Molecular dynamics simulation and experimental study of the surface-display of SPA protein via Lpp-OmpA system for screening of IgG

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Abstract

Staphylococcal protein A (SpA) is a major bacterial virulence factor of *Staphylococcus aureus*. *S. aureus* is capable of escaping from immune system recognition by the surface display of protein A. The SpA protein is broadly used to purify immunoglobulin G (IgG) antibodies. This study investigates the ability of the fusion of Lpp'-OmpA (46 − 159) to anchor and display five repeat domains of protein A with 295 residues length (SpA295) of S. aureus on the *Escherichia coli* cell surface to develop a novel bioadsorbent.

First, the binding between Lpp'-OmpA-SPA295 and IgGFc and anticipation of this structure was investigated using molecular dynamics simulation. Then high IgG recovery from human serum by the surface-displayed system of Lpp'-OmpA-SPA295 performed experimentally.

In silico analysis showed the binding potential of SPA295 to IgG after surface expression on LPP-OmpA. Surface-engineered *E. coli* displaying SpA protein and IgG-binding assay with SDS-PAGE analysis exhibited the high potential of the expressed complex on the surface of *E. coli* for IgG recapture from human serum which is applicable to conventional immune precipitation.

Introduction

S. aureus protein A (SpA) is a surface component of the bacteria (Ton-That et al. 1999) which play a role as a key virulence factor for the *S. aureus* pathogenesis. This is achieved through tightly binding of the SpA to the Fcγ domain of various species of IgG (Sjodahl 1977) and interaction of the Fab domain of VH3-type B cell receptors (Cary et al. 1999). A few of the SpA mutants unable to bind to the IgG-Fcγ, and cross-link B cell receptors have been previously used as a subunit vaccine thereby inducing protein A neutralizing antibodies and were protective against *S. aureus* infections in mice. (Kim et al. 2010). SpA is comprised of five Ig-binding domains arranged as (IgBDs) E, D, A, B, and C (Boyle 1990). Protein A is able to bind, with high affinity, to most IgG subclasses of human, cows, pigs, hamsters, horses, pigs, and rabbits and with low affinity to chicken, goat, rat IgG subclasses (Hadji-Ghasemi et al. 2003). This protein has been widely used for quantitative and qualitative immunological techniques including different kinds of ELISA (Lofdahl et al. 1983; Tashiro and Montelione. 1995).

Display of heterologous proteins on the bacterial surface has been demonstrated as a multi-strategy approach to develop an efficient vaccine for *S. aureus* development (Kalyanasundram et al. 2015), screening of antibody libraries (Cavallari 2017), development of whole-cell bioadsorbents (Tafakori et al. 2012), and biosensors (Furst et al. 2017). Chimeric protein system of the Lpp'-OmpA is used as an anchor and loads heterologous proteins onto the Gram-negative bacterial surface (Yang et al. Georgiou et al. 1996). Lpp'-OmpA consists of the first nine aminoacids of the *E. coli* lipoprotein (Lpp) which is fused to the residues 46–159 of the OmpA porin protein family to anchor bacterial cell wall envelope (Francisco et al. Tafakori et al. 2014).

We examined the possibility of surface displaying of SpA295 via a Lpp'-OmpA system and its binding capability to IgG_{FC} using bioinformatics and computational tools, which was confirmed by the experimental methods. SpA protein was successfully immobilized on the *E. coli* surface using an Lpp'-OmpA (46 − 159) fusion system to develop an efficient method for purification and immunoprecipitation of IgG antibodies.

Materials And Methods

2.1. Construction of Computational Modeling

The structure of SpA protein according to the amino acid sequence in this study that comprises five repeat domains of 295 amino acid residues in length (SpA_{295}) was predicted by ModWeb server (Pieper at al., 2014). The nucleotide sequence of the Lpp'-ompA-Spa construct was submitted in the genebank with the accession number: MT680197. The Geometric coordinates of X-ray crystallography of IgG were obtained from RCSB protein data bank with the access code: 4ZNC.

