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Computational Approaches to Identify Common Subunit Vaccine Candidates against Bacterial Meningitis

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Abstract: Bacterial meningitis, an infection of the membranes (meninges) and cerebrospinal fluid (CSF) surrounding the brain and spinal cord, is a major cause of death and disability all over the world. From perinatal period to adult, four common organisms responsible for most of the bacterial meningitis are *Streptococcus pneumonia, Neisseria meningitidis, Haemophilus influenza* and *Staphylococcus aureus*. As the disease is caused by more organisms, currently available vaccines for bacterial meningitis are specific and restricted to some of the serogroups or serotypes of each bacterium. In an effort to design common vaccine against bacterial meningitis, proteomes of the four pathogens were compared to extract seven common surface exposed ABC transporter proteins. ProPred server was used to investigate the seven surface exposed proteins for promiscuous T-cell epitopes prediction. Predicted 22 T-cell epitopes were validated through published positive control, SYFPEITHI and immune epitope database to reduce the epitope dataset into seven. T-cell epitope 162-FMILPIFNV-170 of spermidine/putrescine ABC transporter permease (potH) protein was conserved across the four selected pathogens of bacterial meningitis. Hence, structural analysis was extended for epitope 162-FMILPIFNV-170. Crystal structures of HLA-DRB alleles were retrieved and structure of potH was modeled using Prime v3.0 for structural analysis. Computational docking of HLA-DRB alleles and epitope 162-FMILPIFNV-170 of potH was performed using Glide v5.7. RMSD and RMSF of simulation studies were analyzed by Desmond v3.2. The docking and simulation results revealed that the HLA-DRB-epitope complex was stable with interaction repressive function of HLA. Thus, the epitope would be ideal candidate for T-cell driven subunit vaccine design against bacterial meningitis.
Key words: bacterial meningitis, T-cell epitope, MHC class II molecule, subunit vaccine, epitope based docking, molecular dynamics.

1 Introduction

Meningitis is inflammation of the protective membranes covering the brain and spinal cord, collectively known as the meninges (Saez-Llorens *et al.*, 2003). The inflammation is generally caused by infection of viruses or bacteria (Ginsberg, 2004). Viral meningitis is generally less severe and resolves without specific treatment, while bacterial meningitis can be quite severe and may result in brain damage, hearing loss or learning disability (Tunkel *et al.*, 2004).

Bacterial meningitis is a common disease with high morbidity and mortality rate with one million cases and 200,000 deaths annually (World Health Organization, 1999). Case-fatality rates vary with age at the time of illness and the species of bacterium causing infection, typically range from 3% to 19% in developed countries while higher case-fatality rates (37-60%) have been reported in developing countries (Centers for Disease Control, 1998). Bacterial meningitis spreads through respiratory tract (Tunkel *et al.*, 2004). Bacteria reach the central nervous system (CNS) either by hematogenous spread or by direct extension from a contiguous site. In neonates, pathogens are acquired from nonsterile maternal genital secretions. In infants, children and adults, organisms that cause bacterial meningitis colonize the upper respiratory tract. Direct inoculation of bacteria into the CNS can result from trauma, skull defects with CSF (Cerebrospinal fluid) leaks, congenital dura defects such as a dermal sinuses or meningomyelocele, or extension from a suppurative parameningeal focus (Saez-Llorens and McCracken, 2003). After bacteremia, pathogens penetrate the blood-brain barrier (BBB) to enter the subarachnoid space and lead to inflammation of the brain and spinal cord (Huang and Jong, 2001). Up to 54% of survivors are left with

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disability due to bacterial meningitis, which includes deafness, mental retardation, neurological sequelae, inflammatory response of sub-arachnoid and ventricular space, breakdown of the BBB, subsequent brain edema and vasculitis of the blood vessels (Tauber *et al.*, 1997).

Causative organisms of bacterial meningitis differ with age groups. *Streptococcus pneumonia*, *Neisseria meningitides*, *Haemophilus influenza*, *Streptococcus agalactiae* (group B Streptococci), *Escherichia coli*, *Listeria monocytogenes* and *Staphylococcus aureus* are common in infants. In elderly individuals *Streptococcus pneumonia*, *Neisseria meningitides*, *Haemophilus influenza*, *Streptococcus aureus*, coagulase-negative staphylococci, aerobic Gram-negative bacilli, *Pseudomonas aeruginosa* and *Propionibacterium acnes* are cause of bacterial meningitis. The common pathogens causing bacterial meningitis in infants and adults are *Streptococcus pneumonia*, *Neisseria meningitidis*, *Haemophilus influenza*, *Staphylococcus aureus etc.* (Schlech *et al.*, 1985; Bandaru *et al.*, 1998). Ten years retrospective study in South India (Mani *et al.*, 2007) and patient records in Sri Venkateswara Institute of Medical Sciences (SVIMS hospital, Tirupati) of Rayalaseema region also reported that these organisms were the most common in bacterial meningitis.

Hib (Haemophilus Influenzae Type B) conjugate, PCV (Pneumococcal conjugate vaccine), meningococcal polysaccharide and conjugate vaccines (MCV4 and MPSV4) against bacterial meningitis are currently available (Ramakrishnan *et al.*, 2009), but the protection afforded by each vaccine is specific to each bacterium and restricted to some of the serogroups or serotypes of each bacterium (World Health Organization, 1999). These organisms are more sensitive to penicillin, vancomycin and resistant to the drugs like ofloxacin, cotrimxazole, cefrotaxime, ceftriaxone trtracycline respectively (Mani *et al.*, 2007). Therefore, it is imperative to design common vaccines for organisms causing bacterial meningitis.

The genome and proteome information available allows studying vaccine development through computational approach, without cultivating the pathogens, known as reverse vaccinology. Potential surface-exposed proteins can be identified in a reverse manner, through a computer aided protocol starting from the genome rather than from the microorganism (Rappuoli, 2001). The comparison of multiple strains of a single species has resulted in the definition of the species pangenomic reverse vaccinology (Tettelin, 2009). These approaches highlight the potential of looking at more than one genome of bacterial species to overcome the problems represented by gene presence and variability (Mora *et al.*, 2005; Serruto *et al.*, 2006). In light of this knowledge, our research group worked on the idea of comparative proteome analysis of several pathogens (of different genus group) causing a single disease to find common surface exposed proteins and explore these proteins for T-cell driven subunit vaccine design (Rakesh *et al.*, 2009; Umamaheswari *et al.*, 2011).

