ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

An *In Silico* Approach To Multiepitope Vaccine Design Against Sea Anemone Toxins.

Sriparna Ray[1] and Jayanta Sinha[2*].

[1]State Aided College Teacher – 1, Department of Zoology, Bidhan Chandra College, Asansol, West Bengal, India.
[*]Ex-Associate Professor, Department of Zoology, Vivekananda College, Kolkata, West Bengal, India.

ABSTRACT

The venom of sea anemone is a mixture of toxins including neurotoxins which acts on voltage gated sodium and potassium channel and in turn these channels assist in excitability of most animals. Generally handling for such issues involves removing stings, using of tropical antibiotics and consuming oral painkillers. Diagnosis is usually sympathetic with this treatment but some highly toxic species causes severe damages and are potentially lethal and therefore vaccine therapy becomes essential. To design computational vaccine against sea anemone toxins IEDB analysis resource server is used to predict epitope and MHC-I (CTL), MHC-II (HTL) molecule. These epitopes are fused with many linkers along with adjuvant and finally yield a chimeric vaccine. For maximal protein expression, the Java Codon Adaptation tool (Jcat) was used to optimize codon usage of the vaccine construct in E coli (strain K12) and C-ImmSim server is used to yield actual immune response of this vaccine. This work is an attempt to respond to the need for novel tools to achieve the success against sea anemone bites. Here we recommend the improvement of a multi-epitope vaccine candidate which could guide to the generation of a more potent protective immune response.

Keywords: in silico, computational vaccine, docking, codon optimization, and immune response

https://doi.org/10.33887/rjpbcs/2024.15.3.5

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Sea anemones are a group of water-dwelling, carnivorous animals of the order Actiniaria. Sea anemones are classified in the phylum Cnidaria, class Anthozoa and subclass Hexacorallia [1]. They are originated in both temperate and tropical seas in many different habitats in both high and low currents. Tropical anemones have a propensity to be larger in size than the temperate ones. In sea anemone, the mouth and the anus are present in the core of the oral disc which are bordered by tentacles and carried with many defensive cnidocytes cells which are used to capture prey. Cnidocytes contain stinging nematocysts which is capsule-like organ and capable of emerging out suddenly [2]. A small toxin vesicle filled with actinotoxins, an inner filament and an external sensory hair is present in each nematocyst. When a touch is administered to the hair, it automatically triggers a cell explosion which launches a harpoon-like structure which attaches to the organism that triggered it and injects an amount of toxin in the flesh of the prey. The venom of sea anemone is a mixture of toxins including neurotoxins (that paralyze the prey) which act on voltage gated sodium and potassium channel and in turn these channels assist in excitability of most animals because they are dependable for activating the initiation, mediation and propagation of action potentials. Some cytolytic sea anemone toxins act cause hemolysis. Phospholipase A2 type sea anemone toxins endorse swelling and pain. Overloaded level of Phospholipase A2 causes vascular inflammation which is correlated with coronary artery diseases and acute coronary syndrome. Generally handling for such issues involves removing stings, using of tropical antibiotics and consuming oral painkillers. Prognosis is generally favorable with this treatment but some highly toxic species (*Actidodendron*, *Phyllodiscus* and *Stichodactyla*) have caused severe injuries and are potentially lethal [3] and therefore vaccine therapy becomes essential. Current developments in the ground of computational tools help us to calculate epitope from antigenic proteins in a definite way that assist to make a specific, tenable and optimized peptide-based vaccine design planning. Consequently, it is very effortless to predict the peptide binding leukocyte antigen (HLA) alleles using structural and modeling methodologies. To design vaccine against sea anemone toxins IEDB analysis resource server is used to epitope and MHC-I, MHC-II molecule. To predict allergenicity Aller Hunter server is used. Haddock 2.2 server was used to predict *in silico* docking of toxins with HLA alleles. Lower energy score represents good binding capability of receptor and ligand. This work is an attempt to respond to the need for novel tools to achieve the success against sea anemone bites. Here we recommend the improvement of a multi-epitope vaccine candidate which could guide to the generation of a more potent protective immune response.

MATERIALS AND METHODS

Collection of protein sequence: The five types of sea anemone toxins were retrieved from Uniprot database [4].

Linear – B-cell epitope prediction: Linear – B-cell epitope of sea anemone toxins were predicted by BepiPred 2.0 server [5].

Cytotoxic T- lymphocytes (CTL) epitope prediction: Cytotoxic T - Lymphocytes of sea anemone toxins were predicted through NetCTL1.2 server [6].

Helper T-lymphocytes (HTL) epitope prediction: Helper T-lymphocytes of sea anemone toxins were predicted by Net MHCII2.2 web server [7].

Antigenicity and allergenicity prediction of the vaccine candidate: Antigenicity of predicted vaccine was predicted by VaxiJen 2.0 server [8]. AllerTOPv.2 [9] and Allergen FP [10] servers were used to predict allergenicity of vaccine candidate.

Physiochemical properties and solubility prediction: Physiochemical properties of chimeric protein were predicted by Protparam server [11] and Protein sol server [12] was used to predict solubility of predicted vaccine.

Secondary structure prediction: Secondary structure of chimeric vaccine was analyzed by GORIV server [13].

ISSN: 0975-8585

Tertiary structure prediction and tertiary structure validation: Tertiary structure of vaccine was analyzed by Swissmodel server [14] and that tertiary structure was validated by ANOLEA [15], PROS [16] and verifies 3D server [17].

Molecular docking of subunit vaccine with human immune receptor (TLR4): Molecular docking of subunit vaccine with human immune receptor (TLR4) was carried out by Cluspro 2.0 serve [18] and the binding affinity was predicted by Prodigy Server (Xue et al. 2016) [19].

Amino acid interaction prediction: Amino acid interaction of subunit vaccine and human immune receptor was analyzed by Ligplot+ server (Wallace et al 1996) [20].

