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A Computational Approach for Protein-Protein Interactions of Bacterial Surface Layer Protein with Human Erb3 and αIIB-β3 Receptors

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Abstract: Host microbial interactions had significant factors in maintains homeostasis and immune-related activity. One such interaction made by Lactobacillus sp. with Surface layer proteins (Slps) had been studied through a computational approach. Erb3 and αIIB-β3, which are epithelial surface layer receptors, are subjected to interact with the Slp homology model. Both cell surface receptors were subjected to interact through computational docking, followed by molecular dynamics simulations through the coarse-grain method to explore the conformational stability. Through the implementation of the molecular docking for the surface layer protein A, we have shown the surface layer protein A, protein-protein interactions are higher in cellular receptors with epidermal growth factor receptor at an -34.45 ΔG and -51.19 ΔG through molecular docking with Erb3 and αIIB-β3. This study shows the unique interaction of Slp with the epithelial surface receptors like Erb3 and αIIB-β3, which are multipurpose applications in microbial-based drug therapeutics.

Keywords: anti-cancer proteins; host-microbial interactions; immunomodulators; molecular docking protein-protein interactions.

1. Introduction

The upregulation and the downregulation of the cytosolic epithelial markers are the primary sources for current cancer diagnosis [1,2]. Based on the hallmark, are two main protein components are usually studied in cancer progression, which includes the predominant epithelial markers like epidermal growth factor receptor (EGFR/ Erb3) and Platelet integrin (αIIB-β3) [3]. The ErbB family tyrosine kinase (EGFR related protein) is crucial in the diagnosis of many cancers [4] and potential targeting drug receptors in the pharma [5-7]. In contrast, αIIB-β3 belongs to the tyrosine phosphatase family who expresses antigens presence, leading to T-cells [8,9]. Due to the increasing need for reliable drug assessment and development, many studies progress on these two receptors (EGFR and αIIB-β3) [10,11].

There is a considerable presence of normal flora through the evolution of human existence through symbiotic relationships [12,13]. These human microbial interactions are now growing a vital interest in understating the immune system's regulation as immune modulators and metabolic regulators from the past few decades [14-16]. One of the associations includes the gut microbial interactions in humans by the *Lactobacillus* species was well studied at the molecular level. *Lactobacillus* sp. is essential in human intestinal colonization; besides, they possess significant protein structure components called surface layer proteins (Slp) [17]. These proteins are outer structures of cell envelope identified in numerous other domains of Bacteria and Archaea. Slps are recognized in many species of Lactobacillus sp. such as *L. acidophilus*, *L. buchneri*, *L. helveticus*, *L. bulgaricus*, and *L. brevis* [18,19]. These proteins are monomolecular crystalline arrays consisting of proteins or glycoproteins subunits, whose molecular weights range from 40 - 200 KDa [16].

Most scientific reports convey the critical importance of Slps as human immunomodulators and transducers [20-22]. The molecular mechanisms of cross-link between bacteria and the host organism system will understand the benefits and potential risks associated with the bacterial combined therapies. The immune-modulatory effect of the Slp had a significant impact on the human immune system [23,24]. Some research studies show the epithelial and macrophage cell line model's role in awaking innate immunity [25,26]. The high potency of Lactobacilli as affiliates to normal intestinal microbiota and their potential biotechnological applications has been well recognized [27,28]. Recent studies showed that the bacterial strain's binding activity with the help of Slp triggers the immune effects mainly through the host cell system's cell wall integrity by TLR4 receptors [29,30]. The proinflammatory activity of the Slp of *Lactobacillus* sp. may exhibit the efficacy of maintaining the human homeostasis of apoptosis and enhancing the Immunomodulation like pro-inflammations activating the macrophages. Considering the exclusive nature of the binding efficacy of the Slp protein towards the host gastrointestinal tract (GIT) and providing the host immunomodulatory effects, the molecular interactions between these host-microbial protein-protein interactions (PPI) are poorly understood [31,32].

In the present study, we showed the molecular PPI in *Lactobacillus brevis* surface layer protein A, with the upregulated cancer cell receptors Erb3 and αIIB-β3. This may provide a piece of important information in the cellular targeting of the bacterial surface layer protein's cell-specific activity towards understanding the cancers and their immune modulations during the host-microbial interactions.

2. Materials and Methods

2.1. Homology modeling of the surface layer protein A (SlpA).

Due course, in the search for the surface layer protein X-Ray and NMR models from the protein data bank (rcsb.org) [33], it doesn't provide satisfactory results us choose for the alternative method for protein structure. We used the *L. brevis* KB290 SlpA protein chain as a model sequence from the NCBI database (ncbi.nlm.nih.gov) [34, 35]. Swiss-model (swissmodel.expasy.org) [36] was chosen to deduce the homology model for the SlpA through the first approach mode with auto coordinates unavailability of the reference template. The generated model was recorded and analyzed for stability and nativity through the Molprobity [37], Qmean [38], and ProSA [39] online servers for the reliability of the structure for further study.

2.2. Receptors and ligand preparation.

Erb3 (2L9U) [40] and αIIB-β3 (2KNC) [41] protein cell receptors were searched and adopted from the rcsb.org (protein data bank), based on the Lactobacillus sp. bound to the GIT. The receptors are directly downloaded to the discovery studio client [42] and Swiss-Pdb viewer [43] through the application search. The protein structures (2L9U and 2KNC as the receptor and SlpA as a ligand) are refined and energy minimized through the application tools by removing the heteroatoms and water molecules to achieve the absolute structures for molecular docking.

2.3. Molecular docking.

Considering the large volume of atomic events for PPI, the molecularly docking was done using Cluspro 2.0, a CAPRI-based docking assessment [44], and PatchDock is a surface geometry-based ranking system [45] web server application. The generated results from the respective web servers are verified for the ranking patterns and analyzed for the effective PPI process.

2.4. Molecular simulations.

The flexibility and rigidity of the protein and PPI are the most critical factors in establishing the protein's stability during the interactions under physiological conditions. Based on the assessment of B-factors and rmsf values for a given protein, residual fluctuations provide detailed evidence of conformational stability. This operation was done using the CABS-Flex 2.0 webserver [46].

