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In Silico Investigation of ESAT-6–β2-Microglobulin and PknG–AKT Interactions in *Mycobacterium Tuberculosis*–Human Crosstalk

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ABSTRACT

ESAT-6 or Early Secreted Antigenic target 6 is a small protein of 6kDa molecular weight, secreted by *Mycobacterium tuberculosis* (Mtb), which causes tuberculosis. It is a major factor of such bacterial cells, responsible for imparting the virulence property to the bacterial cell. It primarily stimulates the T cells of the host immune system. It interacts with the host protein beta-2-microglobulin (β2M), which is responsible for presenting antigens on the cell surface. Such an interaction may potentially hamper the ability of immune cells to respond to Mtb. On the other hand, pKnG is a serine/threonine protein kinase that can manipulate autophagy or other cellular pathways and promotes bacterial survival and hinders pathogen clearance. It can interact with several host proteins, such as Rab, AKT proteins of the host cell, and is considered a crucial virulence factor for mycobacterial cells. In our study, we studied the protein-protein interactions between ESAT-6 and β2M of human, and pKnG and AKT of human using the Hdock tool. We analysed the structure of each protein by checking its two- dimensional structures from PSIPRED and checked for its physicochemical parameters using Expasy ProtParam. The quality of each protein was computed from SAVES, which includes the ERRAT score, Ramachandran plot, etc. The interacting amino acid residues were analysed by DIMPLOT.

Keywords: protein interactions, ESAT-6, pKnG, AKT, B-2-B, Hdock, virulence

Introduction

Mycobacterium tuberculosis (Mtb) is a gram-positive bacterium cell which may be characterised by a unique and complex cell wall, which is a key factor in pathogenicity and makes it resistant to antibiotics and host immune response. The cell wall and cell surface contain certain proteins, which, along with the cell wall, protect the bacteria from harsh conditions and allow them to survive and replicate within the host. The proteins of Mtb interact with each other to form complex networks, which are essential for bacterial survival, replication, and virulence [1-4]. These proteins interact with human proteins, which then play key roles in Mtb's ability to infect and persist within macrophages. For instance, PE/PPE proteins are involved in host-pathogen interactions and are one of the major factors for the ability of Mtb to evade the host's immune defences. ESAT-6 protein is secreted by Mtb cells, which interacts with beta-2-microglobulin (β2M) of the host (human) and aids in the proper folding or surface expression of MHC class I molecules [5-7]. This particular interaction occurs mainly in the endoplasmic reticulum and disrupts the formation of MHC class I-β2 M complexes. Such an interaction inhibits antigen presentation to the immune system and thus allows Mtb to successfully evade the host's adaptive immune response and establish a successful infection. β2M is an essential factor for the proper folding and surface expression of MHC class I molecules; sequestration of β2M in the ER, ESAT-6 prevents it from associating with MHC class I molecules and prevents formation of class I-β2 M molecules [8- 10]. This prevention leads to a weakened immune response, which leads to less activation of cytotoxic T-cells for the effective destruction of infected cells.

On the other hand, pknG of Mtb acts as a serine/threonine kinase that interacts with AKT protein of the host cell to manipulate autophagy and promote the survival of the pathogen within the host. On blocking autophagosome formation, pKnG effectively inhibits the overall autophagy flux, preventing the host from clearing the bacteria effectively. This allows Mtb to survive and replicate within the host [9-11].

In our study, we thus aimed to determine the amino acids that take part in protein-protein interactions between Mtb ESAT-6 and β2M protein of human, pKnG of Mtb, and AKT of the host using a computational approach. Future studies on such protein-ligand/protein-protein interactions will help to unleash the possible molecular mechanisms behind the cause of infectious diseases.

Materials and Methods

Retrieval of three-dimensional structure of proteins pKnG, ESAT-6, AKT, and β2M from the PDB database

The three-dimensional structures or PDB structures of the respective proteins pKnG, ESAT- 6, AKT, and β2M were retrieved from the PDB database (the respective PDB Ids are pKnG:**4Y0X**, ESAT-6: **3OGI**, AKT: **8QAT,** and β2M:**6M1B**) [12] and analysed for their quality before being taken for interaction analysis.