Codon optimization prediction: In order to optimize codon usage of the vaccine construct in *E coli*, the Java codon Adaptation tool (JCat) (Grote et al. 2005) [21] was used and recombinant plasmid was designed by SnapGene software (https://www.snapgene.com/snapgene-server) [22].

Immune simulation: C-ImmSim server was used to yield actual immune responses after injecting secondary dose (https://kraken.iac.rm.cnr.it/C-IMMSIM/) [23].

RESULTS

The amino sequences for sea anemone toxins were retrieved from Uniprot database. These five toxins were the representatives of five types of sea anemone toxins. They were selected according to their greatest length. Table 1 represents brief description of five types of sea anemone toxins.

Table 1: Brief description of five type Sea anemone toxin family

Toxin name (Uniprot Accession no.)	Species name	Amino acid length	Protein sequence
NaV blocker toxin family - Am-1 [**24**] (P69929)	*Antheopsis maculata*	233	MKRIFIVALLFATCLVNAKPSINDADIKRE PEPNVAVPPCGDCYQQVGNTCVRVPSLC PSRKREPEPNVAVPPCGDCYQQVGNTCV RVPSLCPSRKREPEPNVAVPPCGDCYQQV GNTCVRVPSLCPSRKREPEPNVAVPPCGD CYQQVGNTCVRVPSLCPSRKREPEPNVA VPPCGDCYQQVGNTCVRVPSLCPSRKREP EPNVAVPPCGDCYQQGNTCVRVPSLPSR KR
Kv blocker toxin family - Toxin MsePTx1 [**25**] (P0DMD7)	*Metridium senile*	90	MKLQLIVVLLIALVIVSLVDGEMLEKRCK NRLKKCTSNSECCDKKDRGGRKLRCLTC DERHCLTYKQCLFYPGLQKVKRLCFTYCR CTI
Cytolysin toxin family- Toxin PsTX-20A [**26**] (Q8IAE2)	*Phyllodiscus semoni*	226	MRHFVVFLYMFLALSIPTAFAKKHIVTKK GNHQDITNDNEGENAEKKSAAVAGAVIA GGELALKILTKILDEIGKIDRKIAIGVDNES GLKWTALNTYYKSGASDVTLPYEVENSK ALLYTARKSKGPVARGAVGVLAYKMSSG NTLAVMFSVPFDYNLYTNWWNVKIYDG EKKADEKMYNELYNNNNPIKPSIWEKRD LGQDGLKLRGFMTSNGDAKLVIHIEKS

ISSN: 0975-8585

Toxin name (Uniprot Accession no.)	Species name	Amino acid length	Protein sequence
Other toxin family - Nematocyte Protein expressed Protein 6 [**27**] (K7Z9Q9)	*Nematostella vectensis*	287	MKGFIFAGVLVSALICLAEGKPFDNLELV EDDMLMTKEQKEAYLAHQNGRVRRAAL RDRYLWPQGKIPYTFSDDIDQAGRELAE RAMNHWMSRTCLRFSPRRREHAYIEFQY DGRCRARVGYTGEARQKVSIGSALDPCPL GSVIHELGHGIGFFHEHSRPDRDEYVNIN VNNMREGAESNFRKDNGYFVDSRGQDY DYGSIMHYSKYQGNNAFNAVVMEPIQRG AEIGQRDGLSAGDIRQTNLMYKCNAQGD SELQPVNDEDEDKDGGDSKKKPDPKGPK PGEIEE
Phospholipase A2 family - Phospholipase A2 [**28**] (Q8WS88)	*Adamsia palliata*	156	MQLYTYFFTFSLVLILALADQENKSLDFT QEGGIAKRGAFQFSYLIKKYTGRNPLDYW GYGCWCGLGGKGTPVDGVDWCCYHHD MCFNSITQGPRPTCSKNAPYHKNYYFSGL KCSTGWLTSKCGRAICACDIAAVKCFMR NHFNNKYQNYKKNIC

Linear B-cell epitope prediction

Linear B-cell epitopes of varying residue lengths were predicted with BepiPred 2.0 server. Total 36 epitopes were predicted and described and represented in Table 2.

Table 2: Predicted B-cell epitopes of five type sea anemone toxins

Sl. no	Start	End	Peptides	Length (AA)
			B-cell Epitope of Am-1(NaV blocker sea anemone toxin)	
1	6	9	IVAL	4
2	12	38	ATCLVNAKPSINDADIKREPEPNVAVP	27
3	41	70	GDCYQQVGNTCVRVPSLCPSRKREPEPNVA	30
4	75	120	GDCYQQVGNTCVRVPSLCPSRKREPEPNVAVPPCGDCYQQVGNTCV	46
5	124	154	SLCPSRKREPEPNVAVPPCGDCYQQVGNTCV	31
6	159	172	LCPSRKREPEPNVA	14
7	177	208	GDCYQQVGNTCVRVPSLCPSRKREPEPNVAVP	32
8	211	228	GDCYQQGNTCVRVPSLPS	18
			B-cell Epitope of Toxin MsePTx1(Kv blocker sea anemone toxin)	
1	20	53	DGEMLEKRCKNRLKKCTSNSECCDKKDRGGRKLR	34
2	60	60	R	1
3	62	65	CLTY	4
4	71	78	YPGLQKVK	8
			Toxin PsTX-20A (Cytolysin type sea anemone toxin)	
1	21	47	AKKHIVTKKGNHQDITNDNEGENAEKK	27
2	60	61	EL	2
3	74	75	GK	2
4	103	117	ASDVTLPYEVENSKA	15
5	123	130	RKSKGPVA	8
6	170	178	DGEKKADEK	9
7	184	204	YNNNNPIKPSIWEKRDLGQDG	21
			B-cell Epitope of Nematocyte Protein expressed Protein 6	
1	17		LAEGKPFDNLELVE	14
2	36	63	TKEQKEAYLAHQNGRVRRAALRDRYLWP	28

