RESEARCH

T-cell epitope-based vaccine prediction against *Aspergillus fumigatus*: a harmful causative agent of aspergillosis

Darakshan Jabin and Ajay Kumar*

Abstract

Background: Among the most common causes of invasive aspergillosis and acute bronchopulmonary aspergillosis is *Aspergillus fumigatus*. Transmission with *A. fumigatus* produces aggressive aspergillosis in allogeneic haematopoietic stem cell transplant recipients, HIV patients, and cancer patients. Asthmatics and cystic fibrosis patients are allergic to *A. fumigatus*. MHC class-II binding epitopes can initiate immunogenic responses in patients. In this study, we deployed immunoinformatic study to reveal epitopes from fungal proteins.

Results: In modern research, we found multiple epitopes ITLKLLHRYSYKLAG, KLVLRAFPNHFRGDS, RYSYKLAGVN-QVDVV, GKSFELNQAARAVTQ, and LHRYSYKLAGVNQVD from crucial proteins of *A. fumigatus* 5,8-linoleate diol synthase (ACO55067.2) and ChainB-chitinase A1 (2XVN_B). RYSYKLAGVNQVDVV, GKSFELNQAARAVTQ, and LHRYSYKLAGVN-QVD epitopes interact with HLA-DRB01_0101, while ITLKLLHRYSYKLAG and KLVLRAFPNHFRGDS epitopes interact with HLA-DRB01_1501. Molecular docking analysis reveals atomic contact energy (ACE) value for these five epitopes shown below −5 Kcal/mol in docked state.

Conclusions: The invasive aspergillosis and acute bronchopulmonary aspergillosis are caused by harmful fungal pathogen *Aspergillus fumigatus*. Our modern immunoinformatic research shows ITLKLLHRYSYKLAG, KLVLRAFPN-HFRGDS, RYSYKLAGVNQVDVV, GKSFELNQAARAVTQ, and LHRYSYKLAGVNQVD epitopes could bind to MHC-II HLA allelic determinants and can initiate immunogenic response in patients affected by *Aspergillus fumigatus*.

Keywords: Aspergillosis, Immunoinformatics, Epitope, Molecular docking, MD simulation
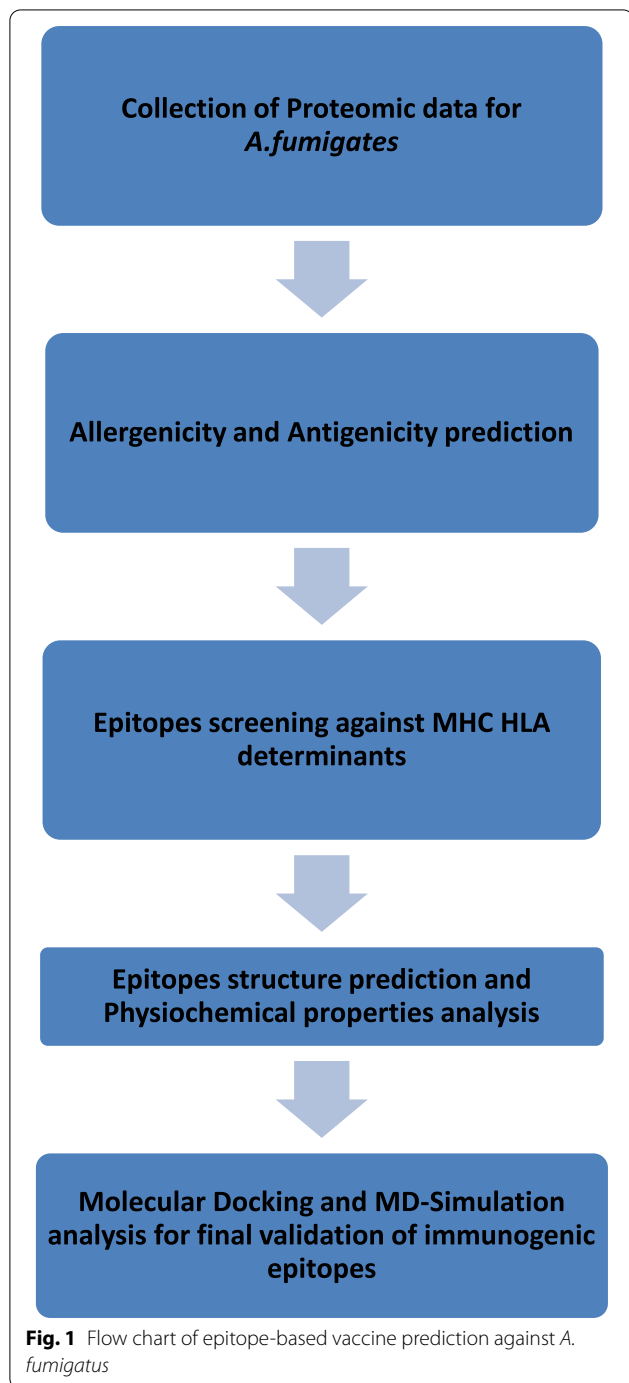
Background

Immunizing the immunosuppressed population vulnerable to opportunistic infections like aspergillosis may appear challenging; however, that could be considered as a first step and as the least immunosuppressed, most worthy prospects, including such granulomatous patients, living donor applicants before graft, leukaemic after effective initial treatment, solid tumour patients at diagnostic test, and healthcare workers with aspergillosis [1]. The far more frequent microbe fungal diseases are *Aspergillus* spp. Among the most common causes of invasive aspergillosis and acute bronchopulmonary aspergillosis is *Aspergillus fumigatus* [2]. Transmission with *A. fumigatus* produces aggressive aspergillosis in allogeneic hematopoietic stem cell transplant recipients, HIV patients, and cancer patients. Asthmatics and cystic fibrosis patients are allergic to *A. fumigatus* [3, 4]. In individuals with atopic asthma or cystic fibrosis, allergy is caused by a hypersensitivity response to *Aspergillus* allergens. When compared to other fungal allergens, diseases related with *A. fumigatus* allergens are on the rise, and it also complicates life-threatening infections in immunocompromised individuals such as cancer patients, HIV patients, and organ transplant recipients [5].

