

# STUDY OF MOLECULAR SURFACE PROPERTY OF PHOSPHOLIPASE A2 (PLA2) FROM NAJA NAJA

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**Abstract-** This study was designed to elucidate the signalling pathways by which secretory phospholipase a2 (sPLA2s) induce in vitro neutrophil migration and PLA2 inhibits platelet aggregation in PRP and explains the decreased clot retraction and retarded and compromised elasticity build up. *Naja naja* venoms are complex and contain several toxic components, including neurotoxins and phospholipases A2 that cause post-synaptic neuromuscular blockade with respiratory paralysis and cardiac arrest. The antigenic epitopes on phospholipase a2 (Pla2) are important determinant of protection against venom. In this analysis, we found the antigenic epitopes 29-GRGGSGTPVDD-39, 77-TCKGDNNACA-86, of protein called the antigenic determinant or the epitope is sufficient for eliciting the desired immune response. Also predict the MHC binder and these MHC Class peptide segments are from a set of aligned peptides known to bind to a given major histocompatibility complex (MHC) molecule as the predictor of MHC-peptide binding. Binding ability prediction of antigen peptides to MHC class molecules is important in vaccine development. The method integrates prediction of peptide MHC class binding; proteasomal C terminal cleavage efficiency of protein.

**Keywords-** phospholipases a2, epitope, hydrophobicity, MHC, surface activity, SVM, PSSM, peptide vaccine

*Abbreviations: Major Histocompatibility Complex, (MHC); Position Specific Scoring Matrices, (PSSMs); Support Vector Machine, (SVM)*

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## I. Introduction

Venomous animals contain concentrates of biologically active substances developed to block vital physiological and biochemical functions of the victims. This has contrasting human health concerns, provide important pharmacological raw material and pose a serious threat to human life and health in tropical and subtropical regions<sup>1</sup>. Phospholipase A2 (PLA2) is the superfamily consists of many different groups of enzymes that catalyze the hydrolysis of the sn-2 ester bond in a variety of different phospholipids. This reaction produces a free fatty acid and lysophospholipid have many different important physiological roles<sup>2</sup>. Phospholipase A2 (PLA2) enzymes from snake venom are toxic and induce a wide spectrum of pharmacological effects, also similarity in primary, secondary and tertiary structures and common catalytic properties. The structure-function relationships and the mechanism of this group of small proteins are subtle, complex and intriguing challenges. It is strongly anticoagulant CM-IV binding properties to factor Xa (its target protein) through the specific anticoagulant site on its surface. In contrast, weakly anticoagulant enzymes, which lack the anticoagulant region fail to bind specifically to the target protein, factor Xa in the coagulation cascade. Our research studies strongly define the target model, which suggests protein-protein interaction rather than protein-phospholipid interaction determines the

pharmacological specificity of PLA2 enzymes<sup>3,4</sup>. Peptide fragments from Phospholipase A2 involved multiple antigenic components directing and empowering the immune system to protect the host from infection. MHC molecules are cell surface proteins, which take active part in host immune reactions and involvement of MHC class-I & II in response to almost all antigens. So a small fragment of antigen can induce immune response against whole antigen. This theme is implemented in designing subunit and synthetic peptide vaccines. The sequence analysis method allows potential drug targets to identify active sites against diseases. The method integrates prediction of peptide MHC class binding affinity, hydrophobicity and relative flexibility of given protein molecule<sup>5,6</sup>.

## II. Methodology

In this research work antigenic epitopes of phospholipase A2 protein from *Naja naja* is determined using the Gomase in 2007, Hopp and Woods, Welling, Parker and Protrusion Index (Thornton) antigenicity<sup>7-9</sup>. The major histocompatibility complex (MHC) peptide binding of phospholipase A2 protein is predicted using neural networks trained on C terminals of known epitopes. In analysis predicted MHC/peptide binding of phospholipase A2 protein is a log-transformed value related to the IC50 values in nM units. MHC2Pred predicts peptide binders to MHC I and MHC II molecules from protein

sequences or sequence alignments using Position Specific Scoring Matrices (PSSMs). Support Vector Machine (SVM) based method for prediction of promiscuous MHC class II binding peptides. SVM has been trained on the binary input of single amino acid sequence<sup>10-15</sup>. In addition, we predict those MHC ligands from whose C-terminal end is likely to be the result of proteosomal cleavage<sup>16,17</sup>.

