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DRUG INTERFACE RESIDUES OF PENICILLIN BINDING PROTEIN 2A: AN INSILICO STRUCTURAL ANALYSIS AND DOCKING STUDIES FOR POTENTIAL DRUGS ON MRSA

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ABSTRACT: Protein-protein interactions play an essential role in microbial metabolism and molecular syntheses for their lead functions and constant survival. Altogether they will also interact with many other ligands, small chemical structures, designed small proteins and specific drugs. Besides these interactions, identifying the specific protein inhibitors to end microbial resistivity over a wide range of modern antibiotics are also helpful. In the present study, PBP2A protein sequence of *Staphylococcus aureus* was retrieved for Homology modelling, Active site predictions, Pocket Identification, Protein-Protein interaction network, Pathway identification, Protein-protein docking and identification of target interface residues were performed for developing target based inhibitors and their efficacy against multi drug resistant pathogens. The divisome complex of all the pathogens have similar protein domains for Peptidoglycan synthesis, Transpeptidase activity, Gycosyl transferase activity and so on. The functional inhibition of this complex leads to destroy the antibiotic resistant phenomena and finally kills the pathogens.

KEYWORDS: Homology modelling, Active site prediction, Penicillin binding protein 2A, *Staphylococcus aureus*, Protein-protein interactions and docking, Interface residues.

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1. INTRODUCTION

Molecular and metabolic protein interactions with extensive experimental supports are key sources for the drug designing studies. Among protein interactions, proteins with proteins are the chief for finding structural analogues as either drug targets or inhibitors. The *in-silico* identification of target residues are competent with the involvement of protein-protein interaction networks, protein pathways and correspondingly a strong literature with experimental evidences. Based on the extensive literature reviews the fundamental principles of protein interactions are included with standard reports as follows. In gram-negative bacteria, periplasm and outer membrane communicates by active protein-protein interactions such as PBPs and their enzyme β-Lactamase are accessed through the compartments as shown in figure 1A. This complex organization permits the management of cytoskeleton and synthesis of protein precursors in cytoplasm, their transport across the inner membrane with layered peptidoglycan (PG) synthesis and entire coordination introverts with outer membrane [1]. In case of gram-positive bacteria such as *Staphylococcus aureus* doesn't contains the outer membrane, but complexed with heavy peptidoglycan layer, in turn PBPs streams over the plasma membrane and involves in PG synthesis [2].

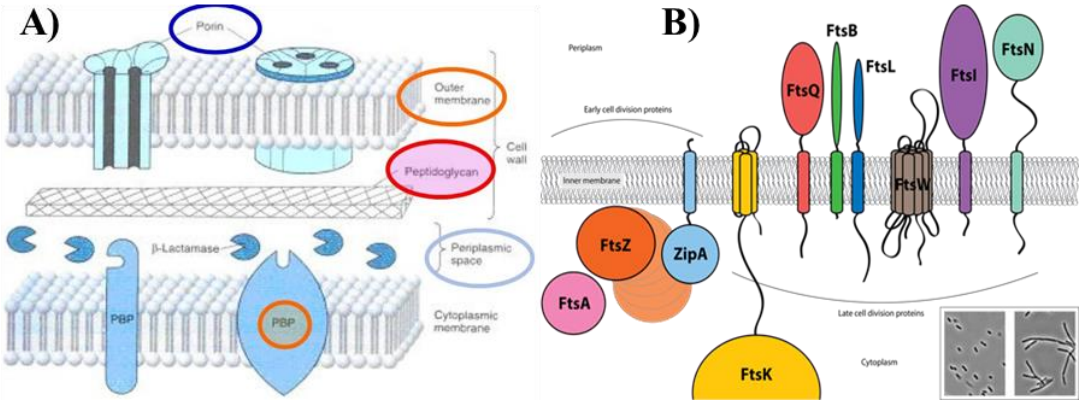


Fig. 1. A) Gram negative bacterial representation of PBPs. B) Complex of proteins for peptidoglycan biosynthesis other than PBPs (Photo copyrights reserved to Marjolein Glas *et al.*, 2015).

The Divisome complex in *E. coli* has similar interacting proteins as in other gram positive bacteria that begins with the formation of FtsZ-ring in cytoplasm and anchors in the inner membrane interacting with FtsA and ZipA. The inner membrane rings interacts with the Fts complex proteins (cell division proteins) such as FtsK, FtsQ, FtsB, FtsL, FtsW, FtsI, and FtsN that spams across the membrane as shown in figure 1B. FtsQ is the central protein, and intermediates with inner and periplasmic protein networks and play an enigmatic role for assembling the divisome complex through various transitory interactions [3, 4]. The cell division machinery with glycosylating protein complex of *L. rhamnosus* shows schematic overview of PBP1A, PBP1B, PBPB2A and MurG are projected to be putative GTs (Glycosyl Transferases or Transglycosylase). The analyzed network-based PBP3, FtsI and PBP2B acts as the substrates for the designated GTs. The cell wall hydrolase Msp1 is an practically proved glycoprotein of *L. rhamnosus* GG readily interacts with substrate

protein complex as shown in figure 2A [5, 6]. The complex PBPs are collective interacting proteins that allows the peptidoglycan synthesis and leads to the formation of thick cell wall in all kind of gram-positive bacteria. Based on these protein and substrate interactions and their inhibitor identification plays lead role in these complex protein inhibitions make the bacterium more susceptible for the new generation antibiotics.

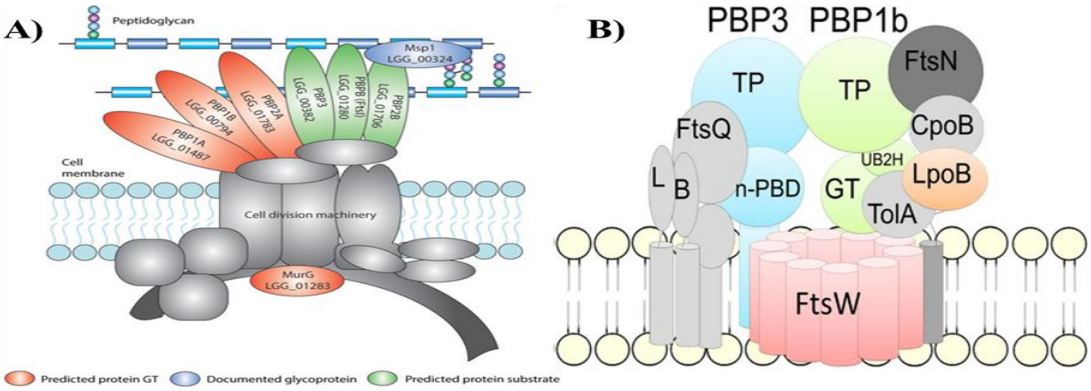


Fig. 2. A) Class of PBPs interactions and their role of PG synthesis that occurs in all kind of bacteria (Photo copyrights reserved to Aminael Sanchez-Rodriguez *et al.*, 2014).

B) Divisome complex of *E. coli* with subsets of protein and PBPs and other enzymes for PG synthesis (Photo copyrights reserved to Sophie Leclercq *et al.*, 2017).

In Escherichia coli, divisome complex persists 20 different protein subunits that accumulate in the order and frames out in two interaction steps; firstly, a tubulin like subunits framing a complex such as FtsZ, ZipA, FtsA, ZapA to E and FtsE to X limits to the underneath of inner side of cytoplasmic membrane. Secondly, the significant components like FtsK, FtsQ, FtsL, FtsB, FtsW, FtsI as PBP3 complex that mitigates the interaction with each other and PBP1b complex by joining with FtsN as a signal inducer [7, 8] to construct the mature divisome. PG GTase/TPase PBP1b complexes are the bifunctional and LpoB, CpoB, TolA are their regulators to associate with divisome complex. For the layered interactions, PBP1b needs PG TPase of PBP3 which itself requires the membrance complex FtsW for forming a complex network of proteins for peptidoglycan machinery. Presently, it is evidented that PG synthase activities are completely regulated by these protein interaction subsets in the divisome that directs the progression of cell cycle conducted by these protein-protein interactions, the depth molecular insists of these interaction routes remained unknown till date. The entire *E. coli* peptidoglycan synthesis machineries of the divisome embraces the sets of FtsQ, FtsL, FtsB; FtsW, PBP3; PBP1b- FtsN, LpoB, CpoB and TolA as shown in figure 2B [9]. Based on these extensive information on protein-protein interactions and their role in PG synthesis both in Gram negative and Gram positive bacterium shown large subsets of these proteins. According to the above interactions observed in *E. coli* the similar interactions were found to be true as shown in figure 2A for the Gram positive bacteria, therefore in the present study, the interaction sets like PBP2A with PBP3 and PBP2A with TG have considered for protein-protein interactions and their docking studies

for target residues for high affinity inhibitor molecules or drugs.

