Supplementary Information

Designing novel epitope-based polyvalent vaccines against herpes simplex virus-1 and 2 exploiting the immunoinformatics approach

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Tables

Supplementary Table S1. The antigenicity determination and physicochemical property analysis of the selected viral proteins. AN; antigenicity, pI; Isoelectric point, AI; aliphatic index, GRAVY; grand average of hydropathicity.

ID of the protein sequence	AN	Total amino acids	pI	Ext. coefficient (in M^{-1} cm^{-1})	Est. half-life (in mammalian cell)	AI	GRAVY
P04488	Antigen	550	5.74	104110	30 h	74.15	-0.255
P10211	Antigen	904	8.30	105255	30 h	70.83	-0.403
Q69091	Antigen	394	7.64	58705	30 h	89.42	-0.143
Q703F0	Antigen	552	5.61	105600	30 h	73.88	-0.250
P06436	Antigen	903	7.89	105255	30 h	70.91	-0.392
Q05059	Antigen	394	8.10	58705	30 h	88.93	-0.151
P89475	Antigen	545	6.03	114080	30 h	78.97	-0.183
P08666	Antigen	904	8.57	95870	30 h	73.52	-0.340
Q69467	Antigen	393	7.65	57215	30 h	91.15	-0.124
P06763	Antigen	904	8.65	95870	30 h	73.63	-0.337
P03172	Antigen	393	8.11	57215	30 h	91.15	-0.127

Supplementary Table S2. MHC class-I epitope prediction and topology, antigenicity, allergenicity, toxicity, conservancy, and human homology analysis of the epitopes of EG-E. AN; antigenicity, AG; allergenicity, CN; conservancy.

Epitope	Topology	AN	AG	Toxicity	CN	Human Homology
YSMDVVWLR	Inside	Antigen	Allergen	Non-toxic	Non-conserved	Non-homolog
YTLSVGDIK	Inside	Antigen	Non-allergen	Non-toxic	Conserved	Non-homolog
ITISTAAQY	Inside	Antigen	Non-allergen	Non-toxic	Conserved	Non-homolog
TISTAAQYR	Inside	Antigen	Allergen	Non-toxic	Conserved	Non-homolog
VSCLAGTPK	Inside	Antigen	Allergen	Non-toxic	Non-conserved	Non-homolog
RTDFVWQER	Inside	Non-antigen	Allergen	Non-toxic	Non-conserved	Non-homolog
RIYESCLYH	Inside	Non-antigen	Allergen	Non-toxic	Non-conserved	Non-homolog
VVEQPLPQR	Outside	Non-antigen	Non-allergen	Non-toxic	Conserved	Non-homolog
AVKSRASGK	Inside	Antigen	Non-allergen	Non-toxic	Conserved	Non-homolog
GTPKTSWRR	Inside	Non-antigen	Non-allergen	Non-toxic	Non-conserved	Non-homolog

Supplementary Table S3. MHC class-II epitope prediction and topology, antigenicity, allergenicity, toxicity, conservancy, cytokine production, and human homology analysis of the epitopes of EG-E. AN; antigenicity, AG; allergenicity, CN; conservancy.

Epitope	Topology	AN	AG	Toxicity	CN	IFN-gamma inducing capacity	IL-4 inducing capacity	IL-10 inducing capacity	Human homology
GAALLLSALGLSVWA	Outside	Non-antigen	Non-allergen	Non toxic	Non-conserved	Non-inducer	Non-inducer	Non-inducer	Non-homolog
MGAALLLSALGLSVW	Outside	Antigen	Non-allergen	Non toxic	Non-conserved	Non-inducer	Non-inducer	Non-inducer	Non-homolog
VMGAALLLSALGLSV	Outside	Antigen	Non-allergen	Non toxic	Non-conserved	Non-inducer	Non-inducer	Non-inducer	Non-homolog
AALLLSALGLSVWAC	Outside	Non-antigen	Non-allergen	Non toxic	Non-conserved	Inducer	Non-inducer	Non-inducer	Non-homolog
ALLLSALGLSVWACM	Outside	Non-antigen	Non-allergen	Non toxic	Non-conserved	Inducer	Non-inducer	Non-inducer	Non-homolog
GAVMGAALLLSALGL	Outside	Antigen	Non-allergen	Non toxic	Conserved	Non-inducer	Non-inducer	Inducer	Non-homolog
AVMGAALLLSALGLS	Outside	Antigen	Non-allergen	Non toxic	Conserved	Non-inducer	Non-inducer	Inducer	Non-homolog
DQTYSMDVVWLRFDV	inside	Antigen	Allergen	Non toxic	Conserved	Non-inducer	Non-inducer	Non-inducer	Non-homolog
AWGHITISTAAQYRN	inside	Antigen	Allergen	Non toxic	Conserved	Non-inducer	Inducer	Non-inducer	Non-homolog
GHITISTAAQYRNAV	inside	Antigen	Allergen	Non toxic	Conserved	Inducer	Inducer	Non-inducer	Non-homolog

Supplementary Table S4. MHC class-I epitope prediction and topology, antigenicity, allergenicity, conservancy, and human homology analysis of the epitopes of EG-B. AN; antigenicity, AG; allergenicity, CN; conservancy.

Epitope	Topology	AN	AG	Toxicity	CN	Human Homology
KVTDMVMRK	Inside	Antigen	Non-allergen	Non-toxic	Conserved	Non-homolog
GTSALLSAK	Outside	Non-antigen	Non-allergen	Non-toxic	Non-Conserved	Non-homolog
LGENNELRL	Inside	Non-antigen	Non-allergen	Non-toxic	Conserved	Non-homolog
AIASATVGR	Inside	Antigen	Non-allergen	Non-toxic	Non-conserved	Non-homolog
TVAWDWVPK	Outside	Non-antigen	Non-allergen	Non-toxic	100%	Non-homolog
SAMERTEHK	Inside	Antigen	Allergen	Non-toxic	Conserved	Non-homolog
YAYSHQLSR	Inside	Antigen	Non-allergen	Non-toxic	Conserved	Non-homolog
FTFGGGYVY	Outside	Non-antigen	Allergen	Non-toxic	Conserved	Non-homolog
ASANASVER	Inside	Antigen	Non-allergen	Non-toxic	Conserved	Non-homolog
ADIDTVIHA	Inside	Non-antigen	Allergen	Non-toxic	Non-conserved	Non-homolog

Supplementary Table S5. MHC class-II epitope prediction and topology, antigenicity, allergenicity, conservancy, cytokine production, and human homology analysis of the epitopes of EG-B. AN; antigenicity, AG; allergenicity, CN; conservancy.

