Table 1. Prediction of the subcellular localization of eukaryotic proteins using DeepLoc server which differentiates between 10 different localizations

PV_{AO}	Localization	Cytoplasm	Peroxisome	Mitochondria	Lysosome	Cell membrane	Extracellular	Plastid	Endoplasmic reticulum	Golgi apparatus	Nucleus
	Likelihood	0.4748	0.1458	0.128	0.0886	0.0608	0.0464	0.0244	0.02	0.006	0.0051
	Type	Soluble	Membrane								
	Likelihood	0.8146	0.1854								
PV_{HSP}	Localization	Cytoplasm	Peroxisome	Lysosome	Cell membrane	Plastid	Endoplasmic reticulum	Mitochondria	Nucleus	Extracellular	Golgi apparatus
	Likelihood	0.3292	0.2826	0.1197	0.0745	0.0525	0.0436	0.0418	0.0247	0.0178	0.0136
	Type	Soluble	Membrane								
	Likelihood	0.6503	0.3497								
SOD	Localization	Mitochondria	Peroxisome	Extracellular	Cytoplasm	Plastid					
	Likelihood	0.9993	0.0002	0.0002	0.0002	0.0001					
	Type	Soluble	Membrane								
	Likelihood	0.7678	0.2322								
GST	Localization	Cytoplasm	Cell membrane	Mitochondria	Peroxisome	Nucleus	Endoplasmic reticulum	Golgi apparatus	Lysosome		
	Likelihood	0.8887	0.0715	0.012	0.0078	0.0074	0.0072	0.0039	0.0009		
	Type	Soluble	Membrane								
	Likelihood	0.936	0.064								
GPx	Localization	Cytoplasm	Mitochondrion	Cytoplasm	Endoplasmic reticulum	Golgi apparatus					
	Likelihood	0.8812	0.0913	0.021	0.0026	0.0039					
	Type	Soluble	Membrane								
	Likelihood	0.8593	0.1407								
HSP70	Localization	Cytoplasm	Peroxisome	Mitochondria	Plastid	Nucleus	Lysosome	Endoplasmic reticulum	Cell membrane	Golgi apparatus	
	Likelihood	0.9096	0.0537	0.0133	0.0063	0.006	0.0053	0.0034	0.0014	0.0007	
	Type	Soluble	Membrane								
	Likelihood	0.9902	0.0098								
HSP90	Localization	Cytoplasm	Nucleus	Endoplasmic reticulum	Lysosome	Peroxisome	Cell membrane	Golgi apparatus	Mitochondria	Extracellular	
	Likelihood	0.9611	0.0266	0.0066	0.0037	0.0006	0.0004	0.0004	0.0003	0.0003	
	Type	Soluble	Membrane								
	Likelihood	0.9991	0.0009								
HSP110	Localization	Cytoplasm	Nucleus	Lysosome	Peroxisome	Endoplasmic reticulum	Mitochondria	Plastid	Cell membrane	Golgi apparatus	Extracellular
	Likelihood	0.852	0.1293	0.0054	0.0039	0.0028	0.0025	0.0016	0.0013	0.0007	0.0004
	Type	Soluble	Membrane								
	Likelihood	0.9943	0.0057								

Table 2. Shared identity of the selected human anti-oxidant and heat shock proteins with vaccine construct.

S.N.	Vaccine construct	Human protein	Identity (%)	Similarity (%)	Matching Score
1	PV_{AO}	GST	6.20	9.4	62.2
2		SOD	11.2	14.4	73.4
3		GPx	8.0	11.2	77.9
4	PV_{HSP}	HSP70	11.5	17.8	91.0
5		HSP90	4.5	5.8	55.5
6		HSP110	9.0	14.5	52.0

Table 3. Evaluation of Antigenicity, Allerginicity and Physiochemical properties of Antioxidant and Heat shock proteins

Name of protein	AntigenPro score	Solubility	No. of amino acids	Molecular weight (Da)	Theoretical isoelectric Point(PI)	Formula	Total no. of atoms	Estimate half life	Instability index	Aliphatic index	GRAVY
SOD	0.826141	0.901803	223	25097.14	8.81	C 1136 H 1788 N 304 O 317 S 10	3555	30 hours (mammalian reticulocytes, in vitro). >20 hours (yeast, in vivo). >10 hours (Escherichia coli, in vivo).	30.97	96.68	-0.174
GPx	0.818261	0.543441	220	24880.56	7.59	C 1130 H 1728 N 290 O 322 S 11	3481	30 hours (mammalian reticulocytes, in vitro). >20 hours (yeast, in vivo) >10 hours (Escherichia coli, in vivo)	26.91	78.86	-0.240
GST	0.517046	0.901803	208	24226.81	6.79	C 1090 H 1697 N 297 O 311 S 9	3404	30 hours (mammalian reticulocytes, in vitro). >20 hours (yeast, in vivo). >10 hours (Escherichia coli, in vivo).	30.29	86.73	-0.523
HSP110	0.915828	0.901803	817	92521.91	5.48	C 4090 H 6471 N 1119 O 1265 S 30	12975	30 hours (mammalian reticulocytes, in vitro). >20 hours (yeast, in vivo). >10 hours (Escherichia coli, in vivo).	39.85	76.38	-0.571
HSP90	0.677248	0.901803	717	82604.71	4.98	C 3651 H 5813 N 957 O 1162 S S 28	11611	30 hours (mammalian reticulocytes, in vitro). >20 hours (yeast, in vivo). >10 hours (Escherichia coli, in vivo).	40.90	80.63	-0.637
HSP70	0.923227	0.90180	645	70356.50	5.56	C 3078 H 4947 N 859 O 983 S 20	9887	30 hours (mammalian reticulocytes, in vitro). >20 hours (yeast, in vivo). >10 hours (Escherichia coli, in vivo).	39.95	79.27	-0.446

Table 4. IFN-γ epitope of antioxidant and heat shock proteins of filarial parasite

Peptide	**Sequence**	**Method**	**Result**	**Score**
SOD	LRGDAGVSGIIYFQQGSGGS	MERCI	NEGATIVE	1
	NGCTSAGDHY	MERCI	NEGATIVE	1
	DIKLRGPLSVIGHSLVVH	MERCI	NEGATIVE	2
	ITTISGSVS	SVM	NEGATIVE	-0.43281269
	SGSVSGLTPGLHGFHVHQYG	SVM	POSITIVE	1.5438308
	DQT	SVM	POSITIVE	1.3440322
	AGDHYNP	SVM	NEGATIVE	-0.14867243
	NPFGKTHGGPNDRIKHIGDL	SVM	POSITIVE	0.94673579
	GNIVAGANG	SVM	NEGATIVE	-0.21317333
	ANTDDLGQGTGNMREESLK	SVM	NEGATIVE	-0.021679592
	TGNAGSRLAC	SVM	NEGATIVE	-0.59914591
GPx	YEFTVKDI	MERCI	POSITIVE	1
	CKSGATDKNYRQLQEMHTRL	MERCI	POSITIVE	1
	NGVDVSLEKYRGHVCLIVNV	MERCI	NEGATIVE	2
	VNGSDADDLY	MERCI	NEGATIVE	1
	NQFGGQEPWAEAEIKKFVTE	SVM	POSITIVE	1.133996
	KYGVQFDMFSKIKV	SVM	NEGATIVE	-0.49531073
	FLKSRQHGTLTNNIKWNFSK	SVM	POSITIVE	1.329577

	QPVKRYSPTTAPYDIEGDIM	SVM	NEGATIVE	-0.14343861
GST	DRINASDWPSMKSHFHFGQ	MERCI	POSITIVE	1
	LPCLYDGDHQIVQSG	MERCI	POSITIVE	1
	PIRLVLVDQGIKFTD	MERCI	NEGATIVE	1
	AILRHLA	SVM	NEGATIVE	-0.022975403
	RKHNLNGGNELETTHID	SVM	NEGATIVE	-0.2516892
	MFCEGVRDLHTKYTKMIYQA	SVM	POSITIVE	1.0405944
	ILPVELAKFE	SVM	NEGATIVE	-0.43792163
	KLLATRD	SVM	POSITIVE	0.093108711
	DGKNFILGEKISYVDFVLFE	SVM	POSITIVE	1.7967633
	CLDKFPLLKAYHQRMEDRPG	SVM	POSITIVE	1.4030477
HSP 110	RVRILLTNLLRESGKKAEEV	MERCI	POSITIVE	1
	AAMKCAILSPAFKVRDFSVK	MERCI	NEGATIVE	1
	PPKAEPAKEPEKGSDGQKGE	SVM	POSITIVE	0.570793
	ETDDTKG	SVM	POSITIVE	0.046773
	NQEKEENTNAPSNDVVLP	SVM	POSITIVE	0.505780
	AFTTRGRSMGVAARQQLNTN	SVM	POSITIVE	0.502655
HSP 90	DDEAEEE	MERCI	POSITIVE	1
	DEEEDKKEKDKK	SVM	NEGATIVE	-0.930605
	GEIEDVGE	SVM	POSITIVE	0.7048250
	KKDEDKEKKE	SVM	NEGATIVE	-0.784628
	LAVKHFSVEGQLEFRALLFV	SVM	POSITIVE	1.261721
HSP 70	KSTAGDTHLGGEDFDNRMVN	MERCI	POSITIVE	5
	MPGGMPSGAPGAGSTGGGPT	MERCI	NEGATIVE	5
	SAGGMPGG	SVM	POSITIVE	0.5289680
	AVITVPAYFNDSQRQATKDS	SVM	POSITIVE	0.8848280

