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Original Article

PREDICTION OF PROMISCUOUS EPITOPE STUDIES OF SPA ANTIGEN IN *STAPHYLOCOCCUS AUREUS:* AN INSIGHT ON PEPTIDE-BASED VACCINE

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ABSTRACT

Objective: Owing to the difficulty in providing drug therapies against Methicillin-resistant *Staphylococcus aureus* (MRSA), the development of an effective and promising vaccine is an immense challenge in combatingMRSA infections. The present work focuses on the development of a peptide-based vaccine, by identifying the epitopes from SPA antigen.

Methods: The epitopes were identified based on different properties, such that they are capable of eliciting broadly neutralizing immune responses. The identified epitopes were subjected for peptide docking using Glide and antibody-antigen docking using ClusPro.

Results: By *in silico* approach two epitopes "NLNEEQRNG" and "LKDDPSQSAN" were identified for SPA protein with sequence lengths of nine and ten respectively. The least energy for the peptide docking was observed for NLNEEQRNG sequence and the amino acid residues of this peptide share similar interaction with antibody-antigen docking.

Conclusion: Based on the properties and docking studies the best-ranked epitope sequence is 'NLNEEQRNG'. Further studies on this peptide sequence might be helpful for alternative therapy of MRSA infections.

Keywords: Epitope, SPA antigen, IgG antibody, Docking studies

INTRODUCTION

Staphylococcus aureus infections are commonly known as Staph infections, which causes many diseases, in some cases leading to life-threatening conditions. The infections caused are resistant to antibiotics and are known asMethicillin-resistant *Staphylococcus aureus* (MRSA). Bacteremia incidence by Staph ranges between 20-50 persons among 100,000 populations annually, of which approximately 10-30% patients die [1]. The staph infections are most commonly spread either by infections HA-MRSA (hospital acquired) or nosocomial MRSA and CA-MRSA (community acquired). There are multiple factors involved in influencing the outcomes of staph infections, but these infections are most commonly observed in infants and old. The methicillin, oxacillin, vancomycin-like antibiotics are resistant to staph which provides an immense challenge for developing new therapeutics to combat staph infections.

The best way for combating the infections is via vaccination process, as vaccines elicit the immune response and provide humoral immunity. Vaccine technology mainly relies on the antigen that has the ability to boost the immune system. About 20 different antigens were evaluated clinically accounting for 19 different vaccines from *S. aureus* [2]. Although the vaccine studies were successful in animals and in preclinical toxicity studies, they were insufficient in providing an immune response in humans. Hence, many of the vaccine developments couldn't advance phase 2 trials, and to date, there are no available vaccines against staph infections. The antigens in the study mostly included surface proteins and a class of microbial surface components recognizing adhesive matrix molecules (MSCRAMM). One of the antigen that was not yet studied from this class is Peptidoglycan-binding Lysm protein or also known as *Staphylococcus aureus* Protein A (SPA) or IgG binding protein.

The MSCRAMM account for about 20 potential antigens that are anchored to peptidoglycan layer by sortase enzyme [3]. MSCRAMMs are essentially important in anchoring the desquamated epithelial cells of human and help in colonization. The clumping factor B bind to the fibrinogen of human [4]and other proteins SdrC/D, SasG/Pls, IsdA help in colonization on the surface of epithelial cells. Whereas the SPA binds to Fab region of antibody IgG [5] and activates the immune response of host leading to the disruption of cell mediated by classical and alternative pathways as depicted in fig. 1. The SPA has the capability of binding Fab region of the B-cell and inducing the programmed cell death process and hence is also known as B-cell superantigen [6]. In addition SPA even function as binding to Fc region [7], which prevents normal phagocytosis process. In *in vitro* conditions, it is probably known that SPA binding inhibits the opsonophagocytosis [8]. All these works indicate that the SPA is potent in altering the immune responses of the host.

Thus, we have focused on the B-cell superantigen SPA for the present study, as it is potent for developing a vaccine. In the early ages of vaccine development, the vaccines were developed by either attenuated or killed pathogens. But the present advancement in vaccine technology has proposed new targets for vaccines which include synthetic peptides that comprehend B-cell and T-cell epitopes [9]. The most important features concerned with the use of peptide-based vaccines rather than pathogen as whole include the following, they uniquely target on specified epitopes; they are even capable of skipping of immune evasion, non-protective responses, and autoimmunity. Furthermore, peptide-based vaccines can safely induce tolerance to specific allergens [10]. The accessibility of advanced computational algorithms and immunoinformatics databases with experimental validations helps in developing the antigen-specific peptide-based vaccines [9]. In this study, we have applied immunoinformatics tools for identifying the epitopes and molecular docking for predicting the best epitope. Identifying the potential epitope will help in developing a peptide-based vaccine, which could certainly be one of the best approaches in the alternative treatment of staph infections.

