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LuxS gene: Molecular docking and virtual screen analysis of *Staphylococcus hominis*

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Staphylococcus hominis plays a vital role in causing pathogenic infections in marine animals. In recent years, natural compounds from marine resources have gained interest owing to their potential effect against multidrug-resistant bacteria. LuxS gene is an important virulence factor needed to coordinate the biofilm production but no structural information is available for LuxS protein. To identify the homology model and to validate LuxS protein structure, an investigation was carried out using Modeller software. Molecular dynamics analysis was performed using a Desmond protocol. Molecular docking studies were carried out using marine compounds to suppress the LuxS protein, and antibiotics were also docked. Virtual screening was performed with LuxS protein against binding (CID-11446) dock score (-9.647) and Maybridge databases (CID-9017) docking score (-9.820) to find the potential compounds which provide better results than marine compounds. On the basis of the findings, it is concluded that the marine compound Aerobactin (CID-123762) is a potential inhibitor for LuxS protein in *S. hominis* with the highest dock score of -10.337, having eight hydrogen bonding interactions. Hence the compound could be further exploited for producing a drug against *S. hominis*.

[**Keywords**: *Staphylococcus hominis*; LuxS; Biofilm; Aerobactin; Maybridge database]

Introduction

The LuxS QS system is present in a variety of Gram-negative and Gram-positive bacteria. In several pathogens it is involved in virulence, but it appears to be nonfunctional or not involved in virulence in some others[1]. The biochemical function of the LuxS protein of *Staphylococcus aureus* in producing AI-2 autoinducer has been demonstrated earlier[2]. The LuxS mutant strain formed a thicker and more compact biofilm compared to the wild-type strain and was a more successful colonizer in an animal model of central venous catheter infection. Sequence analysis of completed genomes revealed that *Staphylococcus* spp., like many other bacteria, also contain a LuxS gene and therefore may employ a second signaling system based on the furan one derivative, autoinducer 2 (AI-2).[3] The genome of a wide variety of prokaryotes contains LuxS gene homolog, which encodes for the protein S-ribosylhomocysteinelyase (LuxS). This protein is responsible for production of the quorum-sensing molecule AI-2 and has been implicated in a variety of functions, such as flagellar motility, metabolic regulation, toxin production, and even in pathogenicity[4]. LuxS in *Staphylococcus epidermidis* is functional and LuxS-dependent gene regulation represses biofilm formation *in vitro* and pathogen success during biofilm-associated infection. Although regulating different biofilm factors, the two QS systems of *Staphylococcus* sp., agr and LuxS, have similar effects on the biofilm mode of growth[5].

The ultimate goal of molecular docking can be defined as the prediction of the donor-acceptor complex structure, where the receptor is usually a protein and the binder is a small natural or synthetic molecule, peptides, or proteins. In docking, the search algorithm explores different positions for the ligand in the receptor's active site, using its translational, rotational, and conformational degrees of freedom[6]. There are two key points in any docking program: The search for the "best" conformation resulting from the formation of the protein-ligand complex and the calculation of free energy for its association[7].

The need for a rapid search for small molecules that may bind to targets of biological interest is of crucial importance in the drug discovery process. One way of achieving this is the *in silico* or virtual screening (VS) of huge compound collections to identify a subset of compounds that contains relatively many hits against the target, compared to a random selection from the collection. The compounds that are virtually screened can stem from corporate or commercial compound collections, or from virtual compound libraries. If a three-dimensional (3D) structure of the target is available, a commonly used technique is structure-based virtual screening (SBVS)[8].

The construction of reliable scoring functions is fast enough to evaluate hundreds or thousands of ligands in a few minutes. This has led to the development of a large number of functions that make use of approximations for assessment of the affinity constant of a receptor-ligand complex[9]. The aim of computer simulations of molecular systems is to compute macroscopic behavior from microscopic interactions.[10] The present study aims at characterization of virulent protein-LuxS through 3D structure prediction and validation, molecular docking, and dynamic simulation, and VS analysis in *Staphylococcus hominis* by Maybridge and Binding databases against LuxS protein.

Materials and Methods

Template selection of LuxS protein

The amino acid sequence of LuxS protein from *S. hominis* was obtained in FASTA format from the UniProtKB database (accession number C2LXE0). The retrieved protein sequence was submitted to a Blastp search against the Protein Data Bank (PDB) for getting the best structural homology of the protein LuxS. From the results based on the high sequence identity and query coverage, the protein structure with PDB ID: 1J6X was selected as a template.