2.2. Computational condition of docking and molecular dynamic simulation

To provide the stable structure of Lpp'-OmpA-SPA295, this complex was subjected to molecular dynamic simulation for 30 ns. MD simulation was performed by GROMACS 5.0.5 software (van Der Spoel et al., 2005) and OPLSAA force field similar to that the previous study (Ghahremanifard et al., Hashemzadeh et al., Fasehee et al., 2018) The molecules were placed in a dodecahedron box containing the water molecule in TIP3P model. In order to create the ionic conditions of 0.15 molar, water molecules were replaced with Na^+ and Cl^- ions and the total charge of system was neutralized. The initial energy minimization was performed using the steepest descent algorithm. After that, the NVT simulation was performed for 50 ps and followed by the NPT ensemble for 30 ns. In order to maintain the temperature of 300° K and the pressure of 1 bar, nose-hover thermostat and berendsen barostat were used, respectively. r = 1.2 was considered for electrostatic and van der Waals interactions.

After the structure stabilized, HDOCK server (Yan et al., 2014) was employed to investigate SpA295-IgG interaction according to default parameters of protein–protein free docking hybrid algorithm of template-based modeling. The crystallography structure of the protein–protein complexes of SpA-IgG divided into individual files as a starting structure for docking simulation.

Then the complex obtained from the HDOCK server was subjected to 30 ns molecular dynamic simulation under the conditions mentioned above.

All structures visualized by the Discovery studio. The number of hydrogen bond (H-bond) formed between acceptor and donor atoms is measured by using the geometrical criteria of a donor-acceptor distance less than 3.5 Å by RING 2.0 web server (The RING 2.0 web server for high quality residue interaction networks).

2.3. Materials used in Experimental Model

List of the primer pairs, bacterial strains and plasmids which were used in this study are mentioned in Table 1. The SpA genes were PCR-amplified from the genomic DNA of *S. aureus* (ATCC 6538) as a template, the Pfu DNA polymerase (Fermentas, Germany) and primers shown in Table 1. For designing the growth curves and optimization tests, bacterial cultures were grown in Luria-Bertani (LB) medium containing of 50 mg/ml kanamycin sulfate. Isopropyl beta-D-thiogalactopyranoside (IPTG) was utilized for inducing recombinant protein expression. Human serum were used for binding analysis in this study.

2.4. Plasmids construction and protein expression

Lpp'-OmpA fragment was amplified by PCR using LPOA1 and LPOTA primers to make plasmid pLOAa, previously made plasmid pET-LOA, containing the fusion of the first nine amino acids of N- terminal the Lpp and amino acids 46 to 159 of OmpA were used as a template. The 381 bp product was digested with *Nde*I-*Eco*RI restriction Enzymes, followed by ligation into the pET26b vector previously digested. PCR was carried on as followed: for 5 min at 94 °C, 30 cycles of 45 s at 94 °C, 45 s at 68 °C, 60 s at 72 °C, and 10 min at 72 °C on an MWG AG, Biotech, Primus 96 system (Germany). For gene cloning, the truncated SpA gene was amplified using primers PAF, PAR from ~ 890 bp, S. *aureus* genome as a template (100 ng/25 µl), and purified. To prepare the final construct of plasmid, SpA fragment (890 bp) and pLOAa were digested by *Eco*RI and *Xho*I and followed by ligation into the plasmid pLOAa. The vector is called pLOA-PA. Transformation of the vector into *E. coli* was carried out. It used CaCl2-mediated procedure. Overnight cultures of recombinant bacterial inoculated into 1 liter of fresh LB medium which contains 50 µg/ml kanamycin sulfate. To express fusion proteins, cultures were induced by using IPTG 0.1 mM, for 16 h. Centrifugation was used at 10000 g for 2 minute to harvest the cells.

2.5. Preparation of surface-engineered *E. coli* displaying SpA protein

A single colony of the recombinant *E. coli* harboring plasmids pLOA-PA were grown overnight in the LB media containing 35 µg/ml of kanamycin and was then inoculated into the fresh medium and continued for 8hr further incubation.

The bacterial culture was collected by centrifugation at 5000Xg for 10 min followed by a washing step with 10 ml PBS buffer by resuspended in PBS following a washing step. This was repeated for three more times.

The last time the bacteria was resuspended in 10 ml of PBS + 0.02% sodium azide, transferred to a 250 mL erlenmeyer flask and stirred at room temperature. Formaldehyde solution was added to give 1.5% final concentration and stirring was continued for 80 minutes at room temperature. Formaldehyde was removed by washing the suspension with 15 ml of 1X PBS. After discarding the

supernatant, the pellet was washed in PBS + 0.02% sodium azide as a 10% (w/v) followed by a 5 min centrifugation at 5000Xg. After resuspension of the bacterial pellet in a 100 mM Tris-HCl buffer pH 8 containing 10% glycerol, it was stored at 4 ºC.