Physicochemical parameters and secondary structure

The physicochemical parameters, such as molecular weight, theoretical pI, number of positively charged amino acids and number of negatively charged amino acids, instability index, and GRAVY values of each protein were computed from ExPasy Protparam. The secondary or 2D structures of each protein were analysed by PSIPRED [13, 14].

Validation of protein structures

The PDB structures of each protein were validated by ERRAT scores and Ramachandran plots, which were computed from the SAVES server [15]. Higher ERRAT scores and the number of allowed regions in the Ramachandran plot, computed from ProCheck, meant a higher quality model of protein obtained.

Protein-protein interaction using Hdock and DIMPLOT

The respective protein-protein interactions were checked using the Hdock web server. The interacting partners were ESAT-6- β2M and pKnG-AKT. The highest quality model was retrieved from Hdock with the most negative docking score and the highest confidence score [16, 17]. Thereafter after the interacting amino acid residues between interactive partners were analysed from DIMPLOT.

Results and Discussion

The three-dimensional structures of the four respective proteins were retrieved from PDB and visualised in RasMol (Fig. 1).

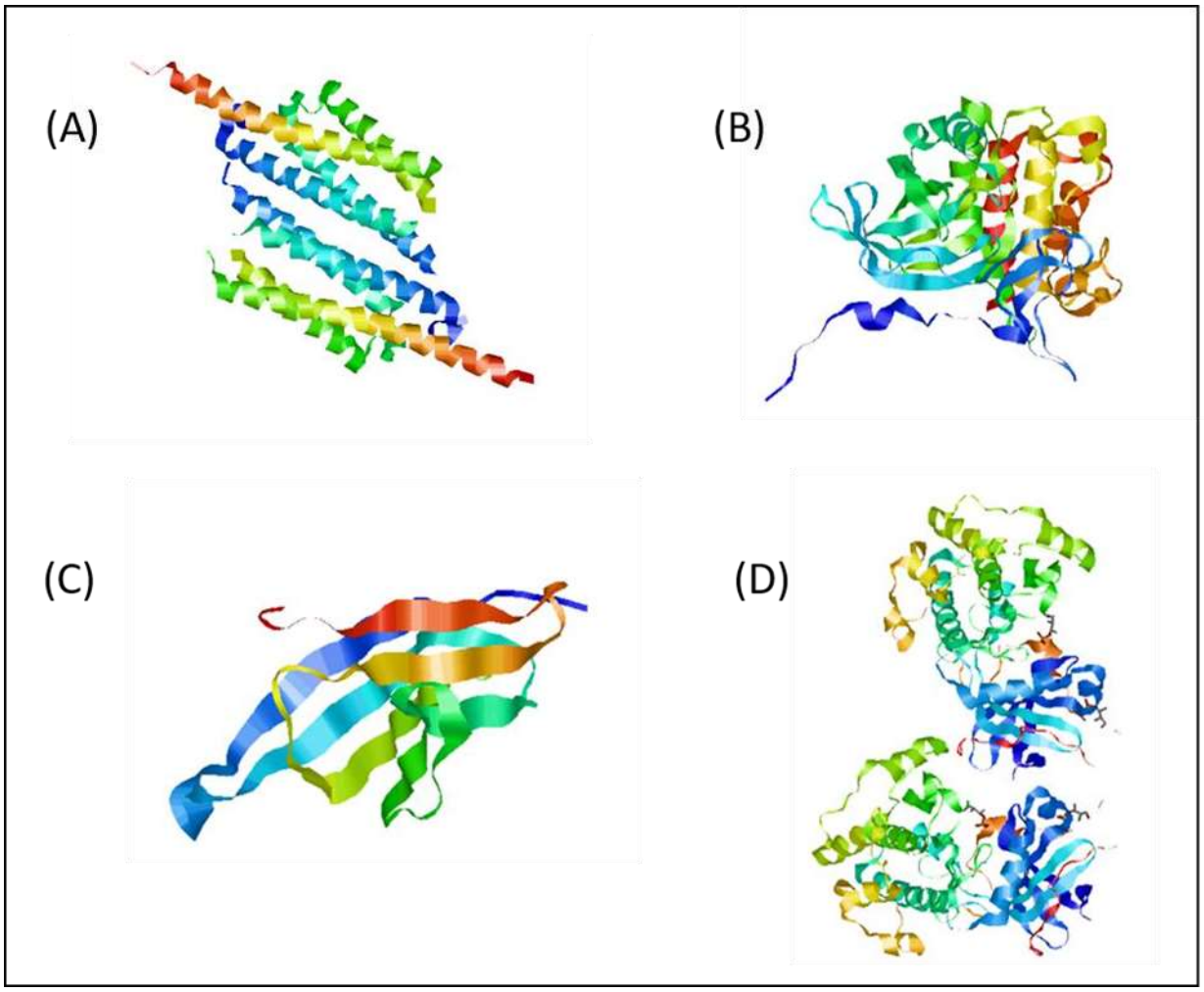

Fig 1: The PDB structures of each protein visualised in RasMol. A. **ESAT-6,** B. **pKnG,** C. **β2M,** D.**AKT**

The ERRAT score of each protein was near about 100% which revealed a good quality model of the protein (Table 1). Moreover, the Ramachandran plots of each protein structure revealed a greater amount of allowed regions, which validated the better quality of the structures (Table 1, Fig. 2)

Table 1: The names of interacting proteins with their ERRAT scores and allowed regions computed from the SAVES server.

Name of Protein	ERRAT score	No of core allowed regions (%)	No of disallowed regions
ESAT-6	100	96.7	0.0
β2M	100	92.2	0.0
pKnG	95.83	92.1	0.0
AKT	94.97	90.0	0.7

