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Homology Modeling and Molecular Docking of the Antibacterial Protein SPLUNC1/ BPIFA1

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Author`s Contribution

[SA] Conception and design, Collection and assembly of data, [A]Analysis and interpretation of the data, Statistical expertise, [S]Final approval and guarantor of the article

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A B S T R A C T

Background: Short palate and nasal epithelial clone 1 (SPLUNC1), also known as the bacterial permeability family member a1 (BPIFA1), is encoded by the lung-specific x gene (LUNX). It is an innate immune defense protein secreted against bacterial infections, especially gram-negative bacteria.

Methodology: The nucleotide sequence of LUNX gene was obtained from NCBI (Accession No. Aaf70860.1). Protein translation, determination of molecular mass, prediction of disulfide bonds, and homology modeling were performed using the Expasy server, ClustalW, NCBI BLAST-2, protein calculator, and PDCP. Molecular operating environment (MOE) software was used for molecular docking of SPLUNC1 protein. The London dg and force-field-based gbvi/wsa dg scoring functions were used for 50 poses/runs.

Results: Sequence analysis showed that the cDNA of the mature lunx was 711 bp in length, encoding 237 amino acids. PDCP predicted the presence of a single disulfide bond at position C161-C205. Homology modeling results showed significant similarity of this protein with PLUNC like proteins from calithrix jacchus (96%) and pongo abelii (94%). Two cysteine residues, C161 and C205, are conserved in both species. Multiple sequence alignment showed the highest sequence similarity of human SPLUNC1 with that of chimpanzees (score 98.96) and rhesus monkeys (score 90.04). Molecular docking analysis confirmed the binding of SPLUNC1 protein with hydrophobic ligands specifically phosphatidyl ethanolamine, D-phosphatidyl choline, L-phosphatidyl choline, dipalmitoylphosphatidylcholine and phosphatidyl glycerol. SPLUNC1 did not bind to the sphingomyelin.

Conclusion: The SPLUNC1 protein showed significant homology with PLUNC like proteins from Calithrix jacchus (96%) and Pongo abelii (94%). Molecular docking confirmed its binding to the hydrophobic ligands.

Key words: SPLUNC1, PLUNC, Upper Respiratory Tract, Homology Modeling, Molecular Docking

Introduction

Short palate and nasal epithelial clone 1 (SPLUNC1) is also known as bacterial permeability family member A1 (BPIFA1), encoded by the lung-specific X gene (*LUNX*). This multifunctional protein is secreted by the upper respiratory tract and is known for its antibacterial activity.[1,2] It belongs to the human PLUNC-like gene subfamily which is located on chromosome 20q11 and contains 8 members. PLUNC family of proteins contains at least 10 related PLUNC family members. It has four short proteins called SPLUNC 1, 2, 3 and 4 with only three fully identified to have 256, 249 and 253 amino acids and 6 distinct long proteins called LPLUNC 1, 2, 3, 4, 5 and 6; four of which have been identified to contain 484, 458, 463 and 469 amino acids respectively. SPLUNC1 is the product of *LUNX* gene and is also called PLUNC. Short proteins have sequence identity with only the N-terminal domain of BPI, whereas long proteins have an identity with both the N- and C-terminal domains of BPI and LBP.[3-6]

These proteins are expressed in submucosal glands of respiratory tract, epithelium of nasal, laryngeal, pharyngeal,

tracheal, and bronchial airway passages. SPLUNC1 is secreted exclusively from the serous cells of upper respiratory tract.[7-10]

SPLUNC1 is an innate immune defense protein that is secreted against bacterial infections, especially gram-negative bacteria.[6] Its levels have been shown to increase exponentially after irritation, stress, invasive surgery, chronic obstructive lung disease, non-small cell lung carcinoma (NSCLC), inflammatory airway diseases and cystic fibrosis. A recent study investigated its antiviral effects and reported its significant role in inhibiting the entry and binding of influenza A virus in the airway epithelium.[11] Previously, it was identified as a superior biomarker for the diagnosis of micro metastasis in peripheral blood of NSCLC patients with highest sensitivity and specificity for lung cancer.[12] SPLUNC1 expression has been found to be decreased in the airways of patients with asthma. Recently, SPLUNC1 was found to inhibit Orai1 and play a role in reducing the inflammation in a murine asthma model.[13]

Expression of SPLUNC1 mRNA has been shown to increase in 84% of patients with NSCLC, and 80% of patients with micro metastasis have higher expression levels of *LUNX* than normal patients. The expression increases greatly with increasing disease severity but decreases shortly after treatment. These findings suggest the use of SPLUNC1 as candidate biomarker for early diagnosis, to assess the therapeutic effects and to analyze the prognosis of NSCLC.[12] Present study was designed to analyze the sequence characteristics, homology modeling to unveil its evolutionary relationships and to predict the possible ligands binding to the protein which would lead to the exploration of its antibacterial action.