MATERIALS AND METHODS

The main objective of immunoinformatics studies on SPA is to identify the epitopes that can mount an immune response against staph infections. This study could cater a most promising alternative in combating infections than that of conventional methods.

Protein sequence retrieval

The protein sequence of SPA was retrieved from the National Centre for Biotechnology Information (NCBI) database with ID: A0A0E8ISN [12]. NCBI is a database that hosts the information related to sequences of protein, DNA and RNA which are known as RefSeq (Reference Sequence). The data are mined taxonomically, being non-redundant and that which are highly annotated.

The data set hosted by NCBI are continuously shared with other data hosting servers like European Nucleotide Archive and the DNA Database of Japan via INSDC (International Nucleotide Sequence Database Collaboration) [13].

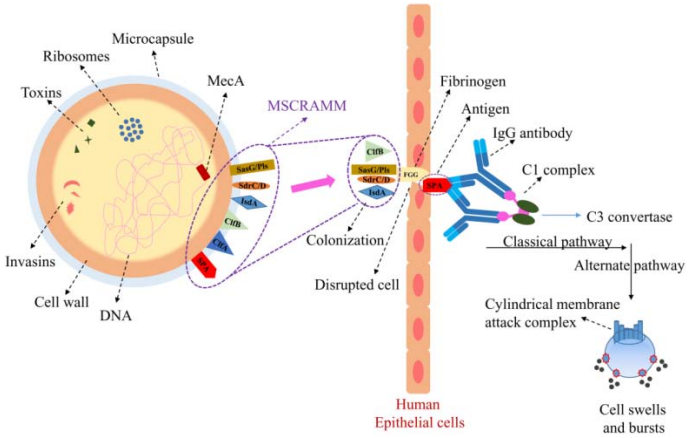

Fig. 1: *Staphylococcus aureus* cell components and its adhesion on disrupted human epithelial cells (source: William *et al.*, 2015 [11])

Protein domain prediction

InterPro is a repository for protein related information like domain architecture, motifs, Gene Ontologies, etc. which are known as signatures. These signatures are integrated from eleven different sources *viz.,* ProDom, PRINTS, PROSITE, Pfam, CATH-Gene3D, SMART, TIGRFAM, HAMAP, PIRSF and PANTHER. With the different signatures integrated into InterPro database, it provides a hub to predict domains, motifs, etc. via its search system InterProScan. Hence, protein domains for SPA antigen were predicted by InterProScan [14].

Epitopes prediction

Epitopes for SPA antigen were predicted by considering the properties of amino acid residues through available online web servers. The immune epitope database (IEDB) analysis resource system [15] was used to predict the epitopes by six different parameters. Six parameters applied here includes antigenicity, B-cell linear epitopes, surface accessibility, the number of beta turns, flexible residues, and hydrophilicity. The antigenic peptides were predicted by applying Kolaskar and Tongaonkar [16] antigenicity scale (threshold=1.00). It integrates the semi-empirical approach and uses physicochemical properties of amino acids for predictions. The hidden markov models and propensity scales were applied to identify the linear epitopes of B-Cell by Bepipred [17] (threshold=0.350). The Emini surface accessibility scale [18] was used in order to identify the residues that are accessible to solvent. The surface accessibility is calculated by the formula Sn = (i−1Π6δn+4+i) × $(0.37)^{-6}$, and when Sn value for hexapeptide is greater than 1 indicates the probability of surface accessible residues. The beta turns are predicted by Chou and Fasman [19]a semi-empirical method which takes into account the relative frequencies of amino acids. Karplus and Schulz scale [20] of flexibility (threshold=1) were applied predicting the flexible residues and this algorithm uses the knowledge of 31 proteins whose B-factor temperatures are derived. Hydrophobic and polar nature of amino acids were predicted by Parker Hydrophilicity Prediction [21] and the predictions by this scale relies on the experimental calculation of the retention time of a peptide while high-performance liquid chromatography (HPLC).