### III. Results and Interpretations

We found binding of peptides to a number of different alleles using Position Specific Scoring Matrix. A phospholipase A2 protein sequence is 119 residues long, having antigenic MHC binding peptides. MHC molecules are cell surface glycoproteins, which take active part in host immune reactions and involvement of MHC class-I and MHC II in response to almost all antigens (Fig. 7). PSSM based server predict the peptide binders to MHC I molecules of phospholipase A2 protein sequence are as 11mer\_H2\_Db, 10mer\_H2\_Db, 9mer\_H2\_Db, 8mer\_H2\_Db and also peptide binders to MHC II molecules of phospholipase A2 protein sequence as I\_Ab.p, I\_Ad.p, analysis found antigenic epitopes region in putative phospholipase A2 protein (Table 1). We also found the SVM based MHCII-IAb peptide regions; MHCII-IAd peptide regions; MHCII-IAg7 peptide regions and MHCII- RT1.B peptide regions, which represented predicted binders from phospholipase A2 protein (Table 2). The predicted binding affinity is normalized by the 1% fractil. We describe an improved method for predicting linear epitopes (Table 2). The region of maximal hydrophilicity is likely to be an antigenic site, having hydrophobic characteristics, because terminal regions of phospholipase A2 protein is solvent accessible and unstructured, antibodies against those regions are also likely to recognize the native protein (Fig. 1, 2, 5, 6). It was shown that a phospholipase A2 protein is hydrophobic in nature and contains segments of low complexity and high-predicted flexibility (Fig. 3, 4). Predicted antigenic fragments can bind to MHC molecule is the first bottlenecks in vaccine design.

### IV. Conclusion

A phospholipase A2 protein from *Naja naja* peptide nonamers are from a set of aligned peptides known to bind to a given MHC molecule as the predictor of MHC-peptide binding. MHC II molecules bind peptides in similar yet different modes and alignments of MHCII-ligands were obtained to be consistent with the binding mode

of the peptides to their MHC class, this means the increase in affinity of MHC binding peptides may result in enhancement of immunogenicity of phospholipase A2 protein. These predicted of phospholipase A2 protein antigenic peptides to MHC class molecules are important in vaccine development from *Naja naja*.

### V. References

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Table 1- PSSM based prediction of MHC ligands, from whose C-terminal ends are proteosomal cleavage sites

MHC-I	POS.	N	Sequence	C	MW (Da)	Score	% OPT.
9mer_H2_Db	107	PYN	DNNYNIDLK	ARC	1090.15	13.298	26.40 %
9mer_H2_Db	102	CFA	GAPYNDNNY	NID	1009.0	12.554	24.93 %
10mer_H2_Db	48	QVH	DNCYNEAEKI	SGC	1180.26	22.756	38.66 %
10mer_H2_Db	58	EKI	SGCWPFYFKTY	SYE	1210.41	15.882	26.98 %
11mer_H2_Db	95	CDR	LAAICFAGAPY	NDN	1078.31	15.928	20.04 %
11mer_H2_Db	90	ASV	CDCDRLAAICF	AGA	1211.45	13.598	17.11 %

Table 2- SVM based prediction of MHC class II binding peptides from phospholipase A2 protein

MHC ALLELE	Rank	Sequence	Residue No.	Peptide Score
I-Ab	1	GTPVDDLDR	34	0.749
I-Ab	2	GAPYNDNNY	102	0.597
I-Ab	3	PYFKTYSYE	62	0.518
I-Ab	4	IKCTVPSRS	9	0.464
I-Ad	1	ADYGICYCGR	22	0.524
I-Ad	2	LDRCCQVHD	40	0.519
I-Ad	3	CYCGRGGSG	26	0.468
I-Ad	4	GRGGSGTPV	29	0.455
I-Ag7	1	GDNNACAAS	80	1.741
I-Ag7	2	WDFADYGCY	19	1.517
I-Ag7	3	YGCYCGRGG	24	1.432
I-Ag7	4	KGDNNACAA	79	1.418
RT1.B	1	KTYSYECSQ	65	0.722
RT1.B	2	SYECSQGTL	68	0.547
RT1.B	3	NNACAASVC	82	0.419
RT1.B	4	YECSQGTLT	69	0.403