Target-template alignment

The sequence data of the target and template were subjected to sequence alignment which was performed by CLUSTAL OMEGA. A global dynamic programming algorithm was used to construct an alignment for full length of the sequences. In the CLUSTAL OMEGA program, the pairwise distances are used to locally adjust the gap opening calculated using a fast approximate method. These multiple sequence alignments provide structural and functional information[11].

Homology modeling of LuxS

Modeller 9.17 program (http://www.salilab.org/modeller/) was employed to generate the initial 3D models of LuxS. It generates 3D models by optimization of molecular probability density functions. The optimization process consists of applying the variable target function as well as conjugated gradients and molecular dynamics (MD) with simulated annealing. The final homology model was selected on the basis of DOPE score and GA341 score.

Model validation of LuxS protein 3D structure

The 3D models of LuxS protein were verified using PROCHECK program of Structural Analysis and Verification Server (SAVS). The overall stereochemical quality of the protein was assessed by Ramachandran plot analysis. The quality of the modeled protein LuxS was also validated by the ProSA server, available at https://prosa.services.came.sbg.ac.at/prosa.php. The ProSA provided the *Z*-score of the LuxS model. The ProSA program was employed to evaluate the quality of consistency between the native fold and the sequence and examine the energy of residue-residue interactions using a distance-based pair potential. The energy was transformed to a score called *Z*-score. Residues with negative *Z*-score indicate reasonable side-chain interactions. The ESPript program (http://espript.ibcp.fr/ESPript) was used to generate figures of aligned sequences with secondary structure information of the modeled protein LuxS. The program is written in Fortran and can be executed locally on Linux or Unix machines or on a web server via a CGI interface[12].

Virtual screening

Virtual screeing (VS) was performed to identify possible lead compounds from the Maybridge *HitFinder*™ database and the binding database. The ligand-based VS of inhibitor compound database with prepared protein was performed with the OPLS3 force field using the virtual screening workflow (VSW) module of the Schrodinger suite. The Maybridge *Hit Finder*™ sets are structural representatives of large non-redundant chemical libraries. This collection includes 14,400 compounds that represent the drug-like diversity of the Maybridge screening collection (~56,000 compounds). The Maybridge *HitFinder*™ set was obtained from http://www.maybridge.com. The ligand files were prepared for docking using Schrodinger LigPrep software. In addition to the generation of energy-minimized 3D structures, Schrodinger LigPrep was also used for addition of hydrogen and desalting of metal ions[13]. The binding database (http://www.bindingdb.org) is a publicly accessible database currently containing 20,000 experimentally determined binding affinities of protein-ligand complexes for 110 protein targets including isoforms and mutational variants and 11,000 small molecule ligands. The data were extracted from scientific literature, data collection focusing on proteins that are drug-targets or candidate drug-targets, for which structural data are present in the PDB. The data in the binding DB are linked to structural data in the PDB via PDB IDs. Also the chemical and sequence searches were referred to the literature in PubMed[14]. A total of 47 compounds were identified.

Molecular docking

Twenty-eight marine compounds were downloaded from Pubchem in the SDF file format. All the compounds were prepared by using the LigPrep module in Schrodinger 2015. The main objective of using LigPrep was to obtain low-energy 3D structures of the set of ligands in the library, for use in further computational studies. The OPLS3 force field was utilized to optimize the geometry and minimize the energy. Force field parameters were assigned to the ligand atoms using default treatment for possible tautomers, ionization at a selected pH range (7 ± 2 by default), and ring conformations (1 ring conformer by default). The co-crystallized ligand was considered as the reference molecule and a grid-enclosing box was centered at the co-crystallized ligand. The grid box was generated around the ligand binding site of the screened targets. The position of grid box was set as *XYZ* axis with radius 2.0 Å and the van der Waals (VDW) radii of the receptor atoms as 1.00 Å with a partial charge cutoff of 0.25 Å to soften the potential for the nonpolar part of the receptor. The LuxS was docked onto the ligand-binding site of the screened targets using Glide extra-precision (XP) docking (Glide, version 6.6, Schrödinger, LLC, New York, NY, 2015). Glide score (a modified and extended version of the empirically base function), Glide energy (modified Coulomb–VDW interaction energy), hydrogen bond interaction, and hydrophobic interactions were considered to investigate the therapeutic effect of LuxS[15].