Peptide docking

The predicted epitopes were then subjected to peptide docking against human IgG protein (antibody). The peptide docking was performed using the peptide docking panel from Bioluminate module [22] of Schrodinger. In this panel, the grid for the antibody is specified by selecting the binding site residues, and the peptide sequence is uploaded. The grid calculations are performed by Glide [23] and the peptide is modeled by MacroModel and conformations are generated by ConfGen [24]. Prior to setting up docking, the antibody was prepared by subjecting it to a protein preparation wizard. The docking was performed by SP-peptide approach from a glide, and the poses were then scored by MMGBSA (Molecular Mechanics, the Generalized Born model, and Solvent Accessibility) [25].

Antibody-antigen docking

To interpret that the epitopes predicted are potentially capable of binding to the antibody, we have considered the protein structure of SPA. The PDB search for the SPA antigen has retrieved the partial structure of the protein, which includes two chains of IgG binding domain. The availability of 3D structure of antigen, we subjected IgG and SPA to ClusPro [26] and was set to antibody-antigen docking. The additional parameters like binding site amino acids were provided. ClusPro is a web server that provides a bench for different macromolecular dockings, like protein-protein, antibody-antigen, multimer docking, etc. ClusPro at its back end uses PIPER program which applies Fast Fourier Transform (FFT) correlation function with structure-based pairwise intermolecular potentials Decoys As the Reference State (DARS) [27]. The docking interactions were analyzed using Bioluminate module of Schrodinger and visualized by Chimera. Further, the binding residues of epitopes by peptide docking and SPA by antibody-antigen docking were compared, to predict the potential epitope.

RESULTS

The potential epitopes that can elicit the immune responses were predicted by B-Cell epitope prediction tool IEDB. We have employed about six different algorithms for the computational screening of epitopes in SPA antigen. All these algorithms predicted epitopes are spanning in different regions; the best-scored epitopes were selected. The domain analysis of SPA antigen has indicated to have five domains (table 1). The epitopes that could score well when all the six scale are considered were selected as potential epitopes. A continuous stretch of 100 residues from 330-420 amino acids has observed to be an epitope by all the scales, excluding the antigenicity property and lacks IgG binding domain. This region is made up of an octapeptide repeat whose function is not yet known and hence was discarded for further study. The regions that were found to be potential when all the scales considered were spanning in

between 80-100, 174-182, 187-196and 460-480 (fig. 2). The other setback was found with the peptide sequence region 460-480 as it is not the part of IgG binding domain and was also discarded in docking studies. When compared to the peptide sequences of 174-182 and 187-196 the peptide region of 80-100 have shown low levels of antigenicity. Hence, the final peptide sequences considered as epitopes were 174-182 and 187-196 (table 2).

Table 1: Domain analysis of SPA antigen

S. No.	Domains	Region	Function
1.	YSIRK Signaling Peptide	1-40	Substrate recognition
2.	IgG binding domain	41-330	Capable of binding Fab and Fc region of IgG antibody
3.	Octapeptide	331-420	Unknown
4.	LysM (lysine motif) domain	421-460	Peptidoglycan binding
5.	LPXTG domain	461-517	Cell wall anchoring

The table shows major portion of antigen constitutes of five repeats of IgG domain.