*Correspondence: ajaymtech@gmail.com

Department of Biotechnology, Faculty of Engineering and Technology, Rama University, G.T. Road, Kanpur 209217, India

Only a few drugs (such as voriconazole and amphotericin B) are now available to treat this invasive condition, and even these have restrictions owing to potential risks, and so, these drugs hold longer duration of treatment with side effects [6], so we tried to explore more best possible options of epitopes by deploying approaches of immunoinformatics. In current study, we targeted variety of proteins from *A. fumigatus* to screen out immunogenic T-cell epitopes against *A. fumigatus*

Table 1 Proteins of *A. fumigatus* with accession number

S. no.	Protein name (no. of amino acids)	GenBank accession no.
1	Beta-tubulin (62 aa[a])	AOH95126.1
2	r-ASP-4 (286 aa)	CAA04959.1
3	ChainB-chitinase A1 (309 aa[a])	2XVN_B
4	1,4-glucan branching enzyme (56 aa[a])	AAY83208.1
5	5,8-linoleate diol synthase (1079 aa[a])	ACO55067.2

[a] *aa* amino acids

fungi. In Fig. 1, detailed stepwise outline of epitope-based vaccine prediction strategies is provided.

Methods

Screening of epitopes from proteome of *A. fumigatus*
Protein database like NCBI GenBank, EMBL, and DDBJ was deployed for checking proteins of *A. fumigatus* with various accession numbers/GenBank ID. Proteins of *A. fumigatus* fungi under consideration were enlisted in Table 1.

Allergenicity and antigenicity prediction of proteins
AllergenFP tool [7] was deployed for prediction allergenicity on the basis of Tanimoto similarity index; also, VaxiJen ver.2.0 tool [8] was used for prediction of antigenicity of epitopes of proteins after successful epitopes screening.

Epitope screening from proteins of *A. fumigatus*
Epitopes were screened by using NetMHCIIpan ver.3.2 server [9, 10], which screens epitopes from proteins of *A. fumigatus* via ANN algorithms. Also, affinity scores assisted in predicting binding of these epitopes to MHC-II allelic determinants. MHC-II HLA determinants were screened by using IEDB database.

Physiochemical properties analysis
All properties of screened epitopes were determined by using ExPASy tools like ProtParam, which assisted us in

Fig. 1 Flow chart of epitope-based vaccine prediction against *A. fumigatus*

Table 2 Allergenicity of core proteins of *A. fumigatus* with Tanimoto similarity index

S. no.	Protein name	Tanimoto similarity index (Allergenicity)
1	Beta-tubulin	0.79 (non-allergen)
2	r-ASP-4	1.0 (allergen)
3	ChainB-chitinase A1	0.8 (non-allergen)
4	1,4-glucan branching enzyme	0.74 (non-allergen)
5	5,8-linoleate diol synthase	0.83 (non-allergen)

finding isoelectric point (pI), GRAVY score, instability score, half-life and molecular weight, etc. [11].

Epitope structure prediction

Many latest tools like I-TASSER [12], SWISS-PROT [13], PEP-FOLD ver.3.5 [14], and Phyre2.0 [15] tools were used for 3D structure prediction of proteins.

Table 3 NetMHCIIpan 3.2 scores for HLA-DRB01_0101

S. no.	Protein GenBank_ID	Epitope	DRB1_0101		
			1-log50k	nM	Rank
1	pdb_2XVN_B	VPERKFYL-SAAPQCI	0.793	9.41	0.4
2	pdb_2XVN_B	PERKFYLSAAPQCII	0.824	6.74	0.09
3	pdb_2XVN_B	ERKFYLSAAPQCIIP	0.827	6.52	0.07
4	pdb_2XVN_B	RKFYLSAAPQCI-IPD	0.821	6.94	0.1
5	pdb_2XVN_B	KFYLSAAPQCI-IPDA	0.787	10.07	0.5
6	ACO55067.2	LHVPTVFRSIEALGI	0.796	9.12	0.4
7	ACO55067.2	HVPTVFRSIEALGIQ	0.821	6.91	0.1
8	ACO55067.2	VPTVFRSIEALGIQQ	0.828	6.41	0.06
9	ACO55067.2	PTVFRSIEAL-GIQQA	0.834	6.05	0.05
10	ACO55067.2	TVFRSIEAL-GIQQAR	0.833	6.11	0.05
11	ACO55067.2	VFRSIEALGIQQARS	0.816	7.3	0.15
12	ACO55067.2	GLCTNFTISRAILSD	0.767	12.49	1
13	ACO55067.2	LCTNFTISRAILSDA	0.788	9.87	0.5
14	ACO55067.2	CTNFTISRAILSDAV	0.792	9.45	0.4
15	ACO55067.2	TNFTISRAILSDAVA	0.792	9.53	0.4
16	ACO55067.2	NFTISRAILSDAVAL	0.77	12.08	0.9
17	ACO55067.2	LHRYSYKLAGVN-QVD	0.78	10.82	0.7
18	ACO55067.2	HRYSYKLAGVN-QVDV	0.803	8.43	0.25
19	ACO55067.2	RYSYKLAGVN-QVDVV	0.812	7.68	0.17
20	ACO55067.2	YSYKLAGVN-QVDVVR	0.789	9.78	0.5
21	ACO55067.2	DIGKSFEL-NQAARAV	0.792	9.53	0.4
22	ACO55067.2	IGKSFELNQAAR-AVT	0.805	8.23	0.25
23	ACO55067.2	GKSFELNQAARA-VTQ	0.805	8.23	0.25
24	ACO55067.2	KSFELNQAARA-VTQQ	0.798	8.93	0.3
25	ACO55067.2	AKTGFIAN-LVNSLHR	0.788	9.94	0.5
26	ACO55067.2	KTGFIANLVNSL-HRH	0.793	9.42	0.4
27	ACO55067.2	TGFIANLVNSL-HRHD	0.774	11.5	0.8

Table 4 NetMHCIIpan 3.2 scores for HLA-DRB01_1501

S. no.	Protein GenBank_ID	Epitope	DRB1_1501		
			1-log50k	nM	Rank
1	ACO55067.2	GVVLIMFNRFH-NYVV	0.66	39.47	0.8
2	ACO55067.2	VVLIMFNRFHNY-VVE	0.661	39.34	0.7
3	ACO55067.2	VLIMFNRFHNYV-VEK	0.667	36.78	0.6
4	ACO55067.2	LIMFNRFHNYV-VEKL	0.66	39.6	0.8
5	ACO55067.2	IMFNRFHNYV-VEKLA	0.649	44.78	1
6	ACO55067.2	VFYKLVLRAFP-NHFR	0.703	24.78	0.15
7	ACO55067.2	FYKLVLRAFPN-HFRG	0.715	21.86	0.08
8	ACO55067.2	YKLVLRAFPN-HFRGD	0.697	26.39	0.2
9	ACO55067.2	KLVLRAFPN-HFRGDS	0.682	31.29	0.4
10	ACO55067.2	ITLKLLHRYSYKLAG	0.651	43.63	1
11	ACO55067.2	TLKLL-HRYSYKLAGV	0.659	40.07	0.8
12	ACO55067.2	LKLL-HRYSYKLAGVN	0.649	44.65	1