The T-cell immune responses are driven by the recognition of peptide antigens (T-cell epitopes) that are bound to MHC molecules thus contingent on several events, including appropriate and effective processing of the peptide from its protein source, stable peptide binding to the MHC molecule and recognition of the MHC-bound peptide by the T-cell receptor. MHC-peptide binding is the most selective event that determines T-cell epitopes. The T-cell epitopes are usually between 8 and 10 amino acids in length (Oftung *et al.*, 1997), and such peptides could easily be synthesized *in vitro* to overcome the problems associated with full-length proteins (Mustafa and Shaban, 2006). Surface-located proteins are favored for T-cell driven subunit vaccine development as they are the most easily accessible to host the immune system (Zagursky and Russell, 2001; Serruto *et al.*, 2009; Rakesh *et al.*, 2009). Therefore, prediction of MHC-peptide binding constitutes the principal basis for anticipating potential T-cell epitopes. The tremendous relevance of epitope identification in vaccine design and in the monitoring of T-cell responses has spurred the development of many computational methods (Singh and Raghava, 2001; Rammensee *et al.*, 1999; Peters *et al.*, 2005) for predicting MHC-peptide binding that improves the efficiency and economics of T-cell epitope identification.

T-cell epitope driven vaccine was successfully pursued in *Leptospira interrogans* (Rakesh *et al.*, 2009; Umamaheswari *et al.*, 2011), *Chlamydia trachomatis*, *Chlamydia pneumoniae* (Barker *et al.*, 2008) and *Mycobacterium tuberculosis* (Mwangi *et al.*, 2007; Panigada *et al.*, 2002). In the present study, proteome of pathogens of bacterial meningitis were selected and T-cell epitope binding affinity was explored at sequence level and structure level.

2 Methodology

2.1 Comparative proteomic analysis

The complete proteome of four common pathogens of bacterial meningitis (adult and pediatric) *Streptococcus pneumonia*, *Neisseria meningitidis*, *Haemophilus influenza*, *Staphylococcus aureus* were retrieved from the institute of genomic research comprehensive microbial resource (TIGR CMR) at http://tigr.org/. *Streptococcus pneumonia* was selected as reference organism, as it is the most predominant pathogen of bacterial meningitis (Bharathi *et al.*, 2003; Mani *et al.*, 2007) and identifies homologous proteins from common pathogens of bacterial meningitis. The common surface exposed proteins (homologous proteins) of the selected organisms were screened for further studies.

2.2 Identification of T-cell epitopes

The surface exposed protein dataset was analyzed to predict T-cell epitopes using ProPred server (Singh and Raghava, 2001). ProPred server uses physico-chemical properties of amino acids to determine antigen region in the membrane proteins and is also used for the prediction of MHC class II HLA-DRB allele binding regions in the identified antigenic regions (Das and Deepika, 2008). ProPred was derived from the TEPITOPE, a matrix based computer program. It was developed for 51 HLA-DR alleles by extracting the matrices from pocket profile database. The server employs amino acid position coefficient deduced from literature by linear prediction model (Wang *et al.*, 2008). A threshold of 3% was fixed to reduce the rate of false positives.

In ProPred, the T-cell epitope prediction from surface exposed proteins were restricted to ten HLA-DR alleles (HLA-DRB1*0101, HLA-DRB1*0301, HLA-DRB1*0401, HLA-DRB1*0701, HLA-DRB1*0801, HLA-DRB1*1101, HLA-DRB1*1301, HLA-DRB1*1302, HLA-DRB1*1501 and HLA-DRB5*0101) (Barker *et al.*, 2008; Umamaheswari *et al.*, 2011) due to their inclusion in an HLA-DR supertype (Southwood *et al.*, 1998) or their inclusion in other promiscuous epitope studies (Texier *et al.*, 2000; Panigada *et al.*, 2002; Doolan *et al.*, 2000). Average ProPred scores were calculated for the epitopes with good binding affinity with ten selected HLA-DRB alleles and validated based on average ProPred score of *Influenza haemagglutinin* epitope (HA307-PKYVKQNTLKLAT-319) which is cited and used as a positive T-cell epitope control (Barker *et al.*, 2008; Panigada *et al.*, 2002). Subsequently, independent algorithms such as SYFPEITHI (http://www.syfpeithi.de/) (Rammensee *et al.*, 1999) and immune epitope database (IEDB) (Peters and Sette, 2007; Peters *et al.*, 2005) were employed to verify the validity of predicted T-cell epitopes.

Further, multiple sequence alignment was performed to identify putative subunit vaccine peptides using CLUSTAL X (Thompson *et al.*, 1997) and to discover the conserved epitopes among selected common pathogens of bacterial meningitis. The T-cell epitope 162-FMILPIFNV-170 from spermidine/putrescine ABC transporter permease (potH) of *Streptococcus pneumonia* was identified to be conserved across four selected pathogens of bacterial meningitis. The conserved epitope structure was modeled by using prime, computational docking analysis using Schrodinger 2011 software suite and cross checked with Hex 6.3.

2.3 Binding of T-cell epitope with HLA-DRBs

2.3.1 T-cell epitope structures

Comparative protein modeling method using Prime v3.0 was used to predict 3D structure of spermidine/putrescine ABC transporter permease (potH) of *Streptococcus pneumonia*. 'Search' option of Prime was implemented with BLAST (Altschul *et al.*, 1997) web server against the PDB to obtain high resolution crystal structure of 3DHW as structural template (Supplementary Fig. 1). Target-template alignment was constructed including secondary structural elements in Prime. "Build structure" of Prime option was used to predict the potH structure with parameters omitting structural discontinuities of more than 20 residues and to optimize the side chains of the protein model. The potH protein model was generated and quality evaluation was done on the basis of Ramachandran plot using PROCHECK (Laskowski *et al.*, 1993). The structures of HLA-DRBs and T-cell epitope were prepared before docking as it is important to obtain reliable binding interaction.

