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An Immuno-informatics driven Epitope study from the molecular interaction of JEV non-structural (NS) proteins with Ribophorin (RPN)

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Abstract:
Japanese encephalitis (JE) is an acute viral infection of the central nervous system where the JE virus infects the lumen of the endoplasmic reticulum (ER) and rapidly accumulates substantial amount of seven different nonstructural proteins (NS). These NS proteins tend to bind on a glycoprotein receptor, ribophorin (RPN) resulting in the malfunctioning of ER in host cells, subsequently triggering an unfolded protein response. Therefore, it is of interest to predict the best possible antigenic determinants in the NS protein capable of eliciting immune response as a strategy to combat JE. Hence, it is our interest to explore the most potent NS protein among all showing the best possible molecular interaction with the RPN receptor present on ER. However, the structures of these NS protein and RPN are currently unknown. Thus, we modeled their structures using the established homology modeling techniques in the MODELLER 9v10 software. The molecular docking of NS proteins with RPN was subsequently completed using the Discovery Studio 2.5 software suite. The docked conformations of RPN with NS were further analyzed and its graphical interpretations were presented for identifying the most potential NS protein for efficient epitope activity. Further, the B cell epitopes were mapped using BCPred and the predicted epitope regions are documented. The data presented in this report provides useful insights towards the design and development of potential epitopes to generate a vaccine candidate against JEV.

Keywords: JEV, Non structural protein (NS), Ribophorin (RPN), B cell epitopes, homology modeling

Background:
Japanese encephalitis virus (JEV) belongs to the Flaviviridae family of dengue virus and yellow fever virus. It is one of the major causes of encephalitis in Eastern and Southern Asia. JEV infection of host cells produces three structural and seven non-structural proteins (NS). The structural proteins consist of positive sense single stranded RNA genome which is packaged in the capsid and formed by the capsid protein, acting as a major antigen used to draw out neutralizing antibody response and protective immunity in hosts **[2].** On the other hand, the non-structural, nucleocapsid protein is the most important protein of the virion. JEV is known to infect the lumen of the endoplasmic reticulum (ER) **[1]** thereby accumulating significant amount of nonstructural viral protein. The genome of JEV encodes several nonstructural proteins which are differentiated into NS1, NS2a, NS2b, NS3, N4a, NS4b and NS5. NS3 is a putative helicase, and NS5 is the viral polymerase. These NS proteins tend to bind on a glycoprotein receptor, ribophorin (RPN) present on ER resulting in its malfunctioning and ultimately triggering an unfolded protein response **[3]**.

In response to JEV infection, the host cell produces virus neutralizing antibodies and cytotoxic T cells (CTLs). It has been shown that defense against JEV infection is primarily antibody dependent, and virus-neutralizing antibodies lacking help are sufficient to convey protection **[4, 5].** It is known that small segments of protein called the antigenic determinants or the epitopes are limiting for eliciting the preferred immune

response. Thus, the B-cell epitopes on JEV NS protein can be developed as important determinants as vaccine candidates against viral infection. Since peptide vaccines in which small peptides derived from target protein epitopes are used to aggravate an immune reaction, peptide(s) from JEV protein that forms the virus-neutralizing epitope (s) could, therefore be used for neutralizing antibodies produced against JEV **[6].**

The current developments and continued use of computational tools and techniques for vaccine design help to decrease the time essential to recognize the contender peptide as vaccine by providing data related to its structure function association of virus proteins. Therefore, it is of interest to the explore molecular interaction and binding mechanism of several NS proteins present in JEV with RPN in an attempt characterize the interface residues between interacting molecules with useful insights in predicting epitopes for vaccine design against JEV.