3. Results and Discussion

The structural data, as shown in Figure 1, of *L. brevis* KB290 from the NCBI and Swiss-Model database, shows that it is a polypeptide with a single chain consists of 469 amino acid with a large part of A (Alanine), T (Threonine), S (Serine), G (Glycine), V (Valine), K (Lysine) and Y (Tyrosine) amino acids, which contain 90% of β-sheet secondary structure, 8% loops and 2% of α-helix in the SlpA protein structure. The Swiss-model prediction of SlpA structure shows a factor of Z= -5.6 conformational confidence and 65% coverage of structural database for proteins. The conformational stability of the SlpA was verified by the results of the Ramachandran plot of the protein (SlpA.pdb file) generated from the Swiss-Model with Qmean integration provided the basic aspects of the protein structure in its tertiary form with allowed 97.9% conformation was established with highly favored regions around 90.6% of the structure which are shown in Fig ESM_1 and Fig ESM_2.

The docking scores generated from simple CPU-based program execution with little manual inference in ClusPro 2.0 webserver provided the results organized in ranked by the program to its lowest energy scores among all the clusters for each interaction to compare and illustrate the interactions. The Lactobacillus sp. binding to the host intestinal receptors was still poorly understood [32,47]. Here in this study, we approach getting the protein, which involves the signal transduction's surface receptors, affecting cells' growth and development, including the Erb3 and αIIB-β3.

The Erb3 (PDB ID 2L9U) transmembrane heterodimeric protein, a tyrosine kinase receptor, belongs to the epidermal growth factor receptor (EGFR/ERBB) family domain helps

in the regulation of cell growth and development. The scores from the interaction of SlpA to Erb3 include the lowest energy scores based on the coefficient weights of balanced, electrostatic, hydrophobic and Van der Waals interactions; these scores were −1048, −1120, −1507and−138.4, respectively. While the αIIB-β3 (PDB ID 2KNC) platelet integrin transmembrane heterodimeric protein, which is a platelet cell membrane receptor for platelet function and homeostasis. The scores from the interaction of SlpA to Erb3 include the lowest energy scores based on the coefficient weights of balanced, electrostatic, hydrophobic and Van der Waals interactions; these scores were −1111.6, −1082, −1714, and −435, respectively, the compared results are mentioned in Table 1.

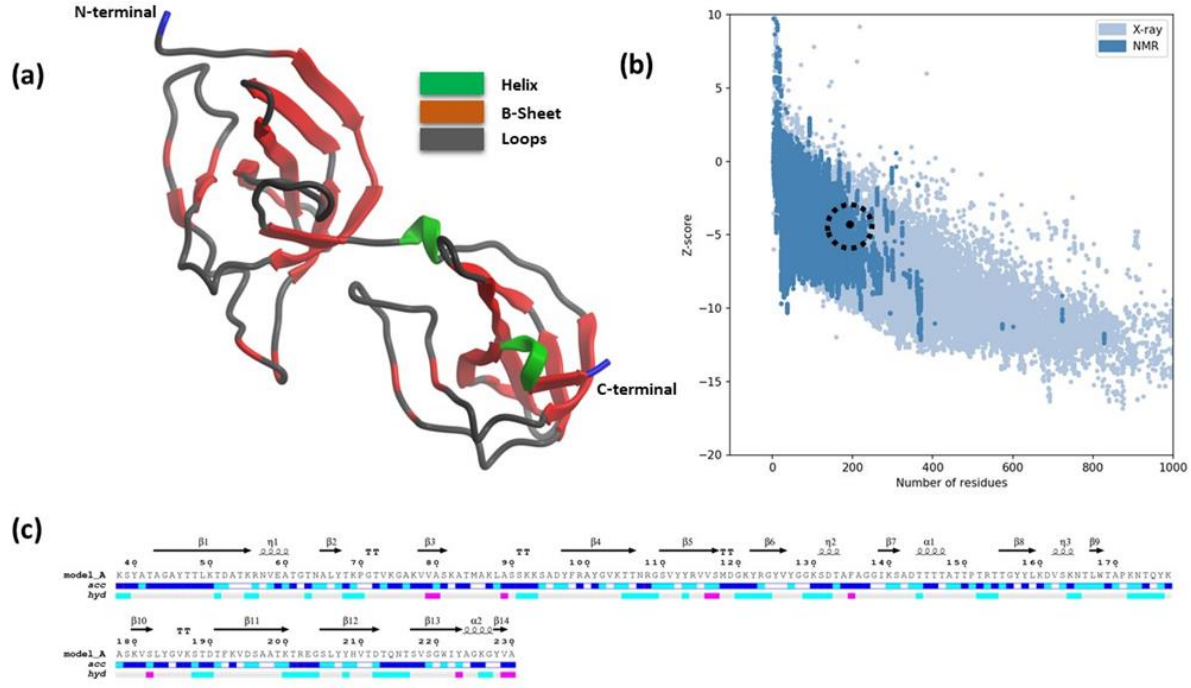

Figure 1. Molecular illustration of the Surface layer protein of *Lactobacillus brevis* KB290. (**a**) Cartoon image of SlpA with color impression showing the secondary structure in its native conformations; (**b**) ProSA based theoretical deduction of conformational validity with lower errors, the protein is shown at the subset X-ray and NMR protein database; (**c**) Primary structure representation with support to the above cartoon image.

The measured bond lengths between the protein's receptor and ligand protein-protein interactions were 3.0 to 4.0 Å at phenylalanine of 2L9U (PHE-663) glycine of SlpA (GLY-109), as shown in Figure 2. While the interactions of methionine of 2KNC (MET-686) with tyrosine of SlpA (TYR-127), as shown in Figure 3. This *in-silico* binding experiment showed that SlpA could strongly interfere in the binding of Erb2 and αIIB-β3 at position alanine (ALA-136) phenylalanine (PHE- 135). Besides, the binding sites of SlpA to Erb2 and αIIB-β3 at amino acid residues 168–228 could prevent the binding of SlpA by the respective receptors. Although the mode of action of SlpA was reported as membrane dissociation of the microorganism, this *in-silico* binding result suggested an additional effort of SlpA via Erb2 and αIIB-β3. Along with Cluspro interaction, a different PatchDock molecular docking server was used to deduce the global energies for the PPI interaction of the SlpA base on the surface geometries. The results are quite impressive as we expected for the Cluspro 2.0 webserver data was matched with the data from the PatchDock for the SlpA interaction with Erb2 showed the ΔG (free energies) -34.45 Kcal/mol and SlpA interaction with αIIB-β3 showed the ΔG -51.19 Kcal/mol, the results are compared in Table 2.