Table 5. Predicted Discontinuous B-cell epitopes of PV_{AO} and PV_{HSP}

No. PV_{AO}	**Residues**	**Number of residues**	**Score**
1	$E^{33}, T^{35}, A^{36}, A^{37}, A^{38}, P^{39}, V^{40}, A^{41}, V^{42}, A^{43}, A^{44}, A^{45}, G^{46}, A^{47}, A^{48}, P^{49}, A^{50}, G^{51}, A^{52}, A^{53}, V^{54}, E^{55}, A^{56}, A^{57}, E^{58}, E^{59}, Q^{60}, S^{61}, E^{62}, F^{63}, D^{64}, V^{65}, I^{66}, L^{67}, E^{68}, A^{69}, A^{70}, G^{71}, D^{72}, K^{73}, K^{74}, I^{75}, G^{76}$, V77, $I^{78}, K^{79}, V^{80}, V^{81}, R^{82}, E^{83}, I^{84}, A^{100}, P^{101}, K^{102}, P^{103}, L^{104}, L^{105}, E^{106}, K^{107}$	59	0.772
2	$G^{667}, P^{668}, G^{669}, M^{670}, F^{671}, C^{672}, E^{673}, G^{674}, V^{675}, R^{676}, D^{677}, L^{678}, H^{679}, T^{680}, K^{681}, Y^{682}, T^{683}, K^{684}, M^{685}, I^{686}, Y^{687}, Q^{688}, A^{689}, G^{690}, I^{695}, L^{696}, G^{705}, P^{706}, :G^{707}, P^{708}, G^{709}, C^{710}, L^{711}, D^{712}, K^{713}, F^{714}, P^{715}, L^{716}, L^{717}, K^{718}, A^{719}, Y^{720}, H^{721}, Q^{722}, R^{723}, D^{726}, R^{727}, P^{728}, G^{746}, D^{747}, G^{748}, K^{749}, N^{750}, F^{751}, I^{752}, L^{753}, G^{754}, E^{755}, K^{756}, I^{757}, S^{758}, Y^{759}, V^{760}, D^{761}, F^{762}, V^{763}, L^{764}, F^{765}, E^{766}, G^{767}, P^{768}, G^{769}, P^{770}, G^{771}, I^{772}, I^{773}, Y^{774}, A^{775}, A^{776}, Y^{777}, V^{778}, A^{779}, E^{780}, V^{781}, Y^{782}, A^{783}, Y^{786}, A^{790}, T^{793}, E^{794}, K^{795}, D^{796}, S^{797}, Y^{798}, A^{799}, A^{800}, Y^{801}, H^{802}, H^{803}, H^{804}, H^{805}, H^{806}, H^{807}$	103	0.765
3	$P^{255}, G^{256}, P^{257}, G^{258}, N^{259}, G^{260}, C^{261}, T^{262}, S^{263}, A^{264}, G^{265}, D^{266}, H^{267}, Y^{268}, G^{269}, P^{270}, G^{271}, P^{272}, G^{273}, N^{274}, P^{275}, F^{276}, G^{277}, T^{279}, H^{280}, G^{283}$	26	0.735
4	$Y^{464}, G^{465}, V^{466}, Q^{467}, F^{468}, D^{469}, M^{470}, F^{471}, S^{472}, K^{473}, I^{474}, K^{475}, G^{476}, P^{477}, G^{478}, P^{479}, G^{480}, F^{481}, L^{482}, K^{483}, S^{484}, R^{485}, Q^{486}, H^{487}, G^{488}, T^{489}, L^{490}, N^{493}, K^{495}, W^{496}, N^{497}, G^{505}, V^{506}, N^{507}, G^{508}, S^{509}, D^{510}, A^{511}, D^{512}, D^{513}, L^{514}, Y^{515}, K^{516}, G^{517}, P^{518}, G^{519}, P^{520}, G^{521}, F^{522}, L^{523}, K^{524}, S^{525}, R^{526}, G^{546}, Q^{547}, P^{548}, V^{549}, K^{550}, R^{551}, Y^{552}, S^{553}, P^{554}, T^{555}, T^{556}, A^{557}, P^{558}, Y^{559}, D^{560}, I^{561}, E^{562}, G^{563}, D^{564}, I^{565}, M^{566}, G^{567}, P^{568}, G^{569}, F^{584}, T^{585}, G^{587}, P^{588}, G^{589}, P^{590}, G^{591}, D^{592}, R^{593}, I^{594}, N^{595}, A^{596}, S^{597}, D^{598}, W^{599}, P^{600}, S^{601}, M^{602}, G^{611}, P^{612}, G^{613}, P^{614}, G^{615}, L^{616}, P^{617}, C^{618}, L^{619}, Y^{620}, D^{623}, G^{631}, P^{632}, G^{633}, P^{634}, G^{635}, A^{636}, I^{637}, L^{638}, R^{639}, H^{640}, L^{641}, A^{642}, G^{643}, P^{644}, G^{645}, P^{646}, G^{647}, R^{648}, K^{649}, H^{650}, N^{651}, L^{652}, N^{653}$	129	0.685

5		$K^{414}, S^{415}, G^{416}, A^{417}, T^{418}, D^{419}, K^{420}, N^{421}, Y^{422}, R^{423}, L^{425}, Q^{426}, E^{427}, M^{428}, H^{429}, T^{430}, R^{431}$	17	0.681
6		$G^{287}, D^{290}, R^{291}, I^{292}, K^{293}, H^{294}, I^{295}, G^{296}, D^{297}, L^{298}, G^{299}, N^{300}, I^{301}, V^{302}, A^{303}, G^{304}, A^{305}, N^{306}, G^{307}, G^{308}, P^{309}, G^{310}, P^{311}, G^{312}, D^{313}, V^{329}, H^{330}, A^{331}, G^{332}, P^{333}, G^{334}, P^{335}, G^{336}, A^{337}, N^{338}, D^{339}, D^{340}, L^{341}, G^{342}, Q^{343}, G^{344}, G^{345}, P^{346}, G^{347}, P^{348}, G^{349}, T^{350}, G^{374}, Y^{375}, E^{376}, F^{377}, T^{378}, Q^{424}$	53	0.628
7		$P^{162}, G^{163}, P^{164}, G^{165}, F^{166}, I^{168}, R^{169}, G^{170}, A^{172}, E^{173}, G^{174}, P^{175}, G^{176}, P^{177}, G^{178}, D^{179}, D^{180}, P^{199}, G^{200}, I^{201}, I^{202}, Y^{203}, F^{204}, Q^{205}, Q^{206}, G^{207}, S^{208}, G^{209}, G^{210}, S^{211}, I^{212}, T^{213}, T^{214}, I^{215}, P^{232}, G^{233}, P^{234}, G^{235}, L^{236}, H^{237}, G^{238}, F^{239}, H^{240}, G^{241}, P^{242}$	45	0.62
8		$E^{121}, A^{122}, A^{123}, G^{124}, A^{125}, T^{126}, V^{127}, T^{128}, V^{129}, K^{130}, E^{131}$	11	0.563
1	PV_{HSP}	$N^{263}, P^{451}, G^{452}, P^{453}, G^{454}, V^{455}, R^{456}, I^{457}, L^{458}, L^{459}, I^{472}, P^{474}, N^{475}, K^{476}, A^{477}, G^{478}, P^{479}, G^{480}, P^{481}, G^{482}, E^{483}, R^{484}, I^{485}, M^{486}, K^{487}, A^{488}, Q^{489}, A^{490}, L^{491}, A^{492}, A^{493}, Y^{494}, F^{495}, S^{496}, K^{497}, L^{498}, F^{499}, Y^{500}, D^{501}, T^{502}$, Y503, $A^{504}, A^{505}, Y^{506}, K^{507}, L^{508}, T^{509}, V^{510}, L^{511}, N^{519}, I^{526}, I^{527}, A^{528}, A^{529}, Y^{530}, M^{531}, S^{532}, E^{533}, E^{534}, M^{535}, N^{536}, G^{537}, E^{538}, T^{539}, A^{540}, {}^{A541}, Y^{542}, Q^{543}, L^{544}, E^{545}, F^{546}, N^{560}, T^{561}, F^{562}, Y^{563}, A^{564}, A^{565}, Y^{566}, L^{567}, M^{568}, S^{569}, L^{570}, I^{571}, I^{572}, N^{573}, T^{574}, F^{575}, A^{576}, A^{577}, Y^{578}, E^{579}, T^{580}, A^{581}, L^{582}, L^{583}, S^{584}, S^{585}, G^{586}, F^{587}, A^{588}, A^{589}, Y^{590}, V^{591}, K^{592}, D^{593}, L^{594}, V^{595}, V^{596}, L^{597}, L^{598}, A^{600}, A^{601}, Y^{602}, M^{603}, K^{604}, N^{606}, Q^{607}, K^{608}, Q^{609}, I^{610}, Y^{611}, A^{612}, A^{613}, Y^{614}, G^{617}, T^{618}, K^{619}, I^{620}, T^{621}, L^{622}, Y^{623}, A^{624}, A^{625}, Y^{626}, Y^{627}, T^{629}, T^{655}, A^{657}, F^{659}, A^{660}, Y^{662}, S^{663}, S^{664}, S^{665}, Q^{666}, A^{667}, S^{668}, I^{669}, E^{670}, I^{671}, H^{672}, H^{673}, H^{674}, H^{675}, H^{676}, H^{677}$	156	0.734
2		$V^{54}, E^{55}, A^{56}, A^{57}, E^{58}, E^{59}, Q^{60}, S^{61}, E^{62}, F^{63}, D^{64}, V^{65}, I^{66}, L^{67}, E^{68}, A^{69}, A^{70}, G^{71}, D^{72}, {}^{K73}, K^{74}, I^{75}, G^{76}, V^{77}$, I78, $K^{94}, D^{95}, V^{97}, D^{98}, G^{99}, A^{100}, P^{101}, K^{102}, P^{103}, L^{104}, L^{105}, K^{107}, A^{109}, K^{110}, A^{113}, D^{114}, A^{116}, K^{117}, A^{118}, K^{119}, L^{120}, E^{121}, A^{122}, A^{123}, G^{124}, A^{125}, T^{126}, V^{127}, T^{128}, V^{129}, K^{130}, E^{131}, A^{132}, A^{133}, A^{134}, K^{135}, P^{136}, P^{137}, K^{138}, A^{139}, E^{140}, P^{141}, A^{142}, K^{143}, E^{144}, P^{145}, E^{146}, K^{147}, G^{148}, S^{149}, D^{150}, G^{151}, Q^{152}, K^{153}, G^{154}, E^{155}, G^{156}, P^{157}, G^{158}, P^{159}, G^{160}, E^{161}, T^{162}, D1^{63}, D^{164}, T^{165}, K^{166}, G^{167}, G^{168}, P^{169}, G^{170}, P^{171}, G^{172}, N^{173}, E^{175}, K^{176}, E^{177}, N^{179}, T^{180}, N^{181}, A^{182}, P^{183}, S^{184}$	108	0.72
3		$M^{1}, A^{2},:K^{3}, L^{4}, S^{5}, T^{6}, D^{7}, E^{8}, L^{9}, L^{10}, D^{11}, A^{12}, F^{13}, K^{14}, E^{15}, M^{16}, T^{17}, L^{18}, L^{19}, E^{20}, L^{21}, S^{22}, D^{23}, V^{25}, K^{26}, K^{27}, E^{29}, E^{30}, A^{32}, E^{33}, V^{34}, T^{35}, A^{36}, A^{37}, A^{38}, P^{39}, V^{40}, R^{221}, G^{285}, P^{286}, G^{287}, D^{288}, D^{289}, G^{299}, G^{300}, E^{301}, I^{302}, E^{303}, D^{304}, V^{305}, G^{306}, E^{307}, G^{308}, P^{309}, G^{310}, P^{311}, G^{312}, K^{313}, K^{314}, D^{315}, E^{316}, D^{317}, K^{318}, E^{319}, K^{321}, A^{329}, V^{330}, K^{331}, H^{332}, S^{334}, G^{337}, Q^{338}, R^{342}, A^{343}, L^{344}, L^{345}, G^{363}, P^{364}, G^{365}, M^{366}, P^{367}, G^{368}, G^{369}, P^{371}, S^{372}, G^{373}, A^{374}, P^{375}, G^{376}, A^{377}, G^{378}, S^{379}, T^{380}, G^{381}, G^{382}, G^{383}, P^{384}, T^{385}, G^{386}, P^{387}, G^{388}, K^{391}, S^{392}$	103	0.656