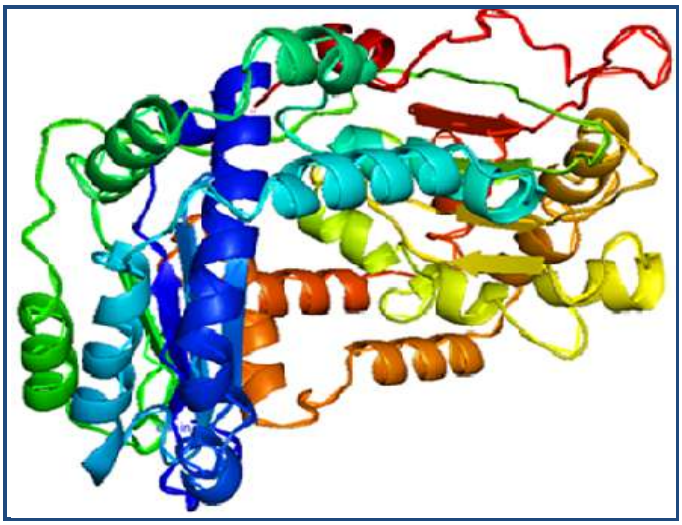

Figure 1: Homology 3D structure model of RPN2 (Ribophorin II) visualized by Pymol

Methodology:

Collection of NS protein sequences

The full length protein sequences of nonstructural (NS) proteins of JEV were retrieved from the NCBI protein database. It is known that JEV infection of host cells produces seven NS proteins namely, (NS) *viz* NS1 (NP_775667.1), NS2A (NP_775668.1), NS2B (NP_775669.1), NS3 (NP_775670.1), NSA (NP_775671.1), NS4B (NP_775673.1) and NS5 (NP_775674.1).

Protein 3D structure modeling of NS and RPN

The 3D structures of NS and RPN are not yet known. Therefore, we generated their homology models using homology modeling **[7]** with Modeller 9v10 **[8]** and SWISS-MODEL from ExPASy server **[9, 10].**

Protein-Protein Docking data

It is known that NS proteins preliminarily interact with RPN of Endoplasmic reticulum. Therefore, it is of interest to explore the interacting interface residues between them. Hence, we explored Protein- Protein interaction of NS class of proteins NS1 Vs RPN1, NS1 Vs RPN2, NS2A Vs RPN1, NS2A Vs RPN2, NS2B Vs RPN1, NS3 Vs RPN1, NS3 Vs RPN2, NS4A Vs RPN1, NS4A Vs RPN2, NS4B Vs RPN1, NS4B Vs RPN2, NS5 Vs RPN1 using PDBe PISA, an interactive tool for the exploration of macromolecular (protein, DNA/RNA and ligand) interfaces residues **[11]** and Discovery studio 2.5 Zdock (Dock Proteins) module for protein-protein docking **[12].**

ZDOCK calculations

ZDOCK is an initial stage rigid body molecular docking algorithm that uses a fast Fourier transform (FFT) method to improve performance for searching in translational space **[12].** All of the available structures were used to calculate the docking poses and the structures obtained were subjected to energy minimization using the smart minimize algorithm (Max steps 200, RMS gradient 0.01) in the program Accelrys Discovery studio 2.5. The resulting **Zdock** scores with the highest value were used as appropriate conformational pose **[13].** After obtaining all protein-protein docking scores we screened the highest NS interaction with RPN score for further analysis.

BCPred

The identification and characterization of B-cell epitopes play an important role in vaccine design, immunodiagnostic tests, and antibody production. Therefore, computational tools for reliably predicting B-cell epitopes in protein sequences are highly desirable. Because it is often valuable to compare predictions of multiple methods, and consensus predictions are more reliable than individual predictions, the BCPREDs server allows users to choose the method for predicting B-cell epitopes among several developed prediction methods. The current implementation of BCPREDS allows the user to select among three prediction methods: (i) our implementation of AAP method **[14]** (ii) BCPred **[15]** (iii) FBCPred **[16].**

Prediction of antigenic peptides

The antigenic peptides server was employed to predict segments from a protein sequence that are likely to be antigenic by eliciting an antibody response. Antigenic peptides were determined using the method of Kolaskar and Tongaonkar **[17]** (www.mifoundation.org). Predictions are based on a table that reflects the occurrence of amino acid residues in experimentally known segmental epitopes. Segments are only reported if they have a minimum size of 8 residues.