2.6. IgG-binding assay

The affinity and IgG-binding ability of the protein A-displaying E. coli was examined using IgG-binding assay with rabbit sera. The surface-engineered *E. coli* were washed with 1 mL of suspension buffer (100 mM Tris-HCl pH 8). The pH of rabbit sera was increased to 7.5–8 by 1M Tris of pH 8 and it was then added to the bacterial suspension followed by incubation for one hour at 4°C. The surface-engineered bacteria were washed with 100 mM Tris-HCl of pH 8. Elution buffer (100 mM glycine of pH 3 containing 1M KCl was used to release the bound IgGs from surface of the bacterial. Eluted fraction was dialyzed in 10 mM Tris-HCl and then resolved on SDS-PAGE prepared according to Laemmli (1970) followed by staining with Coomassie Brilliant Blue R-250. The *E. coli* transformed with the parental plasmid pLpp'-OmpA without IgG-binding domains of SpA was used as a negative control.

Results

3.1. Complex Binding of SpA with IgG_{FC}

We analyzed and evaluated the complex formation of the pLpp'-OmpA-SpA_{295}-IgG_{FC} (Fig. 1) by a high-throughput computational approach. The root mean square deviation (RMSD) and the predicted structure of Lpp'-OmpA-SpA_{295} presented in Fig. 2a. RMSD plot shows the stability of the system and this structure after 30 ns of MD simulation. Binding site of this complex obtained from molecular dynamic to IgG_{FC} determined by docking. The docking cluster scoring models are according to the energy range from the minimum energy interaction of protein-protein complexes. We chose low free energy of the IgG_{Fc} complex with pLpp'-OmpA-SpA_{295} from docking to subject to molecular dynamic simulation for 30 ns that the result structure is in Fig. 2b.

The H-bonds among SpA_{295} with IgG_{Fc} were displayed in Fig. 3. Pro59, Asp99, Asn142, Ala44, Ala63, Gln64, Asn67, Glu9 in SPA involved in H- bond with IgG. Vdw and ionic binding between IgGFc and SpA295 are also seen in the Fig. 3. Glu9, Asp99, Glu145 make ionic bond with IgG.

Hydrophobic sites on the surface of SpA in the region that interact with IgG (Fig. 4a) and the surface electrostatic potential of SpA are observed in Fig. 4b. As can be seen, the number of hydrophilic amino acids and also, negatively charged amino acids are more common in the interaction site

3.2. Construction of Plasmids, transformation and Protein Expression

Surface attachment of the protein A (SpA) containing E, D, A, B and C domains (Fig. 1S) on the surface of *E. coli* BL21 (DE3) was successfully done using the Lpp'-OmpA system. The construct of Lpp'-ompA-Spa in pET26b plasmid was made. DNA sequence of p Lpp'-ompA-Spa construct cloned in pET26b, and protein sequence of p Lpp'-ompA-Spa construct are in Fig. 2S.

Construction of the pET26b plasmid was verified by restriction enzyme digestions (Fig. 3S, 4S and 5S), and DNA sequencing. Amplification of protein A performed using PCR reactions at different temperatures (Fig. 6S). detection of non-recombinant and recombinant plasmids pET26 was performed by enzymatic digestion (Fig. 7S) and electrophoretic mobility shift assay (Fig. 8s).

The expression of the fusion truncated SpA and the control protein was carried out using *E. coli* BL21 DE3 and IPTG as an inducer. The protein expression by *E. coli* transformed with recombinant plasmids pET-LOA and pLOA-PA was evaluated using SDS-PAGE analysis (Fig. 9S and Fig. 10S).

The recombinant truncated Protein A contains five Ig-binding regions of protein A and a 6x His-tag on the C-terminus. To prevent non-specific binding to IgG, the albumin binding region and other regions present in SpA was removed to ensure the specific IgG binding. Immobilized metal affinity chromatography (IMAC) was used to purify the recombinant C-terminus 6X his-tag fusion of the SpA. Meanwhile, using anti-His-tag antibody the fusion protein A can be detected. The recombinant Protein A is ideal for

immunoprecipitation and purification of antibodies as it is able to binds to most human and mouse IgG subclasses of human and mouse.

3.3. Binding assay for SpA-displayed recombinant *E. coli*

The recombinant SpA-displaying *E. coli* adsorbent that developed in this work was used for purification of the IgG from rabbit serum. After binding and two steps of washing to remove nonspecific proteins, the protein A eluted from the bioabsorbent contained mainly IgG molecules.