Table 6. Promiscuous epitopes of multiepitope vaccine constructs

PV_{HSP}	HLA-A1	MAKLSTDELLDAFKEMTLLELSDFVKKFEETFEVTAAAPVAVAAAGAAPAGAAVEAAEEQSEFDVILEAAGDKKIGVIKVVREIVSGLGLKEAKDLVDGAPKPLLEKVAKEAADEAKAKLEAAGATVTVKEAAAKPPKAEPAKEPEKGSDGQKGEGPGPGETDDTKGGPGPGNQEKEENTNAPSNDVVLPGPGPGAAMKCAILSPAFKVRDFSVKGPGPGRVRILLTNLLRESGKKAEEVGPGPGAFTTRGRSMGVAARQQLNTNGPGPGDEEEDKKEKDKKGPGPGDDEAEEEGPGPGGEIEDVGEGPGPGKKDEDKEKKEGPGPGLAVKHFSVEGQLEFRALLFVGPGPGSAGGMPGGGPGPGMPGGMPSGAPGAGSTGGGPTGPGPGKSTAGDTHLGGEDFDNRMVNGPGPGAVITVPAYFNDSQRQATKDSGPGPGAILSPAFKVGPGPGVRILLTNLLGPGPGYIKITPNKAGPGPGERIMKAQALAAYFSKLFYDTYAAYKLTVLGATYAAYNTNIKNTIIAAYMSEEMNGETAAYQLEFRALLFAAYMSLIINTFYAAYLMSLIINTFAAYETALLSSGFAAYVKDLVVLLFAAYMKENQKQIYAAYTRGTKITLYAAYYMTDPIDEYAAYYSDNQPGVLAAYYTNITRARFAAYSSSQASIEIHHHHHH
	HLA-A2	MAKLSTDELLDAFKEMTLLELSDFVKKFEETFEVTAAAPVAVAAAGAAPAGAAVEAAEEQSEFDVILEAAGDKKIGVIKVVREIVSGLGLKEAKDLVDGAPKPLLEKVAKEAADEAKAKLEAAGATVTVKEAAAKPPKAEPAKEPEKGSDGQKGEGPGPGETDDTKGGPGPGNQE

		KEENTNAPSNDVVLPGPGPGAAMKCAILSPAFKVRDFSVKGPGPGRVRILLTNLLRESGKKAEEVGPGPGAFTTRGRSMGVAARQQLNTNGPGPGDEEEDKKEKDKKGPGPGDDEAEEEGPGPGGEIEDVGEGPGPGKKDEDKEKKEGPGPGLAVKHFSVEGQLEFRALLFVGPGPGSAGGMPGGGPGPGMPGGMPSGAPGAGSTGGGPTGPGPGKSTAGDTHLGGEDFDNRMVNGPGPGAVITVPAYFNDSQRQATKDSGPGPGAILSPAFKVGPGPGVRILLTNLLGPGPGYIKITPNKAGPGPGERIMKAQALAAYFSKLFYDTYAAYKLTVLGATYAAYNTNIKNTIIAAYMSEEMNGETAAYQLEFRALLFAAYMSLIINTFYAAYLMSLIINTFAAYETALLSSGFAAYVKDLVVLLFAAYMKENQKQIYAAYTRGTKITLYAAYYMTDPIDEYAAYYSDNQPGVLAAYYTNITRARFAAYSSSQASIEIHHHHHH
	HLA-A*0201	MAKLSTDELLDAFKEMTLLELSDFVKKFEETFEVTAAAPVAVAAAGAAPAGAAVEAAEEQSEFDVILEAAGDKKIGVIKVVREIVSGLGLKEAKDLVDGAPKPLLEKVAKEAADEAKAKLEAAGATVTVKEAAAKPPKAEPAKEPEKGSDGQKGEGPGPGETDDTKGGPGPGNQEKEENTNAPSNDVVLPGPGPGAAMKCAILSPAFKVRDFSVKGPGPGRVRILLTNLLRESGKKAEEVGPGPGAFTTRGRSMGVAARQQLNTNGPGPGDEEEDKKEKDKKGPGPGDDEAEEEGPGPGGEIEDVGEGPGPGKKDEDKEKKEGPGPGLAVKHFSVEGQLEFRALLFVGPGPGSAGGMPGGGPGPGMPGGMPSGAPGAGSTGGGPTGPGPGKSTAGDTHLGGEDFDNRMVNGPGPGAVITVPAYFNDSQRQATKDSGPGPGAILSPAFKVGPGPGVRILLTNLLGPGPGYIKITPNKAGPGPGERIMKAQALAAYFSKLFYDTYAAYKLTVLGATYAAYNTNIKNTIIAAYMSEEMNGETAAYQLEFRALLFAAYMSLIINTFYAAYLMSLIINTFAAYETALLSSGFAAYVKDLVVLLFAAYMKENQKQIYAAYTRGTKITLYAAYYMTDPIDEYAAYYSDNQPGVLAAYYTNITRARFAAYSSSQASIEIHHHHHH
	HLA-A*0205	MAKLSTDELLDAFKEMTLLELSDFVKKFEETFEVTAAAPVAVAAAGAAPAGAAVEAAEEQSEFDVILEAAGDKKIGVIKVVREIVSGLGLKEAKDLVDGAPKPLLEKVAKEAADEAKAKLEAAGATVTVKEAAAKPPKAEPAKEPEKGSDGQKGEGPGPGETDDTKGGPGPGNQEKEENTNAPSNDVVLPGPGPGAAMKCAILSPAFKVRDFSVKGPGPGRVRILLTNLLRESGKKAEEVGPGPGAFTTRGRSMGVAARQQLNTNGPGPGDEEEDKKEKDKKGPGPGDDEAEEEGPGPGGEIEDVGEGPGPGKKDEDKEKKEGPGPGLAVKHFSVEGQLEFRALLFVGPGPGSAGGMPGGGPGPGMPGGMPSGAPGAGSTGGGPTGPGPGKSTAGDTHLGGEDFDNRMVNGPGPGAVITVPAYFNDSQRQATKDSGPGPGAILSPAFKVGPGPGVRILLTNLLGPGPGYIKITPNKAGPGPGERIMKAQALAAYFSKLFYDTYAAYKLTVLGATYAAYNTNIKNTIIAAYMSEEMNGETAAYQLEFRALLFAAYMSLIINTFYAAYLMSLIINTFAAYETALLSSGFAAYVKDLVVLLFAAYMKENQKQIYAAYTRGTKITLYAAYYMTDPIDEYAAYYSDNQPGVLAAYYTNITRARFAAYSSSQASIEIHHHHHH
PV$_{HSP}$	HLA-A1	MAKLSTDELLDAFKEMTLLELSDFVKKFEETFEVTAAAPVAVAAAGAAPAGAAVEAAEEQSEFDVILEAAGDKKIGVIKVVREIVSGLGLKEAKDLVDGAPKPLLEKVAKEAADEAKAKLEAAGATVTVKEAAAKNSYGPGPGNTGPGPGVGKGLRILAFGPGPGFPIRGLAEGPGPGDDGGPGPGLRGDAGVSGGPGPGIIYFQQGSGGSITTIGPGPGSGSVSGLTGPGPGPGLHGFHGPGPGVHQYGDQTGPGPGNGCTSAGDHYGPGPGNPFGKTHGGGPGPGPNDRIKHIGDLGNIVAGANGGPGPGDIKLRGPLSVIGHSLVVHAGPGPGANDDLGQGGPGPGTGNMREESLKTGNAGSRLACGPGPGYEFTVKDIGPGPGNGVDVSLEKYRGHVCLIVNVGPGPGCKSGATDKNYRQLQEMHTRLGPGPGNQFGGQEPWAEAEIGPGPGKKFVTEKYGVQFDMFSKIKGPGPGFLKSRQHGTLTNNIKWNFSKGPGPGVNGSDADDLYKGPGPGFLKSRQHGTLTNNIKWNFSKGPGPGQPVKRYSPTTAPYDIEGDIMGPGPGPIRLVLVDQGIKFTDGPGPGDRINASDWPSMKSHFHFGQGPGPGLPCLYDGDHQIVQSGGPGPGAILRHLAGPGPGRKHNLNGGNELETTHIDGPGPGMFCEGVRDLHTKYTKMIYQAGPGPGILPVELAKFEGPGPGCLDKFPLLKAYHQRMEDRPGGPGPGKLLATRDGPGPGDGKNFILGEKISYVDFVLFEGPGPGIIYAAYVAEVYAAYYAAYAYDTEKDSYAAYHHHHHH
	HLA-A2	MAKLSTDELLDAFKEMTLLELSDFVKKFEETFEVTAAAPVAVAAAGAAPAGAAVEAAEEQSEFDVILEAAGDKKIGVIKVVREIVSGLGLKEAKDLVDGAPKPLLEKVAKEAADEAKAKLEAAGATVTVKEAAAKNSYGPGPGNTGPGPGVGKGLRILAFGPGPGFPIRGLAEGPGPGDDGGPGPGLRGDAGVSGGPGPGIIYFQQGSGGSITTIGPGPGSGSVSGLTGPGPGPGLH

	GFHGPGPGVHQYGDQTGPGPGNGCTSAGDHYGPGPGNPFGKTHGGGPGPGPNDRIKHIGDLGNIVAGANGGPGPGDIKLRGPLSVIGHSLVVHAGPGPGANDDLGQGGPGPGTGNMREESLKTGNAGSRLACGPGPGYEFTVKDIGPGPGNGVDVSLEKYRGHVCLIVNVGPGPGCKSGATDKNYRQLQEMHTRLGPGPGNQFGGQEPWAEAEIGPGPGKKFVTEKYGVQFDMFSKIKGPGPGFLKSRQHGTLTNNIKWNFSKGPGPGVNGSDADDLYKGPGPGFLKSRQHGTLTNNIKWNFSKGPGPGQPVKRYSPTTAPYDIEGDIMGPGPGPIRLVLVDQGIKFTDGPGPGDRINASDWPSMKSHFHFGQGPGPGLPCLYDGDHQIVQSGGPGPGAILRHLAGPGPGRKHNLNGGNELETTHIDGPGPGMFCEGVRDLHTKYTKMIYQAGPGPGILPVELAKFEGPGPGCLDKFPLLKAYHQRMEDRPGGPGPGKLLATRDGPGPGDGKNFILGEKISYVDFVLFEGPGPGIIYAAYVAEVYAAYYAAYAYDTEKDSYAAYHHHHHH
HLA-A*0201	MAKLSTDELLDAFKEMTLLELSDFVKKFEETFEVTAAAPVAVAAAGAAPAGAAVEAAEEQSEFDVILEAAGDKKIGVIKVVREIVSGLGLKEAKDLVDGAPKPLLEKVAKEAADEAKAKLEAAGATVTVKEAAAKNSYGPGPGNTGPGPGVGKGLRILAFGPGPGFPIRGLAEGPGPGDDGGPGPGLRGDAGVSGGPGPGIIYFQQGSGGSITTIGPGPGSGSVSGLTGPGPGPGLHGFHGPGPGVHQYGDQTGPGPGNGCTSAGDHYGPGPGNPFGKTHGGGPGPGPNDRIKHIGDLGNIVAGANGGPGPGDIKLRGPLSVIGHSLVVHAGPGPGANDDLGQGGPGPGTGNMREESLKTGNAGSRLACGPGPGYEFTVKDIGPGPGNGVDVSLEKYRGHVCLIVNVGPGPGCKSGATDKNYRQLQEMHTRLGPGPGNQFGGQEPWAEAEIGPGPGKKFVTEKYGVQFDMFSKIKGPGPGFLKSRQHGTLTNNIKWNFSKGPGPGVNGSDADDLYKGPGPGFLKSRQHGTLTNNIKWNFSKGPGPGQPVKRYSPTTAPYDIEGDIMGPGPGPIRLVLVDQGIKFTDGPGPGDRINASDWPSMKSHFHFGQGPGPGLPCLYDGDHQIVQSGGPGPGAILRHLAGPGPGRKHNLNGGNELETTHIDGPGPGMFCEGVRDLHTKYTKMIYQAGPGPGILPVELAKFEGPGPGCLDKFPLLKAYHQRMEDRPGGPGPGKLLATRDGPGPGDGKNFILGEKISYVDFVLFEGPGPGIIYAAYVAEVYAAYYAAYAYDTEKDSYAAYHHHHHH
HLA-A*0205	MAKLSTDELLDAFKEMTLLELSDFVKKFEETFEVTAAAPVAVAAAGAAPAGAAVEAAEEQSEFDVILEAAGDKKIGVIKVVREIVSGLGLKEAKDLVDGAPKPLLEKVAKEAADEAKAKLEAAGATVTVKEAAAKNSYGPGPGNTGPGPGVGKGLRILAFGPGPGFPIRGLAEGPGPGDDGGPGPGLRGDAGVSGGPGPGIIYFQQGSGGSITTIGPGPGSGSVSGLTGPGPGPGLHGFHGPGPGVHQYGDQTGPGPGNGCTSAGDHYGPGPGNPFGKTHGGGPGPGPNDRIKHIGDLGNIVAGANGGPGPGDIKLRGPLSVIGHSLVVHAGPGPGANDDLGQGGPGPGTGNMREESLKTGNAGSRLACGPGPGYEFTVKDIGPGPGNGVDVSLEKYRGHVCLIVNVGPGPGCKSGATDKNYRQLQEMHTRLGPGPGNQFGGQEPWAEAEIGPGPGKKFVTEKYGVQFDMFSKIKGPGPGFLKSRQHGTLTNNIKWNFSKGPGPGVNGSDADDLYKGPGPGFLKSRQHGTLTNNIKWNFSKGPGPGQPVKRYSPTTAPYDIEGDIMGPGPGPIRLVLVDQGIKFTDGPGPGDRINASDWPSMKSHFHFGQGPGPGLPCLYDGDHQIVQSGGPGPGAILRHLAGPGPGRKHNLNGGNELETTHIDGPGPGMFCEGVRDLHTKYTKMIYQAGPGPGILPVELAKFEGPGPGCLDKFPLLKAYHQRMEDRPGGPGPGKLLATRDGPGPGDGKNFILGEKISYVDFVLFEGPGPGIIYAAYVAEVYAAYYAAYAYDTEKDSYAAYHHHHHH