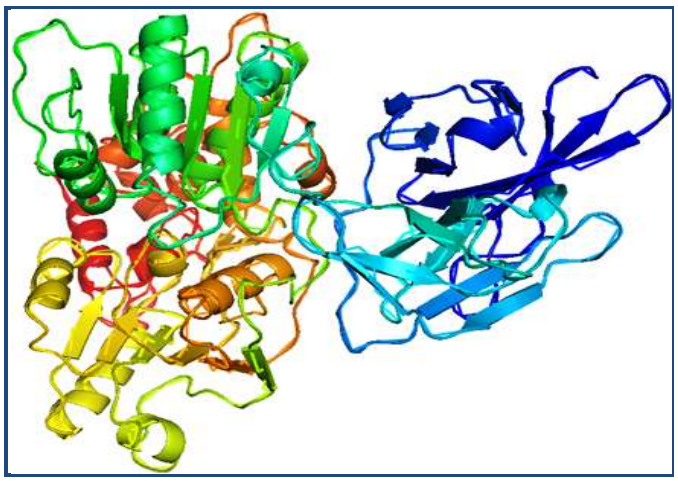

Figure 2: Homology 3D structure model of JEV NS3 visualized by Pymol.

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Results & Discussion:

Prediction and Validation Studies of 3D structures of NS and Ribophorin

Three dimensional structures of NS and RPN were successfully built using Modeller 9v10 **[8]** and SWISS-MODEL from ExPASy server as shown in **Figure 1 & 2 [9].** Suitable templates for structure prediction were obtained using BLASTp program against PDB. Generated 3D structures were further set for validation run using RAMPAGE server from crystallography and Bioinformatics Group, University of Cambridge (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php) on the basis of Ramchandran Plot analysis.

The validation studies of the generated model of RPN and NS showed most of the residues of the modeled proteins in most favored regions, whereas 0.0% of amino acid residues were found in the disallowed region as represented **(Figure 3 & 4), Table 1 (see supplementary material).**

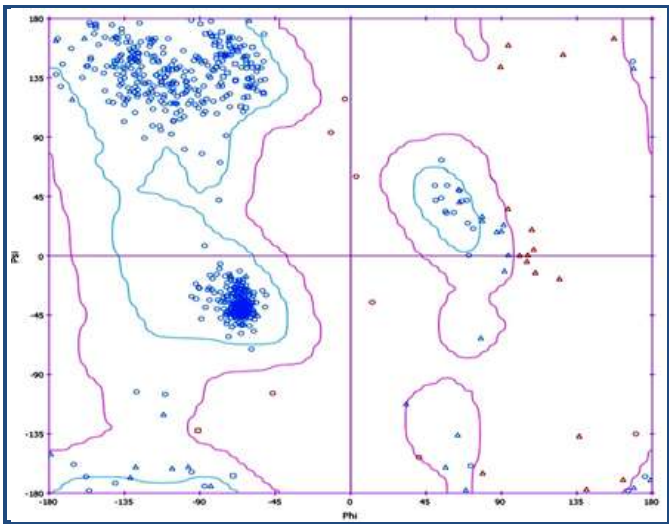

Figure 3: Structural validation of modeled RPN2 protein using Ramachandran plot. Number of residues in favored region (~98.0% expected): 385 (87.7%), Number of residues in allowed region (~2.0% expected): 32 (7.3%) Number of residues in outlier region: 22 (5.0%).