Molecular dynamics

MD simulations were performed using the program Desmond. The initial coordinates for the MD calculations were taken from the modeled protein. The OPLS-2005 force field was used to model all amino acid interactions in the protein. Using System Builder, a 10 Å orthorhombic box with periodic boundary condition was constructed with a Four-Point Transferable Inter-molecular Potential (TIP4P) water model. A short energy minimization was performed via the steepest descent method, followed by a limited memory variation of the Broyden-Fletcher-Goldfarb-Shanno (LBFGS) algorithm. Salt concentrations of 0.15 M of Na^+ or Cl^- molecules were added to balance the net charge of the system. Before continuing with the production phase of MD simulations, the system was minimized with the default parameter set. The covalent bonds involving H atoms were constrained using the SHAKE algorithm and Particle Mesh Ewald (PME) method for electrostatics. The temperature was maintained at 300 K using the Nose-Hoover coupling algorithm and pressure of 1 bar was maintained through the Martyna-Tobias-Klein method. During MD simulation, all protein-ligand complexes were simulated for 10 ns. Energy and atomic coordinate trajectories were recorded every 3 ns. The root mean square deviation (RMSD), root mean square fluctuation (RMSF), and protein-ligand contacts in each trajectory were analyzed with respect to a time scale. The plots were graphically analyzed using OriginPro.

Results and Discussion

Homology modeling

The homology modeling is to select an appropriate template structure for constructing the target model. The sequence encoding for LuxS protein with UniProtKB ID: C2LXE0 was retrieved from the UniProt database. A Blastp search against PDB proteins confirmed that several PDB structures could serve as the potential template for building the LuxS model. LuxS has 65% sequence identity and 84% sequence similarity with the crystal structure PDB ID: 1J6X. Sequence data of target and template were aligned using the program CLUSTALW. The alignment between target and template sequence contains gaps. Here the pairwise sequence alignment between the target (C2LXE0) and template (1J6X) from residues 1 to 156 were aligned using CLUSTAL OMEGA. Among the available potential templates, the crystal structure of 1J6X was eventually selected as the template structure to construct the LuxS model (Fig. 1) in terms of the earlier criteria. The Vc-HlyU has identified Blast search for the