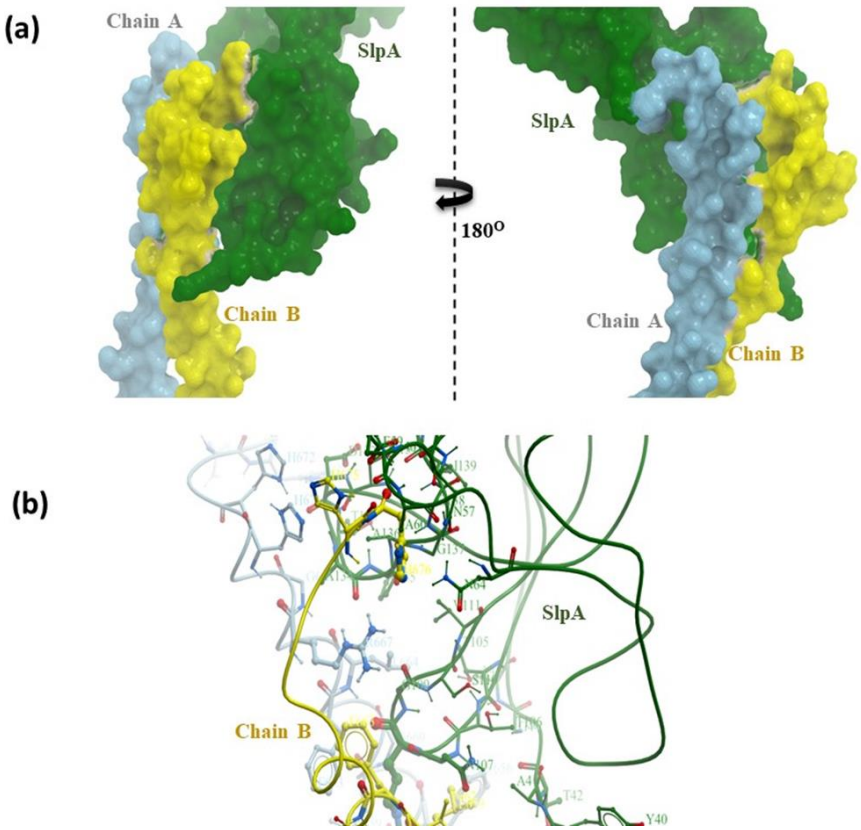

Figure 2. SlpA docking (color-coded for dark green) with Erb2 (EGFR/ERBB) functional transmembrane domain (which represented the heterodimeric protein chains in color-coded with yellow [chain A] and cyan blue [chain B]). (**a**) Surface representation of the molecular docking, contact surface was represented with pale yellow color-coded; (**b**) cartoon representation with primary structure showed the interacted amino acids at their respective distances.

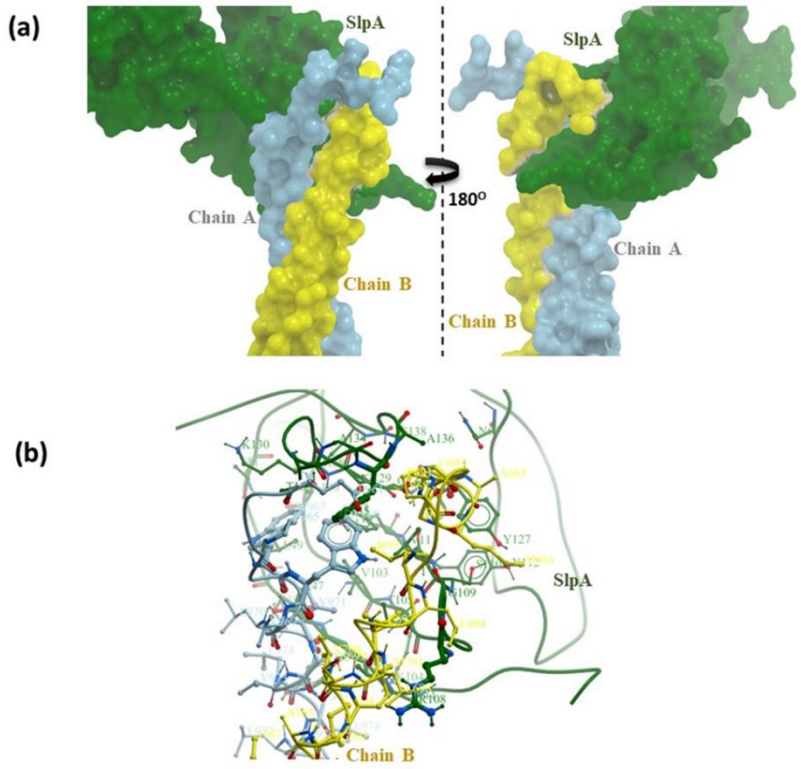

Figure 3. SlpA docking (color-coded for dark green) with the αIIB-β3 functional transmembrane domain of platelets (which represented the heterodimeric protein chains in color-coded with yellow [chain A] and cyan blue [chain B]). (**a**) Surface representation of the molecular docking, contact surface was represented with pale yellow color-coded; (**b**) cartoon representation with primary structure showed the interacted amino acids at their respective distances.

In support of the molecular docking, the root-mean-square fluctuation was presented in Figure 4 for Erb2 and Figure 5 for αIIB-β3 through the CABS flex 2.0 web server, which showed the information on the flexibility and rigidity of the PPI through the coarse-grained protein modeling. The output results are plotted concerning the SlpA bounded receptor, and unbounded receptors are compared, showing the considerable fluctuations in the SlpA structure in bounded form rather than in a free state. The residual contact points are shown in Fig ESM_3. This suggests the higher conformations rigidity of the SlpA suitable for the binding associations mentioned in the above molecular docking concept.