Table 5 NetMHCIIpan 3.2 scores for HLA-DRB01_1101

S. no.	Protein GenBank_ID	Epitope	DRB1_1101		
			1-log50k	nM	Rank
1	ACO55067.2	THVFYKLVL-RAFPNH	0.667	36.66	1.3
2	ACO55067.2	HVFYKLVLRAFP-NHF	0.673	34.51	1.2
3	ACO55067.2	VFYKLVLRAFP-NHFR	0.689	29.05	0.8
4	ACO55067.2	FYKLVLRAFPN-HFRG	0.69	28.52	0.8
5	ACO55067.2	LLLRYFMEGARIRSS	0.668	36.44	1.3
6	ACO55067.2	LLRYFMEG-ARIRSSV	0.676	33.27	1.1
7	ACO55067.2	LRYFMEG-ARIRSSVA	0.671	34.98	1.2
8	ACO55067.2	GARIRSSVAL-PRVVA	0.663	38.35	1.4
9	ACO55067.2	LTTMLKVI-GRLDNLR	0.663	38.15	1.4
10	ACO55067.2	TTMLKVIGRLDN-LRR	0.669	35.96	1.3
11	ACO55067.2	TMLKVIGRLDN-LRRA	0.665	37.69	1.4

Table 6 Epitope screening based on antigenicity scores (threshold value ≥ 0.4)

HLA determinant	Epitope	GenBank_ID	VaxiJen score	Antigenicity
DRB1_0101	VPERKFYLSAAPQCI	pdb_2XVN_B	0.461	Antigen
	LHRYSYKLAGVNQVD	ACO55067.2	0.554	Antigen
	HRYSYKLAGVNQVDV	ACO55067.2	0.834	Antigen
	RYSYKLAGVNQVDVV	ACO55067.2	0.559	Antigen
	GKSFELNQAARAVTQ	ACO55067.2	0.632	Antigen
	KSFELNQAARAVTQQ	ACO55067.2	0.485	Antigen
DRB1_1501	VFYKLVLRAFPNHFR	ACO55067.2	0.482	Antigen
	YKLVLRAFPNHFRGD	ACO55067.2	0.586	Antigen
	KLVLRAFPNHFRGDS	ACO55067.2	0.603	Antigen
	ITLKLLHRYSYKLAG	ACO55067.2	1.034	Antigen
	TLKLLHRYSYKLAGV	ACO55067.2	0.599	Antigen
	LKLLHRYSYKLAGVN	ACO55067.2	0.878	Antigen
DRB1_1101	HVFYKLVLRAFPNHF	ACO55067.2	0.511	Antigen
	VFYKLVLRAFPNHFR	ACO55067.2	0.482	Antigen
	LLLRYFMEGARIRSS	ACO55067.2	0.868	Antigen
	GARIRSSVALPRVVA	ACO55067.2	0.582	Antigen

Table 7 Epitopes further screening based on physiochemical properties

Epitope	Mol. wt.	Isoelectric point	Half-life	Instability index	GRAVY score	Inference
VPERKFYLSAAPQCI	1722	8.74	100 h	76.5 (unstable)	0.047	Not selected
LHRYSYKLAGVNQVD	1762	8.5	5.5 h	−21.43 (stable)	−0.54	Selected
RYSYKLAGVNQVDVV	1710	8.5	1 h	−21.43 (stable)	−0.020	Selected
GKSFELNQAARAVTQ	1619	8.75	30 h	−12.04 (stable)	−0.54	Selected
VFYKLVLRAFPNHFR	1907	11	100 h	44.17 (partially stable)	0.247	Not selected
KLVLRAFPNHFRGDS	1757	10.84	3 h	29.62 (stable)	−0.447	Selected
ITLKLLHRYSYKLAG	1776	10	20 h	−12.67(stable)	0.1	Selected
HVFYKLVLRAFPNHF	1888	9.99	3.5 h	44.17 (partially stable)	0.333	Not selected
LLLRYFMEGARIRSS	1812	10.74	5.5 h	53.62 (unstable)	0.14	Not selected
GARIRSSVALPRVVA	1551	12.3	30 h	53.62 (unstable)	0.613	Not selected

Molecular docking

Molecular docking by using latest tools assisted us in finding binding scores, binding pocket, and H-bonds between epitopes and MHC-II HLA determinants. The latest tool PatchDock [16] free server is easily accessible and deployed here for docking analysis. For analysing, docked complex visualization tools like Chimera tool and PyMOL tool were used. Docked complex structural validation by using ProSA [17] and MolProbity [18] was conducted. These tools assist in determining Z-score and Ramachandran plot for protein complexes.
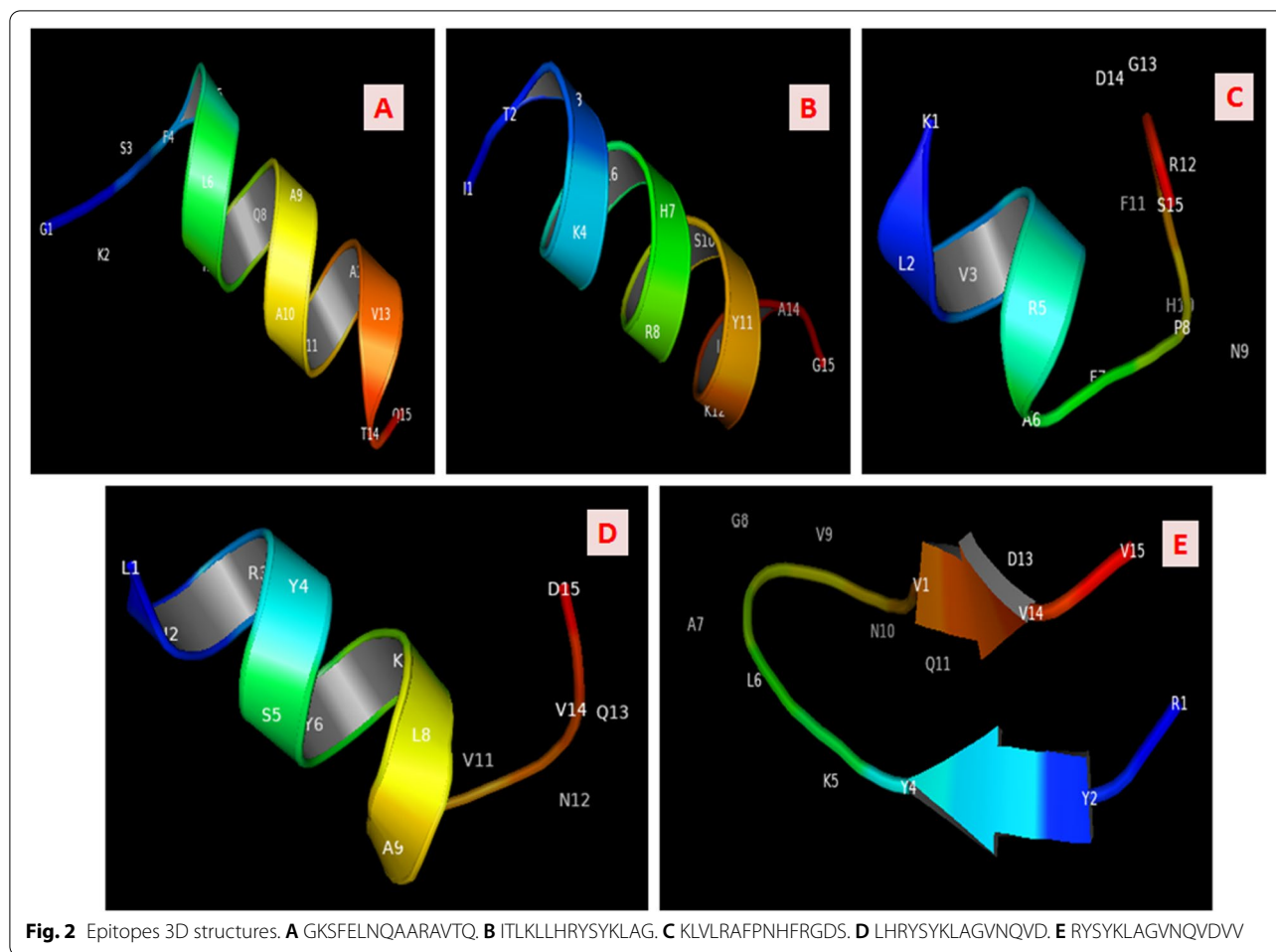
Molecular dynamic and simulation

