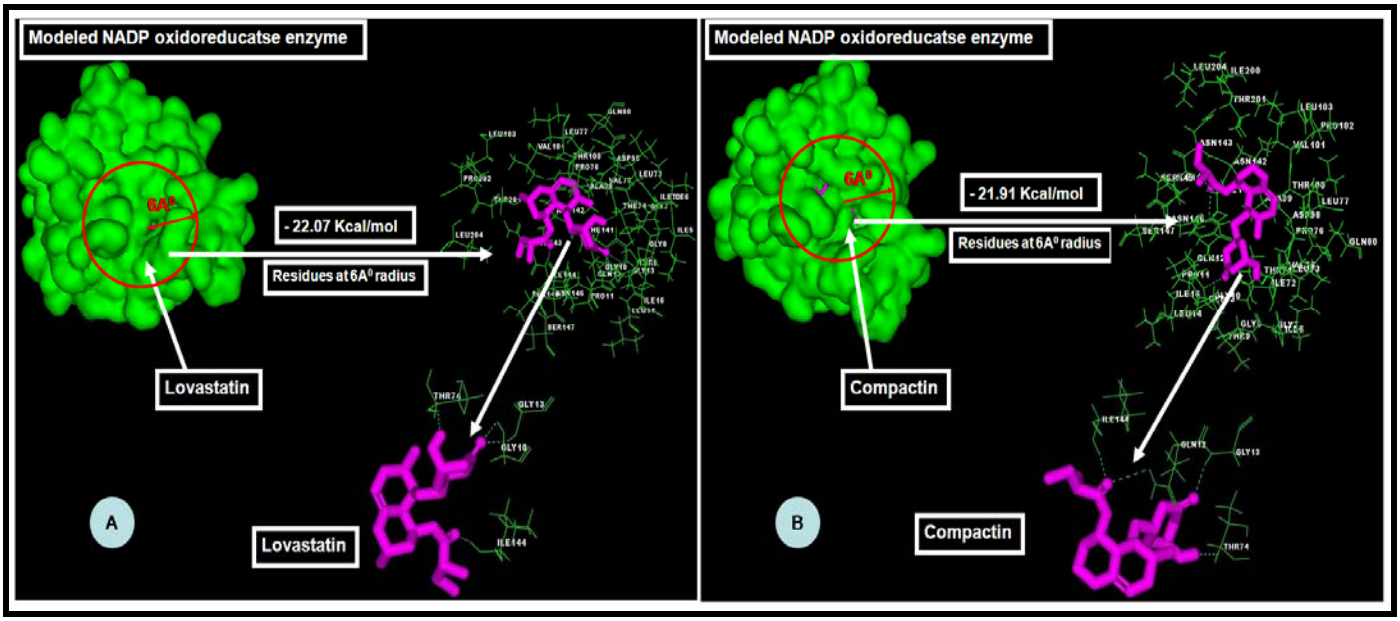

Figure 6: The figure showed (A) Lovastatin inhibitor (pink) binds at the cavity of modeled structure (green) of F420-dependent NADP oxidoreductase protein and residues surrounding the Lovastatin ligand at 6A0 radius. The residues GLY10, GLY13 THR74 and ILE144 make hydrogen bonds (blue) with Lovastatin. (B) Compactin (Mevastatin) inhibitor (pink) binds at the cavity of modeled structure (green) of F420-dependent NADP oxidoreductase protein and residues surrounding the Compactin (Mevastatin) ligand at 6A0 radius. The residues GLN12, GLY13, THR74 and ILE144 make hydrogen bonds (blue) with Compactin**.**

Docking with inhibitors:

Docking via Patchdock produced higher docking score for Lovastatin (score 4852) and Compactin (Mevastatin) (score 4778) along with oxidoreductase natural ligand F420 (score 6150) **(Figure 4)**. Refined docking (making grid map on previously predicted active site) also produced lower docking energy for Lovastatin -22.07 Kcal/mol and Compactin (Mevastatin) -21.91 Kcal/mol which is much lower then oxidoreducatse natural ligand F420, -14.40 Kcal/mol **(Figure 5 & 6)**. Both the inhibitors bind at the cavity of modeled F420 dependent NADP oxidoreducatse protein and show higher affinity as compared to F420 **(Figure 5 & 6).** Our research establishes that Lovastatin and Compactin (Mevastatin) may act as potent inhibitor for F420 dependent NADP oxidoreducatse protein in order to block its active site.Based on the matches, we propose the following putative functional role of the target: The target may participate in hydride transfer of DNA photolyase reaction. The N terminal of the protein may have dinucleotide binding Rossmann fold domain. The target may be a homodimer catalyzing the reversible reduction of NADP+ by the help of F420H2. It may have NADP binding site at N terminal domain and F420 binding at the C terminal domain.

Discussion:

In our study, we determine the 3D model structure of the F420-dependent NADP oxidoreductase from *Methanobrevibacter smithii*. We investigate that Swiss model software develop more accurate protein model for our query sequence as compare to ESyPred3D. It also verifies by PROCHECK and ProSA analysis. These methods confirm the accuracy of our protein model. Structure based functional site prediction methods enumerate the putative amino acid residues from our protein model of F420- dependent NADP oxidoreductase enzyme. We detect that these residues are making ligand binding site pocket. Therefore, in extension of our study, we determine the substrate and inhibitor for our model protein via docking. We find that ligand F420 binds at protein cavity. Results from functional site prediction and residues selection from docking experiment are complementary to each other. In addition, the inhibitor compounds Lovastatin (-22.07 Kcal/mol) and Compactin (Mevastatin) (-21.91 Kcal/mol) show more affinity for model protein as compare to natural ligand F420 (-14.40 Kcal/mol). They share the same cavity as by F420 and surround by similar residues. The Lovastatin is a fungal metabolite isolated from cultures of *Aspergillus terreus* and Compactin (Mevastatin) is an antifungal metabolite from *Penicillium brevicopactum.* Our research establishes that Lovastatin and Compactin (Mevastatin) may act as potent inhibitor for F420 dependent NADP oxidoreducatse protein in order to block its active site.

Conclusion:

The sequence and functional site prediction servers confirmed that the query sequence is putative F420-dependent NADP oxidoreductase protein. Model development and functional site prediction of F420-dependent NADP oxidoreductase protein will give greater insight in understanding the role of amino acids for catalyzing the reaction mechanism. Docking experiments detected putative inhibitor of F420-dependent NADP oxidoreductase protein which may help in blocking the methane production function of protein. As the production of methane in excess amount is harmful for the environment therefore blocking the active site by Lovastatin and Compactin (Mevastatin) inhibitors render the enzyme unable to bind with the substrate which results in decreased methane production and reduction in environment pollution. Our prediction may help the biologist to design putative inhibitor against F420-dependent NADP oxidoreductase protein for regulating its active related to global environment concern.

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