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Redox Partners in Proximity to CYP51B1 of Mycobacterium Tuberculosis: An in Silico Approach Confirming in Vitro Elec-tron Transfer

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ABSTRACT

Enzymes of the cytochrome P450 (CYP) superfamily of heme-thiolate proteins are found in most biological species from all five kingdoms. The discovery of a prokaryotic CYP51 was an exciting discovery in the P450 field because of the soluble nature of CYP51B1. The different sorts of redox partners interactions utilized in cytochrome P450 system are depicted. The redox reactions are a family of reactions that are concerned with the transfer of electrons between species. The 3D structure of Ferredoxin1(Fd1), Ferredoxin2(Fd2) and FerredoxinReductase (Fdr) from Mycobacterium tuberculosis have not been solved and published in any online databases. Data mining has been done using two online databases, tuberculist and PDB. Then, homology modeling was performed using two different softwares and verify 3D was used to evaluate the models. Docking analysis to compare redox partner protein models for distances and interactions to CYP51B1. As a conclusion, 3D structures of redox partners Fd1, Fd2 and Fdr were successfully built. Their electron transfer process in proximity to the heme domain was elucidated for a better understanding of CYP51 interactions with its redox partner proteins complementing experimental results

Keywords:
Cytochrome P450, CYP51B1, Redox partners, Mycobacterium tuberculosis, Ferredoxins, Ferredoxinreductase, Electrostatic, Hydrogen bond

1. Introduction

Enzymes of the cytochrome P450 (CYP) superfamily of heme-thiolate proteins are found in most biological species from all five kingdoms (Lewis &Hlavica, 2000). The amino acid sequence identity is the main point that has been used to classify the cytochrome P450 into families and sub-families [1]. When cytochrome P450 meet ≥40% amino acid sequence identity and members of the same family regularly displayed comparative substrate selectivity, the P450s was classified into the same family[2]. The discovery of a prokaryotic CYP51 was an exciting discovery in the P450 field because of the soluble nature of CYP51B1. The redox reactions are a family of reactions that are concerned with the transfer of electrons between species. Redox responses are a group of responses that are concerned with the exchange of electrons between species. Either NADPH or NADH is the supplier of reducing equivalents for the P450-catalyzed reaction. The electron transfer is mediated by two co-factors. One of which is FAD and the other one will be either FMN or an iron-sulfur redoxin. The main purpose of this research is to model the 3D structure of redox partners Fd1, Fd2 and Fdr then demonstrate the electron transfer process of redox partners in proximity to CYP51B1 for a better understanding to complement wet lab results done by [1] which pointed out the need of either one redox protein to support electron transport from the NADH donor to the heme domain in CYP51B1.

2. Methods

Gene names (Rv0762c, Rv3106, Rv0688, Rv0763c and Rv1786) were entered on Tuberculist to search for details. The gene's details such as predicted structures from Protein Data Bank (PDB), gene length, protein length, sequences and annotations were obtained. The peptide sequences of

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the genes which do not have solved structures were copied to generate the models from RaptorX and SWISS-MODEL [3][4][5]. The PDB identifier obtained from TubercuList was used to search the PDB for solved crystal structures. The .pdb file format was downloaded for analysis. The other redox partner proteins i.e. fd1, fd2, and fdr from Mtb were searched for solved crystal structures in PDB to confirm the existence of solved structures [6]. The peptide sequences which were copied from TubercuList database were uploaded on to the SWISS-MODEL dialogue box to generate the 3D models. The solved 3D structures were notified by email with analysis details of the structures. The best model structure with highest sequence percentage was chosen and the structure was saved in .pdb file format [7][8]. The obtained peptide sequences were then run on the RaptorX program to obtain a predicted 3D structure of the peptide. The program generated the 3D structures as well as a detailed analysis on the structures including residue type, disorder prediction, sequence alignments as well as function annotation; the results are collected and tabulated for comparison (Raptorx.uchicago.edu, 2015).

The generated models by SWISS-MODEL and RaptorX were uploaded on Verify 3D server to evaluate the quality of the models. The 3D-1D profile scores were given in percentage [9]. The PDB file was submitted to RAMPAGE server. The server displayed the output results with evaluation of residues together with number of residues in favoured region, number of residues in allowed region, and number of residues in outlier region [10]. Using ClusPro website, options to use the server without the benefits of our own account was chosen and the job name was entered. This was followed by uploading the proteins in .pdb file format. The job identifier was given and results were accessed using the same job identifier. Then 30 models were displayed as best models and first 10 models were used in this study to be further analyzed [11][12].