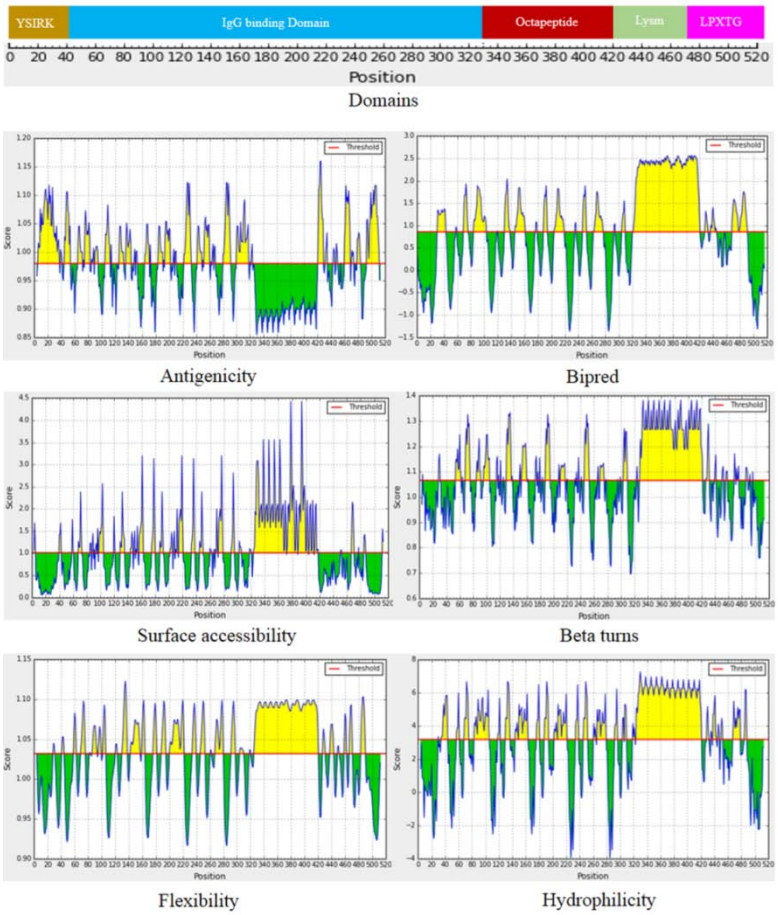

Fig. 2: Epitope prediction of SPA

Table 2: The best potential epitopes predicted

S. No.	Peptide sequence	Region
1.	NLNEEQRNG	174-182
2.	LKDDPSQSAN	187-196

The table 2 shows potential epitopes predicted based on the domains and considering different parameters like antigenicity, linear epitope, beta turns, hydrophilicity, surface accessibility and flexibility.

These predicted epitopes were subjected to peptide docking against the human IgG protein (PDB: 1HZH [28]). To the antibody, the grid box was prepared on one light chain (L) and one heavy chain (H). The peptide sequences were uploaded to peptide dock panel and were subjected sequential 3D generation of peptides followed by docking. The generated peptides were sequentially docked to the established grid of antibody and different poses were generated for each peptide. These poses were then scored on the basis of the MMGBSA, and the best-scored poses are given in table 3. The binding interactions of peptide and antibody were analyzed by protein-protein interaction analysis wizard (table 4). The intermolecular interactions between the peptide and antibody are non-covalent hydrogen bonds. The common interacting residues of the antibody with two peptides are Ser 94 and Glu 1 of the light chain (L) (fig. 3,4).

Table 3: Docking scores of best poses

S. No.	Peptide sequence	Docking score	Glide score	Prime energy	MMGBSA
1.	NLNEEQRNG	-5.278	-5.278	-54817.9	-70.60
2.	LKDDPSQSAN	-5.204	-5.204	-53831.2	-69.16

The table shows best pose for the two peptides based on MMGBSA and socking scores.

Table 4: Antibody-peptide interaction analysis

S. No.	Protein (Antibody)	Peptide (Epitope)	Interacting residues		Distance	Type of interaction
			Protein	Peptide		
1.	IgG-human	NLNEEQRNG	L: Ser 30	X: Asn 3	2.7	Hydrogen bond
			L: Ser 94	X: Arg 7	3.2	Hydrogen bond
			L: Glu 1	X: Asn 8	2.9	Hydrogen bond
			H: Ala 61	X: Gly 9	2.8	Hydrogen bond
2.	IgG-human	LKDDPSQSAN	L: Ser 95	X: Asp 4	2.8	Hydrogen bond
			L: Ser 94	X: Ser 6	2.7	Hydrogen bond
			L: Glu 1	X: Gln 7	2.8	Hydrogen bond
			H: Phe 45	X: Asn 10	2.9	Hydrogen bond

The table shows interactions between antibody and antigen were analyzed, indicating most of the interactions to be non-covalent hydrogen bonds.

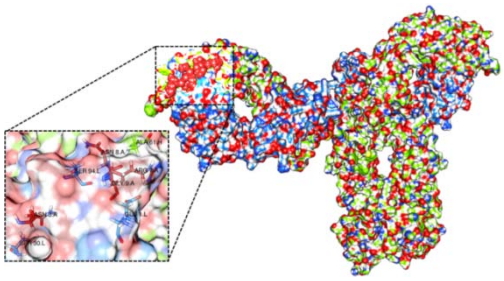

Fig. 3: Peptide docking interaction analysis of NLNEEQRNG against IgG

Heavy chains of IgG are represented in green and light chains in blue whereas epitope in brick red color