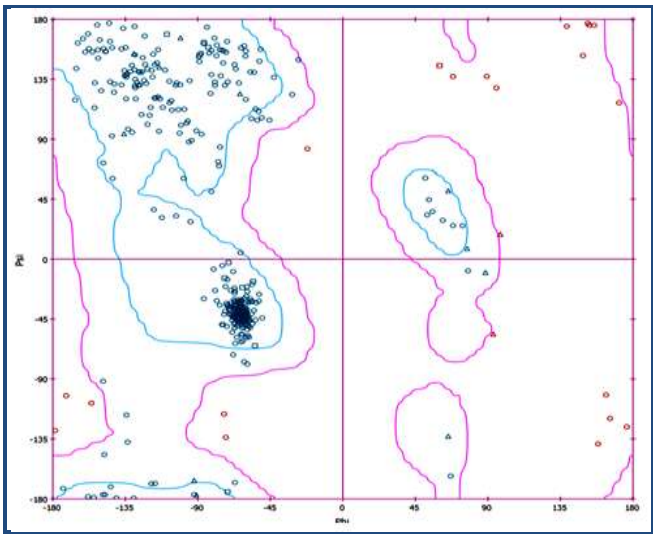

Figure 4: Structural validation of modeled JEV NS3 protein using Ramachandran plot. Number of residues in favored region (~98.0% expected): 587 (95.1%), number of residues in allowed region (~2.0% expected): 21 (3.4%), number of residues in outlier region: 9 (1.5%).

ProSA - Protein Structure Analysis server (https://prosa.services.came.sbg.ac.at/prosa.php) **[18]** has also been used to evaluate energy pattern and verify the structure using Z score, representing the taken as a whole eminence and measures the variation of total energy **[19].** The Z score value of the obtained models of RPN and NS were well within the acceptable range −10 to 10. It has been reported that the Z score is dependent on the length of the protein and negative Z-scores are very good for a reliable model (**Figure 5).**

Protein-Protein docking studies

The binding efficiency between the Nonstructural Proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) and Ribophorins (RPN1 and RPN2) were calculated using ZDOCK. The docked poses were analyzed and the top 10 complexes on the basis of their ER-scores were selected **(Table 2).** 2000 different poses were generated, which were further subjected for refinement using RDOCK, where top 10 poses were selected for further analysis. Finally top 3 complexes on the basis of their binding energy obtained using ER_scores were selected, which includes NS1-RPN2, NS4A-RPN2 and NS3-RPN2, which interacts with binding energies of -42.685 kcal/mol, -45.6684 kcal/mol and -51.0376 kcal/mol respectively (Table 2). Our results revealed that NS3 was found to be interacting most efficiently against RPN2, with highest ER-score i.e. -51.0376 and with maximum hydrogen bonding residues between both the molecules (**Figure 6 & Table 2).**

B cell predicted epitope of NS3

It has already been reported that protection against JEV infection is mainly antibody dependent and thus the prediction of B cell epitopes on JEV nucleocapsid protein may provide important determinants of protection against virus. Subsequently BCPred server was employed which allows users to choose the method for predicting B-cell epitopes among several developed prediction methods **[14].** Based on the protein docking scores obtained above B cell epitopes of specifically NS3 nucleocapsid protein was predicted as presented in **Table 3 (see supplementary material).**

Following were the B cell epitopes predicted having highest sc ore position **Table 4 (see supplementary material).**

(1) 'IFMTATPPGTTDPFPDSNAP'(313) and
(2) 'SAIVQGDRQEEPVPEAYTPN'(163)

These small segments of NS3 protein called antigenic determinants or epitopes obtained above were supposed to be sufficient for eliciting the desired immune response.

Prediction of antigenic peptides

The Nucleocapsid protein (*Japanese encephalitis*) NS3 sequence is 618 residues long. There were 22 antigenic determinants predicted in the sequence **(Table 3).** The highest pick was at start position 537 to end position 558. The antigenic sequence is - NFLELLRTADLPVWLAYKVASN. The average for the whole protein is above 1.0 then all residues having above 1.0 are potentially antigenic. Average antigenic propensity for this

protein was found to be 1.0121. The conformation of the peptide is an important determinant of its immunogenicity, and it may determine whether the anti-peptide antibodies would also recognize the native protein from which the peptide was derived **[20].** Thus, to improve chances of producing anti-peptide antibodies proficient of recognizing JEV nucleo-capsid protein segments from a protein sequence that are likely to be antigenic by eliciting an antibody response has been predicted.