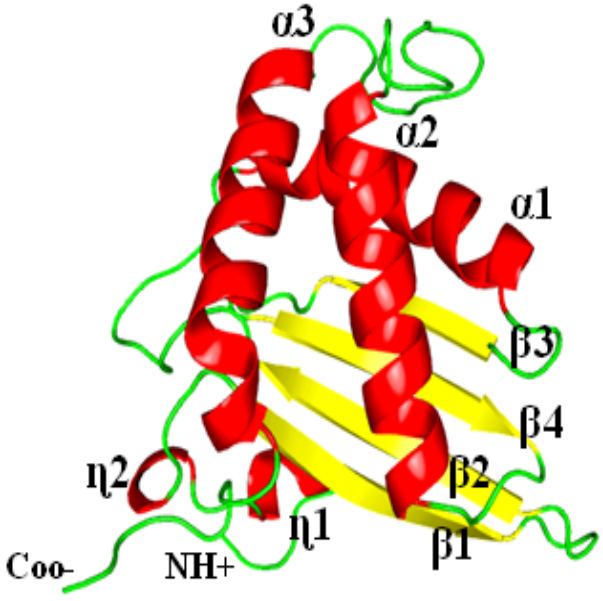


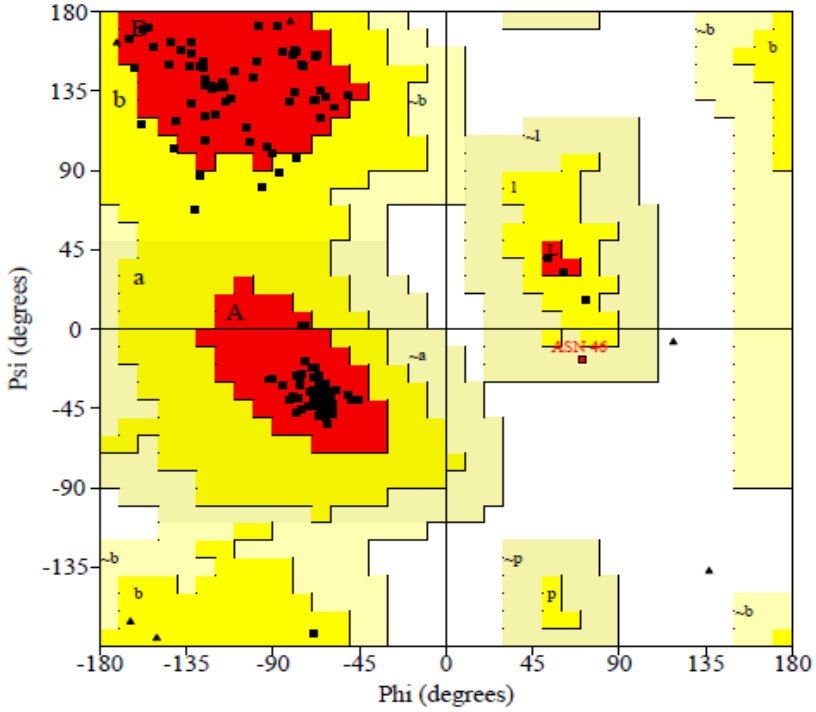
Fig. 1 — Ribbon schematic representation of modeled LuxS protein.

proteins with similar sequence and known 3D structure using the 108-residue-long Vc-HlyU sequence (SWISS-PROT: P52695)[15]. The modeling approaches of the proteins CadC, CzrA from *S. aureus* pI258, and SmtB from *Synechococcus* sp have been previously documented[16].

Model validation by SAVS

The Modeller-generated models were statistically analyzed by SAVS. The structures submitted were validated and the final protein structures selected after analysis in SAVS. Figure 2 shows the Ramachandran plot of the modeled protein LuxS. In the Ramachandran plot analysis, the residues were classified according to their regions in the quadrangle. Becker *et al.*[17] reported that the stereochemistry of the four models of human p-glycoprotein was assessed with PROCHECK. The Ramachandran plots showed a high percentage of the residues in the allowed regions: 99.7% and 98.3% for the nucleotide-bound models built with the SAV1866 and MsbA *Salmonella typhimurium* structures[18].

In the present study also, the stereochemical quality of the model was assessed with the PROCHECK program which predicted the model using the Ramachandran plot that 100% of the non-glycine residues and non-proline residues of the model had dihedral angles (the angle between two intersecting planes) in the most favored and additionally allowed region. No residues were found in disallowed regions



Plot statistics

Residues in most favoured regions [A,B,L]	129	92.1%
Residues in additional allowed regions [a,b,l,p]	10	7.1%
Residues in generously allowed regions [~a,~b,~l,~p]	1	0.7%
Residues in disallowed regions	0	0.0%
Number of non-glycine and non-proline residues	140	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	9	
Number of proline residues	5	
Total number of residues	156	

Fig. 2 — Ramachandran plot of the modeled protein LuxS

and the plot value was found to be 92.1% with 129 residues in the favored region. Such a percentage distribution determined by Ramachandran plot shows that the predicted model is quite satisfactory.

ProSA

The ProSA analysis showed that the *Z*-score in the LuxS model was negative in most residues. The *Z*-score of ProSA indicates overall model quality and measures the deviation of the total energy of the structure with respect to an energy distribution derived from random conformations. The *Z*-scores outside a range characteristic for native proteins indicate erroneous structures. To facilitate interpretation of the *Z*-score of the specified protein, its particular value is displayed in a plot that contains the *Z*-scores of the modeled protein[19]. In the present study, very few residues display negative interaction energies. It can be seen from Figure 3 that the overall results of LuxS were quite similar to those of the templates.

ESpript

ESpript was used in this study and the output obtained for the modeled protein LuxS is shown in Figure 4. Secondary structure elements are presented in the top panel: Helices are presented with squiggles, beta strands with arrows, and turns with TT letters. Strictly conserved residues are highlighted in red and partially conserved residues in yellow boxes. Sequence alignment between LdDCP (accession no. AAV80217) and EcDCP (accession no. P24171) sequences were produced by CLUSTALW. Alignment length:

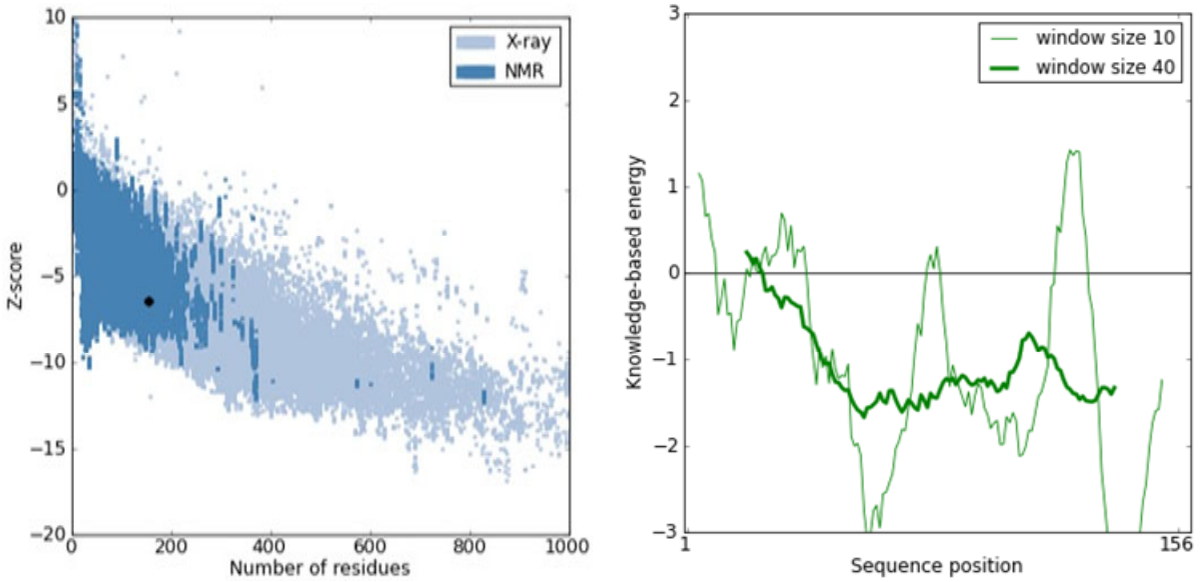


Fig. 3 — ProSA *Z*-score with respect to residue (window size=40). The scores of C2LXE0 and 1J6X are shown in dark green solid lines and light green solid lines, separately.

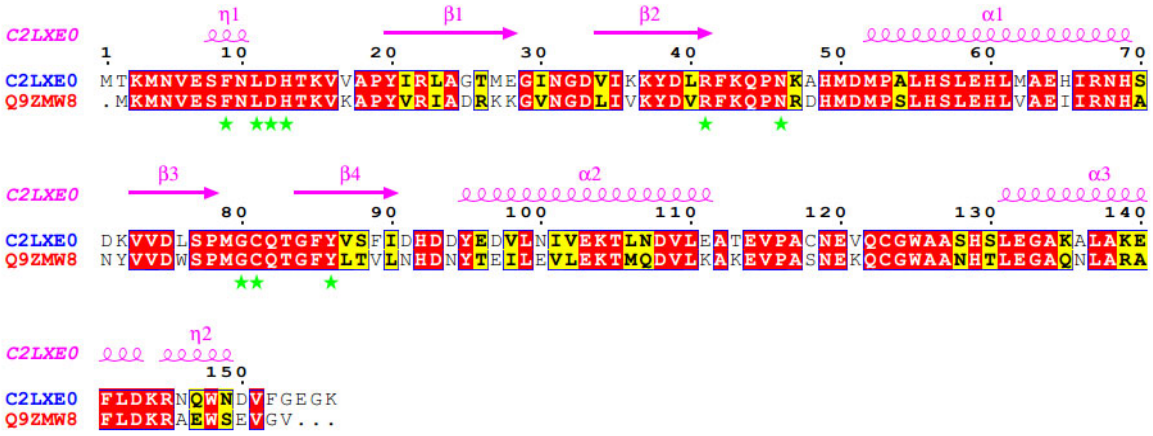


Fig. 4 — Secondary structure elements of the modeled protein LuxS

conserved regions are represented by black boxes. The secondary structure of EcDCP is demonstrated with arrows for β-sheet and spiral for α-helices. Zinc binding motif is represented by the asterisk[20].