Table 7. PV$_{AO}$ Toxicity prediction of selected epitopes of vaccine constructs

Vaccine epitope	Sequence	SVM Score	Prediction	Hydrophobicity	Hydropathicity	Hydrophilicity	Charge	Mol wt
B-cell epitope								
seq1	LRGDAGVSG	-1.14	Non-Toxin	-0.10	-0.02	0.28	0.00	831.02
seq10	ANTDDLGQG	-0.82	Non-Toxin	-0.21	-1.10	0.41	-2.00	890.00
seq11	TGNMREESLKTGNAGSRLAC	-0.90	Non-Toxin	-0.28	-0.77	0.41	1.00	2095.62
seq12	YEFTVKDI	-0.94	Non-Toxin	-0.09	-0.18	0.06	-1.00	1014.25

seq13	NGVDVSLEKYRGHVCLIVNV	-1.05	Non-Toxin	-0.06	0.35	-0.20	0.50	2214.88
seq14	CKSGATDKNYRQLQEMHTRL	-0.29	Non-Toxin	-0.42	-1.38	0.44	2.50	2379.99
seq15	NQFGGQEPWAEAEI	-0.94	Non-Toxin	-0.10	-0.96	0.06	-3.00	1575.86
seq16	KKFVTEKYGVQFDMFSKIK	-1.25	Non-Toxin	-0.20	-0.53	0.27	3.00	2324.06
seq17	VNGSDADDLYK	-0.68	Non-Toxin	-0.24	-0.96	0.58	-2.00	1196.38
seq18	FLKSRQHGTLTNNIKWNFSK	-1.52	Non-Toxin	-0.27	-1.00	-0.09	4.50	2420.08
seq19	QPVKRYSPTTAPYDIEGDIM	-0.75	Non-Toxin	-0.19	-0.77	0.16	-1.00	2281.85
seq2	IIYFQQGSGGSITTI	-1.53	Non-Toxin	0.12	0.55	-0.79	0.00	1585.03
seq20	PIRLVLVDQGIKFTD	-0.99	Non-Toxin	-0.06	0.41	-0.06	0.00	1714.27
seq21	DRINASDWPSMKSHFHFGQ	-1.02	Non-Toxin	-0.23	-1.07	0.02	1.00	2260.74
seq22	LPCLYDGDHQIVQSG	0.24	Non-Toxin	-0.06	-0.19	-0.27	-1.50	1645.05
seq23	AILRHLA	-0.96	Non-Toxin	0.02	1.14	-0.56	1.50	793.08
seq24	RKHNLNGGNELETTHID	-0.95	Non-Toxin	-0.34	-1.52	0.49	0.00	1948.35
seq25	MFCEGVRDLHTKYTKMIYQA	-0.76	Non-Toxin	-0.17	-0.35	-0.12	1.50	2435.17
seq26	ILPVELAKFE	-0.79	Non-Toxin	0.08	0.84	-0.09	-1.00	1158.55
seq27	KLLATRD	-0.80	Non-Toxin	-0.35	-0.46	0.64	1.00	816.06
seq28	DGKNFILGEKISYVDFVLFE	-1.66	Non-Toxin	-0.00	0.26	-0.07	-2.00	2333.97
seq29	CLDKFPLLKAYHQRMEDRPG	-1.02	Non-Toxin	-0.30	-0.93	0.39	1.50	2418.12
seq3	SGSVSGLT	-0.93	Non-Toxin	0.05	0.51	-0.35	0.00	706.86
seq4	PGLHGFH	-0.84	Non-Toxin	0.08	-0.31	-0.76	1.00	763.96
seq5	VHQYGDQT	-0.63	Non-Toxin	-0.24	-1.49	-0.16	-0.50	947.09
seq6	NGCTSAGDHY	-0.69	Non-Toxin	-0.16	-0.95	-0.12	-0.50	1024.16
seq7	NPFGKTHGG	-0.86	Non-Toxin	-0.14	-1.26	-0.02	1.50	914.12
seq8	PNDRIKHIGDLGNIVAGANG	-1.16	Non-Toxin	-0.11	-0.36	0.12	0.50	2031.57
seq9	DIKLRGPLSVIGHSLVVHA	-1.07	Non-Toxin	0.01	0.66	-0.28	2.00	2011.70
MHC-I toxicity								
seq1	VAEVYINSY	-0.88	Non-Toxin	0.06	0.48	-0.71	-1.00	1057.29
seq2	DAGVSGIIY	-1.35	Non-Toxin	0.18	0.96	-0.51	-1.00	894.12
seq3	MTAIKADDY	-1.02	Non-Toxin	-0.13	-0.32	0.24	-1.00	1027.27
seq4	LTNSNYTEL	-1.12	Non-Toxin	-0.16	-0.71	-0.33	-1.00	1054.25
seq5	GKDVSLEKY	-1.08	Non-Toxin	-0.28	-1.03	0.74	0.00	1038.29
seq6	YTKMIYQAY	-0.95	Non-Toxin	-0.07	-0.42	-0.86	1.00	1180.51
seq7	HTKYTKMIY	-1.14	Non-Toxin	-0.21	-0.96	-0.33	2.50	1184.55
seq8	FTDDRINAS	-1.07	Non-Toxin	-0.30	-0.82	0.48	-1.00	1038.19
seq9	AYDTEKDSY	-1.12	Non-Toxin	-0.37	-1.86	0.76	-2.00	1091.20
MHC class II								
seq1	KNFILGEKI	-1.29	Non-Toxin	-0.08	0.04	0.14	1.00	1061.43
seq2	DIKLRGPLS	-1.04	Non-Toxin	-0.22	-0.29	0.43	1.00	998.32

seq3	PLSVIGHSL	-0.88	Non-Toxin	0.17	1.06	-0.76	0.50	922.23
seq4	LSVIGHSLV	-0.96	Non-Toxin	0.23	1.70	-0.92	0.50	924.25
PV$_{HSP}$ toxicity prediction								
B- cell epitopes								
seq1	AFTTRGRSMGVAARQQLNTN	-1.09	Non-Toxin	-0.29	-0.66	0.01	3.00	2179.73
seq2	RVRILLTNLLRESGKKAEEV	-1.19	Non-Toxin	-0.30	-0.36	0.58	2.00	2325.06
seq3	AAMKCAILSPAFKVRDFSVK	-1.07	Non-Toxin	-0.08	0.55	-0.01	3.00	2182.94
seq4	ETDDTKG	-0.98	Non-Toxin	-0.48	-2.31	1.60	-2.00	764.83
seq5	NQEKEENTNAPSNDVVLP	-0.83	Non-Toxin	-0.31	-1.45	0.59	-3.00	1998.35
seq6	PPKAEPAKEPEKGSDGQKGE	-0.34	Non-Toxin	-0.39	-2.07	1.32	-1.00	2079.53
seq7	DDEAEEE	-0.81	Non-Toxin	-0.52	-2.74	2.50	-6.00	835.81
seq8	KKDEDKEKKE	-0.71	Non-Toxin	-0.88	-3.70	3.00	0.00	1276.54
seq9	GEIEDVGE	-0.93	Non-Toxin	-0.12	-0.76	1.09	-4.00	846.96
seq10	DEEEDKKEKDKK	-0.90	Non-Toxin	-0.85	-3.67	3.00	-2.00	1520.77
seq11	LAVKHFSVEGQLEFRALLFV	-1.39	Non-Toxin	0.04	0.82	-0.41	0.50	2304.04
seq12	SAGGMPGG	-1.03	Non-Toxin	0.10	-0.04	-0.19	0.00	632.80
seq13	MPGGMPSGAPGAGSTGGGPT	-1.49	Non-Toxin	0.06	-0.26	-0.19	0.00	1644.10
seq14	KSTAGDTHLGGEDFDNRMVN	-1.18	Non-Toxin	-0.26	-1.08	0.49	-1.50	2164.58
seq15	AVITVPAYFNDSQRQATKDS	-1.76	Non-Toxin	-0.20	-0.54	0.06	0.00	2211.69
MHC class-I epitope								
seq1	FSKLFYDTY	-0.67	Non-Toxin	-0.05	-0.23	-0.61	0.00	1183.44
seq2	KLTVLGATY	-0.70	Non-Toxin	0.06	0.73	-0.63	1.00	965.29
seq3	NTNIKNTII	-0.80	Non-Toxin	-0.13	-0.26	-0.29	1.00	1030.33
seq4	MSEEMNGET	-0.73	Non-Toxin	-0.25	-1.34	0.72	-3.00	1027.21
seq5	QLEFRALLF	-1.18	Non-Toxin	-0.00	0.81	-0.52	0.00	1136.49
seq6	MSLIINTFY	-1.19	Non-Toxin	0.20	1.24	-1.27	0.00	1101.46
seq7	LMSLIINTF	-1.01	Non-Toxin	0.26	1.81	-1.21	0.00	1051.45
seq8	ETALLSSGF	-0.56	Non-Toxin	0.08	0.67	-0.38	-1.00	924.14
seq9	VKDLVVLLF	-1.35	Non-Toxin	0.22	2.16	-0.71	0.00	1045.47
seq10	MKENQKQIY	-0.93	Non-Toxin	-0.43	-1.86	0.47	1.00	1181.51
seq11	TRGTKITLY	-1.20	Non-Toxin	-0.22	-0.43	-0.12	2.00	1052.37
seq12	YMTDPIDEY	-0.77	Non-Toxin	-0.14	-1.00	0.10	-3.00	1146.35
seq13	YSDNQPGVL	-1.57	Non-Toxin	-0.13	-0.73	-0.21	-1.00	992.18
seq14	YTNITRARF	-0.81	Non-Toxin	-0.32	-0.68	-0.19	2.00	1141.41
seq15	SSSQASIEI	-0.89	Non-Toxin	-0.07	0.07	0.03	-1.00	921.09
MHC class-II epitope								
seq1	YIKITPNKA	-1.00	Non-Toxin	-0.15	-0.46	-0.07	2.00	1047.39
seq2	ERIMKAQAL	-1.45	Non-Toxin	-0.24	-0.18	0.37	1.00	1059.42
seq3	AILSPAFKV	-1.05	Non-Toxin	0.16	1.40	-0.59	1.00	945.29
seq4	VRILLTNLL	-1.31	Non-Toxin	0.09	1.69	-0.86	1.00	1054.49

Table 8. Residues pair selected for disulfide engineering.