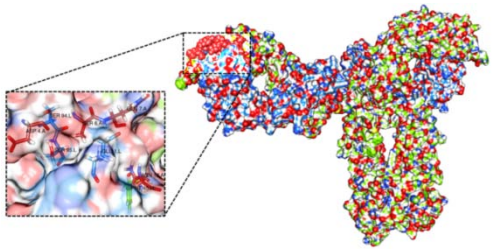

Fig. 4: Peptide docking interaction analysis of LKDDPSQSAN against IgG

Heavy chains of IgG are represented in green and light chains in blue whereas epitope in brick red color

To identify the potential epitope we have then considered the experimental 3D structure of the SPA antigen(PDB: 4NPF) [29]and subjected to antibody-antigen docking. The 3D structure of the protein 4NPF constitute of two chains belonging to the IgG domain. The sequence of 4NPF was manually observed and the epitopes were found on both the chains spanning between residues 22-28 (NLNEEQRNG) and 33-43 (LKDDPSQSAN) and hence only one chain was selected. The docking was performed using ClusPro using antibody mode. A cluster of 20 poses was generated with the similar kind of interaction. Of the cluster, the lowest energy pose-425.3 Kcal/mol was analyzed using protein interaction analysis wizard (table 5). Similarly, to peptide docking the antibody-antigen docking also show non-covalent hydrogen bonds between the interacting residues (fig. 5). The antigen interaction residues are similar to that of the peptide sequence NLNEEQRNG. The results here indicate that the NLNEEQRNG to be one of the potential epitope and the further studies could be undertaken with this epitope to develop a potential peptide-based vaccine.

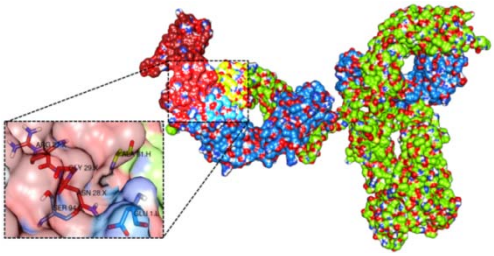

Fig. 5: Antibody-antigen docking interactions

Heavy chains of IgG are represented in green and light chains in blue whereas antigen in brick red color

Table 5: Antibody-peptide interaction analysis

S. No.	Interacting Residues		Distance	Type of interaction
	Antibody (1HZH)	Antigen (4NPF)		
1.	L: Ser 94	X: Arg 27	2.9	Hydrogen bond
	L: Glu 1	X: Asn 28	3.1	Hydrogen bond
	H: Ala 61	X: Gly 29	3.4	Hydrogen bond

The table shows interactions between antibody and antigen were analyzed, indicating most of the interactions to be non-covalent hydrogen bonds.

DISCUSSION

The identification of epitopes on the surface of antigens can be widely used in diagnostics and mainly in designing the peptide vaccines[30]. We have selected an IgG binding antigen for identification of epitopes which can elicit B-cell responses, as the identification of B-cell epitopes has been attracted by several scientists for developing promising vaccines [31]. The most vital

process in epitope-based vaccine design is a prediction of epitopes and mapping them on the surface of the antigen. In the present study, we have applied basic physicochemical properties of amino acids as parameters for predicting the epitopes.

The antigenicity scale was used to determine the antigenic sites as they can bind to the antibody and are capable of eliciting the immune responses [32]. We have identified the linear B-cell epitopes despite many of the B-cell epitopes are conformational because linear epitopes are easier to identify and synthesize[33]. In predictions, the hydrophobic scale and surface accessibility are considered as the preferable interaction of amino acids occur within hydrophobic residues and those which are solvent accessible [34]. Further, it is known that the epitopes are present in between the turns of two parallel arranged beta sheets [35] and hence were predicted. The flexibility of peptides was also predicted as they are important in attaining the secondary structure and allows the segmental motion in antigenic site to bind the antibody [36].