Pymol viewer was used to view the generated 3D models as well as the docked models. The models were viewed in cartoon format, which made the display clearer using secondary structures. The stick format was used to find the distances and the polar contacts [13].

3. Research Methodology

3.1.Generated 3D Models

Figure 1 below shows the models generated using two softwares; RaptorX and SWISS-MODEL. The sequence similarity of the Fdr generated using RaptorX is 96.80% and the sequence similarity percentage of model generated using SWISS-MODEL is 92.49%. The Fd1 model generated using RaptorX, where the sequence similarity is 88.24% and the Fd1 model generated using SWISS-MODEL has 79.69%. Finally using RaptorX the sequence similarity for Fd2 model was generated to be 58.21% and the Fd2 model generated by SWISS-MODEL has 38.71% of sequence similarity.

According to the Figure 1 all three models generated by RaptorX had better and higher quality compared with the models generated using SWISS-MODEL software. Besides that, the sequence similarity of the models generated by RaptorX had higher percentage than the models generated by SWISS-MODELS.

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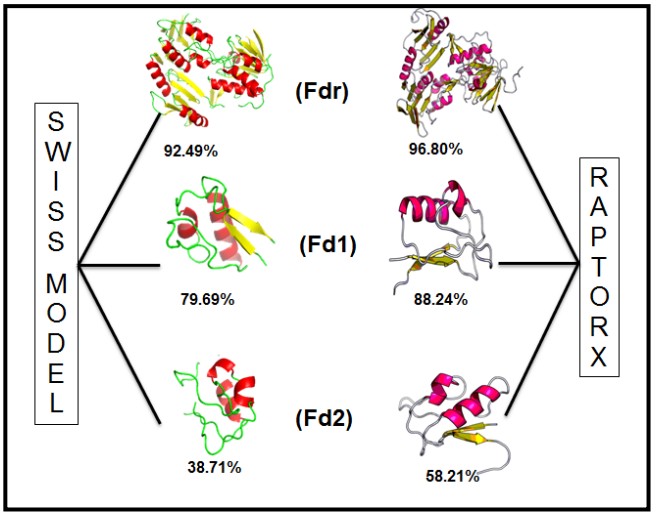

Figure 1: Generated 3D Models

The models on the left were generated by SWISS-MODEL server while the models on the right were generated by RaptorXserver. The models generated by SWISS-MODEL is colour coded i.e. green represents the coils; red - alpha helix, yellow - the beta sheet. The models generated by RaptorX can be seen by white representing the coils; pink - alpha helix, yellow - beta sheet.

By utilizing the 3D profiles, verify3D assesses the protein structures. The program breaks down the similarity of the atomic model (3D) and its amino acid arrangement (1D). A structural class based on every residue is assigned on its environment and location such as alpha, beta, loop, polar, nonpolar and so on. The scores were ranged from -1 to +1. Where, the -1 is a bad score and the +1 is a great score.

The 3D structures of Fdr generated in SWISS-MODEL indicated 3D-1D scores of 0.9249 whereas the 3D structure generated in RaptorX indicated 3D-1D scores of 0.9680(maximum is 1). Next, the 3D structure of Fd1 generated in SWISS-MODEL indicated 3D-1D scores of 0.7969 whereas the 3D structure generated in RaptorX indicated 3D-1D scores of 0.8824. Lastly, the 3D of Fd2 generated in SWISS-MODEL indicated 3D-1D scores of 0.3871 whereas the 3D structure generated in RaptorX indicated 0.5821.