ISSN: 0975-8585

Sl. no	Start	End	Peptides	Length (AA)
3	74	82	DIDQAGREL	9
4	84	84	E	1
5	100	105	PRRREH	6
6	137	138	DP	2
7	158	164	HSRPDRD	7
8	176	197	EGAESNFRKDNGYFVDSRGQDY	22
9	209	234	QGNNAFNAVVMEPIQRGAEIGQRDGL	26
10	248	284	CNAQGDSELQPVNDEDEDKDGGDSKKKPDPKGPKPGE	37
			B-cell Epitope of Phospholipase A2 type sea anemone toxin	
1	20	37	DQENKSLDFTQEGGIAKR	18
2	50	56	TGRNPLD	7
3	66	83	LGGKGTPVDGVDWCCYHH	18
4	93	110	GPRPTCSKNAPYHKNYYF	18
5	116	116	S	1
6	118	121	GWLT	4
7	143	153	HFNNKYQNYKK	11

Cytotoxic T Lymphocytes (CTL) epitopes prediction

A total of 100 CTL (9 mer) ligand was predicted for the five selected toxins using NetCTL1.2 server set at the default threshold score for epitope identification. From these, 22 epitopes were selected either on the basis of their high scores or on their overlap with predicted linear B-cell epitope. Table 3 represents total number of cytotoxic T lymphocyte (CTL epitopes) of sea anemone toxins.

Table 3: Predicted CTL and HTL epitopes of five type sea anemone toxin family

Toxin name	CTL	HTL
	NET1.2CTL server	NETMHCII2.3 server
Am-1(NaV blocker) - 233 aa	Nil	Nil
Toxin MsePTx1(Kv blocker) - 90 aa	LVDGEMLEK(18) LTYKQCLFY(63)	Nil
Toxin PsTX-20A(Cytolysin) - 226 aa	MRHFVVFLY(1) WTALNTYYK(92) GASDVTLPY(102) VENSKALLY(112) MSSGNTLAV(141) VMFSVPFDY(149) SVPFDYNLY(152) YTNWWNVKI(160)	VVFLYMFLALSIPTA(5) VFLYMFLALSIPTAF(6) FLYMFLALSIPTAFA(7) LYMFLALSIPTAFAK(8) YMFLALSIPTAFAKK(9) MFLALSIPTAFAKKH(10) FLALSIPTAFAKKHI(11) VLAYKMSSGNTLAVM(136) LAYKMSSGNTLAVMF(137)
Nematocyte Protein expressed Protein 6(Other) - 287 aa	MTKEQKEAY(35) YLAHQNGRV(43) HSRPDRDEY(158) FVDSRGQDY(189) DSRGQDYDY(191) GSIMHYSKY(200) DIRQTNLMY(238)	Nil
Phospholipase A2- 156aa	FTFSLVLIL(8) YTGRNPLDY(49) PTCSKNAPY(96) NAPYHKNYY(101) MRNHFNNKY(140)	Nil
Total Epitopes	22	9

ISSN: 0975-8585

Helper T - Lymphocytes (HTL) epitopes prediction

High binding MHC-II epitopes for human alleles HLA-DR, HLA-DQ and HLA-DP, predicted with the NetMHCII2.2 web server based on their IC50 scores, were defined as HTL epitopes. A total of 9 high binding HTL epitopes were selected for the final vaccine peptides some of the predicted B-cell epitopes overlapped with HTL epitopes.

Construction of multi-epitope subunit vaccine

The total numbers of predicted epitopes used in designing the vaccine were 36 linear B-cell epitope, 22 CTL epitopes and 9 HTL epitopes. The predicted vaccine sequences contain linear B-cell epitopes and T-cell epitopes which were fused using GPGPG and AAY linkers. In order to potentiate antigen specific responses, the TLR4 (PDB Id: 4G8A) agonist and 50s ribosomal L7/L12 (Locus RL7_MYCTU, accession number P9WHE3) were chosen as an adjuvant which was added to the amino terminus of the vaccine peptide using an EAAAK linker. An extra 6xHis tag was attached to the C-terminal to help in protein purification and identification. The final vaccine peptide generated consisted of 1176 amino acid residues. Figure 1 represents the final multiepitope vaccine peptide.

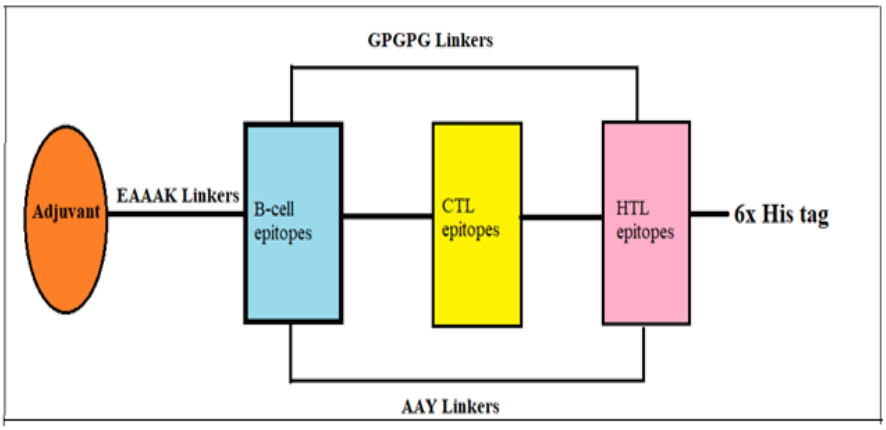

Figure 1: Schematic presentation of the final multiepitope vaccine peptide. The 1176 amino acid long peptide sequence containing an adjuvant (orange) at the amino terminal end linked with the multiepitope sequence through an EAAAK linker. B epitope (blue) and HTL epitopes (pink) are linked using GPGPG linkers while the CTL epitopes (yellow) are linked with the help of AAY linkers. A 6x- His tag is added at the Carboxy terminus for purification and identification purposes.