2.3.2 Preparation of HLA-DRB structures

The protein data bank (PDB) was explored to retrieve experimental structures of ten HLA-DRBs. Eleven alleles of five HLA-DRBs (HLA-DRB1*0101, HLA-DRB1*0301, HLA-DRB1*401, HLA-DRB1*1501 and HLA-DRB5*0101) (Supplementary Table 2) were available in PDB (Umamaheswari *et al.*, 2011). Therefore, structural analysis of T-cell epitope was restricted to eleven crystal structure of five HLA-DRBs. The HLA-DRBs were prepared using protein preparation wizard of Schrodinger 2011. During preparation all hydrogens were added; orientation of hydroxyl groups, Asn, Gln and protonation state of His were optimized; constrained refinement was performed with the *impref* utility, setting the maximum RMSD of 0.30 Å. The *impref* utility consists of a cycle of energy minimizations based on the *impact* molecular mechanics engine and on the OPLS_2005 force field (Schrödinger, LLC, New York). The first minimization was performed constraining the heavy atoms with the hydrogen torsion parameters turned off, to allow free rotation of the hydrogens. Subsequently minimizations were performed gradually decreasing the constraints on the positions of the heavy atoms. A grid was generated on the prepared protein structures by selecting peptide binding site residues in B chain of HLA-DRBs (within 4 Å residues) and excluding co-crystallized peptide, with bounding box dimensions set to 20 Å × 20 Å ×20 Å (Friesner *et al.*, 2004; Halgren *et al.*, 2004).

2.3.3 Preparation of T-cell epitope

The T-cell epitope 162-FMILPIFNV-170 (ninemer) peptide was extracted from potH homology model and defined as ligand. Multiple conformations of the peptide were generated at pH of 7.0 ± 2.0. OPLS_2005 force field was used for each state of peptide to clean the structure, minimize conformation, design high quality 3D structure and multiple protonation/tautomerization states (Brooks *et al.*, 2008).

2.3.4 Docking of T-cell epitope with HLA-DRB

Flexible docking was performed using the extra

precision (XP) feature of Glide module (Friesner *et al.*, 2006). The van der Waals radii scaling factor of 0.80 and partial cutoff charge of 0.15 was set to decrease the penalties for close contacts. The core pattern comparison and similarity mode were not used since the aim was to study the binding of T-cell epitope to the peptide binding site of HLA-DRB. The XP GScore, H-bonds and van der Waals contacts of the docking complexes were visualized using default settings of Maestro v9.2.

2.4 Molecular dynamic simulations

The molecular dynamic simulations have provided significant new information on the nature of proteins. RMSD measures the accuracy and dynamic fluctuations (RMSF) of proteins around their average conformations, acts as an important indicator of many biological processes in complex formations. Molecular dynamic simulations were conducted with Desmond V3.2 utilizing the 3D structure of HLA-DRB-peptide docked complex with the OPLS_2005 force field (Jorgensen *et al.*, 1996; Kaminski *et al.*, 2001). The complex was solvated in a simple point charge (SPC) (Essmann *et al.*, 1995) water box with a 10 Å buffer using the 'System Builder' module in Maestro, resulting in simulation systems of 10,8160 atoms respectively. The solvated system was relaxed through multistage protocol starting with restrained minimization using a force constant of 50.0 kcal/mol Å applied on solute heavy atoms. The successive simulation heat the system from 10 to 300 K while maintaining the restraint on solute heavy atoms followed by a final 10 ps preproduction simulation run at 300 K using periodic boundary conditions at a constant pressure of 1 atm using the Martyna-Tobias-Klein barostat method. Finally, the simulations were run for 1 ns each at 300 K using the Nose-Hoover thermostat method. Van der Waals and short range electrostatic interactions were cut off at 9 Å, and the long range electrostatic interactions were computed using the particle mesh Ewald method. 5 ns MD simulations were performed with a 2 fs time step at the isothermic-isobaric (NTP) canonical ensemble and under the periodic boundary conditions, and conformations were saved every 4.8 ps, accounting for a total of 5,000 conformers. The root mean square deviation (RMSD) and root mean square fluctuation (RMSF) from the MHC-peptide complex was monitored during the molecular dynamic simulation studies.

3 Results and discussion

3.1 Prediction of T-cell epitope

The available proteome of the pathogens and comparative proteomic tools open up opportunities to explore common proteins of several pathogens causing a disease. These sorts of analysis could be directed at designing common vaccine candidates for several pathogens in a reverse manner, a step ahead from pan-genome reverse vaccinology. In contrast to pan-genomic approach (Serruto *et al.*, 2009), comparing multiple genomes from the same bacterial species will identify gene presence and variability between isolates of the species. In the present study, homologous sequences from several pathogens belonging to different disease causing genera were explored instead of different strains of single pathogens for common subunit vaccine identification.

The idea of designing T-cell based common subunit vaccine by exploring proteome of four pathogenic genus groups was implemented in the study. 250 homologous common proteins were identified by comparative analysis of four common pathogens of bacterial meningitis at TIGR CMR. Out of them seven proteins were found to be surface exposed ABC transporter proteins (Table 1). The ABC transporter proteins constitute a large gene family that uses the energy from ATP hydrolysis to translocate a wide variety of substances across biological membranes (Choudhuri *et al.*, 2006). ABC transporter proteins are predominantly involved in nutrient uptake, although they also participate in the export of bacterial toxins and harmful substances, contributing to bacterial multidrug resistance (Locher*et al.*, 2002).

