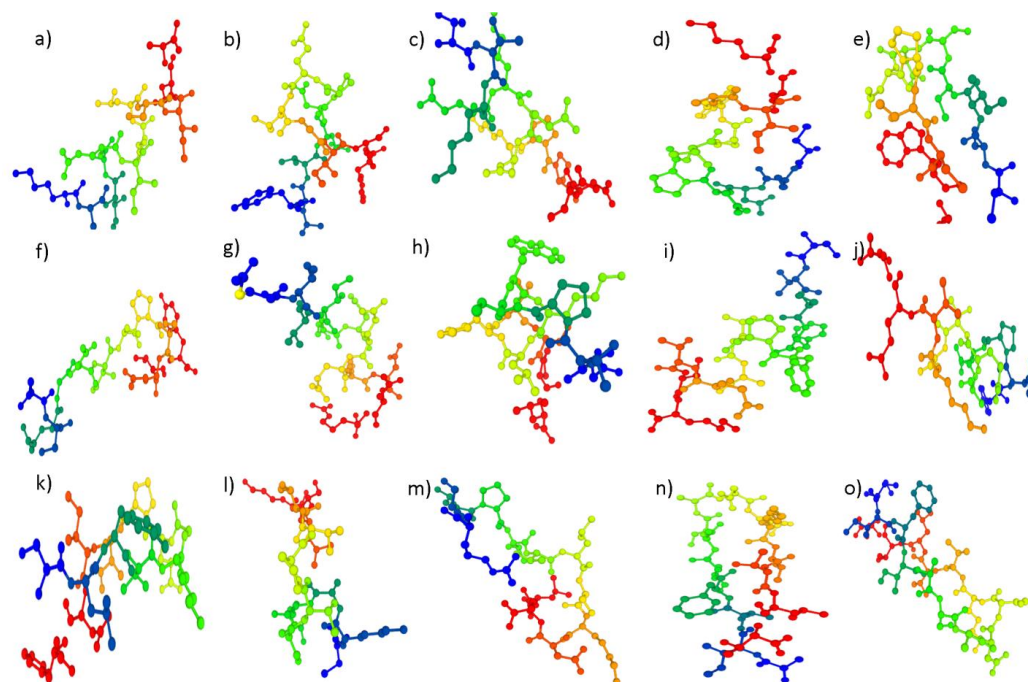
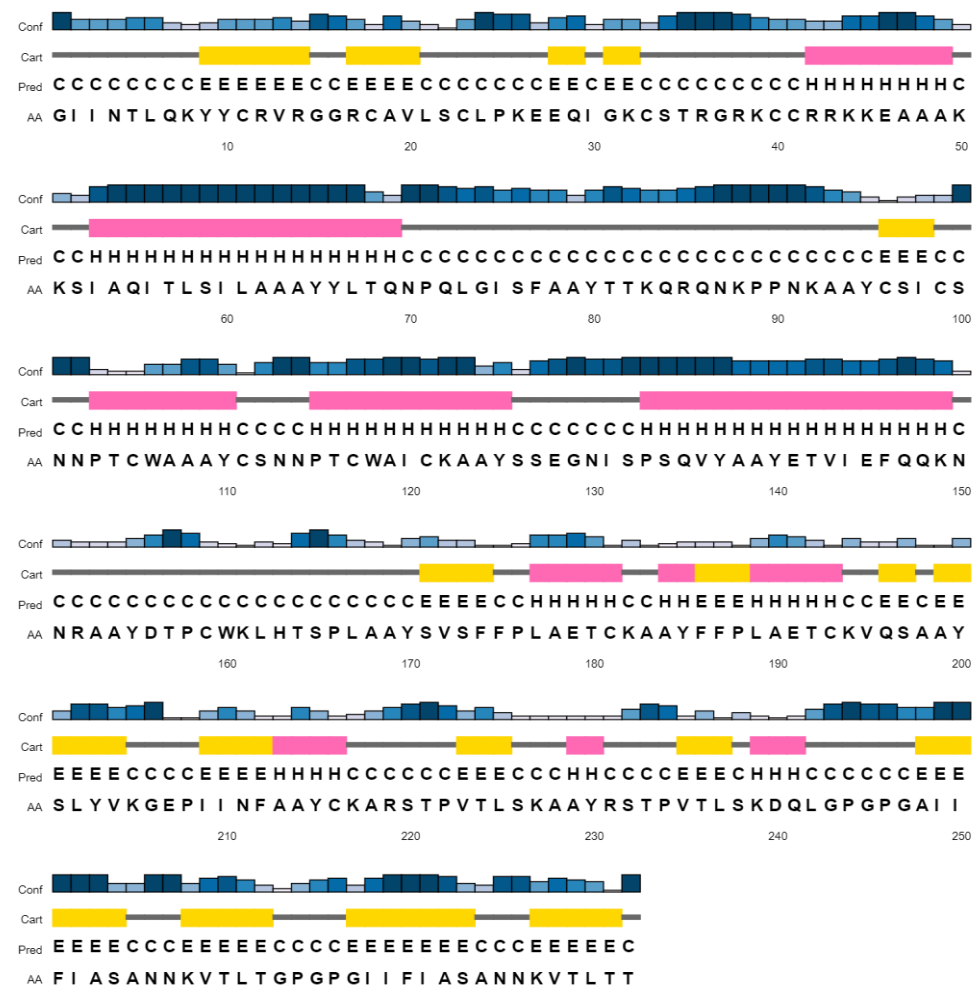