Methodology

Nucleotide sequence of the human SPLUNC1 gene was downloaded from NCBI (Accession No. AAF70860.1). Protein translation, determination of molecular mass, isoelectric point and disulfide bonds of PLUNC protein were analyzed using ExpASy server, and NCBI BLAST 2 computer software, Protein Calculator and PDCP. Homology modeling was performed using CLUSTAL W software to find multiple sequence alignments of the SPLUNC1 protein between different species. Molecular Operating Environment (MOE) software was used for molecular docking of SPLUNC1 protein. Hydrophobic ligands were selected based on the available literature to determine binding affinity with the SPLUNC1 protein. London dg and force-field-based GBVI/WSA dG scoring functions were used for 50 poses/runs.

Results

Sequence analysis showed that the cDNA of mature *LUNX* was composed of 711 bp, containing an intact open reading frame encoding 237 amino acids (excluding the 19 amino acid signal peptide) and an in-frame stop codon (TAA). The Molecular weight of the protein was calculated using the Protein Calculator software and was found to be 24.6kDa, Isoelectric point of the protein was ~6.0 as obtained from the same software.

Prediction of disulfide bonds

A web server for Disulfide Bonding Connectivity Pattern (DBCP) prediction was used to analyze the number and position of disulfide bonds in the protein, showing the presence of a single disulfide bond at position C_{161}-C_{205}.

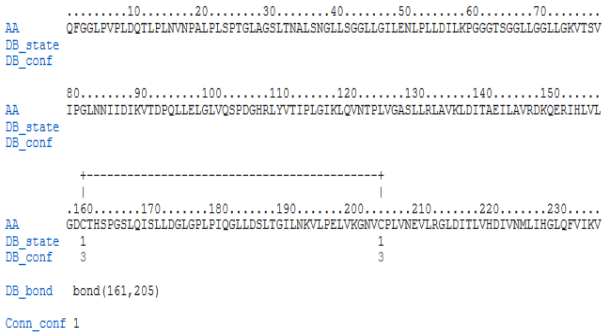

Figure. 1. Disulfide bond predicted by *PSI PRED* software, AA, Amino acid; DB state, disulfide bonding state (1 yes, 0 No); DB_conf, confidence of disulfide bond prediction. It shows the presence of disulfide bond at the position 161-205 with confidence of 3.

Homology Modeling

The deduced amino acid sequence of the mature PLUNC (excluding signal peptide) was compared against non-redundant proteins in the BLAST search tool and found significant homology with PLUNC from different species and also found many human PLUNC protein precursors.