Epitope	Topology	AN	AG	Toxicity	CN	IFN-gamma inducing capacity	IL-4 inducing capacity	IL-10 inducing capacity	Human homolog
GFLIAYQPLLSNTLA	Outside	Non-antigen	Non-allergen	Non toxic	Non-conserved	Non-inducer	Non-inducer	Inducer	Non-homolog
AVGLLVLAGLAAAFF	Outside	Antigen	Non-allergen	Non toxic	Conserved	Non-inducer	Non-inducer	Non-inducer	Non-homolog
VGLLVLAGLAAAFFA	Outside	Antigen	Non-allergen	Non toxic	Conserved	Non-inducer	Non-inducer	Non-inducer	Non-homolog
REMIRYMALVSAMER	Inside	Non-antigen	Allergen	Non toxic	Non-conserved	Non-inducer	Non-inducer	Non-inducer	Non-homolog
EMIRYMALVSAMERT	Inside	Antigen	Allergen	Non toxic	Non-conserved	Inducer	Non-inducer	Non-inducer	Non-homolog
MIRYMALVSAMERTE	Inside	Antigen	Allergen	Non toxic	Non-conserved	Inducer	Non-inducer	Non-inducer	Non-homolog
IRYMALVSAMERTEH	Inside	Antigen	Non-allergen	Non toxic	Conserved	Inducer	Non-inducer	Non-inducer	Non-homolog
GGFLIAYQPLLSNTL	Outside	Non-antigen	Non-allergen	Non toxic	Conserved	Non-inducer	Non-inducer	Inducer	Non-homolog
ATMYYKDVTVSQVWF	Inside	Antigen	Non-allergen	Non toxic	Non-conserved	Non-inducer	Non-inducer	Non-inducer	Non-homolog
FKATMYYKDVTVSQV	Inside	Antigen	Allergen	Non toxic	Conserved	Non-inducer	Inducer	Non-inducer	Non-homolog

Supplementary Table S6. MHC class-I epitope prediction and topology, antigenicity, allergenicity, conservancy, and human homology analysis of the epitopes of EG-D. AN; antigenicity, AG; allergenicity, CN; conservancy.

Epitope	Topology	AN	AG	Toxicity	CN	Human Homology
RTVAVYSLK	Inside	Antigen	Non-allergen	Non-toxic	Conserved	Homolog
TVYYAVLER	Inside	Non-antigen	Allergen	Non-toxic	Conserved	Homolog
RTQPRWNYY	Inside	Antigen	Allergen	Non-toxic	Non-conserved	Homolog
KMADPNRFR	Inside	Antigen	Non-allergen	Non-toxic	Conserved	Non-homolog
LTDPPGVRR	Inside	Non-antigen	Allergen	Non-toxic	Conserved	Non-homolog
KIAGWHGPK	Inside	Non-antigen	Non-antigen	Non-toxic	Non-conserved	Homolog
AIPITVMEY	Inside	Non-antigen	Non-allergen	Non-toxic	Conserved	Homolog
SIQDAATPY	Inside	Antigen	Non-allergen	Non-toxic	Conserved	Non-homolog
ITQFILEHR	Inside	Antigen	Allergen	Non-toxic	Conserved	Homolog

Supplementary Table S7. MHC class-II epitope prediction and topology, antigenicity, allergenicity, conservancy, cytokine production, and human homology analysis of the epitopes of EG-D. AN; antigenicity, AG; allergenicity, CN; conservancy.

Epitope	Topology	AN	AG	Toxicity	CN	IFN-gamma inducing capacity	IL-4 inducing capacity	IL-10 inducing capacity	Human homology
PRWNYYDSFSAVSED	Outside	Antigen	Non-allergen	Non toxic	Non-conserved	Inducer	Non-inducer	Inducer	Homolog
QPRWNYYDSFSAVSE	Inside	Antigen	Non-allergen	Non toxic	Non-conserved	Inducer	Inducer	Inducer	Homolog
RTQPRWNYYDSFSAV	Inside	Antigen	Allergen	Non toxic	Non-conserved	Inducer	Inducer	Inducer	Non-homolog
RWNYYDSFSAVSEDN	Inside	Antigen	Allergen	Non toxic	Non-conserved	Inducer	Non-inducer	Inducer	Homolog
TQPRWNYYDSFSAVS	Inside	Antigen	Allergen	Non toxic	Conserved	Inducer	Inducer	Inducer	Homolog
ILFVVIVGLHGVRSK	Outside	Antigen	Non-allergen	Non toxic	Conserved	Inducer	Non-inducer	Non-inducer	Non-homolog
GAVILFVVIVGLHGV	Outside	Antigen	Non-allergen	Non toxic	Conserved	Inducer	Non-inducer	Non-inducer	Non-homolog
VILFVVIVGLHGVRS	Outside	Antigen	Non-allergen	Non toxic	Conserved	Inducer	Non-inducer	Non-inducer	Non-homolog
AVILFVVIVGLHGVR	Outside	Antigen	Non-allergen	Non toxic	Conserved	Inducer	Non-inducer	Non-inducer	Non-homolog
FVVIVGLHGVRSKYA	Outside	Antigen	Non-allergen	Non toxic	Conserved	Inducer	Non-inducer	Non-inducer	Non-homolog

Supplementary Table S8. B-cell epitope prediction and antigenicity, allergenicity, toxicity, conservancy, human homology, and topology analysis of the epitopes of the three selected proteins. AN; antigenicity, AG; allergenicity.

Envelope glycoprotein E							Envelope glycoprotein B							Envelope glycoprotein D						
Epitope	AN	AG	Topology	Toxicity		Human homology	Epitope	AN	AG	Topology	Toxicity		Human Homology	Epitope	AN	AG	Topology	Toxicity		Human homology
PECLS PADA PCAA ST	Antigen	Non-allergen	Outside	Non-toxic		Non-homolog	EQPR RCPT RPEG QNYT	Non-antigen	Allergen	Inside	Non-toxic		Non-homolog	GLP DPF QPP SLP	Antigen	Non-allergen	Outside	Non-toxic		Homolog
VVEQ PLPQR GADL AEPT HPHV GAPP HAPP THG	Antigen	Non-allergen	Outside	Non-toxic		Non-homolog	TTKA RATA PTTR N	Antigen	Non-allergen	Inside	Non-toxic		Non-homolog	APS EAP QIV RG ASE DV RK QP	Antigen	Allergen	Outside	Non-toxic		Homolog
AYAP PAPSA TGGL	Antigen	Non-allergen	Outside	Non-toxic		Non-homolog	REQS RKPP NPTPP PPGAS ANAS	Antigen	Non-allergen	Inside	Non-toxic		Non-homolog	SAC LSP QA YQ QG VT	Antigen	Non-allergen	Inside	Non-toxic		Homolog
YADW SSDSE GERD QVPW	Antigen	Non-allergen	Outside	Non-toxic		Non-homolog	YEDQ GPLV EGQL GEN	Non-antigen	Allergen	Outside	Non-toxic		Non-homolog	WH GPK APY TST	Antigen	Non-allergen	Outside	Non-toxic		Homolog

LAPPE RPDSP STNG SG													LLP PEL SET PNA TQP ELA PED PED SAL LED PVG TV APQ PPN WHI PSI QD AA TPY HPP ATP NN						
-	-	-	-	-	-	-	RTEH KAKK KG	Antigen	Non-allergen	Outside	Non-toxic		Non-homolog	IRE DD QPS SHQ	Antigen	Non-allergen	Inside	Non-toxic		Non-homolog

Supplementary Table S9. Results of the secondary structure analysis of the vaccine constructs.

| Name of the vaccine	Alpha helix (percentage of amino acids)	Beta sheet (percentage of amino acids)	Coil structure (percentage of amino acids)
HV-1	22.0%	14.6%	63.4%
HV-2	29.2%	12.3%	58.5%
HV-3	39.8%	6.6%	53.6%

Supplementary Table S10. Results of the tertiary structure analysis of the vaccine constructs.

| Name of the vaccine	Number of the domains	p-value
HV-1	3	1.67e-04
HV-2	3	8.91e-05
HV-3	3	1.73e-04

Supplementary Table S11. The list of the predicted conformational B-cell epitopes of the best

vaccine, HV-1 with their scores.