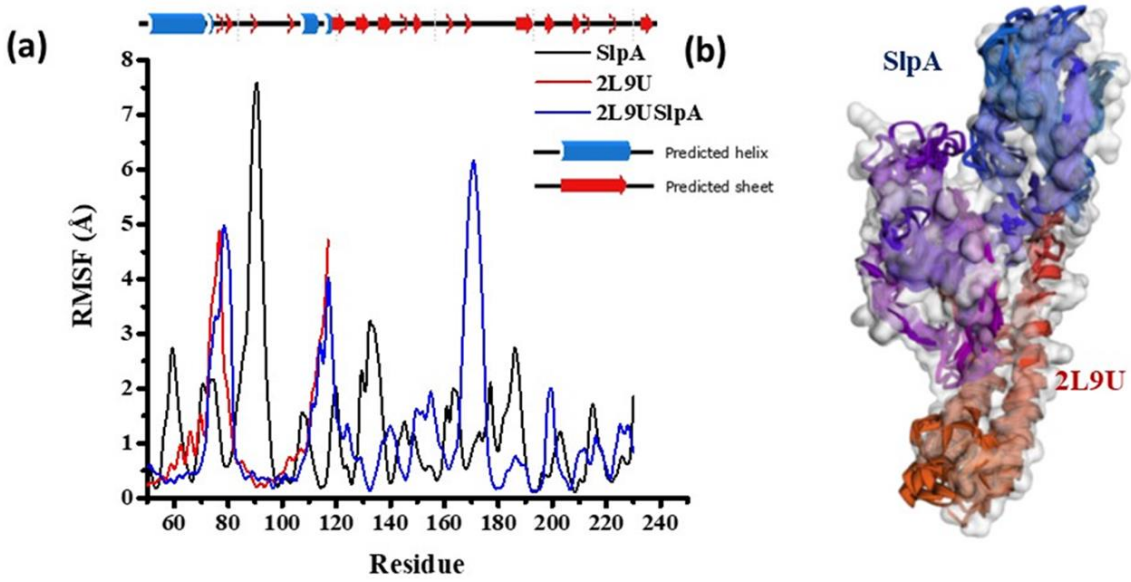

Figure 4. Molecular dynamics with CABS flex server 2.0 representations for rmsf based conformational coarse grain fluctuations for the flexibility and rigidity of the SlpA protein bounded and unbounded form with the receptors Erb2 transmembrane domain. (**a**) rmsf vs residual plots with lower flexibilities at their bounded form of SlpA protein; (**b**) Cartoon.

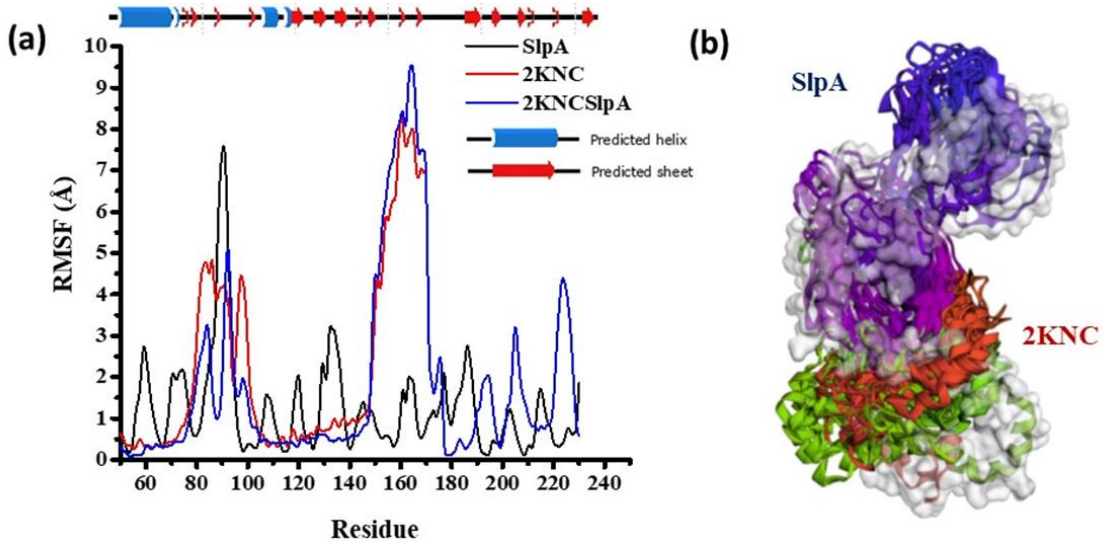

Figure 5. Molecular dynamics with CABS flex server 2.0 representations for rmsf based conformational coarse grain fluctuations for the flexibility and rigidity of the SlpA protein bounded and unbounded form with the receptors αIIB-β3 transmembrane domain. (**a**) rmsf vs. residual plots with lower flexibilities at their bounded form of SlpA protein; (**b**) Cartoon /Surface representations of initial to final fluctuations shown in the group embed form of the PPI.

Based on the results, as mentioned above. The interaction perspectives and application of the SlpA PPI, few works of literature have said this study's interaction. Erb2 and Epidermal

growth factor receptors are the potential receptors in treating cancer and related infections. The outer surfaces of the cells in cancer are upregulated, providing a broad scope to the activity of the drugs specific to the EGFR [47, 48]. *Lactobacillus*, one of the gut microorganisms, can access the EGFR, virtually shown in this docking study. The anti-inflammatory effect of the SlpA had been proved by binding to the αIIB-β3 receptor of platelet cells. Studies from Rubio *et al.* (2017) discussed the importance of the surface layer protein, which shows cytotoxicity when surface layer protein was incubated with the breast cancer cells MDA-MB-231 through the epidermal growth receptors [49-51]. Altin *et al.* (1997) shown the effect of Slps in T-cell activation through the similar receptor mechanism [17], Li *et al.* (2011) suggested the inhibition of the Caco-2 cells [46], Zhong *et al.* (2014) demonstrated the lactic acid bacteria shows the anti-tumor activity towards the colorectal cancers through the PPI [31]. The human blood group A, trisaccharide, acts as a receptor for the Slp of a human *L. brevis* considering the nine N-terminal amino acids of the Slp [28].