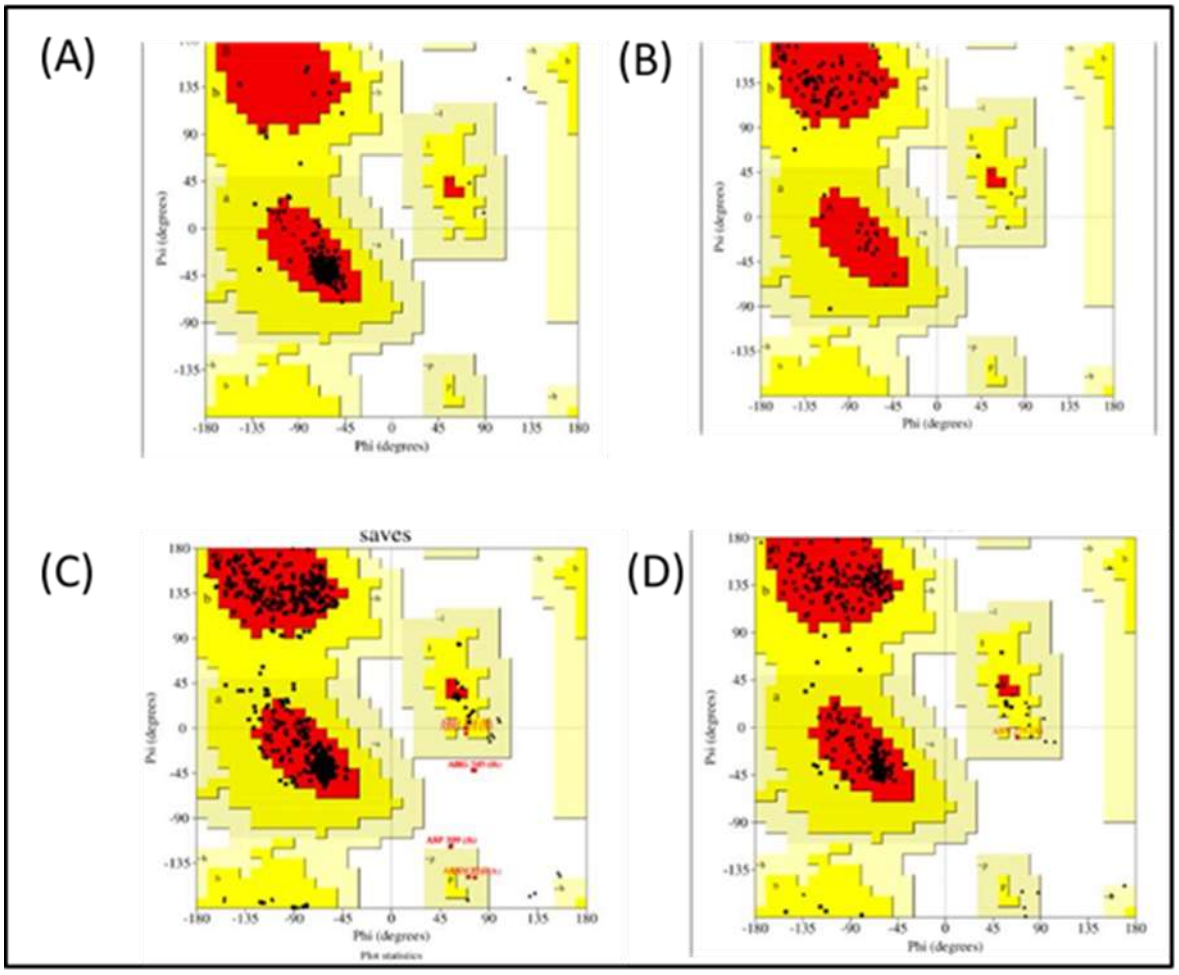

Fig 2. Ramachandran plots for the respective proteins computed from Procheck: A. ESAT-6, B. β2M, C. pKnG, D. AKT

A validation of protein structure is a vital requirement before being subjected to interaction studies. A better quality reveals that no problem would be faced for performing further studies [18-20]. The secondary structures, as revealed from PSIPRED, showed a higher amount of alpha helices for all four proteins, which determined the stability of each protein (Fig. 3).