Res1 Seq #	Res1 AA	Res2 Seq #	Res2 AA	$\chi 3$	Energy	Sum B-Factors
28	PHE	37	ALA	111.66	3.37	19.65
29	GLU	37	ALA	-99.5	4.45	19.67
64	ASP	129	VAL	118.32	4.92	19.11
68	GLU	127	VAL	116.39	4.19	19.7
71	GLY	72	ASP	-105.29	3.97	23.35
76	GLY	123	ALA	80.13	1.32	19.25
89	GLY	92	GLU	81.62	1.74	19.51
108	VAL	112	ALA	-106.12	3.27	20.1
152	GLN	154	GLY	93.03	2.66	26.31
158	GLY	160	GLY	-91.92	5.63	29.98
170	GLY	174	GLN	-88.46	5.25	26.41
186	ASP	415	GLY	-114.11	5.13	18.91
188	VAL	209	VAL	116.11	3.13	19.09
191	GLY	641	ASP/	-57.31	6.28	19.18
194	PRO	277	LYS	-90.95	1.25	19.05
194	PRO	278	GLU	-114.89	4.79	19.04
200	CYS	637	ALA	122.13	3.56	18.89
202	ILE	641	ASP	-62.28	4.73	19.12
203	LEU	208	LYS	85.1	5.1	19.48
208	LYS	405	ASP	72.13	4.34	19.55
210	ARG	405	ASP	-77.13	5.87	19.14
213	SER	395	GLY	120	5.18	21.45
215	LYS	216	GLY	-99.84	0.79	22.26
216	GLY	217	PRO	107.45	1.73	23.71
225	LEU	407	ARG	72.08	1.9	19.05
232	GLU	256	VAL	-109.56	5.49	21.45
242	PRO	259	ARG	113.48	1.85	18.9
244	PRO	258	ALA	99.05	1.08	18.96
244	PRO	271	ASP	-92.18	5.62	18.84
245	GLY	275	ASP	-106.82	3.78	19.2
246	ALA	258	ALA	85.82	0.71	19.04
276	LYS	435	SER	106.32	3.03	19.12
283	GLY	427	SER	111.42	6.5	19.04
286	PRO	308	GLY	90.92	4.97	19.76
317	ASP	382	GLY	-109.94	2.59	25.49
324	PRO	339	LEU	109.67	1.92	21.14
334	SER	337	GLY	111.13	3.37	19.72
344	LEU	378	GLY	124.29	3.52	23.44
357	MET	359	GLY	122.96	6.84	21.64
378	GLY	380	THR	-112.44	3.55	26.66
416	ALA	444	SER	-92.41	2.63	18.99
421	PRO	467	PRO	-104.78	4.61	18.8
423	TYR	515	TYR	83.01	3.53	18.99
424	PHE	428	GLN	91.36	4.05	18.89
448	LYS	643	GLN	93.6	4.39	19.15
462	LEU	469	TYR	70.56	6.76	19.07
468	GLY	601	ALA	-62.85	3.92	19.22
483	GLU	487	LYS	118.41	4.59	20.1

512	GLY	612	ALA	-94.46	1.49	19.03
528	ALA	547	ARG	75.39	4.02	19.11
535	MET	543	GLN	119.58	6.45	19.13
541	ALA	575	PHE	126.32	6.26	19.36
541	ALA	577	ALA	-77.58	7.04	19.55
553	ALA	558	ILE	-116.66	7.32	18.98
571	ILE	577	ALA	81.75	0.72	19.56
597	LEU	604	LYS	125.94	5.4	19.22
629	THR	630	ASP	-88.27	5.24	22.08
646	VAL	652	THR	114.51	3.65	19.22
659	PHE	676	HIS	107.41	5.44	19.83
660	ALA	675	HIS	96.61	4.39	19.59
PVAO						
6	THR	143	GLY	121.78	4.74	0
10	LEU	143	GLY	100.89	5.18	0
12	ALA	16	MET	99	1.21	0
16	MET	20	GLU	70.69	4.12	0
27	LYS	30	GLU	96.07	4.33	0
43	ALA	55	GLU	-110.96	4.26	0
53	ALA	86	SER	-76.27	3.94	0
56	ALA	58	GLU	-80.58	3.24	0
65	VAL	76	GLY	80.18	5.17	0
83	GLU	89	GLY	67.16	7.26	0
97	VAL	115	GLU	116.57	3.2	0
99	GLY	100	ALA	117.18	5.97	0
121	GLU	161	GLY	-71.83	3.87	0
137	SER	185	PRO	-97.01	4.25	0
139	GLY	151	VAL	-77.9	3.65	0
140	PRO	188	ARG	-101.26	3.28	0
156	ARG	196	GLY	-83.54	1.82	0
159	ALA	193	VAL	-66.74	1.47	0
159	ALA	196	GLY	113.26	3.06	0
175	PRO	216	GLY	112.95	4.55	0
191	ALA	223	SER	88.21	3.1	0
194	SER	224	VAL	123.81	4.19	0
204	PHE	217	PRO	85.1	5.01	0
218	GLY	241	GLY	-82.9	3.21	0
222	GLY	245	GLY	-100.43	3.63	0
247	HIS	281	GLY	-90.24	8.17	0
255	PRO	256	GLY	108.82	1.28	0
257	PRO	260	GLY	124.31	2.81	0
264	ALA	286	PRO	-58.9	2.34	0
268	TYR	283	GLY	-97.65	3.89	0
276	PHE	278	LYS	-103.19	1.45	0
284	PRO	322	VAL	-59.46	7.15	0
288	PRO	325	HIS	-96.51	3.64	0
290	ASP	295	ILE	114.71	5.52	0
291	ARG	292	ILE	108.71	3.56	0
299	GLY	326	SER	-101.11	5.77	0
310	GLY	348	PRO	119.93	4.11	0

324	GLY	367	LEU	75.57	4.81	0
328	VAL	370	GLY	-78.2	4.44	0
333	PRO	336	GLY	-78.57	3.33	0
344	GLY	347	GLY	-79.73	5.39	0
365	SER	403	LEU	104.31	0.28	0
372	GLY	379	VAL	99.66	5.79	0
383	GLY	404	ILE	-86.34	2.99	0
386	PRO	396	LYS	104.63	1.19	0
386	PRO	398	ARG	93.17	1.63	0
391	ASP	395	GLU	125.17	6.97	0
393	SER	394	LEU	-109.96	2.36	0
405	VAL	454	GLY	-60.21	3.17	0
413	CYS	463	LYS	-70.48	4.09	0
414	LYS	422	TYR	108.55	5.19	0
423	ARG	426	GLN	-104.93	5.4	0
425	LEU	429	HIS	-105.7	2.22	0
448	GLU	451	ILE	95.9	0.72	0
456	GLY	501	GLY	69.39	4.74	0
466	VAL	467	GLN	106.75	3.04	0
468	PHE	470	MET	-114.38	4.31	0
496	TRP	542	GLY	87.2	2	0
533	ASN	573	ILE	-95.93	3.86	0
557	ALA	558	PRO	-69.01	4.98	0
610	GLN	611	GLY	108.94	3.62	0
620	TYR	623	ASP	123.66	8.38	0
645	GLY	656	ASN	76.84	2.46	0
647	GLY	653	ASN	106.22	4.07	0
650	HIS	653	ASN	86.64	1.96	0
668	PRO	704	GLU	-94.47	5.78	0
673	GLU	674	GLY	-110.51	1.28	0
675	VAL	713	LYS	-83.32	1.6	0
686	ILE	720	TYR	85.05	4.25	0
692	GLY	695	ILE	-88.71	5.42	0
693	PRO	731	PRO	-95.35	1.13	0
699	GLU	739	THR	-89.93	4.66	0
706	PRO	746	GLY	-66.55	6.66	0
749	LYS	793	THR	89	2.7	0
750	ASN	751	PHE	124.48	7.62	0
753	LEU	789	TYR	110.87	0.84	0
773	ILE	778	VAL	-89.43	4.33	0
793	THR	796	ASP	-90.1	5.05	0

Table 9. Molecular docking of MEVs-TLR4 complexes showing H-bonds

Vaccine	Ligand	Interacting residues
PV$_{AO}$	TLR4	LYS^{119}:HZ3 - GLN^{129}:OE1, ASP^{792}:HN - ASP^{95}:OD1, GLN^{39}:HE22 - THR^{145}:0, ASN^{58}:HD22 - GLU^{115}:OE2, ASP^{95}:HN - ASP^{792}:OD2, GLN^{99}:HE21 - PRO^{733}:O, GLY^{120}:HN - ALA^{788}:O, GLN^{129}:HE21 - LYS^{119}:O, LEU^{19}:HA - GLU^{27}:OE1, VAL^{42}:HA-GLN^{599}:O, GLU^{121}:HA - GLU^{154}:OE1, GLY^{150}:HA1 - ASP^{60}:OD2, GLY^{200}:HA2 - GLU^{266}:OE1, GLY^{734}:HA1 - GLN^{99}:O, ASP^{792}:HA - ASP^{95}:OD2, PRO^{28}:HA - LEU^{18}:O, VAL^{30}:HA - LEU^{21}:O, GLY^{96}:HA2 - ASP^{741}:OD1, SER^{102}:HB2-GLY^{730}:O, SER^{123}:HB2 - ALA^{784}:O, LYS^{153}:HE1 - LYS^{119}:O, HIS^{179}:HE1 - PHE^{160}:O LEU^{600}:HA - ALA^{41}:O
PV$_{HSP}$	TLT4	LYS^{74}:NZ - GLU^{266}:OE2, LYS^{487}:NZ – GLU^{27}:OE1, LYS^{153}:NZ - LEU^{269}:O, GLY^{158}:H - THR^{319}:OG1, THR^{162}:OG1 - SER^{317}:OG, GLY^{373}:H - ASN^{531}:OD1, LYS^{497}:NZ - VAL^{32}:O, ARG^{264}:NE - PRO^{159}:O, ASN2^{65}:N - ASP^{153}:OD1, THR^{319}:OG1 - GLY^{158}:O, HIS^{529}:ND1 - PRO^{183}:O, ASN^{531}:ND2 - SER^{372}:OG, SER^{552}:OG - ASN^{181}:OG1, LYS^{153}:CE - GLU^{270}:O, GLY^{352}:CA - ASN^{579}:O, SER^{353}:CB - GLN^{578}OE1, GLY^{369}:CA - HIS^{529}:O, LYS^{487}:CE - PRO^{28}:O, SER^{317}:CB - GLY^{160}:O, HIS^{458}:CE1 - GLU^{30}:O, GLN^{507}:CA - SER^{372}:OG, HIS^{555}:CE1 - ALA^{354}:O