**Figure 1.** 3D structural representation of RSV antigenic proteins: (A) G protein and (B) F protein. RaptorX was used to predict these structures. These 3D structures were predicted to forecast conformational B-cell epitopes from target proteins and identifying their particular positions on proteins structures.



**Figure 2.** 3D structures of MHC Class I and MHC Class II epitopes. These 3D structures were predicted by PEP-FOLD v3.0 server and used as ligands in molecular docking against their respective HLA alleles. .



**Figure 3.** Secondary structure analyses of RSV MEV construct. Yellow bars are representing strands, pink bars are representing helices and grey lines are representing coils.

**Table 1.** RSV antigenic proteins physiochemical properties.

Proteins	Molecular Weight	Theoretical pI	Instability index	Half-Life	Stability Profiling	Aliphatic Index	Grand Average of Hydropathy
Glycoprotein	32767.53	9.85	37.25	30 hours (mammalian reticulocytes, in vitro). >20 hours (yeast, in vivo). >10 hours (Escherichia coli, in vivo).	stable	75.64	-0.520
Fusion protein	63334.16	9.01	40.42	30 hours (mammalian reticulocytes, in vitro). >20 hours (yeast, in vivo). >10 hours (Escherichia coli, in vivo).	unstable	101.50	-0.007

**Table 2.** Prediction of RSV proteins Secondary structure through SOPMA.

Proteins	Sequence Length	$\alpha$ -helix	$\beta$ -Turn	Random Coils
Glycoprotein	298	19.80%	3.36%	52.35%
Fusion protein	574	36.76%	8.71%	31.18%

**Table 3.** Predicted CTL Epitopes. The epitopes listed in the table showed 100% conservancy among the both protein sequences included in the present study.

Proteins	Peptide	Position	Allele	Antigenicity
Glycoprotein	HFEVFNFPVPCSI	164-175	HLA-B*40:01	0.7
	NLKSIAQITLSI	34-45	HLA-A*32:01	1.0
	KSIAQITLSILA	36-47	HLA-A*32:01	0.6
	GNPEHTSQEETL	54-65	HLA-B*38:01	0.6
	NPEHTSQEETLH	55-66	HLA-B*38:01	0.7
	LKSIAQITLSIL	35-46	HLA-A*32:01	0.6
			HLA-B*44:03	
			HLA-A*01:01	
			HLA-B*44:02	
	SSEGNISPSQVY	269-280	HLA-B*15:25	0.7
			HLA-A*30:02	
			HLA-B*58:02	
			HLA-B*15:01	
			HLA-A*30:02	
			HLA-B*15:25	
	ATSQIKNTTPTY	79-90	HLA-B*15:01	0.8
			HLA-B*58:02	
			HLA-A*01:01	
			HLA-B*15:01	
			HLA-A*30:02	
	ISPSQVYTTSEY	274-285	HLA-B*35:01	0.6
			HLA-A*01:01	
			HLA-B*15:25	
			HLA-B*58:01	
	CSICSNNPTCWA	173-184		0.6
	AAIIFIASANNK	57-68	HLA-A*11:01	0.7
	NNPTCWAICKRI	178-189	HLA-B*51:01	0.9
	TTKQRQNKPPNK	147-158	HLA-A*30:01	0.7
	NNDFHFEVFNFV	160-171	HLA-B*38:01	1.14
	CSNNPTCWAICK	176-186	HLA-A*11:01	0.6
	YLTQNPQLGISF	90-101	HLA-B*15:02	1.31
	LPIVNBKQSCSIS	204-215	HLA-B*51:01	1.05