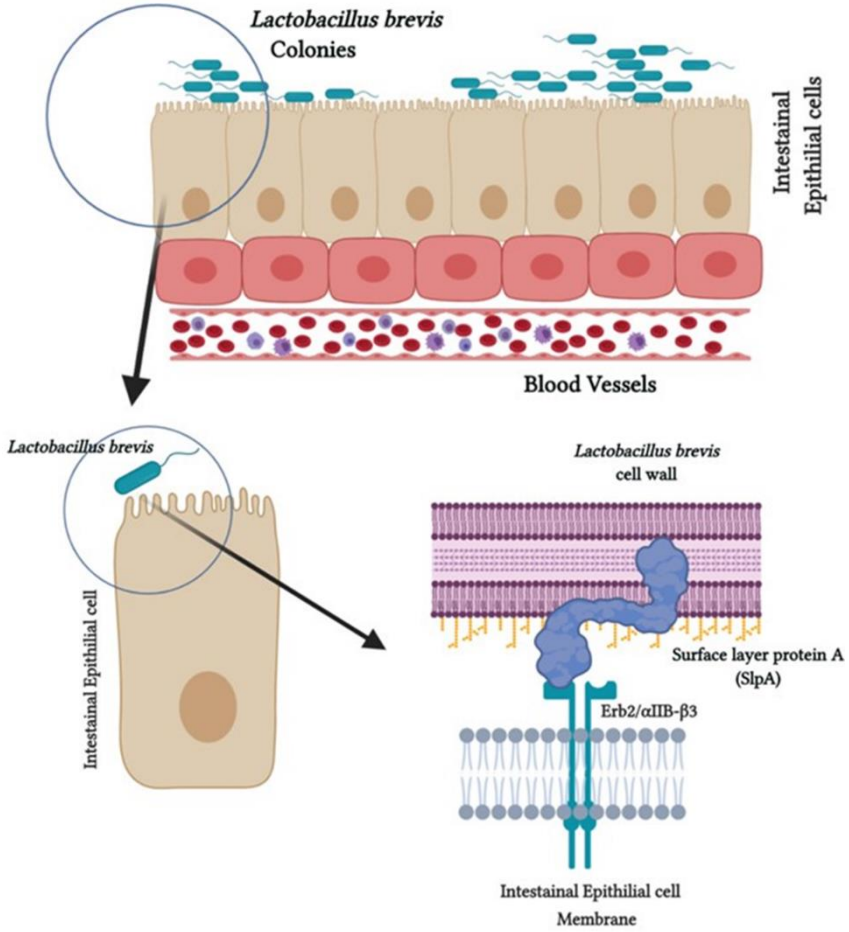

Figure 6. Graphical abstract of the SlpA protein's theoretical binding with the Erb2/ αIIB-β3 transmembrane domain at their respective resolutions.

Table 1. Molecular docking solutions of ClusPro 2.0 coefficient weights through lowest energies of PPI clusters

Protein-protein Interactions	Balanced	Electrostatic	Hydrophobic	Van der Waals
2L9U_SlpA	−1048	−1120	−1507	−138.4
2KNC_SlpA	−1111.6	−1082	−1714	−435

When involved in the expression of several proinflammatory cytokines and a therapeutic target in a wide range of autoinflammatory diseases in the presence of *L. helveticus* MIMLh5 in Caco-2 (the human epithelial cell line) results in reduced levels of transcriptional factor NF-κB activity [50], and that other studies revealed the significant scope of Lactobacillus

sp. This supports that the movement of the SlpA was so peculiar in compatible binding towards the upregulation or downregulation of the specific markers of the cancers [52-54]. These PPI interactions are shown here through this computational study. Further work is in progress in developing the reaction mechanism towards inhibition or cytotoxicity, leading to the treatment of the cancers based on the comprehensive discussion on the activity of the bacterial protein SlpA and other probiotics[55-58]. This study can help understand the host-microbial relationships in treating dreadful diseases like cancers and other GIT disorders with normal microbiota. Further proof of concept in this study's PPI is needed through wet lab analysis to observe the further applications in the host-microbial interactions [59,60].

Table 2. Molecular docking solutions of PatchDock coefficients through free energies of PPI

Protein-protein Interactions	ΔG	ACE
2L9U_SlpA	-34.45	-2.49
2KNC_SlpA	-51.19	-7.97

4. Conclusions

In conclusion, for this study, *Lactobacillus* is considered the probiotic bacteria involved in the GIT tract's homeostasis through symbiotic and normal microbial relationships in human beings and other higher-order species. Based on the literature, the *L. brevis* and other lactobacillus species' binding activity involves regulating the cell surface receptor to activate proinflammatory and immunomodulatory effects. This study made an addon note for the molecular interactions through the molecular docking and simulation studies in a fundamental understating of the SlpA PPI concept.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Haraguchi, N.; Inoue, H.; Tanaka, F.; Mimori, K.; Utsunomiya, T.; Sasaki, A.; Mori, M. Cancer stem cells in human gastrointestinal cancers. *Human cell* **2006**,*19*, 24-29. https://doi.org/10.1111/j.1749-0774.2005.00004.x.
2. Spratlin, J.L.; Serkova, N.J.; Eckhardt, S.G. Clinical applications of metabolomics in oncology: a review. *Clinical cancer research* **2009**, *15*, 431-440. https://doi.org/10.1158/1078-0432.CCR-08-1059.
3. Kovacs, E.; Zorn, J.A.; Huang, Y.; Barros, T.; Kuriyan, J. A structural perspective on the regulation of the epidermal growth factor receptor. *Annual review of biochemistry* **2015** *84*, 739-764. https://doi.org/10.1146/annurev-biochem-060614-034402.
4. Huifang, W.; Yuhan, N.; Jie P.; Qinpei, L.; Rongrong, L.Antibacterial effects of lactobacillus acidophilus surface-layer protein in combination with nisin against staphylococcus aureus. *LWT* **2020,** *124*, 109208. https://doi.org/10.1016/j.lwt.2020.109208.