Prediction of the antigenicity and allergenicity of the multiepitope vaccine

The Antigenicity of the final sequence (including adjuvant sequence) was predicted by the Vaxijen 2.0 server to be 0.7028 with a bacterial model and 0.5781 with a parasitic model at a threshold of 0.5 and 0.884446 with Antigen Pro. The main vaccine sequence (without adjuvant) gave scores of 0.6936 and 0.5871 with bacteria and parasite models respectively of 0.5 on Vaxijen 2.0 and 0.842020 on Antigen Pro. The results indicated that the generated sequences (with and without adjuvant) are both antigenic in nature. The vaccine sequence with and without adjuvant were both predicted to be non-allergic on both the Aller Topv.2 and Allergen FP server.

Table 4: Predicted Antigenicity and allergenicity of the multiepitope vaccine

Predicted Multiepitope vaccine	Vaxijen 2.0 server		AllerTopv.2 server	Allergen FP server
	Bacterial model	Parasitic model		
With Adjuvant	0.7028	0.884446	Non allergen	Non allergen
Without Adjuvant	0.6936	0.5871		

ISSN: 0975-8585

Physiochemical properties and solubility prediction

Table 5 represents physiochemical parameters of multiepitope vaccine. The molecular weight of the predicted vaccine is 132136.49KD with a theoretical isoelectric point value (pI) of 8.06. The protein is then predicted to be basic in nature based on the pI. The estimated half-life is 1 hour (mammalian reticulocyte), 30 minutes (yeast in vivo), and 10 hours (*E coli* in vivo). An instability index (II) of 38.76 was predicted classifying the protein as stable (II of .40 indicates instability). The estimated aliphatic index was predicted to be 70.92, indicating thermos-stability. The predicted Grand Average of hydropathicity (GRAVY) was -0.5111. The negative value of protein indicates the protein is hydrophilic in nature and this protein may be interacting with water molecules. 25.85 mol% aliphatic and 12.245 mol% aromatic amino acid are predicted in this vaccine. The mol % of non-polar amino acid is 54.167 mol % of polar amino acid is 45.833. 27.041 mol % of charged and 15.051 mol % basic amino acid is predicted n this vaccine. Extinction of co-efficient of protein is a measurement of how strongly a protein absorbs light at a given wavelength. Hence the predicted value is 139360$M^{-1}cm^{-1}$ i.e. this predicted vaccine strongly absorbs light at a given wavelength. If expression as inclusion bodies is 1, then it can be predicted that expression of proteins that are toxic to the host cell. Here predicted inclusion bodies are 0.806. That means predicted vaccine is not toxic to the host cell.

Table 5: Physiochemical parameters of multiepitope vaccine

Physiochemical parameters of multiepitope vaccine							
Molecular weight(MW)	**Residue**	**Isoelectric point (pI)**	**Extinction coefficient**	**Inclusion bodies**	**Aliphatic (Mol%)**	**Aromatic (Mol%)**	**Non polar (Mol%)**
132136.49	1176	8.0610	139360M^{-1} cm^{-1}	0.806	25.850	12.245	54.167
Polar (Mol%)	**Charged (Mol%)**	**Basic (Mol%)**	**Acidic (Mol%)**	**Instability index**	**Aliphatic index**	**GRAVY**	**Solubility**
45.833	27.041	15.051	11.990	38.76	70.92	-0.511	100%

Secondary Structure prediction

The final predicted vaccine was predicted to contain 28.66% alpha helix, 19.98% Extended strand and 51.36% random coil (Table 6). Graphical representation is shown in Figure 2.

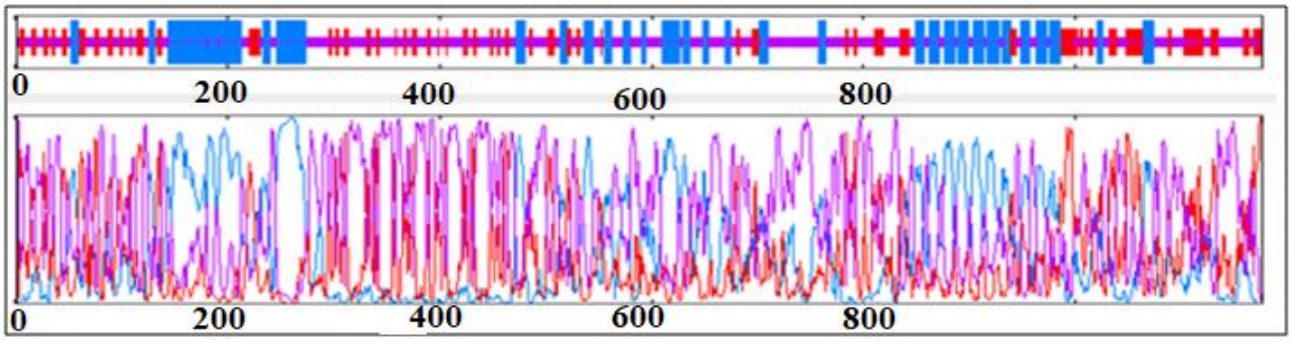

Figure 2: Graphical representation of secondary structure of multiepitope vaccine

Table 6: Secondary structure prediction of multiepitope vaccine

Secondary structure prediction of multiepitope vaccine									
α helix %	**3_{10} helix %**	**Pi helix %**	**β bridge %**	**Extended strand %**	**β turn %**	**Bend region %**	**Random coil %**	**Ambiguous %**	**Other States %**
28.66	0.00	0.00	0.00	19.98	0.00	0.00	51.36	0.00	0.00

ISSN: 0975-8585

Tertiary structure modelling

The tertiary structure of predicted vaccine was carried out by Swissmodel server. The Swissmodel server predicted three tertiary structure models of predicted vaccine based on three templates (4WDC.1A, 4V4P.1.J, 3ULA.1. B) of which 3ULA.1. B gives 99% sequence identity. Figure 3 reflects the Homology model of the tertiary structure of the predicted vaccine supported by the Ramchandran plot analysis. Ramachandran plot analysis reveals 82.4% ,16% and 0% of protein residues in favoured, allowed and disallowed region respectively.