Table 10. Details of immune simulation results of PV$_{AO}$ and PV$_{HSP}$ vaccine constructs

S.N	**Types of immune response**	**PV$_{AO}$**	**PV$_{HSP}$**
1	Antigen count (Ist dose)	Decrease to zero count after 5th day of injection	Decrease to zero count after 5th day of injection
2	Antigen count (IInd and IIIrd dose)	Decrease to zero after 1st day of injection	Decrease to zero after 2nd day of injection
3	Antibody titers (IgG + IgM and IgG1 + IgG2)	Elicited high level of antibody titer	Elicited high level of antibody titer
4	Total B cell population per state at end of IIIrd dose (cells per mm3)	580	500
5	Active B cell population at end of IIIrd dose (cells per mm3)	480	320
6	Plasma B lymphocytes at end of IIIrd dose (IgG1)	9	6
7	IFN-γ (ng/ml)	4.3×10^{5}	4.2×10^{5}
8	TGF-β (ng/ml)	1.1×10^{4}	7.5×10^{4}
9	IL-2 (ng/ml)	3.0×10^{5}	2.0×10^{5}
10	IL-10 (ng/ml)	4×10^{4}	4×10^{4}
11	IL-12 (ng/ml)	2×10^{3}	2×10^{4}
11	Memory T-helper lymphocytes count (cells,per mm3)	4000	~ 3200
13	Active T-cytotoxic lymphocytes population	2200	~ 850

	per state (cells per mm3)		
14	Active macrophages (cells per mm3)	100	100
15	Macrophages presenting (cells per mm3)	200	200

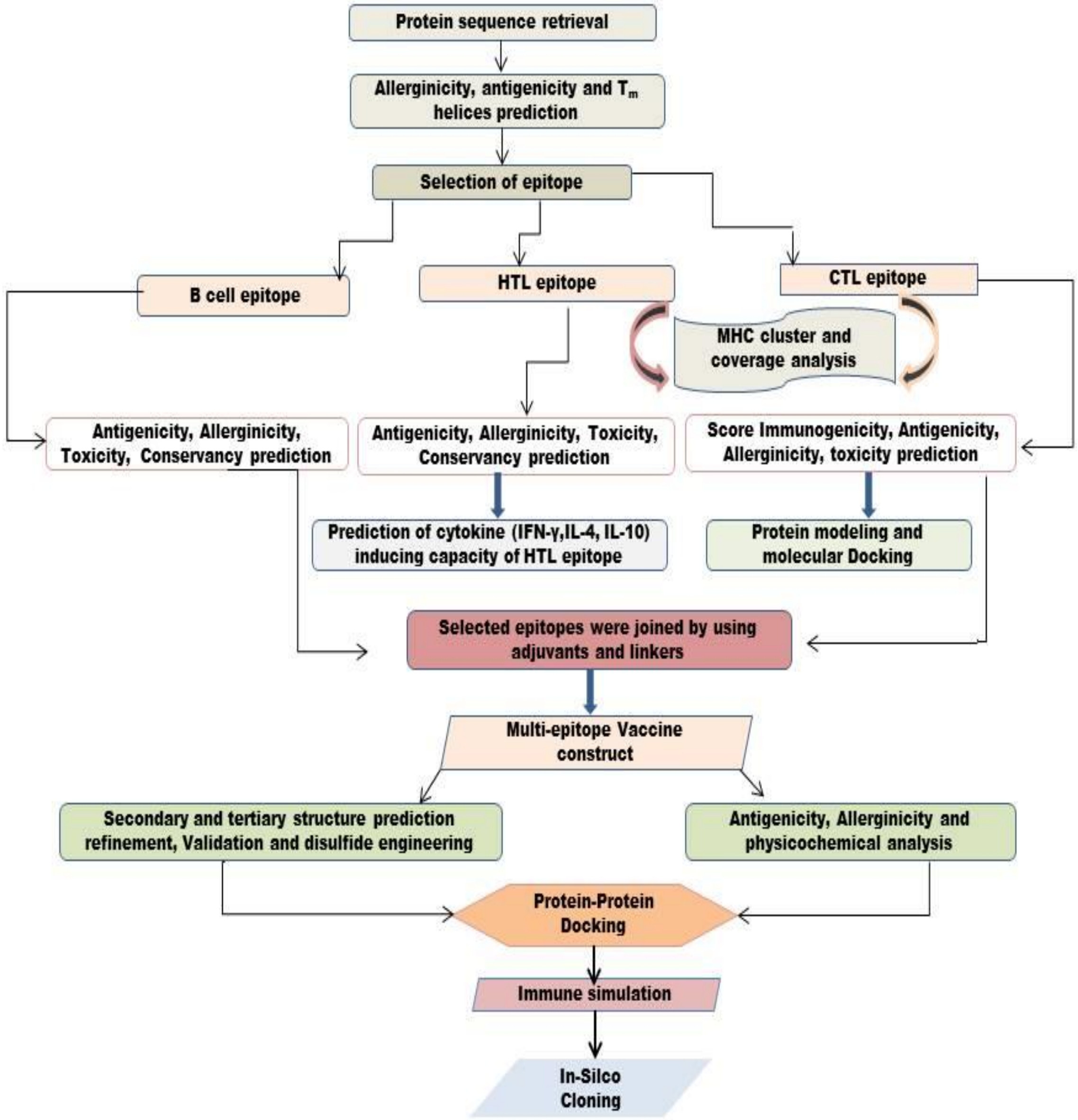


Figure 1. Methodology for multiepitope vaccine construction, Docking, Simulation and Cloning

MAKLSTDELLDAFKEMTLLELSDFVKKFEETFEVTAAAPVAVAAAGAAPAGAAVEAAEEQSEF
DVILEAAGDKKIGVIKVVREIVSGLGLKEAKDLVDGAPKPLLEKVAKEAADEAKAKLEAAGATV
TVKEAAAKNSYGPGPGNTGPGPGVGKGLRILAFGPGPGFPIRGLAEGPGPGDDGGPGPGLRGDA
GVSGGPGPGIIYFQQGSGGSITTIGPGPGSGSVSGLTGPGPGPGLHGFHGPGPGVHQYGDQTGPGP
GNGCTSAGDHYGPGPGNPFGKTHGGGPGPGPNDRIKHIGDLGNIVAGANGGPGPGDIKLRGPLS
VIGHSLVVHAGPGPGANDDLGQGGPGPGTGNMREESLKTGNAGSRLACGPGPGYEFTVKDIGPG
PGNGVDVSLEKYRGHVCLIVNVGPGPGCKSGATDKNYRQLQEMHTRLGPGPGNQFGGQEPWA
EAEIGPGPGKKFVTEKYGVQFDMFSKIKGPGPGFLKSRQHGTLTNNIKWNFSKGPGPGVNGSDA
DDLYKGPGPGFLKSRQHGTLTNNIKWNFSKGPGPGQPVKRYSPTTAPYDIEGDIMGPGPGPIRLV
LVDQGIKFTDGPGPGDRINASDWPSMKSHFHFGQGPGPGLPCLYDGDHQIVQSGGPGPGAILRHL
AGPGPGRKHNLNGGNELETTHIDGPGPGMFCEGVRDLHTKYTKMIYQAGPGPGILPVELAKFEG
PGPGCLDKFPLLKAYHQRMEDRPGGPGPGKLLATRDGPGPGDGKNFILGEKISYVDFVLFEGPGP
GIIYAAYVAEVYAAYYAAYAYDTEKDSYAAYHHHHHH

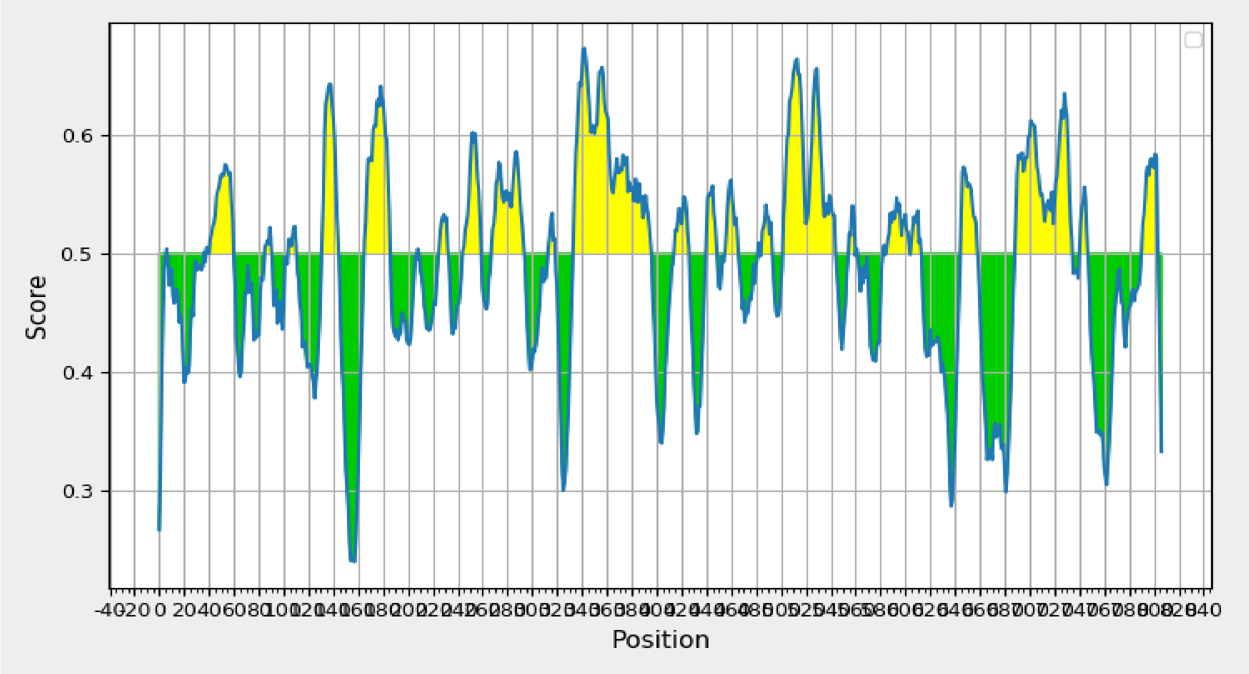


Figure 2. Visualization of vaccine constructs epitopic regions (A) Amino acids sequence of the PV_{AO} adjuvant (yellow) B-cell and HTL (green) CTL (purple) and 6 His tag sky color (B) Yellow peaks represent epitopic regions and green non-epitopic regions.