VS with Maybridge and binding database

Computer-based strategies for structure-based drug discovery presents a valuable alternative to the costly and time-consuming process of random screening. In the present study, two different VS databases were used. This was done to ensure that the ligands shortlisted were actually docking into the binding site of interest[13]. On the basis of these criteria, the best five compounds from each of the binding and Maybridge databases were selected. The compounds selected from Maybridge database, namely, CID-9017, CID-6856, CID-2988, CID-9269, and CID-9270 were docked with the lowest binding energy of -9.820, -8.816, -8.567, -8.421, and -8.932, respectively and those from the Binding database, namely, CID-11446, CID-30686, CID-19559, CID-27386, and CID-347272 were docked with the lowest binding energy of -9.647, -9.114, -9.106, -8.988, and -8.897, respectively.

VSW uses Glide docking to rank the best compound which utilizes the scoring functions, high throughput virtual screening (HTVS), standard precision (SP), and XP. While HTVS and SP modes are used for a large set of ligands, XP docking is more accurate than these two methods. It uses the ligand poses that have a high score from SP docking. The XP GlideScore scoring function was used to order the best ranked compounds and specific interactions for example pi-cation and pi-pi stacking were analyzed using the XP visualizer in the Glide module[21].

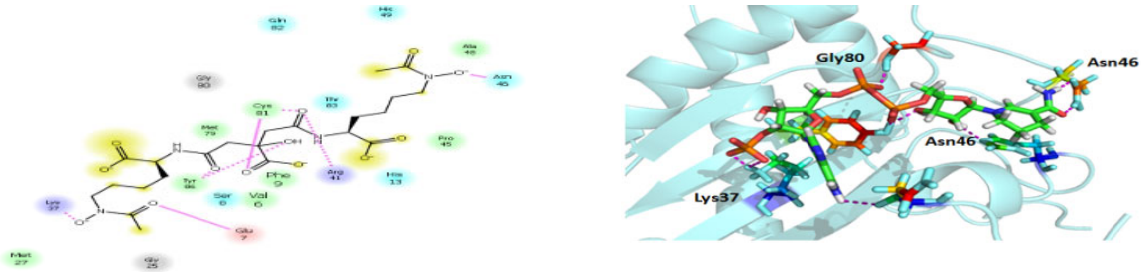
In this study, five compounds were selected from the Maybridge and binding databases on the basis of best docking score and Glide energy. The 3D (left panel) and 2D (right panels) diagrams in Figure 5 show protein-ligand interactions between LuxS and the five hit compounds. The hit compounds, the amino acid residue involved in the interaction with the hit compound, and other residues around the binding pocket were represented in stick and line forms, respectively. The hydrogen bond interactions between the compounds and the binding residues are shown as violet dashed lines.

Molecular docking

The geometries of the compounds were optimized by adding hydrogen and eliminating unwanted structures using the LigPrep module, and a database of chemical compounds was created using the Schrodinger suite; docking was performed using Glide. The 28 marine compounds, namely, camphor (CID-2537), baicalin (CID-64982), 4-hydroxy benzamide (CID-65052), cyanidin (CID-68247), aquayamycin (CID-73441), griseoluteic acid (CID-120266), vibrioferrin (CID-197680), cichoriin (CID-442101), acacetin (CID-5280442), apigenin (CID-5280443), baicalein (CID-5281605), daidzein (CID-5281708), prodigiosin (CID-5351169), fluvibactin (CID-5487127), anguibactin (CID-5487148), vulnibactin (CID-5487539), vibriobactin (CID-5487798), aerobactin (CID-123762), andrimid (CID-6439264), cycloprodigiosin (CID-6439795), magnesidin (CID-6443586), vibrindole A (CID-6452189), holomycin (CID-10262683), moiramide B (CID-11744644), vanchrobactin (CID-16658367), chembl 220619 (CID-23246249), trivanchrobactin (CID-46849168), and divanchrobactin (CID-46849169) were prepared separately by LigPrep and were docked into the binding pockets of the LuxS protein.