5. Wang, H.; Zhang, L.; Li, Q.; Xu, S.; Lu, R. Surface-layer protein produced by Lactobacillus crispatus JCM 2009 ameliorates lipopolysaccharide-induced inflammation through autophagy cross-talk with the NF-κB signaling pathway. *International Journal of Biological Macromolecules* **2021,** *166*, 633-640. https://doi.org/10.1016/j.ijbiomac.2020.10.221.
6. Meng, J.; Wang, Y.Y.; Hao, Y.P. Protective function of surface layer protein from Lactobacillus casei fb05 against intestinal pathogens in vitro. *Biochemical and Biophysical Research Communications* **2021,** *546*, 15-20. https://doi.org/10.1016/j.bbrc.2021.01.101.
7. Herbst, R.S. Review of epidermal growth factor receptor biology. International Journal of Radiation *Oncology Biology Physics* **2004**, *59*, S21-S26. https://doi.org/10.1016/j.ijrobp.2003.11.041.
8. Wieduwilt, M.; Moasser, M. The epidermal growth factor receptor family: biology driving targeted therapeutics. *Cellular and Molecular Life Sciences* **2008**, *65*, 1566-1584. https://doi.org/10.1007/s00018-008-7440-8.
9. Yewale, C.; Baradia, D.; Vhora, I.; Patil, S.; Misra, A. Epidermal growth factor receptor targeting in cancer: a review of trends and strategies. *Biomaterials* **2013**, *34*, 8690-707. https://doi.org/10.1016/j.biomaterials.2013.07.100
10. Altin, J.G.; Sloan, E.K. The role of CD45 and CD45-associated molecules in T cell activation. *Immunology and cell biology* **1997**, *75*, 430-445. https://doi.org/10.1038/icb.1997.68.
11. Holmes, N. CD45: all is not yet crystal clear. *Immunology* **2006**, *117*, 145-155. https://doi.org/10.1111/j.1365-2567.2005.02265.x.
12. Yang, M.; Liu, P.; Huang, P. Cancer stem cells, metabolism, and therapeutic significance. *Tumor Biology*, **2016**, *37*, 5735-5742. https://doi.org/10.1007/s13277-016-4945-x.
13. Zhang, T.; Pan, D.; Yang, Y.; Jiang, X.; Zhang, J.; Zeng, X.; Wu, Z.; Sun, Y.; Guo, Y. Effect of Lactobacillus acidophilus CICC 6074 S-layer protein on colon cancer HT-29 cell proliferation and apoptosis. *Journal of agricultural and food chemistry* **2020,** *68*, 2639-2647. https://doi.org/10.1021/acs.jafc.9b06909.
14. Qi, S.R.; Cui, Y.J.; Liu, J.X.; Luo, X.; Wang, H.F. Lactobacillus rhamnosus GG components, SLP, gDNA and CpG, exert protective effects on mouse macrophages upon lipopolysaccharide challenge. *Letters in applied microbiology*, **2020,** *70*, 118-127. https://doi.org/10.1111/lam.13255.
15. Ge, Y.; Gong, M.; Zadeh, M.; Li, J.; Abbott, J.R.; Li, W.; Morel, L.; Sonon, R.; Supekar, N.T.; Azadi, P.; Wang, Y. Regulating colonic dendritic cells by commensal glycosylated large surface layer protein A to sustain gut homeostasis against pathogenic inflammation. *Mucosal Immunol* **2020,** *13*, 34-46. https://doi.org/10.1038/s41385-019-0210-0.
16. Clarke, M.B.; Sperandio, V. Events at the host-microbial interface of the gastrointestinal tract III. Cell-to-cell signaling among microbial flora, host, and pathogens: there is a whole lot of talking going on. *American Journal of Physiology-Gastrointestinal and Liver Physiology* **2005**, *288*, G1105-G1109. https://doi.org/10.1152/ajpgi.00572.2004.
17. Moran, A.; Gupta, A.; Joshi, L. Sweet-talk: role of host glycosylation in bacterial pathogenesis of the gastrointestinal tract. *Gut* **2011**, *60*, 1412-25. https://doi.org/10.1136/gut.2010.212704.
18. Duncan, H.E.; Edberg, S.C. Host-microbe interaction in the gastrointestinal tract. *Critical reviews in microbiolog* **1995**, *21*, 85-100. https://doi.org/10.3109/10408419509113535.
19. Åvall-Jääskeläinen, S.; Kylä-Nikkilä, K.; Kahala, M.; Miikkulainen-Lahti, T.; Palva, A. Surface display of foreign epitopes on the Lactobacillus brevis S-layer. *Applied and Environmental Microbiology* **2002**, *68*, 5943-51. https://doi.org/10.1128/AEM.68.12.5943-5951.2002.
20. Zhang, T.; Pan, D.; Yang, Y.; Jiang, X.; Zhang, J.; Zeng, X.; Wu, Z.; Sun, Y.; Guo, Y. Effect of Lactobacillus acidophilus CICC 6074 S-layer protein on colon cancer HT-29 cell proliferation and apoptosis. *Journal of agricultural and food chemistry* **2020,** *68*, 2639-2647. https://doi.org/10.1021/acs.jafc.9b06909.
21. Qi, S.R.; Cui, Y.J.; Liu, J.X.; Luo, X.; Wang, H.F. Lactobacillus rhamnosus GG components, SLP, gDNA and CpG, exert protective effects on mouse macrophages upon lipopolysaccharide challenge. *Letters in applied microbiology* **2020,** *70*, 118-127. https://doi.org/10.1111/lam.13255.
22. Åvall-Jääskeläinen, S.; Palva, A. Lactobacillus surface layers and their applications. *FEMS Microbiology Review* **2005**, *29*, 511-529. https://doi.org/10.1016/j.fmrre.2005.04.003.
23. Hynönen, U.; Palva, A. Lactobacillus surface layer proteins: structure, function and applications. *Applied microbiology and biotechnology* **2013**, *97*, 5225-5243. https://doi.org/10.1007/s00253-013-4962-2.
24. Taverniti, V.; Stuknyte, M.; Minuzzo, M.; Arioli, S.; De Noni, I.; Scabiosi, C.; et al. S-layer protein mediates the stimulatory effect of Lactobacillus helveticus MIMLh5 on innate immunity. *Applied and environmental microbiology* **2013**, *79*, 1221-1231. https://doi.org/10.1128/AEM.03056-12.