2. MATERIALS AND METHODS

Datamining and retrieval of PBP2A sequence for protein modelling

The molecular interactions of pathogenic microorganisms such as protein-protein interactions has major role in identifying the inhibitor or drug targets. The target PBP2A has identified as major interaction among cell division and peptidoglycan synthesis. Sequence of PBP2A has retrieved from UniProt database for structure development. PBP3 of *Staphylococcus aureus* has no structure in PDB database hence subjected for modelling. Another interacting protein TG has retrieved from PDB database [10]. As per the molecular interaction mentioned in literature, further modelling, interactions and docking studies were performed.

Structural template search for Homology Modelling

Template structures are the standard X-ray crystallographic structures available in PDB database used for the computational protein modelling. Template search will be done in two methods as follows

Non-automated template search

The non-automated template search will be performed by retrieving the target model sequence and used in protein BLAST [11] with the database selecting option as “Protein databank database”. The BLAST hit will provides number of target based template structure results. The structure hits found in this approach should have minimum similarity above 50% and with 50% query coverage.

Automated template search

Automated template search performed on online programmes such as Swiss Modeller [12], this is performed by providing the target sequence for model generation. Swiss Modeller is the automated and online homology modelling tool that develops the accurate and auto loop refined structures.

Homology Modelling

Protein modelling dates back to 1980’s which has revolutionized and used in the pharmacophore studies and development of targeted drugs. As the protein PBP2A of *Staphylococcus aureus* doesn’t have crystalized structures in PDB database, offline and online based protein modelling was performed. Homology modelling was performed by GUI based offline programme EasyModeller 4.0 [13], the template structures were analysed and compared by both non-automated and automated methods. Every selected protein template should have minimum 50% and above identity and its query coverage should be above 30% for developing good resolute protein models. Finally the original structure with PDB ID- 1MWT [14] from automated template search was retrieved. Similarly, online homology modelling was performed through Swiss Model server by providing the target protein sequence with automated template search. EasyModeller 4.0 has a little drawback in loop refinement such as loop folds will be observed out of the protein conformation, where manual refinement is needed in this programme, hence the selected proteins were modelled using Swiss

Modeller [12]. The modelled protein structures by both methods were compared how the loop refinement will be observed and the final Swiss Models were further submitted for model validation and verification server.

Model Validation and Verification

Modelled protein structures were subjected for stereo chemical quality on SAVES server [15] with development of Ramachandran plots for analysing the favoured regions of all amino acids of the structure. The quality of the structure will be verified and validated based on the percentage of most favoured regions depicted in the Ramachandran plot. In this method the Psi and Phi angles of each peptide represents the quality of three dimensional protein structures.

Active site prediction of the target protein

Proteins have the definite binding sites that are occupied by the various ligands or substrates and other allosteric analogues. The automated active site prediction, poses of the target molecule for probable ligand binding sites or active sites. The SCF Bio Active Site Prediction server [16] computes the cavities in a given protein. PBP2A has submitted for active site prediction and binding sites evaluation.

Pocket Identification of the target protein

Pockets of the protein are different from active sites, where in these are the binding sites of various surface molecules, maximum proteins and peptides will bind with in these regions. The pockets of PBP2A has found by submitting its protein sequence to the GHECOM server: a grid-based protein pocket identifying tool [17]. The programme runs based on the algorithm using a 3D grid depiction of proteins and probes, and their theory of mathematical expressions leads to develop the high accurate amino acid identification as binding pockets.

Protein-Protein interaction network by STRING

Protein interactions are essential in every molecular, metabolic and physiological functions of universal organisms that lead life on earth. Proteins have different functions with distinct molecular networks such as ligands, peptides, metal ions, small proteins and various chemical structures. Protein-protein interaction networks are major among these connections, due to their everlasting functional relationships with high-end signalling activities. The target protein PBP2A interaction network was developed in STRING database [18] and the network maps were retrieved for the important linked functions among other proteins. STRING provides the predicted protein-protein interactions based on direct (physical) and indirect (functional) associated interactions [19]. STRING retrieves the data from five data sets like Genomic Context Predictions, High-throughput Lab Experiments, Co-Expression studies (Conserved), Automated Text mining (Literature databases) and Previous Knowledge in Databases (BioGRID) [20]. Based on these interaction hits, the confined molecular pathway studies were performed.

KEGG molecular pathway identification of target protein

Kyoto Encyclopedia of Genes and Genomes (KEGG), is a database with molecular-level information of sympathetic and complex biological functions with their utilities, such as cells, organism and ecosystem. Especially a large-scale molecular datasets generated by genome sequencing and other high-throughput experimental technologies [21]. A sequence based pathway search was performed and generated the pathway of peptidoglycan (PG) biosynthesis and traced the major roles of Penicillin Binding Protein class. The pathway depicts the important interaction subsets and their role in PG synthesis.

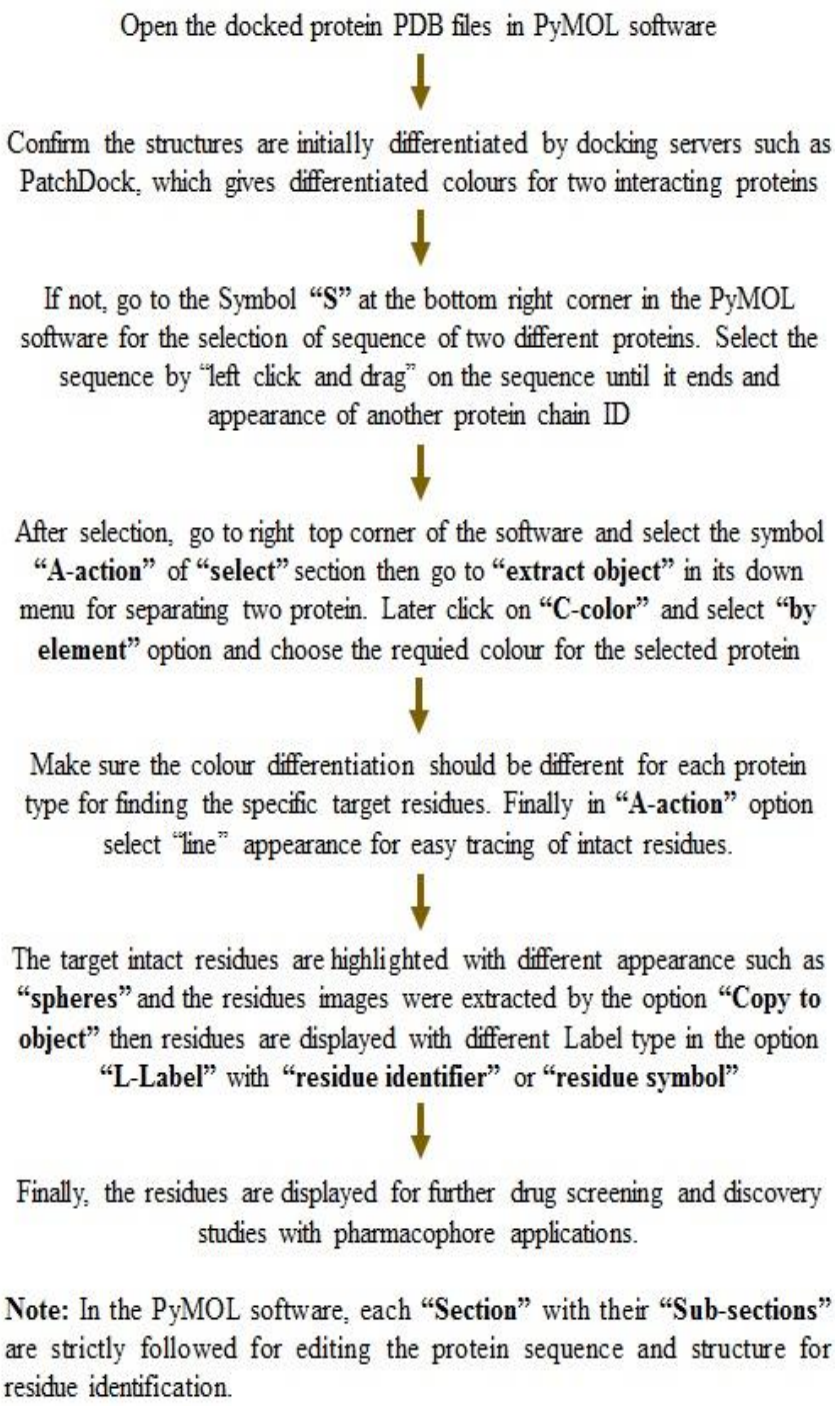
Protein-protein docking by GRAMM-X and PatchDOCK

Protein interactions are major among all prokaryotic and eukaryotic cells, which also acts as work horses of the cells for chief molecular functions. Based on the pathway analysis, interaction network from STRING database and with a strong *in-vitro* molecular interaction studies as mentioned in relevant literature, thus protein-protein docking studies were performed for homology modelled proteins. PBP2A acts as dock target, which is submitted as receptor that interacting with PBP3 and TG proteins. GRAMM-X server [22] has been used to for developing protein-protein docking studies, as this sever develops the surface interactions without intact residues, therefore PatchDock sever [23] was used for sorting the intact interface residues as major drug targets. GRAMM-X runs based on the FFT (Fast Fourier Transform) for the global search out of best stiff protein conformations, whereas PatchDock server works based on the algorithm inspired by object recognition and image subdivision techniques used in the Computer Vision in turn that increases the intactness among two interacting protein's amino acids which are the lead drug targets for Pharmacophore studies.