No	Residues	Number of residues	Score	Figure
01	T452, T453, R454, K456, K457, T459, E460, H461, K462, A463, K464, K465, K466, G467, K468, K469, R470, E471, Q472, S473, R474, K475, P476, P477, N478, P479, T480, P481, P482, P483, P484, G485, A486, S487, A488, N489, A490, S491, K492, K493, I494, R495, E496, D497, D498, Q499, P500, S501, S502, H503, Q504, K505, K506, A507, K508, F509, V510, A511, A512, W513, T514, L515, K516, A517, A518, A519, G520, G521, G522, G523, S524	71	0.823	Supplementary Figure S8 (a)
02	N162, R165, G166, G167, G168, G169, S170, S171, I172, Q173, D174, A175, A176, T177, P178, Y179, G180, P181, G182, P183, G184, G185, A186, V187, M188, G189, A190, A191, L192, L193, L194, S195, A196, L197, G198, L199, G200, P201, G202, P203, G204, A205, V206, M207, G208, A209, A210, L211, L212, L213, S214, A215, L216, G217, L218, S219, G220, P221, G222, P223, G224, I225, R226, Y227, M228, A229, L230, V231, S232, A233, M234, E235, R236, T237, E238, H239, G240, P241, G242, P243, G244, I245, L246, F247, V248, G252	86	0.771	Supplementary Figure S8 (b)
03	E1, A2, A3, A4, K5, G6, I7, I8, N9, T10, L11, Q12, K13, Y14, Y15, C16, R17, V18, R19, G20, G21, R22, C23, A24, V25, L26, S27, C28, L29, P30, K31, E32, Q34, I35, G36, K37, C38, S39, T40, R41, G42, R43, K44, C45, C46, R47, R48, K50, E51, A52, A53, A54, K55, A56, K57, F58, V59, A60, A61, A62, T63, L64, K65, A66, A67, A68, G69, G70, G71, S72, Y73, T74, L75, S76, V77, G78, D79, I80, K81, G82, G83, G84, G85, S90, T91, A92, A93, Q94, Y95, G96, G97, G98, A106, G112, G113, K115	96	0.656	Supplementary Figure S8 (c)
04	G314, L315, H316, G317, V318, R319, G320, P321, G322, P323, G324, F325, V326, V327, I328, V329, A361, P362, P363, A364, P365	21	0.555	Supplementary Figure S8 (d)