The presence of the peptides corresponding to the IgG heavy and light chains (about 50 kDa and 25 kDa, respectively) on polyacrylamide gel further verifies that IgG has adhered to the protein A immobilized on the recombinant *E. coli* surface (Fig. 5). The results of the IgG purification were also comparable with that of the one achieved by commercial protein A- agarose in which the SpA is immobilized on the surface of this polymer (Fig. 6). Eluted IgG from protein A-agarose support prepared in this study was qualitatively comparable to purified IgG using immobilized protein A agarose matrix supports by SDS-PAGE analysis as shown in Fig. 6. It is notable that protein A bind and extracts the intact form of IgG from serum by attaching to its Fc region, without changing the conformation and structure of IgG as it occurs when a reducing agent such as DTT which breaks S–S bond is present in the buffers and cause dissociation of heavy chain and light chain of IgG.

Discussion

One of the most common antibody purification techniques used SpA immobilized on various solid supports that can be used in industrial application. Changes in SpA immobility methods for improving antibody extraction is still ongoing (Rigi, G. et al. 2019).

Increasing stability of the SPA protein in various harsh environment is one of the main factors in designing of IgG screening (Gerald Cherf and Jennifer R. 2015). One of the major strategies to increase proteins stability is display of proteins on the surface of a live cell as an alternative to classic protein immobilization approach (Mateja Lozancic, et al. 2019; Yadveer S. Grewal, et al. 2016). Classic immobilization of recombinant proteins onto the surface of a matrix (Khodaei, S., 2018) is sometimes challenging as the protein might lose its conformation and consequently its function. However, anchoring of the proteins is a mild approach to immobilize the heterologous proteins to the cell outer membranes. In this way the host cell produces the heterologous protein and at the same time covalently attach it on its surface. In this article, we have used the E.coli surface displaying method to express SPA protein for IgG separation. The immunoadsorbent generated from *E. coli* surface display, in addition to the other benefits mentioned, can be rapidly generated in a cost-effective way and stored lyophilized at room temperature, which will be stable for several months that reduces the cost of downstream processes in the industry as well.

The efficiency of surface display systems and the correct and efficient protein folding and its stability is highly related to the specifications of the carrier protein, the passenger protein, and the fusion method (Yang, Z., et al. 2008 ; Barrett, T., et al. 2019). LPP-ompA is an efficient surface display system developed and applied for various applications (Fasehee, H., et al. 2018; Rigi, G., et al. 2014 ;Tafakori, V., et al. 2012). In this research project for applying Lpp'-OmpA as an anchor for SPA, in the initial design, we first performed physico-chemical and structural studies on the fusion chimeric protein using molecular dynamics tools to ensure the strength and stability of this novel structure on the cell surface.

Computational analysis showed that the surface expression of SpA_{295} creates a stable structure and does not form undesirable bonds with the surface protein Lpp'-OmpA, and maintain its binding structure to IgG_{Fc}. Furthermore, the analysis displayed in the binding of Lpp'-OmpA-SpA297 complex with $IgG_{F}c$ which aminoacids involve in Vander Waals interaction, hydrogen binding and ionic binding.

In the experimental work, surface expression of this novel system of the recombinant protein by five repeat domains of protein A on the surface of *Escherichia coli* BL21 and the power of IgG separation confirmed computer simulation findings. The amount of uptake under five SpA repeat domains system was extremely high. The higher IgG-binding yield demonstrated through SDS-PAGE analysis which can be used as a good replacement for the conventional immune precipitates.

The system used in our work, is shown to be a consistent and convenient tool for engineering *E. coli* cell-surface to display various ligands in their active form. An advantage of the present system to the conventional commercially available immobilization matrices is simplicity, high production rates, easier production and handling processes, and the lower cost of preparing the matrix.

Our matrix was able to purify IgG from human sera as a functional assay and when taking into consideration the yield, purity of IgG and the cost of production of matrix, the system can be used to develop an efficient immunoadsorbent.

We showed that the displayed protein domains with distinct functions of IgG purification at the cellular surface are accessible for the binding partner in binding studies. Furthermore, with the whole cell as a matrix, the proteins have proven to be more stable, therefore making downstream processes of associated preparations and protein purification redundant.

Further investigation on this system is required to achieve higher production rates and specificity at the industrial scale.

Abbreviations

Staphylococcus aureus protein A (SpA), immunoglobulin G (IgG), outer membrane protein A (ompA), truncated outer membrane protein A (Lpp'-ompA), isopropylthiogalactopyranoside (IPTG), Ig-binding domains (IgBDs), Luria
Bertani medium (LB medium).