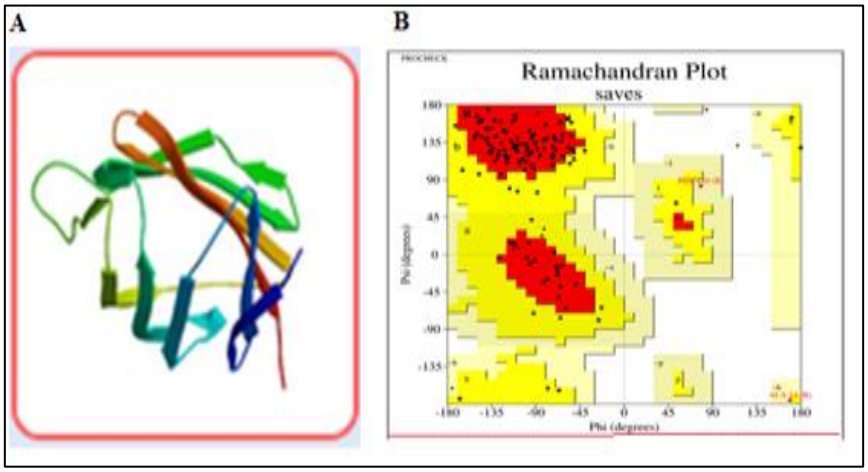

Figure 3: Protein modelling and refinement of multiepitope vaccine. A. The final 3d homology model of multi-epitope vaccine. B. Ramachandran plot analysis.

Validation of tertiary structure

Validation of tertiary structure was validated by three servers, named -Verify 3D, Anolea and Prosa server. The z-score of Anolea (-1.72) and Prosa (-5.18) indicates the better model on the basis of lower value (Table 7). The Verify 3D also evaluates the better model quality.

Table 7: Validation of tertiary structure of multiepitope vaccine

Anolea server	Prosa II server	Verify 3D server
-1.72	-5.18	80.71% of the residue have average 3D-1Dscore > = 0.2

Docking prediction and Amino acid interaction prediction

The predicted vaccine was docked with human TLR4 (4N7N) receptor. It was done by Cluspro 2.0 server. The scoring function predicted was done by Prodigy server. The docked molecule showed -11.2Kcal mol^{-1} free energy and 5.7E-09 Kd(M) value at 25^0 C (Table 8). This result showed satisfactory docking was performed. Ligplot+ server predicted that Asn31, His46, Asp83, Ser87, Tyr 86, Asp85, ser29, Gly94, Lys93, Arg52, Tyr26 amino acids of predicted vaccine involved in docking. The above amino acids of vaccine make H-bonded contact with human TLR4. Besides H-bonded contacts Phe48, Arg90, Leu92, Cys89, Glu95, Gln5, Ser82, Lys4, Asp84, Tyr6, Trp7, Ile50 and Pro27 amino acids were involved in hydrophobic contacts. Figure 4 shows amino acid interaction of the docked molecule.

Table 8: Scoring function prediction of docked model (multiepitope vaccine - Human TLR4)

ΔG (kcal mol^{-1})	K_d (M) at 25.0
-11.2	5.7E-09

ISSN: 0975-8585

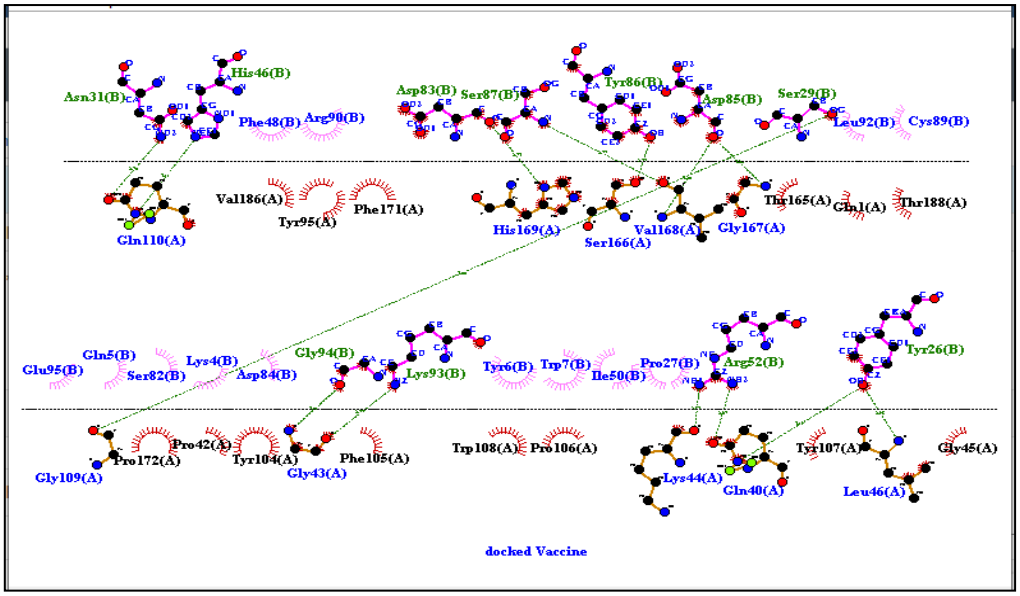

Figure 4: Predicted amino acid interaction by Ligplot+ server.

Codon optimization of the final vaccine construct

For maximal protein expression, the Java Codon Adaptation tool (Jcat) was used to optimize codon usage of the vaccine construct in *E. coli* (strain K12). The length of the optimized codon sequence was 4357 bp. The codon Adaptation index (CAI) of the optimized nucleotide sequence was 0.99 and the average GC content of the adapted sequence was 50.25% showing the possibility of good expression of the vaccine candidate in the *E. coli* host. Finally the sequence of the recombinant plasmid was designed by inserting the adapted codon sequence into pET-21c(+) vector using Snapgene software. Figure 5 represents *in silico* restriction cloned structure of final vaccine and pET21c(+) expression vector.