Along with other reported homologies we revealed significant homology of PLUNC with PLUNC like protein from *Calithrix jacchus,* a new world monkey, having protein Accession No. XP_002747323.1, showing 96% sequence homology, which has not yet been reported in literature. Two cysteine residues, C_{161} and C_{205}, which participate in the disulfide bond only, were conserved among both species.

```
Query  1    QFGGLPVPLDQTLPLNVNPALPLSPTGLAGSLTNALSNGLLSGGLLGILENLPLLDILKP  60
            Q+GGLP+PLDQ LP NVNPALPL+PTGLAG LTNALSNGLLSGGLLGILENLPLLDILKP
Subject 20  QYGGLPIPLDQALPSNVNPALPLNPTGLAGILTNALSNGLLSGGLLGILENLPLLDILKP  79

Query  61   GGGTSGGLLGGLLGKVTSVIPGLNNIIDIKVTDPQLLELGLVQSPDGHRLYVTIPLGIKL  120
            GGGTSGGLLGGLLGKVTSVIPGLN IIDIKVTDPQLLELGLVQSPDGHRLYVTIPLGIKL
Subject 80  GGGTSGGLLGGLLGKVTSVIPGLN-IIDIKVTDPQLLELGLVQSPDGHRLYVTIPLGIKL  138

Query  121  QVNTPLVGASLLRLAVKLDITAEILAVRDKQERIHLVLGDCTHSPGSLQISLLDGLGPLP  180
            QVNTPLVGA+LLRLAVKL+ITAEILAVRDKQERIHLVLGDCTHSPGSLQISLLDGL PLP
Subject 139 QVNTPLVGANLLRLAVKLNITAEILAVRDKQERIHLVLGDCTHSPGSLQISLLDGLSPLP  198

Query  181  IQGLLDSLTGILNKVLPELVKGNVCPLVNEVLRGLDITLVHDIVNMLIHGLQFVIKV  237
            IQ LLDSLTGILNKVLPELV+G VCPLVNEVL GLDITLVHDI NMLIHGLQFVIKV
Subject 199 IQSLLDSLTGILNKVLPELVQGKVCPLVNEVLSGLDITLVHDIANMLIHGLQFVIKV  255
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Figure. 2. Amino acid sequence alignment of Human PLUNC (Query 1) with accession no. AAF70860_1 with *Calithrix jacchus* (subject) with Accession No. XP_002747323.1. showed 96% homology, with a score of 909. (Yellow=variations among the species, green=conserved cysteine residues that participate in disulfide bond formation).

```
Query  1    QFGGLPVPLDQTLPLNVNPA--------LPLSPTGLAGSLTNALSNGLLSGGLLGILENL  52
            QF GLPVPLDQTLPLNVNPA        LPLSP GLAGSLTNALSNGLLSGGLLGILENL
Sbjct  20   QFEGLPVPLDQTLPLNVNPARPWPVKPALPLSPIGLAGSLTNALSNGLLSGGLLGILENL  79

Query  53   PLLDILKPGGGTSGGLLGGLLGKVTSVIPGLNNIIDIKVTDPQLLELGLVQSPDGHRLYV  112
            PLLDILKPGGG SGGLLGGLLGKVTSVIPGLNNIIDIKVTDPQLLELGLVQSPDGHRLYV
Sbjct  80   PLLDILKPGGGISGGLLGGLLGKVTSVIPGLNNIIDIKVTDPQLLELGLVQSPDGHRLYV  139

Query  113  TIPLGIKLQVNTPLVGASLLRLAVKLDITAEILAVRDKQERIHLVLGDCTHSPGSLQISL  172
            TIPLGIKLQVNTPLVGASLLRLAVKL+ITAEILAVRDKQERIHLVLGDC HSPGSLQISL
Sbjct  140  TIPLGIKLQVNTPLVGASLLRLAVKLNITAEILAVRDKQERIHLVLGDCIHSPGSLQISL  199

Query  173  LDGLGPLPIQGLLDSLTGILNKVLPELVKGNVCPLVNEVLRGLDITLVHDIVNMLIHGLQ  232
            L GLGPLPIQGLLDSLTGILNKVLPELV+GNVCPLVNEVL GLDITLVHDIVNMLIHGLQ
Sbjct  200  LHGLGPLPIQGLLDSLTGILNKVLPELVQGNVCPLVNEVLSGLDITLVHDIVNMLIHGLQ  259