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

Not applicable

Competing interests

not applicable

Authors' contributions

Fatemeh Ramezani performed computational simulation. Vahed conducted most of the experiments. Tafakori is contributed to this work by doing some experiments and data analysis. Najafi collected the bioinformatics data and helped as a technical assistant. Ahmadian (corresponding author) designed the experiments, supported the project, analyzed data and wrote the final manuscript t. All authors read and approved the final manuscript.

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Tables

Table 1. Primers, plasmids and strains used in this study

Primer, plasmid or strains	Description or Genotype	Source or reference
Primer PAF - EcoRI	GGGG G AAT TC T GCA AAT GCTGCGCAACAC	MWG
PAR - XhoI	GGGG CTCGAG TTTTGGTGCTTGAGCATCGT	MWG
P1 (LPO1-F) NdeI	GGGGCATATGAAAGCTACTAAACTGGTACTGGGCAACCCGTATGTTGGCTTTGAAATGGG	Tafakori et al. (2012)
LPOTA, EcoRI	GGGGGAATTCGCTCCCGGAATGCCGTTGTCCGGACGAGTG CC	Tafakori et al. (2012)
pET 26b pET 26b-lOAE (pLOAa)	T7 promoter, an N-terminal pelB signal sequence for potential periplasmic localization, plus optional C-terminal His·Tag Vector for construction and expressing of chimeric protein containing lpp'-ompA, Elongatus and Chitin Binding domain	Qiagen Novagene Constructed in this study Constructed in this study
Strain BL21 DE3	F– ompT gal dcm lon hsdSB (rB- mB-) λ (DE3 [lacI lacUV5-T7 gene 1 ind1 sam7 nin5]	Stratagene
Top 10 Staphylococcus aureus	F'[laclq Tn10 (tetR)] mcrA Δ (mrr-hsdRMS-mcrBC) $_{\phi}$ 80lacZΔM15 ΔlacX74 deoR nupG recA1 araD139 Δ(araleu) 7697 galU galK rpsL(StrR) endA1 λ–	Invitrogen

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Figures

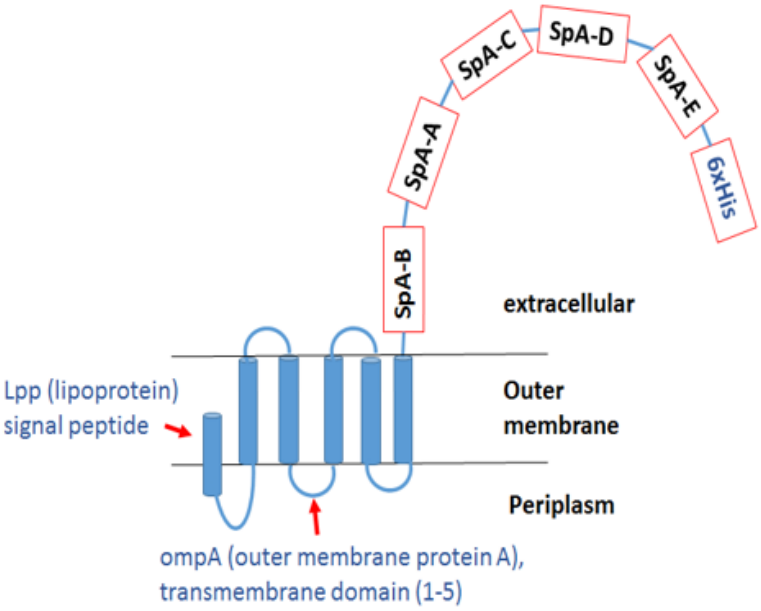

Figure 1

Schematic illustration of Lpp'-OmpA, SpA295 complex.