Table 1: Model evaluation of proteins from various programs

Protein	Modeling Program	Evaluation Program	3D-1D profile	RAMPAGE %
FerredoxinReductase (Fdr)	SWISS MODEL	Verify3D	0.9249	90.6
	RaptorX		0.9680	94.3
Ferredoxin1 (Fd1)	SWISS MODEL	Verify3D	0.7969	95.2
	RaptorX		0.8824	98.5
Ferredoxin2 (Fd2)	SWISS MODEL	Verify3D	0.3871	90.0
	RaptorX		0.5821	93.8

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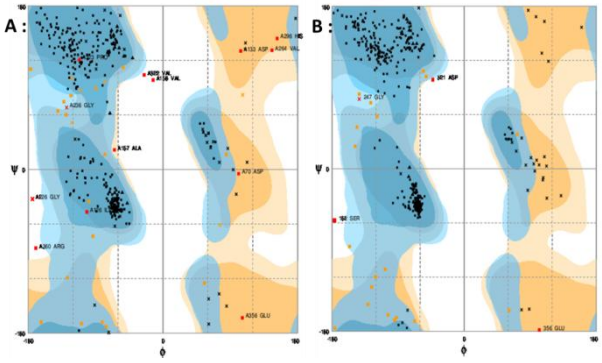
All the six 3D models produced by RAMPAGE program demonstrated that the residues were found in the most favorable areas using Ramachandran Plot Analysis (Figure 4.2, 4.3 and 4.4).
Ramachandran (1962) used computer models of small polypeptides to systematically vary phi and psi with the objective of finding stable conformations. For each conformation, the structure was examined for close contacts between atoms. Atoms were treated as hard spheres with dimensions corresponding to their van der Waals radii. Therefore, phi and psi angles which cause spheres to collide correspond to sterically disallowed conformations of the polypeptide backbone.
The 3D model of Fdr generated by SWISS-MODEL has 90.6% of the residues in the favoured area (Figure 2A). Ramachandran plot analysis for RaptorXFdr model (Figure 2B), brought about 94.3% of the residues in the favored area. These results affirm that the evaluation analysis done by Verify3D demonstrates that the models predicted by the SWISS-MODEL server lacks the number of residues in the expected favoured areas compared to models generated by the RaptorX server. Therefore, it shows that the RaptorX server generated good and better quality 3D structure of Fdr protein from Mtb.
The 3D model of Fd1 that generated by SWISS-MODEL has 95.2% of the residues in the favored area (Figure 2C). Ramachandran plot analysis for RaptorX Fd1 model (Figure 2D), brought about 98.5% of the residues in the favored area. These results affirm that the evaluation analysis done by Verify3D demonstrating that the models predicted by the SWISS-MODEL server lacking the number residues in the expected favored areas compared to models generated by the RaptorX server. Therefore, it shows that the RaptorX server generated good and quality 3D structure of Fd1 protein from Mtb.
The 3D model of Fd2 that generated by SWISS-MODEL has 90.0% of the residues in the favored area (Figure 2E). Ramachandranplot analysis for RaptorX Fd2 model (Figure 2F), brought about 93.8% of the residues in the favored area. These results affirm that the evaluation analysis done by Verify3D demonstrating that the models predicted by the SWISS-MODEL server lacking the number residues in the expected favored areas compared to models generated by the RaptorX server. Therefore, it shows that the RaptorX server generated good and quality 3D structure of Fd2 protein from Mtb.



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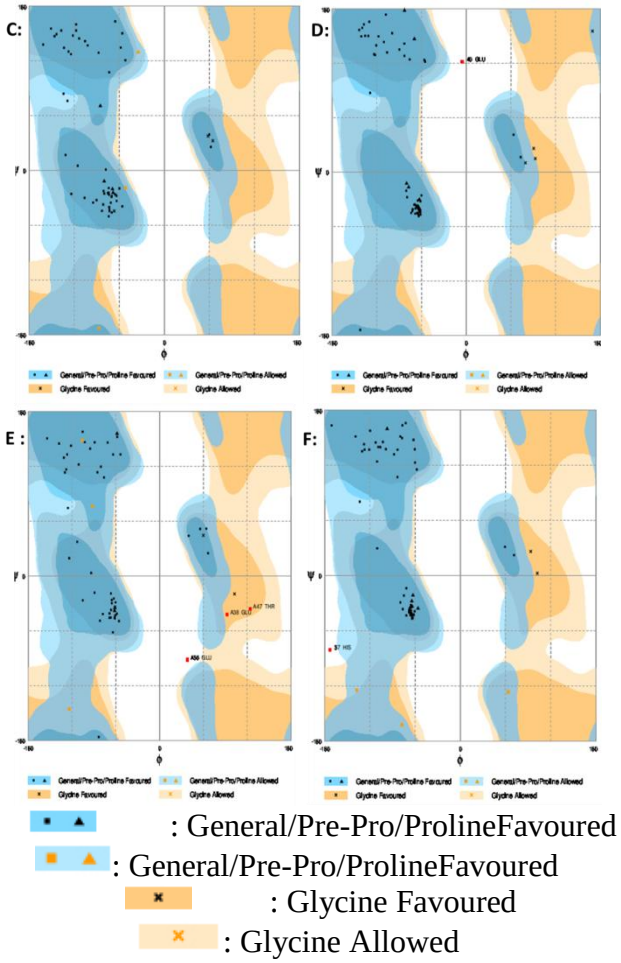