Identification of intact interface residues between target and other interacting proteins

Drug targets are the binding residues readily interacts with other proteins, small peptides, metabolites and other small ligands. The target residues involves in three different mode of interaction such as distant, moderate and intact or contact residues, this differentiation is based on the bonding types and their strength of interaction. Intact residues are the major targets among all the pharmacophore studies, since they have stronger covalent interactions that requires higher energy and efficiency of drug molecules to break these type of interactions. There are few servers which give the predicted interacting residues but not based on this differentiation, hence manual selection of intact residues could give a platform for efficient drug screening. After the selection of efficient protein-protein docking studies, PyMOL programme [24] was used to search for the selection of intact residues by 3D rotation and visualization of intactness of the opposite residues of docked proteins. In this method, initial protein editing is required before sorting for the intact residues, as shown in the following flowchart 1.

Flowchart 1. Methodology for selecting the intact interface residues by PyMOL software



Note: In the PyMOL software, each **"Section"** with their **"Sub-sections"** are strictly followed for editing the protein sequence and structure for residue identification.

3. RESULTS AND DISCUSSION

Retrieval of target sequences and interacting protein structures

The selection of target protein for drug designing is a crucial step in pharmacophore studies. In case of protein drug targets, its three dimensional structure is essential. Proteins that doesn't have X-ray crystallographic structures, sequence based homology modelling studies should be carried out. Penicillin binding protein complex has many protein subunits with different functions, among PBP2A has a major interaction with its adjacent subunit PBP3 during peptidoglycan synthesis in all multi drug resistant pathogens. Henceforth, PBP2A and PBP3 of *Staphylococcus aureus* sequences

were retrieved from UniProt database as shown in table 1 with accession and definition line. Transglycolase structure has retrieved from PDB database (1qsa) for protein-protein docking studies as shown in figure 3.

Table 1. Sequence accession and definition line of retrieval from UniProt for modelleling

>tr|Q6I7E7|Beta-lactam-inducible penicillin-binding protein 2A OS=Staphylococcus aureus
>tr|A0A1K9IMW8|Cell division protein FtsI [Peptidoglycan synthetase] / Transpeptidase, Penicillin-

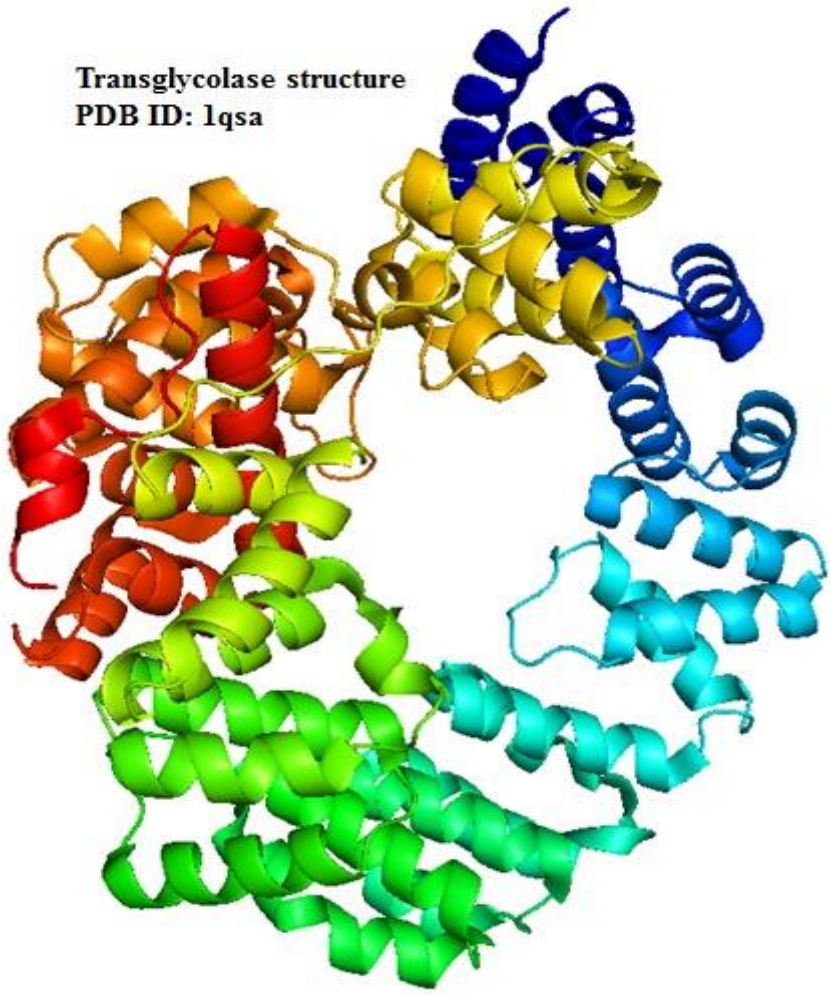


Fig. 3. Structure of Transglycolase (1qsa) as interacting protein of PBP2A

Template identification

Template identification has done in two ways such as non-automated template search through PDB-BLAST as shown in figure 4 and another automated template search by Swiss Model programme which is more accurate than the non-automated template identification as shown in figure 5. Templates through Swiss Model has been considered for homology modelling and has much accuracy with structure validation through Ramachandran plots [25]. The templates above 50% identity were considered to be more reliable and generate the accurate models [26].

Sequences producing significant alignments:

Select: All None Selected:18

Alignments Download GenPept Graphics Distance tree of results Multiple alignment

	Description	Max score	Total score	Query cover	E value	Ident	Accession
☑	Chain A, Structure Of Penicillin Binding Protein 2a From Methicillin Resistant Staphylococcus Aureus Strain 27r At 1.80 A Resolution	1293	1293	96%	0.0	99%	1VQQ_A
☑	Chain A, Crystal Structure Of Pbp2a From Mrsa In Complex With Peptidoglycan Analogue At Allosteric	1291	1291	96%	0.0	99%	3ZG5_A
☑	Chain A, Structure Of Nitrocefin Acyl-Penicillin Binding Protein 2a From Methicillin Resistant Staphylococcus Aureus Strain 27r At 2.00 A Resolution	1290	1290	96%	0.0	99%	1MWS_A
☑	Chain A, Crystal Structure Of Pbp2a Clinical Mutant E150k From Mrsa	1290	1290	96%	0.0	99%	4BL2_A
☑	Chain A, Crystal Structure Of Pbp2a Double Clinical Mutant N146k- E150k From Mrsa	1288	1288	96%	0.0	99%	4CPK_A
☑	Chain A, Crystal Structure Of Pbp2a Clinical Mutant N146k From Mrsa	1279	1279	96%	0.0	99%	4BL3_A
☑	Chain A, Structure Of Semet Penicillin Binding Protein 2a From Methicillin Resistant Staphylococcus Aureus Strain 27r (Trigonal Form) At 2.45 A Resolution	1249	1249	96%	0.0	97%	1MWR_A
☑	Chain A, 2.3 Angstrom Crystal Structure Of The Monomeric Form Of Penicillin Binding Protein 2 Prime From Enterococcus Faecium	347	347	88%	3e-110	36%	5E31_A
☑	Chain A, Penicillin-Binding Protein (PBP2) from Helicobacter pylori	141	141	76%	3e-35	27%	5LP4_A
☑	Chain A, Crystal Structure Of D,d-transpeptidase Domain Of Peptidoglycan Glycosyltransferase From Eggerthella Lenta	95.1	95.1	59%	3e-20	24%	4MNR_A
☑	Chain A, Crystal Structure Of E. Coli Penicillin Binding Protein 3	91.7	91.7	76%	5e-19	22%	4BJP_A
☑	Chain A, Crystal Structure Of Penicillin-binding Protein 2 From Neisseria Gonorrhoeae Containing Four Mutations Associated With Penicillin Resistance	85.9	85.9	81%	3e-17	22%	3EQV_A
☑	Chain A, Crystal Structure Of Penicillin-binding Protein 2 From Neisseria Gonorrhoeae Containing An A501t Mutation Associated With Cephalosporin Resistance	85.1	85.1	81%	6e-17	22%	5KSH_A
☑	Chain A, Crystal Structure Of A Peptidoglycan Glycosyltransferase From Burkholderia Ambifaria	84.7	84.7	76%	9e-17	22%	5UY7_A
☑	Chain A, Crystal Structure Of Penicillin-Binding Protein 2 From Neisseria Gonorrhoeae	79.0	79.0	64%	6e-15	22%	3EQU_A
☑	Chain A, Structure of a putative peptidoglycan glycosyltransferase from Atopobium parvulum in complex with penicillin G	52.0	52.0	38%	1e-06	24%	4N1X_A
☑	Chain A, Crystal structure of peptidoglycan glycosyltransferase from Atopobium parvulum DSM 20469	52.0	52.0	38%	2e-06	24%	4JBF_A
☑	Chain A, Structure of a putative peptidoglycan glycosyltransferase from Atopobium parvulum in complex with nafcillin	51.6	51.6	38%	2e-06	24%	4RA7_A