Query  233  FVIKV  237
            FVIKV
Sbjct  260  FVIKV  264
```

Figure. 3. Comparison of amino acid sequences of Human PLUNC (Subject) having Accession No. AAF70860_1 with the PLUNC like protein of Pongo abelii (Query) having Accession No. XP_002830251.1. by using NCBI-BLAST tools. It shows 94% homology with a final score of 929.

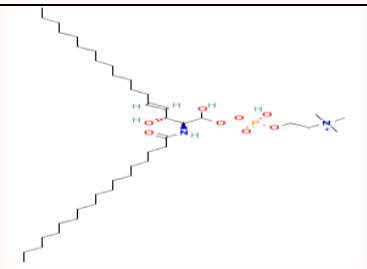
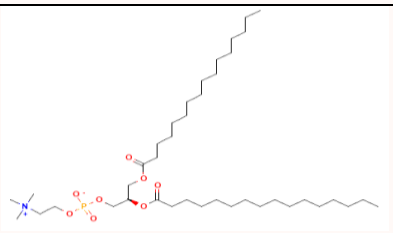
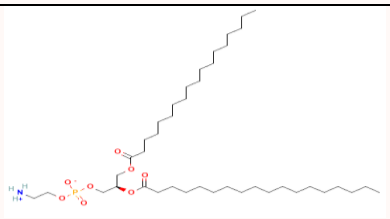
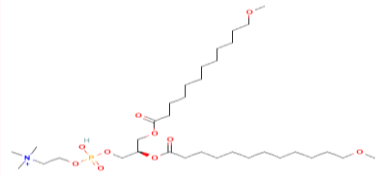
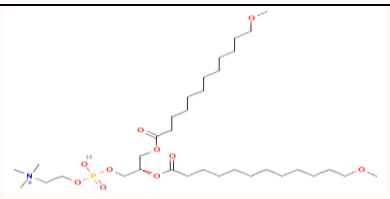
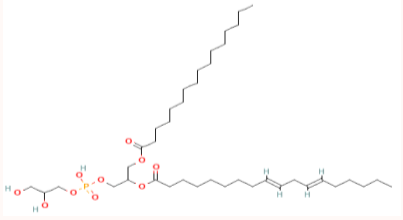
Multiple Alignment of LUNX (SPLUNC1)

The PLUNC amino acid sequence was aligned multiple times using NCBI-CLUSTALW 2 software to analyze the sequence homology of seven different species.

Molecular Docking of SPLUNC1

Molecular docking of SPLUNC1 protein was performed to model the interaction of SPLUNC1 protein with small molecules at atomic level. Crystal structure of maltose bind protein (MBP) fused SPLUNC1 (Figure 5) was downloaded from protein data bank (PDB ID: 4N4X) for molecular docking. Six possible ligands of SPLUNC1 were selected for molecular docking. Selected ligands are given in Table 1 along with their PubChem IDs and molecular structures.

Table I: List of possible ligands selected for molecular docking analysis.

Sr. No.	Ligand name	PubChem ID	Molecular Structure
1	Sphingomyelin	92043678	
2	dipalmitoylphosphatidylcholine	452110	
3	Phosphatidyl ethanolamine	15061530	
4	D-Phosphatidyl choline	454703	
5	L-Phosphatidyl choline	454702	
6	Phosphatidyl glycerol	44566653	

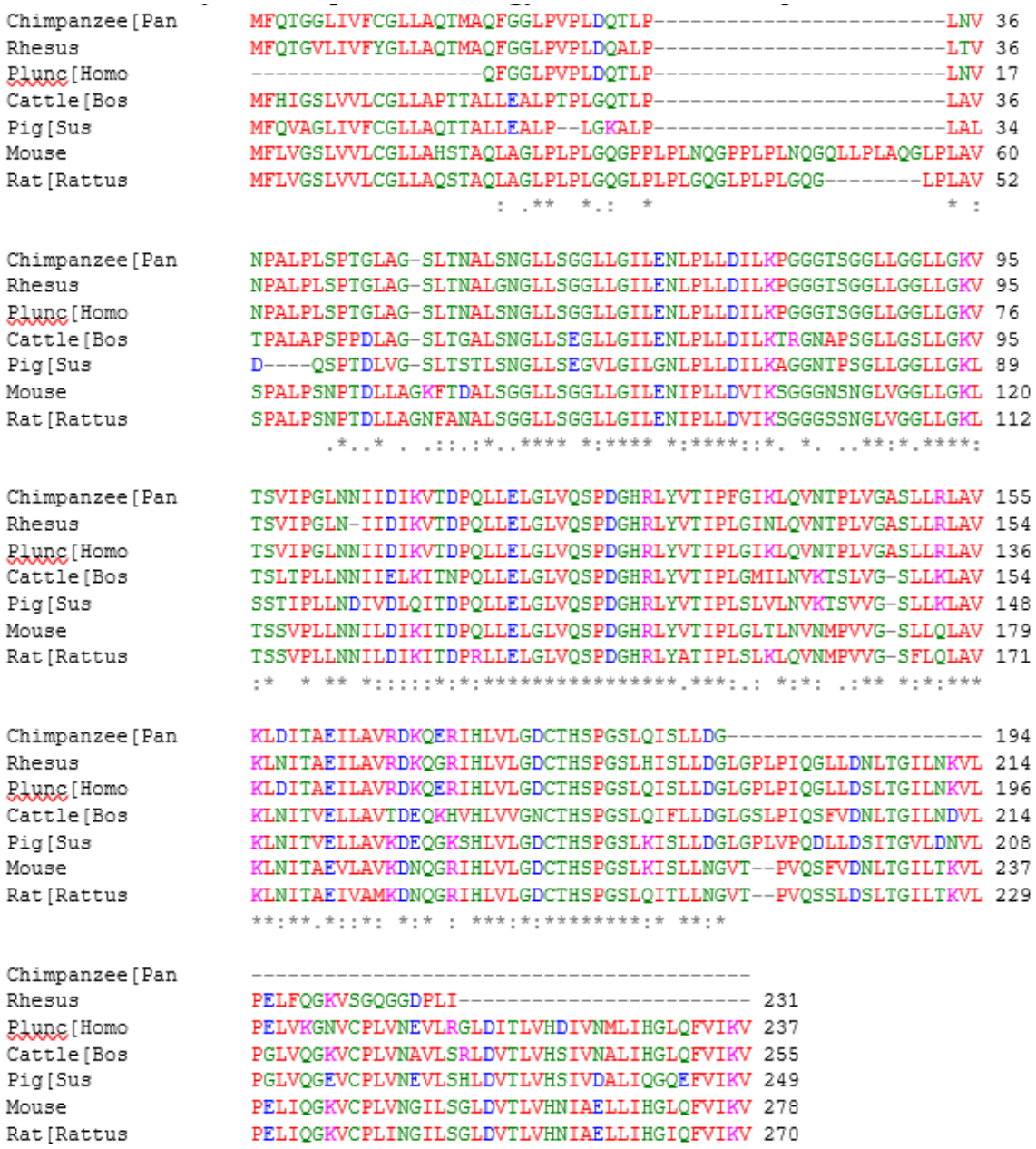


Figure. 4. Amino acid multiple sequence alignment of human PLUNC with chimpanzee pan-troglodytes (Accession No. XP_514582.2), rhesus monkey *Macca mulatta* (Accession No. XP_001104307.2); Cattle, *Bos Taurus* (Accession No. AAI14804.1); Pig, *Sus scrofa* (Accession No. NP_001005727.1); Mouse, *Mus musculus* (Accession No. AAK63069.1) and Rat, *Rattus nervegicus* (Accession No. AAM73687.1), using the CLUSTALW2 multiple alignment program.

Multiple sequence alignment showed the highest sequence similarity of human PLUNC with chimpanzees (score 98.96) and rhesus monkeys (score 90.04). It is more related to the Cattle then Pig; rat and mouse were more related to each other similarly chimpanzee and rhesus monkey also have significant similarity to each other as well as to human PLUNC.