Molecular docking simulations of the 28 compounds active against the protein resulted in a few best compounds that were evaluated on the basis of their binding compatibility [docked energy (kcal/mol)] with the receptor. The final G-scores were analyzed on the basis of the conformation at which the ligands formed hydrogen bonds with at least one of the active-site amino acid residues of the corresponding 3D structure of the modeled protein with optimal binding affinity. The Glide scores and energies, including the VDW and electrostatic energies, were calculated for all the ligands against LuxS. On analyzing the docking score and the Glide energy, it was found that the ligands vibriobactin (-10.232, -65.556) and aerobactin (-10.337, -62.918) possessed better scores than the other compounds. Kirubakaran *et al.*[22] reported the *in silico* studies on marine actinomycetes as potential inhibitors for *Glioblastoma multiforme* (GBM). In this study, three proteins were considered to be the potential therapeutic targets for GBM. Among these, EphA2 was reported to be overexpressed in ~90% of GBM[22]. In the present case, *in silico* docking studies show that the marine compound aerobactin has an inhibitory effect on LuxS protein. MD simulation was performed to check the stability of the protein-ligand complex. The results show that the compound aerobactin is a potential inhibitor of LuxS protein.

CID-11446



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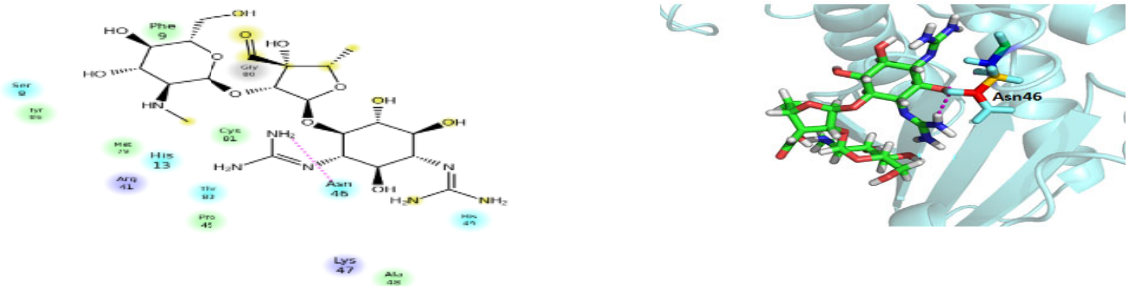

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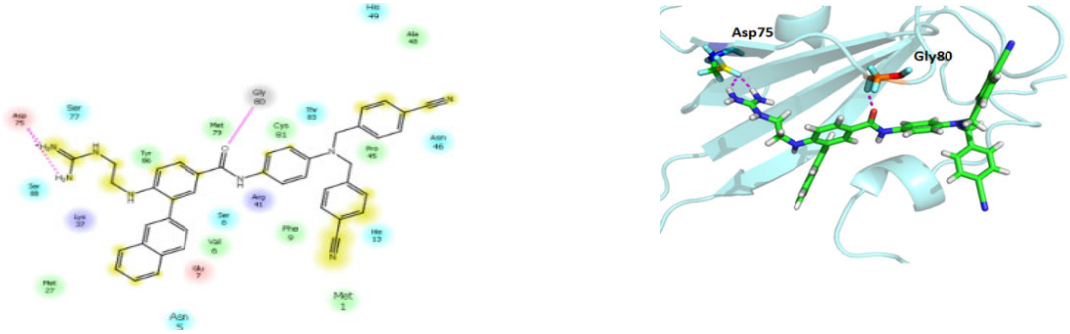

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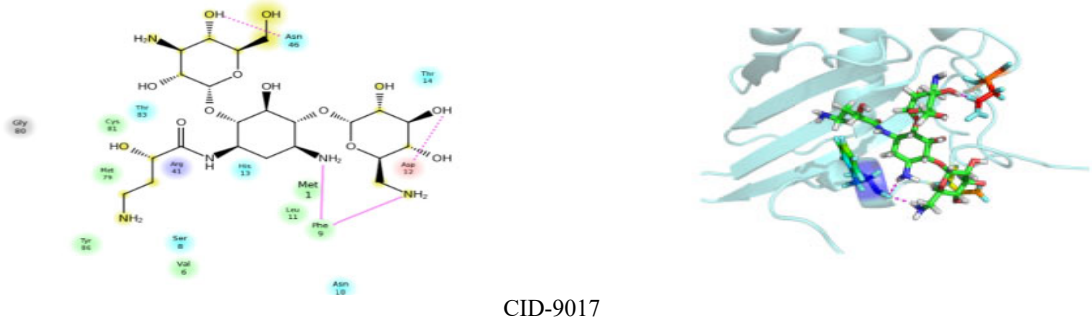