Figures
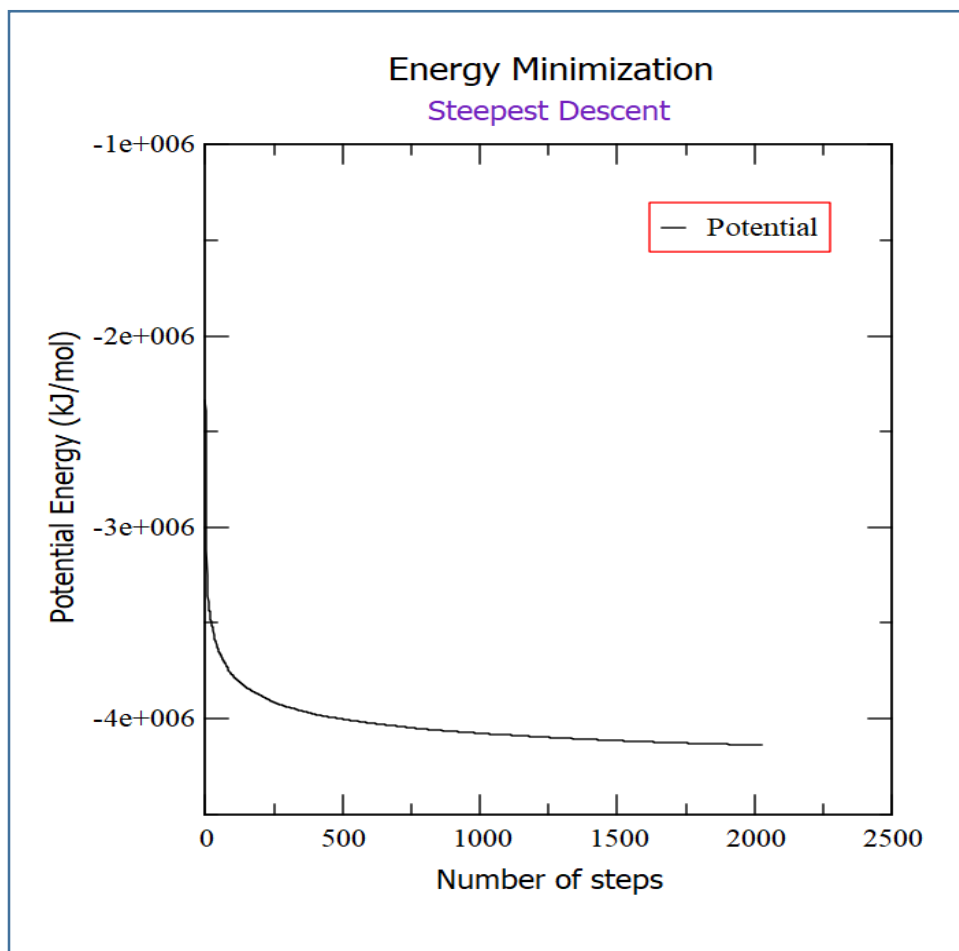
Supplementary Figure S1. Potential Energy calculated at the steepest descent**.**
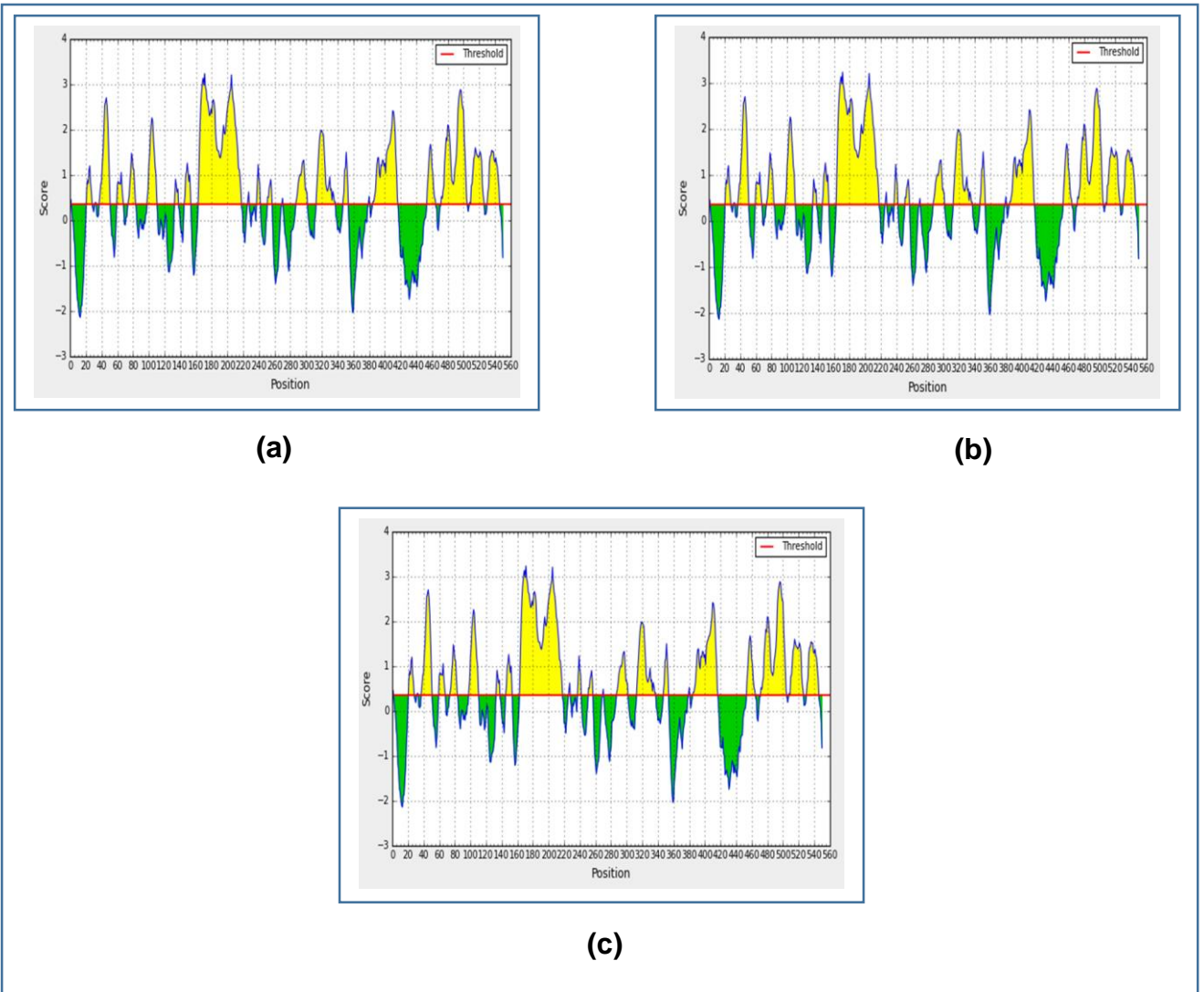
Supplementary Figure S2. Figure showing the graphs of the B-cell epitope prediction of the three selected proteins using BepiPred linear epitope prediction method. Here, (a) is the graph of epitope prediction for envelope protein E, (b) is the graph of epitope prediction for envelope glycoprotein B and (c) is the graph of epitope prediction for envelope protein D.
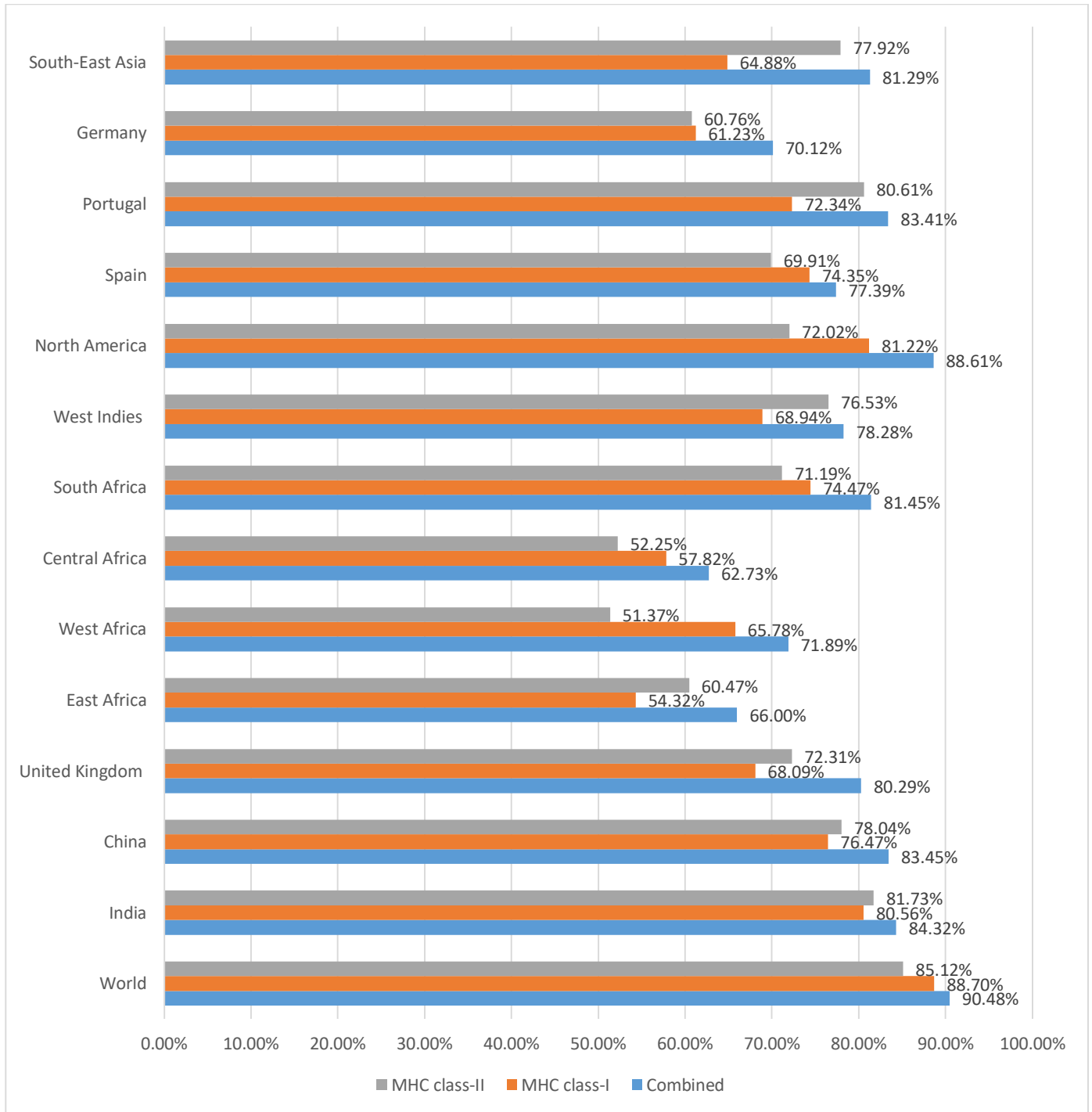
Supplementary Figure S3. The result of the population coverage analysis of the best selected epitopes and their selected MHC alleles.

(a)

(c)

(b)

(d)

Supplementary Figure S4. The results of the MHC cluster analysis. Here, (a) is the heat map (left) and the tree map (right) of MHC class-I cluster analysis, (b) is the heat map (left) and the tree map (right) of MHC class-II cluster analysis.