Two peptide sequences (table 2) were found to be potentially obeying all these parameters indicating that they might influence in eliciting the immune responses. The potency of predicted epitopes was analyzed by molecular docking strategies. To avoid the blind dock, the grid was prepared on the antibody by selecting one light chain and heavy as they serve as the binding sites for the antigen. The peptide docking results indicate that the peptide NLNEEQRNGserve as the potent epitopes than that of LKDDPSQSANas it has the least binding energy, dock score andMMGBSA score (table 3). Further to understand the best among them the experimental structure of the antigen (4NPF) was considered. The SPA protein has five small three-helix-bundle domains which are linked by flexible linkers [29]. The complete crystal structure of SPA is not available, the only experimental structure of SPA is PDB: 4NPF. This structure has two repeats of IgG binding domain as two chains. The antibody-antigen docking was carried out to understand the actual interacting residues. This analysis has indicated that the amino acid residues Arg 27, Asn 28, Gly29 were found to be interacting with the antibody (table 5). These residues are similar from the tail end of the peptide sequence NLNEEQRNG. These studies indicate that peptide sequence NLNEEQRNG as a potential epitope and further studies on it may lead to the development of an alternative treatment against the staph infections.

CONCLUSION

The present study was focused on the identification of potential B-cell epitope by immunoinformatics and molecular docking strategies against the SPA antigen. Two epitopes from the sequence of antigen were identified by six different basic physicochemical parameters. The docking studies suggest that the peptide sequence NLNEEQRNG of length nine residues may serve as the potent epitope in synthesizing a synthetic peptide vaccine. The current finding may further help the scientific communities and pharmacologists in developing a potent vaccine against staph infections.

CONFLICT OF INTERESTS

Declared none

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REFERENCES

1. Van Hal SJ, Jensen SO, Vaska VL, Espedido BA, Paterson DL, Gosbell IB. Predictors of mortality in staphylococcus aureus bacteremia. Clin Microbiol Rev 2012;25:362–86.
2. Yeaman MR, Filler SG, Schmidt CS, Ibrahim AS, Edwards JE, Hennessey JP. Applying convergent immunity to innovative vaccines targeting staphylococcus aureus. Front Immunol 2014;5:463.
3. Navarre WW, Schneewind O. Proteolytic cleavage, and cell wall anchoring at the LPXTG motif of surface proteins in gram-positive bacteria. Mol Microbiol 1994;14:115–21.
4. McDevitt D, Francois P, Vaudaux P, Foster TJ. Molecular characterization of the clumping factor (fibrinogen receptor) of Staphylococcus aureus. Mol Microbiol 1994;11:237–48.
5. Atkins KL, Burman JD, Chamberlain ES, Cooper JE, Poutrel B, Bagby S, *et al.* S. aureus IgG-binding proteins SpA and Sbi: host specificity and mechanisms of immune complex formation. Mol Immunol 2008;45:1600–11.
6. Graille M, Stura EA, Corper AL, Sutton BJ, Taussig MJ, Charbonnier JB, *et al.* Crystal structure of a staphylococcus aureus protein A domain complexed with the Fab fragment of a human IgM antibody: structural basis for recognition of B-cell receptors and superantigen activity. Proc Natl Acad Sci USA 2000;97:5399–404.
7. Romagnani S, Giudizi MG, del Prete G, Maggi E, Biagiotti R, Almerigogna F, *et al.* Demonstration on protein A of two distinct immunoglobulin-binding sites and their role in the mitogenic activity of Staphylococcus aureus Cowan I on human B cells. J Immunol 1982;129:596–602.
8. Dossett JH, Kronvall G, Williams RC, Quie PG. Antiphagocytic effects of staphylococcal protein A. J Immunol 1969;103:1405–10.
9. Flower DR. Designing immunogenic peptides. Nat Chem Biol 2013;9:749–53.
10. Hailemichael Y, Overwijk WW. Peptide-based anticancer vaccines: the making and unmaking of a T-cell graveyard. Oncoimmunology 2013;2:e24743.
11. Sause WE, Buckley PT, Strohl WR, Lynch AS, Torres VJ. Antibody-based biologics and their promise to combat staphylococcus aureus infections. Trends Pharmacol Sci 2015;37:231–41.
12. Tatusova T, Ciufo S, Fedorov B, O'Neill K, Tolstoy I. Ref Seq microbial genomes database: new representation and annotation strategy. Nucleic Acids Res 2014;42:D553–9.
13. Pruitt K, Brown G, Tatusova T, Maglott D. The reference sequence (RefSeq) Database [Internet]. National Center for Biotechnology Information (US); 2012. Available: http:// www.ncbi.nlm.nih.gov/books/NBK21091. [Last accessed on 01 Feb 2015].
14. Mitchell A, Chang HY, Daugherty L, Fraser M, Hunter S, Lopez R, *et al.* The InterPro protein families database: the classification resource after 15 y. Nucleic Acids Res 2014;43:D213–21.
15. Vita R, Overton JA, Greenbaum JA, Ponomarenko J, Clark JD, Cantrell JR, *et al.* The immune epitope database (IEDB) 3.0. Nucleic Acids Res 2015;43:D405–12.
16. Kolaskar AS, Tongaonkar PC. A semi-empirical method for prediction of antigenic determinants on protein antigens. FEBS Lett 1990;276:172–4.
17. Larsen JEP, Lund O, Nielsen M. Improved method for predicting linear B-cell epitopes. Immunome Res 2006;2:2.
18. Emini EA, Hughes JV, Perlow DS, Boger J. Induction of hepatitis a virus-neutralizing antibody by a virus-specific synthetic peptide. J Virol 1985;55:836–9.
19. Chou PY, Fasman GD. Prediction of the secondary structure of proteins from their amino acid sequence. Adv Enzymol Relat Areas Mol Biol 1978;47:45–148.
20. Karplus PA, Schulz GE. Prediction of chain flexibility in proteins. Naturwissenschaften 1985;72:212–3.
21. Parker JM, Guo D, Hodges RS. New hydrophilicity scale derived from high-performance liquid chromatography peptide retention data: correlation of predicted surface residues with antigenicity and X-ray-derived accessible sites. Biochemistry 1986;25:5425–32.
22. Zhu K, Day T, Warshaviak D, Murrett C, Friesner R, Pearlman D. Antibody structure determination using a combination of homology modeling, energy-based refinement, and loop prediction. Proteins 2014;82:1646–55.
23. Friesner RA, Banks JL, Murphy RB, Halgren TA, Klicic JJ, Mainz DT, *et al.* Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. J Med Chem 2004;47:1739–49.
24. Watts KS, Dalal P, Murphy RB, Sherman W, Friesner RA, Shelley JC. ConfGen: a conformational search method for efficient