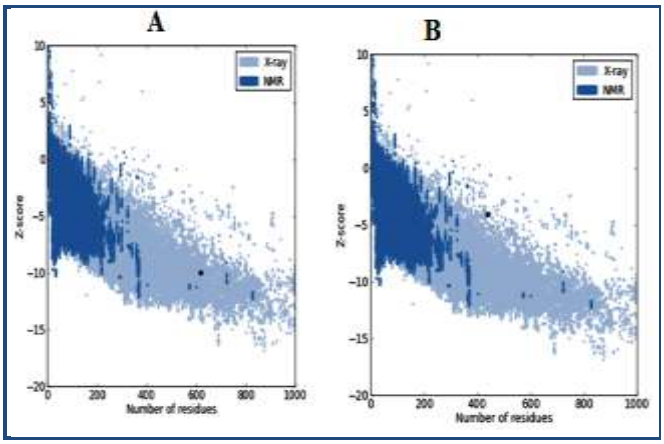

Figure 5: Energy plot of modeled protein (a) NS3 and (b) RPN2 obtained by ProSA web server and corresponding Graph showing Z score of modeled protein.

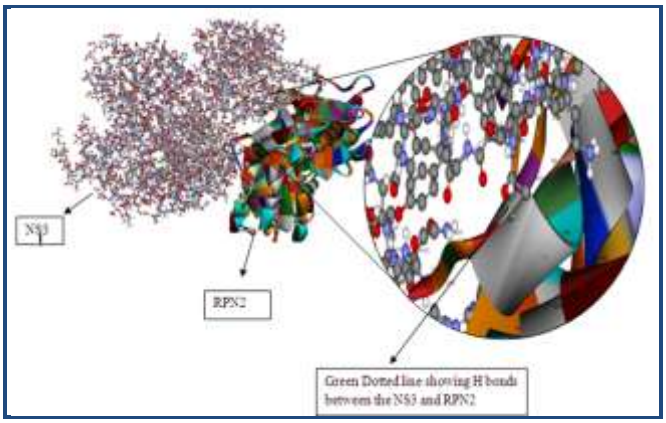

Figure 6: Protein-protein interaction model of JEV-NS3 and RPN2 receptor of Endoplasmic reticulum visualized by Discovery Studio Visualizer.

Conclusion:

The design and development of short peptides as vaccine candidate for JEV is gaining momentum in recent years. Therefore, Thus in the percent study we document predicted epitope like region in the NS3 protein having RPN interaction. Hence, these data could be useful in designing candidates capable of producing antipeptide antibodies which are competent of recognizing JEV specific nucleocapsid protein..

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Supplementary material:

Table 1: Ramachandran plot calculation of 3–D models of NS and RPN protein.

Ramachandran plot statistics	NS1	NS2a	NS2b	NS3	NS4a	NS4b	NS5	RPN1	RPN2
% Residues in most favoured regions	89.0%	92.7%	82.1%	95.1%	98.6%	92.9%	86.6%	92.8%	87.7%
% Residues in allowed region	8.3%	2.8%	14.7%	3.4%	1.4%	3.6%	8.5%	4.2%	7.3%
% Residues in outlier region	2.8%	4.6%	3.2%	1.5%	0.0%	3.6%	4.9%	3.0%	5.0%

Table 2: Docked ER_Scores from different conformations of Non-structural proteins Vs Ribophorin obtained from Protein-Protein Interaction studies.