Fig. 4. Non-automated template search, pBLAST with PDB database

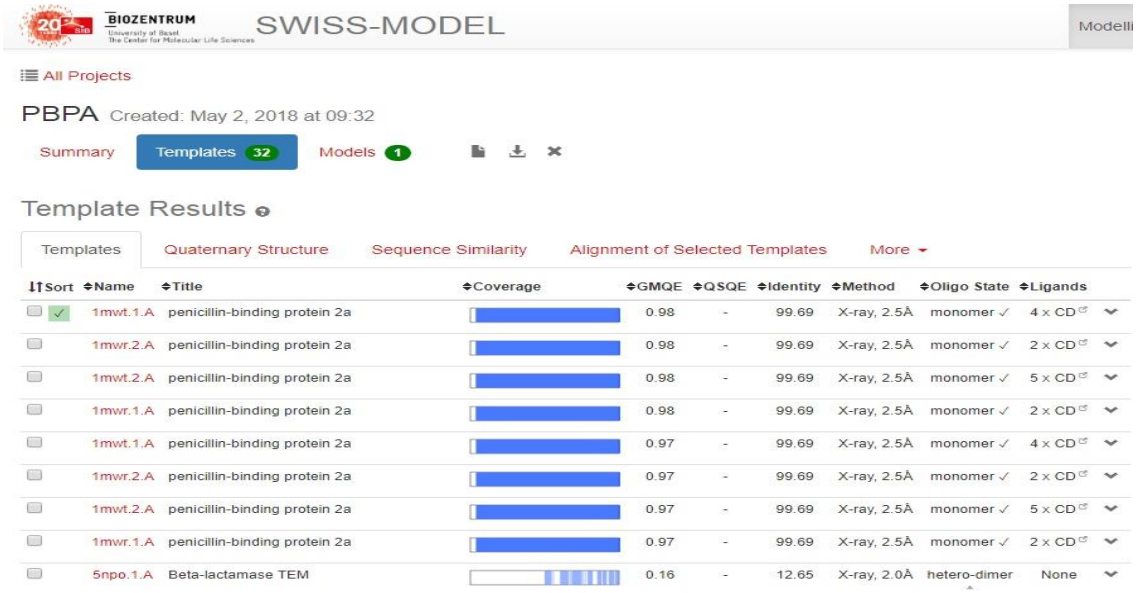


Fig. 5. Automated template search, Swiss Model

Homology modelling

The Homology modelling of PBP2A protein by Easy Modeller 4.0 and Swiss Modeller has provided two different protein modelled structures with the use of same template both offline and online platforms as shown in figure 6. Easy Modeller 4.0 has provided with unrefined loops in the structure whereas Swiss Modeller has provided auto-refined protein structure. Henceforth, PBP3 protein has also modelled through Swiss Modeller as shown in figure 7. Further the structure validation through Ramachandran plot [25] revealed the structures from Swiss modeller has more accurate with good

stereo chemical quality (27).

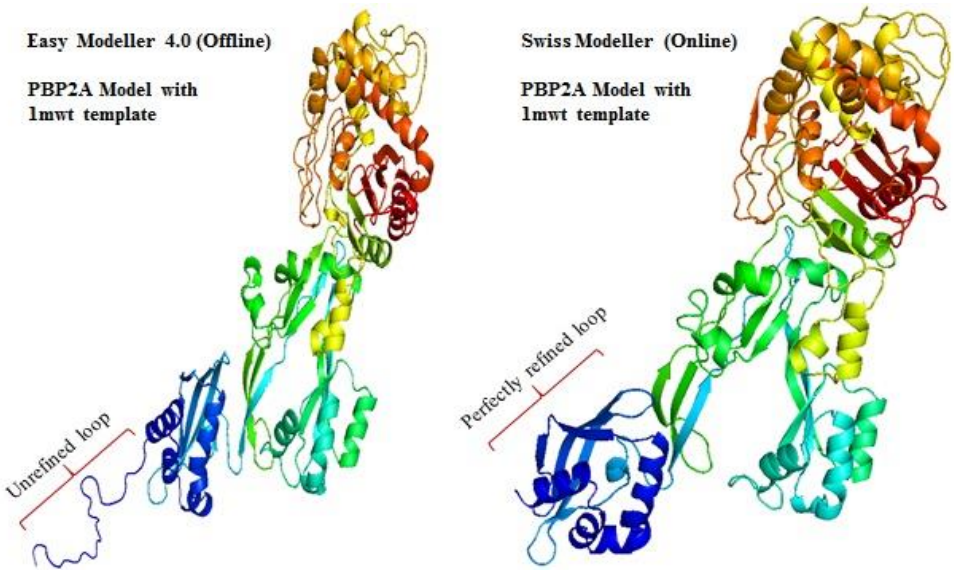


Fig. 6. 3D model structure of protein PBP2A by Easy Modeller 4.0 and Swiss Modeller

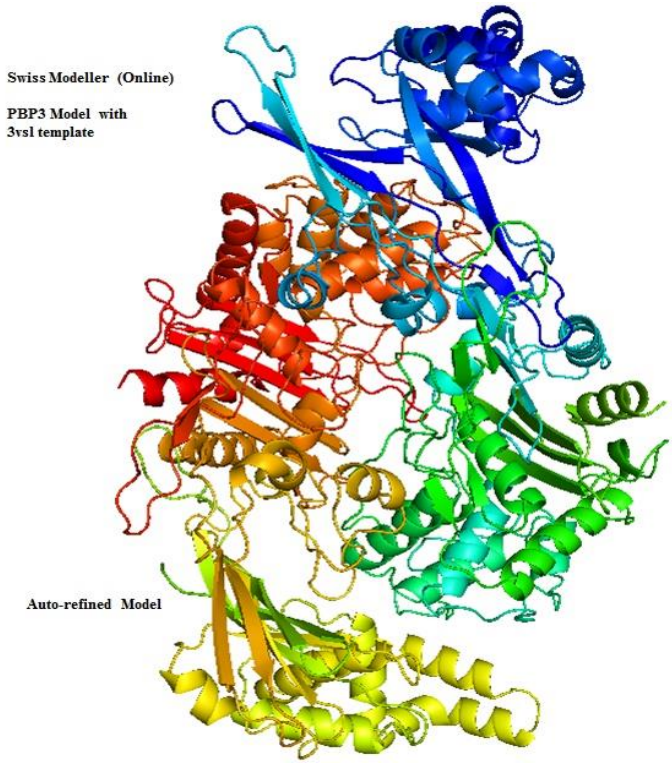


Fig. 7. 3D model structure of protein PBP3 by Swiss Modeller

Model validation and verification

The SAVES results reveal the quality of the modelled proteins PBP2A and PBP3 using Ramachandran plots development. The quality of every protein though these plots decides based on the Residues in most favoured regions (A, B, L) should show above 90% for high protein structure quality [28]. The average above 85 to 90% will depicts good models but not with high quality. If the modelled protein has less than this percentages will not be considered as good refined models and should not be used for further drug designing related studies. PBP2A protein has shown very high

quality structure with 92.9% and PBP3 showing the good quality with 88.7% as shown in figure 8, which are best feasible to use for the drug discovery studies.