Figure 2: (A) Ramachandran plot of the SWISS-MODEL Fdr model, (B) Ramachandran plot of RaptorXFdr model, (C) Ramachandran plot of the SWISS-MODEL Fd1 model, (D) Ramachandran plot of RaptorX Fd1 model.

3.2.Docking Analysis of Redox Partners to CYP51B1

Protein-protein docking is the computational prediction of protein complex structures using individually solved component protein structures. Results of which is important for understanding physiochemical forces that underlie macromolecular interactions for modeling protein complex structures in a reaction mixture (Wiehe, 2015). The interactions of P450 proteins to their redox partners especially to their cofactors are necessary to drive electron transfer from the NADH donor through the partner proteins to the heme domain[1]. Since CYP51 is a target molecule for drug design in candidiasis infections, analysis of the 3D structure with its redox partners may assume greater relevance in drug design for resistant strains (Olivia, Chermak&Cavallo, 2015). The proximity of redox partners to the heme domain, CYP51B1 in this instance, is shown to occur by electrostatic forces as found from 30 docking models generated. This was shown by interactions of adjacent amino acids for example of arginine which is a basic amino acid to glutamate (acidic) and aspartic acid (acidic) to lysine (basic) on the surfaces of CYP51B1 and FprA respectively. An example of this model is illustrated in Figure 3 below.

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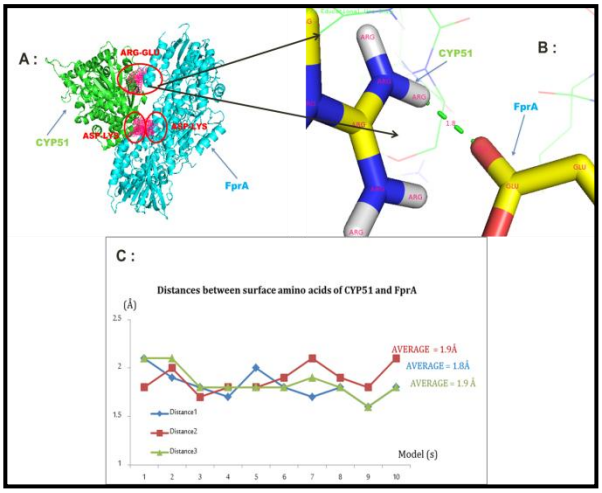

Figure 3: Illustration and docking analysis between CYP51B1 and redox partner FprA surface amino acids. A and B (zoomed in) are pymol snapshots of interactions between Arg-Glu and Asp-Lys of CYP51B1-FprA. C shows the results of 10 possible docking models generated between CYP51B1-FprA with the average distance of 1.8Å -1.9Å at all possibilities.

The number of docking models or possibilities generated was 30 and out of them the top 10 possibilities (Figure 3C) showed amino acid interactions from the surfaces of CYP51B1 and FprA with electrostatic interactions (53%) and hydrogen bonding (47%). These distances between amino acids at the surface of CYP51B1-FprA in Figure 3C, 1.8Å -1.9Å were supportive of the distance promoting electrostatic interactions. These top 10 docking possibilities of CYP51B1 to the surface amino acids of FprA, Fdr, Fd1 and Fd2 are summarized in Table 2-5 below. It is interesting to note that FprA (53%), Fdr (57%) and Fd1 (73%) CYP51B1 redox partner interactions are highest through electrostatic forces which is supportive of electron transfer experiments done by [1]. The experiments noted all positive electron transfer when CYP51B1 was in the presence of either FprA or Fdr without the need of Fd1 or Fd2 while no electron transfer was observed with CYP51B1 in the presence of either Fd1 or Fd2 alone without the reductase partner proteins[1]. This suggest that the proximity of the reductases (FprA and Fdr) to CYP51B1 can alone support electron transfer from NADH electron donor without the need of the ferredoxin partners (Fd1 and Fd2) although CYP51B1-Fd1 demonstrated 73% electrostatic forces while CYP51B1-Fd2 only had 27% electrostatic forces. Hydrogen bonds also play vital roles (almost 50% for CYP51B1-FprA and CYP51B1-Fdr) in supporting electron drive from NADH via ferredoxinreductases to the heme domain in CYP51B1.