Screening of the possible ligands was done by MOE software depending upon their binding energies and RMSD values. Results of molecular docking are given in Table II which shows binding energies (Kcal/mol) as well as RMSD values obtained for each possible ligand. Our results showed that phosphatidyl ethanolamine was the strongest binding ligand of SPLUNC1, with a highly negative binding energy (-10.0038 kcal/mol) and an RMSD value less than 2 (1.8948 Å). Other ligands found to bind significantly to SPLUNC1 were D-Phosphatidyl glycerol (binding energy: -9.79789 Kcal/mol), D-phosphatidyl choline

Table II: Screening of ligands through Molecular docking analysis using MOE software.

Sr. No.	Ligand name	PubChem ID	Pose #	Binding Energy (kcal/mol)	RMSD (Å)
1	Sphingomyelin	92043678	1	-9.4689	2.8427
2	Dipalmitoylphosphatidylcholine	452110	18	-8.30166	1.8291
3	Phosphatidyl ethanolamine	15061530	4	-10.0038	1.8948
4	D-Phosphatidyl choline	454703	3	-9.4959	1.909
5	L-Phosphatidyl choline	454702	24	-7.7729	1.8159
6	Phosphatidyl glycerol	44566653	12	-9.79789	1.9015

(binding energy: -9.4959 Kcal/mol) and Dipalmitoylphosphatidylcholine (binding energy: -8.30166). Sphingomyelin failed to bind to SPLUNC1, as it showed an RMSD value >2 (RMSD:2.8427 Å). D-phosphatidyl choline and L-phosphatidyl choline were found to bind the SPLUNC1 protein with different binding energies. 2-D visualization of compounds obtained by molecular docking analysis is shown in Figure 6

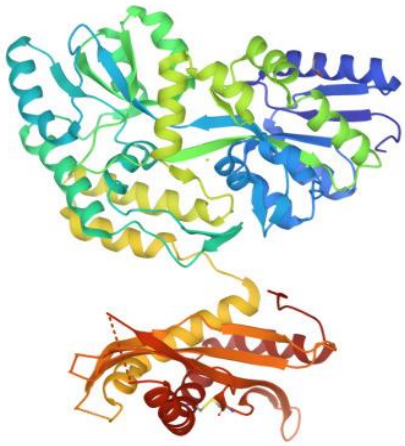


Figure 5. Crystal Structure of the MBP fused human SPLUNC1 (native form) obtained from Protein Data Bank with PDB ID: 4N4X

Discussion

Present study reports the homology modeling and molecular docking of SPLUNC1 protein for the first time. Previous studies have shown the presence of PLUNC family proteins in the mouse and rat genomes.[14] We identified the 94% sequence homology of human SPLUNC1 protein with Sumatran orangutan. Two cysteine residues are conserved between the two species. Multiple alignment of SPLUNC1 protein is similar to of that chimpanzees, rhesus monkeys, cattle, pigs, mice, and rats. This homology of SPLUNC1 protein indicates rapid evolution of PLUNC family proteins and presence of conserved regions. Studies have shown variability in the N-terminal part of the protein and second exon of the gene. [14,15] SPLUNC1 is a novel surfactant protein that exhibits anti biofilm activity. It plays a primary role in activating the innate immune system of mammals, especially against bacteria. It is known for its immunomodulatory and antibacterial activities, but mechanisms of adaptive evolution are poorly described in literature.[16] We therefore, performed the molecular docking of SPLUNC1