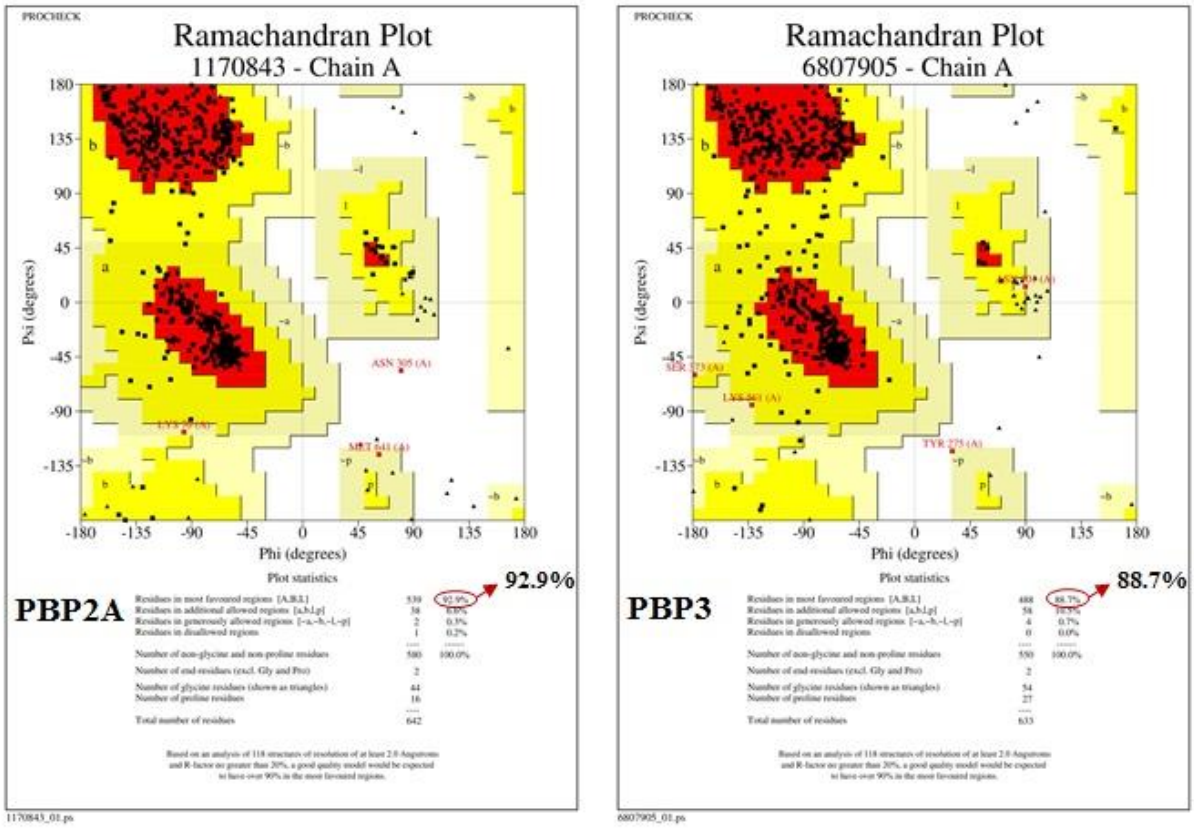


Fig. 8. SAVES Ramachandran plots for quality check of modelled proteins PBP2A and PBP3

Active site prediction

Prediction of active sites on an enzyme or protein reveals the specific binding site of proteins or other peptide residues. These sites are more specific for substrates in case of enzymes, for protein-protein interactions active sites are not feasible for binding and cope up the interactive functions [29]. The SCFbio Active site prediction sever predicted a list of active site on PBP2A protein as shown in table 2. Totally 30 different predicted active sites has shown, out them 6 peptides are lengthy (which are highlighted in red colour) and have more probabilities of largest active sites for binding the other protein molecules for targeted functions.

Binding pockets identification

Protein binding pockets are designated sites for binding proteins, peptides, metal ions, and small chemical ligands [30]. Binding pockets identified by GHECOM sever represents in two types of results such as Pocket grids with clustered colours within the 3D protein structure with all selected number of pockets identified as shown in figure 9. Secondly these pockets are represented graphically by residue based pocketness. This indicates, among the number of identified pockets, the high affinity of binding pocketness signifies the strength of the coloured lines in the graphical

representation. The highest pocket residues were observed in Pocketness for cluster1 and cluster2 as shown in the figure 10. Based on the Rinaccess option, plenty of binding pockets of small segments were identified, in turn these results will help in docking studies and selection of interface residues as drug target identifiers.

Table 2. Active sites of PBP2A protein for specific analogue binding studies

Cavity No.	Amino acid sequence of the determined active site on PBP2A	Cavity No.	Amino acid sequence of the determined active site on PBP2A
1	MYKTGVEDNFPLIRAS	16	KDGEQIHLSRYAV
2	**GDKEHSQLRYNTIAVPMWF**	17	NEDLKTQGVFI
3	**QISLPKEYWGTNAHDRVM**	18	QEHPKDNTYMALS
4	**RDVNKQAFIPHSGLTMWYE**	19	NKTDEHQRILV
5	**EIVKFQTSHDYWGNARM**	20	DINERYKLVAS
6	VKEPGMINYTFDLS	21	**VNKQTWAIGPHESDLF**
7	YKILPDEFGQHNV	22	DKIWENFMTQ
8	**GQAHRTVMKIDNELSY**	23	SEKINYMGVDP
9	QINSTLPKEYGDAM	24	DNQAYSLRKI
10	HSAVKYLDFPENTM	25	LQGYHKASV
11	ITAYNPWQLSEKG	26	NSGIKLMQY
12	LDEVANIKTPQGYS	27	FTLKIVGNAHY
13	FLTNIVDAWKQY	28	KEVGQILSN
14	LKTDEVSGYFRWIQ	29	IDLYQT
15	MLYSIQTAFPWG	30	NDRVPHGTILS

Pockets calculated by GHECOM(0XXX)