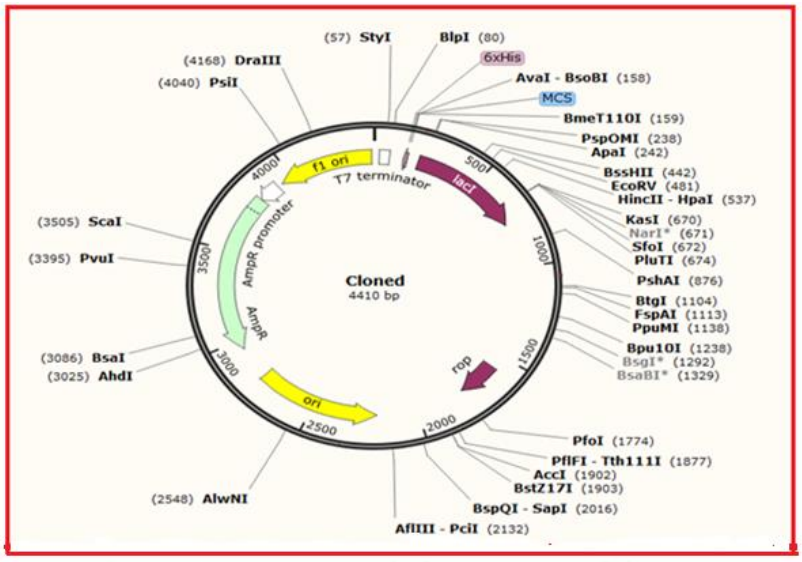

Figure 5: In silico restriction cloning of the final vaccine sequence into the pET21c(+) expression vector. The His-tag is located at the Carboxy terminal end.

Immune simulation

C-ImmSim is an immune simulation server which yielded results that are consistent with actual immune responses because it is evidenced by a universal marked increase in the generation of secondary responses. The primary response was characterized by high levels of IgM + IgG. The secondary responses

ISSN: 0975-8585

(after 30 days) show marked increases in B-cell population and in levels of IgG + IgM, IgM antibodies with a corresponding decrease in antigen concentration. This result indicates development of immune memory and consequently increased clearance of the antigen upon subsequent exposures. A similarity high response was seen in the T_H (helper) and Tc (cytotoxic) cell populations with corresponding memory development. It was also observed that the production of IFN-δ was stimulated after immunization. The C-ImmSim online server, which can be accessed at https://150.146.2.1/C-IMMSIM/index.php, was used to calculate the virtual immune simulation process. The predictions were based on a position-specific scoring matrix (PSSM) for machine learning methods, and the output indicated stimulation in three regions: bone marrow, thymus, and lymph node. The simulation was conducted using default parameters, including a random seed of 12345, a simulation volume of 10, and 100 simulation steps [29]. The result is illustrated in Figure 6 (a – e).