25. Hollmann, A.; Delfederico, L.; Miyoshi, A.; Disalvo, E.A.; DeAntoni, G.; Semorile, L.; et al. S-layer proteins from lactobacilli as vaccine delivery systems. *International Journal of Microbiology Research* **2010**, *2*, 30-43. https://doi.org/10.9735/0975-5276.2.2.30-43.
26. Åvall-Jääskeläinen, S.; Hynönen, U.; Ilk, N.; Pum, D.; Sleytr, U.B.; Palva, A. Identification and characterization of domains responsible for self-assembly and cell wall binding of the surface layer protein of Lactobacillus brevisATCC 8287. *BMC microbiology* **2008,** *8*. https://doi.org/10.1186/1471-2180-8-165.
27. Ryan, A.; Lynch, M.; Smith, S.M.; Amu, S.; Nel, H.J.; McCoy, C.E.; et al. A role for TLR4 in Clostridium difficile infection and the recognition of surface layer proteins. *PLoS Pathog* **2011**, *7*, e1002076. https://doi.org/10.1371/journal.ppat.1002076.
28. Fagan, R.P.; Fairweather, N.F. Biogenesis and functions of bacterial S-layers. *Nature Reviews Microbiology* **2014**, *12*, 211-222. https://doi.org/10.1038/nrmicro3213.
29. Zhong, L.; Zhang, X.; Covasa, M. Emerging roles of lactic acid bacteria in protection against colorectal cancer. *World J Gastroenterol* **2014**, *20*, 7878-7886. https://doi.org/10.3748/wjg.v20.i24.7878.
30. Gerbino, E.; Carasi, P.; Mobili, P.; Serradell, M.; Gómez-Zavaglia, A. Role of S-layer proteins in bacteria. *World Journal of Microbiology and Biotechnology* **2015**, *31*, 1877-8187. https://doi.org/10.1007/s11274-015-1952-9.
31. Burley, S.K.; Berman, M.; Bhikadiya, C.; Bi, C.; Chen, L.; Di Costanzo, L.; et al. RCSB Protein Data Bank: biological macromolecular structures enabling research and education in fundamental biology, biomedicine, biotechnology and energy. *Nucleic acids research* **2019**, *47*, D464-D474. https://doi.org/10.1093/nar/gky1004.
32. Jenuth, J.P. The NCBI. *Bioinformatics Methods and Protocols: Springer* **2000**, 301-312. https://doi.org/10.1385/1592591922.
33. Yakabe, T.; Moore, E.; Yokota, S.; Sui, H.; Nobuta, Y.; Fukao, M.; et al. Safety assessment of Lactobacillus brevis KB290 as a probiotic strain. *Food and chemical toxicology* **2009**, *47*, 2450-2453. https://doi.org/10.1016/j.fct.2009.07.001.
34. Kiefer, F.; Arnold, K.; Künzli, M.; Bordoli, L.; Schwede, T. The SWISS-MODEL Repository and associated resources. *Nucleic acids research* **2009**, *37*, D387-D392. https://doi.org/10.1093/nar/gkn750.
35. Davis, I.W.; Leaver-Fay, A.; Chen, V.B.; Block, J.N.; Kapral, G.J.; Wang, X.; et al. MolProbity: all-atom contacts and structure validation for proteins and nucleic acids. *Nucleic acids research* **2007**, *35*, W375-W383. https://doi.org/10.1093/nar/gkm216.
36. Benkert, P.; Künzli, M.; Schwede, T. QMEAN server for protein model quality estimation. *Nucleic acids research*, **2009**, *37*, W510-W514. https://doi.org/10.1093/nar/gkp322.
37. Wiederstein, M.; Sippl, M.J. ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins. *Nucleic acids research* **2007**, *35*, W407-W410. https://doi.org/10.1093/nar/gkm290.
38. Mineev, K.; Khabibullina, N.; Lyukmanova, E.; Dolgikh, D.; Kirpichnikov, M.; Arseniev, A.S. Spatial structure and dimer–monomer equilibrium of the ErbB3 transmembrane domain in DPC micelles. B*iochimica et Biophysica Acta (BBA)-Biomembranes* **2011**, *1808*, 2081-2088. https://doi.org/10.1016/j.bbamem.2011.04.017.
39. Yang, J.; Ma, Y-Q.; Page, R.C.; Misra, S.; Plow, E.F.; Qin, J. Structure of an integrin αIIbβ3 transmembrane-cytoplasmic heterocomplex provides insight into integrin activation. *Proceedings of the National Academy of Sciences* **2009**, *106*, 17729-17734. https://doi.org/10.1073/pnas.0909589106.
40. BIOVIA DS. Discovery Studio Client; 2.5. 0.9164. Dassault Systèmes: San Diego, CA, USA. 2005.
41. Guex, N.; Peitsch, M.C. SWISS-MODEL and the Swiss-Pdb Viewer: an environment for comparative protein modeling. *Electrophoresis* **1997**, *18*, 2714-2723. https://doi.org/10.1002/elps.1150181505.
42. Schneidman-Duhovny, D.; Inbar, Y.; Nussinov, R.; Wolfson, H.J. PatchDock and SymmDock: servers for rigid and symmetric docking. *Nucleic acids research* **2005,** *33*, W363-W367. https://doi.org/10.1093/nar/gki481.
43. Kuriata, A.; Gierut, A.M.; Oleniecki, T.; Ciemny, M.P.; Kolinski, A.; Kurcinski, M.; Kmiecik, S. CABS-flex 2.0: a web server for fast simulations of flexibility of protein structures. *Nucleic acids research* **2018**, *46*, W338-W343. https://doi.org/10.1093/nar/gky356.
44. Li, P.; Yin, Y.; Yu, Q.; Yang, Q. Lactobacillus acidophilus S-layer protein-mediated inhibition of Salmonella-induced apoptosis in Caco-2 cells. *Biochemical and biophysical research communi*cations **2011**, *409*, 142-147. https://doi.org/10.1016/j.bbrc.2011.04.131.