Docked complexes were used to analyse undersimulation by deploying GROMACS tool [19], which assisted us in

determining stability of complex by notifying RMSD and RMSF plots. We employed an OPLS-AA force field for MD analysis, which was defined by computing the structural energy of biological and biochemical systems for 100 ns.

Results

Protein selection and allergenicity analysis

Core proteins of *A. fumigatus* were downloaded in fast format from GenBank-NCBI database and subjected to allergenicity analysis by using AllergenFP tool. This server produced TSI (Tanimoto similarity index) for defining resemblance of given protein sequence to reveal allergen or non-allergen nature of the given proteins (Table 2). Non-allergenic proteins were selected and further used to identify epitopes from them.

Fig. 2 Epitopes 3D structures. **A** GKSFELNQAARAVTQ. **B** ITLKLLHRYSYKLAG. **C** KLVLRAFPNHFRGDS. **D** LHRYSYKLAGVNQVD. **E** RYSYKLAGVNQVDVV

Table 8 Molecular docking analysis: receptor and ligand docking scores

HLA-allelic determinant (receptor)	Epitope of interest (Ligand)	Atomic contact energy (Kcal/mol)
1XR9	ITLKLLHRYSYKLAG	−6.903
1XR9	KLVLRAFPNHFRGDS	−6.405
1AQD	RYSYKLAGVNQVDVV	−5.525
1AQD	GKSFELNQAARAVTQ	−5.452
1AQD	LHRYSYKLAGVNQVD	−6.325

Epitopes screening and antigenicity analysis

NetMHCIIpan 3.2 server was used to find epitopes of *A. fumigatus* core proteins that can bind to MHC-II HLA-DRB molecules. Considered HLA-DRB proteins were HLA-DRB01_0101, HLA-DRB01_1501, and HLA-DRB01_1101 that were used against each FASTA sequence provided to this server which is based on ANN algorithm. This server generates 1-log50K score,

binding affinity in nm, and ranks. Threshold of rank under 1.5 was considered for selection of epitopes, to reveal perfect epitopes that are able to bind MHC-II allelic determinants. HLA-DRB01_0101 shows maximum binding with a total of 27 epitopes (Table 3), HLA-DRB01_1501 shows maximum binding with total of 12 epitopes (Table 4), and HLA-DRB01_1101 shows maximum binding with total of 11 epitopes (Table 5), each of 15 amino residues in length.

VaxiJen ver.2.0 tool was used to determine antigenicity of selected epitopes with threshold of 0.4 (Table 6). Antigenic epitopes were used for further physiochemical screening of epitopes.

Physiochemical analysis of epitopes

Screened antigenic proteins were further analysed for physiochemical properties to screen epitopes on the basis of stability, half-life, isoelectric point, and GRAVY score (grand average of hydropathicity) (Table 7). Instability index defines protein structure to be unstable if greater than 50%, and half-life was calculated as per

action data against mammalian reticulocytes by deploying ProtParam server of ExPASy tools. Similar epitopes with single or dual amino acid change were also removed from screened data, which finalizes 5 epitopes for further structural and docking analysis.

Structure prediction for selected epitopes

Structural alphabet approach of de novo prediction was deployed to model the finalized epitopes structures. The PEP-FOLD ver.3.5 tool uses 5 to 50 amino residues for structure modelling and also performs 100 short simulations before conformation finalization for the provided sequence data, as this tool uses machine learning algorithms. Structures of epitopes modelled (Fig. 2) were used for further molecular docking studies with known crystal structures of HLA-allelic determinants that were downloaded from RCSB-PDB database; for HLA DRB01_0101 retrieval, PDB_ID is 1AQD, and for HLA DRB01_1501 retrieval, PDB_ID is 1XR9.

Molecular docking

HLA alleles HLA-DRB01:0101 and HLA-DRB01:15:01 were docked with epitopes that show interaction as per NetMHCIIpan 3.2 scores and previous screening in present research context. For molecular docking, PatchDock and FireDock tools were used. The atomic contact energy (ACE value) for docked complexes was provided in Table 8. ACE value for GKSFELNQAARAVTQ, ITLKLLHRYSYKLAG, KLVLRAFPNHFRGDS, LHRYSYKLAGVNQVD, and RYSYKLAGVNQVDVV epitopes show values less than −5 Kcal/mol in docked state with HLA allelic determinants. In Fig. 3, all the 5 docked complexes were shown that reveals fine interactions between receptor and ligand (epitopes).