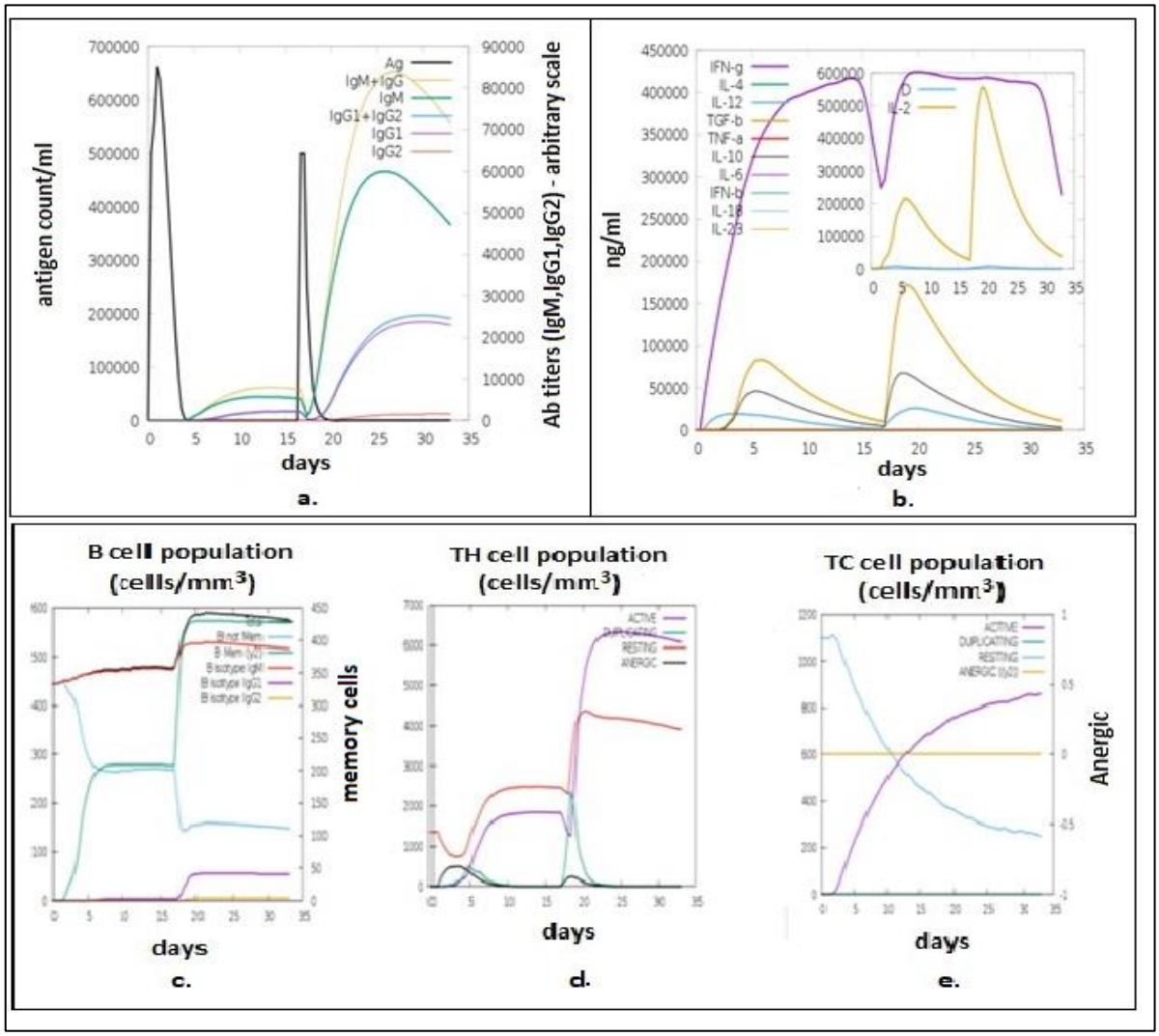

Figure 6: C-ImmSim presentation of an in silico immune simulation with the chimeric vaccine (a) Immunoglobin production in response to antigen injections (black vertical line) specific subclasses are indicated as coloured peaks. (b) C-ImmSim simulation of the Cytokine level induced by two injections given 30 days apart. The insert plot shows IL-2 level with the Simpson index; D indicated by the dotted line. Increase in D overtime indicates emergence of different epitope-specific dominant clones of T-cells. The smaller the D- value, the lower the diversity. (c) The evolution of B-cell populations after two injections (d) The evolution of T-helper and (e) T-cytotoxic cell populations per state after the injections. The resting state represents cells not presented with the antigen while the anergic state represents tolerance of the T-cells to the antigen due to repeated exposures.

DISCUSSION

This work is focussed on the *in silico* design and development of a potential multiepitope vaccine against five types of sea anemone toxins. As any vaccine generated may have both prophylactic and therapeutic potentials, these toxins are preferentially targeted in vaccine development approaches. The potential for cross-protection also exists as the selected proteins and predicted epitopes used in generating the computational vaccine exhibited considerable conservation across the related cnidarians analysed.

The molecular weight of predicted vaccine is 132136.49 and average isoelectric point is 8.061. So, it was predicted that the input sequence has a 100 % chance of solubility when over expressed in *E coli*. Vaccine solubility helps in better interaction with host body [30]. As, pI value is 8.061, that indicate this recombinant protein is basic in nature. An instability index of 38.76 was predicted which classify the protein as stable upon expression, thus further strengthening is potential for use. The aliphatic protein is thermally stable. Secondary structure analyses indicate the recombinant protein is predominant in coils (51.36%) with 32.31% overall disorderedness. The 3D structure of predicted vaccine showed desirable properties based on Ramachandran Plot prediction. To predict potential immune interaction between TLR4 and the recombinant vaccine peptide was carried out since a TLR4 agonist was used as an adjuvant in the designed chimera. The predicted binding energies and binding strength indicated satisfactory binding efficacy. Codon optimization was carried out to achieve high level expression of our recombinant vaccine protein in *E coli* (strain K12). Both the codon adaptability index (0.99) and the GC content (50.25%) were favourable for high level expression of the protein in *E coli*.

Immune simulation shows results consistent with typical immune responses. A general increase in the generated immune responses was followed by repeated exposure to the antigen. The development of memory B-cells and T-cells was evident with memory in B-cells lasting several months. Helper T-cells were particularly simulated. Another interesting observation was that the level of INF-δ and IL-2 rose after 1st injection and remained at peak levels following repeated exposures to the antigen. This indicates high levels of T_H cells and consequently efficient Ig production supporting a hormonal response. The Simpson index, D for investigation of clonal specificity suggests a possible diverse immune response. This type of work was done in 2019 where Ropon-Palacios *et al.* (2019) investigated the *in silico* binding of a novel multi-component vaccine (32.5 kDa), intended by four conserved epitopes from Latin American species such as *Leishmania panamensis, Leishmania mexicana*, *Leishmania braziliensis*, and *Leishmania guyanensis*, with the TLR4/MD2 receptor complex, showing a stable interaction [31].

CONCLUSION

The safety and logical design of the multi-epitope vaccines lead researchers to contrive stronger, stable, and efficient vaccine candidates against many pathogens and diseases. Thus, this new field of immunoinformatics accumulates time and experimental resources and justify further exploration. The objective of the present study was to design a novel Multiepitope vaccine against sea anemone toxins. Inducing T-cell epitopes derived from five type toxins of sea anemone are arranged together by different linkers (GPGPG, AAY, EAAAK) and a TLR-4 agonist as adjuvant (50S ribosomal L7/L12). This recombinant vaccine model in the present study demonstrated is antigenic, allergenic, solubility, safety, and physico-chemical properties. Furthermore, sufficient binding scores and members were predicted between the vaccine candidate and the human TLR-4 receptor, along with stronger cell-mediated immune stimulation. Finally in this study Immunoinformatics tools were employed to design a potential vaccine peptide again sea anemone toxins. The chimeric vaccine peptide could potentially be used as complementary tool to achieve success against sea anemone bites.

ACKNOWLEDGEMENT

The Author Sriparna Ray acknowledges Principal of Bidhan.Chandra. College, Asansol.

REFERENCES

[1] Daly M, Brugler RM, Cartwright P, Collins AG, Dawson MN, Fautin DG, France SC, McFadden CS, Opresko DM, Rodriguez E, Romano SL, Stake JL. The phylum Cnidaria: A review of Phylogenetic patterns and diversity 300 years after Linnaeus. Zootaxa 2007; 1668: 127–182.
[2] Barnes RD. Invertebrate Zoology. Philadelphia, PA: Holt-Saunders International 1982; 150–157.