(a)

```
Conf:
Cart:
Pred: CHHHHHHHHHHHHHHEEEECCEEEEEECCCCHHHCCCCCCCCHHHHHH
AA:   EAAAKGIINTLQKYYCRVRGGRCAVLSCLPKEEQIGKCSTRGRKCCRRKK
              10        20        30        40        50

Conf:
Cart:
Pred: HHHHHHHEEEEEHHHHHCCCEEEEEECCCCCCEEEEECCEECCCCCCC
AA:   EAAAKAKFVAAWTLKAAAGGGSYTLSVGDIKGGGSITISTAAQYGGGSAV
              60        70        80        90       100

Conf:
Cart:
Pred: CCCCCCCCCCCCHHHHEECCCCCEEECEEECCCCCCCCCCCCEEECCCCC
AA:   KSRASGKGGGSKVTDMVMRKGGGSYAYSHQLSRGGGSASANASVERGGGS
             110       120       130       140       150

Conf:
Cart:
Pred: CCCCCCCCCCCCCCCCCCCCCCCCCCHHHCCCCCCCCCCCCCCCCCCCEEE
AA:   KMADPNRFRGGGSKIAGWHGPKGGGSSIQDAATPYGPGPGVRMETPEAIL
             160       170       180       190       200

Conf:
Cart:
Pred: ECCCCCCCCCCCCCCHHHCCCCCCCCCEECCHHHCCCCCCCEEEEEEEE
AA:   FSGPGPGLRFDVPTSCAEMGPGPGPSWVSLMPPKQVGPGPGTMYYKDVTV
             210       220       230       240       250

Conf:
Cart:
Pred: EEECCCCCHHHCCCCCCCCCCCCCEEEEECCCCHHCCCCCHHHHCCCE
AA:   SQVGPGPGYARDLTTKARATGPGPGVMRLQSNPMKALGPGPGQAYQQGVT
             260       270       280       290       300

Conf:
Cart:
Pred: ECCCCCCCHHHHHHCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
AA:   VDSIGPGPGYWMRRHTQKAPKGPGPGVLLNAPSEAPQIKKPECLSPADAP
             310       320       330       340       350

Conf:
Cart:
Pred: CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCHHHHHHHHHCCCC
AA:   CAASTKKAYAPPAPSATGGLKKTTKARATAPTTRNKKRTEHKAKKKGKKG
             360       370       380       390       400

Conf:
Cart:
Pred: CCCCCCCCCCCCCCCCHHHHHHCCCHHHCCCCCCHHHHHHHHEEHHH
AA:   LPDPFQPPSLPKKSACLSPQAYQQGVTKKIREDDQPSSHQKKAKFVAAWT
             410       420       430       440       450

Conf:
Cart:
Pred: HHHHHCCCC
AA:   LKAAAGGGS
             460       470       480       490       500
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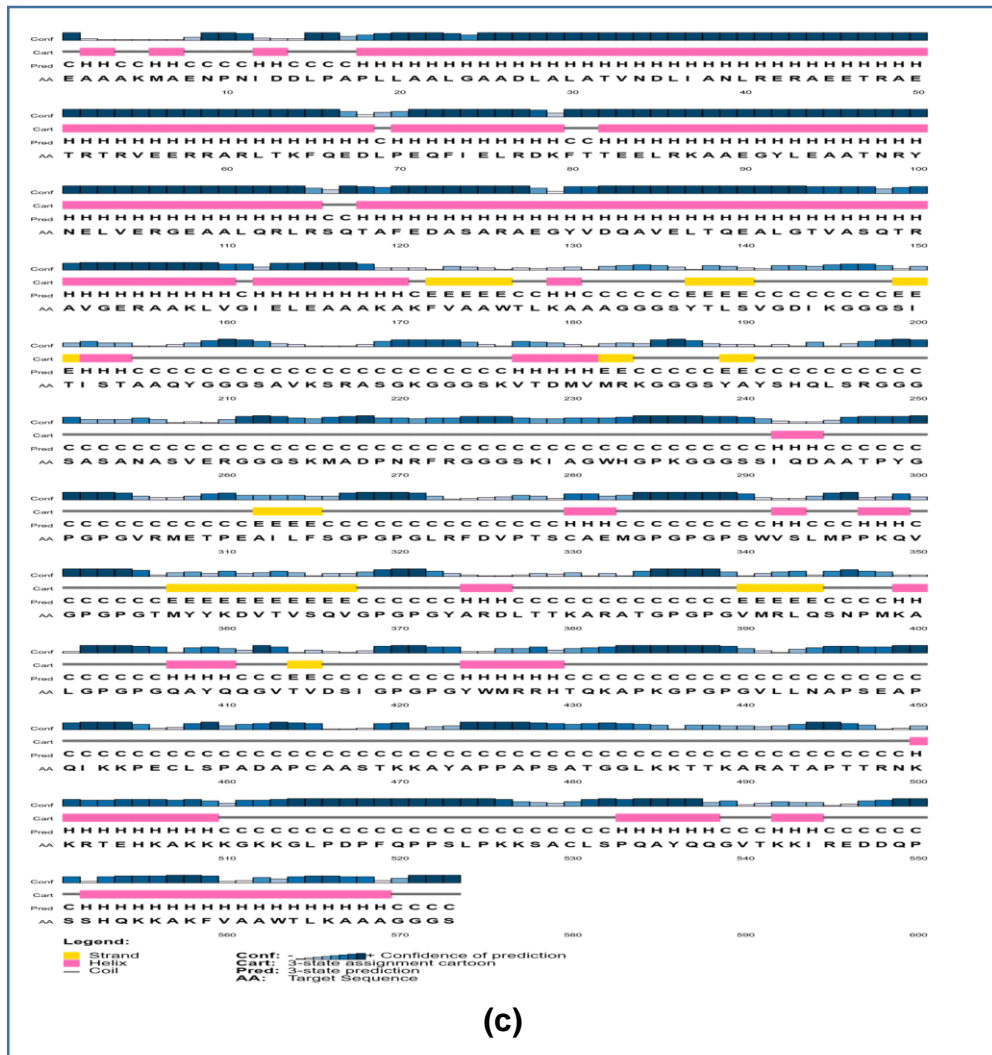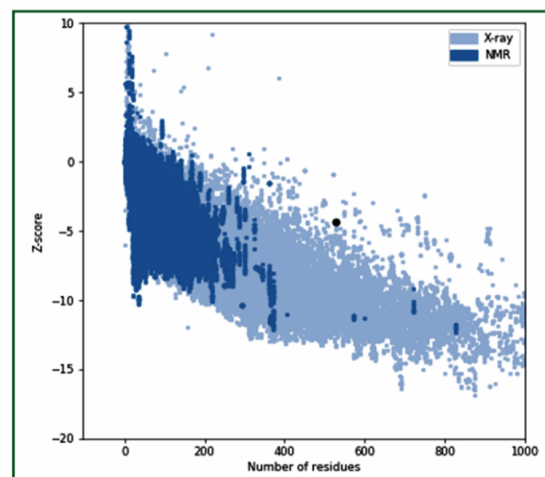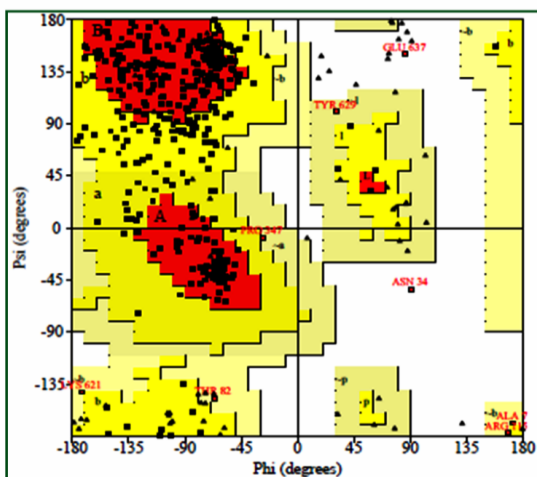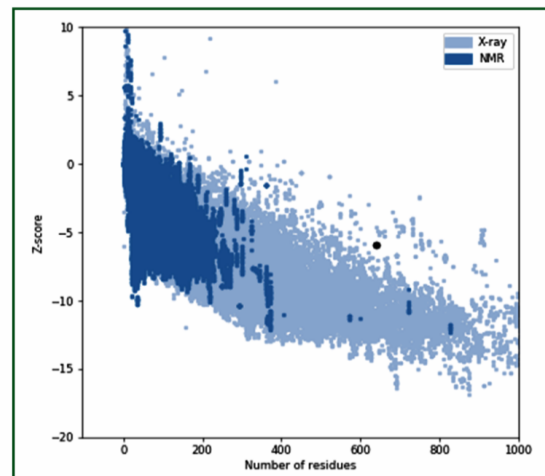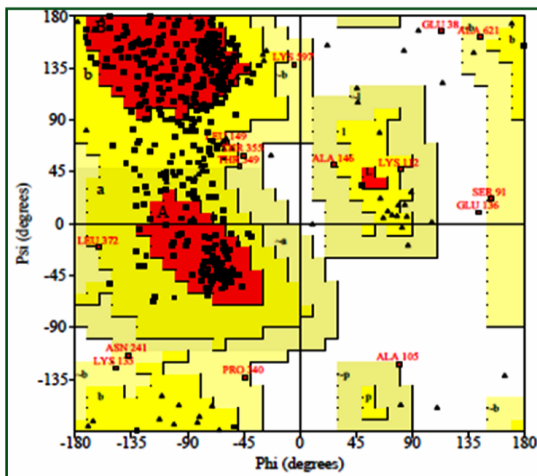
Legend:
- Strand
- Helix
- Coil

Conf: ___ + Confidence of prediction
Cart: 3-state assignment cartoon
Pred: 3-state prediction
AA: Target Sequence

(b)