<b>Fusion protein</b>	DTVSVGNTLYYV	448-459	HLA-A*26:01 HLA-A*68:02, HLA-A*29:02, HLA-A*25:01 HLA-A*01:01 HLA-A*26:01	0.73
	VDTVSVGNTLYY	447-458	HLA-A*01:01 HLA-A*29:02, HLA-A*25:01 HLA-A*01:01	0.6
	LLSLIAVGLLLY	538-549	HLA-A*29:02, HLA-A*30:02 HLA-A*11:01,	0.7
	SLIAVGLLLYCK	540-551	HLA-A*03:01, HLA-A*29:02, HLA-A*03:01	0.8
	YQSTCSAVSKGY	33-44	HLA-B*15:01, HLA-B*15:02 HLA-A*01:01, HLA-A*29:02,	1.03
	KGYLSALRTGWY	42-53	HLA-B*58:01, HLA-B*57:01, HLA-A*30:02	0.5
	FFPLAETCKVQS	351-362	HLA-B*51:01, HLA-B*35:03 HLA-A*01:01,	0.6
	GVDTVSVGNTLY	446-457	HLA-A*26:01, HLA-A*29:02 HLA-A*25:01,	0.7
	YVKGEPIINFYD	468-479	HLA-A*26:01 HLA-A*30:01 HLA-A*11:01	1.2
	CKARSTPVTLSK	550-561	HLA-B*08:01, HLA-B*07:02	0.6
	DTPCWKLHTSPL	310-321	HLA-B*48:01, HLA-A*11:01	0.8
	RQQSYSIMSIK	282-293		

WYCDNAGSVSFF	341-352	HLA-A*24:02 HLA-A*23:01 HLA-B*38:01 HLA-A*03:01,	0.6
SLSNGVSVLTSK	180-191	HLA-A*74:01, HLA-A*11:01	0.7
RSTPVTLSKDQL	553-564	HLA-B*58:01, HLA-B*58:02	0.7
LLLYCKARSTPV	546-557	HLA-B*08:01, HLA-A*02:01 HLA-A*01:01 HLA-A*29:02	1.09
LYVKGEPIINFY	467-478	HLA-A*23:01, HLA-A*26:01 HLA-A*25:01	0.6
NTKEGSNICLTR	325-336	HLA-A*68:01 HLA-A*33:03	1.0
SLYVKGEPIINF	466-477	HLA-A*23:01 HLA-B*15:02	0.7
LRTGWYTSVITI	48-59	HLA-A*32:01, HLA-B*27:02, HLA-B*27:05	0.6
SVSFFPLAETCK	348-359	HLA-A*03:01, HLA-A*11:01	0.7
LSLIAVGLLLYC	539-550	HLA-A*29:02, HLA-A*01:01	0.8
TELQLLMQSTPA	91-102	HLA-B*18:01, HLA-B*40:02	0.7
ETVIEFQQKNNR	218-229	HLA-A*68:01, HLA-A*33:03	1.3
TPCWKLHTSPLC	311-322	HLA-B*08:01 HLA-B*35:03, HLA-B*07:02	0.9
NDMPITNDQKKL	262-273	HLA-B*35:03, HLA-B*37:01	0.6

**Table 4.** Predicted HTL Epitopes. The boxes colored with blue, light grey and black shows the strong, intermediate and non-binding affinities towards the respective human HLA alleles.

[illegible]