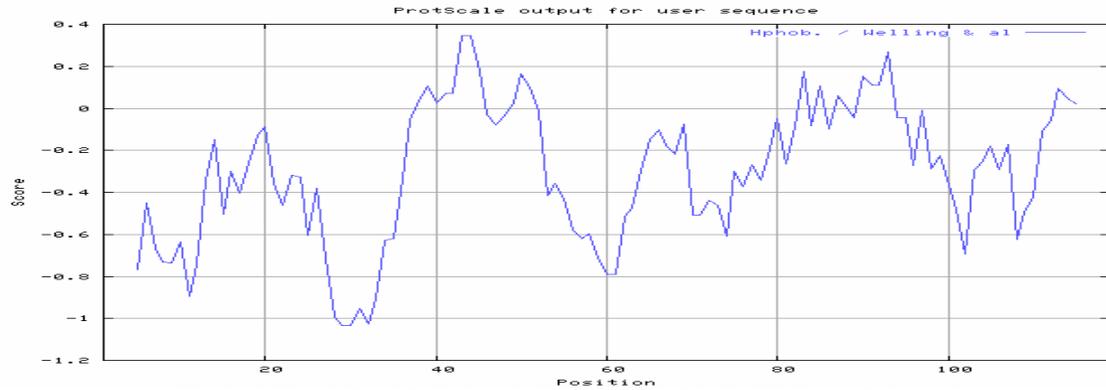


Fig. 1- Antigenicity plot of phospholipase A2 protein by Welling, et al., scale

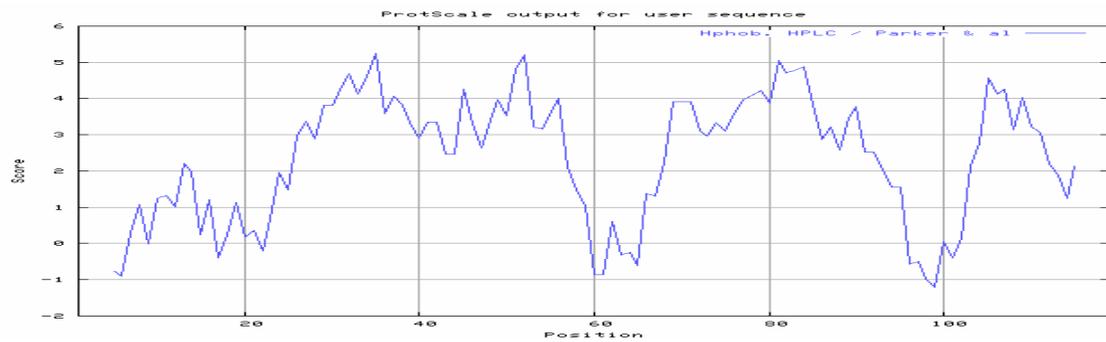


Fig. 2- Antigenicity plot of phospholipase A2 protein by HPLC / Parker, et al., scale

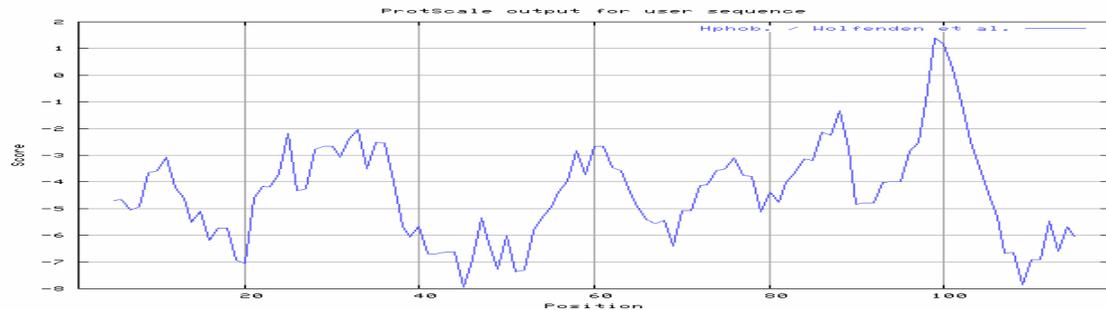


Fig. 3- Hydrophobicity plot of phospholipase A2 protein by Wolfenden, et al., scale