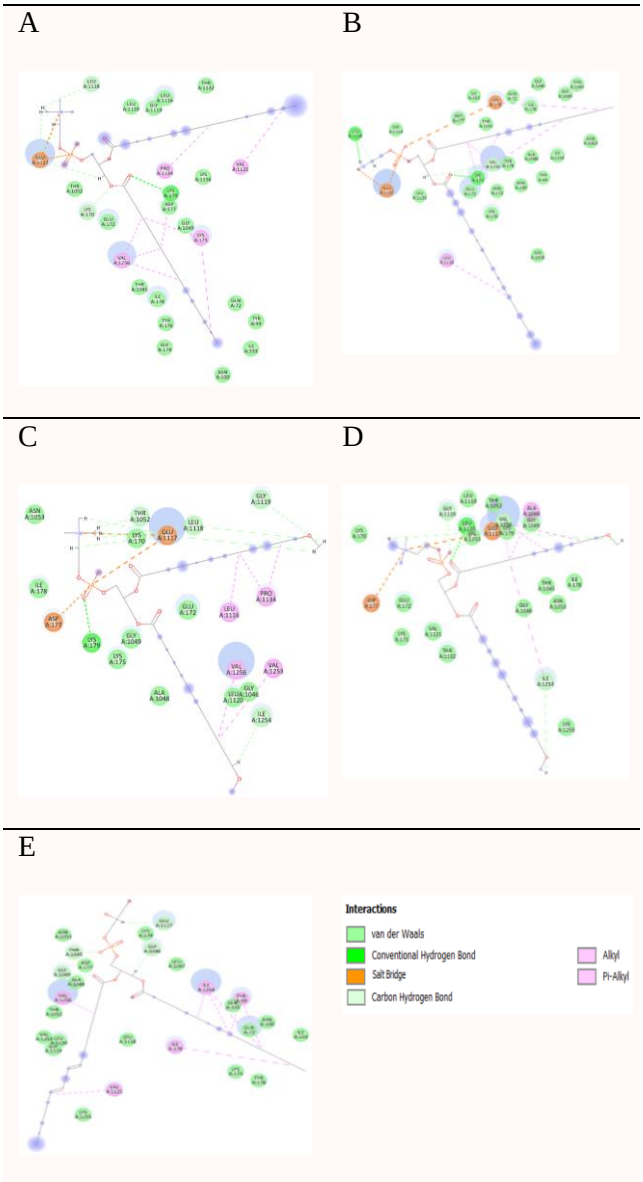


Figure 6:2D visualization of the compounds using Discovery Studio software screened through molecular docking.

Figure A-E respectively represent 452110, 15061530, 454703, 454702, and 44566653 compounds docked with the SPLUNC1 protein. Here, the compounds have been represented in a 'line' display format surrounded by interacting and vicinity amino acid residues of the protein. Amino acid residues of protein have been displayed as discs. "A" in the discs represents chain A of the protein. The dark blue dots/circles on the compound (s) structure represent the solvent exposure. Here, the abbreviations used are Glu = Glutamic acid, Asn = Asparagine, Thr = Threonine, Lys = Lysine, Leu = Leucine, Ile = Isoleucine, Gly = Glycine, Tyr = Tyrosine, Ala = Alanine, Asp = Aspartic acid, Val = Valine, Pro = Proline, Gln = Glutamine.

protein to understand the mechanism of its antibacterial and anti-biofilm activities and its evolutionary pattern. Results found SPLUNC1 binds all lipid ligands except sphingomyelin. It is hypothesized that the LBP/BPI regions bind to the lipid A component of the outer envelope of gram-negative bacteria and initiate early events of the innate immune response by causing an inflammatory response against invading microorganism.[17,18] LPS from gram-negative bacteria binds to the secreted protein and is recognized by the LPS receptor. As there is homology between SPLUNC1 and LPS binding proteins, it is considered a member of the innate immune response. Rat SPLUNC and parotid secretory protein (PSP) interact with bacterial membranes.[19,20] Homology modeling revealed its evolutionary relationship with *Calithrix jacchus,* a new world monkey with 96% homology. Two cysteine residues (C_{161} and C_{205}) were found to be conserved in both species. SPLUNC1 was found to have 94% homology with *Pongo abelii* which is Sumatran orangutan. Results also showed its evolutionary relationship with chimpanzees (score 98.96) and rhesus monkeys (score 90.04). PLUNC family proteins, especially SPLUNC1 probably evolve by gene duplication rather than gene dispersion. Further studies involving genome wide sequence analysis are suggested to uncover the conservation of exons in above mentioned species.

Conclusion