Docked complexes structural validation

Z-score indicates stability of structure and overall quality of the structure modelled with available datasets of X-ray and NMR models. The calculated Z-scores for complexes are as follows: (1) 1AQD-GKSFELNQAARA-VTQ: −5.71; (2) 1AQD-LHRYSYKLAGVNQVD: −5.72; (3) 1AQD-RYSYKLAGVNQVDVV: −5.71; (4) 1XR9-ITLKLLHRYSYKLAG: −8.96; and (5) 1XR9-KLVLRAFP-NHFRGDS: −8.96. Figure 4 indicates Z-plots for all the docked complex structures.
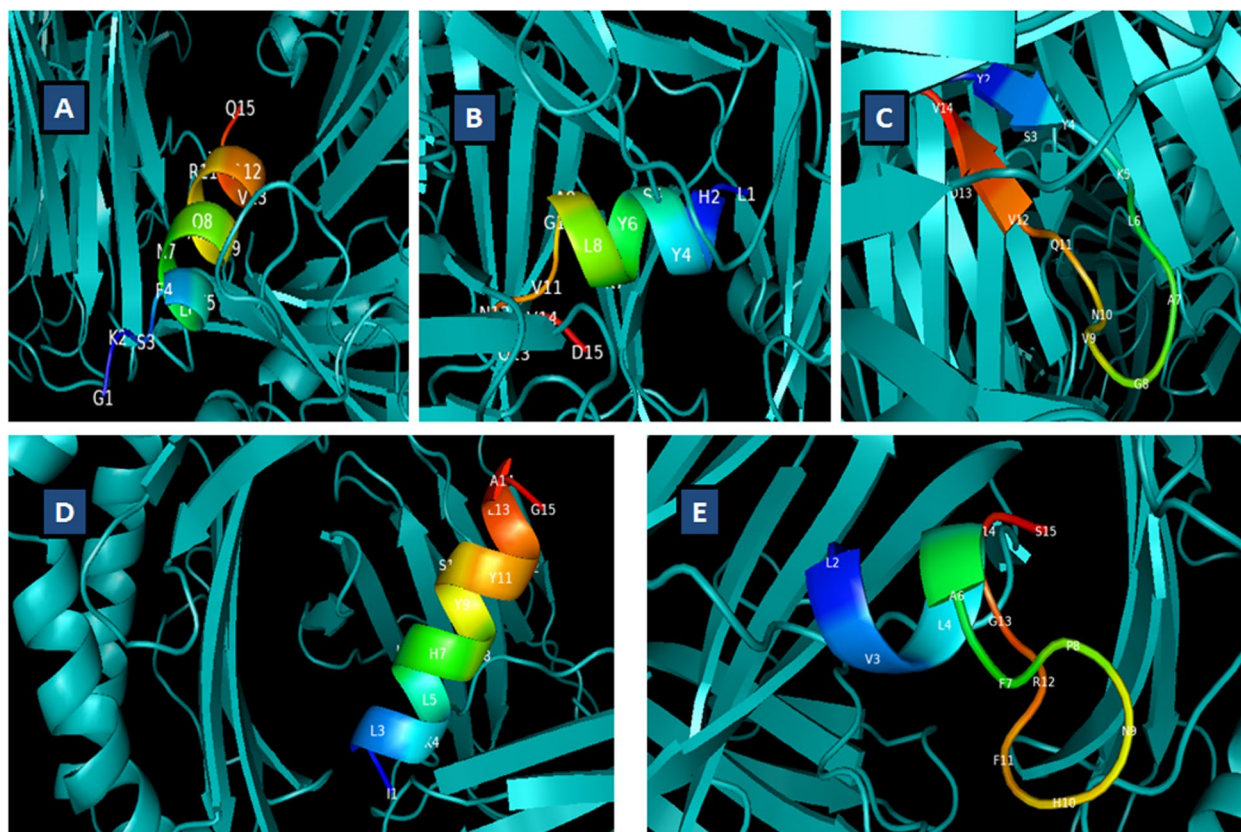
Fig. 3 Docked complexes of epitopes with HLA-allelic determinants. **A** 1AQD-GKSFELNQAARAVTQ. **B** 1AQD-LHRYSYKLAGVNQVD. **C** 1AQD-RYSYKLAGVNQVDVV. **D** 1XR9-ITLKLLHRYSYKLAG. **E** 1XR9-KLVLRAFPNHFRGDS
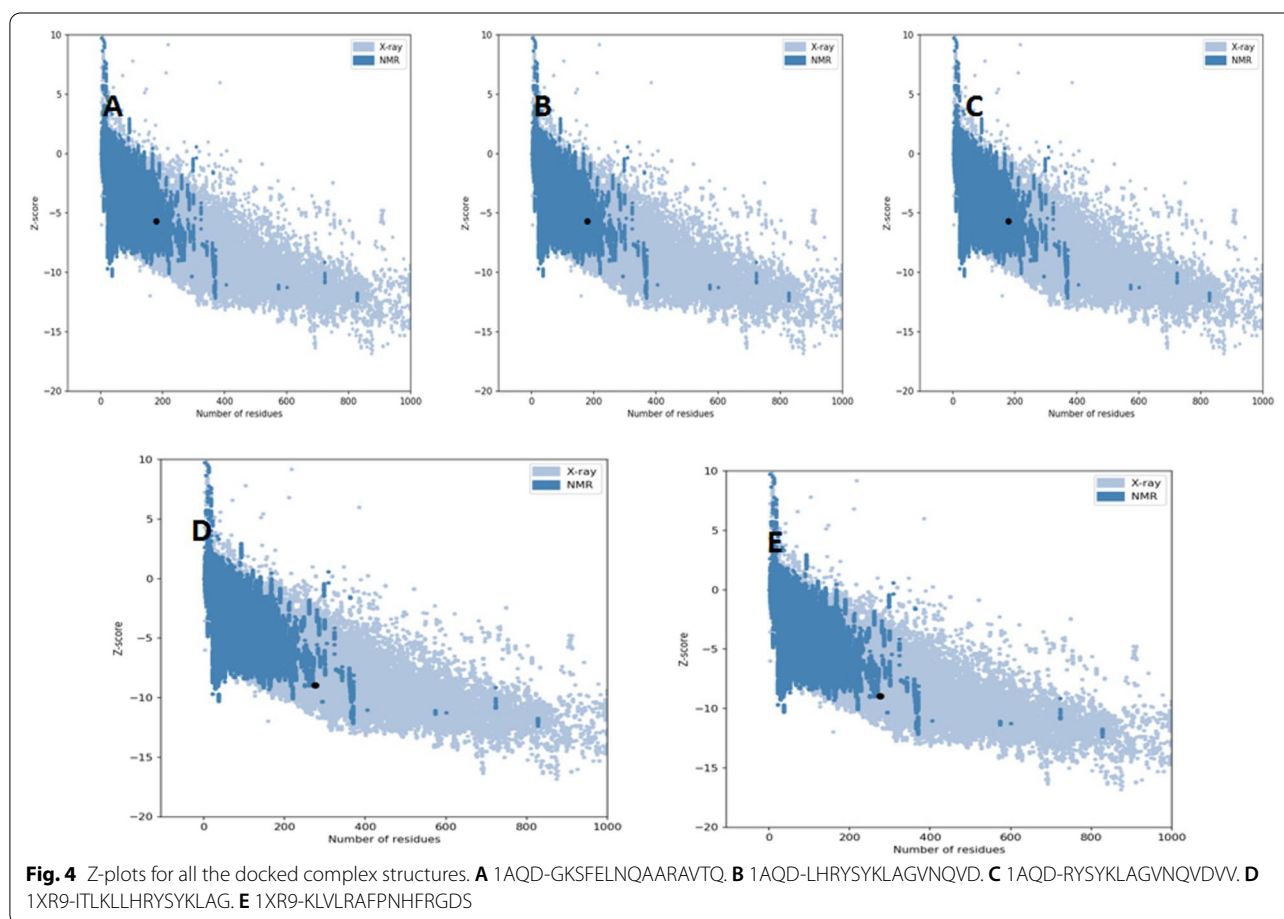
Ramachandran plot analysis of docked complexes