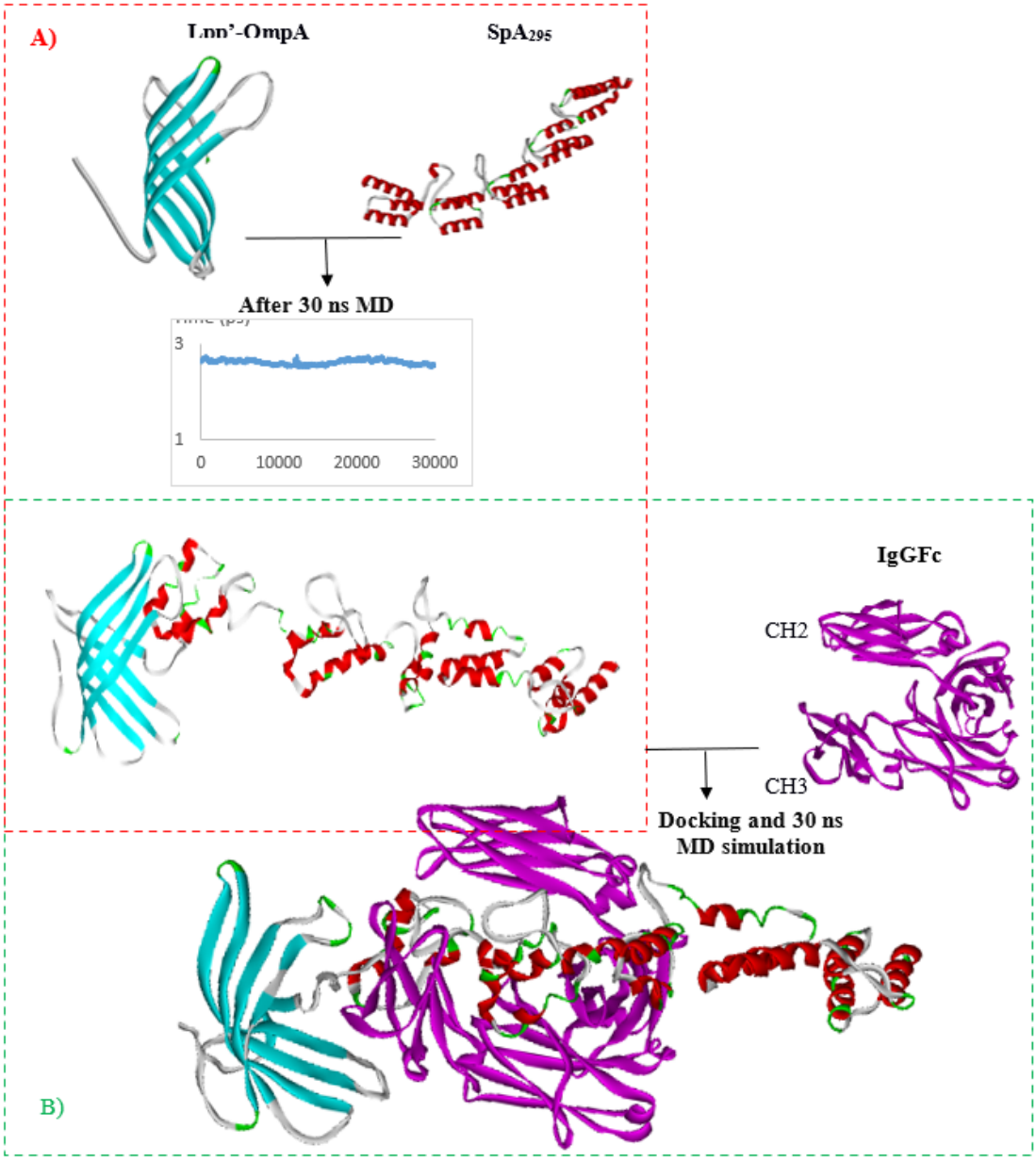

Figure 2

A) The primary structure of Lpp'-OmpA, SpA295 and its complex after 30 ns molecular dynamics, RMSD plot of protein structure compared to the primary structure. B) Lpp'-OmpA-Spa295 ¬and IgGFC and their cpmlex after docking and 30 ns molecular dynamics simulation.