[3] Mizuno M, Ito Y, Morgan BP. Exploiting the Nephrotoxic Effects of Venom from the Sea Anemone, *Phyllodiscus semoni*, to create a Hemolytic Uremic Syndrome Model in the Rat. Marine Drugs 2012; 10 (7): 1582–1604.
[4] Uniprot Consortium, UniProt: a hub for protein information, Nucleic Acids Research 2014; 43(D1) :D204-D212.
[5] Jespersen MC, Peters B, Nielsen M, Marcatili P. BepiPred-2.0: improving sequence-based B-cell epitope prediction using conformational epitopes. Nucleic Acids Res 2017; (Web Server issue): W24-W29.
[6] Larsen MV, Lundegaard C, Lamberth K, Buus S, Lund O and Nielsen M. Large-scale validation of methods for cytotoxic T-lymphocyte epitope prediction. BMC Bioinformatics 2007; 8:424.
[7] Jensen KK, Andreatta M, Marcatili P, Buus S, Greenbaum JA, Yan Z, Sette A, Peters B, Nielsen M. Improved methods for predicting peptide binding affinity to MHC class II molecules. Immunology 2018; 154(3);394-406.
[8] Doytchinova IA, Flower DR. VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccine. BMC Bioinformatics 2007; 8:4.
[9] Dimitrov I , Bangov I, Flower DR, Doytchinova I. AllerTOP v.2--a server for in silico prediction of allergens. Journal of Molecular Modeling 2014 J;20(6):2278.
[10] Dimitrov I, Naneva L, Doytchinova I, Bangov I. AllergenFP: allergenicity prediction by descriptor fingerprints. Bioinformatics. 2014; 30(6): 846–851.
[11] Gasteiger E, Hoogland C, Gattiker A, Duvaud S, Wilkins MR, Appel RD, Bairoch A. Protein Identification and Analysis Tools on the ExPASy Server.(In) John M. Walker (ed): The Proteomics Protocols Handbook, Humana Press (2005). pp. 571-607
[12] Hebditch M, Carballo-Amador MA, Charonis S, Curtis R,Warwicker J. Protein-Sol: a web tool for predicting protein solubility from sequence. Bioinformatics 2017; 33(19):3098-3100.
[13] Garnier J, Gibrat JF, Robson B. GOR secondary structure prediction method versionIV. Methods in Enzymology. R.F. Doolittle Ed. 1996; 266, 540-553.
[14] Arnold K, Bordoli L, Kopp J and Schwede T. The SWISS-MODEL Workspace: A web-based environment for protein structure homology modeling. Bioinformatics 2006; 22 (2): 195-201.
[15] Melo F, Devos D, Depiereux E, Feytmans E. ANOLEA: a www server to assess protein structures. Proceedings. International Conference on Intelligent Systems for Molecular Biology 1997; 5:187-90.
[16] Wiederstein M, Sippl MJ. ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins. Nucleic Acids Research 2007; 35(2): W407-410.
[17] Bowie JU, Luthy R and Eisenberg D. A method to identify protein sequences that fold into a novel three-dimensional structure. Science 1991; 253 (5016):164-170.
[18] Kozakov D, Hall DR, Xia B, Porter KA, Padhorny D, Yueh C, Beglov D, Vajda S. The Cluspro web server for protein protein docking. Nature Protocols 2017; 12(2): 255-278.
[19] Xue L, Rodrigues J, Kastritis PL , Bonvin AMJJ, Vangone A. PRODIGY: a web-server for predicting the binding affinity in protein-protein complexes. Bioinformatics 2016; 32 (23): 3676–3678.
[20] Wallace AC, Laskowski RA. Ligplot: A program to generate schematic diagrams of protein-ligand interactions. Thornton JM. Protein Engineering 1996; 8 (2):127-134.
[21] Grote A, Hiller K, Scheer M, Munch R, Nortemann B, Hempel DC, Jahn D. JCat: a novel tool to adapt codon usage of a target gene to its potential expression host. Nucleic Acids Research 2005; 33(Web server issue): W526-31.
[22] (https://www.snapgene.com/snapgene-server)
[23] Rapin N, Lund O, Bernaschi M, Castiglione F. Computational Immunology Meets Bioinformatics: The Use of Prediction Tools for Molecular Binding in the Simulation of the Immune System. PLoSONE 2010; 5(4): e9862.
[24] Honma T, Minagawa S, Nagai H, Ishida M, Nagashima Y and Shiomi K. Novel peptide toxins from acrorhagi, aggressive organs of the sea anemone *Actinia equina*. Toxicon 2005; 46(7): 768–774.
[25] Orts DJ, Peigneur S, Madio B, Cassoli JS, Montandon GG, Pimenta AM, Bicudo JE, Freitas JC, Zaharenko AJ and Tytgat J. Biochemical and electrophysiological characterization of two sea anemone type 1potassium toxins from a geographically distant population of *Bunodosoma caissarum*. Marine Drugs 2013; 11:655-679.
[26] Nagai H, Oshiro N, Takuwa-Kuroda K, Iwanaga S, Nozaki M and Nakajima T. Novel proteinaceous toxins from the nematocyst venom of the Okinawan Sea anemone *Phyllodiscus semoni* Kwietniewski. Biochemical and Biophysical Research Communities 2002; 294(4):760–763.

[27] Moran Y, Praher D, Schlesinger A, Ayalon A, Tal Y and Technau U. Analysis of soluble protein contents from the nematocysts of a model sea anemone sheds light on venom evolution. Marine Biotechnology (NY) 2013; 15(3): 329-339.
[28] Talvinen KA and Nevalainen TJ. Cloning of a novel phospholipase A2 from the cnidarian Adamsia carciniopados. Comparative Biochemistry and Physiology. Part B, Biochemistry and Molecular Biology 2002; 132(3): 571–578.
[29] Rapin N, Lund O, Bernaschi M, Castiglione F. Computational immunology meets bioinformatics: the use of prediction tools for molecular binding in the simulation of the immune system. PLoS One 2010; 5: e9862.
[30] Franck CO, Fanslau L, Bistrovic Popov A, Tyagi P, Fruk L. Biopolymer-based carriers for DNA vaccine design. Angewandte Chemie International Edition 2021; 60:13225–13243.
[31] Ropón-Palacios G, Chenet-Zuta ME, Otazu K, Olivos-Ramirez G.E, Camps I. Novel multi-epitope protein containing conserved epitopes from different Leishmania species as potential vaccine candidate: Integrated immunoinformatics and molecular dynamics approach. Computional Biology and Chemistry 2019; 83.