Table 1 Identification of novel common T-cell epitopes from four bacterial meningitis pathogens

Sl. No.	Name of bacterial meningitis pathogen	No. of gene products	No. of Common proteins	No. of ABC transporter proteins
1	*Streptococcus pneumoniae* TIGR4	2234	250	7
2	*Staphylococcus aureus* subsp. *aureus* MRSA	1059		
3	*Haemophilus influenzae* KW20 Rd	478		
4	*Neisseria meningitidis* MC58	462		

The seven ABC transporter proteins from the reference organism *Streptococcus pneumonia* were analyzed using ProPred with ten selected HLA-DRB alleles to check their HLA-DRB binding affinity and subsequently to predict efficient epitopes based on ProPred score (Table 2). Twenty-two T-cell epitopes were determined from seven ABC transporter proteins with positive binding affinity towards all ten selected HLA-DRBs. Further, a screening parameter based on average ProPred score of a published positive control (34.05) (Barker *et al.*, 2008; Panigada *et al.*, 2002) was used to select more effective epitope candidates. Seven T-cell epitopes had better binding affinity towards HLA-DRBs than the positive control. These seven epitopes also showed good binding affinity towards HLA-DRBs using SYFPEITHI and IEDB. It confirmed that

Table 2 Predicted common novel T-cell epitopes of ABC transporter proteins

No.	ABC transporter proteins	UniProt ID	Ninemer sequence	Avg. ProPred score
1	Excinuclease ABC subunit A (uvrA)	P63384	LRIAEGYVI	**35.756**
			LRLLYARVG	32.083
			LQILAPVIR	28.551
			LVIEHNLDV	28.757
			YVIIDTMDD	**41.628**
			IVIHGARAH	33.036
			IRLATQIGS	29.714
			IRLATQIGS	31.177
2	ABC transporter ATP-binding protein	Q97QX3	No epitopes	–
3	ABC transporter ATP-binding protein	Q97QT5	LRKMNAVAI	**34.187**
			MIILQANKI	**35.321**
			MRQIESLEA	30.718
			FVSHDRYFI	31.494
			INRVATHVL	17.198
			VRASTTKRA	18.107
4	Excinuclease ABC subunit B (uvrB)	Q54986	LVIAHNKTL	29.39
			IVVASVSCI	28.025
			VVASVSCIY	14.877
5	Spermidine / putrescine ABC transporter permease protein (potH)	Q97Q43	FVLAPLVLI	**34.969**
			FMILPIFNV	**36.969**
			VILILTMFI	**39.214**
6	Amino acid ABC transporter ATP-binding protein	Q97PY0	VALARALAM	17.523
7	Manganese ABC transporter ATP-binding protein (psaB)	Q97PG9	VLIARCLVQ	26.357

the seven predicted epitopes were true irrespective of the types of algorithm used. Spermidine/putrescine ABC transporter permease protein (potH) was found to have three epitopes, while ABC transporter ATP-binding protein and Excinuclease ABC subunit A protein (urvA) had two antigenic peptides. Each of the seven epitopes had good correlation in term of HLA-DRB binding to previously published subunit vaccines (Barker *et al.*, 2008; Panigada *et al.*, 2002). Therefore, the seven epitopes have the potential to act as potent subunit vaccine against bacterial meningitis.

Multiple sequence alignment of all seven epitopes across four selected bacterial meningitis pathogens was performed and the only one epitope 162-FMILPIFNV-170 was found to be 100% similarity (Fig. 1). Sequence based studies have been reported recently for potential subunit vaccine design against bacterial pathogens (Mustafa *et al.*, 2006; Dass and Deepika, 2008). In our previous works on leptospirosis we extended reverse vaccinology approach into structure level analysis by implementing computational program such as Modeller 9v7 and Hex v6.3 for epitope modeling and protein-protein interaction studies respectively (Umamaheswari *et al.*, 2011). In the present study, the work was also extended to structural level analysis of epitope peptide and protein HLA-DRBs in Schrodinger 2011 work environment, where Prime was used for epitope structure modeling and Glide, an improved algorithm used for protein-peptide docking.

3.2 Structure of T-cell epitope

Prime based homology model of potH (Fig. 2) was validated using PROCHECK and 97.6% residues were found in allowed regions (Supplementary Fig. 2). Importantly, all residues of epitopes 162-FMILPIFNV-170 were present in most favorable region of Ramachandran plot (Supplementary Fig. 3). Therefore, the predicted epitope structure is of good quality for structural analysis.

3.3 Protein-epitope docking

The docking complex of T-cell epitope was found to interact well with HLA-DRB alleles (HLA-DRB1*0101, HLA-DRB1*0301, HLA-DRB1*0401, HLA-DRB1*1501 and HLA-DRB5*0101) by forming hydrogen bonds with good binding energies (Supplementary Table 2). Three parameters were considered, *i.e.* XP-Gscore, H-bonds and van der Waals (vdW) interactions. On the basis of these parameters the binding affinity of epitope towards HLA-DRB protein was discussed. The more negative value of XP-Gscore indicates good binding affinity of the epitope with protein. More H-bonds and good vdW interactions strengthen the affinity of docking complex with proper binding orientation.

Epitope of 162-FMILPIFNV-170 docking with HLA-DRB1*0101 (2G9H) shows the highest affinity and the least docking score of −11.615 kcal/mol (Fig. 3) and HLA-DRB1*1501 (1BX2) shows docking score of −7.239 kcal/mol followed by HLA-DRB1 * 0101 (1AQD, −11.373 kcal/mol), HLA-DRB1 * 0101 (1T5W, −10.869 kcal/mol), HLA-DRB5 * 0101 (1H15, −10.784 kcal/mol), HLA-DRB1 * 0101 (1SJE, −10.392 kcal/mol), HLA-DRB5*0101 (1FV1, −9.782 kcal/mol), HLA-DRB1 * 0101 (1KLG, −9.364 kcal/mol), HLA-DRB1 * 0101 (2FSE, −8.771 kcal/mol), HLA-DRB1*0401 (2SEB, −7.770 kcal/mol), HLA-DRB1*0301 (1A6A, −7.729 kcal/mol) and HLA-DRB1 * 1501 (1BX2, −7.239 kcal/mol) (Table 3). The efficiency of peptide to interact with HLA-DRB alleles with low free energy justifies its ability as a T-cell driven subunit vaccine against bacterial meningitis.