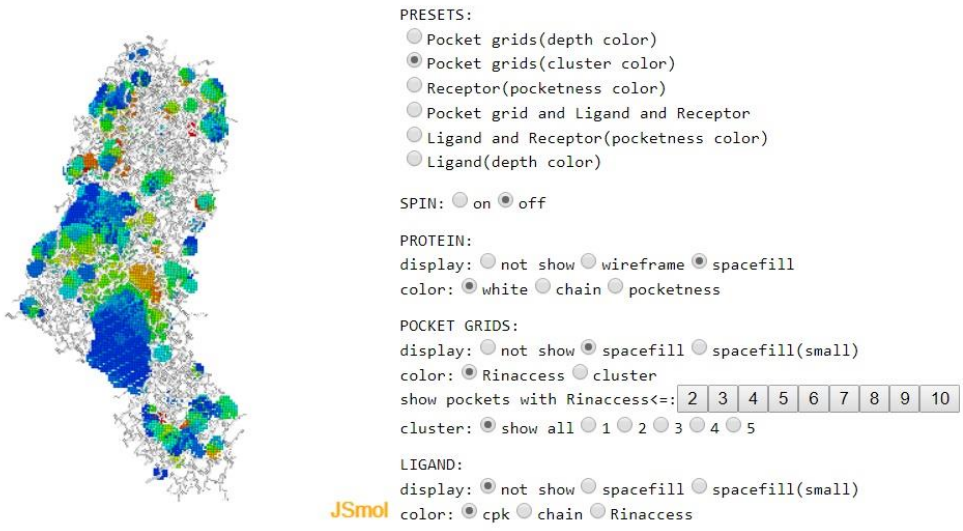


Fig. 9. Visual prediction of identified pockets of PBP2A protein

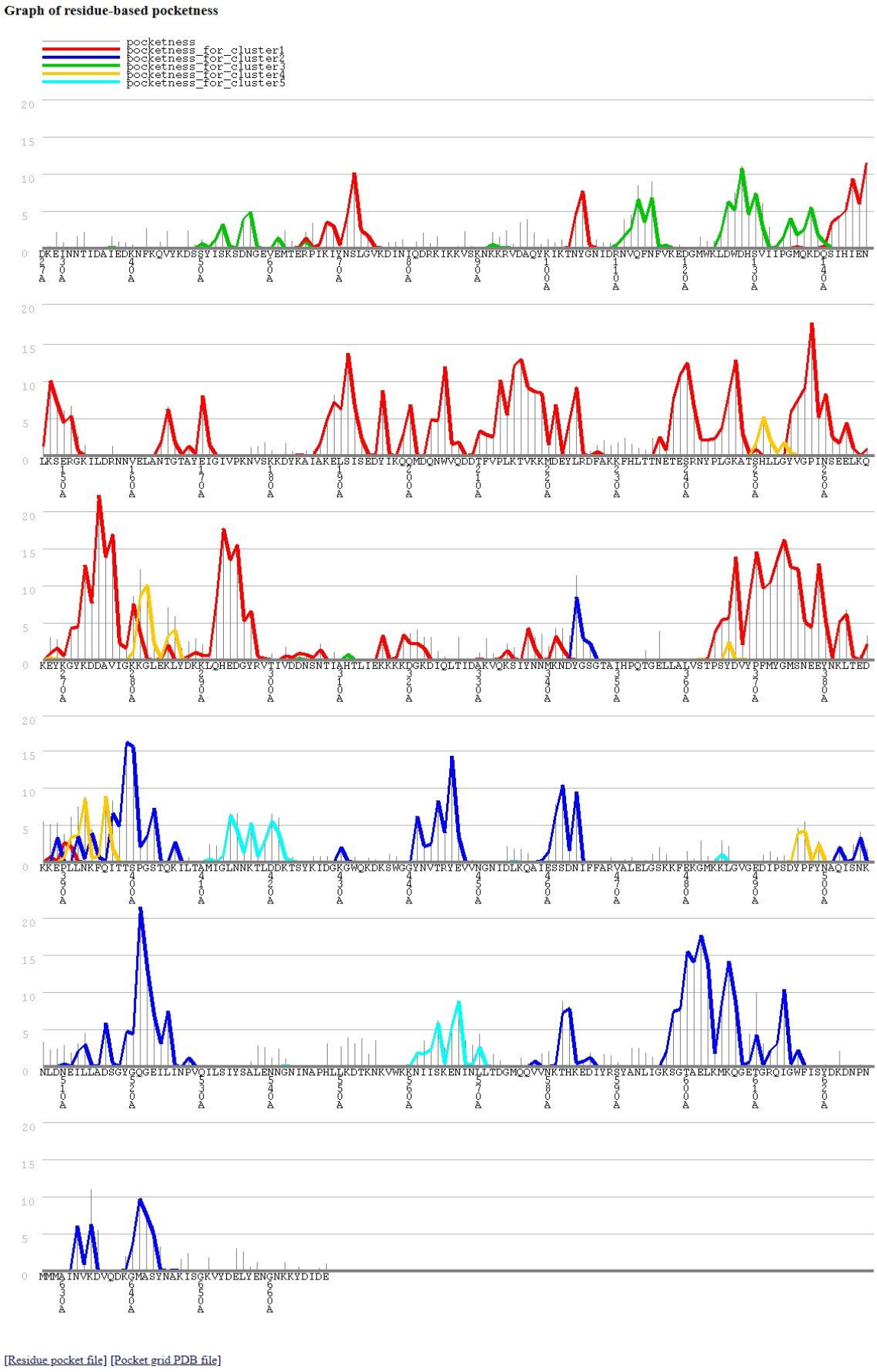


Fig. 10. Graphical representation of binding pocket residues and their pocketness in clusters with colour representations (as shown in left corner of the figure)

Protein-protein interaction network

Identification of number of protein-protein interaction networks among the selected protein targets has lead role in finding the targeted drugs and protein inhibitors to stop the protein molecular interactions for aimed functions. STRING is a sole and single database that represents graphical and 3D structures with in the interacting protein partners available through PDB. The interacting partners and their number of functional predictions are represented in coloured line between them, the more lines indicates more functional interactions among input target protein sequence. Proteins pbp2, ftsW, murC, murG and SACOL1122 are showing more functional interactions as shown in figure 11, among which pbp2 has direct interactions with both ftsW and target input sequence of PBP2A of *Staphylococcus aureus*. Based on the auto detect option for selection of organisms will represents the perfect functional partners. The list of interacting partners and their score of interaction is represented at the bottom of the graphical representation as "Your input" and "Predicted functional partners", highest the score represents the close functional partners and their role in interaction [31]. The overall outline of these interactions depicted lead to consider PBP2A as drug target protein for protein-protein docking studies.

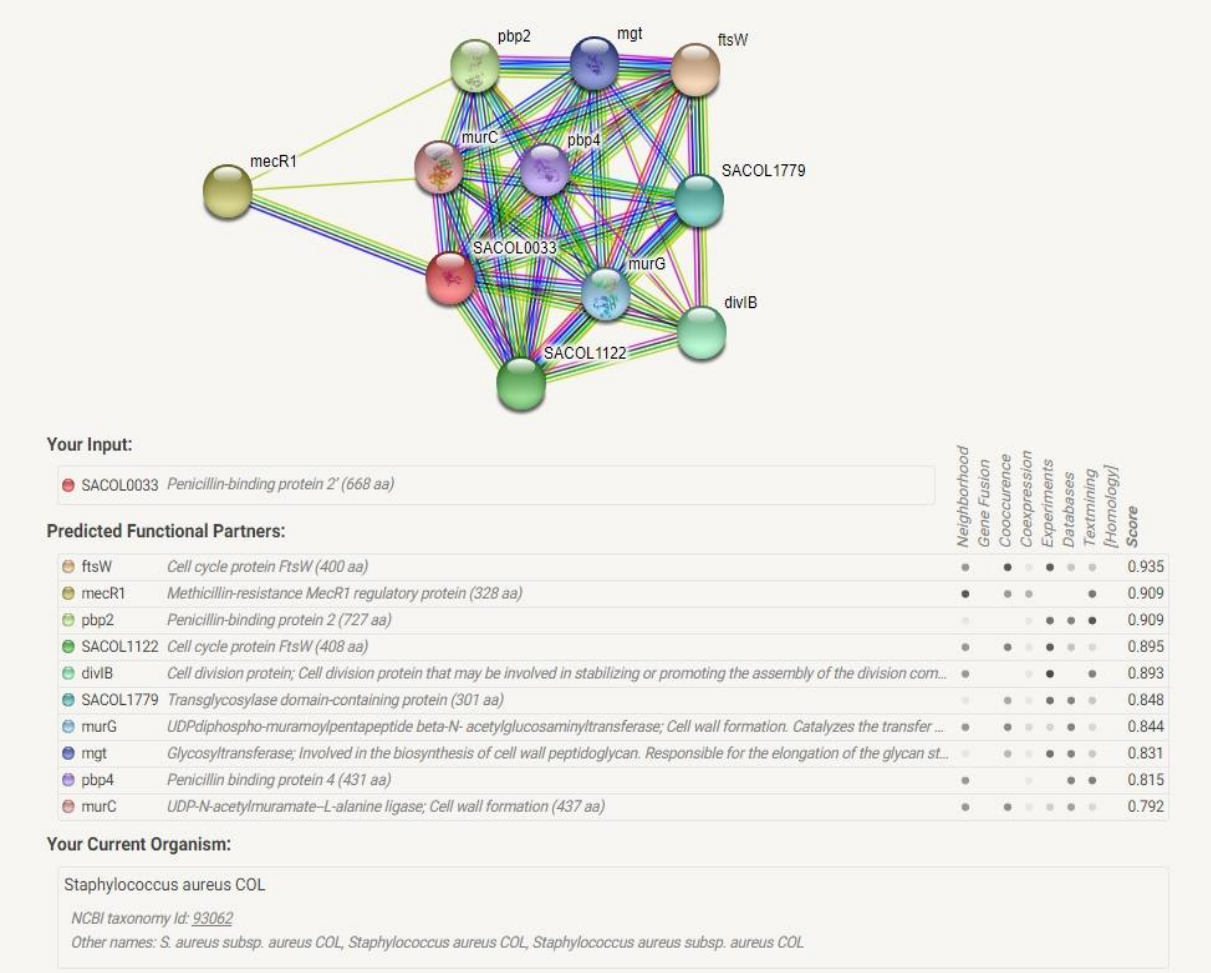


Fig. 11. Protein-protein networks and their functional partners from STRING database

Molecular pathway identification

The sequence based pathway prediction in *Staphylococcus aureus* reveals the metabolic interaction partners. The pathway analysis by KEGG database provides the information about the stage of interaction and their forwarded reactive metabolic products. PBP2A pathway [32] identification showed, these penicillin binding protein complex involves in the biosynthesis of peptidoglycan among all pathogenic microorganisms to protect themselves from lead β-Lactam kind and other antibiotics represents how the todays multi drug resistance phenomenon has increased. The pathway begins by Aminosugar UDP-GlcNAc with a series of Mur class of metabolic enzymes up to the conversion of Und-PP-MurNAc-GlcNAc. Later another class of enzymes called Fem (X, A, B) converts Und-PP-MurNAc-GlcNAc to L-Lys-(L-Gly)-5 in turn leads the signal to the Glycosyl transferase or Tranglycolase to bind to the Class A PBP protein for the reaction steps corresponding to Transpeptidase and Carboxyl peptidase activities and finally involves in synthesis of Lys-type-peptidoglycans of *Staphylococcus aureus* as shown in figure 12. Hence these protein-protein interactions leads to cell wall formation for antibiotic resistivity. Pathways analysis revealed the class of Mur and Fem genes involves in the amino sugar conversion, but they don't have any lead protein-protein interactions. The major interactions are observed from Transglycolase activity with PBP complex, thus this information implied to consider these interaction subsets for functional inhibitors or drug targets [33] by protein-protein docking studies.