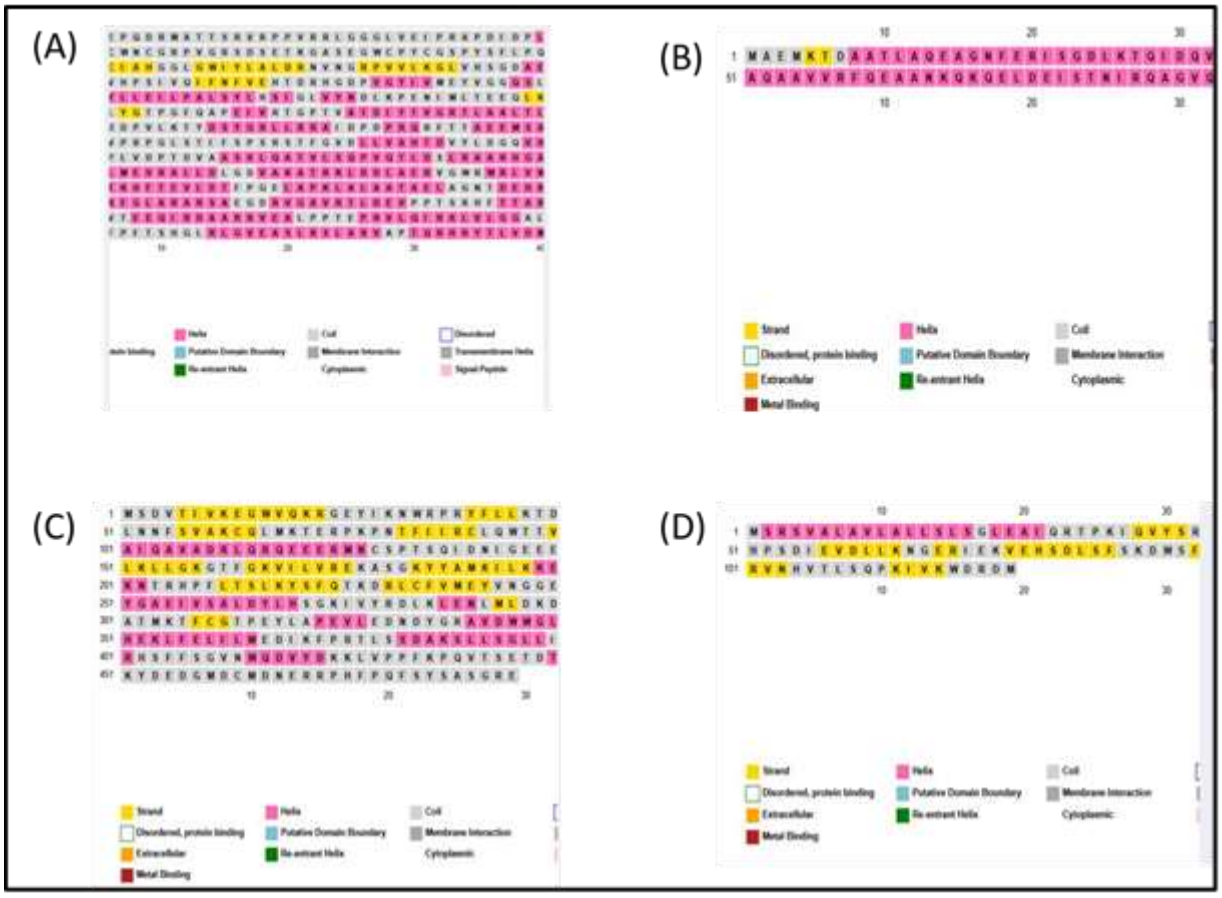

Fig 3. The secondary structures of each protein visualised by PSIPRED: A**. pKnG,** B**. ESAT-6,** C**. AKT, D. β2M**

A comparison of the physicochemical parameters for each protein was computed from ExPasy Protparam computed the stabilities of each protein to be less than 40, which therefore confirmed that the proteins are stable and good to be moved ahead for further interaction studies (Table 2) [21,22].

Table 2. A comparison of physicochemical parameters for each protein

Name of protein	No of amino acids	Theoretical pI	Molecular weight	Total number of negatively charged residues	Total number of positively charged residues	Extinction coefficient	Instability index	Aliphatic index	GRAVY
ESAT-6	95	4.48	9905.87	9	4	17990	36.36	72.21	-0.256
pKnG	750	5.52	81577.9	93	76	74050	40.25	90.12	-0.193
AKT	479	5.72	55774.53	78	68	68800	34.70	71.82	-0.599
β2M	119	6.06	13714.54	16	14	20065	33.82	85.97	-0.376

Interaction studies are solely performed to determine the mechanism of each molecular interaction behind each signalling pathway in our system. We had thus undertaken an approach to determine the underlying mechanism of bacteria-host protein interactions. An interaction between ESAT-6 (Mtb)and β2M (host), pKnG(Mtb) and AKT(host) protein had been performed using Hdock, and the best quality model had been visualised in UCSF Chimera (Fig. 4), with the most negative docking score and greatest confidence score (Table 3). Thereafter, the respective interactions had been visualised from DIMPLOT (Fig. 5). The interacting residues that were seen are given in Table 3. The no of interacting amino acid residues that were predominant were hydrophobic, especially isoleucine, alanine, and phenylalanine between both sets of interacting protein partners.