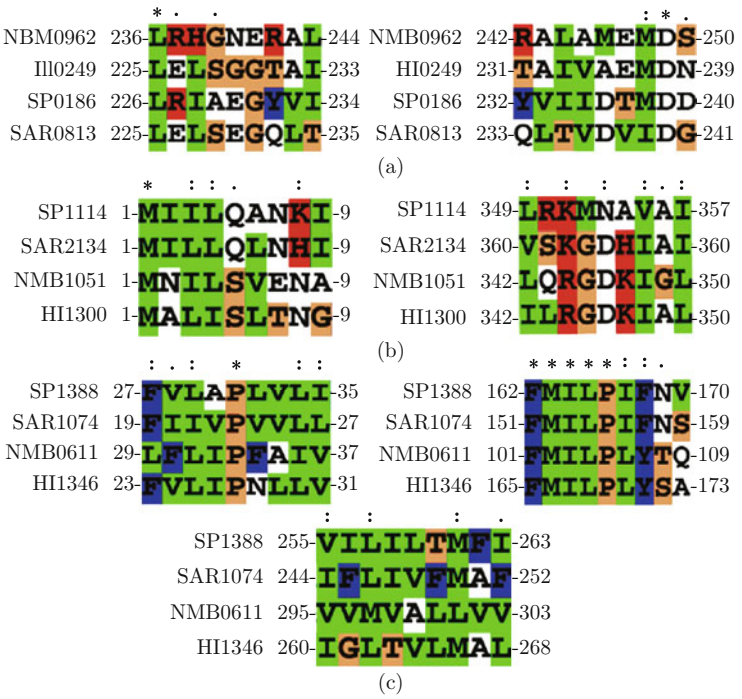

Fig. 1 Multiple sequence alignment of seven epitopes. (a) Excinuclease ABC subunit A protein (urvA). (b) ABC transporter ATP-binding protein. (c) Spermidine/putrescine ABC transporter permease protein (potH).

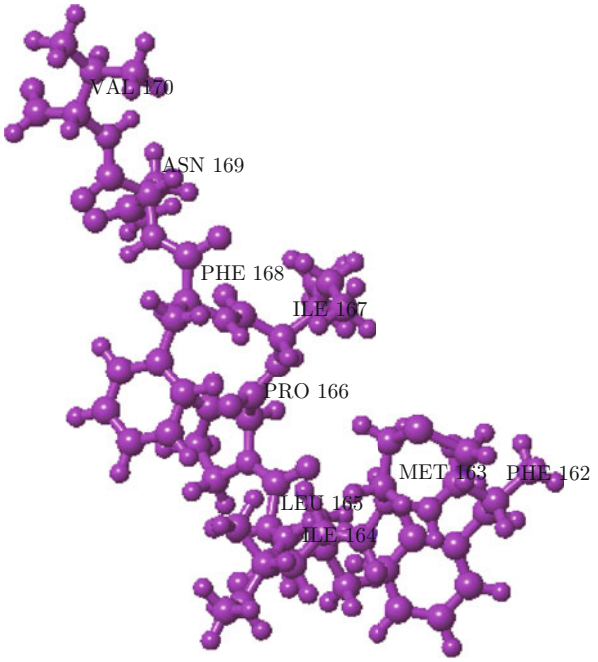

Fig. 2 Predicted 3D structure of potH ninemer (162-FMILPIFNV-170).

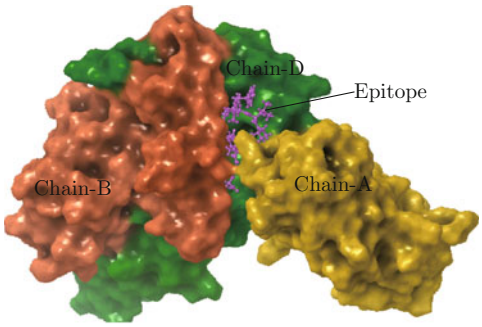

Fig. 3 Docking complex of HLA-DRB1*0101 with potH ninemer.

3.4 Validation of docking results

Analysis of epitope of potH with HLA-DRB*101 (2G9H) docking complex revealed that the residues Phe32, Trp43, Ala52, Ser53, Phe54, Gln9, Val65, Ala61, Glu11, Gly58, Asn62, Phe22, Phe24 of A chain (Supplementary Table 2A), Val85, Asn82, His81, Thr77, Ala74, Gln70, Asp66, Arg71, Leu67, Gln64, Tyr60, Thr47, Trp61, Cys30, Trp9, Leu11, Phe13, Tyr78, Glu28 of B chain (Supplementary Table 2B), and Trp100, His105, Asn98, Thr107, Lys106, Lys104 (Supplementary Table 2C) of D chain had participated in the vdW interactions (Fig. 4). Epitope 162-FMILPIFNV-170 formed four hydrogen bonds with HLA-DRB*101 B chain (Glu28, Arg71, Asn82, and Thr77) and one hydrogen bond with HLA-DRB*101 D chain (Asn98) (Fig. 4). Analysis of original HLA-DRB*101 (2G9H) - peptide crystal structure corroborated well with HLA-DRB*101 (2G9H) and epitope 162-FMILPIFNV-170 docking complex with similar vdW interaction and hydrogen bond.

The HLA-DRB protein-epitope docking was further performed using Hex6.3 (Singh *et al.*, 2009) to com-