MolProbity tool was deployed to reveal the validation of secondary structures of docked complexes by generating Ramachandran plots (Fig. 5), and it was noted that all the residues (above 90%) were found to be in favourable region or allowed region.
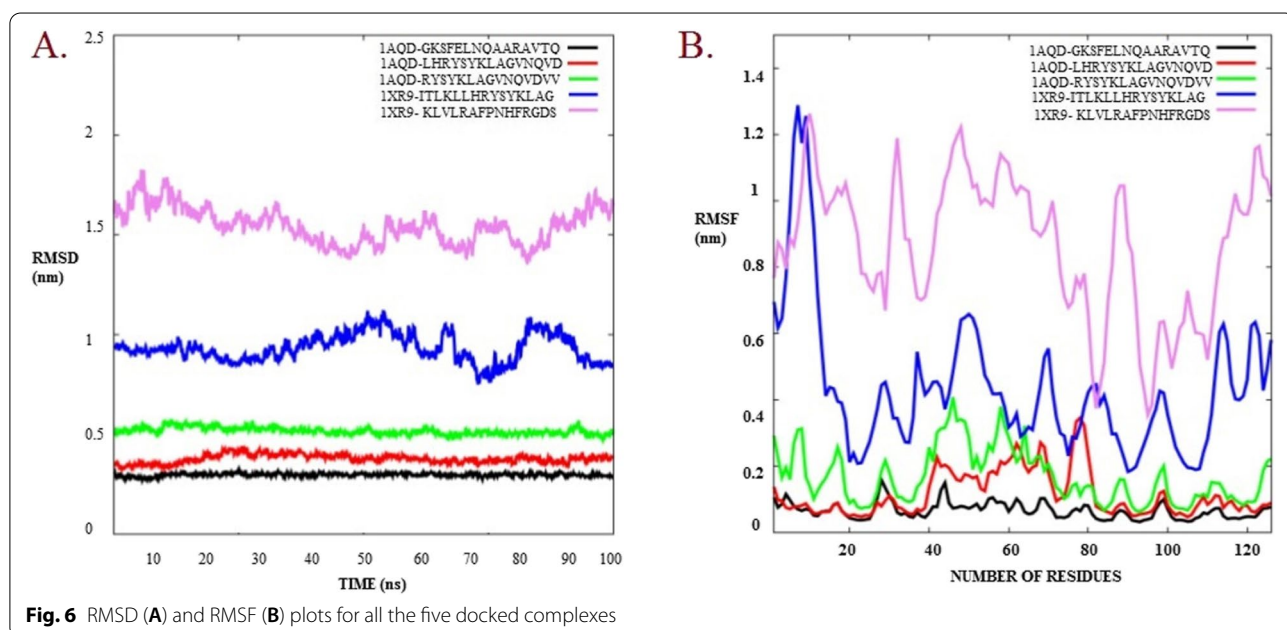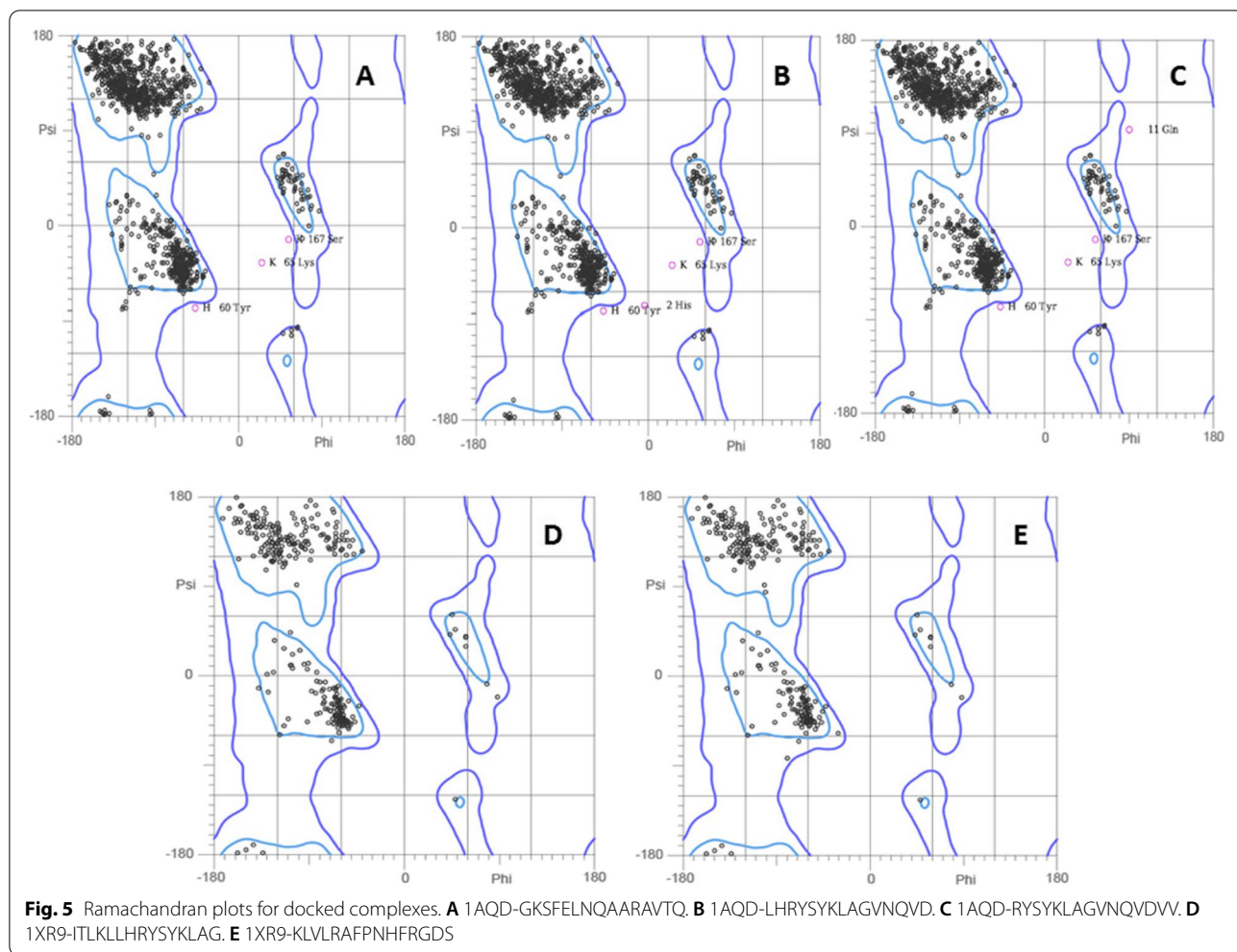
MD-simulation analysis