MAKLSTDELLDAFKEMTLLELSDFVKKFEETFEVTAAAPVAVAAAGAAPAGAAVEAAEEQSEF
DVILEAAGDKKIGVIKVVREIVSGLGLKEAKDLVDGAPKPLLEKVAKEAADEAKAKLEAAGATV
TVKEAAAKPPKAEPAKEPEKGSDGQKGEGPGPGETDDTKGGPGPGNQEKEENTNAPSNDVVLP
GPGPGAAMKCAILSPAFKVRDFSVKGPGPGRVRILLTNLLRESGKKAEEVGPGPGAFTTRGRSM
GVAARQQLNTNGPGPGDEEEDKKEKDKKGPGPGDDEAEEEGPGPGGEIEDVGEGPGPGKKDED
KEKKEGPGPGLAVKHFSVEGQLEFRALLFVGPGPGSAGGMPGGGPGPGMPGGMPSGAPGAGST
GGGPTGPGPGKSTAGDTHLGGEDFDNRMVNGPGPGAVITVPAYFNDSQRQATKDSGPGPGAILS
PAFKVGPGPGVRILLTNLLGPGPGYIKITPNKAGPGPGERIMKAQALAAYFSKLFYDTYAAYKLT
VLGATYAAYNTNIKNTIIAAYMSEEMNGETAAYQLEFRALLFAAYMSLIINTFYAAYLMSLIINTF
AAYETALLSSGFAAYVKDLVVLLFAAYMKENQKQIYAAYTRGTKITLYAAYYMTDPIDEYAAY
YSDNQPGVLAAYYTNITRARFAAYSSSQASIEIHHHHHH

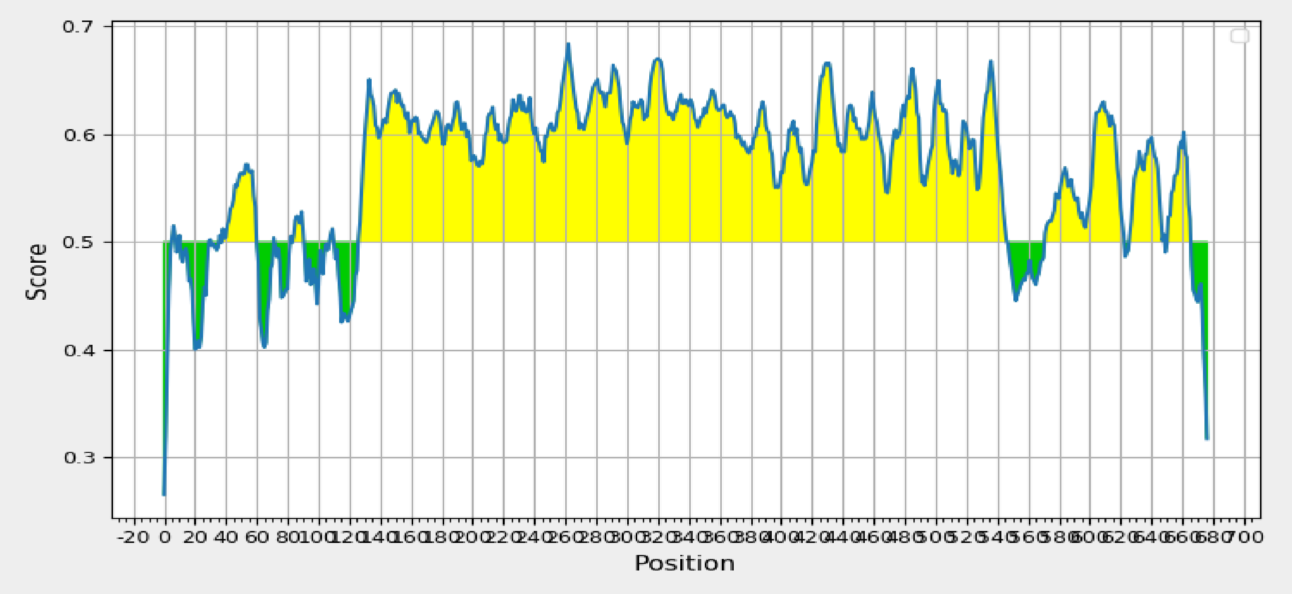
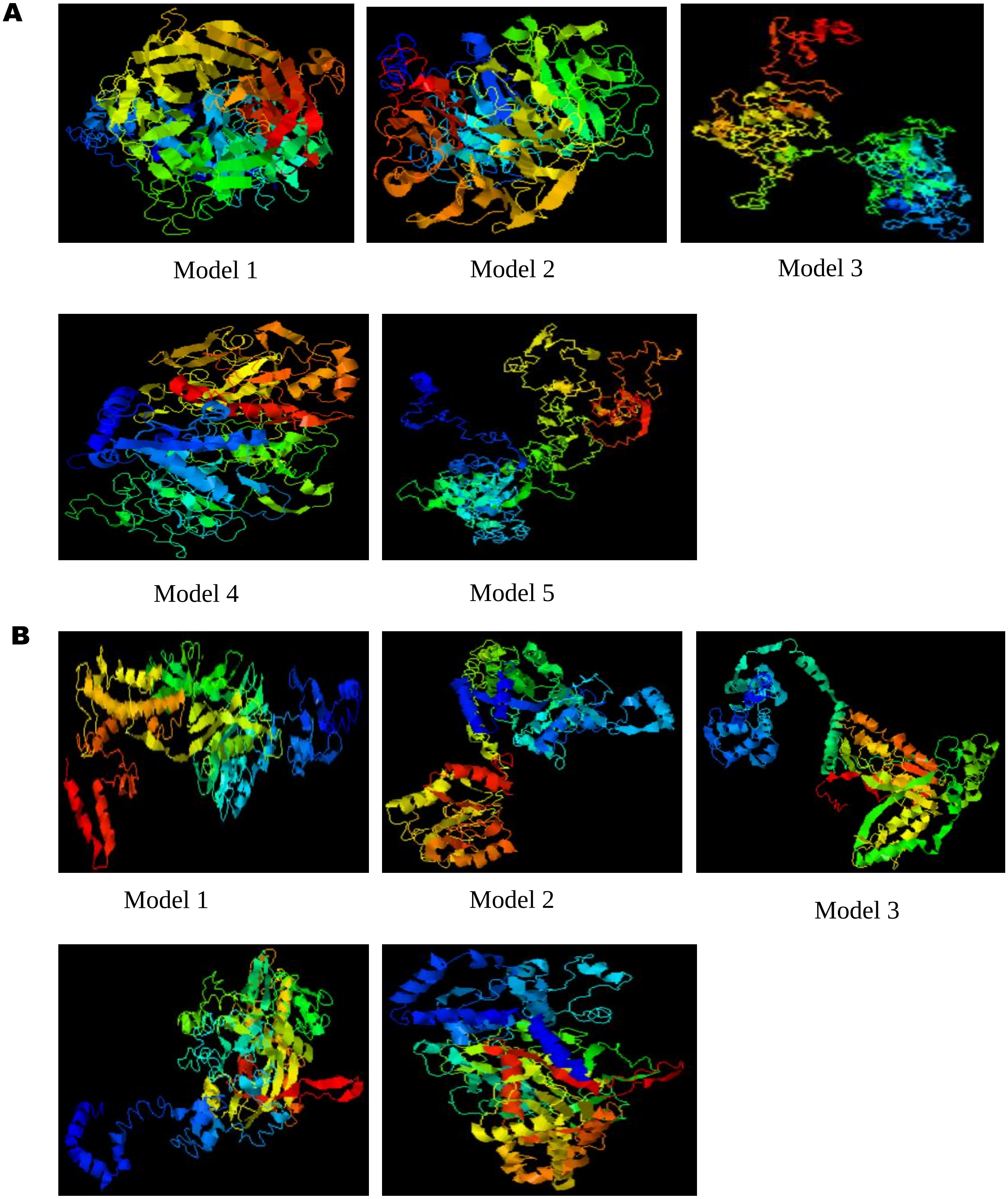


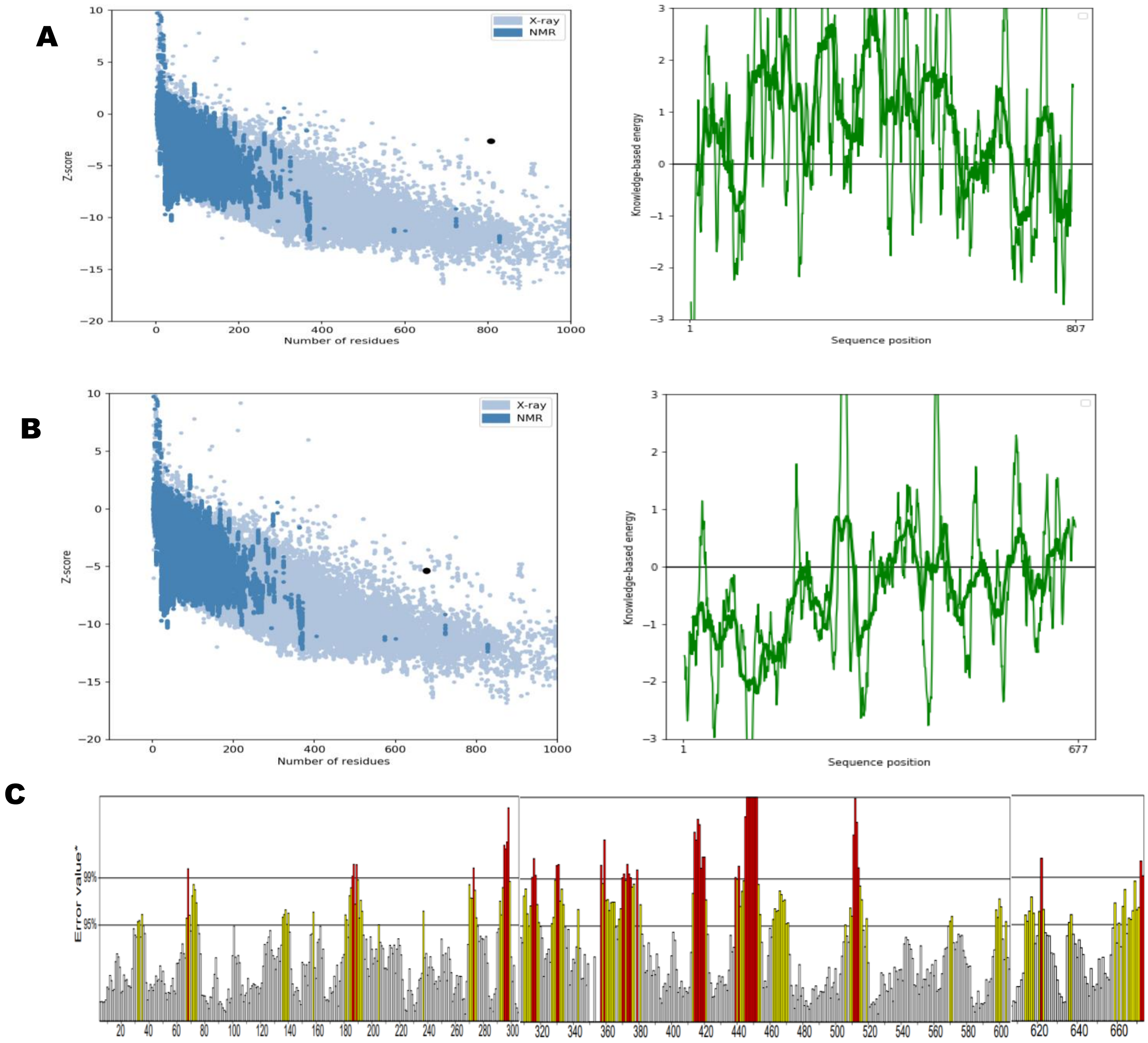
Figure 3. Visualization of vaccine constructs epitopic regions (A) Amino acids sequence of the PV_{HSP} adjuvant (yellow) B-cell and HTL (green) CTL (purple) and 6 His tag sky color (B) Yellow peaks represent epitopic regions and green non-epitopic regions.