CID-9017

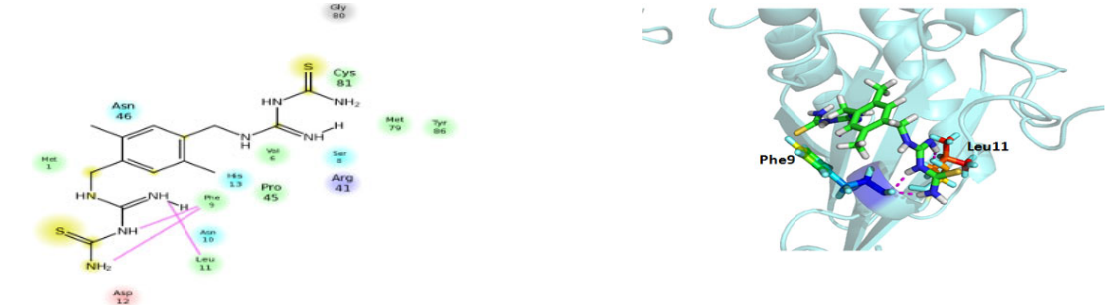

Fig. 5 — Ligand interactions and residue in ligand binding

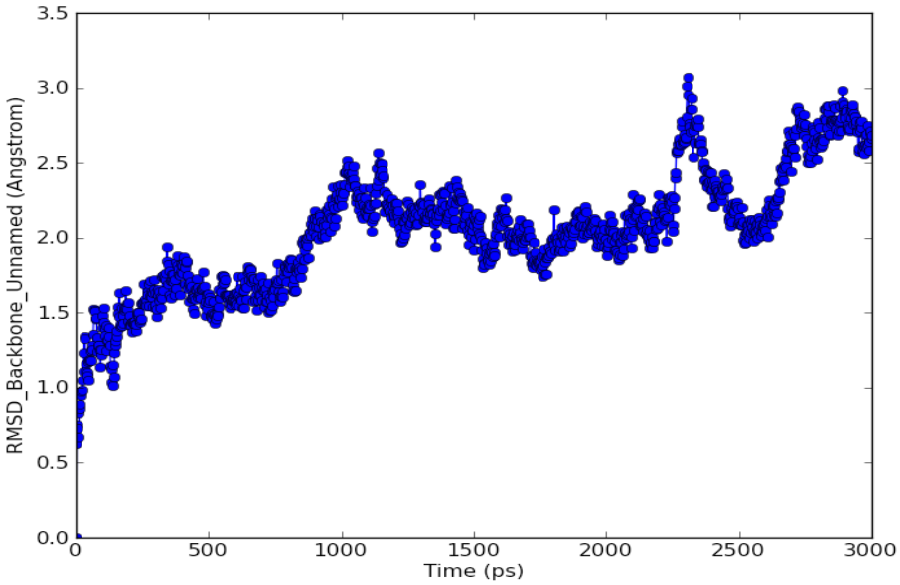

Fig. 6 — RMSD of the backbone atoms of the modeled protein over a time period of 3 ns.

Molecular docking simulation analysis

The docking of the ligands to the active site is shown in Figure 6. Out of the 10 best compounds, the marine compound aerobactin (CID-123762) was obtained from the XP output on the basis of its good interaction, formation of hydrogen bonds with the active site residues Asn46, Arg41, Cys81, Tyr 86, Glu7, and Lys37, and Glide score of −10.337. Hydrogen bonding measures the intermolecular interaction between the protein and ligands. So the compound aerobactin (CID-123762) was taken for MD. The potential energy, temperature, pressure, and volume and time dependence of the RMSD (A˚) from the TvCK homology model for the backbone atoms in 1000 ps MD simulation were evaluated. The backbone atoms in 1000 ps MD simulation suggest that the model is stable[23]. In the present study, MD simulation was also performed for the complex for 3 ns. The results suggest that the complex is stable.

Conclusion

LuxS protein is a virulent factor responsible for pathogenecity of the isolated bacteria *S. hominis*. Our study has provided the 3D structure of LuxS protein through a homology modeling approach. Aerobactin has high affinity to inhibit the LuxS protein in *S. hominis* with the highest dock score of −10.337. On the basis of the docking results, we identified that the lead molecule aerobactin has a better binding affinity against LuxS protein which was analyzed through its docking score and Glide energy. Further analysis of the binding conformation of the lead molecules revealed that LuxS protein plays an important role in protein-ligand interactions. However, further experimental studies are required to validate *in silico* analysis for the therapeutic potential of this lead molecule.

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