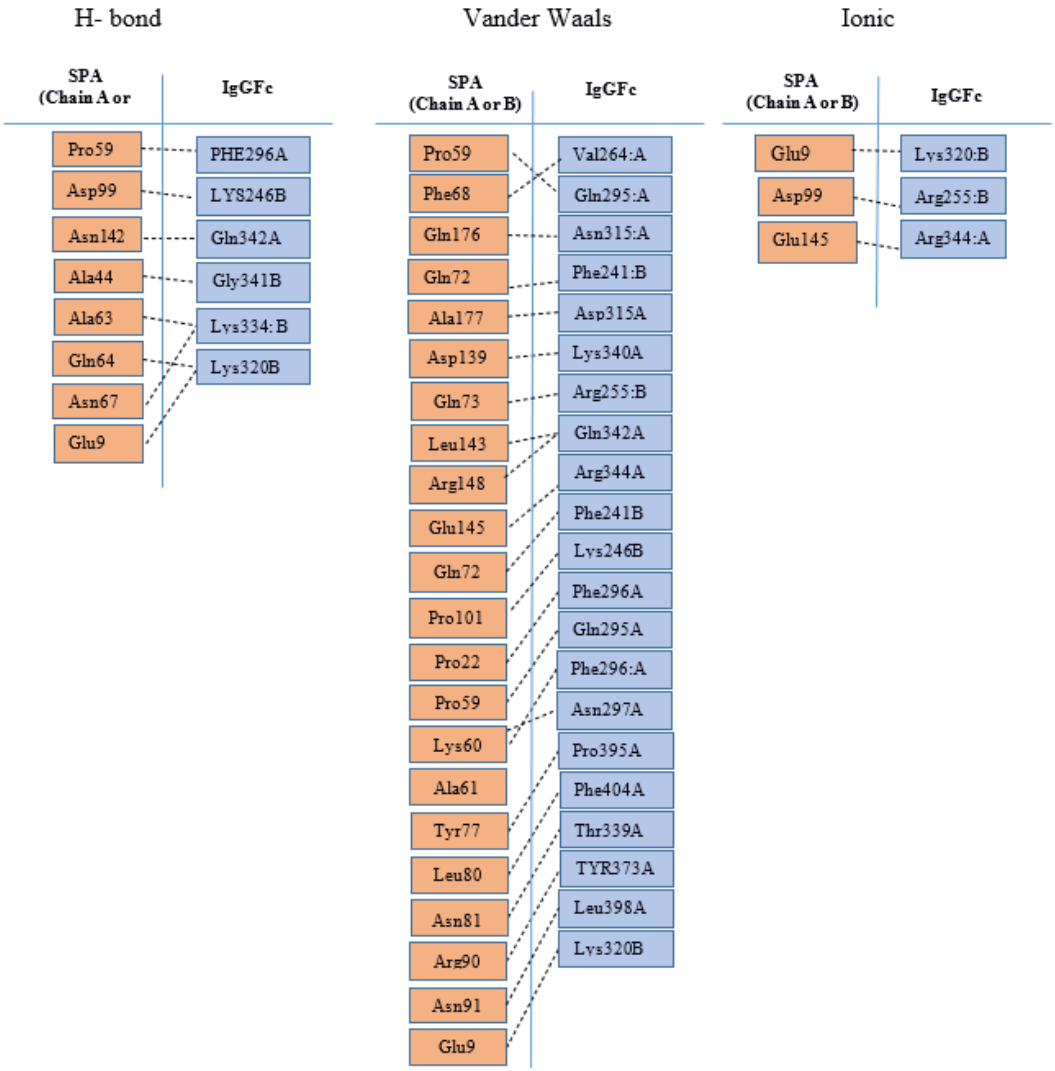

Figure 3

Illustration of H-bonds, Vander Waals and ionic interaction of SpA295 with IgGFc observed after 30ns molecular dynamic simulation (yellow broken lines represent H-bonds between two residues).

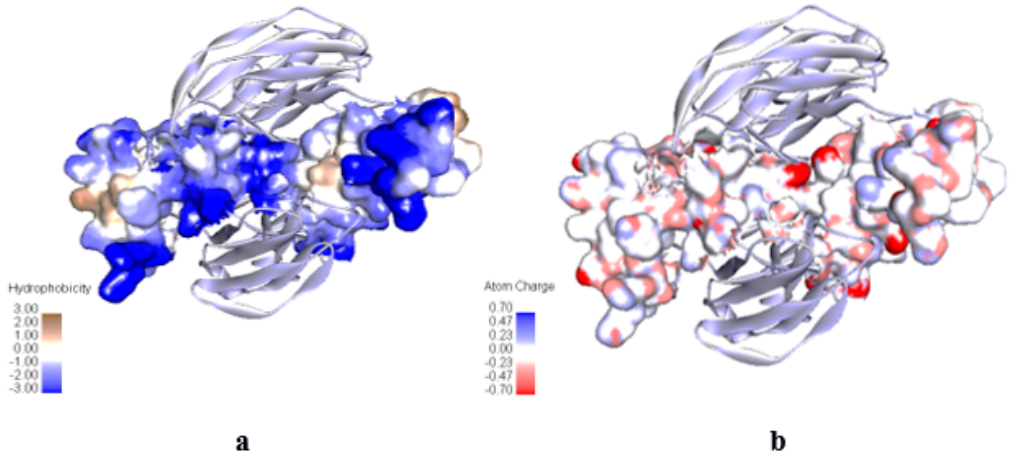

Figure 4

(a) The hydrophobic surface display of interaction of SpA with IgGFc, (b) The electrostatic surface display of interactions SpA with IgGFc.