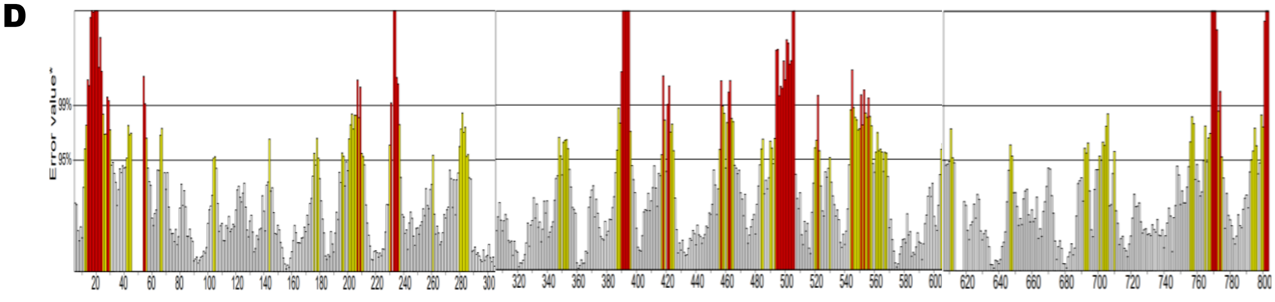
A: Model 1, Model 2, Model 3, Model 4, Model 5

B: Model 1, Model 2, Model 3, Model 4, Model 5

Figure 4. I-TASSER model of PV_{AO} and PV_{HSP} vaccine candidate for model 1 to 5 (A) PV_{AO} vaccine candidate models and (B) PV_{HSP} vaccine candidate models

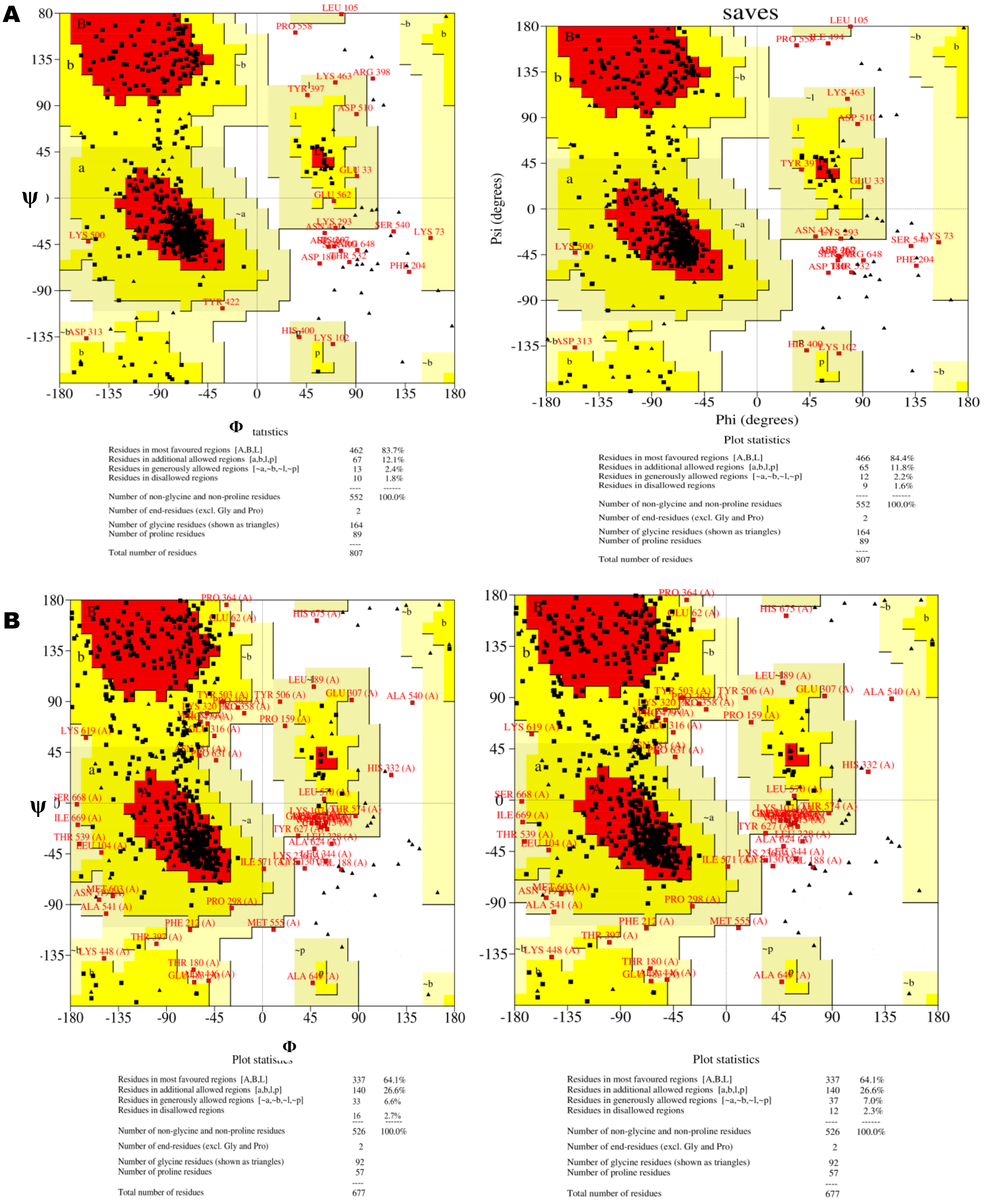


Residue # (window center) Overall quality factor of PV$_{HSP}$ 76.097



Residue # (window center) Overall quality factor of PV$_{AO}$ 77.423

Figure 5. Protein quality structure of PV$_{AO}$ and PV$_{HSP}$ predicted by ProSA Z-score overall model quality. (A) PV$_{AO}$ vaccine candidate and (B) PV$_{HSP}$ vaccine candidate (C) ERRAT PV$_{HSP}$ and (D) ERRAT PV$_{AO}$



tatistics

Residues in most favoured regions [A,B,L]	462	83.7%
Residues in additional allowed regions [a,b,l,p]	67	12.1%
Residues in generously allowed regions [~a,~b,~l,~p]	13	2.4%
Residues in disallowed regions	10	1.8%
	----	------
Number of non-glycine and non-proline residues	552	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	164	
Number of proline residues	89	

Total number of residues	807	

Plot statistics

Residues in most favoured regions [A,B,L]	466	84.4%
Residues in additional allowed regions [a,b,l,p]	65	11.8%
Residues in generously allowed regions [~a,~b,~l,~p]	12	2.2%
Residues in disallowed regions	9	1.6%
	----	------
Number of non-glycine and non-proline residues	552	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	164	
Number of proline residues	89	

Total number of residues	807	

Plot statistics

Residues in most favoured regions [A,B,L]	337	64.1%
Residues in additional allowed regions [a,b,l,p]	140	26.6%
Residues in generously allowed regions [~a,~b,~l,~p]	33	6.6%
Residues in disallowed regions	16	2.7%
	----	------
Number of non-glycine and non-proline residues	526	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	92	
Number of proline residues	57	

Total number of residues	677	

Plot statistics

Residues in most favoured regions [A,B,L]	337	64.1%
Residues in additional allowed regions [a,b,l,p]	140	26.6%
Residues in generously allowed regions [~a,~b,~l,~p]	37	7.0%
Residues in disallowed regions	12	2.3%
	----	------
Number of non-glycine and non-proline residues	526	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	92	
Number of proline residues	57	

Total number of residues	677	

Figure 6. PROCHECK results of PV_{AO} and PV_{HSP} candidates (A) PV_{AO} (before and after refinement) (B) PV_{HSP}, and (before and after refinement) showing the amino acid residues in Ramachandran Plot before and after refining process

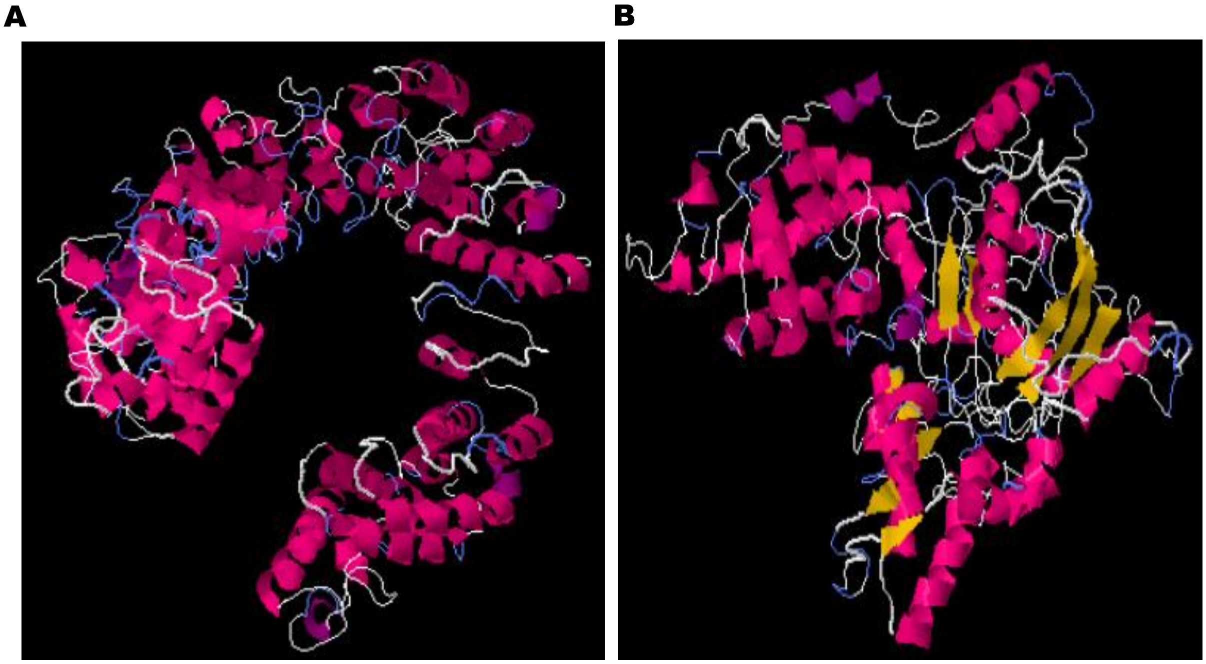


Figure 7. Disulfide bond-bridge (white lines) of vaccine constructs is shown (A) PV_{AO} and (B) PV_{HSP}