45. Pawson, T.; Scott, J.D. Signaling through scaffold, anchoring, and adaptor proteins. *Science* **1997**, *278*, 2075-2080. https://doi.org/10.1126/science.278.5346.2075.
46. Marinissen, M.J.; Gutkind, J.S. G-protein-coupled receptors and signaling networks: emerging paradigms. *Trends in pharmacological sciences* **2001**, *22*, 368-376. https://doi.org/10.1016/S0165-6147(00)01678-3.
47. Rubio, V.P.; Bravo, A.; Olmos, J. Identification of a Bacillus thuringiensis surface layer protein with cytotoxic activity against MDA-MB-231 Breast Cancer Cells. *J Microbiol Biotechnol* **2017**, *27*, 36-42. https://doi.org/10.4014/jmb.1607.07020.
48. Ausiello, C.M.; Cerquetti, M.; Fedele, G.; Spensieri, F.; Palazzo, R.; Nasso, M.; et al. Surface layer proteins from Clostridium difficile induce inflammatory and regulatory cytokines in human monocytes and dendritic cells. *Microbes and infection* **2006**, *8*, 2640-2646. https://doi.org/10.1016/j.micinf.2006.07.009.
49. Partrick, K.A.; Rosenhauer, A.M.; Auger, J.; Arnold, A.R.; Ronczkowski, N.M.; Jackson, L.M.; Lord, M.N.; Abdulla, S.M.; Chassaing, B.; Huhman, K.L.; Ingestion of probiotic (Lactobacillus helveticus and Bifidobacterium longum) alters intestinal microbial structure and behavioral expression following social defeat stress. *Scientific Reports*, **2021**, 11(1), 1-12. https://doi.org/10.1038/s41598-021-83284-z
50. Cai, Z.; Wang, P.; Guo, Y.; Zeng, X.; Wu, Z.; Pan, D. S-layer protein modulates the stimulatory effects of Lactobacillus acidophilus CICC 6074 by triggering PKC signaling cascade in RAW 264.7 cells. *Journal of Functional Foods* **2020,** *67*, 103841. https://doi.org/10.1016/j.jff.2020.103841.
51. Wakai, T.; Kano, C.; Karsens, H.; Kok, J.; Yamamoto, N. Functional role of surface layer proteins of Lactobacillus acidophilus L-92 in stress tolerance and binding to host cell proteins. *Bioscience of Microbiota, Food and Health* **2020**, *40*, 33-42. https://doi.org/10.12938/bmfh.2020-005.
52. Mobarak Qamsari, E.; Kasra Kermanshahi, R.; Erfan, M.; Ghadam, P. Microencapsulation of Omeprazole by Lactobacillus acidophilus ATCC 4356 surface layer protein and evaluation of its stability in acidic condition. *Iranian Journal of Pharmaceutical Research* **2020,** *19*, 240-254. https://doi.org/10.22037/ijpr.2019.111681.13306.
53. Liu, Q.; Jiang, Y.; Yang, W.; Liu, Y.; Shi, C.; Liu, J.; Gao, X.; Huang, H.; Niu, T., Yang, G.; Wang, C. Protective effects of a food-grade recombinant Lactobacillus plantarum with surface displayed AMA1 and EtMIC2 proteins of Eimeria tenella in broiler chickens. *Microbial cell factories* **2020,** *19*. https://doi.org/10.1186/s12934-020-1297-4.
54. Zhuo, S.; Xun, M.; Li, M.; Kong, X.; Shao, R.; Zheng, T.; Pan, D.; Li, J.; Li, Q. Rapid and label-free optical assay of S-layer protein with high sensitivity using TiO2-coated porous silicon-based microfluidic biosensor. *Sensors and Actuators B: Chemical* **2020,** *321*, 128524. https://doi.org/10.1016/j.snb.2020.128524.
55. Klotz, C.; Goh, Y.J.; O'Flaherty, S.; Barrangou, R. S-layer associated proteins contribute to the adhesive and immunomodulatory properties of Lactobacillus acidophilus NCFM. *BMC microbiology* **2020,** *20*, 1-13. https://doi.org/10.1186/s12866-020-01908-2.
56. Gordillo, T.B.; Palumbo, M.C.; Allievi, M.C.; Do Porto, D.A.F.; Ruzal, S.M.; Palomino, M.M. Strategies to display heterologous proteins on the cell surface of lactic acid bacteria using as anchor the C-terminal domain of Lactobacillus acidophilus SlpA. *World Journal of Microbiology and Biotechnology* **2020,** *36*, 1-11. https://doi.org/10.1007/s11274-020-02945-9.
57. Bolla, P.A.; Sanz, A.; Huggias, S.; Ruggera, J.F.; Serradell, M.A.; Casella, M.L. Regular arrangement of Pt nanoparticles on S-layer proteins isolated from Lactobacillus kefiri: Synthesis and catalytic application. *Molecular Catalysis* **2020**, *481*, 110262. https://doi.org/10.1016/j.mcat.2018.12.011.
58. Chen, Y.; Huang, K.; Chen, L.K.; Wu, H.Y.; Hsu, C.Y.; Tsai, Y.S.; Ko, W.C.; Tsai, P.J. Membrane cholesterol is crucial for Clostridium difficile surface layer protein binding and triggering inflammasome activation. *Frontiers in Immunology* **2020,** *11*. https://doi.org/10.3389/fimmu.2020.01675.
59. Mindur, J.E.; Yadav, S.K.; Ito, N.; Senoh, M.; Kato, H.; Dhib-Jalbut, S.; Ito, K. Surface Layer Protein A Expressed in Clostridioides difficile DJNS06-36 Possesses an Encephalitogenic Mimotope of Myelin Basic Protein. *Microorganisms* **2021,** *9*, 34. https://doi.org/10.3390/microorganisms9010034.