**Table 5.** Predicted linear B-cell epitopes of RSV proteins.

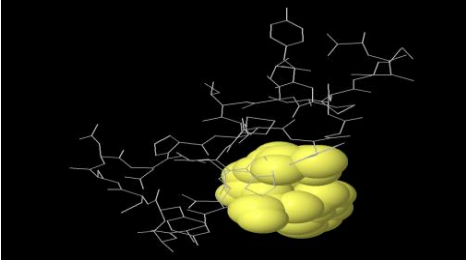
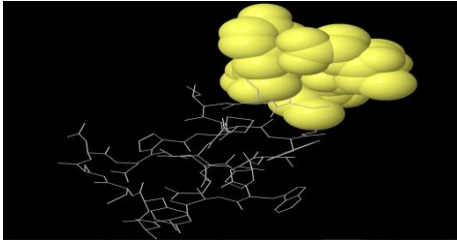
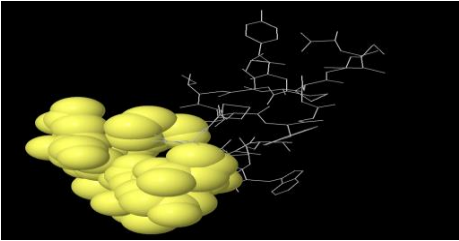
Proteins	B Cell Epitope (Position)	Antigenicity
Glycoprotein	TQIQPSKPTTKQRQNK 139	0.68
	TSQIKNTTPTYLTQNP 80	0.75
	KPTIKTTKKDLKPQTT 205	1.19
	CSICSNNPTCWAICKR 173	0.83
	TKDQRTAKTLEKTWDT 4	0.56
	PNIRTTLLTNSTTGNP 241	0.67
	ESILQSTTVKTKNTTT 123	0.59
	SGTTSQTTAILALTTP 105	0.55
	SQEETLHSTSSEGNIS 260	0.53
	HSTSSEGNISPSQVYT 266	0.51
	PTTKQRQNKPPNKPNN 146	0.79
	VKTKNTTTTQIQPSKP 131	0.83
	SCLYKLNLSIAQITL 28	0.77
	TYLTQNPQLGISFFNL 89	1.17
	HLLFISSCLYKLNLS 22	0.50
	LGISFFNLSTTSQTT 97	1.30
	LLTNSTTGNPEHTSQE 247	0.69
	AIIFIASANNKVTLT 58	0.63
	KPPNKPNNDFHFEVFN 154	0.58
	KSIAQITLSILAMIIS 36	0.54
	YGVIDTPCWKLHTSPL 306	0.56
	NIDIFNPKYDCKIMTS 383	0.78
	GSNICLTRDRGWYCD 329	0.83
	SCSISNIETVIEFQQK 211	1.03
	TSVITIELSNIKENKC 54	0.61
	LLEITREFSVNAGVTT 230	0.60
Fusion protein	CKARSTPVTLSKDQLS 550	0.83
	LIAGVGLLYCKARSTP 541	1.06
	SPLCTTNTKEGSNICL 319	1.37
	GSVSFFPLAETCKVQS 347	0.73
	TRDRGWYCDNAGSVS 335	0.97
	TNAITAILAAVTLCA 8	0.50
	TVSVGNTLYYVKNQEG 449	0.83

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TCSAVSKGYLSALRTG 36	0.71
FIRKSDELLHNVNAGK 505	0.51
NEKINQSLAFIRKSDE 496	0.72
YVSNKGVDTVSVGNTL 441	0.73
IETVIEFQQKNNRLE 217	0.88
SLGAIVSCYGKTKCTA 409	0.77
NIKENKCNGTDAKVKL 63	0.52
VKGEPINFYDPLVFP 469	0.55
QEGKSLYVKGEPIINF 462	0.50
GSAIASGIAVSKVLHL 145	0.58
PRFMNYTLNNTKNTNV 112	0.89
GVSVLTSKVLDLKNYI 184	0.76
SDEFDASISQVNEKIN 485	0.66
PIVNBKQSCSISNIETV 205	0.74
TLYYVNBKQEGKSLYVK 455	0.52
NVTLSKKRKRRLGFL 126	1.20
NVNAGKSTTNIMITTI 515	0.73
PVTLSKDQLSGINNIA 556	0.62

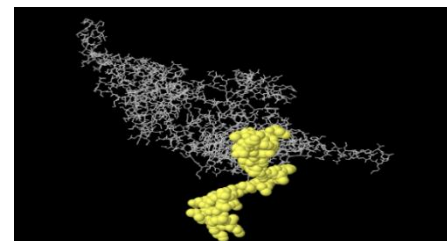
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Table 6. Predicted conformational B-cell epitopes of RSV proteins.

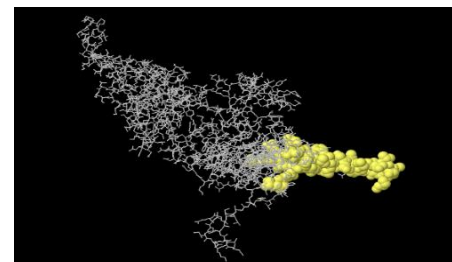
Protein	Conformational B-Cell Epitopes	3D Structure
Glycoprotein	C:T104, C:C105, C:W106	
	C:A94, C:Y95, C:C121, C:K122, C:A123, C:A124	
	C:N101, C:A107, C:A108, C:A109, C:Y110, C:C111, C:S112, C:N113, C:N114, C:P115	