Fig. 4- Hydrophobicity plot of phospholipase A2 protein by Bull & Breese scale

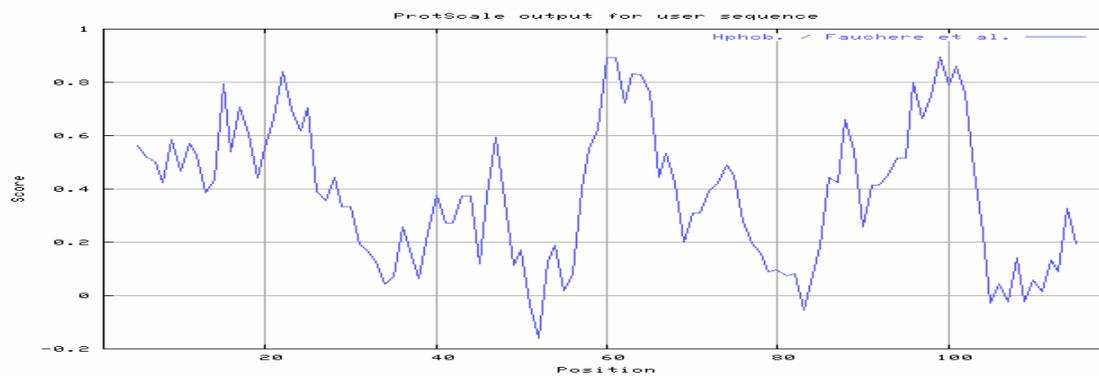


Fig. 5-Hydrophobicity plot of phospholipase A2 protein by Fauchere et al., scale

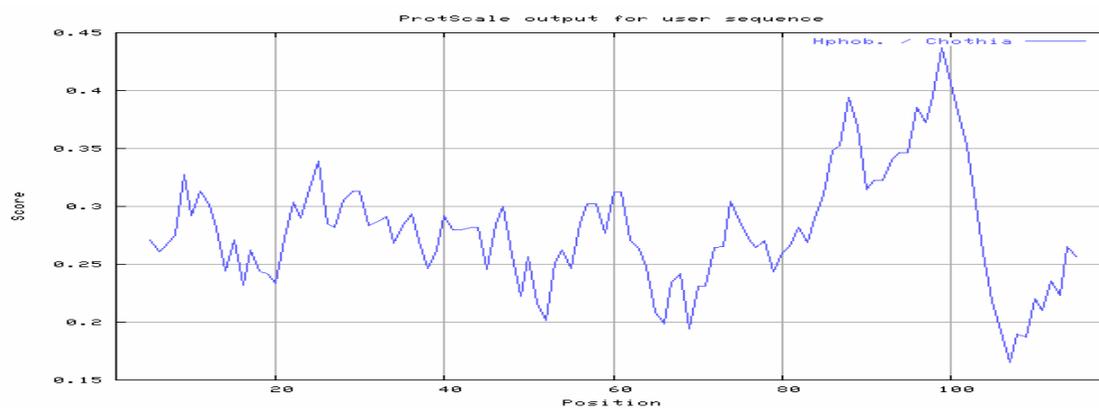


Fig. 6-Hydrophobicity plot of phospholipase A2 protein by Chothia scale

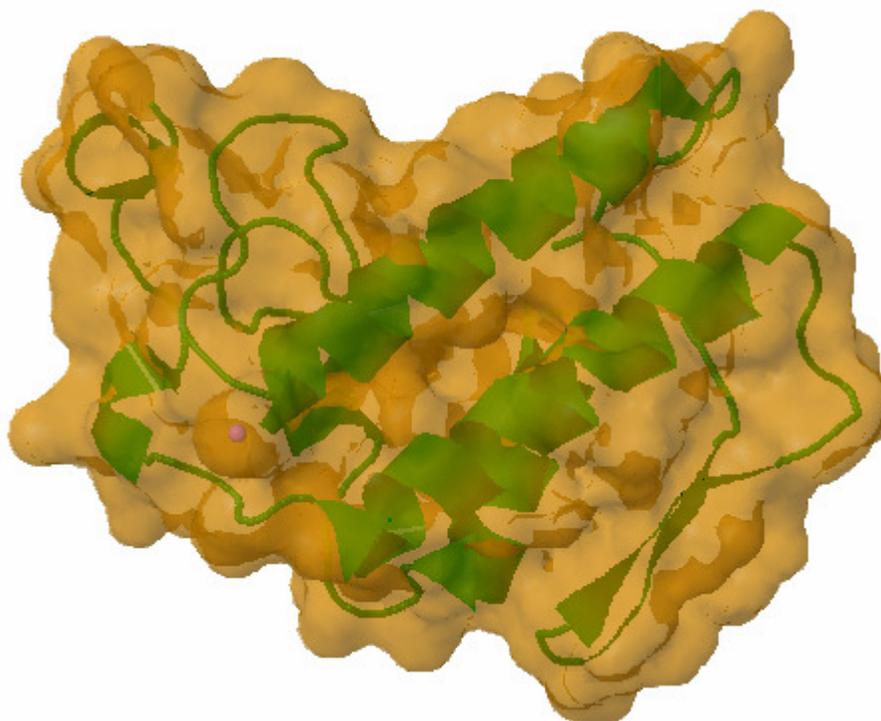


Fig. 7- Phospholipase A2 protein structure of 1A3D