We conclude that SPLUNC1 is an antibacterial protein with significant sequence homology among Sumatran orangutans, chimpanzees, rhesus monkeys, cattle, pigs, mice, and rats. This indicates an evolutionary relationship between human PLUNC and other species. Molecular docking analysis confirmed the binding of SPLUNC1 with hydrophobic ligands, specifically phosphatidyl ethanolamine, D-phosphatidyl choline, L-phosphatidyl choline, dipalmitoylphosphatidylcholine, and phosphatidyl glycerol. SPLUNC1 did not bind to the sphingomyelin.

References

1. Wu T, Huang J, Moore PJ, Little MS, Walton WG, Fellner RC, et al. Identification of BPIFA1/SPLUNC1 as an epithelium-derived smooth muscle relaxing factor. Nat Commun 2017;8(1):14118. http://dx.doi.org/10.1038/ncomms14118
2. Liu H, Zhang X, Wu J, French SW, He Z. New insights on the palate, lung, and nasal epithelium clone (PLUNC) proteins: Based on molecular and functional analysis of its homolog of YH1/SPLUNC1. Exp Mol Pathol 2016;100(3):363–9. http://dx.doi.org/10.1016/j.yexmp.2015.12.002
3. Campos MA, Abreu AR, Nlend MC, Cobas MA, Conner GE, Whitney PL. Purification and characterization of PLUNC from human tracheobronchial secretions. Am J Respir Cell Mol Biol 2004;30(2):184–92. http://dx.doi.org/10.1165/rcmb.2003-0142OC
4. Ghafouri B, Kihlström E, Ståhlbom B, Tagesson C, Lindahl M. PLUNC (palate, lung and nasal epithelial clone) proteins in human nasal lavage fluid. Biochem Soc Trans 2003;31(Pt 4):810–4. http://dx.doi.org/10.1042/bst0310810
5. Liu Y, Bartlett JA, Di ME, Bomberger JM, Chan YR, Gakhar L, et al. SPLUNC1/BPIFA1 contributes to pulmonary host defense against Klebsiella pneumoniae respiratory infection. Am J Pathol 2013;182(5):1519–31. http://dx.doi.org/10.1016/j.ajpath.2013.01.050
6. Di YP. Functional roles of SPLUNC1 in the innate immune response against Gram-negative bacteria. Biochem Soc Trans 2011;39(4):1051–5. http://dx.doi.org/10.1042/BST0391051
7. Di Y-P, Harper R, Zhao Y, Pahlavan N, Finkbeiner W, Wu R. Molecular cloning and characterization of spurt, a human novel gene that is retinoic acid-inducible and encodes a secretory protein specific in upper respiratory tracts. J Biol Chem 2003;278(2):1165–73. http://dx.doi.org/10.1074/jbc.M210523200
8. Leclair EE. Four BPI (bactericidal/permeability-increasing protein)-like genes expressed in the mouse nasal, oral, airway and digestive epithelia. Biochem Soc Trans 2003;31(Pt 4):801–5. http://dx.doi.org/10.1042/bst0310801
9. LeClair EE, Nomellini V, Bahena M, Singleton V, Bingle L, Craven CJ, et al. Cloning and expression of a mouse member of the PLUNC protein family exclusively expressed in tongue epithelium. Genomics 2004;83(4):658–66. http://dx.doi.org/10.1016/j.ygeno.2003.09.015