```
Conf:
Cart:
Pred: CHHHHHHCCCHHHHHHHHHHHCCHHHHHHHHHHHHHHHCCCCCCCHHHHHH
AA:   EAAAKMAKLSTDELLDAFKEMTLLELSDFVKKFEETFEVTAAAPVAVAAA
              10        20        30        40        50

Conf:
Cart:
Pred: CCCCCHHHHHHHHHCEEEEEEECCCHHHHHHHHHHHHHHCCCCHHHHHH
AA:   GAAPAGAAVEAAEEQSEFDVILEAAGDKKIGVIKVVREIVSGLGLKEAKD
              60        70        80        90       100

Conf:
Cart:
Pred: HHCCCCHHHHCCCCHHHHHHHHHHHHHHCCCEEEEEHHHHHCEEEEEEEEH
AA:   LVDGAPKPLLEKVAKEAADEAKAKLEAAGATVTVKEAAAKAKFVAAWTLK
             110       120       130       140       150

Conf:
Cart:
Pred: HHHCCCEEEEEEECCCCCCEEEEECCEECCCCCCCCCCCCCCCCCCCCC
AA:   AAAGGGSYTLSVGDIKGGGSITISTAAQYGGGSAVKSRASGKGGGSKVTD
             160       170       180       190       200

Conf:
Cart:
Pred: HEEECCCCCEEECEEECCCCCCCCCCCEEECCCCCCCCCCCCCCCCCCCC
AA:   MVMRKGGGSYAYSHQLSRGGGSASANASVERGGGSKMADPNRFRGGGSKI
             210       220       230       240       250

Conf:
Cart:
Pred: CCCCCCCCCCCCHHHCCCCCCCCCCCCCCCCCCEEEECCCCCCCCCCCCC
AA:   AGWHGPKGGGSSIQDAATPYGPGPGVRMETPEAILFSGPGPGLRFDVPTS
             260       270       280       290       300

Conf:
Cart:
Pred: HHHCCCCCCCCCCCCCHHHCCCCCCCEEEEEEEEEEEECCCCCCHHHCCC
AA:   CAEMGPGPGPSWVSLMPPKQVGPGPGTMYYKDVTVSQVGPGPGYARDLTT
             310       320       330       340       350

Conf:
Cart:
Pred: CCCCCCCCCCEEEEECCCCHHCCCCCCHHHHCCCEECCCCCCCCHHHHHH
AA:   KARATGPGPGVMRLQSNPMKALGPGPGQAYQQGVTVDSIGPGPGYWMRRH
             360       370       380       390       400

Conf:
Cart:
Pred: CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
AA:   TQKAPKGPGPGVLLNAPSEAPQIKKPECLSPADAPCAASTKKAYAPPAPS
             410       420       430       440       450

Conf:
Cart:
Pred: CCCCCCCCCCCCCCCCCCCCHHHHHHHHHCCCCCCCCCCCCCCCCCCCC
AA:   ATGGLKKTTKARATAPTTRNKKRTEHKAKKKGKKGLPDPFQPPSLPKKSA
             460       470       480       490       500