generation of bioactive conformers. J Chem Inf Model 2010;50:534–46.
25. Tubert-Brohman I, Sherman W, Repasky M, Beuming T. Improved docking of polypeptides with Glide. J Chem Inf Model 2013;53:1689–99.
26. Comeau SR, Gatchell DW, Vajda S, Camacho CJ. ClusPro: an automated docking and discrimination method for the prediction of protein complexes. Bioinformatics 2004;20:45–50.
27. Kozakov D, Brenke R, Comeau SR, Vajda S. PIPER: an FFT-based protein docking program with pairwise potentials. Proteins 2006;65:392–406.
28. Saphire EO, Parren PW, Pantophlet R, Zwick MB, Morris GM, Rudd PM, *et al.* Crystal structure of a neutralizing human IGG against HIV-1:a template for vaccine design. Science 2001;293:1155–9.
29. Deis LN, Pemble CW, Qi Y, Hagarman A, Richardson DC, Richardson JS, *et al.* Multiscale conformational heterogeneity in staphylococcal protein a: possible determinant of functional plasticity. Structure 2014;22:1467–77.
30. Schmidt MA. Development and application of synthetic peptides as vaccines. Biotechnol Adv 1989;7:187–213.
31. Wang JG, Jansen RW, Brown EA, Lemon SM. Immunogenic domains of hepatitis delta virus antigen: peptide mapping of epitopes recognized by human and woodchuck antibodies. J Virol 1990;64:1108–16.
32. Gavin AL, Hoebe K, Duong B, Ota T, Martin C, Beutler B, *et al.* Adjuvant-enhanced antibody responses in the absence of toll-like receptor signaling. Science 2006;314:1936–8.
33. Flower DR. Immunoinformatics and the *in silico* prediction of immunogenicity. An introduction. Methods Mol Biol 2007;409:1–15.
34. Jha AN, Vishveshwara S, Banavar JR. Amino acid interaction preferences in proteins. Protein Sci 2010;19:603–16.
35. Schulze-Gahmen U, Prinz H, Glatter U, Beyreuther K. Towards assignment of secondary structures by anti-peptide antibodies. Specificity of the immune response to a beta-turn. EMBO J 1985;4:1731–7.
36. Fieser TM, Tainer JA, Geysen HM, Houghten RA, Lerner RA. Influence of protein flexibility and peptide conformation on the reactivity of monoclonal anti-peptide antibodies with a protein alpha-helix. Proc Natl Acad Sci USA 1987;84:8568–72.

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