Protein-protein docking studies

The functional protein interactions are the primary sources to perform the protein-protein docking studies. There are several hundreds of protein-protein docking online servers, among them GRAMM-X and PatchDock are used to study the protein docking comparative studies based on the types of interface residues they generate. The preliminary investigations such as literature survey, database mining for protein functions and interaction subsets, protein-protein interaction network studies, Protein pathway analysis confirms the type of proteins to select for the protein-protein docking [34]. Without these fundamental approaches, blind prediction cannot be considered for protein docking and drug designing studies. Since GRAMM-X sever revealed the large sets of moderate interface residues, PatchDock server was used for analysing the intact interface residues as the potential drug targets or sites for inhibitors molecules [35]. Docking of PBP2A and PBP3 through GRAMM-X resulted the moderate kind of residues as shown in figure 13A, similarly PatchDock sever revealed the highest intact residues and are highlighted in coloured spheres as shown in figure 13B. The interaction of PBP2A with TG reveals the same interface subsets as shown in figure 14A and 14B. The protein-protein docking is an *in-silico* prediction, based on the binding orientations and confirmations that they strictly bind within the best top 10 resolute solutions provided by these severs, out of these solutions manually selected best conformations has taken for further investigations as shown in figures 13A, B and 14A, B respectively.

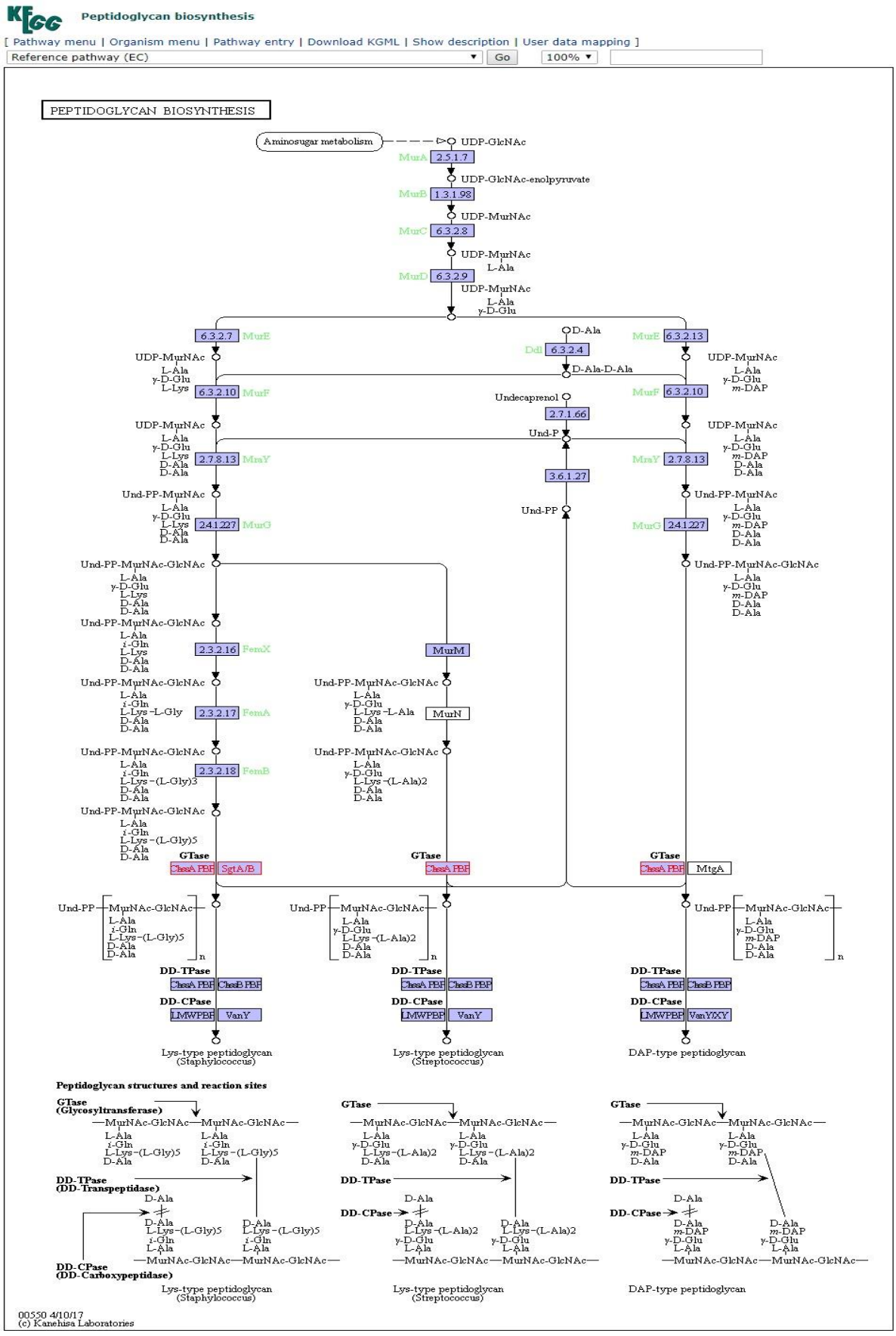


Fig. 12. PBP2A protein pathway analysis by KEGG reference pathways

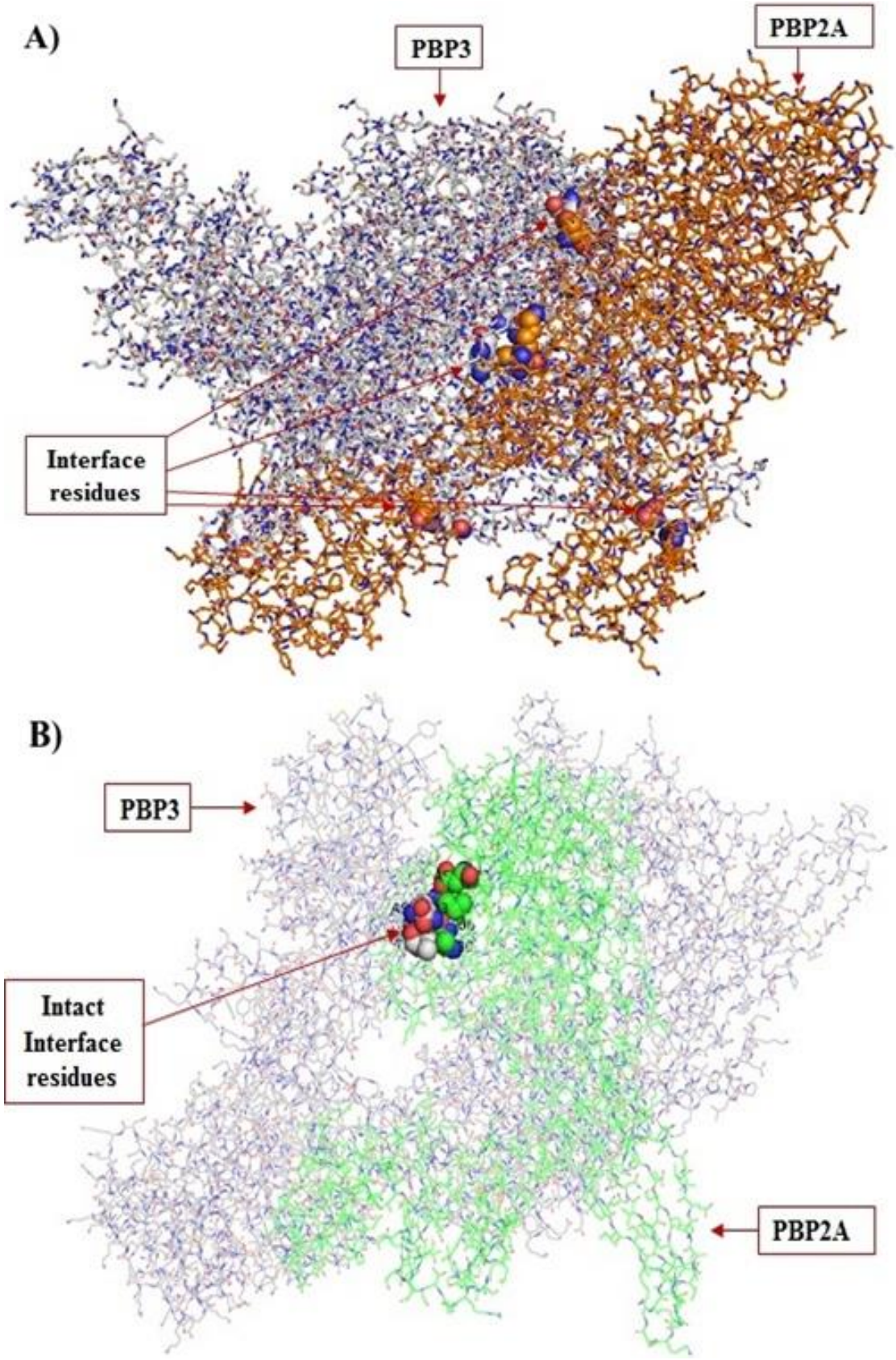


Fig. 13. A) GRAMM-X protein-protein docking of PBP2A and PBP3 with moderate interfaces. B) PatchDock protein-protein docking of PBP2A and PBP3 with intact interfaces.