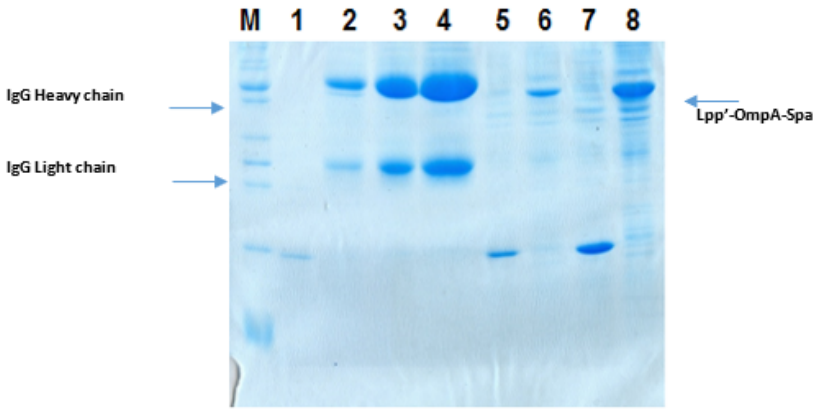

Figure 5

SDS-PAGE analysis showing efficiency of E. coli Lpp'-ompA-SpA compared to the Protein A-agarose, stained by Coomassie Brilliant Blue R.

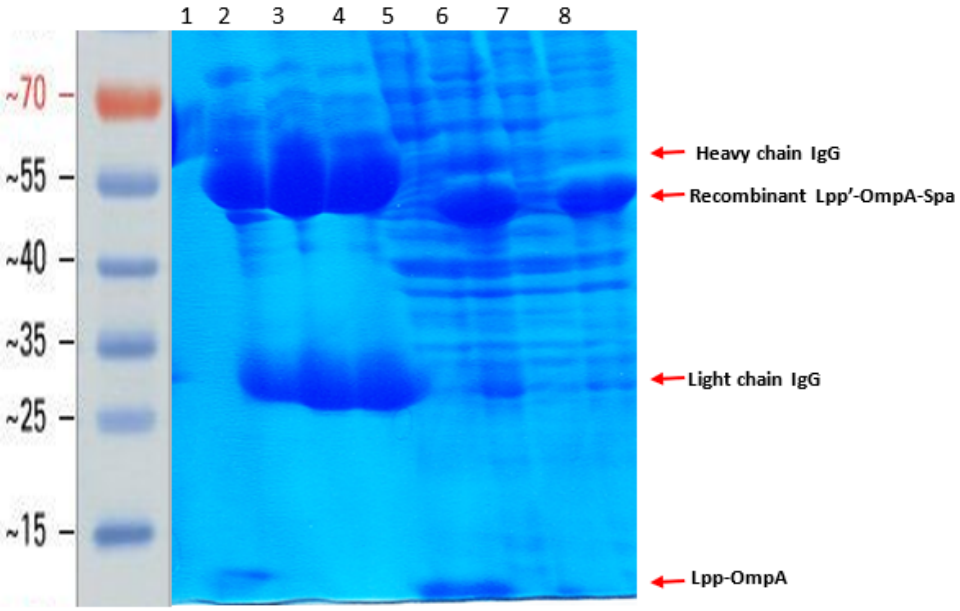

Figure 6

Functional IgG-binding assay of the various constructs. The lanes are loaded as follows: 1: Supernatant of E. coli 1 Lpp'-OmpA incubation with serum after elution. 2: Supernatant of E. coli 1 Lpp'-OmpA-Spa incubation with serum after elution. 3: Supernatant of E. coli 1 Lpp'-OmpA-Spa incubation with serum after elution. 4: Supernatant of Protein A agarose1 incubation with serum after elution. 5: E. coli Lpp'-OmpA pellet after binding to IgG without elution. 6: E. coli Lpp'-OmpA-Spa pellet after binding to IgG without elution 7: E. coli Lpp'-OmpA pellet after binding to IgG following elution of the bound IgG. 8: E. coli Lpp'-OmpA-Spa pellet after binding to IgG following elution of the bound IgG.

Supplementary Files

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- SupplementaryFile.docx