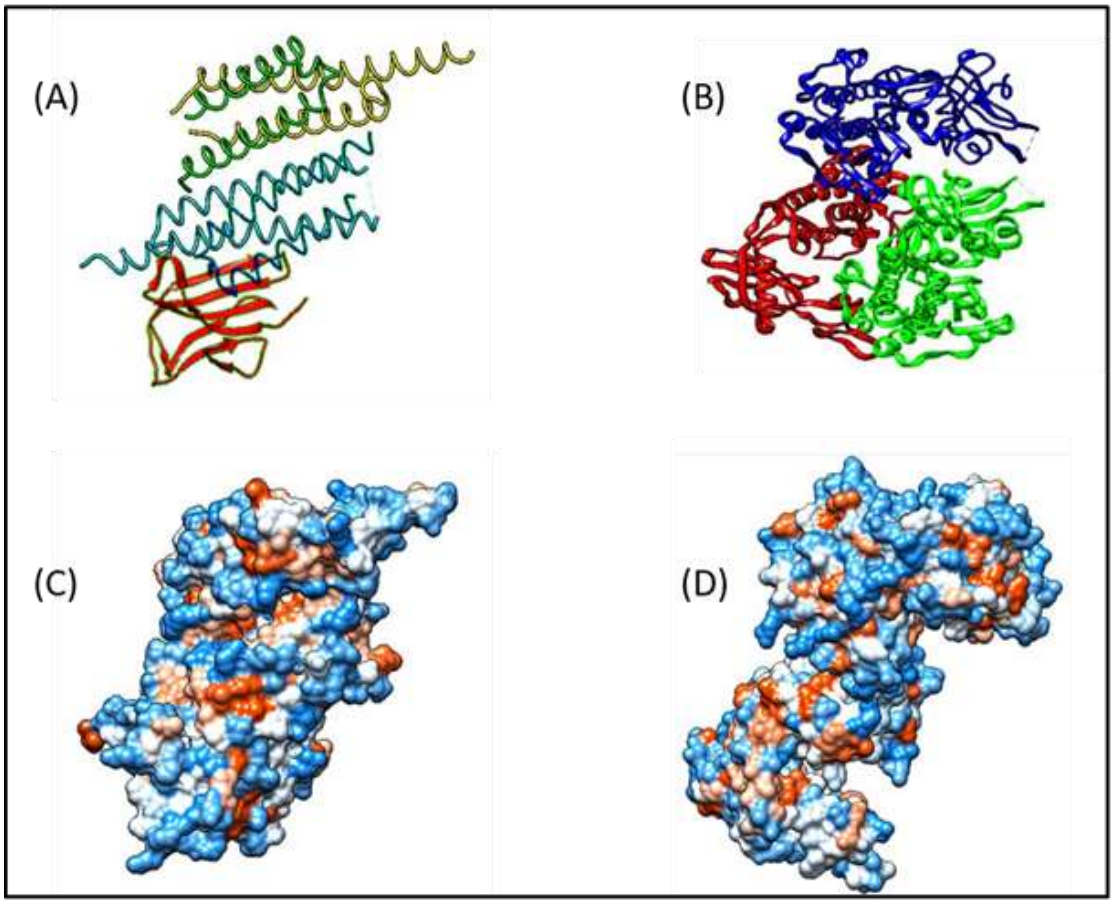

Fig 4: The interacting models of each interacting partner obtained from Hdock **A: ESAT-6** and **β2M, B. AKT** and **pKnG.** Hydrophobic surfaces of each interacting model C. **A: ESAT- 6** and **β2M D. AKT** and **pKnG**

Table 3: The respective docking score, confidence score, and interacting residues for each interacting protein

Name of protein	**Docking score**	**Confidence score**	**Interacting residues**
ESAT-6-β2M	-274.21	0.9230	**ESAT-6** - Ile64, Leu24, Leu23, Ala68, Asp40, Phe41, Ala38,Leu57, Gln67 Ile31, Phe53, Trp42, Ile17 **β2M**- Ile67, Phe64, Ala64, Phe5, Phe19, Ser36, Gly77, Asp81, Met51, Met70, Val74, Thr56, Arg18,Thr25, Val174,
pKnG-AKT	-259.71	0.8997	**pKnG** – His355, Lys191, Asp192, Ala190, Thr162, Phe310, Phe359, Gln356, Gln353, Asp354 **AKT**- Thr174, Lys146, Thr148, Asn150, Ile479, Ser478, Ala477