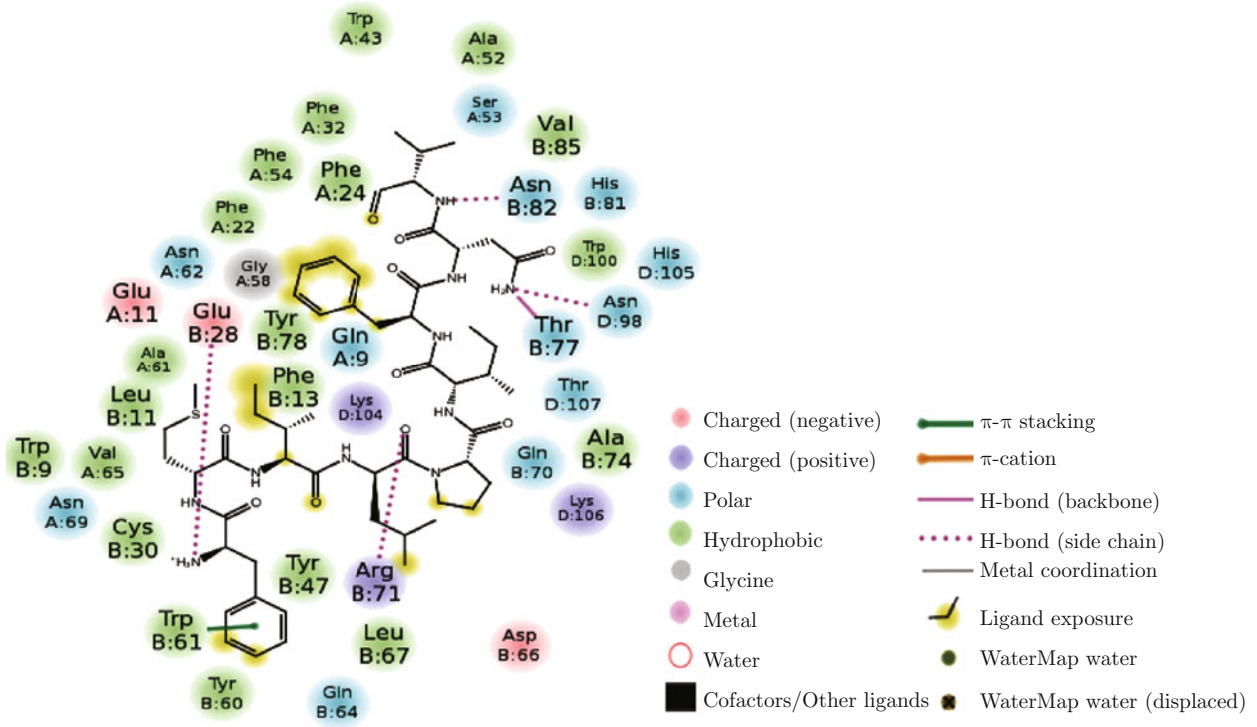

Fig. 4 Interactions of HLA-DRB1*0101 to ninemer.

Table 3 Docking scores

HLA-DRBs	HLA-DRB PDB ID	XP G score (kcal/mol)
HLA-DRB1* 0101	1SJE	−10.392
	2FSE	−8.771
	1KLG	−9.364
	2G9H*	**−11.615**
	1AQD	−11.373
	1T5W	10.869
HLA-DRB1* 0301	1A6A	−7.729
HLA-DRB1* 0401	2SEB	−7.770
HLA-DRB1* 1501	1BX2	−7.239
HLA-DRB5* 0101	1FV1	−9.782
	1H15	−10.784

pare binding orientations of HLA-DRBs with epitope 162-FMILPIFNV-170 docking complex using different docking algorithms. The result is encouraging with docking score of -371.80 kJ/mol and forming three hydrogen bonds with Arg71, Asn82 of B chain, and Asn98 of D chain. The binding orientation from hex results coincides well with HLA-DRB*101 (2G9H) - peptide co-crystal structure and epitope 162-FMILPIFNV-170 docking complex obtained through Glide, with similar hydrogen bonding pattern and good vdW interaction. In each case, the epitope 162-FMILPIFNV-170 formed hydrogen bonds with Arg71 and Asn82 of B chain of HLA-DRB1*0101.

3.5 Structural fluctuations

The changes in structural conformation were monitored in terms of RMSD and RMSF. The characterization of HLA-DRB*101 (2G9H) - epitope complex was analyzed by simulation studies. The time-dependent RMSD values for the HLA-DRB1*0101 backbone atoms and the heavy atoms of epitope during the production phase relative to the starting structures

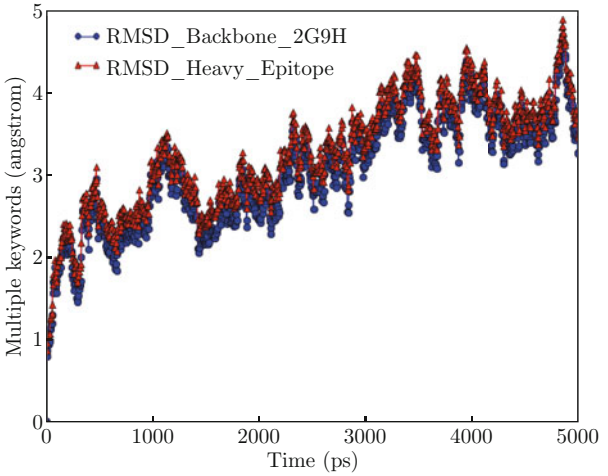

Fig. 5 RMSD of HLA-DRB1*0101 backbone atoms and epitope (162-FMILPIFNV-170) atoms with respect to time over the course of the 5000 ps MD simulation run.

were determined and plotted (Fig. 5). The average of RMSD for backbone atoms was 3.24 Å and for heavy atoms was 3.04 Å. The RMSF values of backbone and side chain atoms were assessed for each residue of HLA-DRB1*0101 (Fig. 6). The side chain and backbone fluctuations of residues were observed with average of 3.66 Å, and 3.44 Å respectively. The trajectory of RMSD and RMSF plots shows that the energy HLA-DRB*101 (2G9H) - epitope complex system is relatively stable during a 5 ns MD simulation run.

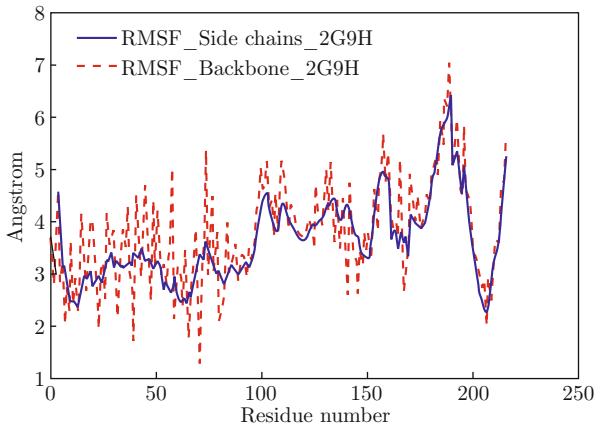

Fig. 6 RMSF for the backbone and side chains of HLA-DRB1*0101-epitope docking complex during the course of MD simulation time (5 ns).

Thus, the sequence based, structure based computational docking and molecular dynamic simulation analysis has consistent results, drawing epitope 162-FMILPIFNV-170 as a potential subunit vaccine against common bacterial meningitis. The combined *in silico* approaches might be encouraging for the development of this exciting novel class of subunit vaccines for other diseases of interest.