The experimental characteristics of the dimension of sustainability and thermodynamics stages were reproduced using this force field for 100 ns. Moreover, within those sorts of simulations, where we examine behaviour at temperatures beyond 300 K, the choice of the water model is critical. TIP4P, a four-water system, was identified as the fine water model for this research. Here docked complexes were analysed for good interaction studies. An RMSD and RMSF plot clearly indicates that all the complexes hold values under 0 to 2.5 nm and 0 to 1.4 nm respectively, as provided in Fig. 6. Such scores indicate stability of complexes under longer durations.

Discussion

Among the most common causes of invasive aspergillosis and acute bronchopulmonary aspergillosis is *Aspergillus fumigatus* [2]. Transmission with *A. fumigatus* produces aggressive aspergillosis in allogeneic hematopoietic stem cell transplant recipients, HIV patients, and cancer patients. Asthmatics and cystic fibrosis patients are allergic to *A. fumigatus* [3, 4]. The majority of T cells might belong to one of two subsets, which are attributed to the presence of one of two glycoproteins on their surface, labelled as CD8 or CD4. CD4 T cells serve as T-helper (Th) cells, recognizing peptides on MHC-II determinants [20]. The immune system's hierarchical and combinatorial features contribute to its complexity. As a result, a massive quantity of data about immune systems is being created. This intricacy must be addressed in immunologic research. In current research, we found multiple epitopes: ITLKLLHRYSYKLAG, KLVLRAFPNHFRGDS, RYSYKLAGVNQVDVV, GKS-FELNQAARAVTQ, and LHRYSYKLAGVNQVD from crucial proteins of *A. fumigatus* 5,8-linoleate diol synthase (ACO55067.2). ChainB-chitinase A1 (2XVN_B),

Fig. 4 Z-plots for all the docked complex structures. **A** 1AQD-GKSFELNQAARAVTQ. **B** 1AQD-LHRYSYKLAGVNQVD. **C** 1AQD-RYSYKLAGVNQVDVV. **D** 1XR9-ITLKLLHRYSYKLAG. **E** 1XR9-KLVLRAFPNHFRGDS

Fig. 5 Ramachandran plots for docked complexes. **A** 1AQD-GKSFELNQAARAVTQ. **B** 1AQD-LHRYSYKLAGVNQVD. **C** 1AQD-RYSYKLAGVNQVDVV. **D** 1XR9-ITLKLLHRYSYKLAG. **E** 1XR9-KLVLRAFPNHFRGDS

Fig. 6 RMSD (**A**) and RMSF (**B**) plots for all the five docked complexes

RYSYKLAGVNQVDVV, GKSFELNQAARAVTQ, and LHRYSYKLAGVNQVD epitopes interact with HLA-DRB01_0101, while ITLKLLHRYSYKLAG and KLVLRAFPNHFRGDS epitopes interact with HLA-DRB01_1501. Molecular docking analysis reveals atomic contact energy (ACE) value for these five epitopes shown below −5 Kcal/mol in docked state. Also, docked complex was analysed for simulation analysis, and it was found that they show stable interaction pattern as per the RMSD and RMSF plots. Many previous studies show the importance of immunoinformatic study to support our analysis on fungal epitope determination likewise for *Candida auris*, *Tropheryma whipplei* [21, 22], dengue [23], human cytomegalovirus [24], and chikungunya [25]. Modern chemi-informatic and immunoinformatics study not only supports rapid vaccine prediction but also provides efficient economic resource management [26–28], although immunoinformatic requires wet-lab support as future perspectives for epitope synthesis and animal cell line-dependent validations.

Conclusions

The invasive aspergillosis and acute bronchopulmonary aspergillosis are caused by harmful fungal pathogen *Aspergillus fumigatus*. Our modern immunoinformatic research shows ITLKLLHRYSYKLAG, KLVLRAFPN-HFRGDS, RYSYKLAGVNQVDVV, GKSFELNQAARA-VTQ, and LHRYSYKLAGVNQVD epitopes could bind to MHC-II HLA allelic determinants and can initiate immunogenic response in patients affected by *Aspergillus fumigatus*.

Abbreviations
RMSD: Root-mean-square deviation; RMSF: Root-mean-square fluctuation; MD: Molecular dynamics; ACE: Atomic contact energy.

Authors' contributions
AK and DJ conceived and conducted this research study. AK and DJ: designing and resolving the research problem. All authors have read and approved the final manuscript.

Availability of data and materials
All data is provided in manuscript.

Declarations

Ethics approval and consent to participate
Not applicable. No impact on ethical standards in this study, and there is no human or animal involvement.

Consent for publication
Not applicable.

Competing interests
All authors declare that they have no competing interests.