Conf:
Cart:
Pred: CCCHHHHHCCCHHHCCCCCCHHHHHHHHHHHHHHHHHHHCCCC
AA:   CLSPQAYQQGVTKKIREDDQPSSHQKKAKFVAAWTLKAAAGGGS
             510       520       530       540       550
```

Legend:
- Strand
- Helix
- Coil

Conf: ___ + Confidence of prediction
Cart: 3-state assignment cartoon
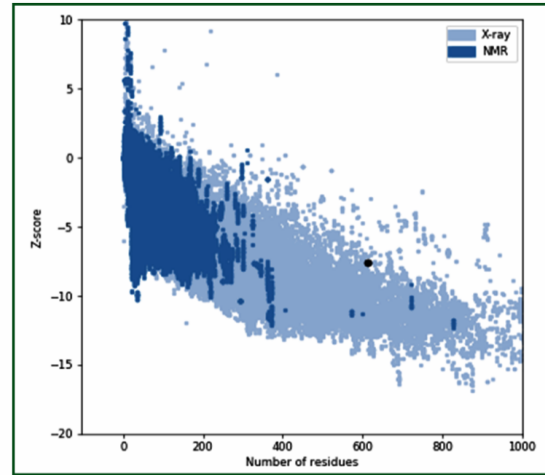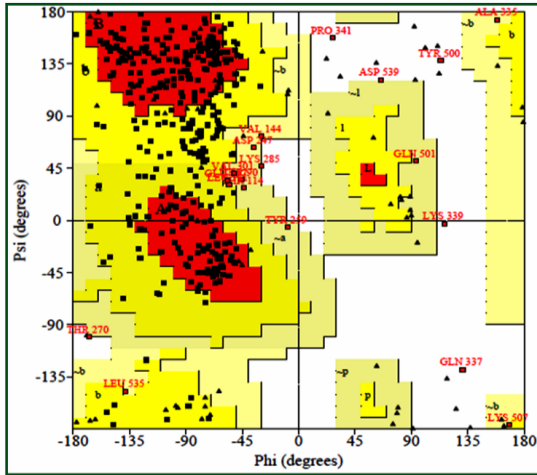Pred: 3-state prediction
AA: Target Sequence

(c)

Supplementary Figure S5. The results of the secondary structure prediction of the constructed HSV vaccines. Here, (a) is HV-1, (b) is HV-2, (c) is HV-3.

**Ramachandran plots**   **Z-score graphs**

Supplementary Figure S6. The results of the Ramachandran plot analysis generated by PROCHECK server (left) and quality score or z-score graphs (right) generated by the ProSA-web server of the three refined vaccine constructs, (a) HV-1, (b) HV-2, (c) HV-3. In the Ramachandran plots, the orange and deep yellow colored regions are the allowed regions, the light yellow regions are the generously allowed regions and the white regions are the outlier regions and the glycine residues are represented as triangles. All the three vaccine constructs were predicted to have quite good structural quality.