4 Conclusions

Meningitis is a life-threatening disease because of the inflammations proximal to the brain and spinal cord; therefore the condition is classified as a medical emergency. No effective vaccines for bacterial meningitis which cover all age groups are known. The pilot work for screening of putative epitope (162-FMILPIFNV-170) using sequenced and structure based immunoinformatics tools suggests that potH protein of *S. pneumonia* could be used for preparation of immunological constructs. We reported single epitope which exhibits good binding affinity with HLA-DRB alleles. Further molecular dynamic simulations revealed that the HLA-DRB-epitope complex was stable. The putative epitope is present on the surface exposed to immunogenic functions of common bacterial meningitis pathogens and it is non-homologues to human proteome. This peptide could be used in designing a subunit vaccine candidate against common bacterial meningitis.

Electronic Supplementary Material

Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s12539-013-0161-1 and is accessible for authorized users.

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References

[1] Altschul, S.F., Thomas, L.M., Alejandro, A.S., Jinghui, Z., Zheng, Z., Webb, M., David J.L. 1997. Gapped BLAST and PSI BLAST: A new generation of protein database search programs. Nucl Acid Res 25, 3389-3402.

[2] Bandaru, N.R., Ibrahim, M.K., Nuri, M.S., Suliman, M.E. 1998. Etiology and occurrence of acute meningitis in children Benghazi, Libyan Arab Jamahiriya. East Mediterr Health J 4, 50-57.

[3] Barker, C.J., Beagley, K.W., Hafner, L.M., Timms, P. 2008. *In silico* identification and *in vivo* analysis of a novel T-cell antigen from *Chlamydia*, NrdB. Vaccine 26, 1285-1296.

[4] Bharathi, M.J., Ramakrishnan, R., Vasu, S., Meenakshi, R., Shivkumar, C.R., Palaniappan, R. 2003. Epidemiology of bacterial keratitis in a referral centre in south india. Indian J Med Microbiol 21, 239-245.

[5] Brooks, W., Daniel, K., Sung, S., Guida, W. 2008. Computational validation of the importance of absolute stereochemistry in virtual screening. J Chem Inf Model 48, 639-645.

[6] Choudhuri, S. 2006. Structure, function, expression, genomic organization, and single nucleotide polymorphisms of human ABCB1 (MDR1), ABCC (MRP), and ABCG2 (BCRP) efflux transporters. Int J of Toxicol 25, 231-259.

[7] Dass, J.F.P., Deepika, V. 2008. Implications from predictions of HLA-DRB1 binding peptides in the membrane proteins of *Corynebacterium diphtheriae*. Bioinformation 3, 111-113.

[8] Doolan, D.L., Southwood, S., Chesnut, R., Appella, E., Gomez, E., Richards, A., Higashimoto, Y.I., Maewal, A., Sidney, J., Gramzinski, R.A., Mason, C., Koech, D., Hoffman, S.L., Sette, A. 2000. HLA-DR-promiscuous T cell epitopes from *Plasmodium falciparum* preerythrocytic - stage antigens restricted by multiple HLA class II alleles. J Immunol 165, 1123-1137.

[9] Essmann, U., Perera, L., Berkowitz, M.L., Darden, T., Lee, H., Pedersen, L.G. 1995. A smooth particle mesh Ewald method. J Chem Phys 103, 8577-8593.

[10] Friesner, R.A., Banks, J.L., Murphy, R.B., Halgren, T.A., Klicic, J.J., Mainz, D.T., Repasky, M.P., Knoll, E.H., Shelley, M., Perry, J.K., Shaw, D.E., Francis, P., Shenkin, P.S. 2004. Glide: A new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. J Med Chem 47, 1739-1749.

[11] Friesner, R.A., Murphy, R.B., Repasky, M.P., Frye, L.L., Greenwood, J.R., Halgren, T.A., Sanschagrin, P.C., Mainz, D.T. 2006. Extra precision glide docking and scoring incorporating a model of hydrophobic enclosure for protein-ligand complexes. J Med Chem 49, 6177-6196.

[12] Ginsberg, L. 2004. Difficult and recurrent meningitis. J Neurol Neurosurg Psychiatry 75, 16-21.

[13] Halgren, T.A., Murphy, R.B., Friesner, R.A., Beard, H.S., Frye, L.L., Pollard, W.T., Banks, J.L. 2004. Glide: A new approach for rapid, accurate docking and scoring. 2. Enrichment factors in database screening. J Med Chem 47, 1750-1759.

[14] Huang, S.H., Jong, A.Y. 2001. Cellular mechanisms of microbial proteins contributing to invasion of the blood-brain barrier. Cell Microbiol 3, 277-287.

[15] Jorgensen, W.L., Maxwell, D.S., TiradoRives, J. 1996. Development and testing of the OPLS all-atom force field on conformational energetics and properties of organic liquids. J Am Chem Soc 118, 11225-11236.

[16] Kaminski, G.A., Friesner, R.A., Tirado-Rives, J., Jorgensen, W.L. 2001. Evaluation and reparametrization of the OPLS-AA force field for proteins via comparison with accurate quantum chemical calculations on peptides. J Phys Chem B 105, 6474-6487.

[17] Laskowski, R.A., MacArthur, M.W., Moss, D.S., Thornton, J.M. 1993. PROCHECK: A program to check the stereochemical quality of protein structures. J Appl Cryst 26, 283-291.

[18] Locher, K.P., Lee, A.T., Rees, D.C. 2002. The *E. coli* BtuCD structure: A framework for ABC transporter architecture and mechanism. Science 296, 1091-1098.

[19] Maestro v9.2, 2011. Schrödinger, LLC, New York.

[20] Mani, R., Pradhan, S., Nagarathna, S., Wasiulla, R., Chandramuki, A. 2007. Bacteriological profile of community acquired acute bacterial meningitis: A ten-year retrospective study in a tertiary neurocare centre in South India. Indian J Med Microbiol 25, 108-114.

[21] Mora, M., Bensi, G., Capo, S., Falugi, F., Zingaretti, C., Manetti, A.G., Maggi, T., Taddei, A.R., Grandi, G., Telford, J.L. 2005. Group A Streptococcus produce pilus-like structures containing protective antigens and Lancefield T antigens. Proc Natl Acad Sci 102, 15641-15646.