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Table 2: Docking analysis between CYP51B1 and FprA at the molecular surface distance of 1Å-3Å shows 53% are through electrostatic forces (shaded cells) while 47% are through hydrogen bonds.

	DISTANCE 1(Å)	DISTANCE 2(Å)	DISTANCE 3(Å)
MODEL 1	2.1 (GLU → GLY)	1.8 (ASN → LYS)	2.0 (GLN → GLY)
MODEL 2	1.9 (ASP → LYS)	2.0 (GLN → VAL)	2.1 (GLU → GLY)
MODEL 3	1.8 (ASP → LYS)	1.7 (ASP → LYS)	1.8 (ARG → GLU)
MODEL 4	1.7 (ASP → LYS)	1.8 (ARG → ALA)	1.8 (GLU → LYS)
MODEL 5	2.0 (ASP → THR)	1.8 (GLU → LYS)	1.8 (GLN → LYS)
MODEL 6	1.8 (THR → LYS)	2.6 (ASP → LYS)	1.8 (ASP → LYS)
MODEL 7	1.7 (GLU → LYS)	2.1 (GLN → ALA)	1.9 (GLU → LYS)
MODEL 8	1.8 (ARG → GLU)	1.9 (GLN → LYS)	1.8 (GLU → LYS)
MODEL 9	1.6 (GLN → LYS)	1.8 (GLU → LYS)	1.6 (GLN → LYS)
MODEL 10	1.8 (ASP → LYS)	2.1 (GLU → GLN)	1.8 (ASP → LYS)

Table 3: Docking analysis between CYP51B1 and Fdr at the molecular surface distance of 1Å-3Å shows 57% are through electrostatic forces (shaded cells) while 43% are through hydrogen bonds.

	DISTANCE 1(Å)	DISTANCE 2(Å)	DISTANCE 3(Å)
MODEL 1	2.5 (ASP → ARG)	1.9 (ASN → ARG)	1.8 (GLU → ARG)
MODEL 2	2.0 (GLU → ARG)	2.0 (ARG → ASP)	1.8 (ARG → GLU)
MODEL 3	1.7 (ARG → ALA)	2.3 (GLN → GLU)	2.0 (GLY → ASN)
MODEL 4	1.9 (GLN → ARG)	2.1 (GLU → SER)	2.0 (ARG → GLU)
MODEL 5	2.5 (LYS → GLU)	1.8 (ARG → GLU)	1.8 (ASP → LYS)
MODEL 6	2.0 (GLN → THR)	2.0 (ARG → ASP)	2.1 (ASN → ASP)
MODEL 7	2.1 (ASN → GLU)	2.0 (GLU → SER)	1.8 (ARG → GLU)
MODEL 8	2.1 (GLN → GLY)	1.9 (LYS → ASP)	1.8 (GLU → ARG)
MODEL 9	1.9 (LYS → GLU)	1.8 (LYS → ASP)	2.0 (ASP → ARG)
MODEL 10	1.8 (ARG → GLY)	2.1 (ARG → GLU)	2.0 (ARG → GLN)

Table 4: Docking analysis between CYP51B1 and Fd1 at the molecular surface distance of 1Å-3Å shows 73% are through electrostatic forces (shaded cells) while 27% are through hydrogen bonds.