**Fusion Protein**

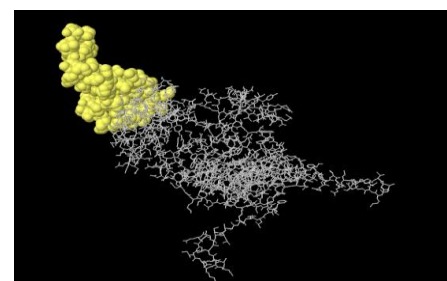
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\_:C21, \_:F22, \_:A23, \_:S24, \_:S25, \_:Q26, \_:N27, \_:I28, \_:T29,  
\_:E30, \_:E31, \_:F32, \_:Y33, \_:Q34, \_:S35, \_:T36, \_:C37, \_:S38,  
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\_:L48, \_:K421



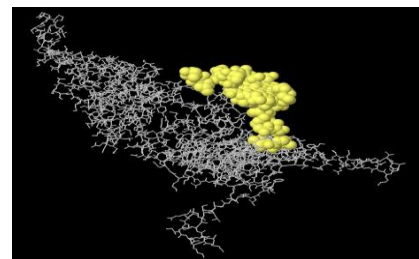
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\_:L512, \_:L513, \_:H514, \_:N515, \_:V516, \_:N517, \_:A518,  
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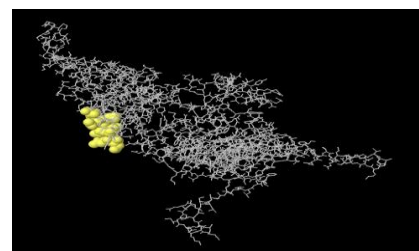
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\_:K168, \_:S169, \_:A170, \_:L171, \_:L172, \_:S173, \_:T174,  
\_:N175, \_:K176, \_:A177, \_:V178, \_:V179, \_:S180, \_:L181,  
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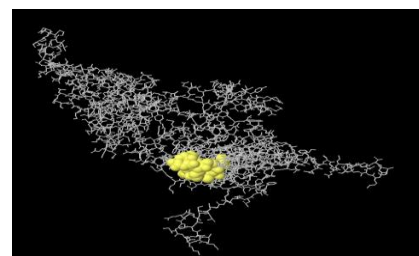
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\_:A107, \_:R108, \_:R109, \_:E110, \_:L111, \_:P112, \_:R113, \_:F114,  
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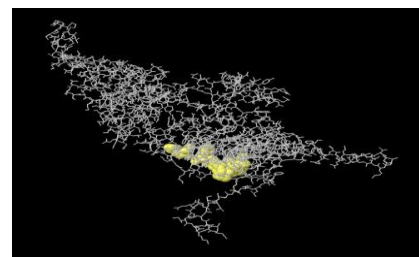
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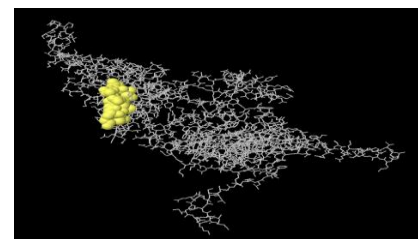
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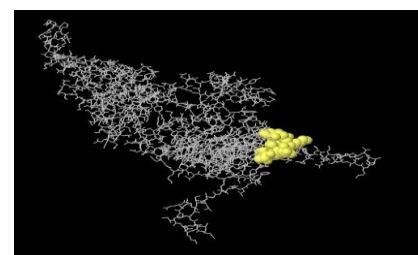
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\_:D385, \_:I386, \_:N388, \_:P389, \_:K390



C69, \_:N70, \_:G71, \_:T72, \_:D73, \_:A74, \_:K75, \_:V76, \_:K77



:T323, \_:T324, \_:N325, \_:T326, \_:K327, \_:E328, \_:G329, \_:S330,  
\_:N331, \_:K399



**Table 7.** Codon optimized nucleotide sequence of MEV construct for cloning in *E. coli* strain K12. Red color bold sequence at 5' site (N-terminal) is representing HindIII restriction enzyme site, while green color bold sequence at 3' site (C-terminal) is representing BamHI restriction enzyme site.

```
AAGCTTGGTATCATCAACACCCTGCAGAAATACTACTGCCGTGTTTCGTGGTGGTCGTTGCGCTGTTCTGTCTTGCCTGCCGAAAGAAGAACAGATCG
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AGTTACCCTGACCGGTCCGGGTCCGGGTATCATCTTCATCGCTTCTGCTAACAACAAAGTTACCCTGACCACCGGATCC
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