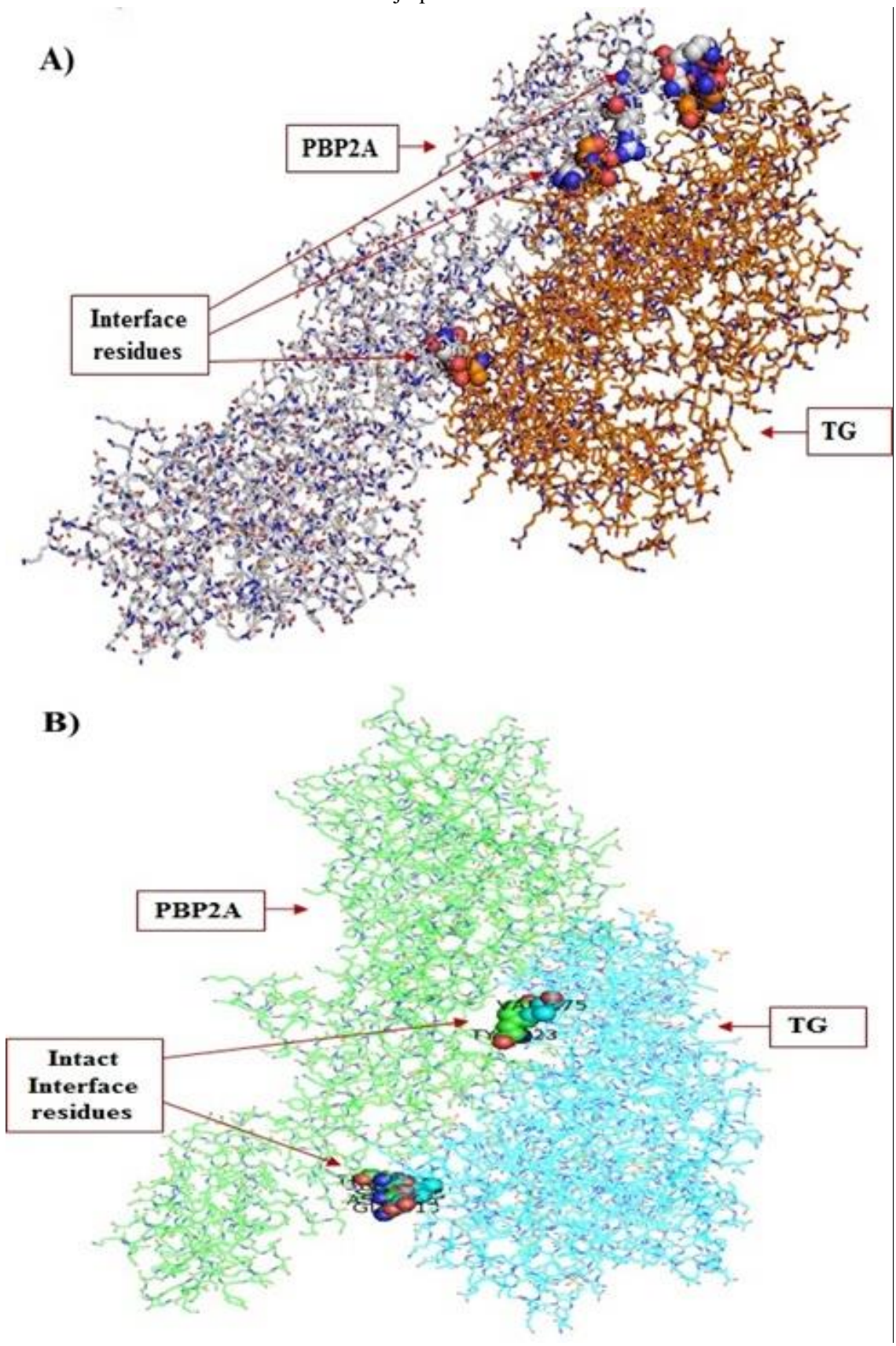


Fig. 14. A) GRAMM-X protein-protein docking of PBP2A and TG with moderate interfaces. B) PatchDock protein-protein docking of PBP2A and TG with intact interfaces.

Target interface residues for inhibitor and drug designing

Large sets of drugs or inhibitors are essential for binding the target sites, but there are many natural protein modification that happens from every generation to generation and microbial evolutions [36]. Targeting the unchanged protein domains during these evolutions is an important step in protein based drug designing. However, the protein conformations also changes due their molecular modifications and their functionality, hence the intact interfaces of the protein-protein docking plays a major role for inhibiting the protein interactions at a particular metabolic stage [37]. Finding these intact residues has achieved by manual progression of docked protein structure using PyMOL software, which has revealed the intact residue subsets as shown in figure 15A and 15B. Basically three types of interfaces will be observed such as distant, moderate and intact, among these distant and moderate are easily get changed through other factors, in turn their bonds will be in weak state hence they can be modified easily. The intact residues are covalent in nature that represents overlapping interactions as shown in figure 15A. The residues represented here are the major target groups for both PBP2A-PBP3 and PBP2A-TG interactions during biosynthesis of peptidoglycans [38].

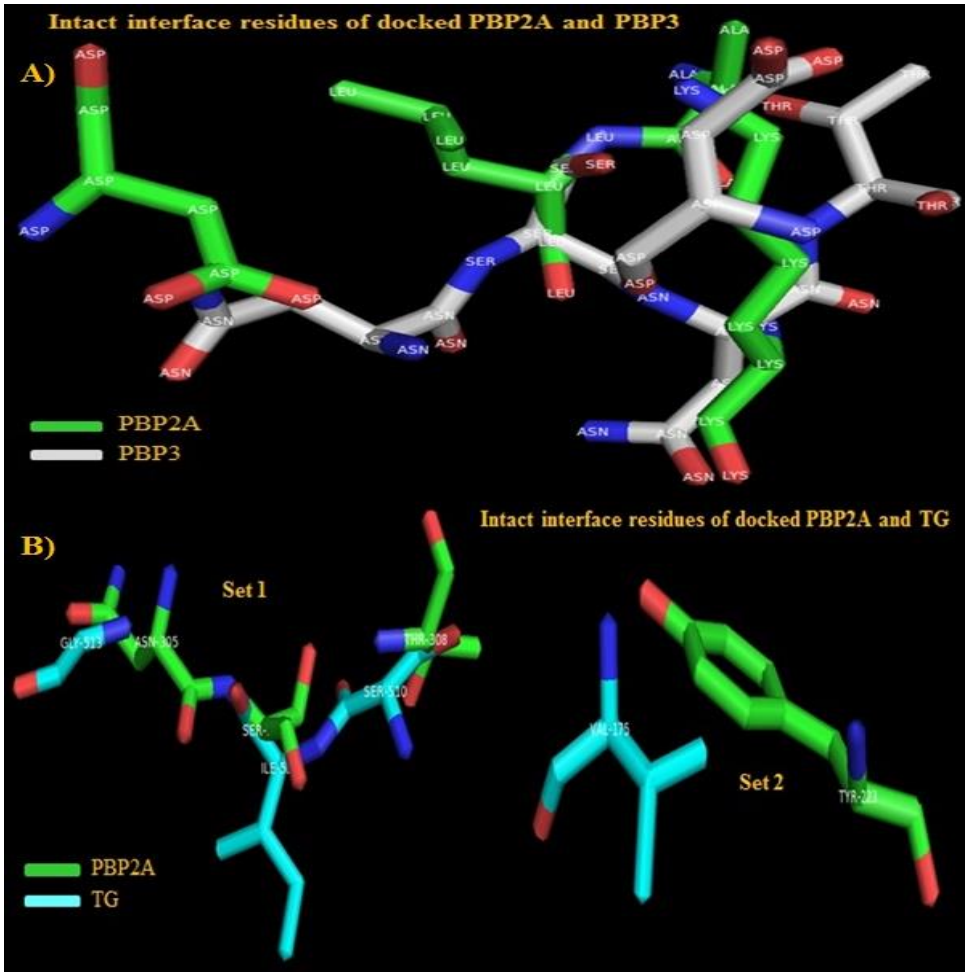


Fig. 15. A) PyMOL intact interfaces and target residues of PBP2A and PBP3

B) PyMOL intact interface and target residues of PBP2A and TG

4. CONCLUSION

The interface amino acids of PBP2A and PBP3/TG are the most interacting interface drug targets which are used for inhibitor binding activities. As per the vigorous literature survey, there are no reports available on *in-silico* protein interaction studies on penicillin binding proteins. Based on the basic construction explained in the introduction section, if these interaction subsets are stopped during cell wall formation, the pathogenic bacteria will be killed very easily with the existing antibiotics by losing the antibiotic resistant capacity. These target residue information has quite big role in drug discovery studies by means of drug screening and development strategies.

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CONFLICT OF INTEREST

Authors state no conflict of interest exists regarding the present research work.

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