	DISTANCE 1(Å)	DISTANCE 2(Å)	DISTANCE 3(Å)
MODEL 1	2.6 (GLY → GLU)	1.9 (ARG → GLU)	2.0 (ASP → LYS)
MODEL 2	1.8 (GLU → LYS)	1.8 (LYS → GLU)	1.9 (ARG → GLU)
MODEL 3	2.0 (ARG → GLU)	2.2 (ARG → ASP)	1.7 (GLN → LYS)
MODEL 4	1.8 (GLU → LYS)	1.8 (LYS → ASP)	1.9 (SER → GLU)
MODEL 5	1.7 (GLU → LYS)	1.9 (ARG → GLU)	2.0 (ARG → ASP)
MODEL 6	1.3 (ARG → GLU)	2.8 (ASP → LYS)	2.4 (ASN → ASP)
MODEL 7	1.9 (ARG → GLU)	1.7 (SER → LYS)	2.4 (ARG → GLY)
MODEL 8	2.7 (GLN → ARG)	1.8 (ARG → GLU)	2.0 (ARG → ASP)
MODEL 9	1.9 (ARG → GLU)	1.7 (ASP → LYS)	1.9 (ASP → ARG)
MODEL 10	1.8 (ARG → GLU)	1.9 (PHE → GLU)	2.1 (ARG → ASP)

Table 5: Docking analysis between CYP51B1 and Fd2 at the molecular surface distance of 1Å-3Å shows 27% are through electrostatic forces (shaded cells) while 73% are through hydrogen bonds.

	DISTANCE 1(Å)	DISTANCE 2(Å)	DISTANCE 3(Å)
MODEL 1	1.7 (ARG → GLY)	2.1 (ARG → PRO)	2.2 (LEU → GLN)
MODEL 2	1.9 (ARG → ASP)	1.9 (ARG → ARG)	1.7 (LYS → GLN)
MODEL 3	1.7 (LYS →ALA)	1.7 (LYS → ALA)	1.9 (ASN → ASP)
MODEL 4	1.9 (ARG → GLN)	2.2 (GLN → GLU)	2.0 (LEU → TYR)
MODEL 5	1.8 (ARG → GLN)	1.9 (ARG → ASP)	2.0 (GLN → ASP)
MODEL 6	2.0 (ARG → GLN)	2.0 (ARG → ASP)	2.1 (ARG → TYR)
MODEL 7	1.9 (GLY → ASP)	2.0 (ARG → GLN)	1.8 (ARG → GLU)
MODEL 8	1.8 (ASP → ARG)	2.0 (ALA → ASP)	2.6 (GLN → ASP)
MODEL 9	2.0 (ARG → ASP)	1.8 (ARG → GLU)	2.0 (ASN → GLN)
MODEL 10	2.2 (ARG → PRO)	2.1 (GLY → ASP)	2.3 (GLN → SER)

[1]also noted that the rate of formation of the P450 species with the presence of CYP51B1-FprA alone is very fast because while modeling the 3D docking model, it was found that FprA was in the dimer formation containing two cofactors (FAD) one from each monomer. Because of this, we suggest that even without the ferredoxins, Fd2 or Fd1, the formation of a P450 species occurred relatively faster compared to the presence of the Fe-S proteins. Consistent with the ability of these two reductases, Fdr and FprA, to reduce CYP51B1 in isolation without the

ferredoxin partners. These findings suggest that the Fd1 and Fd2 do not play vital roles in transferring electrons while the reductases, FprA and Fdr, facilitate faster electron transfer.
It is also important to note that the in silico docking results show that although Fd1 and Fd2 will support electron transfer process as electrostatic interactions to CYP51B1 are present in the form of electrostatic bonds between acidic and basic amino acids and the presence of hydrogen bonds which facilitate electron transfer process, this can only be done in the presence of either FprA or Fdr.

4. Conclusion

Essentially the 3D structures of Fdr, Fd1 and Fd2 from Mycobacterium tuberculosis were predicted from structural comparisons with existing similar sequence structures from the PDB. All three models were generated successfully with good and acceptable quality. This study demonstrated that the electron transfer process using redox partner models of ferredoxinreductases (FprA and Fdr) in proximity to CYP51B1 amino acids were identified. While the most relevant interactions between CYP51B1 and the redox partner proteins were proposed in order to support electron transfer from FAD in FprA/ Fdr to Fe-Heme of CYP51B1 but not via the Fe-S cluster of ferredoxins. It was also suggested that the distance between cofactors & proteins in support of electron transfer can be measure between surface amino acids by surface protein interactions such as electrostatic forces and hydrogen bonds to support P450 species formation which is a reflection of electron transfer.

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