10. Di YP. Functional roles of SPLUNC1 in the innate immune response against Gram-negative bacteria.

Biochem Soc Trans 2011;39(4):1051–5. http://dx.doi.org/10.1042/BST0391051
11. Akram KM, Moyo NA, Leeming GH, Bingle L, Jasim S, Hussain S, et al. Erratum: An innate defense peptide BPIFA1/SPLUNC1 restricts influenza A virus infection. Mucosal Immunol 2018;11(3):1008. http://dx.doi.org/10.1038/mi.2017.109
12. Miyoshi Y, Iwao K, Egawa C, Noguchi S. Association of centrosomal kinase STK15/BTAK mRNA expression with chromosomal instability in human breast cancers. Int J Cancer 2001;92(3):370–3. http://dx.doi.org/10.1002/ijc.1200
13. Wrennall JA, Ahmad S, Worthington EN, Wu T, Goriounova AS, Voeller AS, et al. A SPLUNC1 peptidomimetic inhibits Orai1 and reduces inflammation in a Murine allergic asthma model. Am J Respir Cell Mol Biol 2022;66(3):271–82. http://dx.doi.org/10.1165/rcmb.2020-0452OC
14. Bingle CD, LeClair EE, Havard S, Bingle L, Gillingham P, Craven CJ. Phylogenetic and evolutionary analysis of the PLUNC gene family. Protein Sci 2004;13(2):422–30. http://dx.doi.org/10.1110/ps.03332704
15. Mashbat B, Bellos E, Hodeib S, Bidmos F, Thwaites RS, Lu Y, et al. A rare mutation in SPLUNC1 affects bacterial adherence and invasion in meningococcal disease. Clin Infect Dis 2020;70(10):2045–53. http://dx.doi.org/10.1093/cid/ciz600
16. Gakhar L, Bartlett JA, Penterman J, Mizrachi D, Singh PK, Mallampalli RK, et al. PLUNC is a novel airway surfactant protein with anti-biofilm activity. PLoS One 2010;5(2):e9098. http://dx.doi.org/10.1371/journal.pone.0009098
17. Raetz CR, Whitfield C. Annual review of biochemistry. 2002;71:635–700.
18. Jaiswal AK, Yadav J, Makhija S, Sandey M, Suryawanshi A, Mitra AK, et al. Short palate, lung, and nasal epithelial clone 1 (SPLUNC1) level determines steroid-resistant airway inflammation in aging. Am J Physiol Lung Cell Mol Physiol 2022;322(1):L102–15. http://dx.doi.org/10.1152/ajplung.00315.2021
19. Robinson CP, Bounous DI, Alford CE, Nguyen KH, Nanni JM, Peck AB, et al. PSP expression in murine lacrimal glands and function as a bacteria binding protein in exocrine secretions. Am J Physiol 1997;272(4 Pt 1):G863-71. http://dx.doi.org/10.1152/ajpgi.1997.272.4.G863
20. Ahmad HI, Khan FA, Khan MA, Imran S, Akhtar RW, Pandupuspitasari NS, et al. Molecular evolution of the bactericidal/permeability-increasing protein (BPIFA1) regulating the innate immune responses in mammals. Genes (Basel) 2022;14(1):15. http://dx.doi.org/10.3390/genes14010015