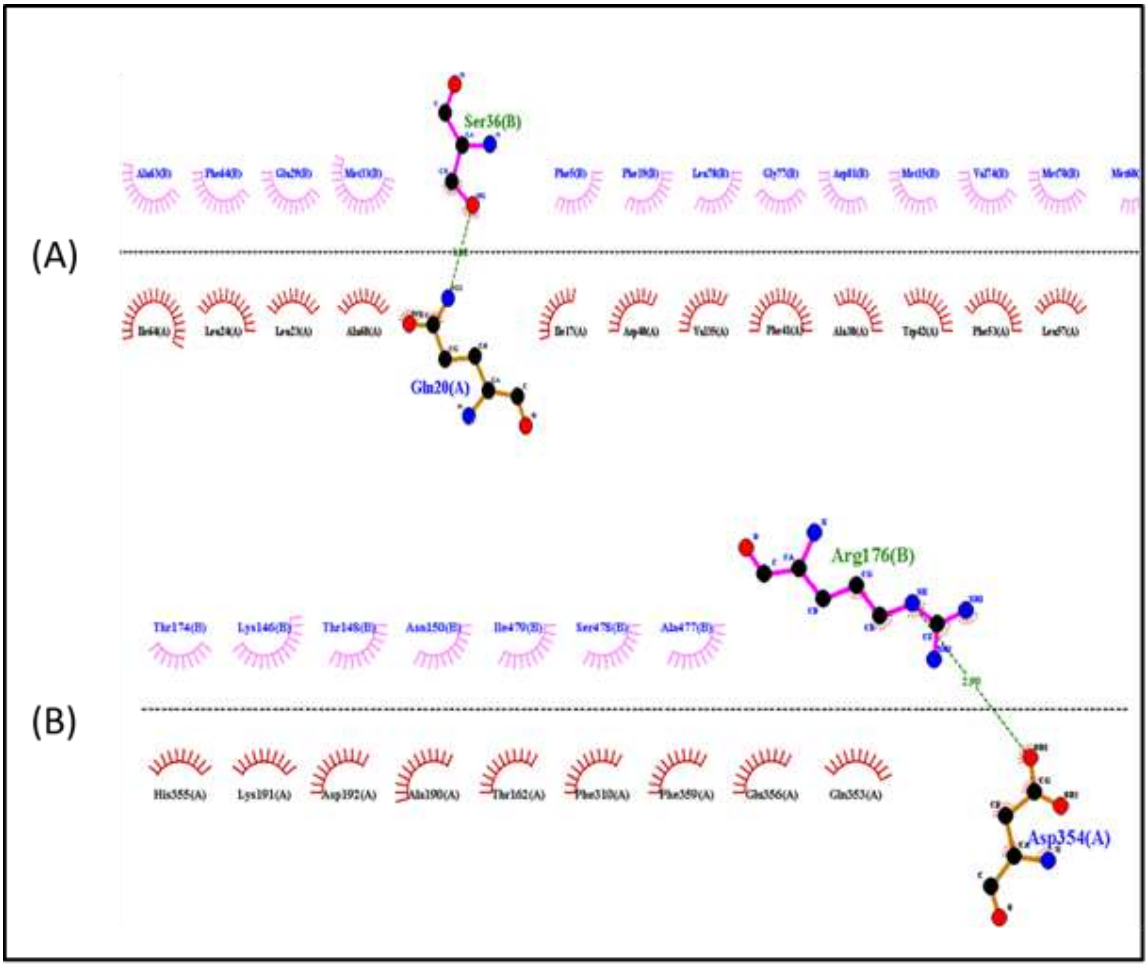

Fig 5: The interacting residues visualised in DIMPLOT A: **ESAT-6** and **β2M** B. **AKT** and **pKnG**

Previous studies performed on host-bacterial protein interactions had confirmed that isoleucine and alanine, the hydrophobic amino acids interactions are found to be crucial for bacteria to adhere to and colonize human tissues, form protective biofilms, and manipulate host functions. The underlying reason is the hydrophobic effect, a thermodynamic phenomenon where nonpolar molecules aggregate in water to minimize their unfavorable contact with the surrounding hydrogen-bonded water molecules [22, 23].

Such a "water-fearing" behavior provides the key energetic drive for bacteria-human interactions, which are otherwise limited by the repulsive forces of the body's aqueous environment. Cell surface hydrophobicity plays a crucial role in attachment or detachment from the surfaces. Host proteins present in macrophages can act against these targeted proteins secreted from microorganisms, but even then, the detailed mechanistic knowledge about such protein interaction studies is still limited in observing the impact of hydrophobicity on adhesion, flocculation, and microbial adhesion [23-25]. The future research would seem to find a possible in manage of microbial adhesion process by steering cell surface hydrophobicity in the upcoming days.

Conclusion

A lot of protein-protein interaction studies have been performed with bacterial-human proteins. We had performed the study to determine such interactions between two specified proteins of Mtb, which take part in imparting virulence to the host cell, with the help of computational tools, and checked that hydrophobic amino acids are taking part in the interactions, which should mainly be targeted in future research, as confirmed by previous studies. Hydrophobic amino acids play a role in cell adhesion to the host or detachment and mainly take part in inducing infection in a host cell. Thus, if we can find out the specific amino acids taking part in virulence of a bacterium cell, it would be easy to determine the unknown molecular mechanisms behind the virulent property of any microbial cell such as bacterium or viral cell and by targeting such factors, an onset of any unknown infectious disease might be prevented from occurring in future. Measurements to suppress the virulent property of a microbe to some extent would help mankind and hold great prospects in future research.

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