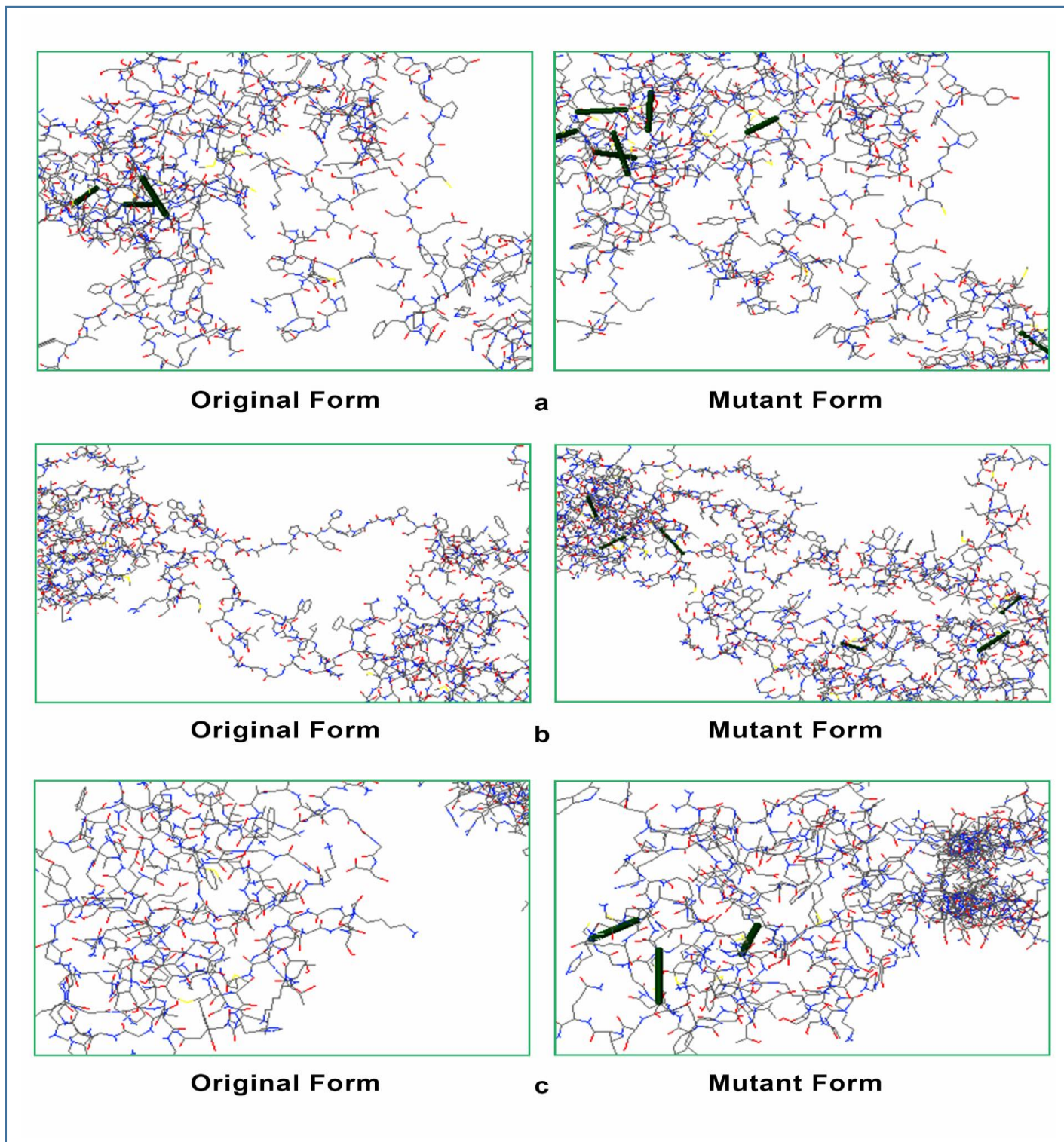
Supplementary Figure S7. The disulfide engineering of the three vaccine constructs, both the original (left) and mutant (right) forms are shown. Here, (a) HV-1, (b) HV-2, (c) HV-3.
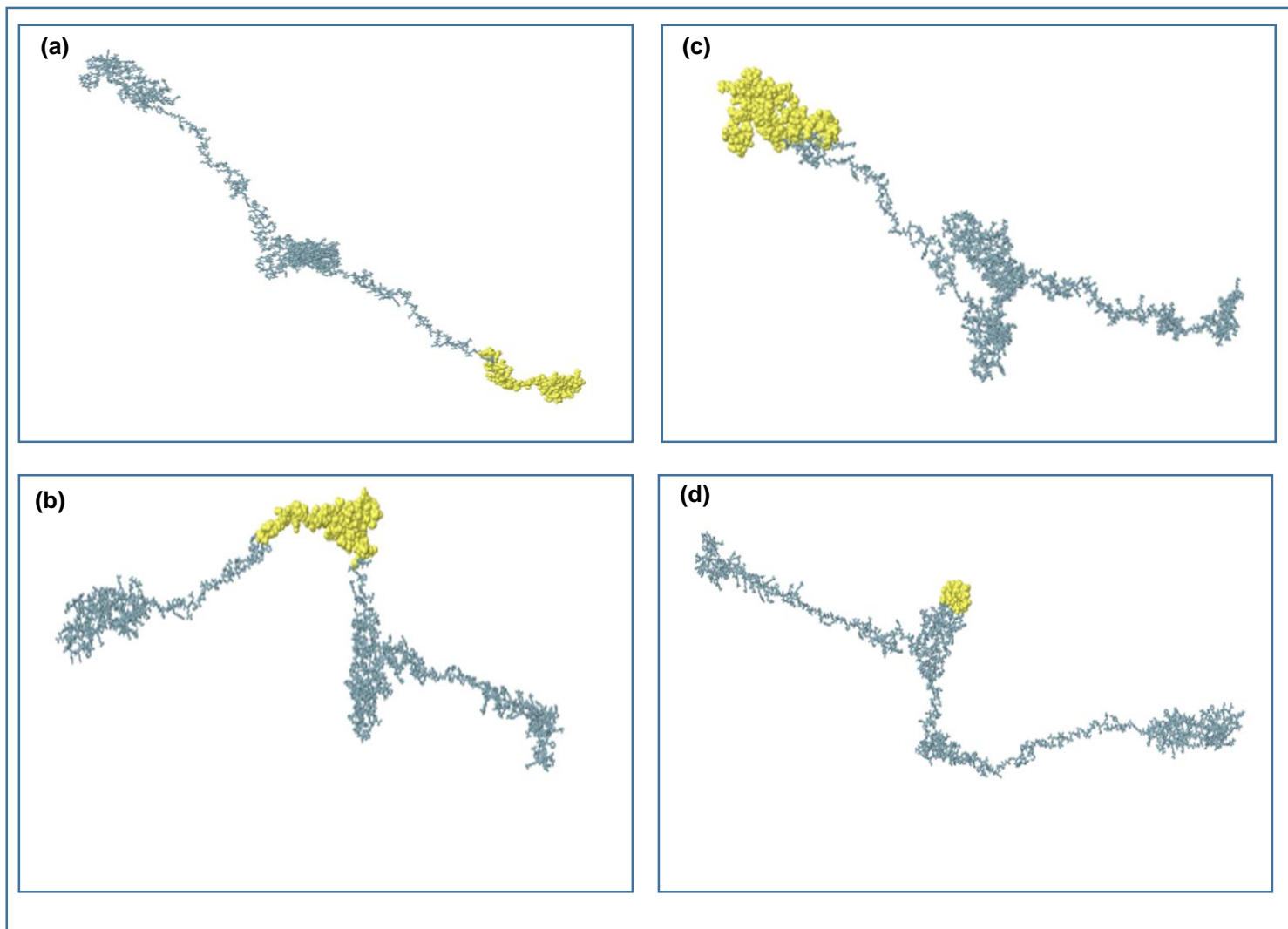
Supplementary Figure S8. Graphical representations of the five predicted conformational B-cell epitopes of HV-1 vaccine construct. The B-cell epitopes are indicated by yellow colored ball-shaped structures.
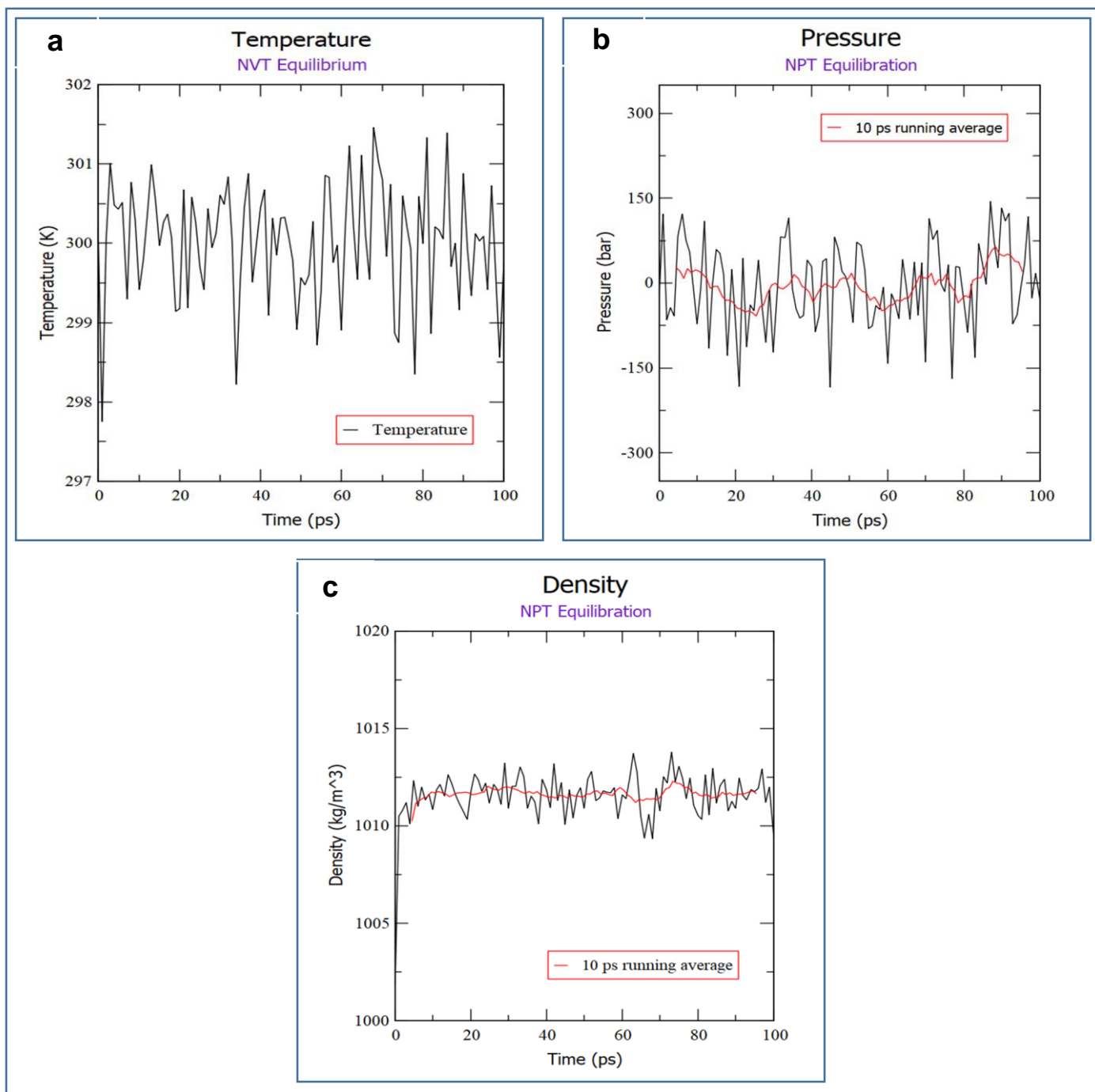
Supplementary Figure S9. (a) Temperature variations during simulation. Temperature had reached 300K and showed minimum fluctuations afterwards. (b) Pressure variations along with a running average with 10 ps window. (c) Density variations over 100 ps with a running average plot with 10 ps window.
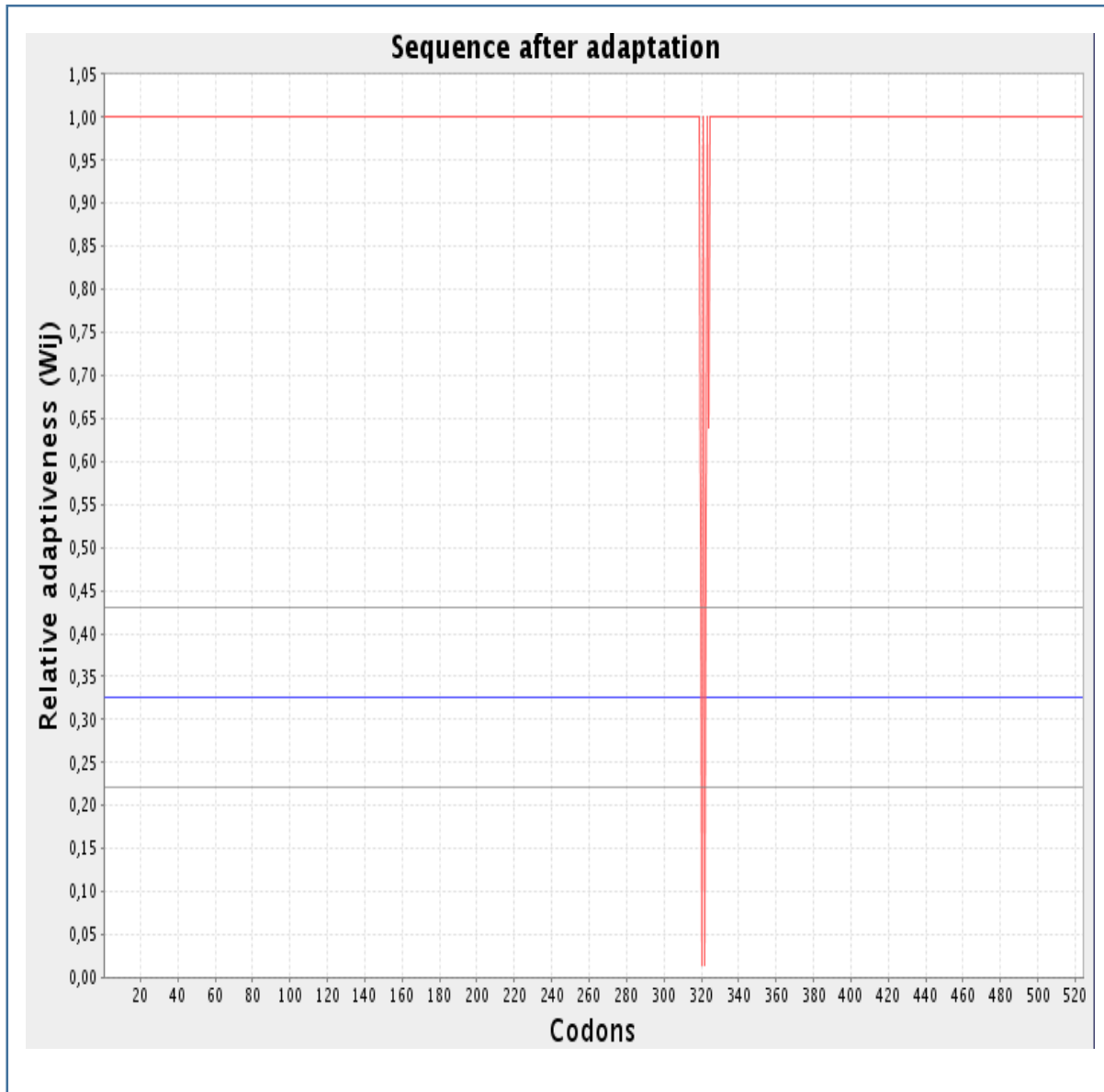
Supplementary Figure S10. Figure showing the codon adaptation graph of the HV-1 vaccine.