[22] Mustafa, A.S., Shaban, F.A. 2006. ProPred analysis and experimental evaluation of promiscuous T-cell epitopes of three major secreted antigens of Mycobacterium tuberculosis. Tuberculosis 86, 115-124.

[23] Mwangi, M.M., Wu, S.W., Zhou, Y., Sieradzki, K., de Lencastre, H., Richardson, P., Bruce, D., Rubin, E., Myers, E., Siggia, E.D., Tomasz, A. 2007. Tracking the *in vivo* evolution of multidrug resistance in Staphylococcus aureus by whole-genome sequencing. Proc Natl Acad Sci 104, 9451-9456.

[24] Oftung, F., Lundin, K.E.A., Geluk, A., Shinnick, T.M., Meloen, R., Mustafa, A.S. 1997. Primary structure and MHC restriction of peptide defined T cell epitopes from recombinantly expressed mycobacterial protein antigens. Med Princples Pract 6, 66-73.

[25] Panigada, M., Sturniolo, T., Besozzi, G., Boccieri, M.G., Sinigaglia, F., Grassi, G.G., Grassi, F. 2002. Identification of a promiscuous T-cell epitope in *Mycobacterium tuberculosis* Mce proteins. Infect Immun 70, 79-85.

[26] Peters, B., Sette, A. 2007. Integrating epitope data into the emerging web of biomedical knowledge resources. Nat Rev Immunol 7, 485-490.

[27] Peters, B., Sidney, J., Bourne, P., Bui, H.H., Buus, S., Doh, G., Fleri, W., Kronenberg, M., Kubo, R., Lund, O., Nemazee, D., Ponomarenko, J.V., Sathiamurthy, M., Schoenberger, S., Stewart, S., Surko, P., Way, S., Wilson, S., Sette, A. 2005. The immune epitope database and analysis resource: From vision to blueprint. PLoS Biol 3, e91.

[28] Porter, V. 2011. Bacterial meningitis: A deadly but preventable disease. Prevention: Where do we stand with vaccination efforts? http://www.medscape.com/viewarticle/481019_6

[29] Rakesh, S., Pradhan, D., Umamaheswari A. 2009. *In silico* approach for future development of subunit vaccines against *Leptospira interrogans* serovar Lai. Int J Bioinform Res 1, 85-92.

[30] Ramakrishnan, M., Ulland, A.J., Steinhardt, L.C., Moïsi, J.C., Were, F., Levine, O.S. 2009. Sequelae due to bacterial meningitis among African children: A systematic literature review. BMC Med 14, 47.

[31] Rammensee, H., Bachmann, J., Emmerich, N.P., Bachor, O.A., Stevanovic, S., 1999. SYFPEITHI: Database for MHC ligands and peptide motifs. Immunogenetics 50, 213-219.

[32] Rappuoli, R. 2001. Reverse vaccinology, a genome-based approach to vaccine development. Vaccine 19, 2688-2691.

[33] Saez-Llorens, X., McCracken, G.H.Jr. 2003. Bacterial meningitis in children. Lancet 361, 2139-2148.

[34] Schlech, W.F., Ward, J.I., Band, J.D., Hightower, A., Fraser, D.W., Broome, C.V. 1985. Bacterial meningitis in the United States, 1978 through 1981. The National Bacterial Meningitis Surveillance Study. J Am Med Assoc 253, 1749-1754.

[35] Segal, S., Pollard, A.J. 2004. Vaccines against bacterial meningitis. Brit Med Bull 72, 65-81.

[36] Serruto, D., Rappuoli, R. 2006. Post-genomic vaccine development. FEBS Lett 580, 2985-2992.

[37] Serruto, D., Serino, L., Masignani, V., Pizza, M. 2009. Genome-based approaches to develop vaccines against bacterial pathogens. Vaccine 27, 3245-3250.

[38] Singh, H., Raghava, G.P. 2001. ProPred: Prediction of HLA-DR binding sites. Bioinformatics 17, 1236-1237.

[39] Singh, P., Suman, S., Chandna, S., Das, T.K. 2009. Possible role of amyloid-beta, adenine nucleotide translocase and cyclophilin-D interaction in mitochondrial dysfunction of Alzheimer's disease. Bioinformation 3, 440-445.

[40] Southwood, S., Sidney, J., Kondo, A., del Guercio, M.F., Appella, E., Hoffman, S., Kubo, R.T., Chesnut, R.W., Grey, H.M., Sette, A. 1998. Several common HLA-DR types share largely overlapping peptide binding repertoires. J Immunol 160, 3363-3373.

[41] Tauber, M.G., Kim, Y.S., Leib, S.L. 1997. Neuronal injury in meningitis. In: Peterson, P.K., Remington, J.S. (Eds.) Defense of the Brain, Blackwell Science, Malden, Mass, 124-143.

[42] Tettelin, H. 2009. The bacterial pan-genome and reverse vaccinology. Genome Dyn 6, 35-47.

[43] Texier, C., Pouvelle, S., Busson, M., Hervé, M., Charron, D., Ménez, A., Maillère B. 2000. HLA-DR restricted peptide candidates for bee venom immunotherapy. J Immunol 164, 3177-3184.

[44] Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G. 1997. The CLUSTAL_X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucl Acid Res 25, 4876-4882.

[45] Tunkel, A.R., Hartman, B.J., Kaplan, S.L., Kaufman, B.A., Roos, K.L., Scheld, W.M., Whitley, R.J. 2004. Practice guidelines for the management of bacterial meningitis. Clin Infect Dis 39, 1267-1284.

[46] Umamaheswari, A., Pradhan, D., Hemanthkumar, M. 2012. Computer aided subunit vaccine design against pathogenic Leptospira serovars. Interdisciplinary Sci Comput Life Sci 4, 38-45.

[47] Wang, P., Sidney, J., Dow, C., Mothé, B., Sette, A., Peters, B. 2008. A systematic assessment of MHC class II peptide binding predictions and evaluation of a consensus approach. PLoS Comput Biol 4, e1000048.

[48] Zagursky, R., Russell, D. 2001. Bioinformatics: Use in bacterial vaccine discovery. Biotechniques 31, 636-659.