References

1. Stevens DA, Clemons KV, Liu M (2011) Developing a vaccine against aspergillosis. Med Mycol 49(S1):S170–S176. https://doi.org/10.3109/13693786.2010.497775
2. Thakur R, Anand R, Tiwari S, Singh AP, Tiwary BN, Shankar J (2015) Cytokines induce effector T-helper cells during invasive aspergillosis; what we have learned about T-helper cells? Front Microbiol 6. https://doi.org/10.3389/fmicb.2015.00429
3. Chaudhary N, Marr KA (2011) Impact of Aspergillus fumigatus in allergic airway diseases. Clin Transl Allergy 1(1):4. https://doi.org/10.1186/2045-7022-1-4
4. Shah A, Panjabi C (2016) Allergic bronchopulmonary aspergillosis: a perplexing clinical entity. Allergy Asthma Immunol Res 8(4):282. https://doi.org/10.4168/aair.2016.8.4.282
5. Castellano-Gonzalez G, Clancy LE, Gottlieb D (2017) Prospects for adoptive T-cell therapy for invasive fungal disease. Curr Opin Infect Dis 30(6):518–527. https://doi.org/10.1097/qco.0000000000000403
6. Low YS, Garcia MD, Lonhienne T, Fraser JA, Schenk G, Guddat LW (2021) Triazolopyrimidine herbicides are potent inhibitors of Aspergillus fumigatus acetohydroxyacid synthase and potential antifungal drug leads. Sci Rep 11(1). https://doi.org/10.1038/s41598-021-00349-9
7. Dimitrov I, Naneva L, Doytchinova I, Bangov I (2013) AllergenFP: allergenicity prediction by descriptor fingerprints. Bioinformatics 30(6):846–851. https://doi.org/10.1093/bioinformatics/btt619
8. Doytchinova IA, Flower DR (2007) VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines. BMC Bioinformatics 8(1). https://doi.org/10.1186/1471-2105-8-4
9. Lundegaard C, Lund O, Nielsen M (2011) Prediction of epitopes using neural network based methods. J Immunol Methods 374(1-2):26–34. https://doi.org/10.1016/j.jim.2010.10.011
10. Jensen KK, Andreatta M, Marcatili P, Buus S, Greenbaum JA, Yan Z et al (2018) Improved methods for predicting peptide binding affinity to MHC class II molecules. Immunology 154(3):394–406. https://doi.org/10.1111/imm.12889
11. Artimo P, Jonnalagedda M, Arnold K, Baratin D, Csardi G, de Castro E et al (2012) ExPASy: SIB bioinformatics resource portal. Nucleic Acids Res 40(W1):W597–W603. https://doi.org/10.1093/nar/gks400
12. Yang J, Yan R, Roy A, Xu D, Poisson J, Zhang Y (2014) The I-TASSER suite: protein structure and function prediction. Nat Methods 12(1):7–8. https://doi.org/10.1038/nmeth.3213
13. Boeckmann B (2003) The SWISS-PROT protein knowledgebase and its supplement TrEMBL in 2003. Nucleic Acids Res 31(1):365–370. https://doi.org/10.1093/nar/gkg095
14. Thevenet P, Shen Y, Maupetit J, Guyon F, Derreumaux P, Tuffery P (2012) PEP-FOLD: an updated de novo structure prediction server for both linear and disulfide bonded cyclic peptides. Nucleic Acids Res 40(W1):W288–W293. https://doi.org/10.1093/nar/gks419
15. Kelley LA, Mezulis S, Yates CM, Wass MN, Sternberg MJE (2015) The Phyre2 web portal for protein modeling, prediction and analysis. Nat Protoc 10(6):845–858. https://doi.org/10.1038/nprot.2015.053
16. Schneidman-Duhovny D, Inbar Y, Nussinov R, Wolfson HJ (2005) PatchDock and SymmDock: servers for rigid and symmetric docking. Nucleic Acids Res 33(Web Server):W363–W367. https://doi.org/10.1093/nar/gki481
17. Wiederstein M, Sippl MJ (2007) ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins. Nucleic Acids Res 35(Web Server):W407–W410. https://doi.org/10.1093/nar/gkm290
18. Chen VB, Arendall WB, Headd JJ, Keedy DA, Immormino RM, Kapral GJ et al (2009) MolProbity: all-atom structure validation for macromolecular

crystallography. Acta Crystallogr D Biol Crystallogr 66(1):12–21. https://doi.org/10.1107/s0907444909042073

19. Abraham MJ, Murtola T, Schulz R, Páll S, Smith JC, Hess B, Lindahl E (2015) GROMACS: high performance molecular simulations through multi-level parallelism from laptops to supercomputers. SoftwareX 1-2:19–25. https://doi.org/10.1016/j.softx.2015.06.001

20. Tomar N, De RK (2014) Immunoinformatics: a brief review. Immunoinformatics 23–55. https://doi.org/10.1007/978-1-4939-1115-8_3

21. Joshi A, Kaushik V (2020) In-silico proteomic exploratory quest: crafting t-cell epitope vaccine against Whipple's disease. Int J Pept Res Ther 27(1):169–179. https://doi.org/10.1007/s10989-020-10077-9

22. Joshi A, Krishnan S, Kaushik V (2022) Codon usage studies and epitope-based peptide vaccine prediction against Tropheryma whipplei. J Genet Eng Biotechnol 20(1). https://doi.org/10.1186/s43141-022-00324-5

23. Krishnan GS, Joshi A, Akhtar N, Kaushik V (2021) Immunoinformatics designed T cell multi epitope dengue peptide vaccine derived from non structural proteome. Microb Pathog 150:104728. https://doi.org/10.1016/j.micpath.2020.104728

24. Akhtar N, Joshi A, Singh J, Kaushik V (2021) Design of a novel and potent multivalent epitope based human cytomegalovirus peptide vaccine: an immunoinformatics approach. J Mol Liq 335:116586. https://doi.org/10.1016/j.molliq.2021.116586

25. Sharma P, Sharma P, Ahmad S, Kumar A (2022) Chikungunya virus vaccine development: through computational proteome exploration for finding of HLA and cTAP binding novel epitopes as vaccine candidates. Int J Pept Res Ther 28(2). https://doi.org/10.1007/s10989-021-10347-0

26. Joshi A, Sunil Krishnan G, Kaushik V (2020) Molecular docking and simulation investigation: effect of beta-sesquiphellandrene with ionic integration on SARS-CoV2 and SFTS viruses. J Genet Eng Biotechnol 18(1). https://doi.org/10.1186/s43141-020-00095-x

27. Jain P, Joshi A, Akhtar N, Krishnan S, Kaushik V (2021) An immunoinformatics study: designing multivalent T-cell epitope vaccine against canine circovirus. J Genet Eng Biotechnol 19(1). https://doi.org/10.1186/s43141-021-00220-4

28. Joshi A, Pathak DC, Mannan MA, Kaushik V (2021) In-silico designing of epitope-based vaccine against the seven banded grouper nervous necrosis virus affecting fish species. Netw Model Anal Health Inform Bioinform 10(1). https://doi.org/10.1007/s13721-021-00315-5

Publisher's Note