S.No.	Proteins	Residues involved in H bonding	H Bond Distance (A)	PosNum	ZDock Score	ZRank Score	ER Dock
1	NS1 vs RPN2	ARG190:HH21 - :MET113:SD	2.48096	27	14.56	-88.096	-42.6825
		:ASN457:HN - :HIS22:NE2	2.17859				
		:LYS80:HZ1 - :GLU182:OE2	2.15617				
		:ASN91:HN - :SER153:OG	2.49234				
		:ASN91:HD21 - :SER153:OG	2.49266				
		:ARG96:HH11 - :SER153:OG	2.22892				
		:ARG96:HH21 - :THR149:OG1	2.35745				
		:ARG103:HH12 - :GLU182:OE2	2.22825				
		:LYS116:HN - :GLU183:OE1	2.23274				
		:LYS116:HN - :GLU183:OE2	2.31051				
		:SER121:HG - :GLU471:OE1	2.40299				
2	NS3 vs RPN2	:LYS69:HZ1 - :LEU65:O	1.80352	15	19.52	-89.307	-51.0376
		:GLN200:HE22 - :THR66:OG1	2.31508				
		:THR207:HN - :LEU307:O	2.04321				
		:LYS244:HZ1 - :ALA302:O	2.45368				
		:LYS244:HZ1 - :GLU306:OE1	1.85926				
		:LYS244:HZ2 - :THR303:O	2.30141				
		:GLY2:HN - :GLU183:OE1	2.13856				
		:VAL3:HN - :GLU183:OE1	2.28959				
		:TYR68:HH - :GLU247:OE1	2.38334				
		:ARG187:HH11 - :SER59:OG	2.06368				
		:GLN188:HE22 - :SER248:O	2.05679				
	NS4a vs RPN2	:ILE102:HN - :SER1:O	2.15287	1	11.6	-130.798	-45.6684
		:ARG266:HH22 - :GLU22:O	2.28829				
		:SER4:HN - :CYS100:O	2.21099				
		:LYS19:HZ1 - :GLU156:OE1	2.3189				
		:LYS19:HZ2 - :SER153:O	2.30926				
		:LYS19:HZ3 - :SER153:O	2.38227				

Table 3: Antigenic determinants in NS3 sequence

S.No.	Start Position	Sequence	End Position
1	30	ILGTYQAGVGVMYENVFHTL	49
2	64	KLTPYWG	70
3	93	DDVQVIVVEPGKAAVNI	109
4	111	TKPGVFR	117
5	119	PFGEVGAVSLD	129
6	158	DGSYVSAIVQ	167
7	188	QMTVLDLHP	196
8	202	RKILPQIIKD	211
9	217	LRTAVLAPTRVVAA	230
10	235	ALRGLPVRYQTSAVQR	250
11	256	EIVDVMCHATLTHR	269
12	277	PNYNLFVMD	285
13	289	FTDPASIAARG	299
14	301	IATKVELGEAAAIFM	315
15	330	NAPIHDLQD	338
16	356	AGKTVWFVAS	365

17	379	AGKKVIQLN	387
18	419	GASRVIDCRKSVKPT	433
19	440	GRVILGN	446
20	501	MPNGLVAQLY	510
21	533	NFLELLRTADLPVWLAYKVASN	554
22	597	LDARVYADHQALKW	610

Table 4: Predicted B cell epitopes in NS3 protein

Position	Epitope	Score
313	IFMTATPPGTTDPFPDSNAP	1
163	SAIVQGDRQEEPVPEAYTPN	1
432	PTILEEGEGRVILGNPSPIT	0.998
6	DTPSPKPCSKGDTTTGVYRI	0.997
129	DYPRGTSGSPILDSNGDIIG	0.997
457	QRRGRVGRNPNQVGDEYHYG	0.995
237	RGLPVRYQTSAVQREHQGNE	0.979
108	NIQTKPGVFRTPFGEVGAVS	0.978
87	RKWNGTDDVQVIVVEPGKAA	0.969
386	LNRKSYDTEYPKCKNGDWDF	0.941
566	DGPRTNAILEDNTEVEIVTR	0.939
29	GILGTYQAGVGVMYENVFHT	0.938
510	YGPEREKAFTMDGEYRLRGE	0.886
334	HDLQDEIPDRAWSSGYEWIT	0.828
481	EDDSNLAHWTEAKIMLDNIH	0.799
587	GERKILKPRWLDARVYADHQ	0.742
407	ITTDISEMGANFGASRVIDC	0.738